Sunday, April 27, 2025

TUMOR BIOLOGY: Cancer Stem Cells
Poster Session

#1 Androgens in glioblastoma: effect of testosterone on the maintenance of glioma stem-like cells.
Juan Carlos Quintero-Gallegos[1], Karina Hernandez-Ortega[2], Martha Robles-Flores[3], Nestor Fabian Diaz[4], Ignacio Camacho-Arroyo[1]

[1]Unidad de Investigacion en Reproduccion Humana, Instituto Nacional de Perinatologia- Facultad de Quimica, Universidad Nacional Autonoma de Mexico, Mexico City, Mexico,[2]Departamento de Biologia, Facultad de Quimica, Universidad Nacional Autonoma de Mexico, Mexico City, Mexico,[3]Departamento de Bioquimica, Facultad de Medicina, Universidad Nacional Autonoma de Mexico, Mexico City, Mexico,[4]Departamento de Fisiologia y Desarrollo Celular, Instituto Nacional de Perinatologia, Mexico City, Mexico

Glioblastoma is the most common and lethal primary malignant tumor of the central nervous system, with a prevalence 1.6 times more frequent in men than in women, suggesting that sex hormones such as androgens play an important role in tumor progression. Poor prognosis has been associated with the presence of cancer stem cells that in glioblastoma are known as glioma stem cells. There is evidence that patients with glioblastoma have a higher serum concentration of testosterone, as well as a higher expression of the androgen receptor. In addition, the use of androgen receptor antagonists reduces the percentage of cancer stem cells. However, the role of testosterone in regulating stemness is unknown. In this study we analyzed the role of testosterone (100 nM) in processes associated with stem cell maintenance in the human glioblastoma cell lines U251 and T98G. Analyzing the presence of the androgen receptor, a higher expression was found in stem cell-enriched suspension cultures compared to monolayer cultures. The sequences of the stemness-associated genes PROM1, SOX2 and NES presented androgen response elements in their promoter regions. Consistently, testosterone increased the expression of these genes in stem cell-enriched cultures. Additionally, the self-renewal was evaluated by neurosphere generation assays, finding that testosterone favored the generation of primary and secondary neurospheres. Taken together, these results suggest that testosterone regulates the growth of cancer stem cells present in human glioblastoma by promoting the expression of stemness-associated genes as well as their self-renewal.
This research was funded by DGAPA-PAPIIT, grant number: IN201823, UNAM, México

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#100 L1CAM(CD171) can be highly expressed in many tumor types but lacks strong associations with tumor phenotype.

Seyma Buyucek[1], Magali Lurati[1], Katharina Moller[1], Florian Viehweger[1], Ria Schlichter[1], Anne Menz[1], Andreas M Luebke[1], Viktor Reiswich[1], Martina Kluth[1], Elena Bady[1], Lisa Hornsteiner[1], Claudia Hube-Magg[1], Andrea Hinsch[1], Florian Lutz[1], Soren Weidemann[1], Frank Jacobsen[1], David Dum[1], Christian Bernreuther[1], Patrick Lebok[1], Guido Sauter[1], Andreas H Marx[2], Ronald Simon[1], Christoph Fraune[1], Natalia Gorbokon[1], Eike Burandt[3], Sarah Minner[1], Stefan Steurer[1], Till S Clauditz[3], Till Krech[1], Viktoria Chirico[1], **Maximilian Lennartz**[1]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany,[2]Academic Hospital Fuerth, Fuerth, Germany,[3]Miamedes Health Group, Hamburg, Germany

The cell adhesions molecule L1 (CD171; L1CAM) was initially characterized as an essential protein for neural development but subsequent studies have pinpointed towards a critical role in cancer. Because of its expression in only few normal tissues and its membranous nature L1CAM is a promising drug target. To better comprehend the role of L1CAM expression in different cancer types, a tissue microarray containing 20,079 samples from 135 different tumor entities and 608 samples of 76 different normal tissue types was analyzed. A membranous L1CAM staining was found in 1175 (9.1%) of the 12888 interpretable tumor samples, including 301 (2.3%) with weak, 569 (4.4%) with moderate, and 305 (2.4%) with strong positivity. A total of 73 of 135 tumor entities showed L1CAM expression in at least one case, and 36 tumor categories included at least one case with strong L1CAM staining. The frequency of L1CAM positivity was particularly high in several subtypes of neural and neuroendocrine neoplasms (11.1-100%), endometrium carcinoma (15.4-54.8%), ovarian cancer (22.0-44.6%), cervical adenocarcinoma (27.3%), malignant melanoma (25.3-31.0%), malignant mesothelioma (20.0-29.2%), adenocarcinomas of the gastrointestinal and the biliopancreatic tractus (9.1-18.9%), and in advanced urothelial tumors (13.0-18.2%). In urinary bladder cancer, there was a significant association between L1CAM expression and invasive tumor growth (pTa vs. pT2-4, $p<0.0001$). In colorectal adenocarcinoma, L1CAM positivity was linked to left-sided tumor location and microsatellite instability. However, L1CAM staining was unrelated to tumor phenotype in muscle-invasive urothelial carcinoma, clear cell and papillary renal cell carcinoma, serous high-grade carcinoma of the ovary, endometrioid endometrium carcinoma, gastric adenocarcinoma, and in pancreatic adenocarcinoma. It is concluded that many different tumor entities could benefit from L1CAM targeting drugs once these should provide sufficiently safe and efficient. However, L1CAM expression does not appear to confer an aggressive phenotype to affected cancer cells.

POPULATION SCIENCES: Genomic Diversity and Disparities in Cancer: Global Insights into Epidemiology, Risk, Outcomes, and Precision Medicine
Poster Session

#1000 Impact of *GSTP1 Ile105Val* genetic polymorphism on cervical cancer risk among Bangladeshi female population.

Md. Mustafizur Rahman[1], Md Mehedi Hasan[1], Abdur Rahman Munna[1], Amir Hossain[2]

[1]Pharmacy Discipline, Khulna University, Khulna, Bangladesh,[2]Department of Pharmacy, Dhaka International University, Dhaka, Bangladesh

Background and aim: Worldwide, cervical cancer is both the fourth-most common cause of cancer and the fourth-most common cause of death from cancer in women. Approximately 90% of deaths from cervical cancer occurred in low- and middle-income countries. Glutathione S-transferase P1 (GSTP1) is a xenobiotic metabolizing (detoxifying) enzyme. Studies have suggested that GSTP1 genetic polymorphism in exon 5 (Ile105Val, *GSTP1 A313G*) may play important role in determining susceptibility to cervical cancer, though the data have often been inconsistent.

Objective: This study aimed to investigate the association of the *GSTP1* Ile105Val polymorphism with the prevalence and risk of cervical cancer in Bangladeshi females. We also aimed at finding any correlation between demographic variables and polymorphic genotypes in cervical cancer patients.

Materials and Methods: Polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) were performed to genotype GSTP1 polymorphism (Ile105Val). Genomic DNA was isolated from venous blood collected from 117 pathologically diagnosed squamous cell cervical cancer patients and 150 healthy females.

Results: The homozygous variant (G/G) genotypes and heterozygous plus variant (A/G+G/G) genotypes showed 3.17 fold (OR= 3.1746, 95% CI= 1.037 to 9.7187, p=0.043) and 1.71 fold (OR= 1.7143, 95% CI= 1.0425 to 2.8189, p=0.0337) increased risk of cervical cancer when compared to homozygous wild type (A/A) genotypes, respectively. The allele frequency distribution of A and G in cases and controls also associated with increased cervical cancer risk (OR= 1.677, 95% CI= 1.1129 to 2.5271) and the result is statistically significant ($p<0.05$). However, there was no significant combined effects of demographic variables ($p>0.05$) and *GSTP1* genotypes in cancer development.

Conclusion: *GSTP1*genetic polymorphism (Ile105Val) could influence susceptibility to cervical cancer among Bangladeshi women.

#1001 Leveraging Electronic Health Records to examine the real-world rates of cancer genetics referrals in a Singapore healthcare cluster.

Jonathan Jian Hao Soon[1], Jianglei Wu[1], Nur Diana Binte Ishak[1], See Wei Qiang[2], Hing Pong Matthew Che[2], Michael Dorosan[2], Jeanette Yuen[1], Andrea Wan Ling Tan[1], Marcus Eng Hock Ong[2], Sean Shao Wei Lam[2], Hwee Lin Wee[3], Jianbang Chiang[1], Joanne Ngeow[1]

[1]Cancer Genetics Service, National Cancer Centre Singapore, Singapore, Singapore,[2]Health Services and Systems Research, Duke-NUS Medical School, Singapore, Singapore,[3]Saw Swee Hock School of Public Health, National University of Singapore, Singapore, Singapore

Identifying patients with hereditary cancer syndromes through genetics referral enhances early detection and reduces healthcare costs. Despite potential benefits, genetics referral rates globally, including Singapore, remain low. This study investigates the real-world rates of genetics referrals in eligible cancer patients at Singapore's largest healthcare cluster using Electronic Health Records. Referral criteria for genetics referrals were based on international guidelines. The institution's data repository was queried for eligible patients with relevant diagnosis codes from 2017 to 2021. We assessed genetics clinic attendance among eligible patients to evaluate referral rates. Variations in referral rates over time were analyzed using linear regression and two-tailed t-test. Of the 10080 patients eligible for a genetics referral, 17.1% (1719) were referred to a cancer genetics clinic. Breast, ovarian, colorectal, and endometrial cancers accounted for 42.9%, 33.5%, 11.3%, and 8.6% of referrals, respectively. Other tumor types accounted for 3.7% of referrals. Referral rates for suspected Hereditary Breast and Ovarian Cancer syndrome (HBOC)-related cancers were higher (19.7%) than referrals for suspected Lynch syndrome (12.2%). Among HBOC referrals, women (20.7%) were more likely to be referred than males (7.8%). From 2017 to 2021, the referral rates among eligible patients steadily increased from 11.6% (483/4174) in 2017 to 23.6% (403/1705) in 2021, an increase of 12%, p=0.005. From 2017 to 2021, we found an increase in referral rates for HBOC (12.8% to 28.6 %, p= 0.005) but not for Lynch syndrome-related indications (8% to 12.6%, p=NS). The increase in referral rates for suspected HBOC in women was more significant than in men (p=0.03) This study found lower referral rates for Lynch syndrome than HBOC, and identified a gender discrepancy, with men with HBOC being less likely to be referred. Efforts to increase referral rates should include raising clinician awareness and electronically identifying suspected cases, especially for male breast cancer and Lynch Syndrome.

#1002 BRCA2, ATM, and POT1 genes mutations in Chilean hereditary gastric cancer families.

Graciela Adriana Molina Fuentes[1], Ana Patricia Estrada-Florez[2], Paul Lott[2], Osvaldo Torres[3], Cedric Adelsdorfer[4], Alejandro H Corvalan[5], Luis Carvajal-Carmona[2]

[1]Escuela de Medicina. Facultad de Ciencias de la Salud, Universidad Autonoma de Chile, Santiago, Chile,[2]Genome Center and Department of Biochemistry and Molecular Medicine, School of Medicine, University of California Davis, Davis, CA,[3]Servicio de Cirugia. Equipo Cirugia Digestiva Alta, Hospital Regional Dr. Guillermo Grant Benavente de Concepcion, Concepcion, Chile,[4]Servicio de Cirugia, Hospital Dr. Gustavo Fricke de Vina del Mar, Vina del Mar, Chile,[5]Departamento de Hematologia y Oncologia., Pontificia Universidad Catolica de Chile and Advanced Center for Chronic Diseases, Santiago, Chile

The purpose of this study is to describe Chilean families having hereditary Gastric Cancer (GC) presenting pathogenic mutations in BRCA 2 (c.4740_4741dup NP_000050.3:p.Glu1581fs), ATM (c.3381_3384del NP_000042.3:p.Gln1128fs) and Protector of Telomers 1 (POT1, 1087C>T:p.Arg363Ter) genes. Only one hereditary diffuse GC (HDGC) Chilean family with a pathogenic *CDH1* mutation has been reported, suggesting a low frequency of pathogenic mutation detection in this population. Whole exome sequencing (WES) was performed using the Agilent SureSelect Human All Exome V7 kit and sequenced in a NovaSeq6000 (Illumina, Inc.) S4 150PE aiming 100x. Alignment and variant calling were performed using Illumina Dragen v3.8. Variant calls were re-calibrated using GATK v4 variant recalibration and annotated with Illumina Nirvana and Annovar. Genotypification of the index patient and their family was performed using KASP-PCR. The index patient having a BRCA2 mutation had metastatic HDGC and his mother had asynchronous breast cancer and GC. The family presenting an ATM gene mutation had three GC-affected subjects, the index patient, and both parents. The index patient of the family having a POT1 mutation presented a CG at 47 yr., and two of her children died of CG at 24 yr. All families show an incomplete penetrance and variable expressivity. We are searching for the second hit in the index patient´s tumor samples. This is the first report of a POT1 gene mutation detected in a hereditary GC. POT1 belongs to the shelterin complex that protects telomeres. This mutation affects the binding to the shelterin complex, and it was previously reported in colorectal cancer. Beca Chile Postdoctorado 74190063, CONICYT-FONDAP 15130011 /ANID-FONDAP APOYO 1523A0008, NIH R01CA223978, R21CA199631, U54CA233306, and P30CA093373.

#1003 Cascade genetic testing for hereditary cancer syndromes in Algerian population: pathways to precision medicine.

Farid Cherbal[1], Chiraz Mehemmai[1], Mouchira Saadi[1], Djamel-Eddine Seddik[1], Hassen Mahfouf[2], Wassila Benbrahim[3], Mohammed Oukkal[4]

[1]Molecular Genetics Team, LBCM-Tamayouz, Faculty of Biological Sci, USTHB, Algiers, Algeria,[2]Mohamed El Kolli Public Hospital, Academic Medical Oncology Services, Rouiba, School. of Medecine, University of Algiers-1, Algiers, Algeria,[3]Medical Oncology Services, Center Anti Cancer Batna, University Batna2 Chahid Mostefa Ben Boulaid, Batna, Algeria,[4]Clinic of Medical Oncology Amine Zirout, University Hospital of Beni-Messous, School of Medicine, University. of Algiers-1, Algiers, Algeria

Background: Cascade genetic testing helps to identify at risk relatives within hereditary cancer syndromes families with a known germline pathogenic variant in cancer predisposition genes (CPG). The detection of high-risk germline CPG variants in index cases and relatives makes available cancer prevention and precision medicine, respectively. To date, in Algeria, there is a limited access in clinical genetic services, mainly due to the costs and the availability of genetic testing. Here, we report cascade genetic testing in at risk relatives within hereditary breast and ovarian cancer syndrome (HBOC), Lynch syndrome (LS) and familial adenomatous polyposis syndrome (FAP) families, respectively.

Material and Methods: Our study included 7 HBOC families, 4 LS families and 3 FAP families. We screened 34 high risk relatives using PCR-Sanger sequencing for the following CPG germline pathogenic variants: c.181T>G, c.798_799delTT, c.2125-2126insA, c.5332+1G>A in *BRCA1* gene, c.1310_1313delAAGA, c.8940delA in *BRCA2*gene, c.1546C>T in *MLH1* gene, c.1605dupA, c.2544dupA and c.3584delT in *APC* gene, respectively.

Results: We detected *BRCA1* c.181T>G in the brother of the index case. *BRCA1* c.798_799delTT has been detected in the sister of the index case. *BRCA1* c..2125_2126insA has been detected in two unrelated families and it has been identified in 4 relatives in the first family and 4 relatives in the second family, respectively. In the first family, the sister, the father, the paternal aunt and her son have been tested positive for the variant. The father and the cousin of the index case were disease free. In the second family, the father, the paternal aunt and two brothers of the index case have been tested positive for the variant and were disease free. *BRCA1* c.5332+1G>A has been detected in 2 relatives. *BRCA2* c.1310_1313delAAGA has been detected in the sister and the father of the proband, while *BRCA2* c.8940delA has been identified in the mother and the sister of the proband. Interestingly, the mother was disease free at age 78 years in this family. For LS, *MLH1* c.1546C>T detected in 3 related families has been identified in 9 relatives in the first family, 2 relatives in the second family and 3 relatives in the third family. In the third family, *MLH1* c.1546C>T has been detected in both parents (mother and father) and in one brother. Interestingly, in this family, a young female sister (not tested) developed glioblastoma and died at age 4 years. As obligate carrier, we can conclude that the young sister developed a CMMRD syndrome. In regard to FAP syndrome, *APC* c.1605dupA has been detected in 3 sisters and one brother of the proband, *APC* c.2544dupA has been detected in one relative and *APC* c.3784delT has been identified in two relatives, respectively.

Conclusion: Cascade genetic testing in Algerian population will help to implement strategies to reduce the risk of cancer in families, promote public health and precision medicine.

#1004 Genome-wide analysis of the interaction between physical activity and genetic susceptibility on colorectal cancer incidence: A nested case-control study in the UK Biobank.

Sooyoung Cho[1], Aesun Shin[2]

[1]Genomic Medicine Institute, Medical Research Center, Seoul, Korea, Republic of,[2]Department of Preventive Medicine, Seoul National University College of Medicine, Seoul, Korea, Republic of

Background: Colorectal cancer (CRC) risk is influenced by a complex interplay between genetic predisposition and lifestyle factors, such as physical activity (PA). To investigate potential gene-environment interactions, we conducted a genome-wide interaction study (GWIS) using data from the UK Biobank (proposal #94695).

Methods: Participants with prior cancer diagnoses, missing genotyping data, missing PA data, or missing covariates (e.g., deprivation index, BMI, smoking, alcohol) were excluded. Genotyping data underwent stringent quality control with thresholds set for minor allele frequency (MAF > 0.03), genotyping call rate (> 99%), and Hardy-Weinberg equilibrium ($p > 1 \times 10^{-6}$). After exclusions, 325,858 participants with the required covariates were included as the base population. From this cohort, 3,607 CRC cases were matched to 14,155 controls using a 1:4 incidence density matching approach based on sex, age at recruitment (±5 years), and follow-up duration (±6 months). PA was categorized based on whether participants met the World Health Organization guidelines: engaging in at least 150 minutes of moderate-intensity activity or 75 minutes of vigorous-intensity activity per week. Conditional logistic regression models were used to evaluate SNP × PA interactions, adjusting for BMI, smoking, alcohol consumption, deprivation index, the first ten genetic principal components, and batch effects. Interaction p-values ($P_{interaction}$) were derived from the statistical significance of the SNP × PA interaction term in the model.

Results: After quality control, 402,069 SNPs were retained for analysis. We could not find any SNPs that met the genome-wide significance threshold of $p < 1 \times 10^{-7}$. However, seven SNPs met the suggestive significance threshold of $p < 1 \times 10^{-5}$, including rs6791309 ($P_{interaction} = 1.20 \times 10^{-6}$), which had the lowest interaction p-value, and rs6770805 (3.65×10^{-6}), which is located within the *ADCY5* gene. Other suggestive variants were identified in genes such as *KCNIP4*, *CDH18* and *LOC124904472*.

Conclusions: This GWIS identified SNPs with suggestive evidence of interaction with PA in relation to CRC risk, although no variants achieved genome-wide significance. Variants in genes such as *ADCY5, KCNIP4*, *CDH18* and *LOC124904472* highlight loci that may contribute to CRC risk through gene-environment interactions. Further replication studies and functional analyses are necessary to validate these findings and uncover the underlying biological mechanisms.

#1005 Germline-somatic interactions in non-small cell lung cancer (NSCLC) patients from Latin America.

Javiera Garrido[1], Evelin Gonzalez[2], Alejandro Blanco[2], Gonzalo Sepulveda-Hermosilla[2], Matias Freire[3], Solange Rivas[2], Katherine Marcelain[4], Gareth I. Owen[5], Carolina Ibañez[5], Alejandro Corvalan[5], Marcelo Garrido[1], Rodrigo Assar[3], Rodrigo Lizana[3], Javier Caceres-Molina[3], Diego Ampuero[3], Liliana Ramos[3], Paola Perez[6], Osvaldo Aren[7], Cristina Fernandez[8], Maria Loreto Spencer[9], Jacqueline Flores[10], Giuliano Bernal[11], Monica Ahumada Olea[12], German Rasse[13], Carolina Sanchez[14], Maria Galli de Amorim[15], Emmanuel Dias-Neto[15], Helano C. Freitas[15], NIRVANA team, Ricardo Armisen[2]

[1]Centro de Oncologia de Precision, Universidad Mayor, Santiago, Chile,[2]Centro Genetica y Genomica, Instituto de Ciencias e Innovacion en Medicina, Facultad de Medicina, Universidad del Desarrollo, Santiago, Chile,[3]CORFO Center of Excellence in Precision Medicine, Pfizer, Santiago, Chile,[4]Departamento de Oncologia Basico Clinica, Facultad de Medicina, Universidad de Chile, Santiago, Chile,[5]Advanced Center for Chronic Diseases and Department of Hematology and Oncology, Pontificia Universidad Catolica de Chile, Santiago, Chile,[6]National Institute of Health, NIDCR, Bethesda, MD,[7]Centro de Oncologia de Precision, Centro de Investigacion Clinica Bradford Hill, Santiago, Chile,[8]Instituto Nacional del Torax, Santiago, Chile,[9]Department of Pathology, Hospital Clinico Regional de Concepcion Dr. Guillermo Grant Benavente, Santiago, Chile,[10]Departamento de Salud Publica, Facultad de Medicina, Universidad Catolica del Norte, Antofagasta, Chile,[11]Departamento de Ciencias Biomedicas, Facultad de Medicina, Universidad Catolica del Norte, Antofagasta, Chile,[12]Servicio de Oncologia, Departamento de Medicina Interna, Hospital Clinico de la Universidad de Chile, Santiago, Chile,[13]Hospital de Puerto Montt, Puerto Montt, Chile,[14]Centro de Genomica y Bioinformatica, Universidad Mayor, Santiago, Chile,[15]Laboratory of Medical Genomics, A. C. Camargo Cancer Center, Sao Paulo, Brazil

Motivation: Tumors of non-small cell lung cancer (NSCLC) patients are heterogeneous entities. Genomic landscapes are influenced by genetic constitution, environmental exposures and the complex interaction between the two. In particular, it has been reported that germline variability specific to Latin American (LA) populations play an important role modulating the presence of acquired somatic alterations in *EGFR* and *KRAS,* two of the most clinically relevant actionable genes for lung cancer (LC). The influence of these specific genetic differences appears to be independent of smoking status, a key exogenous agent also known to influence LC genomic heterogeneity. However, LA patients are strikingly diverse and further studies are needed to better understand the influence of germline-somatic interaction on tumor variability across populations with different patterns of environmental exposures.

Methodology: The population derived from the study protocol *Characterization and Validation of Molecular Diagnostic Technologies for LC Patients from Chile, Brazil, and Peru* (clinicaltrials.gov NCT03220230). The recruitment period was July 2015 to October 2018 and encompassed 37 centers. From NSCLC samples, genomics profiles were assessed using the Oncomine Focus Assay (OFA), with QC-approved assays obtained for 1864 subjects. Relevant covariates were interrogated at enrollment. In the absence of matched normal tissue samples, an in-house algorithm was developed to distinguish germline from somatic variation. Descriptive statistics, Principal Component Analyses (PCA) and logistic regression were used to evaluate germline-somatic interactions.

Results and Conclusions: The in-house algorithm detected 738 unique germline variants and 6636 unique somatic alterations. The majority of the germline variants (80.8%, n=597) are reported in other populations with a Minor Allele Frequency (MAF) ≥ 1%; only a minority (19.1%, n=141) report a MAF either <1% or unknown. These rare germline variants are present in 98 study participants (5.3%) and are more common in the group of Chilean smoker patients with adenocarcinoma and advanced disease, and without a personal and family history of cancer (FHC). Statistical significance was found for cancer stage (stage IV: 80.8% [n=63] vs. 70.8% [n=823], p-value<0.05) and FHC (positive: 20.5% [n=16] vs. 31.9% [n=371], p-value<0.05). Regarding the relationship between germline and somatic alterations, we found a positive association for *EGFR*, *PIK3CA*, *ALK*, *FGFR4* and *ERBB2* and a negative association for the remaining genes (n=46). These findings provide novel insights into the influence of inherited genetic variability on the clinical presentation and somatic landscape of an underrepresented population. Identification of the underlying germline alleles can improve our understanding of the affected biological pathways for the subset of LCs driven by these alterations.

#1006 Local genetic ancestry among breast cancer rare mutation carriers from Lima, Peru.

Luqi Wang[1], Xiaosong Huang[1], Paul Lott[1], Elad Ziv[2], Susan L. Neuhausen[3], Tatiana Vidaurre[4], Laura Fejerman[1]

[1]University of California Davis, Davis, CA,[2]University of California San Francisco, San Francisco, CA,[3]City of Hope Comprehensive Cancer Center, Duarte, CA,[4]Instituto Nacional de Enfermedades Neoplasicas, Lima, Peru

Genetic predisposition to breast cancer is understudied in populations of high Indigenous American (IA) genetic ancestry. This gap in knowledge can lead to lack of equity in risk assessment and prediction. This study aimed to identify pathogenic/likely pathogenic (P/LP) variants and variants of unknown significance (VUS) in known cancer susceptibility genes in women with breast cancer from Lima, Peru, and test their ancestry-specificity. Whole exome sequencing was performed on 40 susceptibility genes in 1,364 women from the Peruvian Genomics of Breast Cancer (PEGEN-BC) study. Variants were called by GATK HaplotypeCaller and annotated by ANNOVAR with the ClinVar database (v20240611). Global ancestry proportions (Indigenous American, European, African, and East Asian) were estimated using the program Admixture (v1.3.0, k = 4), and local ancestry inference was performed with RFMix v2 to determine the ancestral origins of specific chromosomal segments at each locus of identified variants. Among the 1,364 individuals analyzed, 135 (9.90%) carried P/LP variants, including 128 with one variant and 7 with two. The most frequently affected genes were *BRCA1* (29/142, 20.42%), *BRCA2* (24/142, 16.90%), *ATM* (20/142, 14.08%), *PALB2* (18/142, 12.68%), and *CHEK2* (14/142, 9.86%). VUSs were identified in 679 individuals (49.78%), with 503 carrying one VUS and 176 carrying multiple VUSs (2 to 5). The most commonly affected genes with VUSs were *BARD1* (189/888, 28.28%), *NF1* (96/888, 10.81%), *ATM* (67/888, 7.55%), and *BRIP1* (43/888, 4.84%). Seven P/LP variants showed significant differences between the observed and expected ancestry of haplotypes when comparing IA to all other ancestries, with four of these variants located in *BRCA1*. Additionally, 36 VUSs exhibited significant differences in ancestry distribution, with *CDH1* harboring the most variants (5), followed by *CHEK2* and *MSH6* (3 each). For all P/LP variants with a significant difference between the observed and expected haplotypes of IA and other ancestries, the observed frequency of IA haplotypes was lower than expected, whereas the observed frequency of haplotypes from other ancestries was higher than expected. A similar trend was observed for VUSs, except for three mutations where the observed IA haplotype frequency was higher than expected and the frequency of other haplotypes was lower than expected. Our findings suggest that population admixture influences the distribution of P/LP variants and VUSs in Latin American populations. The occurrence of breast cancer in these populations may be partly influenced by the infusion of non-IA haplotypes harboring risk associated variants to the local genomes.

#1007 Screening pathogenic germline variants in prostate cancer predisposition genes in an ethnically diverse cohort of unselected prostate cancer patients.

Vinicius Marques Rocha[1], Vitoria Pelegrino do Val[2], Maria Lucia Hirata Katayama[3], Tais Vaz[4], Simone Maistro[4], Isabela Arcanjo Nunez Arcanjo Nunez[2], Luciane Sussuchi da Silva[2], Fernando Marcon Passos[2], Jaqueline Wang[2], Rosimeire Aparecida Roela[4], Fatima Solange Pasini[4], Glaucia Fernanda de Lima Pereira[4], Rossana Veronica Mendonza Lopez[4], Jose Pontes Junior[5], Fabio Pescarmona Gallucci[5], Juliana Macedo dos Santos[4], Leticia Montanha de Assis[4], Elisa Mei Utsunomiya[1], Beatriz Fernandes de Souza[1], Rafael Lara Nohmi[1], Gabriel Ficagna Zaccaron[6], Luiz Antonio Senna Leite[7], Maria del Pilar Estevez-Diz[8], Cristovam Scapulatempo Neto[2], Roger Chammas[4], William Carlos Nahas[5], Guilherme Lopes Yamamoto[2], Maria Aparecida Azevedo Koike Folgueira[4]

[1]Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil,[2]Dasa Genomica, Sao Paulo, Brazil,[3]Depto Radiologia e Oncologia, Centro de Investigacao Translacional em Oncologia, Instituto do Cancer do Estado de Sao Paulo, Hospital das Clinicas, Faculdade de Medicina, Universidade de Sao Paulo, Comprehensive Center for Precision Oncology (C2PO), Sao Paulo, Brazil,[4]Depto Radiologia e Oncologia, Centro de Investigacao Translacional em Oncologia, Instituto do Cancer do Estado de Sao Paulo, Hospital das Clinicas, Faculdade de Medicina, Universidade de Sao Paulo. Comprehensive Center for Precision Oncology (C2PO), Sao Paulo, Brazil,[5]Departamento de Urologia, Instituto do Cancer do Estado de Sao Paulo, Hospital das Clinicas, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil,[6]Faculdade de Medicina, Universidade Sao Paulo, Sao Paulo, Brazil,[7]Instituto do Cancer do Estado de Sao Paulo, Hospital das Clinicas, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil,[8]Departamento de Urologia, Instituto do Cancer do Estado de Sao Paulo, Hospital das Clinicas, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil

Introduction: There is limited data on the heritability of PrCa in Brazil, a country with a miscegenated ethnic composition. We enrolled participants in a case-control study aimed at calibrating a polygenic risk score. We now present preliminary results of 557 PrCa patients whose samples were analyzed using whole genome sequencing (WGS). We estimate genetic ancestry and assess the prevalence of pathogenic genetic variants (PGVs) in PrCa-related genes.

Methods: We included unselected PrCa patients. Previously we performed in silico random downsampling of WGS data initially at 30x depth to 15x, using samples from 3 Brazilian people, to estimate percentages of variant similarities and rare variants missed due to lower coverage. Only high-quality, rare variants (≤ 1% in population databases), likely functional (exonic or splicing regions) were considered, excluding chromosomes X, Y, and mitochondrial DNA. Sequencing at 15x depth identified 94.2% to 97.1% of heterozygous variants detected at 30x, with a mean expected variant loss of 4.3%. Subsequently, we performed WGS at 15x depth on 557 samples of PrCa patients using Illumina technology. We screened data for germline mutations in a panel of 16 genes (including *HOXB13*) associated with high/moderate risk for PrCa. Ancestry admixture proportions were also estimated.

Results: A total of 557 PrCa patients were included. The median age at diagnosis was 64.6 years (41—90y). Self-reported skin color consisted of White (44.8%), Brown (37.4%), and Black (13.2%). Most patients were diagnosed with clinical stages II (32.8%) and III (43.1%), and ISUP grades 2 (40.2%) and 3 (22.9%). There was significant ancestry admixture, with predominant EUR genetic composition (61.4%), followed by AFR (24.6%), INDIG (7.7%), Asian (1.6%), and Middle Eastern (4.7%). PGVs in high/moderate risk genes for PrCa were identified in 29 out of 557 patients (5.2%), including *BRCA2* (n=8), *CHEK2* (n=5), *ATM*, *BRCA1*, *BRIP1*, *MLH1*, *PALB2*, *PMS2* (n=2 each), and *MSH2*, *MSH6*, *NBN*, *RAD51C*, and *TP53* (n=1 each). One patient carried PGVs in both *PALB2* and *TP53*. Variants included 9 frameshift, 4 splice site, and 17 missense mutations. All these variants will be confirmed in subsequent evaluations.

Conclusion: In a predominantly mixed-population setting, WGS at 15x depth appears appropriate for screening high-risk variants. Although there is a small chance (~5%) of missing rare PGVs or detecting false positives, from a population perspective, downsampling seems to be acceptable, comparing the benefits of sequencing at 15x twice the number of samples than 30x. Preliminary data suggest that the prevalence of PGVs in high/moderate-risk genes in PrCa patients is similar to other populations worldwide. The PGV spectrum identified is consistent with those described in Brazilian cancer patients. Supported by CNPQ

#1008 Genetic predisposition to breast cancer in Senegal: mutations of the *BRCA*1 and *BRCA2* genes.

Jean Pascal Demba Diop

Universite Cheikh Anta Diop de Dakar, Dakar, Senegal

Background: *BRCA1* and *BRCA2* are the most incriminated genes in inherited breast and ovarian cancers. Several pathogenic variants of these genes conferring genetic predisposition have been described in different populations but rarely in subsaharan Africa. The objectives of this study were to identify pathogenic variants of the *BRCA* genes involved in hereditary breast cancer in Senegal and to search for a founder effect.

Methods: We recruited after free informed consent, 46 unrelated index cases diagnosed with breast cancer and each having a family history, a control population of 90 healthy women and 80 women diagnosed with sporadic breast cancer and 36 patients diagnosed without a known family history of breast cancer at under 50 years All *BRCA1* and *BRCA2* exons were Sanger sequenced for the first 15 index cases recruited. For the following 31 index cases and healthy relatives and controls, the recurrent pathogenic variant of *BRCA1* identified in the first group was genotyped by PCR. To search for a founder effect of the recurrent pathogenic variant of *BRCA1*, haplotype analysis of 7 microsatellites encompassing the gene was performed in 10 selected index cases with the pathogenic variant and 15 unrelated healthy controls. Mutation age was estimated using the following equation: $G = \log\delta / \log(1 - \theta)$.

Results: Mutation screening identified a recurrent duplication of 10 nucleotides c.815_824dupAGCCATGTGG, (p.Thr276Alafs) of *BRCA1*, in 26 index. Allelic frequency was estimated at 28, 26% compared to sporadic breast cancer cases (5%) healthy controls (0.55%) The variant was also found in 8 out of 36 patients diagnosed without a known family history of breast cancer at under 50 years of age (allelic frequency estimated at 11.11%) Haplotype analysis showed a shared haplotype encompassing approximately 400 kb. Estimation of the age of the pathogenic variant suggested that it occurred approximatively 1400 years ago and confirm a West African origin. The variant was also detected in healthy relatives of selected families. The genotyping method is now available as a first screening test in women at risk. For *BRCA2* gene, a novel pathogenic variant, c.5219 T >133 G; p.(Leu1740Ter) was identified in a proband from a consanguineous family along with three sisters and one daughter of the index case.

Conclusion: Our study identified a founder pathogenic variant of *BRCA1* predisposing to breast cancer and enabled the establishment of an affordable genetic test as a mean of prevention for Senegalese women at risk. We will screen for the variant *BRCA1*: c.815_824dup in Senegalese patient diagnosed with breast, ovarian, prostate and pancreatic cancers.

Keywords: Hereditary Breast Cancer, *BRCA1, BRCA2*, pathogenic variant, Senegal.

#1009 DNA methylation-predicted plasma proteins and risk of colorectal cancer recurrence: Findings from the ColoCare Study.

Alicia Richards[1], Esther Jean-Baptiste[1], Timothy Barrow[2], Sheetal Hardikar[3], Biljana Gigic[4], Jane Figueiredo[5], Christopher Li[6], David Shibata[7], Adetunji T. Toriola[8], Doratha A. Byrd[1], Cornelia M. Ulrich[3], Paul Stewart[1], Erin M. Siegel[1], **Jacob K. Kresovich**[1]

[1]H. Lee Moffitt Cancer Center, Tampa, FL,[2]University of Essex, Essex, United Kingdom,[3]Huntsman Cancer Institute, Salt Lake City, UT,[4]Heidelberg University Hospital, Heidelberg, Germany,[5]Cedars-Sinai Medical Center, Los Angeles, CA,[6]Fred Hutchinson Cancer Center, Seattle, WA,[7]University of Tennessee Health Science Center, Memphis, TN,[8]Washington University School of Medicine, St. Louis, MO

Introduction: DNA methylation (DNAm)-based predictors of individual proteins (e.g., C-reactive Protein, Interleukin-6) show better predictive performance for various health outcomes compared to direct measurements. This improved performance may be due to the higher reliability and temporal stability of DNAm profiles than direct protein measurements. Building on these findings, a library of 109 DNAm-predicted plasma proteins, called “Protein EpiScores,” was developed using paired plasma proteomic and blood DNAm data from thousands of individuals. Initial studies of Protein EpiScores identified associations with CRC risk, suggesting their potential as novel biomarkers for CRC prediction and as indicators of mechanisms underlying CRC development. Here, we extend the investigation of Protein EpiScores to cancer survivorship by investigating their associations with risk of recurrence and all-cause mortality in newly diagnosed CRC patients.

Methods: The ColoCare Study is an international, multi-center, prospective cohort of newly diagnosed CRC patients. For this study, we selected a nested case-control sample of 137 patients enrolled at Moffitt Cancer Center, matching recurrence cases 1:3 to recurrence-free controls by age, sex, body mass index, stage, and follow-up time. Pre-treatment whole blood DNA methylation profiles were assayed and used to calculate 109 Protein EpiScores. Logistic regression models, adjusted for matching factors and tumor location, estimated odds ratios (ORs) for associations between each Protein EpiScore (per 1-standard deviation [SD] increase) and risks of CRC recurrence and all-cause mortality.

Results: Patients had a mean age of 62.7 years at diagnosis (SD= 13), with a majority being male (57%), overweight (78%), and White (90%). During follow-up, 35 (25%) patients experienced disease recurrence, and 47 (34%) died. Sixteen Protein EpiScores were associated with CRC recurrence risk (HRs ranging from 0.55 to 1.88; all $P< 0.05$). These included six Protein EpiScores designed to predict proteins related to immune response and inflammation (CCL11, PIGR, CCL17, MMP12, OSM, PAPPA), with all showing positive associations except for PAPPA. Ten Protein EpiScores were positively associated with all-cause mortality (all $P< 0.05$), including five immune response and inflammation Protein EpiScores (OSM, S100A12, CCL22, PIGR, S100A9).

Conclusions: Multiple Protein EpiScores measured in pre-treatment blood samples were associated with CRC recurrence risk and mortality. Many of these Protein EpiScores were designed to predict proteins involved in immune response and inflammation, suggesting that changes in peripheral immunity contribute to disease progression.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#101 KPNA2 promotes the progression of lung adenocarcinoma.

Seongran Cho[1], Inyoung Cheon[1], Seoree Kim[2], Yoon Ho Ko[3], Young-Ho Ahn[1]

[1]Ewha Womans University, Seoul, Korea, Republic of,[2]The Catholic University of Korea, Bucheon, Korea, Republic of,[3]The Catholic University of Korea, Seoul, Korea, Republic of

KPNA2 (Importin α1) is a nuclear transporter that mediates the nuclear import of proteins, such as transcription factors and nuclear proteins. Previous studies have highlighted KPNA2 as an important factor influencing CAF activity, prompting us to explore its role and mechanisms in the migration, invasion, and metastasis of lung cancer cells. Immunohistochemistry on tumor samples from lung cancer patients revealed that higher expression of KPNA2 was linked to poorer survival outcomes. To further investigate its function, we used shRNAs to knock down KPNA2 in human and mouse lung cancer cells. This led to a reduction in cancer cell migration, invasion, and metastasis. In contrast, overexpressing KPNA2 enhanced cancer cell migration and invasion. RNA sequencing analysis showed that KPNA2 regulates focal adhesion and NF-κB signaling pathways. Additionally, protein microarray analysis identified Golgi-associated and RNA-associated proteins as potential KPNA2-binding partners, which require further study. These results indicate that KPNA2 plays a crucial role in promoting cancer cell migration, invasion, and metastasis, making it a promising therapeutic target in lung adenocarcinoma.

POPULATION SCIENCES: Genomic Diversity and Disparities in Cancer: Global Insights into Epidemiology, Risk, Outcomes, and Precision Medicine
Poster Session

#1011 Transposable element patterns across genetic ancestries in prostate cancer patients.

Nicholas Korvink, Lucas Ueta, Hannah Park, Vinay Kumar, Farah Rahmatpanah

Pathology and Laboratory Medicine, University of California, Irvine, Irvine, CA

Introduction: Prostate cancer (PCa) is a significant burden to American men with African ancestry (AA). The mechanism of how ancestry contributes to aggressive PCa in AA patients is not well understood. We have identified a difference between races in endogenous retroviruses (ERVs) expression. In prostate and cervical cancer, we have demonstrated that ERV expression signatures are useful for identifying patients at the greatest risk of disease progression. There is consistency in mortality rates for black men in the US, Caribbean, and Sub-Saharan Africa suggesting that shared ancestry is a common risk factor. Using ERVcaller we investigated polymorphic transposon elements (TE) and ERV insertions unique to tumor cells compared to normal blood in prostate cancer patients of African (AF) and European ancestry (EU).
Method: ERVcaller software detects retrotransposons including Alu, LINE1, SVA, and HERV-K types using whole genome sequencing (WGS). We gathered publicly available WGS data (FASTQ files) from the European Nucleotide Archive (SRA accession SRP119289). These were derived from paired blood and tumor samples from 6 AF and 9 EU PCa patients. We processed this data with ERVcaller, revealing TE insertions present in each patient sample (i.e., blood or tumor). We identified tumor-exclusive and blood-exclusive insertions by excluding overlapping TE insertions between tumor and blood samples. These insertions were then analyzed for differences using a T-test in R Studio.
Results: Statistical analysis revealed significant differences in the number of TE insertions in the tumors of AF patients (mean = 50.00) compared to their EU (mean = 31.89) counterparts (p-value < 0.0038). In addition, we found no significant differences in TE insertions in blood samples of AF patients (mean = 35.50) relative to EU patients (mean = 32.78; p-value > 0.56), suggesting that this differential insertion pattern is isolated to the affected tumor region.
Conclusion: ERVs have previously demonstrated important roles in the development of various diseases. Our findings demonstrate significant variations in TE insertions in tumors of PCa patients across genetic ancestries.

#1012 Investigating genetic and transcriptomic drivers of endometrial cancer disparities in Black/African American women.

Danyelle Paine[1], Yuxin Jin[1], Javier Arias-Stella III[2], David Craig[1], John Carpten[1]

[1]Integrative Translational Science, Beckman Research Institute of The City of Hope, Duarte, CA,[2]Department of Pathology, The City of Hope, Duarte, CA

Endometrial cancer (EC) is the sixth most common gynecological malignancy globally, with an increasing incidence over the last two decades. Black/African American (BAA) women are disproportionately affected, experiencing higher rates of high-grade EC and associated mortality compared to Non-Hispanic White (NHW) women. These disparities likely result from complex interactions between social determinants and biological factors. Notably, BAA women are 2-3 times more likely to be diagnosed with aggressive, high-grade EC and have significantly higher mortality rates, underscoring an urgent need to address this disparity. Uterine EC disparities arise from intricate tumor microenvironments characterized by cellular niches with distinct genotypes, phenotypes, and spatial transcriptomic landscapes. In estrogen-dependent EC tumorigenesis, the endometrium undergoes unregulated proliferation, progressing from normal tissue to atypical hyperplasia and ultimately carcinoma. The heterogeneity of EC tumors presents challenges for therapeutic targeting, contributing to poor outcomes. This study investigates the genetic and transcriptomic features within the tumor microenvironment of BAA EC patients to uncover drivers of these disparities. Our cohort includes primary, metastatic, and normal uterine tissues classified as uterine endometrial adenocarcinoma, along with two serous papillary carcinoma cases of varying tumor grades. RNA-sequencing analysis revealed differential gene expression enriched in pathways related to cell proliferation, epithelial-to-mesenchymal transition (EMT), DNA damage response, and immune modulation. Preliminary whole-exome sequencing (WES) data implicate both established and potentially novel mutations in genes associated with cell cycle regulation, angiogenesis, chromatin remodeling, and cell signaling. Paired WES analysis identified somatic variants and copy number alterations in key genes, correlating with known EC-driven genetic alterations documented in the literature. Spatial transcriptomics is being applied to characterize gene signatures across cellular neighborhoods within the tumor microenvironment. This approach elucidates the spatial architecture and cellular niches driving immune modulation and tumor progression. By integrating genomic and spatial transcriptomic data, we aim to provide a comprehensive understanding of the tumor microenvironment in BAA EC patients. This study represents a significant step toward identifying genetic and transcriptomic drivers of EC disparities in BAA women, with the goal of informing the development of improved diagnostic and therapeutic strategies to reduce these inequities.

#1014 Detection of reportable germline *BAP1* mutations in patients with uveal melanoma.

Amy C. Schefler[1], Julia Litvinov[2], Jason Maarsing[2], Kasia Wozny[2], Olga Zolochevska[2]

[1]Retina Consultants of Texas, Houston, TX,[2]Castle Biosciences, Inc., Friendswood, TX

Purpose: The reported rate of pathogenic germline mutations in the *BAP1* gene range from 2% in unselected population to up to 50% in patients with familial uveal melanoma (UM) and are associated with an increased risk of developing *BAP1*-Tumor Predisposition Syndrome (TPDS)-related cancers. The primary objective of this study was to determine more accurately the rate of germline *BAP1* mutations in a clinically representative sample population of UM patients with a known somatic mutation in *BAP1,* using clinically available 7-gene UM next-generation sequencing (NGS) panel DecisionDx-UM*Seq*.

Methods: Blood and buccal swab samples were prospectively collected from 45 adult participants (median age at diagnosis 63 y.o., range 35-92) at a single center under an IRB-approved protocol. All participants had a prior diagnosis of primary UM and had at least one reportable *BAP1* mutation previously identified in their tumors. Both samples (blood and buccal swab) were analyzed using the DecisionDx-UM*Seq* test. Results of all 3 assays (tumor, blood, and buccal swab) were analyzed for the presence of *BAP1* mutations.

Results: All reportable germline mutations detected in blood and buccal swab samples were previously identified in the primary tumor samples by DecisionDx-UM*Seq,* with 100% concordance in mutation calls between blood and swab samples and allele frequencies (AFs) within a 5% range. Out of 45 samples analyzed, 11 (24.4%) had reportable germline mutations classified as either Variants of Uncertain Significance (VUS) or pathogenic according to the ClinVar database. The remaining 34 samples had no reportable germline mutations. When comparing the AFs of *BAP1* mutations in blood and buccal swab to those in tumor samples, 4/11 (36.4%) had matching AFs, while 7/11 (63.6%) exhibited higher AFs in the tumor samples than in blood or buccal swabs. Among the detected mutations, four were classified as pathogenic and seven as VUS. Tumors in this cohort tended to be large, consistent with a high-risk molecular profile, with an average diameter of 14.5±3.53 mm and average tumor thickness of 6.14±3.48 mm.

Conclusions: The DecisionDx-UM*Seq* panel accurately identified reportable germline *BAP1* mutations in both buccal swab and blood samples from patients with previously reported somatic *BAP1* mutations. A quarter of patients in this cohort had a reportable germline mutation, including patients without a family history suggestive of typical *BAP1*-TPDS cancers, thus indicating that the rate of *BAP1* germline mutations may be higher in this cohort than reported elsewhere. This study supports the routine implementation of the DecisionDx-UM*Seq* panel for *BAP1* germline testing in all patients with a somatic *BAP1* mutation, enabling improved critical clinical decision-making and genetic counseling for patients and family members at risk of *BAP1*-related TPDS.

#1015 Real-world evaluation of androgen receptor (AR) ligand-binding domain (LBD) mutations in Chinese patients with metastatic castration-resistant prostate cancer (mCRPC).

Y. Chen[1], B. Dong[1], D. Li[1], Y. Xiao[1], J. Markensohn[2], E. Dettman[3], C. Pena[2], **X. Liu**[4];
[1]MSD R&D (China) Co., Ltd., Beijing, China, [2]Merck Sharp & Dohme LLC, Rahway, NJ, [3]Merck Sharp & Dohme LLC, North Wales, PA, [4]Merck & Co., Inc, Rahway, NJ

Purpose: While AR LBD mutations have been extensively studied in Caucasian populations, there is a lack of research in Chinese populations. This study aimed to investigate the prevalence of AR LBD activated mutations in Chinese mCRPC patients using real-world databases.
Methods: This observational study analyzed two databases of Chinese mCRPC patients. The Gloriousmed database included 2,030 patients with circulating-tumor DNA (ctDNA) collected between 2016-2023 and tested using Gloriousmed's Pushengtai (86 genes) /Qianxianan panel (693 genes), while the Geneseeq database included 91 patients with tissue and/or ctDNA collected between 2020-2022 and tested using Geneseeq Prime panel (425 genes). Activated AR LBD mutation and other prostate driver mutations were assessed in patients with novel hormonal agents (NHA) treatment information.
Results: In Gloriousmed's ctDNA database, 633 (31.2%) were NHA-naïve (not received any NHA treatment), and 1,397 (68.8%) were NHA-treated (undergone NHA treatment before sample collection). The prevalence of AR LBD mutations in NHA-treated patients (20.5%, 287/1397) was significantly higher than that in NHA-naïve patients (15.2%, 96/633) (Fisher test P=0.004). Regarding specific activated point mutations in the AR LBD, W742C (7.74%) and W742L (6.32%) were the two most common mutations in NHA-naïve populations, and significantly higher prevalence of L702H (5.44% vs 2.37%) and T878A (9.45% vs 3.48%) mutations were found in NHA-treated patients compared to NHA-naïve patients (Fisher test P<0.01).. In Geneseeq database, 21 (23.1%) subjects were NHA-naïve, while 70 (76.9%) were NHA-treated. The prevalence of AR LBD mutations in ctDNA was numerically higher in NHA-treated (21.3%, 13/61) than in NHA-naïve patients (5%, 1/20) (Fisher test P=0.17). Notably, the prevalence of AR LBD mutations in ctDNA was significantly higher than that in tissue (14/81 vs. 3/57, Fisher test P=0.04).
Conclusions: This study found consistent prevalence of AR LBD activated mutations (20.5% and 21.3%) in ctDNA from Chinese mCRPC patients after NHA treatment using two real-world datasets. Higher prevalence of AR LBD mutations was observed in NHA-treated patients compared to NHA-naïve patients and in ctDNA compared to tissue samples. Different patterns of specific AR LBD mutations were also identified in NHA-naïve and NHA-treated patients. These findings provide valuable insights into the overall prevalence of AR LBD mutations, as well as specific point mutations frequency that potentially associated with different treatment in real-world datasets of Chinese mCRPC patients.

#1016 Reduction of social disparities in clinical cancer feature prediction via somatic evolutionary embedding of mutational data.

Nic Fisk[1], Jeffrey P. Townsend[2]

[1]Cell and Molecular Biology, University of Rhode Island, Kingston, RI,[2]Biostatistics, Yale School of Public Health, New Haven, CT

Whole exome and whole genome sequencing (WES/WGS) of tumors provide rich repositories of information towards understanding the etiology of various cancer and how best to prevent and intervene in its progression. Studies utilizing these technologies, however, notably skew towards the inclusion of patients from white and well-resourced backgrounds. Considerable and ongoing effort has gone into reducing the disparate representation of WES/WGS data from diverse and global populations in the collective digital tumor corpus; however, the rapid development and deployment of machine learning models for cancer has not paused in wait of data reflective of the populace. This dissonance has resulted in imbalanced and, in an absolute sense, small WES/WGS data being used by increasingly complex machine learning models. Targeted sequencing panels, such as those hosted by AACR Project GENIE, are still skewed in a relative sense, but contain many more samples from all populations due to their routine clinical deployment and comparatively inexpensive. However, panel sequencing data is fundamentally limited in the amount of information captured. It is incumbent, then, to maximize the information that can be derived from WES/WGS data as it applies to somatic mutations assayed in these abundant targeted sequencing panels.

As cancer is ultimately a disease of somatic evolution, with even social and germline contributors to cancer often actualized via somatic mutation, impact scores—derived from observed evolutionary conservation over extended organismal timescales—are frequently used to contextualize somatic mutations for input into predictive machine learning models. We show that cancer effect size, a disease-specific, empirical measure of evolutionary benefit conferred to the tumor by a mutation, matches or outperforms impact measures to this end across a wide range of clinical features of cancer. Further, we apply a transfer learning approach to leverage the feature importance discovered by the cancer effect size annotated WES/WGS models to train models using targeted panel data. We find this embedding to not only improve the performance on predictive tasks overall, but also that it disproportionately improves performance for underrepresented groups. Lastly, we show that, while improvements to models and clever embedding of data undoubtedly can reduce disparities in model performance, the simple inclusion of samples from more diverse, representative individuals reduces that disparity more effectively.

#1017 Causal attribution in Korean cancer patients who underwent genetic testing: What caused my cancer?.

M.-C. Kang[1], M.-A. Jang[2], S.-Y. Park[1], S.-Y. Jung[1], S. Lee[1], J.-K. Kim[1], B. Park[3], S.-Y. Kong[1];
[1]National Cancer Center - Korea, Goyang-si, Korea, Republic of, [2]Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of, [3]Hanyang University College of Medicine, Seoul, Korea, Republic of

Introduction: Perception of patient's causal attribution is crucial for developing effective prevention strategies and improving overall health management approaches. Causal attributions of cancer patients have been well investigated in Western populations, while relatively few studies have conducted on East Asian populations. This study aimed to explore the perceptions of causal attributions among Korean cancer patients who underwent genetic testing.
Methods: Patients who met the criteria of hereditary cancer underwent germline genetic testing were recruited at the National Cancer Center in Korea between July 2023 and October 2024 (CRIS No. KCT0009460). Causal attributions were assessed through an open-ended item included in the Korean version of the Brief Illness Perception Questionnaire (BIPQ-K): "Please list in rank-order the three most important factors that you believe caused your illness." Responses were considered valid if they answered more than once in the three factors.
Results: Of the 958 cancer patients analyzed (97.9% female; mean age±SD, 52.7±9.9 years), breast cancer (531, 55.4%), ovarian cancer (390, 40.7%), other types of cancer (51, 5.3%), and multiple organ cancers (80, 8.4%) were included. First cancer diagnosis age less than the age of 40 were 194 (20.3%) patients and family history of cancer within second-degree relatives were observed in 748 (78.1%) patients. Their genetic testing results frequency were as following: pathogenic variants (PV), 196 (20.5%); variants of uncertain significance (VUS), 357 (37.3%); benign variants (BV), 405 (42.3%). The top five perceived risk factors were stress (718, 74.9%), dietary habits (385, 40.2%), genetics or family history (177, 18.5%), lack of weight management (167, 17.4%), and sleep habits (152, 15.9%). In the PV group, genetics or family history was reported more frequently compared to the VUS and BV groups (39.3% vs. 13.1%; $p<0.001$).
Conclusion: Korean cancer patients tended to overattribute stress as a cause of cancer than Western population, and this tendency was similar in PV group. Additionally, 10 respondents attributed their cancer to COVID-19 or COVID-19 vaccination, reflecting the influence of health anxieties during the COVID-19 pandemic period. The association between clinical and demographic characteristics and causal attributions will be analyzed. This work was supported by the grant of the Korean Cancer Survivors Healthcare R&D Project through the National Cancer Center, funded by the Ministry of Health & Welfare, Republic of Korea (Grant No. RS-2023-CC139201).

#1018 Genetic landscape and functional exploration of kidney cancer predisposition in cross-ancestral populations.

H. Dai, X. Chu, H. Du, S. Yang, Y. Yao, X. Yu, Y. Zhao, Z. Lyu, W. Wang, C. Sheng, H. Zheng, F. Song, F. Song, M. Li, K. Chen; Tianjin Medical Univ. Cancer Inst. & Hospital, Tianjin, China

The genetic architecture of renal cell carcinoma (RCC) has not been fully characterized, particularly in Asian populations. Here, we conducted a genome-wide association study (GWAS) of 4,692 RCC patients and 10,116 controls of Chinese ancestry, extending to a multi-ancestry cohort totaling 879,498 participants. Our approach unveiled 10 novel RCC-associated loci and a Chinese-specific locus at 12p13.33 with genome-wide significance. Utilizing GWAS results and cross-ancestry expression quantitative trait loci (eQTLs), we achieved a refined identification of RCC-related candidate causal variants and genes. These findings were substantiated through CRISPR-based screenings and multiplexed single-cell perturbations. Functionally, we established a novel association between rs28684409 and the oncogene RPL4 at the complex genetic locus 15q22.31. This top-down genetic study of RCC underscores the utility of integrating cross-ancestry GWASs, QTLs, and functional screens to deeply elucidate complex diseases and identify potential therapeutic targets.

#1019 HRD status and biallelic loss of BRCA1/2 in breast cancer patients with germline BRCA1/2 mutation assessed by whole genome sequencing.

Ji Soo Park[1], Jeongsoo Won[2], Tae Yong Kweon[3], Sangwoo Kim[2], Hyung Seok Park[4]

[1]Hereditary Cancer Clinic, Cancer Prevention Center, Yonsei Cancer Center, Seoul, Korea, Republic of,[2]Department of Biomedical Systems Informatics, Yonsei University College of Medicine, Seoul, Korea, Republic of,[3]Robot Mastectomy, Hereditary Breast and Ovarian Cancer Syndrome, & Translational Research (PROHBOT), Yonsei University College of Medicine, Seoul, Korea, Republic of,[4]Department of Surgery, Yonsei University College of Medicine, Seoul, Korea, Republic of

Background: Beyond the properties of hereditary cancers with *BRCA1* and *BRCA2* mutations, the concept of BRCAness is used to predict hypersensitivity to PARP inhibitors by synthetic lethality. In addition to detecting deleterious mutation in homologous recombination (HR) - related genes and genomic instability score (GIS), the application of a mutational signature has been tried using whole genome sequencing (WGS). Although a BRCA-associated cancer arose in the heterozygous carrier with germline likely pathogenic/ likely pathogenic variant (PGVs) in *BRCA1/2* (g*BRCA1/2* mutant) had generally been considered as be in HRD status, some BRCA-associated cancer with g*BRCA1/2* mutation were classified into homologous recombination proficient (HRP) status by HRD assays. In this study, we tried to investigate the discordance in aspects of assay tools and bi-allelicity.

Methods: We collected WGS data from prospectively enrolled clinical database (N=18) and public database (TCGA, N=37) of g*BRCA1/2* mutant breast cancer patients. We compared the HRD status analyzed using HRDetect, CHORD, and HRD score (calculated by GIS) method, and investigated the association with biallelic or monoallelic alteration.

Results: Of all the 55 WGS data from the clinical and public database, 39 (71 %), 39 (71 %), 44 (80 %) were predicted to be HRD by HRD score, CHORD, and HRDetect, respectively. HRDetect showed 94.9 % positive percent agreement (PPA) and 56.3 % negative percent agreement (NPA) to CHORD, and HRDscore showed 86.4 % PPA and 90.9 % NPA to HRDetect. Most biallelic losses of *BRCA1/2* were identified in the tumor with germline mutation and LOH of wild type allele (40 of 43, 93.0%). For the HRD assay using HRDetect, 43 tumors with biallelic loss in *BRCA1/2* showed significant correlation with HRD compared to those with monoallelic loss in *BRCA1/2* (p value < 0.0001).

Conclusions: Even among the g*BRCA1/2* mutant breast cancers, HRD status showed some discordance according to the prediction tools and biallelic loss in *BRCA1/2* genes. Clinical utility of HRD tumors with biallelic loss in *BRCA1/2* need to be further investigated.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#102 Identifying predictors of liver metastasis in small cell lung cancer using gene expression profiling.

George Chrisafis[1], Mohit Arora[2], Nobuyuki Takahashi[3], Anish Thomas[2]

[1]Internal Medicine, University of Michigan Medical School, Ann Arbor, MI,[2]National Cancer Institute, Bethesda, MD,[3]National Cancer Center Hospital East, Kashiwa, Japan

Small cell lung cancer (SCLC) is a highly aggressive, treatment-resistant form of lung cancer which carries an exceptionally poor prognosis. Patients with SCLC often present at an advanced stage due to the propensity of SCLC for early metastasis, with common sites including the brain, liver, adrenal glands, and bone. Liver metastasis has been identified as an independent prognostic factor for unfavorable response to chemotherapy, but specific steps in the liver metastatic cascade are yet to be described. Here, we seek to better characterize SCLC liver metastases by analyzing gene expression data from cell lines and patient biopsy samples. We analyzed data published in MetMap (Jin et al., 2020), which estimated organ-specific 'metastatic potential' of a variety of cell lines from the Cancer Cell Line Encyclopedia (CCLE). Gene set enrichment analysis revealed that SCLC cell lines with high liver metastatic potential exhibit significant positive enrichment of MYC signaling, oxidative phosphorylation, and Wnt/Beta-catenin signaling with negative enrichment of TNF-alpha signaling, inflammatory response, interferon response, and epithelial-to-mesenchymal transition. This pattern appears unique to SCLC cells compared with liver metastatic non-small cell lung cancer and colorectal cancer cells. Genes with differentially higher expression in SCLC lines with high liver metastatic potential were also found to play roles in embryonic organ and lung development. Cell lines with low liver metastatic potential exhibited increased expression of genes involved in pathways related to natural killer (NK) cell mediated immunity, suggesting the important role for innate immunity in preventing liver metastasis. This observation was confirmed in multiple datasets of SCLC patient biopsies, with higher expression of NK cell activating genes associated with a more favorable prognosis. Additionally, chromosome 3p deletion was more frequently observed in liver metastatic cell lines, and in primary patient tumors, the presence of a 3p deletion (plus 5q non-deletion) was associated with the presence of liver metastasis. Genes and pathways identified by this analysis, following experimental validation, could serve as potential therapeutic targets to predict and disrupt the liver metastatic cascade.

POPULATION SCIENCES: Genomic Diversity and Disparities in Cancer: Global Insights into Epidemiology, Risk, Outcomes, and Precision Medicine
Poster Session

#1020 A cancer patient survival study facilitated by an integrated enrichment workflow streamlined DNA and RNA sequencing of biopsy samples.

Junhua Zhao, Benjamin J. Krajacich, Kevin Green, Xiaodong Qi, Shawn Levy

Element Biosciences, Inc., San Diego, CA

RNA sequencing provides valuable insights into gene expression alterations in response to drug-treatments, mutations, and gene fusions in cancer research. Combined analyses of RNA transcript variations and comprehensive genomic sequencing allows for cross-validation of somatic mutations, improvement of accuracy and clinical sensitivity, and understanding of the mutation effects on gene expression, yielding essential transcriptomic/genomic data that inform individual patient's responses to therapeutic interventions. However, challenges in sample and library preparation, along with the complexity of conducting multiple target enrichments for RNA and DNA samples, often are costly and time consuming. In this research study, we employed the novel integrated target capture technology, Trinity^{TM}, to simultaneously capture both RNA and DNA libraries in a single hybridization reaction, thereby streamlining the process and reducing overall costs. This optimized workflow significantly decreases sample-to-answer time below 21 hours, with 1 hour hybridization time and direct loading onto sequencing instrument. This improvement contrasts drastically to traditional multi-day procedures. Using this new approach, we processed matched DNA and RNA sequencing of blood and formalin-fixed paraffin-embedded (FFPE) tissue biopsy samples from eight prostate cancer patients collected pre- and post-intervention for treatment. As a result, the assay showed ~ 10% higher mean target coverage at equivalent read depths compared to data generated from the same samples processed using conventional target capture workflow. Additionally, the coverage depth for Indels was significantly better and generated >0.99 Indel F1 scores with the exome panel in the benchmarking data of reference genome NA12878. Similarly, the RNA data obtained in the study increased sequencing efficiency and generated more usable RNA seq data than traditional bulk-RNA sequencing. We performed a comprehensive evaluation on intervention effect for treatment in both expression changes and mutation profiling and showed the feasibility to apply this assay to larger sample sizes. In summary, using Trinity^{TM} for combined DNA/RNA hybrid capture workflow leads to a rapid, cost-effective workflow to simultaneously unlock both DNA and RNA biomarker insights. This advancement could accelerate multi-omic studies and contribute to research that could help the design of targeted therapies, ultimately benefiting the precision of cancer treatment.

#1021 Predictors of receipt of guideline-concordant chemotherapy among ovarian cancer patients in the Kaiser Permanente Research on Ovarian Cancer Survival (KPROCS) Study.

Elisa V. Bandera[1], Lindsay J. Collin[2], Valerie S. Lee[3], Juraj Kavecansky[4], Farzin Khosrow-Khavar[5], Scarlett L. Gomez[6], Jennifer A. Doherty[7], Lawrence H. Kushi[3]

[1]Rutgers Cancer Institute, New Brunswick, NJ,[2]Emory Univesity, Atlanta, GA,[3]Kaiser Permanente Northern California, Pleasanton, CA,[4]Kaiser Permanente Northern California, Antioch, CA,[5]Rutgers University, New Brunswick, NJ,[6]University of California at San Francisco, San Francisco, CA,[7]Huntsman Cancer Institute, Salt Lake City, UT

Background: Ovarian cancer is the fifth leading cause of cancer death among women in the US. Standard of care for ovarian cancer includes cytoreduction followed by paclitaxel-based chemotherapy. Herein, we aimed to assess patient-level factors affecting receipt of guideline concordant chemotherapy among patients diagnosed with epithelial ovarian cancer in an integrated health system.
Methods: This preliminary analysis included 5,327 patients in the ongoing Kaiser Permanente Research on Ovarian Cancer Survival (KPROCS) Cohort Study, who were diagnosed with epithelial ovarian cancer (2000-2022) and treated at Kaiser Permanente Northern California. Women were included if they were ≥18 years at diagnosis, resided in California, and received chemotherapy. Treatment and other sociodemographic and clinical information, including specific chemotherapy agents and whether neoadjuvant treatment was given were ascertained from electronic health records, and assessed for concordance against National Comprehensive Cancer Network guidelines that were in place at the time of treatment and as appropriate for clinical characteristics such as stage, grade, histotype, and comorbidities. We used logistic regression to compute odds ratios (ORs) and 95% confidence intervals (95%CI) to estimate associations of demographic and clinical characteristics with receipt of guideline concordant chemotherapy, in multivariable models including age, stage, histotype, and Charlson Comorbidity Index (CCI).
Results: We included 3,472 Non-Hispanic White (NHW) women, 297 Non-Hispanic Black (NHB) women, 771 Asian American and Pacific Islander (AAPI) women, 696 Hispanic women, and 91 women of other racial and ethnic groups. Overall, 48% of patients received guideline concordant care. In multivariable-adjusted models, NHB individuals were less likely to receive guideline care compared with NHW individuals (OR=0.76, 95%CI: 0.58-0.99), whereas AAPI individuals were more likely to receive guideline care (OR=1.13, 95%CI: 0.95-1.35) and Hispanic individuals were equally likely (OR=1.04, 95%CI: 0.87-1.24). Women were less likely to receive guideline-concordant chemotherapy if they were older (>70 vs. <50 years, OR=0.63, 95%CI: 0.52-0.78), had higher CCI (CCI 3+ vs CCI=0, OR=0.56, 95%CI: 0.46-0.68), later stage at diagnosis (Stage IV vs Stage 1; OR=0.40, 95%CI: 0.33-0.48), were diagnosed with histotype of carcinoma not otherwise specified vs. endometrioid (OR=0.28, 95%CI: 0.22-0.38), or had high body mass index (≥40 vs <25 kg/m^2; OR=0.58, 95%CI:0.44-0.77).
Conclusions: We found that in a managed care setting, ovarian cancer treatment disparities persisted across racial and ethnic groups, and those with higher comorbidity, later stage at diagnosis, and greater obesity were less likely to receive guideline-concordant care.

#1022 Tumor-associated microbiome association with colorectal cancer-specific mortality in a racially and ethnic diverse patient population.

C. E. Thomas[1], N. C. Lorona[2], S. D. LaBrie[1], K. R. Curtis[1], H. Yin[1], N. Ma[1], T. Randolph[1], C. Qu[1], J. Huyghe[1], S. Thomas[1], L. Hsu[1], A. L. Koehne[1], S. Nayemi[1], H. Ammar[1], O. Kahsai[1], D. Redwood[3], C. I. Li[1], L. Li[4], U. Peters[1], J. C. Figueiredo[2], T. K. Thomas[3], A. I. Phipps[1], M. A. J. Hullar[1];

[1]Fred Hutchinson Cancer Center, Seattle, WA, [2]Cedars-Sinai Medical Center, Los Angeles, CA, [3]Alaska Native Tribal Health Consortium, Anchorage, AK, [4]Ochsner Health System, New Orleans, LA

Background: The tumor-associated microbiome is an important component of the tumor microenvironment implicated in colorectal cancer (CRC) prognosis. However, it remains unclear which phyla or genera are associated with CRC-specific mortality, particularly in understudied racial and ethnic patient populations.

Methods: We included 382 patients with CRC (90 African American, 95 Alaska Native, 71 Hispanic, and 126 non-Hispanic White) from the Translational Research Program in Colorectal Cancer Disparities (TRPCD). We conducted V_4 16S rRNA gene sequencing of genomic DNA extracted from formalin-fixed paraffin embedded (FFPE) tumor tissue. Sequences were processed bioinformatically in QIIME 2.0 to classify the relative abundance of different bacterial genera and phyla in tumor tissue. All batches include positive, no template and tumor sample block wax controls that are extracted and sequenced and from which contaminants are identified and removed via bioinformatics. Patients who died of CRC were matched to patients who did not die of CRC by age, sex, tumor site, tumor stage, year at diagnosis, and racial and ethnic group. We used unconditional logistic regression to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between positivity (non-zero/zero) of phyla or genera with CRC-specific mortality, adjusted for all matching factors. As a sensitivity analysis, we also examined associations comparing those with high levels of a phyla or genera (among those non-zero, at or above the median) versus those with no such bacteria detected, or zero.

Results: After 16S rRNA gene sequencing, 8 phyla and 48 genera were available for analysis. No phyla or genera were statistically significantly associated with CRC-specific mortality status after accounting for multiple testing. The strongest associations were for the genera Enterococcus, where positivity was inversely associated with CRC-specific mortality [OR positive versus zero (95% CI) = 0.52 (0.30, 0.87)], and the genera Cloacibacterium, where positivity was associated with greater odds of CRC-specific mortality [OR positive versus zero (95% CI) = 2.40 (1.14, 5.16)]. Comparing high levels to zero, the strongest association was for the genera Leptotrichia, where high levels were associated with greater odds of CRC-specific mortality [OR high versus zero (95% CI) = 2.60 (1.24, 5.54)].

Conclusions: While no phyla or genera were statistically significantly associated with CRC-specific mortality after accounting for multiple testing, there are several genera that have strong associations and warrant further investigation. This work may be useful identifying pathogens or protective agents that impact CRC-specific mortality in understudied populations.

PREVENTION / EARLY DETECTION / INTERCEPTION: Behavioral Sciences and Prevention, Screening, and Early Detection Poster Session

#1026 Multi-modal liquid biopsy analysis of early-stage breast cancer patients.

Kaylee J. Kamalanathan[1], Alec Horrmann[1], Nathaniel R. Bristow[1], Yash Travadi[1], Kevin Mallery[1], Catalina Galeano-Garces[1], Song Yi Bae[1], Alexa Hesch[1], Grant Schaap[1], Yuliya Olimpiadi[2], Sarah Pederson[2], Jiarong Hong[1], Justin Drake[1], Jayant Parthasarathy[1], Badrinath R. Konety[1]

[1]Astrin Biosciences, Inc., Saint Paul, MN,[2]Allina Health Cancer Institute, Minneapolis, MN

The 5-year breast cancer survival rate in the United States is 91% overall with a 99% survival rate for localized disease. Early detection via mammograms has largely been responsible for the success of finding breast cancer early; however, mammograms have drastically decreased effectiveness in women with dense breasts tissue which is approximately half of the US population. To better serve these women, an alternative screening method is necessary. Astrin Biosciences has developed a multi-modal liquid biopsy-based alternative for breast cancer screening in this population.

Recent literature demonstrates that cancer cells disseminate into blood vessels even at early stages. These early-stage cells are a potential source of late recurrence and therapy resistance but have strong potential benefit in early detection as well as provide opportunities to determine possibly curative interventions. Although these cell assays have a huge potential benefit, they generally leave some to be desired as far as sensitivity due to the rarity of these cells with less than 10 cells per 10 mL blood tube from circulation. To overcome this limitation, Astin's algorithm pairs our AI-empowered proprietary holographic imaging platform with plasma proteomics. This pairing provides increased sensitivity over a cell-based assay alone while maintaining the ability to detect disseminating cells for further analysis in patients with enough cells present.

This combined technology was evaluated in a cohort of more than 100 newly diagnosed early-stage breast cancer patients (Stage 0, 1, and 2). Blood samples were collected prior to initiation of therapy and the plasma and cell fractions were separated. Proteomic analysis was performed on the plasma sample while Astrin's holographic imaging platform combined with in-flow protein marker expression was used to analyze the samples for circulating tumor cells. Preliminary data from this study demonstrates evidence of early dissemination detectable in the majority of breast cancer patients. GSEA analysis of protein expression revealed well known pathways associated with cancer biology. Astrin Biosciences' combined assay has the ability to detect cancer in women with dense breast, increasing the likelihood cancer is detected early in this population.

#1027 A multicenter clinical performance evaluation of a liquid biopsy for earlier brain cancer detection.

James M. Cameron[1], Georgios Antoniou[1], Paul Brennan[2], Holly Butler[1], David Eustace[1], Asgeir Jakola[3], Michael Jenkinson[4], Senada Koljenovic[5], Alan Lazarus[1], Ryan Mathew[6], David Palmer[1], Isaac Phang[7], Alexandra Sala[1], Tobias Weiss[8], Matthew Baker[1]

[1]Dxcover Ltd., Glasgow, United Kingdom,[2]University of Edinburgh, Edinburgh, United Kingdom,[3]University of Gothenburg - The Sahlgrenska Academy, Gothenburg, Sweden,[4]The Walton Centre, Liverpool, United Kingdom,[5]University Hospital Antwerp, Antwerp, Belgium,[6]Leeds Teaching Hospitals, Leeds, United Kingdom,[7]Royal Preston Hospital, Preston, United Kingdom,[8]Universitatsspital Zürich, Zurich, Switzerland

Diagnostic delays impact the quality of life and survival of patients with brain cancer. Currently, with no blood test to assist them, clinicians must make their referral decision based on symptoms. Existing symptom-based referral guidelines inadequately stratify patients for brain imaging based on suspicion of cancer. A simple, rapid liquid biopsy placed in a primary care setting would enable more efficient triage of patients with non-specific symptoms related to brain cancer.

The Dxcover® Liquid Biopsy is a rapid multi-omic liquid biopsy that interrogates a blood sample with infrared radiation and produces a distinctive signature that represents the whole biomolecular profile of the sample, and is sensitive to the hallmarks of brain cancer. The nature of the technology also allows for the diagnostic models to be tailored towards higher sensitivity (or specificity) depending on clinical priorities and international healthcare markets. In initial feasibility studies, we recruited 988 patients prospectively with non-specific symptoms associated with a brain tumor, and the algorithm detected 96% of the patients with brain tumors and 100% of glioblastomas (GBM), when tuned for greater sensitivity.

EMBRACE is a prospective, observational, multicenter study, currently recruiting patients across seven sites in the United Kingdom, Belgium, Sweden and Switzerland. The study duration is 24 months and will recruit a minimum of 2200 participants by the end of 2024. The target population is comprised of patients presenting to primary care with non-specific symptoms that are associated with brain cancer, such as headache and new onset neurological defect. The primary objective is to determine the clinical performance of the liquid biopsy for patients with brain cancer, in terms of diagnostic sensitivity and specificity. The test performance will be determined by comparing the liquid biopsy result to diagnostic imaging. The results of the EMBRACE study will be shared for the first time at the meeting.

Earlier and expeditious diagnoses in these patients are crucial to reduce the morbidities and mortalities associated with brain cancer. This simple, non-invasive liquid biopsy would facilitate the triage of brain tumor patients for rapid imaging.

#1028 Evaluating amplitude modulated radiofrequency electromagnetic fields as a cancer screening modality.

Callum T. McGrath[1], Hugo Jimenez[2], **Boris C. Pasche**[2]

[1]Wake Forest University School of Medicine, Winston Salem, NC,[2]Department of Oncology, Wayne State University School of Medicine, Detroit, MI

Background: Tumor-specific Amplitude Modulation Radiofrequency Electromagnetic Fields (AM RF EMF) have been identified in over 15 different tumor types. Tumor specific frequencies were identified by exposing patients with a known diagnosis of cancer to an AM RF EMF frequency sweep ranging from 0.1 Hz to 150 kHz. Frequencies that elicited an increase in pulse amplitude were registered as tumor-specific frequencies. We hypothesized that this mechanism could be used to screen for cancer, in which patients with an unknown diagnosis are exposed to previously identified tumor-specific frequencies and monitored for any change in hemodynamics.
Methods: 17 patients with biopsy-proven hepatocellular carcinoma (HCC), 14 patients with biopsy-proven breast cancer (BC), and 31 healthy controls were recruited for the study and had their systolic blood pressure (sBP), diastolic blood pressure (dBP), pulse pressure (PP), cardiac output (CO), heart rate (HR) R-R interval (RRI), stroke volume (SV), and total peripheral resistance (TPR) measured while exposed to random (non-tumor-specific) AM RF EMF, HCC-specific AM RF EMF, and BC-specific AM RF EMF. Patient data was then used to train a one-dimensional convolutional neural network (1D CNN) to classify each dataset as control, HCC, or BC.
Results: Overall accuracy for the model was 81.17%. AUC for the ROC curve for control, HCC, and BC was 0.91, 0.96, and 0.96 respectively.
Conclusion: Our model demonstrates that AM RF EMF has the potential to be used as a cancer screening modality. Further studies will need to be performed with larger cohorts to validate the findings of this study and to improve the accuracy of the model. Additionally, future studies should investigate the potential for AM RF EMF-based screening for pancreatic cancer, as tumor-specific frequencies have previously been identified for the malady.

#1029 Prevalence of mammographically dense breasts in Guam.

Reina Faye P. Evangelista[1], Louis Jane B. Dulana[1], **Rodney Teria**[1], Su Bin Jin[1], Gian Paras[1], Rose Grino[2], John Shepherd[3], Grazyna Badowski[1]

[1]University of Guam, Mangilao, Guam,[2]FHP Health Center, Tamuning, Guam,[3]University of Hawai'i Cancer Center, Honolulu, HI

Background: Breast cancer is the most diagnosed cancer among women in Guam. Mammographic breast density is a critical factor in assessing breast cancer risk and the effectiveness of screening. Guam's female population is diverse, comprising CHamoru (37.3%), Filipino (26.3%), Mixed (9.5%), White (7.1%), Chuukese (7%), and other Pacific Islander and Asian ethnicities. Understanding breast density patterns among these groups is essential for developing tailored public health strategies. However, specific data on the distribution of breast density among women in Guam is currently lacking. This study, conducted through the Hawai'i and Pacific Islands Mammography Registry (HIPIMR), addresses this gap by estimating the prevalence of dense breasts among women in Guam aged 40 years and older.

Methods: The mammographic breast density distribution by age and body mass index (BMI) was determined using data from 1,940 mammograms conducted at the FHP Health Center in Guam between October 2023 and September 2024 for women aged 40 and older (N = 1,920). These breast density distributions were applied to age- and BMI-specific counts of the female population in Guam, derived from the 2020 Guam Census and the Guam Behavioral Risk Factor Surveillance System (BRFSS), to estimate the number of women with dense breasts. Ethnic variations in breast density distributions were examined using a chi-square test. Results: Among the 1,920 women analyzed, breast density was categorized as BI-RADS A (9.2%), BI-RADS B (19.2%), BI-RADS C (17.2%), and BI-RADS D (54.1%). BMI distribution included under/normal weight (27.2%), overweight (26.7%), obese (31.2%), and cases with missing BMI data (14.9%). Filipino (81.1%) and Asian (84.0%) women had significantly higher combined proportions of dense breasts compared to CHamoru (63.6%) and Micronesian (52%) women ($P < 0.001$).Overall, 74.8% (95% CI: 74.3%-75.3%) of women aged 40-74 in Guam were classified as having heterogeneously or extremely dense breasts with the prevalence inversely associated with age and BMI. Based on the age and BMI distribution of Guam's female population, an estimated 22,185 (95% CI: 22,043-22,340) women aged 40-74 have heterogeneously or extremely dense breasts, with women aged 40-49 accounting for 82.5% of this group.

Conclusion: The prevalence of dense breasts among women in Guam is high (74.8%) compared to approximately 43% in the U.S., with Filipino and Asian populations showing the highest proportions. These findings underscore the need for breast density notification policies and enhanced access to supplemental screening for high-risk groups to improve early detection in Guam's diverse population.

Acknowledgement: This research was funded by the National Cancer Institute: The University of Guam/University of Hawaii Cancer Center Partnership to Advance Cancer Health Equity, Grant U54CA143728 (University of Guam, YCP)/Grant U54CA143727 (University of Hawaii Cancer Center).

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#103 Syndecan-1 Is a key mediator of entosis and cellular competition in pancreatic cancer.

Kara Li[1], Shigeaki Umeda[2], Chenyang Li[3], Rajya Kappagantula[4], Christine A. Iacobuzio-Donahue[4], Wantong Yao[1]

[1]Translational Molecular Pathology, UT MD Anderson Cancer Center, Houston, TX,[2]Donahue-Zuckerman Research Center, Memorial Sloan Kettering Cancer Center, New York, NY,[3]MD Anderson Cancer Center, Houston, TX,[4]Donahue-Zuckerman Research Center, Memorial Sloane Kettering Cancer Center, New York, NY

Entosis, a form of cell-in-cell (CIC) structure formation where one cell invades and becomes internalized by another, has long been observed in various malignancies and is considered a critical cellular process in cancer progression, with potential impacts on tumor heterogeneity, survival, and metastasis. Syndecan-1 (SDC1), a transmembrane proteoglycan involved in cell adhesion and communication, plays a pivotal role in these processes, yet its specific function in entosis within pancreatic ductal adenocarcinoma (PDAC) remains underexplored. This study aimed to investigate whether SDC1 facilitates entosis in PDAC cells and to assess its impact on tumor aggressiveness and patient outcomes. Using immunohistochemistry, flow cytometry, and entosis assays, our findings reveal that SDC1 prominently localizes at cell-cell contact points, facilitating stable intercellular adhesion and promoting entotic activity. Knockdown experiments reveal that reduced SDC1 expression significantly diminishes CIC formation, implicating SDC1 as a critical mediator of this process. Analysis of clinical samples revealed that high SDC1 expression correlates with increased entotic activity in PDAC tumors and is associated with decreased patient survival. These results suggest that SDC1-mediated entosis enhances tumor aggressiveness by contributing to cellular competition and heterogeneity. Our study sheds light on the critical role of SDC1 in promoting CIC formation and cancer progression, highlighting its potential as a therapeutic target to disrupt entotic mechanisms in PDAC.

PREVENTION / EARLY DETECTION / INTERCEPTION: Behavioral Sciences and Prevention, Screening, and Early Detection Poster Session

#1030 Effects of clearing heat and removing phlegm prescription on inhibiting proliferation and secreted protein expression of human esophageal carcinoma cells.

Fuchun Si[1], Gao Si[2], Xuejie Song[1]

[1]Hunan University of Chinese Medicine, Henan, China,[2]Peking University Third Hospital, Beijing, China

Background: Esophageal carcinoma(EC) is the sixth most common cause of cancer death worldwide. It is still a key problem to find early diagnostic targets and effective treatment medicine in the study of EC. This study aims to screen out serum differentially expressed proteins from EC patients, and explore the inhibitory effects of Clearing Heat and Removing Phlegm prescription(CHRF) on the proliferation of EC cells and serum secretory proteins expression.
Methods: Serum samples of 21 EC patients and 7 healthy normal people were collected. Raybiotech AAH BLG-1 protein chip and data analysis software were used to profile serum differentially expressed proteins of EC. CHRF was developed in our previous research for the treatment of EC. Effects of CHRF on the proliferation, cell cycle and screened serum secretory protein expression of four EC cell lines EC-1, Eca109, EC9706, TE-1 were investigated by MTT assay, flow cytometry assay and Western blot analysis.
Results: 37 differentially expressed proteins were screened out from EC patients compared with the normal control group, among which 22 were significantly up-regulated, 17 were significantly down-regulated. These differential expression proteins mainly involved in cytokine-cytokine receptor interaction, PI3K-Akt signaling pathway, Jak-STAT signaling pathway, chemokine signaling pathway, which have the function of promoting tumor development, tumor growth, angiogenesis, growth and development of tissue. 22 up-regulated serum secretory proteins were selected to detect in four EC cells and observe the inhibitory effects of CHRF. CHRF could obviously inhibit the proliferation of EC-1, EC9706, Eca109 and TE-1 cells in dose-dependent and time-dependent manner. IC50 values of CHRF for EC9706, Eca109, TE-1 and EC-1 were 33.31μg/ml, 20.70μg/ml, 21.93μg/ml, 33.47μg/ml respectively. CHRF induced G2/M phase arrest in all four EC cells. MMP1, MMP14, OPG, FBLN5, PECAM-1 proteins were expressed in all four EC cells and down-regulated by CHRF.
Conclusion: Serum secretory proteins MMP1, MMP14, OPG, FBLN5, PECAM-1 were significantly up-regulated in esophageal cancer cells, which could be considered as the potential markers for EC diagnosis; the inhibitory effects of CHRF on the proliferation of Eca109, EC9706, TE-1 and EC-1 cells have relationship with down-regulating MMP1, MMP14, OPG, FBLN5 and PECAM-1 protein expression, which not only provides new basis for the in-depth study of EC pathogenesis, but also provides foundation for the further study of new traditional Chinese medicine prescriptions for EC treatment.

#1031 Cervical cancer awareness program in Rio Grande Valley (RGV) colonias: empowering communities through our cervical cancer prevention action network (CCPAN) program.

Swati Mohan[1], Bhupendra B. Srivastava[1], Murali M. Yallapu[2], Subhash C. Chauhan[2]

[1]School of Integrative Biological and Chemical Sciences, The University of Texas Rio Grande Valley, Edinburg, TX,[2]School of Medicine, The University of Texas Rio Grande Valley, Edinburg, TX

Cervical cancer remains a significant health challenge, particularly in underserved communities of US-Mexico trans-border Rio Grande Valley (RGV) region. This region represents remarkably higher cervical cancer health disparities. Thus, to improve awareness about cervical cancer in the RGV Colonias by providing crucial evidence-based information on the prevention, early detection, and available clinical resources is utmost desirable in this RGV region. Towards this, we have established a unique cervical cancer prevention action network (CCPAN) program to cater to this need of the community. Our approach towards the awareness of cervical cancer through community engagement and by explaining what cervical cancer is, its risk factors, symptoms, importance of Human Papillomavirus (HPV) vaccination, and right dose schedule of HPV vaccination in our visits to underserved Hispanic population of RGV Colonias. We also explain etiology and progression of HPV infection through our small dramatic representation, videos and cartoons. Additionally, we provide information to the residents of Colonia about the importance of regular Pap tests and HPV tests in early detection of cervical cancer. Moreover, we also provide patients navigation information to local clinics and healthcare facilities that offer cervical cancer, HPV screenings and HPV vaccinations. We encourage people to get vaccinated, get screened, and spread the word at grass root level in the communities. Through our CCPAN program, we are also developing a database regarding Demographics, Knowledge and Awareness, Screening and Prevention, Attitudes and Beliefs, and Awareness of Local Resources. So far, the data that we have collected is alarming and is very different compared to nationwide data. Our collected data from RGV colonias suggest that communities have very limited information related to cervical cancer (30%), causes of cervical cancer (5%), awareness of HPV vaccine (30%) and timely receiving of HPV vaccine (30%). This situation appears alarming and RGV colonias communities required urgent community support to reduce cervical cancer health disparities in the region. We believe that our unique CCPAN program has very high potential to educate and empower the RGV colonias communities to take proactive steps in preventing cervical cancer, that can ultimately lead to reduce cervical cancer health disparities in the RGV.

#1032 Knowledge, attitude and practices regarding human papillomavirus (HPV) and HPV vaccination among medical undergraduate students in Delhi: A cross-sectional study.

Srishty Srishty[1], Rajesh Kumar[1], Bratati Banerjee[1], Amod Laxmikant Borle[1], Aseem Aseem[2]

[1]Department of Community Medicine, Maulana Azad Medical College, New Delhi, India,[2]University of Illinois Chicago, Chicago, IL

Introduction: Human papillomavirus (HPV), a prevalent sexually transmitted infection, is a leading cause of cervical cancer, with high-risk strains such as HPV 16 and 18 contributing significantly to disease burden. Globally, cervical cancer is the fourth most common cancer in women, while in India, it ranks third, with an incidence rate of 18.3% (123,907 cases) and a mortality rate of 9.1% (GLOBOCAN, 2020). Previous studies have highlighted significant gaps in knowledge, attitudes, and practices related to HPV and its vaccine. Furthermore, evidence indicates that targeted educational interventions can significantly enhance vaccine uptake and empower students to serve as effective advocates for vaccination.

Objectives: This study aims to assess medical undergraduate students' knowledge about HPV and its vaccine, their vaccination status, and their attitudes towards HPV vaccination.

Methodology: A cross-sectional study was performed at a medical college attached to a tertiary care hospital (Maulana Azad Medical College), Delhi, India. A total of 415 medical undergraduate students were enrolled via systematic random sampling. These participants were interviewed using a self-designed, pretested, semi-structured questionnaire including sections on sociodemographic data, knowledge about HPV and its vaccination, attitude toward the vaccine and actual vaccination practices among the study participants. Data was collected using Epicollect5 software. Data analysis was done using SAS software. Associations using Chi-square test ($p<0.05$).

Results: The mean age of participants was 21 years (SD = 1.74), ranging from 18 to 27 years. Of the 415 participants, 46.5% (n = 193) were female, and 53.5% (n = 222) were male, with most from an upper-middle socioeconomic background. The mean knowledge scores for HPV vaccination and cervical cancer were 10.77 (SD = 4.10) out of 20 and 12.28 (SD = 3.47) out of 25, respectively. While two-thirds demonstrated adequate knowledge about cervical cancer, awareness of its causal link to HPV was limited. The mean attitude score was 41.59 (SD = 4.93) out of 50, reflecting a generally positive outlook, though concerns about side effects, cost, and cultural acceptance persisted. Only 12.5% (n = 52) were vaccinated, with lack of awareness, cost, and perceived lack of need as key barriers. Notably, 60% expressed willingness to vaccinate if provided at subsidized rates or through university health programs.

Conclusion: The study highlights both opportunities and challenges in promoting HPV vaccination among medical undergraduates in Delhi. Despite positive knowledge and attitudes, vaccination uptake was low. Most students expressed willingness to vaccinate if offered at a subsidized cost or through accessible programs, suggesting cost-effective strategies and institutional support could improve coverage.

#1035 Western lifestyle leads to obesity and cancer via a common factor FTO.

Dong Wu, Ying Qing, Keith Leung, Meiling Chen, Zhicong Zhao, Yangchan Li, Li Han, Wei Li, Zhenhua Chen, Chao Shen, Yuan Che, Tingting Tang, Kitty Wang, Lillian Sau, Zhaoxu Xu, Xiaolan Deng, Jianjun Chen

Department of Systems Biology and Center for RNA Biology and Therapeutics, Beckman Research Institute of City of Hope, Duarte, CA

FTO, the first identified RNA N^6-methyladenosine (m^6A) demethylase, has been reported by our group to play an oncogenic role in acute myeloid leukemia (AML) and by others in various solid tumors, including colorectal and liver cancers. Additionally, FTO was the first gene identified through genome-wide association studies (GWAS) to be associated with obesity risk, with evidence linking elevated FTO expression to increased food intake and obesity development. While obesity is a recognized risk factor for cancer, whether FTO serves as a shared factor driving both obesity and cancer remains unclear. To address this knowledge gap, we employed *in vivo* mouse models with Fto expression manipulated through genetic knockout or pharmacological inhibition to investigate its respective roles in tumorigenesis and obesity. In an ENU-treated spontaneous tumor model, we confirmed that *Fto*-WT mice showed greater susceptibility to tumorigenesis than *Fto*-KO mice. When fed a Western diet high in sugar and fat, *Fto*-KO mice exhibited resistance to diet-induced obesity compared to *Fto*-WT mice. Similar protective effects against diet-induced obesity were observed in models treated with CS1, a potent small-molecule FTO inhibitor developed by our group. To explore the interplay between obesity and cancer, we established combined obesity-tumor models. Western diet-fed obese mice were either treated with ENU to induce spontaneous tumors or transplanted with MC-38 cells. In both scenarios, CS1 treatment simultaneously reduced obesity and tumorigenesis significantly. Mechanistically, FTO inhibition partially reversed Western diet-induced alterations in immune cell populations, indicating an immune-modulatory role for FTO in the pathogenesis of obesity and cancer. Together, our findings identify FTO as a common factor in the development of obesity and tumor and highlight the potential of targeting FTO for the simultaneous prevention and intervention of these conditions.

#1036 Gender differences in recruitment, retention, and preliminary outcomes in a nutrition education program for Latino families.

Melissa Gaviria[1], Bharat Narang[1], Ashley M. Rodriguez[1], Jessica Llamozas[1], Frankie J. Gonzalez[2], Robert Payano[1], Jocelyn Galindo Viayra[1], Luis E. Banegas Ruiz[1], Alexandra Burgos[3], Josana Tonda[1], Vanessa Lucero Cruz[1], Francesca Gany[1], Jennifer Leng[1]

[1]Immigrant Health and Cancer Disparities Service, Memorial Sloan Kettering Cancer Center, New York, NY,[2]City College of New York/CUNY School of Medicine, New York, NY,[3]The University of Texas at Austin, Austin, TX

Background/Objective: Latinos experience a disproportionately high prevalence of obesity and its associated increased risks for cardiovascular disease and certain cancers compared to non-Hispanic whites. Family-centered lifestyle interventions must engage both men and women to increase nutrition knowledge, better distribute care responsibilities, and increase support for parents and caregivers in Latino communities. This study assesses gender differences in recruitment, retention, and preliminary outcomes in Family COMIDA, a pilot community-engaged culturally and linguistically tailored nutrition education program for Latino families.
Methods: Outreach for participation in Family COMIDA was conducted among Latino adults visiting the New York City Mexican Consulate's 'Ventanillas de Salud' (Health Windows) program. Eligible adults were Spanish-speaking, overweight or obese (BMI ≥ 25 kg/m^2), and were the primary caregivers for at least 1 child < 18. Participants received written and digital educational materials. We measured weight and administered the California Health Interview Survey (to assess self-reported dietary behaviors) at baseline and 3 months. We assessed gender differences in recruitment (eligible individuals who consented to participate); retention (participants who completed the 3-month follow-up); and preliminary outcomes (changes in weight and self-reported dietary behaviors).
Results: A total of 76 individuals were interested in participation, among whom 74 completed the eligibility screener (77% women, 23% men). Among them, 65 were eligible, and 51 enrolled (80% (41/51) of women and 71% (10/14) of men). Mean age was 44 (SD 7.3), and 51% had a primary school education or less. 80% of men and 37% of women were employed. At month 3, the retention rate was 98% for women and 60% for men. Mean weight loss among men was 6.1 lbs (SD 6.3), and 5.4 lbs (SD 5.9) among women. Men showed greater improvements in dietary behaviors in many categories, including fruit (+28% men, +15% women), ice cream (-12% men, -8% women), and fried food (-59% men, -35% women) consumption.
Conclusion: Recruitment rates were similar for men and women, with men demonstrating greater weight loss and more substantial dietary behavior improvements in several categories. However, fewer men completed the eligibility screener, and retention rates were lower for men, suggesting barriers to male participation. To address barriers to recruitment and retention of Latino men, outreach efforts should include tailoring to Latino men (e.g., scheduling health promotion/outreach events specifically for fathers, adjusting recruitment hours to after-work and weekends to accommodate job demands), and partnering with Hispanic-serving faith- and community-based organizations to highlight the value of participation to Latino men and encourage engagement with all family members.

#1037 Deep learning-derived dietary patterns and gastric cancer risk in women: A large-scale prospective cohort study.

Hyobin Lee[1], Dongseok Heo[2], Sukhong Min[1], Sinyoung Cho[1], Bongwon Suh[3], Daehee Kang[1]

[1]Department of Preventive Medicine, Seoul National University College of Medicine, Seoul, Korea, Republic of,[2]Interdisciplinary Program in Artificial Intelligence, Seoul National University Graduate School, Seoul, Korea, Republic of,[3]Department of Intelligence and Information, Seoul National University, Seoul, Korea, Republic of

Background & Aims: Machine learning can address limitations of principal components analysis by capturing nonlinear relationships, enhancing dietary pattern identification. This study aimed to identify dietary patterns using deep learning and cluster analysis and to assess their association with gastric cancer incidence in a prospective cohort study.
Methods: Data were obtained from participants of the large-scale prospective cohort study, Health Examinees-Gem (HEXA-G) study, with baseline surveys conducted between 2004 and 2013. Cancer diagnosis information was retrieved from the Korea Central Cancer Registry up to December 31, 2018. The final analysis included 84,725 women. Dietary intake was assessed using a validated semiquantitative food frequency questionnaire at baseline. Dimensionality reduction was performed using an autoencoder, followed by k-means clustering. The optimal cluster number was determined using the silhouette coefficient. Multivariable Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for gastric cancer risk associated with the dietary patterns.
Results: Ten dietary patterns were identified: ‘Common’, ‘Low calorie’, ‘Rice’, ‘High calorie’, ‘Traditional’, ‘Dairy’, ‘Meat’, ‘Processed’, 'Noodles' and ‘Salty’ patterns. A total of 558 cases of gastric cancer were identified during median 9.4 years of follow-up. Compared to the ‘High calorie’ pattern (reference), the ‘Noodles’ (HR 1.97, 95% CI 1.05-3.70), and ‘Low calorie’ (HR 1.91, 95% CI 1.23-2.97) were associated with higher incidence of gastric cancer after adjusting for potential confounders. When stratified by age, ‘Salty’ pattern was associated with 4.34 times higher risk of gastric cancer (HR 4.34, 95% CI 1.49-12.60) among participants aged 40-49.
Conclusion: Our results suggest that relative to the ‘High calorie’ pattern, the ‘Noodles’, ‘Low calorie’, and ‘Salty’ patterns are associated with increased risk of gastric cancer among Korean women. While Helicobacter pylori infection—a major risk factor for gastric cancer—was not accounted for in this study, our findings highlight the importance of dietary interventions to reduce high-risk patterns, such as salty or noodle-based diets, to lower the risk of gastric cancer.

#1038 Examining racial disparities in the context of food security, poverty, and allostatic load.

Jennifer M. Crook[1], Eunkyung Lee[2], Rui Xie[3], Ladda Thiamwong[1], Victoria Loerzel[1]

[1]Nursing, University of Central Florida, Orlando, FL,[2]Health Sciences, University of Central Florida, Orlando, FL,[3]Statistics and Data Science, University of Central Florida, Orlando, FL

Intro: Disparities in cancer incidence and mortality remain a complex and elusive problem with diet well-studied as a contributing factor. However, it is unclear the impact of food and nutritional security on cancer risk. To fully appreciate nutritional inequities of both food insecurity (FI) and poverty within cancer, there remains a need to more closely examine racial/ethnic disparities in chronic inflammatory indicators of allostatic load. We have previously identified nutritional discrepancies between dietary intake report and plasma nutritional and inflammatory statuses. This study aimed to more clearly identify racial/ethnic disparities within FI, poverty, and allostatic load to develop targeted public health nutritional cancer interventions.
Methods: The Vitamin/Mineral and Inflammatory Correlations To Understand Chronic Stress in the U.S.: The VICTUS Study is a cross-sectional study utilizing data from the 2017/2018 National Health and Nutrition Examination Surveys (NHANES) as a representative sample of U.S. adults. Subjective survey data was interrogated with objective laboratory analyses to observe associations between demographic, socioeconomic, health behaviors, nutrition status, and biomarkers of allostatic load (defined as systolic and diastolic blood pressure, body mass index, glucose, albumin, creatinine, HDL, LDL, triglycerides, hs-CRP, red cell distribution width, and cholesterol) to identify racial/ethnic disparities. Pearson's chi-square, correlation, and ANOVA models were constructed.
Results: From 3083 participants, 38.2% other Hispanic, 37.3% multi-racial, 35.2% Mexican American, and 26.4% non-Hispanic Black adults reported FI, while only 22.5% non-Hispanic White and 5.8% of Asian adults did. Allostatic load and inflammatory biomarkers of SBP, DBP, BMI, HDL, tri, RDW, hs-CRP, alb, creat, ($p<0.001$), and chol ($p<0.05$) exhibited significant racial/ethnic disparities. When surveying poverty, Mexican American ($p < 0.001$), multi-racial ($p < 0.01$), NH Black and other Hispanic ($p < 0.05$) exhibited increased rates when compared to NH White participants. There were also clear racial/ethnic disparities between FI report and plasma nutritional status with significant deficient and inadequate statuses seen in the following micronutrients B, C, D ($p < 0.001$), E ($p < 0.01$), Na, and Ca ($p < 0.05$).
Discussion: This study found clear racial/ethnic disparities in FI, poverty, nutritional status, and inflammatory indicators of acute and chronic inflammation (allostatic load). Also identified were incongruences between self-reported FI and plasma nutritional status of many micronutrients with significant associations with race/ethnicity suggesting that current FI assessment may neither be adequately identifying nor intervening in those at highest risk of chronic inflammatory conditions contributing to increased cancer risk.

#1039 Bridging gaps in cancer care: Utilizing large language models for accessible dietary recommendations.

J. A. Logan[1], N. L. Simone[1], S. Sadhu[1], C. Hazlewood[1], S. Burke[1], H. Nourzadeh[1], Y. Vinogradskiy[1], W. Choi[1], A. P. Dicker[1], M. Denton[2], C. Black[2], C. Ciaverelli[2], J. Stulb[2], A. Leader[1];

[1]Sidney Kimmel Medical College, Philadelphia, PA, [2]Thomas Jefferson University Hospitals, Philadelphia, PA

Background: Weight management is directly linked to cancer recurrence and survival; however, oncology nutritional counseling is not typically covered by insurance, creating a disparity among patients without nutritional education and easy food access. Novel ways of imparting personalized nutrition advice are imperative to address this issue. Large language models offer a promising and sustainable path toward dietary advice tailored to individual needs.

Objective: This study aimed to assess the capacity of large language models to offer personalized dietary advice to patients with breast cancer.

Methods: Thirty-one prompt templates were designed to evaluate the dietary recommendations generated by ChatGPT and Gemini with variations within eight categorical variables, including cancer stage, comorbidity, location, culture, age, dietary guideline, budget, and store. Seven prompts were selected for response from four board-certified dietitians. The responses were then evaluated based on qualitative observations of the large language model-generated grocery lists and meal plans and a quantitative comparison of the calories and macronutrients of large language model- and dietitian-generated meal plans to the Acceptable Macronutrient Distribution Ranges and United States Department of Agriculture daily calorie standards.

Results: Large language models generated personalized grocery lists and meal plans, adapting to location, culture, and budget, but not age, disease stage, comorbidities, or dietary guidelines. Gemini provided more comprehensive responses, including visuals and specific prices. While dietitian-generated diets offered more adherent total daily calorie contents to the United States Department of Agriculture's estimated calorie needs, ChatGPT and Gemini offered more adherent macronutrient ratios to the Acceptable Macronutrient Distribution Range.

Conclusions: Large language models can provide personalized dietary advice to cancer patients who may lack access to this care. Recommendations apply to patients with variable food access, socioeconomic means, and cultural preferences and can be a tool to increase health equity.

#1040 Voices of resilience: Analysing #MetastaticBreastCancer content on TikTok.

N. Morena[1], **C. Herman**[1], J. Schneidman[1], E. Belzile[2], A. N. Meguerditchian[2];
[1]McGill University, Montreal, QC, Canada, [2]St Mary's Research Centre, Montreal, QC, Canada

INTRODUCTION:
Young women with metastatic breast cancer (YWMBC) have unique biopsychosocial challenges, for which they often turn to social media for information and support. The aim of this study is to understand YWMBC engagement on TikTok and analyze content about MBC.
METHODS:
Videos tagged with #metastaticbreastcancer on TikTok were collected in 07/24. Reviewers gathered video characteristics: username, user profile, caption, length, date posted, number of followers, likes, views, shares, comments, and presence of sponsorship. Creator demographics were noted. Explanations, communication style, themes, and topics discussed were assessed. Spearman's correlation coefficient was calculated between variables.
RESULTS:
336 English language TikToks were included. Most videos (41%) were posted in 2024, 97% were from individuals. 1% included sponsorship. Average video length was 1:46 minutes. 161 used music. Mean follower number was 61,927 (SD 350,900). Mean number of views, likes, and comments was 209,882 (SD 625,961), 15,541 (SD 134,070), and 622 (SD 3899), respectively. The majority of videos were filmed at home (56%), specifically in the bedroom. 21% were filmed in a hospital setting. Average age at diagnosis was 31.4 years old (SD 4.3). Emotion was explicitly present in 24% of videos. Topics discussed included: daily life with MBC (70%), diagnosis (42%), treatment options (38% systemic, 26% surgery and 16% radiation). Themes are addressed in Table 1. Most relevant correlations for engagement (likes, views) included display of surgical scars, explicit expression of emotions, and diagnosis story. Predictors specific to more comments included fear of recurrence, grief and motherhood.
CONCLUSION:
TikToks from YWMBC present personal narratives of the daily experience of MBC. They are minimally sponsored and generate higher engagement when personal content/emotions are displayed. They may represent a journaling equivalent and could potentially have a beneficial effect on patients.
Table 1 :

Themes	**N (%)**
General experience of treatment	175 (52.2)
Path to diagnosis	166 (49.4)
Side effects (fatigue, nausea, etc)	91 (27.1)
Motherhood	69 (20.5)
Appearance	68 (20.2)
Mental health	61 (18.2)
Treatment choices	54 (16.1)
Spousal relationship	49 (14.6)
Fear of recurrence	37 (11.0)
Family relationship	29 (8.6)
Grief	22 (6.5)
Fertility	15 (4.5)
Social commentary	12 (3.6)
Treatment toxicity	12 (3.6)
Preparing for end of life	9 (2.7)
Sexuality	3 (0.9)

#1041 Active interception to modulate the systemic cancer-permissive microenvironment and reduce cancer incidence in companion dogs with elevated cancer risk.

Ashley J. Schulte[1], Rose Dicovitsky[2], Taylor A. DePauw[1], Amber L. Winter[2], Kathleen L. Stuebner[1], Andrea Chehadeh[2], Sara Pracht[2], Antonella Borgatti[2], Ali Khammanivong[1], Mitzi Lewellen[1], Lauren E. Burt[1], Daniel A. Vallera[1], Michael S. Henson[2], Erin B. Dickerson[1], Christopher Ober[2], Kari L. Anderson[2], Esther Nell[2], Aaron L. Sarver[1], Gary R. Cutter[3], Jaime F. Modiano[1]

[1]University of Minnesota, Minneapolis, MN,[2]University of Minnesota, St. Paul, MN,[3]University of Alabama at Birmingham, Birmingham, AL

Companion dogs have a lifetime cancer risk similar to that of humans. This excess risk, as compared to what is seen in other species, seems to be due to our recent success in outliving our evolutionarily adapted lifespan. For cancers to develop, a cancer-permissive environment must be present, which may exist months or even years before a tumor becomes detectable. We have developed the Shine On Suspicion (SOS) test, a flow cytometry based test to detect these changes that lead to a cancer-permissive environment. To make this test actionable, we combined it with a novel agent for cancer interception (secondary prevention) called eBAT. eBAT is a bispecific ligand targeted toxin consisting of truncated, deimmunized *Pseudomonas* exotoxin fused to epidermal growth factor (EGF) and the amino terminal fragment of urokinase. This drug was designed to use epidermal growth factor receptors (EGFR) and urokinase-type plasminogen receptors (uPAR) as baits to deliver the lethal toxin into the cells, rather than disrupting receptor signaling. eBAT has self-limiting, well-tolerated side effects and is safe enough for administration to otherwise healthy individuals. To date, eBAT has been administered to 11 healthy dogs that were determined to have elevated cancer risk based on the SOS test, with only 1 of the 11 dogs developing cancer more than 2 years after treatment. A hazard ratio calculated with a 95% confidence interval shows that dogs receiving eBAT interception had nearly 4 times reduction in risk of a cancer diagnosis compared to controls. Ongoing research aims to explore the identity and function of niche-forming cells to improve cancer prevention strategies and their applicability to humans.

#1042 MAGE-A3 and PD-L1 protein co-expression in lung cancer a look at the immune cells' tumor microenvironment.

Aimee Icaza Galarza[1], Rachel M. Gonzalez[1], YiChen Guo[1], Dehe Kong[1], Bailey Gilmore[1], Tianli Qu[1], Alex Strom[1], Zhaoying Guo[1], Qi Ren[2], Xiaomin Hu[2], Ranran Zhang[2], Zhaohui Wu[2], Jessi Chen[1], Xuan Liu[1], Wei Fu[1]

[1]Immunology, OriGene Technologies, Inc., Rockville, MD,[2]Immunology, OriGene Wuxi Biotechnology Co., Wuxi, China

This lab's previous studies highlight the co-expression of PD-L1 (CD274) and MAGE-A3/A4 in non-small cell lung cancer (NSCLC) and squamous cell lung cancer, with around 50% of the screened tumors exhibiting this co-expression. Additionally, this study also identified MAGE-A3/A4 as a secreted protein using CytoSections. A sandwich ELISA was developed for MAGE-A3/A4 and demonstrated that serum levels of MAGE-A3 protein were significantly elevated in lung cancer patients compared to matched controls. This statistically significant difference suggests that MAGE-A3 could serve as a valuable biomarker for lung cancer diagnosis and prognosis. Such findings may also provide insights into the tumor microenvironment and the immune evasion mechanisms facilitated by PD-L1 and MAGE-A3 co-expression, potentially guiding the development of targeted therapies or combination strategies in the treatment of lung cancer. In this study, the lung cancers were screened with CD1C, CD163, CD20, CD31, CD3E, CD4, CD68, CD8A, FOXP3 immune markers previously evaluated with PD-L1 to characterize the tumor micro-environment where PDL1 and MAGE-A3 protein are co-expressed versus when only MAGE-A3 is expressed in lung cancer. MAGE-A3 was detected on positive CD3e and suggesting a role in regulating immune response. This study offers insights into the tumor microenvironment, a new view of immune escape in lung cancer, and the potential of MAGE-A3 as a diagnostic/prognostic biomarker and therapeutic target, particularly in combination with immune checkpoint inhibitors.

#1043 Efficacy and mechanistic insights of Shengyang Qushi Decoction in managing surufatinib-associated diarrhea.

Huangying Tan, Shaobo Hu

Integrative Oncology, China-Japan Friendship Hospital, Beijing, China

Background: Surufatinib, a novel TKI targeting VEGFRs, FGFR1, and CSF1R, treats advanced neuroendocrine tumors but often causes diarrhea, which impairs patients' health and anti-tumor efficacy. We've successfully established a rat model of surufatinib-induced diarrhea. This study aims to investigate Shengyang Qushi Decoction's mechanism in treating this diarrhea, focusing on intestinal mucosal barrier and inflammation, for clinical application.

Methods: Sixty male rats were randomly assigned to five groups after a 3-day acclimation period: control, model, low-dose decoction (12g/kg), high-dose decoction (24g/kg), and positive control (Loperamide 0.36mg/kg) group. Except for the control group, all received oral administration of surufatinib at a dose of 110 mg/kg, followed by corresponding treatment after 12 hours. We monitored body weight, general condition and assessed diarrhea. Histological changes in the colon were evaluated. ELISA measured plasma diamine oxidase (DAO) and D-lactate levels, as well as serum inflammatory cytokines (TNF-α, IL-1β, IL-6). Western blotting assessed the expression of tight junction proteins, inflammatory cytokines (TNF-α, IL-1β), and key proteins in the NF-κB pathway (IKK-α, IκB-α, NF-κB p65) in colon tissue.

Results: Rats in the high-dose decoction group had slower weight gain than the control but more than other groups, with improved physical appearance and activity levels, and food and water intake similar to the control. Diarrhea rates were 50%, 17%, and 42% in the low-dose decoction, high-dose decoction, and positive control groups, respectively. Hematoxylin-eosin staining revealed intact colonic structure in the control group with orderly mucosal epithelia and no abnormalities. In the high-dose decoction group showed intact colon mucosa with minimal inflammation and no congestion or edema. DAO levels were not significantly different among groups, but D-lactate levels were significantly higher in the model group ($P < 0.01$) ,and decreased after intervention, most notably in the high-dose decoction group. After drug intervention, the colonic tight junction proteins increased, most notably in the high-dose decoction group ($P < 0.01$ vs. model). Serum TNF-α, IL-1β, and IL-6 levels declined, with the high-dose decoction group showing the greatest reduction ($P < 0.01$). And the colon's TNF-α and IL-1β proteins followed the same trend ($P < 0.01$). Western blotting showed reduced expression of phosphorylated NF-κB pathway proteins (p-IKK-α, p-IκB-α, p-p65) after intervention, with the high-dose decoction group showing the greatest decrease ($P < 0.01$).

Conclusions: Animal experiments confirm Shengyang Qushi Decoction's efficacy in treating surufatinib-induced diarrhea. It mitigates colonic tight junction damage, reduces mucosal permeability, inhibits NF-κB signaling pathway activation, and alleviates colonic inflammation.

#1044 Patient acceptability of human papillomavirus (HPV) self-sampling for cervical cancer screening to reach under-screened women who present to the emergency department.

Sara Heinert[1], Ronald Ikechi[2], Eileen Hsieh[3], Neha Maddali[3], Rufine Kendra Soh[3], Yugledys Hoyos[4], Giana Oelkers[4], Camille Grullon[4], Maya Iglesias[3], Pamela Ohman-Strickland[5], Jonathan McCoy[1], Christine Ramdin[2], Racquel Kelly Kohler[6]

[1]Department of Emergency Medicine, Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ,[2]Department of Emergency Medicine, Rutgers New Jersey Medical School, Newark, NJ,[3]Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ,[4]Rutgers University, New Brunswick, NJ,[5]Department of Biostatistics and Epidemiology, Rutgers School of Public Health, Piscataway, NJ,[6]Rutgers Cancer Institute, New Brunswick, NJ

Despite advancements in cervical cancer screening, treatment, and preventive measures, marked disparities exist among vulnerable populations. While several factors contribute to disparities, over half of cervical cancer cases are diagnosed in women who rarely or never screen. Emergency departments (EDs) tend to serve in a safety net capacity for underserved patients and thus can be an effective site to reach patients overdue for cancer screening. HPV self-sampling, where women use a swab to collect their own cervicovaginal sample, has similar accuracy to clinician-collected samples, has recently been FDA approved, and addresses barriers to screening. However, the optimal kit distribution strategy remains unknown. ED-based in-person distribution with same-day return of self-sampling kits has the potential to alleviate issues of prior trials mailing kits to women's homes. As a first step to implementing such a program, we surveyed women eligible for cervical cancer screening in two large EDs who presented with non-critical conditions to determine patient acceptability and preferences for HPV self-sampling in the ED. We conducted 118 surveys. Average participant age was 44 years (SD=10), 9% were Asian, 31% were Black, and 35% were Hispanic. Over half (55%) of participants had private insurance, 23% had Medicaid, 18% had no insurance; 28% had no primary care doctor. Fourteen percent of participants were overdue for cervical cancer screening (last Pap was >3 years ago or their Pap/HPV co-test was >5 years ago). Most participants (88% overall, 87% of overdue women) said they were willing to use a HPV self-sampling kit if it was offered acutely in the ED, and 89% overall and 87% of overdue women were confident in their ability to collect their sample correctly. The most prevalent concerns of using the kit were: that the test was not done right (50%) and not knowing how to perform the test (31%). Most women (71% overall, 80% overdue) said they would feel comfortable using the self-sampling kit in an ED bathroom. Of all women not comfortable, the most common reason was that it was a public place/no privacy (27%). When given the choice, 36% of all women would prefer to self-sample in the ED bathroom, 34% would prefer to take the kit home to complete and mail it back, and 30% would prefer collection by a physician or nurse. Preference for collection in an ED bathroom was higher (47%) for overdue women. Our survey showed strong patient acceptability of HPV self-sampling in the ED. Our next step is to conduct a pilot trial implementing HPV self-sampling kit distribution in the ED and navigation to follow-up care to improve cervical cancer screening access. Participants' most prevalent concern of not performing the test correctly suggests that education and instructions for self-sampling prior to use will be an important component to successful roll-out.

BIOINFORMATICS / COMPUTATIONAL BIOLOGY / SYSTEMS BIOLOGY / CONVERGENT SCIENCE: Bioinformatics, Computational Biology, and Systems Biology for Healthcare Equity
Poster Session

#1047 Addressing data disparity in pre-clinical research using a generative approach.

Oleksandr Narykov, Alexander Partin, Yitan Zhu, Priyanka Vasanthakumari, Thomas Brettin, Varuni Sastry, Rick L. Stevens

Argonne National Laboratory, Lemont, IL

Anti-cancer drug response prediction is of paramount importance to drug development and patient treatment design. However, publicly available drug screening datasets on cell lines and patient-derived organoids or xenografts contain highly unbalanced information with most experiments corresponding to non-responsive drug and tumor combinations. While previous research explored augmenting tumor gene expression for cancer type classification, we target a more challenging problem - a guided generation of the responder genetic makeup for specified drugs and sample populations. To tackle this task, we employ a combination of cutting-edge artificial intelligence techniques - consistency models (CM), biology-informed dynamic neural networks (NN), contrastive learning (CL), and transformers. Recent work from OpenAI has addressed the convergence and speed limitation of generative adversarial networks (GAN) by introducing CMs, a novel class of generative models. This development provides a framework for generating synthetic cell line data under complex, biologically relevant conditions for subsequent analysis and optimization. We have modified the CM architecture for the efficient, one-shot generation of RNA-Seq samples. However, the raw outputs of the model mimic the original data distribution and produce unbalanced responder and non-responder data. We employ CL between RNA-seq samples and compounds to guide the model to a desirable outcome. This component integrates hierarchical biological knowledge from the KEGG database with convolutional filters to achieve parameter efficiency and transformer-based encoding of drugs to match responders' genetic makeup with the corresponding drug to which it is susceptible. Unlike traditional fixed architectures, RNA-Seq encoder adaptively modifies its topology based on biological relationships in the data and leverages BRITE hierarchical relationships to guide the formation of neural connections in a biologically-informed manner. The proposed method allows for the generation of responder data that can be conditioned on both selected drugs and RNA-Seq samples to produce data with specific traits of demographics of interest. We apply popular clustering algorithms on a combination of real-world data and synthetic samples generated by our model. Multiple metrics, including adjusted random score, adjusted mutual information, and homogeneity score, are used for examining the mixing of real-world and synthetic data. The results demonstrate that ground truth and augmented data are nearly indistinguishable.

#1048 Unraveling cancer disparities in African Americans: Socioeconomic, healthcare, and environmental contributions in UChicago and Baylor catchment areas.

Ahmed Fadiel, Fadiel A.1, Fadel S. 1, Oluyomi A. 2, Rosales O. 2, Eichenbaum K.3, Abbasi M., Elbialy A., Odunsi K1, Saleh Fadel, Kunle Odunsi

University of Chicago, Chicago, IL

Background: Cancer incidence rates are disproportionately high among African Americans for several cancers, including breast and prostate cancer. Reasons for these disparities may take root from a complex set of social, economic, and environmental factors. This study examines disparities in cancer incidence among African Americans between the University of Chicago and Baylor College of Medicine catchment areas, focusing on identifying modifiable factors.
Methods: Data were obtained from SEER, CDC WONDER, ACS, and Policy Map. We used our intelligent Catchment Analysis Tool, or iCAT, to examine the incidence of cancers with demographic, socioeconomic, and healthcare utilization variables, environmental factors, pollution, and water quality. iCAT helped precisely delineate catchment areas to analyze referral patterns of hospitals and population density. Statistical analysis using multivariate logistic regression and spatial analysis enabled us to identify clusters of disparities. Data visualizations were developed to illustrate relationships, including geographic heatmaps and scatter plots.
Results: Our findings demonstrated that cancer incidence rates were dependent on socioeconomic and environmental inequalities, with distinct differences in patterns at U. Chicago and Baylor catchment areas. Communities characterized by higher poverty levels, lower educational attainment, and limited access to healthcare showed increased cancer incidence rates. Environmental factors, especially air quality, further accentuated these disparities.
Conclusions: The findings underscore the urgent need for multilevel interventions to target socioeconomic inequities, improve healthcare access, and reduce environmental risks to decrease the cancer burden among African Americans. This study provides a basis for working toward tailored public health strategies in the U. Chicago and Baylor catchment areas, contributing to health equity and improved cancer outcomes.

#1049 Advancing health equity in oncology through AI, GIS, and data-driven tools.

Ahmed Fadiel, Fadiel A.1, Fadel S. 1, Butt W. 1, Eichenbaum, KD.2 Abbasi M. 1, Elbialy A. 1, and Odunsi K. 1, Kunle Odunsi, Saleh Fadel

University of Chicago, Chicago, IL

Cancer outcomes are influenced by geographic, socioeconomic, and environmental factors, especially in rural and underserved urban areas. Such regions often lack appropriate screening and specialized care, leading to late-stage diagnoses and poor overall outcomes. Key socioeconomic determinants influencing disparities in cancer care include income, education, environmental conditions, insurance status, and geographic proximity to qualified healthcare providers. To address these inequities, sophisticated tools are needed to identify high-risk areas and to improve resource allocation. To address this need, we developed the Intelligent Cancer Catchment Area Tool (iCAT), a GIS-enabled platform enhanced by artificial intelligence (AI). iCAT integrates public health sources and health systems data to generate a comprehensive, geographically diverse analysis of cancer disparities. On this platform, mapping capabilities are analyzed with advanced statistics and machine learning algorithms to derive actionable insights into cancer social and environmental drivers of gaps in healthcare. Two recent module additions to iCAT are 1. integration of authoritative datasets, including SEER, CDC, and other trusted sources, and 2. advanced preprocessing, in which LLMs are used to clean data and handle confounders. We have improved the analytics package to enable univariate and multivariate analyses to find more complex relationships among the variables. We are adding AI-assisted literature matching to help formulate and suggest evidence-based research questions. Future directions for iCAT include quantum-enhanced predictive modeling, real-time analytics with biometric data from wearable devices, and hyper-localized risk assessment using augmented reality visualizations. We also plan to incorporate blockchain for data security and AI-driven optimization algorithms that guarantee privacy while enhancing intervention planning. We are developing iCAT as a tool for healthcare providers, policymakers, and researchers to make evidence-based decisions. With further development, we anticipate use cases ranging from deploying mobile screening units to culturally adapted educational programs and forming policy reforms. This new approach is a key milestone toward eliminating health disparities and redefinition of equitable cancer care. "

Keywords: Health Equity, Oncology, AI, GIS, and Decision Making

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#105 Netrin-1 activates Yap1 to promote pancreatic cancer metastasis and mutant Kras-independence.

Chris R. Harris, Muhammad Sadeqi Nezhad, Shubhrika Jain, Wade Narrow, Darren R. Carpizo

University of Rochester Medical Center, Rochester, NY

Although pancreatic ductal adenocarcinoma (PDAC) is only the 10th most common cancer, it is the 3rd leading cause of cancer deaths due to its high incidence of metastasis. In patient samples, PDAC metastasis associates with expression of the secreted protein Netrin-1, and indeed metastasis of pancreatic adenocarcinomas in mouse models is sharply reduced upon genetic or pharmacological ablation of Ntn1 signaling. Upon binding to its Unc5b receptor, Ntn1 activates the survival factor Yap1, which then promotes resistance to anoikis, which is a key trait of metastatic tumors. But metastatic PDACs are not the only expressers of Ntn1: indeed, primary pancreatic tumors can also derepress Ntn1 if selected to grow in the absence of mutant Kras. These $Kras^{mut}$-independent tumors become dependent upon activation of Yap1 by Ntn1/Unc5b signaling, and suggest that patients with pancreatic cancer may benefit from combined pharmacological targeting of mutant Kras and Ntn1.

BIOINFORMATICS / COMPUTATIONAL BIOLOGY / SYSTEMS BIOLOGY / CONVERGENT SCIENCE: Bioinformatics, Computational Biology, and Systems Biology for Healthcare Equity
Poster Session

#1050 Geographic disparities in the incidence of breast and prostate cancers among African Americans: A comparative analysis between the UChicago and Baylor catchment areas.

Ahmed Fadiel, Fadiel A.1, Fadel S. 1, Oluyomi A. 2, Rosales O. 2, Eichenbaum K.D. 3, Abbasi M. 1, Elbialy A. 1, an, kunle Odunsi, Saleh Fadel

University of Chicago, Chicago, IL

Background: Cancer disparities continue to be a significant challenge for public health across the nation, especially among African Americans. Causes include socioeconomic factors, environmental conditions, and limited healthcare access. We are exploring data-driven studies of geographic disparities in the incidence rates for breast and prostate cancers across African American populations in catchment areas around the University of Chicago and Baylor College of Medicine.
Methods: Catchment areas were identified using our intelligent Catchment Analysis Tool, or iCAT, to map physical boundaries, referral patterns, distances, and population densities. Data were extracted from the SEER Program, CDC WONDER, and the American Community Survey (ACS). The variables analyzed included cancer incidence rates, demographic and socioeconomic factors, healthcare access and utilization, and environmental exposures, including air pollution and water quality. Chi-square tests, t-tests, logistic regression, and multivariate analysis were also performed to identify key drivers of disparities. The results were displayed on geographic maps, charts, and graphs, highlighting spatial clusters and relationships between cancer incidence and contributing factors.
Results: Our preliminary findings indicate large disparities in cancer incidence rates between the two catchment areas. We investigate associations between socioeconomic inequality, healthcare access, and environmental exposures. African Americans living in areas of the lowest socioeconomic status and decreased healthcare access demonstrated higher incidence rates.
Conclusions: This study can catalyze a focus on systemic inequity in socioeconomic conditions, healthcare accessibility, and environmental quality that influence cancer disparities among African Americans. These findings suggest actionable insights toward targeted interventions and policymaking within the UChicago and Baylor catchment areas.

#1051 Predicting stroke as a cause of death in Black cancer patients using ensemble machine learning models.

Raghad Wesamah Alrawashdeh[1], Nour Wesamah Alrawashdeh[2]

[1]Yarmouk University, Irbid, Jordan,[2]Jordan University of Science and Technology, Irbid, Jordan

Objective: No studies have sought to identify the risk factors associated with cerebrovascular diseases (CVD) as a cause of death in black cancer patients. This study aimed to use advanced machine learning techniques to predict CVD mortality in Black cancer patients and to identify significant risk factors.
Methods: We utilized The Surveillance, Epidemiology, and End Results 17 registry to predict CVD as a cause of death in Black cancer patients from January 1, 2000, to December 31, 2020. Various machine learning models were employed, including Random Forest, Logistic Regression, XGBoost, LightGBM, and Gradient Boosting. The dataset included features such as age, sex, cancer type, year of diagnosis, and treatment modalities including adjuvant chemotherapy, adjuvant radiotherapy, adjuvant chemoradiotherapy, surgery alone, primary chemotherapy, primary radiotherapy, primary chemoradiotherapy, and no treatment. Data preprocessing involved cleaning, feature selection, and 80-20 stratified train-test splitting. The models were combined into a voting ensemble to leverage their strengths. The performance of each model was evaluated using metrics including accuracy, AUC (macro, micro, weighted), precision, recall, and F1 scores. Feature importance was analyzed to identify the most contributing variables in predicting CVD.
Results: A total of 292,680 black cancer patients were included, with a mean age of 57.36 ± 13.68 years. The cohort comprised 152,991 males (52.27%) and 139,689 females (47.73%). Among them, 5,874 patients (2.01%) died from cerebrovascular diseases, while 286,806 patients are alive. The voting ensemble model achieved an accuracy of 0.98, with an AUC macro of 0.88, AUC micro of 0.99, and AUC weighted of 0.88. The average precision scores were 0.64 (macro), 0.99 (micro), and 0.98 (weighted). The F1 scores were 0.64 (macro), 0.98 (micro), and 0.97 (weighted). The precision scores were 0.78 (macro), 0.98 (micro), and 0.97 (weighted). Significant predictors of CVD included an earlier year of diagnosis (with patients diagnosed earlier in the study period from 2000 to 2020 having a higher risk of CVD), older age, lack of any cancer treatment, and specific cancer types (notably gastrointestinal and male genital cancers).
Conclusions: Our findings suggest that the voting ensemble machine learning model can effectively predict stroke mortality in black cancer patients, with high accuracy and robust performance metrics.

#1052 Integrating geographical and socioeconomic data with genomic information for enhanced prediction of cancer risk using large language models and knowledge graphs.

Ofek Mendel[1], **Michael S. Sinclair**[2], Kathleen M. Jagodnik[2], Till Krenz[2], Erin Kobetz[2], Beronica A. Ocasio[2], Jeronimo Pissinis[2], Vasileios Stathias[2], Alon Bartal[1], Stephan Schurer[2]

[1]The School of Business Administration, Bar-Ilan University, Ramat Gan, Israel,[2]Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL

Cancer remains a leading cause of mortality worldwide, with risk and survival outcomes influenced by a complex interplay of genetic, environmental, and socioeconomic factors. Traditional cancer risk prediction models often focus on genetic, lifestyle, and family history factors but frequently overlook the critical roles of geographical location and social determinants of health (SDoH). This study introduces a novel approach that integrates diverse multimodal datasets—including environmental exposures, healthcare access, socioeconomic conditions, and genomic data—within a geographic framework to develop more comprehensive and spatially contextualized cancer risk prediction and survival models. First, we developed a data model to integrate the various data modalities. We leverage our internal Sylvester Data Portal Informatics Platform to manage and map the various data types and metadata. Using the GPT-4o Large Language Model, we identified entities and their relationships from semi-structured and unstructured data sources, and used them to construct a multimodal knowledge graph (KG). Our KG comprises 700,000+ nodes spanning 32 entity types (e.g., genes, 5 cancer types) and 2.134 million edges/links across 107 relationship types (e.g., has_genotype, from_race, from_sex). Graph Neural Networks (GNNs) were then trained on this KG to predict cancer risk and survival outcomes based on demographic factors, location, and tumor genetics. Querying our KG using Graph Retrieval Augmented Generation (GraphRAG) and predicting links using our trained GNN, we uncovered novel insights into the relationships among genetics, demographics, environmental exposures, and cancer statistics, highlighting potential complex gene-environment interactions that contribute to cancer risk and survival disparities, and suggesting directions for future research to reduce cancer risk and improve health outcomes.

#1053 Cellular heterogeneity and stress response in lung tumors: understanding cancer disparities through spatial transcriptomics.

Sabrina Akter[1], Aiman Soliman[2], Zeynep Madak-Erdogan[1], Sage J. Kim[3]

[1]Food Science & Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, IL,[2]National Center for Supercomputing Applications, University of Illinois at Urbana-Champaign, Urbana, IL,[3]School of Public Health, University of Illinois Chicago, Chicago, IL

Background: Despite having lower smoking rates, Black men have a higher incidence of lung cancer. Previously, we showed that social stressors, such as neighborhood violent crime, may influence tumor biology through stress response pathways. In this study, we investigated the relationship between neighborhood violent crime rates and glucocorticoid biosynthesis in lung tumors. Specifically, we examined how cell populations change in tumor regions with high glucocorticoid metabolism and receptor activity.
Methods: We analyzed spatial transcriptomic data from 15 lung tumor samples. Pathway activity and cell type associations were examined using gene set co-regulation analysis (GESECA). We computed univariate and bivariate local Moran's I to assess spatial co-localization at $p < 0.05$. To validate these spatial associations, we performed unsupervised cell-type deconvolution using STdeconvolve and applied spatial interpolation to enhance the resolution of cell-type distributions.
Results: We identified a positive correlation between neighborhood violent crime rates and glucocorticoid biosynthesis pathway activity ($p < 0.05$). Several epithelial cell types, including adventitial fibroblasts, basal cells, club cells, and mesothelial cells, were significantly associated with glucocorticoid biosynthesis-enriched regions in lung tumor samples from high-crime neighborhoods. Cell type deconvolution and spatial interpolation confirmed localized clustering of these epithelial cells within glucocorticoid biosynthesis-active regions at high resolution.
Conclusions: Our findings suggest that social stressors, such as neighborhood violent crime, may contribute to lung cancer disparities by influencing tumor development through stress-induced pathways. This study highlights the importance of understanding cellular heterogeneity within the tumor microenvironment, which could inform targeted cancer prevention and control interventions.

#1054 A combined genome-wide-association and admixture analysis of prostate cancer risk in men of African ancestry in the *All of Us* Research Program.

William Letsou, David Mushiyev, Daniel Galvin Gusmano

New York Institute of Technology, Old Westbury, NY

Men of African ancestry are known to suffer increased rates of prostate cancer incidence and mortality. While many studies have identified common variants that are specific to, or at elevated frequency in African populations, the genetic basis of this healthcare disparity remains to be fully worked out. Admixture analysis is a computational tool for identifying local genetic ancestry across individual genomes, and is potentially useful for detecting shared, disease-associated haplotypes in populations, such as African Americans, who derive from multiple ancestry groups. Our hypothesis was that genome-wide admixture mapping could be used as a proxy for a genome-wide linkage scan in *unrelated* individuals, so that a direct comparison of the loci implicated by genome-wide association studies (GWAS) and linkage analysis could be made. To this end, we conducted a case-control GWAS, and a cases-only admixture mapping in 8,957 men at least fifty years-old of African ancestry (578 cases, 8,379 controls) in the *All of Us* Research Program. In total, we tested 5,856,945 common polymorphisms and indels (MAF ≥ 0.05), and found a genome-wide significant variant rs73705708 ($P = 8.8 \times 10^{-13}$) at the previously implicated 8q24 locus. We then conducted admixture analysis using RFMix v. 2.0, with phased input being supplied by Beagle v. 5.4, and detected a statistically significant increase in local European ancestry ($Z < -6$) in the 14q23 region near the *GPHN* gene, as well as a statistically significant increase in local African ancestry ($Z > 4$) near the HLA region at 6p22; neither region was implicated by the GWAS. And although we observed a nominally significant peak ($Z > 3$) as expected in the 8q24 region, we overall found little correlation ($R^2 = 0.0003$) between GWAS *P*-value and admixture Z-score in our cohort. These results support the hypothesis that regions of the genome inherited from a common ancestral population represent a distinct set of risk loci to those identified by GWAS, and suggest that the genetic basis of prostate cancer risk and disparities may have additional unidentified contributions. The authors of this study gratefully acknowledge *All of Us* participants for their contributions, without whom this research would not have been possible, and the National Institutes of Health's *All of Us* Research Program, for making available the participant data examined in this study. All data used in this study came from *All of Us* Research Program's Controlled Tier Dataset v. 7, available to authorized users on the Researcher Workbench.

#1055 Inclusion of large genomics databases in disease modeling improves outcomes across ancestral populations.

Leslie A. Smith[1], Franco Krepel[1], James A. Cahill[2], Ji-Hyun Lee[3], Kiley Graim[1]

[1]Department of Computer & Information Science & Engineering, University of Florida, Gainesville, FL,[2]Environmental Engineering Sciences Department, University of Florida, Gainesville, FL,[3]Department of Biostatistics, University of Florida, Gainesville, FL

Rapid advancements in genomic sequencing technologies have revolutionized the study and treatment of human diseases. Even with the wealth of genomic information available, successful application of developed treatments is often limited to the human populations represented in the original study, suggesting that these studies are overfitting to their data and thus limiting scientific understanding of complex traits and diseases. Human populations have developed population-specific variants in response to evolutionary changes as a result of human migrations and other stressors which can impact disease occurrence and outcome. Lack of sufficient sampling for equal representation of this variation in disease studies has led to disparate outcomes and treatments. While obtaining a representative study population for a disease is often unrealistic, equitable machine learning approaches can bridge the gap. Here, we demonstrate an equitable machine learning approach, PhyloFrame, showing that the inclusion of publicly available genomic resources in disease modeling methods can force machine learning and artificial intelligence models to be more robust to data distribution shifts caused by unequal sampling of human populations, thereby increasing model generalizability. We demonstrate PhyloFrame's utility across several diseases at high risk of ancestral bias, which we identified via an analysis of heritable disease signatures. We first perform statistical enrichment of ancestry-specific alleles within existing disease signatures for Mendelian disorders by calculating variant frequency enrichment for each of the populations in gnomADv4.1 and the 1000 Genomes Project. After identifying the diseases at highest risk for ancestral bias, we apply our PhyloFrame method to RNA-seq datasets from those diseases and contrast PhyloFrame's performance in each ancestry group to a comparable benchmark model that does not incorporate equitable machine learning techniques. Our results demonstrate that incorporating publicly available genomic data into disease modeling methods improves outcomes for all. Growing resources for publicly available large-scale genomic databases provide unprecedented opportunities to understand disease. Resulting analysis of variation across human populations is a powerful approach to supplement disease models. Here we demonstrate how to incorporate this information into disease modeling, and show it is a unique and feasible approach to advancing equity in precision medicine.

#1056 Exploring the impact of ancestry on metabolic dysregulation in lung adenocarcinoma: Implications for racial health inequities and targeted therapeutics.

Benecia A. Jackson, Yuxin Jin, Yonatan Amzaleg, Angel Perez Hunt, Nikita Jinna, Dan Raz, Loretta Erhunmwunsee, John Carpten, Aaron Neely

City of Hope National Medical Center, Duarte, CA

Introduction. The occurrence of lung adenocarcinoma (LUAD) is approximately 68.3 per capita for Black men and 61.5 per capita for White men, reflecting an 11% higher rate in the former. Black patients experience a poorer prognosis and significantly higher mortality rates compared to their White counterparts. *Purpose.* Understanding the genetic and molecular foundations that underly racial health inequities is essential for developing comprehensive interventions and advancing more precise therapeutic modalities for diverse patient populations. Single Nucleotide Polymorphisms (SNPs) can influence oncogenic transformation and may be associated with distinct molecular landscapes in tumors and adjacent TME. Furthermore, ancestry may play a pivotal role in shaping individual susceptibility and risk for mitochondrial dysfunction and ensuing oncogenic transformation.
Methods. We employed germline VCFs for ancestry analysis of 36 LUAD patients (18 Black and 18 White) from the ORIEN data set, while genotype data from the 1000 Genomes Project phase 3, grouped by super population served as reference. Oncoplots were generated using a combination of patients' somatic VCF and metadata from ORIEN. Regression analysis adjusting for age, staging, and gender was performed to determine association between ancestry and gene mutations. Global admixture was estimated using STRUCTURE v2.3.4 with k=5, NUMREPS=2000, and BURNIN=50000. VCFs were subset and merged by 1126 ancestry-informative markers (AIMs) and converted to STRUCTURE format with PLINK. PCA was conducted using R. Local ancestry analysis was performed with LAMP-LD v1.3 using a window length of 50 SNPs and 20 states, based on 20,803 SNPs. Results were visualized with ggplot2 in R.
Results. In Black patients, admixture analysis showed predominant AFR genetic similarity with varying proportions of EUR, AMR and SAS similarity. Local ancestry analysis using LAMPLD reveals admixture across different regions of the genome illustrating the variation in ancestral contributions within and between individuals of the same cohort and compared to counterparts in other populations. Mutations in ACADVL, HK2, SREBF1, COX6L, BID, POLG2, SLC2A2, SLC22A5, and ACKR1 are associated with AFR status >/= 0.5. Mutations in ACADSB, HK3, PPARGC1A are associated with AFR status < 0.5.
Conclusions. Mutations in metabolic and mitochondrial genes emerged as being highly enriched in LUAD patients with high relative to low African genetic ancestry, suggesting opportunities for improved risk-prediction and therapeutic targeting in this subpopulation.

#1057 Addressing challenges in integrating dietary factors into machine learning models for gastrointestinal cancer prediction in South Korean cohort.

Daina Baublyte, Jeonghee Lee, Madhawa Gunathilake, **Jeongseon Kim**

National Cancer Center - Korea, Goyang-si, Korea, Republic of

Introduction: Dietary factors are integral to cancer etiology but are underrepresented in predictive models for gastrointestinal (GI) cancer risk. Challenges such as limited availability and variability of dietary intake data hinder the development of effective machine learning (ML) models. This study investigates these challenges and develops practical solutions to enhance the integration of dietary data into GI cancer risk prediction using a South Korean cohort.
Methods: We analyzed data from the Cancer Screenee Cohort, following 7,862 adults (158 GI cancer cases and 7,704 controls) over 16 years. Dietary data were collected using a validated food frequency questionnaire (FFQ), supplemented by clinical biomarkers and anthropometric measurements. ML models—logistic regression, random forests, gradient boosting machines, and support vector machines—were trained and evaluated using precision and F1 scores, focusing on predicting the minority class (GI cancer cases). A tailored data sampling and model training strategy was developed to address class imbalance and improve predictive performance.
Results: Initial ML models faced performance challenges due to class imbalance and limited sample size, achieving zero precision for the case class and strong performance only for control class (control precision = 0.98). The proposed sampling and model training strategy enhanced minority-class predictions achieving a case class prediction precision of 0.78—a 78% improvement—while maintaining an overall F1 score of 0.75, reflecting a strong balance between overall model precision and recall.
Conclusions: This study highlights the challenges of incorporating dietary factors into GI cancer prediction and underscores the importance of tailored approaches to address class imbalance in ML models. The proposed strategy substantially improved predictions for the minority class, offering practical insights for enhancing GI cancer screening and prevention through data-driven methods. Future work could explore incorporating additional dietary factors and testing the approach in larger populations to further validate its applicability.

#1058 The prediction of positive prostate biopsies in men is significantly enhanced when PVT1 Exon 4A is combined with serum Prostate Specific Antigen (PSA)..

Tinovimba L. Hove[1], Rachel E. Sexton[2], Michael Liss[3], Robin Leach[3], Emmanuel Asante-Asamani[1], Olorunseun O. Ogunwobi[2]

[1]Clarkson University, Potsdam, NY,[2]Michigan State University, East Lansing, MI,[3]University of Texas Health, San Antonio, TX

Prostate cancer (PCa) remains a significant public health challenge, ranking as the second leading cause of cancer-related deaths among men in the United States. Current diagnostic approaches, predominantly centered on prostate-specific antigen (PSA) testing, often lead to overdiagnosis and unnecessary biopsies. The long nonprotein coding gene, PVT1 (plasmacytoma variant translocation 1), has shown promise as a biomarker, being upregulated in PCa and associated with disease progression and metastasis. This study investigates the potential of incorporating PVT1 biomarkers to improve diagnostic accuracy for PCa detection, focusing on both the general population and men of African ancestry, who are disproportionately impacted by PCa incidence and mortality.

A total of 144 serum samples were analyzed, with 72 samples from men diagnosed with PCa and 72 from men without cancer. Serum levels of PVT1 Exons 4A, 4B, and 9 were quantified using digital PCR. Multiple logistic regression models were developed to predict cancer status based on PSA alone, PVT1 biomarkers alone, and combinations of PSA with PVT1 biomarkers. Model performance was assessed using stratified k-fold cross-validation, with comparisons made using likelihood ratio tests, and z-tests for statistical significance.

The results show that combining PSA with PVT1 Exon 4A improves specificity in predicting cancer status in the general population from 0.45 to 0.54 while maintaining sensitivity at 0.81. Within the subpopulation of men of African Ancestry, PVT1 Exon 4A alone achieves an accuracy of 0.75 with a sensitivity of 1.0 and specificity of 0.5 compared to PSA alone which had an accuracy of 0.38, sensitivity of 0.5 and specificity of 0.25.

This study underscores the potential clinical utility of integrating PVT1 biomarkers into prostate cancer screening programs. The inclusion of Exon 4A enhanced PSA-based prediction by addressing its limitations in overdiagnosis and unnecessary biopsies, providing a balanced improvement in diagnostic performance. By improving the accuracy of PCa detection while reducing the burden of overdiagnosis, this approach provides significant value for healthcare providers, diagnostic laboratories, and insurers aiming to enhance early detection and equitable screening outcomes.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#106 Growth-associated protein 43 (GAP-43): the link to Ewing sarcoma bone metastasis.
Tyler Biermann[1], Nicholas Cohen[2], Yasmine Wazni[3], Stefano Vicini[4], Jason Tilan[2], Joanna Kitlinska[1]

[1]Department of Biochemistry and Molecular & Cellular Biology, Georgetown University Medical Center, Washington, DC,[2]Department of Human Science, Georgetown University School of Health, Washington, DC,[3]Department of Global Health, Georgetown University School of Health, Washington, DC,[4]Department of Pharmacology & Physiology, Georgetown University Medical Center, Washington, DC

Ewing Sarcoma (ES) is an aggressive pediatric tumor that arises in bone or soft tissues. ES bone metastases do not respond well to routine therapies and their frequency markedly increases upon disease recurrence, which results in a lower survival rate in patients with osseous metastases as compared to those with pulmonary dissemination. Our previous studies have determined that ES bone metastasis is initiated by hypoxia in the primary tumor site, which leads to the formation of polyploid tumor cells, the progeny of which, named HYP-4n cells, exhibit high levels of chromosomal instability. These cells metastasize almost exclusively to bone and are less sensitive to cytostatic agents. In this study, we demonstrate that HYP-4n cells exhibit an upregulation in several neuronal proteins, specifically growth-associated protein 43 (GAP-43), a membrane protein promoting axon growth in neurons and formation of microtubes connecting glioblastoma cells into invasive and therapy-resistant cellular network. Similar phenotype characterized by the presence of microtube-like cell-cell connections was also observed in ES HYP-4n cells. GAP-43 immunostaining in HYP-4n cells revealed a punctuated pattern along the protrusions that is similar to its localization in neurons. Western Blot analysis performed on HYP-4n cells revealed a membranous localization of GAP-43, which was not detectable in their normoxic diploid counterparts. The functional role of the GAP-43-positive protrusions in connecting HYP-4n cells was confirmed by the gap-junction permeable dye transfer between the cells and ion transfer detected by a patch clamp assay. DAPI staining also displayed nuclear fragments within the protrusions, suggesting the transfer of genetic material. The suppression of GAP-43 expression via siRNA was further shown to inhibit HYP-4n cell migration measured via transwell assay. In immunostained ES xenografts, GAP-43 expression was shown in HYP-4n primary and osseous metastatic tumors, with the highest membranous expression in the ES cells on the invading edge of the tumors. A similar pattern of expression of GAP-43 was noted in the human samples of ES bone metastases. This study indicates that GAP-43 is active in the membrane in HYP-4n cells and drives the formation of microtube networks promoting cell invasion. Further studies are required to prove directly the role of GAP-43 in ES bone metastasis and chemoresistance.

BIOINFORMATICS / COMPUTATIONAL BIOLOGY / SYSTEMS BIOLOGY / CONVERGENT SCIENCE: Database Resources Poster Session

#1061 Post-doctoral fellow.

kang-lin Hsieh, Sankar Maity, Ganiraju Manya, Bradley Broom, Christopher Logothetis, Patrick Pilie

The University of Texas MD Anderson Cancer Center, Houston, TX

Introduction: Prostate cancer research spans genetics, proteomics, metabolomics, cell signaling, and tumor microenvironment domains. While this data is valuable, integrating clinical and basic research findings presents challenges. The Prostate Cancer Cartography Initiative (PCCI) offers a unified data model for efficient research across clinical, co-clinical, and mechanistic studies. The data model divides cases into anatomic and biological groups, facilitating cross-domain knowledge translation and hypothesis generation through efficient data integration.
Materials & Methods: Our database incorporates data from 972 patients across three complementary sources: TCGA (primary prostate cancer), SU2C (metastatic patients), and MDA trials (intermediate-risk prostate cancer). For reproducibility and data consistency, we use NF core pipelines to harmonize data and derive copy loss, Single Nucleotide Variants/Insertions/Deletions (SNV & Indel), and gene expression. After analyzing this information, we group patients based on clinical information such as Gleason scores or genomic information such as copy loss and SNV/Indel to evaluate how these conditions affect gene expression and pathways.
Results: In our initial work, we focused on TCGA patients with Gleason scores, categorizing them into three groups (3+3, 3+4/4+3, and 8 or higher). We further subdivided each category based on genetic alterations: patients with no copy loss, patients with at least one copy loss, and patients with Single Nucleotide Variants or Insertions/Deletions (SNV & Indel). We analyzed key genes including TP53 across these subgroups. Our initial results showed that medium-risk patients (Gleason scores 3+4/4+3) had significantly lower TP53 gene expression when they had both copy loss and SNV & Indel. Patients with SNV & Indel alone also showed lower TP53 gene expression, though this finding was not statistically significant ($p = 0.06$). High-risk patients (Gleason scores 8 or higher) demonstrated similar patterns. When analyzing how copy loss or SNV & Indel affect genes near TP53, we found reduced expression in nearby genes—ALOX15B (logFC: -1.0153, $p<0.001$), TMEM220 (logFC: -0.719, $p<0.001$), and MYO1C (logFC: -0.348, $p<0.001$)—in patients with TP53 copy loss or SNV & Indel. This pattern was confirmed in both medium-risk and high-risk groups. In our pathway analysis, including AD-target and homologous recombination pathways, patients without TP53 copy loss or SNV & Indel showed higher Enrichment Scores.
Discussion: The PCCI provides a platform to integrate these data and generate hypotheses. Our results show that genetic alterations—such as copy loss and SNV & Indel—affect not only the TP53 but also nearby genes ALOX15B, TMEM220, and MYO1C. Notably, ALOX15B and MYO1C are tumor suppressor genes, which raises the question of how TP53 alteration leads to network changes in tumor suppressor genes. These findings further demonstrate the necessity of PCCI in facilitating hypothesis generation.

#1062 Cancer Complexity Knowledge Portal: a centralized web portal for finding cancer related data, software tools, and other resources.

Orion Banks, Ashley Clayton, Aditi Gopalan, Amber Nelson, Stockard Simon, Verena Chung, Amy Heiser, Jay Hodgson, Aditya Nath, Adam Hindman, Milen Nikolov, Adam Taylor, James Eddy, Susheel Varma, Jineta Banerjee

Sage Bionetworks, Seattle, WA

Applying artificial intelligence (AI) and machine learning (ML) techniques to biomedical problems is predicated on access to clean, high-quality data and reusable software tools. Data and tool repositories offer a vital infrastructure for centralizing, storing, and sharing such resources with the global research community of cross-disciplinary scientists. The Cancer Complexity Knowledge Portal (CCKP) is a NIH-listed domain-specific repository that makes oncology data findable and accessible. The CCKP is developed and maintained by the Multi-Consortia Coordinating (MC^2) Center, led by Sage Bionetworks. The MC^2 Center supports resource coordination among six cancer-focused interdisciplinary research consortia funded by the National Cancer Institute (NCI). Through this work, the CCKP has emerged as a significant repository for oncology data and tool sharing.

To define the metadata standards for sharing resources on the CCKP, we host data models for various data modalities including genomics and imaging, and are developing new models for emerging multimodal data types like spatial transcriptomics. The data models undergo iterative development based on emerging community needs, with versioned releases maintained in a public GitHub repository. These data models power established data management tools developed by Sage Bionetworks, including the Schematic Python package and Data Curator App, to support annotation of data according to FAIR standards. The data models are designed to help researchers link research outputs (e.g., publications, datasets), thus helping the CCKP synthesize and highlight the activities and outputs of the NCI-funded cancer research programs in a meaningful way. The CCKP provides search and filtering capability to accelerate discovery and collaboration in the cancer research community. As of November 2024, the portal hosts curated information on 3,786 publications, 904 datasets, and 292 computational tools emerging from over 140 research grants. Our data models also incorporate elements of the Cancer Research Data Commons (CRDC) Data Hub data model to support integration and reuse of datasets within the CRDC ecosystem.

The recent advances in computational oncology showcase the strength of secondary use of research data and highlight an unmet need to ensure that data and tools available through repositories are reliable, and reusable. We are engaging with scientists, clinicians, and patient advocates, and leveraging user-centered design approaches and structured data models to make cancer data and tools more findable, accessible, and reusable. These ongoing improvements of the portal aim to increase its usability, bringing experimental labs generating data and computational labs using data closer together, to fuel the iterative cycle of scientific discovery.

#1063 CellMap: a comprehensive human single cell gene expression reference for automated cell annotation and cancer cell-of-origin analysis.

Yuping Zhang, Gabriel Cruz, Hanbyul Cho, Chandan Kumar-Sinha, Rahul Mannan, Jin Chen, Xuhong Cao, Saravana M. Dhanasekaran, Arul M. Chinnaiyan

University of Michigan, Ann Arbor, MI

In single-cell transcriptomic analysis, accurate cell type annotation forms the essential basis for all downstream analysis and data interpretation. While time consuming manual annotation requires extensive prior knowledge, available automated cell annotation tools lack a unified, curated human cell reference to ensure successful identification of all cell types present in any given dataset. To fill in the gap we create “CellMap” a comprehensive single cell gene expression reference database of known cell types across most human tissues by compiling, curating and integrating single cell datasets from multiple sources including Human Protein Atlas, Tabula Sapiens, ArrayExpress and GEO. CellMap contains 151 different celltypes/states including 94 epithelial, 31 brain, 4 from soft tissues, 8 stromal and 15 major immune celltypes from 42 human tissues. Projecting pseudo-bulk profiles of the 151 celltypes into 2-dimensional UMAP revealed major clusters of cells from the nervous system, respiratory system, gastrointestinal tract, proximal digestive system, female tissues and endocrine tissues. Immune cells and stromal cells are generally clustered together regardless of their tissue origin. We also curated about 200 immune cell phenotypes from the ImmGen database for fine-tuning annotation of immune cell subpopulations. Importantly by combining CellMap and the SingleR algorithm, we successfully identified the cell lineage of the tumor and minor cell types originated from the biopsy site of a breast cancer metastatic sample; the latter were left unannotated with reference derived from breast tissue or misclassified using Human Primary Cell Atlas. In addition, CellMap allowed us to identify highly specific tissue markers and cell type markers. Based on 1241 identified marker genes, we trained a random forest model to predict cancer types using 80% of the TCGA tumor samples of each cancer type (n = 7160) and tested on a 20% hold-out testing set (n = 1774), achieving a median of 95.4% accuracy across cancer types. Cancer types with low accuracy include esophageal carcinoma (mostly mis-classified as head and neck squamous cell carcinoma) and rectum adenocarcinoma (mostly mis-classified as colon adenocarcinoma). The misclassification could be explained by the high similarity between these cancer types in terms of cell-of-origin.

Finally a webportal for exploring celltypes in CellMap and an open-source R-package for automated cell annotation of single cell data and cell lineage prediction for bulk RNA-seq data were developed. This work provides valuable resources for conducting single-cell analysis and cancer cell-of-origin analysis. Additionally, it helps identify closely related cell types across tissue types, which is particularly useful since diseases originating from related cell types may share similar therapeutic targets.

#1064 Cancer data aggregator: a new cancer data discovery tool.

Amanda Charbonneau[1], Arthur Brady[1], Tanner Coon[2], Rachel Kutner[3], Surya Saha[4], Katherine Thayer[3], Alexander Baumann[3], Todd Phil[5], Henry Schaefer[5], Heather Creasy[6], Jack DiGiovanna[4], David Pot[1], Bing-Xing Huo[3]

[1]General Dynamics Information Technology, Inc. (GDIT), Rockville, MD,[2]Blue Halo, Arlington, VA,[3]Broad Institute, Boston, MA,[4]Velsera, Boston, MA,[5]Frederick National Laboratory, Rockville, MD,[6]National Cancer Institute, Bethesda, MD

Cancer research data is generated and managed in silos. Data repositories frequently use different models and vocabularies to describe their data holdings, and many specialize in certain data types, necessitating that data from a large integrative project be spread across several repositories. For researchers who want to reuse existing cancer data, just navigating this ecosystem of repositories, formats and models can be overwhelming. The Cancer Data Aggregator (CDA) is a new free National Cancer Institute (NCI) service that aims to increase the discoverability of cancer datasets by making them searchable from a common interface, using a single model and vocabulary. We combine metadata for thousands of studies hosted at multiple data repositories across the NCI, and make that metadata available for search from a unified database, so researchers can more easily find and reuse existing cancer research data.

The CDA ensures that researchers can find what they're looking for using a single set of search terms by thoroughly cleaning, harmonizing, and cross-referencing the study metadata. This allows researchers to find subjects that have participated in multiple studies, discover data from a disease that was originally described in different ways at each repository, or compile all the data from a large consortium project like CPTAC - no matter how fragmented it ended up.

With the CDA, one set of search terms can return results from data repositories including the Genomic Data Commons, the Proteomic Data Commons, the Imaging Data Commons, Cancer Data Services, and the Integrated Canine Data Commons. Results are displayed in a simple table format (tsv) that is compatible with all standard spreadsheet apps (Excel, Google sheets, Numbers, etc) or loaded directly for analysis in a Jupyter notebook. Results include relevant clinical, phenotypic, genotypic values and provenance details, as well as information about where data files can be downloaded from using the GA4GH DRS API.

The Cancer Data Aggregator publishes a new release at the end of each month, and only publishes public data. This ensures that all researchers can use the CDA anonymously without any special privileges, and can always expect data to be up to date. Non-computational users can search the CDA using fill-in-the-blank style queries, while power users can create software for custom searches using our Python library. Visit https://cda.readthedocs.io/ to get started.

#1065 Exploring differential gene expression in head and neck squamous cell carcinoma using TCGA data.

Donghoon Shin[1], Ji Hyung Hong[2]

[1]MetroWest Medical Center, Framingham, MA,[2]Division of Medical Oncology, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea, Republic of

Introduction: The prognosis for head and neck squamous cell carcinoma (HNSCC) has shown limited advancements, and our understanding of how gene expression relates to patient outcomes remains unclear. With advancements in bioinformatics, the characterization of molecular abnormalities and the identification of prognostic signatures have become increasingly important for risk stratification, survival prediction, and treatment management of HNSCC patients. However, due to its lower prevalence, challenges remain in utilizing genetic markers to better understand prognosis. In this study, we performed a differential gene expression analysis (DEGs) to identify genes associated with overall survival.
Methods: RNA sequencing data from TCGA, including gene expression profiles and clinical information (tumor stage, overall survival), were analyzed to identify DEGs in HNSCC. DEGs associated with overall survival were identified using the R package DESeq2, and an integrated prognostic signature was developed. Kaplan-Meier survival analysis and log-rank tests were used to evaluate the prognostic value of DEGs, stratifying patients into high- and low-risk groups based on expression levels. A multivariate Cox regression analysis was performed to calculate the hazard ratios (HRs) for each gene, which were then used to construct a risk score.
Results: RNA expression data from 521 HNSCC patients were obtained from the TCGA database, comprising 111 early-stage (stage I, II) and 410 advanced-stage (stage III, IV) patients. DEG analysis comparing early-stage and advanced-stage tumors identified 51 genes, including 45 upregulated and 6 downregulated genes. Among these 51 genes, patients were stratified into high- and low-expression groups for each of the genes based on median expression levels. Kaplan-Meier survival analysis revealed that three genes—BRDT ($p = 0.0870$), ZFP42 ($p = 0.0213$), and FDCSP ($p = 0.0343$)—were significantly associated with overall survival. The HRs obtained from the multivariate Cox regression model are as follows: BRDT (HR = 1.51, 95% CI: 1.11-2.06, $p = 0.009$), ZFP42 (HR = 1.43, 95% CI: 1.05-1.94, $p = 0.022$), and FDCSP (HR = 0.72, 95% CI: 0.53-0.98, $p = 0.035$).
Conclusions: The study identified a novel 3-gene signature that can stratify HNSCC patients into distinct risk groups based on overall survival. Through DEG analysis, key genes linked to the prognosis of HNSCC were identified, providing valuable insights into the prognostic factors of the disease.

#1066 OncoKB^{TM}, MSK's precision oncology knowledge base: 2024 updates.

Moriah H. Nissan, Sarah P. Suehnholz, Hongxin Zhang, Ritika Kundra, Calvin Lu, Amanda Dhaneshwar, Nicole Fernandez, Kelly Cavender, Benjamin Preiser, John Konecny, Kseniya Petrova, Mark Ewalt, Maria E. Arcila, Marc Ladanyi, Michael F. Berger, Anoop Balakrishnan-Rema, Aijazuddin Syed, A. Rose Brannon, Ahmet Dogan, Diana Mandelker, Zsofia Stadler, Alexander Drilon, David B. Solit, Ross Levine, Nikolaus Schultz, Debyani Chakravarty

Memorial Sloan Kettering Cancer Center, New York, NY

OncoKB, Memorial Sloan Kettering Cancer Center's (MSK) precision oncology knowledge base, contains evidence-based information about the oncogenic effect and therapeutic implications of somatic alterations in cancer. OncoKB currently includes annotation for >8000 alterations in 900 cancer-associated genes and is the only somatic cancer variant database partially recognized by the US-FDA. OncoKB supports variant interpretation in the cBioPortal for Cancer Genomics, is used to annotate >17,000 MSK patient sequencing reports annually and is publicly available through its website (www.oncokb.org). Access to OncoKB, including its web-based API, is freely available to users in an academic setting, while users from commercial and hospital settings require a fee-based license. OncoKB's Therapeutic Levels of Evidence system classifies variants based on tumor type-specific sensitivity or resistance to targeted therapies. To date, OncoKB includes 53 Level 1 genes as well as MSI-H and TMB-H (included in the FDA drug label), 7 Level 2 genes (included in professional guidelines), 14 Level 3A genes (predictive of response in well-powered clinical studies), 10 Level 4 genes (predictive of response based on compelling biological evidence), and 12 R1/R2 resistance genes. In 2024, OncoKB updated its content to include notable precision oncology drug development changes. OncoKB promoted BRAF fusions to Level 1 following inclusion as patient eligibility criteria in the FDA drug label for tovorafenib (low-grade glioma). Additionally, OncoKB included KRAS G12C in colorectal cancer and IDH1 mutations in myelodysplastic syndromes as Level 1 following FDA approval of adagrasib + cetuximab and ivosidenib, respectively. NCCN guidelines for colorectal cancer and small bowel cancer listed immune-checkpoint inhibitors for tumors harboring POLE or POLD1 oncogenic exonuclease domain missense mutations, making them Level 2 in these indications. Lastly, novel biomarkers including FBXW7 and PPP2R1A alterations (endometrial and ovarian cancer), SMARCA4 mutations (non-small cell lung cancer and esophageal adenocarcinoma) and MTAP deletions (all solid tumors) were included in OncoKB based on compelling preclinical and emerging clinical evidence in association with lunresertib + camonsertib, PRT3789, and AMG193 and MRTX1719, respectively. In sum, 17 novel clinically actionable biomarkers (Levels 1-4) and 26 follow-on precision oncology therapies for existing leveled biomarkers were added to OncoKB in 2024. Additionally in 2024, OncoKB prepared to incorporate germline variants into the knowledge base, a feature expected to be publicly available in 2025. OncoKB also implemented major software updates to support data integration into the EPIC platform. Future OncoKB efforts are focused on whole genome/exome curation, inclusion of biomarkers for non-NGS-based precision oncology therapies, and the development of a clinical trial matching system.

#1067 The ISB Cancer Gateway in the Cloud (ISB-CGC): access, explore and analyze large-scale cancer data through the Google Cloud.

Fabian Seidl[1], Lauren Hagen[2], Jacob Wilson[1], Boris Aguilar[2], Deena Bleich[1], Lauren Wolfe[2], Poojitha Gundluru[1], Suzanne Paquette[2], Elaine Lee[2], Danna Huffman[1], William Longabaugh[2], David Pot[1]

[1]GDIT, Rockville, MD,[2]ISB, Seattle, WA

Rapid growth of cancer data in recent decades has made data discovery and wrangling difficult for the average cancer research lab. Our mission at the ISB Cancer Gateway in the Cloud (ISB-CGC), part of the NCI's Cancer Research Data Commons ecosystem, is to democratize access to large cancer datasets. Funded by the NCI, we have performed ETL processes on data from GDC and PDC projects such as TCGA, TARGET, and CPTAC. We generated hundreds of publicly available BigQuery tables containing data such as mutations, gene expression, and protein abundance, which enable data analysis in the cloud via SQL. BigQuery analyses are inexpensive and rapid even when scaled to petabyte sized inputs, for example in one analysis 6.6 billion correlations were computed in 2.5 hours with a total cost of about one dollar. These data can also be accessed affordably from Google Cloud VMs where researchers can develop analysis pipelines in Python, R, and workflow languages. We will also highlight recently improved accessibility to the Mitelman Database, added and expanded HTAN single cell data and notebook selections, and updates to our BigQuery Table Search tool.

#1068 Navigating next-generation pre-clinical models of drug discovery & development with RNA-seq.

A. P. Singh[1], R. R. Khairi[1], A. L. Reese[2], J. Kirkland[2], J. Duncan[2], N. Wax[2], R. Marlow[2], S. King[2], A. Fernandes[2], J. L. Jacobs[1]; [1]American Type Culture Collection, Gaithersburg, MD, [2]American Type Culture Collection, Manassas, VA

Cell models paired with curated gene expression data are vital for preclinical research. Transcriptomic data from traceable, authenticated cell lines streamline hypothesis development and experimental design while illuminating underlying biological mechanisms. In cancer research, these models have notably identified therapeutic targets and biomarkers. To advance cancer research, ATCC® sequenced the transcriptomes of 141 immune cancer cell lines, accessible through ATCC Cell Line Land, encompassing various hematopoietic lineages. This effort provided insights into gene expression patterns, revealing potential biomarkers and gene regulatory networks tailored to specific cell lines. Utilizing this data, we conducted a comparative analysis, pinpointing genes and pathways governing cellular traits. This informed our evaluation of therapeutic molecules like the Curcumin analog EF-24. Our analysis of EF-24's effects on leukemia cell lines K562 (ATCC® CCL-243™, HL-60 (ATCC® CCL-240™), THP-1 (ATCC® TIB-202™), Kasumi-1 (ATCC® CRL-2724™) demonstrated increased cell death post-treatment. EF-24 activated pathways associated with cell survival and death regulation, notably impacting STAT1-induced cell death, and enhancing innate immune system genes. This research offers a detailed understanding of EF-24's molecular actions in tumors, emphasizing the significance of STAT1 signaling in its antitumorigenic effects. The profound impact of EF-24 on inducing cell death across multiple leukemia cell lines underscores its potential as a therapeutic agent. By elucidating its specific molecular actions and highlighting the pivotal role of STAT1 signaling, this study not only deepens our comprehension of EF-24's mechanisms but also underscores its promising value in targeted antitumorigenic strategies.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#107 Galectin-1 plays a critical role in normal and neoplastic mammary gland morphogenesis and metastatic spreading in breast cancer.

Magali Berton, Ramiro Perrotta, Tomas Dalotto-Moreno, Mahmoud D. Yamil, Sabrina Gatto, Rosa Morales, Gabriel A. Rabinovich, Mariana Salatino

IBYME-CONICET (Institute of Biology and Experimental Medicine), Buenos Aires, Argentina

Galectins are a family of glycan-binding proteins that regulate neoplastic transformation, angiogenesis, cell adhesion and tumor-immune escape. Galectin-1 (Gal-1), a prototype member of this family, binds to multiple *N*-acetyllactosamine units in N- and O-glycans on cell surface glycoproteins. Biologically, Gal-1 plays a key role in the creation of an immunosuppressed tumor microenvironment (TME) by acting as a negative regulator of the immune response, fostering tumor immune-escape, stimulating an invasive, stem-like phenotype and enhancing metastasis in several tumors. In this work we focus on the relevance of the Gal-1-glycan axis in the TME, metastatic progression and antitumor immune response, and we aim to address the role that Gal-1 plays in normal and transformed mammary gland development. First, we observed that C57BL/6 female mice deficient for Gal-1 (*Lgals1*$^{-/-}$ KO) have an impaired mammary branch morphogenesis in response to progesterone stimulus as compared to their C57BL/6 WT counterparts. To tackle the mechanism underlying this deficiency in mammary branching, we performed cell sorting based on surface markers (EpCAM and CD49f)to categorize the isolated mammary cells from C57BL/6 WT and C57BL/6 Lgals*1*$^{-/-}$ KO mice into four main populations: luminal (EpCAM^{high}-CD49f^{low}), basal (EpCAM^{low}-CD49f^{med}), stromal (EpCAM^{neg}-CD49f^{neg}), and mammary stem cells (MaSC) (EpCAM^{low}-CD49f^{high}). Each population's purity was evaluated by Real Time PCR (qPCR) using specific markers (PR, ER, Vim, a-SMA, Nanog) and we observed that *Lgals1*$^{-/-}$ KO mice present a decreased percentage of luminal and MaSC cells (Spectral CyTEK) in comparison to the Gal-1 sufficient strain, which may contribute to the reduced branching. To then analyze the role of Gal-1 in mammary tumorigenesis and metastasis, we developed a MMTV-PyMT transgenic mouse model WT or KO for Gal-1 (*lgals1*$^{-/-}$).Using this novel experimental model, we observed that that PyMT-Gal1-KO mice present a delay in tumor burden and a longer tumor-free survival time, with an overall lower number of transformed ducts in the mammary gland and reduced lung metastasis, alongside a reduced mammary gland branching morphogenesis. Our findings highlight the relevance of Gal-1 in regulating normal mammary gland morphogenesis and assert its critical role in metastatic spreading in breast cancer. From a therapeutical point of view, Gal-1 can be considered as a promising target in metastatic breast cancer.

BIOINFORMATICS / COMPUTATIONAL BIOLOGY / SYSTEMS BIOLOGY / CONVERGENT SCIENCE: Database Resources Poster Session

#1070 siderGrimoire: a database of retroCNVs in human tumors and populations.

Thiago Luiz Araujo Miller, Helena Beatriz da Conceicao, Pedro Alexandre Favoretto Galante

Sirio-Libanes Hospital Brazil, Sao Paulo, Brazil

Background: Retrocopies, also known as processed pseudogenes, are structural variations resulting from the duplication of protein-coding genes via the reverse transcription of their mature messenger RNA by the LINE-1 enzymatic machinery. These retrocopies can be fixed, present in all genomes of a given species, or unfixed—classified here as retroCNVs—when they are polymorphic, either germline or somatic. While fixed retrocopies have been extensively studied, the understanding of retroCNVs remains limited due to the lack of specialized bioinformatics tools for their detection and annotation in DNA sequencing data, as well as curated databases to share findings with the scientific community.
Objective: To address this gap, we developed the sideRETRO, a robust computational tool for the identification and annotation of retroCNVs in whole-genome and exome sequencing data. The results of this tool will be made accessible through siderGrimoire, an online, curated database integrating retroCNVs from human populations and cancer samples.
Methods: The sideRETRO pipeline, published in Bioinformatics, offers a dedicated approach to detecting non-fixed retroCNVs. Using datasets from the 1000 Genomes Project and The Cancer Genome Atlas (TCGA), siderGrimoire curates retroCNVs across human populations and tumor samples, providing detailed annotations and a user-friendly interface.
Results: Initial analyses reveal a comprehensive landscape of retroCNVs, uncovering their prevalence and patterns of events in diverse human populations and cancer types. This resource bridges a critical gap in the availability of retroCNV-specific data for the research community.
Conclusion: The siderGrimoire database represents a significant advancement in retroCNV research, providing a valuable tool for studying the role of retrocopies in genetic variation and cancer biology. By integrating large-scale data from key genomic projects, this platform enables new insights into the genomic impacts of retroCNVs, fostering further exploration in human genetics and oncology.

#1071 Online resources from the Allen Institute for Immunology: longitudinal profiling of multiple myeloma treatment and the Human Immune System Explorer platform.

Lucas Graybuck[1], Christian M. La France[1], Nicole Howard[1], Jessica Liang[1], Autumn Kelsey[1], Lauren Y. Okada[1], Zachary J. Thomson[1], Aishwarya Chander[1], Patrick I. McGrath[1], Love Tätting[2], Yudong D. He[1], Emma L. Kuan[1], Marla C. Glass[1], Samir Rachid Zaim[1], Palak C. Genge[1], Medbh A. Dillon[1], Cole G. Phalen[1], Peter J. Wittig[1], Alexander T. Heubeck[1], Julian Reading[1], Charles R. Roll[1], Veronica Hernandez[1], Vaishnavi Parthasarathy[1], Tyanna J. Stuckey[1], Blessing Musgrove[1], Elliott Swanson[1], Morgan D. A. Weiss[1], Jessica Garber[1], Upaasana Krishnan[1], Yousef Aggoune[1], Madeline Ambrose[1], Aldan Beaubien[1], James Harvey[1], Neelima Inala[1], Ed Johnson[1], Stark Pister[1], Sathya Subramanian[1], Vitalii Tereshchenko[1], Anne Vetto[1], Xiaoling Song[3], Philip D. Greenberg[4], Ananda W. Goldrath[1], Mackenzie S. Kopp[1], Evan W. Newell[1], Damian J. Green[5], Troy R. Torgerson[1], Mary Kwok[3], Xiao-jun Li[1], Melinda L. Angus-Hill[1], Paul Meijer[1]

[1]Allen Institute for Immunology, Seattle, WA,[2]Department of Haematology in Linkoping and Department of Biomedical and Clinical Sciences, Linkoping University, Linkoping, Sweden,[3]Fred Hutchinson Cancer Center, Seattle, WA,[4]Translational Science and Therapeutics Division, Fred Hutchinson Cancer Center, Seattle, WA,[5]Sylvester Comprehensive Cancer Center, Miami, FL

The Allen Institute for Immunology was founded in 2018 to deeply profile the human immune system in health and disease. As part of our mission to understand how the immune system changes over time in the setting of oncology, we recruited a cohort of patients at diagnosis for multiple myeloma (MM), and followed these subjects throughout induction therapy, autologous stem cell transplant, and after transplant recovery. We sampled the same subjects longitudinally, then performed immune profiling using scRNA-seq on peripheral blood cells, CITE-seq on bone marrow cells, multiple high-dimensional flow cytometry panels, plasma and bone marrow interstitial fluid (BMIF) proteomics, and clinical lab tests. To process, analyze, and distribute this data, we developed the Human Immune System Explorer (HISE) platform, a flexible, scalable, cloud-based framework to enable storage, interactive analysis, visualization, and generation of Certificates of Reproducibility that enable inspection and replay of any step of an analysis workflow. We joined our robust, large-scale analytical platform to public-facing tools, scientific context, and data releases, which now include interactive visualization tools for exploring the effects of MM treatment in detail. We invite oncologists, immunologists, and clinicians to explore this resource and our expanding library of immunology data, insights, and tools at https://explore.allenimmunology.org/.

#1072 MOSAIC Window: Spatial intra-tumoral heterogeneity characterization through large-scale spatial and single-nuclei multi-omics profiling.

The MOSAIC Consortium (Owkin, FR; Charite, DE; CHUV, CH; GR, FR; UKER, DE; UPMC, USA), **Presenter: Caroline Hoffmann**

Owkin, PARIS, France

INTRODUCTION- MOSAIC (Multi-Omics Spatial Atlas in Cancer) is a multicenter clinical omics study aimed at deciphering cancer biology. We systematically profile thousands of cancer samples, focusing on spatial transcriptomics and single-nuclei RNA sequencing (snRNAseq) data across multiple tumor types.Here, we present the MOSAIC Window initiative, our first open science dataset. This multi-omics dataset can be leveraged to explore tumor heterogeneity and biomarker discovery within the tumor microenvironment (TME).
METHODS- The MOSAIC Window dataset includes 60 samples from patients with Muscle Invasive Bladder Cancer (n = 15), Ovarian cancer (n = 15), Diffuse-large B-cell Lymphoma, Mesothelioma and Glioblastoma (n = 10 each).Each sample underwent Visium spatial transcriptomics, Chromium Flex snRNAseq, bulk RNAseq, whole-exome sequencing, and hematoxylin-eosin histology scans. We collected extensive clinical information, including age; gender; BMI; risk factors; cancer treatments; clinical, pathological and molecular information; and progression-free and overall survival.We applied strict quality control metrics in each data modality which we are reporting. Tumor heterogeneity was measured using snRNAseq and spatial transcriptomics integration. First, scANVI method was used on snRNAseq data to identify and annotate sub-populations cells from the TME. For cancer cell population, shared nearest neighbour (SNN) modularity optimization based clustering algorithm was used to separate clusters of cancer cell sub-populations. Differential expression analysis was used between these sub-populations to generate references. Then, cell type deconvolution was performed using cell2location and the references generated from snRNAseq data.
RESULTS- The analysis of the spatial distribution of sub-population of cancer cells revealed distinct sub-populations of cancer cells, corresponding to different molecular sub-clones and/or TME cell composition (niches). Our computational approach highlighted significant variability in intra-tumoral heterogeneity across cancer types and patients. Samples ranged from having a single homogeneous niche to multiple distinct niches, each characterized by specific malignant cell subtypes and their associated TME, highlighting the interplay between these two compartments, which we are detailing and quantifying. These results provide important insights into potential mechanisms of treatment resistance, biomarker discovery, patient subgroup identification, and potential combination treatment strategies.
CONCLUSION- The open science MOSAIC Window dataset demonstrates how integrating spatial and single-nuclei data provides key insights understanding of intra- and inter-patient tumor heterogeneity, paving the way for novel therapeutic strategies. Data is accessible on demand on EGA.

#1074 CTCFDB: a systematic framework and comprehensive data resource for CTCF binding in the human genome.

Shengyuan Wang, Zhenjia Wang, Grant Atwood, Yanbin Xu, Chongzhi Zang

Department of Genome Sciences, University of Virginia, Charlottesville, VA

CCTCC-binding factor (CTCF), a highly conserved zinc finger protein, plays a crucial role in the three-dimensional (3D) genome organization by mediating DNA loops and topologically associating domains (TADs). Variations in CTCF binding across different cell types can lead to changes in cell type-specific chromatin conformation, altering gene expression and contributing to diseases such as cancer. Despite its importance, an up-to-date and comprehensive database for human CTCF binding sites has been lacking. To fill this gap, we developed CTCFDB, a curated data resource encompassing 2,251 high-quality CTCF ChIP-seq datasets from NCBI GEO across 344 human cell types. We identified 510,675 high-confidence CTCF binding sites in the human genome. Among those, 22,722 were charactered as constitutive binding sites that are conserved across cell types. Using binding profiles across high-confidence CTCF binding sites, we performed unsupervised clustering analyses on all samples and found that the majority of cell types were clustered together, indicating cell type-specific binding patterns. Besides, we characterized 65,697 cell type-specific gained and 18,990 lost CTCF sites by focusing on individual union sites, further demonstrating the cell type specificity of CTCF binding. CTCFDB, with its systematic approach and user-friendly interface, provides an invaluable resource for advancing research on 3D genomics and gene regulation. This framework can be extended to generate similar data resources for other DNA-associating proteins, facilitating broader insights into functional genomics and cancer research.

#1075 Piloting NTRK fusion-specific oncogenicity guidelines - lessons learned.

Jason Saliba[1], Shivani Golem[2], Arpad Danos[1], Laura B. Corson[3], Elan Hahn[4], Morteza Seifi[5], Emma G. Sullivan[6], Jan Clement A. Santiago[7], Valentina Nardi[8], Theodore W. Laetsch[9], Marilyn M. Li[9], Obi L. Griffith[1], Malachi Griffith[1], Gordana Raca[10], Larissa V. Furtado[11], Alanna J. Church[12], Angshumoy Roy[13]

[1]Washington University School of Medicine, St. Louis, MO,[2]University of Kansas Medical Center, Kansas City, KS,[3]Oncology Consultant, Boston, MA,[4]Mount Sinai Hospital and the University of Toronto, Toronto, ON, Canada,[5]University of Wisconsin-Madison, Madison, WI,[6]Michael G. DeGroote School of Medicine, Hamilton, ON, Canada,[7]University of Washington, Seattle, WA,[8]Massachusetts General Hospital and Harvard Medical School, Boston, MA,[9]Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia, PA,[10]Children's Hospital Los Angeles, Los Angeles, CA,[11]St. Jude Children's Research Hospital, Memphis, TN,[12]Boston Children's Hospital and Harvard Medical School, Boston, MA,[13]Baylor College of Medicine and Texas Children's Hospital, Houston, TX

Gene fusions involving neurotrophic receptor tyrosine kinase genes (NTRK1, NTRK2, & NTRK3) are well-established oncogenic drivers and serve as critical diagnostic and therapeutic markers in cancer. Interpreting their clinical significance is a high priority given FDA approval of TRK inhibitors; however, this remains challenging due to the rapid pace of fusion discovery, the diversity of fusion partners and tumor types, inconsistent and incomplete reporting of fusion data elements, and the lack of standardized fusion-specific classification guidelines. The Clinical Genome Resource (ClinGen) NTRK Fusions Somatic Cancer Variant Curation Expert Panel (SC-VCEP) is addressing these challenges and creating a publicly available resource of high-quality clinically significant NTRK fusion classifications in the Clinical Interpretation of Variants in Cancer (CIViC; civicdb.org) knowledgebase to support patient care.
Our NTRK fusion-specific oncogenicity guidelines (approved April 2022) classify NTRK fusions as Oncogenic, Likely Oncogenic, Fusion of Unknown Significance (FUS), or Benign based on four categories: Fusion Gene Structure (orientation/breakpoints/reading frame), Cancer Association (number of unique cases), Clinical Validity (response to targeted inhibitors), and Functional Status (pathway activation or expression). Piloting our guidelines on a range of common to rare NTRK fusions found in cancers resulted in 12 Oncogenic Assertions (6 Oncogenic, 1 Likely Oncogenic, 2 FUS, 3 Benign), 5 Diagnostic Assertions covering 3 cancers, and 10 Predictive Assertions supporting sensitivity to FDA approved TRK inhibitors, larotrectinib or entrectinib. This pilot introduced several modifications to our oncogenicity guidance including: 1) reducing case numbers required to reach cancer association or clinical validity due to the rarity of reported NTRK-positive tumors; 2) further clarifying NTRK fusion structure, e.g., both gene partners must contribute to the fusion protein sequence; 3) requiring fusions to be reported in the published literature as databases may lack vetting; 4) expanding our NTRK-associated tumor list as more tumors are screened for the presence of NTRK fusions; 5) strengthening Benign support by requiring the confirmed lack of both RNA and protein fusion expression. This process was a valuable step in enhancing the consistency and clarity of the ClinGen NTRK SC-VCEP fusion-specific oncogenicity guidelines to aid their widespread adoption. Future efforts will focus on the Oncogenic classification of the over 80 known NTRK fusions found in cancers and their distribution to the public through the CIViC knowledgebase.

#1076 Sharing data from the Human Tumor Atlas Network through standards, infrastructure, and community engagement.

I. de Bruijn[1], M. Nikolov[2], C. Lau[3], A. Clayton[2], D. L. Gibbs[3], E. Mitraka[2], **D. Y. Pozhidayeva**[3], A. Lash[4], S. Sumer[1], J. Altreuter[4], K. Anton[5], M. DeFelice[2], X. Li[1], A. Lisman[1], W. J. R. Longabaugh[3], J. Muhlich[6], S. Santagata[6], S. Nandakumar[1], P. K. Sorger[6], C. Suver[2], X. Doan[2], J. Guinney[2], N. Schultz[1], A. J. Taylor[2], V. Thorsson[3], E. Cerami[4], J. A. Eddy[2];

[1]Memorial Sloan Kettering Cancer Center, New York, NY, [2]Sage Bionetworks, Seattle, WA, [3]Institute for Systems Biology, Seattle, WA, [4]Dana-Farber Cancer Institute, Boston, MA, [5]University of North Carolina at Chapel Hill, Chapel Hill, NC, [6]Harvard Medical School, Boston, MA

In September 2018, the National Cancer Institute (NCI) launched the first phase of the Human Tumor Atlas Network (HTAN) under the U.S. Cancer Moonshot program with the goal of generating three-dimensional molecular and spatial atlases of diverse human tumors and characterizing crucial transitions in cancer progression and treatment. With over 30 assay types including sequencing, imaging, and spatial transcriptomics spanning 58 cancers across 21 organ types, HTAN has delivered a substantial resource to the broader cancer research community and become the basis for a multitude of publications. Although HTAN is similar to previous large cancer data sharing efforts, new and unique challenges have emerged. First, each HTAN atlas consists of a unique collection of data focused on answering different hypotheses regarding cancer progression. Second, many of the experimental assays used within HTAN—particularly spatial profiling assays—are cutting-edge, requiring their own custom bioinformatics pipelines to perform analyses. Third, HTAN is focused on understanding temporal changes in cancer, and the HTAN data model must therefore be capable of capturing longitudinal clinical/phenotype and profiling data. Fourth, the multi-modal nature of HTAN data requires multiple visualization and data access resources, each of which must be tailored to individual data types or end-users.

To address the unique challenges of HTAN data, the network supports a dedicated Data Coordinating Center (DCC) which is currently managed by personnel from four institutions: Dana-Farber Cancer Institute, Sage Bionetworks, Memorial Sloan Kettering Cancer Center, and the Institute for Systems Biology. The DCC develops infrastructure and tools to ingest, curate, explore, and share these data in a findable, accessible, interoperable, and reusable (FAIR) manner. To this end, the DCC manages the HTAN Data Portal, which provides community access to the atlases and enables filtering of released atlas data based on metadata fields, and pointers to data availability. Additionally, the DCC leads community-driven metadata schema development through a Request for Comments (RFC) process. These activities are critical to ensuring that the wealth of data generated by HTAN is available for use by the broader scientific community. As we transition into the second phase, we describe insights from HTAN phase I, the multiple ways users can access HTAN data and metadata, the associated data standards, the enabling technical infrastructure and governance approaches underlying the DCC, and how community engagement is maintained throughout.

#1077 Visualization and analysis of cancer genomics data using UCSC Xena.

Mary Goldman[1], Brian Craft[1], Cally Lin[2], Jingchun Zhu[1]

[1]UC Santa Cruz Genomics Institute, Santa Cruz, CA,[2]Stanford University, Stanford, CA

UCSC Xena (http://xena.ucsc.edu/) is a web-based visual integration and exploration tool for both bulk and single-cell multi-omic data, and associated clinical and phenotypic annotations. Researchers can easily view and explore public data, their own private data (bulk only), or both using the Xena Browser. Private data are kept on the researcher's computer and are never uploaded to our public servers. We support Mac, Windows, and Linux.

Questions Xena can help you answer:

* Is overexpression of this gene associated with survival differences?
* What genes are differentially expressed between these two groups of samples?
* What is the relationship between mutation, copy number, expression, etc for this gene?

Xena visualizes seminal cancer genomics datasets from TCGA, the Pan-Cancer Atlas, GDC (a complete update from 2024, including new data from CPTAC3 and HCMI), PCAWG, ICGC, and more; a total of more than 1500 datasets across 50 cancer types. We support virtually any type of functional genomics data: SNPs, INDELs, copy number variation, gene expression, ATAC-seq, DNA methylation, exon-, transcript-, miRNA-, lncRNA-expression and structural variants. We also support clinical data such as phenotype information, subtype classifications and biomarkers. All of our data is available for download via python or R APIs, or using our URL links.

Our signature Visual Spreadsheet view shows multiple data types side-by-side enabling discovery of correlations across and within genes and genomic regions. We also have dynamic Kaplan-Meier survival analysis, powerful filtering and subgrouping, differential gene expression analysis, GSEA, charts, statistical analyses, genomic signatures, and the ability to generate URLs to live views.

We now visualize single-cell data, primarily gene, transcript, and protein expression, from spatially-resolved and disassociated single cell datasets. Using Xena, researchers can visualize 2D or 3D embedding views, such as tSNE and UMAP as well as the spatial imaging views, such as H&E, multiplex immunofluorescence, and co-registration of both. Crucially, we overlay genomic data — such as gene and protein expression, genomic signature scores, cell annotations, and label transfer information — on top of these images dynamically. We support a diverse range of data modalities ranging from 10x Chromium and Smartseq2 platforms for scRNA-seq and 10x scATAC-seq, to spatial transcriptomics platforms of Visium, Visium HD, Xenium, CosMx, and MERFISH/Vizgen, to imaging proteomics such as t-CycIF, CODEX, and MIBI-TOF. We showcase data from the HTAN data portal.

If you use us please cite our publication in Nature Biotechnology: https://www.nature.com/articles/s41587-020-0546-8

#1078 Enhancing reusability and adoption of cancer research tools: Development of the Cancer Complexity Toolkit.

Aditi Gopalan[1], Brad Macdonald[1], James Eddy[2], Thomas Yu[1], Susheel Varma[1], Jineta Banerjee[1], Jineta Banerjee[1]

[1]Sage Bionetworks, Seattle, WA,[2]ARPA-H, Bethesda, MD

The Cancer Complexity Knowledge Portal (CCKP) is an open-access data repository that stores and shares resources from multiple cancer research programs. Maintained by Sage Bionetworks, in collaboration with The Multi-Consortia Coordinating (MC2) Center, which supports six NCI Division of Cancer Biology (DCB) funded programs made up of interdisciplinary communities of scientists, the CCKP centralizes access to cancer research tools and data, improving their discoverability and reuse.Reusing tools has proven to be challenging for various reasons. Therefore, we aim to enhance the reusability of the tools available on CCKP. To achieve this, we are designing and implementing a Cancer Complexity Toolkit to validate tool reusability and increase their adoption. Building on existing efforts to curate tools generated by the cancer research community, the 'Cancer Complexity Toolkit' aims to achieve three key objectives: (1) enhancing the discovery and reuse of cancer research tools through rich metadata and standardized evaluation frameworks, (2) creating a catalog of cancer research-focused tools to improve accessibility and confidence in their use, and (3) enabling standardized reporting and validation of tool functionality. Ultimately, we aim to provide a robust platform for discovering, validating, and integrating computational tools in cancer research.To achieve these goals, we are (1) refining a standardized schema to capture metadata on tool parameters and usage, (2) building infrastructure for automated checks to assess documentation, test coverage, and community metrics, and (3) developing tool cards to display relevant metrics and metadata, helping researchers select the right tools and allowing developers to highlight their tools' impact.In Phase 1, we refined the tool metadata schema and developed a Python-based testing infrastructure to check for documentation, testing files, and deployment on non-native Linux environments. We selected ten tools from the CCKP for initial testing, generating logs that assess their reusability.

#1079 L2S2: Chemical perturbation and CRISPR KO LINCS L1000 signature search engine.

G. B. Marino, J. E. Evangelista, D. J. B. Clarke, A. Ma'ayan;
Icahn School of Medicine at Mount Sinai, New York, NY

The Library of Integrated Network-Based Cellular Signatures (LINCS) Common Fund program was established to construct a reference database of human cancer cell lines responses to diverse multi-omics and phenotypic perturbations. As part of the LINCS initiative, 248 cell lines were profiled with the L1000 transcriptomics assay to measure the response to 33,621 small molecules and 7,508 single gene CRISPR knockouts (KOs). From this massive dataset, we computed sets of up- and down-regulated genes. These gene sets are served for search by the LINCS L1000 Signature Search (L2S2) platform. L2S2 provides instant results when searching across over 1.678 million chemical perturbations and CRISPR KO signatures. The platform includes filters for FDA-approved drugs and signature directionality. With the L2S2 search engine, users can identify small molecules and single gene CRISPR KOs that produce gene expression profiles similar or opposite to their submitted gene sets. Significant CRISPR KO signatures can reveal potential driver genes and drug targets. Similarly, approved drugs and preclinical small molecule signatures that might reverse or mimic a desired expression pattern can provide hypotheses for preclinical experimental validation studies. L2S2 also includes a consensus search feature which ranks specific perturbations across cellular contexts, time points, and concentrations. L2S2 was systematically applied to the CPTAC3 cohort to prioritize potential targets, approved drugs, and preclinical compounds for all previously identified multi-omic cancer cohort subtypes. Overall, L2S2 provides cancer researchers with a powerful tool to further understand and personalize treatments for cancer subtypes. L2S2 is available from: https://l2s2.maayanlab.cloud.

#1080 Transforming cancer genomics research: A platform for integrated exploration of COSMIC resources.

Helder Pedro, Zbyslaw Sondka

Wellcome Sanger Institute, Cambridge, United Kingdom

The Catalogue of Somatic Mutations in Cancer (COSMIC) is a vital resource for cancer genomics, offering extensive data on somatic mutations, cell lines, and mutation signatures. While the existing COSMIC dataset provides wealth of diverse, high-quality information, accessing and fully utilising it requires significant processing and expertise in data analysis. To address this, we are developing a new suite of tools to enhance COSMIC data integration, usability and exploration.The Cell Line Explorer serves as a starting point for this suite, enabling users to easily access and analyse COSMIC data related to specific cell lines. This tool integrates genetic variants with annotations and reference datasets, providing dynamic insights into clinical significance. Beyond the cell lines mutation catalogue, the platform connects to key COSMIC resources, including the Cancer Gene Census, Actionability, Cancer Mutation Census, Mutational Signatures, and COSMIC3D. This integration allows users to derive deeper insights, and easily correlate mutation data with functional impact and potential clinical significance.The tools are designed for flexibility and scalability, meeting the needs of diverse users, from computational researchers, clinicians seeking actionable insights, educators incorporating bioinformatics into their teaching, and individuals with limited bioinformatics expertise who require intuitive solutions.With its intuitive interface and robust integration of COSMIC resources, this platform lowers the barriers to access and streamline complex analyses, providing researchers with powerful tools to explore and analyse cancer genomic data at unprecedented depth.

#1081 Sylvester Data Portal: Streamlined access to standardized clinicogenomic data.

Jeronimo I. Pissinis, Oliver Mazariegos, Marcin Pilarczyk, Michael S. Sinclair, Lukas Rupprecht, Caty Chung, Franklin Sotolongo, Gabriel Lichtenstein, Kathleen M. Jagodnik, Joanita Figueredo, Pearl Seo, Carlos J. Obregon, Marcin Blicharz, Piotr Smol, Michal Pyrkosz, Mateusz Jura, Vasileios Stathias, Stephan Schurer

SCCC-Data Science, Univ. of Miami Sylvester Comprehensive Cancer Ctr., Miami, FL

The Sylvester Data Portal (SDP), developed at the University of Miami (UM) Sylvester Comprehensive Cancer Center, is an advanced multi-omics platform that streamlines the management of real-world and research data. SDP facilitates the standardization and integration of data from major genomic profiling vendors, including Caris Life Sciences, Foundation Medicine, and NeoGenomics. SDP utilizes an event-driven ETL pipeline to process, validate, and standardize genomic reports (XML, PDF) and files (FASTQ, BAM, VCF, TSV) from Whole Exome Sequencing (WES), Targeted Sequencing (TS), and Whole Transcriptome Sequencing (WTS) tests. Metadata are harmonized into a unified format, enabling cross-vendor and cross-test-type queries. The platform also supports the preparation of data and metadata for submission to the AACR Project GENIE.A LIMS-like module within SDP is operationally integrated with data generation at the Onco-Genomics Shared Resource (OGSR). This module tracks sequencing workflows, performs automated quality control (QC), and manages the release of high-quality datasets to users, including their secure long-term storage. These functionalities enhance the reliability and accessibility of sequencing data for translational research. SDP also supports interactive data exploration and analysis.The platform enables containerized and reproducible bioinformatics workflows, supporting tasks like demultiplexing, as well as single-cell and bulk RNA-seq analyses. Future functionalities will include handling additional data types such as ATAC-seq and ChIP-seq, alongside integrated DNA analysis of germline and somatic variants.To ensure secure and tailored access to clinical data, SDP provides three user interfaces: (1) The Clinical Dashboard offers aggregated patient statistics, such as demographics and summaries of available data; (2) The Clinical Browser grants access to de-identified subject-level data upon institutional approval; and (3) Clinical Collections include PHI and NGS data, accessible after IRB and Data Broker approval. These interfaces facilitate data sharing within the Sylvester community, advancing translational research while adhering to data governance and regulatory policies.

#1082 A comprehensive platform for stage-specific analysis of colon cancer: integrating transcriptomic and clinical data for biomarker discovery.

Aron Baratha, **Balazs Gyorffy**

Semmelweis University, Budapest, Hungary

Introduction: Understanding the molecular mechanisms underlying stage-specific colon cancer progression is essential for identifying biomarkers and developing effective therapies.
Aim: To address this need, we extended our previously established comprehensive database with stage-specific clinical and transcriptomic data. The new updated database was employed to mine stage-specific patterns and explore functional trends across stage-stratified colon cancer cohorts.
Methods: We used data from GEO (gene arrays), TCGA, and GTEx (RNA-seq) databases. A total of 1,926 colon cancer samples from gene chip studies were manually curated from GEO, and RNA-seq data comprising 469 samples were included from TCGA. To identify differentially expressed genes across stages, we first performed ANOVA tests, adjusting p-values using the Benjamini-Hochberg method with a cutoff of 0.01. Effect size was then estimated using the eta squared approach, applying a moderate effect size threshold of 0.06.
Results: Stage-specific data were incorporated into our previously established colon cancer dataset, including 37 (array) and 75 (RNAseq) Stage I samples, 291 (array) and 176 (RNAseq) Stage II samples, 197 (array) and 128 (RNAseq) Stage III samples, and 123 (array) and 64 (RNAseq) Stage IV samples. The expanded dataset facilitates a detailed investigation of gene expression changes across colon cancer stages, uncovering stage-dependent molecular signatures. We utilized the integrated database to uncover genes with consistent expression change across tumor progression. We identified 94 stage-specific genes with significant differential expression and at least a moderate effect size. These genes were primarily associated with transcription and phosphorylation. Among them, *COG5* (pAdj = 8.17E-09), *AMMECR1* (pAdj = 9.76E-10), and *SOWAHC* (pAdj = 8.51E-10) showed the most consistent differences across stages. The new integrated platform with all transcriptomic and clinical data is accessible at www.tnmplot.com
Conclusion: Our enriched platform provides a powerful resource for the comprehensive exploration of stage-stratified colon cancer data, offering valuable insights into cancer biology and supporting biomarker discovery.

#1083 T cell receptor-associated genes as biomarkers of survival in anti-PD-1 treated cancer patients.

Szonja Anna Kovacs, **Balazs Gyorffy**

Semmelweis University, Budapest, Hungary

Background: Immune checkpoint inhibitors (ICIs) aim to restore T-cell function in the tumor microenvironment, but predicting patient response remains challenging. T-cell abundance and T-cell receptor (TCR) signaling are critical for the effectiveness of ICI therapies. While PD-L1 expression is a commonly used biomarker, its predictive accuracy is inconsistent. Exploring alternative biomarkers could improve response prediction and patient outcomes.
Methods: We integrated multiple gene expression cohorts from cancer patients treated with immune checkpoint inhibitors (anti-PD-1, anti-PD-L1, and anti-CTLA-4). Corresponding clinical annotation data were incorporated for survival and biomarker studies. Survival analysis was performed using Cox regression, and Gene Ontology (GO) enrichment analysis was conducted on both pre-treatment (samples taken before ICI therapy) and on-treatment (samples collected during or after therapy) groups. Published survival data were excluded if no progression or death occurred, and follow-up ended within 12 months.
Results: The complete database includes 1,297 patients with relapse-free survival data and 1,554 patients with overall survival (OS) data. Patients with higher expression of genes associated with the CD4+ or CD8+ T-cell lineage commitment pathway (GO:0043369) demonstrated improved OS. Notably, elevated expression of T-cell receptor beta constant 2 (TRBC2; HR=0.38, log-rank P=1E-03, FDR=5%), Cluster of Differentiation 247 (CD247; HR=0.37, log-rank P=1.7E-03, FDR=5%), and Cluster of Differentiation 3E (CD3E; HR=0.35, log-rank P=1.4E-03, FDR=5%) correlated with better OS after anti-PD-1 therapy. To determine whether these findings reflect a pre-existing immune-infiltrated tumor microenvironment or an immune system boost induced by anti-PD-1 therapy, we also analyzed pre-treatment samples. Higher expression of TRBC2 (HR=0.49, log-rank P=4.4E-05, FDR=1%), CD247 (HR=0.49, log-rank P=4.9E-07, FDR=1%), and CD3E (HR=0.42, log-rank P=9.5E-07, FDR=1%) in pre-treatment samples were also associated with better survival. PD-L1 (CD274) expression is a commonly used but controversial biomarker for ICI therapy. Patients with high PD-L1 expression before anti-PD-1 treatment showed better relapse-free survival (HR=0.61, log-rank P=4.1E-10, FDR=1%) and overall survival (HR=0.61, log-rank P=5.6E-04, FDR=5%). These results demonstrate that, as expected, an already immunologically "hot" tumor predicts a better response and, consequently, improved survival with anti-PD-1 immunotherapy. The complete ICI cohort was integrated into our established web platform (www.kmplot.com) to enable independent future biomarker discovery.
Conclusions: T-cell receptor complex-associated genes, such as TRBC2, CD247, and CD3E, are robust predictors of survival both before and after anti-PD-1 therapy.

#1084 Analyzing artificial intelligence (AI) Resources for Cancer Research Data Programs.

Juergen Klenk[1], Dina Mikdadi[1], Bhavani Singh[1], **Chelsea Owens**[1], Eric Barner[1], Ross Campbell[1], Mary Sears[1], Ina Felau[2], Erika Kim[2], Tanja Davidsen[2]

[1]Deloitte Consulting LLC, Rosslyn, VA,[2]Data Ecosystems Branch, National Cancer Institute, Rockville, MD

Purpose: The National Cancer Institute's (NCI) Cancer Research Data Commons (CRDC) supports the cancer research community by providing cloud-based, secure storage and analytic tools for multiple cancer data types (e.g., genomic, proteomic, imaging, and clinical trial data). CRDC is beginning to integrate artificial intelligence (AI) solutions, such as using AI to annotate medical images and making AI models and resources available for cancer researchers. As AI policies emerge, CRDC must track relevant guidelines to determine how AI can safely and compliantly unlock value for researchers.
Methods: CRDC conducted a landscape analysis to inform CRDC of potential AI risks and policies across three branches of the Federal government and international agencies. CRDC identified and analyzed policies from organizations focused on AI risk mitigation measures, general AI guidance, and AI strategy as relates to federal health agencies.
Results: The following policies have the most implications for CRDC. In the Executive Branch, the Executive Order on Safe, Secure, and Trustworthy AI mandates federal agencies to ensure AI safety and trustworthiness. The National Institute for Standards and Technology developed AI evaluation criteria, risk assessments, and tools to help assess AI models. CRDC can follow this mandate and use the tools to begin developing an AI strategy. In the Legislative Branch, the Senate AI Working Group and House AI Task Force are starting to discuss specific AI legislation. Although legislation hasn't passed, CRDC should track legislative updates that could impact CRDC compliance. In the Judicial Branch, the Supreme Court overturned the Chevron Deference. Although not directly related to AI, the decision could affect how CRDC interprets and acts upon AI guidance. Internationally, European countries developed accountability frameworks, evaluation criteria, tools, and risk assessments to identify roles and responsibilities and help assess AI models. While NCI is not obligated to follow European AI policies, these artifacts can inform CRDC in developing an AI strategy.
Conclusion: AI policies continue to emerge and evolve. As guidance is solidified, it is important that CRDC continues to monitor and analyze requirements to mitigate risks and ensure responsible use of AI in cancer research. This ongoing analysis will inform CRDC, enhance trust within their user community, increase timely access to cancer data analytical tools, and contribute to the advancement of cancer therapies.

#1085 Analyzing economic storage solutions for cancer research data.

Juergen Klenk[1], **Dina Mikdadi**[1], Bhavani Singh[1], Chelsea Owens[1], Eric Barner[1], Ross Campbell[1], Mary Sears[1], Ina Felau[2], Michael Warfe[2], Erika Kim[3], Tanja Davidsen[2]

[1]Deloitte Consulting LLP, Arlington, VA,[2]Data Ecosystems Branch, National Cancer Institute, Rockville, MD,[3]National Cancer Institute, Rockville, MD

Purpose: The National Cancer Institute's (NCI) Cancer Research Data Commons (CRDC) is a cloud-based data ecosystem that allows researchers to share and access clinical, genomic, proteomic, and imaging data. CRDC currently houses more than 10 petabytes (PB) of data (predominantly genomic). The volume of genomic data in CRDC has more than doubled since 2022 from 3.7 PB to 8.8 PB. To address the escalating data storage costs, CRDC must identify economic genomic data storage/compression strategies to achieve long-term sustainability.

Methods: To evaluate the impact of genomic compression algorithms and cloud storage solutions, a data compression and storage pilot study was conducted based on two CRDC data sources:185GB from 1000 Genomes Project, and 151GB from the Integrated Canine Data Commons (ICDC). Four compression algorithms - PetaGene, CRAM, PigZ, and Genozip - were chosen based on their current use within the cancer research community. The study consisted of three parts:

Compression-Only: four compression algorithms were evaluated for efficiency and cost-effectiveness

Cloud Storage+Compression: compressed data were placed in various AWS S3 storage tiers including AWS Intelligent-Tiering (Assumption: no monitoring and automation costs for intelligent tiering)

AWS HealthOmics: a range of possible storage costs were based on two scenarios- (1) data accessed monthly, and (2) data never accessed. (Assumptions: 4 gigabases per gigabyte, no egress costs when moving data off HealthOmics, each genome is downloaded in 500 parts generating 500 GET API calls)

Results: Of the four algorithms tested, PetaGene performed best on both datasets. For 1000 Genomes data, PetaGene compressed data by 76% in ~70 minutes for a one-time cost of $2.86. For ICDC data, PetaGene compressed data by 83% in ~63 minutes for $2.57.

It is broadly accepted that tiered storage yields cost savings. When compressed data was placed in tiered storage, yet more savings were realized. For both datasets, the most cost-effective strategy was S3 Intelligent-Tiering of PetaGene compressed data. The annual cost for PetaGene compressed data stored in intelligent tiering ranges from $2.10 - $6.90 (ICDC) and $3.70 - $12.14 (1000 Genomes).

The annual cost to store data in AWS HealthOmics is $11.15 - $41.82 (ICDC) and $13.66 - $51.23 (1000 Genomes) for data accessed monthly.

Conclusion: Significant cost savings can be achieved using effective genomic data compression tools paired with intelligent tiering storage solutions. There were two main limitations. Data license costs of compression algorithms were not studied, and while the frequency of data access should be considered for real world application, this was not part of the scope of this study. Both these factors will need to be considered as CRDC selects strategies to mitigate cost and inform overall infrastructure.

#1086 Introducing the Florida Cancer Research (FL CARES) Network and the Platform for Accelerating Collaborative Computational Cancer Research (PAC3R).

Michael Sinclair[1], Carlos Obregon[1], Caty Chung[1], Dusica Vidovic[1], Lukas Rupprecht[1], Jeronimo Pissinis[1], Marcin Pilarczyk[1], Franklin Sotolongo[1], Kathleen M. Jagodnik[1], Till Krenz[1], Umamaheswari Natarajan[2], Erin Kobetz[1], Mondal M. Ananda[3], Ling Cen[4], Xuefeng Wang[4], Appu Rathinavelu[2], Stuart Chalk[5], Jiang Bian[6], Ji-Hyun Lee[6], Qianqian Song[6], Vasileios Stathias[1], **Stephan C. Schurer**[1]

[1]University of Miami Miller School of Medicine, Miami, FL,[2]Nova Southeastern University, Fort Lauderdale, FL,[3]Florida International University, Miami, FL,[4]Moffitt Cancer Center, Tampa, FL,[5]University of North Florida, Jacksonville, FL,[6]University of Florida, Gainesville, FL

Florida ranks second in cancer burden in the United States. The Florida CAncer RESearch (FL CARES) Network (https://floridacancernetwork.org/) is a statewide collaboration dedicated to advancing cancer research and improving patient outcomes through education, collaborative research initiatives, and a focus on reducing health disparities by leveraging advanced computational approaches. Funded by the State of Florida Bankhead-Coley Cancer Research Program, FL CARES comprises six research organizations, including Florida's three NCI-designated Cancer Centers. The Network has established a collaborative research consortium with unified policies, coordinated data and metadata standards, and diverse datasets. It incorporates tools like SCAN360, developed at Sylvester Comprehensive Cancer Center, to explore the impact of social determinants of health and environmental factors on cancer outcomes across Florida communities. To further collaborative computational research, FL CARES is developing the Platform for Accelerating Collaborative Computational Cancer Research (PAC3R). This advanced informatics system enables FAIR (findable, accessible, interoperable, reusable) data management, including standardization, harmonization, and integration of multimodal cancer datasets. PAC3R facilitates secure data sharing, scalable bioinformatics tools' deployment, and collaborative analyses across the Network. Built on the Sylvester Data Portal (https://sdp.miami.edu/), a cloud-based multi-omics platform managing clinicogenomic and research data, PAC3R integrates diverse datasets such as transcriptional perturbation signatures, cell sensitivity data, small molecule interactions, and large-scale genomics from The Cancer Genome Atlas (TCGA) and Clinical Proteomic Tumor Analysis Consortium (CPTAC).

#1087 The evolving landscape of cancer transcriptomics data.

Valentin Bernu, Helia Brull Corretger, Charles Lescure, **Abdelkader Behdenna**, Julien Haziza, Clemence Petit, Akpeli Nordor

Epigene Labs, Paris, France

Secondary analysis of omic datasets holds great potential in accelerating precision oncology research and drug development. Yet, scientists lack efficient tools to assess the scope and volume of those datasets. Data heterogeneity and limited browsing features of public databases make it challenging to efficiently search for relevant datasets based on specific criteria, such as experimental designs. We introduce the latest iteration of our solution to map and navigate public databases.

Our solution extracts and preprocesses clinical data that serves as input for our classification models, which range from rule-based algorithms developed in collaboration with domain experts to large language models. We categorize datasets by cancer type, treatment modality, sequencing resolution, cell line identity, mutational status, and drug information. This iteration introduces models for predicting mutation and drug-related data and continuously updates mappings for existing models—cancer type, technology, and treatment—by incorporating new entries. Additionally, this version integrates two databases, namely “Gene Expression Omnibus” or “GEO” and ArrayExpress, and includes a dashboard that allows querying and visualization of the automatically tagged datasets.

We identified 24,131 cancer-related, patient-derived omic datasets from GEO. Our models estimate the following:

- Cancer types: the 5 most frequent indications (breast cancer, brain cancer, non-small cell lung cancer, acute myeloid leukemia, colorectal cancer) constitute about 60% of datasets.
- Technology: RNA-Seq accounts for 29% of datasets, with an estimation of 24% being single-cell and 76% bulk RNA-Seq. RNA-Seq has become the dominant technology: in 2012, the number of microarray studies published that year outnumbered RNA-Seq studies 10:1; by 2023, RNA-Seq studies published annually had overtaken microarray studies by a comparable margin. Today, the total volume of accumulated studies for each approach is nearly equal.
- Treatment: approximately 25% of datasets include treatment information; post-treatment samples are estimated to represent 15%.
- Mutations: information is available in 26% of datasets. Notably, 26% of colorectal cancer datasets contain mutation information, of which 35% report KRAS mutations.
- Drugs: information related to antineoplastic agents is found in 11% of GEO cancer datasets.

The analysis of ArrayExpress found 16,255 cancer-related datasets, 77% of which also exist on GEO.

Our unified data mapping approach streamlines the identification and querying of datasets across multiple public databases. Additionally, it uncovers otherwise hard-to-detect information and reveals meaningful trends, thereby providing researchers with valuable insights for conducting informed secondary data analyses.

#1088 LiverSCA 2.0: An enhanced and comprehensive cell atlas of human hepatocellular carcinoma, intrahepatic cholangiocarcinoma and metabolic dysfunction-associated steatotic liver disease/steatohepatitis.

Fanhong Zeng, Renwen Long, Tina Suoangbaj, Irene Oi-Lin Ng, Daniel Wai-Hung Ho

The University of Hong Kong, Hong Kong, China

Hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC) are two distinct types of primary liver cancer (PLC) characterized by a considerable degree of cellular and molecular heterogeneity. We recently developed a web-based cell atlas called LiverSCA with a user-friendly interface, comprehensive functionality, and a complete set of analytical pipelines. In the previous version, LiverSCA includes 40 subjects (35 HCC patients with various etiological risk factors and five healthy controls) with a total cell count of more than 140,000 cells. It facilitates the exploration of gene expression patterns, cellular composition, and intercellular communication in the liver and PLC tumor microenvironment, aiming to help researchers discover new mechanistic insights into the complex tumor microenvironment of HCC. To further enhance the comprehensiveness of LiverSCA, we have expanded its catalog with additional datasets of different phenotypes/subtypes of liver and PLC, such as intrahepatic cholangiocarcinoma (ICC) and metabolic dysfunction-associated fatty liver disease/steatosis (MASLD/MASH). The current enhanced version of the LiverSCA cell atlas contains six phenotypes (normal, HBV-HCC, HCV-HCC, non-viral HCC, ICC, and MASH), encompassing 63 patients and more than 248,000 cells. In addition, we have incorporated a comparative visualization method that allows users to simultaneously examine and compare gene expression levels between two different phenotypes. We are committed to the continued development of LiverSCA to narrow the knowledge gap between different research fields and accelerate knowledge transfer in the scientific community. We envision that it will become a valuable resource that supports researchers to conveniently study the cellular and molecular landscape of the liver and PLCs. In addition, as scRNA-seq technology is still rapidly evolving, more features, such as spatial transcriptomics and multimodal omics, will be considered in future updates.

#1089 Developing subtype-specific pediatric cancer molecular targets by aggregating diverse genomic data resources to the pediatric cancer (PeCan) knowledge base portal.

David B. Finkelstein, Delaram Rahbarinia, Ramzi Alsallaq, Stephanie Sandor, Michael Macias, Jian Wang, Sue Qiu, Brian Curran, Michael Edmonson, Andrew Thrasher, Jobin Sunny, James Madson, Scott Foy, Brent Orr, Mark R. Wilkinson, Clay McLeod, Michael Rusch, Xiaotu Ma, **Jinghui Zhang**

Computational Biology, St. Jude Children's Research Hospital, Memphis, TN

Knowledge about molecular targets for pediatric cancer has accelerated exponentially in recent years thanks to the increased application of multi-omics profiling in both research and clinical settings. The efficacy of genomic-based clinical interventions may depend on whether observed genomic abnormalities are fundamental to the pathogenesis of a specific cancer subtype. At present such information is limited in pediatric cancer due to the rapid evolution of subtype discovery and classification as well as disease heterogeneity owing to the presence of many rare cancer types. To develop a comprehensive resource of molecular targets in pediatric cancer subtypes, we have integrated somatic variants in pediatric cancer samples (n=~5,500) currently hosted in PeCan Portal (https://pecan.stjude.cloud/) with those (n=~1,400) profiled by NCI's Childhood Cancer Data Initiative (CCDI) molecular characterization program. To leverage the recently developed WHO CNS5 classification of CNS tumors, we reclassified 2,167 relevant samples based on molecular drivers and histology as a major update to our pediatric cancer ontology definitions, which have recently become a shared standard in the CCDI community. To determine driver genes in disease pathways relevant to specific cancer types, we analyzed the pathogenicity of SNVs and indels, CNVs, SVs and gene fusions and integrated the findings with driver genes reported in 176 publications. A total of 469 distinct driver genes were mapped to 40 pathways across the 22 major pediatric cancer types, which include 3 types of hematological malignancies (HM), 10 types of CNS tumor and 9 types of solid tumor (ST). Pathways with driver genes disrupted across the broad spectrum of cancer types include cell cycle, chromatin remodeling, PI-3K, RAS signaling and telomere maintenance. By contrast the vast majority (>90%) of the transcription factors (TF) are lineage-specific— among the 128 TF genes identified as driver genes, only 11 affect multiple lineages. These include *MYC/MYCN* which affect HM, ST and CNS tumors; *ERG*, *EWSR1*, *FLI1* and *WT1* which affect HM and ST; and *KLF4*, *MED12*, *MN1*, *MYB* and *TCF4* which affect CNS and HM. These results highlight the importance of developing an infrastructure for exploring mutation prevalence in driver genes and pathways at the cancer subtype level, which will be supported in the upcoming release of the PeCan portal. This, coupled with new features for dynamic exploration of mutational signatures, global expression maps and gene-level transcription across the cancer subtype classification tree, will greatly expand our knowledge of molecular targets and vulnerabilities in pediatric cancer, paving the way for novel therapeutic advancements

#1090 Multiomic analysis of Taiwanese NSCLC: insights from a predominantly non-smoking cohort.

Hsu-Ching Huang[1], Samira Masoudi[2], Daniel Halmos[3], Chien-jung Huang[4], Yu-Chao Wang[4], Yi-Chen Yeh[5], Yung-Hung Luo[1], Han-Shui Hsu[6], Yuh-Min Chen[7], Chun-Ku Chen[8], Sandip Patel[9], Albert Hsiao[10]

[1]Division of Thoracic Oncology, Department of Chest Medicine, Taipei Veterans General Hospital, Taipei, Taiwan,[2]Halıcıoğlu Data Science Institute, University of California, San Diego, San Diego, CA,[3]Bioinformatics and Systems Biology Graduate Program, University of California, San Diego, San Diego, CA,[4]Institute of Bioinformatics, National Yang Ming Chiao Tung University, Taipei, Taiwan,[5]Department of Pathology and Laboratory Medicine, Taipei Veterans General Hospital, Taipei, Taiwan,[6]Division of Thoracic Surgery, Department of Surgery, Taipei Veterans General Hospital, Taipei, Taiwan,[7]Department of Chest Medicine, Taipei Veterans General Hospital, Taipei, Taiwan,[8]Division of Cardiopulmonary Radiology, Department of Radiology, Taipei Veterans General Hospital, Taipei, Taiwan,[9]Center for Personalized Cancer Therapy, Division of Hematology and Oncology, UC San Diego Moores Cancer Center, San Diego, CA,[10]Department of Radiology, University of California, San Diego, San Diego, CA

Introduction: We curated multiomic data including whole exome sequencing (WES) and high-resolution chest CT from patients diagnosed with NSCLC, and evaluated the potential for a multitask deep learning algorithm to predict tumor mutational status and WES signatures from CT.

Materials and Methods: Taipei Veterans General Hospital (TVGH) initiated a multi-omic databank integrating clinical, radiological, pathological, and genomic data from NSCLC patients. A predefined pipeline identified INDEL and SNP mutations with high or moderate impact using Ensembl Variant Effect Predictor (VEP). PCA reduced dimensionality of the WES binary data, and t-SNE was used for clustering and visualization. Chest CT was performed using scanners with ≥ 20 detector rows, at end-inspiration with 120 kV, automated exposure control, and a 50 cm field of view. Patients were split 80% for training and 20% for validation. 3D CT image data centered on lesions were rotated, resampled and cropped. A 3D ResNet-18 network, pre-trained on Kinetics-400, was used for predicting common NSCLC mutations (EGFR, TP53), and the first and second principal component coefficients.

Results: A total of 317 patients were included, with 74% non-smokers.(Table 1) Of 109 LDCT detected nodules, only 27 were from smokers. PCA of 12,023 gene mutations identified five principal components. t-SNE visualized the clustered distribution of the top three genes with the highest weights: EGFR, TP53, and RBM10, along with tumor mutation burden (TMB) and EGFR co-mutation status. Performance of the deep learning algorithm in the validation set (n=63) showed: EGFR mutation prediction: AUROC 71%, PPV 71%; TP53 mutation prediction: AUROC 76%, NPV 94%, sensitivity 0.84, specificity 0.72.Pearson correlation for prediction of the coefficients of the genetic signature principal components from CT images were 0.37 and 0.47.

Conclusion: Deep learning algorithms can infer genetic mutation signatures from CT radiologic features. Further optimization is needed.

Demographic distribution of 317 NSCLC patients.

Age(range)		61(11)	
Sex	Male	104	33%
	Female	214	67%
Tabaco use history	Never	236	74%
	Current or Previous	81	25%
Chest CT feature	pure GGO	104	33%
	part-solid	93	29%
	solid	121	38%
Family history of lung cancer	present	49	15%
	absence	268	84%
Stage	Tis	97	31%
	stage IA (MIA)	144	45%
	stage IB	23	7%
	stage II	21	7%
	stage III	26	8%
	stage IV	5	2%
Histology	Adenocarcinoma	300	94%
	Squamous cell carcinoma	9	3%
	adenosquamous cell carcinoma	5	2%
	others*	3	1%
WHO grading of adenocarcinoma	grade 1	184	58%
	grade 2	37	12%
	grade 3	79	25%
	NA	17	5%

BIOINFORMATICS / COMPUTATIONAL BIOLOGY / SYSTEMS BIOLOGY / CONVERGENT SCIENCE: Tumor Subtypes and Biomarker Discovery
Poster Session

#1095 Machine learning models predict tumor-specific total mRNA expression associated with clinical outcomes across 14 cancer types.

A. Paul[1], S. Ji[1], J. C. Lal[1], C. Fong[2], S. Vinod[3], H. Hu[4], K. Chen[1], M. Montierth[1], Q. Tran[1], W. Wang[1];
[1]UT MD Anderson Cancer Center, Houston, TX, [2]School of Engineering and Computer Science, Baylor University, Waco, TX, [3]School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, [4]Department of Statistics, Rice University, Houston, TX

Understanding unique cancer tumor phenotypes is critical in determining disease progression, drug response, and clinical outcomes. Tumor cell-specific total mRNA expression (TmS) which quantifies total mRNA content within tumor cells, has been shown as a robust biomarker to unravel genetic makeup of tumors and their surrounding environments. Currently, quantifying TmS from transcriptomic data is time consuming and resource intensive. Moreover, the unavailability of matched DNA/RNAseq data with clinical annotations limits the translation of TmS to clinical settings. Machine learning (ML) is powerful to create blackbox models that utilize raw high-dimensional omic data to predict clinical outcomes. In this work, we propose TmSNet, TmS prediction by ensemble Network. It is an ML framework that extracts cancer-type-specific gene expression, methylation, and miRNA profiles from 14 cancer types in TCGA cohort (Train =70%, test =30%) to predict TmS. For each cancer type, we extracted relevant features with an ensemble feature extractor block, using random forest (RF), gradient boosting (GB), lasso, and elastic net (EN) models. The extracted features from each model were used to train an ensemble of ML models consisting of variational auto encoder (VAE), model agnostic meta learning (MAML), support vector machine (SVM), and multi-layer perceptron (MLP). TmSNet achieved strong predictive performance across all cancer types (Table 1). Our findings suggest that TmSNet effectively predicts TmS from limited data, with strong performance and limited overfitting. The predicted TmS values from TmSNet successfully stratifies patient survival within early and late-stage cancer. This can serve as a valuable prognostic tool for multi-omic analyses in cancer, with implications for tumor phenotyping, cancer screening, and therapeutic intervention.

No.	Cancer Type	Root Mean Squared Error (RMSE) (average = 0.14)	Feature Extraction Model	Prediction Model
1.	Bladder urothelial carcinoma (n=337)	0.15	Lasso	VAE
2.	Head and neck squamous cell carcinoma (n=469)	0.18	RF	VAE
3.	Prostate adenocarcinoma (n=283)	0.17	EN	VAE
4.	Kidney renal papillary cell carcinoma (n=177)	0.13	EN	VAE
5.	Kidney renal clear cell carcinoma (n=274)	0.06	EN	MAML
6.	Colorectal cancer (n=335)	0.15	EN	VAE
7.	Liver hepatocellular carcinoma (n=353)	0.15	EN	VAE
8.	Lung squamous cell carcinoma (n=354)	0.12	EN	MLP
9.	Lung adenocarcinoma (n=375)	0.15	Lasso	VAE
10.	Pancreatic adenocarcinoma (n=102)	0.14	EN	MAML
11.	Thyroid carcinoma (n=220)	0.15	Lasso	VAE
12.	Breast cancer (n=714)	0.14	EN	MAML
13.	Stomach adenocarcinoma (n=252)	0.14	EN	VAE
14.	Uterine corpus endometrial carcinoma (n=305)	0.17	Lasso	MAML

Table 1. RMSE obtained for each cancer type using the best feature extraction and prediction model in TmSNet.

#1096 Estimating risk factors and predicting various cancer types using integrated real-world data: An AI-based approach.

Md Ashad Alam[1], Nick Duesbery[1], Daniel Fort[2]

[1]Ochsner Health System, New Orleans, LA,[2]Ochsner Health Systerm, New Orleans, LA

Cancer remains a critical global health issue with numerous risk factors. However, comprehensive large-scale studies in the United States, particularly those examining cancer-related medical conditions, are limited. This study aims to develop AI-driven precision medicine algorithms by integrating data from electronic health records (EHR) to identify patients at elevated risk for various cancers, using age-, gender-, race-, and area deprivation index (ADI)-stratified data. By employing disease, demographic, and ADI data as covariates, we apply interpretable machine learning models to uncover insights into cancer risk factors. We explored eight machine and deep learning algorithms including logistic regression (LR) and random forest (RF) using 1,229 truncated unique ICD-10-CM codes (selected through related risk analysis) as binary features across a total pool of 1.6 million patients with 5-fold cross-validation. First, we applied both LR and RF methods for all patients. Using LR, we observed that conditions such as nicotine dependence, irregular menstruation, and urinary disorders were highly associated with subsequent cancer diagnoses, while acute pharyngitis and cardiac murmurs were associated with a decreased risk, achieving an overall accuracy of 86%. RF further identified conditions, including acute pharyngitis, nicotine dependence, and other soft tissue disorders with high predictive value, achieving 96% training accuracy and 86% test accuracy. Finally, eight machine and deep learning algorithms were evaluated on various sample sizes, ranging from 200 to 20,000, assessing accuracy, precision, recall, F1 score, ROC AUC, and MCC. Notably, RF accuracy increased from 69% to 78%, and ROC AUC from 79% to 86% as sample size grew, underscoring the model's robustness with larger datasets. Our findings contribute to early cancer detection and provide insights into disease progression. This comprehensive analysis has the potential to improve early detection, diagnostic pathways, and understanding of the pathological processes underlying cancer worldwide, as well as highlight variable performance across methodologies on this kind of data.

#1097 Tumor purity (TP) as a predictive biomarker for adjuvant radiotherapy (ART) in stage IIIAN2 non-small cell lung cancer (NSCLC): An ancillary analysis of Lung ART-IFCT 0503.

Wael Salem Zrafi[1], Victor Albarran-Artahona[2], Maria-Rosa Ghigna[3], Nicolas Signolle[4], Nathalie Cozic[5], Filippo Gustavo Dall'Olio[2], Julien Adam[6], Ludovic Lacroix[7], Cecile Le Pechoux[8], Antonin Levy[8], Daniel Gautheret[9], Benjamin Besse[2]

[1]Department of Biostatistics and Bioinformatics, Gustave Roussy, Villejuif, France,[2]Department of Medical Oncology, Gustave Roussy, Villejuif, France,[3]Department of Pathology, Gustave Roussy, Villejuif, France,[4]UMS AMMICa PETRA, CNRS UAR3655/ INSERM US23, Gustave Roussy, Villejuif, France,[5]Department of Biostatistics, Gustave Roussy, Villejuif, France,[6]INSERM U1186, Gustave Roussy, Villejuif, France,[7]Biopath department, Gustave Roussy, Villejuif, France,[8]Department of Radiation Oncology, Gustave Roussy, Villejuif, France,[9]I2BC, Universite Paris-Saclay, CNRS, Gif sur Yvette, France

BACKGROUND: TP is defined as the proportion of cancer cells in the tumor tissue and serves as a key characteristic of the tumor microenvironment .ESTIMATE algorithm infers TP on samples using gene expression. Its association with the effects of radiotherapy has not yet been explored.
METHODS: In the Lung ART trial (NCT00410683), 501 patients (pts) with completely resected stage IIIN2 NSCLC (AJCC 7th), were randomized to ART or observation (Obs). (Neo)adjuvant chemotherapy was permitted. RNA libraries were successfully extracted from 285 FFPE surgical primary, captured using SureSelect XT HS2 RNA Agilent kit, paired-end sequencing was conducted. Molecular barcode (MBC) was preprocessed using AGeNT tools and quantification was achieved using Kallisto. Gene level TPM quantification was used for TP estimation, then assessed as a prognostic and predictive biomarker using uni and multivariable Cox and Kaplan-Meier analyses.
RESULTS: Among the 285 pts, 141 (49.5%) received ART. Clinical baseline characteristics were well balanced between ART and Obs arms, 61 years as a median age, 30-31% female, 77-83% of non-squamous histology respectively. TP was higher in the ART arm (0.65) Vs (0.63) in Obs p=0.006. TP was positively associated with cancer cell percentage assessed by pathologist on Hematoxylin and Eosin (HE) slides (rho 0.23, p 7.2e-5). Higher TP was significantly associated with lower T cells infiltrate, $CD3^+$ and $CD8^+$ cell density on IHC (rho -0.52, -0.45 respectively $p < 2e-16$, p 1.1e-14). Higher TP was associated with higher necrosis percentage if not exceeding 50% of the slide (rho 0.3 p 4.2e-6). No significant drop in TP was found after neoadjuvant treatment. Patients with high TP, according to the median value in each arm, had worse overall survival (OS) and disease-free survival (DFS) with a median OS of 48.5 vs. 106.5 months (m) for low TP patients (p=0.0008) and median DFS of 18.4 Vs 48 m (p=0.017). The predictive role of TP was amplified in the ART arm, with median OS of 38.9 m (high TP) vs not reached (low TP; p=0.0017) and median DFS of 24 (high TP) vs 58.7 m (low TP; p=0.044). A multivariable cox model showed a significant interaction between TP and ART (HR 71.3 for OS p=0.04 and 33 for DFS p=0.07).
CONCLUSIONS: Our results show that higher TP is significantly associated with poorer outcomes in Stage IIIAN2 NSCLC. ART may have better efficacy in tumors with lower TP. TP is a potential new predictive biomarker for ART. Confirmatory studies are needed.

#1098 Combining random projection and stacking learning for NSCLC subtype classification based on transcriptomic data.

X. Wu, J. Wang, S. Wan;
University of Nebraska Medical Center, Omaha, NE

Lung cancer is the leading cause of cancer-related death and the second most diagnosed cancer worldwide. Non-small cell lung carcinoma (NSCLC), the predominant type of lung cancer, presents diverse pathological features and cellular heterogeneity. As the two major NSCLC subtypes, lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) differ in their cells of origin and exhibit distinct disease progression, which, however, are difficult to distinguish solely based on conventional experimental methods like histological examination. Previous studies indicated that predicting subtypes based on transcriptomic profiles is a feasible and promising method. However, existing computational approaches may not fully capture information from transcriptomics data, and they are not effective in dealing with high dimensionality data. To address these concerns, we present a novel method called RPSLearner that combines Random Projection (RP) with Stacking Learning, to efficiently aggregate diverse information from base models for accurate identification of NSCLC subtypes. Specifically, we applied multiple RPs to reduce the dimensionality of transcriptomic profiles, which were then combined to capture diverse feature information. RP could efficiently reduce the dimensionality of the high-dimensional omics data while preserving distances between data points. Subsequently, the dimension-reduced feature vectors were classified by ten machine learning base models, which were further integrated by a stacking meta-learner. The stacking strategy combines the prediction results from various base models using a multi-layer perceptron (MLP) to boost the prediction performance. Experimental results based on 1,333 lung cancer patient samples from The Cancer Genome Atlas Program (TCGA) database suggested that RPSLearner significantly outperformed existing state-of-the-art methods. We further benchmarked RPSLearner against different cases including using only base classifiers, ensemble random projection with classifiers, and score ensemble methods, and demonstrated that RPSLearner achieved the best performance across multiple metrics including accuracy, AUC and F1-score. We believe that RPSLearner has direct positive impacts on downstream lung cancer risk stratification and tailored treatment design. We also expect that RPSLearner can be customized and extensible to identify subtypes of other types of cancer.

#1099 Leveraging RNA-seq for breast cancer survival analysis and risk stratification.

P. Naseri-Nosar, Z. Eftekhari, C. Wong;
City of Hope National Medical Center, Duarte, CA

Advancements in breast cancer treatment have significantly improved patient survival rates; however, the increasing incidence and stratified mortality risks underscore the critical need for Precision Medicine and Artificial Intelligence (AI) tools to provide clinicians with more accurate assessments of patient status. In this study, we analyzed solid tumor RNA-seq data from over 1,800 breast cancer patients treated at the City of Hope National Medical Center. We employed both supervised and unsupervised machine learning methodologies to develop models aimed at improving the understanding and prediction of patient survival outcomes.

Unsupervised clustering of RNA-seq data revealed that, despite conventional clinical groupings (HR+/ER+, HER2+, HR+/HER2+, and TNEG), breast cancer patients may segregate into distinct subgroups with statistically significant differences in survival rates. Notably, HR+/ER+ patients, typically associated with better survival outcomes, were observed to cluster alongside high-risk TNEG patients, challenging conventional survival expectations.

Building on these insights, we developed two classes of predictive survival models: (i) binary classifiers to predict survival beyond specific time thresholds (e.g., 3- and 5-year survival) and (ii) probabilistic survival models that estimate survival probabilities for clinician-specified timeframes. Evaluation on a 10% hold-out test set demonstrated strong model performance. The top-performing 3-year survival model achieved AUC's of 0.88 and 0.84 for the ROC and Precision-Recall curves, respectively, while the 5-year survival model reported elevated AUC's exceeding 0.95 and 0.98. Furthermore, the probabilistic survival model achieved a robust concordance index (C-index) of 0.83.

Our findings, in their current form, may equip clinicians with data-driven solutions to support more accurate diagnoses and better-informed personalized treatment planning. Further integration of these models with EMR data may provide deeper insights into the biological and clinical factors underlying the observed survival disparities among breast cancer patients. These insights could inform both improvements to current clinical practices and the development of novel therapeutic approaches.

TUMOR BIOLOGY: Cancer Stem Cells
Poster Session

#11 Overexpression of midasin drives cancer stem cell dynamics in *ESR1* mutant breast cancer.

Mounika Pamukuntla[1], Manasa Kotina[1], Bipika Banjara[1], Afia Ohemeng[1], Alicia Hudson[1], A. Michael Davidson[1], Jullian Pope[2], Selina Darlin-Reed[1], Simak Ali[3], Syreeta L. Tilghman[1]

[1]Basic Pharmaceutical Sciences, Florida A&M University College of Pharmacy & Pharmaceutical Sciences, Tallahassee, FL,[2]Departemnt of Biological Sciences, Florida A&M University College of Pharmacy & Pharmaceutical Sciences, Tallahassee, FL,[3]Department of Surgery and Cancer, Imperial College Of London, London, United Kingdom

While most cases of estrogen receptor-positive (ER+) breast cancer initially respond to endocrine therapy, many women acquire resistance. Two major hurdles hampering the response of metastatic breast cancer patients to endocrine therapy is constitutively active somatic mutations in the ER ligand binding domain (LBD) and the presence of cancer stem cells (CSCs). We previously identified midasin, a novel ribosomal protein, which was significantly overexpressed in letrozole-resistant MCF-7 cells enriched with CSCs. However, the consequences of midasin (MDN1) expression, stemness, and *ESR1* mutations are unclear. We hypothesize that *ESR1* mutations along with MDN1 cooperate to alter the microenvironment and position breast cancer cells for a survival advantage through increased stemness. Here, the objective is to evaluate the significance of midasin inhibition on CSCs formation in MCF-7 cells with *ESR1* LBD point mutations. RNA-Seq studies were conducted in MCF-7 parental cells (MCF-7^{LUC}) and cells containing ESR1 LBD point mutations (MCF-7^{Y537S} and MCF-7^{D538G}) and MDN1 levels were similar. Western blots were conducted and MCF-7^{D538G} cells expressed the highest level of MDN1 followed by MCF-7^{Y537S} and MCF-7^{LUC}. ER levels were highest in the MCF-7^{D538G} followed by the MCF-7^{LUC} and MCF-7^{Y537S}. Viability studies were performed using 1-12 µM ribozinoindole-2 (Rbin-2) (ie., midasin inhibitor) and while all cells were responsive, the MCF-7^{LUC} and MCF-7^{D538G} cells were more sensitive. After 48 hours of Rbin-2 treatment MDN1 protein expression was decreased in all cell lines. Wound healing assays were conducted and the MCF-7^{D538G} cells were the most migratory followed by the MCF-7^{LUC} and MCF-7^{Y537S} (ie., motility was inhibited by 48-55%). As there is a functional link between stemness and motility, mammosphere formation assay were performed. Interestingly, the MCF-7^{D538G} cells formedlarge, dense spheres with an average length of (480.3 ± 7.4 µm), followed bythe MCF-7^{Y537S} which formed large and hollow spheres (435.7 ± 0.52 µm),and the MCF-7^{LUC} cells which formed small, dense spheres (372. ± 2.7µm). Rbin-2 treatment induced a 57%decrease in MCF-7^{LUC} mammosphere formation, while the *ESR1*mutant mammospheres disintegrated, and sizes ranged from (101.8 ± 1.52 - 112.5 ±2.2 µm). Western blot studies were conducted in the mammospheres, and both MCF-7^{D538G} and MCF-7^{LUC} cells were $CD44^{high}/CD24^{low}$ and the MCF-7^{Y537S} cells were $CD44^{low}/CD24^{low}$. The cells were treated with Rbin-2 and CD44 expression was decreased while CD24 was unaltered. Likewise Rbin-2 treatment inhibited the mammosphere expression of MDN1 by 69-86%. Based on the preliminary results, higher levels of midasin and ER are associated with denser mammosphere that exhibit more stemness as indicated by the $CD44^{high}/CD24^{low}$ profile. Together, this may contribute to the highly migratory phenotype and survival advantaged which may be reversed by targeting midasin.

BIOINFORMATICS / COMPUTATIONAL BIOLOGY / SYSTEMS BIOLOGY / CONVERGENT SCIENCE: Tumor Subtypes and Biomarker Discovery
Poster Session

#1101 A novel machine learning classifier for SCLC transcriptional subtypes.

Simon Heeke[1], Ivan Valiev[2], Mikhail Kleimenov[2], Anastasia Sobol[2], Evgeny P. Barykin[2], Mikhail V. Slizen[2], Anastasiya O. Evdokimova[2], Vladimir Kushnarev[2], Artem V. Kosmin[2], Anna A. Butusova[2], Patrick Clayton[2], Francesca Paradiso[2], Sheila Yong[2], Alexander Bagaev[2], Lauren Byers[1], John Heymach[1], **Carl M. Gay**[1]

[1]Department of Thoracic/Head & Neck Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, TX,[2]BostonGene, Corp., Waltham, MA

Introduction: Small cell lung cancer (SCLC) exhibits significant molecular and clinical heterogeneity, leading to suboptimal clinical outcomes. To improve patient stratification and treatment selection, Gay et al. (2021) developed a transcriptional classification system that identified four distinct subtypes: SCLC-A (ASCL1), N (NEUROD1), P (POU2F3), and I (Inflamed). In particular, SCLC-I showed higher frequency of an inflamed gene signature than SCLC-A, N, and P, along with longer duration of response to immunotherapy when added to chemotherapy (e.g., IMpower133 and CASPIAN). However, the SCLC subtype clustering methods used in the original analyses by Gay et al. are not ideal for patient selection and stratification. While Heeke et al. (2024) created a bioinformatics tool for assigning individual patient samples to specific SCLC transcriptional subtypes, its implementation in a clinically applicable assay is still challenging. To address this unmet clinical need, we enhanced the bioinformatics classification approach by creating a machine learning SCLC transcriptional classifier for assigning individual patient tumor samples to subtypes A, N, P, and I.
Methods: In total, 167 RNA-seq samples from George et al. (2015) and Heeke et al. (2024) were used as ground truth and split into training (n=125) and tuning (n=42) sets. Our classifier consisted of three hierarchy-arranged gradient boosting binary models, each trained to distinguish between specific SCLC transcriptomic patterns. The first model distinguished between SCLC-P and non-P (SCLC-A, I, and N), the second between SCLC-I and A plus N, and the third between SCLC-A and N. Obtained probabilities of these six pre-subtypes (two from each model) were harmonized to generate four final probabilities, one for each SCLC subtype, the sum of which equaled one. The classifier was evaluated on the tuning set and then tested on a metacohort of 271 publicly available SCLC samples. The expression profile of classified samples was compared to samples of corresponding subtypes in the tuning set. Gene expression-based tumor microenvironment (TME) classification (Immune-enriched fibrotic [IE/F], Immune-enriched non-fibrotic [IE], Immune desert [ID], and Fibrotic [F]; Bagaev et al. 2021) was also applied to the SCLC metacohort.
Results: The SCLC classifier achieved 0.86 sensitivity and 0.95 specificity on the tuning set. For the evaluation samples, the classifier revealed gene expression patterns that were consistent with those in the tuning set. SCLC subtypes were also associated with specific TME subtypes (Chi-squared, $p = 1.14e-07$). SCLC-I correlated with IE (Pearson correlation, $p = 1.5e-15$) and IE/F TMEs ($p = 0.004$), while SCLC-A and N correlated with the ID TME ($p = 0.005$ and 0.001 respectively).
Conclusion: The proposed transcriptomic tool for subtyping and target expression will be applied to stratify patients across treatment arms in an upcoming clinical trial.

#1102 Integrated (epi)genomic atlas for identification of biomarkers in pediatric gliomas.

Devishi Kesar, Michaela Kristina Keck, Robert J. Autry, David T. W. Jones

DKFZ, Heidelberg, Germany

Pediatric gliomas are a heterogeneous group of brain tumors that affect children and adolescents. Despite recent advances in understanding the genetic and molecular underpinnings of these tumors, the precise mechanisms driving their pathogenesis remain elusive. Thus, we comprehensively investigated the regulatory landscape of pediatric gliomas, focusing on the identification of super enhancer regions and novel transcription factors implicated in the development and progression of both low and high grade gliomas. Using chromatin immunoprecipitation sequencing (ChIP-Seq) data we identified super-enhancer regions, which are key regulators of gene expression and to potentially identify novel enhancer hijacking events that have been shown to be important in oncogenic signaling. By leveraging a large dataset of pediatric gliomas omics data, we systematically identified how these super enhancer regions are associated with specific glioma subtypes. Furthermore, to gain a comprehensive understanding of the molecular landscape of pediatric gliomas, integrating ChIP-Seq data with multi-omic datasets, including RNA-Seq and copy number alterations, revealed key driver genes and pathways. This integrative approach allowed us to uncover potential driver genes and signaling pathways associated with pediatric gliomas. These findings offer a comprehensive view of the regulatory mechanisms in pediatric gliomas, highlighting potential biomarkers and therapeutic targets for personalized treatment approaches.

#1103 Multi-modal machine learning identifies predictive biomarkers of response to neoadjuvant axitinib in renal cell carcinoma with venous tumor thrombus.

Rebecca Wray[1], Hania Paverd[1], Ines Machado[1], Johanna Barbieri[1], Farhana Easita[1], Abigail R. Edwards[1], Ferdia A. Gallagher[1], Iosif A. Mendichovszky[1], Thomas J. Mitchell[1], Maike De La Roche[1], Jacqueline D. Shields[2], Stephan Ursprung[1], Lauren Wallis[1], Anne Y. Warren[1], Sarah J. Welsh[3], Mireia Crispin-Ortuzar[1], Grant D. Stewart[1], James O. Jones[1]

[1]University of Cambridge, Cambridge, United Kingdom,[2]University of Nottingham, Nottingham, United Kingdom,[3]Royal Devon University Healthcare NHS Foundation Trust, Devon, United Kingdom

Venous tumor thrombus (VTT) is present in 10-15% patients with renal cell carcinoma (RCC), where the primary tumor invades the renal vein and inferior vena cava, increasing the morbidity and mortality of curative surgery. Neoadjuvant anti-angiogenic therapies have shown potential to reduce VTT length and improve nephrectomy success rates. The NAXIVA trial, a phase II study of neoadjuvant axitinib in 20 RCC patients with VTT, demonstrated that 35% of patients experienced significant downstaging of VTT. However, the biological mechanisms driving response or resistance in the remaining 65% are unclear. Machine learning has the potential to uncover mechanistic relationships between features in these patients and guide biomarker discovery for early response.In this study, we present a predictive machine learning framework based on multi-modal integration of digital pathology, flow cytometry and plasma cytokine profiling, using tissue and blood samples from the NAXIVA trial. A machine learning model incorporating recursive feature elimination and logistic regression with stochastic gradient descent was developed to predict response. To avoid overfitting, a leave-one-out nested cross validation approach was used for 20 iterations, leaving one patient out at a time and training the model on the remaining 19 patients. Two separate model ensembles were produced, using baseline features (N=62) and features from both baseline and two weeks post-treatment initiation (N=69) respectively. We used a statistical consensus approach to identify key predictive factors.The baseline model achieved an AUC of 0.868, identifying tissue microvessel density, plasma IL-12p70 and plasma CCL17 as key predictive factors. Responding patients had lower plasma IL-12p70, lower CCL17 and higher microvessel density than non-responding patients. Bulk RNA-seq data from pre-treatment tumor biopsies revealed elevated *IL-12R* expression in non-responders. Incorporating features post-treatment initiation improved model performance (AUC = 0.945), with fold changes in PlGF and sTie-2, alongside baseline IL-12p70 and CCL17, emerging as key predictors. Responders showed greater PlGF and sTie-2 induction following treatment onset.In conclusion, our machine learning framework accurately predicts response to neoadjuvant axitinib in RCC patients with VTT. The high microvessel density and growth factor induction in responders suggests they exhibit an “angiogenic” phenotype (clusters 1/2 from IMmotion151), while higher cytokine levels in non-responders suggest an “immunogenic” phenotype (cluster 4). Early changes in PlGF and sTie-2 may serve as actionable biomarkers for patient stratification. Further validation in independent datasets is essential to facilitate clinical integration and improve personalized treatment strategies for RCC patients with VTT.

#1104 Identifying pancreatic cancer subtypes by a novel meta-learning model.

Nick Boos Peterson, Mengtao Sun, Jieqiong Wang, **Shibiao Wan**

University of Nebraska Medical Center, Omaha, NE

Due to the difficulty of both early diagnosis and treatment, pancreatic cancer (PC) is the third leading cause of cancer related deaths in the United States and is projected to become the second by 2030. As a heterogeneous disease, PC can be divided into four main subtypes, with each having differing molecular pathways, survival rates, and responses to therapy. Traditional wet lab approaches to identify PC subtypes, such as histopathological studies or molecular profiling, are costly, laborious, and time-consuming. Previous studies indicate that predicting PC subtypes based on transcriptomic profiling is a feasible and promising method. However, these methods are unable to capture the full complexity of the data and may lead to biased and unstable results. To this end, we present a novel meta-learning algorithm for PC subtype identification based on RNA-seq data only. Specifically, after some basic gene filtering and preprocessing, the transcriptome-based feature vectors were classified by multiple base machine learning (ML) classifiers like logistic regression, linear support vector machine (SVM), and radial basis function (RBF) SVM, whose outputs were then combined with the initial filtered feature vectors to constitute a new set of ensemble feature vectors for a meta-learning model. Our meta-learning model could learn and leverage the diversity of different base classifiers to boost the prediction performance beyond any single ML model. Results based on 100 times leave-one-out cross validation tests on a dataset with 96 PC patients demonstrated that our proposed method could achieve an average AUC of 0.99, which was significantly better than existing state-of-the-art methods. In addition, our meta-learning model remarkably outperformed each individual base classifier, demonstrating that our model could combine diverse results from multiple base classifiers to boost the ensemble performance. We expect that our method will provide a complementary way to characterize different PC subtypes, bringing positive impacts on downstream pancreatic cancer risk stratification and tailored treatment design. Additionally, we believe our meta-learning model can also be extensible to identify subtypes of other types of cancer.

#1105 Development of an in-house methodology to analyze large clinical cancer biomarkers datasets.

Tariq Arshad, Nikhil Wilkins, Gilles Tapolsky, Mike Gazda

Bexion Pharmaceuticals, Inc, San Ramon, CA

Background: BXQ-350 is a novel biologic that modulates sphingolipid metabolism. BXQ-350 is currently being investigated in several clinical studies. Multiple biomarker samples are collected in the course of these studies to support clinical development and illustrate the mechanism of action, support patient selection, and associate response with trends. The resulting large data set associated with these different biomarkers requires an analytical tool to process the data. To meet those ends, Bexion Pharmaceuticals developed an in-house analytical method to perform analysis on these data points. The platform performs data wrangling, processing, calculations, and produces statistical and graphical outputs from multiple different input sources.

Methods: The analytical methodology was developed using Python v3.13 and open-source modules. Windows Excel files are used for the input and are formatted in Excel to be properly read. The outputs are statistical Excel files and graphical visualization of the data.

Analytical Methodology 1. Import Excel file and create a pandas dataframe object 2. Merge dataframes based on patient identification 3. Perform data wrangling on the merged dataframe Create or update a MySQL table if applicable 4. Process the data and perform calculations, including QC samples incorporated in each analysis 5. Perform statistical analysis of QC samples to determine which changes would be statistically significant 6. Perform a second round of processing and calculations on data that passed QC 7. Group and separate data for output handling 8. Create graphs and tables

Results: The analytical methodology was designed to analyze any type of biomarker data set, allowing the comparison of results across biomarkers and supporting analysis of potential correlations. Serial analysis can compare trends in biomarkers results and clinical outcome(s). In addition, statistics are derived for both individual patients and groups to stratify the data.

Conclusion: Large biomarker data sets are collected in the course of clinical studies to support clinical development. Furthermore, such analyses can support understanding the mechanism of action and guide patient selection. The method developed allows for flexible and granular analysis of biomarkers and correlative analysis across different groups of biomarkers or individual patients and groups of patients

#1106 RNA-based risk model for nasopharyngeal carcinoma distant metastasis.

Zhaozheng Hou[1], Ping Feng[2], Kazi Anisha Islam[1], Songran Liu[1], Ying Wang[2], Yingpei Zhang[1], Michael King-yung Chung[1], Ngar-Woon Kam[1], Zi-Lu Huang[2], Victor Ho-Fun Lee[1], Anne WM Lee[1], Dora LW Kwong[1], Wai-Tong Ng[1], Yun-Fei Xia[2], Wei Dai[1]

[1]University of Hong Kong, The - Li Ka Shing Faculty of Medicine, Hong Kong, Hong Kong,[2]Sun Yat-sen University Cancer Centre, Guangzhou, China

Background: With advancements in the treatment of nasopharyngeal carcinoma (NPC), the five-year relative survival rate for patients has the potential to reach upwards of 90%. However, patients who develop distant metastases after receiving primary treatment suffer from a significantly reduced survival rate.
Method: We analyzed bulk-RNA sequencing of primary NPC biopsies and clinical characteristics from 177 patients across multiple centers. We established a prediction model for distant metastasis and patient stratification in NPC. Patients diagnosed with NPC at four Hong Kong hospitals (Queen Mary, Queen Elizabeth, Princess Margaret, and Pamela Youde Nethersole Eastern Hospitals) and at Sun Yat-sen University Cancer Center in Guangzhou were selected. Metastatic NPC (MET-NPC) patients were defined as those who developed distant metastasis within three years after standard treatment, while other patients were grouped into non-metastatic NPC (nonMET-NPC). We evaluated the prediction accuracy using the area under the curve (AUC) index with two-way cross-cohort validation and applied Pareto optimization to select the final models. Histological analysis was applied to evaluate the number of Tertiary Lymphoid Structures (TLS) formation in NPC clinical tissues.
Results: Our RNA-based risk score for NPC distant metastasis prediction significantly improved the AUC to 90.4% when combined with a model based on known and identified prognostic clinical parameters including TNM stage, body-mass-index, and plasma EBV DNA level. Pathway-level analysis indicated that the benefit of the proposed risk score might derive from its correlation with the development and activities of B cells and T helper 17 (Th17) cells in the tumor microenvironment, which are highly linked to TLS formation. Multiplex IHC staining further confirmed that the number of TLS was highly correlated with the RNA-based risk score and this number was significantly reduced in MET-NPC.
Conclusions: This study effectively improves the accuracy of NPC metastasis prediction and introduces a new perspective for understanding and forecasting metastasis. We highlighted the potential association of immunosuppression characterized by dysregulated B cell and Th17 function, along with reduced TLS formation, with metastasis. This encourages future studies on understanding how NPC interacts with its tumor immune microenvironment to contribute to metastasis.
Acknowledgements: This work was supported by General Research Fund (17102619) and Theme-based Research Scheme (T12-703/22-R; T123-70323-N) from Research Grant Council, and Health and Medical Research Grant from Health Bureau (08192296) from Hong Kong (SAR), P. R. China, Science and Technology Program of Guangdong Province in China (2021A0505110010).

#1107 Feature selection and machine learning strategies optimize an affordable molecular assay for cholangiocarcinoma subtype.

Ellen Larson, Erik Jessen, Dong-Gi Mun, Jennifer Tomlinson, Amro Abdelrahman, Danielle Carlson, Hojjat Salehinejad, Rory Smoot

Mayo Clinic, Rochester, MN

Background: Cholangiocarcinoma is a molecularly heterogenous cancer arising from the biliary epithelium. Individual omic techniques have identified clinically relevant, targetable subtypes but most tumors have no targetable alterations based on exome or transcriptomic evaluation. Integrated multiomics approaches incorporating transcriptomic, proteomic, and phosphoproteomic characterization provide deeper understanding and identify non-mutated, activated pathways that can be targeted. To optimize the potential clinical utility of these approaches, minimal classifier features must be defined.
Methods: Approximately 60,000 RNA, protein, and phosphoprotein features were extracted from 35 cholangiocarcinomas treated at the primary US study site. 206 patients treated and profiled at an institution in China were also included. Using the Multiomics Factor Analysis clustering method, patients were sorted into 3 distinct molecular profiles. Important molecular features were selected using competing feature selection (FS) methods: Boruta, RreliefF, variable selection using random forests (RF), recursive factor elimination (RFE), median decrease accuracy in RF, and Concrete Autoencoders. Machine learning classification prediction models including Extreme Gradient Boosting, gradient-boosted trees, multinomial logit, and support vector machine (SVM) were tested on each FS. Each combination of FS and predictive model was tested with 10 replicates of 10-fold cross-validation of a randomly-selected balanced test set and an oversampled, balanced training subset. Accuracy, F1 score, AUC, precision, and recall were assessed for each FS and predictive model combination. A custom function of physical assay cost and classification accuracy was optimized to find the best assay. KEGG pathway overrepresentation analysis was used to analyze feature subsets.
Results: A consistent set of 50 important RNA and protein features was identified, including previously reported prognostic factors, like SpryD4 protein expression, as well as previously unreported pathway surrogates, such as Slc2A2 or Pipox protein. Pathway overrepresentation analysis found insulin signaling and resistance and amino acid biosynthesis/metabolism pathways were overrepresented in the discriminative feature set. The gradient-boosted trees algorithm combined with the RFE FS method had the best performance, with a multiclass macro average (±sd) cross-validation accuracy 89% (±6%), F1 score 89% (±8%), AUC 0.98 (±0.02), precision 90% (±10%), recall 89% (±6%).
Conclusions: Feature selection methods and predictive machine learning models work synchronously to develop accurate molecular subtype assays based on just 50 protein and RNA levels. This strategy has the potential to make molecular subtyping for cholangiocarcinoma and other molecularly complex cancers fast and widely available.

#1108 Tissue and circulating DNA methylation profiling can recapitulate SCLC heterogeneity to enable identification of new biomarkers of clinical relevance.

Siyu Liu[1], Mingchao Xie[1], Alexandra Clipson[2], Simon Pearce[2], Brychan Manry[3], Steven Criscione[1], Megan Callahan[1], Priti Chugh[1], Annabel Peachey[2], Ana Ortega-Franco[2], Steven Hill[2], Florent Mouliere[2], Dominic Rothwell[2], James Hadfield[4], Caroline Dive[2], Yashaswi Shrestha[3]

[1]AstraZeneca, Waltham, MA,[2]University of Manchester, Manchester, United Kingdom,[3]AstraZeneca, Gaithersburg, MD,[4]AstraZeneca, Cambridge, United Kingdom

Background: Small-cell lung cancer (SCLC), an aggressive lung cancer type, presents challenges in management due to limited treatment options. In the CASPIAN study (NCT03043872) assessing durvalumab (D) ± tremelimumab (T) and platinum-based chemotherapy in extensive-stage SCLC, improved survival with D + chemotherapy was revealed. Ongoing research exploring SCLC subtypes, treatment responses, epigenetic modifications like DNA methylation, and association with treatment outcomes, is offering insights into tailored treatment and disease progression. Recent studies highlighted the promise of liquid biopsies for sensitive detection and molecular subtyping of SCLC, but the lack of a comprehensive investigation comparing matched tissue and liquid biopsy in SCLC limits a thorough assessment of their effectiveness.

Methods: DNA was extracted from 175 FFPE samples in CASPIAN and TWIST methylome panel analysis, which covers 3.98M CpG sites, was performed to characterize their methylation profiles at promoters and CpG islands. DNA methylation patterns were compared with corresponding RNA-seq gene expression data, and association of methylation with somatic alterations and expression of key SCLC genes was evaluated. A total of 146 CASPIAN serum samples were used to capture ctDNA methylation profiles with T7-MBD-seq methodology.

Results: We uncovered 4 major tissue methylation-based clusters through unsupervised clustering of the top 5% most variable promoter methylation regions. Three clusters were enriched with neuroendocrine (NE) features while one was non-NE driven. Notably, these clusters showed high correlation with RNA-based subtypes and offered distinct insights into clinical outcomes. By integrating promoter methylation levels with other multi-omics datasets, we revealed biological features of each subtype and discovered novel methylation biomarkers, such as ZNF717 and REC8, which were negatively associated with corresponding gene expressions, suggesting that methylation-based profiling could enhance and even expand the current heterogeneity characterization. Other than tissue methylation, ctDNA tumor fractions predicted using copy number alterations served as a prognostic signature for patient outcomes in SCLC. High concordance between ctDNA and tissue methylation provided more confidence in personalized treatment opportunities based on SCLC heterogeneity for this otherwise difficult-to-treat malignancy.

Conclusions: Tissue methylation-based heterogeneity can reveal clinically relevant biomarkers for SCLC, and these clusters are crucial for understanding disease mechanisms and enabling personalized treatment. Additionally, liquid biopsies offer a non-invasive method for subtyping SCLC, potentially improving personalized treatment feasibility.

#1109 BN2 subtype of diffuse large B-cell lymphoma is characterized by distinct alternative splicing.

Sanyeowool An[1], Ja Min Byun[2], Youngil Koh[2], Sung-Soo Yoon[2]

[1]Seoul National University, Seoul, Korea, Republic of,[2]Department of Internal Medicine, Seoul National University Hospital, Seoul, Korea, Republic of

Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous cancer with unique genetic subtypes and clinical profiles. Alternative splicing (AS) is pivotal in regulating cellular growth, tissue invasion, disease progression, and immune evasion in cancer. However, its specific contributions and associations with the molecular subtypes of DLBCL, remain inadequately characterized.Using RNA sequencing (RNA-Seq) and whole-exome sequencing (WES) data from 620 DLBCL patients, we analyzed splicing activity across genetic subtypes classified by the LymphGen algorithm. Splicing-related pathways were assessed using mean z-scores calculated from normalized expression values ($\log_2$(TPM + 0.01)). rMATS was used to quantify splicing events in terms of percent spliced in (PSI) values by comparing each subtype with the combined group of all other subtypes. Notably, splicing-related pathways showed relative activation in the BN2 subtype compared to other subtypes, suggesting a distinct role for splicing dysregulation in its molecular phenotype. Principal component analysis (PCA) of PSI values across all AS events further highlighted BN2's distinct molecular profile, with BN2 samples forming a separated cluster from other subtypes. These findings suggest that splicing dysregulation in the BN2 subtype, defined by translocations in *BCL6*, a well-established transcriptional regulator, may potentiate the downstream effects of disrupted transcriptional control.rMATS analysis revealed significant splicing alterations in RPLP0 (retained introns), *ACTG1* (alternative 5' splice sites and retained introns), *ACTB* (retained introns), and *CD74* (skipped exons), highlighting disruptions in ribosomal stability, cytoskeletal organization, and immune regulation (P-value < 0.05). Differential Transcript Usage (DTU) analysis further validated that isoform-level changes in BN2 corresponded to significant events identified by rMATS. Splicing alterations in *RPLP0*, a ribosomal stability regulator, suggest potential impairments in protein synthesis and cellular homeostasis, while dysregulation of *ACTB* and *ACTG1*, critical cytoskeletal proteins, may enhance cellular motility and invasiveness. These housekeeping genes showed disease-specific splicing in BN2, highlighting their biomarker potential and role in pathogenesis. Similarly, *CD74*, which plays a key role in antigen presentation and immune modulation, showed splicing changes that may contribute to the unique molecular and immune characteristics of BN2.By integrating pathway analyses, PCA, and splicing data, this study highlights BN2-specific molecular vulnerabilities and provides insights into the development of splicing-targeted therapeutic interventions. Future research should focus on validating these findings in preclinical models to evaluate their potential as diagnostic markers or therapeutic targets in DLBCL.

TUMOR BIOLOGY: Stem Cells, Development, and Cancer
Poster Session

#111 Detection of clonal hematopoietic APOBEC3-mediated splicing deregulation.

Inge van der Werf[1], Jane Isquith[1], Emma Klacking[1], Jessica Pham[1], Thomas Whisenant[2], Ludmil Alexandrov[3], Catriona Jamieson[1]

[1]Division of Regenerative Medicine, Department of Medicine, Sanford Stem Cell Institute, University of California San Diego, La Jolla, CA,[2]Center for Computational Biology and Bioinformatics, University of California San Diego, La Jolla, CA,[3]Department of Cellular and Molecular Medicine and Department of Bioengineering, University of California San Diego, La Jolla, CA

Myeloproliferative neoplasms (MPNs) are clonal hematopoietic stem cell (HSC)-derived disorders that often arise as a result of JAK2/STAT3 signal activating mutations. In the setting of microenvironmental inflammatory cytokines, MPNs also acquire splicing alterations that are associated with progression to rapidly lethal secondary acute myeloid leukemia (sAML) (reviewed in *Jamieson, Weisman. NEJM. 2023*). By performing whole genome and RNA sequencing analyses of MPN hematopoietic stem and progenitor populations (HSPCs), we discovered that inflammatory cytokine inducible APOBEC3C upregulation was associated with IL6ST and ADAR1p150 splice isoform overexpressing progenitor proliferation and progression to sAML (*Jiang⋯Jamieson. Cell Reports. 2021*). Thus, we sought to investigate the role of APOBEC3 activation as a driver of clonal hematopoietic progenitor splicing deregulation and propagation.

We cloned human APOBEC3 family genes into pCDH lentiviral vectors and transduced cord blood derived immunomagnetic bead selected $CD34^+$ cells with APOBEC3A, B, C, D, G or H, or pCDH lentiviral backbone controls for whole genome and whole transcriptome analyses. Additionally, CD34+ cells derived from aged normal bone marrow (ABM), collected according to IRB approved protocols, were transduced with APOBEC3C for whole genome and whole transcriptome analyses, coupled with functional assays to assess the effects of APOBEC3 overexpression on RNA splicing and editing deregulation.

Following lentiviral APOBEC3B, APOBEC3C, and APOBEC3G transduction of $CD34^+$ cells, gene set enrichment analysis (GSEA) revealed splicing pathway alterations. Significantly altered APOBEC3-responsive pathways involved genes crucial for hematopoiesis, including ZRSR2, U2AF1, and SRSF2. Additional analysis of RNA sequencing data of CD34+ HSPCs derived from aged normal bone marrow overexpressing APOBEC3C, and CD34+ HSPCs derived from MPNs further confirmed these splicing pathway alterations and revealed a correlation with APOBEC expression levels. Moreover, we observed that lentiviral APOBEC3C wild-type overexpression in ABM-derived CD34+ cells resulted in differential exon usage using splicing reporter assays. Together, these results suggest that detection of APOBEC3-mediated splicing deregulation may inform the development of diagnostic and therapeutic strategies aimed at preventing splicing-mediated MPN progression.

BIOINFORMATICS / COMPUTATIONAL BIOLOGY / SYSTEMS BIOLOGY / CONVERGENT SCIENCE: Tumor Subtypes and Biomarker Discovery
Poster Session

#1110 Longitudinal and multi-regional analysis of cancer phenotypes in hepatocellular carcinoma and intrahepatic cholangiocarcinoma.

Javier Villela Castrejon[1], Dongya Jia[2], Jason T. George[3]

[1]Department of Translational Medical Sciences, Texas A&M University, Houston, TX,[2]Immunodynamics Group, Laboratory of Integrative Cancer Immunology, Center for Cancer Research, National Cancer Institute, Bethesda, MD,[3]Department of Biomedical Engineering, Texas A&M University, Houston, TX

Cancer cells exhibit abnormal metabolic behavior, a feature that allows cancer cells to utilize different metabolic pathways and substrates (such as glucose, fatty acids, and glutamine) to fulfill their energy and biosynthetic requirements. This study aims to explore the spatial and temporal variations in cancer phenotypes in hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA) using single-cell RNA sequencing data.
We utilized single-cell RNA sequencing data from the Sequential NCI-CLARITY cohort (GSE229772). Tumor biopsies were collected longitudinally. We also used the Multi-Regional Cohort (GSE189903), where single cells were isolated from multiple regions (tumor cores, borders, and adjacent normal tissues). Gene signatures in the anabolic and catabolic axis were applied to classify cells into four distinct metabolic clusters: O, W, W/O, and Q. To predict metabolic fluxome and variations, we used FLUXestimator, an online platform that implements single cell flux estimation analysis (scFEA). This unsupervised approach uses a neural network architecture to estimate reaction rates from transcriptomics data. Additionally, pathway enrichment analysis was conducted to corroborate our findings.
Our analysis identified the four distinct metabolic clusters across both cohorts. Briefly, the O cluster is characterized by high oxidative phosphorylation and is associated with better clinical outcomes. The W cluster is defined by high glycolytic activity and is linked to aggressive tumor behavior. The W/O cluster represents a hybrid state with both high glycolytic and oxidative activities, contributing to metabolic flexibility and tumor aggressiveness. The Q cluster relies heavily on glutamine metabolism and is associated with poor prognosis and drug resistance. Normal liver cells were predominantly grouped into the O cluster, with hepatocytes displaying low HIF1, AMPK, and MYC activity, indicative of normal metabolic function. Similarly, border cancer cells were conformed in a distinct cluster, characterized by a low HIF1 score, but a higher AMPK score. Temporal analysis from the Sequential NCI-CLARITY cohort revealed dynamic changes in the distribution of these clusters over time, reflecting the evolution of cancer phenotypes. FLUXestimator predictions and pathway enrichment analysis indicated significant changes in metabolic pathways, supporting the observed phenotypic shifts. This approach validates our comprehensive network model and suggests a method for developing new metabolic networks for cancer modeling based on key features observed during cancer progression.
This study reveals metabolic diversity in HCC and iCCA tumors, identifying clusters linked to tumor progression stages. These phenotypic traits may serve as a potential biomarker for better clinical outcomes.

#1112 Gene expression characteristics of EBV-positive smooth muscle tumors compared to EBV-positive NPC and LELC.

Harold Yu Chung Chan[1], Yi Ren[1], Teo Wei Keat[1], Joseph W. Foley[1], Loh Kwok Seng[1], Raymond KY Tsang[1], Han Shuting[2], Jain Amit[2], Toh Han Chong[2], Joshua K. Tay[1], Victor Lee[1]

[1]National University Hospital Singapore, Singapore, Singapore,[2]National Cancer Centre Singapore, Singapore, Singapore

Background: EBV SMTs are rare stromal malignancies associated with immunosuppression. Despite diversity in their anatomical location and clinical presentation, EBV SMTs likely share common pathways of EBV-driven tumorigenesis. We aim to evaluate the gene expression characteristics of EBV SMTs and compare them with EBV-negative sarcomas and other EBV-positive tumors such as nasopharyngeal carcinoma (NPC) and lymphoepithelial-like carcinoma (LELC).

Methodology: Formalin-fixed paraffin embedded (FFPE) tissue sections were obtained from patients with EBV SMTs, non EBV-SMTs and NPC tumors treated at the National University Hospital, Singapore. FFPE sections for LELC tumors were retrieved from the National Cancer Centre, Singapore. To ensure histological purity, laser-capture microdissection (LCM) was performed for tumor epithelial regions for each tumor specimen. Gene expression libraries were prepared using a specialized mRNA-Seq technique optimized for tiny amounts of FFPE material, followed by next generation sequencing. Differential gene expression analysis and geneset enrichment analysis was performed.

Results: Our preliminary cohort comprised 230 gene expression libraries, including 9 EBV SMT, 16 EBV-negative sarcoma, 161 NPC and 44 LELC libraries. Principal component analysis showed distinct clustering of EBV SMTs separate from EBV-negative sarcomas. EBV SMTs also clustered distinctly when compared to NPC and LELC tumors. Compared to EBV-negative sarcomas, EBV SMTs were enriched for immune genes including CD40 (TNF receptor found on antigen presenting cells, p-adj = 1.33e-13) and MHC Class II genes (HLA-DQA1, HLADQA2, p-adj < 0.005 for both). In contrast, pathways involved in epithelial mesenchymal transition (NES = -1.57, p-adj = 0.0429) were enriched in EBV-negative sarcomas. Although EBV SMTs were more inflamed than their EBV-negative counterparts, we observed that NPC and LELC tumors were further enriched for inflammatory processes compared to EBV SMTs. Biological processes that were upregulated in NPC and LELC tumors included neutrophil chemotaxis (NES = 1.46, p-adj = 0.0325 and antimicrobial humoral immune response mediated by antimicrobial peptide (NES = 1.63 , p-adj = 0.0317). Deconvolution of immune cell types with ESTIMATE, showed that NPC and LELC tumors had the highest immune cell score, followed by EBV SMTs and EBV-negative sarcomas (median score = 656, 595 and 329 respectively, p = 0.006).

Conclusion: Immune processes, including antigen presentation, are enriched in EBV SMTs compared to EBV-negative sarcomas. However, the extent of inflammation appears limited compared to other EBV-positive cancers. Our current work includes expanding our cohort of EBV SMTs for genomic profiling and identifying potential vulnerabilities for targeted treatment.

AACR PROJECT GENIE: AACR Project GENIE Use Cases
Poster Session

#1115 PM-AI agent: A conversational artificial intelligence system for precision medicine and advancing health equity through integrative clinical, genomic and social determinants of health data analysis.

Enrique I. Velazquez-Villarreal[1], Ei-Wen Yang[2]

[1]Integrative Translational Sciences, City of Hope Comprehensive Cancer Ctr., Duarte, CA,[2]Polyagent, San Francisco, CA

Introduction: The integration of clinical, genomic, and social determinants of health (SDoH) data is essential for advancing precision medicine. However, significant challenges remain due to the complexity of cancer research workflows, the technical expertise required, and the pressing need to address cancer health disparities. To overcome these barriers, we developed the Precision Medicine AI Agent (PM-AI)—an innovative conversational AI system that enables users to conduct integrative data analyses through natural language inquiries.
Methods: PM-AI leverages large language models (LLMs) to convert user instructions into executable code and autonomously analyze local stored datasets. The system performs advanced tasks such as subset comparisons, clinical prevalence studies using odds ratio tests, and survival analyses that provide survival curves and hazard ratios. Additionally, PM-AI delivers detailed analytic reports with actionable insights to support decision-making. To validate its utility, PM-AI was applied to a case-control study using data from TCGA and cBioPortal (including the AACR GENIE dataset). The study examined RAS mutations and their impact on the response to FOLFOX (folinic acid, 5-fluorouracil, and oxaliplatin) in colorectal cancer patients. Notably, the analysis incorporated race, ethnicity, and SDoH data, adhering to NASEM recommendations for population descriptors in genomics research, ensuring equitable and representative insights.
Results: PM-AI successfully compared FOLFOX-treated patients with or without RAS mutations and revealed a significant difference in progression-free survival. The result show that KRAS mutations predict a poorer response to FOLFOX and an increased risk of recurrence. These findings were consistent with those reported in the literature, validating PM-AI's independent analytical capabilities.
Conclusion: The development of PM-AI represents a transformative advancement in precision medicine research, addressing key challenges in the integration of clinical, genomic, population-specific variables (race, ethnicity, etc.), and SDoH, into analytical pipelines. By eliminating the need for programming, PM-AI bridges domain expertise with autonomous workflows, empowering researchers across disciplines. Its versatility in handling cross-domain datasets highlights its adaptability to diverse precision medicine applications. Overall, PM-AI ensures equitable and actionable insights, positioning this advance AI system as a scalable tool for advancing precision medicine and promoting health equity in cancer research.

#1116 AI framework for imputing genome-wide somatic mutations in pan-cancer targeted sequencing data for biomarker discovery.

Amirsaman Ashtari, Mohammad Shahrokh Esfahani

Stanford University, Palo Alto, CA

Background: Tumor genotyping via high-throughput sequencing has revolutionized our understanding of various cancer types. The genotyping of tumor biopsies or liquid biopsies has now become an integral part of clinical routines to survey tumors and to identify potential markers for treatment management. This has led to the development of many assays that are either commercially or institutionally available for such testing, where they often target less than 0.5% of the entire genome, focusing on known and recurrent genes for cancer-specific panels, pan-cancer solid tumor panels, or heme panels targeting blood cancers. This enrichment of certain regions has allowed us to find mutations that occur at lower allele fractions. Nonetheless, this approach results in missing hundreds or even thousands of potential mutations, some of which could be potential biomarkers for prognostic or predictive models, or even potential targets for future drug developments. Here, we hypothesize that an artificial intelligence (AI) framework can aid us in unraveling the complex interactions between mutations and thereby impute the off-target space.
Methods: Here, we introduce SPARK (Somatic mutation Prediction via Artificial intelligence for Rapid discovery and Knowledge translation), an algorithm for imputing missing genomic loci in existing genomic panels. SPARK relies on the assumption that there is a latent space in which mutations co-occur or are mutually exclusive across multiple malignancies. This AI-based framework utilizes other data modalities (e.g., gene expression) to train a unified model for solid tumors. During training, we use somatic mutations and RNA expression data, and then employ another network to transfer knowledge, enabling us to take only a small number of mutations from any sequencing panel and impute the rest.
Results: We used genotyping and RNA expression data from TCGA across multiple carcinomas to train the model (n>2000 samples). This pre-trained model is then fine-tuned for each panel for further optimization. To evaluate model performance, we focused on the genes present on the MSK-IMPACT panels obtained from the GENIE Project v16. We found a number of genes that occur with over 1% frequency across multiple carcinomas in TCGA but were not covered by the MSK-IMPACT panels. We then aimed to impute these genes and achieved a precision ranging from 60% to 100% for a minority of genes (e.g., ETV4 and ETV5). The precision was much lower (less than 10%) for many genes, suggesting that those mutations are likely not functional and do not show any strong complex association with the genes in the panel. As the next step, we plan to apply this model to the entire set of samples from the GENIE Project and impute the genes that showed acceptable precision in our TCGA analysis. This larger imputed dataset will enable us to find potential biomarkers from a large number of samples in the GENIE Project.

#1117 cBioPortal for cancer genomics.

Ino de Bruijn[1], Tali Mazor[2], Rima AlHamad[1], Calla Chennault[1], Corey Dubin[3], Jeremy Easton-Marks[2], Zhaoyuan Fu[2], Benjamin Gross[1], Charles Haynes[4], David M. Higgins[4], Jason Hwee[1], Prasanna K. Jagannathan[5], Mirella Kalafati[6], Karthik Kalletla[3], Zeynep Karagoz[6], James Ko[1], Tim Kuijpers[6], Sowmiyaa Kumar[6], Priti Kumari[3], Ritika Kundra[1], Bryan Lai[1], Xiang Li[1], James Lindsay[2], Aaron Lisman[1], Qi-Xuan Lu[3], Ramyasree Madupuri[1], Zain-ul-Abideen Nasir[1], Angelica Ochoa[1], Yusuf Ziya Ozgul[7], Oleguer Plantalech[6], Matthijs N. Pon[6], Baby A. Satravada[1], Jessica Singh[6], Selcuk Onur Sumer[1], Pim van Nierop[6], Floris Vleugels[6], Avery Wang[1], Manda Wilson[1], Hongxin Zhang[1], Gaofei Zhao[1], Ugur Dogrusoz[7], Allison Heath[4], Adam Resnick[4], Trevor J. Pugh[5], Chris Sander[8], Ethan Cerami[2], JianJiong Gao[1], Nikolaus Schultz[1]

[1]Memorial Sloan Kettering Cancer Center, New York City, NY,[2]Dana-Farber Cancer Institute, Boston, MA,[3]Caris Life Sciences, Irving, TX,[4]Children's Hospital of Philadelphia, Philadelphia, PA,[5]Princess Margaret Cancer Centre, Toronto, ON, Canada,[6]The Hyve, Utrecht, Netherlands,[7]Bilkent University, Ankara, Turkey,[8]Harvard Medical School, Boston, MA

cBioPortal for Cancer Genomics is a widely used platform for exploratory, interactive visualization and analysis of large-scale clinico-genomic datasets. cBioPortal provides a range of visualizations and analyses including interactive cohort exploration, OncoPrints, mutation “lollipop” plots, survival analysis, alteration enrichment analysis, and detailed patient-level visualizations. cBioPortal also integrates variant annotations from a variety of sources to facilitate interpretation.

The public cBioPortal (https://www.cbioportal.org) is accessed by >40,000 unique visitors each month and hosts data from >460 studies. All data is also available in the cBioPortal Datahub: https://github.com/cBioPortal/datahub. In 2024 we added 76 new studies (~30,000 samples), including data from the NCI Genomic Data Commons. In addition, >94 instances of cBioPortal are installed at academic institutions and companies worldwide.

cBioPortal partners with AACR Project GENIE to provide access to the GENIE cohort in a dedicated instance (https://genie.cbioportal.org). Users can explore the full GENIE cohort of >229,000 clinically sequenced samples from 19 institutions, as well as cohorts with comprehensive clinical annotations including response, outcome, and treatment history, from the GENIE Biopharma Collaborative (BPC). BPC cohorts for NSCLC (~2,000 samples) and colorectal cancer (~1,500 samples) are available, with more to come.

The past year has brought a variety of enhancements to cBioPortal. A new data type selector on the home page enables users to find studies with specific types of data. The interactive cohort exploration has new ways to explore data with the addition of gene-specific charts to summarize the types of mutations in a gene and the integration of the Plots tab for customizable graphs of any two data attributes. The OncoPrint can now display per group alteration frequency based on any categorical attribute. Variant interpretation is enhanced with the integration of AlphaMissense as a novel annotation source and an update to the latest MutationAssessor data. The patient page also has new visualizations, including mutational signatures and the integration of Chromoscope to visualize structural variations.

We also made significant changes to the backend code to improve both the developer and user experience. The backend code was repackaged and upgraded to simplify and improve the development process. In addition, we are working on switching to an Online Analytical Processing (OLAP) database which will bring significant performance improvements.

cBioPortal is open source: https://github.com/cBioPortal. Development is a collaborative effort among groups at Memorial Sloan Kettering Cancer Center, Dana-Farber Cancer Institute, Children’s Hospital of Philadelphia, Princess Margaret Cancer Centre, Caris Life Sciences, Bilkent University, SE4BIO and The Hyve. We welcome open source contributions from others in the cancer research community.

#1118 The GENIE BPC BRCA Cohort: a real-world repository of standardized clinical and genomic data for young patients with breast cancer.

Evan Seffar[1], Brooke Mastrogiacomo[1], Alex Paynter[2], Protiva Rahman[3], Jesus Fuentes Antras[4], Sonya A. Reid[5], Nikolaus Schultz[1], Michael J. Hassett[6], Ben Park[5], Shawn Sweeney[7], Walid K. Chatila[1], Pedram Razavi[1]

[1]Memorial Sloan Kettering Cancer Center, New York, NY,[2]Sage Bionetworks, Seattle, WA,[3]Vanderbilt University Medical Center, Nashville, TN,[4]Princess Margaret Cancer Centre, Toronto, ON, Canada,[5]Vanderbilt-Ingram Cancer Center, Nashville, TN,[6]Dana-Farber Cancer Institute, Boston, MA,[7]American Association for Cancer Research, Philadelphia, PA

Purpose: The first comprehensive analysis of the detailed clinico-genomic landscape of the breast cancer (BC) cohort of the American Association for Cancer Research (AACR) Project Genomics Evidence Neoplasia Information Exchange (GENIE) Biopharma Collaborative (BPC).

Methods: We analyzed 1,045 BC patients treated at three GENIE-participating institutions. Breast cancer patients in the GENIE registry aged 18-56 at sequencing and with tumor(s) sequenced between 2013 and 2018 were randomly chosen for curation using the PRISSMM framework. Sites of distant metastasis were captured from imaging and pathology reports. A previously published cohort of older patients was used as a point of comparison in identifying age-related differences. Overall survival (OS) was estimated for patients with advanced disease.

Results: Clinical and genomic features were compared across the four subtypes HR+/ HER2 - (N=660), HR+/HER2+ (N=138), HR-/HER2+ (N= 74), and TNBC (n = 173). The age distribution was similar across subtypes, HR+/HER2+ had the highest percentage of patients ages 18-39 (HR+/HER2-: 28%, HR+/HER2+: 39%, TNBC: 33%, HR-/HER2+ 35%). The most frequently altered genes in the cohort were TP53 (HR+/HER2-: 33%, HR+/HER2+: 58%, TNBC: 95%, HR-/HER2+ 80%), PIK3CA (HR+/HER2-: 35%, HR+/HER2+: 33%, TNBC: 8%, HR-/HER2+ 15%), and ERBB2 (HR+/HER2-: 3%, HR+/HER2+: 71%, TNBC: 2%, HR-/HER2+ 84%).

Analysis of patients with metastatic disease (n=818) revealed robust associations with OS and TP53 (HR = 1.87, SF = 100%), ERBB2 amplification (HR = 0.61, SF = 98%), MYC (HR = 1.09, SF = 52%), FGFR2 (HR=0.89, SF = 49.3%), MAP3K1 (HR = 0.94, SF = 35%), DDR2 (HR = 1.09, SF = 33%) and ESR1 (HR=1.07, SF = 32.7%).

In a comparison of older and younger patients with HR+/HER2- subtype, we found that mutations in TP53 (18-39: 35% vs >60: 22%), GATA3 (18-39: 21% vs >60: 10%), and MAP2K4 (18-39: 10% vs >60: 4%) were significantly ($q<0.1$) more prevalent among younger patients. Conversely, PIK3CA (18-39: 31% v >60: 48%), CDH1 (18-39: 3% v >60: 21%), and MAP3K1 (18-39: 5% v >60: 12%) showed higher alteration rates in seniors. These trends were consistent at pathway level with higher alteration of p53 (18-39: 44% vs >60: 29%) and PI3K (18-39: 46% vs >60: 62%).

In patients with any distant metastases, we calculated the metastatic burden, or the number of distant metastases reported in key sites of interest. Patients with a history of adrenal

Metastasis had a higher metastatic burden (49/61, 80.3% with at least 4 distinct sites recorded) than those with no adrenal metastasis recorded (326/764, 42.6% with at least 4 distinct sites recorded).

Conclusions: The GENIE BPC cohort provides a comprehensive clinico-genomic dataset enriched with younger patients with BC, providing a valuable source to improve our understanding of age-related clinical and genomic characteristics and their impact on real-world patient outcomes.

#1119 SoMatt: A foundation model for the tumor genome.

David Andreas Arredondo[1], Ala Jararweh[1], Luis Tafoya[2], Avinash Sahu[1]

[1]University of New Mexico Health Sciences Ctr., Albuquerque, NM,[2]University of New Mexico, Albuquerque, NM

Somatic mutations in cancer cells can lead to diverse phenotypic consequences due to variations in mutation types and locations within the same gene. Interactions between mutations across different genes further contribute to tumor heterogeneity, affecting pathology, phenotype, and clinical outcomes. Current computational methods often treat all mutations within a gene as functionally equivalent and overlook combinatorial effects between genes. Additionally, the diversity of sequencing platforms and gene panels poses significant challenges for integrative genomic analysis across datasets.

Here, we introduce Somatic Attention (SoMatt), a foundation model for tumor genomes that leverages self-attention mechanisms to model interactions among mutations within a tumor. Trained on 167,335 tumors from 149,831 unique cancer patients from AACR Project GENIE, SoMatt generates comprehensive tumor embeddings suitable for various downstream tasks. The model addresses three critical challenges in tumor genome analysis: (a) distinguishing the effects of different mutations within the same gene, (b) capturing complex interactions among multiple mutations within a tumor, and (c) integrating data from diverse targeted mutation panels without requiring uniformity.

We demonstrate that SoMatt accurately predicts patient survival outcomes, achieving a concordance index (C-index) of 0.76 across 90 cancer types, and classifies cancer types with an accuracy of 59%, outperforming baseline models in both tasks. Notably, SoMatt also enables patient stratification beyond traditional subtyping methods by identifying previously unrecognized, molecularly defined patient subgroups with significantly different outcomes, suggesting a novel approach to cancer subtype classification based on tumor molecular profiles.

SoMatt produce a robust and scalable framework for comprehensive modeling of tumor genomes, addressing key challenges in cancer genomics and advancing the potential for precision oncology.

TUMOR BIOLOGY: Stem Cells, Development, and Cancer
Poster Session

#112 ROR1 drives tumor-initiating cells in pancreatic cancer.

Masaya Yamazaki, Takatsugu Ishimoto

Japanese Foundation for Cancer Research, Tokyo, Japan

Tumor-initiating cells are key players in driving chemoresistance and metastasis, making them promising targets for cancer therapy. However, the identity of tumor-imitating cells and molecular mechanisms underlying their capacity in human pancreatic ductal adenocarcinoma (PDAC) remains unclear. To address this, we conducted single-cell RNA sequencing and functional studies in human PDAC xenografts to identify targetable tumor-initiating cells. We found that a subpopulation characterized by a partial epithelial-mesenchymal transition (EMT)-like signature and high expression of receptor tyrosine kinase-like orphan receptor 1 (ROR1) serves as the origin of intratumor heterogeneity in PDAC. Functional analyses demonstrated that ROR1^{high} cells have high tumorigenicity and ROR1 promotes tumor growth, relapse following chemotherapy, and metastasis. We also showed that ROR1 prevents PDAC progression. Mechanistically, ROR1 activates the E2F transcriptional network via c-Myc, accelerating tumor cell hyperproliferation by inducing Aurora kinase B (AURKB). Moreover, epigenomic analyses revealed that *ROR1* expression is flexibly controlled by the enhancer through the YAP/BRD4 axis. Targeting this pathway with a BET inhibitor effectively downregulated *ROR1* expression and suppressed PDAC organoid growth. Collectively, our findings implicate ROR1^{high} cells as tumor-initiating cells in PDAC and illuminate the therapeutic potential of ROR1 as a promising strategy for PDAC.

AACR PROJECT GENIE: AACR Project GENIE Use Cases
Poster Session

#1120 Baseline biomarker analyses of patients with chemorefractory *KRAS* G12C-mutated metastatic colorectal cancer (mCRC) from the phase 3 CodeBreaK 300 study.

M. Fakih[1], Y. Katlinskaya[2], Y. Kuboki[3], E. Chan[2], A. Anderson[2], A. Hindoyan[2], A. Pati[4], A. Reddy[4], L. Salvatore[5];
[1]City of Hope Comprehensive Cancer Center, Duarte, CA, [2]Amgen, Thousand Oaks, CA, [3]National Cancer Center Hospital East, Kashiwa, Japan, [4]Amgen, San Francisco, CA, [5]Universita Cattolica del Sacro Cuore; Comprehensive Cancer Center, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy

Background: The genetic landscape of CRC tumors may influence responses to targeted therapies. In CodeBreaK 300 (NCT05198934), sotorasib (soto) 960 mg plus panitumumab (pani) showed significant improvement in progression-free survival (PFS) compared with trifluridine/tipiracil (T/T) or regorafenib (rego) (hazard ratio, 0.48 [95% CI, 0.30-0.78]; $P = 0.005$) in patients with chemorefractory *KRAS* G12C-mutated mCRC. Here, we evaluated baseline genetic co-occurring alterations and their impact on the efficacy of soto plus pani in CodeBreaK 300.
Methods: Study procedures and eligibility criteria have been previously reported. Next-generation sequencing was performed on baseline tumor and plasma circulating tumor DNA (ctDNA) samples with Tempus xT and Guardant Infinity platforms, respectively. Biomarker status was correlated with clinical outcomes.
Results: A total of 140 available tumor samples and 154 plasma samples were examined. The prevalence of individual gene alterations was similar between arms for both tumor and ctDNA assessments The most common pathogenic KRAS G12C co-alterations occurred in *APC* (82.1%), *TP53* (72.1%), *SMAD4* (23.6%), and *PIK3CA* (17.9%) in tumor samples and in *TP53* (73.4%), *APC* (65.5%), *DNMT3A* (39%), *TET2* (22.8%), and *SMAD4* (21.4%) in plasma samples. An exploratory analysis to determine associations between baseline molecular alterations with ≥10% prevalence and clinical outcomes showed that TGF-β pathway mutations were associated with shorter overall survival and time to progression (TTP) and *TP53* mutation status was associated with shorter TTP. Among the most prevalent co-alterations, clinical benefit with soto 960 mg plus pani vs T/T or rego was observed irrespective of biomarker subgroup. A hypothesis-driven analysis indicated that alterations in *ARID1A* in a few patients were associated with shorter PFS. In patients receiving soto 960 mg plus pani, median PFS was 2.07 months (95% CI, 1.84-3.84; n = 3) for those with *ARID1A* alterations and 5.78 months (95% CI, 4.24-7.59; n = 49) for those without.
Conclusion: Genetic alteration rates in CodeBreaK 300 were similar across all treatment arms and comparable to those observed in CodeBreaK 100 and 101 and *KRAS* G12C+ cases in AACR Genie BPC v1.1. Soto 960 mg plus pani demonstrated consistent clinical benefits compared with T/T or rego across various molecularly defined subgroups. While no additional strong biomarkers associated with response were identified, this study supported the potential association of *ARID1A* alterations with shorter PFS as previously reported in CodeBreaK 101. However, the overall low prevalence of pathogenic *ARID1A* alterations largely limited the interpretation of these findings.

#1121 Pan-cancer prevalence of unusual MTAP alterations: data from the AACR GENIE database.

Mohamed A. Gouda, Camila B. Xavier, Carlos Torrado, Pooja A. Shah, Kunal Rai, Natalie Vokes, Omar Alhalabi, Mark T. Bedford, Jianjun Gao, Nazanin K. Majd, Jenny Li, Milind Javle, Funda Meric-Bernstam, Jordi Rodon

The University of Texas MD Anderson Cancer Center, Houston, TX

Background: PRMT5 inhibitors has shown promising clinical activity in MTAP deficient tumors. Ongoing studies focus on tumors with homozygous *MTAP* deletions; but that doesn't span the whole spectrum of MTAP dysfunctional tumors. Herein, we tried to characterize the pan-cancer prevalence of *MTAP* oncogenic alterations.

Methods: We used the AACR GENIE database v.16.1 to extract data on the prevalence of *MTAP* alterations across different tumor types. OncoKB was used to obtain data on functional significance.

Results: We reviewed data from 214,475 samples obtained from 184,980 patients. Structural variants, mutations, and copy number alterations in *MTAP* were profiled in 55,021, 58,144, and 57,382 samples across different cancers; respetively. A total of 46 *MTAP* fusions were identified; all were likely oncogenic per OncoKB including 12 with level 4 alterations. The most common fusions were *MTAP* intragenic, *MTAP*::*CDKN2A*, and *MTAP*::*CDKN2B* fusions. The highest rates of *MTAP* structural variants were observed in CNS cancers (0.4%; n=1), gastrointestinal neuroendocrine tumor (0.3%; n=1), hepatobiliary cancer (0.2%; n=3), melanoma (0.2%; n=3), and glioma (0.1%; n=5). A total of 356 *MTAP* mutations were identified; including 82 that were likely oncogenic per OncoKB. The most common mutations that were annotated as likely oncogenic were *MTAP* A191Rfs*6, *MTAP* X231_splice, and *MTAP* X116_splice. The highest rates of *MTAP* mutations were observed in endometrial cancer (1.6%; n=36), small bowel cancer (1.6%; n=3), non-melanoma skin cancer, (1.5%; n=6), colorectal cancer (1.2%; n=72), and bladder cancer (1%; n=20). The highest rates of *MTAP* homozygous deletions were observed in glioma (24.6%; n=899), blastic plasmacytoid dendritic cell neoplasm (23.1%; n=3), nerve sheath tumor (18.3%; n=28), mesothelioma (17.7%; n=79), and bladder cancer (16.9%; n=350) (Table 1).

Conclusions: The spectrum of *MTAP* alterations expands beyond *MTAP* deletions; and could be explored in clinical trials targeting *MTAP* loss.

MTAP deletions			**MTAP mutations MTAP structural variants**					
Disease	**%**	**N**	**Disease**	**%**	**Disease**	**%**	**N**	**N**
Glioma	24.6%	899	Endometrial Cancer	1.6%	CNS Cancer	0.4%	1	1
Blastic Plasmacytoid Dendritic Cell Neoplasm	23.1%	3	Small Bowel Cancer	1.6%	Gastrointestinal Neuroendocrine Tumor	0.3%	1	1
Nerve Sheath Tumor	18.3%	28	Skin Cancer, Non-Melanoma	1.5%	Hepatobiliary Cancer	0.2%	3	3
Mesothelioma	17.7%	79	Colorectal Cancer	1.2%	Melanoma	0.2%	3	3
Bladder Cancer	16.9%	350	Bladder Cancer	1%	Glioma	0.1%	5	5
Pancreatic Cancer	10.8%	385	Leukemia	0.9%	Pancreatic Cancer	0.1%	4	4
Melanoma	9.8%	181	Small Cell Lung Cancer	0.9%	Renal Cell Carcinoma	0.1%	1	1
Gastrointestinal Stromal Tumor	9.2%	47	Glioma	0.8%	Endometrial Cancer	0.1%	2	2
Bone Cancer	8.4%	33	Cancer of Unknown Primary	0.7%	Non-Small Cell Lung Cancer	0.1%	8	8
Cancer of Unknown Primary	8.2%	185	Uterine Sarcoma	0.7%	Soft Tissue Sarcoma	0.1%	2	2

#1122 Pan-cancer clinical and molecular landscape of MTAP deletion in nationwide and international comprehensive genomic data.

Hiroaki Ikushima, Kousuke Watanabe, Aya Shinozaki-Ushiku, Katsutoshi Oda, Hidenori Kage

University of Tokyo, Tokyo, Japan

Methylthioadenosine phosphorylase (MTAP) deletion in cancer cells induces vulnerability to protein arginine methyltransferase 5 (PRMT5) inhibition, offering a synthetic lethal target. Early-phase clinical trials have shown promising efficacy of PRMT5 inhibitors in MTAP-deleted tumors, but the full spectrum of patients with MTAP-deleted tumors and their molecular characteristics remain unclear. We report a pan-cancer landscape of MTAP-deleted tumors using comprehensive genomic profiling (CGP) data of 51,828 cases in the Center for Cancer Genomics and Advanced Therapeutics (C-CAT), covering 99.7% patients who underwent CGP in Japan. Findings were validated using TCGA (n = 9,896) and GENIE (n = 178,034) data. MTAP deletion was observed in 9.6% of the C-CAT cohort, 8.9% of TCGA, and 6.6% of GENIE. A machine learning prediction model using pre-CGP clinical data, combined with SHapley Additive exPlanations (SHAP) analysis, identified clinical factors associated with MTAP deletion: sex, age, cancer type, and time intervals from cancer diagnosis to CGP. In C-CAT, MTAP-deleted patients were predominantly male (56.0% vs 47.8%, $p < 0.0001$) and slightly older (mean: 62.4 vs 59.8 years, $p < 0.0001$). MTAP deletion rates were high in pleura (33.1%), bladder/urinary tract (23.8%), brain (19.0%), pancreas (18.4%), biliary tract (15.6%), and lung (14.3%) cancers, but low in prostate (1.2%), bowel (1.7%), cervix (3.6%), uterus (4.0%), ovary/fallopian tube (4.3%), and breast (4.9%) cancers. The TCGA and GENIE cohorts showed similar trends, indicating the broad applicability of therapeutic strategies targeting MTAP deletion across different demographic groups. MTAP-deleted tumors demonstrated a shorter time to standard therapy failure in pan-cancer C-CAT data (median: 380.0 vs 567.0 days, HR: 1.34 [1.30-1.38], $p < 0.0001$) and worse overall survival in TCGA (median: 26.7 vs 93.0 months, HR: 2.36 [2.14-2.61], $p < 0.0001$).

Comprehensive molecular analyses revealed that CDKN2A/B loss was an almost necessary, but not sufficient, condition for MTAP deletion. MTAP deletion was more frequent in KRAS-mutant pancreatic cancer (19.8% vs 8.9%), suggesting a cooperative role of MTAP and KRAS and synergistic effects of PRMT5 and KRAS inhibitors. MTAP deletion was less common in RB1-mutated tumors across multiple cancer types, including pancreatic (7.6% vs 18.7%), lung (2.5% vs 16.1%), and biliary (5.4% vs 16.0%) cancers. In lung adenocarcinoma, MTAP deletion was found in 22.8% of EGFR-mutated tumors, 25.0% of ALK-translocated tumors, and 20.8% of ROS1-translocated tumors, indicating that MTAP deletion is not mutually exclusive with druggable mutations and that PRMT5 inhibitors might benefit patients with known driver mutations.

Our findings define the characteristics of MTAP-deleted cancers, providing a basis for refinement and expansion of synthetic lethal strategies in precision oncology.

#1123 Comprehensive molecular characterization reveals hallmarks and therapeutic insights of collecting duct carcinoma.

Xiaofan Lu[1], Wenxuan Cheng[1], Nassim Tawanaie Pour Sedehi[1], Li Xu[1], Xiaoping Su[2], Roger Mouawad[3], Nizar M. Tannir[4], Gabriel G. Malouf[1]

[1]Department of Cancer and Functional Genomics, IGBMC - Univ. of Strasbourg, Illkirch-Graffenstaden, France,[2]Department of Bioinformatics and Computational Biology, The University of Texas MD Anderson Cancer Center, Houston, TX,[3]Department of Medical Oncology, Groupe Hospitalier Pitie-Salpetriere, Paris, France,[4]Department of Genitourinary Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX

Purpose: Collecting duct carcinoma (CDC) is a rare and aggressive subtype of renal carcinoma, posing significant challenges in diagnosis and treatment. Current treatment relies on platinum-based chemotherapy, yet clinical outcomes remain poor. While targeted therapies and immune checkpoint inhibitors (ICIs) show promise in clear-cell renal cell carcinoma (ccRCC), they have not been evaluated in CDC, and their efficacy remains speculative. This underscores a pressing need for personalized treatment approaches, driven by an in-depth molecular understanding of the disease.

Experimental Design: We conducted a comprehensive molecular characterization of 22 CDC cases using whole-exome sequencing, RNA-seq, and DNA methylation profiling. Renal medullary carcinoma, ccRCC, type 2 papillary RCC, and chromophobe RCC were included as comparators. To verify mutations of interest, we retrieved 25 CDC samples from GENIE (v15.0). We also assessed the association of genomic alterations with response to ICIs using data from the BIONIKK trial (NCT02960906) and the published CheckMate (-009, -010, -025) trials.

Results: We identified frequent mutations in *NF2* (20%), *LZTR1* (20%), and *SMARCB1* (10%), all located on chromosome 22q, along with 22q copy number loss (52%). Mutations in *NF2*, *SAV1*, and *WWC1*—key components of the Hippo signaling pathway—were observed, implicating its disruption (33%) and leading to overexpression of the YAP signature. Notably, a mutually exclusive pattern between DNA damage-related (DDR) mutations (33%) and 22q loss was observed. Transcriptomic analysis highlighted a cell cycle and immune cell enrichment signature in CDC, distinct from other RCC comparators. TME deconvolution revealed two CDC phenotypes tightly associated with genomic alterations. The immune-hot CDC was characterized by enrichment of CD8 T cells, B cells, and tertiary lymphoid structure signatures, and was enriched in DDR mutations. In contrast, the immune-cold CDC was marked by 22q loss and overexpression of the YAP signature. Furthermore, post-hoc analyses using BIONIKK and CheckMate data demonstrated that combining 22q loss and DDR mutations improves the precision of ICI response predictions. We also found a correlation between 22q loss and a CpG Island Methylator Phenotype (CIMP) pattern in CDC, which resembles the aggressive, immune-evasive CIMP subgroup observed in type 2 papillary RCC.

Conclusions: Our comprehensive characterization broadens the molecular understanding of CDC, revealing actionable vulnerabilities such as DDR mutations for immunotherapy and YAP activation for targeted interventions. These findings redefine CDC's therapeutic possibilities, offering a roadmap for precision oncology in this rare malignancy.

#1124 Mining the Kras-p53 mutational interface for synthetic lethality (SL) in pancreatic adenocarcinoma (PA).

Nishanth Thalambedu[1], Nadia Safar[2], Ahmad Mazin M. Safar[1]

[1]University of Arkansas for Medical Sciences, Little Rock, AR,[2]Beth Israel Deaconess Medical Center, Boston, MA

Kras activating mutations are frequent in cancer but are insufficient alone for full malignant transformation. Mutations in the tumor suppressor (TSG) p53 cooperate with mutant Kras and permit full display of oncogenic Ras, both in clinical cases and in experimental models of Kras tumorigenesis. Interestingly, p53 mutations almost never fully abrogate its function, suggesting to us a possible essential role for the (either directly or indirectly) retained transcriptional targets within the p53 network. The phenomenon of oncogene addiction may be the result of this rigid but sufficient TSG output providing adaptations to constant oncogene signaling. It is also plausible that each mutation might provide different adaptations and therefore be differentially selected for in various cases. Drugging the dependency on this adaptation machinery provides an opportunity for SL sparing normal cells. This approach is especially valuable given the limited therapeutic impact of available Kras inhibitors (in scope or in potency). We have shown that specific mutations in p53 with their unique transcriptional lesions cooperate with various Kras mutants in tissue-specific manner. In this work we provide a broader and comparative assessment in PA. We hypothesize that frequency of the top 8 p53 mutational hotspots in PA is non-random with recurring comparable frequencies across independent public datasets (project GENIE, MSK and TCGA). Our work included 4 Kras PA mutants.
Results: Table reports on the frequency of respective mutational pairs.
Conclusion: The available cases in TCGA were limited in number. In the 2 more comprehensive datasets, the frequency of certain pairs between Kras and p53 is remarkably close suggesting a mechanistic justification. For SL considerations, therefore, one needs to examine the transcriptional spectra within defined pairs of Kras-p53 to nominate the appropriate genes for multiplexed gene editing experiments and inform drug discovery.

KRAS	TP53*	GENIE n [%]	MSK n [%]	TCGA n [%]
G12D	R175	154 [23.6%]	53 [23.1%]	-
	R248	129 [19.8%]	45 [19.6%]	2 [22.2%]
	R273	125 [19.2%]	49 [21.4%]	3 [33.3%]
	R282	62 [9.5%]	21 [9.2%]	2 [22.2%]
	G245	55 [8.4%]	13 [5.7%]	-
	Y220	45 [6.9%]	17 [7.4%]	1 [11.1%]
	R196	43 [6.6%]	15 [6.5%]	1 [11.1%]
	R213	39 [5.9%]	16 [6.9%]	-
	R175	44 [21.1%]	21 [23.9%]	1 [12.5%]
G12R	R248	35 [16.8%]	16 [18.2%]	2 [25%]
	R273	41 [19.7%]	18 [20.4%]	1 [12.5%]
	R282	27 [12.9%]	10 [11.4%]	-
	G245	22 [10.6%]	11 [12.5%]	1 [12.5%]
	Y220	8 [3.8%]	4 [4.5%]	1 [12.5%]
	R196	10 [4.8%]	5 [5.7%]	-
	R213	21 [10.1%]	3 [3.4%]	2 [25%]
	R175	101 [20.4%]	40 [20.6%]	3 [27.3%]
G12V	R248	74 [14.9%]	29 [14.9%]	3 [27.3%]
	R273	114 [23.1%]	40 [20.6%]	2 [18.2%]
	R282	55 [11.1%]	21 [10.8%]	-
	G245	39 [7.9%]	14 [7.2%]	1 [9.1%]
	Y220	44 [8.9%]	25 [12.9%]	1 [9.1%]
	R196	33 [6.7%]	14 [7.2%]	1 [9.1%]
	R213	34 [6.9%]	11 [5.7%]	-
	R175	16 [26.7%]	5 [27.8%]	-
Q61H	R248	10 [16.7%]	4 [22.2%]	-
	R273	12 [20%]	3 [16.7%]	-
	R282	3 [5%]	-	-
	G245	3 [5%]	1 [5.6%]	-
	Y220	5 [8.3%]	1 [5.6%]	-
	R196	7 [11.7%]	3 [16.7%]	1 [100%]
	R213	4 [6.7%]	1 [5.6%]	-
	*: Site of the mutation in p53			

#1125 Analysis of the Genie database for TC-NER deficient cancers sensitive to ITX-0121.

Michael J. Kelner

UC San Diego, La Jolla, CA

Cells possess a complex DNA damage response (DDR) system, to prevent detrimental mutations from accumulating and being passed on. The genes encoding various DDR components are frequently mutated in cancer cells and provide a potential site for therapeutic intervention. DNA damaging agents, with one exception, are repaired by the global genome repair (GG-NER). The one exception are Illudofulvenes, which hide from the GG-NER and can only be repaired by the transcriptional-coupled repair (TC-NER) pathway. Many cancers are deficient in the TC-NER pathway and cannot properly repair DNA damage. In TC-NER deficient cancer cells the TC-NER polymerase complex becomes blocked by the illudofulvene ITX-0121, disengages from DNA, and the irreversible process of apoptosis. These cancers are 10-fold more sensitive to ITX-0121 than are cancer cells with a functional TC-NER pathway. In contrast, non-replicating or normal cells are minimally affected by and this is reflected in the lack of systemic toxicity in humans administered an illudofulvene. Analysis of the GENIE Database indicates the incidence of TC-NER deficient cancers in solid tumors is ~13%, and includes all major histological classifications (or ~175,000 new patients annually). The highest incidence of TC-NER gene mutations/deletions was for the ERCC2 and ERCC5 genes with an incidence of 2.1% for each. Surprisingly, many patients with an ERCC2 or ERCC5 mutation had a second mutation in another ERCC gene (often ERCC6). The ERCC8 gene has the lowest incidence (0.4%) and the ERCC1 gene had the second lowest incidence of mutations (0.5%), but 65% of ERCC1 mutations were associated with a mutation in another ERCC gene. Based on studies involving double knockouts of TC-NER genes, these cancers have a 20-fold increase in sensitivity to ITX-0121. Histological classifications of cancers with the highest incidence of TC-NER deficiency (>25%) in the GENIE database included bladder, colorectal, endometrial, ovarian, and melanoma. In contrast, bone, renal, and thyroid cancers had a relatively low incidence of <8%. We developed a companion diagnostic to identify TC-NER deficient patients based on this data. Based on prior studies, we believe the MTD of ITX-0121 in humans to be >1.2 mg/kg, but only 0.4 mg/kg will be sufficient for clinical efficacy in TC-NER deficient cancers. Hence, despite administering a cytotoxic agent, one will treat TC-NER deficient cancer patients at a non-toxic dose. Toxicity (if it occurs) should be limited to transient thrombocytopenia based on prior studies. In summary, we are utilizing a Precision Medicine approach to develop a drug for an indication defined by a specific molecular alteration across cancer types, which in this case is the presence of TC-NER deficiency as identified by monoallelic mutations in specific genes coding for proteins in the TC-NER pathway.

#1126 CLSP-1025, a first-in-class precision T cell engager for the treatment of p53 mutant cancers.

M. F. Maloney[1], J. X. Caushi[1], A. R. Andrews[1], J. B. Bingham[1], R. Copeland[1], L. Cullen[1], J. DeSander[1], S. Devens[1], V. S. Dobrev[1], A. Ford[1], A. S. Gizzi[1], L. C. Harshman[1], G. A. Kingsbury[1], K. Krapfl[1], D. Orlando[2], J. Shepherd[1], M. Curtis Siok[1], C. Souza[1], K. L. Stokes[1], S. A. Underwood[1], V. Suri[1], **K. McEachern**[1];

[1]Clasp Therapeutics, Inc., Cambridge, MA, [2]Orlando Data Science, LLC, Cambridge, MA

Oncogenic driver mutations in p53 occur frequently in cancer. T cell engagers that specifically target tumor cells harboring p53 mutations have the potential to enable durable efficacy while sparing normal tissue. CLSP-1025 leverages this specificity by targeting tumors that express the p53 R175H mutant protein in HLA-A*02:01 positive patients. The preclinical characterization provides a foundation for advancing CLSP-1025 to a first-in-human (FIH) study.

CLSP-1025 is a half-life extended single-chain diabody IgG4 Fc fusion protein that binds to the $p53(R175H)_{168\text{-}176}$ peptide presented by HLA-A*02:01 on cancer cells and to CD3ε on T cells with nM affinity. Preclinical studies show that CLSP-1025 induces potent and targeted T cell activation (EC_{50} = 0.8 nM), leading to effective tumor cell killing in vitro (EC_{90} = 10 nM) and regression of KMS-26 tumor xenografts in vivo.

The safety assessment of CLSP-1025 included various in vitro and ex vivo assays using human cells and tissues. CLSP-1025 did not show significant reactivity towards any non-target HLA-A*02:01-presented human proteome derived peptide. In an evaluation of cross-reactivity to other HLA alleles, CLSP-1025 cross reacted with 8 of 264 HLA alleles tested and patients with these alleles will be excluded in the FIH study. In a tissue cross-reactivity study, CLSP-1025 demonstrated no off-target binding to the 39 normal human tissues tested. Additionally, assays with human whole blood and PBMCs revealed no significant CLSP-1025-dependent T-cell activation, cytokine production, or platelet activation.

To define the addressable patient population, genomic data was analyzed from over 180,000 tumor samples (AACR Project GENIE Consortium, v16.1) to determine the frequency of the p53 R175H mutation. Overall, the mutation was present in 2% of patient samples, with gastrointestinal cancers having the highest frequencies: colorectal (6.3%), pancreatic (4.3%), and esophageal (4.9%). Gynecological cancers also showed notable frequencies, with ovarian at 2.8% and endometrial at 2.4%. The population frequency of HLA-A*02:01 in the US is ~41% (Gragert 2013).

A mechanistically based, CLSP-1025-specific quantitative systems pharmacology (QSP) model incorporating target binding affinities, CD3 receptor occupancy, in vitro pharmacodynamics, and in vivo rodent PK profiles was developed to predict an efficacious dose range to inform the FIH study. The model predicted a terminal half-life for CLSP-1025 of approximately 16 days and the potential for tumor regression at target peptide-HLA densities as low as 2 molecules/cell.

Clinical evaluation of CLSP-1025 will be initiated in early 2025 in the GUARDIAN-101 study, a FIH dose escalation and expansion trial to evaluate the safety, pharmacokinetics, pharmacodynamics, and preliminary antitumor activity of CLSP-1025 in HLA-A*02:01 positive patients with advanced solid tumors harboring the p53 R175H mutation.

#1127 Differences in the clinical and genomic landscape in young onset (YO) gastroesophageal cancer (GEC): an analysis from AACR Project GENIE.

Ronan Andrew McLaughlin, Yvonne Bach, Esraa Mahmoud, Nadia Ghazali, Sheeraz Ali, Harry Harvey, Zac Coyne, Ekaterina Kosyachkova, Carly Barron, Hiroko Aoyama, Sokaina Akhtar, Raymond Jang, Eric Chen, Kevin Wang, Lucy Ma, Sangeetha Kalimuthu, Aruz Mesci, Rebecca Wong, Elliot Wakeam, Jonathan Yeung, Philippe Bedard, Elena Elimova

UHN Princess Margaret Cancer Centre, Toronto, ON, Canada

Background: Although GEC is most common in the elderly population, there is a rising incidence of YO GEC defined as occurring in patients ≤50 years of age. The clinical and genomic differences between YO and older onset disease remain unclear. We aim to comprehensively explore the clinical and genomic landscape of YO GEC and compare with older onset disease (>50 years) to elucidate potential molecular differences between the two age groups that may guide therapeutic approaches in the future.
Methods: Data from the Association for Cancer Research Project Genomics Evidence Neoplasia Information Exchange (AACR-GENIE) registry (version 16.1 for somatic mutation analysis and version 13.1 for survival analysis) to study patients diagnosed with GEC was queried. Patients with GEC were stratified by age at time of tumor sequencing. cBioPortal was used to analyze the differences in genetic mutation frequencies and differences were examined using chi-squared test. Overall survival (OS) between the groups was calculated using the Kaplan Meir method.
Results: The AACR-GENIE registry included 5933 samples from 5553 patients; 892 (16%) patients were ≤50 years old and 4661 (84%) were >50 years old. This cohort includes patients across all stages of disease. 64% were male in YO group versus 73% in group > 50 years. 11% of patients in YO group were of Asian race compared with 6% in older group. The older onset group demonstrated a significantly higher gene altered frequency ($p<0.001$). At a sample level analysis, significant genomic alterations between both age cohorts were identified in 21 genes. In particular, there were only two genes, CDH1 ($p<0.001$) and CCNE1 ($p<0.001$), found to be significantly observed more frequently in YO patients compared with the older onset group. These mutations have been associated with hereditary gastric cancer syndromes, aggressive disease and poorer survival. Amongst the 19 genes significantly observed more frequently in the older onset group was the hereditary APC gene $p<0.001$ and TP53 (<0.001). Potentially targetable markers such as CDKN2A ($p<0.001$), KRAS ($p<0.001$), FGF4 ($p<0.001$), and FGFR3 ($p<0.001$) were also significantly observed more frequently in patients >50 years old. In the earlier analysis of GENIE (Version 13.1) with 2874 patients median OS (all stages) in YO GEC was 37 months (26.6-59.5) versus 28.9 months (26.3- 32.5) (95% CI) in older onset patients, however this was not significant ($p=0.057$).
Conclusions: The GENIE analysis provides a comprehensive landscape of the clinical and genomic data in patients with YO and older onset GEC. Fewer somatic alterations were observed in YO GEC. Further exploration of these results and updated survival are vital to better understand the biological mechanisms and differences observed and to pinpoint potential biomarkers for diagnostic assays and personalized therapy.

#1128 Comprehensive multi-omics analysis of colorectal cancer tumors in diverse populations.

Brigette Waldrup[1], Francisco G. Carranza[1], Yuxin Jin[1], Yonatan Amzaleg[1], Mackenzie Postel[2], David W. Craig[1], John D. Carpten[1], Boudoir Salhia[3], Charite N. Ricker[4], Julie O. Culver[5], Carmen E. Chavez[5], Mariana C. Stern[6], Lourdes Baezconde-Garbanati[6], Heinz-Josef Lenz[4], Enrique I. Velazquez-Villarreal[1]

[1]Integrative Translational Sciences, Beckman Research Institute of The City of Hope, Duarte, CA,[2]Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA,[3]Translational Genomics, University of Southern California, Keck School of Medicine, Los Angeles, CA,[4]Medical Oncology, University of Southern California Keck School of Medicine, Los Angeles, CA,[5]University of Southern California Norris Comprehensive Cancer Center, Los Angeles, CA,[6]Population and Public Health Sciences, University of Southern California Keck School of Medicine, Los Angeles, CA

Introduction: Colorectal cancer is a leading contributor to cancer-related mortality and health disparities within the Hispanic and Latino community. To uncover the biological and genetic factors underlying these inequities, we conducted an analysis of key colorectal cancer molecular characteristics, focusing on somatic alterations, gene expression and genetic similarity (NASEM's term for genetic ancestry).
Methods: As part of the Cancer Moonshot PE-CGS network, we obtained and performed a comprehensive genome-scale analysis of 131 primary CRC Tumor/Normal samples within the Hispanic and Latino population from the Los Angeles area. For comparison, we analyzed 3,920 Non-Hispanic White (NHW) samples obtained from public databases, including AACR-GENIE. Our methodology included DNA exome sequencing to evaluate somatic mutations, somatic copy number alterations, and genetic similarity, as well as RNA sequencing to investigate differential gene expression, cellular pathways, and gene fusions. Insights from Hispanic and Latino samples were systematically compared with those derived from NHW cohorts. In alignment with the NASEM report and terminology, we adhered to best practices for the use of race, ethnicity, genetic ancestry, and other population descriptors in genetics and genomics research.
Results: Our analysis revealed distinct genetic similarity patterns, with a notable prevalence of Peruvian-from-Lima-like (1KG-PEL-like) genetic similarity, as defined by the 1000 Genomes Project. These patterns correlated with microsatellite stability (MSI) status, age at diagnosis, and tumor location. Significant mutations were identified in key colorectal cancer genes, including APC, TP53, and KRAS. Comparative analyses highlighted CRC-related genes with significant differences between Hispanic and Latino samples and NHW cohorts. Drug-targetable amplifications were also identified, while gene fusion analyses uncovered targetable genes such as ALK, FGFR1, RAF1, and PTPRK. Notably, PTPRK was observed in samples with the highest 1KG-PEL-like genetic similarity proportions.
Conclusion: This study advances the molecular profiling of colorectal cancer in Hispanics and Latinos, addressing an underrepresented population in cancer research. As one of the first Cancer Moonshot projects focused on CRC in Hispanics and Latinos with a substantial sample size and among the pioneering efforts to utilize multi-omics for molecular characterization, it sets a significant precedent. Genetic similarity is identified as a critical factor in colorectal carcinogenesis, providing essential insights into the biological drivers of cancer health disparities. By establishing a comprehensive framework for analyzing this underserved group, this research lays the groundwork for future studies, fostering advancements in precision medicine and supporting the development of tailored therapeutic strategies.

#1129 Interrogating the molecular profile of pancreatic cancer tumors in Hispanics living in Puerto Rico.

Ingrid M. Montes-Rodriguez, Veroushka Ballester-Vargas, Sol Perez-Martir, Hilamaris Centeno-Girona, Marcia Cruz-Correa, Julie Dutil

University of Puerto Rico Comprehensive Cancer Center, San Juan, Puerto Rico

Pancreatic adenocarcinoma (PDAC) is projected to become the second leading cause of cancer-related death in the U.S. by 2040. With a low survival rate at advanced stages and an increasing number of patients, PDAC poses a significant public health challenge. The five-year survival rate for localized PDAC is 44%, but it drops to 15% for regional and just 3% for distant disease. There are clear disparities in PDAC outcomes based on race and ethnicity; however, published data often lack racial and ethnic diversity, particularly regarding non-black minority groups like Hispanics. To address this gap, we evaluated the mutational profiles of PDAC tumors from 48 Hispanic patients living in Puerto Rico (PRH) who underwent next-generation sequencing (NGS) testing between 2015 and 2020. Data were provided by the Precision Oncology Alliance (POA) using the CARIS Life Sciences NGS platform. We estimated the prevalence of somatic mutations in PRH PDAC tumors and compared these profiles with those from the TCGA Pan-Cancer Atlas and the AACR Project GENIE, both accessible via the cBioPortal for Cancer Genomics. Notable differences were observed when compared to TCGA and GENIE. For instance, PRH samples exhibited higher mutational frequencies of *BRCA1/2*, which are actionable genes that could make these patients candidates for targeted therapies such as PARP inhibitors. Additionally, elevated frequencies of mutations in *ARID1A* and *TP53* in PRH suggest a distinct mutational landscape not typically represented in non-Hispanic populations. These findings underscore the need for population-specific molecular profiling to uncover optimal targeted therapeutic approaches for Hispanic patients.

TUMOR BIOLOGY: Stem Cells, Development, and Cancer
Poster Session

#113 Decoding developmental programs driving tumorigenesis and immunotherapy resistance in triple-negative breast cancer.

Rachel Gleyzer[1], Farnaz Khameneh[1], Zhen Qi[1], Wubing Zhang[1], Chloe B. Steen[1], Minji Kang[1], Maya Maalouf[2], Melanis Ghadimi[2], Makenna Lindsey[2], Sally Bobo[1], Thomas J. Lomis[2], Frederick M. Dirbas[1], Michael F. Clarke[1], Aaron M. Newman[1]

[1]Stanford University School of Medicine, Palo Alto, CA,[2]Valley Presbyterian Hospital, Van Nuys, CA

Background: Triple-negative breast cancer (TNBC), defined by a lack of hormone receptors and lack of HER2 amplification, is the deadliest subtype of breast cancer. TNBC kills 1 in 3 patients and while promising new drugs based on PARP inhibition and anti-PD1 immunotherapy can extend survival in selected patients, 30-40% of patients relapse or fail therapy. Increasing evidence suggests that TNBC tumors harbor stem-like tumorigenic cancer cells with high plasticity, capable of replenishing cancer cell populations and linked to adverse outcomes. Unfortunately, the phenotypic diversity, microenvironmental contexture, and functional significance of these cells remain poorly understood and available marker genes are not specific, precluding rational drug development.
Methods: We created a multimodal single-cell RNA-seq atlas of 80 TNBC patients, including clinical covariates such as BRCA1/2 mutations, race/ethnicity, and prior treatment status. We then defined differentiation-associated malignant cell states using a novel interpretable deep learning approach for determining single-cell potency. We subsequently interrogated geospatial features, including localized microenvironments of less differentiated malignant cells, and linked them to immune checkpoint inhibitor (ICI) response.
Results: Our quantitative analysis shows that TNBC is enriched in cancer cells with high developmental potency as compared to other breast cancer subtypes, consistent with its aggressive phenotype. Critically, we observed a strong association between enrichment of high potency cells and resistance to ICI treatment across human and mouse bulk expression data, both within TNBC and across other cancer types, a finding that we experimentally validated in a murine model of TNBC. In scRNA-seq and spatial transcriptomics data, we defined striking relationships between less differentiated cancer cells and context-dependent T cell states indicative of strong immunosuppressive capability. In line with this, we found that knockout of genes associated with immature cancer cells promotes TNBC cell killing by T cells in a co-culture CRISPR screen. Ongoing experiments include perturbations of novel therapeutic targets emerging from this work, with the goal of improving immunotherapy responses and ultimately extending patient survival.
Conclusion: We anticipate that our approach will expand our understanding of developmental landscapes in TNBC and lead to new opportunities for developing targeted drugs in this devastating disease.

AACR PROJECT GENIE: AACR Project GENIE Use Cases
Poster Session

#1130 Race and sex differences in TP53 mutation in head and neck cancer.

Nosayaba Osazuwa-Peters, May Z. Gao, Tammara L. Watts, Rong Jiang

Duke University School of Medicine, Durham, NC

Importance: Head and neck cancer (HNC) is one of the most disparate cancers, with the Black vs. White survival difference being the third worst survival gap of all cancers. It is also a male-dominated cancer, with up to 75% of new cases in males. There remains several gaps in our understanding of the genetic basis for race and sex disparity in HNC, including paucity in characterizing differences in common genetic mutations in HNC, such as TP53 mutations.
Objective: To characterize the genetic differences in HNC by race and sex through 1) examining prevalence of TP53 mutation by race, and 2) differences in mutation by sex.
Design: Retrospective cohort study.
Setting: The AACR GENIE consortium level v17.4 dataset for patients with confirmed diagnosis of HNC, independent of HPV status.
Main Outcomes and Measures: First, we estimated the rate of TP53 mutation in our HNC cohort (n = 2,352). We also estimated relative risk (RR) for TP53 mutation by race, and then adjusted age, and sex. We also estimated differences in TP53 mutation by sex, reporting false discovery (q-value) rate as appropriate. Finally, we estimated adjusted hazard ratio for mortality for TP53, adjusting for clinical and nonclinical variables in the model.
Results: In the entire cohort, the rate of TP53 mutation was 48.7%. However, there were only 109 patients in our cohort of 2,352 patients that self-identified as Black patients, and among these, 60 (55.1%) had TP53 mutation. After adjusting for age and sex, compared to White patients, we found aRR for TP53 = 1.17 for Black patients (95% CI: 0.98, 1.39), and for other minority race and ethnic groups together aRR = 1.17 (95% CI: 1.06, 1.29). By sex, we found a significantly higher prevalence of mutant TP53 in female vs. males patients (57.8% vs. 45.3%, q-value <0.0001). TP53 mutation was associated with significantly increased hazard of mortality (aHR = 1.58; 95% CI: 1.30, 1.91).
Conclusion and Relevance: TP53 mutation is very common in HNC, and about one out of every two patients with HNC may have a TP53 mutation based on our results. There may be differences in TP53 mutations in HNC by race and sex, an important finding given the mortality implications of TP53 mutations in HNC.

#1131 Comprehensive genomic profiling in osteosarcoma: Insights from Japanese cohorts and the AACR Project GENIE.

Suehara Yoshiyuki[1], Rina Kitada[1], Masachika Ikegami[1], Satoshi Kamio[1], Koichi Ogura[2], Shintaro Iwata[2], Eisuke Kobayashi[2], Akira Kawai[2], Shinji Khosaka[1]

[1]National Cancer Center Research Institute, Tokyo, Japan,[2]National Cancer Center Hospital, Tokyo, Japan

Background: Osteosarcoma (OS), the most common primary malignant bone tumor, presents challenges in treatment, especially in metastatic or recurrent cases. Genomic profiling may offer novel therapeutic approaches by identifying actionable mutations. This study utilizes data from the Japanese Center for Cancer Genomics and Advanced Therapeutics (C-CAT) and AACR Project GENIE, comparing findings with the MSK-IMPACT dataset to understand differences in genomic landscapes and identify clinically actionable alterations.
Methods: We analyzed 223 high-grade OS samples from the C-CAT database, comparing findings to the GENIE cohort and MSK-IMPACT data. Tumor DNA sequencing was performed using FoundationOne® CDx and OncoGuide™ NCC Oncopanel. Genomic alterations were categorized by levels A-F per Cancer Knowledge Database (CKDB) and compared with public databases (cBioPortal).
Results: In the C-CAT cohort, 42.2% of samples had actionable mutations (CKDB Levels A-C), including two NTRK fusions (0.9%), one TMB-high case (0.4%), and recurrent ERBB, CDK4, PTEN, and PDGFRA gene alterations. Co-occurring KIT, KDR, and PDGFRA amplifications were observed at 4q12 in 3.6% of cases, while VEGFA and CCND3 co-amplifications at 6p12-21 occurred in 14.8% of cases, suggesting multi-kinase inhibitors as potential treatments. Differences from the MSK-IMPACT dataset (Suehara Y et al, Clin Cancer Res. 2019 25(21):6346-6356), where mutually exclusive amplifications were more pronounced, highlight the genetic diversity between Japanese and U.S. cohorts. Analysis with GENIE and MSK-IMPACT indicates a lower incidence of mutually exclusive alterations in the Japanese cohort.
Conclusions: Targetable alterations were present in 42.2% of Japanese OS cases, with a distinct genomic profile compared to U.S. cohorts. Amplifications in 4q12 and 6p12-21, which may be treatable with multi-kinase inhibitors, were found in both datasets, yet mutual exclusivity patterns varied. This study underscores the potential of precision oncology in OS, encouraging continued exploration of genomic data in diverse populations to expand treatment options.

#1132 Quantifying selection intensity of driver genes in primary and metastatic thyroid cancer.

Moein Rajaei, Andrew Ju, Jeffrey P. Townsend

Yale University, New Haven, CT

The mutational profiles of primary and metastatic thyroid cancer (THCA) have been identified by comparing genetic alterations between the two stages, using mutation prevalence and *P* values to identify significant differences. However, these metrics do not quantify the cancer effects of variants. In this study, we quantified somatic selection on variants and genes using exome and targeted sequencing data from 2,145 primary and 1,374 metastatic tumors, derived from previous studies as well as GENIE, and TCGA databases, utilizing cancereffectsizeR to measure the cancer effects of new mutations. Comparisons of trinucleotide mutation profile revealed a relatively similar mutational landscape between primary and metastatic THCA, with notable differences such as GTG → GAG, TTG → TCG, and ATG → ACG mutations, which were more prevalent in metastatic tissues. While it is shown that *TERT* genetic alterations are more frequent in metastatic papillary thyroid carcinomas (PTCs) compared to primary PTCs, our analysis showed that the strength of somatic selection on *TERT* coding mutations was lower during the span from primary to metastatic THCA than during the span from thyroid organogenesis to primary THCA. Conversely, *RET* mutations, which is also shown to be more prevalent in metastatic tumors, were highly selected during the progression from primary to metastatic THCA, in comparison from organogenesis to primary THCA, aligning with the broader spectrum of *RET* mutations observed in metastatic medullary thyroid carcinoma. Furthermore, the selection intensity for mutations in genes such as *BRAF*, *NRAS*, *TP53*, *ATM*, *KMT2D*, *KMT2C*, *NF1*, *NF2*, *PTEN*, and *NKX2-1* was higher during the transition from organogenesis to primary THCA compared to the progression from primary to metastatic THCA. In conclusion, our application of cancer effect size analyses revealed that the strengths of selection on mutations vary dynamically across the trajectories from organogenesis to primary THCA and from primary to metastatic THCA. We identified key genes driving THCA initiation and progression, providing a quantitative understanding of the evolutionary trajectory of mutations in this disease. These insights shed light on the determinants of THCA pathogenesis and metastasis and hold potential to inform future precision treatment strategies.

#1133 Molecular evolution patterns in primary versus recurrent adult gliomas: A multi-institutional study from Project GENIE.

Abhishek Bhattacharya, Anand Kornepati

Internal Medicine, NYU Langone Health, New York, NY

Introduction: Gliomas are the most common primary malignant brain tumors in adults, with high recurrence rates despite multimodal treatment. While previous multi-institutional genomic studies have revealed molecular differences between primary and recurrent tumors, the AACR Project GENIE dataset provides another valuable resource to investigate these distinctions.
Methods: We analyzed 8,959 adult glioma cases from Project GENIE v16.1 (2024), selecting cases with complete mutation and copy number alteration (CNA) data. Tumors were stratified by Isocitrate dehydrogenase (IDH) mutation status, as IDH-wildtype and IDH-mutant gliomas have distinct molecular profiles and patterns of progression. The IDH-mutant cohort (n=2,635) comprised 79.9% primary tumors (n=2,097) and 20.1% recurrent tumors (n=538). The IDH-wildtype cohort (n=5,885) included 89.8% primary tumors (n=5,290) and 10.2% recurrent tumors (n=595). We examined the top 10 mutated somatic genes and compared mutational frequencies and copy number variations between primary and recurrent tumors using Fisher's exact test with Benjamini-Hochberg correction.
Results: In IDH-mutant gliomas, recurrent tumors showed increased mutations across multiple genes, particularly in KMT2D (7.8% to 20.6%, OR 3.06, $p<0.001$), NF1 (5.25% to 13.8%, OR 2.79, $p<0.001$) and NOTCH1 (12.4% to 23.6%, OR 2.18, $p<0.001$). In IDH-wildtype gliomas, the most notable changes were observed in CIC (21.65% to 32.71%, OR 2.12, $p<0.001$) and SMARCA4 (6.82% to 13.75%, OR 2.36, $p<0.001$). Oncogenic drivers like TP53 ($p>0.05$) and PTEN ($p>0.05$) maintained stable mutation frequencies between primary and recurrent tumors in both IDH cohorts. Copy number alterations for all examined genes showed no significant differences between primary and recurrent tumors in either IDH group.
Conclusion: Analysis of this large real-world dataset reveals molecular evolution patterns that align with previous observations, particularly in the stability of copy number alterations in driver genes between primary and recurrent tumors across both IDH groups. Similarly, oncogenic drivers like TP53 and PTEN maintained stable mutation frequencies, indicating preservation of critical growth-promoting pathways during glioma progression. Recurrent IDH-mutant tumors showed high mutational variance in chromatin regulators like KMT2D as well as developmental pathway gene NOTCH1. Recurrent IDH-wildtype gliomas showed more modest increases in mutational frequencies compared to primary counterparts, indicating less selective pressure from treatment in these more aggressive tumors. While the unbalanced distribution of primary versus recurrent samples and potential sampling biases inherent in real-world data limit the generalizability of these findings, these patterns may inform future therapies. Further validation in independent cohorts is warranted.

TUMOR BIOLOGY: Stem Cells, Development, and Cancer
Poster Session

#114 Unraveling the origin of Ewing sarcoma using zebrafish transgenic models.

Elena Vasileva[1], Claire Arata[2], Gage Crump[1], James Amatruda[1]

[1]Children's Hospital Los Angeles, Los Angeles, CA,[2]Keck School of Medicine, University of Southern California, Los Angeles, CA

Ewing sarcoma (ES) is a pediatric cancer of bones and soft tissues with enigmatic origin. Ewing sarcoma cells are characterized by the presence of a driver fusion oncogene, most frequently *EWSR1-FLI1*. Previous reports have suggested that bone-marrow mesenchyme or/and neural crest cells may serve as potential cells of origin for Ewing sarcoma. These hypotheses have never been tested in vivo, largely due to the absence of a representative mouse model as the EWSR1-FLI1 fusion causes severe developmental toxicity in most cell types. To address these questions, we developed a stable zebrafish transgenic model enabling tissue-specific expression of the human EWSR1-FLI1 oncofusion in neural crest cells (Vasileva et al., 2024). Using this model, we demonstrated that expression of human EWSR1-FLI1 oncofusion in neural crest cells can lead to their transformation and the development of tumors in vivo. Single-cell analysis of tumor initiation shows that EWSR1::FLI1 reprograms neural crest-derived cells to a mesoderm-like state, strikingly resulting in ectopic fins formation throughout the body. EWSR1-FLI1 hindered the normal differentiation of neural crest cells into glial and neuronal lineages. Instead, EWSR1-FLI1 strongly reprogrammed neural crest cells by hijacking developmental enhancers and upregulating the expression of mesodermal regulators, including the key mesodermal regulator tbxta (Brachyury or T) transcription factor. *tbxta/TBXT* expression was maintained in subset of zebrafish and human Ewing sarcomas. Our model provides a mechanism by which a neural crest cell lineage can be transformed to Ewing sarcoma, a malignancy with predominant mesenchymal features. Taken together, these findings show how a single mutation profoundly alters embryonic cell fate decisions reprogramming neural crest cells to a mesoderm-like state to initiate a devastating childhood cancer.

#115 S-adenosyl-methionine (SAM) synthesis protects ovarian cancer from chemotherapy induced DNA damage and contributes to cancer stem cell enrichment.

Shu Zhang[1], Tara X. Metcalfe[1], Zhen Fu[2], Christiane Hassel[1], Heather M. O'Hagan[1], Kenneth P. Nephew[1]

[1]Indiana University Bloomington, Bloomington, IN,[2]Van Andel Institute, Grand Rapids, MI

High-grade serous ovarian cancer (HGSOC) is a deadly gynecological malignancy characterized by tumor recurrence and chemoresistance to first-line therapies, including platinum (Pt)-based chemotherapy, and Pt-induced increase in OC stem cells (OCSCs). Methionine (Met) has been shown to maintain CSC phenotypes, but its role in Pt-enriched OCSCs remains unclear. HGSOC cell lines were sorted using FACS into OCSCs and non-CSCs. Expression of methionine adenosyltransferase 2A (MAT2A), the enzyme converting Met to S-adenosyl-methionine (SAM), was higher ($p<0.01$) in OCSCs compared to non-CSCs. Supplementation of extracellular SAM (50μM) increased ($p<0.05$) the percentage of OCSCs, whereas inhibiting MAT2A by siRNA knockdown or the activity inhibitor prevented ($p<0.05$) the cisplatin (IC50, 16hr) induced increase in both OCSCs population and spheroid formation, demonstrating the importance of SAM biosynthesis in OCSC enrichment. The combination of cisplatin and siMAT2A also enhanced ($p<0.01$) DNA damage and apoptotic cell death compared to cisplatin. Mechanistically, siMAT2A inhibited ($p<0.05$) proteins involved in the DNA damage response and repair, including yH2Ax, ATM, ATR, Chk1 and FANCD2. MAT2A knockdown also suppressed ($p<0.05$) the cisplatin-induced upregulation of cyclin E/CDK2 which increased the percentage of cells by-passing the cell cycle checkpoint for DNA repair. Furthermore, siMAT2A increased ($p<0.05$) the binding of SAM sensor protein BMT2 on GATOR1, the negative regulatory complex of mTORC1, attenuating p70-S6 kinase and potentially contributing to downregulate cyclin E. Notably, the effects of siMAT2A were independent of the status of Met salvage enzyme methylthioadenosine phosphorylase. As SAM is a universal methyl donor for various methylation processes and given the established link between OCSC enrichment and aberrant DNA methylation, we used Infinium MethylationEPIC array v2.0 BeadChip to investigate genome-wide changes in DNA methylation using HGSOC cell line OVCAR3 treated with vehicle (control), cisplatin (15μM, 16hr) and/or siMAT2A (48hr). DNA methylation analysis identified differential methylated regions (DMRs) between three comparisons. Cisplatin alone increased ($p<0.05$) hypermethylated DMRs compared to the control. The combination of cisplatin and siMAT2A resulted in an increased ($p<0.05$) hypomethylated DMRs compared to cisplatin only, demonstrating a critical role of SAM synthesis in cisplatin-altered DNA methylation patterns. Enrichment analysis identified genes associated with stemness, differentiation, and cell death in the combination. Collectively, our study demonstrates a new role of SAM synthesis in Pt-induced DNA damage repair and OCSCs enrichment, providing a potential therapeutic target for improving chemotherapy outcomes and preventing OCSC enrichment in ovarian cancer.

#116 CD73$^+$cancer-associated fibroblasts augment cancer stemness and drug resistance in hepatocellular carcinoma through adenosine accumulation.

Hui Hai Yang, Carmen Oi Ning Leung, **Terence Kin-Wah Lee**

Hong Kong Polytechnic University, Hong Kong, Hong Kong

Background: Hepatocellular carcinoma (HCC) exhibits significant therapeutic resistance due to its high cancer stemness, which is strongly influenced by the tumor microenvironment (TME). Extracellular adenosine (eAdo), a critical metabolic regulator within the TME, is primarily generated from extracellular ATP via the enzymatic activity of CD73 (NT5E), which is highly expressed in cancer-associated fibroblasts (CAFs). In our pilot study, we found that the CAF populations marked by CD73 were drastically induced in a lenvatinib-resistant immune-competent mouse HCC model. Based on this data, we hypothesize that CD73$^+$ CAFs confer cancer stemness-driven drug resistance via eAdo accumulation in the TME. **Methodology:** Liquid chromatography-mass spectrometry (LC-MS) was used to quantify adenosine levels, whereas flow cytometry was used to assess stemness-related surface markers. Tumor-initiating frequencies were evaluated using a limiting dilution assay, and CRISPR-Cas9-mediated CD73 knockdown in CAFs, the precursors of CAFs, was employed to validate CD73's functional role. Western blot analysis was conducted to investigate the pathway activation.**Results:** Our results showed that CD73 is a functional marker of CAFs, and its overexpression is significantly correlated with poor prognosis of HCC patients. LC-MS detected a significant amount of adenosine in CAFs when compared with HCC cells, while a notable reduction in adenosine levels was observed in the conditioned medium (CM) of CD73-knockout CAFs. Interestingly, ATP-supplemented CM of CAF augments the cancer stemness of HCC cells, while its effect is offset upon CD73 knockout in CAFs. Echoed to this finding, adenosine treatment (10 μM) exerted similar effects, confirming the critical role of CD73 in CAF-driven cancer stemness via adenosine accumulation. Mechanistic studies have demonstrated that adenosine promotes stemness through activation of the A2AR/AKT/mTOR signaling pathway in HCC cells. **Conclusion**: This study establishes that CD73$^+$ CAFs drive HCC stemness and drug resistance by modulating the TME through eAdo accumulation. Targeting CD73 and its downstream signaling pathways represents a promising therapeutic strategy to overcome drug resistance which potentially opens a new therapeutic avenue for HCC treatment.

#119 A multipotent PROX1+ tumor stem/progenitor cell population emerges during intestinal tumorigenesis and mediates radioresistance in CRC.

Pauliina Kallio[1], Cinzia Bessone[1], Fatemeh Seyednasrollah[1], Jefim Brodkin[1], Marika Lassila[1], Jenny Hogstrom[2], Alejandra Gonzalez-Loyola[3], Tatiana V. Petrova[4], Caj Haglund[1], Kari Alitalo[1]

[1]University of Helsinki, Helsinki, Finland,[2]Cancer Research Institute, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA,[3]Aragon Health Institute of Research IISA, University of Zaragoza, Zaragoza, Spain,[4]Department of Oncology, University of Lausanne, Lausanne, Switzerland

Colorectal carcinoma (CRC) progression is associated with an increase in PROX1+ tumor cells, which exhibit features of CRC stem cells and contribute to metastasis. Here, we aimed to provide a better understanding to the function of PROX1+ cells in CRC, investigating their progeny and their role in therapy resistance. PROX1+ cells in intestinal adenomas of ApcMin/+ mice expressed intestinal epithelial and CRC stem cell markers, and cells with high PROX1 expression could both self-renew tumor stem/progenitor cells and contribute to differentiated tumor cells. Most PROX1-lineage traced tumor cells were stem/progenitor cells, which can supply cells to multiple intestinal tumor cell lineages, whereas most lineage-traced LGR5+ tumor cells were enterocytes, indicating that PROX1+ and LGR5+ tumor stem cells have distinct differentiation programs. Although the PROX1+ tumor cells proliferated slower than PROX1- cells, irradiation increased the proportion of PROX1+ cells in human CRC cell lines, patient-derived organoids, and tumor xenografts. Furthermore, transcripts related to DNA damage repair (DDR) were enriched in PROX1+ vs. PROX1- cells in adenomas and in CRC tumor cells from patients. Experiments with PROX1 silencing and overexpression indicated that PROX1 expression enhances CRC cell colony formation following irradiation. PROX1 interacted with DDR proteins, including components of non-homologous end-joining (NHEJ) and base excision repair, and inhibition of NHEJ repair led to a decreased proportion of PROX1+ cells following irradiation. In conclusion, PROX1+ cells are irradiation-resistant tumor stem/progenitor cells capable of self-renewal and differentiation. DDR inhibitors could represent a strategy to target the treatment-resistant PROX1+ tumor stem cells.

TUMOR BIOLOGY: Cancer Stem Cells
Poster Session

#12 Deciphering the role of oncogenic integrin alpha 6B in aged mouse mammary epithelial cells.

Thangarajeswari Mohan, Morgana McLaughlin, Jacob Mackay, Luzhe Sun

UT Health Science Center at San Antonio, San Antonio, TX

Breast cancer (BC) is the most diagnosed cancer in women with aging being the major risk factor. According to American Cancer Society (2024), an estimation of 310,720 invasive BC and about 56,500 cases of DCIS diagnosed among women in the USA, and the women die from BC are likely among age groups of 70 or older. Our previous report demonstrated that aging was associated with an increased number of basal epithelial cells (CD49f^{hi}CD24^{low}) and reduced luminal epithelial cells (CD49f^{low}CD24^{hi}) in mice. The CD49f (Integrin α6), a cell surface marker of mammary stem cells (MaSc), is enriched in the basal cell population of the mammary gland (MG). These cells have oligo-lineage differentiation potential, especially when transplanted into the cleared mammary fat pad (CFP). Integrin α6 has two variants with a different cytoplasmic domain, produced *via* alternative splicing (Isoforms: α6A & α6B). Recent scientific evidence suggests that α6B produced in transformed mammary cell lines was oncogenic. Previous studies from us and others showed CD49f^{hi} aberrant stem cell-like cells in the luminal epithelium display more hyperplastic duct in the old mice, as well as tumor adjacent normal tissue from women with breast cancer. The mechanism underlying the expansion of CD49f^{hi} cells during aging is unclear. It could be due to the proliferation of basal cells, or trans-differentiation of luminal cells. Henceforward, this study examines changes in the CD49f expression (and its isoforms) in the basal and luminal cell populations with aging in mice, hypothesizing that luminal cell attains basal cell signature by expressing more CD49f that contributes to hyperplasia in the aged mammary glands. To explore this hypothesis, we evaluated the gene and protein expression of Integrin α6 isoforms (α6A & α6B) across basal and luminal populations in FVB/NJ and C57Bl/6 aging mice using RT-PCR and Western blotting. Mammary cells were gated and sorted in FACS and used for RNA and protein studies. Our results revealed that aged basal and luminal cells express higher levels of ITGA6B mRNA transcript compared to ITGA6A in both the mice stains. Cell cycle analysis was performed using Hoechst to evaluate the cell cycle phase of each population using flow cytometry. The results showed that luminal cells from aged mice have a higher fraction of G2/M phase cells than those from young mice, suggesting an increased cell cycle in the aged luminal cells, which may be due to increased Integrin α6B. We are currently developing a method to measure Integrin α6B protein levels in young and aged mouse mammary luminal and basal cells. Our study provides the first evidence that aged CD49f^{hi} cells, particularly those expressing α6B exhibit increased cell proliferation potential, revealing a potential target for breast cancer prevention.

TUMOR BIOLOGY: Stem Cells, Development, and Cancer
Poster Session

#120 Developing a novel intra-portal stem cell hydrogel carrier system for enhancing liver remnant hypertrophy and improving surgical eligibility for hepatocellular carcinoma: preclinical assessment in a murine model of portal vein blockade.

Dominic Karl M. Bolinas, Allan John R. Barcena, Archana Mishra, Marvin R. Bernardino, Steven Y. Huang, Marites P. Melancon

The University of Texas MD Anderson Cancer Center, Houston, TX

Purpose: Hepatocellular carcinoma (HCC) is definitively treated with surgical resection of the affected lobe. However, 32% of patients are ineligible due to insufficient future liver remnant, which increases the risk of post-hepatectomy liver failure. To address this, portal vein blockade with mesenchymal stem cell (MSC) augmentation is used to induce pre-operative liver hypertrophy. However, 20-30% of patients fail to achieve adequate growth due to the rapid washout of MSCs from the liver. Therefore, we pioneered the use of hydrogels as stem cell carrier systems to enhance the retention, survival, and efficacy of MSCs in the liver to maximize hypertrophy.
Materials and Methods: MSCs were loaded in 1.5% sodium alginate solution and cross-linked with $CaCl_2$ to form hydrogels. The biocompatibility of the hydrogels and the release of MSCs were evaluated for 2 weeks. For in vivo modeling, portal vein blockade was achieved via left portal vein ligation (PVL) in cirrhotic Sprague-Dawley rats, effectively cutting off circulation into the ipsilateral lobe. The treatment groups ($n \geq 4$) are untreated control, PVL (surgery only), PVL+MSC (surgery with intraportal $1x\ 10^6$ MSCs injection), and PVL+MSC+hydrogel (surgery with intraportal injection of hydrogel carrier system loaded with 1×10^6 MSCs). The treatment effect was monitored using CT volumetry and liver tests. After two weeks, the animals were sacrificed and livers were collected for liver growth calculation, collagen staining, and MSC immunostaining. Statistical significance is defined as $p<0.05$.
Results: The hydrogel carrier system showed excellent biocompatibility by maintaining high MSC viability (89.0 ± 3.0 %) for 2 weeks. The system demonstrated an initial burst release over 7 days (64.6 ± 4.5%) followed by a more gradual release up to 2 weeks (87.0 ± 2.0%), which is ideal for the controlled delivery of therapeutic MSCs. On CT volumetry, PVL+MSC+hydrogel showed the highest liver growth among the treatment groups at 79.61% from baseline. Furthermore, PVL+MSC+hydrogel improved serum liver markers, such as AST, ALP, and ALT, compared to the PVL and PVL+MSC. On collagen staining, PVL+MSC+hydrogel revealed the highest reduction in fibrosis at 27.4%. Lastly, MSC retention with the alginate hydrogel carrier system in the liver remnant was 41.8% higher than PVL+MSC.
Conclusion: We successfully developed a stem cell hydrogel carrier system with ideal physicochemical and biological properties. The cell-loaded carrier system allowed the improvement of the structure and function of the liver. This is crucial for sustaining therapeutic and regenerative effects of MSCs for liver hypertrophy. Adapting this technology will enhance future liver remnant growth and thus, improve survivability among patients with HCC.

#121 Iqgap3 and Myc interaction regulates damaged stomach repair and early gastric carcinogenesis.

Junichi Matsuo[1], Mitsuhiro Shimura[1], Jungwon Lee[1], Linda S. H. Chuang[1], Wei Peng Yong[2], Yoshiaki Ito[1]

[1]Cancer Science Institute of Singapore, National University of Singapore (NUS), Singapore, Singapore,[2]National University Cancer Institute, National University Health System, Singapore, Singapore

The gastrointestinal tract is frequently subjected to tissue damage caused by various environmental stressors. Adult stem cells conduct tissue repair through regenerative programs with striking parallels to carcinogenesis, implying that dysregulated repair mechanisms initiate cancer development. Previously, we identified Iqgap3 as a marker of rapidly proliferating stem cells within the isthmus of gastric glands, a region essential for maintaining tissue homeostasis. Following tissue injury, we observed upregulation of Iqgap3 expression and activation of Myc signaling pathways, particularly within the fully differentiated chief cell population. Given that Myc is a major oncoprotein, our findings implicate Iqgap3 as a critical mediator in the stomach repair process and carcinogenesis. To understand how the role of Iqgap3 in tissue repair may be hijacked for carcinogenesis, we developed a conditional knockout (cKO) mouse model that attenuates Iqgap3 expression in gastric epithelial cells. Tissue damage and Iqgap3 deletion were induced by high-dose tamoxifen (HDT) treatment. Iqgap3-cKO mice were markedly compromised in tissue repair, exhibiting reduced cellular proliferation and chief cell population even one month after tissue injury. Transcriptomic analyses revealed that Iqgap3 deficiency suppressed the Myc signaling pathway and stem cell activity in the stomach, indicating a causal link between Iqgap3 and Myc during tissue regeneration. Furthermore, Iqgap3 depletion in oncogenic KrasG12D-induced metaplasia mouse model and KrasG12D-induction with Apc-depleted dysplasia mouse model protected against formation of metaplasia and dysplasia. Mechanistically, Iqgap3 deficiency led to reduced expression of neoplastic factors, such as CD44, Aqp5 and Trop2. Interestingly, Myc phosphorylation was attenuated in Iqgap3-cKO mice. We further showed that Iqgap3 directly interacted with Myc, which reinforces a role of Iqgap3 in enhancing Myc activity. Our findings highlight the critical roles of the Iqgap3-Myc axis in the regulation of stem cell activity, gastric tissue regeneration and precancerous metaplasia and dysplasia development. These insights provide a mechanistic framework for understanding the intersection of tissue repair and early carcinogenic processes.

#122 Novel role of SLC22A3 in serotonin transport and subsequent regulation of the pancreatic cancer stemness machinery.

Nivedeta Krishna Kumar, Bhuvaneshwari Seshadri, Venkatesh Varadharaj, Neelanjana Gayen, Palanisamy Nallasamy, Zahraa Wajih I. Alsafwani, Kirtana Arikath, Annant Bir Kaur, Surinder K. Batra, Moorthy P. Ponnusamy

Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE

Cancer stem cells (CSCs) are a small subpopulation within tumors that possess self-renewal and differentiation capabilities, driving tumor recurrence, therapy resistance, disease aggressiveness, and metastasis. In pancreatic cancer (PC), the mechanisms governing CSC self-renewal remain poorly understood, despite their critical role in malignancy. Emerging evidence indicates that normal embryonic and adult progenitor stem cells share molecular characteristics with CSCs, suggesting that early pluripotency programs may be reactivated during oncogenesis, contributing to cancer aggressiveness. In this study, we investigated novel stemness regulators that drive CSC self-renewal in PC. By performing computational analyses of gene signatures shared between embryonic stem cells, CSCs, and PC cells using publicly available datasets, we identified several putative stemness-associated genes, including SLC22A3/OCT3. Our results reveal that SLC22A3/OCT3 is highly expressed in CSC sub-type population and pancreatic tumor tissues and organoids derived from Kras; PdxCre (KC) and Kras; p53; PdxCre (KPC) mouse models, while its expression is minimal or absent in normal pancreatic tissues. Functional studies demonstrated that knockdown (KD) of SLC22A3/OCT3 in PC cell lines significantly reduced spheroid size in serum-free 3D cultures, indicating a decline in the CSC population. Flow cytometry further confirmed a marked reduction in the side population (SP) fraction in SLC22A3/OCT3 KD clones compared to controls. *In vivo*, SLC22A3/OCT3 depletion led to reduced tumor burden and impaired oncogenic dedifferentiation, a hallmark of CSCs in PC. SLC22A3/OCT3, a transmembrane transporter, facilitates the uptake and efflux of organic cations, including monoamine neurotransmitters such as serotonin, dopamine, and norepinephrine. Interestingly, serotonin supplementation increased stemness potential in PC cells, but this effect was absent in SLC22A3/OCT3 KD clones, implicating serotonin transport as a key mechanism in CSC regulation. Mechanistically, we propose that SLC22A3/OCT3 regulates serotonin transport in pancreatic CSCs, activating downstream pathways that enhance transcription factor binding and upregulate self-renewal and pluripotency-related genes. This process may involve serotonin-mediated epigenetic modifications, including serotonylation, to maintain CSC stemness and drive pancreatic tumor aggressiveness. Our findings highlight SLC22A3/OCT3 as a critical regulator of serotonin-driven stemness in PC. Understanding its role in CSC biology may provide novel insights into pancreatic cancer pathogenesis and open avenues for the development of targeted therapies aimed at disrupting CSC-mediated tumor progression.

#123 Induced natural killer cells: A next-generation platform for allogeneic immune cell therapies.

H.-S. Kim, J. Kim, B. Seol, J. Choi, J.-Y. lee, **Y. Cho**;
Korea Research Institute of Bioscience & Biotechnology (KRIBB), Daejeon, Korea, Republic of

Background: Natural Killer (NK) cells hold significant promise for effective and safe allogeneic immune cell therapies, primarily due to their ability to selectively target and eliminate cancer and other abnormal cells. Key advantages, including off-the-shelf availability and a low risk of graft-versus-host disease, cytokine release syndrome, and immune effector cell-associated neurotoxicity syndrome, make them ideal candidate for immunotherapy. However, conventional NK cell sources derived from blood or stem/progenitor cells face challenges such as high heterogeneity, limited scalability, and high production costs, underscoring the need for innovative and robust NK cell sources.

Methods: We previously developed induced NK (iNK) cells *via* OSKM transcription factor-mediated direct reprogramming as an alternative allogeneic NK cell source. To explore their therapeutic potential, we compared the phenotypic and functional characteristics of iNK cells, including receptor expression, cytotoxicity, degranulation, cytokine production, and antibody-dependent cellular cytotoxicity (ADCC), with those of control NK cells (PBMC-NK cells and NK-92). Furthermore, CRISPR/Cas9 screening was performed on pancreatic cancer (PC) cells using a human whole-genome dual-gRNA knockout library to identify genes critical for NK cell-mediated targeting and killing.

Results: iNK cells demonstrated high purity ($CD56^+CD16^+$, 93.1±3.4%) and homogeneity, along with elevated expression of activating receptors (CD69, NKG2D, DNAM-1) and natural cytotoxicity receptors (NKp30, NKp44, and NKp46) while showing reduced KIR expression (2DL1, 2DL2 and 3DL1) compared to control NK cells. Functionally, iNK cells displayed robust cytotoxicity against a broad range of human solid cancers, including PC, through cytokine secretion (IFN-γ, granzyme B, and TNF-α), CD107a degranulation, and ADCC. CRISPR/Cas9 screening identified 500 depleted genes in PC cells, with key targets such as EIF2AK3, PRKAA1, IRAK3, and NEK2, validated as contributors to NK cell-mediated cytotoxicity.

Conclusions: iNK cells represent a scalable and homogeneous alternative to conventional NK cell sources, overcoming limitations such as donor dependency, variability, and production inefficiencies. With their enhanced cytotoxic potential and the identification of key gene targets critical for NK cell-mediated killing, induced NK cells provide a robust and promising platform for the development of “off-the-shelf” allogeneic immunotherapies.

#124 Cell state regulation in early colorectal cancer through transcription factor and chromatin action.

Sweta Sharma, Alireza Lorzadeh, George Ye, Unmesh Jadhav

Department of Stem Cell Biology and Regenerative Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the United States, despite established treatment options and knowledge of causal mutations. CRC involves significant rewiring of cellular chromatin alongside mutational accumulation, which fuel aberrant cancer transcriptional programs. Many developmental gene regulatory mechanisms are also activated during early tumorigenesis. However, details of molecular controls in early tumor development and evolution are not fully understood. Mutations in tumor suppressor APC gene are essential for cancer initiation in the majority (>70%) of CRC patients. We ablated Apc in the mouse gastrointestinal stem cells resulting in their transformation leading to adenoma formation. We purified the transformed epithelial cells in adenoma using FACS and performed single nuclei ATAC and RNA sequencing to generate paired chromatin accessibility and transcriptional profiles using 10X genomics single cell multiome (scMultiome-seq) platform. By profiling the expression of known intestinal cell markers and comparing adenoma cells to wild type cells, we identified cell Apc-null cell populations with transcriptomic and chromatin landscape distinct from those of the wild-type cells. We identified differentially accessible genomic regions among wild-type and adenoma cells, the majority of which were located outside of gene promoters. This suggested changes in the cis-regulatory gene enhancer circuits potentially through altered transcription factor activity. MOTIF analysis using the differentially accessible peaks in adenoma cells revealed significant enrichment of several development specific transcription factors (TFs), including Sox17 and Foxa2. Such reappearance of developmental TFs in early adenomas may drive formation of unique cell states and cancer progression. Interestingly, immunostaining for Sox17 at various stages of adenoma development revealed heterogeneous enrichment within adenomas suggesting its involvement in early adenoma evolution. By profiling the histone modifications and transcription factor binding through chromatin immunoprecipitation sequencing, Sox17 directly binds to uniquely accessible enhancers in adenoma cells and promotes the activation of genes associated with cell-cell adhesion and migration. Our results using single-cell transcriptomic and epigenomic approaches reveal novel cell states formed during the birth and early growth of adenomas and how transformed cells utilize transcription factor activity to alter the cellular enhancer landscape as well as cellular properties during tumor evolution.

#125 Targeting colorectal cancer stem cells through GUCY2C expression.

Jess Kopenhaver, Scott A. Waldman, Adam E. Snook, Trevor Baybutt, Lara Cheslow, Ross Staudt, Jasmine Alvarez

Thomas Jefferson University, Philadelphia, PA

The intestinal cell surface receptor GUCY2C is universally over-expressed by colorectal tumors. Loss of expression of the intestinal hormones regulating GUCY2C silences this signaling pathway which dysregulates the cell cycle and DNA damage repair contributing to intestinal tumorigenesis. Thus, reconstituting GUCY2C activity with oral hormone replacement is being explored in clinical trials as a novel chemoprevention approach for colorectal cancer (CRC). Moreover, GUCY2C is being explored in clinical trials as an immunotherapeutic target reflecting its universal over-expression by metastatic CRC tumors. Here, we explore the expression and activity of GUCY2C in tumor stem cells (SCs) to define its potential as a therapeutic target to prevent therapeutic escape and recurrence in colorectal cancer. CRC-SCs were isolated from a library of patient-derived xenografts (PDXs) and in vitro cell lines by flow cytometry using aldehyde dehydrogenase (ALDH) activity and EpCAM expression. The $ALDH^{HI}EpCAM^{HI}$ CRC-SC fraction comprised ~1-10% of bulk tumor cells, and expressed the cardinal features of cancer SCs, including non-adherent tumor sphere and colony formation in vitro and tumor formation in immunodeficient mice compared to $ALDH^{LO}EpCAM^{HI}$ differentiated tumor cells. $ALDH^{HI}EpCAM^{+}$ SCs universally express GUCY2C mRNA and protein, and expression levels are positively correlated to bulk PDX tumors of origin. GUCY2C is expressed on the surface of $ALDH^{HI}EpCAM^{+}$ SCs, which respond to the GUCY2C agonist linaclotide (*Linzess™*) by producing and secreting cyclic (c)GMP. Unexpectedly, $ALDH^{HI}EpCAM^{+}$ SC are hyper-responsive to linaclotide, and their hormone-induced cGMP accumulation is 5- to 10-fold greater than $ALDH^{LOW}EpCAM^{HI}$ differentiated tumor cells. Universal expression, with amplified signaling, in CRC-SCs suggest that GUCY2C might be utilized as a therapeutic target. Indeed, linaclotide induces a durable block of proliferation in, and expansion of, $ALDH^{HI}EpCAM^{+}$ SCs, quantified by colony formation and expression of Ki67 in vitro. Similarly, $ALDH^{HI}EpCAM^{+}$ CRC-SCs are completely eliminated by GUCY2C-targeted chimeric antigen receptor (CAR) T cells in vitro. Taken together, these observations suggest that, beyond bulk tumor cells, GUCYC may have dual utilities as a therapeutic target in CRC-SCs. Thus, amplified GUCY2C signaling could be activated by agonists, like linaclotide, to suppress SC persistence and resistance to chemotherapy and potentially shift them to a differentiated phenotype susceptible to treatment. Moreover, metastatic CRC-SCs could be eliminated by GUCY2C-targeted immunotherapies, like CAR T cells, to prevent tumor persistence and recurrence.

#126 Targeting PAR1 in an aggressive blood cancer: A novel strategy to eradicate leukemic stem cells.

Nunki Hassan, Kaitlyn Trajcevski, Jenny Wang

University of Sydney, Camperdown, Australia

Acute Myeloid Leukemia (AML) remains a challenging hematologic malignancy with poor prognosis due to the persistence of therapy-resistant leukemic stem cells (LSCs). Current therapeutic strategies often fail to completely eradicate LSCs, leading to relapse. One promising therapeutic target is protease-activated receptor 1 (PAR1), a G-protein-coupled receptor selectively expressed in LSCs. Our research investigates the potential of targeting PAR1 to eliminate LSCs and improve treatment outcomes in AML. High PAR1 expression in a cohort of AML patients had a reduced overall survival (n=163, P<0.001). Lentiviral transduction of PAR1 knockdown and overexpression in mouse LSCs was established. PAR1 and its downstream gene and protein targets were validated utilising RT-qPCR and Western blot, respectively. PAR1 function was assessed with knockdown and PAR1 inhibitor treatment independently, which concurrently reduced colony formation, increased apoptosis, induced cell differentiation, and decreased expression of key self-renewal targets (i.e., ß-catenin and transcriptional factor Tcf7L2) and Gα subunits in LSCs. PAR1 overexpression in pre-LSCs was consistent with knockdown studies, demonstrating significantly increased colony formation and increased expression of key self-renewal genes (i.e. *Tcf7l2*, *Ccnd1*). PAR1 inhibitor showcased a dose and time-dependent early and late-stage apoptosis in LSCs, while in parallel reduced self-renewal signature markers and increased pro-apoptotic markers (i.e., cleaved caspase 3, Txnip). Murine models included intravenous transplantation of LSCs and pre-LSCs to evaluate PAR1 function *in vivo*. Overexpression of PAR1 in pre-LSC accelerated leukemogenesis compared to controls *in vivo*. Murine models demonstrated a marked reduction in leukemic burden in PAR1 knockdown mice, with prolonged survival compared to controls further enhancing LSC eradication and mitigating relapse *in vivo*. This innovative study is the first to establish PAR1 as a critical regulator of LSC self-renewal and survival, offering a potential breakthrough in AML therapy. These findings support the clinical development of PAR1 inhibitors as a next-generation treatment in eradicating LSCs, with the potential to overcome current therapeutic limitations and reduce relapse rates in AML patients, ultimately uniting cancer biology and therapeutic innovation.

TUMOR BIOLOGY: Cancer Stem Cells
Poster Session

#13 A disease-linked mutant reveals a critical N-terminal influence of SOX2 on translation.

Meric Ataman[1], Nitish Mittal[1], Hui Wang[2], Sergiy Velychko[3], Tata Nageswara Rao[4], Jordan Loliger[5], Jonas Lotscher[5], Sarah Roffeis[5], Christoph Hess[6], Hans R. Scholer[7], Ed Hurt[8], Matthias P. Wymann[5], Mihaela Zavolan[1], Claudia Lengerke[9], **Thorsten Schaefer**[5]

[1]University of Basel, Biozentrum, Basel, Switzerland,[2]Shanghai University of Medicine and Health Sciences, Shanghai, China,[3]Harvard Medical School, Department of Genetics, Boston, MA,[4]Cantonal Hospital St. Gallen, Medical Research Center & University of Bern, Institute of Pharmacology, St. Gallen/Bern, Switzerland,[5]University of Basel & University Hospital Basel, Department of Biomedicine, Basel, Switzerland,[6]University of Basel & University Hospital Basel, Department of Biomedicine & University of Cambridge, Department of Medicine, CITIID, Cambridge, United Kingdom,[7]Max Planck Institute for Molecular Biomedicine, Munster, Germany,[8]University of Heidelberg, Biochemistry Center Heidelberg, Heidelberg, Germany,[9]University Hospital Tubingen, Internal Medicine II, Tubingen, Germany

Changes in cell fate are primarily attributed to the spatio-temporal influence of transcription factors, but the ultimate level of expression control remains translation. Some transcription factors (such as YB-1, RfaH and Bicoid) play complementary roles in translation, and we have recently reported that also the pluripotency-inducing transcription factor SOX2 has ribosomal affinity and modulates transcript selection (*Schaefer et al., Cell Reports 2024*). Although this post-transcriptional layer of regulation mainly reflects SOX2‘s C-terminal influence in the cytosol, we here present evidence for a further involvement also of N-terminal SOX2 in translation.
Ribosomal affinity within N-terminal parts of SOX2 first showed in domain separation analyses in breast cancer-derived cell lines. However, although SOX2 fragments (1-119) and (1-179) co-sedimented with 60S ribosomes in sucrose density gradients, they imposed no translational fingerprint in polysome profiles as otherwise seen for full-length or C-terminal SOX2. Nonetheless, the synthesis of bulk protein (indicated by OPP incorporation into nascent polypeptides) did change and hundreds of mRNAs experienced a translational regulation upon SOX2 N-terminus induction. Among these, 65 differentially regulated factors stood out as co-regulated by full-length or N-terminal SOX2, but unaltered or counter-regulated in SOX2 C-terminus induced cells. Overrepresentation analyses within STRING and TISSUES databases indicated a preferential expression in the reproductive tract for 35 of these hits, and also under more stringent inclusion criteria, a functional assignment to the reactomes mitosis and reproduction was reconfirmed. In phenotypic correlation, we indeed noted a co-regulation of cell growth in full-length or N-terminus induced T47D and MDA-MB468 reporter cells, while the C-terminus recapitulated the proliferation rate of control cells. Real-life evidence for a SOX2 N-terminal contribution to translation, however, came from a disease-linked human mutant, SOX2(L97P), originally identified in the context of eye development. When expressed in reporter cells the L97P mutation severely impaired 60S binding, so that neither the polysome profile nor the phosphorylation status of RPS6 changed as otherwise seen for wild-type SOX2.
Our study highlights that, despite decades of preceding research, the protein biochemistry of even key pluripotency factors remains underexplored and still holds unforeseen learning. In summary, we present here extended evidence for a translational significance of SOX2 that (i) involves N-terminal contributions, (ii) arises in physical contact with ribosomes, and (iii) takes influence on cell proliferation. A better understanding of the underlying molecular principles may thus pave the way to a targeted control of cell division, selectively in SOX2-driven reprogramming and cancer.

TUMOR BIOLOGY: The Structural Tumor Microenvironment and Integrin Interactions in Cancer
Poster Session

#130 Matrix remodeling promotes macrophage recruitment in breast cancer.

Ioannis K. Zervantonakis, Youngbin Cho

University of Pittsburgh, Pittsburgh, PA

A high number of tumor-associated macrophages (TAM) in breast tumors is associated with poor patient outcomes. Furthermore, activation of myosin II, a key cytoskeletal regulator, has been identified as a predictor of distant metastasis in triple negative breast cancer (TNBC). However, the relationship between myosin II activation in TNBC cancer cells and macrophage motility remains poorly understood. Here we employed a novel 3D microfluidic model of tumor-macrophage co-culture to study macrophage infiltration toward cancer cells with high spatiotemporal resolution and evaluation of extracellular matrix remodeling. Using a panel of TNBC cell lines we found that cancer cells with higher macrophage recruitment potential in a 3D matrix exhibited higher levels of myosin II activity. We found that macrophage infiltration was higher in decellurized extracellular matrices seeded with the high-macrophage recruiting cancer cell line EMT6 compared to the low-macrophage recruiting cancer cells E0771. Functional contractility analysis using traction microscopy revealed that EMT6 exerted higher matrix displacements compared to E0771 that could be suppressed by pharmacologically blocking myosin II in cancer cells or genetic silencing of the myosin regulatory light polypeptide 9. Consistent with these suppressive effects of targeting actomyosin contractility on matrix remodeling we also found that macrophage recruitment was reduced. These studies reveal the critical role of cancer cell-driven matrix remodeling in promoting macrophage infiltration that can be therapeutically targeted to revere the establishment of a macrophage-rich pro-tumorigenic tumor microenvironment.

#131 PDAC tumor growth suppression by targeting the CD49b collagen receptor through modulating the CD8$^+$ T cell infiltration.

Ibrahim R. Eissa, Motaz Qadan, Sonia Cohen, Genevieve M. Boland, Tatyana Sharova, Kenneth K. Tanabe

Surgery Department, Gastrointestinal & Oncologic Surgery Division, Massachusetts General Hospital /Harvard Medical School, Boston, MA

Background:Pancreatic ductal adenocarcinoma (PDAC) has a desmoplastic extracellular matrix enriched with collagen type I (COL1) and poor CD8$^+$ tumor-infiltrating lymphocyte (TIL) infiltration. CD49b, a key collagen receptor, has been implicated in PDAC progression, but its role in immune exclusion remains unclear.
Objective: This study investigates the functional role of CD49b in modulating anti-tumor immune responses and collagen architecture in PDAC, while exploring its therapeutic potential against PDAC. Methods: Collagen receptors expression in PDAC was analyzed using flow cytometry on treatment-naïve human PDAC samples, Western blotting on PDAC cell lines including KPC cell lines, immunofluorescence on both human and mouse specimens, and analysis of single-cell RNA sequencing (scRNA-seq) on KPC models and human PDAC sample databases. The ITGA2 (CD49b) receptor was knocked out in KPC and Pan02 cells using CRISPR-Cas9. Tumor growth was evaluated in immunocompetent and immunodeficient mouse models, with or without CD8+ T cell depletion. Tumor-infiltrating lymphocytes (TILs) were assessed using FACs, and downstream signaling pathways were analyzed using RNA sequencing and Western blotting. Collagen fiber alignment was evaluated using CT-FIRE. Functional experiments included FAK overexpression to rescue signaling in CD49b-knockout models. Additionally, the consequence of blocking CD49b in vivo was examined.
Results: CD49b was selectively expressed in PDAC cancer cells in both mouse and human specimens. Loss of CD49b impaired tumor adhesion to collagen and significantly suppressed tumor growth in an immunocompetent model by increasing activated intratumoral CD8$^+$ T cell infiltration. Experimental depletion of CD8$^+$ T cells abrogated tumor suppression in CD49b-deficient tumors, demonstrating the importance of CD8$^+$ T cells in the anti-tumor response. Regarding affected signaling pathways, CD49b loss disrupted focal adhesion kinase (FAK) and pFAK signaling, increased CXCL10 chemokine expression, and altered collagen fiber alignment, creating a microenvironment permissive to immune infiltration. A negative correlation between CD49b expression and CD8$^+$ T cell infiltration was observed between the tumor core and edge. FAK overexpression restored tumor growth in CD49b-deficient tumors. Furthermore, vatelizumab, a CD49b-blocking antibody, effectively inhibited PDAC-collagen adhesion and reduced tumor growth in hCD49b-expressing KPC tumor.
Conclusion: This study reveals CD49b as a potent mediator of immune exclusion and collagen remodeling in PDAC and highlights its role in modulating FAK signaling and collagen alignment. Targeting CD49b , such as with vatelizumab, represents a novel and promising approach to overcome immune resistance and improve therapeutic efficacy in PDAC.

#132 Collagen I triggers a GCN2-mediated integrated stress response to promote cancer cell survival.

Mark Ellie De Boton Alonzo[1], Ethan Jadon Subel[2], Hongbo (Beth) Gao[2], Stephen QR Wong[2], Yung Hsing Huang[2], David Chang[2], Grace Wong[2], Mustafa Karabicici[3], Sung Yun Jung[4], Fotis Nikolos[2], Keith Syson Chan[2]

[1]Graduate School of Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA,[2]Department of Urology, Houston Methodist Research Institute, Houston, TX,[3]Department of Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA,[4]Baylor College of Medicine, Houston, TX

Bladder cancer (BLCA) is the 6th most commonly diagnosed cancer in the US. Despite recent advances, BLCA has a high recurrence rate. Increased deposition of the extracellular matrix Collagen I (Col I) is a hallmark of cancer progression and correlates with poor patient survival in BLCA. Recent studies have showcased the dynamic nature of Col I, which signals key pro-tumoral processes like immune evasion and metastasis. Interestingly, Col I limits nutrient access and oxygen to adjacent tumors in the tumor microenvironment. Cancer cells adapt to disadvantageous conditions by activating pathways such as the integrated stress response (ISR) to downregulate mRNA translation to promote survival. High activity of ISR and its related proteins in cancer cells also increase chemoresistance, dormancy and metastasis. How Col I plays a role in activating pro-survival ISR in BLCA remains to be investigated. In this study, we describe the direct role of Col I in exploiting ISR in BLCA, increasing its tumorigenic capacity. We uncover that Col I rapidly downregulates global mRNA translation by inducing ISR, while promoting spheroid size *in vitro* and tumor growth *in vivo*. Mechanistically, Col I induces intracellular amino acid deprivation thereby activating GCN2, an important amino acid sensor and a key kinase mediating ISR. Inhibition of GCN2 and subsequent ISR activity is an effective therapeutic target in multiple cancer types. Here, we show pharmaceutical inhibition of GCN2 disrupts ISR, rescuing translation while decreasing Col I-induced spheroid and tumor growth. Clinically, analysis of the TCGA BLCA dataset reveal both high expression of ISR-associated genes and a high GCN2-mediated signaling pathway score correlate with poor overall survival. GCN2 and ISR pathway scores also correlate with high Col I expression, further indicating their involvement in promoting BLCA tumorigenesis. These findings unravel a novel mechanism by which Col I utilizes the ISR pathway to promote BLCA tumorigenesis and provides a potential therapeutic targets for BLCA patients in the future.

#133 From waste to nutrient: ammonia's recycling by fibroblasts to sustain collagen production.

Julien Guillard, Shan Cao, Marta Storl-Desmond, Brandon Faubert, Simon Schworer

Section of Hematology/Oncology, Department of Medicine, Biological Sciences Division, The University of Chicago, Chicago, IL

Collagen and other extracellular matrix (ECM) proteins are highly abundant in tumors, including pancreatic and breast cancers, and are involved in tumor progression and therapy resistance. Given its amino acid composition, collagen production imposes unique biosynthetic demands on cancer-associated fibroblasts (CAFs), particularly a high need for proline. Understanding how CAFs meet the metabolic requirements for collagen synthesis could provide opportunities to modulate ECM production in cancer. CAFs synthesize proline from glutamine; however, glutamine and other nutrients are often depleted in poorly perfused tumors. How CAFs maintain collagen synthesis under such nutrient-limiting conditions remains unclear, and addressing this question may reveal unique metabolic vulnerabilities in cancer. Previous work from our laboratory showed that CAFs recycle extracellular lactate as an alternative carbon source to sustain non-essential amino acid (NEAA) biosynthesis for collagen production. However, NEAA synthesis also requires nitrogen, and how CAFs fulfill the nitrogen demands for NEAA synthesis under nutrient limitations remains poorly understood. To address this gap, this study aims to identify the nitrogen source used by CAFs for collagen production. To define the nitrogen source for collagen synthesis, we performed metabolic tracing of potential nitrogen donors using fibroblasts stimulated to deposit ECM in the presence of 15N-labeled nutrients. Stable isotope tracing with GC-MS was used to quantify 15N-labeled hydroxyproline in the decellularized and hydrolyzed ECM. We further used CRISPR/Cas9 to delete rate-limiting enzymes to elucidate the pathways involved. Our findings indicate that ammonia, a waste product of cellular metabolism, is recycled for NEAA biosynthesis. Ammonia’s contribution to collagen was dependent on the concentrations of glutamine and ammonia, and could rise to up to 50% of nitrogen in ECM hydroxyproline. All tested fibroblast cell lines exhibited similar ammonia-derived nitrogen labeling. Through genetic and chemical inhibition, we identified glutamate dehydrogenase (GDH) as a key enzyme in ammonia recycling via reductive amination. In conclusion, ammonia, a waste product of cancer metabolism, can be recycled by CAFs to sustain collagen production. This discovery highlights the metabolic plasticity of fibroblasts to maintain ECM synthesis under nutrient-limited conditions and offers new avenues for targeting adaptive pathways to reduce CAF-mediated pro-tumoral effects.

#134 Integrating spatial transcriptomics and second harmonic generation imaging of collagen identifies genes associated with discoidin domain receptor 1 and collagen alignment in colorectal cancer.

Maxwell Stuart Hamilton[1], Harsimran Kaur[2], Alan J. Simmons[2], Ken S. Lau[2], Ambra Pozzi[3], Robery J. Coffey[3]

[1]Cancer Biology, Vanderbilt University, Nashville, TN,[2]Vanderbilt University, Nashville, TN,[3]Vanderbilt University Medical Center, Nashville, TN

Immune checkpoint blockade (ICB) has been shown to be an effective treatment for colorectal cancers (CRCs) highly infiltrated by $CD8^+$ T cells. However, there is a paucity of $CD8^+$ T-cell infiltration in the majority of CRCs. Tumors with highly aligned collagen fibers have been shown to have reduced $CD8^+$ T-cell infiltration. We previously identified a four-gene signature associated with T-cell exclusion in CRC. One of the genes, Transforming Growth Factor Beta Induced (TGFBi), is known to be involved in extracellular matrix regulation. Another, Discoidin Domain Receptor 1 (DDR1), promotes collagen fiber alignment in breast cancer. To determine if TGFBi and DDR1 are associated with collagen alignment in CRC, as well as to identify additional genes involved in collagen alignment, we have developed a method to spatially resolve collagen fiber alignment and integrate it with spatial transcriptomics. To do this, 30 µm thick CRC sections are imaged using a Nikon AX multiphoton microscope by second harmonic generation imaging, which permits label-free imaging of collagen fibers. Spatial heatmaps of the collagen alignment are generated from the resulting images. Spatial transcriptomics is performed on a serial tumor section using the Visium v2 platform and the gene expression matrix is integrated with the collagen alignment heatmap data. As proof-of-principle, two CRC tumors have been analyzed using this method. Highly aligned regions were found to be associated with expression of genes involved in extracellular matrix organization, keloidal collagen and specific fibroblast subtypes. The genes that correlate with collagen alignment will be confirmed at the protein level by multiplexed immunofluorescence on serial sections. This method will provide a rich dataset permitting elucidation of the complex interactions between T-cell exclusion, gene expression and collagen fiber alignment and may support the discovery of new strategies to overcome immune exclusion in CRC.

#135 Myosin 10 suppresses integrin α6β4 expression and drives invasive force generation in Non-small cell lung cancer leader cells.

Raaven Goffe[1], Maia Vierengel[2], Hiroaki Ogasawara[2], Henry Claussen[3], Robert A. Arthur[3], Khalid Salaita[2], Adam I. Marcus[4]

[1]Emory University School of Medicine, Atlanta, GA,[2]Department of Chemistry, Emory University, Atlanta, GA,[3]Emory Integrated Computational Core, Emory University, Atlanta, GA,[4]Winship Cancer Institute, Atlanta, GA

A major contributor to lung cancer mortality is the late stage at which it is diagnosed. Currently, 5-year survival rates for persons with regional and distant lung cancer metastasis at diagnosis are 29.7% and 4.7%, respectively, compared to 56.3% for persons with localized (non-metastatic) disease. Identifying and therapeutically targeting drivers of lung cancer metastasis can improve overall survival, but few models exist where subpopulations with stable high and low invasive potentials can be easily and reliably isolated. Previous work from our group isolated two stable subpopulations from the H1299 Non-Small Cell Lung Cancer (NSCLC) cell line, highly invasive cells at the front of a pack termed Leaders, and significantly less invasive cells that trail behind termed Followers. H1299 Leaders and Followers have heterogenous transcriptional profiles, biomarkers, genomic methylation patterns, metabolic states, and morphologies. H1299 Leaders express high levels of Myosin 10 (MYO10), an actin-bound myosin motor protein that functions in filopodia and drives collective invasion. To investigate how *MYO10* contributes to Leader cell biology and invasion, we generated MYO10 knockout single-cell derived Leader clones utilizing CRISPR-Cas9. Three (3) $MYO10^{-/-}$ knockout and two (2) $MYO10^{+/+}$ wildtype single-cell derived clones were generated suggesting that *MYO10* is not essential for Leader cell survival. In validated clones, WST-8 assays confirmed that proliferation was slightly decreased in $MYO10^{-/-}$ clones relative to their $MYO10^{+/+}$ controls. Bulk RNA sequencing demonstrated distinct transcriptional profiles of $MYO10^{-/-}$ and $MYO10^{+/+}$ clones with gene set enrichment analysis revealing activation of extracellular matrix and external encapsulating structure pathways in $MYO10^{-/-}$ clones relative to controls. Western blot of integrin subunits showed increased expression of α6 and β4 subunits with decreased β3 expression in MYO10 knockout clones. Further, DNA-based tension gauge tether (TGT) probes were used to assess cell spread area and force generation, functional readouts of integrin activity. Of note, $MYO10^{-/-}$ clones demonstrate dramatically reduced cell area and tension forces when compared to $MYO10^{+/+}$ clones. Taken together, these data suggest MYO10 supports integrin-based adhesion and force generation in Leader clones which likely contributes to the highly invasive H1299 Leader cell phenotype. This works serves as a foundation for future studies which aim to elucidate MYO10-dependent mechanisms that drive metastasis in NSCLC.

#136 Discerning the role of the extracellular matrix on epithelial-to-mesenchymal transition and invasiveness of glioblastoma multiforme using a xeno-free 3D hydrogel system.

Alejandra Ferrer Diaz, John Huang

TheWell Bioscience, Monmouth Junction, NJ

The invasive nature of cancer, driven by epithelial-to-mesenchymal transition (EMT), is a critical process influenced by external cues from the tumor microenvironment, such as extracellular matrix (ECM) composition. The ECM primes cells for EMT and metastasis, making it a key topic in understanding disease progression. For example, in glioblastoma multiforme (GBM), a grade IV astrocytoma and the most aggressive brain malignancy, EMT contributes to the highly invasive behavior of tumor cells, limiting patient survival to less than five years. Thus, developing *in vitro* models that can accurately examine how the ECM contributes to GBM invasiveness and survival is vital. Currently, most *in vitro* studies evaluating cell invasion and EMT are conducted in two-dimensions (2D), disregarding the microenvironment's complexity and the ECM role in such processes. The limited case studies on three-dimensional (3D) cancer invasion and EMT models heavily rely on animal-based ECM, such as Matrigel or collagen. These matrices, due to their inherent instability, introduce variability, compromise experimental consistency, and make it challenging to draw robust conclusions. In this study, we use the synthetic and biofunctional VitroGel® system to evaluate cell invasion and EMT in 3D GBM models. The fully characterized VitroGel system allow us to study the effect of the mechanical strength and biochemical composition of the ECM on cell invasion and EMT. In this study, GBM spheroids were formed in an ultra-low attachment plate, and hydrogels functionalized with ligands such as RGD peptides, matrix metalloproteinases, collagen-mimetic peptides, and others were gently added to the cultures to evaluate their role in cell invasion and EMT. The findings showed that the functional ligands stimulate spheroid invasion and promote EMT, evidenced by the rapid proliferation of the spheroid and engulfment of the matrix, resulting in the formation of tumoroid-like structures. Furthermore, we evaluated whether the mechanical strength of the ECM affects spheroid invasion and EMT by adjusting the stiffness of the matrix. Indeed, we showed that less stiff matrices propel spheroid invasion and EMT, indicating that the physical properties of the ECM influence migration. Overall, this study demonstrated that using the xeno-free 3D hydrogel system is an excellent approach to evaluate cancer invasiveness and EMT, and a promising tool for drug-screening applications.

#137 Type XII collagen is elevated in serum from patients with solid tumors: A non-invasive biomarker of activated fibroblasts.

Marina Crespo-Bravo[1], Annika Hettich[1], Jeppe Thorlacius-Ussing[1], Thomas R. Cox[2], Morten A. Karsdal[1], Nicholas Willumsen[1]

[1]Nordic Bioscience, Herlev, Denmark,[2]Garvan Institute of Medical Research, Darlinghurst, Australia

Background: The tumor microenvironment (TME) and extracellular matrix (ECM) are critical to tumor progression and treatment outcomes. Collagens, the main ECM components, influence cell signaling, migration, and tumor invasion. Type XII collagen is essential for type I collagen assembly and stabilizes the ECM. It is predominantly produced by cancer-associated fibroblasts (CAFs), and its upregulation is linked to poor survival in several cancers. This study developed and validated an ELISA to measure circulating type XII collagen as a potential cancer biomarker.
Methods: A monoclonal antibody targeting the C-terminal of type XII collagen was used to develop a competitive ELISA (nordicPRO-C12) for detecting type XII collagen fragments in serum from cancer patients (n=230) and healthy controls (n=33). Type XII collagen expression was also assessed by Western blot in supernatants and lysates from CAFs and normal fibroblasts (NFs) derived from pancreas, lung, colon, and breast tissues, under both TGFβ-stimulated and non-stimulated conditions.
Results: The nordicPRO-C12 assay showed high specificity and robustness, detecting significantly elevated serum PRO-C12 levels in patients with prostate, breast, melanoma, lung, gastric, pancreatic, head and neck, and colorectal cancers ($p < 0.005$ for all). The assay effectively distinguished cancer patients from healthy controls, with high AUROCs for breast (0.95), prostate (0.92), lung (0.92), gastric (0.88), melanoma (0.83), pancreatic (0.80), and head and neck cancers (0.76). These findings were supported by COL12A1 expression data from TCGA and GTEx. Western blot analysis confirmed increased type XII collagen expression in both CAFs and NFs after TGFβ stimulation, highlighting TGFβ's role in ECM remodeling and type XII collagen regulation in cancer and normal microenvironments.
Conclusion: This study highlights PRO-C12 as a promising non-invasive biomarker for cancer detection and monitoring. The ability to quantify type XII collagen fragments provides insights into cancer progression. While these results are encouraging, further studies are required to validate PRO-C12's diagnostic and prognostic potential across cancers, potentially expanding its application to clinical practice.

#138 Lysyl oxidases suppress pancreatic cancer progression by inhibiting focal adhesion kinase signaling.

Lijuan Sun[1], Jean Albrengues[2], John E. Wilkinson[3], Sarah L. Dallas[4], Valerie M. Weaver[5], Mikala Egeblad[1], Mario A. Shields[6]

[1]Johns Hopkins University School of Medicine, Baltimore, MD,[2]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY,[3]University of Michigan Medical School, Ann Arbor, MI,[4]University of Missouri-Kansas City, Kansas City, MO,[5]University of California, San Francisco, San Francisco, CA,[6]Stony Brook University Renaissance School of Medicine, Stony Brook, NY

Lysyl oxidases crosslink type I collagen to promote fibrosis and cancer progression in mouse mammary tumor models. Pancreatic ductal adenocarcinomas (PDACs) are highly fibrotic and contain abundant type I collagen with elevated expression of lysyl oxidases. Indeed, inhibition of lysyl oxidases constitute an attractive anti-tumor therapeutic strategy, with several reported preclinical studies demonstrating efficacy at reducing PDAC fibrosis and progression. Yet, lysyl oxidase was first described as an anti-oncogene through its effect of directly suppressing cell transformation by mutant Ras which is present in around 90% of human pancreatic tumors. These prior studies highlight the dual functions, anti-ras and pro-fibrotic, of lysyl oxidases in pancreatic cancer. As a result, clinical trials targeting lysyl oxidase in cancers have demonstrated limited efficacy. Here we examined the effects of perturbation of lysyl oxidase activity or expression using syngeneic orthotopic transplantation models expressing mutant Ras and intravital imaging. Unexpectedly, genetic or pharmacological inhibition of lysyl oxidases increased invasion along collagen fibers and distant metastasis. Furthermore, inhibition of lysyl oxidases promoted focal adhesion kinase (FAK) activity which was required for metastasis. We found that mutant Kras status dictated lysyl oxidase-mediated suppression on FAK signaling in both mouse and human pancreatic cancer cells. These results suggest that the effect of lysyl oxidase on metastasis are dependent on signaling from Ras and FAK. These results strongly caution against inhibiting lysyl oxidases for cancers driven by mutant Ras.

#139 The nutrient-dictated ECM regulates cancer cell growth via the itga2-FAK-YAP axis.

Kristof Turan, Julien Guillard, Simon Schwoerer

University of Chicago, Chicago, IL

Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer-related deaths in the United States. PDAC tumors are characterized by a dense desmoplastic stroma and altered nutrient availability that promote aggressive cancer cell behavior. A collagen-rich extracellular matrix (ECM) produced by cancer-associated fibroblasts (CAFs) is a key feature of PDAC's stroma and associated with tumor progression and therapy resistance. How altered nutrient availability and ECM production are integrated to modulate PDAC cell biology is not well understood. To address this gap, we develop a spheroid outgrowth assay on ECM produced by CAFs under altered availability of glutamine, an amino acid shown to be depleted in PDAC. We find that glutamine availability dictates the composition of CAF-derived ECM. The glutamine-dictated ECM differentially modulates PDAC cell behavior, with ECM produced under high glutamine (HQ) availability restraining PDAC cell growth but promoting PDAC cell migration. We link these phenotypes to collagen I abundance, which is depleted in ECM produced under low glutamine (LQ) availability. Mechanistically, we find that increased stiffness of HQ ECM underlies its growth restraining properties, while its activation of focal adhesion kinase (FAK) signaling in PDAC cells supports migration. Specifically, collagen I in HQ ECM binds to integrin α2 (Itga2), activating FAK and downstream YAP/TAZ signaling, both of which are required for PDAC cell migration. In contrast, LQ ECM fails to activate YAP/TAZ signaling in PDAC cells. Activation of YAP/TAZ signaling alone was sufficient to rescue PDAC cell migration on LQ ECM. These findings demonstrate that tumor nutrient availability alters ECM composition, which modulates PDAC biology via Itga2-FAK-YAP signaling.

TUMOR BIOLOGY: Cancer Stem Cells
Poster Session

#14 Molecular drivers of lineage bias in TP53-mutant therapy-related acute leukemias.

Diren Arda Karaoglu[1], Ibrahim Aldoss[2], Ann-Kathrin Eisfeld[3], Madina Sukhanova[4], Yasmin Abaza[4], Peng Wang[1], Melissa Tjota[1], Jeremy Segal[1], Angela Lager[1], Carrie Fitzpatrick[1], Jason Xiaojun Cheng[1], Sandeep Gurbuxani[1], Girish Venkataraman[1], Anand Ashwin Patel[1], Adam S. Duvall[1], Michael W Drazer[1], Todd C Knepper[5], Daniel A. Arber[1], Toyosi Odenike[1], Richard A. Larson[1], Guido Marcucci[6], Bijal D Shah[7], Wendy Stock[1], Caner Saygin[1]

[1]The University of Chicago Medicine Comprehensive Cancer Center, Chicago, IL,[2]Department of Hematology & Hematopoietic Cell Transplantation, City of Hope, Duarte, Duarte, CA,[3]The Ohio State University, Columbus, OH,[4]Northwestern University, Chicago, IL,[5]H Lee Moffitt Cancer Center and Research Institute, Tampa, FL,[6]City of Hope Medical Center and Beckman Research Institute, Duarte, CA,[7]University of South Florida Morsani College of Medicine, Tampa, FL

Clonal hematopoiesis (CH) is a precursor for acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). Therapy-related (tr-) leukemias are long-term complications of genotoxic therapies for cancer or autoimmune disease. They often present with a myeloid phenotype, but a subset of patients (pts) may develop tr-ALL. Molecular mechanisms driving the lineage bias of tr-leukemogenesis originating from CH are unknown.We studied 345 tr-MN and 217 tr-ALL pts from six centers in North America. Multiple myeloma (MM) was the most common antecedent malignancy in tr-ALL (25% of pts), and only 4% of tr-MN pts had MM ($p< 0.01$). 33% of tr-MN pts had complex karyotype, 24% of tr-ALL pts had low hypodiploidy. Other most frequent genetic abnormalities had similar frequencies between tr-MN vs tr-ALL, including mutations in *TP53* (30% vs 33%), *DNMT3A* (16% vs 11%), and *KMT2A*-rearrangements (8% vs 15%). Given the overlap for common genetic drivers in tr-MN vs tr-ALL, we focused on the most frequent subtype of *TP53*-mutant (*TP53*-m) tr-leukemias. We hypothesized that *TP53*-m CH progresses to tr-AML vs tr-ALL via divergent molecular pathways.To elucidate the molecular differences between tr-*TP53*-m leukemias, we performed scRNA-seq of bone marrows from healthy individuals (n=4), *TP53*-m CH (n= 3), *TP53*-m tr-AML (n= 7) and *TP53*-m tr-ALL (n= 6). A total of 120,726 cells were analyzed. We compared gene signatures and transcription factor (TF) activities of hematopoietic stem cells (HSCs) vs leukemic blasts. In GSEA, *TP53*-m HSCs from CH were enriched in fat metabolism pathways (i.e., fatty acid, arachidonic acid, peroxisomal lipid metabolism) when compared to *TP53*-wild type HSCs from healthy individuals. These metabolic pathways were further enriched in *TP53*-m AML blasts when compared to CH and healthy stem cells, suggesting a switch in energy metabolism associated with myeloid lineage commitment. AML blasts also had high NLRP3-inflammasome and tumor necrosis factor (TNF) signaling. AML blasts showed higher TF activity of GATA2, CEBPA, LMO2 and KAT6A, which may indicate a block in myeloid differentiation and altered chromatin remodeling. ALL blasts showed increased activity of transcription factors involved in NF-κB signaling, including NFKB, RELA, JUN and AP1 that may promote cell survival and resistance to apoptosis.In interactome analysis, *TP53*-m AML blasts were characterized by their integrin cell surface interactions, including ITGA5 interaction with CD40LG on T cells, and ITGAV interaction with TGF-β secreted from CD8+ T, NK, and Treg cells. In *TP53*-m ALL, upregulated TNFα response resulted in increased HLA antigen expression.Collectively, tr-AML and tr-ALL have overlapping genetic characteristics, dominated by *TP53*-m and aneuploidy. Our data identified key molecular pathways associated with myeloid vs lymphoid bias of leukemogenesis.

TUMOR BIOLOGY: The Structural Tumor Microenvironment and Integrin Interactions in Cancer
Poster Session

#140 Profiling ECM biomarkers PRO-C3 and PRO-C5 in plasma from patients with esophagogastric cancer.

Eleonora Moroncini[1], Andrea Ponzetta[2], Magnus Nilsson[2], Fredrik Klevebro[2], Rebecca Maltez de Sousa[2], Nora Noren[2], Morten Karsdal[1], Nicholas Willumsen[1]

[1]Nordic Bioscience, Copenhagen, Denmark,[2]Karolinska Institute, Stockholm, Sweden

Background: Esophagogastric cancer encompasses malignancies of the esophagus, esophagogastric junction, and stomach. These cancers involve distinct regions of the esophagus and gastroesophageal junction (GEJ) and exhibit unique clinicopathologic, histologic, and mutational features. The major constituents of the tumor microenvironment (TME) are besides the tumor cells themselves, cancer associated fibroblasts (CAFs), stromal cells and the extracellular matrix (ECM). CAFs synthesize excess amounts of collagens leading to a fibrotic TME with poor response to anti-cancer treatment; it was previously found that among other types of collagens COL3A1 and COL5A1 are expressed in most of activated CAFs. The aim of this study was to investigate the relevance of non-invasive serological biomarkers of type III collagen synthesis (PRO-C3) and type V collagen synthesis (PRO-C5) in patients with GEAC and ESCC.
Methods: Levels of PRO-C3 and PRO-C5 were measured non-invasively using competitive enzyme-linked immunosorbent assays in EDTA Plasma from patients diagnosed with esophageal squamous cell carcinoma (ESCC) (n=27), gastroesophageal adenocarcinoma (GEAC) (n=165) and compared to healthy controls (n=20). Blood samples were obtained during biopsy, and the biomarker measurements reflect baseline levels. PRO-C3 and PRO-C5 levels in patients with GEAC and ESCC were assessed according to disease stage, treatment type and resection status.
Results: Circulating PRO-C3 levels were significantly elevated in both GEAC ($p = 0.0025$) and ESCC ($p = 0.0022$), while PRO-C5 levels were markedly increased in both subtypes (GEAC: $p = 0.0012$; ESCC: $p = 0.0002$). PRO-C5 levels were higher in ESCC compared to GEAC ($p = 0.0023$), whereas PRO-C3 levels were similar between the two ($p = 0.441$). Stratifying by treatment intention showed no significant differences in PRO-C3 or PRO-C5 levels in ESCC patients receiving curative or palliative care. In GEAC, PRO-C5 levels were higher in palliative treatment patients ($p = 0.01$). For resection status, neither PRO-C3 nor PRO-C5 levels varied significantly in GEAC. In ESCC, PRO-C3 levels were elevated in non-resected samples ($p = 0.002$). Stratification by cancer stage revealed significant increases in PRO-C5 levels in GEAC patients at Stage III ($p = 0.002$) and Stage IV ($p = 0.004$).
Conclusion: The non-invasive fibrosis-associated biomarkers PRO-C3 and PRO-C5, indicative of type III and type V collagen synthesis mediated by activated CAFs, are elevated in patients with ESCC and GEAC. These biomarkers show promise as non-invasive tools for stratifying and monitoring patients across different subtypes of esophagogastric cancer.

#141 Utilizing multiplex imaging to characterize the extracellular matrix in cancer.

Anton Villamejor, Joseph Lownik, Ben Falk, Larry Milshteyn, Akil Merchant

Cedars-Sinai Medical Center, Los Angeles, CA

The tumor microenvironment (TME) is known to be an essential component in the response to multiple therapeutics, including immunotherapies. The TME is comprised of the tumor itself, infiltrating immune cells, and the extracellular matrix (ECM). ECM has been shown to play a critical role in tumorigenesis, affect the efficacy of certain treatments, and regulate anti-tumor immunity. However, methods to study ECM have typically been limited to single-marker studies or have used dissociated methods which lose spatial context. Additionally, fluorescence-based approaches to study the ECM are challenging due to the auto-fluorescence properties of multiple ECM components. We chose to utilize the multiplex capabilities of Imaging Mass Cytometry (IMC) for its ability to detect up to 40 different proteins via metal-tagging and absence of auto-fluorescence. We selected and validated 40 markers which we believe play an integral role in ECM, including structural proteins as well as immune markers and metabolic enzymes. This 40-plex panel was validated using a tissue microarray (TMA) comprised of various healthy and diseased tissue samples such as Lung, Colon, Breast, Prostate, Kidney, and Liver and their respective tumor counterparts. After staining, our results underwent rigorous analysis through a multi-step image comparison of our staining results to those in the current literature, ensuring our staining was specific. We found significant differences in the protein expression of various ECM markers between the healthy tissue and their tumor counterpart. We found structural variations of multiple ECM proteins between the normal tissue and tumor tissue. However, the expression patterns of these ECM elements were not uniform between tumor types, with overexpression of HAPLN1 in breast cancer while decreased in colon cancer. The included immune markers allow for spatial studies to examine the role of ECM in regulating immune responses, such as CD8+ T-cell infiltration. Additionally, we found that tumors from non-small cell lung cancer (NSCLC) patients exhibit significant ECM heterogeneity. We used a patch-based analysis approach to examine the ECM phenotypes of a cohort of 35 NSCLC patients. Preliminary assessment demonstrates unique clusters of ECM phenotypes have a utility for predicting patient outcomes. Further analysis of this cohort will be presented. Overall, we present a highly-multiplexed IMC panel which allows for detailed assessment of the ECM.

#142 Integrin α6β4 upregulates IDO1 expression and decreases IFNγ-mediated T cell growth in ER-negative breast cancer.

Parvanee Karimpour[1], Oscar Hanson[2], Andrew Elliott[3], Min Chen[4], Kathleen O'Connor[1]

[1]Molecular & Cellular Biochemistry, University of Kentucky, Lexington, KY,[2]Chemistry, Berea College, Berea, KY,[3]Markey Comprehensive Cancer Center, University of Kentucky, Lexington, KY,[4]Toxicology & Cancer Biology, University of Kentucky, Lexington, KY

The kynurenine metabolic pathway degrades tryptophan into immunosuppressive metabolites following pro-inflammatory cytokine stimulation and is upregulated in ER-negative (ER(-)) breast cancers, where it is associated with a worse prognosis. The laminin receptor integrin α6β4 is expressed in a majority of hormone receptor negative breast cancers, where it is known to promote an aggressive phenotype by regulating cellular signaling and epigenetics. Here, we seek to identify how integrin α6β4 regulates the IFNγ-mediated induction of the first kynurenine pathway enzyme, IDO1, and its impact on immunosuppression in ER(-) breast cancer. Integrin β4 only pairs with integrin α6; thus, expression of the β4 subunit is representative of integrin α6β4 when integrin α6 is expressed. We demonstrate that stable expression of integrin β4 in BT549 cells regulates IDO1 expression by altering methylation of the IDO1 promoter and gene body compared to empty vector (EV) control, which resulted in increased IDO1 expression determined using high throughput sequencing, qPCR, and immunoblot analyses. Likewise, knockdown of integrin β4 by shRNA or knockout by CRISPR in HCC1806 and HCC1954 cells decreased IDO1 expression. Gene set enrichment analysis demonstrated an increased IFNγ response in BT549 cells expressing integrin β4 compared to EV control. Moreover, using the TCGA Breast Invasive Carcinoma dataset, we show that expression of IFNγ, a known inducer of IDO1, is positively associated with IDO1 expression in breast cancer. Upon IFNγ stimulation, the expression of integrin β4 resulted in a more dramatic upregulation of IDO1 at the mRNA and protein level, which was accompanied by alterations in STAT signaling. Furthermore, integrin α6β4 signaling significantly increased secretion of the immunosuppressive metabolite kynurenine following IFNγ stimulation, effects which were abrogated by pharmacologic IDO1 inhibition. Using conditioned media transfer onto Jurkat cells, we show that conditioned media from integrin β4-expressing cells stimulated with IFNγ significantly reduced T cell growth compared to media from IFNγ stimulated EV cells or unstimulated β4 cells. In summary, our data suggest that integrin α6β4 increases IDO1 induction in ER(-) breast cancer cells, amplifies kynurenine secretion, and suppresses T cell growth in the presence of the inflammatory cytokine IFNγ. This study suggests a novel role of integrin α6β4 in limiting T cell-mediated antitumor immunity and highlights the need to elucidate the detailed mechanisms governing integrin α6β4-mediated immunosuppression in ER(-) breast cancers.

#143 Cilengitide sensitivity is predicted by overall integrin expression in breast cancer.

Nomeda Girnius[1], Aylin Z. Henstridge[1], Benjamin Marks[1], Jeffrey K. Yu[1], G. Kenneth Gray[1], Chris Sander[1], Ioannis Zervantonakis[2], **Augustin Luna**[3]

[1]Harvard Medical School, Boston, MA,[2]University of Pittsburgh, Pittsburgh, PA,[3]National Institutes of Health, Bethesda, MD

Background: Treatment options for triple-negative breast cancer (TNBC) are limited and patients face a poor prognosis. Here, we sought to identify drugs that target TNBC vulnerabilities and understand the biology underlying these responses. We analyzed the Broad Institute DepMap to identify recurrent TNBC vulnerabilities and performed a 45-compound screen on vulnerability-related pathways on a set of up to 8 TNBC cell lines. We identified a subset of cell lines with an ITGAV vulnerability and a differential sensitivity to cilengitide, an integrin inhibitor targeting ITGAV:ITGB3 and ITGAV:ITGB5. Next, we sought to understand cilengitide resistance and response biomarkers. Clinical trials targeting integrins continue enrolling patients, necessitating an understanding of how these drugs affect tumors.

Methods: We combined in vitro assays with computational approaches to systematically explore the differential sensitivity to cilengitide and resistance mechanisms. We tested an additional pan-ITGAV inhibitor (GLPG0187) to determine how generalizable our findings on cilengitide sensitivity might be to integrin inhibition. ITGB4, ITGA3, and ITGA6 knockdown experiments assessed the importance of integrin monomers in cell attachment during cilengitide treatment. Also, we explored the role of extracellular matrix (ECM) proteins in cilengitide response by performing cell replating experiments and by culturing on collagen, fibronectin, or laminin coated plates.

Results: We discovered that cell-derived ECM modulates cilengitide sensitivity and exogenous fibronectin addition conferred resistance to all sensitive TNBC cell lines, though fibronectin expression did not correlate with sensitivity. Instead, elevated overall integrin protein levels, not specific integrins, in TNBC cells positively correlated with resistance. This suggested that high pan-integrin expression promotes cilengitide resistance. Thus, we tested cilengitide in six luminal breast cancer cell lines (which have low integrin levels); all were sensitive. Also, pan-ITGAV inhibitor, GLPG0187, showed the same sensitivity profile across our TNBC cell lines, suggesting our findings apply to other integrin inhibitors.

Conclusions: Integrin inhibitors are appealing candidates to pursue as anti-cancer drugs because they are generally well-tolerated, but their efficacy is mixed, possibly due to the absence of predictive markers. Cilengitide induces death in breast cancer cells with low integrin abundance, where complementary ECM promotes survival. Thus, integrin inhibition in breast cancer warrants further study.

#144 A bispecific integrin α5β1/αv antibody downregulates c-myc/E2F and TGF-β signaling and induces immunological control of basal-type prostate cancer cells.

R. Joshi[1], M. Zhou[1], J. Lin[1], C. Morrissey[2], K. Hu[1], A. Poltorak[3], P. Mathew[1];
[1]Tufts Medical Center, Boston, MA, [2]University of Washington, Seattle, WA, [3]Tufts University, Boston, MA

Background: Prior studies demonstrated that integrin α5β1 and αv crosstalk in chemotaxis and clonogenic survival of prostate cancer cells is abrogated by a bispecific α5β1/αv antibody (BsAbα5β1/αv), which uniquely induces internalization and lysosomal degradation of target integrins (Joshi, MCR 2020). We hypothesized that the BsAbα5β1/αv would inactivate pathological signaling pathways which would be validated in the control of tumor xenografts *in vivo* and in correlative studies from patient samples.
Methods and Results: In basal-type prostate cancer (BTPC) cells, the BsAbα5β1/αv uniquely abrogated nuclear localization of YAP, active β-catenin, FAK and EGFR, compared to monospecific integrin α5β1 and αv antibodies (MsAbs), individually or combined. Dual integrin αv and α5 knockdown alone phenocopied the BsAbα5β1/αv effect. *In vivo,* clonogenic survival of BTPC cells (PC-3, DU-145) was eliminated by the BsAbα5β1/αv in nude mice, with complete regressions in up to 50% of established xenografts, associated with mononuclear infiltration. Tumor response and survival with BsAbα5β1/αv-treatment was superior to MsAbs, individually or combined. *In vitro*, RNA-seq of BsAb-treated BTPC cells revealed control of c-myc/E2F and EMT signaling with upregulation of Type I/II interferon pathways. Concordantly, multiplex ELISA of BsAb-treated BTPC supernatants showed induction of CXCL10 and CCL5 cytokines, with influx of natural killer (NK) cells, monocytes and macrophages into BsAb-treated xenografts confirmed by flow cytometry. Tumor control by the BsAbα5β1/αv was abrogated by NK cell depletion, highlighting their critical role. In a rapid autopsy TMA of metastatic castration-resistant prostate cancer (CRPC), integrin αv was highly expressed in osseous and non-osseous metastases and co-expressed with integrin α5 in bone metastases, osteoblasts and endothelial cells. Relevant to the osteoblastic phenotype of metastatic prostate cancer, the BsAbα5β1/αv strongly downregulated osteogenic differentiation of human bone marrow-derived mesenchymal stromal cells, superior to individual MsAbs. RNA-seq from rapid autopsy samples correlated integrin αv expression with c-myc/E2F signaling, consistent with data from SU2C CRPC and DKFZ early-onset prostate cancer cohorts.
Conclusions: The BsAbα5β1/αv represents a novel therapeutic strategy to reprogram multiple hallmark pathological pathways including c-myc/E2F, TGF-β and EMT. It induces immunologic control of basal-type prostate cancers which are a paradigm of stem-like treatment resistance and immune evasion. Further translational development of the BsAbα5β1/αv therapeutic strategy in prostate cancer is warranted.

#145 Engineered hydrogel platform reveals integrin-ECM mechanisms and therapeutic targets in esophageal adenocarcinoma.

Daniel E. Garcia, Esha A. Uddin, Noriyuki Nishiwaki, Yasuto Tomita, Joel T. Gabre, Hiroshi Nakagawa, **Ricardo Cruz-Acuna**

Columbia University Irving Medical Center, New York, NY

INTRODUCTION: Esophageal adenocarcinoma (EAC) is an aggressive cancer with poor prognosis, partly due to its complex interaction with the extracellular matrix (ECM), which influences disease progression and therapy resistance. However, the underlying ECM-driven mechanisms of EAC growth and therapeutic resistance remain unknown. Traditional *in vitro* models are limited by their use of cancer cell lines and lack of 3D cellular interactions. To address this gap, we developed a bioengineered hydrogel system integrated with patient-derived 3D organoids (PDOs) to better replicate the native ECM environment in EAC.

METHODS: We engineered a hyaluronic acid (HA)-based hydrogel with tunable physicochemical properties to support the culture of EAC PDOs. To study ECM component-specific influences on EAC mechanobiology, we tethered individual adhesive peptides corresponding to key ECM components onto the hydrogel backbone: RGD (fibronectin), GFOGER (collagen), and YIGSR, AG73, or IKVAV (laminin). By independently varying the type and density of each peptide, we examined their effects on PDO growth, proliferation, and therapeutic resistance. Additionally, we conducted inhibition studies using integrin-targeting small molecule inhibitors to explore the role of integrin-mediated adhesion in sensitizing PDOs to chemotherapy.

RESULTS: Among the five adhesive peptides tested, RGD had the most significant effect on PDO formation, growth, and proliferation. Transcriptional and protein analyses revealed elevated expression of integrin αv, integrin β1, and Yap in PDOs cultured in NorHA-RGD hydrogels compared to other peptides. These findings were validated in EAC patient tissue samples. Additional experiments demonstrated a concentration-dependent effect of RGD on PDO growth and Yap activation, mirroring the increased fibronectin deposition observed in EAC tissue. Treatment with cilengitide, an αvβ3 integrin inhibitor, in RGD-modified hydrogels significantly reduced PDO formation, and enhanced the sensitivity of PDOs to standard chemotherapy.

CONCLUSION: This study establishes an engineered biomaterial platform that recapitulates key ECM biochemical properties, providing a foundation for pre-clinical drug testing and mechanistic studies. Our data indicate that αvβ3 integrin-mediated adhesion is critical for EAC PDO growth and activation of oncogenic pathways. The efficacy of cilengitide within this model suggests that targeting fibronectin-integrin αvβ3 interactions could be a viable therapeutic strategy for EAC when combined with chemotherapy. Ongoing work will extend this platform to *in vivo* models to investigate chemoresistance mechanisms and characterize αvβ3+ cancer stem cell populations.

#146 Studying the effect of compressive solid stress on macrophage phenotype and function in the context of glioblastoma.

Alice Burchett, Hao Chen, Scott Howard, Meenal Datta

University of Notre Dame, Notre Dame, IN

Glioblastoma (GBM) is one of the most aggressive forms of brain cancer, with an overall survival of less than two years. One barrier to therapeutic efficacy is the extremely immunosuppressive GBM tumor microenvironment (TME). Myeloid cells, such as macrophages, accumulate in GBM tumors and can comprise up to half of the tumor bulk. Macrophage phenotype typically ranges from pro-inflammatory (M1-like) to anti-inflammatory (M2-like). In GBM, they adopt a pathological, pro-tumor phenotype that thwarts anti-tumor immune responses. The GBM TME also features solid stress, which arises as the tumor expands and presses against the surrounding tissue. As residents of the tumor, macrophages therefore experience chronic compression. Here, we utilize 2D and 3D models to interrogate the macrophage response to and contribution to solid stress as seen in GBM.

Murine macrophages were seeded in a cell culture insert and compression was applied with a 3D printed weight. An agarose cushion between the cells and the weight served as a media reservoir and prevented direct contact between the cells and the rigid weight. We measured the resulting changes in metabolic state, gene expression, nitric oxide production, phagocytosis, and influence on GBM cell proliferation. Using fluorescence lifetime imaging, we found that compression caused an increase in average cell fluorescence lifetime, indicating a shift towards increased oxidative phosphorylation. This is consistent with an M2-like, or anti-inflammatory phenotype. However, compressed macrophages also produced significantly more nitric oxide, a hallmark of pro-inflammatory macrophage function. Compressed macrophages upregulated both nitric oxide synthase and arginase-1, markers of canonically pro- and anti-inflammatory phenotype, respectively. They also had increased phagocytosis of microbeads. These results suggest that chronic compression results in a more complex phenotype than is represented on the traditional pro- and anti-inflammatory axis. Macrophages can also generate de novo solid stress in a confining 3-D hydrogel, with stress generation and phenotype responsive to physical and biochemical cues. This indicates that there may be reciprocal regulation between solid stress and macrophage phenotype in GBM, representing a promising therapeutic target to alleviate pathological solid stress and immunosuppression.

Macrophages in GBM tumors also display elements of classical pro- and anti-inflammatory phenotypes. These results suggest that mechanical compression alone, independent from tumor-derived biochemical cues, may contribute to this pathological phenotype. Future work will elucidate targetable pathways involved in the macrophage response to chronic solid stress, with the aim of normalizing the tumor immune microenvironment to improve the efficacy of the natural or engineered anti-tumor immune response.

TUMOR BIOLOGY: Cancer Stem Cells
Poster Session

#15 Midasin is required for mammosphere stability in Letrozole resistant breast cancer.

Bipika Banjara[1], Mounika Pamukuntla[1], Manasa Kotina[1], Afia Ohemeng[1], Alicia Hudson[1], Selina Darling-Reed[1], Jillian L. Pope[2], Michael Davidson[1], Syreeta L. Tilghman[1]

[1]Pharmacy and Pharmaceutical Sciences, Florida Agricultural and Mechanical University Foundation, Inc., Tallahassee, FL,[2]Biological Sciences, Florida Agricultural and Mechanical University Foundation, Inc., Tallahassee, FL

While aromatase inhibitors (AI) (i.e., Letrozole) are first-line therapy for postmenopausal women with hormone dependent breast cancer, long-term treatment leads to resistance accompanied by the emergence of breast cancer stem cells (CSCs). Previously, our lab demonstrated that midasin, a novel chaperone protein involved in ribosomal biogenesis was increased 35-fold in the letrozole-resistant mammospheres and high levels of midasin was associated with increased relapse free survival in endocrine resistant breast tumors. Here, we hypothesize that elevated midasin expression poises AI-resistant cells for increased stemness, proliferation, and metastasis. To this end, we cultured AI-sensitive (AC-1) and AI-resistant (LTLT-Ca) cells two- and three dimensionally to study the consequences of midasin alteration on cellular behavior by utilizing a midasin inhibitor (i.e., ribozinoindole-2). Although results from proliferation assays indicate that 1-18 μM Rbin-2 treatment caused a 35-60% reduction in cell proliferation, the AC-1 cells were more sensitive. Similar results were observed when colony formation assays were performed. Overall, the LTLT-Ca cells expressed higher levels of midasin compared to the AC-1 cells, and 18μM Rbin-2 treatment reduced midasin expression in both cell lines. To understand the impact of midasin on the biology of endocrine resistance, epithelial to mesenchymal markers were examined and compared to AC-1 cells, LTLT-Ca cells expressed 3-fold higher levels of N-cadherin which were reduced by Rbin-2 treatment. Although the LTLT-Ca exhibited more mesenchymal properties and were more migratory, Rbin-2 treatment caused a similar dose-dependent decrease in wound healing in both cell lines. Interestingly, when mammosphere formation assays were conducted, Rbin-2 treatment induced mammosphere disintegration in both cell lines. When AC-1 and LTLT-Ca cells were treated with 18 μM Rbin-2, the AC-1 spheres were denser and larger (41.73 μm) while the LTLT-Ca spheres were hollow and smaller (5.80 μm), suggesting that midasin is required for stemness. LTLT-Ca mammospheres exhibited higher levels of N-cadherin and vimentin that were decreased with Rbin-2 treatment. Taken together, these changes suggest that increased midasin expression induces an EMT-like state which may in part be responsible for the mammosphere integrity, and enhanced migration which ultimately aids in maintenance of endocrine resistance. Midasin inhibition reverses this phenotype and may provide a novel strategy to target certain endocrine resistant breast tumors.

TUMOR BIOLOGY: Where's Waldo: Spatial Complexity of the Tumor Microenvironment 1
Poster Session

#150 Integrating spatial metabolomics and spatial transcriptomics on the same cancer tissue sections to detect gene-metabolite correlations.

Trevor M. Godfrey[1], Yasmin Shanneik[1], Wanqiu Zhang[2], Thao Tran[2], Nico Verbeeck[2], Nathan H. Patterson[2], Faith E. Jackobs[1], Maheswhari Ramineni[3], Chandandeep Nagi[1], Livia S. Eberlin[1]

[1]Department of Surgery, Baylor College of Medicine, Houston, TX,[2]Aspect Analytics NV, Genk, Belgium,[3]Department of Pathology, Baylor College of Medicine, Houston, TX

The combination of spatial metabolomics and spatial transcriptomics can reveal powerful connections between gene expression and metabolism in heterogeneous tissues. However, section-to-section variability can convolute data integration. We present a novel approach, termed Desium, which integrates spatial metabolomics by Desorption Electrospray Ionization Mass Spectrometry Imaging (DESI-MSI) and Visium Spatial Transcriptomics (VST) on the same tissue section. This work demonstrates that DESI-MSI does not significantly impact RNA quality, enabling robust analysis of gene-metabolite relationships in human breast and lung cancer tissues. The Desium workflow involves sequentially performing DESI-MSI at 100 µm spatial resolution, hematoxylin and eosin (H&E) staining, and VST CytAssist analyses on the same tissue section. We applied this workflow to six human cancer tissues (3 breast, 3 lung) and analyzed a serial section of two samples (1 breast, 1 lung) with standard VST CytAssist as a control. H&E and VST data were automatically aligned, while DESI-MSI and H&E data were coregistered manually using nonrigid registration. The MSI data was then converted into VST spot coordinates using a Gaussian granularity matching algorithm. Pearson correlation scores were calculated for each tissue to link gene expression and metabolite abundances. Comparing sequencing results between Desium and the standard VST protocol using UMI counts/gene, we found a correlation coefficient of 1 for both lung and breast samples, indicating minimal impact of DESI-MSI on gene expression measurements. Across all samples, we identified 15,111 transcript-metabolite correlations from 1,136 transcripts and 439 metabolites, with correlation scores above 0.5 or below -0.5. Notably, we observed a 0.82 correlation between glycerophosphorylethanolamine and XBP1 in breast cancer and a 0.77 correlation between phosphatidylglycerol PG 22:6_22:5 and MSLN in lung cancer. Our study demonstrates that Desium enables unambiguous spatial correlations between metabolites and RNA transcripts, uncovering novel relationships and unique patterns of intratumor heterogeneity not evident through histologic analysis. These findings suggest that Desium could serve as a powerful tool to explore the gene expression-metabolic phenotype relationships within the complex tumor microenvironment.

#151 Spatial patterns of APOBEC mutagenesis in the tumor microenvironment of Asian breast cancer.

Z. Tan[1], Y. Wu[2], Z. Neo[2], M. Lau[2], S. Chang[3], J. Yeong[2], S.-H. Teo[1], J. Pan[1];
[1]Cancer Research Malaysia, Subang Jaya, Malaysia, [2]Agency for Science, Technology and Research (A*STAR), Singapore, Singapore, [3]Universiti Malaya, Wilayah Persekutuan Kuala Lumpur, Malaysia

A common germline polymorphism in chromosome 22 involves the deletion of a large portion of the *APOBEC3B* gene that results in an *APOBEC3A-B* hybrid transcript. The hybrid transcript increases *APOBEC3A* activity and therefore increases APOBEC-associated mutagenesis, which is a common and important mutational signature in cancers. This deletion polymorphism is found with higher prevalence in Asians and is associated with increased risk of breast cancer in Asian women, as well as development of hypermutated cancers with greater immune cell infiltration. We used Stereo-seq spatial transcriptomics to study the relationship between *APOBEC3B* germline deletion, APOBEC mutagenesis, and the tumor immune microenvironment in Asian breast cancers with *APOBEC3B* germline deletion (carriers, either $A3B^{del/del}$ or $A3B^{del/WT}$), and compared them to non-carriers. To study APOBEC mutagenesis, we constructed new bioinformatics pipelines to conduct variant calling and neoantigen prediction from Stereo-seq data whilst retaining spatial information. Unsupervised neighborhood clustering and cell type deconvolution revealed that the carriers have overall higher immune infiltration into the tumor compared to non-carriers. Somatic C>T mutations, the hallmark of APOBEC mutagenesis, were more prevalent in $A3B^{del/del}$ tumors. One sample showed a high overlap between somatic YTCA mutations, which are associated with *APOBEC3A* activity, and cancer associated fibroblasts (CAFs). Neoantigens originating from somatic mutations, specifically those arising from C>T mutations, were also more prevalent in $A3B^{del/del}$ tumors, but relatively few neoantigens arose from TCA or YTCA mutations. Our data suggest that biologically meaningful spatial resolution of APOBEC-associated somatic variants and neoantigens in tumor samples is possible using Stereo-seq technology.

#153 High-resolution profiling of the tumor microenvironment with spatial ecotypes.

W. Zhang[1], E. L. Brown[1], A. Usmani[2], N. Earland[2], H. Jeon[1], I. Alahi[3], N. P. Semenkovich[4], J. C. Schwab[1], C. B. Steen[5], C. M. Sachs[2], B. Gungoren[6], F. Qaium[7], P. K. Harris[2], J. Mudd[2], Q. Cai[8], A. Bacchiocchi[9], R. C. Fields[2], M. Sznol[9], R. Halaban[9], D. Y. Chen[2], A. A. Chaudhuri[7], A. M. Newman[1];

[1]Stanford University, Palo Alto, CA, [2]Washington University School of Medicine, St. Louis, MO, [3]Washington University, St. Louis, MO, [4]Medical College of Wisconsin, Milwaukee, WI, [5]Oslo University Hospital Institute of Clinical Medicine, Oslo, Norway, [6]California Institute of Technology, Pasadena, CA, [7]Mayo Clinic, Rochester, MN, [8]Peking University, Beijing, China, [9]Yale University, New Haven, CT

Background: Multicellular programs in the tumor microenvironment (TME) drive cancer pathogenesis and response to therapy but remain challenging to identify and profile clinically. Here, we present Spatial EcoTyper, a multimodal machine learning framework for large-scale profiling of spatially dependent cell states and multicellular ecosystems, termed spatial ecotypes (SEs).

Methods and Findings: By integrating five million single-cell and spot-level spatial transcriptomes from 10 diverse human neoplasms, including carcinomas and melanomas, we identified a rich tapestry of conserved regional plasticity across nine TME cell types. We then applied Spatial EcoTyper to deeply dissect these data, revealing nine novel SEs with broad conservation - each with unique biology, TME cell states, geospatial features, and clinical outcome associations. SEs were linked to specific biogeographic domains, including the tumor core, margin, and adjacent stroma, and were remarkably well-validated in 235 held-out tumor samples profiled by either single-cell RNA sequencing, sequencing-based spatial transcriptomics (10x Visium), or high-resolution imaging-based spatial transcriptomics (Vizgen MERSCOPE and NanoString CosMx). By deconvolving SEs from over 1,200 bulk tumor RNA-seq profiles, we discovered striking associations between SE levels and response to immune checkpoint inhibitors (ICIs), outperforming nearly 40 potential correlates of ICI response, including previously described signatures of tertiary lymphoid structures and tumor tissue PD-L1 expression. Notably, baseline levels of a proinflammatory SE - localized to the tumor core and defined by a hub of unique T cell, macrophage, fibroblast, and endothelial cell states - strongly forecasted ICI benefit, while another SE - localized to the tumor margin and defined by myofibroblast and hypoxia-associated endothelial cell states - portended ICI resistance.

Conclusion: Our data reveal fundamental units of TME organization and demonstrate a platform for large-scale profiling of spatial cellular ecosystems in any tissue, with implications for improved forecasting of immunotherapy response and the development of novel therapeutic strategies.

#154 Single-cell spatial transcriptomics unravels cell states and ecosystems associated with clinical response to immunotherapy.

Ziena Abdulrahman[1], Roderick C. Slieker[1], Daniel McGuire[2], Marij J.P. Welters[1], Mariette I.E. van Poelgeest[1], Sjoerd H. van der Burg[1]

[1]Leiden University Medical Center (LUMC), Leiden, Netherlands,[2]Nanostring Technologies, Seattle, WA

The tumor microenvironment (TME) is a highly dynamic and complex ecosystem, where the diversity of cell types, their functional states, and spatial interactions determine responses to immunotherapy. Despite its critical role, the precise mechanisms through which these factors influence clinical outcomes remain poorly understood. In this study, we leveraged single-cell spatial transcriptomics to systematically dissect the TME and identify determinants of immunotherapeutic response in vulvar high-grade squamous intraepithelial lesions (vHSIL).

We analyzed 20 treatment-naïve vHSIL lesions, stratified based on clinical response to tumor-specific peptide vaccination into: complete responders (CR, 100% lesion clearance, n=6), partial responders (PR, >50% lesion clearance, n=7), and non-responders (NR, <50% lesion clearance, n=7). Using a 1000-gene panel, we mapped over 274,000 single cells *in situ*, revealing 18 cell clusters that were further delineated into 99 distinct non-epithelial cell states. Validation against public single-cell transcriptomic datasets confirmed the broader relevance of these findings across tumor types.

Our findings demonstrated profound heterogeneity within the TME across response groups. Notably, CR lesions exhibited a significantly higher ratio of immune-supportive to immune-suppressive cells compared to PR and NR lesions—a pattern mirrored in other solid tumors following neoadjuvant checkpoint blockade therapy (anti-PD1, anti-PDL1, anti-CTLA4). Key immune populations enriched in CR lesions included CD4+CD161+ effector T cells and chemotactic CD4+ and CD8+ T cells, implicating these populations as critical drivers of favorable therapeutic outcomes. Conversely, PR lesions were characterized by increased proportions of T helper 2 cells and CCL18-expressing macrophages, which are associated with the recruitment of type 2 T cells and regulatory T cells. NR lesions displayed preferential infiltration with immunosuppressive fibroblasts. Distinct spatial immune ecosystems further defined response groups: type 1 effector cells dominated interactions in CR lesions, type 2 cells were prominently interacting in PR lesions, while NR lesions lacked organized immune cell interactions.

Thus, these findings emphasize the dual importance of cellular composition and spatial organization in steering response to immunotherapy. By elucidating the mechanisms underlying clinical responses to immunotherapeutic vaccination, this study provides actionable insights to advance precision immunotherapy.

#155 Spatial analysis of tumor-immune-stromal cellular phenotypes and microenvironments in pancreatic cancer.

Noor Shakfa[1], Ferris Nowlan[2], Elizabeth Sunnucks[1], Sibyl Drissler[2], Tiak Ju Tan[3], Chengxin Yu[2], Jennifer L. Gorman[1], Michael J. Geuenich[2], Sheng-Ben Liang[4], Edward L. Y. Chen[1], Golnaz Abazari[2], Barbara Gruenwald[5], Ayelet Borgida[6], Cassandra J. Wong[7], Brendon Seale[7], Zhen Yuan Lin[7], Miralem Mrkonjic[8], Julie M. Wilson[9], Kieran R. Campbell[2], Anne-Claude Gringas[1], Grianne M. O'Kane[9], Faiyaz Notta[9], Steven Gallinger[10], Hartland W. Jackson[1]

[1]Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada,[2]Molecular Genetics, University of Toronto, Toronto, ON, Canada,[3]Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada,[4]UHN Program in BioSpecimen Sciences, University Health Network, Toronto, ON, Canada,[5]Tumor Heterogeneity and Translational Systems Biology, West German Cancer Center, Essen, Germany,[6]Medical Oncology and Hematology, Princess Margaret Cancer Centre, Toronto, ON, Canada,[7]NBCC Proteomics Facility, Mount Sinai Hospital, Toronto, ON, Canada,[8]Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, ON, Canada,[9]PanCuRx Translational Research Initiative, Ontario Institute for Cancer Research, Toronto, ON, Canada,[10]Hepatobiliary/Pancreatic Surgery, University Health Network, Toronto, ON, Canada

Pancreatic ductal adenocarcinoma (PDAC) is notoriously resistant or refractory to both modern and conventional therapies. These tumors are characterized by heterogeneous malignant epithelial lesions and immune cells embedded within a highly dense and complex stroma. The unique, multi-compartmental tumor microenvironment (TME) —encompassing diverse lineages such as fibroblasts, mural cells, endothelial cells, lymphoid and myeloid populations, cancer cells, and a rich extracellular matrix—indicates the presence of intricate cellular networks that drive tumor progression. Efforts to profile and therapeutically target specific compartments or cellular phenotypes have often led to unintended adverse effects on neighboring cells and their microenvironments, compromising drug efficacy. To identify vulnerabilities in these interactions and precisely quantify intra- and inter-tumoral spatial heterogeneity, we utilized single-cell RNA sequencing data from eight distinct datasets to design separate custom multiplexed histopathology panels for imaging mass cytometry against tumor, immune, and stromal targets. Three serial sections of a tissue microarray containing 4 cores from 221 patients on each were stained with compartment-specific panels. Cell segmentation and clustering of 7.9 million cells in our dataset revealed 104 unique cell types, their functional states along with biophysical responses. We identified the presence of multiple epithelial subtypes within a single patient, along with single-compartment cellular organization and phenotypes associated with patient outcomes. Advanced image alignment using pixel classification and image transformation across serial sections enabled integration of TME-wide information, defining reproducible microenvironments neighboring malignant ducts. These microenvironments demonstrated immune and stromal patterns closely associated with specific epithelial phenotypes, carrying significant prognostic implications. Laser capture microdissection combined with mass spectrometry provided deeper insights into the biological processes within our microenvironments. We further identified associations between our microenvironments with bulk-RNAseq and specific genomic aberrations. These findings contribute to a nuanced understanding of the TME in PDAC and highlight the therapeutic potential in addressing this complexity to overcome the challenges in treating patients with PDAC.

#156 Tumor microenvironment profiling of multiplexed images in head and neck cancer reveals spatial features associated with immunotherapy response.

Ettai Markovits[1], James Monkman[2], Aaron Kilgallon[2], Thazin Nwe Aung[3], David Rimm[3], Arutha Kulasinghe[2]

[1]Nucleai, Tel Aviv, Israel,[2]Frazer Institute, Faculty of Medicine, The University of Queensland, Brisbane, Australia,[3]Department of Pathology, Yale University School of Medicine, New Haven, CT

Introduction: Current objective response rates of recurrent or metastatic head and neck squamous cell carcinoma (RM HNSCC) to immune checkpoint inhibition (ICI) remain less than 20%, highlighting the innate variability of patient tumors that preclude their immunogenic clearance. Such outcomes in HNSCC have driven the exploration of the immune and tumor centric properties that drive such refractory responses to ICIs. This study expands on this knowledge base by leveraging highly multiplexed imaging combined with a deep learning cell type classification pipeline to comprehensively evaluate the spatial properties of tumor and immune cells associated with ICI response.

Methods: 65 RM HNSCC pre-ICI biopsies on tissue microarrays were imaged using highly multiplexed immunofluorescence performed on the Akoya PhenoCycler platform. Cell type classification was performed using a deep learning platform developed by Nucleai. Marker positivity was used to further assign cell type functional states. Supervised spatial clustering and tumor cell density contours were used to define tissue regions in TMA cores. Cell frequencies, tumor-immune cell ratios and cell-cell interactions were computed within these regions as input for tests against clinical correlates.

Results: 95,000 tumor cells and 76,000 immune cells were phenotyped for both functional and metabolic states. Macrophages and CD4 T cells were the most abundant immune cell types in the cohort, with both CD4 and CD8 T cells demonstrating highly $OXPHOS^+$ metabolically active states. Univariate analysis for progression free survival indicated CD8 T cells in tumor regions were associated with better PFS, while CD4 Tregs expressing ICOS in stromal regions correlated with poorer PFS. Interestingly, both B cells and Granulocytes at the tumor-stroma interface correlated with poorer PFS. Furthermore, the interaction between tumor cells and highly metabolically active fibroblasts within tumor regions was also associated with poorer PFS.

Conclusions and future directions: Our study interrogated spatial compartments in HNSCC tissues for cellular features that may predict response to ICI therapy. Such approaches are limited by the provision of sufficient cells to derive meaningful biology. Future aims for this study include deep learning methods to contrast tissue subgraphs to identify regions that differ significantly between patients, and thereby provide novel insights into the relevant spatial communities that drive cancer immunotherapy responses in patient biopsies.

#157 Exploratory analysis of tumor microenvironment using scRNA, scTCR, and spatial transcriptomics in salivary gland cancer with surgical sample after neoadjuvant immuno-chemotherapy.

Hyunsu Kim[1], Sehhoon Park[1], Jin Woo Oh[2], Soohyun Hwang[2], Jinyoung Kim[1], Eun-hye Kim[3], Nayeon Choi[3], Junhun Cho[4], Hyun-Ae Jung[1], Dongryul Oh[5], Se-Hoon Lee[1], Yong Chan Ahn[5], Han-Sin Jeong[3], Chang Ho Ahn[2], Chan-Young Ock[2], Myung-Ju Ahn[1]

[1]Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center, Seoul, Korea, Republic of,[2]Lunit, Seoul, Korea, Republic of,[3]Department of Otorhinolaryngology-Head and Neck Surgery, Samsung Medical Center, Seoul, Korea, Republic of,[4]Department of Pathology and Translational Genomics, Samsung Medical Center, Seoul, Korea, Republic of,[5]Department of Radiation Oncology, Samsung Medical Center, Seoul, Korea, Republic of

Background: High-grade resectable salivary gland cancer (SGC) is a rare malignancy, with surgery as the standard primary treatment. However, patients with high-grade histology face a significant risk of regional and distant metastasis, often leading to treatment failure. As part of the ONO-4538-X78 clinical trial, three cycles of neoadjuvant nivolumab combined with cytotoxic chemotherapy were administered prior to surgery. This study aimed to conduct exploratory biomarker analyses using surgical samples to investigate tumor microenvironment (TME) changes after neoadjuvant immunotherapy.
Methods: Single-cell RNA sequencing (scRNA-seq) and single-cell T cell receptor (TCR) sequencing were performed on 17 samples from 14 SGC patients, including 3 adjacent normal tissues, 2 pre-treatment biopsies, and 12 post-treatment surgical specimens. Additionally, spatial transcriptomics (Xenium) and histological analyses (H&E via Lunit SCOPE IO) were conducted on 16 post-treatment surgical samples. Responders were defined as patients exhibiting ≤10% viable tumor in surgical specimens.
Results: scRNA-seq analysis identified 32,489 T and NK cells, which were subclustered to reveal cell types associated with treatment response. Non-responders exhibited a predominance of CD4+ memory T cells, while responders demonstrated enrichment of CD8+ dysfunctional T cells and CD8+ memory T cells. Despite statistical insignificance of increased CD8+ T cell subtypes in responders, accompanied by elevated TCR clonality and reduced TCR diversity, suggestive of clonal expansion. Myeloid cell analysis revealed a predominance of tumor-associated macrophages in non-responders, while responders were enriched in dendritic cells and neutrophils. Morphological profiling via Lunit SCOPE IO identified endothelial cells, fibroblasts, lymphocytes, macrophages, tumor cells, and other cell types. Integration of scRNA annotations with Xenium data validated gene expression patterns and identified detailed subtypes. In cancer regions, responders exhibited significantly higher densities of "other" cell populations, predominantly myeloid and stromal cells, including monocyte-derived macrophages, neutrophils, and smooth muscle cells.
Conclusions: This study employed an integrative approach combining scRNA-seq, Xenium spatial transcriptomics, and advanced morphological profiling to characterize the TME and its association with neoadjuvant therapy response in SGC. This multi-modal methodology provided enhanced resolution, enabling the identification of distinct cellular compositions and spatial patterns in cancer areas that differed between responders and non-responders. These findings offer valuable insights into the mechanisms of neoadjuvant therapy and may inform future therapeutic strategies for SGC.

#158 Spatial heterogeneity of tumor cell states in primary cutaneous melanoma.

Tuulia Vallius[1], Yingxiao Shi[1], Edward Novikov[1], Ajit Nirmal Johnson[2], Roxanne Pelletier[1], Shishir Pant[1], Zoltan Maliga[1], David Liu[3], Guihong Wan[4], Yevgeniy Semenov[4], Christine Lian[5], George Murphy[5], Sandro Santagata[5], Peter Sorger[6]

[1]Harvard Medical School, Boston, MA,[2]Department of Dermatology, Brigham and Women's Hospital, Boston, MA,[3]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA,[4]Department of Dermatology, Massachusetts General Hospital, Boston, MA,[5]Department of Pathology, Brigham and Women's Hospital, Boston, MA,[6]Department of Systems Biology, Harvard Medical School, Boston, MA

Cutaneous melanoma develops from precursor cells that progress to melanoma *in situ* (MIS) and eventually to invasive disease. In metastatic tumors, cells switch from a melanocytic to an undifferentiated, and neural crest (NC)-like state, which is associated with metastasis and therapeutic resistance. However, it is unclear whether these cell states or gene expression changes occur at the premetastatic stage or if they are associated with features of the local tumor microenvironment (TME). We analyzed a cohort of 62 patients with primary cutaneous melanoma (stages 0-III), using cyclic multiplex immunofluorescence imaging (CyCIF), and spatial transcriptomics to assess the molecular and TME changes that occur during the transitions from normal to precursor lesions, MIS and invasive melanoma. The loss of MART1 was associated with increased NGFR expression and elevated proliferation (KI67) in thicker tumors compared to thin (<1mm) lesions. Various tumor cell states coexisted in close proximity within early-stage, untreated melanomas. These tumor states were not arranged by depth of invasion but were associated with specific immune and stromal cell niches. Using CyCIF, we quantified the spatial heterogeneity of tumor cell states at whole-slide, microregion, and single-cell levels by calculating spatial entropy. We found that samples with a range of spatial entropy values contained *both* regions of high and low local neighborhood Shannon entropy at the single cell level. High spatial heterogeneity at the protein level was associated with enrichment of interferon gamma and alpha pathway transcripts. We also identified that transcriptomic heterogeneity within a single primary melanoma sample could be just as varied as across the entire cohort, with microregions showing distinct morphologic and spatial features. To understand the gene expression changes leading to intratumoral heterogeneity, we applied a self-organizing map to the spatial transcriptomic data to determine a developmental trajectory from melanocytic atypia to invasion. We discovered that tumor cells in invasive regions followed two developmental branches, characterized by epithelial-to-mesenchymal transition, angiogenesis, interferon response pathways, and dedifferentiation signatures. These developmental branches also showed differences in the local TME composition and the depth of invasion. Our multi-omic analyses reveal that a spectrum of tumor states exists even in early-stage primary cutaneous melanomas, suggesting an association between these tumor states and TME niches.

#159 Spatial multimodal understanding of disease heterogeneity and aggressiveness in renal cell carcinoma.

Yize Li[1], Wagma Caravan[1], Yuwei Zhang[1], Andrew Houston[1], Siqi Chen[1], Chia-Kuei Mo[1], Preet Lal[1], Xiangwei Fang[1], Xiyi Wei[1], Ruiyang Liu[1], Ilya Strunilin[1], Yizhe Song[1], Song Cao[1], Cody Weimholt[1], Hui Zhang[2], Feng Chen[1], Li Ding[1]

[1]Washington University in St. Louis, St. Louis, MO,[2]Johns Hopkins University, Baltimore, MD

Renal cell carcinomas (RCC) are highly heterogeneous and aggressive cancers with diverse histological subtypes, molecular profiles, tumor microenvironments (TMEs), and clinical behaviors. Intratumoral heterogeneity (ITH), a hallmark of RCC, arises from distinct subclones within the same tumor that vary histopathologically and molecularly. To elucidate the biological mechanisms underlying RCC tumorigenesis at single-cell resolution, we examined 155 tumor specimens from 78 cases, encompassing clear cell RCC (ccRCC), papillary RCC (pRCC), chromophobe RCC (chRCC), and rare subtypes. We utilized 80 single-nucleus RNA sequencing (snRNA-seq) and 55 single-nucleus ATAC sequencing (snATAC-seq) datasets alongside advanced spatial biology techniques, including 27 Xenium In Situ, 12 Visium spatial transcriptomics (ST), and 7 co-detection by indexing (CODEX) samples. The connection of matched bulk proteogenomic data, clinical annotations, and histopathological evaluations from H&E staining further enhanced our spatial multimodal investigation. From single-nucleus sequencing of over half a million nuclei, we identified specific transcriptional programs and chromatin accessibility patterns among RCC subtypes, including HIF pathway dysregulation in ccRCC and MET pathway activation in pRCC. We also identified markers and constructed signature scores for rare but high-grade phenotypes, such as the aggressive rhabdoid and sarcomatoid features in ccRCC. Spatial analyses confirmed the utility of these feature-associated signatures for detection, quantification, and potential therapeutic targeting. Furthermore, we characterized the spatial organization and tumor-TME interactions, providing critical insights into spatially diverse ITH in RCC. Notably, a Grade-4 ccRCC sample exhibited three distinct tumor subclones and regions of necrosis characterized by Xenium, CODEX, and Visium profiling. Among these, a rhabdoid-associated subclone showed tumor-intrinsic signatures of elevated cell cycle activity and epithelial-mesenchymal transition (EMT), distinguishing it from conventional ccRCC subclones and indicating higher metastatic potential. We further revealed a unique macrophage subpopulation closely interacting with the rhabdoid subclone, forming an immunosuppressive niche that likely contributes to immune evasion, resistance to immunotherapies, and disease recurrence. Collectively, our spatial multimodal findings delineate the molecular signatures and mechanisms underlying RCC heterogeneity and aggressive features. By linking spatially distinct subclones to tumor progression and therapeutic resistance, this study provides valuable insights for developing personalized and effective therapeutic strategies to improve outcomes for RCC patients.

TUMOR BIOLOGY: Cancer Stem Cells
Poster Session

#16 Differential stemness properties in bone marrow-derived mesenchymal stem cells from healthy subjects and from patients with acute myeloid leukemia.

Souraya Ismail[1], Fouad Al Feghali[2], Rana Abdel-Samad[3], Abdullah A. Shaito[4], Hiba El Hajj[5], Carine Deleuze-Masquefa[6], Pierre-Antoine Bonnet[6], Marwan El-Sabban[7], **Jessica Saliba**[8]

[1]Biology, Lebanese University, Fanar, Lebanon,[2]Medical Laboratory Sciences, University of Balamand, Sin el Fil, Lebanon,[3]Biology, Saint Mary's College, South Bend,, IN,[4]5Biomedical Research Center and Department of Biomedical Sciences at College of Health Sciences, Qatar University, Doha, Qatar,[5]Experimental Pathology, Immunology and Microbiology, American University of Beirut, Beirut, Lebanon,[6]Institute of Biomolecules Max Mousseron (IBMM), Balard Chemistry Unit, Montpellier University, Montpellier, France,[7]Anatomy, Cell Biology and Physiological Sciences, American University of Beirut, Beirut, Lebanon,[8]Public Health, University of Balamand, Sin el Fil, Lebanon

Study purpose: The imidazoquinoxaline EAPB0503, an imiquimod analogue, exhibits immunomodulatory and anti-cancer properties against several blood malignancies, including acute myeloid leukemia (AML). The bone marrow (BM) niche plays a pivotal role in normal hematopoiesis and in leukemogenesis. To characterize the molecular potency of EAPB0503 in AML, we compared its effect on the expression profile of genes involved in the function and differentiation potential of BM-derived mesenchymal stem cells (BM-MSCs) from a healthy subject and a patient with AML.
Experimental procedures: Transcriptional levels of stemness markers and genes potentially relevant to niche function were evaluated by quantitative real-time polymerase chain reaction in BM-MSCs isolated from BM aspirates of subjects undergoing BM analysis and exposed to EAPB0503. Differentiation potential was assessed in BM-MSCs exposed to EAPB0503 and induced into osteogenic differentiation using a mixture of dexamethasone, ascorbic acid and β-glycerophosphate (DAG). A colorimetric assay was used to detect early-stage osteogenic differentiation by evaluating alkaline phosphatase (ALP) activity.
Summary of results: EAPB0503 treatment induced a differential expression of functionally important marker genes between BM-MSCs from the healthy subject and the patient with AML. As such, levels of Connexin 43 (*GJA1*) decreased in healthy BM-MSCs and increased in AML-derived BM-MSCs following treatment with EAPB0503. In contrast, transcriptional levels of multi-drug resistance 1 (*MDR1* or *ABCB1*) markedly increased in healthy BM-MSCs and remained stable in AML-derived BM-MSCs. Vascular Endothelial Growth Factor (*VEGF*) and N-cadherin (*CDH2*) transcriptional levels increased in AML-derived BM-MSCs, with modest changes in healthy MSCs. In addition, AML-derived BM-MSCs do not seem to respond to differentiation signals in a manner similar to healthy BM-MSCs, as shown by ALP activity.
Conclusions: These preliminary findings underscore the differential transcriptional profile in BM-MSCs derived from a healthy subject or a patient with AML. Importantly, a differential gene expression profile was also obtained in response to the therapeutic drug EAPB0503. Our preliminary findings implicate the BM niche in AML leukemogenesis and response to drugs.

TUMOR BIOLOGY: Where's Waldo: Spatial Complexity of the Tumor Microenvironment 1
Poster Session

#160 A high-throughput in vivo platform (Perturb-map) accurately models patient immunophenotypes and identifies therapeutic targets.

Maxime Dhainaut, Lucas Cavalcante, Hargita Kaplan, Carl Ebeling, Dexter Antonio, Rodney Collins, Francis Fernandez, Eric Siefkas, Anastasia Mavropoulos, Joy Tea, Nicole Snell, Tyler Van Hensbergen, Daniel Bear, Michela Meister, Eshed Margalit, Jake Schmidt, Yubin Xie, Yu (Phoebe) Guo, Jeremie Decalf, Rob Schiemann, Shafique Virani, Jacob Rinaldi, Lacey Padron, Ron W. Alfa

Noetik, South San Francisco, CA

Targeting the right patient population with any given therapeutic agent largely remains an unsolved problem, and successes in pre-clinical models often do not translate into positive outcomes for patients. Recent advances in multi-omics spatial profiling have highlighted the diversity of tumor microenvironment compositions and architectures across patients. Our deepened understanding of human disease also further demonstrates that the handful of pre-clinical models used for a large proportion of pre-clinical studies seldom recapitulate human biology and are unreliable predictors of clinical success.
To bridge the gap between pre-clinical models of disease and human biology, we developed a high throughput in vivo platform that can reproduce in mice the diversity of tumor phenotypes seen in patients. Our platform leverages Perturb-map, a spatial functional genomics technology compatible with high content multimodal phenotyping of 100s of tumor clones (and their associated genetic perturbations) in the same animal. A key feature of Perturb-map is that genetic perturbations can be identified by reading a protein barcode using multiplex imaging, a scalable read out. Our current custom imaging pipeline can analyze the impact of 100+ genetic perturbations - and the equivalent of 1,000s of individual tumors - in a few weeks, while simultaneously profiling the tumor microenvironment.
To date, we have used this platform to generate a dataset encompassing 600+ genetic perturbations (CRISPR KO) in a mouse model of NSCLC (KRASG12D Tp53-/-). For each perturbation, >50 tumors are profiled at baseline, and in the context of immune checkpoint blockade. We have found that, unlike mouse models traditionally used for discovery, our in vivo dataset reproduces a broad spectrum of patient immunotypes, including T cell deserts, highly infiltrated tumors, and more specific profiles of immune infiltration. Moreover, we have identified genetic perturbations that drive the establishment of these immunotypes. For example, loss of STK11 in the mouse tumors lead to a local increase in neutrophil infiltration, a feature that is also associated with STK11 mutations in the patient population. Among the 600+ genetic perturbations, we also identify specific genes that promote local inflammation or tumor immunoediting, and therefore constitute potential therapeutic targets.
Altogether, we have demonstrated that our ability to scale up in vivo experimentation using Perturb-map addresses current limitations in drug development, as we can (i) generate at scale models that better reflect the various facets of human tumor biology and (ii) perform target evaluation at a high throughput without compromising phenotypic readouts.

#161 High-resolution spatial proteomics characterizes colorectal cancer consensus molecular subtypes.

Batuhan Kisakol[1], Anna Matveeva[1], Sanghee Cho[2], Mohammedreza Azimi[1], Andreas U. Lindner[1], Manuela Salvucci[1], Elizabeth McDonough[2], Joanna Fay[3], Tony O'Grady[3], Niamh McCawley[4], John P. Burke[4], Deborah McNamara[4], John Graf[2], Simon McDade[5], Daniel B. Longley[5], Fiona Ginty[2], Jochen H. M. Prehn[1]

[1]Department of Physiology and Medical Physics & RCSI Centre for Systems Medicine, Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland,[2]GE Healtcare, Niskayuna, NY,[3]Department of Pathology, Beaumont Hospital, Dublin, Ireland,[4]Department of Colorectal Surgery, Beaumont Hospital, Dublin, Ireland,[5]School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Belfast, United Kingdom

Background: Identification of the consensus molecular subtypes (CMS) opened significant potential for understanding the tumor biology and intertumoral heterogeneity of colorectal cancer (CRC). Molecular subtyping in CRC traditionally relies on bulk transcriptomics. However, single-cell transcriptomics studies have shown that CRC tumors may be composed of tumor cells displaying different CMS traits, suggesting additional intratumoral heterogeneity.
Methods: To investigate intratumoral heterogeneity at protein level, we constructed tissue microarrays using tumor cores from 222 CRC patients. Arrays were stained and imaged with Cell DIVE multiplexed immunofluorescence (MxIF) imaging using 54 selected protein markers, ranging from cell identity markers to cancer hallmark proteins of metabolism, proliferation, cell death and differentiation. RNA sequencing data and CMS classification were also available for all patients. After segmentation of tumor, stromal and immune cells, we investigated intratumoral heterogeneity within CMS subtypes using spatially resolved single-cell protein profiling (>2 million cells). We compared cell types, their spatial organization and their expression of cancer hallmark-related proteins in CMS 1-4 subtypes.
Results: We revealed tissue atlases illustrating the cell types/states, spatial heterogeneity, cellular neighborhoods, cellular network, and single-cell protein profiles of CMS tumors. CMS1 tumors had more $CD3^+$, $CD8^+$, and $PD1^+$ immune cells that were found in the epithelial layer more frequently than in the other subtypes. CMS1 was also associated with higher levels of metabolic reprogramming markers such as upregulated glycolysis. CMS2 showed immune segregation, reactive stroma patterns, and higher levels of apoptotic and proliferative signaling proteins. CMS3 exhibited tightly clustered cancer cells with high RIP3 and low FLIP levels, suggesting a pro-inflammatory microenvironment driven by intense cell-cell interactions and stromal hubs. CMS4 displayed stromal-centric and immune-evasive tumors characterized by decreased HLA-1 levels and regulatory T cell exclusion from epithelium.
Conclusion: We present the first spatial atlas of CRC tumors with regard to their molecular subtypes at single-cell protein resolution and demonstrate new spatial aspects of tumor structures in CRC.

#162 Mapping the complex interplay between vasculogenic mimicry and immune response in lung cancer using digital spatial profiling.

Chun-Hui Lee[1], Chun-Hua Hung[2], Shu-Hsien Wang[3], Wei-Pang Chung[1], Yu-Min Yeh[1], Peng-Chan Lin[1], Wu-Chou Su[1]

[1]Department of Oncology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan,[2]Department of Bioengineering, College of Engineering, National Cheng Kung University, Tainan, Taiwan,[3]Department of Pathology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan

Background: Vasculogenic mimicry (VM) represents an alternative vascularization mechanism contributing to cancer progression and treatment resistance. Despite its clinical significance, the spatial and molecular relationships between VM networks and immune components remain poorly characterized in non-small cell lung cancer (NSCLC).
Methods: We conducted a comprehensive spatial analysis of treatment-naïve, surgically resectable advanced non-small cell lung cancer (NSCLC) specimens (n=4), encompassing both EGFR-mutant and wild-type variants. High-dimensional spatial phenotyping was performed using PhenoCycler-Fusion multiplexed immunofluorescence technology, enabling precise visualization and quantification of vasculogenic mimicry (VM) architectures and immune cell infiltrates at subcellular resolution. Complementary molecular characterization of discrete tumor microenvironmental regions was achieved through GeoMx Digital Spatial Profiling platform. Spatial transcriptomics analysis was subsequently employed to validate primary observations and elucidate region-specific transcriptional signatures.
Results: Through high-dimensional spatial analysis, we identified a previously undescribed vessel-like population characterized by CD31-VEGFR1+PanCK+ expression, representing vasculogenic mimicry (VM) structures in non-small cell lung cancer (NSCLC). Comprehensive spatial mapping revealed distinct topographical relationships between VM networks and immune cell populations, including CD4+ and CD8+ T lymphocytes, as well as functionally diverse macrophage subsets (CD68+, CD206+, iNOS+, and HLA-DR+). Molecular characterization through digital spatial profiling demonstrated enhanced transcriptional heterogeneity at the tumor margin, with significant enrichment of pathways involved in extracellular matrix (ECM) organization and hypoxia response (FDR < 0.01). Integration of spatial transcriptomics with an established 70-gene VM signature revealed a core set of 10 genes operating at the intersection of VM and ECM remodeling programs. This gene module, predominantly expressed at the tumor-stromal interface, represents a previously unrecognized regulatory hub coordinating VM formation through ECM modification.
Conclusions: Our integrated analysis reveals a novel VM phenotype in NSCLC and identifies a core gene signature linking VM formation to ECM remodeling at the invasive front. These findings provide new insights into tumor-stromal-immune interactions and suggest potential therapeutic strategies targeting the VM-ECM-immune axis.

#163 Spatiotemporal multi-omics uncover tumor ecosystem dynamics driving metastatic colonization.

Liang Wu[1], Yu Zhong[1], Shang liu[1], Zefan Zhang[2], Chunqing Wang[3], Yunfan Sun[2], Jia Fan[2], Jian Zhou[2], Xun Xu[4]

[1]BGI Research, Chongqing, China,[2]Department of Liver Surgery, Liver Cancer Institute, Zhongshan Hospital, Fudan University, Shanghai, China,[3]College of Life Sciences, University of Chinese Academy of Sciences, Beijing, China,[4]BGI Research, Shenzhen, China

The mechanisms underlying the interaction between disseminated tumor cells (DTCs) and their tissue microenvironment during metastasis colonization are poorly understood. We integrated multimodal single-cell and spatial profiling from liver cancer mouse models and human metastases to track the spatiotemporal dynamics of DTCs and their microenvironments across nine sequential time points from single-cell seeding until overt lung metastasis. We identified a residual population of quiescent *Phgdh*^{high} DTCs that survive initial innate immune clearance and become transiently enriched in micrometastases. These cells shape an immune-scarce microenvironment through PHGDH-dependent, H3K27me3-mediated epigenetic silencing of chemokine transcription, promoting metastatic expansion. *Cx3cr1*^{high} interstitial macrophages were also found to be transiently enriched before DTCs expansion, creating an immune-privileged niche for metastatic outgrowth by recruiting immunosuppressive cells. Inactivating the Phgdh-H3K27me3 axis in DTCs or depleting interstitial macrophages restored immune surveillance and inhibited metastatic colonization. These findings provide new therapeutic concepts for developing micrometastasis-targeting regimens.

#164 Heterogeneous dormant breast cancer cells leverage bone marrow cell plasticity at the single-cell level to drive metastasis initiation.

Noriko Gotoh

Cancer Research Institute and Institute for Frontier Science Initiative, Kanazawa University, Kanazawa, Japan

Breast cancer remains a leading cause of cancer-related deaths among women worldwide, largely due to the high risk of recurrence and metastasis in HER2-negative and hormone receptor-negative triple-negative breast cancer (TNBC) patients. While bone metastasis is common, the molecular mechanisms driving its initiation remain poorly understood. We recently identified a subpopulation of cancer stem cells (CSCs) with ancestral features, marked by the FXYD domain-containing ion transport regulator 3 (FXYD3), a Na+/K+ pump component. These “ancestor-like CSCs” persist in breast tissues during neoadjuvant chemotherapy (NAC), linking them to drug-tolerant persisters (DTPs) and positioning them as critical therapeutic targets for preventing local recurrence (Li M, Gotoh N et al., J Clin Invest, 2023). Despite this, the mechanisms underlying DTP dormancy in the bone marrow and their transformation into bone metastasis-initiating cells remain unclear. Given the stromal richness of TNBC tissues, we explored interactions between cancer-associated fibroblasts (CAFs) and breast cancer cells using a co-culture system. Single-cell analysis revealed a strong upregulation of granulocyte colony-stimulating factor (GCSF) in co-cultured CAFs. Notably, we identified FXYD3+ and GCSF receptor-positive cancer cells as a distinct subpopulation of CSCs with bone metastasis initiation potential. To investigate the temporal-spatial dynamics of bone metastasis initiation, we performed single-cell RNA sequencing (10x Genomics) and multiplex immunofluorescent imaging (PhenoCycler) using metastatic bone marrow from the patient-derived xenograft (PDX) models. These analyses revealed how bone metastasis-initiating CSCs exploit the plasticity of bone marrow cells—particularly hematopoietic and mesenchymal stem cells—to establish a CSC niche at the single-cell level. Our findings provide critical insights into the mechanisms of recurrence and metastasis in breast cancer, offering novel therapeutic opportunities to target ancestor-like CSCs and prevent disease progression.

#165 Spatially resolved mechanostress effects on tumor microenvironments: Tumor cells and fibroblasts in focus.

Wooseok Kim[1], Hyunseok Kim[1], Minki Kim[2], Zhangxian Lan[3], Hyung-Soon Park[2], Mi Ryung Roh[4], Joon Kim[1], Jong-Eun Park[1]

[1]Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, Daejeon, Korea, Republic of,[2]Department of Mechanical Engineering, Korea Advanced Institute of Science and Technology, Daejeon, Korea, Republic of,[3]Oral Cancer Research Institute, College of Dentistry, Yonsei University, Seoul, Korea, Republic of,[4]Department of Dermatology, Yonsei University College of Medicine, Seoul, Korea, Republic of

Tumorigenesis is influenced by various environmental factors, including growth-induced solid stress and fluid pressure, both of which are key drivers of tumor progression and resistance to therapy. While certain tumors arise in regions exposed to significant weight-pressure, how macroscopic mechanostress affects the tumor microenvironment remains largely unclear. In this study, we showed that macroscopic mechanostress disrupts nuclear envelope integrity. Single-cell RNA sequencing data demonstrated that the macroscopic pressure shows changes in gene expression within tumor microenvironment cells and reshaped fibroblast subtypes.In spatial transcriptomics, using a distance-based tumor border detection model, we can determine spatially resolved fine layers of tumor margin and peritumor regions. We found an increased proportion of fibroblasts, especially Lrrc15-positive myofibroblasts, located in peritumor region facing the tumor border. We also showed upregulated metastasis signature and immune inhibitory signature indicating that mechanostress-driven transcriptional changes promote metastasis and resistance to immunotherapy. Using a mouse plantar melanoma model, we found that exogenous mechanostress, mimicked by genetic disruption of the nuclear lamina, induces nuclear envelope rupture in melanoma cells and increases the number of Lrrc15-positive myofibroblasts. Interestingly, these myofibroblasts further contribute to nuclear envelope rupture in melanoma cells, creating a feedback loop. Together, our findings highlight a critical role for mechanostress in cancer progression, driven by nuclear envelope damage and shifts in tumor-stromal interactions.

#166 Spatial subtypes and cellular interactions of cancer-associated fibroblasts revealed by single-cell spatial omics.

Yunhe Liu[1], Ansam Sinjab[1], Jimin Min[1], Guangchun Han[1], Francesca Paradiso[1], Yuanyuan Zhang[1], Yang Liu[1], Ruiping Wang[1], Guangsheng Pei[1], Kyung Serk Cho[1], Enyu Dai[1], Akshay Basi[1], Jared K. Burks[1], Kimal I. Rajapakshe[1], Yanshuo Chu[1], Jiahui Jiang[1], Daiwei Zhang[2], Xinmiao Yan[1], Paola A. Guerrero[1], Alejandra Serrano[1], Mingyao Li[2], Tae Hyun Hwang[3], Andrew Futreal[1], Jaffer A. Ajani[1], Luisa M Solis Soto[1], Amir A. Jazaeri[1], Humam Kadara[1], Anirban Maitra[1], Linghua Wang[1]

[1]The University of Texas MD Anderson Cancer Center, Houston, TX,[2]University of Pennsylvania Perelman School of Medicine, Pennsylvania, PA,[3]Department of Artificial Intelligence and Informatics, Mayo Clinic Rochester, Rochester, MN

Background: Cancer-associated fibroblasts (CAFs) are a highly diverse cell population that plays a crucial role in shaping the tumor microenvironment (TME). Their spatial interactions within the TME remain to be systematically characterized, and the factors driving their phenotypic heterogeneity are not yet fully understood. Recent advancements in single-cell spatial transcriptomics technologies and cell segmentation approaches enable the precise measurement of thousands of transcripts at subcellular resolution. Combined with innovative bioinformatics, these advancements have unlocked unprecedented opportunities for in-depth characterization of CAFs and their interactions within the TME.

Methods: We conducted a comprehensive spatial transcriptomic analysis of CAFs across 8 cancer types, using the CosMx and MERSCOPE platforms to profile nearly 6 million cells as part of our discovery cohorts. We developed a novel computational method to spatially cluster CAF subpopulations and defined four distinct spatial CAF subtypes. We further examined their phenotypic differences, spatial niche organization, and interactions with neighboring cells. Additionally, we investigated the influence of CAF subtypes on immune cell phenotypes and infiltration levels. To validate our findings, we employed multiple spatial platforms, including Visium, Xenium, COMET, CODEX, and IMC, to ensure reproducibility across diverse technologies. Moreover, we performed correlative studies in large-cohorts with rich clinical data to evaluate the clinical significance of these CAF subtypes.

Results: Our approach provided an unprecedented high-resolution examination of the spatial phenotypes of CAFs in large, complex tissues, uncovering novel insights. We comprehensively characterized CAFs across various solid tumor types and identified four conserved spatial CAF subtypes. These subtypes are characterized by distinct cell organizational patterns, neighboring cell compositions, interaction networks, and transcriptomic features. Moreover, they demonstrate significant correlations with the abundance, distribution, and phenotypes of tumor-infiltrating immune cells, as well as associations with tumor histopathological features and clinical outcomes.

Conclusions: Our study provides a pan-cancer, high-resolution characterization of CAF spatial organization and interactions in solid tumors and identified conserved spatial CAF subtypes linked to distinct organizational patterns. Our findings reveal that the transcriptomic states and phenotypes of CAFs are significantly shaped by their interactions with neighboring cancer and immune cells. Beyond offering novel insights into the spatial heterogeneity of CAFs, this work paves the way for developing strategies to effectively target and modulate CAFs for therapeutic benefit.

#167 Novel spatial tagging of single nuclei spatializes single-cell ATAC-seq and V(D)J sequencing with gene expression for tumor microenvironment analysis.

Wanxin Wang, Julie Wilhelmy, Christina Chang, Christina Fan

Research and Development, Curio Bioscience, Palo Alto, CA

Advances in single-cell multiomic technologies such as RNA-seq, ATAC-seq, or V(D)J sequencing have enriched our understanding of tumor gene expression, chromatin accessibility, and immune receptor diversity, but they fall short in capturing the critical spatial context of cells within the tumor microenvironment (TME). This limitation hinders our understanding of how tumor cells organize and interact with surrounding cells during tumor progression, metastasis, and treatment response. Existing spatial methods, whether microscopy- or sequencing-based, primarily focus on gene or protein expression only. Additionally, they often lack true single-cell resolution, requiring computationally involved cell segmentation or deconvolution, and offer limited molecular complexity. To address this gap, Curio Trekker single-cell spatial mapping kit, based on Slide-tags technology, provides a paradigm-shifting solution by transforming single-cell sequencing assays into spatially resolved single-cell data. Curio Trekker spatially labels individual cells within a tissue section by releasing the DNA barcodes from a spatially indexed bead array surface into nearby nuclei. The spatially tagged nuclei are then dissociated from the array and processed on conventional single-cell sequencing assays and platforms. The technique expands spatial measurement beyond gene expression alone, enabling spatial multiomic analyses such as chromatin and V(D)J profiling. Moreover, given the spatial measurements are at true single-cell resolution, the resulting datasets are easier to analyze and interpret. In this study, we applied Curio Trekker to existing single-nuclei (sn) ATACseq and sn-V(D)J assays combined with gene expression to spatially analyze human melanoma fresh frozen tissues. These previously unattainable combinations enable the simultaneous spatial exploration of gene expression and epigenetic regulation within the spatial context of tumors and the mapping of immune receptor clones within TME. Joint unsupervised clustering on sn-RNAseq and sn-ATACseq data identified spatially defined tumor, stromal, immune, and vasculature cell types driven by both transcriptomic and epigenetic signatures. These cell types were further classified spatially into tumor zones and tumor-free areas, determined by their spatial locations and CNV profiles. We linked tumor-zone-specific transcriptional factors with accessible chromatin domains and binding motifs. Tumor-stromal interactions were inferred within their native spatial context using spatially aware ligand-receptor analysis. V(D)J and immune composition analysis revealed spatial distribution of different T cell clones. Our findings demonstrate the potential of Curio Trekker to advance oncology and immunotherapy by providing a comprehensive spatial view of tumor ecosystems.

#168 Genomic heterogeneity drives distinct infiltration patterns in glioblastoma.

Dylan Harwood[1], Sara Blaabjerg Artzi[1], Vilde Pedersen[1], Alessio Locallo[1], Maya Jeje Schuang Lu[1], David Scheie[2], Anne Dorte Schou Noroxe[2], Nadine Margaretha Hammouda[1], Ulrik Lassen[2], Joachim Lutken Weischenfeldt[1], Bjarne Winther Kristensen[1]

[1]University of Copenhagen, Copenhagen, Denmark,[2]Rigshospitalet, Copenhagen, Denmark

Glioblastoma is the most common and malignant type of primary brain cancer with a median patient survival of only 15 months. Tumors are typically categorized into three molecular subtypes—proneural, classical, and mesenchymal—each linked to specific genomic and microenvironmental factors. However, the functional and clinical implications of these subtypes remain poorly understood. Standard treatment involves surgical resection followed by chemotherapy and radiotherapy. Despite this, malignant cells have already at the time point of diagnosis infiltrated healthy brain tissue, driving tumor recurrence. Infiltrating cells have been found to maintain high proliferative rates while invading the brain (termed “go-and-grow”) but have also been shown be non-proliferative (termed “go-or-grow”). Here, we perform computational analyses on transcriptomic data from over 600 patients, supplemented with genomic data, to investigate infiltration patterns in glioblastoma tumors. We identify a proliferative astrocytic state strongly associated with the classical subtype and EGFR amplification and distinct from a non-proliferative astrocytic state. Further analysis showed subtype-specific infiltrative patterns between the tumor core and adjacent brain tissue. To validate these findings, we performed single-cell spatial transcriptomics on patient samples, revealing that classical tumors exhibit a “go-or-grow” phenotype, where core regions are proliferative and infiltrative regions are dormant. In contrast, proneural tumors maintain consistent malignant states and proliferation rates, reflecting a “go-and-grow” phenotype. These findings indicate a direct relationship between the genomic status of glioblastoma tumors and the transcriptomic profiles of their infiltrating malignant cells. Together, our study provides insights into genomically-driven infiltration patterns in proneural and classical glioblastomas, with potential implications for clinical treatment.

#169 Tumor heterogeneity and differential pathway activation in melanoma: insights from spatial profiling of AXL and NGFR upregulated regions.

Mark Woodnorth, Giuseppe Tarantino, Anne Zaremba, Tuulia Vallius, Florian Rambow, Lisa Zimmer, Antje Sucker, Elisabeth Livingstone, Eva Hadaschik, Roxanne Pelletier, Yingxiao Shi, Mariana Lopez, Samira Makhzami, Christine Lian, George Murphy, Genevieve Boland, Peter Sorger, Dirk Schadendorf, David Liu

Dana-Farber Cancer Institute, Boston, MA

Tumor heterogeneity remains an obstacle in the treatment of melanoma, with unique tumor microenvironments contributing to therapy resistance and differential tumor growth. Dedifferentiated melanoma states have been associated with resistance to targeted and immunotherapy. AXL and NGFR are dedifferentiation markers linked to these states; however, the mechanisms by which they interact or result in distinct tumor programs remain unclear. Both AXL and NGFR have been linked to therapy resistance. AXL has been associated with cell survival, proliferation and immune suppression and NGFR with tumor progression, invasion, metastasis and therapy resistance. Understanding these interactions is critical for identifying therapeutic strategies and improving patient outcomes. In a melanoma patient with longitudinal tumor samples, we used highly multiplexed cyclic immunofluorescence (CyCIF) and matched H&E to study the tumor and immune states associated with progression. Spatial transcriptomic profiling was performed using GeoMX on selected regions of interest to further study tumor states characterized by different morphology and proteomic differentiation markers. In the primary and in-transit metastasis, we observed intratumoral heterogeneity, with histologically distinct tumor nodules. CyCIF of the in-transit sample showed upregulation of AXL in one nodule and NGFR in the other. The NGFR nodule was characterized by densely packed tumor cells, while the AXL nodule featured an inner cleft region encased by dense peripheral tumor cells. In the NGFR nodule, differential expression analysis (DEA) revealed upregulation of EPAS1, HIF1A, ERBB3 and VEGFA suggesting hypoxic stress, cell signaling, active angiogenesis and cell migration. Transcription factor analysis (TFA) showed upregulation of HDGF, TGFB1I1, HOXA7, IRF6 and SRSF2, TFs associated with proliferation, differentiation and increased translational activity. Downregulated HIP2K and PAWR suggest apoptosis inhibition, further facilitating tumor survival and expansion. In the AXL nodule, DEA revealed increased CDKN1A, indicating stress response and altered cell cycle regulation. Gene set enrichment analysis (GSEA) highlighted WNT and Hedgehog signaling, hormonal responses (e.g., estrogen signaling), and metabolic alterations (bile acid metabolism, myogenesis). EMT upregulation suggested a mesenchymal-like phenotype associated with resistance. TFA showed elevated KLF8 and TGFB1I1 driving EMT and metastasis, while SRSF2, HSF4, CXXC1, and NR1H2 suggested stress resilience, apoptosis regulation, and ER stress mitigation. Distinct signaling in AXL and NGFR regions suggests NGFR drives metabolic activity and proliferation, while AXL promotes resilience via stress and hormonal signaling. These findings offer insights into therapies targeting melanoma heterogeneity and resistance.

TUMOR BIOLOGY: Cancer Stem Cells
Poster Session

#17 hiPSCs-derived *PTEN* deletion suppresses cardiomyocyte differentiation and beating.

Thang Huu Nguyen[1], Chaeyong Jung[2], Sang Wook Park[3], Seung-Rock Lee[1]

[1]Department of Biochemistry, Department of Biomedical Sciences, Chonnam National University Medical School, Gwangju, Korea, Republic of,[2]Department of Anatomy, Chonnam National University Medical School, Gwangju, Korea, Republic of,[3]Department of Oral Biochemistry, School of Dentistry, Chonnam National University, Gwangju, Korea, Republic of

Human-induced pluripotent stem cells (hiPSCs) have been increasingly used for cardiac tissue engineering and cell-based therapies for heart regeneration because they possess self-renewal capability *in vitro* and can differentiate into most cardiovascular cell types, including cardiomyocytes. The phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a potent tumor suppressor frequently being deficient in human cancer. It is also important for cardiac functions and embryonic development since the loss of *PTEN* induced embryonic lethality; however, the underlying mechanisms are not fully understood. Here, to understand the function of PTEN in cardiomyocyte differentiation, we generated *PTEN* deletion in hiPSCs by using CRISPR/Cas9 system. *PTEN* ablation suppressed cardiomyocyte differentiation, which was shown by decreases in cardiac markers *cTNT* and *Myh6* compared with *PTEN* WT. Immunofluorescence staining and flow cytometry analysis using α-actinin and cTNT supported the suppression of cardiomyocyte differentiation induced by $PTEN^{-/-}$ hiPSCs. q-PCR analysis was performed using representative markers for differentiation steps including mesoderm, cardiac mesoderm, cardiac progenitor, and cardiomyocyte. $PTEN^{-/-}$ hiPSC decreased mesoderm markers (*Brachyury, Dkk1, Fox C1*) on the 2nd day of differentiation, suggesting that PTEN is important for early cardiac differentiation. Western blot analysis revealed that Gsk3β and mTOR phosphorylation levels were increased due to an activation of Akt, which may involve $PTEN^{-/-}$-induced mesoderm abnormality. In addition, PTEN deletion also inhibited cardiac beating capability, which may explain $PTEN^{-/-}$-induced embryonic lethality.

TUMOR BIOLOGY: Where's Waldo: Spatial Complexity of the Tumor Microenvironment 1
Poster Session

#170 A novel protease-free workflow for spatial multiomics analysis of the tumor microenvironment in breast cancer.
Patrick Savickas

HistoWiz, Brooklyn, NY, NY

Introduction: Spatial multiomics is revolutionizing our understanding of the tumor microenvironment (TME) by enabling simultaneous analysis of multiple analytes within their tissue context. Combining in situ hybridization with immunofluorescence enables detection of key RNA and protein markers, together, on the same section. However, traditional ISH protocols that use enzyme-based pretreatment were incompatible with certain IHC/IF antibodies. Here, we present a novel protease-free workflow for a spatial multiomics panel, applied to human breast cancer samples, to investigate the interplay between immune cell infiltration and tumor cell signaling.
Methods: Formalin-fixed paraffin-embedded (FFPE) breast cancer tissue sections underwent a protease-free staining protocol. The panel comprised three protein markers: CD3 (all T cells), CD8 (cytotoxic T cells), and PanCK (tumor cells). The RNAscope™ Multiomic LS in situ hybridization (ISH) assay was employed to detect IFNG (T cell activation), CXCL9 (chemokine produced by tumor cells), and CXCR3 (receptor for CXCL9 on CD8+ T cells). Spatial analysis algorithms were used to quantify co-expression and proximity of these markers.
Results: Our protease-free workflow enabled simultaneous visualization of protein and RNA targets with high sensitivity and specificity. Preliminary data demonstrate distinct spatial patterns of immune cell infiltration and activation within the breast TME. We observed correlations between IFNG expression in CD8+ T cells and CXCL9 expression in PanCK+ tumor cells, suggesting a potential mechanism of T cell recruitment and activation.
Conclusions: This novel protease-free spatial multiomics panel provides a powerful tool for dissecting the complex interactions between tumor cells and the immune system in breast cancer. By preserving both protein and RNA integrity, this approach offers valuable insights into the spatial organization of the TME and may identify novel therapeutic targets and biomarkers.

#171 A novel single-cell level approach integrating artificial intelligence (AI)-powered histomorphology labeling and spatial transcriptomics enables biomarker identification of treatment-resistance in salivary gland cancer (SGC).

Jin Woo Oh[1], Sanghoon Song[1], Soohyun Hwang[1], Mingu Kang[1], Jinhee Lee[1], Sergio Pereira[1], Hyunsu Kim[2], Sehhoon Park[2], Junhun Cho[3], Se-Hoon Lee[2], Han-Sin Jeong[4], Chang Ho Ahn[1], Siraj Ali[1], Chan-Young Ock[1], Myung-Ju Ahn[2]

[1]Oncology, Lunit Inc., Seoul, Korea, Republic of,[2]Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center, Seoul, Korea, Republic of,[3]Department of Pathology and Translational Genomics, Samsung Medical Center, Seoul, Korea, Republic of,[4]Department of Otorhinolaryngology-Head and Neck Surgery, Samsung Medical Center, Seoul, Korea, Republic of

Introduction

We integrated H&E-AI analysis and spatial transcriptomics (ST) to provide a synergistic analysis of tumor microenvironments (TME) beyond each technology alone. In this study, we focused on SGC, which encompasses several rare tumor types with unmet need.

Methods

We developed an integrated analysis pipeline to combine cell- and tissue-level morphological information from H&E-AI (Lunit SCOPE IO, AI-powered H&E image analyzer) and gene expression profiles from ST (10x Genomics Xenium). We then analyzed 12 tumor samples provided by 10x Genomics for validation and, as a test set, 16 surgically resected SGC including 11 salivary duct carcinoma (SDC) treated with neoadjuvant nivolumab and cetuximab.

Results

We first reciprocally validated H&E-AI and ST by confirming that ST captures well established cell-specific gene expression that corresponds to AI-predicted cell types (EPCAM, VWF, and TRAC mark carcinoma cells, endothelial cells, and lymphocytes with F1 = 0.88, 0.81, and 0.72, respectively). In the test SGC cohort (total 915,215 cells), we identified 20 cell type groups with distinct gene expression patterns. Due to high gene expression heterogeneity among putative tumor cell subtypes, ST based tumor cell detection using reference gene expression profiles was insufficient and required complementary morphological analysis from H&E-AI, which identified 10 groups as tumor cell subtypes harboring diverse morphologies (mean nuclear size per subtype: 27.14 - 40.92 μm^2). Two patients with SDC had the same dominant tumor subtype (largest nucleus size, HER2/FOXA1/GATA3 positive), yet exhibited discordant responses to neoadjuvant therapy. Tumor cells from the SDC patient with recurrence (SDCw/Rec), compared to those from the SDC patient without recurrence (SDCw/oRec), exhibited upregulation of genes associated with immune evasion or epithelial-to-mesenchymal transition (EMT) such as MYC, TNC, and JUN (174%, 197%, 77% higher). Moreover, SDCw/Rec also had significantly fewer tumor-infiltrating lymphocytes (TIL) than SDCw/oRec (515 vs. 2,306 /mm^2). Interestingly, the proportion of TIL with CXCL9 expression was far lower in SDCw/Rec [19.7% (1,636/8,312)] compared to SDCw/oRec [67.0% (25,658/38,267)].

Conclusion

An integrated ST and H&E-AI analysis allowed parallel transcriptomic and subcellular-level histomorphologic characterization of individual cells from tumor specimens at the micron scale, providing a highly visualizable solution for discovering novel resistance mechanisms. Based on this novel approach, we reported a SDC pair discrepant for recurrence after neoadjuvant therapy, where the recurred case exhibited 1) high intrinsic tumor signatures of EMT and immune evasion and 2) a low level of TILs along with low CXCL9 expression in each TIL.

#172 Spatial profiling of stage III melanoma microenvironment reveals tertiary lymphoid structures as markers of long-term outcomes.

Angel Guan[1], Xinyu Bai[1], Nurudeen A. Adegoke[1], Shila Ghazanfar[2], Nigel G. Maher[1], Alexander M. Menzies[1], Ines Pires da Silva[1], Richard A. Scolyer[1], Georgina V. Long[1], James S. Wilmott[1], Camelia Quek[1]

[1]Melanoma Institute Australia, The University of Sydney, Sydney, Australia,[2]School of Mathematics and Statistics, The University of Sydney, Sydney, Australia

The recent neoadjuvant anti-PD-1 and anti-CTLA4 therapy, such as the PRADO trial (NCT02977052), has shown a high pathological response rate in many melanoma patients. In resectable macroscopic stage III melanoma, PRADO achieved a 72% pathological response rate. However, recurrence remains a challenge, and the biological mechanisms behind durable responses are unclear. Tertiary lymphoid structures (TLSs), immune sites that enhance antigen presentation, cytokine signaling, and antibody release, are present in several cancers, including melanoma. Their role in the efficacy of neoadjuvant therapy, however, is not fully understood. This study quantifies and characterizes TLS formation in melanoma patients treated in the neoadjuvant setting. Using the most recent data with a median follow-up of 55 months, 17 patients involved in this study were grouped into non-recurrence (n=11) and recurrence (n=6) groups. We performed 41-plex PhenoCycler Fusion imaging on 26 formalin-fixed, paraffin-embedded patient index lymph node samples from the PRADO cohort, including 17 baseline samples and nine Week 6 post-treatment samples. Among these, eight patients had paired baseline and Week 6 samples. Bioinformatic analysis was used to quantify cells, characterize TLS molecular features, and analyze interactions within the tumor microenvironment (TME) associated with recurrence-free survival (RFS). The presence of TLSs—consisting of B cells, CD4+ and CD8+ T cells, myeloid cells, and vessels—in baseline samples was associated with longer RFS ($p = 0.0498$). At Week 6, TLSs were present in 4 out of 5 non-recurrence patient samples and 1 out of 4 recurrence patient samples. Moreover, the TLSs in the recurrence patient were relatively less mature than those in non-recurrence patients. These findings indicate mature TLS presence at 6 weeks post-treatment may be associated with durable outcomes, and quantification of TLSs is underway. Notably, TLSs were observed in some matched baseline and Week 6 samples in non-recurrence patients. The structural organization of immune cells at baseline compared to Week 6 demonstrates patterns of increased immune activation, where the early TLSs evolved into more mature-like structures with increased immune cell diversity. Molecular profiling of these immune structures is underway to characterize the TME changes associated with neoadjuvant treatment and to identify features of durable immune protection in patients with prolonged RFS. This 41-plex spatial imaging study highlights the immune-responsive features of TLSs in melanoma, shedding light on their mechanisms of promoting durable responses to neoadjuvant therapy and providing a foundation for future translational research.

#173 Advancements in 3D high-multiplex imaging using the stellaris confocal platform.

L. Alvarez, I. Steinmetz, T. Straka, F. Hecht, M. J. Roberti

Leica Microsystems GmbH, Mannheim, Germany

In cancer immunology research, evaluating tissue changes and cell interactions is crucial for identifying specific cell types, assessing the activation status of immune cells, monitoring the expression of certain proteins and genetic markers, and characterizing the tumor microenvironment. Traditional imaging techniques often fall short in providing comprehensive spatial and functional insights due to their limitations in multiplexing and three-dimensional (3D) analysis. Here, we introduce an innovative high-multiplexing workflow on the STELLARIS confocal platform, utilizing a new functionality called SpectraPlex for advanced 3D imaging (Kunz L., et al. Nat. Methods, 2024). SpectraPlex significantly enhances the capabilities of spatial biology by streamlining the steps from panel creation to image acquisition and unmixing through advanced unmixing algorithms. In addition to the traditional single references and semi-blind unmixing for 15-plex experiments, we introduce a “group” approach that allows users to generate 15 references using only 3 samples, thereby saving preparation and imaging time and preserving a larger amount of samples for experimental steps. This workflow was validated on a cancer model where murine pancreatic ductal adenocarcinoma cells (KPC-4662) were injected into C57Bl/6 mice and allowed to grow for three weeks. Tumor tissues were harvested, fixed, and processed into 70-µm-thick sections for imaging. A 15-plex panel, including markers for T cells (CD4, CD8), B cells (B220), myeloid cells (CD11b, MHC II, CD11c), and other functional and structural markers (TCF1, FoxP3, PD1, Ki67, α-SMA, MHC I, E-cadherin, Tenascin C, CD31), was used to visualize the resulting tumor infiltration. High-resolution 3D imaging was performed on a STELLARIS confocal system, capturing comprehensive spatial information and enabling the identification of immune cell subtypes, functional states, and structural markers within the tumor microenvironment. All 15 markers were present and imaged in a single acquisition, allowing further probing in areas of interest. We also demonstrate how this general approach is easily adapted to investigate the immune landscape of murine lymph nodes (Roberti M. J., et al, Nat. Methods, 2024). The implementation of the 3D high-multiplexing workflow on the STELLARIS confocal platform represents a significant advancement in the ability to interrogate the tumor microenvironment in detail. The SpectraPlex toolbox not only streamlines the imaging process but also ensures high data quality and reliability, setting a new standard for future spatial biology research in cancer.

#174 Multimodal mass spectrometry imaging of immune cell glycosylation across three immunosuppressive tumor microenvironments.

Lyndsay E. A. Young[1], Caroline G. Kittrell[1], Kameisha Rashford[1], Grace Grimsley[1], David N. Lewin[2], Anand S. Mehta[1], Peggi M. Angel[1], Kristin Wallace[3], Richard R. Drake[1]

[1]Pharmacology and Immunology, The Medical University of South Carolina (MUSC), Charleston, SC,[2]Pathology and Laboratory Medicine, The Medical University of South Carolina (MUSC), Charleston, SC,[3]Public Health Sciences, The Medical University of South Carolina (MUSC), Charleston, SC

Glycosylation is essential for the development, trafficking, and activation of the immune system. The complex interaction between the immune system and tumor cells within the tumor microenvironment is crucial to understand cancer progression and improve therapeutic interventions. Immunotherapy is an emerging tool in cancer treatment, showing promise across various malignancies, however; its effectiveness in solid tumors remains limited and inconsistent. Colorectal carcinoma (CRC) has demonstrated enhanced sensitivity to immunotherapy, particularly in cases with high microsatellite instability or mismatch repair deficiency. In contrast, the immune cold prostate (PCa) and pancreatic ductal adenocarcinoma (PDAC) cancers are typically less responsive to immunotherapy due to an immunosuppressive tumor microenvironment. Emerging evidence for the role of N-glycosylation modulating activity in the tumor microenvironment suggests it could be an indicator of response to immunotherapy. We aimed to establish spatial profiles of immune cells surrounding and within tumor tissues of CRC, PCa, and PDAC using Matrix-Assisted Laser Desorption-Ionization Mass Spectrometry Imaging (MALDI-MSI) for N-glycomic profiling and MALDI-immunohistochemistry to identify the cellular compositions of the tumor microenvironment. Utilizing 73 tissue samples collected from patients with varying stages of CRC, PCa, and PDAC, our lab has characterized the N-linked glycan profile of over 250 immune cell clusters within and surrounding these tumors. Using an established photocleavable-mass tagged antibody panel (CD20, CD3, CD8, CD44, CD11b, CD68) we broadly characterized the immune microenvironment. In CRC, we identified immune clusters of CD20+ B cells and CD3/CD44+ T cells distinctive and predictive with signature profiles of bisecting and branched N-glycans. In PCa and PDAC, we identified a similar cellular makeup for the immune clusters as seen in CRC; however, high mannose and core fucosylated N-glycans were more prevalent within and surrounding these tumors. Additionally, N-glycan signatures varied depending on spatial proximity to the tumor in PCa and PDAC tissues. Our innovative, two dimensional approach provides critical insights into the cellular composition and metabolic complexity of the tumor microenvironment. Importantly, establishing a cellular and metabolic profile of the tumor immune microenvironment may offer valuable indicators of immunotherapy effectiveness, paving the way for improved personalized medicine.

#175 Characterising head and neck tumour microenvironment using high plex spatial omics technologies and analyses.

Chin Wee Tan[1], Naomi Berrell[2], Meg L. Donovan[2], James Monkman[3], Habib Sadeghirad[3], Vahid Yaghoubi Naei[3], Jinjin Chen[1], Alyssa Rossenbloom[4], Shilah Bonnett[4], Mark Conner[4], Brian Filanoski[4], Christine Kang[4], Joseph M. Beechem[4], John F. Fraser[2], Catherine Barnett[3], Rahul Ladwa[5], Brett G. M. Hughes[3], **Arutha Kulasinghe**[3]

[1]The Walter & Eliza Hall Institute of Medical Research (WEHI), Melbourne, Australia,[2]Wesley Research Institute, Brisbane, Australia,[3]The University of Queensland, Brisbane, Australia,[4]Nanostring Technologies, a Bruker company, Seattle, WA,[5]Princess Alexandra Hospital, Brisbane, Australia

Head and neck squamous cell carcinoma (HNSCC) is the cancer of different parts of the head and neck region originating from epithelial cells of the mucosal layer. Globally, it is the 7th most common cancer with over 89 thousand new cases each year. Its prognosis has not changed significantly in recent years despite improvements in treatments options like immunotherapy, radiotherapy (RT), chemotherapy (CT) and surgery. Using the latest spatial omics technologies and approaches, this work aims to develop a better insight of the tumour microenvironment (TME), particularly the association with typical clinical endpoints including Disease-Free Survival (DFS) and Overall Survival (OS). In this study, we utilised the latest ultra-high plex (580-proteins) spatial protein technology (GeoMx Immuno-Oncology Proteome Atlas, IPA) and full transcriptome profiling (18,000 transcripts, Whole Transcriptome Atlas, WTA) from Bruker Spatial Biology (Nanostring Technologies) to analysed 84 mucosal HNSCC patient specimens collected during resection. Each patient tissue sample was subdivided into tumour and stromal regions prior to digital spatial profiling. Patients had either underwent CT and/or RT and samples were stratified as either tumour or stromal regions before processing. Till this end, results suggest that patient DFS and OS were associates with anatomical locality and stages of the tumour. We uncovered unique protein and transcript features that's associated with either DFS and/or OS. We validated these findings (specifically CD34 and CD44) using an independent approach (i.e. single-cell proteomics using PhenoCycler-Fusion from Akoya Biosciences) and uncovered cell types associated with patient survival by applying cell type deconvolution on transcript data. This study demonstrated an integrated analysis workflow using high plex proteomics and transcriptomics profiling data for uncovering markers of clinical significance in mucosal HNSCC.

#176 Simultaneous spatial characterisation of copy number change and immune cell profiling using DNA FISH and multiplexed immunofluorescence.

Eleanor M. O'Roberts[1], Pranauti Panshikar[1], Xiaoze Li-Wang[2], Quentin Verron[2], Shahrzad Shirazi Fard[3], Magda Bienko[2], Charlotte Stadler[1]

[1]KTH, Department of Protein Science, SciLifeLab, Stockholm, Sweden,[2]Department of Molecular, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden,[3]Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden

Background: Recent advances in spatial characterization of protein expression has greatly improved our understanding of tissue architecture, cell-cell interactions and their function. In this work, we aim to further improve cell characterization by combining the detection of DNA and proteins in the same sample in an automated and cyclic manner. Single molecule DNA FISH can identify cells with genetic aberrations linked to different tumor types or aggressiveness, however as a stand-alone method this technique lacks spatial context. We set out to use the microfluidic Phenocycler-Fusion platform from Akoya Biosciences to detect single molecule DNA FISH signals, as well as multiplexed protein detection, in order to gain multiple layers of spatial information from one sample and the possibility to explore clonal heterogeneity within tumor sections.
Methods: The Phenocycler-Fusion involves the use of antibodies conjugated to oligonucleotide barcodes. In each cycle, the microfluidics instrument adds up to three reporters labelled with distinct fluorophores (AF488/AF750, Atto550, AF647), these are complementary to individual barcodes. The fluorophores are then imaged and the reporters are eluted ready for the next round of reporter addition, imaging and elution. Our integrated workflow for detecting DNA and protein involves hybridization of primary DNA FISH probes downstream of antibody staining before the sample is loaded onto the Phenocycler-Fusion instrument for cyclic addition of secondary fluorescent DNA FISH probes and reporters. The data is processed so that images from each cycle are collated into a single image meaning that DNA and protein signals can be spatially analyzed simultaneously.
Results: The protocol was developed using fixed cells to identify the optimal conditions for combined DNA and protein detection and we successfully executed the same protocol in fresh frozen tissue sections. We have obtained proof of concept data illustrating the possibility of imaging chromosomal regions simultaneously with immune cell and structural markers such as CD45, pan-cytokeratin and Ki67. We have also shown that DNA targets can be detected across imaging cycles along with protein detection and thereby demonstrate the multiplexing capacity of our integrated protocol on the Phenocycler-Fusion platform in a fully automated manner. The combined method can be optimized and applied to various cancer samples to investigate the tumor microenvironment along with chromosomal aberrations or copy number variations of specific genes. Overall, these promising results show the possibility of using a commercially available system for detecting multiplexed DNA and protein simultaneously across a large imaging area.

#177 Increasing throughput of high-quality spatial biology data via low magnification, high NA scanning on the CellScape platform.

J Spencer Schwarz[1], Charles E. Jackson[1], Kayla E. Cashion[1], Mathew H. Ingalls[1], Brian J. Lane[1], Joseph Lownik[2], **Oliver Braubach**[1]

[1]Bruker Spatial Biology, St. Louis, MO,[2]Cedars-Sinai Medical Center, Los Angeles, CA

The etiology of solid tumor formation is diverse and complex, and studies that better incorporate diversity in sample selection will result in more robust patient stratification and treatment. This reasoning applies to spatial biology studies as well, but increasing sample diversity can quickly lead to added technical challenges, more cost, and more time to data. There is then an obvious benefit to any innovation that increases the throughput of sample processing without unacceptable degradation in data quality. The CellScape™ Precise Spatial Proteomics platform offers spatial resolution at 182 nm/pixel while sampling 0.815 mm^2 per field of view (FOV). This provides a balance of speed and resolution optimal for many applications. However, there are use cases where fast collection is more important than high resolution. To serve these, we explored fitting the CellScape with a primary 10x 0.45 NA objective. The instrument can then collect 3.18 mm^2 of sample area per FOV, quickly scanning through large specimens such as whole embryo mounts, resection biopsies, and tissue micro arrays (TMAs). Combining this large scanning area with the CellScape whole-slide imaging chamber (WSIC) tissue window of 44 mm x 16 mm provides a very efficient workflow for gathering multiplexed immunofluorescence (mIF) data on large area samples. Here we provide data describing the efficiency and data quality of such a system. We found that a complete tonsil resection measuring >57 mm2 can be captured in less than 20 FOVs. And that a single FOV can capture an entire 1.6 mm diameter core without cropping. Further maximization of scanning efficiency was provided by a modification to the typical spatial biology imaging workflow. During TMA scanning, replacing grid-based scanning, we place each FOV at the center of each core to avoid any unnecessary scanning of blank area or double-scanning of single cores. We deployed a 40-plex assay on a 96-core TMA grid using a combination of large-FOV, high-numerical-aperture scanning and on-center FOV placement. We assessed the time required to process the samples from archived FFPE TMA block to image collection and found that using the WSIC and the CellScape platform optimized the mIF assay for rapid data collection. Importantly, we also illustrated the preservation of high-quality imaging data provided by a digital sampling rate of 364 nm per pixel, maintaining compatibility with downstream data analysis pipelines including image segmentation, cell phenotyping, and cellular neighborhood assignment. When speed outweighs resolution, the increased efficiency demonstrated here applies to most mIF spatial biology applications. Large area samples require less FOVs to capture. Small area samples can be batched on the same slide. And small, single samples can be captured without image stitching. All these examples benefit from efficient placement of large FOVs.

#178 Automating a spatial profiling workflow to explore the effects of hypoxia in the tumor microenvironment in head and neck cancer.

Kyla Teplitz[1], David Mason[2], **Regan Baird**[3], Kelly Hunter[4], Joana Campos[4], Jill Brooks[5]

[1]Bruker Spatial Biology, Seattle, WA,[2]Visiopharm, Horslom, Denmark,[3]Visiopharm, Arvada, CO,[4]Propath UK, Hereford, United Kingdom,[5]University of Birmingham, Birmingham, United Kingdom

Background: Head and neck cancer (HNC) encompasses a diverse group of malignancies originating from the mucosal surfaces of the upper aerodigestive tract. The tumor microenvironment (TME) in HNC is marked by immune cells, stromal cells, and extracellular matrix components. Hypoxia, a key feature of the TME, drives tumor growth, invasion, and metastasis by modulating gene expression related to angiogenesis, cell survival, and metabolism. Understanding the interplay between hypoxia and immune infiltrates in the TME is essential for developing new therapeutic strategies for HNC. While whole transcriptome analysis via digital spatial profiling is a powerful method for probing the TME, it is time-consuming for large cohorts. Automating the profiling workflow can reduce hands-on time and selection bias in regions of interest (ROIs), facilitating the exploration of large cohorts to identify mechanisms of action, potential drug targets, and biomarkers.
Methods: We developed an optimized spatial multi-omic workflow for high-throughput spatial analysis using the GeoMx® Digital Spatial Profiler (DSP) with the Whole Transcriptome Atlas (WTA) and immunofluorescent morphology markers: SYTO82 (nuclei), CAIX (hypoxia), pan-cytokeratin (epithelium), and CD3 (T-cells). AI-based analysis (Oncotopix® Discovery) of serial section H&E and GeoMx IF images was employed to identify ROIs for GeoMx collection. Immune hot and cold selections were based on leukocyte density, while tumor/stromal interface selections focused on epithelial areas. Areas of illumination (AOI) were chosen using concentric CAIX expression gradients. Integrated analysis of digital images and the whole transcriptome was conducted to assess the TME compartments.
Results: Automated ROI placement based on tumor/stroma, hypoxia, and immune infiltration, along with AI/Deep Learning-based AOI segmentation, reduced AOI selection time and improved tissue compartment enrichment accuracy across samples and tissue types. The development of hypoxia gradient-based AOI selection enabled a strategy not possible with standard DSP software. Cell phenotyping using IF morphology scans supervised cell deconvolution. DSP results showed strong correlation with patient outcomes.
Conclusions: This study demonstrates that ROI-based spatial analyses can effectively explore the impact of hypoxia on immune infiltration in HNC. Automated AI-based ROI selection offers an unbiased, reproducible method for sampling relevant tumor subtypes based on hypoxia and immune infiltrate criteria. This approach provides a standardized, automated method for selecting ROIs and segmenting AOIs across diverse tissue types and pathological subtypes, enhancing throughput.

#179 Spatial and multiomics analysis reveals immune interactions as key drivers of immunotherapy outcomes in melanoma patients with in-transit metastases.

X. Bai[1], C. Quek[1], G. Al-Eryani[2], S. Ramkomuth[2], L. Baldwin[2], J. Tian[1], N. A. Adegoke[1], I. A. Vergara[1], F. Newell[3], K. Harvey[4], J. Reeves[2], A. J. Spillane[1], N. Waddell[3], S. N. Lo[1], A. M. Menzies[1], I. P. da Silva[1], U. Palendira[1], G. V. Long[1], R. A. Scolyer[1], A. Swarbrick[2], J. S. Wilmott[1];

[1]Melanoma Institute Australia, The University of Sydney, Sydney, Australia, [2]Garvan Institute of Medical Research, University of New South Wales, Sydney, Australia, [3]QIMR Berghofer Medical Research Institute, Brisbane, Australia, [4]Garvan Institute of Medical Research, Sydney, Australia

Background: In-transit metastasis (ITM) of melanoma occurs between the primary tumor and draining lymph nodes. ITM can be managed by by adjuvant immunotherapy after resection, or immunotherapy alone when unresectable. Nevertheless, nearly half of ITM patients are resistant, which constitutes a clinical challenge. Multiomic spatial profiling offers insights into features influencing immunotherapy response, but the tumor microenvironment (TME) and biological determinants of ITM remain poorly defined. This study aims to characterize molecular and spatial TME attributes in ITM patients to improve understanding of immunotherapy response and resistance.
Methods: Fifty-four samples from ITM patients treated with immunotherapy were collected at pre-treatment (PRE) and progression (PROG). Patients with a complete or partial response in the advanced setting, or recurrence-free survival over 12 months in the adjuvant setting, were classified as "responsive," and others as "resistant". Single-cell sequencing and 40-marker imaging were conducted on 18 matched tumor dissociates and FFPE samples at PRE (n=3 resistant, n=5 responsive) and PROG (n=10). Whole genome sequencing on 12 fresh frozen samples (n=4 resistant, n=8 responsive at PRE) were used to identify somatic variants.
Results: While regional immune cell proportions were broadly similar across response groups, PRE responders showed increased intra- and peri-tumoral T cells, and surprisingly, reduced CD141+ dendritic cells (cDC1s) compared to resistant patients (P=0.024). K-means clustering identified 7 spatially resolved cellular neighborhoods (CNs) across all ITMs with heterogeneous cellular constitution; nevertheless, lymphocyte-enriched CNs were more dispersed in PRE responders and interacted with other immune and tumor CNs, while remaining peripheral in PRE resistant tumors. Despite having a lower DC density, pairwise interaction analysis highlighted significant interactions between cDC1s and CD14+ macrophages in PRE responders, and between macrophages and IDO1+ CD4+ T cells or Granzyme B+ CD8+ T cells ($P<0.05$, log2 fold change>0.2), suggesting enhanced macrophage-driven local T cell stimulation in the absence of DCs. Single-cell transcriptomics analysis comfirmed the reduced proportion of cDC1s in PRE responders compared to resistant ITM (P=0.006). Genomic profiling revealed *EREG* mutations exclusively in resistant ITMs, which has been linked to immunoregulationand tumor invasion through the EGFR pathway. Ongoing analysis explores interactions among DCs, macrophages, T cells, B cells, melanocytes, and endothelial cells to elucidate TME dynamics associated with immunotherapy outcomes.
Conclusion: These findings deepen our understanding of tumor-immune interactions in ITM patients and inform personalized immunotherapy approaches tailored to their unique TME.

TUMOR BIOLOGY: Cancer Stem Cells
Poster Session

#18 Fisetin effectively inhibits cancer stem cell properties in pancreatic ductal adenocarcinoma.

Anjali Aggarwal[1], Vinit Sharma[1], Rajesh Gupta[2], Mayank Sharma[1], Ankita Semwal[1]

[1]Anatomy, Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India,[2]Surgical Gastroenterology, Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India

Introduction: Cancer stem cells (CSCs) are crucial in the aggressiveness and chemoresistance of pancreatic ductal adenocarcinoma (PDAC). Targeting CSCs is essential for effective treatment and preventing relapse. Fisetin, a natural flavonoid, has demonstrated anticancer, anti-inflammatory, and antioxidant properties. This study investigates the anti-CSC effects of fisetin in PDAC.
Methods: CSCs were isolated from Panc-1 cells and explant cultures of PDAC tissue using fluorescence-activated cell sorting (FACS) with specific surface markers (CD44, CD24, CD133, CD326/ESA). The isolated CSCs were treated with various doses of fisetin for 24 and 48 hours, and the half-maximal inhibitory concentration (IC50) was determined using the MTT assay. The effects of fisetin on colony formation, cell cycle, apoptosis, migration, invasion , and sphere formation were evaluated. Additionally, the expression of epithelial-mesenchymal transition (EMT) markers and stemness markers was assessed at both gene and protein levels. The molecular mechanisms underlying the anti-CSC effects of fisetin were investigated through mRNA sequencing, followed by validation using ELISA.
Results: Fisetin significantly reduced viability and colony formation of CSCs in a dose- and time-dependent manner. The apoptotic cells increased from 2.06% in untreated to 50.9% in fisetin-treated CSCs. This was further supported by enhanced expression of apoptosis markers Bid and Bcl-2 in fisetin-treated CSCs. Migration capacity was significantly reduced, with the wound closure area increasing from 1.18% to 29.5%. Fisetin also induced cell cycle arrest, with a notable increase in the percentage of cells in the G2 and S phases. The size of tumor spheroids was significantly reduced after treatment. Additionally, fisetin elevated the expression of the epithelial marker E-cadherin and reduced the level of the mesenchymal marker N-cadherin. The expression of transcription factors SOX2 and OCT4, which are associated with the maintenance of CSCs, was decreased. Reactive oxygen species (ROS) levels in CSCs were significantly reduced. Fisetin treatment led to the downregulation of genes involved in inflammatory signalling pathways, including BCL2A1 (NF-κB target gene), CXCL1, CXCL8, CX3CL1, and CCL2. KEGG pathway analysis showed that the NF-κB and cytokine-cytokine receptor signalling pathways were significantly downregulated following fisetin treatment. ELISA further confirmed the significant downregulation of NF-κB p65 signaling ($p = 0.013$) in fisetin-treated CSCs.
Conclusion: Fisetin effectively targets and inhibits CSCs in PDAC, modulating key markers of epithelial-mesenchymal transition and stemness, while downregulating NF-κB signaling. These results support the need for further validation in in vivo models.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Cell Signaling and Tumor-Stroma Cell Interactions
Poster Session

#183 High-content screening reveals kinase regulators of Dvl2 phase separation in Wnt signaling: Implications for targeted cancer therapy.

Antonia Schubert[1], Florian Heigwer[1], Christian Scheeder[1], Oksana Voloshanenko[1], Dominique Kranz[1], Thilo Miersch[1], Barbara Schmitt[1], Melanie Kuhse[1], Franziska Ragaller[1], Nadine Winkler[1], Anastasija Paneva[1], Daniel Gimenes[2], Diana Ordonez-Rueda[2], Jennifer Schwarz[3], Frank Stein[3], Dirk Jager[4], Ulrike Engel[5], Michael Boutros[1]

[1]Signaling and Functional Genomics, DKFZ German Cancer Research Center, Heidelberg, Germany,[2]Flow Cytometry Core Facility, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany,[3]Proteomics Core Facility, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany,[4]Medical Oncology, National Center for Tumor Diseases (NCT), University Hospital Heidelberg, Heidelberg, Germany,[5]Nikon Imaging Center (NIC), BioQuant and Centre for Organismal Studies (COS), Heidelberg, Germany

Wnt signaling controls cell proliferation, differentiation, and migration, with critical roles in diseases such as cancer. It was proposed that Wnt signaling relies on the formation of dynamic biomolecular condensates, such as the “signalosome” and the “destruction complex”, with a potential to modify the spatial and temporal regulation of signaling outcomes. To investigate the role of phase separation in Wnt signaling, we generated a cell line with endogenously tagged Dvl2, a central Wnt scaffolding protein, using the mEos3.2 fluorophore. Applying live-cell and single-molecule localization microscopy, we could demonstrate that Dvl2 forms condensates at the centrosome in a Wnt- and cell-cycle-dependent manner in HEK293T cells (Schubert et al. PNAS, 2022).
Here, we used image-enabled cell sorting to separate cell pools based on these endogenous Dvl2 condensates, followed by temporal and phosphoproteomic analysis to further understand their functional significance. Condensate-positive cells were enriched with proteins associated with planar cell polarity (PCP) Wnt signaling and proteins of the G2/M cell cycle phase were differentially phosphorylated, suggesting distinct signaling and cell-cycle states.
We then conducted a high-throughput, image-based screen of more than 600 kinase inhibitors to identify modulators of Dvl2 condensate dynamics. Several kinases influenced Dvl2 phase separation, revealing a correlation of condensate formation and canonical Wnt signaling activation. Notably, a candidate inhibitor disrupted both Dvl2 condensates and Wnt signaling, affecting cell division, most likely through microtubule regulation. Further phosphoproteomic and chemical-genetic interaction analysis applying CRISPR/Cas9 targeting the Wnt scaffolds APC, Axin1, and MCC, alongside kinase inhibition, revealed distinct roles for these scaffolds in condensate regulation and, interestingly, pathway outcomes.
This work underscores the regulatory complexity of Wnt condensates, highlighting post-translational modifications, cellular context, and scaffold roles in Wnt signaling. Our study introduces a high-content screening approach to intracellularly study phase-separated systems. Targeting condensates may represent a novel therapeutic strategy in cancers with aberrant Wnt signaling.

#184 PHF6-LEO1 axis depletion drives aggressive colorectal cancer through WNT activation and metabolic reprogramming with therapeutic implications.

Su Chan Park[1], Shinae Park[2], Ji-Yeon Lee[1], Taehan Yoon[1], So Hyun Kwon[1], Jung-Shin Lee[2], Eun Jung Park[3], Ji Min Lee[1]

[1]Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Korea, Republic of,[2]Department of Molecular Bioscience, College of Biomedical Science, Kangwon National University, Chuncheon, Korea, Republic of,[3]Department of Colon and Rectal Surgery, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, Republic of

Colorectal cancer (CRC) progression is closely linked to alterations in key signaling pathways and metabolic reprogramming. The WNT signaling pathway plays a critical role in regulating cell development and proliferation, and its abnormal activation is a hallmark of CRC. Plant Homeodomain 6 (PHF6), a chromatin-associated factor, is known for its roles in clonal hematopoiesis, ribosome biogenesis, DNA repair, and aging, and is frequently mutated in lymphoid cancers and leukemia. In this study, RNA-seq and ATAC-seq were conducted to investigate the function of PHF6, we identified PHF6 as a novel regulator of the WNT signaling pathway. We found that PHF6 depletion activate WNT signaling and proliferation of colon cancer cells. In the AOM/DSS model, PHF6 knockout mice exhibited larger polyp formation compared to wild type mice, with polyps extending into the proximal region. Proximal CRC is more prevalent and aggressive and has distinct genetic features compared to distal CRC. Alterations in proximal CRC include the PIK3CA mutation and CpG island methylator phenotype (CIMP)-high frequency, the development of CIMP is followed by global gene expression alteration and metabolic changes. However, the molecular mechanisms of this type of cancer are not fully understood. We observed a reduced levels of LEO1, the transcriptional elongation partner of PHF6 within the PAF1 complex, in the proximal region of PHF6 KO mice. We identified LEO1 and SETD1A as epigenetic regulators of lipid homeostasis in colon cancer cells and patient samples with matched normal. Dysregulation of lipid metabolism in cancer cells impacts tumor initiation, progression, and metastasis. LEO1 KO cells exhibited reduced expression and activation of SREBP1, a master regulator of lipid metabolism, under glucose starvation. These effects were further enhanced by SETD1A knockdown. LEO1 was found to localize to the nucleus and is required for the nuclear localization of SETD1A under nutrient stress. These cells showed an altered cell cycle, resulting in reduced proliferation but increased metastatic potential. These findings suggest that the PHF6-LEO1 axis plays a crucial role in the regional development and metastasis, underscoring its importance in advancing our understanding of tumor biology and guiding targeted therapy development.

#185 Alternative NF-kB inhibits MAPK activity to promote quiescence and drug resistance in ovarian cancer.

Cecilia Gallo, Mikella Robinson, Jacqueline Lara, Omar Lujano Olazaba, Carrie D. House

Biology, San Diego State University, San Diego, CA

Ovarian Cancer (OC) is the most lethal gynecological malignancy in the United States and despite good initial response to chemotherapy, approximately 80% of late-stage patients relapse. Recent studies suggest that a subpopulation of tumor cells with stem-like characteristics, called Cancer Stem-like Cells (CSCs), resist treatment and drive recurrence. Several signaling pathways, including MAPK and NF-kB, are known for their roles in tumor initiation, disease progression, and drug resistance in ovarian cancer. We previously showed that the canonical NF-kB factor, RelA, primarily promotes proliferation, while the non-canonical NF-kB factor, RelB, promotes quiescence. Additionally, activation of MAPK/ERK has been implicated in proliferation of ovarian cancer cells. NF-kB and MAPK signaling cascades are both critical for OC progression, although their precise roles in maintaining CSCs remain unclear. Our preliminary data showed that knockdown (KD) of RelB in vivo following chemotherapy did not decrease growth of residual tumors, whereas KD of RelA or inhibition of MAPK alone reduced growth of residual tumors. However, combining RelB KD with MAPK inhibition reduced growth of residual tumors comparable to RelA KD or MAPK inhibition alone. Therefore, we hypothesize that RelB supports quiescence in CSCs by downregulating MAPK activity. Western Blot analysis indicated increased MAPK activity in the absence of RelB in two high grade serous ovarian cancer (HGSOC) cell lines. Moreover, in vitro cell cycle studies revealed an increase in the percentage of CSCs in the S phase following RelB KD and combining RelB KD with MAPK inhibition rescued this phenotype. Taken together, our findings suggest that RelB downregulates MAPK activity to support quiescence in ovarian CSCs. Ongoing studies are investigating the expression of S phase specific cyclins in the absence of RelB to further elucidate mechanisms of proliferation and cell cycle progression regulated by RelB and MAPK. Additionally, immunohistochemistry (IHC) analysis using tissues derived from control, RelB KD, and RelB KD with MAPK inhibition tumors will be used to evaluate changes in NF-kB, MAPK and cell cycle proteins in residual and regrown tumors. This work reveals novel mechanisms of how RelB supports drug resistance in ovarian CSCs and information gleaned from this study could inform future therapeutic studies.

#186 Tumor cell-intrinsic STAT2 signaling contributes to mitochondrial energetics in colorectal cancer.

J. Canar[1], S. Fossati[1], M. Slifker[2], A. M. Gamero[1];
[1]Temple Univ. Lewis Katz School of Medicine, Philadelphia, PA, [2]Fox Chase Cancer Center, Philadelphia, PA

Colorectal cancer (CRC) is the 3rd most common cancer type in the US, with an alarming increase in incidence rates in young adults that is preventable when detected early. Identifying the molecular culprits involved in promoting CRC is urgently needed to design novel therapeutic interventions to combat this disease. Signal transducer and activator of transcription 2 (STAT2) is a key transcription factor that uniquely mediates the antitumor effects of type I and III interferons (IFNs), a family of pleiotropic cytokines, by activating the transcription of genes involved in restricting tumor growth. Much of what is known about STAT2 is in the context of IFN signaling. Yet, the precise function of STAT2 in cancer is understudied. New evidence shows that STAT2 has opposing biological effects in cancer. We and others have reported that STAT2 inhibits tumor cell proliferation and induces apoptosis in response to IFNs. Conversely, our new data finds that STAT2 promotes tumor growth in pre-clinical models of CRC (spontaneous and tumor xenografts) by a mechanism that is still unidentified. To begin to elucidate the pro-tumorigenic effects of STAT2, we analyzed the human colon adenocarcinoma TCGA datasets for an association with overall survival and alterations in transcriptional signatures between high and low STAT2 expression levels. First, we found a correlation between high STAT2 levels and poor survival. Second, out of the 42 hallmark gene set pathways significantly enriched, oxidative phosphorylation (OXPHOS) was strikingly downregulated in STAT2-high tumors. We performed mitochondrial and glycolysis stress tests to determine changes in cellular energetics between parental and STAT2 deficient (STAT2KO) human HCT116 colon adenocarcinoma cells. Compared to parental tumor cells, STAT2 KO tumor cells had lower glycolytic capacity but increased maximal respiration and spare respiratory capacity. We next assessed the role of STAT2 in mitochondrial homeostasis because loss of STAT2 had been previously reported to interfere with mitochondrial fission. We determined by western blot analysis the activation status of Dynamin-related protein-1 (Drp1), a key protein that regulates mitochondrial fission. We found impaired activation of Drp1 in STAT2 KO tumor cells. Drp1 phosphorylation was reduced at activatory serine 616 and increased at inhibitory serine 627. Overall, our findings are first to indicate that STAT2 in CRC may be involved in disrupting mitochondrial respiration and favoring glycolysis, a hallmark of cancer, to promote CRC. This paves the way for determining whether the tumor promoting effects of STAT2 are dictated at the mitochondrial level by disrupting OXPHOS and whether this is occurring independently of IFN signaling. Studies are ongoing to determine whether other components of mitochondrial fission and kinases involved in Drp1 activation are also regulated by STAT2.

#187 STAT1 and GBP1 promote proliferation in postpartum breast epithelium by downregulating cyclin-dependent kinase inhibitors: implications for increased postpartum breast cancer risk.

Joshua Ogony[1], Melody Stallings-Mann[1], Sarah McLaughlin[2], Mark Sherman[3], **Derek C. Radisky**[1]

[1]Department of Cancer Biology, Mayo Clinic Florida, Jacksonville, FL,[2]Department of Surgery, Mayo Clinic Florida, Jacksonville, FL,[3]Department of Quantitative Health Sciences, Mayo Clinic Florida, Jacksonville, FL

Background: Postpartum breast cancer (PPBC) is associated with increased morbidity and mortality among women within 5 to 10 years following childbirth. The postpartum breast undergoes involution, involving tissue remodeling and immune cell infiltration, potentially creating a pro-tumorigenic microenvironment. The molecular mechanisms underlying this elevated risk remain poorly understood. In this study, we compared normal breast tissue samples and human mammary epithelial cells (HMECs) from postpartum women to those from age- and BMI-matched nulliparous women to elucidate potential molecular contributors to PPBC risk.
Methods: Transcriptomic profiling was performed on RNA extracted from FFPE normal breast tissues using the NanoString IO 360 and BC 360 platforms to examine gene expression in nulliparous and postpartum women under 45 years of age. HMECs were isolated from organoids derived from breast tissue samples and used for next-generation sequencing and knockdown experiments targeting STAT1 and GBP1. Gene set enrichment analysis (GSEA) was applied to NanoString and RNA-seq datasets to identify differentially enriched genes between postpartum and nulliparous women.
Results: Transcriptomic analysis revealed significant upregulation of interferon-stimulated genes, including STAT1 and GBP1, in postpartum breast tissues, consistent with increased immune responses during involution. NanoString data showed that markers of cell proliferation, such as Ki67, STAT1, and GBP1, were significantly higher in samples from parous compared to nulliparous women ($p < 0.05$). GSEA revealed that cell cycle-promoting genes (GBP1, cyclins, CDKs) were upregulated, while cyclin-dependent kinase inhibitors (CDKNs) were downregulated in postpartum breast tissues. Immunohistochemistry confirmed significantly higher expression of Ki67, STAT1, and GBP1 in postpartum versus nulliparous breast tissues ($p < 0.0001$). Silencing STAT1 and GBP1 in HMECs resulted in decreased cell proliferation, G1 cell cycle arrest, and increased expression of CDKN1A and CDKN1C.
Conclusion: These findings suggest that STAT1 and GBP1 promote proliferation in postpartum breast epithelial cells by downregulating cyclin-dependent kinase inhibitors, potentially contributing to the increased risk of PPBC. Targeting these pathways may offer novel strategies for mitigating postpartum breast cancer risk.

#188 Phosphorylation of StarD10 advances alcohol induced breast cancer progression.

M. Dagar, A. Floris, Y. Lim, S. Chandla, M. Justo, N. Mavila, K. Ramani, M. Tomasi;
Cedars-Sinai Medical Center, Los Angeles, CA

Introduction: Breast cancer is the second most common cancer among women, and excessive alcohol consumption is a well-established risk factor for various cancers, including breast cancer. Even moderate alcohol consumption (one drink per day) increases breast cancer risk by 10% compared to non-drinking women. The STAR-related lipid transfer domain-containing protein 10 (StarD10), a phosphoprotein, is overexpressed in 35-40% of primary human breast cancers and interacts with the ErbB2 signalling pathway. Our recent studies demonstrated that ethanol administration promotes StarD10 dephosphorylation and increases its activity, as well as enhances ErbB2 expression, which is strongly correlated with increased cell malignancy and aggressiveness. However, the precise role of StarD10 as a subcellular lipid transporter in the regulation of ErbB2 signalling remains unclear. This study aimed to elucidate ethanol-induced changes in StarD10 activity, with the inhibition of its phosphorylation being a potential target for mitigating alcohol-induced breast cancer progression.

Materials and Methods: To identify specific phosphorylation sites, phospho-peptide mapping of StarD10 from MCF-7 and SKBR-3 cells was performed. The identified phosphorylation site in StarD10 was further validated by western blot and co-immunoprecipitation studies. A lipid overlay assay was performed to assess StarD10 binding to phospholipids.

Results: Phospho-peptide mapping identified threonine 288 (T288) as a specific phosphorylation site, regulated by casein kinase 1α (CK1α). Silencing CK1α blocked StarD10 phosphorylation, mimicking the dephosphorylation effects induced by ethanol. Lipid overlay assay demonstrated that StarD10 binds to a range of phospholipids. We found the interaction of StarD10 with PI(3)P, PI(4)P, PI(5)P, $PI(3,4)P_2$, $PI(3,5)P_2$, $PI(4,5)P_2$, $PI(3,4,5)P_2$ in both MCF-7 and SKBR-3 cells. PP2A and PP1, serine/threonine phosphatases regulate protein function by dephosphorylation impacting in several critical cellular pathways. We found that treatment with okadaic acid (a PP2A inhibitor) and calyculin A (a PP1/PP2A inhibitor) blocked ethanol-induced StarD10 dephosphorylation in both MCF-7 and SKBR-3 cells. Also, this treatment inhibited the membrane fluidity as compared to ethanol. It suggests a regulatory role of PP1/PP2A in the StarD10 function.

Conclusions: The role of phosphorylated StarD10 in alcohol-induced breast cancer progression is mediated by the phosphorylation of T288 by CK1α. PP1/PP2A acts as a positive regulator of StarD10 activity, further implicating these phosphatases in the progression of ethanol-induced breast cancer.

Acknowledgments: R01 AA030576 (NIAAA), Role of StAR-related lipid transfer protein 10 in alcohol-induced breast cancer progression.

#189 The role of DEPTOR in promoting breast cancer initiation and progression.

A. Nam, B. Xiao, V. Sanguin-Gendreau, D. Zuo, W. J. Muller;
McGill Univ. Goodman Cancer Ctr., Montreal, QC, Canada

Breast cancer is one of the most common malignancies in the world, with 2.3 million women diagnosed in 2022. It is largely driven by dysregulation of cellular signaling pathways such as mTOR, which, as a key regulator of cellular growth, is frequently hyperactivated in many cancers. Despite evidence implicating mTOR as a promising target for anti-cancer therapies, many inhibitors in clinical trials yield unfavorable outputs, largely due to its complex regulatory mechanisms that are yet fully understood. DEPTOR, a recently identified direct inhibitor of mTOR, has thus become an intriguing focus of study. Interestingly, while many tumors exhibit high mTOR activity and, as expected, also show low DEPTOR expression, paradoxically, high DEPTOR levels in several cancers corresponds with the worst prognoses for patients. These observations point to dual roles for this protein, and unknown pro-tumor functions that are potentially independent of mTOR. Our work aims to expand our understanding of the functions and interacting partners of DEPTOR, and characterize its role in breast cancer, about which knowledge is currently lacking. In a mouse model of luminal B breast cancer, we observe a severe defect in early mammary cell expansion, delay in tumor onset, and reduction in tumor penetrance upon genetic ablation of DEPTOR, indicating DEPTOR acts in an oncogenic capacity in this system. However, these results are not accompanied by a significant change in activity of major mTOR effectors, and gene expression profiles indicate changes in factors related to transcription and chromatin accessibility but not translation, despite the latter being largely modulated by mTOR to drive cell growth. Additionally, while the innate proliferative ability of early DEPTOR-deficient mammary cancer cells appears unchanged, their immune microenvironment (TIME) is altered to favour an anti-tumor immune response. Thus, we will investigate how DEPTOR-dependent transcriptional regulation may reprogram the TIME to influence tumor initiation and progression. Considering mTOR’s ubiquity in cancer, elucidating the activities of a core regulatory component such as DEPTOR will provide important insights for its therapeutic targeting. Further, by investigating DEPTOR’s mTOR-independent roles, this work will highlight the scope of a poorly understood but widely acting player in cancer and its potential as a novel therapeutic target.

TUMOR BIOLOGY: Cancer Stem Cells
Poster Session

#19 ACTL6A promotes the progression of prostate cancer by modulating GLI1 expression in the Hedgehog signaling pathway.
L.-H. Cheng, K. Tsai;
Taipei Medical University, Taipei, Taiwan

The disruption of tissue organization is a major contributing factor to the malignant transformation of glandular epithelium, including prostate glands. Three-dimensional organotypic cultures have facilitated the discovery of molecular mechanisms underlying malignant progression. Using a robotic-assisted MicroWestern Array-based proteomics screening, we identified that the protein abundance level of the chromatin remodeling factor ACTL6A is upregulated in disorganized prostate epithelial organoids. We showed that ACTL6A critically regulates prostate glandular morphogenesis as its overexpression led to glrandular transformation. Consistent with this finding, ACTL6A expression is higher in human prostate cancer compared to normal prostate tissue and is associated with poor clinical prognosis. At the functional level, tthe knockdown of *ACTL6A* expression in PCa cells attenuated stemness and tumorigenesis *in vitro* and PCa progression in an orthotopic mouse tumor model. Intriguingly, screening of development-associated signaling pathways identified the Hedgehog (Hh) pathway, whose activity and the target genes were reduced by the loss of *ACTL6A* expression in PCa cells. Further mechanistic and molecular studies revealed that ACTL6A regulates the protein abundance level of GLI1, the key transcriptional factor in Hh-pathway, PCa cells by disabling its ubiquitin-dependent proteosomal degradation. Our study thus illuminated a novel oncogenic mechanism mediated by the ACTL6A-GLI1-Hh signaling axis in PCa, which may have diagnostic and therapeutic implications.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Cell Signaling and Tumor-Stroma Cell Interactions
Poster Session

#190 Effects of bisphenol compounds on gene expression and cellular outcomes in MDA-MB-231 breast cancer cells mediated via the G protein-coupled estrogen receptor.

Norman R. Estes, Cameron Sheeler, Olivia Merriam

University of Alabama at Birmingham, Birmingham, AL

Bisphenol A (BPA) is used in the manufacturing of various consumer products such as plastic bottles, epoxy resins in food cans, thermal printed paper, and dental sealants. BPA has been identified as an endocrine disruptor due to its ability to mimic estrogen by activating estrogen receptor signaling pathways and regulating estrogen-responsive genes through a classical estrogen receptor-mediated pathway. Concerningly, BPA is linked to health risks, such as hormone-dependent cancers, metabolic diseases, and developmental defects. What remains unclear is the effect BPA and BPA alternatives have on gene expression and cellular outcomes via interaction with a membrane-bound estrogen receptor that functions as a G protein-coupled receptor (GPER). MDA-MB-231 triple-negative breast cancer cells (TNBC) express GPER but lack the classical estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). These studies seek to determine if BPA or alternatives, functioning through GPER, differentially affect gene expression, cell proliferation, and cell migration through GPER. Preliminary studies using total RNA sequencing (RNA-Seq) identified differential gene expression patterns in cells treated with BPA or 17β -estradiol including changes in cancer-related pathways, CREB signaling, and G-protein coupled receptor-mediated pathways. Cell migration assays performed in MDA-MB-231 cells treated with BPA, BPB, BPC, or 17β -estradiol suggest that treatment with these compounds differentially affects migration, with the highest degree of migration being induced by BPC. These studies indicate that BPA and BPA alternatives differentially affect gene expression and cell migration in triple negative MDA-MB-231 breast cancer cells and exposure to these compounds may affect therapeutic outcomes in TNBC patients. Future studies will seek to clarify the role of select genes and related pathways as identified through RNA-Seq analysis in TNBC cells treated with BPA or BPA alternatives in order to clarify the role of GPER-mediated signaling in TNBC cells.

#191 Functional role of chromosome 1q loci on breast cancer progression.

A. Bera[1], E. Russ[1], H. Hu[2], C. D. Shriver[3], **M. Srivastava**[1];
[1]Uniformed Services University of the Health Sciences, Bethesda, MD, [2]Chan Soon-Shiong Institute of Molecular Medicine, Windber, PA, [3]Murtha Cancer Center, Walter Reed National Military Medical Center, Bethesda, MD

Several studies have identified differential expression at various loci on the long arm of chromosome 1 (1q) as the most frequent cytogenetic abnormalities associated with human breast carcinoma. To investigate the impact of genetic alterations on chromosome 1q, we analyzed data from over thirteen thousand human breast tumors (n=13,146 samples; corresponding to 12,229 patients across 30 studies) using cBioPortal, which included comprehensive datasets like The Cancer Genome Atlas(TCGA). Our findings revealed that eight genes exhibit significant copy number alterations (12-15%), predominantly amplifications. These genes include *TRIM67* (1q42.2, 14%), *DISC1* (1q42.2, 14%), *REN* (1q32.1, 15%), *DNM3* (1q24.3, 12%), *ATP1B1* (1q24.2,12%), *VSIG8* (1q23.2, 14%), *SPTA1* (1q23.1, 14%) and *MUC1* (1q22, 14%). In contrast, analysis across more than 100,000 samples from over 35 different cancers (n=105,424samples; 101,597 patients across 226 studies) showed that these genes have much lower alteration frequencies, approximately 3%. Besides, these genes also exhibited notably low expression levels at both RNA and protein levels in normal breast tissues. We further explored the clinical relevance of these genes, examining their roles in breast cancer prognosis and survival outcomes. Our mutual exclusivity and co-occurrence analyses revealed distinct patterns of genetic interplay, indicating a probable collective gain-of-function that drives breast cancer progression. Specifically,TP53 was found to co-occur with *MUC1, SPTA1,* and *VSIG8,* while *REN* exhibited mutual exclusivity. The driver gene *PIK3CA* co-occurred with *DISC1, TRIM67*, and *REN*, whereas *ERBB2* showed co-occurrence with all eight genes. These findings suggest a complex network of genetic interactions that may underlie invasive and metastatic breast cancer phenotypes.

Additionally, we observed that some of the above mentioned gene products (proteins) including *REN* (Renin) are detectable in serum, suggesting potential as biomarkers for recurrence or prognosis. Our ongoing studies are focused on validating these proteins as diagnostic and therapeutic targets. Collectively, this study underscores the importance of multi-locus evaluation on chromosome 1q, which could pave the way for novel diagnostic and therapeutic strategies in breast cancer management.

#192 Honokiol effectively induces apoptosis by targeting the hedgehog signaling pathways in breast cancer.

Santosh K. Singh, Rajesh Singh

Microbiology, Biochemistry & Immunology, Morehouse School of Medicine, Atlanta, GA

Breast cancer (BrCa) is a heterogeneous and deadly cancer affecting women, involving various pathways regulating the growth and proliferation of cancer cells. Upon diagnosis, BrCa is typically treated according to the molecular subtypes such as estrogen receptor (ER), progesterone receptor (PR), or human epidermal growth factor receptor 2 (HER2). In recent years, chemotherapy and targeted therapy have improved the 5-year survival rate for patients with receptor-positive BrCa. However, the patients diagnosed with triple-negative breast cancer (TNBC) remain unsatisfactory. The existing therapies, such as chemotherapy, cause drug resistance, toxicity, and relapse. Therefore, identifying the alternative approach and molecular targets responsible for BrCa development is crucial for improved therapy. One such target is Sonic Hedgehog (Shh) signaling molecules, which have been linked to the activation of several pathways important for developing BrCa. Shh is a critical component of the Hedgehog (Hh) pathway known for cell differentiation and tissue development. In this study, we investigated the effect of Honokiol (HNK), a dietary photochemical isolated from the *Magnolia officinalis* tree, on inhibiting the progression of TNBC cells (MDA MB231, MDA MB468) and inducing apoptosis *via* the Shh signaling pathway. HNK possesses potent anti-angiogenic, anti-microbial, anti-inflammatory, and antitumoral activities against aggressive cancers, including BrCa. However, the effect of HNK in targeting Hh signaling in TNBC cells remains limited. To determine the IC50 values of HNK, an MTT assay was performed for different time intervals with various concentrations (5-100μM). HNK cytotoxicity was estimated using live/ dead cell assay, scratch assay, and clonogenic assays. The expression of Hh signaling molecules and apoptotic markers were quantitated with real-time PCR and western blot assay, and an immunofluorescence assay was performed to observe the Shh expression in both cell lines. Our results indicate that HNK led to lower cell viability at 48 hours of treatment compared to control. The flow cytometric analysis showed that HNK enhanced the apoptosis of TNBC cells. Furthermore, HNK increased the expression of cell cycle inhibitor p21WAF1/CIP1, which induces cell cycle arrest by controlling the downstream molecules, CDK4 and cyclin D1. Additionally, HNK suppresses the expression of NF-kB and Hh molecules (Shh, Gli1, and Ptch1) in both TNBC cells. These findings suggest that HNK may directly affect the Shh signaling pathway by interfering with NF-κB crosstalk activity, thereby preventing the communication between tumor cells and surrounding tissue. The migration assay further supported these results. Overall findings indicate a promising role of HNK in targeting the Shh pathway in TNBC type of breast cancer, which could improve the understanding for further translational research.

#193 Notch pathway activation predicts poor prognosis in combined hepatocellular carcinoma-cholangiocarcinoma.

Seokhwi Kim[1], Chan Mo Yang[2], Jaehong Lim[3], Ara Ko[3], Taebum Lee[4], Young-Bae Kim[1], Dakeun Lee[1]

[1]Department of Pathology, Ajou University School of Medicine, Suwon, Korea, Republic of,[2]Department of Biomedical Science, Ajou University Graduate School of Medicine, Suwon, Korea, Republic of,[3]Ajou University School of Medicine, Suwon, Korea, Republic of,[4]Lunit, Seoul, Korea, Republic of

Background: Combined hepatocellular carcinoma and cholangiocarcinoma (cHCC-CCC) is a distinct type of tumor that differs from both hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCC). Although the involvement of the Notch signaling pathway in its development is recognized, its role in clinical specimens has not been extensively studied.
Methods: We analyzed surgically resected specimens from 38 HCCs, 32 CCCs, and 42 cHCC-CCCs obtained between 2017 and 2023 at Ajou University Hospital. The expression levels of the HCC marker Glypican-3, the CCC markers CK7 and CK19, and the hepatobiliary progenitor cell markers CK19, EpCAM, and CD13 were investigated through immunohistochemistry (IHC). The expression of Notch receptor subtypes (Notch1-4) and the downstream transcription factors Hes5 and Ascl1 were analyzed using qRT-PCR. Additionally, NOTCH1 and Hes5 protein expression levels were evaluated via IHC.
Results: Compared to HCC, cHCC-CCC exhibited reduced expression of Glypican-3 (p<0.0001) and increased expression of CK7 (p<0.0001), CK19 (p<0.0001), and EpCAM (p<0.0001). When compared to CCC, cHCC-CCC displayed significantly elevated levels of Glypican-3 (p<0.0001) and CD13 (p<0.0001). Among the four Notch receptor subtypes, the mRNA level of Notch1 was preferentially elevated in cHCC-CCC compared to HCC or CCC. Similarly, the mRNA expression levels of Hes5 and Ascl1 were higher in cHCC-CCC than in HCC (p=0.0920 for Hes5; p=0.5453 for Ascl1) and CCC (p=0.0586 for Hes5; p=0.0759 for Ascl1). Higher NOTCH1 protein expression was observed in cHCC-CCC compared to HCC (mean H-score: 43.9 vs. 24.0, p<0.01) and CCC (mean H-score: 43.9 vs. 29.4, p<0.05). Similarly, Hes5 expression in cHCC-CCC was substantially greater than in HCC (mean H-score: 52.7 vs. 8.6, p<0.001). A scatter plot analysis of NOTCH1 and Hes5 expression levels in cHCC-CCC revealed three distinct subgroups based on their expression profiles. Group 1 (n=28) was characterized by low levels of NOTCH1 expression (Notch-inactivated). Group 2 (n=5) exhibited elevated expression of both NOTCH1 and its downstream target (Notch-responsive). Group 3 (n=8) demonstrated high NOTCH1 expression but very low levels of Hes5 expression (Notch-non-responsive). Survival analysis indicated that Group 1 and Group 3 cHCC-CCCs had overall survival and progression-free survival (PFS) rates similar to those of HCCs. In contrast, Group 2 (Notch-responsive subtype) exhibited a significantly worse PFS (hazard ratio [HR] 7.708 [95% confidence interval (CI) 0.7807-76.11], p=0.0002), which was comparable to the PFS of CCC (HR 5.355 [95% CI 2.376-12.10], p<0.0001).
Conclusion: The Notch signaling pathway is differentially expressed in cHCC-CCC. Tumors with activated Notch signaling components are associated with poorer prognostic outcomes within the cHCC-CCC cohort.

#194 Angiopoietin-like 4 induces proliferation and migration of head and neck squamous cell carcinoma cells via p38 kinase and NRP1/ABL1/PXN pathway.

Ajay Kumar

Oncology and Diagnostic Sciences, University of Maryland, Baltimore, Baltimore, MD

Background: The molecular mechanisms in which angiopoietin-like 4 (ANGPTL4), a pluripotent protein implicated in cancer development, contribute to head and neck squamous cell carcinoma (HNSCC) growth and dissemination are unclear.
Methods: We analyzed ANGPTL4 expression in human normal oral keratinocytes (NOKs), dysplastic oral keratinocytes (DOKs), oral leukoplakia cells (LEUK1), and HNSCC cell lines, HN13, HN6, HN4, HN12, and CAL27, as well as in tissue biopsies from patients with oral dysplasia, and primary and metastatic HNSCC. We further examined the contribution of ANGPTL4 cancer progression in an HNSCC orthotopic floor-of-mouth tumors model and upstream and downstream signaling pathways linking ANGPTL4 to cancer cell proliferation and migration.
Results: ANGPTL4 expression was upregulated in premalignant DOKs and HNSCC cell lines compared to NOKs as well as in tissue biopsies from patients with oral dysplasia, and primary and metastatic HNSCC. Downregulation of ANGPTL4 expression inhibited primary and metastatic cancer growth in an HNSCC orthotopic tumor model. Interestingly, ANGPTL4 binding to the neuropilin 1 (NRP1) receptor led to phosphorylation of the focal adhesion protein, paxillin (PXN), and tumor cell migration; this was dependent on the tyrosine kinase ABL1. Further, we examined theextracellular signaling associated with ANGPTL4 expression in HNSCC. Intriguingly, amphiregulin (AREG), an epidermal growth factor receptor ligand, induced ANGPTL4 expression in NOKs and DOKs. AREG-induced activation of cell proliferation was dependent on ANGPTL4. We show that the proliferative potential of AREG in HNSCC is, in part, mediated by ANGPTL4. We also observed a role for p38 MAPK as an important downstream effector for the pro-tumorigenic effects ofANGPTL4 and an upregulation of a wide array of proliferative, proangiogenic, and proinflammatory factors upon treatment of NOKs with this adipokine. This suggests that ANGPTL4 may induce HNSCC tumorigenesis through both autocrine and paracrine pathways.
Conclusion: Our findings identify novel opportunities for the development of therapies targeting the ANGPTL4-induced signaling pathways for the treatment of patients with HNSCC.

#195 The transmembrane mucin MUC1 modulates MAPK signaling dynamics in pancreatic cancer cells.

Seth Thesing, **Ryan Lee Hanson**

Biology and Microbiology, South Dakota State University, Brookings, SD

The transmembrane mucin MUC1 plays several roles in the progression of pancreatic ductal adenocarcinoma (PDAC) and is associated with aggressive disease. One function of MUC1 is the regulation of intracellular signal transduction through association with transmembrane receptors like EGFR and Src. While multiple studies have shown that MUC1 can potentiate signaling mitogen activated protein kinases, such as ERK, it's unclear how dynamic this process is in PDAC. Previous studies examining ERK signaling have shown that dynamics can play a major role in the overall response to cells to growth factor signaling. To examine the influence of MUC1 on dynamic signal transduction we established cell lines expressing fluorescent biosensors of mitogen activated protein kinase activity (ERK, JNK, and p38) using cell lines derived from either wildtype KPC tumors or MUC1 knockout tumors. Morphologically, MUC1 expressing KPC cells appeared more mesenchymal consistent with MUC1's roles in mediating migration and invasion of tumor cells. By comparison, MUC1 knockout cells readily formed monolayers with epithelial morphology. Using time-lapse fluorescence microscopy, we examined whether these cells also exhibited differences in MAPK signaling over time and in response to EGF stimulation. At baseline, KPC wildtype cells showed sustained ERK activation consistent with constitutive activation via K-Ras G12D. In comparison, MUC1 KO cells while still exhibiting high levels of ERK activity would also undergo periodic pulses of on and off states suggesting that MUC1 contributes to sustained ERK activation in tumor cells. EGF stimulation was capable of inducing ERK activation in both KPC wildtype and MUC1 KO cells, but as before KPC wildtype cells exhibited sustained activation while MUC1 KO cells pulsed. Given the high constitutive ERK activity in KPC wildtype cells we hypothesized these cells may be more reliant on ERK activation to survive. Interestingly, while treatment with 25 nM trametinib had a stronger inhibitory effect on MUC1 knockout cells, viability assays suggest that MEK inhibition reduces KPC wildtype growth more strongly than in MUC1 KO cells. These findings suggest that the sustained ERK signaling promoted by MUC1 expression may make tumor cells more reliant on ERK signaling and more prone to MEK inhibition.

#196 Acetylcholine enhances HIF-1α expression in pancreatic cancer cells under hypoxia through nAchR-α7/PDPK1/YAP signaling pathway.

Y. Cho, E.-T. Oh, H. Kim, H. Park;
Inha University College of Medicine, Incheon, Korea, Republic of

The pancreas has a high distribution of the autonomic nervous system. The role of the autonomic nervous system in pancreatic ductal adenocarcinoma development has been addressed in recent studies. HIF-1 regulates the expression of genes involved in angiogenesis, metastasis, tumor growth, chemoresistance and radioresistance, underscoring the growing interest in targeting HIF-1 for cancer control. In this study, we investigated the molecular mechanisms underlying the neurotransmitter acetylcholine-enhanced HIF-1α expression in pancreatic cancer cells under hypoxia, leading to the growth of pancreatic cancer organoids. In vitro assays showed that, under hypoxic conditions, acetylcholine caused a concentration-dependent increase in nAChR-α7-mediated HIF-1α expression, enhancing expression of its target genes in pancreatic cancer cells. We further found that acetylcholine increased HIF-1α protein stability in pancreatic cancer cells under hypoxic conditions. This effect was mediated by PDPK1/YAP signaling pathway, as evidenced by the fact that acetylcholine-induced enhancement of HIF-1α expression was blocked by siRNA-mediated knockdown of PDPK1/YAP signaling pathway. Consistent with these findings, an investigation of the relationship between nAChR-α7 and clinicopathological features in pancreatic cancer patients using publicly available datasets showed that high levels of nAChR-α7 are correlated with a poorer prognosis in pancreatic cancer patients. Subsequently, nAChR-α7 specific inhibitor α-bungarotoxin efficiently inhibited the acetylcholine-induced increase of growth of pancreatic cancer organoids under hypoxia. Collectively, these findings identify novel mechanisms by which acetylcholine enhances HIF-1α expression through PDPK1/YAP signaling pathway and provide further evidence that HIF-1α is a potential anticancer target in acetylcholine-associated pancreatic cancer.

#197 Effects of three nucleoside reverse transcriptase inhibitor analogs on Stat3, NFκB, and JNK/c-Jun signaling in bone metastatic prostate cancer.

Wishrawana Sarathi Ratnayake, Luke Lajmi, Sloan Breedy, Aaron Todman, Karimeh Hagani, Kirpal Bisht, Mildred Acevedo-Duncan

Chemistry, University of South Florida, Tampa, FL

Bone metastatic prostate cancer (BMPC) is the primary cause of fatality in patients with prostate cancer, and there is currently no effective treatment available. The role of the bone microenvironment in this process has led to an increased focus on the interactions between cancer cells and the bone microenvironment, particularly with osteoblasts. Our study delved into the mechanisms of interactions between prostate cancer cells (DU-145 and PC-3) and the hFOB 1.19 osteoblast cell line. We are investigating the broader effects of three different nucleoside reverse transcriptase inhibitor (NRTI) analogs, namely KBMD-E, KBMD-G, and KBMD-H, for their potential development as anti-cancer drugs. Our previous findings indicated that aPKC-ι and ζ play a role in the progression and metastasis of prostate cancer and that NRTI analogs were capable of inducing apoptosis and pyroptosis in DU-145 and PC-3 cells while showing less pronounced effects on the hFOB.1.19 cell line. Our findings revealed that osteoblasts play a crucial role in helping prostate cells thrive in the bone microenvironment by producing chemokines that promote metastasis. Current data suggested that all three NRTI analogs negatively affect three oncogenic pathways, Stat3, NFκB, and JNK/c-Jun pathways, thereby inducing apoptosis and diminishing metastatic stimulating signals in DU-145 and PC-3. Transcription factors Stat3, c-Jun, and NFκB seem to be critical in establishing the prostate cancer cells in bones. Also, data suggested that these three pathways work in tandem. Data shows that KBMD-E/G/H upregulated interleukin (IL)-18 while downregulating IL-8 and IL-6. KBMD-E/G/H can be used to disrupt critical steps of these metastatic cascades of prostate cancer cells, which can be targeted to develop customized, tailored therapies for bone metastatic prostate cancer, which merit further research.

#199 Clinicopathological significance of stromal enzymes lysyl oxidase like 1, 3, and 4 in gastric cancer.

H. Kasashima, Y. Fukui, I. Omori, Z. Wang, S. Ozawa, T. Ishidate, K. Yonemitsu, Y. Seki, Y. Miki, M. Yoshii, T. Fukuoka, T. Tamura, M. Shibutani, T. Toyokawa, S. Lee, M. Yashiro, K. Maeda;
Osaka Metropolitan University, Osaka, Japan

[Background and Objective]
The Lysyl oxidase (LOX) family comprises enzymes that physiologically participate in the cross-linking of extracellular matrix components such as collagen and elastin, thereby playing roles in cell differentiation and tissue formation. In the tumor microenvironment, LOX is known to contribute to the remodeling of the cancer stroma and to influence cancer cell metastasis and differentiation. Our previous studies have reported associations between LOX, LOX-like (LOXL)2, and prognosis in gastric cancer. In this study, we investigated the clinicopathological significance of LOXL1, 3, and 4 in gastric cancer through immunohistochemical staining.
[Methods]
Immunohistochemical staining was performed to analyze the relationship between LOXL1, LOXL3, LOXL4 expression and the clinicopathological characteristics of 597 gastric cancer patients. Additionally, the influence of TGF-β on the expression of LOXL1, LOXL3, and LOXL4 was examined in vitro using diffuse-type gastric cancer cell lines (OCUM-2M, OCUM-12, KATO-III), gastric cancer lymph node metastatic cell lines (OCUM-2MLN), and gastric cancer peritoneal dissemination cell lines (OCUM-2MD3).
[Results]
LOXL1, LOXL3, and LOXL4 expression was significantly associated with T-factor, lymph node metastasis, lymphatic invasion, and venous invasion. LOXL1 expression was more common in the intestinal-type histological subtype ($p < 0.001$). Gastric cancer patients positive for LOXL1, LOXL3, or LOXL4 had a lower overall survival rate compared to negative patients ($p < 0.001$ for each). In vitro analysis revealed that LOXL1 mRNA expression was significantly higher in gastric cancer cell lines compared to lymph node metastatic and peritoneal dissemination cell lines, while LOXL3 and LOXL4 were significantly more expressed in metastatic and peritoneal dissemination cell lines. Western blot analysis showed similar results. When TGF-β was added to the gastric cancer cell lines, LOXL1 expression decreased, while LOXL3 and LOXL4 expression increased.
[Discussion]
The expression of LOXL1, LOXL3, and LOXL4 in gastric cancer is suggested to be associated with distant metastasis and poorer prognosis. TGF-β may influence the expression of LOXL1, LOXL3, and LOXL4 and potentially correlate with histological subtypes of gastric cancer.

TUMOR BIOLOGY: Cancer Stem Cells
Poster Session

#2 CAV1-ITGA3 axis attenuates cancer stemness in a preclinical murine model of triple-negative breast cancer.

Naveen Chintala Ramulu[1], Shilpa Thota[1], Rizwana Begum[1], Dhirendra Pratap Singh[2], Biplov Sapkota[1], Dayanidhi Raman[3], Joseph Francis[1]

[1]Comparative Biomedical Sciences, Louisiana State University, Baton Rouge, LA,[2]Krannert Cardiovascular Research Center, Indiana University School of Medicine, Indianapolis, IN,[3]Cell & Cancer Biology, University of Toledo, Toledo, OH

Triple-negative breast cancer (TNBC) is a notoriously aggressive phenotype with enhanced metastatic and relapse potential. Caveolin-1 (CAV1), a structural component of caveolae is linked to multiple processes involved in primary tumor progression and metastasis. Notably, an upregulation of CAV1 expression level enhances the metastatic capability of TNBC. Previous findings from our lab have demonstrated that CRISPR/Cas9-mediated knockout (KO) of CAV1 mitigates BC metastasis to the lungs in a 4T1 mouse model of mammary carcinoma via integrin alpha 3 (ITGA3) dysregulation. Breast cancer is a heterogenous disease with cancer stem-like cells of high plasticity that recapitulate the primary tumor at a distant metastatic site, CAV1-KO 4T1 cells may likely have an impact on regulation of cancer stemness. In order to investigate this, we evaluated one of the cancer stemness markers, ALDH1A1; pluripotency transcription factors (SOX2, OCT4 and NANOG); and drug efflux transporters (ABCB1, ABCC1, and ABCG2). In support of our hypothesis, data from *in vitro* CAV1-KO cells and the *in vivo* CAV1-KO tumors demonstrated a significant downregulation of cancer stemness parameters as compared to control tumors. Based on these findings, we conclude that CAV1-KO may regulate cancer stemness in a 4T1 mouse model of TNBC. Isolation of breast cancer stem cells (BCSCs) based on ALDH1 activity, morphological characterization, and 3D-functional assays will further solidify the role of CAV1 in cancer stemness and its invasive potential. Overall, we identified a novel role for CAV1 in regulating cancer stemness. CAV1-based targeted therapy has tremendous potential to augment treatment outcomes for TNBC.
Keywords: Triple-negative breast cancer, Caveolin-1, and breast cancer stem-like cells.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Cell Signaling and Tumor-Stroma Cell Interactions
Poster Session

#200 Role of transforming growth factor beta signaling in mediating cancer-stromal interaction and its inhibition by apigenin in ovarian cancer.

Hyunjin Seo, Soo-Jung Lee, Somin Lee, **Youngjoo Kwon**

Ewha Womans University, Seoul, Korea, Republic of

Tumor microenvironment (TME) plays crucial roles in regulating tumor progression. Fibroblasts as predominant component in the TME have shown to promote growth of cancer cells. Transforming growth factor beta (TGF-β) regulates many cellular functions, including cell growth, differentiation, and apoptosis in a context-dependent manner. Growing evidence suggests that TGF-β mediates interaction between cancer cells and their associated fibroblasts (CAFs) to facilitate malignant progression. Polyphenols are widely present in plant-based foods and have diverse health promoting effects via modulating multiple cellular signaling pathways in various cell types. The present study investigated if growth-stimulating tumor-stromal interaction is mediated by TGF-β derived from ovarian CAFs and if food polyphenols inhibit this interaction. Ovarian cancer cells, OVCAR3 and OVCAR4, were cultured in conditioned media (CM) derived from CAFs (CAF-CON) in the presence and absence of SB525334 (an inhibitor of TGF-β receptor 1) to test the involvement of TGF-β in CAF-CON-induced cell growth. Effects of mitogen-activated protein kinase and AKT pathways on TGF-β signaling pathways were examined using their specific inhibitors. Various food polyphenols (5 μM) were treated to CAFs while preparing CM and evaluated their effects on TGF-β1 secretion and cancer cell growth induced by CAFs. Further, the effect of apigenin (API), the most effective compound, on TGF-β signaling pathway was evaluated using immunoblotting. OVCAR3 cells were transplanted into SCID mice. Once tumors were palpable, mice were either treated with vehicle control (VEH) or API (2.5 mg/kg bw/day) by oral gavage while concentrated CAF-CON, CM derived from API-treated CAFs (CAF-API), or phosphate buffered saline were injected onto formed tumors. Treatment with SB525334 effectively inhibited CAF-CON-induced growth increase in both OVCAR3 and OVCAR4 cells. Notably, inhibition of p38 activity suppressed CAF-CON-induced activation of Smad3 and AKT in ovarian cancer cells, suggesting that p38 activation induced by CAF-CON enhances TGF-β1 and AKT signaling pathways. Among various CM obtained from CAFs treated with diverse polyphenols, CAF-API exhibited the greatest inhibitory effect on cell proliferation in both OVCAR3 and OVCAR4 cells (> 50% reduction relative to CAF-CON) and the lowest TGF-β1 levels (<40% reduction relative to CAF-CON). Smad activation was induced in the presence of CAF-CON, whereas it was reduced in CAF-API. OVCAR3-derived tumor growth was significantly increased by treatment with CAF-CON (40% relative to VEH) but not with CAF-API or CAF-CON with simultaneous oral administration of API. Therefore, the present study suggests that TGF-β1 contributes to interplay between ovarian cancer cells and their stroma, and API can inhibit this interaction.

#201 Enhanced motility of cancer-associated fibroblasts is mediated by HIF-1α signaling in the lung tumor microenvironment.

Jihye Park[1], Sieun Lee[1], Jonathan M. Kurie[2], Young-Ho Ahn[1]

[1]Department of Molecular Medicine, Ewha Womans University College of Medicine, Seoul, Korea, Republic of,[2]Department of Thoracic/Head and Neck Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, TX

Cancer-associated fibroblasts (CAFs) are key contributors to promote cancer progression in the lung tumor microenvironment via interactions with cancer cells and other cell types. In a 3-D culture system, we classified CAFs into two subgroups based on their motility, motile and static CAFs, and isolated these subgroups using the photoconvertible fluorescent protein Dendra2. RNA sequencing and subsequent analyses revealed the upregulation of hypoxia- and glycolysis-related genes in motile CAFs. Activation of HIF-1α increased CAF motility in both mono- and co-culture systems. Knockdown of HIF-1α downstream genes, ALDOA and LDHA, reduced CAF motility. Interestingly, HIF-1α activity and CAF motility were heightened when co-cultured with mesenchymal-like lung cancer cells. Mass spectrometry analysis identified ceruloplasmin (CP) as a secretory protein derived from mesenchymal-like lung cancer cells. Knockdown of CP in mesenchymal-like lung cancer cells attenuated CAF motility in co-culture systems and lung tumor metastasis in a mouse model. Collectively, these findings demonstrated that CAF motility is regulated by HIF-1α signaling, which is modulated by lung cancer cell-derived CP.

#203 TCF12 regulates PD-L1 expression through TGF-beta signaling and modulates the immune microenvironment in micropapillary lung cancer.

Yifei Liu[1], Ting Wang[2], Tingting Bian[1], Jia Feng[1], Qiushi Lin[3], Xiaoqun Dong[3]

[1]Affiliated Hospital of Nantong University, Nantong, China,[2]Radiation Oncology, The Affiliated Cancer Hospital of Zhengzhou University & Henan Cancer Hospital, Zhengzhou, China,[3]Precision Health Program, College of Human Medicine, Michigan State University, Lansing, MI

Background: The prognosis of lung adenocarcinoma (LUAD) is strongly influenced by its histologic growth patterns, with the micropapillary component (MPC) being a poor prognostic indicator. However, the molecular mechanisms driving the poor prognosis of the MP subtype remain unclear, particularly regarding spatial transcriptomic (ST) insights.
Methods: Spatial transcriptomics (10X Genomics) was performed on 4 LUAD cases to map gene expression. Targeted next-generation sequencing (NGS) on 72 LUAD samples evaluated genetic alterations. The roles of TCF12 and its regulation via the TGF-β pathway were explored through in vitro and in vivo studies, including CCK-8 assays for cell proliferation, Transwell migration and invasion assays, and sphere formation assays to assess tumorigenic potential. Western blotting and PCR were used to analyze protein and gene expression levels, respectively. Immunohistochemistry of 160 LUAD samples validated TCF12 and PD-L1 expression correlations. Furthermore, mouse xenograft tumor models were employed to verify the effects of TCF12 on tumor growth and its interaction with the TGF-β pathway and PD-L1 expression in vivo.
Results: The first spatial transcriptomic map of LUAD revealed significant spatial heterogeneity, with the MP pattern strongly linked to TGF-β signaling and immune responses. TCF12 was highly expressed in MP regions, activating TGF-β signaling and upregulating PD-L1 expression. Functional assays demonstrated that TCF12 overexpression promoted proliferation, invasion, and angiogenesis in LUAD, which were suppressed by TGF-βRI kinase inhibition. In animal models, dual inhibition of TGF-β and PD-L1 significantly reduced tumor growth in TCF12-overexpressing tumors compared to single-drug and control groups. Immunohistochemistry showed that increased TCF12 expression correlated with poor survival, lymph node metastasis, and MP pattern presence.
Conclusions: TCF12 drives PD-L1 expression via TGF-β signaling, fostering an immunosuppressive microenvironment in LUAD. Dual inhibition of TGF-β and PD-L1 restores immune function, offering a promising strategy for MP-LUAD precision therapy.
Keywords: TCF12, Spatial transcriptomics, TGF-β signaling, PD-L1, Lung adenocarcinoma, Micropapillary component

#204 Targeting tumor-stroma interactions: Prognostic and therapeutic implications of the ACVR1-BMP7 axis in colorectal cancer.

Norikatsu Miyoshi[1], Shinya Kato[2], Shiki Fujino[2], Soichiro Minami[2], Rie Mizumoto[2], Rie Hayashi[2], Kengo Haruma[2]

[1]Gastroenterological Surgery, Osaka University, Osaka, Japan,[2]Innovative Oncology Research and Regenerative Medicine, Osaka International Cancer Institute, Osaka, Japan

The tumor microenvironment plays a critical role in colorectal cancer (CRC) progression, with stromal signatures closely linked to poor prognoses and resistance to chemotherapy. These stromal factors significantly influence tumor dynamics, promoting progression, relapse, and overall worse outcomes. Among these, cancer-associated fibroblasts (CAFs) have gained attention due to their heterogeneity and functional contributions to the tumor microenvironment. Although single-cell analysis has identified distinct CAF subpopulations, molecular-targeted therapies against CAFs remain lacking, highlighting a critical clinical need. To address this, the study utilized organoid culture methods replicating CRC histological architecture. Two-dimensional organoids (2DO) provided a novel platform to study tumor-stromal interactions, facilitating the investigation of cancer-fibroblast crosstalk. The focus was on signaling pathways, specifically the interplay between Activin receptor type I (ACVR1) in fibroblasts and Bone morphogenetic protein 7 (BMP7) in cancer cells, as potential therapeutic targets. Using single-cell analysis and patient-derived models, the study examined the prognostic significance of ACVR1 and BMP7 expression in CRC, especially in stage II patients. Single-cell RNA sequencing identified fibroblast subpopulations, revealing a previously unexplored BMP7-ACVR1 signaling axis. High BMP7 and ACVR1 expression levels were significantly associated with poorer disease-free survival (DFS) and overall survival (OS), emphasizing their prognostic value. Clinical data from stage II patients supported this finding, linking elevated BMP7 and ACVR1 levels to adverse outcomes. The functional role of the BMP7-ACVR1 axis was validated with a 2DO-based mouse xenograft model, which reflected the stromal heterogeneity of human CRC tumors. Tumor growth was significantly inhibited by administering an ACVR1 inhibitor during 2DO-mouse stromal cell co-culture, highlighting the therapeutic potential of targeting ACVR1. This underscores the critical role of CAFs in tumor progression and their potential as therapeutic targets. In conclusion, the study identifies ACVR1 as a promising therapeutic target regulating CAF proliferation, influencing tumor growth and patient outcomes. The prognostic significance of BMP7 and ACVR1 further underscores their potential as biomarkers in stage II CRC.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Comprehensive Multiomic Profiling of Chromatin Dynamics, DNA Methylation, and Tumor Microenvironments
Poster Session

#207 snNOMeHiC: joint profiling of 3D genome, DNA methylation, and chromatin accessibility from the same nuclei at flash-frozen tissue and primary tumor sample.

Hailu Fu[1], Jiayan Ma[1], Eric Tong[1], Mark Youngblood[2], Harrshavasan Congivaram[2], Mateo Gomez[1], Juan Wang[1], Feng Yue[1], Li Wang[1], Yaping Liu[1]

[1]Department of Biochemistry and Molecular Genetics, Northwestern University - Chicago, Chicago, IL,[2]Department of Neurological Surgery, Northwestern University - Chicago, Chicago, IL

Epigenetic marks, including DNA methylation, chromatin accessibility, and three-dimensional (3D) genome organization, play instrumental roles in gene regulation in mammals. Gene activation or repression potential, however, cannot be entirely predicted by looking at a single modality. Accurate predictive models require multiple modalities simultaneously. We previously developed single-cell Methyl-HiC technology to jointly profile DNA methylation and 3D genome from the same cells. Further, we incorporated an additional footprint of GpC methyltransferase activity (i.e., chromatin accessibility) into the assay, named NOMe-HiC, but at the bulk level. Here, we extended NOMe-HiC technology to the single-cell level to enable the joint profiling of the 3D genome, DNA methylation, and chromatin accessibility from the same nuclei. We performed snNOMe-HiC at IMR-90 cell lines and found high concordance across all three modalities with the bulk NOMe-HiC by pooling the single cells. We further prepared a mixture of GM12878 and IMR-90 cells and processed the cell mixture with snNOMe-HiC. snNOMe-HiC can distinguish the two cell types using each of the three epigenetic features, respectively. To benchmark the performance of snNOMe-HiC at flash-frozen heterogeneous tissues, we generated snNOMe-HiC data from ~2000 nuclei at flash-frozen mice cortex. We identified all the major known cell types in the previous studies across three modalities and showed reproducible results across replicates. Finally, we applied snNOMe-HiC to a primary meningioma sample. We identified previously known copy number alterations in tumor-related cell clusters and found concordant changes of epigenetic heterogeneity across cell populations, which can potentially reveal the crosstalk between genetics and multiple epigenetic marks during the clonal evolution in cancer progression. In summary, snNOMe-HiC is a robust technology that can profile three epigenetic modalities and genetic aberrations in the same nuclei from flash-frozen tissues and primary tumors.

#208 Epigenomic characterization of keratinocyte carcinomas in xeroderma pigmentosum.

Bea Riebesehl[1], Lea S. Beensen[1], Felix Bormann[2], Wilfried Roth[3], Carlos F. M. Menck[4], Thomas G. Hofmann[3], Frank Lyko[1], **Manuel Rodriguez Paredes**[1]

[1]German Cancer Research Center, Heidelberg, Germany,[2]Bioinformatics.Expert UG, Berlin, Germany,[3]Johannes Gutenberg University, Mainz, Germany,[4]University of Sao Paulo, Sao Paulo, Brazil

Keratinocyte carcinomas (KCs), comprising basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (cSCC), arise in the epidermis due to chronic exposure to solar ultraviolet (UV) radiation. KCs account for 99% of skin neoplasms and represent the most prevalent form of cancer worldwide. DNA methylation, an epigenetic modification of CpG dinucleotides, modulates gene expression, thereby defining cell identity and mediating cellular responses to external stimuli. Aging and environmental stressors, including UV radiation, progressively alter epidermal methylomes, thus influencing KC development and progression. Xeroderma pigmentosum (XP) is a rare genetic disorder characterized by mutations in genes of major cellular response mechanisms to UV-induced DNA damage, such as the nucleotide excision repair (NER) system or the translesion synthesis (TLS) DNA polymerase η. Consequently, XP patients exhibit a more than 10,000-fold increase in KC incidence, with tumors potentially manifesting as early as childhood. Epigenomic analysis of tumors derived from XP patients could provide valuable insights into the deregulation of DNA methylation in KC tumorigenesis, thereby contributing to the development of novel antitumor therapies for both XP patients and the broader population. Here, we used *Infinium* MethylationEPIC BeadChips to assess the DNA methylation status of 30 epidermal samples from XP patients with mutations in the *XPC* and *POLH* genes, representing roughly 50% of all XP cases and significantly impacted by KCs. Our sample set comprised BCC and cSCC tumors, non-tumorous epidermis, and actinic keratosis (AK), a precancerous lesion that frequently progresses to cSCC. DNA methylation analysis of XP samples clearly distinguished between BCC and cSCC tumors, whereas non-tumorous epidermis unexpectedly clustered with AK samples, reflecting substantial methylome dysregulation that is consistent with the heightened risk of KC development in XP patients. In line with this, further examination of non-tumorous XP methylomes relative to non-XP counterparts revealed features indicative of premature aging, including significant erosion of DNA methylation profiles and increased mitotic rates. Analysis of XP-derived BCC and cSCC methylation profiles also identified a higher frequency of copy number variation (CNV) events and a potential bias towards an epidermal stem cell (EpSC)-like cell-of-origin in cSCC tumors. These findings may suggest an elevated aggressiveness of XP tumors, which we now aim to verify using cellular models of KC deficient in key XP genes.

#209 Differentially expressed genes associated with disease-specific chromatin changes in oral cancer have a significant correlation with overall survival.

Diane Terry[1], Andrey Loginov[1], Jennifer Coleman[2], Amy Plotkin[2], Ashley Johnson[2], Carol Robles[2], Joshua Lubek[3], Donita Dyalram[3], Kyle Hatten[3], Daria Gaykalova[4]

[1]University of Maryland School of Medicine, Baltimore, MD,[2]Pathology and Biorepository Shared Service, Marlene & Stewart Greenebaum Comprehensive Cancer Center, University of Maryland Medical Center, Baltimore, MD,[3]Otorhinolaryngology, Head and Neck Surgery, Marlene & Stewart Greenebaum Comprehensive Cancer Center, University of Maryland Medical Center, Baltimore, MD,[4]IGS, University of Maryland School of Medicine, Baltimore, MD

Globally, there are ~400,000 Oral Squamous Cell Carcinoma (OSCC) cases diagnosed each year and ~200,000 deaths. The 5-year survival rate for OSCC is only ~50%, and this has not improved in decades. In other cancers, recent studies have shown that chromatin structure changes, impacting gene regulatory regions known as enhancers, can play a key role in gene expression dysregulation. Enhancers, with their transcription factor binding sites, attract transcription factors that, along with coactivators, assemble the transcription machinery. Super-enhancers, dense clusters of enhancers, amplify this assembly. The addition of coactivators like histone acetyltransferases and methyltransferases remodels chromatin, affecting DNA accessibility. Research has not been done using primary OSCC tissues. I propose that in OSCC, specific aberrations in chromatin organization lead to alterations in gene expression associated with cancer. Furthermore, I hypothesize that these chromatin anomalies are not random but concentrated at certain genomic loci, which act as 'hotspots' for regulatory disruption. Chromatin immunoprecipitation sequencing (ChIP-seq) for the active enhancer marker H3K27ac was performed using 20 pairs of OSCC-tongue patient matched tumor and normal tissues. From the H3K27ac ChIP-seq data, disease-specific enhancers and super-enhancers, which are present either only in tumor or normal chromatin, were identified. There were 22,336 “hot spots” found where the disease-specific change is in more than one patient. To identify differentially expressed genes (DEGs) regulated by those (super)enhancers, RNA extracted from these tissues was submitted for sequencing (RNA-seq) and analyzed. DEGs that are within the regulatory range of disease-specific enhancers and super-enhancers were identified and evaluated for their impact on OSCC. Tumor-specific enhancers cause upregulation of the target gene, whereas normal-specific enhancers that are absent in the tumor are associated with a gene downregulated in the tumor. Many DEGs in the regulatory range of disease-specific enhancers and super-enhancers were found to have a significant association with overall survival. Such elucidation of the role of chromatin structural alterations in OSCC paves the way for a deeper comprehension of gene expression dysregulation and heralds a new frontier in the development of innovative therapeutic strategies.

#210 Direct multiomic mapping of chromatin proteins and DNA methylation.

Jen Spengler[1], Bryan J. Venters[1], Vishnu U. Sunitha Kumary[1], Allison Hickman[1], Anup Vaidya[1], Jonathan M. Burg[1], Louise Williams[2], Louise Williams[2], Chaithanya Ponnaluri[2], Pierre-Olivier Esteve[2], Issac Meek[2], Sriharsa Pradhan[2], Zu-Wen Sun[1], Martis W. Cowles[1], Michael-Christopher Keogh[1]

[1]Epicypher, Durham, NC,[2]New England Biolabs, Ipswich, MA

DNA methylation (DNAme) is a class of epigenetic marks that includes the methylation of cytosine residues (5mC) within CpG islands. In addition to well characterized roles regulating gene expression, imprinting, and silencing parasitic DNA elements, misregulation of DNAme is implicated in multiple cancers. Evidence is emerging that DNAme is not an independent epigenetic mark but rather closely linked to chromatin proteins. However, examining the relationships between DNAme and chromatin proteins is hampered by correlative analyses that cannot establish a direct mechanistic link. Furthermore, the traditional approach to measure DNAme relies upon harsh bisulfite chemical conversion, which introduces DNA breaks and systemic biases. To address these limitations, we developed CUT&RUN-EM: an ultra-sensitive multiomic technology that integrates EpiCypher's CUT&RUN genomic mapping with New England Biolab's Enzymatic Methyl-seq (EM-seq). Together, these technologies permit high-resolution, simultaneous profiling of chromatin proteins and DNAme, enabling direct interrogation of the complex molecular crosstalk between these genomic features. To quantify the capabilities and limitations of the assay, we tested CUT&RUN-EM across chromatin proteins and cell lines. We show that CUT&RUN-EM is highly reproducible, specific, sensitive, and efficient, requiring as few as 5 million short-read sequences per reaction (10-100x less than whole-genome DNAme sequencing). The advent of CUT&RUN-EM will enable detailed interrogation of the cancer epigenome, providing insights into new therapeutic targets.

#211 Epstein-Barr virus (EBV)–human host chromatin interactions rearrange spatial DNA genome organisation and hijack KDM5B to distant metastasis proneness in EBV-associated nasopharyngeal carcinoma (NPC).

Lai Shun Dittman Chung[1], Zhaozheng Hou[1], Ying Wang[2], Kazi Anisha Islam[1], Songran Liu[1], Jiayan Liu[3], Larry Ka-Yue Chow[1], Cyrus Pakk-Ting Chak[1], Yingpei Zhang[1], Lanqi Gong[1], Karl Kui-Wai Cheng[1], Zhuoyou Yu[1], Ping Feng[4], Zilu Huang[4], Roger Kai-Cheong Ngan[1], Xinyuan Guan[1], Wai-Tong Ng[1], Zhonghua Liu[5], Anna Chi-Man Tsang[1], Dora Lai-Wan Kwong[1], Annie Wing-Mui Lee[1], Victor Ho-Fun Lee[1], Honglin Chen[3], Yunfei Xia[4], Wei Dai[1]

[1]Department of Clinical Oncology, School of Clinical Medicine, LKS Faculty of Medicine, Hong Kong, Hong Kong,[2]Department of Radiation Oncology, Sun Yat-sen University Cancer Centre, Guangzhou, PR China, Guangzhou, China,[3]Department of Microbiology, University of Hong Kong, Hong Kong (SAR), PR China, Hong Kong, Hong Kong,[4]Department of Radiation Oncology, Sun Yat-sen University Cancer Centre, Guangzhou, PR China, Hong Kong, Hong Kong,[5]Department of Biostatistics, Columbia University, New York, NY, USA., Hong Kong, NY

Introduction: Epstein-Barr virus (EBV), the first identified human DNA tumour virus, has been detected in nasopharyngeal carcinoma (NPC) over 40 years and over 90% of NPC is strongly associated with EBV infection in the endemic regions. However, the detailed roles of EBV in NPC pathogenesis and disease progression remain inconclusive. We hypothesise that EBV–human chromatin interactions induce the spatial reorganisation of compartments and chromatin loops facilitating NPC tumour progression.
Methods: Chromosome conformation capture sequencing (Hi-C), transposase-accessible chromatin with sequencing (ATAC-seq), and Cleavage Under Targets and Release Using Nuclease with sequencing (CUT&RUN-seq) were performed in EBV-positive (EBV+) and EBV-negative NPC cell lines, EBV+ patient-derived xenografts (PDXs) and EBV+ clinical samples, followed by single-cell RNA sequencing (scRNA-seq), GeoMx Digital Spatial Profiler, and Spatial Molecular Imaging platform using the clinical samples. To evaluate the clinical impact of the high-order epigenomic changes associated with EBV, whole exome sequencing (WES) and bulk RNA sequencing (RNA-seq) were conducted for NPC biopsies in the multi-center multi-omics study.
Results: EBV modulates 3D human genome by cross-species chromatin interactions. Two paired NPC cell lines demonstrated EBV infection causes 3D genome reorganisation. The PDXs and one clinical sample showed distinctive compartmentalisation compared to normal epithelial (NPE) cells. We discovered that the EBV episomes could tether to the human genome through EBV-interacting regions (EBVIRs), particularly related to inactive-to-active compartment transition. These regions have shown an increase in H3K27-acetylation, suggesting enhancer infestation by the EBV-host chromatin interactions, resulting in aberrant upregulation of KDM5B and its targets (EBVIR-enhancer-KDM5B signature). The spatial transcriptome data revealed that the cancer cells with higher expression of EBV latent genes had increased EBVIR-enahncer-KDM5B signature with metastatic potential. Similarly, the bulk RNA-seq data indicated this signature was highly associated with development of distant metastasis in two independent patient cohorts. The combination of KDM5B pathway activity with plasma EBV DNA level at baseline or after treatment suggests this can be a promising predictor of metastatic risk, achieving AUC = 0.77 and AUC = 0.82, respectively.
Conclusion: EBV, as episomes, tethers to the human genome to reorganise 3D genome and epigenome. Through this mechanism, it regulates cancer-related genes and promotes distant metastasis in NPC. Our discovery of EBVIR-enhancer-KDM5B signature can be used as a promising metastatic predictor in the future.

#212 Disruption of the menin-MLL complex impedes HCC maintenance and increases sensitivity to NF-Y loss.

Margarita M. Dzama, Peyton C. Kuhlers, Jesse R. Raab

UNC Chapel Hill, Chapel Hill, NC

Most patients with hepatocellular carcinoma (HCC) are diagnosed at advanced stages, and there are only a few moderately effective systemic therapies available. Despite the decreased mortality rate for most cancer types over the last decade, liver cancer, with HCC being the prevailing type (~90%), showed an increase, making this disease the third most common cancer-related cause of death. Since ~50% liver cancer samples carry mutations in epigenetic modifiers and the imbalance in the transcriptional landscape can be potentially restored using epigenetic drugs, we focused on identifying new therapeutic targets of HCC among chromatin modifiers and understanding their role in HCC maintenance. Using our epigenome-focused CRISPR library of 6000 gRNAs targeting 737 chromatin-related genes, we performed screenings in two- (2D) and three-dimensional (3D) cultures using multiple HCC cell lines (HLF and PLC/PRF5). Our screen identified several potential new targets essential for HCC survival, including several core subunits of the H3K4me3-mediating menin-MLL complex (*MEN1*, *ASH2L*, *KMT2A*). We validated HCC dependency on these targets by performing a negative selection CRISPR/Cas9 competitive growth assay in HLF and PLC/PRF5 cell lines. Moreover, treatment of HCC cell lines with the next-generation menin inhibitor SNDX5613 in 2D and 3D settings revealed a dose-dependent reduction in cell proliferation. We assessed the changes in the chromatin binding profile of menin (*MEN1*) and MLL (*KMT2A*) as well as changes in H3K4me3 levels upon treatment with 5μM SNDX5613 by CUT&RUN. While menin peaks showed a ~90% reduction upon treatment, MLL and H3K4me3 peaks exhibited a ~3- and ~2-fold reduction, respectively. Next, we evaluated transcriptional changes associated with disrupting the menin-MLL complex by performing RNA sequencing of HCC cells following either *MEN1* gene knockout or treatment with 5μM SNDX5613. Integrated CUT&RUN and RNA-seq data revealed genes involved in the PI3K/AKT signaling pathway, whose independent knockouts affected HCC survival. ATAC sequencing of HLF cells treated with 5μM SNDX5613 revealed a connection between closed genomic sites and loss of menin-MLL complex binding, while newly accessible chromatin was enriched for NF-Y transcriptional factor motifs and were independent of menin binding. Loss of *NFYA* or *NFYB* significantly affected cell growth, supporting their role in HCC survival. The combined *NFYB* knockout and SNDX5613 treatment showed a significantly decreased HCC cell proliferation compared to a single menin inhibition. We propose inhibition of the menin-MLL interaction disrupts HCC homeostasis through changes in the expression of direct target genes within the PI3K/AKT pathway and alters chromatin accessibility, creating sensitivity to *NFYB* gene loss. We believe targeting core subunits of the menin-MLL complex represents an appealing therapeutic strategy for HCC treatment.

#213 Epigenomic dissection of histology-specific drivers of testicular germ cell tumors.

Esme Wheeler[1], Alok K. Tewari[1], Anis A. Hamid[2], Henry Long[1], Christopher Sweeney[1], Myles Brown[1], Eliezer Van Allen[1]

[1]Dana-Farber Cancer Institute, Boston, MA,[2]Memorial Sloan Kettering Cancer Center, New York, NY

Outcomes of testicular germ cell tumors (TGCT), which are the most common malignancies in adolescents and young adult males, are paradoxical; although highly curable with platinum based chemotherapy, testicular cancer is also the cause of the highest number of years of life lost of any adult malignancy. Major barriers to research aiming to uncover novel therapeutic approaches for chemotherapy-resistant TGCTs include its relatively low incidence, histologic diversity (seminoma vs multiple non-seminoma histologies), and the rarity of tissue biopsies in the metastatic setting. As epigenomic alterations play a key role in germ cell development, and there is a paucity of genomic alterations associated with the initiation and development of therapeutic resistance in TGCT, we hypothesized that epigenomic analysis integrated with transcriptomics in a cohort of pre- and post-treatment TGCTs may reveal novel targetable pathways in TGCT. We collected a cohort of 24 TGCT patients with multiple pre- and post-treatment formalin fixed paraffin embedded (FFPE) tissue, and macrodissected histology-specific tumor foci from surrounding stroma. This tissue was subjected to chromatin-immunoprecipitation (ChIP-seq) of the H3K27Ac enhancer chromatin mark and transcriptional profiling (RNA-seq). Histology-specific cell lines were subjected to the same molecular assays. As an initial analysis, we compared differential enhancer marks (ChIP-seq peaks) and transcripts between specific histologies to identify putative drivers of tumor phenotype and tested the function of these candidate drivers in cell lines. Of 52 histology-specific foci dissected from FFPE slides, we generated matched H3K27Ac and RNA data from 19 samples, H3K27Ac data only from 15 samples including two choriocarcinomas, and RNA only data from 3 samples. Principal component analysis demonstrated that epigenomic and transcriptomic profiles clustered by histology rather than by patient. Superenhancer and motif analysis of enhancer marks suggested various histology-specific transcriptional drivers including POU5F1 and NANOG in EC and SEM, E2F in SEM, AP-1 in TER, the HNF and WNT factors in YST, and GRHL2 in choriocarcinoma. A CRISPR-knockout screen in EC cells in the presence of cisplatin and etoposide indicated that POU5F1 and NANOG promote chemotherapy sensitivity in EC cells. Integrated chromatin and transcriptional analyses suggested multiple potential up- and down-stream effectors of WNT signaling in YST, including multiple extracellular matrix proteins. Collectively, our chromatin, transcriptomic and CRISPR assays in TGCT samples have nominated multiple histology-specific drivers of tumor phenotype. Further analysis of pre- vs post- treatment samples and broader validation of these findings is ongoing.

#214 Chromatin profiling from formalin-fixed paraffin-embedded samples for precision oncology.

Ellen N. Weinzapfel[1], Alysha E. Simmons[1], Eva Brill[1], Vishnu U. Sunitha Kumary[1], Eva Oswald[2], Georg Kuales[2], Julia B. Schueler[2], Martis W. Cowles[1], Bryan J. Venters[1], Michael-Christopher Keogh[1]

[1]EpiCypher, Durham, NC,[2]Charles River Laboratories, Inc., Freiburg, Germany

As the oncology field moves towards a precision medicine model of patient care, understanding and profiling the epigenetic landscape is increasingly important. Gene expression and cell function are regulated by epigenomic features, including histone post-translational modifications (PTMs), transcription factors, and other chromatin proteins. Mapping the location of these features provides a powerful approach to study chromatin mechanisms driving disease and can be leveraged for biomarker and drug development.

Formalin-fixed paraffin-embedded (FFPE) tissues banked from cancer clinical trials could be a rich resource for retrospective biomarker studies, due to their association with drug response and disease progression data. However, using FFPE tissues for chromatin mapping assays has been technically challenging for multiple reasons. For instance, the heavy cross-linking conditions make FFPE samples unsuitable for chromatin accessibility profiling (ATAC-seq), while the small amounts of chromatin extracted from FFPE tissues make standard epigenomic mapping assays (ChIP-seq) impractical. Combined, these difficulties have precluded widespread application of FFPE tissues for epigenomics research.

To better leverage chromatin profiling for translational research, we are developing a modified CUT&Tag workflow that is compatible with FFPE samples (CUT&Tag-FFPE). CUT&Tag (Cleavage Under Targets and Tagmentation) is an immunotethering-based strategy that uses pAG-Tn5 to selectively cleave and ligate sequencing adapters at antibody-bound chromatin. The entire assay takes place in intact cells or nuclei bound to a solid support, enabling a highly streamlined, 2-day workflow. Compared to ChIP-seq, CUT&Tag requires 10-1,000X fewer cells, 5-10X reduced sequencing reads, and is scalable for large studies. The improved sensitivity of CUT&Tag makes it an attractive technique in fields limited by cell number, particularly preclinical research studies.

Here, we will discuss optimization of upstream sample processing steps for efficient extraction of nuclei from heavily fixed cells and FFPE tissue. We have applied these modifications to successfully map RNA polymerase II (RNAPII) in heavily fixed cell lines, resulting in high quality chromatin profiles comparable to native controls. We are currently applying this workflow to map RNA Pol II in FFPE liver tissue samples. Future work will be done to validate additional chromatin targets using matched FFPE and fresh frozen tissue samples from various tissues. In parallel, we are optimizing workflows to pair with downstream single cell and spatial platforms to expand the impact of this technology. We envision that CUT&Tag-FFPE, and the associated automated assay service we are developing for large scale clinical trials, will accelerate the discovery of epigenetic drug targets and biomarkers to advance precision medicine.

#215 A novel framework for deciphering 3D genome structure in colorectal cancer.

Amin Nooranikhojasteh, Jakob Zerbs, Ghazaleh Tavallaee, Elias Orouji

Princess Margaret Cancer Centre, University Health Network (UHN), Toronto, ON, Canada

Background: The spatial organization of chromatin is a fundamental determinant of epigenomic landscape which is widely investigated using high-throughput chromosome conformation capture (HiC) technique. To address challenges associated with the resolution and variability of HiC technique, we applied a novel workflow, HiC-ECC (HiC Enhance, Compare, and Call). This approach not only enhances HiC data quality but also enables the identification of biologically significant chromatin interactions and genome structural features. By integrating advanced computational tools with a focus on colorectal cancer (CRC) biology, this study sheds light on the 3D genome architecture of CRC organoids and its implications for patient stratification and therapeutic targeting.
Methods: We applied the HiC-ECC workflow to a cohort of 15 patient-derived CRC organoid models, including samples derived from primary tumors and metastatic sites in the lung and liver. The workflow involved initial data preprocessing to generate high-quality chromatin contact matrices and ensure rigorous quality control. To enhance the resolution of HiC datasets, we utilized deep learning-based methods, enabling more accurate identification of chromatin interactions. Comparative analyses were conducted to identify differential chromatin interaction patterns and conserved structural features across the cohort. Chromatin architecture was further characterized by delineating topologically associating domains (TADs) and clustering them to reveal common and sample-specific interaction networks, offering insights into the hierarchical organization of 3D genome structures.
Results: Our analysis revealed four distinct subtypes of CRC organoids based on their 3D genome architecture, revealing a novel approach to interrogate heterogeneity within CRC. This subtyping was driven by differential chromatin interaction patterns and structural features, including unique TADs and conserved regulatory elements. Comparative analysis highlighted subtype-specific chromatin interaction networks, suggesting distinct epigenomic landscapes that correlate with tumor progression and metastatic potential.
Conclusion: This study enhances our understanding of CRC epigenomics and lays the groundwork for integrating chromatin architecture into patient stratification and precision therapy.

#216 CRISPR screen validates RIC-seq revealed eRNA-uaRNA interactions and transcriptome changes in gastric cancer.

Xiangnan Zhang, Yanxin Yao, Hongji Dai, Wei Wang, **Kexin Chen**, Ben Liu

Tianjin Medical University Cancer Institute and Hospital, Tianjin, China

Introduction: Enhancer RNA (eRNA) is generated by the transcription of activated enhancers and belongs to non-coding RNA, regulating epigenetic activities and significantly affecting the transcription and activating target genes. We use RIC-seq (RNA in situ conformation sequencing) to simultaneously identify RNA-RNA interactions and loop structure between enhancers and promoters in the genome. RIC-seq also characterizes hubRNAs, which are transcribed from motifs similar to hubs that interact with globally distinct RNA fragments and transcriptionally robust and structurally conserved.

Methods: We performed RIC-seq analysis of GC cell lines and normal gastric mucosa cell lines to capture RNA high-level structures, and identify sites of molecular enhancer RNA-uaRNA (promoter region transcribed RNAs) interactions and annotation information at the cellular in situ level. Several CRISPR libraries, including CRISPRa/i and CRISPR-Cas13 libraries were designed to validate these interactions in two GC cell lines. CROP-seq was used to identify transcriptome-level expression patterns generated from eRNA or uaRNA changes.

Results: Based on RIC-seq, we identified direct or RBP-mediated indirect RNA-RNA proximity interactions and global enhancer-promoter loops among GC chromatin structure and specific spatial contacts. We found 88,696 significant pairs of RNA-RNA interactions in the GC cell line, while 96,990 significant pairs in the normal gastric mucosa cell line. Then, 831 pairs of RNA-RNA interactions and 146 eRNA-uaRNA interactions showed significantly differential expression between such two cell lines. All hubRNAs matched by RIC-seq were categorized and demonstrated that hubRNAs were mainly concentrated in protein-coding RNAs, antisense RNAs, and lincRNAs. From all these differentially expressed interactions, we constructed CRISPRa/i libraries using 10 relatively high expressed eRNAs and 116 target genes corresponding to uaRNAs respectively. Three hubRNAs and thirty-eight eRNAs were selected to construct CRISPR-Cas13 library for further validation of interested interactions. We found and validated eRNAs and target genes which were significantly related to GC cell proliferation, and resistance of target genes of 5-Fluorouracil (5-FU) and Oxaliplatin (Oxa). Through CROP-seq, we obtained transcriptome changes at the single-cell level caused by changes in target genes, further revealing the mechanisms underlying the influence of eRNA-uaRNA interactions on transcriptional regulation.

Conclusion: We explored the mechanism of enhancer-promoter interactions by capturing all direct eRNA-uaRNA pairs in GC obtained by RIC-seq, and validation by CRISPR library screen combined with CROP-seq, yielding potential biomarkers and therapeutic targets in GC treatment.

#217 Analysis of chromatin accessibility profiling reveals epigenetic features of azacitidine response in high-risk myelodysplastic syndrome.

Dayoung Kim[1], Silvia Park[2], Yoo Jin Kim[2], Ryan Corces[3], Seung Woo Cho[1]

[1]Ulsan National Institute of Science & Technology, Ulsan, Korea, Republic of,[2]Department of Hematology, Seoul St. Mary's Hematology Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea, Republic of,[3]Gladstone Institute of Neurological Disease, San Francisco, CA

Azacitidine, a hypomethylating agent, is currently represented as initial therapy in most cases of high-risk myelodysplastic syndromes (MDS), yet only 40-60% of patients achieve therapeutic response. Non-responders face poor outcomes and disease progression to secondary acute myeloid leukemia. To improve treatment outcomes, understanding the pre-treatment molecular determinants of azacitidine response is critical. Despite the fundamental role of epigenetic regulation and cellular composition in MDS, the complex interplay between epigenetic regulation and therapeutic response has not been investigated. Here, we analyzed chromatin accessibility landscapes of 23 high-risk MDS patients before getting azacitidine, using Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq). We identified 1,940 distinct chromatin accessibility features strongly associated with azacitidine response, suggesting gene regulatory elements like enhancers as key determinants of it. Also, non-responders exhibited significant myeloid lineage commitment, while responders showed elevated accessibility in regions governing immune activation and T cell differentiation pathways. Notably, T cell subsets of non-responders displayed distinct chromatin states associated with impaired cellular differentiation compared to responders and healthy donors. This study first establishes chromatin accessibility features as potential biomarkers for azacitidine response in high-risk MDS and provides novel insights for personalized treatment optimization.

#218 Long-read nanopore sequencing in pursuit of complete methylation profiling of Hungarian colorectal cancer patients.

Kristof R. Rada, Nikolett Szakallas, Barbara Kinga Bartak, Alexandra Kalmar, Istvan Takacs, Bela Molnar

Semmelweis University, Budapest, Hungary

Introduction: Variations of the epigenetic landscape are the hallmark of many pathologies, including cancer. We investigated the Hungarian colorectal cancer cohort *via* Oxford Nanopore's long-read whole-genome sequencing (WGS) platform to provide for the first time a genome-spanning methylation report of the country. A prominent benefit of the method is native, and so, bias-free detection of modified or alternative nucleotides.

Aims & Methods: We selected 136 tissue biopsy, resection, normal adjacent tissue (NAT) and peripheral buffy coat (BC) samples from patients of Semmelweis University, to represent the range of pathological progression: healthy - adenoma (AD) - carcinoma (CRC). After thorough quality control by fragment length analysis (Tape Station), WGS was carried out on Nanopore's PromethION platform in simplex basecalling mode. Bioinformatic analysis utilized the Nanopore software package, Dorado basecaller and our custom scripts. We focused on methylation and hydroxymethylation of cytosine residues as the most abundant form of epigenetic marks.

Results: Coverage is 19.3x (±0.13), with an average read length and precision of 4.3 (±0.16) kb and 98% (±0.01), respectively; median PHRED is 16.03 (±1.18) and N50 is 7807 (±2262). Average GC ratio is 47% (±7%), island-mapped CpG sites show 69% (±5%) average methylation, of which 5hmC accounts for 5.68% (±1.88%), showing significant variations between groups: from 71% (healthy individuals), average methylation drops by 4.1% (AD, $p=0.013$), 8.8% (CRC, $p=0.007$), and 12.9% (resection, $p<0.001$) in solid tumors; by 0.1% (AD) and 2.5% (CRC) in buffy coats (not significant); and by 2.7% (CRC, $p=0.01$), and 4.6% (resection, $p<0.001$) in NAT samples. Solid samples always show significant hypomethylation compared to buffy coats. Contrasting this, 5hmC reveals a different pattern: it is relatively stable in solid samples and buffy coats, but rises by 47% (CRC, $p<0.001$) and 56% (resection, $p<0.001$) in normal adjacent tissues. When matching the tumor and healthy tissue of the same patient, we also see significant hypomethylation: 5mC decreases by 7% (±2.1%, $p=0.005$), and 5hmC by 31.4% (±3.9%, $p<0.001$) with very few exceptions.

Conclusion: long-read WGS is a strong tool for studying methylation in solid and liquid samples of colorectal cancer patients, revealing marked differences between solid and liquid samples, and also between tumor and healthy tissue. We corroborated the general cytosine hypomethylation associated with cancer, while also observing new trends in hydroxymethylation.

#219 Uncovering synergistic and antagonistic interactions in epigenetic therapeutics using perturb-seq.

Changde Cheng, Rui Lu, Sajesan Aryal, Doug A. Welsch

University of Alabama at Birmingham, Birmingham, AL

Acute myeloid leukemia (AML) is a heterogeneous malignancy characterized by genetic and epigenetic dysregulation, including aberrant activity of chromatin modifiers. The complex interactions among these modifiers and regulators in AML pathogenesis remain poorly understood, posing significant challenges to developing effective combination therapies. To address this, we used Perturb-seq, a CRISPR-based single-cell transcriptomics approach, to profile 16 key genes from the Menin-MLL, Polycomb, and histone acetyltransferase (HAT) complexes in the MOLM-13 AML cell line. We developed a machine learning framework called Composer, which predicts the combinatorial effects of perturbations by analyzing their impact on the transcriptome. We identified cooperative regulation of oncogenic pathways by Menin-MLL and KAT6A, while PRC1 components, such as PCGF1, were found to antagonize DOT1L activity. These predictions were validated using bulk RNA-seq and experiments combining perturbations through small molecule inhibitors and gene knockouts, demonstrating that dual inhibition of Menin-MLL and KAT6A, or DOT1L and KAT6A, significantly reduced AML cell viability. This study underscores the power of combining AI with single-cell Perturb-seq to provide novel insight into the epigenetic landscape of AML and identify promising therapeutic targets to overcome treatment resistance.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#22 Engineering KRAS-mutant-organoids from pancreatic ductal adenocarcinoma for advanced drug screening applications *in-vitro* and *in-vivo*.

A. Savadipour, K. Tummala, E. Park, V. Vidimar, M. Veling, M. Buchanan, E. Muise, G. Hebert, B. Long, N. Solban;
Merck & Co., Inc., Boston, MA

Background: Organoids are powerful tumor models for cancer research surpassing 2D-cultures in maintaining tumor heterogeneity and genetic fidelity. However, working with organoids encounters challenges in imaging, drug-screening, and in-vivo efficacy studies, due to their structural complexity. Additionally, the absence of other relevant cell types including cancer-associated-fibroblasts (CAFs) and immune cells, limits their ability to fully recapitulate the tumor-microenvironment (TME). To overcome these limitations, we developed GFP- or RFP-expressing KRAS-mutant-PDAC-organoids for efficient in-vitro and in-vivo drug-screening in mono- and co-culture systems.
Methods: We developed KRAS-mutant-PDAC-organoids that overexpress GFP or RFP using lentiviral-transduction. The growth and gene expression profile of these engineered organoid lines were compared to the parental line using Incucyte, DRUG-seq, and immunoblotting. These modifications enabled in-vitro mechanistic studies and drug sensitivity assays via confocal-microscopy and cell viability assays, as well as in-vivo quantification of tumor growth, drug-response, and gene expression.
Results: Antibiotic-sensitivity assays showed that 10 µM of antibiotic induces complete cell death within 5 days. Based on this, we stably overexpressed GFP or RFP in organoid lines, with strong GFP and RFP signals confirmed through microscopy. Optimizing culture conditions revealed that the addition of growth-factors (EGF and FGF) is crucial in initial phases of growth, though they can be removed later for drug-screening without impacting the organoids growth curve. This is important for accurate drug-screening, particularly for growth-factor pathway inhibitors. Further, we compared the growth rates and protein expression of the engineered organoids to their parental lines and observed no substantial differences. In high-throughput drug screening, we tested the effects of chemotherapy drugs (Gemcitabine and SN38) on organoids at varying seeding densities. While lentiviral transduction did not significantly affect the IC50 values, increasing the number of cells significantly raised the IC50 values (e.g., IC50 for Gemcitabine increased from 6-70 nM with 1k cells to 32-517 nM with 5k cells).
Conclusion: We successfully engineered KRAS-mutant-PDAC-organoids, which will serve as invaluable tools for in-vitro and in vivo efficacy experiments. Engineered organoids will simplify detection in co-culture systems and eliminate the need for additional staining. Future studies will focus on evaluating the growth and gene expression of engineered lines in-vivo compared to their parental lines. Additionally, these novel co-culture systems are being used to investigate the effects of novel KRAS inhibitors on organoid growth in-vitro and in-vivo.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Comprehensive Multiomic Profiling of Chromatin Dynamics, DNA Methylation, and Tumor Microenvironments
Poster Session

#220 Early methylome-level alterations in colorectal adenocarcinoma.

Dalma Muller, **Balazs Gyorffy**

Semmelweis University, Budapest, Hungary

Introduction: Methylation-based biomarkers represent a promising tool for early tumor detection in colorectal cancer. Genome-wide methylation analysis of large cohorts is an effective way of for the discovery of new biomarker candidates. For such purposes, Illumina arrays are today the most popular sources of methylation data.
Purpose: We aimed to analyze the methylation pattern differences between different groups, especially those involved in normal-adenoma and adenoma-adenocarcinoma transition. In order to enable straightforward exploration of the database we also aimed to establish an interactive web-based platform.
Methods: Raw files of 2,356 samples, out of which 1,273 were from normal mucosa, 815 from adenocarcinoma, and 258 from precancerous adenoma tissues were processed in R using the *minfi* and the *watermelon* libraries. The dataset contains data from both Illumina HumanMethylation450K and EPIC microarray platforms, which were analyzed separately. Based on a matrix of β-values, methylated regions and CpG loci were identified using Kruskal-Wallis-test complemented with ROC analysis. For data visualization we created www.epigenplot.com, a web platform designed for the comprehensive analysis of our database.
Results: When compared to normal tissues, the average methylation of both adenoma and adenocarcinoma samples was significantly lower in both HM450K and EPIC arrays (Kruskal-Wallis, $p<0.001$). The majority of the significant ($\Delta\beta >= 0.2$, $p < 0.001$) methylation changes discovered in adenocarcinomas were already detectable in adenoma tissues. This occurred more often for hyper- than hypomethylation (HM450K: 80.5% and 78.58%; EPIC: 79% and 41%, respectively). Out of 75 genes previously proposed in the literature as CRC biomarker candidates, 49 and 45 (HM450K and EPIC) were significantly differentially methylated in adenocarcinoma tissues. Notably, for all these genes, the change was already present in adenoma tissues. When the difference between normal and adenocarcinoma tissues was analyzed, the methylation of the first exon of the *C1orf70* gene reached the highest AUC 0.98 at both platforms. For this region, the AUC for normal and adenoma tissues was 0.97 in HM450K and 0.99 in the EPIC dataset.
Conclusions: Alternations of the methylome are early events of tumorigenesis. The changes detected by methylation-based biomarkers are already present in precancerous lesions. Our web tool can be utilized for the discovery and validation of biomarkers suitable the early detection of CRC using genome-wide data.

#221 Investigating sex disparities in bladder cancer using carcinogen induced bladder cancer mouse model.

Bhavisha Doshi[1], Eduardo Cortes[2], Bryan M. Gillard[3], Ellen Karasik[1], Norbert Sule[4], Barbara A. Foster[1], Jianmin Wang[5], Wendy J. Huss[6], Anna Woloszynska[1]

[1]Pharmacology & Therapeutics, Roswell Park Comprehensive Cancer Center, Buffalo, NY,[2]Biostatistics & Bioinformatics, Roswell Park Comprehensive Cancer Center, University at Buffalo, Buffalo, NY,[3]Roswell Park Comprehensive Cancer Center, Buffalo, NY,[4]Pathology, Roswell Park Comprehensive Cancer Center, Buffalo, NY,[5]Biostatistics & Bioinformatics, Roswell Park Comprehensive Cancer Center, Buffalo, NY,[6]Dermatology, Roswell Park Comprehensive Cancer Center, Buffalo, NY

DNA methylation plays a crucial role in regulating gene expression; thereby influencing tumor growth, progression, and response to treatment in muscle-invasive bladder cancer (MIBC). Intriguingly, methylation patterns may also underlie the pronounced sex disparities in MIBC incidence and outcomes; where men show a fourfold higher incidence rate. Still, female patients face higher risks of progression, recurrence, and mortality. Despite substantial documentation of DNA methylation patterns in patient tumors, translating these findings into actionable treatments requires reliable preclinical models that recapitulate the human methylation landscape. Such models offer a controlled environment to explore methylation-driven gene expression changes and evaluate potential therapeutic approaches that are otherwise challenging in clinical settings. Our research focuses on the BBN (N-butyl-N-(4-hydroxybutyl) nitrosamine)-induced bladder cancer mouse model. The BBN model mimics the histological and mutational features of human MIBC. We investigated whether the BBN induced bladder cancer model recapitulates the DNA methylation patterns found in clinical bladder cancer, and the use of the model to shed light on methylation-driven shifts in gene expression and pathways. By employing methylation arrays and RNA sequencing, we systematically compared DNA methylation profiles and gene expression patterns in MIBC tumors between BBN-induced mouse bladder tumors and human clinical samples. We identified 4,250 common differentially methylated genes (DMGs) and 512 shared differentially expressed genes (DEGs) between human and BBN-induced mouse tumors. Notably, 280 sex-specific DMGs and four conserved sex-specific DEGs, including XIST, KDM5D, UTY, and DDX3Y, were identified, highlighting the ability of the BBN model to demonstrate sex-based epigenetic characteristics observed in human MIBC. Pathway analysis revealed that genes associated with mTOR signaling were hypomethylated and upregulated in male tumors. While the EMT pathway was upregulated in female tumors across both species. These findings confirm that the BBN model not only recapitulates the methylation patterns of human MIBC but can also serve as a valuable tool to investigate sex-specific methylation mechanisms in disease progression. The BBN model shows significant promise for advancing MIBC biology and guiding the development of sex-specific, methylation-targeted therapies. This abstract was reviewed and refined using AI-based tools to check grammar and improve clarity.

#222 Unraveling tumor progression in breast cancer: The impact of differential DNA methylation.

N. Jayaswal, A. N. Tegge;
Virginia Tech Carilion School Of Medicine, Roanoke, VA

Breast cancer affects over 300,000 Americans annually, with 42,780 deaths projected in 2024. DNA methylation, an epigenetic modification, has been associated with breast cancer progression (e.g., BRCA1 gene promoter hypermethylation). We hypothesize that we can identify differentially methylated CpG sites linked to cancer progression, thereby revealing the biological functions and pathways involved in tumor progression.We used DNA methylation data characterizing over 487,000 CpG sites from 1,032 female breast cancer patients in the Cancer Genome Atlas. CpG methylation was quantified using M values for more accurate homoscedastic statistical analysis. To focus on CpG sites with high methylation variability, sites were filtered by removing those whose variance in the tumor samples were lower than the normal tissue sample. Differential methylation across cancer stages was determined using an analysis of variance (ANOVA; $p \leq 1\times10\text{-}4$). Statistically significant differentially methylated CpG sites were annotated to genes, and these genes were used to perform functional enrichment analysis in DAVID.We identified 473 CpG sites with significant differential methylation across cancer stages that were annotated to 556 genes. Functional enrichment analysis revealed seven significantly enriched pathways, including non-small cell lung cancer (NSCLC; hsa05223, adj. $p = 0.0027$), gastric acid secretion (hsa04971, adj. $p = 0.0032$), insulin signaling (hsa04910, adj. $p = 0.0066$), aldosterone synthesis and secretion (hsa04925, adj. $p = 0.0070$), viral myocarditis (hsa05416, adj. $p = 0.0090$), long-term potentiation (hsa04720, adj. $p = 0.0090$), and propanoate metabolism (hsa00640, adj. $p = 0.0091$). In the NSCLC pathway, five genes were identified: KRAS, a well-known oncogene promoting cell proliferation; ERBB2 (HER2), driving aggressive HER2-positive breast cancer growth; FHIT, a tumor suppressor gene regulating apoptosis and cell cycle; CASP9, a pro-apoptotic tumor suppressor; and RARB, a tumor suppressor regulating cell growth and differentiation. Methylation differences related to tumor progression have been documented in the literature for several of these genes. Our study identified significant CpG sites and corresponding genes associated with breast cancer progression via differential methylation analysis. These results provide valuable insights into the biological mechanisms driving cancer progression and highlight the potential of DNA methylation as predictive biomarkers. Further research is needed to validate and expand these findings, and to explore their clinical implications in breast cancer surveillance and therapeutic interventions.

#223 Evaluating the therapeutic potential of MDM2 inhibitor RG-7388 and epigenetic modulator CM-272 in cisplatin-resistant ovarian cancer.

Fatimah AlssamaniI[1], Umamaheswari Natarajan[2], Shyam Jaganathan[2], Appu Rathinavelu[2]

[1]Rumbaugh-Goodwin Institute for cancer research, Barry & Judy Silverman Collage of Pharmacy, College of Clinical Pharmacy, King Faisal University, Nova Southeastern University, Fort Lauderdale, FL,[2]Rumbaugh-Goodwin Institute for cancer research, Barry & Judy Silverman Collage of Pharmacy, Nova Southeastern University, Fort Lauderdale, FL

Ovarian cancer (OC) is the deadliest gynecological malignancy, often presenting late diagnosis and exhibiting treatment resistance, partly due to dysregulation of the crucial tumor suppressor p53. The oncoprotein MDM2 also negatively regulates p53, and therefore, MDM2 specific inhibitors may offer potential therapeutic avenues through reactivation of p53 and reversing adverse epigenetic modifications causing growth or resistance. In this study, we investigated the therapeutic potential of the MDM2 inhibitor RG-7388, chemotherapeutic agent cisplatin, and the epigenetic modulator CM-272 in cisplatin-resistant A2780cis ovarian cancer cells. We first characterized the growth kinetics of cisplatin sensitive and resistant A2780 cell lines, which confirmed significantly longer doubling times in the resistant cells (approximately 35 hours versus 20 hours in sensitive cells). Treatment efficacy was evaluated through cell viability and cell death assays, along with the expression levels of DNMT1 and EZH2, which are key epigenetic regulators. Treatment of A2780cis cells with RG-7388 demonstrated significant anti-cancer activity, by reducing live cell populations by 42-48% through increased cell death. Despite resistance, qRT-PCR analysis revealed that both cisplatin and CM-272 treatments significantly suppressed DNMT1 expression by 96%A2780cis cells. Western blot analysis also confirmed that both RG-7388 and cisplatin reduced DNMT1 protein levels in A2780cis cells along with the most pronounced reduction of DNMT1 after CM-272 treatment. Notably, CM-272 significantly decreased the levels of EZH2 also while RG-7388 did not impact the EZH2 protein levels. Interestingly, treatments with RG-7388 and CM-272 drug combinations showed varied effects, while maintaining strong inhibition of PARP. Overall, our findings suggest that the MDM2 inhibitor RG-7388 could be a promising therapeutic agent for cisplatin-resistant ovarian cancer, particularly because of its ability to modulateepigenetic regulators like DNMT1. However, the complex interplay between cell survival and epigenetic regulation in drug resistant cells warrant further investigation to optimize the outcomes of combination treatment strategies. Acknowledgement: This project was supported by the Royal Dames of Cancer Research Inc. Ft. Lauderdale, Florida

#224 Methyl-CpG-binding domain protein 2 (Mbd2) as a therapeutic target in breast cancer.

Olivia Dumas[1], Niaz Mahmood[2], Ani Arakelian[2], Moshe Szyf[3], Shafaat Rabbani[4]

[1]Department of Physiology, McGill University, Montreal, QC, Canada,[2]Department of Medicine, McGill University, Montreal, QC, Canada,[3]HKG Epitherapeutics, Shatin, N.T., Hong Kong,[4]Department of Physiology & Department of Medicine, McGill University, Montreal, QC, Canada

Abnormal DNA methylation is frequently observed in the cancer epigenome, rendering it a prime target for pharmacological manipulation. Methyl-CpG-binding domain protein 2 (Mbd2), a reader of DNA methylation, has been shown to be strongly linked to various malignancies, including breast cancer, establishing *Mbd2* as a viable and effective therapeutic target. Our previous studies have demonstrated that genetic deletion of *Mbd2* significantly reduces primary breast tumor growth and lung metastasis in a well-established transgenic mouse model of breast cancer. In the current study, we examined the effect of pharmacological inhibition of *Mbd2* on breast cancer progression and metastasis to non skeletal and skeletal sites. Several small-molecule inhibitors for *Mbd2* have been described, and among these, KCC-07 was shown to significantly reduce medulloblastoma *in vitro* and *in vivo*. Treatment of mouse luminal B breast cancer cell lines (PyMT-R221A, E0071) with KCC-07 resulted in a time- and dose-dependent decrease in tumor cell proliferation and reduced the invasiveness of the cells *in vitro*. These results were recapitulated *in vivo*, where PyMT-R221A cells inoculated via mammary fat pad (m.f.p) and intra-tibial (i.t) routes into female FVB mice and treated with vehicle alone or KCC-07 (100.0 mg/kg) 3QW via the intraperitoneal route for three weeks showed a decrease in tumor volume and skeletal metastasis. Further immunohistochemical analyses of the tumor tissues using markers of tumor proliferation (Ki67), angiogenesis (CD31), and bone resorption (TRAP), as well as any potential side effects on animal toxicity and global transcriptomic changes of the PyMT-R221A cells induced by treatment with KCC-07, will be presented and discussed. In other studies, PyMT-R221A cells were treated with the most effective dose of *Mbd2* siRNA and KCC-07 (40μM) and cellular RNA from control and experimental groups was extracted and subjected to RNA-seq analysis, which is now being analyzed and validated in depth. Results from these analyses will identify key *Mbd2*-mediated signaling pathways and genes that play an important role in breast cancer. Taken together, the results of this study will provide mechanistic insights into the therapeutic potential of targeting *Mbd2* with KCC-07 to block breast cancer growth and metastasis in a preclinical setting. These findings could be highly valuable for the future evaluation of selective inhibitors of *Mbd2* to treat breast cancer patients.

#225 Single Cell DNA methylomes from multiple tissues demonstrate tissue heterogeneity and target enrichment as a driver of read utility.

Austin Holmes, Camden Jansen, Eric Pu, Sanika Khare, Dominic Skinner, Bryce Alves, Jerushah Thomas, Dmitry Pokholok, Felix Schlesinger

Scale Biosciences, Inc., San Diego, CA

Comparative analysis of single cell gene expression technologies is helpful in choosing a platform that fits experimental needs. However, emerging single cell (sc) technologies, like scDNA methylation, are currently unavailable across platforms and are unable to benefit from such analysis. Rather than comparing the performance of one tissue across multiple technologies, here we present one technology and a comparative analysis across multiple tissues, and sequencing read depths, with target enrichment. We integrate bulk-DNA methylation with single cell and transform methylation data to gene level metrics on par with current technologies. These datasets give guidelines on how to maximize the data per cell without excessive sequencing, allowing experiments to scale up. Single cell DNA methylation adds value to current genetic and epigenetic modalities as an orthogonal dataset that further characterizes functional genomics. Scale Bio offers a kitted platform for library generation and analysis of thousands of single cell methylomes. DNA methylation from bulk samples has been widely demonstrated to be a gene regulatory mechanism in health and disease, however large-scale single cell datasets are limited. At the onset of new technologies sequencing depth and cell number often exceed requirements for the most basic single cell analyses such as cell clustering, cell annotation and calling differentially methylated regions. At these high read depths and high cell # per sample, sample multiplexing in a biologically relevant way becomes difficult. We investigated six single cell methylomes (solid tumor, blood tumor, young PBMCs, old PBMCs, human brain and mouse brain) generated with Scale Bio scMet kit. For the tumor samples, we conducted copy number variation analysis alongside down-sampled read depth and cell number. For all samples, we cross compared cell clustering and cell annotation/recovery at various sequencing depths and cell number demonstrating that brain tissue tolerates much lower read depths. Each of these libraries were further enriched using the Twist Human methylome panel, demonstrating that an enrichment of regulatory regions allows for even lower read depths while maintaining a high number of significant DMRs. Lastly, we generated a bulk-DNA methylation library from the blood tumor sample to overlap with the most differentially methylated regions resolved at the single cell, revealing the limitations of bulk DNA compared to single cell. In conclusion these datasets and analyses conclude that tissue heterogeneity and the enrichment of known differentially methylated regions is a critical factor when considering sequencing depth required and the cell number per sample. However, emerging single cell methylomes continue to fine tune known DNA regulatory elements as well as facilitate discovery of de novo DNA methylation events at the single cell level.

#226 Methylation specific silencing of miR-9 is associated with an epithelial-mesenchymal transition (EMT) phenotype in laryngeal squamous cell carcinoma.

Christina Gobin, Matthew Chang, Marie Gauthier, Chayil C. Lattimore, Michael Kladde, Kristianna Fredenburg

University of Florida, Gainesville, FL

Background: Advanced stage laryngeal squamous cell carcinoma (LSCC) carries one of the highest mortality rates of all head and neck cancers. We previously demonstrated that low miR-9 expression impacts invasiveness and resistance to cisplatin in advanced stage LSCC. Here, we seek to further elucidate how low miR-9 influences LSCC pathogenesis.
Methods: Gene expression and clinicopathologic data were obtained for 116 LSCC patients from The Cancer Genome Atlas (TCGA) and used to evaluate predictors of LSCC overall survival. A simple logistic regression combined with a Receiver Operating Characteristic (ROC) curve was used to determine a cutoff for categorizing LSCC patients into low and high miR-9 expression groups. A panel of epithelial-mesenchymal transition (EMT) related genes (MAP1B, DOCK10, FGFR1, FGF1, ZEB1, VCAN, WNT5A, SRGN, THEM30B, CDH1 and EGFR) and methylated miR-9 transcripts were assessed between low and high miR-9 expressing LSCC patients via Principal Component Analysis. *In vitro* validation of an EMT phenotype and miR-9 promoter hypermethylation was performed using four advanced stage LSCC cell lines with variable miR-9 expression. Cell line EMT gene expression was evaluated via RNA sequencing and validated by qPCR. Methylation and chromatin accessibility of the three miR-9 promoter regions were quantified in each LSCC cell line through PacBio sequencing. Low miR-9 expressing cell lines were treated with the global demethylating agent, 5-Azacytidine (0μM, 0.1μM, 0.5μM and 1μM), and differences in EMT-related gene expression were assessed via qPCR.
Results: Low miR-9 and methylation of miR-9 were found to be significant predictors of poor LSCC survival ($p < 0.05$). Gene signatures representing a high EMT phenotype and high methylated miR-9 status were identified as unique classifiers of low compared with high miR-9 expressing LSCC patients. Differential expression of EMT related genes was detected in low compared with high miR-9 expressing cell lines; low miR-9 cell lines had significantly higher baseline expression of MAP1B, DOCK10, FGFR1, FGF1, and ZEB1 (>2-fold, $p < 0.0001$) and significantly lower baseline expression of CDH1 and EGFR (>2-fold, $p < 0.0001$). Low miR-9 expressing cell lines also had significantly greater methylation of miR-9 promoter regions ($p < 0.0001$) as well as significantly fewer chromatin accessibility sites open for miR-9 transcription ($p < 0.0001$). Global demethylation treatment of the low miR-9 expressing cell lines dose dependently restored miR-9 expression (>2-fold, $p < 0.0001$) and resulted in inverse expression of MAP1B, DOCK10, FGFR1, FGF1, ZEB1, CDH1 and EGFR at the 1μM dose (>2-fold, $p < 0.0001$).
Conclusion: Our findings indicate that methylation specific silencing of miR-9 may be a mechanism that yields a high EMT phenotype, predictive of poor overall survival in LSCC.

#227 How arsenic and particulate matter affect epigenetic gene regulation and cancer: bridging the gap between the environment and health: a review.

Bianca Ehling

Olentangy Orange High School, Lewis Center, OH

There are numerous environmental factors that influence epigenetic gene regulation and can thus lead to the development of cancer. Two of these factors include metals and air pollutants. This review examines how arsenic and particulate matter affect epigenetic gene regulation and can lead to cancer. It is important to continue to learn about and understand these mechanisms in a preventative effort to reduce the risk of developing cancer, especially since wildfires are projected to increase in frequency, and they are a source of particulate matter (PM). Arsenic is widespread in the environment and it can be present in PM as well. The references for this review were collected by conducting database searches (PubMed and ScienceDirect) using keywords such as metals, air pollutants, epigenetics, and cancer. Arsenic has been found to be associated with DNA demethylation and the formation of malignancies in vitro in rats, which could possibly be due to the universal methyl group donor (SAM) methylating arsenic and thus having limited availability to methylate DNA and histones. Arsenic can also promote the creation of reactive oxygen species that can damage DNA and hinder its ability to be methylated. Arsenic can lead to the methylation-induced transcriptional silencing of tumor-suppressor genes, as well. In addition, the presence of arsenic is associated with the methylation of transposable elements, which is correlated with colon cancer. Arsenic can also lead to the activation of certain histone kinases, which can potentially demethylate certain histones and eventually lead to proto-oncogenes being induced. Arsenic has also been found to induce the “marking” of certain silent genes, to the point where they are not yet activated, but have the ability to be quickly activated. In addition, since alternative splicing of RNA (specifically, which parts of the RNA will become exons) is dependent on DNA methylation and histone modifications, exposure to arsenic could disrupt normal RNA alternative splicing patterns. Arsenic has also been found to potentially alter miRNA expression. PM can potentially contribute to DNA hypermethylation and thus the onset of lung cancer. The alteration of DNA methylation has also been associated with dysregulation of apoptosis. PM has been found to potentially decrease the expression of histone demethylase, which may lead to increased methylation of histone modifications. PM can also potentially suppress the expression of tumor suppressor miRNAs, enabling lung cancer to develop. Since environmental factors can often be confounding variables, especially when the exposome is taken into account, it is often difficult to discern which environmental factor is causing which epigenetic effect. To combat this, data science methods could be implemented in future research endeavors.

#228 Loss of *TET2* and *TET3* mediated DNA demethylation drives intestinal tumor formation.

Oliver Gilliam[1], Ihab Ansari[2], Nikolaos Georgakopoulos[1], Manuel Mastel[1], Rene Jackstadt[1], Sebastian Belle[3], Yehudit Bergman[2], Frank Lyko[1]

[1]German Cancer Research Center, Heidelberg, Germany,[2]Department of Developmental Biology and Cancer Research, Institute for Medical Research Israel-Canada, Hebrew University Medical School, Jerusalem, Israel,[3]Department of Medicine II, University Medical Center Mannheim, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

Non-mutational epigenetic reprogramming is a widely recognized hallmark of cancer, which emphasizes the relevance of epigenetic modifications for cancer development. The discovery of the CpG island methylator phenotype in colorectal cancer (CRC) and promotor hypermethylation of tumor suppressor genes further raised the interest in DNA methylation changes during tumor development. However, despite decades of cancer epigenetics research, our understanding of the function of aberrant DNA methylation remains limited. Thus, new models are needed to study the link between global DNA methylation changes and tumorigenesis. The Ten-eleven translocator (*TET)* enzymes are critical mediators of active demethylation by oxidizing the C^5-methylcytosine, thereby playing a vital role in regulating DNA methylation. Here, we report an intestinal epithelium-specific *TET2/3* double knockout (dko) mouse model, which shows invasive adenocarcinomas in the small intestine at high penetrance. We performed a detailed DNA methylation analysis and identified enhancers as the main target of hypermethylation in the tumors. Additionally, we observed substantial overlap in methylation patterns with the fetal developmental stage of the mouse intestine. Subsequent analysis also revealed a fetal gene expression signature and activation of *YAP1* signaling, suggesting oncofetal reprogramming. Since fetal reprogramming is enriched in the serrated route to colorectal cancer, we compared our model with models of the classical (*APC*) and serrated (*BRAF*- and *KRAS*-derived) route of CRC. We found similar methylation patterns between the *TET2/3* dko tumors and *BRAF*- and *KRAS*-derived tumors. To investigate the clinical relevance of our findings, we collected human small bowel adenomas and integrated their methylation profiles with available datasets of colorectal adenomas and carcinomas. We observed notable similarities between adenomas of the small bowel and serrated adenomas of the colon. This finding further confirms the similarities between our *TET*-deficient mouse model and the serrated route to colorectal cancer and suggests that non-mutational *TET* deficiency could be an important driver of intestinal cancer.

#229 Oncogenic cell growth linked with hypermethylation of CpG sites within the p53 pathway, and its effects on subgroups.

Roy Khalife, Ethan Grebler, **Anthony Magliocco**

Protean BioDiagnostics Inc, Orlando, FL

Methylation of tumor-suppressor genes is a recognized oncogenic mechanism. This study focuses on determining the extent of CpG site methylation within genes of the p53 pathway and assessing how patient sex influences these methylation patterns, which could aid in cancer identification through p53 pathway methylation analysis. Given that mutations in p53 occur in over half of all human cancers, their pro-apoptotic properties are vital for halting unchecked cell growth. We analyzed the methylation status of p53-regulated transcriptional target genes using FFPE samples from various primary brain cancers. Methylation data, generated using the Infinium MethylationEPIC v2.0 array, was visualized through heat maps. Among all samples, the most hypermethylated genes (m-value >2) were identified in descending order as SFN, CASP9, TP53, APAF1, GTSE1, TSC2, and SESN3. Interestingly, TSC2 and SESN3 did not exhibit hypermethylation in male samples, suggesting potential sex-specific epigenetic regulation. The observed trend of hypermethylation—exceeding 80% CpG site methylation—within key inhibitory, apoptotic, and repair genes underscores their silencing and involvement in tumor development. Additionally, the absence of hypermethylation in canonical TP53 targets such as WAF1/p21 highlights the need for further investigation into pathway-specific methylation patterns, potentially uncovering alternative or complementary mechanisms of tumor suppression evasion.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Growth Factors, Receptors, and GTPase Signaling
Poster Session

#233 Targeting Rho GTPases RAC1 and CDC42 in advanced prostate cancer cells.

Wilfred Fonseca Ferrer[1], Valeria Torres Matos[2], Carolina Restrepo Soto[3], Jessica Colon Gonzalez[4], Suranganie Dharmawardhane Flanagan[4], Cornelis P. Vlaar Stoop[5], Carlos Joel Diaz Osterman[6]

[1]School of Pharmacy, UPR - Medical Sciences Campus, San Juan, Puerto Rico,[2]Biology, UPR - Rio Piedras Campus, San Juan, Puerto Rico,[3]Mathematics, UPR - Rio Piedras Campus, San Juan, Puerto Rico,[4]Biochemistry, UPR - Medical Sciences Campus, San Juan, Puerto Rico,[5]UPR - Molecular Sciences Center, San Juan, Puerto Rico,[6]Clinical and Translational Research, UPR - Comprehensive Cancer Center, San Juan, Puerto Rico

BACKGROUND: The androgen receptor (AR) plays a central role in the development and progression of prostate cancer. Standard treatment for advanced prostate cancer targets the AR using androgen deprivation therapy. Despite initial responses, patients eventually progress to androgen-independent disease with metastasis, raising the need for novel treatment options. GTPases regulate many metastasis-associated processes, including cell division, cell growth, cell migration, and cell adhesion. Novel inhibitors for GTPases are currently being evaluated for efficacy in metastatic breast cancer. However, little is known about their utility for other hormone-dependent cancers such as prostate cancer. We hypothesize that Rho GTPase inhibitor efficacy will vary between prostate cancer cell models based on AR expression status and metastatic potential. This study aims to determine the efficacy of nine compounds targeting the GTPase pathway in the setting of AR-positive compared to AR-negative prostate cancer cells. METHODS: AR-positive (LNCaP, 22rv1) and AR-negative (PC3, DU145) prostate cancer cell lines were used to evaluate the efficacy of nine novel Rho GTPase inhibitors. MTT viability assays were performed following exposure of cells to 0-100μM compound for 72 hours. Migration assays were performed using sublethal doses of select compounds for 3-5 days. Intracellular expression of Rho GTPase-associated proteins was evaluated using Western blot. RESULTS: Of the compounds analyzed, six had an IC50 in the nanomolar range. The most effective compound overall was CPV-337 (IC50: 6.5-108.6nM). AR-negative cell lines exhibited a higher sensitivity to EHOP-097 and MBQ-168 than AR-positive cell lines. Proteins associated with mechanotransduction were elevated in AR-negative compared to AR-positive cells. CONCLUSION: Our study demonstrates a central role for Rho GTPases in prostate cancer metastatic potential in the context of AR independence.
ACKNOWLEDGEMENTS: These studies were supported by start-up funds provided to C.J.D.O. by the University of Puerto Rico Comprehensive Cancer Center in San Juan, Puerto Rico.

#234 $G\alpha_i2$ induces cell migration in prostate cancer cells in the absence of rac1 activation.

Rarnice Johnson[1], Silvia Caggia[2], Shafiq Khan[1]

[1]Biological Sciences, Clark Atlanta University, Atlanta, GA,[2]Department of microbiology and immunology, University of Miami, Miami, FL

Prostate cancers can grow slowly and are confined to the prostate gland; However, metastatic prostate cancers are aggressive and can spread quickly. $G\alpha_i2$ is a heterotrimeric G protein subunit and its essential for cell migration in prostate cancer cells, in response to both oxytocin and EGF, acting via GPCR and PTKR, respectively. The Rho family of GTPases includes RhoA, Rac1 and Cdc42, these are intracellular protein that regulate cell migration. In the previous studies we seen that the knockdown of $G\alpha_i2$ reduces cell migration and lamellipodia formation at leading edge of PC3 cell overexpressing constitutively active Rac1, therefore $G\alpha_i2$ may be involved in the regulation of cell motility and lamellipodia formation at a step which is independent or downstream of Rac1 activation. We hypothesize that $G\alpha_i2$ is required for the activation of the downstream proteins of Rac1 leading to lamellipodia formation at the leading edge of migrating cells. We overexpressed constitutively active form of Gαi2 (Gαi2-Q205L) in PC3 parental cell lines. The result showed that when PC3 cells are transfected with Gαi2-Q205L the migration rate in these cells is significantly increased. In the parallel experiment we used a specific Gαi2 inhibitor, which resulted in attenuation of Giα2-Q205L effects on cell migration in PC3 cells. Furthermore, Rac1 inhibitor did not block increased cell migration in PC3 cells overexpressing constitutively active Gαi2. We studied $G\alpha_i2$ effects on proteins activated by Rac1, specifically Wave2 and Arp 2/3. We transfected PC3 empty vector and PC3 Rac1 constitutively active stable cells with control siRNA or Gαi2 siRNA to knockdown endogenous Gαi2 expression. The western blot analysis showed Rac1 dependent activation of Wave2 and Arp 2/3 are impaired in the absence of Gαi2. We conclude activated Gαi2 plays a crucial role in cell migration in prostate cancer cells which is independent of Rac1 activation.

Acknowledgement: These studies were supported by the NIH/NIMHD/RCMI under Award Number U54MD007590.

#235 Targeting NOS sensitizes the NRAS-mutant melanomas to MEK inhibition and induces immunogenic cell death.

Jyoti Srivastava, Vipin Kumar Yadav, Rachel V. Jimenez, Bin Fang, Alvaro de Mingo Pulido, John M. Koomen, Kenneth Y. Tsai, Keiran SM Smalley, Paulo C. Rodriguez, Sanjay Premi

H. Lee Moffitt Cancer Center, Tampa, FL

Background: Activating NRAS mutations occur in 15-25% of melanomas; however, this subtype is highly aggressive and refractory to existing therapeutic approaches. Consequently, the identification of novel molecular mechanisms is of critical clinical importance. Melanomas harbor hyperactive nitric oxide synthases (NOS) that strongly correlate with poor prognosis. Here, we investigated the role of nitrosylation, a nitric oxide-induced posttranslational modification, in regulating the oncogenic MEK/ERK signaling pathway and Immunogenic Cell Death (ICD) in NRAS-mutant melanomas.
Methods: We employed various cellular and mouse models to investigate the effect of combinatorial inhibition of NOS and MEK in NRAS-mutant melanomas. We also profiled global nitrosylation by mass-spectrometry and validated the nitrosylation status of various MEK-ERK regulators and ICD markers using Biotin-switch method. Using site-directed mutagenesis, we characterized potential nitrosylation sites on the MEK and ERK proteins. Additionally, we characterized the nitrosylated forms of potential regulators of ICD andanti-melanoma immunity using cell-based assays and immunogenic mouse models. The immunomodulatory role of nitrosylation was further characterized using the dendritic cells(DCs): CD8+ T-cell cocultures.
Results: By inhibiting nitrosylation, we sensitized the NRAS-mutant melanomas to MEK inhibitors. We observed a strong correlation between global de-nitrosylation of MEK-ERK cascade proteins, and that of death-associated molecular patterns (DAMPs) which induce ICD.We also identified potential nitrosylation sites on MEK and ERK proteins with a pivotal role in melanoma cell proliferation. Further, we observed elevated nitrosylation and induction of oncogenic proteins upon MEKi acquired resistance, which was abrogated upon NOS inhibition. Using an immunocompetent mouse model, we demonstrated marked restriction of tumor growth in Nras-mutant melanoma upon inhibition of nitrosylation and MEK. Both *in vivo* and *in vitro* models showed extracellular release of ICD hallmark DAMPs, such as calreticulin, phospho-eIF2α, and HMGB1, which is a pre-requisite for ICD induction. This release triggered the activation of dendritic cells, leading to the proliferation and activation of a functional CD8+ T-cell repertoire in the tumor microenvironment.
Conclusions: We conclude that nitrosylation hyperactivates the MEK-ERK pathway and suppresses the anti-melanoma immune response, thereby promoting tumor progression and Meki resistance. Inhibiting nitrosylation not only enhances MEKi efficacy but also induces ICD. Further investigations on underlying mechanism(s) will identify novel therapeutic vulnerabilities against the yet untreatable, NRAS-mutant melanoma.

#236 Selective inhibition of NRAS-GTP in cancer models with small molecule NEOS-223.

Harish Potu[1], **Sara A. Little**[1], Stephanie Fedorchak[1], Sumathi Chittamuru[1], Timothy M. Murphy[1], Sarah Di Croce[1], Melanie George[1], Jeremy Harvey[2], Neil Rory McFarlane[2], Piet Christiaens[3], Timothy Fisher[1], Laxman S. Desai[1]

[1]NeoSome Life Sciences, Boston, MA,[2]KU Leuven, Leuven, MA,[3]Silverbridge Consulting, Heverlee, MA

NRAS is one of the second most mutated RAS oncogene followed by KRAS in cancer, and efforts to target directly inhibit its function have been continuing for years. Strategies targeting oncogenic RAS pathway include direct and indirect approaches. However, despite aggressive efforts in recent years, there are no clinical or on the market that directly target NRAS and inhibit its aberrant function in various cancers. The recent success of KRAS G12C inhibitors has demonstrated clinical efficacy in patients and approved for the treatment of non-small cell lung cancer. Despite the promising success of targeting KRAS, there remains no available inhibitor that targets NRAS alone. In pursuit of NRAS inhibition and we have developed a small molecule potent selective non-covalent inhibitor that inhibits NRAS-GTPase activity of both mutant and wild type of NRAS while sparing KRAS and HRAS. Our molecular dynamics simulation studies reveal the structural basis of inhibitor binding at the switch II pocket in NRAS. NEOS-223 demonstrated potent activity against cancer models with NRAS Q61K and inhibited cancer growth. Treatment with NEOS-223 led to tumor growth blockage and well tolerated in diverse RAS-addicted preclinical cancer models. NEOS-223 has a greater synergistic effect on inhibition of cell proliferation combined with specific targeted inhibitors such as gefitinib (EGFR), sotorasib (KRAS G12C), vemurafenib (BRAF V600E) and MRTX1133 (KRAS G12D) correlating with stronger suppression of EGFR-RAS-MAPK signaling. Resistance to KRAS inhibitors (G12C, G12D) seemed to be driven by EGFR mediated upregulation of wild type RAS activity. Feedback reactivation of adaptive RAS family members including NRAS (WT) occurs following KRAS inhibition, a key mediator of resistance. Co-targeting with NEOS-223 abrogates the EGFR-mediated feedback NRAS reactivation enhancing the antitumor effects improving the efficacy in KRAS mutant tumors with KRAS inhibition and similar effects were also observed in BRAF mutant colon cancer with inhibition of NRAS with NEOS-223 plus vemurafenib. Our study highlights the feasibility of developing NRAS selective inhibitor for therapeutic efforts and these results suggest that NRAS plus KRAS to be a promising strategy for treating both EGFR and RAS mutant tumors. Overall, these data provide important mechanistic insight to guide therapeutic strategies targeting NRAS mutant, dependent and resistance tumors.

#237 Species-specific role of RAS in malignant transformation.

Nikhil Gupta[1], Milla Majaniemi[1], Otto Kauko[1], Tero Aittokallio[1], Vera Gorbunova[2], Jukka Westermarck[1]

[1]University of Turku, Turku, Finland,[2]University of Rochester, Rochester, NY

Malignant transformation is a complex, multi-step process driven by the interplay between oncogene activation and tumor suppressor inhibition. The role of RAS in this process has been shown to exhibit striking species-specific differences. In mouse cells, RAS can effectively induce transformation with the inhibition of a single tumor suppressor. However, in human cells, RAS-driven transformation uniquely requires the inhibition of Protein Phosphatase 2A (PP2A). Despite over 20 years of investigation, the molecular explanation for this radical difference remains elusive. To address this fundamental gap in understanding, we performed a comprehensive phosphoproteogenomics analysis comparing mouse and human cells overexpressing oncogenic RAS.Transcriptomics, proteomics, and phosphoproteomics analyses revealed remarkably significant intra- and interspecies heterogeneity, highlighting the profound species-specific differences in RAS-induced signaling. Strikingly, while mTOR, CDK4-E2F1, and MYC signaling pathways were robustly activated by RAS in mouse cells, these activations were notably absent in human cell models. However, co-inhibition of the tumor suppressor phosphatase PP2A rendered human cells permissive to RAS-induced malignant transformation and restored pathway activities overlapping with RAS-only transformed mouse cells. Intriguingly, our findings suggest that RAS overexpression in human cells opposes phosphosignaling and pathway-targeted therapy responses, a phenomenon absent in mouse cells. This highlights a unique buffering role for RAS against transformation in human cells. Moreover, whereas RAS induced substantial signaling pathway connectivity in mouse cells, similar connectivity in human cells was observed only upon PP2A co-inhibition.Collectively, these findings reveal that, unlike in immortalized mouse cells, RAS signaling in human cells acts as a buffer against transformation unless PP2A is inhibited. This underscores the inadequacy of mouse models in representing RAS-driven signaling and transformation in human cells. Our results highlight the need for human cancer models to revolutionize the cancer therapies.

#239 RCZY-690 and RCZY-680: Novel, highly potent, and orally bioavailable, tri-complex pan RAS-MULTI(ON) inhibitors with distinctive oral pharmacokinetic profiles, exhibiting broad anti-tumor activities in CDX models.

Celia X.-J. Chen, Lin Wang, Yu Bai, Xiaohong Liu, Qiaoni You, Jianming Bao

Rongchang Pharmaceuticals, Ltd., Shanghai, China

Alterations in RAS proteins, including KRAS, HRAS, and NRAS, are common in cancer. KRAS mutations drive 85% of RAS-driven cancers, including lethal types like pancreatic ductal adenocarcinoma (PDAC), non-small-cell lung cancer (NSCLC), and colorectal cancer (CRC). These mutations impair GTP hydrolysis, locking RAS in an active GTP-bound state that promotes unchecked cell proliferation and survival. Although KRAS G12C inhibitors like sotorasib and adagrasib show promise for NSCLC, resistance develops rapidly, and effective therapies are still lacking for over 85% of RAS-driven or RAS-amplified cancers.
Herein, we report RCZY-690 and RCZY-680 as novel and potent tri-complex RAS-MULTI(ON) inhibitors. These compounds form a tri-complex with active GTP-bound RAS proteins and cyclophilin A (CyPA) through a RAS-targeting strategy pioneered by Revolution Medicine. This approach, exemplified by the lead compound RMC-6236, has shown promise in KRAS-mutant PDAC and NSCLC patients in phase 1 study. RCZY-690 and RCZY-680 demonstrated high binding affinity to CyPA, as confirmed by surface plasmon resonance assays, and potent nanomolar EC_{50} values for binding to CyPA and KRAS mutants (G12C, G12V, G12D) in HTRF assays. Both compounds achieved potent IC_{50} values in disrupting interactions between NRAS, HRAS, and various KRAS subtypes (e.g., G12C, G12D, G12V, G12R, G12S, G13C, G61L) with the CRAF-RAS binding domain, as determined by HTRF. Their efficacy in blocking the RAS/RAF/MAPK pathway was demonstrated by sub-nanomolar IC_{50} values for suppressing p-ERK in cancer cell lines with diverse RAS mutations, including HPAC and Capan-2. CellTiter-Glo assays further revealed sub-nanomolar IC_{50} values for cellular cytotoxicity across multiple RAS-mutant human cancer cell lines. As expected, RCZY-690 and RCZY-680 were inactive in BRAF V600E-mutated and RAS-independent cancer cell lines. Both compounds exhibited favorable in vitro ADME properties. They have excellent and yet distinctive oral pharmacokinetic profiles across multiple species. At tolerable doses, oral administration of RCZY-690 and RCZY-680 showed potent anti-tumor efficacy in a KRAS G12D-mutant HPAC pancreatic xenograft model, with sustained inhibition of intratumoral p-ERK and DUSP6 levels. The superior anti-tumor efficacy may be attributed to their highly potent cellular cytotoxicity, long mouse half-lives (10.1 h and 4.8 h, respectively), and high oral exposure levels. In conclusion, RCZY-690 and RCZY-680 are tri-complex pan RAS-MULTI(ON) inhibitors with best-in-class clinical potential for treating RAS-driven solid tumors.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#24 Influence of culture conditions and donor material on phenotypic features of PDX-derived breast cancer tumoroid lines.
Ellen Klaproth[1], Julia Thiel[2], Jannik Scherer[1], Meng Dong[2], Kanstantsin Lashuk[1], **Julia B. Schueler**[1]

[1]Charles River Laboratories, Inc., Freiburg, Germany,[2]Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology and University of Tubingen, Stuttgart, Germany

Introduction: Breast cancer is one of the most common and deadly cancers among women, highlighting the need for reliable in vitro models to study tumor biology and assess therapeutic strategies. In this project, we established and characterized three breast cancer tumoroid lines (two triple negative and one HER2+), derived from patient-derived xenograft models as a robust in vitro system for cancer research: MAXFHER 2500, MAXFT 583 and MAXFTN 2988. The tumoroids were maintained under various conditions, including long-term culture, cryopreservation, and suspension adaptation, to evaluate their morphological stability and functional fidelity over time. They were characterized by histology, multiplex IHC and QuantiGene analysis. To evaluate predictivity for preclinical in vivo as well as clinical outcomes, four standard of care compounds were evaluated in the HER2 positive breast cancer model MAXFHER 2500 and compared to in vivo data of the corresponding PDX. To be able to determine tumor growth as well as efficacy of test compounds an automated image analysis script was developed to quantify tumoroid growth.
Results: The three lines showed very similar expression levels of Ki67, Her2 (both IHC) and histological architecture as compared to the donor PDX and the donor patient. The expression levels of Estrogen as well as Progestogen receptor, EpCAM and Cytokeratin 18 were comparable in the tumoroid lines and the PDX as confirmed by multiplex IHC. The different culture techniques, dome (high Matrigel concentration) vs suspension method (low Matrigel concentration) did not influence the expression of the above-mentioned markers or the histological structure of the tumoroids. It was possible to create tumoroid lines from fresh PDX as well as from viably frozen material. Cisplatin was highly active in all three tested culture conditions with IC50 values ranging from 2.9 - 9.6 µM. In contrast, the sensitivity towards Oxaliplatin was influenced by the culture conditions with IC50s ranging from 8.2 - 94.6 µM. Gemcitabine and Cisplatin efficacy in vitro was predictive for antitumoral activity in vivo. However, Paclitaxel was inactive in vitro but showed some efficacy in vivo. Discussion: Overall, this study demonstrates that tumoroid lines derived from PDX models retain key tumor characteristics, making them a valuable tool for cancer research. Although the breast cancer tumoroid lines preserved morphological and functional features under various conditions there were indications that some culture conditions do influence the sensitivity towards anticancer treatment. The observed variability underscores the importance of carefully selecting and optimizing culture conditions to maintain the predictive value of PDX derived tumoroids for breast cancer drug development.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Growth Factors, Receptors, and GTPase Signaling
Poster Session

#240 Study of MUC16/ERBB axis in pancreatic cancer.

Amina Baniya, Michael (Tony) A. Hollingsworth

University of Nebraska Medical Center, Omaha, NE

Pancreatic cancer is the third leading cause of cancer-related deaths due to its poor prognosis and highly malignant nature. About 65% of pancreatic cancer cases express the biomarker CA125, encoded by the *MUC16* gene. MUC16 is a member of mucin family of glycoproteins known to line epithelial cells for protection and lubrication and is overexpressed in several cancer types including pancreatic cancer, often linked with enhanced disease progression, metastasis, and reduced patient survival. MUC16 has a large extracellular domain containing unstructured N-terminal domain and variable number tandem repeat domain with SEA domain, and short carboxy terminal domain. TR1.2 is a recombinant peptide containing SEA domain 5. Circulating levels of MUC16/CA125 has been used as a biomarker to monitor adenocarcinoma for decades. In serum, CA125 sizes range from 200-2000 kDa and contain SEA domains. However, functional roles of CA125/MUC16 have not been elucidated. We present evidence that in addition to serving as a biomarker, circulating MUC16 has pro-tumorigenic activities by engaging ERBB receptors. We hypothesize that isoforms of MUC16 engage ErbB3 receptors, enhancing ERBB2-EGFR heterodimerization upon ligand binding to redirect oncogenic signaling in pancreatic cancer, with concomitant activation of downstream signaling cascades that include Akt. We evaluated the influence of MUC16 (TR1.2) on ErbB receptor activation, and its capacity to activate downstream signaling pathways using pancreatic cell line models. We used alphafold3 to model the binding of TR1.2 to different ERBB receptors. We found that in serum starved conditions, TR1.2 induced a transient, yet significant downregulation of ERBB3 phosphorylation, accompanied by a slight reduction in ERBB2 phosphorylation, with no observable changes in EGFR phosphorylation in time- and dose- dependent manner. By 15 hours, phosphorylation levels returned to baseline, indicating the transient changes in serum-starved conditions. In contrast, in the presence of serum-containing media, we found significant increases in ERBB2 and EGFR phosphorylation after 15 hours treatment. Alphafold3 predicted that TR1.2 binds to domain 1 of ERBB3 causing conformational changes leading to blocking of dimerization. Together, our results suggest that TR1.2 directly binds to ERBB3 to enforce a monomer conformation. This in turn allows ERBB2 to preferentially heterodimerize with EGFR in the presence of EGF-type ligands in complete media, thereby enhancing downstream oncogenic signaling through AKT. Our future directions include validation of binding of TR1.2 to ERBB3 receptor and investing the activation of alternative pathways (such as MAPK) in pancreatic cancer cell line-based models. Understanding the biological activity of MUC16 and its cleaved product (CA125) provide insight into the role of MUC16 in the autocrine setting, the tumor microenvironment and at distant organ sites to influence the metastatic niche.

#241 Clec4f is expressed in a subset of PDAC metaplastic neuroendocrine cells and alters tumor cell growth.

Bailey A. Bye[1], R. McKinnon Walsh[1], Austin E. Eades[1], Kathleen E. DelGiorno[2], Michael N. VanSaun[1]

[1]Department of Cancer Biology, University of Kansas Medical Center, Kansas City, KS,[2]Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN

Pancreatic ductal adenocarcinoma (PDAC) is a deadly disease with a 5-year survival rate of just 13%. Poor survival is due in part to late detection, as well as resistance to current standard-of-care therapeutics. PDAC tumorigenesis is a result of exocrine cell metaplasia: when stressed, acinar cells transdifferentiate to form metaplastic ducts in a process called acinar-to-ductal metaplasia (ADM). ADM is normally protective, but if metaplastic ductal cells develop a mutation in the proto-oncogene *Kras*, they can become trapped in the transdifferentiated state and form neoplastic lesions (PanINs), which can eventually develop into PDAC. Metaplastic ducts contain a myriad of cell types, including metaplastic neuroendocrine cells, which have been shown to promote neoplastic growth and PDAC tumorigenesis. In a subset of metaplastic neuroendocrine cells, we have identified upregulation of carbohydrate receptor Clec4f. In humans, Clec4f is upregulated in PDAC tumors compared to normal pancreas and correlates with poor prognosis when elevated in early stage disease. While Clec4f's function in mice has been primarily associated with glycan recognition for antigen clearance by mouse liver macrophages, its function in PDAC tumor cells is unknown. Since we have identified Clec4f expression in a discrete subpopulation of pancreatic epithelial cells associated with early stage disease, we hypothesize that Clec4f plays a novel role in regulating PDAC tumorigenesis in response to binding glycans in the tumor microenvironment. Ectopic overexpression of Clec4f (Clec4f-oe) in murine PDAC cells revealed prominent reduction of classically mitogenic pathways including MAPK (via phosphorylated ERK) and PI3K (via phosphorylated AKT) as well as elevated levels of phosphorylated c-Myc (S62), which has been shown to play a role in regulating ductal-to-neuroendocrine lineage plasticity and therapeutic resistance. Clec4f-oe also preliminarily protected PDAC tumor cells from cell death via Gemcitabine or a D-galactose-based senescence model. In accordance with other studies in macrophages, Clec4f expression in PDAC tumor cells appears to be niche dependent. We found that, in Clec4f-oe tumor cells, incubation with galactose stabilizes Clec4f expression and Clec4f-associated signaling alterations. These data suggest that carbohydrate binding by Clec4f alters growth and plasticity phenotypes in PDAC tumor cells. Future experiments will investigate the intracellular binding partners for Clec4f to interrogate its signaling function in metaplastic neuroendocrine cells in early PDAC.

#242 Differential activation of TAM receptors by Gas6 and protein S.

Ziren Wang, Samhita Bapat, Varsha Gadiyar, Christopher Varsanyi, Rachael Pulica, Trevor Frederick, Ahmed Aquib, Raymond Birge

Rutgers Graduate School of Biomedical Sciences, Newark, NJ

Tyro-3, Axl, and Mertk (abbreviated TAM receptors) comprise a homologous family of type I receptor tyrosine kinases (RTKs) have been shown to have increasingly important roles for both homeostasis, particularly for anti-inflammation and the resolution of inflammation. While previous studies have shown that TAM receptors are differentially expressed on immune cells, surprisingly much less is currently known about the differential functional activities and expression of the major TAM ligands, Growth Arrest Factor-specific gene 6 (Gas6) and Protein S (Pros1), particularly whether they have overlapping or distinct biochemical and cellular functions, and how they regulate the interplay between inflammation and coagulation. To evaluate the functions of Gas6 and Pros1, we first cloned and expressed human proteins for testing on TAM-IFNgR1 reporter cell lines. While both Gas6 and Pros1 require post-translational gamma-carboxylation of their Gla domains for biological activity and binding to phosphatidylserine (PS), they possess differential capacity to bind soluble Axl and C4BP. Moreover, both Gas6 and Pros1 require the 4 tandem EGF-like domains for their activity, and both appear to require extracellular protein disulfide isomerase activity for receptor activation. Taken together, we have identified a novel mechanism for TAM activation by Gas6 and Pros1, as well as both overlapping and non-overlapping activities with respect to ligand-dependent receptor activation. These results have potential implications in both cancer and inflammation.

#243 FLT4, a novel regulator of renal cell carcinoma progression.

Djazia Haferssas[1], Nathalie Henley[2], Jonatan Barrera Chimal[2], Casimiro Gerarduzzi[1]

[1]Pharmacology and Physiology, University of Montreal, Montreal, QC, Canada,[2]Centre de Recherche de l'hopital Maisonneuve Rosemont, Montreal, QC, Canada

Background: Clear Cell Renal Cell Carcinoma (ccRCC) is one of the ten most frequently diagnosed malignancies worldwide and constitutes 2-3% of all adult cancers. Disruption of p53 function, a critical tumor suppressor, plays a pivotal role in cancer development and resistance to chemotherapy. While p53 gene mutations are rare in ccRCC, these tumors often resist DNA-damage- based therapies, suggesting alternative mechanisms suppressing wild-type p53 in ccRCC. Therefore, reactivating this pathway represents a potential therapeutic strategy. p53 is tightly regulated by MDM2 and MDMX which are frequently elevated in ccRCC patients. However, the molecular mechanisms underlying MDM2/MDMX-mediated p53 regulation in ccRCC remain unclear. Our study identified the Receptor Tyrosine Kinase FLT4 as a novel regulator of MDM2/MDMX and p53 activity. Notably, both FLT4 and its ligand, VEGFC, are overexpressed in ccRCC patients.
Hypothesis: We propose that FLT4 activation increases the stability of the MDM2/MDMX complex, resulting in p53 inactivation and promoting ccRCC tumorigenesis.
Methods: 1) We analyzed Bulk and single cell RNAseq data of ccRCC patients from the Cancer Genome Atlas (TCGA) to assess the expression of FLT4 and VEGFC. 2) The ccRCC cell lines ACHN and Caki-2 were used for *in vitro* experiments, including VEGFC treatment for functional assays (proliferation, migration, and survival) and MDM2/MDMX/p53 protein analysis. 3) ACHN and Caki-2 cells were transduced with Luciferase and FLT4 and injected into immunodeficient NOD/SCID mice to study tumor development and metastasis using *In vivo* Imaging System (IVIS).
Results: FLT4 and VEGFC are overexpressed in ccRCC patients and correlate with cancer progression and poor survival. In addition, FLT4 activation promotes proliferation and migration both *in vitro* and *in vivo* and reduces the chemosensitivity of ccRCC cells mainly through the modulation of p53 levels. Mechanistically, FLT4 activation decreases p53 stability and activity by increasing MDM2/MDMX levels through post-translational modifications.
Conclusion: Aberrant FLT4 signaling drives ccRCC progression by suppressing p53 through the upregulation of its negative regulators MDM2 and MDMX. These findings highlight FLT4 as a potential target for restoring p53 activity and improving ccRCC outcomes.

#244 Identification of proteins in the oncogenic FLT3-ITD signaling pathway using the APEX2 system.

O. Tatli[1], M. E. Jakobsson[2], G. Mitsa[2], J. U. Kazi[3], **L. Ronnstrand**[3];
[1]Istanbul Medeniyet University, Istanbul, Turkey, [2]Acrivon AB, Lund, Sweden, [3]Lund University Faculty of Medicine, Lund, Sweden

The FLT3-ITD oncogenic mutation is commonly found in acute myeloid leukemia (AML) and correlated with a poor prognosis. In order to identify proteins within the FLT3-ITD pathway, we employed the engineered ascorbate peroxidase 2 system (APEX2). Monoclonal Ba/F3 cells stably expressing the APEX2-FLT3-ITD fusion protein were incubated with biotin-phenol and treated with H_2O_2, enabling the APEX2 enzyme to catalyze the biotinylation of proteins in close proximity to FLT3-ITD. Given that quizartinib, an FLT3 inhibitor, alters the localization of FLT3-ITD protein to the cell surface, a quizartinib-treated group was used to mimic wild-type FLT3 and designated as pseudo-wild-type FLT3. Biotinylated proteins were selectively enriched using streptavidin beads to obtain proximity proteome of FLT3-ITD. Enriched proteins were analyzed by state-of-the-art data independent acquisition (DIA) liquid chromatography-mass spectrometry (LC-MS/MS), uncovering differential associations between wild-type and oncogenic FLT3-ITD. After quantitation by mass spectrometry, the proteins were classified by KEGG enrichment analysis. Interestingly, the protein export group (mmu03060) was enriched in only FLT3-ITD protein interactors. Given that activation of differential signaling cascades relies upon the subcellular localization of FLT3, this set of proteins suggests a possible nuclear localization of FLT3. Upon categorizing the identified proteins based on their molecular function, a significant number of FLT3-ITD interactor proteins were found to be engaged in nuclear events, supporting that FLT3-ITD protein is translocated to the nucleus. Subsequently, we grouped the identified FLT3-ITD interactors based on the domains that they harbor. We selected some specific candidates of particular interest for validation by reciprocal co-immunoprecipitation (co-IP) assays. Protein kinases CDK5 and CHEK1, small GTP binding protein GSPT1, and the nuclear export protein XPO1 were tested for their interaction with FLT3-ITD. Plasmids encoding these proteins with a C-terminal FLAG-tag were co-transfected into human kidney cells with FLT3-ITD. Of these, CDK5 displayed a robust interaction with FLT3-ITD in reciprocal co-IP experiments. Besides, the CDK5 interaction of FLT3-ITD was significantly greater than that of wild-type FLT3. Thereof, we surveyed the phosphorylation dependence of FLT3/CDK5 interaction by suppressing FLT3 phosphorylation in both wild-type FLT3 and oncogenic FLT3-ITD. The inhibition of FLT3 kinase activity with quizartinib drastically reduced CDK5 interaction, revealing that CDK5/FLT3 interaction is dependent of FLT3 phosphorylation. To summarize, we leveraged exploratory proteomics to uncover a panel of candidate FLT3-ITD effectors based on their association or proximity with FLT3-ITD. Future studies will investigate the role of these proteins in FLT3-ITD-induced transformation.

#245 Preclinical development of HDM2020, a novel ADC targeting FGFR2b, in gastric cancer (GC) and squamous non-small cell lung cancer (sq-NSCLC) xenograft models.

Tingyang Wang, Liubin Guo, Kuan Lu, Hao Pan, Yang Chen, Zhaoxing Yu, Yayun Zhao, Shengxing Zhao, Qingli We, Ruizhe Wang, Yan Xia, Hongwen Li, Dongzhou Jeffrey Liu

Huadong Medicine Co., Ltd., Hangzhou, China

Introduction: FGFR2b is a novel protein biomarker detected by IHC with the overexpression rate of ~30% (any 2+/3+) in gastric or gastroesophageal junction cancer(GC/GEJC) patients, and among those patients ~40% are not eligible for currently approved targeted therapies (HER2, PD-L1, MMR and CLDN18.2)[1]. HDM2020 is a topoisomerase Inhibitor based DAR8 ADC developed using a novel Ab targeted against FGFR2b, which has the potential to treat FGFR2b overexpressing tumor with reasonable safety window[2].
Methods: *In vivo* cell line (CDX) and patient derived xenograft (PDX) models were conducted to validate the therapeutic potential of HDM2020 against GC and sq-NSCLC. FGFR2b expression were detected by IHC using various tumor paraffin sections.
Results: Combination of HDM2020 and avastin or paclitaxel demonstrated a synergistic effect in GC CDX model. Single-agent therapeutic activity of HDM2020 was demonstrated in two FGFR2b-positive GC PDX models with TP53 or BCOR gene mutation and at least 1st line chemotherapy. At a dose of 10mg/kg, the maximum tumor growth inhibition rate were 98% and 99%. In FGFR2b middle expressed sq-NSCLC PDX model, complete response was observed at single dose of 10mg/kg. The minimum effective doses were between 2.5-3mg/kg in above three models. FGFR2b is overexpressed in GC, sq-NSCLC, TNBC and may other tumor types.
Conclusion: Based on the clinical validation of FGFR2b in GC and signs of early preclinical activity in sq-NSCLC with significant unmet need, HDM2020 might has promising efficacy in these indications. HDM2020 is expected to enter clinical development in 2025.
References:1: Seiya Sato, Shannon L Rhodes, et al. Fibroblast Growth Factor Receptor 2 Isoform IIIb (FGFR2b) Protein Overexpression and Biomarker Overlap in Patients With Advanced Gastric or Gastroesophageal Junction Cancer(GC/GEJC). ESMO,2024.2: Tingyang Wang, Liubin Guo, et al. Development of a novel FGFR2b-targeting ADC with robust anti-tumor efficacy in preclinical evaluation. World ADC SAN DIEGO 2024.

#246 Innovative use of a fluorescent probe and 3D spheroids to study A3 adenosine receptor-targeted compounds in cancer cell lines.

Katerina Jecmenova, Alzběta Srovnalova, Marian Hajduch, Petr Dzubak

Palacky University in Olomouc, Olomouc, Czech Republic

Introduction: The A3 adenosine receptor (A3AR) is a promising cancer therapy targeted due to its role in tumor progression, immune regulation, and apoptosis. Its selective expression in tumors like the liver and pancreas makes it ideal for therapies with minimal off-target effects. However, live-cell imaging techniques to study A3AR, including receptor expression, membrane localization, and interactions with AR modulators, are limited. Here, we present CELT-228, an A3AR-specific fluorescent probe, as a tool for visualization and functional analysis in live cells and 3D spheroid cultures. This method also enables competitive binding assays to identify and validate new A3AR agonists and antagonists.
Material and methods: The reporter cell line ES-012-A, overexpressing A3AR, was treated with the CELT-228 (Celtarys, Spain) and imaged via spinning disc confocal microscopy (Yokogawa CV7000). Fluorescent signal localization and intensity were analyzed using Signal Image Artist (Revity). Competitive binding assays using CELT-228 were developed for High Content Screening (HCS) of newly synthesized nucleoside-based compounds and for studying A3AR interactions in cancer cell lines of diverse origins. Additionally, CELT-228 staining was applied to 3D spheroids from Hep-3B cells to investigate receptor localization under varying microenvironmental conditions.
Results: In 2D and 3D models, CELT-228 predominantly localized to cell membranes of reporter and cancer cell lines. Competitive assays showed that new compounds bind to the orthosteric site, competing effectively with CELT-228. In 3D spheroids, staining revealed A3AR distribution across different regions, including cell membranes and intracellular compartments. A3AR expression patterns varied in hypoxic, nutrient-deprived, and proliferative regions, providing insights into microenvironmental influences on A3AR regulation.
Conclusion: CELT-228 is a versatile tool for studying A3AR in 2D and 3D models, enabling receptor localization, competitive binding assays, and receptor mapping in spheroids. This approach holds great potential for A3AR-targeted drug discovery and advancing cancer therapies.
Supported by the MEYS Czech Republic: CZ-OPENSCREEN (LM2023052), EATRIS-CZ (LM2023053), SALVAGE (811100711/37), National Institute for Cancer Research (Program EXCELES, ID Project No. LX22NPO5102) and IGA_LF_2024_038.

#247 Investigating the impact of HER4 mutations on intracellular signalling pathways.

Denis M. Collins[1], Marta Valenti[2], Johanna Gaubatz[1], Birgit Bossenmaier[3], Bryan Hennessy[4], John Crown[5]

[1]Dublin City University, Dublin, Ireland,[2]Cancer Biotherapeutics Research Group, Life Sciences Institute, School of Biotechnology, Dublin City University, Dublin, Ireland,[3]Bossenmaier Consulting, Munich, Germany,[4]Department of Medical Oncology, Beaumont Hospital, Dublin, Ireland,[5]Department of Medical Oncology, Saint Vincent's University Hospital, Dublin, Ireland

Background: HER4 is the least studied member of the HER/ErbB receptor tyrosine kinase family that also includes EGFR, HER2 and HER3. HER4 is frequently mutated in skin, lung and gastro-intestinal tumours but there are limited data on the impact of HER4 point mutations (MUTs) on intracellular signalling pathways. This study identifies 8 HER4 point MUTs (2 extracellular domain (ECD) and 6 intracellular domain (ICD)) and utilises plasmid-based overexpression to assess the impact of the HER4 point MUTs on intracellular signalling pathways in HEK293 cells.

Methods: 8 HER4 point MUTs were identified using publicly available Catalogue Of Somatic Mutations in Cancer (COSMIC) data and Provean/SIFT analysis. 4 HER4 MUTs were deemed deleterious/damaging and 4 MUTs were classified as neutral or tolerated. MUT1 (p.E202D) and 2 (p.P224R) are ECD, MUT3-8 (p.T1036I, p.Y1242C, p.H1245N, p.S1246R, p.L1247M, p.H1255N) are ICD. Mutant primers were designed and constructed (Sigma Aldrich). Site-directed mutagenesis (QuikChange Lightning kit) was carried out using the pRK5 HER4 wild-type (WT) plasmid as template creating 8 HER4 MUT plasmids. Sequences were validated (SequiServe). HEK293 cells were transfected (Fugene) with either empty vector, GFP control, WT HER4 or the HER4 MUT plasmids. Transfection was confirmed by Western blot. Plasmid transfected HEK293 protein lysates were analysed in biological triplicate by reverse phase protein array (RPPA) to determine the levels of 50 total proteins and 34 phospho-proteins (n= 84 targets). Increases or decreases in protein levels were determined as significant ($p<0.05$, Student's t test) vs appropriate control, WT HER4 vs. empty vector or MUT HER4 vs WT HER4.

Results: HEK293 cells express WT EGFR and negligible levels of HER3 and HER4. Total EGFR levels were unchanged in response to WT HER4 transfection but EGFR phosphorylation at Y992 ($p=0.01$), and Y1068 ($p<0.001$) increased 15-fold and 2-fold, respectively. Total and phosphorylated Akt (S473 and T308) and MAPK-ERK1/2 (T202/Y204) were not impacted by WT HER4 transfection. Overexpression of WT HER4 resulted in increased levels of phosphorylated p38 MAPK (T180/Y182) ($p=0.04$), Src (Y416) ($p<0.001$) and STAT3 (Y705) ($p<0.001$). The 8 HER4 MUTs assessed had no impact on the activity or total expression of EGFR, Akt and Stat3. MUT2 decreased phosphorylated MAPK-ERK1/2 ($p=0.01$) and total p38 MAPK levels ($p=0.04$). MUT4 decreased phosphorylated MAPK-ERK1/2 levels ($p<0.001$). MUT6 decreased total p38 MAPK levels ($p=0.03$) and increased phosphorylated Src levels ($p=0.01$). MUT7 decreased levels of total MAPK-ERK1/2 ($p=0.03$) and total p38 MAPK ($p<0.001$).

Conclusions: WT HER4 activates EGFR and downstream signaling through p38 MAPK, Src and STAT3. The HER MUTs investigated affected MAPK-ERK1/2, p38 MAPK and Src signaling pathways, suggesting phenotypic impact that supports a role in tumorigenesis.

#248 Dissecting therapeutic mechanisms in EGFR and HER2 signaling pathways using Lumit cellular immunoassays.
Matthew R. Swiatnicki, Mindy Lin, Hicham Zegzouti

Promega Corporation, Fitchburg, WI

Due to the importance of aberrant receptor tyrosine kinase (RTK) regulation in many cancers, we sought to employ the Lumit Immunoassay Cellular System (Lumit ICS) in order to characterize signaling nodes within important RTK signaling pathways. Lumit ICS is a homogenous immunoassay for detecting targets of interest in cellular lysate. Phosphorylated or total protein can be detected in as little as two hours with a simple add and read format. In this study, we generated several Lumit assays to explore whether Lumit was capable of teasing out mechanistic differences between various drug treatment modalities. These assays include p-EGFR, p-HER2, p-cMET, and various downstream nodes such as p-AKT and p-H2AX. In *EGFR* WT cell lines, we observed increased phosphorylation of EGFR when treating cells with the ligand, EGF. This increased phosphorylation was inhibited with both small molecule inhibitors, such as erlotinib, and monoclonal antibodies, such as cetuximab. However, in mutant *EGFR* cell lines that have a level of basal EGFR phosphorylation, only small molecule inhibitors could completely inhibit EGFR phosphorylation. Cetuximab was only capable of inhibiting EGFR phosphorylation stemming from extracellular stimulation (EGF) and not basal p-EGFR. Within the *HER2* overexpressed cell line BT474, we detected a high level of basal HER2 phosphorylation. This increased phosphorylation could be inhibited by the small molecule inhibitor CP-724714. However, when treating BT474 cells with monoclonal therapies such as trastuzumab and pertuzumab, we saw a robust increase in HER2 phosphorylation at early timepoints and a more modest increase at longer treatment timepoints. We also noted differences in other cell signaling nodes when treating HER2 overexpressing cells with varying treatment modalities. When treating cells with the antibody drug conjugate (ADC) DS-8201a, we noted increased HER2 phosphorylation, as well as increased phosphorylation of the DNA damage response (DDR) mark, p-H2AX. Increased p-H2AX was not seen when treating the same cell line with trastuzumab. An increase in DNA damage response with DS-8201a treatment fits within the context of DS-8201a delivering a topoisomerase inhibitor as its payload. Finally, we noted a loss of AKT phosphorylation when treating BT474 cells with trastuzumab, but not DS-8201a. While we have yet to explore the reason for this difference in AKT phosphorylation, this result highlights the divergent effects of varying treatment modalities on downstream signaling. Overall, these data exemplify the complexity of cell signaling pathway inhibition and demonstrate Lumit ICS as being an assay capable of teasing out some of that complexity.

#249 EGFR activation reverses gastrin suppression by MENIN by inducing its nuclear export.

Uloma B. Elvis-Offiah[1], Suzann Duan[2], Sulaiman Sheriff[2], Juanita L. Merchant[3]

[1]Program in Cancer Biology, University of Arizona, Tucson, AZ,[2]Department of Medicine, Division of Gastroenterology, University of Arizona College of Medicine, University of Arizona, Tucson, AZ,[3]Program in Cancer Biology; Department of Medicine, Division of Gastroenterology, University of Arizona, Tucson, AZ

Introduction: Gastroenteropancreatic neuroendocrine tumors (GEP-NETs) are rapidly increasing malignancies in the US. They are strongly associated with loss-of-function mutations in the *Multiple Endocrine Neoplasia type 1 (MEN1)* gene which encodes the tumor suppressor MENIN. Gastrinomas secrete excess gastrin, representing the most malignant subtype of sporadic or hereditary GEP-NETs. Although MENIN epigenetically inhibits *gastrin* gene expression, ligand-receptor activation inducing *GASTRIN* removes MENIN suppression. However, the mechanism by which MENIN suppression is alleviated remains poorly defined. Exon 10 encodes all three nuclear localization signals (NLSs) of MENIN within its C-terminal domain. Moreover, NLS1 harbors a phosphorylation motif for protein kinase B, which mediates epidermal growth factor receptor (EGFR) signaling. Since MEN1-gastrinomas predominantly arise within the submucosal Brunner's glands rich in pro-proliferative EGFR ligands like epiregulin (EREG), we hypothesized that EGFR activation induces *GASTRIN* by reducing nuclear MENIN.

Methods: We subcloned wild-type (WT) *MENIN* cDNA and the sequential C-terminal deletions into pcDNA carrying the FLAG epitope and validated with sequencing. Regulation of endogenous *GASTRIN* mRNA was determined after transfection of MENIN vectors into two human gastric cancer cell lines—AGS and KATOIII. The effect of EGFR signaling on MENIN's subcellular localization, stability, and repression of the gastrin gene was determined by immunoblotting, subcellular fractionation, immunofluorescent staining, and RT-qPCR.

Results: WT MENIN and constructs retaining 2 or 3 nuclear localization signals (NLSs) suppressed 50% of *GASTRIN* expression under basal conditions. Moreover, deleting all three NLSs abolished MENIN suppression. EREG treatment induced *GASTRIN* in the absence of the presence of WT or MENIN without 1-2 NLS. However, no induction of GASTRIN was observed with all 3 NLSs deleted. Constructs lacking all 3 NLSs exhibited reduced protein expression, which was reversed by the proteasome inhibitor MG132, suggesting decreased protein stability. NLS-deficient MENIN was highly localized to the cytoplasm whereas WT MENIN and deletions with >1 NLSs were distributed between the nucleus and cytoplasm under basal conditions. However, EREG treatment induced their cytoplasmic translocation, suggesting that EGFR signaling blocks MENIN's nuclear localization.

Conclusion: EGFR activation induces *GASTRIN* gene expression by preventing nuclear import which requires the C-terminal NLSs.

#250 TMEM97/sigma 2 receptor increases androgen receptor activities to regulate prostate cancer cell growth and sensitivity to castration therapy.

Daotai Nie, Xiangwei Fang, Yuanqin Zhang, Jiuhui Wang

Southern Illinois University School of Medicine, Springfield, IL

Prostate cancer is one of the most common cancers in men. Suppression of androgen receptor (AR) activity by androgen depletion or direct AR antagonist treatment, although initially effective, leads to emergence of castration-resistant prostate cancer. Sigma 2 receptor (σ2R) has been implicated in carcinogenesis including prostate cancers. Its molecular identity remained elusive until 2017 that transmembrane protein 97 (TMEM97) was identified as the *bona fide* sigma 2 receptor. Here we report that TMEM97/σ2R expression was elevated in prostate tumors of higher malignancy and advanced stages, and was associated with reduced survival of prostate cancer patients. Via in-vitro studies, increased expression of TMEM97 enhanced prostate cancer cell proliferation, clonogenicity, and resistance to enzalutamide. Knockdown of σ2R expression reduced prostate cancer cell growth and clonogenicity, and sensitized LNCaP and castration resistant 22Rv1 cells towards enzalutamide. There was a positive correlation between TMEM97 and AR expressions in prostate tumors. Knockdown of σ2R expression reduced the protein levels of AR and its AR-V7 variant under hormone deprivation condition. Decreased TMEM97 expression also downregulated AR activities as evidenced by reduced expression of AR target genes. PB-28, a known σ2R agonist, can stimulate AR target genes expressions while antagonist decreased them. SM21, a σ2R antagonist, presented synergism when combined with enzalutamide in the treatment of prostate cancer cells. Our findings suggest a potential approach targeting TMEM97/σ2R to reduce castration resistance.

#251 Resistance to androgen receptor pathway inhibitors is mediated by a pro-survival stress response converging on altered mitochondrial energetics.

Shibjyoti Debnath[1], Kathryn Ware[1], Bea Thomas[1], Kelsey H Fisher Wellman[2], Andrew Armstrong[1], Jason Somarelli[1]

[1]Duke University, Durham, NC,[2]East Carolina University, Greenville, NC

Purpose: The current front-line therapies for men with metastatic prostate cancer are novel androgen receptor pathway inhibitors (ARPIs), such as abiraterone acetate or enzalutamide, which target the androgen receptor (AR) pathway. These therapies significantly prolong overall survival; however, acquired resistance to these drugs is common over several years. Therefore, there is a major unmet clinical need to identify mechanisms of resistance and innovative therapies to treat this therapy-resistant, metastatic disease.
Experimental Procedures: To identify common molecular mechanisms of acquired ARPI resistance we developed a panel of three enzalutamide-resistant (enzaR) cell lines, LNCaP-enzaR, CS2-enzaR, and LN95-enzaR, by chronic, long-term exposure to increasing doses of enzalutamide. Analysis of the genome, transcriptome and phospho-proteome of enzaR prostate cancer cell lines were evaluated by comparative genomic hybridization (aCGH), RNA-seq and reverse phase phospho-protein arrays. Oxygen consumption rate was measured by Seahorse assays. To decipher the correlation between stress response and ATP hydrolysis, we have utilized small molecule p38 inhibitor (SB203580) to test if p38 is related to ATP hydrolysis and validated the result by western blot. The functional role ATPIF1 was deciphered by ATPIF1 siRNA transfection in LNCaP cells.
Results: Our genomic and functional studies have pinpointed a pro-survival cellular stress response as convergently activated during the acquisition of AR-dependent enzalutamide resistance. This pro-survival stress response leads to induction of metabolic quiescence and activation of p38/MAPK signaling, which triggers reversal of mitochondrial ATP synthase. Instead of canonical “forward” ATP synthase activity to generate ATP, the ATP synthase runs in “reverse”, hydrolyzing ATP to maintain a proton gradient across the mitochondrial membrane and permit survival. These features create collateral sensitivity to organic cations that target non-canonical polarization of the mitochondrial membrane that is critical to cellular persistence. In short, our findings support the hypothesis that AR inhibition induces a pro-survival response characterized by mitochondrial energetic changes that can be targeted as novel treatments for AR therapy-resistant prostate cancer.
Conclusion: We have discovered non-canonical ATP synthase reversal as a key targetable mechanism by which cancer cells maintain mitochondrial polarization in the face of therapy-induced resource depletion to sustain cell survival. Future studies are aimed at clinical translation of novel agents to target these persister populations.

#252 IRS2-driven modulation of PI3K/AKT signaling and tumorigenesis in estrogen receptor-positive breast cancer.

Seonmin Lee[1], Ju Hee Kim[2], Han-Byoel Lee[3], Wonshik Han[3]

[1]Seoul National University Cancer Research Institute, Seoul, Korea, Republic of,[2]Biomedical Research Institute, Seoul National University Hospital, Seoul, Korea, Republic of,[3]Department of Surgery, Seoul National University College of Medicine, Seoul, Korea, Republic of

Introduction
Insulin receptor substrate 2 (IRS2) is a critical adaptor protein mediating intracellular signaling pathways downstream of insulin and insulin-like growth factor 1 (IGF1) receptors. Through its involvement in the PI3K/AKT signaling cascade, IRS2 regulates key processes essential for tumor growth and progression. While high IRS2 expression is linked to tumor progression, low IRS2 expression is associated with poorer overall survival, particularly in estrogen receptor-positive (ER+) breast cancer. However, the underlying mechanisms remain unclear, prompting this investigation into the role of IRS2 in ER+ breast cancer.
Method
IRS2 expression levels and overall survival across subtypes were analyzed using TCGA and METABRIC public data. IRS2 expression levels were analyzed by Western blot and qRT-PCR. In the ER+ MCF7 cell line, IRS2 knockdown was performed using shRNA, and its effects on tumor cell proliferation, migration, and invasion were assessed via MTT, wound healing and transwell assays. To examine the impact of IRS2 on the IGF1 pathway, cells were treated with IGF1, and Western blot was used to analyze alterations in PI3K/AKT signaling and EMT marker expression following IRS2 knockdown. Tumorigenesis and metastasis were studied using fat pad and tail vein injections in NOD.SCID mice.
Results
Analysis of METABRIC and TCGA patient data revealed that in the Luminal A subtype, lower IRS2 expression correlated with poorer overall survival. Among the 12 breast cancer cell lines, the ER+ MCF7 cell line, which exhibited relatively high IRS2 expression, was selected for shRNA knockdown experiments. IRS2 knockdown resulted in reduced cell proliferation, migration, and invasion. Time-dependent IGF1 treatment revealed reduced p-AKT levels following IRS2 knockdown, demonstrating suppression of IGF1-mediated PI3K/AKT signaling. EMT marker analysis revealed reduced expression of mesenchymal markers associated with cancer metastasis. Animal experiments suggested a potential reduction in tumorigenesis and metastasis; however, results were inconclusive and required further validation.
Conclusion
Our findings demonstrate that IRS2 knockdown reduces proliferation, migration, and invasion, emphasizing its critical role in tumor cell functions. IRS2 depletion also suppressed IGF1-induced activation of the PI3K/AKT pathway and downregulated mesenchymal EMT markers, highlighting its role in cancer progression and metastasis. However, the significant correlation between low IRS2 expression and poorer patient outcomes underscores the need for further investigation into its clinical implications.

#253 Diet induced insulin resistance is due to induction of expression of tumor suppressor pten.

Radha Mukherjee, Priya Pancholi, Malvika Sharma, Hilla Solomon, Merna N. Timaul, Vladimir Markov, Elisa de Stanchina de Stanchina, Kiran Vanaja, Neal Rosen

Memorial Sloan Kettering Cancer Center, New York, NY

The PI3K/AKT/mTOR pathway is a key regulator of insulin and metabolic homeostasis, proliferation, growth, and apoptosis. While AKT regulates key processes like glucose uptake, mTOR is a master regulator of cap-dependent translation through its substrates S6K and 4E-BP. This pathway also plays a key role in tumorigenesis- with high incidence of activating mutations in the oncogene *PIK3CA* and loss of function alterations of tumor suppressor and lipid phosphatase *PTEN*. **Most recently our work led to the discovery that the translation of PTEN lipid phosphatase, is regulated by PI3K-mTOR regulated cap dependent translation, constituting a feedback loop.** Pharmacologic inhibition by PI3K inhibitors reduces PTEN protein leading to a rebound in PI3K signaling that reduces the efficacy of the PI3K inhibitors. Insulin resistance is a condition which arises due to inadequate response of the PI3K/AKT signaling to insulin, demanding increasing amount of insulin to elicit the same degree of AKT activation. Onset of insulin resistance is closely associated with a variety metabolic aberrations like type 2 diabetes, obesity and liver steatosis. Insulin resistance and Type 2 Diabetes are characterized by hyperinsulinemia, hyperglycemia with severe cases ending up in insulin deficiency due to pancreatic beta cell exhaustion. There is also a strong association of insulin resistance and increased risk of developing cancer and resistance to cancer therapy especially in breast and endometrial cancers that are mutant for PI3K/PTEN. Currently, little is known about the early kinetics and molecular changes in PI3K/AKT signaling that may allow for the reduced insulin action and therefore progression of insulin resistance. We speculated that insulin resistance is due to insulin dependent induction of its negative regulator PTEN protein that prevent further increases in PI3K signaling. Here we show that in a diet induced model of obesity and insulin resistance, mouse fed on high fat diet, PTEN levels are increased in fat, muscle and liver tissues. Onset of hyperinsulinemia and PTEN induction in tissue is followed by hyperglycemia, hepatic steatosis and severe glucose intolerance. Treatment with a PTEN phosphatase inhibitor prevents and reverses these phenotypes, whereas an mTORC1 kinase inhibitor reverse all but the hepatic steatosis. These data suggest that induction of PTEN by increasing levels of insulin elevates feedback inhibition of the pathway to a point where downstream PI3K signaling is reduced and hyperglycemia ensues. PTEN induction is thus necessary for insulin resistance and the type 2 diabetes phenotype and a potential therapeutic target. This was accompanied by a significant increase in weight, hyperinsulinemia, hyperglycemia, insulin resistance and liver lipidosis. Inhibition of PTEN activity or its translation by inhibiting mTOR, both prevented and reversed the development of insulin resistance and obesity phenotypes

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Mechanisms of Oncogenesis and Tumor Suppression
Poster Session

#257 The E3 ligase HECTD4 regulates COX-2 dependent tumor progression and metastasis.
J. Vuille, **C. Tanriover**, D. S. Micalizzi, R. Y. Ebright, S. Animesh, R. Morris, S. Hajizadeh, Z. J. Nicholson, H. C. Russell, E. F. Zaniewski, B. S. Wittner, B. K. Wesley, J. Grunewald, R. N. Szalay, E. Antmen, D. B. Fox, M. Yang, J. Joung, D. C. Gulhan, A. E. H. Elia, W. Haas, E. Oh, S. Maheswaran, D. A. Haber;
MGH/Harvard Medical School, Boston, MA

E3 ubiquitin ligases mediating turnover of proteins engaged in cancer progression point to key regulatory nodes. To uncover modifiers of metastatic competency, we conducted an *in vivo* genome-wide CRISPR-inactivation screen using cultured breast circulating tumor cells, following intravascular seeding and lung colonization. We identified *HECTD4,* a previously uncharacterized gene encoding a conserved potential HECT domain-containing ubiquitin transferase, as a potent tumor suppressor. We show that purified HECTD4 mediates ubiquitin conjugation *in vitro,* and proteomic studies combined with ubiquitin remnant profiling identify a major degradation target as the prostaglandin synthetic enzyme cyclooxygenase-2 (COX-2; *PTGS2*). In addition to COX-2 itself, HECTD4 targets its regulatory kinase MKK7. In breast cancer models, HECTD4 expression is induced as cells lose adherence to the matrix, and its depletion massively increases COX-2 expression, enhancing anchorage-independent proliferation and tumorigenesis. Genetic or pharmacologic suppression of COX-2 reverses the pro-tumorigenic phenotype of *HECTD4*-depleted cells. Thus, *HECTD4* encodes an E3 ubiquitin ligase that downregulates COX-2 suppressing anchorage-independence in epithelial cancer cells.

#258 RUNDC1 is required for p53-mediated tumor suppression.

Atul Ranjan[1], Elizabeth Thoenen[1], Mrinalini Bhosale[1], Dipesh Thapa[2], Alejandro Parrales[1], Tomoo Iwakuma[1]

[1]Division of Hematology & Oncology, Department of Pediatrics, Children's Mercy Research Institute, Kansas City, MO,[2]Department of Cancer Biology, The University of Kansas Medical Center, Kansas City, KS

The tumor suppressor p53 is a transcription factor whose downstream target genes are involved in the regulation of the cell cycle and apoptosis, thus leading to tumor suppression. In many cancers, wild-type p53 activity is inhibited. Small molecule inhibitors of MDM2 (a negative regulator of p53), like Nutlin-3a which activates p53 by binding to MDM2, induce tumor suppression. Although p53-dependent tumor suppression is well studied, crucial factors required for the efficacy of p53-activating agents remain unknown. Toward this goal, we conducted a human whole-genome lentiviral shRNA screening in wild-type p53 carrying cells treated with Nutlin-3a. We identified RUNDC1 (Run domain containing 1) as one of crucial factors that were required for Nutlin-3a-mediated inhibition of colony formation in a p53-dependent manner. RUNDC1 is a poorly characterized protein, and its role in cancer is still unknown. Here, we demonstrated that downregulation of RUNDC1 attenuated p53-mediated cell cycle arrest. As a mechanism, RUNDC1 bound to p53 and enhanced p53's binding to the p53-responsive elements in the promoter regions of p53 target genes. Our study, for the first time, reveals the biological function of RUNDC1, and its functional association with p53, suggesting the possibility that RUNDC1 contributes to p53-mediated tumor suppression.

#259 Germline p53 R181 mutants and DNA binding cooperativity in tumorigenesis.

R. Moses[1], A. Indeglia[2], A. S. Levine[3], R. Hausler[1], G. Kelly[1], S. A. Miller[4], M. Heller[1], A. N. Le[5], W. Kohlmann[6], A. Naumer[6], J. Vagher[6], S. H. Cahill[3], L. D. Maese[6], J. Karanicolas[4], J. E. Garber[3], M. E. Murphy[7], K. N. Maxwell[1];
[1]University of Pennsylvania, Philadelphia, PA, [2]Harvard University, Boston, MA, [3]Dana-Farber Cancer Institute, Boston, MA, [4]Fox Chase Cancer Center, Philadelphia, PA, [5]Philadelphia College of Osteopathic Medicine, Philadelphia, PA, [6]Huntsman Cancer Institute, Salt Lake City, UT, [7]Wistar Institute, Philadelphia, PA

TP53 is the most frequently mutated gene in cancer, and its encoded protein p53 has many tumor-suppressive functions. There are several different classes of mutant p53 acquired in human cancer and inherited in cancer-prone families with Li-Fraumeni Syndrome, where individuals have an 80-90% increased risk of cancer. These include structural mutations that are generally misfolded (R175H), DNA contact mutations (R273H); and oligomerization mutations (A347D). A new class of variants called “cooperativity” mutations affect the binding of the p53 tetramer to DNA via disruption of a salt bridge formed by the negatively charged glutamic acid (E) 180 residue of one p53 monomer and the positively charged arginine (R) 181 residue of another. p53 cooperativity mutations at the R181 residue (R181H and R181C) have been increasingly identified in cancer-prone families undergoing genetic testing; however, the mechanism by which these variants disrupt p53 tumor suppression in humans is not understood. We show that R181H and R181C have significantly diminished ability to transactivate a curated set of ~300 known p53 target genes in CRISPR knock-in colorectal and breast cancer cell lines. This loss of transactivation ability is not explained by defects in p53 structure or oligomerization, but through reduced cooperative binding to p53 target sites on DNA as determined by fluorescence polarization and chromatin-immunoprecipitation experiments. Interestingly, we observe residual apoptotic activity in R181H and R181C mutant cells upon DNA damage, despite the poor transcriptional activation of p53’s proapoptotic targets. We observe increased p53 at the mitochondria for transcription-independent apoptosis in R181-mutant cells. Understanding the tumor suppressive activities that are lost versus retained will guide the definition of the pathogenicity of R181 variants, which account for 5000 cancer cases per year in the United States.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#26 Developing PDX derived organoid models for efficacy evaluation of anticancer therapies.
W. Zhang, X. Zhao, Y. Chang, Y. Jiang, F. Jia, Z. Sun, J. Li, X. Zhang, X. Yan, Q. Zhou, D. Zhang, Y. Guan, X. Mei, F. Yan, C. Zhao, J. Liao, Z. Liu, Q. Mei, J. Zhang, Y. He, X. Zhang, J. Zhang, W. Sun, B. Cao;
Shanghai Medicilon Inc., Shanghai, China

BACKGROUND AND PURPOSE: Developing the most fitting models that best reflect real patient tumor system is crucial for advancing cancer mechanistic research and mediating drug discovery. Patient derived xenograft (PDX) models can accurately reflect cancer responses to therapies, thus being recognized as a gold standard in oncology drug development. However, PDX models can be labor intensive, time-consuming and costly. Cancer organoid is 3D infinitesimal derived from cancer stem cells, which can faithfully recapitulate genetic profile, mutational landscape, phenotypic and histo-pathological features and responses to therapies of patient tumors. Organoids derived from PDX tumors, namely PDXOs, are suitable for in vitro anticancer drug efficacy evaluation, forming good combinations with their in vivo PDX counterparts. This work aims to develop PDXO models from our PDX biobank for anti-cancer drug efficacy evaluation uses.
EXPERIMENTAL APPROACH: PDXOs were developed using Matrigel dome embedding approach with fresh cell digests from PDX tumor tissues. The model development included continuous expansion for >3 passages followed with cryopreservation and downstream analysis. Each model was assessed using bright field imaging (BFI) and H&E staining. Whole exome sequencing (WES) and mRNAseq were used to assess the genetic status. Immunocytochemical staining (ICC) and flow cytometry were used to assess the biomarker expression. CellTiter-Glo (CTG) assay and LDH release assay were used for drug efficacy evaluation.
KEY RESULTS: By 2024-Oct, we have established >20 PDXOs, covering different cancer types, including colon cancer, gastric cancer, pancreatic cancer, lung cancer, liver cancer, etc. We have shown that the exonic mutational status and drug response behavior of PDX and PDXO counterparts were consistent, and CTG assay and BFI were suitable for drug efficacy evaluation of PDXOs. We have also shown that PDXOs with particular protein expression features were suitable for evaluating the corresponding targeted therapies. Moreover, co-culture with PBMCs or other immune cells could be used to assess the anti-cancer efficacy of immune therapies on PDXOs.
CONCLUSIONS AND IMPLICATIONS: Our work illustrates the significant potential and value of PDXO models in assessing various kinds of anti-cancer therapies. An integrated platform combining PDX and PDXO models will advance cancer pharmacological research and drug discovery.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Mechanisms of Oncogenesis and Tumor Suppression
Poster Session

#260 PDCD10 regulates Wnt activation-induced intestinal adenoma formation by promoting Wnt dependent stem cell function.

Trisha Mondal[1], Sneha Kesaraju[1], Li Dong[1], Alex Ellerstein[1], Tong-Chuan He[2], Evgeny Izumchenko[3], Christopher R. Weber[1], Le Shen[1]

[1]Department of Pathology, The University of Chicago, Chicago, IL,[2]Department of Orthopedic Surgery and Rehabilitation Medicine, The University of Chicago, Chicago, IL,[3]Section of Hematology and Oncology, Department of Medicine, The University of Chicago, Chicago, IL

The goal of this study is to understand how PDCD10 regulates early steps of tumor development by using genetically modified mice and organoids.

BACKGROUND: Programmed cell death 10 (PDCD10) is an adaptor protein critical for vascular development. Germline loss of function mutations of PDCD10 cause familial cerebral cavernous malformation. While some studies show that upregulated PDCD10 increases cancer aggressiveness, others show that the loss of PDCD10 promotes tumor progression. Given these conflicting reports, the role PDCD10 plays in tumorigenesis and cancer aggressiveness remains largely elusive. Furthermore, as most of the previous studies were performed using established 2D adapted tumor cell lines, how PDCD10 affects the early stages of tumorigenesis has not yet been investigated. In this study, we aimed to delineate how PDCD10 expression affects early stage of tumor development in the intestine using genetically modified mice.

METHODS: Intestinal stem cell (ISC) and intestinal epithelial cell (IEC) specific *Pdcd10* deletion mice were generated by crossing *Olfm4-EGFP-CreERT2* and *Vil-CreERT2* mice with *Pdcd10*floxed mice, respectively. *Ctnnb1* (β-catenin encoding gene) exon 3 floxed mice were crossed with these mice to induce Wnt activation. IEC organoids from these mice were generated and characterized. Histological and molecular analyses were performed using samples obtained from mice and organoids.

RESULTS: Tamoxifen-induced Wnt activation in *Olfm4-EGFP-CreERT2 Ctnnb1* exon 3 floxed mice caused progressive weight loss, leading to animal death. These changes were limited by *Pdcd10* deletion. Histological assessment revealed that while Wnt activation induced significant adenoma formation, particularly in proximal small intestine, very limited adenoma was present in Wnt activated mice with *Pdcd10* deletion in ISCs. Lineage tracing studies using mice with tamoxifen-induced IEC specific *Pdcd10* deletion showed that *Pdcd10* deletion reduced ISC function. This ISC defect was associated with reduced expression of intestinal stem cell markers, including *Lgr5*, *Olfm4*, and *Lrig1*, along with decreased expression of *Axin2* and other Wnt signaling marker genes. Studies using IEC organoids derived from *Pdcd10* floxed mice indicated that 4-hydroxytamoxifen-induced *Pdcd10* deletion limited organoid growth, and reduced ISC marker and Wnt signaling marker gene expression. Induced Wnt activation in organoids by CHIR99021 rescued *Pdcd10* deletion-induced organoid growth defect, and lineage tracing studies showed that *Ctnnb1* exon 3 deletion reverses ISC defects in *Pdcd10* deleted mice.

CONCLUSION: PDCD10 is required for optimal Wnt signaling to maintain ISC function and participates in Wnt-induced intestinal adenoma formation. These results demonstrate that targeting PDCD10 function and related signaling pathways may serve as therapeutic targets to limit cancer progression.

#261 Blocking AKT2 downregulates SOX9/Notch signaling inhibiting PTEN-null driven liver tumorigenesis.

Qi Tang, Jingyu Chen, Guo Zhang, Ielyzaveta Slarve, Ni Zeng, Lina He, Diala Alhousari, Phillip Nguyen, Shunan Hu, Shefali Chopra, Liyun Yuan, Bangyan L. Stiles

USC - University of Southern California, Los Angeles, CA

Primary liver cancer, including hepatocellular carcinoma (HCC) and Cholangiocarcinoma (CCA), arise from the neoplastic transformation of hepatocytes and cholangiocytes in the liver. Their association with liver cancer stem-like cell (LCSC) makers presents a more aggressive behavior and high lethal rate. PTEN is a tumor suppressor gene that negatively regulates the PI3K/AKT pathway. Downregulation of *Pten* is observed in up to 70% of the cases in CCA and 50% of the cases in HCC. In mouse models lacking PTEN, CCA develops in 100% of the mice at 12 months of age regardless of whether the whole liver cells (LiPTEN, $Pten^{loxP/loxP}$; Alb-Cre^{+}), hepatocytes-only (HpPTEN, $Pten^{loxP/loxP}$; $R26R^{YFP}$; AAV8-TBG-Cre), or cholangiocytes-only (ChoPten, $Pten^{loxP/loxP;}$ $R26R^{YFP}$; SOX9-$Cre^{ERT)}$ are targeted. Treatment of these mice with DDC at 3 months to obstruct the bile duct leads to an earlier CCA oncogenesis at 6 months of age. Using YFP to follow the lineage of tumor cells in the HpPTEN and ChoPTEN mice, we found that PTEN loss allows for transdifferentiation from cholangiocytes to hepatocytes or vice versa. To explore the mechanism for how PTEN regulates cell fate, we focused on SOX9, a transcription factor that plays a critical role in embryonic development and is believed to be the downstream target of NOTCH signaling. Hyperactivation of the SOX9 and NOTCH signaling are observed at the tumor stage of all the PTEN loss mouse models. We showed that knocking down SOX9 with siRNA reduced the sphere-forming ability of liver cells established from the mice mentioned above, indicating SOX9 promotes LCSC proliferation and sustains the tumorigenic potential of these liver cells. Gain-of-function assays of the Notch signaling through exposure to Jag1 ligand-coated extracellular matrix or transfection of Notch intracellular domain (NICD) both induced the expression of SOX9 significantly. The treatment of *Pten*-deleted mice with DAPT to inhibit NOTCH signaling showed concurrently decreased expression of Notch target Hes1 and SOX9. We also addressed the roles of AKTs in this PTEN-regulated CCA development. Our data show that deletion of *Akt2* in the LiPTEN mice (LiPTEN-A2, $Akt2^{loxP/loxP}$; $Pten^{loxP/loxP}$; Alb-Cre^{+}) significantly attenuates tumor development. Compared with the LiPTEN mice, loss of AKT2 in LiPTEN-A2 mice robustly and significantly reduces the expression of SOX9/NOTCH signaling and manifests a bile duct malformation at 12 months of age. Together, this finding suggests NOTCH activation regulates SOX9 and collaborates with PTEN loss to drive liver cancer progression, and inhibiting AKT2 specifically can block HCC-CCA formation through SOX9/NOTCH downregulation.

#262 APOBEC4, a novel regulator of p53-dependent cell cycle arrest, senescence, and apoptosis.

Dipesh Thapa[1], Atul Ranjan[2], Elizabeth Thoenen[2], Alejandro Parrales[2], Shigeto Nishikawa[3], Jay Vivian[2], Tomoo Iwakuma[2]

[1]Department of Cancer Biology, The University of Kansas Medical Center, Kansas City, KS,[2]Department of Pediatrics, Children's Mercy Research Institute - Kansas City, Kansas City, MO,[3]Department of Thoracic Oncology, Kyoto University, Kyoto, Japan

Introduction: The tumor suppressor protein p53 (p53) is a transcription factor that can transactivate downstream target genes like *p21, PUMA, NOXA, BAX,* and *GADD45a* to cause cell cycle arrest, senescence, apoptosis, and other biological processes. However, its function is frequently compromised in tumorigenesis, with approximately half of human cancers harboring mutations in the TP53 gene. Notably, even in cases retaining wild-type p53 (wtp53), it gets degraded or inhibited through various mechanisms, including, the overexpression of its E3 ubiquitin ligase MDM2. MDM2 antagonists such as Nutlin-3a are promising strategies to restore p53 function and suppress tumor growth. However, the factors/biomarkers regulating the efficacy of MDM2 antagonists and p53-mediated tumor suppression remain unclear. Identifying such factors and understanding their functions are important to develop optimal therapeutic strategies targeting p53.

Experimental procedures: Through a human whole-genome shRNA library screen, we attempted to identify factors regulating p53-mediated tumor suppression by treating osteosarcoma U2OS cells with an MDM2 antagonist. This screening and subsequent validation identified APOBEC4 as a factor that is required for p53-mediated suppression of colony formation following Nultin-3a treatment.

Summary of data: Knocking down *APOBEC4* mitigated Nutlin-3a-induced activation of p53 as a transcription factor, as well as p53-mediated cell cycle arrest and apoptosis in multiple p53-proficient cancer cells. Cellular senescence induced by H_2O_2 was also reduced upon APOBEC4 knockdown. Moreover, APOBEC4 overexpression enhanced x-ray irradiation-induced apoptosis in a manner dependent on p53.

Conclusion: Our data suggest that APOBEC4 is a novel regulator required for p53-dependent cell cycle arrest, cellular senescence, and apoptosis.

#263 Histone hypoacetylation of CD9 mediates differentiation arrest and immune evasion in pediatric acute myeloid leukemia.

Yaqun Xu[1], Kathy Yuen Yee Chan[1], Qiwei Sun[1], Yixuan Wu[1], Siu Ping Fok[1], Qi Zhao[2], Gernot Schabbauer[3], Kam Tong Leung[1]

[1]The Chinese University of Hong Kong, Shatin, NT, Hong Kong,[2]University of Macau, Taipa, Macao,[3]Medical University Vienna, Vienna, Austria

Acute myeloid leukemia (AML) is an aggressive hematologic malignancy characterized by impaired terminal differentiation. Despite intensive treatment regimes, the clinical outcomes remain suboptimal. Here, we report a new mechanism involving the tetraspanin protein CD9 in orchestrating differentiation arrest and immune escape in pediatric AML.

The expression of CD9 on blasts of AML patients (12.5%, n=115) was significantly lower than those of ALL patients (93.6%, n=343, $P<0.0001$) or hematopoietic stem cells from normal bone marrow donors (48.4%, n=22, $P=0.004$). Among AML cases, 43 patients (37.4%) were CD9+, with 5-year event-free survival rate significantly higher than CD9- patients (58.1% *vs*. 38.3%, $P=0.040$).

A marked decrease of H3K9/27Ac occupancy in the *CD9* locus was observed in AML than in ALL cells, which strongly correlated with CD9 repression ($r=0.585$-0.719, $P<0.01$). Exposure of CD9- AML to the HDAC inhibitor panobinostat significantly elevated CD9 mRNA and protein level, restored histone acetylation marks, and suppressed myeloblast proliferation *ex vivo* (median IC50: 21.4 nM). Compound library screen with a collection of 896 epigenetic agents confirmed focal hypoacetylation as the key driver of CD9 silencing. Enforced CD9 expression in MV4-11 cells significantly suppressed proliferation and colony formation. NOD/SCID mice transplanted with CD9+ cells exhibited reduced leukemic load, prolonged survival duration ($P<0.001$), and a marked regression of extramedullary sarcoma.

RNA-seq of 114 pediatric AML revealed decreased stemness and increased monocyte gene signatures in CD9+ samples. Concordantly, we observed a profound up-regulation of CD9 with the appearance of lineage markers (CD14, CD36, CD86, CD115, CD300e) in monocytic-supportive *ex vivo* culture. The gain of CD9 in primary myeloblasts (n=13) consistently augmented monocytic markers. Transcriptional network analysis revealed CD9+ AML exhibited enhanced antigen processing and presentation. Confirmatory overexpression of CD9 promoted basal and IFNγ-induced MHC-I/II expression through JAK2/STAT5 axis. Interestingly, CD9 physically bound to MHC-I/II and formed an immune complex on the membrane. In NSG mice, co-transplantation of human PBMCs mounted an effective immunity against CD9+ but not CD9- AML, concomitant with a robust BM infiltration of GZMB+ cytotoxic T cells as revealed by single-cell transcriptomics and CyTOF deep T cell profiling. Importantly, panobinostat priming rejuvenates immune sensitization via CD9 transcriptional activation in humanized mice, resulting in robust leukemia clearance.

Taken together, our data provided molecular, cellular and clinical evidence showing CD9 as a key driver intertwining monocytic differentiation and immune recognition in pediatric AML, and inspired a new combinatorial epigenetic/immunotherapy for this rare but aggressive malignancy.

#264 The E3 ubiquitin ligase WWP2 regulates stability of the chromatin remodeler ARID1B.

Pradipta Hore, Sandipkumar Bambhaniya, **Murali Dharan Bashyam**

Ctr. for DNA Fingerprinting & Diagnostics (CDFD), Hyderabad, India

The Switch/sucrose-non-fermentable (SWI/SNF; also known as BRG1 associated factors (BAF)) chromatin remodeling complex functions to slide/evict nucleosomes thus making DNA available for multiple DNA-protein interactions to facilitate cardinal nuclear processes. Work during past two decades has revealed the cardinal role of the BAF complex in modulating tumorigenesis. ARID1B, a well-known tumor suppressor and a key subunit of the cBAF complex, is frequently mutated across various cancer types. Although the downregulation of *ARID1B* transcript levels has been observed in many cancers, our knowledge about the regulation of ARID1B at the protein level remains meagre. Here, using BioGRID (Biological General Repository for Interaction Datasets) and PDC (Proteomic Data Commons) datasets, multiple ubiquitination sites were predicted to be localized at the C-terminal end of ARID1B. We performed affinity purification followed by mass spectrometry for ARID1B and further mined previously published ARID1B interactome to identify a comprehensive list of potential ARID1B interactors that included several hitherto unidentified proteins. More importantly, this analysis identified WWP1 and WWP2, well characterized E3 ubiquitin ligases, as interacting partners of ARID1B. Interestingly, WWP2 is known to modulate levels of BAF155, another SWI/SNF subunit, via poly-ubiquitination mediated proteasomal degradation. We confirmed the interaction of ARID1B with both WWP1 and WWP2 through bi-directional pull down-immunoblotting assays. Domain mapping of the interaction is currently underway. Ectopic expression of WWP2 resulted in significant downregulation of ARID1B levels. Further, cycloheximide chase assay revealed that wild-type (but not catalytically inactive) WWP2 modulates ARID1B protein stability. Specifically, WWP2 facilitates the K48-linked ubiquitination of ARID1B, leading to the latter's subsequent proteasomal degradation. Interestingly, silencing WWP2 expression results in a decrease in ubiquitination and a subsequent increase in endogenous ARID1B protein levels. Our preliminary observations suggest that ARID1B protein level varies during cell cycle progression. Work is currently underway to determine a possible oncogenic function of WWP2 via downregulation of ARID1B mediated tumor suppression. Our results therefore highlight a novel mechanism of post-translational regulation of ARID1B, which may have implications in the regulation of ARID1B-mediated tumor suppression and cell cycle progression. Given the recent interest to target BAF complex as a therapeutic option in many cancers, our results on regulation of ARID1B protein stability form a foundation for development of possible targeted therapy in cancer.

#265 NF1 is a novel MAP that modulates microtubule damage repair: rediscovering a tumor suppressor.

Eleonora Messuti[1], Giulia Tini[1], Alessia Castiglioni[1], Ambra Dondi[1], Giuseppe Ciossani[1], Silvia Monzani[1], Daria Khuntsariya[2], Bruno Achutti Duso[1], Marcus Braun[2], Zdenek Lansky[2], Simona Rodighiero[1], Luigi Scietti[1], Luca Mazzarella[1]

[1]IEO - European Institute of Oncology, Milan, Italy,[2]Institute of Biotechnology CAS - BIOCEV, Vestec, Czech Republic

Neurofibromin 1 (NF1) is a tumor suppressor widely mutated across several cancers and primarily recognized as a GTPase-activating protein (GAP) that inhibits RAS signaling. Other potential functions have been suggested but still not fully explored, due to its large size and complex domain structure. It remains unclear whether somatic mutations in NF1 can be used to inform clinical decision-making. We previously showed that NF1 plays a novel RAS-independent role in microtubule damage repair in response to the microtubule destabilizing drug Mertansine (DM1), the payload in the commonly used Antibody-Drug Conjugate T-DM1. We showed that NF1 exhibits biochemical properties of a *bona fide* Microtubule-Associated Protein (MAP) and specifically enhances microtubule (MT) repair, a recently discovered phenomenon whose regulation remains poorly characterized. A key unresolved question is whether the MT-repairing function is dependent or uncoupled from the GAP activity; this requires the development of novel methods to quantify MT damage *in vitro* and *in vivo*. We developed a novel analysis method to systematically identify and quantify areas of decreased tubulin intensity in MT stabilized *in vitro* in conditions known to lead to intratubular damage, using TIRF microscopy. Our method allowed us to asses MT repair as the amount of free-tubulin incorporation spatially overlapping with damaged sites. DM1 increased the number of intra-lattice discontinuities and abolished repair of damaged sites. This was rescued by the addition of NF1, which led to a strong increase in the fraction of repaired MTs, with or without DM1.To further confirm the role of NF1 in modulating DM1-dependent microtubule damage, we quantified tubulin staining patterns by high-resolution immunofluorescence (MT length, mean staining intensity and other derived parameters) in HER2+ breast cancer cell lines, in which we managed to obtain, for the first time, stable expression of WT NF1 in a KO background by lentiviral transduction. NF1 ablation or rescue did not lead to visible differences in MT signal in the absence of treatment. Upon addition of DM1, MTs were significantly shorter and more damaged in NF1 KO cells; this was completely rescued by WT NF1 re-expression. This system allows to directly measure the activity of NF1 or its mutants on MT damage, allowing the quantification of structure-function relationships in a relevant cellular system. We generated a collection of NF1 mutants, selected based on clinical associations and prior literature, that impact various domains of NF1, either affecting its GAP activity, its MT binding or its structural stability. Preliminary results suggest that GAP and MT-repairing activity can be partially but not entirely uncoupled. We speculate that some effects of NF1 somatic loss in cancer may be revisited as due to MT dysfunction and not to altered RAS signaling. Funded by Next Generation EU- PNRR-MAD-2022-12376934

#266 Androgen regulated ECD overexpression promotes prostate cancer tumorigenesis through increased glycolysis.

Mohsin Raza[1], **Asher Rajkumar Rajan**[1], Benjamin B. Kennedy[1], Timothy E. Reznicek[1], Farshid Oruji[1], Sameer Mirza[1], M Jordan Rowley[1], Glen Kristiansen[2], Bhopal C. Mohapatra[1], Hamid Band[3], Vimla Band[1]

[1]Genetics Cell Biology and Anatomy, University of Nebraska Medical Center, Omaha, NE,[2]Institute for Pathology, University Hospital "Carl-Gustav-Carus", University of Technology, Dresden, Institute for Pathology, University Hospital "Carl-Gustav-Carus", University of Technology, Dresden, Fetscherstrasse 74, Germany,[3]Fred & Pamela Buffett Cancer Center, University of Nebraska Medical Center, Omaha, NE

Introduction: Prostate cancer (PC) is the most diagnosed cancer in males. Androgen receptor (AR) signaling is essential for PC progression. ECD (Ecdysoneless) protein participates in cell cycle progression and cell survival and is overexpressed in non-PC cancers. Current study explored the role and function of ECD in PC progression.
Methods: TCGA Prostate Adenocarcinoma database was used to assess correlation between ECD and AR expression, and ECD's expression in PC dataset. Bioinformatic analysis was performed to predict androgen response elements in the ECD promoter. Chromatin immunoprecipitation (ChIP) and Promoter-Luciferase assays were used to assess AR recruitment to the ECD promoter. ECD was overexpressed, as well as crispr knocked out in hormone-dependent LNCaP and hormone-independent C4-2B PC cell lines. RNA-seq analysis was performed to assess transcriptomics changes in ECD-overexpressing (ECD-OE) tumors. RNA immunoprecipitation (RIP) was performed to assess ECD protein association to RNAs. Glucose uptake, and glycolytic rates assays were carried out to assess glycolysis.
Results: ECD is overexpressed in PC patients' tissues as compared to hyperplasia prostate samples, and ECD overexpression is associated with short patient survival. A significant positive correlation was observed between ECD and AR expression. Consensus androgen response elements in the ECD promoter showed androgen dependent recruitment of AR to the ECD promoter by ChIP assay. Luciferase assays showed androgen-dependent increase in ECD-luciferase activity. ECD overexpression and knockout in both LNCaP and C4-2B PC cell lines showed enhanced and decreased oncogenic traits, respectively. *In vivo* mice xenograft experiments showed ECD overexpression in LNCaP led to a significant increase in tumorigenesis. RNA-seq showed ECD overexpression led to increased levels of key glycolytic genes, which was confirmed by qRT-PCR. RIP assay revealed that ECD associates with the mRNA of these key glycolytic genes and enhances their stability. Functionally, ECD overexpressing PC cells showed higher glucose uptake and glycolytic rates, as compared to vector cells.
Conclusion: Overall, our results demonstrate a novel role for androgen-regulated ECD overexpression in PC tumorigenesis through direct binding and stabilization of mRNAs of key glycolytic genes.

#267 ECD co-operates with ERBB2 to promote tumorigenesis through upregulation of unfolded protein response and glycolysis.

Benjamin B. Kennedy[1], **Mohsin Raza**[1], Sameer Mirza[1], Asher Rajkumar Rajan[1], Farshid Oruji[1], Matthew M. Storck[2], Subodh M. Lele[3], Timothy E. Reznicek[1], Lusheng Li[1], M. Jordan Rowley[1], Shibiao Wan[1], Bhopal C. Mohapatra[1], Hamid Band[2], Vimla Band[1]

[1]Department of Genetics, Cell Biology, and Anatomy, University of Nebraska Medical Center, Omaha, NE,[2]Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE,[3]Department of Pathology, Microbiology, and Immunology, University of Nebraska Medical Center, Omaha, NE

Introduction: The mammalian ortholog of the Drosophila ecdysoneless (ECD) protein regulates cell cycle progression and cell survival. ECD levels are overexpressed in breast cancer (BC), and its overexpression correlates with poor prognosis and short patient survival, particularly in ERBB2-positive BC. This study explores the cooperative oncogenic mechanisms of ECD and ERBB2 to enhance cancer progression.

Methods: We generated immortal human mammary immortalized epithelial cell lines (hMECs) overexpressing ECD, ERBB2, or both. Additionally, we developed transgenic mice overexpressing ECD and/or ERBB2 (huHER2) in mammary epithelium under the control of the MMTV promoter (*ECD; HER2*Tg) to elucidate the co-oncogenic role and mechanisms underlying this cooperation.

Results: hMECs co-expressing ECD and ERBB2 demonstrated a significant increase in *in vitro* oncogenic traits as compared to cells expressing either ECD or ERBB2. Similarly, while *huHER2*Tg mice developed more homogenous solid nodular carcinomas, double transgenic mice (*ECD; huHER2*Tg) developed heterogenous and histologically aggressive mammary tumors with basal-like phenotype and epithelial mesenchymal transition (EMT) features, resembling tumors seen in *ECD*Tg mice. RNA sequencing revealed significant upregulation of two major oncogenic pathways, unfolded protein response (UPR) and glycolysis in ECD+ERBB2 expressing cells as compared to ECD or ERBB2 overexpressing hMECs. Consistently, transcriptomic profile of *ECD; huHER2*Tg tumors also demonstrated upregulation of glycolysis and UPR pathways. RNA immunoprecipitation assays showed ECD associates to mRNAs of three key glycolytic enzymes (LDHA, PKM2, and HK2) and the major UPR-regulated gene GRP78. This association resulted in increased mRNA stability, leading to higher expression of these genes in ECD+ERBB2-expressing cells. Consequently, ECD+ERBB2-overexpressing cells exhibited higher glucose uptake, and a higher glycolytic rate compared to cells overexpressing either ECD or ERBB2 alone.

Conclusions: Our findings support a co-oncogenic role of ECD and ERBB2 in ERBB2-driven BC oncogenesis by enhancing two major oncogenic pathways, UPR and glycolysis.

#268 Ryanodine receptor-1 upregulation alters organellar calcium signaling, modulates gene expression and contributes to ovarian cancer development.

Chi-Lam Au-Yeung[1], Kay-Pong Yip[2], Wan-Yee Tang[3], Samuel C. Mok[1], James S.K. Sham[4]

[1]UT MD Anderson Cancer Center, Houston, TX,[2]University of South Florida, Tampa, FL,[3]University of Pittsburg, Pittsburg, PA,[4]Johns Hopkins University School of Medicine, Baltimore, MD

Ca^{2+} signaling plays crucial roles in cancer development through Ca^{2+}-dependent regulation of gene transcription. However, specific changes of Ca^{2+} signaling in HGSOC are unclear. Analysis of RNAseq data from TCGA and GEO database and our patient samples identified ryanodine receptor 1 (RYR1) over-expression was associated with poor survival of HGSOC patients. RYR1 expression was also markedly higher in several HGSOC cell lines compared to normal human control cells. To examine Ca^{2+} signaling in HGSOC cells, cytosolic, nucleoplasmic, and mitochondrial [Ca^{2+}] were monitored by confocal imaging of Ca^{2+} fluorescence dyes and organelle specific Ca^{2+} biosenors. Resting cytosolic [Ca^{2+}] and $[Ca^{2+}]_{nucleus}$ was significantly elevated. Ca^{2+} signals triggered by the RYR-agonist 4-chloro-m-cresol (4-cmc) in cytosol, nucleus, and mitochondria were significantly higher in OVCA432 cells compared to the control HOSE cells. The elevated resting [Ca^{2+}] and the 4-cmc-induced Ca^{2+} signals in various intracellular compartments were reduced to the control level by RYR1-shRNA in OVCA432 cells. Gene Set Enrichment Analysis of RNAseq data revealed that RYR1 silencing suppressed gene sets related to mitochondrial proteins, mitochondrial membrane potential, fatty acid metabolism and oxidoreductase activity, as well as activated gene sets related to histone 4 lysine acetyltransferase, H2A ubiquitination and protein localization to chromatin, suggesting RYR1 in HGSOC cells plays important roles in modulating mitochondrial functions and epigenetic regulation of gene expression. Furthermore, shRNA knockdown or inhibition of RYR1 with the RYR1 blocker dantrolene suppressed HGSOC proliferation and migration, and significant reduced OC tumor evaluated by bioluminescence and tumor weight in OVCA432 cells injected athymic nude mice These observations are the first direct evidence that RYR1 alters cytosolic, nucleoplasmic and mitochondrial Ca^{2+} signaling in HGSOC cells, and these changes may contribute to the malignant phenotype for HGSOC development.

#269 TRPM8 as a testosterone receptor: Exploring its role in prostate cancer and male behavior.

Erick Benjamin Saldes[1], Alexandra Erdmier[1], Jai Velpula[2], Benny Tom[1], Timothy E. Koeltzow[3], Don Vander Griend[4], Swapna Asuthkar[1]

[1]University of Illinois College of Medicine (Peoria), Peoria, IL,[2]Pringle Robotics, Peoria, IL,[3]Department of Psychology, Bradley University, Peoria, IL,[4]University of Illinois at Chicago, Chicago, IL

The transient receptor potential melastatin 8 (TRPM8) channel has emerged as a critical mediator of testosterone signaling in prostate cancer (PC), influencing both tumor progression and sexually dimorphic behaviors. Our research expands upon TRPM8's role as a testosterone receptor, facilitating calcium influx that modulates cell proliferation and apoptosis in PC cells. Notably, TRPM8 mRNA is overexpressed in androgen-dependent benign PC tumors, but exhibits decreased expression in metastatic tumors, indicating a potential tumor-suppressive function in early-stage PC. This suggests that TRPM8 may serve as a biomarker for disease progression and a therapeutic target in androgen-regulated PC. Moreover, our findings suggest that TRPM8 is involved in sexually dimorphic behavioral responses, particularly in the context of age-dependent testosterone’s effects on male physiology. The absence of TRPM8 has been shown to result in significant alterations in male-specific behaviors, highlighting its role in mediating testosterone's influence on male reproductive and territorial behaviors. The male TRPM8 knock out (TRPM8 KO) mice showed increased serum testosterone when compared to wild type. Together, increased testosterone in the male TRPM8 KO mice is associated with aberrant anxiety and depression-like behavioral responses. This data position TRPM8 as a promising therapeutic target in PC and a key player in the regulation of male-specific behaviors. Further studies are warranted to elucidate the mechanisms by which TRPM8 influences both cancer progression and sexually dimorphic behaviors, particularly in the context of androgen signaling.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#27 Orthotopic tumor models using luciferized patient-derived organoids for investigating EGFR-MAPK pathway inhibition in colorectal and pancreatic cancer.

K. S. Tummala, M. Chalishazar, M. Angagaw, A. Savadipour, Q. Yan, C. Long, M. Buchanan, P. N. Lalagkas, E. Muise, L. Chen, K. Cheng, E. Park, J. Piesvaux, E. Leccese, B. Long, N. Solban;
Merck & Co., Inc, Boston, MA

The EGFR-MAPK signaling pathway is frequently activated in cancers, particularly due to KRAS gain-of-function mutations. Certain types, such as pancreatic ductal adenocarcinoma (PDAC) with KRAS mutations and cholangiocarcinoma with FGFR2 fusions, show dependency on EGFR. Recent studies demonstrate promising outcomes in metastatic colorectal cancer by combining an irreversible $KRAS^{G12C}$ inhibitor with an EGFR inhibitor. To explore therapeutic benefits across tumor types, we analyzed the expression of EGFR and co-receptors (ERBB) in TCGA samples and CCLE models with different KRAS mutations. We also verified the reliance on ERBB receptors using DepMap data.
Patient-derived organoid (PDO) models from PDAC, colorectal cancer (CRC), and CRC liver metastases were utilized to develop orthotopic tumor models. These PDO lines were characterized through mutational profiling, cell signaling analysis, RNA-sequencing, and whole exome sequencing, providing insights into their molecular features. Higher EGFR expression levels at both protein and RNA levels were observed in specific CRC PDO models, consistent in in-vitro PDO models and in-vivo PDO-derived Xenograft (PDOX) tissues from subcutaneous implantation in immunocompromised mice.
Additionally, the PDO models were evaluated for sensitivity to standard of care (SOC) drugs and EGFR inhibitors, revealing EGFR expression-independent and tumor type-dependent sensitivities (e.g., Afatinib IC50: PDAC (128nM+/-123) vs CRC (2776nM+/-3099)). Surface expression levels of ERBBs were measured in in-vivo PDOX tissues to assess the comparative use case for targeting the EGFR pathway via inhibition or antibody-drug conjugate (ADC) molecules.
Furthermore, we generated and validated stable tumoroid models expressing firefly luciferase in PDOs derived from primary CRC, CRC metastasis to the liver, and primary PDAC. To further validate the potential for in-vivo models, orthotopic tumor models were established in immunocompromised mice for each PDO line, monitoring tumor burden using bioluminescence imaging. Correlation with MRI and ultrasound imaging was conducted for PDAC and liver tumor burden, respectively, with significant correlation observed.
Overall, this study aims to enhance understanding of tumor biology and response to treatment through the development and validation of PDO-based orthotopic models, with future work involving validation of EGFR inhibitors targeting the pathway and ADCs based on ERBBs targeting tumor cells expressing these proteins. In vivo luciferase reactivation in our models will allow us to dissect the mechanisms of resistance to these agents using physiologically relevant tumor models and single-cell RNA-sequencing analysis.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Mechanisms of Oncogenesis and Tumor Suppression
Poster Session

#271 BMH-21, an RNA polymerase I inhibitor provides a novel mechanism to target Δp53 and R248Wp53 driven pancreatic cancers.

Mudassier Ahmad[1], Sahir Sultan Alvi[1], Haider Ahsan[1], Carlos Perez[1], Andrew Massey[2], Vivek Kashyap[1], Neeraj Chauhan[1], Emmanuel Anning[1], Manish Tripathi[1], Dae Joon Kim[1], Nirakar Sahoo[3], Tamer Oraby[4], Mohammad Moshahid Khan[5], Manu Sebastian[6], Subhash C. Chauhan[1], Bilal Bin Hafeez[1]

[1]University of Texas Rio Grande Valley, McAllen, TX,[2]National Institute of Biomedical Imaging and Bioengineering, Section on Mechanobiology, Bethesda, MD,[3]School of Integrative Biological and Chemical Sciences, University of Texas Rio Grande Valley, Edinburg, TX,[4]School of Mathematical and Statistical Sciences, University of Texas Rio Grande Valley, McAllen, TX,[5]Department of Neurology, College of Medicine, UTHSC, Memphis, TN,[6]Department of Veterinary Medicine & Surgery, The University of Texas MD Anderson Cancer Center, Houston, TX

p53 mutations occur in 50-75 % of pancreatic cancer cases. p53 mutations promote the protein turnover and increase the dependence of cancer cells on ribosome biogenesis as compared to normal cells. We report that BMH-21, which is an RNA Polymerase I inhibitor can be used to inhibit the growth of Δp53 (truncated p53) and R248Wp53 driven pancreatic cancers. Our studies show that BMH-21 induced degradation of RPA194 (a catalytic subunit of RNA Polymerase I) has a very less cytotoxic effect on normal human pancreatic ductal epithelial cells. The p53 mutations in PanCa cells were found to play an important role in the recruitment of catalytic subunits of RNA polymerase I (RPA194 and RPA135) on ribosomal DNA (rDNA). BMH-21 decreases the recruitment of RPA194 on rDNA in Capan-2 cells that express p53 wild type and recruitment of both RPA194 and RPA135 on rDNA in MIA PaCa-2 cells that express R248W p53. We found that p53 wild type (WT) and mutant p53 physically interact with both the catalytic subunits of RNA polymerase I. BMH-21 induced degradation of RPA194 was found to expose Δp53 and R248Wp53 for ubiquitination. No ubiquitination of p53 WT was observed in BMH-21 treated Capan-2 cells. RNA polymerase inhibitors induce nucleolar stress that promotes p53 expression. BMH-21 induced ubiquitination of p53 prevents increases in expression of Δp53 and R248Wp53p53 which restricts the role of mutant p53s in the proliferation of PanCa cells. BMH-21 treatment reduces the growth of orthotopic xenograft pancreatic tumors in athymic nude mice with no observed toxicity. These findings suggest that BMH-21 is a promising therapeutic agent for treatment of PanCa.

#272 Loss of Rab25 and associated mechanisms in cancerous transformation of melanocytes.

Samikshya Kandel, Krishna Rao

Southern Illinois University School of Medicine, Springfield, IL

Background: Rab25 is a member of RAS protein superfamily of small GTPases that belong to RAB11 subfamily located on 1q22 of chromosome. It is generally expressed by epithelial cells and its expression level varies by tissue types. From our previous research, Rab25 has been shown to be a significant player in moderating transformation of human mammary epithelial cells, as its loss promotes cellular transformation particularly in presence of RAS mutation. Rab25 is an ambiguous protein in cancer research as its role is cancer type dependent and remains unknown in melanoma. There is deep deletion of Rab25 in a section of melanomas (only~3% profiled) in databases like the Cancer Genome Atlas and Cbioportal. Additionally, Rab25 shares a common consensus motif with the binding site of mutant H-RAS, Q61L. NRAS is mutated in 80% of RAS activated melanomas. Therefore, we are investigating the role of Rab25 loss in the development of NRAS mutant melanoma.

Objectives: To study the status of Rab25 and its role in the transformation of melanocytes, we screened for Rab25 expression and protein level in different melanoma cell lines. We further did immunohistochemistry to examine the Rab25 expression in biopsy-proven melanoma sections. To uncover the function of Rab25, we overexpressed Rab25 in NRAS mutant melanoma cell lines and knocked down Rab25 in non-tumorigenic immortalized melanocytes. Further, we will use liposomal encapsulation technique to deliver a six-peptide sequence intracellularly to target NRAS.

Results: There was significant loss of Rab25 in mRNA and protein level in melanoma cell lines. Rab25 is found in normal skin tissues but was significantly lost in melanoma sections and associated with reduced survival. NRAS mutant human melanoma cells transduced with Rab25 showed reduced proliferation, migration tendency, and loss of anchorage-independence growth as compared to control cell lines. Additionally, knockdown of Rab25 in non-cancerous immortalized human melanocytes showed resulted in enhanced proliferative behavior and gain of anchorage-independent growth in soft agar colony formation assay.

Conclusion: These observations suggest that Rab25 loss results in gain of migratory potential, anchorage-independent growth, and enhanced proliferation potential which are requisite events in melanoma-genesis. Further studies on associated functional proteins of Rab GTPase family will be conducted to strengthen this data.

#273 Decipher actionable tumor suppressor defects in prostate cancer for precision medicine.

F. Chen, **D. Zhao**;
UT MD Anderson Cancer Center, Houston, TX

Chromatin remodeling proteins contribute to DNA replication, transcription, repair, and recombination. The chromodomain helicase DNA-binding (CHD) family of remodelers plays crucial roles in embryonic development, neurogenesis, and carcinogenesis. As the founding member, CHD1 is capable of assembling nucleosomes, remodeling chromatin structure, and regulating gene transcription. Dysregulation of CHD1 at genetic, epigenetic, and post-translational levels is common in prostate cancer and other malignancies. Through interacting with different genetic alterations, CHD1 possesses the capabilities to exert oncogenic or tumor-suppressive functions in context-dependent manners. In the past decade, my study uncovered the novel cell-intrinsic and -extrinsic roles of CHD1 during prostate cancer development, progression, and therapy resistance (*Nature 2017, Cancer Discover 2020, Cancer Research 2022, Nature Cancer 2024*). Cancer genome studies have uncovered a unique subtype of prostate cancer characterized by speckle-type BTB/POZ protein (SPOP) mutations, which stabilize AR and its co-activators and have been associated with prolonged responses to androgen deprivation therapies and favorable prognoses. Loss of CHD1 frequently co-occurs with SPOP mutations; however, how concurrent CHD1 loss impacts SPOP-mutated prostate cancer remains unclear. Most recently, by generating the first-in-the-field genetically engineered mouse models, we provided genetic evidence supporting that CHD1 loss accelerates SPOP-mutated prostate cancer progression and confers resistance to castration in this context. We uncover a non-canonical epigenetic function of CHD1 and demonstrate that CHD1 reprograms lipid metabolism by governing SREBPs. CHD1 loss-induced lipid reprogramming supplies intratumoral androgen biosynthesis and retains AR transcriptional activity in SPOP-mutated prostate tumors upon castration. Furthermore, the mechanistic studies facilitate the development of a novel biomarker-driven combinatorial therapy for castration-resistant prostate cancer harboring CHD1 loss and SPOP mutations. These findings advance our understanding of an emerging prostate cancer subtype and mechanism of action and provide novel therapeutic strategies for men with advanced prostate cancer.

#274 Resistance to VEGFR inhibitors converges from molecular diversity in angiosarcoma cells.

Eslenur Nipa, Donghee Lee, Sun Hee Choi, Jong Hyuk Kim

Small Animal Clinical Science, University of Florida, Gainesville, FL

Angiosarcomas are aggressive soft tissue sarcomas that form malignant endothelium. Standard care treatment for angiosarcoma patients includes surgery, radiation, and chemotherapy, but outcomes remain poor, especially in unresectable or metastatic cases. These endothelial malignancies activate angiogenic and vascular pathways, implying VEGF/VEGFR and Tie2 signaling as key drivers of tumor progression. However, therapeutic antibodies, such as bevacizumab (Avastin), and chemical inhibitors targeting VEGF pathways have shown limited clinical benefits in angiosarcomas. The molecular mechanisms that establish resistance to VEGF inhibition in angiosarcomas are virtually unknown. In this study, we used five human angiosarcoma cell lines - KU-CAS3, KU-CAS5, HAMON, ISO-HAS-B, and AS5 - for functional characterization. We first determined cell surface expression of four endothelial markers: CD31, neurophilin-1, VEGFR2, and Tie2. Our flow cytometry data revealed that CD31 expression was markedly elevated in nearly 100% of cells in four of the five angiosarcoma cell lines, with the exception of AS5, which showed 26.1% expression. Neurophilin-1 expression varied across the cell lines: 5.6% in KU-CAS3, 11.8% in AS5, 31.1% in ISO-HAS-B, 60.0% in HAMON, and 99.7% in KU-CAS5. We also found that three cell lines - HAMON (95.2%), KU-CAS3 (72.6%) and KU-CAS5 (92.7%) - showed high expression of VEGFR2, while two cell lines - ISO-HAS-B (14.0%) and AS5 (14.8%) - exhibited low expression. The expression levels of Tie2 were abundant, ranging from 74.8% to 96.4% in four cell lines, with modest expression in AS5 (47.8%). Then, we examined cellular response to VEGFR2 (Regorafenib, Sorafenib) and Tie2 inhibitors (Rebastinib, Pexmetinib) using cell growth inhibition assays. All five angiosarcoma cell lines exhibited IC50 values higher than 1 uM against both VEGFR2 inhibitors, Regorafenib and Sorafenib, except for KU-CAS5, which showed an IC50 of 0.44 uM. Both Tie2 inhibitors, Rebastinib and Pexmetinib, were also ineffective in decreasing cell proliferation in most of the cell lines, with two exceptions showing submicromolar IC50 values of 0.91 uM and 0.25 uM in KU-CAS3 and AS5, respectively. In summary, our data revealed that the robust expression of endothelial and vascular markers in angiosarcoma cells indicates a conserved endothelial phenotype under in vitro conditions. However, none of the cell lines exhibited significant sensitivity to VEGFR and Tie2 inhibitors in terms of clinical potential. It demonstrates broad resistance to VEGFR2 and Tie2 inhibition, independent of VEGFR2 and Tie2 expression in angiosarcoma cells. Furthermore, our results suggest angiosarcoma cells establish a functional convergence that potentiates the signaling activation of VEGF and Tie2 pathways, despite their molecular divergence. Our ongoing work aims to explore the molecular mechanisms promoting resistance to VEGF and Tie2 inhibitors.

#275 Increased expression levels of *KISS1* in IDCC breast cancer induced by melatonin treatment.

Kelly P. Ferreira, Bruna C. Almeida, Edmund C. Baracat, **Katia C. Carvalho**

University of Sao Paulo, Faculty of Medicine, Sao Paulo, Brazil

Introduction: Tumor suppressors encode proteins commonly expressed in non-cancer cells that avoid abnormal events that culminate to neoplasms development. In a deregulated expression case, they are useful as biomarkers for pathological stages or tumor progression. *KISS1* (*KiSS-1 metastasis suppressor*), is a known metastatic suppressor and its expression is commonly downregulated in the metastatic tumors of various origins, including breast cancer. In this context, some researches showed that melatonin (Mel) can improve survival rates, suggesting that its application may be a tool for therapeutic use in some breast tumors. Thereby, we aimed to identify mRNA levels of *KISS1* in invasive ductal carcinoma cells (IDCC) with metastasis in pleural effusion (MCF7) treated with Mel and compare with MCF7 non-treated cells (CC).

Methods: MCF7 (ER+) cell line was cultured with RPMI 1640 (Sigma-Aldrich, Germany), with 10% fetal bovine serum (FBS - Gibco, USA) treated with activated charcoal, inactivated, sodium bicarbonate, pyruvic acid and antibiotics as recommended by ATCC and subsequently treated with 3000 µM Mel. After treatment the RNA was extracted using mirVana miRNA Isolation Kit (Ambion, USA) and then, purity and concentration of total RNA were accessed. The Reverse transcription synthesis was performed using High-Capacity cDNA Reverse Transcription Kit (ThermoFisher Scientific, Lithuania). Expression profile of 91 mRNA, including *KISS1* and, 5 housekeeping mRNAs was performed by TaqMan Array Plates using Real-Time PCR (Applied Biosystems, USA). Data analysis was obtained by ExpressionSuit Software v1.3 (ThermoFisher Scientific).

Results: The concentration of melatonin tested in MCF7 cells (ER+) from metastasis showed, a significant increase of mRNA *KISS1* expression levels when compared to the levels presented in MCF7 cells that did not receive melatonin treatment (control cell - CC).

Conclusion: Melatonin treatment was able to increase the expression of *KISS1* in MCF7 cells, indicating that its use may bring benefits associated to an improved prognosis for similar tumors. Further studies are ongoing to confirm and expand our results.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Metabolomics
Poster Session

#279 Unraveling biological differences of high grade serous ovarian cancer subtypes by metabolomics.

Zhanjun Hou[1], Madelyn Brzezinski[1], Carrie O'Connor[1], Xun Bao[1], Ayesha B. Alvero[2], Radhika Gogoi[1], Ramandeep Rattan[3], Gil Mor[2], Jing Li[1], Aleem Gangjee[4], Larry H. Matherly[1]

[1]Barbara Ann Karmanos Cancer Institute, Detroit, MI,[2]Wayne State University School of Medicine, Detroit, MI,[3]Henry Ford Cancer Institute and Henry Ford Health System, Detroit, MI,[4]Duquesne University Division of Medicinal Chemistry, Pittsburgh, PA

Epithelial ovarian cancer (EOC) accounts for ~90% of ovarian cancers and continues to be a deadly disease. High grade serous ovarian cancer (HGSOC) is the most common subtype of EOC. PARP inhibitors (PARPis) are important drugs for treating recurrent ovarian cancer post-platinum-based chemotherapy and recently this has been applied to first-line maintenance treatment after chemotherapy. Meta-analysis suggested that PARPis are most effective for patients with *Brca* mutations. Studies also suggested better efficacy of PARPis in HGSOC patients with *Brca2* mutations than patients with *Brca1* mutations. However, only ~15-20% of HGSOC tumors carry germline *Brca1* or *Brca2* mutations and somatic mutations are found in ~8% of cases. In addition, the increasing use of PARPis for treating HGSOC has raised concerns about PARPi resistance in clinical practice. Clearly, there is an urgent need to identify new metabolic vulnerabilities in HGSOC in general, and in *Brca1*/*Brca2* mutant subtypes in particular, that can be targeted alone, or in combination with existing medications including PARPis. The ID8 HGSOC model is the most widely used transplantable mouse syngeneic model of ovarian cancer. ID8 variants including single (*Trp53*$^{-/-}$) or double (*Trp53*$^{-/-}$/*Brca1*$^{-/-}$ or *Trp53*$^{-/-}$/*Brca2*$^{-/-}$) gene deletions recapulate characteristics of human HGSOC subtypes including the expected patterns of sensitivity to cisplatin and PARPis (i.e., olaparib). Our previous studies showed that in primary patient specimens [including normal ovary (n=8) and HGSOC (n=40)], transcripts for one-carbon (C1) genes in mitochondria (*SLC25A32*, *SHMT2*, *MTHFD2*, *MTHFD1L*) and in cytosol (*GARFTase*, *AICARFTase*) increased significantly over normal ovaries. We performed untargeted metabolomics studies (~2000 metabolites) with the aforementioned ID8 sublines. The results showed significant quantitative differences between wild-type ID8 and ID8/*Trp53*$^{-/-}$, ID8/*Trp53*$^{-/-}$/*Brca1*$^{-/-}$ or ID8/*Trp53*$^{-/-}$/*Brca2*$^{-/-}$ cells, as well as between ID8/*Trp53*$^{-/-}$/*Brca1*$^{-/-}$ and ID8/*Trp53*$^{-/-}$/*Brca2*$^{-/-}$ cells, involving C1 metabolism including metabolites of glycine, serine, threonine, cysteine, methionine, pyrimidines and purines. Targeted metabolomics and flux analysis with [^{13}C (U)]serine and LC-MS/MS revealed enhanced mitochondrial C1 flux in the ID8 variants, reflected in levels of total serine and serine isotopomers (ID8 > ID8/*Trp53*$^{-/-}$ = ID8/*Trp53*$^{-/-}$/*Brca1*$^{-/-}$ > ID8/*Trp53*$^{-/-}$/*Brca2*$^{-/-}$). C1 flux from [^{13}C (U)]serine in the cytosol was also significantly elevated in ID8 variants, reflected in a dramatic decrease of glycinamide ribonucleotide (GAR) (M+0, M+2) in *de novo* purine biosynthesis compared to wild-type ID8 cells. Collectively, our results suggest that C1 metabolism in both the mitochondria and cytosol is a bona fide metabolic vulnerability in HGSOC that can be therapeutically exploited.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#28 Novel organoid-based exploration reveals the role of LMTK3/FADS2 signaling in metastatic breast cancer progression in cats and humans.

H. Yamamoto[1], M. Elbadawy[2], T. Usui[3], K. Sasaki[3];

[1]Haru Yamamoto (Individual), Fuchu, Japan, [2]University of Georgia, Athens, GA, [3]Tokyo University of Agriculture and Technology, Fuchu, Japan

Cat breast cancer (CBC) is the third most common type of feline neoplasm. It constitutes approximately 17% of feline tumors, with an estimated annual incidence of between 13 and 25/100,000 cats. CBC shares similar histology, genetics, behavior, and clinicopathological characteristics with human BC. Despite advancements in human breast cancer (BC) treatment, outcomes in human and veterinary medicine remain suboptimal. Spontaneously developed BC in CBC is highly malignant, intractable, and has the potential to become a valuable translational model for human metastatic BC (HBC). We developed primary CBC organoids and analyzed their morphology, gene expression patterns, and response to anti-cancer drugs. CBC organoids recapitulated the structure and behavior of tumor cells, exhibiting diverse hormone receptor expressions and tumorigenic potential. Moreover, varying sensitivities to chemotherapies and targeted drugs were evident across CBC organoid lines. Furthermore, LMTK3 was significantly upregulated in CBC organoids compared to normal organoids. Further, LMTK3/FADS2 pathway was revealed to be implicated in progression and metastasis by influencing cell proliferation, invasion, and apoptosis of CBC organoids. Cell viability of human BC organoids was also prevented by treatment of LMTK3 inhibitor. Survival time of human BC patients with high co-expression of LMTK3 and FADS2 was shorter than that with low co-expression. These findings highlight the importance of LMTK3/FADS2 pathway in BC progression and indicate that companion animal cancer organoid research can model HBC.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Metabolomics
Poster Session

#280 ALDH2 knockout dysregulates arginine and proline metabolism in SK-BR-3 breast cancer cells.

Ming Zhao[1], Blake R. Rushing[2], Zhikun Ma[1], Rachel A. Coble[2], Amanda B. Parris[1], Sergey Krupenko[2], Susan J. Sumner[2], **Xiaohe Yang**[1]

[1]BBRI and Department of Biological and Biomedical Sciences, North Carolina Central University, Kannapolis, NC,[2]Department of Nutrition and Nutrition Research Institute, UNC Chapel Hill, Kannapolis, NC

Aldehyde dehydrogenase 2 (ALDH2) mitigates cellular stress by detoxifying reactive aldehydes produced during alcohol exposure and endogenous metabolic processes. The ALDH2*2 variant, prevalent in East Asian populations, has decreased catalytic activity, which is linked to an increased risk of cancer and other diseases. While the effects of ALDH2 deficiency on cellular stress responses are well documented, the specific metabolic dysregulations arising from this deficiency require further investigation. To explore the impact of ALDH2 deficiency on metabolism and cellular stress in breast cancer, we generated the ALDH2 knockout subline of the SKBR-3 cell line (SKBR-3/ADKO) using CRISPR/Cas9 technology. Our findings indicate that ALDH2 knockout leads to heightened cellular stress, evidenced by elevated levels of 8-OHdG, reactive oxygen species (ROS), and malondialdehyde (MDA). Untargeted metabolomic analysis of SKBR-3 and SKBR-3/ADKO cells using Ultra High Performance Liquid Chromatography/High Resolution Mass Spectrometry assigned a total of 5,033 metabolites, with 615 metabolites being significantly different (fold change ≥ 2, p-value < 0.05) between the two cell lines. The Mummichog approach has identified six key metabolic pathways altered upon ALDH2 knockout, including: arginine biosynthesis; D-amino acid metabolism; arginine and proline metabolism; alanine, aspartate and glutamate metabolism; pentose and glucuronate interconversions; pantothenate and CoA biosynthesis. The most significantly dysregulated pathway in response to the ALDH2 loss was arginine and proline metabolism, which is an integral pathway to redox homeostasis, ROS production, and cellular stress responses. Our study provides a novel insight into the role of ALDH2 in cancer metabolism, which might have a broader implication for cancer biology.

#281 Plasma metabolomic profiling identifies novel biomarkers for gastric cancer diagnosis and prognosis.

Zun Li, Xiangnan Zhang, Yanxin Yao, Yubei Huang, Zhangyan Lv, Hongji Dai, **Kexin Chen**, Ben Liu

Tianjin Medical University Cancer Institute and Hospital, Tianjin, China

Gastric cancer (GC) remains a significant global health challenge, representing the fifth leading cause of cancer-related mortality. Altered metabolism plays a crucial role in GC development and progression. This study aimed to identify plasma metabolic biomarkers for improved GC diagnosis and prognosis prediction. We performed untargeted metabolomic profiling of plasma samples from 105 GC patients and 120 healthy controls using ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS). Following rigorous quality control, orthogonal partial least squares discriminant analysis (OPLS-DA) identified 562 differentially abundant metabolites between groups. Pathway enrichment analysis revealed significant alterations in metabolic pathways associated with amino acid metabolism, lipid metabolism, and hormone signaling. Machine learning algorithms were employed to develop diagnostic and prognostic models based on these differentially abundant metabolites. A four-metabolite diagnostic model achieved high sensitivity (0.86) and specificity (0.95) in an independent validation cohort, significantly outperforming conventional protein-based diagnostic markers. Furthermore, our prognostic model demonstrated superior performance compared to established clinical parameters, effectively stratifying patients into distinct risk groups for personalized therapeutic strategies. This comprehensive metabolomic analysis provides novel insights into the metabolic landscape of GC and identifies promising biomarkers for improved clinical management.

#282 Pre-diagnostic circulating metabolites and risk of overall, intermediate-risk, high-risk and lethal prostate cancer: a systematic review andmeta-analysis of untargeted metabolomic studies.

Harriett Fuller, Orietta P. Agasaro, Burcu F. Darst

Fred Hutchinson Cancer Center, Seattle, WA

Prostate cancer (PCa) is the 2nd most common cancer in men. Metabolomic dysregulation is expected to contribute to PCa pathogenesis; however, pre-diagnostic evidence of metabolite-PCa associations has not been quantitatively aggregated. This study aimed to quantitatively assess metabolite-PCa associations, compare these associations between distinct PCa outcomes, and evaluate sources of bias in PCa metabolomic epidemiology studies. We conducted a systematic review (October 17th, 2024) and meta-analysis to identify pre-diagnostic untargeted serum metabolomic studies of PCa risk. Restricted maximum likelihood (REML) was used to conduct meta-analyses to quantify associations between metabolites and risk of overall PCa, low- to intermediate-risk PCa, high- to very high-risk PCa and lethal PCa, as defined by the NCCN. Statistical significance was defined as FDR-adjusted P< 0.05. Enrichment analyses were conducted to identify biologically relevant pathways. The DrugBank Pharmaco-metabolomics database was used to determine whether identified metabolites are drug modifiable. Thirteen predominantly European ancestry studies were identified (10,467 cases/10,833 controls) and 602 metabolite biomarker associations were meta-analyzed. Three, three, eleven and nineteen metabolites were significantly associated with risk of overall, low- to intermediate-risk, high- to very high-risk and lethal PCa, respectively. No metabolite was significantly associated with >1 PCa outcome; however, 12 metabolites were nominally associated (P<0.05) with >1 outcome. Effect sizes between metabolites associations with overall and high- to very high-risk PCa were significantly correlated when considering all 144 metabolites (r=0.19, P=0.023) and 10 metabolites nominally associated with both outcomes (r=0.87, P=0.001). Metabolite effects associated with lethal PCa risk were largely distinct from other outcomes. Metabolites significantly associated with risk of high- to very high-risk PCa were significantly enriched for lipids (P=0.03). Using DrugBank, we found that levels of 11 significant metabolites are drug modifiable. Key sources of bias identified included the selective reporting of results, inconsistent metabolite naming, limited adjustment for fasting status and limited diversity, which could impact the generalizability of findings. In conclusion, pre-diagnostic levels of 36 circulating metabolites were associated with PCa risk, 30 of which were associated with high- to very high-risk or lethal PCa. The metabolomic profile of lethal PCa risk was largely distinct from other PCa outcomes. Future work is needed to further investigate how pre-diagnostic metabolite profiles differ by PCa aggressiveness and to evaluate the role of identified metabolites as PCa biomarkers across populations.

#283 Cholesterol($C_{27}H_{46}O$)identified by metabolomics enhance lipid raft composition for cell proliferation in epithelial ovarian cancer.

Hitomi Sakaguchi-Mukaida, Kosuke Hiramatsu, Tatsuo Masuda, Mamoru Kakuda, Satoshi Nakagawa, Tadashi Iwamiya, Shinya Matsuzaki, Yutaka Ueda

Osaka University, Suita, Japan

Objective: We previously reported that lipoprotein promoted cell proliferation of epithelial ovarian cancer (EOC) cells in vitro. Moreover, we demonstrated High-Fat Diet (HFD) strongly promoted the tumor growth of EOC in vivo and HFD mouse serum promoted cell proliferation in vitro. These results suggest that lipid molecules derived from HFD contribute to cell proliferation of EOC. In this study, we aimed to identify the lipid molecules in HFD mouse serum which contribute to cell proliferation of EOC cells by metabolomics and figure out the function of the lipid molecule.
Methods: We obtained serum of HFD and normal diet (ND) fed mouse and performed metabolomics to identify the lipid molecules which contribute to cell proliferation of EOC. In vitro, we validated that lipid molecules in HFD mouse serum promote the cell proliferation and analyzed which signaling pathway was activated. In vivo, we evaluated the effect of lipid molecules-rich diet in tumor growth. We also evaluate the association of lipid molecules and lipid rafts in EOC cells.
Results: HFD significantly promoted the tumor growth EOC in vivo ($p < 0.05$), and HFD mouse serum also promoted cell proliferation of EOC cells in vitro ($p < 0.05$). Metabolomics showed obvious different metabolic profiles of HFD and ND mouse serum (non-tumor bearing). Partial least squares-discriminant analysis revealed that cholesterol ($C_{27}H_{46}O$) ranked highest as the lipid molecule in HFD mouse serum. In vitro, cholesterol significantly promoted cell proliferation ($p < 0.05$) via Akt/mTOR signaling pathway. Moreover, we demonstrated that cholesterol-rich diet promoted the tumor growth of EOC ($p < 0.05$). Finally, we evaluated that lipid raft inhibitor blocked cholesterol induced cell proliferation ($p < 0.05$).
Conclusions: Cholesterol ($C_{27}H_{46}O$) uptake lead to the cell proliferation of epithelial ovarian cancer via stimulation Akt/mTOR signaling pathway following lipid raft.

#285 Integration of serum and tumor metabolomics reveals distinct metabolic profiles in early onset colorectal cancer.

D. Vadehra[1], S. Rosario[1], T. J. Brown[2], H.- . Hsaio[1], S. Mukherjee[1];
[1]Roswell Park Comprehensive Cancer Center, Buffalo, NY, [2]UTSW Simmons Comprehensive Cancer Center, Dallas, TX

Introduction: Early onset colorectal cancer (EOCRC) is rising at an alarming rate and is increasingly recognized as a distinct clinical entity from average onset (AO) CRC. However, the molecular underpinnings remain largely unknown. We found key differences at the transcriptomic level that highlighted the enrichment of several metabolic pathways that are distinct in the EOCRC (<50 years old) compared to the AOCRC population, prompting further study of the association between age of onset and role of metabolomics. To this end, we sought to highlight differences in the metabolic profile of EOCRC patients compared to AOCRC.
Methods: Banked serum samples from the Roswell Park Data Bank and biorepository were obtained from EOCRC (n=25) and AOCRC (n=25) patients, as well as age-matched non-disease (n=10) populations. Further, matched tumor samples from AOCRC (n=25) and EOCRC (n=25) were obtained. Comprehensive metabolomics via mass spectrometry was conducted on both the serum and matched tumors. Metabolite abundances from serum and tumors were assessed independently. Abundances were log2 transformed to achieve a normal distribution. Prior to differential metabolite abundance, patient data were assessed for outliers and necessary co-variates (e.g., sex, race, etc.). Differential abundance was assessed via the generation of linear mixed models and analyses of variances with $p \leq 0.05$ set for statistical significance.
Results: Samples were well-matched at baseline for relevant demographics aside from age. When integrating serum and tumor metabolites, after adjusting for biological sex, there were eight significantly altered metabolites. These were primarily lipids, including phosphatidylcholines, lysophosphatidylcholines, sphingolipids, and ceramides. All of these were increased in AOCRC compared to EOCRC. Conversely, hypoxanthine and hexosylceramides were found to be increased in the EOCRC compared to AOCRC. Further, we observed significant differences in metabolite expression by biological sex, with AO female tumor samples also enriched for lipids like phosphatidylcholine, sphingolipids, and ceramides, as compared to AO males.
Conclusion: Metabolomics assessment of CRC patients demonstrated significant differences in metabolite concentrations between EOCRC and AOCRC patients in both serum and tumor samples. Further, these differences were strongly correlated with patients' sex, suggesting a biological influence of sex on CRC pathogenesis. Altogether, these results imply a distinct metabolic phenotype among the EOCRC population. Given the reported role of hypoxanthine and hexosylceramides in CRC metastasis, further studies should investigate their influence on clinical outcomes and treatment response.

#286 Metabolic alterations in skeletal muscle and serum of tumor-bearing cachectic mice.

Kuo-Chu Lai

Department of Physiology and Pharmacology, College of Medicine, Chang Gung University, Taoyuan, Taiwan

Cancer cachexia, a multifactorial syndrome characterized by systemic inflammation, high energy expenditure, and progressive skeletal muscle loss, poses a significant challenge in cancer management due to limited therapeutic options. Understanding the metabolic shifts associated with cachexia-related muscle wasting could provide critical insights into identifying potential biomarkers and therapeutic targets. In this study, we conducted targeted metabolomics to investigate metabolic alterations in the skeletal muscle and serum of tumor-bearing cachectic mice. To facilitate a comprehensive analysis, skeletal muscle samples from non-cachectic tumor-bearing mice were included to explore metabolic changes associated with tumor growth and its progression to cachexia. We found that tumor-bearing, non-cachectic muscles exhibited significantly higher levels of glucose-6-phosphate (G6P), fructose-6-phosphate with glucose-1-phosphate (F6P + G1P), glyceraldehyde-3-phosphate (G3P), glycerol-3-phosphate (Glycerol-3P), and phosphoenolpyruvate (PEP) compared to control muscles. However, no significant differences were observed between non-cachectic muscles and cachectic muscles. These findings indicate that increased glycolysis is more critical for tumor growth than for the progression to cachexia. Amino acids such as asparagine, alanine, and serine were significantly increased in cachectic muscle. Compared to non-cachectic muscle, levels of 2-hydroxyglutarate (2-HG) and CoA were decreased in cachectic skeletal muscle. Notably, alterations in 2-HG and CoA were consistent between muscle and serum, highlighting their potential as systemic biomarkers. These results reveal key metabolic alterations in skeletal muscle and serum associated with cancer cachexia, offering novel insights into the pathogenesis of muscle wasting and providing potential avenues for biomarker discovery and therapeutic intervention.

#287 Circulating metabolites associated with response to immune checkpoint blockade in melanoma.

Nazli Dizman, Iqbal Mahmud, Julie Simon, Elizabeth Sirmans, Hussein A. Tawbi, Jeffrey Gershenwald, Michael Davies, Nadim Ajami, Carrie Daniel-MacDougall, Jennifer Wargo, Philip Lorenzi, Jennifer McQuade

UT MD Anderson Cancer Center, Houston, TX

Introduction: Metabolism plays a crucial role in tumor microenvironment dynamics and may influence response to immune checkpoint blockade (ICB). We examined the relationship between the serum metabolomic and lipidomic profiles and outcomes with ICBs in melanoma.

Methods: Patients with melanoma initiating standard of care anti-PD1-based ICB therapy with neoadjuvant or palliative intent were prospectively enrolled. Clinical characteristics were abstracted and survival and objective response using RECIST 1.1 were assessed. Patients with complete or partial response were classified as the response (R), and the remainder as the non-response (NR) cohort. Baseline serum metabolome profiling was conducted at the University of Texas MD Anderson metabolomics core using high-resolution mass spectrometry to assess global lipids and semi-targeted metabolites using in-house standard metabolite library and compared between R and NR.

Results: A total of 67 patients (median age 61 years, 70% male) were included. The majority (67%) received ICB for stage IV disease while 34% were treated in the neoadjuvant setting. The most common ICB regimen was anti-PD-1 and anti-CTLA4 combination (55.2%), followed by anti-PD-1 and anti-LAG3 combination (35.8%). Overall objective response rate was 52.5%. A total of 1,161 lipid metabolites and 120 semi-targeted metabolites were identified and analyzed. Global metabolite profiles of the R and NR cohorts suggested up-regulation of pentose phosphate pathway metabolites and downstream nucleotide precursors in the R cohort. Global lipidome profile demonstrated similarities between groups, although a degree of clustering effect by response status was observed in principal component analysis (data will be presented). In the R cohort, differentially overrepresented lipid classes included plasma membrane lipids ($p<0.001$, false detection rate [FDR] q-value=0.004) and inflammation-associated lipids such as sphingolipids ($p<0.005$, $q=0.006$), diacylceramides ($p=0.001$, $q=0.008$), diradylglycerols ($p=0.003$, $q=0.016$), diacylglycerols ($p<0.001$ $q=0.004$), and sphingosine-containing lipids ($p=0.003$, $q=0.017$). Additionally, polyunsaturated fatty acid (PUFA)-containing lipids in general were down-regulated ($p<0.001$, $q<0.001$), with notable down-regulation of PUFA-enriched triacylglycerides (TAGs) such as TAG 52:7, TAG 50:5, TAG 54:7, TAG 56:9, TAG 50:4, TAG 54:8 ($p<0.005$, $q<0.05$).

Conclusion: Distinct lipid class differences were observed between R and NR to ICB in melanoma. Together, these observations prompt the hypothesis that melanomas that are highly responsive to immunotherapy may efflux metabolites representing up-regulated nucleotide synthesis and dysregulated lipid metabolism representing a highly inflammatory state and potentially up-regulated autophagy.

#288 Metabolic profiling of MEKi-treated fusion-negative rhabdomyosarcoma cell lines suggests a role for glutamine antagonism as a preclinical therapeutic approach.

Konstantinos Flamourakis, Lior Levy, Jingdong Tan, Kathryn M. Lemberg

Johns Hopkins University School of Medicine, Baltimore, MD

Improved genetic characterization of rhabdomyosarcoma (RMS) subtypes, including PAX3/PAX7 fusion-negative RMS (FN-RMS) with RAS pathway mutations [1], has led to hope for individualized treatments with fewer side effects than conventional chemotherapy. Understanding the metabolic vulnerabilities of RMS remains an area with room for improvement. We hypothesized that determining the metabolic effects of RAS pathway inhibition in RAS-active RMS could inform new therapeutic combinations. We performed polar metabolomic profiling comparing trametinib and DMSO-treated FN-RMS cell lines, including two cell types with RAS mutations (SMS-CTR and RD) and one with an FGFR1 amplification (RMS-YM). Although inhibition of phospho-ERK was observed after 6 hours of treatment with trametinib in cells, limited effects on polar metabolites were observed at this time point. Following 24h of trametinib treatment, we observed alterations in purine and pyrimidine metabolism, amino acid metabolism, and central carbon metabolism. Based on these findings and our prior research on metabolic vulnerabilities other RAS-active sarcomas [2], we investigated RMS cell dependence on glutamine, the most abundant amino acid in serum and a crucial metabolic contributor of nitrogen and carbon substrates to these pathways [3]. FN-RMS cell lines demonstrated reduced colony formation in media with low glutamine. The clinical glutamine antagonist, DRP-104 [4], inhibited the growth of RAS-RMS cells with IC50 values in the low micromolar range. We investigated the effects of DRP-104 on markers of tumor growth, DNA damage, and cell death by western blotting. As our own and other data support a role for MEKi and glutamine antagonism in RAS-active tumor models [5], future investigations will seek to combine DRP-104 with trametinib in cell-based and in vivo RAS-RMS models.

References [1]Shern, J. F., et al. (2015). "Pediatric Rhabdomyosarcoma." Crit Rev Oncog **20**(3-4): 227-243.[2]Lemberg, K. M., et al. (2023). "Pro-905, a Novel Purine Antimetabolite, Combines with Glutamine Amidotransferase Inhibition to Suppress Growth of Malignant Peripheral Nerve Sheath Tumor." Mol Cancer Ther **22**(12): 1390-1403.[3]Yoo, H. C., et al. (2020). "Glutamine reliance in cell metabolism." Exp Mol Med **52**(9): 1496-1516.[4]Rais, R., et al. (2022). "Discovery of DRP-104, a tumor-targeted metabolic inhibitor prodrug." Sci Adv **8**(46): eabq5925.[5]Encarnacion-Rosado, J., et al. (2024). "Targeting pancreatic cancer metabolic dependencies through glutamine antagonism." Nat Cancer **5**(1): 85-99.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#29 The utilization of patient-derived organoids as a platform for drug screening: Chemotherapy, targeted therapy, and immunotherapy.

N.-G. Lee, M. Park, S.-E. Park, H.-A. Kim, S.-y. Lee, J. Rheey, **J.-Y. Park**;
Gradiant Bioconvergence, Seoul, Korea, Republic of

Cancer is one of a leading cause of mortality worldwide, with current treatments facing limitations such as drug resistance and relapse. To address these issues, the use of advanced in vitro models, such as patient-derived organoids (PDOs), is crucial. Unlike conventional 2D cultures, PDOs closely resemble the biological characteristics of tumors, thereby allowing the prediction of responses to drugs and supporting personalized therapies. Gradiant Bioconvergence (GBCC) has established a biobank comprising over 800 PDOs derived from tissue or metastatic samples of patients with lung, colorectal, gastric, pancreatic, and ovarian cancers. The biobank also includes NGS datasets of genomics and transcriptomics, as well as clinical information, including treatment history, and autologous peripheral blood mononuclear cells (PBMCs) or tumor infiltrating lymphocytes (TILs). PDOs are highly effective models for evaluating drug efficacy in vitro, capable of predicting clinical drug responses and providing valuable insights for the development of new therapeutics. GBCC has developed two distinct organoid-based drug screening platforms for evaluating various cancer therapies. The first drug screening platform employs organoids alone to evaluate anti-cancer drugs, including chemotherapy and targeted therapy. Additionally, the platform is capable of supporting mono or combination drug screenings. Of note, lung PDOs with driver mutations, such as EGFR and KRAS, demonstrated responsiveness to targeted drugs, with results that aligned with the patient's treatment history. Antibody-drug conjugates (ADCs), including Trastuzumab Deruxtecan (T-DXd), exhibited sensitivity in HER2-overexpressing gastric cancer organoids and lung cancer organoids with HER2 mutations. To evaluate immune checkpoint inhibitors (ICIs), the GBCC has developed a second drug screening platform using autologous T cell co-culture with organoid models. Flow cytometry was employed to ascertain organoid viability and cytokine release by T cells, thereby confirming that the viability of cancer organoids was reduced in the presence of ICIs. The results demonstrate that our organoid-based drug screening platforms can be utilized to assess drug responsiveness, thus representing a valuable tool for the preclinical evaluation of drugs, and supporting the advancement of precision medicine and the discovery of new anti-cancer drugs.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Senescence
Poster Session

#293 DNMT1 promotes escape from chemotherapy-induced senescence in acute myeloid leukemia.

Manna Ahmed[1], Dharmendra Kashyap[1], Hannah Kim[2], **Cihangir Duy**[1]

[1]Fox Chase Cancer Center, Philadelphia, PA,[2]Temple University, Philadelphia, PA

In addition to inducing cell death, chemotherapy can trigger cellular senescence in cancers as a side effect that is typically viewed beneficial for hindering cancer progression. However, acute myeloid leukemia (AML) cells can evade chemotherapy-induced senescence (CIS), leading to therapy failure and disease recurrence. To reduce the risk of AML recurrence, we investigated the mechanisms underlying CIS escape in AML that remain largely elusive. To achieve this, we developed an *ex vivo* AML recurrence model using primary patient-derived AML cells following exposure to chemotherapy (Ara-C). To minimize potential confounding effects caused by genetic heterogeneity, we utilized single-cell-expanded monoclonal AML populations. These populations were analyzed following CIS escape through integrative transcriptome and DNA methylome profiling. Our analyses revealed that, beside random DNA methylation changes, AML clones gained gene-specific promoter DNA hypermethylation sites associated with transcriptional silencing after CIS escape. This gained transcriptional silencing was conserved across five genetically independent AML specimens, suggesting a shared mechanism underlying AML recurrence after CIS. Using CRISPR-based epigenome-editing tools, we demonstrated that these acquired promoter DNA hypermethylation sites enhanced the survival fitness of AML cells during CIS. To identify the DNA methyltransferase (DNMT) responsible for this epigenetic silencing, we conducted knockdown experiments and identified DNMT1 as the key mediator. Both genetic and pharmacological inhibition of DNMT1 effectively blocked AML cells from escaping CIS. Overall, targeting DNMT1 disrupted CIS escape in AML cells, offering a therapeutic strategy to mitigate the risk of relapse driven by senescence-like leukemia cells.

#294 TFEB nuclear persistence predicts sensitivity to lysosomal inhibition in therapy-induced senescent thyroid cancer cells.
Beatrice Mazzoleni, Mara Mazzoni, Angela Greco

Experimental Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

Therapy-induced senescence (TIS), is a potential outcome of anti-cancer treatments, characterized by a stable cell cycle arrest. However, it is now widely accepted that this process acts as a double-edged sword: in facts, senescent cells are active drivers of cancer relapse, aggressiveness and metastasis, through the release of various pro-inflammatory factors, and the ability to resume proliferation. Therefore, selectively targeting TIS cells, a strategy named one-two punch approach, is crucial to avoid their harmful effects. To this purpose, identifying targetable characteristics of TIS cells is essential for the development of new senotherapeutics. One of the peculiarities of senescent cells is the expansion of the lysosomal compartment. To assess whether lysosomes may be a valid target for new senotherapies, we employed as a TIS model different cell lines of anaplastic thyroid cancer, an aggressive and untreatable type of tumor, undergoing senescence upon either CDK4/6 inhibitor Palbociclib or ionizing radiation treatment. Senescence induction and lysosomal expansion were assessed by a multi-marker approach, comprising β-gal assay, γH2AX nuclear foci quantification, p21 and HMGA2 expression levels, and lysosomal staining. Our findings indicated that the response to TIS varied among cell lines regarding lysosomal content increase. Although the autophagic flux, that relies on lysosomes, was elevated across all cell lines, its functionality was line-specific, as evidenced by differential p62 protein turnover. Targeting autophagy with the lysosomal inhibitor Chloroquine exhibited a senolytic effect only on senescent cells with an expanded lysosomal compartment and functional autophagy. Conversely, the lysosome-sparing autophagy inhibitor LY294002 did not replicate these results, suggesting that the senolytic effect is ascribable to lysosomal rather than autophagy inhibition. To further explore the differential expansion of the lysosomal compartment, we analyzed the localization of TFEB, the master regulator of lysosomal biogenesis, following TIS induction, through immunofluorescence. Six days after treatment, TFEB maintained a nuclear localization exclusively in the cell line with functional autophagy. Interestingly, this was associated with significantly lower protein levels of Exportin-1, an export receptor responsible for TFEB nuclear extrusion.
Our results indicate that lysosomes represent a promising target for developing new senotherapeutics, but TFEB's ability to enter and persist at the nuclear level appears to be crucial in determining this sensitivity. Understanding the molecular differences underlying the varying functionality of the lysosomal biogenesis machinery, often defective in cancer, may pave the way for the future introduction of this one-two punch approach in the clinical setting.

#295 Induction of senescent cancer associated fibroblasts promotes cancer cell growth.

Shengliang Zhang, Lanlan Zhou, Leiqing Zhang, Wafik S. El-Deiry

Brown University, Providence, RI

Cancer associated fibroblasts (CAF) are heterogenous and plastic population in the tumor microenvironment (TME), and each of the CAF subtypes plays a different role in cancer progression and suppression. Chemotherapy can induce CAF senescence, and cell senescence has been known to promote tumor growth and drug resistance. To investigate the role and molecular mechanism of the senescent CAF to promote tumor growth, we set up methods to induce CAF senescence by Chemotherapy or Radiation therapy using two gastrointestinal CAF cell lines CK4520 and CK8612. The flow cytometry showed around 40% of the cells with beta-gal staining in both CK8612 and CK4520 by the Etoposide (40 uM) treatment, and around 80-90% of the cells with beta-gal staining by the X-ray (20 Gy) treatment in both CK8612 and CK4520. Western blot assay showed an increase in p21 and p53 at protein level, and a decrease in Lamin B1 at the protein level in both CK8612 and CK4520 by the X-ray treatment. The cytokine profiling showed an increase in IL6, IL8, and CXCL2 and other factors related to senescence in CK4520 cells treated with X-ray and Etoposide. These results indicate the CAF senescence. In addition, the cytokine profiling also showed the different senescence-associated secretory phenotype (SASP) in CK4520 cells between X-ray and Etoposide treatment, and the different SASP also observed between CK4520 and CK8612 upon the X-ray treatment. To determine if the senescent CAF promotes tumor growth, we generated the conditioned medium using the senescent CK4520. Cancer cells cultured with the conditioned medium showed an increase in cell viability in different cancer cell lines. These results suggest that SASP of the senescent CAF promotes cancer cell growth.

#296 Identification of novel senolytic targets and development of conditionally active biologic-based-drug conjugates for targeted senescence-associated secretory phenotype elimination *in vivo*.

Jian Chen, Jing Wang, Haizheng Liu, Gerhard Frey, Cathy Chang, William J. Boyle, Jay M. Short

R&D, BioAtla, Inc., San Diego, CA

Elimination of senescent cells has been reported to improve normal and pathological changes associated with aging and cancer in mice. However, most senolytic agents inhibit anti-apoptotic pathways that do not differentiate between hyper-inflamed senescence-associated secretory phenotype (SASP) cells from relatively non-inflamed, valuable functional senescent cells, leading to off-target effects in normal tissues. Conditionally Active Biologics (CAB) technology is a proprietary platform unique in its ability to be selectively active in the context of diseased, acidic tissues (*e.g.* inflamed tissue and tumor microenvironment), but not in alkaline normal tissues. The aberrant accumulation of senescent cells in aged and cancerous tissue triggers increasing inflammatory signaling through SASP, promoting aging and tumor progression. Since these cells have an acidic surface environment, similar to cancer cells, we explored whether CAB technology allows selective removal of senescent cells in SASP-associated microenvironments. In addition, to complement this selectivity, we identified more specific senolytic targets to further reduce side effects and increase the efficiency of senescence cell removal and SASP reduction. In this study we identified several novel senescence-specific surface antigens as senolytic targets both i*n vitro* and *in vivo*. For the *in vivo* studies, we developed several fibrosis models in mice, including unilateral ureteral obstruction (UUO) in kidneys, bleomycin- or silica-induced pulmonary fibrosis in lung, high-fat diet (HFD)+CCl4-induced non-alcoholic steatohepatitis (NASH) in liver, and isoproterenol-induced cardiac fibrosis. We confirmed that the novel senescence-specific antigens we identified in our *in vitro* system are highly expressed in fibrotic tissues with newly induced senescent cells and associated with inflammation in *in vivo* models. Furthermore, these antigens were co-expressed with senescence makers SA-β-Gal, GL13, p16 and p21. Finally, we developed duocarmycin-based CAB-antibody drug conjugates (CAB-ADC) to study whether CAB-ADC reduces senescent cell occurrence *in vivo*. Mice treated with CAB-ADC targeting novel senescence-specific antigens showed a significant reduction of inflammatory cells infiltrated in kidneys compared to mice treated with benchmark and isotype ADCs. Thus, the CAB antibody-based therapeutic is a promising strategy for senescent cell removal in an inflammatory microenvironment using these newly identified senescence-associated targets. In conclusion, CAB technology provides a new generation of biologics with an increased safety margin and therapeutic index, selectively targeting acidic senescence cells and SASP in cancer and age-related diseases.

#297 Targeting splicing factor SF3B4 induces cancer cell senescence by elevating p21 mRNA levels.

Donghee Kang, Hyun Jung Hwang, Min-Ji Kim, Jae-Seon Lee

Inha University College of Medicine, Incheon, Korea, Republic of

SF3B4, a component of U2 snRNP complex, is amplified in various cancers and contributes to cancer cell proliferation, migration and invasion by regulating alternative gene splicing. Despite its potential significance, the mechanisms by which SF3B4 regulates nonsense-mediated mRNA decay (NMD) and cancer cell senescence remain poorly understood. In this study, we reveal a novel regulatory role of SF3B4 in the regulation of cancer cell senescence by targeting *p21* mRNA. Depletion of SF3B4 leads to reduced cancer cell proliferation, increased SA-β-Gal positivity, and elevated p21 expression independent of p53, ultimately inducing cellular senescence. Mechanistically, SF3B4 directly binds to the 3'UTR of *p21* mRNA, negatively regulating its stability. We demonstrate that SF3B4 plays a crucial role in recruiting key components of the NMD pathway, such as UPF1, MAGOH and RNPS1, to promote the degradation of *p21* mRNA. Depletion of SF3B4 disrupted the association of NMD factors from the 3'UTR of *p21* mRNA. Taken together, our results reveal a novel regulatory role in which SF3B4 collaborates with NMD factors to regulate *p21* mRNA stability and cancer cell senescence.

#298 Senescence-associated gene expression in head and neck cancer in TCGA samples.

Sanjana Arji, Beverly R. Wuertz, Frank G. Ondrey

Otolaryngology, University of Minnesota-Twin Cities, Minneapolis, MN

Introduction: Senescent cells in tumors constitute an important cell population that may be both anti- and pro-tumorigenic. Head and neck cancer (HNC) is associated with treatment resistance, recurrence, and decreased survival rates that are somewhat stagnant. We examined the TCGA for differences in senescence-associated genes using various parameters.
Methods: 83 established HNC senescence-associated genes were examined in the TCGA Pancancer Atlas dataset via CBioPortal. mRNA expression (RSEM; Batch normalized from Illumina HiSeq_RNASeqV2) for each of the genes of interest was examined. Demographic information, disease stage, and HPV subtype data were also collected. Student's t-tests were conducted using RStudio's *tidyverse* package to determine statistically differentially expressed genes ($p < 0.05$). Then, Gene Ontology, through DAVID Bioinformatics, was conducted on the set of statistically significant genes found from each comparison to determine overexpressed pathways and adjusted p-values post-Benjamini-Hochberg correction ($q < 0.05$).
Results: Considering HPV Status: 65/83 of the tested genes statistically differed between HPV+ and HPV-. Out of the 65 significant genes, 6 were related to peptidyl-serine phosphorylation: TOP1, NEK6, LATS2, MAPK1, MAP4K1, TGFBR1($p = 4.3E-5$; $q = 1.7E-2$), 5 were related to the G1/S transition of the mitotic cell cycle: CCND1, CDK6, CDKN1A, EZH2, LATS2 ($p = 2.7E-5$; $q = 1.7E-2$), and 5 were related to canonical WNT signaling pathway: YAP1, CAV1, LATS1, PTEN, SIAH1($p = 1.7e-4$; $q = 3.5E-2$). Considering Caucasian vs African American, HSPA5 ($p=0.02$), NEK6 ($p = 0.0163$), and SMURF1 ($p = 0.0015$) were all found to be statistically significant. SMURF1 and HSPA5 were found to negatively regulate the TGF-beta receptor signaling pathway ($p = 1.3e-2$; $q = 7.6e-1$). Considering 1st vs 4th quartile age: SERPINE1 ($p = 0.00301$), POU5F1 ($p = 0.0063$) and CDKN2A ($p = 0.0653$) were differentially expressed. No overexpressed pathways were noted in the Gene Ontology Biological Process Analysis. Considering tumor stage: AREG ($p=0.01405$), GAPDH ($p = 0.01475$), PSMB5 ($p = 0.04848$), LIMA1 ($p = 0.04709$), EHF ($p = 0.03858$), and DPY30 ($p = 0.003711$) were found to be differentially expressed. However, no overexpressed pathways were found in Gene Ontology Biological Process Analysis.
Conclusions: We identified various statistically different senescence-associated markers between various parameters in our analysis of TCGA-associated data. Furthermore, four overexpressed pathways were identified through gene ontology of statistically significant genes between HPV status and race. Identifying these pathways and genes may help better understand variations in HNC presentation and create more definitive senescent markers to determine prognosis based on these parameters. Further analysis of the genes with higher stringency thresholds and consideration of epigenetic factors must be done to solidify the findings.

#299 Characterization of a senescence phenotype induced by advanced glycation end products in prostate cancer.

Luisa Bacca[1], Tarek Hallal[2], Carole Luthold[3], Nadia Boufaied[4], Francois Bordeleau[3], David Labbe[5]

[1]Experimental Medicine, McGill University, Montreal, QC, Canada,[2]Department of Anatomy and Cell Biology, McGill University, Montreal, QC, Canada,[3]Departement de biologie moleculaire, de biochimie medicale et de pathologie, Universite Laval, Quebec City, QC, Canada,[4]Cancer Research Program, Research Institute of the McGill University Health Centre, Montreal, QC, Canada,[5]Department of Surgery, McGill University, Montreal, QC, Canada

Prostate cancer (PCa) represents a worldwide leading cause of cancer-related mortality in men. Diabetes is also a highly prevalent and a rapidly growing pathology. Intriguingly, diabetes is associated with decreasing PCa incidence, and at the same time, it is linked to increased mortality rates for men already afflicted by the disease. A plausible mediator causing this effect are Advanced Glycation End Products (AGEs), products of the Maillard reaction, occurring between sugars and proteins at body temperature. AGEs, known to be elevated in diabetic patients, have been reported to increase the Extracellular Matrix (ECM) stiffness via ECM-crosslinking, potentially explaining the tumor-promoting effects of diabetes in cancer progression. Nonetheless, the protective role of diabetes in prostate cancer is not yet fully understood. Interestingly, we demonstrated that AGEs, produced by mixing sugars and an abundant circulating protein in the blood decreased PCa cell proliferation *in vitro* in a dose-dependent and irreversible manner across a panel of human PCa cell lines. Importantly, through transcriptomic analysis, we found that AGEs-mediated cell proliferation blocking correlate with a senescence transcriptional signature. Accordingly, treatment with AGEs lead to increased beta-galactosidase activity, a hallmark of senescence, and to the upregulation of senescent markers linked to cell cycle arrest ($p21^{WAF1/Cip1}$) and pro-inflammatory cytokines (IL-8) at the transcriptional and translational levels. We also confirmed the expression of the senescence-associated secretory phenotype (SASP, *e.g.*, IL, CXCL, CCL) in AGEs-treated PCa cells. Finally, our validation of key transcriptomics analysis confirmed the upregulation of HMOX1, an enzyme reported to be induced during cellular senescence, upon AGEs treatment. Altogether, our results suggest that AGEs-mediated senescence could underlie the lower incidence of PCa among diabetic patients. This sets the stage for combination treatments between AGEs and senolytics, drugs that selectively target and induce cell death in senescent cells, as a novel therapeutic strategy in PCa.

TUMOR BIOLOGY: Cancer Stem Cells
Poster Session

#3 TIP60 modulates colorectal cancer stemness through cd44 expression.

Mohammad Asad, Sudhakar Jha

Physiological Sciences, Oklahoma State University, Stillwater, OK

Objective: To identify the functional role of TIP60 in regulating colorectal cancer (CRC) stemness.
Materials and Methods: The HCT116 CRC cell lines were cultured in DMEM (high glucose) supplemented with 10% FBS. ShRNA-mediated depletion of TIP60 was generated using lentiviral vectors. To confirm TIP60 knockdown efficiency and CD44 expression levels, qPCR analyses were performed. For the colony formation assay, 1000 cells of HCT116 ShLuc control and ShTIP60 were seeded in 6-well plates and incubated for 11 days. For immunofluorescence staining, cells were grown on cover slips, fixed with 100% methanol for 20 minutes, and blocked with 3% BSA. They were then incubated with E-cadherin primary antibody at a 1:500 dilution and secondary antibody Alexa Fluor-488 at a 1:1000 dilution. Cells were mounted with Vectashield mounting medium containing DAPI. Spheroid culture was conducted in 96-well ultra-low attachment plates, with 100 cells of HCT116 ShLuc control and ShTIP60 seeded in each well and incubated for 7 days. The 3D invasion assay was performed in 96-well ultra-low round-bottom plates supplemented with 3D basement membrane extract (BME) invasion matrix.
Findings: This study uncovered the pivotal role of HIV Tat-interactive protein (TIP60) in regulating CRC stemness. TIP60 depletion resulted in a significant decrease in cellular proliferation, highlighting the importance of TIP60 in CRC growth. Furthermore, TIP60 depletion altered colony formation, transitioning from densely packed formations to scattered spindle networks implying its involvement in the epithelial-mesenchymal transition (EMT). 3D culture models demonstrated that TIP60 is essential for spheroids formation, emphasizing its significance in promoting cancer stemness. TIP60-depleted cells exhibited enhanced invasion in a 3D BME invasion matrix, accentuating its crucial contribution to cellular invasiveness. Furthermore, we have identified that TIP60 undergoes liquid-liquid phase separation (LLPS). TIP60 harbors an intrinsically disordered region (IDR), which is essential for inducing LLPS in CRC. We have also discovered that TIP60 IDR regulates CRC stemness and EMT in CRC. In addition, the reduction of TIP60 was linked to a decrease in the expression of CD44, which is a crucial indicator for cancer stem cells (CSCs). Significantly, CD44 overexpression reinstated the efficiency of spheroid formation and reversed the EMT phenotype.
Conclusions and Clinical Implications: Our findings suggest that TIP60 has CSC characteristics in CRC, and targeting TIP60 will offer a novel therapeutic approach against colorectal cancer stem cells.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#30 Establishment of a chemical carcinogen-induced bladder cancer model using healthy canine bladder organoids.

Yuko Nagashima, Haru Yamamoto, Mohamed Elbadawy, Amira Abugomaa, Tatsuya Usui, Kazuaki Sasaki

Tokyo University of Agriculture and Technology, Tokyo, Japan

Background Canine bladder cancer (BC) accounts for approximately 2% of all spontaneous canine cancers, and the majority (over 90%) of these are moderate to highly invasive bladder cancers. In terms of biological behavior, canine BC is said to be similar to the cytological and molecular characteristics of human muscle-invasive BC, especially high-grade invasive transitional cell carcinoma. In recent years, cancer research in dogs has been used to define genetic and environmental risks and evaluate prevention and treatment approaches that will benefit not only dogs but also humans. An organoid is a miniaturized and simplified version of an organ produced ex vivo in three dimensions, mimicking the key functional, structural, and biological complexity of the actual organ. Organoids are attracting attention as complementary models to traditional animal models, and are often used in human and animal cancer research. In the current study, we developed a carcinogen-induced BC organoid model using organoids generated from healthy canine bladder tissue.
Materials and methods Canine normal bladder organoids (CNBOs) were established using bladder epithelial cells collected using a urinary catheter from a healthy beagle dog in a non-invasive manner. The CNBOs were treated with 1 μM 2-Acetylaminofluorene (2-AAF), a known bladder carcinogen, and then transplanted subcutaneously into immunodeficient mice. As a result, a mass was formed, confirmed to be a benign bladder tumor (canine bladder benign tumor: CBB) by histopathological analysis. When organoids were developed again from CBB (CBBO) and transplanted under the skin of immunodeficient mice, the tumor was reformed. The re-formed tumor was confirmed to be BC (Canine bladder carcinoma: CBC), and organoids were created from CBC (CBCO), and comparative analysis was conducted on CNBO, CBBO, and CBCO.
Results The expression of CK5 and UPK3A, was confirmed in CNBO, CBBO, and CBCO. In addition, observations using electron microscopy revealed findings suggestive of a normal bladder in CNBO and findings suggestive of a malignant tumor in CBCO. RNA sequencing analysis showed significant differences in gene expression in CNBO, CBBO, and CBCO. Gene set enrichment analysis revealed that the epithelial-mesenchymal transition pathway was enhanced in CBCO compared to CNBO. CNBO showed high cell proliferation in cell proliferation assays. The anti-cancer drug sensitivity test showed different sensitivities to treatment with vinblastine, mitoxantrone, carboplatin, and lapatinib.
Discussions In this study, chemical carcinogen-induced canine BC organoids were generated by treating CNBO with bladder carcinogen (2-AAF) and transplanting it into mice twice. In the future, we plan to perform cancer-related gene mutation analysis and proteomic analysis of CNBO, CBBO, and CBCO to clarify further the detailed molecular mechanisms of carcinogenesis.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Senescence
Poster Session

#300 Senescence and secreted factors: implication of DNA damage response pathways.

Jordan Desmul, Nicolas Malaquin, Francis Rodier

CRCHUM, Montreal, QC, Canada

Introduction Senescence is a tumor-suppressive mechanism characterized by a halt in cellular proliferation. It can be triggered by various genotoxic sources, such as exposure to ionizing radiation during anticancer therapies to prevent the proliferation of cells with DNA damage. These cells adopt a senescence-associated secretory phenotype (SASP), which consists of hundreds of molecules, including cytokines, chemokines, and other pro-inflammatory factors. These secretions have beneficial anti-proliferative effects. However, their long-term accumulation can lead to detrimental outcomes, such as aging and tumorigenesis, driven by chronic inflammation. The senescence mechanism develops progressively in multiple steps over several days following genotoxic stress. In our model of irradiation-induced senescence (IR-10Gy) in human fibroblasts (BJ, HCA2), IR activates DNA damage response (DDR) pathways. These cells enter senescence 7 to 10 days post-exposure and develop their SASP. It is well established that there is a link between DDR activation and SASP induction. The genetic depletion of key DDR players (ATM, CHK2, NBS1) results in SASP suppression. However, the timing of these processes does not coincide. While DDR pathways are activated within minutes of DNA damage, the SASP takes several days to develop. Thus, the mechanism by which DDR influences SASP activation remains poorly understood. We identified a late non-canonical branch of the DDR (ncDDR) that appears to activate several days after the initial stress and is associated with the late pro-inflammatory SASP. In contrast, the canonical DDR pathway seems responsible for early non-inflammatory secretions. These findings suggest that the composition of senescent cell secretions evolves over time.
Objective: We hypothesize that during the multi-step process of senescence establishment, the composition of the SASP evolves based on the activation or deactivation of DDR pathways. Modulating DDR activation through irradiation or genetic/pharmacological approaches will allow us to understand its impact on SASP. Our objective is to determine how the activation of different DDR pathways drives SASP induction and how these pathways influence its composition over time.
Results: We irradiated fibroblasts with increasing doses of irradiation (10, 20, 40, 80 Gy) to sustain a high level of canonical DDR activation. At day 10 post-IR, we observed: • a dose-dependent decrease in the number of cells with genomic instability, • an almost complete suppression of the pro-inflammatory factor IL-6, • a significant increase in the secretion of GDF15, another SASP factor. Interestingly, these results were reversed when analyzing the secretome 24 hours post-irradiation. In conclusion, increased DDR activation induced a significant increase in various components of the early SASP and demonstrated distinct regulation between the early and late SASP.

#301 PARP inhibitor-induced senescent fibroblasts enhance prostate cancer cell proliferation and suppress NK cell-mediated cytotoxicity.

Maximillian Pinho-Schwermann[1], **Claire Lin**[2], Shaolei Lu[3], Shengliang Zhang[3], Praveen Srinivasan[3], William MacDonald[3], Connor Purcell[3], Matthew J. Hadfield[4], Galina Lagos[4], Vida Tajiknia[3], Nolan Stubbs[3], Kimberly Meza[3], Dragan Golijanin[5], Elias Hyams[5], Anthony Mega[4], Wafik S. El-Deiry[3], Benedito A. Carneiro[4]

[1]Department of Surgery, The Warren Alpert Medical School of Brown University, Providence, RI,[2]The Warren Alpert Medical School of Brown University, Providence, RI,[3]Department of Pathology and Laboratory Medicine, Brown University, Providence, RI,[4]Hematology-Oncology Division, Brown University Health, Providence, RI,[5]Urology Division, Brown University Health, Providence, RI

Introduction: Prostate cancer (PCa) is a leading cause of cancer-related morbidity and mortality in men. Despite initial tumor response to androgen deprivation therapy (ADT), most patients with PCa progress to a castrate-resistant disease state. Therapy-induced cellular senescence modulates the tumor microenvironment (TME) through the senescence-associated secretory phenotype (SASP), contributing to tumor growth, progression, and treatment resistance. We have demonstrated increased patient serum levels of SASP-related cytokines following treatment with ADT (Schwermann, AACR 2024). We investigated the ability of PARP inhibitors (PARPi) to induce senescence of fibroblasts and its impact on the proliferation of PCa cell lines and NK cell-mediated cytotoxicity.
Methods: Senescence of fibroblasts (IMR-90) was induced by treatment with etoposide (40uM), olaparib (15uM), and talazoparib (20uM) for 3 weeks. The senescent population was stained with the SPiDER-Gal live cell marker and sorted (BD FACS Aria). Cytokines (SASP) from senescent fibroblasts were characterized using bead-based multiplex assay (Luminex® xMAP®). Senescent fibroblasts were co-cultured with PCa cell lines (LNCaP, DU145, 22Rv1, PC3) in a 10:1 ratio over 12 days. The growth rate of PCa cells was assessed by colony assay formation. Senescent fibroblasts were co-cultured with NK cells (1:30 ratio) over 5 days. The NK proliferation status and cytotoxic markers were analyzed by flow cytometry. NK cells exposed to senescent fibroblasts were incubated with fluorescently labeled PCa cells.
Results: PCa cell lines displayed increased proliferation when co-cultured with senescent fibroblasts (PC3+IMR-90 [C] = 59.6±4.72, PC3+etoposide treated IMR-90 [T1]=111.7±10.6, PC3+olaparib treated IMR-90 [T2]=97±11.3, $p<0.005$, LNCaP+[C]=16.67±5.6, LNCaP+[T1]=86.33±5.1, LNCaP +[T2]=65.3±8.3, $p<0.001$). Senescent fibroblasts induced by etoposide, olaparib, and talazoparib displayed an increased secretion of the SASP IL-6, IL-8, IL-1, IL-15, CCL2, CCL3, and CCL20. NK cells exhibited a lower proliferation rate when co-cultured with senescent fibroblasts (Ki67% [C]:71.6±3.5, [T1]: 26.3±5.1, [T2]: 41.5±3.5, $p<0.002$) and reduced IFN-γ secretion ([C]:32±4, [T1]: 26±3.1, [T2]: 29±6.5, $p=0.32$). NK cells co-cultured with senescent fibroblasts also exhibited reduced killing of PCa cells (tumor-NK cell ratio 10:1 and 20:1).
Conclusions: Etoposide and PARPi-induced senescent fibroblasts displayed increased SASP production and promoted the proliferation of PCa cells in vitro. These senescent fibroblasts also limited NK cell proliferation and reduced their cytotoxicity. Future experiments will investigate the impact of therapy-induced senescent fibroblasts *in vivo* growth of PCa tumors.

#302 Study of treatment-induced senescence in head and neck squamous cell carcinoma according to HPV status.

Karim Bouhjar[1], Carine Michiels[2], Francis Rodier[1]

[1]CrCHUM, ICM, University of Montreal, Montreal, QC, Canada,[2]URBC, Narilis, University of Namur, Namur, Belgium

Introduction: Head and neck cancer (HNC) is the sixth most common cancer worldwide, with an incidence of approximately 600,000 new cases each year. Within this type of cancer, two main categories should be distinguished: HPV-negative and HPV-positive tumors. In this context, the tumorigenesis process differs, and the prognosis associated with each subtype is variable. Despite this difference, the therapeutic management of patients is identical. The aim of this research project is to demonstrate that this management should take HPV status into account and that the associated treatments must be adapted accordingly. Indeed, preliminary results show that depending on the HPV status, the response to treatments induces either a pro-apoptotic or pro-senescence response. This research project therefore investigates the biological and molecular mechanisms by which the HPV status influences cell fate decisions following treatment.

Materials and Methods: Different characteristics of senescent cells have been assessed; morphology, positivity for SA-β-gal, SASP expression, cell cycle assessment and proliferation. The preliminary results suggest an induction of cellular senescence in most of the cell lines following treatment. Following this, a new aspect of the thesis has been recently opened through a mass spectrometry approach. In parallel, HPV-negative cell lines expressing key viral factors are currently being generated to create a more relevant model, combined with the use of patient tissues that will be analyzed by multiplexed immunofluorescence.

Discussion: The first results have fully characterized the induction of the senescent phenotype following treatment, depending on HPV status, in head and neck cancer cell lines. The unbiased proteomic approach will help to identify promising candidates involved in this senescence induction according to HPV status. Simultaneously, access to the CrCHUM biobank will allow testing of the various hypotheses in situ.

#303 Discovery of autophagy-inhibiting compounds as a novel class of senolytics.

Martin Mistrik, Matthew Lacey, Lucie Beresova, Alzbeta Srovnalova, Josef Srovnal, Petr Dzubak, Marian Hajduch

Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky Univer, Palacky University in Olomouc, Olomouc, Czech Republic

Introduction: Senolytics are compounds capable of selectively eliminating senescent cells, holding promise for therapies targeting age-related diseases and cancer. To identify novel senolytic agents, we screened a chemical library using cellular models of replicative senescence induced by replication stress. This screen yielded several hits, including MCOPPB, a known nociceptin receptor agonist. Subsequent in vivo studies in a murine model confirmed the potent senolytic activity of MCOPPB. Surprisingly, further investigations revealed that this activity was independent of nociceptin receptor signaling, suggesting an off-target mechanism. Methods: To explore the underlying mechanism, we performed a cell-painting assay, which phenotypically links compounds based on their cellular effects. MCOPPB clustered closely with another senolytic hit from the screen, Daurisolin, a natural autophagy inhibitor. Autophagy inhibition by MCOPPB was validated through assays measuring LC3 accumulation and other markers. Additional studies with known autophagy inhibitors, including Dauricin and Chloroquine, demonstrated both senolytic activity and clustering with MCOPPB in the cell-painting assay.
Results: Our findings establish autophagy inhibition as the common mechanism of senolytic action for this newly identified class of compounds. MCOPPB, Daurisolin, and related inhibitors displayed consistent phenotypes in cell-painting assays and induced selective clearance of senescent cells. The clustering of these compounds in phenotypic assays provides a powerful approach for identifying senolytics with similar mechanisms of action.
Conclusion: This study highlights autophagy inhibition as a novel senolytic mechanism and identifies a unique chemical cluster of autophagy-inhibiting senolytic compounds. These insights could drive the development of targeted therapies for conditions associated with cellular senescence.
Acknowledgements: This work was supported by the Technology agency of the Czech Republic project PERMED: T2BA (TN02000109) and the National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102). Infrastructure was supported by projects CZ-OPENSCREEN (LM2023052), EATRIS-CZ (LM2023053) and Czech Bioimaging (LM2023050)

#304 Profiling the acute effects of androgen receptor inhibition in hormone-sensitive prostate cancer.

Julian Brandariz[1], Lara de Llobet Cucalon[1], Daniel Aguilar[1], Andrei Salca[1], Victor Esquefa[1], Youhao Chen[1], Gisela Mir[1], Anna Oliveira[1], Irene Casanova-Salas[1], Alba Escriche[1], Manuel Jara Espejo[2], Jose Alberto Lopez-Dominguez[3], Victoria Cid[4], Marco Piva[4], Maria Abad[1], Francisco Martinez-Jimenez[2], Raquel Perez-Lopez[1], Manuel Serrano[5], Arkaitz Carracedo[4], Nicolas Herranz[1], Joaquin Mateo[1]

[1]Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain,[2]Systems Oncology Program, Vall d'Hebron Institute of Oncology (VHIO), Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain,[3]Instituto de Biologia Molecular y Celular del Cancer and Centro de Investigacion del Cancer of Salamanca, University of Salamanca-CSIC, Campus Unamuno s/n, 37007, Salamanca, Spain,[4]Translational Prostate Cancer Research Laboratory, CIC bioGUNE-Basurto, Biobizkaia Health Research Institute, Barakaldo, Bizkaia, Spain,[5]Institute for Research in Biomedicine (IRB Barcelona), Barcelona, Spain

Introduction: Metastatic prostate cancer (mPC) is driven by androgen signaling. Standard treatment involves androgen-deprivation therapy (ADT) and androgen receptor (AR) inhibitors (e.g. enzalutamide), but resistance eventually arises, leading to metastatic castration-resistant prostate cancer (mCRPC). This study investigated the acute effects of AR inhibition in hormone-sensitive mPC to identify therapeutic targets to prevent resistance.

Methods: We analyzed RNAseq before and after 3 or 6 months of AR inhibition from two neoadjuvant enzalutamide clinical trials in PC (NCT03297385, *n=39*; NCT02430480, *n=37*) using Hallmark pathways, and with a focus on senescence and senescence associated secretory phenotype (SASP) signatures. *In vitro*, we treated human (LNCaP, VCaP, LAPC4) and murine (Myc-CaP, *Pten*$^{pc-/-}$*Lkb1*$^{pc-/-}$) mPC cells with enzalutamide and we assessed proliferation (CCK8, BrdU), viability (AnnexinV/PI), cell cycle distribution, SAβGAL activity, cyclin-dependent kinase (CDK) inhibitors, and RNAseq. We derived an RNA hormonal therapy-induced senescence (HTIS) signature from LNCaP cells treated with enzalutamide for 2 days vs 2 weeks or 6 weeks. ssGSEA analysis assigned a senescence score to patient samples for clinical outcome correlations. *In vivo*, HTIS was validated in LNCaP and mPC patient-derived xenografts (PDX) models using SAβGAL activity, Ki67 (proliferation), p16/p21, and RNAseq.

Results: Upon enzalutamide, GSEA analysis of patient samples (NCT03297385, NCT02430480) revealed downregulated Hallmark gene sets linked to proliferation/AR signaling, and upregulated pathways related to protein secretion, inflammation, TNFα-NFκB, and IL6-JAK-STAT3 signaling. Senescence and SASP signatures were enriched in post-enzalutamide biopsies. *In vitro,* AR inhibition in LNCaP, VCaP, LAPC4, Myc-CaP and *Pten*$^{pc-/-}$*Lkb1*$^{pc-/-}$ induced G_0/G_1 proliferation arrest without affecting cell viability, alongside senescence features such as increased SAβGAL activity, and upregulation of p16/p21 and SASP genes. HTIS signature from LNCaP cells, which incorporated downregulated genes linked to proliferation and upregulated genes linked to inflammation was validated *in vivo*, where castration or enzalutamide in LNCaP xenografts and PDXs tumors resulted in decreased proliferation (Ki67), increased SAβGAL activity, upregulation of p16 or p21, and enrichment for senescence and SASP signatures. Last, patients from the NCT03297385 dataset with high HTIS signature scores showed greater correlation with other senescence signatures and lower rates of biochemical relapse (high = 28% (7/25); mid = 42.9% (3/7); low = 85.7% (6/7)).

Conclusions: We demonstrate that AR inhibition induces a senescent phenotype in hormone-naïve prostate cancer models, including PDXs. RNAseq of patient biopsies suggest this adaptive phenotype is present also in patients and has prognostic implications.

#305 Cellular senescence in human liver under normal aging and cancer.

Xiang Li[1], Alla Karpova[1], Chien-Wei Peng[1], Daniel R. Rapp[2], Kelsey L. Gallant[1], Andrew J. Houston[1], Andre Luiz N. Targino da Costa[1], Michael D. Iglesia[1], John M. Herndon[2], Kathleen Byrnes[3], Nataly Naser Al Deen[1], Wagma Caravan[1], Preet Lal[4], Reyka G. Jayasinghe[1], Jeffrey A. Blatnik[5], William G. Hawkins[6], Dominic E. Sanford[6], J. Chris Eagon[5], Natasha Leigh[7], Maria Bernadette M. Doyle[5], L. Michael Brunt[5], William C. Chapman[5], Roheena Z. Panni[5], Darren R. Cullinan[5], Sherri R. Davies[8], Matthew A. Wyczalkowski[1], Michael C. Wendl[1], Hong Zhang[9], Milan G. Chheda[10], Sheila A. Stewart[11], Feng Chen[4], Ryan C. Fields[2], Li Ding[12]

[1]Department of Medicine, McDonnell Genome Institute, Washington University in St. Louis, Saint Louis, MO,[2]Department of Surgery, Siteman Cancer Center, Washington University in St. Louis, Saint Louis, MO,[3]Department of Pathology and Immunology, Washington University in St. Louis, Saint Louis, MO,[4]Department of Medicine, Siteman Cancer Center, Washington University in St. Louis, Saint Louis, MO,[5]Department of Surgery, Washington University in St. Louis, Saint Louis, MO,[6]Siteman Cancer Center, Washington University in St. Louis, Saint Louis, MO,[7]Siteman Cancer Center, Department of Surgery, Washington University in St. Louis, Saint Louis, MO,[8]Department of Medicine, Washington University in St. Louis, Saint Louis, MO,[9]Department of Pediatrics, University of Massachusetts Medical School, Worcester, MA,[10]Department of Medicine, Siteman Cancer Center, Department of Neurology, Washington University in St. Louis, Saint Louis, MO,[11]Department of Cell Biology and Physiology, Washington University in St. Louis, Saint Louis, MO,[12]Department of Medicine, McDonnell Genome Institute, Siteman Cancer Center, Department of Genetics, Washington University in St. Louis, Saint Louis, MO

Liver senescence involves the gradual deterioration of hepatic cell function and reduction of regenerative capacity due to aging, chronic liver disease, and cancer. To elucidate the role of liver senescence in aging and cancer, we employed single-cell sequencing, spatial omics, and imaging approaches, collectively over 100 assays, to characterize 43 normal liver samples from a cohort of 95 donors, across ages and selected based on histology and clinical data. We conducted similar assays on samples from 24 patients with colorectal cancer metastasis in the liver. Our study identified CDKN1A+ senescent cells across four liver-resident cell types, namely hepatocytes, fibroblasts, cholangiocytes, and liver sinusoidal endothelial cells, with their prevalence significantly associated with age or liver disease. We observed distinct senescent populations in fibrotic compared to aged livers, indicating differential mechanisms of senescence in these conditions. Fibrosis was related to the expansion of a specific niche residing in portal triads and fibrous septae including immune cells and senescent fibroblasts, which exhibited abundant and strong cellular interactions. Notably, chemotherapy exacerbated senescence, increasing the number of senescent hepatocytes by five-fold compared to aging alone, while the effect of cancer itself was moderate. Other cell types affected by chemotherapy included liver sinusoidal endothelial cells and stellate cells, while CDKN1A+ portal fibroblasts and cholangiocytes were not affected as much. Cancer appeared to induce senescence in nearby hepatocytes, whereas chemotherapy severely affected more distant normal liver tissue. Cancer patients also demonstrated unique populations of CDKN2A+ hepatocytes and cholangiocytes with a distinct secretory phenotype. Senescent cells in liver shared core features, such as AP-1, NFkB activity, SERPINE1, CCL2, COL4A secretion, and anti-apoptotic protein expression, offering new insights into the cellular and molecular mechanisms underlying liver senescence. These findings underscore the complexity and heterogeneity of liver senescence and may have implications for our understanding of liver health and disease.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Antireceptors and Other Biological Therapeutic Agents
Poster Session

#309 Adding nadunolimab to chemotherapy or antibody drug-conjugates (ADCs) may improve antitumor efficacy and counteract peripheral neuropathy.

Samuel Le Sourd[1], Rocio Garcia-Carbonero[2], Sandrine Hiret[3], **Elin Jaensson Gyllenback**[4], Camilla Rydberg Millrud[4], Lars Thorsson[4], Svetlana Shatunova[5], Irina Vetter[5], Hana Starobova[5], David Liberg[4]

[1]Centre Eugene Marquis, Rennes, France,[2]12 Oct Hospital, Madrid, Spain,[3]Institut de Cancerologie de l'Ouest ICO, Saint-Herblain, France,[4]Cantargia AB, Lund, Sweden,[5]The University of Queensland, Institute for Molecular Bioscience, Saint Lucia, Australia

Background: Nadunolimab, a fully humanized ADCC-enhanced, monoclonal IgG1 antibody targets IL-1 receptor accessory protein (IL1RAP) and blocks IL-1 signaling. In pts, nadunolimab with standard chemotherapies showed good safety and promising efficacy. In 73 advanced pancreatic ductal carcinoma (PDAC) pts, combination with nab-paclitaxel and gemcitabine showed 33% ORR and 13.2mo OS, with 48% ORR and 14.2mo OS in the IL1RAP-high subgroup. In 40 1st or 2nd line non-small cell lung cancer pts, combination with platinum-doublet showed 55% ORR and 13.7mo OS, with 91% ORR and 26.7mo OS in the 2nd line non-squamous subgroup. Interestingly, in addition to promising anti-tumor effects, a significantly delayed onset and reduced incidence of nab-paclitaxel/gemcitabine-related chemotherapy-induced peripheral neuropathy (CIPN) were observed in pts receiving higher doses of nadunolimab compared to lower doses (36% vs 60%). CIPN is a neuroinflammatory side effect driven by immune activation and cytokine release such as IL-1 that limits treatment and severely impairs quality of life. Building on these promising findings, our aim was to further investigate nadunolimab's additional potential in mitigating neuropathies associated with chemotherapy and ADCs.
Methods: The potential neuroprotective effect of nadunolimab was analyzed in two clinical trials: one involving 14 pts with 8 different cancer indications treated with FOLFOX and nadunolimab at 1 mg/kg (n=7) or 0.5 mg/kg (n=7), and another involving 18 PDAC pts treated with FOLFIRINOX and nadunolimab at doses of 0.5 mg/kg (n=8), 1 mg/kg (n=3), 1.5 mg/kg (n=4), and 2.5 mg/kg (n=3). In mice, neuropathy was induced by vincristine, nab-paclitaxel, nab-paclitaxel plus gemcitabine, or ADC payloads. The effects of nadunolimab surrogate antibody (10 mg/kg) on neuropathy were evaluated using electronic von Frey apparatus, parallel rod floor apparatus, and grip strength meter. Tissue samples were analyzed for inflammatory markers and immune cell infiltration.
Results: We observed a lower incidence of any-grade FOLFOX-induced CIPN in the 1 mg/kg nadunolimab dose group compared to the 0.5 mg/kg dose group (43% vs 100%) and a significantly longer time to onset (median not estimable vs 4 days; HR=0.15, p=0.0004). Similar trends were observed for FOLFIRINOX-induced CIPN in PDAC pts. In mice, CIPN was linked to infiltration of myeloid cells and inflammatory cytokines in dorsal root ganglia. Co-administration of the nadunolimab surrogate completely abrogated CIPN. Ongoing studies aim to confirm the effects of the nadunolimab surrogate on ADC payload-induced neuropathy.
Conclusion: Overall, our current findings in conjunction with previous results, suggest that nadunolimab may not only offer anti-tumor benefits but also protection against the neuro-inflammatory component driving chemotherapy- and ADC-induced neuropathy.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#31 Characterization and cross comparison of cancer xenografts in humanized mice.

Ulrike Litzenburger, Stephanie Stahnke, Oliver von Ahsen, Simon Heller, Philipp Renke, Natalie Altenburg, Ellen Hauke, Rene Zernicke, Carlo Stresemann

Nuvisan, Berlin, Germany

Immunotherapy has emerged as a promising cancer treatment strategy, including approaches such as immune checkpoint inhibitors (ICIs), cytokine-based therapies, chimeric antigen receptor (CAR) T-cell therapies, oncolytic viruses, cancer vaccines, and combination treatments. ICIs and CAR-T-cells have revolutionized cancer therapy, significantly enhancing the treatment landscape for a variety of cancers. However, despite encouraging results in preclinical models, translating novel therapies into clinical success remains challenging. Traditional rodent models, which have long been fundamental in cancer research, have limitations in mimicking human immune responses. To address these challenges, humanized mouse models have recently emerged as a critical tool. These models incorporate human immune components, providing a more accurate representation of how human immune systems interact with tumors. In this study, we explored the differences in the longitudinal dynamics of human immune cell populations across two distinct humanization mouse models. One model was generated using CD34+ hematopoietic stem cells (HSC) derived from cord blood donors, while the other was created by injecting human PBMCs. The PBMC-based model included characterization of several different PBMC donors, whereas the CD34+ HSC-based model involved evaluation of distinct stem cell donors across various CDX models.Our findings reveal dynamic changes in immune cell composition and behavior in humanized mouse models. In CD34+ HSC-humanized mice, the overall amount of human CD45+ cells in peripheral mouse blood declines over time including a shift of immune cell populations. B cells are initially predominant but decrease as the T cell compartment increases. In PBMC-humanized mice, the proportion of human immune cells in the circulation starts low (1-3% of total CD45+ cells) but increases significantly over time, reaching up to 70%, depending on the donor. These human immune cells are mainly comprised of T cells, whereas other hematopoietic compartments, like B cells or Monocytes are quickly lost.
In both humanized mouse models, PBMC and CD34+ HSC, expression of the activation marker PD-1 on T cells increases over time. Notably, the CD4:CD8 T cell ratio, as well as overall numbers, are different for each model.Based on our studies tumor characteristics, rather than the type of PBMC or stem cell donor, are the primary drivers of immune cell infiltration. We found that tumor-specific factorsare key in shaping immune responses. This highlights the critical role of intrinsic tumor properties in modulating the immune microenvironment and influencing therapeutic outcomes.
These results provide valuable insights into the selection of appropriate humanized models and can inform experimental timing for testing novel immuno-oncology therapies. This may ultimately lead to more personalized and effective cancer treatment strategies.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Antireceptors and Other Biological Therapeutic Agents
Poster Session

#310 Deciphering the molecular basis for pan-HLA recognition of divarasib-modified pMHCs by newly discovered hit-to-lead antibody AETX-R302.

Lorenzo Maso, Weifeng Liu, Angelina Pizzo, Sarah Mosure, Matthew Southard, Lauren Stopfer, Samantha Sze, Diamond Mensah, Sergio Rodriguez, Anusha Keerthi, Sean Toenjes, Brian Vash, Paul Da Silva Jardine, Rajesh Chopra, Christoph Rader

Aethon Therapeutics, Long Island City, NY

The practicality of combining targeted therapies using covalent inhibitors of driver oncoproteins with specifically engineered antibodies to selectively kill drug-resistant cancer cells was recently demonstrated (Hattori, Maso et al., *Cancer Discov* 2023; Maso et al., *PNAS* 2024). Covalent inhibitors, once bound to their intracellular targets, can survive proteasomal degradation and are subsequently presented by class I MHCs as tumor-specific synthetic neoantigens. Companion antibodies of high specificity and affinity are then generated to selectively recognize these hapten-peptide MHCs (hapten-pMHCs), exploiting the high dissimilarity of hapten-pMHCs from MHCs presenting self-peptides. Therefore, combining the benefits of both targeted and immune therapy, overcomes their weaknesses as monotherapy. The potential of exploiting synthetic neoantigens is well demonstrated by antibody AETX-R114, which recognizes multiple hapten-peptides (sotorasib-conjugated $KRAS^{G12C}$ peptides) presented by different HLAs. When reformatted as a T cell engager (TCE), AETX-R114 potently and selectively kills sotorasib-resistant $KRAS^{G12C}$ cancer cells expressing different HLAs upon sotorasib treatment *in vitro* and *in vivo*. However, its high-affinity hapten-pMHCs cross reactivity, and related cancer cell killing, is restricted to hapten-peptides presented by HLAs belonging to the same HLA superfamily. Here we present the discovery and structural characterization of hit-to-lead antibody AETX-R302, which was raised against hapten-pMHCs presenting divarasib-conjugated $KRAS^{G12C}$ peptides (diva-pMHCs). AETX-R302 reveals specific, high affinity (K_D ranging between 0.78 and 2.75 nM), and pan-HLA recognition of diva-pMHCs across HLAs A*02 (A2 supertype) and A*03/A*11 (A3 supertype), with minimal free drug competition (IC_{50} >30 μM), demonstrating that antibodies developed through this platform can effectively break down the supertype barrier while maintaining MHC-restricted recognition. High-resolution cryo-EM structures of AETX-R302 in complex with diva-pMHCs revealed an epitope that involves all three components of the synthetic neoantigen (HLA, hapten, and peptide), exemplifying the three-layer specificity that can be achieved with such antibodies. Its unique binding geometry enables the recognition of HLA residues that are almost completely conserved among HLAs A*02, A*03, and A*11, while allowing a comparable recognition of the hapten when presented by different $KRAS^{G12C}$-derived peptides. Collectively, the cryo-EM structures explain AETX-R302's unprecedented A2/A3 supertype cross-reactivity and its high specificity and affinity. This information will be exploited to develop TCEs that engage tumor-specific synthetic neoantigen targets with pan-HLA recognition.

#311 Long-term tumor growth inhibition and extended survival with TD001, a novel optimized PSMA-targeting ADC, in PSMA-expressing CRPC CDX castrated mouse models.

Daniela Impellizzieri[1], Elisa Storelli[1], Atik Balla[1], Simone Mosole[1], Cristina Dongilli[1], Federico Jauk[1], Roberta Frapolli[2], Lavinia Morosi[3], Maurizio D'Incalci[3], Jemila Houacine[4], Morris Rosenberg[4], Mohamed Bekradda[4], Esteban Cvitkovic[4], **Carlo V. Catapano**[1]

[1]Institute of Oncology Research (IOR), Bellinzona, Switzerland,[2]Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy,[3]IRCCS Humanitas Research Hospital, Milan, Italy,[4]T.O.A.D. Oncology SA, Geneva, Switzerland

Patients with metastatic castration-resistant prostate cancer (mCRPC) who progress on androgen deprivation therapy (ADT) have limited therapeutic options. Prostate-specific membrane antigen (PSMA) is a type II transmembrane glycoprotein highly expressed in mCRPC and is a validated target for precision medicine approaches. TD001 is a novel antibody-drug conjugate (ADC) composed of a deimmunized anti-PSMA IgG1 monoclonal antibody (HuJ591) conjugated to a highly stable protease-cleavable topoisomerase I inhibitor (exatecan) proprietary linker-payload (LD038) with a drug-to-antibody ratio (DAR) of 8. TD001 was evaluated in vivo in human cell line-derived xenograft (CDX) models with high PSMA expression (LNCaP-abl; ~10^4 ligands/cell) or intermediate/heterogeneous PSMA expression (22Rv1; ~10^2-10^4 ligands/cell) in castrated NRG mice, and in the high PSMA-expressing (~10^5 ligands/cell) patient-derived xenograft (PDX) model C5 in non-castrated NRG mice. Of note, PSMA expression increased upon castration in both CDX models, mimicking the clinical condition of the target patient population. After single IV administration across a wide range of doses (up to 20 mg/kg), TD001 achieved dose-dependent, potent tumor growth inhibition (TGI) not only in the high-PSMA LNCaP-abl CDX and C5 PDX models but also in the intermediate-PSMA 22Rv1 CDX model. Rapid responses and high TGI (>90%) after a single administration at optimal doses were seen across all models. Upon tumor regrowth, PSMA expression was unchanged between treated and vehicle control groups, indicating no impact on target expression. Recurrent tumors remained similarly responsive to rechallenge with TD001. Of note, fractionation of a single TD001 dose into 2 or 3 doses administered every 10 days resulted in persistent tumor regression (TGI ≥90% for 60-70 days) and extended survival compared to the equivalent higher single dose. TD001 was well tolerated with no body weight loss or other signs of toxicity, nor mortality at all doses after single and repeated dosing regimens. Alternative dosing schedules, dose response, and tumor/plasma ratio result in a 2-3 fold increase in therapeutic index (TGI ≥90% for up to 60 days). TD001 appears as a best-in-class anti-PSMA ADC with its novel linker-payload (LD038) and exhibits potent antitumor activity in preclinical models with a broad range of PSMA expression, representing a highly promising treatment for PSMA-expressing CRPC patients. The efficacy of TD001 in preclinical in vivo castrated CDX and PDX models supports further investigation in clinical settings.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Drug Resistance in Molecular Targeted Therapies 1
Poster Session

#3116 High-definition spatial transcriptomics reveals microenvironment features associated with resistance to anti-PD1 therapies in lung cancer.

Corinne Ramos, Sandra Delebecq, Richard Ruez, Victor Senechal

R&D, Aliri, Lille, France

Introduction: Primary resistance to immune checkpoint inhibitors (anti PD1) presents a significant hurdle in lung cancer treatment. Tumor microenvironment (TME) features, particularly within the immune compartment, are central to shaping therapeutic response. This study employs high-definition spatial transcriptomics to identify biomarkers and mechanisms underlying resistance.
Methods: Formalin-fixed, paraffin-embedded (FFPE) lung cancer tissues from patients resistant to anti PD1 therapies were analyzed using the Visium HD spatial transcriptomics platform. Unsupervised clustering of spatial transcriptomics data was performed to identify distinct gene expression patterns within the immune compartment. Tissue morphology was integrated with gene expression profiles to map spatial heterogeneity.
Results: Our in-depth analysis of lung cancer tumor samples, utilizing high-throughput spatial transcriptomics and multiplexed immunohistofluorescence (IHF), revealed that primary resistance to PD1 PDL1 therapies is associated with a distinct tumor microenvironment. Unsupervised clustering of spatial transcriptomics data identified clear groupings that differentiated responders from resistant patients, based on immune compartment gene expression profiles. This analysis highlighted a suppression of immune-activating pathways and enrichment of immunosuppressive signals in resistant tumors, particularly within peritumoral regions. These findings underscore the pivotal role of the tumor microenvironment in modulating the likelihood of response to PD-1 blockade.
Conclusion: This study underscores the transformative potential of high-definition, single-cell scale spatial transcriptomics in revealing microenvironmental features associated with primary resistance to PD1 therapies in lung cancer. By enabling precise spatial evaluation of the tumor microenvironment, the identified biomarkers offer a robust foundation for developing predictive tools and personalizing immunotherapy strategies for improved patient outcomes.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Antireceptors and Other Biological Therapeutic Agents
Poster Session

#312 Comprehensive characterization of DT-7012, a differentiated CCR8-depleting antibody for the treatment of solid tumors.

Helene Lelievre, Melanie Frauli, Maria Dolores Garcia Fernandez, Dounia Chraa, Luc Baron, Aurelie Janvier, Iseulys Richert, Solene Rose, Katia Saulnier, Malaury Schappler, Christel Franchet, Orphee Blanchard, Thibaut Brugat, Stephan Schann, **Nathalie Lenne**

Domain Therapeutics, Illkirch-Strasbourg, France

Background: CCR8 is a chemokine G-protein coupled receptor predominantly expressed on tumor-resident regulatory T cells (Tregs), which suppress anti-tumor immunity. DT-7012 is a highly differentiated, fully humanized monoclonal antibody designed to deplete CCR8+ Tregs through Fc-mediated mechanisms, including antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis (ADCP). This study aimed to characterize the binding properties, effector functions, and Treg-depleting activity of DT-7012 to support its clinical development, planned for early 2025.
Methods: Binding assays: The binding affinity of DT-7012 to human CCR8 was assessed using flow cytometry on a variety of CCR8-expressing cell lines and native Tregs. Specificity against native circulating immune cells as well as membrane and secreted targets have been investigated. Functional assays: ADCC and ADCP activities were evaluated using gene reporter assays or various target:effector killing assays. Treg depletion: Ex vivo studies were conducted using blood samples from patients with non-small cell lung cancer (NSCLC) to quantify DT-7012-mediated depletion of CCR8+ Tregs.Results: DT-7012 displayed high-affinity binding to human CCR8 and selectively recognized CCR8 without cross-reactivity to other targets including chemokine receptors. In vitro, DT-7012 induced robust ADCC and ADCP activities on all tested CCR8-expressing cells, including in presence of CCL1. Ex vivo treatment with DT-7012 resulted in significant depletion of CCR8+ Tregs from NSCLC patient samples without affecting other immune cell populations. Together, these results demonstrate the high potency and selectivity of DT-7012. Preliminary safety studies confirmed the good tolerability of DT-7012 in rat and monkey. Additional data are ongoing and will be presented.
Conclusions: DT-7012 exhibits high-affinity binding to CCR8, potent Fc-mediated effector functions, and selective depletion of CCR8+ Tregs in NSCLC patient samples as well as a good safety profile. These findings highlight the therapeutic potential of DT-7012 as a novel strategy to enhance anti-tumor immunity and support its upcoming clinical development.

#313 Discovery of novel CXCR4 targeting compounds with antitumor activity in preclinical models of diffuse large B-cell lymphoma.

Miranda Fernandez-Serrano[1], Nuria Profitos-Peleja[1], Judith Camalo-Vila[2], Miquel Martinez-Asensio[2], Jordi Teixido[2], Jose I. Borrell[2], Albert Gibert[2], Raimon Puig de la Bellacasa[2], Roger Estrada-Tejedor[2], Gael Roue[1]

[1]Josep Carreras Leukaemia Research Institute, Badalona (Barcelona), Spain,[2]Pharmaceutical Chemistry Group, IQS School of Engineering, Ramon Llull University, Barcelona, Spain

The constitutive activation of CXC motif chemokine receptor 4 (CXCR4) is strongly implicated in tumor progression, metastasis, invasion, and chemotherapy resistance. In diffuse large B-cell lymphoma (DLBCL), the most common subtype of mature B-cell non-Hodgkin lymphoma, CXCR4 activation is associated with poor prognosis. Previous attempts to target CXCR4 in this disease using tetraamine-based inhibitors (CXCR4-i) have been limited by suboptimal pharmacological properties. Using structure-based drug design, we identified novel CXCR4-i candidates with potential anti-lymphoma activity. A library of 31 highly pure compounds was synthesized and evaluated for cytotoxicity in DLBCL cell lines via the MTT assay. Four compounds showed concentration-dependent antiproliferative effects, with 46-59% and 57-96% inhibition at 30 μM and 50 μM, respectively. The most potent compound, IQS-022, achieved up to 96% viability reduction at 50 μM, with an IC_{50} of 45.5 μM at 24 hours across seven DLBCL cell lines representing the ABC and GCB subtypes. In an immunocompetent chicken chorioallantoic membrane (CAM) DLBCL xenograft model, 50 μM IQS-022 reduced tumor area by 36.5% and decreased tumor B-cell infiltration in the heart and bone marrow by 54.7% and 74.2%, respectively. However, treatment led to >30% embryo weight loss, indicating limited selectivity, as corroborated by off-target effects in human peripheral blood mononuclear cells (PBMCs). To address this limitation, five new tetraamine derivatives were synthesized. Among these, IQS-036 demonstrated similar potency (IC_{50} = 38.14 μM at 24 hours) but significantly improved selectivity, reducing DLBCL cell viability by 52% at 30 μM while sparing PBMCs (10% viability reduction). Flow cytometry revealed that 94-97% of DLBCL cells underwent apoptosis after 24-hour exposure to 50 μM IQS-036, whereas the standard CXCR4-i, Plerixafor, showed minimal apoptogenic effects. IQS-036 exhibited concentration-dependent CXCR4 occupancy (64% at 20 μM) and decreased sensitivity in CRISPR-engineered NALM6-$CXCR4^{KO}$ cells compared to wild-type counterparts. In vivo, 30 μM IQS-036 inhibited tumor growth by 42% in NALM6-$CXCR4^{WT}$ xenografts without systemic toxicity, whereas CXCR4-knockout tumors were unresponsive. These findings establish CXCR4 as a viable therapeutic target in DLBCL and identify IQS-036 as a potent, selective, and specific CXCR4 inhibitor with significant antitumor activity in lymphoid neoplasms.

#314 Improved tumor penetration and cytotoxic payload release with TD001, a novel PSMA-targeting ADC with optimized linker-payload composition, in PSMA-expressing CRPC CDX castrated mouse models.

Daniela Impellizzieri[1], Elisa Storelli[1], Atik Balla[1], Simone Mosole[1], Cristina Dongilli[1], Federico Jauk[1], Roberta Frapolli[2], Lavinia Morosi[3], Maurizio D'Incalci[3], Jemila Houacine[4], Morris Rosenberg[4], Berangere Deleglise[4], Esteban Cvitkovic[4], Carlo V. Catapano[1]

[1]Institute of Oncology Research (IOR), Bellinzona, Switzerland,[2]Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy,[3]IRCCS Humanitas Research Hospital, Milan, Italy,[4]T.O.A.D. Oncology SA, Geneva, Switzerland

Prostate-specific membrane antigen (PSMA), a type II transmembrane glycoprotein belonging to the folate receptor family, is highly expressed in metastatic castration-resistant prostate cancer (mCRPC), and increases following androgen deprivation therapy (ADT). PSMA is a validated diagnostic and therapeutic target for radioligands and antibody-drug conjugates (ADCs). TD001 is a novel ADC composed of a deimmunized anti-PSMA IgG1 monoclonal antibody (HuJ591) conjugated to a highly stable protease-cleavable proprietary linker and the potent Topo I inhibitor exatecan payload. The LD038 linker-payload leverages exatecan's ability to stabilize Topo I-DNA complexes and induce DNA damage and cell death. We tested TD001 in 2D and 3D cell cultures and in vivo in human CRPC xenograft models with different levels of PSMA expression. We evaluated cell internalization, payload release, and drug activity by flow cytometry, immunohistochemistry, and immunofluorescence microscopy. Free payload levels were measured 24 h post treatment in plasma and tumors by LC-MS/MS. TD001 showed efficient binding and rapid internalization within 1-3 h followed by lysosomal localization and payload release in PSMA-expressing cells. Due to the quick and effective payload release, TD001 exhibited selective and potent activity with the induction of Topo I-mediated DNA damage (gamma-H2AX) and apoptosis (cleaved caspase 3). TD001 had no effect on PSMA-negative cells. This was supported by 3D cell culture models, which demonstrated rapid diffusion into the extracellular matrix, and rapid internalization of TD001 into PSMA-expressing tumor cells. TD001 was evaluated in vivo in three CRPC xenograft models with different PSMA expression patterns: the high/homogenous (~10^4 ligands/cell) PSMA-expressing LNCaP-abl cell line-derived xenograft (CDX) castrated mouse model, the intermediate/heterogeneous (~10^2-10^4 ligands/cell) PSMA-expressing 22Rv1 CDX castrated mouse model, and the AR-positive, high/homogenous (~10^5 ligands/cell)-PSMA-expressing patient-derived xenograft (PDX) C5 mouse model. The LD038 linker-payload in TD001 resulted in high free payload release within tumor tissue, triggering DNA damage, cell death, and growth arrest. Notably, tumor uptake and a very high tumor/plasma ratio (70-100 fold) of exatecan were similar 24 h after IV administration in all three in vivo models despite substantial differences in PSMA expression and heterogeneity. These findings highlight the ability of TD001 to deliver effectively and selectively the payload exatecan to PSMA-positive prostate tumors, resulting in potent antitumor activity in different CRPC models, including those with intermediate and heterogenous PSMA expression.

#315 Dose-response and tumor size at time of treatment with TD001, a novel ADC against PSMA, drive potent tumor growth inhibition in a CRPC CDX castrated mouse model.

Roberta Frapolli[1], Ezia Bello[1], Marina Meroni[1], Daniela Impellizzieri[2], Elisa Storelli[2], Carlo V. Catapano[2], Lavinia Morosi[3], Maurizio D'Incalci[3], Jemila Houacine[4], Esteban Cvitkovic[4]

[1]Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy,[2]Institute of Oncology Research (IOR), Bellinzona, Switzerland,[3]IRCCS Humanitas Research Hospital, Milan, Italy,[4]T.O.A.D Oncology SA, Geneva, Switzerland

Prostate-specific membrane antigen (PSMA), a type II transmembrane glycoprotein belonging to the folate receptor family, is highly expressed in metastatic castrate-resistant prostate cancer (CRPC), and increases following androgen deprivation therapy. TD001 is a novel antibody-drug conjugate (ADC) composed of a deimmunized anti-PSMA IgG1 monoclonal antibody (HuJ591), which binds to the extracellular domain of PSMA, conjugated to a proprietary cleavable topoisomerase I inhibitor exatecan linker-payload (LD038), with a drug-to-antibody ratio (DAR) of 8. Flow cytometry and immunofluorescence microscopy show TD001 is internalized and cleaved rapidly, colocalizing with lysosomes in PSMA-expressing tumor cells. TD001 (10-20 mg/kg IV) was evaluated in a human CRPC cell line-derived xenograft (CDX) model, LNCaP-abl with high PSMA expression, in castrated NSG mice, with a range of tumor sizes (from 150 to 700 mm3) at the time of treatment. TD001 gave dose-dependent and sustained potent activity (tumor growth inhibition [TGI] >80%) with single dosing. Sustained inhibition of regrowth for 20 days was observed after TD001 10 mg/kg even in large tumors up to 400 mm^3. Strong antitumoral activity of a single dose of TD001 10 mg/kg was also observed even in tumors >400 mm^3 at the time of treatment. Tumor regrowth was faster in larger tumors (mean volume ~300-400 mm3) compared to smaller tumors (mean volume ~200 mm3). PSMA expression was unchanged in tumor regrowth between treated and vehicle control in the CDX castrated mouse model after treatment with TD001 up to 20 mg/kg, indicating no impact on target expression. Fractionation of a single TD001 dose into 2 or 3 doses administered every 10 days resulted in a 2-3 fold higher therapeutic index (TGI >90%) compared to the equivalent higher single isodose. No mortality or weight loss was reported. Pharmacokinetics/pharmacodynamic correlations of TD001 are described separately. TD001 appears as a best-in-class anti-PSMA Topo I inhibitor ADC (exatecan payload), and exhibited potent and sustained antitumor activity, including in large tumors, and represents a promising new treatment for PSMA-expressing CRPC patients.

#316 Translational studies of HDM2012, a novel topoisomerase inhibitor ADC targeting MUC17, in patient derived GC, CRC, PDAC tumor models.

Qinyu Shu, zhaofeng qin, Shengxing Zhao, Yang Chen, Kuan Lu, Qian Zhang, Liubin Guo, Qingli Wei, Hao Pan, **Yan Xia**, Hongwen Li, Dongzhou Jeffery Liu

Huadong Medicine Co., Ltd., Hangzhou, China

Introduction: Mucin-17 (MUC17) is a transmembrane glycoprotein, expressed on the apical membrane of normal gastrointestinal mucosal epithelial cells, play important roles in preventing infection at mucosal surfaces[1-2]. MUC17 is a promising target for ADC development due to its highly expressed in gastric, colon and pancreatic cancers, but limit normal organs expression.
Methods: HDM2012 is a MUC17 targeting ADC, consisting of a topoisomerase inhibitor via a cleavable liker with average drug antibody ratio (DAR) 8. The efficacy and safety of HDM2012 was tested in patient derived xenograft (PDX) models and monkeys, respectively.
Results: HDM2012 showed target dependent anti-tumor activity and by-stander effect in vitro. HDM2012 exhibits good efficacy in PDX and CDX models. In gastric PDX models, HDM2012 demonstrated dose dependent efficacy, with TGI (tumor growth inhibition) value of 82-93%, regardless of prior Ironitecan and Top-I ADC treatment. In heavily pre-treated colon cancer PDX models, HDM2012 induced prolonged tumor regression, with TGI value of 94.6%. Furthermore, in MUC17 low expression pancreatic PDX models, HDM2012 could induce sustained tumor regression, with TGI value of 91.5%. In exploratory toxicology study, HDM2012 is well tolerated by cynomolgus monkeys at 45 mpk.
Conclusion: Here, we reporting a novel ADC product HDM2012 targeting MUC17, is a potential therapeutic approach for MUC17 positive GC, CRC, PDAC cancer treatment. All the experiment data indicated HDM2012 may be a promising candidate for GI cancer treatment.
Reference: 1. Gum JR, et al. Biochem Biophys Res Commun.2002;291:466-475. 2. Van Putten JPM, et al. J Innate Immun. 2017;9:281-299.

#317 Discovery of HDM2017, a CDH17-targeting ADC for colorectal cancers.

Chen Lin, **Yan Xia**, Liubin Guo, Yang Chen, Tingyang Wang, Kuan Lu, Hao Pan, Yayun Zhao, Jie Fang, Hongwen Li, Dongzhou Jeffery Liu

Huadong Medicine Co., Ltd., Hangzhou, China

Introduction: Cadherin 17 (CDH17) is a cell-to-cell adhesion protein that is a member of the cadherin superfamily. CDH17 is frequently overexpressed in colorectal cancer (CRC), gastric cancer (GC) and pancreas cancer (PC)1. HDM2017 ADC comprises a human monoclonal antibody specifically targeting CDH17 and chemically conjugated to CPT-derivative payloads. HDM2017 is undergoing IND-enabling studies.
Methods: In vitro and in vivo preclinical studies were conducted in the discovery of HDM2017 against gastrointestinal cancers. Initial investigations on plasma stability were performed. Exploratory toxicity assessments were carried out in cynomolgus monkeys and rats.
Results: HDM2017 demonstrates excellent cell binding activities with CRC and GC cells, with an EC50 ranging between 0.1-1.0nM. Additionally, HDM2017 exhibits strong target-mediated internalization activities, achieving an internalization efficiency of 43-75%. The HDM2017 ADC displays potent expression-dependent in vitro anti-tumor activity in a range of CDH17 expressing tumor cell lines. Treatment with HDM2017 ADC resulted in significant inhibition of tumor growth in xenograft models of CDH17+ human CRC (71~97% TGI) and GC (71~90% TGI). HDM2017 ADC exhibited excellent stability in human, monkey, and rat plasma with a drug shedding rate below 0.5%. Toxicity studies primarily revealed payload-related toxicity profile, which supports further development of the ADC molecule. Analysis using CDH17 IHC assay on patient tissues showed detectable expression of CDH17 in 90% (29/29) cases of colorectal cancer, 70% (7/10) cases of gastric cancer and 65% (13/20) cases of pancreas cancer.
Conclusion: HDM2017ADC exhibited potent cytotoxicity in vitro and in vivo anti-tumor activity in various gastrointestinal cancer models. The preclinical activity and safety profile warrants further clinical development with biomarker strategy. Overall, the preclinical data presented here suggests that HDM2017 ADC has the potential to provide clinical benefit to gastrointestinal cancer patients.

#318 Optimal free exatecan payload tumor/plasma ratio of TD001, a new ADC against PSMA, in CRPC CDX mouse models.

Lavinia Morosi[1], Daniela Impellizzieri[2], Roberta Frapolli[3], Elisa Storelli[2], Atik Balla[2], Carlo V. Catapano[2], Jemila Houacine[4], Esteban Cvitkovic[4], **Maurizio D'Incalci**[1]

[1]IRCCS Humanitas Research Hospital, Milan, Italy,[2]Institute of Oncology Research (IOR), Bellinzona, Switzerland,[3]Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy,[4]T.O.A.D. Oncology SA, Geneva, Switzerland

Prostate-specific membrane antigen (PSMA), a type II transmembrane glycoprotein folate receptor, is highly expressed in metastatic castrate-resistant prostate cancer (CRPC) and expression increases following androgen deprivation therapy. TD001 is a novel antibody-drug conjugate (ADC) composed of a deimmunized anti-PSMA IgG1 monoclonal antibody (HuJ591), conjugated to a highly stable, protease-cleavable proprietary Topo I inhibitor exatecan linker-payload (LD038), with a drug-to-antibody ratio (DAR) of 8. Free payload and total ADC were evaluated in mouse plasma, tumor, and liver, after TD001 10 mg/kg IV (which has potent antitumor activity in vivo) in 2 human CRPC cell line-derived xenograft (CDX) models, LNCaP-abl (high PSMA expression ~10^4 ligands/cell) and 22Rv1 (intermediate/heterogeneous PSMA ~10^2-10^4 ligands/cell) in castrated NRG/NSG mice. The TD001 PK profile was characterized across a wide temporal span post treatment, in LNCaP-abl (5 min to 10 days) and 22Rv1 (2 h to 6 days). The PK/PD profile of a range of doses (3-20 mg/kg) was characterized in both models. Dose proportional free exatecan concentrations were observed in tumor and plasma, and a consistently high tumor/plasma (T/P) ratio (~70-100 fold) was seen in both tumor models across the range of TD001 doses tested. Potent tumor growth inhibition (TGI) was dose dependent up to 20 mg/kg. PK/PD and the high therapeutic index with different dosing schedules are presented separately. Free exatecan was detected in tumors 5 min post treatment in LNCaP-abl. Free exatecan concentrations were approximately 80x higher in tumor compared to plasma 2 h post treatment, and at least 100x higher from 6 h to 6 days post treatment, showing rapid and high distribution of TD001 to the targeted tumor tissue regardless of PSMA expression levels. The concentration of total ADC (assuming constant DAR 8) was ~200 µg/mL (5 min post treatment), remained high for up to 6 days post treatment, while decreasing gradually from 24 h allowing for prolonged tumor exposure to TD001. The high and prolonged T/P ratio supports the excellent therapeutic index observed in TGI studies in both CDX castrated mice models, with TGI >90% and good tolerance (described separately). Of note, exatecan plasma levels rapidly declined under 0.5 ng/mL (from 24 h) and were under the limit of quantification (0.1 ng/mL) 10 days post treatment, preventing systemic toxicity. No free exatecan accumulation was observed after repeat dosing (described separately). TD001 effectively and preferentially delivers the potent exatecan payload to tumor tissue, with minimal systemic exposure, contributing to an excellent therapeutic index and strong antitumor activity in multiple CRPC models with high or intermediate PSMA expression. Our preclinical PK data support clinical development of TD001 in PSMA-expressing CRPC patients.

#319 Testing of trispecific antibodies for treating multiple myeloma using the Membrane Proteome Array.

Lewis Joe Stafford, J. Tabb Sullivan, Carmen T, Navia, Amelia M. Snyder, Diana M. Norden, Benjamin J. Doranz

Integral Molecular, Philadelphia, PA

Background: Assessment of off-target antibody reactivity is a regulatory requirement prior to clinical development; however, conventional screening methods are frequently ineffective in screening newer therapeutic modalities including multispecifics and CAR-T cell therapies. This presents an issue for T-cell engaging therapeutics that require exquisite specificity, as even minimal cross-reactivity could result in non-target cell killing accompanied by serious or even life-threatening consequences.
Methods: We developed the Membrane Proteome Array (MPA)—containing 6,000 human proteins—as a comprehensive approach to identify off-target protein interactions. The MPA is a cell-based protein array that assesses binding interactions in native, unfixed cells, allowing for quantitative and high sensitivity detection using flow cytometry. The MPA has been used to test specificity of over 1000 therapeutic molecules, including conventional MAbs and newer biotherapeutic modalities, including scFv, VH/VHH, and bispecific molecules, as well as other protein modalities.
Results: We used the MPA to evaluate the specificity of several multispecific antibody candidates targeting the G protein-coupled receptor GPRC5D, a promising target for multiple myeloma. Here we tested a panel of GPRC5D bispecifics using a CD3 targeting arm, as well as trispecific antibodies targeting GPRC5D, BCMA, and CD3. The MPA was successfully able to assess specificity of these molecules, showing compatibility with screening trispecifics. As a result, lead candidates devoid of cross-reactivity were identified for further development. The MPA has also been used to rapidly evaluate the specificity of novel CAR-T cell therapies for cancer and autoimmunity.
Conclusions: The MPA is compatible with screening of multispecifics, has been used for lead selection, and has been used in numerous successful regulatory applications for antibody-based therapies. The MPA is currently being developed as an FDA-qualified Drug Development Tool.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#32 Efficacy and tolerability of a enantiomer of Edelfosine (S-CP201) in pancreatic cancer PDX models as a single agent and in combination with approved drugs in clinical use.

Michael Ahrweiler, Wolfgang Meyer, Axel Zander, Daniel Perrissoud

RDP Pharma AG, Romanshorn, Switzerland

The anticancer alkylphospholipid Edelfosine incorporates into cell membrane lipid rafts, inducing apoptosis via CD95 and inhibiting AKT3/PI3K, phospholipase C, and protein kinase C. It disrupts cholesterol and phosphatidylcholine metabolism, and has antiangiogenic and antimetastatic effects. It's preferentially taken up by malignant cells, showed efficacy in xenografts (including metastatic breast and prostate cancer), and phase 2 trials across tumors. The main adverse events were reversible, dose-dependent gastrointestinal toxicity. Due to PK and PD advantages of Edelfosine's S-enantiomer (S-CP201), further development has focused on S-CP201. Inter alia its efficacy was tested in patient-derived xenograft (PDX) pancreatic cancer models in immunodeficient mice. Initial testing used NSG mice with s.c. implanted PNX0001 pancreatic cancer PDX cells. Treatment groups included S-CP201, R-CP201 (20 mg/kg p.o. each; given for 5 days/week over 3 w.), Gemcitabine (Gem.;) (50 mg/kg i.p., 3 d./w. over 3 w.), and vehicle. S-CP201, R-CP201, and Gem. showed tumor growth inhibition (TGI) of 57%, 33%, and 40%, respectively, with all mice on active treatment surviving the treatment period. Treatments were well tolerated. In a similar study, Gem. and S-CP201 were combined. Three groups of mice were allocated: 5 mg/kg p.o. for S-CP201 plus i.p. Gem.; 20 mg/kg S-CP201 plus i.p. Gem.; Gem. doses see above; vehicle; 3 w. treatment. The 20 mg/kg combination group exhibited the best TGI (85%), equal to stable disease in patients. The tolerability of the active groups was comparable. The antitumor efficacy of S-CP201 (20 mg/kg p.o. over 3 w.) was assessed when given in triple combination with Gem. and albumin-bound Paclitaxel in clinically relevant doses with a TGI of 67% (matching partial response in patients), compared to 57% for Gem. and albumin-bound Paclitaxel. Additional studies with S-CP201 (20 mg/kg daily p.o. over 3 w.) and Gem. (240 mg/kg, 1x/w. over 3 w.) testing 9 pancreatic adenocarcinoma PDX models in NMRI mice showed limited efficacy for S-CP201 alone, with modest activity in PAXF2141 and PAXF2146 (tumor/control ratio [T/C] ~57%). Gem. alone was modestly effective only in PAXF2196 (T/C ~50%). Combination therapy achieved borderline efficacy (T/C 51%-59%) in models PAXF2141, PAXF2146, and PAXF1986, and significant TGI in PAXF1900, PAXF2196, and PAXF2116, with T/C values of 31%, 35%, and 47% ($p<0.05$). PAXF2141, PAXF2146, and PAXF2116 harbor Ras mutations: (G12R, G12V, G12R respectively). Weight loss was minor but rose in 4 combination groups. Survival rates stayed high across all models. These results support clinical trials of S-CP201 as an effective combination partner for Gem. and/or albumin-bound Paclitaxel in pancreatic adenocarcinoma, including in patients with tumors containing mutated Ras. An IND enabling data package is underway.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Antireceptors and Other Biological Therapeutic Agents
Poster Session

#320 CRISPR-mediated glypican-3 genome editing via ultra-large porous silica nanoparticles in hepatocellular carcinoma therapy.

S. Lee[1], S. Lee[2], H. Lee[2], S. Kim[3], D.-H. Kim[1];

[1]Feinberg School of Medicine, Northwestern University, Chicago, IL, [2]Korea Institute of Science and Technology (KIST), Seoul, Korea, Republic of, [3]Gyeongsang National University, Jinju, Korea, Republic of

Purpose: We aim to develop non-viral nanocarriers to deliver ribonucleoproteins (RNP) targeting Glypican-3 (GPC3) and investigate the biological and immunological therapeutic effects from CRISPR-Cas9 GPC3 gene editing in hepatocellular carcinoma (HCC).
Methods: Ultra-large porous silica nanoparticles (ULSN) were synthesized following established methods, with morphology confirmed via electron microscopy. ULSN-loaded Cas9-RNP was characterized using Zetasizer NS90, and RNP content measured by NanoDrop and BCA assays (ThermoFisher). *In vitro* experiments were performed on HepG2 (HB-8065, ATCC) and Hepa1-6 (CRL-1830, ATCC) cells. Gene deletion was assessed via RT-PCR, deep sequencing (Illumina MiniSeq), and cell viability. Western blotting analyzed key signaling pathways. *In vivo* GPC3 genome editing was evaluated in Hepa1-6 orthotopic HCC models (C57BL6, male, 6-7 weeks) using T7E1 assay and T cell infiltration analysis. GPC3 targeting therapeutic effects were compared with Codrituzumab (anti-GPC3) treatment in HepG2-bearing HCC models (Balb/c, male, 6-7 weeks) by measuring tumor volume, immunohistology (H&E, Ki67, and GPC3), serum GPC3, and T cell populations from tumor-draining lymph nodes using flow cytometry (BD).
Results: ULSN exhibited a size of 300 nm, pore size of 22.86 nm, and surface area of 525.9 m^2/g as determined by TEM and BET analyses. Its ultra-large pores allowed a 27% loading capacity for Cas9-RNP, with a loading efficiency of 98% for both sgRNA and Cas9, compared to 48% and 52% for Lipofectamine (commercial control), respectively. In HepG2 cells, GPC3 deletion showed 67% reduction in RT-PCR, 15% indel frequency (vs. 3% for Lipofectamine), and reduced cell viability (55% vs. 94%). To analyze the molecular effects of GPC3 gene, Wnt and Hippo/YAP signaling were examined. Western blotting revealed downregulation of Wnt signaling (lower level of Wnt, p-LRP, and β-catenin), upregulation of Hippo signaling through increased LATS phosphorylation, and decreased YAP phosphorylation, potentially inhibiting tumor cell proliferation. In orthotopic HCC models, ULSN showed targeted accumulation in liver tissues, successfully achieving GPC3 deletion with complete tumor regression 14 days post-procedure. Tumor cell death led to enhanced $CD4^+$ and $CD8^+$ T cell infiltration, alongside a decrease in regulatory T cells. Additionally, ULSN-mediated GPC3 gene editing outperformed Codrituzumab (GPC3 blockade) by reducing tumor establishment, Ki67 and GPC3 expression levels in tumor cells.
Conclusions: ULSN presents strong potential as a non-viral delivery system for CRISPR-Cas9 RNP targeting GPC3 in HCC, offering improved gene delivery efficiency over lipid-based nanomaterials. This approach not only enhances tumor growth suppression, but also promotes T cell infiltration, ensuring effective and lasting therapeutic outcomes through GPC3 deletion.

#321 BTE-EN1, a novel acting heterobifunctional compound inhibiting bone destruction by established prostate cancer bone metastasis.

Fangfang Qiao[1], Ryan Gordon[2], Abhinandan Pattanayak[3], Katelyn O'Neill[1], Weining Chen[1], Prathap R. Mukthapuram[1], Amar Natarajan[1], Raymond Bergan[1]

[1]Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE,[2]Division of Hematology/Oncology, Knight Cancer Institute, Oregon Health & Science University, Portland, NE,[3]Oregon Health & Science University, Portland, OR

Bone destruction by prostate cancer (PCa) metastasis is a major cause of morbidity and mortality. In order to destroy bone, PCa and bone cells stimulate each other in a process that requires their coordinated movement within bone itself. To therapeutically inhibit this process, we synthesized the first-in-class heterobifunctional compound, BTE-EN1. One group is a selective inhibitor of cancer cell motility, discovered by us (Nature Communications 2018). It is chemically coupled to a bisphosphonate group, which binds to bone mineral and enables targeted delivery of the therapeutic to bone-destructive lesions. We sought to test whether BTE-EN1 would be active against well-established bone-destructive PCa metastasis and to evaluate its potential as a therapeutic.

We used the state-of-the-art caudal artery injection model to selectively deposit human PCa cells into the long bones of the leg in mice and let them grow for two weeks, thus emulating the scenario of established disease in patients. We then began weekly administration of BTE-EN1 for six weeks, quantified bone destruction by CT imaging and demonstrated a significant decrease in bone destruction by 62%, compared to control. In a separate study, BTE-EN1 treatment prior to intra-cardiac injection of PCa cells led to dose-dependent significant prolongation of life, a finding not seen with bisphosphonate and denosumab therapies. Comprehensive in vitro studies demonstrate that BTE-EN1 retains the cellular and molecular pharmacologic and pharmacodynamic properties of its two functional groups. BTE-EN1 exhibited no toxicity when mice were treated at a dose 4000-fold higher than that showing efficacy and lacked toxicity in the NCI-60 cell line panel screen. Additional studies supporting translation to the clinic include non-interference with therapy used to treat advanced PCa, as well as inhibition of cell motility across a panel of human cancer cell types associated with bone destruction.

In conclusion, BTE-EN1 is a first-in-class highly active heterobifunctional compound that inhibits bone destruction in established PCa bone metastasis and is well tolerated. Our data strongly suggests that BTE-EN1 has high potential for translation into humans and constitutes a novel therapeutic strategy.

#322 Nonclinical characterization of the secondary mechanisms of action of PF-08046054 (PDL1V), a novel vedotin ADC directed to PD-L1 that selectively depletes tumor cells without depleting healthy immune cells.

Megan Atkins Ramirez, Midori Clarke, Steven Jin, Ellie Moskovitz, Andres Forero-Torres, Garrett Cornelison

Pfizer Oncology, Bothell, WA

PDL1V is a novel vedotin antibody drug-conjugate (ADC) currently in Phase I clinical development for advanced solid tumors as a monotherapy or in combination with pembrolizumab (NCT05208762). The primary mechanism of action (MOA) of PDL1V is direct cytotoxicity to PD-L1-expressing tumor cells via the intracellular delivery of MMAE. While PD-L1 is an established checkpoint protein, pharmacokinetic data suggest steric checkpoint inhibition by PDL1V may be limited by the dose and schedule being evaluated in the clinic. Here, we characterized additional antitumor MOAs of PDL1V, including bystander killing and immunogenic cell death (ICD), and evaluated the effects of PDL1V on immune cells.

PDL1V is potently cytotoxic to a variety of PD-L1-positive cancer cell lines *in vitro.* Using a flow cytometry-based viability assay, we showed that PDL1V also drives dose-dependent bystander effect-based cytotoxicity of PD-L1-negative tumor cells when co-cultured with PD-L1-positive tumor cells. ICD, a regulated activation of immune cell recruitment to the TME, is caused by tumor cell release of damage-associated molecular patterns (DAMPs) such as cell surface chaperone calreticulin (CRT), extracellular adenosine triphosphate (eATP), and High Mobility Group Box 1 protein (HMGB1). PD-L1-positive cancer cells treated with PDL1V *in vitro* released all three DAMPs, suggesting that targeted delivery of the MMAE payload drives induction of ICD.

As an immune checkpoint protein, PD-L1 is expressed on antigen presenting cells and T cells. Depletion of these cells could affect the tolerability of PDL1V as a monotherapy, or limit efficacy in combination with anti-PD-1 therapies. To investigate the potential for immune cell depletion, macrophages, CD4+ T cells, and CD8+ T cells were evaluated in an *in vitro* fluorescence-based cytotoxicity assay. PDL1V had minimal cytotoxic activity across all three cell-types, despite expression of PD-L1. These data support PDL1V as selectively cytotoxic to PD-L1-positive and negative cancer cells of the tumor microenvironment, and not cytotoxic to PD-L1-positive immune cells involved in natural antitumor immunity. Since T cell-driven antitumor immunity is a key driver for the efficacy of PD-(L)1 blockade, these data suggest PDL1V is a promising combination partner with anti-PD-1 immunotherapies such as pembrolizumab. Future studies will evaluate the effects of PDL1V on immunosuppressive cells, including regulatory T cells and myeloid derived suppressor cells, and the pharmacodynamic effects of PDL1V in syngeneic mouse xenograft models.

Ethics Approval: All procedures performed on animals were in accordance with regulations and established guidelines and were reviewed and approved by an Institutional Animal Care and Use Committee or through an ethical review process.

#323 Preclinical evaluation of SDV2102, a novel PSMA-targeting DAR1 ADC for mCRPC treatment.

Damien Dattler, Fabian Schlimpen, Constantin Semenchenko, Oleksandr Koniev, **Sergii Kolodych**

Syndivia, Strasbourg, France

Metastatic castration-resistant prostate cancer (mCRPC) presents a significant therapeutic challenge due to limited treatment options. Prostate-specific membrane antigen (PSMA) is overexpressed in mCRPC tumors, making it a viable target for antibody-drug conjugates (ADCs). We developed SDV2102, a novel PSMA-targeting ADC designed to enhance tumor penetration and efficacy. SDV2102 utilizes a novel anti-PSMA antibody, D10, selected for its superior antigen-binding properties. The D10 antibody is conjugated with monomethyl auristatin E (MMAE), a tubulin-binding cytotoxic agent to which mCRPC tumors are inherently more sensitive than to topoisomerase I inhibitors. Notably, SDV2102 has a drug-to-antibody ratio (DAR) of 1, allowing for higher dosing and increased efficacy without proportional increases in systemic toxicity, thereby improving the therapeutic index. In vivo efficacy was assessed using the C4-2 cell-derived xenograft (CDX) model, characterized by high PSMA expression. SDV2102 demonstrated significantly greater anti-tumor efficacy than classical DAR4 ADCs constructed with either the generic J591 antibody or the D10 antibody when administered at equivalent doses of MMAE. Additionally, SDV2102 showed potent activity in patient-derived xenograft (PDX) models with low to moderate PSMA expression levels, with some tumors expressing as few as 25,000 receptors per cell. This indicates that SDV2102 may be effective across a spectrum of PSMA expression in mCRPC tumors. Pharmacokinetic studies in rats revealed that SDV2102 has a favorable profile, closely mirroring that of the unconjugated D10 antibody, suggesting maintained antibody-like characteristics in circulation. Dose-response studies in the C4-2 model identified a minimum effective dose of 2.5 mg/kg. Toxicology studies in non-human primates showed no major toxicities at doses up to 24 mg/kg, with the maximum tolerated dose (MTD) not reached, indicating a wide therapeutic window. SDV2102 achieved a therapeutic index of 38, representing a substantial safety margin. These preclinical results support the advancement of SDV2102 into clinical trials as a promising therapeutic candidate for the treatment of mCRPC.

#324 PF-08052666 (SGN-MesoC2; HBM9033), a first-in-class topoisomerase 1 inhibitor-based ADC targeting MSLN, demonstrates potent antitumor activity in preclinical models of ovarian, lung, and colorectal cancers.

Garrett L. Cornelison[1], Meihong Zhang[2], Cui Nie[2], Guangbei Zhu[2]

[1]Pfizer Oncology, Bothell, WA,[2]Nona Biosciences, Natick, MA

Mesothelin (MSLN) is a cell surface molecule that is expressed in its mature form as a 40 kDa protein anchored to the cell membrane through a glycosylphosphatidylinositol linkage. While its expression is limited in normal tissues, MSLN is overexpressed in numerous solid tumors, including ovarian, pancreatic, endometrial, lung and colorectal cancers. Since its discovery, MSLN has been a target of interest for the development of anti-tumor therapeutics across a variety of modalities, including antibodies, CAR-T, and multiple antibody-drug conjugates (ADCs). To-date, anti-MSLN ADCs have focused on the delivery of anti-tubulin payloads, including DM4, MMAE, and tubulysin. While these ADCs have had manageable safety profiles in the clinic, efficacy has been modest. Here we present the nonclinical characterization of PF-08052666 (SGN-MesoC2; HBM9033), a first-in-class topoisomerase 1 inhibitor (TOP1i)-based ADC targeting MSLN. PF-08052666 has been designed to overcome the shortcomings of the previous generation of anti-MSLN ADCs through a novel antibody, differentiated payload, and a higher drug-to-antibody ratio (DAR). PF-08052666 consists of a human IgG1 monoclonal antibody conjugated to a potent camptothecin-based TOP1i payload with a protease cleavable linker at an average DAR of 8. *In vitro*, PF-08052666 drives direct cytotoxicity through delivery of payload to MSLN-positive cells, bystander killing activity on co-cultured MSLN-negative cells, and retains cytotoxicity in the presence of physiologically relevant concentrations of soluble MSLN. *In vivo*, PF-08052666 outperformed a DM4-based anti-MSLN benchmark ADC in cell-line and patient-derived xenograft models across various tumor types, including ovarian, lung, and colorectal cancers. PF-08052666 also outperformed the DM4-based anti-MSLN benchmark ADC in heterogenous xenograft models consisting of ad-mixed MSLN-positive and MSLN-negative cells, demonstrating the increased bystander activity of the TOP1i-based payload of PF-08052666. These data support the ongoing first-in-human phase 1 clinical trial of PF-08052666 in patients with advanced solid tumors, which is currently enrolling (NCT06466187).
Note: All procedures performed on animals were in accordance with regulations and established guidelines and were reviewed and approved by an Institutional Animal Care and Use Committee or through an ethical review process.

#325 Innovative approach in evaluating gap junction functionality using co-cultured spheroids.

Tomas Lugo, Annelise Nguyen, Stephanie Myers

Texas Tech University, Lubbock, TX

In recent years, significant advancements were made in colorectal cancer research, particularly in understanding the role of gap junctions in cancer development and progression. However, challenges persisted in effectively evaluating gap junction functionality within 3D structures without disrupting spheroids or relying on dye-addition methods to the media, which often had limitations. To address these issues, we developed an innovative method for microinjecting dye into a single cell within a multicellular spheroid, enabling real-time analysis of gap junction functionality in living cells. Building on this approach, we aimed to create a more physiologically relevant tumor microenvironment by co-culturing spheroids with fibroblast cells. This setup better replicated the complexity of in-vivo conditions. To further enhance our analysis, we fixed these spheroids at various time points and used immunohistochemistry (IHC) to evaluate dye penetration into the spheroid core. The experimental method involved co-culturing fibroblasts (CCD-112CoN) with colorectal cancer epithelial cells (Caco2, SW620, SW480, or HT-29) in a CelVivo Clinostar reactor to produce spheroids larger than 300 µm in diameter. These spheroids were then microinjected using an Eppendorf FemtoJet 4i Microinjector with a dye mixture containing Texas Red (high molecular weight, 40,000 Da) and Lucifer Yellow (low molecular weight) to assess gap junction activity using a Leica confocal microscope. Additionally, we examined gap junction proteins using an immunofluorescence assay between co-cultured and single-cell cultured spheroids. The results indicated that microinjection was a reliable method for evaluating gap junction functionality within spheroids. Notably, different stages of colorectal cancer modulated dye absorption and transfer, with SW620 spheroids exhibiting lower transfer through cells, while HT-29 spheroids showed increased dye transfer between cells. Furthermore, co-cultured spheroids demonstrated enhanced dye transfer compared to single-cell-type spheroids of the same type. There was an increase in connexin 43 expression based on immunofluorescence in Caco2 with CCD-112CoN spheroids compared to Caco2 and CCD-112CoN single-cell cultures. In conclusion, to our knowledge, this study introduced a novel approach to measuring gap junction activity for spheroids. Furthermore, it highlighted the significant role fibroblasts may play in influencing gap junction dynamics in colorectal cancer cells within complex 3D structures.

#326 Chemotherapies change the NRF2 levels and impact the differentiation and growth of human high grade serous ovarian cancer.

Chelsea Katz[1], Helen Toma[1], Yaas Azmoudeh[2], Nasrine Bendjilali[3], Huseyin Karaduman[4], Hadi Shojaei[5], Gord Zhu[5], Lauren Krill[6], Chu Christina[6], David P. Warshal[6], Yemin Wang[7], Samera Hamad[1]

[1]Surgery Department, Cooper University Health Care, Camden, NJ,[2]Cooper Medical School of Rowan University, Camden, NJ,[3]College of Science and Mathematics, Rowan University, Glassboro, NJ,[4]North Carolina State University, Raleigh, NC,[5]Department of Pathology and Laboratory Medicine, Cooper University Health Care, Camden, NJ,[6]MD Anderson Cancer Center at Cooper, Camden, NJ,[7]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada

NRF2, an antioxidant gene and anti-inflammatory pathway, is altered in about 50% of high grade serous ovarian cancer (HGSOC), the most aggressive type of ovarian cancer. NRF2 is implicated in the resistance to chemotherapies in several types of cancers. We aimed to understand if treatment with carboplatin or paclitaxel impacts the levels of NRF2 and if that changes the differentiation, growth, and metastasis of HGSOC. We used OVCAR8, a human HGSOC cell line with a basal level of NRF2, and induced overexpression of NRF2 using pIND20 system with $NRF2^{E79Q}$ mutation (one of the most common mutations of NRF2 found in human tumors). Our results showed high growth and invasion in high-NRF2 tumor cells compared to low-NRF2 tumor cells with and without chemotherapies. Treatment with carboplatin inhibits the levels of NRF2 in low-and high-NRF2 versions of OVCAR8. Carboplatin also inhibits the MYC, P63, and CD274 (PD-L1) protein levels, and the epithelial to mesenchymal transition (EMT) pathway (Vimentin). Additionally, in high-NRF2 tumor cells, paclitaxel increases the levels of NRF2, MYC, CD274 (PD-L1), and Vimentin, and decreases the levels of P63. In contrast, low-NRF2 tumor cells treated with paclitaxel show decreased MYC, CD274 (PD-L1) and Vimentin and increased P63. These changes impact the growth and metastasis (by scratch test) of OVCAR8. We found that carboplatin inhibits the growth and invasion while paclitaxel increases the growth and invasion in the high-NRF2 version of OVCAR8. These studies show that carboplatin might be a good option as a single treatment compared to paclitaxel. In addition to the increase in NRF2 and differentiation markers that support the tumor cell growth, paclitaxel also increased the PD-L1 protein levels in high-NRF2 tumor cells which might enhance the efficacy of anti-PDL1. Therefore, combination immunotherapy (anti-PDL1) with paclitaxel may have therapeutic benefit in patients with high-NRF2 HGSOC.

#328 A novel human-like asparaginase - indication expansion made possible.

Amanda M. Schalk[1], Maaike Van Trimpont[2], Ying Su[1], Ashley De Loera[1], Alyssa Garcia[1], Tim Lammens[2], Steven Goossens[2], Arnon Lavie[1]

[1]University of Illinois College of Med. at Chicago, Chicago, IL,[2]Cancer Research Institute Ghent (CRIG), Ghent, Belgium

L-asparaginase (ASNase) has been a cornerstone of pediatric acute lymphoblastic leukemia (ALL) treatment since the 1970s, leveraging a unique mechanism that exploits the metabolic vulnerability of leukemic cells. Cancer cells deficient in asparagine synthetase (ASNS), which rely on blood asparagine, are starved when ASNase depletes asparagine in the bloodstream. While this approach is effective in ALL, preclinical studies suggest that ASNase could also target other ASNS-deficient ($ASNS^{low}$) cancers, including acute myeloid leukemia (AML) and subsets of solid tumors like liver and colorectal cancers. However, the severe toxicity of existing ASNase formulations, largely due to off-target glutaminase activity and immunogenicity, has limited its broader clinical application. To overcome these barriers, we developed a novel human-like mammalian ASNase designed to minimize immunogenicity and eliminate glutaminase-driven toxicity while maintaining high specificity for asparagine. This engineered ASNase demonstrated robust in vitro and in vivo efficacy against both T-cell and B-cell ALL. Notably, it also showed potent activity as a monotherapy in AML, eradicating tumor cells in blood and bone marrow. Beyond blood cancers, this ASNase displayed significant anti-tumor effects against a CRISPR/CAS-9-engineered melanoma model with genetic loss of ASNS and a hepatocellular carcinoma model with low endogenous ASNS expression, achieving durable tumor suppression. This demonstrates that for $ASNS^{low}$ tumors the glutaminase activity is not required for efficacy. Crucially, the novel ASNase was well tolerated in animal studies, enabling prolonged asparagine depletion—an essential factor for its striking anti-cancer effects. With its enhanced safety profile and specificity, this humanized ASNase represents a transformative advancement, extending the benefits of metabolic tumor starvation to a broader range of cancers. Its predictable mechanism of action, guided by biomarkers of $ASNS^{low}$ status, positions it as a promising therapeutic breakthrough for targeting diverse malignancies. The authors used GPT-4.o in order to rephrase certain sentences. After using this tool or service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

#329 Proliferative inhibition of pancreatic cancer cells treated with pancreatic cancer - specific amplitude-modulated radiofrequency electromagnetic fields.

Hugo Jimenez[1], Callum McGrath[2], Liyue Zhang[1], Grayson Barker[1], Allan M. Johansen[2], Janaka S. S. Liyanage[1], Mohammed N. Al Hallack[1], Anthony F. Shields[1], Wasif Saif[1], Husain Y. Khan[1], Amro Aboukameel[1], Hafiz Uddin[1], Sahar F. Bannoura[1], Ramzi M. Mohammad[1], Alexandre Barbault[3], Carl F. Blackman[1], Ravi K. Paluri[4], Asfar Azmi[1], Boris Pasche[1]

[1]Oncology, Wayne State University School of Medicine / Karmanos Cancer Institute, Detroit, MI,[2]Cancer Biology, Wake Forest University School of Medicine, Winston-Salem, NC,[3]TheraBionic GmbH, Ettlingen, Germany,[4]Medicine, Atrium Health Wake Forest Baptist Hospital, Winston-Salem, NC

Background: Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related mortality, and about 50% of PDC patients present with metastatic disease. Despite advances in systemic therapy, the prognosis for PDC is dismal, with a five-year overall survival (OS) for metastatic PDC of about 5%. The most used regimens for advanced pancreatic cancer include gemcitabine/nab-paclitaxel (Gem/NabP) and FOLFIRINOX, with the recent addition of NALIRIFOX. All these regimens result in median survival rates under one year. Amplitude-modulated 27.12 MHz radiofrequency electromagnetic fields (AM RF EMF) delivered via a spoon-shaped antenna placed on the patient's tongue results in shrinkage of the primary and metastatic tumors in patients with cancer. The mechanism by which AM RF EMF have direct antiproliferative effect has been found to be dependent on the influx of extracellular Ca2+ into the cancer cell via Cav3.2 (gene name CACNA1H) T-type voltage gated calcium channel. Here we present, for the first time, data showing that Pancreatic cancer-specific AM RF EMF (PancMF) is effective in pancreatic cancer cells.
Methods: MIAPaCa-2 and PANC-1 cell lines were exposed either to no treatment (SHAM) to PancMF three hours per day for seven days total. Cell lines were exposed using systems replicating human exposure levels and treatment duration. Ethosuximide (Ethos), a pan t-type voltage gated calcium channel (VGCC) blocker was used to inhibit the effects of PancMF. In vitro experiments, (proliferation) Tritiated Thymidine incorporation and (stemness) tumor sphere formation were performed. ANOVA and Two-tailed Student's t-test were used for comparison.
Results: Panc1 cell line: Proliferation was inhibited 38.51% by PancMF treatment (p-value: <0.0001) but in the presence of ethosuximide PancMF proliferation inhibition was blocked (p-value: 0.9897). Stemness was inhibited 41.02% by PancMF treatment (p-value < 0.0001). MiaPaca2 cell line: Proliferation was inhibited 20.67% by PancMF treatment (p-value: 0.0208) but in the presence of ethosuximide PancMF proliferation inhibition was blocked (p-value: 0.8933). Stemness was inhibited 26.54% by PancMF treatment (p-value < 0.0001).
Conclusions: Proliferation and Stemness of Panc1 and MiaPaca2 cell were inhibited following PancMF treatment, however, the presence of ethosuximide blocked the anti-proliferative effects of PancMF. Results indicate that the t-type VGCC mediates the anti-proliferative effect. This results trend with previously published data showing that Cav3.2 is the bioantenna and mediator for tumor-specific AM RF EMF treatment.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#33 Development of a humanized mouse model for γδT cells.

Lydia Blot[1], Lopes Joaquim[1], Emerson Serres[1], Vanessa Croize[1], Herve Barret[1], Pauline Rettman[1], Clement Barjon[2], Dorothee Bourges[1], Antoine Lhermitte[1], Valeria Fantin[3], **Sukhvinder Sidhu**[1], Loreley Calvet[1]

[1]Oncology, Sanofi, Vitry sur Seine, France,[2]Oncology, Servier, Saclay, France,[3]Oncology, Sanofi, cambridge, MA

Unconventional T cells such as gamma delta (γδ) T cells have garnered significant interest in cancer immunotherapy due to their potent anti-tumor properties including strong cytotoxic capabilities in hematological malignancies and their ability to recognize a broad range of tumor antigens independent of the classical major histocompatibility complex (MHC) molecules. Given the interest in engaging these immune cell populations in cancer therapy, we established a new in vivo humanized mouse model through the adoptive transfer of human γδT cells to support this emerging area of therapeutic interest. This model was created by transplanting γδT cells sorted from PBMC donors into Fc-resolv huIL15 NOG (functional knock-out of murine Fcγ receptors to minimize the confounding impact of mouse immune cells) mice, genetically engineered to express human IL-15. We demonstrate that various γδT cell populations, including Vδ1 and Vδ2, can be successfully maintained over a long period of time, preserving the expression of critical functional biomarkers such as NKG2D, CD69 or CD16, under various engraftment conditions. Additionally, we showed that hu γδT cells display functional engagement in vivo, achieving high efficacy with epcoritamab (CD20-CD3 Ab) against disseminated tumors in the hu γδT cell adoptive transfer mouse model.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Antireceptors and Other Biological Therapeutic Agents
Poster Session

#330 Treatment of fibrolamellar carcinoma with hepatocellular carcinoma -specific amplitude-modulated radiofrequency electromagnetic fields.

Hugo Jimenez[1], Liyue Zhang[1], Grayson Barker[1], Callum McGrath[2], Allan M. Johansen[2], Janaka S. S. Liyanage[1], Mohammed N. Al Hallak[1], Anthony F. Shields[1], Wasif Saif[1], Husian Y. Khan[1], Amro aboukameel[1], Hafiz Uddin[1], Sahar F. Bannoura[1], Ramzi Mohammad[1], Sean Ronnekleiv-Kelly[3], Alexandre Barbault[4], Carl F. Blackman[1], Asfar Azmi[1], Boris Pasche[1]

[1]Oncology, Wayne State University School of Medicine / Karmanos Cancer Institute, Detroit, MI,[2]Cancer Biology, Wake Forest University School of Medicine, Winston-Salem, NC,[3]Surgery, University of Wisconsin - Madison, Detroit, WI,[4]TheraBionic GmbH, Ettlingen, Germany

Background: Amplitude-modulated 27.12 MHz radiofrequency electromagnetic fields (AM RF EMF) delivered via a spoon-shaped antenna placed on the patient's tongue result in shrinkage of the primary and metastatic tumors in patients with advanced hepatocellular carcinoma (HCC). The mechanism by which AM RF EMF has a direct antiproliferative effect has been found to be dependent on the influx of extracellular Ca^{2+} into the cancer cell via $Ca_V3.2$ (gene name CACNA1H) T-type voltage-gated calcium channel. Fibrolamellar Carcinoma (FLC), a rare subtype of HCC, has few options available for treatment. Here we present, for the first time, data showing that HCC-specific AM RF EMF (HCCMF) are effective in FLC.
Methods: HepG2 is an HCC cell line and H33 ($HepG2^{DNAJB1\text{-}PRKACA}$) was generated by using the HepG2 cell line and generating the *DNAJB1-PRKACA* gene fusion at the native locus (CRISPR-based 400kbp excision). Cells either received no treatment (SHAM) or were exposed to HCCMF three hours per day for seven days total. HepG2 and H33 cells were exposed using systems replicating human exposure levels and treatment duration. *In vitro* experiments (colony formation and tumor sphere formation) with HepG2 and H33 cells were performed. Two-tailed Student's t-test was used for statistical comparison.
Results: Proliferation (colony formation assay) of HepG2 and H33 is inhibited by 37.54% (p-value: 0.0023, N=6) and 27.08% (p-value: 0.0006, N=6), respectively. Stemness (tumor sphere formation assay) of HepG2 and H33 is inhibited by 33.17% (p-value < 0.0001, N=8 SHAM and N=7 HCCMF) and 43.95% (p-value< 0.0001, N=7 SHAM and N=8 HCCMF), respectively.
Conclusions: Proliferation and stemness of the HepG2 and H33 [HepG2 (DNAJB1-PRKACA)] cell lines were inhibited following HCCMF treatment. Results show the presence of the FLC fusion protein (DNAJB1-PRKACA) does not inhibit the potential of HCCMF as a novel systemic targeted therapy for FLC. This proof-of-principle data shows significant potential for the treatment of FLC.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Biochemical Modulators of Cancer / Differentiation Therapeutic Strategies
Poster Session

#334 proPBD-ADCs: optimization of therapeutic potential of ADCs based on capped PBD payload with attenuated potency.

Anette Sommer, Remon van Geel, Sander van Berkel, Elias Post, Jorin Hoogenboom, Floris van Delft

Synaffix - A Lonza company, Oss, Netherlands

Antibody-drug conjugates (ADCs) with pyrrolobenzodiazepine (PBD) dimer payloads have been explored preclinically and several PBD-based ADCs entered clinical trials. PBD dimers produce highly cytotoxic DNA crosslinks and thus have a mode-of-action differentiated from microtubule inhibitors, TOPO1 inhibitors and other DNA targeting warheads. In April 2021, the FDA approved the first PBD-based ADC, the CD19 targeting loncastuximab tesirine, for treatment of adult patients with relapsed or refractory large B-cell lymphoma, including DLBCL, after 2 or more lines of therapy. However, loncastuximab tesirine carries safety warnings - for example for effusion and edema, myelosuppression, and cutaneous reactions.
While PBD-based ADCs show efficacy in the clinic, they belong to a highly potent payload class with IC50 values on human cancer cell lines in the picomolar range. In fact, the ultrapotent character of the vast majority of PBD-based ADCs has highly limited the administration at clinically suitable dosing regimens. Therefore, increasing the therapeutic dose and at the same time improving the therapeutic windows of PBD-based ADCs will be key for the next generation of PBD-ADCs.
Although conjugation of linker-payload to the antibody is typically based on random conjugation to cysteine or lysine, we have shown that the native glycan of monoclonal antibodies is a privileged site for attachment of cytotoxic payloads[1] (GlycoConnect® technology), while the therapeutic index is further elevated by a highly polar spacer technology (HydraSpace®)[2]. Complemented by a broad choice of payloads with various mode-of-action (toxSYN® platform), these joint technologies have been applied in multiple clinical programs up to phase 3.
Our strategy to optimize the therapeutic potential of PBD-ADCs that incorporate GlycoConnect® and HydraSpace® technology further, is two-fold: (a) apply PBD dimer payload with reduced potency versus common clinical structures and (b) incorporate a pro-drug concept (proPBD-ADC) by shielding the PBD payload by a capping structure that is stable in circulation but at the same time conditionally released by tumor-associated proteases or under reducing conditions.
Data from efficacy studies with HER2-proPBD-ADCs in CDX tumor bearing mouse models will be shared, as well as tolerability studies performed both in rats and in the chicken egg chorioallantoic membrane (CAM) model. The data suggest that different cap structures of proPBD-ADCs, as well as the use of a PBD structure with attenuated potency, have a differential impact on efficacy and tolerability.

[1] (a) van Geel *et al*. *Bioconj. Chem.* **2015**, *26*, 2233-2242. (b) Wijdeven *et al. MAbs* **2022**, DOI: 10.1080/19420862.2022.2078466. [2] Verkade *et al*. *Antibodies* **2018**, *7*, 12, doi:10.3390/antib7010012.

#337 Highly potent, bispecific ADCs by application of clinical stage GlycoConnect® technology to bYlok® antibodies.

Floris van Delft, Lianne Lelieveldt, Jasper van de Sande, Anette Sommer

Synaffix - A Lonza company, Oss, Netherlands

Bispecific antibodies (bsAbs), with the ability to bind selectively to two different tumor-associated antigens in concerted fashion, have been increasingly successful for the treatment of cancer patients, delivering increased therapeutic potency compared to traditional mAbs, whilst also contributing to a reduction in the development of clinical resistance. Unfortunately, production of bsAbs is often plagued by HC-LC mispairing or by reduced titers, binding affinity, thermal stability or immunogenicity. Recently, we demonstrated that bsAbs generated using Lonza's bYlok® modification display a high percentage (>95%) of correct HC-LC pairing and can be purified to high levels using standard downstream purification (https://www.lonza.com/biologics/expression-technologies/bYlok).

Another increasing successful modality for anti-cancer therapy is the antibody-drug conjugate (ADC). Although typically based on random conjugation to cysteine or lysine, we have shown that the native glycan of monoclonal antibodies is a privileged site for attachment of cytotoxic payloads[1], while the therapeutic index is further elevated by a highly polar spacer technology (HydraSpace®)[2]. Complemented by a broad choice of payloads with various mode-of-action (toxSYN® platform), these joint technologies have been applied in multiple clinical programs up to phase 3 (see: https://synaffix.com/partnered-pipeline/).

Here we will demonstrate how bYlok® and GlycoConnect® technology are seamlessly combined to generate bispecific ADCs (bsADCs) with high yield and purity. Various bsADCs, featuring cytotoxic payloads with multiple MOA, including topoisomerase 1 inhibitors and tubulin binders, were fully analytically characterized and functionally evaluated, showing compelling tumor regression in multiple in vivo xenograft models. Combination of the technologies was further expanded to provide first-in-class bispecific ADCs with dual mode-of-action (dual warhead bsADCs).

[1] (a) van Geel *et al*. Chemoenzymatic Conjugation of Toxic Payloads to the Globally Conserved NGlycan of Native mAbs Provides Homogeneous and Highly Efficacious Antibody–Drug Conjugates. *Bioconj. Chem.* **2015**, *26*, 2233-2242. (b) Wijdeven *et al.* Enzymatic glycan remodeling–metal free click (GlycoConnect™) provides homogenous antibody-drug conjugates with improved stability and therapeutic index without sequence engineering. *MAbs* **2022**, DOI: 10.1080/19420862.2022.2078466.[2] Verkade *et al*. A Polar Sulfamide Spacer Significantly Enhances the Manufacturability, Stability, and Therapeutic Index of Antibody–Drug Conjugates. *Antibodies* **2018**, *7*, 12, doi:10.3390/antib7010012.

#338 Innovative strategy for enhancing the reach of HER3 antibody-drug conjugates: A novel drug combination approach.

Kathryn E. Vanderlaag, Anukriti Dhar, Arisa Paul, Molly I. Volkman, Erik M. Werner, Transon V. Nguyen, Kamran A. Ali

Eigen Therapeutics Inc., Redwood City, CA

Antibody-drug conjugates (ADC) and radioligand therapies (RLT) have emerged as promising treatment modalities for various cancers. In recent years, there have been numerous approvals of ADCs and RLTs by regulatory agencies, highlighting their clinical success. Despite their success, they still suffer from a common challenge: tumor heterogeneity. Tumor heterogeneity limits not only the patient response, but also the eligible patient population for these therapies. To address this challenge, we have developed a high throughput screening platform to identify small molecule “priming” agents that selectively upregulate tumor antigens in cancer, but not in normal cells. We have demonstrated that by increasing expression of target antigens on cancer cells, priming agents sensitize tumors to ADCs and can thus improve their efficacy and therapeutic window. Our priming screens thus far included evaluating priming agents for HER3, B7-H4, FR-A, TROP2, HER2, NECTIN-4, and CD123. Additionally, DLL3, c-MET, and SSTR2 are scheduled for early next year.

Our lead program focuses on HER3, where we have utilized our library to identify priming agents that upregulate HER3 cell surface expression by 2-3 fold across breast and ovarian cancer cell lines, demonstrating potential for indication-agnostic applications. Mechanistic validation has been achieved through the use of multiple inhibitors within the same pathway, which exhibit similar priming effects. Notably, HER3 upregulation is cancer-selective, with expression levels remaining low in normal cells. Ongoing experiments aim to further elucidate the underlying mechanism, and we have initiated medicinal chemistry efforts to advance this program.

Utilizing a priming approach in conjunction with an ADC or radiopharmaceutical offers three distinct advantages that lead to best-in-class or first-in-class therapies:

1. Patient Population Expansion: The priming approach enables the treatment of a broader range of cancer patients by overcoming tumor heterogeneity.
2. Reduced Toxicity: The ability to lower the dose of the ADC or radiopharmaceutical while maintaining efficacy reduces the risk of adverse effects and enhances patient safety.
3. Improved Durability of Responses: By addressing tumor heterogeneity, the priming approach enhances the durability of patient responses, leading to longer-lasting remissions.

By leveraging the potential of ADC and RLT (radioligand therapy), the priming approach opens up new avenues for more effective and personalized cancer treatments.

#339 Development of ^{212}Pb-based Radio-DARPin therapy (RDT) for the treatment of mesothelin (MSLN)-positive solid tumors.

Stephan Wullschleger[1], Amal Saidi[2], Delphine Buffet[1], Tania Stallons[2], Stefanie Riesenberg[1], Amy Wong[2], Maria Paladino[1], Federico Rojas[2], Mischa Muller[1], Christel Herzog[1], Justin Walter[1], Denis Villemagne[1], Yvonne Kaufmann[1], Nicole Pina[1], Henri Fernandez[1], Tamara Lekishvili[1], Eleni Tselempi[1], Jacqueline Blunschi[1], Liridon Abduli[1], Chloe Iss[1], Amelie Croset[1], Clara Domke[1], Aaron Schatzmann[2], Anne Goubier[1], Julien Torgue[2], Daniel Steiner[1]

[1]Molecular Partners, Zurich-Schlieren, Switzerland,[2]Orano Med LLC, Plano, TX

Radioligand therapies have emerged as promising treatment modality for certain cancers. This therapeutic approach relies on a suitable tumor-targeting moiety labelled with a radioactive payload to allow the delivery of the radionuclide to cancer cells with high specificity. Due to its expression profile in tumor vs healthy tissues, MSLN is an attractive protein for targeted therapies. MSLN is a GPI-anchored membrane protein which is overexpressed in many tumor types, including ovarian cancer and pancreatic adenocarcinoma. Therapeutic approaches targeting MSLN have encountered obstacles due to the high levels of its proteolytically cleaved soluble form (sMSLN), which can sequester anti-MSLN therapeutics and potentially diminish their efficacy. To circumvent this caveat, we sought to generate a Designed Ankyrin Repeat Protein (DARPin) that binds to a membrane proximal epitope of MSLN and therefore avoids binding to sMSLN. Thanks to their small size and high affinity, DARPins are ideal vectors for targeted radiotherapies, dubbed as Radio-DARPin therapies (RDT). As radioactive payload we used ^{212}Pb, an alpha particle-emitting radionuclide with a short half-life and a favorable decay profile which allows high energy deposition on tumor in a short time frame. Herein we report for the first time the development and initial preclinical characterization of our anti-MSLN ^{212}Pb-RDT approach. *In vitro* assays revealed that the membrane proximal MSLN targeting DARPin specifically interacts with MSLN expressed on cancer cells with high affinity, and cell binding was not affected by the presence of sMSLN. The DARPin was then labelled with ^{212}Pb and its preclinical pharmacokinetic and biodistribution profile was evaluated in MSLN-expressing xenograft tumor models. In these tumor bearing mice, the anti-MSLN ^{212}Pb Radio-DARPin demonstrated substantial uptake into MSLN-positive tumors, while other organs only showed limited accumulation. We designed an approach to specifically target MSLN expressed on cancer cells employing DARPin technology combined with ^{212}Pb. Our early preclinical results indicative of a favorable biodistribution profile of the ^{212}Pb labelled MSLN DARPin motivate further development of this RDT for the treatment of patients with MSLN-positive solid tumors.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#34 From fluid to function: Leveraging ascites to build orthotopic ovarian cancer models.

Cheryl Davis, Melissa Tran, Helen Ketteringham, Corrine Silvio, Ben Hoerner, Marcus Josey, Victoria Caruso, Kathleen Hutchinson, Dawn Lusk, Chris Holding, Shorena Nadaraia-Hoke

Reaction Biology Corporation, Hummelstown, PA

Often referred to as the “silent killer”, ovarian cancer poses significant challenges due to its asymptomatic nature and lack of reliable early detection methods, leading to late-stage diagnoses in over 70% of cases. As the eighth most common cancer among women worldwide, robust translational models for preclinical testing of ovarian cancer are essential. Unfortunately, the limited availability of orthotopic in vivo models presents a significant challenge in accurately replicating ovarian cancer for research purposes. By harnessing the ascitic nature of two well-established ovarian adenocarcinoma cell lines, we have successfully generated robust orthotopic ovarian in vivo models, which we can now offer as a valuable platform for preclinical research. Both our intra-peritoneal and intra-bursal models exhibit predictable growth patterns, thus contributing to the advancement of ovarian cancer treatments by enabling the exploration of tumor biology, drug resistance mechanisms, and therapeutic efficacy. The innovative models presented here are powerful tools for investigating tumor biology and advancing preclinical drug development, with the ultimate goal of improving therapeutic outcomes for patients with ovarian cancer.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Biochemical Modulators of Cancer / Differentiation Therapeutic Strategies
Poster Session

#340 GenSci139, a highly differentiated EGFR×HER2 bispecific ADC, for the treatment of multiple solid tumors.

Xiaojuan Chai, Fu Li, Hongmei Xie, Yuting Lu, Nan Li, Hanxi Yu, Li Tong, Weiming He, Wenqiang Zhai, Fanglong Yang, John L. Xu

GeneScience Pharmaceutical Co., Ltd., Shanghai, China

Background: Enhertu®/DS-8201, a HER2-directed ADC, has been used for the treatment of multiple tumor types, including HER2-positive and HER2-low breast cancer, HER2-mutant NSCLC, HER2-positive gastric cancer, etc. Despite Enhertu's success as a breakthrough therapy, significant unmet need remains in the HER2-low settings and in the occurrence of secondary resistance. Dual targeting of EGFR and HER2 represents a promising approach to the treatment of HER2-low patients and to overcoming drug resistance, as they are both oncogenic drivers and widely co-expressed. We developed GenSci139, an EGFR×HER2 bispecific ADC with a highly stable but cleavable linker and a potent topoisomerase 1 inhibitor payload, using our proprietary platform technologies. Preclinical studies are underway to evaluate the potential of GenSci139 as a novel targeted therapy across multiple solid tumor types.

Methods: GenSci139's plasma stability, *in vitro* cellular binding, internalization, cytotoxicity, and bystander effect, and blocking activity of GenSci139 or its parental bispecific antibody on EGFR/HER2 signaling in a number of human cancer cell lines were characterized. *In vivo* anti-tumor activity of GenSci139 was evaluated in a number of human cancer CDX models in mice. In addition, the ability of GenSci139 to induce immunogenic cell death (ICD) in tumor cell cultures was assessed.

Results: GenSci139 showed much better *in vitro* plasma stability than DS-8201, suggesting a favorable PK and safety profile. GenSci139 exhibited stronger *in vitro* tumor cell binding and internalization activities than DS-8201 and potent direct cytotoxicity and bystander killing effect in tumor cell lines. The parental bispecific antibody of GenSci139 inhibited EGF-induced signaling and cell proliferation in tumor cell culture. GenSci139 demonstrated superior *in vivo* anti-tumor efficacy *versus* DS-8201 in a number of CDX models in mice. In addition, GenSci139 was shown to induce hallmarks of ICD in tumor cells, providing a complementary anti-tumor mechanism when in a combination therapy regimen with immune checkpoint inhibitors.

Conclusion: These data suggest the potential of GenSci139 as a novel and effective therapy across a variety of tumor types, including urothelial cancer, lung cancer, gastric cancer, colorectal cancer, ovarian cancer, breast cancer, etc., and support its further evaluation in IND-enabling studies.

#341 VBC108: a first-in-class CDH17/CLDN18.2 targeted bispecific antibody drug conjugate (ADC) to overcome tumor heterogeneity of gastrointestinal cancers (GC, PADC, CRC, etc.).

Wei (Vivian) Wang[1], Lingyu Guan[2], Man Xu[1], Qun Yin[1], Chen Dong[1], Jing Li[1]

[1]VelaVigo (Hong Kong) Limited, Pudong, China,[2]VelaVigo (Shanghai) Limited, Pudong, China

CLDN18.2 is a promising target for GI cancer therapy, with various modalities under clinical evaluation. However, these therapies face challenges due to the heterogeneous expression of CLDN18.2, leading to inconsistent efficacy and the need of patient stratification, as observed with Zolbetuximab, AZD0901 and IBI343. Besides, these treatments may induce drug resistance by downregulating CLDN18.2. Recently, CDH17 (Cadherin 17) has emerged as a novel target for biologics, often overexpressed in GI cancers. But in normal tissues, it remains inaccessible as it is hidden within the tight junctions of intestinal epithelial cells, which makes it a favorable target for cancer therapy. More importantly, systematic analysis of expression data reveals high co-expression or complementary expression of CDH17 with CLDN18.2 in GI cancers, with no co-expression in other major organs. This discovery presents an opportunity for a bispecific anti-CDH17/CLDN18.2 therapy, potentially addressing the limitation of targeting CLDN18.2 alone. VBC108 is a bispecific ADC designed to target both CDH17 and CLDN18.2 with TOPO1i payload. The unique design maximizes efficacy while minimizing safety risks. VBC108 distinguishes itself from other CLDN18.2-targeting therapies through the following features. VBC108 targets both CDH17 and CLDN18.2, potentially overcoming tumor heterogeneity and covering over 80% of gastric and 50% of pancreatic cancers without CLDN18.2 IHC selection. It has high affinity to CDH17 and CLDN18.2, enhanced binding avidity, internalization and cytotoxicity. Moreover, it has a strong bystander effect to help further overcome the heterogeneity of tumor antigen expression. In CDH17 single-positive CDX models, which represent clinical scenarios lacking targeted therapies, VBC108 exhibited strong efficacy, whereas AZD0901 did not inhibit tumor growth at all. In CDH17/CLDN18.2 double-positive CDX models, representing clinical scenarios with target expression heterogeneity, VBC108 further showed its superiority. In CLDN18.2 single-positive CDX models, reflecting clinical scenarios with available CLDN18.2-targeted therapies, VBC108 demonstrated comparable efficacy with different payload mechanisms. In the PDX (GC, PADC, CRC) studies, especially in the GC model with low to medium expression of CDH17/CLDN18.2, it achieved a 100% CR. Furthermore, preliminary toxicity studies in Cyno-monkeys showed good tolerability with repeated doses. VBC108 also exhibited excellent developability in a comprehensive developability assessment (DA) test, making it a strong candidate for further CMC development. In summary, VBC108, with its unique design, shows the potential as a first-in-class ADC for GI cancer and supports the advancement to clinical trials.

#342 First-in-class click chemistry-based pre-targeting of CEACAM5 tumor antigen selectively activates an MMAE payload at tumors.

George Coricor, Masa Aleckovic, Sangeetha Srinivasan, Jesse McFarland, Stefanie Wagner, Nathan A. Yee, Tri-Hung Nguyen, Michael Zakharain, Travis Biechele, Jose M. Mejia Oneto

Shasqi, San Francisco, CA

Targeted delivery technologies (ADC, TRT, etc.) are commonly dose-limited by off-target toxicities. Less than 1% of the dose is delivered to the tumor while the remainder follows the normal catabolic elimination route of the antigen-binder resulting in significant normal tissue exposure and toxicity. A pre-targeting approach such as Shasqi's Click Activated Protodrugs Against Cancer (CAPAC®) technology, that separates the antigen-binder from the payload and reunites them only at the antigen is needed to overcome this limitation. CAPAC utilizes in-vivo click chemistry to selectively and rapidly reunite antigen-binders and payloads. Here we present preclinical data on a clickable CEACAM5 antigen-binder and a clickable monomethyl auristatin E (MMAE) payload or protodrug. We synthesized a clickable binder for CEACAM5 consisting of fragment antibodies covalently conjugated with the activating agent. The microtubule-disruptor, MMAE, was chemically attenuated with *trans*-cyclooctene to develop the protodrug, SQP22 (clickable payload). Both the clickable binder and protodrug were tested individually *in vitro*, with or without cells. They are currently being tested *in vivo*, individually or together, for efficacy and tolerability in naive tumor-free or tumor bearing CEACAM5-expressing cell-derived xenograft (CDX) rodent models. SQP22 showed a high level of attenuation (up to ~700x) in cytotoxicity assays *in vitro* compared to the active payload and cleared rapidly *in vivo*, both important features of CAPAC protodrugs. Additionally, the drug metabolism and pharmacokinetic characterization showed a favorable drug profile for SQP22, supporting its further development as a clinical agent of the CAPAC platform. The CEACAM5-targeted Fab has a binding affinity (KD) of 0.046 nM and a strong binding potency of 0.78 nM (EC50) to a CEACAM5-expressing cell line. Efficacy data from gastric and pancreatic CDX models shows that the clickable binder with SQP22 significantly improves tumor growth inhibition over a clickable non-binding isotype control with SQP22 and other negative controls. Tolerability studies in rats at various dose levels are ongoing. We have developed a novel, first-in-class, CEACAM5 clickable binder that can selectively activate an MMAE payload using click chemistry-based pre-targeting. Our results show that the protodrug and the clickable binder have favorable characteristics. Moreover, efficacy data indicate a meaningful benefit in cancer CDX models over negative controls. These data support the continued development of CEACAM5 clickable binder in combination with MMAE clickable payload for the treatment of CEACAM5 expressing cancers.

#343 GenSci143, a novel B7-H3×PSMA bispecific and potential best-in-class ADC, for the treatment of metastatic castration-resistant prostate cancer.

Yao Xu, **Fu Li**, Zhiyu Cui, Hongmei Xie, Wei Yan, Yan Li, Weiming He, Wenqiang Zhai, Fanglong Yang, John L. Xu

GeneScience Pharmaceutical Co., Ltd., Shanghai, China

Background: PCa is the most common cancer among men worldwide. Once patients have progressed to castration resistance, treatment options become scarce, leaving a substantial unmet need in the mCRPC settings. Both B7-H3 and PSMA are tumor-associated antigens that exhibit high levels of expression in PCa, and dual targeting of them with a single agent represents a promising therapeutic approach to the treatment of mCRPC. We developed GenSci143, a novel B7-H3×PSMA-directed bispecific ADC comprised of a bispecific antibody against B7-H3 and PSMA, a highly stable but cleavable linker, and a potent topoisomerase 1 inhibitor payload. Potential of GenSci143 as a novel targeted cancer therapy is currently evaluated in preclinical studies.

Methods: Membrane expression of B7-H3 and PSMA in tissue microarrays of PCa biopsies was examined by immunohistochemistry analysis. Cell surface levels of B7-H3 and PSMA in PCa cell lines were quantified by flow cytometry using Quantibrite beads. Binding affinity of GenSci143 to B7-H3- and/or PSMA-expressing tumor cells was also determined using flow cytometry. To assess internalization, cells were treated with pHrodo^{TM}-labelled antibodies, and fluorescence intensity was recorded. *In vitro* cytotoxic effect of GenSci143 on PCa cell lines was evaluated using a Luminescent Cell Viability assay. *In vivo* anti-tumor activity of GenSci143 was assessed in CDX models of PCa and compared to that of the DS-7300 and ARX517 analogs.

Results: B7-H3 and PSMA were highly co-expressed in PCa tissues (N=102), lending strong support to our dual-targeting therapeutic approach for mCRPC. GenSci143 showed strong *in vitro* binding, internalization, and cytotoxicity in tumor cells expressing either B7-H3 or PSMA or both TAAs. In human CDX models of PCa in mice, GenSci143 effectively induced tumor regression across different expression levels of B7-H3 and PSMA and demonstrated superior anti-tumor efficacy *versus* the DS-7300 and ARX517 analogs. Moreover, GenSci143 showed much better *in vitro* plasma stability than DS-7300. Following intravenous injection at 10 mg/kg in cynomolgus monkeys, GenSci143 exhibited a favorable pharmacokinetic profile, with serum concentration of free payload lower than 1.0 ng/mL at any time examined. In a pilot non-GLP repeat dose toxicity study in cynomolgus monkeys, GenSci143 was well tolerated, demonstrating a significantly higher therapeutic index than DS-7300 based on PK-PD modeling and simulation.

Conclusion: These results suggest that GenSci143 has the potential to become an effective therapeutic option for mCRPC patients and support its further evaluation in IND-enabling studies.

#344 IBI3014, a TROP2xPD-L1 bi-specific ADC integrating ADC killing with checkpoint blockade within one molecule, exhibits promising efficacy and safety in preclinical models.

Xiguang Zhang, Yanping Jin, Jinchang Lu, Ninghuan Li, Zhihai Wu, Min Wu, Yan Bian, Fenggen Fu, Weiwei Wu, Shuaixiang Zhou, Huizhong Xiong

Innovent Biologics (Suzhou) Co. Ltd, Jiangsu, China

Antibody-drug conjugates (ADCs) have emerged as a distinguished class of anticancer therapeutics. ADCs targeting Trophoblast Cell Surface Antigen 2 (TROP2) have shown efficacy in treating various epithelial tumors, such as non-small cell lung cancer (NSCLC) and triple-negative breast cancer (TNBC). Programmed Death Ligand 1 (PD-L1) serves as both an immune checkpoint and a tumor-associated antigen (TAA), commonly overexpressed in diverse tumor types. Growing evidence showed that ADCs can increase the efficacy of immunotherapeutic agents by multiple mechanisms, such as induction of immunogenic cell death, dendritic cell maturation, increase of T cell infiltration and expression of immune-regulatory proteins including PD-L1 and MHC. Both preclinical and early clinical studies suggest that ADCs and immunotherapy are promising combination options for tumor treatment. IBI3014 is an innovative TROP2 and PD-L1 dual-targeting ADC, comprising a humanized bispecific antibody linked to a novel DNA topoisomerase I inhibitor, NT1, via a cleavable linker. *In vitro* studies demonstrated the unconjugated bispecific antibody of IBI3014 has potent internalization activity potent immune checkpoint blocking activity. IBI3014 demonstrated superior cytotoxicity to benchmark and parental ADCs across several cell line-derived xenograft (CDX) models with different TROP2 and PD-L1 expression pattern, offering broader tumor type coverage. IBI3014 showed acceptable stability profile in mice and monkeys and can be well tolerated in monkeys with a highest non-severely toxic dose (HNSTD) of 50 mg/kg. Conclusion: IBI3014 represents a first-in-class bispecific ADC that integrates targeted cytotoxicity with checkpoint blockade within a single molecule. This dual mechanism of action not only enhances therapeutic efficacy but also maintains a favorable safety profile, which provides a promising approach for cancer therapy.

#345 Trop2 and B7H4 bi-specific ADC with improved efficacy and safety for gynecologic cancers.

Jing Chen, Chenjuan Zhu, Chenchen Zhu, Zhongmin Liu, Min Wu, Weiwei Wu, Junjie Deng, Yi Wang, **Xiguang Zhang**, Yiming Li

Innovent Biologics (Suzhou) Co. Ltd, Jiangsu, China

The effectiveness of Antibody-Drug Conjugates (ADCs) is closely associated with the level of tumor antigen expression and the administered drug dose, as observed in both preclinical and clinical settings. Trop2-targeted ADCs represent a promising therapeutic approach for various solid tumors; however, their efficacy is constrained by the expression levels of Trop2 and the off-tumor, on-target toxicity. To address these limitations, we have developed a bi-specific ADC that targets both Trop2 and B7H4, aiming to increase the overall tumor antigen expression level, and reduce the off-tumor toxicity associated with Trop2 by incorporating a single-arm Trop2 antibody and employing a less toxic Topoi NT3 linker payload in the ADC. Our research has confirmed the co-expression of Trop2 and B7H4 on tumor cells in Triple Negative Breast Cancer, ovarian cancer, and endometrial cancer, while not on normal mucosal epithelial cells. This co-expression profile supports the use of our bi-specific approach to improve cancer targeting efficiency while minimizing toxicity to normal tissues. In vitro studies demonstrate that our bi-specific Trop2/B7H4 ADC (IBI3022) exhibits superior cytotoxicity in HT29 cells overexpressing Trop2 and B7H4 compared to mono-specific ADC benchmarks targeting Trop2 or B7H4. Additionally, IBI3022 shows comparable efficacy to B7H4 ADC in B7H4-positive HCC1569 cells and to Trop2 ADC IBI130 in Trop2-positive BxPC3 and HCC1954 cells. In vivo, IBI3022 demonstrates enhanced tumor suppression compared to mono-specific ADC benchmarks in various tumor models with differing levels of Trop2 and B7H4 expression, including MDA-MB-468, HT29-Trop2-B7H4, HCC1806, JIMT1, and NUGC4. The half-life of IBI3022 in mice is 113 hours, supporting its sustained in vivo efficacy. The developability profile of IBI3022 is favorable, and notably, it is well-tolerated in cynomolgus macaques at doses up to 50 mg/kg. Overall, IBI3022 represents a promising bi-specific ADC for the treatment of gynecologic cancers, offering improved efficacy and safety profiles.

#346 MP0712, the first anti-DLL3 212Pb radio-DARPin (RDT) candidate for targeted radiotherapy of small cell lung cancer (SCLC).

Amelie Croset[1], Amal Saidi[2], Francesca Malvezzi[1], Tania Stallons[2], Madlaina Mettier[1], Amy Wong[2], Jitka Rantanen[1], Federico Rojas[2], Stephan Wullschleger[1], Eleni Tselempi[1], Yvonne Kaufmann[1], Tamar Lekishvili[1], Stefanie Riesenberg[1], Nicole Pina[1], Jacqueline Blunschi[1], Liridon Abduli[1], Christian Reichen[1], Christian Lizak[1], Aaron Schatzmann[2], Anne Goubier[1], Julien Torgue[2], Daniel Steiner[1]

[1]Molecular Partners AG, Zurich-Schlieren, Switzerland,[2]Orano Med LLC, Plano, TX

Radioligand therapy has shown strong clinical potential for the treatment of certain neuroendocrine and prostate tumors. Nevertheless, effective strategies in various cancer types are currently restricted by the lack of appropriate targeting agents for suitable tumor-associated antigens. Not expressed in healthy tissue, DLL3 is one of these promising targets highly upregulated in SCLC and other high-grade neuroendocrine tumors. Herein we present preclinical results of our DLL3-targeting 212Pb-based Radio-DARPin Therapeutic (RDT) combining the advantages of a small protein-based delivery vector and the short-lived alpha particle-emitting radioisotope 212Pb. Designed Ankyrin Repeat Proteins (DARPins) are a class of binding proteins with high specificity and affinity that can be generated against a broad range of tumor targets. Leveraging the learnings from our DARPin platform optimization allowed us to achieve efficient tumor uptake and penetration, while limiting exposure of healthy tissues. Our Radio-DARPin platform was optimized to effectively reduce DARPin uptake to kidneys, a major drawback of all small-size polypeptide vectors. This can be addressed by engineering the DARPin scaffold surface in conjunction with half-life extension (HLE) through serum albumin binding. 212Pb is a radioisotope with a short half-life of 11h and a favorable decay chain, allowing high energy deposition on tumor in a short time frame. By combining 212Pb with a HLE anti-DLL3 DARPin candidate, we aimed to generate a targeted RDT with high efficacy and a favorable safety profile. After several screening rounds of anti-DLL3-binding DARPins combined with HLE in mice, MP0712 was selected as lead candidate for further development. This molecule showed specific binding to lung cancer cells in vitro (~2nM on NCI-H82 ± HSA) and high affinity to human DLL3 (0.2nM by Surface Plasmon Resonance). MP0712 biodistributions were assessed in different mouse xenograft tumor models, matching clinically relevant DLL3 expression levels or overexpression of DLL3, and reached tumor:kidney ratios >2. Double xenografted mouse models showed selective uptake in DLL3-expressing tumors compared with tumors not expressing DLL3, confirming high target specificity of MP0712. The anti-tumor activity of MP0712 in different mouse xenograft tumor models showed promising efficacy. MP0712 led to tumor stabilization and significant effects on tumor versus necrotic tissue. In NCI-H82 tumors, median survival duration increased up to 3-fold with MP0317 compared with the buffer control. MP0712 also showed a favorable safety profile in vivo up to 30µCi. These preclinical results support our 212Pb-based RDT against DLL3 as a promising treatment option for SCLC, with encouraging in vivo antitumor activity and a good safety profile for our first DLL3-targeting 212Pb-RDT candidate for further development into the clinic.

#347 Novel EGFR x cMET bispecific ADC GQ1033 and DLL3-ADC GQ1030 demonstrated promising therapeutic efficacy in preclinical studies.

Sipeng Li, Yajun Sun, Meijun Xiong, Chong Liu, Yanwen Feng, Zengyan Mu, Xinju Gao, Yu Han, Paul H. Song, Gang Qin

GeneQuantum Healthcare (Suzhou) Co., Ltd., Suzhou, China

Background: Lung cancer is the leading cause of cancer deaths globally, with NSCLC comprising around 85% and SCLC about 15%. Several tyrosine kinase inhibitors (TKIs) have been approved for NSCLC. However, TKIs favor the emergence of resistant cells, ultimately resulting in tumor relapse. EGFR and cMET are co-expressed oncoproteins, with cMET playing a pivotal role in resistance to EGFR TKI in NSCLC. SCLC commonly develops disease relapse and resistance following first-line platinum-based chemotherapy, leading to a poor 5-year overall survival of below 7%. DLL3, an inhibitory Notch ligand, is selectively expressed on the surface of SCLC cells and represents a validated therapeutic target.

Methods: Traditional ADC discovery relies on rational design, which often limits diversity and may exclude promising candidates due to the complexity of ADCs. Existing conjugation technologies are not well-suited for diversity-oriented synthesis (DOS), as they involve multi-step processes, low versatility, and inconsistent quality. Our proprietary enzymatic site-specific conjugation platforms, iGDC™ and iLDC™, enable high-throughput synthesis of diverse ADC libraries. Using the platforms, we created a broad ADC library targeting EGFR x cMET and DLL3, combining antibodies with various formats, different linkers, and payloads like MMAE, Eribulin, Exatecan, Lurbinectedin, and TopoIx. The resulting ADCs were rigorously evaluated using in vitro cytotoxicity assays and in vivo PDX subclinical trials. Through this process, we developed GQ1033 and GQ1030, next-generation ADCs specifically targeting EGFR x cMET and DLL3, respectively.

Results: GQ1033 showed potent cytotoxicity in the HCC827 cell line and complete tumor regression at a single 4 mg/kg dose in the NCI-H1975 CDX model. In an osimertinib-resistant NSCLC PDX trial, involving ~10 models with diverse EGFR/cMET expression levels, GQ1033 demonstrated robust antitumor activity with an overall ORR of ~70%. GQ1030 displayed strong cytotoxicity in DLL3-positive cancer cell lines, with no off-target effects in DLL3-negative cells. In vivo, GQ1030 exhibited robust antitumor activity in the SHP-77 CDX model at a single dose of 2.5 mg/kg. In a PDX trial involving over 10 SCLC models randomly chosen without consideration of DLL3 expression, GQ1030 demonstrated best-in-class potential with an ORR of ~80%. The selected lead ADCs did not necessarily possess the highest antibody affinities or the most potent payloads. Furthermore, ADCs with higher DARs did not consistently demonstrate significant efficacy advantages.

Conclusions: GQ1033 and GQ1030, developed through high-throughput screening leveraging our iGDC™ and iLDC™ platforms, have demonstrated encouraging efficacy in preclinical studies, suggesting a broader therapeutic index. These next-gen ADCs hold potential as innovative treatments for lung cancers.

#348 Development of an anti-TF ADC with a potentially improved therapeutic index.

Xiaodong Xiao[1], Zhidi Pan[2], Zhangyi Song[1], Haiyang Yin[2], Hua Jiang[1], Yueqing Xie[1], Jianwei Zhu[1]

[1]Jecho Laboratories, Frederick, MD,[2]Jecho Institute, Shanghai, China

Tissue factor (TF) plays important roles in the hemostasis process. Through forming complex with factor (F) VII/VIIa, TF helps to initiate the blood coagulation process that protects vital organs from excessive bleeding. Under pathological conditions TF could also be involved in thrombosis. Furthermore, TF has functions beyond hemostasis such as signaling. TF is involved in many aspects of cancer transformation such as aberrant signaling, metastasis, and tumor angiogenesis. TF has been found to overexpress in multiple cancers including cervical, pancreatic, lung, head and neck, and prostate cancers, etc. Therapeutics targeting TF has achieved success with most notably the recent approval of Tisotumab Vedotin against cervical cancer. We report here the early phase development of an anti-TF ADC. Our in-house phage display library was employed to isolate a panel of fully human anti-TF antibodies. They vary in binding epitope and affinity allowing the opportunity to evaluate the optimum combination of both to achieve superior efficacy and safety. We found that the binding epitope plays a significant role in the antibody-mediated internalization, whereas within the same binding bin, antibody affinity and internalization may not have a direct correlation. One such antibody, 1E11, has a similar epitope to Tisotumab. It has a modest binding affinity (KD=68 nM) compared to other internal candidates as well as Tisotumab, yet it displayed similar or superior internalization efficiency and ADC mediated cell killing against multiple TF positive cell lines *in vitro*. In several CDX *in vivo* models tested so far, 1E11 based ADC showed comparable or superior activities to Tisotumab carrying the same linker/payload. Significantly, while Tisotumab displayed significant disruption of TF/factor VII interaction, a potential cause for excessive bleeding observed in clinics using Tisotumab Vedotin, 1E11 showed little or no such activities. These highly relevant function and safety features of 1E11-based ADC plus its excellent developability profile suggest that it is a promising candidate for further development into various ADC based therapeutics either used alone or in combination with other therapeutics.

#349 GenSci140, a potential best-in-class FRα-directed biparatopic ADC, for the treatment of platinum-resistant ovarian cancer.

Meihan Li, Fu Li, Yingying Sun, Qiuru Che, Na Dong, Li Tong, Weiming He, Wenqiang Zhai, Peng Qi, Min Zhang, Fanglong Yang, John L. Xu

GeneScience Pharmaceutical Co., Ltd., Changchun, China

Background: Folate receptor alpha (FRα) is a GPI-anchored cell-surface glycoprotein overexpressed in a variety of solid tumors, including ovarian cancer, non-small cell lung cancer (NSCLC), triple-negative breast cancer (TNBC), and endometrial cancer. It exhibits limited expression in normal tissues, making it an ideal target for the development of cancer therapy. IMGN853/ELAHERE^{TM}, a FRα-directed ADC, has been approved for platinum-resistant epithelial ovarian cancer (PROC) patients with high levels of FRα expression. Despite this advancement, it is estimated that around 60% of PROC patients with medium to low FRα expression do not benefit from this therapy. In addition, occurrence of ocular toxicity has been reported in approximately 40% of patients treated with ELAHERE^{TM}. In order to address unmet need in broader patient populations and to minimize toxicity, we developed GenSci140, a novel FRα-directed biparatopic ADC with a potent Topo1 inhibitor payload and a stable but cleavable linker at the drug-to-antibody ratio (DAR) of 6.
Materials and Methods: A novel FRα-directed biparatopic antibody was constructed from a monoclonal antibody and a VHH domain antibody to bind with high and low affinity, respectively, to two distinct epitopes on the tumor antigen, and used to generate GenSci140 with our proprietary linker-payload technology. *In vitro* tumor cell binding and internalization of GenSci140 were characterized by flow cytometry; cytotoxicity and bystander killing activity were assessed in Cell Titer-Glo assay and in luciferase assay, respectively. *In vivo* anti-tumor efficacy of GenSci140 was assessed in human cancer cell line-derived xenograft (CDX) models in mice, and pharmacokinetic and toxicology profiles were evaluated in cynomolgus monkeys.
Results: GenSci140 exhibited significantly stronger *in vitro* tumor cell binding and more efficient internalization than IMGN853. GenSci140 displayed potent cytotoxicity in FRα-positive tumor cells and effective bystander killing of FRα-negative cells in a FRα^{+}/FRα^{-} tumor cell co-culture system. GenSci140 demonstrated superior *in vivo* anti-tumor efficacy compared to ELAHERE^{TM} and IMGN151 (competitor's next-generation FRα-directed biparatopic ADC product) across a variety of CDX models including ovarian cancer, NSCLC, and TNBC, irrespective of their FRα expression levels. Furthermore, GenSci140 featured a favorable pharmacokinetic profile and was well-tolerated in a non-GLP repeat dose toxicity study in NHPs.
Conclusion: These data suggest that GenSci140 could be a more effective and safer treatment option than the current standard of care for 2L PROC, potentially addressing the unmet need of patients with varying levels of FRα expression. Therapeutic potential of this molecule is currently further evaluated in IND-enabling studies.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#35 Hydrogel-based microwell chip for improved organoid culture and on-chip imaging.
Thi Kim Ngan Ngo, Ting-Yuan Tu

Department of biomedical engineering, National Cheng Kung University, Tainan, Taiwan

Organoids have become essential tools for modeling complex biological systems, driving significant advancements in drug screening, cancer research, and regenerative medicine. However, traditional dome-shaped organoid culture methods come with notable limitations. These include variability in organoid size, structural heterogeneity, and difficulties in achieving high-resolution imaging due to overlapping positions and artifacts from the surrounding extracellular matrix (ECM). Such issues often hinder the reproducibility and precision of experimental outcomes, especially in long-term culture settings. To overcome these challenges, we developed a hydrogel-based microwell chip designed to standardize organoid culture and enhance imaging quality. The chip contains a grid of 100 microwells, each optimized to support the formation of up to 100 uniform spheroids or organoids. It is designed with a main microwell channel along with two inlet and outlet channels for hydrogel injection, which can be connected to a pump to enable dynamic culture conditions. Using this system, we successfully generated uniform spheroids from various cancer cell lines and consistent mouse intestinal organoids that retained characteristics comparable to those produced with the dome-shaped method. Importantly, the optimized size of the microwells reduces the volume of culture medium and cell suspension required, offering a more cost-effective and resource-efficient solution. This efficiency is particularly advantageous for drug screening, large-scale studies, or research involving precious samples, such as patient-derived organoids. Another notable advantage of our microwell chip is its compatibility with high-quality, live on-chip imaging throughout the culture period. This capability is essential for real-time studies of dynamic processes in organoid models, such as tissue morphogenesis, drug responses, and disease progression. Furthermore, the chip's glass-bottom design, combined with a thin hydrogel layer, facilitates confocal imaging either with the hydrogel in place or after it has been dissolved. Overall, our hydrogel-based microwell chip represents a robust and versatile platform that not only standardizes organoid culture but also improves the quality of imaging and data collection. By enhancing reproducibility, efficiency, and throughput, this technology holds promise for applications in precision medicine, drug testing, cancer research, and beyond.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Biochemical Modulators of Cancer / Differentiation Therapeutic Strategies
Poster Session

#350 AUTX-703, a novel and potent KAT2A and KAT2B protein degrader, induces differentiation and provides a potential therapeutic opportunity in subtypes of prostate cancer.

S. L. Sinicropi-Yao[1], J. DeBartolo[1], U. Sharif[2], Z. Boudhraa[3], M. El Ezzy[3], S. Sharma[1], M. Bhatta[1], C. Lee[1], B. Chan[1], H. Wilson[1], J. Neef[1], M. Bittenger[1], L. Antipov[1], T. G. Graeber[1], D. S. Millan[1], K. E. Yen[1], K. S. Straley[1];
[1]Auron Therapeutics, Newton, MA, [2]Sygnature Discovery, Cheshire, United Kingdom, [3]Paraza Pharma Inc, Laval, QC, Canada

Introduction: Phenotypic plasticity, or the ability of cancer cells to switch between cell states, is a hallmark of cancer. Cancer cells can hijack normal developmental pathways and push cells to a more proliferative, plastic cell state similar to those found in early development. To identify therapeutic targets driving tumor plasticity, we utilized our proprietary AI-ML platform to create a high resolution atlas of normal human development. Tumors were mapped to the atlas to identify the genes driving their oncogenic cell state. Our platform identified KAT2A, a lysine acetyltransferase, as a key driver in neuroendocrine prostate cancer (NEPC). Additionally in castration resistant prostate cancer (CRPC), elevated expression of KAT2A has been reported in patients with high grade disease or biochemical recurrence and is associated with poor clinical survival. Literature has demonstrated that shKAT2A can modify resistance to anti-androgens and suggests that KAT2A can play a role in the localization of androgen receptor (AR). Our studies investigate the opportunity for targeting KAT2A in treatment refractory prostate cancer (NEPC and CRPC).
Methods: To pharmacologically target KAT2A, and its paralog KAT2B, we developed AUTX-703, a novel, potent and orally bioavailable heterobifunctional protein degrader. The effects of AUTX-703 on cellular differentiation and growth inhibition were evaluated in NEPC cell lines and patient derived organoids models. The effects of AUTX-703 on AR localization and signaling were also evaluated in cell lines resistant to anti-androgens.
Results: Treatment of the NEPC cell line, LASCPC01 and two NEPC organoid models with AUTX-703 degraded KAT2A and KAT2B, induced differentiation toward an epithelial cell state and inhibited growth, in vitro. In AR positive treatment refractory prostate adenocarcinoma models, AUTX-703 alone or in combination with anti-androgens reduced cell growth, decreased levels of nuclear AR, and decreased levels of AR and Myc signaling. Unlike anti-androgen treatment which shifted cells toward a more stem-like state, treatment with AUTX-703 alone or in combination with anti-androgens shifted cells towards a more differentiated epithelial cell state.
Conclusion: Our studies validate KAT2A and KAT2B as key drivers of tumor cell state plasticity. Degradation of KAT2A and KAT2B with AUTX-703 induces a more differentiated cell state and inhibits growth in NEPC and CRPC models. These data support progression of AUTX-703 into clinical development for NEPC and targeting KAT2A and KAT2B in the treatment of anti-androgen resistant prostate cancer.

#351 Preclinical development of MHB009C: A novel anti-B7-H4 ADC with a potent topoisomerase I inhibitor.

L. Zhu, B. Liu, Y. Chen, X. Meng, A. Li, L. Liu, Z. Yao, Y. Wang, X. Weng, X. Zhu, Y. Liu, S. Zhou, J. Shi, **G. Cao**;
Minghui Pharmaceutical, Hangzhou, China

B7-H4, a member of B7 family, is a transmembrane protein that inhibits T cell immunity. It is overexpressed in various malignancies, including breast, ovarian, and endometrial cancers, as well as cholangiocarcinoma, where it has been associated with tumor progression and poor patient outcomes. Antibody-drug conjugates (ADC) targeting B7-H4 have demonstrated promising clinical activity. Here, we present the development and preclinical characterization of MHB009C, a novel B7-H4 ADC.

MHB009C comprises a human IgG1 antibody against B7-H4, conjugated to the SuperTopoi™ payload, a novel DNA topoisomerase I inhibitor that is 3~5 times more potent than DXd, with a drug-antibody ratio of ~4.0. Fc function was silenced to minimize nonspecific ADC uptake, and the linker was designed to enhance hydrophilicity and prevent the retro-Michael reaction, improving plasma stability. MHB009C demonstrated strong binding affinity to B7-H4 and was efficiently internalized by SK-BR-3 cells. It exhibited potent cytotoxicity in B7-H4-positive breast cancer cell lines and showed significant antitumor activity in an MX-1 breast cancer xenograft model. GLP study in monkeys revealed a favorable safety profile with no off-target toxicities, and the highest non-severely toxic dose (HNSTD) was determined to be 30 mg/kg Q3W, the maximum dose tested.

In conclusion, MHB009C demonstrated potent anti-tumor activity both *in vitro* and *in vivo*, along with a favorable safety profile, supporting further clinical development.

#352 Preclinical development of MHB042C: A next-generation anti-c-Met ADC conjugating VHH-Fc fusion protein with a novel topoisomerase I inhibitor.

Bo Liu, Liyuan Zhu, Yile Chen, Xiang Meng, Ao Li, Li Liu, Zhili Yao, Yan Wang, Xialian Weng, Xuefeng Zhu, Yang Liu, Shiyu Zhou, Junwei Shi, **Guoqing Cao**

Minghui Pharmaceutical, Hangzhou, China

c-MET, a receptor tyrosine kinase encoded by the MET proto-oncogene, is widely expressed in various solid tumors. Antibody-drug conjugates (ADC) targeting c-Met have shown promising clinical activity in patients with high c-MET expression, yet the therapeutic benefit remains limited for those with moderate or low expression levels. Here, we present the development and preclinical characterization of MHB042C, a next-generation anti-c-Met ADC.

Unlike conventional monoclonal antibody (mAb), MHB042C utilizes a humanized VHH targeting c-Met, fused with human IgG1 Fc to deliver the cytotoxic payload. The smaller VHH-Fc fusion protein offers higher potential for tumor tissue penetration compared to traditional mAb. This unique configuration streamlines the conjugation process, achieving a uniform drug-to-antibody ratio (DAR) of 4. Additionally, Fc function was silenced to minimize nonspecific ADC uptake. The payload, SuperTopoi^{TM}, a novel DNA topoisomerase I inhibitor, was 3 to 5 times more potent than DXd. The linker was designed to enhance hydrophilicity and to resist the retro-Michael reaction, thereby improving plasma stability. In preclinical assays, MHB042C demonstrated stronger cellular binding than competitor antibodies, reaching an internalization rate of approximately 60% in Hs746t cells within one hour. MHB042C exhibited potent cytotoxicity across c-Met-positive cancer cell lines and showed marked antitumor activity in an EBC-1 Squamous NSCLC xenograft model. GLP study in monkeys revealed a favorable safety profile with no off-target toxicities. The highest non-severely toxic dose (HNSTD) was determined to be 15 mg/kg Q3W, the maximum dose tested.

In conclusion, MHB042C demonstrated potent antitumor activity both *in vitro* and *in vivo*, along with a favorable safety profile, supporting advancement to clinical development.

#353 Preclinical development of MHB048C: A novel anti-PSMA ADC with a potent topoisomerase I inhibitor.

Yile Chen, Ao Li, Bo Liu, Liyuan Zhu, Xiang Meng, Zhili Yao, Yan Wang, Xialian Weng, Xuefeng Zhu, Shiyu Zhou, Junwei Shi, **Guoqing Cao**

Minghui Pharmaceutical, Hangzhou, China

Prostate-specific membrane antigen (PSMA), a type II transmembrane glycoprotein, is highly and specifically expressed in prostate cancer while showing limited expression in normal tissues. The FDA's approval of (177) Lu-PSMA-617 has validated PSMA as a therapeutic target for treating metastatic castration-resistant prostate cancer (mCRPC). Here, we present the development and preclinical characterization of MHB048C, a novel PSMA specific antibody-drug conjugate (ADC).

MHB048C comprises a human IgG1 antibody against PSMA, conjugated to the SuperTopoi^{TM} payload, a novel DNA topoisomerase I inhibitor that is 3~5 times more potent than DXd. The drug-antibody ratio is approximately 4.0. Fc function was silenced to minimize nonspecific ADC uptake, and the linker was designed to enhance hydrophilicity and prevent the retro-Michael reaction, improving plasma stability. The antibody preferentially binds to the PSMA dimer rather than the monomer, as only dimers display the native conformation. MHB048C showed strong binding affinity in protein and cell-level assays and was efficiently internalized by LNCaP cells, reaching an internalization rate of ~70% within 1 hour. It exhibited potent cytotoxicity in PSMA-positive prostate cancer cell lines with varying PSMA expression levels and demonstrated significant antitumor activity in an LNCaP FGC prostate cancer xenograft model. In GLP monkey studies, MHB048C exhibited a favorable safety profile with no off-target toxicities. The highest non-severely toxic dose (HNSTD) was determined to be 45 mg/kg Q3W, the maximum dose tested.

In conclusion, MHB048C demonstrated potent anti-tumor activity both in vitro and in vivo, along with a favorable safety profile, supporting advancement to clinical development.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Cell Cycle Effects of Anticancer Drugs
Poster Session

#357 Detailed mechanistic understanding of ACR-2316, a novel, clinical-stage WEE1/PKMYT1 inhibitor, rationally designed for superior single-agent activity through potent activation of CDK1, CDK2, and PLK1 using Acrivon's machine learning-driven AP3 platform.

Lei Shi[1], Martina Pasetto[2], Mohamed Eldeeb[2], Reina Improgo[1], Shahrzad Rafiei[1], Maria Rodriguez Zabala[1], Calvin Yang[1], Ahmed Youssef[1], Georgia Mitsa[2], Nina Lipjankić[2], Anna-Maria Alves[1], Portia Lombardo[1], Jessica Hopkins[1], Bryan Thornlow[1], William Dahlberg[1], Everett Hay[1], Ignacio Arribas Diez[2], Marc Isaksson[2], Zachary Best[1], Chris S. Balagtas[3], Corey Xu[1], Magnus E. Jakobsson[2], Helen Nilsson[2], Joon Jung[1], Caroline Wigerup[2], David A. Proia[1], Kristina Masson[2], Peter Blume-Jensen[1]

[1]Acrivon Therapeutics Inc., Watertown, MA,[2]Acrivon AB, Medicon Village, Lund, Sweden,[3]Active Motif, Carlsbad, CA

Introduction: WEE1 and PKMYT1 are essential kinases that safeguard genomic integrity by regulating CDK1 and CDK2. ACR-2316 is a potent, selective WEE1/PKMYT1 inhibitor rationally designed using Acrivon's Predictive Precision Proteomics (AP3) platform. ACR-2316 was uniquely designed, optimized, and advanced from initial lead to first-in-human dosing in < 15 months by AP3 to deliver complete tumor regression and pro-apoptotic tumor cell death through potent activation of CDK1, CDK2, and, importantly, PLK1. Here, we leveraged AP3-based global differential phosphoproteomic profiling and computational analyses to map and quantify the ACR-2316-regulated CDK1/2 and PLK1 substrates for unbiased understanding of drug action.
Results: AP3-based data-independent acquisition mass spectrometry was conducted using human cancer cells treated with vehicle or ACR-2316, -/+ CDK1 or CDK2 inhibition. Kinase activity inference analyzed by AP3 machine learning leveraging curated kinase-substrate relationships revealed that ACR-2316 strongly activated CDK1 and CDK2 followed by mitotic and DNA damage response kinases. We quantified hundreds of CDK1 and CDK2 substrates, regulated by ACR-2316, associated with S-phase DNA replication and replicative stress (CDK2) and with G2/M phase mitotic chromosome re-organization and segregation (CDK1). Multiparametric flow cytometry confirmed that ACR-2316 triggers DNA damage, premature mitosis, and S/G2-M cell cycle arrest. CellTiter-Glo and live cell imaging assays demonstrated robust inhibition of cell proliferation and potent cell death across human cancer cell lines. Inhibition of CDK1 or CDK2 partially rescued these phenotypes, confirming on-target mechanism of action identified through AP3. Chromatin immunoprecipitation sequencing showed unique intronic regions were susceptible to ACR-2316-induced double strand breaks. Finally, multiplexed immunofluorescence for alpha-tubulin and DNA revealed a large ACR-2316-induced increase in the number of mitotic aberrations in human cancer cells compared to vehicle control, and inhibition of only WEE1 or PKMYT1.
Conclusions: The unique capabilities of our generative AI-driven AP3 platform used to rationally design ACR-2316 enabled a comprehensive analysis of ACR-2316-regulated CDK1/2-and PLK1-induced pathways underlying its differentiated, potent anticancer activity, as demonstrated in head:head preclinical studies against clinical benchmark inhibitors. ACR-2316 has advanced into the clinic ahead of schedule uniquely enabled by AP3 and is currently in a Phase 1 clinical trial in subjects with AP3-selected advanced solid tumors. Acrivon anticipates reporting initial ACR-2316 clinical Phase 1 data second half of 2025.

#358 INK4A/B as predictive biomarkers for enhanced efficacy of dual WEE1 and PKMYT1 inhibition in CDK4/6 inhibitor-resistant breast cancer.

Mei-Kuang Chen[1], Xiaoting Jiang[1], Xiayu Rao[2], Tuyen Bui[1], Yan Wang[1], Jing Wang[2], Grace DeAlessandro[1], Nicole L. Massoumi[1], Rachel Y. Kim[1], Roanna A. Debler[1], Serena Kim[1], Kelly K. Hunt[3], Felicia Gray[4], Shaoxian Sun[4], Khandan Keyomarsi[1]

[1]Experimental Radiation Oncology, UT MD Anderson Cancer Center, Houston, TX,[2]Department of Bioinformatics and Computational Biology, UT MD Anderson Cancer Center, Houston, TX,[3]Department of Breast Surgical Oncology, UT MD Anderson Cancer Center, Houston, TX,[4]Schrodinger, Inc., New York, NY

Purpose: Although CDK4/6 inhibitors combined with endocrine therapy represent the standard of care for ER+/HER2- advanced breast cancer, resistance inevitably develops in a significant subset of patients, limiting effective treatment options. Similarly, patients with triple-negative breast cancer (TNBC) lacking BRCA mutations face an absence of targeted therapeutic strategies. These challenges underscore the urgent need for biomarker-driven targeted therapies in approximately 30% of breast cancer patients. Both CDK4/6 inhibitor-resistant (CDK4/6i-R) breast cancer and TNBC exhibit increased replication stress and cell cycle dysregulation, suggesting potential sensitivity to interventions targeting G2/M transition regulation by kinases like WEE1 and PKMYT1. Prior findings show that WEE1 and PKMYT1 inhibition disrupts TNBC cell survival by forcing cell cycle progression without adequate DNA repair and chromosome segregation. Our findings further indicate that WEE1 or PKMYT1 inhibition can effectively suppress CDK4/6i-R tumor growth. Additionally, PKMYT1 upregulation mediates resistance to WEE1 inhibition, suggesting that dual WEE1/PKMYT1 inhibition may provide synergistic tumor suppression in CDK4/6i-R and TNBC contexts. This study aimed to identify predictive biomarkers of response to WEE1/PKMYT1 dual inhibition while maintaining manageable toxicity.
Results: Evaluating WEE1 inhibitors (adavosertib or azenosertib) in combination with the PKMYT1 inhibitor (lunresertib) across CDK4/6i-R and TNBC models, including patient-derived xenografts (PDXs) and organoids, demonstrated significant tumor suppression, with the novel dual WEE1/PKMYT1 inhibitor SGR-3515 showing comparable efficacy. RNA sequencing analysis revealed a positive association between high INK4B (p15) expression and robust treatment response, while baseline expression data from the Cancer Cell Line Encyclopedia indicated a positive correlation between both INK4A (p16) and INK4B expression and responsiveness to dual WEE1/PKMYT1 inhibition across ER-positive and TNBC subtypes. Notably, forced overexpression of INK4B sensitized cells to dual inhibition, whereas INK4B knockdown increased resistance, underscoring its pivotal role in therapeutic response. Higher baseline levels of INK4A and INK4B in CDK4/6i-R and TNBC PDX models further correlated with improved treatment outcomes.
Conclusion: Dual inhibition of WEE1 and PKMYT1 presents a compelling therapeutic strategy for CDK4/6i-R breast cancer and TNBC. Elevated baseline levels of INK4A and INK4B are strongly associated with enhanced treatment responses, highlighting their potential as predictive biomarkers for selecting patients likely to benefit from WEE1/PKMYT1 dual inhibition therapy.

#359 Rb loss-related DNA damage repair deficiency mediates the synthetically lethal effect of ATR/PKMYT1 co-inhibition in TNBC and CDK4/6i-resistant ER+ breast cancer.

X.-T. Jiang, M.-K. Chen, J. Lee, X. Rao, J. Wang, K. Hunt, K. Keyomarsi;
The University of Texas MD Anderson Cancer Center, Houston, TX

Purpose: This study explores the therapeutic potential of dual ATR and PKMYT1 inhibition for treatment-resistant breast cancers, particularly triple-negative breast cancer (TNBC) and ER-positive breast cancer resistant to cyclin-dependent kinase 4/6 inhibitors (CDK4/6i). By investigating the synthetic lethality of ATR/PKMYT1 co-inhibition, we aimed to identify biomarkers predictive of treatment response, specifically examining Rb status and its effect on DNA damage repair mechanisms.
Methods: The study tested the combination of ATR inhibitor (RP3500) and PKMYT1 inhibitor (RP6306) in TNBC and ER-positive parental and palbociclib-resistant breast cancer cell lines, as well as in patient-derived xenograft (PDX) models. RNA sequencing (RNAseq), reverse phase protein array (RPPA) analyses, and western blot were employed to evaluate treatment efficacy, synergy, and biomarkers associated with response, particularly focusing on Rb1 expression.
Results: In vitro, both TNBC and palbociclib-resistant breast cancer cells—characterized by higher replicative stress—showed heightened dependency on ATR and PKMYT1 pathways. ATR inhibition disrupted DNA replication, while PKMYT1 inhibition induced premature mitosis, collectively leading to increased DNA damage and apoptosis in these models. In vivo, the combination treatment produced significant anti-tumor effects in TNBC and palbociclib-resistant PDX models. The loss of Rb strongly predicted treatment response; Rb-deficient cell lines (MCF-7 palbociclib-resistant, BT-549, and MB-436) and PDX models (PR4-T141 and TNBC2-BCX070) demonstrated pronounced apoptosis and tumor regression. Conversely, Rb-proficient MCF-7 parental cells, MB-231 and MB453 were less responsive. Rb1 re-expression in sensitive BT-549 cells mitigated apoptosis under the combination treatment, while Rb1 knockdown in MB-231 and MCF-7 cells increased sensitivity to the therapy. Comparative RNAseq and RPPA analyses showed diminished DNA damage repair activity in Rb-deficient cell lines and PDX models compared to Rb-proficient models, suggesting an Rb-dependent regulation of various DNA damage repair pathways. This was further supported by neutral comet assays in the MB-231 parental and Rb1 knockdown cell lines, which demonstrated longer DNA double stranded-break repair times with Rb1 knockdown.
Conclusions: ATR and PKMYT1 co-inhibition is a promising therapeutic strategy for TNBC and CDK4/6i-resistant breast cancer. The synthetic lethality of this combination is enhanced in Rb-deficient models, where Rb1 loss impairs DNA damage repair capabilities, amplifying the anti-tumor effects.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#36 A patient-centered approach to 3D *in vitro* modeling of pancreatic ductal adenocarcinoma.
Jade Vipond Cotter, Katie Reindl

Biological Sciences, North Dakota State Univ., Fargo, ND

Matrigel® is the most commonly used hydrogel product for developing and testing 3-D *in vitro* pancreatic ductal adenocarcinoma (PDAC) models; however, its composition is not reflective of the ECM content of most PDAC tumors. To remedy this pitfall, we have developed a novel approach for generating patient-derived 3D tumor organoids grown in customized hydrogels that better reflect their original tumor tissue. Using surgically resected original patient tissue, we measured the relative area covered by the major ECM constituents in PDAC: collagens I, III, IV, and laminin via IHC. Cells collected from PDX-propagated patient tissues were then embedded in the custom hydrogel comprised of ECM proteins in concentration that mirrored the original patient tissue. Organoids will then be monitored for size and rate of proliferation via MTT assay. Finally, to ensure transmembrane interactions with the customized hydrogel, expression of integrins α1, α2, α6, which bind the aforementioned hydrogel components, will be measured via IHC. We anticipate organoids grown in these custom hydrogels will demonstrate a greater likelihood of successful model development and higher growth rate than those grown in Matrigel®. Additionally, we expect organoids grown by this method will show increased expression of integrins α1, α2, and α6. These results provide preliminary evidence that the composition of the hydrogel used to generate 3D *in vitro* models may have an impact on their phenotypic expression. Further development and characterization of these models could result in better reflection of *in vivo* conditions in preclinical therapy testing.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Cell Cycle Effects of Anticancer Drugs
Poster Session

#360 Discovery of selective and orally bioavailable PKMYT1 inhibitor: characterization of anti-tumor activities as single and dual agents.

Xiaobin Summer Wang[1], Sining Zhu[1], Haili Ding[1], Yang Tang[2], Minrui Weng[3], Kelly Chen[4], Yanna Ma[3], Pan Wang[3], Yifan Tu[4], Juhao Yang[4], Jun Wu[5], Dan Zhao[5], Xuefei Tan[5], Ying Ji[6], Xiangyu Yao[1], Zhipeng Yan[1]

[1]Oncology Discovery, China Innovation Center of Roche, Shanghai, China,[2]Lead Discovery, China Innovation Center of Roche, Shanghai, China,[3]iSafe, China Innovation Center of Roche, Shanghai, China,[4]Pharmaceutical Sciences, China Innovation Center of Roche, Shanghai, China,[5]Medicinal Chemistry, China Innovation Center of Roche, Shanghai, China,[6]Formulation, China Innovation Center of Roche, Shanghai, China

Advancing effective cancer therapies requires innovative approaches to better understand complex biological interactions. PKMYT1, a crucial regulator of the cell cycle involved in the G2/M transition through inhibitory phosphorylation of CDK1, has emerged as a promising therapeutic target. Inactivation of PKMYT1 is synthetically lethal in tumor cells with genomic alterations leading to high replication stress, such as CCNE1 amplification, which is frequently found in high-grade serous ovarian, uterine, and gastro-esophageal cancers. In this report, we present a series of potent PKMYT1 inhibitors, emphasizing their pharmacokinetic profiles and anti-tumor activities, characterized as either single agents or in combination. Furthermore, we explored the potential of combination therapy using these profiled PKMYT1 inhibitors. We hypothesized that inhibition of the DNA damage response kinase ATR and its downstream pathway may further enhance the tumor cytotoxicity of PKMYT1 inhibitors. Preclinical validation of this hypothesis demonstrated that combining PKMYT1 inhibitors with ATR inhibitors, such as prexasertib and RP-3500, resulted in enhanced antitumor activity in CCNE1-amplified cancer models. Additionally, we observed synergistic effects when combining PKMYT1 inhibitors with standard-of-care chemotherapies that induce DNA damage, such as gemcitabine and hydroxyurea. We also identified novel synergistic partners from library screening, featuring GSK3B inhibitors. These findings were confirmed in cell lines, organoid models, and in vivo CDX models, highlighting the potential of PKMYT1 inhibitors as part of combination therapy strategies. In conclusion, our study underscores the therapeutic potential of selective PKMYT1 inhibitors in CCNE1-amplified cancers and provides a strong rationale for their combination with ATR inhibitors and DNA-damaging chemotherapies. These findings support the continued development and clinical evaluation of PKMYT1 inhibitors as a novel approach to cancer treatment.

#361 Allosteric ClpP agonist ONC206 alters mitochondrial metabolism, stress response, chromatin accessibility and cell cycle to elicit apoptosis in pheochromocytoma.

Varun V. Prabhu, Cristina Maranto, Andrew Lee, Scott Foster, Joshua E. Allen

Chimerix, Inc., Durham, NC

Dordaviprone (ONC201), an allosteric caseinolytic protease P (ClpP) agonist and dopamine receptor D2/3 (DRD2/3) antagonist, has demonstrated durable responses in recurrent H3 K27M-mutant glioma patients. ONC206, a derivative of dordaviprone with nanomolar potency for DRD2/3 and ClpP, is in Phase I trials. Dordaviprone demonstrated responses in patients with neuroendocrine pheochromocytoma-paraganglioma (PC-PG) including tumors with SDHB/FH loss-of-function driver mutations. We characterized ClpP binding, in vitro efficacy, comparison to standard of care (SOC) treatments, downstream signaling and response determinants for ONC206 in PC. Co-crystallization with ClpP that assembles as a tetradecameric complex revealed an allosteric ligand interaction with distinctions in the ONC206-ClpP resolved crystal structure relative to the dordaviprone-bound or apo complex, including an increase in the resolved pore size and decreased complex height. ONC206 was more potent than dordaviprone in cell-free human ClpP casein/peptide degradation assays. Accordingly, PC lines (hPheo1, PC12, MPC10) exhibited increased nanomolar sensitivity to ONC206 (~6 fold) relative to dordaviprone in cell viability assays. ONC206 induced dose- and time-dependent apoptosis in PC but not fibroblast (HFF-1, MRC-5) cells as measured with caspase Glo and/or annexin V assays. ONC206 generally required a shorter duration of exposure (~24h) and induced enhanced apoptosis in PC cell lines relative to dordaviprone at therapeutically relevant concentrations. ONC206 also demonstrated superior cell viability inhibition and apoptosis induction in PC cell lines relative to temozolomide and sunitinib at equivalent concentrations. CRISPR-mediated SDHB or FH knockout hPheo1 cells were equally sensitive to ONC206 as parental cells. Unlike DRD2, CRISPR-mediated ClpP knockout in hPheo1 cells impaired ONC206 sensitivity. Proteomics indicated inhibition of mitochondrial metabolism including OXPHOS and TCA cycle in hPheo1 cells. Accordingly, metabolomics revealed ONC206 elevated α-ketoglutaric acid, 2-hydroxyglutaric acid and reduced succinic acid, fumaric acid in a ClpP-dependent manner in hPheo1 cells. Consistent with epigenetic regulation by metabolites, ATACseq revealed ONC206 altered chromatin accessibility in hPheo1 cells. RNAseq demonstrated ONC206 upregulated stress response, apoptosis and downregulated cell cycle, metabolism-related pathways in hPheo1 cells. Accordingly, western blot showed ONC206 downregulated mitochondrial proteins (ClpX, SDHB), neuroendocrine markers (tyrosine hydroxylase, enolase-2, synaptophysin) and upregulated stress response (ATF4) in hPheo1 cells. Thus, ONC206 is a potent novel agent that is superior to SOC and dordaviprone that have shown clinical activity in PC-PG.

#362 Prevention of centrosome clustering by targeting the motor protein X: A promising therapeutic strategy for small cell lung cancer.

N. Nakagawa[1], M. Tokunaga[1], M. Ka[1], T. Iida[1], T. Ando[1], A. Kunita[1], K. Watanabe[1], Y. Ohta[2], X. Liu[3], E. Dmitrovsky[3], H. Kage[1], M. Kawakami[1];

[1]Graduate School of Medicine, The University of Tokyo, Tokyo, Japan, [2]Research Hospital, Institute of Medical Science, University of Tokyo, Tokyo, Japan, [3]Frederick National Laboratory for Cancer Research, Frederick, MD

Cancer cells often have more than two centrosomes (centrosome amplification). This is a cancer hallmark. Cancer cells cluster supernumerary centrosomes during mitosis so that they can undergo bipolar mitosis, similar to normal cells. We previously reported that inhibition of centrosome clustering by CDK2 antagonism in non-small lung cell cancer caused multipolar cell division and apoptotic death, termed anaphase catastrophe. Anaphase catastrophe preferentially affects cancer cells, sparing normal cells with two centrosomes. This therapeutic window implicates triggering anaphase catastrophe as a promising antineoplastic pathway. Motor protein X is reported to affect centrosome clustering but is not essential in normal cells. Small cell lung cancer (SCLC) has p53 inactivation and a poor prognosis with limited therapeutic options. Loss of p53 function disrupts the cell cycle checkpoint and is associated with centrosome amplification. We hypothesized that SCLC is vulnerable to mitotic abnormalities, including anaphase catastrophe. We explore here protein X as a novel therapeutic target that triggers anaphase catastrophe and apoptosis in SCLC. In vitro studies of multiple SCLC cell lines were performed. Immunofluorescence staining of pericentrin revealed higher frequency of centrosome amplification in SCLC than in normal human bronchial epithelial (NHBE) cells. Immunoblotting indicated that motor protein X was over-expressed in SCLC cells compared to NHBE cells. Functional analyses of protein X was done using siRNAs, CRISPR-Cas9 system, and a specific inhibitor. Protein X inhibition reduced cell proliferation and triggered apoptosis in the examined SCLC cell lines. In NHBE cells with infrequent centrosome amplification, these effects were rarely observed. To elucidate the mechanism for these antineoplastic effects, the mitotic status of SCLC cells was studied by staining for α-tubulin, pericentrin, and DAPI. Cells with clustered supernumerary centrosomes were statistically significantly decreased, while the number of multipolar mitotic cells were statistically significantly increased upon protein X inhibition. This indicated that targeting the protein X produced anaphase catastrophe in SCLC cells. We confirmed and extended these in vitro findings to the in vivo setting. The protein X inhibitor statistically significantly suppressed the growth of NCI-H146 tumor xenografts in mice. Pericentrin staining of resected treated xenografts revealed that the protein X inhibitor antagonized clustering of supernumerary centrosomes and led to multipolar mitosis in vivo. Taken together, prevention of supernumerary centrosome clustering by targeting the motor protein X is an attractive therapeutic strategy for SCLC.

#363 Exploiting Rb-deficiency: Dual inhibition of TRIP13 and Aurora A as a path to mitotic catastrophe.

Lacin Yapindi[1], Soma Ghosh[1], Li Shen[2], Jing Wang[2], Faye M. Johnson[1]

[1]Thoracic/Head & Neck Medical Oncology, UT MD Anderson Cancer Center, Houston, TX,[2]Bioinformatics and Computational Biology, UT MD Anderson Cancer Center, Houston, TX

Deregulation of the Rb pathway is found in approximately one-third of all solid tumors. Despite the high prevalence, there are still no clinically established therapies selectively targeting Rb-deficient cancers. Therefore, understanding how Rb function loss can be leveraged is crucial for developing novel, biomarker-driven, targeted therapeutic strategies. We and others have previously shown that Rb-deficient cancer cells depend on Aurora A kinases to bypass the primed spindle assembly checkpoint (SAC) and survive due to overexpression of the mitotic checkpoint protein Mad2. Thyroid hormone receptor interactor 13 (TRIP13) is a mitotic checkpoint modulatory protein that catalyzes the ATP-dependent conformational conversion of Mad2 to promote SAC silencing. Our previous research demonstrated that the combination of Aurora A inhibition using specific inhibitor alisertib and TRIP13 depletion induced synthetic lethality selectively in Rb-deficient cancer cells where the combination induced mitotic catastrophe. The combination also significantly increased the number of mitotic cells with an abnormal mitotic morphology by causing mitotic spindle defects and abnormalities in chromosomal alignment. Additionally, the combination led to prominent G2/M arrest in Rb-deficient cancer cells, while it only transiently delayed cell cycle progression in Rb-proficient cancer cells. Our live-cell imaging experiments using three Rb-deficient cell line models (HeLa, UMSCC47, ME180) showed that the combination induces marked prolonged arrest in mitosis and results in mitotic cell death. Furthermore, using auxin-inducible degron system and siRNA-mediated protein depletion, we showed that TRIP13 plays a modulatory role in mitotic exit as its depletion induced transient mitotic arrest and enhanced alisertib-induced lethality by further extending the mitotic arrest. Since mitotic catastrophe can cause DNA damage and this damage can be sensed by the inducers of immunogenic cell death (ICD), we also tested the effect of the combination on DNA damage and ICD. The combination caused marked DNA damage in mitotic Rb-deficient cancer cells, as measured by the increased γH2AX protein levels and tail-length using the neutral comet assay. Similarly, it increased the protein levels of ICD potentiators, cGAS and cleaved-gasdermin E. The mitotic entry was shown to be pivotal to the lethal effects of the combination, since the inhibition of mitotic entry with concurrent CDK1 inhibitor, RO-3306, prevented apoptosis and DNA damage, as determined by APO-DIRECT assay and immunoblotting. This is the first study to elucidate the mechanism behind the synthetic lethality of TRIP13 and Aurora A in Rb-deficient cancer cells. These findings may provide important insight into how to rationally design novel therapeutic strategies targeting mitotic regulators by harnessing the therapeutic vulnerability of Rb-deficient cancer cells.

#364 Oxaliplatin and 5-fluorouracil cause therapy-induced senescence in low-grade serous ovarian cancer cells.

Rewati Prakash[1], Alicia A. Goyeneche[2], Edith Zorychta[3], Shuk On Annie Leung[4], Lucy Gilbert[4], Carlos Telleria[1]

[1]Pathology, McGill University, Montreal, QC, Canada,[2]Research Institute of the McGill University Health Centre, Montreal, QC, Canada,[3]McGill University, Montreal, QC, Canada,[4]Gynecologic-oncology, Research Institute of the McGill University Health Centre, Montreal, QC, Canada

A critical concern with managing low-grade serous ovarian cancer (LGSOC) is the lack of response to standard carboplatin-paclitaxel chemotherapy; less than 30% of patients respond to such initial chemotherapy. Oxaliplatin (OXP) and 5-Fluorouracil (5-FU) combination treatment is a standard treatment regimen against colorectal cancer (CRC); however, it could be repurposed for use against LGSOC. p53 wildtype CRC tumours have been shown to respond better to OXP & 5-FU suggesting that LGSOC, which is well-characterized to also be p53 wildtype, would also respond effectively to this treatment. Consequently, in this study, we utilized three LGSOC cell lines: VOA6406, VOA7681, and VOA1056. We determined the cytotoxic effects of treatment with OXP & 5-FU; viability, proliferation, clonogenic capacity, and drug-withdrawal recovery were assessed. Results showed a dramatic decrease in cell proliferation in the short-and-long term, without a reduction in cell viability. This effect was confirmed further by measuring Ki67, a marker of cell proliferation; OXP & 5-FU-treated cells showed a significant reduction in Ki67 labelling. The persistence in viability alongside the reduced cell proliferation in cells treated with OXP & 5-FU led us to hypothesize that this drug combination induces senescence in LGSOC cells. A critical aspect of senescence is the arrest of the cell cycle. A propidium iodide-based cell cycle assay, along with western blotting to measure expression of p53, p21, and pRb, demonstrated that the LGSOC cells treated with OXP & 5-FU were arresting at the G1-S phase. Morphology alterations and lysosomal swelling were measured via expression of senescence-associated β-galactosidase (SA-β-gal); OXP & 5-FU-treated LGSOC cells demonstrated a marked increase in SA-β-gal as well as a distinct flattening of cell morphology. Consistent with hallmarks of senescence, anti-apoptotic proteins Bcl-2 and Mcl-1, were elevated upon treatment with OXP & 5-FU. A compelling increase in reactive oxygen species (ROS) production, as well as an elevated expression of caveolin-1, suggest a senescence-induction pathway dependent on oxidative stress. The use of a known ROS quencher, α-tocopherol, confirmed this pathway, as it partially rescued the senescence phenotype induced by OXP & 5-FU. The use of OXP & 5-FU to induce senescence in LGSOC shows promise as an alternate treatment option for this rare disease. We will further study if facilitating a “one-two punch theory” could be used against LGSOC, whereby OXP & 5-FU induces senescence, which could be subsequently “cleared” by a senolytic drug.

#365 A dual mechanism of sensitivity to PLK4 inhibition by RP-1664 in neuroblastoma.

Isabel Soria-Bretones[1], Matias Casas-Selves[1], Elliot Goodfellow[1], Ariya Shiwram[1], Nancy Laterreur[1], Li Li[1], Cathy Carron[1], Julian Bowlan[2], Hyeyeon Kim[1], Danielle Henry[1], Alejandro Alvarez-Quilon[1], Frederic Vallee[1], Artur Veloso[2], Jordan T. F. Young[1], Stephen J. Morris[1], C. Gary Marshall[2], **Michal Zimmermann**[1]

[1]Repare Therapeutics Inc., St. Laurent, QC, Canada,[2]Repare Therapeutics Inc., Cambridge, MA

Introduction:

High-risk neuroblastoma (HRNB) is a pediatric tumor with poor prognosis and limited treatment options. Recently, a novel therapeutic hypothesis for HRNB was proposed based on synthetic lethality between inhibition of polo-like kinase 4 (PLK4) and high expression of *TRIM37* - a gene encoded on chromosome 17q, whose gain is among the most frequent genomic alterations in HRNB[1,2]. This synthetic lethality depends on PLK4 inhibition-driven loss of centrioles, which in *TRIM37*-overexpressing tumor cells disrupts mitotic spindle assembly, and causes cell death through mitotic delay and p53 activation. Interestingly, partial PLK4 inactivation by low doses of pharmacological inhibitors is known to have an opposite effect on centrioles - increasing their number[3]. Here we report that both centriole amplification and centriole loss contribute to an exquisite sensitivity of HRNB models to RP-1664, a first-in-class selective and orally available inhibitor of PLK4.

Methods:

RP-1664 sensitivity and cellular consequences of PLK4 inhibition were evaluated in a panel of HRNB cell lines and normal immortalized cells with or without *TRIM37* overexpression and functional *TP53*. To that end, cell growth assays, immunofluorescence, immunoblotting and live cell imaging were used. CRISPR/Cas9 genomic screening was employed to map genes modulating RP-1664 sensitivity. RP-1664 *in vivo* efficacy was assessed in HRNB mouse xenograft models.

Results:

RP-1664 induced expected phenotypes of PLK4 inhibition in human cells, including an increase in PLK4 protein level, p53/p21 activation, centriole amplification at lower concentrations, and centriole loss at higher concentrations. HRNB cell lines showed general hypersensitivity to RP-1664, including at concentrations leading to centriole amplification. While sensitivity at higher RP-1664 concentrations depended on *TRIM37* and *TP53*, and was associated with a delay in mitosis, sensitivity at lower concentrations was *TRIM37*- and *TP53*-independent. CRISPR screens and live cell imaging after low-dose RP-1664 treatment revealed that HRNB cells, unlike normal immortalized cells, were unable to compensate for excess centrioles by their inactivation or clustering, and succumbed to multipolar mitoses. *In vivo*, RP-1664 showed robust efficacy at well tolerated doses in HRNB models and at plasma exposures consistent with centriole amplification.

Conclusion:

HRNB cell models are hypersensitive to centriole amplification induced by low levels of PLK4 inhibition as well as to centriole loss at higher PLK4 inhibitor levels, warranting the investigation of RP-1664 in clinical trials with neuroblastoma patients.

1. Yeow, Z. Y. *et al.,* Nature 585, 447-452 (2020).

2. Meitinger, F. *et al.,* Nature 585, 440-446 (2020).

3. Tkach, J. M. *et al.,* Elife 11, e73944 (2022).

#366 CDK7 inhibition as a synthetic lethal strategy in Rb-deficient ER+/HER2- breast cancer and sarcoma cells resistant to CDK4/6 inhibitors.

Rezvan Esmaeili[1], Tuyen N Bui[1], Tuyen Duong Thanh Nguyen[1], Jim Joseph[2], Fred Aswad[2], Kelly K Hunt[3], Elena Fonfria[4], Khandan Keyomarsi[1]

[1]Department of Experimental Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX,[2]Apeiron Therapeutics, Burlingame, CA,[3]Department of Breast Surgical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX,[4]Recursion, Oxford, United Kingdom

Purpose: Resistance to CDK4/6 inhibitors (CDK4/6i) presents a significant barrier in the treatment of ER+/HER2-breast cancers, affecting approximately 50%-60% of patients initially responsive to therapy. Moreover, soft tissue sarcomas (STS), representing over 13,000 new cases annually in the U.S. alone, are frequently characterized by Rb pathway disruption and have poor survival outcomes due to limited therapeutic options. Given the role of CDK7 as an upstream regulator of CDK2, and emerging evidence that Rb deficient cells are sensitive to CDK7 inhibition, we hypothesize that the novel CDK7 inhibitor REC-617may act as a synthetic lethal agent in Rb-deficient, CDK4/6i-resistant cancer models. This study aims to evaluate the efficacy of REC-617 in both ER+/HER2-breast cancer and STS cell lines, especially within Rb-deficient contexts, as a therapeutic strategy for overcoming resistance and broadening targeted treatment options.

Methods: CDK4/6i-resistant cell lines were generated by progressively exposing MCF7 breast cancer cells to increasing concentrations of palbociclib (1.2-4.8 µmol/L), ribociclib (1.2-4.8 µmol/L), and abemaciclib (0.5 and 1 µmol/L). CRISPR-Cas9 technology was used to create Rb-deficient models in resistant cells to assess REC-617's impact. Syngeneic and non-syngeneic sarcoma cell lines with differential Rb status were also treated to evaluate the drug's broader applicability in Rb-deficient cancers. Cell cycle progression, apoptosis, and toxicity were analyzed, and Western blotting was performed to monitor changes in cell cycle proteins, DNA damage markers, and signaling pathways.

Results: REC-617 induced a marked G2 phase arrest and increased apoptosis in CDK4/6i-resistant MCF7 breast cancer cells compared to parental cells. This effect correlated with decreases in CDK1 and CDC25C and an increase in cyclin B1, confirming G2/M arrest. DNA damage response was heightened, as shown by elevated γH2AX levels, while Rb and p53BP1 levels were diminished. Notably, both Rb-deficient CRISPR-engineered breast cancer cells and Rb-deficient sarcoma models exhibited heightened sensitivity to REC-617 compared to their Rb-intact counterparts. This increased susceptibility was consistently observed across palbociclib-, abemaciclib-, and palbociclib-resistant cells, further validating the relevance of Rb status in CDK7-targeted responses.

Conclusion: These findings demonstrate that CDK7 inhibition via REC-617 offers a potent synthetic lethal approach in Rb-deficient, CDK4/6i-resistant ER+/HER2-breast cancer and soft tissue sarcomas. Reduced Rb expression may be predictive of the level of response to REC-617, thus providing a targeted treatment option for overcoming resistance in both cancer types and addressing a critical need in resistant ER+/HER2-breast cancers and soft tissue sarcomas.

#367 Temporal dynamics of the senescence-associated secretory phenotype triggered by CDK4/6 inhibitor or doxorubicin treatment in liposarcoma.

Joanna Lan Hing Yeung[1], Justin Rendleman[1], Nicole Pagane[1], Irene Duba[1], Andrew Scortea[1], Lauren Anderson[1], Ria Hosuru[1], Bat Ider Tumenbayar[1], Andrew Koff[2], Viviana Risca[1]

[1]The Rockefeller University, New York, NY,[2]Memorial Sloan Kettering Cancer Centre, New York, NY

Liposarcoma is a rare cancer that frequently exhibits amplification of the cyclin-dependent kinase CDK4 gene. CDK4/6 inhibitors (CDK4/6i) represent a promising targeted therapy to halt proliferation in these tumors, particularly when therapeutic options are limited, and surgical resection is no longer viable. CDK4/6 inhibition can result in either reversible or irreversible exit from the cell cycle (quiescence or senescence, respectively), but the mechanisms driving these outcomes remain unclear. Since CDK4/6 inhibitors appear to induce cell cycle arrest without causing extensive DNA damage, we hypothesize that the senescence-associated secretory phenotype (SASP)—a collection of secreted proinflammatory molecules—induced by CDK4/6i differs significantly from the SASP triggered by cytotoxic chemotherapeutics such as doxorubicin. To investigate this, we characterized differences in SASP gene expression and its upstream regulators using RNA-seq and ATAC-seq in a 28 day time-course study of a patient-derived liposarcoma cell line responsive to both drugs. Our findings reveal that extracellular matrix (ECM) genes are upregulated early in CDK4/6i treatment, a response largely absent in doxorubicin-treated cells. Many of these ECM genes are previously identified SASP components, suggesting a CDK4/6i-specific SASP module. We identified the Hippo pathway regulator TEAD as a key player in shaping this response. Pharmacological inhibition of TEAD suppresses the upregulation of key SASP genes, *ANGPTL4* and *IGFBP3*, which are more strongly induced by CDK4/6i compared to doxorubicin. While the SASP profiles differ between drugs early in treatment, we observe emergence of a shared SASP signature characterized by NF-κB signaling at later time points. This is indicated by upregulation of NF-κB target genes and enrichment of NF-κB motifs in the regions with increased chromatin accessibility under both drugs. In both cases, we detect increased nuclear localization of p65, a subunit of NF-κB, confirming NF-κB activation. Furthermore, addition of an NF-κB inhibitor suppresses the upregulation of NF-κB target SASP genes in both treatments. These results highlight how the composition of therapy-induced SASP evolves over time and contains both common and treatment-specific modules. We also demonstrate that NF-κB activity contributes to shaping part of the CDK4/6i-induced senescence response, addressing an area of ongoing debate in the field. Our study provides new insights into how cancer therapies regulate the SASP profile, with implications for understanding the effects of therapy-induced senescence on the tumor microenvironment.

#368 Unique breast cancer CDX panels for KAT6, ER, and CDK4/6.

Tianyu Pang, Haiting Dai, Ziwei Li, Wenbo Du, **Wen-Jen Yu**

ICE Bioscience INC, Beijing, China

Estrogen receptor-positive (ER+) breast cancer accounts for approximately 75% of all breast cancers. Over-expression of lysine acetyltransferase 6A (KAT6A), a histone acetyltransferase (HAT), shared a proportion of 10-15% in breast cancer patients and increased the tumor growth through binding the promoter region to enhance ER expression level. Inhibitors of KAT6A were thought to be anti-tumorigenic by deregulation of ER expression. In addition, the cyclin-dependent kinases 4/6-cyclinD-Rb (CDK4/6-cyclinD-Rb) pathway is very important and crucial for ER downstream signaling in ER+ breast cancers. To accelerate the discovery of anti-ER+ breast cancer drugs through KAT6A and CDK4/6 pathways, we generated four specific CDX models which cover the most popular ER+/KAT6A cell lines (BT-474, ZR-75-1) and ER+/ CDK4/6 cell lines (MCF-7, HCC1428) and revealed the efficacy of KAT6A inhibitor (PF-9363), CDK4/6 inhibitor (Palbociclib), or ER inhibitor (Fulvestrant) on these breast CDX models. The in vivo MOA of these inhibitors were also evaluated by Western Blotting and pathological examination.

#369 A novel class of multitarget small molecule PI3K-CDK4/6-CDK9-AURAKA/B inhibitor harnesses Warburg effect against non-small cell lung cancer.

Anna V. Ivanina Foureau, Cody C. McHale, Hailey L. Dryden, Krishnaiah Maddeboina, David M. Foureau, Fei Guo, Donald L. Durden, Kathryn F. Mileham

Levine Cancer Institute, Charlotte, NC

Introduction: *In silico* designed novel multitarget small molecule PI3K-CDK4/6-CDK9-AURKA/B inhibitor LCI139 is nanomolar potent against NSCLC cells of mixed histologic and mutational backgrounds. We previously established that higher NSCLC cells metabolic activity promotes LCI139 potency. Here, we describe how LCI139 harnesses the Warburg effect to induce synthetic lethality in NSCLC.
Methods: LCI139-sensitive (NCI-H1703) and resistant (NCI-H1781) human NSCLC cells, identified from prior IC_{50} experiments, were treated with LCI139 (0.25 and 0.5μM) or single agent inhibitors PI3Ki (BKM120), CDK4/6i (Ribociclib), CDK9i (AZD4573), and AURKAi (Alisertib). Apoptosis and senescence were measured by flow cytometry (Annexin V/7-AAD or β-gal). Mitochondrial respiration and mitochondrial complexes activity was measured with Clark-type electrode. Target inhibition, glycolysis and OXPHOS enzymes, AMPK-FoxO3 signaling, Bcl2-family proteins, and caspase expression/activation were evaluated by Western blot and qRT-PCR on whole cellular extract or isolated mitochondria.
Results: LCI139 induced caspase-dependent apoptosis in sensitive H1703 cells by promoting Cas8 activation, BIM expression, Bax-Bak complex formation, mitochondrial fission and apoptosome formation (Cas9). In resistant H1781 cells, LCI139 promoted cellular senescence by inducing mitochondrial respiration and oxidative stress (ROS-forming complexes I-III activity), while repressing caspase activation. Bioenergetic stress sensor AMP-activated protein kinase (AMPK) and downstream forkhead box O3 transcription factor (FOXO3) signaling played a key role in LCI139 response. Upon treatment of H1703 sensitive cells, pAMPK levels increased and FoxO3 was expressed/stabilized, leading to subsequent pro-apoptotic Cas8/BIM expression. Compared with H1781 resistant cells, H1703 cells had greater baseline glycolytic activity and mitochondrial respiration. Consequently, LCI139 profoundly disrupted both glycolytic-ATP (glucose uptake [Glut1] and catabolism [PFK, LDH] reduction), and OXPHOS-ATP production (mitochondrial complexes I-IV loss) in H1703 sensitive but not H1781 resistant cells. Single target inhibition experiments showed that the metabolic stress response to LCI139 was primarily driven by PI3Ki and CDK4/6i warheads and promoted both Cas8 and BIM. Inhibition of CDK9 alone was a potent inducer of subsequent Cas8 activation and, together with AURKAi, promoted cellular apoptosis in H1703 sensitive cells
Conclusions: Multitarget inhibitor, LCI139 is uniquely designed to harness the Warburg effect to induce synthetic lethality in glycolysis-reliant NSCLC by 1) inducing metabolic stress (pAMPK), 2) lowering the threshold to metabolic stress response (FoxO3 stabilization), and 3) enhancing caspase-dependent cell death non-canonical Cas8 activation.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#37 Characterization of cervical cancer patient derived organoid.

Rui Wang, Dalissa Negron-Figueroa, Xiaogang Wu, Bo Jiang, Barrett C. Lawson, Timothy A. Harris, Maura Gillison, Lauren Colbert

UT MD Anderson Cancer Center, Houston, TX

Background: HPV-related cervical cancers pose challenges in murine models due to human-specific viral carcinogenesis. Patient-derived organoids (PDOs) enable to incorporate extracellular matrix and tumor microenvironment, and mirror human tumor features.
Methods: Tumor and adjacent tissues were sampled via cytobrushes and resections, and then dissociated, and purified using a Lymphoprep gradient. One million cells were used for scRNA sequencing, while the rest formed PDOs. Baseline tumor and buccal swabs served as genomic controls. PDO identity was validated through immunofluorescent staining, bulk RNA sequencing, and whole exome sequencing (WES). Cell viability assays assessed PDO responses to bacterial metabolites, radiation, and chemotherapy.
Results: We established five PDO lines from cervical squamous cell carcinoma (CSCC) and cervical adenocarcinoma (CAC), including one normal PDO from CAC-adjacent tissue. Two primary cell lines were also developed: one from CAC and one from CSCC. Early cultures showed differential KI67 staining: CAC1101, CAC1446, and normal PDOs were positive, while CAC1237 and CSCC1188 were negative. Later cultures showed KI67 positivity across all lines. P63 staining was positive in early CAC1101, CAC1446-adjacent normal, and CSCC1188 PDOs but became negative in late CSCC1188. Only CAC1101 PDOs were PAX8-positive, and PAS staining was observed in all CAC-derived PDOs, including CAC1446-adjacent normal. Somatic mutation analysis revealed 22-46 mutations across most samples, except for Day-14 CAC1101 PDOs, which had 536 mutations. Day-21 CAC1101 PDOs showed 32.6%-54.5% similarity to their tumor swab. CSCC1188 samples exhibited consistent similarity with their tumor swabs (25.9%-40.0%) and within PDOs (37.0%-64.0%). Germline mutation analysis showed 1,045-1,918 mutations in most samples, except for Day-14 CAC1101 PDOs, which had 38 mutations. CSCC1188 samples showed higher similarity with their buccal swabs (71.3%-74.0%) and within PDOs (70.4%-78.4%) compared to CAC1101. Comparison of RNA sequencing data of CAC1101 and CSCC1188 showed samples from same patient sharing similar profiles. Cell viability assays indicated that bacterial metabolites increased resistance to radiation and cisplatin in CSCC1188-derived primary cells but had no effect on CAC1237-derived cells.
Conclusion: We developed a workflow for generating and expanding PDOs from surgical and non-invasive cytobrush samples while preserving tumor characteristics. PDOs recapitulated the features of their original tumors, offering insights into tumor-microenvironment interactions and variable responses to chemoradiation therapy.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Cell Cycle Effects of Anticancer Drugs
Poster Session

#370 Resistance to sotorasib does not confer resistance to adagrasib: Why?.

Atish Ranjan Mohanty[1], Baiyi Quan[2], Supriyo Bhattacharya[1], Dana Do[1], Sravani Ramisetty[1], Tsui-Fen Chou[3], John Orban[4], Prakash Kulkarni[1], Ravi Salgia[1]

[1]City of Hope National Medical Center, Monrovia, CA,[2]Proteome Exploration Laboratory, California Institute of Technology, Pasadena, CA,[3]California Institute of Technology, Monrovia, CA,[4]University of Maryland, Baltimore, MD

Introduction: KRAS mutations are among the most common gain-of-function alterations, found in approximately 30% of lung adenocarcinomas. These mutations primarily occur at codon 12, leading to amino acid substitutions that impair KRAS's intrinsic GTPase activity, rendering the oncoprotein constitutively active. For decades, KRAS was considered "undruggable" due to the absence of a suitable drug-binding pocket. This paradigm shifted with the development of sotorasib, a covalent inhibitor that irreversibly binds to a cysteine residue, locking KRAS in its inactive GDP-bound state. Despite this breakthrough, the CodeBreaK 100 Phase I/II clinical trial revealed that the objective response rate to sotorasib was only 36%, with a median progression-free survival (PFS) of 7.6 months. These findings underscore that 64% of patients with advanced NSCLC exhibited innate resistance to sotorasib and those who responded eventually developed acquired resistance. Thus, in this study, we aim to explore the mechanisms of sotorasib resistance and evaluate the response of resistant cells to adagrasib using proteomic data.
Methods: KRAS G12C NSCLC cell lines were screened for sotorasib sensitivity, and isogenic-resistant lines were developed through incremental exposure. These lines were tested for adagrasib sensitivity. Proteomic changes in parental and resistant cells, untreated or after treatment, were analyzed via mass spectrometry.
Results: Incremental sotorasib treatment of sensitive cells generated isogenic resistant lines capable of tolerating sotorasib concentrations up to 20 μM. Exome analysis of resistant cells revealed no acquired driver mutations, which could be correlated with sotorasib resistance. However, these cells exhibited increased expression of ITGB4 and WNT2, leading to activation of PI3K/Akt and Wnt/β-catenin signaling pathways, as confirmed by western blot analysis. Proteomic profiling identified an upregulation of proteins involved in biological processes like cell adhesion and signal transduction, consistent with the activation of ITGB4 and Wnt signaling. Notably, adagrasib treatment significantly suppressed these biological processes and activated metabolic processes. Live-cell imaging and cell cycle analysis further demonstrated that adagrasib induced a more robust cell cycle arrest than sotorasib.
Conclusion: This study concludes that sotorasib resistance can arise through the activation of alternative signaling pathways, underscoring the adaptability of biological networks in cancer cells. However, this non-genetic resistance mechanism to sotorasib does not confer cross-resistance to adagrasib, indicating that the fitness acquired by cancer cells to tolerate sotorasib is ineffective against adagrasib. These findings suggest that patients who progress on sotorasib treatment may still benefit from adagrasib, highlighting its therapeutic potential in overcoming resistance.

#371 Co-existent *PIK3CA* and *ARID1A* mutations lead to enhanced sensitivity to PI3K inhibition through PUMA induction by FOXO3a and p53/p21.

Shirsa Udgata, Jordan N. Stoecker, Xingqi Shen, Cheri A. Pasch, Dustin A. Deming

University of Wisconsin-Madison, Madison, WI

Background: *PIK3CA* (PK) mutations occur in ~18% of colorectal cancer (CRC) cases. However, an effective means to target these cancers in clinic remains a challenge. Co-alterations in *PIK3CA* and *ARID1A* (AD) occur in ~1% of all cancer cases. The Z1F arm of the NCI MATCH clinical trial demonstrated an improved response rate for the pan-PI3K inhibitor, copanlisib (cop) in subjects with PK and AD mutant cancers. Recent studies have shown that cop treatment leads to p53 independent induction of PUMA by FOXO3a in CRC cell lines. Furthermore, ARID1A is suspected to regulate the levels of p53/p21 and c-MYC, which are important for the induction of apoptosis. Here we further evaluate this potential mechanism of action.

Methods: AD was knocked out from human 2D CRC cells SW48 and SW48$^{PIK3CA\text{-}H1047R}$ (SW48PK) using CRISPR as previously described (SW48AD; SW48PKAD). Flow cytometry (FC) was performed for apoptosis (Annexin V and PI) after 48 hours of cop treatment at 10nM and 50nM (PI3K and MTOR IC_{50} respectively). Western blot (WB) was performed after cop treatment for 6 hours and 24 hours to determine the protein levels of the anti-apoptotic protein MCL-1, pro-apoptotic protein PUMA, and proteins that activate PUMA (FOXO3a, p53, p21 and c-MYC).

Results: An increase in apoptosis was observed in SW48PKAD cells compared to SW48PK with 50nM cop treatments for 48 hours ($p=0.02$). MCL-1 protein expression at baseline was lower in SW48PKAD compared to SW48PK cells. This also decreased significantly by 6 hours with cop ($p<0.05$). PUMA expression was significantly increased in SW48PKAD after 6 hours cop 10nM ($p<0.05$) and 50nM ($p<0.005$) compared to SW48PK, where there was a delayed increase in PUMA levels by 24 hours (50nM; $p<0.005$). FOXO3a was significantly increased in SW48PKAD at 6 hours (10 and 50nM; $p<0.005$), indicating the potential for the induction of PUMA to be secondary to FOXO3a. Among FOXO3a targets, c-MYC showed no difference in its expression but there was higher expression of p53 in SW48PKAD compared to SW48PK without cop which significantly decreased after 24 hours of cop at 50nM ($p<0.005$). p21, which is present downstream of p53 and helps induce apoptosis in both p53-dependent and independent manners, was also highly expressed without cop and decreased after 24 hours in SW48PKAD compared to SW48PK ($p<0.005$).

Conclusions: Co-alterations in *PIK3CA* and *ARID1A* lead to enhanced sensitivity to PI3K inhibition through PUMA induction by FOXO3a due to aberrant expression of p53/p21 caused by ARID1A loss. Further validation studies are underway utilizing PK and AD altered patient-derived cancer organoids.

#372 Loss of *RB1* sensitizes *TP53*-mutated cancer cells to WEE1 inhibition by azenosertib.

Daehwan Kim, Olivier Harismendy, Catherine Lee, Erika Cabrera, Danielle Jandial, Doris Kim, Mark R. Lackner, Jianhui Ma

Zentalis Pharmaceuticals, San Diego, CA

The retinoblastoma protein 1 (RB1), encoded by *RB1* gene, plays a critical role in regulating the G1 to S-phase transition of the cell cycle through its interaction with the E2F family of transcription factors. In addition, RB1 has been shown to directly regulate DNA damage response and apoptosis. Loss of RB1 function, predominately via gene deletion, occurs frequently in various human cancers, with the highest prevalence in small cell lung cancer (almost ubiquitous) and basal-like breast cancer (up to 72%). *RB1* loss is often associated with aggressive phenotypes, especially in cancers that harbor concurrent *TP53* mutations. In *TP53* mutated cancer cells, further loss of *RB1* can drive uncontrolled entry into S phase, leading to increased replication stress and DNA damage. WEE1 kinase regulates essential cell cycle checkpoints, preventing premature mitosis and allowing time for DNA repair. Therefore, we hypothesized that loss of *RB1* would sensitize *TP53*-mutant cancer cells to the selective WEE1 inhibitor azenosertib. We tested this hypothesis using small cell lung cancer (SCLC) and triple-negative breast cancer (TNBC) models. In *TP53*-mutant SCLC (N=9) and TNBC (N=3) cell lines, concurrent *RB1* loss increased sensitivity to azenosertib, as evidenced by greater cell growth inhibition compared to cells with wild-type *RB1* (median GR_{max} = -0.23 vs -0.81, 0.01 vs -0.55, respectively). Knockdown of *RB1* in *RB1* wild-type DMS53 (SCLC) and MDA-MB-231 (TNBC) cell lines sensitized them to azenosertib (GR_{max} = -0.47 vs -0.94, -0.02 vs -0.5, respectively). Downstream signaling analysis revealed that azenosertib treatment induced higher levels of DNA damage and apoptosis, as shown by the significant increase in γH2AX and cleaved-Caspase 3 levels in *RB1* knockdown cells compared to control cells. Conversely, overexpression of *RB1* in *RB1*-null cell lines significantly desensitized the cells to azenosertib with GR_{max} increased from -0.81 to -0.45 in NCI-H1048 (SCLC), and from -0.63 to -0.34 in MDA-MB-468 (TNBC), respectively. Consistently, *RB1* overexpressed cells demonstrated lower levels of DNA damage and apoptosis after azenosertib treatment. In xenograft models, azenosertib demonstrated enhanced tumor growth inhibition (TGI) in models with both *TP53* and *RB1* loss (NCI-H146 [SCLC]: 90%, MDA-MB-468 [TNBC], 87%) compared to those with *TP53* mutation alone (DMS53 [SCLC]: 49%, MDA-MB-231 [TNBC] 50%). Together, our data demonstrates loss of *RB1* sensitizes *TP53*-mutant cancer cells to WEE1 inhibition in multiple models of SCLC and TNBC, suggesting *RB1* loss can be used as a predictive marker to identify *TP53*-mutated patients of these cancers who are likely to benefit from WEE1 inhibition by azenosertib.

#373 Comparison of the anti-cancer mechanism of tolfenamic acid and its copper (II) complex.

Hayden Flume[1], Riva Sarkar[2], Tanaya Washington[3], Ethan Schroeder[3], Sakariyau A. Waheed[3], Kaylin L. Thomas[3], Stephen Beebe[3], Riyaz Basha[1], Alvin Holder[3], Umesh T. Sankpal[1]

[1]Texas College of Osteopathic Medicine, University of North Texas Health Science Ctr., Fort Worth, TX,[2]George Mason University, Fairfax, VA,[3]Old Dominion University, Norfolk, VA

Tolfenamic acid (TA), a non-steroidal anti-inflammatory drug, has demonstrated anti-cancer properties across multiple cancer models including prostate, pancreatic, ovarian, colon, leukemia, lung, and medulloblastoma. Studies have identified potential targets of TA including transcription factors Sp1, Sp3 and Sp4, anti-apoptotic proteins survivin and Bcl2, anti-angiogenic protein VEGF, and NFkB gene products. These interactions result in inhibition of cell proliferation, induction of apoptosis, and suppression of tumor growth in mouse models. To enhance the efficacy of TA, a novel copper (II) complex of TA ($[Cu(TA)_2(bpy)]$, bpy = 2,2'-bipyridine, Cu(II)-TA) was synthesized. Cell viability assays revealed that Cu(II)-TA exhibited greater anti-proliferative activity than TA, as indicated by lower IC_{50} values. However, the mechanism underlying this increased activity remains unclear. In this study, we compared the mechanism of action of TA and Cu(II)-TA in medulloblastoma cells. The anti-proliferative activity of the two compounds was assessed using a cell viability assay and the apoptotic activity was evaluated by caspase 3/7 activity assay. Cell cycle effects were analyzed by flow cytometry and DNA damage was assessed by measuring levels of phospho-H2AX using western blot and immunofluorescence microscopy. Consistent with previous findings, we observed increased anti-proliferative effects with Cu(II)-TA compared to TA in medulloblastoma cells. This enhanced activity was accompanied by increased apoptosis as indicated by higher caspase 3/7 activity. Cell cycle analysis by flow cytometry revealed distinct profiles for the inhibitors. TA treatment resulted in increased G2/M population, while Cu(II)-TA did not show a prominent or dose-dependent increase with treatment. Interestingly, TA induced greater DNA damage than Cu(II)-TA as indicated by increased phospho-H2AX levels detected by western blotting and immunofluorescence staining. These findings suggest that while Cu(II) enhances the anti-proliferative activity of TA, the underlying mechanisms differ, likely involving distinct proteins and pathways.

#374 OMX-0407 - A spectrum selective kinase inhibitor shows preclinical efficacy in RCC as well as sarcomas.

Ilona Petra Maser, Carolin Strobl, Bettina Bauer, Andreas Schirmer, Moritz Zulley, Carmen Amerhauser, Marisa Stebegg-Wagner, Tiantom Jarutat, Hannes Loferer, Stefan Bissinger

iOmx Therapeutics AG, Martinsried/Planegg, Germany

OMX-0407 is an orally available spectrum-selective kinase inhibitor targeting key oncology-relevant tyrosine kinases and the salt-inducible kinase family. OMX-0407 offers a dual mode of action (MoA) in cancer by inducing cell cycle arrest in tumor cells and sensitizing the tumor environment to immune cell-mediated tumor cell killing. It is being developed as first-in-class treatment for solid tumor indications with high unmet medical need.

Key oncogenic signaling pathways of tyrosine kinases drive cancer progression by regulating cell cycle and growth signaling cascades. Dysregulation of these kinases, such as platelet-derived growth factor receptors, Eph receptors, and Src family kinases, contributes to cancer progression and therapy resistance. OMX-0407 effectively disrupts these processes through potent inhibition of these kinases and their downstream signaling cascades. A comprehensive viability screen of over 380 human cancer cell lines revealed a selective anti-tumor activity across a subset of cancers, including in renal cell carcinomas (RCC) and sarcomas.

Pharmacodynamics studies via phospho-proteomics, Simple-Western or functional kinase activity assays revealed that OMX-0407 induces a G1 cell cycle arrest, accompanied by dose-dependent downregulation of cell cycle-associated proteins and kinase signaling pathways. These effects have been confirmed across in vitro cell line studies, in vivo cell line-derived models or even patient-derived xenograft models.

Besides its direct effect on tumor cells, OMX-0407 is repolarizing the tumor microenvironment by reducing immunosuppressive regulatory T cells in the tumor bed and shifting toward a pro-inflammatory state. This dual MoA contributes to the potent anti-tumor effects in various syngeneic animal studies. In a human patient-derived xenograft of epithelioid angiosarcoma (AS), derived from a 9-year-old patient, OMX-0407 demonstrated remarkable dose-dependent anti-tumor efficacy as a single agent. The strong anti-tumor efficacy, with tumor reduction in individual animals, was associated with dose-dependent downregulation of phosphoproteins and significant inactivation of key OMX-0407 regulated signaling pathways on kinase level. Clinical data from a patient, diagnosed with secondary radiation-induced AS, resistant to prior lines of chemotherapy, demonstrated that OMX-0407 was well tolerated and achieved a complete response at doses of up to 60 mg twice daily, which remains ongoing at 14 month.

These findings support OMX-0407 as a promising first-in-class therapeutic candidate for the treatment of AS and RCC, with a dual mechanism combining direct anti-tumor effects with repolarization of an immunosuppressive tumor microenvironment. As of September 2024, Ph1b expansion cohorts of the first-in-human trial (NCT05826600) were initiated for patients with unresectable or metastatic AS or clear cell RCC.

#375 Cancer cells may utilize centrosome amplification to promote survival as a response to chemotherapeutic stress.

Madison M. Purkerson, Sarah R. Amend, Kenneth J. Pienta

Johns Hopkins University School of Medicine, Baltimore, MD

Therapy resistance is one of the most common underlying causes of poor prognosis in cancer patients, ultimately driving fatal outcome. We have previously demonstrated that following chemotherapeutic stress, a subset of cancer cells undergo a mitotic skip and enter an endocycle, causing whole-genome duplication without division. This endocycling cell state is resistant to cytotoxic therapies and thus is an actuator of therapy resistance. Cells in the endocycling state eventually undergo depolyploidization and repopulate the tumor, observed clinically as a recurrence. A common feature of cancers is centrosome amplification (CA) that includes aberrations in centrosome number (>2 centrosomes/cell), size, shape, and/or positioning. Our preliminary data has shown that endocycling cancer cells exhibit CA following treatment across multiple types of chemotherapies. Based upon this data, we hypothesize that studying centrosome dynamics and abnormalities presents a window into better understanding endocycle biology and this novel mechanism of therapy resistance.We have found that the population of prostate cancer and breast cancer cells possessing CA following treatment with LD50 dose of cisplatin or docetaxel increased as a function of recovery time to over 95% by day 10, with nearly all cells clustering extra centrosomes together. Of note, the number of mitotic skips an endocycling cell undergoes has not been found to be associated with the number of centrosomes the cell has. PLK4 is a kinase essential for centriole duplication and its overexpression causes CA. Immunofluorescence analysis has revealed that endocycling cancer cells exhibit an increase in PLK4 protein expression. Endocycling cancer cells also exhibit increased protein expression of KIFC1, a mediator of centrosome clustering. Both PLK4 and KIFC1 protein expression have been found to positively correlate with DNA content. We hypothesize that endocycling cells may use clustered, supernumerary centrosomes as platforms to locally concentrate signaling molecules, lowering the activation threshold for downstream effectors. Published data from bioID proteomics of centrosomal proteins has revealed an enormous unexplored protein landscape around these organelles. Because of this, CA may be a critical mechanism for endocycling cancer cells to survive and maintain this resistant cell state.

#376 Characterization of PARP inhibitor response in prostate tumor cells reveals drug tolerant persistent phenotype.

Akshaya Karthikeyan, Love A. Moore, Bryan Correa Gonzalez, Anamitra Bhaumik, Ethan Sandoval, Alan P. Lombard

UCD School of Medicine & Medical Science, Davis, CA

Background: PARP inhibitors (PARPi) have improved management of prostate cancer, but acquired resistance and progression remain inevitable. Understanding treatment failure will improve outcomes. Drug tolerant persistence (DTP) is characterized by a small population of tumor cells which survive treatment through transient acquisition of insensitivity. DTP cells are not intrinsically resistant but may slowly proliferate, allowing for mutation accumulation, development of resistance, and progression. DTP cells may also increase mutability, further driving this process. Characterization of drug tolerant persistence and mechanisms enabling a DTP state under PARP inhibition remain poorly understood in prostate cancer.

Methods: Viability assays determined efficacious doses of olaparib and rucaparib in PARPi sensitive C4-2B metastatic castration-resistant prostate cancer cells and the C4-2B abiraterone-resistant derivative cells, AbiR. Phase contrast microscopy, western blots, beta-galactosidase activity assays, and flow cytometry characterized response to olaparib and rucaparib. RNA-seq determined the transcriptomic profile of PARPi DTP cells.

Results: C4-2B and AbiR response to olaparib and rucaparib is heterogeneous, characterized by cell death and cytostasis. Despite prolonged exposure to high dose PARPi, we observe a small minority of cells which persist, display varying altered morphologies, and altered cell cycle dynamics. While most of these cells appear arrested, we consistently note the presence of a subset which form colonies. C4-2B and AbiR cells exposed to high PARPi dosing for 9 days followed by drug holiday regain normal, parental cell morphology and become re-sensitized to treatment in line with acquisition of a DTP phenotype. RNA-seq reveals transcriptomic characteristics of drug tolerant persistence including evidence for increased mutability, altered metabolism including fatty acid metabolism and lysosomal activity, altered inflammatory signaling, and cell identity changes.

Conclusions: Our data suggest that transient, drug tolerant persistence may mediate survival of a minority of tumor cells via modulating metabolic and immune-associated pathways. Overtime, these persisters may acquire resistance and fuel progression due to increased mutability via down-regulation of DNA repair mechanisms. Future efforts will characterize this phenotype further and investigate vulnerabilities that target survivors to improve treatment efficacy.

#378 Nicotine replacement therapy may interfere with cisplatin efficacy and promote tumor progression.

Gautham Chengizkhan[1], Vengatesh Ganapathy[1], Mayilvanan Chinnaiyan[1], Lurdes Queimado[2]

[1]Department of Otolaryngology - Head and Neck Surgery, University of Oklahoma Health Sciences Center, Oklahoma City, OK,[2]Dept. of Otolaryngology-Head & Neck Surgery, Cell Biology, TSET Health Promotion Research Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK

Background: Smoking during cancer treatment is associated with significant risks, including treatment failure, metastasis, recurrence, and reduced quality of life. Continued smoking during therapy remains the strongest adverse predictor of survival in cancer patients. Nicotine replacement therapy (NRT) is commonly used to manage cravings in cancer patients undergoing treatment. Although nicotine has been proposed to promote cellular proliferation and tumor progression, the impact of NRT during cisplatin treatment has not been investigated.
Methods: Human oral cancer cell lines (WSU-HN6 and WSU-HN30) were subjected to various treatment combinations to explore the effects of NRT on cisplatin efficacy. Cells were first exposed to nicotine (10, 20, or 40 ng/mL, consistent with plasma concentrations) for 48 hours, followed by treatment with cisplatin at its 50% inhibitory concentration (IC50) in the presence or absence of nicotine for an additional 48 hours. Then, cell viability was assessed using the MTT assay, and clonogenic survival of single cells was evaluated. Controls included exposure to nicotine only, cisplatin only, and mainstream cigarette smoke. Additionally, cells were exposed to varying concentrations of nicotine, treated with cisplatin, and then fixed with 70% ethanol, and analyzed by flow cytometry for cell cycle progression. Statistical significance between groups was determined using Student's t-test.
Results: Cisplatin treatment of cancer cells exposed to nicotine at concentrations similar to those observed in the plasma of individuals undergoing NRT, resulted in a significant decrease in cell viability but did not achieve the anticipated 50% reduction observed with cisplatin treatment alone. To explore this further, cells were exposed to nicotine for 48 hours, followed by cisplatin treatment for an additional 48 hours in the presence or absence of nicotine. The presence of nicotine during cisplatin treatment significantly impaired the efficacy of cisplatin to induce cancer cell death in a concentration-dependent manner. The nicotine presence during cisplatin treatment also altered cell cycle progression and increased clonogenic survival in both oral cancer cell lines.
Conclusion: This study shows that nicotine, even at very lower concentrations, significantly impairs the efficacy of cisplatin, as evidenced by increased cell viability and altered cell cycle progression and increases clonogenic survival in both oral cancer cell lines. These findings pinpoint the potential of NRT to compromise cancer treatment outcomes by interfering with the cytotoxic effects of cisplatin and increasing the number of cancer cells able to proliferate indefinitely. Addressing nicotine exposure during cancer therapy is critical for improving treatment efficacy and patient outcomes.
Grant Support: NIH/NCI (R01CA242168, Queimado); TSET HPRC (Queimado).

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#38 Augmenting drug responses to PARP inhibitors in high grade serous cancer with inhibitors of the anti-apoptotic protein Bcl-XL.

Alla Buzina, Wiebke Schormann, Lilian Gien, Helen MacKay, **David W. Andrews**

Sunnybrook Health Sciences Centre, Toronto, ON, Canada

While most patients with high grade serous ovarian cancer (HGSC) respond to platinum-based chemotherapy, the response is rarely durable and recurrence almost inevitable. A characteristic of HGSC is defective DNA repair. A class of drugs called PARP inhibitors (PARPi) exploit this vulnerability and have proven useful in delaying recurrence. However, resistance is inevitable. In models of HGSC a protein that frequently confers resistance is called Bcl-xL, a member of the Bcl-2 family of proteins that prevent apoptosis. If treating the patients with a PARPi makes cancer cells dependent on Bcl-xL then adding an inhibitor of Bcl-xL to their treatment might overcome resistance to Olaparib and cause the cancer cells to die.
To test this, we have initiated companion studies for a clinical trial in which patients that have had a recurrence after receiving platinum-based therapy will first be treated with the PARPi Olaparib and then, an inhibitor of Bcl-xL, Navitoclax, will be added to their course of treatment. Navitoclax has a well-known on-target side effect of thrombocytopenia. We show that with continuous dosing of Navitoclax, platelet counts recover and are maintained. Our preliminary data from the first few patients enrolled in the trial suggest that the combination is well tolerated and for several participants there was a reduction in tumor burden. However, Bcl-xL is only one of the five known inhibitors of apoptosis. To identify which women will benefit most from adding Navitoclax to their treatment we need a biomarker(s).
Our hypothesis is that patient derived organoids can be used as a pragmatic way to identify for patient cohorts which Bcl-2 protein inhibitor will synergize with a PARPi to optimize treatment.
By combining novel methods for cell aggregation and hydrogel based synthetic ECM supports we can reproducibly generate HGSC patient-specific tumor organoids models in weeks with greater than 90% success. Organoids grown 384 well format are stained with novel non-toxic dyes enabling live cell painting of chemoresponses to drugs alone and in combination with drugs targeting anti-apoptotic proteins. Our data suggest that this approach captures the inherent heterogeneity of the disease, albeit local to the sampled site. We are employing deep learning AI algorithms to enable automated analyses of 3D confocal image stacks of organoids to infer drug responses that will be compared to patient responses in ongoing clinical trials.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Degraders and Glues 1
Poster Session

#382 TRX-214-1002, An antibody drug conjugate (ADC) targeting CD33 with a novel GSPT1 molecular glue for treatment of acute myeloid leukemia.

H. Lee, S. Kim, D. Kim, S. Choi, J. Lee, S. Lee, E. Choi, S. Park, K. Chun, K. Lee;
Therapex, Seoul, Korea, Republic of

Several promising therapies for acute myeloid leukemia (AML) targeting specific genetic and epigenetic mutations have been reported. However, there remains a critical need for novel therapies with higher efficacy and favorable safety profiles particularly for patients with poor-risk cytogenetic features, such as TP53 mutation, or those with relapsed and refractory AML. G1 to S phase transition 1 (GSPT1) is a small GTPase that interacts with eRF1, the translation termination factor, to facilitate the process of translation termination. GSPT1 has been well known to play a critical role in the proliferation of AML and MYC-driven lung cancer. Recently, molecular glue degraders (MGDs) targeting GSPT1 have emerged as a new class of cancer therapies and have shown early promise in clinical trials for AML. However, clinical development of GSPT1 MGDs, such as CC-885 and CC-90009, has been hampered by off-target toxicities. To improve both target specificity and the therapeutic window, we discovered TRX-214-1002, a CD33-targeting ADC that used the same monoclonal antibody (mAb) as Mylotarg, conjugated with a novel proprietary GSPT1 MGD as the payload. The *in vitro* and *in vivo* pharmacology of TRX-214-1002 were compared with ORM-6151 (another GSPT1 MG degrader-based CD33 ADC) and Mylotarg. TRX-214-1002 demonstrated superior and sustained degradability against GSPT1 compared to ORM-6151, and it exhibited potent anti-leukemic activity with picomolar GI_{50} values in TP53 or FLT3 mutated AML cell lines. Importantly, it highly spared CD33-negative leukemia cell and human PBMC, with an *in vitro* therapeutic index greater than 100-fold. We also observed that TRX-214-1002 significantly upregulated integrated stress response markers (ATF4, CHOP, ATF3) and impaired neo-protein synthesis, consistent with apoptosis induction. Interestingly, TRX-214-1002 maintained potent anti-leukemic activity even in venetoclax-resistant HL-60 cells and in drug-resistant milieu derived from bone marrow stromal HS-5 cells. Furthermore, a single 1 mg/kg dose of TRX-214-1002 completely regressed tumors in MV4-11 and HL-60 xenograft models without tumor regrowth until day 42, whereas the ORM-6151-treated group led to transient tumor regression. Pharmacodynamic studies in the HL-60 tumor model showed dose-dependent degradation of GSPT1. In addition, we estimated that the minimum therapeutically effective dose was less than single 1 mg/kg in a disseminated tumor model. Collectively, TRX-214-1002 highlighted the noticeable GSPT1 degradability and enhanced antileukemic activity, particularly in TP53 mutated and venetoclax-resistant AML cell lines, along with significant antitumor activity superior to competitor ADCs. Our preclinical data suggest that TRX-214-1002 has the high potential to provide a novel treatment strategy for CD33 positive relapse/refractory AML patients.

#383 Discovery of potent, selective, and orally bioavailable GSPT1 molecular glue degraders (MGDs) for the treatment of MYC-driven tumors.

Zhimin Zhang, Min Chen, Cancan Cai, Li Teng, Sirui Liu, Yujie Chang, Xinxin Jin, Xiaohua Zhang, **Dongzhou Liu**

Huadong Medicine Co.,Ltd, Hangzhou, China

Background: MYC is a well-recognized proto-oncogene, which is mutated and overexpressed in various tumors. However, MYC is challenging to target due to its disordered structure and nuclear localization. There is a significant unmet clinical need for the effective treatments for MYC-driven tumors. G1 to S phase transition 1 (GSPT1) is a translation termination factor regulating protein expression. GSPT1 is involved in many biological processes, including the regulation of cell proliferation, migration, growth cycle and apoptosis. MYC-driven tumors are highly dependent on protein translation to sustain their growth, creating a reliance on the GSPT1 activity. Recent studies have shown that molecular glue degraders (MGDs) targeting GSPT1 affect protein synthesis, thus interfering with the expression of MYC and its downstream target genes. Therefore, GSPT1 is a potential therapeutic target for MYC-driven tumors.
Results: Through a comprehensive artificial intelligence-driven drug design and structure-based approach, we designed and synthesized a series of GSPT1 degraders. The most potent degrader effectively degrades GSPT1 proteins in the MDA-MB-468 cells in a dose-dependent manner and achieves a DC_{50} value of less than 2 nM. The maximum degradation (D_{max}) value observed is >95%. The degradation of GSPT1 induced the associated downregulation of MYC (EC_{50}<10 nM), resulting in preferential anti-proliferative activity in MYC-driven cancer cells with IC_{50} values of 3.28 nM in MDA-MB-468 cells and 0.82 nM in NCI-H1155 cell lines. PK studies revealed low clearance, high plasma exposure, and oral bioavailability in mice. Furthermore, the MGD induced robust and statistically significant tumor growth inhibition (TGI) in the NCI-H1155 and MDA-MB-468 Xenograft Models, with TGI values of 99.57% and 97.93%, respectively.
Conclusion: A novel, selective, potent oral GSPT1 degrader was discovered and demonstrated robust anti-tumor activity in mouse xenograft tumor models as a single agent. Collectively, we have identified novel GSPT1 MGDs as promising candidates for the treatment of MYC-driven tumors.

#384 IPS-06061, a novel TPD molecular glue degrader targeting KRAS G12D.

Incheol Kang[1], Seungoh Seo[2], Seong Hyeok Cho[1]

[1]InnoPharmaScreen/Hoseo University, Incheon, Korea, Republic of,[2]InnoPharmaScreen, Incheon, Korea, Republic of

We have discovered a novel KRAS G12D-degrading Molecular Glue using IPS's cutting-edge ProGlu AI-based drug discovery platform and PPIExplorer, an IPS-Chip based Proteomics technology. ProGlu accurately predicts PPI between E3 ligase and KRAS G12D through MD simulation, enabling de novo design of MG degraders. One such degrader, namely IPS-06061, was successfully synthesized and confirmed a strong anti-tumor efficacy using a xenograft animal model. These data demonstrate that KRAS MG will be a promising new therapeutic modality for the treatment of PDA, CRC, and NSCLC.

#385 Discovery of OST08267, an orally available PDE3A-SLFN12 molecular glue for the treatment of gastrointestinal stromal tumors with tyrosine kinase inhibitors (TKIs) resistance.

F. Wu, L. Deng, Z. Ma, Z. Lu, G. Deng;
Open Source Therapeutics, Suzhou, China

Gastrointestinal stromal tumor (GIST) is the critically mesenchymal malignancy inhabiting the GI tract, which crucially induced by receptor tyrosine kinase (KIT) gene mutation. Although the tyrosine kinase inhibitors (TKIs), such as imatinib has prolonged survival significantly, the intrinsic and acquired resistance still disturbed more than 20% patients. Therefore, significant unmet medical remains needed. phosphodiesterase 3A (PDE3A)- Schlafen family member 12 (SLFN12) molecular glue is a novel anti-tumor targeted drug, mainly by mediating the formation of a complex between PDE3A and SLFN12, ultimately leading to the tumor apoptosis. In this study, we present OST08267, an orally available PDE3A-SLFN12 molecular glue tailored from the FDA-approved PDE3A inhibitor. Our transcriptional profiling of over 2,000 patient samples revealed high expression levels of PDE3A and SLFN12 in GIST tumors, suggesting that GIST is a promising indication for this treatment with a high responsive rate of up to 60%. In vitro, OST08267 demonstrated significant anti-tumor activity, irrespective of KIT mutation status by testing against multiple GIST patient-derived organoids. In vivo, OST08267 causes dose-dependent regression of tumor volume in the GIST patient-derived xenograft (PDX) model with imatinib resistance, with 4 mg/kg/day dose resulting in deeper and durable tumor regressions that were maintained for at least a month even after the dosing had discontinued.Moreover, OST08267 exhibited favorable pharmacokinetic and toxicity properties. These promising preclinical results strongly support the potential of OST08267 to address unmet needs in GIST treatment, justifying its further clinical development.

#386 High CRBN expression in neuroendocrine cancer creates a vulnerability to GSPT1 molecular glue degraders: Target indication selection for CYRS1542 development.

Jaewoo Park[1], **Min Sung Joo**[2], JaeYung Lee[2], Eun-Jung Kim[2], Dong Hyuk Ki[2], Hunmi Choi[2], Wooseok Han[2], Keon Wook Kang[1]

[1]Seoul National University, Seoul, Korea, Republic of,[2]Cyrus Therapeutics, Seoul, Korea, Republic of

Multiple companies have been developing GSPT1-targeted molecular glue degraders (MGDs) for neuroendocrine (NE) cancers, a rare and aggressive cancer type. CYRS1542, a potent and orally bioavailable GSPT1 MGD with a superior therapeutic index, was identified and derived from CYRS381 (originally known as SJ6986) in our previous work (Joo et al., AACR 2024). In this study, we investigated the mechanistic basis of GSPT1 MGD sensitivity in NE cancers, using CYRS381 as a tool compound to assess selective susceptibilities. We found that CYRS381-induced GSPT1 degradation was selectively effective in the NE cancers with high CRBN expression, while the non-NE cancers with a low level of CRBN showed limited response. NE cancer cells positive for NE markers exhibited both higher CRBN expression and greater sensitivity to CYRS381, showing rapid GSPT1 degradation upon GSPT1 MGD treatment compared to non-NE cancer cells. Further analysis of TCGA and DepMap expression data revealed a significant positive correlation between high CRBN levels and NE characteristics, whereas GSPT1 levels showed no such correlation. Consistently, overexpressing CRBN in non-NE cancer cells increased sensitivity to GSPT1 MGD, and introducing NE-driving factors in non-NE cancer cells elevated CRBN expression, further enhancing the response to GSPT1 MGD. Finally, CYRS1542 demonstrated effects similar to CYRS381 in comparable experiments, further supporting the selection of NE cancers as the lead indication for its clinical trials. Overall, these findings highlight CRBN as a key determinant of GSPT1 MGD efficacy and underscore the potential of CYRS1542 as a targeted therapeutic option for aggressive NE cancers.

#387 Preclinical biological screening and evaluation of KRAS molecular glues.

Zhaoxia Yin, Lili Chai, Xian Wu, Tao Li, Xiaojian Wang, **Tj (Tiejun) Bing**

ICE Bioscience, Inc., Beijing, China

The KRAS protein is a signal transduction molecule associated with various types of cancer. Due to its high mutation rate and key role in tumor development, KRAS has become an important target for anti-cancer drug development. Activating mutations in KRAS, such as KRASG12C, are prevalent in about one-third of all human cancers and significantly contribute to tumor initiation, progression, and resistance to therapy. In recent years, with the continuous deepening of research related to KRAS, a new therapeutic strategy targeting the KRAS protein has been discovered, known as KRAS molecular glue. KRAS molecular glue specifically binds to particular mutation sites on the KRAS protein, such as the G12C mutation, thereby preventing its interaction with downstream signaling molecules and inhibiting the proliferation and survival of tumor cells.

In this context, we have developed a comprehensive screening and evaluation platform for KRAS molecular glue, covering studies from in vitro to in vivo. The platform includes a series of assays at both the biochemical and cellular levels. It utilizes biochemical and biophysical methods for the screening of POI binders, detection of KRAS(ON)/CypA/cRAF binary complexes, and KRAS(ON)/CypA ternary complexes, among others. Cell-based functional assays such as 3D cell proliferation and detection of ERK phosphorylation, a marker related to signaling pathways, are also included. Additionally, we offer p-ERK panel and 2D/3D cell panel including pan KRAS cell panel, KRAS G12D cell panel, KRAS G12C cell panel, KRAS G12V cell panel, pan NRAS cell panel and pan HRAS cell panel tests for KRAS molecular glue, and we can also supply KRAS G12C mutation-resistant cell lines for related testing. Of course, it also includes the efficacy assessment of the CDX model in vivo. Overall, we provide a comprehensive platform for the screening and evaluation of KRAS molecular glue, offering a scientific basis for the clinical application of KRAS molecular glue.

#388 HZ-V068, an oral, highly potent pan-Ras molecular glue inhibitor demonstrated robust potency in pancreatic ductal adenocarcinoma and colorectal cancer models.

Yizhe Wu, Jiangfeng Xie, Jinglai Huang, Xinglu Zhou

HealZen Therapeutics Co., Ltd., Hangzhou, China

Introduction: Ras oncogenes are frequently mutated in approximately 30% of all human cancers, which drive tumor growth and metastasis. The current approved Kras-targeted drugs are Kras G12C inhibitors. However, Kras G12C is only prevalent in part of lung cancer. Most of common mutations in pancreatic ductal adenocarcinoma (PDAC) or colorectal cancers (CRC) including Kras G12D, G12V, G12R are still lack of approved drugs. RMC6236, a next generation of novel pan-Ras^{Multi}(on) inhibitor which functions as molecular glue to form binary complex with CYPA and Ras^{Multi} and sterically blocks Ras binding to effectors, has been validated as a superior strategy targeting Ras in clinical study. Given GTP-bound RAS proteins as a promising new approach to enhance clinical benefits, we have developed a novel oral available pan-Ras molecular glue——HZ-V068 based on in-house Rational Molecular Glue Design Platform, which exhibits significantly binding affinity to CYPA, enriched tumor exposure and prolonged Ras inhibition. HZ-V068 has been validated as a promising pre-clinical candidate for further clinical development.

Results: 1. In biochemical assays, HZ-V068 more potently disrupted the binding of $KRAS^{Mut}$(On) to BRAF in a CypA-dependent manner. 2. HZ-V068 suppressed the growth of $Kras^{Multi}$-BaF3 cell-line covering various mutation types (G12C/D/V/R, G13C/D, Q61K, A146V) at picomolar level. Moreover, HZ-V068 demonstrated 5-10 folds stronger potency in $Kras^{Multi}$-addicted cancer cell lines compared to RMC-6236. 3. In a single-dose PK/PD study, HZ-V068 demonstrated rapid and more sustained intratumoral pERK inhibition, improved bioavailability, and enhanced tumor distribution. 4. In multiple KrasMulti-driven PDAC or CRC CDX models, oral administration of HZ-V068 results in robust tumor regression at lower doses, demonstrating efficacy that is significantly superior to that of RMC6236

Conclusion: In summary, HZ-V068 is a promising targeted therapeutic option for PDAC and CRC. Based on these promising results, we are advancing HZ-V068 into clinical study.

#389 GFH276: A molecular glue panRAS(on) inhibitor with broad spectrum anti-tumor activities.

Feng Yan, Jichen Zhao, Jingyang Zhang, Hongcan Ren, Siyuan Le, Fusheng Zhou, Jiong Lan, Qiang Lu

GenFleet Therapeutics, Shanghai, China

KRAS is the most frequently mutated oncogene but had long been considered to be undruggable until the approval of the first KRAS G12C inhibitor Sotorasib for NSCLC treatment in 2021. However, the clinical performance of Sotorasib and other SIIP-based KRAS inhibitors is hindered by their susceptibility to adaptive resistance and rapidly emerging, diverse acquired resistance. Therefore, developing a novel RAS inhibitor being able to overcome the above limitations holds the potential of making the most of RAS-targeted therapies. Here we report the characterization of a preclinical candidate molecular glue panRAS(on) inhibitor- GFH276, designed to target multiple RAS mutants of active, GTP-bound state by recruiting intracellular cyclophilin A (CypA) protein. In biochemical assays, GFH276 disrupted the binding of GMPPNP-bound KRAS G12C, G12D or G12V mutants or wild type KRAS, HRAS or NRAS proteins to RAF1 in a CypA-dependent manner. In cellular assays, GFH276 suppressed phosphor-ERK1/2 (p-ERK) level in KRAS G12C, G12D and G12V-mutated cells with IC50s < 1 nM, and demonstrated potent tumor-suppressive effect in a panel of cell lines carrying different KRAS (G12A, G12C, G12V, G12R, G12S, G13D or Q61K), NRAS (G12D) or HRAS (Q61H) mutations, with IC50s of 0.07-73 nM. In addition, GFH276 was also active in tumor cell lines carrying EGFR or FGFR2/3 alterations but showed no activity in cell lines carrying mutations on downstream BRAF component. In KRAS-mutated xenograft models, oral administration of GFH276 elicited rapid and deep inhibition on p-ERK level in tumor tissues, demonstrated dose-dependent anti-tumor activity and induced tumor regression at dose level as low as 1 mg/kg QD. Compared to SIIP-based KRAS G12C inhibitors Sotorasib, Adagrasib and Divarasib, GFH276 was not susceptible to upstream RTK-reactivation by EGF stimulation and remained effective against cell lines carrying second KRAS mutations or RTK alterations or with induced resistance to Sotorasib. GFH276 showed no off-target activity in 72-kinase selectivity and SafetyScreen44 panel tests. GFH276 showed high bioavailability in mice, rats and dogs (39%-113%). Taken together, GFH276 represents a new generation of RAS inhibitors that are superior to the mainstream SIIP-based KRAS inhibitors in overcoming adaptive and acquired resistance. GFH276 demonstrated strong therapeutic activities in tumors with activating RAS and RTK alterations. Encouraged by these promising results, we are advancing GFH276 into the Phase 1 clinical trial by conducting IND-enabling studies and the IND submission is planned in Q2 2025.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#39 Digital pathology foundation models enable accurate tumor organoid classification.

Adi Orlyanchik[1], Matteo Sacco[1], James Dolezal[2], Joseph Kainov[1], Piao Zhao[1], Mohammed Aziz Khan[1], Marina Garassino[1], Marc Bissonnette[1], Le Shen[1], Alexander T. Pearson[1], Christopher Weber[1]

[1]BSD, The University of Chicago Medicine Comprehensive Cancer Center, Chicago, IL,[2]Geisinger Cancer Institute, Danville, PA

This study develops an efficient and accurate pipeline for classifying tumor-derived organoid candidates as benign or malignant, addressing challenges posed by organoid culture and advancing personalized medicine applications.
Background: Tumor-derived organoids replicate tumor architecture and molecular features, providing controlled environments to study tumor behavior and therapeutic responses. A key challenge in using organoid models is classifying cellular aggregates as benign or malignant. Benign cells in a tumor source can sometimes outcompete malignant tumor cells in culture, misrepresenting the tumor's characteristics. Also, histological characterization of organoids is challenging due to the absence of histologic clues from tissue organization. Current approaches, such as genomic sequencing, effectively resolve such uncertainties but are costly and time-intensive.
Hypothesis: We hypothesized that a deep learning (DL) based computational image analysis classification pipeline could improve the accuracy and efficiency of distinguishing organoids as benign or malignant.
Methods: We developed a novel pipeline to enhance organoid classification methods using ML. Our pipeline was developed and validated on H&E-stained slides obtained from organoids derived from 17 colorectal cancer patients (26 slides: 11 tumor, 15 normal) with various degrees of differentiation and paired normal controls. Ground truth data were established using a comprehensive genomic profiling panel to confirm that tumor organoids matched primary tumor features and, for tumors with high microsatellite instability, mismatch repair protein status. We first trained a segmentation model using a Human-in-the-Loop approach for precise identification of regions of interest. We extracted non overlapping 256x256 tiles at 20x magnification. We used the UNI foundation model to extract (k=1024) relevant features from each tile. We fitted Linear Discriminant Analysis (LDA) models on the UNI feature vectors using leave-one-patient-out cross validation according to patients. Each slide's final classification score was determined by averaging the LDA scores across all tiles from that slide.
Results: The LDA classifier achieved 92.3% accuracy and AUROC of 0.964 (0.907-1.000), matching gastrointestinal pathologist performance. Additionally, we visualized the UNI feature vectors through UMAP plots. Plot evaluation demonstrates distinct clustering of unsupervised features into tumor and normal groups.
Conclusions: These findings demonstrate the feasibility of coupling organoids with DL methods to facilitate organoid classification. Our approach offers an efficient new strategy for studying organoid morphology and advancing organoid models. Adaptations of this model are expected to be valuable in understanding organoid responses to various therapies, paving the way for improved personalized cancer care.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Degraders and Glues 1
Poster Session

#390 ERAS-0015 is a pan-RAS molecular glue with best-in-class potential in RAS mutant solid tumors.

Erin D. Lew, Alexei Brooun, Justine Lam, Joanne Oh, Taylor Sullivan, Corine Holub, Jae Hyun Bae, Bingzhen Lin, Brian Qua, Alex Stromitis, Sarah M. Griffith, Andrea Shen, Dawei Xuan, Robert F. Shoemaker

Erasca, Inc., San Diego, CA

As a key node of the canonical RTK-RAS-MAPK signaling cascade, the RAS family of GTPases plays an essential role in numerous physiological processes including cellular proliferation, migration, survival, and immune cell activation. However, under pathological conditions, oncogenic RAS mutations, particularly in hotspot codons 12, 13, and 61, affect RAS intrinsic hydrolysis and/or GEF-mediated exchange resulting in a shift from GDP-bound to GTP-bound RAS. This shift to the GTP-bound RAS state leads to recruitment of effector proteins through the RAS-binding domains (RBDs) and ultimately leads to prolonged intracellular activation and modulation of the tumor microenvironment (TME).
ERAS-0015 is a pan-RAS molecular glue with best-in-class potential designed to target RAS in the GTP-bound state and shut down cellular signaling mediated by both mutant and wild-type RAS. ERAS-0015 exhibited 3-7-fold more potent inhibition of cellular proliferation relative to RMC-6236, a RAS molecular glue comparator, across multiple RAS mutant cell lines harboring G12X/G13X/Q61X mutations. ERAS-0015's roughly 8-20-fold higher binding affinity to cyclophilin A (CYPA) is thought to contribute to its higher *in vitro* cellular potency as well as favorable ADME and PK properties resulting in a longer residence time and greater exposure in tumor tissues as compared to RMC-6236. These favorable properties enabled ERAS-0015 to achieve comparable tumor growth inhibition (TGI) relative to RMC-6236 at $1/8^{th}$-$1/10^{th}$ the dose across multiple KRAS mutant CDX models irrespective of histology or specific mutation.
ERAS-0015 demonstrated promising preclinical activity across multiple RAS mutant models supporting IND filing in 1H 2025.

#391 Pharmacological suppression of neuroblastoma growth via RBM39 degradation by the molecular glue PPI-011.

Yu Cao[1], Qilei Han[1], Penxuan Ren[1], Bin Zhang[2], Lin Wang[1], Xianglei Zhang[1], Lei Zhou[3], Song Feng[3], **James X. Rong**[3], Lianghe Mei[2], Kai Li[4], Fang Bai[1]

[1]School of Life Science and Technology, and Shanghai Institute for Advanced Immunochemical Studies, ShanghaiTech University, Shanghai, China,[2]Suzhou Institute of Drug Innovation, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Suzhou, China,[3]Peak Perform Innova Co., Ltd., Shanghai, China,[4]Department of Pediatric Surgery, Children's Hospital of Fudan University, Shanghai, China

High-risk neuroblastoma, often driven by the amplification or overexpression of MYC family oncogenes (*MYCN* and *MYC*), remains a significant cause of pediatric cancer mortality, contributing to 15% of tumor-related deaths in children. Despite multimodal treatment approaches, prognosis remains poor, underscoring an urgent need for new therapies. The current study targets the degradation of RBM39, an RNA splicing factor linked to splicing irregularities in MYC-driven tumors. We identified PPI-011, a proprietary molecular glue, using an AI-assisted integrated drug design platform. Demonstrated by the Time-Resolved Förster's Resonance Energy Transfer recruitment assay, PPI-011 promotes the formation of the ternary complex involving RBM39 and DDB1-DCAF15, a substrate adaptor in the RBX-CUL4-DDA1-DDB1-DCAF15 E3 ligase. PPI-011 induced dose-dependent degradation of RBM39 across multiple neuroblastoma cell lines, a mechanism confirmed by the inhibitory effects of a neddylation blocker and DCAF15 knockout, validating CUL4-DCAF15-mediated proteasomal degradation as the underlying pathway. RBM39 degradation resulted in splicing changes in multiple genes, including CDK4 and PEX3, and led to cancer cell death. Preliminary pharmacokinetic studies indicated that PPI-011 possessed good PK profiles, with oral bioavailability of ~100% and high exposure. In vivo, daily oral administration of PPI-011 (15 mg/kg) suppressed tumor growth by 90% ($P < 0.001$ vs. vehicle) in IMR-32 neuroblastoma xenografts in balb/c nude mice, with almost complete tumor elimination at 30 and 50 mg/kg within 28 days, and no associated weight loss at any dose. The suppressed tumor growth was associated with decreased RBM39 protein in the xenografts. Notably, tumor suppression at 50 mg/kg was sustained for 72 days even after treatment discontinued. Similarly, PPI-011 significantly ($P < 0.001$ vs. vehicle) inhibited the growth of LAN-5 xenografts, another neuroblastoma model, at doses of 3, 10, and 30 mg/kg in a dose-dependent manner. In a patient-derived xenograft model, using cells from a chemotherapy-relapsed neuroblastoma, PPI-011 achieved 88% tumor suppression ($P < 0.001$ vs. vehicle) at 30 mg/kg, and complete tumor clearance at 90 mg/kg within 21 days. Furthermore, PPI-011 has no apparent safety liabilities, supported by in vitro genetic toxicology and hERG assays, warranting further in vivo toxicological analysis. Our findings demonstrate that PPI-011, developed through an innovative drug design platform targeting previously undruggable targets, effectively inhibits neuroblastoma growth by inducing RBM39 degradation. This suggests PPI-011 as a promising new therapeutic strategy for MYC-driven malignancies, including neuroblastoma.

#392 Pyrimethamine inhibits NRF2^{Mut} in esophageal squamous cell carcinoma as a molecular glue.

Zhaohui Xiong[1], Chorlada Paiboonrungruang[1], Boopathi Subramaniyan[2], Candice Bui-Linh[1], Yahui Li[2], Huan Li[2], Francis Spitz[2], Xiaoxin Chen[1]

[1]Coriell Institute for Medical Research, Camden, NJ,[2]Cooper University Health Care, Camden, NJ

Esophageal squamous cell carcinoma (ESCC) is a deadly disease and one of the most aggressive cancers of the gastrointestinal tract. As a master transcription factor regulating the stress response, nuclear factor erythroid 2-related factor 2 (NRF2) is often mutated and becomes hyperactive, and thus causes chemo-radioresistance and poor survival in human ESCC. In our previous study, we have identified pyrimethamine (PYR) as an NRF2 inhibitor using a small molecule screening, and further validated it as an inhibitor of NRF2 expression in *NRF2^{Mut}* ESCC cells *in vitro* and *Nrf2^{E79Q}*-driven esophageal phenotype in mice. As a result, PYR has moved into a Phase I window of opportunity trial (NCT05678348). This study is aimed to investigate the molecular and phenotypic consequences of NRF2 hyperactivation in ESCC, and elucidate the mechanism of action of PYR. We first generated isogenic human ESCC cells through CRISPR-Cas9 knockout for comparison with its parental cells. RNAseq and Western blotting showed that NRF2^{Mut} overexpression downreulated the expression of squamous differential markers and upregulated the expression of proliferation markers, while NRF2^{WT} overexpression showed different patterns. Flow cytometry and live cell imaging further showed that NRF2 activation modulated cell cycle distribution and proliferation, but not apoptosis of human ESCC cells. PYR increased chemosensitivity and radiosensitivity in *NRF2^{Mut}* ESCC cells. Co-immunoprecipitation (Co-IP) and proximity ligation assay (PLA) suggested that PYR promoted NRF2-KEAP1 binding as a molecular glue in *NRF2^{Mut}* ESCC cells, but not in *NRF2^{WT}* ESCC cells. In summary, NRF2 hyperactivation differentially modulated squamous differentiation and cell proliferation in *NRF2^{Mut}* and *NRF2^{WT}* ESCC cells. PYR enhanced chemo-radiosensitivity by acting as a molecular glue specifically in *NRF2^{Mut}* ESCC cells, supporting PYR as a novel therapy for *NRF2^{Mut}* ESCC.

#393 Discovery of novel, potent and orally active GSPT1 molecular glue degraders.

Chi-Chung Chan, Chun-Sing Li, Lihong Hu, Wenxi Li, Zhenzhen Zhu, Shuhui Chen

Prospect Therapeutics, Shanghai, China

Molecular glue degrader (MGD) is an emerging therapeutic strategy offering an exciting alternative to traditional small molecules exploring new target space. These small molecules interact with the ubiquitin-proteasome system (UPS) to promote degradation of protein of interest (POI), especially those considered to be challenging targets (e.g., GSPT1, VAV1, WIZ, BCL6, STAT6, IKZFs). In fact, clinical proof of concept has been obtained with immunomodulatory drugs such as thalidomide, lenalidomide and pomalidomide, which degrade IKZF1/IKZF3. GSPT1 (G1 to S phase transition protein 1) is a challenging POI engaged in regulation of cell cycle progression at the G1-S transition and in mRNA translation termination, playing a key role in cancer progression. Recently, encouraging results have been obtained with different modalities targeting GSPT1. GSPT1 degrader antibody conjugates are now in clinical trials for breast cancer (ORM-5029) and AML (BMS-986497/ORM-6151). Of interest, an orally active GSPT1 degrader (MRT-2359) is in Phase 2 trial for MYC-driven tumors including NSCLC and SCLC, with 2 PR, 1 SD in six biomarker positive patients (September 2024). Safety profile at this stage supports further clinical development. Preclinically, MRT-2359 is a potent GSPT1 degrader with nanomolar *in vitro* IC50 and oral bioavailability of ~50%. It shows anti-tumor activity in NSCLC xenograft model starting at 1 mg/kg PO, QD. These encouraging results have prompted us to explore a series of GSPT1 MGDs.

In the present study, we describe the discovery of a series of novel, potent and orally active GSPT1 MGD targeting tumors with high GSPT1 expression. In the BT-747 human breast cancer cell assay, IC50s for serval compounds are between 5 - 50 nM, in the same range as that of MRT-2359 and other comparators in the same assay. Significant GSPT1 protein degradation of these compounds is observed showing on-target mechanism of action, without affecting protein degradation in a panel of proteins. A representative compound in the series shows a promising pharmacokinetic profile (PO at 3 mg/kg; IV at 1 mg/kg) with an oral bioavailability of 55 %, Cmax of 0.78 μM, t1/2 of 2.3 hour and Vdss of 1.82 L/kg in mouse. This compound demonstrates *in vivo* efficacy in CDX model with a favorable profile comparable to that of MRT-2359. In summary, a series of orally active GSPT1 degraders have been discovered with preclinical profile suitable for further development to manage cancers with high GSPT1 expression.

#394 Bridged proteolysis targeting chimera (PROTAC) enables degradation of the cyclin D undruggable protein.

Y. Zhong[1], Y. Xiong[1], H. Yim[1], X. Yang[1], K.-S. Park[1], L. Xie[2], P. Poulikakos[1], X. Chen[2], J. Liu[1], J. Jin[1];
[1]Icahn School of Medicine at Mount Sinai, New York City, NY, [2]University of North Carolina at Chapel Hill, Chapel Hill, NC

Proteolysis targeting chimeras (PROTACs) are attractive therapeutic modalities for degrading disease-causing proteins. While significant progress has been made in developing PROTACs for various protein targets, current small-molecule PROTAC approaches cannot target undruggable proteins lacking small-molecule binders. To overcome this limitation, we present a novel PROTAC approach, termed bridged PROTAC. This approach utilizes a small-molecule binder of the target protein's binding partner to recruit the protein complex into proximity with an E3 ubiquitin ligase, enabling the degradation of the target undruggable protein. Applying this bridged PROTAC strategy, we discovered MS28, the first-in-class degrader of cyclin D1. MS28 effectively degraded cyclin D1 prior to CDK4/6 and with superior degradation potency through recruiting the CDK4/6-cyclin D1 complex to the von Hippel-Lindau (VHL) E3 ligase. MS28 suppressed the proliferation of lung and breast cancer cells more effectively than CDK4/6 inhibitor and degraders. Notably, while triple negative breast cancer (TNBC) cell lines were mostly insensitive to CDK4/6 inhibitor and degrader treatment, they exhibited potent sensitivity to MS28. RNA-sequencing analysis revealed that while MS28 and CDK4/6 inhibitor and degrader shared downregulated genes associated with cell cycle regulation and DNA replication, MS28 upregulated a unique set of genes such as those involved in the unfolded protein response and endoplasmic reticulum stress pathway. Altogether, the bridged PROTAC strategy could provide a generalizable platform for targeting undruggable proteins, thereby offering invaluable chemical tools for the study of these proteins, and potentially delivering greater therapeutic efficacy than small-molecule inhibitors or degraders targeting alternative components within the signaling pathway.

#395 Triamterene and Asinex 1 target DLG and ETGE mutants of NRF2 in esophageal squamous cell carcinoma.

Zachary A. Ladd[1], Candice Bui-Linh[2], Chorlada Paiboonrungruang[2], Boopathi Subramaniyan[3], Zhaohui Xiong[2], Tao Gao[3], Haining Wang[4], Francis Spitz[3], Xiaoxin Chen[3]

[1]Cooper Medical School of Rowan University (CMSRU), Camden, NJ,[2]Coriell Institute for Medical Research, Camden, NJ,[3]Surgery, Cooper University Health Care, Camden, NJ,[4]Insilico Medicine, Cambridge, MA

Introduction: NRF2 is a master transcription factor that regulates oxidative stress response. Esophageal squamous cell carcinoma (ESCC) frequently harbors gain-of-function NRF2 mutations that cause NRF2 overexpression, drive chemo- and radio-resistance, and lead to poor survival in human patients. NRF2 interacts with KEAP1's Kelch domain through the DLG (Trp^{24}-Leu^{30}) and ETGE (Leu^{76}-Leu^{84}) motifs, which are mutation hotspots in human ESCC. Hence, there is a need to develop targeted therapies against $NRF2^{Mut}$-ESCC carrying DLG or ETGE mutations, without inhibiting $NRF2^{WT}$. Our previous study has validated two FDA-approved drugs (Pyrimethamine and Mitoxantrone) as $NRF2^{Mut}$inhibitors in human ESCC cells and a genetically modified murine model (*Redox Biology 2023; 67:102901*). This study aims to further identify inhibitors that target DLG and ETGE mutants of NRF2 in ESCC.

Methods: The effects of Triamterene (FDA-approved diuretic) and Asinex 1 (novel compound) on NRF2 expression, half-life, and ubiquitination were examined in $NRF2^{W24C}$-KYSE70, $NRF2^{D77V}$-KYSE180, $NRF2^{D29H}$-TE14, $NRF2^{G81S}$-OE21, $NRF2^{WT}$-KYSE450, and $KEAP1^{Null}$-KYSE450 cells. Proximity ligation assay (PLA) was performed to investigate the KEAP1-NRF2 interaction. Seven Triamterene analogues were synthesized for a structure-activity relationship study.

Results: Both Triamterene and Asinex 1 displayed dose- and time-dependent inhibitory effects on $NRF2^{W24C}$ and $NRF2^{D77V}$ expression, respectively, with limited cytotoxicity. Both compounds did not impact $NRF2^{WT}$ expression in $NRF2^{WT}$-KYSE450 and $KEAP1^{Null}$-KYSE450 cells, indicating their selective inhibition of $NRF2^{Mut}$. Triamterene and Asinex 1 significantly reduced the half-life and enhanced ubiquitination of $NRF2^{W24C}$ and $NRF2^{D77V}$, respectively. In addition, Triamterene and Asinex 1 significantly inhibited the expression of $NRF2^{D29H}$ in TE14 cells and $NRF2^{G81S}$ in OE21 cells, respectively. PLA showed a significant increase in KEAP1-$NRF2^{W24C}$ interaction by Triamterene. The structure-activity relationship study and computer modeling suggested that Triamterene binds KEAP1 (in particular Arg^{415}), thus modifies the conformation of Kelch domain to fit DLG^{W24C}.

Conclusion: Triamterene and Asinex 1 inhibit $NRF2^{Mut}$ in ESCC cells with specificity for DLG mutants and ETGE mutants, respectively. Both compounds are being further investigated for their mechanisms of action as molecular glues and efficacy in genetically modified mice carrying DLG and ETGE mutations.

#396 Discovery of SHR3591, a highly potent and orally bioavailable AR PROTAC for the treatment of prostate cancer.

Minqiang Jia, Limin Zhang, Fanglong Yang, Gang Chen, Shouli Zhao, Weimin Wang, Jiangtao Chi, Wei Peng, Luona Wang, Huishuang Miao, Zhibin Xu, Yuchang Mao, Xun Li, Yi Mao, Shuyu Cai, Jun Feng, Min Hu, Xin Li, Feng He

Shanghai Hengrui Pharmaceutical CO., LTD., Shanghai, China

Prostate cancer is the second most common cancer in men after lung cancer. Androgen receptor (AR) signaling plays a key role in the development and progression of prostate cancer. A number of AR-centered therapies have been developed for the treatment of prostate cancer, such as CYP17A1 inhibitor abiraterone, which blocks the androgen synthesis pathway, and a series of second-generation AR antagonists. Unfortunately, resistance mechanisms to these AR-signaling targeted agents usually develop within 18 months of treatment, including AR amplification, various ligand-binding domain (LBD) mutations and splicing variants. However, AR signaling still plays an important role in these resistant settings. Therefore, it's urgent to develop new therapeutic strategies targeting AR, particularly for the treatment of metastatic castration-resistant prostate cancer (mCRPC). To meet this challenge, proteolysis-targeting chimera (PROTAC) technology provides a possible solution. We disclose herein SHR3591 as an orally bioavailable AR PROTAC for the treatment of prostate cancer, which is potent in degrading both AR wildtype and LBD mutations.SHR3591 demonstrated nanomolar AR degradation activities in both LNCaP (DC_{50}: 7.9 nM) and AR-amplified VCaP cells (DC_{50}: 7.5 nM). To our delight, SHR3591 also degraded various clinically relevant AR mutants, including H875Y, T878A, W742L, F877L and M896V with single-digit nanomolar potency. One of the most common mutant L702H could also be degraded efficiently with a DC_{50} value less than 20 nM. Meanwhile, no obvious degradation of neosubstrates of common CRBN binders, such as IKZF1, IKZF3 or GSPT1, were observed (DC_{50}>10000 nM), displaying good selectivity. The *in vivo* PD experiment was carried out in castrated male mice harboring VCaP tumors with 10 mg/kg of SHR3591 treatment (po, qd×3). Over 90% of AR proteins were degraded by SHR3591 treatment. Furthermore, in a VCaP xenograft model, SHR3591 showed robust anti-tumor activity in a dose-dependent manner, leading to tumor regression (TGI > 130%) at as low as 3 mg/kg (po, qd), without obvious influences in mouse body weight during 25 days of treatment. Additionally, SHR3591 also displayed favorable pharmacokinetic profiles across preclinical species with oral bioavailability more than 20%. In conclusion, SHR3591 has been developed as a highly potent and orally bioavailable AR PROTAC, exhibiting superior AR degradation activities and good selectivity *in vitro*. SHR3591 could efficiently degrade AR WT and clinically relevant mutants including L702H. Furthermore, SHR3591 displayed excellent PD effect and robust tumor inhibition activity *in vivo*. SHR3591 showed favorable pharmacokinetic profiles across different preclinical species as well. SHR3591 has advanced into phase1/1b clinical studies in patients with mCRPC.

#397 Combinatorial use of VHL and KEAP1-based PROTACs reveals unexpected synergy and hook effect relief.

Sehbanul Islam[1], Haihong Jin[2], Dong Liu[2], Dan Lu[2], Yunkai Zhang[2], Renxu Chang[1], Joel Austin[2], Thomas Beer[3], Hsin-Yao Tang[3], Lan Huang[2], James Tonra[2], Luca Busino[1]

[1]University of Pennsylvania, Philadelphia, PA,[2]SEED Therapeutics Inc., King of Prussia, PA,[3]The Wistar Institute, Philadelphia, PA

Proteolysis-targeting chimeras (PROTACs) are bifunctional molecules bridging a target protein with an E3 ubiquitin ligase, promoting its ubiquitylation and degradation. Unlike traditional inhibitors, PROTACs exploit transient interactions, enabling the selective degradation of "undruggable" proteins, thereby expanding the landscape of druggable targets. However, PROTACs are not without limitations. The "hook effect"—a phenomenon where high PROTAC concentrations reduce efficacy due to inactive binary complex formation—compromises their clinical utility. Additionally, single PROTACs may fail to achieve complete degradation due to insufficient polyubiquitination or suboptimal E3 ligase recruitment, limiting their therapeutic efficacy. These challenges highlight the need for strategies to enhance PROTAC performance and so therapeutic applicability. In this study, we introduce a novel dual-PROTAC strategy that recruits two distinct E3 ligases, such as KEAP1 and VHL, to cooperatively degrade KRAS(G12D) and Androgen Receptor (AR). Our data demonstrate that combining KEAP1- and VHL-recruiting PROTACs synergistically enhances target degradation, reduces the required dosing, and minimizes off-target effects. Notably, KEAP1 recruitment offers the unique advantage to favour polyubiquitin chains elongation, thereby improving proteasomal recognition. In conclusion, dual-targeting approach represents a promising avenue for optimizing PROTAC-based therapeutics.

#398 Systematic identification and translation of novel targeted protein degradation mechanisms.

Christian Dillon

PhoreMost Limited, Pampisford, United Kingdom

The rise of Targeted Protein Degradation (TPD) is rapidly changing perceptions about therapeutic target druggability and drug design. However, the majority of degraders in clinical and preclinical development rely on the recruitment of a single E3 ligase, Cereblon, highlighting a pressing need to identify and translate novel mechanisms. Moreover, the discovery of molecular glue degraders is hampered by the prevailing serendipitous nature of their discovery. To unlock the potential of this modality we have developed proprietary cell-based screening platforms, SITESEEKER® and GlueSEEKER™, to uncover novel TPD mechanisms. These technologies exploit vast libraries of computationally-derived coded peptide fragments within the context of a pooled degradation-based phenotypic screen. Specifically, SITESEEKER® screens have identified a cache of degrader mechanisms that rely on hijacking components of the UPS machinery. GlueSEEKER™ utilizes peptide libraries to create a diverse suite of surface-edited E3 ligases to systematically explore neo-substrate degradation across a variety of E3 ligases and Proteins-of-Interest (POI).PhoreMost is progressing a pipeline of monovalent and heterobifunctional oncology degrader programmes arising from its platforms. We describe a novel set of small molecule heterobifunctional degraders that operate via an E3 ligase distinct from Cereblon or VHL-based PROTACs. Mechanistically, these degraders function via a CUL1-SKP1 SCF complex FBOX substrate receptor and demonstrate potential for enhanced cancer-selective degradation of POIs. Finally, we uncover neomorphic interactions revealed by GlueSEEKER™ that provide translational insights for the degradation of both precedented and unprecedented substrates of the E3 ligase, Cereblon.

#399 Targeted protein degradation of the androgen receptor by ARCC-4 and ARD-2128: proteomic and therapeutic potential in AR-driven cancers.

Elsa Liu[1], Judy Fu[1], Lori Fan[1], Luciano Galdieri[2], Justin Lipner[2], Steven Garner[2], Brogan Epkins[2], Emily Schultz[2], Daria Clucas[2], Kaitlyne Powers[2], Julie Chung[1], Ian Huang[1], Pei Ching Cheng[1], Phil Lin[1], Alastair J. King[2], ChunYao Lee[1]

[1]Eurofins Discovery, New Taipei City, Taiwan,[2]Eurofins Discovery, St. Charles, MO

Background: The androgen receptor (AR) plays a central role in the progression of prostate cancer, where AR signaling drives tumor cell growth and survival. Current treatment strategies, including androgen deprivation therapy and AR antagonists like enzalutamide, aim to reduce AR activation. However, resistance often develops partly due to compensatory upregulation of the AR protein. Targeted protein degradation (TPD) represents an alternative strategy to overcome the limitations of traditional therapies by degrading the AR protein rather than merely inhibiting its function.

Methods: This study investigated the AR-targeting degraders, ARCC-4 and ARD-2128, and their effects on AR nuclear translocation and transactivation in AR-expressed CHO-K1 cells. Additionally, *in vitro* efficacy was evaluated in cell proliferation assays, notably using the LNCaP clone FGC prostate cancer cell line. Proteomic analysis was conducted to examine global protein profiles and signaling pathways impacted by each degrader.

Results: ARCC-4 and ARD-2128, which act via distinct E3 ligase pathways (VHL and CRBN, respectively), demonstrated significant AR degradation, with ARD-2128 achieving greater potency in reducing AR nuclear translocation and transactivation. Both degraders showed lower GI_{50} values and greater efficacy in suppressing cell proliferation in LNCaP clone FGC cells when compared with enzalutamide. Additionally, proteomic analysis highlighted distinct molecular signatures associated with each degrader, revealing differential effects on AR network proteins and broader signaling pathways.

Conclusion: The potent growth inhibition observed with ARCC-4 and ARD-2128, as compared with enzalutamide, underscores the therapeutic potential of TPD in AR-driven cancers. These findings also potentially support TPD as a robust approach for overcoming resistance mechanisms associated with AR-targeted therapies. Broader profiling using the OncoPanel® platform would further demonstrate the therapeutic potential and selectivity of AR degraders such as ARCC-4 and ARD-2128. Investigation in additional prostate and breast cancer cell lines with diverse nuclear hormone receptor expression would be particularly relevant and may offer insights into the application of AR-targeted degradation across different cancer types, potentially leading to more effective treatment options.

TUMOR BIOLOGY: Cancer Stem Cells
Poster Session

#4 Vitamin D blocks platinum-induced ovarian cancer stem cell plasticity.

Tara X. Metcalfe[1], Sophie Xanders[2], Shu Zhang[3], Christiane Hassel[4], Heather M. O'Hagan[1], Kenneth P. Nephew[5]

[1]School of Medicine, Indiana University, Bloomington, IN,[2]Jesse H. and Beulah Chanley Cox Scholars Program, Indiana University, Bloomington, IN,[3]Biology, Indiana University, Bloomington, IN,[4]Flow Cytometry Core Facility, Indiana University, Bloomington, IN,[5]Melvin and Bren Simon Comprehensive Cancer Center, Indiana University, Indianapolis, IN

Most women diagnosed with late-stage high grade serous ovarian cancer (HGSOC) develop recurrent, platinum-resistant tumors. Ovarian cancer stem cells (OCSCs) are hypothesized to contribute to the emergence of these tumors. CSCs reside in a plastic state, with the ability to convert between non-CSC to CSC. This process of dedifferentiation is thought to continue during tumor development and chemotherapeutic agents such as platinum can exaggerate CSC plasticity. However, the underlying mechanism has not been established. Furthermore, how platinum transforms non-OCSCs into OCSCs to contribute to this subpopulation of cells remains unclear. To examine OCSC plasticity, aldehyde dehydrogenase (ALDH; functional marker) and fluorescence activated cell sorting were used to isolate OCSCs (ALDH+) and non-OCSCs (ALDH-) from HGSOC cell lines, OVCAR5 and OVCAR3. To determine if platinum induced conversion of non-OCSC to OCSC, ALDH- cells were cultured for 3-10 days after sorting. At timepoints examined, ALDH- cells remained ALDH-, (>99% were ALDH- after 10 days). ALDH- cells were then treated with cisplatin (OVCAR5: 12μM,16h, OVCAR3: 15μM 16h), and the percent of ALDH+ cells was measured using flow cytometry. Treatment of cisplatin resulted in conversion of approximately 4% and 10% of ALDH- cells into ALDH+ cells ($p<0.05$) in OVCAR5 and OVCAR3 cells, respectively. Furthermore, converted OCSCs displayed increased ($p<0.05$) expression of stemness genes (BMI1, NANOG, OCT4, SOX2) and spheroid formation compared to parental ALDH+, demonstrating that platinum induced the observed differences in the stemness phenotype. With the goal of targeting key genes and pathways to inhibit platinum-induced OCSC conversion, RNA-sequencing was performed on ALDH- and ALDH+ cells before and after cisplatin treatment. Vitamin D receptor (VDR)/retinoid x receptor (RXR) activation was a major pathway in the platinum-induced converted OCSC transcriptome, which was of interest because VDR signaling plays a role in differentiation, and its key binding partner, RXR and retinoic acid receptor (RAR) are linked to stemness. To determine the effect of vitamin D on OCSC conversion, ALDH- cells were treated with vitamin D for 24h (OVCAR5: 34nM, OVCAR3: 56nM) and/or cisplatin and the ALDH activity was measured with flow cytometry. Vitamin D treatment in combination with cisplatin significantly decreased the platinum-induced percent of converting cells ($p<0.05$). Additionally, vitamin D treatment decreased expression of stemness genes and spheroid formation in both whole cell and sorted cell populations. Currently, we are further investigating the relationship between cisplatin, RAR/RXR signaling and stemness. Collectively, these results demonstrate a role for VDR signaling in platinum-induced OCSC conversionproviding further support for the development targeted therapies to block the persistence of OCSCs and ultimately reduce patient mortality.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#40 Organoid models of ovarian clear cell carcinoma for evaluating therapeutic sensitivity.

Clare E. Padrick[1], Magdalena Zielinska[1], Minh Ha[1], Aisha L. Saldanha[1], Cam Anh Tran[1], Brendan Shay[2], Daohong Zhou[3], Kristopher A. Sarosiek[4], Cloud P. Paweletz[1], Ursula A. Matulonis[2], Joyce F. Liu[2], **Elena V. Ivanova**[1], Elizabeth H. Stover[2]

[1]Belfer Center for Applied Cancer Science, Dana-Farber Cancer Institute, Boston, MA,[2]Medical Oncology, Dana-Farber Cancer Institute, Boston, MA,[3]Biochemistry & Structural Biology, U.T. Health San Antonio, San Antonio, TX,[4]Environmental Health, Harvard School of Public Health, Boston, MA

Ovarian clear cell carcinoma (OCCC) is a rare subtype of ovarian cancer, often resistant to platinum-based chemotherapy. OCCC is characterized by ARID1A mutations, PI3K and RAS/MAPK pathway alterations, and activation of the HIF signaling pathway. We and others showed that OCCC are sensitive to inhibition of BCL-XL, a pro-survival protein in the intrinsic pathway of apoptosis. Patient-derived models of OCCC could potentially accelerate pre-clinical testing of therapeutics in this difficult-to-treat cancer, but relatively few patient-derived organoid (PDO) or xenograft models of OCCC have been reported. We describe the establishment and propagation of two PDO models of OCCC. Patient 1 had recurrent OCCC with PIK3CA mutation and HER2 amplification. Patient 2 had recurrent OCCC with CDKN2A/B deletion. Both patients underwent secondary cytoreductive surgery for recurrent disease. Fresh tissue was collected under IRB-approved protocols and tumor cells were plated in Matrigel and formed PDOs. Patient 1 OCCC cells have been propagated for over 35 passages, and patient 2 cells for 9 passages to date. Immunohistochemistry to confirm fidelity to the original tumor is in progress. As a proof-of-concept for evaluating therapeutics in OCCC PDOs, we used a 3D microfluidic device (AIM Biotech) to evaluate drug responses in patient 1 PDO. PDO cells were seeded in the microfluidic device and expanded for one week, treated with drugs within the device, then stained at day 6 with fluorescence markers for live (TMRM, Thermo Fisher), apoptotic (CellEvent Caspase-3/7, Thermo Fisher), and dead (DRAQ7, CST) cells. Quantitative imaging was performed to determine the cell area positive for each marker. The OCCC PDO was treated with standard-of-care chemotherapies carboplatin and paclitaxel; BCL-XL inhibitor A1331852 (Selleckchem); a novel BCL-XL PROTAC degrader DT2216 (Dialectic Therapeutics); or combinations of the BCL-XL inhibitor/degrader with paclitaxel. Treatment with A1331852 or DT2216 induced apoptosis and death in the OCCC PDO cells compared to control. While paclitaxel alone also induced some cell death, the combination of DT2216 (1μM) and paclitaxel (500nM) was highly potent, resulting in elimination of almost all the OCCC cells (Bliss synergy score 0.28±0.08, p=0.013). These data suggest that BCL-XL inhibition/degradation combined with paclitaxel may be a promising treatment strategy for OCCC. Our study also demonstrates the successful establishment of OCCC PDOs and application of a microfluidic device for evaluating novel therapeutic strategies in ovarian cancer.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Degraders and Glues 1
Poster Session

#400 Preclinical development of TQB3019: A potent and selective oral BTK degrader leveraging the OAPD® platform to overcome acquired resistance mutations.

Jing Ren, Jinan Wang, Sheng Xu, Jin Liu, Ying Zhang, Xiaojin Wang, Baomin Liu, Wei Shi, Hongjiang Xu, Ling Yang, Wei Zhao, Xiquan Zhang

Chia Tai Tianqing Pharmaceutical Group Co., Ltd., Nanjing, China

Background: Bruton's tyrosine kinase (BTK) is a clinically validated target for the treatment of hematological malignancies. Small molecule inhibitors targeting BTK have been used in the clinic to manage B-cell malignancies. However, the emergence of BTK mutations, such as BTK C481S, ect., can lead to drug resistance to both covalent and non-covalent inhibitors. To address the acquired resistance mutation issues, here we report TQB3019, a novel BTK degrader, discovered using Chia Tai Tianqing's proprietary Platform for Orally Available Protein Degrader (OAPD®).

Result: TQB3019, a highly potent oral BTK degrader, induced robust BTK degradation with a DC_{50} of less than 0.1 nM and D_{max} of greater than 95% in DLBCL cell line OCI-LY10 at 6 hours. Proteomics studies revealed excellent degradation selectivity, then no Ikaros and Aiolos degradation was further confirmed in OCI-LY10 cells and human T cells. TQB3019 also exhibited efficient degradation kinetics in cells harboring both wild-type and C481S mutation, achieving 80% of BTK degradation within 2 hours at 10 nM. Importantly, TQB3019 induced strong cell proliferation inhibition in cells carrying wild-type BTK (IC_{50} of sub-nanomolar range) and BTK C481S mutation (IC_{50} of single-digit nanomolar range). TQB3019 exhibits favorable kinase selectivity of BTK over EGFR, and did not degrade EGFR in lung cancer cell line A431. Unlike covalent inhibitor ibrutinib, TQB3019 also demonstrated improved selectivity for BTK over TEC. TQB3019 using a novel designed CRBN ligand showed over 14-fold higher binding affinity to CRBN protein compared to pomalidomide. Additionally, TQB3019 showed weak inhibition of hERG and cytochrome P450 activities (CYPs) (hERG IC_{50} >30 μM and CYPs IC_{50} >10 μM). TQB3019 demonstrated promising metabolic stability *in vivo* with a mean residence time (MRT) of over 10 hours in dogs, and exhibited good oral bioavailability in both rodents and dogs. Furthermore, oral administration of TQB3019 led to dose-dependent tumor growth inhibition (TGI>95% at 15mg/kg) even complete tumor regression (30mg/kg) in a BTK wild-type TMD8 xenograft tumor model, without significant weight loss.

Conclusion: Developed using Chia Tai Tianqing's OAPD® platform, TQB3019 is an orally available BTK degrader with promising potency, superior selectivity, and favorable pharmacological properties. With IND (Investigational New Drug) application forthcoming, TQB3019 represents a promising therapeutic candidate to address resistance challenges associated with traditional BTK inhibitors.

#401 HDBNJ3049: A novel EGFR-targeting PROTAC to overcome C797S-mediated osimertinib resistance in NSCLC.

Mohammad Hassan Baig[1], Yun Seong Jo[1], Sagar Dattatraya Nale[2], Chang Joong Kim[2], Tae Hwan Park[2], Ju Han Bok[3], Dong Min Kim[3], Ji Min Park[3], Hye Mi Kim[3], Jae June Dong[1], Byoung Gon Moon[3]

[1]Yonsei University College of Medicine, Seoul, Korea, Republic of,[2]BNJ Biopharma Inc., Incheon, Korea, Republic of,[3]Handok Inc., Seoul, Korea, Republic of

The emergence of C797S-driven Osimertinib resistance represents a major hurdle in the treatment of non-small cell lung cancer (NSCLC). This study presents a novel series of proteolysis-targeting chimeras (PROTACs) designed to overcome resistance by potently and selectively degrading EGFR mutants, including the C797S variant. These next-generation EGFR PROTACs demonstrated significant anti-proliferative activity across a broad spectrum of EGFR mutations, achieving subnanomolar potency in cellular proliferation and protein degradation assays alike. Notably, these compounds effectively targeted not only C797S mutants but also other clinically relevant mutations such as Exon19Del and L858R/T790M variants. The lead candidate, HDBNJ3049, exhibited superior activity across all tested EGFR mutant cell lines, including potent growth inhibition of C797S-mutant Ba/F3, and H1975 cells (GI_{50} as low as 17 nM and 50 nM for L858R/T790M/C797S Ba/F3 and H1975, respectively), and robust degradation of Del19/T790M/C797S mutant EGFR (DC_{50}: 13 nM, D_{max}: 98%). *In vivo* pharmacokinetic and pharmacodynamic studies further validated the therapeutic potential of HDBNJ3049, establishing it as a highly promising lead candidate for overcoming osimertinib resistance in NSCLC and offering new hope for patients with limited treatment options.

#402 BCL6 degrader with novel CRBN ligand demonstrates promising selectivity and preclinical efficacy alone or in combination with BCL2 inhibitor.

Jun Xu, Yangguang Li, Ju Wang, Pingmin Tang, Yuting Liao, Chen Zhang, Pangke Yan

Haisco Pharmaceutical Group Co Ltd., Chengdu, China

The B-cell lymphoma 6 (BCL6) transcription repressor, a protooncogene particularly significant in diffuse large B-cell lymphoma (DLBCL), is known to confer survival, protection, and maintenance of lymphoma cells. Its expression in normal B cells is crucial for the initiation and sustenance of germinal centers (GC). Somatic mutations of the BCL6 gene, including chromosomal translocations, have been linked to the deregulation of BCL6 expression, contributing to a subset of aggressive double- and triple-hit lymphomas with poor outcomes with conventional therapy. Addressing this critical clinical need, we have developed HSK47977, a highly specific, potent, and orally bioavailable BCL6 protein degrader with a novel CRBN ligand. Unlike traditional IMiDs, this new CRBN ligand alone had no IMiD activity and barely induced protein degradation in PBMC cells. Meanwhile, HSK47977 baring this ligand has shown remarkable in vitro and in vivo efficacy across multiple pre-clinical DLBCL models. HSK47977 achieves over 90% BCL6 degradation at 10 nM and a DC50 of less than 1 nM in the OCI-Ly1 model. Time-course studies revealed that over 90% BCL6 degradation was achieved within 1 hour and sustained up to 24 hours. Meanwhile, HSK47977 minimally induced the degradation of first-generation IMIDs targets, including GSPT1, SALL4, CK1α, Aiolos, and Ikaros, suggesting its safety profile. The optimization of the CRBN ligand has also significantly bolstered the pharmacokinetic (PK) profile of HSK47977, endowing it with impressive oral bioavailability and enhanced in vivo exposure following oral administration. In addition to this, we have devised a mechanism-based combination strategy aimed at amplifying the therapeutic potency of HSK47977. Given that BCL6 functions as a transcriptional repressor, the degradation of BCL6 could potentially upregulate the expression of the anti-apoptotic protein BCL2, providing the rational for combined inhibition of both. Indeed, we proved that the synergistic effects of of HSK47977 and a BCL2 inhibitor was achieved in both in vitro and in vivo. This combination therapy demonstrated that a minimal dose of HSK47977, when paired with a BCL2 inhibitor, was adequate to elicit tumor regression in CDX models. In summary, these findings underscore HSK47977 as a potent and orally bioavailable BCL6 degrader, distinguished by its novel CRBN ligand. Moreover, the strategic combination with a BCL2 inhibitor is poised to significantly augment the therapeutic efficacy, offering a promising approach for the treatment of diffuse large B-cell lymphoma.

#403 Characterization of PROTACs for targeting tissue transglutaminase 2 (TG2) in cancer.

Andres Felipe Valdivia[1], Purav Vagadia[2], Natalia Masnica[3], Joshua Li Zhu[2], Vanessa Hernandez[4], Ana Maria Isac[4], Gary Schiltz[2], Daniela Matei[4]

[1]Medicine, Northwestern Univ. Feinberg School of Medicine, Chicago, IL,[2]Department of Chemistry, Northwestern Univ., Evanston, IL,[3]Northwestern Univ., Evanston, IL,[4]OBY/GYN, Northwestern Univ. Feinberg School of Medicine, Chicago, IL

Introduction: Tissue transglutaminase (TG2) is a multifunctional enzyme involved in the interaction between fibronectin and integrins, as well as in GTP hydrolysis. TG2 is upregulated and active in several cancers, including ovarian cancer (OC). Due to its multifunctional nature and involvement in various processes that promote cancer progression, targeting TG2 therapeutically has proven challenging. Here, we explore the development of PROteolysis TArgeting Chimeras (PROTACs) against TG2 as a strategy to induce protein degradation and target TG2 in cancer.

Materials and Methods: OVCAR5 or SKOV3 OC cells expressing TG2 were treated with different concentrations of PROTACs for 6 or 24 hours. TG2 expression was measured via Western blot (WB) and TG2 activity was assessed by using an enzymatic activity assay. PROTACs that induced TG2 degradation were further validated through reverse phase protein array (RPPA) analysis, adhesion assays, migration assays, and in-vivo omentum adhesion assays.

Results: Over 50 compounds were screened. WB analysis showed significant ($p < 0.05$) degradation of TG2 using three PROTACs: P74, P404, and P405, in both SKOV3 and OVCAR5 cells at 24 hours of treatment, without decreased cell viability ($p < 0.05$). Degradation was stable at 48 hours. Migration and adhesion assays revealed that PROTACs reduced both adhesion and migration capabilities of the cells at 24 hours ($p < 0.005$). Additionally, PROTACs reduced the enzymatic activity of TG2, demonstrating the ability to disrupt other TG2 functions ($p < 0.001$). MG132 treatment prevented PROTAC induced degradation of TG2 showing that the degradation is proteasome dependent. ($p < 0.05$). Reverse phase proteomic analysis (RPPA) of P74-treated cells identified 136 differentially expressed proteins, including a reduction in TG2 and an increase in VHL expression. The most affected pathways in PROTAC-treated cells included cell adhesion, the PI3K/AKT pathway, and others (FDR < 0.05, fold change < 0.5). PROTACs P74 and P405 also reduced in vivo adhesion of OVCAR5 cells to the omentum in an intraperitoneal omental colonization assay ($p < 0.05$).

Conclusion: Treatment of OC cells with PROTACs P74, P404, and P405 significantly reduced TG2 expression levels, disrupted migration and adhesion, and impaired TG2 enzymatic activity. Furthermore, PROTACs reduced cell adhesion in-vivo, highlighting their potential as therapeutic agents

#404 Mechanistic characterization of selective monovalent direct degraders of SMARCA2.

Julia Toth, Gregory Parker, Geoffray Leriche, Tayor Kampert, Aleksandar Jamborcic, Gabrielle Blanco, Kenneth Steadman, Duc Tran, Shu You, Luis Lopez, Farhana Barmare, Kyohei Hayashi, Gang Liu, Alex Campos, Meg McCarrick, Kevin Freeman-Cook, Peggy Thompson

Plexium, San Diego, CA

SMARCA2 and SMARCA4 are essential, redundant, catalytic subunits of the multi-subunit BRG1/BRM-associated factor (BAF) complex. This complex regulates gene expression and DNA repair through chromatin remodeling activity. The paralogues SMARCA2 and SMARCA4 bind acetylated histones through their highly conserved bromodomains and alter DNA-histone contacts using ATP-dependent helicase activity. Recently, mutations in specific components of the BAF complex have been identified. Of great interest are loss of function mutations in SMARCA4, found in 5-7% of all human cancers and occur with high prevalence (~10%) in non-small cell lung cancer (NSCLC), making these SMARCA4-deficient tumor cells highly dependent on SMARCA2 for viability. This synthetic lethal relationship may provide a unique opportunity for SMARCA2-selective degraders to block the growth of SMARCA4 deficient tumors, while sparing normal tissues. To enable degrader discovery, we focused our efforts on a monovalent direct degrader approach, where small molecules are designed to bind the target protein and induce its degradation through the recruitment of an E3 ligase complex. Initial screening hits from distinct chemical series were optimized to produce potent and selective SMARCA2 degraders. Co-treatment with a proteasome inhibitor confirmed that degradation was mediated via the ubiquitin proteasome system (UPS), and a UPS-focused CRISPR screen identified the E3 ligase responsible for compound-induced degradation of SMARCA2. Cell based protein-protein interaction (PPI) studies advanced our understanding of compound-mediated E3 ligase recruitment and provided mechanistic insights into the observed selectivity for SMARCA2 over SMARCA4. Ternary complex dynamics were further investigated *in vitro* via surface plasmon resonance (SPR) studies. Using a combination of co-immunoprecipitation, mass spectrometry, and point mutant studies, specific amino acids that are required for compound-induced PPI and subsequent degradation of SMARCA2 were identified. Collectively, these results highlight the discovery and characterization of novel small molecule monovalent direct degraders of SMARCA2. The detailed description of the degradation mechanism presented here provides critical insights into an efficient approach to eliminating pathogenic proteins through targeted protein degradation.

#405 Discovery of GLR203101, a selective and orally bioavailable PROTAC of SMARCA2 for the treatment of SMARCA4 mutant tumors.

Xiaoguang Li, Siyu Gu, Yongzheng Ding, Jing Cheng, Kanghua Wang, Weiwei Yu, Wenmin Chen, **Fuming Xu**

Gan & Lee Pharmaceuticals, Beijing, China

The SWI/SNF (mammalian SWItch/Sucrose Non-Fermentable) helicase SMARCA4 is frequently mutated in cancer. Its inactivation results in a cellular dependence on its paralog, SMARCA2, for survival. Therefore, SMARCA2 constitutes an attractive therapeutic synthetic lethality target. However, the co-deletion of Smarca2 and Smarca4 in adult mice was lethal, highlighting the necessity for designing molecules that target SMARCA2 selectively. This objective is rather challenging due to the high homology of SMARCA2/4. Few SMARCA2 selective compounds have entered clinical trials, including PROTAC molecule PRT-3789 administered by intravenous injection in phase 2 clinical trials.

Herein, we report the discovery of a selective and orally bioavailable SMARCA2 PROTAC degrader, GLR203101. Notably, our lead compound GLR203101 possesses a favorable molecular weight (< 650), contributing to excellent physical and chemical properties. GLR203101 exhibited robust degradation of SMARCA2 (DC_{50} < 10 nM, D_{max} > 80%) over SMARCA4 (D_{max} < 10%) in Hela cell line using HiBiT assay. The degradation selectivity was also validated by western blot in several SMARCA4 wild-type cell lines, including Hela, MV-4-11, LNCaP and SW1573. GLR203101 also selectively inhibited the viability of SMARCA4-deficient cancer cells with low nanomolar IC_{50} values, showing >1000-fold selectivity over SMARCA4 wild-type cell lines. In addition, GLR203101 exhibited favorable PK properties with high exposure and moderate oral bioavailability in mice and SD rats. After 24 hours of oral administration of GLR203101, a robust degradation of SMARCA2 while sparing SMARCA4 was observed in transplanted tumors in the xenograft model. GLR203101 is being evaluated as a preclinical candidate.

#406 Targeting NSD2 aberrations in hematological malignancies with a potent and selective NSD2 PROTAC degrader.

Yihan Liu[1], Abhijit Parolia[1], Lianchao Liu[2], Tongchen He[1], Yuanyuan Qiao[1], Sanjana Eyunni[1], Jie Luo[1], Zhen Wang[2], Arul Chinnaiyan[1], Ke Ding[2]

[1]Michigan Center for Translational Pathology, University of Michigan, Ann Arbor, MI,[2]Shanghai Institute of Organic Chemistry, Shanghai, China

Nuclear receptor-binding SET domain-containing 2 (NSD2) is a methyltransferase that predominantly catalyzes the dimethylation of lysine 36 on histone 3 (H3K36me2). It has been implicated as an oncogene, with a hotspot gain-of-function mutation frequently identified in B-cell acute lymphoblastic leukemia (B-ALL) and chromosomal translocations involving NSD2 reported in approximately 20% of multiple myeloma cases. Despite its clinical significance, existing NSD2 inhibitors suffer from low activity or inferior selectivity, and none can simultaneously remove the methyltransferase activity and chromatin binding function of NSD2. To address these limitations, we developed LLC0424, a novel NSD2 degrader engineered using proteolysis-targeting chimera (PROTAC) technology based on a PWWP1 domain binder. LLC0424 demonstrated potent degradation of NSD2 protein in acute lymphoblastic leukemia (ALL) RPMI-8402 cells, with a DC50 value of 20 nM and a Dmax value of 96%. TMT quantitative proteomics confirmed LLC0424's high selectivity for NSD2, underscoring its specificity. Intravenous or intraperitoneal administration of LLC0424 achieved robust NSD2 degradation in vivo, validating its systemic effectiveness. In B-ALL cells harboring the NSD2 point mutation, LLC0424 treatment led to sustained downregulation of H3K36me2, a critical epigenetic mark associated with NSD2 activity. Additionally, LLC0424 exhibited cytotoxicity in ALL cell lines with NSD2 mutations, inhibiting cell growth and reducing colony formation capacity. Overall, these findings highlight the therapeutic potential of NSD2 degradation as a novel strategy to target cancers driven by NSD2 aberrations.

#407 Development of an integrated multi-modal platform for comprehensive analysis of targeted protein degraders in a drug discovery setting.

Magdalena Kieltyka[1], Maciej Olszewski[2], Marta Smejda[2], Kamila Zaremba[2], Katarzyna Nosek[2], Magdalena Malik[2], Katarzyna Szafran[2]

[1]Selvita Inc, Boston, MA,[2]Selvita SA, Krakow, Poland

With growth in the area of targeted protein degradation (TPD), there has been increasing interest in the development of analytical platforms which can facilitate efficient drug discovery processes. We provide a fully developed high throughput analytical platform to study and optimize protein degrader candidates in a comprehensive manner. Our platform consists of multiple orthogonal technologies that generate major mechanistic information and help advance TPD drugs rapidly through the preclinical development stages. Our platform is based on a well-defined degrader development strategy using advanced technologies and automated processes. Major analytical features include: • Binding and binary complex formation: assessment of warheads. Monitoring target protein binding interactions in both cell free and cellular systems, using surface plasmon resonance (SPR), isothermal titration calorimetry (ITC) and NanoBRET, respectively. • Ternary complex formation evaluation: assessment of degrader-induced protein-protein interactions in both cell free and cell-based systems, using homogeneous time-resolved fluorescence (HTRF) and NanoBRET respectively. • Cellular degradation profiling via a variety of methods, including automated capillary Western blotting, HiBiT and High Content Screening, enabling precise quantification of degradation kinetics and efficacy. • Proteome-wide specificity assessment using mass spectrometry. In our integrated data processing pipeline, we correlate physicochemical properties with degradation efficiency, to support SAR analysis and enhance the rational optimization of degraders. The platform has been proven to work across multiple E3 ligase systems, including CRBN, VHL and other tissue-specific ligases, thus supporting a wide range of TPD programs. Comparative case studies from several client-based programs exemplify the versatility of our platform and its capability to generate comprehensive data for effective decision making. Notably, we achieved high concordance between orthogonal methods. Modular design of the platform enables its segmentation to fit the needs of the clients' individual specifications whilst retaining standardized work procedures for effective delivery of the project. This comprehensive analytical strategy addresses the challenges of TPD drug discovery and provides the scalability and reliability required for contract research organizations supporting multiple concurrent programs.

#408 CETSA-based evaluation of non-degraded PROTAC targets.

Tomas Friman, Tuomas Tolvanen, Alexey Chernobrovkin, **Daniel Martinez Molina**

Pelago Bioscience AB, Solna, Sweden

Protein degradation is a non-classical modality with the potential to tackle therapeutically interesting proteins, even those previously deemed undruggable. The molecular effect that a productive interaction between target proteins and a bifunctional degrader/PROTAC can easily be monitored by measuring protein degradation. However, not all proteins that bind degraders are in fact degraded, which suggests that not all effects of PROTACs are based on the degradation of targets, but also the effects of traditional binary protein - ligand interactions. The Cellular Thermal Shift Assay (CETSA) is a powerful technology for identifying the interactions between compounds and their cognate protein targets. Coupling CETSA with mass spectrometric (MS) readout allows for profiling of binding selectivity of both the warhead that binds the protein of interest (POI) but also to investigate the selectivity for different PROTAC scaffolds. CETSA can be carried out in both lysates and intact cells, always without the need to modify the compound, the proteins or the cellular environment. In a typical CETSA workflow we can address both the degradation profile of proteins in the proteome that is accessible by MS, and also provide insights into the binding of the degrader to both the E3 ligase and the POI. This allows us to identify any disconnect between degradation and binding of degrader as well as to follow the downstream cellular and molecular effects. Here we will exemplify how CETSA can be applied in deconvoluting protein binding of a CDK9 targeting PROTAC: THAL SNS 032, which is composed of the cereblon (E3-ligase) binder Thalidomide and the CDK (POI) binder SNS 032. THAL SNS 032 induces degradation of the annotated target CDK9 and its associated protein Cyclin-T1, but target engagement can also be observed for additional proteins, including other kinases. In turn, the non-productive interactions from a degradation point of view can still result in biological effects, as exemplified here where GSK3A and GSK3B as well as their downstream substrate FOXK1 are thermally shifted but not degraded by the PROTAC. The effects on the GSK3 - FOXK1 signaling axis would have been missed if only protein degradation had been considered. By combining mass spectrometric data from CETSA experiments with degradation readouts it is possible to correlate cellular target engagement potencies with degradation efficiency and importantly, also identify any drug - protein binding that do not result in degradation

#409 Targeted degradation of FAK and PYK2 in cancer.

Christina C. Kuismi[1], Yuan Jiang[2], Baishan Jiang[2], Behnam Nabet[1]

[1]Human Biology, Fred Hutchinson Cancer Center, Seattle, WA,[2]Department of Radiation and Medical Oncology, Medical Research Institute, Zhongnan Hospital, Wuhan University, Wuhan, China

Focal adhesion kinase (FAK) and its paralog proline-rich tyrosine kinase 2 (PYK2) are non-receptor kinases that are critical in the initiation, progression, and metastasis of several cancers, including triple-negative breast cancer and pancreatic ductal adenocarcinoma. FAK and PYK2 are overexpressed in these cancers and have diverse activities in the cell, including functioning as kinases, serving as scaffolding proteins, and regulating transcriptional programs. Consequently, they have emerged as important drug targets in cancer. Despite the critical need to advance novel therapeutic approaches in triple-negative breast cancer and pancreatic ductal adenocarcinoma to improve survival rates, the development of selective agents to target FAK or PYK2 in isolation has remained challenging. In this study, we hypothesized that abolishing all functions of FAK and/or PYK2 using targeted degradation approaches would be advantageous over small molecule kinase inhibitors. Building on our prior work to develop FAK-selective heterobifunctional degraders known as PROTACs, we endeavored on a campaign to develop PYK2-selective and FAK/PYK2-dual targeting PROTACs. We synthesized a series of PROTACs that engage FAK and/or PYK2 and recruit an E3 ubiquitin ligase to achieve target protein degradation. These compounds, which vary in their kinase binding warheads, linkers, and E3 ubiquitin ligase binders, were evaluated through assays to measure target engagement in cells, protein levels using immunoblotting, selectivity using quantitative proteomics, and impact on phenotypes including 3D-spheroid viability and cell migration. This pipeline led to the identification of potent (< 250 nM) and selective degraders of PYK2 and FAK/PYK2 that rapidly (< 2 hours) diminish protein levels. These PROTACs require the ubiquitin-proteasome machinery to achieve degradation and display proteome-wide selectivity towards the intended kinases. In pancreatic cancer and breast cancer cell lines, degradation of both FAK/PYK2 led to significant reductions in 3D-spheroid viability and migration. These effects were improved compared to FAK or PYK2 inhibition or degradation in isolation. In addition, targeted destruction of FAK, PYK2, or FAK/PYK2 led to substantially improved reductions in 3D-spheroid viability and migration compared to kinase inhibition, highlighting the importance of disabling the enzymatic and non-enzymatic roles of FAK and PYK2. Together, our work establishes a chemical toolbox to degrade FAK, PYK2, or FAK/PYK2 to uncover FAK and PYK2 shared and distinct roles and advances targeted degradation of these kinases as a potential therapeutic approach in cancer.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#41 Generation and multiomic profiling of a*APC/TP53*double knockout normal colon organoid.

Younghee Choi, Eunju Kim, Jihyun Park, Sang-Hyun Song, Tae-You Kim

Seoul National University, Jongno-gu, Korea, Republic of

Colorectal cancer frequently arises from early tumorigenic events driven by mutations in the APC and TP53 genes. To elucidate mechanisms underlying early colon tumorigenesis, we established normal colon organoids with APC and TP53 double knockouts (DKO). Through integrated multi-omics analyses, including RNA-seq, ATAC-seq, methylation-seq, and H3K27ac ChIP-seq, we identified significant upregulation of the KIT gene in DKO organoids compared to controls. Subsequent KIT knockout generated APC/TP53/KIT triple knockout (TKO) organoids, which exhibited markedly reduced migratory capacity and Ki-67 tumor intensity, highlighting a pivotal role for KIT in early tumorigenic processes. Proteomic analysis further revealed that GSK3β phosphorylation was significantly reduced in TKO organoids compared to DKO organoids, suggesting a mechanistic link between KIT expression and downstream signaling pathways critical for early tumor progression. Notably, treatment with the KIT inhibitor imatinib phenocopied the effects of KIT knockout, reinforcing KIT's role as a driver of early colon tumorigenesis. These findings provide critical insights into the molecular mechanisms of early tumorigenesis in APC and TP53 mutant contexts, identifying KIT as a promising therapeutic target for intervention in early-stage colorectal cancer.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Degraders and Glues 1
Poster Session

#410 The BTK degrader BGB-16673 shows anti-tumor activity in marginal zone lymphoma models.

Alberto J. Arribas, Elisa Civanelli, Camilla Scalise, Luciano Cascione, Andrea Rinaldi, Davide Rossi, Emanuele Zucca, Francesco Bertoni

IOR - Institute of Oncology Research, Bellinzona, Switzerland

Introduction. Targeting the B cell receptor (BCR) signaling with BTK and PI3K inhibitors is efficacious in treating patients with lymphoid tumors, including marginal zone lymphoma (MZL). Unfortunately, resistance occurs, indicating the need for combinations and novel approaches. BGB-16673 is a first-in-class cereblon-mediated BTK degrader currently in phase 1 for refractory/relapsed B cell lymphoma patients, including MZL. Here, we assessed BGB-16673 in MZL models as a single agent to provide the rationale for future trials in MZL patients.
Methods. Effect on cell viability was assessed by MTT assay after 5 days of exposure to increasing concentrations of compounds or DMSO (control) in MZL cell lines and in cell lines with secondary resistance to idelalisib (n.=2), copanlisib (n.=1), ibrutinib (n.=2), and copanlisib/venetoclax. Analyses included apoptosis and cell cycle analysis by flow cytometry, immunoblotting, and RNA-Seq.
Results. Six MZL cell lines (SSK41, Karpas1718, VL51, HC1, HAIRM, and ESKOL) were treated with the BTK degrader BGB-16673 at a concentration of up to 10μM. The BTK degrader was very active in 2 models (SSK41, IC50=0.1 nM; Karpas1718, 1.7 nM). VL51 achieved a maximal reduction in proliferation of 25% at 10 nM. Less than 20% of the reduction was observed in HC1, HAIRM and ESKOL. The activity of BGB-16673 did not differ from the BTK inhibitor zanubrutinib and, accordingly, was limited in cells resistant to ibrutinib (n.=2), idelalisib (n.=2), copanlisib (n.=1), and copanlisib/venetoclax (n.=2). BGB-16673 induced apoptosis and cell cycle arrest in sensitive Karpas1718 and SSK41. It importantly inhibited p-BTK(Tyr223) and decreased total BTK protein levels in all six cell lines. RNA-Seq was performed on Karpas1718 exposed to BGB-16673, zanubrutinib or DMSO. Both molecules down-regulated the BCR-TLR-NF-κB signaling pathway, MYC target genes, and upregulated genes involved in DNA damage. Oxidative phosphorylation, commonly associated with resistance to multiple therapies, was repressed by BGB-16673 but upregulated by zanubrutinib.
Conclusions. Its ability to degrade BTK protein and its antitumor activity position the BTK degrader BGB-16673 as a promising candidate for further development in MZL patients.

#411 Selective degradation of SMARCA2 and dual degradation of SMARCA2 and SMARCA4 has strong antitumor activity in marginal zone lymphoma (MZL).

Alberto J. Arribas[1], Eleonora Cannas[1], Stefano Pileri[2], Davide Rossi[1], Diane Heiser[3], Francesco Bertoni[1]

[1]IOR - Institute of Oncology Research, Bellinzona, Switzerland,[2]Division of Diagnostic Haematopathology, European Institute of Oncology, Milan, Italy,[3]Prelude Therapeutics Inc, Wilmington, DE

Background: The SWI/SNF chromatin remodeling complex is crucial for normal B cell development. Its importance is underlined by the occurrence of somatic mutations in genes coding for subunits of the complex, such as ARID1A and SMARCA4, in lymphomas. PRT3789 is a novel bifunctional small molecule containing a SMARCA2-bromodomain binder linked to a VHL E3 ubiquitin ligase-binding moiety, with preclinical activity in SMARCA4-deficient cancer cells but not SMARCA4 wt cells. PRT3789 is currently in phase 1 as a single agent or in combination with docetaxel for patients with solid tumors with loss of SMARCA4. Here, we assessed the antitumor activity of the SMARCA2 degrader PRT3789 and a novel dual SMARCA2/4 degrader in MZL preclinical models, including derivatives with acquired resistance to BTK, PI3K, and BCL2 inhibitors.
Methods: VL51, Karpas1718, SSK41, ESKOL, HC1, HAIRM and derivatives with resistance to targeted agents obtained by long drug exposure (3 from VL51, 1 from Karpas1718 and SSK41) were exposed to increasing concentrations of the compounds or DMSO, as control. Anti-proliferative activity was assessed by MTT assay after 6 days of exposure. Cell cycle and apoptosis assays were performed.
Results: Antitumor activity was seen in the nM range with both compounds. PRT3789 had a median IC50 of 13.27 nM (95%CI, 3.91-41.19). The dual SMARCA2/4 degrader was more active, with a median IC50 of 0.93 nM (95%CI, 0.18-1.92). The activity was maintained in cells with acquired resistance to BTK, PI3K, and BCL2 inhibitors. None of the models had mutations in the coding regions of ARID1A or SMARCA4.Adding individual degraders to the BTK inhibitor ibrutinib, the PI3Kδ inhibitor idelalisib, or to the combination of the PI3K inhibitor copanlisib with the BCL2 inhibitor venetoclax led to additivity/synergism. Adding a low concentration of the degraders (1-10nM) overcame resistance to either copanlisib/venetoclax, ibrutinib, or idelalisib. Adding the dual SMARCA2/4 degrader increased copanlisib/venetoclax efficacy in resistant SSK41 and was strongly synergistic in resistant VL51. Adding PRT3789 enhanced copanlisib/venetoclax and ibrutinib potencies and idelalisib efficacy in the corresponding resistant cells. When we exposed Karpas1718 and SSK41 to the two degraders as single agents or in combination with ibrutinib, we observed that the addition of the compounds improved the cytotoxic activity of ibrutinib in both cell lines by inducing apoptosis.
Conclusions: The single SMARCA2 degrader PRT3789 and the dual SMARCA2/4 degrader showed strong dose-dependent cytotoxic activity in MZL cells, showing activity in the low nM range. Both compounds induced apoptosis as single agents and improved the activity of a BTK inhibitor in the two cell lines tested. Interestingly, the antitumor activity was maintained in MZL cell lines with acquired resistance to BTK, PI3K, and BCL2 inhibitors.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Drug Resistance in Molecular Targeted Therapies 1
Poster Session

#415 Metformin sensitizes non-small cell lung cancer to CDK4/6 inhibitor treatment by reducing lysosomal trapping.

Yaqi Liu[1], Yalei Wang[1], Ni Zhang[1], Haijuan Xiong[1], Lipan Zhao[1], Jiping Xie[2], Xiaofeng Duan[3], Dongsheng Yue[2], **Shuo Qie**[1]

[1]Department of Pathology, Tianjin Medical Univ. Cancer Inst. & Hospital, Tianjin, China,[2]Department of Lung Cancer, Tianjin Medical Univ. Cancer Inst. & Hospital, Tianjin, China,[3]Department of Minimally Invasive Esophageal Surgery, Tianjin Medical Univ. Cancer Inst. & Hospital, Tianjin, China

Hyperactivation of cyclin D1-CDK4/6 signaling is correlated with tumour metastasis, and poor prognosis in patients with non-small cell lung cancer (NSCLC), suggesting the therapeutical potential of targeting CDK4/6 to treat NSCLC. Our preliminary data suggest CDK4/6 inhibitors are sequestered in lysosomes in NSCLC cells, a phenomenon termed lysosomal trapping that is correlated with intrinsic resistance. It's promising to search for new compounds that could be co-administered with CDK4/6 inhibitors to reduce lysosomal trapping, hence enhancing the sensitivity of NSCLC cells. Here, we find metformin reduces the activity of lysosomal pathway through genome-wise analyses; in accordance, metformin efficiently reduces lysosomal trapping of CDK4/6 inhibitors in NSCLC cells. Biologically, metformin enhances the suppressing effects of CDK4/6 inhibitors on cell cycle progression, cell proliferation, and colony formation. Mechanistically, metformin suppresses lysosomal trapping by: 1) reducing translocation of TFEB into nuclei that leads to decreased lysosome biogenesis, and 2) disrupting lysosomal acidic environment through reduced V-ATPase activity and decreased interaction between LAMP1/LAMP2 and TMEM175. Clinically, combined treatment with metformin and CDK4/6 inhibitors significantly retards the growth of mouse xenografts and tumour cell organoids. Taken together, our study provides a new mechanistic dimension on utilizing metformin to treat NSCLC by reducing lysosomal trapping, therefore, increasing cellular sensitivity to CDK4/6 inhibitors.

#416 Spatial transcriptomic analysis of breast cancer patients with early and late progression of disease after endocrine and CDK4/6 treatments: deconvoluting the tumor microenvironment.

Sunil S. Badve[1], Shihan Liu[2], Madhubanti Basu[3], Chiao-Wang Sun[3], Shanrun Liu[3], Jian Hu[4], **Yesim Gokmen-Polar**[1]

[1]Pathology and Laboratory Medicine; Winship Cancer Institute, Emory University School of Medicine, Atlanta, GA,[2]Biostatistics and Bioinformatics, Rollins School of Public Health, Emory University, Atlanta, GA,[3]Flow Cytometry and Single Cell Core Facility, University of Alabama at Birmingham, Birmingham, AL,[4]Department of Human Genetics, Emory University School of Medicine, Atlanta, GA

Background: Cyclin-dependent kinase inhibitors, CDK4/6i, have been one of the most significant practice-changing advancements for the estrogen receptor positive (ER+) breast cancer therapy in the recent decade. However, no molecular biomarkers are successful in predicting sensitivity/resistance to CDK4/6 inhibitors in clinical trials. It is believed that the heterogeneity of the tumor microenvironment (TME) plays a key role in resistance and metastasis. Deconvolution of TME in CDK4/6 treated samples may provide novel tumor-specific strategies to overcome resistance and reduce mortality in ER+ breast cancer.
Methods: Samples of primary tumors from patients treated for metastatic ER+ breast cancer with CDK4/6 inhibitors were identified from the database of Emory University hospital. Archival FFPE sections were analyzed using 10X CytAssist-enabled Visium Spatial transcriptomics using the UAB Flow Cytometry and Single Cell Core Facility. IRIS and cell type deconvolution analyses were performed to identify TME parameters (immune markers and cell types) associated with time to progression on CDK4/6i.
Results: The analysis was performed on 10 patients who were all treated with Palbociclib and endocrine therapy. The early time to progression (<6 months; n=6) exhibited differential cellular phenotypes (Tumor, Stroma, and Immune Cells) in comparison to late time of progression (>30 months; n=4). The study revealed that both B cells, and CD4+ and CD8+ T cells, are present in significantly higher proportions in the progression of the <6 months group, suggesting a role for immune-related mechanisms in the development of resistance. In addition to lymphocytes, cancer-associated fibroblasts (CAFs) were more prominent in early progression cases suggesting the involvement of tumor microenvironment. Additional confirmatory analyses are on-going.
Conclusion: Deconvolution of the TME using spatial transcriptomics will be a powerful tool for understanding the tumor heterogeneity. Better understanding of the cellular interactions could provide novel strategies to prevent CDK4/6 inhibitor resistance.

#417 Identification of novel inhibitors of the deubiquitinase USP14 for targeting cisplatin-resistant ovarian carcinoma.

C. Corno[1], D. Russo[2], F. Pignotti[3], F. De Giorgi[2], I. Penna[2], F. Saccoliti[2], M. Costantino[1], L. Mirra[1], P. Pettinari[1], N. Perta[3], C. M. Ciniselli[1], P. Pratesi[1], R. Scarpelli[2], F. Bertozzi[2], P. Verderio[1], G. L. Beretta[1], D. Di Marino[3], T. Bandiera[2], **P. Perego**[1];
[1]Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, [2]Istituto Italiano di Tecnologia, Genoa, Italy, [3]Polytechnic University of Marche, Ancona, Italy

Deubiquitinases (DUBs) including the proteasome-associated USP14 have emerged as drug targets in cancer and may contribute to drug resistance. Since DUBs are deregulated in ovarian carcinoma, the disease management may benefit from novel inhibitor development. The aim of this study was to identify novel USP14 inhibitors (USP14i) to test in preclinical ovarian cancer models. Cellular pharmacology approaches were employed to examine USP14 role in cisplatin-sensitive and resistant cells. An assay for compound screening, previously developed by Lee and colleagues (PMID: 23788557), was optimized using Ub-Rho110-G. Molecular docking was used to evaluate the best ligand interaction of putative inhibitors. In 134 specimens representative of the frequency occurrence of the histological ovarian carcinoma subtypes, USP14 mRNA levels were associated with tumor grade. Exogenous over-expression of USP14 increased survival upon cisplatin exposure in cisplatin-resistant IGROV-1/Pt1 cells, but not in cisplatin-sensitive IGROV-1 cells, supporting a role for this DUB in the cisplatin-resistant phenotype. Knockdown of USP14 in IGROV-1/Pt1 cells by small-interfering RNA transfection impaired cell aggressive features such as proliferative, migratory and invasive abilities; sensitization to cisplatin was evident in IGROV-1/Pt1 but not in IGROV-1 cells, suggesting that USP14 acts on resistance-relevant substrates. A diversity set of 1056 small molecules from a library of ca. 11,000 compounds was screened. Setting a relatively low threshold of inhibition of USP14 activity, 5 initial hits were identified. The most interesting compound, referred to as Cpd1, displayed an IC_{50} of 18.4 μM. As Cpd1 represents a novel chemical class of USP14i, a docking protocol targeting the USP domain of USP14 was employed to identify its potential binding modes (BMs). Molecular dynamics simulations were then performed using the best docking results to assess the stability of the identified BMs and evaluate the interactions between Cpd1 and USP14. The simulations revealed excellent stability for two BMs and provided insights into the interaction mechanism. In cells expressing a proteasome sensor vector, Cpd1 displayed proteasome inhibiting activity, also evident in intact cells by active-site direct labeling with HA Ub-vinyl sulfone. The identified hit represents a new chemical class of USP14i and is a starting point for future chemical optimization aiming at identifying more potent compounds. In conclusion, USP14 appears to be a relevant and druggable target in ovarian carcinoma.

#418 Modelling PARP inhibitor resistance using CRISPR/Cas9 genome engineering in a PARP inhibitor-sensitive organoid model identified by organoid panel screening.

Lenno Krenning[1], Dorrith Verstegen[1], Irene Drosou[1], **Marten Hornsveld**[1], Mariusz Madej[1], Hester Bange[1], Peter van Schaik[1], Ludovic Bourre[2], Marrit Putker[1]

[1]Crown Bioscience Netherlands B.V., Leiden, Netherlands,[2]Crown Bioscience, San Diego, CA

Background: Inhibition of poly(ADP-ribose)polymerase (PARP) is synthetically lethal in cells with impaired homologous recombination (HR) DNA repair Hence, PARP-inhibitors (PARPi) are used to treat tumors with disruptive mutations in HR factors. However, both pre-existing and acquired resistance mechanisms limit the clinical success of PARPi, highlighting the need to develop new drugs and/or treatment strategies.

Methods: PARPi efficacy was tested in a CellTiterGlo (CTG)-based OrganoidXplore™ organoid screening panel of 50 organoid models, spanning 7 cancer indications, including models with mutations in HR genes. CRISPR/Cas9-based genome editing was used to knock-out SLFN11 in an originally PARPi-sensitive organoid model, to mimic clinical PARPi resistance. Lastly, isogenic pairs of PARPi sensitive and resistant organoids were used in drug efficacy studies to assess treatment efficacy of multiple therapies.

Results: The OrganoidXplore panel screening captured the heterogeneous response to talazoparib that is also observed in patients. 19 models demonstrated sensitivity to talazoparib (IC_{50}s <1 µM), including models carrying inactivating mutations in HR genes (BRCA1, BRCA2 and RAD51C). However, not all models that carry mutations in HR genes displayed PARPi sensitivity and not all sensitive organoid models carry mutations in known HR genes, stressing the importance of *in vitro* efficacy testing. Inactivating mutations into SLFN11, a well-known clinical biomarker for PARPi and chemotherapy response, was introduced in one of the most PARPi-sensitive models, using CRISPR/Cas9. The isogenic SLFN11 wild-type and knockout pair were used to assess single agent sensitivity to talazoparib, and the chemotherapeutic drugs etoposide, doxorubicin and camptothecin (the parent compound to irinotecan and topotecan), and found that SLFN11 loss causes resistance to all tested agents.

Conclusion: *In vitro* organoid screening successfully identified PARPi-sensitive organoid models containing known and unknown mechanisms of PARPi sensitivity. Further investigation is required to determine the sensitivity of certain models, as they may bear novel biomarkers of PARPi sensitivity, or possess unknown intrinsic resistance mechanisms. Furthermore, CRISPR/Cas-mediated genome editing can be used to establish isogenic organoid pairs that can subsequently be used for single agent as well as combination therapy efficacy testing *in vitro*.

#419 Chromosomal instability and oncogene dosage variations underlying KRAS G12C inhibition therapy resistance in lung cancer.

Dingcheng Gao[1], Yingzhuo Liu[1], Yi Ban[2]

[1]Weill Cornell Medicine, New York, NY,[2]NYU, New York, NY

KRAS G12C inhibitors (G12Ci), including the FDA-approved Sotorasib and Adagrasib, represent a significant advancement in targeted cancer therapies. As their use in non-small cell lung cancer patients expands, addressing the pervasive challenge of drug resistance is critical. Resistance mechanisms can be innate, acquired, or both, often coexisting within complex scenarios. Recent studies have highlighted various resistance mechanisms to G12Ci, such as oncogene amplification, activation of alternative signaling pathways, additional mutations in KRAS or its associated networks, and adaptive processes like epithelial-to-mesenchymal transition. Identifying the primary drivers and predominant mechanisms of resistance is crucial for developing effective countermeasures. To tackle these challenges, we developed Oligo-CALL, an innovative cellular barcoding technology that employs antisense oligonucleotide-inducible CRISPRa for lineage labeling. This technique allows for precise clonal tracking and the retrospective isolation of clones. Using Oligo-CALL, we analyzed tumor clonal heterogeneity and traced the evolution of resistance in lung cancer cells treated with G12Ci. Treatment with Sotorasib resulted in reduced clonal diversity, indicative of strong selective pressure. Similar clonal compositions across different replicates suggest that intrinsic mechanisms significantly contribute to G12Ci resistance. Notably, about 30% of resistant clones showed increased expression of the Kras G12C allele, primarily due to genomic gains, pointing to chromosomal instability (CIN) as a key factor in resistance development. Comprehensive analyses including fluorescent karyotyping, whole-genome, and transcriptome sequencing of isolated tumor clone pairs, revealed extensive genomic complexity and increased heterogeneity under G12Ci treatment. Resistant clones exhibited specific increases in copy number variations, chromosomal structure variations, and additional single nucleotide mutations, further underscoring CIN as a driving force behind G12Ci resistance. Clones that underwent drug treatment displayed higher CIN scores compared to their treatment-naïve counterparts. Intriguingly, cells resistant to G12Ci demonstrated increased sensitivity to PARP inhibitors, suggesting that targeting DNA repair pathways could induce synthetic lethality, offering a potential strategy to overcome resistance in lung cancer cells.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#42 Non-small cell lung cancer (NSCLC) patient derived xenograft organoids (PDX-O) as a model for testing novel therapeutic approaches.

Maia G. Clare, Robert B. Cameron, Nia G. Hammond, Massar Alsamraae, Brandon Faubert

The University of Chicago Medicine Comprehensive Cancer Center, Chicago, IL

Despite advances in therapy and early screening, non-small cell lung cancer (NSCLC) remains responsible for the most cancer-related deaths worldwide. There is an urgent need to elucidate the drivers of NSCLC progression to develop better treatment options, but these efforts are often limited by a lack of clinically relevant model systems. To address this need, we developed a series of patient-derived xenografts (PDX) from a clinical study of tumor metabolism. We confirmed that these models retain the genetic, metabolic, and metastatic phenotypes of the patient tumor. Furthermore, we developed patient-derived organoids (PDOs) from these systems by growing and passaging PDX cells in Matrigel droplets with the ThermoFisher OncoPro System. Preliminary validation studies have revealed these organoids have expected morphology, high viability, and maintenance of NSCLC expression markers. We have used these novel model systems to identify heterogeneous metabolic phenotypes and identify targetable vulnerabilities. Specifically, we have used a drug screening approach to combine metabolic inhibitors with standard of care chemotherapies to evaluate combinatorial treatment strategies in NSCLC by cancer cell death. In summary, we have developed a bedside-to-bench approach of identifying patient-relevant tumor biology, in tractable, testable systems, and we use these models to identify novel therapeutic approaches in NSCLC.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Drug Resistance in Molecular Targeted Therapies 1
Poster Session

#420 On-target mutations confer resistance to WRN helicase inhibitors in Microsatellite Unstable Cancer Cells.

Gabriele Picco[1], Yanhua Rao[2], Angham Al Saedi[1], Samantha J. Walker[1], Shriram Bhosle[1], Yang Lee[3], Sara F. Vieira[1], Gilberto Valdes Garcia[2], Kieron May[4], Francesco Sassi[5], Mamta Sharma[1], Cansu Dincer[1], Inigo Barrio Hernandez[1], Theo Bell[1], Anastasia Kavasakali[1], Maria Garcia-Casado[1], Ray Shenje[2], Cuthbert Martyr[2], Edward Brnardic[2], Nanhua Deng[3], Hristina Grigorova Dimitrova[1], Emre Karakoc[1], Emma Hitch[1], Katrina McCarten[1], Zoe Hewitson[1], Howard Lightfoot[1], Syd Barthorpe[1], Michael P. DeMartino[2], Phil Landis[2], Emanuel Goncalves[6], Andrea Bertotti[5], Livio Trusolino[5], Geeta Sharma[3], Matthew A. Coelho[1], Jonathan Houseley[4], Benjamin Schwartz[3], Mathew J. Garnett[1]

[1]Wellcome Sanger Institute, Hinxton, United Kingdom,[2]GSK, Upper Providence, PA, US 19426, PA,[3]GSK, Cambridge, MA, US 02139, MA,[4]Babraham Institute, Babraham Institute, Cambridge, UK., United Kingdom,[5]Candiolo Cancer Institute, 10060 Candiolo, Torino,, Italy,[6]Instituto Superior Tecnico (IST), Universidade de Lisboa, 1049-001 Lisboa, Portugal, United Kingdom

Werner helicase inhibitors (WRNi) are in clinical development for treating patients with microsatellite-unstable (MSI) tumors characterized by defective DNA mismatch repair. Here, we investigate the impact of cancer cell evolutionary adaptation on WRN pharmacological inhibition with implications for understanding drug selectivity and potential clinical resistance. Coupling genome-wide CRISPR screens with WRN gene knockout, no suppressors of WRN dependency were identified, underscoring WRN's essential non-redundant function in MSI cells. Pharmacogenomic screens pinpointed modulators of WRNi sensitivity, including SMARCAL1, linking it to WRN-MSI synthetic lethality. Semi-saturation mutagenesis of WRN and prolonged drug treatment identified on-target WRN mutations driving acquired resistance to multiple WRNi in vitro and in vivo. Specific resistance mutations induce cross-resistance to WRN inhibitors, whereas others preserve sensitivity to alternative WRNi. Our work guides next-generation strategies for targeting MSI cancers, enabling cross-resistance studies to evaluate current and novel WRNi efficacy and informing future clinical trial design.

#421 Targeting WEE1 enhances the antitumor effect of *KRAS* mutated non-small cell lung cancer harboring *TP53* mutations.

K. Fukuda[1], S. Takeuchi[1], S. Sato[1], S. Arai[2], S. Nanjo[3], H. Taniguchi[1], S. Yano[3];
[1]Kanazawa University Cancer Research Institute, Kanazawa-shi, Japan, [2]Kanazawa University Nano Life Science institute, Kanazawa-shi, Japan, [3]Kanazawa University Department of Respiratory Medicine, Faculty of Medicine, Institute of Medical, Pharmaceutical, and Health Sciences, Kanazawa-shi, Japan

While *KRAS*-G12C inhibitors are clinically approved, the efficacy of sotorasib alone remains limited due to challenges such as co-mutations, intrinsic resistance, and acquired resistance. Overcoming these barriers requires innovative strategies to enhance apoptosis in *KRAS*-mutant non-small cell lung cancer (NSCLC).

To identify genes whose loss induces apoptosis in *KRAS*-mutant NSCLC, we performed a CRISPR-knockout screen using a crRNA library targeting 746 protein kinase genes. This high-throughput approach identified WEE1 as a critical kinase whose inhibition significantly enhances apoptosis. Drug screening with a custom library of 432 kinase inhibitors also validated WEE1, CHK1, and CDK inhibitors as effective, with WEE1 emerging as the most potent target, particularly in *TP53*-mutant cells.

WEE1's primary role is to prevent cells with damaged DNA from entering mitosis by regulating the cell cycle through DNA damage checkpoints. Mechanistic analyses revealed that WEE1 inhibition accelerates the G2/M transition and markedly reduces the expression of CHK2 and Rad51, two critical regulators of the DNA damage response (DDR) pathway. This indicates that WEE1 operates upstream of the DDR pathway, playing a crucial role in DNA repair and cell cycle regulation in *KRAS*-mutant NSCLC cells.

To further evaluate therapeutic potential, we investigated the combined effects of sotorasib and WEE1 inhibitors. Combined treatment significantly suppressed cell viability and enhanced c-caspase-3/7 activity in both sotorasib-sensitive and sotorasib-resistant cells. Multidimensional two-drug synergy assays revealed Bliss scores of 11.105 for H358 (sotorasib-sensitive) and 11.226 for H2122 (sotorasib-resistant) cells, indicating a potent synergistic effect across different resistance profiles. In patient-derived xenograft (PDX) models established from a patient with KRAS-G12C mutation and primary resistance to sotorasib, the combination of sotorasib and the WEE1 inhibitor, ZN-c3, resulted in near-complete tumor regression. This effect was consistently observed across four in vivo models, highlighting the translational potential of this combination therapy.

Our findings highlight WEE1 inhibition as a promising therapeutic strategy for *KRAS*-mutant NSCLC with *TP53* mutations. This approach provides a foundation for future clinical investigations to overcome initial resistance to KRAS-G12C inhibitors and improve outcomes for patients with *KRAS*-mutant NSCLC.

#422 Aurora kinase A inhibition overcome adaptive resistance to KRAS G12C inhibitor by replication stress-induced genomic instability and mitotic cell death.

C. Li[1], V. Nangia[2], C. J. Graser[3], J. Chang[4], A. Nimbalkar[1], M. U. Syed[1], M. Vieira[1], Y. Shen[1], S. Altschuler[4], L. Wu[4], F. Michor[3], S. L. Spencer[2], A. N. Hata[1];

[1]Harvard Medical School/Massachusetts General Hospital, Boston, MA, [2]University of Colorado, Boulder, CO, [3]Dana-Farber Cancer Institute, Boston, MA, [4]University of California San Francisco, San Francisco, CA

Cancer cells develop drug-tolerant states, enabling them to evade therapies. In response to KRAS G12C inhibitors (G12Ci), non-small cell lung cancer (NSCLC) cells harboring KRAS mutations sustain a drug-tolerant phenotype through the activation of aurora kinase A (AURKA). AURKA phosphorylates c-Raf, thereby sustaining KRAS signaling under G12Ci treatment. However, the molecular mechanism by which G12Ci treatment induces dependency on AURKA remains poorly understood, and there is currently a lack of molecular biomarkers that predict sensitivity to combined G12Ci and AURKA inhibitor (AURKAi) therapy. We show that the selective AURKAi (LSN3321213) induces mitotic catastrophe and subsequent cell death in NSCLC cells resistant to the G12Ci (LY3499446). Machine learning-based single-cell image tracking revealed that G12Ci-treated cancer cells overcome G1 arrest-induced apoptosis. When treated with AURKAi, these cells undergo mitotic stalling and subsequent mitotic slippage, allowing them to evade cell death. In contrast, simultaneous treatment with both inhibitors results in catastrophic mitotic cell death. G12Ci induces DNA damage as a result of replication stress, leading to increased apoptotic priming during mitosis, which may shift the cellular response towards mitotic catastrophe rather than slippage. Approximately 40% of genetic alterations in NSCLC are associated with replication stress, including MYC amplification. Our findings indicate that MYC amplification is associated with enhanced synergy between G12Ci and AURKAi in NSCLC. Furthermore, induction of CMYC expression in CMYC wild-type cell lines leads to mitotic catastrophe in response to G12Ci + AURKAi, suggesting that CMYC induces replication stress, thereby promoting a dependency on AURKAi. This study reveals a novel relationship between replication stress-induced genomic instability and mitotic defects in KRAS G12C NSCLC and identifies MYC amplification as a potential biomarker for guiding the selection of combined G12Ci and AURKAi therapy in patients with KRAS mutations and MYC amplification.

#423 CDK2 activation mediates response of acute myeloid leukemia to CHK1, ATR and WEE1 inhibitors.

Annapoorna Venkatachalam, Husheng Ding, Nicole Vincelette, Cordelia McGehee, Kevin Peterson, Paula Schneider, Cristina Correia, Hu Li, Scott Kaufmann

Mayo Clinic, Rochester, MN

Background: Replication checkpoint modulators (RCMs), including inhibitors of CHK1, ATR, and WEE1, have been widely studied for their ability to inhibit cell cycle checkpoints and enhance the toxicity of agents that stall replication forks. In preclinical studies each of these inhibitors also demonstrates notable activity as a single agent against relapsed/refractory acute myeloid leukemia (R/R AML). In particular, our prior study (PMID: 33414171) demonstrated that several myeloid cell lines underwent cell death at nanomolar concentrations, with a median IC_{50} of 9 nM irrespective of the *TP53* status. Examination of pathways involved in RCM-induced leukemia cell killing demonstrated that CHK1 inhibition induces CDC25A upregulation accompanied by activation of a death program that involves a CDK2-initiated 10- to 50-fold upregulation of TNFα and downstream engagement of the extrinsic apoptotic pathway. Here we have comprehensively characterized the processes that occur between the interruption of replication checkpoints and leukemic cell death in these sensitive cells versus cells selected for RCM resistance.
Methods: Using the CHK1 inhibitors MK8776 and prexasertib, ATR inhibitors berzosertib and ceralasertib, and WEE1 inhibitor adavosertib as paradigm drugs, we examined signaling in two AML cell lines, U937 and THP.1, that were selected for CHK1 inhibitor resistance, examined cross-resistance patterns, and assessed the biochemical basis for leukemic cell death. Changes in gene expression and signaling induced by RCMs were analyzed in isogenic pairs using RNA sequencing, flow cytometry and immunoblotting.
Results: In contrast to parental U937 and THP.1 cells, CHK1 inhibitor-selected U937 and THP.1 cell exhibited a lack of TNFα mRNA induction and did not undergo cell death in response to multiple CHK1 inhibitors. RNA sequencing of CHK1 inhibitor-selected AML lines revealed an almost complete absence of induction of CDK2-activated genes following prexasertib treatment. Interestingly, unlike solid tumors, AML cell lines that were resistant to CHK1 inhibitors exhibited hypersensitivity to WEE1 or ATR inhibition. Further studies demonstrated that CHK1 inhibitor response was varied in primary AML samples, with 50% of primary AML specimens resistant to CHK1i monotherapy, again without any relationship to *TP53* status. The common features among sensitive vs. resistant leukemias was the successful inhibition of CDK2 phosphorylation and the associated induction of TNFα.
Conclusions: Our findings reveals critical new insights into the factors that influence the response to CHK1 inhibitors in AML. Importantly, we found no evidence of cross-resistance to inhibitors of other replication checkpoint proteins, enabling alternative options.

#424 Chromosomal instability leads to EGFR-TKI refractoriness via cGAS-STING activation in EGFR-mutated non-small cell lung cancer.

K. Yonesaka[1], T. Kurosaki[1], J. Tanizaki[1], H. Kawakami[1], K. Tanaka[1], O. Maenishi[1], S. Takamura[1], K. Sakai[1], Y. Chiba[1], T. Teramura[1], H. Goto[2], E. Otsuka[2], H. Okida[2], M. Funabashi[2], Y. Hashimoto[2], K. Hirotani[3], Y. Kamai[3], T. Kagari[2], K. Nishio[1], K. Kakimi[1], H. Hayashi[1]; [1]Kindai University, Faculty of Medicine, Osaka, Japan, [2]Daiichi Sankyo Co., Ltd., Tokyo, Japan, [3]Daiichi Sankyo Co.; Ltd., Tokyo, Japan

Background: Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) are a standard therapy for EGFR-mutated non-small cell lung cancer (NSCLC); however, their efficacy is inconsistent. Tumor inflammation is observed in subpopulations of EGFR-mutated NSCLC tumors, which potentially influence the efficacy of anti-cancer agents. Chromosomal instability (CIN) is a trigger of tumor inflammation in malignancies. CIN causes accumulation of cytoplasmic DNA, which subsequently activates cGAS-STING signaling, resulting in tumor inflammation. However, it is unclear whether CIN affects susceptibility to EGFR-TKIs.
Materials and methods: The current study was based on the genomic and transcriptomic analyses of 67 tissue samples obtained from patients with EGFR-mutated NSCLC before EGFR-TKI treatment (n = 32) and after the acquisition of EGFR-TKI resistance (n = 35). RNA-Seq data were analyzed using the CIBERSORTx, GenePattern for ssGSEA, Carcinoma EcoTyper, and Tumor Immune Dysfunction and Exclusion (TIDE). The CIN score was calculated as previously reported (S. L. Carter et al. Nat. Genet. 2006), using the mean of the normalized gene expression data for the 70 genes comprising the signature.
Results: CIN is associated with poor outcomes of EGFR-TKI treatment in patients with EGFR-mutated NSCLC (CIN-low group vs CIN-high group; median PFS 16.7 vs. 11.0 months, HR 3.274, p = 0.005). Transcriptomic analysis revealed that cGAS-STING signaling is enhanced and the innate immune response is prominent in chromosomally unstable cancers. Consistently, EGFR-mutated H1975dnMCAK cells, which frequently underwent chromosomal mis-segregation, demonstrated a refractoriness to EGFR-TKI osimertinib compared to control cells in vitro growth inhibition assay (IC50 values; 6 vs 0.005 μM, respectively). H1975dnMCAK cells showed an activated signaling of cGAS-STING and an increase of its downstream IL-6. Several protumor signaling, including IL-6 and NF-κB, was activated in chromosomally unstable EGFR-mutated NSCLC tumor. Finally, chromosomally unstable H1975dnMCAK cells showed enhanced epithelial-mesenchymal transition (EMT) and blockade of cGAS-STING-TBK1 signaling reversed EMT, and consequently restored susceptibility to EGFR-TKIs both in vitro and in vivo.
Conclusion: These results suggest that CIN may induce EGFR-TKI refractoriness via cGAS-STING activation in EGFR-mutated NSCLC. Furthermore, cGAS-STING-TBK1 inhibitors, in combination with EGFR-TKIs, represent a rational therapeutic strategy that should be evaluated in patients with CIN-induced EGFR-TKI resistant NSCLC.

#425 Overcoming PARP inhibitor resistance in triple-negative breast cancer models through olaparib and RAD51 inhibition with TGF-beta 2 downregulation.

TaeYeong Kim[1], Jeong Dong Lee[2], Won-Ji Ryu[3], Cheol-Hwa Hong[3], Hyun Myoung Yun[3], Yumi Hwang[3], Hyunju Han[3], Gun Min Kim[3], Joohyuk Sohn[3], Min Hwan Kim[3]

[1]Internal medicine, Yonsei University College of Medicine, Seoul, Korea, Republic of,[2]Biomedical Research Institute, Seoul National University Hospital, Seoul, Korea, Republic of,[3]Division of Medical Oncology, Department of Internal Medicine, Yonsei Cancer Center, Yonsei University College of Medicine, Seoul, Korea, Republic of

Background: Recent studies have shown that RAD51 involves resistance to the PARP inhibitor in triple-negative breast cancer (TNBC) cells with *BRCA1* mutations. RAD51 protein has been reported to be recruited to the double-stranded DNA cleavage site to form homo-polymeric filaments that function as templates for double strand break repair. This study aimed to characterize the mechanisms of PARP inhibitor resistance and the therapeutic relevance of RAD51 using a *BRCA1*-mutant triple-negative breast cancer (TNBC) patient-derived xenograft (PDX) model.

Method: We used three PDX models derived from tumor tissues of TNBC patients with *BRCA1* c.5339T>C(L1780P) and c3627 dupA mutation, as well as wild-type *BRCA1* status. We administered Olaparib (PARP inhibitor), RI-1 (RAD51 inhibitor), and their combination for 6 weeks to PDX models for tumor growth assay. After 6 weeks, tumor tissues (n=3, each) was collected and subjected to RNA sequencing and RAD51 immunohistochemistry (IHC) staining. To investigate the role of TGF-β in RAD51-mediated resistance, we assessed TGF-β2 expression levels after RAD51 inhibitor treatment. We also established acquired Olaparib resistance model by chronic drug treatment in TNBC cell lines with mutant BRCA1 (L1780P) stable transfection. Combination treatment of RI-1 and LY2109761 (TGF-β receptor inhibitor) was performed in these Olaparib-resistant TNBC cells.

Results: In the three TNBC PDX models, *BRCA1* c.5339T>C model showed sensitivity to Olaparib treatment, while *BRCA1* wild-type and *BRCA1* L1780P-mutant PDX models showed resistance to Olaparib treatment. Notably, combination treatment with RI-1 and Olaparib synergistically inhibited growth of *BRCA1* wild-type and L1780P mutant PDX model. In the IHC assay of tumor tissues, we observed that RAD51 expression was downregulated in tumors after PARP and RAD51 inhibitor combination treatment. RNA sequencing analysis of tumors identified TGFB2 as downregulated in the RAD51 inhibitor-treated tumors, suggesting TGF-β2 suppression may involves overcoming Olaparib resistance mechanisms. We subsequently found RI-1 and LY2109761 combination treatment induces upregulation of P53 protein expression and decreased RAD51 expression in BRCA1-mutant TNBC cells. RI-1 and LY2109761 combination treatment also decreased cell growth Olaparib-resistant TNBC cells.

Conclusion: RAD51 and PARP combinatory inhibition effectively overcomes Olaparib resistance in BRCA1-mutatnt and wild-type TNBC PDX models. RAD51 inhibition decreased expression of TGF-β2 in TNBC tumors, suggesting targeting RAD51 and TGF-β2 may be a potential therapeutic strategy to overcome Olaparib resistance in triple-negative breast cancer.

#426 Dual CDK4/6 and CDK7 inhibition: A synergistic strategy to overcome resistance and enhance anti-tumor immunity in breast cancer.

Sungsoo Kim, Eugene Son, Haram Park, Minah Kim, **Hee Won Yang**

Columbia University Irving Medical Center, New York, NY

Cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) have significantly improved outcomes for patients with hormone receptor-positive (HR^+) breast cancer. However, resistance to CDK4/6i limits their long-term efficacy, and their monotherapy has shown limited impact in triple-negative breast cancer (TNBC). Here, we present a novel therapeutic strategy combining CDK4/6i and CDK7 inhibitors (CDK7i) to address these limitations. Using a comprehensive suite of approaches—including live-cell imaging, RNA sequencing, and preclinical tumor models—we demonstrate the efficacy of this combination in overcoming drug resistance and enhancing anti-tumor immunity.Kinetic analyses revealed that CDK7i primarily suppresses RNA polymerase II-mediated transcription, a key driver of CDK4/6i resistance. Mechanistically, CDK7i prevents E2F activity amplification following retinoblastoma protein (Rb) degradation, a critical step in the development of resistance. Consequently, combining CDK4/6i and CDK7i synergistically suppresses E2F-driven transcriptional programs, resulting in robust inhibition of tumor proliferation in both HR^+ and TNBC models.In vivo studies using syngeneic and patient-derived xenograft organoid models showed that this combination therapy not only suppresses tumor growth but also reprograms the tumor microenvironment (TME). RNA sequencing of treated tumors revealed upregulation of immune response pathways, including IFN-γ and TNF-α signaling, and increased expression of cytokines such as Cxcl9, Cxcl10, and Ciita. Flow cytometry and immunohistochemistry further confirmed enhanced infiltration of $CD8^+$ T cells and natural killer cells into the TME, accompanied by a reduction in immunosuppressive macrophages. These changes collectively amplify anti-tumor immunity, providing an additional therapeutic benefit.This dual CDK4/6 and CDK7 inhibition approach represents a promising first-line strategy for TNBC and a second-line option for HR+ breast cancer resistant to CDK4/6i and endocrine therapy. These findings not only provide a strong rationale for advancing CDK7i into clinical trials but also highlight the therapeutic potential of targeting transcriptional regulation to overcome resistance in breast cancer.

#427 Genome-wide CRISPR knockout screen identifies novel determinant of PARP inhibitor resistance in small-cell lung cancer.

Jiaqi Xiong[1], Mansi Aparnathi[1], Tony Yu[1], Lifang Song[1], Jonathan St-Germain[1], Brian Raught[1], Troy Ketela[1], Sami Ul Haq[2], Vivek Philip[1], Benjamin Lok[1]

[1]UHN Princess Margaret Cancer Centre, Toronto, ON, Canada,[2]Schulich School of Medicine & Dentistry, London, ON, Canada

Introduction: Small cell lung cancer (SCLC) is an aggressive tumor with poor prognosis. The standard-of-care treatment (chemotherapy +/- radiation) has seen little advancement over three decades, apart from the addition of immunotherapy for extensive-stage patients. PARP inhibitors (PARPi) have emerged as a promising therapeutic option in SCLC; however, their clinical application remains challenging due to variable patient responses. The biological determinants of PARPi response in SCLC are not fully understood. This study aims to identify novel biological factors that predict PARPi response in SCLC.

Methods: A genome-wide CRISPR-Cas9 knockout (KO) screen was conducted in the SBC-5 SCLC cell line using the Toronto Knockout v1 (TKOv1) library with PARPi olaparib (5 μM) as the selection pressure. Cells were collected on days 25 and 35 for genomic sequencing to determine sgRNA enrichment. Isogenic *FBXO11* and *LacZ* (negative control) KO cell lines were generated, and cell viability was assessed after 7-day PARPi (olaparib, talazoparib) treatment. Immunofluorescence staining of γH2AX foci was performed in isogenic *FBXO11* KO SBC-5 cells after 24hr talazoparib (100 nM) treatment. Proximity-dependent biotin labeling combined with LC-MS protein identification (BioID) was performed in FlpIn HEK293 cells expressing BirA*-FBXO11 fusion protein. KEGG pathway analysis of putative interactors was performed to examine enrichment of relevant biological pathways. Protein-protein interaction was assessed using proximity ligation assays (PLA). LC-MS-based global proteomic profiling was conducted in isogenic *FBXO11* KO SBC-5 cells (+/- olaparib), and gene ontology (GO) analysis was performed to identify differentially regulated pathways.

Results: The genome-wide CRISPR-Cas9 KO screen revealed that *FBXO11* loss provides a survival advantage under stringent PARPi selection. *FBXO11* KO resulted in a 2- to 20-fold increase in the IC50 of olaparib and talazoparib in three SCLC cell lines (NCI-H196, SBC-5, NCI-H69). *FBXO11* KO cells exhibited fewer γH2AX foci after PARPi treatment compared to LacZ KO ($p < 0.0001$). BioID of FBXO11 identified putative protein interactors from a pre-mRNA splicing complex (XAB2, ISY1, AQR). PLA showed a significantly reduced interaction between XAB2 and AQR in *FBXO11* KO cells ($p < 0.0001$), suggesting that FBXO11 affects pre-mRNA splicing complex formation. GO analysis of global proteomics showed an upregulation of pathways involved in apoptosis, mRNA splicing, and spliceosomal complex assembly after olaparib treatment exclusively in *LacZ* KO cells, but not *FBXO11* KO cells, which may explain the differential response to PARPi.

Conclusion: We identified a novel gene, *FBXO11*, whose loss results in PARPi resistance in SCLC cell lines. BioID of FBXO11 and global proteomic profiling suggests that pre-mRNA splicing may play a role in mediating drug resistance.

#428 Low cellular pH promotes survival in CDK4/6 inhibitor resistant ER+ breast cancer.

Erin R. Scheidemann, **Ayesha N. Shajahan-Haq**

Oncology, Georgetown Lombardi Comprehensive Cancer Ctr., Washington, DC

Cyclin dependent kinase 4 and 6 inhibitors (CDK4/6i) are part of standard of care therapy in combination with antiestrogens for the treatment of metastatic estrogen receptor positive (ER+) breast cancer. However, resistance to CDK4/6i is prevalent and advanced metastatic ER+ breast cancer remains incurable. Beside inhibiting the cell cycle, CDK4/6i are known to induce accumulation of lysosomes in sensitive cells but the role of lysosomes in CDK4/6i resistance remains elusive. Recently, we have shown that CDK4/6i resistant breast cancer cells gain metastatic potential relative to sensitive cells and this is potentially due to deregulated lysosomes. We have shown that all three CDK4/6i induce rapid cellular acidification in sensitive cells, which suggests a role of cellular pH in CDK4/6i-mediated cell death. Particularly, in abemaciclib resistant cells, intracellular pH is lower (pH=6.9) than that of sensitive cells (pH=7.4). Therefore, we hypothesize that a lower pH in CDK4/6i resistant cells promotes survival and this pathway can be leveraged to target resistance. Interestingly, protein levels of carbonic anhydrase IX (CA9), an important regulator of cellular pH, are significantly elevated in resistant cells. Increased CA9 levels correlate with impaired overall survival in breast and other cancers. Small molecule inhibitor of CA9, S4 or the clinical-grade drug SLC-0111, inhibits growth and invasive potential in resistant cells when combined with CDK4/6i plus an antiestrogen. CDK4/6-induced cellular acidity correlated with increased levels of hypoxia-inducible factor 2-alpha (HIF2α) protein in sensitive cells. Moreover, basal HIF2α was increased in resistant cells compared with sensitive cells. Furthermore, knockdown of HIF2α reduces CA9 protein expression and inhibits proliferation in resistant cells. Thus, our study highlights the role of the modulation of pH homeostasis as a mechanism for CDK4/6i resistant breast cancer and identifies CA9 and HIF2α as novel targets in treating advanced ER+ breast cancer.

#429 Targeting translesion synthesis to sensitize ovarian cancer cells to PARP inhibitor treatment.

Na Li, Qianyun Ge, Yajing Yang, Linzhou Wang, Xiaoli Zhang, Junran Zhang, Zaibo Li, Floor Backes, Qi-En Wang

The Ohio State University, Columbus, OH

BRCA-deficient tumor cells, characterized by defective homologous recombination (HR) repair, show high sensitivity to poly (ADP-ribose) polymerase inhibitors (PARPi). The mechanism is that PARPi blocks PARP and traps them at DNA damage sites (PARP-DNA complex), inducing replication fork stalling, leading to cancer cell death with BRCA mutation due to the accumulation of unrepaired DNA double-strand breaks. At present, PARP inhibitors have been widely used to treat ovarian cancer patients with deficient HR. However, like other chemotherapy agents, many patients acquire PARPi resistance. Replication fork protection is a key mechanism driving resistance to PARPi. Translesion synthesis (TLS) is an error-prone DNA damage tolerance pathway that can help cancer cells bypass DNA lesions and rescue replication fork stalling with Y-family DNA polymerases. Thus, we hypothesized that enhanced TLS enables BRCA-mutated ovarian cancer cells to overcome PARPi treatment via bypassing PARP-DNA complexes, and rescuing stalled replication forks, eventually leading to BRCA secondary mutations and partial restoration of HR function. To test this hypothesis, we first confirmed that PARPi can promote TLS activity by increasing the expression of monoubiquitinated PCNA in BRCA-mutated ovarian cancer cells. In addition, replication fork stalling has been observed following treatment with PARP inhibitors, as demonstrated by DNA fiber assays. Blocking TLS by knocking down REV1 exacerbates replication fork stalling, highlighting the interplay between PARPi treatment and TLS pathways. To further investigate the role of TLS polymerases, we individually knocked down TLS polymerases and evaluated their effects. Knocking down POLH (encode Polη), exacerbated Olaparib-induced replication fork stalling, irrespective of BRCA status. Functional analyses revealed that POLH knockdown sensitized BRCA-mutated cells to Olaparib but had no significant effect on BRCA wild-type cells. Next, we utilized a newly developed ChIP-based assay to assess TLS efficiency. We demonstrated that TLS inhibition through knocking down POLH or using a TLS inhibitor JH-RE-06 impairs DNA replication across Olaparib-induced PARP1-DNA complexes. Furthermore, JH-RE-06 sensitized HR-deficient ovarian cancer cell lines and patient-derived organoids to Olaparib in vitro. It also enhanced the efficacy of Olaparib in vivo, both in NSG mice bearing human BRCA1-mutated ovarian cancer cells and C57BL/6 mice with murine ovarian cancer cells harboring $P53^{-/-}$ and $BRCA1^{-/-}$ genotypes. In summary, our findings reveal that TLS facilitates replication fork recovery by enabling DNA replication across PARPi-induced PARP-DNA complexes, with Polη playing a key role. Importantly, JH-RE-06 represents a promising strategy to enhance the therapeutic sensitivity of HR-deficient ovarian cancer cells to PARP inhibitors like Olaparib.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#43 Establishment and characterization of prostate adenocarcinoma XPDX models from naïve and pretreated patients.

Johnnie Flores[1], Alyssa Simonson[1], Anna Stackpole[1], Jennifer Garcia[1], Shaquille Johnson[1], Pedro Cesenes[1], Mia Lopez[1], Peter Forofontov[1], Lisa Gonzales[1], Garrett Mercer[1], Amarissa Rojas[1], Nehal Lakhani[2], Emiliano Calvo[3], Andrew Cunningham[1], Michael J. Wick[1]

[1]XenoSTART, San Antonio, TX,[2]START Midwest, Grand Rapids, MI,[3]START Madrid, Madrid, Spain

Background: Preclinical models of prostate adenocarcinoma (PAC) are challenging to develop and maintain. We previously reported (AACR2022/ENA2023) the successful establishment and characterization of four parental and three conditioned prostate adenocarcinoma models in intact immunodeficient male mice. Using this experience, we have expanded our platform with six additional PAC models designated ST6084, ST6838, ST7482, ST7695, STF1159 and STM344. These models were characterized for receptor expression, genomic alterations, and in vivo drug sensitivity to relevant therapies.
Methods: XPDX models representing prostate cancer were established from primary (ST6084, ST6838, ST7695, STF1159) or metastatic (ST7482, STM344) samples implanted into intact CB-17 SCID mice. Resulting models were passaged serially and further developed in both intact and castrated mice until growth stabilization. Resulting models were characterized using genomic analysis, including WES and RNA_{seq}, receptor expression, and in vivo drug sensitivity studies. For in vivo studies, docetaxel, abiraterone and enzalutamide were evaluated at standard treatment regimens. Study endpoints included tumor volume and time from treatment initiation with %T/C values and tumor regression reported at study completion; a %T/C of ≤ 20% versus control was considered sensitive. Tumor regression (%T/C<0%) versus Day 0 tumor volume was also reported.
Results: Most models lacked AR or PSMA staining but ST6838 and STM344 were positive for AR-V7. Genomic characterization identified KDM6A loss in ST7482, a model from a patient post-apalutamide. An ATM aberration was present in both ST7482 and STM344, the latter derived from a patient post-chemo and targeted therapies including a five-month response on an ATR inhibitor. No tested models were sensitive to abiraterone or enzalutamide (%T/C>20%) and only ST7482 was sensitive to docetaxel.
Conclusion: We have established and characterized an expanded panel of PAC XPDX models. These models can be utilized as a valuable tool in better understanding prostate cancer and in developing novel therapies for drug-resistant patients.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Drug Resistance in Molecular Targeted Therapies 1
Poster Session

#430 Therapeutic benefits of maintaining CDK4/6 inhibitors and incorporating CDK2 inhibitors beyond progression in breast cancer.

Jessica Armand, Sungsoo Kim, Kibum Kim, Eugene Son, Minah Kim, Hee Won Yang

Pathology and Cell Biology, Columbia University Irving Medical Center, New York City, NY

The combination of CDK4/6 inhibitors (CDK4/6i) and endocrine therapy has revolutionized treatment for hormone receptor-positive (HR+) metastatic breast cancer. However, the emergence of resistance in most patients often leads to treatment discontinuation with no consensus on effective second-line therapies. The therapeutic benefits of maintaining CDK4/6i or incorporating CDK2 inhibitors (CDK2i) after disease progression remain unclear. Here, we demonstrate that sustained CDK4/6i therapy, either alone or combined with CDK2i, significantly suppresses the growth of drug-resistant HR+ breast cancer. Continued CDK4/6i treatment induces a noncanonical pathway for retinoblastoma protein (Rb) inactivation via post-translational degradation, resulting in diminished E2F activity and delayed G1 progression. Importantly, our data highlight that CDK2i should be combined with CDK4/6i to effectively suppress CDK2 activity and overcome resistance. We also identify cyclin E overexpression as a key driver of resistance to CDK4/6 and CDK2 inhibition. These findings provide crucial insights into overcoming resistance in HR+ breast cancer, supporting the continued use of CDK4/6i and the strategic incorporation of CDK2i to improve therapeutic outcomes.

#431 Novel WRN drug resistant CDX models.

Haiting Dai, Tiejun Bing, Huayi Qu, Huawei Pan, **Wen-Jen Yu**

ICE Bioscience INC, Beijing, China

Microsatellite Instability (MSI) is a condition characterized by the presence of mutations in microsatellite regions of DNA. This can lead to the accumulation of mutations in microsatellite regions, resulting in genomic instability and happened in various cancer types. Werner helicase (WRN) plays an important role in highly MSI (MSI-H) cancer cells and cause synthetic lethality when WRN was inhibited or knockout. Few WRN inhibitor like HRO761 and VVD-214 were revealed effectively killing the MSI-H cancer cells such as human colorectal cell lines HCT116 and SW48. In this study, to face the new unmet need that WRN inhibitors anti-cancer drugs may occurred, a WRN inhibitor resistant cell line against HRO761 and VVD-214 were established through the combination drug tolerance in vivo and in vitro was established. The drug resistant tumor tissues were collected after the in vivo drug resistance appeared as regrowth of tumors under 20 mg/kg HRO761 and 5 mg/kg VVD-214 treatment and dissected for primary cell culture to get the drug resistant cell line by escalating drug concentrations in vitro. The WRN inhibitors resistant cell lines were genotype and phenotype checked by STR and IC50 of cell growth when reached the drug resistant index more than 5 times of that of parental cells and indicated that different WRN inhibitors resistant HCT116 cell lines were successfully established and helpful for the discovery of anti-cancer drugs targeted on resistant to WRN inhibitors.

#432 Evolution of drug tolerance and resistance to endocrine therapy and CDK4/6 inhibitors in ER+ breast cancer.

Justin Rendleman[1], Matthew Pressler[1], Joanna Yeung[1], Lauren Anderson[1], Arnold Ou[1], Junyue Cao[1], Andrew Koff[2], Sarat Chandarlapaty[2], Viviana Risca[1]

[1]The Rockefeller University, New York, NY,[2]Memorial Sloan Kettering Cancer Center, New York, NY

Inhibitors of CDK4/6 kinases provide a significant clinical benefit among estrogen receptor positive (ER+) breast cancer patients with advanced disease when combined with endocrine therapies (e.g., fulvestrant, letrozole). However, drug resistance to this combination therapy arises in many individuals. Some drivers have been defined that are responsible for a fraction of refractory disease, but for >50% of cases, the underlying cause of disease progression remains unknown. While inducing cell death in cancer cells is an ideal drug outcome, growing evidence indicates CDK4/6 inhibitors (CDK4/6i) can contribute to disease management by forcing cancer cells into permanent cell cycle arrest through a process known as geroconversion. We hypothesize that acquired resistance can arise from cells that are drug-tolerant and fail to complete geroconverstion, instead only entering a state of quiescence for a limited time before returning to proliferation.

Here we investigate the molecular networks and their regulators that are responsible for differential outcomes in combined hormone and CDK4/6i treatment, and the synergistic effects of these drugs on geroconversion in ER+ breast cancer models. Employing a combination of bulk and single-cell RNA- and ATAC-seq, we have generated temporal maps of the transcriptional evolution of underlying regulator networks that cultivate a resistant state in MCF7 ER+ breast cancer cells. We validated our top findings in patient tumor samples and propose targets for novel therapeutic approaches.

Our phenotypic and bulk sequencing assays displayed synergistic effects between drugs, resulting in a more rapid repression of cell cycle genes in combined therapy during early timepoints (<5 day), as well as a more durable suppression of S-phase entry at later time points (>15 days). By applying sci-RNA-seq we identified gene expression patterns that are specific to these drug-tolerant subpopulations, which we have validated at the protein level. Interestingly, we found that endocrine therapy imposed a distinct chromatin and transcriptional landscape detectable as early as 9 days of treatment which persisted through 40 days of treatment. Activated genes included cell surface proteins that could distinguish this drug-tolerant population and demarcate the bottleneck period between initial response and inevitable progression.

Our evidence suggests that while endocrine therapy alone can induce both apoptosis and senescence, the additional layer of proliferative suppression afforded by CDK4/6i further delays the evolution and expansion of endocrine resistant cells that emerge. Future directions aim to validate our putative biomarkers of drug resistance using 3D patient-derived organoid models, *in vivo* xenografts, and additional patient clinical data to assess the robustness of the drug resistance signatures we have identified.

#433 Clarification and overcoming the resistance mechanisms to inotuzumab ozogamicin and ponatinib in B-ALL.

N. Ida[1], M. Okura[2], N. Hosono[1], T. Yamauchi[1];
[1]University of Fukui Hospital, Fukui, Japan, [2]Dokkyo Medical University Saitama Medical Center, Saitama, Japan

[Introduction] Recently, the prognosis of B-cell acute lymphoblastic leukemia (B-ALL) has significantly improved with the appearance of novel drugs, including inotuzumab ozogamicin (IO) and ponatinib. IO is an anti-CD22 antibody conjugated with calicheamicin and highly effective for B-ALL. Ponatinib, a third-generation tyrosine kinase inhibitor for Philadelphia chromosome (Ph)-positive ALL. The acquisition of resistance to these latest drugs is a major clinical hurdle. In the previous study, we showed that the addition of polyadenosine diphosphate-ribose polymerase (PARP) inhibitors synergistically enhanced the cytotoxicity of IO via the inhibition of the leukemic DNA damage repair mechanism. The present research attempted to clarify and overcome the resistance mechanism to IO and ponatinib using newly established drug-resistant cell lines.
[Method] Gene expression were analyzed using DNA microarray. Protein expression was analyzed by western blotting. Quantitation of DNA damage and repair was measured using the alkaline comet assay. Additional aberrations of Ph were examined by G-banding and FISH analysis. *BCR::ABL* point mutations were analyzed by direct sequencing.
[Results] We successfully established 6 IO-resistant cell lines that were 60-fold more resistant to IO than the parental cell line. All 6 cell lines were also resistant to apoptosis. In resistant cells, DNA microarray showed that *ABCB1* gene encoding p-gp, drug efflux pump, was overexpressed, and western blotting revealed the overexpression of p-gp protein. The combination of IO and a p-gp inhibitor, PSC-833, decreased the IC_{50} values of IO by 90% and increased apoptosis. Alkaline comet assay revealed increased DNA damage in the combination. When combined with IO and PARP inhibitors, olaparib and talazoparib, increased the IO sensitivity of resistant cell lines by 50 to 90%. The triple combination of p-gp inhibitor, PARP inhibitors and IO improved the IO sensitivity to the same level as the parental cell line. The alkaline comet assay indicated DNA damage observed at 1 hour after administration of IO and restored after 6 hours. However, in the triple combination, DNA damage that was observed 1 hour more increased after 6 hours. In the ponatinib-resistant cell line, the IC_{50} value of the ponatinib was 100-fold higher than in the parental cell line. No additional chromosomal abnormalities other than Ph were detected in G-banding in the ponatinib-resistant cell line. The *BCR::ABL* breakpoint was the same , and no point mutations were detected in parental and resistant cell lines. DNA microarray showed that *JAK2* expression was upregulated in resistant cell line.
[Conclusion] The present study indicated that inhibition of drug efflux and DNA damage repair overcame the resistance to IO in Ph-negative B-ALL. Moreover, *JAK2* might be the therapeutic target to overcome the resistance to ponatinib in Ph-positive B-ALL.

#434 Synergistic suppression of gemcitabine resistance in PDAC by targeting STAT3 and ABCB11.

Tai-Lin Chen[1], Chi-Wei Chen[2]

[1]Department of Post-Baccalaureate Medicine, National Chung Hsing University, Taichung, Taiwan,[2]Department of Biology, Appalachian State University, Boone, NC

Gemcitabine resistance is a critical challenge in treating pancreatic ductal adenocarcinoma (PDAC). STAT3 is implicated in tumor progression and chemotherapy resistance. This study investigates the role of STAT3 in gemcitabine-resistant PDAC and evaluates the potential of a STAT3 inhibitor (napabucasin) to enhance gemcitabine efficacy. Gemcitabine-resistant PDAC cells were developed via long-term in vitro culture and in vivo xenograft models. The expression of STAT3 and its phosphorylated form (p-STAT3) was analyzed in resistant cells. Cytotoxicity assays evaluated the sensitivity of resistant and non-resistant PDAC cells to gemcitabine, napabucasin, and an EGFR inhibitor (gefitinib). RNA sequencing revealed upregulation of ABCB11, correlating with p-STAT3 levels, suggesting STAT3-driven multidrug resistance. Combination therapies were assessed in vitro using colony formation assays and in vivo using a PDAC xenograft mouse model treated with gemcitabine, napabucasin, and/or the ABCB11 inhibitor glibenclamide. Gemcitabine-resistant PDAC cells (BxPC3-GR and BxPC3-T1-GR) exhibited elevated STAT3 and p-STAT3 levels. Cytotoxicity assays demonstrated resistance to gemcitabine and gefitinib but increased sensitivity to napabucasin. Combination treatment with gemcitabine and napabucasin significantly suppressed colony formation in vitro. In vivo, combining gemcitabine with napabucasin and glibenclamide led to significant tumor growth inhibition in gemcitabine-resistant PDAC xenografts. The combination of gemcitabine, napabucasin, and glibenclamide synergistically suppresses tumor growth in resistant models, highlighting a potential therapeutic strategy to overcome gemcitabine resistance. Further studies are warranted to optimize combination therapies for clinical application.

#435 JAK/STAT1-interferon-ISGylation networks in breast cancer resistance to inhibitors of FOXM1 and CDK4/6.

Benita S. Katzenellenbogen[1], Yvonne Ziegler[1], Sandeep Kumar[1], Blake N. Plotner[1], Carlos M. Saeh[1], Grace O. Pento[1], Sung Hoon Kim[1], Rachel Schiff[2], John A. Katzenellenbogen[1]

[1]University of Illinois at Urbana-Champaign, Urbana, IL,[2]Baylor College of Medicine, Houston, TX

Resistance to targeted therapies often develops in advanced estrogen receptor (ER)-positive breast cancer, but the mechanisms underlying this resistance are still not fully known. We show that ER-positive FOXM1 inhibitor resistant cells and CDK4/6 inhibitor (Palbociclib and Abemaciclib) resistant cells all exhibit an increased JAK/STAT1-interferon responsive gene and protein signaling network with elevated interferon stimulated gene15 (ISG15). ISG15 protein is present as high intracellular free ISG15 and also as increased ISGylated protein conjugates that can be markedly reduced by treatment of resistant cells with the JAK1/2 inhibitor ruxolitinib. Likewise, resistant cells contain increased levels of ubiquitin-like conjugating enzymes HERC5 and HERC6 and ubiquitin ligases (e.g., UBE2L6) known to facilitate the transfer of ISG15 onto cell proteins. Notably, elevated ISG15 and elevated HERC5 and HERC6 are all associated with poorer relapse-free and overall patient survival. Breast cancer cells resistant to the CDK4/6 inhibitors Palbo or Abema, which are often used in first line treatment of patients with HR-positive breast cancer, and cells resistant to FOXM1 inhibitor show some similarities in the ISGylated protein patterns. However, there are also some differences observed in these patterns between Palbo and Abema and FOXM1 inhibitor (NB73) resistant cells. Upregulation of this STAT1-interferon-ISGylation network in ER-positive breast cancers displaying resistance to three different drugs suggests it may be centrally involved in supporting the drug-resistant state. Importantly, Palbo and Abema resistant cells and organoids can still be effectively growth inhibited by FOXM1 inhibitor NB73, and likewise, FOXM1 inhibitor resistant cells and organoids are sensitive to suppression of viability by Palbo or Abema. This suggests that sequential treatment approaches might be effective in overcoming resistance and enabling the suppression of these drug-resistant cancers.(Supported by Breast Cancer Research Foundation grant BCRF-083 and NIH R01CA220284 to BSK and BCRF grant BCRF-145 to RS)

#436 NRF2 activity impairs the efficacy of sotorasib in human KRAS-mutant lung adenocarcinoma.

T. Hess[1], Hansa Joshi[1], Yaas Azmoudeh[2], Gord Zhu[3], Francis Spitz[1], David Shersher[1], Samera Hamad[2]

[1]Surgery, Cooper Medical School of Rowan University (CMSRU), Camden, NJ,[2]Cooper Medical School of Rowan University (CMSRU), Camden, NJ,[3]Pathology, Cooper Medical School of Rowan University (CMSRU), Camden, NJ

Lung cancer continues to be the leading cause of cancer-related mortality in the United States, with lung adenocarcinoma (LUAD) representing the most common histological subtype. Clinical evidence has demonstrated that tumor plasticity—, the transformation between lung cancer subtypes—, plays a crucial role in the development of resistance to chemotherapy and targeted therapies. Hyperactivation of NRF2, a transcription factor that normally protects cells from oxidative damage, is implicated in the resistance to various types of therapies including chemotherapies and targeted therapies. This study aimed to identify if NRF2 induces the resistance to carboplatin, and/or to sotorasib, a targeted therapy of KRAS-mutant LUAD, through histological transformation. We used KRAS-mutant human lung adenocarcinoma cell lines, high $NRF2^{WT}$-A549, and H358 with $NRF2^{E79Q}$ activating mutation. Our results show that treatment of high-NRF2 LUAD cell lines with sotorasib induces the transformation to neuroendocrine tumors (NE) and augments the NRF2 levels in vitro. Further, this transformation increased the growth and invasion of the transformed tumor cells. Interestingly, the low-NRF2 version of H358 (without $NRF2^{E79Q}$ activating mutation) did not have these changes. Further, LUAD cell lines had good responses to carboplatin with and without NRF2 activation. This NRF2-treatment interaction study shows that carboplatin might be a good option in NRF2-high LUAD with KRAS mutation and that targeting NRF2 in $NRF2^{high}$ LUAD tumors could enhance the efficacy of sotorasib and prevent the transformation after treatment. Current studies in our lab are testing these changes in vivo using immunodeficient mice.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#44 PDX-derived cellular models for pharmacological screening and functional studies.

Lea Sinayen[1], Enora Le Ven[1], Emilie Indersie[1], Ester Morgado[2], Laurent Pouyet[2], Francois Romagne[2], **Olivier Deas**[1]

[1]XenTech, Evry-Courcouronnes, France,[2]MImAbs, Marseille, France

Despite substantial advancements in understanding the biology and genetics of cancer, the development of effective therapies is hindered by the lack of adequate experimental models that replicate the genetic diversity of this disease. Patient-derived xenografts (PDXs) have become the gold standard in preclinical oncology for the evaluation of new anticancer drug candidates. The faithful reproduction of patients' cancer features, and the ability to generate models that recapitulate the genetic heterogeneity of the patient population, confer PDXs with a critical added value in the preclinical field. Over the past 15 years, we have generated and characterized a collection of over 200 PDXs from various solid tumors that accurately reproduce the histological and molecular heterogeneity of original tumors. This panel has enabled the preclinical validation of several anticancer drugs that are now used in the clinic. Although an indispensable tool for conducting preclinical studies, the use of in vivo PDX systems for large-scale screening during early drug discovery is restricted by ethical, economic, and throughput limitations that limit the number of drug candidates tested. To address this challenge, we have developed a panel of PDX-derived cells (PDXDCs) that we propose as a time- and cost-effective, medium-throughput screening tool to profile the anticancer activity of early test compounds. To date, over 50 PDXDCs from various indications such as breast, prostate, lung, and several others have been generated and tested for their in vitro response to standard of care, targeted anticancer agents, and immunotherapies, used as monotherapy or in combination to study potential synergistic effects. PDXDCs were obtained from dissociated PDX tumors cultured under various media and matrix conditions. They were characterized by comparison with the parental PDX through short tandem repeat (STR) profiling prior to establishing a master bank. PDXDC RNA and exome sequencing data faithfully match the parental PDX features, and by modulating experimental parameters, such as 2D or 3D growth conditions, drug exposure duration, and endpoint readouts, we could phenocopy in vitro the corresponding PDXs' sensitivities to drug candidates. These results support our PDXDCs panel as a valuable in vitro tool for drug screening to help selecting drug candidates for further in vivo validation, as well as the most appropriate in vivo PDX models to use.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Epigenetic Targets
Poster Session

#440 Dual epigenetic targeting of hepatocellular carcinoma.

Kyounghyun Kim, Ponmari Guruvaiya

University of Arkansas for Medical Sciences, Little Rock, AR

Despite advancements in medical treatment, hepatocellular carcinoma (HCC) remains a leading cause of cancer-related mortality globally. Combination therapies involving multiple targeted agents are increasingly recognized as effective strategies to improve treatment outcomes and overcome the limitations of single-agent therapies. While single-target approaches have shown specificity and potency, they face challenges such as drug resistance, limited pharmacokinetics, and patient adherence. Given the complex nature of HCC, targeting multiple molecular pathways through combination strategies holds promise for more robust therapeutic outcomes. Epigenetic therapies offer a distinct advantage in treating HCC by targeting reversible modifications to reprogram aberrant gene expression and address tumor heterogeneity. These therapies can enhance the efficacy of existing treatments, overcome resistance mechanisms, selectively target cancer cells, and modulate the immune microenvironment, making them a promising avenue for improving HCC outcomes.
EZH2 (Enhancer of Zeste Homolog 2) is a histone methyltransferase that catalyzes the trimethylation of histone H3 at lysine 27 (H3K27me3), leading to the silencing of tumor suppressor genes and the promotion of stem cell-like features. LSD1 (Lysine-specific demethylase 1) is a histone demethylase that modulates gene expression to promote tumor progression, cancer stemness, drug resistance, and epithelial-mesenchymal transition (EMT). These epigenetic enzymes form a corepressor complex to inhibit tumor suppressor gene expression. Both EZH2 and LSD1 are overexpressed in HCC and are strongly associated with poor clinical outcomes. Patients with high expression levels of both EZH2 and LSD1 exhibit the poorest survival, suggesting that dual inhibition of these enzymes may offer a more effective therapeutic strategy than monotherapy in HCC.
Dual inhibition of EZH2 and LSD1 using GSK126 (an EZH2 inhibitor) and SP2509 (an LSD1 inhibitor) more effectively suppresses liver cancer cell proliferation compared to single-agent treatments. This combination reduces cell proliferation markers, including PCNA and Cyclin D1, and is associated with enhanced programmed cell death. Additionally, we observed decreased cell migration, invasion potential, cancer stem cell characteristics, and drug resistance. Gene Set Enrichment Analysis (GSEA) revealed that high expression of either EZH2 or LSD1 is linked to crucial processes such as cell proliferation, migration, invasion, cancer stemness, and EMT, reinforcing the potential of dual epigenetic targeting. Although EZH2 and LSD1 inhibitors are currently undergoing clinical trials for various cancers, their combination has not yet been explored in HCC. Therefore, evaluating this dual epigenetic therapy for HCC treatment represents a promising direction for future research with immediate translational potential.

#441 Evaluation of the effects of MDM2 inhibitor and epigenetic modifiers in combination with AURKB inhibitors for treating lung cancer.

Neha Tumati[1], Umamaheswari Natarajan[2], Shyam Sundar Jaganathan[3], Appu Rathinavelu[4]

[1]Rumbaugh-Goodwin Institute for Cancer Research, Farquhar Honors College, Halmos College of Arts and Science, Nova Southeastern University, Fort Lauderdale, FL,[2]Rumbaugh-Goodwin Institute for Cancer Research, Barry and Judy Silverman College of Pharmacy, Farquhar Honors College, Halmos College of Arts and Science, Nova Southeastern University, Fort Lauderdale, FL,[3]Rumbaugh-Goodwin Institute for Cancer Research, Barry and Judy Silverman College of Pharmacy, Nova Southeastern University, Fort Lauderdale, FL,[4]Rumbaugh-Goodwin Institute for Cancer Research, Barry and Judy Silverman College of Pharmacy, Farquhar Honors College, Nova Southeastern University, Fort Lauderdale, FL

Lung cancer remains one of the leading causes of cancer-related deaths globally, primarily due to its aggressive nature, high metastatic potential and resistance to treatment. It is classified into two main subtypes: non-small cell lung cancer (NSCLC), which accounts for approximately 85% of cases, and small cell lung cancer (SCLC), making up the remaining 15%. Despite significant advances in early detection and treatment, lung cancer continues to pose a major challenge because of its resistance to therapy and its ability to metastasize rapidly. A promising avenue for more effective treatments lies in understanding the molecular mechanisms that can trigger lung cancer progression. Specifically, Aurora kinase B (AURKB) has emerged as an important oncogenic protein involved in regulating cell cycle progression, and its dysregulation is implicated in various cancers, including lung cancer. The interaction of AURKB with MDM2 is of interest due to its potential role in tumor progression and resistance to chemotherapy. In this study, we aimed to explore the relationship between AURKB and MDM2 in lung cancer cells, to determine where and how these proteins interact to support cancer growth. We also examined the anti-cancer effects of RG-7388, SAHA, CM-272, and Barasertib (an AURKB inhibitor), in modulating cell cycle regulation. Our results demonstrate that inhibition of AURKB induces cell cycle arrest and apoptosis in lung cancer cells. Additionally, treatment with RG-7388 activates the p53 pathway, leading to upregulation of p21 and subsequent cell cycle arrest and cell death. Interestingly, SAHA and CM272 both exhibited inhibitory effects on tumor growth by modulating AURKB through other regulatory proteins. Western blot and Immunofluorescence analysis of A549 and H460 cells after drug treatments, revealed differential responses of protein involved in cell cycle regulation, Cell Death, and epigenetic alterations. Interestingly, treatment of A549 and H460 cells with RG-7388, CM-272, and SAHA) led to upregulation of p53, p21, p27, ALDH1A1, PARP, BAX, Caspases 3, and Caspases 7. On the other hand, MDM2, AURKB, CDC25C, Cyclin A, Cyclin D, CDK4, CDK6, BCl-XL, and XIAP levels were downregulated after above mentioned treatments. Our findings confirm that AURKB levels can be down-regulated by using the combination of MDM2 inhibitors and epigenetic regulators such as SAHA and CM-272. However, the therapeutic efficiency of these combinations needs to be verified using *in vivo* experimental models.

Funding & Acknowledgements: This work was supported by the President's Faculty Research and Development Grant and also the authors would like to thank Royal Dames of Cancer Research, Inc. (Ft. Lauderdale, FL, USA) for their financial support in conducting this research.

#442 Dual KAT6/7 inhibition disrupts epigenetic programs that promote tumor evolution and adaptive drug resistance.

Manav Gupta, Pranav Gupta, Scott R. Tyler, Nour Ghaddar, Steven L. Spivak, Zabrisky Roland, Katelyn N. Lukas, Ivan G. Shabalin, Jonathon S. Ryan, Carl Schultz, Daniel M. Walden, Santosh Keshipeddy, Muzaffar Alam, Daniel Severance, Anjali Bisaria, Kelly S. Tego, Parker Y. Jameson, Mason J. Appel, Brittany Cruzan, Jay Prakash Jain, Michael E. Dalziel, Sunjay Sethi, Rebeca Choy, Richard Zang, Xin Linghu, Yuchen Bai, Peter Teriete, Diana M. Munoz, Christian R. Frey, Claire L. Neilan, Josh Taygerly, Jacob Burch-Konda, Paul A. Barsanti, Michael A. White, Brian T. Jones

Ideaya Biosciences, South San Francisco, CA

The formation of cancer and responsiveness to therapy is governed by a constantly evolving balance between somatic variation and dynamic epigenetic regulation. This evolution has been recognized as a significant obstacle confronting the development of durable cancer therapies. Thus, targeting epigenetic reprogramming in human cancers is a key focus for the design of new drugs to potentially deliver robust and durable anti-tumor activity. Lysine acetyltransferases (KATs) are a promising class of epigenetic targets that operate within interchangeable multi-subunit complexes to regulate cell cycle, pluripotency and lineage determination. Within this class, KAT6A and KAT7 have been recognized as mechanistically intertwined modulators of stem cell identity and lineage commitment through partially overlapping control of chromatin organization specified by histone H3 acetylation of lysine 23 (H3K23) and lysine 14 (H3K14). We noted multiple lines of orthogonal genetic evidence for a “paralog” relationship between these enzymes to support cancer cell autonomous survival in lineage-enriched subsets of the CCLE associated with predictive molecular features. These observations prompted evaluation of concomitant inhibition of KAT6A and KAT7 as a therapeutic approach in biomarker-defined patient populations. Here, we report an equipotent KAT6/KAT7 dual inhibitor with high selectivity versus the structurally similar KAT family members KAT5 and KAT8. We observed synergistic impact on cell viability and pharmacodynamic (PD) modulation of H3K23/H3K14 acetylation in biomarker positive breast, lung, and esophageal cancers with dual KAT6/7 inhibition versus selective inhibition of KAT6 or KAT7. *In vitro* transcriptomic evaluation of dual KAT6/7 drug effects in ER+ metastatic breast cancer models recapitulated convergent paralog behavior observed using genetic suppression with RNAi. Quantitative transcriptomic and epigenomic profiling of cancer cell responses to dual KAT6/7 inhibition confirmed downregulation of gene expression programs supporting lineage specification, stemness, and WNT signaling. *In vivo* efficacy studies in 8p11-amplified breast and lung xenografts confirmed enhanced tumor growth inhibition and PD modulation responses for dual KAT6/7 versus selective KAT6 inhibition. Notably, dual KAT6/7 inhibition also promoted improved durable and stable anti-tumor responses in endocrine therapy resistant and ESR1-mutant breast cancer patient derived xenografts that could not be achieved with selective KAT6 inhibition alone. Taken together, these observations support evaluation of dual KAT6/7 inhibition in biomarker-selected patient populations.

#443 Targeting therapeutic vulnerabilities mediated by epigenetic reprogramming in ARID1A and ARID1B dual-deficient gynecologic cancers.

Rebecca Ho, Bengul Gokbayrak, Eunice Li, Shary Chen, Emma Guo, Chae Young Shin, David Huntsman, Nathan Lack, Yemin Wang

University of British Columbia, Vancouver, BC, Canada

Background: Dedifferentiated carcinoma of the uterus and the ovary (DDEC collectively) are rare gynecologic cancers made of low-grade differentiated adenocarcinoma of the uterus or ovary juxtaposed against undifferentiated carcinoma. The two components are clonally related, and previous studies have suggested that loss of various switch/sucrose non-fermentable (SWI/SNF) proteins may contribute to the transformation of differentiated cancer cells to an undifferentiated state. Specifically, a third of all DDECs have co-inactivation of cBAF-specific subunits ARID1A and ARID1B. cBAF is one of the three major SWI/SNF complex subfamilies, and loss of ARID1A/B disrupts the chromatin remodeling function of cBAF. We hypothesize that epigenetic reprogramming in response to loss of cBAF activity will alter residual SWI/SNF complex assembly, creating vulnerabilities in DDEC that will allow for novel therapeutic opportunities. **Methods:** We generated an isogenic cell line with ARID1A/B dual loss to narrow down vulnerabilities in dual-deficient cells. Using this isogenic pair, we conducted a drop-out screen using EPIKOL, a CRISPR knockout library targeting epigenetic modifiers and cofactors. To validate the results, we genomically depleted and treated cells with small molecules to inhibit protein function. Additionally, to further understand the changes in SWI/SNF complex assembly when cBAF is lost, we conducted density gradient sedimentation assays with nuclear protein extracts isolated from the isogenic cell line pair. Finally, SWI/SNF inhibitors were added to bona fide ARID1A/B dual-deficient dedifferentiated carcinoma of the uterus and the ovary cell lines and xenografted tumors to test the generalizability of our findings. **Results:** EPIKOL screening identified that ARID1B-dependent, ARID1A-deficient cancer cells have increased reliance on ARID2 and PBRM1, two PBAF-specific subunits, upon ARID1B deletion. When *ARID2* and *PBRM1* were knocked out in ARID1A proficient and ARID1A/B dual-deficient cells, the latter had significant growth impairment. Furthermore, when treating the isogenic cell line pair with SWI/SNF inhibitors, we also noted increased sensitivity in the dual-deficient cell line. Moreover, we observed a shift towards PBAF assembly in the cells with ARID1A/B dual-loss, further supporting an increased dependency on the remaining SWI/SNF complexes in dual-deficient cells. Finally, we observed increased sensitivities towards SWI/SNF subunit inhibitors in other ARID1A/B dual-deficient ovarian and endometrial cancer cell lines and their xenografted tumors. **Conclusion:** ARID1A/B dual-deficient cancer cells have an increased reliance on the remaining SWI/SNF complexes for survival, thus highlighting a novel therapeutic option for DDEC with ARID1A/B dual loss.

#444 ASXL1 mutations in AML are associated with a distinct epigenetic state that results in vulnerabilities to epigenetic-targeted agents.

Christopher P. Mill[1], Warren C. Fiskus[1], Christine E. Birdwell[1], John A. Davis[1], Kaberia Das[1], Hanxi Hou[1], Surbhi Sharma[1], Koji Sasaki[1], Sanam Loghavi[1], Tapan M. Kadia[1], Naval Daver[1], Courtney D. DiNardo[1], Lu Wang[2], Kapil N. Bhalla[1]

[1]UT MD Anderson Cancer Center, Houston, TX,[2]Northwestern University, Chicago, IL

ASXL1 is frequently mutated in myeloid malignancies, often causing a stabilization of ASXL1 and the polycomb-repressive deubiquitinase (PR-DUB) complex, that results in a global reduction in H2AK119Ub and increased mRNA transcription. Poorer prognosis of AML with mutant (mt) ASXL1 creates an unmet need to identify and test novel therapies for this AML sub-type. We generated two knock-in (KI) models of the clinically relevant mtASXL1 Y591* into the AML cell lines OCI-AML3 and UCSD-AML1. Notably, ASXL1/BAP1 stabilization caused an increased expression of c-Myc at both the RNA and protein level. We observed via ChIP-Seq reduced H2AK119Ub at H3K27Ac-marked chromatin on Wnt/β-catenin stem cell pathway genes that resulted in increased mRNA expression of FZD5, TCF7L2, and WNT5A. KI of mtASXL1 in OCI-AML3 cells also increased G0/G1 with a concomitant reduction in S and G2/M phases of the cell-cycle. RNA-Seq analysis showed reduced expression of the REACTOME cell-cycle mitotic gene-set, associated with depletion of E2F1, AURKA/B, and PLK1 mRNA. A CRISPR screen in parental and KI cells revealed SPI1, CDK9, and EP300 among the top dependencies specific to mtASXL1 AML. Notably, compared to parental cells, OCIAML3 ASXL1 Y591* cells exhibited reduced sensitivity to standard anti-AML, chemotherapeutic agents, including cytarabine, etoposide and daunorubicin. Our findings confirm the previously published discovery that the presence of mtASXL1 confers an increased sensitivity to BETi inhibitors, e.g., pelabresib or birabresib. These cells were also more sensitive to other epigenetically targeted agents, including HATi (GNE-781 or CBP-IN-1), CDK8/19 mediator kinase inhibitor (SEL120-34A/RVU120) and the dual HAT/BETi (NEO2734/EP31670). Treatment with NEO2734 reduced the permissive chromatin marks especially on c-Myc and SPI1 super enhancers. There was also a significant reduction in mRNA and protein expressions of c-Myc, c-Myb, and PU.1. RNA-seq and mass-spectrometric analyses revealed that treatment with NEO2734 negatively enriched gene sets of c-Myc targets, rRNA processing, mRNA splicing, and translation. Treatment with SEL120 globally reduced active chromatin marks in the mtASXL1 expressing AML cells. This was associated with reduced protein expression of BAP1, pSTAT1 (S727), pSTAT5 (S726), MEIS1 and HOXA9, exclusively in mtASXL1 expressing cells. Co-treatment with pelabresib and NEO2734 or SEL120 and NEO2734 synergistically induced apoptosis in mtASXL1-expressing cells. Importantly, in the NSG mice engrafted with OCIAML3 ASXL1 Y591*, monotherapy with NEO2734, pelabresib or SEL120 significantly reduced AML burden. Collectively these findings highlight previously uncharacterized biologic effects of the presence of mtASXL1 and support the rationale for further evaluating AML therapies incorporating BETi, HAT-BETi or mediator-kinase inhibitor.

#445 Epigenetic modification induced by DNMTs inhibitor enhances apoptosis through p21 pathway in neuroblastoma cells.

Arun Jagarlamudi[1], Nicolas Tapia Stoll[1], Shyam Sundar Jaganathan[2], Umamaheswari Natarajan[3], Appu Rathinavelu[2]

[1]Rumbaugh-Goodwin Institute for Cancer Research, Halmos College of Arts and Sciences, Nova Southeastern University, Fort Lauderdale, FL,[2]Rumbaugh-Goodwin Institute for Cancer Research, Barry & Judy Silverman College of Pharmacy, Nova Southeastern University, Fort Lauderdale, FL,[3]Rumbaugh-Goodwin Institute for Cancer Research, Barry & Judy Silverman College of Pharmacy, Halmos College of Arts and Sciences, Nova Southeastern University, Fort Lauderdale, FL

Introduction: Neuroblastoma, a prevalent and aggressive pediatric cancer, poses significant treatment challenges due to its aggressive nature, rapid proliferation, and tumor heterogeneity, particularly in high-risk cases. While genetic mutations and chromosomal abnormalities play a key role in the development and progression of neuroblastoma, there is growing evidence to confirm that epigenetic changes are equally important in influencing tumor behavior, treatment responses, and patient outcomes. Emerging evidence indicates that the DNMTs inhibitor (CM-272) may facilitate cell cycle arrest and induce apoptosis by modulating critical regulatory pathways. Therefore, our hypothesis is that CM-272 treatment could lead to apoptosis mediated cell death in SK-N-SH neuroblastoma cells through the upregulation of p21 transcription.
Methods: To test our hypothesis, SK-N-SH cells were treated with 2 μM of CM-272 with untreated cells serving as control. Cell viability was evaluated using the Trypan Blue Dye Exclusion (TBDE) assay. Both qRT-PCR and western blot analyses were conducted to assess the expression levels of cell cycle, apoptotic, and epigenetic markers such as p53, p21, PARP, BAX, BCL-XL and DNMT1. The RT^2 Profiler PCR array method was used for determining the expression profile of the cell cycle pathway genes.
Results: Our study results confirmed that SK-N-SH cells treated with CM-272 undergo increased cell death. Consistent with our hypothesis, we observed an increase and decrease in BAX, BCL-XL levels, respectively, suggesting that DNMT1 inhibition might have facilitated the induction of apoptosis through a p21-driven mechanism.
Conclusion: Our results highlight the potential use of CM-272 to induce apoptosis in SK-N-SH cells through p21 upregulation and an increase in BAX levels. Furthermore, our findings suggest that targeting DNMT1 may offer an alternative pathway for inducing cell death through elevation of p21 in neuroblastoma that may have p53 mutations. If validated in further studies, DNMTs-targeted therapies could represent a significant advancement in developing new treatments for aggressive cancers.
Acknowledgements: This project was supported by the National Pediatric Cancer Foundation (NPCF). This research was also partially funded by the Bankhead Coley Infrastructure Development Grant from the Florida Department of Health that was awarded to A.R. through the Department of Pharmacology of the University of Miami (Coral Gables, Florida USA). The authors would like to thank Royal Dames of Cancer Research Inc. (Ft. Lauderdale, Florida) for their financial support in conducting this research.

#446 Embryonic ectoderm development protein (EED) inhibitor APG-5918 exhibits potent antitumor activity and synergizes with androgen receptor (AR) inhibitor enzalutamide in preclinical prostate cancer (PCa) models.

Yan Yin[1], Zhiyan Liang[2], Baisong Li[1], Yun Yang[1], Dajun Yang[1], Yifan Zhai[1]

[1]Ascentage Pharma (Suzhou) Co., Ltd., Suzhou, China,[2]Ascentage Pharma Group Inc., Rockville, MD

Background: Frequent PRC2 dysregulation in PCa is associated with poor prognosis. As a core PRC2 component, EED is essential for histone methyltransferase activity. The potential antitumor effects of APG-5918 in combination with enzalutamide were evaluated in PCa models.

Methods: Human AR-dependent LNCaP and AR-independent castration-resistant C4-2B and 22Rv1 PCa cell lines were used for *in vitro* antiproliferation assays. Cell viability was assessed using CellTiter-Glo® luminescent assay. Subcutaneous LNCaP cell-derived xenograft (CDX) and 22Rv1 CDX models in castrated nude mice were established to evaluate *in vivo* antitumor effects.

Results: APG-5918 showed superior inhibitory effects on cellular proliferation *in vitro,* with IC_{50} values of 10.16 nM in LNCaP, 26.32 nM in C4-2B, and 70.32 nM in 22RV1 cells after 14-day continuous treatment. APG-5918 appeared to be more potent than two EZH2 inhibitors (tazemetostat, PF-06821497) and EED inhibitor ORIC-944 under the same experimental condition. APG-5918 in combination with enzalutamide synergistically suppressed cellular proliferation. APG-5918 induced dose-dependent cell cycle arrest in LNCaP and C4-2B cells, increasing the proportion of cells in G0/G1 phase while decreasing those in S- and G2/M-phase by flow cytometry. Combining enzalutamide with APG-5918 deepened G0/G1 cell cycle arrest. In the LNCaP CDX model, APG-5918 100 mg/kg administered orally once daily for 28 days exerted significant antitumor activity (T/C = 40.78%; $p < 0.001$ vs. vehicle), whereas enzalutamide 20 mg/kg showed limited antitumor activity (T/C = 81.56%). In the 22Rv1 CDX model, APG-5918 100 mg/kg or 50 mg/kg had superior antitumor activity, with T/C values of 41.57% ($p < 0.001$ vs. vehicle) and 33.78% ($p < 0.001$ vs. vehicle), respectively, whereas enzalutamide 30 mg/kg for 41 days had minimal antitumor effects (T/C = 87.03%). No significant differences in body weight changes were observed between treatment and vehicle groups (all p values > 0.1 vs. vehicle), indicating a favorable safety profile. Western blot analysis showed that APG-5918 downregulated key on-target pharmacodynamic markers (e.g., H3K27me3, EED, EZH2, SUZ12) and AR pathway proteins. APG-5918 also suppressed oncogenic driver ERG, DNA methylation factors (UHRF1, DNMT1), and antiapoptotic protein MCL-1, and reduced levels of pRb, CDK4, and cyclin D1—crucial mediators of cell cycle regulation. APG-5918 combined with enzalutamide further enhanced the downregulation of PRC2 components, AR pathway proteins, cell cycle regulators, oncogenic drivers, and DNA methylation factors.

Conclusions: APG-5918 alone or in combination with enzalutamide exhibited antitumor activity in preclinical PCa models, supporting EED inhibition (± AR antagonists) as a therapeutic strategy for PCa.

#447 Preclinical development and characterization of IACS-16559, a dual bromodomain inhibitor of CBP/EP300, and its potential in AML subtypes.

Ciro Zanca, Michael Soth, Ajay Saw, Uttara Saran, Guang Gao, Jason Gay, Ningping Feng, Christopher Bristow, Joseph Marszalek, Kunal Rai, Virginia Giuliani, Tim Heffernan

UT MD Anderson Cancer Center, Houston, TX

Paralogs CBP and EP300 are transcriptional co-regulators, with a mechanism of action involving regulation of chromatin accessibility, direct acetylation of master transcriptional factors and bridging enhancers to activated promoters. They possess both reader and writer of the histone code due to the bromodomain and histone acetyltransferase domain, respectively. Aberrant transcription in cancer can occur at multiple stages, contributing to proliferation, therapeutic resistance, and escape from induced cell death, making it a good target for therapeutic intervention. Clinical validation of bromodomain inhibition of paralogs CBP and EP300 is undergoing in multiple clinical trials, including both hematological diseases and solid tumors, using EP31670, CCS1477 and TT125-802, while selective CBP or EP300 inhibition is being explored preclinically. We recently developed a dual bromodomain inhibitor for CBP and EP300, IACS-16559 with selectivity over BRD4, pharmacokinetic properties showing low clearances, high oral exposures and predicted QD dosing in human. IACS-16559 toxicity profile in rats and dogs was in line with previous reports for dual CBP/EP300 bromodomain inhibition. Repeat dose studies in rats and dogs of IACS-16559 was tolerated, with dose-dependent changes affecting bone marrow and lymphoid tissues, testes, GI tract at high doses. All the toxicology findings were reversable once the drug treatment is discontinued, and the animals allowed to recover. Given the potential for anticancer activity mediated by dual CBP/EP300 inhibition, we evaluated the response in selected subtypes of AML, both *in vitro* and *in vivo*. Single agent inhibition prolongs survival of the mice bearing orthotopic leukemia xenografts driven by several alterations, such as AML1-ETO, MLL-AF9, NPM1c, RUNX1mut. Mechanistically, CBP/EP300 dual inhibition caused disruption of the MYC and MYB oncogenic network in multiple models, required for the AML proliferation. Interestingly, the combination of IACS-16559 and MLL-Menin inhibition demonstrated synergistic effects *in vivo* with the OCI-AML3 (NPM1c) model, significantly prolonging the survival of experimental animals. However, in contrast, long-term treatment in the MLL-AF9 (MOLM14) model resulted in antagonistic effects. These findings underscore the complexities of combinatorial therapeutic development and emphasize the critical need for careful selection of robust therapeutic combinations and consideration of the genetic background in the target disease.

#449 Small molecular inhibitors of PRC1 demonstrate potent activity in acute leukemia models.

SeRa Park, Yiwu Yao, Sydney Musser, Miranda Simes, Haiqing He, Hongzhi Miao, Alyssa Winkler, Trupta Purohit, Xiaotian Zhang, Jolanta Grembecka, Tomasz Cierpicki

University of Michigan, Ann Arbor, MI

Background: Polycomb repressive complex 1 (PRC1) is an epigenetic regulatory complex that monoubiquitinates H2A on lysine 119 and is involved in gene regulation and development, resulting in the repression of target genes important for cellular identity, stemness and differentiation. Activity of PRC1 is maintained by E3 ligases RING1A or RING1B. Recent studies implicate RING1B in various cancers and highlights the importance of PRC1 function in the maintenance and proliferation of leukemic stem cells. This demonstrated that PRC1 has role in acute leukemias, supporting the need to develop PRC1 inhibitors. Therefore, development of potent small molecule inhibitors targeting PRC1 can provide novel therapeutic new approach for leukemia treatment.
Results: We have developed potent PRC1 inhibitors represented by our lead compound RB-231. We found that treatment with RB-231 induces potent anti-proliferative effects on cell growth at mid-nanomolar concentrations in multiple acute leukemia cell lines. Analysis of H2A ubiquitination using Western Blots have shown that activity of RB-231 is correlated with global reduction of H2Aub levels. Profiling of gene expression in various AML and ALL cell lines demonstrates de-repression of many genes. Through mechanistic studies using Cut&Run, we validated decrease in H2Aub signal at multiple gene loci, including CDKN1A. We performed Flow cytometry to verify apoptosis and cell differentiation in AML cells. These studies revealed that RB-231 treatment induces cellular differentiation as well as apoptosis in leukemia cells. We also tested the activity of RB-231 in primary AML patient samples. Treatment with RB-231 decreased colony formation in patient samples and induced morphological changes, suggesting differentiation of these cells from leukemic blasts to more mature hematopoietic cells. We also evaluated the activity of RB-231 in normal human CD34+ bone marrow cells to confirm no toxicity. We assessed efficacy of RB-231 *in vivo* using MV4;11, Jurkat and Reh xenografts. Treatment with PRC1 inhibitor resulted in pronounced reduction of leukemia burden and increase in survival. In accompanying pharmacodynamics PD experiments, RB-231 showed reduced leukemia hCD45+ blast level in blood, bone marrow and spleen compared to the vehicle control, and increased CDKN1A expression.
Conclusion: We developed a first- in class inhibitor directly targeting the E3 ligase activity of PRC1. Treatment with our PRC1 inhibitors in leukemic cells elicits an anti-proliferative effect, blocks ubiquitination of H2A, and results in the de-repression of many genes repressed by PRC1. Our data suggests that multiple subtypes of acute leukemia are sensitive to inhibition of PRC1. Furthermore, this study demonstrated that RB-231 is potent PRC1 inhibitor suitable for evaluation *in vitro* and *in vivo* models of acute leukemia.
Acknowledgement: This research was funded by R01CA282082 grant from NIH.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#45 PDX technology transfer: NCI cancer center to HBCU.

Teni O. Akin-Olugbemi (equal contribution)[1], De'llaijah Lucas (equal contribution)[2], Romell Stone[1], Esther Frimpong[1], Raviteja Bulusu[1], Hernan Flores-Rozas[1], Edward Agyare[1], Tanise Jackson[1], Renee Reams[1], Seth Ablordeppey[1], Brooke Hensel[3], Jose Trevino[4], Steven J. Hughes[2], Han Song[2], Christina Gobin[2], Bodour Salhia[5], Paul Okunieff[2], Kristianna Fredenburg[2], John M. Allen[3], Bereket Mochona[1], Diana J. Wilkie[2]

[1]Florida A&M University, Tallahassee, FL,[2]University of Florida, Gainesville, FL,[3]University of Florida, Orlando, FL,[4]Virginia Commonwealth University, Richmond, VA,[5]University of Southern California, Los Angeles, CA

We profile a technology transfer process between two partnering research institutions and the early outcomes. The Partnerships to Advance Cancer Health Equity (PACHE) program seeks to increase cancer research at HBCUs via collaborations with NCI-designated cancer centers. One such partnership, the Florida-California Cancer Research Education and Engagement (CaRE^2) Health Equity Center, completed a series of studies with Patient Derived Xenograft (PDX) models to test novel compounds and delivery systems as treatment for pancreatic cancer. PDX models involve the implantation of human tumor tissues into immune incompetent mice for studies that mirror the human context. Few pancreatic cancer PDX models exist for Black patients, at high risk for pancreatic cancer incidence and mortality. For early studies, the cancer center Tissue Modeling & Drug Development Core (TMDDC) shipped the PDX models to the HBCU after implantation in the 25 mice needed for each study. To increase research capacity at the HBCU after these successful studies, the HBCU investigators, students, and a veterinarian were trained at the cancer center lab to implant the PDX tumor tissue in immune incompetent mice. The TMDDC shipped frozen PDX tumor tissue from a Black male patient to the HBCU, which the veterinarian implanted in 3 mice (Generation 1; Gen1). Tumor growth occurred in 1 of 3 mice (33%). The Gen1 tumor is now being expanded by implantation into 5 additional mice (Gen2) for future implantation in the 25 mice (Gen3) that are needed for the planned studies. The TMDDC shipped tissue from a Black female patient's PDX tumor to the HBCU for replication of Gen1-3 procedures. In support of 2 CaRE^2 projects, the 50 mice (25 from a Black male, 25 from a Black female) with the Gen3 PDX tumors will be used at the HBCU to (1) test effects of a modified gemcitabine compound delivered by nanotechnology, and (2) test a cfDNA biomarker of tumor response. Two postbaccalaureate trainees (1 at cancer center, 1 at HBCU) worked with the TMDDC at each institution as part of the technology transfer from the cancer center to the HBCU. The trainees' important contribution to the technology transfer process involved documentation of the standard operating procedures for receiving the PDX tissues, implementing the expansion Gen1-3 steps for research at the HBCU, and building a well-annotated, living repository of pancreatic PDX tissues at the HBCU for ongoing cancer health disparities research studies and for training of the HBCU graduate students in the use of these technologies. This successful technology transfer enables cutting edge training for the HBCU students and facilitates their success as they enter the cancer health disparities research workforce. Furthermore, this project demonstrates the hallmark of a successful PACHE partnership, specifically the complementary collaboration among the CaRE^2 Projects, Research Education Core, TMDDC, and Administrative Core to enable this technology transfer to the HBCU.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Epigenetic Targets
Poster Session

#450 Discovery of a highly potent KAT6 Inhibitor for potential treatment of advanced, metastatic and refractory ER+Her2- breast cancers.

Congcong Cai, Shiya Zhu, Linjun Wang, Shu Cai, Hongkun Li, Min Wen, Mingxin Qian, Shifeng Liu, **Bin Shi**, Hancheng Zhang

Hangzhou Innogate Pharma Co., Ltd, Hangzhou, China

Frequent genomic alteration of KAT6A and KAT6B have been found in a variety of cancer types. Histone acetyltransferases (HAT) KAT6A and KAT6B can acetylate lysine residues of histone H3 (including H3K23), leading to a relaxing state of chromatin and active transcription of genes regulating cell cycle, cellular senescence, and tissue development; to name a few, genes encoding ER (estrogen receptor), Myc, and Cyclin D1. Inhibition of these processes exerts an anti-tumor effect in many cancer types. We, Innogate Pharma (Innogate), have discovered a novel and highly potent series of small molecule KAT6 inhibitors with strong in vitro potency and robust in vivo anti-tumor efficacy.
Innogate's KAT6 preclinical candidate compound (PCC) exerts single digit nM IC_{50} activities against KAT6A/6B enzymes with good selectivity over KAT5, KAT7, KAT8. The molecule also demonstrated anti-proliferation activity at sub-nM IC_{50} in ZR-75-1 and T47D. We also tested enzyme kinetics and found the molecule have favorable binding profile to KAT6A in a fast-on-slow-off manner. The PCC molecule can be converted to salts with potential advantages in CMC development and showed a favorable solubility profile independent of GI pH variation. In ER+Her2-breast cancer ZR-75-1 xenograft models, the molecule at 0.3 mg/kg /PO/QD for 28 days leads to significant tumor growth inhibition and regression with TGI 129% at the termination day 28. Furthermore, in a fulvestrant resistant, ER+HER2- breast cancer PDX model, the molecule displayed excellent anti-tumor efficacy at 10 mg/kg /PO/QD, with tumors completely disappeared on day 22 and without safety issues observed, while the positive control compound still had palpable tumors on termination day 28 at the same dose. In other preclinical studies, the molecule holds good in vitro ADME properties and PK profiles in mice, rats and beagle dogs, favoring a potential once daily oral dose regime in the future clinical setting. The molecule also exhibited good in vitro safety profile in hERG and well tolerated in rodent study. Collectively, these results provide preclinical evidence that our PCC molecule could be a superior KAT6A/6B inhibitor to treat patients with advanced, metastatic, refractory, fulvestrant-sensitive or-resistant, ER+Her2- breast cancers.

#451 Superior preclinical efficacy of BRG1/BRM inhibitor combined with BET inhibitor or decitabine against MECOM-rearranged AML.

Warren C. Fiskus[1], Christopher P. Mill[1], Jessica Piel[2], Murphy Hentemann[2], Christine E. Birdwell[1], Kaberi Das[1], Hanxi Hou[1], John A. Davis[1], Surbhi Sharma[1], Tapan M. Kadia[1], Naval Daver[1], Sanam Loghavi[1], Courtney D. DiNardo[1], Kapil N. Bhalla[1]

[1]UT MD Anderson Cancer Center, Houston, TX,[2]Foghorn Therapeutics, Cambridge, MA

AML with MECOM rearrangement (r) involving chromosome 3q26.2, specifically inv(3)(q21q26.2) or a translocation t(3;3)(q21;q26.2), accounts for approximately 1% of newly diagnosed AML. They involve aberrant relocation of the distal GATA2 hematopoietic enhancer with MYB binding sites, leading to EVI1 overexpression from the MDS1-EVI1 complex (MECOM) locus. EVI1 is a transcriptional regulator that plays a role in proliferation, differentiation and maintenance of the AML stem cell-like phenotype. In MECOM-r AML, due to the GATA2 haplo-insufficiency with overexpression and increased activity of EVI1, AML stem/progenitor cells exhibit aggressive proliferation, are therapy refractory and display poor overall survival. Therefore, there is an unmet need to develop and test novel targeted therapies to improve outcomes in AML with MECOM-r and EVI1 overexpression. BRG1 (SMARCA4) and BRM (SMARCA2) are the core ATPases within the chromatin remodeling BAF complexes that regulate gene transcription. FHD-286 is a potent, selective, first in the class inhibitor of BRG1/BRM in clinical development as an AML therapy. Our present studies demonstrate that exposure to FHD-286 (10 to 100 nM) for 4 to 7 days induced CD11b expression with morphologic features of differentiation leading to loss of viability in AML cell lines and patient-derived (PD) AML cells with MECOM-r. FHD-286 treatment caused whole-genome, concordant, up- or down-regulations in H3K27Ac ChIP-Seq peaks and the RNA-Seq-determined mRNA expressions of specific loci, including significant reduction in the gene-sets of MYC targets and repression of EVI1. Immunoblot analysis showed that FHD-286 treatment reduced EVI1 protein expression. Additionally, mass spectrometry on MECOM-r AML cells treated with FHD-286 showed log2 fold-reductions in EVI1, c-Myc, CD44, c-KIT, CD93, CDK4 and PLK1 protein levels. In mass cytometry-determined, phenotypically defined AML stem/progenitor cells, FHD-286 treatment also reduced EVI1 expression. Co-treatment with FHD-286 and decitabine, BET inhibitor (OTX015), or HAT inhibitor GNE-781 exerted synergistic in vitro lethality against MECOM-r AML cell lines and PD AML cells (Delta synergy scores > 5 by the ZIP method). Finally, as compared to treatment with each drug alone or vehicle control, in PD xenograft (PDX) models of AML cells with MECOM-r, co-treatment with FHD-286 and decitabine or OTX015 significantly reduced AML burden and improved overall survival of the NSG mice, without inducing significant toxicity. Taken together, these findings highlight the promise of FHD-286 treatment alone and in rational combinations in exerting significant anti-AML efficacy against cellular models of AML, especially those with MECOM-r and EVI1 overexpression.

#452 ORIC-944, a PRC2 inhibitor with best-in-class properties, restores luminal features and restricts adaptation in prostate cancer models, conferring synergy with AR pathway inhibitors.

Anneleen Daemen, Alexandr Pankov, Natalie Yuen, Amber Wang, Livia Ulicna, Xi Chen, Frank L. Duong, Jason E. Long, Robert Warne, Lori S. Friedman, **Melissa R. Junttila**

ORIC Pharmaceuticals, Inc., South San Francisco, CA

Polycomb repressive complex 2 (PRC2) silences transcription by tri-methylating histone H3 at lysine 27 (H3K27me3), regulating cell growth and differentiation. The EED subunit is required for chromatin recruitment and histone methyltransferase activity. PRC2 dysregulation is linked to poor prostate cancer prognosis, promotes lineage plasticity and therapeutic resistance. Combining androgen receptor pathway inhibitors (ARPI) with PRC2 inhibitors may overcome or prevent resistance, with clinical trials showing promise in metastatic castration-resistant prostate cancer. First-generation PRC2 inhibitors exhibit poor drug properties and short half-lives, requiring at least twice daily dosing at high doses. ORIC-944 is a second-generation allosteric inhibitor of PRC2 that binds EED and exemplifies best-in-class drug properties including potency, solubility, and ADME/PK, with half-life supporting once daily dosing. The mechanism of PRC2 in prostate cancer lineage plasticity was explored with RNA-seq, H3K27me3 ChIP-seq, and ATAC-seq data from in vitro and in vivo studies of ORIC-944 as a single agent and combined with ARPI. No evidence for a non-canonical EZH2 role was found comparing EZH2 and EED inhibitors by RNAseq. ORIC-944 or EZH2 inhibition led to robust PRC2 inhibition, luminal state transition, and increased AR signaling in a spectrum of AR-positive in vivo prostate cancer models. In vivo kinetic studies revealed that luminal cell state transition and increased AR signaling are early events induced by ORIC-944. The luminal cell state acquired with PRC2 inhibition was confirmed by IHC, and is highly dependent on AR signaling in vivo, indicative of a functionally relevant cell state. Continuous ORIC-944 administration was necessary to maintain the luminal state and antitumor efficacy. Surprisingly, analysis of chromatin revealed that ORIC-944 specifically restricts accessibility to lineage-associated transcription factors known to impart cellular plasticity in prostate cancer. This plasticity-restricted luminal state conferred by PRC2 inhibition is dependent on AR, leading to antitumor activity either in the hormone-depleted context or in the setting of ARPI combination. Together these data indicate that ORIC-944 reinforces a luminal cell state and, notably, restricts access to plasticity genes leading to a highly AR-dependent state in prostate models. This mechanism supports the observed preclinical synergy between PRC2 inhibition and ARPI combination in both ARPI-sensitive and ARPI-resistant settings. These results position ORIC-944 as a potential best-in-class PRC2 inhibitor that blocks prostate tumor adaptation, restores luminal features, and sensitizes tumors to ARPI. A phase 1b trial of ORIC-944 in combination with ARPIs is ongoing in metastatic prostate cancer (NCT05413421).

#453 Discovery of potent, selective, and orally bioavailable small molecule inhibitors of YTHDC1 for the treatment of MYC-driven cancers.

Richard C. Centore[1], Mark Charles[2], Mansi Arora[1], Marius Rebmann[2], Jerome Cattin[2], Xuejing Yang[3], Emily Batchelor[3], Matthew Watson[2], Nagakumar Bharatham[2], Prathima Radhakrishanan[1], Alex Howarth[2], Seema Qamar[2], Laura Andraghetti[2], Martin Kulander[2], Tuomas Knowles[2], Michael G. Kharas[3], **Shilpi Arora**[1]

[1]Transition Bio, Watertown, MA,[2]Transition Bio, Cambridge, United Kingdom,[3]Memorial Sloan Kettering Cancer Center, New York, NY

The MYC family of oncogenes (including c-MYC, L-MYC, and N-MYC) has been considered one of the most sought-after drug targets in oncology. These transcription factors are critical cancer drivers but have remained difficult to drug directly with small molecules; thus, alternative approaches to regulate MYC expression or activity are of high interest. We have previously demonstrated that MYC family members are regulated by YTHDC1. In AML cells, *MYC* mRNA is post-transcriptionally modified with methylation of adenosine at the N6 position (m^6A), and m^6A-marked *MYC* transcripts localize within phase-separated assemblies, termed biomolecular condensates, with the m^6A reader, YTHDC1. YTHDC1 condensates regulate m^6A-marked *MYC* and other oncogenic transcripts and promote cancer cell proliferation including in MYC-driven AML and small cell lung cancer (SCLC). Here, we describe the identification of YTHDC1 inhibitors optimized utilizing structure-based drug design (SBDD). This series of inhibitors occupies the RNA binding pocket of YTHDC1 and disrupts its interaction with m^6A-RNA at nanomolar potencies in biochemical and cellular assays. The inhibitors are selective for YTHDC1 over other YTH family members and selectively dissolve YTHDC1 condensates over other condensate systems. Profiling in broader kinase and safety panels also suggests that these YTHDC1 inhibitors are selective with a favorable *in vitro* safety profile. Our lead molecule is drug-like, orally bioavailable, has suitable ADME properties and is well-tolerated *in vivo*. In *MYC*- and *MYCL*-driven AML and SCLC cells, respectively, we demonstrate the presence of YTHDC1 condensates, which are abrogated by YTHDC1 inhibition. Consistent with the genetic perturbation of YTHDC1, pharmacologic inhibition of YTHDC1 reduces MYC expression and results in anti-proliferative and pro-apoptotic phenotypes. Induction of myeloid differentiation markers is also observed in AML cells upon YTHDC1 inhibition. We evaluate the effects of YTHDC1 inhibition on primary patient AML samples. In addition, we demonstrated no impact on normal CD34+ progenitor cells. YTHDC1 inhibition results in *in vivo* efficacy at well-tolerated doses in multiple AML and SCLC xenograft models. Further, we demonstrate a dose-dependent PK/PD/efficacy relationship, suggesting on-target YTHDC1 activity through suppression of the MYC pathway. Thus, our data demonstrate that targeting YTHDC1 condensates represents a novel approach to the treatment of MYC-driven tumors.

#454 Leveraging cell cycle to enhance differentiation therapy in acute myeloid leukemia.

Riccardo Valzelli[1], Kourosh Hayatigolkhatmi[1], Amir Hosseini[2], Elena Ceccacci[1], Giorgia Parodi[3], Angela Bachi[4], Saverio Minucci[1]

[1]European Institute of Oncology (IEO), Milan, Italy,[2]Ludwig Institute for Cancer Research, Oxford, United Kingdom,[3]Cogentech, Milan, Italy,[4]IFOM ETS - The AIRC Institute of Molecular Oncology, Milan, Italy

Acute myeloid leukemia (AML) is a highly heterogeneous hematological malignancy characterized by diverse genetic and epigenetic aberrations, making therapeutic interventions particularly challenging. We have identified a novel combination therapy that leverages differences in cell cycle dynamics to induce AML cell differentiation, and found AML cells which were highly sensitive to inhibitors of the histone demethylase LSD1/KDM1A depending on their cell cycle profile. Here, we aim at identifying other potential epigenetic vulnerabilities of AML cell lines exposed to the treatment with different CDK inhibitors. Given the heterogeneity of the epigenetic aberrations found in AML, targeting additional chromatin regulators could improve the clinical applicability of this combination therapy. Thus, we started an in vitro CRISPR-screening approach, targeting over 600 chromatin-related genes. Following optimization of Cas9-expressing clones and evaluation of knockout (KO) efficiency, we confirmed that the best-performing clone retained phenotypic consistency with parental cells under combination therapy. Preliminary data validated the effectiveness of our screening platform, as sgRNAs targeting LSD1 recapitulated the phenotype observed with CDK inhibitors treatment, thereby demonstrating the feasibility of the approach. In parallel, we investigated the molecular link between cell cycle manipulation and chromatin remodeling. We conducted a nuclear phospho-proteomics experiment on cells treated with CDK inhibitors. This analysis identified differential phosphorylation of several proteins, which are currently under investigation. Additionally, gene ontology analysis revealed enrichment in pathways regulating RNA metabolism, transcription, and DNA damage response. By integrating cell cycle modulation with epigenetic targeting, our work offers new insights into AML differentiation therapy, with the potential to improve therapeutic outcomes in diverse AML subtypes.

#455 Targeting CARM1- expressing ovarian cancers with HSP-70 inhibitors.

S. Kumari, S. Gupta, A. Jamil, S. Karakashev;
Temple Univ. Lewis Katz School of Medicine, Philadelphia, PA

Epithelial ovarian cancer (EOC) is the leading cause of gynecological cancer-related deaths in the United States, characterized by its genetic heterogeneity and the urgent need to develop novel therapeutic strategies in a personalized manner. Coactivator-associated arginine methyltransferase 1 (CARM1) is an enzyme responsible for post-translational modifications of protein substrates on arginine residues. CARM1 is often overexpressed and functions as an oncogene in several cancer types. Notably, high-grade serous ovarian carcinoma (HGSOC) exhibits the highest rates of CARM1 amplification, with approximately 20% of cases showing overexpression, which correlates with poor survival outcomes in EOC patients. This study aimed to identify potential therapeutic targets for EOC cells with CARM1 amplification. We conducted an unbiased evaluation of a custom set of 27 small-molecule inhibitors to identify agents selectively effective against CARM1-expressing EOC cells, leading to the identification of the HSP-70 inhibitor AP-4-139B as the most potent candidate. HSP-70 is a crucial part of the protein folding machinery, and its levels are upregulated in multiple types of cancer and serve as a substrate for CARM1 enzymatic activity; however, the implications of HSP-70 modification by CARM1 remain unclear. The CRISPR-Cas9-based knockout technology was employed to analyze the effect of CARM1 knockout on cell proliferation in EOC cells, which demonstrated that CARM1 knockout significantly slowed cell growth in EOC cells. Synthetic lethality of CARM1-expressing cells upon HSP-70 inhibition by AP-4-139B was validated in both the ovarian cancer cell model system and the xenograft model. Mechanistically, AP-4-139B treatment induced apoptotic cell death in CARM1-high EOC cells through activation of the apoptotic pathway as analyzed by cell proliferation assay, apoptotic assay, and immunoblotting of apoptotic markers. Our findings indicate that CARM1-high cells are selectively sensitive to HSP-70 inhibitors as a single agent. HSP-70 inhibition alone will be sufficient to induce apoptosis and inhibit tumor growth in a CARM1-dependent manner *ex vivo* and *in vivo*. Thus, these findings will have far-reaching implications for developing personalized therapeutic strategies based on CARM1 amplification. The therapeutic and mechanistic rationale for targeted therapy on patients with CARM1 high EOC who are most likely to benefit from HSP-70 inhibitor treatment.

#456 An ultrahigh throughput phenotypic screening platform to discover novel inhibitors of methyl transferases.

Nathan G. Hedrick, Maina Ndungu, Ivy Nai-Jung Hung, Devin K. Porter, Elliot Imler, Gopi Chandran RaviChandran, Aurelien Lauerre, Chris M. Glinkerman, Martin Smrcina, Mark R. Hansen, Allan Nojadera, Alexandra Cole, Cindy Huynh, Michael Miller, Dmitri rozanov, Logan Van Meter, Nicholas Sm-Soon, Steven Brown, Yi Zhang, **Vikas K. Goel**

Zafrens Inc., San Diego, CA

Discovering drug-like molecules for hard-to-target proteins remains a significant challenge. Methyltransferases (DNA, RNA, and protein) are among such targets. At Zafrens, we have developed an ultra-high-throughput discovery platform that leverages large arrays of isolated nano-wells and single-bead-single-compound DNA-encoded library (DELs) to uncover target-specific inhibitory impacts on cells. Using a combination of next-generation sequencing and spatially indexed optical imaging of biomarkers and cell paint probes, we successfully linked transcriptomic signatures and phenotypic responses to a combinatorial chemical library of over 15,000 compounds, achieving approximately fivefold hit coverage. Incorporation of known inhibitors of a methyltransferase into the chemical library revealed that both transcriptome-driven clustering and machine learning-driven linkage of compound SMILES with cell phenotype could successfully identify reproducible signatures that were aligned well with public databases, including expected methyltransferase-dependent genes such as MTF1. The robustness and consistency of our platform have allowed us to identify several novel compounds that mirrored the transcriptomic and phenotypic profiles of the control compounds. Subsequent biochemical assays confirmed that many of these were bona fide inhibitors of the target methyltransferase. Among these, ZF165 emerged as a promising candidate due to its high hit frequency across multiple screens and across two cell lines. ZF165 was then resynthesized and further validated by both immunofluorescence- and western blot analyses for its impact on biomarker responses. Bulk transcriptomic mRNA sequencing analysis confirmed common profiles between ZF165 and a control methyltransferase inhibitor. Furthermore, ZF165 demonstrated initial anti-tumor cell activity with a potency comparable to known pre-clinical compounds, further supporting its potential as a lead molecule. With ZF165 as the new series lead, we designed a secondary DEL library featuring novel warheads and improved physicochemical properties. The secondary library, ZEL29, was synthesized and screened, yielding new hits as additional (or) providing SAR, with enhanced potency and better drug-like properties relative to ZF165. These results support the utility of our platform in both the initial hit discovery and subsequent hit optimization toward lead compounds. Validation-design cycles for lead optimization are currently ongoing. By coupling this powerful screening platform with robust data analytics, we aim to revolutionize the discovery of novel therapeutics to address other challenging drug targets.

#457 QLS1304, a highly potent and selective KAT6A/B inhibitor treating $ER^{+}HER2^{-}$ breast cancer.

Yuxing Zhang, Chundao Yang, Huijie Guo, Changliang He, Lan Zhang, Ling Li, Mengmeng Zhang, Xuejian Zheng, Ping Chen, Dong Yang, Dongdong Wu, Guqin Shi, Daqing Sun, Wenyuan Qian, Weimei Sun, Weikang Tao

Shanghai Qilu Pharmaceutical Research and Development Center Ltd., Shanghai, China

KAT6A and KAT6B, members of the MYST family of lysine acetyltransferases, are implicated in estrogen receptor-positive (ER+) breast cancer. KAT6A amplification occurs in about 10% of these patients, linking to overexpression and poorer outcomes. KAT6B is known to be functionally redundant with KAT6A in regulating levels of H3K23Ac, although its specific role in ER+HER2- breast cancer is not well understood. The simultaneous inhibition of KAT6A and KAT6B may lead to a more profound suppression of histone acetylation. Thus, the development of a dual inhibitor targeting KAT6A and KAT6B has been aggressively pursued, with the goal to offer an effective therapeutic solution for patients with ER+ breast cancer. QLS1304 is a highly potent and selective inhibitor of KAT6A/B (KAT6A Ki: 0.2 nM, KAT6B Ki: 0.4 nM; and >200-fold selectivity over KAT5/KAT7/KAT8). H3K23 acetylation was dose-dependently inhibited in ER+HER2- breast cancer cell lines, while ER expression was only suppressed in KAT6A-high cells. QLS1304 demonstrates strong in vitro and in vivo efficacy in multiple ER+HER2- breast cancer CDX models, either as a single agent or in various combinations with current standard therapies. Notably, QLS1304 has demonstrated efficacy in an ESR1 mutant ER+HER2- PDX model derived from a patient resistant to SoC. QLS1304 also exhibits desirable drug-like properties with an excellent pharmacokinetics profile across different animal species, and preclinical safety assessment also indicates a wide therapeutic window. Collectively, QLS1304 appears to be a promising candidate for the treatment of ER+HER2- breast cancer given its efficacy in both SoC-sensitive and SoC-resistant models. Moving it forward into clinical trials is warranted both as single agent and combining with other therapies.

#459 Discovery and proteomic characterization of a novel KAT6A/B inhibitor.

Gali Arad[1], Avital Hay-Koren[1], Sagie Brodsky[1], Andrew Morley[2], Iris Alchanati[1], Galina Otonin[1], Yonatan Katzenelenbogen[1], Barr Tivon[1], Alon Shtrikman[1], Dimitri Kovalerchik[1], Anjana Shenoy[1], Katharina Lange[1], Nitzan Simchi[1], Eran Seger[1], Kirill Pevzner[1]

[1]Protai, Ramat Gan, Israel,[2]O2H Discovery, Cambridge, United Kingdom

Lysine histone acetyltransferase 6 (KAT6A/B) is a MYST family member of histone acetyltransferases (HATs), known to regulate gene expression by acetylation of both histone and non-histone substrates. KAT6A/B is a promising cancer drug target, specifically in estrogen receptor positive (ER+) breast cancer, but anti-cancer activity in other cancer indications has also been shown, suggesting a potential benefit for wider patient populations. Despite antitumor activity observed in clinical trials, KAT6i inhibitors are also reported to cause several high grade adverse events, including dysgeusia, neutropenia, and anemia, in some cases leading to treatment discontinuation. The efficacy alongside tolerability profiles of KAT6i reflects the need for improved therapeutic window by developing cleaner, more selective drugs; as well as by identification of additional responsive populations.

By applying computational and medicinal chemistry approaches we discovered a novel, highly potent inhibitor of KAT6A/B, demonstrating anti-tumor activity using both in-vitro cell viability assays and tumor growth inhibition in xenograft models. We combined drug sensitivity with basal and perturbational proteomics data of cancer cell lines, in order to provide a mechanistic explanation of KAT6A/Bi mode of action. Proteomic analysis of KAT6i sensitive and resistant cell lines highlighted several candidates for KAT6 predictive biomarker and shed light on the proteome-wide effects of KAT6 inhibition. In summary, these results support KAT6i drug development both within and outside ER+ breast cancer, as well as optimization of more selective KAT6 inhibitors to improve tolerability and therapeutic window.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#46 Alternative lengthening of telomeres in patient-derived xenograft models of soft tissue sarcomas with complex genomic profile.

Agnieszka Wozniak[1], Nicola Biondi[2], Kimberly Verbeeck[1], Oliver Bechter[3], Priya Chudasama[2], Patrick Schoffski[3]

[1]Laboratory of Experimental Oncology, KU Leuven, Leuven, Belgium,[2]Precision Sarcoma Research Group, German Cancer Research Center, Heidelberg, Germany,[3]Department of General Medical Oncology, University Hospitals Leuven, Leuven, Belgium

Background: Cancer cells require telomere maintenance mechanisms (TMM) to achieve replicative immortality. About 85% of cancers utilize telomerase-mediated elongation of telomeres, and 15% of cancers use alternative lengthening of telomeres (ALT). In sarcoma ALT positivity (ALT+) is observed in 10-80% of samples, mainly in tumors with high genomic instability. They are characterized by the presence of extra-chromosomal telomeric repeats called C-circles and frequent loss of function of chromatin remodeling proteins alpha-thalassemia/mental retardation syndrome X-linked (ATRX) and/or death domain-associated protein (DAXX). To expand the utility of patient-derived xenografts (PDX) to therapeutic approaches targeting ALT pathway we aimed to characterize the type of TMM and related characteristics in PDX of sarcoma with high genomic instability.
Methods: Out of 92 established and well-characterized PDX from 27 subtypes of soft tissue sarcoma (STS) from our XenoSarc panel 22 models with complex genomic profile were selected for the analysis: 7 models of leiomyosarcoma (LMS), 6 of dedifferentiated liposarcoma (DDLPS), 4 of undifferentiated pleomorphic sarcoma (UPS) and myxofibrosarcoma (MFS), and 1 model of high grade sarcoma not otherwise specified (HG). The level of C-circles as an ALT surogate marker was quantified based on detection of products of rolling circle amplification of C-circles by Phi-polymerase. Samples with C-circle levels ≥ 0.1 were classified as ALT+. Expression levels of ATRX and DAXX were evaluated using immunohistochemistry (IHC) and low coverage whole genome sequencing (lcWGS) was performed to compare potential differences in genomic landscapes of ALT+ and ALT- PDX models.
Results: C-circle analysis revealed 14 out of 22 PDX models analyzed to be ALT+ (63%), including 5/7 LMS, 2/6 DDLPS, 4/4 UPS, 2/4 MFS, and 1/1 HG models. Out of 14 ALT+ models, loss of ATRX and DAXX expression was detected in 9 and 2 PDXs, respectively. High quality lcWGS data were successfully generated, which are currently being analyzed to detect differences between ALT+ and ALT- PDX, and will be integrated with IHC results.
Conclusion: We documented a high prevalence of ALT+ in STS PDX models with complex genomic profile. An on-going molecular characterization of these models will further support genotype-specific evaluation of TMM-associated characteristics of PDXs, such as telomeric features, will add to the validity of these ALT+ models. We consider our PDX models an appropriate platform to study candidate drugs that can target ALT pathway in STS.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Epigenetic Targets
Poster Session

#461 A screening platform to target intrinsically disordered regions (IDRs) of chromatin factors in disease.

Laura Hsieh, Philamer Calses, Muryam Gourdet

TippingPoint Biosciences, Inc., San Francisco, CA

Aberrant chromatin is expanding as a new class of disease and resistance driver for multiple disease pathologies. While billions of dollars have been poured into drugging chromatin regulatory factors, the toxicity of previous approaches limits potential and promise. Conventional drug discovery strategies have focused on targeting only the stable interfaces of chromatin regulators. However, the specific regulation of chromatin function occurs in large part from the unstructured intrinsically disordered regions (IDRs). IDRs help specify the functionality of structured domains and play a critical role in regulating both physiological and disease-driving multi-protein assemblies. TippingPoint Biosciences has developed a first-of-a-kind screening platform that now enables targeting of IDRs in chromatin proteins by exploiting unique inter-molecular interfaces only found in disease driving chromatin states. TippingPoint's approach should increase specificity, reduce toxicity and be less susceptible to drug resistance. To validate our drug discovery approach we are focusing our efforts on developing novel small molecule therapeutics for a rare pediatric brain tumor, Diffuse Intrinsic Pontine Glioma (DIPG), a disease driven by a very specific chromatin mutation that is difficult to target using conventional approaches. DIPG is driven by the H3.3 K27M mutation. Recognition of H3.3K27M by Polycomb Repressive Complex (PRC2), renders PRC2 inactive leading to a reduction in global H3K27me3. As a result, there are increased levels of H3K27ac and altered gene expression. Using our platform we generated a synthetic DIPG chromatin state, mimicking the stabilized interactions between PRC2-H3.3K27M chromatin IDRs. We successfully screened thousands of molecules against the DIPG chromatin state and identified molecules that disrupted PRC2 and H3K27M IDR interactions. These small molecule hits partially restored H3K27me3 levels, reduced H3K27ac levels, and decreased oligodendrocyte transcription factor 2 (OLIG2) expression in DIPG cell-lines to promote differentiation. These results successfully show that our platform can identify small molecules that uniquely target aberrant chromatin to rescue disease pathology.

#462 TNG456 is a next-generation, brain-penetrant, MTA-cooperative PRMT5 inhibitor for the treatment of solid tumors with MTAP loss.

Kimberly J. Briggs, Alice Tsai, Minjie Zhang, Wenhai Zhang, Alan Huang, Jannik N. Andersen, Kevin M. Cottrell

Tango Therapeutics, Boston, MA

MTAP deletions occur in 10-15% of all human cancers, providing one of the largest precision oncology patient populations. MTA-cooperative PRMT5 inhibitors leverage the well-characterized synthetic lethal relationship between PRMT5 inhibition and MTAP deletion. TNG908, TNG462, AMG 193, BMS-986504, and AZD3470 are clinical-stage MTA-cooperative PRMT5 inhibitors for the treatment of solid tumors with MTAP loss, though only TNG908 and AMG 193 are reported to be brain-penetrant. TNG456 is a next-generation, highly potent and selective MTA-cooperative PRMT5 inhibitor designed for patients with MTAP-null cancers including gliomas and other tumors which frequently metastasize to the brain, such as NSCLC. In vitro, TNG456 is 55X selective for MTAP-null cancer cell lines over isogenic MTAP WT cell lines (compared to 15X for TNG908) and has marked selectivity for MTAP-null cancer cell lines independent of lineage in a large, diverse cell line panel. TNG456 is brain-penetrant in preclinical species with a Kpuu range of 0.5-1.1 in non-human primates and dogs. Oral administration of TNG456 drives dose-dependent antitumor activity including durable tumor regressions and complete responses in multiple cell line- and patient-derived xenograft models. With enhanced potency and selectivity for MTAP-null cancer cells, and strong preclinical evidence of brain-penetrance, TNG456 has the potential for broad clinical activity in MTAP-null solid tumors including gliomas and CNS metastases.

#463 Preclinical evaluation of CNS drug penetration of a novel series of MTA-cooperative PRMT5 inhibitors including TNG456.

Alice Tsai, Charles B. Davis, Kimberly J. Briggs, Kevin M. Cottrell, John P. Maxwell, Adam S. Crystsal, Ellen Hooper, Jannik N. Andersen

Tango Therapeutics, Boston, MA

Treatment of brain tumors and brain metastases remains a significant unmet medical need. This is especially true for high-grade gliomas, where up to 50% of cases exhibit loss of MTAP, creating a dependency on PRMT5 and a susceptibility to MTA-cooperative PRMT5 inhibitors. For preclinical drug discovery, the accurate prediction of drug exposure in the human CNS compartment is therefore of critical importance. This study evaluates the utility of preclinical models, specifically the monkey cerebrospinal fluid (CSF) model, alongside the physicochemical properties of compounds, including permeability and efflux characteristics, to predict free brain drug exposure in humans. The non-terminal monkey CSF model permits assessment of CNS penetration with minimal invasiveness. Complementary to this, as part of the 14-day terminal dose range finding toxicology studies, brain tissues were collected at necropsy to verify and correlate predicted brain penetration with actual exposure levels. Our findings demonstrate a strong correlation between CSF concentrations in the monkey model and free brain drug exposure, suggesting that incorporating these datapoints, in addition to optimizing permeability and efflux properties, enhances the reliability of CNS drug delivery predictions. This research highlights the importance of integrating non-terminal preclinical models and physicochemical analysis to improve the safety and efficacy of CNS-targeted therapeutics. In addition, utility of rodent terminal brain drug exposure models and the importance of deriving unbound free brain drug exposures will be discussed.

#464 Discovery and development of IST-477, a selective KAT6A histone acetyl transferase inhibitor and characterization of its anti-tumor efficacy in ER+ HER2- breast cancer and other tumor indications.

Roopa Rai[1], M.v. Sreedhara[2], Samir K. Pawar[3], Rahul R. Nagawade[3], Krishna A. Putta[2], Mahaboobi Jaleel[2], Jianhong Wang[1], Alison L. Hannah[1], **Leslie J. Holsinger**[1]

[1]Isosterix, Inc., San Carlos, CA,[2]Biology, Sai Life Sciences Limited, Medchal-Malkajgiri District Telangana, India,[3]Discovery Chemistry, Sai Life Sciences Limited, Medchal-Malkajgiri District Telangana, India

KAT6A and its paralog KAT6B are histone acetyltransferases (HATs) which direct chromatin structural rearrangement and subsequent changes in gene transcription through acetylation of histones, primarily specific lysine residues on histone H3. Dysregulation of this epigenetic target is known to drive gene expression and tumorigenic progression in cancers and is commonly amplified at the genomic level and/or overexpressed in a number of solid tumors. An inhibitor of KAT6A and KAT6B, PF-07248144, has entered phase 2 clinical studies with durable partial responses reported in estrogen-receptor positive (ER+) HER2- breast cancer patients who had progressed on a prior endocrine therapy and CDK4/6 inhibitor (Mukohara et al, 2024, Nature Medicine 30:2242). In addition to estrogen receptor expression, transcriptional regulation of other pathways involved in tumorigenesis is known to involve KAT6A, expanding the potential for KAT6A inhibitors to be used in other indications.

Isosterix has developed a series of structurally diverse small molecule KAT6 inhibitors which are potent and selective for the KAT6A paralog. Potency directed to the KAT6A paralog is believed to offer the potential for reduced off-target toxicity. Lead optimization led to a clinical development candidate IST-477, which has entered IND-enabling studies. IST-477 inhibits KAT6A-mediated histone acetylation in a variety of tumor cell lines. It reduces cell proliferation and is additive when treated in combination with CDK4/6 inhibition. Excellent anti-tumor potency has been achieved in ER+ HER2- CDx and PDx breast cancer models with this KAT6A-selective inhibitor. IST-477 has high aqueous solubility, good PK properties, including %F>50 in mice/rats/dogs with an estimated clearance of 0.26 mL/min/kg and half-life of 10.2 hours in humans, predicted by multi-species allometric scaling. Early repeat-dose toxicology studies indicate excellent tolerability.

Efficacy of IST-477 in pharmacology models outside of breast cancer support clinical investigation of this therapeutic in other indications. IST-477 demonstrates single-agent efficacy in a number of solid tumor models including an ovarian CDx and an esophageal PDx tumor model. Pharmacologic efficacy of this targeted therapeutic will be discussed with an analysis of the relationship to KAT6A and 6B expression in these models. All IND-enabling studies to support clinical progression of IST-477 are planned for completion in mid-2025.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Gene and Vector-Based Therapy
Poster Session

#467 Programmable transcription factors as protein adjuvants for cancer immunotherapy.
Nicholas Truex

University of South Carolina, Columbia, SC

Immunotherapies are transforming clinical oncology by exploiting immune pathways to improve cancer treatment outcomes and provide long-term remission. Anticancer immune pathways are typically activated with dose regimens of antibodies, engineered cytokines, salt or oil emulsions, oligonucleotides, or lipid nanoparticles, which ultimately induce transcription and translation of interferon-stimulated genes that instruct the immune system to respond to tumors. These and other molecular adjuvants are widely used to stimulate broad inflammatory signals but are also associated with abnormal cytokine expression levels and tissue toxicity. To address the challenge of nonspecific activation and to improve antitumor immunity, our group is developing artificial transcription factor (ATF) proteins as a generalizable technology platform to target and control specific immune signaling pathways. This presentation will describe the design and characterization of these ATF proteins, including biophysical, biological, and antitumor properties, and demonstrate their therapeutic potential as gene- and protein-based adjuvants for cancer immunotherapy.

#468 A novel gene therapy approach for treating prostate cancer through the overexpression of mutant androgen receptor.
S.-h. Jeong, S. Hong, H. Yuk, C. Kwak;
Seoul National University, Seoul, Korea, Republic of

Prostate cancer is the second most common cancer in men and one of the leading cause of cancer related death. In metastatic prostate cancer androgen deprivation therapy (ADT) is the main treatment strategy. However within one to two years it progresses into castration resistant prostate cancer (CRPC) through various mechanisms including androgen receptor (AR) mutations and splicing variants. In this study we produced non-functioning AR gene incorporated into plasmid vector enabling constant amplification in mammalian cells. The reconstructed plasmid was encapsulated within PLGA nanoparticle which harboring PSMA antibody on its surfaces through PEGylation. The therapeutic effect of non-functioning AR amplification was evaluated in this study We developed AR gene sequence with 3 base pair deletion in DNA binding domain which confers non-functioning AR. The mutated AR was incorporated into plasmid vector under CMV promotor which enables constant amplification of non-functioning AR in mammalian cells. To enhance the delivery of the reconstructed plasmid to prostate cancer cells, PLGA nanoparticle was utilized. PLGA nanoparticle was PEGlyated with PSMA antibody on its surfaces. The reconstructed plasmid was contained in PSMA-PLGA nanoparticle (mAR-PSMA-PLGA). Prostate cancer celling 22Rv1 was used for the experiment and enzalutamide resistant 22Rv1 (EnzR-22Rv1) was produced through serial treatment of enzalutamide on 22Rv1 cells. Xenograft prostate cancer model was developed using nude mice and treatment effect was evaluated We confirmed the specificity of PSMA-PLGA nanoparticle on prostate cancer cells. When LNCaP prostate cancer cells were co-cultured myoblast cells, GFP producing PSMA-PLGA nanoparticle was treated. Only LNCaP cells were positive for GFP signal which means that PSMA-PLGA nanoparticle attaches to prostate cancer cells specifically. 22Rv1 cells were treated with mAR-PSMA-PLGA and showed constantly enhanced non-functioning AR production without activating subsequent genes including IGF1 and PSA. The mAR-PSMA-PLGA also suppressed proliferation of 22Rv1 and EnzR-22Rv1 cells. Furthermore in xenograft model using EnzR-22Rv1, mAR-PSMA-PLGA reduced tumor growth significantly and reversed enzalutamide resistance showing synergistic effect with enzalutamide treatment. PSMA-PLGA nanoparticle system efficiently deliver reconstructed plasmid in vitro and in vivo which enabling targeted gene therapy. Amplification of non-functioning AR using mAR-PSMA-PLGA significantly suppress tumor growth and diminishes enzalutamide resistance.

#469 Bladder cancer gene therapy via Nectin-4-targeted PLGA nanoparticles delivering CRISPR/Cas9 for double-strand break induction.

Seung-hwan Jeong, Seokbong Hong, Hyeong Dong Yuk, Cheol Kwak

Urology, Seoul National University, Seoul, Korea, Republic of

Nectin-4-targeted antibody-drug conjugates (ADCs) linked with monomethyl auristatin E (MMAE) have revolutionized the treatment paradigm for metastatic bladder cancer. In combination with pembrolizumab, this therapy has doubled overall survival compared to conventional platinum-based chemotherapy. However, challenges such as drug toxicity and the accumulation of resistance to MMAE limit its therapeutic potential. In this study, we introduce a novel Nectin-4-targeted gene therapy strategy employing the CRISPR/Cas9 system. This advanced gene-editing tool, known for its precise induction of double-strand DNA breaks, was adapted to create over 50 random double-strand breaks (referred to as MT50) in target cells, leading to apoptotic cell death. To address the critical challenge of delivering gene-editing tools efficiently and specifically to cancer cells, we developed a highly efficient nucleic acid encapsulation system with poly(lactic-co-glycolic acid) (PLGA) nanoparticle. This platform demonstrated high nucleic acid encapsulation efficiency, exceeding 60% for plasmids (~1 kb) and 80% for shorter RNAs, such as siRNA. The system was further optimized for encapsulating CRISPR/Cas9 components, including guide RNA (gRNA) and Cas9 mRNA. The T24 bladder cancer cell line, characterized by high Nectin-4 expression, was used as the experimental model. In vitro experiments demonstrated that MT50 delivery via Nectin-4-conjugated PLGA nanoparticles inhibited cellular proliferation by over 70%. In vivo, the nanoparticles were administered systemically through intraperitoneal injection into a murine model bearing T24 bladder cancer tumors. Tumor volume decreased by 70% following MT50 treatment, with significant downregulation of Nectin-4 expression observed in treated tumors compared to controls. While this experiment was conducted in immunocompromised mice, precluding the assessment of immunogenic cell death or synergistic effects with immune checkpoint inhibitors, we anticipate enhanced anti-tumor efficacy in immunocompetent models. Specifically, combining MT50 treatment with immune checkpoint inhibitors is expected to amplify therapeutic outcomes. This study highlights the successful development of a Nectin-4-targeted PLGA nanoparticle system capable of efficiently encapsulating and delivering CRISPR/Cas9 components to bladder cancer cells. The MT50 delivery system demonstrated significant anti-tumor effects both in vitro and in vivo, offering a promising alternative to MMAE-linked ADCs. By circumventing cumulative resistance and leveraging the precision of gene editing, this approach has the potential to redefine bladder cancer therapeutics.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#47 Optimizing in vivo establishment of hormone receptor-positive breast cancer patient-derived xenografts for therapeutic assessment.

Abigail M. Fielder, Manohar Ratnam, Lisa Polin, Julie Boerner, Seongho Kim, Kristen Purrington

Oncology, Wayne State University School of Medicine, Detroit, MI

Patient-derived xenograft (PDX) models are invaluable tools for studying the heterogeneity of primary human tumors and preclinical drug responses. However, traditional PDX models for estrogen receptor-positive (ER+) breast cancer face notable challenges. These models involve implanting tumor tissue into mice with the aim of achieving tumor "engraftment," a process that typically requires 2 to 6 months and often longer for the tumor to grow to a size adequate for passaging. This extended timeframe, combined with the relatively indolent and less aggressive nature of hormone receptor-positive (HR+) breast cancers, results in lower success rates for tumor engraftment and passage. Additionally, these slow-growing tumors are prone to clonal selection during long-term growth, further reducing the utility of traditional PDX models for timely therapeutic studies. To address these challenges, we developed a streamlined PDX model optimized for HR+ breast cancer. Tumors were resected from deidentified patients at Karmanos Cancer Institute meeting specific eligibility criteria: ER or PR-positive, HER2-negative, and no prior neoadjuvant or endocrine therapy. Tumor specimens were aseptically implanted into the mammary fat pads of female SCID mice, which were supplemented with slow-release estradiol pellets to maintain estrogen levels. Tumor growth was monitored using caliper measurements, and tumor presence and vascularization were confirmed at 14 days post-implantation using Susceptibility Weighted Imaging (SWI). Following confirmation of tumor establishment, mice were randomized into two groups: a Tamoxifen-treated cohort (100 mg/kg orally for three days) and an untreated control group. Tumors were subsequently harvested for molecular and histological analyses. Our preliminary findings indicate that tumor engraftment and vascularization can be reliably achieved within two weeks, supporting the feasibility of this approach for time-sensitive therapeutic studies. Importantly, tumors remained present following treatment, providing adequate samples for downstream analyses after the experiment. This model circumvents many limitations of traditional PDX systems, including slow tumor growth and clonal selection, while providing a practical platform for studying endocrine therapy responses in HR+ breast cancer. By minimizing reliance on long-term engraftment and optimizing conditions for short-term assessment, this refined system holds promise for advancing translational research and improving preclinical evaluations of HR+ breast cancer therapies.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Gene and Vector-Based Therapy
Poster Session

#470 Appropriateness of long-term culture of patient-derived tumor organoid for colorectal cancer modeling through genetic and phenotypic profiling.

Jaemin Cho[1], Eun Ah Jung[2], Hyowon Hong[3], Sang Joon Byun[4], Sung Wook Bae[5], Inchul Seo[6], Jumgsook Ha[7], Hyewon Lee[1], Shin Kim[1]

[1]Pathology, Keimyung University School of Medicine, Daegu, Korea, Republic of,[2]Anatomy, Keimyung University School of Medicine, Daegu, Korea, Republic of,[3]Research Affairs, Keimyung University School of Medicine, Daegu, Korea, Republic of,[4]radiation omcology, Keimyung University School of Medicine, Daegu, Korea, Republic of,[5]colorectal surgery, Keimyung University School of Medicine, Daegu, Korea, Republic of,[6]immune, Keimyung University School of Medicine, Daegu, Korea, Republic of,[7]Laboratory Medicine, Keimyung University School of Medicine, Daegu, Korea, Republic of

Background and Objective: Colorectal cancer (CRC) is one of the leading causes of cancer-related morbidity and mortality worldwide, requiring effective disease models to study its progression and develop novel therapeutics. This study aimed to establish a long-term patient-derived tumor organoid (PDO) model and evaluate its suitability for colorectal cancer modeling through comprehensive genetic, morphological, and molecular analyses.
Methods: PDOs were cultured for 1, 3, and 6 months to mimic the progression of CRC. Chromosomal instability was assessed using karyotyping, and morphological changes were evaluated through hematoxylin and eosin (H&E) staining and bright-field imaging. Immunofluorescence staining was performed for the colorectal stem cell marker LGR5 and the epithelial differentiation marker CDX2, which is specifically expressed in the small intestine and colon epithelium and plays a key role in epithelial growth and differentiation. Temporal changes in genetic instability, structural characteristics, and marker expression were analyzed to validate the model.
Results: Karyotyping revealed a progressive increase in chromosomal instability across the 1-, 3-, and 6-month PDOs, consistent with CRC-associated genetic alterations. Morphological analysis using H&E staining and bright-field imaging demonstrated gradual structural changes, with the 6-month PDOs displaying histological features characteristic of CRC. Immunofluorescence staining showed a decline in LGR5 expression over time, reflecting reduced stemness in advanced stages, while CDX2 expression was maintained across all time points, indicating its critical role in epithelial differentiation during cancer progression.
Conclusion:The long-term PDO-based model successfully recapitulates the genetic, morphological, and molecular hallmarks of colorectal cancer progression. This model captures key transitions, including chromosomal instability, structural alterations, and dynamic marker expression, while retaining essential epithelial markers like CDX2. These findings underscore the potential of PDOs as a robust in vitro platform for studying colorectal cancer development and progression and advancing therapeutic research.

#471 CRISPR-Cas9 as a novel cancer gene therapy approach against pancreatic cancers.

Selina Shiqing K. Teh, Akhil Kotwal, Kirsten Bowland, Alexis Bennett, Eitan Halper-Stromberg, Laura Morsberger, Sarah Wheelan, Nicholas J. Roberts, Chien-Fu Hung, Michael Goldstein, Ying S. Zou, James R. Eshleman

Johns Hopkins University School of Medicine, Baltimore, MD

Pancreatic cancer (PC) is the third leading cause of cancer deaths in US with a 5-year survival rate of 13%. Most patients are diagnosed at advanced stages, and conventional treatments remain ineffective and highly toxic to these patients as both chemotherapy and radiation affect normal, healthy cells, leading to adverse side effects. We have adapted CRISPR-Cas9 as a cancer-specific killing strategy that spares normal cells. CRISPR-Cas9 is a molecular scissor that induces DNA double strand breaks (DSBs) at its targeted genomic locations. We use CRISPR-Cas9 to target the subset of somatic mutations that create protospacer adjacent motifs (PAMs). Using *S. pyogenes* Cas9 that recognizes a 5'-NGG-3' PAM, we identified somatic single base substitutions (SBSs) that give rise to novel PAMs (e.g. NCG→NGG), and use the upstream sequence to design sgRNAs for cancer-targeting. We have discovered that ~13% of SBSs in PC form novel PAMs, and these cancer-specific sgRNAs exhibited on-target activity and lack of off-target events. Notably, while examining WGS data from rapid autopsy samples, we found that 90% of PAMs were maintained among primary carcinomas and metastases. Using multi-target sgRNAs targeting 2-16 sites in the non-coding regions of human genome, we demonstrate a dose-response relationship in which >10 target sites are required to achieve >99% cell elimination. Moreover, we established that simultaneous induction of multiple DSBs by CRISPR-Cas9 causes extreme chromosomal instability that ultimately leads to cell death. We detected massive chromosomal aberrations, including dicentrics, ring chromosomes, and polyploidization. Surprisingly, CRISPR-Cas9-induced DSBs are significantly more cytotoxic in PCs than a comparable number of radiation-induced DSBs, in which the number of DSBs required to achieve equitoxic effects was ~3 times higher for radiation than CRISPR-Cas9. Finally, we have developed a pipeline to screen for the most toxic sgRNAs in a given PC for selective killing. Using our PAM-based approach, we identified >100 Panc10.05-specific sgRNAs that have no off-target activity at 0- and 1-mismatch sites. We cloned them into lentiviral vectors and transduced in bulk, followed by NGS to discover sgRNAs that decreased in representation in the targeted cell line but not in the controls. We selected the top sgRNAs that have 100% variant allele frequency (VAF), cloned 4 of them into a multi-sgRNA expression vector, and transduced it into co-cultures consisting of two PC cell lines. Greater than 85% selective reduction of the targeted cell line was detected. Then, we selected nine top sgRNAs, transduced them as a pool into Panc10.05 cell line, and injected these cells into athymic, nude mice for tumor growth. We observed >90% tumor growth inhibition with these cancer-specific sgRNAs. Overall, our study highlights the therapeutic potential of CRISPR-Cas9 as an anti-cancer strategy and supports the genetic targeting of PCs.

#472 A simple, rapid, and robust bioluminescent assay for detecting anti-AAV neutralizing antibodies in serum samples.

Morten Seirup, Trish Hoang, **Jeff Nelson**, Brock Binkowski, Marjeta Urh

Promega, Madison, WI

Adeno-associated viruses (AAVs) are widely used as vectors in gene therapy, including for the delivery of therapeutic genes in cancer treatments. In the context of cancer therapeutics, AAVs can be engineered to deliver tumor-suppressing genes or immune-modulating factors directly to cancer cells, enhancing the efficacy of immunotherapy and oncolytic strategies. However, these treatments can be compromised by pre-existing neutralizing antibodies (NAbs) in human serum. To address the need for a sensitive and efficient screening method, we present a novel cell-based transduction inhibition (TI) assay using NanoLuc luciferase, an engineered enzyme known for its high brightness and stability. This assay enables rapid and sensitive detection of NAb activity, utilizing low multiplicities of infection (MOI: 100-10,000) and incubation times as short as 4 hours. Our workflow employs a straightforward add-mix-read format with a luminescent detection reagent, facilitating luminescence measurements with high reproducibility and minimal hands-on time. The assay's ability to tolerate high serum concentrations, allowing dilutions as low as 1:3, significantly enhances sensitivity for detecting neutralizing activity. Comparative analysis with firefly luciferase demonstrated that NanoLuc produces over 700-fold brighter signals, providing superior detection at lower MOIs. We validated the assay using HEK293 cells, achieving consistent intra-assay variability (%CV <18%) and precise neutralizing dose (ND50) determinations in a 3-fold dilution series of 60 human serum samples. Single-dilution screening highlighted the impact of serum dilution on hit rates, while robust 4-parameter logistic (4PL) curve fitting enabled reliable ND50 determinations for 28 of the tested samples. The assay’s simplicity, high sensitivity, and rapid turnaround make it an invaluable tool for the preclinical and clinical evaluation of NAbs against AAV serotypes, thereby supporting patient eligibility assessments and therapeutic development. These findings underscore the NanoLuc-based assay as a transformative solution for advancing AAV-based gene therapy, particularly in the realm of cancer therapeutics, where precise and rapid NAb screening is crucial for successful treatment outcomes.

#473 Inhibition of SUMOylation reverses T-cell metabolic exhaustion in large B-cell lymphoma.

Joseph Lownik, Larry Milshteyn, Anton Villamejor, Akil Merchant

Cedars-Sinai Medical Center, Los Angeles, CA

Immune based therapies for large B cell lymphoma (LBCL) have transformed our approach to this disease. Therapies such as CD3 bispecific T cell engagers and chimeric antigen receptor (CAR) T cells, have diverse mechanisms of action, yet they all depend on activation of T cells to affect an anti-cancer response. While the often-dramatic responses seen to immune therapies have captured wide-spread attention, the majority of patients treated with immune based therapies for relapsed lymphoma will not achieve long term cure, and poor T cell function is a common mechanism for treatment failure. Several seminal studies have identified T-cell metabolic reprogramming as a key mechanism behind T-cell exhaustion. Mechanisms to reverse metabolic exhaustion and reinvigorate T-cells has been shown to lead to increased anti-tumor responses. Small ubiquitin-like modifiers (SUMO) proteins regulate a variety of cellular processes in tumor and immune cells. Inhibitors of SUMOylation (SUMOi) have shown anti-tumor activity in a CD8 T-cell dependent manner, however, the mechanism behind this T-cell dependence is unknown.

We show that a novel covalent SUMOi, SB-4826 leads to a robust anti-tumor response which is dependent on CD8+ T-cells in lymphoma. Using single cell metabolic studies, we demonstrate that SUMOi increases the metabolic rate, glycolytic dependency and proliferative capacity of tumor infiltrating PD1+ CD8+ T cells. We further show that SUMOylation is increased following T-cell activation, and exhausted CD8+ TILs have the highest levels of total SUMOylation of T-cell subsets. We validate these findings of increased SUMOylation in CD8+ TILs in human lymphoma samples. In a CD19 CAR T-cell model, we show that pulse treatment of CAR T-cells with SUMOi leads to enhanced anti-lymphoma activity in vitro.

Using a mass-spectroscopy/immunoprecipitation approach, we identified more than 1,000 proteins which are SUMOylated in T-cells. We found that multiple T-cell transcriptional regulators, including TBET, LEF1 and TCF1 are all SUMOylated either during a resting or activation state in CD8+ T-cells. Together, these findings suggest a novel role for SUMOylation in regulating T-cell effector activation and exhaustion, by modulating metabolic state likely through the regulation of key transcriptional T-cell programs.

#474 IL-12 immunotherapy using a novel gene delivery platform for RNA payloads achieves effective antitumor responses without systemic toxicity in an aggressive CT26 murine cancer model.

Laura Strauss[1], Carli Jones Burns[1], Akihito Inagaki[1], Stephanie Lees[2], Noriyuki Kasahara[3], Cecilia Roh[2], Robert G. Johnson[1]

[1]Pre-Clinical Immuno-Oncology, GenVivo, Inc., San Marino, CA,[2]R&D, GenVivo, Inc., San Marino, CA,[3]Brain Tumor Center, UCSF, San Francisco, CA

Background: IL-12 exhibits potent anti-tumor properties, which enhance cytolytic activity and tumor infiltration of natural killer (NK) cells and cytotoxic T lymphocytes (CTLs) and activates Th-1-type immune responses [1-2]. However, systemic administration of recombinant IL-12 protein is still a challenge due to serious toxicity resulting from interferon (IFN)-γ overproduction [3-5]. Viral and non-viral vectors for IL-12 gene therapy represent an alternative therapeutic strategy, which can be combined with pro-drug activated cancer killing gene therapy approaches. Combined gene delivery of IL-12 and herpes simplex virus thymidine kinase (HSV-TK) significantly boosts anti-tumor efficacy in animal studies and has been shown to be safe in human clinical studies when administered intratumorally [6-12]. Here we present a novel gene therapy approach for *in vivo* gene delivery of IL-12 combined with an enhanced gain of function-variant of HSV-TK (v-eTK).

Methods: Non-replicating retrovectors encoding murine IL-12 only (mGEN1018, encoding both p35 and p40 subunits, as described previously [13-14], or in combination with v-eTK (mGEN1013), were administered intratumorally or systemically to a subcutaneous tumor model of CT26 murine colorectal cancer in syngeneic BALB/c mice. Mice receiving mGEN1013 were subsequently treated with Ganciclovir (GCV) pro-drug. General health and body weight as well as tumor growth and survival were monitored over time, and immunophenotyping of peripheral blood, spleen, and tumor-infiltrating immune cells was performed.

Results: Our data show that in subcutaneous CT26 tumor models, intratumoral and systemic gene delivery of IL-12 by the mGEN1018 vector induces both local and systemic anti-tumor immune responses primarily by stimulating production of IFN-γ from NK cells and CTLs. Gene delivery of IL-12 in combination with v-eTK by the mGEN1013 vector followed by GCV pro-drug showed enhanced tumor growth inhibition and increased survival compared to untreated controls. Systemic anti-tumor effects of IL-12 gene therapy were not associated with visible signs of toxicity or significant body weight loss.

Conclusion: Gene delivery of v-eTK followed by GCV treatment results in tumor cell lysis and exposure of tumor antigens, while simultaneously, IL-12 gene delivery enhances anti-tumor immune responses though upregulation of inflammatory cytokines. These results show the potential of mGEN1013 as a novel RNA payload delivery platform for combined GCV pro-drug and more effective tumor-localized IL-12 immunotherapy without systemic toxicity.

#475 Autophagy inhibition enhances the antitumor effect of p53-armed oncolytic virotherapy against pancreatic cancer.

Yosuke Takahashi[1], Hiroshi Tazawa[1], Satoru Kikuchi[1], Shinji Kuroda[1], Yasuo Urata[2], Shunsuke Kagawa[1], Toshiyoshi Fujiwara[1]

[1]Gastroenterological Surgery, Okayama Univ. Graduate School of Med., Dentistry & Pharm. Sci., Okayama, Japan,[2]Oncolys BioPharma, Inc., Tokyo, Japan

Background: Autophagy is a survival mechanism that transports intracellular materials to lysosomes for degradation to provide energy or macromolecular precursors in response to cellular stresses such as hypoxia or starvation. Pancreatic ductal adenocarcinoma (PDAC) is characterized by high levels of autophagy, which plays a crucial role for cancer survival. Recently, autophagy has been shown to promote immune evasion by lysosomal degradation of MHC-1 expression and has been proposed as a therapeutic target in combination with immunotherapy. We have previously developed OBP-702, a tumor-specific replication-competent oncolytic adenovirus expressing the p53 tumor suppressor gene and demonstrated that OBP-702 exhibited direct cytotoxicity via p53-mediated induction of apoptosis and indirect immune activation via p53-mediated enhancement of immunogenic cell death (ICD). In this study, we examined the therapeutic potential of autophagy inhibition on the cytotoxicity and ICD of OBP-702 and MHC-I expression in PDAC cells.
Methods: Murine PDAC cell line PAN02 was used in this study. The cytotoxic activity of autophagy inhibitor, chloroquine (CQ), and OBP-702 in monotherapy and combination therapy was analyzed using XTT assay. The combination index was calculated using CalcuSyn software. The apoptosis-inducing effect of CQ and OBP-702 in monotherapy and combination therapy was analyzed using apoptosis assay. The effect of CQ on virus-induced cell death and the molecular mechanism were investigated by Western blot analysis. The levels of extracellular ATP as ICD marker were analyzed following OBP-702 infection with or without CQ using ELISA assay. We further analyzed whether CQ enhances the expression level of MHC-1 by FACS analysis.
Results: CQ and OBP-702 suppressed the viability of PAN02 cells in a dose-dependent manner. The calculation of combination index demonstrated that combination of CQ and OBP-702 showed profound synergistic effect, in which CQ enhanced the apoptosis-inducing effect of OBP-702. Moreover, CQ increased the levels of extracellular ATP followed by OBP-702 infection, suggesting that CQ enhances OBP-702-mediated ICD induction. CQ also increased the expression level of MHC-I on PAN02 cells.
Conclusions: Our data suggest that autophagy inhibition by CQ enhances the induction of apoptosis and ICD by OBP-702 and the level of MHC-I expression on PDAC cells, probably promoting the antitumor immune responses against PDAC cells. Therefore, we are currently evaluating the combined effect of CQ and OBP-702 using the syngeneic PAN02 subcutaneous tumor models.

#476 *In vivo* CD47-mediated targeted oligonucleotide delivery for endolysosomal escape in cancer treatment.

M. Jo[1], Y. Jeong[1], Y. Kim[2], S. Song[2], H. Joo[3], Y. Choi[2], I. Lee[2], M. Kwon[1], G. Kim[2], I.-S. Kim[3], G.-H. Nam[1];
[1]Korea University, Seoul, Korea, Republic of, [2]SHIFTBIO INC., Seoul, Korea, Republic of, [3]KU-KIST Graduate School of Converging Science and Technology, Korea University, Seoul, Korea, Republic of

Therapeutic oligonucleotides, such as small interfering RNAs (siRNAs) and antisense oligonucleotides (ASOs), hold promise for suppressing pathogenic genes, including mutated KRAS, a key driver in many cancers. However, their systemic administration is challenged by rapid degradation and poor cellular uptake. Existing delivery systems, like lipid nanoparticles, face limitations in safety, extrahepatic delivery, and evading endolysosomal degradation. To address these limitations, we developed and evaluated an extracellular vesicle (EV)-based delivery platform for CD47-mediated oligonucleotide delivery *in vivo*. EVs, known for their biocompatibility, were engineered to display the SIRPα membrane protein on their surface, creating CD47-targeting SIRPα-EVs (S-EVs). These S-EVs were designed to preferentially accumulate in CD47-overexpressing pathological tissues, such as inflamed and tumor sites, as confirmed by *in vivo* imaging systems, flow cytometry, and intravital live imaging. To ensure consistent oligonucleotide encapsulation and batch-to-batch stability, we employed a cholesterol-based exogenous loading technique. Mechanistically, S-EVs entered target cells through CD47-mediated endocytosis, bypassing endolysosomal degradation and delivering oligonucleotides efficiently. Our results demonstrated that S-EVs accumulated selectively in CD47 overexpressing tissues and delivered oligonucleotides more efficiently than lipid-based transfection reagents or naïve EVs, significantly enhancing therapeutic efficacy. When administered systemically in an LPS-induced inflammatory model, S-EVs loaded with NLRP3-ASOs effectively reduced inflammation, outperforming naïve EVs. In a KRAS-mutated tumor model, KRAS siRNA-loaded S-EVs (siKRAS-S-EVs) suppressed KRAS expression in tumor tissues and increased $CD8^+$ T cell infiltration into the tumor microenvironment, demonstrating significant antitumor effects. When combined with PD-1 blockade therapy, siKRAS-S-EVs led to substantial survival improvements, with 60% of treated animals achieving complete tumor remission. These findings emphasize the advantages of CD47-mediated EV delivery, enabling selective targeting of pathological tissues and efficient intracellular delivery of therapeutic oligonucleotides by bypassing endolysosomal degradation. This study highlights the potential of EV-based CD47-mediated delivery platforms as next-generation systems for treating cancers and other CD47-overexpressing diseases, addressing the limitations of current therapeutic approaches.

#477 The Nanoprimer: Overcoming the liver challenge to increase the efficacy of IV-administered biologic therapies.

matthieu Germain, Julie Devalliere, Laurence Poul, Maxime Bergere, Francis Mpambani, Andromeda Savari

Nanobiotix, Paris, France

Despite progress in the design of therapies administered by intravenous (IV) route, the liver remains one of the main challenges for treatment delivery. The hepatic clearance is responsible for the low delivery of treatment to the target site and the limited efficacy outcome. Moreover, the unintended liver distribution could cause harmful side effects. Curadigm's innovative Nanoprimer technology aims to shift the balance of therapeutic agents' bioavailability and toxicity. The platform is designed to decrease therapeutic agents' liver trapping, to increase their systemic bioavailability for optimal accumulation in target tissues. This technology has the potential to redefine the benefit/risk ratio of drugs, improving their clinical outcomes and treatment value. The Nanoprimer is an engineered, biocompatible liposome that transiently and specifically occupies the liver clearance pathways responsible for sub-optimal therapeutic bioavailability. The Nanoprimer is administered just before the treatment. By interacting specifically with the receptors of the mononuclear phagocytic system cells, the Nanoprimer enables a temporary reduction of the drug clearance. Multiple proofs of concept were realized, showing the ability of the Nanoprimer to improve the efficacy of different natures of treatments including nanomedicines such as irinotecan-loaded liposomes and nucleic acid-loaded lipid nanoparticles in various therapeutic areas. Evaluation of the Nanoprimer has now been extended to biologic therapies: - Protein-based therapy: E. Coli asparaginase is used to treat Acute Lymphoblastic Leukemia (ALL). This protein depletes asparagine from the blood leading to tumor cell death since they are not able to synthetize this amino acid contrary to healthy cells. Bioavailability evaluation performed on a mouse model showed that the Nanoprimer increases by 3-fold the systemic bioavailability of the asparaginase. Adding the Nanoprimer in ALL treatments could allow a decrease of the number of asparaginase injections, potentially reducing the related toxicity for the same efficacy. - Oncolytic viruses: The use of oncolytic viruses by IV route is highly limited by the liver that removes up to 90% of the administered dose. In a collaborative study, we evaluated the impact of the Nanoprimer on the biodistribution of a herpes simplex virus-1 in a mouse model xenografted with a B-16 tumor model. The addition of the Nanoprimer led to a 10-fold increase in the viral copy number in the tumor, opening perspectives for cancer treatment by IV route administered oncolytic viruses. Altogether these data demonstrate that the ubiquitous mode of action of the Nanoprimer allows its application to a broad spectrum of drugs ranging from nanomedicine to biologic therapies.

#478 Engineering viruses for the next generation targeted cancer therapy.

Ikeda Trashi[1], Jeremiah Gassensmith[2]

[1]University of Texas at Dallas, Richardson, TX,[2]University of Texas at Dallas, Richardson, TX

The development of targeted therapies, including monoclonal antibodies, CAR-T cells, nanobodies, and combination treatments, has significantly advanced cancer management, particularly for HER2-positive tumors. Despite these advancements, challenges such as instability, high toxicity, immune responses, rapid clearance, drug resistance, and exorbitant manufacturing costs persist. A major contributor to these costs is the reliance on mammalian cell systems for production. Additionally, minimizing off-target effects remains a critical concern for targeted therapies.

In this study, we engineered virus-like particles (VLPs) to selectively target HER2-positive tumors with minimal off-target effects. These VLPs were further designed to carry genetically expressed RNA interference (RNAi) systems for in situ gene silencing. Cryo-electron microscopy (Cryo-EM) was employed to characterize the functionalization of the VLP exterior and verify the packaging of the RNAi cargo. This novel single-cell approach combines targeted tumor homing with siRNA delivery, enabling simultaneous tumor targeting and silencing of the PDL1 gene. The multivalent nature of VLPs allows for the incorporation of multiple peptides, enhancing tumor affinity and specificity. With a size of approximately 35 nm, VLPs exhibit favorable pharmacokinetics, including reduced clearance and effective tumor homing. A key advantage of our approach is the ability to express VLPs in Escherichia coli systems, significantly reducing production costs compared to traditional mammalian cell-based systems, making the therapy more accessible.

Our findings demonstrate that intravenously administered HER2-targeting VLPs achieve high specificity and affinity for HER2-positive tumors. Once localized, the VLPs penetrate deeply into tumor tissue, delivering RNAi cargo to silence PDL1 expression. Light sheet microscopy will be used to visualize tumor architecture in three dimensions to further investigate tumor penetration, offering a more comprehensive view than traditional cryosections. By inhibiting tumor growth and recruiting immune cells, this approach offers a dual mechanism of action: direct tumor suppression and enhanced immune-mediated clearance. Furthermore, VLPs provide unique advantages such as efficient cell penetration, scalable production, and robust cargo delivery, positioning them as a promising platform for cost-effective and effective cancer therapies.

#479 Engineering LNP formulations for mRNA delivery to macrophages.

Yasir A. Alshehry, Raneem R. Aldaqqa, Matthew E. Fernandez, Asma M. Al-Terawi, Douglas H. Sweet, Sandro R.P. da Rocha

Department of Pharmaceutics and Center for Pharmaceutical Engineering and Sciences, Virginia Commonwealth University - VCU, Richmond, VA

Macrophages play a pivotal role in the microenvironment of solid tumors, contributing to cancer progression and metastases through various functions. mRNA technologies may afford opportunities to reprogram these cells towards an anti-tumorigenic phenotype for therapeutic interventions in cancer. This study focuses on optimizing lipid nanoparticle (LNP) formulations for mRNA delivery to macrophages. We first investigated the impact of six ionizable lipids, three helper lipids, and two sterols on the transfection efficiency of mRNA-LNPs in RAW264.7 macrophages in vitro. Among the ionizable lipids, SM-102 demonstrated the highest reporter protein expression, significantly outperforming other lipids except ALC-0315, with the ranking as follows: SM-102 > ALC-0315 = OF-C4-Deg-Lin > CL1 > cKK-E12 > MC3. For helper lipids, DOPE yielded higher protein expression compared to DSPC and 9A1P9, though the difference was not statistically significant relative to DSPC. Among the sterols tested, increasing the proportion of β-sitosterol in β-sitosterol:cholesterol mixtures resulted in a corresponding increase in protein expression. The formulation comprising SM-102, DOPE, and β-sitosterol emerged as the top candidate, surpassing formulations prepared with the same composition as commercially available LNPs, with a 700-fold and 250-fold increase in reporter luciferase (Fluc) and eGFP expression, respectively. We also evaluated the extent of particle internalization, which did not correlate with protein production. Our analysis revealed that nearly 100% of macrophages took up the LNPs with the optimum formulation within 4 hours, with the peak in protein expression observed at 24 hours post-transfection. To extend these findings, the optimum formulation was tested on murine bone marrow-derived monocytes differentiated into M0 [F4/80+], M1-like [F4/80+CD80+CD206-], and M2-like [F4/80+CD206+CD80-] macrophages, as well as RAW264.7 cells polarized into these same subtypes. The results confirmed the superior transfection efficiency of the optimum formulation across all tested phenotypes. To evaluate in vivo performance, we quantified the bioluminescence expression of Fluc mRNA in BALB/c mice following an intraperitoneal injection of 0.5 µg of the mRNA-loaded LNPs. Bioluminescence was measured 6 hours post-injection for whole-body imaging and organs *ex vivo*. The optimum formulation demonstrated efficient mRNA delivery without any observed toxicity. Organ distribution studies indicated accumulation of the LNPs in the liver and spleen. Overall, our findings demonstrate the significant effect of LNP formulation on its ability to transfect macrophages, achieving efficient transfection in M0, M1-like, and M2-like macrophage subtypes *in vitro* and effective mRNA expression *in vivo*. These results highlight the potential of optimized LNPs to advance macrophage-targeted mRNA therapies for the treatment of solid tumors.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#48 Enhanced patient derived xenotransplantation of TP53 mutant and splicing factor mutant acute myeloid leukemia in super immunodeficient NOG-EXL mice.

Tanmoy Sarkar[1], Sarah Skuli[1], Nicolas Skuli[1], Martin Keogh[1], Anthony Secreto[1], Moriah Jacobson[2], Martin Carroll[1]

[1]University of Pennsylvania, Philadelphia, PA,[2]Taconic Biosciences, Inc., Rensselaer, NY

Xenotransplantation of acute myeloid leukemia (AML) cells is an established model for preclinical development and understanding of AML biology. AML xenotransplantation has been well characterized in highly immunocompromised NSG mice, but time to engraftment in this strain is often prolonged and some AML types engraft poorly. In particular, we have found that TP53 mutant AML and splicing factor mutant AML engraft poorly in NSG mice. Alternative mouse models are NSG-SGM3 (SGM3) and NOG-EXL mice, which combine severe immunodeficiency with transgenic expression of human myeloid stimulatory cytokines. However, the SGM3 strain is prone to highly variable time to and level of engraftment as well as spontaneous animal death. To address these concerns, we compared AML primary sample engraftment in the NSG, SGM3, and NOG-EXL. First, we studied overall survival in SGM3 mice compared to NOG-EXL mice engrafted with 4 separate AML's (n=5 per cohort). Consistent with previous results, 25% of SGM3 mice engrafted with AML became ill and required sacrifice approximately 5 weeks after injection. This did not correlate with the level of AML engraftment. In contrast, only one NOG-EXL mouse became ill during 12 weeks of observation. We next studied relative engraftment in all 3 strains of five TP53 mutant AML samples that had either previously not engrafted or engrafted poorly in NSG mice. We aspirated bone marrow on all mice five weeks after injection. Level of engraftment across strains varied from not detectable in NSG mice to over 80% in NOG-EXL and SGM3 mice. Mice were observed until symptoms of leukemia engraftment developed (42 to 77 days) at which time mice were sacrificed and analyzed for leukemic burden by measuring huCD45+huCD33+ cells in bone marrow and spleen. Interestingly, bone marrow burden increased in NSG mice over time although total mouse leukemic burden in NSG mice was consistently lower than in the SGM3 and NOG-EXL mice. Comparing leukemic burden in NOG-EXL to SGM3 mice showed a modestly higher leukemic burden in NOG-EXL at time of sacrifice. Human CD45+ and human CD33+ cell expression, as assessed by flow cytometry, were higher in SGM3 and NOG-EXL compared to NSG mice. We also found that splicing factor mutant AML engrafted poorly in NSG mice, and preliminary analysis suggests similar results with shorter time to engraftment and higher levels of engraftment in NOG-EXL and SGM3 mice. Again, no early deaths were seen in the NOG-EXL strain. Overall, these results support the hypothesis that the NOG-EXL strain is an improved strain for use in AML xenotransplantation compared to NSG and NSG-SGM3, with enhanced levels of engraftment without spontaneous mortality. Data on relative response to cytotoxic and venetoclax therapies is ongoing.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Gene and Vector-Based Therapy
Poster Session

#480 An engineered bacterial delivery platform, BacID, selectively delivers activated Caspase-3 into the cytoplasm of cancer cells, eliminating tumors and reducing metastatic burden.

Vishnu Raman[1], Christopher L. Hall[1], Victoria E. Wetherby[1], Neil S. Forbes[2], Nele Van Dessel[1]

[1]Ernest Pharmaceuticals, Hadley, MA,[2]University of Massachusetts, Amherst, Amherst, MA

Most cytotoxic therapies, either administered as a compound or incorporated as cargo into a targeted platform (nanoparticles, antibody drug conjugates etc.), only become effective after reaching the intended target inside the tumor cell cytoplasm. Intracellular targeting faces multiple challenges, including tumor-specific receptor-binding, crossing the cell membrane, and endosomal escape into the cytoplasm. Each of these limitations reduce tumor accumulation while increasing systemic toxicity of the drug. Here, we have created a clinically-ready, non-pathogenic strain of bacteria, BacID, that accumulates and grows exponentially in tumors. The bacteria have a 5,000-fold selectivity for tumors over healthy organs and enter into cancer cells without requiring any tumor-specific receptor engagement. Once intracellular, BacID lyse and deliver a cytotoxic payload into the cytoplasm. The resulting cell death creates an immunostimulatory microenvironment that facilitates tumor clearance. The BacID strain is engineered to be safe by attenuating the external lipopolysaccharide layer, flagella expression and utilizing the intracellular lysis system that enables both delivery inside cancer cells and autonomous clearance after treatment. The bacteria is well tolerated after systemic administration in mice. To target liver cancer and metastases, The BacID strain was engineered to deliver activated Caspase-3 (EBT-002) to cancer cells. After systemic administration, the bacteria colonize tumors and rapidly clear from healthy organs. Three days after injection, bacterial concentrations in tumors peak. At this point, aspirin is administered to activate expression of flagella and the type 3 secretion system, which triggers the BacID strain to invade cancer cells. After invasion, the bacteria enter a modified endosome within the cell, lyse, and deliver the activated Caspase-3 payload into the cytoplasm, inducing cell death. Delivery of Caspase-3 with BacID enables tumor clearance, in part, by activating a 12-fold increase in intratumoral innate immunity, followed by an adaptive immune response. EBT-002 also targets and eliminates liver and lung metastases of breast cancer as small as 100 µm in diameter. After systemic administration, EBT-002 reduces lung metastatic burden by over 80% compared to a standard-of-care chemotherapeutic regimen. The BacID platform enables delivery of several biological therapies selectively inside tumor cells due to the natural tumor tropism of the platform. Moreover, the BacID strain can express and deliver DNA, RNA and protein-based therapies into tumor cells without any need for tumor-specific receptor engagement due to the natural cell invasion mechanisms inherent to the platform. These qualities make the BacID platform a universal delivery vehicle to treat solid tumors.

#481 Development of a rapid method for the detection of neutralizing antibodies in sera of primates for viral gene therapy stratification.

Gregor Sommerkamp, Eva M. Egenter, Katharina Schaich, Daniel Feger, Sebastian Dempe, Jan E. Ehlert

Reaction Biology Europe GmbH, Freiburg, Germany

Gene therapeutical intervention using viral vectors is becoming increasingly relevant for the treatment of various diseases, including cancer. A critical factor for a successful viral therapy is that the patient has not previously developed neutralizing antibodies to the respective viral serotype in the context of a naturally occurring anti-infection immune response. Such antibodies would impose the risk to neutralize and thus jeopardize the intended therapeutic response. This is especially relevant in therapies with adeno-associated virus (AAV), as immunity against AAV-serotypes is very common. We here report on the development and validation of a straightforward method to functionally detect neutralizing antibodies in primate sera, employing a transduction method on a human cell line using AAVs of different serotype coding for an easily detectable transgene. The reported methodology will be helpful for the stratification of primates and patients exploiting their sera for the prevalence of neutralizing antibodies prior to the initiation of the AAV treatment. In addition, our assay has the potential to be applied to other viral treatment strategies also.

#483 RNA-missplicing dependent nucleic acid therapeutics for SF3B1-mutant cancers.

Benjamin G. Dubner[1], Jian Xiang Peng[2], Rebecca Foerstch[3], In-Young Choi[1], Panagiotis Tsakiroglou[1], Ilias Sinanidis[1], Fatemeh Rajaii[4], Ben Park[5], Jonathan Ling[6], William Dalton[1]

[1]Hematologic Malignancies, Johns Hopkins University School of Medicine, Baltimore, MD,[2]Duke University, Durham, NC,[3]Oregon Health & Sciences University, Portland, OR,[4]Ophthalmology, Johns Hopkins University School of Medicine, Baltimore, MD,[5]Vanderbilt School of Medicine, Nashville, TN,[6]Pathology, Johns Hopkins University School of Medicine, Baltimore, MD

Cancer therapies face the challenge of specificity-limiting toxicity to normal cells and maximizing it in malignant ones. The last two decades have seen significant advancements in therapeutics that increase specificity; however, these remain elusive for the many cancerous aberrations that affect nonenzymatic, intracellular proteins.

One such protein, SF3B1, mediates branch point selection in pre-mRNA splicing as part of the U2snRNP complex. Its mutation in various cancers-from hematologic malignancies to pancreatic carcinomas-creates conserved missplicing events, including alternative 3' splice acceptors, exon exclusion/inclusion, and intron retention. Here, we sought to create nucleic acid therapeutics that leverage missplicing to express targetable or intrinsically toxic cargo in SF3B1-mutant cancers.

We previously reported a highly specific missplicing event caused by mutant SF3B1: the inclusion of a cryptic exon in the UBA1 gene. Using a fragment containing this exon, we engineered a plasmid to switch between dsRed with canonical splicing and eGFP with cryptic splicing. We also created a splicing dependent iCaspase9 plasmid that induced mutant-specific cell death with transient transfection. However, when using more clinically relevant delivery with viral vectors, we discovered that expression was too low to produce a significant effect. We therefore engineered more efficient and potent plasmids that could induce toxicity even with low expression.

We first tested a variety of constitutive promoters driving expression of our fluorescent switch. Across separate transient transfections in triplicate, flow cytometry consistently showed that the promoter from human elongation factor 1 alpha (EF1a) resulted in the highest ratio of cryptic to canonical fluorescence.

In isogenic acute myeloid leukemia (K562) and uveal melanoma (Mel202) models, we further optimized plasmids with our synthetic UBA1 gene fragment and CD19 or diphtheria toxin A subunit (DTA) cargo. For CD19, we created a fusion of the CD44 signal peptide and CD19 extracellular domain that demonstrated high specificity for SF3B1-mutant clones through anti-CD19 staining and flow cytometry. For DTA, our plasmid with an attenuated version of the toxin induced statistically significant, highly mutant-specific cell death, both through transient transfection and delivery with adeno-associated viral (AAV) vectors.

Using the cryptic exon inclusion event from UBA1, we have selectively killed SF3B1-mutant cells in 2 distinct isogenic cell models *in vitro*. Next, we hope to further optimize plasmid delivery and splicing specificity, especially *in vivo*. While recent literature has shown the efficacy of a different cryptic intron inclusion event in inducing SF3B1 mutant-specific death, our approach may result in greater splicing specificity. Combined, our results underscore the promise of mutant-SF3B1-targeting nucleic acid therapies.

#484 Mechanisms of synergy between TGF-beta inhibitor and IL12 expression from the systemically deliverable clinical oncolytic immunotherapy VET3-TGI.

Steve Thorne

Kalivir Immunotherapeutics LLC, Pittsburgh, PA

Oncolytic vaccinia virus therapies actively recruit T-cells towards the tumor microenvironment. These can be combined with IL-12 expression to help enhance the immune response by stimulating Th1 cells. However, IL-12 mediated induction of IFN-gamma was also found to increase TGF-beta signaling within the tumor microenvironment.TGF-beta mediated resistance is one of the major mechanisms of immune suppression utilized across multiple solid tumor types. Blockade of TGF-beta (TGFBi) will therefore likely improve response to immunotherapy. Combining TGF-beta inhibition with IL-12 expression can maximize therapeutic benefits through simultaneously reducing immunosuppression and enhancing anti-tumor immune response. Current studies describe a vaccinia-based immunotherapy, VET3-TGI, combining enhanced systemic virus delivery to tumors and locally expressed IL-12 and TGFBi within the tumor microenvironment, for efficient control of multiple tumor models. *In vivo* mouse studies demonstrated potent therapeutic activity, including many CRs, even at doses several logs below equivalent clinical doses after systemic (intravenous) delivery. In addition, toxicity studies demonstrated the safety of VET3-TGI in mouse models. This data led to the selection of VET3-TGI as a lead clinical candidate and clinical testing has begun.

#485 Using the Celigo™ Image Cytometer for effectively screening rAAV gene delivery methods.

Mackenzie Pierce, **Yongyang Huang**, Bo Lin

Revvity Health Sciences, Inc., Lawrence, MA

To develop effective rAAV gene delivery vectors, scientists need to consider multiple elements, such as the promoter, insertion location, genetic target, and target gene to name a few. Green fluorescent protein (GFP), or other detection markers are often used to measure vector transduction and gene expression efficiency. Transduced GFP samples can be analyzed using fluorescent microscopy and flow cytometry, however, both methods can be quite time consuming, labor intensive, and lack high-throughput capabilities to kinetically monitor gene expression. In this work, we developed and employed the Celigo™ Image Cytometer to screen different gene delivery vectors by comparing GFP expression level in SupT1 cells at 24-, 48-, 72-, and 96-hours following transduction. Using the Celigo™ we were able to monitor transient and integrated GFP expression amongst multiple gene delivery vectors. For transient expression monitoring, SB-P-AA-101-07 displayed stronger expression levels at 24- and 48-hours. For integrated expression monitoring, both plasmid and rAAV with integration element exhibited over ~90%+ GFP transduction efficiency by 96-hours. In summary, the Celigo™ may prove useful for researchers developing and screening gene delivery vectors in a high throughput manner.

Note: SB-P-AA-101-07 (from Revvity Health Sciences, Inc.) is for research use only. Not for use in diagnostic procedures.

#486 Intratumor gene therapy designed to induce both local and systemic tumor directed immune responses.

Richard Heller[1], Loree C. Heller[1], Jody Synowiec[1], Julie Singh[1], Alex Otten[2], Mark J. Jaroszeski[2]

[1]USF Health Morsani College of Medicine, Tampa, FL,[2]Medical Engineering, University of South Florida, Tampa, FL

Immune checkpoint inhibitors (ICIs) have provided a major breakthrough for the treatment of multiple cancer types. While there have been significant clinical benefits, the therapy has been limited by both primary and acquired resistance. The immune state of the tumor microenvironment (TME) can play a significant role in ICI effectiveness. Modifying the TME to increase lymphocytic infiltrate and/or increasing expression of checkpoints can improve ICI efficacy. Multiple studies have been initiated to modify the TME to enhance the effectiveness of ICIs. We previously demonstrated that the intratumor delivery of a plasmid encoding interleukin-12 (pIL-12) resulted in a significant increase in T effector cells and a reduction in T regulatory cells and myeloid derived suppressor cells within the TME, inducing a robust systemic immune response when combined with ICIs. The key to make this an effective therapy is to deliver these agents in a manner that has minimal adverse and off target effects while still inducing the desired response. We have designed a novel approach to efficiently deliver multiple plasmids directly to solid tumors. The rationale was based on the premise that by performing intratumor delivery, stimulation would be directed against the specific antigens within the TME to produce an effective local response while stimulating a systemic response, resulting in an abscopal effect. This delivery utilizes a next generation electrotransfer device that enables the use of significantly reduced voltages and includes a means to monitor when successful delivery has occurred. We tested this concept in the B16.F10 mouse melanoma model. Intratumor delivery of pIL-12 resulted in effective control of the treated tumor in over 90% of the mice and generated resistance to challenge. To fully test this approach, we next evaluated a combination therapy delivering both pIL-12 combined with a plasmid encoding a PD1 peptide, an alternative form of checkpoint inhibitor. The therapy was tested in a multi-tumor model which included a subcutaneous tumor and intraperitoneal injection of tumor cells. The plasmids were delivered to the subcutaneous tumor; tumors generated by intraperitoneal injection were untreated. Peritoneal tumor growth was not affected by single plasmid delivery or when both plasmids were delivered using other delivery approaches or older electrotransfer technology. However, this new therapy induced complete regression of the subcutaneous tumor and blockage of peritoneal tumor growth as assessed via in vivo imaging. Work is ongoing to translate this approach to clinical evaluation.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Molecular Classification of Tumors for Diagnostics, Prognostics, and Therapeutic Outcomes
Poster Session

#489 Unveiling markers of response to ATR inhibitor and TMZ combinations in rare tumor PDXs.

Yingdong Zhao[1], **Mariam M. Konate**[1], Ming-Chung Li[1], Peter I. Wu[2], Li Chen[2], Lindsay Dutko[2], Alyssa Chapman[2], Nikitha Nair[2], Melinda G. Hollingshead[3], Shahanawaz Jiwani[2], Biswajit Das[2], Julia Krushkal[1], Andrea R. Voth[4], Larry V. Rubinstein[1], Howard Stotler[4], Shannon Uzelac[4], Justine Mills[4], Tiffanie Chase-Miner[4], Suzanne D. Borgel[4], Cindy R. Timme[4], Lijun Chen[5], Yingwei Hu[5], Hui Zhang[5], Alice P. Chen[6], Chris A. Karlovich[2], P. Mickey Williams[2], Yvonne A. Evrard[4], Lisa M. McShane[1], James H. Doroshow[6]

[1]Biometric Research Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Rockville, MD,[2]Molecular Characterization Laboratory, Frederick National Laboratory for Cancer Research, Leidos Biomedical Research, Inc., Frederick, MD,[3]Biological Testing Branch, Developmental Therapeutics Program, National Cancer Institute at Frederick, Frederick, MD,[4]Advanced Development Research Directorate, Frederick National Laboratory for Cancer Research, Leidos Biomedical Research, Inc., Frederick, MD,[5]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD,[6]Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, MD

Rare tumors are unique cancers that often present distinct biological behaviors and treatment responses. Researchers face significant challenges in the study of those tumors due to limited patient samples, lack of comprehensive genomic data, and difficulties in establishing appropriate preclinical models for effective drug testing. The identification of effective drug combinations for cancer treatment remains a major challenge, largely due to tumor heterogeneity and variable therapeutic responses. ATR is a key kinase involved in the DNA damage response. The combination of ATR inhibitors with temozolomide (TMZ) [ATRi+TMZ] enhances the cytotoxic effects of TMZ by targeting DNA damage response pathways, particularly in cancer cells with impaired DNA repair mechanisms, offering a promising therapeutic strategy for tumors resistant to conventional treatments. This study utilized gene expression profiling of 38 rare tumor patient-derived xenograft (PDX) models available in the NCI Patient-Derived Models Repository (PDMR) (https://pdmr.cancer.gov) to identify potential biomarkers that predict responses to TMZ combined with either of two ATR inhibitors, i.e., AZD6738 (Ceralasertib) and BAY1895344 (Elimusertib). We conducted comprehensive analyses including gene expression correlation with response, network interaction and gene set enrichment analysis. Our study revealed that lower MGMT expression (i.e., $MGMT^{low}$, normalized count <50) in PDX models was associated with sensitivity to ATRi+TMZ combination therapy as expected; 3/4 and 4/4 $MGMT^{low}$ models were sensitive to AZD6738+TMZ and BAY1895344+TMZ combination, respectively. However, $MGMT^{high}$ status did not necessarily denote resistance; a portion of $MGMT^{high}$ models were also sensitive to the ATRi+TMZ combinations. In $MGMT^{high}$ PDXs, network analysis and GSEA revealed that ATRi+TMZ sensitive models had significant ($p<0.001$) enrichment of highly expressed DNA replication genes such as MCM2 compared to resistant models. Moreover, elevated MCM2 expression was associated with sensitivity to ATRi+TMZ: 8/8 and 7/9 $MGMT^{high}/MCM2^{high}$ models were sensitive to AZD6738+TMZ and BAY1895344+TMZ, respectively. Correspondingly, 14/18 and 24/24 $MGMT^{high}/MCM2^{low}$ PDX models were resistant to AZD6738+TMZ and BAY1895344+TMZ, respectively. We used mass spectrometry-based proteomics data from a subset of rare tumor models in our RNAseq-based dataset to validate the MGMT/MCM2 expression association with ATRi+TMZ response, confirming a similar trend. Our findings revealed distinct expression signatures associated with favorable responses to two ATR inhibitors when individually combined with TMZ, highlighting candidate markers that could guide treatment decisions. Additional validation in a larger study would establish the potential utility of these candidate genes for patient selection in clinical trials.
*First 5 authors contributed equally

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#49 Genetic, genomic, and pharmacologic characterization of a new cohort of ancestrally diverse patient-derived xenograft models of cancer.

Nina M. Dashti-Gibson, Amy L. Olex, Katarzyna M. Tyc, Carson J. Walker, Oniya M. Smith, Rachel K. Myrick, Bin Hu, Praveen Bhoopathi, Madhavi Puchalapalli, Li Wang, Yue Zhang, Brydon P. G. Wall, Valentina Robila, Mikhail G. Dozmorov, Jinze Liu, Jennifer E. Koblinski, Jose G. Trevino, J. Chuck Harrell

Virginia Commonwealth University, Richmond, VA

Introduction: Patient derived xenografts (PDX) are important preclinical models of cancer as they faithfully recapitulate many aspects of human tumors. Currently, models derived from patients of non-European genetic ancestry are underrepresented, limiting the potential of precision medicine advancements to equitably improve patient outcomes. The United for Health Equity Living PDX Program (U4HELPP) seeks to address this need by generating new PDX models representative of the diverse population served by the VCU Health System. This study aims to 1) characterize the histologic, genomic, and transcriptomic features of a new cohort of ancestrally diverse PDX models of breast, pancreatic, colon, and lung cancer, and 2) identify drug sensitivities and response markers that will be targeted in ongoing studies utilizing these models.
Methods: Tumor fragments were implanted in the bilateral mammary glands or subcutaneous flank of NSG mice, and growth rates were defined. Tissue was fixed and embedded for histological staining to identify the cancer type and subtype. Whole genome and bulk RNA sequencing were performed to assess the mutational profile, genetic ancestry, and global transcriptomic landscape. Single cell RNA sequencing was utilized to capture the transcriptomic heterogeneity within each model. Finally, high throughput drug screening with the NCI NExT Oncology Interrogation Tools Set of 555 agents was performed in vitro to assess the drug sensitivities of each PDX.
Results: Whole genome sequencing and bulk RNA sequencing positively identified distinct genetic ancestries within the cohort. Histopathologic analysis revealed a rare metaplastic breast carcinoma, and immunohistochemical staining stratified breast cancer models into estrogen receptor positive and triple negative subtypes. Bulk RNA sequencing was used to subtype pancreatic ductal adenocarcinoma models as basal-like, classical, or intermediate, and single-cell RNA sequencing confirmed the presence of mixed subtype populations within these samples. High throughput drug screening identified sensitivities that correlated with mutations detected through whole genome sequencing; overall, several classes of drugs including proteasome and cyclin-dependent kinase inhibitors were found to be highly cytotoxic in all cancer types tested.
Conclusion: This study integrates multiple modalities to characterize the histological features, molecular signatures, and cellular heterogeneity of this new cohort of PDXs, which will provide researchers with extensive data on which to base future studies utilizing these models. Furthermore, it identifies promising therapeutic agents and explores genomic markers of drug sensitivity which could be used to predict patient response to novel treatments.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Molecular Classification of Tumors for Diagnostics, Prognostics, and Therapeutic Outcomes
Poster Session

#491 Molecular correlates of drug response to identify novel therapeutic combinations in triple-negative breast cancer.

Nathan M. Merrill, Hamadi Madhi, Nathalie Vandecan, Athena Apfel, Habib Serhan, Peter J. Ulintz, Liwei Bao, Xu Cheng, Aki Morikawa, Matthew B. Soellner, Sofia D. Merajver

University of Michigan, Ann Arbor, MI

TNBC accounts for 10-20% of total breast cancer cases in the US and is more aggressive, higher grade, and has a poorer prognosis than other forms of breast cancer. TNBC is more likely to metastasize within 3-5 years than other forms of breast cancer and there is an urgent need to effectively implement precision medicine to improve outcomes for patients with TNBC, beyond unranked lists of drugs that may affect pathways related to genomic alterations. To this end, we have developed a precision-medicine-platform (PMP) to identify robust predictors of drug response in TNBC. We assembled a panel of 35 TNBC models that were screened with a curated library of 359 compounds comprising a broad array of approved and experimental small-molecule inhibitors. Additionally, we completed multiomic characterization (DNA sequencing, RNA sequencing, RNA targeted panels, and Reverse Phase Protein Array, RPPA) of passage-matched material. Integrating drug response readouts with molecular characterization allowed us to identify statistically significant molecular correlates of response to therapy. Further, co-targeting correlating pathways has proven to be a reliable method to identify robust synergistic combinations. Following screening, we prioritized 35 drugs on the basis of their activity and variance of response, identifying significant molecular correlates of drug response for 27 prioritized compounds. Grouping together chemotherapeutic agents, we were able to identify a signature of response generalized to chemotherapeutics. From our molecular correlate signatures, we identified a robust and novel correlation between combretastatin A4 (CA4) response and expression of EGFR at both the RNA and protein levels. Combining CA4 and EGFR inhibitors resulted in robust synergy in cell lines, *ex vivo* patient-derived xenograft models, and patient-derived organoid models. We finally validated this synergy using *in vivo* models of TNBC. Moving forward, we are working to evaluate the CA4 and EGFR inhibitor combination using additional *in vivo* models of TNBC. In conclusion, our PMP has allowed us to identify molecular correlates of response to therapy. Leveraging these molecular correlations, we identified robust novel combinations. This strategy will be critical in helping to translate precision-medicine to the clinic for patients with TNBC. Moreover, our strategy could allow us to revive repurposing strategies that previously failed clinical trials by pairing the right patients with the right drug and combination.

#493 Cingulin plays a role in PDAC organoid polarity and subtype.

Mariaelena Nabors, Sandra Zarmer, Yi Xu, Jen Jen Yeh

University of North Carolina at Chapel Hill, Chapel Hill, NC

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers with a dismal five-year survival rate of 12.5%. Our lab previously identified two transcriptomic PDAC tumor subtypes, termed basal-like and classical. Basal-like tumors are more aggressive while classical tumors have a better prognosis and are more sensitive to chemotherapy. However, many of the biological differences and drivers of the two subtypes remain unknown. We defined the proteomic landscape of PDAC tumors and found that classical tumors are enriched for cell polarity processes compared to basal-like tumors, and that one of the significantly enriched polarity proteins in classical PDAC tumors was cingulin (CGN). Classical organoids recapitulate the well-differentiated architecture of classical PDAC with well-organized hollow lumens. We found that CGN is required for the well-differentiated architecture of classical PDAC organoids and overexpression of CGN is sufficient to drive basal-like organoids to a more well-differentiated state. Additionally, overexpression of CGN in basal-like cell lines decreased invasion. Our results indicate CGN plays a role in PDAC organoid polarity, subtype, and aggression and further study could increase our understanding of the biological differences between PDAC subtypes.

#494 Identifying vulnerabilities in basal-like pancreatic adenocarcinoma (PDAC).

Sandra Zarmer, Ian McCabe, Jen Jen Yeh

UNC Chapel Hill, Chapel Hill, NC

Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal disease, with a 5-year survival of only 12.5%. There are two transcriptomic PDAC tumor subtypes: classical and basal-like. Basal-like PDAC is associated with worse patient outcomes and does not respond to the standard-of-care chemotherapeutic regimen FOLFIRINOX; new therapeutic strategies effective in basal-like PDAC are needed. To identify essential targets in basal-like PDAC, we designed a CRISPR library targeting 394 highly expressed basal-like exemplar genes and performed the screen basal-like cell lines derived from PDAC patient-derived xenograft (PDX) tumors. We found 46% of the library genes were essential (181/394, $p<0.05$), with the top Reactome pathways represented by essential genes being polo-like kinase mediated events, cell cycle checkpoints, formation of the cornified envelope, and Type I hemidesmosome assembly. Of 8 top lethal genes, 7 were validated. One of these validated targets was integrin subunit alpha 3 (ITGA3). Integrins are cell surface receptors that interact with extracellular matrix proteins, the cytoskeleton, as well as downstream effectors important in cancer signaling. ITGA3 was required for colony formation in basal-like PDX-derived cell lines. In basal-like organoids, shRNA knockdown of ITGA3 reduced growth compared to classical organoids. We report promising targets for basal-like PDAC that may play a role in subtype-specific biology and therapeutic response.

#496 Clinical, biologic, and immunogenic characteristics of gastro-esophageal cancers (GEC) harboring *CLDN18::ARHGAP* fusions.

Axel Grothey[1], Amber Fifield[2], Kieran Sweeney[2], Heinz-Josef Lenz[3], Matthew Oberley[2], Sanjay Goel[4], Shuanzeng Wei[5], Andrew Elliott[2], Bradley Somer[1]

[1]The West Clinic, Memphis, TN,[2]Caris Life Sciences, Irving, TX,[3]USC Norris Cancer Center, Los Angeles, CA,[4]Rutgers Cancer Institute, New Brunswick, NJ,[5]Fox Chase Cancer Center, Philadelphia, PA

Background: Claudin 18.2 (*CLDN18.2*) overexpression has recently emerged as a therapeutic target in advanced gastro-esophageal cancer (GEC). *CLDN18::ARHGAP* fusions are pathognomonic for GEC, but their biologic relevance in this malignancy is poorly understood. We analyzed the correlation of *CLDN18::ARHGAP* fusions with other molecular parameters, tumor microenvironment (TME) parameters, patient characteristics, and outcomes data in a large clinico-genomic database.

Methods: DNA and RNA (whole transcriptome) sequencing was performed for patient tumor samples submitted to Caris Life Sciences. *CLDN18::ARHGAP* fusions were identified from RNA transcripts. PD-L1 (28-8) and CLDN18 (43-14A) protein levels were determined by immunohistochemistry (IHC). TMB-High (TMB-H) was defined as ≥10 mutations/Mb. Significance was tested using Mann-Whitney, Fisher's Exact, or Chi-squared tests with multiple comparisons correction applied as appropriate. TME cell fractions were estimated by RNA deconvolution using quanTIseq. Real-world overall survival (OS) and nivolumab time on treatment (ToT) were obtained from insurance claims data and calculated from collection date to time of last contact and first of nivolumab to last of nivolumab, respectively; associated hazard ratios (HR) and p-values were calculated using the Cox proportional hazard model.

Results: Among 4430 GEC tumors, 262 tumors (6.1%) harbored a *CLDN18::ARHGAP* fusion, primarily *CLDN18::ARHGAP26* (N=226). Patients with fusion+ tumors were younger (median age: 58 vs 66 years, p<0.0001) and more frequently female (64.1% vs 41.1%, p<0.0001). Fusion+ tumors had more frequent *HER2* (9.7% vs 3.4%, p<0.0001) and *HER3* (10.5% vs 3.8% p<0.0001) mutations and less frequent *RHOA* (0% vs 7.5%, p=0.0009), *CDH1*(3.9% vs 20.4%, p<0.0001), and *KRAS* (2.7% vs 10.0%, p=0.0033) mutations. Fusion+ tumors had a lower prevalence of the IO-related biomarkers TMB-H (4.8% vs 13.9%, p=0.0030), dMMR/MSI-H (3.9% vs 9.5%, p=0.0416), and PD-L1+ (12.5% vs 32.5%, p=0.0001), lower infiltration of CD8+ T cells (0% vs 0.2%, p=0.0016) and M1 macrophages (3.0% vs 3.9%, p<0.0001), and higher infiltration of M2 macrophages (5.4% vs 3.9%, p<0.0001). Using either standard (≥2+, ≥75%) or reduced (≥2+, ≥40%) IHC cut-offs, fusion+ tumors were more frequently CLDN18 IHC+ than fusion- tumors (79.5% vs 47.9%, p<0.0001 and 86.4% vs 54.5%, p<0.0001, respectively). Comparing fusion+ vs fusion- cancers, there was no significant difference in median OS (15.6 vs 13.6 months; HR=0.892, p=0.1986) or nivolumab ToT (7.4 vs 4.8 months, HR=0.751, p=0.1359).

Conclusions: *CLDN18::ARHGAP* fusions characterize GECs with distinct clinical and biologic features that suggest lower immunogenicity. Fusions were positively correlated with a higher CLDN18 protein expression

#497 Real world experience of Aspyre Lung (Tissue) LDT: Simplified genomic profiling.

Elizabeth Gillon-Zhang[1], Eleanor Gray[2], Ryan Evans[1], Julia Brown[1], Candace King[1], Katherine Knudsen[1], Ethan Clark[1], Mary Beth Rossi[1], Cory Kiser[1], Amanda Green[1], Honey V. Reddi[1], Barnaby Balmforth[2], **Jeffrey Gregg**[1], Wendy Levin[1], Shari Brown[1]

[1]Biofidelity Inc, Morrisville, NC,[2]Biofidelity Ltd, Cambridge, United Kingdom

Aspyre Clinical Test for Lung (Tissue) tests lung FFPE and blood samples for actionable biomarkers across 11 genes and 114 variants associated with NSCLC (*ALK*, *BRAF*, *EGFR*, *ERBB2*, *KRAS*, *MET*, *RET*, *ROS1*, *NTRK1*/*2*/*3*). The assay uses standard PCR equipment and interrogates RNA and DNA directly for clinically actionable alterations in a cost effective and rapid manner. We present results from the initial cohort of clinical samples sent to Biofidelity Inc. Laboratory (a CAP/CLIA site). De-identified demographic and clinical data were obtained, and collated alongside laboratory data. Clinical sample and internal laboratory processing characteristics analyzed included biopsy type, pathology diagnosis, tumor content, % necrosis, number of slides used, turnaround time (TAT), variant(s) identified, and assay validity. Samples that did not meet all validated requirements were also analyzed separately. 113 clinical samples were analyzed. At the time of data cut-off, mean patient age was 65.9 yrs (range 38-94 yrs), 58 Male, 55 Female, mean tumor content 46% (range 0-95%), mean necrosis 5% (range 0-80%), 15 were resections, 18 FNA, 35 CNB, 4 cytology, 19 cryobiopsies, 11 transbronchial biopsies, 11 unknown. 92% of samples were processed within 2 business days, with an average of 5.7 slides (cut at 5 µm) used (median 4). 64 samples were adenocarcinoma/ adenosquamous, 24 samples were squamous cell carcinoma, 1 spindle cell carcinoma, 7 NSCLC, 1 small cell carcinoma and 16 not known or no pathology report. Overall, 49 samples were positive for a variant, and 64 had no variant identified. Variants included SNV in *BRAF*, *EGFR*, and *KRAS*, *EGFR* exon 19 deletions and exon 20 insertions, *MET* exon 14 skipping and gene fusions. Aspyre Clinical Test for Lung has a high assay success rate and proven rapid 2-day TAT. This makes it suitable as a first-line testing option, or for samples that are scant, of low quality, or fail next-generation sequencing (NGS) quality control, and can provide a large fraction of NSCLC patients with actionable biomarker information. The Aspyre assay is uniquely suited to inform patient decisions using very small samples that are not otherwise amenable to comprehensive genomic profiling by NGS. Aspyre Clinical Test for Lung simplified genomic profiling is transformative for cancer care management, and enables more patients with NSCLC to benefit from more effective and better tolerated targeted therapies.

#498 In melanoma, disruption of the NF1/SPRED1 RAS inactivating complex cooperates with mutant KIT necessitating combined KIT and MEK inhibition to suppress tumor growth.

M. Tee[1], J. Yang[1], J. Ma[1], M. Wang[1], D. Schadendorf[2], B. C. Bastian[1], **I. Yeh**[1];
[1]UCSF - University of California San Francisco, San Francisco, CA, [2]University Hospital Essen and German Cancer Consortium (DKTK), Essen, Germany

We previously identified concurrent loss of function mutations of either *NF1* or *SPRED1* with activating mutations in *KIT* in primary acral and mucosal melanomas. We hypothesized the positive selection for NF1 or SPRED1 inactivation in the context of KIT mutations reflects a functional cooperation that may impact response to therapy targeting KIT. We find that contrary to each of the alterations alone, combined loss of either NF1 or SPRED1 in the presence of mutant KIT transforms immortalized mouse melanocytes. Because loss of NF1 and SPRED1 each activates RAS acting downstream of KIT, it could subvert the effect of KIT inhibitors. In patient-derived melanoma cell lines with KIT mutations and loss of NF1 or SPRED1, combination treatment with a KIT inhibitor (imatinib or avapritinib) and a MEK inhibitor (trametinib) acted synergistically. Combined KIT and MEK inhibition also reduced tumor growth and prolonged survival of mice xenografted with KIT-mutant melanoma cell lines. Our findings suggest that combination therapy with KIT and MEK inhibitors would be beneficial for melanoma patients with activating KIT mutations and concomitant loss of function NF1/SPRED1 mutations.

#499 Spatial RNA sequencing supports programmed death ligand 2 (PD-L2) as a potential immunotherapy target in high grade serous ovarian carcinoma (HGSOC).

Elise Yates[1], Cailin O'Connell[2], Yin Zheng[3], Hong Zhao[4], Aparna Kamat[5], Biana Godin[6]

[1]Obstetrics and Gynecology, Houston Methodist Hospital, Houston, TX,[2]Texas A&M University School of Engineering Medicine, College Station, TX,[3]Biomedical Infromatics Service Core, Houston Methodist Research Institute, Houston, TX,[4]Advanced Cellular and Tissue Microscopy Core, Houston Methodist Research Institute, Houston, TX,[5]Gynecology Oncology, Houston Methodist Hospital, Houston, TX,[6]Department of Nanomedicine, Department of Ob/Gyn and Cancer Center, Houston Methodist Research Institute, Houston, TX

Currently available PD-1 and PD-L1 immune checkpoint inhibitors have shown limited success in HGSOC. There is increasing evidence for the significance of PD-L2, a PD-1 ligand expressed by antigen presenting cells (APC), and mainly by tumor associated M2 macrophages (CD163+), as a key player in the HGSOC immune microenvironment. Here we aimed to utilize the GeoMx Digital Spatial Profiling system to evaluate the presence of PD-L2 and APC in HGSOC across clinical stages and in normal adjacent tissue (NAT)
GeoMx spatial sequencing protocols were applied to HGSOC tumor microarrays (TMA) of different stages and whole transcriptome (RNA) was quantified using Illumina next generation sequencing (NGS). 47 cores were analyzed, including NAT (n=8), local (IA-IB, n=8), regional (IC-IIC, n=17), and distant HGSOC (IIIA+, n=14). The data was then imported into the GeoMx analysis pipeline and Q3 normalized data was used to perform immune deconvolution analysis. GeoMx SpatialDecon was used to estimate the abundance of immune cells using whole transcriptome analysis RNA expression as a proxy for cell type. Differences across stages were analyzed using a linear mixed model with BH multiple test correction and adjusted for region of interest (ROI) to account for inter-patient heterogeneity. A Kaplan-Meier plot of PD-L2 (PDCD1-LG2) progressionfree survival outcomes for all histologic subtypes of ovarian cancer (OvCa) was also created using kmplot.com.
Results: Expression of M2-macrophage marker CD163 and PD-L2 were significantly increased in the stroma among all stages of HGSOC compared to NAT (P=<0.05) Generic macrophage marker CD68 was increased in local and distant stages. PD-1 (CD274) was not significantly different from NAT in the stroma among any stage of HGSOC. However, PD-1 expression was found to be increased in the NAT epithelium group when compared to local HGSOC epithelium. A Kaplan-Meier plot of PD-L2 expression in all subtypes and treatment pathways of OvCa demonstrated that high expressors have a much lower progression free survival versus low expressors (HR=1.41, p = 0.0022).
We found that macrophages are increasingly present in the HGSOC stroma across stages. Additionally, CD163 expression is increased versus NAT, demonstrating the importance of the M2-like macrophage phenotype in OvCa progression. Interestingly, we found that PD-L2, but not PD-L1 transcription, is significantly increased in the stroma of all stages of HGSOC versus NAT stroma. These findings were supported by a Kaplan-Meier plot of PD-L2 expression in all OvCa subtypes showing that the prognosis (progression-free survival) is poorer in high PD-L2 expressors. Therefore, PD-L2 serves as a potential immunotherapy marker for HGSOC. Future efforts should investigate the impact of PD-L2 expression on the success of anti-PD-1 therapies and the role of PD-L2 as a potential immunotherapy target.

TUMOR BIOLOGY: Cancer Stem Cells
Poster Session

#5 Noncanonical NF-kB and notch signaling activity supports ovarian cancer stem-like cells following chemotherapy.

Gregory J. Jordan, Cecilia Gallo, Mikella Robinson, Omar Lujano-Olazaba, Carrie D. House

San Diego State University, San Diego, CA

In the United States, ovarian cancer is the most lethal gynecological malignancy and the fifth most lethal cancer for those at risk. Although patients typically respond well to initial chemotherapy treatments, approximately 80% of patients will relapse with chemotherapy-resistant disease. Studies suggest a subpopulation of ovarian cancer cells, termed cancer stem-like cells (CSCs), can resist chemotherapy and repopulate tumors following treatment. The mechanisms enabling these cells to survive chemotherapy and re-establish heterogeneous tumors are unclear but likely involve activation of multiple survival and developmental pathways. Our previous work shows that the noncanonical NF-κB transcription factor, RelB, supports ovarian CSCs, and our preliminary data suggests RelB regulates the expression of Notch pathway genes. Notably, Notch is upregulated in patient-matched tumors following chemotherapy relative to pre-treatment samples. We hypothesize that RelB-mediated upregulation of Notch signaling is required to maintain CSCs and facilitate tumor regrowth after chemotherapy. To test this hypothesis, we first used qRT-PCR and western blot to assess the expression of different Notch receptors and ligands under spheroid-forming 3D culture conditions relative to monolayer 2D conditions. We observed increased expression of most Notch genes in 3D conditions. Interestingly, in sorted CD117+ CSCs, Notch receptor expression (Notch 1 and Notch 3) was maintained, while expression of Notch ligands (DLL1 and DLL3) was reduced, and Jag2 expression was elevated relative to CD117- bulk tumor cells. Additionally, we observed a consistent increase in the Notch target gene *CDKN1A* in 3D growth conditions relative to 2D. To confirm and further assess Notch activity, we are currently using Notch reporter cells under various conditions, including spheroid culture, treatment with carboplatin and paclitaxel, RelB knockdown, and RelB activation. Our preliminary *in vitro* data suggests that RelB upregulates Notch signaling in CSCs to enhance stemness and tumor-initiation following chemotherapy treatment. These findings will be validated in an *in vivo* relapse model to assess the requirement for Notch signaling during relapse. This work highlights a novel collaboration between RelB and Notch activity in CSCs, which may guide the design of more effective therapeutic strategies to reduce relapse and improve survival outcomes for ovarian cancer patients.

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#50 Development of paired enfortumab vedotin-resistant urothelial cancer models using organoid and PDX.

Claire Beraud[1], **Emilie Decaup**[1], Anne-Sophie Bajeot[2], Celine Rouget[1], Karine Rollet[1], Mathieu Roumiguie[2], Xavier Game[2], Philippe Lluel[1], Nadege Bidan[1]

[1]Urosphere SAS, Toulouse, France,[2]Toulouse Hospital, Toulouse, France

Nectin-4 has emerged as a promising target for antibody-drug conjugates (ADCs) due to its high expression in bladder cancer (BC) cells. Enfortumab vedotin (EV), an anti-Nectin-4 ADC conjugated with MMAE toxin, has demonstrated potent therapeutic activity, particularly in combination with pembrolizumab. However, resistance to EV presents a significant clinical challenge. This study aims to develop EV resistant models among Urosphere's biobank of BC patient-derived xenografts (PDX) and organoids for screening anti-Nectin-4 compounds and investigating mechanisms of resistance to EV. RNA sequencing/Affymetrix and immunohistochemistry (IHC) were performed to quantify Nectin-4 expression. Sixteen PDX models were treated with EV (4 mg/kg, twice) to evaluate antitumor activity. Two PDX models (coded as B521 and F659) highly responsive to EV were selected to generate acquired-resistant models. They were treated with repeated EV treatments until tumors no longer regressed. Subsequently, tumor fragments from both PDX models were implanted into new NMRI mice and treated with EV to validate the resistance acquisition. Starting from PDX tumors no longer responding to EV, organoid model was established and treated to EV from 0.003 up to 10 µg/mL for five days, followed by cell viability assessment using the CellTiter-Glo® 3D assay to confirm their resistance. Urosphere's PDX biobank encompasses diverse Nectin-4 expression profiles. EV efficacy *in vivo* was not strictly correlated with Nectin-4 expression. B521 did not acquire any resistance whereas F659 PDX model developed resistance after ~200 days of repeated administration. We established the corresponding organoids and we showed that IC50 and Emax were significantly different between sensitive and resistant models. Nectin-4 expression remained stable in resistant models (mRNA and protein levels), indicating that resistance was unrelated to target loss. Transcriptomic analyses highlighted several potential mechanisms, including the upregulation of efflux transporters such as MDR1 and MRP3, which may contribute to resistance. Urosphere's PDX and organoid biobank serves as a powerful platform for advancing research on Nectin-4-targeted therapies. From the F659 PDX model, we successfully developed paired *in vitro* and *in vivo* models resistant to EV. These novel models provide valuable tools for assessing innovative therapeutic approaches for resistant tumors. Additionally, the EV-resistant models underscore the potential role of ABC transporter upregulation in resistance mechanisms, offering critical insights for the development of alternative treatment strategies.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Molecular Classification of Tumors for Diagnostics, Prognostics, and Therapeutic Outcomes
Poster Session

#500 A molecular comparison reveals distinct transcriptomic differences between uterine carcinosarcoma and papillary serous carcinoma distinguishable by DNA damage.

Kevin J. Lee[1], Tanvi Joshi[1], Sagar Chokshi[1], Mackenzie Cummings[1], Jennifer Scalici[2], Nathaniel Jones[1]

[1]Gynecologic Oncology, USA Health Mitchell Cancer Institute, Mobile, AL,[2]Gynecologic Oncology, Emory University School of Medicine, Atlanta, GA

Background: Uterine papillary serous carcinoma (UPSC) and uterine carcinosarcoma (UCS) are rare subtypes of endometrial cancer (EMCA) with poor prognosis. Both are high risk histotypes, though their distinct molecular and genomic profiles are undefined. Recent clinical trials such as RUBY, Keynote 775, DUO-E and NRG-GY018 have shown survival benefit in subsets of EMCA with mismatch repair deficiency (MMRd), however, small numbers of patients with UPSC and UCS were included. The role of MMRd or DNA damage & response (DDR) mechanisms in general are under-explored in these EMCA subtypes. We therefore sought to characterize the immune, inflammatory, and DNA damage profiles of UPSCs and UCSs to better define the role of immunotherapy (IO) in these tumors.
Methods: Total DNA damage was analyzed in 53 UPSC and 43 UCS FFPE samples collected at our institution using repair-assisted damage-detection (RADD). RNA was isolated from a subset of patients (n = 18/group) and expression (50 ng/per sample) was analyzed using the nCounter PanCancer IO 360 panel from Nanostring comprising 770 gene expression markers. Data was analyzed using the Rosalind Bioinformatics in conjunction with Nanostring's IO360 analysis team.
Results: We have previously shown a 3.6 fold decrease ($p<0.0001$) of unrepaired DNA damage between UCS samples compared to UPSC, suggesting greater DNA repair capacity in UCS than UPSC. Consistent with this data, transcriptional analysis revealed increased expression of DNA repair genes in UCS such as POLD1, MSH2, BRIP1, BIRC5, and CDK6, among others. Interestingly, decreased expression of DNA repair genes was noted in UPSC despite nearly 4 times the amount of unrepaired DNA damage. As expected, the immune system was activated in response to this increased DNA damage with higher levels of T cells, NK cells, IL-10, cytotoxic T cells, and Th1 cells. However, there was also increased expression of immune evasive genes such as PD-1, CD47, and VTCN1, among others. Additionally, expression of NK CD56dim was also upregulated, a known marker of immune exhaustion. This suggests DNA damage tolerance and immune exhaustion are hallmarks of UPSC.
Conclusions: We present here a detailed, treatment-oriented molecular comparison between two histologically distinct EMCA subtypes, UPSC and UCS. Assessment of unrepaired DNA damage and, by proxy, DNA repair capacity, gives context to the immune transcriptomic landscape. Our data suggests that the immune exhausted molecular landscape of UPSC is more amenable to IO than that of UCS, while the DNA repair-robust UCS may be more vulnerable to agents targeting DNA Damage repair such as PARP inhibitors. Further, our data suggests that estimation of DNA repair capacity via RADD may be as treatment informative as molecular sequencing.

#501 APC-loss as a potential biomarker for NT219 treatment in colorectal cancer (CRC).

Hadas Reuveni, Hava Ben David, Tomer Meirson

Purple Biotech Ltd., Rehovot, Israel

Background: NT219 is a small molecule dual inhibitor of STAT3 and insulin receptor substrate 1/2 (IRS), covalently binds to IRS1/2 leading to its degradation. IRS regulates PI3K/AKT and the β-catenin pathways. It was demonstrated that NT219 inhibits the IRS2-mediated β-Catenin signaling and synergizes with 5FU to suppress colorectal cancer (CRC) brain metastasis and increase survival. In an APC loss-driven CRC mouse model (CPC-APC), where IGF1R and IRS1/2 are upregulated, the dual inhibitor caused a substantial reduction in tumor burden and metastasis by affecting the tumor and the TME. Here we present the effect of NT219 in CRC patient-derived specimens and suggest upregulation of wnt/β-catenin or loss of its negative regulator APC as a potential biomarker for NT219 therapy.

Method: Six CRC ex vivo patient-derived tumor explants (PDEs), involving resected tumor fragments retaining TME native architecture, tumor heterogeneity and proliferative capacity were treated with NT219 and analyzed for efficacy. Genomic and transcriptomic analyses were performed on responding and non-responding biopsies to identify potential predictive biomarkers. Phospo-STAT3(Y705) immunohistochemistry assessed the immediate and sustained effects of NT219. Additional PDEs harboring TP53(D259Y), PIK3CA(E110del) gain-of-function, and APC(s1355fs*60) loss-of-function (LoF), were treated with NT219 w/wo TAS-102. A FOLFOX-resistant patient derived xenograft (PDX) with an APC R1450* LoF mutation received NT219 (60mg/kg, twice weekly) to detect tumor growth.

Results: CRC PDEs treated with NT219 showed >75% cell death in 2/6 (33%) samples. Genomic analysis identified APC(E1138fs) LoF mutation (1/2 responders, 0/4 non-responders) and CNV associated with the wnt/ β-catenin pathway (2/2 responders, 0/4 non-responders). Transcriptomic analysis showed differential patterns of genes associated with up-regulation of wnt/β-catenin pathways, such as WNT7A/10A and DDR2. Sustained inhibition of STAT3 phosphorylation was exclusive for responders. Genomic analysis of one biopsy pre vs post- NT219 treatment revealed the APC LoF mutation Y917fs in the pretreatment biopsy which was undetectable post-treatment, suggesting elimination of APC LoF cells. The anti-tumor activity was confirmed in a PDX CRC model harboring APC R1450* in which NT219 demonstrated a single agent sustained tumor growth inhibition (86%, p=0.001). NT219 with TAS-102, induced significant and synergistic cell death (91%) in TAS-102 resistant CRC PDE harboring APC (s1355fs*60) LoF.

Conclusion: The anti-tumor activity in CRC explants along with genomic and transcriptomic analysis propose that the response to NT219 is associated with enhanced wnt/β-catenin signaling or APC loss. The results suggest APC-loss as a potential biomarker for NT219 treatment in CRC.

#502 Exploring STEAP1 expression in prostate cancer cells in response to androgen deprivation and in small extracellular vesicles.

Candice L. Bizzaro[1], Camilla A. Bach[1], Ricardo A. Santos[1], Cecilia E. Verrillo[1], Nicole M. Naranjo[1], Ishan Chaudhari[1], Waleed Iqbal[2], Alessandro Fatatis[2], Ada G. Blidner[3], Gabriel A. Rabinovic[3], William Kevin Kelly[4], Lucia R. Languino[1]

[1]Department of Pharmacology, Physiology and Cancer Biology, Thomas Jefferson University, Philadelphia, PA,[2]Department of Pharmacology and Physiology, Drexel University, Philadelphia, PA,[3]Laboratory of Glycomedicine, Institute of Biology and Experimental Medicine, Buenos Aires, Argentina,[4]Department of Medical Oncology, Thomas Jefferson University, Philadelphia, PA

The Six Transmembrane Epithelial Antigen of the Prostate (STEAP1 and 2) metalloreductases are therapeutic targets for advanced PCa, and their expression has been linked to androgen receptor (AR) signaling; however, the regulatory mechanism and function of STEAP in PCa progression remains elusive. In this study, we explore how *in vitro* androgen modulation influences STEAP family expression in cell lines with varying reliance on androgen signaling. Our data show that in response to androgen deprivation, *STEAP1* and *STEAP2* exhibit elevated transcript levels, while *STEAP4* mirrors the expression profile of *KLK3* (Kallikrein Related Peptidase 3), showing reduced expression. Further, we found that an AR splice variant, AR-V7, which emerges in aggressive PCa, correlates with lower STEAP1 expression under androgen deprivation compared to full hormone conditions. Given their role in the exocytic pathway, we evaluated STEAP1 and STEAP2 expression profiles in hormone refractory PCa cells and their derived circulating small extracellular vesicles (sEVs). STEAP1, but not STEAP2, is upregulated in sEVs from AR-negative cells with low STEAP1 levels (DU145 and PC3) and AR-positive cells with higher STEAP1 levels (C4-2B). These results indicate selective packaging of STEAP1 in PCa cell sEVs, irrespective of AR status and cellular STEAP1 expression levels. We have previously shown that β1 integrins in the prostate tumor epithelium are necessary for sEVs to support anchorage-independent growth. Here, *ex vivo* analysis of circulating sEVs from genetically engineered mice carrying PCa show that STEAP1 is found in the sEV cargo, independent of β1 integrins. Our findings underscore the nuanced interplay between androgen signaling and STEAP1 expression and highlight the potential use of STEAP1 in sEVs as a biomarker in PCa to inform diagnostic and therapeutic strategies.

#503 Tracking evolution of transcriptional landscapes during colorectal cancer development and progression.

Sudhir B. Malla[1], Natalie Fisher[1], Raheleh Amirkhah[1], Joanne Edwards[2], Owen Sansom[3], Philip D. Dunne[1]

[1]Queen's University Belfast, Belfast, United Kingdom,[2]Wolfson Wohl Cancer Research Centre, School of Cancer Sciences, University of Glasgow, Glasgow, United Kingdom,[3]Cancer Research UK Scotland Institute, Glasgow, United Kingdom

Colorectal cancer (CRC) has been classified into molecular subtypes using gene-level data from both bulk and single-cell RNA sequencing (scRNA-seq), leading to the development of the consensus molecular subtypes (CMS) and intrinsic CMS (iCMS). In addition to CRC studies, scRNA-seq and spatial transcriptomics characterization of human polyps, aligned with the conventional adenoma and serrated polyps, identified distinct driver mutations, cells-of-origin and iCMS traits associated with each polyp type. These gene-level transcriptional classification approaches were both strongly aligned to mutations underpinning the Vogelstein model of CRC development.

However, when viewed using pathway-level information, the most dominant mechanistic signaling cascades in CRC were completely independent of these same mutational drivers. Therefore, to deliver a holistic landscape-view of the tumor ecosystem, we proposed that pathway-level subtyping could offer a broader understanding of non-genetic phenotypic signaling in CRC, leading to more informative stratification and therapeutic decision-making.

Using this new approach in combination with bulk transcriptional data from n=1200+ tumors, alongside n=29,452 scRNA-seq profiles derived from murine organoids, and n=49,155 epithelial cells derived from n=63 human tumor samples, we identified three pathway-derived subtypes (PDS1-3), revealing previously overlooked changes in epithelial cell identities and phenotypic states. PDS2 represents tumors with stroma/immune-dense, regenerative stem-like features. PDS1 were associated with elevated MYC signaling, proliferation and canonical stem-cells with good prognosis, while PDS3 displayed a differentiated-like phenotype associated with reduced polycomb repressive complex 2 (PRC)-mediated gene repression and poorer prognosis.

The inverse relationship between MYC/PRC targets within PDS1/PDS3 tumors supported the development of stem maturation index, which was directly related to cellular entropy and differentiation status of colonic cells allowing our tumor data to be projected onto a Waddington's landscape. Given these associations with developmental patterns in established tumors, we next utilized the same pathway-level approach in combination with transcriptional data derived from n=2641 human polyps, where we observe that PDS traits can be established early in precancer development. Using a combination of trajectory analyzes, lineage identities and cellular phenotyping, these approaches have again revealed that, even in very early precancers, there is no association between Vogelstein-related mutational drivers and prominent phenotypic signaling cascades.

While work is ongoing, the use of pathway-level information provides a unique insight into the dynamic nature of phenotypic evolution across early-to-late stages of colorectal precancers and tumors.

#504 A comparative analysis of statistical and machine learning approaches to predict drug resistance based on synergistic genetic alterations.

R. Nair[1], N. R. Mistry[1], R. Khalife[2], A. M. Magliocco[2];
[1]University of Central Florida, Orlando, FL, [2]Protean BioDiagnostics, Orlando, FL

Introduction: This study evaluates the utility of a machine learning approach to predict drug resistance in breast cancer (BC), based on comparison to an automated statistical model, using data from cBioPortal.

Methods: Our *in silico* approach investigated the association between synergistic genetic alterations in BC and survival data with various treatments based on one study (TCGA, PanCancer Atlas 2018). A set of 491 commonly altered genes in cancer (based on TSO500) was analyzed for this study. We programmed a statistical model to automatically select pairs of genes whose alterations (mutations, structural variants/fusions, copy number alterations) correlated with significantly worse five-year overall survival (OS) upon synergistic alterations, compared to one gene alone ($p<0.05$; $HR\geq1$). The results were analyzed via subgroup analysis of six treatment groups according to cBioPortal (chemotherapy, ancillary, hormone, immunotherapy, radiation, other) to predict potential drug resistance if HR significantly changed based on treatment. We compared our results with a machine learning approach. The model first computed relevancy/attention scores on treatments based on order, duration and type. This allowed the model to consider chronological order and simultaneous treatment administration. We then fed this into a model that returned a classification score (probability of death before 60 months) with ~90% accuracy, and another model that returned a regression score (expected survival in months) score, with mean squared error<0.02.

Results: A statistical brute force approach tested each gene's influence on another, and each treatment category's influence on a gene pair. Permutations of pairs from the geneset (240590 total) were generated for each treatment; 570 corresponded with significantly worse OS ($HR\geq1$) with chemotherapy, 392 with hormone therapy, 124 with immunotherapy and 614 with radiation therapy. Less than five patients had ancillary or other treatment, preventing us from generating statistically significant results. Permutations were used since the order of genes matters during statistical analysis for alterations to one gene versus synergistic alterations. The machine learning approach yielded treatment rankings for each gene permutation based on the classification score. The latest test demonstrated that most gene permutations correspond with a high classification score with hormone therapy (48%), and most gene permutations correspond with worse total OS with chemotherapy (86%). Rankings show promise that some marked values from statistical analysis are shared with the machine learning model, but further work is needed to improve stability to make accurate predictions for survival outcomes.

Conclusion: Our findings suggest the possible utility of an automated approach to predict gene pairs that confer drug resistance in BC.

#505 Differences in gene expression across races in pediatric T-cell acute lymphoblastic leukemia (T-ALL).

Rishabh Gaur[1], Irina Pushel[2], Midhat Farooqi[2]

[1]University of Missouri-Kansas City School of Medicine, Kansas City, MO,[2]Children's Mercy Hospital, Kansas City, MO

T-cell acute lymphoblastic leukemia (T-ALL) is a malignancy of immature T-cells characterized by rapid proliferation of leukemic cells in the bone marrow and blood. Despite significant advancements in the understanding and treatment of B-cell acute lymphoblastic leukemia (B-ALL), T-ALL remains comparatively underexplored, with limited data on the underlying genomic alterations driving disease progression. T-ALL constitutes 12-15% of pediatric and adult ALL cases, with poorer outcomes than B-ALL. Understanding differential gene expression in T-ALL is critical, especially with respect to how these may differ across racial groups, as African Americans and Asians have been shown to have lower survival than Caucasians.

In this study, we analyzed RNA sequencing data from a cohort of pediatric T-ALL patients to identify differences in gene expression patterns between racial groups, specifically focusing on Caucasian, Asian, and African American patients. Using DESeq2, differential gene expression analysis was performed across racial categories. A total of 37 patients with genomic data were included, with expression counts normalized, and statistical significance was determined based on adjusted p-values <0.05. We also examined potential correlations between gene expression and clinical outcomes such as relapse status. Preliminary results identified several genes exhibiting significant differential expression between racial groups. 250 genes, mostly related to the adaptive immune system, were significantly differentially expressed in Caucasian vs African American patients. 261 genes, mostly related to homeostasis and cellular development, showed differential expression in Caucasian vs Asian patients. There was an overlap of 17 genes which were differentially expressed in all three races. Importantly, FAT1 was one of these 17 genes, as it is a gene that has previously been implicated in T-ALL but not studied with regard to differences in expression by race. Preliminary analyses also found differentially expressed genes between African American vs Asian and relapse vs non-relapse. Additional studies are ongoing to determine the specificity of all these genes to T-ALL and to compare the genes that were differentially expressed across various races to the genes differentially expressed across outcomes.

This study highlights the importance of examining racial differences in gene expression within T-ALL, a leukemia subtype that has not been extensively understood. The identification of race-specific gene expression profiles may provide insights into the biological mechanisms that contribute to differences in clinical outcomes and response to treatment. Future work will focus on validating these findings in larger, independent cohorts and exploring how these genetic differences may influence disease progression, ultimately reducing racial disparities in T-ALL survival.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Repurposing New and Existing Drugs for Cancer Treatment
Poster Session

#507 Nebivolol induces autophagy and inhibits cell proliferation in triple negative breast cancer.

Wangjia Cao[1], Tagari Samanta[2], Benny A. Kaipparettu[2], Meghana V. Trivedi[1]

[1]Department of Pharmacological & Pharmaceutical Sciences, University of Houston, Houston, TX,[2]Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX

Background: Among various types of breast cancer (BC), triple negative BC (TNBC) has a high-risk of recurrence and poorer prognosis. Chemotherapy, which is associated with many acute and chronic toxicities, remains the primary treatment for TNBC. Despite aggressive therapy, about one-third of early-stage TNBC patients develop metastasis within 5 years of diagnosis and have a median survival of only 12 months. Therefore, identifying safe and effective novel treatment for TNBC is of utmost importance. Using 2 cell models, our group recently reported the repurposing potential of nebivolol (NEB) in TNBC cells. Here, we aimed to confirm the effects of NEB using a panel of 10 TNBC cell lines and identify its mechanism of action.
Methods: The half-maximal inhibitory concentrations of NEB (IC50) were determined across 10 cell lines by a concentration-response curve using non-linear regression analysis fitted to a 3-parameter logistic equation. MDA-MB-231 and SUM159 cells were chosen for subsequent experiments. RNA-seq followed by enrichment analysis using KEGG database and reverse phase protein array (RPPA) followed by two-way Analysis of Variance (ANOVA) with multiple comparisons were conducted to identify downstream pathways and target proteins impacted by NEB. To assess the effect on autophagy, lysosome formation, and cell proliferation, autophagy and lysosome fluorescent dyes and EdU proliferation assay were used, respectively, after treating the cells with various NEB concentrations. Fluorescent images from these experiments were imaged using a Nikon confocal microscope and analyzed in ImageJ. Differential expression of protein candidates from RPPA and autophagy related markers was confirmed using western blotting (WB). A p-value < 0.05 was considered statistically significant by student's t-test and one-way ANOVA, as appropriate.
Results: NEB inhibited the growth of all 10 TNBC cell lines in a concentration-dependent manner. The IC50 of NEB for TNBC cell lines ranged from 6.1 to 13.9 µM. Integrative RNA-seq analysis of both cell line models suggested significant upregulation of autophagy pathway (FDR < 0.05) after NEB treatment. NEB induced autophagy and lysosome formation in a concentration- and time-dependent manner, significantly inhibited cell proliferation, and significantly upregulated autophagy markers p62 and LC3B-II. In RPPA, DNMT3B and pTRAP220/MED1 were the most significantly downregulated proteins after NEB treatment in both cell lines (FDR < 0.05), which was further confirmed using WB.
Conclusion: NEB inhibits TNBC growth and proliferation and induces autophagy and lysosome formation by modulating key proteins. Ongoing studies include the analysis of NEB's effects on inducing apoptosis and its role in clonogenic and tumorigenic potential of TNBC cells.

#508 Triple negative breast cancer growth and immune responses to metronomic chemotherapy regimens with varied drugs and dosages.

Amanda I. Rakoski, Sarah Y. Neshat, Joshua C. Doloff

Biomedical Engineering, Johns Hopkins University, Baltimore, MD

Despite an increasing number of chemotherapy options, triple-negative breast cancer (TNBC) has arisen as the subtype that causes the most breast cancer mortality as it is not sensitive to drugs targeting estrogen, progesterone, and epidermal growth factor 1 receptors. The current standard of care for cancer, utilizing maximum tolerated dose with a following long break for patient recovery, has not been optimal as it causes temporary tumor regression. We propose utilizing metronomic dose scheduling where a reduced amount of drug is given on a schedule with intermittent drug-free breaks. Due to findings on the utility of metronomic chemotherapy against glioblastoma (JCD, et al. Molecular Cancer) where cyclical immune cell infiltrations have been seen, we are applying a similar 6-day schedule on aggressive TNBC mouse models to investigate if we can cause similar induction of antitumor immunity and tumor regression. For our experiments, 4T1 TNBC cells syngeneic to BALB/c mice were injected orthotopically into the right mammary fat pad. Once tumors were ~200 mm3, treatment was initiated. Various clinically relevant chemotherapies at doses shown in the literature to affect cancer growth were dissolved in vehicles and dispensed based on mouse body weight (mg/kg). Every 6 days, drug solutions were injected intraperitoneally (IP). We additionally injected 200 µg anti-PD1 (aPD1) IP starting at day -3 and every 6 days after that for chosen drugs at 100 mg/kg. Mice were sacrificed and tissues collected after 4 rounds of chemotherapy. Comparing six chemotherapies, we found no tumor regression, but tumor stasis was instead achieved in gemcitabine (GEM) and cyclophosphamide (CPA)-treated mice. After increasing GEM up to 150 mg/kg, CPA up to 200 mg/kg, and adding aPD1 to GEM and CPA at 100 mg/kg, the tumor volume still remained static vs. GEM and CPA at 100 mg/kg alone. To investigate local immune responses, qPCR was performed on bulk tumor tissues, measuring RNA expression of gene markers for immune cells. Immune trends comparing GEM and CPA varied based on drug mechanism of action. Anti-metabolite GEM caused more expression of markers associated with the innate immune system, seen with natural killer cell marker NKp46. Alkylating agent CPA-treated tumors experienced more favorable adaptive immune cell RNA expression such as increased CD8+ T cell marker CD8a and decreased FoxP3 marker seen in Tregs. Generally, decreased immune marker gene expression was seen when the mice were given higher doses compared to 100 mg/kg, suggesting dose-limiting immune cytotoxicity. aPD1 co-treated tumors also had increased immune marker expression but not enough to change tumor stasis to regression. Ultimately, these findings showcase that metronomic chemotherapy can bring upon immune cell responses, but drug dose and activation of immune cells need to be carefully considered for potential in achieving tumor regression.

#509 Synergistic inhibition of TNBC via enhancer of zeste homologue 2 (EZH2) inhibition and D1 receptor agonism.

Rajni Kant Shukla[1], Kate Ormiston[2], Gautam Sarathy[3], Shivani Dhekne[3], Sanjay Gupta[4], Daniel Stover[2], Christian Rolfo[2], **Eswar Shankar**[2]

[1]Cardiology, Oklahoma Health Science Center, Columbus, OK,[2]Internal Medicine, The Ohio State University Wexner Medical Ctr., Columbus, OH,[3]The Ohio State University Wexner Medical Ctr., Columbus, OH,[4]Urology, Case Western Reserve University School of Medicine, Columbus, OH

EZH2, a histone methyltransferase linked to poor outcomes in cancer, is overexpressed in triple-negative breast cancer (TNBC), disproportionately affecting African-American and Hispanic women. EZH2 regulates the tumor immune microenvironment (TIME) by inhibiting MHC-I antigen presentation, upregulating PD-L1, leading to reduced antitumor immune responses. Current EZH2 inhibitors target its catalytic activity; however, the development of resistance mechanisms in cancer cells frequently undermines their effectiveness. The neurotransmitter dopamine, through activation of its D1 receptor, promotes apoptosis and autophagy while suppressing cancer cell invasion. Dopamine D1 receptor signaling mitigates the immunosuppressive effects of myeloid-derived suppressor cells (MDSCs) on T cell proliferation and IFN-γ production. Using in silico approaches such as cryptic pocket identification, docking, and molecular dynamics (MD) simulations, initially we assessed the binding affinity of the DRD1 agonist to EZH2. Our in-silico analyses uncovered stable binding profiles, indicating novel interactions at previously uncharacterized sites within the CXC domain and the EED-binding region for both GSK126 and A77636. Next, we performed in vivo experiments combining GSK126 and A77636 to evaluate their effect on tumor growth and the TIME using an NSG mouse model. MDA-MB-231 cells were injected into the breast fat pads of 4-6-week-old female NSG mice. Once tumors were palpated, the mice were randomized into four groups: vehicle treatment, GSK126 (2 mg/kg), A77636 (50 mg/kg), and the combination of both, with treatments administered intraperitoneally for four weeks. Tumor growth was monitored throughout the treatment period, and at the termination, blood, tumor, and spleen were collected and processed into single-cell suspensions for high-dimensional flow cytometry analysis. GSK126 and A77636 treatment in combination significantly reduced tumor volume and decreased EZH2 expression in monocytes and neutrophils within the blood and tumor microenvironment and lowering the levels of pro-inflammatory cytokine IL-1β. In summary, this approach unveils new possibilities for treating TNBC by concurrently targeting EZH2 and DRD1 through a combinatorial strategy. (This work is supported by DOD: W81XWH2010065, for Eswar Shankar).

TUMOR BIOLOGY: Human and PDX Models 1
Poster Session

#51 10 second classification of spontaneous lymphoma in patient-derived tumor xenograft models.
N.-A. Pham, L. A. Ye, J. Froment, A. Zarrine-Afsar;
University Health Network, Toronto, ON, Canada

Patient-derived tumor xenografts (PDX) propagated in immunodeficient murine hosts have become effective preclinical models used in drug development and biological studies. PDX models have been established across tumor types with varying degrees of success. A main factor of engraftment failure is formation of spontaneous lymphocytic growths that may occur in murine hosts at the implant site of the patient tumor fragments or serial PDX fragments. Continuous monitoring of fidelity of PDXs to their parent patient tumors is by histological slide review of collected tumors. Processing for histological reviews could range from several hours to days, thus lacking in real-time decision-making support for utility of specimens.
Method: Ambient Picosecond InfraRed Laser (PIRL) ablation soft ionization Mass Spectrometry (MS) was evaluated as a potential tool for rapid classification of spontaneous lymphocytic growth compared to PDX tumor growths. This method uses a 10-second sampling and analysis duration of 3 mm^3 tumor fragments.
Results: PIRL MS analyses across 5 common tumor types of pancreas, lung, colon, ovarian and head and neck with a total of 90 PDX specimens and 20 samples of H&E verified lymphocytic growths was used to train a Principal Component Analysis Linear Discriminate Analysis (PCA-LDA) model to discriminate lymphocytic growths from tumors. Cross-validation using a 20% leave-out test resulted in >94% accuracy for correctly classifying lymphocytic or tumor growths. To ensure the validity of this observation, a permutated version of this model with false annotation resulted in prediction accuracy of ~50% in a 20% leave-out cross-validation. A test set of 90 specimens comprised of 15 lymphomas and 75 tumours was blindly sampled and subjected to the aforementioned model for prediction of specimen type. This blind assessment resulted in 99% sensitivity and 99% specificity values for either of the lymphoma or tumor classes.
Conclusion: This proof-of-principle evaluation suggests that 10-second PIRL MS analysis is able to differentiate between lymphocytic and true tumor growths, further highlighting distinct mass to charge (*m/z*) values specific to each tissue type. PIRL-MS can therefore enable real-time decision-making with respect to the utility of PDX tissue collection for immediate downstream laboratory use, and to further minimize the cost associated with unintentional use of non-tumor outgrowths. The next steps involve determining the molecular identities of lymphoma classifying *m/z* values and to assess the PCA-LDA model's sensitivity to engrafts that contain both lymphocytic and cancer growths.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Repurposing New and Existing Drugs for Cancer Treatment
Poster Session

#510 Neurotransmitter receptor antagonists and metronomic chemotherapy: A promising novel approach for brain metastases in breast cancer.

Arianna Bandini[1], Paola Orlandi[1], Marta Banchi[1], Giulio Francia[2], Guido Bocci[1]

[1]Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy,[2]Border Biomedical Research Center, University of Texas at El Paso, El Paso, TX

Brain metastases (BM) from breast cancer are a significant clinical issue with a poor prognosis. New therapies targeting the tumor microenvironment are promising. Various types of neurotransmitter (NT) receptors (e.g., glutamatergic, dopaminergic, serotoninergic receptors) are expressed on breast cancer (BC) cells, modulating cell proliferation, survival, and migration. We propose a novel approach for BM treatment using NT receptor antagonists in combination with metronomic chemotherapy (mCHEMO) - the frequent and regular administration of drug doses, for extended time, without breaks - to block the pro-tumoral interaction between cancer cells and the brain microenvironment, increasing therapeutic efficacy and reducing toxicity. The Kaplan-Meier plotter website was used to assess the impact of the NT receptor gene expression of our interest on overall survival (OS) in metastatic BC patients. Real-Time PCR assessed NT receptor gene expression in monolayer and spheroid cultures of MCF-7 (ER+), MDA-MB-231 (triple-negative), and MDA-MB-361 (HER2+) BC cell lines. Proliferation assays were performed on BC cell lines exposed to metronomic temozolomide (TMZ) and NT receptor antagonists (memantine, ML00253764, SB-269970, clozapine) for 144h daily treatments. The synergism of the concomitant combinations between low-dose TMZ and NT receptor antagonists was evaluated by the combination index method.The Kaplan-Meier analysis showed that a higher level of NT receptors expression is associated with worse OS rates in metastatic BC, particularly with elevated *GRIN1* and *MC4R*. Indeed, genes of NT receptors were highly expressed in BC cell lines. The MC4R antagonist ML00253764 was active on tumor cell lines with a strong antiproliferative effect on MDA-MB-231, showing IC_{50} value of 4.72 nM. The NMDA antagonist memantine was very effective on MDA-MB-361 with a concentration- and time-dependent effect (IC_{50} at 144h 168.1 nM), while the 5-HT_7 receptor antagonist SB-269970 showed encouraging antiproliferative results on MCF-7 (IC_{50} 9.61 nM). Metronomic TMZ decreased cell proliferation in tumor cell lines. The concomitant combinations of TMZ+ML00253764, TMZ+memantine, and TMZ+SB-269970 showed marked synergisms, notably on MDA-MB-361 cells. These findings build rational bases for the promising therapeutic approach, including NT receptor antagonists and metronomic TMZ, for breast cancer BM. Moreover, *in vitro* experiments demonstrated a key role of NT receptor on cell proliferation due to the activity of pharmacological antagonists alone or with mCHEMO. These results support further *in vitro* 3D investigation on multicellular spheroids and *in vivo* experiments on BM models to validate our pioneering therapeutic hypothesis, which could be easily translated into future clinical trials.

#511 Neratinib monotherapy in advanced non-small cell lung cancer with EGFR exon 18 mutations in China.

Wenfeng Fang[1], Ning Li[2], Runxiang Yang[3], Ying Cheng[4], Baogang Liu[5], Haiyong Wang[6], Lin Wu[7], Qiming Wang[8], **Steve Shen**[9]

[1]Sun Yat-sen University Cancer Center, Guangzhou, China,[2]Cancer Hospital Chinese Academy of Medical Sciences, Beijing, China,[3]Yunan Cancer Hospital, Kunming, China,[4]Jilin Cancer Hospital, Changchun, China,[5]Harbin Medical University Cancer Hospital, Harbin, China,[6]Shandong Cancer Hospital, Changsha, China,[7]Hunan Cancer Hospital, Changsha, China,[8]Henan Cancer Hospital, Changsha, China,[9]Convalife (Shanghai) Co., Ltd., Shanghai, China

Background: In NSCLC patients, EGFR exon 18 mutations account for approximately 3-5% and are associated with a poor prognosis. In phase 2 SUMMIT global trial, 29 metastatic NSCLC patients with EGFR exon 18 mutations received neratinib monotherapy, having good result. The objective response rate (ORR) was 35%. However, only 1 (4.5%) Asian individual is enrolled in study. Therefore, there is an urgent need to explore the effect of neratinib monotherapy in Chinese NSCLC patients with EGFR exon 18 mutation.
Methods: Planned 14 patients with EGFR exon 18-mutated NSCLC who had failed first-line standard chemotherapy were enrolled in this study(NCT06029816) . The primary endpoint is the ORR by the investigator according to RECISTv1.1 and the secondary endpoints are DCR (Disease Control Rate), PFS (Progression-Free Survival).
Results: To date, 7 advanced NSCLC patients with exon 18 mutations after receiving Neratinib treatment following the failure of standard first-line therapy. The ORR is 42.9% (3 PR, 4 SD) and DCR is 100.0%. The common 1-2 grade treatment-related adverse events (AEs) occur in 82% of patients. 28.6% of patients have Diarrhea (CTCAE Grade 2) , and that can be managed after loperamide intervention within 3 days. To date, there have been no grade 4- 5 AEs in any of the patients.
Conclusion: Neratinib monotherapy has demonstrated tolerable safety and encouraging preliminary efficacy in NSCLC with EGFR exon 18 mutations. This study is expected to provide more treatment options for Chinese patients with NSCLC who have EGFR exon 18 mutations.
Keywords: Neratinib; EGFR mutations; NSCLC

#512 Inhibition of ATR enhances the anticancer activity of ciclopirox in triple-negative breast cancer.

Bing Cheng[1], Chinedum Okafor[2], Jehan Abdulsattar[2], Bolin Liu[3], **Shile Huang**[4]

[1]Biochemistry and Molecular Biology, LSU Health Sciences Center - Shreveport, Shreveport, LA,[2]Pathology, LSU Health Sciences Center - Shreveport, Shreveport, LA,[3]LSU Health Sciences Center - New Orleans, New Orleans, LA,[4]LSU Health Sciences Center - Shreveport, Shreveport, LA

Triple-negative breast cancer (TNBC) is a deadly and difficult-to-treat cancer, partly due to drug resistance, which links to enhanced DNA damage response (DDR). Our previous studies have shown that ciclopirox olamine (CPX), an off-patent fungicide, inhibits cell proliferation and induces apoptosis of various tumor cells *in vitro* and *in vivo*. Mechanistically, this is partly associated with inhibition of the mTOR signaling in tumor cells. Meanwhile, CPX can induce DDR by activating the ATR-Chk1 pathway. AZD6738 (ATR inhibitor) is currently under clinical trials for treatment of solid tumors including TNBC. This study aimed at investigating whether AZD6738 can enhance the anticancer activity of CPX in TNBC. We found that AZD6738 potentiated CPX-induced inhibition of cell proliferation and induction of apoptosis in TNBC cells. CPX treatment decreased the levels of cyclins, CDKs and phospho-Rb and increased the levels of cleaved caspase-3 and γH2AX in TNBC cells, which could be strengthened by addition of AZD6738. In addition, we observed that the combined treatment with CPX/AZD6738 did not exhibit significant toxicity in nude mice, but inhibited tumor growth more potently than the single treatment with CPX or AZD6738 in MDA-MB-231 xenografts. The results indicate that inhibition of ATR with AZD6738 can potentiate the anticancer activity of CPX in TNBC cells *in vitro* and *in vivo*. Our finding suggests that combination of CPX and AZD6738 may represent a novel therapeutic approach for TNBC.

#514 Therapeutic potential of antidepressant amitriptyline in medulloblastoma.

Frank B. Wang[1], Prabhakar Pitta Venkata[1], Andrew Brenner[2], William Kelly[2], Gangadhara R. Sareddy[3], Manjeet K. Rao[1]

[1]Greehey Children's Cancer Research Institute - UT Health San Antonio, San Antonio, TX,[2]Mays Cancer Center - UT Health San Antonio, San Antonio, TX,[3]Department of Obstetrics & Gynecology, UT Health San Antonio, San Antonio, TX

Introduction: Medulloblastoma, the most common malignant brain tumor in children, primarily arises in the posterior fossa, predominantly affecting the cerebellum. Accounting for approximately 20% of all pediatric brain tumors, medulloblastoma has an annual incidence of about 500 cases in the United States, with a peak age of diagnosis between 5 and 7 years and a slight male predominance. Advances in genomic and epigenomic profiling have identified four distinct subgroups—WNT, Sonic Hedgehog (SHH), Group 3, and Group 4—each characterized by unique molecular features, patient demographics, and prognostic outcomes. Current treatment strategies, including surgical resection, craniospinal irradiation, and adjuvant chemotherapy, have improved survival rates; however, up to 40% of patients experience incurable recurrence or metastasis, the leading cause of mortality. Furthermore, survivors often endure debilitating long-term side effects from these aggressive therapies, underscoring the urgent need for more effective and less toxic therapeutic approaches.
Methods: To evaluate the anticancer activity of Amitriptyline, we utilized SHH (DAOY) and Group 3 (HDMB03, D556, D425) medulloblastoma cell lines. Cell viability, survival, and apoptosis were assessed through established functional assays. The impact of Amitriptyline on cancer stemness was investigated using sphere formation and extreme limiting dilution assays. Mechanistic studies were conducted through RNA sequencing, quantitative RT-PCR, and Western blot analysis to elucidate molecular pathways. The therapeutic potential of Amitriptyline was further validated in vivo using orthotopic xenograft mouse models of medulloblastoma.
Results: Amitriptyline treatment significantly inhibited cell viability, colony formation, and migratory capacity while promoting apoptosis in medulloblastoma cell lines. Additionally, Amitriptyline reduced the viability and sphere-forming ability of medulloblastoma stem cells, indicating a potent effect on cancer stemness. Notably, Amitriptyline enhanced the therapeutic efficacy of Vincristine in medulloblastoma cell lines. *In vivo* studies demonstrated that Amitriptyline treatment significantly prolonged survival in the orthotopic xenograft mouse model, with no sign of toxicity, highlighting its therapeutic potential.
Conclusion: Collectively, our findings suggest that Amitriptyline may serve as a viable therapeutic option for medulloblastoma, providing a basis for potential clinical application.

#515 Repurposing nebivolol with dasatinib and CPI-613: A synergistic strategy to combat triple-negative breast cancer.

Mikayla Skillman[1], Airong Li[1], Fatima Dagher[1], Diana Chow[1], Meghana Trivedi[2]

[1]Department of Pharmacological & Pharmaceutical Sciences, University of Houston, Houston, TX,[2]Department of Pharmacy Practice and Translational Research, University of Houston, Houston, TX

Triple-negative breast cancer (TNBC) is a relentless and aggressive disease, posing significant challenges due to its high recurrence rates and poor prognosis. Existing treatments, such as chemotherapy and radiation, often cause long-term side effects, and over half of patients experience relapse within a few years of diagnosis. This study investigates the novel approach of repurposing nebivolol (NEB), a beta-blocker with a well-established safety profile, in combination with dasatinib (DAS) or Devimistat (CPI) to potentially enhance anti-tumor efficacy in TNBC. Dasatinib targets multiple kinases involved in cancer cell proliferation and metastasis, while CPI-613 induces metabolic stress and apoptosis in cancer cells. We evaluated the cytotoxic effects of NEB, DAS, and CPI, both individually and in combination, across TNBC cell lines (MDA-MB-231, 4T1, BT-549) using MTT assays. IC_{50} values were determined, and optimal drug ratios were identified using the Chou-Talalay method and 3D surface plots. Drug ranges were selected below and above determined IC_{50} values and used in a two-system matrix design. Combination Index (CI) plots were generated to assess synergistic interactions. Additionally, a migration assay was performed to evaluate the effects of the combinations on cell migration. NEB, DAS, and CPI individually reduced cell viability with IC_{50} values of 6.3 µM, 76.4 nM, and 71.1 µM in MDA-MB-231 cells; 12.8 µM, 91.2 nM, and 41.4 µM in 4T1 cells; and 2.7 µM, 33.2 nM, and 24.8 µM in BT-549 cells. The NEB-DAS combination exhibited significant synergy in BT-549 cells, with a CI of 0.35 at 800 nM NEB and 3 nM DAS ($p < 0.002$ compared to each drug alone). Similarly, NEB-CPI demonstrated additive effects in BT-549 cells, with a CI of 1.0 at 800 nM NEB and 20 µM CPI ($p < 0.003$ compared to each drug alone). In MDA-MB-231 cells, the NEB (3 µM)-DAS (50 nM) combination showed significant synergy only with NEB ($p = 0.0007$, CI 0.85), while the NEB (3 µM) -CPI (15 µM) combination was synergistic compared to either drug alone ($p < 0.001$, CI 0.49). For 4T1 cells, NEB (6 µM) and CPI (20 µM) was significantly more potent compared to each drug alone ($p < 0.0001$, CI 0.32), and NEB (3 µM) and DAS (30 nM) was more potent compared to each drug alone ($p < 0.004$). Most combinations using Chou-Talalay had CI values predominantly below 1, confirming synergy. The migration assay indicated that NEB and CPI significantly reduced migration in MB-231 cells compared to either drug alone ($p < 0.03$). Interestingly, the NEB-DAS combination did not show a significant difference compared to DAS alone ($p=0.17$) but was more effective than NEB alone ($p = 0.0009$). These findings highlight the potential of NEB in combination with DAS or CPI as a promising therapeutic strategy for TNBC. Ongoing *in vivo* studies using a TNBC mouse model aim to validate these findings and further explore the clinical potential of these combinations to improve TNBC patient outcomes.

#516 SOX4 dictates gastric cancer progression by modulating the tumor immune microenvironment.

G. Zou[1], K. Yoshimura[2], Y. Fan[1], R. Wang[1], K. Yamashita[1], S. Shao[1], A. Scott[1], J. Jin[1], M. P. Pizzi[1], M. Silva[1], X. Yao[3], C. Brown[1], R. Waters[1], S. Song[3], L. Wang[1], S. Dhar[1], J. A. Ajani[1];

[1]UT MD Anderson Cancer Center, Houston, TX, [2]Hamamatsu University School of Medicine, Hamamatsu, Japan, [3]Coriell Institute for Medical Research, Camden, NJ

SOX4 upregulation is common in multiple tumors, yet its role in shaping the immune microenvironment of gastric adenocarcinoma (GAC) remains unclear. This study investigates the influence of SOX4 on GAC progression and immune modulation. Using single-cell RNA sequencing, patient-derived models, and functional analyses, we examined SOX4 expression and its target genes in GAC. SOX4 was found to be highly expressed in both primary and metastatic GAC tissues, correlating with poor prognosis. Knockdown of SOX4 in GAC cells reduced tumor growth, migration, and colony formation and suppressed metastasis. Furthermore, SOX4 depletion enhanced T-cell cytokine production and promoted M2-macrophage repolarization in cocultures with patient-derived PBMCs and U937 cells. Conversely, SOX4 overexpression reinforced these aggressive phenotypes. RNA-seq analysis of SOX4 wildtype and knockout cells led to the identification of six drugs that could mimic the effects of SOX4 deletion, with Cucurbitacin-I and Tunicamycin emerging as potent inhibitors of GAC cell growth in patient-derived models. These findings highlight SOX4's role in driving GAC progression and immune evasion, offering potential therapeutic avenues for targeting SOX4 in GAC treatment.

#517 YAP-TEAD-mediated tumor suppression and immune modulation of gastric adenocarcinoma by HSP90 inhibition.

K. Yoshimura[1], **G. Zou**[2], Y. Fan[2], K. Yamashita[2], J. Wu[2], R. Wang[2], S. Shao[2], A. Scott[2], J. Jin[2], M. Pizzi[2], X. Yao[2], C. Brown[2], L. Wang[2], L. Wang[2], Q. Gan[2], R. Waters[2], Y. Feng[2], S. Song[3], S. Dhar[2], J. Ajani[2];

[1]Hamamatsu University School of Medicine, Hamamatsu, Japan, [2]UT MD Anderson Cancer Center, Houston, TX, [3]Coriell Institute for Medical Research, Camden, NJ

Heat shock protein 90 (HSP90), a vital chaperone involved in the folding and stabilization of various cellular proteins, regulates key functions in many tumor cells. In the context of gastric adenocarcinoma (GAC), where HSP90's role remains largely unexplored, we aimed to investigate the significance of HSP90 inhibitor, AUY922, in regulating the YAP1/TEAD pathway and its association with the tumor immune microenvironment (TME). Our results showed that AUY922 effectively inhibited GAC aggressiveness in both the in-vitro and in-vivo models, induced apoptosis, and cell-cycle arrest. Various functional assays elucidated that AUY922 potently inhibited the expression and interaction among YAP1/TEAD and HSP90, evoking in down-regulation of target functional genes. AUY922 additionally altered the TME into an inflamed state with increased cytokine production in T-cells and inhibition of M2-polarization of tumor-associated macrophages, rendering it a favorable partner for immune checkpoint inhibition. Our findings highlighted the suggestion of targeting HSP90 in GAC therapy via down-regulating YAP1/TEAD signaling. Further, results suggest that AUY922's ability to reshape the GAC TME favoring the host sets the stage for a clinical trial that combines HSP90 and checkpoint inhibition, where HSP90 could serve as a biomarker for patient selection.

#518 Repurposing of pralatrexate in gastric cancer: Inducing cell death via SMO inhibition and GLI1 suppression.

Hyemi Yun[1], Bo Ram Kim[2], Bu Gyeom Kim[2], Dae Yeong Kim[2], Okhyeon Kim[1], Yoon Namgung[3], Jun Woo Bong[4], Sanghee Kang[4], Sun Il Lee[4], Sang Cheul Oh[2]

[1]Biomedical Sciences, Korea University College of Medicine, Seoul, Korea, Republic of,[2]Department of Oncology, Korea University Guro Hospital, Seoul, Korea, Republic of,[3]Division of Oncology/Hematology, Department of Internal Medicine, Korea University Guro Hospital, Seoul, Korea, Republic of,[4]Surgery, Korea University Guro Hospital, Seoul, Korea, Republic of

Gastric cancer (GC) is the fifth most common cancer and the fourth leading cause of cancer-related deaths worldwide. Currently, GC has serious drug resistance and side effects to existing treatments, and the development of new treatments is urgently needed. Abnormal activation of Hedgehog (Hh) signaling can promote the proliferation, metastasis, and drug resistance of GC cells and inhibit apoptosis. In gastrointestinal cancer, the expression of components of the Hh signaling pathway and their target genes are upregulated. Through TCGA analysis, we found that among the five types of digestive cancers: cholangial, colorectal, gastric, hepatocellular, and pancreatic, GC is most affected by Hh signaling. Pralatrexate is an FDA-approved drug originally used to treat relapsed or refractory peripheral T-cell lymphoma (PTCL). Research has been conducted on its anticancer efficacy in solid carcinomas such as Non-small cell lung cancer (NSCLC), but its efficacy in GC remains unexplored. In a previous study, screening results using an FDA-approved drug library showed that Pralatrexate inhibits GLI-dependent luciferase activity. In this study, we demonstrated that Pralatrexate canonically regulates Hh signaling by downregulating SMO. Structural predictions using AI modeling indicated that Pralatrexate binds to a site similar to the SMO antagonist ANTA-XV, distinct from Cyclopamine's binding pocket. It was confirmed that Pralatrexate causes degradation of SMO, and GLI1 was subsequently degraded by lysosome. This suppression of Hedgehog (Hh) signaling results in reduced cell proliferation and the induction of apoptosis. In summary, Pralatrexate canonically regulates Hh signaling by inhibiting SMO and suppressing GLI1, ultimately reducing cell proliferation and causing apoptosis in GC. Our findings uncover a novel anticancer mechanism of Pralatrexate in GC, providing a potential basis for its clinical application in GC therapy.

#519 CDK4/6 inhibitors and endocrine therapies induce senescence with drug- and cell line-specific vulnerabilities in ERα+ breast cancer.

Lena Batoon, Angelo J. Guilatco, Anna Detry, Xiyin Wang, Megan M. Weivoda, John R. Hawse

Mayo Clinic, Rochester, MN

Estrogen receptor alpha (ERα)-positive breast cancer accounts for up to 80% of cases. Despite advances in CDK4/6 inhibitors (CDK4/6i) and endocrine therapies, recurrence and late relapse remain significant challenges. Recurrence often arises from disseminated cancer cells that persist despite 5-10 years of systemic adjuvant treatment. While CDK4/6i and endocrine therapies induce prolonged growth arrest resembling senescence, they do not significantly kill cancer cells. Senescent/pseudo-senescent cancer cells are implicated in aggressive variant formation, recurrence, and metastasis. We explored eliminating breast cancer cells via senolysis while they are in therapy-induced senescent state to prevent their potential to escape, repopulate, or cause long-term adverse effects. Live-cell imaging of MCF7 and T47D cells treated with abemaciclib and endocrine therapies (endoxifen or estrogen deprivation to mimic aromatase inhibition) at clinically relevant concentrations for eight days demonstrated prolonged growth cessation and G1 cell cycle arrest even two weeks post-drug withdrawal. These cells displayed a senescence-like gene expression profile, with increased *CDKN2B* (p15), *CDKN1A* (p21), *BCL2*, reduced *KI67*, and increased *TGFβ* expression, a known Senescence-Associated Secretory Phenotype (SASP) factor linked to deep senescence. These results confirm effective senescence induction by various CDK4/6i and endocrine therapy combinations. Further analysis using established senescence markers (e.g., nuclear size, telomere-associated foci, satellite distension, and loss of nuclear HMGB1 and LaminB1) revealed unique senescence phenotypes across therapy combinations. Cell viability assays and live imaging of Caspase 3/7 activation demonstrated differential vulnerabilities of senescent/senescent-like ERα+ cancer cells (MCF7, T47D, UCD4, UCD12) to 13 senolytics (1 μM), including natural flavonoids and inhibitors targeting pro-survival factors, tyrosine kinases, and the Notch pathway. For example, MCF7 and UCD4 cells responded to MCL1i and RSL3, T47D to BCLXLi and RSL3, and UCD12 to BCLXLi and Navitoclax. Notably, senolytic cocktails (e.g., RSL3, GNF7, Venetoclax, Dasatinib) at reduced concentrations (0.25 μM each) effectively eliminated senescent/senescent-like ERα+ cancer cells across all cancer cell lines, achieving 100% elimination in T47D cells. Collectively, these findings demonstrate that despite variability in therapy-induced senescence, senolytic combinations effectively target senescent ERα+ cancer cells. This provides preliminary evidence for the potential integration of this strategy into standard breast cancer therapy with a goal to improve recurrence-free survival and increase the cure rates for ERα+ breast cancer patients. In vivo studies are underway to evaluate this strategy in preclinical models.

#520 Repurposing verteporfin: anti-metastatic properties associate with extracellular matrix modulation in osteosarcoma.

Danny Carreira[1], Anita K. Luu[1], Carey E. Dougan[2], Shelly R. Peyton[2], Geoffrey A. Wood[3], Brenda L. Coomber[1], Alicia M. Viloria-Petit[1]

[1]Biomedical Sciences, University of Guelph, Guelph, ON, Canada,[2]Chemical Engineering, University of Massachusetts Amherst, Amherst, MA,[3]Pathobiology, University of Guelph, Guelph, ON, Canada

Osteosarcoma (OS) is the most common primary bone cancer; 20% of OS patients present with metastasis at diagnosis, with more than 80% of those cases having spread to the lungs. Lack of treatments for metastatic disease leads to poor survival outcomes, making it imperative to find better therapies. Verteporfin (VP), a photosensitizer used for the treatment of age-related macular degeneration, targets the hippo signaling pathway via YAP1, while also inhibiting autophagy mediators through the formation of crosslinked oligomers, all without direct photoactivation. The purpose of this study was to assess VP's effect on OS lung colonization, biomechanical properties, and extracellular matrix modulation independent of photoactivation.

K7M2 OS cells or PBS were tail vein injected into BALB/c mice, and K7M2 injected mice were assigned to receive intraperitoneal 20 mg/kg VP or vehicle twice a week, starting at day 2 post-injection. Histological analysis of H&E and Masson's trichrome stained sections was performed using QuPath on lung tissue collected on days 4, 8, 16, and 24 to assess metastatic burden and collagen content, respectively. Biomechanical analysis was performed with a custom indenter setup on lung samples with and without lesions to measure tissue stiffness. Insoluble protein contents were analyzed via mass spectrometry. Subsequently, in-vitro analysis was performed in K7M2 cells following treatment with 0.09 μM, 1.5 μM, 7.5 μM VP or vehicle for 24 hours, controlling for light. Fibronectin (FN) and tumor-related FN isoform (EIIIA, EIIIB, and IIICS) levels were assessed at the mRNA level via RT-qPCR on cell and lung samples, and FN protein expression was analyzed through immunofluorescence microscopy of cultured K7M2 cells.

Histological analysis of day 24 lung samples highlighted a significant reduction in metastatic burden following VP treatment. Interestingly, stiffness was unchanged across day 4 and 24 in lung samples from vehicle-treated mice; however, VP treated samples showed a small yet significant increase. Collagen levels in metastases and stroma from vehicle and VP injected mice were unchanged throughout OS progression, though mass spectrometry identified FN enrichment in lung tissue from VP treated mice. FN mRNA levels were unchanged in cell lysates; conversely, lung samples highlighted reduced FN mRNA with metastasis, with VP restoring levels to that of normal lung. EIIIB FN was found to be decreased with metastasis, though largely variable with VP treatment. In cultured cells, FN protein expression increased parallel to VP concentration, with unchanged distribution. Results point towards VP effectively reducing OS metastasis to the lungs in parallel to increases in stiffness and FN expression. Further research on the role of the ECM, including FN and its isoforms, will further our understanding of the anti-metastatic properties of VP.

#521 Drug repurposing via ultra-high throughput 3D spheroids screening in rare cancer treatment.

M. G. Yonk[1], M. S. Tora[1], M. A. Lim[2], T. Federici[1], H. Fu[1], Y. Du[1], B. Yao[1], N. Boulis[1], K. Lei[1];
[1]Emory University School of Medicine, Atlanta, GA, [2]University of Illinois Urbana Champaign, Champaign, IL

Spinal cord gliomas (SCG) are rare cancers, and relevant pharmaceutical research and treatments are limited. While drug repurposing of FDA-approved compounds has shown promise for orphan diseases, its potential for SCG remains uncertain. Our research team has recently developed an SCG model in minipigs, offering anatomical, immunological, developmental, and genetic similarities to humans, which makes it an ideal model for translational precision medicine. In this study, we used a novel drug repurposing strategy using 3D spheroids from our recently established large animal SCG model as a case study. First, we established a miniaturized 3D cell culture platform for ultra-high-throughput screening (uHTS). After screening the FDA-approved and Bioactive compound library with minipig SCG 3D spheroids, Clofoctol, showed potential as a glioma treatment by targeting genes in the p53 pathway. Evaluation in a xenograft model demonstrated Clofoctol's ability to suppress tumor growth over a 10-day treatment period, followed by immunohistochemistry and RNA-seq. These findings suggested Clofoctol is a promising therapeutic for glioma treatment. Thus, our study shows the feasibility of 3D spheroids uHTS to expedite orphan disease research, providing a strategy for future drug repurposing for rare cancer patients.

#522 One drug- Two targets in osteosarcoma.

Marina Curcic, Sanjay K. Srivastava

Texas Tech University Health Sciences Center, Lubbock, TX

Osteosarcoma, the most common primary malignant bone tumor, mostly affects adolescents and older adults, with limited therapeutic advances leading to poor survival rates for metastatic or recurrent cases. Current treatments often fail due to high mutational burdens and chemoresistance, necessitating new targeted therapies. In this study, we investigated the potential of PCT, a kinase inhibitor recently approved for hematological malignancies, for its anticancer effect against osteosarcoma. Our comprehensive in vitro analysis demonstrated that PCT inhibits cell viability, induces apoptosis, and causes G2/M arrest in multiple osteosarcoma cells. Gene expression analysis indicated that at very low micromolar concentrations, PCT inhibits the expression of AXL, β-Catenin and key players of the pathway at protein and mRNA level. Molecular docking analysis indicated PCT's high binding affinity to AXL confirming our observations that PCT suppresses AXL/β-Catenin expression and transcriptional activity. Oral administration of PCT significantly reduced osteosarcoma tumor growth in murine models without any observable toxicity. The tumors of PCT treated mice exhibited inhibition of AXL and β-Catenin signalling axis and induction of apoptosis. Our findings suggest PCT as a dual inhibitor of AXL and β-catenin, directly affecting osteosarcoma progression. This research highlights PCT's potential as a targeted therapeutic for osteosarcoma, offering new avenues to improve outcomes for patients with this aggressive malignancy.

#523 Minnelide as a potential treatment strategy for CIC::DUX4 sarcoma.

MaKenna R. Browne[1], Peter G. Hendrickson[2], David G. Kirsch[3]

[1]UHN Princess Margaret Cancer Centre, Toronto, ON, Canada,[2]Duke Universtiy, Durham, NC,[3]Radiation Medicine, UHN Princess Margaret Cancer Centre, Toronto, ON, Canada

Background: CIC::DUX4 Sarcoma (CDS) is driven by t(4;19) or t(10;19) chromosomal translocations and is associated with high rates of metastasis, chemoresistance, and poor outcomes. Previously classified as “Ewing-like sarcoma” due to overlapping morphological and immunobiological features, CDS differs from Ewing sarcoma in several key aspects: it rarely arises in bone, lacks the Ewing-defining ETS gene fusion, and has a more aggressive clinical course. Despite recent reclassification by the World Health Organization as its own entity, CDS patients are still treated with ineffective Ewing-based chemotherapies. This study evaluates the therapeutic potential of 160 compounds in human CDS cells, identifying Triptolide as a top candidate. Minnelide, the water-soluble prodrug of Triptolide, is currently undergoing Phase I/II trials for various cancers, including pancreatic and gastric cancer.

Methods: We conducted a viability drug screen on human CDS cells using a Tocriscreen compound library. The top hit, Triptolide, was subsequently validated using its water-soluble prodrug, Minnelide, which replicated the screen results. To investigate the mechanism of action, cells were treated for 72 hours at the IC_{50} concentration and processed for RNA sequencing. Finally, we modeled CDS by generating subcutaneous xenograft tumors in immunodeficient NSG mice. Mice were transplanted with CDS tumor-derived cells and treated with Minnelide (or vehicle control) once daily for 21 days. The drug doses (0.21 mg/kg and 0.27 mg/kg) were selected based on bioavailability data and maximum tolerability observed in Phase I/II clinical trials.

Results: Minnelide treatment decreased CDS cell viability and significantly reduced the growth of CIC::DUX4 sarcoma xenografts within 21 days. The Phase I clinical trial data indicates that the recommended starting dose for patients is 0.67 mg/m², which corresponds to a dosage of 0.21 mg/kg in mice. The average tumor volume of the 0.21 mg/kg Minnelide treatment group was significantly (two-way ANOVA, $P = 0.0001$) lower than that of the control group at day 21 post-treatment initiation. Moreover, drug treatment was not associated with any adverse side effects. RNA sequencing and western blot analysis of CDS cells treated with Minnelide revealed downregulation of many CIC::DUX4 target genes, such as ETV1/4/5 and DUSP6. These results suggest that Minnelide alters the CDS transcriptional program and could represent an efficacious treatment alternative.

Conclusion: Therapeutic advancements for CIC::DUX4 sarcoma have been limited to date, and there are currently no clinical trials available for CDS patients. Our findings demonstrate that Minnelide effectively reduces CDS cell viability and xenograft growth, while downregulating key CIC::DUX4 target genes. Given the role of CIC::DUX4 as a potent transcriptional activator, Minnelide's disruption of this activation may offer a promising therapeutic approach for CDS.

#524 An integrative approach to unveiling biomarkers and therapeutic targets identifies novel treatment strategy for advanced prostate cancer.

Shreya Shyam Sundar[1], Ephraim J. Gardner[1], Surendra Gulla[2], Remi Adelaiye-Ogala[2]

[1]Pharmacology, University at Buffalo, State University of New York, Buffalo, NY,[2]Department of Medicine, University at Buffalo, State University of New York, Buffalo, NY

Advanced prostate cancer (PCa) is one of the leading causes of cancer-related deaths in men in United states. Despite advances in treatment modalities, patients still develop resistance to treatment. Limited studies have focused on targeted therapeutic approach addressing the heterogeneity of the disease. However, a comprehensive multi-omics approach that integrates transcriptomic and proteomic profiles can discover pathways associated with the development of both primary and acquired resistance and remains a critical factor in identifying predictive markers of treatment efficacy to investigate new therapeutic options. In this study, we performed LC-MS/MS and downstream analysis in established patient derived xenograft (PDX) models of advanced castrated resistant PCa (CRPC) to detect protein abundance and enriched pathway. All terms and pathways with an FDR <0.05 after BH adjustment were used. To integrate and determine concordance, transcriptomic followed by enrichment pathway analysis was performed, revealing commonly enriched pathways at the proteomics and transcriptomics level. These data formed the bases for a biomarker-specified library of 100 FDA approved drugs for high throughput screening. Amongst all drugs tested, Trifluridine, previously FDA approved for viral eye infection and in combination with Tipiracil, approved for metastatic colorectal cancer, emerged as a promising candidate. Trifluridine in combination with enzalutamide, showed reduced cell proliferation in CRPC models. The potential of Trifluridine was further highlighted in CRPC cells exhibiting synergism when combined with PARP inhibitors. The synergistic impact of trifluridine alone or in combination will potentially enhance treatment efficacy in overcoming drug resistance by perhaps targeting the DNA repair mechanism. Therefore, our hypothesis is that repurposing Trifluridine alone or in combination with standard of care will be benefitable for treatment-resistant prostate cancers. Our current study has shown that changes in the proteomic landscape that are in concordance with changes at the transcriptomic level are associated with treatment resistance and can direct the best treatment option. This approach identified an FDA approved drug, trifluridine, that can be repurposed for treatment of CRPC, offering a promising treatment strategy for advanced disease. In summary, our studies highlight the unique benefits of using an integrative approach to unveil novel biomarkers and targets for enhanced therapeutic strategies for CRPC.

#525 From unbiased genetic screen to therapy: Combination of 5-ALA and artesunate for the treatment of primary glioblastoma multiforme.

Michael Orthofer[1], Marianna Rozsova[1], Ferdinand Baumgartner[1], Barabara Kiesel[2], Georg Widhalm[2], **Moritz Horn**[1], Josef M. Penninger[3]

[1]JLP Health GmbH, Vienna, Austria,[2]Medical University Vienna, Vienna, Austria,[3]Helmholtz Centre for Infection Research, Braunschweig, Germany

The natural compound Artemisinin and its derivative Artesunate are widely used anti-malarial drugs. Due to their cytotoxic activity, they have also been investigated as anti-cancer agents, while their precise mechanism of action and key host cell targets have remained largely elusive. Using an innovative forward genetic screening approach, we identified porphyrin biosynthesis as the critical pathway governing Artemisinin's cytotoxicity. Genetic or pharmacological modulation of porphyrin production, e.g. by supplementation of the porphyrin precursor 5-aminolevulinic acid (5-ALA), defines Artesunate's cytotoxicity in multiple eukaryotic cells, including human cancer cells. 5-ALA, a clinically approved photodynamic porphyrin enhancer, is used as a surgical fluorescence marker, which specifically labels tumorigenic brain areas due to differential uptake and metabolism. Combining Artesunate with 5-ALA therefore provides an additional level of selectivity towards malignant tissue. We translated the screening results into clinically relevant model systems of brain tumor development: glioblastoma models in engineered cerebral organoids, patient-derived brain tumor spheroids, and orthotopic xenograft models using freshly isolated patient cells. The 5-ALA-Artesunate combination showed a strong and selective antineoplastic activity in all three model systems. At AACR 2025 we will present a comprehensive preclinical data-package, including a new delivery system with animal pharmacokinetics and efficacy data, paving the way for the clinical application of this promising therapeutic approach.

#526 Leveraging germline genetics to predict the efficacy of repurposing approved cancer therapies for cancer prevention.

Chibuzor F. Ogamba[1], Elio Riboli[2], James Yarmolinsky[2], Philip C. Haycock[3], Ruth C. Travis[1], Karl Smith-Byrne[1]

[1]Nuffield Department of Population Health, University of Oxford, Oxford, United Kingdom,[2]Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, United Kingdom,[3]Bristol Population Health Science Institute, MRC Integrative Epidemiology Unit, University of Bristol, Bristol, United Kingdom

Background: The high costs and failure rates of drug development have hindered progress in new cancer therapies, driving interest in systematic drug repurposing. Recent studies suggest that genetic evidence can improve the success rate of drug development. We aimed to investigate the yield of germline genetics in recapitulating the efficacy of approved cancer therapies for preventing the same cancer.

Methods: We mapped 3,081 plasma protein cis-protein quantitative trait loci (pQTLs) from UK Biobank Olink and Iceland SomaLogic GWAS data to approved and currently marketed cancer drug information on the Open Targets Platform. Using publicly available GWAS summary statistics for 39 cancer subtypes, we performed cis-pQTL Mendelian randomization (MR) analyses to estimate the association between drug-target protein concentrations and corresponding cancer risk. Odds ratios (ORs) were oriented to the drug's action on the protein i.e., a drug inhibiting a risk-increasing protein would correspond to a reduced risk. Using our MR results, we evaluated the success of genetic evidence in repurposing cancer treatments for prevention, relative to the approval rate of new drugs progressing from phase 1 trials. We quantified this using the relative probability with 95% confidence intervals (CI) that a target-indication (T-I) pair approved for treatment will have genetic evidence for use in prevention (R(G)), using the ratio of the probability of a directionally consistent significant MR result (P(G)) to the probability of approval for pairs that completed phase 1 trials (P(D)). We tested the sensitivity of R(G) by cancer type and targeted pathways.

Results: Of the 298 cancer T-I pairs available for MR analyses, we found 49 significant protein-cancer associations representing 28/72 targets across 22/39 cancer subtypes. Of these, 36 were directionally congruent with the drug's action on the protein, representing 20/72 targets of 54/139 drugs across 19/39 cancer subtypes. MR-significance and directionality of T-I pairs did not differ significantly by the presence of a targeted somatic mutation in the protein. We estimated the relative probability (R(G)) that a T-I pair approved for treating cancer has genetic support for prevention to be 1.95 (95% CI: 1.46-2.60). R(G) was significantly higher for 8/18 cancers [pancreatic cancer (3.7, 1.1-12.1) to brain cancer (67.5, 18.6-244.6)], significantly lower for thyroid cancer, and not significantly different for 9/18 cancers. Furthermore, R(G) was significantly higher for T-I pairs mapped to 9/12 pathways, including extracellular matrix organization, transcription and signal transduction.

Conclusion: Our findings indicate that certain targets of existing cancer therapies may be repurposed for cancer prevention and demonstrate a potentially high yield in the utility of genetics in improving target identification for drug repurposing in cancer.

#527 Atovaquone upregulates PD-L1 expression via activation of the ATM/ATR DNA damage response pathway.

Sejal Tarun Sharma, Maria Virumbrales-Munoz, Mayra Betancourt Ponce, Lisa Barroilhet, Manish Patankar

Department of Obstetrics & Gynecology, University of Wisconsin-Madison, Madison, WI

Background: Ovarian cancer is one of the deadliest gynecological malignancies, typically diagnosed at an advanced stage complicating standard treatments and shows a need for new treatment options. Previously our lab has shown that atovaquone, a known anti-malarial drug, reduces ovarian cancer proliferation under *in vitro* and *in vivo* conditions. Atovaquone is a potent inhibitor of oxidative phosphorylation (OXPHOS) and as a result causes a significant increase in intracellular oxygen radicals. The oxidative stress mediated by atovaquone causes double strand DNA breaks. Here, we examine if the DNA damage by atovaquone leads to activation of the ATM/ATR pathway and, consequently, upregulate PD-L1. Increased PD-L1 expression in ovarian cancer patients after treatment with atovaquone will provide a rationale in combining atovaquone with anti -PDL1 therapy to enhance anti-tumor response and improve overall treatment outcome.

Methods: For all experiments, ovarian cancer cells (OVCAR-5 and ID8) were treated with atovaquone at IC50 concentrations (10-30 μM). DNA damage was assessed by staining with picogreen and monitoring the cells by fluorescent microscopy as well as 8-OHdG (marker of DNA damage due to oxidative stress) flow cytometry. Activation of the ATM/ATR pathway was evaluated by western blotting using cell lysates for the expression of phosphorylated ATM, ATR, CHK1, CHK2, JAK1, STAT1 and IRF1 proteins. Flow cytometry was used to monitor expression of PD-L1 on the tumor cells. In some experiments, the ATM/ATR inhibitor, AZD1056, was added to the media at 2.5 μM-5 μM concentration for 5 h prior to determining the effect of this treatment on PD-L1 expression.

Results: A 75% increase in abnormal nuclei with DNA damage was observed after 72 h treatment of OVCAR-5 and ID8 with atovaquone. Mild dysfunction in nuclear morphology was visible at the 24 h. Increase in expression of 8-OHdG was observed after atovaquone treatment confirming DNA damage. Exposure to atovaquone for 72 h resulted in significant upregulation of phosphorylated ATM, ATR, CHK1, CHK2, JAK1 and STAT1 proteins. Flow cytometry data demonstrated a (20-55%) increase in the level of PDL1 expression after 48h to 72 h atovaquone treatment in OVCAR 5 and ID8 cells. A (55-65%) increase in the number of both OVCAR 5 and ID8 cells expressing PDL1 expression was also observed. Inhibition of ATM/ATR signaling by pretreatment with AZD1056 reversed the level of increase in PDL1 expression in atovaquone treated OVCAR-5 and ID8 cells.

Conclusion: These findings underscores ATM/ATR pathway as a novel molecular mechanism of PDL1 upregulation in atovaquone-treated ovarian tumors. Thus, our next step is to investigate if combining atovaquone with anti-PD1/PDL1 therapy will be effective in *in vivo* models of ovarian cancer.

#529 Obatoclax, a safe BH3-mimetic, is noninferior to neurotoxic oxaliplatin when combined with adenovirus to treat colorectal carcinoma.

John Milburn Jessup[1], Sameer Tiwari[1], Ali Andalibi[2], Yungtao Wu[1]

[1]George Mason University, Manassas, VA,[2]Charles R. Drew University, Los Angeles, CA

Oxaliplatin (Ox) in an important inducer of Immunogenic Cell Death (ICD) that causes endoplasmic reticulum (ER) stress and externalizes Calreticulin (ectoCALR) to attract and activate immune cells agnostically. However, since OX is quite toxic, we hypothesize that Obatoclax (OB), a clinically safe BH3 mimetic, that causes ER stress through mitochondrial stress can replace OX in our vaccine model. Our model involves a single intralesional injection of 80 μM OX combined with an oncolytic chimeric Ad5/3-NP2.ADP (ADP) adenovirus into > 6 mm CT26 colorectal carcinomas (CRC) dwelling in BALB/c hosts for 14 or more days. Treatment causes 90% regression with 20% durable complete responses - without checkpoint blockade. Treatment is accompanied by a four-fold increase in intratumoral CD8+ T cells from 99% PD-1^{Hi}EOMES$^{Int\text{-}High}$in control tumor bearers to 79% PD-1^{Lo}EOMES^{Lo} or PD-1^{Lo}EOMES^{Int} CD8+ T cells in treated mice along with new *in vitro* cytotoxicity to CT26 cells and rejection of distant CT26 cells in survivors. We postulate that OB synergizes with ADP to increase ectoCALR, enhance viral infectivity and inhibit the growth of murine and human CRC *in vitro* that can be used *in vivo* as a vaccine. The approach used cytometry to quantify proliferation, CALR protein expression, viral production, autophagy and ER stress, and their response to inhibitors. In both murine CT26 and human Clone A (DLD-1) CRC cells, OB induces more ectoCALR than OX as a monotherapy within 2 hours at dosages equivalent to 50% of clinical Cmax for each agent. OB increased superoxide production with an EC50 50.2 nM in CT26 cells confirming mitochondrial stress. OB and OX both cause necroptosis in CT26 and Clone A cells which is critical for ICD. OB synergizes with ADP to inhibit growth *in vitro* in CT26 cells (OB alone IC50 1.1 nM, ADP IC50 for Multiplicity of Infection (MOI) 69.2 MOI which decreases to an IC50 of 0.3 MOI with OB at 1 nM). Similar synergy occurred with Clone A cells. Clone A was used In further studies for interaction with ADP because human adenovirus does not replicate in mouse cells. Both OB and OX activate PERK, increase DDIT3/CHOP expression, and enhance red/green fluorescence ratio (R/GFIR) in acridine orange staining. PERK (PERK-IN-2) and ER stress (4-phenylbutyric Acid) inhibitors decrease these responses and ectoCALR expression but increase proliferation of Clone A cells. Ecto-CALR expression with OB-ADP on Clone A cells was higher than that of Clone A cells after OX-ADP after three days *in vitro* with a similar growth inhibition. In summary, OB is an agent that rapidly initiates ICD and supports viral infectivity in CRC cells to achieve ICD with necroptosis and autophagy. OB-ADP treatment is noninferior to OX-AdV therapy *in vitro* for initiation of ICD. These results support using OB in an agnostic therapeutic vaccine *in vivo*, particularly due to its systemic injection capability.

#530 A personalized HGSC organoid platform for repurposing lysosomal death inducing drugs.

A. Skorda[1], B. S. Rasmussen[1], M. Lund Bay[1], D. Afedeva[2], A. Rossberg Lauridsen[1], Y. Li[2], E. Valkonen[3], A. Abdul-Al[1], M. Casasnovas Ventura[1], L. Fuso[1], J. Roca Arias[1], W. Senkowski[4], K. Wennerberg[4], O. Carpen[2], A. Virtanen[5], A. Lahtinen[2], J. Oikkonen[2], J. Hynninen[3], S. Hautaniemi[2], T. A. Kallunki[1];

[1]Danish Cancer Society, Copenhagen, Denmark, [2]University of Helsinki, Helsinki, Finland, [3]University of Turku, Turku, Finland, [4]Biotech Research and Innovation Centre, Copenhagen, Denmark, [5]University of Helsinki, Helsinki, Denmark

Introduction: Among the gynecological malignancies, the most prevalent and lethal is the high-grade serous ovarian cancer (HGSC), accounting for 70-80% of ovarian cancer mortality. Tumor evolution studies suggests that high molecular heterogeneity prevails long before treatment intervention, evincing that patients would not benefit from a universal treatment but would rather benefit personalized treatments. Here, we established a prospective HGSC patient-derived tumor organoid biobank, where we are testing lysosome-targeting FDA approved medicines, to evaluate their cytotoxicity as standard treatment alternatives and/or supplements.

Materials and Methods: HGSC patient tissues from various anatomical sites (multiregional) and different courses of treatment (longitudinal) were grown as long-term 3D tumor organoid cultures (PDOs). Relevant biomarker proteins were qualitative and quantitative evaluated via immunofluorescent labeling and drug profiling was studied via cytotoxicity assays, measurement of proliferative index and lysosomal membrane permeabilization using 3D high-throughput high-content imaging and image analysis. Genomic and transcriptomic data were derived from whole-genome sequencing, whole exome sequencing and RNA sequencing of the organoid cultures and corresponding parental tumors. PDX models were used for in vivo drug testing.

Results and Discussion:Organoids were established as self-renewable, robust cultures with stemness-like characteristics that mirrored the genomic profile of the parental tissue, thus being ideal model systems for preclinical studies. We identified a cohort of 22 samples derived from 17 patients and tested sensitivity towards clinically approved lysosomal-targeting drugs. Samples were categorized as sensitive or non-sensitive to the treatment and gene-expression analysis indicated a pathway possibly involved in the resistance mechanism of the non-sensitive group. Inhibition of this pathway enhanced the initially observed cytotoxicity. Treatment efficiently inhibited the growth of engrafted sensitive samples in vivo.

Conclusion: To maximize survival potential of HGSC patients, functional drug testing on patient-derived cancer models is essential. Here, our platform is a powerful tool to study a broad spectrum of alternative drug combinations to accordingly direct clinical decisions. It also gives the possibility to investigate intensively the mechanisms that lie behind the onset and the refractory of the HGSC.

#531 Treatment of pancreatic adenocarcinoma with omeprazole.

Karl N. Thomas[1], Rohit Singh[1], William E. Grizzle[2], Lacey R. McNally[1]

[1]OU Health Stephenson Cancer Center, Oklahoma City, OK,[2]University of Alabama at Birmigham, Birmingham, AL

Background: Pancreatic adenocarcinoma (PADC) is associated with poor clinical outcomes that show resistance to apoptosis. Therefore, we explored an off-label use of Omeprazole to discover its effect on PADC. Our goal was to expose underlying cellular processes, namely autophagy, which are differentially regulated by Omeprazole to aid in treatment design and improve patient outcomes.
Methods: Freshly cultured S2VP10 and Panc1 cells were given 50uM and 500uM Omeprazole treatment for 4-hours. Neutral Red (NR), Acridine Orange (AO), and LysoTracker stains were used to assess viability and lysosomal activity. Immunocytochemistry (ICC) in S2VP10 and Suit2 Cells was used to examine the activity of proton-sensing ion channels ASIC1 and ASIC3 following 50uM Omeprazole treatment. For AO, LysoTracker, and ICC experiments, analysis of fluorescence was performed using Nikon NIS Elements to trace relevant regions and normalize to region area. Significance was determined using Kruskal-Wallis tests followed by pairwise Wilcoxon pos hoc tests. Differences were considered statistically significant at $p<0.05$.
Results: Following Omeprazole treatment, mean NR fluorescence decreased by 54.5% and 6.0% respectively for 500uM treatment of S2VP10 and Panc1 cells. AO staining of S2VP10 cells after 4-hour 500uM treatment showed a 58.2% increase in red fluorescence indicative of autophagosomes and lysosomes. This finding is supported by qualitative analysis of LysoTracker staining. Significantly increased ASIC1 and ASIC3 staining was observed for the 4-hour treatment groups compared to controls in S2VP10 and Suit2 cells lines.
Discussion: Our results show that Omeprazole, a proton pump inhibitor, has a significant effect on the proton compartmentalization within pancreatic cancer. Treatment of PDAC remains challenging due to its enhanced autophagy expression and low extracellular pH which enables cell survival despite nutrient deprivation. We have shown that use of proton inhibitors like Omeprazole disrupt autophagy and cytoprotective mechanisms. Exploiting this, may help to reduce chemoresistance in PDAC.

#532 Single-cell analysis reveals neuroblastoma heterogeneity and therapeutic opportunities.

Michele Tomanelli[1], Jennifer Hoti[1], Matteo Saettone[1], Daniele Reverberi[2], Adriana Bajetto[1], Tullio Florio[1], Aldo Pagano[2]

[1]University of Genoa, Genova, Italy,[2]IRCCS Policlinico San Martino, Genova, Italy

Neuroblastoma is the most common extracranial tumor found in neonatal patients, arising from neural crest cells. By utilizing specific Neuroblastoma cell lines, we have developed clones that express the non-coding RNA NDM29. These investigations have enabled the assessment of NDM29's role in tumor differentiation, identifying new therapeutic targets for controlling tumor malignancy. Further studies highlighted the presence of different cell subpopulations in this model by performing a single-cell sequencing analysis. These subpopulations were isolated with FACS analysis. The cellular heterogeneity characterizing this model reflects what is observed in patients. The gene expression of potential therapeutic targets identified in the heterogeneous model was evaluated in individual subpopulations. The individual subpopulations were pharmacologically treated by inhibiting some of these targets. These treatments revealed subpopulations that were either more sensitive or more resistant to specific inhibitors. By performing gene ontology analyses using sequencing data, we identified potential new pharmacological targets to enhance the mortality of even the resistant cells.

#533 Disruption of cholinergic signaling via inhibition of the M3 muscarinic acetylcholine receptor reduces glioblastoma stemness and enhances cell death.

Godwin Peasah-Darkwah[1], Ayanfeoluwa S. Kolawole[2], Tingting Huang[3], Grace M. Rosner[1], Johnathan D. Rodgers Gochicoa[4], Matthew C. Havrda[1], Kelli B. Pointer[3]

[1]Molecular and Cellular Biology, Dartmouth College, Hanover, NH,[2]Geisel School of Medicine, Hanover, NH,[3]Radiation Oncology and Applied Sciences, Dartmouth Cancer Center, Lebanon, NH,[4]Integrated Neuroscience, Dartmouth College, Hanover, NH

Background: Glioblastoma (GBM) is the most aggressive primary brain tumor in adults, with a median survival of less than two years despite treatment with surgery, chemotherapy, and radiation. Tumor recurrence, driven in part by treatment-resistant glioblastoma stem cells (GSCs), contributes to this poor prognosis. Understanding the mechanisms that promote GSC resistance may aid in developing targeted therapies. GBMs frequently occur in acetylcholine (ACh)-rich brain regions. ACh is a neurotransmitter responsible for cholinergic signaling through muscarinic acetylcholine receptors (mAChRs) and has been shown to regulate stemness in healthy oligodendrocyte progenitor cells. The M3 mAChR has also been shown to hold potential prognostic value in GBMs. Taken together, we investigated the role of ACh and the M3 mAChR in promoting GSC-like states and therapeutic resistance.
Methods: Human GBM LN-229 cells were cultured under stem-like conditions. CRISPR/Cas9 was used to generate an M3 mAChR knockout line. To assess the role of cholinergic signaling in promoting stemness, cell lines were treated with vehicle control or 3μM of carbachol, an ACh mimetic, and sphere formation assays and immunoblotting for stem-like markers (Nestin and CD44) were performed. To assess if M3 mAChR inhibition decreases stemness and tumor cell viability, cell lines were treated with benztropine (BZT; 1 μM and 3 μM), a muscarinic antagonist, and MTT assay for cell viability was performed. All experiments were performed in triplicates.
Results: Carbachol treatment increased the number of spheres compared to vehicle control (mean: 34 vs. 20, $p=0.0141$), as well as the sizes of the spheres (mean: 192 μM vs. 100 μM, $p<0.0001$). Immunoblotting after carbachol treatment increased protein expression of stemness markers and the M3 mAChR. The M3 mAChR knockout cell line demonstrated a decrease in cell viability after 48 hours of treatment compared to wildtype control ($p<0.0001$). Also, there was a decrease in protein expression of stemness markers in the M3 mAChR knockout cell line compared to wildtype control. Additionally, BZT treatment reduced cell viability compared to vehicle control in the MTT assay at 1 μM ($p<0.0001$) and 3 μM ($p<0.0001$).
Conclusion: ACh signaling via M3 mAChR promotes stemness in GBM and disruption of cholinergic signaling through knockout of the M3 receptor decreases stemness and tumor cell viability. Also, pharmacological inhibition of cholinergic signaling with a muscarinic antagonist such as BZT may be useful to target GSCs with the ultimate goal of improving patient outcomes.

#534 Sotorasib-resveratrol combination induces synergistic cytotoxicity by downregulating K-RAS signaling in K-RAS mutant lung cancer cell lines.

Pedro Barrios Bernal, Norma Hernandez-Pedro, Jose Lucio-Lozada, Eunice Romero-Nunez, Cesar Castillo-Ruiz, Brandon Lopez, Sandra Hernandez-Sanchez, Oscar Arrieta

Instituto Nacional de Cancerologia, Mexico, Mexico City, Mexico

Background: K-RAS mutations are the most frequent (35-50%) in patients with non-small cell lung cancer (NSCLC) who have a smoking history. These mutations promote cancer development through constitutive activation of poliferative signaling pathways, metabolic modifications and mechanisms of apoptosis evasion. Sotorasib is a covalent inhibitor targeting KRAS G12C; however, the primary challenge lies in acquired resistance. Resveratrol is a natural compound that promotes apoptosis and inhibits proliferation through the MAPK and Wnt/β-catenin pathways in cancer cells. Therefore, this project proposes using resveratrol as an adjuvant therapy to sotorasib in cell lines with KRAS mutations.
Methods: Dose-response curves were generated using MTT assays to determine the IC50 for sotorasib and the IC10 for resveratrol, in addition to determine the type of interaction between both drugs in the K-RAS mutant cell lines A549 (G12S) and H23 (G12C). Crystal violet assays were performed to measure the effect on cell proliferation. Mitochondrial membrane potential was assessed via flow cytometry. Finally, protein expression, activation, and inactivation were analyzed using western blot assays (K-RAS, BRAF, C-RAF, p-CRAF, GLUT1, GLUT3, HXK 1, AMPK and p-AMPK).
Results: Our results revealed a synergistic effect of the combined sotorasib-resveratrol treatment in the H23 cell line, while an additive or antagonic effect was observed in the A549 cell line. Cell proliferation assays demonstrated significant growth inhibition in both cell lines when treated with sotorasib alone or in combination with resveratrol. Additionally, mitochondrial matrix collapse was predominantly observed with the combined treatments. Western blot analysis showed strong inhibition of the K-RAS signaling pathway and glucose transporters (GLUTs), along with AMPK activation, particularly in treatments involving resveratrol. Furthermore, the induced cytotoxicity was linked to the inhibition of K-RAS signaling, GLUTs, Hexokinase 1, and AMPK reactivation.
Conclusions: The addition of resveratrol to sotorasib enhanced growth inhibition, independent of KRAS mutations, primarily through apoptotic mechanisms and the downregulation of the glucose metabolic pathway.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Reversal of Drug Resistance 1
Poster Session

#539 Secretory EMT fuels ERBB3 axis activation in resistance to EGFR inhibitors.

Alessandra Morselli[1], Donatella Romaniello[1], Chiara Miroglio[1], Martina Mazzeschi[1], Michela Sgarzi[1], Federica Pagano[1], Cinzia Girone[1], Francesca Ambrosi[1], William Kothalawala[2], Gyorffy Balazs[3], Andrea De Giglio[1], Michelangelo Fiorentino[1], Andrea Ardizzoni[1], Yosef Yarden[4], **Mattia Lauriola**[1]

[1]University of Bologna, Bologna, Italy,[2]Semmelweis University, Budapest, Hungary,[3]Dept. of Bioinformatics, Semmelweis University, Budapest, Hungary,[4]Weizmann Institute of Science, Rehovot, Israel

Non-small cell lung cancer (NSCLC) patients with activating EGFR mutations, such as exon 19 deletions (del746-750) and the L858R point mutation in exon 21, respond well to third-generation tyrosine kinase inhibitors (TKIs) like osimertinib. However, resistance inevitably emerges, limiting the long-term efficacy of these therapies. In this study, we investigated non-genomic mechanisms contributing to TKI resistance, focusing on the activation of bypass pathways that facilitate drug tolerance. These pathways enable drug tolerant persister cells to survive EGFR inhibition by exploiting alternative signaling routes. Among these, the upregulation of HER3 has been identified as a key mechanism. Clinical evidence has demonstrated the therapeutic potential of targeting HER3, notably with antibody-drug conjugates (ADCs) like patritumab deruxtecan. However, the precise molecular and cellular basis for HER3 dependency in NSCLC patients who progress on TKI therapy remains poorly understood. To address this gap, we employed a combination of immortalized and patient-derived cell lines, alongside advanced single-cell sequencing technologies, to elucidate the underlying biology. Our findings reveal that HER3 dependency post-TKI treatment is specifically enriched in pulmonary alveolar type I and II cells. This dependency is driven and maintained by paracrine signaling involving secreted factors, with neuregulin-1 (NRG1) playing a central role. NRG1 is primarily secreted by the tumor stroma and by cancer cells undergoing epithelial-to-mesenchymal transition (EMT) or dedifferentiation. Prolonged drug exposure further amplifies NRG1 secretion, which not only sustains HER3 activation but also promotes genetic and phenotypic plasticity. This is accompanied by EMT-associated morphological changes that enhance invasiveness and metastatic potential. Notably, our preclinical models demonstrated that the combination of an NRG1-neutralizing antibody with a dual EGFR blockade—achieved through a TKI and an anti-EGFR antibody—completely eradicated tumors in vivo. These results highlight the critical role of HER3 signaling and its interplay with the tumor microenvironment in mediating TKI resistance and suggest a compelling therapeutic strategy for overcoming resistance in NSCLC.

TUMOR BIOLOGY: Imaging in Animal Models
Poster Session

#54 Comparative preclinical antitumor assessment of 5FU versus anti-PD1 using in vivo imaging system in MC38 orthotopic tumor model.

Balaji Ramachandran, Ravindra Ghodke, Satheeshkumar Rajendiran, Bhanupranav Skanda, Krishnappa Haladasappa, Sachin Joshi

Pharmacology, Adgyl Lifesciences Private Limited, Bangalore, India

Immunotherapy has evolved as potential and practical therapeutic options for primary and metastatic tumors. Orthotopically implanted tumor models are clinically relevant and however growth monitoring of orthotopic tumor & assessing efficacy outcomes poses a practical challenge. Optical imaging by *In Vivo* Imaging System (IVIS) is a valuable method to monitor the real time progression of in vivo tumor growth without implying the need for interim termination during the study. In the present study, we have evaluated the preclinical efficacy of 5- Fluorouracil in combination with mouse anti-PD1 antibody in orthotopic MC38 model in immunocompetent mice. Subcutaneous tumor was generated in donor mice by inoculation of MC38 luciferase reporter cell line. Tumor fragments (~30mg) harvested from the donor mice were orthotopically implanted on the subserosal layer of cecum of C57BL/6 mice. Tumor growth was monitored twice weekly by IVIS optical imaging system. Mice were randomized based on total flux signal intensity (1-3 X 10^6 photons/second). Standalone & combination treatment was initiated with 5-FU; 25 mg/kg, i.p; Q2D and anti-PD-1 at 5 mg/kg, ip; Q4D x 4 doses. Total flux (photons/second), changes in body weight, clinicals signs, mortality were monitored twice weekly up to 2 weeks. At the end of the study, ex vivo bioluminescence signal intensity and tumor weights were recorded. In the current MC38 orthotopic tumor model, standalone treatment of anti-PD-1 and 5-FU resulted in moderate tumor regression. However, combination of 5-FU with anti-PD1 resulted in improved efficacy when compared with individual treatments which was evident from the reduction in signal intensity. In conclusion, evaluating efficacy of combinations treatment in preclinical orthotopic tumor models using IVIS imaging will be helpful tool for an accurate, non-invasive longitudinal monitoring of tumor growth over extended duration.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Reversal of Drug Resistance 1
Poster Session

#540 AVJ16 alleviates ABCB1-mediated doxorubicin chemoresistance via IGF2BP1 inhibition.

Eveiyn Weng Yan Phoon, Kowit-Yu Chong

Division of Biotechnology, Graduate Institute of Biomedical Sciences, Chang Gung University, Taoyuan City, Taiwan

Background: The efficacy of chemotherapy is often compromised by multidrug resistance (MDR), driven by the overexpression of ATP-binding Cassette Sub-family B Member 1 (ABCB1). While ABCB1 inhibitors are urgently needed, toxicity and pharmacokinetic challenges have limited clinical progress. Dysregulation of N6-Methyladenosine (m^6A)—a key mRNA modification involved in gene regulation—is linked to chemoresistance, presenting a potential target to counteract ABCB1-mediated MDR.
Aim: To examine m^6A's role in ABCB1-driven chemoresistance in uterine sarcoma and breast cancer and evaluate alternative ABCB1 inhibitors.
Methods: RT-qPCR analysis identified *Insulin-like Growth Factor 2 mRNA-binding protein 1 (IGF2BP1)*—an m^6A reader—as the most upregulated m^6A regulator in MDR uterine sarcoma cells. Expression levels were validated in parental and MDR uterine sarcoma and breast cancer cells. GEO dataset of breast cancer patients treated with neoadjuvant chemotherapy was analyzed *in silico* to determine *IGF2BP1-ABCB1* correlation. Functional assays were performed to evaluate IGF2BP1 knockdown and inhibition effects by AVJ16 (an IGF2BP1 inhibitor) on ABCB1 expression and doxorubicin resistance. Additionally, 3-deazaadenosine (DAA), an m^6A inhibitor, was added to MDR cells with IGF2BP1 knockdown or AVJ16 treatment to confirm IGF2BP1's role in m^6A-dependent regulation of ABCB1 and chemoresistance.
Results: IGF2BP1 and ABCB1 were significantly upregulated in MDR uterine sarcoma and breast cancer cells. *In silico* analysis confirmed a positive correlation between *IGF2BP1* and *ABCB1* expression, linking IGF2BP1 to ABCB1-driven chemoresistance clinically. IGF2BP1 knockdown in MDR uterine sarcoma cells reduced ABCB1 expression and doxorubicin resistance, evidenced by lower cell viability and colony formation. Moreover, AVJ16 significantly decreased doxorubicin's half-maximal inhibitory concentration (IC_{50}) in MDR uterine sarcoma by downregulating ABCB1 expression. DAA, combined with IGF2BP1 knockdown or AVJ16, markedly enhanced doxorubicin sensitivity, supporting IGF2BP1's m^6A-dependent role in MDR.
Conclusion: IGF2BP1 is a novel m^6A-dependent regulator of ABCB1-driven chemoresistance. These findings highlight AVJ16 as a promising therapeutic candidate for overcoming ABCB1-driven MDR cancers.

#541 PARP and autophagy inhibition synergy in small cell lung cancer.

T. Yu, R. Barayan, L. Song, V. Venkatasubramanian, S. N. Nair, V. Philip, B. H. Lok;
Princess Margaret Cancer Centre, Toronto, ON, Canada

Purpose: Small cell lung cancer (SCLC) is a high-grade neuroendocrine carcinoma comprising 15% of lung cancers. First-line treatment with platinum and etoposide chemotherapy—and radiotherapy for limited stage disease—produces good initial response, but most patients suffer treatment-resistant relapse within 2 years. The median survival is under 10 months, with immunotherapy increasing this by about 2 months. This regimen has changed minimally in 3 decades, fueling a need for more effective therapies. Poly (ADP-ribose) polymerase (PARP) inhibitors (PARPi) effectively induce DNA damage in SCLC and have shown potential in this setting, but response is variable, so we aimed to identify mechanisms through which SCLC may be sensitized to PARPi therapy.
Methods: CRISPR dropout screens were conducted using the Toronto KnockOut v1 (TKOv1) CRISPR library in the SBC5 and H82 SCLC cell lines, with the PARPi, olaparib, as the selection pressure. DNA sequencing was performed at days 25 and 35 for SBC5 and days 28 and 39 for H82. Top hits were identified by gene dropout in the olaparib condition versus the control, with a false discovery rate (FDR) cutoff of 0.05. Gene ontology analysis was used to identify critical pathways. Stable shRNA knockdown cell lines were generated using lentiviral transduction and validated by Western blot. Cells were treated with olaparib and assayed for viability with CellTiter-Glo 2.0. In wild-type cell lines, therapeutic mTOR activation with MHY1485 was validated by Western blot and therapeutic autophagy inhibition with chloroquine (CQ) or GNS561 was validated by Western blot after 3 hours starvation in EBSS medium. Efficacy of autophagy inhibitors alone and in combination with PARPi was assayed by cell viability, and SynergyFinder+ was used to quantify synergism of the combination.
Results: CRISPR screening identified mTOR pathway regulators, including components of the TSC and GATOR1 complexes, and gene ontology analysis indicated downregulation of TOR signaling and upregulation of autophagy as key pathways which confer PARPi sensitivity when lost. *TSC1/2* knockdown cell lines exhibit reduced viability after PARPi treatment. MHY1485 treatment blocked autophagy as indicated by LC3B-II accumulation, but combination therapy with MHY1485 and olaparib was antagonistic due to proliferative effects of MHY1485. CQ or GNS561 treatment also blocked autophagy, and SCLC cell lines had varying sensitivity to these agents as monotherapies. Autophagy inhibition synergized with PARPi, sensitizing SCLC cell lines to PARPi therapy.
Conclusions: *TSC1/2* knockdown sensitizes SCLC cell lines to PARPi in concordance with our model that mTOR downregulation promotes autophagy and cell survival after PARPi therapy. While mTOR upregulation was antagonistic with PARPi and promoted cell growth, autophagy inhibitors were a superior therapeutic approach, synergizing with PARPi *in vitro*.

#542 Chronic gastroesophageal reflux promotes chemoresistance in esophageal adenocarcinoma through APE1-redox-dependent PRDX2 activation and ferroptosis inhibition.

Lei Chen[1], Heng Lu[1], Farah Ballout[1], Dunfa Peng[1], Zheng Chen[1], Jianwen Que[2], Oliver G. McDonald[1], Alexander Zaika[1], Wael El-Rifai[1]

[1]University of Miami Miller School of Medicine, Miami, FL,[2]Columbia University, New York, NY

Background: The incidence of esophageal adenocarcinoma (EAC) is rapidly increasing in Western countries. Most EACs originate from Barrett's esophagus (BE), a precancerous lesion initiated by chronic reflux of gastrointestinal contents, especially acidic bile salts (ABS). Several chemotherapeutics induce high reactive oxygen species (ROS) levels to eliminate tumor growth. Ferroptosis is a recently identified cell death mode triggered by ROS and subsequent lipid peroxidation. However, cancer cells develop adaptive abilities to recalibrate redox balance via hijacking the antioxidant systems, resulting in drug resistance. PRDX2 protein is a member of the peroxiredoxin family of antioxidant enzymes. We hypothesized that PRDX2 may be a critical mediator in redox-associated chemoresistance under reflux conditions.

Methods: We analyzed RNA sequencing data and public databases to unveil the aberrant overexpression and potential chemoresistance function of PRDX2 in EAC. To simulate both acute and chronic clinical conditions of gastroesophageal reflux disease (GERD) in vitro, we employed transient and repeated ABS exposure (rABS). EAC cells and 3D organotypic culture (OTC) served as platforms to explore the anti-ferroptosis function and regulatory mechanisms of PRDX2. Our initial findings were validated in patient-derived xenografts (PDX), and human EAC tissue microarrays (TMA).

Results: Aberrant expression of PRDX2 was detected in both human EAC tissues and cell lines exhibiting resistance to oxaliplatin treatment. Knockdown of PRDX2 impaired the recovery of EAC cells from ABS-induced ROS-dependent lipid peroxidation. Moreover, silencing PRDX2 sensitized the chemo-resistant EAC cells to oxaliplatin. Chromatin immunoprecipitation (ChIP) assay identified that NF-kB activated PRDX2 transcription by directly binding to its promoter region. We also found that APE1-redox-mediated NF-kB activation is crucial for ABS-induced PRDX2. We further discovered that PRDX2 contributes to chemoresistance by upregulating GPX4 and suppressing ferroptosis. Furthermore, immunofluorescent staining in the 3D model (OTC) and immunohistochemistry staining in human TMA confirmed co-overexpression of APE1 and PRDX2. In addition, APX2009, an APE1-redox-specific inhibitor, significantly sensitized EAC cells to oxaliplatin by inhibiting PRDX2 expression and inducing ferroptosis. Notably, we observed synergistic effects of the combination of Oxaliplatin and APX2009 in the xenografts derived from a chemo-resistant EAC cell line.

Conclusion: Our findings revealed a novel link between reflux-induced redox unbalance and ferroptosis-related chemoresistance in EAC via APE1/redox/NF-kB/PRDX2/GPX4 axis. Targeting PRDX2 by APE1-redox-specific inhibitors is a potential novel strategy for drug combination in refractory EAC.

#543 SF2523, a dual PI3K-BRD4 inhibitor, enhances KRAS (G12C) or EGFR (exon 19/21) mutant targeted inhibitors in non-small-cell lung cancer (NSCLC).

Baskaran Subramani[1], Patrick Joseph Conway[2], Kun Zhang[1], Guillermo A. Morales[3], Joseph R. Garlich[4], **Daruka Mahadevan**[1]

[1]UT Health Science Center at San Antonio, San Antonio, TX,[2]Keiser University, Palm Beach Garden, FL,[3]Innoventyx LLC, Baton Rouge, LA,[4]SignalRx, San Diego, CA

KRAS and EGFR mutations are common oncogenic drivers in NSCLC. Osimertinib is a first line therapy for EGFR exon 19 and 21 mutant NSCLC. Sotorasib and Adagrasib are a second line therapy for KRAS G12C mutant NSCLC. Single agent inhibitors targeting mutant EGFR or KRAS develop drug resistance leading to relapsed/refractory NSCLC. SF2523 is a dual PI3K-bromodomain-containing protein 4 (BRD4) small molecule inhibitor being evaluated to mitigate KRAS or EGFR inhibitor induced drug resistance. Co-inhibition of PI3K/BRD4 blocks MYC expression, activation and enhances MYC degradation leading to inhibition of cancer growth and metastasis. We investigated SF2523 in EGFR mutant (HCC2935 and HCC827) and KRAS mutant (NCI H2030 & NCI H23) NSCLC cells lines with single agent or combination with Osimertinib or Sotorasib for anti-tumor activity, respectively. SF2523 IC_{50} in EGFR mutated NSCLC cells ranged from 90-230nM, while in KRAS mutated NSCLC cells ranged from 144-217nM versus Buparlisib plus JQ1 in the µM range. SF2523 plus Osimertinib decreased the IC_{50} to 1.2-1.4nM and SF2523 plus Sotorasib 1.6-13nM, respectively. In contrast, combinations of SF2523 plus Nindetinib (VEGFR inhibitor) or Rapamycin (mTOR inhibitor) were additive in potency with EGFR and KRAS mutant NSCLC cell lines. In KRAS mutant NSCLC treatment with SF2523 or Sotorasib increased PTEN mRNA expression and the combination therapy was additive. In EGFR mutant NSCLC treatment with SF2523 or Osimertinib or in combination decreased MYC, PIK3CA and AKT1, respectively. In a SCID H2030 KRAS G12C mouse xenograft model, SF2523 at a dose of 150 mg/kg was well tolerated without weight loss with tumor growth inhibition of >60% at 6 weeks. In conclusion, our results demonstrate SF2523 is active alone and is synergistic with Osimertinib or Sotorasib in inhibiting EGFR or KRAS mutant NSCLC cell lines and is active as a single agent in a KRAS G12C mutated H2030 CDX NSCLC mouse model.

#545 RFX5 knockdown restores sensitivity to cellular cyclophosphamide in triple-negative breast cancer cell lines.

Elizabeth Katherine Molchan, Kathleen Streeks, Alyssa Ohaegbulam, Mohammed Gbadamosi

University of Florida, Gainesville, FL

Cyclophosphamide (CTX) is a widely used chemotherapeutic agent in breast cancer (BC) treatment regimens. Despite its frequent use, patient responses vary significantly. While several studies have identified clinically relevant genetic variants associated with CTX metabolism to phosphoramide mustard (PM)—the active cellular anti-tumor metabolite—the factors influencing sensitivity at the cellular level remain poorly characterized. We previously performed a genome-wide association study (GWAS) of lymphoblastoid cell lines identified the SNP rs12408401 as associated with PM resistance. Employing the 15-state chromatin prediction model, ChromHMM, and other *in silico* tools, we identified an association between this SNP and the disruption of a repressive CTCF binding domain in multiple tissue types, including breast. This leads to an upregulation of several nearby genes regulated by this motif, including *RFX5*. Analysis of TCGA BC patient data revealed that increased RFX5 expression correlates with reduced disease-free interval in breast cancer patients treated with CTX (HR = 5.32, P = 0.028), but not in other BC patients. *RFX5* is a transcription factor that typically regulates the expression of MHC II-related genes. However, *RFX5* has been reported to exhibit upregulation in several malignancies and off-target transcriptional activity. Herein, we aim to functionally validate the role of *RFX5* in mediating CTX resistance, hypothesizing that decreased *RFX5* expression will decrease drug resistance *in vitro*. To test this hypothesis, we achieved gene knockdown through the administration of silencing RNA (siRNA) targeting RFX5 then assessed sensitivity by treating cells with PM, determining cell viability to quantify IC_{50}. As determined by qPCR, knockdown of RFX5 expression in the cell line MDA-MB-468 resulted in a 91% reduction in RFX5 expression compared to cells treated with scrambled siRNA ($p = 0.004$). At a PM concentration of 0.4 mM, RFX5 knockdown cells showed significantly reduced viability compared to scramble control cells ($33.3 \pm 4.5\%$ vs. $49.6 \pm 5.4\%$; $p = 0.031$). Furthermore, there was a 15% reduction in the IC_{50} of knockdown cells compared to control cells (0.273 ± 0.016 mM versus 0.346 ± 0.020 mM; $P = 0.004$). Together, these results provide strong evidence that RFX5 significantly attenuates CTX resistance. This work aims to link SNP genotype, gene expression, and drug sensitivity to improve predictive models of patient response and support targeted therapies enhancing CTX efficacy. Ongoing studies aim to delineate the pathways mediating aberrant RFX5 activity and validate its role in PM sensitivity across additional TNBC cell lines, with a focus on using CRISPRi to investigate the impact of genetic variation on the regulation *RFX5* expression and *RFX5* target genes.

#546 A novel approach to treat relapsed/refractory acute myeloid leukemia by targeting nucleolin as an innovative and promising biomarker.

Jihyun Um[1], Kyung Jin Kim[1], Eun-Hye Huh[2], Eun Jung Shin[1], Yumi Ji[1], Sung Hwan Moon[1], Je-Hwan Lee[2], Soo Jin Lee[1]

[1]Aptabio Therapeutics Inc., Yongin, Korea, Republic of,[2]Department of Hematology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, Republic of

Acute myeloid leukemia (AML) is a rapidly growing cancer of abnormal white blood cells accompanied by various genetic alteranation. Cytarabine (Ara-C) and daunorubicin are treated as the standard first-line chemotherapy for AML but approximately 30% of the patients are refractory and more than 50% of them face a relapse because of resistance. Moreover, FLT3 inhibitors, the only targeted therapy for AML, can treat only 20% of patients and resistance develops within a few months. Therefore, there is a strong unmet to develop a next-generation targeted therapy to overcome resistance. Nucleolin (NCL) is a protein highly expressed and localized on the cell surface of cancer cells. In this study, we confirmed that NCL was highly expressed in various human AML cell lines and patient-derived bone marrow cells compared to normal cells. Furthermore, it was particularly overexpressed in drug-resistant AML cells and refractory/relapsed (R/R) AML patient-derived bone marrow cells. NCL is a promising target for cancer treatment as mentioned above, but to date, no therapy has been developed to specifically target NCL. APTA-16 is a first-in-class AML treatment that specifically targets NCL developed by aptamer-drug conjugation (APTA-DC) technology. APTA-16 showed an outstanding anti-leukemic activity in drug-resistant AML cells through NCL inhibition whereas standard chemotherapies showed no efficacy *in vitro* and *in vivo*. We also identified the resistance-overcoming mechanism of APTA-16 in terms of intracellular drug metabolism and biological signaling pathway. The alternative activies of equilibrative nucleoside transporter (ENT) and deoxycytidine kinase (dCK) are main mechanisms by which cancer cells acquire Ara-C resistance. APTA-16 can avoid this process by entering cells via NCL on the surface of AML cells. We also figured out that NCL-DNMT1-axis signaling pathway plays an important role in drug-resistant AML cells and APTA-16 treatment significantly regulated the pathway. Collectively, our findings show that NCL can be an innovative biomarker targeting entire population of R/R AML patients regardless of their genetic defects and APTA-16 has can be developed as the first innovative NCL-targeted therapy. Based on the excellent *ex vivo* results using patient-derived AML cells, successful clinical results are expected. GLP safety/toxicity studies of APTA-16 have been completed and orphan drug designation (ODD) was grantd by FDA in 2021.

#547 HPB-092: A novel FLT3 and IRAK4 dual inhibitor for the treatments of AML and MDS.

Suyue Wang[1], Lei Wu[2], Rui Yang[2], He Zhou[2], Bing Zhang[2], Wenyuan Xu[2], Michael Xiang[1], Camille Xiang[1], Brent Meadows[1], Jason Xiang[1]

[1]Polymed Biopharmaceuticals, Cambridge, MA,[2]Polymed Biopharmaceuticals, Hangzhou, China

Activating mutations in the Tyrosine kinase FLT3 occur in about 25% of acute myeloid leukemias (AML)[1] and selective FLT3 inhibitors are clinically effective and have been recently approved by FDA for patients with relapsed/refractory AML with FLT3 mutations. While effective, a significant challenge with current FLT3 inhibitors is the rapid development of resistance through mechanisms including additional FLT3 mutations, CYP3A4 upregulation, or adaptive resistance through activation of parallel signaling pathways. It has been recently shown that an important mechanism of adaptive resistance to FLT3 inhibitors involves activation via Interleukin-1 receptor-associated kinase 4 (IRAK4) pathway[2]. Concomitant inhibition of FLT3 and IRAK4 could, therefore, enhance efficacy and durability in FLT3 mutant AML. We have developed HPB-092, a highly selective, novel FLT3/IRAK4 dual inhibitor. In biochemical assays, HPB-092 demonstrated potent inhibitory activity against FLT3-ITD (IC50 = 0.24 nM), FLT3-D835Y (IC50 = 0.18 nM), and IRAK4 (IC50 = 3.1 nM). HPB-092 effectively inhibited the proliferation of MV4-11, a FLT3-ITD mutant cell line at IC50 = 6.5 nM, and displayed excellent kinase selectivity in the Eurofins ScanEDGE assay at 1 uM. HPB-092 did not inhibit CYP3A4 up to 50 uM or hERG at concentrations up to 10 uM. In mouse tumor xenograft models of FLT3 mutant leukemias (MV4-11 and MOLM-13 cells), HPB-092 exhibited dose dependent efficacy and regression of established tumors. A 28-day GLP-compliant toxicity study showed HPB-092 was well tolerated in rats (NOAEL: 75 mg/kg/day) and dogs (NOAEL: 24 mg/kg/day), with no test article-related genotoxicity or safety pharmacology findings. Compared to the approved FLT3 inhibitors gilteritinib and quizartinib, and the clinical-stage FLT3/IRAK4 dual inhibitor CA-4948, HPB-092 showed comparable or superior inhibitory potency against mutated forms of FLT3, improved IRAK4 inhibition, better selectivity, minimal inhibition of CYP3A4, no hERG activity, and an overall favorable safety profile, potentially benefiting a broader range of hematological malignancies. HPB-092 has received FDA clearance for a phase 1 dose-escalation and expansion study to assess its safety, tolerability, pharmacokinetics, pharmacodynamics, and preliminary efficacy in adult patients with relapsed or refractory AML, particularly those with FLT3 mutations and U2AF1 or SF3B1 mutations, at a leading cancer center in the US. 1https://pubmed.ncbi.nlm.nih.gov/31972687/ 2https://www.sciencedirect.com/science/article/pii/S0006497118618622

#548 Histone deacetylase inhibitor, Vorinostat, reverses EGFR-TKI resistance in lung adenocarcinoma via degradation of MET.

Choudhary Harsha[1], Yuan Liu[1], Whitney S. Brandt[1], Di He[1], Manendra B. Lankadasari[1], Stella Tsui[1], Nanruoyi Zhou[1], Brooke Mastrogiacomo[2], Tetsuhiko Asao[1], David R. Jones[1]

[1]Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY,[2]Center for Molecular Oncology, Memorial Sloan Kettering Cancer Center, New York, NY

Background: *Epidermal growth factor receptor* (*EGFR*) mutations occur in 15-50% of patients worldwide with lung adenocarcinoma (LUAD). While tyrosine kinase inhibitors (TKIs) that directly target activating *EGFR* mutations prolong the survival of these LUAD patients, most tumors inevitably develop acquired resistance to TKI therapy. The second most frequent cause of such acquired TKI resistance is the amplification of the *MET* oncogene, which occurs in about 20% of *EGFR*-mutated LUAD samples with acquired TKI resistance. Our prior study showed that Vorinostat (VS), a potent HDAC inhibitor, induced cell death in NSCLC cell lines, and its sensitivity was correlated with *MET* levels in these cells. Therefore, we hypothesize that VS can re-sensitize *EGFR*-mutant LUAD with *MET* amplification-induced acquired TKI resistance to TKI treatment.

Methods: The human LUAD cells HCC827 parental ($EGFR^{Exon19Del}$) and HCC827-R (Osimertinib resistant with *MET* amplification) were used to test our hypothesis. To confirm if VS induced cell death in a *MET*-dependent manner, we conducted cell viability assays in HCC827 and HCC827-R cells after treatment with escalating doses of VS. Next, to explore the mechanism(s) through which VS enhances MET-dependent cell death, we performed several experiments including 1) real-time RT-PCRs and western blots to discern if VS regulates the expression of MET at transcriptional or translational level; 2) cycloheximide chase assays to examine the impact of VS on MET protein stability; 3) treatment with RGFP109, a HDAC1/HDAC3 selective inhibitor, to specify which HDAC(s) may be involved in this process. Finally, cell viability assays and clonogenic assays were performed using HCC827 and HCC827-R cells treated with VS alone, Osimertinib alone, or both, to investigate if VS re-sensitizes TKI-resistant cells to the treatment of Osimertinib.

Results: Our results showed that VS inhibited the viability of both HCC827 and HCC827-R cells in a dose-dependent manner with an IC50 of ~3μM. Unexpectedly, VS failed to affect *MET* mRNA, but significantly reduced the protein level of MET, compared to DMSO treatment. Moreover, the treatment with VS induced MET protein degradation in a MET level-dependent manner. Treatment with RGFP109 also induced MET protein degradation, suggesting that HDAC1 and/or HDAC3 might be involved in VS induced MET degradation. Interestingly, we observed that VS rescued sensitivity to Osimertinib in both HCC827 cells pre-treated with HGF and HCC827-R cells.

Conclusion: Our study suggests that VS promotes cell death of LUAD cells with amplification of *MET* via inducing HDAC1/3-mediated MET protein degradation. In combination with Osimertinib, VS reverses the amplification of *MET*-induced TKI resistance in LUAD cells. This combination might be therapeutically exploitable to overcome acquired TKI resistance caused by *MET* amplification in NSCLC patients.

#549 Biologically informed machine learning for discovery of protein assemblies underlying cancer therapeutic resistance.

Oliver Priebe[1], Eric Ho[1], Fransisco Guedes[1], Chandni Khandwala[1], Eduardo Farias[1], Trey Ideker[2], Adam Yaari[1], Maxwell Sherman[1]

[1]Serinus Biosciences Inc., New York, NY,[2]University of California, San Diego, La Jolla, CA

Despite substantial advances in cancer therapeutics, the near inevitable emergence of treatment resistance limits overall survival of advanced cancer patients. Genomic drivers of intrinsic and acquired resistance are highly multifaceted. Extensive profiling of tumors has demonstrated that the disparate resistance driving alterations often converge onto shared cellular processes. Motivated by this observation, we developed an intrinsically interpretable, gradient descent-based algorithm to learn assemblies of physically and functionally interacting proteins that are jointly predictive of tumor sensitivity to chemical and genomic perturbations. Importantly – and unlike existing solutions – the model learns protein assemblies de novo during training rather than being limited to rigid gene sets from public databases or literature. By co-embedding experimentally derived PPI maps with sample-specific multi-omics, the model to identifies interacting proteins that predict perturbation response. Once identified, protein assemblies can be used as compound biomarkers, for new target identification, for drug-target deconvolution, and for combination design amongst other therapeutic applications.

We benchmarked the predictive accuracy of our method using a panel of 23 clinical oncology drugs and related genetic perturbations targeting multiple areas of cell biology, including Ras/Raf signaling, G1/S checkpoint control, and DNA damage response. Our single model, trained on all 23 perturbations, outperformed state-of-the-art methods optimized for each individual perturbation in a held-out response prediction task (Mean accuracy: Random Forest, 0.41; Elastic Net, 0.37; FCNN, 0.33; this work, 0.43, highest accuracy for 15/23 perturbations). Our method achieved increased predictive accuracy while providing interpretable importance statistics for the members of each protein assembly. To predict CDK4/6 inhibitor response, the model learned a G1/S checkpoint assembly including CDK4 ($P=1.4\times10^{-8}$), CDK6 ($P=4.5\times10^{-8}$), CCNE1 ($P=7.0\times10^{-15}$), and RB1 ($P=4.7\times10^{-17}$). To demonstrate the application of our method to target discovery, we performed a synthetic lethality screen in cell lines with high and low activation of a G2/M protein assembly predictive of Nutlin-3A response ($P=3.1\times10^{-34}$). As expected given p53's central role in the protein assembly ($P=3.6\times10^{-56}$), top hits included known TP53-driven lethalities including TP53BP1 ($P=2.8\times10^{-34}$) and USP28 ($P=2.8\times10^{-27}$). Moreover, we identified a synthetic dependency on FBXO42 ($P=5.2\times10^{-8}$) associated not only with TP53 loss-of-function but also with two other assembly members involved in apoptosis regulation.

These results demonstrate the potential for biologically informed machine learning models to discover protein assemblies that explain drug resistance and enable new therapeutic avenues.

TUMOR BIOLOGY: Imaging in Animal Models
Poster Session

#55 A claudin-1 near-infrared fluorescent antibody conjugate for in vivo primary and metastatic colorectal cancer detection.

Mark Primeaux[1], Ritika Gupta[1], Iram Fatima[1], Sumbal Talib[1], Aaron Mohs[1], Michael Bouvet[2], Punita Dhawan[1]

[1]University of Nebraska Medical Center, Omaha, NE,[2]University of California San Diego, San Diego, CA

Background: Claudins are a large family of tight junction (TJ) proteins being investigated as biomarkers and therapeutic targets in cancer. Their availability at the cell surface, tissue-specific expression, and upregulation in some carcinomas make them amenable to monoclonal antibody targeting. In addition, epitope masking within the intact TJs of healthy tissue limits off-target binding. Claudin-1 (CLDN1) is upregulated in colorectal cancer (CRC) and promotes therapy resistance and metastasis.
Significance of Problem: CRC is the second deadliest cancer in the United States. While localized CRC can be treated surgically and has a 5-year survival rate of 91.1%, the survival rate of metastatic CRC falls to 15.7%. Due to resistance to current systemic treatments, surgery is the only curative option for CRC. However, tumors recur after ~30% of curative surgeries for stage II or III CRC. The rate of positive surgical margins for CRC has been reported at 6.83% and is associated with poor prognosis and metastasis. Fluorescence-guided surgery using targeted near-infrared (NIR) dyes allows surgeons to more accurately identify tumor margins.
METHODS: scRNAseq was used to characterize CLDN1 expression in CRC tumors. A NIR fluorescent dye, IRDye800 NHS-Ester, was conjugated to an anti-CLDN1 mAb (1C5-D9, CLDN1-IR800) and an IgG isotype control (IgG-IR800). Specificity was evaluated using fluorescent microscopy and Western blot in CLDN1-overexpression and knockout cell lines. The antibody was then tested *in vivo* using CRC cell line xenograft, patient-derived organoid (PDO) xenograft, and orthotopic syngeneic mouse models. Animals were injected IV and imaged using the LI-COR Pearl Trilogy System at multiple time points post-injection. Finally, an *ex vivo* panel of PDOs representing different ethnic and mutational backgrounds was used to examine the conjugate's translation potential.
Results: CLDN1 is highly expressed in CRC tumor cells, with low levels in the healthy colon. CLDN1-IR800 showed specific binding and robust fluorescence in CLDN1-expressing CRC cells compared to non-expressing cell lines or IgG-800. *In vivo*, CLDN1-IR800 showed significantly higher signal in CLDN1-expressing CRC cell line and PDO xenografts when compared to CLDN1 knockout tumors or IgG-IR800-injected mice. Biodistribution upon necropsy showed a significantly higher signal in the tumor compared to other organs. In an orthotopic syngeneic mouse model, both primary and metastatic lesions were detectable. Finally, *ex vivo* imaging showed significant signal in a panel of PDOs with diverse mutational and racial backgrounds.
Conclusions: CLDN1's selective expression on CRC tumor cells makes it an ideal candidate for *in vivo* tumor detection. In this study, we demonstrate the translation potential in targeting CLDN1 for tumor detection using multiple *in vitro, in vivo*, and *ex vivo* models.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Reversal of Drug Resistance 1
Poster Session

#550 Patient-derived organoids as a screening platform to identify mechanisms of osimertinib resistance in non-small cell lung cancer.

Yifan Yu, Samir H. Barghout, Nikolina Radulovich, Geoffrey Liu, Ming Tsao

University Health Network, Toronto, ON, Canada

Background: Epidermal growth factor receptor (*EGFR*)-positive non-small cell lung cancer (NSCLC) represents a large subset of NSCLC, accounting for approximately 30% of cases. Despite the initial effectiveness of osimertinib, the frontline modality in *EGFR*-positive NSCLC, resistance remains a major therapeutic challenge. To address osimertinib resistance, preclinical models that accurately represent the complexity of tumor biology are needed. Patient- and xenograft-derived organoids (PDOs and XDOs) offer a promising option as they preserve the genetic and phenotypic characteristics of the original tumor, allowing for more clinically relevant studies. We developed a panel of *EGFR*-positive PDOs and XDOs with fully annotated molecular, genetic, clinical and treatment profiles, and will utilize them to investigate osimertinib resistance.
Method: To demonstrate the utility of these models as a screening platform to identify osimertinib resistance mechanisms, we conducted a pilot single-agent screen in 6 PDOs/XDOs using osimertinib and a panel of small-molecule inhibitors that target relevant targets in NSCLC including Aurora kinase B, CDK4/6, c-MET, ALK, RET, ROS1, MEK, and BRD4. Viability has been assessed by the ATP luminescence assay CellTiter-Glo. Subsequently, we performed a high-throughput combinatorial screen where 2 osimertinib resistant models (LPTO357 & LPTO362) were treated with osimertinib alone and in combination with a library of 1144 FDA-approved and investigational drugs.
Result: We identified several compounds that displayed efficacy and potency in osimertinib-resistant models. The c-MET inhibitor capmatinib was specifically potent in LPTO245 but not in other models. This finding was confirmed using savolitinib, another selective c-MET inhibitor, suggesting the observed effects were due to on-target c-MET inhibition. Using whole exome sequencing, we confirmed the presence of c-MET amplification in LPTO245. Additionally, we identified an inverse sensitivity correlation between osimertinib and the Aurora kinase B inhibitor AZD2811 across all 6 models (r = -0.64; p = 0.03). The following analysis of our RNAseq data has shown a positive correlation between AURKB RNA expression and osimertinib IC_{50} values in 9 PDOs/XDOs. These data corroborate previously reported role of c-MET and Aurora kinase B as osimertinib resistance mechanisms and demonstrate the utility of the platform. Subsequently, our high-throughput combinatorial screen has identified 125 hits that potentiate/synergize the action of osimertinib including drugs targeting topoisomerases, JAK, PLK1, and PI3K.
Conclusion: Our screening platform can provide novel biological insights into osimertinib resistance. Moreover, it can potentially identify drug combinations with immediate clinical utility in NSCLC, particularly with approved and clinical-grade drugs.

#551 The FOXC2 transcription factor inhibitor MC-1-F2 alters the chemosensitivity of B16-F10 melanoma cells.

B. F. Jenkins, K. M. Hargadon;
Hampden-Sydney College, Hampden-Sydney, VA

Melanoma, the most aggressive form of skin cancer, is highly resistant to diverse classes of chemotherapeutics. The FOXC2 transcription factor, which is dysregulated in many cancers, has been linked with drug resistance in cancers of epithelial origin. We have recently identified a role for FOXC2 in the progression of melanoma. To investigate whether FOXC2 contributes to chemoresistance in this cancer, we have evaluated B16-F10 melanoma cell susceptibility to diverse classes of chemotherapeutic agents following treatment with the FOXC2 inhibitor MC-1-F2. We report increased anti-tumor activity when MC-1-F2 is used in combination with 5-fluorouracil, doxorubicin-HCl, or gemcitabine, highlighting the potential of this inhibitor as a novel chemosensitizing anti-cancer agent.

#552 Fenretinide enhances trastuzumab efficacy in trastuzumab-resistant HER2+ breast cancer cell lines.

Debbie O'Reilly[1], John Crown[2], Denis M. Collins[1]

[1]School of Biotechnology, Dublin City University, Dublin, Ireland,[2]Department of Medical Oncology, St Vincent's University Hospital, Dublin, Ireland

Introduction: HER2-positive (HER2+) breast cancer (BC) treatments have significantly improved patient outcomes, but treatment resistance remains a critical challenge, often driven by breast cancer stem cells (BCSCs). These resilient cells evade targeted therapies, leading to disease relapse and progression. Fenretinide (Fen), a synthetic retinoic acid (RA) derivative, has shown promise in acute myeloid leukemia by promoting cellular differentiation. Given BCSCs' similarities to embryonic cells, RA-based approaches may induce BCSC differentiation, reducing their resistance to HER2-targeted therapies. This project aims to identify BCSC markers in resistant HER2+ BC cell lines and evaluate the effects of Fen induced differentiation.
Methods: RNA-Seq gene expression data was downloaded from CCLE and analysed using RStudio (R version 4.1.2). HER2+ BC cell lines with innate (HCC1569, HCC1954) or acquired (SKBR3-T, BT474-T) trastuzumab (Tras) resistance were studied. Acquired resistance was induced by continuous exposure to 10 ng/mL Tras for 6 months. Cells were treated with 1 µM Fen for 24 hours. qPCR analysis employed the comparative Ct method (ΔΔCt), expressed as fold change to control. Statistical significance was determined using the Student's t-test or one-way ANOVA with Dunnett's multiple comparison. Results are presented as the mean of biological triplicate experiments ± standard deviation.
Results: DepMap analysis showed enhanced CD44 expression in innate-resistant HCC1569 and HCC1954 compared to parental SKBR3 and BT474. qPCR confirmed significantly higher CD44 levels in HCC1569 than SKBR3 ($p = 0.0318$) and significantly lower CD24 levels in HCC1569 and HCC1954 compared to SKBR3 ($p = 0.008$ for both). Acquired-resistance models SKBR3-T and BT474-T exhibited reduced BCSC markers compared to their parental lines. Fen had no significant effect on the expression of BCSC markers in innate or acquired resistant cell lines. Fen significantly decreased colony formation (CFA) across all cell lines and enhanced the growth-inhibitory effect of Tras in the SKBR3-T cell line in a dose-dependent manner.
Conclusions: Preliminary findings indicate that Fen influences CFA in both innate and acquired Tras-resistant HER2+ BC cell lines and enhances Tras efficacy in acquired resistant SKBR3-T. However, its impact on BCSC markers is limited. Further investigation is needed to elucidate the role of Fen in overcoming Tras resistance mechanisms in HER2+ BC.

#553 Drp1 knockdown impairs mitophagy and sensitizes AML cells to venetoclax by inducing apoptosis.

Priyanka Sharma[1], Hiroki Akiyama[1], M. Anna Zal[2], Bing Z. Carter[1], Michael Andreeff[1], Gautam Borthakur[1]

[1]Department of Leukemia, UT MD Anderson Cancer Center, Houston, TX,[2]Epigenet & Mol Carcinogenesis, UT MD Anderson Cancer Center, Houston, TX

Background: Mitochondrial structural dynamic of fission and fusion impacts mitochondrial function as well as mitochondrial quality control by facilitating mitophagy and ROS mitigation. Mitophagy as well as the expression of DRP1, a dynamin related protein driving mitochondrial fission, are increased in acute myelogenous leukemia (AML) resistant to Bcl-2 inhibitor venetoclax. Our previous findings demonstrated that ERK1/2 inhibition reduced the phosphorylation of Drp1, a dynamin-like GTPase involved in mitochondrial fission, and overcame resistance to venetoclax in acute myeloid leukemia (AML). While Drp1-driven mitochondrial fission acts as a mitophagy checkpoint, the precise relationship between Drp1 and mitophagy is not fully elucidated in AML.
Methods: Effect of doxycycline induced Drp1 knock down on viable cell count and apoptosis in AML cells was determined by bead-based annexin-V assay. Mitochondrial length was assessed using super resolution imaging with OMX Blaze. Mitochondrial ROS levels, mitochondrial mass, LC3 lipidation (autophagosome maturation), mitophagy and autophagosome content were determined using flowcytometry.
Results: Mitochondrial fission driven by Drp1 acts as a mitophagic checkpoint to regulate mitochondrial quality. Drp1 overexpression in OCIAML3 cells resulted in smaller mitochondria and higher LC3 lipidation, while knockdown increased mitochondrial length with a decrease in autophagic vesicles and LC3B lipidation suggesting decreased autophagy. Conditional knockdown of Drp1 inhibited mitophagy ($p<0.0001$), as detected with flowcytometry using the mitophagy dye that detects mitochondrial acidification resulting from fusion of mitochondria to lysosomes. Additionally, Drp1 knockdown significantly increased mitochondrial ROS with no change in mitochondrial mass. Knockdown of Drp1 significantly inhibited the clonogenic potential of AML cells, leading to a marked reduction in colony formation compared to control cells supporting that mitochondrial fission, ROS mitigation and mitophagy are crucial for preserving the stem-like properties of leukemia stem cells in AML. Moreover, conditional knockdown of Drp1 sensitized OCIAML3 cells to venetoclax ($p<0.0001$) as well as Idarubicin ($p<0.0001$) as demonstrated by a reduction in viable cell count and an increase in apoptosis.
Conclusion: Drp1 knockdown reduces mitochondrial fission and mitophagy in AML cells and sensitizes AML cells to venetoclax. These results further elucidate the role of Drp1 in mitochondria and offer new mechanistic insights into the interplay between mitophagy and mitochondrial fission in AML, highlighting the potential therapeutic benefit of co-targeting Drp1 in venetoclax-based regimens to eliminate leukemia stem cells. Ongoing studies are investigating the molecular mechanisms and in vivo efficacy of Drp1 knockdown in AML.

#554 Nanoparticle enabled miR-205 restoration in docetaxel resistant prostate cancer.

Neeraj Chauhan, Eswara NHK Ghali, Rahul Tiwari, Iris Enriquez, Rajasekhar Baru, Subhash C. Chauhan, **Murali M. Yallapu**

University of Texas Rio Grande Valley, McAllen, TX

Background: Prostate cancer (PrCa) is the second most diagnosed malignancy and a leading cause of cancer-related death in men worldwide. Docetaxel (Dtxl)-based chemotherapy remains a cornerstone of treatment for PrCa. However, most patients develop resistance to Dtxl treatment, leaving limited therapeutic options. Although cabazitaxel offers a secondary line of therapy, it is associated with many severe adverse effects. Restoring miR-205, a tumor-suppressor microRNA frequently downregulated in PrCa, has emerged as a promising strategy to reverse Dtxl resistance and achieve tumor regression.
Methods: A novel nanoformulation (MNP-miR-205) was developed using iron oxide magnetic nanoparticles (MNPs) layered with polyethylene imine (PEI) polymer to deliver miR-205. The optimized nanoformulation of MNP-miR-205 was characterized by size, surface charge, biochemical properties using DLS, gel retardation, and biochemical analyses. Chemo-sensitizing and anti-cancerous effects of MNP-miR-205 were evaluated in Dtxl-resistant PrCa cell lines and corresponding xenograft mouse models. Biodistribution of the formulation was evaluated by incorporation of indocyanine green (ICG) dye to track tumor accumulation.
Results: The MNP-miR-205 nanoformulation demonstrated optimal particle size and zeta potential, ensuring efficient cancer cell delivery. Hemocompatibility studies confirmed its safety profile. The formulation achieved an excellent cellular uptake *via* endocytosis, by passing endosomal and lysosomal degradation pathways. In Dtxl resistant PrCa cells, MNP-miR-205 enhanced chemosensitivity to Dtxl, as evident by increased apoptosis, Caspase 3/7 activity, and reduced mitochondrial membrane potential. In vivo studies confirmed that MNP-miR-205 exhibited superior tumor accumulation, significantly reducing tumor volume and weight, and prolonging overall mice survival in xenograft mouse models.
Conclusion: This innovative MNP-miR-205 nanoformulation offers a groundbreaking theranostic approach to address Dtxl resistance in prostate cancer. By enhancing chemo-sensitization and providing a safer imaging platform, it holds significant potential to improve prostate cancer management.

#555 ERBB4 upregulation in a taxol-resistant triple-negative breast cancer cell line.

Ling-Huei Yih, Hsiao-Hui Kuo, Tsai-Ming Lu

Academia Sinica - Institute of Cellular & Organismic Biology, Taipei, Taiwan

Resistance to chemotherapy remains an unfavorable obstacle to effective cancer treatment. Although paclitaxel is one of commonly used chemotherapeutic agents in the treatment of triple-negative breast cancer (TNBC), resistance mechanisms are not fully understood. In this study, we found that ErbB2 Receptor Tyrosine Kinase 4 (ERBB4) was substantially upregulated in T50RN, a paclitaxel-resistant cell clone established from the human TNBC cell line MDA-MD-231. We thus aimed to explore whether and how ERBB4 may modulate T50RN cell resistance to paclitaxel. The T50RN cell was obtained by exposing human TNBC cell line MDA-MB-231 to stepwise escalating paclitaxel concentration from 0.5 to 50 nM and then the resistant cell line T50RN was selected and expanded. The IC50 of paclitaxel was 2 and >100 nM in MDA-MB-231 and T50RN cells respectively. Treatment of the cells with ERBB inhibitors, sfatinib, allitinib, or PF299804, in sub-lethal concentration only induced a small additive effect on paclitaxel cytotoxicity in MDA-MB-231 cells, but dramatically enhanced paclitaxel cytotoxicity in T50RN cells. Since only the expression of *ERBB4* was significantly upregulated in T50RN cells, the expression of *EGFR1* was equally high in MDA-MB-231 and T50RN cells, and *ERBB2* and *ERBB3* were low in both cells, *ERBB4* might play a critical role on the effect of the three ERBB inhibitors. In addition, the three inhibitors also significantly enhanced paclitaxel-induced spindle abnormalities in T50RN cells but had no effect in MDA-MB-231 cells. Depletion of ERBB4 with shRNA also significantly reduced the cell growth ability of T50RN but not MDA-MB-231 on soft agar. These results suggest that ERBB4 may play a critical role in paclitaxel resistance in T50RN cells. Further elucidation of how ERBB4 is induced in T50RN cells and how ERBB4 regulates cell resistance might provide leads to overcome paclitaxel resistance.

#556 The glucocorticoid axis serves as biomarkers for supraphysiological androgen therapy response in enzalutamide-resistant prostate cancer.

J. Li, Z. Liu, M. Sands, S. Adams, J. Lee, M. Wang, T. Walsh, M. Haerens, M. Li, L. Leon, A. Zablah;
University of Notre Dame, Notre Dame, IN

Next-generation androgen receptor (AR) antagonist, like enzalutamide (Enz) has revolutionized the treatment of castration-resistant prostate cancer (CRPC), the lethal form of the disease, by effectively suppressing the androgen axis. However, resistance to Enz eventually develops in nearly all patients. Recent studies indicate that increased glucocorticoid receptor (GR) expression contributes to Enz resistance. Our prior work in prostate cancer models and patient tissues suggests that Enz decreases 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2, inactivates cortisol) and increases hexose-6-phosphate dehydrogenase (H6PD, promotes cortisol production) expression in tumor, which then sustains high levels of cortisol, leading to overstimulation of GR and resistance to Enz. Collectively, the glucocorticoid axis, including GR expression level and cortisol concentration controlled by 11β-HSD2 and H6PD, plays a critical role in Enz-resistant CRPC. Studies suggest AR activation reduces GR expression in PC, and SPA counteracts glucocorticoid-induced muscle atrophy, indicating AR activation inhibits GR functions in various contexts. Patient and mouse studies show that supraphysiologic androgen (SPA) reverses Enz resistance, and ~30% of patients respond to SPA; however, we lack a biomarker that identifies which patients will respond. A clinical study revealed that Enz treatment increases serum cortisol by suppressing11β-HSD2 expression, which is correlated with patient blood pressure. Taking all these together, we hypothesized that SPA reverses Enz through attenuation of the GR pathway by affecting GR expression and cortisol level. By using qPCR, immunoblot, high-performance liquid chromatography, and live cell imaging analysis, we observed that SPA decreases GR expression, accelerates cortisol metabolism by decreasing H6PD and increasing 11β-HSD2, and suppresses glucocorticoid-induced cell growth in Enz-resistant prostate cancer cell lines. Next, to further test our hypothesis that the glucocorticoid axis and blood pressure can serve as biomarkers for SPA therapy response. We will first confirm our cell level findings with cell-derived and patient-derived xenograft studies, then by collaborating with physician scientists, we will further evaluate the biomarker roles of the glucocorticoid axis for SPA therapy response in Enz-resistant prostate cancer by analyzing serum cortisol, expression of GR, H6PD, and 11β-HSD2 in circulating tumor cells, and blood pressure of patients who have undergone SPA treatment. This research holds the promise of not only confirming and solidifying both SPA's clinical benefits and the GR pathway's clinical significance in Enz resistance but also setting the foundation for our goal of developing the first non-invasive biomarkers for SPA therapy in patients with disease resistant to Enz.

#557 A novel tumor targeted extracellular vesicle based iron nanoparticle delivery system to induce ferroptosis and overcome chemoresistance in lung cancer.

Anjugam Paramanantham, Rahmat Asfiya N/A, Akhil Srivastava

University of Missouri - Columbia, Columbia, MO

Introduction: Cisplatin (CDDP) and its analogs are widely used in lung cancer (LC) treatment. However, despite their effectiveness, advanced LC has a poor 5-year survival rate of less than 10% due to chemoresistance. This resistance is often driven by disrupting apoptosis, a key hallmark of cancer. Ferroptosis, an iron-dependent, non-apoptotic cell death pathway driven by lipid peroxidation, presents a promising alternative to bypass apoptotic resistance. In this study, we developed a novel, tumor-specific delivery system, ExoFeR^{TF}, based on extracellular vesicles (EVs) functionalized with transferrin (TF) and loaded with iron oxide nanoparticles (IONPs). This system is designed to target transferrin receptors on LC cells to induce ferroptosis, thereby overcoming chemoresistance in a targeted manner.

Methods: ExoFeR^{TF} was synthesized by loading IONPs into TF-decorated EVs derived from MRC-9 lung fibroblast cells. The structural and functional integrity of ExoFeR^{TF} was confirmed through physicochemical characterization. To assess its therapeutic efficacy in reversing chemoresistance, ExoFeR^{TF} was tested on CDDP-resistant LC cells, patient-derived tumoroids (PDTs), and circulating tumor cell (CTC)derived xenograft (cDXT) cells and xenograft mouse models. We evaluated cell viability, mitochondrial damage, glutathione (GSH) levels, and expression changes in key ferroptosis markers (xCT/SLC7A11, GPX4). Molecular mechanisms of ferroptosis induction were also explored. In vivo, ExoFeR^{TF} tumor targeting was confirmed in LC xenograft models using Magnetic Resonance Imaging and Prussian Blue staining.

Results: ExoFeR^{TF} demonstrated robust structural stability, targeted tumor localization, and effective induction of ferroptosis in CDDP-resistant LC cells, PDTs, cDXT cells and xerograph mouse models. Treatment with ExoFeR^{TF} led to mitochondrial disruption, GSH depletion, and downregulation of ferroptosis markers. Additionally, Nrf2, a key regulator of antioxidant responses and chemoresistance, was downregulated with reduced intracellular translocation in ExoFeR^{TF}-treated cells. Subsequent CDDP treatment further sensitized the cells, indicating a reversal of chemoresistance.

Conclusion: ExoFeR^{TF} offers a promising, tumor-targeted approach to overcoming chemoresistance in lung cancer by inducing ferroptosis, a non-apoptotic cell death mechanism. These findings support the potential of ExoFeR^{TF} as a novel therapeutic strategy for treating resistant lung cancer, providing a foundation for future clinical applications in combatting chemoresistant cancers.

#558 MUC1*-deruxtecan kills tumors that have acquired resistance to trastuzumab containing therapeutics.

Cynthia C. Bamdad, Benoit J. Smagghe, Scott T. Moe, Kevin R. Yi, Trevor J. Grant, Mark G. Carter, Daniel S. Miller, Michael J. Nash, Jacy P. Marquez, Natalie K. Miller, JacLeen S. S. Nash, Robert McDermot, Andrew K. Stewart

Minerva Biotechnologies, Waltham, MA

Purpose: To develop a treatment for HER2 positive patients whose tumors have stopped responding to Enhertu
Methods: HER2 positive BT474 breast cancer cells were induced to become Herceptin resistant (trastuzumab resistant) in vitro via prolonged exposure to sub-lethal levels of the drug. Analysis of the trastuzumab-resistant cells showed that they had greatly increased expression of MUC1*, known to have anti-apoptotic and pro-growth properties. Recall that MUC1* (muk 1 star) is a growth factor receptor created after cleavage and release of the N-terminal portion of MUC1. The targeting antibody, huMNC2, has already demonstrated safety and cancer selectivity in a 1st-in-human CAR-T clinical trial for treatment of metastatic breast cancers. MNC2 is an IgG1 antibody that only recognizes the conformational epitope created when MUC1 is cleaved by specific tumor-associated enzymes. Confocal microscopy and pHrodo show that MNC2 is rapidly and robustly internalized by the cancer cells. We conjugated MNC2 to deruxtecan to yield an MNC2-deruxtecan with a DAR of 7.4. Female NSG mice were xenografted with either BT474-wt cells or the trastuzumab-resistant cells, BT474-Res2. Tumors were allowed to engraft then treated with trastuzumab or MNC2-deruxtecan.
Results: Herceptin had little to no effect on BT474-Res2 implanted tumors. However, MNC2-deruxtecan potently killed the trastuzumab resistant tumors in a matter of weeks. MNC2 conjugated to other linker-payloads similarly killed the trastuzumab resistant tumors. Treated animals showed no obvious signs of toxicities.
Conclusions: Patients with HER2 positive breast cancers have benefitted greatly from the new ADC Enhertu (fam-trastuzumab deruxtecan). However, most patients, develop resistance to HER2-directed therapies and experience progressive disease leaving them with few treatment options. Here we've shown that HER2 positive breast cancer cells acquire resistance to trastuzumab by increasing expression of the powerful growth factor receptor MUC1*. These MUC1*-targeting ADCs offer an effective treatment for these patients. These data support a conclusion that a MUC1*-ADC is safe and could have high therapeutic value for patients who have stopped responding to Enhertu.

#559 Targeting MCL1 or SOS1 can overcome lenvatinib resistance in HCC.

Kevin Chau[1], Martina S. J. McDermott[2], Dennis J. Slamon[2]

[1]Medicine - Molecular and Medical Pharmacology, UCLA - University of California Los Angeles, Los Angeles, CA,[2]Medicine - Hematology Oncology, UCLA - University of California Los Angeles, Los Angeles, CA

Lenvatinib (LEN), a front-line treatment for advanced liver cancer, has a short benefit of 13.6 months median overall survival. Understanding how resistance emerges may provide insight into LEN-combined therapies that might boost its clinical efficacy. LEN-sensitive liver cell lines were conditioned with LEN in culture to establish this study's three LEN-resistant (LR) models. All LR models exhibited a stable right shift in the LEN dose-response curve. The LR models were cross-resistant to other FGFR inhibitors and expressed markers linked to cancer aggressiveness. Pro-apoptosis signals, induced by LEN treatment in Hep3B parental cells, were no longer induced in Hep3B LR cells. Compared to parental cells, Hep3B LR and JHH4 LR cells were no longer Annexin-V positive with LEN treatment, supporting their ability to evade apoptosis. A validated HTS method, using Cell-Titer Glo to measure the cell viability of Hep3B and JHH4 models tested across 440 small molecule inhibitors and LEN at a single concentration, identified nine synergistic combinations. Vetting these leads using a multidose titration assay showed synergy with LEN across all three LR models for two drugs: MRTX0902 targeting SOS1, an intracellular RTK signaling protein, and S63845 targeting MCL1, an anti-apoptosis protein. Multiple RTKs were upregulated in the LR models, thus making SOS1 an attractive target for blocking convergent signals. LEN treatment of parental models increased the expression of pAKT, pERK, and MCL1. LEN with S63845 in Hep3B parental cells reduced pAKT, pERK, and MCL1 and increased γ-H2AX. LEN with S63845 induced more apoptosis than either single agents in parental models or Hep3B LR cells. Enhanced apoptosis was not observed using MRTX0902 alone or with LEN. MRTX0902 alone reduced pAKT and pERK in Hep3B parental cells and was able to offset LEN's accumulation of these proteins in combination. We performed RNA-seq analysis of our model systems at baseline and with LEN treatment. LEN-treated parental cells exhibited a statistically significant increase in MCL1 and SOS1 transcripts. GSEA analysis of LEN-treated parental cells revealed positive MAPK pathway enrichment consistent with SOS1 function. It also identified negative enrichment of genes involved in DNA replication and G2M checkpoint, a p53-regulated process. This novel finding suggested that LEN induces genotoxic stress. Indeed, immunofluorescent foci staining of γ-H2AX increased with LEN treatment of parental Hep3B cells and remained elevated in Hep3B LR cells. Therefore, SOS1 and MCL1 may have a role in overcoming genotoxic stress induced by LEN. Overall, our research has generated multiple LR models and used them to identify resistance components. We also showed that targeting these components (SOS1 and MCL1) synergizes with LEN and inhibits cancer growth. These findings may become relevant patient treatments to extend patient survival against liver cancer.

TUMOR BIOLOGY: Imaging in Animal Models
Poster Session

#56 Multi-omic profiling of PSMA heterogeneity in prostate cancer liver metastases identifies new metabolomic targets.

Martin K. Bakht, Yasutaka Yamada, Sheng-Yu Ku, Anthony P. Belanger, Quang-De Nguyen, Himisha Beltran

Dana-Farber Cancer Institute, Boston, MA

Background: Prostate-specific membrane antigen (PSMA) is a metabolic enzyme involved in glutamate and folate pathways. Being a cell surface protein, PSMA serves as a theranostic target in castration-resistant prostate cancer (CRPC) with PSMA-radioligand therapy (PSMA-RLT). However, not all patients with PSMA-PET positive tumors respond to PSMA-RLT, partly due to heterogeneous expression of PSMA. We hypothesize that the metabolic features of the tumor microenvironment contribute to PSMA expression and function.
Methods: We profiled CRPC autopsy tumors from five patients and a series of orthotopic PSMA-positive metastatic CRPC models using mass spectrometry (MS), H3K27ac ChIP-seq, and spatial whole transcriptomics by Visium CytAssist platform.
Results: Spatial transcriptomic mapping indicated enrichment of amino acid transport and biosynthesis pathways in PSMA-suppressed liver metastases versus other sites of disease. Similarly, MS metabolomics revealed elevated levels of neutral amino acids, such as L-Leucine, in liver metastases. H3K27ac ChIP-seq showed that only liver metastatic tumors in the 22Rv1 CRPC model exhibited an H3K27ac peak at the SLC7A5 (LAT1) gene locus, correlating with low PSMA expression, not seen in primary tumors. A PSMA-suppressed 22Rv1 liver metastasis-derived cell line demonstrated sensitivity to LAT1 inhibition (V-9302), suggesting its potential as a therapeutic target.
Conclusions: Multi-omic profiling of patient tumors and prostate cancer models point to PSMA-suppression and heterogeneity in liver metastases associated with potentially targetable epigenetic and metabolomic alterations.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Reversal of Drug Resistance 1
Poster Session

#560 Characterizing the role of TIAM1-Rac1 signaling in advanced prostate cancers.

James P. Maine, Masuda Sharifi, Shu Ning, Amy R. Leslie, Zachary A. Schaaf, Wei Lou, Allen C. Gao

Urologic Surgery, University of California, Davis, Davis, CA

Background: Roughly 20-30% of patients with castration-resistant prostate cancer (CRPC) will progress to neuroendocrine prostate cancer (NEPC) following treatment of androgen receptor signaling inhibitors (ARSIs) such as enzalutamide and abiraterone. NEPC is an aggressive, treatment-resistant form of prostate cancer (PCa) with an average survival time of ~7 months post-diagnosis. Poor understanding of acquired resistance mechanisms and disease progression prevents the development of efficacious treatments for advanced PCa (NEPC and CRPC). The Rac1-associated guanine nucleotide exchange factor TIAM1—identified as a novel neural lineage signature in enzalutamide-resistant PCa cells—promotes the survival of neuroendocrine small-cell lung cancer. Here, we interrogate how TIAM1 contributes to acquired resistance and disease progression in advanced PCa.
Methods: RNA-seq, GEO, and cBioPortal datasets were analyzed to assess the relationships between TIAM1 expression and enzalutamide treatment, patient survival, and androgen receptor dependency. Cell growth assays determined the effects of TIAM1-specific RNA interference and TIAM1 overexpression in CRPC and NEPC-like cell lines. TIAM1 expression was evaluated using real-time quantitative PCR.
Results: Analysis of publicly available datasets revealed patients and mice treated with enzalutamide showed increased TIAM1 mRNA expression and that high relative TIAM1 mRNA expression significantly correlated to poor disease-free survival. NEPC and NEPC-like cell lines exhibited higher average TIAM1 mRNA expression than CRPC cells. siRNA TIAM1 depletion in drug-resistant NEPC-like cells showed moderate re-sensitization to the ARSIs enzalutamide and abiraterone. Transient TIAM1 overexpression in CRPC cells conferred partial tolerance to enzalutamide compared to vehicle-treated cells.
Conclusions: These results establish the clinical relevancy of TIAM1 in PCa models. Long-term treatment with enzalutamide increased TIAM1 expression in patient and murine samples, possibly contributing to acquired resistance mechanisms as demonstrated by increased ARSI sensitivity upon TIAM1 depletion. Current efforts in our lab focus on elucidating the mechanistic contributions of the TIAM1-Rac1 axis in acquired ARSI resistance to facilitate synergistic treatment of advanced PCa.

#561 Targeting mTOR/S6 signaling reverses acquired resistance to MTRX849 in KRAS^{G12C}-mutant lung adenocarcinoma cell lines.

Athena M. Madrid[1], Gabriel Choi[1], Jenny J. Hong[2], Sahel Hazeghsa[1], Jenny Z. Zhang[3], Kenny Castro[1], Neil A. O'Brien[1], Dennis J. Slamon[1]

[1]UCLA - University of California Los Angeles, Los Angeles, CA,[2]Chicago Medical School at the Rosalind Franklin University of Medicine and Science, North Chicago, IL,[3]Weill Cornell Graduate School of Medical Sciences, New York, NY

Background: *KRAS* is a commonly mutated oncogene long considered undruggable. Recent discoveries found compounds capable of binding to KRAS^{G12C} that inhibit KRAS-dependent signaling by locking the protein in its inactive state. However, clinical efficacy of these molecules has been limited by high rates of innate and acquired therapeutic resistance. Here, we developed cell line models of acquired resistance to the KRAS-G12C inhibitor MRTX849 (adagrasib) to study the mechanisms that underlie resistance which may help in the identification of novel therapeutic strategies to overcome resistance.

Materials and Methods: KRAS^{G12C}-mutant H358 and H1373 lung cancer cell lines were treated through long-term exposure of increasing doses of the MRTX849 to generate the resistant clones, H358-R and H1373-R. Antiproliferative IC50s were determined by treating cells with serially diluted drug for 6 days and cells were quantified using CellVista® at Day 1 and Day 6. Resistant cells were genotyped by PCR amplification. Changes in protein signaling were determined by immunoblotting and changes in mRNA signaling were measured using RNA-seq analysis.

Results: A 15-fold shift in IC50 was observed between H358-R and parental H358 (p-value = 0.029), and between H1373-R and parental H1373 (p-value = 0.022). Genotyping of the cell lines indicated that mutant *KRAS^{G12C}* was preserved in both resistant cell lines. RNA-seq analysis revealed a significant log-fold increase in *RPS6* expression between H358-R and parental cells treated with MRTX849 (p-value = 6.06E-6). Treatment with MRTX849 inhibited downstream signaling of ERK1/2 and RSK in both parental and resistant cell lines. However, signaling downstream of mTORC1 through pS6 only remained active in the presence of MRTX849 in the resistant cells. Treatment with the mTORC1 inhibitor, RAD001, induced partial inhibition of pS6 in the resistant cells, whereas combined treatment with MRTX849 and RAD001 induced complete and sustained inhibition of resistant pS6 signaling. Combination treatment with MRTX849 and RAD001 also produced significantly improved inhibition of proliferation in both the acquired resistant clones relative to treatment with either single agent alone; H358-R (p-value = 0.0099) and H1373-R (p-value = 0.018).

Discussion: In this study, we confirmed mTOR/S6 signaling as a potential escape pathway for KRAS-G12C inhibition. Targeting mTORC1 in combination with MRTX849 induced a sustained knockdown of S6-signaling and reversed resistance to MRTX849. Further genomic, transcriptomic and proteomic profiling of the generated resistant cell lines may identify additional mechanisms responsible for acquired KRAS-G12C resistance.

#562 Role of kinesin and Bcl-2 protein association in prostate cancer therapeutic resistance to taxane chemotherapy.

Maddison Archer[1], Hamam Said[2], Sudeh Izadmehr[1], Nabila Zaman[1], Prerna R. Nepali[1], Goutam Chakraborty[1], Matthew D. Galsky[1], Wei Yang[2], Natasha Kyprianou[1]

[1]Icahn School of Medicine at Mount Sinai, New York, NY,[2]Stony Brook University, Stony Brook, NY

Introduction: Therapeutic resistance to taxane chemotherapy remains a key contributor to lethality in patients with metastatic castration resistant prostate cancer (mCRPC). Understanding the mechanisms that drive therapeutic resistance is critical to reduce prostate cancer mortality. Our previous studies demonstrated overexpression of the mitotic spindle protein kinesin (HSET) and the anti-apoptotic Bcl-2 in taxane resistant human prostate cancer cells, C4-2BDR. This study aims to understand the contribution of Bcl-2 and kinesin protein interactions and their co-targeting by cabazitaxel (2nd line taxane chemotherapy) and kinesin inhibitor ispinesib, to overcome therapeutic resistance in prostate cancer.

Methods: Two prostate cancer cell lines were used, the LNCaP-Bcl-2 and the C4-2BDR. To investigate the role of Bcl-2 in resistance, LNCaP cells overexpressing Bcl-2 were treated with cabazitaxel and ispinesib as monotherapies and in combination. Treatment response was analyzed based on cell viability studies, and expression of HSET and Bcl-2 was assessed via Western blot. Immunoprecipitation of Bcl-2 was performed for proteomic analysis in C4-2BDR cells following cabazitaxel treatment. We subsequently evaluated the *in vivo* effect cabazitaxel in combination with ispinesib, on C4-2B xenograft growth and tumor evaluation by immunohistochemistry.

Results: Time course and dose response studies revealed that overexpression of Bcl-2 in LNCaP cells *in vitro* conferred resistance to both cabazitaxel and ispinesib as monotherapies. Transient increase in expression of HSET was observed, while Bcl-2 expression was unchanged by treatment. However, the association between HSET and Bcl-2 was targeted by cabazitaxel after 24 hours in both parental and LNCaP-Bcl-2 cells. Proteomic analysis of Bcl-2 binding partners in C4-2BDR cells, revealed upregulation of pathways involved in cytoskeleton remodeling, ribosomal and translation pathways, and cell adhesions in response to treatment. In C4-2B xenograft models, combination therapy of cabazitaxel and ispinesib reduced tumor growth, compared to each drug alone. Tumors treated with combination therapy exhibited higher E-cadherin, and reduced vimentin and HSET immunoreactivity compared to other treatments, suggesting EMT reversion towards an epithelial phenotype that correlated with response.

Conclusions: These studies suggest a functional Bcl-2 association with mitotic spindle protein, kinesin and proteins involved in cytoskeleton organization and remodeling, that might result in resistance to taxane chemotherapy. These findings are of therapeutic significance in identifying a potential targeting value of Bcl-2 association with HSET to increase the therapeutic vulnerability in advanced prostate tumors.

#563 AR-dependent castration-resistant prostate cancer relies on histone methyltransferase KMT2D for cell growth.

A. Bononi[1], P. Redford[2], W. Zhang[3], G. Cifelli[3], S. Kim[3], I. Gao[3], K. Werth[3], P. Carman[3], G. Valdes Garcia[3], Z. Taylor[3], R. Totoritis[3], J. Schneck[3], P. Bamborough[2], M. Queisser[2], G. Maundrill[2], D. Thomas[2], H. Tran[3], S. Varma[1], B. Mangatt[3], M. McCabe[3], B. Schwartz[1], K. Choi[1];

[1]GlaxoSmithKline, Cambridge, MA, [2]GlaxoSmithKline, Stevenage, United Kingdom, [3]GlaxoSmithKline, Upper Providence, PA

The KMT2D histone H3 lysine 4 (H3K4) methyltransferase plays critical roles in enhancer-regulated gene activation. It is a large multi-domain protein with its C-terminal SET domain being responsible for this activity. High KMT2D expression is associated with reduced patient survival in prostate cancer. Cancer Dependency Map data show a correlation between KMT2D gene dependency and FOXA1 expression in prostate cancer cell lines. FOXA1 is a major regulator of Androgen Receptor (AR) transcription and recent findings showed that KMT2D establishes the chromatin competency to recruit AR and for FOXA1 to activate AR-dependent transcription. Since FOXA1 is predicted to be poorly tractable, we aimed to target the more tractable KMT2D in FOXA1-overexpressing AR-dependent castration-resistant prostate cancer (CRPC). CRPC cell lines with high FOXA1 ($FOXA1^{high}$) and high AR (AR^{high}) and/or AR constitutively active splice variants, such as AR-V7 (AR-$V7^{+}$) were found to express high levels of KMT2D. KMT2D knockout (KO) reduced cell growth in these cells and in isogenic AR-$V7^{+}$ 22Rv1 cells lacking full length AR. Additionally, KMT2D KO modulated AR target genes as demonstrated by reduced PSA mRNA and increased mRNA for IGFBP3 and EFNB2. Inactivating SET domain mutations demonstrated that these effects were mediated by KMT2D's catalytic function thus supporting SET-domain inhibition as the desired target modality. This study highlights the potential of KMT2D as a therapeutic target for AR-dependent CRPC patients with constitutively active AR splice variants who have high unmet medical need due to AR variants being undruggable with currently available therapeutic agents.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Theranostics and Radiotheranostics
Poster Session

#566 Preclinical and translational pharmacokinetic (PK) modeling of the self-assembling and disassembling (SADA) bispecific fusion protein CD38-SADA for first-in-human (FIH) pretargeted radioimmunotherapy (PRIT).

Brian H. Santich[1], Charlotte A. Russell[2], LinLin Wang[1], Maria During[2], Lionel Renaud[3], Matthias Machacek[3], Norman LaFrance[1]

[1]Y-mAbs Therapeutics, Inc., New York, NY,[2]Y-mAbs Therapeutics, A/S, Horsholm, Denmark,[3]LYO-X AG, Basel, Switzerland

Background: PRIT is designed to deliver tumor-targeted radiation while limiting exposure to healthy tissues. CD38-SADA binds CD38 and select radioisotopes chelated to tetraxetan (DOTA) and contains a p53-derived domain mediating the self-assembly of tetramers from monomers in a reversible, concentration-dependent manner. Preclinical studies have demonstrated dose-dependent anti-tumor efficacy of two-step CD38-SADA PRIT with ^{177}Lu-DOTA administered 48h after an initial CD38-SADA infusion. Here, we report preclinical PK models and human simulations that informed the FIH study design and dosing regimen.
Methods: Total plasma concentrations of CD38-SADA (not cross-reactive with mouse CD38) were measured in 2 repeat dose studies following administration on D1, 8, and 15 in transgenic mice expressing human CD38 (huCD38 tg) and in a single dose study in huCD38 tg and wild-type (WT) mice. A PK model with Michaelis-Menten approximation of target-mediated drug disposition (TMDD) was fitted to the combined data. The PK model also incorporated *in vitro* mass photometry estimates of the CD38-SADA tetramer: monomer equilibrium. The model was scaled for human PK simulations using body weight-based allometry and theoretical scaling coefficients. For TMDD scaling, 2 scenarios were simulated: 1) Same CD38-mediated elimination in human as estimated in huCD38 tg mice. 2) No CD38-mediated elimination, *i.e.*, the most conservative case resulting in the highest predicted CD38-SADA exposure.
Results: The mouse PK model adequately described the observed CD38-SADA plasma concentration-time profiles. C_{max} was dose-proportional, with faster elimination observed in huCD38 tg mice vs WT mice at low doses, consistent with TMDD through binding to CD38 receptors. CD38-SADA monomers and tetramers demonstrated parallel linear and CD38-mediated nonlinear elimination from the central compartment. Linear clearance of CD38-SADA monomers was approximately 20 times faster than CD38-SADA tetramers (CL_{mon} 1.21 vs CL_{tet} 0.058 mL/h). At the planned FIH starting dose of 3 mg/kg, the predicted exposure provided a sufficient toxicological margin with/without TMDD scaling: C_{max} = 57.68 vs 58.71 µg/mL; $AUC_{0\text{-}inf}$ = 1070 vs 3020 µg·h/mL, respectively. The predicted tetrameric prevalence was ≥90% at the C_{max} concentrations and >50% at total plasma CD38-SADA concentrations >1 µg/mL, which was predicted to last for 40 to 136 hours depending on the TMDD scaling scenario.
Conclusions: A 2-compartmental PK model with nonlinear CD38-mediated elimination of CD38-SADA described the observed total CD38-SADA plasma concentration-time in huCD38 tg mice. PK simulations supported the initial FIH dosing for trial NCT05994157, CD38-SADA/^{177}Lu-DOTA PRIT in patients with relapsed/refractory non-Hodgkin lymphoma.

#567 Effectiveness of [^{225}Ac]Ac-labeled anti-MUC-16 radioimmunconjugate against CA125 expressing pancreatic and ovarian cancer xenografts.

Emmanuel Nwangele[1], Hanan Babeker[2], Alyssar Monzer[2], Fabrice Ngoh Njotu[3], Jessica Pougoue Ketchemen[3], Alireza Doroudi[4], Florence-Anjong Tikum[4], Nikita Henning[4], Emina Torlakovic[5], Maruti Uppalapati[6], Humphrey Fonge[7]

[1]Faculte de pharmacie, CHU de Quebec - Universite Laval, Quebec, QC, Canada,[2]College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada,[3]Faculte de pharmacie, CHU de Quebec-Universite Laval, Quebec, QC, Canada,[4]College of medicine, University of Saskatchewan, Saskatoon, SK, Canada,[5]Department of Pathology, Royal University Hospital, Saskatoon, SK, Canada,[6]Department of Pathology and Lab. Medicine, University of Saskatchewan, Saskatoon, SK, Canada,[7]Faculte de Pharmacie, Centre de Recherche du CHU de Quebec-Universite Laval, Quebec, QC, Canada

Purpose: Cancer biomarkers like CA125 (MUC-16) are targets for the selective delivery of toxic radiation and/or cytotoxic molecules to tumor loci. MUC-16 is overexpressed in 80% of epithelial ovarian cancer (EOC) and 65% of pancreatic ductal adenocarcinomas (PDAC). Prognosis of EOC and PDAC has remained poor with 5-year survival rate of between 30-50% and 15-20% for EOC & PDAC respectively, hence, the need for new treatment approaches. Our lab has developed a fully human monoclonal antibody (mAb) that binds specifically to MUC-16 and demonstrated the effectiveness of [^{89}Zr]Zr-DFO-MUC-16 as a PET imaging agent. Here, we have developed [^{225}Ac]Ac-Macropa-MUC-16 and investigated its effectiveness against CA125 expressing EOC and PDAC cell derived xenografts (CDX) and patient derived xenografts (PDX).

Method: The anti-MUC-16 mAb was conjugated to macrocyclic bifunctional chelator Macropa-NCS and radiolabeled with [^{225}Ac]$AcNO_3$ to produce [^{225}Ac]Ac-Macropa-MUC-16 radioimmunoconjugate (RIC). Quality control assays were conducted to evaluate the integrity of the antibody, stability of the RIC and binding to MUC-16. We developed MUC-16 positive PDAC CDX (SW1990) and PDX (medium MUC-16 expression) and EOC PDXs (high and low MUC-16 expression).Biodistribution studies of [^{225}Ac]Ac-Macropa-MUC-16 was performed in non-tumor bearing mice at different timepoints and used to project organ doses using OLINDA. Groups of tumor-bearing mice were treated using 2 x of 13 kBq of the RIC at day 0 and 10 and tumor growth was monitored. [^{225}Ac]Ac-Macropa-Rituximab was used as a control RIC.Additionally, safety of the RIC was assessed after administration of 1 or 2 doses of the agent to healthy mice followed by clinical chemistry, CBC and histopathology.

Results: 16.67% of CDX (SW1990) treated with 2 x 13 kBq of the RIC had complete remission (CR). The remaining 83.33 % in this group had tumor growth suppression for ≥ 50 days post treatment and never reached endpoint (≥ 1500 mm^3), while saline and control RIC groups reached end point by day 33.[^{225}Ac]Ac-Macropa-MUC-16 was very effective against high and medium MUC16 expressing PDAC and EOC PDXs, respectively heading to 100% CRs while median survival for control groups was 36 (PDAC) and 47 (EOC) days. The RIC was less effective at inhibiting EOC PDX with low MUC-16 expression. For this, the % tumor growth inhibition (% TGI) was 94.5±6.8% and 84.7±11% at the day 12 and 21, respectively. Although CBC & clinical chemistry show no safety issues, histopathological studies showed mild to moderate pathologies in the liver and spleen for mice exposed to 2 doses of 15 kBq of the RIC. .

Conclusion: [^{225}Ac]Ac-Macropa-MUC-16 is effective against MUC-16 expressing EOC and PDAC xenografts. The exciting therapeutic potential is however dependent on the expression level of MUC-16 and points to the significance of heterogeneity within and between tumor types in cancers.

#568 Click chemistry for theranostic approaches in triple negative breast cancer.

Cristina Simo, Shayla Shmuel, Alex Vanover, Patricia M. R. Pereira

Washington University School of Medicine in St. Louis, St. Louis, MO

Introduction: Immunotherapies are part of the rapidly expanding landscape of targeted cancer treatments, with market sizes reaching over $150 billion in 2024. However, further understanding of tumor biology is crucial to developing effective therapies that minimize resistance. *N*-linked glycosylation, a post-translational membrane modification, is well known for its critical role in tumor growth and resistance, its impact on antibody tumor accumulation, and its effect on tumor response to targeted therapies. Despite its significance, methods to image and target (theranostic approaches) *N*-glycosylation have still not been fully developed. In this study, we use biorthogonal (click) chemistry to visualize and target *N*-glycans on triple negative breast cancer (TNBC) and exploit them to enhance immunotherapy.

Methods: 4T1 TNBC cells were inoculated subcutaneously in Balb/c mice. Glycans visualization: Mice were administered intravenously with tetraacetyl-*N*-azidoacetylmannosamine (mannose-N_3; 40 mg/kg) or PBS (control) once daily for three consecutive days. At 24 h post the last injection, AZDye-680 DBCO (5 mg/kg) was intravenously injected. At 24 h post-AZDye-680 DBCO administration, the organs were harvested, scanned, homogenized, and lysed for fluorescence and protein measurements. Glycans targeting to enhance anti-PD-L1 antibody uptake: Mice were administered mannose-N_3 or PBS as described before. Mice were then intravenously injected with the anti-PD-L1 antibody avelumab radiolabeled with copper-64 [^{64}Cu]Cu-NOTA-avelumab-DBCO (6.7 MBq, 40 µg). Mice were imaged with positron emission tomography (PET) at 24 and 48 h.

Results: Glycans visualization: 4T1 tumors exhibit high levels of *N*-glycosylation as visualized through metabolic glycoengineering using a mannose-N_3 derivative and AZDye-680 DBCO fluorescent dye. First, mannose-N_3 is administered, introducing azide functional groups to cell-surface *N*-glycoproteins. Next, the AZDye-680 DBCO is administered, clicking with azide groups on the cancer cell's surface. When compared with PBS, we observed a 3.5-fold increase in tumor uptake in animals administered mannose-N_3. Glycans targeting to enhance anti-PD-L1 antibody uptake: Previous studies demonstrated that PD-L1 is highly *N*-glycosylated in cancer cells, affecting antibody accumulation in tumors. Using immuno-PET with [^{64}Cu]Cu-NOTA-avelumab-DBCO, we showed a 1.9-fold increase in avelumab accumulation in mannose-administered mice compared to PBS controls.

Conclusions: In this study, we applied click chemistry to not only visualize *N*-glycans within tumors but also to exploit their presence to enhance antibody therapy. Our ongoing studies are focused on using metabolic glycoengineering to enhance anti-PD-L1 and anti-PD-1 therapy in TNBC.

TUMOR BIOLOGY: Imaging in Animal Models
Poster Session

#57 Membrane-bound heat shock protein Hsp70 as a new target in oncology: from theory to practice.

Maxim Shevtsov[1], Natalia Yudintceva[2], Danila Bobkov[2], Ruslana Likhomanova[2], Anastasiya Nechaeva[2], Anastasiya Lukacheva[2], Natalia Mikhailova[2], Viacheslav Fedorov[2], Elena Oganesyan[2], Oleg Rozanov[2], Konstantin Samochernykh[3], Alexey Ulitin[3], Alexander Kim[3], Elena Mikhailova[2], Rustam Ziganshin[4], Emil Pitkin[5], Stephanie E. Combs[1]

[1]Klinikum rechts der Isar, Technical University of Munich, Munich, Germany,[2]Personalized Medicine Centre, Almazov National Medical Research Centre, St.Petersburg, Russian Federation,[3]Polenov Neurosurgical Institute, Almazov National Medical Research Centre, St.Petersburg, Russian Federation,[4]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry Russian Academy of Sciences (RAS), Moscow, Russian Federation,[5]Department of Statistics and Data Science, The Wharton School, University of Pennsylvania, Philadelphia, PA

Heat shock proteins (HSP), particularly 70 kDa heat shock protein (Hsp70), apart from their cytoplasmic localization in cancer cells, can be exposed on the surface of the plasma membrane. We report that the membrane-bound protein mHsp70 of malignant brain tumors is required for high migratory and invasive activity of cancer cells. Live-cell inverted confocal microscopy of adult and pediatric brain tumor samples (n=32) showed mHsp70 overexpression on the membrane, especially in the perifocal zone. Mass-spectrometry analysis of lipid rafts isolated from brain tumor cells confirmed the presence of the protein in HSP cluster (including representatives of other families such as HSP70, HSP90), which in turn, during interactome analysis, was associated with proteins involved in cell migration and invasion (i.e., Rac1, RhoC, myosin-9). Application of small-molecule inhibitors of HSP70 (JG-98, PES) led to a significant decrease in the invasive potential of cells isolated from a tumor sample of patients, which indicates the role of the chaperone in invasion. Moreover, the use of chaperone inhibitors in orthotopic brain tumors model in rodents significantly decreased tumor progression, which was accompanied by an increased overall survival. Data demonstrate that chaperone inhibitors, particularly JG-98, disrupt the function of mHsp70, thereby providing an opportunity to better understand the diverse functions of this protein and offer aid in the development of novel cancer therapies. Apart from chaperone inhibitors we developed mHsp70-targeted peptide that could efficiently cross the blood-brain barrier accumulating in the glioblastoma cells in vivo. Subsequently, in the orthotopic GL261 mice and C6 rat glioma, intravenous administration of the fluorescently labeled peptide resulted in the tumor retention of agent (further confirmed by histological studies) 24 hours following i.v. injection. Topical application of the mHsp70-targeted peptide (sprayed over the freshly dissected brain tissues) helped to delineate the tumors in glioma-bearing animals employing intraoperative fluorescent imaging system. In conclusion, peptide recognizing mHsp70 demonstrated high targeting properties which could be employed for the intraoperative visualization as well as for developing of a potential carrier for drug delivery.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Theranostics and Radiotheranostics
Poster Session

#570 Preclinical insights into ^{161}Tb-trastuzumab and immune checkpoint combination therapy for cancer in a HER2-expressing murine cancer model.

Sigrid Cold[1], Pragalath Sadasivam[2], Myrto Ischyropoulou[2], Sakeerthi K. Vinethkanth[2], Emma Papin[2], Jacob Hald[2], Sebastian Gnosa[2], Trine B. Engel[2], Lene Jessen[2], Andreas Kjaer[3], **Carsten H. Nielsen**[2], Lotte K. Kristensen[2]

[1]Cluster for Molecular Imaging, Cluster for Molecular Imaging, Copenhagen University Hospital - Rigshospitalet; University of Copenhagen; Minerva Imaging ApS, Copenhagen, Denmark,[2]Minerva Imaging ApS, Oelstykke, Denmark,[3]Department of Biomedical Sciences, Cluster for Molecular Imaging, Copenhagen University Hospital - Rigshospitalet; University of Copenhagen, Copenhagen, Denmark

Introduction: Targeted radionuclide therapy (TRT) is a unique treatment option allowing for *in vivo* imaging and delivery of lethal radiation directly and specifically to solid cancers. As TRT is generally well-tolerated with limited side effects, it holds a significant potential for use in combination with other therapies such as immunotherapy. However, preclinical assessment of this combinational pair has been limited by the lack of relevant animal models. To address this, we established a fully immunocompetent animal model bearing murine tumors expressing a human target making the model eligible for both TRT and immune checkpoint inhibitor (ICI) therapy. Subsequently, the model was used for evaluating the *in vivo* biodistribution, tolerability and efficacy of Tb-161 trastuzumab in combination with anti-PD-1 (aPD-1) therapy. In addition, whole-body immune status following therapy was evaluated with CD8 ImmunoPET with 89Zr-Df-IAB42M1-14 (ImaginAb) and tumor microenvironment was profiled by flow cytometry.
Methods: The murine EMT-6 breast cancer cell line was modified to express HER2 by lentiviral transduction with expression confirmed by flow cytometry. The engineered EMT-6-HER2 (1+) were implanted on Balb/c mice, which subsequently were included in a theranostic study receiving either vehicle, ^{161}Tb-Trastuzumab, aPD-1 or a combination of ^{161}Tb-Trastuzumab and aPD-1. SPECT/CT imaging was performed 72 and 168 hours post dosing of TRT single therapy to evaluate biodistribution. Eight days after treatment initiation, PET/CT imaging of CD8 T cells was performed 24 hours after injection 89Zr-Df-IAB42M1-14 (4 MBq, 0.2 MBq/µg) in all treatment groups. Autoradiography and IHC were performed on tumors to evaluate co-localization of target expression and compound accumulation.
Results: Animals were included with a tumor volume of 101.5 mm^3 (n=12-16/ group). ^{161}Tb-Trastuzumab SPECT/CT imaging showed high tumor uptake (>15 %ID/g) and limited uptake in non-target expressing organs. Animals receiving ^{161}Tb-Trastuzumab as a single therapy or in combination with aPD-1 had significantly smaller tumor volumes when compared to the vehicle control (Dunnett's, p=0.003) with the smallest tumor volume observed in the combinatory treatment group. PET/CT imaging of 89Zr-Df-IAB42M1-14 targeting CD8 T cells revealed tumor uptake of 5-10 %ID/g. Autoradiography and IHC confirmed tumor border T cell infiltration and subcellular overlay between HER2 target expression and Tb-161.
Conclusion: This study demonstrated an evaluation of the efficacy of TRT and ICI, both as single therapies and in combination in a relevant animal model. We furthermore confirmed targeting of ^{161}Tb-Trastuzumab *in vivo* and showed that the immune response and CD8 T cell infiltration could be monitored with PET/CT imaging in animals previously dosed with Tb-161 radiolabeled trastuzumab.

#571 Novel somatostatin receptor type 2 targeted radiopharmaceuticals with improved renal clearance and enhanced tumor retention relative to DOTATATE.

Nicole Bakas, Alain Noncovich, Andrew Hudson, Su-Tang Lo, Lea Nyiranshuti, Patrick Napier, Nicholas Smith, Steven Horton, Samantha Richardson, Terra Miller, Ron Deocareza, Katrina Salvador, Tarsicio Juarez, Charlotte Lorenz, Abhijit Bhat

RayzeBio, San Diego, CA

Introduction: Somatostatin receptor type 2 (SSTR2) is highly expressed on a majority of well-differentiated gastroenteropancreatic neuroendocrine tumors (GEP-NETs) and is also expressed in other solid tumors such as small-cell lung cancer (SCLC) and breast cancer, albeit with a lower prevalence. [^{177}Lu]Lu-DOTATATE is an eight amino acid peptide with high affinity for SSTR2 receptor used as a radiopharmaceutical therapy (RPT) for treatment of SSTR2 expressing solid tumors. One limitation of DOTATATE-based RPT is the uptake and extended retention of radiolabeled peptides in the proximal renal tubule cells, increasing the risk for radiation-induced nephropathy. The kidney retention of DOTATATE limits the administered radioactivity and effective radiation dose delivered to the tumors. We report the development of a novel SSTR2 targeted RPT agents, RYZ-SSAs, designed to have enhanced renal clearance and improved tumor exposure compared to [^{177}Lu]Lu-DOTATATE; thereby maximizing the therapeutic potential of SSTR2 RPT by effectively delivering higher radiation doses to tumors while minimizing renal exposure.

Methods: RYZ-SSAs are highly selective, potent SSTR2 agonists conjugated to a DOTA chelator, and capable of binding various radioisotopes. The binding affinity to the SSTR1-5 proteins, and the cell binding and internalization in SSTR2 expressing cell lines were determined by radioligand binding assays. Further evaluation by cAMP and b-arrestin recruitment assays were performed in SSTR2 overexpressing CHO cells. Biodistribution studies using SPECT imaging and *ex vivo* quantification, and efficacy were performed in SSTR2 expressing xenograft models.

Results: RYZ-SSAs show high binding affinity to human SSTR2, with sub-nanomolar SSTR2 Ki's and selectivity of >200-fold against SSTR1/3/4/5 isoforms. The IC50s and EC50s for RYZ-SSAs ranged from single digit nanomolar to sub-nanomolar. In *ex vivo* mouse biodistribution studies, [^{177}Lu]Lu-RYZ-SSAs were compared with [^{177}Lu]Lu-DOTATATE in a SSTR2 positive NSCLC xenograft. The tumor to kidney ratio based on the mTIACs were 2.1 for DOTATATE and 10.5 for RYZ-SSA1. RYZ-SSA1 demonstrated greater than two-fold increase in tumor retention, and two-fold decrease in renal exposure relative to DOTATATE. A single injection of [^{225}Ac]Ac-RYZ-SSA1 resulted in significant tumor growth inhibition in a NSCLC xenograft model with durable regression and prolonged survival. All treatments were well tolerated.

Conclusion: Preclinical *in vitro* and *in vivo* data demonstrate that RYZ-SSAs have an improved therapeutic window relative to [^{177}Lu]Lu-DOTATATE. RYZ-SSA1 has the potential to deliver higher radiation doses to tumors while sparing the kidneys without co-administration of amino acids, paving the way for a more effective and convenient RPT treatment regimen for SSTR2 expressing solid tumors.

#572 Development of a targeted copper theranostic to fibroblast activation protein (FAP) which shows high uptake and long-term retention at the tumor site.

Jaclyn L. Lange[1], Lachlan E. McInnes[1], Jessica Van Zuylekom[2], Benjamin Blyth[2], Stacey E. Rudd[3], Paul S. Donnelly[3], Aidan Ingham[4], Jason S. Lewis[4], **Matthew Harris**[1]

[1]Clarity Pharmaceuticals, Sydney, Australia,[2]Peter MacCallum Cancer Centre, Melbourne, Australia,[3]School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Melbourne, Australia,[4]Memorial Sloan Kettering Cancer Centre, New York, NY

Background and Purpose: Fibroblast Activation Protein (FAP), a membrane-bound enzyme and cell surface protein, is up-regulated in cancer-associated fibroblasts and is present in the tumor microenvironment across numerous cancers. There is low to no expression of FAP in healthy tissue. Targeted Copper Theranostics (TCTs) are radiopharmaceuticals that utilize copper-64 (Cu-64) for PET imaging and copper-67 (Cu-67) for therapy. Herein, we report preclinical evaluation of a mono- and bis-FAP targeting agent using Cu-64 in two FAP-positive tumor xenograft models. These agents enable the visualization of FAP as a potential pan-cancer imaging agent and therapy.

Methods: We evaluated the imaging characteristics of SAR-FAP and a dimer, SAR-bisFAP, which utilize the FAP inhibitor (UAMC1110) conjugated to a sarcophagine chelator (SAR). We tested the compounds in two xenograft models which have high expression levels of FAP: NSG mice bearing a SK-MEL-187 xenograft (a human-derived metastatic cell line); or athymic nude mice bearing a U87MG xenograft (a human-derived glioblastoma cell line). The mice were administered ~5 MBq of [^{64}Cu]Cu-SAR-FAP or [^{64}Cu]Cu-SAR-bisFAP and the biodistribution and tumor uptake assessed via microPET/CT imaging and *ex vivo* biodistribution at 1, 4 and 24 h post i.v. injection. A comparison study with [^{68}Ga]Ga-FAPi-46 along with blocking studies with FAPi-46 (an industry standard) were also completed.

Results: Imaging and an ex-vivo biodistribution study of the SK-MEL-187 xenograft NSG mice showed that both SAR-FAP tracers have specific binding to FAP-positive SK-MEL-187 tumors and higher uptake compared to ^{68}Ga-FAPi-46 at 1 h. Additionally, both SAR-FAP tracers show FAPi-46 blockable uptake, confirming the tracer is binding specifically to FAP. Overall, the [^{64}Cu]Cu-SAR-bisFAP has better retention at 24 h. In the U87MG xenografted athymic nude mice, the tumor uptake, as measured via ex vivo biodistribution for the [^{64}Cu]Cu-SAR-FAP tracer was consistent over 4 hours (4.8% IA/g) which thereafter decreased to 0.8% IA/g at 24 hours. We were again able to observe higher uptake and long retention in the tumor after 24 hours (6.2% IA/g) for the [^{64}Cu]Cu-SAR-bisFAP.

Conclusion: Imaging and biodistribution studies demonstrated high tumor-specific uptake for [^{64}Cu]Cu-SAR-FAP and [^{64}Cu]Cu-SAR-bisFAP tracers in the SK-MEL-187 and U87MG tumor xenograft mouse models. The uptake and 24-hour retention of the [^{64}Cu]Cu-SAR-bisFAP is very encouraging and indicates potential for therapeutic benefit with this tracer. Efficacy studies are underway to ascertain the therapeutic potential of [^{67}Cu]Cu-SAR-bisFAP as a TCT.

#573 Radioimmunotherapy using $^{64/67}$copper labelled trastuzumab in mice bearing HER2-positve xenografts as a model for breast cancer therapy.

Stacey E. Rudd[1], Jessica Van Zuylekom[2], Benjamin Blyth[2], **Matthew Harris**[3], Paul S. Donnelly[1]

[1]School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Melbourne, Australia, [2]Peter MacCallum Cancer Centre, Melbourne, Australia,[3]Clarity Pharmaceuticals, Sydney, Australia

Background and Purpose: Human epidermal growth factor receptor 2 (HER2) is overexpressed in several cancers including breast cancer. In the treatment of HER2 positive breast cancer, patients have shown significant improvements with trastuzumab therapy. Radioimmunotherapy (RIT) utilizes an antibody targeted to a receptor overexpressed on tumors to deliver radiation to the target. RIT utilizing trastuzumab could provide an additional treatment option for HER2+ breast cancer patients. We have produced the theranostic pair [^{64}Cu]Cu-MeCOSar-trastuzumab, for PET imaging, and [^{67}Cu]Cu-MeCOSar-trastuzumab, for therapy via beta emission, to evaluate this Targeted Copper Theranostic (TCT) approach as an effective radioimmunotherapy.

Methods: The MeCOSar chelator was conjugated to trastuzumab and subsequently radiolabeled with either copper-64 (Cu-64) or copper-67 (Cu-67). Proof of concept studies were performed using SKOV-3 tumor bearing mice, injected with one dose of [^{64}Cu]Cu-MeCOSar-trastuzumab or [^{67}Cu]Cu-MeCOSar-trastuzumab to compare biodistribution of the theranostic pair. Tumor uptake was assessed using PET/CT imaging and/or ex vivo biodistribution studies. In therapy studies, a single dose of saline, MeCOSar-trastuzumab, or [^{67}Cu]Cu-MeCOSar-trastuzumab was administered and endpoints assessed.

Results: [^{64}Cu]Cu-MeCOSar-trastuzumab and [^{67}Cu]Cu-MeCOSar-trastuzumab showed comparable high tumor-specific uptake in biodistribution studies, with tumor uptake at 48 hours of 61 ± 6 % IA/g for [^{64}Cu]Cu-MeCOSar-trastuzumab and 59 ± 7 % IA/g for [^{67}Cu]Cu-MeCOSar-trastuzumab. Mice treated with [^{67}Cu]Cu-MeCOSar-trastuzumab tolerated the treatment well with limited side effects or AEs. Treated mice also showed significant tumor growth inhibition and extended survival in a dose dependent manner compared with the control groups. The median survival was 32 days in the control groups and 102 days in the 9 MBq group.

Conclusion: These results show high tumor uptake for [^{64}Cu]Cu-MeCOSar-trastuzumab and [^{67}Cu]Cu-MeCOSar-trastuzumab with very similar biodistribution profiles at 48 hours in SKOV-3 xenograft model. [^{67}Cu]Cu-MeCOSar-trastuzumab demonstrated significant tumor growth inhibition compared to the control groups and effectively increased the survival of the treated group. This study demonstrates the potential of [^{67}Cu]Cu-MeCOSar-trastuzumab for clinical translation.

#574 Detection of tumor cell surface calreticulin after doxorubicin treatment using a novel radiolabeled nanobody.

Rachael Guenter[1], Yuvasri Golivi[1], Lucinda Hall[1], Amro Abdelrahman[2], Chloe La Prairie[3], Caleb Miller[1], Tejeshwar C. Rao[4], Danielle M. Carlson[5], Mark J. Truty[2], Benjamin Larimer[6], J. Bart Rose[1]

[1]Surgery, University of Alabama at Birmingham, Birmingham, AL,[2]Surgery, Mayo Clinic, Rochester, MN,[3]Pediatric Dentistry, University of Alabama at Birmingham, Birmingham, AL,[4]Pharmacological and Pharmaceutical Sciences, University of Houston, Houston, TX,[5]Internal Medicine, Mayo Clinic, Rochester, MN,[6]Radiology, University of Alabama at Birmingham, Birmingham, AL

Background: Targeted agents, such as nanobodies, can be covalently linked to radionuclide chelators for theranostics. Nanobodies are single-domain immunoglobulin variable regions with a short blood half-lives and high specificity. Calreticulin (CALR) is endoplasmic reticular (ER) protein that translocates to the cell surface upon induction with certain agents, namely doxorubicin. We hypothesized surface CALR on tumors could be targeted using a novel nanobody after induction with doxorubicin.

Methods: Studies were conducted using *in vitro* and *in vivo* models of pancreatic ductal adenocarcinoma (PDAC). Surface CALR expression was determined by western blot on fractionated lysates, flow cytometry, or immunofluorescence (IF). Doxorubicin was administered by intraperitoneal injection to mouse models of PDAC. The anti-CALR nanobody was developed via the panning of a synthetic nanobody phage display library. The top CALR-binding nanobody was confirmed by ELISA, immunohistochemistry (IHC), and flow cytometry. For *in vivo* studies, the nanobody was conjugated with the chelator NOTA and radiolabeled with ^{64}Cu. PET images were acquired 1-hour after injection. Significance was determined using an independent t-test and p values < 0.05 were considered statistically significant.

Results: PDAC cells treated with doxorubicin showed peak surface CALR expression between 24 and 48 hours. Flow cytometry revealed that 61.7% of live PDAC cultured cells treated for 24 hours expressed surface CALR, compared to 2.2% in controls ($p < 0.001$). In a subcutaneous syngeneic PDAC mouse model, two doses of doxorubicin 48 hours apart significantly increased surface CALR, with 30.4% of live-cells expressing CALR in treated tumors versus 1.5% in controls ($p < 0.001$). Similarly, doxorubicin-treated tumors from a PDAC patient derived xenograft (PDX) model had a 6.7-fold increase in live-cells expressing surface CALR compared to controls ($p < 0.01$). IF confirmed a 1.4-fold increase in CALR expression in doxorubicin-treated tumors from the subcutaneous PDAC mouse model ($p < 0.01$) and a 1.7-fold increase in the PDAC PDX model ($p < 0.01$). Our anti-CALR nanobody has an apparent 37 nM affinity for recombinant CALR and successfully detected surface CALR by IHC and flow cytometry. *In vivo* PET imaging showed significant increased uptake of the ^{64}Cu-NOTA-nanobody in tumors from doxorubicin-treated mice over controls, with no significant changes in heart, kidney, or liver accumulation by PET quantification and flow cytometry ($p < 0.05$).

Conclusions: Systemic doxorubicin treatment induces CALR surface translocation in tumors that can be successfully targeted with a radiolabeled nanobody for PET imaging. Targeting chemotherapy-induced surface CALR using our radiolabeled nanobody could provide a theragnostic strategy for the detection and treatment of PDAC.

#575 ^{225}Ac-SSO110 as next-generation SSTR2 targeting radiopharmaceutical for small cell lung cancer.

Anika Jaekel, Prachi Desai, Manuel Sturzbecher-Hoehne, Dennis Mewis, Germo Gericke, Manfred Ruediger

Ariceum Therapeutics GmbH, Berlin, Germany

SSO110 (DOTA-JR11) is a somatostatin receptor 2 (SSTR2) antagonist currently under clinical development in small cell lung cancer (SCLC). SSO110 targets a higher number of SSTR2 binding sites and shows longer tumor retention than the SSTR2 agonist DOTATATE translating into higher anti-tumor efficacy of ^{177}Lu-SSO110 compared to ^{177}Lu-DOTATATE. The aim of this study was to identify the most potent radioisotope (^{225}Ac, ^{212}Pb, ^{161}Tb, ^{177}Lu) for SSO110 in comparison to DOTATATE to guide further clinical development. Balb/c or Swiss nude mice were engrafted with SSTR2-positive xenografts NCI-H69 (SCLC model) or AR42J (pancreatic cancer model). The therapeutic efficacy of ^{225}Ac, ^{161}Tb-, ^{212}Pb-, and ^{177}Lu-labelled SSO110 was compared to ^{225}Ac- and ^{161}Tb-labelled DOTATATE after single-dose intravenous injection. In the NCI-H69 SCLC model, 20 MBq ^{177}Lu-SSO110 and 21 kBq ^{225}Ac-SSO110 demonstrated significantly better anti-tumor efficacy than 42 kBq ^{225}Ac-DOTATATE, 20 MBq ^{161}Tb-DOTATATE, or 20 MBq ^{161}Tb-SSO110. Most remarkably, a single dose of only 30 kBq ^{225}Ac-SSO110 (human equivalent dose: 7.3 MBq) induced complete tumor remissions and long-term survival. Various doses of ^{212}Pb-SSO110 were less or equally effective than ^{177}Lu-SSO110. In contrast to treatment with ^{225}Ac-SSO110, no complete remissions were induced with ^{212}Pb-SSO110. In the AR42J model, 37 kBq ^{225}Ac-SSO110 induced tumor growth inhibition immediately after treatment and tumor volume remained low and constant leading to 100% survival of all animals. Histopathological assessment of the tumors showed a lower mitotic index, higher necrosis and a regressing phenotype of neoplastic cells in comparison to tumors from the vehicle group (median survival of 11 days). 37 kBq ^{225}Ac-DOTATATE could not control tumor growth and median survival was reached after 33 days. 20 MBq ^{161}Tb-SSO110 induced a slightly stronger tumor growth delay than 20 MBq ^{177}Lu-SSO110 (median survival 36 vs 30.5 days). All treatments were well-tolerated. The preclinical in vivo comparison demonstrates superior efficacy of SSO110 over DOTATATE across all isotopes tested (Lu-177, Ac-225, Tb-161). Our results highlight the exceptionally strong anti-tumor efficacy of ^{225}Ac-SSO110 in vivo even at low, single doses. Interestingly, ^{212}Pb-SSO110 was not superior to ^{177}Lu-SSO110 and ^{225}Ac-SSO110, most likely due to the long tumor half-life of SSO110 (>7 days) that matches better with the long-lived isotopes Ac-225 and Lu-177. ^{161}Tb-SSO110 demonstrated modestly better efficacy than ^{177}Lu-SSO110, but only in the AR42J model with high and homogenous target expression. Based on promising emerging clinical data from the LuSato1 study with ^{177}Lu-SSO110 and the outstanding preclinical activity of ^{225}Ac-SSO110, a global phase I/II clinical study with ^{225}Ac-SSO110 in patients with ES-SCLC or Merkel Cell Carcinoma on Immune-Checkpoint Inhibitor monotherapy is being initiated.

#576 Discovery of FL801, a B7-H3-targeting nanobody-based radioligand vector, using Clear-XTM linker technology.

Xiaozhu Wang, Ming-Jin Jheng, Pengyun Li, Dian Li, Yihu Xie, Biao Guo, Ruyi Hu, Fa Liu

Full-Life Technologies Limited, Shanghai, China

B7-H3, also known as CD276, is a type I transmembrane protein and a member of the B7 family, which includes immune checkpoints such as PD-L1. B7-H3 is over-expressed in multiple solid tumors including lung, esophageal, prostate and breast cancers. B7-H3 antibody-drug conjugates (ADCs) have demonstrated promising early clinical antitumor activity. In contrast to ADCs, radionuclide drug conjugates (RDCs) offer not only a different therapeutic approach but also allow tumor imaging. VHH (variable heavy domain of heavy chain) is a highly attractive binder modality, however, one persistent challenge of VHH-based RDCs is the low tumor uptake together with a high kidney uptake prohibiting its application in RDC therapy. Our Clear-X™ linker technology was developed to specifically address such therapeutically sub-optional biodistribution profiles that are nearly without exception shared by all VHHs and other similar-sized protein-based vectors such as affibodies and DARPins. Using Clear-X™, we developed our lead B7-H3 radioligand vector FL801. The binding affinity of FL801 was assessed in vitro. In the cell-based binding assays, FL801 exhibited specific B7-H3 binding with sub-nanomolar affinity. The radiolabeling of FL801 with ^{177}Lu resulted in radiochemical purities (RCP) of >98% and molar activities of 18.5 MBq/nmol. [^{177}Lu]Lu-FL801 was found to be stable in the formulation buffers at 4°C for 5 days. The in vivo biodistribution profile of [^{177}Lu]Lu-FL801 was characterized by SPECT/CT imaging and cut-and-count biodistribution studies in B7-H3-positive tumor-bearing mice. The SPECT/CT imaging of 11.1 MBq [^{177}Lu]Lu-FL801 revealed an intense tumor uptake that was significantly higher than in all normal organs at 24 and 72 hour. In the cut-and-count biodistribution studies, [^{177}Lu]Lu-FL801 demonstrated a therapeutically favorable profile including a tumor uptake of >30% ID/g and a tumor-to-kidney (T/K) ratio of >2.0 at 24 hour. This was a significant improvement over our starting-point VHH lead without Clear-X™ modification which exhibited a tumor uptake of 2.3% ID/g and a T/K ratio of <0.01 at 24 hour. In addition, the anti-tumor activity of [^{177}Lu]Lu-FL801was evaluated in the same model using a single dose of 11.1 MBq [^{177}Lu]Lu-FL801 per mice. It was found that the treatment was well tolerated, and all treated animals achieved a complete remission of the tumor. In summary, the preclinical data package collectively supports to proceed towards clinical studies of [^{177}Lu]Lu-FL801 as a potential therapeutic option for B7-H3 positive tumors. In addition, this work validated the effectiveness of Clear-X™ linker technology in optimizing VHH-RDCs, and we expect Clear-X™ is also applicable to other protein-based RDCs with similar molecular sizes.

#577 First-in-class Claudin 18.2 targeting radiopeptide, ^{64}Cu-PD-29875 and ^{177}Lu-PD-29875, exhibit promising theranostic potential in a pancreatic cancer xenograft model.

Shota Tsuchida[1], Yoshihide Mizukoshi[1], Shintaro Kato[1], Hiroko Inaba[1], Rie Komura[1], Miho Satake[2], Yuki Sato[2], Naoki Kanazawa[2], Hayato Yanagida[1], Hidetomo Kitamura[1], Kentaro Suzuki[2], Keisuke Matsuhara[2], Satoshi Matsushima[2], Masato Murakami[1]

[1]PeptiDream Inc., Kawasaki, Japan,[2]PDRadiopharma Inc., Sammu, Japan

Introduction: Claudin 18.2 (CLDN18.2) is one of the tight junction proteins essential for maintaining the gastric mucosal barrier, preventing gastric acid leakage through paracellular space. Although CLDN18.2 is physiologically expressed in gastric mucosal cells and restricted localization at tight junction, it is overexpressed and exposed on the surface of cancer cells in several tumor types, including pancreatic, esophageal, and gastric cancers, which indicates CLDN18.2 is attractive molecule for cancer targeting. PD-29875 is a novel macrocyclic peptide targeting CLDN18.2 and can be labeled with radionuclides such as cupper (^{64}Cu) and lutetium (^{177}Lu), which enables tumor-specific PET imaging and therapy. This study reports the theranostic potential of PD-29875 for a pancreatic cancer xenograft model.

Materials and Methods: PD-29875 was discovered using Peptide Discovery Platform System (PDPS), a proprietary screening system of PeptiDream. Binding affinity and CLDN18.2 selectivity of PD-29875 was measured by cell-based assay. For in vivo evaluation, we used a xenograft model in which BxPC3 (PDAC cell line) which overexpressed CLDN18.2 was subcutaneously transplanted into nude mice. To assess biodistribution, ^{64}Cu-PD-29875 or ^{177}Lu-PD-29875 was dosed to BxPC3 xenograft mice, and the percentage of injected dose in tumors and major organs was quantified by cut and count method. To evaluate whether PET imaging can detect tumors, ^{64}Cu-PD-29875 was dosed to BxPC3 xenograft mice and PET scanning was performed. For therapeutic experiment, ^{177}Lu-PD-29875 was dosed to BxPC3 xenograft mice and tumor volume and body weight were measured for 48 days after dosing.

Results: Cell-based assay revealed that ^{64}Cu-PD-29875 exhibit a strong binding to HEK293-hCLDN18.2 OE cell line, but not HEK293-hCLDN18.1 OE and parental HEK293 cell line, demonstrating that PD-29875 is selectively binding to hCLDN18.2. In *in vivo* biodistribution experiment, ^{64}Cu-PD-29875 and ^{177}Lu-PD-29875 showed a strong accumulation in the tumors and were still highly retained in the tumor at 24 hours after dosing, in contrast, accumulation in the normal stomach was markedly lower, suggesting PD-29875 distinguishes physiological and tumor-expressed CLDN18.2. In PET imaging, ^{64}Cu-PD-29875 clearly detected tumors, consistent with the biodistribution data. In therapeutic experiment, ^{177}Lu-PD-29875 was well tolerated and improved mouse overall survival compared with control animals for 62 days after transplantation, which suggests a robust tumor growth inhibition of ^{177}Lu-PD-29875 administration.

Conclusion: PD-29875 has preferable properties for imaging and therapy with radionuclides for theranostic use in CLDN18.2 expressing solid tumors including pancreatic cancer.

#578 ATNM-400 is a novel Actinium-225 antibody radioconjugate with strong efficacy in preclinical models of prostate cancer.

Denis Beckford, Sumit Mukherjee, Debbie Lewis, Jason Li, Dina Yang, Karina Peregrina, Amanda Chin, Le-Cun Xu, Monideepa Roy, **Adeela Kamal**

Actinium Pharmaceuticals, Inc., New York, NY

Introduction: In 2024, an estimated 299,010 new prostate cancer (PCa) cases will be diagnosed in the US, with 35,250 deaths. While PCa initially responds to androgen deprivation therapy (ADT), it often progresses to metastatic castration-resistant prostate cancer (mCRPC), a challenging condition with limited treatment options. Targeted radiotherapies like Pluvicto, a beta-emitting treatment targeting prostate-specific membrane antigen (PSMA), have shown promise but face limitations due to radiation properties and variability in PSMA expression. mCRPC is driven by persistent androgen receptor signaling and other pathways, often mediated by overexpressed cell surface receptors. We designed ATNM-400 against a target highly expressed in mCRPC that has been previously targeted with naked antibodies or antibody drug conjugates but have been ineffective clinically. Here, we tested ATNM-400, a first-in-class actinium-225 (Ac-225) antibody radioconjugate (ARC) in preclinical models of PCa with the hypothesis that targeted delivery of an alpha-emitter would offer stronger therapeutic potential than existing therapies to address a critical unmet need in mCRPC patients.
Methods: ATNM-400 was synthesized by conjugating ATNM400 antibody with p-SCN-Bn-DOTA, followed by radiolabeling with Ac-225. Binding affinity and internalization in PCa cell lines were evaluated using flow cytometry and radioactivity measurement. The biodistribution (BioD), pharmacokinetics (PK), and efficacy of ATNM-400 were tested in preclinical mouse models using 22Rv1 and DU145 PCa cell lines. The potential to overcome Pluvicto resistance in mCRPC was also studied both in vitro and in vivo.
Results: ATNM-400 demonstrated over 98% radiochemical purity and rapid internalization into PCa cells. Treatment of PCa cells with ATNM-400 demonstrated potent cytotoxic effects in a dose-dependent manner. In vivo BioD and PK studies in PCa models showed that ATNM-400 accumulated in tumors for up to 144 hours, with minimal accumulation in normal organs. Small animal SPECT/CT images in mouse xenograft models of PCa showed that Indium-111 radiolabeled ATNM400 selectively accumulated in tumors and cleared from healthy tissues. The radiation dose to organs, extrapolated from mouse dosimetry, suggested an acceptable safety profile. In vivo studies in multiple PCa models (22RV1 and DU145) using a single-dose of ATNM-400, showed a potent tumor growth inhibition of 68.5% at 20 µCi/kg and 99.8% at 40 µCi/kg, with all doses well tolerated. Also, ATNM-400 is being tested in Pluvicto-resistant PCa preclinical models, with results forthcoming.
Conclusions: ATNM-400 showed strong anti-tumor efficacy and a favorable safety profile in preclinical PCa models. These results highlight ATNM-400's potential as a transformative therapeutic option for PCa patients with unmet clinical needs.

#579 A novel nanobody-based radionuclide drug conjugate (RDC) platform through introducing a human serum albumin (HSA) binder by the intelligent ligase-dependent conjugation (iLDC) technology: an innovative and versatile approach for next-generation RDCs with enhanced pharmacokinetics and druggability.

Chong Liu, Sipeng Li, Zengyan Mu, Yajun Sun, Haibo He, Lili Shi, Paul Song, Gang Qin

GeneQuantum Healthcare (Suzhou) Co., Ltd., Suzhou, China

Background: RDC has become an attractive strategy in cancer treatment, but still faces significant drawbacks due to poor tumor penetration and undesirable pharmacokinetics of the most used targeting carriers, such as peptides, small molecules and nanobodies. To address these limitations, we developed a robust and versatile nanobody-based RDC platform, that site-specifically conjugates an small molecular HSA binder into the nanobody-based RDC to improve its druggability by increasing its circulation half-life. The validation of the RDC platform is exemplified by ^{64}Cu/^{177}Lu-NBC-02, a theranostic anti-PSMA nanobody-based RDC.

Method: Using iLDC technology, a diverse and high-quality RDC library was generated by site-specifically conjugating various stable branch linkers containing different HSA binders and a DOTA into multiple anti-PSMA nanobodies with varying affinities, followed by labeling with ^{64}Cu and ^{177}Lu. Following the screening of those RDCs through PET imaging study and in vivo anti-tumor efficacy study, the theranostic anti-PSMA RDC, ^{64}Cu/^{177}Lu-NBC-02, was developed. Subsequently, an IIT study was conducted to confirm the distribution of ^{177}Lu-NBC-02in mCRPC patients.

Results: PET imaging studies performed in the LNCaP CDX model showed enhanced circulation half-life of ^{64}Cu-NBC-02, which resulted in a higher accumulation and retention over time in the tumor with a much higher tumor-to-background ratio. Remarkably, ^{177}Lu-NBC-02 demonstrated superior antitumor efficacy to ^{177}Lu-PSMA-617 (Pluvicto®) with complete tumor regression in all treated mice. In addition, ^{177}Lu-NBC-02 can reduce tumor volume significantly when the tumors became refractory to Pluvicto®. This demonstrated ^{177}Lu-NBC-02's potential to overcome resistance to Pluvicto®. An IIT study in several mCRPC patients injected with a single dose showed that ^{177}Lu- NBC-02 was well tolerated. SPECT images revealed longer blood circulation of ^{177}Lu- NBC-02 with much higher uptake in tumor lesions and lower uptake in salivary glands compared with published data of Pluvicto®.

Conclusion: The novel RDC platform combining iLDC technology with a unique HSA-binding linker-chelator design can generate ^{177}Lu-NBC-02 with an extended circulation half-life, higher tumor uptake and lower salivary gland uptake in both CDX model and mCRPC patients compared to Pluvicto®. The IND enabling of this potential BIC anti-PSMA RDC is underway for the exploration of therapeutic effects on the treatment of mCRPC. Furthermore, the applied strategy provides a promising next-generation platform for future RDC discovery which requires nanobody as a targeting carrier, hence significantly expanding promise beyond the current format.

TUMOR BIOLOGY: Imaging in Animal Models
Poster Session

#58 Subcutaneous tumor volume measurements: The method matters.

Katarzyna Urbańska, Irmina Przydatek, Agnieszka Drzał, Karina Adamowicz, Fabio dell'Aquila, Magdalena Chwalisz, Miłosz Gawenda, **Martyna Sniegocka**

Selvita S.A., Krakow, Poland

In preclinical studies, tumor volumes are important indicators of cancer progression and the response to therapy with selected drug candidates. The most common method of tumor volume assessment, due to its simplicity and cost-effectiveness, is measuring length, width, and height with a caliper, and calculating the volume using the ellipsoid formula. However, many tumors, even if inoculated by an experienced operator, show irregular shapes that deviate from the idealized ellipsoid volume which may lead to over- or underestimation of the actual tumor volume. In such cases, more specialized techniques like TumorImager® using structured light or ultrasound imaging can potentially be a better solution. The purpose of this study was to compare three different methods of tumor measurements: the caliper, the TumorImager®, and ultrasound imaging, and observe any correlation between the volumes obtained by each technique. CT26 and B16F10 cancer cells were inoculated subcutaneously on the flank of 6-week-old male mice (Balb/c and C57Bl6/J, respectively). Tumors were measured on days 8, 15 and 22 after cancer cell inoculation. Ultrasound, caliper and TumorImager® measurements were always performed by the same person to maintain replicability. The last measurements were performed on the day mouse sacrificed, and data collected was compared with the dissected tumor volume. Obtained tumor volumes were analyzed to compare the differences between the techniques used and determine the level of correlation between them. Our results showed that the measurements obtained with a caliper were similar to those based on an ultrasound 3D model. In this case, the biggest differences observed were of tumors significantly deviating from the ellipsoid shape. Measurements acquired with the TumorImager® differed significantly from those taken with calipers and ultrasound imaging. This study emphasizes the importance of choosing the right method of tumor measurement and understanding the differences between them. With an experienced operator, choosing either the caliper or ultrasound imaging is the right choice; the decision should be made based on information that needs to be obtained. Ultrasound imaging is the most informative method since it can be used, for example, to visualize the vascularity of the tumor. However, it has the greatest impact on animal welfare because the animals need to be anesthetized for the procedure. Moreover, it is important to acknowledge that the formula chosen for the study considers the tumor height, which is crucial in the case of tumors growing deeply into the animal's body. The TumorImager® does not record this height dimension and therefore the highest deviations in relation to ultrasound were observed. However, based on a correlation between the techniques deployed. we were able to find constant coefficients enabling the scaling of TumorImager® volumes to the true volume of a tumor.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Theranostics and Radiotheranostics
Poster Session

#580 ^{111}In/^{225}Ac-ABD320, a novel 5T4-targeted radiopharmaceutical with favorable tumor-to-normal tissue biodistribution and single-dose efficacy in preclinical cancer models.

E. S. Melese, E. Cummins, A. Mandel, L. Li, D. Namjoshi, M. Čučuk, N. Menon, B. Harbourne, P. Tao, B. McLeod, T. Osborne, A. Roman, K. Lassueur, A. McDonagh, M. Abrams, A. Judge, L. Janes, **I. Kulić**;
Abdera Therapeutics Inc., South San Francisco, CA

Radiopharmaceutical therapies offer a promising, targeted approach for treating cancers with limited options. The 5T4/trophoblast glycoprotein (encoded by *TPBG)* is an oncofetal cell surface antigen found on various cancer cells, including gastrointestinal, head and neck, and non-small cell lung cancers. 5T4 is associated with cancer stem cell properties, metastatic spread and poor clinical outcomes in cancer patients, while it is minimally expressed or absent in healthy adult tissue. Hence, 5T4 is an ideal target for tumor-directed radiotherapy, particularly since targeting 5T4 has no clear therapeutic benefit without a cytotoxic payload in preclinical models. ABD320, a humanized camelid heavy chain only antibody (VHH) fused to an engineered Fc with altered Fc-gamma and neonatal Fc receptor binding, uses a novel linker-chelator to deliver targeted ^{111}In and ^{225}Ac to 5T4 positive cancer cells. The ABD320 VHHFc binds human 5T4 with high affinity and specificity and is internalized by 5T4-expressing cancer cells. ABD320 is designed to minimize systemic and whole-body exposure through fast blood clearance and minimal normal tissue exposure. Tumor targeting was evaluated using ^{111}In-ABD320 in murine models bearing human xenografts. In the NCI-H1975 non-small cell lung cancer model, a single intravenous administration of ^{111}In-ABD320 was rapidly cleared from blood and normal tissues. Tumor accumulation peaked at 40% ID/g, despite low expression (29,000 receptors/cell) corresponding to high tumor to normal tissue exposure ratios (kidney 6, liver 11) assessed between 1 to 240 hours after ^{111}In-ABD320 administration. ABD320 was well tolerated when used to deliver 12 to 20 kBq of the therapeutic radionuclide ^{225}Ac to 5T4-expressing tumors. ^{225} Ac-ABD320 achieved significant tumor regression, suppression of regrowth and extension of survival. Within-mouse dosimetry estimates a tumor inferred dose of 50 to 80 Gy corresponding to efficacious doses of ^{225} Ac-ABD320. In conclusion, ABD320, which employs a novel VHH-binder and linker-chelator, selectively and preferentially delivers radionuclides to 5T4 expressing tumors, demonstrates potent anticancer activity and is a promising candidate for further development.

#581 Better together: Biparatopic [^{225}Ac]Ac-labeled anti-HER2 antibody-drug radioconjugates using two domain-specific antibodies for the treatment of trastuzumab and T-DM1 resistant HER2-positive breast cancer xenografts.

Jessica Pougoue Ketchemen[1], Alissar Monzer[2], Fabrice Ngoh Njotu[3], Hanan Babeker[2], Emmanuel Nwangele[3], Anjong Florence Tikum[2], Nikita Henning[2], Nava Hassani[2], Alireza Doroudi[2], Humphrey Fonge[1]

[1]CHU de Quebec - Universite Laval, Quebec, QC, Canada,[2]University of Saskatchewan, Saskatoon, SK, Canada,[3]Universite Laval, Quebec, QC, Canada

Purpose: HER2 is amplified and overexpressed in breast cancer (BC) and other cancers where it promotes proliferation and mediates resistance to targeted therapies. A combination of anti-HER2 trastuzumab and pertuzumab is approved for treatment.

Aim: To develop and evaluate a combination antibody-drug radioconjugates (ADRs) [^{225}Ac]Ac-Macropa-trastuzumab-PEG$_6$-DM1 ([^{225}Ac]Ac-Macropa-T-PEG$_6$-DM1) and [^{225}Ac]Ac-Macropa-pertuzumab-PEG$_6$-DM1 ([^{225}Ac]Ac-Macropa-P-PEG$_6$-DM1) against HER2-positive BC.

Experimental Design: Therapeutics [^{225}Ac]Ac-Macropa-T-PEG$_6$-DM1 and [^{225}Ac]Ac-Macropa-P-PEG$_6$-DM1 and their respective imaging pairs [^{89}Zr]Zr-DFO-trastuzumab-PEG$_6$-DM1 and [^{67}Cu]Cu-DOTA-pertuzumab-PEG$_6$-DM1 were developed and characterized. Biodistribution and toxicological evaluation of [^{225}Ac]Ac-Macropa-T-PEG$_6$-DM1 and [^{225}Ac]Ac-Macropa-P-PEG$_6$-DM1 were carried out in healthy female Balb/C mice. Dual microPET/SPECT/CT imaging and biodistribution using [^{89}Zr]Zr-DFO-T-PEG$_6$-DM1 and [^{67}Cu]Cu-DOTA-P-PEG$_6$-DM1 was performed in female athymic Balb/C nude mice bearing HCC1954 and JIMT-1 xenografts. Therapy was carried out using ADCs (T-PEG$_6$-DM1 + P-PEG$_6$-DM1) and ADRs ([^{225}Ac]Ac-Macropa-T-PEG$_6$-DM1 + [^{225}Ac]Ac-Macropa-P-PEG$_6$-DM1 = [^{225}Ac]Ac-Macropa-(T+P)-PEG$_6$-DM1) in athymic Balb/C nude mice bearing trastuzumab-resistant HCC1954 (high HER2 density) and T-DM1/trastuzumab-resistant JIMT-1 (medium HER2 density) tumors.

Results: Internalization in HCC1954 and JIMT-1 cells was HER2 density-dependent, with the highest total red object area values recorded with the ADC combination (T-ADC + P-ADC) compared with the single agents ($p < 0.0001$). Dual microPET/SPECT/CT imaging and biodistribution of [^{89}Zr]Zr-DFO-T-PEG$_6$-DM1 and [^{67}Cu]Cu-DOTA-P-PEG$_6$-DM1 showed very high tumor uptake in both HCC1954 and JIMT-1 xenografts with high tumor-non-target ratios. The combination of ADCs (T-ADC + P-ADC) and ADRs ([^{225}Ac]Ac-Macropa-(T+P)-PEG$_6$-DM1) administered separately in healthy Balb/C mice, 10 days apart was well tolerated biochemically and haematologically for 20 days (d). Compared with the individual agents, there was improved non-target clearance using the combined ADRs [^{225}Ac]Ac-Macropa-(T+P)-PEG$_6$-DM1. In HCC1954-tumor bearing mice, all treatment groups had complete remission (CR) after 29d and 22d for (T-PEG$_6$-DM1 + P-PEG$_6$-DM1) and ADRs ([^{225}Ac]Ac-Macropa-(T+P)-PEG$_6$-DM1) respectively. Interestingly, in JIMT-1 xenografts at 60d post treatment, no CR was observed for the ADCs group whereas in the ADRs group, the average tumor volume was 11.5 ± 4.4 mm^3 with 3/8 CRs.

Conclusion: [^{225}Ac]Ac-Macropa-(T+P)-PEG$_6$-DM1 is more potent than their respective combined ADCs against HER2-positive BC and warrants further evaluation.

#583 Preclinical development of [^{68}Ga]Ga/[^{225}Ac]Ac-FL-031 theranostic pair for the treatment of SSTR2-positive cancers.

Junyu Yang, Taishan Hu, Lanjiao Zhao, Yihu Xie, Juan Zhang, **Fa Liu**

Full-Life Technologies Limited, Shanghai, China

Somatostatin receptor 2 (SSTR2) is frequently overexpressed in solid tumors including neuroendocrine tumors (NETs) and small cell lung cancer (SCLC), making it a promising target for radionuclide drug conjugates (RDC) based cancer imaging and therapy. Gallium-68 and Lutetium-177 labeled somatostatin analogs [^{68}Ga]Ga/[^{177}Lu]Lu-DOTATATE, a theranostic pair, have been approved by FDA for detecting and treating gastroenteropancreatic (GEP)-NETs. Currently, [^{225}Ac]Ac-DOTATATE is under clinical development for the treatment of SSTR2-positive cancers. To further optimize the theranostic profile of SSTR2-targeting RDC, we have applied our proprietary UniRDC^{TM} platform technologies to discover FL-031, a novel and potentially next generation SSTR2-targeting RDC vector. We then evaluated the feasibility of PET/CT imaging with [^{68}Ga]Ga-FL-031, as well as the *in vivo* biodistribution and efficacy profiles of [^{225}Ac]Ac-FL-031 in SSTR2^{+} xenograft mouse models. The radiochemical purity (RCP) of radiolabeled FL-031 was determined by radio high-performance liquid chromatography (radio-HPLC). FL-031 was successfully labeled with ^{68}Ga and ^{225}Ac, achieving an RCP of over 98% and specific activities of 18.5 MBq/nmol and 185 kBq/nmol, respectively. Subsequently, PET/CT imaging and *in vivo* biodistribution as well as efficacy was conducted in SSTR2-expressing SCLC and NET tumor-xenograft models. After administration of 3.7 MBq [^{68}Ga]Ga -FL-031 in mice implanted with SCLC-21H SCLC xenografts, PET/CT images demonstrated intense tumor uptake which was significantly higher than in normal organs at 1- and 3-hour post-dosing. In the *in vivo* biodistribution study, [^{225}Ac]Ac-FL-031 showed high and durable tumor uptake in mice implanted with AR42J NET xenografts with fast clearance in normal organs. Importantly, in SSTR2^{+} xenograft models, [^{225}Ac]Ac-FL-031 exhibited significant anti-tumor activity at dose levels of 37 kBq or lower without notable changes in body weight and hematology parameters. In summary, [^{68}Ga]Ga-FL-031 PEC/CT proved suitable in detecting SSTR2-positive tumors in preclinical models. [^{225}Ac]Ac-FL-031 demonstrated a promising *in vivo* biodistribution profile and anti-tumor activity in SSTR2-expressing xenograft models. In conclusion, our data suggest that [^{68}Ga]Ga/[^{225}Ac]Ac-FL-031 is a promising theranostic pair for further development as a diagnostic imaging and RDC therapy for SSTR2-positive cancers.

#584 Preclinical efficacy and safety of [^{161}Tb]Tb-labeled anti-nectin-4 radioimmunoconjugate as theranostic against triple-negative breast cancer (TNBC) and non-small cell lung cancer (NSCLC).

Fabrice Ngoh Njotu[1], Hanan Babeker[2], Jessica Pougoue Ketchemen[1], Emmanuel Nwangele[1], Anjong Tikum[2], Nikita Henning[2], He Dong[2], Nava Hassani[2], Alissar Monzer[2], Dede Api Fon[1], Chrysantus Njobinkir Bimela[2], Therese Mercado[2], Franco Vizeacoumar[2], Dennis Elema[3], Michiel Van de Voorde[3], Maarten Ooms[3], Maruti Chandra Uppalapati[2], Humphrey Fonge[1]

[1]Pharmacy, Universite Laval, Quebec, QC, Canada,[2]University of Saskatchewan, Saskatoon, SK, Canada,[3]SCK CEN- Belgian Nuclear Research Center, Mol, Belgium

Purpose: Targeted radioligand therapy (RLT) and radioimmunotherapy (RIT) have emerged as effective and safe treatment modalities for metastatic cancers. Nectin-4 is a Ca^{2+}-independent immunoglobulin-like cell adhesion molecule. Aberrant expression of Nectin-4 is observed in many cancers notably TNBC and NSCLC. No RLT/RIT is approved/in advanced clinical development against TNBC and NSCLC. For the first time, we report the efficacy and safety of ^{161}Tb-labeled anti-Nectin-4 antibody ([^{161}Tb]Tb-DOTA-N4MU01) against Nectin-4 positive TNBC and NSCLC mouse models.

Procedures: Nectin-4-expressing mouse syngeneic cell lines for TNBC (4T1.$_{Nectin-4}$ and E0771.$_{Nectin-4}$) and NSCLC (LLC.$_{Nectin-4}$ and CMT167.$_{Nectin-4}$) were obtained by stable transduction or transfection. Nectin-4 expression and binding affinity of immunoconjugates were assessed by flow cytometry and radioligand binding assays, with internalization studied using live-cell imaging. Pharmacokinetics and safety of the radioligand were evaluated in healthy female Balb/C mice, with tumor uptake assessed by SPECT/CT and biodistribution in tumor-bearing mice. The efficacy of the radioligand was assessed in mouse xenograft models of Nectin-4-expressing human cell line, MDA-MB468, and mouse syngeneic cell lines 4T1.$_{Nectin-4}$ (immune checkpoint blockade therapy (ICBT) resistant), E0771.$_{Nectin-4}$, CMT167.$_{Nectin-4}$, and LLC.$_{Nectin-4}$.

Results: Nectin-4 expression on syngeneic cell lines was high. DOTA conjugation did not affect the internalization of N4MU01 *($p > 0.999$*) while immunoconjugates retained strong binding affinities to human Nectin-4 (≤ 11 nM). ^{161}Tb was stably chelated to DOTA-N4MU01 with yield and purity > 95 %. The clearance half-life of [^{161}Tb]Tb-DOTA-N4MU01 was 120 ± 20 h while a dose of 2x 5 MBq administered intravenously, and 7 d apart was well tolerated over 28 d. Tumor targeting was specific with tumor-to-muscle uptake ratios of 7.7 and 6.7 for CMT167.$_{Nectin-4}$ and 4T1.$_{Nectin-4}$ at 24 h post-injection. In 4T1.$_{Nectin-4}$ model, single dose radioligand at 2.5 MBq and 5 MBq and a repeated dose at 2x 5 MBq showed significant dose-dependent anti-tumor efficacy and survival (*$p \leq 0.0021$*) compared with saline and N4MU01 (25 µg), with 2x 5MBq of radioligand producing complete remission in 20 % of mice. Anti-PD-L1 ICBT had no anti-tumor effect (*$p = 1166$*) on this model. A dose of 2x 5 MBq of radioligand resulted in significant anti-tumor effects and survival compared with saline and N4MU01 (25 µg), in aggressive E0771.$_{Nectin-4}$ (*$p \leq 0.0316$*), CMT167.$_{Nectin-4}$ (*$p \leq 0.0236$*), LLC.$_{Nectin-4}$ (*$p \leq 0.0097$*), and MDA-MB-468 (*$p \leq 0.0.0017$*) xenograft models.

Conclusions: Our findings reveal the clinical potential of [^{161}Tb]Tb-DOTA-N4MU01 as an effective and safe theranostic against Nectin-4-expressing TNBC and NSCLC, even for patients who do not respond to ICBT

#585 Utilizing CAR T cell-derived exosomes for radiotheranostic applications.

Sarah Qureshy[1], Leah Gajecki[2], Darren Veach[2], Sang Gyu Lee[2], Irina Matei[3], Lukas Carter[4], David Lyden[3], David Scheinberg[1], Simone Krebs[5]

[1]Molecular Pharmacology Program, Memorial Sloan Kettering Cancer Center, New York, NY,[2]Radiochemistry and Imaging Sciences Service, Department of Radiology, Memorial Sloan Kettering Cancer Center, New York, NY,[3]Departments of Pediatrics, Cell and Developmental Biology, Drukier Institute for Children's Health, Weill Cornell Medicine, New York, NY,[4]Department of Medical Physics, Memorial Sloan Kettering Cancer Center, New York, NY,[5]Molecular Imaging and Therapy Service, Department of Radiology, Memorial Sloan Kettering Cancer Center, New York, NY

Chimeric antigen receptor (CAR) T cell therapy is limited by antigen escape, poor solid tumor penetration, and an off-tumor toxicity profile. CAR T cell-derived exosomes have been shown to express the parental CAR construct, allowing tumor targeting but limited inherent anti-tumor effects alone. Further engineering CAR T cells to modify their exosome cargo, such as to bind anti-tumor drugs or radionuclides, may allow for the generation of novel targeted delivery vehicles for cancer therapy. We previously engineered humanized C825 (huC825), an anti-metal-DOTA-hapten single chain variable fragment (scfv), into multiple different constructs, one of which is the 4h11 CAR against MUC16/Cancer Antigen 125, developed by Brenjens lab at Memorial Sloan Kettering Cancer Center. We showed that huC825 allows for theranostic potential based on high-affinity binding to various chelated radionuclides, such as for alpha or beta particle-based radiotherapy or diagnostic imaging. We derived exosomes from 4h11 and huC825-4h11 CAR T cells, as well as untransduced T cells, and characterized them for CAR and huC825 expression using Western blot and radio thin layer chromatography (radio-TLC), respectively, as well as with direct stochastic optical reconstruction microscopy (dSTORM). We performed binding and cytotoxicity assays in vitro after radiolabeling and purifying the exosomes with size-exclusion chromatography (SEC), or using a pretargeted approach, with various chelated radiotracers. 4h11 and huC825-4h11 CAR T cell exosomes were reliably manufactured from parental CAR T cells with high transduction rates (80-95%). Western blot using CD81 and ALIX exosomal markers illustrates that 4h11 and huC825-4h11 CAR T cell exosomes and their parental CAR T cells are positive for the 4h11 CAR. dSTORM imaging shows huC825-4h11 exosomes are positive for both 4h11 and huC825. Radio-TLC illustrates that radionuclides chelated by Y-aminobenzyl-1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid, further referred to as aminobenzyl-DOTA (ABD), such as [^{161}Tb]Tb-ABD (therapeutic metal) or [^{177}Lu]Lu-ABD (diagnostic and therapeutic), exhibit significant binding to huC825-4h11 CAR T cell exosomes compared to controls. We optimized a method for radiolabeling huC825-4h11 exosomes and purifying the product with SEC using various DOTA-chelated radionuclides. In vitro radiotracer binding assays using SKOV3 human ovarian cancer cells overexpressing MUC16 illustrate that only huC825-4h11 CAR T cell-derived exosomes selectively bind both MUC16-expressing tumor cells and DOTA-chelated radionuclides. Our work illustrates the potential of huC825-4h11 CAR T cell-derived exosomes as delivery vehicles for radionuclides for imaging or targeted therapy. Their 50-160 nm size may allow better solid tumor penetration in vivo than current cellular treatment modalities. Future directions include in vivo imaging and radiotherapy studies.

#586 Combining advanced peptide screening methods to identify candidates for PDC/RDC/POC development.

Weiliang Xu, Jin Zhang

PepLib, Metuchen, NJ

Peptide-based therapeutics, including peptide-drug conjugates (PDCs), peptide-radionuclide conjugates (RDCs), and peptide oligonucleotides (POCs), are gaining recognition as highly promising targeted treatment options due to their exceptional specificity, low toxicity, and flexible pharmacokinetic profiles. To expedite the identification of effective peptide candidates, we have developed an advanced peptide discovery platform that combines Peptide Information Compression Technology (PICT), Disulfide-Rich Peptides (DRPs) phage display, animal toxin libraries, nano macrocyclic peptides, and virtual peptide libraries. This cutting-edge platform efficiently discovers multicyclic peptide hits with antibody-like binding affinity (sub-nM to pM), enhanced serum stability (lasting from hours to days), and bi-functional properties optimized to engage multiple receptor targets within a six-month timeframe. Our integrated screening approach employs high-throughput techniques to swiftly identify peptide leads that meet the stringent criteria required for PDC, RDC, and POC development. We have successfully discovered peptide leads with high binding affinity and endocytosis on over 40 targets, including tumor biomarkers such as FAP, Nectin-4, Trop2, GRPR, FGFR2, GPC3 and DLL3. The platform's combination of diverse peptide libraries and sophisticated computational modeling enhances the selection of candidates with superior binding affinities, structural stability, and functional adaptability. By leveraging these advanced screening technologies, we address the limitations of traditional peptide discovery methods, shortening development timelines and reducing the reliance on extensive medicinal chemistry optimization. Our findings illustrate that this platform significantly streamlines the discovery process for peptide-based therapeutics, establishing a powerful pipeline for the development of next-generation bi-functional peptides with broad applications in oncology, inflammation, and other therapeutic areas.

#588 Preclinical evaluation of a novel ^{212}Pb-based, PSMA-targeted radioligand, ^{212}Pb-ADVC001, for prostate cancer treatment.

Melissa E. Monterosso, Feifei Liu, Didier Boucher, Stelle Shakti, Kwong Ching Li, Aimee Horsfall, Saawan Kumar, Johannes Koehbach, Kevin Kuan, William Tieu, Stephen Rose, Anna Karmann, Gary Li, Simon Puttick, Thomas Kryza

AdvanCell Isotopes Pty Limited, Sydney, Australia

Introduction: PSMA-targeted radioligand therapy (RLT) with lutetium-177 (^{177}Lu), a beta-emitter, has been clinically proven to be efficacious for the treatment of prostate cancer (PC). Although emerging clinical data indicate that better efficacy can be achieved by alpha-emitters, the toxicities associated with actinium-225 (^{225}Ac) may limit its broad application especially in early lines of treatment. Lead-212 (^{212}Pb), is a promising isotope for alpha therapy with a high linear energy transfer, a half-life of 10.6 hours, and a simple decay scheme. These properties promote efficient targeting of PSMA+ PC cells while limiting off-target toxicities. ^{212}Pb-ADVC001 is a novel ^{212}Pb-based PSMA-targeted RLT currently in Phase Ib/IIa clinical trial (NCT05720130) for patients with metastatic castration-resistant prostate cancer (mCRPC).

Methods: The affinity of ADVC001 and ^{212}Pb-ADVC001 to PSMA was determined by SPR and radioligand-binding assays in PSMA+ PC cell lines. Cytotoxic activity was measured by cell viability and clonogenic assays. *In vivo* biodistribution and anti-tumor efficacy studies were performed in human-derived PC-bearing mice. The intrinsic molecular and cellular mechanisms associated with ^{212}Pb-ADVC001 mediated cell-death were assessed using transcriptomics and proteomics.

Results: ADVC001 binds human, mouse and rat PSMA with comparable nanomolar activities, and efficiently internalizes upon binding to PSMA+ PC cells. *In vitro*, ^{212}Pb-ADVC001 showed specific and potent cytotoxic activity against multiple PSMA+ human PC cell lines, while sparing PSMA- cells. *In vivo* biodistribution of ^{212}Pb-ADVC001 showed tumor uptake at 19.6, 20.9, 9.4 and 5.4 %ID/g at 1, 3, 24 and 48 h, respectively, with retention out to 72 h. High tumor uptake and fast renal clearance resulted in favorable tumor/kidney ratios. Minimal uptake was observed in other normal tissues. Single administration of ^{212}Pb-ADVC001 in PSMA+ PC xenografts led to dose-dependent anti-tumor response and significant survival benefit. Transcriptomic analysis of *in vitro* treated PC cells and proteomic analysis of tumors harvested longitudinally revealed multiple mechanisms of action involving DNA damage, cell cycle arrest and cell death, and immune response modulation.

Conclusion: Preclinical data demonstrated the potential of ^{212}Pb-ADVC001 for the treatment of patients with PSMA+ prostate cancer. Transcriptomic and proteomic analyses indicate multiple mechanisms of action collectively resulting in effective cancer therapy.

TUMOR BIOLOGY: Imaging in Animal Models
Poster Session

#59 CD4-targeted peptides: Discovery, *in vitro* characterization, and preclinical PET tracer evaluation in a humanized CD4 knock-in mouse model.

Anders Wilgaard Sinkjaer[1], Ida Vang Andersen[2], Anne Skovsbo Clausen[1], Line Bruhn Schneider Knudsen[1], Camilla Stavnsbjerg[1], Andreas Kjaer[1]

[1]Department of Clinical Physiology and Nuclear Medicine & Department of Biomedical Sciences, Copenhagen University Hospital - Rigshospitalet & University of Copenhagen, Copenhagen, Denmark,[2]University of Copenhagen, Copenhagen, Denmark

Introduction: Immune checkpoint inhibitors (ICIs) have revolutionized cancer treatment, but their efficacy can vary greatly among patients. This variation may be due to the level of immune infiltration in the tumor microenvironment. Immune surveillance, particularly of tumor-infiltrating leukocytes like CD4+ T-cells, can help predict ICI efficacy. This work presents the discovery and preclinical characterization of two CD4-targeted peptide PET tracers.
Methods: Peptide discovery was based on a peptide phage display biopanning, and selected peptides were commercially synthesized (>95% purity). The binding of all peptides to human CD4 (hCD4) was characterized using surface plasmon resonance (SPR) on a Biacore X100. Peptides exhibiting binding to hCD4 were also evaluated for binding to a negative control protein and select peptides to murine CD4 (mCD4). All SPR experiments were analyzed via reference and blank subtraction, and fitted to a 1:1 Langmuir model. The peptides' ability to interact with hCD4 was evaluated using a Sypro Orange thermal shift assay (TSA) on an MxPro3005 qPCR instrument, followed by determination of the midpoint temperature (T_m) via a Boltzmann fit. Peptides 11 (P11) and 17 (P17) underwent DOTA-conjugation (>95% purity) and radiolabeling with copper-64 (^{64}Cu) (>95% radiochemical purity) for *in vivo* PET/CT imaging of C57BL/6J hCD4 knock-in (KI) and control (wild-type) models on a Siemens Inveon PET/CT scanner. The mice were imaged at 1 and 3 hours post-injection (p.i.), and the PET signal of select organs was decay-corrected and quantified as percentage injected dose per gram (%ID/g). The spleen was the organ of interest due to its relatively high population of CD4+ T-cells.
Results: Twenty peptides were synthesized and tested on SPR. P11 and P17 showed selective hCD4 binding on SPR with affinities of K_D=0.96 µM and 2.39 µM, respectively. P11 induced a significant T_m shift (57.3±0.28°C, p<0.0001) and [^{64}Cu]Cu-DOTA-P11 PET/CT imaging showed high spleen uptake at 1 and 3 hours p.i. in both models. P11 binding to mCD4 was estimated to K_D =26.6 nM. [^{64}Cu]Cu-DOTA-P17 PET/CT imaging showed significantly higher spleen uptake (0.71±0.10 %ID/g, p<0.05) at 1 hour p.i. in the hCD4 KI model, but not at later time-points.
Conclusions: P11 bound hCD4 with nanomolar affinity, and a significant T_m increase suggests hCD4-P11 interaction. PET/CT imaging with [^{64}Cu]Cu-DOTA-P11 showed high spleen uptake in both models. This, and P11's nanomolar affinity towards mCD4 indicates cross-reactivity. For future work [^{64}Cu]Cu-DOTA-P11 needs to be evaluated in a different animal model not reliant on differences between hCD4 and mCD4, e.g., in a high and low expressing hCD4 xenograft flank tumor model. P17 bound hCD4 with low micromolar affinity. PET/CT imaging with [^{64}Cu]Cu-DOTA-P17 showed significantly higher uptake in the hCD4 KI spleen at 1 hour.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Theranostics and Radiotheranostics
Poster Session

#590 Prostate specific membrane antigen targeted PRMT5 inhibition re-sensitizes cancer cells to radiation therapies.

Sagnik Sengupta, **Sudarsan Reddy Kasireddy**, Ramesh Mukkamala, Madduri Srinivasarao, Philip S. Low

Chemistry, Purdue University, West Lafayette, IN

Prostate cancer (PCa) is the second most common cause of cancer-related deaths in US men. Although treatment with the targeted radioligand therapy, ^{177}Lu-PSMA-617 (Pluvicto®) has significantly improved outcomes of patients who progress on androgen deprivation therapy, development of resistance to Pluvicto via upregulation of DNA damage repair enzymes has remained a major impediment to achievement of complete responses. To prevent emergence of this resistance, DNA damage repair inhibitors (DDRIs) have been explored with some success, however, their efficacies are limited by significant off-target toxicities. To avoid these toxicities, we have designed prostate specific membrane antigen (PSMA)-targeted DDRIs comprised of the same targeting ligand used in Pluvicto, but linked to a DDRI instead of a radionuclide. In the studies summarized here, we conjugate an inhibitor of protein arginine methyltransferase 5 (PRMT5), i.e. a master regulator of DNA damage repair that is overexpressed in response to ionizing radiation, to the small ligand PSMA-617, and examine its impact on the treatment of PCa tumor bearing mice with ^{177}Lu-Pluvicto.
Briefly, a releasable PSMA-targeted PRMT5 inhibitor (PSMA-PRMT5i) was synthesized and tested for its binding affinity and efficacy in PSMA-expressing LNCaP cells. PSMA-PRMT5i was found to bind PSMA on LNCaP cells in culture with high affinity (K_d = 1.5 nM) and significantly reduce their survival following exposure to 2Gy of beam radiation. Quantitative analysis of the radiation-induced DNA double-strand breaks showed that PSMA-PRMT5i increased the number of DNA double-strand breaks by a factor of two, even at the very low concentration of 1 nM, whereas non-targeted PRMT5 inhibitor had no impact at the same concentration. Finally, analysis of antitumor activity in LNCaP tumor bearing mice demonstrated that the targeted PSMA-PRMT5i reduced tumor growth by 90% when used in combination with ^{177}Lu-Pluvicto, whereas ^{177}Lu-Pluvicto alone suppressed tumor growth by only 50%. Not surprisingly, co-administration of PSMA-PRMT5i similarly increased the efficacy of beam radiation from 50% to 90% tumor suppression at the same concentration (10 nmol/mouse/day). Because no therapeutic benefit could be detected in mice treated with the same concentration of nontargeted PRMT5i, and since no loss in animal body weight was observed in any of the treatment groups, we conclude that low dose PSMA-PRMT5i can significantly improve radiation therapies outcomes with little or no accompanying toxicity.

#591 Exploration of immune effects of [^{177}Lu]Lu-PSMA therapy and rationale for combination with immunotherapy.

Y.-H. Dai, G. Dias, C. Kramer, K. A. Vallis;
University of Oxford, Oxford, United Kingdom

[^{177}Lu]Lu-PSMA therapy has emerged as a promising option for metastatic prostate cancer (PCa). Many ongoing trials are exploring the combination of [^{177}Lu]Lu-PSMA therapy with immunotherapy, highlighting the urgent need to identify potential biomarkers for this therapeutic approach. This study aims to investigate the immune effects of radionuclide therapy using [^{177}Lu]Lu-PSMA-I&T in PCa.We used the RM1-PGLS cell line (a murine PCa cell line with a castration-resistant phenotype, stably transfected with human PSMA) to establish a syngeneic mouse model. RM1-PGLS tumor-bearing male C57BL/6 mice were treated with either 60 MBq of [^{177}Lu]Lu-PSMA-I&T or 5 Gy of external beam radiotherapy (EBRT). Tumors were harvested on day 7 following treatment, and downstream profiling of immune responses was conducted using the NanoString Pancancer Immune panel.In vivo, immune responses were significantly activated on day 7, with 109 differentially expressed genes (DEGs) upregulated after [^{177}Lu]Lu-PSMA-I&T therapy (accounting for 98.2% of all DEGs). Single sample Gene Set Enrichment Analysis revealed that 24 immune-related pathways (curated from MSigDB) were activated compared to the untreated control. Further analysis also showed significantly higher estimated infiltrations of macrophages, neutrophils, and CD56dim NK cells.Among the observed changes, Cxcl10 emerged as the most significantly upregulated gene for both therapies ([^{177}Lu]Lu-PSMA-I&T: log2 fold change [FC] = 2.1, adjusted p = 0.0059; EBRT: log2FC = 2.3, adjusted p = 0.0025). Protein-protein interaction analysis revealed that Cxcl10 ranked among the top three genes with the highest number of interactions with other genes ([^{177}Lu]Lu-PSMA-I&T: 71 edges, top 1; EBRT: 124 edges, top 3), indicating its critical role in the immune response.To investigate whether RM1-PGLS cells can express and release Cxcl10, we treated the cells with 1.2 MBq/ml of [^{177}Lu]Lu-PSMA-I&T (equivalent to 5 Gy EBRT in vitro) and incubated them for 7 days, with the medium replaced every 2 days. Immunofluorescence images demonstrated a significant increase in Cxcl10 gene expression on day 7 ($p < 0.001$), while the Cxcl10 protein release peaked on day 2 ($p < 0.05$) but declined by days 4 and 6, showing no significant difference from baseline. These results suggest that RM1-PGLS cells can express and release Cxcl10 independent of immune cell involvement.Taken together, this study demonstrates that [^{177}Lu]Lu-PSMA-I&T therapy induces a significant immune response characterized by Cxcl10 upregulation and activation of multiple immune pathways. Further studies are warranted to validate the therapeutic potential of these combinations in enhancing anti-tumor immunity and improving clinical outcomes for patients with PCa.

#592 Theranostic ^{68}Ga/^{131}I compounds for PSMA targeted radioligand therapy.

Lars Hvass[1], Marcel Martin[2], Anne Clausen[1], Panagiota Iakovidou[2], Marius Muller[2], Umberto Battisti[2], Vladimir Shalgunov[1], Agustin Lenero[2], Nadia Pedersen[2], Sophie Stotz[1], Matthias Herth[2], Andreas Kjaer[1]

[1]University of Copenhagen and Rigshospitalet, Copenhagen, Denmark,[2]University of Copenhagen, Copenhagen, Denmark

Introduction: Prostate cancer is the most common cancer in men and continues to be a common cause of cancer related death. With progression to castration resistant metastatic prostate cancer, treatment options are scarce. Radioligand therapy has emerged as a successful treatment for these patients, significantly prolonging survival when other treatments fail. Thus far, approved PSMA targeting radiopharmaceuticals mainly use DOTA-based precursors to chelate gallium-68 for diagnosis and lutetium-177 for therapy. Here we suggest a gallium-68/iodine-131 theranostic pair as an alternative radiohalogenated treatment option.

Method: Four compounds based on a common urea PSMA binding motif with additional peptidic linkers were synthesized. These compounds are suitable for both radiohalogenation as well as radiometal labeling via DOTA-chelator(s). For comparative screening, all compounds were labelled with ^{68}Ga in >95% radiochemical purity. Subsequently, BALB/c nude mice carrying LNCaP xenografts were intravenously injected with 8-13 MBq of compound [1]-[4]. Mice were PET/CT scanned one and two hours after injection. After final scan, mice were euthanized and organs harvested for ex vivo biodistribution. Based on tumor and off-target accumulation, a lead compound is selected for iodine-131 labelling by dematallation to assess distribution and dosimetry.

Results: All compounds screened demonstrated favorable tumor uptake and fast clearance. Ex vivo Tumor, blood and kidney accumulation is presented in Table 1, alongside tumor- (T/B) and tumor-to-kidney (T/K) ratios. Notably, compound 3 demonstrated high tumor uptake similar to PSMA-617, as well as low renal retention and faster blood clearance.

Conclusion: Here we tested four different compounds in a mouse model of prostate cancer and identified compound 3 as lead candidate for further translational investigation using iodine-131.

	^{68}Ga[1]	^{68}Ga[2]	^{68}Ga[3]	^{68}Ga[4]	[^{68}Ga]Ga-PSMA617
Tumor %ID/g	6.7	4.9	7.7	6.1	7.4
Blood %ID/g	0.2	2.8	0.2	1.1	0.4
Kidney %ID/g	19.9	22.7	3.8	22.6	2.0
T/B ratio	43.0	1.7	30.8	5.6	16.9
T/K ratio	0.3	0.2	2.0	0.3	3.6

Table 1: %ID/g and T/B and T/K in tumor, blood and kidney of compound 1-4 as well as PSMA-617.

#593 c-MET targeted theranostic Raptamers for early diagnosis of lung cancer.

Sougata Dey, Uksha Saini, Stephanie Vega, Secondra L. Holmes, Nancy Ward, Atul Varadhachary, Dev Chatterjee

Fannin, Houston, TX

Histological screening of tumor biopsies is the gold standard for identifying actionable biomarkers, but is invasive and non-representative, especially for patients with advanced or inaccessible tumors. Imaging with targeted positron emission tomography (PET) may provide a more complete view of the cancer and guide therapy. PET imaging, with a targeting ligand, is preferred for evaluating tumor avidity of new radiolabeled molecular entities. Raptamers (aptamers with amino acid-like functionality) combine attractive attributes of small molecules and monoclonal antibodies (mAbs) in targeted delivery of radionuclides. Like mAbs, Raptamers can provide low nM to pM binding affinities with very low off-rates. Unlike mAbs, they are smaller (with a potentially broader volume of distribution), are manufactured synthetically (less expensive and more consistency), and are more stable. Radionuclide-labeled Raptamers (Radiomers®) can be used for both imaging and therapeutics. We are developing a Radiomer to image, and potentially treat, c-Met over-expressing cancers with minimal off-target effects. c-Met protein is over-expressed in non-small cell lung cancer (NSCLC); with up to 70% in adenocarcinomas (ADCs) and 40% in squamous cell carcinomas (SCCs). Overexpression promotes tumor growth, angiogenesis, invasion and metastasis. The expression level of c-Met correlates with response to c-Met-targeted therapy. The discovery campaign identified multiple high-affinity Raptamers for c-Met, confirmed using biolayer interferometry, and a lead candidate was selected. For surrogate studies, we conjugated Raptamers with a fluorescent tag (Cy5) at the 5’ end. Cy5-Raptamers showed internalization into c-Met+ cells and accumulated, *in vivo*, in c-Met+ tumors in mice. Additional modifications on the 3’ end improved the biodistribution profile. We then optimized click chemistry reaction conditions to conjugate a DOTA chelator at the 5’ end of Raptamers with a high degree of reproducibility and purity. Stability tests confirmed *in vitro* integrity of the DOTA conjugates, followed by successful radiolabeling trials with ^{86}Ga and ^{177}Lu. The tests showed higher loading efficiency of Lu over Ga, but we were able to proceed with further studies using both isotopes. ^{177}Lu-Radiomers demonstrated cell surface binding and rapid clearance in mice. ^{177}Lu-Radiomers also showed promising biodistribution in tumor bearing mouse models. hese studies provide the first proof-of-concept data of Radiomers in targeted molecular imaging of cancer. Molecular imaging can potentially allow detection and monitoring of lung cancers with abnormal alterations of c-Met in real time. Also, Radiomers can focus radioactive decay to the vicinity of or within the tumor cells, leading to therapeutic options. In this way, Radiomers can potentially target any cell-surface neo-antigen and open up new diagnostic and therapeutic paradigms in cancer.

#594 Lintuzumab-Ac225 has potent mutation agnostic antileukemic activity in preclinical models of AML.

Amanda S. Chin[1], Rubin Jiao[2], Kevin J. H. Allen[2], Jason Li[1], Madhuri Vusirikala[1], Avinash Desai[1], Le-Cun Xu[1], Monideepa Roy[1], Mackenzie Malo[2], Denis Beckford-Vera[1], Ekaterina Dadachova[2], Adeela Kamal[1]

[1]Actinium Pharmaceuticals, Inc., New York, NY,[2]University of Saskatchewan, Saskatoon, SK, Canada

Introduction: Acute myeloid leukemia (AML) is a complex and aggressive blood cancer with a poor prognosis, driven by genetic mutations that complicate treatment and often lead to relapse. Lintuzumab-Ac225 is a promising investigational antibody radio-conjugate that targets CD33. The payload, actinium-225 (Ac-225) is an alpha particle-emitting radionuclide that causes lethal DNA damage. In clinical trials, lintuzumab-Ac225 had positive responses when combined with CLAG-M chemotherapy in relapsed/refractory AML patients, including those with high-risk features like venetoclax resistance and TP53 mutations. Here we tested the hypothesis that lintuzumab-Ac225 has anti-leukemic activity in AML cells regardless of mutations (FLT3, TP53, NPM1, and KMT2A). Also, we explored its potential as a single agent or in combination with other therapies to enhance the treatment response for AML.

Methods: Lintuzumab-Ac225 was made by conjugating lintuzumab with *p*-SCN-Bn-DOTA and subsequently radiolabeled with Ac-225. A flow cytometry viability assay was performed in vitro with test drugs to assess the response across genetically altered human AML cell lines (mutant *FLT3*, *KMT2A*: MV-4-11, MOLM-13 cells, mutant *NPM1*: OCI-AML3, and mutant *TP53*: Kasumi-1, HL-60 cells). FLT3 inhibitors (gilteritinib, quizartinib), KMT2A inhibitors (revumenib, ziftomenib) and azacitidine were evaluated as single agents and in combination with Lintuzumab-Ac225. The modulation of DNA damage markers in response to lintuzumab-Ac225 was analyzed by Western blots. The effect of lintuzumab-Ac225 on AML growth in vivo was investigated in subcutaneous MV-4-11 leukemia xenograft models.

Results: Treatment with lintuzumab-Ac225 showed a potent, dose-dependent decrease in leukemia cell viability in vitro, across all tested AML models, regardless of mutation status (FLT3, KMT2A, NPM1, TP53). IC50 values ranged from 0.5 to 5 nCi/mL, showing that lintuzumab-Ac225 induces cell death independent of these mutations. The treatment also triggered DNA damage and apoptosis in mutant cells, with increased phosphorylation of H2A.X and p21. While FLT3 inhibitors, KMT2A inhibitors, and azacitidine showed variable single-agent efficacy in mutation-carrying AML cells, combining these with lintuzumab-Ac225 in vitro enhanced their effects ($p<0.01$). In the MV-4-11 in vivo AML model, lintuzumab-Ac225 combined with FLT3 or KMT2A inhibitors significantly reduced tumor growth compared to monotherapy ($p<0.002$).

Conclusions: Lintuzumab-Ac225 shows broad anti-leukemic activity in AML cell lines, in a mutation (FLT3, KMT2A, NPM1, TP53) agnostic manner. It improves AML control in high-risk cases and enhances response durability when combined with standard of care treatments. These findings support its potential as a backbone therapy for relapsed/refractory AML, warranting further clinical evaluation.

#595 CMOS sensor-based micro-dosimetry for radiation theranostics: A novel approach.

Rao V. Papineni[1], Prem Saganti[2], Huichen Wang[2], Rahman M[2]

[1]University of Kansas Medical Center, Branford, CT,[2]Prarie View A & M University, Prarie View, TX

CMOS Sensor based micro-dosimetry allowing measuring radiation tracks at micron level by the track structure detector (TSD) have been established by us with 1.67 micron / pixel resolution. In radiation theranostics, individual organ dosimetry is critical for predicting absorbed doses to normal organs and target tissue in terms of therapeutic efficacy and toxicity reduction with radiopharmaceutical therapy. We designed and applied our well characterized micro dosimetry system utilizing a CMOS (complementary metal oxide)-based radiation Track Structure Detector (TSD) device to analyze radiation quality and quantity from different forms of radiation and radiopharmaceutical drugs. The TSD system allowed capturing and analyzing heavy ion tracks at cellular dimensions with a 1.67 microns per pixel resolution and more than 10 M pixels on a 0.6 cm x 0.4 cm sensor. Further towards individualized radiopharmaceutical treated patients, we propose Integrated Wearable Micro Dosimetry for Remote Monitoring-VASTRA. Here, we demonstrate the capability to objectively detect, measure, and spatially resolve the imaging radiation activity of radiopharmaceutical therapeutic radionuclides including 18F-FDG, TC99m and validates the VASTRA in a smart phantom. Consequently, the approach is capable to measure patient-specific temporal activity data in radiopharmaceutical therapy. As an integrated ergonomical wearable dosimetry, VASTRA in Post cancer treatment, has unique opportunities and a profound impact on the quality assurance in the cancer treatment.

CHEMISTRY: Natural Products
Poster Session

#599 Oligomeric proanthocyanidins overcome Lenvatinib resistance by targeting ITGA3 and EGFR-AKT pathways in hepatocellular carcinoma.

Takayuki Noma[1], Yuan Li[1], Yuma Wada[2], Katsuki Miyazaki[2], Yuji Morine[2], Mitsuo Shimada[2], Ajay Goel[1]

[1]Molecular Diagnostics and Experimental Therapeutics, Beckman Research Institute of The City of Hope, Monrovia, CA,[2]Department of Surgery, Tokushima University, Tokushima, Japan

Background: Hepatocellular carcinoma (HCC) is the third most common cancer worldwide and is often diagnosed at an advanced stage, with chemotherapy often being the treatment of choice. Lenvatinib, a multi-targeted tyrosine kinase inhibitor, is widely used as a first-line therapy for advanced HCC following the REFLECT trial, demonstrating its non-inferiority to Sorafenib in overall survival. Additionally, combination therapies with immune checkpoint inhibitors or transarterial chemoembolization have shown promising results, with clinical studies indicating improved overall survival compared to monotherapies. However, frequent resistance to Lenvatinib significantly limits its clinical efficacy, necessitating novel strategies to address this challenge. Various pathways have been reported for the mechanisms of Lenvatinib resistance, including activation of EGFR, changes in the immune microenvironment, and activation of tumor stem cells. Oligomeric proanthocyanidins (OPCs), natural polyphenols with broad-spectrum anticancer properties, may enhance Lenvatinib's efficacy by targeting these resistance pathways. This study aims to elucidate the mechanisms of Lenvatinib resistance and evaluate the potential of OPCs to overcome resistance in HCC.
Methods: Lenvatinib-resistant Huh-7 and PLC-PRF-5 cell lines were generated through long-term exposure to increasing concentrations of Lenvatinib. We performed a series of cell culture experiments to evaluate the synergistic anticancer effect of the combination of Lenvatinib and OPCs in Lenvatinib-resistant cell lines. By performing genome-wide transcriptomic profiling, we identified specific growth-signaling pathways targeted by the combined treatment and validated these findings in cell lines.
Results: The combination therapy of Lenvatinib and OPCs yielded promising results, significantly suppressing cell proliferation, invasion, and spheroid formation in Lenvatinib-resistant HCC cells. Transcriptomic analysis revealed that ITGA3 is a critical factor in Lenvatinib resistance, and its upregulation is associated with poor prognosis in HCC patients. Furthermore, high ITGA3 expression in resistant cells was found to promote activation of the EGFR-AKT pathway and enhance resistance to anoikis, contributing to Lenvatinib resistance. Molecular analyses demonstrated that this combination therapy effectively suppresses the ITGA3, EGFR-AKT pathway, thereby overcoming Lenvatinib resistance and supporting the promising results of this treatment.
Conclusion: This study identifies OPCs as a promising therapeutic agent for overcoming Lenvatinib resistance by targeting ITGA3 and the EGFR-AKT pathways. The results emphasize the potential benefits of combining OPCs with Lenvatinib to enhance therapeutic efficacy and improve outcomes in Lenvatinib-resistant HCC.

TUMOR BIOLOGY: Cancer Stem Cells
Poster Session

#6 Targeting eukaryotic elongation factor 2 kinase (eEF2K) to combat cancer stem cells and tumorigenesis in triple-negative breast cancer.

Ezgi Biltekin[1], Nermin Kahraman[1], Yasemin M.Akay[2], Gulperi Oktem[3], Metin Akay[2], Bulent Ozpolat[1]

[1]Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX,[2]Department of Biomedical Engineering, University of Houston, Houston, TX,[3]Department of Histology, Ege University Faculty of Medicine, Izmir, Turkey

Breast cancer remains the most commonly diagnosed cancer and the second leading cause of cancer-related deaths among women. Triple-negative breast cancer (TNBC) accounts for 15-20% of all breast cancers, is a highly aggressive and lethal form of breast cancer (BC) and characterized by the lack of ER, PR, and HER2 receptor expression, early relapses, drug resistance, and the poorest survival outcomes among all BC subtypes. Currently, there are no effective targeted therapies for TNBC, highlighting the urgent need for novel treatment strategies. eEF2K is an atypical alpha kinase that has been shown to promote TNBC cell proliferation, migration, invasion, and tumor growth. We previously validated eEF2K for the first time as a potential molecular target in TNBC and demonstrated that genetic inhibition of eEF2K completely suppresses in vivo TNBC tumor growth in mice. Overall data suggests that eEF2K is a critical oncogenic driver of TNBC tumorigenesis. Cancer stem cells (CSCs), a small but specific group of cancer cells, play a significant role in relapses and resistance to therapeutics including chemo-drugs. CSCs initiate tumor formation, promote tumor growth and cause relapses with their self-renewal and self-proliferation properties. While TNBC is known for the high number of CSCs population, the role of eEF2K in CSCs is unknown. In this study, we report the critical role of eEF2K in TNBC CSCs survival and tumorigenesis and found that the inhibition of eEF2K by siRNA and a small molecule inhibitor that we developed dramatically suppressed CSCs growth, migration, invasion and spheroid formation as well as TNBC cell proliferation and tumor growth in mice multiple TNBC models. Conversely, eEF2K overexpression promoted sphere formation. Our Kaplan-Meier analysis of the Cancer Genome Atlas (TCGA) revealed that high expression of eEF2K and ALDH1a3, a stemness marker, is associated with shorter overall and relapse-free survival. We also found that eEF2K is highly overexpressed in the ALDH-positive TNBC stem cell population. Genetic knockdown and pharmacological inhibition of eEF2K significantly suppressed the expression of key stemness signaling molecules, including ALDH1a3, Notch1, and Notch2. Currently, we are targeting eEF2K in MDA-MB-231 and MDA-MB-436 TNBC orthotopic xenograft and 4T1 mouse mammary tumor models in syngeneic mice to further assess its impact on CSCs-driven tumorigenesis. Our findings suggest that eEF2K plays a pivotal role in promoting TNBC stemness through the modulation of key stemness pathways, including ALDH1a3, Notch1, and Notch2 and tumor growth. Targeting eEF2K may therefore be a promising therapeutic strategy to eradicate TNBC by targeting primary and CSCs populations.

TUMOR BIOLOGY: Imaging in Animal Models
Poster Session

#60 Nanoparticle delivery of cancer-activated DNA constructs for the diagnosis of liver tumors.

Nadege Morisot, Blaine McCarthy, Sushil Lathwal, Dariusz Wodziak, Suthara Ramachandran, Anderson Lee, Dang Dang, Blair Cain, Xiaobin Wu, Robby Chandra, Badriprasad Ananthanarayanan, David Suhy, Christine Peterson

Earli Inc., Redwood City, CA

Early detection of liver cancer has the potential to substantially improve survival rates. Yet, diagnosis of hepatocellular carcinoma (HCC) is hampered by poor sensitivity of traditional imaging techniques - such as ultrasound, CT, and MRI - which often miss small or atypical tumors, particularly in cirrhotic livers with altered anatomy. As a result, more than a third of early-stage HCCs are missed. Earli is addressing these limitations by developing a platform technology that utilizes LNP-delivered recombinant DNA constructs containing cancer-activated synthetic promoters driving the selective expression of proteins such as well defined epitopes for the imaging of tumors or cytokines that have therapeutic potential to treat the tumor. Although non-viral systems like lipid nanoparticles (LNPs) have proven effective for RNA delivery, DNA delivery to tumor cells remains challenging. Earli-derived LNP formulation FRM237 was used to encapsulate a recombinant DNA comprised of a non-specific CAG promoter to drive the expression of a firefly luciferase reporter and was evaluated by ex vivo bioluminescence imaging (BLI) in a subcutaneous Hep3B (liver adenocarcinoma) xenograft model. The FRM237 formulation produced high levels of expression and achieved BLI levels that were 5-fold higher than the same DNA formulated in a multi-component LNP used in a commercial siRNA product. More importantly, engineered LNP enabled 88-fold greater tumor-to-liver BLI ratio, revealing liver tumor tropism. Pharmacokinetic studies show a large increase in circulation LNP-delivered DNA vector copies were consistently 10-fold higher in serum as compared to the competitor formulation. Effective extrahepatic delivery and expression to HCC was further verified in an orthotopic liver xenograft model using a recombinant DNA driven by a synthetic promoter known to be highly active in liver cancer cells. Administered via IV injection to mice with Hep3B tumors, this product produced 96-fold higher expression from those malignant tissues as compared to naïve liver tissues. Likewise, immunohistochemistry (IHC) analyses revealed selective expression of the protein throughout the orthotopic Hep3B tumors with little or no corresponding staining in normal hepatocytes. Finally, intravenously dosed lead formulations were tolerated as evident by modest but transient body weight loss and clinical observations. Overall, our data collectively points to successful delivery of DNA to hepatic tumors via engineered LNP formulations following iv administration. When combined with cancer-activated expression of PET reporter genes and further validation in preclinical mouse models and toxicology studies, this represents a promising diagnostic imaging platform for early detection of liver cancer.

CHEMISTRY: Natural Products
Poster Session

#600 Targeting STAT pathways with oridonin derivatives to suppress triple negative breast cancer.

Gabrielle Vontz[1], Jun Li[2], Zhipin Liang[1], Jia Zhou[2], Qiang Shen[1]

[1]Department of Interdisciplinary Oncology, LSU Health New Orleans, New Orleans, LA,[2]Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, LA

Breast cancer remains the most common cancer type and second leading cause of cancer related deaths among women in the U.S. Despite progress in detection, surveillance, and targeted therapies, aggressive subtypes like triple-negative breast cancer (TNBC), along with challenges such as drug resistance and disease recurrence, remain significant clinical hurdles. To address these unmet needs in TNBC treatment, our group has developed a series of oridonin-derived analogs (oridonalogs), including CYD0618. These analogs exhibit enhanced drug-like properties and significantly improved potency against various cancers in both *in vitro* and *in vivo* models. This study aims to characterize the mechanism of action of CYD0618 against TNBC cells. Our findings demonstrate that CYD0618 effectively reduces the proliferation of TNBC cells *in vitro* with IC_{50}s at the nanomolar level. Additionally, CYD0618 exhibits high potency against TNBC *in vivo*, significantly suppressing tumor growth and prolonging survival in a xenograft mouse model. Preliminary results reveal that CYD0618 inhibits activation of STAT3, a key transcription factor involved in proliferation, survival, and transformation of epithelial cells. Given that STAT3 is constitutively active in ~70% of human cancers, there has been a tremendous effort to develop STAT3 targeting therapies; however, none have achieved FDA approval. In addition to STAT3, our study shows that CYD0618 also suppresses other STAT family transcription factors. Compared to selective STAT3 degradation by PROTAC treatment, poly-STAT targeting by CYD0618 exhibits superior activity against TNBC cells. These findings identify CYD0618 as a novel poly-STAT inhibitor with promising activity against TNBC.

#601 Momordicine-I treatment reduces the triple negative breast cancer growth and alters the M2 phenotype of macrophages in tumor microenvironment.

R. A. Patel, E. T. Tran, K. Kesh, R. B. Ray;
Saint Louis University, St. Louis, MO

Triple negative breast cancer (TNBC) is an aggressive form of breast cancer with a worse prognosis. The goal of this study is to examine whether Momordicine-I (M-I), a key bioactive compound from *Momordica charantia*, displays an anti-cancer activity and the potential mechanism. We treated human and mouse TNBC cell lines, MDA-MB-231 and 4T1, with M-I in a dose-dependent manner for 48 hr. We observed a significant inhibition of cell growth. To understand the potential mechanism, we treated these cells with vehicle or M-I and cell lysates were prepared after 48 hr. We found that M-I inhibited pSTAT3, cyclinD1 and c-Myc expression in M-I treated cells. We next evaluated the anti-tumor efficacy of M-I in orthotopic breast cancer mouse model. MDA-MB-231 cells were implanted into the mammary fat pad of nude mice. Tumor bearing (~80 mm^3 in volume) mice were divided into two groups. One group received M-I (30 mg/kg/day) and the other group received vehicle as a control. Tumor volumes were significantly reduced in the M-I treated group compared to the control group. Our previous study showed that M-I treatment alters macrophage phenotype in a different cancer model. We next examined the status of tumor associated macrophages (TAMs) in TNBC tumors. TAMs are mainly considered M2-like macrophages, which plays a crucial role in mediating the interaction between tumor cells and the immune cells. To examine the specific TAM polarization *in vivo*, RNA from control and treated tumors was analyzed for macrophage polarization using specific primers. Since nude mice contain macrophages, we examined the status of *Arg1* and *IRF-4* (M2 *marker)* from the tumor treated with vehicle or M-I. We found a significant reduction in treated tumors. We also performed an *in vitro* experiment where conditioned media of 4T1 cells with or without M-I treatment were incubated with immortalized bone marrow derived mouse macrophages (iMac) cells and examined the expression of several macrophage markers. We observed that M-I treatment significantly reduced M2 phenotype of iMac cells. Co-culture experiment with or without M-I treated MDA-MB-231 cells with PMA treated THP-1 displayed downregulation of Arg1 and IL-10 expression. Our data demonstrates that M-I has potential as a candidate for TNBC therapy and inhibits the M2 polarization of macrophages. Further work is underway using 4T1 mouse model to understand M-I mediated tumor growth inhibition.

#602 Synergistic anti-tumor effects of andrographis and lactoferrin in colorectal cancer via modulation of AXIN2/Wnt/E-cadherin signaling pathway.

Yuan Li, Caiming Xu, Takayuki Noma, Ajay Goel

City of Hope Comprehensive Cancer Ctr., Duarte, CA

Background: Colorectal cancer (CRC) is one of the most common malignancies worldwide and 20-25% of patients experience metastatic colorectal cancer (mCRC) at initial diagnosis. However, the high mortality rates associated with mCRC are largely due to the inadequate efficacy of current treatments and the development of acquired resistance to conventional chemotherapy. Recent studies have indicated that Lactoferrin is frequently used as a drug transport carrier, enhancing drug absorption. Natural compounds like Andrographis offer cost-effective, multi-targeted treatments for tumors. Therefore, we hypothesize that Lactoferrin might serve as a transport carrier for Andrographis, improving its bioabsorption and in offering a more pronounced anticancer activity in CRC.
Methods: We conducted a series of in-vitro and in-vivo experiments to evaluate the synergistic anticancer effects of Lactoferrin and Andrographis in CRC. Furthermore, we performed a genomewide transcriptomic analysis to identified specific growth-signaling pathways targeted by the combined treatment and subsequently validated these findings in cell lines and patient-derived 3D organoids.
Results: The combined treatment with Lactoferrin and Andrographis exhibited excellent synergistic anti-tumor potential, affecting cell viability, proliferation, migration, and invasion in CRC cells. In CRC, metastasis commonly occurs through the epithelial-mesenchymal transition. This combination treatment exerted the most pronounced effects on β-catenin responsive transcription and E-cadherin promoter activity. Mechanistically, transcriptomic analysis revealed that the combined treatment resulted in the inhibition of AXIN2 which resulted in E-cadherin-mediated inhibition of Wnt signaling ([FC]: 3.46, $P < 0.005$). Additionally, we observed that the expression levels of AXIN2 were significantly higher in cancer vs. normal tissues ($P < 0.005$) in the TCGA dataset. Finally, in 3D organoid models, the combined treatment exhibited superior anti-cancer activity, significantly reducing both the number and size of organoids ($P < 0.05$). These findings highlight the therapeutic potential of targeting the AXIN2/Wnt/E-cadherin axis with Lactoferrin and Andrographis in CRC.
Conclusion: We present evidence that the synergistic anticancer effects of Lactoferrin and Andrographis in CRC are mediated through the AXIN2/Wnt/E-cadherin axis, highlighting their potential as a therapeutic strategy.

#603 Repurposing the plant-derived compound apigenin in anticancer and antiaging pipelines.

Y. Sun[1], H. Zhang[2], Q. Xu[1], R. Sun[3], J. L. Kirkland[4];

[1]Chinese Academy of Sciences, Shanghai, China, [2]Shanghai University of Traditional Chinese Medicine, Shanghai, China, [3]Bioduro-Sundia, Shanghai, China, [4]Cedars-Sinai Medical Center, Los Angeles, CA

Cellular senescence is a cell fate triggered by inherent or environmental stress and characterized by stable cell cycle arrest accompanied by a hypersecretory feature, termed as the senescence-associated secretory phenotype (SASP). Senescent cell burden increases with natural aging, functionally contributing to age-related organ dysfunction and multiple disorders including various malignancies. In this study, we performed a large scale screening of a natural product library for senotherapeutic candidates by assessing their effects on human senescent cells. Apigenin, a dietary flavonoid previously reported with antioxidant and anti-inflammatory activities, exhibited a prominent capacity in targeting senescent cells as a senomorphic agent. In senescent cells, apigenin blocks the interactions between ATM/p38 and HSPA8, thus preventing transition of the acute stress-associated phenotype (ASAP) towards the SASP. Mechanistically, apigenin targets peroxiredoxin 6 (PRDX6), an intracellular redox-active molecule, suppressing the iPLA2 activity of PRDX6 and disrupting downstream reactions underlying the SASP development. Without reversing cellular senescence, apigenin deprives cancer cells of malignancy acquired from senescent stromal cells in culture, while reducing chemoresistance upon combination with chemotherapy in anticancer regimens. In preclinical trials, apigenin administration improves physical function of animals prematurely aged after whole body irradiation, alleviating physical frailty and cognitive impairment. Overall, our study demonstrates the potential of exploiting a naturally derived compound with senomorphic capacity to achieve anticancer and geroprotective effects by modulating the SASP, thus providing a research platform for future exploration of novel natural agents against age-related pathologies, including but not limited to cancers.

#604 Punicic acid from pomegranate seed oil induces cytotoxicity and oxidative stress in MCF-7 breast cancer cells.

Braden Quitmeyer, Shelley A. Phelan

Fairfield University, Fairfield, CT

Punicic Acid is an omega-5 long chain saturated fatty acid found in abundance in the oil of pomegranate seeds. While already known to provide various health benefits from a dietary perspective, punicic acid (PA) has also been shown to be an anti-proliferation agent in various cancer cell lines such as colorectal cancer and breast cancer. In this study, we examined the effects of PA on the MCF-7 breast cancer cell line as compared to the non-cancerous MCF-10A breast epithelial cell line. We found that PA induces dose-dependent cytotoxicity on both lines, with higher cytotoxicity in MCF-10A cells at all concentrations. MCF-7 cells exhibited relatively strong resistance to PA at the 0.4 µg/ml concentration, but increased cytotoxicity at 2 and 10 µg/ml. By contrast, there was notable cell death at all three concentrations within the non-cancerous cell line. Examination of apoptosis confirmed its role in both cell line responses to punicic acid, with significantly higher levels of apoptosis in MCF-10A cells. In order to examine the antioxidant response to punicic acid, we used qPCR to measure the expression of the Peroxiredoxin gene family (Prdx1, Prdx2, Prdx3, Prdx4, Prdx5, and Prdx6) after 48 hours of punicic acid treatment in both lines. We observed significant induction of all six Prdx genes in MCF-7 cells, with over a 4-fold increase in Prdx1, Prdx2 and Prdx6 at the 10ug/ml concentration. Interestingly, MCF-10A cells exhibited considerably higher induction of all six Prdx mRNAs at 10 µg/ml, exceeding a 30-fold induction of Prdx1, Prdx2 and Prdx5. Current work in our lab is focused on determining the role of oxidative stress in the PA-induced cytotoxicity in MCF-7 cells. Our data suggests a dose-dependent increase in mitochondrial superoxide production after 48hrs of punicic acid treatment. Analysis of other ROS markers is ongoing. Together, our data provide important new insights into the mechanisms and cell-specific effects of punicic acid, and its potential preventative and treatment efficacy in breast cancer.

#606 Celastrol treatment induces apoptosis and modulates metabolic pathways in FLT3 - Positive acute myeloid leukemia cells.

Biba Vikas[1], Vasantharaja Raguraman[1], Shanid Mohiyuddin[1], Sofia Vega[1], Zhentian Lei[2], Gerhard Hildebrandt[1], Senthilnathan Palaniyandi[1]

[1]Ellis Fischel Cancer Center, University of Missouri - Columbia, Columbia, MO,[2]Metabolomics Center, University of Missouri - Columbia, Columbia, MO

Acute Myeloid Leukemia (AML) is a hematologic malignancy with poor prognosis. Historically, its treatment is associated with severe toxicity and mortality. Moreover, the success of chemotherapy is often hampered by drug resistance and relapse, affecting treatment response and long-term survival of patients. To find alternative drugs and targeted immunotherapies for AML remains an unmet need. Celastrol is a natural compound isolated from the medicinal plant *Tripterygium wilfordii Hook F*. It is a pentacyclic triterpenoid, that has shown therapeutical potential due to its anti-inflammatory and anti-cancer properties, yet the mechanism is poorly understood. In the present study, we demonstrate the cytotoxic effects of celastrol in FLT3 ITD mutated AML. Molecular docking studies confirmed celastrol's effective binding to the FLT3 protein, suggesting a potential targeted therapy. Cell viability and apoptosis were determined after celastrol treatment in MV-4-11 cells at varying concentrations, and cell viability was hampered in a dose-dependent manner with an IC_{50} value of 0.3μM. Next, Annexin V and PI staining were used to examine whether this inhibitory effect of celastrol on cell viability was related to the induction of apoptosis. Celastrol-induced cell apoptosis was demonstrated ranging from 75 to 78 % along with increased apoptotic markers like caspase 3, caspase 8, and caspase 9 expression, confirming its ability to trigger the apoptotic cascade through intrinsic and extrinsic pathways. Seahorse assay revealed that celastrol treatment significantly suppressed the maximal oxygen consumption rate (OCR) with minimal effect on basal respiration. Interestingly, combination treatment with celastrol and Giltertinib strongly suppressed maximal OCR. In addition, celastrol treatment affected the extracellular acidification rate (ECAR) in MV-4-11 cells showing a compensatory decrease in glycolysis. Furthermore, untargeted metabolomics showed 18 significantly changed metabolites with better variance between the vehicle and celastrol treatment as evidenced by principal component analysis (PCA). Pathway analysis revealed alterations in glutathione, lipid, and energy metabolism after celastrol treatment. This study demonstrated that celastrol treatment targets both glutathione and glycolysis metabolism which are crucial for cell survival and will potentially be a powerful strategy to induce a more selective, metabolically driven toxicity on FLT3-positive AML cells. The ongoing transcriptomics analysis and the in vivo experiments will delineate celastrol's potential role in treating AML.

#607 Andrographis and aronia berry extract synergistically exhibit anticancer effects in colorectal cancer by activating the p-YAP/PUMA signaling pathway.

Kainan Wang, Yuan Li, Caiming Xu, Ajay Goel

City of Hope Comprehensive Cancer Ctr., Monrovia, CA

Background: Colorectal cancer (CRC) is the third leading cause of cancer-related mortality worldwide, posing a persistent global health challenge. Despite advancements in current therapeutic strategies, the emergence of resistance highlights the urgent need for novel treatment approaches to overcome their limited efficacy. Among these, natural products have emerged as a promising resource due to their high efficacy, low toxicity, and potential for integration into dietary regimens. The antioxidant and anti-inflammatory properties of Andrographis (Andro) and Aronia berry extract (ABE) counteract cancer by neutralizing free radicals, reducing inflammation, and enhancing immune function. Herein, we hypothesize that their combined treatment may synergistically regulate multiple oncogenic pathways, leading to enhanced anti-cancer effects in CRC. Methods: The synergistic anticancer effects and mechanisms of Andro and ABE in CRC were evaluated through a combination of functional assays and genome-wide transcriptomic profiling. Clonogenic, cell viability, flow cytometry, and transwell migration assays were employed to assess their impact on cell proliferation, apoptosis, and metastatic potential. Transcriptomic analysis was performed to identify the key signaling pathways modulated by the combination treatment. Finally, these findings were validated using CRC patient-derived 3D organoids (PDOs). Results: The combined treatment with Andro and ABE exhibited synergistic anti-tumor effects in CRC cells by suppressing cell viability, proliferation, migration, and invasion. Transcriptomic profiling revealed a significant upregulation of PUMA expression (Andro vs. Control, log2Foldchange [FC]: 1.29, $P = 0.01$; ABE vs. Control, log2FC: 1.08, $P = 0.001$), which was associated with modulation of the Hippo-YAP pathway (Fold enrichment: 5.06, $P = 0.0009$). Mechanistically, Andro, and ABE synergistically promote YAP phosphorylation (p-YAP), which in turn enhances PUMA expression, thereby driving apoptosis in CRC cells, as confirmed by western blot, qRT-PCR, and flow cytometry analyses. These findings were validated in PDOs, which showed reduced size and number, and increased p-YAP activation and PUMA expression, underscoring the therapeutic potential of the combination treatment. Conclusion: In summary, our research provides strong evidence that the combination of Andro and ABE could be a potent and safe integrative approach for CRC treatment. The synergistic activation of the p-YAP/PUMA signaling pathway by these natural products induces apoptosis in CRC cells, offering a promising new direction for cancer treatment.

#609 Triptolide inhibits ovarian cancer growth by enhancing EZH2 degradation.

Yongxia Zhu[1], Qing Tang[2], Hongtao Xiao[1], Ningyu Wang[2]

[1]Department of Pharmacy, Sichuan Clinical Research Center for Cancer, Sichuan Cancer Hospital & Institute, Sichuan Cancer Center, Affiliated Cancer Hospital of University of Electronic Science and Technology of China, Chengdu, China,[2]School of Life Science and Engineering, Southwest Jiaotong University, Chengdu, China

Ovarian cancer is a lethal gynecological tumor, typically diagnosed at an advanced stage and associated with limited treatment options. The Enhancer of Zeste Homolog 2 (EZH2) is a crucial methyltransferase that serves as a catalytic subunit of the Polycomb Repressive Complex 2 (PRC2), alongside other core subunits (EED, SUZ12, and RbAp46/48). EZH2 contributes to ovarian cancer progression through both its catalytic and non-catalytic functions, making it a promising therapeutic target. Data from the TCGA database and the Human Protein Atlas indicate that EZH2 mRNA and protein levels are elevated in ovarian cancer tissues compared to normal ovary tissues, correlating with poor prognosis in patients. This provides a rationale for targeting EZH2 in ovarian cancer treatment. However, traditional EZH2 enzyme inhibitors only block its catalytic function and are ineffective against ovarian cancer driven by its non-catalytic functions. EZH2 degraders can effectively inhibit both functions. To identify phytochemicals that promote EZH2 degradation, we screened and identified 20 candidates that can directly or indirectly modulate EZH2 expression. Western blot analysis revealed that Triptolide (TPL, 1 μM) significantly degrades EZH2 protein. TPL is a diterpene compound extracted from the roots, leaves, flowers, and fruits of *Tripterygium wilfordii*, known for its anti-inflammatory and anti-tumor properties. However, its role in regulating EZH2 in ovarian cancer has not been previously reported. Our western blot results showed that TPL significantly degrades EZH2 and other core PRC2 subunits (EED and SUZ12) in a concentration-dependent manner, with a half-degradation concentration (DC_{50}) as low as 25 nM in the ovarian cancer cell line A2780. The degradation effect of TPL on EZH2 and PRC2 increased with longer exposure times. Further experiments indicated that TPL induces degradation of EZH2 or the PRC2 complex via the ubiquitin-proteasome pathway. A biotin-labeled TPL probe, Biotin-TPL, used in a pull-down assay, confirmed the interaction between TPL and EZH2. Additionally, TPL effectively inhibits the proliferation of ovarian cancer cells in a time- and concentration-dependent manner, with IC_{50} values in low nanomolar range. Colony formation and 3D cell spheroid formation assays demonstrated that TPL (3.7-33.3 nM) significantly reduces colony formation in A2780 and ES-2 ovarian cancer cells, as well as the formation of ovarian cancer 3D cell spheroids. RNA-seq data and subsequent validation experiments showed that TPL can inhibit signaling pathways regulated by EZH2's non-catalytic functions. Collectively, these findings suggest that TPL may inhibit ovarian cancer growth by degrading the EZH2/PRC2 complex and modulating the signaling pathways associated with EZH2's non-catalytic functions.

Acknowledgements: This work was supported by National Natural Science Foundation of China (82304454).

TUMOR BIOLOGY: Imaging in Animal Models
Poster Session

#61 Establishment of translational luciferase-based cancer models to evaluate antitumoral therapies.

Martin R. Ramos-Gonzalez[1], Nagabhishek S. Natesh[1], Satyanarayana Rachagani[2], James Amos-Landgraft[3], Haval Shirwan[4], Esma S. Yolcu[4], Jorge G. Gomez-Gutierrez[4]

[1]NextGen, University of Missouri - Columbia, Columbia, MO,[2]Veterinary Medicine and Surgery, University of Missouri - Columbia, Columbia, MO,[3]Veterinary Pathobiology, University of Missouri - Columbia, Columbia, MO,[4]Pediatrics, University of Missouri - Columbia, Columbia, MO

There is an increased need for translational cancer models in research that can serve as more clinically relevant platforms to better study how cancer develops, the formation of metastases, and for the evaluation of the efficacy of new antitumoral therapies. Orthotopic cancer models represent a valuable opportunity to better understand how cancer cells behave and produce tumors in their primary organs, as well as how the local microenvironment can affect the tumor development. However, studying a growing tumor inside of an animal's body carries several challenges, like the difficulty to accurately detect the tumor's presence or changes in size since its location is not always easily accessible. To address this situation, we developed luciferase-based cancer cell lines that can produce bioluminescence (BL) and when inoculated orthotopically, they produce solid tumors that can be detected in live animals, allowing for a more accurate model to evaluate antitumoral therapies. We stably transfected the triple negative breast cancer (TNBC) cell line from human origin HCC1937, and the murine cell line 4T1, as well as the murine lung cancer cell line TC-1 with a luciferase expressing plasmid. After transfection, the luciferase activity was tested *in vitro* and once confirmed these luciferase-expressing cell lines were inoculated orthotopically into synergic animals. We successfully established orthotopic BL tumors that could be detected by bioluminescence imaging (BLI), as well as the detection of distal metastases by this method. The signal from the tumors in vivo correlated with the signal detected from the ex vivo organs, confirming the high accuracy and sensitivity of the use of BL as a detection method for cancer models. Interestingly, we confirmed a change in phenotype after the plasmid internalization in the cell line HCC1937/luc2. This cell line modified its response to an oncolytic adenovirus virotherapy, downregulating its antiviral response and becoming more sensitive to be infected and killed by these viruses. This highlights the importance of confirming the desired phenotype of a cell line remains unaltered after a genetic modification, as in our case, a plasmid insertion. In our study, we offer clinically relevant bioluminescent orthotopic cancer models that can be used to accurately evaluate the efficacy of antitumoral therapies by the quantification of BL signal coming from live animals. This method facilitates the monitoring of the tumoral growth and the appearance of metastases, with the advantage of allowing for multiple readings over time on live animals to assess the efficacy of antitumoral therapies.

CHEMISTRY: Natural Products
Poster Session

#610 Manzamine and mitragynine: investigation of beta carboline compounds from marine sponges and kratom leaves as ovarian cancer therapeutics.

Ashley Gabriel, Haifa Sahouri, Helen Fischer, Kimberly A. Gonzalez, Jihad Aburas, **Cai M. Roberts**

Midwestern Univ. - Downers Grove Campus, Downers Grove, IL

Epithelial ovarian cancer (EOC) is the leading cause of death among gynecologic cancers. Five-year survival rates hover around 35%, largely due to widely metastatic and drug-resistant recurrent disease. Prior studies in our laboratory and others have shown that tumor progression, as evidenced by epithelial to mesenchymal transition (EMT) and metastasis, can have a significant impact on the response of cancer cells to treatment. Tumor heterogeneity is a substantial barrier to effective therapy. For instance, stem-like cells can withstand treatment that eliminates faster-growing cells and have the potential to give rise to recurrence. Thus, novel therapies or drug combinations that can eliminate all subpopulations within a tumor are needed. Natural products have a long history in cancer treatment. Prior studies in the natural products space on anti-EMT drugs focused on the beta carboline molecule harmine and its derivatives. While these compounds have shown efficacy, translation of these compounds to the clinic is complicated by their hallucinogenic properties. Therefore, we sought alternatives with similar beta carboline moieties. In the present study, we investigated alkaloids from marine sponges *(Halicloa spp.)* and the kratom plant *(Mitragyna speciosa)* as potential therapeutics for EOC. We tested the kratom compound mitragynine, the marine compound manzamine A, and several of their derivatives and analogues, in multiple EOC cell lines. We found that mitragynine killed EOC cells with an IC50 of ~20 μM. The marine alkaloid manzamine A killed EOC cells with IC50 values in the low micromolar range. Each of these outperformed their respective analogues in cell survival assays. Importantly, both pre- and post-EMT cells, and models of primary and recurrent disease, all responded to manzamine treatment. Our findings also suggest an additive effect when manzamine therapy is combined with conventional chemotherapy. Additional work will be needed to fully elucidate the full mechanism of action of these compounds and whether inclusion of these alkaloids in treatment regimens will be effective in combating drug resistance. However, our results suggest that there is a place for these compounds in our arsenal for the deadliest gynecologic cancers.

#611 Actinobacteria from macroalgae as a source of new anti-lymphoma compounds.

Filippo Spriano[1], Alberto J. Arribas[1], Nikolai Bendik[1], Mariana Girao[2], Ines Ribeiro[2], Maria de Fatima Carvalho[2], Francesco Bertoni[1], Pedro Leao[2]

[1]IOR - Institute of Oncology Research, Bellinzona, Switzerland,[2]CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, Matosinhos, Portugal

Background: Actinomycetota of the genus Streptomyces are well known to produce a wide range of secondary metabolites, many of which have already been developed into antibiotics, antifungals, anti-inflammatory compounds, and anticancer agents. They can be found in the marine environment, including the deep sea, and are frequently associated with marine organisms, such as macroalgae. We studied the anti-lymphoma activity of extracts from Actinomycetota isolated from several deep-sea samples and macroalgae species abundant along the Portuguese coast.
Methods: Organic extracts were made from 7-day cultures using a 1:1 (v/v) methanol:acetone mix. An LC-MS/MS-based dereplication was applied to the extracts presenting the most relevant properties, using GNPS to identify the ones putatively containing novel molecules. Cancer cell lines were treated with each extract for 72h followed by MTT.
Results: Actinomycetota strains were isolated from the tissues of 3 macroalgae species collected on the northern Portuguese coast (*Laminaria ochroleuca*, *Chondrus crispus, Codium tomentosum*) and from sediments, corals, and sponges from deep-sea regions. 147 extracts were tested (1 and 10 µg/ml) in marginal zone lymphoma (MZL) cell lines with acquired resistance to PI3K (n.=2), BTK (n.=1), and PI3K/BCL2 (n.=1) inhibitors and their parental counterparts (n.=2). 18 extracts gave broad-activity in at least 4/6 cell lines tested. Among those, we identified molecules present in more than 1 fraction, such as Apoptolidin A, known to have a cytotoxic effect in cancer cells. All extracts were MS tested to know the compositions of known molecules or analogs. Among the active extracts, we have identified some containing known molecules with unknown functions or unknown molecular entities with a broad anti-cancer effect. Twenty-three extracts showed different activity in parental and resistant cell lines. Thirty extracts with broad anti-cancer activity or different activity between parental and resistant were further validated in a larger dose-response curve. Seven were confirmed to have a higher activity in resistant than parental cells or broad antitumoral activity. Three extracts from seaweeds, M15 and M16 (from Streptomyces) and M28 (Verrucosispora sp) were selected for up-scaling culture and further fractionation in 13, 12, and 11 fractions, respectively. Activity was confirmed in some fractions at 10 µg/ml and for some M28-derived fractions at 1 µg/ml. M28 (containing no known compounds) and M15 showed a higher activity in resistant than parental models.
Conclusions: We identified Actinobacteria extracts with broad anti-lymphoma activity or showing increased activity in MZL cells with acquired resistance to FDA-approved compounds compared to parental cells. Some fractions were composed of new molecular entities, representing potential sources of novel therapeutic compounds.

#612 Antimicrobial peptides with antitumor activity against marginal zone lymphoma cell lines resistant to BTK, PI3K, and BCL2 inhibitors.

Filippo Spriano[1], Alberto J. Arribas[1], Fangwen Zhang[1], Maria Luisa Mangoni[2], Francesco Buonocore[3], Francesco Bertoni[1]

[1]IOR - Institute of Oncology Research, Bellinzona, Switzerland,[2]Department of Biochemical Sciences, Sapienza University of Rome, Laboratory Affiliated to Pasteur Italia-Fondazione Cenci Bolognetti, Rome, Italy,[3]Department for Innovation in Biological, Agrofood and Forest Systems, University of Tuscia, Viterbo, Italy

Background: Antimicrobial peptides (AMPs) are a class of small proteins produced by all living organisms, from prokaryotes to humans. In higher eukaryotes, AMPs contribute to the host's innate immunity, promptly responding against any pathogen. Due to their mechanism of action, represented mainly by their ability to bind and perturb microbial membranes, AMPs are being explored as novel agents against multidrug-resistant bacteria and anti-cancer agents. Natural peptides could be used as a scaffold to design new AMPs with improved effectiveness, stability, and selectivity. Marginal zone lymphoma (MZL) is an indolent yet incurable B-cell lymphoid tumor. Here, we assessed the antitumor activity of seven AMPs in MZL models with acquired resistance to FDA-approved BTK, PI3K, and BCL2 inhibitors.

Methods: Established human cell lines derived from MZL (VL51, Karpas1718) and derivatives with secondary resistance to targeted agents obtained by long drug exposure (n.=3 from VL51, n.=1 from Karpas1718 (Arribas et al, Haematologica 2022; Mol Cancer Ther 2024; ENA 2019; ENA 2020) were exposed to increasing concentrations of AMPs or DMSO, as control. Anti-proliferative activity was assessed by MTT assay after 72 hours of exposure.

Results: MZL cell lines were exposed to AMPs originating from Antarctic fishes (*Chionodraco hamatus*, *Trematomus bernacchii*; trematocines, n.=2; chionodracines, n.=1) and amphibians (temporins, n.=4;). Anti-proliferative activity was observed in parental MZL with two synthetic AMPs: a *Trematomus bernacchii* - derived trematocine mutant (Della Pelle et al, Antibiotics 2020), with IC50 values of 7.5 µM in VL51 and 10 µM in Karpas1718 and the *Rana temporaria* - derived Temporin L (Simmaco et al, Eur J Biochem 1996) with IC50 of 8 µM in VL51 and 15 µM in Karpas1718. Notably, the antiproliferative activity was maintained in all the derivative MZL cell lines with resistance to BTK, PI3K, and BCL2 inhibitors. At higher concentrations, also the other trematocine *(*Karpas1718 models) and all the temporins (VL51 and Karpas1718 models) had anti-proliferative activity (IC50 values 25-50 µM).

Conclusions: AMPs derived from Antarctic fishes and amphibians have in vitro anti-tumor activity in cell lines derived from MZL, including models with acquired resistance to BTK, PI3K, and BCL2 inhibitors. AMPs and their synthetic derivatives can provide novel anti-lymphoma agents with mechanisms of action deeply different from currently used drugs.

#613 Investigating cyclooxygenase isoforms as targets for anticancer compounds isolated from Tulbaghia violacea.

Zodwa Dlamini, Rodney Hull, Mohammed Alaouna

Pan African Cancer Research Institute (PACRI), University of Pretoria, Pretoria, South Africa

Introduction: The medicinal plant Tulbaghia violacea exhibits anticancer properties in triple-negative breast cancer (TNBC) cells, primarily through apoptosis induction. Transcriptomic analyses of treated TNBC cells highlighted altered expression of cyclooxygenase isoforms COX-1 and COX-2. COX-1, with anti-apoptotic properties, is associated with tumor progression, while COX-2, with pro-apoptotic properties, acts as a tumor suppressor.

Methods: Bioactive compounds derived from T. violacea were screened for their potential interaction with cyclooxygenase isoforms using molecular docking approaches. Protein structures for COX-1 (PDB: 6Y3C) and COX-2 (PDB: 5IKT) were retrieved from the Protein Data Bank. Ligand and protein preparation for sixteen compounds was conducted using the Schrödinger Suite. Standard precision (SP) docking was followed by MM-GBSA post-docking and extra precision (XP) docking to refine binding affinity predictions.

Results and Discussion: COX-2 demonstrated the highest average docking scores, indicating stronger binding affinity for the T. violacea-derived compounds. Comparative structural analysis revealed significant differences in binding site residues and global backbone structures between COX-1 and COX-2. SiteMap analysis identified the most druggable regions, with COX-2's larger binding pocket enabling higher binding affinity. Notably, the same compounds were capable of binding both isoforms, although with lower affinity for COX-1. These findings suggest that the anticancer activity of T. violacea compounds may be mediated by their preferential binding to COX-2, which is often upregulated in cancers.

Conclusion: This study highlights the potential of Tulbaghia violacea as a source of bioactive compounds targeting cyclooxygenase isoforms, particularly COX-2. The preferential binding to COX-2 aligns with its pro-apoptotic role and its upregulation in cancers, underscoring its relevance in cancer therapy. Further experimental validation is warranted to confirm these in silico findings and to explore the therapeutic potential of T. violacea-derived compounds.

#614 REPYR-SC1: Hemp extract exhibits potent inhibition of squamous cell carcinoma.

Joel Costoya, Sylvia S. Daunert, Sapna K. Deo, **Joaquin J. Jimenez**

Biochemistry and Molecular Biology, University of Miami Miller School of Medicine, Miami, FL

Squamous cell carcinoma (SCC) is the second most common form of skin cancer in the world, presenting in nearly 1 in 5 US adults by age 70 alongside basal cell carcinoma. Localized low- and high-risk SCC is treated with surgical techniques offering favorable prognoses with recurrence being unlikely. Therapies for non-operable metastatic SCC, such as chemotherapy, possess undesirable side effects, and have yet to achieve similar response rates to surgery. Investigation into pharmacological targeting of cancer using cannabinoids is ongoing, demonstrating pre-clinical efficacy in various cancer models. Additionally, cannabinoids are established immunomodulatory compounds, capable of shifting immune cell populations, regulating immune cell trafficking, and their migration. Our study aimed to investigate the immunomodulatory, and anti-neoplastic capacity of REPYR-SC, a novel hemp extract. SCC-15 cells were used to model SCC, HEKa epidermal keratinocytes were used as a non-cancerous peripheral tissue model, and CTLL-2 T lymphocytes to screen for immune reactivity. REPYR-SC1 was dosed at 0 (control), 0.1, 0.5, 1, and 5 µL/mL and incubated with SCC-15 or HEKa cells for 36 and 72 hours, meanwhile 24 and 48 hours for CTLL-2 cells all at 37°C under 5% CO_2 (initial cell density of 2.5 x 10^5 cells/mL). Cell counts were evaluated pre- and post-treatment with an automated cell counter, and cell viability was determined following treatment via trypan blue exclusion and morphological analysis. REPYR-SC1 induced a significant time- and dose-dependent reduction in proliferation and cell viability ($p<0.001$) in squamous cell carcinoma cell line SCC-15, as well as T lymphocytes. At a dose of 1 and 5 µL/mL at the 36- and 72-hour mark, 100% inhibition of proliferation was observed in SCC-15 cells and 25% at 5 µL/mL - 72 hours for HEKa cells. At a dose of 1 and 5 µL/mL at the 48-hour mark, 100% inhibition of proliferation was observed for CTLL-2 cells. These results support earlier evidence suggesting the immunomodulatory, and anti-neoplastic activity of cannabinoids. Further testing *in-vivo* is warranted, and the mechanism of action for REPYR-SC1 remains unknown. Nonetheless, cannabinoid-based compounds may still represent a novel chemotherapeutic modality with a favorable toxicity profile.

#616 TYK2: A novel therapeutic target in triple-negative breast cancer brain metastasis.

Jayshree Mishra[1], Priyam Kumar[2], Narendra Kumar[1]

[1]Pharmaceutical Sciences, Texas A&M University, Kingsville, TX,[2]University of Pennsylvania, Philadelphia, PA

Breast cancer (BC) is one of the deadliest cancers that affect 300,000 women annually where 15-25% are Triple negative in nature and spreads quickly to the brain causing brain metastasis (TNBC-BM). Triple-negative breast cancer (TNBC) is a heterogeneous disease characterized by lack of hormone receptor expression and is known for high rates of recurrence, distant metastases, and poor clinical outcomes. The 5-year survival rate for TNBC-BM is only 9%. Current standard of care to treat TNBC-BM include whole brain radiation therapy, surgery and chemotherapy which leads to sever side effects, high level of neurotoxicity, neurological complication that leads to low survival rate and low quality of life. Because of such a low survival rate, there is an unmet clinical need to treat these patients differently to decrease neurotoxicity, and TNBC-BM cell death. In our study, we identified and characterized novel therapeutic targets and targeted pharmacologic inhibitors of natural compound origin for the treatment of TNBC-BM. Our preliminary data suggest (1) overexpression of Tyk2 (JAK Family meber) gene in TNBC-BM compared to TNBC and TNBC lung metastasis using a PDX database. Overall Survival analysis of TNBC patients databases suggest that higher expression of Tyk2 gene and it's downstream signaling partner gene STAT5A. Tyk2 is overexpressed in the TNBC-BM metastatic brain sample and TNBC -BM specific cell line (BrM-831). Based on the published literature and our strong preliminary data, we hypothesize that over expression of Tyk2 promotes brain metastasis in TNBC cells through the activation of Tyk2-STA5A signaling pathway stimulating epithelial-to-mesenchymal transition, cell migration and invasion of TNBC cells, leading to tumorigenesis and metastasis in TNBC; Together, this study will demonstrate Tyk2 as a new target and Tyk2 targeted therapeutic agent will be capable of inhibiting TNBC-metastasis through blocking the activation of the Tyk2-/STAT5 molecular targets. In future, we plan to develop Tyk2 targeted drug compounds originated from natural products to treat TNBC-BM.

TUMOR BIOLOGY: Imaging in Animal Models
Poster Session

#62 Tumor targeted contrast agents for pancreatic adenocarcinoma.

Sylvana Knapp[1], Ryan Bynum[1], Molly McNally[1], William E. Grizzle[2], Lacey R. McNally[1]

[1]OU Health Stephenson Cancer Center, Oklahoma City, OK,[2]University of Alabama at Birmigham, Birmingham, AL

Purpose: Pancreatic cancer has a low relative survival rate of only 12.8%, with surgical resection being an option for just 20% of patients. Achieving R0 resection is vital, as it forms the cornerstone of curative treatment strategies. pH low insertion peptides (pHLIPs), a novel class of targeted imaging agents, are being developed for use with multispectral optoacoustic imaging (MSOT) for potential clinical or intraoperative applications. However, existing pHLIPs have limitations in dynamic range and solubility. To address these issues, a new pHLIP variant, V7Phe, was designed to enhance both solubility and dynamic range for identifying the acidic tumor microenvironment in pancreatic cancer.
Methods: V7 and V7F peptides were synthesized using microwave chemistry, then removed from resin, lyophilized, and rehydrated. Conjugation with HiLyte 750 dye via maleimide chemistry was confirmed through spectroscopy. Pancreatic cancer cell lines S2VP10 and S2013 were treated with V7-750 and V7F-750 at pH 7.4 and pH 6.6. Probe uptake was assessed using near-infrared fluorescence imaging.
Results: V7F-750 demonstrated a significantly higher signal in vitro than V7-750 at both pH 7.4 and pH 6.6 ($p<0.01$). V7F-750 also exhibited greater signal in more acidic conditions ($p<0.01$). Although solubility was moderately improved, V7F-750 did not show an enhanced dynamic range compared to V7-750, with the ratio of signal at pH 6.6 to 7.4 being 1.19 for V7F-750 versus 1.39 for V7-750 ($p=0.21$) across both cell lines.
Conclusion: V7F-750 effectively targets the acidic tumor microenvironment, demonstrating improvements in peptide solubility

CLINICAL RESEARCH: Cancer Care for All: Innovations in Geriatric Oncology and Broadening Research Representation Poster Session

#620 Differences in fatigue, sleep disturbance, and quality of life in middle aged and older adults with cancer prior to cancer treatment.

David Asakitogum[1], Arsham Alamian[2], Victoria Loerzel[1]

[1]University of Central Florida, Orlando, FL,[2]University of Miami, Miami, FL

Introduction: Symptom burden and poor quality of life (QOL) are associated with cancer and its treatment. However, there is limited amount of research regarding age differences in common physical symptoms and QOL in adults with cancer prior to cancer treatment. Knowledge of age differences in common physical symptoms in these patients will inform clinical decision on pre-interventions. Therefore, the purposes of this study were to: 1) evaluate age differences in occurrence rates and severity ratings for fatigue, sleep disturbance, and QOL; 2) assess risk factors associated with the severity of each symptom and QOL outcomes.
Methods: Participants (n=111) were dichotomized into middle (50 - 64 years) and older (≥65 years) age groups. Participants completed the Symptom Representation Questionnaire and the European Organization for Research and Treatment of Cancer-Core 30 prior to cancer treatment. Differences in demographic and experiential characteristics, each symptom, and QOL outcomes were evaluated using parametric and non-parametric tests. Logistic procedures were used to identify risk factors associated with the severity of each symptom and QOL outcomes.
Results: Overall, the prevalence of moderate to severe fatigue were 13.51% and 26.12%, tired 21.62% and 40.54%, and sleep disturbance were 12.61% and 26.13% in the middle and older aged patients respectively, prior to the initiation of chemotherapy. Our findings indicate that compared to middle age, older patients experienced similar fatigue (2.67 Vs 3.04), sleep disturbance (1.98 Vs 2.03), and QOL scores (5.62 Vs 5.42) respectively prior to chemotherapy. Holding other variables constant: patients with trade and some college degrees were less likely (OR=0.095) to experience severe fatigue scores while those with cancer stage II-III were less likely (OR=0.129-0.083) to experience moderate fatigue. Compared to females, male patients were less likely (OR=0.166) to experience severe sleep disturbance while older aged were more likely (OR=5.492) to experience moderate sleep disturbance compared to middle age. Patients with breast cancer were more likely (OR=7.835) to experience excellent QOL compared to other cancers.
Conclusion: Approximately 4.50% and 9.01% of middle and 11.71% and 9.01% of older aged patients with cancer experienced severe fatigue and sleep disturbance respectively prior to cancer treatment. The study identified risk factors associated with severe fatigue and sleep disturbance. These findings have implications for oncology clinicians to assess symptoms and provide early intervention for these patients before commencement of cancer treatment so worsening of side effects can be minimized.

#621 Changes in the senescence biomarker p16INK4a among older adults with early breast cancer treated with and without chemotherapy and non-cancer controls.

Chaiyaporn C. Vatanatham[1], Jingran Ji[1], Canlan Sun[2], Marla D. Lipsyc-Sharf[1], Nikita V. Baclig[1], Yuliya A. Zektser[1], Ali Al Saleem[1], Joseph D. Olivera[1], Kelly S. Synold[1], Lauren R. Zelman[1], Hyman B. Muss[3], Mina S. Sedrak[1]

[1]University of California, Los Angeles, Los Angeles, CA,[2]City of Hope, Duarte, CA,[3]University of North Carolina at Chapel Hill, Los Angeles, CA

Background: Our group previously showed that $p16^{INK4a}$ (p16) expression, a biomarker of senescence (a fundamental process of aging), increases after adjuvant breast cancer chemo. However, no study has examined whether this increase in p16 is driven by cancer, chemo, or both. Here, we compare the change in p16 between older adults with early breast cancer who receive chemo, those who do not, and those without cancer.

Methods: We prospectively recruited 701 adults age ≥65 with stage I-III breast cancer (composed of three age-matched cohorts: 501 treated with chemo [BC chemo], 100 treated without chemo [BC control]), and 100 without cancer (NC control). Sociodemographic, functional, and clinical characteristics were collected at baseline. The p16 level in peripheral blood CD3+ T lymphocytes was obtained at two time points: ≤14 days pre-chemo (T1) and ≤30 days post-chemo (or at matched time points in the BC and NC control groups, T2). The primary endpoint was the mean Δp16 level from T1 to T2. Data was analyzed using descriptive statistics, t-tests, and analysis of variance (ANOVA).

Results: There were no differences in T1 age, race/ethnicity, education, comorbidities, or functional status across the three groups. At T1, there was no difference in mean p16 levels across the three groups (p=0.06) (Table). At T2, the BC chemo group had a significantly higher mean p16 level compared to the BC and NC control groups (p<0.01). The mean Δp16 levels from T1 to T2 were significantly different among the three groups (p=0.04). The BC chemo group had a 15% increase in mean Δp16 from T1 to T2 (p<0.01), while the BC and NC control groups had no significant change.

	BC chemo	**BC control**	**NC control**	**Total**	**p-value**
T1 p16 Level (log_2 units)					0.06
No. of participants	317	81	83	481	
Mean (SD)	10.1 (0.91)	10.0 (1.02)	9.9 (1.05)	10.1 (0.96)	
Median	10.2	9.9	9.8	10.1	
95% CI	(9.4-10.9)	(9.1-11.0)	(8.6-11.1)	(9.3-10.8)	
T2 p16 Level (log_2 units)					<0.01
No. of participants	263	76	82	421	
Mean (SD)	10.4 (0.94)	10.0 (0.85)	9.9 (0.85)	10.2 (0.93)	
Median	10.4	10.1	9.9	10.2	
95% CI	(9.7-11.1)	(9.0-10.9)	(9.1-10.6)	(9.5-10.9)	
mean Δp16 Level from T1 to T2 (log_2 units)					0.04
No. of participants	199	67	68	334	
Mean (SD)	0.2 (0.81)	0.1 (0.87)	-0.1 (0.87)	0.1 (0.84)	
Median	0.1	0.1	0.0	0.1	
95% CI	(-0.6-1.0)	(-0.8-0.8)	(-1.1-0.8)	(-0.6-0.8)	

Conclusions: In a cohort of older adults with early breast cancer, the combination of breast cancer and chemo led to increases in p16 levels. Further independent validation of these findings is needed to understand the role of senescence in older adults with early breast cancer treated with chemo.

#622 Age-dependent changes in cell cycle dynamics and hematopoietic responses to chemotherapy.

Jianxing He[1], **Yue Pan**[1], Hao Liu[2], Ying Huang[1], Hongsheng Deng[1], Chao Yang[1], Qi Cai[1], Shan Xiong[1], Huiting Liu[1], Wenhua Liang[1]

[1]The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China,[2]Henan University, Henan, China

Background: Precision medicine emphasizes individualized cancer treatment, particularly for elderly lung cancer patients facing unique chemotherapy challenges. Age-related changes in cell cycle dynamics significantly influence therapeutic outcomes, yet their role in optimizing personalized therapies remains underexplored. This study investigates these age-related dynamics to inform personalized therapeutic strategies for elderly lung cancer patients.

Methods: Single-cell RNA sequencing data from 166 healthy individuals (aged 25-85 years) were analyzed to explore age-associated changes in cell cycle distribution, differentially expressed genes (DEGs), and enriched pathways. Young (9-11 weeks) and aged (17 months) mice received weekly cisplatin for four cycles. Peripheral blood and hematopoietic stem cells (HSCs) were monitored dynamically for cell cycle phases, DNA damage (γ-H2AX), and vascular leakiness using immunofluorescence. RNA sequencing of HSCs identified DEGs and enriched pathways after treatment cycles.

Results: Single-cell analysis revealed a linear decrease in S-phase proportions with age ($r=-0.681$, $p=0.0026$) and identified 28 DEGs, including PAXB-AS1, BABAM1, and DONSON, enriched in immune regulation and cell cycle pathways. In murine models, aged mice showed impaired immune recovery, with persistently higher neutrophil proportions (post-cycle 1: 14.75% vs. 6.1%, $p<0.05$) and lower lymphocyte proportions (post-cycle 1: 65.73% vs. 79.98%, post-cycle 4: 60.5% vs. 63.8%) compared to young mice. Moreover, aged mice exhibited delayed neutrophil recovery (Day 4 vs. Day 2), accompanied by persistent G1-phase arrest in HSCs (Day 4: 86% vs. 82%) and reduced G2/M-phase proportions (Day 4: 3% vs. 6%). RNA sequencing of aged HSCs further revealed significant enrichment in the DNA_DAMAGE_TELOMERE_STRESS_INDUCED_SENESCENCE pathway after four treatment cycles ($|NSE| = 1.98$, $p < 0.05$), a pattern absent in young mice. Finally, γ-H2AX flow cytometry confirmed increased DNA damage in aged HSCs, while immunofluorescence demonstrated heightened bone marrow vascular leakiness in aged mice.

Conclusion: Aging significantly alters cell cycle dynamics, hematopoietic recovery, and chemotherapy responses, highlighting the need to integrate cell cycle-based insights into personalized treatment strategies. These findings provide a foundation for future research on age-specific therapeutic interventions in lung cancer.

#623 Epigenetic age acceleration measures and chemotoxicity in older adults with early breast cancer.

Jingran Ji[1], Canlan Sun[2], Marla Lipsyc-Sharf[1], Nikita V. Baclig[1], Yulia A. Zektser[1], Ali Al Saleem[1], Joseph D. Olivera[1], Kelly S. Synold[1], Lauren R. Zelman[1], Alexandra M. Binder[3], Mina S. Sedrak[1]

[1]UCLA - University of California Los Angeles, Los Angeles, CA,[2]City of Hope, Duarte, CA,[3]University of Hawaii, Honolulu, HI

Background: Older adults with early breast cancer can have very different risks of chemotoxicity despite similar chronological age. This heterogeneity may be due to differences in biological age. However, few studies have evaluated whether epigenetic indicators of biological age can stratify the risk of chemotoxicity in older adults with early breast cancer.
Methods: In a prospective multicenter study of 394 adults age >65 with stage I-III breast cancer treated with neo/adjuvant chemo, we analyzed DNA methylation patterns from peripheral blood to estimate epigenetic age acceleration (EAA) prior to chemo. We tested three generations of epigenetic age algorithms (1st gen: Horvath and Hannum; 2nd gen: PhenoAge and GrimAge; 3rd gen: DunedinPACE). We calculated EAA as the residual that results from regressing epigenetic age on chronological age. The primary endpoint was grade 2+ chemotoxicity (yes/no, yes defined as any grade 2+ toxicity attributed to chemo). Using multivariable logistic regression, we examined the association between EAA (analyzed as continuous and categorical [quartiles] variables) and grade 2+ chemotoxicity, adjusting for demographic, geriatric, and clinical covariates.
Results: The median (range) pre-treatment chronological age was 70 (65-85), 65% had stage II/III disease, 38% had anthracycline, and 75% received G-CSF prophylaxis. A total of 334 (84.8%) participants experienced a grade 2+ toxicity. After adjusting for age, cell composition, stage, race/ethnicity, education, regimen, organ function, and geriatric assessment variables, there was no significant association between the measures of EAA and grade 2+ chemotoxicity (Table).
Conclusions: In this cohort of older adults with early breast cancer, there was no significant association between pre-treatment EAA and grade 2+ chemotoxicity. Further research is needed to examine how measures of biological age can be translated to the clinical care of older patients with breast cancer.

	Multivariable Analysis*	
Measures of EAA	**OR (95% CI)**	**p-value**
Horvath		
Categorical (quartiles, Q4 vs. Q1)	1.35 (0.57-3.16)	0.50
Continuous (each one-year increase)	1.00 (0.94-1.07)	0.96
Hannum		
Categorical (quartiles, Q4 vs. Q1)	1.17 (0.50-2.78)	0.71
Continuous (each one-year increase)	1.00 (0.93-1.08)	0.98
PhenoAge		
Categorical (quartiles, Q4 vs. Q1)	1.41 (0.63-3.16)	0.40
Continuous (each one-year increase)	1.02 (0.96-1.08)	0.59
GrimAge		
Categorical (quartiles, Q4 vs. Q1)	1.12 (0.47-2.67)	0.81
Continuous (each one-year increase)	1.02 (0.94-1.11)	0.66
DunedinPACE		
Categorical (quartiles, Q4 vs. Q1)	1.29 (0.51-3.28)	0.59
Continuous (each one biologic year/chronologic year increase)	1.69 (0.10-28.70)	0.72
*Adjusted for age, cell composition, stage, race/ethnicity, education, regimen, organ function, and geriatric assessment variables.		

#625 Partnering with the community to evaluate policies that regulate cancer clinical trial practice at Henry Ford Health to make it accessible for the Black/African American community in Detroit.

Lisa Lange[1], Donna Harris[2], Martina Caldwell[1], Linda Kaljee[1], Ali Haythem[1], Sophia Chue[3], Valerie Ofori Aboah[1], Harriet Larrious-Lartey[1], Sylvester Antwi[1], Eleanor Walker[1], Dominique Bragg[1], Jorge Chinea[4], Danielle Nelson[1], Camille Romain[1], Denise White-Perkins[1], Evelyn M. Jiagge[1]

[1]Henry Ford Health System, Detroit, MI,[2]Grace Learning Center, Detroit, MI,[3]Caribbean Community Service Center, Detroit, MI,[4]The Center for Latino/a and Latin American Studies, Detroit, MI

Background: Within communities of color, traditional approaches to research have often not benefited and sometimes actually harmed the participants, resulting in a distrust of and reluctance to get involved in clinical trials. The participation of the Black/African American population in clinical trials is essential to develop globally applicable curative, life-extending, life-enhancing cancer therapies using patient centric approaches. The proposed project builds on our Participatory Action for Access to Clinical Trials (PAACT) project which focused on systemic, clinical, and community barriers to Black/African American participation in cancer clinical trials. The proposed project is designed to concurrently address health system policies and programs that affect community engagement, cancer clinical care, and clinical trials.

Method: We documented and assessed the current Henry Ford Health (HFH) policies that govern community engagement, cancer care, and cancer clinical research at Henry Ford Cancer Institute (HFCI). Six main levels of policies were assessed. These include: (1) Cancer clinical trial review processes to determine if the trials are scientifically sound and can be conducted at HFH; (2) Cancer Clinical and Translational Research Office (CCTRO); (3) Feasibility Review Committee (FRC); (4) Scientific Review Committee (SRC); (5) Research Administration; and, (6) Internal & External Institutional Review Boards (IRB). We then engaged with the project Steering Committee consisting of community stakeholders from ten community organizations, other community members, and HFH/HFCI leadership to evaluate the policies and make recommendations for the development of interventions to make the process more acceptable to Black/African American communities.

Results: The interventions that were recommended by the steering committee were put into six categories: (1) Seat at the Table: Valuing People Over the Project; (2) Enhanced Participant Recruitment Approaches; (3) Monitoring Racial/Ethnic Representation at Monthly Clinical Trial Review Meetings; (4) Patient-Centered Informed Consent; (5) Respectful Monetary Incentives for Clinical Trial Participants; (6) Recognition of Healthcare Providers' Time and Effort. We are in the process now of prioritizing and piloting these interventions at HFH.

Conclusion: To increase the participation of *Black/African Americans* in cancer clinical trials, we need to not only focus on patient and community level interventions *ideally,* but, there is the need to evaluate the institutional policies that make the process acceptable to the patients.

#626 Black/African American participation in cancer clinical trials: Healthcare professional perspectives.

Linda Kaljee[1], Sylvester Antwi[1], Doreen Dankerlui[1], Donna Harris[2], Barbara Israel[3], Denise White-Perkins[1], Valerie Ofori Aboah[1], Livingstone Aduse-Poku[4], Harriet Larrious-Lartey[1], Barbara Brush[3], Chris Coombe[3], La'Toshia Patman[5], Nayomi Cawthorne[6], Sophia Chue[7], Zachary Rowe[8], Cassandra Mills[1], Kurt Fernando[3], Gwendolyn Daniels[9], Eleanor Walker[1], Evelyn M. Jiagge[1]

[1]Henry Ford Health System, Detroit, MI,[2]Grace Learning Center, Detroit, MI,[3]University of Michigan, Ann Arbor, MI,[4]University of Florida, Gainesville, FL,[5]Our Wellness Hub, Detroit, MI,[6]Eastside Community Network, Detroit, MI,[7]Caribbean Community Service Center, Detroit, MI,[8]Friends of Parkside, Detroit, MI,[9]Institute for Population Health, Detroit, MI

Purpose: The purpose of this community-based participatory research (CBPR) was to identify barriers and facilitators to Black/African American engagement and inclusion in cancer clinical trials in the Detroit Metropolitan Area. This presentation focuses on data from healthcare professionals at Henry Ford Cancer Institute.
Design: The CBPR approach included the establishment of a Steering Committee composed of researchers, health professionals, and representatives of eight diverse organizations representing African American, African Caribbean, and Ghanaian communities. A qualitative methodology was employed to obtain contextual and experiential data. Fourteen healthcare professionals participated in individual interviews including physicians, surgeons, research nurses and navigators, clinical research coordinators, and cancer trial investigators. Interview guides were developed based on an extensive literature review. Interviews were audio recorded and transcribed. Data were coded and searches were conducted to identify themes within the coded data.
Outcomes: Data were categorized into six constructs: 1. disparities; 2. trust; 3. advantages and disadvantages to trial participation; 4. clinical and system challenges for physicians' trial engagement; 5. trial participant selection criteria; and 6. clinical trial recruitment protocols. Themes within disparities include inequitable access to healthcare and patient selection bias. Patient and community trust and distrust were discussed within historical and experiential abuses and neglect in Black/African American communities. Physicians perceived trial advantages to include access to new therapies and patient-internal rewards, e.g., contributing to wellbeing of future generations. Disadvantages included participants' financial, social, and psychological costs. Physician challenges included awareness of trials, time allocation, and systemic expectations of output. Selection criteria themes were focused on the internal procedures required to identify eligible patients and the barriers associated with trial sponsor requirements. Recruitment barriers included complex consenting procedures and resources to support informed patient decision-making. Study respondents provided recommendations to address barriers for both patients and healthcare providers.
Conclusions: Increasing diversity in clinical trials requires understanding barriers and facilitators at multiple levels including communities, patients and their families, healthcare professionals, health system leadership, and trial sponsors. Recognizing the experiences and perceptions of healthcare professionals is one step toward addressing the complex social environments in which cancer clinical trials reside. Recommendations from this study are being piloted.

#628 Enhancing data integrity in contact registries: Methods for preventing fraudulent enrollment.

Ileana Guzman[1], **Nazleen Del Rio**[1], Brooke Hensel[1], Eduardo Ibarra[2], Carolina Aristizabal[2], John Luque[3], Rosa Barahona[2], Diana Wilkie[4], Mariana C. Stern[2], Lourdes Baezconde-Garbanati[2], Sandra Suther[3], Fern Webb[5]

[1]University of Florida, Orlando, FL,[2]University of Southern California, Los Angeles, CA,[3]Florida A&M University, Tallahassee, FL,[4]University of Florida, Gainesville, FL,[5]University of Florida, Jacksonville, FL

Significant barriers to engaging underrepresented communities in cancer research are the limited access to information, misinformation, mistrust, and, at times, the absence of invitations to participate. Contact registries play a vital role in connecting community members with opportunities in cancer health research, they ensure that more people are informed and included in these efforts. With the use of contact registries, participants gain access to studies aligned with their interests; and have the freedom to choose to participate or not. While contact registries enable the large-scale recruitment of diverse participants, they also have a higher likelihood of attracting fraudulent enrollments. The likelihood drastically increases when an incentive is available upon enrollment. On July 2021 the Florida-California Cancer Research, Education, and Engagement (CaRE^2) Health Equity Center, Community Outreach Core (COC), developed a contact registry designed to engage Black/African American (B/AA) and/or Hispanic/Latinos/a (H/L) individuals in ongoing research studies conducted across our bicoastal center institutions which includes the Florida Agricultural & Mechanical University, the University of Florida, and the University of Southern California. Due to the increased challenges of internet-based recruitment, the study team has implemented methods to filter out fraudulent applications and confirm participant eligibility. The methods include 1) using a REDCap software feature (CAPTCHA) to prevent bots from enrolling, 2) individually reviewing each registry application for any errors, 3) checking for duplicate IP addresses, 4) Using external resources such as voter registration information, social media, and/or Google to verify identity information; and lastly, 5) calling each potential applicant and having them verify the information they provided. Each application is color-coded to identify whether it has been approved (green), denied (red), or pending (purple). Although internet-based recruitment poses a great risk to a study, it in turn provides a time and cost-effective option, allows for recruitment from multiple geographical areas, and facilitates the recruitment of a large number of participants. As of November 8th, 2024, 1,408 participants have enrolled in the registry, representing 70% of the 2,000 enrollment goal. Based on our experience with internet-based recruitment for contact registries, we recommend the following) Implement methods to verify applicant information 2) Utilize security features within the registry application. 3) Incorporate a phone screening process. 4) Consider leveraging external resources, such as Whitepages, for information verification. 5) Maintain detailed records of the verification process.

#629 Providing a safe space for our diverse vulnerable populations to talk about their health concerns across the continuum of cancer care: Lazarex Cancer Wellness HUB pop-up model.

Elena Nieves[1], Rosa Barahona[2], Marya Shegog[3], Dana Dornsife[3], **Lourdes A. Baezconde-Garbanati**[2]

[1]Norris Comprehensive Cancer Center, Keck School of Medicine of USC, Los Angeles, CA,[2]Keck School of Medicine of USC, Los Angeles, CA,[3]Lazarex Cancer Foundation, Danville, CA

Background: The Lazarex Cancer Foundation, in collaboration with USC established the Lazarex Cancer Wellness HUBs pop-up model to provide a welcoming and safe space embracing the cultural and unique cancer challenges of communities that we serve, our diverse populations. A major part of cancer health equity means providing a space representative of the people we serve so we meet them where they are while providing information in a culturally sensitive and language specific way. The HUB offers equitable access to cancer programs and support that could help achieve better results from prevention through treatment and survivorship.
Methods: Between Oct 2021 to July 2024 our HUB has conducted outreach in several YMCAs across LA County with other community-based organizations and churches. We supported community events such as Black Men's Wellness Day, Taste of Soul, and the San Gabriel Lunar Lantern Festival. A series of mindfulness workshops provide interventions that help develop emotional balance and find greater calm and control in the daily lives of those affected by cancer. Our speaker series, facilitated by scientists and invited guest speakers have provided community members with current scientific discoveries including robust overview of cancer, cancer treatment and opportunities available.
Results: Over 8,000+ individuals have received cancer information. Majority have identified as women (over 70%) representing different race/ethnicity designations. Most indicated some level of health insurance with about 50% having a government supported plan. About half shared they received a pre-high school or high school education. Moreover, over 2,000 community members have attended our speaker series that discussed topics such as Oral Care for Cancer Patients, At Your Cervix: How the Pap Smear Helps Prevent and Detect Cancer, LGBTQI+ Identity, Screening, and Risk Factors, and Liver and Hepatitis Cancer. 52 members attended mindfulness workshops that were engaged in mind-body health practices. Finally, a one-day in-person event where 92 community members attended and learned about "The Future in Cancer Therapy: From Participant Engagement to Scientific Discovery" brought together cancer research experts to immerse the community in knowledge.
Conclusion: We created a community outreach approach that allows to be present and visible in our diverse communities in LA County with information tailored culturally and locally. Education and connection to cancer support services are provided to help equip everyone in their cancer journey before, during and beyond. Information provided include cancer screening guidelines, the importance of diversity participation in cancer clinical trials, self or patient advocacy, and access to sources. The actions we are take allow us to help reduce cancer disparities.

TUMOR BIOLOGY: Imaging in Animal Models
Poster Session

#63 Orai1 regulates collagen secretion in pancreatic stellate cells and is enriched in fibrotic cancer-associated fibroblasts in PDAC.

Zoltan Petho[1], Ilka Neumann[1], Andrea Oeckinghaus[2], Albrecht Schwab[1], Rieke Schleinhege[1]

[1]Insitute of Physiology II, University of Munster, Munster, Germany,[2]Insitute of Molecular Tumor Biology, University of Munster, Munster, Germany

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is characterized by a desmoplastic stroma driven by pancreatic stellate cells (PSCs) and cancer-associated fibroblasts (CAFs). The Ca^{2+}-permeable ion channel ORAI1 is known to influence PSC proliferation and activation, but its role in collagen secretion and tumor fibrosis remains unclear.
Experimental Procedures: Using the PSC cell line PS-1, we developed and validated a high-throughput fibrosis assay based on the collagen-binding peptide CNA-35-tdTomato. Collagen secretion was assessed following treatment with transforming growth factor β1 (TGF-β1) and/or vitamin C, with ORAI1 inhibition achieved via siRNA or Synta-66. In vivo, Orai1 expression was examined by immunohistochemistry in the genetically engineered murine PDAC model KPfC ($Kras^{wt/LSL-G12D}$ $Tp53^{fl/+}$ $PDX1^{Cre+}$). The intracellular Ca^{2+} concentration was assessed with the fluorescent Ca^{2+} indicator Fura-2.
Results: TGF-β1 and vitamin C synergistically enhanced collagen deposition in PSCs, a process regulated by ORAI1. Vitamin C induced a significant rise in intracellular Ca^{2+} in PSCs, which was attenuated by the ORAI inhibitor Synta-66. ORAI1 expression was detected in αSMA^{+} CAFs in KPfC-derived PDAC tissue, with a higher prevalence in collagen-rich fibrotic tumor regions compared to non-fibrotic areas or non-tumorous tissue. In addition, vessel-lining endothelial cells also express ORAI1.
Conclusions: Our findings highlight ORAI1 as a regulator of collagen secretion in PSCs and reveal its enrichment in CAFs within fibrotic PDAC stroma, underscoring its potential role in PDAC-associated desmoplasia.

CLINICAL RESEARCH: Cancer Care for All: Innovations in Geriatric Oncology and Broadening Research Representation Poster Session

#630 Directly engaging participants in rare cancer research is feasible: the osteosarcoma and leiomyosarcoma projects.

Julia M. W. Wong[1], David Merrell[1], Eirian Siegal-Botti[1], Noorshifa Arssath[1], Carrie Cibulskis[1], Evelina Ceca[2], Alanna Church[3], Alex Wilson[1], Lorena Lazo De La Vega[2], Jill Stopfer[2], Ellen Sukharevsky[2], Nia Daley[2], Anusha Sharma[2], Sidney Benich[2], Zachary Kahn[2], Lauren Fisher[2], Parker Chastain[1], Brendan Reardon[2], Taisha Hendrickson[1], Colleen Nguyen[1], Melissa Chiumiento[1], Melissa Mirick[1], Noriela Elia[1], Priscilla Merriam[2], Eliezer Van Allen[2], Judy Garber[2], Riaz Gillani[2], Chandrajit Raut[4], Stacey Gabriel[1], Timothy Rebbeck[2], Jason L. Hornick[4], Jennifer Mack[2], Suzanne George[2], Diane Diehl[1], Gad Getz[1], Katherine A. Janeway[2]

[1]Broad Institute of MIT and Harvard, Cambridge, MA,[2]Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Boston, MA,[3]Boston Children's Hospital, Boston, MA,[4]Brigham and Women's Hospital, Boston, MA

Background and Purpose: Osteosarcoma (OS) and Leiomyosarcoma (LMS) are sarcomas with complex genomes for which there has been limited progress in identifying new treatments and improving outcomes. While slow progress is partially due to insufficient genomic characterization, generating large genomic datasets has been challenging because these are rare cancers. The OS and LMS Projects use various approaches to directly engage pediatric and adult participants with OS and LMS in genomics research.
Methods: Working with patients and advocates at the design stage, we created websites (OSProject.org and LMSProject.org) where patients register and consent to participation. Any patient with OS or LMS living in the United States or Canada is eligible. Participant outreach approaches include partnership with advocacy organizations, webinars, social media posts, meeting presentations, stakeholder and physician engagement committees, and direct mailings. Blood and saliva are collected directly from consented participants by mail, archival FFPE tumor samples are obtained from pathology departments and medical records are requested from treating institutions. WES, WGS, DNA panel sequencing and RNASeq of tumor and germline (T/N) is performed. Results are shared with patient, advocacy, physician, and research communities in several ways. Individual participants receive a shared learning report describing the somatic variants identified in their tumor from T/N clinical WES and are offered clinical germline genetic testing and genetic counseling.
Results: The study outreach team has participated in or led a total of 33 online and in person events not including social media posts or stakeholder meetings. So far, in 26 months 515 LMS patients (ages 16-83y; median 55) and 145 OS patients (ages 7-74y; median 20) have consented. Thus far, 274 and 76 tumor samples and 555 and 127 germline samples have been obtained from LMS and OS consented participants, respectively. Analysis of the first 20 LMS participants with T/N WGS showed widespread alteration of TP53 (11 pts), RB1 (8 pts), and PTEN (16 pts), concordant with findings in past studies. Likewise, the first 7 OS pts with T/N WGS demonstrated inactivation of TP53 (4 pts) and RB1 (2 pts), as expected from past OS cohorts.
Conclusions: Using community partnerships and direct outreach to connect and engage with participants and a virtual consenting process for genomics research in rare cancers is feasible. It is possible to obtain germline samples directly from about half of participants and archival tumor samples from treating pathology departments for about one third of participants consented in direct-to-patient online genomics studies. We have been able to utilize archival tumor samples to identify, with T/N WES/WGS, expected genomic events in complex genome cancers. Recruitment and sequencing are ongoing.

#631 Building bridges between communities and cancer researchers through the CaRE^2 Health Equity Center Community Scientist Research Advocacy Program.

Carolina Aristizabal[1], Fern Webb[2], Sandra Suther[3], John Luque[3], Eduardo Ibarra[1], Nazleen Del Rio[2], Ileana Guzman[2], Brooke Hensel[2], Janet Rodriguez[1], Rosa Barahona[4], Mariana Stern[5], Lourdes A. Baezconde-Garbanati[4]

[1]University of Southern California, Los Angeles, CA,[2]University of Florida, Orlando, FL,[3]Florida Agricultural and Mechanical University, Tallahassee, FL,[4]Keck School of Medicine, University of Southern California, Los Angeles, CA,[5]University of Southern California Norris Comprehensive Cancer Center, Los Angeles, CA

Building healthy and trustworthy relationships between communities and researchers is essential for cancer research endeavors. Training community advocates in cancer research can increase capacity among community members to leverage networks, participate in research teams and/or boards, and increase knowledge and insights to I'm prove patient outcomes through increased participation in cancer research. The Florida-California Cancer Research Education and Engagement (CaRE^2) Health Equity Center designed a Community Scientist Research Advocacy (CSRA) program to inform, educate, and empower community members to become cancer research advocates. Our program was specifically tailored for Black/African American (B/AA) and Hispanic/Latino/a (H/L) adults living in Florida or California. Primary program objectives were to increase human capacity for cancer research advocacy and increase multi-directional communication between cancer advocates with cancer survivors, community members, academic scientists, and policy makers. The CSRA program was delivered over a 10-week period, with a hybrid model compromised of 90% virtual sessions via Zoom to allow for joint participation across the two coasts, and one in-person experiential lab day at each of our three institutions from our partnership. Didactic online learning sessions covered topics including cancers that disproportionately affect medically underserved populations, participation in clinical trials, genomics, social determinants of health, tissue donation/biorepository, and ethics in research studies. Participants completed pre- and post-surveys to assess knowledge, self-efficacy, and program satisfaction and evaluation; and they also completed a mentored advocacy project upon completion of the program. We provided simultaneous Spanish interpretation during all sessions, and materials were translated and provided in both English and Spanish. The 2024 CSRA cohort (N=18) included 12 females and 6 males; 11 were B/AA and 7 were H/L, 11 were from Florida and 7 were from California. Participants were required to attend at least 80% of sessions including one mandatory in-person lab visit activity; leading to a 100% program completion. When comparison pre- and post-attendance surveys, participants showed increases in knowledge (p-value = 0.007) and self-efficacy (p-value = 0.3). Program satisfaction and evaluation were rated as high. In summary, our program demonstrates the effectiveness of training community members to become cancer research advocates to help reduce the burden of inequities in cancer research and care. CSRA programs increase communication between scientists and communities to bring education and awareness about clinical studies and cancer research. Thus, promoting diverse participation in cancer studies, and eventually reducing cancer health disparities.

CLINICAL RESEARCH: Combination Immunotherapies
Poster Session

#635 Unraveling the potential of neogenin 1 in non-small cell lung cancer: A path toward innovative combination therapies.

Asmita Pandey[1], Shaoqiu Chen[2], Li Ma[2], Mayumi Jijiwa[2], Masaki Nasu[2], Vedbar Khadka[2], Yu Chen[2], Hua Yang[2], Sudhir Rai[2], Youping Deng[2]

[1]Department of Tropical Medicine, Medical Microbiology, and Pharmacology, John A. Burns School of Medicine University of Hawaii at Manoa, Honolulu, HI,[2]Department of Quantitative Health Sciences, Bioinformatics Core, John A. Burns School of Medicine University of Hawaii at Manoa, Honolulu, HI

Non-small cell lung cancer (NSCLC) has a poor survival rate due to delayed diagnosis and treatment resistance. Currently, immunotherapy has advanced the NSCLC treatment; however, only a small fraction of patients respond to the therapy, demanding the identification of novel biomarkers associated with resistance to develop new therapeutic strategies. Herein, we investigated the role of neogenin 1, a transmembrane protein that belongs to the immunoglobulin superfamily, in seven different human NSCLC cell lines and compared the expression with Jurkat T cell line and human peripheral blood mononuclear cell (PBMC)-derived CD3/CD28-activated CD8+ T cells using qPCR and western blot. We also performed the cell proliferation assay in adenocarcinoma (LUAD) and squamous cell carcinoma (LUSC) human cell lines after knockdown and overexpression of neogenin 1. We found that neogenin 1 was highly expressed in primary CD8+ T cells compared to the cancer cell lines, implying that genetic alteration of neogenin 1 might interfere more with CD8+ T cell effector function. We also discovered that both adenocarcinoma and squamous cell carcinoma cell proliferation ability were influenced by genetic alteration of neogenin 1. In addition, gene ontology enrichment analysis and KEGG pathway analysis indicated that neogenin 1 was involved in the TGF-beta signaling pathway, possibly suggesting that neogenin 1 might play a role in therapeutic resistance, including immunotherapy, in NSCLC. Correlation analysis in LUAD and LUSC in the TCGA dataset also revealed that neogenin 1 was positively correlated with a set of genes, such as IGF1R and USP8, associated with immunotherapy resistance. Further investigation of this potential gene *in vivo* will unfold its role in immunotherapy resistance in NSCLC, paving the way for developing novel and effective combinatorial therapeutic strategies targeting neogenin 1.

#636 ABT-863: The next generation anti-CCR8 immuno-oncology antibody therapeutic.

Pia Kuss, Karen Chiang, Alexander Alexandrov, Le Li, Riccardo Contu, Christopher Roth, Anke Kretz-Rommel, Mauro Mileni

Abilita Therapeutics, San Diego, CA

The chemokine receptor CCR8 has come into focus based on its upregulation on immune suppressive Tregs within the tumor microenvironment in a range of solid tumors, eg breast, colon, and lung cancers. This finding has spurred efforts to develop CCR8 targeting antibodies (mAbs) that can selectively target and deplete this Treg population to augment the antitumor immune response and the efficacy of cancer therapies, including checkpoint inhibitors. To date, the development of several clinical antibodies primarily focuses on the depletion of CCR8-expressing Tregs through enhanced Antibody Dependent Cellular Cytotoxicity and Antibody-Dependent Cellular Phagocytosis. While these antibodies have been reported to compete with CCL1, CCR8's endogenous ligand, the simple inhibition of the receptor, without a depletion mechanism, has failed to demonstrate tumor suppression in mouse models. Overall, the impact of CCR8 expression on the Treg phenotype and its involvement in cancer progression remains to be fully elucidated. Abilita Therapeutics has instead focused on maximizing the inhibition of CCR8 function by developing potent inverse agonists based on single domain heavy chain antibody (VHH) scaffolds that engage the receptor's orthosteric pocket in the transmembrane domain to inhibit both CCL1 agonism and ligand-independent signaling. Our development candidate ABT-863 was discovered using Abilita's proprietary Enabled Membrane Protein (EMP) platform. EMP technology was leveraged to generate a stabilized CCR8 variant, which was key to llama immunization, protein-based antibody selections, and affinity maturation of the humanized VHH. ABT-863 is formatted as a bivalent VHH-Fc fusion that demonstrates excellent affinity, selectivity, and an optimal developability profile. In preclinical studies using human CCR8 knock-in mice, ABT-863 demonstrated significant tumor growth inhibition in both the MC38 (murine colon carcinoma) and CMT167 (murine lung carcinoma) models. Remarkably, an Fc effector-null version of the antibody demonstrated efficacy in absence of Treg depleting activity, both as monotherapy or in synergy with anti-PD1, which contrasts with what others have reported. Because of its distinct pharmacology, this mechanism may offer more robust modulation of the TME and immune responses, potentially enhancing therapeutic efficacy, while avoiding unnecessary safety risks of depleting Tregs in healthy tissues or other CCR8-expressing cell types. In conclusion, ABT-863 is a differentiated first-in-class inverse agonist antibody with the potential to provide superior efficacy, improved safety and more favorable patient outcomes.

#637 Single-cell analysis of systemic immune response to sequential radiation and immune checkpoint blockade in metastatic colorectal cancers.

Joshua Brand[1], Sean G. Kraus[2], Michael Bassetti[3], Dustin A. Deming[2], Huy Q. Dinh[2]

[1]Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison, Madison, WI,[2]Department of Oncology, University of Wisconsin-Madison, Madison, WI,[3]Department of Human Oncology, University of Wisconsin-Madison, Madison, WI

Immune checkpoint blockade (ICB) has shown significant clinical efficacy in mismatch repair deficient colorectal cancers (CRCs). However, an effective means to use these therapies for mismatch repair proficient (pMMR) CRC has yet to be developed. To enhance the efficacy of ICB for patients with oligometastatic pMMR CRC, we performed a clinical trial evaluating the sequential use of stereotactic body radiotherapy (SBRT), pembrolizumab, surgical resection, and then adjuvant pembrolizumab (NCT02837263). The primary endpoint of 1-year recurrence-free survival (1 yr RFS) was met in 60% of trial participants compared to a historical control of 50%. From this trial, 13 subjects could have blood samples collected for peripheral blood mononuclear cell isolation pretreatment and after operative management. We hypothesize that the systemic immune response changes upon combined treatment and is distinct between responders and non-responders. We performed scRNA-Seq on these samples to compare the circulating immune cell populations with clinical outcomes. Despite a small sample size, integrated bioinformatics analysis identified (i) pre-existing CD8+ T cells were observed with treatment response ($p = 0.031$) (ii) combined RT and ICB induces changes in myeloid cell phenotypes, including reduced pro-inflammatory monocytes ($p = 0.038$) and conventional cDC2 ($p = 0.030$) in subjects without recurrence and increasing pDCs ($p = 0.024$) in most patients. CD8+ T cells from those recurrence-free subjects showed terminally differentiated effector phenotypes, expressing *CX3CR1*, *GZMB*, and *KLRG1* before treatment. cDC2s expressed interferon activity markers, including *STAT1*, *IFI44*, and I*RF7,* that presented a more activated state. CD8+ T cell abundance remained stable, with no significant changes before and after treatment. In contrast, myeloid cells showed greater variability, reflecting unique polarization states through differences in cytokine (*CCL3*, *CXCL8*), interferon (*ISG15*, *IFI44*), and complement (*C1QA*, *C1QB*) associated genes. Future work will increase sample size in independent patient cohorts to elucidate the extent to which circulating phenotypes are also identified in tumors. Overall, this study demonstrates pre-existing T cell abundance and changes in myeloid cell phenotypes may be associated with clinical outcomes from combined immunotherapy, which have the potential to serve as predictive biomarkers for therapeutic response.

#638 Discovery of HX016-7, a novel PD-L1 x VEGF BsAb, as a new candidate treatment of solid tumors.

H. Ke[1], T. Yang[1], F. Peng[1], J. Li[1], C. Chen[1], S. Xiong[2], L. Zhang[1], F. Zhang[1], **H. Q. Li**[1];
[1]Hanx Bio, Hangzhou, China, [2]Crown Bioscience, Taicang, China

A first-in-class PD-L1xVEGF bispecific antibody (BsAb), BNT327/PM8002, has demonstrated promising efficacy in treating TNBC and NSCLC cancers in advance stage of clinical development, which prompted us to engineer a novel and potentially best-in-class (BIC) PD-L1xVEGF BsAb by using a validated anti-PD-L1 and a novel anti-VEGF (HX006, a novel VEGF mAb with more favor activity over Avastin) mAb sequences. HX016-7 has 2+2 IgG1 symmetric structure constructed by grafting the PD-L1 binding domain of PD-L1 mAb onto the N-terminus of HX006. HX016-7 binds with similarly affinity to PD-L1 recombinant protein with EC_{50} of 0.27nM (vs. 0.36nM by BNT327/PM8002) and to VEGF recombinant protein with EC_{50} of 0.094nM (vs. 0.063 by BNT327) in ELISA assay. It also binds similarly to the PD-L1 positive cell line as BNT327/PM8002 with comparable affinity. Reporter-based bioassay for PD1/PD-L1 blockade function demonstrated that HX016-7 has EC_{50} of 4.85 nM (vs. 5.13nM for BNT327/PM8002), while reporter-based bioassay for VEGF function demonstrated that HX016-7 has EC_{50} of 11.7nM (vs. 15.8nM for BNT327/PM8002). In a MC38-hPD-L1-hVEGF colorectal cancer syngeneic model implanted in hPD-1 humanized mice, HX016-7 was shown to have comparable anti-tumor activity as BNT327/PM8002, but stronger activity over HX006, which can be attributed to both PD-L1- and VEGF-targeting. In a subcutaneous A549 non-small-cell lung cancer (NSCLC) cell-derived xenograft model in immunocompromised mice, HX016-7 also showed slightly stronger anti-tumor activity over BNT327/PM8002, which can be attributed to anti-VEGF function. We believe these preclinical data warrants the candidacy of HX016-7 to be further developed for the treatment of solid tumors.

#639 Targeting TET3 potentiates an effective anti-tumor immune response after irradiation.

Sera Averbek, Stephen J. Kron

Ludwig Center for Metastasis Research and Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL

The benefits of radiotherapy (RT) have long been appreciated as limited by formation and repair of DNA double-strand breaks (DSBs) in tumor cells, but recent understanding has identified a key role for RT and DNA damage in promoting inflammation that drives anti-tumor immune response. While chromosomal DSBs indeed directly limit cancer cell survival and proliferation, RT-induced damage leads to cytosolic DNA that activates cGAS/STING/IRF3 signaling and drives IFNα/β expression, recruiting cytotoxic T and NK cells to shrink tumors. In turn, along with DSB repair, another determinant of RT resistance is rebound immunosuppression. Here, IFNγ released by the infiltrating lymphocytes activates JAK/STAT to induce interferon-stimulated genes (ISGs) including PD-L1 that can reduce tumor inflammation and restore growth. Though combining PD-1/PD-L1 blockade with RT is sufficient to block this adaptive response in mice, the lack of benefit to patients suggests the need to more broadly target immunosuppressive ISG expression. Targeting IFNγ signaling by blocking JAK/STAT may limit immunosuppressive ISG expression but will impair T and NK cytotoxicity. Looking downstream, blocking JAK/STAT-dependent ISG promoter CpG demethylation by Ten Eleven Translocation (TET) dioxygenase enzymes may prevent PD-L1 expression without affecting cytotoxicity. In this study, we disrupted TET activity with small-molecule inhibitors and CRISPR RNP knockouts (KO) to evaluate effects on radiation response *in vitro* and in tumors. As predicted, TET inhibitors blocked PD-L1 induction in irradiated CT26 murine colon carcinoma cells. Assaying TET1, 2 and/or 3 KO CT26 cells for PD-L1 induction identified TET3 as the critical mediator. TET3 KO cells also exhibited slower DSB repair after irradiation, associated with markedly increased ssDNA resection and cytosolic DNA. The irradiated TET3 KO cells also displayed enhanced IFNα/β expression, suggesting intact cGAS/STING/IRF3 signaling. While TET3 KO CT26 cells formed tumors in syngeneic BALB/c mice much like CT26 controls, RT produced greater NK cell infiltrates by one week and increased tumor elimination by two weeks. TET3 KO 4T1 mammary carcinoma cells formed orthotopic tumors in BALB/c mice but produced fewer lung metastases compared to controls. In conclusion, TET3 may offer an attractive, druggable target for radiosensitization by slowing DSB repair, enhancing tumor inflammation, and blocking immunosuppressive ISG expression.

TUMOR BIOLOGY: Imaging in Animal Models
Poster Session

#64 Depleting CD38 leads to an aggressive phenotype of multiple myeloma.

Michael Riley Dyer, Alexander Zheleznyak, Rui Tang, Julie Prior, Erin Teubner, Brad Manion, Mikhail Y. Berezin, Junwei Du, Julie O'Neal, Monica Shokeen

Radiology, Washington University School of Medicine in St. Louis, St. Louis, MO

Introduction: Multiple myeloma (MM), the second most common hematological cancer in the United States, is characterized by the proliferation of malignant clonal plasma cells within the bone marrow. Since the FDA approval of Daratumumab in 2015, CD38-targeted immunotherapies have significantly advanced the treatment of MM, improving patient response. However, due to the multifunctional role of CD38 in the complex bone microenvironment, its role in MM remains under investigation. This study examined the effect of modulating CD38 expression by using a CD38 knock-out (KO) clone of a human MM cell line xenografted into immunodeficient mice.
Methods: CRISPR/Cas9 gene editing was used to KO CD38 expression in the MM.1S human MM cell line, confirmed with RNA sequencing and flow cytometry. After tumor implantation in mice, bioluminescence imaging (BLI) monitored tumor burden. PET was performed using [^{18}F]-FDG to detect metabolic activity and [^{89}Zr]-Daratumumab to analyze human CD38 expression throughout the body. Post-PET tissue biodistribution and select bone autoradiography were performed to evaluate the specificity of tracer uptake. CT, dual-energy X-ray (DEXA), and immunohistochemistry were used to quantify changes in bone mineral density and osteolytic disease. The imaging results were corroborated with changes in blood composition and cytokine production in the microenvironment.
Results: After the cells were implanted into mice, BLI showed a significant tumor burden increase in CD38 KO MM models compared to WT MM models at 4 weeks. CT and DEXA imaging revealed that the KO mice had significantly decreased bone density, and similarly, planar X-ray imaging indicated severe myeloma bone disease characterized by cortical and trabecular osteolytic lesions in the femur and tibia. [^{89}Zr]-Daratumumab accumulated in the spine, femur, and tibia in WT MM mice, with minimal bone uptake in CD38 KO MM mice. [^{18}F]-FDG PET revealed an increased metabolic activity of CD38 KO MM, with an aggressive kinetic uptake of the tracer. Blood analysis indicated elevated markers of disease progression and renal dysfunction, with reduced leukocyte and thrombocyte production in CD38 KO MM xenografts. Cytokine profiling revealed altered levels of pro-inflammatory cytokines, including IL-6, RANKL, and VEGF-α in the bone microenvironment of MM with depleted CD38. RNA sequencing showed significant transcriptional changes in CD38 KO cells, particularly in cell adhesion and inflammatory pathways.
Conclusions: The findings underscore CD38's critical role in MM progression, where its depletion drives an aggressive phenotype marked by widespread metastasis, extensive bone degradation, renal involvement, and heightened immunosuppression. These results showcase the critical importance of CD38 expression levels and suggest a potential role for CD38 and related transcriptional changes in contributing to the aggressive phenotype of MM.

CLINICAL RESEARCH: Combination Immunotherapies
Poster Session

#640 Augmenting T-cell bispecific antibody therapy in AML through co-stimulatory agonists.

Anne-Sophie Neumann[1], Gerulf Hanel[1], Vesna Kramar[2], Christina Claus[2], Kieron White[1], Amelie Muth[1], Lis Winter[1], Monika Sponheimer[1], Giulia Rappa[1], Sebastian Theurich[1], Veit L. Bucklein[1], Pablo Umana[2], Christian Klein[3], Marion Subklewe[1]

[1]Department of Medicine III, University Hospital LMU Munich, Munich, Germany,[2]Pharmaceutical Research & Early Development, Roche Innovation Center Zurich, Schlieren, Switzerland,[3]Curie.Bio, Zurich, Switzerland

The classical two-signal model states that T-cell activation requires TCR/CD3 engagement (signal 1) and co-stimulation (signal 2) via e.g. CD28 or 4-1BB. T cell bispecific antibodies (TCBs) activate T cells solely through CD3 engagement, bypassing co-stimulatory pathways. However, the efficacy of TCB-mediated cytotoxicity against acute myeloid leukemia (AML) cells is determined by co-stimulatory and co-inhibitory ligands expressed on AML cells (Marcinek et al., 2023). Thus, we hypothesized that TCB-mediated T-cell responses could be enhanced by combination with targeted co-stimulation, particularly in AML cases lacking co-stimulatory ligands. Accordingly, we evaluated the combination of the AML-targeting Wilms tumor 1 (WT1)-TCB with co-stimulatory agonistic bispecific antibodies, targeting 4-1BBL or OX40 and fibroblast activation protein (FAP) expressed on bone marrow stromal cells.Efficacy of WT1-TCB + FAP-4-1BBL or FAP-OX40 was evaluated in co-culture assays with human AML cell lines or murine Ba/F3 cell lines expressing hu4-1BBL or huCD86, healthy donor T cells and an NIH-3T3 fibroblast cell line expressing FAP. In addition to short-term assays (4 days), we co-cultured cells over 28 days to address T-cell exhaustion in the context of continuous target antigen exposure. Lastly, we investigated the impact of co-stimulation in an *ex vivo* setting with primary AML cells (pAML). T-cell activation, phenotype, cytotoxicity, and metabolic fitness were assessed by MPFC, bulk RNA sequencing, and Seahorse assays. Addition of FAP-4-1BBL strongly enhanced WT1-TCB-mediated cytotoxicity against the AML cell line Set-2, which shows a low expression of co-stimulatory ligands. Although FAP-OX40 did not enhance cytotoxicity, it increased T-cell proliferation. T-cell responses against AML cell lines with high expression of co-stimulatory ligands (SKM-1, OCI-AML3, OCI-AML2) did not show any improvement by the combinatorial approaches. The results were confirmed in the murine Ba/F3 system, showing that especially the presence of hu4-1BBL on the cell surface cancels out the positive effect of the combinatorial approaches. Results could be validated using pAML samples. Importantly, considering the continuous application of TCBs in the clinics, long-term cultures over 28 days showed that adding FAP-4-1BBL or FAP-OX40 preserved T-cell function (proliferation, cytokine secretion, cytotoxicity, and metabolic fitness) over time compared to WT1-TCB alone. Improvements were again stronger and more durable for the combination with FAP-4-1BBL than with FAP-OX40. We conclude, that combining a WT1-targeting TCB with a 4-1BB agonist significantly enhanced T-cell activity against AML cell lines and primary AML samples. Notably, this positive immune modulation persisted in long-term cultures, counteracting T-cell exhaustion caused by continuous TCB stimulation.

#641 Identification of anti-tumoral neutrophil phenotypes induced by immunotherapies in head and neck cancer.

Anqi Gao[1], Amy Erbe-Gurel[1], Natalie B. Winn[1], Joel Matthews[1], Kaia N. Heimstreet[1], Wei Wang[2], Paul F. Lambert[1], Paul M. Sondel[1], Huy Q. Dinh[1]

[1]University of Wisconsin-Madison, Madison, WI,[2]Michigan State University, East Lansing, MI

Only ~20% of recurrent and metastatic head and neck squamous cell carcinoma (HNSCC) patients respond to immune-checkpoint blockade (ICB) therapy. A major roadblock is the immunosuppressive nature of the tumor microenvironment. Increased infiltration of neutrophils in blood and tumors of HNSCC patients is associated with worse prognosis and ICB therapy resistance. Depleting neutrophils, however, with or without ICB treatment, has not inhibited tumor growth in mice bearing syngeneic MOC2 tumors, which are phenotypically analogous to immunologically “cold”, aggressive HNSCC tumors. We hypothesize that this is due to the heterogeneity of tumor-infiltrated neutrophils with both pro- and anti-tumoral phenotypes. This study uses multi-omics single-cell profiling, combining RNA and protein, to identify pro- and anti-tumoral neutrophil states in MOC2 tumors treated with recently reported neutrophil-related therapies. Specifically, we tested anti-CD40 agonist, TNF-alpha, and both in combination with cetuximab, an anti-tumor antibody (designated Neutrophil Activating Therapy, NAT) that has been recently reported in other cancers. Cetuximab (an anti-EGFR mAb) was used for MOC2-huEGFR (M2h), an HNSCC cancer model transduced with human-EGFR. We found this treatment induced new neutrophil subsets, defined by scRNA-Seq bioinformatics analysis. One subset has recently reported interferon-stimulated phenotypes, which we found in the anti-CD40 alone, and NAT combinatorial treatment groups; these two groups also showed the best anti-tumor efficacy. Gene signature of this subset is also found in scRNA-Seq of infiltrating neutrophils in our previous K17-KO MOC2 model, which shows spontaneous tumor regression in the absence of therapy. In addition, we identified a neutrophil subset associated with migration-related transcription factors only in NAT and TNFa treatment groups, which might explain why NAT is superior to anti-CD40 alone. Our findings thus far demonstrate the presence of neutrophil heterogeneity, with some phenotypes associated with anti-tumor responses, including a potentially new phenotype, in a murine head and neck cancer model induced by a combination of immunotherapies. Ongoing and future work includes identifying specific synergistic mechanisms of the anti-tumoral neutrophil subpopulations induced by anti-CD40 and TNF-alpha treatment and if these phenotypes are also seen in ICB-treated HNSCC patients who respond to their therapy. We anticipate our characterization of anti-tumoral neutrophil phenotypes induced by NAT in M2h tumors may provide insights into how more effective immunotherapy regimens beyond ICB can be designed to confer tumor clearance and prolonged survival in patients with head and neck cancer.

#642 Peripheral immune cells associated with toxicity to ipilimumab plus nivolumab checkpoint blockade in patients with metastatic melanoma.

Jiajia Chen[1], Joanna Baginska[1], Giuseppe Tarantino[1], Kelly Burke[1], Elad Sharon[2], Anita Giobbie-Hurder[1], Jason L. Weirather[1], Michael Manos[1], Janice D. Russell[1], Ryan Brennick[1], Matthew Nazzaro[1], Emma Hathaway[1], Marta Holovatska[1], Claire Manuszak[1], Srinika Ranashinge[1], David Liu[1], F. Stephen Hodi[1]

[1]Dana-Farber Cancer Institute, Boston, MA,[2]National Institutes of Health, Bethesda, MD

Background: Immune checkpoint blockade (ICB) has revolutionized clinical outcomes in melanoma. Compared with single ICB (aCTLA-4, aPD-1), combination ICB aCTLA-4 plus aPD-1 shows the highest response rate, with 50%-60% patients responding to treatment. However, it also has the highest toxicity rate, with about 50% patients developing high grade toxicity. Identifying circulating immune cells as biomarkers to predict toxicity could guide clinical decisions and shed light on potential mechanisms driving toxicity.
Methods: We identified and quantified circulating immune cells proportions using CyTOF (cytometry by Time-Of-Flight), measured cytokines using Luminex, and collected complete blood counts from 89 patients with metastatic melanoma treated with combination therapy. Samples were obtained from three points in time: pre-treatment, one month and approximately three months after treatment started. We then evaluated the association between circulating immune cells, cytokines, clinical labs values, and high grade toxicities (grade >=3 or grade 2 requiring treatment). We also investigated the co-occurrence patterns of toxicity at various organs systems, as well as the onset timing of different grades of toxicities.
Results: Most high grade toxicities occurred between 3 and 18 weeks after start of therapy, consistent with prior studies. Interestingly, we found a statistically significant mutual exclusivity of high grade lung and gastrointestinal toxicities (Fisher's exact, $p = 0.01$, OR = 0). In contrast to prior results, we found that in pre-treatment blood samples higher naïve T cells, lower central memory T cells (TCM) and effector memory T cells (TEM) are associated with development of high grades toxicities (MWW, $p = 0.002$, $p = 0.043$, $p = 0.009$). A greater increase in CD8+ TEMs from pre to post1 time points (MWW, $p = 0.047$), while a lower increase in basophils (MWW, $p = 0.030$), was associated with patients with high grade toxicities.
Conclusions: Collectively, our findings help gain more insights into the development of toxicities, and could ultimately guide the development of personalized strategies for toxicities prevention, surveillance, and mitigation in melanoma patients treated with combination immunotherapy.

#643 Efficacy and safety comparison of CAR-T and blinatumomab immunotherapy as bridge-to-transplant strategy in relapsed/refractory B cell acute lymphoblastic leukemia.

Wenyue Cao[1], Ningwen Li[2], Na Wang[2], Yang Cao[2]

[1]Texas A&M University, Houston, TX,[2]Department of Hematology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Background: B cell acute lymphoblastic leukemia (B-ALL) remains a therapeutic challenge despite advances. Hematopoietic stem cell transplantation (HSCT) provides a potential cure but is hindered by various limitations. Emerging immunotherapies, including chimeric antigen receptor T cell (CAR-T) therapy and Blinatumomab, have shown potential as bridging strategies to HSCT in relapsed/refractory (R/R) cases.
Methods: A retrospective study was conducted at Tongji Hospital from March 2017 to March 2023, involving 36 R/R B-ALL patients who underwent HSCT. Prior to transplantation, 27 patients received CD19/CD22 CAR-T therapy, while 9 received Blinatumomab. Outcomes assessed included the overall survival (OS), progression-free survival (PFS), graft-versus-host disease-free and relapse-free survival (GRFS), and non-relapse mortality (NRM) , with comparisons made between treatment groups. Hematopoietic reconstitution and transplant-related complications were also evaluated.
Results: The median follow-up time was 28.07 (range: 2.29-92.21) months. The 2-year OS, PFS, GRFS, and NRM of the entire cohort were 76.54%, 54.97%, 40.12%, and 9.93%, respectively. In the CAR-T and the Blinatumomab treatment group before transplantation, the 2-year OS was 73.89% and 88.89% (P=0.862), PFS, was 59.03% and 44.44% (P=0.501), GRFS was 47.86% and 13.89% (P=0.083), and NRM was 8.52% and 11.11% (P=0.713), respectively. Safety profiles were similar with no significant differences observed in hematopoietic reconstitution, infection, the incidence of grade II-IV acute graft-versus-host disease (GVHD) and chronic GVHD incidence between CAR-T and Blinatumomab groups.
Conclusion: CAR-T and Blinatumomab therapies demonstrate comparable safety and efficacy as bridging treatments to HSCT in patients with R/R B-ALL. Further studies are needed to optimize these treatment strategies.

#644 Cytokine-induced memory-like NK cells with tafasitamab show efficacy against B-cell acute lymphoblastic leukemia.

Dimitrios Filioglou, Geovana Leite, Helena Batatinha, Nina Santa-Cruz, Dan Davini, Richard Simpson, Emmanuel Katsanis

University of Arizona, Tucson, AZ

Introduction: While chimeric antigen receptor (CAR)-T cells can be effective in treating B-cell acute lymphoblastic leukemia (B-ALL), Natural Killer (NK) cell therapies are showing promise as an easier to manufacture product with potent activity in early studies. Cytokine-induced memory-like NK cells (CIMLNK) are generated by brief stimulation with the cytokines IL-12, IL-15, and IL-18 and demonstrate enhanced anti-cancer responses. Tafasitamab (TAFA) is an Fc-enhanced humanized anti-CD19 monoclonal antibody designed to enhance anti-tumor activity through antibody-derived cellular cytotoxicity (ADCC), a function primarily mediated by NK cells. Therefore, we hypothesized that combining TAFA with CIMLNK can augment responses against B-ALL.
Methods: NK cells were isolated from peripheral blood mononuclear cells (PBMCs) of healthy volunteers. For the generation of CIMLNK, NK cells were incubated overnight with IL-12, IL-15, and IL-18. For flow cytotoxicity assays, target cells (NALM-6, a B-ALL cell line) were co-incubated with the effectors (NK or CIMLNK) at 2.5:1 effector: target (E: T) ratio, with or without TAFA (1 µg/ml). Degranulation and IFN-γ release assays were performed to examine mechanistic differences between effector responses. CD16 blocking assays were done using a purified anti-CD16 mAb to assess the role of CD16 binding in TAFA-induced ADCC. In vivo, irradiated NSG mice received 1 x 10^4 Nalm6-luc intravenously (i.v.). The next day, 1 x 10^6 CIMLNK were injected i.v., with TAFA given at 0.3 - 0.5 mg/kg, i.v. or intraperitoneally (i.p.) respectively. Statistical analyses were performed using GraphPad Prism v10. P values of < 0.05 were considered significant.
Results: Cytotoxicity assays showed that CIMLNK + TAFA resulted in significantly higher killing of NALM6 compared with CIMLNK alone 84.4% vs 22.7%, (p=0.0005) and NK+TAFA 84.4% vs 38.3%, (p=0.0006). CIMLNK alone demonstrated significantly higher cytotoxicity against NALM6 compared to NK cells, 22.7% vs 1.2% (p=0.02). Cytotoxicity was not observed when TAFA was used alone. Mechanistically, we observed that combining TAFA with CIMLNK led to a significant increase in degranulation, 55.4% vs 13.3%, (p=0.02) and IFN-γ release, 26.7% vs 3.6%, (p=0.03) compared to CIMLNK monotherapy. Moreover, in the presence of TAFA, CIMLNK degranulated significantly more compared to unstimulated NK cells, 55.4% vs 27.7% (p=0.04). The addition of anti-CD16 mAb to CIMLNK abrogated TAFA-induced ADCC. In vivo, combining CIMLNK with TAFA resulted in a median survival of 35 days, which was significantly higher than that seen in untreated mice (28 days, p= 0.01), TAFA monotherapy (28 days, p= 0.003) and CIMLNK monotherapy (26.5 days, p=0.009).
Conclusion: The combination of CIMLNK and TAFA exerts synergistic effects against B-ALL both in vitro and in vivo.

#646 Unravelling the mechanisms of resistance and hyperprogression of renal medullary carcinoma to immune checkpoint therapy.

J. Qian[1], M. Soeung[2], X. Yan[1], K. Yu[1], C. Zanca[1], L. Zhang[1], Z. Chen[1], L. Perelli[1], J. Chen[1], R. S. Tidwell[1], H. Khan[1], F. Duan[1], M. Karki[1], R. He[1], C. N. Le[1], T. N. Lam[1], N. Bhattacharya[1], M. N. Williams[1], D. H. Peng[1], R. Shah[1], I.-L. Ho[1], N. Feng[1], N. M. Zacharias[1], R. A. Sheth[1], T. K. Bathala[1], P. Rao[1], N. C. Daw[1], D. N. Tripathi[3], C. L. Walker[4], R. Wang[1], M. Dang[1], E. Dai[1], F. Peng[1], Y. Liu[1], A. S. Jadhav[1], W. Lang[1], C. A. Arrechedera[1], L. Campos Clemente[1], H. Lu[1], C. L. Haymaker[1], I. I. Wistuba[1], A. Futreal[1], A. Viale[1], T. Heffernan[1], G. F. Draetta[1], N. M. Tannir[1], J. Gao[1], L. Wang[1], G. Genovese[1], P. Msaouel[1];

[1]UT MD Anderson Cancer Center, Houston, TX, [2]Dana-Farber Cancer Institute, Boston, MA, [3]FDA, Silver Spring, MD, [4]Baylor College of Medicine, Houston, TX

Renal medullary carcinoma (RMC) is defined by the loss of the SMARCB1 tumor suppressor and represents one of the most aggressive kidney cancers, predominantly affecting young individuals of African descent with the sickle cell trait. RMC exhibits rapid progression, and poor response to standard therapies, with a median survival of only 13 months following diagnosis. In pursuit of more effective treatments, we launched two clinical trials specifically for patients with RMC evaluating anti-PD1 + anti-CTLA4 immune checkpoint therapy (ICT) with nivolumab + ipilimumab (ClinicalTrials.gov identifier: NCT03274258) and anti-PD1 + anti-LAG3 ICT using nivolumab + relatlimab (ClinicalTrials.gov identifier: NCT05347212). However, both trials were terminated early for futility due to resistance and hyperprogression by the established consensus Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 radiological criteria in Matos et al. Clin Cancer Res. 2020 (PMID: 31757877). Co-clinical experiments testing the same ICT regimens in our immunocompetent somatic mosaic genetically engineered mouse model (GEMM) of RMC demonstrated similar patterns of resistance and hyperprogression with ICT compared with IgG control. The combination of anti-PD1 with either anti-CTLA4 or anti-LAG3 significantly accelerated primary tumor progression and markedly increased metastatic burdens in the lung and liver of RMC mice. Comprehensive analyses, including longitudinally collected single-cell RNA sequencing from human RMC patient samples and RNA sequencing of GEMM RMC tumors revealed significant transcriptional reprogramming, enhanced tumor proliferation, activation of oncogenic pathways, and a shift toward myeloid-specific gene expression by RMC tumor cells in response to ICT. In our RMC mouse models, tumor cells have been engineered to express green fluorescent protein (GFP), enabling us to distinguish them from non-malignant mouse cells. Accordingly, multiplex immunofluorescence (mIF) revealed that tumor tissues from mice treated with ICT showed a significant increase of tumor cells co-expressing GFP and myeloid markers such as CD68, F4/80 and S100A9. Differential patterns of resistance and hyperprogression when RMC mice were treated with monotherapies targeting PD1, CTLA4, or LAG3. To counteract ICT-induced hyperprogression, we tested the CEBPB/p300 complex inhibitor IACS16898 which successfully induced tumor responses when combined with ICT in our RMC GEMM. IACS16898 treatment significantly reduced the expression of myeloid-associated markers on GFP+ tumor cells. Our study not only elucidates a novel mechanism of resistance and hyperprogression to PD1 plus either CTLA4 or LAG3 inhibition in SMARCB1-deficient cancers but also provides a foundation for targeting oncogenic and resistance pathways in RMC treated with distinct immunotherapy regimens.

#647 Intratumoral activation of anti-tumor CD4Th1 cells prior to neoadjuvant chemotherapy eradicate metastasis initiating cancer cells in breast cancer patients.

Ganesan Ramamoorthi[1], Marie Catherine Lee[2], Carly M. Farrell[2], Hyo S. Han[2], Caitlyn N. Balsay-Patel[2], Grace Spiegelhoff[1], Brian J. Czerniecki[2]

[1]Clinical Science and Immunology, Moffitt Cancer Center, Tampa, FL,[2]Department of Breast Oncology, Moffitt Cancer Center, Tampa, FL

Clinical overt metastases emerge from metastasis initiating cancer cells (MICs) disseminated during early tumor progression prior to clinical diagnosis of apparent primary breast cancer (BC). Targeting and inhibiting dissemination of MICs from early tumor lesions can be a potential therapeutic strategy to prevent late recurrence and metastasis in patients. Unfortunately, no such therapy currently exists that inhibits MIC dissemination and metastatic seeding in distant organs. Our recent findings support the critical contribution of intratumoral delivery of type 1 conventional dendritic cells (cDC1) in complete eradication of primary BC in patients. We demonstrated that intratumoral activation of anti-tumor CD4T helper 1 (CD4Th1) cells by tumor antigen targeting cDC1 limits MIC dissemination mechanisms independent of CD8 T cells in BC mouse models. Anti-tumor CD4Th1 cells also restored the loss of nuclear receptor subfamily 2 group F member 1 (NR2F1) in human epidermal growth factor receptor 2 (HER2)+ early tumor lesions, reducing dissemination of MICs. Moreover, CD4Th1 cytokine Interferon gamma inhibited dissemination of MICs from early lesions and primary tumors via the modulation of HER2/progesterone receptor/Wnt4/receptor activator of nuclear factor-κB ligand (RANKL) dissemination mechanisms. Most strikingly, we provide clinical evidence that intratumoral activation of anti-tumor CD4Th1 cells inhibited disseminating MIC phenotypes in the tumor lesion of BC patients. This was further corroborated by the decreased seeding of disseminated MICs in the bone marrow of BC patients treated with intratumoral cDC1 prior to any standard neoadjuvant chemotherapy (NACT) compared to patients treated with standard NACT or patients with no prior treatment. Single cell RNA sequencing (scRNA-seq) of isolated MICs (disseminated cancer cells; DCCs) from bone marrow of BC patients with no prior treatment revealed that these patient MICs were highly enriched for cytokeratin 10/13, heterogeneity, stemness and loss of MHC class II and displayed a unique transcriptome profile. Our ongoing clinical study is investigating isolated MICs from patients treated with intratumoral cDC1 and standard NACT at a single cell level by scRNA-seq. Collectively, these results provide a new strategy to selectively target and inhibit dissemination of MICs from the early tumor lesion and prevent overt metastasis in patients. This is also the first study to delineate the transcriptome profile of MICs (DCCs) from BC patients using scRNA-seq.

#648 Activity of BNT327/PM8002 (PD-L1 x VEGF-A bispecific antibody) in combination with BNT325/DB-1305 (TROP2 ADC) in solid tumors: Early preclinical and clinical evidence to support BNT327 + ADC combinations.

E. P. Hamilton[1], J. Zhu[2], W. Zhuang[3], Y. Cheng[4], J. Shi[5], H. Amin[6], K. Moore[7], H. Yang[8], Y. Sun[9], K. A. Lerro[10], K. Sheng[11], Y. Qiu[11], X. Li[11], C. Li[11], Y. Lou[12], A. Tsun[12], W. Huang[13], S. Peng[13], P. Wang[13], F. Gieseke[14], **S. Tillmanns**[15], D. Akahara[16], S. Koseoglu[16], A. M. Obrate[17], C.-N. Gann[14], M. Wenger[14], I. Celik[14], O. Tureci[14], U. Sahin[14];

[1]Sarah Cannon Research Institute, Nashville, TN, [2]Zhejiang Cancer Hospital, Hangzhou, China, [3]Fujian Cancer Hospital, Fuzhou, China, [4]Jilin Provincial Cancer Hospital, Changchun, China, [5]Linyi Cancer Hospital, Linyi, China, [6]BRCR GLOBAL, Plantation, FL, [7]Stephenson Cancer Center at the University of Oklahoma Medical Center, Oklahoma City, OK, [8]Affiliated Hospital of Hebei University, Baoding, China, [9]Shandong Cancer Hospital and Institute, Jinan, China, [10]Regional Medical Oncology Center, Wilson, NC, [11]Duality Biologics, Shanghai, China, [12]Biotheus Inc., Zhuhai City, Guangdong Province, China, [13]Biotheus (Suzhou) Co., Ltd., Suzhou, China, [14]BioNTech SE, Mainz, Germany, [15]BioNTech SE, Munich, Germany, [16]BioNTech US Inc., Cambridge, MA, [17]BioNTech Pharmaceuticals Spain, S.L., Barcelona, Spain

Emerging data suggest that IO-ADC combinations may lead to better patient outcomes. BNT327 (also known as PM8002) is an investigational anti-PD-L1 x VEGF-A bispecific antibody that has shown encouraging efficacy and tolerability across tumor types, including “cold” tumors not generally responsive to IO therapies (eg, low PD-L1 levels) (Wu J, SABCS 2024 PS3-08; Wu YL, ESMO 2024 1255MO). BNT325 (also known as DB-1305) is an investigational topoisomerase-I-inhibitor-based anti-TROP2 ADC that has so far shown manageable safety and encouraging early clinical activity (Rubinstein M, SGO 2025). The combination of BNT327 with BNT325 may allow for improved efficacy without compounding overlapping toxicities. The monotherapy safety profiles are characterized by low-grade proteinuria, hypertension, and elevated transaminases (BNT327; Guo Y, ASCO 2023 #414802) and stomatitis (BNT325; Marathe O, ESMO 2023 689P). Herein we provide evidence of early preclinical and clinical data to support the novel combination of an anti-PD-L1 x VEGF-A bispecific antibody with an ADC. Preclinical *in vivo* models testing the combination of BNT327 with BNT325 showed superior tumor growth inhibition (TGI) compared with each treatment alone. In a xenograft A375 + PBMC co-injection model (hTROP2^{+}A375 in B.NDG B2M KO plus mice), the combination of BNT325 (1 mg/kg Q2D*5) and BNT327 (20 mg/kg Q2D*2) had TGI of 111.3% vs. 33.6% for BNT325 or 78.2% for BNT327 alone. In a syngeneic hTROP2^{+}MC38 model, BNT325 (20 mg/kg QW*4) combined with a surrogate BNT327 (surBNT327, anti-mPD-L1 x mVEGF) showed TGI of 106.1% and 106.9% for surBNT327 (5 mg/kg Q2D*4 and 20 mg/kg Q2D*4, respectively) vs. 51.4% for BNT325 alone or 47.6% for surBNT327 (5 mg/kg) and 71.4% for surBNT327 (20 mg/kg) alone. In the clinic, as of Nov 21, 2024, 8 patients (breast cancer n=2, ovarian cancer n=2, other n=4) have received the combination of BNT327 and BNT325 in an ongoing first-in-human phase 1/2a study of patients with advanced solid tumors (NCT05438329). No DLTs were reported in the DLT-evaluable patients (first 6 patients). Stomatitis was reported in 6/8 patients (Grade 3 in 2 patients), which led to a dose reduction of BNT325 in 3 patients. There were no adverse events leading to dose discontinuation. Tumor response analyses and biomarker testing are ongoing. Overall BNT327 plus BNT325 demonstrated superior anti-tumor effects preclinically compared to each drug alone. Early clinical data showed successful dose escalation allowing enrollment to NSCLC, TNBC, ovarian, and cervical cancer expansion cohorts, with updated clinical data planned to be presented. Further evaluation is ongoing of BNT327 with ADCs against HER2 (BNT323/DB-1303), B7H3 (BNT324/DB-1311) and HER3 (BNT326/YL202), with a focus on tumors where early monotherapy efficacy has been observed with these ADCs.

TUMOR BIOLOGY: Imaging in Animal Models
Poster Session

#65 Real time imaging of the non-small cell lung cancer immune microenvironment modulation by low dose photodynamic therapy.

Damien N. Marie[1], Louis-Emmanuel Chriqui[1], Alexandros Sifis[1], Etienne Abdelnour[1], Michel Gonzalez[1], Thorsten Krueger[1], Johanna A. Joyce[2], Sabrina Cavin[1], Jean Y. Perentes[1]

[1]Lausanne University Hospital, Lausanne, Switzerland,[2]Department of Oncology, University of Lausanne, Lausanne, Switzerland

Immune checkpoint inhibitors (ICIs) have significantly improved the outcome of non-small cell lung cancers (NSCLCs). However, cancer response to ICIs occurs in a fraction of patients and correlates with the presence of tumor-infiltrating lymphocytes (TILs). Thus, methods to increase the presence of TILs within lung cancer are urgently needed. Previously, in a murine model of malignant pleural mesothelioma, we described a mechanism by which low dose photodynamic therapy (L-PDT) enhanced the vascular expression of E-selectin which favored TILs recruitment and improved tumor control. Here, we hypothesized that a similar mechanism existed in NSCLC and could be further potentiated by ICIs.
We developed a murine model with a chest window allowing real time imaging by two-photon microscopy of lung adenocarcinoma (LUAD, 344-SQ-GFP, $Kras^{G12D}$; $p53^{R172H\Delta G}$ mutant) growing in the lungs of C57BL/6-CD2-dsRed transgenic mice. We determined the impact of L-PDT±E-selectin blocking antibodies (EBA) on CD2+TILs recruitment over 30 days. In a separate experiment, we then assessed the impact of L-PDT±EBA on tumor growth, animal survival and spontaneous metastasis development. Finally, we characterized the LUAD immune microenvironment and TILs immune checkpoint expression 1 and 5 days after L-PDT by 16-colors flow cytometry.
L-PDT significantly enhanced the recruitment of CD2+TILs, with a significant increase observed at day 1, that lasted up to 20 days post-treatment compared to controls. L-PDT was significantly correlated with improved tumor control (-42%), a significant reduction in metastasis development (-50%) and an increase in animal survival (+26%) compared to the control group. Interestingly, this phenotype was abrogated to control levels when L-PDT was combined with EAB. Further analysis of the tumor immune microenvironment confirmed the recruitment of CD8+ T lymphocytes at both 1- and 5-days following L-PDT with increased PD-1 expression on their surface day 5 post-treatment compared to controls.
L-PDT triggers, through vascular E-Selectin, a durable infiltration of NSCLCs by TILs which provide better tumor control, impair metastasis development, and improve animal survival. The increased PD-1 levels on CD8-T cells following L-PDT suggest a favorable combination with PD1/PDL1 inhibitors.

CLINICAL RESEARCH: Combination Immunotherapies
Poster Session

#650 Enhancing antitumor immunity by inducing PANoptosis using a new generation of vesicular stomatitis virus.

F. Chen[1], L. Lang[2], S. Tang[1], F. Yang[1], N. F. Saba[1], M. Luo[3], **Y. Teng**[1];
[1]Emory University, Atlanta, GA, [2]Augusta University, Augusta, GA, [3]Georgia State University, Atlanta, GA

Despite significant advances, the limited therapeutic efficacy of virotherapy remains a major hurdle in the use of oncolytic viruses (OVs) for cancer treatment. To overcome this challenge, we integrated the SMAC/DIABLO gene into the genome of vesicular stomatitis virus (VSV) to create a new generation of VSV, termed VSV-S. In this study, we infected head and neck squamous cell carcinoma (HNSCC) cells and tumors with VSV-S to evaluate its therapeutic efficacy and underlying mechanisms. VSV-S exhibited significantly enhanced antitumor activity in HNSCC cells compared to the parental virus. It not only promoted cancer cell apoptosis through the overexpression of SMAC during VSV infection, but also induced PANoptosis (a combination of pyroptosis, apoptosis, and necroptosis) through the activation of caspase 3, gasdermin D and gasdermin E, and MLKL signaling pathways, which led to enhanced recruitment of CD8+ T cells to the tumor and eventually potentiated T cell-mediated antitumor immunity. In addition, VSV-S reduced PD-L1 levels in HNSCC cells and, when combined with anti-PD-1 antibody, produced a more potent antitumor effect than either treatment alone. These results provide valuable preclinical evidence to support the development of VSV-S-based antitumor therapies that could improve outcomes for patients with HNSCC.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#6509 Carcinogen withdrawal in the BBN induced mouse model of bladder cancer mimics cigarette smoking cessation in humans with decreased tumor progression.

Gabrielle Kennelley[1], Alexandra Pado[2], Rachel Sanyu[2], Norbert Sule[3], Craig Brackett[3], Barbara Foster[3], Wendy Huss[3]

[1]University of Pittsburgh Medical Center, Pittsburgh, PA,[2]University at Buffalo Jacobs School of Medicine and Biosciences, Buffalo, NY,[3]Roswell Park Comprehensive Cancer Center, Buffalo, NY

Field cancerization is associated with greater tumor mutational burden in bladder cancer. Mutations in TRP53 (p53) are early drivers of disease and correlate with tumor stage, grade, and a poor prognosis. In humans, cigarette smoking correlates with a greater frequency of p53 mutations. N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) is a cigarette smoke-mimicking carcinogen that can be used to induce bladder cancer in rodent models. Mirroring real-world phenomena, BBN exposure induces p53 mutations and leads to more rapid tumor initiation and progression in male rodents. Male and female mice were exposed to 0.05% BBN in drinking water for 4, 12, or 20 weeks (wks). Bladder samples were collected immediately after BBN discontinuation or after a period of drinking untreated water. Pathology, proliferation, and mutant p53 expression were analyzed to investigate BBN-induced field cancerization in urothelium, urothelial carcinoma in situ (UCIS), and invasive urothelial carcinoma (iUC). Mice exposed to 4 wks of BBN had groups of proliferative and mutant p53 cells. A continuous basal proliferation pattern was more commonly seen in males compared to females (87% vs. 43%), and a single male progressed to UCIS. Interestingly, by 22 wks (18 wks untreated drinking water after 4 wks BBN), there was no longer detectable proliferation, mutp53 cells, or UCIS. Mice exposed to 12 wks of BBN progressed to UCIS or iUC (55% males vs. 33% females). Most bladders with UCIS had a continuous basal cell proliferation pattern with mutant p53. By 22 wks (10 wks untreated drinking water after 12 wks BBN), bladders with UCIS had limited continuous basal cell proliferation but retained mutant p53, and males had more iUC than females (38% vs. 14%). Additionally, by 52 wks (40 wks untreated drinking water after 12 wks BBN), males more often progressed to iUC compared to females (94% vs. 44%). By 78 wks (66 wks untreated drinking water after 12 wks BBN), 75% of females progressed to iUC. After 20 wks of BBN, all male mice progressed to iUC by 26 wks, whereas iUC development in female mice was delayed until 51 wks. BBN induces p53 mutations associated with the initiation and progression of murine bladder cancer. Data showing mutant p53 without development of UCIS or iUC in mice treated with BBN for 4 wks indicates field cancerization is not maintained after a short exposure to BBN. However, exposure to BBN for 12 wks induces iUC in most males, but less in females, and 20 wks of BBN exposure leads to more rapid iUC development in males than in females. This data mimics research in humans showing a reduced risk of bladder cancer development after cessation of cigarette smoking. The pattern of mutant p53 staining, particularly p53 mutations within proliferating cells, may also correlate with greater field cancerization and likelihood of UCIS and iUC development, particularly in those with a longer history of carcinogen exposure.

CLINICAL RESEARCH: Combination Immunotherapies
Poster Session

#651 Safety and efficacy of pembrolizumab plus personal dendritic cell vaccines in elderly patients with metastatic melanoma.

C. S. Nangia[1], K. L. Lopez[2], G. I. Nistor[2], **R. O. Dillman**[2];
[1]Hoag Hospital, Newport Beach, CA, [2]AIVITA Biomedical, Inc., Newport Beach, CA

Monoclonal antibodies targeting programmed death molecule-1 (anti-PD-1) are an effective immunotherapy for advanced melanoma. An investigational approach that has also produced encouraging long-term survival results in such patients is a patient-specific therapeutic vaccine consisting of autologous dendritic cells (DC) pulsed with autologous tumor antigens (ATA) from autologous tumor cells that are self-renewing in tissue culture. There is a strong rationale for combining these two immunotherapy approaches, but the first step is establishing that it is safe to give both therapies concurrently. Eligible patients had advanced melanoma with at least one resectable lesion and were intended to be treated with an anti-PD-1 monoclonal antibody. There was no upper age limit. Co-administration of anti-CTLA-4 antibody was not allowed. Surgically resected metastatic melanoma tissue was processed into cell suspension, then cultured in liquid media that favors stem cell survival and progenitor cell proliferation. Lysates of Irradiated tumor cells (ITC) were used as ATA. Peripheral blood mononuclear cells were collected by leukapheresis, enriched for monocytes (MC), and then immature DC were differentiated from MC by culturing with granulocyte-macrophage colony-stimulating-factor (GM-CSF) and interleukin-4. Antigen loading was accomplished by incubating DC with ITC lysate. During the 8 to 9 weeks while DC-ATA was being manufactured, patients received standard infusions of anti-PD-1. Over the next 24 weeks, anti-PD-1 was infused at standard intervals, and DC-ATA admixed in GM-CSF was injected s.c. at weeks 1, 2, 3, 8, 12, 16, 20, and 24. Patients were followed for acute, subacute, and chronic toxicity. Four patients (mean age 81.7 years) have been treated (NCT03743298). Three were treated with pembrolizumab. An 89-year-old female with recurrent stage 3 stopped treatment after five vaccine doses due to recurrent dermatologic toxicity that started during pembrolizumab and persisted during concurrent therapy. She remains recurrence-free 26 months after vaccine initiation. Two stage-4 patients received all 8 DC-ATA doses without toxicity. A 73-year-old female with stage 4b remains in complete remission 33 months after vaccine initiation. An 81-year-old male with stage 4b had a partial response, progressed after 6 months, but was still alive after 14 months. An 82-year-old male with stage 4c received pembrolizumab while DC-ATA was manufactured, then because of new brain metastases, received opdualag with DC-ATA. He had a partial response while receiving the eight vaccine doses but later progressed and died 10 months after vaccine initiation. In elderly patients with advanced melanoma, it appears that DC-ATA can be administered concurrently with anti-PD-1 without increased toxicity and with no decrease in efficacy. Additional testing of this combined immunotherapy is warranted.

#652 Exploring the therapeutic benefit of AVA-NP-695 with combination modalities in ICB refractory and rare cancers.

Borja Ruiz-Fernandez de Cordoba[1], Avijit Goswami[2], Sandeep Goyal[2], Kawaljit Singh[2], Princy Khurana[2], Alejandro Sweet-Cordero[3], **Aditya Kulkarni**[2]

[1]Department of Pediatric Oncology, University of California San Francisco, San Francisco, CA,[2]Avammune Therapeutics, Phoenixville, PA,[3]University of California San Francisco, San Francisco, CA

Background: Ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) expression is associated with poor prognosis in many cancers. Previous studies identified ENPP1 as the primary hydrolase of extracellular cGAMP, that activates the anticancer STING pathway. High ENPP1 expression is found in several tumors, including human astrocyte tumors and triple-negative breast cancer (TNBC) cell lines, where it contributes to tumor progression and impedes T cell infiltration. By inhibiting cGAS-STING-mediated immune activation and producing adenosine, an immune suppressor that promotes cell migration, ENPP1 plays a critical role in tumor growth. Interestingly, surface and global proteome analyses identify ENPP1 as potential immunotherapeutic targets in case of rare cancers like sarcomas.

Methods: The inhibition potency of AVA-NP-695 was confirmed by 5'TMP and 2'2'-cGAMP enzymatic assays. Pharmacokinetic profile of AVA-NP-695 in Mice, Rats and Beagle Dogs and the human PK profile was predicted. The efficacy of AVA-NP-695 was demonstrated in 4T1 Tumor bearing BALB/c mice as monotherapy and in combination with anti-PD-L1, Olaparib and Paclitaxel. Finally, *in vivo* the effect of pharmacological inhibition of DDR with Olaparib in combination with AVA-NP-695 was assessed in OS models.

Results: We demonstrate that AVA-NP-695, a selective and potent small molecule ENPP1 inhibitor, exhibited no adverse effects in multiple safety studies in mice and beagle dogs. *In vivo* studies revealed that AVA-NP-695 (6 mg/kg BID) provided superior tumor growth inhibition and reduced metastasis compared to Olaparib and Anti-PD1 in a syngeneic 4T1 breast cancer mouse model. Additionally, AVA-NP-695 demonstrates tumor regression in both paratibial (OT) and metastatic (lung) models of Osteosarcoma (OS). AVA-NP-695 significant increase the cGAMP levels (~2-3 fold) as early as 6h post-treatment, without additional stimulation. A targeted pharmacological screen revealed synthetic lethality when combining AVA-NP-695 with Olaparib (PARPi) *in vitro* OS cell lines. This novel combination increased both intra- and extracellular cGAMP production. Additionally, the pharmacological inhibition of DDR with Olaparib in combination with AVA-NP-695 led to OS tumor regression and enhanced antitumor immune responses in both OT and metastatic contexts. Compassionate use of AVA-NP-695 showed > 80% tumor volume reduction in Canine patients.

Conclusions: The potent anti-tumor efficacy of AVA-NP-695 both as monotherapy and combination along with its safety profile provides a strong rationale for the therapeutic potential of AVA-NP-695 against solids tumors (breast cancer and Osteosarcoma). Our results uncover novel mechanistic vulnerabilities and suggest that treatment with AVA-NP-695 in combination may increase effectiveness in patients, thus preventing local and distant dissemination.

#653 Proved clinical benefit of low-dose anti-CTLA4 + anti-PD-1 immunotherapy versus mono anti-PD-1 therapy in patients unresectable or metastatic melanoma: Phase III OCTAVA trial.

Lev Demidov[1], **Igor Samoylenko**[1], Galina Kharkevich[1], Kristina Orlova[1], Vladimir Moiseenko[2], Igor Utyashev[3], Daniil Stroyakovskiy[4], Vadim Kozlov[5], Anastasia Mochalova[6], Svetlana Demidova[7], Marina Lyadova[8], Andrey Kutkovich[9], Pavel Skopin[10], Nadezhda Kovalenko[11], Sufia Safina[12], Vitaliy Volkov[13], Yulia Semiletova[14], Vera Vaschenko[15], Nikolaiy Kislov[16], Artem Poltoratsky[17], Irina Shumskaya[18], Sergey Kolomiets[19], Alexander Sobolev[20], Igor Belogortsev[21], Svetlana Odintsova[22], Sameer Rastogi[23], Timur Andabekov[24], Anastasia Zimina[25], Konstantin Penkov[26], Anna Semenova[27], Alexey Obukhov[28], Vasiliy Belyakovsky[29], Oleg Gladkov[30], Rakesh Neve[31], Natalia Falaleeva[32], Elena Poddubskaya[33], Amale Vaibhav[34], Dmitriy Kirtbaya[35], Yana Chapko[36], Maria Smagina[37], Irina Sorokina[38], Yulia Linkova[39], Arina Zinkina-Orikhan[39], Fedor Kriukov[39], Anton Lutsky[40], Evgenia Mikhailova[40]

[1]FSBI "N.N. Blokhin National Medical Research Center for Oncology", MoH, Moscow, Russian Federation,[2]SBHI "N.P. Napalkov St. Petersburg Clinical Scientific and Practical Center for Specialized Types of Medical Care (Oncology)", St. Petersburg, Russian Federation,[3]Hadassah Medical LCC, Moscow, Russian Federation,[4]Moscow City Oncology Hospital No. 62, Moscow, Russian Federation,[5]SBHI "Novosibirsk Regional Clinical Oncology Dispensary", Novosibirsk, Russian Federation,[6]JSC Medsi Group of Companies, Moscow, Russian Federation,[7]Health care institution "Minsk City Clinical Oncology Center", Minsk, Belarus,[8]SBHI "City Clinical Oncology Hospital No. 1 of the Moscow City Health Department", Moscow, Russian Federation,[9]LLC "Stepmed Clinic>, St. Petersburg, Russian Federation,[10]FSBEI of Higher Education "National Research Mordovian State University named after N.P. Ogarev", Saransk, Russian Federation,[11]SBHI Volgograd Regional Clinical Oncology Dispensary, Volgograd, Russian Federation,[12]SAHI "Republican Clinical Oncology Dispensary of the Ministry of Health of the Republic of Tatarstan named after Professor M. Z. Sigal", Kazan, Russian Federation,[13]Healthcare Institution "Brest Regional Oncology Dispensary", Brest, Belarus,[14]FSBEI of Higher Education "Saint Petersburg State University", St. Petersburg, Russian Federation,[15]RSBHI "Kostroma Oncology Dispensary", Kostroma, Russian Federation,[16]SBHI "Regional Clinical Oncology Hospital", Yaroslavl, Russian Federation,[17]FSBI "N.N. Petrov National Medical Research Center for Oncology", MoH, St. Petersburg, Russian Federation,[18]SBHI "Nizhny Novgorod Regional Clinical Oncology Dispensary", Nizhny Novgorod, Russian Federation,[19]RSBHI "Altai Regional Oncology Dispensary", Barnaul, Russian Federation,[20]SBHI "Kuzbass clinical oncological dispensary named after M.S. Rappoport", Novokuznetsk, Russian Federation,[21]SBHI , St. Petersburg, Russian Federation,[22]JSC Modern Medical Technologies, St. Petersburg, Russian Federation,[23]All India Institute Of Medical Sciences Delhi, New Delhi, India,[24]Limited Liability Company AV Medical Group, St. Petersburg, Russian Federation,[25]BHI "Clinical Oncology Dispensary", Omsk, Russian Federation,[26]Private Medical Institution "Euromedservice", St. Petersburg, Russian Federation,[27]FSBI "National Medical Research Center of Oncology named after N.N. Petrov" of the Ministry of Health of the Russian Federation, St. Petersburg, Russian Federation,[28]SHI "Vitebsk Regional Clinical Oncology Dispensary", Vitebsk, Belarus,[29]HI "Gomel Regional Clinical Oncology Dispensary", Gomel, Belarus,[30]Limited Liability Company EVIMED, Chelyabinsk, Russian Federation,[31]PDEAS Ayurved Rugnalaya & Steriling Multispeciality Hospital, Pimpri-Chinchwad, India,[32]FSBI "National Medical Research Center of Radiology" of the Ministry of Health of the Russian Federationn Federation, Obninsk, Russian Federation,[33]FSAEI of Higher Education I.M. Sechenov First Moscow Medical University, MoH, Moscow, Russian Federation,[34]Horizon Multispeciality Hospital, Sangli Miraj Kupwad,, India,[35]SBHI "Oncology Dispensary No. 2", MoH of the Krasnodar Territory, Sochi, Russian Federation,[36]State Budgetary Healthcare Institution of the Arkhangelsk Region "Arkhangelsk Clinical Oncology Dispensary", Arkhangelsk, Russian Federation,[37]State Budgetary Healthcare Institution Leningrad Regional Clinical Hospital, St. Petersburg, Russian Federation,[38]BIOCAD, Moscow, Russian Federation,[39]JSC BIOCAD, St. Petersburg, Russian Federation,[40]BIOCAD, St. Petersburg, Russian Federation

Background: BCD-217-2/OCTAVA (NCT05732805) is an international, multi-center, randomized, double-blind, placebo-controlled phase III study conducted to access the efficacy and safety of prolgolimab+nurulimab (BCD-217) combination therapy with continued prolgolimab therapy compared to prolgolimab monotherapy as 1st line treatment for patients (pts) with unresectable or metastatic melanoma (un/mM). BCD-217 is a fixed-dose combination of nurulimab (aCTLA-4, 5 mg/ml) and prolgolimab (aPD-1, 15 mg/ml) was recently approved as the 1st line treatment for un/mM in Russia. Here we present the primary analysis of the study.

Methods: Pts with unresectable or metastatic cutaneous melanoma (IIIC-IVM1a-c) with treatment- *naïve* for unresectable/metastatic disease were randomized in 2 treatment arms: combination drug containing nurulimab (1 mg/kg) and prolgolimab (3 mg/kg) at a dose of 0.2 ml/kg Q3W during the first four blinded infusions (nuru+prolgo arm) and prolgo arm received prolgolimab monotherapy at a dose of 3 mg/kg Q3W during the first four blinded infusions. Then both arms received prolgolimab maintenance up to two years. The primary endpoint of the study was progression-free survival (PFS).

Results: 271 pts were randomized to nuru+prolgo (n=135) or prolgo monotherapy (n=136) arms. After the median of 15.8 mo follow-up the median PFS (mPFS) was 15.4 (10.3; ND) mo in the nuru+prolgo group and 10.8 (4.7; ND) mo in the prolgo monotherapy group (95% CI, HR 0.68 (0.482; 0.957), iRECIST). The mPFS benefit of nuru+prolgo arm compared to prolgo arm are maintained in RECIST 1.1 assessment: 9.9 mo vs 2.8 mo, respectively. ORR and DCR were also higher in NURU+PROLGO arm. mOS was not reached in both groups (95% CI, HR 0,88, (0.50; 1.55)). 12-mos OS was 84% in each arm. Grade 3-4 treatment-related AE were reported in 16.3% of pts in nuru+prolgo arm compared to 14.0% - prolgo arm. Immune-related AEs (irAE) of all grades were reported in 52.6% of cases in nuru+prolgo arm and 32.4% of cases - in prolgo arm (p 0.0007). Majority of them were mild. The proportion of gr.≥3 irAEs was 13.3% vs 5.9% in nuru+prolgo arm and prolgo arm, respectively (p 0.04). Treatment discontinuation due to AE was reported in 9.6% of cases for nuru+prolgo vs 4.4% of cases for prolgo arm.

Conclusions: OCTAVA trial resuts demonstrated that the fixed-dose combination of nurulimab + prolgolimab is significantly more effective than aPD-1 monotherapy without a serious deterioration of the safety profile in patients with metastatic or unresectable cutaneous melanoma as 1st line therapy.

#654 Transcriptomic analysis ofTGF-β-targeting oncolytic adenovirus and immune checkpoint inhibitor therapy in a triple-negative breast cancer mouse model.

N. Nhan[1], B. Filimon[1], H. Fowler[2], S. Ganesan[2], X. Wang[2], B. Brockstein[1], W. Xu[1];
[1]Northshore University Healthsystem, Evanston, IL, [2]Northwestern University Feinberg School of Medicine, Chicago, IL

Immunotherapy has shown promise in cancer treatment, but resistance remains a major challenge. Combining therapies that target specific pathways, such as TGF-β signaling, can enhance the effectiveness of immune checkpoint inhibitors (ICIs). Our oncolytic adenovirus (OAd), mHAdLyp.sT, containing a TGF-β decoy to reverse immune resistance to ICI treatment and the LyP-1 peptide to enhance tumor targeting, has been shown to significantly enhance anti-tumor responses and reduced lung metastasis in a 4T1 triple negative breast cancer (TNBC) mouse model when systemically delivered in combination with anti-PD-1 and anti-CTLA-4 antibodies. We also showed previously that mHAdLyp.sT systemically primes an inflamed immune cell phenotype for ICI treatment by both flow cytometry and mass cytometry (CyTOF). In this study, we investigated how the mHAdLyp.sT-ICIs combination affects the expression of key genes in major signaling and immune regulatory pathways by RNA-seq analysis, both in tumors and systemically in the spleen, at the early response stage (day 16) and experiment terminal point (day 25) in the TNBC mouse model. In tumor, both ICIs and the mHAdLyp.sT-ICIs combination treatments significantly induced immune-related genes beneficial for anti-tumor effects compared to the untreated control group at both day 16 and day 25. However, at day 16, while ICI treatment significantly escalated the expression of several proinflammatory cytokines, such as IL-6, IL-10, and IL-11, which are known to potentiate tumor resistance to immunotherapy, mHAdLyp.sT-ICIs combination treatment did not trigger this response. In the spleen, all treatments exhibited slight effects on the immune response at day 16, while at day 25, mHAdLyp.sT-ICIs combination induced a strong response with extensive rescued changes across various crucial signaling pathways in tumor progression and metastasis, including those involved in TGF-β signaling, cell adhesion, migration, angiogenesis, extracellular matrix organization, cell differentiation, gene expression regulation, protein phosphorylation, inflammation, and apoptosis. Notably, mHAdLyp.sT-ICIs combination treatment more effectively inhibited immunosuppressive genes such as *Myd88* and *Ptgir* than ICI alone, confirming that TGF-β inhibition by mHAdLyp.sT enhanced anti-tumor immune responses and helped overcome ICI resistance. We are confident in advancing this new treatment option for TNBC and other metastatic cancers that are projected to be resistant to immunotherapy in a clinical setting.

#655 Spatiotemporally deciphering the response dynamics of micro-satellite stable rectal cancer to neoadjuvant short-course radiotherapy and PD-1 blockade.

J. Wang, Z. Zhang;
Fudan University Shanghai Cancer Center, Shanghai, China

Neoadjuvant chemoradiotherapy is the stand of care for patients with locally advanced rectal cancer (LARC). For microsatellite-stable (MSS) LARC patients, combining immune checkpoint inhibitors (ICIs) with radiotherapy has shown improved tumor regression, though the mechanisms behind tumor response remain unclear. Here, we analyzed 60 pre-, mid- and post-treatment tumor samples from 39 LARC patients in the TORCH clinical trial, utilizing single-cell sequencing and spatial transcriptomics to explore cell dynamics and their association with therapeutic efficacy. In well responders, combination therapy expanded the $CD8^+$ and $CD4^+$ memory T cell pool, which interacted with type 2 dendritic cells and clustered around non-malignant epithelial cells, forming part of tertiary lymphoid structures, as validated by spatial transcriptomics. In poor responders, the tumor microenvironment was enriched with immunosuppressive cells clustering around cancer-associated fibroblasts. This study uncovers mechanisms underlying the efficacy of radiotherapy combined with ICIs and provides valuable resources for identifying targets in MSS LARC patients.

#656 Enhanced therapeutic effect of combining denosumab with PD-1 inhibitor in advanced KRAS-mutated lung adenocarcinoma.

C. Liu, H. Li, S. Zheng, N. Sun, Y. Wang, **J. He**;
National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Background: Previous studies have suggested that the combination of RANKL inhibitors and immune checkpoint inhibitors (ICIs) may potentiate the therapeutic response in lung adenocarcinoma, particularly in cases with KRAS mutations. The exact mechanisms and clinical validation of this combination therapy are yet to be fully understood.
Methods: We conducted a comprehensive analysis by integrating public datasets and retrospective clinical data to examine the relationship between RANKL alterations and the tumor immune microenvironment in KRAS-mutated lung adenocarcinoma. We also investigated the signaling pathways affected by RANKL manipulation in cancer cell lines and assessed the impact on macrophage function. A prospective clinical trial was carried out to evaluate the potential of the RANKL inhibitor combined with PD-1 inhibitor therapy.
Results: Our analysis revealed that the combination therapy of RANKL inhibitor with PD-1 inhibitor demonstrated improved outcomes in patients with KRAS-mutated lung adenocarcinoma. Transcriptomic data and immunohistochemical staining showed that RANKL was upregulated in these tumors, which was inversely associated with PD-L1 expression and the secretion of CXCL9, CXCL10, and CXCL11, leading to a decrease in $CD8^+$ T cell infiltration and a more immunosuppressive environment. We found that RANKL modulated PD-L1 levels via the PI3K/AKT pathway and reduced CD8+ T cell infiltration by decreasing the expression of CXCL9, CXCL10, and CXCL11. In a mouse model, KRAS-mutated lung adenocarcinoma treated with the combination therapy showed significant tumor growth inhibition compared to PD-1 inhibitor alone. Additionally, the combination therapy significantly enhanced macrophage and $CD8^+$ T cell recruitment. The DEMAND trial confirmed the superior therapeutic efficacy of this combination regimen in patients with KRAS-mutated lung adenocarcinoma.
Conclusion: Our study sheds light on the role of RANKL overexpression in promoting immunosuppression and ICI resistance in KRAS-mutated lung adenocarcinoma. Our results indicate that the combination of RANKL inhibitor and PD-1 inhibitor could be a promising therapeutic strategy for this patient subset.

#658 Triple-negative breast cancer sensitization to anti-metabolite chemotherapy utilizing PARP inhibiting olaparib.

Joshua C. Doloff, Amanda Rakoski, **Abigail Yoo**

Biomedical Engineering, Johns Hopkins University, Baltimore, MD

Triple Negative Breast Cancer (TNBC) is one of the most aggressive breast cancer subtypes as it grows faster than typical invasive carcinoma, leading to poor prognosis and an increased chance of recurrence. It lacks three key hormone receptors, estrogen, progesterone, and HER2 receptors, eliminating the efficacy of receptor-targeted therapies. The first line of treatment is the use of combination chemotherapies given at Maximum Tolerated Dosage (MTD) but this typically leads to higher toxicity and systemic side effects. We investigate Olaparib (OLA) and Gemcitabine (GEM) as a dual chemotherapy. OLA is an FDA-approved PARP inhibitor, facilitating single-stranded DNA breakage by preventing PARP from replacing excised nucleotides. GEM is an anti-metabolite that prevents cancerous cells from continuing mitosis and increasing apoptosis by taking the place of the nucleotide cytidine in the DNA during replication, leading to cell cycle arrest. We propose sensitizing TNBC to GEM by using OLA at lower doses than MTD while causing significant TNBC tumor cell death compared to the drug alone.We used EO771 cells (syngeneic to C57BL/6 mice) to evaluate GEM and OLA. Cells (100,000/well) were plated in 24-well plates and treated with 0, 10, or 30 nM GEM, OLA, or combinations thereof. Crystal violet staining was used to visualize viable cells, which were quantified by absorbance at 550 nm. RNA extracted from 500,000 cells/well in 6-well plates was analyzed via qPCR for stress and cytokine-related gene expression. GEM at 30 nM was more effective than at 10 nM, especially in combination with OLA (10 or 30 nM). Phase-contrast microscopy revealed significant cytotoxicity and cellular detachment with combined treatments, particularly GEM30/OLA10 and GEM30/OLA30. GEM 30/OLA 10 and GEM 30/OLA 30 showed the most significant cell death in response to combined chemotherapy. GEM 30/OLA 10 interestingly exhibited a slightly lower cell viability percentage than GEM 30/OLA 30. GEM30/OLA10 was the most effective combination tested, reducing TNF-α expression and cell viability while minimizing toxicity. The dual combinations suggest that GEM effectively disrupts DNA synthesis and drives single-stranded breakage. Future studies should include in vivo analyses of the tumor microenvironment, ELISAs for protein validation, and additional cell viability assays, such as MTT or XTT, to confirm these findings.

#659 Translational medicine studies of BR111, a biparatopic anti-ROR1 antibody drug conjugate for hematological and solid tumor treatment.

Qinqin Zhuang[1], Yan Sun[1], Lilv Fan[1], Qianqian Tao[1], Xiaobei Zhao[2], Jie Zhu[2], Zhenhua Wu[1], Lei Nie[1], Gang Chen[2], Haibin Wang[1]

[1]Bioray Pharmaceutical Ltd., Hangzhou, China,[2]Bioray Pharmaceutical Co., San Diego, CA

ROR1 is a type I transmembrane protein that belongs to the ROR family. It is a receptor for Wnt family signaling molecules Wnt5a, and plays a critical role in various cellular processes, such as cell proliferation, survival, and migration. Being an oncofetal protein, ROR1 exhibits limited expression in most normal tissues. However, it is abnormally expressed in various hematological and solid cancers, contributing to the development and progression of many types of cancer. Due to its overexpression in cancer, ROR1 presents as a highly attractive target for antibody-drug conjugate (ADC) therapy. The current clinical results of ROR1 ADCs have been promising in treating patients with relapsed and/or refractory (R/R) hematologic malignancies.

BR111 is anti-ROR1 ADC that consists of a biparatopic antibody, stably conjugated to an antimitotic agent. The biparatopic antibodies demonstrate superior binding affinity to ROR1-expressing cancer cells compared to single epitope antibodies. Notably, our unique and innovative conjugation platform CysX™, not only prevents payload detachment during circulation and reducing the off-target toxicity, but also enables a uniformed drug-to-antibody ratio (DAR) of 4.

Recent translational medicine studies showed that ROR1 is relatively highly expressed in the patients of many solid tumors including sarcoma, gastric cancer, TNBC, endometrial cancer, etc. And BR111 has demonstrated efficacy in *in vitro* and *in vivo* models of those indications. BR111 has also showed synergistic efficacy when combined with PD-1/L1 antibodies, SIRPα antibody and several chemotherapeutics. Studies have also shown that ROR1 levels increase while HER2 levels decrease in cell lines treated with DS-8201, and BR111 showed improved efficacy in DS-8201 resistant cell line as well as in combination with DS-8201. The promising outcome holds great potential as a therapeutic treatment for hematological and solid cancers, offering a better safety profile. BR111 has great potential in further clinical evaluations.

#660 Investigating the mechanism of the abscopal effect in combined IRE and anti-PD-1 therapy against PDAC.

Qizhen Cao[1], Yitao Tang[1], Nicholas A. Egan[2], Ping Pan[1], James P. Long[1], Huamin Wang[1], Jared K. Burks[1], Han Liang[1], Weiyi Peng[2], **Chun Li**[1]

[1]UT MD Anderson Cancer Center, Houston, TX,[2]University of Houston, Houston, TX

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is characterized by a high potential for metastatic dissemination, contributing to its poor 5-year survival. Irreversible electroporation (IRE) is an interventional technique that employs short, high-voltage electric pulses to induce cell death. This study was aimed at investigating the abscopal effect of combined IRE and anti-PD-1 therapy in the setting of liver metastasis and possible mechanisms action.

Methods: Female C57BL/6 mice were inoculated with murine Kras* PDAC cells in the pancreas and the liver. The primary tumor in the pancreas was treated with IRE while the distal liver metastasis was untreated by IRE. Following six intraperitoneal doses of anti-PD-1 (100 μg/100 μL/injection, administered every other day), all mice were euthanized 14 days after IRE. Both the IRE-treated pancreatic tumors and the liver metastases were collected for pathological analysis. Tumor tissue microarrays were prepared, and multiplex immunofluorescence staining was performed using the Lunaphore COMET™ system. Additionally, to evaluate the impact of IRE on macrophage phagocytosis of pancreatic tumor cells, macrophages were co-cultured with either control or IRE-treated CFSE-pre-stained pancreatic tumor cells, and phagocytosis effect was assessed using flow cytometry. To explore the impact of IRE on gene expression pathways, Kras* PDAC cells were treated with IRE (400 V, 20 pulses), and cells were collected 4 hours post-treatment. Total RNA was extracted for RNA sequencing analysis.

Results: Results from the PDAC liver metastasis mouse model demonstrated enhanced antitumor activity in both the IRE-treated pancreatic tumors and the untreated liver metastases following combined IRE and anti-PD-1 treatments. Multiplex immunofluorescence analysis of both tumor types revealed a significant accumulation of F4/80+ macrophages in the interface between necrotic tissue (caused by IRE) and viable tumor tissue. Additionally, CD8+GzmB+ T cells and B220+ B cells were significantly increased in the tumor tissues of mice treated with the combination of IRE and αPD-1. In vitro macrophage phagocytosis assays further demonstrated that macrophages were more effective at phagocytosing IRE-treated Kras* PDAC cells compared to untreated control cells. Moreover, RNA sequencing of KRAS* PDAC cells treated with IRE (400 V, 20 pulses) identified 908 differentially expressed genes (DEGs) (fold change > 2, FDR < 0.01) compared to untreated controls. Ingenuity Pathway Analysis revealed that these DEGs were associated with TNFα signaling via the NFκB pathway and IL6-JAK-STAT3 signaling, highlighting the expression of key inflammatory genes essential for immune responses.

Conclusions: These findings indicate that combining IRE with PD-1 blockade enhances a robust antitumor immune response, resulting in systemic antitumor activity and a strong abscopal effect.

#661 LAG-3 blockade enhances the anti-tumor efficacy of PD-1 blockade combined with radiotherapy in a syngeneic murine triple-negative breast cancer model.

Yoomin Kim[1], Seung Hyuck Jeon[2], Nawon Park[1], Seongmin Kim[1], In Ah Kim[1]

[1]Seoul National University, Seoul, Korea, Republic of,[2]Seoul National University Bundang Hospital, Seongnam, Korea, Republic of

The anti-tumor efficacy of PD-1 blockade and local radiotherapy (RT) is often limited by diverse mechanisms that suppress $CD8^+$ T cell responses. Lymphocyte-activation gene 3 (LAG-3) is an inhibitory receptor expressed on exhausted $CD8^+$ T cells and regulatory T cells (Tregs), and its blockade has been shown to enhance the anti-tumor effects of PD-1 blockade. Therefore, we hypothesized that combining LAG-3 blockade with PD-1 blockade and local RT would overcome treatment resistance in triple-negative breast cancer (TNBC). Using the 4T1 syngeneic mouse TNBC model, we evaluated the anti-tumor effects and immune cell phenotypes following treatments with local RT, PD-1 blockade, and LAG-3 blockade. We found that the triple combination of local RT, PD-1 blockade, and LAG-3 blockade resulted in the greatest tumor growth delay in both irradiated (primary) and unirradiated (secondary) tumors, with a significant reduction in metastatic lung nodules. The proportion of tumor-infiltrating $CD8^+$ T cells increased with triple combination therapy while the proportion of Tregs decreased compared to the PD-1 + RT combination in both primary and secondary tumors. Consequently, the $CD8^+$ T cell to Treg ratio was highest in the triple combination group. The proportion of tumor-specific $CD8^+$ T cells increased following triple combination therapy not only in tumor microenvironment but also among splenic $CD8^+$ T cells. Interestingly, the proportions of $CD8^+$ T cells, as well as $CD4^+$ T cells, with an effector memory phenotype were most significantly elevated by the triple combination therapy. In tumor-draining lymph nodes, the proportion of conventional type 1 dendritic cells capable of antigen cross-presentation to $CD8^+$ T cells was increased by the triple combination therapy. These findings suggest that LAG-3 blockade enhances the therapeutic efficacy of local RT and PD-1 blockade by promoting T cell-mediated anti-tumor immune responses. This study highlights the potential of combining LAG-3 blockade with local RT and immune checkpoint inhibitors for the treatment of TNBC. (Work supported by grant from the National Research Foundation of Korea #2023R1A2C3003782)

#662 Clinical and biomarker analyses of SHR-1701 combined with famitinib in patients with previously treated advanced biliary tract cancer or pancreatic ductal adenocarcinoma: A phase II trial.

L. Yi, Z. Meng, J. Xie;
Fudan University Shanghai Cancer Center, Shanghai, China

Background: Advanced biliary tract cancer (BTC) and pancreatic ductal adenocarcinoma (PDAC) are aggressive malignancies with poor prognoses and limited treatment options. This first-in-class phase II study evaluated the efficacy and safety of SHR-1701, a bifunctional fusion protein targeting PD-L1 and TGF-β, combined with famitinib, a multi-targeted receptor tyrosine kinase inhibitor, in patients with advanced BTC or PDAC who failed previous standard treatment.
Methods: In this open-label, single-arm trial (ChiCTR2000037927), patients with advanced BTC or PDAC who had failed at least one line of prior therapy received SHR-1701 (30 mg/kg IV Q3W) plus famitinib (20 mg PO daily). The primary endpoint was objective response rate (ORR) per RECIST v1.1. Secondary endpoints included disease control rate (DCR), progression-free survival (PFS), overall survival (OS), and safety. Exploratory biomarker analyses were performed using RNA sequencing, multiplex immunohistochemistry, and machine learning.
Results: Among 51 patients (BTC, n=25; PDAC, n=20), the BTC cohort showed an ORR of 28% (including 2 complete responses [CRs]) and a DCR of 80%, with a median PFS of 5.1 months and a median OS of 16.0 months. In the PDAC cohort, the ORR was 15% (2 CRs), with a DCR of 60%, and the median PFS and OS were 2.1 and 5.3 months, respectively. Grade 3/4 treatment-related adverse events occurred in 29.4% of patients, with no grade 5 events reported. Primary tumor resection history, peripheral blood immunophenotype changes, and distinct immune-metabolic profiles were associated with treatment benefits, with the TGF-β trap function of SHR-1701 potentially sequestering TGFB1 that was elevated in responders. An immune/metabolism (I/M) score integrating six genes was developed as a predictive biomarker for immunotherapy response in multiple cohorts, allowing to identify patients most likely to benefit from this therapy regimen.
Conclusions: SHR-1701 plus famitinib showed promising efficacy and manageable toxicity in advanced BTC and PDAC, providing proof-of-concept for targeting PD-L1, TGF-β, and angiogenesis pathways simultaneously. The I/M score may help select patients most likely to benefit from this regimen.

#663 A novel combination immunotherapy for p53 mutant metastatic breast cancer leveraging vulnerabilities in the DNA damage response.

Andrei V. Bakin[1], Ashley Guo[1], Priyanka Rajan[1], Mohammed Alruwaili[1], Joseph Barbi[1], Scott I. Abrams[1], Thomas Melendy[2], Christos Fountzilas[1]

[1]Roswell Park Comprehensive Cancer Center, Buffalo, NY,[2]The University at Buffalo, Buffalo, NY

Metastatic breast cancer (MBC) is a life-threatening disease with the lowest 5-year survival rates seen in patients with metastatic triple-negative breast cancer (TNBC). MBCs, including TNBCs, commonly carry mutations in the tumor suppressor p53. Mutant p53 (p53Mut) drives cancer progression and metastasis, in part, by promoting the accumulation of myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs), major enforcers of immune suppression in the tumor microenvironment (TME). At present, there are no effective treatment options for p53mut MBC, while existing chemotherapy-based treatments exhibit low selectivity for p53mut status and frequent adverse side effects. To address this clinical challenge, we developed a novel therapeutic strategy for the selective targeting of p53mut tumors. This novel strategy employs two drugs, one acting as an inducer (TAS102) and the other as an amplifier (PARP inhibitor, PARPi), to achieve selective and potent damage to p53mut tumors. Thymidine nucleotide analogue TAS102 does not block DNA synthesis but activates post-replicative repair leading to DNA breaks, while PARPi blocks repair of these DNA lesions. Post-replicative DNA repair results in DNA breaks and G2-arrest in p53mut cells. In contrast, p53WT cells accumulate in G1-phase and efficiently remove thymidine analogues. Our two-drug strategy showed high efficacy, without adverse effects, in preclinical cancer models of MBC. In this study, we further investigated the impact of the two-drug TAS102-PARPi regimen on the immune landscape in preclinical models of metastatic TNBC. We observed that the TAS102-PARPi combination reduced tumor growth and metastases in the lungs and liver. The two-drug regimen reduced the levels of MDSCs and TAMs, increased the levels of lymphocytes in the TME, and promoted $CD8^+$ T cell-mediated anti-tumor activity that was abrogated by cell depletion. Immunophenotyping showed that the dual agent therapy increased the frequency of intra-tumoral $CD8^+$ T cells expressing the immune checkpoint receptors PD-1 and LAG3, suggesting that the regimen may cooperate with immunotherapies targeting these molecules. Indeed, combining the dual agent therapy with anti-PD-1 or anti-LAG3 antibodies showed a further reduction in tumor growth and metastases. Together, these results demonstrate that the two-drug TAS102-PARPi regimen can be combined with immune checkpoint blockade for effective treatment of MBC, and potentially other p53-mutant cancers, like colorectal and pancreatic cancers.

#664 Integrated scRNAseq and spatial transcriptomic analyses identify immune correlates of response to combined BRAF/MEK/PD-1 inhibition in metastatic colorectal cancer.

Hongcheng Yao[1], Alexander Tang[2], Ryan Corcoran[3], Nir Hacohen[3], Ilya Korsunsky[1], **Jonathan H. Chen**[4]

[1]Brigham and Women's Hospital, Boston, MA,[2]Broad Institute, Cambridge, MA,[3]Massachusetts General Hospital, Boston, MA,[4]Northwestern University - Feinberg School of Medicine, Chicago, IL

Background: Our clinical trial of combined BRAF, MEK, and PD-1 inhibition showed a 24% response rate in BRAF V600E mutant colorectal cancer (CRC) (PMID: 36702949). However, the mechanism underlying this response is unknown.
Methods: We analyzed 327,000 immune and stromal cells from 23 paired scRNAseq profiles of pre- and day 15 on-treatment tumor biopsies. Progression-free survival of 6 months or greater was defined as a clinical response. We performed hierarchical clustering on the scRNAseq data and tracked the TCR repertoire in tumor and blood. Spatial transcriptomic mRNA microscopy (Xenium) was run on 16 biopsies and single cell cluster assignments were mapped onto the microscopy image.
Results: Analysis of scRNAseq data revealed that responders had significant increases in CXCL10+ macrophages and MHCII+ and HOBIT+ CD8 T cells.
Prior analysis of the tumor epithelial cells showed that responders had inhibition of the MAPK pathway (consistent with BRAF/MEK blockade) and increased interferon-stimulated gene (ISG) scores. In contrast, the combination therapy induced more modest shifts in MAPK gene expression scores in immune cells. ISG scores increased across immune cells.
Integration of scRNAseq T cell clusters with TCR clones found a greater influx of new clones (as a fraction of all clones in the activated CD8 T cell clusters) on-treatment in mismatch repair proficient (MMRp) responders versus non-responders ($p=0.02$), with higher normalized counts per new clone in responders versus non-responders ($p=0.03$). Within the blood, TCRs from Tregs and activated CD8 T cells (identified based on scRNAseq signatures in tumor-infiltrating lymphocytes) displayed a burst at two weeks on-treatment but this clonotypic expansion diminished over the next couple of weeks.
Using spatial transcriptomic to localize the populations identified by scRNAseq, we found that SPP1+ macrophages and Tregs were diffusely distributed in tumor areas whereas CXCL10+ macrophages and CD8 T cells formed clusters near the tumor-stromal interface, consistent with tumor immunity hubs.
Conclusions: Altogether, our work suggests that MAPK inhibition in combination with PD1 blockade can induce anti-tumor immune T cell responses, even in MMRp CRC. However, our T cell clonal analysis and subsequent patient cancer progression indicate this response is not sustained. Future work will focus on developing ways to increase the durability of this response, possibly by therapeutically targeting immunity hubs.
Ethics approval: This study was approved by the DF/HCC IRB as protocol 18-144 (see ClinicalTrials.gov NCT03668431).

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Mechanisms of Oncogenesis and Tumor Suppression
Poster Session

#6663 DGAT1 inhibition enhances radiosensitivity in treatment-resistant cervical tumor cells.

Leahan E. Castillo, Marin Guthrie, Naoshad Muhammad, Naomi Msengi, Julie Schwarz

Department of Radiation Oncology, Washington University School of Medicine in St. Louis, ST. Louis, MO

Standard of care treatment for locally advanced cervical cancer has proven to be minimally effective in one third of patients, emphasizing the need for new treatment strategies. Previous research has found that the supplementation with unsaturated free fatty acids may improve tumor response to radiation therapy. Building on this finding, this study investigates if radiation resistant cervical cancers are dependent upon fatty acid metabolism activation of the PI3K pathway. The P13K pathway is responsible for promoting cellular growth, survival and metastasis, thus mutations in this pathway can predict radio-sensitization through the inhibition of DGAT1. DGAT1 is an enzyme involved in cancer cell proliferation and spread by promoting fatty acid metabolism and energy storage, therefore its inhibition can lead to severe oxidative stress, killing cervical cancer cells.
This study investigated the role of DGAT1 inhibition in enhancing radiosensitivity in cervical cancer cells using SiHa parental and SiHa PTEN-null cell lines. Cells were treated with the DGAT1 inhibitors Fenofibrate and A-922500, individually and in combination with radiation therapy (RT). Alamar Blue assays measured cell viability post-treatment, while Western blotting evaluated DGAT1 protein expression following radiation exposure and inhibitor treatment. Flow cytometry was used to quantify apoptosis and reactive oxygen species (ROS), including total, lipid, and mitochondrial ROS, across different treatment conditions. Additionally, colony-forming assays assessed the long-term impact of treatments on cell proliferation and survival.
Results showed that radiation upregulated DGAT1 expression, likely as a protective response to oxidative stress, and that inhibiting DGAT1 significantly decreased cell viability and increased apoptosis when combined with radiation, particularly in PTEN-null cells. These findings suggest that targeting DGAT1 disrupts lipid metabolism and enhances radiosensitivity in cervical cancer cells.
DGAT1 plays a critical role in protecting cervical cancer cells from oxidative damage and maintaining lipid homeostasis during radiation therapy. Inhibition of DGAT1 using Fenofibrate and A-922500 successfully sensitized both SiHa parental and SiHa PTEN-null cells to radiation, with a more pronounced effect observed in the PTEN-null cell line, which is associated with PI3K pathway activation and radio-resistance. These results highlight the therapeutic potential of targeting DGAT1 to overcome radio-resistance in cervical cancer, particularly in tumors with PI3K/Akt pathway activation. Future directions include testing DGAT1 inhibitors in other radioresistant cervical cancer models and conducting in vivo studies to further explore the clinical potential of this approach.

CLINICAL RESEARCH: Diagnostic Biomarkers 2
Poster Session

#668 Systematic and robust prediction of gene expression using comprehensive epigenomic profiling of plasma ctDNA.

Khoi Nguyen[1], Justin Finkle[1], Anthony D'Ippolito[1], Jonathan Beagan[1], Aparna Gorthi[1], Travis A. Clark[1], Amy Donahue[1], Baovy Tran[1], Tyrone Tamakloe[1], Charlene O'Brien[1], Michael Coyne[1], Jenna I. Wurster[1], Kristian Cibulskis[1], Corrie A. Painter[1], Mike Zhong[1], Humphrey Gardner[1], Jacob Berchuck[2], Jamey Guess[1], Jayne Stommel[1], Gordon B. Mills[3], SMMART Clinical Trials Program, J. Carl Barrett[1], Matthew L. Eaton[1]

[1]Precede Biosciences, Inc., Boston, MA,[2]Emory University, Atlanta, GA,[3]OHSU, Portland, OR

Background
Accurate prediction of RNA transcript levels from circulating tumor DNA (ctDNA) offers a promising approach for dynamic noninvasive cancer biomarker characterization and longitudinal monitoring. By leveraging epigenomic signals proximal to individual genes, we developed transcript level machine learning prediction models for breast and prostate cancer using simulated plasma and validated them in patient plasma.
Methods and Results
In breast cancer, our models predicted a panel of 760 key breast cancer oncogenes and transcriptional drivers at simulated ctDNA fractions that capture approximately 45% of stage IV breast cancers profiled, enabling clinically relevant prediction of cancer gene transcription. These models are tuned to separate healthy plasma signal from cancer cells' contribution to the plasma. The panel is enriched for genes important for transcriptional regulatory identity and breast cancer subtypes, as well as targets for antibody drug conjugates (ADCs) and radio immune conjugates (RICs), including HER2, PSMA , Nectin-4, B7-H3, B7-H4, MET, and EGFR. Our models demonstrated direct applicability in patient plasma with orthogonal validation. For example, they accurately predicted prostate cancer PSA and PSMA PET SUVmean, as well as breast cancer HER2, ER, and PR status. Additionally in a cohort of patients with matched tissue RNA-seq there was a strong correlation between RNA-seq and plasma-based predictions. Further improvements were achieved with bespoke feature engineering and multi-gene aggregation. A PSMA predictor refined with locus-specific features improved performance at lower ctDNA fractions for predicting PSMA PET SUVmean. Similarly, a multi-gene DLL3 predictor in lung cancer reduced the ctDNA threshold from 4% to 1.9% by incorporating predicted expression of three highly correlated genes. In predicting ER+ IHC breast cancer, ESR1 transcript prediction alone performed well at 1% ctDNA (AUC=.78) but was significantly improved (AUC=.90) by incorporating predictions of transcription partners FOXA1 and GATA3.
Conclusions
Comprehensive epigenomic profiling enables accurate prediction of tumor-specific RNA transcription in cfDNA, even at low ctDNA levels. This approach enhances ADC target profiling and enables tumor transcriptional profiling in patients from blood. Expanding beyond proof-of-concept models promises improved predictive accuracy at lower tumor fractions and broader applications in diagnostics and therapeutic targeting.

#669 AmoyDx essential NGS panel (LC 10), one solution covering tumor tissue and plasma for detection of EGFR and cMET alterations in NSCLC.

Min Qing[1], Jingxun Wu[2], Yuping Li[3], Jianmin Li[4], Xing Li[5], Cheng Li[5], Yin Shi[5], Xuejun Chen[5], Zhenjun Tan[5], Xiaofang Zhuo[6], Chengjuan Xiong[1], Longen Zhou[1], Flora Berisha[7], **Changbin Zhu**[5]

[1]Janssen Research & Development, Shanghai, China,[2]Department of Oncology, The First Affiliated Hospital of Xiamen University, Xiamen, China,[3]Department of Pulmonary and Critical Care Medicine, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China,[4]Department of Pathology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China,[5]Amoy Diagnostics Co., Ltd., Xiamen, China,[6]Janssen Research & Development, Beijing, China,[7]Janssen Research & Development, Raritan, NJ

Background: Approximately 50% of Asian non-small cell lung cancer (NSCLC) patients carry EGFR and MET alterations with available targeted therapies. LC10 was designed for detection of genetic alterations in 10 driver genes in NSCLC, using FFPE or plasma samples. Notably, current commercial testing systems often use different NGS platforms for tissue and plasma analysis, limiting direct comparability and sample accessibility. This study validates EGFR and cMET alteration detection in tissue and plasma samples using a unified NGS platform (LC10) and assesses its implications for treatment planning in NSCLC patients.
Methods: LC10 is used for detection of 10 lung cancer driver genes by using ddCapture® technology with increased recovery rate and capture specificity. The analytical validation utilized clinical samples with varying dilutions of specific mutations (EGFR ex20ins, common mutations and MET ex14 skipping) and 11 synthesized plasmids in HEK-293T cells with gradient concentrations of 3rd TKI acquired resistance mutations (muts), along with a total of 277 plasma and 163 tissue samples. During clinical validation stage, paired peripheral blood and tumor tissue samples collected from 316 NSCLC patients were analyzed. Both tissue and plasma samples were tested with an average sequencing depth of 10,000X. The recommended input for tissue DNA and plasma ctDNA is 50ng and 30ng respectively.
Results: LC10 showed competent performance in EGFR ex20ins, common muts, 3rd TKI resistance muts and MET ex14 skipping detection with LOD 1% in tissue and 0.3% in plasma analysis. Specifically, a total of 16 different EGFR ex20ins types were validated during analytical validation stage. Among the 316 NSCLC patients (median age: 59 years; 51.6% male), 80.1% were treatment-naïve, while 19.9% had relapsed with drug resistance. Patients from stage IIIB-IV comprised 37.3% of the cohort (n: 118). In these 118 stage IIIB-IV patients, 57 EGFR muts (19del: 21, L858R: 23, T790M: 7, exon20ins: 4, other: 2) and 1 MET ex14 skipping muts were detected in plasma, achieving an overall percent agreement (OPA) of 86.4% with tissue samples. Concordance by muts site was as follows: 19del: 94.9%, L858R: 91.5%, T790M: 94.1%, exon20ins: 100%, and MET exon 14 skipping: 98.3%. Among the 189 stage I-IIIA patients, 12 EGFR muts and 1 MET exon 14 skipping mut were detected in plasma with OPA of 60.31% compared to tissue. Considering the “non-shedding” nature of early-stage lung adenocarcinoma, this concordance rate is comparable to previous studies.
Conclusion: In advanced-NSCLC, plasma is a dependable source for guiding targeted therapies, capturing both primary driver muts and acquired resistance. Employing LC10 provides a one-stop platform for both tissue and plasma testing in NSCLC diagnosis and treatment, which enhances the precision of muts detection with broader sample accessibility.

TUMOR BIOLOGY: Imaging in Animal Models
Poster Session

#67 Deciphering the mechanisms underlying the pathophysiology and chemotherapy resistance in acute myeloid leukemia using intravital imaging and humanized NSGW41IL7 mouse.
R. Niavarani, X. Lei, M. Edson, E. Chen, S. Wang, R. DaCosta;
University Health Network, Toronto, ON, Canada

Introduction: Acute Myeloid Leukemia (AML) is an aggressive & highly heterogeneous malignancy that originates within the bone marrow (BM). A major challenge in treating AML is its resistance to therapy & high relapse rates, driven in part by changes AML induces in the BM microenvironment, promoting immune suppression & creating hypoxic conditions. However, the mechanisms behind therapeutic resistance in AML patients in the presence of hypoxic BM niche, are poorly understood.
Innovation & Clinical Impact: To address this knowledge gap, our study employs two pioneering methods: a humanized NSGW41IL7 mouse model engrafted with human AML cells & the femur window chamber (FWC) technique, enabling unprecedented real-time visualization of AML dynamics within BM. Using advanced intravital imaging, we are the first to observe the intricate, continuous interactions between AML cells & immune components, such as human gamma delta T cells, within the BM microenvironment. Furthermore, we are interested in using our new approach to investigate how standard chemotherapies impact AML cell survival & immune responses under hypoxic conditions over time. Ultimately, our findings will provide new insights into treatment resistance mechanisms & pave the way for innovative therapeutic strategies targeting the BM microenvironment, improving outcomes for AML patients.

CLINICAL RESEARCH: Diagnostic Biomarkers 2
Poster Session

#671 Development of a diagnostic antibody for CD24 targeted therapy.

Peng Chen[1], Jishun Chen[2], Min Deng[1], Rong Guo[1], Ming Song[1], Jay Mei[3], **Bing Hou**[3]

[1]Shanghai Antengene Corporation Limited, Shanghai, China,[2]Antengene (Hangzhou) Biologics Co., Ltd, Hangzhou, China,[3]Antengene Corporation, Shaoxing, China

Background: CD24 is a small yet extensively glycosylated glycosylphosphatidylinositol protein that is overexpressed in many tumor cells. Elevated CD24 levels typically indicate a poorer prognosis, as it facilitates malignant activity and confers resistance to chemotherapies. CD24 is also identified as a surface marker of cancer stem cells across multiple tumor types. It triggers an anti-phagocytic by binding to sialic-acid-binding Ig-like lectin 10 (Siglec10) on tumor-associated macrophages, thereby inhibiting the phagocytosis of tumor cells. CD24 has emerged as a promising therapeutic target for anti-cancer treatment. Several clinical trials are being conducted to evaluate the safety and efficacy of CD24-targeted therapies. Here, we developed and characterized an anti-CD24 diagnostic antibody to enhance the screening and selection of patients based on CD24 expression.
Methods: SJL mice were immunized with 5-10 million HEK293T-hCD24 cells by intraperitoneal injection. Screening and selection of positive hybridoma clones were conducted using ELISA and flow cytometry analysis. Potential immunohistochemistry (IHC) clones were screened by staining formalin-fixed, paraffin-embedded (FFPE) specimens of human normal esophageal tissue using Leica Bond III platforms. The accuracy assessment, sensitivity (selectivity), specificity, and assay precision were evaluated using the lead antibody. Various human tumor tissue microarrays (TMA), including but not limited to breast cancer, ovarian cancer, urothelial cancer, and small cell lung cancer, were evaluated using lead antibody through IHC staining for further validation.
Results: Monoclonal antibody clone ATG1144 binds to the hCD24 core peptide in ELISA with an EC50 of 0.06 nM. Distinct membrane staining on human normal esophageal tissue FFPE specimens can also be observed with IHC staining using ATG1144. For accuracy assessment, six CDX and twenty human specimens, comprising both positive and negative specimens (including solid tumors and B-cell non-Hodgkin lymphomas), were validated. Samples exhibiting high, medium, and low CD24 expression levels were evaluated for sensitivity and specificity, and the interpreted results aligned with the reference outcomes. FFPE tissues from three distinct patients were evaluated for assay precision assessment. The TMA IHC staining result revealed that 50-80% of patients with lung, breast, bladder, ovarian, or liver cancer have CD24 expression on tumor cell surface with low expression in the para-cancerous normal tissue.
Conclusion: ATG1144 specifically binds to human CD24 with high sensitivity as demonstrated by IHC staining. The development and validation of the method have been finalized using Leica Bond III platforms. These data suggest a potential diagnostic use of ATG1144 for identifying human CD24 + patients.

#672 Plasma levels of Endothelin-1 are increased in colorectal cancer patients versus benign disease patients.

C. S. Herath Mudiyanselage[1], Y.-R. Chen[1], N. Mitra[1], H. Miyagaki[2], E. Nilsson Sjolander[1], X. Yan[1], V. Cekic[1], R. L. Whelan[1]; [1]Lenox Hill Hospital, Northwell Health, New York, NY, [2]Otemae Hospital, Osaka, Japan

Introduction: Endothelin-1 (ET-1) belongs to the Endothelin family and exerts its effects via ETA and ETB receptor (ETAR, ETBR) activation. ET-1 is mainly produced by endothelial cells (EC); its receptors are found in EC and vascular smooth muscle cells. ET-1 increases HIF1α stability and results in transcription of VEGFA, VEGFC, and their receptors. ET-1 has anti-apoptotic effects and influences cell survival pathways, including PI3K-dependent AKT activation and NF-κB signaling that are implicated in tumor progression, migration, and metastatic growth. ETAR inhibition impedes tumor growth and promotes apoptosis. ET-1 is overexpressed in many colorectal cancers. Tumor-derived ET-1 modulates MMP's and urokinase-type plasminogen activator that facilitate ECM degradation and tumor cell liberation. ET-1 alone also promotes neovascularization. This study assesses and compares preoperative (PreOp) plasma ET-1 levels in colorectal cancer (CRC) and benign colon pathology (BCP) patients.

Method: CRC and BCP patients (pts.) undergoing elective colorectal resection who were enrolled in an IRB approved tissue/data bank for whom PreOp plasma samples were available were eligible for the study. Demographic and clinical data were collected. PreOp plasma samples were obtained and stored at -80°C. Plasma ET-1 levels (pg/ml) were determined in duplicate via ELISA in and results presented as median +95% CI. ET-1 expression was analyzed in matched CRC and adjacent normal mucosal tissue samples from a subgroup of CRC patients (n=23) via QRT-PCR. Immunohistochemistry (IHC) was performed on formalin-fixed tissue sections (tumor/normal pairs) as per protocol.

Results: A total of 116 CRC pts (68% colon, 32% rectal) and 112 BCD pts (adenoma 46%, diverticulitis 50%, other 4%) were studied. The CRC stage distribution was: Stage (Stg) 1, 30%; Stg 2, 31% Stg 3, 31%; and stg 4,8%. Median plasma ET-1 levels were higher in CRC (1.39, CI: 1.25, 1.56) compared to BCP patients (1.11, CI: 1.021, 1.21p<0.001). A non-significant (ns) increase in mean ET-1 levels were noted in STG IV vs STG I and STG II and STG III was found (30-37%) and the mean levels in rectal cancer patients was 21% higher than those found colon cancer pts. IHC validated the presence of ET-1 protein in CRC tumors relative to normal colon. Increased expression of ET-1 was seen in 39% of tested CRC samples (9/23).The AUC value for the ROC curve was 0.673 (sensitivity 53%, specificity 73%).

Conclusion: The median plasma level of ET-1 in CRC pts. was 26% greater than noted in BCP pts. Increased tumor expression of ET-1 mRNA was identified in 39% of pts. High expression in the tumor may contribute to the elevated plasma levels. The analysis of the ROC curve indicates that ET-1 could serve as a potential candidate for inclusion in a more comprehensive diagnostic panel with other proteins found to be elevated in CRC pts. Further investigation is necessary.

#673 Protein Plus BCT: Ensuring reliable protein biomarker analysis in liquid biopsies by minimizing pre-analytical variations.
Rachel M. Miller, Sama Mehta, Jing Li

Streck LLC.R, La Vista, NE

While plasma protein biomarkers show promise for cancer early detection, diagnosis, prognosis, and monitoring, pre-analytical variation can affect sample integrity and limit the reliability of proteomic analysis. Sample collection, storage, and processing conditions can alter the proteome through *ex vivo* blood cell activation and lysis, which releases proteins that can obscure relevant *in vivo* abundances. This can significantly impact cancer biomarkers, many of which are expressed in white blood cells and platelets. Here, we use both immunoassay- and LC-MS-based proteomics to show that Protein Plus BCT minimizes proteome alterations during whole blood storage and plasma processing. To this end, plasma was isolated using single and double spin protocols from blood collected into EDTA and Protein Plus BCT immediately after draw or after 3 days of storage at either 2 °C, room temperature (22 °C), 30 °C, or 37 °C. When samples were analyzed on the Ella platform for oncology-relevant biomarkers, including TGF-β (relevant in CRC), we observed that the abundance of TGF-β increased by 5- and 4.3-fold on day 3 compared to draw time in plasma isolated from EDTA using single and double spin protocols, respectively. Conversely, there were minimal changes in abundance for plasma isolated from Protein Plus BCT, with no more than 1.5-fold change from day 3 to draw time observed for either single or double-spin processing. When samples were prepared for bottom-up proteomic analysis, we noted 6 times more proteins were significantly elevated in abundance for single spin plasma at draw time for EDTA compared to Protein Plus BCT. This trend persisted after 3 days of whole blood storage, with 11 times more proteins being elevated in abundance for single spin plasma for EDTA compared to Protein Plus BCT, further demonstrating the ability of Protein Plus BCT to maintain proteome stability across diverse sample storage and handling conditions. Specifically, we saw that EDTA samples demonstrated at least a 3 Log_2 fold change increase in S100A8 concentration, a potential cancer biomarker, on day 3 relative to draw time levels regardless of sample processing (single vs double spin) or storage temperature (2 °C, 22 °C, 30 °C, or 37 °C), whereas we saw no increase in abundance in samples collected into Protein Plus BCT on day 3 except for samples stored at 37 °C, which showed a 0.5 Log_2 fold change increase. Taken together, these data indicate that Protein Plus BCT preserves sample integrity and minimizes changes to the proteome, relative to EDTA, by reducing the impact of extended storage, temperature variations, and sample handling on accurate analysis. This offers researchers and assay developers flexibility in sample transport, storage, and processing. Protein Plus BCT is for Research Use Only. Not for use in diagnostic procedures. Protein Plus BCT should only be used for research or the development of new assays.

#674 Functional characterization of SMARCA4 genomic variants.

Shivani Jagannathan Murali, Bharath Kumar karre, Cristina Ivan, Kelly Craven, Stephanie Williams, Shikha Mahajan, Asfetaw Abera, Chao Sima, David Spetzler, Milan Radovich, Heather O'neill, Ravi Chakra Turaga

Department of Precision Medicine, Target & Drug Discovery, Caris Life Sciences, Inc., Chandler, AZ

Purpose: This study aims to carry out a pan cancer analyses of SMARCA4 genomic variants, including fusions, protein truncating variants (PTVs) and non-truncating variants(non-PTVs). Histological correlation of genomic variants for SMARCA4 expression may help identify tumors deficient in SMARCA4, making them potential candidates for treatment with synthetically lethal SMARCA2 inhibitors.
Methods: We analyzed 350,413 samples from Caris Life Sciences for clinical molecular profiling at Caris Life Sciences via Caris MI Tumor Seek (Phoenix, AZ). Gene fusion detection was performed on mRNA from FFPE tumor samples using the Illumina NovaSeq platform. Fusions with ≥ 3 junction reads were reviewed for domain prediction, breakpoints, frame retention, and co-occurring alterations. WES data was also examined for pathogenic mutations to assess fusion oncogenicity. IHC for SMARCA4 (N-terminal & C-terminal) and SMARCA2 was conducted on FFPE sections from 35 patients with SMARCA4 variants, with staining scored by a board-certified pathologist.
Results: Pan-Cancer analysis of SMARCA4 alterations revealed that NSCLC (30.7%) cases harbored the largest percentage of mutations followed by the CRC (15%) and UTNE (10%). Further, analysis through IHC revealed that patients with PTVs such as nonsense and frameshift mutations, were typically characterized by shorter/ truncated SMARCA4 protein and exhibited complete loss of SMARCA4 expression through IHC, often accompanied by an increase in SMARCA2 expression by IHC. In contrast, patients with missense mutations (non-PTVs) retained SMARCA4 expression, with minimal changes in SMARCA2 staining. In cases with SMARCA4 fusions detected, IHC analysis revealed an ambiguous result with some fusions retaining expression of SMARCA4 protein and others that did not. Patient cases harboring SMARCA4 fusions that displayed loss of SMARCA4 and concordant increase in SMARCA2 staining, would make good candidates for SMARCA2 inhibitor treatment. WTS analyses also revealed a decrease in SMARAC4 expression in PTVs, but not in non-PTVs. Further, we noted that other prevalent co-occurring mutations included TP53(55.7%), KRAS(19.7%) and ARID1A(16.7%) to be the most prominent alterations in patients with SMARCA4 alterations. Patients with SMARCA4 PTVs had poor prognosis compared to those without SMARCA4 variants in the NSCLC and UTNE indication.
Conclusion: SMARCA4 mutations, including frameshift and nonsense mutations, are common in NSCLC, UTNE, and OSEC. These alterations, particularly PTVs, make SMARCA4-deficient tumors potential targets for SMARCA2 inhibitor therapy. Some cases with SMARCA4 fusions or missense mutations may also be eligible for treatment, but these should be assessed individually, considering co-occurring mutations, copy number amplifications and fusion partners. This will aid in refining patient selection and improving treatment outcomes using companion diagnostics.

#675 Performance of highly sensitive molecular biomarker for pancreatic cancer detection.

Sophia F. Fang, Neng Yang

Epi One, Inc., Brooklyn, NY

Background: Pancreatic cancer is one of the most lethal malignancies, with resection being the only potentially curative treatment. However, due to the absence of effective early detection or screening, most patients are diagnosed at an advanced stage with a five-year survival rate of only 3%. Several genetic mutation-based biomarkers are already in use for cancer diagnosis. Epigenetic alterations, recognized as early events in tumorigenesis, also play a critical role in cancer development. The integration of mutational and epigenetic biomarkers has significant potential to enhance the accuracy of diagnostic panels for early cancer detection. By applying advanced mathematical models to integrate multi-level genomic and epigenetic data, Epi One has identified highly accurate biomarker panel for the early detection of pancreatic cancer. We developed pancreaScout - a simple, cost-effective, and highly sensitive qPCR assay for detecting the biomarker panel associated with pancreatic cancer. In this report, we present the performance of pancreaScout for the biomarker panel in human tissue and blood samples.

Methods: Eighty-seven human primary tissue samples and 48 blood samples with IRB consent were collected DNA was extracted from human primary samples DNA underwent bisulfite conversion to enable methylation-specific analysis, with quantitative methylation-specific PCR (qMSP) performed to quantify DNA methylation levels. In parallel, quantitative PCR (qPCR) was conducted on untreated DNA to detect genetic mutations. qPCR data analysis was carried out to calculate scores, which reflect the levels of mutation and methylation in samples.

Results: Identification of a pancreatic cancer detection panel with two mutation- and four DNA methylation- biomarkers. Validation of the biomarker panel using qPCR in 87 tissue samples (65 pancreatic cancer patients and 22 adjacent normal controls) , achieving 87% sensitivity and 90% specificity. Validation of the biomarker panel using qPCR in 41 blood samples (including 41 pancreatic cancer patients and 7 normal individuals) and achieved 69% sensitivity and 100% specificity.

Conclusions: pancreaScout is a sensitive and specific diagnostic panel for pancreatic cancer detection for both tissue and blood samples. Compared to sequencing-based methods for early cancer detection, pancreaScout assay achieves comparable sensitivity in blood tests. The simplicity of pancreaScout adds significant value in the fight against pancreatic cancer.

#676 First in man microdose trial of [^{68}Ga]Ga-R54, CXCR4 targeted PET tracer: Comparison with [^{18}F]-FDG and proof of concept in lung cancer models.

Anna Maria Trotta[1], Luigi Portella[1], Michela Aurilio[1], Crescenzo D'Alterio[1], Francesco Manna[1], Costantina Maisto[1], Antonio Luciano[1], Giuseppe Palma[1], Gaetana Di Maiolo[1], Laura D'Ambrosio[1], Francesca Bruzzese[1], Roberta De Marino[1], Aureliana Esposito[1], Luigi Di Caterino[1], Valentina Vozza[1], Elena Sinopoli[1], Gianfranco De Feo[1], Francesco Perrone[1], Salvatore Di Maro[2], Secondo Lastoria[1], **Stefania Scala**[1]

[1]Istituto Nazionale Tumori - IRCCS G. Pascale, Naples, Italy,[2]Universita della Campania "L. Vanvitelli", Caserta, Italy

CXCR4 is overexpressed on the majority of haematological and solid cancers and correlates with metastatic features. CXCR4 is a potential imaging target for the accuracy of diagnosis, staging and predictive treatment efficacy. Despite the development of CXCR4 targeting PET tracers, the sensitivity and specificity toward solid tumors is modest. A new CXCR4-targeting PET tracer, [^{68}Ga]Ga-R54 (PCT/EP2020/087792) specifically accumulated in CXCR4-overexpressing tumors in *in vivo* murine models. Aim of the work is to extend the *in vivo* specificity and imaging efficacy of [^{68}Ga]Ga-R54 in lung cancer and to evaluate the [^{68}Ga]Ga-R54 in humans. Thus, PET/CT imaging and biodistribution was conducted on athymic mice bearing subcutaneous NCI-H69 (SCLC) and H460 (NSCLC) tumors. Biodistribution studies revealed high and specific tumour uptake in both H69 and H460 tumors s after 1 hour post injection (H69-tumor uptake: 12.2±1%ID/g; H460-tumor uptake: 2±0.4%ID/g) and rapid renal excretion. Competition with cold R54 reduced [^{68}Ga]Ga-R54 uptake by approximately 90% and 60% respectively showing CXCR4 specificity. Compared to [^{18}F]-FDG, [^{68}Ga]Ga-R54 showed significantly higher uptake in H69 tumors (12.2±1%ID/g vs 3.75±0.6%ID/g, $p<0.001$). Consistently, PET/CT scans clearly visualized tumors at 1 hour post tracer administration with significant reduction in accumulation due to cold R54. Accordingly, high CXCR4 expression was revealed in H69 human lung cancer cells. Thus, a Microdose clinical study (ICH M3(R2) CPMP/ICH/286/95 Chapter 7.1) was planned to identify Pharmacokinetics/ Pharmacodynamic properties of [^{68}Ga]Ga-R54 in metastatic/ advanced solid tumors. Toxicity and toxicokinetic as GLP-Extended single dose toxicity study in rats demonstrated non-evident toxicity induced by iv injection of 1000x the supposed clinical dose of [^{68}Ga]Ga-R54 at 2 and 14 days post-injection. A monocentric, nonrandomized, open label clinical trial with [^{68}Ga]Ga-R54 (R4-FOUR-PET - EU CT: 2024-513303-14-00) was recently approved on October 2024. R4-FOUR-PET will enrol 5 to 10 patients carrying CXCR4 expressing advanced cancers (breast, colon, lung, pancreas and ovarian) and will be conducted at the Nuclear Medicine of IRCCS-Pascale. Primary objective of the study is to evaluate pharmacokinetics and pharmacodynamics of a single microdose (<100µg) of [^{68}Ga]Ga-R54; comparison with [^{18}F]-FDG and correlation between SUVmax and CXCR4 IHC expression will be conducted. Patient’s enrolment is ongoing.

#677 H-score as a diagnostic tool in HER2 equivocal (IHC 2+) breast cancer.

Tasnem Alsebai[1], Muhammed Yaman Swied[1], Ricardo Cossyleon[2], Kathy Robinson[2], Kristin Delfino[1], John Gao[3], Krishna Rao[2]

[1]Southern Illinois University at School of Medicine, Springfield, IL,[2]Simmons Cancer Institute at Southern Illinois University, Springfield, IL,[3]Department of Pathology, Springfield Memorial Hospital, Springfield, IL

Introduction: Human epidermal growth factor 2 receptor (HER2) overexpression occurs in 15-20% of primary invasive breast cancers, significantly contributing to their aggressive nature and poor prognosis [1]. HER2 status is typically assessed using immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH). IHC results classified as 2+ are considered equivocal, leading to a reliance on FISH for confirmation. However, FISH is costlier and time-consuming, which can delay necessary treatment [2]. This study aims to assess the potential of the histochemical score (H-score), a quantitative measure derived from IHC staining and calculated using ImageJ software, as a reliable tool for classifying HER2 status in cases with equivocal IHC 2+ results. By investigating whether the H-score can accurately predict HER2 status, this study explores the possibility of reducing reliance on FISH for confirmation.
Methods: This retrospective study included adult patients (≥18 years) diagnosed with HER2 IHC 2+ breast cancer from January 2014 to December 2023. Demographic and pathologic data were collected from medical records, and diagnostic biopsy slides were reviewed. Images of IHC specimens were captured at ×10 magnification, standardized, and analyzed using ImageJ software. The H-score was calculated using mean staining intensity and stained area percentage. Binary logistic regression evaluated the predictive power of the continuous H-score on HER2 status by FISH.
Results: The mean H-score of 182 patients was 2.65, with HER2-positive cases showing a higher mean score (3.06) than HER2-negative cases (2.62). ROC analysis yielded an AUC of 0.668 (95% CI 0.523 to 0.812), indicating poor predictive accuracy. Moreover, when calculating the Youden index, the optimal H-score cutoff score (2.6007) provided moderate sensitivity (0.769) but limited specificity (0.580). These results suggest that while the H-score may provide some predictive value, it has limited accuracy for classifying HER2 status independently.
Summary: The H-score lacks accuracy to reliably distinguish HER2 status (positive from negative) in IHC 2+ cases. The moderate AUC and risk of false positives emphasize the need to continue using FISH analysis for confirmation. While moderately correlated with HER2 status, the H-score does not meet clinical precision standards, making FISH essential for accurate classification. Future research should aim to refine the H-score or identify new markers to improve predictive accuracy in equivocal cases.

#678 A 10-second lipidomic based approach to diagnose common spinal tumor types with picosecond infrared laser mass spectrometry.

Alexa Fiorante[1], Michael Woolman[1], David Munoz[2], Taira Kiyota[3], Lan Anna Ye[4], Yasamine Farahmand[5], Darah Vlaminck[1], Francis Talbot[4], Sunit Das[2], Gelareh Zadeh[4], Howard Ginsberg[2], Ahmed Aman[3], Arash Zarrine-Afsar[4]

[1]Medical Biophysics, University of Toronto, Toronto, ON, Canada,[2]Unity Health Toronto, Toronto, ON, Canada,[3]Ontario Institute for Cancer Research, Toronto, ON, Canada,[4]University Health Network, Toronto, ON, Canada,[5]University of Toronto, Toronto, ON, Canada

Intradural extramedullary spinal neoplasms account for ~40% of all diagnosed spinal tumors. Of that, meningioma and schwannoma are the most common. However, improving the surgical outcomes for these spinal neoplasms requires precise intraoperative diagnosis provided by highly trained neuropathologists. Through a retrospective study of n=257 patient specimens, we demonstrate that 10-second picosecond infrared laser mass spectrometry (PIRL-MS) can robustly and objectively diagnose commonly occurring spinal tumor types with the sensitivity and specificity values of (93±1)% and (97±2)%, respectively. This classification utilizes n=41 cellular lipids including phosphatidylcholines, sphingomyelins, phosphatidylethanolamines, and ceramides, whose identities were established using high-resolution tandem mass spectrometry. The identified lipids form a ‘molecular array’ for robust diagnosis of meningioma and schwannoma tumors by non-pathologists in a manner like genomic, transcriptomic, or methylomic arrays used to diagnose brain cancer types, albeit on a faster timescale of seconds as opposed to hours. Furthermore, when subjected to the presence of additional intradural extramedullary spinal tumor types in the differential diagnosis, the generalizability and robustness of the identified molecular array rendered correct classification even in the presence of data not seen previously by the model. PIRL-MS mediated pathology stratifies the resection risk such that complete removal of certain spinal neoplasms such as meningioma tumors with dural excision could be justified to improve the surgical outcomes. Thus, providing informed surgical care even in the absence of intraoperative consults, addressing the human resource limitations in settings that are understaffed in neuropathology. Here, the current state of PIRL-MS device development allows operation by anyone with basic laboratory skills.

#679 Orthogonal confirmation using the Pacbio®two step universal adapter amplicon protocol to validate a comprehensive genomic profiling assay for hematologic malignancies.

Grant Hogg[1], Kimberly A. Holden[1], John Howitt[2], Adib Shafi[1], Amanda Willliamson[2], Tong Liu[1], Helen Cao[1], Xiaojun Guan[1], Michael Mooney[2], Qian Zeng[3], Stan Letovsky[3], Li Cai[2], Eric A. Severson[2], Shakti Ramkissoon[2], Anjen Chenn[2], Marcia Eisenberg[4], Eyad Almasri[1], Taylor J. Jensen[1], Jonathan Williams[1]

[1]Oncology, Labcorp, San Diego, CA,[2]Oncology, Labcorp, Durham, NC,[3]Labcorp, Westborough, MA,[4]Labcorp, Burlington, NC

Next generation sequencing (NGS) using targeted panels is standard of care to aid diagnosis, prognosis and treatment selection for hematologic diseases such as acute myeloid leukemia. We have validated the Labcorp Pan-Heme NGS (LPH) panel that targets 141 clinically relevant genes to detect single nucleotide variants (SNVs), insertions/deletions (indels), *FLT3* internal tandem duplicates (*FLT3*-ITDs) and copy number aberrations (CNAs) in myeloid and lymphoid malignancies. In total, 677 samples were assessed including 315 clinical samples. Analytical sensitivity and specificity experiments estimated that the limit of detection (LoD) for the LPH panel was 3-5% variant allele frequency (VAF). Determining accuracy of SNV/indels in clinical samples requires an independent confirmation. Sanger is the gold standard but has a sensitivity of ~15% VAF. We developed and utilized a custom PacBio multiplexed two-step amplicon sequencing protocol using Sanger sequencing products as template. We determined it to be a sensitive, cost-effective and easily customizable approach to detect variants with VAF <15%. The LPH panel interrogates all coding exons of the 141 genes using a custom hybrid capture-based assay of genomic libraries from 250 ng blood or bone marrow genomic DNA followed by sequencing on Illumina® sequencers. For PacBio confirmation, M13 tagged amplicons with length ranging from 214-678bp were generated using 10-50 ng input DNA, amplified with asymmetric barcodes, and processed for PacBio HiFi sequencing on a Sequel II. PacBio variants were called using Mutect2 (GATK) and/or visual inspection of .bam files by Integrated Genome Viewer (IGV). A subset of variants was called using the PacBio Amplicon Analysis tool (pbAA). 217 amplicons from 126 unique clinical samples were assessed by PacBio with a VAF ranging from 3-100% and comprising 140 SNVs, 63 indels <25bp and 14 indels ≥ 25bp. Concordance between PacBio and the LPH panel was 84.8% (184/217) and 69.4% (66/95) for the subset of variants with VAF between 3-5%. 9 of 184 required pbAA analysis to make a final consensus call. 63 variants were assessed with PacBio by processing the same M13 amplicons previously taken through Sanger analysis. This "double-dip" approach showed an 96.7% agreement (59/61) between PacBio and Sanger. The 2 variants not in agreement had VAF <15%.The PacBio variant calls in this study were incorporated into the results of the LPH panel analytical validation as orthogonal information, yielding an overall PPA for SNVs/indels of 99.1% (1250/1272) and FDR of 0.8% (10/1260) using VAF thresholds of 3% for SNVs and 5% for indels. In conclusion, the PacBio amplicon protocol can utilize amplicons generated for Sanger sequencing with minimal additional wet lab processing offering a flexible approach to confirmation for variants below the threshold of Sanger sensitivity.

TUMOR BIOLOGY: Imaging in Animal Models
Poster Session

#68 Precision medicine in glioblastoma: cortical network disruptions, biomarker development, and multimodal therapeutic strategies.

Tenesha Connor, Frederick Bell, Macit Emre Lacin, Miguel Maldonado, Kemal Ozdemirli, Anthony Sloan, Justin Lathia, **Murat Yildirim**

Cleveland Clinic Lerner Research Institute, Cleveland, OH

Glioblastoma (GBM) is a highly aggressive brain tumor characterized by diffuse infiltration into surrounding brain tissue, disruption of neural circuitry, and interactions among neurons, astrocytes, and blood vessels. This infiltration leads to severe functional impairments, including neuronal hyperexcitability, cognitive deficits, and in some cases seizures. While substantial progress has been made in understanding GBM's molecular mechanisms, less is known about how tumor progression alters brain connectivity, neural oscillations, and behavior. Using advanced transgenic mouse models (e.g., GCaMP6s, GCaMP6f, jGCaMP8m, and Vgat-Cre-ChR2) and custom-built optical microscopes, we performed large-scale, high-speed neural recordings and manipulations across over 10 million neurons spanning 60 brain regions in vivo in pre-clinical GBM models. Our approach integrates deep learning tools for multimodal analysis of brain activity, pupil dynamics, and orofacial behaviors, supported by computational models optimized with quantum computing. Preliminary data reveals significant tumor-induced disruptions in neural and behavioral dynamics. Tumor growth in the mouse striatum is associated with increased pupil size, restricted gaze range, and reduced interhemispheric connectivity. Furthermore, oscillatory activity shows a striking shift, with decreases in delta power accompanied by increases in theta and alpha band power. These findings highlight GBM's capacity to disrupt normal neural rhythms and connectivity. Our work underscores the potential of using behavioral relevant parameters as a non-invasive biomarker for early detection of GBM-induced neural dysfunction. Future studies will focus on leveraging these insights to develop novel therapeutic strategies targeting tumor-induced disruptions in oscillatory activity. By integrating cutting-edge imaging technologies and computational methods, our findings have significant implications for improving early diagnosis and treatment outcomes for GBM patients.

CLINICAL RESEARCH: Diagnostic Biomarkers 2
Poster Session

#680 HPV serology and circulating viral DNA for detection, genotyping, and measurement of disease burden in oropharyngeal cancer.

Lucas Penny[1], Eric Y. Stutheit-Zhao[2], Birgitta E. Michels[3], Fabian Rosing[3], Jinfeng Zou[4], Zhen Zhao[4], Yangqiao Zheng[4], Shao Hui Huang[2], Johnny Carlton[2], Andrew McPartlin[2], John R. de Almeida[5], David Goldstein[5], Andrew Hope[2], Ali Hosni[2], John Kim[2], Fei-Fei Liu[2], C Jillian Tsai[2], John N. Waldron[2], Anna Spreafico[6], Enrique Sanz Garcia[5], Lillian L. Siu[6], Tim Waterboer[3], Geoffrey Liu[1], Scott V. Bratmn[2]

[1]Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada,[2]Department of Radiation Oncology, University of Toronto, Toronto, ON, Canada,[3]Infections and Cancer Epidemiology Division, German Cancer Research Center (DKFZ), Heidelberg, Germany,[4]Princess Margaret Cancer Centre, Toronto, ON, Canada,[5]Department of Otolaryngology-Head & Neck Surgery/Surgical Oncology, Princess Margaret Cancer Centre, Toronto, ON, Canada,[6]Division of Medical Oncology & Hematology, Princess Margaret Cancer Centre, Toronto, ON, Canada

Introduction: The incidence of human papillomavirus-positive (HPV+) oropharyngeal cancer (OPC) has increased rapidly, and HPV early antigen serology has been proposed as a scalable and cost-effective early detection test. HPV seropositivity can precede clinical presentation of OPC by several years, so additional surveillance procedures may be necessary to optimize early cancer detection. The potential for HPV circulating tumor DNA (ctDNA) to confirm a diagnosis of OPC in seropositive individuals is poorly understood. Here, we assess the relationship between HPV serology and HPV ctDNA with disease burden in a large cohort of HPV+ OPC.
Methods: We analyzed pre-treatment peripheral blood plasma from 262 patients with non-metastatic p16+ OPC treated with definitive (chemo)radiotherapy. A multiplex ELISA was used for serologic evidence of HPV proteins from 10 HPV genotypes, quantified by mean fluorescence intensity (MFI). Plasma HPV ctDNA was quantified by whole viral genome sequencing utilizing a custom HPV-targeted capture panel for 38 HPV genotypes. Gross tumor volume (GTV) was obtained from the sum of all target contours on computed tomography scans.
Results: Both assays identified HPV16 as the most prevalent genotype (84%), with results indicating a total of 4 and 6 HPV genotypes for serology and HPV ctDNA, respectively. HPV ctDNA results demonstrated higher sensitivity and lower cross-reactivity between HPV types compared with HPV serology results. Furthermore, HPV ctDNA but not HPV16 E6 antibody levels were positively associated with disease burden as determined by N-category (Table 1) and tumor volume (ctDNA vs GTV, r=0.48 p=6.4e-13; E6 vs GTV, r= -0.079 p=0.26).
Conclusion: This is the largest cohort to compare HPV serology and ctDNA results in OPC. These findings highlight the potential for ctDNA to augment future strategies for blood-based early detection of HPV+ OPC.
Table 1: Comparison of HPV16 E6 serology (MFI) and HPV ctDNA (log[copies/mL]).

N-Category(UICC 8th Ed.)	**N0(n=6)**	**N1 (n=139)**	**N2 (n=42)**	**N3 (n=14)**	**P-value (Anova)**
Serology	7432	8633	8097	5444	**0.038**
ctDNA	2.32	2.63	2.83	3.85	**<0.001**
T-Category	**T1 (n=45)**	**T2 (n=92)**	**T3 (n=32)**	**T4 (n=29)**	**P-value (Anova)**
Serology	9041	8210	7427	8233	**0.36**
ctDNA	2.41	2.79	2.88	2.99	**0.11**

#681 A novel approach of Claudin 18 immunohistochemistry testing demonstrated comparable performance and high concordance compared to Ventana 43-14A in gastric cancer.

Zhenghua Tang[1], peng cheng[2], Xin Liu[3], Huangbin Lu[1], Jingjing Xu[1], Yiling Cai[1], **Changbin Zhu**[2]

[1]Research and Development Department, Amoy Diagnostics Co., Ltd., Xiamen, China,[2]Translational Medicine Department, Amoy Diagnostics Co., Ltd., Xiamen, China,[3]BD Department, Amoy Diagnostics Co., Ltd., Xiamen, China

Background: Claudin(CLND)18 is a tight-junction molecule consisting two Isoforms:CLND18.1 and CLND18.2. Isofrom CLND18.2 is specificly expressed in gastric cancer. Zolbetuximab is the first CLDN18.2 therapeutic monoclonal antibody approved by FDA. VENTANA® CLND18(43-14A), a pan-CLND18 immunohistochemistry (IHC) testing, was approved as companion diagnostics simutanusly. Validation between different Claudin 18 IHC testing approaches could improve the convenience of diagnostics in clinical use.

Methods: A total of 68 gastric cancer samples were enrolled in this study. Expressing status of Claudin 18 was evaluated independently by two IHC approaches. AmoyDx CLDN18 (43-14A) assay was used as testing reagent and VENTANA® CLDN18 (43-14A) RxDx acts as reference reagent. Claudin 18 positive was defined as: tumor with staining intensity of 2+ or 3+(total) in ≥75% of tumor cell.

Results: Validtaion results was shown in Table 1. 21 positive cases and 47 negative cases were identified by two reagents. Standing for a 100%(84.54% - 100%) positive percent agreement (PPA), 100% (92.44% - 100%) negative percent agreement (NPA), and 100% (94.65% - 100%) overall percent agreement (OPA). Poisson distribution analysis of the proportion of cells with four staining intensities(3+, 2+, 1+ and 0) demostrated that the staining intensity of the two approaches was highly consistent ($R = 0.99$-1, $p < 2.26e^{-16}$). When the positive threshold was exploratively adjusted (2+/3+ cells ≥50%), high concordance was still maintained (PPA 100%, NPA 93.55%, OPA 97.06%, Table 1.) except 2 conflict samples, which were close to the exploratively threshold.

Conclusion: Two Claudin 18 testing approaches demonstrated high concordance. It has potential versatility in future clinical practice.

Concordance of two CLND18 testing approaches

2+/3+≥75%	Ventana CLDN18(43-14A)				2+/3+≥50%	Ventana CLDN18(43-14A)			
AmoyDx CLDN18 (43-14A)		Positive	Negative	Total	AmoyDx CLDN18 (43-14A)		Positive	Negative	Total
	Positive	21	0	21		Positive	37	2	39
	Negative	0	47	47		Negative	0	29	29
	Total	21	47	68		Total	37	31	68
PPA	100% (84.54% - 100%)				PPA	100% (90.59% - 100%)			
NPA	100% (92.44% - 100%)				NPA	93.55% (79.28% - 98.21%)			
OPA	100% (94.65% - 100%)				OPA	97.06% (89.90% - 99.19%)			

#682 PCDHGB10: a unique, sensitive, and specific serum cfDNA methylation biomarker for stomach adenocarcinoma diagnosis.

Donghong Zhang[1], Chaoyan Niu[2], Daoyu Yan[3], Ruo-Pan Huang[4]

[1]Donghong Zhang (Individual), Peachtree Corners, GA,[2]RayBiotech Guangzhou Co. Ltd, Guangzhou, China,[3]RayBiotech Guangzhou Co. Ltd., Guangzhou, China,[4]RayBiotech Life Inc, Peachtree Corners, GA

Serum cell-free DNA (cfDNA) methylation has demonstrated its potential in detecting and screening for multiple cancers, offering a critical window for identifying patients eligible for effective treatment. Our objective was to evaluate the efficacy of high methylation-sensitive restriction enzyme combined quantitative PCR (MSRE-qPCR) in identifying a serum cfDNA methylation biomarker for a spectrum of cancer types. We initially developed a 6-gene panel comprising 11 pan-cancer cell-differentially methylated regions across 15 prevalent cancer types, utilizing data from the TCGA methylome database (n = 6981). This panel was then validated using MSRE-qPCR on serum cfDNA samples from 825 participants, encompassing 632 cancer patients across 11 cancer types and 193 healthy controls, collected from multiple cancer centers in China. We assessed the specificity and sensitivity of the panel for diagnosing individual cancers at multiple sites, including the colorectum, esophagus, stomach, liver, lung, ovary, lymphoma, cervix, endometrium, nasopharynx, and pancreas. Among the 11 cancer types, stomach adenocarcinoma (STAD) demonstrated the highest sensitivity and specificity for DNA methylation-based diagnosis in real-world scenarios. PCDHGB10 methylation particularly stood out for STAD diagnosis, achieving a sensitivity of 93.16% and specificity of 88.24% in the training cohort, and 84.09% sensitivity and 96.67% specificity in the validation cohort. For early-stage (I and II) patients, PCDHGB10 sensitivity was 78.48%, rising to 86.89% in late-stage patients. In contrast, PCDHGB10 methylation showed suboptimal diagnostic performance (less than 65% sensitivity) in the other 10 cancer types. This extensive public database and clinical validation study underscores the role of PCDHGB10 methylation as a highly sensitive, specific, and distinctive serum cfDNA methylation biomarker for STAD diagnosis, with particular promise for early-stage detection.

#683 NaDC3: a potential tumor suppressor and biomarker in renal cell carcinoma.

Miswakumari Lad, Tianyu Yang, Karina Aguilar, Vinata Lokeshwar

Augusta University, Augusta, GA

Introduction and Objective: The five-year survival rate for patients with metastatic renal cell carcinoma (mRCC) is less than 10%, with the highest incidence found among African American males. Addressing racial disparities in renal cell carcinoma (RCC) are essential for the development of biomarkers and targeted therapies. NaDC3, a succinate/citrate transporter expressed in kidney epithelial cells, has not yet been studied in relation to any diseases. In our research, we assessed NaDC3 expression in both normal and RCC tissues and examined its correlation with clinical outcomes and racial disparities. Additionally, we investigated the biological functions and molecular signaling pathways influenced by NaDC3 in RCC cells.

Methods: Differences in gene expression in the matched normal and RCC tissues (n=6/category) was evaluated using microarray analysis; results were then validated by QPCR and immunoblotting in tissues from 83 patients (White=46; Hispanic=24; AA=13). VHL+ and VHL- RCC cells were stably transfected with a NaDC3 construct. Transfectants were assessed for cell proliferation, cell cycle, motility, succinate/citrate transport and reactive oxygen species (ROS) measurement assays under normoxic and hypoxic conditions. SDCT2 induction was evaluated following 5-azacytidine plus Trichostatin A treatment.

Results: NaDC3 was 63- and 100-fold downregulated in low- and high-stage RCC tissues respectively. QPCR validation showed 40-fold downregulation of NaDC3 in RCC tissues when compared to normal kidney ($P< 0.0001$). Downregulation was 40-fold in White and Hispanic patients, but 198-fold in AA patients ($P=0.0049$) and correlated with tumor stage and metastasis ($P=0.009$). Under hypoxic conditions, NaDC3 expression caused 3-4-fold inhibition of proliferation, cell-cycle, and motility in both VHL+ and VHL- cells ($P<0.01$); only VHL+ cells were inhibited under normoxia. NaDC3 expression induced ROS levels and succinate transport by >3-fold ($P<0.01$) and activated the p16INK4a-RB pathway and apoptosis (caspase-3 and PARP activation). NaDC3 expression was silenced in patient-derived primary cell lines from AA patients.

Conclusion: This is the first study on a functional biomarker in RCC, NaDC3, that is a possible novel tumor suppressor gene. NaDC3 loss promotes RCC growth and survival and inhibits cellular senescence, while its downregulation correlates with metastasis and racial disparity.

Support: 1R01CA227277-05A1; 1R01CA283699-01

#684 Multi-site external reproducibility of PD-L1 IHC 22C3 pharmDx performance on Dako Omnis and comparison to Autostainer Link 48 for head and neck squamous cell carcinoma using the Combined Positive Score.

Monika D. Polewski[1], Lining Hutchinson[1], Jianan Ren[2], Nichole LaPointe[2], Siena Tabuena-Frolli[2], Kurt Mathews[2], Gregory A. Cherryholmes[1]

[1]Global Clinical Affairs, Organization of the Chief Medical Officer, Agilent Technologies, Inc., Carpinteria, CA,[2]Research and Development, CDx, Agilent Technologies, Inc., Carpinteria, CA

Background: PD-L1 IHC 22C3 pharmDx is a diagnostic assay indicated as an aid in identifying biomarker PD-L1 expression levels for use on the Autostainer Link 48 (ASL48) platform for multiple indications. However, to date PD-L1 IHC 22C3 pharmDx on Dako Omnis platform has only been validated for non-small cell lung cancer. This study sought to demonstrate external reproducibility of PD-L1 IHC 22C3 pharmDx for head and neck squamous cell carcinoma (HNSCC) using Dako Omnis (Investigator Use Only/Performance Evaluation Only). This study also investigated the concordance of PD-L1 IHC 22C3 pharmDx on HNSCC on both ASL48 and Dako Omnis at external sites.

Methods: This study was a three-site, blinded, and randomized study of PD-L1 IHC 22C3 pharmDx on HNSCC specimens. To investigate inter-site and intra-site reproducibility, five replicate sets of pre-qualified unstained specimens were stained using Dako Omnis and evaluated at each of the three testing sites. For the inter-platform performance evaluation, pathologists at each of the three testing sites evaluated one set of HNSCC slides that was pre-stained using PD-L1 IHC 22C3 pharmDx on ASL48. Pathologists determined the PD-L1 expression level using a Combined Positive Score (CPS) at diagnostic cutoffs CPS ≥ 1 and CPS ≥ 20. The analytical agreements of the diagnostic outcome (positive/negative) were estimated by evaluating negative percent agreement (NPA), positive percent agreement (PPA), and overall percent agreement (OA) based on comparisons to the consensus diagnostic outcome with corresponding percentile bootstrap confidence intervals. The pre-determined acceptance criteria (AC) for all endpoints are that the lower-bound (LB) of a two-sided 95% confidence interval (CI) for NPA, PPA, and OA must meet or exceed 85.0%.

Results: The study met all AC for the CPS ≥ 1 and CPS ≥ 20 cutoffs. For CPS ≥ 1, the 95% CI LB of the inter-site reproducibility endpoints were 90.5% for NPA, 98.7% for PPA, and 95.0% for OA and intra-site reproducibility endpoints were 98.9% for NPA, 98.0% for PPA, and 98.5% for OA. For CPS ≥ 20, the 95% CI LB of the inter- site reproducibility endpoints were 87.7% for NPA, 91.1% for PPA, and 91.2% for OA and intra-site reproducibility endpoints were 92.7% for NPA, 95.8% for PPA, and 95.0% for OA. For CPS ≥ 1, the 95% CI LB of the inter-platform performance comparison endpoints were 96.0% for NPA, 98.7% for PPA, and 98.0% for OA. For CPS ≥ 20, the 95% CI LB of the inter-platform performance comparison endpoints were 90.6% for NPA, 94.2% for PPA, and 93.3% for OA.

Conclusions: This study demonstrates reproducibility of PD-L1 IHC 22C3 pharmDx (with Dako Omnis) in HNSCC specimens at the CPS ≥ 1 and CPS ≥ 20 cutoffs. The results of the inter-platform performance comparison study demonstrate concordance between ASL48 and Dako Omnis in HNSCC specimens at the CPS ≥ 1 and CPS ≥ 20 cutoffs.

#685 Integrative screening of DNA methylation markers enables early detection of hepatocellular carcinoma.

Hao Zhuang[1], Bo Wang[2], Yongmei Li[2]

[1]UC San Diego, San Diego, CA,[2]Tianjin Medical University, Tianjin, China

DNA methylation, a pivotal epigenetic modification regulating gene expression, has gained significant attention as a potential diagnostic biomarker for various cancers, including hepatocellular carcinoma (HCC). This study aimed to identify aberrant DNA methylation patterns and protein alterations using integrated methodologies and to develop a robust biomarker panel for blood-based early detection of HCC. Genome-wide methylation profiling was performed to identify hypermethylated CpG sites and genes that distinguish early-stage HCC from non-HCC tissues. Using four distinct strategies, 44 candidate marker genes were identified and subsequently assessed in combination with five protein markers in a cohort of 299 blood samples. Through 5-fold cross-validation, a panel comprising six methylation markers from four different groups achieved an area under the curve (AUC) of 0.938, outperforming panels derived from single-group markers. A final 6-marker panel, combining four methylation markers and two protein markers in a logistic regression (LR) model, was developed. This panel demonstrated a sensitivity of 92.2% at a specificity of 94.3% in the training set, and a sensitivity of 86.4% at a specificity of 97.8% in the validation set. Notably, the 6-marker panel showed superior sensitivity (75.0%) for detecting HCC at Barcelona Clinic Liver Cancer (BCLC) stages 0-A compared to alpha-fetoprotein (AFP) and des-gamma-carboxyprothrombin (DCP). A multi-group approach to selecting methylation markers outperformed single-group strategies. The developed 6-marker panel demonstrates excellent sensitivity and specificity, offering a promising tool for the early detection of HCC, particularly in its earliest stages.

#686 Retrospective analysis of estrogen receptor expression in prostatic neoplasms: Implications across age and racial groups.

Laura B. Pagan[1], Tingting Yang[2], Jinghe Mao[3], Xinchun Zhou[1]

[1]Pathology, The University of Mississippi Medical Center, Jackson, MS,[2]The First Hospital of Jilin University, Changchun, China,[3]Biology, Tougaloo College, Jackson, MS

Background: Prostate cancer (PCa) is the most common cancer in men in the United States, aside from non-melanoma skin cancer. The roles of estrogen receptor α (ERα) and estrogen Receptor β (ERβ) in PCa have not been fully elucidated yet. This study aims to evaluate the expression levels and localizations of ERα and ERβ in benign prostatic tissue (BPT) and PCa across different racial and age groups.
Methods: Immunohistochemistry (IHC) for ERα and ERβ was performed on tissue microarrays (TMA) containing 240 samples of BPT and PCa from Black and White individuals and age groups ≤60 and >60 years. The localization of the IHC signal for ERα and ERβ was determined in glandular and stromal regions. Expression levels of ERα and ERβ were quantified using an IHC score, calculated by multiplying the area score (percentage of stained cells: 0 for no staining, 1 for <10% staining, 2 for 10-50% staining, and 3 for >50% staining) by the intensity score (0 for no signal, 1 for weak, 2 for moderate, and 3 for strong staining). Statistical analysis was performed using Student's t-test; a p-value ≤0.05 was considered significant.
Results: In the entire population, the IHC scores for glandular ERα, stromal ERα, and glandular ERβ were lower in all PCa cases than in BPT, but a significant difference was only seen in glandular ERβ between PCa and BPT (3.43±2.3 vs. 4.86±2.8; p=0.0021). Interestingly, the IHC scores for all glandular ERα, stromal ERα, and glandular ERβ were significantly lower in metastatic PCa than in BPT (p=0.014, p=0.0001, and p=0.0035, respectively). While the entire population was stratified by race, there was no significant difference in IHC score for glandular ERα, stromal ERα, or glandular ERβ in both BPT and PCa between Black and white populations. While entire population was stratified by age, individuals with age >60 years had significantly lower IHC scores for glandular ERα (p=0.0023) and stromal ERα (p=0.03), but not for and glandular ERβ (p=0.1) in BPT; and significantly lower IHC score for stromal ERα (p=0.0036).
Conclusions: These findings suggest that ERα and ERβ could be potentially diagnostic and prognostic biomarkers for PCa. Declined expression levels of glandular ERα, stromal ERα, and glandular ERβ may correlate to the oncogenesis, progression and disparities in race and age PCa.

#687 Development of MAGE-A4 IHC 1F9 pharmDx for melanoma-associated antigen 4 (MAGE-A4) detection in FFPE synovial sarcoma specimens for afamitresgene autoleucel therapy.

Andrea N. Marquez[1], Siena Tabuena-Frolli[1], Sneha L. Koneru[1], Pedro Corona[1], Rose-Ann De Los Santos[1], Adrian Miranda[1], Kristopher Kersch[1], Eric Broxham[2], Regina Singson[2]

[1]Companion Dx, Life Sciences and Diagnostics Markets Group, Agilent Technologies, Inc., Carpinteria, CA, [2]Pathology, TOPA Diagnostics, Thousand Oaks, CA

Introduction: MAGE-A4 IHC 1F9 pharmDx is a qualitative immunohistochemistry assay developed as a companion diagnostic for afamitresgene autoleucel therapy to detect Melanoma-associated antigen 4 (MAGE-A4) expression in synovial sarcoma (SyS) specimens. The assay's analytical performance has been validated on the Autostainer Link 48 Platform using mouse monoclonal anti-MAGE-A4 antibody, clone OTI1F9, in formalin-fixed paraffin-embedded (FFPE) human synovial sarcoma (SyS). Squamous non-small cell lung carcinoma (sqNSCLC) specimens were used in the analytical performance evaluation to supplement sample size and support the use of sqNSCLC as a positive control due to the rarity of SyS. MAGE-A4 positivity was defined as nuclear and/or cytoplasmic staining in viable tumor cells and MAGE-A4 expression was determined using a Tumor Intensity Proportion Score (TIPS) algorithm. Analytical validation of the MAGE-A4 IHC 1F9 pharmDx assay included sensitivity, precision, and robustness testing of MAGE-A4 expression in SyS and sqNSCLC specimens based on the MAGE-A4 TIPS (≥ 2+) ≥ 75% cutoff.
Methods: Sensitivity determined if the assay could detect a range of MAGE-A4 expression levels in SyS and sqNSCLC specimens. Precision testing assessed variability in observers/run/instrument/operator/day/lot. Observer precision was evaluated by inter/intra-observer agreement. Robustness studies evaluated assay performance variability for target retrieval solution (pH, re-use, temperature and time), slide type, and tissue section thickness. SyS and sqNSCLC data were analyzed together using negative percent agreement (NPA), positive percent agreement (PPA), and overall agreement (OA) with two-sided 95% bootstrap confidence intervals (CIs) based on MAGE-A4 binary status (positive/negative) at the cutoff. The following acceptance criterion (AC) was applied: the lower bound (LB) of the two-sided 95% CI computed on percent agreement (NPA, PPA, and OA) ≥ 85%.
Results: MAGE-A4 IHC 1F9 pharmDx detected a dynamic range of staining levels in SyS and sqNSCLC. The prevalence of MAGE-A4-positive cases per cutoff in the commercially procured FFPE SyS specimens tested was 30.3%. MAGE-A4 IHC 1F9 pharmDx demonstrated acceptable performance characteristics (sensitivity, precision, robustness) and met all predefined AC. The NPA 95% CI LB for slide type was 83.9%, however, this result was due to lower-than-expected sample size since the NPA point estimate was 100%. The MAGE-A4 TIPS (≥ 2+) scoring algorithm is practical, transferrable, and reproducible per the assessment of inter/intra-observer percent agreement point estimates being >93%.
Conclusion: These studies demonstrated high analytical assay performance and observer scoring precision of the MAGE-A4 IHC 1F9 pharmDx assay in SyS and combined with sqNSCLC specimens at MAGE-A4 TIPS (≥ 2+) ≥ 75%.

#688 Characterization of microsatellite status in endometrial tumors using comprehensive genomic profiling via the PGDx elio tissue complete.

Darshan Shimoga Chandrashekar[1], Joao Lobo[2], Mehenaz Hanbazazh[3], Kanako Okamoto[1], Jennifer B. Jackson[4], Shuko Harada[1], Alexander C. Mackinnon[1]

[1]Pathology, University of Alabama at Birmingham, Birmingham, AL,[2]Portuguese Oncology Institute of Porto, Porto, Portugal,[3]University of Jeddah, Jeddah, Saudi Arabia,[4]Labcorp, Baltimore, MD

Background: Microsatellite instability (MSI) is a vital genomic biomarker in endometrial cancer (EC), playing a key role in guiding patient management decisions. Microsatellite status can be assessed using various methods, including immunohistochemistry (IHC), polymerase chain reaction (PCR), and next-generation sequencing (NGS). However, accurately determining microsatellite status in EC can be challenging due to the typically weak MSI phenotype observed in this cancer type. This study aims to enhance microsatellite analysis in EC by combining EC mutation profiling with MSI detection techniques into a unified analytical workflow.
Methods: We performed comprehensive genomic profiling on 43 EC samples using the PGDx elio™ tissue complete (ETC), an FDA-cleared NGS test targeting 505 solid tumor related genes. In addition to mutation profile, we obtained microsatellite status and tumor mutational burden for each case. Mismatch repair (MMR) status were also determined for all 43 ECs using IHC (MLH1, PMS2, MSH2 and MSH6). 'maftools' R package was utilized for data processing and visualization, while genomic classifier was developed using 'randomForest' R package.
Results: IHC analysis led to identification of 28 proficient MMR (pMMR) and 15 deficient MMR (dMMR) EC samples. We observed 100% (28/28) concordance for pMMR samples and 73.3% (11/15) concordance for dMMR samples between IHC analysis and NGS assay. Four discordant samples showed low TMB and low-grade instability in 68 mononucleotide tracts considered by NGS assay indicative of weak MSI phenotype. We also identified 15 genes (including *ERBB4, PTPRT, ARID1A, ARID1B*, and *BRD4*) significantly altered in dMMR samples compared to pMMR samples. Random forest classifier was developed considering 88 distinct parameters (including mutation profile of 15 genes, 5 clinicopathologic features and 68-mononucleotide tract status) for all EC samples.
Conclusion: We observed varying degrees of concordance (100% specificity and 73.3% sensitivity) between MSI status by NGS based ETC and MMR status by IHC in a cohort of EC. Targeted genomic profiling via ETC identified significantly altered genes in dMMR endometrial tumors and led to development of random forest classifier to predict MSI EC tumor [which showed AUC value of 0.875, 87.5% specificity and 100% sensitivity for test set]. Integration of mutational profiling with MSI detection can be leveraged to increase sensitivity for MSI detection on the ETC platform.

TUMOR BIOLOGY: Imaging in Animal Models
Poster Session

#69 Integrin $\alpha_V\beta_3$-targeted PET/CT for the assessment of bevacizumab therapy in patient-derived glioblastoma xenografts.

Anne Skovsbo Clausen[1], Ida Vang Andersen[1], Malene Martini Clausen[1], Aleena Azam[1], Julie van Krimpen Mortensen[1], Emilie Graae Lund[1], Thomas Haargaard Urup[2], Andreas Kjaer[1]

[1]Department of Clinical Physiology and Nuclear Medicine & Cluster for Molecular Imaging, University of Copenhagen & Rigshospitalet, Copenhagen, Denmark,[2]Department of Oncology, Rigshospitalet, Copenhagen, Denmark

Introduction: Glioblastoma continues to be the most aggressive type of brain cancer. Standard treatment includes surgical resection, concomitant chemoradiation therapy (CRT) with temozolomide (TMZ) followed by adjuvant TMZ (Stupp's regimen). Bevacizumab (BEV), a monoclonal antibody targeting vascular endothelial growth factor (VEGF) A, is approved for recurrent glioblastoma with no significant benefit in the total population of recurrent glioblastoma patients. However, responders to BEV experience clinical improvement and prolonged survival. Nevertheless, no predictive biomarker for treatment effect has been identified. This study evaluates tumor angiogenesis using [^{68}Ga]Ga-NODAGA-E[c(RGDyK)]$_2$ (RGD_2) PET/CT targeting integrin $\alpha_V\beta_3$ to predict response to BEV combined with conventional therapy in patient-derived xenograft (PDX) models with postoperative primary or recurrent glioblastoma, aiming to guide clinical patient selection.
Experimental procedures: Tumors from four patients with primary or recurrent glioblastoma were enrolled for preclinical efficacy evaluation in PDX models (PDX 1-4) to receive BEV monotherapy, Stupp's plus BEV, Stupp's alone, or saline. PDX patient tissue were selected based on clinical treatment regimen. PET/CT with RGD_2 was performed at baseline and post-therapy. To assess the correlation between treatment efficacy and tracer uptake, tumor uptake of RGD_2 was compared with survival outcomes. Immunohistochemistry (IHC) was used to assess glioblastoma and angiogenesis markers.
Results: PDX models with increased uptake of RGD_2 at baseline (PDX 1: 6.2±0.66 max%ID/g) showed better response rate to Stupp's plus BEV compared with PDX models with low uptake (PDX 3: 2.1±0.25 max%ID/g). PDX models of both primary (PDX 1) and recurrent (PDX 4) glioblastoma had better survival with Stupp's plus BEV therapy compared with BEV monotherapy, Stupp's, and saline. PDX 1 treated with Stupp's plus BEV had better survival with a median survival time of 70 days (95% CI: 63-77 days) compared with BEV monotherapy (p<0.0001), Stupp's (p<0.05), and controls (p<0.0001). Additionally, PDX 4 treated with Stupp's plus BEV had better survival with a median survival time of 49 days (95% CI: 42-56 days) compared with BEV monotherapy (p<0.0001), Stupp's (p=0.0009), and controls (p<0.0001). IHC showed upregulated angiogenesis markers (CD31, CD34, VEGF, and integrin $\alpha_v\beta_3$) in PDX tumors with high RGD_2uptake.
Conclusion: Our results indicate that RGD_2 can be used as a predictive biomarker for treatment response to BEV in PDX models of glioblastoma. Translationally, RGD_2 PET imaging could be used in clinical settings to predict responses to BEV combination therapy. Furthermore, our results support the investigation of BEV in clinical trials as part of first-line adjuvant therapy, potentially advancing its role in improving treatment outcomes in glioblastoma patients.

CLINICAL RESEARCH: Diagnostic Biomarkers 2
Poster Session

#690 Rethinking molecular analysis of gastric cancer: Insight into morphologic phenotype.

Harim Chun[1], Kwangil Yim[2], Sangjeong Ahn[3], Sung Hak Lee[1]

[1]Department of Hospital Pathology, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea, Republic of,[2]Department of Hospital Pathology, Uijeongbu St. Mary Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea, Republic of,[3]Department of Pathology, Korea University Anam Hospital,, College of Medicine, Korea University, Seoul, Korea, Republic of

Introduction: Advanced gastric cancer (GC) is one of the most challenging malignancies, with high mortality rates and a lack of effective treatment strategies. Histomorphological features, termed ‘morphologic phenotypes’, predict therapeutic response, with lymphoepithelial carcinoma showing immunotherapy responsiveness while diffuse-type GC exhibits resistance to immunotherapy and targeted therapies such as HER2 and CLDN18 inhibitors. Despite these associations, comprehensive molecular understanding based on meticulous morphological assessments remains elusive. Herein, we aim to identify robust biomarkers and elucidate the molecular mechanisms underlying GC phenotypes, providing a more precise reflection of the spatial phenotype of GC.
Methods: TCGA stomach adenocarcinoma RNA-sequencing (n=412), WSIs (n=398), and clinical data were retrieved from the GDC Data Portal. Expert gastrointestinal pathologists reviewed H&E-stained WSIs for consensus morphological classifications. RNA-seq data were analyzed using DESeq2 for normalization and differential expression testing. Overall single-cell RNA sequencing (scRNA-seq) analysis was conducted using Seurat to validate findings at the cellular level. Functional analyses, including Gene Set Enrichment Analysis (GSEA) and Over-Representation Analysis (ORA), utilized the MSigDB Hallmark and Gene Ontology Biological Process databases to identify key pathways and biological processes.
Results: A total of 4,342 differentially expressed genes (DEGs) were identified, revealing novel distinctions between diffuse and intestinal GC subtypes. A calcium-dependent adhesion signature showed coordinated upregulation of *CDH2* and *ACTA2*, with *CDH1* downregulation in diffuse-type tumors. Notably, elevated *ACTA2* expression, a marker of *CAF*, suggested enhanced tumor-stromal interaction and potential therapy resistance mechanisms. Further analysis indicated that *CDH1* suppression could be attributed to somatic mutations or epigenetic regulation. Single-cell level transcriptome profiling revealed tumor heterogeneity, with distinct cell populations exhibiting unique molecular signatures in diffuse-type tumors. These findings highlight the *CDH2*/*CDH1* axis as a key determinant of aggressive tumor phenotypes in diffuse-type gastric cancer, offering potential therapeutic insights.
Conclusion: This study underscores the importance of standardized pathological annotations for uncovering phenotype-specific molecular biomarkers in GC. By integrating RNA-seq and scRNA-seq data, we highlighted the *CDH1*/*CDH2* adhesion axis as a critical determinant of diffuse-type tumor aggressiveness. These molecular insights emphasize the significance of phenotype-specific biomarker discovery and reveal potential mechanisms of therapy resistance, providing a foundation for novel therapeutic strategies and improved patient stratification.

#691 Decentralized pan-cancer tumor profiling: a comprehensive performance evaluation of the MSK-IMPACT® Assay with SOPHiA DDM™ .

Julien Pontis[1], Fuad Mohammad[1], Anjali Anjali[1], Yvan Wenger[1], Florian Klemm[1], Anita F. Bowman[2], Brian Houck-Loomis[2], Jacklyn Casanova-Murphy[2], A. Rose Brannon[2], Micheal F. Berger[2], Zhenyu Xu[1]

[1]SOPHiA GENETICS SA, Rolle, Switzerland,[2]Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, NY

Background: Molecular profiling of solid tumors through FFPE (Formalin-Fixed Paraffin-Embedded) tissue samples is crucial for personalized cancer treatment. MSK-IMPACT® (Memorial Sloan Kettering - Integrated Mutation Profiling of Actionable Cancer Targets) is a well-established, high-throughput genomic assay designed to identify known cancer-driving alterations in solid tumors from FFPE tissue samples. SOPHiA GENETICS is developing a decentralized version of this assay, integrating key features of MSK-IMPACT® including comprehensive genomic profiling, the utilization of hybrid capture-based next-generation sequencing, and the identification of actionable somatic mutations. This report presents the results from a performance evaluation study conducted during the development of this product.

Methods: A cohort of 167 FFPE solid tumor samples and matched normal tissue were collected from cancer patients and processed at MSKCC (Memorial Sloan Kettering Cancer Center) according to the validated standard operating procedures for MSK-IMPACT®, which involves DNA extraction, library preparation, hybrid capture targeting 505 actionable cancer genes, and sequencing on an Illumina platform. In parallel, SOPHiA GENETICS processed the same samples using proprietary enrichment techniques, and sequencing was performed on Illumina platforms. Somatic variants were identified using a robust bioinformatics pipeline, and the results were compared with those obtained by MSKCC to assess the accuracy of the decentralized “MSK-IMPACT® powered with SOPHiA DDM™” solution.

Results: A total of 1736 somatic mutations were detected, with variant allele fractions (VAF) as low as 1.1% and a median of 5 variants with coding impact per sample. The positive percent agreement (PPA) for all detected variants with the original MSK-IMPACT® assay is 99.3% for SNV/INDEL with a PPA >98% down to 2.5-6% VAF. Furthermore, PPA for CNVs is 94.6%, and overall percent agreement for MSI and TMB of 99.4% and 98.8%, respectively.

Conclusions: The findings affirm that the decentralized “MSK-IMPACT® powered with SOPHiA DDM™” solution delivers a high level of concordance with the established results by MSK-IMPACT®. Future studies are planned to further verify the analytical and clinical performance and to evaluate the utility in routine clinical practice. This solution equips laboratories with a premier, comprehensive testing capability that identifies SNV/INDEL, CNV, DNA fusion, and the critical immuno-oncology biomarkers TMB and MSI.

#692 Analytical validation of a tissue-free epigenomic assay for circulating tumor DNA (ctDNA)-based molecular residual disease (MRD) detection in early-stage cancer.

Katie Quinn, Amy Wilfert, Ranjani Lakshmin, Sai Chen, Albert Lee, Jun Zhao, Daniel Gaile, Yunpei Chang, Ruth McCole, Jordan Burke, Derek Dustin, Thereasa Rich, Jack Tung, Kristin Price, Darya Chudova

Guardant Health, Redwood City, CA

Introduction: Molecular residual disease (MRD) detection can offer valuable prognostic insights and guide personalized treatment decisions. High sensitivity and specificity are required for accurate MRD assessment, with most methods currently relying on tissue sequencing for informing MRD assay design. However, tissue testing can be logistically challenging and not all patients have accessible tissue. Here, we describe the analytical validation of Guardant Reveal for plasma-only, epigenomic-based detection and quantification of ctDNA in CRC, lung, and breast cancers.

Methods: Guardant Reveal evaluates signals in thousands of differentially methylated regions (DMRs) and classifies samples as ctDNA detected or not detected. Quantitative tumor fraction (TF) is estimated by normalizing selected cancer DMRs with matched control regions. Analytical sensitivity was assessed in titrations of cancer clinical samples and cell lines into non-cancer samples. Analytical specificity was assessed in samples from age-matched self-declared healthy donors. Qualitative precision was assessed in within- and between-batch replicates of ctDNA-positive and ctDNA-negative samples. Accuracy was assessed for concordance to a genomic and methylation-based reference assay.

Results: Analytical sensitivity studies determined the ≥ 95% limit of detection (LoD) for each cancer type to be down to an order of 10^{-5}-10^{-4} at both minimal (3-4ng) and typical (15ng) cfDNA input amounts. The analytical specificity (limit of blank) study using 102 age-matched healthy donor samples demonstrated 100% specificity (ctDNA negative results) for each cancer type caller. Within-batch and between-batch qualitative precision was 100% (81/81) for ctDNA-positive samples across cancer types, and 100% (36/36) for ctDNA-negative samples. In studies using titration replicates, TF demonstrated high quantitative precision and accuracy down to an order of 10^{-4}, a range which encompasses 97% of ctDNA-positive clinical MRD samples. Comparison of ctDNA detection to a reference method demonstrated ≥97% positive percent agreement (PPA), and ≥95% negative percent agreement (NPA) for 153 CRC, 92 lung cancer, and 96 breast cancer samples. All discordant samples were borderline to the detection threshold. TF demonstrated quantitative equivalence to the reference method across the range of TF.

Conclusions: Guardant Reveal demonstrated high analytical sensitivity, specificity, and TF quantitative precision in CRC, lung and breast cancer. This technology demonstrates the potential for ultra-sensitive and accurate MRD detection using a tissue-free assay, enabling precise monitoring for cancer recurrence.

#693 Comparative gene signature between NEPC and CRPC transcriptomics.

Srinivas V. Koduru, Mark Kidd, Abdel Halim

Wren Laboratories, LLC, Branford, CT

Introduction: Prostate cancer (PCa) is the most common cancer in men, with an estimated 299,010 new cases and 32,250 deaths projected in 2024. Approximately 1 in 8 men will be diagnosed with PCa, with the majority (60%) occurring in those aged 65 or older. Castration-resistant prostate cancer (CRPC) represents an advanced stage of PCa, with up to 30% of these cases classified as neuroendocrine prostate cancer (NEPC). Previous studies have demonstrated that DNA methylation, chromatin integrity, and transcriptional regulation play crucial roles in the development of treatment-resistant PCa. In this study, we aim to apply next-generation sequencing (NGS) to identify gene signatures regulated in CRPC and NEPC using whole blood samples from patients to discover circulating RNAs and their dysregulated pathways.

Methods: Whole blood samples were collected from NEPC (n=9), CRPC (n=5), and healthy controls (n=11). Total RNA was extracted using TRIzol and RNeasy kits, with quality control assessed via Bioanalyzer. RNA-seq was performed on a NovaSeq 6000 platform, and data were analyzed using PartekFlow with High Performance computing system (HPC) to identify differential gene expression patterns. Pathway analysis was conducted on the differentially expressed genes.

Results: RNA-seq analysis revealed significant differences in gene expression between NEPC, CRPC, and healthy controls. In total, 4,655 genes were found to be significantly differentially expressed (fold change >1; $P<0.05$) in NEPC, and 2,944 genes in CRPC. Notably, 28% of the statistically significant genes overlapped between NEPC and CRPC. Additionally, analysis of the Wren's PROSTest (27 genes) and NETest (55 genes) signatures identified 25 and 43 genes in NEPC, and 23 and 41 genes in CRPC, respectively, within the differentially expressed gene sets.

Conclusion: Our transcriptomic data reveal that NEPC and CRPC share approximately 28% of their gene signatures, with significant correlation to the PROSTest and NETest gene panels. This highlights the potential of these gene signatures as diagnostic tools in identifying and understanding the progression of advanced prostate cancer.

#694 Brush biopsy miRNA based oral squamous cell carcinoma detection and localization-overcoming barriers to classifier development.

Madeleine W. Fine[1], **Samira Cheruku**[1], Antonia Kolokythas[2], Ashleigh Weyh[3], Joel Schwartz[1], Beth Miloro[1], Guy R. Adami[1]

[1]Oral Medicine and Diagnostic Sciences, University of Illinois Chicago, Chicago, IL,[2]Oral and Maxillofacial Surgery, Vanderbilty University Medical Center, Memphis, TN,[3]Oral and Maxillofacial Surgery, University of Illinois Chicago, Chicago, IL

Cancers of the oral cavity arise from the surface tissues and, despite prominence of these sites, over 50% are not diagnosed until they are in advanced stage. Unfortunately, patients with advanced stage oral cancer have high mortality rates because treatments are usually not successful, complex and have high complication rates. Among the main reasons for delays in diagnosis of squamous cell carcinoma, which make up over 90% of oral cancers, is the resemblance of these lesions to benign lesions. Specifically, oral squamous cell carcinoma (OSCC) is often misdiagnosed as a benign process, most commonly benign leukoplakia (BL) or oral lichen planus (OLP) and is not interrogated for diagnosis confirmation. On the other extreme, patients with BL or OLP that have no malignant potential are subjected to unnecessary stress and surgical interventions when these lesions are mistaken as cancers. Unlike cancers of other organs, no screening protocols or non-invasive diagnostic tests are currently available to reliably detect these cancers early when treatments can save lives. We have developed a noninvasive brush-based tissue collection and miRNA (and not mRNA) analysis method that can differentiate OSCC from other mucosal lesions. This brush based sampling method showed 93% accuracy in differentiating OLP from OSCC based on the expression of as few as 6 miRNAs. We will report on the validation of this classifier with a set of 40 OSCC and OLP samples, and the training of a classifier to differentiate BL from OSCC with over 35 additional samples. We will also discuss the role of technical replicates and clinician calibration in efficient miRNA based classifier development.

#695 Relative importance between DNA methylation and mutation in nonmalignant tissue for cancer risk.

Harumi Yamada[1], Satoshi Yamashita[2], Yu-Yu Liu[1], Toshikazu Ushijima[1]

[1]Hoshi University, Tokyo, Japan,[2]Maebashi Institute of Technology, Maebashi, Japan

Cancer risk is correlated with the accumulation level of genetic (mutation) and epigenetic alterations (DNA methylation). Analysis of these alterations in nonmalignant tissue contributes to assessing cancer risk and to revealing environmental factors that induce the alterations. DNA methylation can be readily measured due to its high accumulation levels, while on the other hand, it is difficult to measure accumulation levels of mutations because of their rarity. To detect rare point mutations accurately and inexpensively, we developed a novel next generation sequencing (NGS) method, EcoSeq [Ueda, PNAS, 119:e2123241119, 2022]. Here, using EcoSeq, we aimed to evaluate the impact of DNA methylation and mutation for gastric cancer risk, and the mutational signatures of *H. pylori* (*HP*) infection, which is a mutagen for gastric cancer. Nonmalignant gastric mucosae were obtained from G1 (low risk; never infected with *HP*), G2 (intermediate; healthy people after *HP* eradication) and G3 (high; cancer patients after *HP* eradication). The mutation frequency was slightly increased by history of *HP* infection (p=<0.0001, G1vsG2), but not associated with cancer risk (p=0.50, G2vsG3). On the other hand, the DNA methylation level of *miR124a-3* was strongly correlated with cancer risk (p<0.0001, G1vsG2; 0.00069, G2vsG3). Mutational signatures of SBS39 were observed in G2 and G3. These results showed that DNA methylation was more useful for assessing gastric cancer risk than point mutation frequency, and suggested that SBS39 is due to *HP* infection.

#696 Shed Trop2 drives prostate cancer progression and Trop2 is a novel tissue prognostic biomarker and a candidate urinary marker for prostate cancer and bladder cancer.

Shiqin Liu

UCLA - University of California Los Angeles, Los Angeles, CA

Distinguishing indolent from clinically significant localized prostate cancer and treatment of metastatic prostate cancer are two major clinical challenges in prostate cancer. The development of novel predictive biomarkers will help with risk stratification, and influence clinical decision-making between treatment and active surveillance, leading to a decrease in over or under-treatment of patients with prostate cancer. Here, we report that Trop2, an oncogenic transmembrane surface protein, is a prognostic tissue biomarker for clinically significant prostate cancer by utilizing the Canary Prostate Cancer Tissue Microarray (CPCTA) cohort composed of over 1100 patients from a multi-institutional study. We demonstrate that higher Trop2 expression is correlated with worse clinical features and elevated Trop2 expression at radical prostatectomy predicts worse overall survival in men undergoing radical prostatectomy. Additionally, we detected shed Trop2 in urine from men with clinically significant prostate cancer and bladder cancer. We further define the functional role of shed Trop2 on metastasis in prostate cancer and identify that shed Trop2 increases cell migration, invasion, metastatic colonization, and spontaneous metastasis *in vitro* and *in vivo*. Proteomic profiling reveals that shed Trop2 modulates a set of proteins associated with invasion, migration, mTOR signaling, and epithelial-to-mesenchymal transition. Shed Trop2 binds to EGFR and results in the activation of the EGFR-PI3K-AKT-mTOR pathway in prostate cancer. Our study reveals a new function of shed Trop2 in driving prostate cancer progression and identifies Trop2 as a novel tissue prognostic biomarker and a candidate non-invasive marker for prostate cancer that could be used to optimize treatment decision-making.

#697 The role of salivary exosomal cargo as diagnostic biomarker for oral squamous cell carcinoma among South African population.

D. O. Temilola, M. Chetty;
University of the Western Cape, Bellville, South Africa

Introduction: Globally, oral cancer remains a non-communicable debilitating disease of major public health concerns in Africa and other low- and middle-income countries (LMICs). South Africa has the highest incidence and prevalence of oral cancer in Africa. More than 90% of all oral cancer are oral squamous cell carcinoma (OSCC). Factors such as the aggressive nature of the disease among Africans, late diagnosis, and access to and limited healthcare resources have contributed to the growing burden of OSCC in Africa and other LMICs. Exosomes play a major intercellular communication role due to their ability to transmit their cargo of proteins, nuclei acids, and lipids from one cell to another.
Aim: The study is focused at investigating the role of salivary exosome cargo as diagnostic biomarkers for OSCC in a South African population.
Methodology: Saliva samples were collected from 10 OSCC patients and 5 healthy individuals that consent to be part of the study. Exosomes were isolated from all saliva samples. Characterization of exosomes size and concentration was done using NanoSight NS300. Exosomes samples were also characterized using transmission electron microscope.
Results: Salivary exosomes from OSCC patients were found to significantly have higher concentration and size than those from healthy individuals. There are no significant differences in size and concentration of exosomes between male and female OSCC patients. ROC analysis showed a maximal sensitivity (100%) and specificity (80%) (AUC of 0.98) at cut-off of 1.99e+9 particles/ml.
Conclusion: Our study showed the potential uses of salivary exosomes morphological characteristic as diagnostic biomarker tool for OSCC.

TUMOR BIOLOGY: Cancer Stem Cells
Poster Session

#7 Lipid-driven oxidative phosphorylation fuels lymph node colonization by esophageal tumor cells.

Shanshan Li[1], Baifeng Zhang[2], Cuicui Huang[2], Yuying Zhao[1], Stephanie Ma[2], Xinyuan Guan[1]

[1]University of Hong Kong-Shenzhen Hospital, Shenzhen, China,[2]University of Hong Kong, Hong Kong, China

Lymph node (LN) metastasis is a pivotal step in the progression of esophageal squamous cell carcinoma (ESCC) and is associated with poor clinical outcomes. Despite its significance, the mechanisms underlying the early colonization of LNs by tumor cells remain insufficiently understood. In this study, we utilized a human xenograft model of spontaneous LN metastasis and performed single-cell RNA sequencing to analyze the transcriptomes of tumor cells from primary tumors and LN metastases. A rare subset of metastasis-initiating cells (MICs) with stem-like characteristics was identified as critical drivers of LN metastasis. These MICs exhibited a unique metabolic adaptation, relying on fatty acid oxidation (FAO)-driven oxidative phosphorylation (OXPHOS) to survive in the lipid-enriched LN microenvironment. Additionally, nuclear factor erythroid 2-related factor 2 (NRF2) was found to play a key role in maintaining redox balance and supporting the metabolic resilience of MICs during metastatic progression. This study underscores the importance of FAO-driven OXPHOS and NRF2-regulated redox homeostasis in LN metastasis and highlights these pathways as promising targets for therapeutic intervention in ESCC.

TUMOR BIOLOGY: Imaging in Animal Models
Poster Session

#70 Novel PSMA+ and FAP+ mouse models to support the development of novel molecular radiotherapy agents.

Sarah Belderbos, Peggy Provent, Solene Fernandez, Maeva Albanese, Marie Grunenwald, Florent Potvain, Kenny Herry, Didier Grillot, Nicolas Ancellin, **Eftychia Koumarianou**

Oncodesign Services, Dijon, France

To evaluate novel molecular radiotherapies preclinically, non-radiosensitive mouse models with optimal target expression levels that recapitulate the human tumor microenvironment are required. Within the past year Oncodesign Services has developed and validated such models for two targets: fibroblast activated protein (FAP) and prostate specific membrane antigen (PSMA), which are of high interest to the oncological and nuclear medicine community.
To assess novel FAP inhibitor (FAPi) tracers, we developed two FAP-overexpressing cell lines. First, HT-1080 human sarcoma (FAP-negative; 0.0% FAP+) and U87-MG human glioblastoma cells (low FAP-positive; 15.0% FAP+) were transduced using a lentiviral vector overexpressing FAP and ZsGreen (VectorBuilder, Neu-Isenburg, Germany). Immunohistochemistry and flow cytometry confirmed the expression of both markers in the transduced cells, with up to 63.4% and 96.0% of FAP-positive cells in the HT-1080-FAP and U87-MG-FAP cells, respectively. Next, Swiss Nude mice (Charles River, Ecully, France) were subcutaneous engrafted with either the FAP-overexpressing HT-1080 or U87-MG cell line. A slow homogeneous tumor growth (humane endpoint of 1500 mm^3 reached after 33-36 days) was noted with U87-MG-FAP tumors, while a faster and more heterogeneous tumor development was observed in mice bearing HT-1080-FAP tumors (humane endpoint reached after 14-18 days). Flow cytometry of the tumors *ex vivo* showed a higher expression of FAP in the U87-MG-FAP tumors (up to 81.5% FAP+ tumor cells) compared to HT-1080-FAP tumors (up to 61.5% FAP+ cells), as previously noted *in vitro*.
In parallel, our team also focused on developing additional models for the assessment of novel molecular radiotherapies targeting PSMA. Male BRGSF mice (GenOway, Lyon, France) were subcutaneously engrafted with either LnCaP C4.2 cells (50%/50% Matrigel) or 22RV1-Luc-mCherry cells. A special enrichment was put in place to allow group housing of the male mice. The growth of LnCaP C4.2 tumors was relatively quick but heterogenous (humane endpoint reached after 19-32 days) and accompanied by a body weight loss that reached 23.6% despite the use of an enriched diet. 22RV1-Luc-mCherry tumor growth was relatively homogeneous and tumor size surpassed the human endpoint of 1500 mm^3 after 29 days. Castration of the BRGSF mice before tumor cell engraftment delayed tumor growth by 3 to 11 days, depending on individual mice. Confirmation of PSMA expression or evaluation of PSMA tracers/molecular radiotherapy is foreseen in future projects.
In conclusion, we designed several new mouse models, which could aid in the development of novel imaging or molecular radiotherapy agents for both FAP and PSMA, two “hot” biomarkers in the field of oncology.

CLINICAL RESEARCH: Precision Medicine in Pediatric Cancer
Poster Session

#701 Understanding the genomic landscape of pediatric cancer predisposition.

Martha O'Brien[1], Robert J. Autry[1], Olaf Witt[2], David Jones[3], Stefan Pfister[1]

[1]Pediatric Neurooncology, Hopp Children's Cancer Center Heidelberg, Heidelberg, Germany,[2]CCU Pediatric Oncology, Hopp Children's Cancer Center Heidelberg, Heidelberg, Germany,[3]Pediatric Glioma Research, Hopp Children's Cancer Center Heidelberg, Heidelberg, Germany

Once thought to be rare, 10% of pediatric cancers can be attributed to mutations in known cancer predisposition genes (CPGs). However, given that many pediatric cancers occur sporadically without germline mutations in the ~114 known CPGs, it is plausible that previously unidentified mutations in novel genes and non-coding regions contribute to pediatric cancer development. This research aims to uncover novel germline mutations, to use somatic “second hits” to prioritize candidates for detailed multi-faceted analysis of downstream effects contributing to pediatric cancer predisposition and pathogenesis. Here we present an analysis of the largest pediatric burden testing cohort to date (>7,000 tumor/normal) of WGS, WES and RNAseq data to explore the genetic landscape of childhood cancers. First, we combined multiple rare-variant burden testing methods, such as TRAPD, SKAT-O, CoCoRV, RV-EXCALIBER, to identify novel germline variants associated with cancer at both within and across pediatric cancer entities. Putative CPGs were prioritized by detecting somatic second hits in tumors, leveraging multiple genomic data types (SNVs, indels, structural variants (SVs), and copy number variations (CNVs). The impact of germline mutations on the somatic landscape was explored by examining how these variants influence tumor mutational profiles, including co-occurring mutations and mutational signatures. Single-cell RNA sequencing data were interrogated when available for pediatric brain tumors and sarcomas to determine how the identified germline genes influence the cell-of-origin and its malignant transformation. We will assess whether these mutations can be observed early in development and their potential causative role in specific tumor types. The findings from our comprehensive approach will deepen our understanding of the interplay between germline and somatic mutations in cancer, enhancing risk prediction and guiding precision oncology efforts.

#702 Payor coverage for laboratory developed genomic testing, a looming challenge for pediatric precision cancer medicine.

Jennifer A. Oberg[1], Susan Hsiao[2], Mahesh Mansukhani[2], James H. Garvin[1], Darrell J. Yamashiro[3]

[1]Pediatrics, Columbia University Irving Medical Center, New York, NY,[2]Pathology and Cell Biology, Columbia University Irving Medical Center, New York, NY,[3]Pediatrics; Pathology and Cell Biology, Columbia University Irving Medical Center, New York, NY

Introduction: Although genomic testing may inform medical decision making for pediatric cancers, inconsistent coverage and variable payment by insurers has made routine utilization in clinical practice challenging. Payer's state insufficient evidence of clinical utility limits their evaluation of medical necessity for genomic testing. Adoption of changes to CPT coding may have furthered an uncertain reimbursement landscape for laboratories. A review of solid and CNS tumor cases sequenced through the Precision in Pediatric Sequencing (PIPseq) program at Columbia University Irving Medical Center (CUIMC) was conducted to describe clinical utility, coverage, and reimbursement for genomic testing.

Methods: Laboratory developed matched tumor-normal whole exome, tumor transcriptome, or 450+ targeted gene panel sequencing was performed in a CLIA-certified laboratory at CUIMC. Claims were submitted April 2014-October 2024 based on CPT coding Pre 2020 (stacked Tier 1/2 Molecular Pathology CPT codes) and Post 2020 (limited Genomic Sequencing Procedure (GSP) CPT codes). Carriers were categorized into commercial, managed government (managed Medicaid), and government (Medicaid) plans and linked to clinical utility, defined as an alteration of diagnostic, prognostic, or therapeutic significance, to demonstrate medical necessity of genomic testing. All costs were covered by PIPseq.

Results: Reimbursement/utility data were available for 226/303 tests; Pre 2020 (n=186), Post 2020 (n=40). Solid tumors (ST), n=135 (2020: Pre n=113, Post n=22); CNS tumors (CNS), n=91 (2020: Pre n=73, Post n=18). Clinical utility was demonstrated in 162 tests (72%): ST, n=87 (64%); CNS, n=75 (82%). RNAseq identified 43% of findings (ST, n=38). Findings of diagnostic (n=61: ST, n=39) or prognostic (n=54: CNS, n=31) significance were identified; 44 were denied coverage (ST, n=20) and 70 received partial coverage (ST, n=41) at an average rate of 57% of the service charge. Targetable alterations were identified in 49 patients; 25 received a matched targeted therapy (CNS, n=15), 8 were denied coverage (CNS, n=5) and 17 received partial coverage (CNS, n=10) at an average rate of 51% of the service charge. Medicaid did not provide coverage for testing (n=11). Commercial and managed government plans Pre 2020 (n=177) covered 42% of the service charge (median, 50%) whereas limited GSP codes Post 2020 (n=38) led to a 48% reduction in reimbursement with insurers covering 21% of the service charge (median, 0%) ($t(213)=3.58$, $p=.001$; $eta^2=.06$).

Conclusions: 72% of samples sequenced had a clinically impactful finding supporting the medical necessity of testing in pediatric solid and CNS tumors. Changes in CPT coding not reflected in updated payer coverage policies reduced insurance coverage by 48%. Revised reimbursement policies are needed to ensure equitable access to genomic testing that benefits most patients.

#703 Epigenome-wide analysis of DNA methylation mediators linking cancer treatments to cardiometabolic risks in survivors of childhood cancer.

Tiffany Eulalio[1], Yoonji Kim[1], Peggy Meng[1], Qian Dong[1], Noel-Marie Plonski[1], Heather Mulder[2], John Easton[2], Emily Walker[3], Geoffrey Neale[3], Deokumar Srivastava[4], Stephanie Dixon[5], Kirsten K. Ness[1], Melissa M. Hudson[5], Gregory T. Armstrong[1], Zhaoming Wang[1]

[1]Epidemiology and Cancer Control, St. Jude Children's Research Hospital, Memphis, TN,[2]Computational Biology, St. Jude Children's Research Hospital, Memphis, TN,[3]Hartwell Center, St. Jude Children's Research Hospital, Memphis, TN,[4]Biostatistics, St. Jude Children's Research Hospital, Memphis, TN,[5]Oncology, St. Jude Children's Research Hospital, Memphis, TN

Background: Cancer treatments engender the risk of long-term cardiometabolic and cardiac conditions among survivors of childhood cancer. However, the underlying biological mechanisms remain unclear. This study aimed to identify DNA methylation (DNAm) CpG sites associated with cardiometabolic risk factors (CMRFs) and adverse cardiac outcomes, and to assess if these DNAm CpG sites mediate associations between specific cancer treatments and these outcomes.

Methods: We included 3,044 survivors (median age at blood draw: 29.6 years) from the St. Jude Lifetime Cohort. Blood-derived DNA was profiled using the EPIC BeadChip. Associations between DNAm and seven clinically assessed outcomes were examined: five CMRFs (abnormal glucose metabolism, hypercholesterolemia, hypertriglyceridemia, hypertension, and obesity), and two cardiac outcomes (cardiomyopathy and myocardial infarction). Treatment-specific epigenome-wide association studies (EWAS) evaluated associations with 10 chemotherapeutic agents and four region-specific radiotherapies.

Results: EWAS identified 1,833 CpG sites associated with cardiometabolic outcomes ($p < 9x10^{-8}$), including obesity (N=1,709), abnormal glucose metabolism (N=141), and hypertriglyceridemia (N=134). A moderate number of CpG sites mapped to *CPT1A*, a key gene in fatty acid metabolism linked to metabolic disorders, with 23 outcome-CpG associations involving 7 unique CpG sites. Most (71%) outcome-associated CpG sites were also associated with cancer treatments, particularly chest- (N=610) and abdominal-radiotherapy (N=599), suggesting potential causal mediation for the treatment-related genotoxicity. Mediation analyses identified 386 CpG sites mediating treatment effects on cardiometabolic outcomes. Asparaginase treatment was significantly associated with abnormal glucose metabolism (Odds ratio [95% CI] = 1.09 [1.06-1.13]). Eighteen CpG sites collectively mediated 26.7% of this effect, with 14 previously implicated in type-2 diabetes. Similarly, alkylating agents were associated with hypertriglyceridemia (1.11 [1.07-1.14]) mediated by DNAm at 46 CpG sites; five independent CpG sites accounted for a substantial 79.8% of such mediation effect. Notably, 39 of these mediator CpG sites were previously associated with C-reactive protein (CRP) levels (two-sided Fisher's test, $p=2.2x10^{-30}$), suggesting potential DNAm-mediated inflammatory pathways connecting alkylating agents to triglyceride dysregulation.

Conclusion: Our findings suggest that DNAm changes may mediate the association between cancer treatments and cardiometabolic risks. These results implicate inflammatory and metabolic pathways in treatment-related adverse outcomes. These insights may inform risk stratification and targeted interventions to improve cardiometabolic and cardiac health in childhood cancer survivors.

#704 Primary versus delayed surgery in fibrolamellar carcinoma.

Paul M. Kent[1], Tomoaki Kato[2], Abhinar Humar[3], Oliver Fisher[4], Matthew Dixon[5], Christopher Seder[6], Tom Stockwell[7]

[1]Medical Director, FibroFighters, River Forest, IL,[2]Hepatobiliary Surgery and Transplant, New York Presbyterian - Columbia, New York, NY,[3]Hepatobiliary Surgery and Liver Transplant, University of Pittsburgh, Pittsburgh, PA,[4]Hepatobiliary Surgery, FibroFighters, St. George Hospital, Australia,[5]Hepatobiliary Surgery, Rush University Medical Center, Chicago, IL,[6]Thoracic Surgery, Rush University Medical Center, Chicago, IL,[7]CEO, FibroFighters, Temacula, CA

Background: Fibrolamellar carcinoma (FLC) is a rare, primary liver cancer of young adults without liver disease, distinct from hepatocellular carcinoma (HCC), usually presenting as advanced stage. FLC has been thought of as a "surgical disease." Because of a lack of data, and a legacy of FLC being mislabeled as “fibrolamellar HCC,” recommendations have been similar to HCC with upfront surgery considered for all new diagnosis and systemic therapy reserved for those deemed inoperable. Our foundation has assembled the largest collection of FLC cases to date. We reviewed surgery data in FLC stratified by stage and systemic therapy.
Methods: We report 216 cases of FLC from our own database and 24 from the medical literature that included: Information about demographics, surgery, systemic therapy, staging, time to relapse and overall survival. We defined “localized” as confined to the liver (AJCC stage 1), “regional” as regional lymph node involvement at surgery (AJCC stage 2 and 3), and “metastatic” (AJCC stage 4) as distant metastasis.
Results: Of 155 patients with initial surgery, 44 (28%) had adjuvant systemic therapy and 85 ‘unresectable’ patients had neo-adjuvant therapy converting 31 patients to have delayed surgery (36%). Relapses were nearly universal (89% at 5 years). There was 100% 10-year survival for localized disease, but frequent relapses nonetheless. Adjuvant therapy gave regional patients a survival advantage. Neo-adjuvant therapy benefited metastatic patients as well as providing as significant delay in time to relapse. Benefit to 54 metastatic patients who had neoadjuvant therapy but no surgery was also seen. [Table1]
Conclusion: Primary surgery for localized FLC offers excellent long-term control, but regional and metastatic FLC should be considered systemic diseases and systemic therapy before surgery should be considered before committing to initial resection. Neoadjuvant therapy can covert unresectable patients to resectable, and adjuvant therapy appears to benefit all non-localized patients.

Primary v. Delayed Surgery in FLC as a Function of Stage and Therapy

Stage	Number	Median Followup (months)	Median Overall Survival (years)	1 yr OS	3 yr OS	5 yr OS	10 yr OS	Median time to progression (months)	1 yr Freedom from Relapse	3 yr Freedom from Relapse	5 yr Freedom from Relapse	10 yr Freedom from Relapse
All Pts. with Initial Surgery	**155**	**54**	9	0.98	0.87	0.72	0.46	21	0.61	0.25	0.17	0.03
All Localized Disease with Initial Surgery	25	98	>10	1.00	1.00	1.00	**1.00**	68	0.99	0.84	0.58	0.19
Localized Disease with Initial Surgery - NO ADJUVANT THERAPY	24		>10	1.00	1.00	1.00	1.00	70	0.95	0.87	0.60	0.20
Localized Disease with Initial Surgery - WITH ADJUVANT THERAPY	1		>10	1.00	1.00	1.00	1.00	18	1.00	0.00	0.00	0.00
All Regional Disease with Initial Surgery	97	60	8.8	0.98	0.90	0.69	0.44	14	0.62	0.17	0.13	0.00
RegionalDisease with Initial Surgery - NO ADJUVANT THERAPY	71		8.5	0.98	0.89	**0.56**	**0.40**	13	**0.57**	**0.13**	0.11	0.00
RegionalDisease with Initial Surgery - WITH ADJUVANT THERAPY	26		7.3	1.00	0.94	**0.93**	**0.60**	15	**0.75**	**0.27**	0.18	0.00
All Metastatic Disease - **Resectable**	33	39	**3.9**	0.97	0.70	0.46	**0.14**	**8**	0.32	0.07	0.00	0.00
MetastaticDisease with Initial Surgery - NO ADJUVANT THERAPY	16		4.5	0.94	0.79	0.50	0.33	**4**	**0.13**	**0.00**	0.00	0.00
Metastatic Disease with Initial Surgery - WITH ADJUVANT THERAPY	17		3.3	1.00	0.56	0.43	0.00	**12**	**0.50**	**0.13**	0.00	0.00

All Metastatic - **Unresectable** - WITH NEO-ADJUVANT THERAPY	**85**	**35**	**4.6**	0.99	0.72	0.46	**0.21**	**19**	0.68	0.06	0.00	0.00
Metastatic, **NEOADJUVANT DELAYED SURGERY**	31		**4.7**	1.00	0.75	0.49	0.25	**22**	0.70	0.17	0.00	0.00
Metastatic - **NEOADJUVANT. NO SURGERY**	54		**4.5**	0.98	0.70	0.44	0.18	**18**	0.67	0.00	0.00	0.00

#706 Impact of symptom clusters on quality of life in pediatric cancer survivors after treatment completion.

Krishtee Napit[1], Don Coulter[1], Katrina Cordts[1], Daisy Dai[1], Evi Farazi[2], Shinobu Watanabe-Galloway[1]

[1]University of Nebraska Medical Center, Omaha, NE,[2]European University Cyprus, Nicosia, Cyprus

Background: The advancement in pediatric cancer treatment led to an increased five-year survival. Symptoms develop due to cancer and its treatment during the treatment phase, but they can persist even after treatment completion. Cancer patients experience multiple interrelated symptoms known as symptom clusters. Compared with a single symptom, symptom clusters synergistically affect quality of life (QoL). Limited studies in pediatric cancer have assessed the association between symptom clusters and quality of life, but none were assessed after the treatment completion.
Objective: To find the association between symptom clusters and QoL among pediatric cancer survivors after treatment completion.
Method: We conducted a cross-sectional study of 136 pediatric cancer survivors aged 10-25 who completed treatment within eight years from 2 major hospitals in Nebraska. We used a survey to collect data during their hospital visit, the validated Memorial Symptom Assessment Scale tool to collect information on symptoms, and the PedsQoL instrument to measure the quality of life. We used exploratory factor analysis to derive symptom clusters. We conducted a multiple linear regression model to find the association between symptom clusters and quality of life at a significance level with a p-value of less than 0.05 and an adjusted beta coefficient.
Results: The mean age was 14.91, the mean year since diagnosis and treatment completion was 4.66 and 3.05, respectively, and the mean number of symptoms was 6.06. Difficulty concentrating (48.53%), lack of energy (45.59%), and feeling drowsy (45.59%) were the most common symptoms. Around 80% of participants experienced more than one symptom. The mean overall quality of life score was 80.84, the psychosocial score was 79.84, and the physical score was 82.72 out of 100. The exploratory analysis derived four symptom clusters: Cluster 1: dry mouth, lack of energy, feeling drowsy, feeling irritable, and difficulty sleeping; Cluster 2: feeling nervous, worrying, feeling sad, and difficulty concentrating; Cluster 3: nausea and lack of appetite; and Cluster 4: pain, cough, numbness in hands/feet, dizziness and sweats. The multiple linear models were adjusted for possible confounders and sociodemographic and clinical characteristics. Cluster 1 (β= -4.74, 95% CI: 7.32, 2.15) and Cluster 2 (β= -3.39, 95% CI: 5.80, -0.98) were associated with lower overall quality of life. Cluster 1 (β= -3.96, 95% CI: -7.47, -0.44) was associated with lower physical quality of life. Cluster 1 (β= -5.15, 95% CI: -7.83, -2.47) and Cluster 2 (β= -4.64, 95% CI: -7.13, -2.14) were associated with lower psychosocial quality of life.
Conclusion: Even after treatment completion, symptom clusters are prevalent in pediatric cancer survivors. Among the four clusters identified, Cluster 1 and Cluster 2, which include mainly psychological symptoms, affect all dimensions of quality of life.

#708 Phase I clinical trial to target oxidative phosphorylation and CREB for children and young adults with relapsed or refractory AML.

K. Mark, N. Lacayo, **K. M. Sakamoto**;
Stanford University, Stanford, CA

The overall survival for pediatric AML is 60-70% and treatment includes intensive chemotherapy and stem cell transplantation, which are associated with significant morbidity and mortality. There are few therapies for relapsed/refractory pediatric AML. We previously demonstrated that the transcription factor CREB is overexpressed and activated in AML blasts and leads to increased proliferation and survival of AML cells. CREB overexpression was associated with an increased risk of relapse and decreased event-free survival in AML patients. Knockdown of CREB in mouse bone marrow transduction/transplantation assays did not affect engraftment of healthy hematopoietic stem or progenitor cells. Therefore, we hypothesized that CREB is a potential target for AML therapy.The CREB inhibitor, XX-650-23, disrupts CREB interaction with its binding partner, CBP/p300 (CREB Binding Protein), which is required for CREB function. Treatment of primary pediatric AML cells and xenograft mouse models demonstrated that XX-650-23 inhibits AML progress *in vitro* and *in vivo*. Since XX-650-23 is not a clinical candidate, we performed a virtual screen of FDA-approved drugs with known safety and pharmacokinetic profiles and similar structure to XX-650-23. Niclosamide is an orally bioavailable drug that was previously used to treat tapeworm in developing countries and is safe for both children and adults. We studied niclosamide in pediatric AML cells and pediatric AML xenograft mouse models. Niclosamide primarily acts through inhibition of oxidative phosphorylation and induces apoptosis of AML blast cells, including leukemia initiating cells, resulting in decreased progression and prolonged survival in mice. Combination of niclosamide and cytarabine showed synergistic effects with combination index of less than 1. Based on our preclinical data, a Phase I clinical trial was initiated for relapsed or refractory AML in children and young adults (clinicaltrials.gov, NCT05188170). The study is a single institution, open label, prospective, dose escalation study. The treatment is 2 weeks of oral niclosamide followed by 5 days of high dose cytarabine. Eligibility is between 2 and 30 years of age with relapsed or refractory AML. The primary objective is to identify and evaluate the safety for Phase 2 dose of niclosamide in combination with cytarabine for relapsed/refractory pediatric AML. The secondary objective is to determine the pharmacodynamic effects of niclosamide in AML cells. We are currently enrolling patients and at the first dose level have not observed any significant toxicities related to niclosamide. Preliminary data have demonstrated decreased AML blasts in the bone marrow. Our results suggest that repurposing niclosamide is potentially safe and effective for treatment of relapsed or refractory pediatric AML.

#709 Gut microbiome, physical activity and cognitive dysfunction among childhood brain tumor survivors.

Jinbing Bai[1], Anna Janss[1], Claire Mazewski[1], Hye In Noh[1], Maria C. Swartz[2], Lisa Ingerski[1], Grace Fong[3]

[1]Emory University, Atlanta, GA,[2]Department of Pediatrics-Research, University of Texas MD Anderson Cancer Center, Houston, TX,[3]Children's Healthcare of Atlanta, Atlanta, GA

Background: Childhood brain tumor survivors (BTS) experience a significant increase in cognitive dysfunction, adversely affecting educational attainment and quality of life. The biological mechanisms of cognitive dysfunction, particularly the gut microbiome (GM) and its function, are rarely studied in childhood BTS. Physical activity (PA) may mitigate cognitive dysfunction by reducing proinflammatory GM. Associations of GM with PA and cognitive dysfunction are unknown in childhood BTS. This study investigated associations of GM with PA and cognitive dysfunction in this population.

Methods: A prospective, observational study was conducted in 28 childhood BTS (60% female, 63% white, mean age 13.7 years) enrolled from Children's Healthcare of Atlanta. 16S rRNA V3V4 gene of fecal specimens was sequenced for GM. PA and cognitive function were self-reported by the Patient-Reported Outcomes Measurement Information System-PA and Cognitive Function scales; cognitive performance (working memory [WM] and processing speed [PS]) was tested by Wechsler Adult Intelligence Scale-IV (18-21 year) or Wechsler Intelligence Scales for Children-V (7-17 year). Chi-square test analyzed the relationship of PA with cognition; Mann-Whitney and PERMANOVA tests were used for diversity analysis, and general linear models for abundance analysis. Function of 16S bacterial communities were analyzed by Tax4fun2.

Results: Most survivors were diagnosed with medulloblastoma (71%) with surgery (98%) and radiotherapy (65%). 61% and 71% of survivors reported low PA and cognitive function (T-score<50); 31% reported low WM and 77% reported low PS. A higher PA indicated better self-report cognitive function (p=0.035). Survivors with normal PA and self-report cognitive function (T-score≥50) showed higher diversity than those with low PA (p=0.086), mild (p=0.026) and moderate (p=0.028) self-report cognitive dysfunction, respectively. Children with normal PA had lower abundance of proinflammatory genera *UBA1819* (p=0.016)*, Ruminococcus gnavus group* (p=0.016)*, Incertae sedis* (p=0.026)*, Alistipes* (p=0.026), but higher anti-inflammatory *Marvinbryantia* (p=0.005). Children with high normal self-report cognitive function had higher *Marvinbryantia* (p=0.007). Survivors with normal WM had lower *Ruminococcus gnavus group* (p=0.085) but higher *Ruminococcus gnavus group* (p=0.086) for normal PS. Fatty acid biosynthesis and vitamin metabolism were associated with PA, while glycan, lipid, and amino acid metabolisms were associated with cognitive dysfunction.

Conclusions: Low PA was associated with lower diversity and more proinflammatory taxa, which are linked with cognitive dysfunction. Promoting PA and anti-inflammatory GM may be useful strategies to improve cognitive function in childhood BTS. Future work is needed to study whether GM mediates the relationship of PA with cognitive dysfunction in a large cohort.

TUMOR BIOLOGY: Imaging in Animal Models
Poster Session

#71 Improving the pH-responsive peptide V3 for pancreatic cancer imaging and identification.

Emma Sanderson[1], Ryan C. Bynum[1], Tereza Husarova[2], Lacey R. McNally[1]

[1]OU Health Stephenson Cancer Center, Oklahoma City, OK,[2]Military University Hospital Prague, Prague, Czech Republic

Introduction: Pancreatic cancer treatment with surgical resection or receptor-targeted therapies is complicated by the heavy vasculature of the organ and the heterogeneity of the tumors. Utilization of pH-responsive peptides in tandem with multispectral optoacoustic tomography may address these issues and offer a treatment solution. Although the V3 pH-responsive peptide has demonstrated its ability to target slightly acidic microenvironment, the V3 peptide struggles to identify highly acidic microenvironment like those found around pancreatic cancer cells. To overcome this limitation, we modified the V3 peptide's original sequence to make the V3-Pro and V3-ProFS variants and then tested the new variants' abilities to target the more acidic microenvironment that is characteristic of pancreatic cancer.
Methods: We designed modifications to the V3 peptide sequence, and the peptide variants were synthesized using the CEM Liberty Blue microwave technology. V3 and the new variants were conjugated to HiLyte Fluor 750 C2 maleimide dye to make the V3-750, V3-Pro-750, and V3-ProFS-750 imaging probes. S2VP10 pancreatic cancer cells were pretreated with 7.4, 6.8, and 6.6 pH media for 30 minutes, and then treated with the peptide imaging probes at 1 micromolar concentration for 1 hour. The cells were then washed with pH-specific PBS and imaged with the Odyssey LiCor near-infrared fluorescence machine. Cells were then scraped for multispectral optoacoustic tomography (MSOT). Scraped cells were loaded into tissue-like phantoms, then imaged with the MSOT software.
Results: The V3-Pro-750 and the V3-ProFS-750 treated S2VP10 cells featured significantly higher signal values in the 6.6 pH environment compared to the 7.4 pH environment ($p<0.01$). Fluorescent images of the V3-Pro-750 and V3-ProFS-750 treated S2VP10 cells featured higher signal values for the 6.6 pH and a larger contrast between 6.6 and 7.4 pHs as compared to fluorescent images of S2VP10 cells treated with V3-750 ($p<0.05$). MSOT data supported this trend, with the V3-Pro-750 and V3-ProFS-750 treated cells at 6.6 pH displaying the highest signal values and visual contrast.
Conclusion: Increased signal values for cells treated with the new V3-Pro-750 and V3-ProFS-750 imaging probes in both fluorescence and MSOT suggest that the modified sequences increased the dynamic range between the 6.6 pH and the 7.4 pH. These results point to the newly designed probes potentially being better suited to identify pancreatic cancer based on its acidic tumor microenvironment. The V3-ProFS-750 is especially appealing because of its increased water solubility and its contrast between the 6.6 and 7.4 pHs, making it promising for the clinical translatability of a pH-responsive peptide suited for targeting pancreatic cancers.

CLINICAL RESEARCH: Precision Medicine in Pediatric Cancer
Poster Session

#710 Germline whole genome sequencing and nanopore long-read sequencing reveal high incidence of pathogenic variants in childhood cancer survivors with subsequent malignant neoplasms.

Eun Seop Seo, Ji Hwan Moon, Woong-Yang Park, Ji Won Lee

Samsung Medical Center, Seoul, Korea, Republic of

Background: Childhood cancer survivors with cancer-predisposing variants are at increased risk of developing subsequent malignant neoplasms (SMNs). Although panel sequencing is commonly used for diagnostics, its overall yield has been lower than expected.
Methods: From 1,435 pediatric solid tumor patients diagnosed or treated between 1994 and 2014, we previously identified 72 individuals who developed SMNs; germline DNA samples were available for 40 of them. Whole genome sequencing (WGS) was performed on these 40 survivors using Illumina sequencing to investigate deleterious germline variants in 205 known cancer predisposition genes (CPGs). We further assessed candidate structural variants with Oxford Nanopore long-read sequencing in 10 patients who showed no pathogenic variants but were highly suspicious for genetic predisposition.
Results: Pathogenic or likely pathogenic (P/LP) variants were identified in 18 patients (45%), with 11 patients (27%) carrying P/LP variants in known autosomal dominant CPGs. Excluding RB1, the incidence remained high at 32% (10 out of 31 patients). Patients with P/LP variants in known CPGs developed a second tumor at a significantly younger age ($P = 0.031$), more pronounced in autosomal dominant CPGs ($P = 0.008$). Excluding RB1 cases, patients with any P/LP variants were more likely to have a family history of cancer among second-degree relatives ($P = 0.034$). Nanopore long-read sequencing revealed pathogenic structural variants in two highly suspicious cases not identified by Illumina sequencing. Specifically, a structural variant in the MLH1 gene was discovered in a patient with colorectal cancer and microsatellite instability, leading to a Lynch syndrome diagnosis. Additionally, a structural variant was found in the RB1 gene of a patient with a family history of retinoblastoma.
Conclusions: Our study demonstrates a high incidence of pathogenic germline variants in childhood cancer survivors who develop SMNs. Integrating long-read sequencing technologies, such as Oxford Nanopore sequencing, enhances the detection of structural variants missed by traditional short-read WGS. These findings underscore the importance of comprehensive genomic profiling—including both short-read and long-read sequencing—in accurately identifying genetic predispositions in high-risk populations. Early identification of these variants can inform surveillance strategies and guide clinical management to improve patient outcomes.

#711 The Medulloblastoma Meta-Analysis Portal for integrative exploration of clinical and genomic data from 898 patients.

Xin Zhou, Karishma Gangwani, Congyu Lu, Sandeep Dhanda, Airen Peraza, Edgar Sioson, Gavriel Matt, Robin Paul, Jian Wang, Colleen Reilly, Alexander Acic, Kyle Smith, Tong Lin, Qian Li, Paul A. Northcott, Giles W. Robinson

St. Jude Children's Research Hospital, Memphis, TN

Medulloblastoma (MB) is one of the most malignant pediatric brain tumors. Molecularly, MB separates into four subgroups—WNT, SHH, Group 3 (G3), and Group 4 (G4)—each with unique clinical features and genetic profiles. WNT tumors have the best survival outcomes and low metastatic potential, whereas G3 tumors exhibit the highest malignancy and poorest outcomes. While WNT and SHH are highly distinct, G3 and G4 tumors are interrelated and when grouped together, these are referred to as G3/G4 tumors. These tumors are genetically complex, with a constellation of molecular features including *MYC/MYCN* amplifications, *OTX2* activation, *KBTBD4* mutations, and 8 molecular subgroupings complicating risk stratification. Multi-omics approaches, especially DNA methylation profiling, have advanced our understanding, but further exploration is needed to clarify the genetic heterogeneity of G3/G4 tumors.

To address this complexity, we developed the Medulloblastoma Meta-Analysis Portal, incorporating data from 898 patients across three major clinical trials (ACNS0331, ACNS0332, SJMB03) to facilitate cross-cohort data exploration. Our portal offers an array of robust tools for investigating genetic, cytogenetic, and clinical features of medulloblastoma, including custom variable creation, cohort filtering, integration of molecular data with tumor methylome t-SNE maps, and on the fly Cox regression. As a use case the portal enables the assessment of *MYC* amplification and isochromosome 17 cytogenetic association with progression-free survival of standard-risk G3/G4 tumors. This exercise reveals that *MYC* amplification is significantly associated with worse outcomes irrespective of isochromosome 17 status suggesting *MYC* amplification as the prognostic marker and justifying its inclusion as a high-risk feature in risk-stratification algorithms. In the second use case, we overlay mutation status on the methylome t-SNE map to reveal complex relationships between genetic alterations and DNA methylomic state. Given that *KBTBD4* is mutated with complex insertions in G3/G4 tumors we explored its relationship among G3/G4 subgroups. By manually reviewing and rescuing mutation calls, the portal uncovered novel mutation patterns associated with distinct methylome subgroups, as well as outlier cases. These findings emphasize the heterogeneity within G3/G4 subgroups and underscore the limitations of current classification methods, which may fail to capture nuanced genetic differences. In summary, the portal is a valuable resource for detailed exploration of medulloblastoma clinical features, methylomic classifications, and driver mutations that can inform both basic research and clinical decision-making. The portal will be freely available at https://proteinpaint.stjude.org/mbmeta.

#712 The genomic landscape of subsequent breast, meningioma, and thyroid neoplasms after treatment for childhood cancer: a report from the Childhood Cancer Survivor Study.

Samuel W. Brady[1], Michael A. Arnold[2], Mingjuan Wang[1], Ramzi Alsallaq[1], Li Dong[1], Mohammad A. Khan[1], Wentao Yang[1], Kayla L. Stratton[3], Wei Liu[1], Yan Chen[1], Emily Plyler[1], Jacob Steele[1], Brent B. Powers[1], David Rosenfeld[1], Michael N. Edmonson[1], Sasi Arunachalam[1], Heather L. Mulder[1], Deo Kumar Srivastava[1], Michael Rusch[1], Vikki Nolan[1], Aaron McDonald[1], Lucie Turcotte[4], Cindy Im[4], John Easton[1], Zhaoming Wang[1], Wendy Leisenring[3], Miriam Conces[5], Joseph P. Neglia[4], Yutaka Yasui[1], Smita Bhatia[6], Jinghui Zhang[1], Gregory T. Armstrong[1]

[1]St. Jude Children's Research Hospital, Memphis, TN,[2]University of Colorado, Anschutz Medical Campus, Aurora, CO,[3]Fred Hutchinson Cancer Center, Seattle, WA,[4]University of Minnesota, Minneapolis, MN,[5]Nationwide Children's Hospital, Columbus, OH,[6]University of Alabama at Birmingham, Birmingham, AL

While >85% of children with cancer will become five-year survivors, they are at substantially increased risk of developing subsequent neoplasms (SNs) in part due to DNA-damaging therapy. However, the effects of primary cancer therapy on the SN genomic landscape are unknown. We utilized biospecimens collected through the Childhood Cancer Survivor Study (CCSS) and employed whole-genome, exome, and RNA sequencing to analyze 199 SNs (median diagnosis age of 37.8 years, range 13.0-54.4) and matched germline tissue from 159 childhood cancer survivors, including 62 breast, 57 meningioma, and 42 thyroid SN patients. Overall, each SN type had similar somatic driver alterations to corresponding *de novo* cancers though at different frequencies, including increased frequency of kinase fusions and copy number alterations in thyroid SNs compared to *de novo* thyroid cancers. Meningioma and thyroid SNs had significantly elevated somatic single-nucleotide variant (SNV) and insertion-deletion (indel) burdens compared to *de novo* tumors from published cohorts such as The Cancer Genome Atlas, while breast SNs and *de novo* breast cancers had similar SNV and indel burdens. Prior treatment with nitrogen mustards, such as cyclophosphamide, was associated with increased levels of ubiquitous clock-like SNV mutational signature SBS5 in breast and meningioma SNs. We confirmed this association experimentally by treating cultured breast epithelial cells with an active cyclophosphamide metabolite followed by WGS, which revealed an SBS5-like signature induced by the treatment. In addition, we observed platinum-induced signatures SBS31 and SBS35 in 4 of 5 meningioma and thyroid SNs previously treated with platinum therapy, and identified and functionally validated *NF2* splice variants which were predicted to be platinum-induced in meningioma based on their occurrence at platinum signature hotspots. Driver alterations occurred evolutionarily early in most breast and thyroid SNs (12 of 14 multi-sample patients) as evidenced by their truncal status (detected in all samples at clonal variant allele fractions), while most meningioma patients (3 of 5) show intrapatient driver divergence, including a lack of shared mutations indicative of genetically independent tumors. Together, these results demonstrate the long-term impact of childhood cancer treatment on the genomes of SNs developing in adulthood, which may guide efforts to treat and prevent SNs.

#713 Utility of next generation sequencing multigene assay for comprehensive molecular profiling and targeted therapy in solid organ malignancies - An Indian experience.

R. Katara, A. Agarwal, D. K. Sharma, V. Kumar, S. S. Mohanty, A. Yadav, S. Sharma, M. Dixit, S. Arora, S. K. Mohanty;
CORE Diagnostics India, Gurgaon, India

Introduction: Next-generation sequencing (NGS) has paved its path and broaden its utility in oncology practice. The dependability, experiments, pipeline building, data annotation, and its interpretation, including the cost have significantly improved in the recent years making its application more universal. As a result, molecular pathology is now essential for making treatment decisions in clinical practice as predictive and prognostic tools. The need of the hour is to identify a reliable and cost effective comprehensive genomic panel which can aid in a) massive parallel sequencing and screening of multiple genes at the same time; b) identifying deleterious somatic aberrations; c) assessing baseline disease progression / recurrence and guidance to therapy; d) conclude druggable tumor specific somatic mutations. This study aims at the validation and implementation of a comprehensive NGS panel in solid tumors.
Methodology: A total of 243 patient samples (Females: 126, Males 117) were included. DNA and RNA were isolated from the tumor sample and subjected to target amplification using an optimized 161 gene panel (Oncomine Somatic 161 Gene Panel and Thermo Fischer Scientific Ion S5™ System). The NGS output sequences of the multigene assay were aligned to the human reference genome hg19 (GRCh37). The alignments and variant calling was done using the ION S5 torrent server. Variants were identified and interpreted using Ion Reporter Software.
Results: The DNA and RNA alterations were more prevalent between 51-60 years in females, while 61-70 years in males. Total positivity rate was found to be 66% in males and 79% in females. The most prevalent mutations observed amongst females were *TP53* (39% in lung cancers), *KRAS* (16% in lung cancers), *PIK3CA* (10% in breast cancers), *NRAS* (3% not specific to any particular cancer), *CTNNB1* (3% not specific to particular cancer) and *ATM* (3% in pancreas cancers) while male preponderance were observed with *EGFR* (7% in lung cancers), CNVs (6% in stomach cancers), *RB1* (3% in gall bladder cancers) and *BRAF* (3% in lung cancers).
Conclusions: The study is unique as it allows thorough genetic profile of individuals by examining 161 genes at the same time, eliminating the need for several tests. The assay aids clinicians in personalizing drug regimens by understanding tumorigenic pathways and timely management of the disease. Comprehensive genomic profiling improves the evaluation of moderate/low penetration genes that are frequently overlooked in clinical genetic settings, resulting in a direct influence on providing individuals with appropriate genetic counselling and clinical care
Keywords: NGS, 161 genes, Comprehensive molecular profiling

CLINICAL RESEARCH: Predictive Biomarkers 2
Poster Session

#716 Discovery of biomarkers of brain metastasis using Genomic Proximity Mapping (GPM) on formalin-fixed paraffin-embedded head and neck squamous cell carcinomas.

Emily Reister[1], Maika Malig[1], Mary Wood[1], Alexander Muratov[1], Ivan Liachko[1], **Stephen Eacker**[1], Ida Deichaite[2]

[1]Phase Genomics, Seattle, WA,[2]University of California, San Diego, San Diego, CA

Introduction: Head and neck squamous cell carcinoma (HNSCC) encompass a heterogenous classification of solid tumors that account for ~5% of the total new cancer cases globally. HNSCC presents as metastatic disease at estimated rates of 4-25%, most commonly in bone, liver and lung. Brain metastasis (BM) is uncommon and is particularly deadly. There is a need to better understand biomarkers that may presage brain metastasis in hopes of identifying those patients requiring aggressive treatment to head-off metastatic disease. Furthermore, identification of common biomarkers may elucidate therapeutic strategies to treat this disease. To compliment ongoing targeted sequencing efforts, we have applied high-resolution cytogenomic analysis using the genomic proximity mapping (GPM) platform, CytoTerra, to investigate chromosomal abnormalities associated with BM HNSCC.
Methods: GPM uses proximity ligation and short-read sequencing to detect alterations in genomic structure. This technology is compatible with formalin-fixed paraffin-embedded tissue samples because it uses formalin fixation to capture genomic proximity information. 15 µm molecular curls were used as inputs for library preparation from 2 primary tumors of BM HNSCC and 3 samples of non-BM HNSCC. Chromosomal abnormalities were detected using the cloud-based CytoTerra analytic platform which utilizes a suite of AI-based and bioinformatic tools.
Results: GPM libraries with sufficient QC statistics were generated from all five samples. Evaluation of HNSCC genomes revealed a wide array of structural alterations commonly observed in cancer, including balanced and unbalanced translocations, inversions, chromothripsis, deletions, duplications, and copy-neutral loss of heterozygosity. Notably structural rearrangements involving the *JAK1* and *EGFR* loci were observed in BM HNSCC samples. In addition we identified a novel *NRG1::FAM110B* fusion in one case of BM HNSCC. None of these rearrangement involved the disruption of the coding sequence of the gene and therefore would escape detection through standard panel sequencing. Non-BM HNSCC showed similar classes of chromosomal abnormalities and included a deletion of *KDM6A*. Variants identified in serial sampling of neighboring HNSCC lesions in non-BM HNSCC demonstrated a high degree of overlap, demonstrating reproducibility. Uniquely identified variants allow inferences on tumor evolution between the two lesions.
Conclusion: GPM with the CytoTerra next generation cytogenomic platform successfully identified chromosome abnormalities in FFPE HNSCC samples. BM HNSCC samples demonstrated structural alterations in known and targetable oncogenes suggesting that CytoTerra can be a useful tool for novel biomarker discovery in solid tumors, including HNSCC.

#717 Pan-cancer analysis of *TRIM37* copy-number and development of fit-for-screening in situ hybridization tools.

Isabel Soria-Bretones[1], Joseph D. Schonhoft[2], Esha Jain[2], Nikita Kotlov[3], Parham Nejad[2], Daria F. Goncharova[3], Oleg A. Baranov[3], Jessica K. Scher[3], Elizabeth A. Sheehan[3], Vladimir A. Kushnarev[3], Konstantin Chernyshov[3], Gary Marshall[2], Ian M. Silverman[2], Victoria Rimkunas[2]

[1]Repare Therapeutics Inc., Saint-Laurent, QC, Canada,[2]Repare Therapeutics Inc., Cambridge, MA,[3]Boston Gene, Watertown, MA

Background: Elevated TRIM37 protein levels, often driven by genomic copy number (CN) gains in cancer cells, lead to loss of the pericentriolar material during mitosis and increased dependence on PLK4-mediated centriole assembly. This synthetic lethal hypothesis is being tested in a phase I clinical trial evaluating the activity of the PLK4 inhibitor, RP-1664, in advanced solid tumors with *TRIM37* CN gains or overexpression (LIONS, NCT06232408). In support of this clinical study, we characterized *TRIM37* CN and RNA expression across a large genomic database and by imaging-based DNA and RNA in-situ hybridization.

Methods: Whole exomes and transcriptomes from >12,000 adult solid tumors were analyzed for *TRIM37* CN gain (CN > ploidy) or amplification (amp, CN ≥ ploidy + 2), respectively, or TRIM37 RNA expression (BostonGene^{TM}). Locus specific probes for fluorescence in-situ hybridization (FISH) were developed for *TRIM37* and centromere 17 (*CEP17*). TRIM37 RNA-ISH was developed using the RNAscope^{TM} system (ACD Bio^{TM}).

Results: Across adult solid tumors, *TRIM37* amps, were most frequent in breast (9%), lung adenocarcinoma (5%), and melanoma (5%). Using the lower cutoff inclusive of *TRIM37* gains, CN events were most often observed in urinary tract cancer (33%), lung adenocarcinoma (32.2%), breast cancer (26%), and squamous cell lung carcinoma (24.2%). *TRIM37* CN levels were correlated with TRIM37 RNA expression in all indications examined. *TRIM37* CN level was inversely correlated with segment length (coef -1.76, p-value = 6.1e-20). *ERBB2* (HER2) is located approximately 19 megabases (mb) from *TRIM37* and was often co-amplified, with 73% of *ERBB2* gain/amps also having *TRIM37* gain/amps. *TRIM37* focal amps (<5.6 mb) were enriched in samples with co-occurring *ERBB2* focal amps, even though these events were on different segments. HER2-enriched breast cancer had the highest percentage of focal *TRIM37* amps (56%) and high-level amps (≥ 5 copies; 60%). To identify TRIM37 high patients clinically, we developed RNA and DNA ISH assays. *TRIM37* DNA FISH ratio and ploidy corrected CN by NGS were strongly correlated (N=16, Pearson correlation = 0.89, p-value = $3.4x10^{-6}$). DNA FISH ratio was positively associated with RNA-ISH scores (Jonckheere-Terpstra test, P = 0.03), and of 16 *TRIM37* FISH positive DNA-FISH samples, 14 were scored 2-3+ (≥4 copies/cell), while 2, scored 1+ (1-3 copies per cell) by RNA-ISH.

Conclusions: A considerable population of adult solid tumors are TRIM37-high. Creation of fit-for-screening tools employing in-situ hybridization techniques may provide enhanced sensitivity for identification of patients with TRIM37-high tumors.

#718 The crucial role of SPARC during the transition from in situ to invasive breast cancer.

Marianela Sciacca[1], **Marcela S. Villaverde**[1], Maria del Pilar Carballo[2], Ezequiel Lacunza[3], Lina Marino[4], Noelia Paola Cardozo[5], Naira Rodriguez Padilla[1], Martin Abba[3], Erica Rojas Bilbao[4], Pablo J Saez[6], Ana Maria Eijan[1], Catalina Lodillinsky[1]

[1]Research Area, Instituto de Oncologia Angel H. Roffo, Universidad de Buenos Aires, CABA, Argentina,[2]Department Pathology, Instituto de Oncologia Angel H. Roffo, Universidad de Buenos Aires, CABA, Argentina,[3]CINIBA, School of Medical Sciences, National University of La Plata, La Plata, Argentina,[4]Department of Pathology, Instituto de Oncologia Angel H. Roffo, Universidad de Buenos Aires, CABA, Argentina,[5]Animal facility, Instituto de Oncologia Angel H. Roffo, Universidad de Buenos Aires, CABA, Argentina,[6]Cell Communication and Migration Laboratory, Institute of Biochemistry and Molecular Cell Biology, Center for Experimental Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Breast carcinomas are classified as ductal carcinoma in situ (DCIS) or invasive carcinoma (IDC) depending on the degree of invasion. DCIS is generally found adjacent to IDC in the primary tumor and is considered a precursor of IDC. Still, it has not been possible to identify features that predict the progression to more advanced stages. We have previously demonstrated that MT1-MMP, a key membrane-associated metalloprotease (MMP), involved in the cleavage of the extracellular matrix is essential for the transition from in situ to invasive tumors using intraductal xenograft model (Lodillinsky, 2016; 2021). In this work, RNAseq analysis from MT1-MMPhigh cells from invasive tumors post-intraductal inoculation was compared against a set of human high-grade DCIS previously described (Abba, 2015). Secreted protein acidic and cysteine-rich (SPARC) emerges as a candidate gene in early breast cancer progression. By IHC, we observed an over-expression of SPARC in neoplastic cells compared to normal tissue from 2 different cohorts of patients with IDC and DCIS foci (PICBIM n=55 and Roffo n=34). In matched samples, SPARC levels were higher in IDC than DCIS tumors ($p<0,0001$) and correlated with MT1-MMP levels. SPARC-positive tumors were higher in high-grade and TNBC IDC tumors ($p=0,02$). In a metastatic cohort of patients (n=57), lymph node metastases did not express SPARC, suggesting its specific role in early breast cancer progression. In the murine cell LM38-LP, MT1-MMP mRNA level and degradative capacity were reduced in KO SPARC cells ($p<0,05$). STRING analysis showed a medium interaction score between SPARC and TGF-B1. LM38-LP cells were sensitive to TGF-B1 pathway regulation ($p<0,05$; $p<0,001$) and KO SPARC LM38-LP cells reduced pSMAD 2/3 ($p=0,003$). SB431542 (20ng/ml) decreased SPARC and MT1-MMP expression in LM38-LP and human MCF10DCIS.com cell lines ($p<0,001$). TGF-B1 (2ng/ml) increased the degradative capacity of LM38-LP whereas, SB431542 and Galunisertib (1µg/ml), reduced it ($p<0,001$; $p<0,05$). On migration assays, we observed that the inhibitory effect of SB431542 was lost in KO SPARC cells ($p<0,0001$). In vivo, Galunisertib (50mg/kg) reduced the proportion and tumor area of IDC ($p= 0,04$) in a new mouse intraductal syngeneic model. Moreover, high SPARC expression was positively correlated with both, TGF-β and TGFBR1 in a basal type of breast cancer collection supporting our findings. This study identifies SPARC as a driver of early tumor progression via a TGF-B1-dependent mechanism, suggesting TGFRI as a target for SPARC-positive patients.

#719 Clinical and molecular differences suggest different responses to immune checkpoint inhibitors in microsatellite-stable solid tumors with high tumor mutational burden.

Tarik Demir[1], Imran Nizamuddin[2], Katrina Dobinda[3], Ruohui Chen[3], Masha Kocherginsky[3], Peter Doukas[2], Neelima Katam[4], Carolyn Moloney[1], Devalingam Mahalingam[1]

[1]Division of Hematology and Oncology Developmental Therapeutics Institute, Northwestern University, Chicago, IL,[2]Division of Hematology and Oncology, Northwestern University, Chicago, IL,[3]Preventive Medicine; Feinberg School of Medicine, Northwestern University, Chicago, IL,[4]Northwestern University, Chicago, IL

Introduction: High tumor mutational burden (TMB) has shown promise in specific tumor types; however, its broad applicability across all solid tumors remains unclear. We aim to evaluate the outcomes of patients with advanced solid tumors exhibiting TMB ≥10 mutations per megabase (mut/Mb) who were treated with immune checkpoint inhibitors (ICIs) and to identify resistance mechanisms.
Methods: Patients who received treatment at the Northwestern University between January 2015 and December 2020 with ICIs and a TMB of ≥10 mut/Mb were identified through an Enterprise Data Warehouse grant and determined by next-generation sequencing. Tumors with microsatellite instability were excluded. Survival curves were calculated using the Kaplan-Meier method, and multivariate analyses were conducted to evaluate the impact of clinical variables on response, with significance defined as $p \leq 0.05$.
Results: 117 patients were enrolled, median age of 68 years, and 61% of the participants were male. The patient cohort was categorized into two distinct groups based on their sensitivity to ICI treatments: Group 1: ICI-sensitive (75%, 88/117 patients). Group 2: Non-ICI-sensitive (25%, 29 /117 patients). Upon evaluation of treatment efficacy (n=105), the overall response rate was recorded at 34%, with 14% of patients achieving a complete response (CR). The median progression-free survival and overall survival were 10.9 months and 29.9 months, respectively. Positive treatment responses were associated with a lack of prior exposure to systemic therapy (p=0.046), specific tumor types (p=0.009), absence of liver metastasis (p=0.015) across all group. Additionally, the presence of POLE mutations was associated with better outcomes (p=0.037), as well as a TMB of ≥15 mut/Mb in non-ICI-sensitive cancer group (p=0.012). However, PD-L1 status, specific ICI regimen, age, and sex did not demonstrate prognostic significance. Regarding genetic pathways, mutations in the TP53 pathway were significantly correlated with poorer treatment response (p=0.018). No significant differences were observed in the RTK/RAS, NRF2, PI3K, TGFβ, WNT, MYC, cell cycle, and Notch pathways. Furthermore, specific mutations in the TP53 ($p = 0.029$) and PIK3CA ($p = 0.033$) genes were linked to diminished responses. A trend was also noted suggesting ROS1 mutations may be associated with a poorer response (p=0.057).
Conclusion: Immunotherapy utilizing ICIs effectively treats TMB-H tumors, which typically demonstrate low CR rates. Enhanced therapeutic responses have been observed in patients who have not previously undergone systemic therapy, do not present with liver metastasis, and possess a TMB level of ≥15 mut/Mb. Mutations in the TP53 pathway are linked to a diminished response, while other pathways analyzed did not exhibit statistically significant differences in response rates.

TUMOR BIOLOGY: Imaging in Animal Models
Poster Session

#72 A case of immune therapy-refractory composite lymphoma: The complex interplay between two distinct tumors within a single spatial microenvironment.

Ashley K. Volaric, James Gerson, Karamatullah Danyal, Rangsinee Nusapan, Seth Frietze

Larner College of Medicine at The University of Vermont, Burlington, VT

Immune checkpoint (IC) therapy with anti-PD1 agents is typically used in treatment-refractory classic Hodgkin lymphoma (CHL). However, we present a rare case of composite classic Follicular lymphoma (cFL) and CHL whereby frontline use of anti-PD1 therapy (Nivolumab) in combination with standard AVD (Doxorubicin, Vinblastine, Dacarbazine) was clinically justified. We performed the Visium spatial transcriptomics assay to gain insight into the unique tumor cell characteristics and immune cell composition of the tumor microenvironment both pre- and post-IC therapy. Our case is a of a 44-year-old female who presented with fever, night sweats, and cervical lymphadenopathy. The patient underwent frontline IC combination therapy with single-agent Nivolumab and standard AVD. She showed nearly complete treatment response after 6 cycles. However, three months post-therapy, she developed new symptoms of fatigue, fever, and inguinal lymphadenopathy. Excisional biopsy of the inguinal lymph node showed recurrence of the cFL with no evidence of the previous CHL. The clinicopathologic findings suggest a primary treatment response of the CHL to IC combination therapy, but refractory response of the cFL. To understand the complex immune cell composition of this rare lymphoma, we performed the 10X Genomics Visium CytAssist assay on a representative tumor area from the formalin-fixed paraffin-embedded tissue block of the initial diagnostic biopsy. The data were analyzed using a bioinformatics workflow for cell deconvolution to identify cellular composition, ligand-receptor interaction analysis to study cell-cell communication, and spatial gene expression analysis to understand the disease context. Pathologic diagnosis of the lymph node excision showed a composite lymphoma comprised of cFL and CHL. Cytogenetic fluorescence *in situ* hybridization (FISH) study for *BCL2* rearrangement was negative. Our analysis revealed distinct transcriptomic profiles associated with cFL and CHL regions within the lymph node excision. We identified unique immune cell infiltration patterns with pronounced CD4(+) T-cell memory signatures in both cFL and CHL regions. We observed transitional zones between the two lymphomas characterized by a mixed expression profile with increased stromal and stem cell markers. Furthermore, we uncovered T-cell immunoregulatory and trafficking signals localized to the CHL and transitional zones, potentially influencing the surrounding tumor microenvironment. In conclusion, this study provides an unprecedented investigation into a rare case of composite lymphoma treated with frontline IC combination therapy through molecular and spatial profiling. Further analysis using the Visium assay is pending on the subsequent post-therapy tissue biopsy which may undoubtedly reveal underlying unique biologic relationships.

CLINICAL RESEARCH: Predictive Biomarkers 2
Poster Session

#720 Clinical actionability of an Asian pan-cancer tissue-based next generation sequencing (NGS) assay.

Jing Yi Lee[1], Aya E. Helali[2], Zi Yi Wan[3], Donald Y. H. Poon[4], Jens Samol[5], Tsz Him So[6], Su Pin Choo[7], Ravindran Kanesvaran[8], Cheng-Vai Hui[9], Joseph S. Au[10], Timothy Yip[3], Ross Soo[11], Michelle Pek[3], Ruifen Weng[12], Bin Tean Teh[13], Min-Han Tan[3], Jonathan Poh[3], Jason Y. Chan[8]

[1]Cancer Stem Cell and Biology, Duke-NUS Medical School, Singapore, Singapore,[2]Department of Clinical Oncology, The University of Hong Kong, Hong Kong, Hong Kong,[3]Lucence Diagnostics Pte Ltd, Singapore, Singapore,[4]Mt Elizabeth Novena Specialist Centre, Singapore, Singapore,[5]Medical Oncology, Tan Tock Seng Hospital, Singapore, Singapore,[6]Hong Kong Integrative Oncology Centre, Hong Kong, Hong Kong,[7]Curie Oncology, Singapore, Singapore,[8]Division of Medical Oncology, National Cancer Centre Singapore, Singapore, Singapore,[9]Department of Oncology, Centro Hospitalar Conde de Sao Januario, Macao, China,[10]Hong Kong Precision Oncology Society, Hong Kong, Hong Kong,[11]Department of Hematology-Oncology, National University Cancer Institute, Singapore, Singapore,[12]Diagnostics Development Hub, Agency for Science, Technology and Research (A*STAR), Singapore, Singapore,[13]Division of Medical Sciences, Duke-NUS Medical School, Singapore, Singapore

Introduction: While traditionally informed by tumor tissue origin and histopathology, advancements in next-generation sequencing (NGS) technology have catalyzed tumor-agnostic approaches which prioritize molecular alterations for personalized treatment. Here, we demonstrate the clinical utility of molecularly guided tumor-agnostic precision medicine in an Asian cohort, leveraging an Asian-centric NGS panel tailored to address the unique genetic landscape of the region.
Methods: 1,167 formalin-fixed paraffin-embedded tissue samples from 1,150 Asian cancer patients across >70 centers in 7 countries underwent real-world NGS testing using an Asian-centric pan-cancer hybrid-capture NGS panel (UNITED), profiling 572 cancer-related genes and 91 RNA fusion partners. Biomarkers were classified as actionable if therapeutic indications were FDA-approved, guideline-recommended, or supported by robust phase III clinical trials.
Results: The cohort encompassed 23 cancer types, with lung (17.7%), colorectal (12.8%), prostate (11.1%), and breast (8.6%) cancers accounting for 50.1% of the clinical volume. Actionable biomarkers were identified in 62.2% of samples, with *KRAS, EGFR,* and *PIK3CA* among the most frequently mutated genes. The likelihood of identifying ≥1 actionable mutation was highest in CNS tumors (83.6%), followed by lung cancer (81.2%), breast cancer (79%), and others. Among 27,552 somatic variants detected, 1,291 (4.7%) were potentially targetable by regulatory-approved therapies. Notably, ≥1 tumor-agnostic biomarker (including TMB-H, dMMR/MSI-H, *NTRK*/*RET* fusions, and *BRAF* V600E) was identified in every cancer type, detected in 98 samples (8.4%). This includes in 16.9% of lung tumors, 12.2% of tumors of unknown primary origin, 11.8% of endometrial tumors, and others. As expected, TMB was significantly associated with MSI ($p<0.0001$). *ERBB2* amplification, a potential surrogate marker for HER2 overexpression, was identified in 42 samples (3.6%) and was most frequently detected in breast tumors (15%), followed by endometrial tumors (11.8%), ovarian tumors (8.9%), and others. Homologous recombination deficiency (HRD) was observed in 407 samples (34.9%) and were present in about half of the tumors of the breast (50%), colon (49%), urothelium (47.1%), endometrium (47.1%) and lung (44%). Notably, HRD-positive tumors exhibited significantly higher TMB compared to HRD-negative tumors (median TMB 5.58 vs. 5.15, $p=0.0001$).
Conclusion: Leveraging an Asian-centric NGS panel, actionable molecular alterations were detected in 62.2% of Asian cancer patients. The high prevalence of actionable biomarkers, coupled with the detection of regulatory-approved tumor-agnostic markers, underscores the significance of comprehensive NGS testing in facilitating tumor-agnostic precision medicine in an Asian setting, thereby improving treatment strategies and patient outcomes.

#721 The role of NCAM1(CD56) in mediating venetoclax resistance.

Bernhard Alber, Tania Q. Vu, Bridget Robinson, Daniel Chandra, Jessica Mulkey, Yoko Kosaka, Evan Lind, Jeffrey W. Tyner, Jennifer N. Saultz

Knight Cancer Institute, Oregon Health & Science University, Portland, OR

Background: NCAM1 (CD56) is a neural cell adhesion molecule that is expressed on immune cells including NK cells, T cells, dendric cells and monocytes. Acute myeloid leukemia (AML) patients also aberrantly express NCAM1 in around 30% of cases which may impact treatment response. Our group recently found that CD56 expression on myeloid blasts is associated with worse overall survival to treatment with the BCL2 inhibitor, Venetoclax (VEN), including VEN combined with hypomethylating agents or with the JAK inhibitor, Ruxolitinib. Within molecular prognostic risk score (mPRS) defined higher and intermediate- risk groups, CD56 positivity further stratified survival in addition to genetics. The functional role of CD56 in VEN resistance is unknown.

Methods: We used 2 different AML cell lines showing high and intermediate expression of CD56, MOLM14 and OCI-AML2 respectively. Utilizing CRISPR Cas9 gene editing technology we generated stable *NCAM1* knockouts. Multiparameter flow cytometry was used to access changes in surface CD56 expression. Ex-vivo drug sensitivity assays were performed on parental, *NCAM1* knockout, and non-targeting cells to investigate VEN response at baseline and upon *NCAM1* knockout. Western blot as well as single-cell imaging analysis were performed to elucidate changes in protein expression.

Results: VEN sensitivity is associated with relative CD56 expression in MOLM14 and OCI-AML2. MOLM14 cell with high CD56 expression are more resistant to VEN than OCI-AML2 showing intermediate expression. Additionally, upon acquisition of VEN resistance through long-term drug exposure, MOLM14 and OCI-AML2 increase expression of CD56, phospho-MAPK1/3 (pERK), and have a higher ratio of MCL1/BCL2. To understand the impact of genetic deletion, we utilized CRISPR Cas9 to knockout *NCAM1* in MOLM14 and OCI-AML2 and found that absence/or knockdown of CD56 sensitizes cells to VEN, as well as decreases protein levels of BCL2, pMAPK1/3, MCL1 and FGFR1. Of note, we saw no changes in surface markers associated with myeloid cell differentiation state (ie. CD11b, CD14, CD117) in our *NCAM1* knockout lines compared to non-targeted control.

Conclusions: Genetic deletion of CD56 sensitizes AML cells to VEN treatment and decreases pMAPK1/3 as well as BCL2 protein levels. CD56 signaling occurs through the PTK2/RAS/MAPK signaling pathway, however a direct regulatory role of CD56 on BCL2 expression has not yet been reported. Future therapeutics targeting CD56 may be able to improve response and survival in patients with CD56 positive AML particularly after VEN resistance. Further research to delineate the underlying mechanisms associated with CD56 and BCL2 family profiles are underway which may enhance current treatment approaches.

#722 Characterization and estimation of treatment response of immunotherapy-based total neoadjuvant therapy (iTNT) for microsatellite stable (MSS) locally advanced rectal cancer.

Yaqi Wang[1], Yang Xu[2], Fan Xia[1], Juefeng Wan[1], Lijun Shen[1], Yan Wang[1], Hui Zhang[1], Hairong Bao[2], **Haimeng Tang**[2], Qiuxiang Ou[2], Hua Bao[2], Yang Shao[2], Sanjun Cai[1], Xinxiang Li[1], Zhen Zhang[1]

[1]Fudan University Shanghai Cancer Center, Shanghai, China,[2]Nanjing Geneseeq Technology Inc., Nanjing, China

Background: Total neoadjuvant therapy (TNT) combined with immunotherapy (iTNT) has shown promising results for locally advanced rectal cancer (LARC), especially in proficient mismatch repair (pMMR)/microsatellite stable (MSS) patients. However, traditional clinical approaches often fail to accurately forecast clinical outcomes, and reliable methods to predict treatment response and recurrence risk are essential for optimizing treatment strategies and maximizing organ preservation.

Methods: We analyzed clinical and genetic data from 63 patients in the TORCH clinical trial who received iTNT. Baseline tissue mutations, magnetic resonance tumor regression grade (mrTRG), and circulating tumor DNA (ctDNA) positivity at different time points were examined for their association with complete response (CR) and recurrence. Predictive models integrating these features were developed to assess their accuracy in distinguishing CR from non-CR patients. Prognostic features were analyzed using COX regression and Kaplan-Meier analyses.

Results: Baseline *SMAD4* mutation was significantly associated with poor response to iTNT (P=0.007), while post-treatment mrTRG (P<0.0001), tumor mutational burden (TMB) (P=0.03), and latest ctDNA status (P=0.03) also correlated with CR rates. Integration of post-treatment mrTRG, *SMAD4* mutation, TMB, and latest ctDNA status significantly improved CR prediction accuracy compared with mrTRG-only model (area under the curve (AUC) 0.92 vs 0.75, P=0.0098). Early prediction models incorporating middle-iTNT mrTRG and these genetic features showed superior accuracy to predict CR than mrTRG-only model (AUC 0.89 vs 0.76, P=0.047). Furthermore, ctDNA positivity at later time points and *SMAD4* mutations correlated with worse disease progression, including local recurrence and distant metastases.

Conclusion: Our findings highlight the predictive power of combining genetic and clinical features, particularly ctDNA and *SMAD4*mutation, for improving CR prediction and early recurrence risk stratification in iTNT-treated LARC. These models could guide more personalized treatment strategies, enabling early identification of patients requiring intensified therapy or nonoperative management.

#723 Tumor microenvironment and epithelial subtypes in nasopharyngeal carcinoma for precision oncology.

Wei Keat Teo[1], Yi Ren[1], Chee Yit Lim[1], Luvita Suryani[1], Joseph W. Foley[1], Bing Cheng Wu[2], Kwok Seng Loh[1], Raymond KY Tsang[1], Joshua K. Tay[1]

[1]Otolaryngology - Head & Neck Surgery, National University of Singapore (NUS), Singapore, Singapore,[2]Pathology, National University of Singapore (NUS), Singapore, Singapore

Nasopharyngeal carcinoma (NPC) is an Epstein-Barr virus (EBV)-associated epithelial malignancy endemic to Southeast Asia, Southern China, and the Middle East. Profiling the gene expression of NPC has been challenging due to its dense inflammatory microenvironment admixed with tumor epithelial cells, as well as limited tissue availability from small 4mm transnasal endoscopic biopsies. We performed laser-capture microdissection on a cohort of formalin-fixed paraffin-embedded (FFPE) biopsy samples obtained from NPC patients (n = 69) treated at the National University Health System Singapore and nasopharyngeal biopsies from healthy controls (n = 7). Primary, pretreatment tumors from patients who developed recurrent or metastatic disease (Group A) were compared with tumors from patients who remained healthy (Group B), matched for stage of disease. Tumor epithelial (TUM) and microenvironment (TME) regions were separately isolated. Gene expression libraries were prepared using a specialized mRNA-Seq technique optimized for tiny amounts of FFPE material, followed by next generation sequencing. Post-filtering, 330 gene expression libraries were analyzed, including 165 TUM, 134 TME, 4 dysplasia, 18 normal-adjacent and 9 normal libraries. Principal component analysis showed distinct clustering of epithelial and microenvironment gene expression libraries, confirming the purity of microdissection. Unsupervised k-means clustering revealed three distinct TME clusters (C1, C2, C3), which were also confirmed by silhouette analysis. Gene set enrichment analysis demonstrated significant enrichment of immune processes in C1, proliferative pathways in C2 and stromal processes in C3. Deconvolution of cell-type signatures with ESTIMATE showed that C1 had the highest immune score (1057, 129 and 733 respectively, $p < 2e-16$) while C2 had the highest tumor epithelial purity score (0.666, 0.852 and 0.715 respectively, $p = 3e-16$). C2 and C3 tumors had a higher recurrence rate compared to C1 (42.85%, 73.07% and 71.43% respectively, $p = 0.004$). Within the tumor epithelial compartment, Group A tumors were enriched for vascular endothelial growth factor signaling (NES = 1.55) and fibroblast growth factor receptor signaling (NES = 1.53), when compared to Group B. Consistent with our previous work interrogating receptor kinase signaling in NPC, FGF2 was significantly upregulated in Group A tumors (log2FC = 3.612). Microdissected gene expression profiling of FFPE patient biopsies identifies biomarkers and microenvironment subtypes important for prognosis and targeted treatment. VEGF and FGF pathway enrichment in high-risk tumors forms the basis of ongoing anti-VEGF clinical trials for NPC at our institution, with the potential of using a gene signature approach for patient selection.

#724 Target identification for antibody-drug-conjugate (ADC) therapy in biliary tract cancers (BTC).

Aruj Dhyani[1], Zeynep Tarcan[2], Joanne Chou[3], Rohit Thummalapalli[1], Walid Chatila[3], Sharanya Nag[4], Ezra Rosen[5], Sydney Bowker[6], Wungki Park[1], Elisa De Stanchina[7], Sarat Chandarlapaty[5], David Solit[4], William Jarnagin[8], Marinela Capanu[3], Eileen M O'Reilly[1], Ghassan K Abou-Alfa[1], Olca Basturk[9], James Harding[1]

[1]GI Oncology-Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY,[2]Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, NY,[3]Epidemiology-Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY,[4]Human Oncology and Pathogenesis, Memorial Sloan Kettering Cancer Center, New York, NY,[5]Breast Oncology-Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY,[6]Research Facilities, Memorial Sloan Kettering Cancer Center, New York, NY,[7]Department of anti-tumor assessment, Memorial Sloan Kettering Cancer Center, New York, NY,[8]Surgery, Memorial Sloan Kettering Cancer Center, New York, NY,[9]Pathology and laboratory medicine, Memorial Sloan Kettering Cancer Center, New York, NY

Background: BTCs are an uncommon malignancy with poor prognosis. ADCs have efficacy in several solid tumors with antigen surface expression serving as a potential biomarker in some instances. Herein, we evaluate the protein expression and heterogeneity of ADC target antigens and their prognostic implications in BTC.
Methods: Utilizing an IRB-approved, retrospective biospecimen protocol, we constructed tissue microarrays (TMA) from resected, early staged intrahepatic cholangiocarcinoma (IHC), extrahepatic cholangiocarcinoma (EHC) and gallbladder cancer (GB). IHC for Nectin-4, TROP2, c-MET, CLDN18.2 was assessed. Positivity defined as ≥10% weakly stained tumor cells and quantified by H-score (intensity × percentage). HER2 IHC scored per standard colon criteria. Clinicopathological factors and outcomes were associated with ADC target expression.
Results: N=65 resected cases were selected (median age 72 years (range 36-92); 55% males, anatomical subtype 40% IHC, 43% EHC, 17% GB; 84% moderately differentiated adenocarcinoma. c-MET and TROP2 were expressed in 75% and 83% of cases with differential expression based on anatomic site (c-MET: IHC: 62%, EHC: 89% vs GB: 73%, p=0.049; TROP2: IHC: 65%, EHC: 93% vs GB: 100%, p=0.010, Table). HER2, CLDN18.2, and Nectin-4 were expressed in 7.7%, 46%, and 66% of cases, and did not differ based on anatomic site. Median RFS and OS were 20 (95%CI 10-NR) and 40 (95%CI 27-NR) months respectively and outcomes were not impacted by expression of HER2, Nectin-4, TROP2, c-MET, or CLDN18.2.
Conclusion: Modest to high expression of Nectin-4, TROP2, c-MET and CLDN18.2 was identified across BTC anatomic subtypes, suggesting a potential role for ADCs to these antigens in patients with BTC. Preclinical validation is ongoing in BTC patient derived xenografts with trastuzumab deruxtecan (HER2 sensitive and HER2 resistant models), enfortumab vendotin (Nectin-4 positive model), and sacituzumab govitecan and these results will be presented at the meeting.

Differential Expression of ADC targets in BTC across anatomic subsite

	cMET			TROP2			CLDN 18.2			Nectin 4			Her2	
	Positive N (%)	H-Score, median (IQR)	H Score >200	Positive N (%)	H-Score, median (IQR)	H Score >200	Positive N (%)	H-Score, median (IQR)	H Score >200	Positive N (%)	H-Score, median (IQR)	H Score >200	Equivocal N (%)	Positive N (%)
Total	49 (75%)	100 (20,200)	3 (4.6%)	54 (83%)	100 (100,200)	8 (12%)	30 (46%)	0 (0,100)	2 (3.1%)	43 (66%)	100 (0,200)	1 (1.5%)	1 (1.5%)	5 (7.7%)
ICC	16 (62%)	65 (0,100)	0 (0%)	17 (65%)	100 (0,150)	3 (12%)	10 (38%)	0 (0,100)	0 (0%)	17 (65%)	100 (0,200)	0 (0%)	1 (3.8%)	2 (7.7%)
ECC	25 (89%)	100 (100,200)	3 (11%)	26 (93%)	100 (100,190)	3 (11%)	16 (57%)	55 (0,100)	2 (7.1%)	20 (71%)	100 (0,200)	1 (3.6%)	0 (0%)	2 (7.1%)
GB	8 (73%)	25 (5,100)	0 (0%)	11 (100%)	170 (140,200)	2 (18%)	4 (36%)	0 (0,30)	0 (0%)	20 (71%)	100 (0,200)	0 (0%)	0 (0%)	1 (9.1%)

#725 An mRNA-based predictor of response to daratumumab in multiple myeloma.

Steen Knudsen[1], Peter Gimsing[1], Henrik Stender[1], Thomas Jensen[1], **Jeremy R. Graff**[2]

[1]Allarity Therapeutics, Inc., Horsholm, Denmark,[2]Allarity Therapeutics, Inc., Tarpon Springs, FL

The purpose of this study was to develop a predictor of response to treatment with daratumumab in multiple myeloma. Daratumumab has considerably improved outcomes for myeloma patients. However, the treatment is expensive and not all myeloma patients benefit from treatment. Therefore, a predictor of daratumumab benefit would focus on treating patients with high likelihood of responding, and avoid treating those who are unlikely to respond or benefit. Daratumumab is a monoclonal antibody that binds to CD38, causing apoptosis via antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cellular cytotoxicity (CDCC). ADCC requires the presence of natural killer cells, and can be measured in vitro with a calcein release assay using multiple myeloma cell lines in the presence of natural killer cells. Measuring the baseline gene expression in the cell lines makes it possible to identify mechanisms of resistance or sensitivity to daratumumab induced ADCC in vitro. We used published data of lysis of 19 multiple myeloma cell lines and B-cell non-Hodgkin's lymphoma cell lines in the presence of daratumumab and natural killer cells from peripheral blood mononuclear cells. Correlating the sensitivity to ADCC with the baseline gene expression of these cell lines, we identified 27 genes correlated to sensitivity and 26 genes correlated to resistance. Among these are genes previously associated with sensitivity to daratumumab, glucocorticoid response, the proteasome, natural killer cell resistance, nuclear transport, myeloma risk classification, and myeloma cancer drivers. The 53 genes collectively constitute a daratumumab drug response predictor (DRP), and a single score of predicted sensitivity to daratumumab can be calculated as the difference between the mean of the positively correlated genes and the mean of the negatively correlated genes. We used published single-cell RNAseq data from the KYDAR trial of multiple myeloma patients treated with daratumumab, carfilzomib, lenalidomide and dexamethasone, to test the 53 gene DRP on clinical samples. The KYDAR trial enrolled 41 newly diagnosed MM patients who either failed to respond, or experienced early relapse, after a bortezomib-containing induction regimen. Baseline single-cell RNA sequencing data and overall response information was available for 17 subjects. The overall response to treatment correlated with the DRP score: Pearson correlation coefficient 0.26 (95% CI 0.15 - 0.83). A cutoff can be applied to separate responding patients from nonresponding patients. These results provide proof-of-concept for an mRNA-based predictor of response in multiple myeloma that warrants further clinical validation in multiple myeloma. When combined with our previously published response signatures for other cancer treatments used in myeloma, a map of predicted sensitivities can be provided for each patient based on a bone marrow sample.

#726 Comprehensive genomic profiling of cell-free DNA and matched white blood cells improves accuracy of ctDNA molecular response in immunotherapy treated NSCLC.

Jennifer B. Jackson[1], Pedro Rocha[2], James White[1], Andrew Georgiadis[1], Ellen Verner[1], Amy Greer[1], Rafael Bach Mora[3], Laura Masfarre[3], Nil Navarro[3], Beatriz Bellosillo[4], Sergi Clave[4], Mark Sausen[1], Edurne Arriola[2]

[1]Labcorp, Baltimore, MD,[2]Cancer Research Program, Hospital del Mar Medical Research Institute, Barcelona, Spain,[3]Medical Oncology Department, Hospital del Mar, Barcelona, Spain,[4]Pathology Department, Hospital del Mar, Barcelona, Spain

Introduction: Assessment of molecular response (mR) to targeted and immunotherapies (IO) in solid cancer is an emerging clinical liquid biopsy application. Current approaches have demonstrated promising results but have predominately relied on tumor-informed strategies and small to intermediate-sized next generation sequencing (NGS) gene panels. Lack of tissue availability and emergence of comprehensive genomic profiling (CGP), however, limit the practicality of implementing these approaches in a clinical setting. Furthermore, biological signals from non-tumor derived alterations arising from clonal hematopoiesis (CH) may confound detection of tumor-specific alterations for therapy selection and longitudinal monitoring. To address these limitations, we evaluated the 521-gene PGDx elio™ plasma complete assay with an integrated matched white blood cell (WBC) analysis algorithm to longitudinally assess cell-free tumor DNA (ctDNA) through a baseline plasma-informed approach and correlate mR to radiographic response and outcomes.
Methods: Plasma from 57 patients with mNSCLC who received IO from 2017-2019 at Hospital del Mar had detectable pre-treatment ctDNA. Blood was collected prior to the start of therapy, early on-treatment (median 4 weeks) and longitudinally throughout clinical follow-up and disease progression. Germline and CH variants were identified and removed through matched WBC NGS analyses. mR was calculated as the percent change in mean variant allele frequency of the tumor-specific alterations assessed at the early on-treatment timepoint compared to the pre-treatment timepoint and then compared to radiographic response (RR) and overall survival (OS).
Results: Thirty percent (17/57) of patients had a complete or partial radiographic response, with median follow up of >12 months. A tumor-specific genomic landscape analysis at baseline demonstrated associations between mR, recurrently mutated genes, and clinical outcomes. Removal of non-tumor derived cell-free DNA (cfDNA) variants improved characterization of mR and associations with radiographic and survival-based endpoints. A mR rate of 33% (19/57) was observed with a median OS of 53 months compared to 8.4 months for patients experiencing molecular progression at the early on-treatment timepoint (HR 4.8, 95% CI 2.2-10.2, $p<0.001$). Of all patients that survived ≤12 months after initiation of treatment, 85.7% (24/28) were categorized as experiencing molecular progression through early on-treatment cfDNA analyses.
Conclusions: CGP of cfDNA with integrated matched WBC analyses improves accuracy of mR and associations with RR and outcomes. This tumor-agnostic approach for assessment of genomic alterations at baseline and mR early on-treatment improves our ability to associate liquid biopsy-based biomarkers with patient and treatment-level outcomes.

#727 Association of baseline tumor and peripheral biomarker features with efficacy in metastatic castration-resistant prostate cancer patients treated with xaluritamig.

Benjamin E. Decato[1], Zhao Yang[1], Isabelita Vengco[2], Katherine Paweletz[2], Amrita Pati[2], Anita Reddy[2], Tara O'Donohue[1], Sheryl Treichel[2], **Kristen M. Smith**[2]

[1]Amgen Oncology, Thousand Oaks, CA,[2]Amgen Oncology, South San Francisco, CA

Xaluritamig, a novel STEAP1 x CD3 XmAb® 2+1 T-cell engager, is showing initial safety and efficacy in patients with metastatic castration-resistant prostate cancer (mCRPC). Here, we report on the association of baseline tumor and peripheral biomarker characteristics with efficacy in the first-in-human dose exploration trial. We examined STEAP1 target expression by immunohistochemistry in fresh and archival tumor biopsies, peripheral immune populations by multiparameter flow cytometry, and tumor somatic mutations via circulating tumor DNA (ctDNA) in plasma. STEAP1 was broadly expressed in mCRPC subjects with greater than 95% of tumor biopsies containing at least 1% STEAP1-positive tumor cells. STEAP1 expression was similar at various metastatic sites examined in the patient cohort and was not impacted by the number or types of prior therapies. Additionally, STEAP1 expression levels were not correlated with PSMA expression. Neither STEAP1 IHC intensity nor the percentage of STEAP1-positive tumor cells correlated with response to xaluritamig. Baseline peripheral immune cell populations and blood chemistry features may potentially associate with response. Patients who achieved a PSA50 response had lower neutrophil counts, neutrophil-to-lymphocyte ratio, and C-reactive protein than non-responding patients. The association between xaluritamig response and baseline tumor T cell infiltration is being assessed. Baseline somatic tumor mutations were determined by analysis of ctDNA. The distribution of somatic tumor mutations was consistent with published mCRPC mutational frequencies, with deleterious alterations in TP53, AR, PTEN and RB1 observed most frequently. Xaluritamig efficacy was observed in patients whose tumors carried these common mCRPC alterations, as well as in patients with detectable TMPRSS2:ERG fusions Taken together, these data suggest that xaluritamig has anti-tumor activity in a broad mCRPC patient population and provide support for enrolling patients with mCRPC in a Phase 3 study of xaluritamig (NCT06691984) regardless of STEAP1 expression levels or tumor mutational status.

#728 Identification of high-risk luminal early breast cancer patients based on a p53 signature.

Florian A. Buttner[1], Stefan Winter[1], Johanna V. Dahlen[2], Thomas E. Murdter[1], Reiner Hoppe[1], Peter Fritz[1], Patrick Danaher[3], Stephen F. Madden[4], Matthias Schwab[5], **Werner Schroth**[1]

[1]Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology and University of Tuebingen, Stuttgart, Germany,[2]Medical Faculty, University of Tubingen, Tubingen, Germany,[3]Bruker Spatial Biology, Seattle, WA,[4]Data Science Center, School of Population Health, Royal College of Surgeons in Ireland, Dublin, Ireland,[5]Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology and Departments of Clinical Pharmacology, Pharmacy and Biochemistry, University of Tuebingen, Stuttgart, Germany

Estrogen receptor (ER) positive luminal early breast cancer (BC) benefits from endocrine therapy based on the selective ER modulator tamoxifen (TAM) and aromatase inhibitors (AI). Yet, tumor recurrence occurs in up to one third of patients due to primary and secondary endocrine treatment resistance (ETR). The causes of primary ETR are not well defined by predictive tumor markers, however, *TP53* mutations have been linked with primary ETR in a preoperative short-term endocrine treatment study. We used a previously learned *TP53* mutation-predictive p53 score as part of the NanoString®BC360 mRNA expression panel to identify patients with p53 mutant-like status, and tested its applicability for the prediction of primary ETR in the adjuvant setting. In the TCGA BRCA cohort (N=1078), the p53 score distribution was analyzed for the presence of different subgroups using Gaussian finite mixture modeling. The same procedure was applied in breast cancer patients from the BreastMark database (N=1936) as an independent test of methodological and clinical validity. An ad-hoc binary classifier was trained to apply the cutoff identified in TCGA patients to patients of a prospectively collected, postmenopausal early breast cancer test cohort (IKP211) of hormone receptor-positive patients treated with TAM and/or AI (median follow up 6 years, N=632; DRKS00000605). Cluster modeling revealed that the p53 score distribution in the TCGA cohort is composed of two different patient groups: 363 patients (33.7%) with higher p53 scores were separated from the remaining patients with lower p53 scores. Likewise, the BreastMark cohort also showed two clusters with 419 patients (21.6%) forming the patient subgroup with high p53 scores. Cancer-specific survival (TCGA) and recurrence-free survival (BreastMark) were significantly shorter in p53-high patients of Luminal A subtype (P=0.0045 and P=0.04, respectively), with no significant differences observed in the remaining subtypes. When applying the binary classifier (mean AUC 0.95) on patients of the IKP211 test cohort, 14 patients (2.2%) were predicted as p53 mutant-like. This p53-positive patient group was strongly associated with higher recurrence or death rates in multivariate Cox regression (HR=3.3, 95% confidence interval 1.5-7.4; P=0.0037). As an independent verification, *TP53* amplicon sequencing of these patients confirmed a positive mutation status in all samples that passed quality control (N=11, 100%). We conclude that this p53-based division of Luminal A patients is useful to predict high-risk hormone receptor-positive early breast cancer patients with p53 mutations upfront, whose tumors likely show primary ETR and who are not sufficiently treated with endocrine agents.

#729 Applying novel transcriptomics-based markers to predict response to durvalumab and olaparib in mTNBC from histopathological slides.

Yaron Kinar[1], Gal Dinstag[1], Omer Tirosh[1], Doreen S. Ben-Zvi[1], SMMART Clinical Trials Program, **Tuvik Beker**[1], Aharonov Ranit[1], Gordon B. Mills[2]

[1]Pangea Biomed, Tel Aviv-Yafo, Israel,[2]Knight Cancer Institute, Portland, OR

Background: Transcriptomics-based predictors show great promise in identifying responders to immune checkpoint inhibitors (ICI) who are rejected by standard biomarkers like PD-L1. However, the reliance on gene-expression data makes these biomarkers less applicable in the clinic. On the other hand, H&E-stained slides are commonly available, so predicting response from slide scans could greatly improve accessibility. We have previously described DeepPT - a deep learning model to infer gene expression from H&E slides. Such a model could bridge that gap and allow clinicians to use transcriptomics-based biomarkers directly with H&E slides.
Methods: We tested two transcriptomics-based biomarkers of response to PD-1/PD-L1 inhibitors on a dataset of 21 mTNBC patients treated with olaparib and durvalumab. The first biomarker was derived using our published ENLIGHT platform, and the second is an XGBoost model on a curated set of 839 genes associated with ICI response, trained on 24 datasets with measured gene-expression and response data. We blindly applied the two models to the pre-treated tumor RNAseq data of the patient tumors, as well as to expression profiles inferred from their H&E slides using DeepPT. We also examined whether combining the scores from multiple slides per sample improves prediction. We then unblinded the outcomes and analyzed the model's ability to predict objective response (RECIST 1.1).
Results: Both models showed high predictivity in distinguishing responders (CR/PR) from non responders (SD/PD) when applied to RNAseq gene-expression profiles: AUC of 0.73 for the ENLIGHT model and 0.85 for the XGB model (Odds ratio for classification of 2.1 and 5.6, respectively). The performance on the gene-expression profiles that were inferred from a single H&E slide was also high - AUC of 0.82 for ENLIGHT and 0.83 for XGB (OR of 44 and 12). The performance of the XGB model improves when we average over all slides that correspond to a single patient (AUC of 0.85, OR of 16.7) while we observe no such improvement for ENLIGHT (AUC of 0.78, OR of 6.7). Interestingly, both models also distinguish stable from progressing disease within the nonresponders group.
Conclusion: We introduce a method to tackle the challenge of applying gene-expression based biomarkers for response to treatment by PD1/PDL1 inhibitors in the clinic. We do this by combining transcriptomics-based models and a deep-learning model for predicting gene-expression from H&E slides. We demonstrate that this method correctly predicts response on an external dataset, favorably comparing with direct application of the models to RNAseq data. This approach may be extended to additional drugs where transcriptomics-based biomarkers exist, and can be further improved by taking into consideration the differential predictivity of genes from H&E slides.

TUMOR BIOLOGY: Imaging in Animal Models
Poster Session

#73 Molecular characterization of senescence induced by PARPi in preclinical xenograft mouse models of ovarian cancer.

Sarah Saoudaoui, Nicolas Malaquin, Isabelle Clement, Tibila Kientega, Erwan Goy, Kim Leclerc-Desaulniers, Anne Marie Mes-Masson, Francis Rodier

CHUM Research Centre, Montreal, QC, Canada

Introduction: Ovarian cancer (OC) is amongst the most lethal cancers in Canada. High-grade serous ovarian cancer (HGSC) usually responds to initial therapy, however resistance to drugs often develops over time. We have described a new two-step OC combo-therapy: 1st, a PARP inhibitor is used to induce a senescence proliferation arrest in cancer cells followed by, 2nd, the induction of senolysis in senescent cancer cells using the Bcl-2 family inhibitor ABT263, which redirects senescent cells towards apoptosis. Our hypothesis is that therapy-induced senescence (TIS) could be beneficial in the clinic, but questions remain on its exact function in this context. Our objective is to characterize the spatiotemporal evolution of senescence during OC treatment using xenograft mouse models to define the senescence peak in tissues and optimize the targeting of senolysis approaches. Methods-results: We generate a tumor biobank consisting of OV1946, OV4453, TOV21G and MDAMB231 preclinical xenograft models treated with combinations of the PARP inhibitor Olaparib and ABT263. In all models, PARPi-ABT263 resulted in a significant reduction in tumor growth compared to each drug alone. To explore spatiotemporal cell fate decisions including senescence, a time course analysis was performed by gene expression analysis, SA-βgal staining and COMET multiplexing. COMET is an immunofluorescence technology that allows up to 40 molecular biomarkers (vasculature, epithelium, senescence phenotypes, DNA damage response) at single-cell resolution on paraffin embedded tumors. Conclusion: We propose that characterizing the cell fate decisions within the tumor including senescence can be used to improve drug administration patterns in combo therapies and reduce resistance. For example, this should refine targets and timing to improve chemotherapies that aim to manipulate senescence.

CLINICAL RESEARCH: Predictive Biomarkers 2
Poster Session

#730 Can we overcome the scarcity of data for building predictive models in cancer?.

Gal Dinstag, Yaron Kinar, Eden Maimon, **Tuvik Beker**, Ranit Aharonov

Pangea Biomed, Tel Aviv-Yafo, Israel

Background: A major aim in precision oncology is establishing predictive biomarkers to enable improved treatment matching for patients. mRNA expression is one of the most available and explored modalities to enable generation of such models. Several works have demonstrated that supervised learning using transcriptomic data can generate predictive biomarkers. However, the scarcity of datasets that couple mRNA with drug response severely limit this approach. In contrast, unsupervised models offer a potential for a broadly applicable approach to develop predictive markers. We have previously described ENLIGHT, an unsupervised approach that generates predictive biomarkers to virtually any targeted cancer drug without any drug-specific data requirements. Here, we perform a large-scale analysis, comparing the supervised and unsupervised approaches. We show that ENLIGHT is able to generate predictive markers on a far larger number of drugs than the supervised approach, and that for those drugs where a supervised approach is feasible, ENLIGHT's performance is comparable.
Methods: We established a list of the 114 approved targeted oncology drugs, and systematically searched for available training and validation data. For all 114 drugs, we generated an unsupervised biomarker using ENLIGHT. For drugs where more than one dataset was available, we trained and validated a logistic regression model with LASSO in a leave-one-dataset-out fashion, and compared its performance with ENLIGHT, which was not trained on any of the datasets. For drugs where only one dataset was available, we only tested ENLIGHT. We evaluated both methods by the odds ratio (OR) to respond at a score threshold that yields 50% sensitivity in each dataset. We then averaged these values across datasets to a single value for comparison.
Results: Of the 114 drugs tested, only 7 had more than one dataset with mRNA and objective responses available, to allow appropriate supervised training and validation. Thus, while we could generate ENLIGHT biomarkers for all 114 drugs, supervised methods allowed generation of a biomarker for only 7 drugs. We could test the ENLIGHT biomarkers for 17 drugs for which at least one dataset was available for validation, and found it was predictive (OR>1) in 16 of those. When focusing on the 7 drugs for which we could also generate supervised biomarkers, we found that ENLIGHT was roughly on par with the supervised models (aggregated OR of 2, p=0.003 on 6 datasets vs. aggregated OR of 2.52, p=0.0001 on all 7 datasets, respectively).
Conclusion: We demonstrate that ENLIGHT can overcome the inherent lack of data for generating predictive models for cancer drugs using a powerful unsupervised approach. While less than 10% of approved targeted cancer drugs have minimal data enabling generation of supervised models, ENLIGHT can generate predictive models for all the targeted drugs, and shows predictive value in almost all cases where validation data exists.

#731 Integrating ctDNA and FDG-PET dynamics to predict pathological response following neoadjuvant systemic therapy in stage III melanoma.

Wei Yen Chan[1], Li Zhou[2], Edward Hsiao[3], Jenny H. Lee[1], Ashleigh Stewart[1], Russell J. Diefenbach[1], George Au-Yeung[4], Maria Gonzalez[2], Andrew J. Spillane[2], Robyn P. M. Saw[2], Sydney Ch'Ng[2], Matteo Carlino[2], Richard A. Scolyer[2], Alexander M. Menzies[2], Georgina V. Long[2], Helen Rizos[1]

[1]Macquarie University, Sydney, Australia,[2]Melanoma Institute Australia, Sydney, Australia,[3]Royal North Shore Hospital, Sydney, Australia,[4]Peter MacCallum Cancer Centre, Melbourne, Australia

Background: Neoadjuvant therapy followed by surgery is the current standard for stage III melanoma, but 60% of patients achieve a major pathological response (MPR) with good long-term outcomes. This raises the potential for modifying treatment strategies to identify patients who may safely omit surgery. Biomarkers such as circulating tumor DNA (ctDNA) and imaging modalities have shown promise in assessing therapeutic response, yet each has limitations when used independently. This study evaluates the relationship between ctDNA clearance and changes in fluorodeoxyglucose(FDG)-positron emission tomography(PET) SUVmax from pre-treatment to pre-surgery, to predict pathological response and the feasibility of non-surgical management in appropriately selected patients.
Methods: In the discovery cohort, plasma ctDNA levels and computed tomography (CT) RECIST data were retrospectively analyzed in stage III melanoma patients from the OpACIN-neo trial (N=9), where two doses of neoadjuvant combination immunotherapy was administered. For validation, FDG-PET SUVmax changes and ctDNA dynamics will be assessed in two trial cohorts: NeoTrio (N=45, pembrolizumab ± dabrafenib and trametinib) and NeoPele (N=20, pembrolizumab and Lenvatinib). PET responses will be categorized into complete metabolic response (CMR), near-CMR (SUVmax reduction >90%), partial metabolic response (PMR), stable metabolic disease (SMD), and progressive metabolic disease (PMD).
Results: In the OpACIN-neo cohort, 7/9 (78%) patients achieved ctDNA clearance pre-surgery and RECIST partial response, with five achieving MPR and two non-MPR. Two patients with persistent ctDNA pre-surgery, which cleared post-surgery, had RECIST stable disease (one achieved MPR and one non-MPR). None of these nine patients experienced clinical recurrence. Preliminary data from the validation cohort shows that all eight patients (8/33; 24%) with CMR or near CMR achieved MPR, while 5/6 (83%) patients with PMD were pathological non-responders. CT RECIST was useful in evaluating disease burden in the discovery cohort; however, PET imaging is expected to provide greater specificity and stronger correlation with pathological outcomes. Furthermore, ctDNA clearance has demonstrated potential in identifying patients who may avoid surgery and remain recurrence-free, highlighting the complementary value of both modalities.
Conclusion: Combining ctDNA dynamics with FDG-PET SUVmax changes can improve the prediction of pathological response to neoadjuvant therapy in stage III melanoma. This integrated approach could help identify patients who will achieve durable responses and can safely avoid surgery, supporting the development of more personalized treatment strategies.

#732 Targeting adenosine A2B receptor using PBF-1129 in treating patients with metastatic non-small cell lung cancer.

Ruohan Wu[1], Dwight Owen[1], Lai Wei[1], Mikhail Dikov[1], Shankar Suman[1], Joseph Amann[1], Shuxiao Guan[1], Po-Lan Su[1], Catherine Schweitzer[1], Michael B. Smith[1], Sarah Ferguson[1], Julio Castro[2], Daniel Spakowicz[1], Erin Bertino[1], Peter Shields[1], Kai He[1], Carolyn J. Presley[1], Gregory A. Otterson[1], William E. Carson[1], David P. Carbone[1]

[1]The Ohio State University Wexner Medical Center, Columbus, OH,[2]Palobiofarma, Barcelona, Spain

Background: Immunotherapy has improved lung cancer treatment drastically, but its effectiveness is limited by the immunosuppressive tumor microenvironment (TME). In the TME, adenosine accumulates from ATP breakdown by ectonucleotidases like CD39 and CD73, recruiting immunosuppressive cells such as Tregs and MDSCs. Adenosine regulates immune cell function by activating four cell surface receptors, with A2B being the only low-affinity receptor primarily triggered by high adenosine levels in cancer, which makes it a favorable drug target. We previously reported the results of a phase 1 dose escalation trial of PBF-1129, a first-in-class A2BAR inhibitor in patients with mNSCLC who progressed on chemotherapy and immune checkpoint inhibitors (ICI, NCT03274479). Patients received escalating doses of PBF-1129 orally daily (40 mg, 80 mg, 160 mg, 320 mg) until disease progression or intolerability. Primary objective was safety and tolerability, followed by PK, ORR, PFS, OS, and immunophenotyping analysis. Here we report correlative analysis of immunophenotyping of peripheral blood changes during treatment. Blood was collected before treatment started, after the first cycle of treatment, and after the second cycle/end of treatment. PBMC was isolated using ficoll gradient.

Results: 21 patients (9 female), median age 61 years (range 49-75) were enrolled, median line of therapy was 4th (range 3-6). As previously reported, no dose-limiting toxicities were observed at any dose level and no maximum tolerated dose was identified. Best response of stable disease was observed. Flow cytometry analysis revealed that the PD-1 exhaustion marker was downregulated in CD8+ T cells following one to two treatment cycles ($p=0.03^*$). Nonsignificant changes indicated a reduction in CD73 expression on various immune subpopulations, in addition to MDSCs and Tregs percentage reduction was observed in most patients. Conclusions: PBF-1129 was safe and tolerable in patients with heavily pretreated mNSCLC, although limited single-agent activity was observed. The treatment improved immune cell function by alleviating PD-1 levels in cytotoxic T cells.

#733 Development and characterization of a negative prediction algorithm for actionable mutations utilizing genomic and epigenomic profiling in cfDNA.

Andrew M. Gross, Katie Quinn, Leslie Bucheit, Jill Tsai, Aaron Hardin, Catalin Barbacioru, Martina Lefterova, Justin Odegaard, Darya Chudova

Guardant Health, Palo Alto, CA

Introduction: Despite success of genomic tumor characterization from plasma, one challenge of cell-free DNA (cfDNA) profiling is an inability to rule out the presence of actionable genomic mutations. This difficulty stems from the inability to assess if known drivers are absent or if the tumor level is insufficient for detection in the assay. Confident negative variant prediction could allow clinicians to expedite clinical action from cfDNA without waiting for tissue biopsy sequencing when no actionable alterations are identified. Here we leverage an assay which simultaneously profiles epigenomic and genomic signals to develop a negative prediction algorithm in advanced cancers.

Methods: We use epigenomic signals to quantify an unbiased tumor fraction (TF) estimate and overcome limitations of genomic-based TF estimation including limit of detection, clonal hematopoiesis, variable copy-number on mutant alleles, and ambiguity of germline/somatic mutation origin. Using population priors, epigenomic TF, and observation of mutant and non-mutant coverage over variants of interest, we developed an algorithm to predict post-test probability of the cfDNA sample harboring guideline recommended genomic biomarkers relevant for treatment selection. This algorithm is applied in multiple tissue-specific diagnostic settings including colorectal cancer where testing for RAS/RAF hotspot mutations and lung cancer where testing multiple biomarkers are needed for optimal therapy selection.

Results: In an analysis of 3775 de-identified advanced stage CRC samples, 1342 (36%) were found to have a reportable *RAS*/*RAF* mutation. Among the remaining samples, 38% had sufficient tumor fraction to confidently assess the sample as *RAS*/*RAF* variant negative (with >99% confidence), which expanded to 46% at >95% confidence. This indicates that the test can confidently assign *RAS*/*RAF* status to >65% of CRC plasma samples. Reasons why the remaining samples could not be confidently assessed as biomarker positive or negative included low tumor shedding (including 15% with nondetectable tumor), low genomic coverage over loci of interest, and mutant allele support below the confident call threshold. Finally, we compare the application of epigenomic TF against genomic tumor-fraction algorithms using either RAS hotspot or a broader subset of cancer mutations, and show the epigenomic method to be much more sensitive and precise for TF estimation than genomic-only methods.

Conclusions: An epigenomics-based approach to enable confident negative prediction may increase the utility of liquid biopsy and expedite clinical decision-making in advanced solid tumors with biomarker-informed treatment pathways. Next steps include characterization in diverse tissue types as well as clinical validation to link negative call status to variant status in tissue biopsies.

#734 Stratification based on PRAME gene expression shows inverse survival associations among histology subtypes in a first-line non-small cell lung cancer real-world cohort.

R. S. Patel, S. Franch-Exposito, P. A. Fields, S. Islam, C. Igartua;
Tempus AI, Inc., Chicago, IL

Background: PRAME (Preferentially Expressed Antigen in Melanoma) is an intracellular cancer-testis antigen over-expressed across solid tumor types. Elevated PRAME expression is associated with poor prognosis, particularly in lung cancer. Further, its selective expression in tumor cells and ability to induce robust T-cell-mediated immune responses make it a promising pan-cancer immunotherapy target. This study investigates the influence of histological subtypes on PRAME expression and its impact on outcome to immunotherapy in first line (1L) lung cancer patients.

Methods: We analyzed 4,519 non-small cell lung cancer patients from the Tempus de-identified real-world database. PRAME expression levels (quantified by the Tempus xR RNA-seq assay), were classified into high and low based on median expression. We included pre-treatment biopsy samples receiving immunotherapy (IO) and chemotherapy (chemo), as individual monotherapies or in combination in the 1L setting. We evaluated PRAME expression by histology, tumor purity and biopsy site using a linear model and compared real-world overall survival and progression-free survival for high versus low expressors in each histological subtype.

Results: PRAME expression displayed a bimodal distribution in both adenocarcinoma (n=3,213) and squamous (n=1,306) histologies, with a significant enrichment of high expressors in the squamous subtype (Chi square $p < 2.22 \times 10^{-16}$). The squamous group exhibited inferior rwOS and rwPFS compared to the adenocarcinoma group across all treatment groups (rwOS: IO+chemo: HR: 1.25, p<0.01, IO: HR: 1.63, p<0.01, chemo: HR: 1.31, p=0.02, rwPFS: IO+chemo: HR: 1.04, p=0.471, IO: HR: 1.13, p<0.01, chemo: HR: 0.88, p=0.297). Notably, high PRAME expressors in the squamous group correlated with improved overall survival only within the IO+chemo treatment group (HR: 1.28, p=0.027), while low PRAME expressors in the adenocarcinoma cohort demonstrated better survival outcomes compared to high expressors (HR: 0.79, p<0.01). PRAME expression was not significantly associated with outcome in the IO mono or chemo mono treatment groups. Similarly for rwPFS, high PRAME expression was only significantly associated with benefit in the IO+chemo group (HR: 0.81, p<0.01, IO: HR: 0.91, p=0.425, chemo: HR: 0.85, p=0.232).

Discussion: Our findings identify PRAME expression as a prognostic biomarker in lung cancer, with distinct implications based on histological context. High *PRAME* expressors may benefit from PRAME-targeted therapies combined with IO and chemo, highlighting the potential for personalized treatment strategies. These results emphasize the utility of PRAME expression in clinical treatment decisions and demonstrate the value of real-world data in identifying patients with unmet need.

#735 Immunologically active phenotype by gene expression profiling predicts clinical benefit from PD-1/PD-L1 inhibitors in a pancancer cohort.

Mehdi Lamkhioued[1], Foy Jean-Philippe[2], Aurelie Swalduz[1], Sandra Ortiz-Cuaran[1], Andy Karabajakian[1], Kim-Arthur Baffert[1], Lucas Michon[1], Adrien Buisson[1], Maurice Perol[1], Sylvie Lantuejoul[1], Loic Verlingue[1], Jerome Fayette[1], **Pierre Saintigny**[1]

[1]Ctr. Leon Berard, Lyon, France,[2]Sorbonne Universite, Hopital Pitie-Salpetriere, Paris, France

Background: Our team recently developed a scoring system called the HOT-score, derived from an unsupervised clustering analysis of 421 HPV-negative head and neck squamous cell carcinomas (HNSCC) from TCGA. The HOT-score is based on a 27-gene signature associated with cytotoxic CD8+ T cell activation, interferon-gamma signaling, and IDO1 expression. These genes were identified through differential expression analysis. The HOT-score is computed as an enrichment score (ES) using Gene Set Variation Analysis (GSVA), providing a bimodal ES distribution ranging from -1 to 1. Tumors are classified either as "hot" phenotypes (ES score > 0) or "cold" phenotypes (ES score < 0). We have previously shown an association between the HOT-score and “hot”/”cold” phenotypes with overall survival (OS) and progression-free survival (PFS) in retrospective cohorts of patients with HNSCC (n=102) and non-small cell lung cancer (NSCLC, n=82) treated with immune checkpoint blockade (ICB). Herein, we evaluated the HOT-score's association with clinical outcomes across a broader range of cancer types in the CRi-Atlas cohort.
Methods: We analyzed pre-treatment tumor samples from the CRi-Atlas cohort (available at https://cri-iatlas.org/) that included cancers for which ICB therapies are approved. Inclusion criteria required samples to have annotations for progression-free survival, overall survival, or response evaluation by RECIST criteria. Associations with survival outcomes were assessed using Cox proportional hazards modeling, and comparisons of HOT-scores by response categories were performed using the Wilcoxon test.
Results: The cohort consisted of 348 bladder carcinomas, 207 kidney clear cell carcinomas, 313 skin cutaneous melanomas, and 45 stomach adenocarcinomas. HOT-scores, analyzed as continuous variables, were significantly lower in patients with progressive disease compared to those with stable disease (P=3.6e-05), partial response (P=3.3e-07), and complete response (P=5.5e-07). HOT-scores were strongly associated with better survival outcomes, including progression-free survival (PFS; n=489, HR 0.67; 95% CI [0.53-0.85]) and overall survival (OS; n=913, HR 0.51; 95% CI [0.41-0.63]). Tumors with "hot" phenotypes exhibited improved survival outcomes compared to those with "cold" phenotypes, with PFS (HR 0.77; 95% CI [0.67-0.97]) and OS (HR 0.54; 95% CI [0.43-0.67]). Notably, in a multivariate analysis accounting for tumor types, both HOT-scores and "hot" phenotypes remained strongly associated with better PFS and OS (P<0.001).
Conclusion: The HOT-score is associated with favorable clinical outcomes, including PFS, OS, and RECIST response, across multiple cancer types. Prospective studies are needed to validate its utility for routine clinical implementation.

#736 Deep learning response score using baseline and 12-week RECIST chest CTs enhances overall survival (OS) prediction in advanced NSCLC: external validation in a trial with dostarlimab.

Brenda F. Kurland[1], Chiharu Sako[2], Jing He[3], Marius de Groot[4], Taly G. Schmidt[2], Arpan A. Patel[5], Dwight H. Owen[6], Arya Amini[7], Ronan J. Kelly[8], Ray D. Page[9], Aurelie Swalduz[10], Jean-Paul Beregi[11], Jan Chrusciel[12], Stephane Sanchez[12], Richard Rosenberg[13], Jakob Weiss[14], An Liu[7], Olivier Gevaert[15], George R. Simon[16], Ravi B. Parikh[17], Alma Hart[18], Petr Jordan[2], Jasper van der Aart[19]

[1]GSK, Collegeville, PA,[2]Onc.AI, San Carlos, CA,[3]GSK, San Francsico, CA,[4]King's College, London, UK, GSK, Stevenage, UK, and Groover Consulting, Rotterdam, Netherlands,[5]University of Rochester Medical Center, Rochester, NY,[6]James Comprehensive Cancer Center, Ohio State University, Columbus, OH,[7]City of Hope, Duarte, CA,[8]Baylor University Medical Center, Dallas, TX,[9]The Center for Cancer and Blood Disorders, Fort Worth, TX,[10]Centre Leon Berard, Lyon, France,[11]CHU Caremeau, Nimes, France,[12]Hopitaux Champagne Sud, Troyes, France,[13]Medicom Technologies and Arizona Oncology, Phoenix, AZ,[14]Mass General Brigham, Harvard Medical School, Boston, MA,[15]Stanford Center for Biomedical Informatics Research, Stanford University, Palo Alto, CA,[16]Ohio Health, Columbus, OH,[17]Emory University School of Medicine, Atlanta, GA,[18]GSK, Stockholm, Sweden,[19]GSK, Cambridge, United Kingdom

Background: Early response assessment and identification of patients (pts) with NSCLC likely to derive long-term benefit from immune checkpoint inhibitors (ICIs) are crucial for treatment planning and drug development.
Methods: We developed a deep learning pipeline using a large multi-institute real-world dataset to generate a serial CT response score (SerialCTRS) from paired pre-treatment and 12wk scans, estimating probability of 1y OS. External validation applied SerialCTRS to an expansion cohort of a single-arm phase 1 clinical trial of dostarlimab as ≥2L therapy for advanced NSCLC (GARNET [cohort E], NCT02715284). OS hazard ratios (HRs) of SerialCTRS were compared to tumor volume change derived from manual segmentations and 12 wk RECIST 1.1 overall response using the same input scans.
Results: Of 67 clinical trial pts, 49 were evaluable for all 3 models predicting OS. Matching group sizes to RECIST overall response, 13 pts (26%, the number with RECIST PD) had low probability of 1y OS (SerialCTRS 0.12-0.64), 21 pts (43%, number with SD) had medium probability (0.64-0.82), and 15 pts (31%, analogous to CR/PR) had high probability of 1y OS (0.83-0.90). HRs of adjacent categories predicting OS showed superior performance of SerialCTRS, especially for SD/medium vs CR/PR/high probability (HR 2.68 [95% CI 1.09-6.59]; Table). Continuous SerialCTRS predicted OS with a concordance index of 0.717 (0.733 in subset with RECIST 12 wk SD) and remained a significant predictor of OS in bivariable survival models controlling for known predictors of OS such as age, ECOG, stage at diagnosis (all n=54), and best overall response from previous therapy (n=49).
Conclusions: Deep learning-based SerialCTRS, without manual annotation, improved prediction of OS vs RECIST and tumor volume change in the NSCLC cohort of the GARNET trial.

SerialCTRS prediction of advanced NSCLC OS compared to 12 wk RECIST response and percentage change in tumor volume

	Concordanceindex	SD/medium (n=21) vsCR/PR/high (n=15)		PD/low (n=13) vsSD/medium (n=21)	
		HR[a] (95% CI)	p-value	HR[a] (95% CI)	p-value
RECIST 1.1	0.686	1.35(0.58–3.16)	0.49	10.87(3.49–33.92)	<0.001
% change in tumor volume	0.610(0.507[b])	0.93(0.40–2.16)	0.87	4.31(1.71–10.86)	0.002
SerialCTRS	0.717(0.733[b])	2.68(1.09–6.59)	0.03	7.83(2.88–21.30)	<0.001

[a]SerialCTRS and volume change categorized to match three RECIST response category proportions for like-to-like comparisons (low vs medium vs high probability of 1y OS corresponding to PD vs SD vs CR/PR). Unadjusted p-values from Wald test for pairwise comparisons in Cox proportional hazards model.[b]Concordance index for continuous marker (% change in tumor volume or SerialCTRS) predicting OS for participants with 12 wk RECIST response of SD

#737 Androgen receptor-indifferent prostate cancer in radical prostatectomy cases detected by single-cell image analysis.

Beatrice S. Knudsen[1], Wei Zhang[2], Mason Hovinga[1], Erika Egal[2], Chance Walker[1], Yosep Chong[3], Galaxy Yang[4], Michael R. Freeman[4], Yi Qiao[5]

[1]Pathology, University of Utah, Salt Lake City, UT,[2]Huntsman Cancer Institute, University of Utah, Salt Lake City, UT,[3]Pathology, St. Mary Hospital, Seoul, Korea, Republic of,[4]Surgery, Cedars Sinai Medical Center, Los Angeles, CA,[5]Biomedical Informatics, University of Utah, Salt Lake City, UT

Rational: A subset of castration-resistant prostate cancer (CRPC) displays an androgen receptor indifferent (ARi) phenotype. In these cases, the nuclear AR fails to promote the expression of transcripts in the AR cistrome, such as KLK3/PSA. Because of the aberrant functionality of AR, the ARi PC may not respond efficiently to hormonal therapies. As the ARi phenotype has only been described in CRPC, we hypothesized that it might exist in primary PC, potentially providing an opportunity for early detection of treatment resistance.
Methods: 36 cases of pT3b prostate cancer with lymph node metastasis are displayed in 10 x 2mm cores per case on tissue microarrays. From each, we sampled 10 tumor regions including lymph node metastases, seminal vesicle and perineural invasion, extraprostatic extension, cribiform and non-cribifrom intraprostatic regions. TMA slides are stained with a multiplex panel of AR, PSA, Nkx3.1, PSMA, High molecular weight cytokeratin (HMW-CK, 34betaE12) and pan-cytokeratin (AE1/AE3) on the Leica Bond Rx and digitized using the Axioscan Z1. Manual annotations, cell and gland segmentation, and single cell protein expression measurements are conducted in QuPath. The clustering analysis is performed using CytoMap. Gene set enrichment analysis is used to determine the ARi phenotype in public RNA expression data sets. Significant differences are determined using T-statistics.
Results: Using normal prostate glands as a reference, we identified the intensity of nuclear AR that is sufficient for PSA expression. We used this threshold to find cancer regions with less than 30% PSA-positive cells despite adequate AR expression levels in more than 50% PC cells, indicative of the ARi phenotype. Five of 36 cases contained regions with an ARi phenotype in at least one of the TMA cores. Out of all ARi regions, 23.9% are in lymph node metastasis and 21.7% in seminal vesicle PC. Within PC cases, percentages of AR positive cancer cells in lymph nodes don't correlate with percentages at other PC locations, while percentages of AR-positive cells in seminal vesicle correlates with those in areas of extraprostatic extension or cribiform PC. Unsupervised k-means clustering using multiplex PC staining data reveals one of five clusters harboring most ARi TMA cores. The AR:PSA ratio is the most important variable for predicting the ARi phenotype, followed in importance by percentages of PSA, PSMA, Nkx3.1 and AR positive PC cells. Differential expression analysis of the TCGA-PRAD cohort cases confirms a subgroup of high AR and low KLK3/PSA cases with significant enrichment of the EMT hallmark gene expression signature, consistent with the ARi phenotype.
Conclusion: Single cell analysis of AR and PSA identifies PC regions with ARi characteristics in primary PC and lymph node metastases, possibly predicting primary resistance to first line PC treatment.

#738 Single-cell trajectories of metastatic urothelial cancer and individual patterns of resistance to immune checkpoint inhibitors.

Ronan Flippot[1], Amelie Roehrig[2], Julien Vibert[3], Nicolas Stransky[4], Luc Cabel[5], Kevin Mulder[6], Benjamin Besse[7], Claudio Nicotra[8], Maud Ngo Camus[3], Christophe Massard[3], Etienne Rouleau[9], Gerome Jules-Clement[10], Alicia Tran-Dien[10], Lambros Tselikas[11], Fabrice Andre[12], Jean-Yves Scoazec[9], Celine Vallot[2], Maud Kamal[12], Eric Letouze[2], Yohann Loriot[12]

[1]Medical Oncology / Immunomonitoring laboratory (CNRS 3655 - INSERM US23), Gustave Roussy, Villejuif, France,[2]One Biosciences, Paris, France,[3]Drug Development Department, Gustave Roussy, Villejuif, France,[4]Celsius Therapeutics, Villejuif, MA,[5]Institut Curie Paris, Paris, France,[6]INSERM UMR1015, Gustave Roussy, Villejuif, France,[7]INSERM U981, Gustave Roussy, Villejuif, France,[8]Drug Development epartment, Gustave Roussy, Villejuif, France,[9]Pathology, Gustave Roussy, Villejuif, France,[10]Bioinformatics Core Facility, Gustave Roussy, Villejuif, France,[11]Radiology, Gustave Roussy, Villejuif, France,[12]IHU PRISM, Gustave Roussy, Villejuif, France

Background. Immune checkpoint inhibitors (ICI) improve survival in patients with metastatic urothelial cancers (mUC) but most patients experience disease progression. The mechanisms driving this progression are mostly unknown. We longitudinally assessed mUC microenvironment at the single-cell level in ICI-treated patients to explore the determinants of the response and adaptive resistance mechanisms on therapy.

Methods. The prospective MATCH-R study (NCT02517892) included patients with mUC treated with single-agent PD-(L)1 inhibitors. Biopsies of metastatic sites were performed at baseline, on therapy and at progression for single-nuclei RNA sequencing (10X genomics, 5' v1.1). After stringent quality control and cell type annotation, we compared the proportions and transcriptomic signatures of tumor and immune cell subsets between responders and non-responders, and explored their evolution during treatment. Pseudobulk analyses were used to classify whole samples according to the consensus molecular classification (Kamoun et al. 2020).

Results. We enrolled 32 patients among whom eight (25%) achieved objective response (OR). All patients underwent biopsies at baseline, 6 (19%) on therapy, 17 (53%) at progression. Tumor cells showed high transcriptomic heterogeneity within the consensus groups, with the coexistence of basal and luminal cell states within the same samples. The proportion of basal cells at baseline, but not the basal sample classification, was associated with an increased OR (p=0.02). Immune features associated with resistance to ICI at baseline involved both myeloid and lymphoid compartments : within myeloid cells, an abundance of pro-tumoral HES1 macrophages and higher expression of immunosuppressive genes *CD163*, *MS4A4A* and *STAB1* ; within T cells, a higher proportion of exhausted CD8+ lymphocytes, and higher expression of immune checkpoint genes including *PDCD1.* Conversely, the baseline abundance of CD4+ naive T helpers was associated with improved OR. Longitudinal sampling showed potential adaptive resistance mechanisms at progression, including downregulation of HLA genes and IFN signaling in tumor cells ; shift from M1 to M2 macrophage polarization within myeloid cells; increased expression of immune checkpoints, downregulation of type-I interferon induced genes and cytotoxicity markers such as *GZMA* in T cells. Individual patient analyses have shown that these mechanisms may coexist with discrete patterns.

Conclusions. We identified potential mechanisms of resistance to ICI in patients with mUC related to both tumor and immune compartments, including specific T cell and macrophage subpopulations. Longitudinal assessments in patients show individual molecular patterns related to progression that may allow for dynamic and tailored therapeutic strategies.

#739 Patient-derived xenografts allow deconvolution and prediction of chemotherapy responses.

Jonathan T. Lei[1], Lacey E. Dobrolecki[1], Chen Huang[2], Ramakrishnan R. Srinivasan[1], Suhas V. Vasaikar[3], Alaina N. Lewis[1], Christina Sallas[1], Na Zhao[1], Jin Cao[4], John D. Landua[1], Chang I. Moon[1], Yuxing Liao[1], Susan G. Hilsenbeck[1], C. K. Osborne[1], Mothaffar F. Rimawi[1], Matthew J. Ellis[5], Varduhi Petrosyan[1], Bo Wen[6], Kai Li[7], Alexander B. Saltzman[1], Antrix Jain[1], Anna Malovannaya[1], Gerburg M. Wulf[8], Elisabetta Marangoni[9], Shunqiang Li[10], Daniel C. Kraushaar[1], Tao Wang[1], Senthil Damodaran[11], Xiaofeng Zheng[11], Funda Meric-Bernstam[11], Gloria V. Echeverria[1], Meenakshi Anurag[1], Xi Chen[11], Bryan E. Welm[12], Alana L. Welm[12], Bing Zhang[1], Michael T. Lewis[1]

[1]Baylor College of Medicine, Houston, TX,[2]University of Alabama at Birmingham, Birmingham, AL,[3]Pfizer, Bothell, WA,[4]Zhejiang University, Hangzhou, China,[5]Guardant Health, Palo Alto, CA,[6]University of Washington, Seattle, WA,[7]University of Michigan, Ann Arbor, MI,[8]Harvard Medical School, Boston, MA,[9]Institut Curie, Paris, France,[10]Washington University in St. Louis, St. Louis, MO,[11]MD Anderson Cancer Center, Houston, TX,[12]University of Utah, Salt Lake City, UT

Introduction: Combination chemotherapy remains essential for clinical management of triple-negative breast cancer (TNBC), making it impossible to assess responses to multiple agents in a single patient. Herein, we conduct multi-omic analyses of TNBC patient-derived xenografts (PDXs) treated with single agent carboplatin and docetaxel, or the combination, to identify candidate mechanisms of resistance, as well as predictive biomarkers to individual treatments, and to develop therapeutic strategies to overcome resistance.
Methods: Genomic, transcriptomic, and proteomic profiles of baseline tumors from 50 TNBC PDXs were associated with responses to human equivalent doses of either single agent carboplatin, docetaxel, and the combination. Integration of external TNBC PDX and clinical datasets was performed using ComBat. Protein Marker Selection (ProMS) tool was used to integrate both RNA and protein data to select 5-10 RNA feature combinations for optimized prediction of chemotherapy response in a logistic regression model.
Results: Combination responses were generally no better than the best single agent, with enhanced response in only ~13% of PDX, and apparent antagonism in a comparable percentage. Single ome comparisons showed largely non-overlapping results between genes associated with single agent and combination treatment that validated in independent patient cohorts. Multi-omic analyses of PDXs identified agent-specific treatment-associated biomarkers and predictive biomarker combinations. Notably, integrating proteomic with mRNA data improved machine learning models that achieved AUROC performance of 0.85 to predict pathologic complete response to combination chemotherapy. These models await further evaluation in datasets that may become available, including data from the BEAUTY (NCT02022202), TBCRC 030 (NCT01982448), and RESPONSE (NCT05020860) clinical trials, or in future experimental settings using PDX/PDX organoids. In PDXs responsive to any treatment, several basal cytokeratins were among the top upregulated proteins compared to PDXs resistant to all treatments. KRT5 was validated by IHC in PDX tumors, achieving an AUROC of 0.83 for predicting responsiveness, indicating its potential as a future chemoresponse marker in TNBC. PDXs refractory to all treatments showed dysregulated mitochondrial function. Treatment with romidepsin, an HDAC inhibitor that targets this process indirectly, increased DNA damage, and enhanced carboplatin response, in a chemoresistant PDX model with high abundance of HDAC proteins.
Conclusion: Multi-omic characterization identifies molecular mechanisms and predictive biomarkers for stratifying TNBC tumors for single or combination chemotherapy treatments, suggests targeted therapies to augment chemotherapy response, and provides a valuable resource for researchers and clinicians.

TUMOR BIOLOGY: Imaging in Animal Models
Poster Session

#74 Hypoxia-fibrosis cycle in pancreatic tumor microenvironment: Implication for diagnosis and treatment.

Nai-Jung Chiang[1], Ya-Chin Hou[2], Jiun-I Lai[1], Lun-De Liao[3], Yan-Shen Shan[4]

[1]Department of Oncology, Taipei Veterans General Hospital, Taipei, Taiwan,[2]Institute of Clinical Medicine, College of Medicine, National Cheng Kung University, Tainan, Taiwan,[3]Institute of Biomedical Engineering and Nanomedicine, National Health Research Institutes, Miaoli, Taiwan,[4]Department of Surgery, National Cheng Kung University Hospital, Tainan, Taiwan

Pancreatic cancer (PC) is difficult to treat and has the lowest five-year survival rate across the world. PC are characterized by a dense fibrotic stroma composed of abundant cancer-associated fibroblasts (CAFs) and excessive extracellular matrix (ECM), which creates a pathological barrier that impedes drug delivery to tumors, leading to suboptimal treatment efficacy and resistance to current therapeutics. Like a vicious cycle, hypoxia-fibrosis cycle consists of dynamic cancer cell-CAF interactions. Currently, most clinical therapeutics aim at tumor cells while overlooking the surrounding tumor microenvironment (TME). The TME comprises all of the physiological and biochemical elements, including, but not limited to, the ECM, CAFs, cancer-associated immune cells, and the hypoxic and acidic environment. Therefore, we aim to investigate intratumor heterogeneity of PC and figure out the tumor- and TME-driven signaling by using multi-omics analysis. The data obtained from bulk and single-cell RNA sequencing, spatial transcriptomics, and tissue microarrays show that hypoxia levels were positively correlated with tumor fibrosis. Hypoxia-fibrosis cycle modulates PC progression and pro-tumoral inflammatory microenvironment, which was associated with activation of hypoxia, KRAS, mTORC1, and inflammatory response pathways. This cycle acts as a poor prognostic indicator in patients with PC. We also established a three-dimensional cell culture model mimicking cancer cell in hypoxia-fibrosis environment to validate the impacts of hypoxia-fibrosis cycle on promoting the malignant phenotype of PC cells. A dual-modality ultrasound (US) and photoacoustic (PA) real-time imaging system is demonstrated to assess tissue oxygen saturation dynamics as well as tumor fibrosis in an orthotopic PC mouse model with/without irinotecan or nanoliposomal irinotecan treatment and highlights the potential for evaluating the efficacy of cancer therapies in preclinical settings. Our work provides useful information for enhancing the effectiveness of current therapies and may have the potential to develop new precision medicines.

CLINICAL RESEARCH: Predictive Biomarkers 2
Poster Session

#740 Standardization and validation of CLDN18 antibodies for CLDN18.2 testing in pancreatic cancer: performance across antibodies and immunohistochemistry platforms.

Gudrun Baenfer, Ali Jason Saleh, Rolf Diezko, Riem Adjam, Hans-Ulrich Schildhaus, Matthias Kirchner, Josef Ruschoff, Michael Friedrich

Discovery Life Sciences Biomarker Services GmbH, Kassel, Germany

Background: Pancreatic cancer, with a 5-year survival rate of just 10%, is one of the most lethal cancers. Claudin 18.2 (CLDN18.2) is a promising therapeutic target under investigation. This study aims to standardize and validate CLDN18.2 IHC testing in pancreatic cancer by: (1) extending a gold-standard CLDN18 IHC method from gastric to pancreatic cancer, (2) validating two CLDN18 antibodies across three IHC platforms, and (3) assessing a CLDN18.2-specific antibody in pancreatic cancer samples.
Methods: The Ventana CLDN18 (43-14A) assay on the Benchmark Ultra platform served as reference standard. Results from CLDN18 antibodies—Abcam 43-14A and LSBio B16145—were evaluated on Benchmark Ultra, Dako AS Link48, and Leica BOND III. Sixty pancreatic cancer samples verified the Ventana assay with inter-rater agreement by two pathologists. Twenty samples reflecting the full CLDN18 expression level (0-100%), were used to validate the two CLDN18 antibodies on the three platforms. Concordance with the reference standard was calculated for system suitability and accuracy. Precision was evaluated with five samples. A sample was deemed CLDN18.2 positive if at least 75% of tumor cells showed ≥2+ membranous CLDN18 staining. In addition, ten samples were used to explore the performance of the CLDN18.2-specific Abcam EPR19202 antibody on the Benchmark Ultra platform using OptiView DAB IHC detection kits.
Results: The verification of the Ventana CLDN18 IHC Assay demonstrated its reliability and reproducibility in determining CLDN18.2 positivity in human pancreatic cancer samples, with an interrater agreement of 92%. This validation study showed that the Abcam 43-14A antibody outperformed the LSBio antibody in concordance analysis with the reference standard. The Benchmark Ultra platform provided the best results with strong, specific membranous CLDN18 staining and minimal background. The Leica platform performed the poorest, with strong cytoplasmic staining and lower specificity. The Dako platform was intermediate, more specific than Leica but weaker than the Benchmark Ultra. The Abcam EPR19202 antibody performed inadequately with OptiView; staining was faint, diffuse, non-specific with significant background, and poor membranous delineation.
Conclusions: This study reveals key variations in anti-CLDN18 antibodies and IHC platforms, crucial for assessing borderline cases at the cutoff used in clinical trials (≥75%, IHC 2+/3+). It underscores the need for selecting suitable antibodies and optimizing antibody-platform combinations for reliable results. The Abcam 43-14A antibody on the Benchmark Ultra platform performed comparably to the Ventana assay. By evaluating multiple combinations, the study helps standardize CLDN18.2 IHC testing, enhancing diagnostic accuracy and healthcare decision-making.

#741 Assessing overall survival relative to LORIS response prediction in patients receiving immunotherapy in a large community-based health system.

Cheryl P. Jones[1], McKenna Deaton[1], Bing Xu[1], **Rachel Elsey**[1], Crystal Hattum[1], Mason Arndt[1], Victoria Moe[2], Alex Mohama[3], Casey Williams[1], Tobias Meissner[1]

[1]Avera Cancer Institute, Sioux Falls, SD,[2]University of South Dakota, Sanford School of Medicine, Vermillion, SD,[3]Science and Behavior, University of Notre Dame, Notre Dame, IN

Purpose: The use of immune checkpoint inhibitors (ICI) has revolutionized outcomes in several cancers. Despite markers such as tumor mutational burden (TMB) and PD-L1 expression, predicting which patients will respond remains a challenge. Recently, a tool was developed using machine learning to predict clinical outcomes from ICI across cancer types known as LORIS (logistic regression-based immunotherapy-response score) that provides a probability of response from 0-100% based on six factors. LORIS values <27% were deemed 'unlikely to respond' and >70% were 'likely to respond' in the originating study. This project evaluates the association of LORIS scores to overall survival (OS) with the aim of understanding its utility in clinical-decision making in a community health-based cancer center.

Methods: We performed a retrospective analysis of 136 patients at the Avera Cancer Institute that received at least one dose of an ICI and possessed all components to calculate LORIS, including age, cancer type, previous therapy, albumin, blood neutrophil-to-lymphocyte ratio (NLR) and TMB, in addition to outcomes. LORIS values were calculated according to the most recent values available prior to initiation of therapy. The association between OS and LORIS was evaluated by Cox proportional hazards model. With no scores >70% in our population (the threshold for likely response), patients were categorized as 'unlikely to respond' or 'not unlikely to respond' based on a score of ≤27% or >27%, respectively, and assessed via Kaplan-Meier.

Results: The median age was 67 years (range 36-87) and 48.5% were female. Lung cancer represented 47% of patients, followed by 15.4% melanoma, 14.7% gynecologic cancer and less than 5 patients each with GI, GU, and head and neck cancers; the remaining were considered "other" per LORIS. The median TMB was 6.1, median albumin was 3.8, and median NLR was 3.8. In contrast to the original LORIS datasets where 72% of patients were previously treated, our population was greater at 84% indicating a more heavily pre-treated group. Only 20 patients (14.7%) had a LORIS ≥27%. The hazard ratio was 0.09 (95% confidence interval 0.017-0.51) and LORIS was statistically significant for impact on OS (p=0.006). The median OS for those unlikely to respond (n=116) was 13.7 mo (9.9 - 18.7) vs. not reached (NR) (15.9 - NR) for those not unlikely to respond (n=20).

Conclusion: Higher scores were associated with longer OS, confirming its ability to predict long-term outcomes. This was upheld despite having few patients with >27% scores and no patients >70%, possibly due to the greater portion that previously received systemic therapy. Future studies will need to address how LORIS can be applied to individual patient scenarios to support clinical decision-making.

#742 Development and evaluation of a non-invasive qPCR assay for sensitive detection of FGFR2/3 alterations in urine samples as a companion diagnostic for erdafitinib.

Meirong Zhang, Mingzhu Li, Yue Zhang, Hang Dong, Haoran Tang, Feng Xie, **Qing Xu**

Huidu Shanghai Medical Sciences, Ltd., Shanghai, China

Introduction: Fibroblast growth factor receptor (FGFR) alterations, including point mutations and gene fusions, play a pivotal role in various cancers, particularly urothelial carcinoma. Erdafitinib, an FDA-approved therapy for locally advanced or metastatic urothelial carcinoma with FGFR2/3 alterations, currently relies on tissue-based diagnostics, which are invasive and may not always be feasible. Non-invasive liquid biopsy approaches, such as qPCR assays using urine cell pellets (UCP), offer significant advantages in terms of patient convenience and safety while providing accurate detection of clinically relevant alterations.

Methods: We developed and evaluated the Predicine FGFR2/3 qPCR Kit, a real-time multiplex PCR assay designed to detect four FGFR3 mutations (R248C, S249C, G370C, Y373C) and five FGFR2/3 fusions (FGFR3-TACC3 V1/V3, FGFR3-BAIAP2L1, FGFR2-BICC1, FGFR2-CASP7) from urine DNA and RNA. The assay integrates proprietary buffers to preserve nucleic acids, allowing simultaneous detection of DNA mutations and RNA fusions. Analytical performance, including sensitivity, specificity, and accuracy, was assessed using gradient dilutions, known positive controls, and clinical urine samples. Detection thresholds were benchmarked against NGS and ddPCR.

Results: The assay demonstrated high sensitivity, detecting DNA variants at allele frequencies as low as 0.25% and RNA fusions at 6-7 copies. Specificity exceeded 98% across all tested alterations in healthy donor samples, and positive percentage agreement (PPA) exceeded 95% for FGFR3 mutations and FGFR2/3 fusions compared to NGS results. In a retrospective cohort of 60 clinical urine samples, mutation detection sensitivity reached 100% for most variants, except for FGFR3-S249C, while specificity ranged from 92% to 100%. UCP-derived nucleic acids enabled streamlined workflows with a single vial of urine. Mutation detection achieved a limit of 0.1% for DNA and 6.25 copies for RNA fusions.

Conclusion: The Predicine FGFR2/3 qPCR Kit offers a rapid, robust, and non-invasive solution for detecting FGFR alterations in urine samples. By providing high sensitivity, specificity, and efficiency, it represents a promising alternative to invasive tissue-based diagnostics for identifying patients eligible for FGFR-targeted therapies such as erdafitinib. These findings highlight its potential utility as a companion diagnostic, with further clinical validation planned to support its integration into routine molecular diagnostics.

#743 A prognostic model based on plasma proteomics for predicting immuno-chemotherapy outcomes in advanced non-small cell lung cancer.

Zhihuang Hu[1], **Jialei Wang**[1], Changbin Zhu[2], Xin Zhang[1], Shenglin Huang[3], Si Sun[1], Zhenhua Wu[1], Yao Zhang[1], Ying Lin[1], Jin Wang[2], Tao Hu[2], Zhan Huang[2], Hui Yu[1], Xinmin Zhao[1], Huijie Wang[1], Xianghua Wu[1]

[1]Department of Medical Oncology, Fudan University Shanghai Cancer Center, Shanghai, China; Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, China; Institute of Thoracic Oncology, Fudan University, Shanghai, China,[2]Department of Medicine, Amoy Diagnostics Co., Ltd., Xiamen, China,[3]Shanghai Key Laboratory of Radiation Oncology, Fudan University Shanghai Cancer Center; Shanghai Key Laboratory of Medical Epigenetics, Institutes of Biomedical Sciences, Fudan University, Shanghai, China

Background: Predicting benefit from immune checkpoint inhibitors in advanced non-small cell lung cancer (aNSCLC) remains challenging. We developed a plasma proteomic model to evaluate immuno-chemotherapy (IC) outcomes.

Methods: Blood samples were collected prior to 1st-line IC for analysis of plasma proteins and peripheral blood mononuclear cells (PBMC) from 194 aNSCLC patients without *EGFR/ALK* mutations. The Olink Immuno-Oncology Panel was used to detect the soluble proteins in plasma, and transcriptome sequencing was conducted on PBMC.

Results: We optimized a prior prognostic model by incorporating a validation cohort and employing the Survival SVM method. This improved model, trained on plasma levels of eight proteins (CD27, TWEAK, CD8A, GZMH, IL6, PGF, TIE2, and TNFRSF12A), independently predicted overall survival (OS). Kaplan-Meier analysis validated significant differences in PFS and OS ($p < 0.001$, respectively). Repeated 10-fold cross-validation in the discovery cohort demonstrated model robustness, with stable predictive power for 12-month OS and PFS (AUCs: 0.86 and 0.68, respectively), which was further supported in the validation cohort (AUCs: 0.78 and 0.63, respectively). The model's independent prognostic value was further substantiated through Cox regression analyses (Table). Our model exceeded the performance of conventional PD-L1 TPS cutoff values of 1% or 50% in stratifying patient risk. Low-risk patients exhibited enriched memory B cells ($p < 0.05$), while high-risk patients showed an upregulation of angiogenesis-related proteins ($p < 0.05$), consistently observed across both cohorts. Validation of the eight proteins is ongoing using ELISA assays to further confirm their clinical utility.

Conclusion: We established and validated an optimized plasma protein-based prognostic model that identifies distinct immune phenotypes and provides critical insights into aNSCLC progression.

PFS and OS by Risk Groups in Discovery and Validation Cohorts

Cohort	Group	PFS/OS	HR (95% CI)	p value	mPFS/OS /(month) (95% CI)	p value
Discovery cohort	High risk vs. Low risk	PFS	4.09(2.28-7.34)	p<0.001	High risk: 5.1 (4.2–9.9) vs. Low risk: 14.8 (12.5–31.9)	p<0.001
		OS	8.33 (4.26-16.28)	p<0.001	High risk: 8 (5.5-13.3) vs. Low risk: 35.3 (28.7-NA)	p<0.001
Validation cohort	High risk vs. Low risk	PFS	2.35 (1.25-4.42)	p=0.008	High risk: 15.1 (7-NA) vs. Low risk: 21.5 (14.9-NA)	p=0.006
		OS	3.16 (1.54-6.47)	p=0.002	High risk: 15.5 (7.4-NA.0) vs. Low risk: NA (NA)	p<0.001
HR, Hazard Ratio; 95% CI, 95% Confidence Interval						

#744 Exploration of potential biomarkers associated with treatment outcomes in locally advanced esophageal squamous cell carcinoma patients receiving neoadjuvant nivolumab plus paclitaxel and cisplatin chemoradiotherapy.

Ta-Chen Huang[1], Jhe-Cyuan Guo[2], Chia-Chi Lin[1], Hung-Yang Kuo[1], Chien-Huai Chuang[2], Jason, Chia-Hsien Cheng[1], Feng-Ming Hsu[1], Jang-Ming Lee[1], Pei-Ming Huang[1], Chih-Hung Hsu[1]

[1]National Taiwan University Hospital, Taipei, Taiwan,[2]National Taiwan University Cancer Center, Taipei, Taiwan

Background: In a phase II trial (neo-NTP-CRT, NCT05130684), the efficacy of adding nivolumab to paclitaxel and cisplatin-based neoadjuvant chemoradiotherapy (CRT) was tested in patients with locally advanced esophageal squamous cell carcinoma (ESCC) (n=17). The study reported a pathological complete response (pCR) rate of 24%, a median relapse-free survival and overall survival (OS) of 8 and 16 months, respectively. We conducted this exploratory analysis to investigate the potential immune-related biomarkers.
Materials and Methods: Pretreatment endoscopy-biopsied tumor tissues were analyzed. Immunohistochemistry (IHC) was used to measure PD-L1 expression by clone SP142 (Ventana, Tucson, AZ, USA) on tumor cells (PD-L1-TC), immune cells (PD-L1-IC), and combined positive score (CPS), and to define mature tertiary lymphoid structure (mTLS) according to CD20 and CD23 expression (n=16). The expression of immune-related genes in ESCC tumor tissues was quantitated by PanCancer Immune Profiling and analyzed by nCounter® Analysis System (nanoString, Seattle, WA, USA) (n=12). 12-chemokine TLS signature was also analyzed. The association of PD-L1-TC, PD-L1-IC, CPS, mTLS, and TLS signature with pCR was examined by Chi-square test; the association with OS was examined by log rank test. The differences of various immune cells and immune-related functions between patients with and without pCR was investigated by t-test.
Results: pCR rate was significantly higher in the patients with positive PD-L1 TC (100% vs 18%, p=0.019), high PD-L1 IC (100% vs 18%, p=0.019), and CPS≥5 (100% vs 10%, p=0.0006), and trended to be higher in patients with mTLS (50% vs 14%, p=0.19) and higher 12-chemokine TLS signature (75% vs 17%, p=0.077). OS trended to be longer in patients with positive PD-L1 TC (median: not reached vs 15 months; HR: 0.24, p=0.05) and CPS≥5 (median: 26 vs 15 months; HR: 0.19, p=0.069), and patients with high PD-L1 IC had numerically longer OS (median: 25 vs 16 months; HR: 0.42, p=0.38). mTLS was not associated with OS (median: 25 vs 25 months; HR: 0.91, p=0.88); however, patients with higher 12-chemokine TLS signature had better OS (median: 25 vs 14 months; HR: 0.23, p=0.042). The analysis of immune-related gene expression revealed patients with pCR had significantly higher abundance of TILs, lower amounts of Treg, exhausted CD8 T cells, and macrophages. Significantly higher B cell function, cytotoxicity function, and Toll-like receptor function was found in tumors of pCR patients.
Conclusion: We found that PD-L1 expression and 12-chemokine TLS signature may be a potential predictor of the response to neoadjuvant nivolumab plus paclitaxel and cisplatin-based CRT. (grants: NCTRC200909, MOST 108-2314-B-002-076-MY3, MOHW 111-TDU-B-221-114006)

#745 Combining circulating inflammatory proteins and thymidine kinase activity to predict survival in melanoma patients receiving immune checkpoint inhibitors.

Suzanne Egyhazi Brage[1], Fernanda Costa Svedman[2], Mattias Bergqvist[3], Hildur Helgadottir[2]

[1]Karolinska Institutet, Stockholm, Sweden,[2]Karolinska University Hospital/Karolinska Institutet, Stockholm, Sweden,[3]Biovica, Uppsala, Sweden

Background: We have demonstrated that increased baseline levels of several inflammatory plasma proteins were associated with poorer clinical outcome in melanoma patients with metastatic cancer treated with immune checkpoint inhibitors (ICIs). Furthermore, the plasma levels and dynamics of thymidine kinase activity (TKa), which is involved in DNA synthesis and cell proliferation, have also been associated with treatment efficacy and patient outcome. The aim of our study was to explore the link between TKa, inflammation, and overall survival (OS) in ICI-treated melanoma patients.
Methods: Fifty-eight patients receiving single anti-PD1 therapy for metastatic disease were treated between 2015 and 2019. There were twenty-one patients with M1a disease, fifteen patients with M1b disease, sixteen patients with M1c disease and six patients with M1d disease. TKa levels were measured with the DiviTum® TKa assay (Biovica) and correlated to 80 inflammation proteins from the Olink® target 96 inflammation platform in pretreatment plasma samples using Qlucore Omics Explorer 3.9.
Results: A cut-off level of TKa at 60 Du/L has previously been determined in metastatic melanoma. Thirty-three out of 80 inflammation proteins were differentially expressed between low and high TKa groups (adjusted p-value<0.05), including IL8, CCL3, HGF and S100A12 which are also associated with shorter OS. We explored if the combination of the five unfavorable biomarkers TKa, IL8, CCL3, HGF and S100A12 could improve risk stratification for survival. When comparing Kaplan-Meier curves we observed a shorter median OS for patients with four-five or two-three markers elevated (10.2 or 18.8 months) compared with patients with one or no marker being elevated (not reached) (log-rank trend test p<0.0001). A pronounced gradual decrease in the proportion of alive patients was associated with an increasing number of unfavorable biomarkers. The five-year OS rates were 83%, 62%, 33% and 11% for the subgroup of patients with no, one, two-three or four-five elevated biomarkers, respectively.
Conclusions: This multi-biomarker panel could serve as a risk stratification tool for melanoma patients to predict survival after anti-PD1 therapy. Our findings demonstrate a complex interplay between cell proliferation and pro-tumorigenic inflammation. Further investigations are warranted to elucidate the underlying biology and if circulating TKa and inflammatory proteins can be utilized jointly as biomarkers for ICI efficacy.

CLINICAL RESEARCH: Spatial Proteomics and Transcriptomics 1
Poster Session

#749 Dynamic change in immune landscape among NSCLC patients who received neo-adjuvant PD-1 treatment.

Zheshen Han[1], Haoting Shi[2], Xue Wang[2], Yufei Wang[3], Ruixin Pan[2], Chao Hu[2], Qing Qu[2], Rong Cai[2]

[1]The University of Hong Kong, Hong Kong, Hong Kong,[2]Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China,[3]Shanghai Jiao Tong University School of Medicine, Shanghai, China

Background: Neoadjuvant immunotherapy in combination with chemotherapy has been approved for resectable lung squamous cell carcinoma (LUSC) patients. However, the dynamic protein changes in the local tumor microenvironment (TME) associated with treatment remains unclear. We sought to spatially profile the treatment-related protein landscape changes in LUSC.
Methods: A total of 11 LUSC patients who received 2 to 4 cycles of neoadjuvant PD-1 inhibitors in combination with chemotherapy (nab-paclitaxel and carboplatin) were recruited. Formalin-fixed paraffin-embedded (FFPE) pre-treatment lung biopsy and paired post-operative surgery samples were collected. To investigate the immune landscape change related to neoadjuvant immunotherapy, we utilized the GeoMx Digital Spatial Profiler (DSP) to spatially profile the expression of >570 proteins in tumor- (pan-CK+), immune- (CD45+), and cancer-associated fibroblast (CAF, a-SMA+) regions in pre- and post-treatment specimens. Differential expression analyses, and GSEA were performed.
Results: We found that active YAP1, BAX, and IRF3 in the tumor region, and CD11a, CXCR3, and CD34 in the immune region were upregulated after treatment, while Syndecan-1, Tuberin, and PAK1 in the immune region and MEK1, CD3G, and VEGFD in the CAF region were downregulated after treatment (all FDR < 0.05). GSEA highlighted that the neoadjuvant immunochemotherapy downregulated differentiation, HIF1, Insulin, and mTOR pathway (NES: -1.70, -1.76, -1.77, and -1.64, respectively) in immune and CAF regions (all adjusted P < 0.05). Findings suggested that neoadjuvant immunochemotherapy may improve oxygenation via the HIF1 pathway, induce metabolic stress and nutrient depletion via the mTOR and insulin pathways, promote dedifferentiation or epithelial-to-mesenchymal transition, as well as reduce immune evasion by modifying the tumor microenvironment. This study provides clues and preliminary evidence to further understand the treatment mechanism of neoadjuvant immunochemotherapy for LUSC.
Conclusion: In this spatial biology study, we found dynamic changes in proteins between pre- and post-neoadjuvant immunochemotherapy among LUSC patients. The dynamic changes revealed some potential impacts of NAT on LUSC, including improving oxygenation, reducing mTOR and insulin activity, and mitigating immune evasion. Understanding these mechanisms can guide the development of combination therapies to improve patient outcome.

#750 Exploring PD1/PDL1 interactions and macrophage-tumor barriers in head and neck cancer: A spatial approach to immunotherapy response.

V. Yaghoubi Naei[1], R. Tubelleza[2], J. Monkman[2], H. Sadeghirad[3], M. L. Donovan[4], T. Blick[2], A. Wicher[5], S. Bodbin[5], S. Basu[5], R. Stad[6], C. Barnett[7], K. O'Byrne[7], R. Ladwa[7], C. Cooper[7], M. Ebrahimi Warkiani[1], B. GM Hughes[8], **A. Kulasinghe**[2];
[1]University of Technology Sydney, Sydney, Australia, [2]The University of Queensland, Brisbane, Australia, [3]The University of Queensland, Brisbane, Brisbane, Australia, [4]Queensland Spatial Biology Centre, Brisbane, Australia, [5]Navinci Diagnostics, Uppsala, Sweden, [6]Akoya Biosciences, California, USA, California, CA, [7]The Princess Alexandra Hospital, Brisbane, Australia, [8]The Royal Brisbane and Women's Hospital, Brisbane, Australia

With nearly a million new cases expected, the global death rate from mucosal head and neck cancer is projected to reach around 50% in 2024. In the U.S., patients with localized tumors have a five-year survival rate of 50-60%. While immune checkpoint inhibitors (ICIs) have shown promise in extending survival, significant challenges remain, particularly the limited effectiveness of PD-1/PD-L1 blockade therapies. Since many studies have shown that PD-L1 protein expression alone may not be a reliable predictor of response to ICI therapy, understanding the spatial context of PD-1/PD-L1 interactions might be crucial for identifying immune evasion mechanisms and predicting responses to ICIs in head and neck cancer. To address this, we aimed to take a more comprehensive approach by mapping PD-1/PD-L1 interactions across n=35 mucosal head and neck squamous cell carcinoma (HNSCC) tissue samples collected before ICI treatment, using a combination of high-plex spatial proteomics and the *in situ* Navinci Proximity Ligation Assay (isPLA). Formalin-fixed, paraffin-embedded (FFPE) tissue samples were stained using multiplex immunofluorescence and the Navinci Diagnostics isPLA assay to detect PD-1/PD-L1 interactions. After incubation with primary anti-PD1 and anti-PD-L1 antibodies, secondary probes tagged with oligonucleotides were applied, enabling PLA to highlight the interactions through a ligation step and an amplification process to enhance the visibility of the protein interactions. Finally, a detection solution was applied to visualize the amplification product at the sites of PD1/PD-L1 interaction.We identified distinct spatial patterns of PD-1/PD-L1 interactions at the tumor-stroma interface, particularly in progressive disease (PD) patients, who exhibited dense layers of isPLA$^+$ macrophages, CD3e T cells, and tumor cells, suggesting an immunosuppressive barrier in this region. In contrast, complete response (CR) patients showed rare or absent barriers, with enriched immune cell aggregates, including B cells and T cells, near the tumor boundary. These findings were corroborated through neighborhood clustering analysis, revealing significant associations between isPLA$^+$ regions and poor prognosis.The spatially resolved nature of our analysis underscores the critical role of PD-1/PD-L1 interactions in shaping the tumor microenvironment (TME) and highlights their potential as predictive biomarkers for ICI therapy efficacy. Unlike methods that detect mere colocalization, isPLA technology enables us to detect true PD-1/PD-L1 interactions, which are more likely to affect immune suppression directly. Our study also emphasizes the need for further exploration of macrophage and T cell subtypes, as well as PD-1/PD-L1 interactions across tumor-stroma boundaries in various cancers to improve immunotherapy strategies.

#751 High-resolution and AI-enabled single-cell spatial transcriptomics and histopathology integrated to reveal tumor differentiation and immune exclusion in skin squamous cell carcinoma.

Patrick Danaher, **Michael Patrick**, Shanshan He, Liang Zhang, Stefan Rogers, Michael Rhodes, Haiyan Zhai, Joseph Beechem

Bruker Spatial Biology, Seattle, WA

Skin squamous cell carcinoma (SCC) is characterized by heterogeneity in differentiation states and immune exclusion within the tumor microenvironment (TME). Using the Bruker Spatial Biology CosMx® Whole Transcriptome (WTX) panel, which profiles approximately 19,000 genes at single-cell resolution, we examined spatial gene expression in FFPE SCC sections. Individual single cell boundaries were defined utilizing a trained AI cell segmentation model. H&E staining on the same tissue provided histopathological context, enabling the integration of molecular and morphological findings. Combining CosMx WTX data with H&E staining, downstream AI models could be used to further predict cell types and transcriptomic signatures directly from H&E images. FFPE SCC samples were subjected to CosMx WTX imaging to generate spatially resolved transcriptomic data. H&E staining, performed after CosMx WTX, was aligned with transcriptomic data using custom software. Histopathological analysis identified keratin pearls, invasive fronts, and stromal compartments. Bioinformatics mapped gene expression signatures of keratinization, immune exclusion, and extracellular matrix remodeling, providing a detailed view of tumor architecture. Thousands of genes were mapped with high resolution, allowing in-depth exploration of the SCC TME. Cell typing identified spatially distinct cancer subpopulations and their relationship with stromal and immune components. Ligand-receptor analysis revealed spatial patterns of cell-cell communication, while proximity analyses highlighted unique immune interactions. Macrophages near cancer cells showed increased transcription of specific genes compared to distant macrophages, suggesting spatially restricted tumor-immune signaling. Integration with H&E staining revealed molecular correlates of keratinization, immune exclusion, and invasive front dynamics, bridging histopathology with spatial transcriptomics. This integration offers the potential to train AI models that predict tumor cell types and molecular profiles directly from H&E images, enabling faster and more accessible analysis. This study integrates single-cell spatial transcriptomics with histopathological features to reveal the molecular mechanisms of tumor differentiation, immune evasion, and stromal remodeling in SCC. The combination of spatial transcriptomics and H&E images holds potential for future AI models that enable non-invasive, real-time tumor profiling, providing a foundation for therapeutic strategies targeting SCC progression and immune suppression.

#752 Hypoxia-driven spatial dynamics of epigenetic and transcriptional cell states in glioblastoma.

Phoebe Lombard, Mark Zaidi, Ronald Wu, Sheila Mansouri, Gelareh Zadeh, Bradly G. Wouters

UHN Princess Margaret Cancer Centre, Toronto, ON, Canada

Glioblastoma (GBM) is an aggressive disease with poor overall survival rates that have not substantially changed in decades. A key feature underlying the lack of therapeutic success in GBM is its inherent morphological and phenotypic diversity. To better understand intra-tumor heterogeneity, single cell sequencing studies have delineated core transcriptional states to characterize GBM cell phenotypes. This study investigates the role of hypoxia in driving the establishment and transformation of GBM cell state. To characterize the spatial organization of cell states relative to hypoxia, spatial transcriptomics (ST) was performed on samples from GBM patients administered pimonidazole prior to surgery as part of a clinical trial. By overlaying serial pimonidazole-stained sections with ST, we constructed a hypoxia gene signature to identify hypoxic regions in ST. Our ST data was integrated with public datasets to create the largest global GBM dataset. In this dataset, we identified novel, ST-specific states that represent recurring cellular phenotypes. We used geographically weighted models to assess hypoxia's spatial colocalization with previously defined cell states and our independently derived states. Finally, we investigated underlying mechanisms by which hypoxia may influence cell state using patient derived glioma stem cells (GSCs) cultured under hypoxic conditions. Using ATAC-seq, ChIP-seq, and bulk RNA-seq, we identified specific genes and chromatin regions differentially regulated under hypoxia. We find that hypoxia demonstrates remarkable spatial correlation with mesenchymal-like cell states, while states reminiscent of neural and glial lineages are notably absent in hypoxic regions. We also identify a leukocyte migratory phenotype strongly spatially correlated with hypoxia, which may indicate immune infiltration in these regions. Epigenetic profiling in GSCs identified specific genomic regions epigenetically regulated by hypoxia. Chromatin regions open under hypoxia with corresponding increased expression are enriched for known hypoxia-associated transcription factors. Conversely, chromatin regions closed and with corresponding decreased expression are enriched for neurodevelopment and differentiation transcription factors. These epigenetically regulated genes are strongly spatially correlated with hypoxic regions in patient ST data and are prognostic as a signature in data from TCGA. Together, our work demonstrates that hypoxia drives remodeling of the transcriptional and epigenetic landscape in GBM. In patients, hypoxia and genes epigenetically regulated under hypoxic conditions associate with specific GBM cell states, suggesting that hypoxia-driven epigenetic remodeling may contribute to these state phenotypes and, consequently, to malignant phenotypes in tumors.

#753 AI-powered deep visual proteomics (DVP) for early pancreatic cancer insights.

Jimin Min[1], Lisa Schweizer[2], Gijs Zonderland[2], Benson Chellakkan Selvanesan[1], Lukas Oldenburg[2], Michael A. Hollingsworth[3], Paul M. Grandgenett[3], Ishani Ummat[2], Maximilian T. Strauss[2], **Andreas Mund**[4], Anirban Maitra[1]

[1]Department of Translational Molecular Pathology, Sheikh Ahmed Center for Pancreatic Cancer Research, MD Anderson Cancer Center, Houston, TX,[2]OmicVision Biosciences, Copenhagen, Denmark,[3]The Eppley Institute for Research in Cancer and Allied Diseases, Fred & Pamela Buffet Cancer Center, University of Nebraska Medical Center, Omaha, NE,[4]Andreas Mund (Individual), Copenhagen, Denmark

Pancreatic ductal adenocarcinoma (PDAC), the most common form of pancreatic cancer, presents a formidable clinical challenge. Patients experience 70-80% post-resection mortality due to recurrence. Identifying aberrant molecular pathways and drivers of pre-cancer progression is an unmet area of importance for developing effective biomarkers and enabling early detection. Pancreatic intraepithelial neoplasia (PanINs) represent the key precursor to PDAC. While genomic and transcriptomic profiling of these lesions has been performed at depth, the deep proteomic profiles of these precursors remains largely unexplored. Deep Visual Proteomics (DVP) combines advanced microscopy, machine learning-based image analysis, automated laser microdissection, and high-sensitivity mass spectrometry to analyze specific cell populations while maintaining their spatial context within tissue samples. Here, we combine DVP with cutting-edge pathology foundation models trained on over 600,000 histopathological slides and data from more than 200,000+ patients, to systematically and precisely characterize key regions of interest (ROIs) in incidental panIN lesions in organ donor samples. We then employ our spatially-resolved mass-spectrometry based proteomics pipeline to compare these pancreatic precursor lesions, including acinar ductal metaplasia and regions of low- and high-grade PanINs, to normal healthy ducts. Hereby we developed the first comprehensive spatial proteomics maps of pancreatic precursor lesions. This AI-powered ROI classification, coupled with our advanced DVP platform, delivers unprecedented molecular insights. Our platform achieves remarkable proteomic depth, identifying ~6,000 proteins from ~100 phenotype-matched cells per sample, revealing stage-specific molecular signatures in PanIN lesions. This first-of-its-kind dataset enables the creation of detailed, phenotype-specific and spatially-resolved proteomic landscapes of PDAC progression. Our innovative approach reveals progressive activation of KRAS signaling across the PanIN-to-PDAC transition, corroborating key drivers of multistep carcinogenesis. Furthermore, we identify several promising biomarkers candidates, including targets that might form the basis for early detection and also provide unprecedented insights into targets for cancer interception. Combining the precision of AI-based pathology with the depth of DVP represents a paradigm shift in the toolkit to study PDAC progression, and generates novel molecular insights in panIN lesions that may enable meaningful intervention in pancreatic cancer.

#754 AI-powered spatial inference platform for qualifying ADCs and multispecific therapeutic targets.

Michael Mints[1], Ettai Markovits[1], Ron Elran[1], Shai Bookstein[1], Yuval Shachaf[1], Elad Amsalem[1], Mor Kenigsbuch[1], Jason W. Reeves[2], Ken Bloom[2], Oscar Puig[2]

[1]Nucleai, Tel Aviv, Israel,[2]Nucleai, Chicago, IL

Over the past two decades, genomic alterations have become important biomarkers for predicting response to targeted therapies; however, the emergence of immunotherapy, antibody-drug conjugates and multispecifics may require a different class of biomarkers that can quantify and localize drug targets within tumor and immune cells. Genomic testing frequently lacks correlation with protein expression, fails to capture membranous versus cytoplasmic localization, and has limited ability to decipher spatial interactions between targets. Multiplex immunofluorescence (mIF) is an established technology capable of quantifying and capturing the subcellular localization of dozens of proteins (>25 proteins per slide), enabling parallel assessment of a large number of drug targets. Here we describe a framework for leveraging high-plex spatial proteomics with an AI-driven platform to develop rational approaches for evaluating individual targets and combinations of targets within and across disease indications. We designed a 30-plex mIF panel to perform sequential immunofluorescence (seqIF™) on the COMET™ platform to profile known tumor antigens and targets (n = 18) currently in development along with the tumor microenvironment (TME), immune cells, and key signaling cascades (n = 12). We tested this panel on 217 samples (>1,700,000 cells) from lung (n = 90), gastric (n = 11), and colorectal (n = 68) cancers, as well as normal tissue specimens (n = 48, 22 sites). Utilizing Nucleai's deep-learning-based multiplex imaging analysis pipeline, we quantified the protein abundance, localization, and interactions among 9 different cell types. Tissue areas were divided into subregions and neighborhoods to characterize localization of these cell types to distinct areas including the tumor core (TC), tumor invasive margin interface (TIM), and stroma. We confirmed known drug targets with historical approvals in each of these indications, which validated that the pipeline was able to correctly identify key targets based on subcellular localization, spatial proximity analysis, and analysis of off-target cell type expression. We also identified potential combination partners for multi-specifics based on target colocalization, which were found to have reductions in off-target cell colocalization, as determined by protein expression in the right compartment and in the expected cell types. Nucleai's platform enables deep spatial proteomic profiling by deriving AI-driven insights for actionable characterization of protein interactions and patient heterogeneity across tissues. We demonstrate that this framework can aid in the prioritization and derisking of potential drug targets used by key classes of therapeutics currently in development.

#755 Identifying the molecular signature of infiltrating edge cells in glioblastoma as drivers of tumor invasion and recurrence.

Alyona Ivanova[1], Shamini Ayyadhury[2], Megan Wu[3], David G. Munoz[4], Trevor J. Pugh[5], Sunit Das[6]

[1]Institute of Medical Science, University of Toronto, Toronto, ON, Canada,[2]The Donnelly Centre, University of Toronto, Toronto, ON, Canada,[3]Brain Tumour Research Centre, The Hospital for Sick Children, Toronto, ON, Canada,[4]St. Michael's Hospital, Toronto, ON, Canada,[5]Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada,[6]St.Michael's Hospital, Toronto, ON, Canada

Background: Glioblastoma (GBM) is the most common malignant brain tumor in adults. Despite extensive research, there haven't been remarkable gains in resolving the seeds of glioblastoma recurrence, and the outcomes for many patients suffering from this devastating disease remain poor. Complete tumor resection in GBM patients is not possible. Residual therapy-resistant cells drive tumor recurrence and infiltrative expansion. Our knowledge on GBM heterogeneity is mostly restricted to the surgically resectable tumor core, while the functional characterization of tumor cells at the infiltrating edge remains largely elusive due to the presence of normal functional brain tissue in the peritumoural lesion. Edge-derived cells exhibit larger capacity for infiltrative expansion and are the main drivers of treatment failure and tumor recurrence, making them action targets for novel treatment approaches.
Methods: To resolve the transcriptional heterogeneity of GBM within the spatial context, we profiled gene expression of tumor regions selected based on histological features ("edge", "core", and "infiltrating zone") obtained from 4 primary and 2 matched pairs of primary/recurrent IDH-WT GBM patients at single-cell resolution with Visium HD.
Results: We show that infiltrative cells are spatially segregated and are characterized by regionally shared distinct transcriptomic signatures which define their cell state and identity. We complement non-spatial leiden clustering approach with BANKSY spatial clustering to augment the features of each cell with both an average of the features of its spatial neighbors along with neighborhood feature gradients. Using pathologically annotated H&E images integrated with spatial gene expression, we identify patterns related to tissue structure and identify transcriptional programs that promote invasiveness and underly disease recurrence in GBM. To further characterize invasive cells at the normal brain-tumor boundary, we identify top spatially variable genes in this cell population using unsupervised cell phenotyping. Upregulated DEGs of tumor edge cells are significantly associated with chemical synaptic transmission, and nervous system development. These modules represent tumor cell hijacking of neuronal programs as described in the context of glioma-neuron synaptic communication and formation of neurite-like tumor microtubes. Upregulation of ion regulation transport at the tumor periphery indicates enhanced neuronal activity and excitability driving infiltrating growth.
Significance: Identifying biomarkers that are specific to malignant edge-derived cells may serve as new diagnostic feature that would help assess treatment response before or within early phases of therapy and allow for individual tailoring of the treatment plan to slow disease progression.

#756 Unveiling the spatial landscape of tumor and stroma heterogeneity in colorectal cancer with spatial transcriptomics and deep learning.

Jie Zhou[1], Todd B. Sheridan[2], Sergii Domanskyi[3], Stephanie L. Cowles[4], Sunghee Park[3], Ilham Putra[4], Olga Anczukow[1], Jeffrey H. Chuang[1], Jill C. Rubinstein[5]

[1]The Jackson Laboratory for Genomic Medicine, Farmington, CT;Department of Genetics and Genome Sciences, Farmington, CT,[2]The Jackson Laboratory for Genomic Medicine, Farmington, CT; Department of Pathology, Hartford hospital, Hartford, CT,[3]The Jackson Laboratory for Genomic Medicine, Farmington, CT,[4]Department of Pathology, Hartford hospital, Hartford, CT,[5]The Jackson Laboratory for Genomic Medicine, Farmington, CT; UCONN School of Medicine, Department of Surgery, Farmington, CT;Hartford HealthCare Cancer Institute at St. Vincent's Medical Center, Bridgeport, CT

Heterogeneity in colorectal cancer (CRC) manifests as inter- and intra-patient diversity in tumor and immune cell populations as well as varied patterns of tumor interaction with surrounding stroma. Spatial transcriptomics (ST) captures gene expression within the native spatial context of tissues, offering a high-resolution approach to dissect cancer molecular landscapes. Prior ST studies in CRC have been constrained by small sample sizes and tissue selection biases, reducing clinical relevance. Here we performed ST (10x Genomics Visium Spatial Gene Expression) on 40 retrospectively-collected CRC samples with available clinicopathological data (stages I-IV: 13, 8, 14, and 5 samples, respectively) and long-term clinical outcomes to define the molecular topography and spatial heterogeneity of tumors with their stromal context. Using ST data, we identified recurring spot-level (55-micron resolution) tumor and stromal sub-populations across the cohort, designating them as T1 (marker genes EPCAM, LGALS4, and FXYD3) and T2 (marker genes KRT8, KRT18, and KRT19) for tumor subtypes, and F1 (marker genes SPARC and LUM) and F2 (marker genes COL3A1 and PTGDS) for stromal subtypes. Comprehensive analysis of tumor/stromal pairs—including copy number variations (CNVs), cell type compositions, pathway activations, metabolic profiles, spatial distributions, and histological characteristics—revealed pathways enriched in T2 compared to T1, including epithelial-mesenchymal transition (EMT), angiogenesis, hypoxia response, TGF-beta signaling, and metabolic pathways supporting the energy and biosynthetic demands of aggressive tumor cells. We also identified recurrent spatially co-localized tumor and stromal sub-clonal pairs—T1&F2 and T2&F1—that consistently preserved their spatial architecture across samples. Notably, the prevalence of T1&F2 was significantly correlated with disease-specific survival (DSS) (log-rank test, p=0.05), highlighting the prognostic importance of tumor-microenvironment interactions. Using a transformer-based image feature encoder, we demonstrated that these clusters are morphologically distinguishable in hematoxylin and eosin (H&E)-stained images co-registered with the ST data, achieving an AUC (the Area Under the ROC Curve) of 0.84 for distinguishing T1 from T2 and an AUC of 0.75 for distinguishing F1 from F2. These clusters were also detected in H&E images from external datasets, including The Cancer Genome Atlas (TCGA, N=232) and an independent validation set (N=107). Deep learning models trained on TCGA data further revealed a trend where higher T2 prevalence or lower T1 prevalence correlated with shorter overall survival. This ST study establishes novel molecular subtypes and their spatial co-occurrence in CRC, linking spatial behaviors to clinically prognostic and H&E-based morphological characteristics.

#757 Decoding resistance: Spatial transcriptomics reveals tumor core and edge dynamics in high-risk cutaneous squamous cell carcinoma treated with neoadjuvant cemiplimab.

Ali Baghian[1], Hugo Lara-Martinez[2], Rania Bassiouni[3], Gino In[4], Homa Dadrastoussi[4], Paniz Zarand[4], Harley Wu[4], Kaijin Wu[4], Brittney K. DeClerck[4], Ashley Gochoco[1], Kevin Kelly[4]

[1]Beth Israel Deaconess Medical Center, Boston, MA,[2]MD Anderson Cancer Center, Houston, TX,[3]City of Hope, Duarte, CA,[4]Keck School of Medicine of USC, Los Angeles, CA

Introduction: Immune checkpoint inhibitor (ICI) therapies have redefined the treatment landscape of advanced cutaneous squamous cell carcinoma (CSCC). NeoPOWER is an ongoing, single-arm, phase II clinical trial studying the role of preoperative cemiplimab in patients with high risk/unresectable CSCC. Based on recent data, patients with CSCC who attain a pathologic complete response with neoadjuvant ICI have a 12-month disease free survival of 92%. Response rates to ICI; however, are approximately 50-60% and CSCC refractory to ICI is associated with an inferior survival, highlighting a need to stratify patients who are most likely to benefit from neoadjuvant cemiplimab. We employ Visium (v2, 10x genomics) to comprehensively analyze the CSCC tumor immune microenvironment of patients with divergent responses to immunotherapy.

Methods: We performed Visium spatial transcriptomics (ST) profiling on formalin fixed paraffin embedded biopsy samples from patients with high risk or unresectable CSCC treated with neoadjuvant cemiplimab. Nine patients were included and divided into two groups as defined by presence of residual disease (RD) or no residual disease (NRD). An integrated RD and NRD pre-treatment (pre-iRD/NRD) analysis (n = 8 samples) was performed to identify factors predictive of treatment response. Separately, an integrated RD pre-treatment and post treatment (iRD) analysis was explored to assess for mechanisms of treatment resistance (n = 7 samples). Gene expression data generated by Visium was imported and analyzed in Seurat.

Results: We observed that 2 distinct clusters identified as CSCC, histologically and transcriptionally, formed a substructure comprised of an outer and inner layer tumor edge (TE) and tumor core (TC), respectively. This association of spatial adjacency was visually evident in both pre-iRD/NRD and iRD groups. Comparative gene set enrichment analysis between pre-iRD/NRD showed alterations in KRAS signaling pathways and down-regulation of multiple immune response pathways, implying fundamental differences in the TC of both response groups. In contrast, comparisons of the iRD TC showed post-treatment enrichment of epithelial mesenchymal transition, hypoxia, and metabolic pathways. Post treatment TE was similarly enriched in EMT and hypoxia with down-regulation of immune response pathways. Alterations in immune cell proportions post-treatment were notable in the iRD group following ICI, as were differences in baseline B and T cell proportions in pre-iRD/NRD tumor clusters. Ligand receptor analysis highlighted key immune-inhibitory interactions within the TE and TC.

Conclusions: ST analysis of the pre- and post-treatment CSCC microenvironment captures tumor edge/core dynamics associated with different response outcomes.

#758 Spatial profiling of matched primary and recurrent glioblastoma samples reveals changes in cellular states and the immune microenvironment.

Sara Artzi[1], Marc N. Klausen[1], Dylan S. L. Harwood[1], Signe R. Michaelsen[1], Simone B. Maarup[2], Alessio Locallo[3], Nicolai S. Bager[1], Nadine M. Hammouda[1], Benedikte Hasselbalch[2], Ulrik Lassen[2], Joachim Weischenfeldt[3], Bjarne W. Kristensen[1]

[1]Department of Pathology, Rigshospitalet, Copenhagen University Hospital, Biotech Research and Innovation Centre (BRIC), University of Copenhagen, Copenhagen, Denmark,[2]Department of Oncology, Rigshospitalet, Copenhagen University Hospital, Copenhagen University Hospital, Copenhagen, Denmark,[3]The Finsen Laboratory, Rigshospitalet, Copenhagen, Biotech Research and Innovation Centre (BRIC), University of Copenhagen, Copenhagen, Denmark

Background: Glioblastoma (GBM), the most aggressive brain tumor, is treated with surgery, chemotherapy, and radiation, yet 90% recur within two years. PD-1 blockade improves outcomes in other cancers, but shows limited effect in GBM, likely due to tumor heterogeneity and an immunosuppressive microenvironment. This study aims to investigate post-treatment transcriptional changes in tumor cells and tumor-associated macrophages (TAMs) in matched primary and recurrent GBM, focusing on neoadjuvant PD-1 blockade.

Methods: We used the GeoMx Digital Spatial Profiler to spatially select and transcriptomically profile tumor cells ($SOX2^+$) and TAMs ($IBA1^+$). Gene expression changes between primary and recurrent GBMs were analyzed in 30 patients (26 IDHwt, 4 IDHm) with 20 paired primary samples. All patients received standard treatment, and 20 also received second-line nivolumab one week before recurrence surgery. Tumor purity was confirmed using InferCNV and cell signatures. Subsequently, differential expression and pathway enrichment analyses were performed to compare recurrent tumors treated with nivolumab to those untreated, as well as primary vs. recurrent tumors. We also evaluated Neftel signatures in matched tumors to assess treatment- and recurrence-associated malignant state changes.

Results: Principal component analysis of all samples revealed distinct transcriptional profiles between tumor cells and TAMs. Separate analyses showed clear differences in tumor cell and TAM segments based on tumor status (primary/recurrent), though no separation related to nivolumab treatment. Similarly, comparing nivolumab-treated recurrent tumors with untreated controls showed no significant changes in malignant states or gene expression in tumor cells or TAMs, consistent with the lack of clinical survival benefit.To investigate overall transcriptional changes induced by standard treatment, differential expression analysis comparing tumor cell segments in paired primary and recurrent tumors revealed few significant genes, likely reflecting high inter-patient variability driven by tumor heterogeneity. Unsupervised clustering of tumor cell segments identified a distinct cluster specific to recurrent tumors, with DEGs highlighting pathways linked to this particular tumor cell phenotype. In TAM segments, increased expression of checkpoint-related genes in recurrent tumors suggests TAM phenotype changes that may influence immune dynamics in the microenvironment.

Conclusion: Spatial profiling of matched primary and recurrent GBM samples reveals distinct transcriptional changes in both tumor cells and TAMs, reflecting the complex microenvironment of recurrent GBM. However, our findings point to a limited impact of neoadjuvant PD-1 blockade on transcriptional activity, highlighting ongoing challenges in targeting GBM with immunotherapy.

#759 Spatial transcriptomics identifies unique tumor and microenvironment pathomic programs that are associated with the lung premalignancy and adenocarcinoma continuum.

Yibo Dai[1], Fuduan Peng[1], Ansam Sinjab[1], Sujuan Yang[1], Minyue Chen[1], Warapen Treekitkarnmongkol[1], Lorena I. Gomez Bolanos[1], Tieling Zhou[1], Alejandra G. Serrano[1], Jianlong Liao[1], Guangsheng Pei[1], Yunhe Liu[1], Yang Liu[1], Jiahui Jiang[1], Kyung Serk Cho[1], Yanshuo Chu[1], Kai Yu[1], Ruiping Wang[1], Jiping Feng[1], Zahraa Rahal[1], Guangchun Han[1], Naoe Jimbo[2], Takuo Hayashi[3], Satsuki Kishikawa[3], Kazuya Takamochi[3], Akshay Basi[1], Avrum Spira[4], Steven Dubinett[5], Tomokazu Itoh[2], Takashi Yao[3], Kenji Suzuki[3], Luisa M. Solis[1], Stephen Swisher[1], Mingyao Li[6], Junya Fujimoto[7], Ignacio I. Wistuba[1], Jared Burks[1], Kadir Akdemir[1], Hind Refai[1], Katy Rezvani[1], Jeffrey Myers[1], Humam Kadara[1], Linghua Wang[1]

[1]The University of Texas MD Anderson Cancer Center, Houston, TX,[2]Kobe University Hospital, Kobe, Japan,[3]Juntendo University Graduate School of Medicine, Tokyo, Japan,[4]Boston University, Boston, MA,[5]The University of California Los Angeles, Los Angeles, CA,[6]University of Pennsylvania, Philadelphia, PA,[7]Hiroshima University Hospital, Hiroshima, Japan

Background: Lung adenocarcinoma (LUAD) is one of the most prevalent and lethal cancer types worldwide. However, our understanding of the transition from normal-appearing tissue (NAT) to adenomatous lung premalignant lesions (aPMLs) and LUADs, particularly within a spatial context, remains limited. This study aims to address this gap by systemically analyzing the pathologic continuum of NAT > aPML > LUAD using spatial transcriptomics (ST).

Methods: High-resolution spatial profiling was conducted on 56 samples from 25 patients with paired aPMLs and LUADs using the Visium ST platform. Non-negative matrix factorization was conducted to identify transcriptional programs for each sample, following clustering analysis to define consensus metaprograms across the cohort. Additionally, spatial molecular imaging was performed on an expanded cohort of 188 cores arranged into eight tissue microarrays using the Xenium in situ platform with a customized lung cancer gene panel to establish a single-cell spatial atlas of the disease continuum and systemically investigate spatial organization, cellular neighborhoods and interactions.

Results: Eight distinct metaprograms (MP1~8) were identified, distinguishing stromal (MP2), myeloid (MP4), lymphoid (MP6), and epithelial (MP3, MP5) compartments. Additionally, metaprograms were identified for the lung capillary bed (MP7), stressed cellular state (MP8), and mosaic cellular patterns (MP1). These metaprograms correlated strongly with pathological annotations and captured fine tissue structures like lymphoid aggregates. LUADs and aPMLs showed distinct MP profiles, with MP3 and MP6 being highly abundant in LUADs, while MP1, MP4, MP5 and MP7 were more abundant in aPMLs. Specifically, we observed positive correlations between MP3 and MP6, as well as between MP4 and MP5 in sample distributions, indicating co-evolution of tumor and immune microenvironments during disease progression. Xenium data complemented these findings by revealing distinct compositions of microenvironmental cellular states and tumor neighborhood structures across aPML and LUAD lesions. A total of 44 cell states were defined. Specifically, NK cells were depleted in aPMLs and LUADs compared with NAT, accompanied by an enrichment of regulatory T cells and myofibroblastic cancer-associated fibroblasts (CAFs) in the latter. Additionally, pro-inflammatory cellular subsets, including *IL1B*$^{+}$ macrophages and *IL6*$^{+}$ inflammatory CAFs were more abundant in tumor neighborhoods of aPMLs compared to LUADs, potentially contributing to the pre-malignant to malignant transition.

Conclusions: This study offers a comprehensive molecular and cellular landscape of aPML and LUAD, laying a crucial foundation for future mechanistic studies aimed at early disease interception.

#760 Cell type resolved spatial proteomics defines the transition of precancerous fallopian tube lesions to invasive ovarian cancer.

Andreas Metousis[1], Hilary Kenny[2], Lisa Schweizer[1], Agnes Bilecz[2], Rahul Krishnan[3], Isabel Alcazar[2], Mallika Ravi[2], Aasa Shimizu[2], Sophia Steigerwald[1], Thierry Nordmann[1], Ricardo Lastra[4], Ernst Lengyel[2], Matthias Mann[1]

[1]Proteomics and signal transduction, Max Planck Institute of Biochemistry, Munich, Germany,[2]Obstetrics and Gynecology, University of Chicago, Chicago, IL,[3]Northwestern Medical Group, Orland Park, IL,[4]Pathology, University of Chicago, Chicago, IL

Serous epithelial ovarian cancer is the most common subtype of ovarian cancer, and it is thought to arise from dysplastic fallopian tube (FT) lesions. Depending on their histopathological characteristics and proliferative potential, precancerous lesions of the FT are categorized as p53 signatures, Serous Tubal Intraepithelial Lesions (STILs) or Serous Tubal Intraepithelial Carcinomas (STICs). In this study, we analyzed protein changes from precancerous lesions through invasive tumor development and metastasis to identify early molecular switches and druggable proteins for ovarian cancer treatment.

We assembled a cohort of 5 p53 signatures, 8 STILs, 5 STICs alone, and 7 STICs with concurrent HGSC and 4 matching omental metastases. We interrogated these samples using Deep Visual Proteomics, an innovative technology we recently developed for spatial and cell-type resolved mass spectrometry-based proteomics. Epithelial cells were segmented using deep learning and classified into normal, dysplastic precursor, tumor, and metastasis. We collected 100-150 cell epithelial cell shapes for each pathologic category and 250 cell shape equivalents of adjacent stroma using laser capture microdissection. The samples were analyzed on a Thermo Orbitrap Astral mass spectrometer coupled to an Evosep One.

Single injections of epithelial samples yielded a median of 6,562 proteins. The epithelial proteomics data validated that the secretory, and not the ciliated, epithelial FT cells are the origin of dysplastic FT lesions. While p53 signatures have at least 12 consecutively mutated cells lining the FT, they are almost identical to normal cells on the proteomic level. However, more than 2,000 proteins are differentially regulated in STIC vs normal secretory cells. Among these proteins, MARCKSL1 (cell motility and adhesion), WLS (Wnt signaling and cell proliferation), and PCNA (DNA repair and replication) are significantly enriched in STIC.

In the stroma, we detected a median of 5,072 proteins. Interestingly, the PCA plot for components 1 and 2 reveals that separating STICs, invasive tumors, and omental metastases is better accomplished using the stroma than the epithelium. Consistent with the epithelial data, the transition from normal to the histologic p53 signature stroma is not accompanied by any proteomic changes in our dataset. However, STIC stroma, when compared to normal, is characterized by a significant loss of most collagens but not COL8, which shows the opposite trend. A massive extracellular matrix (ECM) remodeling is observed in the entire STIC tumor microenvironment, with significant regulation of ECM proteins like VCAN and laminins.

In conclusion, our study provides a deep, cell-type resolved proteomics map of the transition of early FT lesions to invasive cancer and omental metastasis and identifies proteins with potential therapeutic or diagnostic implications.

#761 Shared and heterogeneous structural and transcriptional signatures of pre-cancerous cystic lesions in the development of PDAC.

Austin N. Southard-Smith, Reyka G. Jayasinghe, Andre L. N. Targino da Costa, Liang-I Kang, John M. Herndon, Jacqueline Mudd, Andrew Houston, Xiang Li, Michael C. Wendl, Faria A. Simin, Xiangwei Fang, Feng Chen, Koushik K. Das, Ryan C. Fields, Li Ding

Washington University School of Medicine in St. Louis, St. Louis, MO

Pancreatic ductal adenocarcinoma PDAC has been observed to arise in the context of diverse pre-cancerous cystic (intraductal papillary mucinous neoplasms (IPMN) and mucinous cystic neoplasms (MCNs)) and pre-cystic (pancreatic intraepithelial neoplasia (PanIN)) lesions in the clinic. While mouse models of PDAC and these precancerous lesions have existed for a decade, only recently has the transcriptional signature of dysplastic progression for one of these lesions (PanIN) been thoroughly characterized at single cell resolution. Utilizing a multimodal, spatial interrogation via Xenium and co-detection by indexing (CODEX) of PanIN (n=5), IPMNs (n=5), MCNs (n=2), and PDAC (n=5) at varying stages, we identify transcriptional signatures of each lesion at varying stages of progression and alterations of the pre-cancer microenvironment composition and structure. In doing so, we observed a conserved gastric metaplasia phenotype along the progression from PanIN to IPMN that is maintained in a subset of cells in higher grade and dual-subtype IPMNs. This phenotype is diminished in invasive PDAC. By contrast, MCN cyst epithelium and a small subset of PanIN cells lacked the expression of gastric markers and exhibited a more reactive ductal phenotype. Directly, adjacent to each of the pre-cancerous lesions we observed a thin layer of myCAF-like fibroblasts frequently interspersed with T cells that were otherwise excluded from the region surrounding invasive PDAC while the myCAF layer expanded. In the case of MCNs specifically this thin myCAF-like layer coincided with an ovarian-like expression signature alongside other markers. Our findings offer a high resolution window into the heterogeneity of PDAC precursor lesions, highlighting shared dysplastic expression and structural phenotypes along the progression towards invasive PDAC.

#762 Spatial transcriptomic characterization of uterine carcinosarcomas identifies MECOM as a tumor regulator.

Sanaa N. Borrego, Elizabeth Kertowidjojo, Yan Li, Agnes Bilecz, Bridget Rodig, Lucy Kelliher, Hilary Kenny, Ernst Lengyel, **Katherine C. Kurnit**

University of Chicago, Chicago, IL

Uterine carcinosarcoma is an aggressive subtype of endometrial cancer associated with poor patient outcomes. Less is known about the molecular features of this heterogeneous cancer, which is characterized by both epithelial and mesenchymal components. Our study aimed to use spatial transcriptomics to provide insights into its tumor biology. Spatial transcriptomics was performed using the NanoString DSP's GeoMx 1,800 Cancer Transcriptome Atlas. We evaluated 7 primary tumors, 5 of which had paired lymph node metastases. Planned comparisons were as follows: epithelial vs mesenchymal; epithelial vs stromal; mesenchymal vs stromal; primary vs metastatic tumor. In primary tumor, there was differential expression of cell-cell adhesion genes and keratins (both higher in epithelial), and epithelial-mesenchymal transition (EMT) genes and collagens (both higher in mesenchymal). The stroma had multiple immune genes upregulated, including genes relating to macrophage function, cytokine binding, and complement activation. To further investigate the immune findings, immunohistochemistry (IHC) from 4 cases showed inconsistent immune cell differences between the carcinoma and sarcoma compartments. In 3 of the 4 cases, CD3 (T cell) cells were higher in the carcinoma than in the sarcoma, and 2 of the 4 cases showed higher CD206 (M2 macrophage) in the sarcoma than the carcinoma compartments. Few differences in gene expression were noted between the primary and metastatic tumors. MECOM and SOX17, two stem cell genes found to be associated with more aggressive ovarian cancers, were both upregulated in the epithelial compartment relative to the mesenchymal or stromal compartments on transcriptomics. To further elucidate MECOM's role in uterine carcinosarcoma, we used in situ hybridization and confirmed differential MECOM RNA expression in the tumor compartments of two different uterine carcinosarcoma cases. This was paralleled by higher protein expression in the epithelial relative to the mesenchymal tumor compartments in 12 cases (IHC). Then, MECOM was knocked down in two uterine carcinosarcoma cell lines. While no differences in proliferation were noted, blocking MECOM led to lower expression of EMT-related proteins. These data suggest that MECOM may be linked to EMT characteristics within uterine carcinosarcoma. The immune characterization of uterine carcinosarcoma is likely complex given the inherent heterogeneity of these tumors, and should be further evaluated.

#763 Multimodal spatial-omics atlas of lung premalignancy and adenocarcinoma reveals tumor initiating interplay between alveolar progenitors and inflammation.

Fuduan Peng[1], Ansam Sinjab[1], Sujuan Yang[1], Yibo Dai[1], Minyue Chen[1], Warapen Treekitkarnmongkol[1], Tieling Zhou[1], Alejandra G. Serrano[1], Jianlong Liao[1], GuangSheng Pei[1], Yunhe Liu[1], Yang Liu[1], Jiahui Jiang[1], Kyung Serk Cho[1], Yanshuo Chu[1], Kai Yu[1], Ruiping Wang[1], Jiping Feng[1], Zahraa Rahal[1], Lorena I. Gomez Bolanos[1], Guangchun Han[1], Naoe Jimbo[2], Takuo Hayashi[3], Satsuki Kishikawa[3], Kazuya Takamochi[3], Akshay Basi[1], Avrum Spira[4], Steven Dubinett[5], Tomokazu Itoh[2], Takashi Yao[3], Kenji Suzuki[3], Luisa M. Solis[1], Stephen Swisher[1], Mingyao Li[6], Junya Fujimoto[7], Ignacio I. Wistuba[1], Jared Burks[1], Kadir Akdemir[1], Jeffrey Myers[1], Linghua Wang[1], Humam Kadara[1]

[1]UT MD Anderson Cancer Center, Houston, TX,[2]Kobe University hospital, Kobe, Japan,[3]Juntendo University Graduate School of Medicine, Tokyo, Japan,[4]Boston University, Boston, MA,[5]University of Califormia Los Angeles, Los Angeles, CA,[6]University of Pennsylvania, Philadelphia, PA,[7]Hiroshima University Hospital, Hiroshima, Japan

Background: The events mediating the transition of normal-appearing tissue (NAT) to adenomatous premalignant lesions (aPMLs) and minimally or locally invasive lung adenocarcinoma (LUAD) are very poorly narrated.

Methods: We analyzed matching NATs, aPMLs, and LUADs from 25 patients using integrative approaches including spatial transcriptomics (ST, n=56 tissues), single-cell RNA-sequencing of fixed cells (scFFPE-seq) from matched uninvolved NAT, aPMLs and LUADs (n = 75 tissues), and whole exome sequencing (WES, n=79). A subset of tissues was also analyzed by sequential immunofluorescence (seqIF, COMET) and subcellular spatial gene expression (Xenium, n=41).

Results: We performed multimodal spatial omics analysis of lung tissues along the NAT-aPML-LUAD continuum. After quality control, we retained and analyzed 477,283 spots by ST, 401,636 cells by scFFPE-seq, and more than 1.75 million cells by Xenium. Paired aPMLs and LUADs showed distinct spatial transcriptional patterns, lineage plasticity, and cell states. We derived nine metaprograms using scFFPE-seq data of epithelial cells, which showed robust alignment with sub-cell type and states from the ST data. Integrative analysis of ST and scFFPE-seq demarked metaprograms that denote alveolar type 1 (AT1) and type 2 (AT2), alveolar intermediate cell (AIC), and tumor cells in the cellular path to aPML and LUAD. Both LUAD and aPML regions highly expressed signatures of KRT8+ AICs (KACs), which denoted transitionary states between NATs and aPMLs or LUADs. Trajectory analysis demonstrated two alveolar transition paths, namely AT2-AIC-AT1 and AT2-KAC-lung tumors. In contrast to LUADs, early aPMLs and KAC-rich NAT expressed proinflammatory metaprograms, suggesting that interplay between KAC and inflammation is contextual and likely involved in tumor initiation in the peripheral lung. Spatial single-cell neighborhood analysis by Xenium platform concordantly showed that KACs were proximal to proinflammatory macrophages and cancer-associated fibroblasts (CAFs). Specifically, IL-1B+ proinflammatory macrophages and inflammatory CAFs were observed to be enriched in the neighborhood of tumor cells in aPMLs. Multimodal analysis of a tobacco lung carcinogenesis mouse model showed that KACs displayed increased NF-kB signaling and expression of the *Il1b* receptor *Il1r1*. Importantly, treatment with IL-1b or macrophage co-culture enhanced growth of KAC-enriched organoids, and IL-1b neutralization *in vivo* led to KAC regression.

Conclusions: We generated a multimodal spatial and single-cell atlas of the transition of normal tissue to aPML and LUAD. This comprehensive atlas uncovered critical interactions between tumor progenitors and inflammation that inspire initiation of lung lesions and inform of unique and targetable cellular states for early detection and interception of LUAD.

#764 Molecular diagnosis using spatial analysis of lung tumors with a history of pancreatic cancer.

Kosei Ishimura[1], Kazuhiko Shien[2], Shuta Tomida[3], Hidetaka Yamamoto[4], Hirofumi Inoue[5], Kazuya Hisamitsu[1], Ryota Fujiwara[1], Atsushi Matsuoka[1], Ryunosuke Fujii[1], Ryo Yoshichika[1], Tomohiro Higashihara[1], Naohiro Hayashi[1], Yuma Fukumoto[1], Kazuhiro Okada[1], Fumiaki Mukohara[1], Mao Yoshikawa[1], Ken Suzawa[2], Shinichi Toyooka[2]

[1]Thoracic, Breast and Endocrinological Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan,[2]Thoracic, Breast and Endocrinological Surgery, Okayama University Hospital, Okayama, Japan,[3]Center for Comprehensive Genomic Medicine, Okayama University Hospital, Okayama, Japan,[4]Pathology (Tumor Pathology), Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan,[5]Okayama University Hospital, Okayama, Japan

Background: In pancreatic cancer (PC) patients, the lung is the third most common site of recurrence after the liver and peritoneum. Accurate histological diagnosis of lung tumors is essential for determining treatment strategies. However, pancreatic cancer lung metastases (PCLM) and invasive mucinous adenocarcinoma (IMA), a subtype of primary lung adenocarcinoma, share histopathological features, such as mucin-rich tall columnar epithelial cells growing in glandular or papillary structures with a lepidic growth pattern. Even with immunohistochemical markers like TTF-1 or CK20, differentiation remains challenging. This study aimed to identify molecular markers unique to PCLM and IMA using spatial multi-omics analysis.
Materials and Methods: We performed spatially resolved transcriptomics/proteomics on surgically resected lung tumor specimens obtained from patients with a history of PC. A total of nine patients were included: five diagnosed as possibly PCLM based on clinicopathological features and four with tumors difficult to distinguish between PCLM and primary lung IMA. Regions of interest (ROIs) were selected in each tumor component and the tumor microenvironment (TME). Gene and protein expression profiles were comprehensively analyzed using the GeoMx digital spatial profiler in each ROI. Additionally, primary PC specimens were analyzed in five cases where there were available, and three patients of primary lung IMA without a history of PC were included as controls.
Results: The median age of the nine patients with PC were 67 years (range: 54-80 years), with four males and five females. Histologically, all patients exhibited tall columnar epithelial cells with glandular or papillary structures. Among them, five patients had limited or sparse mucin, leading to a diagnosis of possibly PCLM, while the remaining four were challenging to distinguish. Spatial multi-omics analyses stratified the nine patients into subtypes, revealing distinct molecular signatures. Comparison with IMA-specific signatures identified molecular markers to aid in differentiating PCLM from primary lung IMA. Additionally, differences in gene/protein expression profiles, including within the TME, were observed between primary pancreatic tumors and lung metastases.
Conclusion: This study identified molecular markers that could distinguish PCLM from primary lung IMA despite their similar imaging and histological features. These findings suggest that spatial multi-omics analysis could be a valuable tool for improving the differential diagnosis of PCLM and IMA.

#765 Spatial transcriptomic profiling of normal breast tissue reveals potential mechanisms underlying increased breast cancer risk in Black women.

Camila Pacheco Erak[1], Nicole Cruz-Reyes[1], Pria Westerman[1], Joshua Ogony[1], Jean R. Clemenceau[2], Tae Hyun Hwang[2], Sarah McLaughlin[3], Derek Radisky[1]

[1]Department of Cancer Biology, Mayo Clinic, Jacksonville, FL,[2]Department of Artificial Intelligence and Informatics, Mayo Clinic, Jacksonville, FL,[3]Department of Surgery, Mayo Clinic, Jacksonville, FL

Purpose: Black women are disproportionately affected by aggressive breast cancers compared to white women, suggesting underlying biological differences in normal breast tissue. This study aims to identify molecular disparities in normal breast tissue between premenopausal Black and white women that may contribute to increased breast cancer risk.
Experimental Procedures: Visium spatial transcriptomic analysis was performed on a pilot cohort of normal breast tissue samples from premenopausal Black and white women. Tissue sections were spatially profiled, and capture spots were classified based on predominant cell types: epithelial, stromal, or endothelial. Differential gene expression analysis between racial groups was conducted for each cell type classification. Multiplex immunofluorescence (mIF) was used to validate selected markers in the pilot cohort. Meta-analysis compared differentially expressed genes with published gene sets associated with breast cancer subtypes.
Results: Significant racial differences in gene expression profiles were identified in both epithelial and stromal spots. In epithelial spots, genes upregulated in Black women significantly overlapped with over 20 published gene sets associated with estrogen receptor-negative (ER–) breast cancer, including key genes involved in Wnt signaling inhibition (e.g., WIF1) and basal epithelial markers (e.g., KRT14, KRT15). This suggests alterations in pathways linked to more aggressive breast cancer phenotypes. In stromal spots, differentially expressed genes related to extracellular matrix remodeling and fibroblast activation (e.g., PI16, DPT, EFEMP1) were observed, indicating potential differences in stromal-epithelial interactions. Ongoing deconvolution and spatial analyses are being performed to further elucidate these findings. Preliminary mIF validation confirmed increased expression of PI16 in samples from Black women. Validation of additional markers is underway in a larger cohort of premenopausal Black and white women.
Conclusions: Spatial transcriptomic profiling of normal breast tissue reveals distinct molecular differences between Black and white women, particularly in pathways associated with aggressive breast cancer subtypes and stromal interactions. These findings suggest that inherent variations in the normal breast microenvironment may contribute to the increased risk and aggressiveness of breast cancer in Black women. Further validation and analysis may identify potential biomarkers for risk assessment and inform targeted prevention strategies.

#766 Spatial evaluation of tumor sialylation of N-linked glycans in prostate cancer tissues..

Kameisha Rashford[1], Caroline Kittrell[1], Bettina F. Drake[2], Ariana Ahmed[2], Aleksandra Klim[3], Eric Kim[3], Peggi Angel[1], Joseph E. Ippolito[4], Richard Drake[1]

[1]Immunology and Pharmacology, The Medical University of South Carolina (MUSC), Charleston, SC,[2]Institute of Public Health, Washington University, St.Louis, MO,[3]Division of Urology, Washington University, St.Louis, MO,[4]Department of Radiology, Washington University, St.Louis, MO

Aberrant sialylation is a common feature of prostate cancer. Sialylation is part of the co-translational glycosylation machinery, where sialic acids are enzymatically added to glycoproteins as a quality control mechanism to ensure proper protein folding and stability. However, in solid tumors like prostate cancer, this process becomes dysregulated, resulting in hypersialylation. Tumor cells exploit this alteration to mask their identity, evade immune detection, and promote aggressive tumor growth. In humans, α2,3 and α2,6 isomers of N-acetylneuraminic acid are the most abundant species detected in sialylated N-glycans. The presence of α2,6 sialylated glycans is a well-established marker for PCa aggressiveness, while α2,3 sialylation is a known feature of sialyl Lewis x/a tumor antigens. Distinguishing between these isomers is crucial due to their distinct chemical and biological properties, which can offer deeper insights into disease pathology, thus we used N-glycan mass spectrometry imaging to preserve histology and spatial distribution of both sialic acid isomeric species. In this study, we evaluated N-linked sialylated glycans in a large cohort of 381 primary prostatectomy samples in tissue microarray format. A double amidation chemical treatment to differentially add mass tags specific to each isomer was done, followed by matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI). This platform enabled high-resolution spatial analysis of α2,3 and α2,6 sialylated N-glycan isomers in each tumor core. Initial data grouping compared the intensities of 80 sialylated N-glycan isomers across Gleason scores G6 (n = 134), G7[3+4] (n = 181), and G7[4+3] (n = 66). Significant differences in the levels of specific N-glycans were determined between these groups, including upregulation of α2,3-linked tetra-antennary fucosylated glycans, downregulation of bi-antennary α2,3- and α2,6-linked species, and fluctuations in tri- and tetra-antennary glycans of both isomers, suggesting glycan remodeling with tumor advancement. Additional clinical data assessments are ongoing to evaluate sialylation differences associated with tumor stage, race, BMI, age, and other parameters. The combination of double amidation isomer tagging and MALDI-MSI provides a promising advancement in the differentiation and characterization of previously difficult-to-identify sialic acid isomers, enhancing potential applications in prostate cancer diagnostics.

#767 Discovering the geospatial framework in glioblastoma: Uncovering hotspots for glioma stem cell hide-out and their therapeutic implications.

Shamini Ayyadhury[1], Fatemah Al Solaiman[2], Yuna Lee[1], Alyona Ivanova[3], Ana Nikolic[4], Farzaneh Aboulizadeh[5], Melanie Peralta[6], Patty Sachamitr[7], Michelle M. Kushida[7], Nicole I. Park[7], Fiona J. Coutinho[7], Owen Whitley[1], Panagiotis Prinos[8], Cheryl H. Arrowsmith[8], Sam Weiss[4], Sheila Mansouri[9], Gelareh Zadeh[9], Peter B. Dirks[7], Troy Ketela[5], H. Artee Luchman[4], Gary D. Bader[1], Trevor J. Pugh[9]

[1]University of Toronto, Toronto, ON, Canada,[2]Qatar University, Qatar, Saudi Arabia,[3]The Hospital for Sick Children, Toronto, ON, Canada,[4]University of Calgary, Calgary, AB, Canada,[5]Princess Margaret Genomic Center, University Health Network, Toronto, ON, Canada,[6]Mount Sinai Hospital, Sinai Health, Toronto, ON, Canada,[7]The Author and Sonia Labatt Brain Tumor Research Centre, The Hospital for Sick Children, Toronto, ON, Canada,[8]Structural Genomics Consortium, Toronto, ON, Canada,[9]Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada

Introduction : Glioblastoma or GBM is an aggressive brain cancer with a 5 year survival rate below 10 percent. Glioma stem cells or GSCs drive GBM formation, growth, and resistance. Previously, analysis of 17,601 phase contrast images from 15 GBM patients revealed that neurodevelopmental GSCs form smaller, uniform clusters, while mesenchymal and injury-response GSCs exhibit complex, irregular growth patterns. This new study examines the spatial distribution and local cellular niche of GSCs along a neurodevelopmental gradient in primary GBM. We develop a geospatial map of GSCs in relation to other cell-types, identifying community patterns of organization.

Methods : We profiled 14 primary, treatment-naive GBM tissue sections (5 micrometer thick) on a spatial transcriptomics platform (Xenium), utilizing a custom 414 gene panel to score the GSC, GBM and other cell signatures. We segmented cell boundaries using both Baysor and Proseg algorithms and performed hematoxylin-eosin (HE) staining of tissue sections post-Xenium run. We performed spatial clustering using SpaGCN and Banksy, followed by spatial distribution and spatial point pattern analysis (Ripley's, Moran's I).

Results : Across the 14 tissues, we segmented 5, 705, 825 cells and annotated each cell with a cell-type label using pre-defined gene sets. This resulted in 15.2 percent GSC-like cells, 9.0% oligodendrocytic-OPC-like, 8.7% astrocytic-mesenchymal-like, 6.7% neuronal-like, 15.0% immune, 7.1% endothelial, and the rest of the cells showing mixed signatures. In total we identified 110-140 spatial clusters, which we classified into 8 domains by grouping highly correlated spatial clusters using Pearson correlation.

All cell-types were differentially distributed both proportionally and spatially. Domains with highly correlated grouped clusters exhibited distinct spatial arrangements of cells as well. For instance, GSC-like cells were confined to unique spatial domains with specific geometric properties across cellular hierarchies. Likewise, immune and endothelial cells displayed enrichment in preferred domains, with some co-localized with GSC-enriched regions while others were devoid of these cell types. Therefore, we find that these geospatial topology maps could identify spatial enrichment patterns and distributional trends.

Conclusions : This study deciphers GBM's geospatial topology, where annotated cell-types are mapped to specific niches. Though the ultimate aim of the study will be to eliminate GSC-like cells in GBM, we posit that by revealing cell community patterns, we aim to shift therapeutic strategies from targeting single cells or molecules to biomarkers that address entire cell communities within which these GSCs are spatially and functionally connected to. This framework lays the groundwork for future biomarker discovery and therapeutic interventions in GBM.

#768 Decode the antibody-drug conjugate surfaceome in metastatic urothelial cancer (DECODE-ADC).

C. Kuppe[1], N. Klumper[2], O. Goni[1], P. Kie?ling[1], E. Scheidereit[1], S. Samiei[1], T. Buttner[2], F. Zengerling[3], N. Gaisa[3], C. Bolenz[3], M. Toma[2], S. Zschabitz[4], V. Grunwald[5], M. Holzel[2], M. Iyer[6], N. Kashikar[6], M. Eckstein[7];
[1]University Hospital RWTH Aachen, Aachen, Germany, [2]University Hospital Bonn, Bonn, Germany, [3]University Hospital Ulm, Ulm, Germany, [4]University Hospital Heidelberg, Heidelberg, Germany, [5]University Hospital Essen, Essen, Germany, [6]OMAPiX, Frederick, MD, [7]University Hospital Erlangen, Erlangen, Germany

Background: Antibody-drug conjugate (ADC) therapies like Enfortumab Vedotin (EV), targeting NECTIN4, are transforming cancer treatment, particularly for metastatic urothelial cancer (mUC). While single-agent EV and EV in combination with immunotherapy show promising results, long-term benefits are limited to a subset of patients, underscoring the challenges associated with its non-selective application. All-comer application of EV raises concerns about toxicity and cost, highlighting the necessity for more personalized approaches. ADCs targeting other tumor antigens, such as HER2, HER3 and EGFR, are in late-stage clinical development, emphasizing the importance of understanding spatial molecular target distributions in patient tissues. Spatial multi-omics and single cell technologies applicable to patient tissues like formalin fixed paraffin embedded tissue (FFPE) open the possibility to unlock these spatial molecular profiles which ultimately could be used to identify companion diagnostic biomarkers and providing valuable insights into tumor microenvironment heterogeneity to optimize treatment efficacy and minimize off-target effects.
Methods: The DECODE-ADC project integrates single-cell and spatial multi-omics data from FFPE tissues of EV-treated patients, encompassing matched pairs of primary tumors and metastatic biopsy from a cohort of 20 patients. Single-nucleus RNA sequencing and high-resolution spatial transcriptomics were used to profile various ADC targets with additional markers of the tumor microenvironment and drug resistance (over 5000 gene panels). To bridge translational gaps, we employed machine learning (ML) models, such as variational autoencoders, to predict molecular signatures from routine Hematoxylin and Eosin (H&E) stainings, enabling cost-effective clinical application.
Results: Using spatial single-cell multiomics, we identified novel spatial domains regulating ADC target expression and delineated tumor microenvironment-specific signals. Unique molecular signatures distinguished luminal from basal urothelial cancer subtypes, revealing conserved microdomains across metastatic sites. Importantly, machine learning models transferred high-resolution molecular insights to H&E images, enabling precise, scalable predictions of ADC target distribution.
Conclusions: DECODE-ADC provides unprecedented insights into the spatial dynamics of ADC targets within the tumor microenvironment of mUC. By integrating advanced molecular profiling with ML-driven predictions, this study advances our understanding of ADC target expression and establishes a pathway for rational biomarker selection Our findings propose a foundation for precise ADC biomarker development and stratification strategies to improve treatment ADC responses, which ultimately paves the way for ADC precision oncology.

#769 Spatial proteomics and transcriptomics reveal an altered immune cell landscape in bladder cancer patients unresponsive to BCG treatment.

Trine Strandgaard[1], Tine Ginnerup Andreasen[1], Tessa Jane Divita[1], Liina Salminen[1], Sean Houghton[2], Kasper Thorsen[1], Iver Nordentoft[1], Iyinyeoluwa Okulate[3], Alexander Schmitz[4], Soren Riis Paludan[4], John Sfakianos[3], Amir Horowitz[3], Jorgen Bjerggaard Jensen[1], Lars Dyrskjot[1]

[1]Aarhus University Hospital, Aarhus, Denmark,[2]Weill Cornell Medicine, New York, NY,[3]Icahn School of Medicine at Mount Sinai, New York, NY,[4]Aarhus University, Aarhus, Denmark

Introduction: Patients with high-risk non-muscle invasive bladder cancer (NMIBC) are recommended treatment with Bacillus Calmette-Guérin (BCG). The therapeutic effect of BCG is highly dependent on the host immune system and the tumor microenvironment (TME). The cellular composition and the functional status of the TME have been shown to play crucial roles for treatment efficacy. However, much is still unknown regarding the cellular interactions and mechanisms of BCG. Using spatial proteomics and transcriptomics we investigated the immune cell landscape in a cohort of BCG treated patients.

Materials and methods: A total of 150 tumors from 105 patients with NMIBC treated with BCG were included on a tissue microarray (TMA). The TMA included paired pre- and post-BCG tumors. We analyzed the tumor tissue using Imaging Mass Cytometry (IMC; StandardBioTools) for simultaneous detection of 35 immune and tumor-related proteins at single cell resolution. Ilastik was used to generate probability maps and DeepCell Mesmer was used for cell segmentation. Markers were quantified for the mean intensity per cell. The GeoMX Digital Spatial Profiling (DSP) was used for spatial proteomics and transcriptomics profiling of tumor and stromal areas, targeting 63 protein targets and whole transcriptome amplification, respectively.

Results: After computing a neighborhood graph using the log normalised and z-scaled signal and embedding it using UMAP, leiden clustering was performed on 360,673 cells to detect communities of unique cell types by interrogating the presence of phenotypic markers. We identified major lineages such as macrophages, CD8 T cells, dendritic cells, natural killer (NK) cells and neutrophils in the TME of analyzed tumors. We observed more macrophages, CD4 T cells and CD8 T cells after BCG (p=0.02, p<0.001 and p=0.004, respectively) and similarly, we found enrichment of cellular neighborhoods composed of immune cells after treatment. Patients who progressed to muscle-invasive bladder cancer after BCG had higher numbers of CD4 T cells, CD8 T cells and NK cells prior to BCG (p=0.034, p=0.023 and p<0.001, respectively). Prolonged exposure to interferons has been associated with immune evasion and tumor cell proliferation. In concordance, we found high pretreatment expression of interferon signaling pathways in tumor regions from patients with a signature of CD8 T cell exhaustion after BCG using GeoMx DSP transcriptomic analysis.

Conclusion: The composition and functional status of the TME is associated with clinical and biological features such as immune cell abundance, CD8 T cell exhaustion, and progression. A greater understanding of the TME may help identify patients unresponsive to BCG and improve the understanding of biological differences in tumor development and aggressiveness ultimately improving patient outcomes.

#770 ccRCC metastasis rewires tumor microenvironment to increase immunosuppression and fibrosis.

Yufei Wang[1], Jae-Won Cho[2], Yasmin Nabil Laimon[2], Wenxin Xu[1], Aseman Bagheri Sheshdeh[2], Nithyassree Murugan[1], Hsien-Chi Yuan[1], Jon Wee[2], David Braun[3], Toni Choueiri[1], Catherine Wu[1], Sabina Signoretti[2], Gordon Freeman[1], Martin Hemberg[2], Wayne Marasco[1]

[1]Dana-Farber Cancer Institute, Boston, MA,[2]Brigham and Women's Hospital, Boston, MA,[3]Yale School of Medicine, New Haven, CT

Tumor progression and metastasis is driven by interplays between cancer cells and their surrounding tumor microenvironment (TME). Investigating the spatiotemporal transcriptomic profile of primary and metastatic tumors sheds light on how the differences in spatial architecture shape the TME and promote tumor metastasis. Here, we profiled three primary and three lung metastatic clear cell renal cell carcinoma (ccRCC) samples using the CosMx platform, which provides a 999-gene transcriptome with spatial coordinates. After cell segmentation and annotation, we further incorporate the cellular context and spatial information to identify niches by grouping the cells with similar neighboring cells. Cell-cell communication and architecture were analyzed to understand the spatial organization in primary and metastatic ccRCC. From niche analysis, 12 distinctive niches were identified, including cancer, stroma, alveolar, tertiary lymphoid structure (TLS), and mast niches, formed by 25 different cell types. Analyzing cancer niches, we found that the metastatic TME is much more immunosuppressive compared to the TME in primary ccRCC. There is a higher infiltration of exhausted T cells, Treg and M2-like macrophages in metastases. Moreover, the fibroblasts in metastases have higher expression of VEGF and metalloproteinases, which led to angiogenesis and worse prognosis. Architecture analysis showed that lung metastatic ccRCC cells, surrounded by a stromal barrier, are not directly interacting with tumor infiltrating leukocytes (TILs), whereas primary ccRCC cells interact more with TILs. Moreover, compared to the TLSs in primary ccRCC, the ones in metastases showed features of dysfunction, which was correlated with a worse prognosis. Our results show the impact of spatial architecture on primary and metastatic ccRCC, as well as the relation between spatial organization of the cellular context and cell-cell communication. In addition, it suggests that immune cells and cancer-associated fibroblasts (CAFs) are essential components of the ccRCC TME. Their interaction constitutes a major factor not only for tumor progression but also limiting therapy response in metastatic ccRCC.

#771 Spatiotemporal evolution of the tumor microenvironment predicts treatment response in phase II trial of neoadjuvant chemotherapy for resectable pancreatic cancer.

Choong-kun Lee[1], Changjin Hong[2], Ho Kyoung Hwang[3], Min Hwan Kim[1], Jean Rene Clemenceau[4], Seock-Jin Chung[4], Isabel Barnfather[4], Sumanth Reddy Nakkireddy[4], Jeong Hwan Park[5], Ji-Youn Sung[6], Minji Kim[4], Yooho Choi[4], Inyeop Jang[4], Sunho Park[4], Sung Hyun Kim[3], Chang Moo Kang[3], Hye Jin Choi[1], Tae Hyun Hwang[4]

[1]Division of Medical Oncology, Yonsei University College of Medicine, Seoul, Korea, Republic of,[2]KURE.AI, Seoul, Korea, Republic of,[3]Department of Surgery, Yonsei University College of Medicine, Seoul, Korea, Republic of,[4]Department of Artificial Intelligence and Informatics, Mayo Clinic, Jacksonville, FL,[5]Department of Pathology, SMG-SNU Boramae Medical Center, Seoul National University College of Medicine, Seoul, Korea, Republic of,[6]Department of Pathology, College of Medicine, Kyung Hee University hospital, Kyung Hee University, Seoul, Korea, Republic of

Background: Understanding the spatiotemporal dynamics of the tumor microenvironment (TME) during treatment is crucial for improving outcomes in pancreatic ductal adenocarcinoma (PDAC). This study presents a pre-planned spatiotemporal analysis from a phase II trial evaluating neoadjuvant mFOLFIRINOX in resectable PDAC, aimed at elucidating treatment resistance mechanisms and identifying predictive biomarkers.
Methods: This multi-institutional, single-arm phase II trial enrolled treatment-naïve patients with histologically confirmed resectable PDAC (NCT05066802) who underwent six cycles of neoadjuvant mFOLFIRINOX followed by surgery. The primary endpoint was the R0 resection rate. Matched pre-treatment tumor biopsies and post-treatment surgical specimens were analyzed from selected responders (R) and non-responders (NR) using high-resolution spatial transcriptomics (10X Genomics Visium). This approach enabled comprehensive mapping of TME evolution and treatment-induced changes.
Results: Between May 2020 and February 2022, 27 patients were enrolled. Of these, 24 patients underwent surgery after neoadjuvant treatment (resection rate: 88.9%), and 23 achieved R0 resection (85.2%), meeting the primary endpoint. However, 20 resected patients (83.3%) recurred. Spatiotemporal analysis was performed on matched samples from four R and four NR. In pre-treatment samples from NR group, clusters dominated by fibroblasts (enriched for ECM, EMT, and CAF phenotypes) and B cell subtypes were distinct compared to R group, which exhibited elevated antibody responses and robust T cell and macrophage activity. NR samples showed increased IgM expression but lacked corresponding Fc receptors, suggesting limited immune effector function. Post-treatment NR surgical specimens displayed elevated expression of MUC5AC and CEACAM6 in malignant ductal cells, alongside genes promoting tumor growth. Neoadjuvant mFOLFIRINOX increased fibroblast, while reducing immune cell components. NR tumors formed distinct "resistance niches" after chemotherapy, marked by ECM production, CAF activation, and RTK signaling. Conversely, R tumors showed enhanced epithelial organization with increased type-I hemidesmosome complexes facilitating epithelial adhesion to the basement membrane.
Conclusion: Three months of neoadjuvant mFOLFIRINOX was feasible and tolerable for resectable PDAC but associated with high recurrence rate, emphasizing the need for better patient selection. Spatiotemporal analysis revealed critical differences in TME dynamics according to treatment response, including the emergence of resistance niches and distinct pre-treatment spatial signatures. These findings support the integration of spatial biology into future pancreatic cancer trials to improve stratification and therapy.

#772 Spatial analysis of the DLBCL tumor microenvironment via the novel SignalStarTM multiplex immunohistochemistry assay.

Jennifer Ziello[1], Shuling Zhang[2], Derek Papalegis[1], Lily Vu[1], Gabriella Spang[1], Jean Loebelenz[1], Arvin Ruiz[3], Giorgio Inghirami[3], Sarah Klein[1]

[1]Cell Signaling Technology, Inc., Danvers, MA,[2]Visiopharm (Registered Trademark), Horsholm, Denmark,[3]New York Presbyterian Hospital Weill Cornell, New York, NY

Diffuse Large B-Cell Lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma, accounting for more than 18,000 cases each year within the United States. While roughly 60% of patients with DLBCL achieve durable remissions with existing chemotherapies, the prognosis of patients with refractory DLBCL is poor. The immune landscape of DLBCL is complex and further elucidation of the interplay between T cell activation and immunosuppression within the tumor microenvironment (TME) is necessary in order to better stratify patients and predict outcomes for therapy. This complexity, as well as the relevance of the spatial context of the various cell types within the TME necessitate the ability to simultaneously visualize biomarkers and phenotypic markers via multiplex immunohistochemical (mIHC) assays. Here, we construct a 12-plex SignalStar mIHC panel consisting of T cell phenotypic, activation and suppression markers in order to characterize the TME of FFPE DLBCL cores. We visualize immuno-oncology markers in the context of the tumor and myeloid cells, as defined by CD68. In this assay, all 12 antibodies are applied in one primary incubation, followed by the addition of a network of fluorescently-labeled oligonucleotides used to amplify the signal of 4 antibody-oligo conjugates across 3 rounds of imaging. Following amplification and imaging of the first 4 conjugates, the fluorescent signal was enzymatically removed and the process repeated for two more rounds. The three resulting images were then aligned using Visiopharm's Tissuealign^{TM} platoform. Quantitative analysis, including positive cell counts and marker co-localization, was conducted with Visiopharm's Phenoplex^{TM}. Biomarker expression patterns were also examined using Phenoplex^{TM}. Finally, we correlated these data with patient demographics, disease progression, and treatment responses. Our data demonstrate that the SignalStar mIHC assay is a powerful tool in the elucidation of the complex immunosuppressive TME, enabling researchers to better understand the responses to immunotherapeutic approaches and chemotherapy combination strategies.

#773 High-throughput in situ multiomic profiling of kidney, lung, colon, and breast cancer FFPE samples for profiling of tumor-immune interactions.

Kenneth H. Gouin, Michael Lawson, Andrew Pawlowski, Sabrina Shore, Yuji Ishitsuka, Daan Witters, Eli N. Glezer

Singular Genomics Systems Inc., San Diego, CA

Molecular level spatial profiling of tissue provides crucial insights into tumor biology and immunotherapeutic responses. The G4X Spatial Sequencer, our innovative platform, facilitates extensive translational studies by simultaneously detecting RNA transcripts, proteins, and H&E staining in FFPE tissues. Capable of imaging up to 40 cm^2 of tissue in a single run, it significantly enhances throughput compared to traditional methods and enables 3D reconstruction from serial sections in one run. We designed ~300-gene transcript panels for each of four tissue types: kidney, lung, colon, and breast, with an emphasis on immuno-oncology relevant targets. The panels share a common set of 150 immuno-oncology and 50 stromal genes. Each panel also includes a set of ~100 tissue-specific genes to profile cell types, cancer pathways and tumor microenvironment. We imaged gene and protein expression in 5-µm thick sections of human kidney, lung, colon, and breast, from both healthy and cancer FFPE tissue samples. Using a novel tissue transfer process, we arrayed multiple tissue sections in two formats: a large sample format (10 mm x 10 mm) with 10 sections/slide, and a small sample format (4.5 mm x 4.5 mm) with 32 sections/slide. Each slide was analyzed in three modalities (RNA, proteins, fluorescent H&E). Following antibody staining, probe binding and amplification of targets, images were acquired using 4-color SBS readout, followed by nuclear and cytoplasmic staining to generate a fluorescence-based H&E stain. In FFPE cancer samples and healthy controls, we detected an average of 50-200 transcripts/cell and 20-50 unique genes/cell, with the dynamic range extending to > 500 transcripts/cell. Using 6 rounds of priming, we achieved high transcript density profiling while maintaining a false discovery rate of ~1%. Strong correlation between single-cell transcript and protein expression profiles confirmed specificity of each modality. Comparison across serial sections showed strong concordance in expression profiles for both modalities, demonstrating robust reproducibility. We also show the assembly of serial sections to reconstruct a 3D tumor volume. Finally, we identified distinct tumor-associated cellular phenotypes and spatial neighborhoods using a novel multi-modal graph-based clustering method leveraging RNA, protein, morphology, and spatial information.

#774 Deciphering the biology of colorectal metastasis to the peritoneal surface.

Shahid Umar, Badal C. Roy, Amrita Mitra, Jill Haley, Harsh B. Pathak, Andrew K. Godwin, Luke V. Selby, Mazin Al-Kasspooles

General Surgery, University of Kansas Medical Center, Kansas City, KS

Background. Colorectal cancer (CRC) is the third most frequently diagnosed cancer with an annual incidence of 1.4 million new cases and 700,000 deaths worldwide. The high death toll of CRC patients is due to metastatic disease with liver being the preferred site of metastasis (LM), followed by lung and peritoneal metastasis (PM). Roughly 25% of CRC patients present with, or will eventually develop PM, and while survival for LM and lung metastasis has significantly improved over time, survival following PM remains poor. Mucinous and non-mucinous adenocarcinoma have different propensity to form PM or LM, and surprisingly little is known about the biology of CRC PM. Aims and

Objectives. To perform deep mapping using Nanostring's GeoMx Cancer Transcriptome Atlas assay to measure gene expression in resected normal colon (healthy) primary tumors (primary), LM, and PM samples collected from the KUMC Biospecimen Repository. Morphological staining using Pan-cytokeratin, CD45, & ELA2 was performed to identify epithelial tumor cells, immune cells, and neutrophils, respectively. Data processing and resulting DCC files were imported to the GeoMx DSP software for statistical analyses of the transcriptomic data using a linear mixed model.

Results. We performed spatial transcriptomic analysis on paired tissue samples (surgical tissue from primary and metastatic sites) from 4 patients with stage IV CRC. Our results unveiled diverse gene expression patterns across different PM and LM tumor sub-regions. Cluster analysis revealed significant differences in gene expression between specimen types. Specifically, in mucinous adenocarcinoma samples, *PKM*, *CEACAM6* and *LYZ* were upregulated in primary tumors while *ADH1A/B/C*, *Muc4* and *PCK1* were upregulated in healthy tissues. Interestingly, we also observed significant upregulation of *CEACAM6* and *SERPINA1* in PM vs primary tumor cells. In non-mucinous adenocarcinoma, *CEACAM6* and *H3C2/10* were upregulated in primary vs metastatic tissues. When comparing either primary or metastatic vs healthy stromal areas, *COL1A1* and *COL6A3* were significantly upregulated in primary and or metastatic regions hinting at extracellular matrix remodeling during PM. In LM, *CEACAM6*, *SLC1A5* and *CD74* were significantly upregulated when compared to PM, indicating an inflammatory role in LM. As neutrophil extracellular traps (NETs) promote metastasis, we discovered significant infiltration of ELA2+ neutrophils in the primary mucinous adenocarcinoma while ELA2+ cells were predominantly localized at the leading edge of the non-mucinous adenocarcinoma. These spatio-temporal relationships were further validated in the metastatic sites of the two CRC subtypes.

Conclusions. Mucinous vs non-mucinous adenocarcinomas differ in terms of their proclivity to metastasize to the peritoneal surfaces and that the differences could be attributed to the intra-tumoral vs leading edge distribution of ELA2+ neutrophils.

#776 Identifying gene expression patterns in intraductal carcinoma of the prostate using spatial gene expression analysis: Potential for new biomarkers and treatments.

R. Watanabe[1], H. Arai[1], M. Kurata[1], N. Miura[1], R. Kitazawa[2], T. Kikugawa[1], T. Saika[1];
[1]Ehime University Graduate School of Medicine, Toon, Japan, [2]Ehime University Hospital, Toon, Japan

Introduction and Objective: Intraductal carcinoma of the prostate (IDC-P) is a form of prostate cancer where abnormal cells grow inside the ducts and glands, while the outer basal cell layer stays intact. IDC-P is often linked to certain genetic changes, including TMPRSS2-ERG fusions, as well as mutations or deletions in key genes like TP53, RB1, and PTEN. Given these findings, NCCN guidelines advise early genetic testing for IDC-P patients. Correctly diagnosing IDC-P and applying this knowledge to tailor treatments is essential for improving patient outcomes.
Methods: We performed spatial gene expression analysis (using CytAssist Visium) on prostate cancer tissue samples with IDC-P. The goal was to identify gene abnormalities that could help in diagnosing IDC-P or serve as targets for future treatments. We analyzed how gene expression was distributed in IDC-P regions compared to other parts of prostate cancer tissue.
Results: Our analysis showed that IDC-P regions had a unique gene expression profile, distinct from other prostate cancer areas. Specific genes such as MUC6, MYO16, NPY, and KLK12 were highly expressed in IDC-P, and these genes are associated with more aggressive cancer behavior. Additionally, genes related to hypoxia like HIF1A, BNIP3L, PDK1, and POGLUT1 were highly upregulated in IDC-P areas. At the same time, markers for immune cells (CCR5, FCGR3A) and fibroblasts (CCL5, TRAC) were downregulated. This suggests that IDC-P may be more aggressive due to hypoxia and fewer immune and fibroblast cells in the tumor environment.
Conclusions: This study used spatial gene expression analysis to characterize IDC-P (Watanabe et al., IJMS 2024), providing valuable insights. However, this method also has some challenges, such as capturing signals from different cell types in the same spots, which makes it harder to distinguish between them. In future studies, combining spatial gene analysis with single-cell RNA sequencing or using more advanced techniques like Xenium could address these challenges. We are currently working on those studies and will be able to present the latest data on the day of the presentation. These approaches may help identify new diagnostic markers and lead to more personalized treatment options for patients with IDC-P.

#777 Revealing the transcriptional and proteomic spatial neighborhoods of early prostate cancer and the tumor microenvironment.

Anna Y. Lee[1], Megan Hopkins[1], Linda Liao[1], Vida Talebian[1], Tamara Jamaspishvili[2], David M. Berman[3], Melanie Spears[1], Jane Bayani[1]

[1]Diagnostic Development, Ontario Institute for Cancer Research, Toronto, ON, Canada,[2]Department of Pathology & Laboratory Medicine, SUNY Upstate Medical University, Syracuse, NY,[3]Department of Pathology & Molecular Medicine, Queen's University, Kingston, ON, Canada

Intermediate-risk prostate cancer (PCa) poses a challenge for treatment decisions, made more complex by its heterogeneous nature. Growing evidence highlights the critical role of the tumor microenvironment (TME), in cancer initiation, progression, and response to treatment, underscoring the importance of profiling cancer cells but also the TME within their spatial context. Here, we present single-cell *in situ* transcriptomic and spatial proteomics results from 300 intermediate-risk PCa prostatectomy samples. Multiple regions of the prostate, including regions of the index lesion, secondary lesions, regions of benign and normal were sampled then analyzed using Bruker's CosMx SMI platform for a 6000-plex RNA assay in a subset of patients; in addition to proteomic profiling using the GeoMx DSP platform. Clustering assigned cells into 15 cell types based on their transcriptional profiles. Each cell type was tested for significantly different proportions in tumor versus non-tumor samples. The cell type with the greatest bias had a 9-fold greater proportion in tumor samples (adjusted $P < 0.01$). ERG, largely associated with the TMPRSS2-ERG fusion in PCa, was most specifically expressed in cells of this type. Conversely, the cell type with the greatest bias towards non-tumor samples (3-fold greater proportion, adjusted $P < 0.01$) is characterized most specifically by the expression of MSMB, which encodes an immunoglobulin binding factor previously found to have decreased expression in PCa. Other evidence of intratumoral heterogeneity was found in samples that are not dominated by these stereotypical cell types. For example, a relatively higher-grade sample from an index tumor possessed high proportion of the tumor-dominant cell type whereas a relatively lower-grade sample from the same tumor did not. Similarly, the tumor and non-tumor samples arising from the same patient showed similar cell type composition, highlighting inter-patient heterogeneity. Moreover, we identified proteins with significantly different expression in tumor versus non-tumor samples. In the TME, EpCAM and CD56 were significantly overexpressed and underexpressed in tumor versus non-tumor regions, respectively (adjusted $P < 0.01$). In the epithelium, cleaved caspase-9, which initiates the caspase cascade leading to apoptosis, was the most significantly underexpressed protein in tumor samples (adjusted $P < 0.01$). However, the expression of the mRNA encoding caspase-9 is not highly correlated with the expression of cleaved caspase-9, highlighting the added benefit of protein expression profiling. Taken together, this multi-modal spatial analysis of multiple samples from the same PCa patients facilitates a detailed understanding of early PCa for biomarker discovery and pathways of therapeutic intervention.

#778 Profiling the immune landscape of CDK4/6 inhibitor intrinsic resistance among metastatic HR+ breast cancer.

Haoting Shi[1], Zheshen Han[2], Xue Wang[1], Yufei Wang[3], Chao Hu[1], Ruixin Pan[1], Xiaosong Chen[1], Qing Qu[1], Rong Cai[1], Kunwei Shen[1]

[1]Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China,[2]The University of Hong Kong, Hong Kong, Hong Kong,[3]Shanghai Jiao Tong University School of Medicine, Shanghai, China

Cyclin-dependent kinases 4/6 (CDK4/6) inhibitor is a standard-of-care for hormone receptor-positive/human epidermal growth factor receptor 2 negative metastases breast cancer (HR+/HER2- mBC), while substantial proportion of patients suffer from intrinsic resistance (IR), and the mechanism at the spatial level remains unclear. A total of 10 eligible HR+/HER2- mBC patients (age: median age 63, range 40-67 years) with liver metastases who underwent liver biopsies and received CDK4/6 inhibitor were included. The majority (N = 7, 70%) were of first-line treatment at metastatic disease settings and in combination with fulvestrant (N = 9, 90%). After a median follow-up of 22.2 months, four patients were determined as IR, with a median PFS of 4.2 months. We collected formalin-fixed paraffin-embedded samples from these patients and spatially profiled the expression of more than 570 proteins across tumor (panCK+), immune (CD45+), and cancer-associated fibroblast region (CAF, α-SMA+) regions, respectively using the GeoMx Digital Spatial Profiler (DSP) platform. The differentially expressed proteins (DEPs) were identified and GSEA analyses were performed. Elevated expression of MHC I proteins (HLA B7 and HLA E), extracellular matrix (ECM)-associated protein (FAK, Fibronectin, and Periostin), antioxidant-associated proteins (NQO1 and G6PD), and mucin-related proteins (Muc5AC and Muc1) were observed in tumor regions (all FDR < 0.05) among IR patients. Similarly, HLA B7, Fibronectin, MucAC5 and NQO1 were found to be overexpressed in CAF region. The proteins associated with the target of CDK4/6 inhibitor were down-regulated among IR patients [NF-kB p105, CK8 (phosphor S431), and Cyclin D1], suggesting that the IR could be partially explained by loss-of- drug-target. Meanwhile, downregulation of Caveolin-1 and CD63 were identified in CAF regions, while downregulation of HLA-DR and MHC II proteins were observed in immune regions. GSEA results showed that MHC I antigen presentation pathway was significantly upregulated in CAF regions (NES 1.87, FDR 0.016), while TGF-beta pathway and autophagy pathway were significantly downregulated in CAF regions (NES -1.77 [FDR 0.016] and -1.84 [FDR 0.040], respectively). These findings suggested that IR might be driven by the immune escape associated with CAFs. Our results showed that Bcl-2 expression in tumor region has the highest value in predicting IR (AUROC 0.989). Leveraging high-plex spatial proteomics data from HR+/HER2- mBC patients, the IR of CDK4/6 inhibitor might be associated with loss-of-target in tumor cells, immune escape driven by CAFs, and alterations in the ECM.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#78 Snord67 promotes breast cancer metastasis through U6-mediated alternative splicing.

Katherine Ismei Zhou[1], Yvonne L. Chao[2], Kwame K. Forbes[3], Alessandro Porrello[3], Gabrielle M. Gentile[3], Yinzhou Zhu[1], Aaron C. Chack[3], Dixcy J. S. John Mary[2], Haizhou Liu[2], Eric Cockman[1], Lincy Edatt[3], Grant A. Goda[3], Justin Zhao[1], Hala A. Assi[1], Hannah J. Wiedner[3], Yi-Hsuan Tsai[3], Lily Wilkinson[3], Amanda E. Van Swearingen[1], Lisa A. Carey[3], Jimena Giudice[3], Daniel Dominguez[3], Christopher L. Holley[1], Chad V. Pecot[3]

[1]Duke University Medical Center, Durham, NC,[2]University of Pittsburgh, Pittsburgh, PA,[3]University of North Carolina, Chapel Hill, NC

Small nucleolar RNAs (snoRNAs) are a class of ncRNAs that canonically guide post-transcriptional modifications, including 2'-*O*-methylation, on ribosomal RNA (rRNA) and small nuclear RNA (snRNA). While traditionally considered housekeeping genes, snoRNAs have increasingly been found to function in diverse physiologic and pathologic processes, including cancer. In an immune-competent murine model of triple negative breast cancer (TNBC) lymphatic metastasis, we identified the snoRNA Snord67 as one of the most upregulated noncoding RNAs in axillary LN (AxLN) tumors relative to mammary fat pad (MFP) tumors and lung metastases. Loss of Snord67 resulted in decreased colony formation and spheroid size in murine and human TNBC cell lines, and led to decreased lymph node tumor growth and decreased distant metastases in two immune-competent murine models of TNBC lymphatic dissemination. To determine the mechanism by which Snord67 promotes tumor growth and metastasis, we examined the impact of Snord67 on 2'-*O*-methylation, gene expression, and alternative splicing. Loss of Snord67 in TNBC cell lines led to decreased 2'-*O*-methylation at the C60 nucleotide (Cm60) in the core spliceosome component U6 snRNA. Re-introduction of wild-type Snord67 rescued Cm60 in U6 snRNA and rescued the colony formation and spheroid phenotypes of the Snord67 knockout cell lines. However, a mutant Snord67 incapable of guiding U6 Cm60 only partially rescued these *in vitro* phenotypes, suggesting that Snord67 promotes *in vitro* tumor cell growth at least in part by guiding Cm60 in U6 snRNA. We then performed RNA sequencing of Snord67 knockout and wild-type murine and human TNBC cell lines. We found that loss of Snord67 led to widespread changes in alternative splicing, consistent with its role in guiding 2'-*O*-methylation of the core spliceosome component U6. We further demonstrated that the inclusion of alternatively spliced cassette exons in *MYO18A* and *NFYA* was not only downregulated upon Snord67 knockout in a human TNBC cell line, but also positively correlated with Snord67 expression levels in primary breast tumors and lymph node metastases from breast cancer patients. Based on these results, we propose a model in which Snord67 guides U6 Cm60, which leads to a pro-metastatic alternative splicing program and thereby promotes lymph node tumor growth and distant metastasis.

CLINICAL RESEARCH: Survivorship Research and Supportive Care
Poster Session

#782 Mapping therapy-related clonal hematopoiesis across immune cell populations in long-term survivors of pediatric cancer by single-cell multi-omics profiling.

Sivaraman Natarajan, Nadezhda V. Terekhanova, Kohei Hagiwara, Bensheng Ju, Li Dong, Zhaoming Wang, Jinghui Zhang, John Easton

St. Jude Children's Research Hospital, Memphis, TN

Recently we and others reported high prevalence of clonal hematopoiesis (CH) in long-term survivors of pediatric cancers by performing ultra-deep panel sequencing of peripheral blood samples, a phenomena significantly associated with exposures to radiotherapy and chemotherapeutic agents. Motivated by a preliminary finding that a therapy-related *STAT3* CH mutations were highly enriched in T-cells of 3 Hodgkin's Lymphoma (HL) survivors, we initiated a systematic investigation to map the CH mutations across different immune cells populations. Blood samples of 108 survivors of 13 pediatric cancer types (4 hematological, 8 solid and 1 central nervous system (CNS) tumor types), known to harbor CH mutations discovered by prior bulk sample analysis, were selected for multi-omics single-cell profiling with Mission Bio Tapestri assay. For mutation screening, we designed a panel of 29 amplicons across 12 genes, covering 52 CH mutations detected in bulk samples. Amongst the 23 completed samples, we identified 99 mutations in CH-genes (*DNMT3A*, *STAT3*, *TP53*, *KRAS*, *TET2*, and *ASXL1*), and 67.7% of those were found in all cell types and the rest were present in at least two cell types. We discovered that a subset of CH mutations was enriched (FDR < 0.05) in certain cell types across several samples. For example, *STAT3* Y640F mutations were consistently enriched in CD8 T-cells in 6 out of 7 HL samples with a mean fold change of 18.5. *DNMT3A* mutations were enriched in cells from both myeloid (monocytes) and lymphoid (CD4/8 T-cells and NK-cells) lineages and affected samples from five cancer types. Specifically, we found that *DNMT3A* mutations were enriched in monocytes in 2 HL and 2 acute lymphoblastic leukemia (ALL) cases with an average fold change of 2.3; and in CD8 T-cells in 5 samples from Wilms tumor, soft tissue sarcoma, ALL, HL, and CNS with an average fold change of 4.9. *KRAS* L19F was found to be enriched in monocytes and NK-cells in an ALL sample. By contrast, we observed a uniform distribution of some *TP53* mutations across all cell types, suggesting that they confer comparable selective advantage across all immune cell types, consistent with the biological function of TP53 in cell cycle. To our knowledge, this is the first large-scale multi-omics single-cell profiling of CH mutations in long-term survivors of pediatric cancer. The differential clonal expansion in immune cell populations demonstrates the potential impact of CH mutations on immune cell dysfunction. This suggests that CH mutations may contribute to long-term adverse health outcomes related to the immune system in long-term survivors beyond the direct cause of secondary neoplasms, which will warrant future investigations using the valuable data resource established through this study.

#783 Estrogen Receptor Alpha (ERα) is critical for the maintenance of mitochondrial form and function in the prevention of anthracycline-induced cardiotoxicity.

T. K. King III, N. L. Yang, A. Singh, P. H. Tran, A. Ma, E. Yim, W. C. Tai, E. H. Yang, A. Hevener;
University of California Los Angeles, Los Angeles, CA

Cancer remains a leading cause of mortality in the United States, and women's cancer risk increases after menopause. Anthracyclines (e.g., doxorubicin) are widely used in cancer treatment but their therapeutic value is limited by anthracycline-induced cardiotoxicity (AIC), which can promote irreversible cardiac damage and heart failure. 17-β-estradiol protects against cardiac injury in ovariectomized rodents treated with anthracyclines, thus, we hypothesized that estrogen receptor (ER) α may play a role in safeguarding against AIC. Even without AIC, cardiomyocellular-specific deletion of ERα impairs mitochondrial form and function. This is accompanied by increased fibrosis and reduced cardiac ejection fraction, as determined by echocardiography. Transmission electron microscopy of hearts from ERα knockout mice revealed a marked reduction in mitochondrial cristae density. These cristae are essential structures of the inner mitochondrial membrane, serving as the primary sites for electron transport chain protein localization and oxidative metabolism required for ATP production. Microscopy findings were further supported by RNA sequencing, which indicated differences in the expression of transcripts associated with ATP biosynthesis, oxidative phosphorylation, and mitochondrial respiratory chain complex assembly in the hearts of ERα knockout mice compared to ERα-replete controls. Complementary analysis using Seahorse respirometry on frozen tissue demonstrated a marked reduction in complex I oxygen consumption in ERα knockout mice. With the phenotype observed in heart-specific ERα knockout mice, we hypothesized that doxorubicin-treated HL-1 cardiomyocytes lacking ERα would display increased cell death compared to control cells due to further impairment of mitochondrial function. Indeed, doxorubicin treatment induced a higher rate of cell death in ERα knockout cells vs. control. Key proteins involved in mitochondrial remodeling and mtDNA replication, including Mitofusin 1 (MFN1), Mitofusin 2 (MFN2), Optic Atrophy 1 (OPA1), Dynamin-Related Protein 1 (DRP1), DNA Polymerase Gamma (POLG), Mitochondrial Contact Site and Cristae Organizing System Subunit 25 (MIC25), and Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-Alpha (PGC1α), were significantly reduced in non-treated ERα knockout HL-1 cells compared to cells with ERα. Combining these findings with the observation that ERα overexpression enhances mitochondrial cristae density, the expression of key mitochondrial-related targets, and mtDNA copy number, our results collectively suggest that ERα is essential for mitochondrial function in the heart and could serve as a therapeutic target to protect against AIC.

#784 Cardiovascular disease mortality surpasses cancer mortality in patients with renal cell carcinoma: A U.S. population-based cohort study.

Zhengyi Deng, Minji Jung, Jinhui Li, Mingyi Li, Marvin E. Langston, Benjamin I. Chung

Stanford University School of Medicine, Stanford, CA

Background: Cardiovascular disease (CVD) is the leading cause of non-cancer death among patients diagnosed with renal cell cancer (RCC). There is limited evidence on how CVD and RCC death rates change over time since RCC diagnosis, and evidence on potential risk factors associated with short-term and long-term CVD and RCC deaths.
Methods: This population-based cohort study used data from the Surveillance, Epidemiology, and End Results Program (SEER, 2004-2020). We included adult patients (aged ≥18 years) diagnosed with a first malignant RCC from 2004 to 2019, who survived at least one year since their cancer diagnosis. Poisson regression with natural cubic spline was used to model the trends in RCC and CVD death rates over a 16-year follow-up period and to determine the crossover time where the two curves intersected. Fine and Gray competing risk models were implemented to evaluate time-dependent associations of sociodemographic and clinical characteristics with CVD and RCC deaths at 1, 5, 10, and 16 years of follow-up.
Results: Among 116,836 RCC patients, CVD death rate overtook RCC death rate at 13.7 (95% confidence interval: 12.0-15.4) years after cancer diagnosis. The crossover time occurred earlier in patients who were ≥75 years, female, unmarried, Non-Hispanic Black, or had a RCC of stage I, tumor size ≤7 cm, grade I, or chromophobe histology, compared to others (crossover time ranged from 3.0 to 9.0 years). In contrast, CVD death rate did not exceed RCC death rate in patients with a RCC of stage III/IV, tumor size >7cm, or those receiving chemotherapy/radiotherapy during the study period. Age, sex, and papillary histology were time-dependently associated with RCC deaths, and stage and grade were time-dependently associated with CVD deaths.
Conclusion: In RCC patients, CVD death became dominant over RCC death during survivorship, with patterns varying according to different sociodemographic and clinical characteristics. These findings underscore the need for integrated management strategies addressing both cancer and CVD in RCC patients.

#785 Epidemiology, clinical outcomes, and healthcare resource utilization in multiple myeloma patients with protein-energy malnutrition hospitalized for acute congestive heart failure: A comprehensive population-based analysis.

Adamsegd I. Gebremedhen[1], Abdu Mohammed[2], Mamdouh Souleymane[1], Michael Fatuyi[3], Malik Samardali[4], Kemnasom Nwanwene[5], Toni O. Pacioles[5]

[1]Internal Medicine, Marshall University Joan C. Edwards School of Medicine, Huntington, WV,[2]Internal Medicine, Trinity Health System, Steubenville, OH,[3]Cardiology, University of Wisconsin, Madison, WI,[4]Internal Medicine, St Elizabeth Youngstown Hospital, Youngstown, OH,[5]Hematology and Oncology, Marshall University Joan C. Edwards School of Medicine, Huntington, WV

Introduction Multiple myeloma (MM) is often associated with heart failure, which can result from a high- output state, light-chain cardiac amyloidosis, or treatments such as proteasome inhibitors. Common MM-associated complications like anemia can further exacerbate heart failure, leading to acute decompensation and increased rates of hospitalization. Protein-energy malnutrition (PEM) is common in MM patients and is linked to poorer outcomes and longer hospital stays in various cancers. However, the impact of PEM on MM patients with acute congestive heart failure (ACHF) has not been well studied. This research aims to explore how PEM affects in- hospital outcomes in MM patients with ACHF. Methods We identified adult patients with MM from the National Inpatient Sample who were admitted with ACHF as the principal diagnosis from 2018 to 2021, using ICD-10 codes. The cohort was categorized based on the presence or absence of PEM, and we applied complex sampling weights to ensure national representativeness. Our primary outcome was all-cause mortality, and we analyzed several secondary outcomes. We used multivariate regression models to evaluate outcome disparities between the groups, with statistical significance set at $p < 0.05$. Results We identified a total of 2,390 adults with MM who were admitted with a principal diagnosis of ACHF from 2018 to 2021. Among these, 395 patients (16.5%) had a concurrent diagnosis of PEM. The mortality rate was 5.2% in the entire cohort and 11.4% in the PEM group. The PEM group had a higher proportion of female patients (59.5% vs. 43.9%, p=0.01). Patients with PEM had higher odds of all-cause mortality, with an adjusted odds ratio (aOR) of 2.64 (95% confidence interval [CI] 1.07-6.48). They also had higher odds of experiencing acute kidney injury (aOR 1.86, 95% CI 1.05-3.30), requiring mechanical ventilation (aOR 15.17, 95% CI 1.98-116.22), experiencing cardiogenic shock (aOR 3.73, 95% CI 1.27-10.92), and needing vasopressor support (aOR 4.96, 95% CI 1.37-17.90). Furthermore, patients with PEM faced longer hospital stays (9.8 vs. 5.8 days), with an adjusted incidence rate ratio (aIRR) of 1.55 (95% CI 1.25-1.92), and higher hospitalization charges ($118,775 vs. $59,505), with an aIRR of 1.71 (95% CI 1.28-2.28). Conclusion Our study found that MM patients with PEM admitted for ACHF had significantly higher odds of all-cause mortality and other adverse clinical outcomes compared to those without PEM. In addition, these patients experienced longer hospital stays and higher hospitalization charges. These findings underscore the importance of addressing PEM in the management of MM patients with ACHF to improve clinical outcomes and reduce healthcare resource utilization.

#787 Multi-omics approach to delineate the mechanisms of doxorubicin-induced cardiotoxicity.

Mohamed S. Dabour[1], Ibrahim Y. Abdelgawad[1], Bushra Sadaf[1], Mary R. Daniel[1], Marianne K. O. Grant[1], Anne H. Blaes[2], Pamala A. Jacobson[1], Beshay N. Zordoky[1]

[1]Experimental and Clinical Pharmacology, University of Minnesota, College of Pharmacy, Minneapolis, MN,[2]Division of Hematology/Oncology/Transplantation, Medical School, University of Minnesota, College of Pharmacy, Minneapolis, MN

Introduction: Doxorubicin (DOX) is a highly effective chemotherapeutic agent utilized in the treatment of a wide variety of adult and pediatric malignancies. However, the clinical utility of DOX is limited by its cardiotoxicity. Mechanisms of DOX-induced cardiotoxicity are not fully elucidated, highlighting the need for comprehensive studies. Proteomic and transcriptomic analyses provide an unbiased platform to uncover the molecular pathways underlying drug-induced toxicity. *The objective* of the current work is to delineate the mechanisms of DOX-induced chronic cardiotoxicity in C57BL/6N mice using a multi-omics approach to identify key molecular pathways and potential therapeutic targets.

Methods: Five-week-old C57BL/6N mice were randomized to receive six weekly intraperitoneal injections of either DOX (4 mg/kg/week for 6 weeks) or saline. Three days following the last injection, cardiac function was assessed by trans-thoracic echocardiography. Cardiac proteomic profiling and RNA sequencing were performed using pulverized heart tissues to determine the underlying molecular mechanisms of DOX-induced cardiotoxicity. Identified differentially expressed proteins and genes were validated by western blotting and quantitative real-time PCR, respectively. Key protein biomarkers were measured in plasma samples from breast cancer patients treated with doxorubicin using ELISA. To contextualize our findings, we also compared our data with multiple publicly available proteomic and RNA-sequencing datasets.

Results and Conclusion: DOX treatment induced cardiac dysfunction, evidenced by reductions in left ventricular fraction and fractional shortening. Proteomic analysis of heart tissue identified key proteins related to cardiotoxicity, including serpina3n, thrombospondin-1, and epoxide hydroxylase 1, all of which were significantly upregulated. RNA sequencing revealed the upregulation of Nr1d1, Phlda3, sestrin2 (sesn2), lipocalin2 (Lcn2), and Trp53inp1, with a downregulation of apelin-1. Protein expressions of serpina3n, and thrombospondin-1 were confirmed by immunoblotting, and gene expressions of Nr1d1, Trp53inp1, sestrin2, epoxide hydroxylase 1, and apelin-1 were confirmed by real-time PCR. Integrative analysis of our proteomics, RNA sequencing and other publicly available datasets identified common molecular pathway involving Nr1d1-serpina3n, underscoring its potential role in DOX-induced cardiotoxicity. Furthermore, plasma levels of serpina3 were significantly elevated in breast cancer patients 24 hours after receiving DOX-based chemotherapy compared to baseline, highlighting translational relevance of these findings. In conclusion, our findings provide valuable insights into the molecular mechanisms underlying DOX-induced cardiotoxicity, which could inform future therapeutic strategies to mitigate its adverse effects.

#788 QT prolongation induced by CDK-4/6 inhibitors in breast cancer: A prospective cohort study utilizing 12-hour continuous ECG monitoring.

Chia-Lun Chang[1], Wan-Chen Hsieh[1], Wei-Wen Chang[2], Ping-Kun Hsiao[2], Mao-Chih Hsieh[2], Tzeon-Jye Chiou[1], Tzu-Yao Liao[1], H-Eugene Liu[1], Hsin-Hsien Yu[2], Chih-Hsin Lee[3]

[1]Hemato-oncology, Taipei Municipal Wanfang Hospital (Managed by Taipei University), Taipei City, Taiwan,[2]General surgery, Taipei Municipal Wanfang Hospital (Managed by Taipei University), Taipei City, Taiwan,[3]Pulmonary medicine, Taipei Municipal Wanfang Hospital (Managed by Taipei University), Taipei City, Taiwan

CDK 4/6 inhibitors improve survival in HR+ and HER2 breast cancer patients but carry risks of QT prolongation. Single ECG readings may be unreliable. This study assesses QT prolongation via 12-hour ECG monitoring for three CDK 4/6 inhibitors.
Methods: A prospective cohort study (Apr 2023-Aug 2024) included HR+, HER2- breast cancer patients receiving CDK 4/6 inhibitors. Exclusions were QTc >500 msec and persistent Af. Ribociclib/Palbociclib followed a 21/7 regimen; Abemaciclib was continuous. ECG monitoring occurred pre-treatment and on day 14 (Ribociclib/Palbociclib) or day 14 only (Abemaciclib). ECGs were recorded from 8 PM-8 AM and stored on the PCA 500 cloud. Drug blood concentrations were measured on day 8, with dose adjustments as needed.
Results: From July 16, 2023, to July 31, 2024, 33 patients were included: Ribociclib (N=19), Abemaciclib (N=9), and Palbociclib (N=5). Case 36 underwent testing at 600 and 400 mg Ribociclib due to QT prolongation.

- Ribociclib: Median baseline QTcF was 422 msec, rising to 431 msec (400 mg, N=7) and 440 msec (600 mg, N=13) post-treatment. QTcF Max >500 msec increased from 23% to 54% in the 600 mg group.
- Palbociclib: Median QTcF rose from 431 msec (washout) to 439 msec (100 mg, N=4) and decreased to 421 msec (125 mg, N=4). QTcF Max >500 msec rose from 20% to 40% post-treatment.
- Abemaciclib: Post-treatment QTcF medians were 425 msec (100 mg, N=1), 433 msec (150 mg, N=5), and 434 msec (alternative 150 mg, N=3). QTcF Max >500 msec increased to 56% post-treatment.

Conclusions: Ribociclib and Abemaciclib prolonged QTcF Max >500 msec, especially at higher doses. Palbociclib had less QT prolongation and reduced QTcF at 125 mg. 12-hour ECG monitoring underscores the need for closer cardiac safety measures.

Patient characteristic	Ribociclib 400mg	Ribociclib 600mg	Abemaciclib	Palbociclib
Patients, N	7*	13	9	5
Age, yr- (mean,range)	66 (53-78)	64 (49-90)	49 (37-67)	63 (45-75)
Female, %	7 (100%)	13 (100%)	9 (100%)	5 (100%)
DDI prolong QT, N	1 (14%)	1 (7%)	0	0
eGFR <60, N	1 (14%)	2** (15%)	3 (33%)	0
ALT > 3 UNL, N	0	1 (7%)	0	0
QTcF Max >500 msec. before CDK-4/6i, N (%)	1* (14%)	3 (23%)	-	1 (20%)
QTcF Max >500 msec. after CDK-4/6i, N (%)	1* (14%)	7 (54%)	5 (56%)	2 (40%)

*Case 36 used Ribociclib 600mg initially, then underwent dose reduction to 400mg for*One patient is ESRD under regular hemodialysisYr = year-old, DDI = drug-drug interaction, UNL = upper normal limit, Max = maximum

#789 The role of the cardio-splenic axis in doxorubicin-induced exacerbation of hypertension-associated cardiomyopathy.

MARY RAPHEL DANIEL[1], Benu George[1], Marianne K.O Grant[1], Korbyn J.V. Dahlquist[2], Christina D. Camell[2], Beshay N. Zordoky[1]

[1]Experimental and Clinical Pharmacology, University of Minnesota, College of Pharmacy, Minneapolis, MN,[2]Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN

Introduction: Doxorubicin (DOX), a commonly used chemotherapeutic agent in childhood cancers, is associated with significant cardiotoxic side. Hypertension is a known risk factor for heart failure in DOX-treated childhood cancer survivors. This study utilizes a "two-hit" mouse model, wherein juvenile DOX exposure amplifies adult-onset hypertension-associated cardiomyopathy. We hypothesize that hypertension mobilizes DOX-induced immunosenescent cells from the spleen to the heart via the cardio-splenic axis, contributing to cardiac dysfunction. The primary objective was to delineate the role of the cardio-splenic axis and CCR2+ immune cell recruitment in DOX/hypertension-induced cardiomyopathy.
Methods: Male C57BL/6N mice (5 weeks old) were treated with DOX (4 mg/kg/week) or saline for 3 weeks, followed by a 5-week recovery period. Subsequently, hypertension was induced with angiotensin II (ANG II; 1.4 mg/kg/day) or saline for 14 days. Cardiac function was assessed via echocardiography, and gene expression of inflammatory and immune recruitment markers in the heart and spleen was quantified using real-time PCR. Flow cytometry was performed to evaluate changes in splenic immune cell subpopulations, focusing on CCR2+ immune cells. To assess the functional role of CCR2, the effects of DOX/hypertension on cardiac function were assessed in CCR2 knockout mice.
Results: Low-dose DOX treatment alone did not cause significant cardiac dysfunction in young C57BL/6N mice. However, ANG II-induced hypertension markedly exacerbated cardiac dysfunction in DOX-treated mice, as evidenced by a significant reduction in cardiac output and a remarkable inflammatory/fibrotic remodeling. DOX treatment disrupted splenic immune cell populations, with an increase of CCR2+ immune cells. ANG II-induced hypertension further compounded this effect by causing splenic contraction in DOX-treated mice. ANG II-induced hypertension upregulated gene expression of CCR2 in the heart and reduced it in the spleen of DOX-treated mice, suggesting that CCR2+ cells were mobilized to the heart via the cardio-splenic axis. CCR2 knockout mice were protected against DOX/hypertension-induced cardiac dysfunction, as KO mice maintained cardiac output compared to wild-type mice, underscoring the critical role of CCR2-mediated innate immune responses in the observed cardiac dysfunction.
Conclusions: These findings demonstrate that DOX-induced CCR2+ immune alterations exacerbate hypertension-associated cardiomyopathy via the cardio-splenic axis. Our data suggest that hypertension mobilizes CCR2+ immune cells from the spleen to the heart, driving inflammatory responses and cardiac dysfunction. Genetic deletion of CCR2+ cells protects against these effects, highlighting CCR2 as a potential therapeutic target to mitigate DOX/hypertension-induced cardiac dysfunction.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#79 Pax6 regulates stemness and enhances brain metastases in breast cancer.

Laiba Anwar[1], Asad Ur Rehman[1], Mohammad Abbas Ali Zaidi[1], Parvez Khan[1], Mahek Fatima[1], Md Arafat Khan[1], Aatiya Ahmad[1], Nivedeta Krishna Kumar[1], Moorthy P. Ponnusamy[1], Surinder K. Batra[1], Metin Uz[2], Juan Santamaria-Barria[1], Mohd Wasim Nasser[1]

[1]Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE,[2]Cleveland State University, Cleveland, OH

Brain metastasis (BrM) remains the most severe complication of breast cancer (BC), the most diagnosed cancer in females, associated with an abysmal prognosis. Our poor understanding of the molecular mechanisms driving the disease is one of the major reasons for the dearth of specific therapies. This highlights an urgent clinical need to decipher how BC cells form BrM. BCBrM is preceded by a period of dormancy, during which stem cell-like features of cancer cells may play a role in reactivating them to form metastatic lesions. Cancer stem cells, known as “brain tumor-initiating cells” contribute to tumor formation in the brain and could also lead to radio and chemoresistance in the treatment of brain metastases. We, therefore, conducted a bioinformatic analysis on publicly available transcriptomic datasets of matched primary and brain metastatic patient samples and found that Pax6, a transcription factor, was upregulated in the metastatic samples compared to the primary tumors. In tissue microarray (TMA) belonging to BrM patients, Pax6 expression was evident in the BrM lesions and nearly absent in normal brain tissue. Pax6 was found to be upregulated in BCBrM cell lines compared to primary BC cell lines. Pax6, a master regulator of transcription, essential for normal development of the eyes and central nervous system, has been implicated in cancer progression. Pax6 can promote proliferation, migration, and resistance to apoptosis. To delineate the essentiality of Pax6 in BCBrM, we performed CRISPR/Cas9-mediated Pax6 knockout (KO) in the BCBrM cell lines, which decreased proliferation, wound healing, and migration properties. Intracardiac injection of these Pax6 KO BCBrM cells into immunocompromised mice resulted in a significant reduction of brain metastatic tumor lesions compared to controls. Conversely, overexpression of Pax6 in primary BC cells induced a BrM-like phenotype, enhancing proliferation, migration, and wound healing. Since Pax6 regulates stem cell features we also found that Pax6 KO reduced the expression of stemness-promoting factors such as OCT4, NANOG, and KLF4. Further, to confirm the stemness features we found that in serum-free conditions, Pax6 KO cells significantly lacked mammosphere-forming abilities and side populations. These findings suggest that Pax6 mediates stemness in cancer cells and enhances the metastatic potential of BCBrM cells. Unraveling the role of Pax6 in BCBrM could potentially open new therapeutic avenues for the treatment of BCBrM patients which eventually will improve outcomes of these patients.

CLINICAL RESEARCH: Survivorship Research and Supportive Care
Poster Session

#790 Effect of heart failure on incidence rates of pneumonia, septic shock and overall mortality in patients with myelodysplastic syndrome.

Karnav Modi[1], **Himil Mahadevia**[1], Simran Chandra[1], Ibrahim Khamees[1], Parth Sharma[1], Deepthi Vodnala[2], Taiyeb Khumri[2]

[1]University of Missouri - Kansas City School of Medicine, Kansas City, MO,[2]Saint Luke's Hospital of Kansas City, Kansas City, MO

Introduction: Myelodysplastic syndromes (MDS) refer to disorders originating from clonal stem cells, marked by ineffective hemopoiesis and cytopenias. Patients with MDS have a higher incidence of cardiovascular mortality, with heart failure playing a significant role. This has been linked to persistent anemia, excess iron accumulation and a systemic inflammatory state. Patients with MDS have significantly higher rates of infectious events, with pneumonia (PNA) and sepsis being the most common infections. The influence of systolic heart failure (SHF) or diastolic heart failure (DHF) on the risk of infections in patients with MDS is unclear. We evaluated whether chronic SHF or DHF, as well as acute SHF or DHF in concomitance with MDS, affected the rates of PNA, septic shock and overall mortality.
Method: A retrospective study identified patients with MDS using specific ICD 10 codes (D46.9, D46A-C and D46.22) from the National Inpatient Sample 2019-2021. Patients with acute and chronic SHF or DHF were obtained using particular ICD 10 codes (I50.2 - I50.4). The incidence rates of PNA, septic shock and overall mortality were compared between MDS patients with each of these types of HFs versus those without any HF using multivariate logistic regression adjusting for demographic characteristics, hospital-related factors, and common comorbidities.
Results: A total of 223,975 patients were identified with MDS. Patients with MDS who had chronic SHF (n=11,505) showed increased rates of PNA (OR:1.4, $p<0.001$) and septic shock (OR: 1.4, $p <0.001$) but no significant difference in overall mortality ($p=0.603$) compared to those without any HF. Patients with MDS and acute SHF (n=12,560) had enhanced rates of PNA (OR: 1.4, $p<0.001$), septic shock (OR: 1.9, $p<0.001$), and overall mortality (OR:1.6, $p<0.001$) versus those without any HF. Patients with MDS and chronic DHF (n=18,990) had no difference in rates of PNA ($p=0.2$), septic shock ($p=0.16$), or overall mortality ($p=0.1$). Acute DHF (n=16,700) led to a greater incidence of PNA (OR: 1.5, $p<0.001$) and overall mortality (OR: 1.2, $p<0.001$), but no significant difference in the incidence of septic shock ($p=0.07$) in patients with MDS.
Conclusion: Acute SHF was associated with significantly elevated rates of PNA and septic shock in patients with MDS, whereas acute DHF was linked to an increased incidence of PNA. Chronic SHF demonstrated higher occurrences of both PNA and septic shock. Furthermore, acute SHF and DHF correlated with increased mortality rates. Consequently, fostering early collaboration between hematology specialists and those in cardio-oncology is essential for effectively managing patients who have both MDS and HF. This would enable the implementation of timely preventive measures and therapeutic interventions, leading to more comprehensive care strategies that may improve clinical outcomes for these patients.

#791 Increased aortic stiffening in premenopausal women and triple negative breast cancer patients: impact of biomarkers and breast cancer therapy- findings from the PREVENT Trial (WF-98213).

Lorraine N. Velez-Torres[1], Alexandra Thomas[2], Sarah Hatcher[2], Ralph B. D'Agostino[3], Nathaniel S. O'Conell[3], Carolyn J. Park[3], W. Gregory Hundley[1], Jennifer H. Jordan[1]

[1]Virginia Commonwealth University, Richmond, VA,[2]Duke University, Durham, NC,[3]Wake Forest University, Winston-Salem, NC

Background: Breast cancer (BC) systemic therapies have improved survival but are associated with cardiovascular (CV) risks, particularly in premenopausal women with hormone receptor-positive (HR+) BC under near-complete estrogen deprivation (NCED) and in those with triple-negative BC (TNBC) who may experience chemotherapy-induced menopause. This PREVENT Trial (NCT01988571) sub-analysis investigated the impact of BC treatment, menopausal status, and biomarkers on vascular stiffness over two years.
Methods: This randomized trial included 238 BC patients receiving either atorvastatin (40 mg/day) or placebo for two years (PREVENT Trial WF-98213 Wake Forest NCORP Research Base UG1CA189824, ECOG-ACRIN and Alliance). Aortic pulse wave velocity (PWV) and aortic distensibility (AoD), measured via cardiac magnetic resonance imaging (CMR), were assessed at baseline, 6 months, and 24 months as indicators of vascular stiffness. Longitudinal general linear mixed models adjusted for baseline values, hypertension, body mass index, BC subtype, menopausal status, and statin treatment group evaluated changes in vascular stiffness. Univariate adjustments were performed to assess the influence of biomarkers, including cholesterol, aldosterone, renin, IL-6, TNF-alpha, and CRP.
Results: Of 238 participants, 116 were premenopausal (65% HR+, 35% TNBC) and 122 postmenopausal (58% HR+, 42% TNBC). At baseline, PWV did not differ significantly between BC subtypes but by 24 months, TNBC patients exhibited significantly increased PWV ($p = 0.0298$). Premenopausal women, despite having lower baseline PWV, surpassed postmenopausal levels by 24 months. AoD declined significantly across all subgroups ($p < 0.05$), reflecting increased vascular stiffening. Preliminary analysis links biomarkers with vascular stiffness. In TNBC patients, every 1 ng/mL increase in renin is associated with a 0.18 $mmHg^{-1}$ AoD decrease at 6 months ($p=0.0343$). For IL-6, every 5 pg/mL increase is linked to a 0.145 $mmHg^{-1}$ AoD increase at 6 months and 0.192 $mmHg^{-1}$ at 24 months in TNBC and postmenopausal women ($p=0.0288$ and $p=0.0325$, respectively). For cholesterol, every 10 mg/dL rise in TNBC reduces AoD by 0.261 $mmHg^{-1}$, reflecting higher PWV ($p=0.0298$). Statin treatment did not mitigate aortic stiffening.
Conclusions: This study reveals that premenopausal and TNBC women undergoing anthracycline-based chemotherapy exhibit significant increases in vascular stiffness, highlighting their heightened CV risk. Biomarker interactions differed across subgroups, with renin and cholesterol linked to poorer vascular outcomes in TNBC patients, while IL-6 appeared to promote compensatory vascular remodeling in TNBC patients and postmenopausal women. Enhanced cardiac monitoring and targeted interventions may be essential for these high-risk populations.

#792 Immunoprotective effects of metformin against delayed doxorubicin-induced immune perturbation in C57BL/6 mice.

Ian J. Ahlberg[1], Mary R. Daniel[1], Benu George[1], Marianne K.O. Grant[1], Korbyn J.V. Dahlquist[2], Christina D. Camell[2], Beshay N. Zordoky[1]

[1]Department of Experimental and Clinical Pharmacology, University of Minnesota, Minneapolis, MN,[2]Institute on the Biology of Aging and Metabolism, University of Minnesota, Minneapolis, MN

Introduction: Chronic immunological perturbations that leave cancer survivors with a long list of sequelae, such as an increased risk of death due to infection, remain a major complication of traditional chemotherapy. This risk is particularly pronounced in childhood cancer survivors treated with doxorubicin (DOX), a well-known culprit of immunosuppressive activities that may linger for years following the cessation of chemotherapy. Metformin is a first-line drug against Type-2 Diabetes Mellitus and has recently entered the pharma-oncology limelight due to its senomorphic and immunoprotective properties. In this study, we determined the potential immunoprotective effects of metformin against delayed DOX-induced immune perturbation in a mouse model of childhood cancer survivors.
Methods: Five-week-old C57BL/6N mice were randomly assigned to four groups: Control, Metformin, DOX, and Metformin/DOX. DOX-treated mice received intraperitoneal injections with either DOX (4 mg/kg/week) or saline for three weeks. Metformin-treated mice were administered metformin (250 mg/kg/day) via drinking water. Metformin treatment began three days before the first DOX injection and was continued throughout and five weeks post DOX injections. Five weeks following the last dose of DOX, all mice were humanely euthanized in accordance with institutional guidelines. Blood and bone marrow samples were collected during the necroscopy and analyzed with a Flow Cytometer and a Hematological Analyzer to provide insight into individual immune cell populations.
Results: Metformin alleviated delayed DOX-induced perturbations in several immune subpopulations. In the innate immune system, metformin decreased neutrophil and monocyte frequency in the blood and bone marrow. However, metformin also ameliorated DOX-induced depletion of the $CCR2^+$ activated and $CD86^+$ invasive monocyte frequencies. In the adaptive immune system, metformin increased the frequency of lymphocytes, particularly B cells, in the blood. This trend is supported by the increased population of $Ki67^+$ proliferating B cells in the bone marrow across all stages of maturation.
Conclusion: DOX-induced lingering effects on immune cell populations were partially mitigated by metformin. Metformin improved the functionality of the attenuated monocyte population in the bone marrow and increased the frequency of B lymphocytes in the blood, which can be traced to the expanded population of proliferating B cells in the bone marrow. These results suggest metformin has dual effects on the immune system and may serve as a therapeutic option to mitigate chronic immune perturbations in childhood cancer survivors.

#793 Cardiometabolic risk factors among Puerto Rican men with prostate cancer.

Valeria Torres Matos[1], Katherine Carrero Figueroa[2], Noemi Santiago[3], Julie Dutil[4], William Roman Torreguitart[3], Carlos Joel Diaz Osterman[2]

[1]Biology, UPR - Rio Piedras Campus, San Juan, Puerto Rico,[2]UPR - Comprehensive Cancer Center, San Juan, Puerto Rico,[3]Puerto Rico Urology Group, Mayaguez, Puerto Rico,[4]Clinical and Translational Research, UPR - Comprehensive Cancer Center, San Juan, Puerto Rico

Background: Cardiovascular and metabolic disorders are significant contributors to morbidity and mortality among men with prostate cancer. However, few studies characterize the association between cardiometabolic comorbidities and tumor risk in the post-operative space among high-risk groups. Like African American men, Puerto Rican men have a higher risk of developing and dying from prostate cancer than other racial and ethnic groups in the United States. As cardiovascular and metabolic risk factors are highly prevalent in Puerto Rico, we proposed to investigate coordinated risk evaluation of cardiometabolic and oncologic risk in this population. We hypothesized that a subset of cardiometabolic risk factors consistently correlate with clinically defined aggressive disease.
Methods: The Puerto Rico Urology Group (PRUG) is the largest group urology practice in Puerto Rico. It currently consists of ten urologists serving the Western, Southern, Central, and Metropolitan regions of Puerto Rico including the island municipalities of Vieques and Culebra. Our cohort (n=388) of patients underwent robotic assisted radical prostatectomy for prostate cancer between 2015 and 2022. Clinical data were extracted from consented patients' electronic health records with appropriate IRB approval (Ponce Research Institute IRB no. 2210121134, University of Puerto Rico Comprehensive Cancer Center IRB no. 2023-08-111). Post-surgical cancer of the prostate risk assessment (CAPRA-S) was used to stratify tumors and cardiometabolic risk factors were evaluated across tumor risk strata.
Results: The majority of study participants had low-risk tumors (238/388, 61.3%), with intermediate tumors accounting for 106/388 (27.3%), and high-risk tumors accounting for 44/388 (11.3%). Higher CAPRA-S score was significantly associated with greater tumor mass ($p<0.001$), presence of perineural invasion ($p<0.001$), and presence of hyperlipidemia ($p<0.001$).
Conclusion: The Puerto Rican population represents the second largest Hispanic/Latino subgroup in the United States and has disproportionately high rates of prostate cancer and cardiometabolic comorbidity. Our preliminary findings indicate that the coordinated evaluation of cancer risk in the context of high-risk disease may provide additional insights for clinicians managing prostate cancer among populations with high prevalence of these chronic diseases. These findings provide insights for ongoing studies to evaluate large, multi-institutional cohorts and identify molecular indicators of cardiometabolic risk predictive of prostate cancer aggressiveness among diverse populations.
Acknowledgements: This study was supported by funding from the Puerto Rico IDeA Network of Biomedical Research Excellence (PR-INBRE, grant no. 2P20GM103475-21), awarded to C.J.D.O. through the Developmental Research Projects Program.

#794 Comprehensive outcomes for after cancer health (COACH): Interim analysis of physical function data for individuals with diverse tumor types following primary cancer therapy.

Marilyn Hammer[1], Robin Lally[2], Barbara Halpenny[1], Hajime Uno[1], Ijeoma Eche-Ugwu[1], Whitney Posada[1], Inga Van Wieren[1], Alexi Wright[1], Ayodeji Adeniji[3], Sarah Alzahid[4], Lauren A. Anderson[5], Betty Arceneaux[3], Jerry Armah[4], Elizabeth Arthur[6], Kristen Fessele[7], Laura Flora[6], Alyssa Garcia[5], Anneliese Gonzalez[3], Michael Harrison[8], Brian Hulbert[8], Mariah Jackson[2], Kaukab Jafry[3], Rebecca Johnson-Beller[2], Abbey Kaler[5], Ginny Kirklin[5], Jessica Krok-Schoen[6], Hilda H. Lewis[9], Jennifer Loftis[8], Debra Lyon[4], Kathrin Milbury[5], Ariana Mohan[7], Jasmine Moore[8], Estee Murray-Ross[8], Darcy Ponce[5], Leorey Saligan[10], Elham Samami[4], Rachael Schmidt[2], Grace Smith[5], Angela Starkweather[11], Maria Chang Swartz[5], Martha Thompson[3], Gisele Tlusty[5], Meagan Whisenant[5], Gloria Yang[7], Kelly J. Brassil[8]

[1]Cantor Center for Nursing Research, Dana-Farber Cancer Institute, Boston, MA,[2]University of Nebraska, Omaha, NE,[3]UT Health Houston, Houston, TX,[4]University of Florida College of Nursing, Gainsville, FL,[5]The University of Texas MD Anderson Cancer Center, Houston, TX,[6]Ohio State University, Columbus, OH,[7]Memorial Sloan Kettering Cancer Center, New York, NY,[8]Pack Health, A Quest Diagnostics Company, Birmingham, AL,[9]Montefiore Medical Center, Bronx, NY,[10]National Institute of Nursing Research, Bethesda, MD,[11]Rutgers School of Nursing, Newerk, NJ

Purpose: Wellness interventions are important for improving outcomes, including physical function (PF), in cancer survivors. The COACH study is a randomized, wait-list controlled trial assessing feasibility of a 6-month digital health coaching (DHC) program for individuals within one year of completing primary cancer therapy, and those with metastatic disease. Diverse patient-reported outcome (PRO), wearable, device, gut microbiome, and dietary data are collected up to once monthly from 675 individuals over 12 months. This interim analysis examined change in PF using the PROMIS Short Form v2.0-PF 10a.

Methods: The analysis included a pooled sample of participants who completed baseline and 6-month assessments as of November 2024. Descriptive statistics evaluated trends in physical function by age, sex, enrollment site, metastatic disease, and tumor and primary treatment type.

Summary Data: Among the 352 individuals enrolled across 7 study sites, 177 completed baseline and 6-month assessments to date. Mean age of participants was 59.7 (R 30-83, SD 11.8). Fourteen participants (8%) identified as Black or African American, 9 (5%) as Hispanic or Latino, 147 (83%) as non-Hispanic White, and 7 (4%) as other, including Asian, American Indian, or Alaskan Native. Mean PROMIS PF scores by analysis group are presented in Table 1.

Conclusions: An interim analysis of PF data demonstrated trending improvements across all groups from baseline to 6 months, except at one site enrolling only individuals with metastatic breast cancer, for whom mean difference is lower than in those with non-metastatic disease. Minimally important differences in PROMIS PF for individuals with advanced cancer is 4-6 points. At study completion (July 2026), a full analysis will be conducted to explore differences in PF by various patient characteristics, and if and how DHC may impact these trends over time.

PROMIS Physical Function (PF) Scores by Demographic and Clinical Variables (n= 177)

Variable		N	PROMIS PF (Baseline)		PROMIS PF (6 months)		Difference (6 months-Baseline)	
			Mean	SD	Mean	SD	Mean	SD
Sex	Male	6 (3.4%)	48.85	11.19	50.58	12.66	1.73	4.97
	Female	171 (96.6%)	46.71	7.28	48.76	8.19	2.05	5.38
Cancer Diagnosis	Ovarian	33 (18.6%)	47.17	6.29	50.18	8.16	3.01	5.16
	Breast	109 (61.6%)	46.51	7.81	48.18	8.36	1.66	5.16
	Endometrial	28 (15.8%)	47.45	6.98	49.85	8.09	2.40	6.41
	Other	7 (4%)	46.49	8.81	48.37	10.39	1.89	5.24
Age	<65 years	104 (58.8%)	47.27	7.33	48.64	8.32	1.37	5.93
	>/= 65 years	73 (41.2%)	46.09	7.51	49.08	8.42	2.99	4.29
Metastatic Disease	Yes	10 (5.6%)	48.46	8.64	49.07	11.59	0.61	9.19
	No	166 (93.8%)	46.75	7.31	48.89	8.11	2.14	5.09
Treatment Type	Chemotherapy, Immunotherapy, Biotherapy only	9 (5.1%)	40.56	4.27	43.53	5.45	2.98	3.81
	Surgery only	21 (11.9%)	48.08	8.19	51.30	8.75	3.22	5.29
	Radiation only	24 (13.6%)	46.36	8.66	47.95	9.79	1.59	5.27

	2 treatment modalities	87 (49.2%)	47.66	7.42	49.55	8.36	1.89	5.19
	3 or more treatment modalities	36 (20.3%)	45.74	5.90	47.52	7.08	1.78	6.26
Site	1	47 (26.6%)	47.59	6.38	50.75	8.15	3.16	6.33
	2	33 (18.6%)	46.06	7.68	47.64	8.27	1.58	4.11
	3	9 (5.1%)	45.34	8.52	47.33	10.47	1.99	6.52
	4	76 (42.9%)	46.98	7.64	49.06	8.14	2.09	4.97
	5	12 (6.8%)	45.44	8.76	44.13	7.60	-1.32	4.98

#795 Acute and long-term effect of prepubertal chemotherapy on spermatogenesis, fertility and offspring health.

Meghali Joshi, Duane G. Pereira, Sonia de Assis

Department of Oncology, Georgetown Lombardi Comprehensive Cancer Ctr., Washington, DC

Early detection and treatment of childhood cancers have improved the cure and the survival rates for more than a decade. However, long-term survivors of childhood cancers can experience fertility problems because of chemotherapy treatment. This is a major problem particularly for young boys, for whom no fertility preservation treatment is yet established. Here, we have determined the acute and long-term effect of cyclophosphamide (an alkylating drug commonly used in childhood cancers) on the germ cell development, fertility and on the health of their subsequent generations. We treated the prepubertal mice with clinically relevant dose of either cyclophosphamide (low dose- 100mg/kg and high dose- 200mg/kg), and vehicle control (sterile PBS), respectively. Acute exposure to both low and high doses of cyclophosphamide led to morphological changes in the prepubertal testis. Moreover, prepubertal treatment had long-term effect as the sperm concentration and motility in adult mice decreased in a dose dependent manner. Infertility and sub-fertility were observed in males exposed to high, but not in low dose of cyclophosphamide as compared to control males. There was no difference in the litter size born to cyclophosphamide exposed fathers as compared to controls. However, there was a sex specific increase in the litter weights of the female offspring born to fathers exposed to high dose of cyclophosphamide as compared to controls. Additionally, a high male to female birth sex ratio was found in the litters born to cyclophosphamide exposed fathers as compared to controls, suggesting that the cyclophosphamide exposure may be impacting the paternal X chromosome. We also investigated the adverse effect of paternal cyclophosphamide exposure on the metabolic health of F1 offspring. Female, but not male offspring of fathers exposed to cyclophosphamide, developed severe insulin resistance compared to controls. We have previously shown that sperm small non-coding RNAs contribute to the intergenerational transmission of paternally acquired metabolic disorders. Using small RNA sequencing, we found alterations in the population of small non-coding RNAs in the sperm of cyclophosphamide exposed fathers as compared to controls suggesting that sperm small non-coding RNAs may contribute to the adverse effect of pre-pubertal cyclophosphamide exposure. Overall, our results demonstrate that exposure to clinically relevant dose of cyclophosphamide in prepubertal mice not only affects the reproductive health of exposed mice but could also affect the health of their off-spring by altering the sperm small non-coding RNAs.

#796 Incidence of depression and anxiety in kidney cancer survivors: A nationwide population-based cohort study.

M. Jung[1], M. Li[1], E. Choo[2], S. Lee[2], D. Spiegel[1], M. Baiocchi[1], Z. Deng[1], J. Li[1], M. E. Langston[1], M. L. Bondy[1], B. I. Chung[1]; [1]Stanford University School of Medicine, Stanford, CA, [2]Ajou University, Suwon, Korea, Republic of

Depression and anxiety are highly prevalent among kidney cancer survivors, yet limited evidence exists on their cumulative incidence following kidney cancer diagnosis and their associations with the diagnosis. We conducted a nationwide, population-based retrospective cohort study using the Korean National Health Insurance and Medical Checkup Linkage Database. Adults, over 20 years, diagnosed with kidney cancer from 2010 to 2020 were included, along with age- and sex-matched non-cancer comparators. Individuals with prior cancer, depression, or anxiety were excluded. We 1) quantified the empirical risk trajectories of depression and anxiety in kidney cancer survivors for up to five years post-diagnosis, and 2) estimated the time-dependent associations between kidney cancer diagnosis and the incidence of these conditions within three post-diagnosis intervals (0-1, 1-3, and 3-5 years) using inverse-probability weighted Cox regressions. Until five years post-diagnosis, survivors (n=24,310) consistently had a higher cumulative incidence of depression (2.8% vs 2.2%) and anxiety (3.3% vs 2.6%) compared with comparators (n=173,471). Additionally, survivors (n=16,049) had an increased hazard of depression (hazard ratio (HR)=1.92, 95% confidence interval (CI): 1.52-2.42) and anxiety (HR=1.63, 95% CI: 1.31-2.02) compared to comparators (n=100,782) within the first year. During the subsequent 1-3 years, the hazard of anxiety remained increased (HR=1.32, 95% CI: 1.07-1.62). In conclusion, kidney cancer survivors experienced a higher rate of depression and anxiety, especially in the early post-diagnosis period, compared to the non-cancer population. Our findings emphasize the need for proactive prevention and management of psychiatric disorders in kidney cancer patients.

#798 The genetic link between psychosocial stress and outcome in oral squamous cell carcinoma.

Minh Phuong Dong[1], Gary Yu[2], Michele Arambula[1], Bradley Aouizerat[3], Khanh Nguyen[1], Paul Walker[1], Carissa Thomas[4], Chi Viet[1]

[1]Loma Linda University, Loma Linda, CA,[2]Columbia University, New York, NY,[3]New York University, New York, NY,[4]University of Colorado, Boulder, CO

Psychological well-being predicts future physical health and mortality beyond its association with reduced stress and depression. Studies on psychological well-being in cancer have not evaluated the effect of psychosocial stressors on cancer outcomes. The objective of this study was to investigate the impact of psychosocial stressors and their resultant dysregulated genetic pathways on oral squamous cell carcinoma (OSCC) outcomes. This prospective multi-institutional study enrolled 173 OSCC patients to determine prior and current psychosocial stressors (using the Adverse Childhood Experience - ACE and Life Events Checklist - LEC-5), characterize cancer symptoms and quality of life disturbance (using the UCSF Oral Pain Questionnaire - UCSF-OPQ, modified Brief Pain Inventory - BPI, and EORTC-QLQC30 and H&N35), and collect flash-frozen cancer tissue for RNA-sequencing. Data was analyzed by a biostatistician using Pearson correlation, Student's t-test, one-way ANOVA, Kaplan Meier log-rank test and Cox hazard regression. Our cohort consisted of 99 males, 74 females; racial/ethnic composition included 118 White, 14 Asian, 12 Black, and 28 White-Hispanic. The mean age of the cohort was 64. Patients who had high cancer pain (UCSF-OPQ) had significantly greater physical and psychological interferences (modified BPI; $p<0.05$). Patients with worse pain also had disturbances in all 6 functional categories measured by EORTC-QLQC30, including physical functioning, role functioning, emotional functioning, cognitive function, social functioning, and global health status ($p<0.0001$). In terms of the effect of childhood or adult psychosocial stressors on cancer outcomes, patients with a high ACE or LEC-5 score had worse cancer symptoms (in all symptom scales including modified BPI, EORTC-QLQ, and H&N35) independent of their pathologic stage. A Cox hazard regression model showed that the H&N35 score (HR = 1.969, CI 95% [1.060 - 3.659]) was independent of pathologic stage (HR =1.808, CI 95% [1.244-2.628] and age (HR: 1.669, CI 95% [1.151-2.419]) in predicting survival. Analysis of RNA-seq data in flash-frozen cancer tissue of 50 OSCC patients showed that the extracellular matrix organization and the regulation of integrin-mediated signal pathways were dysregulated in patients with high pain levels. Additionally, patients with psychosocial stress exhibited high expression of genes such as *RLP6*, *RPL11*, and *RPL23*, which are known to play significant roles in lung and gastric cancer but not previously associated with OSCC. Enriched pathways in these patients included ribonucleoprotein complex biogenesis and regulation of p53-mediated signal transduction. Our results demonstrate that psychosocial stressors worsen cancer symptoms, which is independently associated with worse cancer outcomes and survival. We delineate the significant dysregulated pathways linking behavioral health to cancer outcomes.

#799 Comparing the efficacy of infliximab and vedolizumab in treating steroid-refractory immune checkpoint inhibitor-induced colitis.

Irene Li[1], Sandip P. Patel[2]

[1]School of Medicine, University of California San Diego, La Jolla, CA,[2]Moores Cancer Center, University of California San Diego, La Jolla, CA

Purpose: This study aims to evaluate the efficacy of infliximab and vedolizumab in managing corticosteroid-refractory immune checkpoint inhibitor (ICI)-induced colitis. Some patients receiving immunotherapies develop severe, grade 3+ ICI-induced colitis, which often requires second-line treatments such as biologics commonly used for irritable bowel disease. Although there is literature supporting the use of infliximab, limited data exist regarding the efficacy and specific use of vedolizumab for ICI-induced colitis.
Methods: A retrospective analysis was conducted on patients treated with either vedolizumab or infliximab alongside a checkpoint inhibitor over the past five years (March 1, 2019 - March 1, 2024). Patients included were treated with vedolizumab and received one of the following immunotherapies at UC San Diego: pembrolizumab, nivolumab, ipilimumab, atezolizumab, durvalumab, or cemiplimab. Patients treated with infliximab/vedolizumab for other indications, such as conventional irritable bowel syndrome, were excluded.
Results: The database search identified 16 patients on infliximab and 18 on vedolizumab. The final analysis included 9 patients in the infliximab group and 8 in the vedolizumab group. Most patients were white and had various types of cancer, predominantly at advanced stages (III or IV). Of the patients, 41% were receiving PD1-targeted immunotherapy at the onset of colitis symptoms. Patients received an average of 1.7 doses of infliximab (range: 1-8) and 3.5 doses of vedolizumab (range: 1-8). Among infliximab-treated patients, 66% received a single dose during hospitalization without further treatment, compared to 12.5% of vedolizumab-treated patients. Infliximab enabled 66% of patients to successfully complete a steroid taper, while vedolizumab had a success rate of 75% (Chi-square, $p = 0.70$). The average time on steroids was longer in the vedolizumab group (30.21 weeks) than in the infliximab group (17.97 weeks), though this difference was not statistically significant ($p = 0.80$). No significant differences were observed when results were stratified by sex (infliximab, $p = 0.40$; vedolizumab, $p = 0.21$) or type of immunotherapy (infliximab, $p = 0.96$; vedolizumab, $p = 0.21$).
Conclusion: Both infliximab and vedolizumab appear to be viable options for treating ICI-induced colitis, with most patients successfully completing a steroid taper. However, this study does not provide sufficient evidence of superior outcomes for either drug, although some patients responded well to treatment. Therefore, infliximab remains a reasonable first-line treatment for steroid-refractory ICI-induced colitis.

TUMOR BIOLOGY: Cancer Stem Cells
Poster Session

#8 Identifying the role of KIF20A in stemness and metastasis in hepatocellular carcinoma.

Liuxian Ban[1], Xiaona Fang[2], Shan Liu[3], Jie Luo[1], lanqi Gong[1], Hongyu Zhou[1], Beilei Liu[4], Jiao Huang[5], Nanzhou Yu[1], Yingchen Lyu[1], Licheng Tan[1], Qin Liu[1], Cuicui Huang[1], Xin-Yuan Guan[1]

[1]The University of Hong Kong, Hong Kong, China,[2]Sun Yat-sen University Cancer Center, Guang Zhou, China,[3]Sichuan Cancer Hospital & Institute, Sichuan Cancer Center, Sichuan, China,[4]City University of Hong Kong, Hong Kong, China,[5]Cutaneous Biology Research Center, Massachusetts General Hospital, Boston, MA

Hepatocellular carcinoma (HCC) is remarkable for its high recurrence and metastasis rate. According to the cancer stem cell (CSC) model, a small group of stem cells is capable of keeping self-renewal, initiating relapse and metastasis in tumors. However, the mechanism of CSCs in regulating HCC recurrence and metastasis awaits further investigation. We previously established an in vitro human hepatocyte differentiation model and defined four developmental stages: embryonic stem cell (ES), endoderm (EN), liver progenitor cell (LN) and premature hepatocyte (PH). Kinesin family member 20A (KIF20A) was found highly expressed in ES and EN stages while decreasing sharply in LP and PH stages. Besides, in an online GEO dataset, we regrouped single cells into the hepatocyte, stem-like HCC and HCC subclusters according to their expression profiles. KIF20A was spotted mostly in the stem-like HCC cluster with a low proportion, sharing the same expression pattern with CD133, suggesting its potential role in cancer stemness. Moreover, KIF20A expression was positively correlated with stemness markers CD133, Epcam, CK19, and MYCN in our clinical cDNA array. In the TCGA-LIHC dataset, KIF20A expressed higher in tumor tissues than normal counterparts, and related to shorter overall survival in patients. Functionally, KIF20A promoted cell proliferation, induced sorafenib resistance, increased sphere formation, and enhanced cell migration and invasion in vitro. In subcutaneous mouse models, liver orthotopic mouse models and hydrodynamic transfection mouse model of HCC, KIF20A fueled tumorigenicity and lung metastasis significantly. After mRNA sequencing of KIF20A knock-down cells, bioinformatic analysis suggested that KIF20A may modulate metastatic biological processes via the Hippo pathway. Western Bloting further confirmed the result of pathway changes in protein level. The underlying mechanism of how KIF20A interacts with Hippo pathway still needs further investigation. In conclusion, we provided new insights into the role of KIF20A in regulating stemness and metastasis in HCC. KIF20A might be a potential target for HCC treatment in the future.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#80 Integrin driven adhesion in ovarian cancer.

Nazia Bano[1], Isabelle Lewis[2], Malaika Amin[3], Eva M. Schmelz[2]

[1]Virginia Polytechnic Institute & State University, Blacksburg, VA,[2]Human Nutrition, Foods, and Exercise, Virginia Polytechnic Institute & State University, Blacksburg, VA,[3]Hollins University, Roanoke, VA, Roanoke, VA

Ovarian cancer is the fifth leading cause of cancer-related deaths in women due to late-stage diagnosis, reducing the survival rate to 30%. During dissemination, ovarian cancer cellsexfoliate from the primary tumor and aggregate to form spheroids in the peritoneal cavity. Peritonealaggregation enhances the survival of cancer cells by mitophagy, reduced metabolism, andproliferation. However, as spheroids adhere to secondary sites, mitochondrial function is restoredby mitobiogenesis, with increased metabolism and proliferation. These findings strongly suggest aswitch from mitophagy in spheroids to mitobiogenesis upon adhesion. However, the mechanism bywhich adhesion induces mitobiogenesis remains unknown. Therefore, our objective is to identifykey adhesion receptors, with a focus on integrins that activate adhesion and downstream signalingcascades. To investigate changes in integrin expression, we mimicked three major stages ofovarian cancer progression by developing adherent, spheroid, and adherent spheroid stages usingmouse ovarian surface epithelial (MOSE) cell lines. These represented the benign (MOSE-E) stage,as well as slow-developing (MOSE-L) and fast-developing (MOSE-LTIC*v*) disease in biologicallyrelevant conditions. We identified integrin and adhesion-related gene expression levels via RT-qPCR. Western blotting and confocal microscopy were used to observe protein expression andlocalization throughout ovarian cancer progression. Drug treatment was done to observe the effectof integrin inhibition on spheroid aggregation and adhesion to secondary sites. Our finding revealeddistinct expression changes of different integrins during aggregation and adhesion, as well asspecific changes of other adhesion molecules, ECM, and their regulators indicating specific rolesin metastatic dissemination and implantation. This was further confirmed with specific integrininhibitors that were effective in preventing aggregation and spheroid adhesion to secondary sitesindicating distinct roles of integrins during the metastatic stages. Overall, this project will aid inidentifying integrin and integrin drug targets to develop new metastatic treatments, ultimately aimingto reduce mortality in women with ovarian cancer.

Keywords: Spheroids, mitophagy, adherent spheroid, mitobiogenesis, mouse ovarian surfaceepithelial, integrins, metastasis, tumorigenesis, dissemination, implantation

CLINICAL RESEARCH: Survivorship Research and Supportive Care
Poster Session

#800 Safety and efficacy of same-day administration of novel long-acting G-CSF efbemalenograstim α in breast cancer patients undergoing cytotoxic chemotherapy.

Shoubing Zhou[1], Jing Pan[1], Qiujun Zhang[1], Wenyu Li[1], Dandan Hu[1], Qianyu Zhang[1], Shikai Hong[2], Zhengzhi Zhu[2], Hu Liu[3], Xudong Zhang[4], Hua Xie[5], Yueyin Pan[3]

[1]Department of Tumor Biotherapy (5th Ward of the Department of Oncology), Anhui Provincial Cancer Hospital, West District of the First Affiliated Hospital of University of Science and Technology of China, Anhui Provincial Hospital, Hefei City, Anhui Province, China,[2]Department of Breast Tumor，Anhui Provincial Cancer Hospital, West District of the First Affiliated Hospital of University of Science and Technology of China, Anhui Provincial Hospital, Hefei City, Anhui Province, China,[3]Anhui Provincial Cancer Hospital, West District of the First Affiliated Hospital of University of Science and Technology of China, Anhui Provincial Hospital, Hefei City, Anhui Province, China,[4]The Affiliated Chuzhou Hospital of Anhui Medical University, the First People's Hospital of Chuzhou, Chuzhou City, Anhui Province, China,[5]Xuancheng People's Hospital, the Affiliated Xuancheng Hospital of Wannan Medical College, Xuancheng City, Anhui Province, China

Background: There is controversy over whether Granulocyte colony-stimulating factor (G-CSF) can be used within 24 h after chemotherapy. Efbemalenograstim α is a novel long-acting G-CSF approved by FDA in 2023. Guard-02 trial (ChiCTR2300078792) was to evaluate the efficacy and safety of efbemalenograstim α for prevention of chemotherapy-induced neutropenia (CIN) within 24 h after chemotherapy.
Methods: This was a prospective, multicenter, single-arm, two-stage clinical study. Breast cancer patients received chemotherapy with epirubicin (90 mg/m^2) and cyclophosphamide (600 mg/m^2) for 4 cycles were enrolled. If less than 9 cases of grade 3-4 neutropenia occurred in 16 patients in first stage, the toxicity was acceptable and more patients would be recruited in second stage. Patients received 20 mg efbemalenograstim α at 24±4 h (d2) after chemotherapy in first cycle and at 4±1 h (d1) after chemotherapy in subsequent cycles. The primary endpoint was incidence of grade 3-4 (ANC <1×10^9/L) neutropenia in cycle 1-2. The safety profile was evaluated.
Results: The incidence of grade 3-4 neutropenia in cycle 1-2 and cycle 1-4 of 16 patients (median age at 51 years, r.35-60) were 25.00% (90% CI: 9.03%-48.44%) and 31.25% (95% CI: 11.02%-58.66%), both lower than that in phase III trial using efbemalenograstim α at 48 ± 4 h (d3) after EC chemotherapy (NCT04174599, whose incidence of grade 3-4 neutropenia in cycle 1-2 and cycle 1-4 were 34.16% and 36.67%). Using efbemalenograstim α within 24 h after chemotherapy was safe, the most frequently adverse event (AE) related to study drug was muscle pain (6.25%). The incidences of AEs were low and no serious adverse event was related to study drug.
Conclusions: Using long-acting G-CSF on same-day of chemotherapy was as safe and effective as on d3 of chemotherapy in reducing CIN. In patients with daytime chemotherapy, efbemalenograstim α provides oncologists an alternative for simplifying management of CIN.

	Cycle 1-2	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 1-4
The incidence of grade 3-4 neutropenia in each cycle (n/N, %)	4/16(25.00)	1/16 (6.25)	3/16 (18.75)	1/16 (6.25)	1/16 (6.25)	5/16 (31.25)
The incidence of grade 4 neutropenia in each cycle, (n/N, %)	1/16 (6.25)	0/16 (0.00)	1/16 (6.25)	0/16 (0.00)	1/16 (6.25)	2/16 (12.50)
The incidence of febrile neutropenia (FN) in each cycle, (n/N, %)	1/16 (6.25)	0/16 (0.00)	1/16 (6.25)	0/16 (0.00)	0/16 (0.00)	1/16 (6.25)
The duration of grade 3-4 neutropenia in each cycle (median[95% CI], days)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 1)

#801 Mechanism of oxaliplatin-induced cachexia: A transcriptional and physiological perspective.

Junwei Du[1], Rachel L. Mintz[2], Rachael Field[3], Leland C. Sudlow[1], John E. Morley[4], Jonathan R. Brestoff[3], Gwendalyn J. Randolph[3], **Mikhail Y. Berezin**[5]

[1]Radiology, Washington University In St. Louis, St. Louis, MO,[2]Department of Biomedical Engineering, Pathology and Immunology, Washington University In St. Louis, St. Louis, MO,[3]Pathology and Immunology, Washington University In St. Louis, St. Louis, MO,[4]Saint Louis University, St. Louis, MO,[5]Washington University In St. Louis, St. Louis, MO

Background: Cancer cachexia is a multifactorial, largely irreversible metabolic disorder characterized by skeletal muscle and adipose tissue depletion that is responsible for 20% of cancer-related deaths. Oxaliplatin, a chemotherapeutic agent commonly used to treat colorectal cancer and other malignancies, is associated with weight loss, muscle weaknesses, and metabolic dysregulation. Yet it is unknown whether oxaliplatin mediates these effects independently of cancer.
Methods: Non-tumor-bearing immunocompetent C57BL/6 mice were administered a human-equivalent dose of oxaliplatin over 8 weeks. Metabolic parameters were assessed using metabolic cages and EchoMRI, followed by post-mortem bulk RNA sequencing of muscle, adipose tissue, and the hypothalamus. The results were compared to data sets from cachexia models in cancer and fasting.
Results: Oxaliplatin induced significant weight loss (~12%), decreased lean mass, reduced food intake, decreased physical activity, and lowered core body temperature. Adipose tissue exhibited extensive transcriptional dysregulation, with over 2,000 differentially expressed genes (DEGs) affecting metabolic and inflammatory pathways. Muscles exhibited transcriptional evidence of atrophy, supported by histological images. Hypothalamic RNA-seq revealed dysregulated thermogenic and adipocytokine pathways and overexpression of neuropeptides such as neuropeptide Y (NPY), linking peripheral metabolic changes to central regulation. Comparisons with clinical and preclinical cancer and fasting models demonstrated overlapping yet distinct transcriptional signatures.
Conclusion: Our study demonstrates that oxaliplatin independently induces cachexia-like symptoms via transcriptional and metabolic disruptions peripherally in muscle, adipose tissue, and centrally in the hypothalamus. The findings point to the importance of identifying early cachexia diagnostic markers and targeted interventions to stop and reverse cachexia during oxaliplatin treatment.
Significance: Adipokines such as leptin and GDF15 may be potential biomarkers of oxaliplatin-induced cachexia, and the associated metabolic pathways could be targeted therapeutically to improve cancer patient outcomes.

#802 Utilization of emotional support services among breast cancer patients.

Ahmed Allibhai[1], Asiyah Allibhai[1], Zishan Allibhai[2]

[1]Holy Trinity School, Richmond Hill, ON, Canada,[2]Department of Radiation Oncology, Southlake Hospital/Princess Margaret Hospital, Newmarket, ON, Canada

Introduction: Breast cancer patients frequently experience significant psychological distress, with prevalence rates of anxiety and depression reaching as high as 50% in certain studies. This distress is linked to worse treatment adherence and outcomes. Additionally, a recent review demonstrated that psychological interventions in these patients have been found to improve quality of life, decrease distress, and may extend disease-free survival while reducing recurrence and mortality. Despite the benefits, mental health services remain underutilized in this population. This study examined the use of emotional support resources by breast cancer patients using data from a single-institution patient-reported outcomes registry.

Method: Data were collected from our institutional electronic medical records of breast cancer patients treated within the past year. Participants included those reporting moderate to severe symptoms (≥4) on the Edmonton Symptom Assessment Score (ESAS) for depression or anxiety. Utilization of emotional support resources, such as referrals to psychosocial interventions (Psychiatrists, Social Workers), was analyzed. Demographic, clinical, and geographic characteristics were compared between resource users and non-users using chi-square and z-tests.

Results: Among 340 participants with moderate or higher distress, 118 (35.1%) accessed psychosocial resources, such as counseling or support groups. Younger patients showed a trend toward higher utilization ($p=0.068$), and white participants were slightly more likely to use resources ($p=0.229$), though neither finding reached statistical significance. Travel time significantly impacted resource use, with shorter commutes (<30 mins) leading to greater engagement (43.5% vs. 21.7% for 30-60 mins and 12.5% for >60 mins, $p<0.001$). Furthermore, patients with higher ESAS scores, reflecting greater symptom burden, were significantly more likely to engage with support resources ($p<0.001$). Other characteristics, including gender ($p=0.51$) and disease stage ($p=0.432$) were not associated with differences in resource use.

Conclusions: This study highlights factors influencing the utilization of psychosocial support resources among breast cancer patients with moderate or greater distress. While age and race showed nonsignificant trends, travel time and symptom burden were significant predictors of engagement. Some patients may have declined additional referrals due to pre-existing support systems, indicating the need to assess these factors in future studies. Expanding access and addressing barriers through healthcare and advocacy partnerships remains essential. Further research could explore the use of psychosocial resources by patients with mild distress (ESAS scores 1-3).

#803 Elevated neuroinflammation in women with breast cancer: initial insights from [^{11}C]PBR28 PET/MR imaging.

Yehui Zhu[1], Natalie Swanson[1], Mehrbod Mohammadian[1], Jennifer Murphy[1], Minhae Kim[1], Ann Partridge[2], Marilyn Hammer[2], Robert Edwards[3], Kristin Schreiber[3], Marco Loggia[1]

[1]Massachusetts General Hospital, Boston, MA,[2]Dana-Farber Cancer Institute, Boston, MA,[3]Brigham and Women's Hospital, Boston, MA

Introduction: As breast cancer survivorship rises, addressing the long-term side effects of cancer and its treatment is crucial. Chemotherapy has been linked to neuroinflammation, leading to cognitive and neurological impairments. However, other treatments (e.g., surgery, endocrine therapy) or related conditions (e.g., pain, cancer progression) may impact neuroinflammation as well.
Methods: This cross-sectional study used Siemens 3T integrated positron emission tomography-magnetic resonance imaging (PET/MRI) with the radioligand [^{11}C]PBR28, which binds to the neuroinflammation marker 18kDa translocator protein (TSPO), to assess the extent and distribution of neuroinflammation in two age-matched cohorts of women with and without breast cancer. A T1-weighted volume (MEMPRAGE; voxel size = 1x1x1mm3) was acquired simultaneously and used for attenuation correction of PET data and spatial normalization to the MNI space. Standardized uptake values ratios (SUVR) were computed from 60-90 min post-[11C]PBR28 injection data, intensity normalized by whole brain signal, spatially smoothed (FWHM=8mm) and normalized to the MNI template. PET imaging data were analyzed and processed using FSL and FreeSurfer. A mixed effects ordinary least squares analysis, using a cluster-forming threshold of $z=2.3$ and a cluster size threshold of $p<0.05$ was conducted. Mean PET signal in the regional clusters was determined, and an exploratory comparison in neuroinflammation ([^{11}C]PBR28 signal) made between women with and without breast cancer, who did or did not receive chemotherapy (Mann-Whitney U test).
Results: Twelve breast cancer patients (age=63±9) and 6 matched healthy controls (age=57±10) were included in this analysis. Compared to healthy controls, breast cancer patients showed significant increased [^{11}C]PBR28 signal uptake in the middle cingulate cortex and underlying white matter ($SUVR_{BC}$=0.95±0.03, $SUVR_{HC}$=0.85±0.04). No significant differences were observed between women with (n=7) and without (n=5) chemotherapy (U=15.00, p=0.685).
Conclusions: These preliminary findings indicate increased neuroinflammation in breast cancer patients compared to age-matched controls, independent of chemotherapy. These preliminary findings suggest that cancer or other treatments may contribute to neuroinflammation. Larger studies with focused analysis of region(s) of interest are needed to confirm these results.

#804 Gut microbiota, health-promoting lifestyle behaviors, and symptoms of pain and fatigue in hematopoietic cell transplantation survivors.

Elham Samami[1], Angela Starkweather[2], Debra Lyon[1], Debra Kelly[3]

[1]University of Florida, Gainesville, FL,[2]School of Nursing, Rutgers Health, Newark, NJ,[3]Loewenberg College of Nursing, University of Memphis, Memphis, TN

Hematopoietic cell transplantation (HCT) is a critical treatment for hematological malignancies, but survivors often face long-term health challenges, including complications related to immune dysfunction, gut microbiota (GM) alterations, and physical symptoms. Recent research has highlighted the important role of health-promoting lifestyle behaviors (HPLBs), such as diet, physical activity, and stress management, in improving health outcomes among HCT survivors. This study is a post-transplant analysis (30 days) of 55 adults (> 18 years of age) from a longitudinal descriptive study of GM composition diversity using Chao, Shannon, and health-promoting lifestyle profile domains measured by the HPLPII that includes health responsibilities, physical activities, nutrition, spiritual growth, interpersonal relationships, and stress management. Pain and fatigue were measured using the Memorial Symptom Assessment Scale, GM was assessed using 16S ribosomal gene (V4 region) amplicons from Illumina MiSeq. Data analysis was performed with the Python 'pandas' package using robust Pearson correlations for finding linear correlations and production of 95% Credibility Intervals (CI) align with Canonical Correlation using scikit-learn library and Kernel Correlation using rbf_kernel function to find nonlinear associations with ($p<0.05$) significance level. The study sample was comprised of 77% white,19.3% African American or Black, and included 2.5% Hispanic ethnicity. The mean age of participants was 56.21 years + 14.67 years, participants were diagnosed with Lymphoma (46.2%), Leukemia (27.7%), Multiple Myeloma (16.80%) and Myelodysplastic syndrome (9.24%) and the majority received autologous HCT (39.16%). Both Canonical and Kernel correlations between health-promoting lifestyle scores, gut microbiota number of OTUs, and pain-related metrics showed moderate association with 0.60 and 0.4. Pearson correlation revealed a negative association between stress management domain and pain (r= -0.30) pain severity (r= -0.26), fatigue (r= -0.37), fatigue interference (r= -0.44) and negative association between physical activity and pain frequency (r= -0.4) and severity (r= -0.4) and fatigue (r= -0.21). The physical activity domain showed a positive correlation with the Chao index (r= 0.11) and the Shannon index (r= 0.14). The nutrition domain showed a positive association between Chao (r= 0.11) and Shannon (r= 0.07). Also, positive associations between pain frequency (r= 0.10), pain severity (r= 0.19), pain distress (r= 0.20) and the spiritual growth domain were identified. These findings highlight the potential of multi-domain lifestyle interventions in enhancing post-HCT recovery and improving gut microbiota diversity. Further research is warranted to confirm these associations and refine health promotion strategies for HCT survivors.

#805 Macrophages protect salivary glands from ionizing radiation.

Dimitri Scofield, Cristina Paz, Jasmine Robinson, Kwang Nickel, Randy Kimple

Human Oncology, University of Wisconsin-Madison, Madison, WI

Background: Radiation-induced xerostomia (RIX) is a common side-effect of radiation therapy in patients with head and neck cancer. Our group has previously demonstrated that MSC(M) injection into irradiated murine SMG tissue results in an improvement in salivary function and improvement in glandular health (% saliva in MSC v PBS groups). which corresponds to alterations in macrophages. Our clinical studies have demonstrated that MSC(M) injection into salivary glands is safe and effective. However, the full mechanism behind this effect remains unknown. We hypothesized that MSC(M)s in the SMG are affectingimpact tissue-resident macrophage activity and/or promotein macrophage traffickingg the influx of new macrophages to mediate improved salivary function. Here we test whether macrophages modulate the response to radiation in our mouse model of RIX and whether MSC(M)s can modulate salivary tissue resident macrophages.

Methods: Clodronate liposomes delivered via tail vein injection were used to deplete macrophages in C57/BL6 mice prior to irradiation treatment (15 Gy) of SMGs with 15 Gy. Saliva production was monitoredmonitored, and glands were collected for histology at 3-, 7-, and 60-days post-irradiation. Primary, marrow-derived MSCs (MSC(M)) from C57/B6 male mice were isolated, cultured, and characterized using the immune monitoring 48-plex Luminex assay to characterize the secretion profile of immunomodulatory chemokines.

Results: Macrophage depletion was confirmed via flow cytometry of mouse spleens. Clodronate-treated mice produced less saliva than control mice (92% vs 71% percent decrease respectively) consistent with a protective effect of macrophages. Secretome analysis of MSC(M) revealed a robust secretion profile with high levels of macrophage associated proteins including CCL2 (1527 pg/mL), CCL7 (332.18 pg/mL), CXCL1 (311.41 pg/mL), and CCL5 (157.62 pg/mL). These data suggest that MSC(M)s may have the capacity to modulate recruited and tissue-resident SMG macrophages. This is currently being tested.

Conclusions: Our data suggests a beneficial role of macrophages in the natural response to radiation in the murine SMG. Previous data from our lab demonstrates a benefit from MSC injection after RT-exposure, and our secretome analysis of these cells show the immunomodulatory capacity of our MSC line. Future experiments must be done evaluating the effectiveness of MSC injection in our immune depleted models to determine whether MSCs are directly impacting the macrophages in the radiation damaged gland.

#807 RSV vaccine hesitancy among southern rural veterans with cancer in the Arkansas-Louisiana-Texas (ArkLATX) region: The impact of vaccine fatigue.

P. A. Haddad[1], C. Barmer[2], M. Craig[2], S. Hudson[2], A. Gupta[2];

[1]LSUHSC-S, Feist-Weiller Cancer Ctr/Overton Brooks VAMC, Shreveport, LA, [2]Overton Brooks VAMC, Shreveport, LA

Background: Respiratory syncytial virus (RSV) is one of the leading causes of respiratory infections, particularly in immunocompromised older adults. The recently available RSV vaccines offer a tool to reduce the disease burden. However, despite the availability of effective vaccines, vaccine fatigue, a phenomenon characterized by declining enthusiasm and adherence to vaccination campaigns due to the perceived overload of immunization schedules, has emerged as a challenge for vaccine uptake. With the increasing number of recommended vaccines, particularly in the wake of COVID-19 and the influenza vaccination efforts, many patients may experience hesitation toward additional vaccines, including RSV. This quality improvement study aims to explore the prevalence of vaccine fatigue and its effects on the uptake of the RSV vaccine among ArkLATX veterans to identify potential strategies to address this growing concern.

Methods: Between 2023-2024, age-appropriate veterans from 3 Hematology-Oncology clinics at Overton Brooks VAMC were provided the CDC RSV vaccine pamphlet to read along with a provider discussion about the need, indication, and side effects. Afterward, the patients were offered the RSV vaccine. If they declined, they were asked about the reasons. Descriptive statistics, as well as the Chi-square test and logistic regression, were calculated to determine the impact of demographic factors on RSV vaccine uptake.

Results: The sample consisted of 200 veterans from Arkansas, Louisiana, and Texas. The median age was 74, with an age range of 54-93 years. Participants were 93% male, 53% White, 45% Black, and 2% Other. Ninety-one percent were COVID19 vaccinated with 62% receiving both. Thirty-seven percent of the participants declined the RSV vaccine (factoring in 4% RSV vaccine uptake conversion rate during the study period), and 7% received neither. The main reasons for declining were “did not want another shot” (53%), current illness/vaccine reactions (11%), and unknown reasons (36%). There were no statistical differences with respect to age, sex, race, or geography between those who received the RSV vaccine and those who declined. Those who were COVID-19 vaccinated were more likely to have received the RSV vaccine (Odds Ratio=2.4, p=0.02).

Conclusions: Our study reveals that the majority of ArkLATX veterans with cancer are willing to be vaccinated against RSV, though at a lesser rate than previously reported for the same group with the COVID-19 vaccine. The primary reason behind vaccine hesitancy was not wanting another vaccine injected, which was consistent with vaccine fatigue. Fortunately, no one voiced major concerns regarding vaccine safety or efficacy. The results suggest that targeted communication, consistent but not obtrusive reminders, simplifying access, and respecting the patient’s timing and mental readiness could improve uptake.

#808 Healthy dietary patterns and cancer survival: A cohort study.

Z. Bian, X. Xu, S. Zhou, X. Li, X. Wu;
Zhejiang University School of Medicine, Hangzhou, China

Introduction: Diet plays a crucial role in the management and recovery of cancer patients. Making appropriate dietary choices can help provide necessary nutrients, enhance the immune system, maintain energy levels, and promote recovery. To examine the associations of dietary scores for 9 healthy eating patterns with risk of all-cause and cancer mortality in cancer survivors.
Methods: A prospective cohort of cancer survivors (n = 4001; weighted number, 17548466) from the US National Health and Nutrition Examination Survey from 1999 to 2018. Participants were linked to mortality data from their interview and physical examination date through December 31, 2019.
The exposures were Alternative Healthy Eating Index-2010 (AHEI2010), Alternate Mediterranean Diet score (AMED), Dietary Approaches to Stop Hypertension score (DASH), Dietary inflammation index (DII), Healthy dietary score (HDS), Healthy eating index-2020 (HEI2020), Plant-based diet index (PDI), Healthful plant-based diet index (hPDI), and World Cancer Research Fund/American Institute for Cancer Research dietary score (WADS).
Main Outcomes were All-cause and cancer mortality.
Results: The final study sample included 4001 cancer survivors (mean [SD] baseline age,61.86[14.79] years; 2408 (66.37%) females; 2519(82.33) white individuals). During a total of 33130 person-years of follow-up, 1385 Participants died. Of note, when comparing the highest with the lowest tertiles, only AMED (HR (95% CI): 0.80 (0.65-0.98), P = 0.03), HEI2020 (HR (95% CI): 0.81 (0.68-0.96), P = 0.02), and hPDI (HR (95% CI): 0.84 (0.71-0.98), P = 0.03) were significantly associated with all-cause mortality. Also, higher levels of HEI2020 were associated with a lower risk of cancer mortality in cancer survivors (HR (95% CI): 0.70 (0.55-0.88), P = 0.003). All dietary scores had no nonlinear association with all-cause mortality. The inverse associations between these scores and risk of mortality were consistent in subgroups.
Conclusions: In this cohort study of a nationally representative sample of US cancer survivors, embracing a comprehensive and nutritionally balanced dietary pattern, as exemplified by HEI2020, holds tremendous value in promoting the well-being and recovery of cancer survivors.

#809 Food choices of patients with advanced cancer at start of early-phase combination trials with immune checkpoint blockade.

Goldy C. George, Hangil Lee, Sarina A. Piha-Paul, Ecaterina Dumbrava, Apostolia Tsimberidou, Siqing Fu, Grace Appleton, Laila Noor, Jordi Rodon, Timothy A. Yap, Funda Meric-Bernstam, David S. Hong

UT MD Anderson Cancer Center, Houston, TX

Purpose: We examined baseline diet and its association with outcomes and social determinants of health (SDoH) factors among patients (pts) enrolled in early-phase immune checkpoint blockade-included combination trials in a comprehensive cancer center.
Methods: Pts (ECOG 0-1) scheduled to begin an early-phase combination trial with immune checkpoint blockade were recruited into a prospective, longitudinal study design, and completed the validated Multifactor Dietary Screener (0-8, higher score = more frequent use of food during the past 30 days) prior to trial start. Clinical data were extracted from the EMR. Statistics included t-tests and Spearman correlations.
Results: Among 259 pts (52% male, 85% White, age=25-83y, BMI=14-46, median prior lines of treatment=3, 41% had ≥2 metastatic organ involvement), increased intakes of vegetables and whole grain breads were associated with longer trial duration ($P<0.029$ for each). Frequent use of nuts/ seeds was linked to prolonged overall survival ($P=0.014$), and beans to extended progression-free survival ($P=0.039$). Lower regular sugar desserts and white potatoes were associated with superior treatment response (CR / PR) ($P<0.005$ each). Males had bacon/ sausage, hot dogs, red meat, milk, and ice-cream more often than females, and fruits, green leafy vegetables, and other vegetables less often ($P<0.008$ each). White pts had milk and ice-cream more often, and lettuce and leafy vegetables less often than minority pts ($P<0.002$ each). Hispanic pts had beans ($P=0.024$) more often, and cheese, and other vegetables less often than non-Hispanic pts ($P<0.006$ each). Young adults (25y-39y) had pasta more often than older patients ($P=0.006$); pts >70y had shellfish and milk more often than younger pts ($P<0.028$ each). Higher BMI was linked to more frequent intakes of regular fat sausage or bacon, hot dogs, fried potatoes ($P<0.003$ each), and regular fat salad dressing or mayonnaise ($P=0.013$), less frequent intakes of fruit ($P<0.001$), and fewer years of education ($P=0.037$). Less education was associated with more frequent use of bacon/ sausage, doughnuts/ other baked desserts ($P<0.002$ each), regular fat salad dressing, and hot dogs ($P<0.016$ each), and less frequent intakes of whole grain breads, fruits, greens, other vegetables, nuts, yogurt ($P<0.004$ each), and fish ($P=0.013$).
Conclusions: Dietary food choices, increasingly recognized as impacting the hallmarks of cancer, may offer tangible interception of disease outcomes even during treatment in early-phase combination trials incorporating immune checkpoint blockade. Food preferences for pts in trials may be bidirectionally influenced by SDoH factors including educational attainment, gender, race/ ethnicity, and age, and as such, these factors need to be considered when developing wholesome individualized dietary prescriptions and dietary interventions for trial participants.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#81 FOXN3 suppresses FUT5-mediated adhesion of metastatic ovarian cancer cells to mesothelial cells.

Siyu Zhou, Ayon Ahmed Hassan, Alice Sze Tsai Wong

School of Biological Science, The University of Hong Kong, Hong Kong, Hong Kong

Ovarian cancer is the most lethal gynecological malignancy primarily due to widespread peritoneal metastasis at diagnosis, posing significant treatment challenges. Our previous research has indicated that fucosyltransferase 5 (FUT5) is upregulated in metastatic ovarian cancer and is a key contributor to peritoneal metastatic colonization, which represents the initial rate-limiting step in metastasis. However, little is known about the regulation of FUT5. In this study, using a microfluidic chip model with ascitic fluid shear stress perfusion, we found that FUT5 is transcriptionally upregulated in ovarian cancer cells treated with ascitic shear stress, with no changes in degradation rates of FUT5 protein and transcript. Through a comprehensive analysis involving transcriptional factor prediction, biostatistics on TCGA database expression patterns, and sequence analysis using a metastatic cancer model, we identified Forkhead Box N3 (FOXN3) as a potential regulator of FUT5 transcription. Further investigation demonstrated that FOXN3 suppresses FUT5 expression by directly interacting with its promoter. Expression of FOXN3 suppresses tumor-mesothelial adhesion, which can be reversed by FUT5 overexpression. Together, these findings suggest that the FOXN3/FUT5-axis plays a crucial role in ovarian tumor-mesothelial interaction and could serve as a potential therapeutic target (This work is supported by HMRF08192286 and RGC SRFS2223-7S05).

CLINICAL RESEARCH: Targeted Therapies and Combinations 1
Poster Session

#813 *In vitro* and *in vivo* modelling of drug tolerance and minimal residual disease to discover effective therapeutic combination strategies for EGFR-mutated lung cancer.

Kristyna A. Gorospe[1], Ming Li[2], Arundhathi Arivajiagane[2], Nhu-An Pham[2], Roya Navab[2], Kelsie L. Thu[3], Ming-Sound Tsao[2]

[1]Laboratory Medicine and Pathology, University of Toronto, Toronto, ON, Canada,[2]Princess Margaret Cancer Research Tower, Toronto, ON, Canada,[3]Laboratory Medicine and Pathology, St. Michael's Hospital, Toronto, ON, Canada

Resistance to EGFR-targeted therapy is a major barrier to improving survival rates for non-small cell lung cancer (NSCLC) patients whose tumors have activating *EGFR* mutations. Resistance to EGFR tyrosine kinase inhibitors (TKIs) emerges from drug-tolerant persister cells (DTPs) that survive TKI therapy and manifest in patients as minimal residual disease, which inevitably drives fatal tumor recurrences. DTPs are a rare population of cancer cells in a reversible, slowly proliferating state that survives targeted therapy. Upon TKI withdrawal, they exit the DTP state to recommence rapid proliferation and aggressive tumor growth. Since DTPs provide a reservoir of surviving cells from which outright resistance driven by acquired genetic alterations arises, we hypothesize that discovering DTP survival mechanisms will inform vulnerabilities whose inhibition could enhance the efficacy of EGFR TKIs. However, such mechanisms are poorly understood in EGFR-mutant lung adenocarcinoma (LUAD). To address this knowledge gap, this study aims to generate robust *in vitro* and *in vivo* LUAD models of Osimertinib (Osi)-induced drug tolerance. Genetic and functional characterization of these models has the potential to identify mechanisms enabling drug tolerance that could be targeted to prevent TKI resistance from developing. To date, the response of 3 *EGFR*-mutant LUAD models to Osi treatment has been characterized both in vitro and in vivo [PC9, HCC4006, and 1 patient-derived xenograft (PDX)-derived cell line, X137CL]. Osi treatment of these cells increased apoptosis, but also arrested treated cells in G0-G1 phases of the cell cycle, consistent with the slow cycling nature of DTPs. Osi treatment of the xenografts formed by these lines induced strong tumor regressions and tumor replicates relapsed upon drug cessation, indicating these models are appropriate for studying DTPs *in vivo*. These models will be leveraged for molecular, functional, and pharmacological studies to identify shared and distinct mechanisms driving drug tolerance, in *in vitro* and *in vivo* conditions, including comparative single-cell transcriptomic profiling and pathway analysis. Candidate genes and pathways discovered will be functionally validated using CRISPR screens. Our comparative analyses of DTP versus untreated tumors will reveal novel interventions for preventing tumor relapse following TKI therapy in *EGFR*-mutant lung cancers.

#814 A novel small molecule prodrug activated inside cancer cells by a tumor associated enzyme for targeted chemotherapeutics in kidney cancer.

Ross A. Hamilton, Leniher C. Chibas, Uksha Saini, Stephanie Vega, Dev Chatterjee, Atul Varadhachary

Brimstalt Therapeutics, Houston, TX

Significance: Brimstalt Therapeutics is developing a small molecule drug for treatment of cancer patients with tumors that express the tumor associated enzyme (TAE), SULT1A1. The lead compound, BST-12, is a prodrug that is activated by SULT1A1, converting it into a potent non-specific alkylating agent resulting in targeted cancer cell death while leaving normal tissue unaffected. BST-12 belongs to a class of compounds called N-Benzyl Indole Carbinols (N-BICs). N-BICs have been previously identified as compounds that demonstrate potent antitumor effects once activated by SULT1A1, whose expression is both necessary and sufficient for activation. Kidney and renal pelvis cancer is the seventh most common cancer in the US, with an estimated 82,610 new cases and 14,390 deaths in 2024. Renal cell carcinoma (RCC) is the most frequent type of renal cancer; unfortunately, 33% of patients will experience relapse at metastatic sites and, consequently, have significantly poorer outcomes, highlighting the urgent need for novel therapeutic strategies. SULT1A1 is a metabolic sulfotransferase that is normally expressed in the liver, kidney, and GI tract, and either not expressed or expressed at low levels in other tissue types. However, SULT1A1 is a known inducer of carcinogenesis, considered a TAE that is over-expressed in about 8-15% of major cancers, including RCC, breast, and prostate, and its expression correlates with poorer patient prognosis. An IHC study on 233 tumors from 48 tissue types showed that in RCC, SULT1A1 levels were elevated and homogenously expressed; thus, RCC was selected for the initial indication for BST-12 testing.

Results: In preclinical studies, BST-12 demonstrates potent anticancer activity in SULT1A1+ RCC cells (A498 and Caki-1), with IC50s under 250 pM, but has no effect on SULT1A1- cells (ACHN and 786-0). Direct sulfonation assays using recombinant SULT1A1 protein supports the mechanism of action reported with N-BICs. Furthermore, BST-12 treated A498 and Caki-1 cells had increased phospho-H2A.X levels, a DNA-damage marker associated with non-specific alkylation, topoisomerase inhibitors, and ROS inducing agents. In vivo, BST-12 treatment in A498 CDX models rapidly eliminated established tumors. To assess toxicity, the organs (liver, kidney, GI tract), serum liver enzyme levels (AST, ALT), and body weights of male/female rats treated with BST-12 were evaluated and indicated BST-12 has a good tolerability profile.

Conclusions: The development of BST-12 has significant potential to improve survival / quality of life of SULT1A1+ RCC cancer patients. Although RCC is our primary target indication, the initial BST-12 proof-of-concept will support label expansion opportunities in other SULT1A1+ cancer types. Lastly, BST-12 has intriguing promise for bypassing any acquired resistance pathways associated with current standards of care.

#815 AZD5335, a folate receptor alpha-targeted ADC, enhances ovarian cancer treatment with bevacizumab, paclitaxel and/or carboplatin.

Jason J. Zoeller[1], Ravinder Tammali[1], Ana De Almeida[2], Shane Cronin[1], Nancy Lee[1], Alma Andoni[1], Claire Myers[1], Elaine Joseph[2], Michael Lehmann[1], Rebecca Sargeant[3], Ina Bisha[4], Simon Christ[4], Iris Dino[4], Xhenifer Guza[4], Jixin Wang[1], Lisa Godfrey[3], Roger Dodd[3], Neki Patel[5], Edward Rosfjord[1], Marco Gymnopoulos[1], Tim Brier[3], Sabina Cosulich[3], Elaine Hurt[1], Puja Sapra[1]

[1]AstraZeneca US, Gaithersburg, MD,[2]AstraZeneca US, Waltham, MA,[3]AstraZeneca UK, Cambridge, United Kingdom,[4]AstraZeneca GmbH, Munich, Germany,[5]AstraZeneca UK, London, United Kingdom

Elevated expression of folate receptor alpha (FRα) in various cancers, including epithelial ovarian cancer (EOC), highlights FRα as a potential target for the development of novel therapeutics to treat FRα-expressing tumor types. AZD5335, an FRα-targeting antibody conjugated to a topoisomerase 1 inhibitor (TOP1i) payload, is currently being investigated in the FONTANA Phase I/IIa clinical trial (NCT#05797168), either as a single agent or in combination with a PARP1-selective inhibitor (saruparib). Using an FRα-expressing EOC xenograft model (OV90), we evaluate additional AZD5335-relevant combined treatment strategies and report pre-clinical assessment of AZD5335 in combination with bevacizumab, paclitaxel and/or carboplatin. Compared to monotherapy treatment, we successfully demonstrate that combination treatments with AZD5335+bevacizumab, AZD5335+paclitaxel or AZD5335+carboplatin result in significant and durable anti-tumor responses. Using the same EOC xenograft model, we extended these studies to include AZD5335+bevacizumab plus either paclitaxel or carboplatin treatments and demonstrated that these triplet combos resulted in superior and durable anti-tumor activities. Combination data in a number of additional CDX/PDX will be presented. A series of in vitro studies will also define the ADC/SOC drug interactions and further delineate underlying mechanisms driving enhanced efficacy. These studies inform our ongoing clinical development of AZD5335 in ovarian cancer.

#816 Aurora kinase A inhibition overcomes tolerance to panHER inhibitors in HPV positive HNSCC.

Sebastian Cruz-Gomez[1], Jong Woo Lee[1], Julian Barrantes[1], Richard Ivey[2], Sara Savage[3], Pratima Chaurasia[1], Gunner Dickson[1], Dickson Adah[1], Bing Zhang[3], Amanda Paulovich[2], Barbara Burtness[1]

[1]Internal Medicine, Yale Cancer Center, New Haven, CT,[2]Fred Hutchinson Cancer Center, Seattle, WA,[3]Baylor College of Medicine, Houston, TX

The incidence of HPV-associated (HPV+) head and neck squamous cell carcinoma (HNSCC) has been increasing in recent decades. The EGFR inhibitor cetuximab (CTX) is less active in HPV+ than HPV- HNSCC. HPV oncoproteins E6 and E7 induce HER3 overexpression, and EGFR-HER3 heterodimer-driven survival signaling via PIK3, AKT and ERK1-2 contributes to resistance to cetuximab and the EGFR inhibitor erlotinib. We demonstrate that although panHER inhibitors afatinib and dacomitinib more effectively inhibit growth of HPV+ HNSCC cells than do erlotinib or cetuximab, adaptive signaling through the AURKA/PLK1 axis mediates resistance to panHER inhibitors. We hypothesized that combined panHER and AURKA inhibition would synergize to overcome persistence of HPV+ HNSCC. We find that HPV+ HSNCC cells UM-SCC47 and UPCI:SCC154 express both EGFR and HER3. Indeed, cell viability experiments and clonogenic survival assays demonstrate strong synergy between panHER and AURKA inhibitors, confirmed in xenografted tumor models treated with the second generation AURKA inhibitor VIC-1911 and dacomitinib. We then performed RNAseq and shotgun proteomics to understand the cellular mechanisms behind our combination. Dacomitinib had expected effects downregulating Hallmark G2M and EGFR phosphorylation targets, upregulating EMT markers and cell death markers. In contrast, addition of AURKA inhibition upregulated PLK1 and pPLK1 and cell death markers. These findings indicate that combination therapy with AURKA inhibition will mitigate the adaptability to EGFR and panHER inhibitors previously described for HPV+ HNSCC.

#817 A prospective, multi-center, phase IV study to evaluate safety and efficacy of Dr. Reddy's bevacizumab (DRL_BZ) in patients with solid tumors.

Narendra Maharaj[1], Ranjith K[1], Anand Eswaraiah[1], Pramod kumar Reddy[1], Gopichand M[2], Rajender Singh Arora[3], Krishna Chaithanya Puligundla[4], Ashok Kumar Diwan[5], Tanveer Mohibbhai Maksud[6]

[1]Dr. Reddy's Laboratories Ltd, Hyderabad, India,[2]HCG City Cancer Centre- A unit of Health Care Global Enterprises Limited, Vijaywada, India,[3]Sujan Surgical Cancer Hospital- Amravati Cancer Foundation, Amravati, India,[4]MNJ Institute of Oncology and Regional Cancer Center, Hyderabad, India,[5]Government Medical College, Nagpur, India,[6]Unique Hospital Multispecialty & Research Centre, Surat, India

Introduction: DRL_BZ is a biosimilar to the reference product, bevacizumab, and was approved in India and subsequently introduced in other markets for the use in various other solid tumors as single or combination therapy. This study was conducted as part of a post-marketing commitment to regulatory authority aimed to evaluate treatment safety (primary objective), and efficacy (secondary objective) in male and female patients with solid tumors.

Methods: In this prospective, single-arm, multicenter, Phase IV study (BZ-01-002), a total of 200 patients with median age 54.0 years were enrolled with the following indications: mCRC (n = 59); NSCLC (n = 41); epithelial ovarian cancer (n = 27), and cervical cancer (n = 73). Patients received DRL_BZ either with chemotherapy or as monotherapy and followed up to disease progression, unacceptable toxicity, or death due to any cause, or up to 6 months from first dose whichever occurs earlier.

Results: The overall median duration (days) of DRL_BZ treatment exposure for the enrolled patients was 151.5 (1.0 - 253) days. Of the 200 enrolled patients, 117 (58.5%) patients completed the study, while 83 (41.5%) patients were withdrawn from the study due to various reasons (majorly due to COVID-19 pandemic). Out of 454 TEAEs reported from 139 (69.5%) patients, 46 TEAEs in 25 (12.5%) patients were considered related to DRL_BZ administration. Thirteen (6.5%) TEAEs leading to permanent study drug discontinuation. A total of 13 SAEs in 12 (6%) patients were reported and four of them were related to study drug. As per mITT population, 153 patients were evaluable for efficacy, and the reported ORR was 23.1% (3/13) for 2-weekly mCRC patients, 25% (8/32) for 3-weekly mCRC patients, 45.7% (16/35) for NSCLC patients, 72.7% (16/22) for epithelial ovarian cancer patients, and 37.3% (19/51) for cervical cancer patients.

Conclusions: The safety profile of DRL_BZ, in terms of reported adverse events, in patients with solid tumors is comparable and consistent with the known safety profile of bevacizumab in literature. Overall, findings from this Phase IV study indicate that the safety and efficacy of DRL_BZ in patients with solid tumors is as per the approved prescribing information.

#818 A logic-gated chimeric antigen receptor T cell (CAR T) for post-transplant acute myeloid leukemia (AML) that distinguishes donor and recipient hematopoiesis.

D. Manry, B. L. Luna, A. G. Winters, A. D. Flynn, T. K. Gill, M. Mcelvain, A. Sima, J. Wang, A. Zampieri, M. Daris, J. Zhang, S. Martire, B. DiAndreth, C. Netirojjanakul, T. Tokatlian, A. Kamb, J. S. Welch;
A2 Biotherapeutics, Inc, Agoura Hills, CA

Background: AML relapse after a stem cell transplant is associated with dismal outcomes and no current FDA-approved therapeutic options. Cell therapy for AML has been challenging due to a lack of antigens distinguishing malignant from normal hematopoiesis, the potential for antigen escape due to intratumor antigen heterogeneity, and poor T cell function in lymphocytes from AML patients. To overcome these problems, we focus on opportunities in patients who have received human leukocyte antigen (HLA)-A*02 mismatched transplants. We have developed a Tmod HLA-A*02 NOT gate (blocker) that prevents CAR T killing of HLA-A*02 positive cells. In patients who are HLA-A*02 negative and receive an HLA-A*02 positive stem cell transplant, the blocker distinguishes tumor (HLA-A*02 negative) and normal (HLA-A*02 positive) hematopoiesis. A similar Tmod NOT gate has been safe in an ongoing solid tumor clinical trial (NCT05736731) (Grierson, et al. *SITC.* 2024). The blocker is paired with a dual CD33/CLL-1 activator to prevent antigen escape in donor-derived HLA-A*02 positive T cells to mitigate AML patient T-cell dysfunction. Additional blockers targeting HLA-A*03, etc can expand therapeutic opportunity to patients who have received other HLA-mismatch transplants.
Methods: CAR constructs were designed and screened in Jurkat and primary T cell assays. The 50% effective and inhibitory concentration (EC50 and IC50) results were compared to monospecific CAR constructs using benchmark activators based on single-chain variable fragments (scFvs) from M26 (CLL-1) and lintuzumab (CD33) using wild-type A*02-negative and A*02-positive engineered AML cell lines.
Results: 111 tandem CD33/CLL-1 activator CAR constructs were evaluated in Jurkat cells and 23 in conjunction with HLA-A*02 blocker. Six tandem constructs were prioritized for evaluation in primary T cells, identifying one (20I21-1A4 mBAAsh) with IC50 potency similar to monospecific CAR constructs, and selectivity observed between HLA-A*02 positive vs. HLA-A*02 negative cells. Selective protection also was observed in HLA-A*02 positive primary hematopoietic stem cells compared with CAR constructs without the blocker.
Conclusions: We have developed a cell therapy strategy to leverage HLA mismatched stem cell transplants using a Tmod HLA-A*02 blocker. This approach could be applied to patients who have relapsed after a mismatched transplant or integrated into a stem cell transplant to prevent relapse. Pre-clinical data of this design demonstrates selectivity between HLA-A*02 positive and HLA-A*02 negative cells, and dual antigen activity addresses antigen escape. Future studies will interrogate the impact of membrane-tethered cytokines to augment potency and xenografts studies to further define activity.

#819 Comparison of DNA+RNA-NGS and DNA-NGS for detecting targetable driver gene alterations in Chinese patients with advanced NSCLC.

Guohao Xia[1], Ping Liu[1], Lin Shi[1], Gao Ning[2], Ding Zhang[2]

[1]Medical Oncology, Jiangsu Cancer Hospital, Nanjing, China,[2]3D Biomedicine Science & Technology Co., Ltd., Shanghai, China

Background: Patients with advanced non-small cell lung cancer (NSCLC) require genetic testing to enable precision treatment. The conventional method for genetic testing is next-generation sequencing (NGS) using DNA templates (DNA-NGS). However, multiple studies have shown that DNA-NGS often fails to detect fusion mutations, while RNA-based NGS demonstrates superior detection rates for these alterations. This study evaluates the rate of targetable driver gene alterations (AGAs) in NSCLC patients using DNA+RNA-NGS compared to DNA-NGS in real-world settings, providing reliable evidence to support clinical practice.

Methods: This study included 143 Chinese patients with advanced untreated NSCLC from November 2022 to October 2024. Eighty patients underwent DNA-NGS testing, and 63 underwent DNA+RNA-NGS testing. In DNA+RNA-NGS, DNA and RNA were extracted from the same tissue specimens. RNA was reverse transcribed into complementary DNA (cDNA) and combined with DNA. Detection was performed in a single workflow, using DNA for single nucleotide variant (SNV) and insertion/deletion (indel) detection, while RNA was utilized for rearrangement detection.

Results: In the DNA+RNA-NGS cohort, the median age was 68 years (63.5% male), while in the DNA-NGS cohort, it was 64 years (67.5% male). The detection rate of AGAs was higher in the DNA+RNA-NGS cohort (55.6% vs. 45.0%, p=0.21), primarily due to increased detection of rearrangements (14.3% vs. 7.5%, p=0.21). SNV/indel detection rates showed no significant difference (41.3% vs. 37.5%, p=0.65). Among rearrangement AGAs, there were no significant differences in ALK (4.8% vs. 3.8%, p=0.76), RET (1.6% vs. 1.3%, p=0.86), and METex14 skipping (1.6% vs. 2.5%, p=0.71). However, the ROS1 rearrangement detection rate was significantly higher in the DNA+RNA-NGS cohort (6.3% vs. 0%, p=0.02), attributed to the sequence characteristics of the ROS1 gene, which contains two large repetitive fragments (L1PA8A and L1PA10) in intron 31 that DNA probes often miss. Similar challenges occur with NTRK1/2/3 rearrangements, especially at intron 11 and 12 breakpoints in NTRK3, which are hard to capture with DNA probes, leading to missed detections. However, due to the low incidence of NTRK rearrangements, no cases were found in either cohort. In total, 7 (ALK, n=2; ROS1, n=3; RET, n=1; METex14 skipping, n=1) and 3 (ALK, n=3) fusion-positive patients from the DNA+RNA-NGS and DNA-NGS cohorts, respectively, received targeted therapy, with all 10 achieving favorable clinical responses.

Conclusion: DNA+RNA-NGS enhances the detection rate of AGAs compared to DNA-NGS, particularly for rearrangement-related genes in patients with NSCLC. Patients with rearrangements identified by DNA+RNA-NGS who receive targeted therapy can experience significant clinical benefits, thereby improving precision diagnosis and treatment in NSCLC.

#820 Combined HDAC and ATM inhibition potentiates anticancer effects in glioblastoma preclinical models.

Susan J. Histed, Kaitlyn Chung, Gozde Uzunalli, Elizabeth Hayes, Kyria Kelley-Wolfe, Jordon Fakhoury, Tigran Margaryan, Sonam Patel, Nader Sanai, Artak Tovmasyan, An-Chi Tien, Shwetal Mehta

Ivy Brain Tumor Center at Barrow Neurological Institute, Phoenix, AZ

Background: Brain penetrant histone deacetylase inhibitors (HDACi) are promising radiosensitizing agents for glioblastoma (GBM). In addition to suppression of HDAC activity, the antiproliferative effect of HDACi includes downregulation of DNA damage repair (DDR) pathway and persistent DNA damage. Here, we evaluated synergistic effect of combining HDAC and DNA repair enzyme, ataxia telangiectasia mutated (ATM) inhibitors, quisinostat (QST) and AZD1390 to broaden therapeutic window for GBM treatment.
Methods: *In vitro* dose-response assays were performed in patient-derived xenograft (PDX)-derived glioma stem cells (GSCs) with both QST and AZD1390 with or without radiation treatment to assess synergy. Immunoblotting and immunofluorescence assays were performed for histone acetylation, DNA damage (pH2AX), pRAD50, pATM and cleaved caspase 3 to assess pharmacodynamic responses. Quantitative PCR analysis was performed to examine changes in gene expression of key DNA damage pathway genes.
Results: Combined treatment with QST and AZD1390 synergistically inhibit GBM growth across multiple GBM GSC models in combination with radiation. HDACi treatment dampens the DDR response pathway via suppression of key DDR pathway genes resulting in persistent unrepaired DNA damage.
Conclusions: Our data indicate that QST and AZD1390 act synergistically to radiosensitize GBM cells and increase the therapeutic window for treatment. *In vivo* experiments are under way to examine efficacy of this combination using orthotopic PDX GBM models.
Keywords: glioblastoma, ATM, HDAC, DNA damage repair

#821 A potent CDK7 inhibitor TY-2699a suppresses palbociclib-resistant breast cancer and has the potential for combination with chemotherapy or immunotherapy in solid tumors.

Shengli Dong*,#[1], Apeng Liang*,#[1], Shaoqing Chen*[1], Zhengfei Guo[1], Jian Zhu[1], Huan Wang[2], Jing-Lan Mu[2], Yakun Qi[2], Meihua Li[1], Kai Wang[1], Hongqiang Li[1], Xinlong Yang[1], Chao Zhou[1], Zhiyong He[1], Zhengzhou, Henan Niu[3], Mingyu Jiang[2], Xiugui Chen[2], Jun Li[3], Yusheng Wu[3]

[1]TYK Medicines, Inc., Changxing county, Huzhou City, China,[2]TYK Medicines, Inc. (Shanghai), Xuhui District, Shanghai, China,[3]TYK Medicines, Inc. (Zhengzhou), Zhengzhou, Henan, China

Introduction: Development of an effective treatment strategy for CDK4 and CDK6 inhibitors (CDK4/6i)-resistan cancers has become an urgent unmet clinical need. CDK7 is a kinase at the core of transcription and also functions in regulating cell cycle progression. Recent studies have shown that CDK7 is a potential target to overcome CDK4/6i resistance. CDK7 overexpression has been identified in pancreatic cancer. High CDK7 expression blocked apoptosis of pancreatic cancer cells induced by nab-paclitaxel combined with gemcitabine. TY-2699a is a selective CDK7 inhibitor developed by TYK Medicines, Inc. Here we report that TY-2699a demonstrated superior efficacy as well as good tolerability and safety.

Results: TY-2699a was more than 80-, 66- and 86-fold more active than palbociclib in palbociclib-resistant stable cell lines of MCF7, T47D and HCC1428 cells, respectively. Consistent with the *in vitro* results, a 40 mg/kg dose of palbociclib almost completely failed to inhibit tumor growth in the xMCF-7/Palbo-R mouse model of breast cancer. TY-2699a at a dose of 6 mg/kg significantly inhibited tumor growth. The efficacy of CT-7001 monotherapy at a dose of 100 mg/kg was comparable to that of TY-2699a at a dose of 1 mg/kg, which only mildly inhibited tumor growth. Our efficacy data of TY-2669a provides a solid rationale for further evaluation of TY-2699a +/- fulvestrant in clinical HR+Her2- CDK4/6-resistant breast cancer patients. In addition, targeted inhibition of CDK7 not only enhanced the efficacy of paclitaxel or gemcitabine in pancreatic cancer cells, but also enhanced the *in vivo* efficacy of nab-paclitaxel, gemcitabine, and nab-paclitaxel in combination with gemcitabine in mouse pancreatic cancer tumor models. We initiated a phase I, multicenter, open-label study of TY-2699a administered orally to adult patients with locally advanced or metastatic solid tumors in 2023. In the single-agent dose escalation stage, TY-2699a showed better clinical safety than SY-5609 did. Most Adverse events were Grade 1 or 2. Adverse events assessed as grade 3 or 4 were a decrease in PLT, diarrhea, stomatitis, and anemia. We plan to enroll 40 subjects each cohort to evaluate the efficacy of TY-2699a combination treatment in ER+/Her2- breast cancers, pancreatic cancer, and nasopharyngeal carcinoma.

In summary, TY-2699a is a highly selective and potent CDK7 kinase inhibitor, The compound has potential wider therapeutic window than multiple CDK7 inhibitors that are active in the clinical development stages. TY-2699a has shown a manageable clinical safety profile. In addition to its use as monotherapy, TY-2699a has great potential for use as a combination therapy with chemotherapy or immunotherapy.

[#]Shengli Dong and Apeng Liang contributed equally to this work.

* To Whom Correspondence should be addressed to: Shaoqing Chen, Shengli Dong and Apeng liang.

#822 Combination of carboplatin and CHK1 inhibition to overcome platinum resistance in high grade serous ovarian cancer.

Mackenzie Cummings[1], Jaden Williams[2], Jennifer Pierce[1], Nathaniel Jones[1], Kevin Lee[1]

[1]Gynecologic Oncology, USA Health Mitchell Cancer Institute, Mobile, AL,[2]USA Health Mitchell Cancer Institute, Mobile, AL

Platinum based chemotherapy is the most effective treatment for high grade serous ovarian cancer (HGSOC). Resistance in the recurrent setting is a poor prognostic indicator with few treatment options. CHK1 inhibitors have been studied in HGSOC in preclinical and early phase trials alone and in combination with cytotoxic chemotherapy, but have not been studied in combination with platinum to our knowledge. We used the CHK1 inhibitor, prexasertib, as a strategy to overcome platinum resistance by promoting cell cycle progression, limiting repair of platinum induced DNA damage. Sensitivity to carboplatin and prexasertib alone and in combination were measured in a panel of pre-clinical HGSOC cell lines. DNA damage was detected using the Repair Assisted Damage Detection method in untreated and treated cells for 24h with carboplatin and prexasertib alone and in combination. An enzymatic cocktail of UDG, FPG, T4PDG, Endo IV, Endo VIII, and AAG was used to extract DNA damaged lesions. Damaged lesions were labeled for detection with polymerase and a Digoxigenin labeled dUTP. Samples were stained for anti-DIG and anti-pCHK1 S317, imaged on a confocal microscope, and analyzed in Nikon Elements software. Expression of pCHK1 S317 was confirmed via Western Blot. A synergistic effect was observed in all cell lines with the combination of carboplatin and prexasertib. In ES2 cells, carboplatin alone resulted in 15.4% cell death and increased to 72.5% ($p<0.001$) and 86.8% ($p<0.0001$) with the addition of prexasertib 5nM and 10nM, respectively. In SKOV3 cells, carboplatin resulted in 38.2% cell death and increased to 80.0% ($p=0.0002$) and 86.6% ($p<0.0001$). In OvCar4 cells, carboplatin resulted in 53.2% cell death and increased to 90.6% ($p=0.0001$) and 92.4% ($p<0.0001$). In OV90 cells, carboplatin resulted in 25.3% cell death and increased to 56.6% ($p<0.0001$) and 60.1% ($p<0.0001$). In OvCar8 cells, carboplatin resulted in 29.3% cell death and increased to 91.9% ($p<0.0001$) and 95.4% ($p<0.0001$). DNA damage increased in ES2 cells with prexasertib 10 nM + carboplatin (1.4-fold increase, $p=0.03$), SKOV3 with prexasertib 5 nM + carboplatin (257.0-fold increase, $p=0.002$), OvCar4 with prexasertib 10 nM + carboplatin (1.4 fold increase, $p=0.03$) and OvCar8 with prexasertib 10 nM + carboplatin (2.0-fold increase, $p=0.0003$). There was no difference in DNA damage in OV90 cells. pCHK1 S317 was increased significantly in all 5 cell lines treated with combination therapy, consistent with known phosphorylation of CHK1 at S317 by ATR in response to DNA damage and inhibition of p-CHK1 auto-phosphorylation by prexasertib leading to inactivation. The CHK1 inhibitor, prexasertib, demonstrated a synergistic effect when used in combination with carboplatin, correlating with an expected increase in DNA damage and p-CHK1 S317. The use of CHK1 inhibitors with platinum may be a new treatment strategy in platinum resistant HGSOC.

#823 Combination therapy of HDAC inhibitors with HSP90 and TRAP1 targeting the hypoxia pathway in triple-negative breast cancer.

Golnaz Asaadi Tehrani[1], Becca Kubick[2], Andrew Gutierrez[3], Terin D'Amic[3], Hailey Sanders[3], Brian Blagg[3], Bradley Smith[3], Meenal Datta[1]

[1]Aerospace and Mechanical Engineering, University of Notre Dame, South Bend, IN,[2]Department of Biological Sciences, University of Notre Dame, South Bend, IN,[3]Department of Chemistry and Biochemistry, University of Notre Dame, South Bend, IN

Triple-negative breast cancer (TNBC) is one of the deadliest subtypes of breast cancer that more than 50% of cases present with hypoxia, which induces chemotherapy resistance and fuels treatment failure. While various therapies targeting components of the hypoxia signaling cascade have been identified, those focusing on specific targets often fail to produce lasting clinical responses. Therefore, there is a critical need for novel hypoxia inhibitors that combine potency, specificity, low toxicity, and favorable druggability. In this study, we evaluated the efficacy of novel drug combinations involving Heat Shock Protein 90 inhibitor (HSP90i) NDNB-25, Tumor Necrosis Factor Receptor-Associated Protein 1 inhibitor (TRAP1i) NDNT-34, and histone deacetylase inhibitors (HDACis) such as CAY10603, suberoylanilide hydroxamic acid (SAHA), and Valproic acid (VPA). The goal was to suppress the hypoxia pathway in TNBC and assess impacts on cell viability, metastasis, and hypoxia-related gene expression using 2D and 3D TNBC models. Our results demonstrated a strong synergistic effect of SAHA/NDNB-25 and SAHA/NDNT-34 combination therapies. Specifically, NDNB-25/SAHA combination significantly down-regulated *HIF1A* expression in 2D- cultures. While NDNB-25 and NDNT-34 mono-therapies only reduced *HIF2A* and *TRAP1* expression, the NDNB-25/NDNT-34 combination down-regulated *HIF1A*, *HIF2A*, *VEGFA*, *TRAP1*, and *HSP90* in 3D cultures. Moreover, the NDNB-25/NDNT-34 combination, at lower concentrations (250 nM and 5 µM), inhibited cell migration more effectively than mono-therapies at higher concentrations (500 nM and 10 µM). Fluorescent imaging revealed more distribution of hypoxia signals using a labeled hypoxia probe and the localization of TRAP1i, tagged with rhodamine B, in TNBC-derived mammospheres compared with luminal subtype. These findings suggest that combining epi-drugs and targeted therapies to modulate the hypoxia pathway could inform the development of innovative clinical strategies against TNBC.

#824 Comparative pharmacokinetics (PK) of targeted tissue factor tTF-NGR when intravenously applied alone or in combination with preceding trabectedin .

Christian Schwoppe, Caroline Brand, Kathrin Hessling, Heike Hintelmann, Andrew F. Berdel, Georg Lenz, Torsten Kessler, Manfred Fobker, Christoph Schliemann, **Wolfgang E. Berdel**

University of Munster, Muenster, Germany

Trabectedin is a treatment option for relapsed/refractory soft tissue sarcomas (STS). CD13 is a neutral aminopeptidase expressed on invasive endothelial cells (EC) such as in the tumor vasculature. CD13-targeted tissue factor (tTF-NGR) is a recombinant pro-coagulatory fusion protein with a molecular weight of 30381.98 g/mol which accumulates in the tumor vasculature leading to tumor vascular occlusion and tumor infarction. Giving both compounds in sequence could trap trabectedin inside tumors and increase its efficacy. *Vice versa* trabectedin optimizes activity of tTF-NGR by externalizing phosphatidylserine (PS) on the surface of EC. PK of safety patients treated at our hospital within a multicenter trial of a combination of trabectedin and tTF-NGR in advanced STS patients refractory to 1st line systemic therapy (TRABTRAP, EudraCT 2020-005858-21) were compared with PK from our phase I monotherapy study with tTF-NGR (EudraCT 2016-003042-85). We compared standard PK parameters for tTF-NGR at identical dose levels (1.0 to 3.0 mg/m²) from TRABTRAP and phase I within the same laboratory with identical methods and calculations. Although some PK differences between phase I and TRABTRAP were more pronounced at lower dose levels, global values such as Cmax, AUC, t1/2alpha, t1/2term, and Kel indicated an increased AUC with a delayed elimination of tTF-NGR when given after trabectedin. As tTF-NGR was always given daily, we measured the remaining tTF-NGR concentrations before subsequent applications and found significantly increased values in the combination protocol (1 mg/m^2: $p<0.0001$; 3 mg/m^2: $p<0.0002$) when compared with the phase I values, suggesting a possible accumulation of tTF-NGR which we did not observe with monotherapy. A specific way of tTF-NGR elimination in the vascular system is the internalization of the tTF-NGR:CD13 complex into EC. Using flow cytometry and fluorescence labeling with tTF-NGR and EC *in vitro*, expression of CD13 on EC was slightly diminished by trabectedin exposure. Also, tTF-NGR binding to CD13 ($p<0.01$) and internalization ($p=0.01$) decreased when tested on control and trabectedin-exposed EC. Both lead to more tTF-NGR molecules remaining in the circulation, explaining part of the delayed elimination. Further, we compared functionality of tTF-NGR in the plasma of patients from phase I and TRABTRAP. On the basis of equal protein amounts and without influence of confounders such as storage, the ability of factor VIIa:tTF-NGR:CD13 to activate factor X to Xa was higher when trabectedin was given to the patients before. Among possible explanations are the decreased liver protease levels after trabectedin, which could influence the functionality of tTF-NGR. In summary, these results explain why the Maximum Tolerated Dose level for tTF-NGR established in the TRABTRAP safety cohort is lower than the 3 mg/m^2 in the phase I study.

#825 Correlation of clinical and biomarker data in FGFR inhibitor failed metastatic cholangiocarcinoma patients with tumor response to tinengotinib.

Sarina A. Piha-Paul[1], Sanjay Goel[2], Chih-Yi Liao[3], Nashat Gabrail[4], Farshid Dayyani[5], Syed Kazmi[6], Sayeh Lavasani[7], Katie Hennessy[8], Hui Wang[9], Caixia Sun[9], Peng Peng[9], Jean Fan[8], Milind Javle[1]

[1]University of Texas MD Anderson Cancer Center, Houston, TX,[2]Rutgers Cancer Center Institute of New Jersey, New Brunswick, NJ,[3]The University of Chicago Medicine and Biological Sciences, Chicago, IL,[4]Gabrail Cancer Center Research, Canton, OH,[5]University of California, Irvine, Irvine, CA,[6]University of Texas Southwestern, Dallas, TX,[7]City of Hope National Medical Center, Duarte, CA,[8]TransThera Sciences (US), Inc., Gaithersburg, MD,[9]TransThera Sciences (Nanjing), Inc., Nanjing, China

Background: Cholangiocarcinoma (CCA) patients (pts) harboring FGFR fusions treated with FGFR inhibitors (FGFRi) have shown clinical benefit, but often have progression in 5- 7 months. Secondary polyclonal mutations in the FGFR2 kinase domain (KD) may be a mechanism for acquired resistance to FGFRi. Tinengotinib, a spectrum-selective multi-kinase inhibitor with unique binding properties to FGFR2, potently inhibited FGFR2 KD mutations in pre-clinical models and exhibited antitumor activity in CCA pts. Here we present two cases of CCA pts treated with tinengotinib in a phase Ib/II trial.

Methods: Biomarker samples were collected at baseline and C3D1 timepoints as an exploratory endpoint for pts receiving tinengotinib therapy. Efficacy was evaluated by RECIST v1.1. Tissue and blood samples were analyzed by FoundationOne® CDx and FoundationOne® Liquid CDx test, respectively, for FGFR alterations.

Results: Two CCA pts received oral tinengotinib 12mg once daily continuously for 28-day cycles and completed biomarker sampling at baseline and C3D1. Both pts were previously treated with chemotherapy and pemigatinib. Pt A is a 28yr female with CCA metastatic to liver and bone. Tinengotinib was initiated in August 2021. The pt achieved a partial response (PR) with tumor reductions of 29.2%, 36% and 41.6% at C3D1, C4D1 and C5D1, respectively. Pt A was treated for 210 days, discontinuing due to radiographic disease progression following a 17-day dose interruption for suspected grade 3 TSH increase. The progression-free survival (PFS) was 6.9 months. Baseline biomarker data showed presence of KD mutations including: E565G/A, N549H, K659M and I548T. At C3D1, E565G had reduced from 1.36% to 0.09%, and there was disappearance of all other FGFR2 KD mutations. Pt B is a 40yr female with CCA metastatic to liver, lung and lymph nodes. Tinengotinib was initiated in August 2021. The pt achieved a PR with tumor reductions of 23.4% and 48.6% at C3D1 and C5D1, respectively. Pt B was on treatment for 181 days and discontinued due to progression in non-target lesions at C6D1, following a 23-day dose interruption for unrelated grade 3 ALT increase. PFS was 5.9 months. Baseline biomarker data showed presence of KD mutations including: E565A/G/K, V564L/I/F, N549K/T

Conclusions: An inverse correlation between the presence of KD mutations and the response to tinengotinib treatment was observed in these two FGFRi relapsed/refractory metastatic CCA pts. This suggests tinengotinib may overcome acquired resistance to FGFRi in CCA pts. An ongoing randomized phase III study is investigating tinengotinib vs. chemotherapy in FGFRi refractory/relapsed CCA (NCT05948475). Clinical trial information: NCT04742959

#826 Combination of CDK4/6 and CDK2/4/6 inhibitors with novel biguanides for pancreatic cancer treatment.

Samantha A. Melendrez[1], Mario Morales Martinez[1], Eduardo Mauricio Gonzalez[1], Gang Deng[2], Timothy Donahue[3], Michael Jung[2], Jaydutt V. Vadgama[4], Richard Pietras[1], Diana C. Marquez-Garban[1]

[1]Department of Medicine, UCLA Jonsson Comprehensive Cancer Center, Los Angeles, CA,[2]Department of Chemistry and Biochemistry, UCLA Jonsson Comprehensive Cancer Center, Los Angeles, CA,[3]Division of Surgical Oncology, Department of Surgery, Department of Medical Pharmacology, UCLA Jonsson Comprehensive Cancer Center, Los Angeles, CA,[4]Department of Medicine, Charles Drew University of Medicine and Science, UCLA Jonsson Comprehensive Cancer Center, Los Angeles, CA

Pancreatic ductal adenocarcinomas (PDAC) is a leading cause of cancer mortality in the U.S. Men and women of African ancestry have the highest incidence and mortality rates as compared to other racial or ethnic groups. African American (AA) patients present at younger ages, with more advanced disease stage, and the least access to surgical and chemotherapeutic interventions. Risk factors include diabetes mellitus type 2 (DM2), obesity, tobacco use, age, race/ancestry, and genetic mutations. Cytotoxic chemotherapy remains a first-line treatment for many PDAC patients since current therapies are often ineffective, necessitating alternative strategies. KRAS is mutated in about 90% of PDAC cases and is essential for initiating and maintaining PDAC growth. Further, a second key genetic event in PDAC involves deletion or silencing of the CDKN2A tumor-suppressor gene that encodes p16INK4A which in turn inhibits cyclin-dependent kinases CDK4 and CDK6 and complements KRAS activation in promoting PDAC. Of note, independent epidemiologic reports show that DM2 patients treated with the biguanide, metformin, a common medication used to treat DM2, have a reduced risk of PDAC and improved survival rates among those who develop PDAC. The current study investigates the hypothesis that combination of new, more potent biguanide analogues with CDK 4/6 and CDK2/4/6 inhibitors could improve treatment outcomes for PDAC. Our work involved the assessment of a newly-designed biguanide analogue termed JD006, alone and combined with selected CDK 4/6 inhibitors (abemaciclib or ribociclib) or CDK2/4/6 inhibitor PF-0687300, on growth of human PDAC cells (Panc-1, Panc 02.03, CFPac-1) in vitro. Cell proliferation assays and immunoblot analyses were used to assess effects of treatments. Combined therapy with JD006 plus CDK 4/6 or CDK2/4/6 inhibitors significantly reduced PDAC cell proliferation ($P<0.01$); with evidence of an additive effect. Immunoblots revealed enhanced inhibition of cell cycle-related proteins and AKT/mTOR downstream signaling with combination treatment with JD006 and ribociclib, abemaciclib or PF-0687300. In conclusion, combinations of JD006 with CDK 4/6, CDK2/4/6 inhibitors appears to effectively target multiple molecular pathways, suggesting a potential new approach for PDAC treatment. Further studies are needed to compare response to treatment and molecular signatures in preclinical models from patients of different ancestry. Better understanding of these biological differences may help reduce health disparities in patients diagnosed with PDAC. [Funded by NCI 5R21CA176337; NCI U54CA143930, UCLA JCCC Breast Cancer Award, CBCRP B27IB3869, Hickey Foundation]

#827 Correlation among early surrogate endpoints and survival in borderline-resectable pancreatic cancer: A trial-level meta-analysis.

Chanbormey Leatheng[1], Alexander Khamechand[1], Gabriel A. Brooks[2], Hirotaka Miyashita[2]

[1]Medicine, Dartmouth-Hitchcock, Lebanon, NH,[2]Oncology, Dartmouth-Hitchcock, Lebanon, NH

Background: Multiple endpoints are used as surrogates for overall survival (OS) in clinical trials for borderline resectable pancreatic cancer (BRPC), including the R0 resection rate. However, consensus is lacking regarding the validity of this and other surrogate endpoints. This study aims to assess the relationship between common surrogate endpoints and median OS (mOS).
Methods: We conducted a systematic review of PubMed and Web of Science to identify clinical trials investigating neoadjuvant treatment (surgery, chemotherapy, or radiation therapy) of BRPC. Eligible trials reported on OS and at least one surrogate endpoint (resection rate [RR], R0 resection rate [R0], 1-year OS [1yOS], and 2-year OS [2yOS]). We estimated the correlation of OS with each of the surrogate endpoints using both Spearman and Pearson coefficients (r). The confidence intervals (CI) of coefficients were estimated by 2,000 times of bootstrapping resampling. Weighted linear regressions with calculation of R-squared were utilized to estimate correlations adjusted for the sample size in each cohort.
Results: We identified 25 eligible clinical trials with 32 treatment cohorts. The range of mOS was 11 to 41 months, and the median mOS was 23.3 months. R0 and 2yOS were significantly correlated with mOS by the Spearman coefficient (r=0.51 [95% CI: 0.01-0.79] and r=0.74 [0.19-0.95], respectively) but RR and 1yOS were not. All early endpoints (RR, R0, 1yOS and 2yOS) were significantly correlated with mOS by the Pearson coefficient (r=0.49 [0.01-0.79], 0.58 [0.28-0.78], 0.75 [0.12-0.91] and 0.79 [0.43-0.92], respectively). Weighted liner regression revealed that RR, R0, 1yOS and 2yOS were significantly correlated with mOS (R-squared= 0.20, 0.32, 0.43 and 0.57, respectively with $p < 0.05$ for all).
Conclusions: This study demonstrated significant correlations between early surrogate endpoints and mOS in BRPC. However, the correlation between mOS and resection-related endpoints (RR and R0) was modest. These findings provide important context for selection and interpretation of surrogate endpoints in studies of BRPC.

#828 ANS03, a novel, orally bioavailable small-molecule type II ROS1/NTRK inhibitor, effectively overcomes clinically relevant ROS1/NTRK resistance mutations and exhibits potent antitumor activity in preclinical tumor models.

Gong Li[1], Alexander Drilon[2], Shengxiang Ren[3], Peilong Zhang[1], Xiangqiu Li[1], Linna Li[1], Ruiya Zhang[1], Wenli Lan[1], Di Han[1], Weizhe Xue[1], Peipei Yang[1], Shutan Liao[4], Kevin Schaab[5], **Brendan Putz**[6], Hepeng Shi[1]

[1]Shenzhen Avistone Biotechnology Co., Ltd, Beijing, China,[2]Memorial Sloan Kettering Cancer Center and Weill Cornell Medical College, New York, NY,[3]Department of Medical Oncology, Shanghai Pulmonary Hospital, Shanghai, China,[4]Avistone Biotechnology Ltd, Beijing, China, Beijing, China,[5]Avistone Biotech, Inc., Dover, DE,[6]Avistone Biotech, Inc., Raleigh, NC

Background: ROS1 and NTRK are critical driver genes for tumorigenesis and progression, and inhibitors targeted towards their tyrosine kinases (TKIs) are clinically proven anti-cancer agents. Although Type I ROS1/NTRK TKIs like Repotrectinib show clinical benefit, on-target resistance mutations inevitably develop. Hence there is an unmet clinical need for novel Type II ROS1/NTRK TKIs capable of overcoming on-target acquired resistance mutations.
Methods: A comprehensive panel of IND-enabling non-clinical studies was conducted to evaluate the mechanism of action, pharmacologic activity, pharmacokinetics, and the safety profile of ANS03.
Results: ANS03 is a novel Type II TKI targeting both ROS1 and NTRK. Unlike type I TKIs, which engage the active “DFG-in” conformation of the kinase, type II TKIs engage the inactive “DFG-out” conformation of the kinase and also extend into an adjacent allosteric pocket. As a result, ANS03 shows a broad and potent inhibitory spectrum for ROS1/NTRK drug-resistance mutations with $IC_{50's}$ ranging from 0.324 nM to 4.88 nM. In cellular systems, ANS03 showed outstanding antitumor effects against wild-type fusions and diverse acquired drug-resistance mutations, with $IC_{50's}$ ranging from 0.1 nM to 26 nM. For ROS1 SF mutations (G2032R, D2033N), ANS03 was 22.2x and 30.6x more active than Repotrectinib, respectively. ANS03 showed a 10^4x enhancement against ROS1 Cβ6 mutation (L2086F), a 52.5x enhancement against NTRK1 xDFG mutation (G667C), and a 104.4x enhancement against NTRK1 compound mutation (G595R-G667C) vs. Repotrectinib. ANS03 also had better activity against the ROS1 L2086F mutation when compared to both Repotrectinib and NVL-520. *In vivo*, ANS03 exhibited antitumor effects in BALB/c nude mouse subcutaneous xenotransplantation models that were as good- or better than- those of the ROS1/NTRK TKIs Repotrectinib or NVL-520. Notably, it achieved complete tumor regression in Ba/F3-ROS1-G2032R models at a dose of 10 mg/kg and triggered tumor regression in LU-01-0414 (ROS1 wild-type fusion) and KM12-Luc (NTRK wild-type fusion) models at a dose of 3 mg/kg. Moreover, ANS03 significantly improved the survival rates of tumor bearing mice in a dose-dependent manner. Concurrent pharmacokinetic (PK) studies in mice positively correlated plasma exposures of ANS03 to administered doses, with ANS03 showing favorable tumor distribution and detectable brain penetration.
Conclusion: Non-clinical studies of ANS03 showed it is a potent, orally bioavailable Type II ROS1/NTRK inhibitor with remarkable activity against various pathogenetic ROS1/NTRK alterations and with favorable absorption, distribution, pharmacokinetics, efficacy, and tolerability profiles *in vivo*. The compound will enter Phase 1 clinical development in the US and China soon.

#829 *ITGAV* is a targetable vulnerability in epithelial ovarian cancer.

Gilbert K. Salloum

Pathology, Beth Israel Deaconess Medical Center - Harvard Medical School, Boston, MA

Ovarian cancer is a lethal heterogenous disease that is typically diagnosed at late stages. In high-grade serous ovarian carcinoma (HGSOC), the most common subtype, PARP inhibitors and chemotherapy are standard of care. Although HGSOC patients are responsive to these therapies, resistance develops and patients invariably relapse. Alterations in genes within the phosphoinositide 3-kinase (PI3K) and AKT network are highly prevalent. Although specific PI3K and AKT small molecule inhibitors have been developed and clinically evaluated, to date none have been approved for clinical use in ovarian cancer patients. To identify collateral druggable vulnerabilities to AKT hyperactivation and develop novel combination therapies in HGSOC, we have performed a CRISPR-based genome-wide synthetic lethal screen. The goal is to identify genes that upon genetic knockout transform a cytostatic cellular response to PI3Ki/AKTi to a cytotoxic one. Using this platform, we have identified genes that regulate integrin signaling as a major mechanism of synthetic lethality against the PI3Ki pictilisib and the AKTi capivasertib. *ITGAV*, encoding the integrin subunit α_V, was a significant dropout against both kinase inhibitors. Pharmacological targeting of integrin α_V synergized with pictilisib and capivasertib to induce apoptotic cell death in multiple cell line models of ovarian cancer, as well as patient-derived organoids. Combined PI3K/AKT and integrin α_V inhibition induced DNA damage and cell cycle arrest. The mechanisms underlying induction of DNA damage and cell death in response to combination treatment are currently under investigation. The ultimate goal is to bring this combination treatment approach to HGSOC patients and shed new light on mechanisms of resistance to PI3K/AKT pathway inhibitors in ovarian cancer.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#83 Investigating the effect of SERPINB3 and STAT3 on invasion and metastasis in cervical cancer cells.
M. Campos Guerrero, **D. E. Hasanova**, L.-Y. Chen, L. Sherbansky, S. Markovina;
Washington University in St. Louis, Saint Louis, MO

Cervical cancer is the fourth most leading cause of cancer related deaths in women worldwide. Of the women treated for cervical cancer, nearly a third experience recurrence after initial treatment with chemotherapy and radiation. After recurrence, salvage rates are low, thus better treatment options must be developed.
One biomarker in cervical cancer is Squamous Cell Carcinoma Antigen 1, also known as SERPINB3. SERPINB3, a member of the serine protease inhibitor superfamily, has been found in elevated levels in patients with cervical cancer, and correlates with resistance to chemo and ionizing radiation. Signal Transducer and Activator of Transcription 3 (STAT3) activates signaling pathways that lead to tumor progression including cancer cell migration and metastasis.
In this project, we investigated how SERPINB3 and STAT3 correlate to cervical cancer cell migration and metastasis. Our hypothesis is that SERPINB3 interacts with STAT3 to increase metastasis of cervical cancer cells. To test this hypothesis, we used the genetically modified SW-756 cervical cancer cell line with and without SERPINB3 and performed transient transfection to knock down the STAT3 gene using silencing RNA (siRNA).
To analyze the knock down of STAT3, we used immunoblotting and quantitative polymerase chain reaction (qPCR) to determine whether the expression of the STAT3 protein and gene was silenced. To determine metastasis of the cells in vitro, we conducted a wound healing assay, in which a wound is generated in the middle of the tissue culture well, and the closure of the wound is monitored every 24 hours via microscopy. The images were analyzed using FIJI Image J Wound Healing Program.
In this project, we have minimized the expression of STAT3 in our cervical cancer cell lines. We noticed that when we decreased STAT3 expression, SERPINB3 levels had also decreased. This correlates with literature suggesting that STAT3 regulates SERPINB3 expression. The wound healing assays revealed that upon knocking down STAT3 in SERPINB3 knockout cells, cell migration had severely decreased compared to SERPINB3 control cell lines knocked down with STAT3. The knockdown will be optimized and results repeated along with pharmacologic inhibition of STAT signaling to determine the mechanism of B3 in migration and metastasis phenotypes. The future direction of this project is to identify the exact SERPINB3-STAT3 interaction that leads to invasion metastasis in cervical cancer. By doing this, we hope to determine different pathways that may lead to increased invasion and metastasis to develop better treatment options in cervical cancer.

CLINICAL RESEARCH: Targeted Therapies and Combinations 1
Poster Session

#830 Combined a B7-H3-targeting antibody-drug conjugate, 7MW3711, and PARP inhibitors synergistically potentiates the antitumor activity in B7-H3-positive cancers.

Xiaowei Cen[1], Peng Fang[2], Yueyue Yang[1], Dongan Yu[2], Yueqin Yao[1], Fei Mei[2], Xueying Li[1], Meng You[2], Yiyan Zhang[1], Long Yin[2], Xiaowei Sun[2], Xiaoding Tan[2], Wei Zhou[1], Hai Wu[1]

[1]Mabwell (Shanghai) Bioscience Co., Ltd., Shanghai, China,[2]Jiangsu Mabwell Health Pharmaceutical R&D Co., Ltd., Taizhou, China

Introduction: B7-H3, an attractive target for antibody drug conjugate, is overexpressed in multiple tumors. Overexpression of B7-H3 in tumor cells frequently correlates with fewer tumor-infiltrating lymphocytes, faster cancer progression, and poor clinical outcome in several malignancies. Preliminary evidence revealed PARP inhibitors (PARPi) and B7-H3-directed antibody-drug conjugate (ADC) as two practical approaches to overcome chemoresistance in solid cancers. Thus, synthetic lethality exploitation by an anti-B7-H3 ADC plus PARPi might be a promising strategy in solid tumor models. Here, we investigated the activity of 7MW3711, a B7-H3-directed ADC bearing a novel isomerase I inhibitor (TOP1i) payload, alone and in combination with PARPi, in preclinical models to evaluate the potential for future clinical application.
Method: 7MW3711 was evaluated in vitro and vivo efficacy with or without PARPi (Olaparib or AZD5305). In vitro, human cancer cell lines were treated with 7MW3711 and two PARPi to determine the effect on anti-proliferative effects, double-stranded DNA breaks, and cell-cycle arrest. In vivo, mice bearing ovarian and SCLC tumor xenografts were treated with the combination of 7MW3711 and PARPi.
Results: Evaluation of the drug resistance ability in cell lines expressing P-gp revealed 7MW3711 exhibited > 3-fold more potent than DS7300 (B7-H3-directed DXd ADC), indicating that 7MW3711 has a lower efflux efficiency in drug-resistant strains. 7MW3711 combined use of PARPi synergistically potentiated the inhibitory effects of 7MW3711 on the growth of B7-H3-positive cancer cells, inducing DNA damage and apoptosis. The highest level of double-stranded DNA breaks was observed in cells treated with combination which can be determined in the upregulation of γ-H2AX protein. Combination increased both G2/M phase-arrested cells and S-phase cells, reduced G1-phase, indicating that most proliferating cells were arrested. Combining 7MW3711 with olaparib or AZD5305 produced significant antitumor effects and survival benefit above that achieved with monotherapy in various B7-H3-positive solid tumor xenografts. Collectively, our results demonstrate the synergistic antitumor activity of combining 7MW3711 with PARPi, suggesting that DNA damage-repair inhibitors in combination with B7-H3-targeted ADCs is a promising approach for treating B7-H3-positive malignancies.
Conclusion: These data provide evidence for the potential utility of 7MW3711 combination with PARPi for treatment of B7-H3-expressing tumors and support the rationale for further clinical studies.

#831 CPT1A inhibition sensitizes diffuse large B-cell lymphoma to venetoclax treatment.

Sophia Steinlein[1], Heike Horn[1], Emma Attwood[1], Annette M. Staiger[2], Katrin S. Kurz[2], Andreas Rosenwald[3], German Ott[2], Claudia Kalla[1]

[1]Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany,[2]Department of Clinical Pathology, Robert Bosch Hospital, Stuttgart, Germany,[3]Institute of Pathology, University of Wurzburg, Wurzburg, Germany

First-line immunochemotherapy fails to achieve a durable response in up to one third of patients with diffuse large B-cell lymphoma (DLBCL). Therapy resistance is mediated, in part, by ineffective elimination of B-cells protected by a metabolic tumor-stroma interaction. Carnitine palmitoyltransferase 1A (CPT1A), a component of the prognostic DLBCL-LAMI signature, plays a major role in transporting fatty acids - partly provided by stromal cells - into mitochondria to support B-cell metabolism. We investigated the CPT1A inhibitors ST1326 and perhexiline for their ability to eliminate lymphoma cells in the presence of stromal cells and the potential of sensitizing them to the therapeutic BCL2 inhibitor venetoclax.

CPT1A protein expression was evaluated in primary tumor specimens by immunohistochemistry using TMAs. CPT1A inhibitors and venetoclax (ABT-199) were tested on different pre-clinical models: (1) 15 DLBCL cell lines, (2) co-culture models of DLBCL cell lines and either stromal cell line HS-5 or primary DLBCL-cancer associated fibroblasts to evaluate the induction of B-cell death in a stromal environment, and (3) patient-derived DLBCL cells. Therapeutic efficacy was assessed using MTT cytotoxicity and CellTiter Glo 2.0 assays. Effects on lipid metabolism, molecular signaling and induction of cell death were investigated by oil red O staining, immunoblots and AnxV/TMRM flow cytometry, respectively.

CPT1A protein was expressed in 77% (67/87) of primary DLBCL (cytoplasmic staining, cut-off 20%). Both CPT1A inhibitors impaired the viability of lymphoma cells: perhexiline treatment was cytotoxic in 5/5 cell lines (IC50 = 5.5-7.5 μM), ST1326 in 10/15 cell lines (IC50 = 5-18 μM). Viability of normal fibroblasts was not affected by either drug. Perhexiline lost its cytotoxic effect against lymphoma cells when co-cultured with stromal cells. In contrast, ST1326 remained effective in killing DLBCL cells also in stromal environment. Suppression of fatty acid transport into mitochondria by ST1326 resulted in cytoplasmic lipid accumulation, suppression of anti-apoptotic Bcl-XL and phospho-AKT, and induction of apoptotic cell death. The combination of ST1326 with the BCL2 inhibitor venetoclax increased its cytotoxic efficacy. Three DLBCL cell lines that were BCL2-positive but resistant to venetoclax were sensitized by ST1326 to the BCL2 inhibitor, an effect that persisted in the presence of stromal cells. In patient-derived DLBCL models (partly from patients with poor response to standard immunochemotherapy), CPT1A inhibition impaired the viability of primary DLBCL cells and enhanced the antitumor activity of venetoclax.

Targeting fatty acid metabolism using ST1326 effectively eliminates lymphoma cells in the presence of stromal cells and increases significantly the efficacy of the BCL2-specific inhibitor venetoclax, thus providing a new candidate approach in the treatment of DLBCL.

#833 A novel anti-CEACAM6 heavy-chain antibody-drug conjugate for gastrointestinal cancer therapy.

Ming-Heng Wu, Yao-Tsung Tsai, Chee Voon Yap

International Ph.D. Program for Translational Science, Taipei Medical University, Taipei, Taiwan

Antibody-drug conjugates (ADCs) are "biological missiles" that utilize a chemical linker to connect monoclonal antibodies (mAbs) with cytotoxic drugs, which induces the precise killing of targeted tumor cells and causes the death of surrounding untargeted cancer cells through the bystander effect. However, current ADCs are too specific to certain cancer subtypes and not specific enough to accurately target a cancer-specific protein, which limits the therapeutic populations and induces unwanted side effects. We developed a heavy-chain antibody (HCAb) from a llama immune library, which recognizes glycosylated CEACAM6 (Carcinoembryonic antigen-related cell adhesion molecule 6), a broad-surface marker of epithelial-type cancers. The anti-CEACAM6 HCAb showed a higher affinity (KD: sub-nanomolar), better thermal stability, unique tissue penetration and epitope-recognition abilities compared to the conventional antibodies. The anti-CEACAM6 HCAb-drug conjugate (HCAb-ADC), which links the HCAb and tubulin inhibitors via hydrophilic linkers, specifically killed CEACAM6-expressing colorectal (CRC), lung, pancreas, and bile duct carcinoma cells at sub-nanomolar concentrations and had a better anti-cancer effect than current FDA-approved ADCs. In xenograft models, a single administration of HCAb-ADC completely abolished colorectal and pancreatic tumors. Notably, HCAb-ADC can effectively inhibit tumor growth even at very low doses (0.2 mg/Kg) and demonstrated a superior therapeutic effect over current therapeutic regimens. Meanwhile, it exhibited minimal toxicity to normal epithelial and peripheral blood mononuclear cells. In summary, the excellent anti-tumor activity across different epithelial cancers and minimal toxicity to normal tissues support the clinical development of the novel HCAb-ADC for treating multiple epithelial-type cancers, including CRC, lung, pancreatic, and bile duct carcinoma.

#834 *In vivo*, *ex vivo*, and *in vitro* (2D and 3D) tumor models: Uses, strengths and limitations.

B. A. Teicher[1], M. G. Hollingshead[1], N. P. Coussens[2], T. S. Dexheimer[2], T. E. Silvers[3], E. M. Jones[2], L. Rubinstein[1], J. H. Doroshow[4]; [1]National Cancer Institute, Rockville, MD, [2]Frederick National Lab for Cancer Research, Frederick, MD, [3]Frederick National Lab for Cancer Research, Freerick, MD, [4]National Cancer Institute, Bethesda, MD

Preclinical models are critical tools in the war on cancer, used in cancer biology & genetics research, drug/therapeutic discovery & development, and for identification of drugs & drug combinations for use to treat specific tumors & specific patients. Each preclinical model has strengths & limitations & varied endpoints. When driving an SAR, new compounds are compared with each other in solid tumor models & tumor growth delay is the endpoint assessed. To determine tumor-cell kill in vivo in syngeneic or xenograft tumors, ex vivo assays are used after in vivo drug administration. To assess inherent potency & potential depth of tumor cell kill, 3D in vitro models are used. The limitations of in vitro models include that prodrugs are non-functional, there is no normal tissue, & the dynamics of drug metabolism are missing. A major limitation of in vivo tumor models is that the mouse is an inbred host in a controlled environment making it difficult to predict toxicity in patients. It is possible to extrapolate response to a drug or drug combination from a 3D cell culture model to the same model grown as a solid tumor in a mouse. Optimized 3D culture models include tumor cells & normal cells such as endothelial & mesenchymal cells. The log-kill hypothesis holds that 90% kill of tumor cells is required for a partial response, while 99% kill is required for a complete response. The 138582-377-R Merkel cell (PDX) treated with cediranib (6 mg/kg) & erlotinib (90 mg/kg) Q5D for 3 wks resulted in 80% decrease in tumor vol compared with controls & 80% cytotoxicity in complex spheroids from the same tumor. The 712881-215-R penile squamous cell ca PDX achieved a brief disappearance of measurable tumor on a complex regimen of cediranib (6 mg/kg) & erlotinib (90 mg/kg), reaching a tumor vol 1% of the control tumor. In complex spheroids, exposure to the combination of cediranib & erlotinib produced 99% cytotoxicity. When the 138582-377-R Merkel cell PDX was exposed to entinostat (30 mg/kg) Q5D for 3 wks, an 86% decrease in tumor vol was achieved compared with the control tumor vol. Less than 90% cytotoxicity was achieved in the complex spheroids in the estimated clinical Cmax concentration range of entinostat. The combination of ipatasertib (60 mg/kg) & selumetinib (20 mg/kg) administered twice per day for 2-rounds of 10 days was less effective in the 171881-019-R breast ca PDX and the 845751-090-R head & neck squamous ca PDX than would be predicted by the complex spheroid studies. Funded in part by NCI Contract No. 75N91019D00024.

#835 A potent and selective small molecule inhibitor of CD38.

Jinwen Huang, Zhongyuan Li, Zeng Song, Yunli Liu, **Steve Shen**

Convalife (Shanghai) Co., Ltd., Shanghai, China

Background: CD38 is a multifunctional ectoenzyme involved in NAD metabolism and is highly expressed in various hematological malignancies, making it a critical therapeutic target in oncology. By depleting NAD and producing immunosuppressive metabolites such as adenosine, CD38 supports tumor growth and creates an immunosuppressive microenvironment. Compared to CD38 antibodies, small-molecule inhibitor offer advantages, including better tissue penetration, oral bioavailability, and cost-effectiveness. CD38 inhibition may restore NAD levels, reverse immunosuppression, and enhance antitumor immune responses, potentially synergizing with immune checkpoint inhibitors (ICIs), providing a promising strategy for cancer therapy.
Methods and Materials: Accelerated by the "Right 6D+AI" platform, AI-based screening and multi-angle evaluation were conducted on over 5,000 virtual molecules, yielding candidate molecule N066 with IC50 activity below 1 nM. To explore the therapeutic potential, oral administration of N066 in mice assessed dose-dependent effects on NAD+ elevation and ADPR reduction in tissues such as the spleen and liver. In syngeneic cancer models, CD38 protein expression was evaluated using immunohistochemistry (IHC) following ICI treatment. In mouse solid tumor models, N066 was administered as monotherapy or combined with ICIs.
Results: The small-molecule inhibited intracellular CD38 activity in cultured human primary T cells, increasing intracellular NAD+ levels. In mice, oral administration of the inhibitor resulted in a dose-dependent increase in NAD+ levels and a decrease in ADPR levels in tissues such as the spleen and liver. In syngeneic cancer models, monotherapy with the N066 showed antitumor activity correlated with changes in NAD+ and ADPR levels. In B16-F10 tumor-bearing mice, combining N066 with anti-PD-L1 therapy enhanced antitumor activity.
Conclusion: CD38 small-molecule inhibitors N066 demonstrates good antitumor activity, highlighting its therapeutic potential.

#836 A phase Ib/II study of PARPi Mefuparib Hydrochloride (CVL218) in combination with PD-1 inhibitor plus chemotherapy in metastatic or recurrent triple-negative breast cancer (TNBC).

Jian Zhang[1], Rujiao Liu[1], **Steve Shen**[2]

[1]Department of Medical Oncology, Fudan University Shanghai Cancer Center, Shanghai, China,[2]Convalife (Shanghai) Co., Ltd., Shanghai, China

Background: BRCA 1 and /or BRAC 2 mutation accounts for about 15% of all TNBC and remains poor outcomes. Mefuparib hydrochloride (CVL218) is a second generation in PARPi with low hematological toxicity and high ability to cross the blood-brain barrier. This study is to explore efficacy and safety of CVL218 combined with PD-1antibody and paclitaxel nanoparticle albumin-bound in patients with TNBC who have failed previous first-line treatment.
Methods: This open- labeled, phase Ib/II clinical study NCT06078670 explores efficacy and safety of three drugs combination . 500mg BID CVL218 was administered orally in combination with toripalimab 240 mg on Day 1 of every 21-day cycle and paclitaxel nanoparticle albumin-bound 125 mg/m^2 on Day 1 of every 21-day cycle. Patients in cohort A were advanced TNBC with either CPS ≥ 1 or HRD gene pathogenic variants.
Results: From July 2023 to 15 July 2024 data, 6 subjects were enrolled in Phase Ib and 5 subjects were enrolled in Phase II. In phase Ib, The RP2D of CVL218 was identified as 500 mg BID. The preliminary efficacy was perfomed every 6 weeks in the first half a year and then once every 12 weeks thereafter in both phase Ib and II by RECISTv 1.1. Among 11 patients, ORR was 72.7% (8 PR, 2 SD), and DCR (Disease Control Rate) was 90.9%. The most common Grade 3 or higher treatment-related AEs (TRAEs) were hepatic function injury (2.6%), adynamia (1.8%), creatinine increased (0.8%), neutrophils decreased (0.8%), white blood cell decreased (0.8%), exanthema (0.8%), and peripheral sensory nerve disorder (0.8%), No Grade 5 TRAE. A total of 9 treatment-related SAEs in 6 patients (54.5%) were observed, including 2 aspartate aminotransferase increased, 2 alanine aminotransferase increased, 1 creatinine increased, 1 liver function injury, 1 adynamia, 1 neutrophils decreased, 1 fever, and 1 ventricular premature beats. All these SAEs recovered after symptomatic treatment.
Conclusion:CVL218 combined with PD-1 antibody and chemotherapy appears to be well tolerated and has good preliminary efficacy in TNBC patients.

#837 A highly potent and selective small molecule TY-4028 for the targeted therapy of non-small cell lung cancer with EGFR exon 20 or HER2 exon20 insertion mutations.

Chengshan Niu*[1], Shengli Dong*[2], Shaoqing Chen[3], Zhongwei Guo[1], Huan Wang[3], Apeng Liang[2], Kaige Ji[1], Meihua Li[2], Bailu Zheng[3], Yanchao Zhao[1], Ming Wu[3], Mingyu Jiang[3], Xiugui Chen[3], Jun Li*[3], Yusheng Wu[3]

[1]TYK Medicines, Inc. (Zhengzhou), Zhengzhou, Henan, China,[2]TYK Medicines, Inc., Changxing county, Huzhou City, China,[3]TYK Medicines, Inc. (Shanghai), Xuhui District, Shanghai, China

Introduction: Following EGFR exon 19del and EGFR L858R mutations, EGFR exon20 insertion mutations are the third most common type of EGFR mutations, representing up to 12% of all EGFR mutations. Approximately 2% of NSCLC patients bear hotspot mutations in HER2,and over 90% of these HER2 mutations are exon 20 insertion mutations. The FDA-approved 1st to 3rd targeted EGFR inhibitors failed to effectively treat NSCLC harboring constantly active EGFR exon20ins mutations in the clinic. To date, no small molecule targeted therapy has been approved by the FDA for the treatment of EGFR exon20ins in NSCLC. Furthermore, Antibody such as Amivantamab has difficulty penetrating the BBB, brain metastases presents a significant clinical challenge in lung cancer with EGFR exon20ins mutations. There is an urgent unmet medical need to develop targeted therapeutics against EGFR and Her2 exon20ins mutations.
Results: TY-4028 is a novel, potent, and orally available inhibitor targeting EGFR and Her2 exon20ins mutations. It is a highly potent covalent inhibitor with rapid absorption and clearance, which contributes to the decreased toxicity of covalent drugs. Preclinical studies have shown a good PK profile and manageable toxicity with TY-4028. TY-4028 showed remarkable efficacy in *in vitro* and *in vivo* models of EGFR exon20ins and HER2 exon20 Ins. The data showed that the efficacy of TY-4028 was similar to that of Mobocertinib(TAK-788)and better than that of Sunvozertinib (DZD9008), whereas the tolerance of TY-4028 was better than that of Mobocertinib in CDX mouse models. The B/P ratio (brain tissue AUC_{last}/plasma AUC_{last}) of SD rats was 1.63 and 1.04, respectively, suggesting that TY-4028 has good potential to cross the BBB.
Taken together, TY-4028 was fully developed by TYK Medicines, Inc., and the compound has great potential to meet the unmet medical needs of NSCLC patients with EGFR exon20ins mutation or HER2 exon20ins mutation. IND clearances of TY-4028 from the US FDA and CN NMPA has received.
*To Whom Correspondence should be addressed to: Jun Li, Ph.D.; Dong Shengli, Ph.D. and Chengshan Niu, Ph.D.

#838 The novel eIF4A inhibitor synergizes potently with the XPO-1 inhibitor in T-cell lymphoma.

So-Young Seol[1], Chung Hyun Park[2], Soo Jeong Kim[2], Hyunsoo Cho[3], Jin Seok Kim[3], Yu Ri Kim[1]

[1]Department of Internal Medicine, Hematology, Gangnam Severance Hospital, Seoul, Korea, Republic of,[2]Department of Internal Medicine, Hematology, Yongin Severance Hospital, Yongin, Korea, Republic of,[3]Department of Internal Medicine, Hematology, Severance Hospital, Seoul, Korea, Republic of

Malignant lymphomas are categorized into B-cell and T-cell lymphomas. While treatment outcomes for B-cell lymphoma have significantly improved with targeted therapies and cellular treatments, T-cell lymphoma remains a therapeutic challenge due to the lack of standardized first-line treatments. Consequently, T-cell lymphoma exhibits lower remission rates, higher relapse rates, and poor overall prognosis. Although T-cell lymphomas account for only 15-20% of all malignant lymphomas, their incidence is notably higher in Asia compared to Western countries, making Korea an ideal location for research on this disease. Also, T-cell lymphoma (TCL) is an aggressive malignancy with poor prognosis, characterized by high relapse rates and limited therapeutic options. Novel therapeutic approaches are urgently needed to improve outcomes for patients with TCL. Previous studies have demonstrated the efficacy of eIF4A inhibitors in downregulating oncogenic protein synthesis and inducing cell death in various lymphoma models. We hypothesized that the combination of eIF4A inhibitor (CR-1-31-B) and XPO-1 inhibitor (Selinexor) would exhibit synergistic anti-lymphoma activity in TCL and have potential clinical applications. The anti-lymphoma effects of CR-1-31-B in combination with a XPO-1 inhibitor were evaluated in TCL cell lines using flow cytometry. Cell apoptosis assays were performed at increasing concentrations of CR-1-31-B and the XPO-1 inhibitor alone, as well as in combination, over 48 hours. To investigate the molecular mechanisms underlying the observed synergistic effects in TCL, RNA-seq profiling was conducted on cells treated with single agents and the combination therapy. Gene set enrichment analysis (GSEA) was performed to identify pathways involved in the combination's mechanism of action. CR-1-31-B and the XPO-1 inhibitor demonstrated concentration- and time-dependent cytotoxicity in TCL cell lines at low nanomolar concentrations. The combination of the eIF4A inhibitor and the XPO-1 inhibitor showed strong synergistic effects in all tested TCL cell lines ($p<0.0001$). RNA-seq profiling revealed that the transcriptional signature of the combination therapy was distinct from that of single-agent treatments. The combination of eIF4A inhibitor and XPO-1 inhibitor demonstrates potent anti-lymphoma activity in T-cell lymphoma. This novel combination represents a promising therapeutic strategy for patients with TCL, warranting further preclinical investigations and potential clinical translation.

#839 The clinical C/EBPβ antagonist peptide lucicebtide synergizes with molecularly targeted therapies in GBM.

Julia Diehl, Ricardo Ramirez, Mark Koester, Franco Abbate, Lila Ghamsari, Abi Vainstein-Haras, Barry J. Kappel, **Claudio Scuoppo**, Jim A. Rotolo

Sapience Therapeutics, Inc., Tarrytown, NY

Glioblastoma (GBM) is the most common and aggressive malignant brain tumor, with median overall survival (OS) following standard-of-care treatment of 15-18 months and 5-year survival of less than 10%. Despite extensive characterization of the genetic lesions driving GBM, there has been no improvement on the overall natural history of the disease, mostly due to the cellular and genetic heterogeneity of these tumors. CCAAT/Enhancer Binding Protein β (C/EBPβ) is a transcription factor identified as a master regulator of the mesenchymal transition in GBM. Lucicebtide (ST101) is a C/EBPβ antagonist peptide that was evaluated in a Phase 2 clinical study in patients with recurrent and newly diagnosed GBM (NCT04478279) and has shown durable responses in a subset of patients. We have previously demonstrated that lucicebtide anti-tumor activity is due to both direct cancer cell death and immune-activation within the tumor microenvironment. Here we investigated whether lucicebtide may synergize with targeted therapeutic strategies against genetic dependencies or immune checkpoints in GBM. We adopted a synthetic lethal CRISPR screen approach in which gain-of-function drivers that are mutated in more than 5% of GBM patients in publicly available datasets (*EGFR, PDGFRA, PI3KCA, MDM2, MDM4, CDK4)* were suppressed in the presence or absence of lucicebtide in three genetically characterized GBM lines. Our data identified that EGFR suppression by multiple independent sgRNAs was synthetic lethal in the presence of lucicebtide. We confirmed these findings by performing checkerboard assays of lucicebtide in combination with chemically distinct EGFR inhibitors, indicating that lucicebtide synergizes with EGFR inhibition (EGFRi) in GBM. We further characterized the mechanism of action of EGFRi-lucicebtide combination on known signal transducers of EGFR in the presence or absence of ST101. Finally, we explored the relationship between transcriptional signatures of C/EBPβ activity and EGFR mutational profile in publicly available GBM datasets, identifying potential genetic biomarkers for prediction of maximal efficacy of this combination. These studies demonstrate the potential of lucicebtide to enhance the activity of molecularly targeted therapeutics such as EGFRi that are typically not effective when used as monotherapies in GBM. Biomarker analysis utilizing GBM genetics and signatures of C/EBPβ activity was performed to identify potential target populations likely to benefit from ST101 combinations.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#84 Dimerization of MACC1: A newly identified feature and its role in cancer metastasis.

Malti Dumbani[1], Benedikt Kortum[1], Simona Kostova[1], Fabian Zincke[1], Alexandra Forrai[1], Dennis Kobelt[1], Wolfgang Walther[1], Erich Wanker[1], Oliver Daumke[1], Christoph Buhlheller[2], **Ulrike S. Stein**[1]

[1]Max Delbruck Center for Molecular Medicine, Berlin, Germany,[2]Medical University Graz, Graz, Austria

Cancer metastasis remains the most lethal characteristic of tumors mediating the majority of cancer-related deaths and continues to be a leading cause of mortality. Exemplified for colorectal cancer (CRC) metastasis results in around 0.9 million deaths annually worldwide. Identifying key molecules responsible for metastasis, understanding their biological functions and therapeutically targeting these molecules is therefore of tremendous value. Metastasis Associated in Colon Cancer 1 (MACC1), a gene first described in 2009, is such a key driver of metastatic processes, initiating cellular proliferation, migration, invasion, and metastasis in vivo. Since its discovery, the value of MACC1 as a prognostic biomarker has been confirmed and linked to poor overall outcome in more than 20 cancer types. Despite numerous studies identifying MACC1 as a promising target for solid cancer, little is known about the structural features of MACC1 protein and its oligomeric states. Therefore, we asked whether MACC1 can form protein complexes and if so, which region is critical for complexation/oligomerization. Using AlphaFold-Multimer, the MACC1 homodimer structure was predicted and the residues involved in dimerization were evaluated. The notion of MACC1 dimerization was validated further using bioluminescence resonance energy transfer (BRET) in HEK293 and HCT116 cell system. To our surprise, BRET revealed that MACC1 exists as homodimer in living cells. Further, we report that mutation of the predicted dimer interface residues Val212, Ile214 and Cys216 close to the N-terminus of MACC1 in the ZU5 domain hinders homodimerization and affects MACC1-mediated cell migration in vitro and metastasis formation in vivo. Taken together, these findings identify an important role of MACC1 dimers for the MACC1-mediated metastasis phenotype. We provide with this study a framework to better understand the MACC1 signaling landscape and to facilitate the rational development of novel therapies aiming at MACC1 oligomerization to intervene in MACC1-mediated metastasis.

CLINICAL RESEARCH: Targeted Therapies and Combinations 1
Poster Session

#840 The ITCC-P4 consortium: Deep molecular characterization of pediatric cancer xenograft (PDX) models enable prediction of novel personalized combination treatment options for patients with childhood cancer.

D. Gurgen[1], A. Gopisetty[2], E.-M. Rief[2], A. Federico[3], D. A. Zwijnenburg[4], M. Kool[3], A. Saint-Charles[5], G. Schleiermacher[5], G. Vassal[2], J. J. B. Koster[4], S. M. Pfister[3], J. Hoffmann[1];

[1]Experimental Pharmacology and Oncology Berlin-Buch GmbH, Berlin, Germany, [2]ITCC-P4 gGmbH, Heidelberg, Germany, [3]Hopp Children's Cancer Center (KiTZ), Heidelberg, Germany, [4]University of Amsterdam, Amsterdam, Netherlands, [5]PSL Research University, Institut Curie Research Centre, Paris, France

Despite advances in the understanding of the pathogenesis of pediatric cancers, first line treatment of advanced, recurrent and metastatic childhood cancer still remains chemotherapy. Omics data unraveling the molecular pathogenesis together with overall low efficacy of targeted agents in preclinical trials so far did not improve the clinical outcomes of pediatric patients. The EU-IMI-2 funded, public-private partnership project “ITCC-Pediatric Preclinical Proof-of-Concept Platform” (ITCC-P4) has built a large repertoire of more than 400 pediatric patient-derived xenograft (PDX) models, including brain, neuroblastoma, soft tissue and other rare solid pediatric cancers. Tumor and PDX omics and in vivo data are stored and analized utilizing the ITCC-P4 R2 data base (https://r2.amc.nl). Our strategy aimed to utilize deep systems biology data from human tumors and corresponding PDX models to predict more effective drug and superior drug combination treatment of neuroblastoma (NB) and rhabdomyosarcoma (RS).

ITCC-P4 single mouse trial (SMT) screening in 27 PDX models from the NB and 24 RS cohort reveal overall low response rates for single targeted drugs including ALk, MDM2, CHEK, MEK kinase inhibitors and efficacy in 1 out of 3 chemotherapies only. Therefore, we conducted a SMT combination trial with already approved targeted cancer drugs in NB and RS PDX models (n=10 each), including major entity subtypes, e.g. high risk-, MYCN amplified NB and PAX3-Foxo fusion RS. In total, 10 drug combinations were compared to a control and single agent arm of up to four individual drugs. Except one drug combination, all doses and schedules were well tolerated in mice indicating absence of adverse side effects.

Interestingly, similar response pattern were observed in NB and RS PDX regardless of their molecular phenotype. However, the response to single agent treatment according to RECIST criteria was low with only 20% achieving stable disease (SD) or better. In contrast, when combining these less effective single agents, we observed an additive anti-tumoral effect with 40% of PDX models responding with SD or better. Most importantly, a significant and outperforming synergistic effect with 50% complete remission and 20% stable disease was achieved with simultaneous inhibition of ALK / BCl-2 or ALK / RAF signaling, which was observed in NB and RS to similar extend.

Taking together, deep molecular phenotyping together with our extensive *in vivo* PDX toolbox allows prediction and exploration of promising noval treatment options. Our results highlight the great potential of the ITCC-P4 portfolio and the close collaboration with internationally recognized pediatric oncologists and physicians, which enable personalized translation of specific combination treatments for childhood cancers in the near future.

#841 The preclinical pharmacokinetics and pharmacodynamics of AZD0022: A potent, oral KRAS^{G12D}-selective agent exhibiting oral bioavailability and KRAS pathway suppression in the GP2D xenograft model.

Kevin Beaumont[1], Sofia Guzzetti[1], Pablo Morentin-Gutierrez[1], Rhys D. O. Jones[1], Ken Grime[1], Carla Martins[1], Alex Koers[1], Alison Peter[1], Simon T. Barry[1], Susan Critchlow[1], Michele Moschetta[2], Michael Niedbala[3]

[1]DMPK, Oncology Targeted Discovery, AstraZeneca UK Limited, Cambridge, United Kingdom,[2]Early Oncology Clinical, AstraZeneca, Barcelona, Spain,[3]Early Oncology Programs Group, AstraZeneca, Waltham, MA

Objectives: AZD0022 is a small molecule selective inhibitor of KRAS^{G12D}. These studies investigate the preclinical PKPD relationships of AZD0022 to inform clinical development.

Methods: Measurement of plasma and tumor concentrations were completed using standard extraction techniques with quantitation by LC-MS/MS methods. Target modulation (pRSK inhibition) was assessed in Nude mice implanted with GP2D human tumors by Western blot.

Results: The preclinical PK properties of AZD0022 are defined by its physicochemistry (MW 614, extensive lipophilicity with log $D_{(7.4)}$ 2.5, dibasic with pKa values of 8.4 and 7.4). Oral administration studies suggest that AZD0022 is 30 to 70% absorbed from the gastrointestinal tract. AZD0022 exhibits extensive distribution into tissues. It also undergoes time-dependent partitioning into the red blood cell fraction of whole blood, suggesting that whole blood is the matrix that best represents the PK of the molecule. Blood clearance of AZD0022 was 8.2 and 8.6 mL/min/kg in mice and dogs, respectively. The measured Vss values based on blood were 10.8 and 20.4 L/kg, respectively. These characteristics suggest that in human, AZD0022 should be orally absorbed and exhibit a half-life consistent with once daily administration. In the GP2D model, single oral 150 mg/kg doses displayed prolonged plasma and tumour exposure. AZD0022 was estimated to be 18x more bound in tumour tissue than in plasma. Seven days of continuous 150mg/kg BID dosing resulted in up to approx. 75% pRSK inhibition. A dose range of 10, 50 and 150 mg/kg BID over 7 days displayed exposure-dependent pRSK modulation, with maximal of 75% pRSK inhibition. PKPD modelling suggested an unbound IC_{50} of 1.4 nM for pRSK inhibition.

Conclusions: The results presented here support the clinical development of AZD0022 which is currently being tested in a first in human Phase I/IIa study (ALAFOSS-01, NCT06599502)

#842 Early clinical outcomes of pembrolizumab plus neoadjuvant chemotherapy in early stage triple negative breast cancer in a Hispanic majority community.

Aymen Baig, **Erin C. Kaser**, Nico-al Paolo Gotera, Jay Parekh, Ranjit S. Banwait, Marcela Mazo Canola

The University of Texas Health Science Center At San Antonio, San Antonio, TX

Background: Triple-negative breast cancer (TNBC) accounts for 15% of breast cancers, and it has a poor prognosis with a five-year survival of 8 to 16% lower than that of hormone receptor-positive disease. There is a high prevalence of TNBC among Hispanics. In KEYNOTE-522 (KN522), a phase 3 trial, pembrolizumab plus neoadjuvant chemotherapy had been shown to be an effective option for pathological complete response (pCR) in early-stage TNBC as compared to neoadjuvant chemotherapy alone at the time of definitive surgery. While this treatment protocol showed promising data, immune checkpoint inhibitors such as pembrolizumab have been known to cause immune-related adverse effects (iRAEs) such as thyroiditis. There are limited studies on the efficacy of this treatment protocol and its adverse effects in Hispanic communities. Our study aims to present the relationship between pCR and immune-mediated thyroiditis in pembrolizumab plus neoadjuvant therapy for early-stage TNBC in a Hispanic-majority population in South Texas.
Methods: This is a retrospective analysis of patients with stage II or III TNBC at Mays Cancer Center in San Antonio, Texas between January 2020 to November 2024. This KN522 treatment protocol included neoadjuvant therapy with four cycles of pembrolizumab every 3 weeks plus paclitaxel and carboplatin. Patients then received four additional cycles of pembrolizumab and doxorubicin-cyclophosphamide or epirubicin-cyclophosphamide. At the 24-week mark, patients were assessed to see if they had successfully completed the KN522 protocol as planned. Patients were assessed for pCR at the time of definitive surgery. Outcomes include assessing for the development of immune-mediated thyroiditis. Statistical analysis to compare outcomes between Hispanics and non-Hispanics was performed using R v4.2.
Results: 66 patients were originally identified on review. 3 patients were excluded based on incomplete data. 63 patients were identified (68.3% Hispanic, 31.6% Non-Hispanic). At the time of definitive surgery, 55.6 % of patients had pCR (62.9% Hispanic, 37.1% Non-Hispanic) vs 44.4% with no pCR (75.0% Hispanic, 25.0% Non-Hispanic) with a p-value = 0.3. Comparing pCR and the development of immune-mediated thyroiditis, 14 patients with pCR (40%) vs 9 patients with no pCR (32.1%) developed immune-mediated thyroiditis with a p-value = 0.5.
Conclusion: There was no difference in pCR with pembrolizumab plus neoadjuvant chemotherapy between Hispanic and non-Hispanic populations. Furthermore, there was no difference in pCR between patients who developed immune-mediated thyroiditis or not. Our results were notable for occurring in a Hispanic majority population, but our study was limited by a small sample size. More studies are needed with a larger sample size to ascertain if there is a difference in outcomes between Hispanic vs non-Hispanic patients.

CLINICAL RESEARCH: Vaccines and Cell Therapies
Poster Session

#845 Development of enhanced extracellular vesicle-based canine osteosarcoma cancer vaccines via metabolic labeling and click chemistry approach.

Yuan Liu[1], Yusheng Liu[1], Matthew R. Berry[2], Hua Wang[1]

[1]Department of Materials Science and Engineering, University of Illinois at Urbana-Champaign, Urbana, IL,[2]Department of Veterinary Clinical Medicine, University of Illinois at Urbana-Champaign, Urbana, IL

Introduction: Osteosarcoma, characterized by its high metastatic potential, remains a prevalent bone malignancy in canines. Current therapeutic approaches, including amputation and chemotherapy, demonstrate limited efficacy and may compromise the patient's quality of life. This study explores a novel immunotherapeutic strategy utilizing metabolically labeled extracellular vesicles (EVs) conjugated with toll-like receptor (TLR) agonists as a potential cancer vaccine for canine osteosarcoma. The approach involves a facile and universal metabolic labeling technique to introduce azido groups onto the surface of mammalian cells, including cancer cells. Subsequently, nanosized EVs released from these cells inherit the azido groups, enabling conjugation with TLR agonists via click chemistry. Upon internalization by dendritic cells (DCs), the conjugated EVs are hypothesized to undergo lysis, facilitating antigen processing and presentation to effector T cells, while simultaneously activating DCs through TLR stimulation. This dual-action mechanism aims to promote a robust cytotoxic T lymphocyte (CTL) response against osteosarcoma cells.
Methods: In this study, peripheral blood mononuclear cells (PBMCs) were isolated from canine donor blood samples. CD14+ monocytes were subsequently purified using magnetic-activated cell sorting (MACS) with microbeads. The isolated monocytes were cultured for 6 days in the presence of human granulocyte-macrophage colony-stimulating factor (GM-CSF) and canine recombinant interleukin-4 (IL-4) to induce differentiation into monocyte-derived dendritic cells (moDCs). For the conjugation with EVs, a panel of toll-like receptor (TLR) agonists was selected, comprising imiquimod (TLR7 agonist), motolimod (TLR8 agonist), CpG oligodeoxynucleotides (TLR9 agonist), and polyinosinic:polycytidylic acid (poly(I:C), TLR3 agonist).
Results and Conclusion: The selected TLR agonists were successfully conjugated with EVs. Notably, EVs conjugated with imiquimod demonstrated significantly enhanced activation of moDCs compared to EVs alone, imiquimod alone, or a simple mixture of EVs and imiquimod. These findings suggest that the conjugation of TLR agonists, particularly imiquimod, with EVs may potentiate the immunostimulatory effects on moDCs. This approach shows promise for the development of a novel immunotherapeutic strategy against canine osteosarcoma. *In vivo* evaluation of the vaccine's efficacy is currently in preparation, which will provide crucial insights into its potential clinical application.

#846 Circular RNA based cancer vaccine encoding NY-ESO-1 is effective for the treatment of lung cancer.

S. Chen, **M. Qiu**;
Peking University People's Hospital, Beijing, China

Nucleic acid vaccines have undergone rapid development in recent years and are playing an increasingly prominent role in cancer therapy. Circular RNA (circRNA), with its unique closed-loop structure, extended protein expression half-life, enhanced stability, and simplified production process, represents a next-generation RNA carrier that offers significant advantages over traditional mRNA. In this study, we utilized our proprietary circRNA platform (NeoAna) to synthesize circRNA encoding NY-ESO-1, a highly immunogenic cancer-testis antigen expressed in various types of cancer, including lung cancer. This circRNA was delivered via lipid nanoparticles to develop a cancer vaccine. Our in vitro experiments demonstrated that circRNA encoding EGFP which was synthesized by NeoAna platform, achieved higher protein expression levels and prolonged expression duration in various cell lines compared to its mRNA counterpart. Moreover, NY-ESO-1 circRNA was found to effectively promote dendritic cell maturation, as well as T cell proliferation and activation in vitro. To assess the vaccine-induced anti-tumor immune responses, we collected spleens and lymph nodes from C57BL/6 mice following intramuscular vaccination. ELISpot assays and flow cytometry assays revealed that circRNA vaccine significantly increased the CD8/CD4 T cell ratio in these tissues, along with enhanced IFN-γ and TNF-α secretion by CD8+ and CD4+ T cells. Elevated NY-ESO-1-specific IgG antibody and IL-12 were confirmed by ELISA. In a murine lung cancer model established by subcutaneous injection of Lewis lung carcinoma cells overexpressing NY-ESO-1 into C57BL/6 mice, the circRNA vaccine demonstrated superior tumor suppression compared to an mRNA vaccine, with a higher proportion of antigen-specific T cells detected in peripheral blood. Additionally, histological examination of (H&E staining) and blood tests confirmed the safety of the circRNA vaccine for biological applications. In conclusion, our novel circRNA vaccine effectively activates anti-tumor immune responses and inhibits lung cancer progression, thereby providing a promising strategy for the clinical treatment of lung cancer.

#847 Small circular RNA for cancer immunotherapy.
Xiang Liu

Xiang Liu (Individual), Ann Arbor, MI

Introduction: Messenger RNA (mRNA) has emerged as a novel vaccine platform and has proven highly effective during the pandemic. This platform had also found application in the development of cancer vaccines. Despite the encouraging outcomes observed in early-phase clinical trials, certain concerns are still associated with current mRNA-based cancer vaccines such as limited stability, suboptimal tumor therapy potency and durability, and limited safety. Here, we report novel highly stable antigen-encoding small circular mRNA (circRNA) vaccines that elicit potent and durable T cell responses for robust tumor immunotherapy.
Method: Small circRNAs comprise minimal elements: codon-optimized peptide antigen-encoding RNA, a short IRES, and a Kozak consensus sequence that recruits ribosomes for peptide translation. Using MHC-I-restricted ovalbumin$_{257\text{-}264}$ (OVA$_{257\text{-}264}$ or SIINFEKL) as a model antigen, we prepared circRNA as follows: 1) circularizing 5'-phosphate-RNA using T4 RNA ligases and complementary 30-mer DNA splints, 2) removing linear/lariat RNA and DNA using DNase I and exonuclease T or RNase R, 3) purification to remove immunostimulatory small DNA/RNA fragments, enzymes, and nucleotides, and 4) verification by Sanger sequencing and gel electrophoresis.
Results: Compared to modified mRNA and current long circRNA, small circRNA shows superior stability, minimal protein kinase R activation, and low cytotoxicity. This allows small circRNA vaccines to sustain efficient antigen translation over an extended period while activating pattern recognition receptors for innate immunostimulation. Relative to several modified mRNAs, nanocarrier-delivered small circRNA vaccines show superior safety and elicit up to 10-fold antigen-specific T cells, accounting for 25%-75% total peripheral CD8+ T cells over 6 months in mice. circRNA vaccines are applicable for various major histocompatibility complex I/II-restricted tumor and viral (neo)antigens to elicit CD8+ and CD4+ T cell responses, in young adult and immunosenescentaged mice. In mice, mono-/multi-valent circRNA vaccines plus immune checkpoint blockade reduced tumor immunosuppression and eradicated multiple tumors, including B16F10, MC38, TC1, and immunotherapy-resistant BrafV600E melanoma. Overall, small circRNA vaccines represent a promising advancement in the field of tumor immunotherapy, offering optimal stability, safety, and the potential to generate potent and enduring immune responses against a variety of tumors.

#848 Preclinical development of the lipid nanoparticle-based mRNA vaccine with multiple natural T cell epitopes for pancreatic cancer.

Josephine Chang[1], Kevin Zheng[2], Yiming Li[3], Wanting Shan[4], **Lei Zheng**[4], Hai-Quan Mao[4], Juan Fu[4], Jiawei Zhou[4]

[1]Hampton Roads Academy, Newport News, VA,[2]Gilman School, Baltimore, MD,[3]Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins School of Medicine, Baltimore, MD,[4]Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, MD

Background: Immunotherapy has recently emerged as a promising avenue in cancer treatment; however, its effectiveness on pancreatic ductal adenocarcinoma (PDAC) is hindered by the lack of effector T cells in the tumor microenvironment. One of the strategies to overcome the resistance of PDAC to immunotherapy involves identifying immunogenic tumor-associated and specific antigens for the development of personalized cancer vaccines. The mRNA platform has become an attractive platform for antigen presentation. The delivery of mRNA cancer vaccines via lipid nanoparticles (LNPs) enables intracellular delivery and controlled release of the payload, offering improved vaccine efficacy. However, mRNA vaccines presenting human T cell epitopes often lack a preclinical model for antitumor immune efficacy testing.
Methods: A syngeneic mouse model of PDACs was established by implanting KPC tumor cells into the livers by hemispleen surgery. mRNA vaccines expressing five 25-mer peptide regions were synthesized. Two mouse CD8+ T cell epitopes were predicted. Their MHC binding was tested by the T2 binding assay. CD8+ T cells specific for these two epitopes were examined by ELISpot assays and were quantified by ImmunoSpot ELISpot Reader to identify changes in IFN- secretion.
Results: Using mass spectrometry analysis, we successfully identified natural HLA class I and class II-binding epitopes in the neoplastic tissues of human PDACs. For the design of mRNA vaccine, we chose five 25-mer peptide regions that each contains both class I and class II epitopes punctuated by AAY and GPGPG linkers and followed by an MITD sequence, which all aim to improve the efficiency of antigen presentation to dendritic cells. Using NetMHC, we predicted H2Kb or H2Db-binding epitopes LYPFPLAL and SMVQMTFL, within these five human peptide regions. The T2 binding assay showed a lack of evident binding of both peptides to the T2-Kb or T2-Db cells. However, The ELISpot assay showed that LYPFPLAL, but not SMVQMTFL, can induce the IFN- expression from CD8+ T cells derived from splenocytes of the KPC tumor mice vaccinated by a peptide vaccine containing both peptides. Therefore, this result confirmed that LYPFPLAL is a mouse CD8+ T cell epitope and can be used to monitor antigen-specific CD8+ T cell response in response to the mRNA vaccine treatment in the syngeneic mouse model of PDACs.
Conclusions: Antitumor efficacy of our above-designed mRNA vaccine can be tested in the syngeneic mouse hemispleen model of KPC tumors. Mouse LYPFPLAL epitope-specific CD8+ T cell response can serve as a surrogate marker for the antitumor efficacy of this mRNA vaccine in mouse models for PDAC.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#85 Puf60 drives tumor progression and therapy resistance in head and neck squamous cell carcinoma.
Jung Je Park[1], Chae Dong Yim[2], Ji Hyun Seo[3], So Young Cheon[4]

[1]Otorhinolaryngology-head and neck surgery, Gyeongsang National University, Jinju, Korea, Republic of,[2]Otorhinolaryngology-head and neck surgery, Gyeongsang National University Hospital, Jinju, Korea, Republic of,[3]Gyeongsang National University, Jinju, Korea, Republic of,[4]Gyeongsang National University Hospital, Jinju, Korea, Republic of

Background: Head and neck squamous cell carcinoma (HNSCC) is a malignancy with high morbidity and mortality due to its invasive nature and frequent resistance to therapy. PUF60, a multifunctional RNA-binding protein, plays roles in transcription regulation and DNA repair, but its specific contributions to HNSCC pathogenesis remain unclear. This study investigates the role of PUF60 in tumor progression, oxidative stress responses, and resistance to therapeutic interventions.
Methods: HNSCC cell lines were employed to study the functional effects of PUF60 overexpression and knockdown. Cellular assays assessed invasion, migration, and apoptosis under standard conditions and in response to oxidative stress, cisplatin, and radiation. DNA damage was analyzed using flow cytometry and Western blotting, while immunoprecipitation and subcellular fractionation were performed to explore interactions between PUF60 and vital regulatory proteins such as PTEN and Sirt6.
Results: Overexpression of PUF60 significantly enhanced the invasive and migratory capabilities of HNSCC cells, while knockdown reduced these phenotypes. PUF60-overexpressing cells demonstrated resistance to cisplatin-induced apoptosis, accompanied by altered ROS production and decreased markers of DNA damage under oxidative stress. Radiation studies revealed a protective effect of PUF60, with reduced DNA damage and enhanced survival. Mechanistically, PUF60 modulated the subcellular localization of PTEN and interacted with Sirt6, highlighting its role in maintaining tumor cell viability under stress conditions.
Conclusion: PUF60 is a crucial driver of HNSCC progression and therapy resistance, promoting tumor cell invasiveness, modulating responses to oxidative and genotoxic stress, and facilitating survival under therapeutic pressure. Targeting PUF60 may offer a novel approach to overcoming resistance and improving treatment outcomes in HNSCC.

CLINICAL RESEARCH: Vaccines and Cell Therapies
Poster Session

#850 Development of a systemic enveloped immunovirotherapy for targeting metastatic sites.

Yunyi Kang[1], **Duong Nguyen**[1], Trevor Smith[1], Stephanie Songco[1], Forrest Neuharth[1], Ivelina Minev[1], Karolin Streule[2], Selamawit Alemu[2], Lina Schulte[2], Thomas Herrmann[2], Boris Minev[1], Antonio Santidrian[1]

[1]Calidi biotherapeutics, San Diego, CA,[2]StemVac, Bernried, Germany

Background: Systemic virotherapies targeting directly all tumors are a promising form of cancer immunotherapy not only due to their tumor lytic characteristics but also because of their potential to transform tumor immune microenvironment, and targeted delivery of virus-encoded therapeutic payloads into tumors. Developing a platform for systemic delivery would provide a significant advantage in treating both primary tumors and any metastatic lesions. However, systemic administration of virotherapies may pose challenges of increased toxicity and reduced efficacy due to rapid virus clearance. In order to overcome these limitations, we have selected and developed a novel enveloped vaccinia virus platform by isolating a complement-resistant strain called Redtail vaccinia virus (RT). We have further engineered the backbone of the isolated strain to achieve tumor specific targeting and high therapeutic efficacy.
Methods: The RT backbone was genetically engineered by deleting multiple metabolic and immuno-modulatory genes. The replications of lead RT candidates were compared in vitro by xCELLigence real time cell analysis. In addition, therapeutic efficacy and toxicity profile of the candidate RT were monitored through in vivo imaging and clinical observation in both immunocompromised and immunocompetent mouse models.
Results: The newly developed and engineered RT demonstrated significant production of enveloped viruses with improved safety profile and enhanced therapeutic efficacy in metastatic lung cancer mouse models. In the immunodeficient nude mouse model, selected RT modification showed tumor specific targeting and preferential amplification in the tumors. In immunocompetent mouse model, RT lead candidate exhibited significant survival in circulations, tumor targeting, regression of primary and metastatic tumors with acceptable safety profile. In addition, drastic changes in tumor microenvironment were observed. Enveloped RT oncolytic immunotherapy increased cytotoxic T cell infiltration and decreased Tregs at the tumor sites, along with the release of proinflammatory cytokine, ultimately leading to tumor regression.
Conclusions: In summary, we have successfully developed a novel enveloped antitumor virotherepy that could be systemically delivered as a form of cancer immunotherapy in a safe manner.

#851 AdAPT-001 demonstrates potent oncolytic activity, high levels of TGFβ inhibition, and encouraging clinical activity in refractory sarcoma: update of the BETA-PRIME trial and preclinical correlates.

Chris Larson[1], Ana Sanchez[1], Farah Hedjran[1], Garett Soapes[1], Bryan Oronsky[1], Anthony P. Conley[2], Meaghan Stirn[1], Tony Reid[1]

[1]EpicentRx, Inc., La Jolla, CA,[2]MD Anderson Cancer Center, Houston, TX

Soft tissue sarcomas are an exceedingly rare and aggressive malignancy, occurring in about 5000 patients per year with an estimated median overall survival of around 12-18 months. Standard chemotherapy includes doxorubicin alone or with ifosfamide with high toxicity and low response rates. Other approved therapies in sarcoma are trabectedin, eribulin, and pazopanib, but their response rates are less than 10%. Immunotherapy trials have been largely disappointing. In the SARC028 phase 2 clinical trial, the PD-1 inhibitor, pembrolizumab, had limited activity except in undifferentiated pleomorphic sarcoma and liposarcoma. In the Phase 2 Alliance A091401 trial, the response to nivolumab was very low at only 5%, which led the investigators to arrive at a conclusion of medical futility, stating that, “Nivolumab alone does not warrant further study in an unselected sarcoma population given the limited efficacy”. AdAPT-001 is currently under investigation in a Phase 1/2 clinical trial for the treatment of advanced malignant solid tumor(s) for patients who have received all conventional therapies considered appropriate by Investigator. To date, of the 25 patients who received multiple doses of AdAPT-001 plus a checkpoint inhibitor, 14 of them were diagnosed with soft tissue sarcoma. Of these 14 patients, 69.2% (9/14 patients) reached a 3-month PFS and 35.7% (5/14) had an objective response. Most of these sarcomas had previously progressed on a PD-1 or PD-L1 inhibitor or were considered nonimmunogenic and, therefore, unsuitable for checkpoint inhibitor monotherapy. To date, the median PFS is 8.5 months. To further understand the activity of AdAPT-001 in sarcoma, we analyzed the lytic activity, TGFβ trap expression, and checkpoint expression patterns of sarcoma cell lines after infection. We demonstrate robust infection, replication, lysis and high-levels of TGFβ trap expression in sarcoma cell lines and objective responses, including pathologic complete responses, among patients who have progressed on chemotherapy and immunotherapy.

#852 A novel EGFR neoantigen-specific mRNA cancer vaccine demonstrates immunogenicity and anti-tumor efficacy in human.

Wenhua Liang[1], Bo Cheng[1], Jingshu Ma[2], Yan Wu[2], Liang Du[2], Peng Gao[2], Zhenxing Yang[2], Wenjie Song[2], Bo Ying[2], Jianxing He[1]

[1]Department of Thoracic Surgery and Oncology, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China,[2]Suzhou Abogen Biosciences Co., Ltd., Suzhou, China

Activating mutations in the epidermal growth factor receptor (EGFR) gene occur in 10-20% of Caucasian and 40% of Asian non-small cell lung cancer (NSCLC) patients. Predominant ones among these are in-frame deletions in exon 19 and the L858R point mutation in exon 21, which constitute 85-90% of all tyrosine kinase domain mutations. Although targeting these mutations has led to the early success of tyrosine kinase inhibitors, all patients eventually will develop resistance to treatment. Immunotherapy has been extensively explored in NSCLC patients in the recent years, but treatment outcome is still unsatisfying. As peptide-based neoantigens targeting EGFR mutations have demonstrated the potential of eliciting robust T cell responses against cancer cells, the combination of immune checkpoint inhibitor (ICI) and cancer vaccine has become a promising approach in fighting NSCLC. Here, we reported an mRNA-based cancer vaccine targeting mutated EGFR led to great anti-tumor efficacy in patients either in monotherapy or in combination of ICI. ABO2013 is an mRNA-based cancer vaccine targeting three most common EGFR mutations including exon 19 deletions, L858R, and T790M mutations. Mutation-specific T-cell responses induced by ABO2013 were initially tested using human PBMCs and transgenic mice expressing prevalent human HLA-A alleles, then anti-tumor efficacy was assessed in EGFR mutation-bearing mouse models. Intramuscular injection of ABO2013 elicited a robust adaptive T-cell response specifically targeting the three encoded EGFR mutations. The activated T cells demonstrated potent cytotoxic activity against tumor cells harboring the corresponding mutations, as evidenced by both *in vivo* and *in vitro* anti-tumor efficacy assessment. A first-in-human dose-escalation trial was conducted to further evaluate clinical safety and efficacy of ABO2013 in treating NSCLC patients (ChiCTR2300071001). Eligible participants include those carrying EGFR exon 19 deletions and/or L858R and/or T790M mutations. Participants must have also failed of standard of cares. Different doses of ABO2013 monotherapy or in combination with sintilimab 200mg were administered every 3 weeks to evaluate the safety, immunogenicity and antitumor efficacy. A total of 30 patients have been treated with either ABO2013 monotherapy (n=20) or in combination with sintilimab (n=10) by October 29, 2024. No dose-limiting toxicities or grade 3-4 TRAEs have been observed. Notably, robust neoantigen-specific immune responses were detected with patients achieving complete remission or partial response, respectively. ABO2013, as a monotherapy and in combination with sintilimab, has exhibited a favorable safety profile and a robust neoantigen-specific immune response and promising antitumor activity, warranting further investigation in EGFR-mutated NSCLC population.

#853 PDL1-PRAME dual antigen cancer vaccine inhibits melanoma growth in syngeneic mouse model and induces antigen-specific immune responses.

K.-T. Chan[1], H.-W. Chen[2], P.-H. Liang[3], Y.-Y. Lin[3], T. Zhang[1], M. Toh[1];
[1]CK Life Sciences Development Limited, Hong Kong, Hong Kong, [2]National Health Research Institutes, Miaoli, Taiwan, [3]ImmunAdd Inc., Taipei City, Taiwan

Preferentially expressed antigen in melanoma (PRAME) is a cancer/testis antigen frequently expressed in various types of cancer, especially in melanoma, and its overexpression is associated with aggressive tumor progression. PRAME-specific T-cells found in cancer patients recognize and specifically lyse cancer cells, positioning PRAME as a promising target for cancer immunotherapy. PDL1 represents one of the most important immune checkpoint antigens frequently overexpressed on tumor cells to suppress T-cell mediated anti-cancer immunity. Several anti-PDL1 antibodies have been approved for clinical use through passive immunization. We had developed PDL1-PRAME dual antigen cancer vaccines and reported their promising tumor growth inhibition effects against syngeneic mouse hepatocellular carcinoma in 2023. In the present study, anti-cancer efficacy of the PDL1-PRAME dual antigen cancer vaccine was evaluated in an aggressive mouse melanoma model. Subcutaneous administration of the cancer vaccine formulated with alum-CpG oligodeoxynucleotides (ODNs) prior to tumor implantation significantly inhibited tumor growth by 91.5% *(P<0.01)* and prolonged the median survival time from 23.5 days to 40.5 days *(P<0.01),* compared to adjuvant treated control. To further optimize the vaccine formulation, another *in vivo* study was conducted with the PDL1-PRAME vaccine protein formulated with different adjuvants, including a novel synthetic saponin based adjuvant IAS-01, to evaluate the vaccine-induced antigen-specific immune responses in C57BL/6 mice. IAS-01, developed by ImmunAdd, is a liposome-based adjuvant incorporating two potent immunostimulants, a fully synthetic monophosphoryl Lipid A and a fully synthetic truncated QS-21 analogue, IA-05, devoid of hemolytic toxicity. Both alum-CpG ODN and IAS-01 obviously enhanced the vaccine-induced T-cell response against PRAME and elevated the anti-PDL1 antibody response. In particular, the highest anti-PDL1 antibody response was found with the IAS-01 adjuvant, compared to all the other adjuvants tested. Our study highlighted the prospect of developing the PDL1-PRAME dual antigen cancer vaccine for cancer immunotherapy. The vaccine efficacy is being further optimized in terms of its protein structure, adjuvant formulation, and administration route for potential clinical testing.

#854 Targeting Irg1 expressed on macrophages in lymph nodes promotes response to mRNA cancer vaccines.

Chao Wan[1], Wenwen Wei[1], Xiao Yang[1], Yeshan Chen[1], Mengjie Che[1], You Qin[1], Qiao Li[2], Kunyu Yang[1]

[1]Cancer Center, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China,[2]Rogel Cancer Center, University of Michigan, Ann Arbor, MI, United States, Ann Arbor, MI, MI

mRNA cancer vaccines show great promise for tumor therapy, but their therapeutic efficacy is limited. Metabolites play critical roles in immunomodulation, however, their role in mRNA cancer vaccines remains unclear. Using metabolome analysis combined with single-cell RNA sequencing, we found that macrophage-derived itaconate increased markedly after ovalbumin-encoding mRNA-lipid nanoparticle (OVA-LNP) injection in activated ipsilateral lymph nodes compared to that in homeostatic contralateral lymph nodes. Depleting the immune-responsive gene 1(Irg1) in macrophages, which encodes the itaconate-production enzyme aconitate decarboxylase (ACOD1), improved the antigen presentation of dendritic cells and the functions of T cells. Knockdown of Irg1 using small interfering RNA (siRNA) combined with OVA mRNA in LNPs enhanced the therapeutic efficacy of mRNA vaccines alone or combined with an anti-programmed cell death-1 antibody. Taken together, our results reveal a link between itaconate and mRNA cancer vaccines, and suggest that targeting Irg1 via siRNA-LNP may be a promising strategy for improving the therapeutic efficacy of mRNA cancer vaccines.

#855 Dynamics of T cell and T cell receptor following mRNA-4157 (V940) plus pembrolizumab or pembrolizumab alone in resected melanoma from the mRNA-4157-P201 (KEYNOTE-942) trial.

Min Lu[1], **Ryan J. Sullivan**[2], Jacky Chow[1], Janice M. Mehnert[3], Matteo S. Carlino[4], Adnan Khattak[5], George Ansstas[6], Matthew H. Taylor[7], Meredith McKean[8], Georgina V. Long[9], Mark B. Faries[10], Jason J. Luke[11], Anjali Rao[1], Laureen S. Ojalvo[1], Filippos Porichis[1], Igor Feldman[1], Lakshmi Srinivasan[1]

[1]Moderna, Cambridge, MA,[2]Massachusetts General Hospital, Boston, MA,[3]NYU Grossman School of Medicine, New York, NY,[4]Melanoma Institute Australia and Westmead Hospital, Sydney, Australia,[5]Hollywood Private Hospital and Edith Cowan University, Perth, Australia,[6]Washington University School of Medicine, St Louis, MO,[7]Earle A. Chiles Research Institute, Portland, OR,[8]Sarah Cannon Research Institute, Nashville, TN,[9]Melanoma Institute Australia, The University of Sydney, Royal North Shore and Mater Hospitals, Sydney, Australia,[10]The Angeles Clinic and Research Institute, a Cedars-Sinai affiliate, Los Angeles, CA,[11]UPMC Hillman Cancer Center, Pittsburgh, PA

Introduction: mRNA-4157 is a novel mRNA-based individualized neoantigen therapy (INT) designed to enhance endogenous antitumor T-cell responses by targeting unique patient-specific tumor mutations. In the phase 2 mRNA-4157-P201 (KEYNOTE-942) trial, patients with completely resected high-risk stage IIIB-IV melanoma receiving mRNA-4157 + pembrolizumab (pembro) showed prolonged recurrence-free survival (RFS) and distant metastasis-free survival (DMFS) versus those receiving pembro alone (Weber JS, et al. *Lancet* 2024). Here, we characterized the dynamics of T cell and T cell receptor (TCR) repertoire in peripheral blood following mRNA-4157 plus pembro or pembro alone.

Methods: Patients were randomized 2:1 to receive mRNA-4157 + pembro or pembro alone. Whole blood samples from longitudinal time points (baseline: pre-pembro & pre-INT; post-pembro & pre-INT; post-pembro & post-INT; and longer-term follow-up after completion of treatment; 116, 101, 108, and 79 patients respectively) were collected and TCR sequencing was performed. TCR beta chains were down sampled and normalized to the top 10k clones by rank sum unique molecular identifier. Shannon clonality was calculated on down sampled data. Differential clone abundance between screening and on-treatment samples was calculated by binomial test.

Results: Baseline TCR clonality was not associated with RFS of mRNA-4157 + pembro or pembro alone. However, longitudinal analysis of samples post-INT showed clonality was increased in 70% (55 out of 78) and 31% (12 out of 38) of the patients treated with mRNA-4157 + pembro and pembro alone, respectively. Analysis of clonotypes showed median number of novel expanded clonotypes after mRNA-4157 + pembro (n=78) and after pembro alone (n=38) was 38 and 22, respectively, suggesting that mRNA-4157 + pembro was associated with expansion of more novel clonotypes relative to pembro alone. Furthermore, among 78 patients in the mRNA-4157 + pembro arm, patients without recurrence (n=59) had median number of 40 novel expanded clonotypes while patients with recurrence (n=19) had median number of 16 novel expanded clonotypes. Additional analysis of neoantigen-specific T cells is ongoing and will be reported.

Conclusions: Expansion of novel T cell clonotypes in peripheral blood was observed to a greater extent after mRNA-4157 + pembro compared to pembro alone, and the degree of novel expansion was associated with RFS.

#856 Exploiting the molecular mimicry for preventive/therapeutic cancer vaccine development.

Maria Tagliamonte, Beatrice Cavalluzzo, Angela Mauriello, Concetta Ragone, Biancamaria Cembrola, Simona Mangano, **Luigi Buonaguro**

National Cancer Institute Pascale, Naples, Italy

Shared tumor antigens are needed for developing off-the-shelf preventive/therapeutic cancer vaccines. To this aim, shared mutated neoantigens (TSAs) cannot be exploited for highly prevalent tumors in the majority of worldwide cancer patients. Therefore, TAAs must be used, which may suffer from the potential immunological tolerance. Analogue peptides with improved antigenicity and immunogenicity, able to elicit a cross-reactive T cell response are needed. To this aim, non-self-antigens derived from microorganisms (MoAs) may be of great benefit. We will describe several examples of MoAs sharing sequence homology with TAAs (“molecular mimicry”), able to elicit a cross-reacting CD8+ T cell response. In particular, the homology to cancer testis TAA (e.g. MAGE-A1) allows to develop cancer vaccines useful for several tumor types, “one-vaccine-for-many-tumors”. In conclusion, non-self MoAs should be selected and included in preventive/therapeutic cancer vaccines with a more potent anti-tumor efficacy compared to those based on TAAs. This would allow the development of ready to be used “off-the-shelf” cancer vaccines for the global population of patients affected by one or more cancer types (Tagliamonte M, et al., Molecular mimicry and cancer vaccine development. Mol Cancer. 2023 Apr 26;22(1):75. doi: 10.1186/s12943-023-01776-0).

#857 Calcium enhances synthetic HPV long peptide vaccination-mediated anti-tumor immunity and improves tumor control.
Ming-Hung Hu

Johns Hopkins University, Baltimore, MD

Introduction: Transfection efficiency is a critical factor in the successful delivery of tumor vaccines. Intramuscular (IM) injection followed by electroporation is considered a superior approach compared to conventional IM injection alone. However, electroporation's clinical unavailability and feasibility present significant limitations. Calcium, a ubiquitous second messenger, plays a crucial role in regulating various cellular processes, including transcription and cell death. Calcium overload can increase cell membrane permeability, promote reactive oxygen species accumulation, and trigger cell necrosis, leading to the release of intracellular contents such as antigens and damage-associated molecular pattern molecules (DAMPs). Our hypothesis is that calcium administration, by effectively priming immune responses, could serve as a feasible therapeutic strategy to overcome these limitations in cancer vaccine delivery.
Method: The synthetic long-peptide vaccine utilized in this study, E7aa 43-62, comprises a synthetic peptide that mimics the 43-62 amino acid sequence of the HPV-16 E7 antigen. The long-peptide vaccines (20 μg) were administered to evaluate their anti-tumor effects in buccal TC-1 tumor-bearing C57BL/6 mice, with a total of three doses given at 7-day intervals. Vaccination was followed immediately by calcium administration or calcium electroporation. Calcium chloride solution (168 mM) was administered either subcutaneously or intratumorally. Electrical pulses were delivered using Tweezertrodes electrodes connected to a BTX electroporation generator (ECM830; BTX Harvard Apparatus). Eight pulses at 72 V were applied, each lasting 20 ms, with 200-ms intervals between pulses.
Results: In the current study, calcium administration was shown to enhance HPV long-peptide vaccination-mediated anti-tumor immunity. Specifically, calcium administration achieved tumor control and prolonged survival comparable to calcium electroporation, accompanied by elevated levels of serum E7-specific tetramer formation. Additionally, mice receiving calcium injections, either subcutaneously or intratumorally, exhibited improved tumor control and superior survival compared to vaccination alone. Cellular analysis revealed that calcium injection significantly increased the systemic E7-tetramer population. This robust and sustained immunological activation was not only effective against contralateral buccal tumors but also provided protection against TC-1 tumor re-challenge.
Conclusion: Our data demonstrate that calcium administration, either subcutaneously or intratumorally, enhances the anti-tumor immunity elicited by HPV long-peptide vaccination. This treatment effect is evidenced by controlled tumor growth and prolonged survival, accompanied by increased E7-specific tetramer formation.

#858 Development of next generation carbohydrate-based vaccines.

X. Huang;
Michigan State University, East Lansing, MI

The development of an effective vaccine construct against tumor associated carbohydrate antigens (TACAs) is an attractive approach towards cancer treatment and prevention. However, a significant challenge is that TACAs are only weakly immunogenic and direct administration of TACAs cannot elicit a powerful antibody response to protect the host from cancer development. In this presentation, we will present our strategies in engineering virus like particle bacteriophage Qβ to significantly enhance the immunogenicity of TACAs as potential anti-cancer vaccines. With its abilities to display antigens in a highly organized manner, Qβ can be a powerful carrier to deliver the antigen to the immune system and activate adaptive immunity. We have engineered Qβ to reduce the antibody responses against the carrier and incorporate T cell epitopes to induce a comprehensive immune response activating both humoral and cellular immunity. With the new Qβ carrier in hand, ganglioside GD2 has been explored as a representative antigen. Ganglioside GD2 is an appealing TACA due to its overexpression on many types of tumor cells. However, its low immunogenicity and the significant side effects observed with anti-GD2 antibodies present significant obstacles for vaccines. To overcome these, a new GD2 derivative bearing an *N*-acetamide (NHAc) at its non-reducing end neuraminic acid (9NHAc-GD2) has been designed to mimic the 9-*O*-acetylated-GD2 (9OAc-GD2), a GD2 based antigen with a restricted expression on tumor cells. 9NHAc-GD2 was synthesized efficiently via a chemoenzymatic method and subsequently conjugated with a powerful carrier bacteriophage Qβ. Mouse immunization with the Qβ-9NHAc-GD2 conjugate elicited strong and long-lasting IgG antibodies, which were highly selective toward 9NHAc-GD2 with little cross-recognition of GD2. Immunization of canines with Qβ-9NHAc-GD2 showed the construct was immunogenic in canines with little adverse effects, paving the way for future clinical translation to humans. Building on these results, we are further engineering the 9NHAc-GD2 antigen to improve its immunogenicity. Our latest efforts in designing new GD2 antigens and evaluating them in tumor models will be discussed.

#859 Novel oncolytic RNA viruses, IVX037 and IVX055, demonstrate potent antitumor activity.

Darren Shafren, Christine Lee, Jera Peters, Szun Tay, Chloe Evans, Min Quah

ImmVirX, Newcastle, Australia

Background: Oncolytic viruses have emerged as promising therapeutic agents to selectively target, infect and destroy cancer cells while synergizing with novel immunotherapies including Checkpoint inhibitors (CPI) and Chimeric Antigen Receptor (CAR) T- cells to increase anti-tumor efficacy. We developed two novel oncolytic non-enveloped RNA viruses, IVX037 and IVX055, using a proprietary receptor-focused bio-selection platform technology. IVX037 and IVX055 are small, single stranded RNA viruses, utilizing independent cell surface receptors to facilitate tumor targeting, cell entry and subsequent potent lytic replication. Intratumoral (i.t) IVX037 challenge of human microsatellite-stable colorectal cancer (MSS-CRC) xenografts increases expression of PD-L1 and mediates a widespread inflammation phenotype, suggested to allow increased migration of anti-tumor lymphocytes, elevation of cellular targets for immune checkpoint and cell-based anti-cancer therapies.

Methods: *In vitro* monolayer cultures of human cancers were challenged with various input multiplicities of IVX037 or IVX055 with lytic infection assessed by a XTT viability assay. For mouse models, immunocompromised mice (SCID or nu/nu) were seeded with single flank human tumor cell administrations. Following development of palpable tumors, IVX037 was administered via i.v or i.t routes. Calliper measurements were employed to assess tumor burden. Checkerboard titrations of IVX037 and a mesothelin targeted CAR-T were utilized to assess anti-tumor activity in monolayer cultures of human gastric cancer cells.

Results: IVX037 demonstrated potent oncolytic *in vitro* activity in human ovarian, gastric, and hepatocellular cancer (HCC) cell cultures. IVX055 induced rapid cell lysis in non-small cell lung, HCC and human bladder cancer *in vitro* cell preparations. Intratumoral and intravenous administration of IVX037 in human tumor xenograft models using immunocompromised mice were well tolerated and displayed potent anti-tumor activity against ovarian, gastric and HCC cancers. In reference, to cell-based therapies, preliminary *in vitro*combination studies with mesothelin-targeted CAR T-cells and IVX037 suggest enhanced tumor cell killing compared to that of either agent alone in a human gastric cancer cell culture model.

Conclusions: The promising preclinical anti-tumor activity of IVX037 and IVX055 against a wide range of human malignancies warrants further clinical evaluation in combination with novel immunotherapy strategies. To this end, IVX037 is currently being investigated in an ongoing Phase 1b combination trial with sintilimab (anti-PD-1) in patients with advanced MSS-CRC, gastroesophageal, and ovarian cancer (NCT05427487). Future plans for IVX055 include evaluation in combination with immune checkpoint therapy in patients with advanced non-small cell lung cancer (NSCLC) bearing metastatic liver disease.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#86 The role of truncated O-glycans in PDAC progression: Linking C1GALT1 loss to metabolic alterations.

Wyatt Petersen[1], Venkatesh Varadharaj[2], Zahraa I. Alsafwani[2], Surinder K. Batra[2], Moorthy P. Ponnusamy[2]

[1]Cancer Research, University of Nebraska Medical Center, Omaha, NE,[2]Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE

Pancreatic cancer (PC) remains one of the leading causes of cancer death, often advancing undetected until late stages. Aberrant O-glycosylation, including the expression of truncated carbohydrate antigens such as Tn and STn, is a hallmark of PC; however, the mechanisms linking these glycan alterations to tumor progression and metastasis are poorly understood. This study investigates the role of truncated O-glycans, driven by the loss of the O-glycosyltransferase core 1 synthase (C1GALT1), in promoting metabolic reprogramming and early metastatic dissemination in pancreatic cancer. C1GALT1 catalyzes the biosynthesis of mucin-type O-glycans, but its inactivity in PC leads to the accumulation of truncated glycans. Analysis of poorly differentiated human PC tissues revealed a significant loss of C1GALT1 expression. CRISPR/Cas9-mediated knockout (KO) of C1GALT1 in PC cell lines also results in increased Tn and STn glycan expression, enhanced tumorigenic potential, and increased metastatic capacity. Using genetically engineered mouse models (*C1galt1* KO with $Kras^{G12D/+}Trp53^{R172H/+}$ mutations), we observed early-onset and widespread metastasis, mirroring the heightened aggressiveness associated with C1GALT1 loss in pancreatic cancer. Transcriptomic profiling through RNA sequencing and pathway enrichment analysis in C1GALT1 KO PC cells revealed significant upregulation of cholesterol metabolism pathways, suggesting that the loss of C1GALT1 may drive metabolic changes to support the aggressive behavior of PC cells. Supporting this, functional assays demonstrate a significant increase in wound healing and migratory capacity of C1GALT1 KO cells compared to control PC cells, highlighting their enhanced motility and potential for metastasis. These findings underscore the role of C1GALT1 loss in driving aggressive tumor phenotypes, potentially through metabolic and functional adaptations that promote the need for further investigation. This work highlights a previously underexplored link between aberrant glycosylation and metabolic rewiring in pancreatic cancer. By elucidating the role of C1GALT1 loss in driving these processes, our findings provide novel insights into the mechanisms of tumor progression and identify potential vulnerabilities for therapeutic intervention. Targeting the interplay between glycosylation and metabolic pathways offers a promising avenue to disrupt the aggressive phenotype of pancreatic cancer.

CLINICAL RESEARCH: Vaccines and Cell Therapies
Poster Session

#860 BCMA CAR-T cells releasing mutant CST6 ^{N137D} proteins further improve therapeutic effects in multiple myeloma.

Yamin Meng[1], Zijun Zhang[2], Yan Cheng[2], Hongwei Xu[2], Frits van Rhee[2], Guido Tricot[2], John D. Jr Shaughnessy[2], Fenghuang Zhan[2]

[1]University of Arkansas for Medical Sciences, Little Rock, AR,[2]Internal Medicine, University of Arkansas for Medical Sciences, Little Rock, AR

Introduction: Multiple myeloma (MM) is a hematological malignancy characterized by the accumulation of abnormal monoclonal plasma cells and osteolytic bone disease. The latter is a key feature of MM, which is associated with worse clinical outcomes. We have found that high cystatin E/M (CST6) expression is linked to the absence of bone lytic lesions in human MM patients and CST6 suppresses the formation of osteolytic lesions by blocking osteoclast differentiation and function in MM. We have also developed the B cell maturation antigen (BCMA)- chimeric antigen receptor T cell secreting wild-type CST6 proteins (BCMA/CST6^{WT}), which specifically kill MM cells and suppress osteolytic lesions in MM mouse models. However, we also found that CST6^{WT} induced immunosuppression in MM, which is an unwanted effect of MM therapy. The objective of this study is to develop a BCMA-CAR-T engineering a mutant CST6, which still targets both MM cells and osteoclasts but without immunosuppressive function.
Materials and Methods: The different functional mutants of CST6 proteins were purified to test their effects on both osteoclastogenesis and immune cells. The proliferation assays and osteoclast formation assays were conducted to test the mutant CST6 function on immunosuppression and osteoclast formation. the BCMA/CST6 ^{N137D} CAR vector contained BCMA-scFv and the mutant CST6 ^{N137D} was constructed. A P2Aself-cleaving peptide was inserted between CAR and CST6 ^{N137D}. The CBA assays were employed to test the concentration of the cytokines. The ELISA assays were conducted to test the concentration of CST6 protein.
Results: We have generated 6 different CST6 mutants to determine the key domain(s) for the immunosuppressive effects of CST6. By determining $CD8^+$ cell proliferation and performing osteoclast formation assays, we have identified that the non-glycosylated CST6 ^{N137D} proteins lack an immunosuppressive function but maintain the suppressive function of osteoclastogenesis. We further developed the BCMA/CST6 ^{N137D} CAR-T cells releasing a mutant CST6 and found that the BCMA/CST6 ^{N137D} CAR-T cells significantly increased the expression of both IFN-γ and TNF- α cytokines compared with the BCMA-CAR-T cells and BCMA/CST6 ^{WT} -CAR-T cells. The BCMA/CST6^{N137D}CAR-T cells also had a stronger antitumor activity compared with the BCMA-CAR-T cells and BCMA/CST6 ^{WT} -CAR-T cells.
Conclusion: In this study, we have identified a CST6^{N137D} mutant, which could suppress osteoclast differentiation without immunosuppressive effects. Furthermore, we have also developed that the BCMA/CST6^{N137D} CAR-T cells may have a better therapeutic effect by targeting both tumor cells and osteoclasts but without suppression of cytotoxic T cell proliferation in MM.

#862 Dynamic hydrogel drug delivery systems for enhancing tertiary lymphoid structure formation and maturation.

W. Tang[1], C. Kuwentrai[2], M. J. Webber[3], Z. Ye[1], **J. Huang**[2];
[1]University of Hong Kong, Faculty of Dentistry, Pokfulam, Hong Kong, [2]University of Hong Kong, The - Li Ka Shing Faculty of Medicine, Pokfulam, Hong Kong, [3]University of Notre Dame, Notre Dame, IN

Introduction: Tertiary lymphoid structures (TLSs), also known as ectopic lymphoid structures, are organized lymphoid-like aggregates that can form within the tumor microenvironment. TLSs exhibit similar structural and functional characteristics to secondary lymphoid organs, such as lymph nodes, and are associated with improved cancer prognosis and enhanced immune responses, including immune checkpoint blockade (ICB) therapies. However, inducing mature TLSs remains a significant challenge. Our study aims to develop a dynamic hydrogel drug delivery system that stimulates the formation and maturation of TLSs.
Design: The study involved the synthesis of a hydrogel through supramolecular interactions between 4-(4-chlorophenyl)pyridine-modified hyaluronic acid (HA-CPP) and cucurbit[8]uril (CB[8]). The hydrogel was loaded with the cytokines CXCL13 and LIGHT or with liposomes encapsulating the mRNA coding for these two cytokines, and was injected into cancer-bearing mouse models. We assessed the effect of the hydrogel on TLS formation and maturation through immunofluorescence staining. We also investigated tumor growth and survival rates. Furthermore, we explored the combined effect of the hydrogel and anti-PD1 ICB therapy on tumor suppression and TLS formation.
Results and discussion: The study demonstrated that a single injection of the HA-CPP ⊂ CB[8] hydrogel containing CXCL13 and LIGHT effectively increased TLS density, facilitated TLS maturation, suppressed tumor growth, and extended survival in the intraperitoneal melanoma mouse model. Moreover, the combination of the hydrogel-based drug formulation and anti-PD1 ICB therapy resulted in increased tumor suppression, improved survival rates, and further strengthened TLS formation. Furthermore, although TLS formation in some solid tumor locations like subcutaneous areas was significantly suppressed compared to that in intraperitoneal areas, our mRNA-loaded hydrogel still effectively induced TLS and combated tumors in various subcutaneous tumor models, including melanoma, breast cancer, colon cancer, and pancreatic cancer. This effectiveness is likely due to the adaptability, high translational efficiency, and durable bioactivity of mRNA therapies.
Conclusion: The study concludes that the HA-CPP ⊂ CB[8] hydrogel-based drug formulation acts as an effective synthetic immune niche scaffold for promoting mature TLS formation within tumors. This approach provides a promising strategy for advancing tumor immunotherapy by enhancing the immune response against various cancers and improving the efficacy of ICB therapy.

#863 A novel SIRPA based FiCAR T cells therapy targeting TYRP1 to treat melanoma patients.

Henrik Paavilainen[1], Sakari Poysti[1], Veera Nikoskelainen[2], Marie Nyman[1], **Anil K. Thotakura**[1]

[1]Immuno-Oncology, Orion Corporation, Turku, Finland,[2]Protein and Antibody Engineering, Orion Corporation, Turku, Finland

Background: The first-generation immune checkpoint inhibitors anti-PD1/PDL1 and anti-CTLA-4 either alone or in combination have shown tremendous clinical success in BRAF wild type melanoma cancer patients. However, there is still a high unmet need as great proportion of melanoma patients relapsed after treatment with first generation immune checkpoint inhibitors and BRAF mutant patient relapsed after treatment BRAF inhibitors. Chimeric antigen receptor (CAR) expressing T cells have shown clinical success in hematological malignancies, recent studies have shown that CAR Ts cells were detected in circulation a decade after the first CD19 CART cell injection. Here, we generated a novel SIRPa based extracellular extension TYRP1 targeting CAR T cell, here onwards referred as TYRP1 FiCAR, that work independent of the tumor mutation burden and BRAF status. TYRP1 targeting FiCARs could be an alternative and attractive therapeutic strategy for treatment relapsed melanoma patients.

Methods & Results: A well-known anti-TYRP1 binder flanvotumab's scfv was inserted into FiCAR-structure containing CD28 and CD3zeta intra cellular signaling domain and those CAR T cells were studied for both in vitro and in vivo function. TYRP1 based FiCAR grew normally and good transduction rates with low vector copy numbers were achieved. In addition, upon co-culture of TYRP1 FiCAR T cells with tumor cells expressing TYRP1 a rapid and significant release of pro-inflammatory cytokines was detected. Moreover, killing of the target cells were observed by the TYRP1 FiCAR T cells. To study the in vivo functionality of the TYRP1 FiCAR-T cells, NSG mice were inoculated with melanoma cells subcutaneously. Three weeks after the inoculation mice were intravenously injected with a single dose of TYRP1 FiCAR T cells.

Discussion: The data presented here shows a proof-of-concept study for the FiCAR structure to work against TYRP1 antigen. TYRP1 FiCAR T cells upon co-culture with TYRP1 expressing tumor elicited a significant increase in effector cytokines production and has not shown any signs of tonic signaling. Additionally, TYRP1 FiCAR T cells were able to kill tumor cells efficiently reaching 100% maximal killing. In vivo, TYRP1 FiCAR T cells significantly delayed tumor growth. However, full regression of the tumors was not achieved in vivo. Collectively, the in vitro and in vivo data support further optimization of TYRP1 FiCAR T cells to treat melanoma patients refractory to immunotherapy and BRAF inhibitors.

#864 Novel innate immune receptor-based chimeric antigen receptors for *in vivo* programming of myeloid cells show potent anti-tumor activity in preclinical solid tumor models.

Yuxiao Wang[1], Neha Diwanji[1], Shannon Argueta[1], Junming Tong[2], Ewa Grudzien-Nogalska[1], Michael Gorgievski[1], Josephine D'Alessandro[1], Edward Cochran[1], Phillip Darcy[2], Jian Ding[1], Robert Hofmeister[1], Daniel Getts[1]

[1]Myeloid Therapeutics, Cambridge, MA,[2]Peter MacCallum Cancer Centre, Melbourne, Australia

Background: Chimeric antigen receptor (CAR) T cell therapy faces significant challenges in solid tumors due to the immunosuppressive tumor microenvironment (TME). Given their tumor-infiltrating capability, phagocytic activity, and ability to promote adaptive immune responses, CAR-engineered myeloid cells represent a promising therapeutic approach. Here we introduce a novel class of CARs designed for programming myeloid cells *in vivo*. These CARs comprise antibody binders against tumor antigens fused with truncated innate immune receptors CD89 or NKp44. Like their natural counterparts, activation of CD89 and NKp44-based CARs requires association with their cognate signal adapters FcRγ or DAP12, which are only expressed in myeloid/NK cells. Such segregation of antigen binding from cellular activation ensures immune cell-restricted CAR activity, minimizing off-target effects during *in vivo* administration.

Methods: The CAR constructs comprise a single-chain variable fragments (scFv) against HER2 or gp75, fused to truncated CD89 or NKp44. CAR mRNA was encapsulated in lipid nanoparticles (LNPs) to achieve *in vivo* programming. Coding sequences and 3' UTR of the mRNA were optimized for prolonged expression. *In vitro*, CAR expression and function were evaluated in a THP1 reporter cell line and primary monocytes. Anti-tumor efficacy of the CARs was assessed in a human HER2 (hHER2) transgenic mouse model and a syngeneic B16/F10 melanoma model, in which CAR mRNA/LNPs were administered intravenously.

Results: The CD89 or NKp44-based CARs were highly expressed in primary CD14+ monocytes for more than 7 days post electroporation. THP1 reporter cell assays demonstrated CAR-dependent activation of the NFκB pathway. Biochemical studies confirmed specific association and phosphorylation of FcRγ chain or DAP12 with CD89 or NKp44-based CARs, respectively. Transfection of human PBMCs with mRNA/LNP showed preferential CAR expression in myeloid cells. In the hHER2 transgenic mouse model bearing hHER2-MC38 tumors, intravenous administration of mRNA/LNPs encoding HER2-targeting CARs showed potent anti-tumor activity and prolonged survival. In the B16/F10 melanoma model, delivery of gp75-targeting CARs reduced tumor growth and increased $CD8^+$ T cell infiltration into tumors, indicative of TME remodeling toward an immunostimulatory state.

Conclusions: We demonstrated that CD89 and NKp44-based CARs achieve robust expression in myeloid cells. *In vivo* programmed CAR myeloid cells elicit potent anti-tumor activity and promote an T cell-mediated adaptive immune response in syngeneic mouse models. The CARs' reliance on endogenous signaling adaptors, combined with preferential mRNA/LNP uptake by myeloid cells, ensures immune cell-specific activity and provides an enhanced safety profile for *in vivo* myeloid cell engineering.

#865 Engineering a next-generation dual-knockout TIL product with AaCas12b nuclease editing.

Jingwei Sun[1], Yao Sheng[1], Hongwei Zhang[2], Xi Zhu[2], Jingman Wang[1], Ke Liu[1], Shin-Shay Tian[2], **Pin Wang**[3], Xiaoping Zhao[2], Yarong Liu[1]

[1]Grit Biotechnology, Shanghai, China,[2]Shanghai Vitalgen Biopharma, Shanghai, China,[3]USC - University of Southern California, Los Angeles, CA

Advancing cellular therapies, particularly allogeneic and functionally enhanced treatments using gene editing technologies, necessitates rigorous safety and quality assessments to ensure clinical success. Among CRISPR nucleases, Cas12b—a type V CRISPR enzyme—stands out for its high editing fidelity, owing to its stringent mismatch intolerance between single guide RNA (sgRNA) and target DNA, surpassing SpCas9 in precision. An engineered variant of Cas12b from *Alicyclobacillus acidiphilus*, termed AaCas12b^{Max}, has demonstrated exceptional on-target editing efficiency with minimal off-target activity, making it a promising tool for complex therapeutic gene editing. This study employed AaCas12b^{Max} to develop GT300, a next-generation tumor-infiltrating lymphocyte (TIL) product incorporating a dual-gene knockout. Candidate sgRNAs for the target genes were identified through *in silico* analysis to minimize genome-wide mismatches. Top-performing guides, validated in HEK293 cells and human PBMC T cells, were further evaluated in TIL products and compared with SpCas9-mediated editing. The AaCas12b^{Max} system achieved comparable knockout efficiency and target protein reduction to SpCas9, with functional evaluations showing similar cytotoxicity, cytokine secretion in vitro, and antitumor efficacy in a mouse PDX model using autologous TILs. Notably, TIL products edited with AaCas12b^{Max} exhibited 3-5 times greater expansion and enhanced viability during large-scale production compared to those edited with SpCas9. These cells demonstrated improved stemness, reduced exhaustion, and apoptosis, alongside superior expansion post-infusion in vivo, suggesting reduced genetic stress during editing. Comprehensive off-target and chromosomal translocation analyses using SITE-seq, GUIDE-seq, and PEM-seq, validated by Amp-seq and hybrid capture assays, confirmed a superior safety profile for AaCas12b^{Max}-edited GT300 compared to SpCas9. RNA-seq analyses at 6 and 24 hours post-electroporation revealed diminished activation of apoptosis and exhaustion pathways in AaCas12b^{Max}-edited TILs. In conclusion, GT300 edited with AaCas12b^{Max} demonstrated efficient gene editing, a superior safety profile, and significant improvements in cell expansion, viability, and fitness. A first-in-human clinical trial of GT300 is currently underway in China.

#866 Next-generation neoantigen vaccines powered by AI-assisted discovery and APC-targeted delivery platforms.

Jingwei Sun[1], Zhenjiang Liu[1], Ke Liu[1], Xi Zhu[2], Chenxi Hou[2], Hong Chen[2], Xu Wang[1], Jiahui Jin[1], Zhao Xu[1], Stephen Schoenberger[3], **Pin Wang**[4], Yarong Liu[1]

[1]Grit Biotechnology, Shanghai, China,[2]Shanghai Vitalgen Biopharma, Shanghai, China,[3]La Jolla Institute for Immunology, La Jolla, CA,[4]USC - University of Southern California, Los Angeles, CA

Neoantigen-based cancer vaccines have shown promising efficacy in recent clinical trials for treating solid tumors. However, traditional neoantigen prediction methods, which largely rely on HLA-peptide binding databases, often yield false-positive predictions, highlighting the need for more accurate strategies to identify truly immunogenic neoantigens. To address this, we developed *NEOVigator*, an advanced neoantigen discovery platform that combines an AI-driven algorithm with functional validation using tumor-specific T cells derived from autologous tumor-infiltrating lymphocytes (TILs) and peripheral blood mononuclear cells (PBMCs). The *NEOVigator* platform mimics the complete antigen presentation and T-cell response cascade by isolating and expanding autologous antigen-presenting cells (APCs) to evaluate neoantigen expression, processing, MHC binding, and presentation. Patient-derived TILs and PBMC T cells are then used to assess TCR recognition and T-cell responses to APC-presented antigens. Trained on experimentally validated immunogenic neoantigens, the AI algorithm achieves over 40% predictive accuracy across diverse solid tumor types. To enhance neoantigen delivery and maximize antitumor T-cell responses, we developed an APC-targeted lipid nanoparticle (LNP) system. This innovative mRNA delivery platform enables efficient, APC-specific targeting across multiple organs while minimizing off-target expression in non-immune cells, such as hepatocytes. Preclinical toxicology studies in multiple species have demonstrated favorable tolerability for both the ionizable lipid L52715 and the APC-targeted LNP formulation. Our lead neoantigen vaccine candidate, GT601, has successfully entered an investigator-initiated clinical trial in China, marking a major milestone in translating the *NEOVigator* platform into next-generation cancer immunotherapies.

#867 Multi-omic characterization of tumor microenvironment evolution in LBCL across treatment lines: Insights from Atlas classification and axicabtagene ciloleucel trials.

Michael D. Mattie[1], Regis Perbost[2], Aurelie Auguste[2], Alexia Papadopoulos[2], Jenny J. Kim[1], Ioana Kloos[1], Gayatri Tiwari[1], Justin Budka[1], Darawan Rinchai[1], **Simone Filosto**[1], Rhine Shen[1], Davide Bedognetti[1], Jerome Galon[3]

[1]Kite Pharma, Inc., Santa Monica, CA,[2]Veracyte, Inc., Marseille, France,[3]INSERM, Paris, France

Axicabtagene ciloleucel (axi-cel), an autologous anti-CD19 chimeric antigen receptor (CAR) T-cell therapy, was approved for patients with relapsed or refractory large B-cell lymphoma (LBCL) after two or more lines of systemic therapy (ZUMA-1, NCT02348216), as well as for those refractory to or relapsing within 12 months of first-line chemoimmunotherapy (ZUMA-7, NCT03391466). Recent molecular classifications of LBCL based on genetic factors shown to associate with response to R-CHOP and other chemo-immunotherapy based standard of care regimens have not been shown to associate with response to axi-cel in LBCL. Methods: To advance our understanding of the impact of tumor biology on response to axi-cel, we performed a detailed characterization of the tumor microenvironment (TME) using a multi-omic approach, integrating data from proteomics, transcriptomics and genomics offering a comprehensive view of biological systems, which we named Atlas. To construct the LBCL Atlas, we applied multiple technologies to 267 vendor-procured tumor samples from LBCL patients at diagnosis: (i) IHC panels, capturing spatial information at the protein and cellular level by counting and positioning cells of interest, (ii) Transcriptomics via RNA sequencing (RNA-seq) and Nanostring hybridization technology, (iii) Genomics, focusing on somatic mutation analysis through whole-exome sequencing (WES). Advanced mathematical and bioinformatics processing was employed to extract biological insights by combining both linear (multimodal factor analysis) and non-linear (self-organizing maps) methodologies. This effort resulted in a graphical representation of both complementary and overlapping signatures. Using samples with different prognostic outcomes and data from ZUMA-1 and ZUMA-7 axi-cel clinical trials, we further explored the evolution of the TME throughout disease progression and different lines of treatment. We focused on both adaptive immunity (lymphocytes, NK cells, etc.) and innate immunity (macrophages, MDSCs), examining the TME clusters defined by Atlas to characterize distinct tumor microenvironments. This Atlas provided an alternative to the cell-of-origin classification and other genetically based classifications of LBCL, based on TME. We will present evolutionary changes though lines of therapy to less favorable tumor microenvironments and, through the projection of clinical trial samples, demonstrate that the Atlas is a powerful tool for exploring mechanisms behind treatment response and disease progression. This includes the association of immune cell populations, such as T-cell and macrophage phenotypes and overall immune cell contexture within the TME, and other biomarkers with disease progression and treatment response.

#868 Platform for purifying serially cytotoxic T cells: advancing cell engineering and in vivo applications.

Eric Ferguson, John M. Beierle, **Behrad Azimi**

Narwhal Bio Inc., San Diego, CA

Current techniques for the enrichment of engineered T cells rely on surface markers that do not accurately capture a T cell's exhibited cytotoxicity, specificity for antigens, or its temporal state in the activation-exhaustion cycle. While microscopy-based methods have been developed to assay live T cells based on their interaction and cytotoxicity against antigen-presenting cells (APCs), no single technique can isolate serially cytotoxic T cells based on the frequency and the cytotoxic outcome of their interaction with specific APCs. The addition of an isolation step based on cell behavior is imperative for pooled screening for optimized engineering of T-cells as well as for the utilization of the enriched cells in vivo. Narwhal Bio Inc.'s A1 platform, an instrument, software, and reagent combination, seeks to overcome this challenge by in vitro cell identification, tagging, and downstream isolation of live cells based on behaviors assessed through microscopy. This live-cell-in, live-cell-out process could enable downstream applications like animal studies, further cell engineering, and multiomics drug and target discovery. We have applied this system to enrich CAR-T cells involved in repeated cytotoxic events. We assayed and tagged T cells based on their serial cytotoxicity in an APC co-culture in real time. We then isolated the tagged T cells, assessed their activation state using CD69 and CD25, and confirmed enrichment for the CAR representation in the more cytotoxic cell population. We assessed the potency of the enriched T cells after sorting, culture, and subsequent co-culture with APCs, showing >3x improvement in potency. Lastly, we illustrated how the process can be performed on the order of millions of cells in a day for various in vivo and in vitro downstream applications, including pooled screening of T-cell engineering and use in animal models.

#869 Distinct expansion, phenotype, function, and toxicity of cilta-cel vs. ide-cel CAR T cells in the real world.

Djordje Atanackovic[1], Tim Luetkens[2], Dina Schneider[3], Peirong Hu[3], Xu Wang[3], Amol C. Shetty[4], Luke Tallon[4], Imari Patel[1], Rohan Singh[1], Etse Gebru[1], Rediet Mulatu[1], Destiny Omili[1], Daniel Yamoah[1], Xiaoxuan Fan[1], Aerielle Matsangos[1], Patricia Lesho[1], Kenneth A. Dietze[2], Ariel A. Fromowitz[1], Kim Hankey[1], Saurabh Dahiya[5], Jean A. Yared[1], Nancy M. Hardy[1], Rima Koka[2], Michael E. Kallen[2], Ashraf Badros[1], Aaron P. Rapoport[1], Mehmet Kocoglu[1]

[1]University of Maryland Greenebaum Comprehensive Cancer Center, Baltimore, MD,[2]University of Maryland, Baltimore, MD,[3]Lentigen Technology Inc., Gaithersburg, MD,[4]University of Maryland Baltimore, Baltimore, MD,[5]Stanford University, Stanford, MD

Idecabtagene vicleucel (ide-cel) and ciltacabtagene autoleucel (cilta-cel) are approved chimeric antigen receptor T cell (CAR T) therapies for multiple myeloma. Unfortunately, most patients receiving these treatments will experience toxicities and/or relapse highlighting the need for optimizing CAR T strategies. We performed the first in-depth, comparative and prospective biomonitoring of patients (N=39) receiving cilta-cel or ide-cel in the real-world setting. Cilta-cel response rates were higher, although not statistically significant, and atypical neurotoxicities/infections were more frequent in the cilta-cel group. Peak circulating CAR T counts were significantly higher in cilta-cel patients, driven by a pronounced $CD4^+$ CAR T expansion, and correlated with depth of clinical response. The pronounced expansion of cilta-cel CAR T was associated with higher CAR expression and overexpression of CD27. The cilta-cel CAR T expansion was followed by a CAR-specific switch from proliferation-associated genes to genes/surface markers associated with effector/memory (EM) function. The comparably longer persistence of cilta-cell CAR T was associated with increased resistance to exhaustion and increased IL-7R expression; *in vitro* data showed persistent and antigen-independent activation and higher metabolic activity of cilta-cel vs. ide-cel CAR T. Among cilta-cel-treated patients experiencing atypical neurotoxicities, central nervous system (CNS)-infiltrating, effector-type cilta-cel CAR T presented a distinct inflammatory phenotypic/cytokine-expression profile. This first report of in-depth, comparative patient biomonitoring following real-world cilta-cel or ide-cel therapy highlights intrinsic biological differences between these BCMA-targeting CAR T products, potentially explaining differences in clinical activity and toxicity. Our findings may guide the optimization of cellular immunotherapy strategies in myeloma patients.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#87 Targeting POU5F1-expressing circulating tumor cells to prevent metastasis and recurrence in colorectal cancer.
S. Fujino, A. Ito, R. Hayashi, M. Yasui, M. Ohue, N. Miyoshi;
Osaka International Cancer Institute, Osaka, Japan

Background: Recurrence and metastasis following curative treatment remain significant challenges in colorectal cancer (CRC). Circulating tumor cells (CTCs) are pivotal in the dissemination of cancer, leading to distant metastases and eventual recurrence. Despite their importance, the cellular lineages driving these processes in CRC remain poorly defined. POU5F1, a transcription factor associated with stemness, is highly expressed in recurrent CRC tumors and is linked to poor prognosis and a high incidence of liver metastasis.
Methods: We developed a patient-derived cancer cell model, termed isolated tumor-derived cancer cells, cultured as two-dimensional organoids (2DOs). These 2DOs retain tumor heterogeneity and tumor microenvironment interactions, providing a robust platform for investigating treatment-resistant lineages. Using EGFP-reporter 2DOs, POU5F1-expressing cells were isolated and characterized through drug sensitivity assays, single-cell RNA sequencing, immunocytochemistry, and epigenetic analyses. Therapeutic studies were conducted to evaluate the efficacy of targeting key pathways.
Results: POU5F1-expressing cells were highly enriched in blood samples from relapsed CRC patients as CTCs. These cells exhibited cancer stem cell properties, including self-renewal and differentiation, and demonstrated a high capacity for liver metastasis in vivo. Single-cell RNA sequencing revealed that POU5F1-positive cells generate heterogeneous populations enriched in the Wnt signaling pathway. Immunocytochemistry confirmed the co-expression of POU5F1 and CTLA4, with epigenetic analysis showing demethylation at transcriptional start sites of both genes, implicating epigenetic regulation in their aggressiveness. The Wnt/β-catenin pathway inhibitor XAV939 effectively suppressed adhesion and survival of POU5F1-positive cells in vitro. Remarkably, early administration of XAV939 completely inhibited liver metastasis induced by these cells in vivo.
Conclusions: POU5F1-expressing cells are critical drivers of CRC metastasis and recurrence, exhibiting stem-like properties and treatment resistance. Their enrichment in Wnt signaling and CTLA4 expression identifies actionable therapeutic targets. Our findings highlight the potential of Wnt/β-catenin inhibitors like XAV939 to prevent CRC metastasis and recurrence, paving the way for improved therapeutic strategies in high-risk patients.

CLINICAL RESEARCH: Vaccines and Cell Therapies
Poster Session

#870 Engineering of a novel Buddy-CAR system: CAR-NK cells expressing IL7/IL7Rα exhibit superior proliferation and anti-tumor responses while promoting trans-activation of the bystander T cells.

Andreia Maia, Mubin Tarannum, Sara Piccinelli, Mila Stanojevic, Maily Nhuyen, Grace Birch, Alaa Ali, Rizwan Romee

Medical Oncology, Dana-Farber Cancer Institute, Boston, MA

Natural killer (NK) and chimeric antigen receptor (CAR) NK cells offer a promising platform for cellular immunotherapy. However, the immunosuppressive tumor microenvironment (TME), particularly in pancreatic cancer, negatively affects the efficacy of cell therapies. IL7 is an attractive cytokine to module TME as it promotes $CD4^+$ and $CD8^+$ T cell proliferation while maintaining them in a less differentiated state, improving metabolic profile, and preventing exhaustion. Mature NK cells are minimally responsive to IL7 as they lack the expression of IL7Rα. To overcome this, we engineered the Buddy-CAR system, consisting of CAR-NK cells expressing IL7Rα AND secreting IL7 to enhance the activation and proliferation of CAR-NK cells (cis-activation) and modulate the TME by activating the bystander tumor-infiltrating T cells. We designed three CAR constructs: (1) Mesothelin (MSLN)-CAR (M), (2) MSLN-CAR secreting IL7 (M7) and (3) MSLN-CAR expressing IL7Rα AND secreting IL7 (M7R). Peripheral blood-derived NK cells were transduced using Baboon lentivirus achieving a high transduction efficiency and IL7 secretion was assessed by ELISA. M7R cells exhibited a superior proliferation index of 1.9 versus 1.1 for M (p=0.03) on day 9 using CTV flow cytometry assay. M7R showed increased cytotoxicity against $MSLN^+$ ASPC1 cells, 61.4% vs 52.3% for M7 (p=0.712) and 49.8% for M7R (p=0.002) at a 2:1 ratio. In addition, M7R showed a 3.9-fold increased IFN-γ production versus M7 (0.9, p=0.0002) and M (1, p=0.0005). The incorporation of IL7Rα and IL7 also led to increased expression of NKp30, NKp46, pSTAT1 and pSTAT5. We then evaluated the Buddy-CAR system's ability to stimulate bystander T cells by co-culturing IL7-releasing CAR-NK cells (M7 and M7R) with donor-matched MSLN-CAR T cells for 9 days in a 1:1 ratio. In a CTV flow cytometry assay, CAR-T cells showed a higher percentage of cells in >G3 when co-cultured with M7 (26.3%, p=0.048) and M7R (27.6%, p=0.035), versus M (2.72%). Lastly, we assessed the impact of IL7 signaling in CAR NK cells *in vivo* by injecting $6x10^6$ of M7 and M7R cells into NGS-Tg (Hu-L15) mice intravenously. Mice injected with M7R showed a significantly higher percentage of human $CD45^+$ infiltrating cells in the bone marrow (47.6%, p=0.02), liver (66.7%, p<0.0001), and spleen (69.8%, p=0.03), two weeks post-injection compared to M7-NK cells (<3%), indicating superior trafficking and proliferation *in vivo*. Ongoing studies are evaluating tumor control and effects on T cell proliferation and trafficking of the Buddy-CAR system in a xenograft mouse model. The versatility of the Buddy-CAR system allows for targeting different antigens by modifying CAR specificity while also modulating the immunosuppressive TME, offering a powerful approach to significantly improve the efficacy of CAR-based therapies across diverse cancer types.

#871 Quality control aspects of cell therapy products.

Bettina A. Y. Meissburger, Julian S. Thimm, Stefan B. Eichmueller

D210 GMP & T cell therapy, German Cancer Research Center, Heidelberg, Germany

Quality control of cell therapy products is critical to ensure safety and efficacy of these advanced therapeutics. Cell therapy products are characterized by their complexity and variability due to the use of patient-individual living cells as the primary therapeutic agent. Consequently, QC processes must address several unique challenges and parameters. The QC process typically involves a combination of in-process controls and final product testing. In-process controls are essential to monitor critical manufacturing steps and ensure that the process remains within predefined parameters. Final product testing is conducted to confirm that the cell therapy product meets all established quality specifications before release. Key QC criteria for a T cell product include cell number and viability, identity, potency, as well as endotoxin, mycoplasma and microbiological testing. Analytical QC methods need to be extensively validated before routine use. The application of pre-validated QC kits simplifies the validation process while at the same time ensuring fast in-house QC testing. For endotoxin testing we have established a cartridge based testing methodology, which has been validated with the final cell product enabling rapid pre-release testing. Mycoplasm testing can be performed using a Ph.Eur. compliant TaqMan-based real-time qPCR method, which ensures fast and sensitive mycoplasm detection including a DNA extraction step from the cellular product. Pre-tested freeze-dryed antibody cocktails combined with automatic gating strategies ensure robust identity testing by flow cytometry analysis. Additionally, cell counting and viability testing can be integrated into this analysis, thus omitting additional counting methods. Due to the short product shelf-life of living cells, microbiological testing poses a challenge with classical culture methods. Post-release final results might be acceptable taking into account the advantage of direct application of living cells without additional freezing steps. Novel rapid testing methods will be a promising alternative in the future. Potency of the cell product is another challenge, which is often assessed by surrogate markers such as cytokines. In case of known target antigens, killing assays or ELISpot assays are possible. Additionally, multimer staining can test for MHC-antigen recognition of the cell product. In summary, robust QC systems are indispensable for the development and clinical application of cell therapy products, safeguarding patient health and advancing the field of regenerative medicine.

#872 High quality on-demand gene edited human primary T cells.

John Latimer, Daniel Orozco, Paolo Piazza, Alex Brown

EditCo Bio, Inc., Redwood City, CA

EditCo unveils a breakthrough in T cell engineering aimed at benefiting cancer research: a rapid platform delivering premium-quality edited human CD4+ and CD8+ T cells with unprecedented efficiency. The platform achieves knock-out efficiency greater than 80% in less than 2 weeks, with edited cell pools maintaining viability above 70% before cryopreservation. These cells can be thawed and expanded at least 30-fold over 14 days independent of TCR stimulation, with no loss in editing efficiency. The platform's versatility has been demonstrated across multiple unique gene targets critical to cancer research and diverse donor populations, ensuring broad applicability. Notably, edited CD4+ T cells retain robust functionality after cryo-recovery, producing IL2, IFNγ, and TNFα upon stimulation. Similarly, edited CD8+ T cells maintain their endogenous antigen-specific cytotoxic activity, successfully express lentiviral CAR-T constructs, and demonstrate bi-specific engager-mediated cell killing. This comprehensive validation establishes EditCo's platform as a versatile, high-performance solution for T cell editing, maintaining cellular integrity and functionality while delivering consistent results across multiple genetic targets and donor populations.

#873 2-deoxy-D-glucose sensitizes the antigen recognition of chimeric antigen receptor-expressing tumor-infiltrating lymphocytes toward hepatocellular carcinoma cells.

Mingyu Liu, Deping Han, Jean-Paul Thiery, **Xi Zhang**

Biosyngen Pte Ltd, Singapore, Singapore

Background: Tumor-infiltrating lymphocytes (TILs) are heterogeneous populations of lymphocytes residing in tumor tissues, comprising various T-cell clones with specificity towards tumor or viral antigens. TIL therapy has been recently approved for advanced melanoma treatment with high-dose interleukin-2 (IL-2) as a concomitant treatment. However, the accessibility of tumor tissues, the varied abundance and effectiveness of tumor-reactive T-cells, and the toxicity of IL-2 infusion limit the clinical application of TILs toward a broader range of tumor indications. To address the high individual variance, we have engineered TILs expressing chimeric antigen receptor (CAR) constructs targeting glycoproteins on the hepatocellular carcinoma (HCC) cell surface. However, heavy glycosylation of these glycoproteins may mask peptidic epitopes to single-chain antibodies. Previous studies have revealed that the glucose/mannose analog, 2-deoxy-D-glucose (2-DG) treatment of pancreatic cancer cells can disrupt the synthesis of N-glycan moieties on the cell surface and enhance CAR T-cell activity. 2-DG treatment of T cells augmented antitumor activity and cell surface retention of IL-2R of T cells. In this study, we investigated whether the anti-tumor function of CAR-TILs against HCC lines would also benefit from the 2-DG treatment.
Methods: TILs were isolated from fresh or cryopreserved tumor biopsy samples and stimulated with anti-CD3/CD28 antibodies. Thereafter, TILs were lentiviral transduced with Glypican 3 (GPC3) CAR and membrane bound-interleukins, expanded with γ-irradiated feeder cells in an optimized rapid expansion process (REP), and cryopreserved until use. Phenotypic analyses were performed by flow cytometry. HCC lines were pre-treated with or without 2-DG, and the antigen recognition by antibodies targeting GPC3, CD133, and CD44v6 was evaluated by flow cytometry. *In vitro* effector function was assessed by IFN-γ release (ELISA) and killing assay (Incucyte). The anti-tumor efficacy of CAR-TILs with or without 2-DG treatment was further assessed in the immunocompromised xenograft model.
Results: Pre-treatment of HCC cell lines with 2-DG (1~10 mM) enhanced the antibody recognition of GPC3 in a dose-dependent fashion while showing no improvement in mAb binding towards CD133/CD44v6. CAR-TILs showed enhanced killing and more IFNγ release towards 2-DG pre-treated $GPC3^+$ HCC lines. The synergistic effect of 2-DG with CAR-TILs was further validated in the immunocompromised murine model, and no apparent toxicity was observed. The proliferation and the CD4/CD8 ratio of CAR-TILs were not affected by 2-DG treatment.
Conclusion: 2-DG sensitized GPC3 antigen recognition, thus boosting CAR-TIL cytotoxic activity against $GPC3^+$ HCC cells. These data prompt further investigation before pursuing a combination therapy.

IMMUNOLOGY: NK Cells and Innate Immunity
Poster Session

#877 Targeting pancreas cancer liver metastases with B7-H3 tri-specific killer engager and regional delivery of placental-derived NK cellular therapy.

M. Purl[1], T. Mukarrama[1], J. Kim[1], S. M. Cruz[1], J. S. Miller[2], M. Felices[2], W. J. Murphy[3], R. J. Canter[1], S. J. Judge[1];
[1]UC Davis Comprehensive Cancer Center, Sacramento, CA, [2]University of Minnesota Masonic Cancer Center, Minneapolis, MN, [3]UC Davis School of Medicine, Sacramento, CA

Introduction: Pancreatic cancer (PDAC) is a dismal disease characterized by early metastases to the liver and poor overall survival. The success of natural killer (NK) cell therapies for solid tumors has been limited in part due to inadequate cytotoxicity and homing to sites of disease. We set out to evaluate the function of placental-derived NK cells in combination with a tri-specific killer engager (TriKE) targeting B7-H3 with ability to engage CD16 and express IL-15, critical components for NK cytotoxicity against B7-H3-expressing PDAC cells. We also investigated the effect of regional delivery as a method to enhance liver engraftment with the goal of combining regionally delivered placenta NK cells with B7-H3 TriKE platform to treat pancreatic cancer liver metastases.
Materials and Methods: B7-H3 IHC staining was performed on 84 patient samples from resected PDAC to quantify expression on primary tumors. Human NK cells were isolated from human placenta and expanded ex vivo using the established K562.cl9mIL21 feeder line and IL-2. Phenotype was evaluated by flow cytometry and functional assessment by PANC-1 killing with or without B7-H3 TriKE. Nanoparticle labelled and unlabeled ex vivo activated NK cells were adoptively transferred into immunodeficient mice via either tail vein (IV) or portal vein (PV) injection and liver and spleen engraftment was assessed by ultrasound-guided photo-acoustic imaging and flow cytometry.
Results: B7-H3 (>1% expression) was detected on 76/84 (90%) primary resected PDAC samples and median H-score was 12 (range 0-132). In vitro, PANC-1 cells showed 99% expression of B7-H3. Ex vivo expanded placenta NK cells were >90% CD16 positive and showed cytotoxicity against PANC-1 cells after 4 and 8 hours at 10:1 effector-to-target ratio. Addition of B7-H3 TriKE enhanced cytotoxicity in placenta NK cells, at both time points (4 hr: 51.2% ±2.0 vs 27.7% ± 3.0, $P < 0.01$; 8 hr: 76.7% ± 2.6 vs 56.6% ± 2.1, $P < 0.01$). 24 hours after administration, in vivo imaging analysis showed a 2.7-fold higher signal from PV compared to IV in the liver. This was confirmed by flow cytometry with administration by PV having showed significantly higher liver engraftment compared to IV (human CD45 positivity 23.6% ± 7.6 vs 8.8% ± 4.5, $P = 0.03$), while spleen engraftment was minimal and not significantly different between PV and IV administration (0.7% ± 0.4 vs 0.3% ± 0.2).
Conclusion: B7-H3 is expressed on both primary human PDAC and the PANC-1 cell line representing a logical target for novel therapeutic strategies. Ex vivo expanded placenta NK cells demonstrate cytotoxicity against PDAC which is further enhanced through the B7-H3 TriKE. Regional delivery by portal venous administration results in increased liver engraftment and represents a viable and rational strategy for targeting PDAC liver metastases.

#878 Anti-MICA/B antibody promote anti-tumor activity of cytokine expanded tumor infiltrated natural killer (TINK) cells from canine sort tissue sarcomas.

Weiqing Jing[1], Himaly Shinglot[1], Juliana Ng[1], Milan Medd[1], Ali Zhang[1], abhilasha srivastava[1], Shay Bracha[2], Laura Selmic[2], Chelsea Tripp[3], Farres Obeidin[1], Jennifer Wu[4], Seth Pollack[1]

[1]Northwestern University, Chicago, IL,[2]Ohia State University, Columbus, OH,[3]Bridge Animal Referral Center,, Edmonds, WA,[4]Urology, Northwestern University, Chicago, IL

Background: Presence of tumor infiltrating NK (TINK) cells linked to prognosis in many solid tumors, but their function is often constrained by the adverse tumor microenvironment. Although promising antileukemia activity has been shown, the exploitation of adoptive NK cell therapy for solid tumor is still limited and unsatisfactory. Tumor MIC (MHC I chain related molecule) modulates TME and contributes NK cell dysfunction, here we study the role of anti-MICA/B antibody in TINK's function against sarcoma tumors. Because NK cells are prevalent in human Undifferentiated Pleomorphic Sarcomas (UPS), we sought the analogous canine soft tissue sarcomas (STS) as a readily available source to culture and perform intensive study on TINKs.
Methods: Canine TINK cells were expanded form canine sarcoma tumor specimens with IL-2. Human NK cells were expanded from PBMC of healthy donor with K652 feeder cells. In vitro cytotoxic assay against sarcoma was monitored by Incucyte system. Canine or human sarcoma xenograft mouse models were used to evaluate therapy effect of adoptive transferred NK cells, with or without anti-MIC Ab combinatory therapy.
Results: The percentage of CD5-NKp46+ and CD5dimCD8+NKp46+ NK cell within tumor infiltrating CD45 cells in canine STS is significantly higher compared with osteosarcoma and other types of tumors. Even with small STS tumor fragments for TIL expansion, more than 90% of the fragments were expanded sufficiently for analyses. Expanded lymphocytes were mostly NK cells (TINKs) which was confirmed with flow cytometry (NKp46+ CD5(dim) TCRαβ− TCRγδ− CD4− B220−CD1d−) and RNA sequencing. TINKs effectively kill autologous tumor cells and the NK-sensitive canine tumor cell line CTAC and result in a significantly increased expression of Granzyme B and Th1 cytokines (IFN-γ, TNF-α and GM-CSF) upon co-culturing with tumor cells. Although adoptively transferred TINK cells showed a significant inhibition in xenograft CTAC tumor growth, combinatory therapy with huB10G5 further significantly inhibited tumor growth. The anti-MIC antibody huB10G5 showed specific binding to canine sarcoma tumor cell lines, fresh processed tumor cells and tumor tissues. Similar to canine STS, NK infiltration in human UPS is significantly higher than in other types of sarcoma tumors. IHC demonstrated that MIC is broadly expressed in human UPS. Similarly, huB10G5 significantly enhanced the in vivo therapeutic effect of PBMC-expanded human NK cells against human sarcoma xenografts.
Conclusions: Our results demonstrated that functional TINKs can be efficiently expanded from canine sarcoma tumor tissue and demonstrated therapeutic potential in vivo allowing us to better model human disease. Anti-MIC treatment enhanced the efficacy of NK therapy in both canine and human sarcoma xenografts mouse models.

#879 Chemokine engineering significantly enhanced migration and tumor infiltration of *ex vivo*-expanded natural killer cells in osteosarcoma.

Shiori Eguchi[1], Wen Luo[1], Hai M. Hoang[1], Hongwen Zhu[1], Mitchell S. Cairo[2]

[1]Department of Pediatrics, New York Medical College, Valhalla, NY,[2]Department of Pediatrics, Medicine, Pathology, Microbiology and Immunology, Cell Biology and Anatomy, New York Medical College, Valhalla, NY

Metastatic and relapsed osteosarcoma (OS) has poor prognosis despite multiagent chemoradiotherapy. Novel therapies are urgently needed. Natural killer (NK) cells are highly cytotoxic to OS cells. Increased abundance of tumor infiltrating activated NK cells has been correlated with extended overall survival in OS. However, the OS tumor microenvironment (TME) impairs NK cell TME infiltration. We and others found that CXCR3, a receptor for CXCL9, -10, and -11, is highly expressed on *ex vivo*-expanded NK cells. We hypothesize that inducing OS cells to secrete CXCL9, -10, or -11 will enhance NK infiltration into the OS TME and improve outcomes. We expanded NK cells from PBMCs using K562-mbIL21-41BBL feeder cells in the presence of IL-2 (50 IU/mL) and confirmed high CXCR3 expression (98%) on expanded NK cells by flow cytometry. CXCL9, -10, or -11 cDNA was cloned into pMSCVneo to produce lentiviruses for infecting OS 143B cells. ELISA assay showed significantly increased secretion of CXCL9, -10, and -11 from CXCL9, -10, and -11 virus-infected 143B cells compared with wild-type (WT) cells (135.0 ± 51.3 vs. 1.7 ± 1.7 pg/mL, $p < 0.05$; 1165.4 ± 102.4 vs. 0.4 ± 0.4 pg/mL, $p < 0.01$; and 86.1 ± 10.5 vs. 0.0 ± 0.0 pg/mL, $p < 0.01$, respectively). Importantly, we observed significantly enhanced migration of expanded NK cells toward CXCL9, -10, or -11 conditioned media (CM) relative to WT CM (1.6 ± 0.1-fold, $p < 0.001$; 1.7 ± 0.1-fold, $p < 0.05$; and 1.5 ± 0.0-fold, $p < 0.001$, respectively) in transwell assays. Luciferase-based *in vitro* cytotoxicity assay showed that chemokine (CXCL9, -10, or -11)-secreting 143B cells were similarly if not more sensitive than WT cells to NK cells. To compare the migration of expanded NK cells towards WT and chemokine-secreting OS, we inoculated WT and chemokine-secreting 143B cells subcutaneously into separate flanks of the same NSG mice and administered luciferase-expressing human NK cells intraperitoneally after tumor establishment. IVIS imaging of the mice showed significantly enhanced NK migration toward CXCL9, -10, or -11-secreting tumors compared with WT tumors (2.0 ± 0.3-fold, $p < 0.05$; 2.0 ± 0.2-fold, $p < 0.05$; and 2.1 ± 0.1-fold, $p < 0.01$, respectively). To evaluate NK cell tumor infiltration, we injected human NK cells with an IL-15 agonist, NKTR-255, and harvested and dissociated tumors 5-7 days after NK injection for flow cytometry analysis. Intriguingly, we found significantly increased number of human $CD45^+$ cells in CXCL9, -10, or -11-secreting tumors compared with WT tumors (2.4 ± 0.2-fold, $p < 0.05$; 11.3 ± 2.8-fold, $p < 0.05$; and 4.7 ± 1.2-fold, $p < 0.05$, respectively). Our *in vitro* and *in vivo* data demonstrated that secretion of CXCL9, -10, and -11 from OS significantly enhanced NK migration and NK infiltration into the OS TME. We are actively investigating the *in vivo* anti-tumor efficacy of expanded NK cells against CXCL9, -10, or -11-secreting disseminated OS tumors.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#88 Ephrin receptor A4 is a regulator of metastatic colonization in triple negative breast cancer.

Wenchao Liu, Hoi Wan Lee, Madeline H. Bungert, Geetha P. Yerradoddi, Marsha R. Rosner

University of Chicago, Chicago, IL

Triple negative breast cancer (TNBC) is the most aggressive breast cancer subtype, affecting more than 40,000 people in the US. Most TNBC patient deaths are due to distal metastasis, a stage for which effective treatment is not yet available. Metastasis is an inefficient, multi-step process. The bottleneck for establishing the life-threatening macro-metastasis is considered to be metastatic colonization, the step at which cancer cells exit the circulation, survive in the new microenvironment and re-initiate aggressive growth. Numerous studies have found that cancer cell adhesion and migration pathways are required for metastatic colonization. However, the mechanisms by which these pathways are modulated are incompletely understood. Ephrin and Ephrin receptors are a family of proteins with conserved functions related to modulation of cell repulsion and adhesion, particularly in the regulation of pattern formation during development. Here we show that Ephrin Receptor A4, a cell surface receptor tyrosine kinase, regulates cell-cell interactions, migration and metastatic formation in TNBC, similar to its role in development. Depletion of EPHA4 in either human xenograft MDA-MB-231 or mouse syngeneic 4T1 TNBC tumors does not significantly alter tumor growth, but results in differential expression of genes involved in cell-cell adhesion, cell-matrix adhesion, and cell migration. *In vitro* assays suggest that EphA4 loss promotes collective rather than single cell migration, which can lead to increased metastatic formation. Metastatic lung colonization assays in mice indicate that loss of EphA4 increases metastatic lesions and/or metastatic burden in the MDA-MB-231 and 4T1 tumor models. Consistent with these findings, Ephrin Receptor A4 expression in TNBC is downregulated at metastatic sites relevant to the primary tumor. These results suggest that EphA4 regulates cell adhesion in cancer cells to reduce the efficacy of metastatic colonization.

IMMUNOLOGY: NK Cells and Innate Immunity
Poster Session

#880 Accumulation of cytosolic single-stranded DNA triggers innate immune responses via a novel sting-independent mechanism.

Jing Zhang

Hangzhou Institute of Medicine, Chinese Academy of Sciences, Hang Zhou, China

Immune checkpoint blockade (ICB) has shown durable responses in a subset of patients with hypermutated cancers. Although some non-hypermutated cancer patients have also demonstrated clinical responses to ICB, the majority do not respond, prompting research into novel combination immunotherapies. Ongoing clinical trials have explored such combinations, but challenges like hematological toxicities and resistance have limited their effectiveness. Therefore, there is an urgent need for more effective therapies. Recent studies suggest that mislocalized cytoplasmic DNA may serve as a potent mediator of innate immune responses, potentially enhancing ICB efficacy. However, the mechanisms by which intracellular single-stranded DNA (ssDNA) activates the innate immune system remain poorly understood. In this study, we report that accumulation of cytosolic ssDNA in cancer cells by silencing TREX1 improves ICB response and promotes the infiltration of immune cells via DDX3X. Additionally, we further developed mRNA-based therapeutic agents HIM-mRNA-001 to enhance tumor sensitivity to ICB with minimal toxicities. These findings suggest that targeting cytosolic ssDNA accumulation could provide a new therapeutic strategy for non-hypermutated cancers, offering a promising alternative to current immunotherapies.

#881 Innate immune cell-mediated anti-tumor activity against lung cancer and pleural mesothelioma cells.

H. Tomono[1], K. Akagi[1], Y. Umeyama[2], M. Matsuo[1], H. Taniguchi[1], H. Gyotoku[1], S. Takemoto[1], Y. Tanaka[1], H. Mukae[1];
[1]Nagasaki University, Nagasaki, Japan, [2]Sasebo City General Hospital, Nagasaki, Japan

[Objectives] Immune checkpoint inhibitors have played a pivotal role in cancer treatment, and various combination therapies are being developed. However, their add-on effects remain limited, highlighting the urgent need for novel therapeutic modalities. Among various approaches, the adoptive transfer of immune effector cells has attracted particular attention, especially the use of allogeneic immune effector cells due to the frequently immunosuppressed status of cancer patients. We have previously focused on Vδ2 γδ T cells and NK cells, developing methods for their large-scale expansion. These innate immune effector cells are highly suitable for allogeneic adoptive transfer, as they exhibit tumor cytotoxicity independently of the major histocompatibility complex. In this study, we evaluated the cytotoxic properties of these innate immune effector cells, including innate cytotoxicity, antigen-specific cytotoxicity, antibody-dependent cellular cytotoxicity (ADCC), and cytokine production.
[Materials and Methods] After obtaining informed consent, peripheral blood mononuclear cells (PBMCs) were collected from healthy adults, lung cancer patients, and pleural mesothelioma patients to prepare PBMCs. The PBMCs were stimulated with tetrakis-pivaloyloxymethyl 2-(thiazole-2-ylamino) ethylidene-1,1-bisphosphonate (PTA), a bisphosphonate prodrug, along with IL-2 to enable the expansion of Vδ2 γδ T cells. The expanded Vδ2 γδ T cells were then co-cultured with human lung cancer cell lines to evaluate their effector functions in vitro. Additionally, CD3-negative PBMCs were cultured with IL-2 and IL-18 to expand NK cells, which were subsequently co-cultured with human pleural mesothelioma cells to assess the effector functions of NK cells in vitro. PTA was used as the antigen, and anti-EGFR antibody was used for ADCC assays.
[Results] Vδ2 γδ T cells exhibited PTA-dependent cytotoxicity against lung cancer cells. Moreover, Vδ2 γδ T cells with high CD16 expression demonstrated ADCC and IFN-γ production against lung cancer cells. NK cells displayed innate cytotoxicity against pleural mesothelioma cells, and this cytotoxic activity increased in an antibody-dependent manner.
[Conclusion] The use of PTA/IL-2 enabled the high-purity expansion of Vδ2 γδ T cells, which exhibited robust immune effector functions against lung cancer cells via T cell receptor and CD16-dependent mechanisms. Similarly, IL-2/IL-18 facilitated the high-purity expansion of NK cells, which demonstrated strong innate cytotoxicity and ADCC against pleural mesothelioma cells. Moving forward, in vivo studies are planned to advance the potential for infusion therapy with Vδ2 γδ T cells and NK cells.

#882 Soluble TNF blockade enhances trastuzumab-deruxtecan innate antitumor immune response and type-I IFN signalling in HER2-positive breast cancer.

Sofia Bruni[1], Camila Jencquel[2], Martin A. Rivas[1], Roxana Schillaci[2], Maria F. Mercogliano[2]

[1]University of Miami Miller School of Medicine, Miami, FL,[2]Instituto de Biologia y Medicina Experimental, Buenos Aires, Argentina

Since DESTINY-Breast03 results where trastuzumab-deruxtecan (T-DXd) showed remarkable overall survival improvement, this drug is used to treat patients with unresectable or metastatic HER2-positive (HER2+) breast cancer who have received prior anti-HER2-based regimen. One antitumor mechanism of T-DXd is attributed to DXd inhibition of topoisomerase I in the tumor cells that internalized the drug, and to the bystander effect of DXd on neighbor tumor cells**.** Reports describe that topoisomerase I inhibitors produce DNA fragmentation, stimulating cGAS/STING pathway and producing type-I IFN, a key mediator of the innate immune response against tumors. Previously, we reported that soluble TNF (sTNF) overproduction by HER2+ tumors generates resistance to trastuzumab-based therapies. Blocking sTNF with INB03 (DN), a dominant-negative molecule, sensitized tumors to therapy and improved the antitumoral immune response. Here, we explore if blocking sTNF can also improve T-DXd antitumor effect, and its impact on the innate immune response involving cGAS/type-I IFN, in a model with resistance to multiple HER2-targeted therapies.Female nude mice bearing JIMT-1 tumors were treated i.p. twice/week with 5 mg/kg of IgG or 10 mg/kg DN, or with 5, 2.5 or 1.25 mg/kg of T-DXd i.v. on days 0, 7 and 14, or the combination therapies. Tumor volume was measured twice/week for 21 days (n=6-8). Tumor-infiltrating macrophages and NK cells were studied by flow cytometry. ISG15, a downstream mediator of type-I IFN pathway, and cGAS were evaluated by western blot on tumor extracts.The dose-response curves showed a tumor growth inhibition of 83% (T-DXd 5 mg/kg), 61% (T-DXd 2.5 mg/kg) and 37% (T-DXd 1.25 mg/kg) vs. IgG ($p<0.0001$, 0.0001 and 0.05, respectively). Adding DN enhanced this inhibition to 98% (5 mg/kg), 81% (2.5 mg/kg) and 73% (1.25 mg/kg), $p<0.0001$ vs. IgG. T-DXd 1.25 mg/kg +DN achieved an antitumor effect that resembles T-DXd 5 mg/kg alone. Furthermore, addition of DN to T-DXd 1.25 and 5 mg/kg increased macrophage infiltration and polarization to the antitumoral M1-like phenotype. T-DXd 1.25 mg/kg +DN treatment mimics the increase of infiltrating NK cells observed in the higher doses of T-DXd alone. Finally, we observed an increase in cGAS and ISG15 expression in tumor extracts form T-DXd 1.25 mg/kg +DN, compared to T-DXd 1.25 mg/kg alone.Our results suggest that sTNF blockade with DN enhances T-DXd antitumor effect, inducing a reinforced innate immune response with increased infiltration of M1-like macrophages and NK cells, and activation of cGAS and type-I IFN pathway, measured by its surrogate ISG15. Adding DN allows to de-escalate T-DXd dose without impairing the antitumor effect. Neutralizing sTNF could improve the outcome of patients treated with T-DXd by enhancing the antitumor immune response.

#883 Scalable differentiation of cytotoxic natural killer (NK) cells from human pluripotent stem cells.

Marcus Bunn[1], Lindsay Bailey Steinitz[2], Vivek Chandra[2], Michael Akenhead[1], Logan Wilson[1], Erica Heipertz[1], Chris Yankaskas[1], **Colin Paul**[1], Matt Dallas[1], Mark Kennedy[1], David Kuninger[1]

[1]Thermo Fisher Scientific, Frederick, MD,[2]Thermo Fisher Scientific, Carlsbad, CA

Cell therapy is expanding as a treatment for various cancers. FDA-approved autologous therapies, such as chimeric antigen receptor (CAR) T cells and tumor infiltrating lymphocytes (TILs), are used for aggressive or late-stage cancers. However, these therapies face challenges due to lengthy manufacturing times and patient variability. Manufacturing is already a bottleneck, which is expected to worsen as these therapies are tested in earlier cancer stages and become available to more patients. An emerging solution for cancer treatment is the use of natural killer (NK) cells as an "off-the-shelf" therapy. These cells target malignant cells independent of antigens, without needing HLA matching, and have not caused cytokine release syndrome like some T cell therapies. Clinical trials typically administer 5x10^6 to 1x10^8 NK cells per kilogram of body weight per dose. Therefore, developing methods to produce large quantities of functional NK cells is crucial. Current methods, such as sourcing primary cells or differentiating from human pluripotent stem cells (PSCs) in two dimensions (2D), yield variable results, often require co-culture or embryoid body formation, and usually fail to produce enough functional NK cells for therapy. To address these limitations, we developed a scalable 3D PSC culture differentiation protocol to hematopoietic stem cells (iHSCs), a multipotent progenitor cell population, and then to induced natural killer (iNK) cells that primarily uses GMP grade reagents with regulatory documentation. Differentiation occurs in suspension using shake flasks. After 38 days, over 50% of cells are CD56+ CD3-, and can be cryopreserved, thawed, and further expanded. This method, tested in up to 100 mL cultures, produces 5-10x10^6 CD56$^+$CD3$^-$ iNK cells/mL, sufficient to treat one patient at the lower dosing range. Multiple 100 mL cultures can be cultivated in parallel to increase the yield, and future efforts will focus on optimizing the protocol in larger culture volumes. To evaluate iNK cell cytotoxicity, they were co-cultured with three-dimensional (3D) patient-derived cancer organoids, a physiologically relevant in vitro cancer culture that better mimics tumor tissue than traditional 2D cancer cell lines and are harder to kill. iNK cells induced apoptosis in breast, colorectal, and endometrial cancer organoids in a dose-dependent manner. Against the same target colorectal cancer organoids, iNKs were more cytotoxic than the NK-92 cell line at equal doses, and matched primary NK cells at roughly double the dose. In summary, we demonstrate a novel scalable protocol for generating functional iNK cells, showing potency in a cytotoxicity assay with patient-derived cancer organoids, which could be further developed for cell therapy research.

#884 Development of allogeneic persistence-enhanced PB-derived NK cells in treating solid tumors.

C. Wang[1], T. Liu[1], Q. Wang[1], Y. Gong[1], F. Gao[1], F. Zhou[2], **Z. Cao**[1];
[1]Shanghai Simnova Biotechnology Co., Ltd., Shanghai, China, [2]State Key Laboratory of Neurology and Oncology Drug Development, Simcere Zaiming Pharmaceutical Co, Ltd., Shanghai, China

Although chimeric Antigen Receptor (CAR)-T cell therapy has emerged as a promising immunotherapeutic approach to fight cancers, the utilization is limited due to safety concerns such as serious cytokine release syndrome, neurotoxicity, or graft-vs-host-disease. Natural killer (NK) cells can be derived from multiple sources and scaled manufactured as an off-the-shelf product likely with fewer toxicities. While NK cell therapies have been demonstrated to elicit clinical efficacy against hematopoietic malignancies, many challenges exist including issues related to cell persistence, overcoming the immunosuppressive microenvironment, and optimizing specific cytotoxic efficiency. To address those challenges, we used a single retroviral vector to engineer peripheral blood (PB) derived NK to express: soluble IL15 armor for proliferation and activation, a non-cleavable CD16 with high affinity to Fc receptor to enhance ADCC response, alloimmune defense receptor (ADR) targeting alloreactive T cells to overcome host immune cell-mediated rejection. Our preclinical results demonstrated the multiplexed engineered PBNK (eNK) showed improved persistence, resistance to allorejection, as well as anti-tumor efficacy. To evaluate ADRs functions, both anti-rejection CD5 and CD70 ADRs expressing eNK were generated and characterized. eNK with CD5 ADR displayed comparable phenotype and expansion to non-transduced NK, whereas eNK with CD70 ADR showed significantly aberrant growth due to CD70-mediated fratricide. eNKs with CD5 ADR demonstrated better persistence and enhanced tumor cells killing when cocultured with tumor cells and allogeneic T cells in a mixed lymphocyte reaction, compared to eNK cells without CD5 ADR or eNK with CD70 ADR. To determine whether non-cleavable CD16 and IL15 engineering could improve eNK proliferation and ADCC, serial re-stimulation *in vitro* cytotoxicity assays (luciferase-/RTCA-based) were performed in the presence and in the absence of Cetuximab, at a 1:2 E:T ratio. eNK plus Cetuximab showed more potent cytotoxicity against HCT116 colon cancer cells and NCI-H1975 lung cancer cells compared to non-transduced NK combo, eNK only and EGFR targeted CAR-NK cells. Using different xenograft mouse models, we demonstrated eNKs combined with therapeutic antibodies could performed superior tumor inhibition and extended NK persistence. Taken together, these results supported the development of allogeneic PB-derived NK cell therapies armoring with soluble IL15, non-cleavable CD16 and anti-allorejection ADR, and demonstrated the efficacy and safety of eNK mono and combination treatments, significantly enhancing their therapeutic potential in clinical settings.

#885 Generation and characterization of NK-92®(aNK™) cells with hypoimmunogenic modifications.

Ivan Ting Hin Fung, Laurent Boissel, Patrick Soon-Shiong, Hans Klingemann

ImmunityBio, Inc., Culver City, CA

The next generation of NK-92® (also known as aNK™) cells will include inducible kill switches and enhanced cytotoxicity to make them safer and more effective, without the need to irradiate them before infusion into patients. Because of their enhanced longevity *in vivo* and their allogeneic nature, NK-92 cells are susceptible to rejection by the patient's own immune system and need to be equipped with mechanisms to prevent such rejection. We have genetically engineered NK-92 cells by targeting a combination of molecules that allow them to be undetected by the patient's T-cells as well as resistant to lysis by peripheral blood NK cells (PBNKs). HLA-I-deficient NK-92 cells generated by electroporation of Cas9-sgRNA ribonucleoprotein complexes to knockout the common β_2 microglobulin subunit (NK-92_B2M^{KO}) completely inhibited CD8$^+$ T-cell proliferation after 7 days in a mixed lymphocyte reaction with peripheral blood mononuclear cells (PBMCs) isolated from multiple healthy donors. Because downregulation of HLA-I on the cell surface can increase lysis by PBNKs via the 'missing-self' mechanism, we further engineered NK-92_B2M^{KO} cells to re-express the non-classical MHC class I molecule HLA-E (NK-92_B2M^{KO}_HLAE) to deliver an inhibitory signal through engagement with the NKG2A/CD94 heterodimer. Using flow cytometry-based cytotoxicity assays, we confirmed that the lack of HLA-I molecules rendered NK-92_B2M^{KO} cells highly susceptible to lysis by both resting and IL-2-activated PBNKs from healthy donors, relative to wild type NK-92. We also demonstrate that re-expression of HLA-E on NK-92_B2M^{KO} cells provides significant protection against both resting and IL-2 activated PBNKs (42.5% ± 9.0% reduction in killing at 5:1 E:T ratio, p=0.0002). HLA-E can also convey a lysis-activating signal through the NKG2C/CD94 dimer. NKG2C$^+$ NK cells are naturally present at low numbers in blood and can be expanded in individuals who are seropositive for human cytomegalovirus. To determine whether NK-92_B2M^{KO}_HLAE cells are sensitive to killing by NKG2C$^+$ NK cells, we performed co-culture assays using PBNKs and our engineered NK-92 cells and examined CD107a degranulation in different NK subsets. We observed that HLA-E expression completely inhibits degranulation in NKG2A$^+$ NK cells, while it was not increased any further in NKG2C$^+$ cells, suggesting that the re-expression of HLA-E did not make NK-92_B2M^{KO}_HLAE cells more susceptible to NKG2C$^+$ PBNK lysis. We report here that abolishment of HLA class-I and re-expression of HLA-E on NK-92 cells effectively inhibited CD8$^+$ T-cell recognition as well as provided significant protection against resting and IL-2 activated PBNKs. Our results also suggest that these modifications are likely sufficient to allow the engineered NK-92 cells to evade immune alloreactivity in a majority of patients after infusion.

#886 Response to g-NK is associated with higher CD8+ T cells and lower MDSC in bone marrow of multiple myeloma patients.

Aimee M. Merino[1], Zachary Davis[1], Jacob Rugloski[1], Madison Shackelford[1], Matt Collinson-Pautz[2], Austin Bigley[2], Stefanie Mandl-Cashman[2], Jeffrey A. Miller[1]

[1]University of Minnesota, Minneapolis, MN,[2]Indapta Therapeutics, Seattle, WA

Patients with relapsed/refractory (R/R) multiple myeloma (MM) have poor outcomes and no curative therapies. In an ongoing, open-label Phase I/II trial of g-NK cells (IDP-023), we recently reported a mean maximum decrease in serum M-protein or light chain of 73% in eight R/R myeloma patients, with three patients achieving a reduction of 84% or greater (Patel, Merino et al, SITC 2024). G-NK are a subgroup of adaptive NK cells that are epigenetically reprogrammed to exhibit higher antibody-dependent cytotoxicity following engagement of CD16 than conventional NK cells. G-NK also lack the inhibitory checkpoint NKG2A enabling them to kill HLA-E+ target cells. Because the impact of the tumor microenvironment (TME) on outcomes after cellular therapy is poorly understood, we collected bone marrow samples for cytometry time-of-flight (CyTOF) with a commercial 37 marker panel designed to identify immune populations in patients treated at the University of Minnesota. Four patients had bone marrow collected for this analysis. One patient (#004) achieved a partial response (PR) by International Myeloma Working Group (IMWG) criteria in monoclonal protein and had improvement in the activity of lytic lesions on their PET scan thirty days after treatment. One patient (#003) had a minimal response (MR) and the other two patients had stable disease (SD) by IMWG. Interestingly, two of the three patients with relatively stable monoclonal protein in the blood (MR or SD) had significant improvement in PET scans after NK-cell therapy (#003, #001). One of the patients with SD (#001) and improvement in lytic lesions as measured by PET, maintained SD for five months before proceeding to their next therapy. Patients #001 and #004 had post-treatment bone marrow samples showing decreased expression of the exhaustion marker PD1 by CD8+ T cells. The patients who had improvement of their lesions on PET saw decreases in T cell expression of the inflammatory markers CXCR3 and CD38. The patient (#001) with SD and improvement in bone lesions that lasted five months saw a 5x increase in CD8+ T cells in his post-treatment biopsy with near complete clearance of MDSC (<1%). Additionally, our data suggest that the pre-treatment condition of the bone marrow may be predictive of anti-tumor activity. For example, of the four evaluable patients, the patient who achieved a PR had the highest percentage of CD8+ T cells and lowest percentage of myeloid derived suppressor cells (MDSC) at baseline. The patient with SD and no improvement by PET had the highest % of MDSC, the lowest CD8+ T cell percentage, and the highest expression of PD-1 on CD8+ T cells of all the patients. Collectively, these data suggest that g-NK cell therapy can alter the TME and may promote MM control by influx of non-exhausted T cells. Furthermore, the amount of MDSC in the bone marrow at time of treatment may be inversely correlated with anti-tumor activity.

#887 Human microRNA-1250-5p targets IGFBP2 expression to regulate NK cell proliferation in hypoxia.

Kyung-Mi Lee, Minwha shin, Seon Ah Lim

Korea University, Seongbuk-gu, Korea, Republic of

Insulin-like growth factor binding protein 2 (IGFBP2) is involved in many physiological events, such as progression and survival of many types of cells. However, little is known about its role in human natural killer (NK) cells. Here, we report that miR-1250-5p regulates IGFBP2 expression, the most expressed gene in hypoxia-activated NK cells, thereby promotes NK cell proliferation in hypoxia. We found that IGFBP2 was not only highly expressed in cellular level, but also secreted from NK cells exposed to hypoxia. Both endogenous and secreted IGFBP2 are involved in NK cell proliferation in hypoxia. We identified miR-1250-5p directly binds to the 3'untranslated region of IGFBP2, and negatively regulates the expression of IGFBP2 in hypoxia-activated NK cells. Overexpression of miR-1250-5p has decreased NK cell proliferation, whereas inhibition of miR-1250-5p shows dramatically opposite effect. Clinical data showed that IGFBP2 expression was increased in tumor infiltrating NK cells compared with non-tumor infiltrating NK cells in patients of hepatocellular carcinoma (HCC), which is one of the representative tumors of hypoxia. We further demonstrated that miR-1250-5p negatively regulates IGFBP2 expression in tumor infiltrating NK cells of HCC patients. Furthermore, proliferation and survival markers, Ki-67, PCNA, cIAP1, cIAP2 as well as NK cell activating receptor, NKp44, were upregulated, whereas apoptotic and senescence markers, Bax, p53 and p16 were downregulated in tumor infiltrating NK cells in patients of HCC. These results indicate that the proliferation of NK cells in hypoxia is regulated by miR-1250-5p and its target gene IGFBP2, thereby enhancing therapeutic potential of NK cells against hypoxic tumor microenvironment.

#888 NKC and KIR humanized mice: a powerful tool to develop NK-targeting drugs.

Qiurong Wang, Qi Zhang, Na Kang, Huizhen Zhao, Lili Liu, Jiawei Yao, Yanshuang Li

Biocytogen, Waltham, MA

Natural killer (NK) cells are leukocytes within the innate immune system, acting as a first line of defense against cancer and virally infected cells. NK cell responses—whether “killing” or “tolerant”—are regulated by interactions between activating and inhibitory receptors and their ligands. Currently, there remains a need for comprehensive in vivo models featuring humanized NK cell receptors, including key proteins encoded by the KIR and NKC gene clusters.

Killer cell immunoglobulin-like receptors (KIR) form a highly polymorphic gene cluster expressed by NK and certain T cell subtypes, while the Natural Killer Gene Complex (NKC) encodes several C-type lectin-like receptors, such as the NKG2 family, found across various immune cells. To introduce a novel NK gene cluster-related in vivo model, Biocytogen has used advanced gene-editing technology to humanize ~150,000 base pairs of KIR gene cluster DNA (B-hKIR mice) and 0.9 million base pairs of NKC gene cluster DNA (B-hNKC mice) on a C57BL/6 background. In this combined B-hNKC/hKIR mouse model, 29 functional genes have been modified.

The immunophenotype of B-hNKC/hKIR mice is consistent with that of wild-type C57BL/6 mice. Human proteins NKG2A, NKG2C, CD94, NKG2D, and CD161 are detectable on NK cells, while human CLEC9A is observed on CD8a+ and CD103+ dendritic cells in the spleen. Human CLEC12A is expressed on T cells, macrophages, monocytes, and dendritic cells, and human LOX1 is present on activated macrophages, monocytes, and neutrophils in the bone marrow. Additionally, human CD69 is expressed on activated T cells, B cells, NK cells, macrophages, monocytes, neutrophils, and dendritic cells in the spleen. In B-hKIR mice, human KIR2DL1, KIR2DL3, and KIR3DL2 are detectable on NK cells, and mRNA for human KLRF2, CLEC2A, TMEM52B, CLEC1A, and CLEC2D is also expressed in B-hNKC/hKIR mice.

This B-hNKC/hKIR mouse model provides an advanced platform for NK-targeted research and serves as a valuable in vivo system for preclinical studies.

#889 T cell help enhances anti-CD38-mediated NK cell ADCC of multiple myeloma despite expression of CD38 by NK cells.

Reza Amani[1], Jyoti Arora[1], George J. Weiner[2]

[1]Holden Comprehensive Cancer Center, University of Iowa, Iowa City, IA,[2]Department of Internal Medicine, University of Iowa, Iowa City, IA

Anti-CD38 monoclonal antibodies such as daratumumab and isatuximab are effective therapeutic agents for some patients with multiple myeloma (MM) even though CD38 is expressed by B cells, monocytes and a significant subset of NK cells as well as MM cells. Treatment with anti-CD38 antibodies has been shown to deplete NK cells from the peripheral blood. This raises questions concerning the mechanisms of action of anti-CD38 as a treatment for MM such as NK cell mediated antibody dependent cellular cytotoxicity (ADCC). Since many NK cells express CD38, such NK cells could be susceptible to anti-CD38-induced fratricide, where CD38-expressing NK cells are targeted and eliminated. In previous studies in vitro, in animal model and in clinical correlative studies, we demonstrated that T cells play a key role in supporting NK cell viability and cytotoxicity following NK cell activation by anti-CD20 coated target cells. This T cell help is provided largely through IL2 production in the local microenvironment by CD4+ T cells. Here, we evaluated the role of T cell help in anti-CD38 mediated NK cell ADCC. Peripheral Blood Mononuclear Cells (PBMCs) were obtained from normal donors. T cells were depleted and added back to the PBMCs in known numbers to create PBMCs with controlled numbers of T cells. These PBMCs were cultured with MM.1S myeloma cells for 1, 3, or 7 days with and without anti-CD38. The presence of T cells had limited impact on tumor cell numbers or NK cell phenotype in the absence of anti-CD38 confirming modest direct anti-MM effect of T cells in this culture system. CD38 expression by NK cells was decreased after culture with anti-CD38 under all conditions. NK mediated ADCC was observed despite loss of NK cell CD38. This ADCC was dependent on T cell help. MM cells were reduced by day 3 and were largely eliminated by day 7 in samples treated with anti-CD38. Greater anti-MM effects were seen with PBMCs that contained larger numbers of T cells while ADCC was less apparent in samples with fewer numbers of T cells. At day 7, IL2 restored the longer-term anti-MM effect of anti-CD38 in the absence of T cells. A T cell dependent increase in NK cell numbers was also seen at day 7. We conclude that anti-CD38 can induce NK cell mediated ADCC of MM cells despite loss of CD38 expression by NK cells suggesting down-modulation of CD38 on NK cells as opposed to fratricide of CD38-expressing NK cells. T cell help, likely mediated in large part by local production of IL2, enhances anti-CD38-induced NK cell activation, viability and ADCC. These data indicate NK cell ADCC, enhanced by local T cell help, may be an important mechanism of action for anti-CD38 in MM.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#89 Genomic and clinical characteristics associated with metastatic patterns in urothelial carcinoma patients.

Jordan E. Eichholz[1], Henry Walch[1], Jacob Tallman[2], Syed Alam[2], James Rodrigues[3], Kwanghee Kim[4], Christopher Fong[1], Ecenur Turkay[5], Alejandra Lopez Rojas[6], Gopa Iyer[7], Eugene Pietzak[2], Jonathan E. Rosenberg[7], Hikmat Al-Ahmadie[8], Nikolaus Schultz[1], Walid K. Chatila[1], David B. Solit[9]

[1]Computational Oncology, Memorial Sloan Kettering Cancer Center, New York, NY,[2]Urology Service, Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY,[3]Gerstner Sloan Kettering, Memorial Sloan Kettering Cancer Center, New York, NY,[4]Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY,[5]Gerstner Sloan Kettering Graduate School, Memorial Sloan Kettering Cancer Center, New York, NY,[6]Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, NY,[7]Genitourinary Oncology Service, Memorial Sloan Kettering Cancer Center, New York, NY,[8]Pathology, Memorial Sloan Kettering Cancer Center, New York, NY,[9]Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY

Introduction: To identify the patients with urothelial cancer (UC) at greatest risk of metastasis, we performed an integrated analysis of clinicopathologic and somatic mutation data from a prospectively generated tumor genomic sequencing cohort.
Methods: We collected detailed clinicopathologic data from 1,617 patients with bladder and upper tract UC treated at Memorial Sloan Kettering. Tumors were sequenced with MSK-IMPACT, a targeted DNA sequencing assay that identifies genomic alterations in 341-505 genes. Metastatic site involvement was inferred using a natural language processing model, as reported in the MSK-CHORD dataset. Patients were considered never metastatic if there was no evidence of metastases after 2.5 years of follow-up from diagnosis. Comparisons were made between ever-metastatic (EM) and never-metastatic (NM) patients. In a time to metastasis analysis, metastatic patients at diagnosis and patients that had metastasis within 6 months of diagnosis were excluded. Continuous variables were analyzed using Wilcoxon rank sum test and two-sided Fisher's exact test for categorical variables. Univariable Cox proportional hazard models were used to assess time-to-metastasis. Benjamini-Hochberg was used to correct for multiple hypothesis testing and q-values <0.1 were considered significant.
Results: EM patients were more likely to be former smokers (64% vs 50%, q<0.001) and be diagnosed after age 80 (17% vs 10%, q=0.033). NM patients were more likely to be never smokers (48% vs 34%, q<0.001) and diagnosed before age 65 (36% vs 28%, q=0.016). EM tumors had a higher fraction genome altered (0.20 vs 0.10, q<0.001) and higher rates of *TP53* (55% vs 44%, q=0.003), *ELF3* (15% vs 9%, q=0.018), and *CDKN2A* (29% vs 21%, q=0.069) alterations. NM tumors were more likely to have *FGFR3* (18% vs 26%, q=0.002) and *ERCC2* (6% vs 13%, q<0.001) alterations. In a time to metastasis analysis: former smoking (HR: 1.44, q=0.041), *CDKN2A* (HR: 1.59, q=0.005), and *ELF3* (HR: 1.64, q=0.030) alterations were associated with a higher risk of bone metastasis. *CDKN2A* (HR: 1.98, q<0.001) and NRF2 pathway alterations (HR: 2.13 q=0.026) were associated with higher risk of lung metastasis. Former smoking (HR: 1.41, q=0.029), *CDKN2A* alterations (HR: 1.33, q=0.086), *ERBB2* amplifications (HR: 1.81, q=0.009), NRF2 (HR: 1.92 q=0.026) and HIPPO (HR: 1.62 q=0.031) pathway alterations were associated with higher risk of lymph node metastasis. *ERCC2* alterations were associated with lower risk of bone, (HR: 0.33, q=0.017), lung (HR: 0.48, q=0.098), and lymph node metastases (HR: 0.43, q=0.026).
Conclusion: Through integration of tumor genomic profiling data with NLP-derived clinical annotation, we identified clinical and genomic features associated with a higher risk of developing metastatic disease in patients with UC, as well as alterations associated with organ-specific metastatic tropism.

IMMUNOLOGY: NK Cells and Innate Immunity
Poster Session

#890 NK-92® (aNK™) whole cell lysate exerts potent cytotoxic and anti-proliferative activity on tumor cells.

Himani Chinnapen, Laurent Boissel, Thomas Bickett, Courtney Fleenor, Vidya Godbole, Manju Saxena, Patrick Soon-Shiong, Hans Klingemann

ImmunityBio, Inc., Culver City, CA

Lysates from human cells represent biofluids that are used in the biotechnology field for a number of applications, such as biomarker identification and antibody detection. Lysate from human blood platelets is widely used in the clinical setting to control bleeding. We hypothesized that whole cell lysate prepared from the natural killer cell line NK-92® or 'activated' NK (aNK™) has the potential as an immunotherapy modality for local treatment of cancer lesions, as it would be expected to contain an abundance of cytotoxic granule-associated serine proteases (granzymes), the pore-forming cytolytic protein (perforin), as well as an array of chemokines and cytokines that could contribute to a more immune-active tumor microenvironment.
Here, we have generated whole cell lysate from NK-92 cells, and its high-affinity CD16 Fc receptor (158V) and erIL-2 -engineered variant haNK® cells, by repeated freeze/thaw cycles at a density of 10^8 cells/mL in a balanced salt solution (BSS) buffer containing no detergent, protease inhibitors, or EDTA. The resulting whole cell lysates were characterized for composition and cytotoxic activity. Western blot analysis confirmed the presence of perforin, granulysin, exosomal markers, and the full spectrum of granzymes known to be expressed in NK-92 cells. Biomarker profiling by Meso Scale Discovery-based assays revealed the presence of various chemokines and cytokines at significant concentrations in both NK-92 and haNK lysates, notably IL-2 (haNK only), IL-8, IL-10, IL-16, TNF-β, IFN-γ, and MIP-1α and β. A fluorometric assay confirmed enzyme activity of Granzyme B was maintained before and after cryopreservation of the lysate. Incucyte® SX3 live cell imaging assays showed that fresh lysate prepared from haNK cells displays cytotoxic and anti-proliferative activity against human and canine cancer cell lines after only a 15-minute exposure *in vitro*, suggesting cross-species specificity. Importantly, under the same conditions the lysate had no effect on primary human or canine cells. Intra-tumor injection of cryopreserved haNK lysate into intradermal tumors in immunocompetent C57BL/6 mice provided tumor control and clearance in two-thirds of the animals. Strikingly, when re-challenged with the same tumor cells 6-8 weeks later, no tumor growth occurred suggesting a vaccine-like effect of the haNK lysate. In conclusion, we have developed a simple, scalable method to prepare bioactive cell lysates from NK-92 and haNK that show anti-tumor activity and a potential for immune modulation both *in vitro* and *in vivo*.

#891 Therapeutic role of NK cells in treatment of myeloid malignancies using hypomethylating agents.

Junshik Hong[1], Suji Min[1], Jihyun Park[1], Carly Fielder[2], Qianni Hu[2], Seula Kim[1], Sung-Soo Yoon[1], Tae Kon Kim[2]

[1]Department of Internal Medicine, Seoul National University College of Medicine, Jongno-gu, Korea, Republic of,[2]Division of Hematology/Oncology, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN

We previously analyzed serial bone marrow (BM) aspirates from a patient with high-risk myelodysplastic syndrome (HR-MDS) who responded to a hypomethylating agent (HMA), azacitidine using single-cell RNA sequencing. Our finding showed an increased proportion of NK cells in the BM, with elevated CXCR4 expression following azacitidine treatment (Hong J, et al. AACR 2023). Building on these observations, we tested our hypothesis that HMA can potentiate BM homing and cytotoxic effect of NK cells in myeloid leukemia. To test our hypothesis, we will carry out in vitro assays, animal experiments, and patients' serial BM samples. First, we assessed NK cells changes in BM aspirates from AML or HR-MDS patients before and after HMA treatment. Flow cytometric analysis revealed that patients who achieved complete remission after HMA treatment exhibited a significant increase in NK cells proportion in the BM compared to non-responders (add statistics). Additionally, CXCR4 expression on NK cells was higher in patients with a favorable response, suggesting a link between NK cell dynamics and treatment outcomes (add statistics). To carry out mechanistics studies, C1498 mouse AML cells were transplanted into C57/BL6 mice, which were treated with decitabine or vehicle for 5 days. Decitabine not only inhibited in vivo AML proliferation but also increased the proportion of NK cells cells in the BM. Of interest, CXCR4, a chemokine receptor that has been known for BM homing, was also upregulated on the surface of NK cells. In addition, CXCR4 expression was first increased in NK cells, and then NK cell homing was followed. To investigate whether HMA treatment increases CXCR4 expression and induces NK cell migration, an in vitro Transwell migration assay was performed using NK92 cells (a human NK cell line) with AML cell lines (MV4-11 and OCI-AML3) and mesenchymal stromal cells (MSC) from AML patients. CMFDA-labeled NK92 cells showed higher migration and increased CXCR4 expression in decitabine-treated wells compared to vehicle controls, with the effect being more pronounced in the presence of MSCs. Taken together, these findings suggest that HMA-induced NK cell recruitment to the BM via CXCR4 upregulation may play a key role in the anti-leukemic effects of HMAs in AML and HR-MDS. Increased NK cell migration and CXCR4 expression are associated with better treatment responses, highlighting the potential of enhancing NK cell immunity as a therapeutic strategy.

#892 Development of iPSC-derived feeder cells to enhance NK cell expansion and cytotoxicity for cancer therapy.

Z.-Q. Lin[1], M.-T. Hsu[2], Y. Lin[1], L.-F. Chen[1], B.-H. Chen[1], Y.-C. Sun[3], K.-F. Hung[2];
[1]Taipei Veterans General Hospital, Taipei, Taiwan, [2]National Yang Ming Chiao Tung University, Taipei, Taiwan, [3]Taipei Tzu-Chi Hospital, the Buddhist Tzu-Chi Medical Foundation, New taipei City, Taiwan

Natural killer (NK) cells have been considered a promising candidate for cell therapy in cancer treatment. However, since NK cells constitute a minor fraction of peripheral blood mononuclear cells (PBMCs), recent efforts have concentrated on enhancing the efficiency of *in vitro* expansion. Among various approaches, the genetically engineered K562 cell line has shown promising potential in NK cell expansion. Nonetheless, as K562 cells are a cell line derived from human leukemia cells, the use of K562 as feeder cells raises significant safety concerns. To address this issue, we developed a novel feeder cell system by engineering hematopoietic progenitor cells (HPCs), derived from induced pluripotent stem cells (iPSCs), to express co-stimulatory molecules and cytokines on their surface via viral transduction. We showed that iPSC-derived HPCs efficiently prolong the lifespan of primary NK cells beyond two weeks, surpassing the typical half-life of NK cells in regular culture conditions. To further enhance the stimulatory effect of membrane-bound interleukin on the surface of feeder cells, we engineered a bispecific antibody comprising a recombinant cytokine and a single-chain variable fragment (scFv) with binding specificity to feeder cells. Interestingly, we found that the combination of HPC feeder cells and recombinant cytokines significantly enhance NK cell expansion and cytotoxicity. We also found that iPSC-derived feeder cells efficiently sustain the cytotoxicity activity of iPSC-derived NK cells, showing that iPSC-derived cells can serve as a powerful platform for cancer therapy. In conclusion, our novel feeder cell system shows great potential to enhance NK cell proliferation and activation. While further optimization is required, this non-cancerous source of feeder cells offers an appealing alternative to K562 cells, facilitating the development of NK cell-based cancer therapy.

#893 A novel approach to treating HER2-low breast cancer: surface-engineered NK/tz cells targeted immunotherapy.

Maria Jose Godoy Calderon[1], Eric Gauchat[2], VK Gadi[1]

[1]Medicine, University of Illinois at Chicago, Chicago, IL,[2]Cancer Center, University of Illinois at Chicago, Chicago, IL

Background: The majority (60-70%) of metastatic breast cancers (MBC) are HER2-low expressing, and in distinction to HER2+ disease, is not addicted for growth to this oncogene. Trastuzumab-based treatments have failed to improve the outcomes of patients with HER2-low breast cancer. Despite promising results with trastuzumab-deruxtecan (T-DXd), significant systemic toxicity and disease progression were observed highlighting the need to develop safer and more effective, and accessible therapies. This study evaluates the therapeutic potential of surface-engineered NK cells (SE-NK) modified with trastuzumab (Tz) for targeting HER2-low breast cancer cells.
Methods: To establish a preclinical model, the triple-negative murine breast cancer cell line 4T1 was transduced with a lentiviral vector encoding human HER2, generating clones with HER2 expression comparable to HER2-low human breast cancer cells (MDA-MB-175). HER2 expression was validated using flow cytometry, confocal microscopy, and Western blot analysis. SE-NK/Tz cells were generated by embedding 25 ug Tz onto the NK cell surface using a hydrophobic linker. These cells were tested in vitro for HER2-specific cytotoxicity against the engineered 4T1-HER2-low cells.
Results: Transduced 4T1-HER2-low cells exhibited HER2 expression levels consistent with HER2-low human breast cancer cells. In vitro, SE-NK/Tz cells demonstrated significantly enhanced cytotoxicity against 4T1-HER2-low cells (24%, $p < 0.05$) compared to NK cells alone (0%) when an effector to target ratio of 1.25:1 was used.
Conclusions: This study establishes the feasibility of using SE-NK/Tz cells as a novel immunotherapeutic approach for HER2-low breast cancer. We created for the firsttime to our knowledge a murine cell line with human HER2-low status comparable to expression values reported in patients. The preclinical findings support the potential of this strategy to overcome the limitations of current therapies by offering an effective and accessible treatment option. Future studies will explore mechanisms of immune activation and therapeutic efficacy in diverse in vivo HER2-low models.

#894 Generation of cytokine-induced memory-like PD-L1 t-haNK (NK-92®) cells.

Dustin Elwood, Devapriya Segaran, Joseph Thibault, Vidya Godbole, Manju Saxena, Hans Klingemann, **Laurent Boissel**, Patrick Soon-Shiong

ImmunityBio, Inc., Culver City, CA

Cytokine-induced memory-like NK cells have garnered a lot of attention as cellular therapeutic agents due to their much-increased lifespan in patients and their enhanced memory response upon re-challenge by cytokines or target cells. A key part of this memory recall feature is the increased secretion of interferon gamma (IFNγ). This potent immune stimulator can trigger upregulation of MHC-I molecules expression on the surface of tumor cells, thereby pushing the tumor towards an immunologically “hot” status. However, IFNγ also triggers expression of the immune checkpoint molecule PD-L1. We show here that the Natural Killer cell line NK-92®, or ‘activated’ NK (aNK™), can acquire a memory-like phenotype upon overnight induction with IL-12, IL-18, and the IL-15 superagonist N-803, as evidenced by increased steady-state IFNγ secretion (>50-fold increase) and up-regulation of CD25. Although the initial increase in IFNγ secretion subsides after 48hrs, memory-like NK-92 cells display memory recall for several days post-activation, as evidenced by an enhanced IFNγ response upon challenge with K562, Raji, and THP-1 cells compared to non-memory-like cells (2.3, 6.9, and 3.8-fold increases after 1 week, respectively). Further, NK-92 cells engineered to express a high-affinity Fc receptor and an anti-PD-L1 Chimeric Antigen Receptor (PD-L1 t-haNK) maintained their susceptibility to memory-like cytokine induction. Exposure of the NK-resistant cells lines THP-1 and SK-ES-1 to culture supernatant from memory-like PD-L1 t-haNK triggered expression of PD-L1 on their surface, rendering them highly sensitive to PD-L1 CAR-mediated targeted killing by the PD-L1 t-haNK cells in a 4-hour *in vitro* cytotoxicity assay (respectively 91% and 88% killing at E:T ratio of 1.25:1, compared to 54% and 41% for target cells exposed to regular PD-L1 t-haNK supernatant), while not affecting their resistance to NK-92 cells. Infusion of a cellular therapy product that constitutively secretes high IFNγ may cause detrimental side effects. Memory-like PD-L1 t-haNK cells may circumvent this problem through their memory recall ability and deliver increased amount of IFNγ specifically at the tumor site when locally engaging with tumor cells. This increase would then drive upregulation of PD-L1 expression on neighboring tumor cells, creating an expanding wave of targets sensitized to killing by PD-L1 t-haNK cells. A next generation, memory-like PD-L1 t-haNK cell line equipped with inducible safety switch features to remove the need for irradiation and extend their half-life in patients is being developed and would constitute a promising “off-the-shelf” cellular therapy against immunologically cold cancers.

#895 A systemically administrated STING agonist in albumin formulation activated neutrophils for anti-cancer immunotherapy.

Jinsong Tao, Hongyi Zhao, Chengyi Li, Zhongwei Liu, Hanning Wen, Qiuxia Li, Miao He, Bo Wen, Fang Ke, Wei Gao, Duxin Sun

University of Michigan, Ann Arbor, MI

Background: Neutrophils, the most abundant leukocytes (50-70%) in human circulation, are key players in the innate immune response and increasingly recognized for their multifaceted roles in cancer biology. Beyond their established functions in infection control and inflammation, they have been shown to significantly influence tumor progression through complex interactions within the tumor microenvironment. Neutrophils exhibit remarkable phenotypic heterogeneity and plasticity, which allows them to adopt either tumor-suppressing or tumor-promoting functions. How to program neutrophils in vivo for anti-cancer therapy? We developed a systemically administrated STING agonist formulated within albumin formulation to activate neutrophils for anti-cancer immunotherapy.
Methods & Results: We synthesized a novel non-nucleotide STING agonist dimer ZSA-51D encapsulated in albumin nanoparticles (nano ZSA-51D), which exhibited a potent STING pathway activation in THP1-Blue^{TM} ISG cells (EC 50 0.44 nM) and mouse myeloid cells (EC 50 < 10 nM). Interestingly, we found the Nano ZSA-51D highly activated CD101-CD14+ and CD101+CD14+ neutrophils in vitro and in vivo, which was STING pathway dependent. The pharmacokinetic studies in mice shown Nano ZSA-51D highly accumulated in bone marrow (BM). Most of the Nano ZSA-51D were absorbed by neutrophils in BM, blood and Tumor, which resulted in high neutrophil activation in vivo. The Nano ZSA-51D plus α-PD1 achieved complete remission in the MC-38 colon cancer and robust anti-cancer effects in pancreatic cancer model. The tumor immune cell infiltration was examined by flow cytometry at 24 h after treatments. We found the neutrophils were highly activated in BM and blood, then the activated (CD101-CD14+ and CD101+CD14+) neutrophils largely infiltrated into tumor after Nano ZSA-51D treatment. The bulk mRNA sequence analysis showed the neutrophils were activated through Toll-like receptor, TNF-α and NF-κB signaling pathway. Surprisingly, the activated CD101-CD14+ and CD101+CD14+ neutrophils enhanced the antigen presentation of exogenous peptide antigen via MHC class I, further promote the proliferation and activation of T cells, which shown robust anti-cancer effects in vivo after rejected the activated neutrophils into tumor.
Conclusion: In this study, we developed a novel systemically administered STING agonist formulated within albumin formulation (Nano ZSA-51D) to activate neutrophils for anti-cancer immunotherapy. Nano ZSA-51 plus α-PD1, demonstrated superior anticancer efficacy through activating neutrophil to promote the proliferation and activation of T cells in vivo.

#896 Unleashing the power within: enhancing cancer treatment with invariant natural killer T cells.

Daniah Alkassab, Carolina de Amat Herbozo, Thierry Mallevaey

Immunology, University of Toronto, Toronto, ON, Canada

Invariant natural killer T (iNKT) cells are a promising target for adoptive cell therapy (ACT) due to their unique ability to recognize glycolipid antigens presented by the non-polymorphic MHC class I-like CD1d molecule, enabling heterologous application without the risk of alloreactivity. Activated, iNKT cells exhibit potent antitumor activity through cytokine secretion, chemokine release, and cytolysis, making them ideal candidates for off-the-shelf cancer therapies. However, limited knowledge of iNKT cell biology, particularly their functional heterogeneity, has hindered their therapeutic optimization. This study aimed to (a) investigate the functional heterogeneity of iNKT cells, (b) expand iNKT cells from human peripheral blood mononuclear cells (PBMCs), and (c) optimize the generation of cytotoxic iNKT cells by evaluating their phenotypic and functional profiles in response to various cytokine stimulations. PBMCs from 14 healthy donors (7 males and 7 females) were analyzed using spectral flow cytometry to assess the expression of key iNKT cytotoxic markers, along with markers associated with natural killer cells and CD8+ T cells. Phenograph clustering revealed distinct iNKT subpopulations, including one characterized by high expression of cytotoxic markers such as CD57, granzyme B, and granulysin, and another enriched for CD62L, a marker linked to central memory-like phenotypes with the potential to differentiate into cytotoxic subsets with enhanced antitumor activity. To optimize iNKT cell expansion, PBMCs were co-cultured with artificial antigen-presenting cells loaded with the iNKT ligand α-galactosylceramide in the presence of IL-2. iNKT cells were sorted two weeks post-initial stimulation and restimulated every 2-3 weeks using anti-CD3, resulting in the generation of millions of cells. To further enhance cytotoxic potential, iNKT cells were treated with cytokines such as IL-7, IL-15, IL-12, and IL-21. Preliminary findings demonstrated that cytokine stimulation influences the phenotypic profiles of iNKT cells. These findings provide a robust foundation for generating large quantities of cytotoxic iNKT subsets optimized for in vitro and in vivo assays. This work not only enhances the understanding of iNKT cell biology but also represents a significant step toward leveraging iNKT cells as versatile and powerful tools in ACTs.

TUMOR BIOLOGY: Cancer Stem Cells
Poster Session

#9 Genetic and oncogenic viruses as risk factors in Egyptian breast cancer patients.

Sarah Mohamed Mahmoud ElSayed[1], Rania Abdulmonem Eissa Al Najar[1], Nasra Fathy Abdel Fattah[2], Ayman Abdel-Samie Gaber[3], Tarek Hashem[4], Wafaa Khalaf[5], Ahmed Basyony[5], **Mohamed A. Eltokhy**[6], Sahar Mohamed Ramzy Radwan[7], . Amany abdullah EL-Sharif[7], Samah Loutfy[8]

[1]Clinical Pharmacy, Breast Cancer Comprehensive Center, National Cancer Institute, Cairo, Egypt,[2]Virology and Immunology, Cancer Biology Department, National Cancer Institute, Cairo, Egypt,[3]Medical Oncology Department, National Cancer Institute, Cairo, Egypt,[4]Surgical Oncology Department, National Cancer Institute, Cairo, Egypt,[5]Microbiology and Immunology Department, National Cancer Institute, Cairo, Egypt,[6]Texas Tech Health Science Center (TTUHSC), Abilene, TX,[7]Microbiology and Immunology Department, Faculty of Pharmacy (Girls), Al-Azhar University (Cairo), Cairo, Egypt,[8]National Cancer Institute, Cairo, Egypt

Introduction: Breast cancer (BC) is a leading cause of death worldwide. Genetic factors, such as ATM, BRCA1, BRCA2, CHEK2, and PALB2 are detected in 5%-7% of women with breast cancer elevating risk of disease development. The role of environmental factors such as oncogenic viruses remained under investigation. Therefore, we aimed to investigate correlation between variations in some exons of BRCA1, BRCA2, CHEK2, and PALB2 and presence of human papillomavirus (HPV), human mammary tumor virus (HMTV), and Epstein-Barr virus (EBV) DNAs in the blood of BC patients, hoping to understand role of oncogenic viruses in cancer development
Methods: Genomic DNA was extracted from 55 fresh tissues and blood samples of BC patients, as well as ten blood samples from healthy women, served as a control using Gene JET Extraction Kit (Thermo Scientific/US, Canada). PCR assay was employed to detect presence of HPV, HMTV and EBV DNAs. Additionally, variations in BRCA1, BRCA2, PALB2, and CHEK2 genes were screened using high resolution melting assays.
Results: HPV and HMTV DNAs were detected in the blood of 25.5% and 23.6% BC patients, respectively, with 9% of patients exhibiting co-presence of both viruses. No viral DNAs was detected in the healthy controls. In tumor tissues, *HPV* DNA, *HMTV* DNA, and *EBV* DNA were detected in 47.2%, 27.2%, and 25.5% of BC patients (P=0.012). Regarding genetic analysis, variations were detected in BRCA1 25.5%, BRCA2 43.6%, PALB2 42%, and CHEK2 52.7% genes of the blood of 55 BC patients. Our results demonstrated overlapping between PALB2, CHEK2, and BRCA2 variants in BC patients. Among PALB2 carriers, 30% harbored BRCA2 variants, among CHEK2 carriers, 32% harbored BRCA2 variants. Moreover, 25% of PALB2 carriers and 18% of CHEK2 carriers presented with BRCA1 variants. Additionally, our findings revealed co-occurrence of genetic variations among BC patients. Notably, 45.4% with BRCA2 exon11 variants, exhibited variations in both PALB2 exon 4 and CHEK2 exon 10. Additionally, 43% had variations in BRCA1 exon 6, and in CHEK2 exon 10, 57% had variations in BRCA1 exon 9 and PALB2 exon 4.
Results indicated an association between presence of viral DNAs and some genetic variations. Among 26 HPV-positives, 19.2% exhibited variations in exons 11 and 27 of BRCA2, and 50% had variations in PALB2 exon 4. In 15 HMTV-positives, 20% had variations in BRCA2 exon 27, and 66.7% exhibited variations in CHEK2 exon 10.
Conclusion: Results suggest a potential association between genetic variations in *BRCA1, BRCA2,* PALB2, and CHEK2, and presence of oncogenic viruses (HPV, HMTV) in the pathogenesis of BC. Further investigations are required to confirm these observations. Data indicated a correlation between CHEK2, BRCA2, and HMTV, and between PALB2, BRCA1, and HPV. Understanding these associations, especially if present in certain exons may aid in understanding the role of oncogenic viruses and developing new markers for early detection and prognosis of disease.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#90 OTUD7B activates the caspase 8-RIP1-NEMO complex-regulated NF-kB pathway to promote triple-negative breast cancer metastasis.

Hui-Yu Lin[1], Kun-Ju Chen[2], **Yuan-Feng Lin**[2]

[1]Fu-Jen Catholic University, New Taipei City, Taiwan,[2]Taipei Medical University, Taipei, Taiwan

Background: Triple negative breast cancer (TNBC) is a highly metastatic BC subtype and refers to a poorer prognosis of BCs. Distant metastasis of TNBCs to other organs, e.g. lungs and brain, risks the patient's life. However, the molecular mechanism underlying TNBC metastatic progression is still under investigation. The aim of this study was made to delineate the role of ovarian tumor deubiquitinating enzyme 7B (OTUD7B) in the molecular mechanism for TNBC metastasis. **Methods:** Site-directed mutagenesis was performed to confirm that the deubiquitinating activity of OTUD7B is required for promoting TNBC metastatic potentials. Human Ubiquitin Array kit was employed to identify the potential target of OTUD7B in the highly metastatic TNBC cell line MDA-MB231. Pathway enrichment analysis was utilized to predict the possible pathways involved in TNBC metastasis. Pulldown assay was utilized to validate the deubiquitinating activity of OTUD7B towards the identified protein targets. Cycloheximide-chased assay was used to measure the degradation of identified protein targets in response to OTUD7B knockdown in MDA-MB231 cells and its overexpression in poorly metastatic TNBC cell line HCC1937. **Results:** Site-directed mutagenesis indicated that the mutation of Asn193 to Leu or Met, which leads to the loss of OTUD7B deubiquitinating activity, as compared to wild-type gene fails to promote cellular migration ability and lung colony-forming capacity of the tested TNBC cells. The employment of Human Ubiquitin Array kit demonstrated that Caspase-8 and NEMO, also named IKKγ, are the potential targets for OTUD7B deubiquitinating activity in MDA-MB231 cells. Pathway enrichment analysis predicted the pathway in which NF-κB activation through FADD/RIP-1 axis mediated by Caspase 8 is probably activated in the primary tumor from TNBC patients with lymph node metastasis. Pulldown assay further confirmed the deubiquitinating activity of OTUD7B towards Caspase-8 and NEMO in TNBC cells. Cycloheximide-chased assay revealed that OTUD7B knockdown accelerates protein degradation of Caspase-8 and NEMO in MDA-MB231 cells. The pharmaceutical inhibition of NEMO complex with IKKα/β dramatically suppressed NF-κB activity and metastatic potentials in the OTUD7B-overexpressing HCC1937 cells. Importantly the signature of combining lower levels of cleaved/active Caspase-8 and higher levels of pS536-NF-κB was found to highly correlate with lymph node metastasis and a poorer overall survival rate in TNBC patients. **Conclusion:** Our results suggest that OTUD7B upregulation promotes TNBC metastasis by recruiting the Caspase-8-RIP1-NEMO axis-activated NF-κB pathway.

IMMUNOLOGY: Tumor-induced Immune Suppression: Intrinsic and Extrinsic Factors
Poster Session

#900 Melanoma immune escape controlled by cancer cell-intrinsic STING signaling is linked to MHC-I suppression.

L. S. Jensen[1], A. Weiss[2], A. Draghi[2], S. Khan[2], A. S. Hansen[3], A. Etzerodt[1], B. W. Deleuran[1], M. Donia[2], M. R. Jakobsen[1];
[1]Aarhus University, Aarhus, Denmark, [2]Copenhagen University Hospital, Herlev, Denmark, [3]University of Southern Denmark, Odense, Denmark

Background: A crucial aspect of anti-tumor immunity is the infiltration of cytotoxic CD8+ T cells, which eliminate tumor cells by recognizing tumor antigens presented on major histocompatibility complex (MHC) class I molecules. The innate immune pathway regulated by Stimulator of Interferon Genes (STING) plays a significant role in modulating tumor immunity, and pathway activation is evident to impact T cell priming. In cancer cells, genomic instability leads to the accumulation of cytosolic DNA, activating the STING pathway and triggering a cytokine-driven immune response, which supports tumor control. However, to evade immune detection cancer cells continuously evolve mechanisms to evade immune recognition, such as downregulating MHC-I surface expression, thereby reducing the efficacy of CD8+ T cell-mediated cancer cell elimination.
Methods: We utilized a syngeneic mouse model of the murine YUMM.G/YUMMER.G melanoma cells that present high or low somatic mutational burden (SMB), along with a cohort of human melanoma patient-derived cell lines. We investigated the modulating role of cancer cell-intrinsic STING signaling in tumor immunity, focusing on its impact on antigen presentation and immune cell infiltration under varying levels of SMB both *in vitro* and *in vivo*.
Results: We observed that cancer cell-intrinsic STING signaling strongly associates with MHC I expression in our murine melanoma models. Similar correlations were found in a large group (n=76) of human melanoma patient-derived cancer cell lines. Our *in vivo* tumor models demonstrated that loss of STING expression significantly affected tumor control in melanoma models with high SMB. Notably, high SMB melanoma tumors deficient in STING expression escaped immune control by reducing infiltration and activation of cytotoxic CD8+ T cells. Conversely, STING depletion in low SMB melanoma cells had limited impact.
Conclusion: Our findings advocate that STING expression status in melanoma plays a critical role in determining tumorigenicity, with its influence being dependent on the somatic mutational burden of the cancer cells. This highlights the potential of STING expressions within cancer cells as a critical factor for tumor immunogenicity and should be considered as an exploratory biomarker in immunotherapy settings combined with tumor mutational burden.

#901 KLF4-induced melanocyte-schwann plasticity promotes immune escape in melanoma via LAG3 checkpoint.

A. Zhao[1], X. Liu[2], **Z. Zuo**[2];

[1]Zhejiang Cancer Institute, Zhejiang Cancer Hospital, Hangzhou Institute of Medicine, Chinese Academy of Sciences, Hangzhou, China, [2]Sun Yat-sen University Cancer Center (SYSUCC), Guangzhou, China

Precision therapy is crucial to the successful treatment of rare tumors with limited treatment options. Here, we report a rare case of primary anterior mediastinal melanoma with liver metastasis. The patient had previously failed anti-PD1 treatment, leading us to profile the molecular features of the tumor to identify alternative therapeutic targets. Through comprehensive multi-omics analyses, we discovered a high degree of tumor cell plasticity in this rare case, with a subset of tumor cells displaying a Schwann cell phenotype evolved from melanocyte tumor cells. Cell-cell communication analysis revealed that the Schwann-like tumor cells may evade immune surveillance through the LAG3-LGALS3 immune checkpoint. The patient was then recommended for enrollment in a trial using combination therapy with anti-PD1 and anti-LAG3, and the disease remained stable for up to 24 months. Further investigation into the underlying mechanism showed that transcription factor KLF4-mediated transcription reprogramming drives B16 melanoma cell trans-differentiation into a Schwann cell phenotype. This trans-differentiation sensitizes the tumor cells to anti-LAG3 therapy. By analyzing melanoma tissue microarrays and external single-cell sequencing data, we found that this melanocyte-Schwann plasticity (MSP) existed in around 20% of melanoma cases. Moreover, a public immunotherapy cohort and our data indicate that MSP is associated with poor response to anti-PD1 treatment, suggesting that MSP may serve as a predictive biomarker for immunotherapy efficacy. Overall, our study not only provides a paradigm for precision immunotherapy in the context of rare tumors, but also reveals a potentially effective immunotherapeutic strategy for advanced melanoma with melanocyte-Schwann plasticity.

#902 Examining the impact of chromosome 1q-located gamma secretase subunits in the resistance to BCMA-targeted therapies in multiple myeloma patients.

Sejal Chikhale, Mansour Poorebrahim, Holly Lee, Elham Hasheminasabgorji, Anja Barbour, David Jung, Paola Neri, Nizar J. Bahlis

Arnie Charbonneau Cancer Institute, University of Calgary, Calgary, AB, Canada

Multiple Myeloma (MM) is an incurable hematological malignancy originating from abnormal plasma cells in the bone marrow. A common genetic abnormality in MM is the gain or amplification of chromosome arm 1q (chr1q), present in 30-40% of MM patients and typically associated with poor prognosis. Chr1q includes three subunits of the gamma-secretase (GS) enzyme- Nicastrin (NCSTN), Presenilin-2 (PS2), and Anterior Pharynx Defective 1 Homolog A (APH1A)—which catalyze the conversion of membrane-bound BCMA (mBCMA) to soluble BCMA (sBCMA). BCMA is a crucial target receptor for anti-BCMA therapies. Elevated sBCMA levels are linked to poor prognosis and reduced efficacy of BCMA-targeted therapies, apparently due to the sink effect. We hypothesize that chr1q amplification in MM leads to increased GS expression, resulting in reduced mBCMA and elevated sBCMA levels, which may drive resistance to BCMA-targeted therapies and contribute to adverse patient outcomes. To investigate this, we employed cloning and lentiviral transduction techniques to overexpress NCSTN, PS2, and APH1A individually, as well as in various combinations, in OPM2 myeloma cell lines, generating seven novel cell lines. Each subunit was fused with distinct tags to confirm successful transduction and overexpression. Using flow cytometry with BCMA-PE antibodies and sBCMA ELISA, we assessed mBCMA and sBCMA levels across these GS subunit-overexpressing cell lines compared to an empty vector control. Our results demonstrated that all seven GS subunit-overexpressing OPM2 cell lines exhibited decreased mBCMA levels. The triple-overexpressing cell line (NCSTN, PS2, and APH1A) showed a remarkable ten-fold reduction in mBCMA compared to the control. Additionally, differences in sBCMA levels were observed across the GS subunit-overexpressing cell lines relative to the control. These findings underscore the pivotal role of GS subunits encoded by chr1q in driving the poor prognosis associated with chr1q gain or amplification in MM. Ongoing analyses aim to elucidate the resistance mechanisms of these GS subunit-overexpressing OPM2 cell lines to anti-BCMA CAR-T therapies and T-cell engagers (TCEs).

#903 Mechanisms mediating hypoxic memory of type I IFN signaling suppression and implications for immune evasion in luminal breast cancer cells.

R. Marker, A. Moriarty, R. Klotz, M. Yu;
University of Maryland, Baltimore, Baltimore, MD

Solid tumors often contain patches of hypoxic regions that yield aggressive, pro-metastatic phenotypes. Prevention and understanding of metastases is critical for the future of breast cancer research, as metastatic cancer is extremely difficult to manage and treat. Recent work in our lab has shown that long-term hypoxia suppresses type I interferon (IFN) signaling in breast cancer cells. Even upon reoxygenation, these gene expression changes are maintained, indicative of “hypoxic memory.” Breast cancer cells that have disseminated from the primary tumor as circulating tumor cells with this “post-hypoxic” memory phenotype show enhanced metastatic potential. IFNs typically boost and enhance immune cell activity through interferon-stimulated genes (ISGs); thus, hypoxic suppression of IFN signaling yields an immunosuppressive tumor microenvironment. More research is needed to understand how ISGs are downregulated in hypoxia and how those changes in gene expression contribute to immunosuppressive ability of hypoxic and post-hypoxic cells. Interferon-inducible 44-like (IFI44L) is an ISG that has been associated with tumor-suppressive properties, and correlated with presence of tumor-infiltrating lymphocytes in other cancers, but its role in breast cancer has not been studied. We found that IFI44L is significantly suppressed in breast cancer cells in hypoxia and maintained after reoxygenation as a hypoxic memory. To try to understand possible mechanisms of hypoxic suppression, we overexpressed interferon regulatory factors (IRFs), which are positive regulators of the type I IFN pathway. However, this did not rescue hypoxic suppression and memory of IFI44L, suggesting that IRFs are not the sole regulator of IFN and ISG expression in hypoxia. Additional experiments are currently ongoing in the lab to address other possible mechanisms of hypoxic suppression and memory of IFN signaling. Additionally, co-culture experiments of breast cancer cells with knockdown or overexpression of IFI44L with different immune cell populations will shed light on the role of IFI44L in immune interactions in the tumor and in circulation. To better understand implications of maintained IFI44L suppression, we will also examine immune cell activity and metastatic potential using in vivo mouse studies. We expect to see less immune cell effect and enhanced metastasis with IFI44L knockdown and reversed phenotypes with overexpression of IFI44L. Understanding mechanisms of immune evasion mediated via IFI44L can lead to strategies for targeting hypoxia-mediated tumorigenesis and metastasis.

#904 Mapping the T cell-cold phenotype to the colorectal cancer genome identifies POFUT1 as a driver of tumor T cell inhibition.

C. J. Wagner;
Wake Forest University School of Medicine, Winston-Salem, NC

Colorectal cancer (CRC) patients with T cell-cold tumors experience rapid cancer progression and poor survival. While T cell-enhancing immunotherapies have improved outcomes for a subset of CRC patients, most remain nonresponsive. Therefore, better understanding the mechanisms that drive T cell dysfunction in CRC is needed. We used matched gene expression and copy number profiles from TCGA to evaluate CRC tumors (N=373) based on measures of T cell infiltrate, and developed a method to identify genes with potential roles in T cell exclusion. Leveraging the intersect between copy number amplification and gene expression in the T cell-cold state, we pinpointed a region of chromosome 20q11.21 where DNA- and RNA-levels were more strongly correlated with T cell exclusion than anywhere else in the CRC genome. This locus also contained the most frequently amplified and overexpressed genes in the CRC genome. Within this region, Protein O-Fucosyltransferase 1 (POFUT1) was the most distinguished gene, and correlated with CTL inhibition across 12/24 solid tumor types annotated in TCGA (SKCM, THCA, COAD, READ, LUSC, CESC, KIRC, STAD, HNSC, KIRP, LIHC, UCEC; $p<0.0001$). Stable overexpression of POFUT1 (POFUT1-OE) in MC38 mouse colon cancer cells enhanced proliferation relative to MC38-Vector (RTCA $p<0.0001$, CellTiterGlo $p=0.0011$); and in co-culture with c57BL/6 spleen-derived PBMCs, CD8+ T cell populations from MC38-POFUT1-OE co-cultures were significantly reduced over the course of 7 days. This effect persisted when PBMCs were stimulated with IL-2 and anti-CD2/CD3/CD28 in 50% conditioned medium (CM) from MC38-POFUT1-OE, prompting us to profile the CM for differences in secretomes. Of 110 profiled cytokines, two were at significantly higher levels in MC38-POFUT1-OE CM: IL-6 and IL-23 ($p=0.0003$, $p=0.0055$). These results were validated via ELISA and transient knockdown (KD) of POFUT1, where POFUT1-KD resulted in reduced cancer cell proliferation, diminished secretion of IL-6, and increased CD8+ T cell abundance in our CM models. Furthermore, siRNA-mediated KD of POFUT1 in MDST8 and HCT15 human CRC cell lines resulted in significant reductions in secreted IL-6 ($p=0.0003$, $p<0.0001$). Treatment of murine PBMCs with IL-6 (5.0 pg/mL - 50 ng/mL) decreased the proportion of CD8+ T cells to an analogous level as culture with POFUT1-OE CM. The decrease in CD8+ T cell proportion was also associated with less proliferation (CFSE, $p=0.0049$), and reduced surface expression of activating co-receptors CD25 and CD69 ($p=0.1008$, $p=0.0015$). Dosing with anti-IL-6 IgG (10 ng/mL) reversed the decrease in CD8+ T cell proliferation induced by CM from MC38-POFUT1-OE, establishing IL-6 as the downstream mechanism of POFUT1-dependent inhibition of CD8+ T cell activity. Together, these findings link POFUT1 amplification in CRC with dysregulation of CD8+ T cells via upregulation of cancer-intrinsic IL-6 secretion.

#905 SMARCA4 mutation induces tumor cell-intrinsic defects in enhancer landscape and resistance to immunotherapy.

Y. Lissanu, Y. Wang, I. Meraz, M. Qudratullah, S. Kotagiri, Y. Han, J. Roth;
UT MD Anderson Cancer Center, Houston, TX

SMARCA4 is a frequently mutated subunit of the SWI/SNF chromatin remodeling complex. Importantly, clinical reports indicate that SMARCA4-mutant lung cancers respond poorly to immunotherapy and have dismal prognosis. However, the mechanistic basis of immunotherapy resistance is unknown. Here, we corroborated the clinical findings by using immune-humanized, syngeneic, and genetically engineered mouse models of lung cancer harboring SMARCA4 deficiency. Specifically, we show that SMARCA4 loss caused decreased response to anti-PD1 immunotherapy associated with significantly reduced infiltration of dendritic cells (DCs) and CD4+ T cells into the tumor microenvironment (TME). Mechanistically, we show that SMARCA4 loss in tumor cells led to profound downregulation of STING, IL1β and other components of the innate immune system as well as inflammatory cytokines that are required for efficient recruitment and activity of immune cells. We establish that this deregulation of gene expression is caused by cancer cell-intrinsic reprogramming of the enhancer landscape with marked loss of chromatin accessibility at enhancers of genes involved in innate immune response such as STING, IL1β, type I IFN and inflammatory cytokines. Interestingly, we observed that transcription factor NF-κB binding motif was highly enriched in enhancers that lose accessibility upon SMARCA4 deficiency. Finally, we confirmed that SMARCA4 and NF-κB co-occupy the same genomic loci on enhancers associated with STING and IL1β, indicating a functional interplay between SMARCA4 and NF-κB. Taken together, our findings provide the mechanistic basis for the poor response of SMARCA4-mutant tumors to anti-PD1 immunotherapy and establish a functional link between SMARCA4 and NF-κB on innate immune and inflammatory gene expression regulation.

#906 High K+ in tumors impairs T cell effector activity via lysosomal acidification-induced autophagy.

Robert L. Eil, Chelsea Jenkins, Jessica Briones, Matthew McWhorter, Alexandra Bartlett

Knight Cancer Institute, Oregon Health & Science University, Portland, OR

Effective antitumor T cell response necessitates nutrient uptake and utilization. Here, we demonstrate that potassium (K^+) accumulation within the tumor microenvironment (TME) drives adaptive changes in T cell metabolism via K^+-dependent lysosomal acidification. We found that lysosomal acidification promotes a catabolic state in $CD8^+$ T cells that persists independently of extracellular glucose levels (i.e. functional caloric restriction) and limits T cell effector functions while enhancing persistence.We previously identified a specific elevation in extracellular K^+ ($\uparrow[K^+]_{ex}$) within tumors, which dampens mTOR activation and effector functions in both CD8+ and CD4+ T cells (Eil et al, *Nature* 2016). $\uparrow[K^+]_{ex}$ increased intracellular K^+ ($[K^+]_{in}$) and drove selective mitochondrial acetyl-CoA accumulation at the expense of nuclear histone acetylation, enhancing mitochondrial function and limited epigenetic programming of T cells (Eil & Vodnala et al, *Science* 2019). Here, we report that $\uparrow[K^+]_{ex}$ in the TME also produced reduced glycolytic flux, even in the presence of abundant glucose, highlighting a rerouting of nutrient processing under K^+-rich conditions. The prior theoretical model of K^+ as a solely a regulator $V_{m,}$ and thereby TCR induced Ca^{2+} influx, fails to account for the metabolic reprogramming observed under K^+-rich conditions in the TME.To support the development of a new model defining effective ionic T cell responses to the high K^+ TME, we pursued an *in vivo* CRISPR-Cas9 screen targeting ion transporters in a syngeneic melanoma model. Our results pointed to the critical role of lysosomal H^+ importers in $CD8^+$ T cell persistence. Genetic or pharmacologic enhancement of lysosomal H^+ importers augmented autophagy, lysosomal acidification and function, and T cell persistence. Moreover, while $\uparrow[K^+]_{ex}$ enhanced lysosomal acidification and persistence, genetic depletion of $[K^+]_{in}$ produced lysosomal de-acidification, unleashed Akt-mTOR activation, and compromised persistence. These findings reveal lysosomal pH as a key determinant of T cell persistence in the TME, highlighting a mechanism of dynamic metabolic adaptation of T cells required to perform in differential ionic environments.Our findings suggest a model whereby lysosomal ion transport governs T cell adaptation, underscoring nutrient processing, in addition to availability, as a significant mediator of metabolism. The interplay between $[K^+]_{in}$ and lysosomal pH highlights novel intervention points for genetic and pharmacologic interventions modulating T cell function in cancer immunotherapy.

#907 Identification of a stem cell-specific secretory immune regulatory ligand.

Benjamin T. Nicholson, Ama Ofori, Jennifer Good, Weijie Guo, Yuxuan Miao

Ben May Department for Cancer Research, University of Chicago, Chicago, IL

Immunotherapies have transformed cancer treatment, but subsets of patients experience differential responses. Patients with squamous cell carcinomas (SCCs) in various tissues exhibit a high rate of relapse after their initial response to immunotherapies. Recent research highlighted the critical roles of a group of stem cell-like tumor-initiating cells (TICs) in driving the relapse of both cutaneous (CSCCs) and head and neck (HNSCCs). These TICs exhibit superior resistance to anti-tumor immunity despite their intact antigen presentation. Although TICs are the root cause of SCC relapse, they compose less than 5% of the total tumor cell population. Therefore, their unique immune resistance mechanisms have been largely overlooked. Here, we aim to identify the special immune regulatory mechanisms that are specifically activated in TICs endowing them with stronger immune resistance. By analyzing single-cell RNA-seq data profiling the gene signatures of various tumor populations in CSCCs, we identified cytotoxic T lymphocyte antigen 2a (*Ctla2a*) was specifically activated in TICs. Ctla2a is characterized as a cysteine peptidase inhibitor and has high homology to the I29 inhibitory domain in the proregion of mouse and human cathepsins. Silencing *Ctla2a* in SCC cells reduced tumor growth, enhanced CD8+ T cell infiltration, and elevated their antigen-specific cytotoxicity. Importantly, we have found that the conditioned medium collected from SCC cells overexpressing *Ctla2a* is sufficient to blunt the cytokine production of CD8 T cells, suggesting that Ctla2a proteins are secreted from TICs to modulate T cell immunity. Ongoing aims focus on 1) how the peptidase inhibitor activity of Ctla2a reduces cytotoxic cytokine production in CD8+ T cells and 2) whether the I29 inhibitory domain of human cathepsins is capable of conferring the immune-suppressive activity observed in Ctla2a expressing SCCs. Taken together, these results suggest that TICs can secrete these cathepsin inhibitory peptides to perform moonlighting functions and modulate CD8+ T cell anti-tumor cytotoxicity. This interaction may serve as a target for next-generation immunotherapy that is able to blunt TIC-specific immune resistance.

#908 Decoding the role of intrinsic pancreatic cellular signaling in shaping the immunosuppressive tumor microenvironment.

Bushangqing Liu, Ethan Agritelley, Junping Wei, Gangjun Lei, Tao Wang, Jason McBane, Daniel Nussbaum, Zachary Hartman

Duke University School of Medicine, Durham, NC

Background: Pancreatic ductal adenocarcinoma (PDAC) has the highest mortality rate among major cancers, with over 50% of patients having liver metastases at diagnosis. Given that conventional T-cell based immunotherapies have been ineffective against PDAC liver metastases, it is vital to elucidate the mechanisms regulating tumor immunity within the hepatic microenvironment to enhance anti-tumor T cell. Notably, PDAC cells exhibit exceptional plasticity and intrinsic heterogeneity, contributing to the evolution of immunosuppressive primary and early liver PDAC metastases. Our study leverages an array of congenic PDAC clones with varying degrees of immunogenicity to delineate the signaling pathways driving primary and liver immunosuppression.
Methods: We investigate the growth kinetics of PDAC clones both in vitro and in vivo, alongside their transcriptomic variations. Using model antigens (chicken ovalbumin), we assess how different clones modulate the immune microenvironment to elicit antigen-specific adaptive anti-tumor responses. Transcriptomic profiling informs CRISPR-based knockout and overexpression strategies to uncover intrinsic gene contributions to primary anti-tumor immunity in vivo. Additionally, we have developed stable PDAC liver metastasis models through portal vein injection to evaluate each clone's metastatic efficiency and associated immune landscape within the hepatic microenvironment.
Results: In vivo engraftment of PDAC clones revealed differential infiltration of CD4+/CD8+ T cells and neutrophils. While all OVA+ PDAC clones triggered antigen-specific immune responses, only immune-sensitive tumors were eradicated, whereas immune-insensitive tumors persisted. Despite OVA expression, all clones successfully established liver metastases following intraportal injection. RNA-seq analysis identified upregulation of key pancreatic development regulators, including PDX1 and RBPJ, in immune-insensitive clones. CRISPR knockout of these transcription factors in vivo demonstrated altered immune cell composition and reduced tumor growth in immunocompetent mice expressing OVA.
Conclusion: Using diverse PDAC models, we created tumors with immune heterogeneity and altered transcriptional profiles. We identified validated immune-altering cytokines (CXCL1 and CSF3) and immunomodulatory roles of PDX1 and RBPJ. Immune-insensitive clones evaded antigen-specific immunity in both subcutaneous and liver metastasis models, while immune-sensitive clones were eradicated subcutaneously but gained protection in the liver. Ongoing studies confirm the phenotypes of CRISPR-KO clones and delineate the immunomodulatory roles of these transcription factors in liver metastasis.

#909 A new chemokine identified as a key player of tumor immune microenvironment remodeling in β-catenin mutated hepatocellular carcinoma.

Justine Vache[1], Camille Dantzer[2], Isabelle Mahouche[3], Ramazan Akyol[4], Adel Hammoutene[5], Paulette Bouliac-Sage[3], Valerie Paradis[5], Violaine Moreau[3], Clotilde Billottet[1]

[1]Universite de Bordeaux, Inserm U1312 BRIC, Bordeaux, France,[2]University of Stuttgart, Institute of Cell Biology and Immunology, Stuttgart, Germany,[3]Inserm U1312 BRIC, Bordeaux, France,[4]Centre d'Immunologie de Marseille-Luminy, Marseille, France,[5]Hopital Beaujon, Inserm 1149 Research Center on Inflammation, Paris, France

The emergence of immunotherapy has revolutionized the treatment landscape for advanced hepatocellular carcinoma (HCC), significantly enhancing patient survival. However, accumulating evidence indicates that two-thirds of HCC harboring oncogenic mutations in the β-catenin coding gene (*CTNNB1*) exhibit resistance to immunotherapy. These tumors are largely devoid of immune infiltrates, highlighting a critical mechanism of immune escape. To investigate this phenomenon, we developed two 3D liver cancer spheroid models using a β-catenin-wild-type cell line (Huh7) and a β-catenin-mutated cell line (HepG2) co-cultured with PBMC (Peripheral Blood Mononuclear Cells). Flow cytometry analyses revealed that β-catenin mutation significantly impairs immune cell infiltration, particularly by Natural Killer (NK) cells. Our global transcriptomic analysis conducted in HepG2 cell line identified a specific chemokine, referred to here as CXX*, which is downregulated by mutated-β-catenin. In 2D and 3D cultured models, depletion of the mutated β-catenin in HepG2 cells led to an increase in CXX expression and secretion while β-catenin pathway overactivation in Huh7 cells induced its decrease. As CXX is known to recruit NK cells, we investigated the impact of its depletion or overexpression on immune cell infiltration in Huh7 and HepG2 spheroids. Our results demonstrated that CXX indeed plays a key role in the recruitment of NK cells in both models. In silico transcriptomic analysis of HCC patient cohorts corroborated these findings, revealing reduced *CXX* expression and diminished NK cell markers in tumors harboring β-catenin mutations, with these two results being positively correlated. Additionally, we report both a decrease in CXX expression and a reduced infiltration of NK cells in tumor sections from HCC patients with β-catenin mutation. Preliminary data obtained from supernatants of HCC patient Precision-Cut Liver Slices (PCLS) also revealed a reduction of CXX secretion by PCLS obtained from patients bearing the β-catenin mutation. In conclusion, our results identify CXX as a novel negative target of oncogenic β-catenin and provide new insights into how β-catenin mutations reshape the tumor microenvironment of HCC, enabling immune evasion. *A patent is in progress.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#91 Mechanistic insights on the regulation of IL-1β expression by the androgen receptor in prostate cancer cells.

Yetunde Oyende, Jaden Drumm, Anthony DiNatale, Alessandro Fatatis

Pharmacology and Physiology, Drexel University College of Medicine, Philadelphia, PA

Prostate cancer (PCa) in its early stages is driven by the activation of androgen receptor (AR) signaling. Local treatment modalities such as surgery and radiation cure most patients with locally confined or low-grade disease. However, over time, ~20% of patients recur with metastatic tumors and are treated with androgen deprivation therapy (ADT) and AR inhibitors (ARIs) but eventually develop incurable metastatic castration-resistant prostate cancer (mCRPC). A significant fraction of metastatic tumors in PCa harbor cancer cells that lack AR expression (AR^{Neg}). We have previously reported that AR^{Neg} cancer cells express IL-1β, which promotes bone colonization of PCa and other cancers. We have shown that this results from constitutively transcriptional repression by the AR, which binds to a half-ARE on the IL-1β promoter. Thus, we reveal that exposure to ADT and/or ARIs also results in the de-repression of IL-1β transcription in PCa cells that express AR. Here, we extend these studies to other AR variants and show that similarly to AR full-length, AR-V7, a constitutively active AR-variant, also represses the transcription of the IL-1β gene. These findings have high clinical relevance since the current targeting of AR – and possibly of AR-V7, which is detected in ~70% of mCRPC patients, would unleash the pro-metastatic effects of IL-1β. We also present evidence that histone deacetylases directly interact with the AR and act as co-repressors of IL-1β transcription. Furthermore, we found that IL-1β production in PCa cells is independent of inflammasome and caspase 1-8 recruitment. Finally, we show that IL-1β downregulates AR expression and alters EMT markers in PCa cells. Taken together, these findings help delineate a likely scenario of functional interactions occurring in metastatic tumors harboring PCa cells with mixed AR status, either *ab initio* or promoted by AR targeting. We propose that combining ADT/ARIs with inhibitors IL-1β signaling will address both the presence of AR^{Neg} PCa cells and unintended consequences of therapy-induced AR inhibition in the metastatic niche.

IMMUNOLOGY: Tumor-induced Immune Suppression: Intrinsic and Extrinsic Factors
Poster Session

#910 Chromosome 3q amplification in lung squamous cell carcinoma is associated to iron metabolism and immune cell exclusion.

Maria Gutierrez Perez[1], Javier Ramos Paradas[2], David Gomez Sanchez[1], Ricardo Garcia Lujan[3], Jon Zugazagoitia[4], Nuria Carrizo[1], Roxolyana Khoroz[1], Ines Diaz Cano[1], Laura Garcia Redondo[1], Ana B Enguitta[5], Esther Conde[5], Eva M Garrido Martin[6], Alvaro C. Ucero Herreria[7], Luis Paz-Ares[8]

[1]H12O-CNIO Lung Cancer Clinical Research Unit, Health Research Institute Hospital 12 de Octubre (imas12) / Spanish National Cancer Research Centre (CNIO), Madrid, Spain,[2]Yale University, New Haven, CT,[3]Pulmonary Department, 12 de Octubre Hospital, Madrid, Spain,[4]Medical Oncology Department, 12 de Octubre Hospital, Madrid, Spain,[5]Pathology Department, 12 de Octubre Hospital, Madrid, Spain,[6]Cell Biology Department, Universidad Autonoma de Madrid, Madrid, Spain,[7]Departments of Medicine and Physiology, Complutense University of Madrid (UCM) / Health Research Institute Hospital 12 de Octubre (imas12) / Spanish National Cancer Research Centre (CNIO), Madrid, Spain,[8]Medical Oncology Department, 12 de Octubre Hospital / Health Research Institute Hospital 12 de Octubre (imas12) / Spanish National Cancer Research Center (CNIO) / Complutense University of Madrid (UCM), Madrid, Spain

Introduction and purpose:
Lung squamous cell carcinoma (LUSC) is an aggressive tumor type accounting for 30% of all lung cancers, with a roughly 30% 5-year survival. The genetic nature of these tumors precludes the use of targeted therapies and the standard immunotherapy, either alone or in combination with chemotherapy, results in response rates below 50% after biomarker selection. Better patient selection for immunotherapy regimens requires a better definition of the molecular and immune landscape of LUSC. The purpose of this study is to comprehensively characterize the molecular, clinical and immune context of LUSC to identify novel biomarkers that can guide treatment decisions and to find novel therapeutic targets.
Experimental procedures:
A multiparametric study including a targeted DNA and RNA sequencing of genes related to tumor-immune system crosstalk was conducted in a cohort of 98 early-stage LUSC patients. Histopathological features including immune infiltrate and metabolic traits were defined by in the LUSC tissues. Consensus clustering algorithm classified the sample transcriptomes, and all this data was cross-analyzed with clinical, molecular and immune annotations.
Results:
Computational analysis of immuno-oncology gene expression data from 98 LUSC tumors led to the classification in 4 patient subgroups with specific molecular and immune characteristics. Interestingly, we found a genomic amplification of the chromosome region 3q, present in almost 30% of the patients, strongly associated with the low immunogenic, as previously shown, and high tumorigenic subgroup. To know the mechanism behind this robust association and given the nature of relevant genes in this region, we analyzed iron accumulation in the tumor samples. We found that iron-positive tumors showed a reduced infiltration of $CD8^+$ T cells compared to iron-negative tumours. The same tendency was observed in CD4, CD20 and CD68 analyses, further supporting an association of this amplification and iron accumulation with a low immunogenic tumor profile. These results were validated in an independent cohort of early-stage LUSC.
Conclusions:
Taken together, our results support the possible connection between iron accumulation and 3q amplification as a mechanism for immune exclusion in LUSC. We believe that this molecular alteration can be clinically relevant as a biomarker for immunotherapy selection in LUSC, at least in a neoadjuvant setting. Whether this association applies to other squamous carcinomas or might represent novel therapeutic vulnerabilities ensures further research

#911 Cancer stem cells establish intrinsic immune resistance through suppressing IFN-mediated antitumor immunity.
Xuejie Huang, Weijie Guo, Yuxuan Phoenix Miao

University of Chicago, Chicago, IL

Although the cancer immunotherapy has generated promising outcomes in the clinics, a substantial proportion of cancer patients either show resistance to immunotherapy or experience relapse following an initial response. This issue is particularly urgent for patients with squamous cell carcinomas (SCCs), highlighting the critical need to understand the mechanisms behind immune evasion and immunotherapy resistance in these cancers. Recently, our lab identified a subset of tumor-initiating stem cells (tSCs) in skin and head/neck SCCs that respond to transforming growth factor β (TGF-β) and play a key role in driving tumor relapse post-immunotherapy. Notably, these tSCs are resistant to T-cell attacks despite retaining functional antigen presentation, resulting in only transient responses to immune checkpoint blockade therapy across SCC types. Here, my current study has uncovered a central role for Sox2 as a master regulator that drives immune resistance in TGF-β-responsive tSCs. Through RNA-seq and qPCR analyses, we observed that Sox2 amplification in tSCs significantly blunted their interferon-γ (IFN-γ) responsive potentials. Mechanistically, we found that Sox2 neither blocks IFN-γ signaling transduction nor directly binds to the enhancers or promoters of most IFN-γ-induced genes. Instead, we discovered that the reduced IFN-γ response in Sox2-overexpressing cells is likely due to the altered Stat1-mediated transcriptional repression. This study uncovered a stem cell specific transcription regulation that provides new insights in developing new strategies to abolish tSC-specific immune resistance.

#914 Differential expression of Cbl-b in tumor infiltrating immune cells in patients with solid tumors.

Elli Narvi[1], Reetta Virtakoivu[1], Endrit Elbasani[1], Nelson Kibinge[2], Laura Ravanti[2], **Anil K. Thotakura**[1]

[1]Immuno-Oncology, Orion Corporation, Turku, Finland,[2]Orion Corporation, Turku, Finland

Introduction: To mechanistically elucidate the differences in the cancer patient clinical response, several pre-clinical and clinical biomarker studies have suggested the existence of several novel intracellular immune checkpoints. Casitas B cell lymphoma (Cbl-b) is an intracellular immune checkpoint protein that impedes the T cell and NK cell function.

Methods: Immunohistochemistry (IHC) analysis was performed on the tissue micro array (TMA) from five different cancer indications (n=25) and healthy tissue controls (n=5). TMAs were stained utilizing commercially available IHC antibodies for Cbl-b, CD8, CD56 and FoxP3, nuclei were stained with DAPI. For in vitro studies, T cells were activated with anti-CD3/CD28 and NK cells with recombinant human IL-2 and IL-15. SiRNA mediated Cbl-b silencing in primary T and NK cells was also confirmed by western blot analysis and the effects of Cbl-b silencing were evaluated by measuring IFN-γ, IL-2, TNF-α and Granzyme-B accumulation by ELISA. For single-cell analysis, we leveraged publicly available Gene Expression Omnibus (GEO) data to perform an in-depth single-cell analysis of *CBLB* expression in different immune cell-types. This workflow included quality control, normalization, dimensionality reduction, clustering and cell type annotation

Results: Compared to healthy tissue controls, tumors have high number of Cbl-b positive immune cells. The proportion of Cbl-b positives CD8 T cells and CD56 NK cells varied between indications. Interestingly, $FoxP3^+$ regulatory T (Treg) cells have low levels of Cbl-b. The IHC observations was complemented by scRNA sequencing data. Consistent with the published data, Cbl-b silenced immune cells (T and NK cells) showed rapid activation and cytokine production as assed by a significant increase in IFN-γ, IL-2, TNF-α and Granzyme-B levels. Furthermore, Cbl-b silenced T cells are dependent on TCR alone and become independent of CD28 co-stimulation. Interestingly, the basal level of Cbl-b expression is high on NK cells compared to T cells, but upon TCR activation Cbl-b expression in T cells was rapidly increased while in NK cells IL-2 and IL-15 cytokine activation did not alter the CBL-B expression as assessed by western blot.

Conclusion: Previous studies have established the role of Cbl-b in suppressing T cell and NK cells function, however, differential expression of Cbl-b and Cbl-b expression kinetics in different immune cell type remained unknown. Using IHC method and scRNA sequencing, we found that the Cbl-b expression is high in CD8 and NK cells, and low in Treg cells. Furthermore, in vitro Cbl-b silencing and Cbl-b expression in TMA suggest that silencing or inhibiting Cbl-b in T cells and NK cell is a valid approach.

#916 Tumor extracellular vesicles trigger a dendritic cell stress response to promote immune evasion and immunotherapy resistance.

Mandy Wang, Michael Plebanek, Kaylee Villarreal, Nicholas C. DeVito, Bala Theivanthiran, **Brent A. Hanks**

Medicine, Pharmacology and Cancer Biology, Duke University Medical Center, Chapel Hill, NC

Previous studies have demonstrated dendritic cell (DC)-mediated T cell activation to be impaired within the tumor microenvironment (TME). This has included studies showing that activation of XBP1 within the unfolded protein response (UPR) pathway triggers lipid accumulation by DCs within the TME, however the signal mediating XBP1 activation has remained unclear. We have previously demonstrated that fatty acid oxidation (FAO) as well as the activation of the SREBP2-dependent mevalonate biosynthetic pathway leads to the development of mregDCs in the TME, exhibiting impaired antigen cross-presentation and an enhanced capacity to drive regulatory T cell (Treg) differentiation. Despite the critical role of the DC in orchestrating anti-tumor immunity, there are currently no therapeutic strategies to augment their function. Using an autochthonous model of $BRAF^{V600E}$ melanoma, we demonstrate that tumor-dependent generation of extra-cellular vesicles (ECVs) suppresses anti-tumor immunity and supports tumor progression. Using bulk and single-cell RNA sequencing analysis, we show that tumor ECVs upregulate various components of the UPR pathway in conventional DCs (cDCs), including IRE1alpha-XBP1 and SREBP2 activation based on qPCR and Western blot studies. We further show that XBP1 activation leads to PPARalpha nuclear translocation and enhanced FAO. We verify that lymph node DC populations engulfing labeled tumor ECVs harboring a CD81-GFP fusion protein also exhibit both enhanced XBP1/PPARalpha activation as well as the transcriptional signature of mregDCs. Together, these ECV-dependent effects suppress DC-mediated antigen cross-presentation and $CD8^+$ T cell activation while promoting DC-dependent Treg differentiation *in vitro* and *in vivo*. Additional studies were conducted showing similar effects generated by ECVs derived from models of colon and breast cancer. We subsequently found DC-specific knock-out of PPARalpha to partially reverse the effects of tumor ECVs on DC functionality in an engineered transgenic murine model. Indeed, a PPARalpha antagonist, TPST-1120, overcomes resistance to anti-PD-1 immunotherapy in a poorly immunogenic autochthonous melanoma model while suppressing DC lipid stores and DC FAO *in situ*. Together, this work suggests that pharmacologic targeting of pathways elicited by tumor-derived ECVs can reverse DC tolerization in the TME and overcome immunotherapy resistance in select tumors. Understanding which tumors rely on ECVs to suppress anti-tumor immunity will be a critical step in prospectively developing targeted therapeutics to block these immune evasive pathways and enhance the efficacy of checkpoint inhibitor immunotherapy.

#918 Tumor-induced interleukin 16 (IL-16) is associated with suppression of antitumor functions of NK cells in ovarian cancer.

Animesh Barua[1], James T. O'Donnell[2], Dale B. Hales[3], Janice M. Bahr[4]

[1]Oncology, Simmaron Research Inc, Milwaukee, WI,[2]Pharmaconsultant Inc, Barrington, IL,[3]Physiology, Southern Illinois University, Carbondale, IL,[4]Animal Science, University of Illinois at Urbana Champaign, Urbana-Champaign, IL

Background: Natural Killer (NK) cells kill tumor cells directly and provide first line of defense against the development and progression of ovarian cancer (OVCA). OVCA progresses mainly through local diffusion by suppression of NK cells. Factors and mechanism(s) involved in tumor-induced NK suppression are unknown. Such information is critical to develop NK-targeted therapies and OVCA prevention as current immunotherapies including T-cell targeted ones show poor efficacies against OVCA. Ovarian and fimbrial tissues are persistently exposed to inflammation/stress due to ovulation or infection and accompanied with high expression of interleukin 16 (IL-16), a proinflammatory cytokine. IL-16 is suggested to be associated with malignant transformation and secretion of IL-15 by ovarian tumors. IL-16 may play a role in suppression of NK cells by exhausting them through persistent stimulation by IL-15, a stimulator of NK cells. The goal of this study was to examine if tumor-induced IL-16 is associated with suppression of NK cell functions during OVCA development and progression and if so, to determine the mechanism of IL-16-induced suppression of NK cell functions.
Materials and Methods: Association of ovarian/fimbrial tumor-induced IL-16 expression with the suppression of anti-tumor functions of NK cells was examined in an exploratory study using laying hen model of spontaneous OVCA. Fimbrial and ovarian tissues from normal hens (n=10, 3-4 years old), hens with early (n = 8) and late stage OVCA (n = 10) were examined. Tissue expression of IL-16, GRP78 (glucose regulated protein 78, a marker of cellular stress), NK cells (CD56+), IL-10/IL-15 and CISH (a marker of NK cell exhaustion) were examined. Changes in the frequencies of immunopositive cells or intensities of their expression and association among them during OVCA development were determined. Data were analyzed by ANOVA and t-test and significance was taken when $P<0.05$.
Results: Frequency of IL-16-expressing cells and intensities of GRP78 expression were significantly ($P<0.001$) increased during OVCA development. Frequency of NK cells in tumor stroma was significantly higher in hens with early and late stage OVCA than normal hens ($P<0.001$). IL-16 expression was positively correlated with IL-15 expression. However, frequency of intratumoral NK cells was not increased significantly in late stage than early stage OVCA suggesting that OVCA progresses with reduction in intratumoral influx of NK cells. It is possible that consistent exposure of NK cells to persistent high levels of tumor-induced IL-15 might be a reason.
Conclusion: The results of this study suggest that enhanced IL-16 expression during malignant transformation may inhibit intra-tumoral influx of NK cells. Thus, tumor-induced IL-16 offers a potential target for immunotherapy to enhance anti-tumor functions of NK cells.
Support: Ovarian Cancer SPORE, Roswell Park.

#919 Regulation of PS externalization on tumor cells by TMEM16F and P2X7 receptors.

Rachael Pulica[1], Varsha Gadiyar[1], David C. Calianese[2], Mariana De Lorenzo[3], Raymond B. Birge[1]

[1]Microbiology, Biochemistry, and Molecular Genetics, Rutgers Graduate School of Biomedical Sciences, Newark, NJ,[2]Osaka University, Suita, Osaka, Japan,[3]Cell Biology and Molecular Medicine, Rutgers Graduate School of Biomedical Sciences, Newark, NJ

Immune evasion in the tumor microenvironment (TME) is critical for tumor progression, and many therapeutic strategies are under development to activate host anti-tumor immunity. Tumors avoid immune detection and clearance through several mechanisms, one of which is the chronic externalization of the phospholipid phosphatidylserine (PS) to the outer leaflet of the plasma membrane. Externalized PS on tumor cells alters the tumor microenvironment to an immunosuppressive phenotype. Immune cells expressing PS-binding TAM (Tyro3, Axl, MerTK) receptors interact with the tumor-exposed PS and release immunosuppressive cytokines, leading to the polarization of the TME to pro-tumorigenic. While it is understood how PS is externalized by calcium-induced scramblase TMEM16F in non-pathological conditions and caspase-cleaved scramblase Xkr8 in apoptotic conditions, the mechanism through which the constitutive externalization of PS on tumor cells occurs is still unknown. We hypothesize that the increased concentration of calcium in tumor cells activates TMEM16F to externalize PS, and the increased levels of extracellular ATP bind P2X7 receptors (P2X7R), further leading to PS externalization in the TME.

To better understand the mechanisms of PS exposure under oncogenic stress, we have developed both TMEM16F and P2X7R CRISPR knockout EO771 breast cancer cell lines. These cells are unable to externalize PS when subjected to their respective triggers (calcium or ATP). They have additionally been shown to be unable to upregulate immune-suppressive MerTK signaling upon exposure to their PS-externalizing triggers, whereas wild-type EO771 cells show large upregulation in MerTK binding upon treatment. Orthotopic injection into the mammary fat pad of C57/Bl6 mice yielded a reduction in tumor size in TMEM16F knockout tumors compared to the wild-type EO771 tumors (P2X7R knockout tumor experiments are ongoing). Based on these studies, we show that both calcium-induced TMEM16F and ATP-binding P2X7R contribute to the externalization of PS and immune suppression in the TME, resulting in tumor growth and progression. Our studies add new insights to how PS acts as an immunosuppressive signal in the tumor microenvironment.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#92 The role of AKT3 in prostate cancer.

Dineen Vogler[1], Seamus Degan[2], Henry Withers[1], Irwin Gelman[1]

[1]Roswell Park Comprehensive Cancer Center, Buffalo, NY,[2]University of North Carolina at Chapel Hill, Chapel Hill, NC

The five-year survival rate for metastatic prostate cancer (mPC) patients remains significantly lower than that of patients with primary prostate cancer (PC). The loss of PTEN, a lipid phosphatase that negatively regulates PI3K/AKT pathway, is one of the most frequent mutations in prostate cancer. Although AKT inhibitors seem promising for mCRPC patients, they often perform poorly in the clinic as monotherapies. We hypothesize that this poor response to AKT inhibitors may be due to a failure to account for the distinct and opposing roles of AKT isoforms. Previously published data from our lab suggests that AKT2 and possibly AKT3, are the mediators of PC progression while AKT1 is responsible for PC development. To further investigate the mechanisms underlying AKT isoform plasticity in the context of PTEN loss, we conducted a phosphoproteomics analysis that identified *Akt* isoform-specific substrates using T402 (mouse prostate epithelial cells with $PTEN^{-/-}/Rb^{-/-}$) and T402[PTEN] (T402 cells with PTEN re-expression). We identified both canonical (RXRXXS/T*P) and novel non-canonical phosphorylation motifs. Interestingly, more substrates were associated with *Akt3* compared to *Akt1 and Akt2*, indicating that *Akt3* may be an underappreciated driver of PC progression. Furthermore, *Akt3* was found to preferentially phosphorylate substrates with non-canonical motifs. However, the direct mechanisms through which AKT3 and its substrates contribute to PC progression and metastasis remain unclear. From our phosphoproteomics analysis, we identified $PAK4^{S181}$ as a potential *Akt3* direct substrate. Knockdown of PAK4 in PTEN deficient PC cells resulted in a greater reduction in chemotaxis compared to PTEN intact PC cells. We hypothesize that AKT3 directly phosphorylates $PAK4^{S181}$ to promote PC metastatic progression. These studies will provide a functional characterization of the downstream activity of AKT3, through substrate usage, to aid in the development of an AKT3 specific inhibitor. This research highlights the importance of discovering biomarkers to unveil a more targeted approach toward treating advanced stage prostate cancer patients.

IMMUNOLOGY: Tumor-induced Immune Suppression: Intrinsic and Extrinsic Factors
Poster Session

#920 Determining the role of Siglecs in macrophage mediated immune evasion in prostate cancer.

Nathan S. Lam, **Ru M. Wen**, James D. Brooks

Urology, Stanford University School of Medicine, Stanford, CA

Siglecs (sialic acid-binding immunoglobulin-like lectins) are a family of cell surface proteins that are mainly expressed in immune cells. Recent studies show that Siglecs play important roles in immune evasion for tumors. We previously reported that Siglec-7 and Siglec-9 contribute to immune evasion by interacting with their ligands in prostate cancer, and blocking these interactions inhibits prostate cancer tumor progression. Further, Siglec-7 and Siglec-9 are highly expressed in macrophages in metastatic human prostate tumors. However, how macrophages mediate immune evasion via Siglec-dependent pathways remains unknown. Here, we have found that Siglec-7 and Siglec-9 are expressed in monocyte-differentiated macrophages derived from peripheral blood mononuclear (PBMC) cells. We will further profile Siglec expression in macrophages derived from PBMC and tumor tissues from patients with prostate cancer. The Siglecs on macrophages will be modulated, and their function will be evaluated through in vitro and in vivo models. Our study will provide insight into understanding macrophage-mediated immune evasion through Siglec-dependent mechanisms.

#921 SOX11-mediated antigen presentation suppression: A mechanism of immune evasion in triple-negative breast cancer.

Salomat Abdulkhuseynova[1], Ashok Pullikuth[2], Lance Miller[2], Shay Soker[1]

[1]Department of Cancer Biology and Wake Forest Institute for Regenerative Medicine, Wake Forest University School of Medicine, Winston-Salem, NC,[2]Department of Cancer Biology, Wake Forest University School of Medicine, Winston-Salem, NC

Background: Triple-negative breast cancer (TNBC) is an aggressive subtype with no targeted therapies and limited response to immunotherapy. TNBC evades immune surveillance by repressing antigen presentation machinery (APM) genes, reducing MHC Class I expression. Using bioinformatics, we identified SOX11 as a potential transcriptional regulator associated with APM suppression and immune evasion in TNBC.

Methods: Gene expression data from basal breast cancer (BRCA) tumors (n = 189) in TCGA and cancer cell lines (n = 1,189) in DepMap were analyzed. Spearman correlation coefficients were used to evaluate associations between a T-cell infiltration signature (e.g., CD3, CD8) and an APM signature (e.g., TAP1, TAP2, B2M, HLA-ABC). SOX11 was prioritized as it was strongly negatively correlated with both signatures. Differential expression analysis of SOX11-overexpression (OE) and knockdown (KD) datasets confirmed potential downstream targets, including MEX3A. To validate, SOX11 KD was performed in CAL-148 cells (high level of endogenous SOX11) using siRNA for 96 hours. Western blot assessed SOX11 and MEX3A protein levels, while flow cytometry measured surface MHC Class I (HLA-ABC) expression.

Results: SOX11 was strongly inversely correlated (rho < -0.4, adjusted $p < 0.01$) with APM and T-cell infiltration signatures in basal BRCA and DepMap datasets. In basal BRCA tumors, SOX11 expression was enriched in T-cell cold tumors compared to T-cell hot tumors ($p < 0.01$). Reanalysis of SOX11-modulated datasets (KD and OE) confirmed APM-suppressive downstream targets, with MEX3A showing a strong co-expression pattern (Spearman $p<0.05$). SOX11 KD in CAL-148 cells significantly reduced MEX3A protein levels and increased surface HLA-ABC expression. These findings suggest that SOX11 represses antigen presentation pathways, contributing to immune evasion in TNBC.

Conclusions: SOX11 suppresses HLA-A/B/C expression and may contribute to immune evasion in TNBC. Its high expression in T-cell cold tumors and its regulation of APM targets, including MEX3A, highlight its potential as a therapeutic target. Targeting SOX11 could restore antigen presentation, promote immune recognition, and enhance immunotherapy responses in TNBC.

#922 Mutant KRAS enhances immune evasion via epitranscriptomic regulation of NT5E in colon cancer.

Seungjae Shin, Yoojin Yang, Sung-yup Cho

Seoul National University, Jongno-gu, Korea, Republic of

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related deaths worldwide. Mutation in KRAS occurred in approximately 40% of patients, and leading to resistance to FDA approved anti-EGFR agents. Also, CRC patients harboring KRAS mutations do not respond well to immune checkpoint blockades. Therefore, additional therapeutic strategies to overcome KRAS mutations are strongly required. In recent years, N6-methyladenosine (m6A) modification on mRNA has been recognized for its significance in various cancer phenotype. This modification plays a crucial role not only in cancer cell behavior but also in shaping the tumor microenvironment (TME). Many studies about m6A in cancer focus on the direct roles and effects of m6A regulators. These regulators include writers, which attach m6A to RNA; erasers, which remove it; and readers, which bind to m6A to regulate the fate of mRNA. However, how oncogenic mutations affect the landscape of m6A deposition remains poorly understood. To explore the impact of KRAS mutations on m6A modifications, we performed MeRIP-seq and discovered significant changes, particularly in NT5E/CD73, a molecule known for its immune suppressive role in the TME. Our result suggest the potential for developing novel therapies by modulating the KRAS-m6A-NT5E axis in KRAS-mutated colorectal cancer.

#923 The role of NNMT in anti-tumor immunity in LKB1 deficient NSCLCs.

Thi Na Nguyen, Wenjing Na, Pawel Nowialis, Thi Kim Ngan Bui, Kunyang Li, Ryan Clay, Lina Song, Lingtao Jin

Molecular Medicine, UT Health Science Center at San Antonio, San Antonio, TX

Lung cancer is the leading cause of cancer-related deaths with non-small cell lung cancer (NSCLC)accounting for 85% of all lung cancers. Loss or inactivating mutations of serine/threonine kinase STK11 encoding the LKB1 protein occurs in approximately 20% of lung adenocarcinoma (LUAD). Although treatment of NSCLCs with immune checkpoint inhibitors (ICI) has responded in overall survival in a subset of patients, studies have revealed that co-occurring KRAS/LKB1 mutations promote primary resistance to ICIs in NSCLCs, the mechanism of which is still not completely understood. Nicotinamide N-methyltransferase (NNMT) is a metabolic enzyme that catalyzes the methylation of nicotinamide (Nam) using the co-factor S-adenosyl-L-methionine to form 1-methyl-nicotinamide (1-MNA). In this study, we discovered that levels of NNMT and its product 1-MNA are elevated inNSCLCs with loss of function of LKB1. Mechanistically, we found that LKB1-SIK1/2/3 axisdirectly suppresses NNMT expression transcriptionally. Therefore, loss of function of LKB1 leadsto upregulation of NNMT and its products 1-MNA. Interestingly, knockout of NNMT or 1-MNA treatment did not impact cell proliferation or survival in NSCLC cells with or without functionalLKB1. Global metabolomic and transcriptomic studies revealed that 1-MNA promotes FOXP3induction and regulatory T cell (Treg) expansion, consequently leading to reduced CD8 T cellfunction both in vitro and in vivo. Knockout of NNMT in KPL cells ($Kras^{G12D}$Tp53/LKB1 KO)enhanced anti-tumor immune response and promoted ICI efficacy by suppressing Tregaccumulation in vivo. Importantly, NNMT inhibitor improves the therapeutic efficacy of anti-PD-1antibody in KPL model in vivo. The present study revealed that NNMT/1-MNA signaling plays animportant role in regulating Treg function in LKB1 deficient NSCLC. Targeting NNMT with smallmolecular inhibitor may hold promise to improve the efficacy of ICIs for LKB1 deficient NSCLCs.

#924 Tumor-initiating stem cells fine-tune the plasticity of neutrophils to sculpt a protective niche.

Weijie Guo, Jingyun Luan, Yuxuan Miao

University of Chicago, Chicago, IL

The abundant accumulation of neutrophils in various solid cancers has been well recognized, but the functions of tumor-associated neutrophils (TANs) remain controversial. TANs have long been believed to be immune suppressive and have thus been referred to as “myeloid-derived suppressor cells”. However, effective tumor control induced by immunotherapy was recently found to be associated with strong neutrophil signatures. These seemingly contradictory findings highlight the unexpected degree of plasticity and heterogeneity unique to TANs. How the cellular plasticity and functional heterogeneity of TANs are regulated remains unknown. Here, we show that, while anti-PDL1/CD40 agonist immunotherapy can induce interferon responses to reprogram many TANs, allowing them to regain anti-tumor activities in squamous cell carcinomas, a subset of TANs residing at the tumor-stroma interface can preserve their immune suppressive state. Importantly, we identified a group of $Sox2^{Hi}$ tumor-initiating stem cells (tSCs) at the tumor-stroma interface that could upregulate Fatty Acid Desaturase 1 (Fads1) to produce arachidonic acid. This tSC-specific pathway enhances the autocrine prostaglandin E_2 (PGE_2) signaling in TANs, which can disrupt their interferon responsive potentials, preventing the interferon-mediated reprogramming. Thus, by fine-tuning the plasticity of neutrophils, tSCs shape neutrophil heterogeneity, and sculpt a protective micro-niche to survive from immunotherapy and drive cancer relapse.

#925 Golgi-derived extracellular vesicles lack conventional tetraspanins and mediate immune evasion in cancer.

Ikjot Singh Sohal, Sydney N. Shaw, Lauren N. Meeks, Andrea L. Kasinski

Biological Sciences, Purdue University, West Lafayette, IN

Immunotherapy has significantly improved the survival of patients with various malignancies. However, immunotherapies have largely remained ineffective in tumors that have reduced infiltration of T cells, also called immune-cold tumors. Despite an extensive understanding of factors that mediate poor T cell infiltration, current therapies that target these factors have shown modest clinical benefit, indicating that other factors contribute to this process. One such factor that has been shown to contribute to immunosuppression are extracellular vesicles (EVs). However, due to significant EV heterogeneity, discovery of specific EV subpopulations that are responsible for these immunosuppressive effects has remained a challenge. Overcoming this challenge, we first established relevant lung cancer models to study EV-mediated T cell suppression and then identified a previously uncharacterized population of lung cancer-derived EVs that strongly inhibit the proliferation and activation of T cells. We further identified that this novel T cell-suppressive EV subpopulation originates from the *trans*-Golgi network and expresses the classic *trans*-Golgi marker protein, TGN46. In addition, the TGN46+ EV subpopulation lacks conventional EV markers CD81 and CD63. In the immunosuppressive lung cancer model, while total EVs suppress T cells, specifically removing the TGN46+ EV subpopulation ameliorated this effect. Conversely, overexpressing TGN46 in a lung cancer model that had no EV-mediated effect on T cells was sufficient to increase TGN46+ EV release and the ability of the EVs to suppress T cells, underscoring the physiological significance of the TGN46+ EV subpopulation. Analysis of patient data from The Cancer Genome Atlas (TCGA) indicates that high TGN46 expression (both transcript and protein) correlates with worse progression-free survival in lung cancer and overall survival in other cancer types - glioblastoma, lower grade glioma, testicular cancer, and large B-cell lymphoma. Most importantly, tumor-immune association analysis of TCGA data suggests that high tumor TGN46 expression significantly correlates with an immunologically-cold tumor signature, i.e., low infiltration of CD8+ T cells and activated NK cells, and high infiltration of M2 macrophages, across several cancer types, stressing the clinical relevance of our findings. The data elucidates that TGN46+ EVs are a novel factor that potentially contributes to immune-cold tumor microenvironment. How TGN46+ EVs suppress T cells mechanistically and their role in overall tumor microenvironment modulation are currently under investigation, which may inform strategies to enhance immunotherapy effectiveness in immune-cold tumors.

#926 Myeloperoxidase limits tumor-associated neutrophils mediated immunosuppression in pancreatic cancer.

Angisha Basnet, Kaitlyn M. Landreth, Tracy W. Liu

Microbiology, Immunology and Cell Biology, West Virginia University. School of Medicine, Morgantown, WV

Pancreatic ductal adenocarcinoma (PDAC) is a deadly disease with a survival rate under 13%. Treatments like immunotherapy and chemotherapy are ineffective in PDAC due to the immunosuppressive tumor microenvironment, in which tumor-associated neutrophils (TANs) are a major component. The immunosuppressive function of TANs has been shown to depend upon increased production of reactive oxygen species, with myeloperoxidase being a major source of it. We investigated the contribution of myeloperoxidase in mediating tumor-associated neutrophils immunosuppression using orthotopic PDAC models in wild-type and syngeneic myeloperoxidase-deficient mice. We observed a significant delay in tumor growth and improved clinical scores when myeloperoxidase was deficient. Additionally, treatment with the myeloperoxidase-specific inhibitor verdiperstat delayed tumor growth. Flow cytometry analysis of the tumor microenvironment revealed a significant increase in CD8+ T cells and natural killer (NK) cells in MPO-deficient mice, accompanied by elevated granzyme B levels in both cell subsets. Additionally, MPO deficiency was associated with increased expression of activation and memory markers among both CD8+ and CD4+ T cells. *Ex vivo* studies with isolated TANs from tumor-bearing mice showed reduced CD8+ T cell and NK cell suppression, and reactive oxygen species level in the absence of myeloperoxidase. These data suggests that loss of myeloperoxidase in TANs results in decreased immunosuppressive functions and increased cytotoxic and activated T cells and NK cells within the tumor microenvironment. Depletion of NK cells in myeloperoxidase-deficient mice abolished the antitumor effects observed with myeloperoxidase loss, underscoring the critical role of NK cells as key mediators of the antitumor response in the absence of myeloperoxidase. Additionally, we observed an enhanced chemotherapy (Gemcitabine) and immunotherapy (anti-PD1 and ant-CTLA4) response with myeloperoxidase deficiency. Overall, our results demonstrate the contribution of myeloperoxidase in regulating PDAC tumor progression, TAN immunosuppression, treatment response and immune cell infiltration using an orthotopic PDAC model.

#928 Advancing CAR-Ts and anti-immunosuppressive therapy development: Immunosuppressive TME 3D co-culture platform grounded on a fully characterized PDX tumor bank.

Mara Gilardi, Veena Jagannathan, Brandon Walling, BanuPriya Sridharan, Stefano Cairo, Marianna Zipeto, Michael Ritchie

Champions Oncology, Inc., Hackensack, NJ

Solid tumors represent a significant challenge in oncology due to their complex and highly immunosuppressive tumor microenvironment (TME). This hostile environment, shaped by immunosuppressive cytokines, and immune evasion mechanisms, limits the efficacy of immune-based therapies, including checkpoint inhibitors, chimeric antigen receptor (CAR) T cells, and bispecific antibodies. Although therapeutic strategies have been developed to overcome immune suppression, accurate preclinical models with predictive value remain critically needed. To address this, Champions Oncology has developed a robust ex-vivo 3D co-culture platform based on its extensive patient-derived xenograft (PDX) tumor bank, comprising over 1,500 fully characterized models. These models are annotated with comprehensive multi-omic data, including RNA sequencing, proteomic, and phospho-proteomic profiles, enabling the selection of tumor models aligned with specific indications, clinical history, and target expression, mimicking patient cohorts. The platform uses rigorously characterized organoids derived from this tumor bank as its core ensuring fidelity to the original tumor. Developing the ex-vivo immunosuppressive co-culture system requires careful integration of relevant cellular players, including human cancer-associated fibroblasts (CAFs), infiltrating immune cells such as M2-polarized macrophages, and tumor cells from 3D PDX organoids. Co-culture of these cell types creates a biologically accurate representation of the human TME. Here we describe a novel complex co-culture platform, with fully optimized conditions enabling the differentiation of cellular components and functional co-cultures. This system was used to test the potency of diverse CAR-T therapies engineered to overcome immune suppression. A custom high-throughput flow cytometry panel was developed to interrogate the system, measuring both cancer cell killing and immune activation. The co-culture system proved ideal for evaluating CAR-T cancer-killing capacity and understanding mechanisms of immunosuppression-mediated therapeutic resistance. Additionally, the platform supports testing combination therapies, such as checkpoint inhibitors paired with TME reprogramming agents, to combat resistance mechanisms. In conclusion, this ex-vivo co-culture platform represents a transformative tool in preclinical immuno-oncology. Combining a deeply characterized tumor bank with a robust organoid-based system enables testing anti-immunosuppressive therapies in a clinically relevant setting. This innovation promises to accelerate the development of next-generation therapies, offering unparalleled insights into tumor biology and immune dynamics to improve outcomes for patients with immunosuppressive tumors.

#929 Altered lipid metabolism drives immunotherapy resistance via an EGFR/CERS4/PD-L1/PGE2 signaling axis in solid tumors.

Wyatt O. Wofford, Han Gyul Lee, Bryan Granger, Elif Percin, Mohamed Faisal Kassir, Lucy Mulligan, Odai Darawshi, Paramita Chakarborty, Salih Gencer, Natalia Oleinik, Stefano Berto, Shikhar Mehrotra, Besim Ogretmen

The Medical University of South Carolina, Charleston, SC

Immunotherapy has transformed cancer treatment, yet durable responses to immune checkpoint blockade (ICB) are limited to a subset of patients. Identifying tumor-intrinsic resistance mechanisms is critical to improving ICB efficacy and outcomes. We recently identified crosstalk between lipid metabolism via the downregulation of ceramide synthase 4 (CerS4) and internalized programmed death-ligand 1 (PD-L1) signaling, which promotes metastasis and impairs ICB response. Our data also demonstrated that αPD-L1 therapy enhanced internalized PD-L1 signaling, suggesting a role in adaptive ICB resistance. To investigate the underlying mechanism, we established an E0771-allograft-based triple-negative breast cancer (TNBC) model of ICB resistance, E0771-2RA, through serial αPD-L1 treatment and subculturing of resistant tumors. The 2RA model is resistant to both αPD-L1 and αPD-1 therapy *in-vivo*, with reduced CerS4/ceramide levels and increased PD-L1 internalization, consistent with our prior findings. Whole transcriptomic RNA sequencing of E0771 and 2RA tumors revealed enrichment in epidermal growth factor receptor (EGFR) and prostaglandin E2 (PGE2) signaling in 2RA tumors. Mechanistically, we found that (1) EGFR signaling negatively regulates CerS4 expression; (2) CerS4 suppression enhances PD-L1 internalization and subsequent association with Caprin-1; and (3) the intracellular PD-L1-Caprin-1 complex stabilizes prostaglandin-endoperoxide synthase 2 (Ptgs2, Cox2) mRNA to promote PGE2 production. Immune profiling of E0771 and 2RA tumors using flow cytometry and single-nuclei RNA sequencing revealed M2 macrophage polarization, PMN-MDSC recruitment, and T cell dysfunction in 2RA tumors. However, pharmacological targeting of PGE2 signaling and exogenous CerS4/ceramide restoration in ICB-resistant 2RA tumors primarily alleviated T cell dysfunction, suggesting the T cell compartment is the primary target of this signaling axis. Single-nuclei RNA sequencing and CellChat analyses revealed abundant TGF-β signaling in 2RA tumors, which we found to regulate PD-L1 internalization *in-vitro,* presenting a possible therapeutic vulnerability. Thus, to target this mechanism, we combined αPD-1 therapy with TGF-β inhibition *in-vivo*, which restored ICB sensitivity and prolonged survival. Using TCGA and human ICB resistance datasets, we validated the link between EGFR, CerS4, PD-L1, and PGE2 signaling in TNBC, head and neck squamous cell carcinoma (HNSCC), and melanoma. Based on our mechanistic and sequencing data, we additionally curated a gene signature to predict ICB response vs. resistance in solid tumors. Our findings uncover a novel mechanistic link between altered CerS4-dependent lipid metabolism, EGFR, PD-L1, and PGE2 signaling and introduce mechanism-based therapeutic strategies for overcoming ICB resistance in solid tumors.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#93 Alternative splicing reveals epigenetic subtypes in gastric cancer peritoneal metastasis.

Joseph J. Zhao[1], Xiaonan Fan[2], Jia Jia Chan[2], Daryl Kai Ann Chia[3], Robert Walsh[1], Haoran Ma[4], Wei Peng Yong[5], Jimmy So[6], Patrick Tan[4], Yvonne Tay[2], Raghav Sundar[7]

[1]Department of Haematology-Oncology, National University Cancer Institute, Singapore, Singapore,[2]Cancer Science Institute, Singapore, Singapore,[3]Department of Surgery, University Surgical Cluster, National University Health System, Singapore, Singapore,[4]Cancer and Stem Cell Biology Program, Duke-NUS Medical School, Singapore, Singapore, Singapore,[5]National University Cancer Institute, Singapore, Singapore,[6]Department of Surgery, University Surgical Cluster, National University Health System, Singapore, Singapore, Singapore,[7]Yale Cancer Center, New Haven, CT

Background: Gastric cancer (GC) with peritoneal metastases (PM) carries a grim prognosis. Despite progress in elucidating transcriptomic mechanisms of metastasis, epigenetic factors and vulnerabilities are still not fully understood.
Methods: Primary tumor (PT, n=157), primary normal (PN, n=33) and peritoneal metastases (PM, n=97) from patients with GC were retrieved and sequenced with whole transcriptome sequencing. PSI-Sigma was conducted to retrieve alternative pre-mRNA splicing (AS) changes. Differential splicing events (DSE) that were concordant between PT vs PN and PM vs PT were utilized to identify AS event hubs through non-negative matrix factorization. Transcriptomic derivatives such as epithelial-mesenchymal transition (EMT)-scores, mesenchymal subtypes were retrieved utilizing log2(FPKM) gene expression. Epigenetic subtypes of GCPM samples were retrieved through hierarchical clustering of AS event hubs and survival analyses were conducted with a Cox-proportional hazard model.
Results: Nine hundred and nine overlapping DSE were identified between PT vs PN and PM vs PT comparisons, with majority being mutually exclusive exons (44.6%). Of this 909 DSEs, 45 DSE were found to be concordant across comparisons. Unsupervised clustering with gene hubs retrieved from 45 DSEs reveals 2 GCPM epigenetic clusters, characterized by divergent EMT states ($p<0.001$). The clusters were subsequently coined GCPM-Mesenchymal (n=47, mean EMT-score=1.06) and GCPM-Epithelial (n=50, mean EMT-score=0.17). GCPM-Mesenchymal samples were predominantly found to be nested within an inflamed immune-infiltrated peritoneal niche ($p=0.002$) and participants with GCPM-Mesenchymal only samples had significantly poorer survival outcomes upon PM diagnosis (Log-rank, $p=0.037$).
Conclusions: This study sheds light on alternative splicing in transcoelomic metastasis and highlights potential for splicing-based patient stratification in GCPM.

IMMUNOLOGY: Virology and Cancer
Poster Session

#932 Somatic EGFR mutations and oncogenic viruses in NSCLC Egyptian patients.

Shaimaa Saeed[1], Amira Salah El-Din Youssef[1], Ahmed M. Fahmy[2], Nasra F. Abdel Fattah[1], Zeinab K. Hassan[1], Mohamed M. Hafez[1]

[1]Cancer Biology, National Cancer Institute, Cairo University, Cairo, Egypt,[2]Cancer Pathology, National Cancer Institute, Cairo University, Cairo, Egypt

Background: Lung cancer (LC) is the leading cause of cancer-related mortality worldwide, with non-small cell lung cancer (NSCLC) accounting for approximately 75-80% of cases. Genetic alterations, particularly somatic mutations in the *Epidermal Growth Factor Receptor* (*EGFR*) gene, play a critical role in NSCLC tumorigenesis and treatment responsiveness. Oncogenic viruses, such as Human Papillomavirus (HPV) and Epstein-Barr virus (EBV) as environmental factors, have also been implicated in various cancers including lung cancer. This study aimed to investigate *EGFR* somatic mutations profile and its association with theses oncogenic viruses in NSCLC Egyptian patients, hoping to provide new insights on role of *EGFR* mutations associated with oncogenic viruses in lung cancer development.

Methods: This study was conducted on ninety-three formalin-fixed, paraffin-embedded (FFPE) samples of NSCLC Egyptian patients, as well as ten FFPE from safety margins served as control group. The somatic *EGFR* mutations in exon 18(G719A\G719C and G719S), exon 19(19 deletion), exon 20 (insertions, T790M, S768I), and exon 21(, L858R and L861Q) were detected using AmoyDx® EGFR 29 Mutations Detection Kit. Moreover, the HPV DNA was detected by common primers (MY09 and MY11) targeting the L1 gene using conventional PCR assay. The EBV DNA was detected using the Artus EBV TM PCR Kit, targeting the EBNA 1 gene by real-time PCR.

Results: Our results revealed that the *EGFR* mutations were detected in 19 out of 93 NSCLC patients (20.4%); these mutations were distributed in exon 18 (9.5%), exon 19 (42.8%), exon 20 (28.5%), and exon 21(19%). On the other hand, no mutations were detected in the control group. The total number of *EGFR* mutations was 21 per 19 NSCLC patients; where all *EGFR*-mutated NSCLC patients had one mutation, except for two patients had two mutations. The most frequent detected *EGFR* mutations in NSCLC patients were; 19 del (42.8%), T790M (23.8%), L858R (19%), G719S (9.5%), and insertions (4.7%). Furthermore, the DNA of HPV and EBV were detected in 29 (31.1%) and 2 (2.1%) of NSCLC patients, respectively. On the other hand, these viruses were not detected in the control group. Regarding association of oncogenic viruses with somatic *EGFR* Mutations, 26.3% were positive for HPV DNA among *EGFR*-mutated NSCLC patients. No EBV DNA was detected in the *EGFR*-mutated NSCLC patients (0%).

Conclusion: Our findings show that *EGFR* mutations in conjunction with certain oncogenic viruses, may contribute to the pathogenesis among Egyptian NSCLC patients, however a larger sample size is needed to confirm these findings. The prognosis of EGFR-TKI treatment may vary according to different *EGFR* actionable mutations and the co-presence of oncogenic viruses. Thus, understanding this association may provide a relevant modification for developing personalized therapeutic options for treatment of NSCLC patients associated oncogenic viruses.

#933 Effect of mRNA vaccine-induced neutralizing antibodies on human T-cell leukemia virus type-1.

D. Frias[1], N. Banu[2], X. Cheng[2], A. Joseph[2], Z. Liu[2], S. Whelan[2], L. Ratner[2], L. Ratner[2];
[1]Universidad de Puerto Rico, Rio Piedras Campus, San Juan, Puerto Rico, [2]Washington University School of Medicine, St. Louis, MO

Introduction/Background: Human T-cell leukemia virus type 1 (HTLV-1) is a retrovirus linked to severe conditions such as adult T-cell leukemia/lymphoma (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). The absence of a preventative therapy for HTLV-1 represents a significant gap in public health. Our lab has developed a codon-optimized HTLV-1 envelope (Env) mRNA vaccine encapsulated in lipid nanoparticles (LNP), which demonstrated partial protection in a rabbit model. However, further research is required to optimize its efficacy.
Methods: The study involved evaluating the neutralizing antibody response induced by the HTLV-1 mRNA vaccine against HTLV-1a and HTLV-1c in various assays. Western blot analysis assessed the expression of the gp46 envelope glycoprotein and HIV capsid protein p24 in HEK 293T cells transfected with HTLV-1a or HTLV-1c Env plasmids. Fusion assays measured cell-cell fusion using mCherry fluorescent protein in 293T cells, with the effects of IgG serum from infected rabbits assessed. Virus titration involved VSV-HTLV Env and VSV-eGFP to evaluate viral infection inhibition. Neutralizing antibody assays tested IgG from HTLV-infected and non-infected rabbits to determine the extent of viral inhibition.
Results: Expression of gp46 and HIV-1 p24 was confirmed in 293T cells co-transfected with HTLV-1a or HTLV-1c Env plasmids. Fusion assays indicated that IgG from infected rabbits effectively inhibited fusion in HTLV-1a and HTLV-1c Env-expressing cells. Virus titration presented significant inhibition of viral infection by IgG from HTLV-infected rabbits, with an IC50 of 0.05 µg.
Conclusion: The study demonstrates that the HTLV-1 envelope mRNA vaccine induces neutralizing antibodies that can inhibit HTLV-1 infection and fusion processes. These findings support the potential of the mRNA vaccine as a preventive measure against HTLV-1-associated diseases and provide a basis for further research to optimize vaccine efficacy.

#934 Defining the role of Epstein-Barr virus (EBV) in diffuse large B-cell lymphoma (DLBCL) pathogenesis.

Quincy Rosemarie[1], Mitch Hayes[1], Eric C. Johannsen[2]

[1]McArdle Laboratory for Cancer Research, University of Wisconsin - Madison, Madison, WI, [2]Department of Medicine & McArdle Laboratory for Cancer Research, University of Wisconsin - Madison, Madison, WI

Background: Diffuse large B-cell lymphoma (DLBCL) is the most common aggressive lymphoma. DLBCL is not a single disease, but a group of histologically similar yet genetically distinct cancers with unique prognostic and therapeutic features. The recently defined genetic landscape of DLBCL includes at least seven subtypes, but does not consider Epstein-Barr virus (EBV) status, despite up to 14% of DLBCL being EBV-positive and EBV's known role in B cell lymphomagenesis. We performed a reanalysis of published DLBCL genetic data to molecularly define EBV-positive DLBCL, and characterized its cell line models.

Methods: Published whole exome (WES) and RNA sequencing data were analyzed *in silico* to identify EBV-positive (EBV+) DLBCL tumors. Differential analyses of the mutation and transcriptomic profiles of the viral and cellular genes of EBV+ and EBV-negative tumors were performed. WES, RNAseq, and immunoblotting were performed on EBV+ DLBCL cell lines Farage, BCKN1, IBL1, and Val to assess the extent to which they accurately reflect profiles from EBV+ DLBCL tumors.

Results: We identified 20 EBV+ DLBCLs in the NCI cohort (n=481). EBV+ DLBCL is significantly enriched within the BN2 subtype (p=0.0051, Barnard's test) and comprised ~1/3 of EBV+ DLBCLs. The remaining ~2/3 are distinct from currently defined subtypes. Several differentially mutated genes within the BN2 tumors were found to correlate with EBV status. These include subtype-defining genes, genes involved in B cell receptor signaling and cell cycle regulation, and those known to be downstream of EBV. This finding supports our hypothesis that EBV oncogenes can functionally compensate for specific cellular mutations in DLBCL. Preliminary transcriptomic analyses found EBV+ DLBCL to have increased inflammatory and immune signatures, and JAK/STAT and NF-kB signaling. By RNAseq and immunoblot we determined the cell lines Farage, BCKN1, and IBL1 to have EBV Latency III, while Val has Latency II. All lines are infected with EBV Type 1 strain, except IBL1 which harbors EBV Type 2. Genes mutated in BCKN1, IBL1, and Val are consistent with 'Unclassified' subtype, whereas Farage is ST2 subtype.

Conclusion: EBV+ DLBCL, like DLBCL itself, is not a single disease. A subset of EBV+ DLBCL is enriched within, and likely have a shared pathogenesis with, BN2 DLBCL. The remaining EBV+, 'Unclassified' DLBCL may constitute a novel subtype(s). EBV status correlates with distinct mutation and gene expression profiles, including subtype-defining features. Thus, integrating EBV status into the classification of DLBCL is likely to better inform tumor subtyping and molecular studies, and resolve the specific role(s) of EBV in DLBCL. Characterization of EBV+ DLBCL cell lines revealed limited correspondence with EBV+ tumors, underscoring the need for additional cell line models to support in vitro studies of EBV+ DLBCL.

#935 HPV16 E6 splicing induces partial epithelial-mesenchymal transition in oropharyngeal squamous cell carcinoma.

Yvonne Xinyi Lim[1], Min Liu[1], Allison Furgal[2], Jake McGue[3], Shiting Li[4], Bailey Garb[2], Timothy Frankel[3], Veera Baladandayuthapani[3], Laura Rozek[5], Maureen Sartor[2], Nisha D'Silva[1]

[1]University of Michigan School of Dentistry, Ann Arbor, MI,[2]University of Michigan School of Public Health, Ann Arbor, MI,[3]University of Michigan Medical School, Ann Arbor, MI,[4]University of Michigan, Ann Arbor, MI,[5]Georgetown University, Washington, WA

The incidence of HPV16-positive oropharyngeal squamous cell carcinoma (HPV+ OPSCC) is rising at an alarming rate and has overtaken cervical cancer to become the most prevalent HPV+ cancer in the United States. Although most patients with HPV+ OPSCC respond favorably to standard chemoradiation, a substantial proportion of patients have highly aggressive tumors that recur. Biomarkers are needed to reliably stratify patients into risk subgroups. Using multiple complementary models that recapitulate HPV+ OPSCC, our group demonstrated that enhanced splicing of HPV16 E6 oncogene increased migration and invasion. *In vivo*, HPV+OPSCC with higher spliced E6 (E6*) generated more isolated clusters of tumor cells that spread further away from the main tumor relative to the original full-length isoform (E6FL). This is consistent with an adverse phenotype seen in human HPV+ OPSCC. However, the mechanisms of how spliced E6 enhanced invasion remain unclear.Epithelial-mesenchymal transition (EMT) drives invasion in OPSCC, and is commonly characterized by a loss of epithelial genes and gain in mesenchymal genes. However, recent studies also identified a partial EMT (p-EMT) phenotype, where cancer cells co-express both epithelial and mesenchymal genes. In this study, we explored whether spliced E6 enhances invasion via EMT in OPSCC. Immunofluorescence staining of E-cadherin (epithelial phenotype) and vimentin (mesenchymal phenotype) was performed on formalin-fixed paraffin-embedded tissue of 46 human HPV+ OPSCC. Analysis of the tumor regions on whole-slide images showed that patients with increased E6* relative to E6FL had reduced membrane localization of E-cadherin. In contrast, total expression of E-cadherin and vimentin were unchanged. This finding corroborates with our *in vivo* chick chorioallantoic membrane model and *in vitro* studies in OPSCC cell lines, where E-cadherin was more localized at cell-cell junctions in cancer cells with increased E6 splicing. Therefore, we propose that splicing of HPV16 E6 oncogene promotes p-EMT by inhibiting translocation of epithelial E-cadherin from the cell membrane to cytoplasm to reduce cell-cell adhesion. Overall, our findings may shed valuable insights into the mechanisms driving aggressive HPV+ OPSCC and substantiate E6* as a prognostic biomarker to distinguish HPV+ OPSCC risk subgroups for personalized treatment.

#936 HPV-related cancer model development and its application in the efficacy evaluation of a saRNA cancer vaccine.

Zhongbao Song, GuangXu Ding, Anran Xia, Lihuan Bai, Wanqing Ma, Qiuhong Li, Xu Chen, Zhixiang Zhang, Qingyang Gu

WuXi AppTec, Nantong, China

Cancer remains one of the most serious diseases threatening human health, influenced by many factors, such as genetic, physical, chemical and biological factors. Among various cancers, a special subtype is associated with viral infections. Globally, about 10% of all cancers are caused by viral infections including those from HPV, HBV, EBV and KSHV. After infection, these viruses manipulate the proliferation, metabolism, immunity and other life processes of the host cells through various mechanisms, resulting in malignant cell lesions. Therefore, targeting these viruses offers a novel therapeutic approach for virus-related cancers. In this study, codon optimization and site-direct mutagenesis were performed on HPV18 E6 and E7 genes, which were then cloned into a lentivirus vector to establish an HPV18 E6/E7-overexpressing cell line (EMT6-HPV18). EMT6-HPV18 cells stably expressed HPV18 oncogenes E6 and E7 *in vitro* and *in vivo*, and induced tumor formation in mice. To further investigate the potential of this model for evaluating HPV18-related anti-tumor drugs, we designed a self-replicating RNA (saRNA) expressing HPV18 E6 and E7, linked to FLT3L and CCL11 with the 3' and 5' terminal, respectively. Immunogenicity of the saRNA was validated after three rounds of immunizations by ELISpot and ICS flow cytometry, showing a marked increase in E6/E7-specific IFN-γ^+ T cell response. Subsequently, the therapeutic effect was evaluated in the EMT6-HPV18 subcutaneous tumor model, where saRNA treatment remarkably inhibited tumor progression, improved early-stage survival rates, and induced long-term anti-tumor immunity. Immune profiling revealed significant infiltration of immune cells into the tumors post-saRNA treatment, namely $CD4^+$ T cells, $CD8^+$ T cells and DCs with significant increase in E6/E7 specific IFN+ T cell and memory T cell subsets. In conclusion, we have successfully established an HPV18 E6/E7-expressing tumor model, demonstrating that the saRNA therapeutic vaccine effectively inhibits tumor progression in HPV18-related malignancies. Keywords: Oncogenic virus, HPV18, E6, E7, saRNA

#937 mRNA-encoded HPV16/18 therapeutic vaccine with co-stimulator to activate antigen presenting cells and promote T cell-mediated anti-tumor activity.

Qiang Zhang, Beibei Cao, Lei Li, Shaoli Liu, Xiaoju Zhang, Andong Liu, Xiaoyun Ma, Hongya Han, **Wei Xu**

METiS Pharmaceuticals, Hangzhou and Beijing, China

Background: Persistent infection with human papillomavirus (HPV) 16 and 18, two most prevalent high-risk subtypes, is a predominant cause to human cancers such as head and neck, and cervical cancers. Although prophylactic vaccines can provide protection from infection, therapeutic and efficacious drugs for already developed HPV-related cancers are badly needed. The conventional therapeutic vaccines based on protein or peptides have not reached satisfactory efficacy for patients due to a lack of strong immunogenicity to fully unleash the power of T cell immunity. Here, we developed a lipid nanoparticle (LNP)-encapsulated mRNA-encoding for multiple antigenic signals: E6/E7 antigens from both HPV16 and 18, and an optimized co-stimulatory adjuvant to help both antigen presentation and T cell stimulation.

Methods: The mRNA backbone of the vaccine was designed with mutated HPV16/18 E6/E7 at the N-terminus to bypass p53 and pRb degradation, thus avoiding carcinogenic risk. A T2A element is used to space genes from co-stimulatory signals. A suitable LNP was identified both in vitro and in vivo for its expression efficiency and safety profiles. The efficacy and mechanistic studies of the lead mRNA-LNP were further evaluated in both TC-1(HPV16$^+$) and HPV18 transgenic MC38 syngeneic models.

Results: Our lead LNP MLX0487-23 could effectively target the splenic dendritic cells and macrophage, leading to dose- and time-dependent tumor eradication in tumor-bearing mice. The optimized mRNA antigen construct named as P016-2 translated the proteins at high level *in vitro* and as desired, with p53 and pRb remaining intact. Our lead mRNA-LNP showed superior anti-tumor activity both in the HPV16$^+$ TC-1 and the HPV18$^+$ MC38 models, as compared to the benchmark construct lacking co-stimulatory signals OX40L and/or GM-CSF. We observed an expansion of HPV-specific CD8$^+$ T cells and intra-tumoral infiltration of T cells into tumors. After rechallenging with the same TC-1 tumor cells in the contralateral dorsal skin in the complete regression mice induced by P016-2, no tumor was formed, indicating long-term immune memory.

Conclusion: We conclude that MLX0487-23 (LNP) encapsulated P016-2, a 4 antigen and GM-CSF expressing mRNA, effectively expanded HPV-specific CD8$^+$ T cells and mediated potent anti-tumor efficacy in the preclinical models, making it an excellent candidate therapeutic mRNA vaccine for clinical testing its safety and efficacy in HPV-driven malignancies.

#938 HHV-7-mediated downregulation of HLA-F: a mechanism for immune evasion in angiosarcoma.

Bavani Kannan[1], Janos Luka[2], Tun Kiat Ko[1], Jason Chan[1]

[1]National Cancer Centre Singapore, Singapore, Singapore,[2]Kintla Research Technology, Belgrade, MT

Background: Human herpesvirus-7 (HHV-7) is a ubiquitous, CD4+ T cell lymphotropic virus. It establishes lifelong latency and may reactivate, particularly in immunocompromised individuals. We had previously detected the presence of HHV-7 in two-thirds of angiosarcoma clinical samples. HHV-7-positive (HHV-7+) tumors were shown to be relatively enriched for B- and mast cells and had high tumor inflammation signature scores compared to HHV-7-negative tumors.
Objective: To investigate HHV-7 pathogenesis in host cells and its role in angiosarcoma.
Methods: A SupT1 infection model was established. Images and cell pellets were collected at Days 0 and 4 (early infection phase) and Days 8 and 14 (late infection phase) for bulk RNA-seq analysis. Gene expression data was validated with quantitative PCR (qPCR). RNA-seq and Nanostring gene expression profiling with the Pancancer IO360 panel were previously performed on HHV7+ fresh frozen and FFPE angiosarcoma samples respectively.
Results: Infected SupT1 cells exhibited cytopathic effects (CPEs) such as membrane blebbing, syncytia formation and growth arrest during early infection phase. Cell lysis, followed by recovery of cell growth, were observed during late infection phase. RNA-seq revealed significant pathway alterations across the infection course, supporting these observations. Early infection showed notable upregulation in arachidonic acid metabolism and antigen processing and presentation pathways, indicating an anti-viral response in host cells. The downregulation in actin cytoskeleton and cell cycle pathways were seen, corresponding to the observed CPEs. During late infection, there was a significant downregulation in antigen processing and presentation pathways (padj. <0.05), suggesting immune evasion the virus, along with a significant downregulation of tumor suppressor genes such as RNF43 and CBFA2T3. Through the course of infection, a general upward trend of oncogenic and inflammatory pathways was seen, with the latter being observed in HHV-7+ angiosarcomas as well. Narrowing down on specific genes, we observed the significant downregulation of HLA-F and upregulation of HK2 in both late-stage SupT1 infection and HHV-7+ angiosarcomas, implicating these genes in immune evasion and tumor progression.
Conclusions: Our study provides the first RNA-seq profiling of HHV-7 infection in host cells. The study reveals alterations in key cellular pathways that promote an immunosuppressive and pro-inflammatory environment. These changes likely facilitate tumor progression and immune evasion in angiosarcoma.

#939 Development of a dual fluorescent reactivation reporter Epstein-Barr virus (DFRR-EBV) to optimize lytic-induction therapy of EBV-associated cancers.

Emilie Greene, Eric Johannsen, Ahmed Ali

University of Wisconsin-Madison, Madison, WI

Epstein-Barr virus (EBV) is a highly successful γ-herpesvirus, infecting >95% of the human population. Approximately 0.01% of infected individuals will develop an EBV-associated cancer later in life, resulting in 200,000 annual cases globally. EBV-associated cancers, such as Diffuse Large B-Cell Lymphoma, are dependent on the expression of EBV latency genes for their continued growth and survival, making the disruption of latent infection a promising therapeutic target. “Lytic induction therapy” combines small molecules capable of reactivating EBV from latency with anti-viral drugs to increase toxicity to tumor cells and prevent EBV particle production. The full potential of this approach will require the development of second-generation lytic induction agents that induce a much greater proportion of tumor cells to enter the lytic cycle. Efforts to study the mechanisms of EBV reactivation are essential to this endeavor. EBV entry into the lytic phase is controlled by two viral transcription factors, Rta and Zta, and activators of the Rta and Zta promoters have previously been identified using reporter assays. These approaches, however, failed to capture major regulatory mechanisms that govern viral reactivation in EBV-positive tumors, such as long-range enhancer effects and epigenetic regulation of viral transcripts. To overcome these limitations, we constructed a dual fluorescent reactivation reporter virus (DFRR-EBV) that replaces Rta and Zta with GFP and RFP, respectively. This reporter is being used to screen a library of FDA-approved small-molecules to identify agents that reactivate EBV, in addition to a genome-wide unbiased CRISPR/Cas9 screen to identify negative regulators. Significantly, our reporter will distinguish between which small molecules are Rta versus Zta induction agents. Although Zta had been considered the principal regulator of the latent/lytic switch, we and others have demonstrated that expression of both Rta and Zta are essential for EBV reactivation[1,2]. Therefore, the combination of an Rta induction agent with a Zta inducer should act synergistically, offering a significant improvement over the current approach of single-agent lytic induction therapies for EBV-associated cancers.

References:1.Ali, A. *et al.* Rta is the principal activator of Epstein-Barr virus epithelial lytic transcription. *PLoS Pathog* **18**, e1010886 (2022).2.Feederle, R. The Epstein-Barr virus lytic program is controlled by the co-operative functions of two transactivators. *The EMBO Journal* **19**, 3080–3089 (2000).

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#94 Tumor RNA isoforms are associated with recurrence in resected non-small cell lung cancer.

Kelly M. Cagin[1], Arsalan Khan[1], Savan Shah[1], Wara Naeem[1], Minha Ansari[1], Oluwamuyiwa W. Adebayo[1], Hita Moudgalya[2], Michael J. Liptay[1], Christopher W. Seder[1], Jeffrey A. Borgia[1]

[1]Rush University Medical Center, Chicago, IL,[2]Pathology, Ohio State University, Columbus, OH

Introduction: Surgical resection remains a mainstay of treatment for individuals with non-small cell lung cancer (NSCLC). However, disease recurrence rates remain a major concern, standing at 5-19% for stage I disease, 11-27% for stage II disease, and 24-40% for stage III disease. This study focused on identifying tumor transcript variants that allow patients to be risk-stratified for recurrence/disease progression.

Methods: Patients with stage I-IIIA NSCLC tumors ($T_{1-2}N_{0-2}M_0$) who did not receive neoadjuvant therapy and had follow-up clinical data available were included. The primary outcome was event free survival (EFS), defined as time until recurrence or death. Tumor RNAseq data was interrogated for RNA isoforms at the splice variant (n=645 subjects) and fusion transcript (n=555 subjects) levels, identifying 225,896 unique splice variants via FRASER and 1,382 unique fusion transcripts via STAR-Fusion, respectively. Differential analyses were performed via edgeR based on nodal and recurrence status (i.e. N_0 Non-recurrent, N_0 Recurrent, N_{1-2} Non-recurrent, and N_{1-2} Recurrent) at the individual isoform level using mean feature importances of a supervised learning model (3 random forest ensemble) to rank isoforms on prognostic value. Aggregate analyses were also performed, assessing the relationship between recurrence and tumor fusion burden in two ways (sum of normalized counts and sum of unique transcripts).

Results: From the intersection of top features and differential expression analysis comparing the N_0 Non-recurrent group (n=325) to those that recurred/progressed (N_0 Recurrent, N_{1-2} Non-recurrent, and N_{1-2} Recurrent; n=230) we identified differential utilization at multiple splice site loci, including at chr 7: 16,861,429 - 16,861,430 (RGA3 exon 6) that was associated with progression ($q = 2.145 \cdot 10^{-7}$; log fold change = 0.374). Total normalized fusion transcript fragments (as FFPM) per subject pass Kruskal-Wallis test for differences between the four individual groups (p = 0.3126). Tumors with greater than 10 unique fusion transcript variants detected were associated with a superior 5-year EFS (HR = 0.59 (95%CI: 0.4-0.86), p = 0.0069).

Conclusions: These data suggest multiple loci producing splice variants associated with recurrence exist, and a fusion burden >10 unique transcripts correlates with a superior EFS in patients with resected stage I-IIIA NSCLC, which may be reflective of increased neoantigen load and immune activation. Current efforts are focused on validation of these findings in the TracerX cohort and mass spectrometry.

IMMUNOLOGY: Virology and Cancer
Poster Session

#940 Harnessing oncolytic virus VSV and combination therapy to advance treatments for relapsed refractory multiple myeloma using Vk*MYC models.

Yi Ying Ma[1], Lianwen Zhang[1], Rebecca A. Nace[1], Marta Chesi[2], Stephen J. Russell[3], Kah Whye Peng[1]

[1]Molecular Medicine, Mayo Clinic, Rochester, MN,[2]Medicine, Mayo Clinic, Scottsdale, AZ,[3]Vyriad, Rochester, MN

VSV-IFNβ-NIS (VV1) is a Vesicular Stomatitis Virus (VSV) that encodes the interferon beta (IFNβ) and the iodide symporter (NIS) gene. The IFNβ gene confers tumor selectivity and enhances host antitumor immunity while the NIS gene acts as an imaging biomarker, allowing noninvasive imaging of viral infection sites using PET/SPECT. Multiple myeloma (MM) is a cancer of plasma cells and poses a challenge for treatment due to its biological heterogeneity. As MM usually relapses and is refractory, current therapies face the issue of drug resistance and patients may have to turn to novel therapies. Therefore, we set out to understand the activity of VSV which could be a new approach for patients with relapsed refractory MM (RRMM). Our study utilized two unique bortezomib resistant Vk*MYC transplantable models, Vk12598 and Vk12653, with distinct genomic features. Upon re-transplantation of splenocytes from these mice, flow cytometry revealed that Vk12598 and Vk12653 mice had high MM populations in the spleen and bone marrow. To assess VV1 efficacy in tumor bearing mice, we performed a VV1 dose escalation study, showing efficacy in delaying MM development and prolonging survival in both mice models, with Vk12598 being the more responsive model. Later studies also showed that VV1 induces tumor remission in the Vk12598 model when treatment was initiated at low tumor burden (gamma/albumin ratio ~0.2). IFNβ levels increased significantly post 24-72h of VV1 treatment, indicating targeted infection and replication of the virus in MM cells. However, high doses of VV1 led to toxicity, as observed from early mortality and weight loss. To determine the sites of MM metastasis and VV1 infection, timepoint harvests were carried out. Hematoxylin and eosin and immunostainings showed the onset of MM of Vk*MYC in the bone marrow and spleen first, then later in the liver. Immunofluorescent staining confirmed that the higher the tumor burden observed, the more VV1 was present in the tissues. To enhance VV1 efficacy, we performed in vivo combination therapies of VV1 with anti-PD-1, an inhibitor of PD-1 which is overexpressed on MM patient T cells; and bortezomib, which inhibits NF-κB which in turn prevents IFN pathway protein transcription, potentially enhancing the replication of VV1 virus. In Vk12598 mice, VV1 combined with both drugs induced tumor regression and doubled the median survival time compared to treatment with VV1 only or VV1 coupled with either of the drugs individually. Addition of VV1 to either anti-PD-1 or bortezomib alone also increased survival time significantly. Studies are still ongoing to understand the mechanisms of VSV and its combination treatments in Vk*MYC models. This translational study will allow us to gain the knowledge needed to refine VSV treatments and provide novel therapy combinations to improve MM patient survival.

IMMUNOLOGY: Viruses and Bacteria in Cancer Therapy
Poster Session

#944 Evaluation of oncolytic herpes simplex virus overexpressing granulocyte-macrophage colony-stimulating factor as a potential therapeutic against pancreatic ductal adenocarcinoma.

Biplov Sapkota[1], Naveen Chintala Ramulu[1], Shilpa Thota[1], Rizwana Begum[1], Shreya Pokharel[1], Brent Stanfield[2], Dayanidhi Raman[3], Gus Kousoulas[2], Joseph Francis[1]

[1]Department of Comparative Biomedical Sciences, Louisiana State University, Baton Rouge, LA,[2]Department of Pathobiological Sciences, Louisiana State University, Baton Rouge, LA,[3]Department of Cell & Cancer Biology, University of Toledo - Health Science Campus, Toledo, OH

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal and drug-resistant cancers. The 5-year overall survival (OS) for PDAC remains low at 12.8%, largely due to its advanced presentation stage that prevents curative resection, and due to a high tendency for recurrence. The PDAC tumor microenvironment (TME) is desmoplastic and highly immunosuppressive. Oncolytic viruses (OV) are known to transform the TME from ‘cold’ to ‘hot’ by increasing immune cell infiltration within the TME. The primary objective of this study is to investigate the potential of Herpes simplex virus (HSV-1), strain VC2, overexpressing granulocyte-macrophage colony-stimulating factor (GM-CSF), HSV-1 (VC2) GM-CSF, as a potential therapeutic agent in *in vitro* and *in vivo* models of PDAC. We will assess the intricate molecular mechanisms involved in the interaction between OV and PDAC TME and perform immunoprofiling. We hypothesize that the administration of HSV-1 (VC2) GM-CSF as a monotherapy or combination therapy will improve treatment outcomes via cytopathic effects (CPE) of OV against cancer cells and immunomodulation of PDAC TME by GM-CSF. We evaluated the effect of HSV-1 (VC2) GM-CSF on human MIA PaCa-2 (KrasG12C, Trp53R248W) and murine KPC (KrasG12D, Trp53R172H, and Cre control) PDAC cell lines. Both cell types showed notable CPE, including cell aggregation, rounding and eventual detachment, following treatment with HSV-1 (VC2) GM-CSF, in contrast to the effects observed with wild-type HSV-1 (VC2) or in uninfected controls after 24 h of viral exposure. The observed CPE was corelated with cell death using trypan blue staining. We determined the viral titers to be 9.6×10^7 pfu/ml for wild-type HSV-1 (VC2) and 1.2×10^7 pfu/ml for HSV-1 (VC2) GM-CSF. The infection of both human and murine PDAC cells lines with HSV-1 (VC2) GM-CSF at 0.1, 1.0 and 10 MOIs significantly increased the expression of proinflammatory markers (IL-10, COX-2, iNOS) and upregulated Fas ligand (FasL) and PD-L1 expression suggesting immunomodulatory activity. An orthotopic PDAC model in C57BL/6 mice has been established, providing a solid foundation for further research. Ongoing studies employing advanced 2D and 3D cell culture systems, including cell-derived spheroid co-cultures and patient-derived organoid models, along with preclinical evaluation in established orthotopic mouse models, will elucidate the therapeutic potential of HSV-1 (VC2) GM-CSF, both as a standalone treatment and in combination with anti-PD-L1 immunotherapy and Gemcitabine.

#945 Live attenuated MMR vaccines modulate tumor immune cell infiltration and synergize with standard of care to prolong survival in preclinical HCC models.

M. Z. Tesfay[1], A. Cios[1], K. U. Ferdous[1], R. S. Shelton[1], B. Mustafa[1], C. C. Simoes[1], S. Post[1], T. Kelly[1], M. Moaven[2], L. R. Roberts[3], M. J. Borad[4], M. J. Cannon[1], A. Basnakian[1], N. Bolni[1];

[1]University of Arkansas for Medical Sciences, Little Rock, AR, [2]Louisiana State University (LSU) Health-New Orleans, New Orleans, LA, [3]Mayo Clinic, Rochester, MN, [4]Mayo Clinic, Phoenix, AZ

The global burden of hepatocellular carcinoma (HCC) continues to rise, driven by increasing metabolic risk factors such as nonalcoholic fatty liver disease, obesity, and type II diabetes. Patients with advanced HCC have limited therapeutic options and a dismal prognosis. While recent advancements in combination therapies—including immune checkpoint blockade (ICB) with anti-PD-1/PD-L1 and anti-CTLA-4 agents and anti-angiogenics—have improved survival for some, up to 80% of patients fail to respond, underscoring an unmet need for new strategies. Emerging data indicate a strong association between increased tumor-infiltrating lymphocytes (TILs) and better responses in ICB-treated HCC, suggesting TIL enhancement as a promising approach. However, HCC's inherent low immunogenicity and immunosuppressive tumor microenvironment (TME) limit the efficacy of current TIL-promoting strategies. Here, we demonstrate that low-dose intratumoral immunotherapy using the trivalent MMR vaccine—composed of live attenuated measles, mumps, and rubella viruses—modulates TME immune infiltration, increases cytotoxic T cell frequency, and delays tumor growth in multiple murine HCC models. Importantly, intratumoral or systemic MMR administration in combination with dual ICB (anti-mPD-1/anti-mCTLA-4) significantly prolongs survival, promoting tumor-specific and memory immune responses in both subcutaneous and orthotopic HCC models. These findings reveal a unique mechanism of vaccine-induced anti-tumor activity, highlighting MMR as a viable, globally accessible option for enhancing immune responses in advanced HCC therapy.

#946 Novel oncolytic virus targeting GPC3 and CTNNB1 demonstrates complete response in hepatocellular carcinoma (HCC) xenograft with a single, systemic administration.

Chad M. Moles[1], Rupsa Basu[1], Brenda Ho[1], Manal Farhat[1], Jason Kent[1], Peter Weijmarshausen[1], Bruce F. Smith[2], Michael A. Whitt[3]

[1]Humane Genomics Inc., New York, NY,[2]Scott-Ritchey Research Center, Auburn University College of Veterinary Medicine, Auburn, AL,[3]Department of Microbiology, Immunology and Biochemistry, The University of Tennessee Health Science Center College of Medicine, Memphis, TN

Introduction: Oncolytic viral therapies (OVTs) have shown promise in cancer treatment, though efficacy and selectivity challenges remain. HGI627 is an engineered vesicular stomatitis virus (VSV) featuring a synthetic glycoprotein retargeted to glypican-3 (GPC3) and a beta-catenin (CTNNB1)-dependent aptazyme as a genetic ON switch. This dual targeting strategy enhances specificity to liver cancer cells.

Methods: HGI627 was constructed and rescued using a synthetic virology platform [Moles et al., Viruses 2024], and viral titers were measured by $TCID_{50}$ assay on Hep3B cells. Cytotoxicity and selectivity were evaluated using real-time imaging (IncuCyte system) on liver cancer (e.g. Hep3B), off-target (e.g. SW-480), and normal (e.g. THLE-2) cells. To further evaluate this virus *in vivo*, an efficacy study performed in Hep3B xenograft-bearing NSG mice. To evaluate potential acute toxicity and maximum tolerated dose (MTD), a study in NSG mice (n=5/group) was then conducted using single and multiple $1x10^9$ $TCID_{50}$ doses.

Results: HGI627 demonstrated robust lytic activity *in vitro* at low MOIs (0.01) in GPC3+/CTNNB1+ cells with minimal to no cytotoxicity in off-target and normal cells at MOI=10. *In vivo*, a single intraperitoneal (i.p.) injection of 1×10 $TCID_{50}$ HGI627 (n=9) achieved a 100% complete response and significantly extended survival to day 49 (end of study) compared to median survival of 15 days in vehicle group ($p<0.005$). In the safety study, even multiple (n=3) high-dose (1×10 $TCID_{50}$) administrations of HGI627 were well tolerated, and no signs of clinical toxicity were observed up to 14 days; Importantly, this highest dosing group received an equivalent of >100× the proposed first-in-human starting dose without adverse effects.

Conclusion: These results highlight HGI627's potency, selectivity, and safety, overcoming previous OVT challenges. HGI627 exemplifies a promising approach for liver cancer treatment using a dual-targeting mechanism for enhanced precision. Current studies include an orthotopic HepG2-luciferase model to further validate efficacy.

#947 Engineering a synthetic oncolytic virus to overcome apoptotic resistance and induce immunogenic cell death in pancreatic ductal adenocarcinoma.

Aleksandra Cios[1], Khandoker Usran Ferdous[1], Mulu Z. Tesfay[1], Alicja Urbaniak[2], Bahaa Mustafa[3], Steven R. Post[1], Thomas J. Kelly[1], Martin J. Cannon[4], Alexei Basnakian[5], Omeed Moaven[6], Bolni Marius Nagalo[1]

[1]Department of Pathology, University of Arkansas for Medical Sciences, Little Rock, AR,[2]Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR,[3]Department of Pharmaceutical Sciences, University of Arkansas for Medical Sciences, Little Rock, AR,[4]Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, AR,[5]Department of Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR,[6]Division of Surgical Oncology, Department of Surgery, Louisiana State University (LSU) Health, New Orleans, LA

Introduction: Oncolytic viruses, particularly vesicular stomatitis virus (VSV), combat cancer through tumor lysis and immune response enhancement. However, pancreatic ductal adenocarcinoma (PDAC) often resists apoptosis due to p53 mutations and overexpression of anti-apoptotic proteins. We developed an innovative VSV variant, VMG, incorporating the glycoprotein of the Morreton virus (MORV-G) to enhance safety, tumor targeting, and immune engagement. VMG was further engineered to encode a proprietary synthetic caspase-activating protein (VMG-apo), activating both intrinsic and extrinsic apoptotic pathways via ER stress and caspase activation.
Methods: Experiments were conducted on three PDAC cell lines: HPAFII, HS766T, and Panc1. Viral cytotoxicity was analyzed using the xCELLigence system over 40 hours post-infection. Cells were infected at MOI of 1 and harvested at 15 HPI for proteomic analysis. Additional analyses included apoptosis/necrosis assays, calreticulin expression, and Western blotting.
Results: Proteomic analysis revealed distinct responses across cell lines. In Panc1 cells, VMG-apo altered 74 genes, notably downregulating CD9, COL1A1, and NQO1, while inhibiting extracellular matrix remodeling. HPAFII cells showed changes in cell cycle regulation. Both cell lines exhibited strong CASP3 overexpression (fold change >5.60). The resistant HS766T line showed minimal changes. VMG-apo downregulated DAMP and PAMP-associated genes while promoting calreticulin release. Panc1 showed highest sensitivity, with significant cell death at 15 hours post-infection. HPAFII cells underwent primarily apoptosis, while HS766T remained resistant. Calreticulin expression decreased in Panc1 and HPAFII cell lines, while in HS766T remained the same as control. Western blot analyses detected caspase-3 but no cleaved caspase-3.
Conclusions: The results provide insights into immunogenic cell death and apoptotic mechanisms in PDAC following VMG-apo treatment. The differential responses across cell lines underscore VMG-apo's therapeutic potential and complexity. The heterogeneous responses indicate the need for personalized strategies in oncolytic virotherapy. These findings advance the understanding of how oncolytic viruses can enhance both direct tumor cell death and anti-tumor immunity, offering a promising avenue for improving PDAC treatment outcomes.

#948 PAX3-FOXO1-driven oncolytic herpes virus designed for treatment of alveolar rhabdomyosarcoma.

C. W. Peters, T. Nowicki, M. Kawakami, C. Kidd, A. Lechein;
UCLA - University of California Los Angeles, Los Angeles, CA

Background: Alveolar rhabdomyosarcomas (aRMS) often express an oncogenic fusion protein PAX3-FOXO1. PAX3-FOXO1 (P3F) acts as an oncogenic transcription factor, driving aggressive and lethal tumors in children and young adults. P3F has proven elusive to small molecule inhibitors, and the low immunogenicity of aRMS tumors has impaired efforts to treat with immune checkpoint blockade. The absence of an effective targeted treatment for fusion-positive aRMS represents an important an unmet therapeutic need.
Methods: We designed a system to combine and compare sequences which bind PAX3-FOXO1 to drive a transgene reporter system. We then used this system in a variety of rhabdomyosarcoma cell lines which either expressed PAX3-FOXO1, or were fusion negative. We then inserted this P3F-driven promoter into the oncolytic herpes simplex virus (oHSV) G47Δ, driving the cytotoxic ICP34.5 gene, and tested its ability to kill P3F-positive and P3F-negative aRMS cell lines in vitro. To determine the effects of P3F expression on tumor cell response to oHSV infection, we performed RNA sequencing and differential gene expression using DESeq2 to assay alterations between P3F-positive and negative tumor cells during the lifetime of oHSV infection. Enrichment analysis on genes identified as significantly different were then analyzed via EnrichR to evaluate gene ontology and pathways significantly enriched during infection which differed based on PAX3-FOXO1 expression.
Results: We identified two synthetic promoters which exhibited expression of luciferase or GFP reporter genes only in the presence of P3F (T-Test $p<0.05$, $n=10$). We then inserted the cytotoxic HSV1-ICP34.5 gene into the ICP6 gene of the previously validated oHSV G47Δ under this synthetic promoter. These novel G47Δ-P3Fpro:ICP34.5 elicited quicker cell killing in several P3F-positive RMS lines faster than the parental virus, while exhibiting the same cell killing kinetics in matched fusion negative cells (2-way ANOVA $p<0.05$, $n=3$, Tukey multiple comparisons test). Differential gene expression over time demonstrated PAX3-FOXO1 is involved in modulating a substantial amount of immune response genes during oHSV infection including IFI16, SAMD9, and DUSP4. Expression was also significantly altered between fusion positive cells infected with the ICP34.5-armed virus compared to fusion negative cells infected with the same virus.
Conclusions: We demonstrate the synthesis of a novel PAX3-FOXO1-controlled gene expression system, and its implementation within an oHSV to combat PAX3-FOXO1 fusion oncogene-positive aRMS. Our study demonstrates the influence of PAX3-FOXO1 during viral infection with an oHSV and the potential to tailor P3F specific promoters to novel cancer targeting vectors.

#949 Boosting oncolytic virus-mediated antitumor immunity by dendritic cell vaccine therapy via p53-activated immune response.

Naohiro Okada[1], Hiroshi Tazawa[1], Motohiko Yamada[1], Satoru Kikuchi[1], Shinji Kuroda[1], Yasuo Urata[2], Shunsuke Kagawa[1], Toshiyoshi Fujiwara[1]

[1]Department of Gastroenterological Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan,[2]Oncolys BioPharma, Inc., Tokyo, Japan

Background: Dendritic cell (DC) vaccine therapy represents a promising immunotherapeutic approach by inducing tumor antigen (TA)-specific immune responses. The tumor suppressor gene p53 is one of the most frequently mutated genes, and mutant or wild-type p53 epitopes function as TAs. Previous studies have demonstrated that DCs transduced with a non-replicative adenovirus expressing wild-type p53 (Ad-p53) (Ad-p53-DCs) induces p53-specific cytotoxic T lymphocytes (CTLs), leading to antitumor immunity. We have previously developed OBP-702, a tumor-specific replication-competent oncolytic adenovirus expressing p53, and demonstrated that OBP-702 exhibits direct cytotoxicity via p53-mediated induction of apoptosis and indirect immune activation via p53-mediated enhancement of immunogenic cell death (ICD). In this study, we investigated whether Ad-p53-DCs or OBP-702-transduced DCs (OBP-702-DCs) in combination with OBP-702 augment antitumor immune responses in murine colorectal cancer models.
Methods: Bone marrow-derived DCs were harvested from BALB/c mice and cultured with IL-4 and GM-CSF for five days, followed by infection with Ad-p53 or OBP-702 for two days. Maturation of DCs was evaluated by flow cytometric analysis of surface markers CD11c, CD86, CD103, and MHC-class II. RT-PCR confirmed the expression of p53 RNA in p53-expressing virus-transduced DCs. Ad-p53-DCs or OBP-702-DCs were administered subcutaneously weekly for four cycles before CT26 tumor cell inoculation. Tumor-bearing mice were treated with intratumoral injection of OBP-702 weekly for two cycles. Four experimental groups were established: (1) control, (2) p53-expressing virus-transduced DC alone, (3) OBP-702 alone, and (4) combination of p53-expressing virus-transduced DC and OBP-702. Tumor growth was monitored, and the immune microenvironment was analyzed using immunohistochemistry to evaluate the tumor infiltration of CD8+ T cell and CD11c+ DC.
Results: Ad-p53-DCs and OBP-702-DCs displayed significantly increased expression of DC maturation marker compared to control DCs. Combination of Ad-p53-DCs or OBP-702-DCs with OBP-702 markedly suppressed tumor growth compared to OBP-702 monotherapy, demonstrating a synergistic antitumor effect. Immunohistochemical analysis revealed that tumors treated with the combination therapy had significantly increased infiltration of CD8+ T cells and CD11c+ DCs, indicating robust activation of the antitumor immune responses.
Conclusions: Our data suggest that immunization with p53-expressing virus-transduced DCs enhances the antitumor efficacy of p53-armed oncolytic adenovirus OBP-702 in murine colorectal cancer models via induction of p53-specific antitumor immune responses.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#95 MTAP deficiency closely correlates with p16 status in non-small cell lung cancer but is unrelated to tumor aggressiveness or patient prognosis.

Natalia Gorbokon[1], Nina Schraps[1], Katharina Moller[1], Seyma Buyucek[1], Maximilian Lennartz[1], Florian Viehweger[1], Christoph Fraune[1], Christian Bernreuther[1], Elena Bady[1], Lisa Hornsteiner[1], Ronald Simon[1], Guido Sauter[1], Martin Reck[2], Till Olchers[2], Florian Lutz[1], Till S Clauditz[1], Martina Kluth[1], Stefan Steurer[1]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany,[2]German Center for Lung Research, Grosshansdorf, Germany

Loss of S-methyl-5′-thioadenosine phosphorylase (MTAP) expression is often caused by genomic deletions of chromosome 9p21 in non-small cell lung cancer (NSCLC). Complete MTAP loss results in a critical vulnerability of cancer cells towards drugs targeting adenine synthesis and the methionine cycle. To study the clinical impact and heterogeneity of MTAP expression in lung cancer, a tissue microarray containing 470 adenocarcinomas and 235 squamous cell carcinomas, and conventional whole sections from all (5-15, average 7.4) tumor-containing tissue blocks of 5 lung cancers were analyzed by for immunohistochemical (IHC) expression of MTAP. MTAP expression was lost (0+) in 13.2%, weak (1+) in 45.4% and strong (2+/3+) in 41.4% of 423 interpretable adenocarcinomas (ADC), and 0+ in 25.8%, 1+ in 46.4% and 2+/3+ in 27.8% of 209 interpretable squamous cell carcinomas (SQCC) of the lung. MTAP deficiency was unrelated to parameters of tumor aggressiveness, including pT status, pN status, histological grade, and patient survival, in ADC and in SQCC. In 40 analyzed mesotheliomas, a MTAP loss was seen in 45% ($p<0.0001$ for adenocarcinoma vs. mesothelioma). Among the 5 consecutive lung cancers from which a total of 37 whole sections were analyzed, MTAP was homogeneously deficient in 1 case, heterogeneously deficient in another case, and homogeneously retained in the remaining 3 cases. In conclusion, loss of MTAP expression occurs in 13% (ADC) to 25% (SQCC) of NSCLC irrespective of tumor extent or metastasis stage. These findings identify a potential application of MTAP IHC in NSCLC for diagnosis of malignancy and the identification of patients who might benefit from MTAP-based therapies. However, the possible therapeutic efficacy could be impaired in patients with heterogeneous MTAP loss.

IMMUNOLOGY: Viruses and Bacteria in Cancer Therapy
Poster Session

#950 Engineered oncolytic vesiculovirus downsizes pancreatic tumor and boosts immune response.
K. Ferdous, M. Z. Tesfay, A. Cios, I. R. Miousse, B. M. Nagalo;
University of Arkansas for Medical Sciences, Little Rock, AR

Vesicular stomatitis virus (VSV) is one of the leading oncolytic virus prototypes used in both preclinical and clinical cancer research. A newly engineered chimeric vesiculovirus, VMG, has been tested against various cancer types, including pancreatic ductal adenocarcinoma (PDAC). However, the effectiveness of virotherapies for PDAC is often limited by the immunosuppressive factors present in the tumor microenvironment. Moreover, the presence of hyaluronic acid (HA) and collagen (CN) in the microenvironment contributes to increased tumor stiffness and interstitial fluid pressure, which impede the delivery of drugs, oncolytic viruses, and immune cells into the tumor. To address these challenges, we developed VMG expressing bacterial collagenase (CNase), mouse hyaluronidase (mPH20), or a special enzyme (Enz) having both CNase and PH20-like effects; and evaluated their efficacy in a preclinical Pan02 PDAC model. Genetic modifications to VMG did not compromise its oncolytic activity or replicability *in vitro*, as shown by MTS assays and kinetic studies. *In vivo* studies revealed that VMG-Enz significantly reduced tumor volume compared to other synthetic viruses. Immunohistochemistry (IHC) and quantitative polarized light imaging (QPLI) confirms that the hybrid VMG viruses effectively dismantled stromal components, facilitating enhanced viral infection and propagation into the tumor. Flow cytometry analysis indicated a substantial increase in $CD8^+$ T cells, granzyme B^+ T cells, dendritic cells, and natural killer cells, alongside a decrease in M-MDSCs, which are indicators of successful onco-immunotherapy. However, we also noticed an elevation of immune checkpoint markers, including PD-1, PDL-1, PDL-2, and anti-viral resistance genes for IFN-α, IFN-β and Mx-1 after viral treatment which may have limited complete tumor remission by the VMG vectors. We conclude that VMG-Enz successfully enhanced immune response and significantly reduced tumor volume by altering stromal HA and collagen levels. Further studies are needed to explore VMG-Enz's potential as combination therapy with immune checkpoint inhibitors or type I interferon antagonists to achieve complete tumor eradication and enhance survival benefits in experimental animals.

#951 A novel oncolytic adenovirus platform with multi-gene immunomodulation for ovarian cancer treatment.

Er Yue, Edward Wang

Department of Medical Oncology & Therapeutics Research, City of Hope Comprehensive Cancer Center and Beckman Research Institute, Duarte, CA

Ovarian cancer (OC) remains a challenging malignancy with limited treatment options and a poor prognosis, emphasizing the urgent need for innovative therapeutic strategies. We have established a conditionally replicating oncolytic adenovirus (CRAd) platform designed to selectively target CA125 transactivation for OC treatment. To enhance therapeutic efficacy, we engineered the CRAd to include two gene-expression cassettes in the E3 region and each cassette can express two genes linked by a P2A peptide, enabling the incorporation of four exogenous genes in one virus. We rationally chose the following cytotoxic and immunoregulatory genes to integrate into one virus to enhance oncolytic and immunoregulatory capacity and amplify the anti-cancer immune response, full-length tumor necrosis factor-related apoptosis-inducing ligand (fl-TRAIL), CD137L, granulocyte-macrophage colony-stimulating factor (GM-CSF), and interferon alpha-2b (IFN-α2b). We successfully generate the virus by transfecting the recombined Ad/Muc16 genome into HEK293 packaging cells. Ovarian cancer cells expressing CA125 were infected with Ad/Muc16, and expression of all four genes were confirmed via ELISA or flow cytometry. The anti-cancer efficacy of Ad/Muc16 was evaluated in immune-competent syngeneic mouse models of ovarian cancer (ID8/C57BL/6 and STOSE/FVB mice). Mouse ovarian cancer cells (ID8 and STOSE) infected with Ad/Muc16 were served as a whole-cell cancer vaccine in a subcutaneous inoculation model to establish an immune response. The vaccinated mice were re-challenged with parental ovarian cancer cells. Ad/Muc16 treatment elicited a robust anti-cancer immune response, significantly suppressing the growth of re-challenged parental cells and prolonging survival compared to control groups. This CRAd platform integrate with multiple genes demonstrates a novel strategy for specific targeting OC by combining oncolytic virotherapy with immune modulation.

#952 Intravesical instillation of a TME engineering viral vector increases survival in an orthotropic bladder cancer model.

Emma Eriksson, Ioannis Nikoloudis, Mariela Mejia Cordova, Tanja Lovgren, Angelica Loskog

Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden

Gene engineering of the tumor microenvironment (TME) with viral vectors encoding immunostimulatory genes can inflame the TME and create the basis for an immune response against the tumor cells. Adm703 is an adenoviral vector based on the AdEasy system. Adm703 encodes for two murine immunostimulatory genes, trimerized membrane-bound CD40L and 4-1BBL that are expressed by all infected cells (tumor and stroma). The transgenes can induce maturation of dendritic cells and expansion and memory formation of T cells as well as shift the general pro-tumorigenic environment towards anti-tumor state by influencing both tumor and stroma cells. Non-muscle invasive urinary bladder cancer is currently treated with bacillus Calmette-Guérin (BCG) instillations followed by nadofaragene firadenovec-vncg for BCG refractory patients. If the tumor becomes muscle invasive, the patients are subjected to radical cystectomy in which the urinary bladder is removed. This surgery is severely disabling and there is an urgent need to develop a treatment that will lead to a long lasting immune control with a possibility to delay or avoid surgery. We hypothesize that LOAd703, the human equivalent to Adm703, may be an interesting option for these patients. An orthotopic bladder cancer mouse model was treated intravesically with Adm703 to evaluate if Adm703 can control tumor growth. Bladders of female C57Bl/6 mice were incubated with poly-L-lysin to enhance tumor uptake before implantation of MB49 cells. The mice received four intravesical treatments with Adm703 or sodium chlorine (control) on day 1, 8, 15, 22 after tumor instillation. Mice were monitored for hematuria which is an early sign of tumor take. If more than 10 percent weight loss occurred the mice were sacrificed. The mice treated with Adm703 had less hematuria during treatment and had increased survival (mOS=40 days) compared to the control group (mOS=12 days). In conclusion, TME gene engineering with TMZ-CD40L and 4-1BBL increased survival of mice with orthotopic bladder cancer. Further studies investigating Adm703's effect on the immune system are planned.

#953 Oncolytic viruses as in situ personalized tumor vaccines for durable efficacy.

William WEI GUO Jia

Virogin Biotech Ltd, Richmond, BC, Canada

Tumor vaccines have shown clinical promise in treating certain cancers. However, their long-term effectiveness is often hindered by two major challenges:

1. The immune-suppressive tumor microenvironment (TME).
2. Selective targeting of antigen-positive tumor cells, which can lead to the expansion of antigen-negative cells and eventual tumor relapse.

To address these challenges, we explored a combination approach using mRNA tumor vaccines and oncolytic virotherapy in HPV and HER2 mouse tumor models. Our findings reveal that while mRNA vaccines effectively eradicate antigen-positive tumors and suppress tumor growth, resistance quickly emerges, enabling tumors to evade the vaccine's effects. When mRNA vaccination was followed by intratumoral administration of an oncolytic virus—either with or without HPV or HER2 antigen expression—a significantly more durable anti-tumor response was achieved in both models. The oncolytic virus initiated broad tumor antigen presentation by lysing tumor cells, releasing diverse tumor-associated antigens, and increasing the overall antigenic load within the tumor. Furthermore, the lytic activity of the virus reprogrammed the TME, transforming it from an immune-suppressive environment into one more favorable for anti-tumor immune responses. This combination therapy elicited a potent local immune response, leveraging enhanced antigen presentation by the virus. The modified TME allowed the systemic immune response generated by the mRNA vaccine to penetrate and act effectively within the tumor. Additionally, the treatment induced systemic antigen-specific T-cell responses, and treated animals developed resistance to subsequent tumor challenges, demonstrating the establishment of immune memory. Our study highlights the unique role of oncolytic virotherapy as an intratumoral "boost" mechanism. By lysing tumor cells, oncolytic viruses amplify the range of tumor antigens presented to the immune system and reshape the TME to support robust immune activity. This synergy between mRNA vaccination and oncolytic virotherapy—when employed in a prime-boost regimen—produces a powerful and durable anti-tumor immune response that is both localized and systemically protective against recurrence. In conclusion, this research underscores the potential of combining mRNA tumor vaccines with oncolytic virotherapy to overcome immune evasion mechanisms, enhance tumor antigen presentation, and reprogram the TME, providing a promising strategy for achieving long-lasting anti-tumor immunity.

#954 Preoperative p53-armed oncolytic virus therapy enhances the efficacy of postoperative adjuvant ICI therapy.

Daisuke Kadowaki[1], Shinji Kuroda[1], Makoto Matsumoto[1], Hitoshi Minagi[1], Tetsuya Katayama[1], Yu Mikane[1], Shunya Hanzawa[1], Masashi Hashimoto[1], Nobuhiko Kanaya[1], Yoshihiko Kakiuchi[1], Satoru Kikuchi[1], Kazuhiro Noma[1], Hiroshi Tazawa[1], Shunsuke Kagawa[1], Yasuo Urata[2], Toshiyoshi Fujiwara[1]

[1]Okayama Univ. Graduate School of Med., Dentistry & Pharm. Sci., Okayama, Japan,[2]Oncolys BioPharma, Tokyo, Japan

Background : In recent years, the importance of perioperative treatment in cancer therapy has increased, and postoperative adjuvant therapies using immune checkpoint inhibitors (ICIs) have been applied clinically in multiple cancer types. However, the efficacy of ICIs as monotherapy is often limited. We have developed a telomerase-specific oncolytic virus (OV), OBP-301, and its p53 gene-armed derivative, OBP-702. OBP-301 is currently undergoing clinical trials. Previous studies have demonstrated enhanced antitumor immunity, including an increase in CD8+ T cells following OV administration. This study investigates the combination effect of preoperative OV therapy and postoperative adjuvant ICI therapy.

Methods : Experiments were conducted using C57BL/6 mice implanted with MC38 colon cancer cells (p53 mutant/MSI-H) and PAN02 pancreatic cancer cells (p53 wild/MSS). OBP-702 was used as the OV, and anti-PD-1 antibody was used as the ICI. A subcutaneous tumor model in mice was employed to evaluate the therapeutic effects and immune responses using flow cytometry under a protocol combining preoperative OV therapy and postoperative ICI therapy. Additionally, Bulk RNA sequencing was performed to further analyze the mechanisms of enhanced antitumor immunity.

Results : The OV-treated group exhibited significantly enhanced antitumor effects compared to the PBS-treated group. Analysis of tumor-infiltrating lymphocytes (TILs) in MC38 tumors revealed an increase in resident memory CD8+ T cells, PD-1+ CD8+ T cells, and p15E tetramer+ CD8+ T cells. Ex vivo CTL assays showed that TILs induced by OV exhibited stronger tumor specificity and antitumor activity. Additionally, in the OV group, p15E tetramer+ CD8+ T cells persisted significantly in the spleen for three weeks following primary tumor resection. In a rechallenge model where new tumors were established after tumor resection, preoperative OV therapy was shown to suppress postoperative recurrence and tumor growth while enhancing the effect of postoperative ICI therapy. Bulk RNA-seq of OV treated MC38 tumor with reactome pathway analysis revealed the upregulation of pathways associated with enhanced antitumor immunity.

Conclusion : OV therapy not only demonstrated direct antitumor effects but also induced tumor-specific systemic immunity and sustained long-term immune activation. These results suggest that preoperative OV therapy can enhance the effectiveness of postoperative ICI therapy.

#955 Single cell sequencing delineates immune features of oncolytic virus therapeutic response in murine high-grade gliomas.

Jack Hedberg, Alexia Martin, Doyeon Kim, Jaye Navarro, Adithe Rivaldi, Yeaseul Kim, Elizabeth Garfinkle, Katherine Miller, Ravi Dhital, Kevin Cassady, Elaine Mardis

Nationwide Children's Hospital, Columbus, OH

Oncolytic virotherapy (OV) comprises a developing family of cancer treatments that exploit cancer-specific vulnerabilities in viral defense through treatment with therapeutic viruses modified for safety and tumor-selective infection. Beyond direct infection and lysis of cancer cells, OVs are additionally capable of stimulating powerful immune responses that pose an opportunity to redefine the immune system's behavior in the tumor microenvironment. This may hold particular significance for highly immunosuppressive malignancies, including glioblastoma and other high-grade gliomas (HGG). Our labs developed OV C134, now under evaluation in a phase I clinical trial of recurrent glioblastoma (NCT03657576). While additional refinements to the activity and control of OVs within the tumor microenvironment may lead to superior performance, little is known about the exact features or timing of immune changes undergone in the setting of positive therapeutic response to oncolytic virus treatment. Here, using the widely-adopted C57BL/6 syngeneic high-grade glioma model CT2A modified to express luciferase, we applied bioluminescence imaging to measure tumor progression in vivo and identify therapeutic responders after intracranial treatment with C134, C002 (IL-12-expressing C134), or control (saline). We then performed single cell RNA sequencing of CD45-sorted high-grade glioma immune infiltrate (n=10 mice total; n=3 C002 responder and n=3 C002 nonresponder). Analysis of single cell sequencing data revealed that OV treatment produced diverse changes in gene expression consistent with activation of antigen presentation and antigen-specific immune responses throughout a variety of innate and adaptive immune populations. Further, cell type-specific immune signatures of C002 therapeutic responders as compared to nonresponders were identified, including responder-specific increases in major histocompatibility complex class II in macrophages/monocytes, interferon gamma in CD4+ and CD8+ T cells, tumor necrosis factor-alpha in CD4+ T cells, and a decrease in interferon gamma receptor 1 in T regulatory cells. Collectively, our findings contribute to the knowledge of how OV therapy alters tumor immune microenvironments and reveal prioritized candidates whose further study could advance the understanding of how to achieve positive responses to OV therapy in patients.

#956 HB-200: A novel viral vector vaccine platform that can improve immunotherapy treatment in HPV16+ HNSCC.

Sydney Fisher, Matthew Ji, Yuxuan Miao

The University of Chicago, Chicago, IL

Human papillomavirus-associated (HPV+) oropharyngeal squamous cell carcinoma (OSCC) is the most common head and neck cancer in the United States. While many HPV+ OSCC are curable using current standard treatment, the efficacy of these treatments diminishes when the cancers become metastatic. The viral associated malignancy provides unique opportunities for immunotherapy, however, immune checkpoint inhibitors only induce weak and unsustainable immunity against HPV+ cancer. Thus, there remains a need for new treatment modalities that improve outcomes while also reducing adverse effects. Previously, HB-200, a novel HPV-specific therapeutic vaccine containing two replicating live-attenuated vectors expressing the highly antigenic HPV16 E7E6 protein, has been shown to elicit a more robust anti-tumor immune response alongside chemotherapies compared to standard immunotherapies in clinical trial. However, the underlying mechanisms of why this vaccine can induce stronger immune response than immune checkpoint blockade remains unclear. Here, we utilize mouse models of HPV16 E6/E7+ OSCC to elucidate how HB-200 enhances anti-tumor immunity. To achieve this goal, we first established two complementary HPV16 E6/E7+ oral SCC tumor models: 1) grafting syngeneic MOC2 cells expressing the E6 and E7 oncoproteins of HPV16 on immune-competent C57BL/6 mice; 2) exposing the oral cavity of K14CreER; Rosa26-LSL-E6/E7 mice with 4-NQO to induce spontaneous oral SCCs. With these novel models, mice were then treated with either anti-PD-L1 or HB-200 plus chemotherapy to mimic clinical trials in humans. At 4 weeks post-tumor grafting, we profiled the immune landscape of these tumors by flow cytometry to quantify the frequency of specific CD8 T cell, CD4+ Tfh cell, and B cell populations which we hypothesized to be enriched following HB-200 treatment. This study addresses the central question of how viral immunotherapy can induce stronger immune responses than standard immune checkpoint immunotherapy and better enable T cells to achieve tumor control in HPV-associated cancer, providing an important mechanistic understanding of the HB-200 vaccine platform. Thus, findings from this study give critical insights for optimizing HPV-specific immunotherapy with the exciting potential to translate into novel clinical interventions for HPV+ OSCC patients.

#957 A novel therapeutic modality integrating antibody therapy and oncolytic virotherapy for enhanced tumor targeting and treatment.

Wenxin Xu[1], Can Chen[2], Huan Zheng[1], Jieying Xu[1], Mengshi Jiang[1], Li Zhang[1], Deyu Xu[1], Hongjie Cheng[1], Yang Zhang[1], Mengmeng Liu[1], Jinlu Cai[2], Fang Hu[2], Lieming Ding[1]

[1]Betta Pharmaceuticals Co., Ltd., Hangzhou Shi, China,[2]Hangzhou Converd co., Ltd, Hangzhou Shi, China

Background: In contemporary oncopharmacology, antigenic targets remain a pivotal focus, especially within the realm of immunotherapy, where tumor-specific antigens are of critical importance. Extensive research on these antigens has spurred the development of innovative therapeutic modalities, such as antibody therapies, CAR-T/NK cell therapies, XDC conjugate drug therapies, tumor neoantigen therapies, and gene therapies. However, these therapeutic strategies encounter significant challenges related to antigen target specificity. Although engineered antigen-targeting drugs typically provide high specificity and low toxicity, they can still disrupt normal signaling pathways and produce off-target effects, leading to adverse reactions that may compromise treatment duration and patient outcomes.To mitigate these challenges, we have devised a novel oncological strategy that synergizes antibody therapy with oncolytic virotherapy. This approach uses a genetically modified oncolytic virus to present a human-absent antigen on the surface of tumor cells, which is then targeted by a CD3 bispecific antibody to achieve the desired therapeutic effect. This combinatorial approach could expand treatment options for cancer patients, especially those with resistant tumors. It may also alter the tumor microenvironment, creating a bystander effect that boosts treatment effectiveness.

Methods: Through comprehensive mega data analytics, non-primate endogenous antigenic proteins were identified. Advanced genetic engineering techniques were employed to integrate the antigenic complex TT3 into the genome of a genetically modified oncolytic vaccinia virus, ensuring stable expression on the surface of virus-infected tumor cells. Then a TT3/CD3 bispecific antibody, designated BK-001, was developed. The binding affinity of BK-001 was rigorously evaluated using ELISA, FACS, and BLI methodologies. The functional potency was determined using RGA, and in vitro cytotoxicity assessed via RTCA. Anti-tumor efficacy of BK-001 was validated using preclinical models, and a comprehensive non-clinical safety assessment was conducted on cynomolgus monkeys.

Results and Conclusion: BK-001 is a bispecific T-cell engager antibody designed to boost the immune response against tumors infected by oncolytic viruses, promoting the destruction of cancer cells and improving the tumor microenvironment. The activation of BK-001 relies on the presence of TT3 antigen, which guarantees targeted immune response against tumors. In efficacy studies, BK-001 demonstrated robust tumor growth inhibition at minimal doses, with statistically significant differences compared to control groups. Furthermore, BK-001 exhibited prolonged activity and a favorable safety profile, underpinning its clinical development potential when used in conjunction with oncolytic virus therapy.

#958 Myeloid cell populations drive early Vaccinia-induced anti-tumor responses differentially based on tumor cell immunogenicity.

Duale Ahmed[1], Robyn Skillings[1], Omar Abdo[1], Aroosha Fareghdeli[2], Zoya Versey[1], Alicia Boxma[1], Leila Mostaco-Guidolin[2], Edana Cassol[1]

[1]Department of Health Sciences, Carleton University, Ottawa, ON, Canada,[2]Department of Systems and Computer Engineering, Carleton University, Ottawa, ON, Canada

Cancer poses an enormous burden on the healthcare system, with approximately 1 in 5 people worldwide expected to develop cancer in their lifetime according to the World Health Organization. Given this, there is a significant need for new therapeutics that are highly selective and evade tumor resistance to therapy. Oncolytic viruses (OVs) have been shown to selectively target and kill cancer cells while simultaneously activating local and systemic immune responses. Among these, Vaccinia (VACV) virus is a potential OV candidate with a high safety profile and a large genome, providing opportunities for genetic modification. However, in clinical trials it has shown to have limited success. To improve its efficacy, we need a more thorough understanding of VACV's immunomodulatory activity both within and across distinct tumor microenvironments (TME). In these studies, we treated weakly immunogenic (B16-F10) and highly immunogenic (MC38) tumors with a mouse-adapted wild-type VACV (3 intratumoral injections, 48 hours apart). Changes in immune cell infiltration and tumor associated macrophage (TAM) activation were assessed in tissues collected 24 hours after each injection and up to 21 days after completion of the treatment regimen. We found that VACV treatment significantly reduced tumor volume and was associated with an early influx of CD45+ cells across both models. This infiltration occurred earlier, was more pronounced, and lasted longer in MC38 vs. B16-F10 tumors. While both models were associated with a significant decrease in the relative frequency of immunosuppressive TAMs (24 hours after first injection), VACV treatment of MC38 tumors was associated with higher and more sustained levels of newly recruited inflammatory monocytes. Similarly, the MC38 TME maintained higher levels of activated TAMs (↑CD80/CD86 expression) and sustaining this phenotype for a longer period. Importantly, these changes were linked to increased infiltration of CD8 T cells, anti-tumor immune responses and a slower rebound of tumor growth (>2 weeks). Collectively, our results suggest that VACV treatment can help limit tumor growth in both MC38 and B16-F10 models but that the anti-tumor response is more pronounced and sustained in highly vs. weakly immunogenic tumors. This increased activity is not dependent on early loss of immunosuppressive TAMs but is dependent on the increased recruitment of inflammatory monocytes and the maintenance of activated TAMs in the TME. Further studies are required to assess the specific contribution of these cells in driving anti-tumor immune responses and whether sustained viral replication is required for these processes.

#959 Engineering tumor-avid bacteria as anticancer immunotherapy.

Noah Chen[1], Mathieu Rouanne[1], Zaofeng Yang[1], Tal Danino[2], Nicholas Arpaia[1]

[1]Columbia University Irving Medical Center, New York, NY,[2]Columbia University, New York, NY

The observation that bacteria colonize tumors, but not normal organs, after intravenous administration has spurred new interest in bacteria as tumor-targeted delivery systems. Since bacteria replicate within the tumor site, intravenous administration of bacteria leads to specific expression of peptide-based therapeutics at the tumor site. However, most prior bacterial cancer therapies have relied on this natural tumor tropism since the mechanisms behind this tumor-specificity are poorly understood. Additionally, clinical trials with bacterial therapeutics have shown lower tumor colonization rates in humans, which limits their translatability. This study used a pooled barcode library approach to screen the *E. coli* genome for pathways which may influence tumor colonization. We found specific pathways, include glucose catabolism, which promote *E. coli* tumor colonization, and non-glucose catabolic pathways which inhibit *E. coli* tumor colonization. KO of these inhibitory metabolic pathways increase *E. coli* glucose uptake and utilization. This was associated with increased tumor colonization as validated by competitive infection experiments, which was further enhanced when glucose was introduced into the tumor microenvironment. Together, these results support a major role for *E. coli* glucose catabolism in defining bacterial tumor tropism and suggest a promising method by which bacterial tumor tropism can be fine-tuned.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#96 Elucidating the role of CXCL17/GPR35 axis in breast cancer bone metastasis.

Gunjan Sharma, K. M. Abdullah, Seema Singh, Ajay P. Singh, Jawed A. Siddiqui

Cell and Molecular Biology, University of Mississippi Medical Center, Jackson, MS

Breast cancer (BC) has a high propensity to metastasize to the bone, but the molecular mechanisms driving bone metastasis (BoM) are not yet fully understood. BC cells establish bidirectional interactions with the bone microenvironment through altered expression of several chemokines and their receptors to facilitate metastasis and niche formation. Various G protein-coupled receptors are involved in oncogenic processes. Recently, it has been reported that G protein-coupled receptor 35 (GPR35), a cognate receptor for CXCL17, plays a significant role in tumor progression, metastasis, and drug resistance; however, its role in the modulation of bone microenvironment has not been elucidated. Here, we analyzed the expression of GPR35 in BC patients using the TCGA database. Furthermore, we also employed immunohistochemistry to examine the expression profile of GPR35 in BC tissue microarray. Our findings revealed high GPR35 expression in BC relative to the normal breast tissue. High GPR35 expression in BC also correlated positively with poor patient outcomes. Moreover, GPR35 expression was elevated in human as well as syngeneic BC bone metastatic cell lines (SCP2, SCP28, and E0771/Bone) compared to their relative parental cell lines (MDA-MB-231 and E0771P), advocating its significant potential in bone metastasis. Since most of the BC bone metastasis is osteolytic (bone destructive) in nature, and osteoblasts (OBs) regulate the osteoclasts (OCs) functions, therefore we examined the effect of conditioned media (CM) from the BC cells treated with either vehicle or rhCXCL17 on OBs and OCs. BC-CM induced bone cell differentiation, which was remarkably significant in OBs and OCs treated with BC-CM of rhCXCL17-treated cells. Together, these findings highlight the critical role of the CXCL17/GPR35 signaling axis in bone microenvironment remodeling supporting BC cell colonization.

IMMUNOLOGY: Viruses and Bacteria in Cancer Therapy
Poster Session

#961 Scalable isolation of bacterial extracellular vesicles for macrophage-activating immunotherapy.

Irem Karaman, Asmita Pathak, Oriana Teran Pumar, Brandon Emanuel Leon, Jonathan Mitchell, Carlos David Oliva, Selma Maacha, Wael El-Rifai, Defne Bayik, Dionysios C. Watson

University of Miami Miller School of Medicine, Miami, FL

The efficacy of immune checkpoint inhibitors (ICIs) is hampered by tumor-associated immunosuppressive myeloid cells. Secreted nano-sized (~25-100 nm) bacterial extracellular vesicles (bEVs) modulate myeloid cells in the context of infection and colonization, and are being developed for cancer immunotherapy. However, lack of standardized bEV purification strategies limit reproducibility across laboratories and hamper translational development. Herein, we aimed to develop a scalable isolation workflow for bEV therapeutics. We grew the human commensal strain *E. coli Nissle* to early stationary phase to generate bEV-rich conditioned cell culture medium; removed bacteria by centrifugation and filtration; concentrated conditioned medium by tangential flow filtration (TFF; 100 kDa MWCO); and purified bEVs by semi-automated size-exclusion chromatography (SEC). During TFF concentration, we observed conditioned media becoming turbid, suggesting prescipitation of macromolecules, including potentially nucleic acids. Indeed, when we assessed DNA content at sequential purification steps via gel electrophoresis, and found that commercial bacterial culture media contains significant amounts of DNA which co-isolate with bEVs. We thus expanded our workflow to include clearance of baseline culture medium nucleic acid content by 30 kDa ultrafiltration of the culture medium prior to use. In addition, we treated TFF-concentrated conditioned media with the promiscuous DNAse/RNAse Benzonase (25 units/mL) which reduced nucleic acid content of bEVs by 66%, suggesting that Benzonase further increased bEV purity by depleting nuclease-sensitive non-vesicular nucleic acids. After SEC, we identified bEV-containing fractions by DNA/RNA/protein quantification, transmission electron microscopy and nanoparticle tracking analysis. Next, we assessed the immunologic activity of bEVs ex vivo by adding to cultured mouse splenocytes and bone marrow cells, and observed broad effects on myeloid cells and lymphocytes. Our optimized workflow provides a broadly applicable approach for isolating bEVs from species requiring complicated medium. This standardized method will enhance reproducibility and minimize non-specific effects of contaminants, facilitating translational development of bEV immunotherapy. Future experiments will test the role of bEVs from different microbial species in augmenting ICI efficacy in mouse models of cancer.

#962 Activation of natural killer cells by small molecule inhibits growth of BCG-resistant bladder cancer cells.

A. Gupta, S. Verma, S. Gupta;
Case Western Reserve University, Cleveland, OH

Bacillus Calmette-Guérin (BCG) is the standard treatment for non-muscle invasive bladder cancer (NMIBC) patients, but resistance declines its efficacy, emphasizing the need for alternative therapies. BCG therapy triggers a specific cytotoxic immune response by activating and expanding CD56/natural killer (NK) cells and T cells, which effectively target and eliminate bladder cancer cells. This priming of NK and T cells by BCG leads to the release of cytokines such as interferon-gamma (IFNγ) and tumor necrosis factor-alpha (TNFα), which stimulate adaptive immunity and enhance tumor cell destruction. Evidence suggests that impaired T-cell and cytotoxic responses are key factors in immunotherapy failure. We designed the study to identify compounds which have the ability to activate the NK cells and generate a robust immune response within the bladder microenvironment. Our preliminary studies exhibited a notable elevation in HLA-B expression and an increased NK cell presence in recurrent NMIBC patients. Yet, despite the increased NK cell population, our findings suggest that NK cell activation may be insufficient, possibly due to inhibitory signals that suppress NK cell cytotoxicity. Bioinformatics analyses revealed a strong association between HLA-B and the NK cell inhibitory receptor KIR3DL3, prompting us to explore potential inhibitors of this interaction. In-silico screening identified 4′,5,7-trihydroxyflavone, a natural flavone as a small-molecule blocker of the KIR3DL3-HLA-B interaction. We hypothesized that inhibiting this pathway, 4′,5,7-trihydroxyflavone could potentially reduce inhibitory signaling, thus enhancing NK cell-mediated cytotoxic activity against bladder cancer cells. Treatment of BCG-resistant human bladder cancer T24 cells with BCG or 4′,5,7-trihydroxyflavone alone for 8 hours no notable effect was noted on cell viability. Incubation of NK cells with 4′,5,7-trihydroxyflavone, without BCG, resulted in a significant increase in cancer cell death, indicating that the compound alone could induce a potent cytotoxic response. Furthermore, qRT-PCR analysis demonstrated elevated expression of CD244, a type-I transmembrane protein involved in immune system regulation, and CD160, a glycoprotein closely linked to NK cell activity, specifically following treatment with 4′,5,7-trihydroxyflavone but not with BCG. Taken together, our data provide evidence that 4′,5,7-trihydroxyflavone may act as a reactivator of NK and T cell-mediated responses in BCG-resistant bladder cancer cells. By effectively blocking the inhibitory KIR3DL3-HLA-B interaction, this compound enhances NK cell cytotoxicity, resulting in a higher percentage and more sustained immune-mediated cancer cell death. This suggests a promising therapeutic approach for patients who are unresponsive to BCG, potentially leading to improved outcomes in resistant NMIBC.

#963 Evolved therapies specifically activate and expand endogenous γ;δ; T cells to remove the barriers to broadly effective immunotherapies.

Jonathan W. Kotula, Ed Lemmens, Rafael Rivera-Lugo, Ying Feng, Daniel A. Portnoy

Laguna Biotherapeutics, Inc., San Francisco, CA

Live bacterial therapeutics have the potential to generate transformative outcomes for cancer patients. CAR-T cells are a synthetic attempt to combine B and T cell biology to maximize targeted killing and deliver abundant and active cells to overcome the limitations of endogenous immune cells. Although these therapies have provided some immunotherapy success, they are limited by practical challenges such as an inability to manufacture sufficient material, extreme cost, an inability to re-dose, and severe acute toxicities.

In contrast, $\boldsymbol{\gamma}$;$\boldsymbol{\delta}$; T cells have emerged as an exciting alternative to CAR-T. Because they have evolved to fill a unique niche at the interface of the innate and adaptive immune system they may be a solution to the limitations of CAR-T. Where CAR-T cells carry the risk of GvHD, $\boldsymbol{\gamma}$;$\boldsymbol{\delta}$; T cells are MHC-independent, and possess multiple checkpoints that prevent killing healthy cells. The tumor microenvironment prevents CAR-T penetration, but resident $\boldsymbol{\gamma}$;$\boldsymbol{\delta}$; T cells exist within every tissue, awaiting activation and expansion. $\boldsymbol{\gamma}$;$\boldsymbol{\delta}$; T cell dominance of immunotherapy has so far been prevented by our inability to durably or repeatedly expand endogenous cells.

Our solution to these limitations is to exploit the natural properties of Listeria monocytogenes to induce the proliferation and durable activation of endogenous $\boldsymbol{\gamma}$;$\boldsymbol{\delta}$; T cells. Our pre-clinical *in vitro* and *in vivo* data, combined with results from previous clinical studies show that Listeria expanded endogenous $\boldsymbol{\gamma}$;$\boldsymbol{\delta}$; T cell populations to >1x10^9 cells, orders of magnitude more than other approaches. Furthermore, Listeria-activated $\boldsymbol{\gamma}$;$\boldsymbol{\delta}$; T cells are uniquely cytotoxic, durable, and safe. They eliminate cancer cell lines in vitro at E:T ratios of ~1, whereas antibody and cell therapies require E:T ratios >10. They down-regulate exhaustion markers and remain active for multiple rounds of cell killing, and they express 5-fold less IL-6, the primary indicator of CRS toxicity. Listeria-activated $\boldsymbol{\gamma}$;$\boldsymbol{\delta}$; T cells are more abundant, cytotoxic, durable, and less toxic than CAR-T, and they can be re-dosed for persistent efficacy.

To improve on the safety profile of previous Listeria-based therapeutics, we have generated a quadruple attenuated strain (QUAIL) that cannot grow extracellularly. Our *in vitro* and *in vivo* data show that QUAIL cannot grow in human serum or on stents or indwelling ports, where previous therapeutic strains thrived, and cannot persist in RAG1 -/- mice that lack T and B cells, all while maintaining the $\boldsymbol{\gamma}$;$\boldsymbol{\delta}$; T cell activation and expansion that was observed in cancer patients. QUAIL will enter the clinic in multiple myeloma in 2025, and we believe that this novel therapy will generate meaningful and lasting cancer remission where current cell therapies have provided a glimpse of what's possible.

POPULATION SCIENCES: Bridging Gaps in Cancer Care: Disparities, Social Determinants, Innovations, and Strategies Poster Session

#967 Geographic and social determinants of late-stage breast cancer diagnoses in Guam: The role of social vulnerability and access to care.

Michael Basaliza Cajigal, Grazyna Badowski, Louis Jane B. Dulana

University of Guam, Mangilao, GU

Background: Breast cancer is the second leading cause of cancer deaths among women in Guam. Despite a lower incidence than the mainland U.S. (86.6 vs. 126.8), Guam has a higher mortality rate (27.3 vs. 20.3, 2013-2017), suggesting that late-stage diagnoses and social determinants of health (SDOH) contribute to poorer outcomes. Access to mammography screening clinics is uneven across Guam, with most facilities concentrated in the northern and central regions. The Social Vulnerability Index (SVI), a measure of SDOH, offers a framework to explore these disparities. This study investigates geographic variation in late-stage breast cancer rates across Guam's municipalities and the association with municipal-level SVI scores.
Methods: A cross-sectional analysis was conducted using data from the Guam Cancer Registry for all breast cancer cases diagnosed between 1998 and 2021. Late-stage rates were calculated for each municipality. Socioeconomic vulnerability was measured using Guam-specific Social Vulnerability Indices (SVIs), derived from 15 variables reflecting socioeconomic and demographic factors, with adjustments for additional Guam-specific characteristics. The beta regression model with mixed effects was applied to examine the relationships between advanced-stage diagnoses and SVI.
Results: Overall, 36% of breast cancer cases were diagnosed at a late stage, with regional disparities ranging from 14% in certain areas to as high as 40% in others. These variations underscore significant geographical differences in healthcare access and early detection. Beta regression analysis revealed that higher scores on the Social Vulnerability Index (SVI), which captures socioeconomic and environmental risk factors, were significantly associated with increased rates of late-stage diagnosis ($PR = 1.04$, $p = 0.02$). Women residing in the South region of Guam were particularly at risk, showing a 43% higher likelihood of late-stage diagnosis compared to those in the central region ($PR = 1.43$, $p = 0.01$).
Conclusion: Significant disparities in late-stage breast cancer diagnoses exist in Guam, especially among areas with higher social vulnerability. Living in the South region and limited access to mammography services increased the likelihood of late-stage diagnosis. These findings highlight the urgent need for targeted public health interventions to improve early detection, address social inequities, and enhance healthcare access across Guam.
Acknowledgement: This research was funded by the National Cancer Institute: The University of Guam/University of Hawaii Cancer Center Partnership to Advance Cancer Health Equity (PIPCHE), Grant U54CA143728 (University of Guam)/Grant U54CA143727 (University of Hawaii Cancer Center).

#968 Predictors of advance care planning documentation in female patients with breast cancer: A multilevel model analysis.
A. Garcia Sierra, M. Tan;
The University of Chicago Medicine Comprehensive Cancer Center, Chicago, IL

Background: Advance care planning (ACP) is a process that supports adults at any age or stage of health in understanding and sharing their personal values, life goals, and preferences regarding future medical care. Likewise, ACP is associated with multiple benefits, such as lower rates of hospitalizations and reduced futile, aggressive cancer treatment at the end of life. However, ACP rates remain low in the U.S., especially for patients with advanced cancer. Despite being more likely to be diagnosed at advanced stages and hospitalized due to advanced cancer with poor prognosis, Black and Hispanic/Latino populations continue to have very low rates of ACP. Research has shown that both individual-level and systems-level factors contribute to these racial disparities in ACP. Considering these disparities among vulnerable populations, interventions that increase ACP among minority patients with cancer are needed.
Objective: To analyze using multilevel model analysis the predictors of ACP documentation in female patients with breast cancer over 5 years in the UChicago Health System.
Methods: Using the Clinical Data Research Warehouse, this study included women diagnosed with breast cancer (N=7,814) from 2016 to 2021. Descriptive statistics were performed to characterize ACP, and potential differences in ACP rates among control variables were assessed using odds ratio (OR) estimations. Analyses were conducted using random effects (mixed effects) multilevel modeling. To account for individual differences in ACP documentation completeness over time, both intercept and slope were included as random effects. In the multilevel model, level 1 modeled individual ACP documentation and level 2 tested the group effects in ACP documentation. The effect of time was tested in level 1, and the variances in the intercept and slopes were represented in level 2.
Results: 8.74% of women with breast cancer had at least one type of ACP documented in their medical records; 4.25% had a power of attorney and 5.75% had an advanced directive. Age and the time elapsed between diagnosis and ACP completion are related to an increased likelihood of ACP completion over time. Being divorced increased the likelihood of ACP completion at baseline. Outpatient services decreased the likelihood of ACP completion over time. Conclusion: Understanding the factors that are related to ACP documentation will allow us to manage them and improve their completion. These results will help to inform the development of tools to increase the integration of ACP into care, especially those that are technology-based. Further work could include the design of a predictive model to prioritize patients who require ACP. Providing knowledge about ACP in nonclinical areas can lead to increased education about ACP and its benefits to families and communities.

#969 Deciphering sex differences in clear cell renal cell carcinoma at the single-cell resolution.

Xiangwei Fang, Yize Li, Xiyi Wei, Siqi Chen, Chia-Kuei Mo, Wagma Caravan, Preet Lal, Ruiyang Liu, Ilya Strunilin, Yizhe Song, Yuwei Zhang, Song Cao, Cody Weimholt, Feng Chen, Li Ding

Washington University in St. Louis, School of Medicine, St. Louis, MO

Clear cell renal cell carcinoma (ccRCC) is the most prevalent subtype of renal cell carcinoma, accounting for approximately 70% of cases. Strikingly, ccRCC exhibits a marked sex disparity in prevalence, being significantly more common in men than women. This disparity underscores the importance of systematically investigating sex differences to elucidate their implications for the diagnosis, prognosis, and therapeutic strategies of ccRCC. To address this, we generated a comprehensive single-nucleus sequencing atlas (snRNA-seq and snATAC-seq) comprising over 400,000 nuclei from 65 ccRCC cases. This dataset was complemented by paired bulk proteogenomic data, enabling a multi-layered analysis of the molecular landscape. The study cohort, including 21 females and 44 males, reflects the sex distribution observed in ccRCC incidence within the general population. Our analysis uncovered sex-associated molecular differences in key pathways driving ccRCC progression. These include genes regulating the hypoxia-inducible factor (HIF) pathway, a hallmark of ccRCC, as well as lipid metabolism and energy homeostasis pathways, which are critical to ccRCC progression. Furthermore, genomic characterization revealed significant sex differences in tumor mutational burden (TMB) and specific genetic alterations, including higher mutation frequencies in *KDM5C* and *MTOR* in male tumors. These genetic differences were linked to distinct downstream effects, suggesting sex-specific impacts on tumor biology. These findings illuminate new opportunities for developing sex-informed therapeutic strategies and biomarkers, enabling a more precise and tailored approach to ccRCC screening and treatment.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#97 Microtubule dynamics in regulating bone metastasis of breast cancers: a novel function of tubulin β-2 and tubulin β-3.

Shreya Patel[1], Marcus Winogradzki[1], Waddell Holmes[1], Alan Blank[2], Niyati Patel[1], Jitesh Pratap[1]

[1]Anatomy and Cell Biology, Rush Univ. - Rush Medical College, Chicago, IL,[2]Section of Orthopedic Oncology, Rush Univ. - Rush Medical College, Chicago, IL

Introduction: Bone metastases (BM) represent a significant hurdle in advanced cancer management, with median survival rates of 12 to 50 months. Current treatment options, including surgical resection and microtubule (MT)-targeting chemotherapies like paclitaxel, often provide palliative rather than curative outcomes. MTs, composed of α- and β-tubulin heterodimers, are essential for cell division, migration, and trafficking. Dysregulated expression of β-tubulin isotypes, TUBβ2 and TUBβ3, has been implicated in chemoresistance, yet their specific roles in breast cancer bone metastases (BMBC) remain poorly understood. This study explores the novel functions of TUBβ2 and TUBβ3 and investigates their potential as therapeutic targets by disrupting their unique C-terminal domains.
Methods: RNA-Seq datasets and patient survival data from publicly available databases were analyzed to evaluate the expression of TUBβ2 and TUBβ3. Immunohistochemistry (IHC) was performed on matched primary and BM tumor biopsies (n=16) from BC patients. CRISPR/Cas9-mediated knockdowns (KDs) in bone-derived MDA-MB-231 cells were employed to assess cell growth, survival, adhesion, migration, and metastatic properties. MT polymerization rates were evaluated using MT regrowth assays, subcellular trafficking was analyzed via immunofluorescence imaging. Focal adhesion turnover were assessed by western blotting, and IL6 secretion-analyzed by ELISA.
Results:
Elevated expression levels of TUBβ2 and TUBβ3 significantly correlated with poor survival in breast cancer patients and were markedly higher in BM tumors compared to primary and normal breast tissues. CRISPR KD revealed distinct morphological changes, with TUBβ2KD inducing giant cell formation and TUBβ3KD leading to elongated cytoplasmic extensions.

- Metastatic properties: TUBβ2KD reduced adhesion but enhanced migration, while TUBβ3KD diminished migration but increased adhesion. Reduced focal adhesion kinase (FAK) levels were observed in both KDs, impairing adhesion and migration.
- MT dynamics: KDs caused significant delays in MT repolymerization, indicating isotype-specific integration into MT polymers.
- Cytokine secretion: Loss of TUBβ2 led to a 2-fold increase in IL6 production, whereas TUBβ3 loss resulted in a 70% reduction in IL6 secretion.
- Subcellular trafficking: Loss of perinuclear endosomal puncta revealed novel roles for TUBβ2 and TUBβ3 in endosomal trafficking.

Conclusions and Impact: This study identifies TUBβ2 and TUBβ3 as critical regulators of MT dynamics and metastatic properties in BMBC. Their roles in cell adhesion, migration, and trafficking underscore their potential as therapeutic targets. Current clinical approaches to BM focus largely on palliative care, but targeting TUBβ2 and TUBβ3 offers a promising strategy for more precise interventions that could improve patient survival and quality of life.

POPULATION SCIENCES: Bridging Gaps in Cancer Care: Disparities, Social Determinants, Innovations, and Strategies Poster Session

#970 Unveiling cohort profile and familial aggregation in South Asian American women with breast cancer: Insights from the CANSAH-BC study.

Tina Dharamdasani[1], Anusha Addanki[1], Anushyaa Vasudevan[1], Prachi Trivedi[2], Caroline Morales[3], Lisa Paddock[3], Nur Zeinomar[2], Bo Qin[2], Shridar Ganesan[2], Anita Y. Kinney[2], Elisa V. Bandera[2], Jaya M. Satagopan[1]

[1]Rutgers School of Public Health, Piscataway, NJ,[2]Rutgers Cancer Institute of New Jersey, New Brunswick, NJ,[3]New Jersey State Cancer Registry, Trenton, NJ

Background: The Cancer Analytics and South Asian Health - Breast Cancer (CANSAH-BC) pilot study was launched in New Jersey (NJ) in 2022 to evaluate the drivers of aggressive breast cancer, familial aggregation, and quality of life among South Asian American (SAA) females, who are an understudied population.
Purpose: To compare the CANSAH-BC cohort with SAA patterns in the National Health Interview Survey (NHIS), the California Health Interview Survey (CHIS), & breast cancer survivors identified in the Surveillance, Epidemiology, and End Results (SEER) NJ program, and assess familial aggregation.
Methods: Eligible CANSAH-BC participants were SAA females aged 21+, diagnosed with primary invasive breast cancer in NJ (2017-2023), who provided consent. Sociodemographic, lifestyle factors, family, and medical history were collected via interviews, with tumor data from the NJ State Cancer Registry. We compared CANSAH-BC participants with SAA females from NHIS, CHIS, and SAA breast cancer survivors in SEER using descriptive statistics. Familial incidence rate was calculated by dividing breast cancers in relatives by total person-years. Using the Institute of Health Metrics and Evaluation (IHME) population rates for South Asia, we calculated the standardized incidence ratio (SIR) and its 95% confidence interval (CI). Assuming a lognormal distribution for breast cancer risk and leveraging the known relationship between SIR and coefficient of variation of the risk distribution, we estimated the proportion of breast cancers in SAA women at highest and lowest 25% risk.
Results: Among 101 CANSAH-BC participants, most were first-generation immigrants (94%), married (86%), insured (99%), and non-smokers (99%). Compared to NHIS and CHIS, more participants held a college degree (71%, 82%, 87%, respectively), were employed (62%, 68%, 73%, respectively), and had hypertension (17%, 11%, 33%, respectively) or diabetes (9%, 8%, 22%, respectively). Stage (60% localized) and triple-negative subtype (10%) distributions were similar to SEER NJ SAA survivors (62% localized, 9% triple-negative) but CANSAH-BC participants were younger (Mean (SD) = 52 (12) years vs. 56 (13) years in SEER NJ; p = 0.0017). The SIR was 3.86 (95% CI: 3.11 - 4.74), indicating significant familial aggregation. Under a lognormal distribution of risk, 68.7% of breast cancers will occur in SAA women at the top 25% risk group, while only 3.3% in the lowest 25%.
Conclusion: CANSAH-BC participants closely mirror NHIS and CHIS respondents, but are significantly younger than SEER NJ survivors. The significant familial aggregation and likely concentration of breast cancers in the top 25% risk group underscore the predictive influence of shared genetic and environmental factors among relatives of SAA women with breast cancer. Our future direction will investigate these shared factors using germline and genomic markers.

#971 Biological sex disparities in oral HPV infection and vaccination rates in people with HIV in Puerto Rico: a public health concern.

Jurelis Torres-Reyes, Juliana M. Serrano-Rodriguez, Gabriel Borgez-Velez, Jeannette L. Salgado-Montilla, Maria Sanchez-Vazquez, Magaly Martinez-Ferrer, Ana P. Ortiz-Martinez, Josue Perez-Santiago

UPR - Comprehensive Cancer Center, San Juan, Puerto Rico

Background: Human papillomavirus (HPV) is the most common sexually transmitted infection, with estimates for the probability of infection with the virus exceeding 80% for women and 90% for males across their lifetime. People with HIV (PWH) are at a higher risk of acquiring oral HPV infection, despite antiretroviral therapy, making them more susceptible to Oropharyngeal cancer (OPC). Puerto Rico (PR) has a significant burden and disparity of HPV and HIV-related malignancies, and socio-economic disadvantages, which limit access to HPV vaccine. While HPV vaccination rates in the US is 46% in young adults, HPV vaccination rates in PR are limited, particularly in PWH. Here, we described the prevalence of oral HPV infection among PWH in PR and investigated socio-behavioral factors associated with HPV infection.
Methods: We evaluated 135 sexually active PWH with a median age of 48 years old. Oral rinse samples were collected and analyzed for HPV and genotyped using the DNA ELISA kit HPV SPF10 and RHA kit HPV SPF10-LiPA25. An administered questionnaire collected sociodemographic characteristics and lifestyle variables. Statistical analyses were performed in R-statistical software.
Results: There was a 32% prevalence of oral HPV infection, of which 61% were high-risk genotypes, with HPV-18 being the most abundant (39%). The prevalence of oral HPV was higher in males than females (34% vs. 17%), and high-risk genotypes were only present in males. High proportion of our cohort (67%) did not have the HPV vaccine while 60% have the knowledge of HPV infection. Knowledge of HPV infection was higher in males than females (56% vs 33%), but even then, vaccination rates were very low (20% males vs 11% females).
Conclusion: High prevalence of oral HPV infection was observed in PWH, higher than previously observed in PR. Limited HPV vaccination rates may possibly be due to age ineligibility, access, or lack of awareness of the vaccine. Males had a higher prevalence of oral HPV infection and were the group in which high-risk HPV genotypes were detected. Although female vaccination rates were low, the lack of high-risk genotypes may be due to early access to HPV vaccine based on regular established screening protocols for cervical cancer, but these protocols have not been established for high HPV-related cancer risk male populations. The incidence in OPC is increasing and it is expected to exceed that of cervical cancer, these findings highlight the need to establish better preventive public health strategies from an educational standpoint, and medical care access.

#972 Identifying patient navigation needs and gaps in cancer care in Puerto Rico: insights from the START-PR study.

Lorena Gonzalez-Sepulveda[1], Stephanie Cameron-Maldonado[1], Karina M. Rivera-Mejia[1], Natalia E. Dominguez-Betancourt[2], Maria M. Vazquez-Torres[1], Alejandra Diaz-Rohena[3], Liamarys Ortiz-Torres[1], Noedly Ayala-Sanchez[4], Yadielis S. Arce[4], Nancy Cardona-Cordero[1], Marievelisse Soto-Salgado[1]

[1]University of Puerto Rico Comprehensive Cancer Center, San Juan, Puerto Rico,[2]University of Puerto Rico, Rio Piedras Campus, San Juan, Puerto Rico,[3]University of Puerto Rico, Medical Sciences Campus, San Juan, Puerto Rico,[4]University of Puerto Rico, Cayey Campus, Cayey, Puerto Rico

Background: Patient navigation (PN) services in cancer care have significantly benefited cancer patients by reducing disparities in cancer outcomes, screening, and treatment. Identifying the characteristics of cancer patients who need PN services and the services they need may assist in the design of targeted future PN programs. Therefore, our study aims to identify the PN services that are most needed in this population and determine the association between the need for PN services among cancer patients in Puerto Rico and sociodemographic and health-related factors.
Methods: Our ongoing pilot cross-sectional study, START-PR, has recruited cancer patients aged 21+ years who received active treatment in the last year and resided in Puerto Rico, through an online survey. Data on PN services, sociodemographic, and clinical characteristics were assessed in this study. Multivariate logistic regression models, including covariates with p-values < 0.25 in univariate models, were performed to determine associations between the selected characteristics and the need for PN services. McNemar's test was used to determine differences in the proportions between the need and the receipt of PN services.
Results: To date, 547 individuals have accessed the survey and met the eligibility criteria, with 538 (98.4%) completing the survey. Most of our sample were women (69.2%), aged between 40-64 years (56.1%), had a higher educational attainment than high school (72.6%), and had cancer in localized stages (64.4%). About 29% of the patients self-reported needing PN services. Among these patients (n=156), the most commonly needed services were: (1) coordination of tests, procedures, and treatments (36.5%), (2) education (34.6%), and (3) assistance in scheduling appointments or finding a new doctor (29.5%). Participants aged 40 to 64 (34.8%), with education beyond high school (31.4%), with comorbidities (32.1%), without a partner (32.5%), with public health insurance (34.3%), and diagnosed with cancer for more than a year (32.8%) had higher PN service needs than their counterparts ($p<0.25$). After controlling for potential confounders, patients with comorbidities had 2.2 times the odds (95% CI: 1.28, 3.79) of needing PN services compared to those without comorbidities. About 10.8% did not need the PN services but ended up receiving them, while 18.4% needed the PN services but did not receive them ($p=0.001$). Conclusion: The results of this study provide valuable information to guide the design of targeted future PN programs in Puerto Rico. Further studies should include in-depth qualitative methods to better understand the reasons behind these needs and identify the specific barriers patients face to receive cancer care.
Fundings: Partially supported by the Office of Cancer Research Training and Education Coordination of the UPR Comprehensive Cancer Center and the Hispanic ALLIANCE (NIGMS-U54GM133807).

#973 Silent suffering: the urgent need for improved palliative care and opioid access for breast cancer patients in the Caribbean.

Alexandria E. Curry, Advith Suresh, Robin Williams, Samrawit Zinabu, Miriam Michael

Howard University, Washington, DC

This study aims to investigate the availability and accessibility of palliative care and opioid-based pain management for patients with advanced breast cancer in the Caribbean, highlighting regional disparities and barriers to effective end-of-life care. We analyzed the availability of palliative care services and opioid medications in Caribbean countries, focusing on gaps in healthcare infrastructure, regulatory challenges, and cultural or legal barriers to opioid use. We collected data from national reports, healthcare registries, and published literature. Our study found that access to palliative care in the Caribbean is severely limited, with some nations, like Haiti, having no palliative care physicians. The Bahamas, which has the highest breast cancer mortality in the region, only has one. Countries with established programs, like Barbados and the Cayman Islands, struggled to expand services beyond limited geographic and population reach due to funding constraints and a lack of trained professionals. Despite the inclusion of at least two forms of opioid medications in most national formularies, their actual use remains significantly lower than global standards—20 times less than the per capita usage in the United Kingdom and 60 times less than the usage in the United States. Barriers included restrictive laws, negative perceptions of opioids, and logistical inadequacies in medication access. In many countries, opioids could only be obtained from hospital pharmacies, further restricting access and stigma and fear of addiction contributed to underprescription and underuse, even when medications were available. These issues were compounded by a lack of provider education in safe opioid prescribing, resulting in unnecessary suffering for patients with advanced disease. These gaps in palliative care result in inadequate care, leaving many patients without the necessary support to manage their symptoms and maintain quality of life. Access to palliative care and effective pain management for advanced breast cancer patients in the Caribbean is critically inadequate. There is an urgent need for enhanced palliative care services, including professional training, and the development of formal guidelines to address regulatory barriers to opioid access. To address these challenges, we recommend the creation of a resource-specific guide for low- and middle-income countries that focuses on integrating pain medication into palliative care protocols as a central component of treatment. Additionally, we propose a basic level of supportive care for patients with organ-based metastatic disease, which provides site-specific recommendations for managing symptoms and complications in resource-limited settings. These measures are essential to improving the quality of life and ensuring equitable care for patients in the region.

#974 Social determinants in healthcare utilization among women diagnosed with cervical cancer: data from *The All of Us Research Program*.

Brenda C. Torres-Velasquez[1], Josue Perez-Santiago[2], Natalie Alamo-Rodriguez[3], Julie Dutil[2], Vivian Colon-Lopez[3]

[1]Division of Shared Resources and Scientific Operations, University of Puerto Rico Comprehensive Cancer Center, San Juan, Puerto Rico,[2]Division of Clinical and Translational Cancer Research, University of Puerto Rico Comprehensive Cancer Center, San Juan, Puerto Rico,[3]Division of Cancer Control and Population Sciences, University of Puerto Rico Comprehensive Cancer Center, San Juan, Puerto Rico

Despite significant declines in cervical cancer incidence and mortality, disparities persist, particularly among underserved populations. This study aims to investigate how social determinants of health contribute to the barriers faced by women diagnosed with cervical cancer, affecting healthcare utilization. Utilizing the *All of Us Research Program* database, we analyzed a cohort of women diagnosed with malignant tumors of the cervix, including carcinoma in situ, using ICD-10-CM codes C53 and D06. Participants, all identifying as women, completed validated surveys assessing overall health, healthcare access and utilization, personal and family health history, and social determinants of health. Univariate and multivariate logistic regression models were conducted to assess social determinants of health in relation to healthcare access. Key dependent variables included delays in seeking healthcare due to limited time-off, caregiving responsibilities, inadequate transportation, and financial constraints such as difficulties affording medications or copayments. Data was analyzed using RStudio Cloud Environment within All of Us Workbench. Among the 1,310 cervical cancer patients analyzed, 299 (22.8%) identified themselves as Hispanics, 670 (51.1%) as non-Hispanic White, 265 (20.3%) as non-Hispanic Black, and 76 (5.8%) as other or unknown. Educational attainment was high, with 852 (65%) attending or having completed college. Most participants (95%) had some form of insurance; however, (43.7%) reported annual earnings below $50,000. Participants earning between $25,000 and $50,000 were 3.6 times more likely [OR=3.6; 95% CI: 1.2-15.8] to delay seeking healthcare due to time-off issues compared to those earning $200,000 or more. Participants earning $25,000 or less compared to those earning $200,000 or more identified transportation access as a significant barrier to healthcare (OR=6.3 [95% CI: 1.8-40.1]), still were 4.2 times more likely [95% CI: 1.1-17.3] to seek advice from a general doctor. This OR slightly decreased to 4.1 [95% CI: 1.1-17.0] when adjusting for insurance coverage awareness, to 3.8 [95% CI: 1.01-15.5] when considering the nervous emotional state, and returned to 4.2 [95%CI 1.1-17.4] when perceiving easily understandable health information. Income and transportation are critical factors influencing healthcare-seeking behavior among low-income women diagnosed with cervical cancer; however, they do not act in isolation. Barriers related to social determinants significantly affect their ability to maintain appropriate follow-up care. Interestingly, race and ethnicity did not emerge as significant disparities in this cohort. Further investigation into insurance specifics, family dynamics, cultural beliefs, and other social determinants is essential for achieving equity in healthcare access for women with cervical cancer.

#976 Evaluation of causes of late presentation among female cancer patients in a tertiary cancer center in Nepal: Mixed method study.

Shweta Baral[1], **Ashim Khadka**[2], Deep Lamichhane[3]

[1]Bhaktapur Cancer Hospital, Kathmandu, Nepal,[2]Nepal College of Information and Technology, Kathmandu, Nepal,[3]Surgical Oncology, Bhaktapur Cancer Hospital, Kathmandu, Nepal

Introduction: Gender disparity in cancer diagnosis and treatment is prevalent in LMICs. Late presentation among female cancer patients is of the challenging issue here leading to poorer treatment outcomes. This study is first of kind in Nepal to investigate factors contributing to delayed presentation for treatment.
Methodology: A convergent mixed-method design was conducted in a government cancer center to evaluate the factors contributing to the late presentation among female cancer patients with an average age of 54.19 years. The quantitative component surveyed 200 late presenting female cancer patients and 15 patients who were purposefully chosen to participate in a focus group discussion to examine socioeconomic and demographic factors. Descriptive statistics, Chi-square tests and logistic regression were used to identify the factors associated with late presentation.
Results: Among the 200 patients surveyed, 88 presented in a locally advanced stage without much symptoms, and 58% patients (116/200) delayed seeking treatment after being symptomatic. Quantitative analysis showed that the following factors were significantly linked to late presentation among delayed treatment seeking patients : rural residency (Odds Ratio [OR] = 2.15, 95% Confidence Interval [CI]: 1.42-3.25), education below 12th grade (OR = 3.76, 95% CI: 2.10-6.72), extended joint family living (OR = 1.85, 95% CI: 1.12-3.04), and unemployment (OR = 2.54, 95% CI: 1.63-3.97). Chi-square analysis also showed significant associations between late presentation and factors like rural residency ($p < 0.01$), education below 12th grade ($p < 0.01$), joint family living ($p < 0.05$), and unemployment ($p < 0.01$). A majority of late presenters (63.79%, 74/116) hid their symptoms for an average of 15 months, while 24.13% (28/116) postponed their visit due to financial difficulties, and 12.06% (14/116) were ignored by family members until their symptoms worsened. Four major themes emerged from the focus group discussions that contributed to the presentation's delay: 1) fear of destabilizing the family due to illness, 2) financial hardship in covering treatment costs, 3) underestimating the seriousness of symptoms, and 5) fear of abandonment after receiving a cancer diagnosis, especially by spouses and in-laws.
Conclusion: This study highlights the need for interventions addressing both the socioeconomic and cultural barriers that delay timely healthcare access for female cancer patients in Nepal. Enhancing community awareness, improving healthcare accessibility, and providing financial support could mitigate the identified challenges, potentially leading to better outcomes for women.

#977 Metabolomic and proteomic signatures of ultra-processed foods with liver cancer and liver disease.

Longgang Zhao[1], Yun Chen[1], Alyssa Clay-Gilmour[2], Jiajia Zhang[2], Xuehong Zhang[1], Susan Steck[2]

[1]Yale University, School of Nursing, West Haven, CT,[2]Arnold School of Public Health, University of South Carolina, Columbia, SC

Introduction: Higher intake of ultra-processed foods is associated with increased risk of obesity and type 2 diabetes, but evidence is limited on associations of metabolomics and proteomics with ultra-processed foods and liver diseases. We aimed to evaluate associations of metabolomic and proteomic signatures related to ultra-processed food intake with liver cancer and liver disease.
Methods: Data from a total of 173,840 participants aged 40-69 years from the UK Biobank were analyzed. Ultra-processed food intake was assessed by 24-hour dietary recalls. Metabolites were measured by nuclear magnetic resonance spectroscopy and plasma proteome was profiled using the Olink platform. Liver disease outcomes were ascertained using data from the in-hospital, cancer registry, and death registry including metabolic dysfunction-associated steatotic liver disease (MASLD), cirrhosis, liver cancer, and severe liver disease (hepatocellular carcinoma and other severe liver diseases). Elastic net regression with 10-fold cross validation was used to calculate the omics signatures related to ultra-processed food intake. We used Cox proportional hazards regression models to estimate hazard ratios (HR) and 95% confidence intervals (CIs) for ultra-processed food intake and its omics signatures and liver disease outcomes adjusting for multiple potential confounding factors.
Results: With a median of 8.9 years follow-up, ultra-processed food intake was positively associated with risk of MASLD ($HR_{\text{Tertile 3 vs. tertile 1}}$ 1.42; 95% CI 1.21-1.67), cirrhosis (HR 1.25; 95% CI 1.05-1.48), and severe liver disease (HR 1.52; 95% CI 1.21-1.91) but not with liver cancer (HR 1.30; 95% CI 0.89-1.91). The metabolic signature score of ultra-processed foods was positively associated with risk of MASLD (HR 3.25; 95% CI 2.06-5.14). The proteomic signature score of ultra-processed foods was positively associated with risk of MASLD (HR 1.99; 95% CI 1.04-3.80) and cirrhosis (HR 2.00; 95% CI 1.12-3.60). A total of 34 metabolites and 65 proteins were significantly associated with ultra-processed food intake based on the multivariable-adjusted model and elastic net regression. These metabolites and proteins are linked to biological pathways such as lipids metabolism, oxidative stress, and inflammatory response.
Conclusions: Ultra-processed food intake and its metabolic and proteomic signatures are positively associated with risk of adverse liver outcomes.

#979 Caregiver experiences and prostate cancer survivor quality-of-life in the Detroit Research on Cancer Survivors study.

Colleen B. McGrath[1], Jamaica R. M. Robinson[2], Konrad H. Stopsack[1], Julie J. Ruterbusch[2], Michelle O. Sodipo[1], Kathryn L. Penney[1], Briana J. K. Stephenson[3], Laura D. Kubzansky[4], Lorelei A. Mucci[1], Jennifer Beebe-Dimmer[2]

[1]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA,[2]Department of Oncology, Wayne State University School of Medicine, Detroit, MI,[3]Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA,[4]Department of Social and Behavioral Sciences, Harvard T.H. Chan School of Public Health, Boston, MA

Introduction: Cancer survivorship is influenced by informal (unpaid) caregiving received by survivors. However, informal caregivers often experience caregiving-related burden, or strain from providing care, which may, in turn, impact health-related quality-of-life (HRQoL) among cancer survivors. We investigated associations of caregiver burden and involvement intensity with HRQoL of prostate cancer survivors receiving care.

Methods: We included 323 prostate cancer survivor-caregiver dyads who participated in the Detroit Research on Cancer Survivors study, a population-based cohort of Black cancer survivors. Informal caregivers were invited to report on their experiences, including caregiving burden (via Zarit Burden Interview [ZBI]) and involvement intensity (via Level of Care Index); these were measured at caregiver enrollment. Survivors completed enrollment and follow-up (approximately annually) surveys, including measuring HRQoL using the Functional Assessment of Cancer Therapy tool; we measured HRQoL for up to 6 years. We examined overall QoL (range 0-108), subscales of functional, social, physical (each 0-28), and emotional (0-24) wellbeing, and prostate cancer-specific QoL (0-48); higher scores reflect better HRQoL. We used linear mixed effects models including exposure-time interactions to evaluate differences in survivor HRQoL scores at enrollment and over time by each exposure, adjusting for survivor and caregiver sociodemographics, survivor health, and cancer and dyad relationship characteristics.

Results: Survivor median age at diagnosis was 62 years. Most informal caregivers were the survivor's partner (68%) and were women (94%). Most caregivers reported low burden (ZBI < 21; 82%), and most were highly involved (60%). At enrollment, low vs. moderate or higher caregiver burden was associated with better overall QoL (mean difference 8.7, 95% Confidence Interval [CI] 4.1, 13.2), prostate cancer-specific QoL (3.1, 95% CI 0.8, 5.4), functional wellbeing (3.1, 95% CI 1.4, 4.8), and slightly, emotional and physical wellbeing. Having a low vs. high involved caregiver was associated with better overall QoL (7.3, 95% CI 2.8, 11.7), prostate cancer-specific QoL (4.0, 95% CI 1.7, 6.3), functional wellbeing (3.2, 95% CI 1.5, 4.9) and slightly, physical wellbeing at enrollment. On average, the additional change in physical and prostate cancer-specific QoL scores per year comparing survivors with caregivers who have low vs. moderate or higher burden was -0.5 (95% CI -0.8, -0.1) and -1.3 (95% CI -2.4, -0.3) lower, respectively.

Conclusion: While informal caregivers' experiences immediately influenced survivor HRQoL, we also observed that the relationship of caregiver burden with physical forms of wellbeing differed over time. Supporting informal caregivers can impact the care recipients, potentially having long-term influence for cancer survivors.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#98 STK11 loss in KRAS driven lung adenocarcinoma promotes cancer stemness.

Kyla R. Esposito, Shannon M. Prior, Cole M. Royer, David J. Seward, Paula B. Deming, Melissa N. Scheiber

University of Vermont, Burlington, VT

Lung adenocarcinoma (LUAD) is the most common subtype of non-small cell lung cancer. A significant proportion (25% to 30%) of LUAD cases are driven by mutations in the KRAS oncogene, with 10-15% of these patients harboring a loss of function (LoF) mutation in the tumor suppressor gene *STK11* (serine/threonine kinase 11). Loss of STK11 function in lung cancer has been associated with an immunosuppressive environment, altered metabolism, epithelial-mesenchymal transition (EMT), and destabilization of cell polarity. Retrospective patient studies have demonstrated that concurrent oncogenic *KRAS* and *STK11* LoF mutations are associated with metastatic disease, poorer patient survival, and inferior therapeutic response. We hypothesize that STK11 loss in KRAS-driven LUAD enriches the cancer stem cell (CSC) population, resulting in a pro-metastatic phenotype. CSCs represent a small subset of tumor cells capable of self-renewal, asymmetric division, and tumor initiation. Given their role in metastasis, chemoresistance, and disease progression, CSCs could be key contributors to the aggressiveness observed in KRAS-driven STK11 LoF LUAD. To test this hypothesis, we utilized the KRAS-driven LUAD cell line NCI-H2009 and an STK11 knockout variant (ΔSTK11) to assess differences in CSC properties. CD44 is a common biomarker for CSC and is involved in many aspects of cancer progression. Both parental and ΔSTK11 cells grown in 2-D monolayers were found to express similar levels of CD44 of mRNA and protein, as assessed by qRT-PCR and flow cytometry. However, in 3D culture, ΔSTK11 multicellular spheroids (MCTs) showed a significant increase in CD44 mRNA when compared to both monolayers and parental MCTs. We have also observed increased mRNA expression of two additional CSC markers, NANOG and OCT4, in ΔSTK11 MCTs compared to parental MCTs. CD44, plays a critical role in maintaining the stem-like properties of cancer cells, however, the precise mechanisms connecting CD44 expression and STK11 remain unclear. To address this, we are investigating transcription factor pathways, including NF-kB and HIPPO, to elucidate their roles in mediating enhanced CD44 expression upon STK11 loss. To characterize the function of the CSCs in KRAS-driven STK11 LoF LUAD, we have employed an embryonic zebrafish xenograft model. This in vivo system allows for real-time visualization of tumor cell survival and dissemination, providing critical insights into the metastatic behavior of cells. In conclusion, our research highlights the role of STK11 loss in driving CSC enrichment and enhancing metastatic potential in KRAS-driven LUAD. By elucidating the underlying mechanisms, we aim to identify new therapeutic targets to improve outcomes for patients with this aggressive cancer subtype.

POPULATION SCIENCES: Bridging Gaps in Cancer Care: Disparities, Social Determinants, Innovations, and Strategies Poster Session

#980 Deaths from cardiovascular disease in rural and African American men with prostate cancer.

Caroline Himbert[1], Heather M. Munro[2], Adam Bress[1], Philip W. Kantoff[3], Kenneth J. Mukamal[4], Martha J. Shrubsole[2], Konrad H. Stopsack[5]

[1]University of Utah, Salt Lake City, UT,[2]Vanderbilt University Medical Center, Nashville, TN,[3]Convergent Therapeutics Inc, Boston, MA,[4]Beth Israel Deaconess Medical Center, Boston, MA,[5]Leibniz Institute for Prevention Research and Epidemiology, Bremen, Germany

Background: Cardiovascular disease (CVD) is a major cause of morbidity and mortality among men diagnosed prostate cancer. Different CVD risk factor profiles and disparities in healthcare access may place a higher burden on Black men and those living in rural areas diagnosed with prostate cancer.
Methods: This cohort study aimed to evaluate differences in CVD deaths by rurality and race among men with prostate cancer. The Southern Community Cohort Study (SCCS) is a prospective cohort, which enrolled 34,313 racially diverse, predominantly low-income men aged 40-79 in twelve southeastern states of the United States. Between 2002 and 2021, 1,544 men with prostate cancer were free of prevalent CVD (i.e., coronary heart disease and stroke). Prostate cancer cases, vital status, and causes of death were identified through linkages to state cancer registries and the National Death Index as of December 31, 2022. Residence at enrollment was classified into rural and urban using community area codes. Lifetime risk for CVD death from Aalen-Johansen estimators and cause-specific hazard ratios (HRs) were computed.
Results: 356 participants (23%) were classified as rural and 1,174 (76%) self-identified as African American. Over 21 years of follow-up (median 8 years), 618 deaths occurred, of which 149 (24%) were from CVD. The lifetime risk of CVD death was similar comparing men with prostate cancer living in urban and rural areas at about 20%. After adjusting for sociodemographics, cancer, and CVD risk factors, the hazard ratio (HR) for rurality and CVD death was 0.95 (95% confidence interval (CI): 0.66, 1.40). African American men had a higher lifetime risk of CVD death (21%, 95% (CI): 16, 28) than White men with prostate cancer (16%, 95% CI: 11, 24). The cause-specific HR for CVD deaths was 1.36 (95% CI: 0.91, 2.04) in African American men compared to White men. African American men tended to be diagnosed with prostate cancer at a younger age (age adjusted HR: 1.68; 95% CI: 1,12, 2.52). The association was attenuated upon adjustment for social determinants of health reflected in community health center enrollment (HR: 1.02; 95% CI: 0.66, 1.59) and was essentially unchanged after further adjustments (HR: 0.99, 95% CI: 0.63, 1.57). African American men with CVD risk factors at prostate cancer diagnosis (hypertension, diabetes, hypercholesteremia), lower education level, or no insurance or Medicaid/Medicare coverage were at the highest risk for CVD death.
Conclusions: African American men with prostate cancer face a higher age-adjusted risk of death from CVD than White men with prostate cancer. This association could mainly be explained by racial differences in social determinants of health reflected in community health center enrollment. Integrating sociodemographic characteristics into treatment planning and prioritizing CVD prevention and management along prostate cancer care may reduce CVD mortality among men with prostate cancer.

#982 Disparities in allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia are increasing.

Lixin Kang[1], Mark A. Fiala[2], Kimberly J. Johnson[1]

[1]Washington University in St. Louis, St. Louis, MO,[2]Washington University School of Medicine in St. Louis, St. Louis, MO

Introduction: Acute myeloid leukemia (AML) is the most prevalent form of leukemia. Allogeneic hematopoietic cell transplantation (allo-HCT) is the most effective and often the only curative treatment. Due to advancements in donor selection, a suitable donor is available for nearly all patients, and this may have remediated the previously reported disparities in allo-HCT based on race and ethnicity. To date, no studies have comprehensively examined allo-HCT rates and trend disparities after expanded donor availability. Therefore, this study aimed to identify and quantify trends in allo-HCT rates and disparities among different racial and ethnic groups. We hypothesized that transplant disparities have decreased over time between those identified as racial and ethnic minorities and non-Hispanic Whites (NHWs).

Methods: Data on AML patients diagnosed from 2004 to 2021 were obtained from the National Cancer Database Participant User Data File. Utilizing a multiple linear regression model with a difference-in-differences approach, we calculated the annual absolute change in allo-HCT rates and corresponding p-values across racial and ethnic groups to compare trends in allo-HCT rates adjusted for covariates.

Results: Over the study period, non-Hispanic Asian or Pacific Islanders (NHAPIs) exhibited the highest allo-HCT rate at 20.9%, starting at 12.1% in 2004 and influenced by an outlier increase to 34.0% in 2021. NHWs had the second highest rate of 19.7%, increasing from 9.1% in 2004 to 22.9% in 2021. The allo-HCT rate for Hispanics increased from 9.4% in 2004 to 24.3% in 2021; however, a period of low rates from 2006 to 2011 resulted in a lower overall allo-HCT rate of 15.9%. Non-Hispanic Blacks (NHBs) had the lowest allo-HCT rate at 9.8%, with an increase from 2.3% in 2004 to 11.5% in 2021.

From 2004 to 2021, the annual percentage increase in allo-HCT in NHBs was estimated at 0.41% lower than NHW (95% Confidence Interval [CI]: -0.61%, -0.21%; $p<0.01$). Hispanics also had a statistically significant 0.27% lower annual increase in allo-HCT rate compared to NHW (95% CI: -0.51%, -0.03%; $p=0.03$). Although NHAPI exhibited a higher overall allo-HCT rate compared to NHW, the annual rate increase was 0.23% lower than NHW (95% CI: 0.55%, 0.09%; $p=0.16$), which reduced the differences in allo-HCT rates between these groups over the study period.

Conclusion: NHB and Hispanics exhibited lower overall allo-HCT rates than NHW from 2004 to 2021, with evidence of widening disparities. NHAPI had overall higher allo-HCT rate than NHW, but the difference narrowed over time as NHW patients experienced a steeper increase in allo-HCT rates. These results suggest that despite the availability of suitable allo-HCT donors, not all patients have benefited equally. Further research is needed to investigate individual and systemic factors associated with allo-HCT disparities among racial and ethnic minorities.

#983 Characterizing somatic copy number alterations in African American renal cell carcinoma patients.

Musinu Zakari, Egbenoma A. Aigboeghian, Khadijah A. Mitchell

Cancer Prevention and Control Program, Fox Chase Cancer Center, Philadelphia, PA

Background: Renal cell carcinoma (RCC) has one of the highest mortality rates of all urologic cancers in the US. Somatic copy number alteration (SCNA) profiles have been linked with RCCC mortality. African American (AA) RCC patients have higher mortality and lower survival rates when compared to European Americans (EAs) with the disease. AA RCC patients are underrepresented in cancer genomic studies, and RCC mortality and survival disparities may be attributed to SCNA differences. We hypothesize there are distinct population-enriched SCNAs in RCC tumors from AAs compared with EAs.

Methods: DNAs were isolated from fresh frozen primary tumors for each RCC patient in the Geisinger-Cooperative Human Tissue Network discovery cohort (n=20), followed by OncoScan CNV (whole-genome copy number microarray) analyses. SCNAs at the genome-wide, chromosomal, chromosomal region, and gene levels were compared in AA (n=10) and EA (n=10) RCC patients using Chromosome Analysis Suite v7. Population differences were calculated using t-tests with Welch's corrections and multiple unpaired t-tests ($P < 0.05$). STRING was used to identify protein-protein interaction networks and functional enrichment analyses. GraphPad Prism 10 v7 and STRING v12 were used to generate all images.

Results: Genome-wide across 22 autosomes, a combined 397 chromosomal regions (n=292 amplifications; 105 deletions) were altered in AA RCC patients compared to 476 chromosomal regions (n=302 amplifications; 174 deletions) in EA RCC patients. We observed AAs had significantly higher amplifications across 13 chromosomes (3, 4, 6, 7, 9,10, 13-15, 18, 20-22) when compared to their EA counterparts ($P < 0.05$; AA: 164/292, EA: 156/302). A total of 12,114 genes in AAs and 11,320 genes in EAs were amplified. Uniquely amplified genes (n=1,095) in AA RCC patients corresponded with keratin-associated protein (KRTAP) networks (PPI enrichment P value: 3.38e-08). EA RCC patients had 301 uniquely amplified genes that corresponded with trace-amine and natural killer cell lectin-like receptor activities (PPI enrichment P value: 0.000334).

Conclusion: KRTAPs are known RCC oncogenes. KRTAP amplifications may drive expression and help to explain more aggressive tumor biology in AA RCC patients.

Future Direction: Integrate SCNA data with matched bulk mRNA-sequencing data for each RCC patient.

#984 Implementation of tissue next generation sequencing in a real world diverse safety-net setting.

Kavya Athipatla[1], Lindsay C. K. Reynolds[2], Susmita Potti[3], Mitchell S. von Itzstein[4], Janet Miles[5], Kalyani Narra[6]

[1]Carroll High School, Southlake, TX,[2]Office of Clinical Research, John Peter Smith Health Network, Fort Worth, TX,[3]JIPMER, Puducherry, India,[4]Internal Medicine (Division of Hematology-Oncology), University of Texas Southwestern, Dallas, TX,[5]Pathology, John Peter Smith Health Network, Fort Worth, TX,[6]Oncology and Infusion Center, John Peter Smith Health Network, Fort Worth, TX

Background: Next-generation sequencing (NGS) is generally considered the standard of care for molecular testing in advanced cancer cases across professional organization guidelines. Implementing expensive NGS testing in safety-net settings poses significant challenges due to limited resources. John Peter Smith Health Network (JPS) serves a large diverse population of uninsured and underinsured patients, and we addressed this challenge by partnering with ProPath and Bernhardt Laboratories (BHL) to facilitate tissue NGS testing.

Methods: Patients at JPS with tissue NGS tested between 1/1/24 to 9/30/24 using a ProPath authorization program and BHL testing were included. AuraSeq-Comprehensive was the NGS test utilized and includes a panel detecting clinically relevant alterations in 500 cancer-related genes, including base pair substitutions, small insertions and deletions, copy number variations, and gene fusions. Data analysis was performed using Excel.

Results: A total of 152 patients underwent tissue analysis, each patient undergoing a single NGS test during this period. The average age was 62 years and there were 76 (50%) females. 41 (27%) were Hispanic, 54 (36%) non-Hispanic White, 41 (27%) non-Hispanic Black, 16 Asians and others. Excluding tumor mutational burden (TMB), homologous recombinant deficiency (HRD) and Microsatellite instability (MSI), 146 patients had at least one genomic alteration, 118 had two, 87 had three, 55 had four, and 26 had five. TMB testing was conducted on 150 patients and 29 (19%) had high TMB using a cutoff of 10 mut/Mb. HRD testing was positive in 14 out of 150 (9%). MSI was high in 2 out of 15 tested. A total of 5 (10%) *ALK* fusions, 10 (21%) activating *EGFR* alterations, 1 (2%) *MET* exon 14 skipping mutation, and 9 (19%) *KRAS* G12C mutations were noted in 48 non-squamous NSCLC cases. Of the 9 Hispanic patients with non-squamous NSCLC, 2 had *ALK* fusions, 3 had *EGFR* mutations and 1 had *KRAS* G12C. Of the 21 patients with squamous NSCLC, one patient had *BRAF* mutation. Of the 43 patients with gastrointestinal cancers, there were 1 *KRAS* G12C, 2 *BRAF*, 1 *BRCA*2, 2 *ERBB*2 amplifications. Of the 13 breast cancers, there were 3 *PIK3Ca*, 1 *BRCA*2, 1 *ERBB*2 amplification and 1 *ERBB*2 alterations. JPS enrolled the nationwide 1st patient on PrE1702 Real-World Data Study in October 2024 and has opened clinical trials for patients with *KRAS* and *PIK3Ca* alterations.

Conclusions: This study demonstrates the successful implementation of NGS in a safety-net healthcare setting through public-private partnership. Our analysis revealed a high prevalence of actionable genomic alterations across various cancer types, with significant findings in non-squamous NSCLC, especially among Hispanic patients. Future efforts should focus on expanding access to genomic testing to further improve
patient care and outcomes with opportunities for more clinical trials in safety-net settings.

#985 CARIB-CARES: the first exploratory research center addressing the intersection of climate and the cancer control continuum.

Ana P. Ortiz[1], Noreen Michael[2], Nancy R. Cardona-Cordero[1], Marievelisse Soto-Salgado[1], **Polaris N. Torres**[1], LaVerne E. Ragster[2], Leticia Nogueira[3], Frank E. Muller-Karger[4], Zack Guido[5], Tracy E. Crane[6], Pablo Mendez-Lazaro[7]

[1]UPR - Comprehensive Cancer Center, San Juan, Puerto Rico,[2]University of the Virgin Islands, St Thomas, Virgin Islands, U.S.,[3]American Cancer Society, Atlanta, GA,[4]University of South FLorida, St Petersburg, FL,[5]University of Arizona, Tucson, AZ,[6]University of Miami, Miami, FL,[7]UPR- Medical Sciences Campus, San Juan, Puerto Rico

Background: Climate change is a threat to human health, impacting the cancer control continuum. Frequent extreme weather events in the US Caribbean (Puerto Rico [PR] and US Virgin Islands [USVI]) have harmed human health and socioecological ecosystems and have disrupted cancer prevention and control efforts. This has unveiled the vulnerabilities of critical infrastructure systems, which, when disrupted, cause catastrophic failures with fatal and long-lasting consequences. Addressing these challenges is crucial for promoting resilient and healthy island communities, mitigating cancer risk, and sustaining cancer control infrastructure.
Methods: The Caribbean Climate Change, Cancer, and Health Disparities Research Center (CARIB-CARES) is a regional initiative aimed at tackling these critical issues, with the participation of a multidisciplinary team of experts in environmental health, climate, oceanography, and cancer research. A partnership between the University of PR Comprehensive Cancer Center, the University of PR-Medical Sciences Campus, and the University of the Virgin Islands together with other institutions in the continental US, government agencies, health clinics, and non-governmental organizations, CARIB-CARES seeks to address the impact of multi-hazard climate-related stressors on the cancer control continuum and increase research and adaptation capacity in the field.
Results: Funded in September 2024, CARIB-CARES has initiated capacity-building initiatives, delivering webinars on climate change, health, and the cancer control continuum to over 100 community members, health professionals, and students across PR, USVI, and mainland US. Student engagement and mentorship has initiated, supporting more than 15 students from various academic backgrounds (public health, nursing, and biology) and different career levels (undergrad, grad, and post-doc) and from underrepresented populations as mentees, providing them with research opportunities on the intersection of climate change and cancer. Research projects are also underway, evaluating environmental disparities and cancer risk across the US Caribbean and climate change awareness among oncology health professionals. Community partnerships are being reinforced, developing a Community Coalition of Partners that includes academic institutions, nonprofits, and government agencies, leading to actionable collaborations in addressing cancer-related disparities within the context of climate change.
Conclusion: Through its comprehensive approach, CARIB-CARES has begun building a robust framework to advance research, strengthen governance, and build equitable adaptation to effectively address the challenges of climate change and cancer control across vulnerable and underserved small islands in the Caribbean.
Acknowledgments: Funding NIH Award: 1P20CA294096-01.

#986 *SMARCB1* missense mutants destabilize SWI/SNF complex stability and remodeling activity.

Garrett W. Cooper[1], Benjamin Lee[1], Won Kim[2], Eliseo Salas[3], Yongdong Su[1], Victor Chen[1], Xiaoping Yang[4], Robert Lintner[4], Frederica Piccioni[4], Andrew Giacomelli[2], Thomas Howard[2], Karen Conneely[5], David Root[4], William Hahn[2], David Gorkin[6], Bo Liang[3], Jaclyn Biegel[7], Susan Chi[8], Andrew Hong[1]

[1]Department of Pediatrics, Emory University, Atlanta, GA,[2]Dana-Farber Cancer Institute, Boston, MA,[3]Department of Biochemistry, Emory University, Atlanta, GA,[4]Broad Institute of MIT and Harvard, Boston, MA,[5]Department of Human Genetics, Emory University, Atlanta, GA,[6]Department of Biology, Emory University, Atlanta, GA,[7]Department of Pathology, University of Southern California, Los Angeles, CA,[8]Boston Children's Hospital, Boston, MA

SMARCB1-deficient cancers are aggressive and highly lethal pediatric malignancies. Loss of SMARCB1 protein expression, a subunit within the SWI/SNF chromatin remodeling complex, remains the key diagnostic feature of these cancers. This can occur through large deletions, balanced translocations, frameshift mutations, or truncating nonsense mutations. Here, we sought to understand the effect of missense mutations on the tumor suppressor function of SMARCB1 through deep mutational scanning (DMS). Specifically, we developed and introduced a library containing >99% of all possible SMARCB1 amino acid substitutions, including frameshift and nonsense mutants, into three pediatric SMARCB1-deficient cell lines (G401 - malignant rhabdoid tumor of the kidney, BT16 - atypical teratoid/rhabdoid tumor, and CCLF_PEDS9001_T1 - renal medullary carcinoma) and assessed cell fitness after 8-14 days. We observed broad mutational intolerance in three SMARCB1 domains: the winged-helix domain, intrinsically disordered region, and the RPT2 domain. Following our high-throughput study, we then focused on two highly enriched residues predicted to closely interact within the RPT2 domain of SMARCB1. We validated that specific missense mutations in these two residues mimic loss of function while retaining protein expression. Mechanistic studies revealed that these mutations destabilize the SWI/SNF complex, notably resulting in decreased affinity for SWI/SNF subunits known to be associated with cancer pathogenesis. This complex instability leads to diminished nucleosome remodeling and subsequent transcriptional deregulation. These findings challenge our current understanding of what a loss-of-function mutation means in the context of SMARCB1, suggesting that the absence of SMARCB1 protein expression may not be the sole indicator of SMARCB1 deficiency. Furthermore, this dataset provides a valuable resource for researchers to investigate key residues of SMARCB1 that may drive critical intermolecular interactions necessary for proper SWI/SNF complex assembly and function.

#987 Enhancing oncology navigation services: a mixed methods approach to developing an EHR power form to improve metrics and reporting.

Kelsie Campbell[1], Joi Torrence-Hill[2], Karey A. Whitten[2], Melissa Abbud[3], Jynette Querubin[2], Charis Stewart[2], Mariana Dobre[2], Trista A. Beard[1], Fatimata Sanogo[1], Chanita Hughes Halbert[1]

[1]Department of Population and Public Health Sciences, USC - University of Southern California, Los Angeles, CA,[2]Norris Comprehensive Cancer Center, Los Angeles, CA,[3]Keck Hospital of the University of Southern California, Los Angeles, CA

Importance: Pioneered in the 1990s by Dr. Harold P. Freeman, patient navigation interventions are a key strategy for addressing cancer disparities among underserved populations. Despite widespread adoption, the efficacy and cost-effectiveness of oncology navigation interventions require further evaluation. Organizations such as the Academy of Oncology Nurse and Patient Navigators (AONN+) have established evidence-based metrics to demonstrate the value of oncology navigation programs. However, inconsistencies in data collection and the limitations of Electronic Health Record (EHR) systems hinder effective reporting.
Objectives: To identify critical gaps in navigation tracking practices and develop a standardized power form for integration into the Cerner EHR system to improve tracking and reporting of navigation metrics in an established cancer navigation program.
Design, Setting, and Participants: A cohort of 517 patients seen between July 1, 2022, and June 30, 2024, at University of Southern California Norris Comprehensive Cancer Center (USC NCCC). The cohort consisted of 137 breast, 312 lung, and 68 sarcoma cancer patients. We introduced an Excel-based navigation tracking tool in January 2023, adapted from a tool provided for the *American Cancer Society's Navigational Capacity Building Initiative*; shadowed navigators and interviewed with leadership to gather qualitative data on patient flow, tracking protocols, and program priorities.
Main Outcomes/Measures: Primary outcomes included navigation caseload, diagnosis-to-treatment times, and treatment adherence for the cohort at USC NCCC. Qualitative data assessment included the consistency of the documentation of primary outcomes, clinical trial referrals, care transitions, and program goals.
Results: Most patients (82.0%) were over age 50, with 42.4% identifying as White, 15.4% as Asian, and 5.4% as Black/African American. Caseloads increased from 158 in July 2022 to 268 in June 2024, and average diagnosis-to-treatment times decreased from 33 to 22 days. Qualitative data identified critical gaps in tracking practices, such as inconsistent documentation of treatment initiation dates, clinical trial referrals, and care transitions (e.g., transfers to other facilities, transitions to survivorship, or ongoing treatment)
Conclusion: These findings informed the development of a power form to track diagnosis-to-treatment times, treatment adherence, no show rates, clinical trials referrals, and care transitions, to address data collection gaps and align with navigator and leadership priorities. Scheduled for implementation on January 1, 2025, the power form will streamline workflows, enhance data-driven decision making in cancer care, and support reimbursement for navigation services under the new Principal Illness Navigation designation by the Centers for Medicare & Medicaid Services.

#988 Impact of the COVID-19 pandemic on HPV vaccine hesitancy.

Eric Adjei Boakye[1], Jun Jin[1], Mrudula Nair[1], Dakshu Jindal[1], Joel Fokom Domgue[2], Olivia Aspiras[3], Samantha H. Tam[1], Nosayaba Osazuwa-Peters[4]

[1]Henry Ford Health System, Detroit, MI,[2]University of Texas at MD Anderson Cancer Center, Houston, TX,[3]Michigan State University, East Lansing, MI,[4]Duke University School of Medicine, Durham, NC

Background: A major reason for suboptimal HPV vaccination coverage in the US is parental vaccine hesitancy. The COVID-19 pandemic and the vaccine had an impact on public trust in public health authorities, science, and medicine. The novelty of the disease and concerns over safety and efficacy of COVID-19 vaccine led to several people refusing the vaccine and increasing hesitancy towards other vaccines, including the HPV vaccine. We examined if HPV vaccine hesitancy among parents with unvaccinated children have changed due to the COVID-19 pandemic in the US.

Methods: This study used data from the 2018, 2019, 2022, 2023 National Immunization Survey - Teen. Hesitancy was defined by responses to the question, “How likely is it that [your teen] will receive HPV shots in the next 12 months?” as “not too likely,” “not likely at all,” or “not sure/don't know.” The Fisher exact test with FDR control was applied to identify significant predictors among adolescent’s age, gender, race, healthcare engagement, poverty level, doctor recommendation, geographic region; and mother’s age, marital status, education, number of children under 18, COVID-19 phases (2018-2019 as pre-COVID, and 2022-2023 as post-COVID), and their interactions. Next, logistic regression with forward selection was employed to quantify the effects of these predictors.

Results: Analysis included 103,977 unvaccinated adolescents (i.e., had not received any HPV vaccine doses at the time of the survey) whose parents were classified as vaccine hesitant. All demographic predictors and the COVID-19 timeline significantly influenced HPV vaccine hesitancy based on the two-stage estimation. In adjusted difference-in-difference analyses, overall, parental hesitancy during post-COVID years decreased compared to the pre-COVID years (aOR=0.84; 95% CI 0.79, 0.88). The impact of children's age and race and mother’s age showed consistent and significant changes from pre-COVID to post-COVID years. HPV vaccine hesitancy increased from pre-COVID to post-COVID years among parents of adolescents who were 15 (aOR=1.38; 95% CI 1.28, 1.46), 16 (aOR=1.40; 95% CI 1.31, 1.51) and 17 (aOR=1.52; 95% CI 1.40, 1.63), and among mothers who were over 45 years or older (aOR=1.06; 95% CI 1.01, 1.13). Conversely, HPV vaccine hesitancy of parents of non-Hispanic black adolescents (aOR=0.90; 95% CI 0.83, 0.98) decreased from pre-COVID to post-COVID years. Incorporating COVID-related features significantly enhances the ability to predict hesitancy.

Conclusions: HPV vaccine hesitancy among parents of unvaccinated adolescents decreased overall from pre-COVID to post-COVID years. However, hesitancy increased within specific groups, notably among parents of adolescents aged 15 to 17 and mothers aged 45 years and older age. These trends highlight the need for future research to explore the underlying factors driving these differences, enabling more targeted and effective public health interventions.

TUMOR BIOLOGY: Molecular Regulation of Metastasis
Poster Session

#99 HMGB1deficiency occurs in a broad range of human cancers and is often associated with unfavorable tumor phenotype.

Viktoria Chirico[1], Hena Sharifi[1], Maria Christina Tsourlakis[1], Seyma Buyucek[1], Clara von Bargen[1], Katharina Moller[1], Florian Lutz[1], David Dum[1], **Claudia Hube-Magg**[1], Elena Bady[1], Lisa Hornsteiner[1], Georgia Makrypidi-Fraune[1], Piero Caneve[1], Maximilian Lennartz[1], Morton Freytag[1], Sebastian Dwertmann Rico[1], Simon Kind[1], Viktor Reiswich[1], Eike Burandt[1], Till S Clauditz[1], Patrick Lebok[1], Christoph Fraune[1], Till Krech[1], Sarah Minner[1], Andreas H Marx[2], Waldemar Wilczak[1], Ronald Simon[1], Guido Sauter[1], Stefan Steurer[1], Kristina Jansen[1]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany,[2]Academic Hospital Fuerth, Fuerth, Germany

High-mobility group protein B1 (HMGB1) is a chromatin-associated protein with a role in maintenance of nucleosome structure, transcription control, and DNA repair. Both its overexpression and impaired function have been linked cancer development and progression. To better comprehend the role of HMGB1 expression in cancer, a tissue microarray containing 14,966 samples from 134 different tumor entities and 608 samples of 76 different normal tissue types was analyzed. A strong nuclear HMGB1 staining occurred in almost all normal cell types and in most cancers. Of 11,808 evaluable cancers, only 921 (7.8%) showed a complete HMGB1 expression loss (HMGB1 deficiency) while 1,172 (9.9%) showed a 1+, 2,956 (25%) showed a 2+, and 6,759 (57.2%) showed a 3+ HMGB1 positivity. HMGB1 deficiency most frequently occurred in pheochromocytoma (90%), seminoma (72.4%), gastrointestinal stromal tumor (28.6%), adrenal cortical carcinoma (25%), and Hodgkin's lymphoma (25%). Low HMGB1 expression was linked to poor histologic grade ($p<0.0001$), advanced pT stage ($p<0.0001$), high UICC stage ($p<0.0001$), and distant metastasis ($p=0.0413$) in clear cell RCC, invasive tumor growth in urothelial carcinoma (pTa vs. pT2-4, $p<0.0001$), mismatch repair deficiency ($p=0.0167$) in colorectal cancers, and advanced pT stage in breast cancer of no special type (NST) ($p=0.0038$). High HMGB1 expression was linked to nodal metastases in high-grade serous ovarian carcinomas ($p=0.0213$) and colorectal cancers ($p=0.0137$), as well as to high histological grade in squamous cell carcinomas ($p=0.001$). In conclusion, our data show that HMGB1 deficiency and reduced HMGB1 expression occurs in a broad range of different tumor entities and that low (and not high) HMGB1 expression is often linked to an aggressive tumor phenotype. Whether or not HMGB1 deficiency renders cells susceptible to specific drugs remains to be determined.

POPULATION SCIENCES: Bridging Gaps in Cancer Care: Disparities, Social Determinants, Innovations, and Strategies Poster Session

#990 Role pf viral infection in APOBEC3 dysreguation in cancer: a single-cell gene expression profiling approach.

Mohadeseh Soleimanpour-Moghadam, diako Ebrahimi

Texas Biomedical Research Institute, San Antonio, TX

The Cancer Genome Atlas (TCGA) provides comprehensive genomic and transcriptomic data from over 10,000 cancer patients, along with metadata including viral infection status and survival information. We investigated the role of viral infections in dysregulating the expression of A3 enzymes (A3A/B/C/D/F/G/H). These enzymes primarily function to restrict viral infections by introducing C-to-U mutations in viral genomes. However, certain members (notably A3A and A3B) can also mutate cellular DNA, contributing to tumor initiation and progression. Cancers such as breast, bladder, cervical, head & neck, and skin exhibit characteristic C-to-T mutations attributed to the aberrant expression/activity of A3 enzymes. Accurate quantification of A3 dysregulation in tumor cells using TCGA bulk RNAseq data is complicated by the ubiquitous expression of these enzymes in infiltrating immune cells. This highlights a broader challenge in analyzing heterogeneous tumor biopsies to uncover virus-specific dysregulated processes in cancer. To address this challenge, we employed Nonnegative Matrix Factorization (NMF) to deconvolute A3 expression profiles from TCGA bulk RNA-seq data, i.e. to separate tumor cell profiles from those of the tumor microenvironment. We validated our NMF-extracted profiles by analyzing publicly available single-cell RNA-seq datasets from approximately 3,000 cancer projects in the Gene Expression Omnibus (GEO). Additionally, to identify cell types represented by these profiles, we compared them with experimentally obtained A3 expressions from purified cell types. Our analyses identified up to four distinct A3 profiles: two associated with T cells and NK cells, one linked to dendritic cells and macrophages, and one corresponding to cancerous epithelial cells. Notably, the A3 profiles derived from our analysis of single-cell RNA-seq data closely matched those deconvoluted from TCGA bulk RNA-seq data, demonstrating that our bulk RNA-seq deconvolution effectively captures single-cell population-level profiles. We found that both tumor-specific and immune cell-specific A3 profiles correlate with viral infection; however, significant variations were observed across different viral types and tumor types. None of these profiles were correlated with the extent of A3 mutational signatures SBS2 and SBS13.In conclusion, our study demonstrates that deconvolution of bulk RNA-seq datasets can reveal single-cell population-level insights into the cellular composition of tumor biopsies. Moreover, this approach offers the resolution needed to pinpoint virus-specific dysregulated processes within the complex and heterogeneous tumor microenvironment.

#991 PARADIGM: an embeddings-based multimodal learning framework with foundation models and graph neural networks.

Asim Waqas[1], Aakash Tripathi[1], Mia Naeini[2], Paul A. Stewart[1], Matthew B. Schabath[1], **Ghulam Rasool**[1]

[1]Moffitt Cancer Center, Tampa, FL,[2]Electrical Engineering, University of South Florida, Tampa, FL

Introduction: Cancer research faces significant challenges in integrating heterogeneous data across varying spatial and temporal scales, limiting the ability to gain a comprehensive understanding of the disease. PARADIGM(Pan-Cancer Embeddings Representation using Advanced Multimodal Learning with Graph-based Modeling) addresses this challenge by providing a framework leveraging foundation models (FMs) and Graph Neural Networks (GNN). PARADIGM framework generates embeddings from multi-resolution datasets using modality-specific FMs, aggregates sample embeddings, fuses them into a unified graph representation, and uses GNNs for survival prediction.

Methods: We applied PARADIGM for survival analysis of five squamous cell carcinomas; head and neck (527), lung (496), bladder (408), cervical (294), and esophageal (184) cancers, and five adenocarcinomas; colon (393), lung (549), prostate (490), rectum (157), and stomach (406) cancers. Additionally, we validated using lung squamous cell data from Moffitt Cancer Center (103 patients). Multimodal data for training and evaluation included EHR data (e.g., age, gender, and smoking status), whole slide images (WSIs), pathology reports, and molecular data (e.g., gene expression, miRNA, and protein expression). Model performance was assessed using concordance index (C-index) for survival prediction, with 7-fold cross-validation ensuring robustness.

Results & Conclusions: PARADIGM demonstrated significant improvements in survival prediction compared to unimodal and multimodal approaches such as multilayer perceptron, Transformers, and XGBoost (Table 1). Our findings indicate that integrating diverse data modalities into a cohesive representation yields more accurate and insightful predictions. This approach highlights the potential of embeddings-based multimodal learning to foster improved outcome predictions and provide actionable insights for personalized treatment strategies.

Table 1.

		C-Index						
		Four Modalities	**Three Modalities**			**Two Modalities**		
Model	**Cancer**		**Clin, Path Rep, WSI**	**Clin, Path Rep, Molec**	**Clin, Molec, WSI**	**Clin, WSI**	**Clin, Path Rep**	**Clin, Molec**
MLP	LUSC	0.48	0.49	0.52	0.50	0.48	0.54	0.49
	HNSC	0.49	0.51	0.52	0.48	0.49	0.51	0.50
	ESCA	0.48	0.49	0.47	0.45	0.48	0.50	0.44
	CESC	0.52	0.49	0.54	0.51	0.48	0.54	0.58
	BLCA	0.53	0.52	0.51	0.52	0.53	0.54	0.48
	PanSCC	0.60	0.50	0.61	0.57	0.50	0.59	0.59
SVM	LUSC	0.49	0.48	0.57	0.47	0.48	0.53	0.56
	HNSC	0.49	0.51	0.56	0.49	0.50	0.54	0.58
	ESCA	0.48	0.49	0.55	0.50	0.47	0.50	0.56
	CESC	0.51	0.49	0.61	0.52	0.48	0.55	0.61
	BLCA	0.51	0.51	0.55	0.53	0.51	0.52	0.58
	PanSCC	0.54	0.50	0.59	0.53	0.50	0.52	0.58
XGBoost	LUSC	0.62	0.61	0.63	0.60	0.59	0.63	0.59
	HNSC	0.58	0.57	0.57	0.58	0.57	0.56	0.58
	ESCA	0.50	0.50	0.50	0.51	0.50	0.49	0.55
	CESC	0.59	0.58	0.58	0.58	0.60	0.60	0.60
	BLCA	0.59	0.57	0.58	0.60	0.59	0.59	0.58
	PanSCC	0.62	0.60	0.62	0.61	0.60	0.60	0.61
Transformers	LUSC	0.50	0.51	0.50	0.50	0.51	0.51	0.50
	HNSC	0.50	0.50	0.48	0.50	0.50	0.48	0.50
	ESCA	0.50	0.50	0.48	0.50	0.49	0.51	0.50
	CESC	0.51	0.50	0.50	0.49	0.49	0.49	0.49
	BLCA	0.50	0.50	0.51	0.50	0.51	0.50	0.50
	PanSCC	0.50	0.50	0.50	0.500	0.50	0.50	0.49
GNN	LUSC	**0.72**	**0.69**	**0.70**	**0.70**	**0.70**	**0.70**	**0.73**
	HNSC	**0.67**	**0.66**	**0.70**	**0.66**	**0.68**	**0.69**	**0.71**
	ESCA	**0.70**	**0.69**	**0.74**	**0.69**	**0.67**	**0.73**	**0.72**
	CESC	**0.67**	**0.68**	**0.72**	**0.71**	**0.71**	**0.70**	**0.72**
	BLCA	**0.70**	**0.68**	**0.70**	**0.67**	**0.65**	**0.69**	**0.71**
	PanSCC	**0.70**	**0.70**	**0.72**	**0.70**	**0.69**	**0.71**	**0.74**

#992 Distinct body composition profiles in early- vs. late-onset locally advanced rectal cancer - Results from the ColoCare Study.

Mmadili N. Ilozumba[1], Lia Whisenant[1], Britton Trabert[1], Jeffrey T. Yap[1], Tengda Lin[1], Vaia Florou[1], Mark A. Lewis[2], Patricia A. Erickson[1], Nicole C. Lorona[3], Anita R. Peoples[4], Joseph Letourneau[5], Manju George[6], Sheetal Hardikar[1], Caroline Himbert[1], Maria Gomez[7], Biljana Gigic[8], Christopher I. Li[9], Adetunji T. Toriola[10], Jane C. Figueiredo[3], David Shibata[11], Erin M. Siegel[12], Cornelia M. Ulrich[1], Doratha A. Byrd[7], Ramya Thota[13], **Jennifer Ose**[14]

[1]Huntsman Cancer Institute, Salt Lake City, UT,[2]Intermountain Health Care, Murray, UT,[3]Medicine, Cedars-Sinai Medical Center, Los Angeles, CA,[4]American Cancer Society, Atlanta, GA,[5]University of Utah, Salt Lake City, UT,[6]Paltown Development Foundation, Crownswille, MD,[7]Epidemiology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL,[8]General, Visceral and Transplantation Surgery, Heidelberg University Hospital, Heidelberg, Germany,[9]Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA,[10]Surgery, Washington University St. Louis, St. Louis, MO,[11]Department of Surgery, University of Tennessee Health Science Center, Memphis, TN,[12]Division of Cancer Control and Population Sciences, National Cancer Institute, Bethesda, MD,[13]Intermountain Health Care, Salt Lake City, UT,[14]Information and Communication, University of Applied Sciences and Arts, Hannover, Hannover, Germany

Introduction: Muscle mass and fat distribution are key components of body composition and have been linked to treatment response, treatment toxicity, and survival in cancer patients. Few studies have investigated body composition parameters in relation with rectal cancer, with no study evaluating body composition profiles in early-onset rectal cancer (EORC; diagnosed <50 years) and late-onset rectal cancer (LORC; diagnosed ≥65 years).

Methods: We utilized pre-treatment computed-tomography scans from 191 patients diagnosed with stage II/III rectal cancer recruited in Utah and Florida (two study sites of the ColoCare Study). Body composition parameters were assessed at L3 vertebral level including visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT), skeletal muscle tissue (SKM), intramuscular adipose tissue (IMAT), body mass index (BMI; kg/m²; continuous), BMI categories, and sarcopenia (yes/no). Multinomial logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (95% CI) for associations of body composition parameters with EORC and LORC, adjusted for sex, surgery, tumor stage, and study site, compared with average-onset rectal cancer (AORC; 50-64 years) as the reference group.

Results: Twenty-seven percent and 28% of patients were diagnosed with EORC and LORC, respectively. A one-unit increase in log-transformed IMAT area was associated with increased odds of LORC (OR 3.21 [1.46-7.08]) and decreased odds of EORC (OR 0.27 [0.13-0.59], p_{het}=<0.05) compared to AORC. A similar pattern emerged for sarcopenia (yes/no: OR_{LORC} 1.76 [0.86-3.62]; OR_{EORC} 0.43 [0.19-0.96], p_{het}=0.05) and VAT: OR_{LORC}1.03 [0.95-1.12]) and OR_{EORC} (0.91 [0.84-0.99], p_{het}=0.01) compared to AORC. The opposite pattern and magnitude of associations was observed for a one-unit increase in log-transformed SKM (OR_{LORC} 0.04 [0.004-0.31]; OR_{EORC} 9.49 [1.06-84.60], p_{het}<0.05). SAT area and BMI were not associated with onset of EORC or LORC.

Discussion: This study is the first analysis of a comprehensive panel of body composition parameters in EORC and LORC. LORC patients had higher IMAT or VAT areas and sarcopenia, consistent with associations in healthy older individuals. Similarly, EOCRC patients had greater SKM area, compared to AOCRC. It will be important to evaluate the associations of body composition parameters within each group with clinical outcomes and evaluate whether integrating body composition assessment into patient care may help optimize treatment response, reduce toxicities and improve outcomes.

#993 Exploring the tumor suppressive role of LRIG1 in breast cancer disparities.

A. H. Pajimola, B. Jenkins-Lord;
Johns Hopkins Bloomberg Sch. of Public Health, Baltimore, MD

Background: Breast cancer (BC) is the second most common cause of cancer-related death among women in the United States (U.S.). Despite a lower incidence rate, African American (AA) women suffer a persistent 40% higher BC mortality rate compared to European American (EA) women. The aggressive breast tumor biology experienced by AA women is rooted in both genetic and environmental causes and we are interested in investigating the social and molecular drivers influencing this complex health disparities problem. In our recent study using breast tumor DNA extracted from a diverse cohort of women from the Baltimore, Maryland area, we performed a methylation array to compare differences in neighborhood conditions to DNA methylation levels, including differences by race. From this analysis, we identified the tumor suppressor LRIG1 as significantly hypomethylated among AA patients with deprived neighborhood conditions. This gene encodes a transmembrane protein predicted to downregulate growth receptor signaling, with lower expression associated with decreased survival. These findings were unique to the tumor, which points to epigenetic changes to LRIG1 impacting tumor mutational burden, gene expression, and protein expression.
Objective: The goal of this project is to assess the relationship between LRIG1 genetic variants and molecular, clinical, and sociodemographic data in a diverse historical cohort of BC cases and controls. We hypothesize that LRIG1 single nucleotide polymorphism (SNP) genotypes will be more strongly linked to increased BC susceptibility and adverse clinical outcomes in AA women compared to EA women.
Methods: We obtained breast tissue DNA from our previous cohort, including 216 breast tumors (AA=135, EA=81) and 203 normal breast tissues (AA=115, EA=88). We conducted DNA genotyping on a clinically relevant ancestrally informative SNP of LRIG1 (rs2306272) using PCR amplification and detection by fluorescent reporters. We analyzed the associations between LRIG1 rs2306272 SNP genotypes and race, BC risk factors, and clinical data.
Results: Our experimental minor allele frequencies were in line with what is expected for AA and EA populations for this LRIG1 SNP. Chi-square tests showed associations between the LRIG1 rs2306272 genotypes and self-reported race ($p<0.0001$), family history of BC ($p=0.0037$), and breast tumor grade ($p=0.0768$).
Conclusions: This is the first data showing clinical significance between rs2306272, self-reported race, and BC characteristics in patient samples. We will continue analyzing the rs2306272 SNP genotypes in our DNA cohort in addition to a second ancestrally relevant LRIG1 SNP, rs1403626. Furthermore, using matched methylation data, we will perform analyses to identify differentially methylated CpG sites for LRIG1 that may be influenced by genetic ancestry. Our investigations will further interrogate the impact of LRIG1 regulation in differential BC risk and outcomes.

#994 Advancing health equity and reducing disparities in prostate cancer in Black men: iCCaRE.

Kimlin Tam Ashing[1], Timethia Bonner[2], Ewan K. Cobran[3], Folakemi Odedina[4], Aj Merriweather[4], Ernie Kaninjing[4], Floyd Willis[4], Cassandra Moore[4], Joanne Oliver[4], Fornati Bedell[1], John McCall[5]

[1]City of Hope National Medical Center, Duarte, CA,[2]Mayo Clinic, Rochester, MN,[3]Mayo Clinic, Phoenix, AZ,[4]Mayo Clinic, Jacksonville, FL,[5]Temple Beth Baptist Church, Pasadena, CA

In the US and globally, Black men experience the highest prostate cancer burden among all racial and ethnic groups, underscoring the urgent need for research to guide the development and implementation of culturally tailored strategies aimed at reducing disparities and improving outcomes. In response, the Inclusive Cancer Care Research Equity (iCCaRE) Consortium was created to advance health equity and reduce disparities in prostate cancer. Importantly, a Survivorship Care Plan (SCP) is recommended to educate and empower patients, enabling them to be better informed about their cancer diagnosis, treatment, and survivorship care recommendations. To date, there is no SCP targeted to Black prostate cancer survivors (CaPS) who have the greatest disparities with the poorest survivorship outcomes (ACS F&F; AACR HD Report; Fullwood, 2024). Therefore, we aimed to develop a SCP tailored for Black men. Addressing the unique needs of Black men require a genuine bidirectional relationship between scientist and the community rooted in community engaged research (CER). The SCP was first informed by the literature (Bonner, 2024) and the ASCO template (canceradvocacy.org). With input from the iCCaRE leadership and the Partnership Engagement Services (PES) Core, the Whole Person SCP better responds to the informational, clinical and quality of life needs of Black Prostate Cancer survivors. Based on CER, we included 22 diverse, Black CaPs to further guide and tailor the iCCaRE. Our findings indicate that the iCCaRE Survivorship Care Plan (SCP) enhanced the ASCO template by incorporating and expanding several unique domains, including family history, care team contacts, follow-up and surveillance, symptoms, quality of life, survivor concerns, major comorbidities, health advisories, and culturally relevant resources, creating a comprehensive, whole-person SCP tailored specifically for Black men. It is crucial to continue to improve support for clinical research and advocate for Black men and their communities to reduce prostate cancer burden. Prostate cancer also needs to include incidence, mortality, and disparities for Black men to raise awareness of scarcely studied field of Black men and prostate cancer survivorship.

#996 Dietary patterns in plasma cell disorders: Opportunities for targeted interventions.

Kadiatou Traore[1], Francesca Castro[1], Alankrita Taneja[2], Elan Gorshein[3], Andriy Derkach[1], Jorge Arturo Hurtado Martinez[4], Edith Serrano[1], Alexander Lesokhin[1], Jennifer M. Ahlstrom[4], Torin Block[5], Jay R. Hydren[4], Ana M. Sahagun Sanchez Aldana[4], Patricia Alejandra Flores Perez[4], Cynthia A. Thomson[6], Saad Z. Usmani[1], Urvi A. Shah[1]

[1]Memorial Sloan Kettering Cancer Center, New York, NY,[2]Hematology, Thomas Jefferson University Hospitals, Philadephia, PA,[3]Yale New Haven Health, Guilford, CT,[4]HealthTree Foundation, Lehi, UT,[5]NutritionQuest, Berkeley, CA,[6]University of Arizona, Tucson, AZ

Background: Multiple myeloma (MM), the second most common hematologic malignancy in the US, has seen improved survival with therapeutic advancements. This presents an opportunity to enhance outcomes through diet and lifestyle. Poor dietary habits and obesity are linked to adverse outcomes in MM and its precursors—monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM)—potentially mediated by intestinal dysbiosis which affects the immune microenvironment. High Healthy Eating Index (HEI) scores, which reflect better adherence to the Dietary Guidelines for Americans, are associated with reduced mortality. However, data on the dietary habits of individuals with plasma cell disorders (PCDs) remains limited.
Methods: We surveyed 443 individuals with MGUS, SMM, or MM on HealthTree Cure Hub using the Block Food Frequency Questionnaire (2022- 2023). We calculated HEI scores and analyzed by age, sex, race, BMI, PCD diagnosis, and education level. We also compared participants' scores to the general US population by age, sex, and race.
Results: Average HEI score was higher for participants (69) than for the US population (58) across all subgroups ($p<0.001$). Lower education was associated with lower HEI scores ($p<0.001$) and trended toward higher fruit and lower added sugar intake. Higher BMI trended toward lower HEI scores ($p=0.052$) and significantly increased fatty acid intake. Males consumed more calories with less vegetables, sodium, and fatty acid intake. Non-Hispanic Asians and Blacks consumed fewer calories but more fatty acids. MGUS and SMM participants consumed more vegetables and lower added sugar, but more sodium compared to MM patients.
Conclusion: Individuals with PCDs have more healthful diets than the general US population. However, females, non-Hispanic Asians, non-Hispanic Blacks, individuals with higher BMIs, and those with less education may benefit from targeted dietary interventions to improve health outcomes.

TABLE: Average Daily Food and Micronutrient Intake for Patients with Plasma Cell Disorders

		Fruit	Vegetable	Added Sugar	Fatty Acids	Sodium	Total Calories
	Mean	**1.0**	**1.6**	**5.1**	**1.6**	**1.8**	**1535**
Age	**31-59**	1.0	1.6	5.0	1.6	1.8	1560
	60-70	1.0	1.7	5.0	1.6	1.7	1523
	71+	1.1	1.5	5.3	1.6	1.7	1526
	P-value	0.490	0.423	0.525	0.054	0.410	0.717
Sex	**Male**	1.0	1.3	5.1	1.4	1.7	1680
	Female	1.1	1.8	5.1	1.7	1.8	1457
	P-value	0.313	**<0.001**	0.222	**<0.001**	**<0.001**	**<0.001**
Race	**Hispanic**	0.9	1.4	4.4	1.6	1.9	1467
	Non-Hispanic Asian	0.9	1.7	3.5	2.5	1.8	1177
	Non-Hispanic White	1.0	1.6	5.4	1.5	1.7	1553
	Non-Hispanic Black	0.9	1.2	5.1	2.3	1.7	1427
	Other race	1.0	1.3	6.6	1.3	1.5	1698
	Missing	1.1	1.8	4.4	1.7	1.7	1548
	P-value	0.783	0.140	0.101	**0.001**	0.244	**0.019**
BMI	**Underweight (<18.5)**	1.1	1.8	4.4	1.5	1.7	1612
	Healthy (18.5-24.9)	1.1	1.7	4.7	1.8	1.7	1510
	Overweight (25-29.9)	1.0	1.6	4.9	1.5	1.8	1583
	Obese (>30)	0.9	1.4	6.3	1.4	1.8	1515
	Missing	0.9	1.6	5.0	1.9	1.8	1489
	P-value	0.167	0.054	0.061	**0.001**	0.527	0.735
PCD Type	**MM Precursor States**	0.9	1.8	4.8	1.6	1.8	1522
	MM	1.0	1.5	5.3	1.6	1.7	1539
	Missing	1.1	1.7	4.8	1.7	1.7	1534
	P-value	0.149	**0.032**	**0.037**	0.091	**0.040**	0.615
Education	**Less than college**	1.0	1.4	6.0	1.6	1.7	1465
	Bachelor's or equivalent	1.0	1.5	5.2	1.6	1.8	1495
	Graduate/Professional	1.1	1.5	5.0	1.6	1.7	1587
	Missing	1.0	1.8	4.3	1.7	1.7	1549
	P-value	0.089	0.067	0.093	0.348	0.452	0.253

Fruit= total fruit (cup equiv./1000 kcal); vegetable= total vegetable (cup equiv./1000 kcal); added sugar (tbsp equiv./1000 kcal); fatty acids (total grams of PUFA + MUFA)/grams of sat fat); sodium (g/1000 kcal); total calories (kcal/day); BMI= Body mass index; PCD= Plasma cell disorder; MM Precursor states= Smoldering multiple myeloma and Monoclonal gammopathy of undetermined significance; MM= Multiple myeloma; P-value indicates the main effect of a factor using Kruskal-Wallis statistical method

POPULATION SCIENCES: Genomic Diversity and Disparities in Cancer: Global Insights into Epidemiology, Risk, Outcomes, and Precision Medicine
Poster Session

#999 Spectrum of germline BRCA1/2 gene mutations in Nigerian breast cancer patients.

A. F. Onyia[1], P. Jibrin[2], T. Olatunji-Agunbiade[3], A. Oyekan[3], A. Lawal[4], A. Alabi[4], A. C. Sowunmi[4], E. A. Aje[4], O. B. Ogunniyi[1], E. S. Nkom[5], O. C. De Campos[1], O. A. Rotimi[1], J. O. Oyelade[1], **S. O. Rotimi**[1];
[1]Covenant University, Ota, Nigeria, [2]National Hospital, Abuja, Nigeria, [3]Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria, [4]Lagos University Teaching Hospital, Idi-Araba, Lagos, Nigeria, [5]Federal Medical Centre, Abeokuta, Nigeria

Breast cancer (BC) is the leading cause of cancer-related deaths in Nigerian women, with triple-negative breast cancer (TNBC) being the most prevalent. The TNBC subtype is characterized by mutations in BRCA1 and BRCA2 genes, and germline pathogenic carriers of these mutations have an increased risk for BC. Despite these challenges, the prevalence and spectrum of BRCA1/2 pathogenic variants in the Nigerian population differ, and there is a margin in the local capacity to characterize these variations. Therefore, this study aimed to identify and characterize germline variations in BRCA 1/2 genes in Nigerian BC patients and healthy aged-matched controls to understand the genetic risk profiles of BC in this population. Forty-five BC patients were recruited across four major hospitals in Nigeria and aged-matched with 51 healthy female controls. DNA was extracted from blood samples, followed by targeted sequencing of BRCA 1/2 intronic and exonic regions using the Ampliseq for BRCA panel and the Illumina Miseq Platform. Variant calling was performed, and the clinical significance of identified variants was evaluated on the ClinVar and BRCA exchange databases. Variants of unknown significance (VUS) were assessed using known *in silico* prediction software, and haplotype analysis was carried out using the Haploview 4.2 software. Pathogenic variants were identified in 6.7% of cases, all exclusive to BC patients. These variants included two BRCA1 variants (3: c.133_134delAA (p.Lys45fs) and c.5324T>A (21: p.Met1775Lys), and one BRCA 2 variant (22: c.8817_8820del (p.Lys2939fs) all found in patients with the TNBC subtype. Additionally, 97 benign or likely benign BRCA1/2 variants were found in both BC and control groups, with notable variants such as the rs799917 identified as a surrogate indicator of ancestry. Eighteen VUS were identified, with four predicted to be damaging by three *in silico* prediction software. The results of haplotype analysis identified distinct BC haplotypes in Nigerian BC patients. The identification of BRCA1/2 pathogenic variants in Nigerian BC patients, especially those with TNBC, suggests a potential for targeted therapies, such as PARP inhibitors, to improve treatment outcomes in this population. This further highlights the need for increased population-specific screening and the integration of genetic screening into BC management strategies, which could facilitate early detection, personalized treatment plans, and genetic counseling for Nigerian BC patients.

Monday, April 28, 2025

BIOINFORMATICS / COMPUTATIONAL BIOLOGY / SYSTEMS BIOLOGY / CONVERGENT SCIENCE: Application of Bioinformatics to Cancer Biology
Poster Session

#1073 Integrated single cell atlas of human breast cancer enables highly-resolved analysis of tumor heterogeneity and metabolic comorbidities.

Andrew D. Chen[1], Christina Ennis[2], Lina Kroehling[1], Gerald Denis[2], Stefano Monti[3]

[1]Bioinformatics, Boston University, Boston, MA,[2]BMC Cancer Center, Boston University, Boston, MA,[3]Computational Biomedicine, Boston University, Boston, MA

Single cell transcriptomics (scRNAseq) remains the method of choice to quantify and annotate cellular heterogeneity in the tumor and its microenvironment (TME), yet most studies are under-powered to associate changes in tumor heterogeneity with phenotypes such as tumor subtype, grade, and patient age, among others. In this study, we created an integrated atlas of human breast cancer (BC), the largest resource of its kind, totaling more than 700,000 cells across 145 patients, and optimized computational methods to benchmark integration performance, and robustly perform hierarchical cell type annotation. By combining single profiles, a higher-resolution annotation of immune, stromal, and epithelial cell types was achieved. Further, using this integrated atlas, differential abundance analyses identified significant changes in immune and stromal cell type composition associated with tumor grade, subtype, and inferred metabolic comorbidities. For insulin resistance and obesity, we identified significant expansion in exhausted CD4 T Cell populations and interferon signalling in CD8 and macrophage populations. These changes in TME heterogeneity were not discernible or had effects in discordant directions when the analysis was limited to individual BC studies, highlighting the need for atlas-based mega-analysis approaches. This highly-resolved integrated scRNAseq BC atlas represents a valuable resource for hypothesis-driven analyses of tumor heterogeneity.

#1094 Harnessing neural networks for cancer prediction: clinical staging of head and neck cancer.

Amrit Menon[1], Nathan DeMichaelis[2], Dalton Schutte[3], Frank G. Ondrey[4]

[1]University of Chicago, Chicago, IL,[2]Dartmouth College, Hanover, NH,[3]Department of Surgery, University of Minnesota Medical School, Minneapolis, MN,[4]Department of Otolaryngology - Head & Neck Surgery, University of Minnesota Medical School, Minneapolis, MN

Introduction: Predictive oncology through the use of deep learning offers potential to both classify and assist in the treatment of head and neck cancer, a disease that has a relatively static rate of cure. More specifically, neural networks may allow for the classification of genomic expression data in a more complex manner. The present study applies neural networks to the larger NCI Cancer Genome Atlas (TCGA) and our previously published smaller U133A Affymetrix gene expression dataset.
Methods: The models were constructed using Pytorch, built from modular blocks of neural networks (Chunks) to facilitate extensive combinations of layers. Every Chunk consisted of a linear layer and optional predefined layers: rectifier (ReLU), normalization, and random neuron dropout (to prevent overfitting). The DeepChunk model (consisting of multiple Chunks) had additional tunable hyperparameters (optimization function, learning rate, etc.) The neural networks were trained to classify T and N staging data separately for both TCGA (split by HPV status) and Affymetrix chip datasets between early (T1/2) versus advanced (T3+) T stages, N0 versus N1+, respectively. Models were trained on larger datasets (HPV- T and HPV N, n >= 350) using randomly split data with 80% reserved for training and 20% reserved for testing, with proportional staging data across the split. The remaining datasets (n <= 50) had a split of 70% training and 30% testing. Hyperparameters (tuned for both the model and specific chunks) were assessed (by test accuracy and area under receiver operator characteristic curve, AUC-ROC) on the whole gene expression data. Settings selected during the hyperparameter screen were assessed with 20 trials using randomized initialization and data splits.
Results: The models (test accuracy [95% CI], AUC-ROC [95% CI]) trained on the TCGA HPV- N (0.619 [0.601, 0.637], 0.63 [0.602, 0.659]) and TCGA HPV+ T (0.72 [0.672, 0.768], 0.733 [0.671, 0.794]) datasets performed significantly better than the other models trained. The remaining models failed to significantly predict above random chance (with 95% CIs overlapping with 0.5). Unstable performance from these models could be attributed to overfitting or quickly converging during training with lack of improvement afterwards. Another potential cause involves high variance from the smaller size of training data.
Conclusions: The process used to tune and train the neural networks harbors potential in the analysis of large gene datasets. At the same time, it may benefit from feature reduction or functional annotations as the one dimensional feature lists are not inherently ordered or clustered, as is the case with two dimensional image neural networks.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Novel Antitumor Agents 1
Poster Session

#1171 M9466 (HRS-1167): A highly potent and selective PARP1 inhibitor with DNA trapping activity, monotherapy antitumor activity and synergistic potential with DDR inhibitors and chemotherapies.

Heike Dahmen[1], Ada Sala Hojman[1], Steffie Revia[1], Xin Li[2], Ting Zhang[2], Nina Berges[1], **Astrid Zimmermann**[1], Thomas Grombacher[1], Brian Elenbaas[3]

[1]The healthcare business of Merck KGaA, Darmstadt, Germany,[2]Jiangsu Hengrui Pharmaceuticals Co., Ltd., Shanghai, China,[3]EMD Serono, Billerica, MA

Poly(ADP-ribose) polymerase 1 (PARP1) plays a key role in the repair of single-stranded DNA breaks and DNA damage caused by endogenous replication and repair errors or by exogenous DNA damaging agents. Currently approved PARP inhibitors (PARPis) such as olaparib, niraparib and talazoparib show synthetic lethality in homologous recombination deficient (HRD) cancers including those with *BRCA1/2* mutations. Although approved PARPis provide a major therapeutic advance for patients with these cancers, they are associated with hematologic toxicities which limit their therapeutic window and their combinability. All currently approved PARPis non-selectively inhibit PARP1, PARP2 and often additional members of the PARP family. However, inhibition of PARP1 appears to be sufficient for antitumor activity, while PARP2 inhibition is associated with hematologic toxicities in preclinical models. M9466 is a highly potent and selective PARP1 inhibitor with DNA trapping activity. It shows a highly potent antiproliferative efficacy in the DLD-1 (BRCA2 KO) cell line, but not in the DLD-1 wild-type cell line. M9466 exhibited selective antiproliferative activity in a large panel of 96 cancer cell lines *in vitro* including several *BRCA1/2* mutant / PARP-sensitive models. M9466 also selectively inhibited PARP1 vs PARP2 (672-fold) in A549 cells as shown by PARylation inhibition assays after shRNA knockdown of individual PARP genes. M9466 strongly reduced tumor growth in a *BRCA1* mutant breast cancer tumor xenograft model *in vivo* with once daily, oral dosing. To identify top synergistic combination partners for M9466, a large *in vitro* drug combination screen was conducted with 101 compounds in 35 cancer cell lines. The screen highlighted synergistic activity of M9466 with certain DDR inhibitors (ATR and ATM kinase inhibitors) and with topoisomerase I (Topo I) inhibitors and temozolomide. The combination potential was further evaluated and confirmed in a series of *in vivo* tumor xenograft studies. Here, M9466 enhanced the antitumor efficacy of the ATR inhibitor tuvusertib and the chemotherapy carboplatin. Thus, M9466 has strong single agent synthetic lethal activity in HRD models and combinability with selected DDRis and chemotherapies. Based on these data, Phase I clinical trials are evaluating M9466-based combinations in patients with advanced solid tumors, and colorectal cancer (NCT06421935, NCT06509906).

TUMOR BIOLOGY: 3D Models and Bioprinting
Poster Session

#1217 Modelling GBM-vasculature interaction on chip.

Promise Emeh[1], Kevin Jimenez-Cowell[2], Martine Lamfers[2], Clemens Dirven[2], **Nick Saites**[1], Todd Burton[3], Karla Queiroz[3]

[1]MIMETAS, Oegstgeest, Netherlands,[2]Department of NeuroSurgery, Erasmus MC-University Medical Center Rotterdam, Rotterdam, Netherlands,[3]MIMETAS, oegstgeest, Netherlands

The tumor microenvironment (TME) in glioblastoma (GBM) is remarkably intricate, characterized by its dynamic nature and high heterogeneity that significantly influence tumor progression and treatment resistance. The heterogeneous TME comprises of blood vessels, immune cells, neurons, and the blood-brain barrier (BBB) presenting distinct tumor compartments that influence the tumor behavior. A recent classification based on the heterogeneous TME identified three distinct subtypes TME^{Low}, TME^{Med}, and TME^{High} distinguished by varying endothelial and immune cell populations. This study explores the complex interactions between glioblastoma (GBM) and its tumor microenvironment (TME), focusing specifically on the blood-brain barrier (BBB) and vascular compartment. To evaluate GBM's impact on the BBB, a compartmentalized model was developed that prevents direct cell-to-cell contact, allowing the observation of paracrine signaling. Using this model, distinct effects were identified across different GBM cell lines: one cell line caused significant BBB permeability with low transendothelial electrical resistance (TEER) values, while another had minimal impact on barrier integrity. To further investigate GBM-BBB interactions, a vascular network model incorporating brain endothelial cells, stromal cells and GBM cells within an extracellular matrix was developed. This model revealed consistent differences between the cell lines, aligning with the initial findings and reflected in endothelial cell morphology and vascular network density. Analysis of cytokines secreted by the GBM cell lines provided further insights into the observed changes in vascular organization. Ongoing investigation aims to use patient derived cells in this model to pick up interpatient variability and specifically look at the differences between the TME subtypes. This approach allows for the in vitro modelling and characterization of GBM TME subtypes and potentially the identification of novel druggable targets in the GBM TME.

#1218 A comprehensive and scalable human liver-on-a-chip model forfibrosis drug discovery and gene delivery applications.

Flavio Bonanini, Roelof Dinkelberg, Tessa Hagens, Nienke Kortekaas, **Nick Saites**, Manuel Torregrosa, Dorota Kurek, Paul Vulto, Kristin Bircsak

MIMETAS, Oegstgeest, Netherlands

Liver cancer, or hepatocellular carcinoma (HCC), is a formidable malignancy characterized by the uncontrolled growth of cells within the liver tissue. It represents a global health concern, with risk factors including chronic viral infections (such as hepatitis B and C), excessive alcohol consumption, fatty liver disease, and certain genetic conditions. The silent progression of HCC complicates early detection, emphasizing the need for a deeper understanding of the liver tumor microenvironment, which plays a crucial role in disease development. The liver model presented here incorporates a wide array of relevant liver cell types, including hepatocytes, endothelial cells, stellate cells, and resident immune cells. This is complemented by a complete vascularization system, allowing for fluid flow across a micro vascular network that faithfully models the dimensions of liver sinusoids. All these elements are seamlessly integrated into an automated and high throughput platform known as the OrganoPlate®. To model the liver tumor microenvironment, hepatocellular carcinoma cells were integrated into healthy liver tissue model. The resulting model allowed for the observation of integrated cancer colonies, providing a more realistic platform for therapy development compared to monocultures. Simultaneously, the liver's particle clearance mechanisms were investigated using fluorescent imaging. Endothelial cell phagocytosis of E. coli particles and resident macrophages' internalization of dextran molecules were studied to explore hepatic delivery of macromolecules and nanoparticles via the systemic circulation. The integration of hepatocellular carcinoma cells within healthy liver tissue revealed the formation of integrated cancer colonies, offering insights into the complex interactions within the liver tumor microenvironment. Fluorescent imaging showcased endothelial cell phagocytosis of E. coli particles and resident macrophages' internalization of fluorescent molecules. These findings elucidate the liver's particle clearance mechanisms, providing a foundation for understanding immunological processes and designing efficient drug delivery systems. Understanding the liver tumor microenvironment is critical for developing effective therapies for HCC. The integration of hepatocellular carcinoma cells within healthy liver tissue offers a more realistic platform for therapy development compared to traditional monocultures. We believe this system holds the potential for a groundbreaking progress in liver modelling which, besides including functional hepatic cells, also reflects the cellular organization and interactions found within the liver lobule. In conjunction with its high throughput capability, this has the potential to revolutionize drug discovery and develop therapies for complex liver diseases.

#1220 Cryopreserved 3D human tumor spheroid platform in 96-well format for robust & scalable discovery and screening.

Madhu Nag, Simon Strobel, Simon Hutter, Tala Issa, Laure-Anne Ligeon, Nils Goedecke, Olivier Frey

InSphero AG, Schlieren, Switzerland

Development of new therapies relies on rigorous testing in physiologically relevant, predictive in vitro models. This approach reduces the attrition of preclinical candidates going into animal studies and onto Phase 1 trials. While 2D tumor cell cultures have traditionally been the norm in early drug screening due to their automation compatibility and ease of use, they are no longer the optimal choice for drug development. This is due to their lack in translatability to human physiology, which is essential for assessing aspects of the cellular microenvironment, intercellular interactions, and the role of immune cells. The generation of 3D in vitro tumor spheroids for high-throughput screening campaigns can be labor-intensive, particularly at the lead discovery stage. The availability of a constant, reproducible supply of a panel of tumor spheroids for target ID and target validation studies represents a significant time and resource saving. To provide more flexibility in large-scale screening for therapeutic candidates in oncology, we developed a new platform offering a wide range of cryopreserved 3D tumor models in microplate format, enabling off-the-shelf high-content screening. The platform allows users to begin testing compounds within 3-5 days by just thawing an ARCTis™ plate (Always Ready Cryo Tissues) and following a few instructions. Another advantage of these cryopreserved 3D models is that they are manufactured in larger batches under highly standardized processes, ensuring a consistent starting point for compound testing. This results in enhanced data quality, an extended test window length, and data that is comparable across workgroups. Our current portfolio comprises 20 tumor cell lines from ATCC, representing the most common solid tumors. Staurosporine has been used as a positive control to detect a dose-dependent effect on cell viability. Following this treatment the dose-response indicated growth inhibition, shrinkage or tumor cell killing effects based on size and metabolic analysis (ATP). Furthermore, we demonstrated the robust performance of these assays for plates stored up to 6 months at -80 °C. This study demonstrates the efficacy of selected small molecules to show the functionality of the spheroids and to compare their functionality across different tumor models. Therefore, we will present applications for ARCTis™ plates that proof their relevance for pre-clinical testing. In conclusion, this innovative approach addresses the limitations of traditional 2D cell cultures by providing a high-throughput, in-plate frozen, 3D cell culture platform and access to a large tumor panel for parallel testing, offering a more physiologically relevant representation of tumor diversity. Our robust frozen flexible assay platform offers a promising new approach to advancing cancer drug discovery, streamlining the exploration of complex drug testing screens in vitro.

#1221 Modelling the heterogeneity of high grade serous ovarian tumors in 3D to study its metabolic response to atovaquone.

Paula M. Manan-Mejias[1], Nicha Boonpattrawong[2], Manish Patankar[1], Maria Virumbrales Munoz[1]

[1]University of Wisconsin - Madison, Madison, WI,[2]University of Wisconsin - Oshkosh, Oshkosh, WI

Ovarian cancers are the 8th most common cause of cancer related deaths in women, which outcomes have not meaningfully improved since the 1980s. High-grade serous ovarian carcinoma (HGSOC), is the most common subtype of ovarian cancer and the deadliest, responsible for 70-80% of ovarian cancer deaths. HGSOC is typically diagnosed at advanced stages, due to lack of reliable tests and vague external symptoms. When detected, treatment options are limited and result in a 60% recurrence rate. Consequently, there is an urgent need for new therapeutic agents for HGSOC. Atovaquone is an FDA-approved drug for malaria that has been effective against HGSOC in 2D models. Atovaquone (ATO) is an OXPHOS inhibitor that induces reactive oxygen species (ROS) by binding to complex III, leading to apoptosis. However, the mechanism of action of Atovaquone is not well understood, arguably limited to using traditional HGSOC models in mechanistic studies. For example, 2D *in-vitro* models fail to include oxygen and nutrient gradients, as well as relevant mitochondrial organization for 3D migration processes, whereas *in-vivo* models make real-time cell inspection challenging. To bridge this gap, we have designed a microphysiological system (MPS) to investigate the role of the 3D tumor microenvironment (TME) in the HGSOC response to therapeutics. Our MPS includes an HGSOC cell spheroid that models intra-tumor metabolic heterogeneity, by recapitulating a necrotic core, quiescent area, and proliferative edge. Adjacent to the tumor spheroid, we can include 3D tubular vessels to study the delivery and effects of atovaquone in lymphatic drainage. Migration and invasion of HGSOC spheroids decrease with ATO treatment. We also showed that atovaquone alters mitochondrial dynamics within 48-72 h of treatment. In addition, a non-destructive imaging technique capable of assessing real-time metabolic profiles in spheroids, revealed that ATO is capable of shifting cancer cell metabolism in spheroids, impeding ATP production. Using this HGSOC MPS, we aim to better understand the mechanism of action of atovaquone and accelerate its translation into the clinic as a sole or combination therapy agent.

#1222 Integrating i*n vitro* and *in vivo* data with algorithm-driven PK/PD modeling: a fast and efficient tool for evaluating cancer therapies in preclinical models.

Karina Adamowicz, David Cebrian, Fabio dell' Aquila, Leanne Doyle, Agnieszka Drzał, Simone Esposito, Bartosz Płociennik, Katarzyna Urbańska, **Martyna Sniegocka**

Selvita S.A., Krakow, Poland

Preclinical *in vivo* PK/PD studies of cancer therapies are invaluable for cancer drug discovery and the translation of therapies from bench to bedside. However, conventional *in vivo* experimentation are often time-intensive, resource-demanding, and may require redesign due to inefficient and/or toxic range of concentrations, or suboptimal study parameters. To address these challenges, we developed an integrated approach combining *in vitro* and *in vivo* data to generate *in silico* PK/PD models of anti-cancer compounds. Our algorithm was verified using standard chemotherapies, including Cisplatin and Dacarbazine, against tumoral cell lines for e.g. B16F10 and LLC. The algorithm was trained with the cells doubling time *in vitro* and with the tumor growth rate in mice. We then conducted an *in vitro* time-course experiment using a range of concentrations to assess cellular growth inhibition. This data was integrated into the algorithm thus it can calculate the IC_{50}, the drug plasma concentration, and the efficacy over-time of each drug against the engrafted cell lines in mice. Finally, we validated our model *in vivo* using selected doses from the calculated efficacy ranges, achieving tumor volume reductions comparable to the model's projections for the tested drugs. In conclusion, we generated a fast and resource-efficient method for PK/PD studies, broadly applicable to cancer drug candidates with minimal prior *in vivo* efficacy data.

#1223 A novel 3D high-throughput screen identified drugs that re-epithelialize colorectal cancer cells and enhance response to chemotherapy.

Sarah J. Harmych[1], Thomas P. Hasaka[1], Chelsie K. Sievers[2], Seung W. Kang[1], Vivian T. Jones[1], Zhiguo Zhao[2], Oleg Kovtun[1], Claudia C. Wahoski[1], Ken S. Lau[1], Robert J. Coffey[2], Joshua A. Bauer[1], **Bhuminder Singh**[2]

[1]Vanderbilt University, Nashville, TN,[2]Vanderbilt University Medical Center, Nashville, TN

Loss of polarity is a hallmark of cancer, and the related epithelial-to-mesenchymal transition (EMT) phenotype also impacts prognosis and therapy outcomes, particularly in colorectal cancer (CRC). However, the mechanisms and drugs that impact these morphologic changes are understudied, in part, due the complete failure of typical live/dead 2D high-throughput screens (HTS) to capture morphology or lack of robustness of 3D screens. Here, we designed an HTS using 3D type I collagen cultures of CRC cells to assess morphological changes in colonies and identified several FDA-approved drugs that re-epithelialize CRC colonies. One of these drugs, Azithromycin, increased colony circularity, enhanced E-cadherin membrane localization, and surprisingly, also elevated sensitivity to the chemotherapeutic, Irinotecan. A retrospective study of patient data demonstrated that the combination of Azithromycin with Irinotecan improved the 5-year survival of patients with CRC. These results highlight the importance of morphologic screens to identify novel drug candidates and synergistic mechanisms.

#1224 Unlocking the potential of 3D patient-derived tumor microtissues as reliable and scalable *in vitro* pre-clinical models.

Laure-Anne Ligeon, Charlotte Veser, Michal Rudnik, matthias Zbinden, Agnieszka Pawlowska, Simon Hutter, Tala Issa, Irina Agarkova, sue grepper, Nils Goedecke, Madhu Nag

InSphero AG, Schlieren, Switzerland

Over the last decade remarkable advances have been made in our understanding of tumor biology's microenvironment, allowing for the development of innovative drugs. However, the vast majority of oncology drug candidates entering clinical trials often fail due to the lack of clinical efficacy. This high rate of attrition is in large part due to the lack of physiologically relevant, clinically translatable, preclinical models. Indeed, current preclinical models often inadequately reflect tumor heterogeneity and intricate complexities of the tumor microenvironment (TME), which are important determinants of both the sensitivity and the acquired resistance of any tumor. In an effort to tackle both these issues, we developed a robust preclinical model based on 3D patient-derived tumor microtissues. While there are a few 3D *in vitro/ex vivo* models incorporating primary tumor tissue, such as patient-derived organoids, PDX-derived organoids, and organotypic slice cultures, our 3D InSight™ Primary Tumor platform provides the preserved original patient tumor microenvironment at a scale that no one else is able to offer, thereby making this platform amenable for both screening and validation. This platform also addresses the research field's challenge of mirroring patient heterogeneity in a plate. We successfully established uniform 3D patient-derived tumor microtissues from fresh surgically resected tissues from colorectal or breast cancer, with a success rate of 70%. The newly formed 3D patient-derived tumor microtissues were characterized physiologically. We were able to accurately identify the cell types comprising the tumor and the TME by flow cytometry after tissue dissociation. Furthermore, these 3D patient-derived tumor microtissues were treated with standard of care chemotherapeutic drugs, and mimicked the *in vivo* response which we would expect to see with the same original tumor. Therefore, our 3D InSight ™ Primary Tumor platform is a solution that allows for the testing of multiple therapeutics as single agents/combinations, in a model that very closely resembles its *in vivo* counterpart. The ability to do this at scale has never previously been offered on any platform. Furthermore, this platform will pave the way for the development of therapeutics in oncology that are truly tailored to specific patient profiles, thereby greatly improving the prognosis and quality of life for patients suffering from cancer.

#1225 Phenotypically-relevant patient-derived 3D cell models to interrogate personalized medicine approaches at scale.

Thomas Grundy[1], Peilin Tan[2], Joanna Wasielewska[1], Christine Yee[1], Tania Fowke[1], Sean Porazinski[1]

[1]Inventia Life Science, Sydney, Australia,[2]UNSW Sydney, Kensington, Australia

The complexity and heterogeneity of cancers both intra- and inter-patient have led to the precision medicine paradigm of cancer therapy where treatment is personalized to the individual patient. Recent clinical trials such as the I-PREDICT study [1] have underscored the benefits of approaches matching combination therapies to the patient's molecular alterations, leading to improved disease control and survival rates. Tumoroids are patient-derived cancer cells that grow as 3D self-organized, multicellular organoids that maintain key characteristics of the patient tumor of origin including molecular features such as genotype and gene expression, and biological behaviors. Whilst these models serve as valuable tools for studying tumor biology and responses of patient-derived cells to various anti-cancer therapies, the establishment and maintenance of tumoroids has historically been difficult and labor intensive, limiting more widespread use. Here, we utilize the Inventia RASTRUM^{TM} platform and OncoPro^{TM} colorectal cancer (CRC) Tumoroid Cell Lines (ThermoFisher Scientific [2]) to easily create plug and play patient-derived 3D cell models based on synthetic PEG-based hydrogel matrices, which were tuned to mimic the tissue stiffness and ECM composition of CRC tumors. We used this workflow to evaluate how these 3D models maintain the clinicopathological features of patient-derived tumoroid cells, as well as molecular features and cellular signaling native to the tumor and its microenvironment. We further demonstrate the utility of this approach for throughput drug screening and predicting personalized therapy responses. Overall, our approach provides a scalable framework for evaluating precision medicine approaches using clinically-relevant patient-derived tumoroids. Future work will focus on the development of processes to support direct dissociation and generation of patient-derived models from biopsies from a variety of cancer types and larger scale utility of the RASTRUM platform in workflows that could be leveraged to inform treatment decision making.

References

[1] Sicklick JK et al. Molecular profiling of cancer patients enables personalized combination therapy: the I-PREDICT study. Nat Med. 2019 May;25(5):744-750.[2] Paul CD et al. Long-term maintenance of patient-specific characteristics in tumoroids from six cancer indications in a common base culture media system. bioRxiv 2024. doi: https://doi.org/10.1101/2024.06.10.598331

#1226 Advancing CAR-T cell therapies using Nilogen's ex vivo 3D tumoroid platform.

Vanessa Garrido, Jared Ehrhart, **Rikhia Chakraborty**

Nilogen Oncosystems, Tampa, FL

Background: Advancing CAR-T cell therapies for solid tumors remain a significant challenge due to the complexities of the tumor microenvironment, which impacts therapeutic efficacy, operational mechanisms, and biomarker discovery. Existing preclinical models, including patient-derived xenografts and traditional organoids, often fail to replicate the physiological and immunological intricacies of solid tumors and require extensive time for establishment. Nilogen's ex vivo 3D tumoroid platform overcomes these limitations by preserving the tumor-immune microenvironment and extracellular matrix (ECM) observed in patient tumors. This platform enables robust therapeutic evaluation in a physiologically relevant context. Renal cell carcinoma (RCC), which constitutes 3% of adult cancers, has seen an increasing focus on immunotherapies and combination regimens. CD70, a highly expressed antigen in RCC, represents a promising target for CAR-T cell therapy, currently in phase I clinical trials. However, patient-specific variability in CD70 expression highlights the need for models like tumoroids to capture this heterogeneity for robust assessment of CAR-T efficacy in a patient-specific manner.
Methods: This study assessed the performance and functionality of second-generation anti-CD70 CAR-T cell therapy in RCC tumoroids. We explored the impact of immune checkpoint inhibitor combination therapy on CAR-T cell function. Key evaluations included tumor cell apoptosis (measured through time-lapse imaging), T cell activation (monitored using CD69, CD25, 4-1BB, and OX40 expression), T cell exhaustion (monitored using PD-1, LAG-3, and Tim-3 expression), and inflammatory secretome focusing on Th1/Th2 cytokines. In addition, we explored the impact of immune checkpoint inhibitor combination therapy on CAR-T cell functional efficacy.
Results: In tumoroids derived from 13 RCC patients, CD70 CAR-T cell therapy showed variable tumor-killing efficacy, with approximately 50% of samples exhibiting a positive response. Activated CAR-T cells displayed expected activation markers upon CD70 recognition and co-expressed exhaustion markers in both responders and non-responders. Cytokine profiling and the evaluation of combination therapies provided further insights into additional opportunities for optimizing CAR-T cell therapeutic performance.
Conclusions: Nilogen's tumoroid platform provides a physiologically relevant system to evaluate CAR-T cell functionality in solid tumors, enabling the assessment of patient-specific variability in response to therapy. These findings underscore the potential of tumoroids to refine and accelerate the development of CAR-T therapies, which, in turn, enhances their clinical success, particularly for RCC and other solid tumors.

#1227 Cancer cell-specific killing by reduced dose of chemotherapeutic agents in combination with immune cell therapy may be optimized in 3D BioSPHEER™ cancer models.

Boye Schnack Nielsen, Natasha H. Madsen, Isabella Skandorff, Jesper Larsen, Kim Holmstrom, Monika Gad

Cellular Engineering & Disease Modeling, Bioneer A/S, Copenhagen N., Denmark

3D cancer models consisting of cancer and fibroblast cell lines (BioSPHEERs™) exhibit complex morphology depending on the cell lines used. We recently reported that such 3D cancer models can be used for drug testing by measuring cell killing and cell survival [Nielsen et al 2024, doi: 10.3389/fonc.2024.1386097]. Chemotherapeutic agents, like 5-FU, cisplatin and cyclophosphamide, at a certain dose, kill the majority of cancer cells, but also normal cells in the body, thus causing unappreciated discomfort and side effects. In contrast, therapeutic immune cells, like TILs and CAR-T cells perform cancer cell specific killing. Importantly, our 3D cancer models also enable studies on T cell infiltration and T cell directed cell killing. PBMC-derived T cells added to heterospheroids infiltrated the structures without excessive killing, unless the T cells had been activated by CD3/CD28 beads, or primed with the respective cancer cell line ahead of mixing. In this study we first combined low (sub-lethal) dose of the chemotherapeutic agent 5-FU with subsequent administration of PBMCs. 5-FU was added to spheroids, consisting of mCherry-engineered HT29 cells and 1BR3G fibroblast, at day 7 after seeding and after 24 hours the 5-FU was removed and PBMCs were added. 5-FU added at 500 µM (high dose) caused dramatic killing measured by a luciferase assay, whereas the drug at 100 µM (low dose) appeared quite harmless to the cells in the spheroids. We next treated spheroids with the low dose of 5-FU and after 24 hours added T cells. After additional 72 hours, histological examination showed that the T cells themselves partly infiltrated the vehicle-treated spheroids, but caused little killing. However, the same T cells added to the 5-FU-treated spheroids caused excess killing, suggesting a synergistic effect of the two agents. We are currently testing more PBMC donors in combination with other therapeutic agents, including antibody drug conjugates (ADC). Our observations suggest that well-known chemotherapeutic agents applied at sub-lethal dose may enhance the effect of immune cell therapies. The combination treatment may retain or improve drug efficacy and at the same time reduce severe side effects caused by the chemotherapeutic agent. We propose that the use of 3D BioSPHEER™ cancer models to study combination treatment, will allow better optimization of dosage and timing of combined chemo-immuno-therapy.

#1228 High throughput phenotyping and drug discovery using the TumorGraft3D co-culture platform and flow cytometry.

Sumanun Suwunnakorn, Fu-Ju Chou, Elena Bruni, Karolina Pilipow, Xiangyang Zhang, Chelsea Riveley, Mara Gilardi, Stefano Cairo, **Brandon Walling**

Champions Oncology, Inc., Hackensack, NJ

Many anticancer drugs fail in clinical trials due to issues resulting from inadequate efficacy and/or high toxicity emphasizing the need for improving clinically relevant, preclinical models for high throughput drug screening. Currently, 3D models have emerged as a highly promising tool for initial drug screening that closely mimics *in vivo* tumors through preserved morphology and molecular mechanisms. To further recapitulate the *in vivo* models, interactions between immune and tumor cells can be performed via co-culture models. As the complexity of the models has increased, the analytical methods have had to evolve as well. In this work, we have developed a high throughput flow cytometry (FlowHT) assay using Champions' TumorGraft3D (CTG3D) models. CTG3D are 3D organoids derived from our highly characterized and clinically annotated Patient Derived Xenograft (PDX) models. We have developed a method of TumorGraft3D dissociation which preserves cell viability and epitope stability. We further optimized a robust FlowHT workflow that allows for the detection of low-frequency targets and the characterization of unique subpopulations in modular multicolor panels to distinguish immune cells and CTG3D in mixed populations. Using this method, we have validated that the CTG3D models retain their original phenotype with minimal differences observed between source PDX tumors and expanded ex-vivo 3D models. At co-culture endpoints, our assay was highly sensitive to immune cell-mediated tumor killing as well as SOC drug responses. Interrogation in an allogenic PBMC coculture revealed the ability to detect cytotoxic T cells (Tc) and helper T cells (Th) activation and immune checkpoint markers such as CD69, TIM-3, and PD-1 among others. Collectively, we have developed a quantitative, sensitive, and robust assay that can be utilized as an analytical endpoint for both monoculture and coculture studies to enhance the interrogation of immune-oncology agents for tumor killing, ADCC effects, increasing T cell activation, reversal of T cell exhaustion and identifying specific markers across multiple cell types.

#1229 3D-bioprinted cancer models for prediction of drug response driven by H&E-based AI algorithm.

Ronit Satchi-Fainaro[1], Anne Krinsky[1], Anshika Katyal[1], Opal Avramoff[1], Yulia Liubomirski[1], Gal Dinstag[2], Omer Tirosh[2], Ranit Aharonov[2], Tuvik Beker[2], Iris Barshack[3], Shaked Lev-Ari[4], Shirly Grynberg[4], Ronnie Shapira-Frommer[4]

[1]Tel Aviv University, Tel Aviv, Israel,[2]Pangea Biomed, Tel Aviv, Israel,[3]Tel Aviv University, Sheba Medical Center, Ramat Gan, Israel,[4]Sheba Medical Center, Ramat Gan, Israel

Many drugs show promising results in laboratory research but eventually fail to show clinical benefit. One main reason for this translational gap is that the cancer models used are inadequate. Most models lack the complex tumor-stromal-immune cell interactions with their tumor microenvironment (TME) that are required for progression. Conventional 2D models, where cells grow on rigid plastic plates mainly as mono-cultures, fail to recapitulate these complex interactions. Hence, we developed two platforms of 3D cancer models that better mimic the TME: (i) a 3D tumoroid model consisting of all the cells from the patient's tumor tissue, following cell suspension, and isolated peripheral blood mononuclear cells (PBMC) from the same patient's blood, and (ii) a vascularized, hydrogel-based 3D-bioprinted tumor model consisting of patient-derived tumor, PBMCs and TME cells. Our 3D models are based on a library of hydrogels that we developed as scaffolds for different tumor types according to the mechanical properties of the tissue of origin which dictates the proteo-genomic signature and, hence, the response to different therapies. The patient-derived models consist of cells isolated from a freshly-resected tumor mass and include PBMCs that either surround the 3D tumoroids or flown through 3D-bioprinted functional blood vessels connected to a pump. Using these unique high throughput 3D models, we are currently validating their ability to mimic patient-specific tumors and TME, in order to serve as a tool to predict patient response to different treatments (e.g., chemotherapy, immunotherapy, and targeted therapies). To decide which therapies to test on our 3D platforms, we consider cancer specific standard-of-care (SoC), investigational therapies as well as therapies proposed by ENLIGHT-DP, an AI-based algorithm developed by Pangea Biomed that utilizes H&E slides to generate individual drug response scores for targeted and immune therapies. The ENLIGHT-DP first predicts gene expression from H&E slides using a deep-learning AI algorithm and then uses the predicted expression to generate response scores for a variety of therapies. To validate the ability of our models to predict patient responses, we are currently conducting an 80-patient clinical trial (IRB approved) testing different treatments on samples covering 7 different cancer types. An interesting case from this trial is a mucosal melanoma patient who failed SoC treatments correlated with failure to respond in our models. Interestingly, ENLIGHT-DP predicted that the patient would respond to regorafenib, a suggestion corroborated by our 3D model. Following this promising outcome, the patient received it as compassionate use, resulting in an immediate partial response lasting for 6 months so far. These unique 3D models have the potential to facilitate target discovery and drug development, as well as to serve as a reliable system for precision medicine.

#1230 High throughput quantitative molecular characterization of cytotoxic antibody-drug conjugates in spheroid models for improved functional characterization, screening and candidate selection.

Meghan Hogan[1], Andrea Hernandez Rojas[1], Araba Sagoe-Wagner[1], Jodi Wong[1], Sarah Church[1], Brigitte Lovell[2], Sergio Hernandez[2], Lakshmi Chandramohan[2], Anna Juncker-Jensen[2], **Kirsteen Maclean**[2]

[1]Zymeworks, Vancouver, BC, Canada,[2]Neogenomics, Fort Myers, FL

Background: Antibody-drug conjugates (ADCs) are a class of cancer therapeutics comprised of a linker-payload conjugated to a monoclonal antibody targeting a tumor-associated antigen (TAA), to enable the delivery of the cytotoxic payload to cancer cells. Presently, there is a need for improved in vitro models that better recapitulate in vivo tumor tissue complexity to aid in the screening and evaluation of novel ADCs during preclinical development. Specifically, we have developed in vitro 3D models from cancer cell lines yielding spheroids in a rapid, robust and uniform manner. Using these spheroid models, we have developed cell-based assays to functionally evaluate the cytotoxic activity of ADCs *in vitro*. For further comprehensive characterization of ADC activity in 3D cell line models, we utilized the nCounter® ADC Development Panel, a specialized gene expression tool for molecular characterization of biological function, with customizable gene content to address complex questions important for the success of ADCs throughout discovery, pre-clinical and clinical development.
Methods: Spheroids were generated by seeding ovarian cancer cell lines into microtiter plates treated with ultra-low attachment coating, using automated liquid-handling robots, followed by incubation with ADCs under standard culturing conditions, prior to functional transcriptomic evaluation. The nCounter ADC Development Panel was analytically validated and included performance specification of accuracy, precision, analytical specificity, and RNA input range of the panel to directly profile 770 genes addressing essential biological questions relevant to ADC activity, including tumor targeting and antigen expression; ADC internalization; payload release; drug mechanisms of action; target cell death; immunogenic cell death; and mechanisms of resistance.
Results: The combination of high-throughput spheroid assays and comprehensive transcriptomic analysis presented herein highlight an important and streamlined workflow to aid in the screening and selection of therapeutic cytotoxic ADC candidates for the treatment of solid tumors.

#1231 Exploring drivers of variability in patient tumor responses to HIF-2α inhibitor using the Xsphera patient-derived organotypic tumor spheroid (PDOTS) platform.

Alyssa Martin[1], Chunxiao Cui[1], Aaron Goldman[2], **Michael A. Perricone**[1]

[1]XSphera Biosciences, Inc., Boston, MA,[2]Department of Medicine, Harvard, Boston, MA

Background: The hypoxia-inducible factor 2 alpha (HIF-2α) complex is a critical driver of clear cell renal cell carcinoma (ccRCC), with FDA-approved inhibitor PT2977 (belzutifan) demonstrating promising clinical efficacy. Xsphera's patient-derived organotypic tumor spheroid (PDOTS) platform offers an innovative ex vivo approach to recapitulate the tumor microenvironment (TME) and assess patient-specific responses to cancer therapeutics. This study highlights how the PDOTS platform can bridge the gap between preclinical and clinical data, providing insights into patient-specific tumor responses and mechanisms of resistance.
Methods: Resected patient RCC tumors (n=7) were processed into PDOTS and cultured within a microfluidic device to preserve native TME. Baseline immune cell profiles were characterized using flow cytometry on dissociated single-cell sub-fractions. PDOTS from each patient tumor were treated with the HIF-2α inhibitors PT2977 (7.5-750 ng/mL) and PT2385 (50-5000 ng/mL), or vehicle control. Tumor responses were evaluated at day 3 post-treatment using Hoechst Propidium Iodide (HPI) automated image analysis. RNA was extracted post-treatment, and gene expression was profiled using the NanoString IO360 panel. Integrated analyses of immune cell composition, cytotoxicity, and gene expression were performed to explore potential correlations with response.
Results: Cytotoxicity: PT2385 induced dose-dependent cytotoxicity, in 4 of 7 samples (57%), while PT2977 demonstrated activity in 2 of 7 (28%) patient tumors without a clear dose-response relationship. Immune Profiles: No significant associations were observed between baseline immune cell composition and tumor response. Gene Expression: Preliminary NanoString analysis revealed distinct gene expression changes in responsive versus non-responsive tumors. Responsive tumors exhibited downregulation of pro-tumorigenic pathways, including cell proliferation and G2M checkpoint-related genes, relative to controls. Interestingly, markers associated with myeloid cells, such as CD163 and LILRB4, were elevated in responsive tumors following treatment, but remained unchanged in non-responsive tumors.
Conclusions: Xsphera's PDOTS platform effectively mirrors the clinical response rates to HIF-2α inhibitors, offering actionable insights into tumor variability and resistance mechanisms. By combining cytotoxicity, immune profiling, and transcriptomic analyses, the PDOTS platform provides a robust predictive and exploratory tool for oncology drug development. These findings underscore the utility of this platform in tailoring therapeutic strategies for ccRCC and other cancer types.

#1232 A 3D bioprinted prostate cancer ex vivo model with nanoengineered hydrogel for potential drug screening.

Anirud Chakravarthi Arava[1], Camar Ameril[2], Ananya Aggarwal[1], Gnanasekar Munirathinam[1], Mohammad Fazle Alam[1]

[1]University of Illinois College of Medicine (Rockford), Rockford, IL,[2]University of the Philippines, Los Banos, Philippines

Prostate cancer (PCa) is one of the most lethal cancers in men worldwide. As of 2024 there have been 2,001,140 new cases reported. The tumor microenvironment (TME) is a dynamic niche where tumor cells, stromal, and immune cell types interact within the tumor-specific extracellular matrix (ECM), playing a fundamental role in regulating tumorigenesis and dissemination. TME promotes signaling between tumor cells and non-malignant cells, which leads to tumorigenesis that results in metastasis. An *Ex vivo* model is required for understanding the tumor microenvironment and to study the changes that occur during metastasis but it remains challenging to achieve due to the complexity posed by the disease's heterogeneity. Currently, the latest advancement in 3D bioprinting and organoid technology offers to fabricate a physiologically relevant ex-vivo cancer model. However, challenges remain in terms of the choice of suitable bioink. In this study, we developed a nanoengineered hydrogel (NOEH) by mixing different ratios of Laponite with Gelatin Methacrylate (GelMA). After mixing, we characterized the rheological and swelling ratio, parameters. We observed that the viscoelastic property of Nanoengineered hydrogel (NOEH) gets enhanced as the ratio of laponite. To analyze the biocompatibility and proliferation of NOEH, we embedded the prostate cancer cells (WPMY-1 and PC3), mesenchymal cells (WPMY-1), and Human umbilical vein endothelial cells (HUVECs) and observed NOEH are compatible with all the cells. We optimized the printing parameter of NOEH to fabricate the 3D prostate cancer scaffold and mimic the TME by adding different proportions of cells. The outcome of the study revealed that the NOEH supports cellular properties like viability, migration, and proliferation in 3D bioprinted scaffolds. The combination of Laponite with GelMA enhances viscoelastic properties and swelling capacity, promoting cellular interactions for the co-culture of normal and cancer cells of PCa-TME. The combination of GelMA-Laponite also resulted in higher viability as the cell in co-culture maintained high viability for 10 days. This novel 3D-bio printed PCa-TME model is progressing towards a platform for drug testing personalized medicine but needs further analysis to understand complex interactions of the cellular and non-cellular components. The PCa-TME model is being utilized to explore how the stiffening of the Extracellular Matrix contributes to chemoresistance and the potential role of efflux pumps in this process.

#1233 Construction and application of an immune microphysiological model for lung cancer.

Jianli Dai, Qianqian Wang, Junhong Li, Yang Li, Wenbo Ma, Qing Zhang, Jingyu Yang, Panglian Xu, **Yanmei Zhang**

Institute of New Materials and Advanced Manufacturing, Beijing Academy of Science and Technology, Beijing, China

Tumor models are important tools for cancer research and drug development. The existing tumor models are unable to reconstruct the microphysiological processes of tumor immune responses in vivo, thus limiting their applications in the development of tumor immunological drugs and tumor immunotherapy. How to rapidly and accurately construct tumor models with a tumor immune microenvironment and an immune microphysiological system is a critical problem that urgently needs to be solved in the field of tumor immunology research and therapy. In this study, innovatively based on bio-3D printing and organ-on-a-chip technology, a lung cancer immune microphysiological system model(LCIMS) integrating the tumor area, blood vessels and lymphatic area was precisely constructed. Firstly, we screened a variety of biohydrogel materials where cells could coexist, and the hydrogel was mixed with the immune cells and fibroblastic reticular cells derived from the draining lymph nodes of lung cancer patients to generate a printable bioink. Then, lymphoid organoids with germinal centers were constructed through extrusion 3D printing. In addition, based on droplet microfluidic technology, we constructed lung cancer assembloids with intra-batch consistency and tumor microenvironment. Further, the lung cancer assembloids and lymphoid immune organoids were integrated together in an organ-on-a-chip with vascular channels, forming an immune microphysiological system with the tumor area, lymphatic area and vascular channels. In this LCIMS model, the cancer assembloids in the tumor area have similar molecular, cellular and histopathological characteristics to primary tumors, and the immune organoids in the lymphatic area have T cell and B cell regions. The results of single-cell sequencing and flow cytometry show that the organoids have similar cell subtypes and cell interaction characteristics to the patient's lymph nodes and can generate antibody responses under the stimulation of viral vaccine. In order to further explore the application value of the model in immunodrug screening, we evaluated the role of immune checkpoint inhibitor PD-1 on lung tumors. The activation of CD8+ T cells in the lymphatic area, their directed chemotaxis towards tumors, infiltration into tumors and tumor killing can be observed. In addition, the effects of CAR-T cells on cancer were tested in this model, compared to traditional tumor organoid models, the release of interleukin-6 and interferon γ in the lymphatic area can be clearly observed under the action of CAR-T cells in the model. This highlights the potential of our model to mimic the cytokine storm caused by cellular immunotherapy and its impact on tumor killing. In conclusion, the LCIMS model is a more ideal tumor immune model and has important application value in the development of tumor immunobiological drugs and the evaluation of tumor immunotherapy.

#1234 Mechanical control of cancer-associated adipocyte plasticity orchestrates pancreatic cancer progression.

Bo Han[1], Zhi Yang[1], Shuqing Zhao[1], Thomas Schmittgen[2], Jamel Ali[3], Jonathan Barajas[1], Carolyn Ruiz-Rivera[1], Ba Xuan Hoang[1]

[1]Keck School of Medicine of USC, Los Angeles, CA,[2]University of Florida, Gainesville, FL,[3]Florida A&M Univeresity, Tallahassee, FL

Cancer-associated adipocytes (CAAs) are key components of the pancreatic tumor microenvironment, yet how their phenotype and function are regulated by environmental conditions remains poorly understood. Here, we demonstrate that oxygen tension and matrix stiffness dramatically alter CAA behavior and their subsequent effects on pancreatic ductal adenocarcinoma (PDAC) progression. Under normoxic conditions and soft matrix, CAAs exhibit an activated phenotype characterized by extended podia formation and enhanced secretion of matrix-degrading enzymes, particularly MMP-2 and MMP-9, leading to dissolution of organoid extracellular matrix (ECM) and restriction of PDAC cell growth through direct cell-cell contact. In contrast, when cultured within stiff ECM environments (~30 kPa), CAAs maintain a rounded, stem cell-like morphology without matrix-degrading activity, but notably promote PDAC cell migration. Gene expression confirmed that ECM-primed CAAs revealed upregulation of stemness markers including OCT4, SOX2, and SOX9, along with enhanced expression of pro-inflammatory cytokines. Using ectopic pancreatic tumor models, we found that CAAs primed in stiff ECM significantly accelerate tumor progression and invasion compared to controls, with increased tumor volume and invasion into the muscle layer 4 weeks post-implantation. Mechanistically, stiff ECM-primed CAAs showed distinct stem cell-like phenotype and altered secretome profiles. When cocultured with PDAC cells, their crosstalk promoted increased secretion of pro-inflammatory cytokines IL-6 and TNF-α while downregulating anti-inflammatory cytokine IL-10, creating a more tumor-supportive microenvironment. IHC reveals macrophage F4/80 showed no significant difference between groups, however, CD163+ macrophages were more prevalent at the early stage in the inactivated CAA-PDAC group. These findings reveal previously unrecognized plasticity in CAA function regulated by mechanical and oxygen tension cues, suggesting that the spatial distribution of these environmental factors within pancreatic tumors may create distinct zones of tumor promotion versus restriction. Our results highlight the complex role of CAAs in PDAC progression and suggest that targeting ECM stiffness could be a strategy to modify CAA behavior for therapeutic benefit. Understanding this mechanical control of CAA function could lead to novel therapeutic strategies for PDAC treatment.

#1235 Complex in vitro tumor models screen NK therapy and reveal mode of action.

Bin Xue[1], Christopher Harrod[1], Long Le[1], Julia Schueler[2], **Kolin C. Hribar**[1]

[1]Cypre Inc., San Francisco, CA,[2]Charles River Laboratories, Freiburg, Germany

NK cell therapy is an emerging and increasingly promising modality for various cancers. Whereas T cell-based adoptive cell therapy (e.g. tumor infiltrating lymphocytes or TILs, and CAR-T) is mainly limited to patient's autologous cells due to concerns around allogeneic responses with MHC (major histocompatibility complex) mismatch, NK therapies do not suffer from this due to their low risk of introducing GvHD (Graft-versus-host disease), enabling the development of NK therapies using either patient-sourced NK cells or allogeneic, healthy donor-sourced cells. NK therapies have demonstrated early success in some hematologic cancer, however, the inadequate infiltration and persistence in the tumor microenvironment (TME) limits their application in solid tumors. Therefore, the ability to model solid tumors in vitro to screen efficacy and study mode of action would be highly beneficial to gaining early and actionable insights for clinical translation. Here, we developed several patient-derived xenograft (PDX) 3D in vitro non-small cell lung or colorectal cancer models using a proprietary hydrogel platform. The platform resembles the TME to enable consistent growth of tumor cells, fibroblast elongation, and immune cell interactions. An activated NK cell population ("NK therapy"), featuring $CD56^{bright}$ CD16+ (~50%) and $CD56^{bright}$ CD16- (~50%) phenotypes by flow cytometry, was highly potent in the 3D models. These NK therapy cells demonstrated a significant increase in tumor infiltration and tumor cytotoxicity compared to the non-activated NK ("NK control") counterparts from the same donor. Interestingly, key cytokines like IFNg were downregulated in the NK therapy as compared to the NK control. These data demonstrate a robust and comprehensive in vitro analysis for NK therapy, offering a new preclinical tool for validating their effectiveness and mode of action.

#1236 Immune competent 3D pancreatic adenocarcinoma (PDAC)-on-chip model for preclinical drug discovery.

Alina Deipenbrock[1], Ben Eric Wilmes[1], Kyra Moustakas[1], Irene Esposito[2], **Thomas Sommermann**[3], Martin Raasch[3], Nicole Teusch[1]

[1]Institute of Pharmaceutical Biology and Biotechnology, Heinrich-Heine-Universitat Dusseldorf, Dusseldorf, Germany,[2]Institute of Pathology, University Hospital Dusseldorf, Dusseldorf, Germany,[3]Dynamic42, Jena, Germany

Representing the most common malignancy of the pancreas, pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive cancer type with a five-year survival rate of less than 13%. To date, targeted treatment regiments are not available, leaving only cytotoxic chemotherapy with gemcitabine or FOLFIRINOX as the first-line-treatment for patients who are ineligible for surgical resection. One factor which contributes to the lack of efficient treatment options is the presence of a highly fibrotic and immunosuppressive tumor microenvironment (TME), mainly consisting of cancer associated fibroblasts (CAF) and tumor associated macrophages (TAM). Furthermore, effective treatments require drug candidates to pass the endothelium into the tumor. This barrier is not addressed in most *in vitro* models traditionally used in drug screening. Thus, novel PDAC models reflecting, the cellular heterogeneity within the TME including a vascular linkage will be essential to find efficacious treatment strategies. Using the Dynamic42 biochip platform, which allows dynamic culturing of cells in three channels each separated by porous membranes, we generated a fit-for-purpose 3D model of human PDAC. The model combines a perfused vasculature with an underlying tumor tissue consisting of tumor spheroids with a CAF hull embedded into an extracellular matrix (ECM). We first used this PDAC-on-chip model as a proof-of-principle to deliver small molecule drug candidates via a vasculature to the 3D spheroids. As one example, we identified vorinostat, a histone deacetylase inhibitor already approved for cutaneous T cell lymphoma, significantly reducing PDAC spheroid viability in a dose dependent manner without impacting the vascular integrity. We further expanded the PDAC-on-chip model by introducing peripheral blood mononuclear cells (PBMC)-derived monocytes. We show that monocytes applied through the vasculature invade through the endothelial barrier and migrate into CAF rich areas, where they differentiate to M2-like macrophages upregulating CD163 and loosing CD86 and HLA-DR expression. We now use this system to study T cell invasion, as well as direct vascularization of PDAC tumors.

TUMOR BIOLOGY: Cancer Evolution In Response to Therapy
Poster Session

#1240 Genomic characterization of changes in the molecular profile of locally advanced rectal cancer treated with neoadjuvant therapy.

Sharafudeen D. Abubakar, Christina I. Lee, Farheen Shah, Chin-Tung Chen, Paolo Manca, Henry Walch, Walid K. Chatila, Rona Yaeger, Julio Garcia-Aguilar, Francisco Sanchez-Vega

Memorial Sloan-Kettering Cancer Center, New York, NY

Background: Locally advanced rectal cancer (LARC) patients exhibit a widely heterogeneous degree of response to neoadjuvant therapy (NAT) and many experience disease relapse. By examining tumors at multiple timepoints before and after NAT, we investigated 1) pre-treatment genomic predictors of metastasis to specific anatomic sites that could potentially inform future therapeutic strategies and 2) genomic mechanisms driving tumor evolution under NAT stress. Methods: We analyzed 687 specimens from LARC patients treated at Memorial Sloan Kettering Cancer Center (MSKCC). This included 473 endoscopic biopsies collected before NAT and 214 samples after NAT. Post-NAT specimens included 99 surgical resections (SR), 19 local recurrences (LR) and 96 distant recurrences (DR). The DR set included 33 liver, 33 lung, and 30 metastases located in other organs. All samples underwent targeted DNA sequencing with MSK-IMPACT, an assay that identifies genomic alterations in 341-505 genes. We assessed somatic DNA alterations at the gene and the pathway levels, tumor mutational burden (TMB), fraction genome altered by copy number changes (FGA), tumor purity and whole-genome doubling (WGD) status. Results: Pre-NAT biopsies from patients who never developed any metastasis had higher Wnt pathway alterations than those whole developed metastases (85.9% vs 73.2%, p=0.0055), driven by APC alterations (84.6 vs 71.1%, p=0.0046), and higher rates of FBXW7 (17.8 vs 9.3%, p=0.044) and BCL2L1 alterations (6.7 vs 1%, p=0.01). In contrast, patients who develop metastases tend to have more alterations in KRAS (51.6 vs 39.6%, p=0.038) and PTEN (6.2 vs 1.1%, p = 0.0065). Liver metastases tend to occur earlier than lung metastases (median 12.9 vs 16.1 months from end of NAT, p=0.034). Pre-NAT specimens had higher rates of APC alterations than post-NAT specimens (81.8% vs 74.3%, p=0.025), while TGFβ pathway alteration rate was higher post-NAT (15.4% vs 24.3%, p=0.0074), driven by SMAD4 inactivation (10.8 vs 17.8%, p=0.014). LR specimens exhibited higher FGA (median 0.8 vs 0.55, p<0.001), higher rates of WGD (68.8% vs 37.5%, p=0.017), and lower rates of APC (52.6% vs 80.2%, p=0.007) than non-LR specimens. Among DR samples, KRAS alterations were more frequent in lung than liver metastases (60.6% vs 39.4%, p=0.14), while the opposite trend was observed for SMAD4 alterations (9.1% vs 30.3%, p=0.06). Conclusion: We observed significant differences in alteration rates in several driver genes in LARC specimens sequenced before and after NAT, which might be explained by NAT stress driving tumor evolution. The distinct genomic profile of LRs may be indicative of differences in their biological mechanisms of emergence. Differences in the somatic landscape of liver and lung metastases may have translational implications for the treatment of patients with advanced disease.

#1241 Deciphering heterogeneity and tumor evolution in bladder cancer using multi-omics and liquid biopsies from rapid autopsies.

Pushpa Itagi[1], Samantha Schuster[1], Sonali Arora[1], Patricia Galipeau[1], Thomas Persse[1], Funda Vakar-Lopez[1], John K. Lee[2], Petros Grivas[3], Robert Montgomery[1], Jonathan Wright[1], Hung-Ming Lam[1], Andrew Hsieh[1], Gavin Ha[1]

[1]Fred Hutchinson Cancer Center, Seattle, WA,[2]Human Biology Division, Fred Hutchinson Cancer Center, Seattle, WA,[3]Department of Medicine, University of Washington, Seattle, WA

Introduction: Metastatic bladder cancer (mBLCA) exhibits significant heterogeneity at the genomic, transcriptomic, molecular, and epigenetic levels, contributing to challenges in diagnosis, prognosis, and treatment. While 75% of mBLCA cases are urothelial carcinomas, 25% show divergent differentiation with variant histological features. This work characterizes intra-patient and inter-patient heterogeneity through comprehensive genomic and transcriptomic analyses of a rapid autopsy cohort of 20 patients with multiple tumors, matched normal tissue, and cell-free DNA (cfDNA). Our findings provide critical insights into tumor heterogeneity, clonal evolution, metastatic seeding, and the tumor microenvironment.

Approach: Using bioinformatics tools, we reconstructed clonal evolution trees from whole-genome sequencing (WGS) analysis of tumors and inferred migration patterns to determine metastatic seeding. COSMIC mutational signatures were examined to correlate histological subtypes with clinical history. Single-nucleus RNA sequencing (snRNA-seq) revealed cell types and transcriptional heterogeneity using the human protein atlas and known marker annotations. To investigate alterations and clonality in tumors and their matched cfDNA, we used custom methodologies to evaluate mutations, CNAs, SVs, and rearrangements.

Results: The clonal evolution trees and bulk RNA-seq highlight cisplatin resistance in the plasmacytoid urothelial carcinoma subtype, driven by upregulation of the DNA damage response pathway. Significant mutational heterogeneity (~20-30% subclonal) emerges in later stages of tumor evolution, potentially contributing to therapy resistance. Additionally, a substantial proportion (60-70%) of structural variants arise later in tumor progression, particularly in driver genes. snRNA-seq revealed distinct clusters of epithelial cells, macrophages, fibroblasts, and other cell types. cfDNA analysis captured over 80% of subclonal deleterious tumor mutations in BLCA driver genes. Nucleosome profiling of cfDNA helps distinguish mBLCA from healthy samples and identify key transcription factors to differentiate between variants.

Conclusion: This comprehensive study of mBLCA, leveraging a rapid autopsy dataset, uncovers critical insights into variant histologies across multiple omics layers. Integrating cfDNA analysis captures intra-patient and inter-patient tumor heterogeneity, providing a holistic view of clonal dynamics. By elucidating the patterns of clonal evolution, this work advances our understanding of tumor progression and establishes a foundation for precision therapies targeting adaptive and evolving clones.

#1243 The pro-metastatic phenotype of chemoresistant breast cancer is stable and transcriptionally defined.

Alberto Dalmasso, Andrea Cossa, Ilaria Servidio, Guido Campani, Elisa Bugani, Francesco Antonio Tucci, Lucrezia Stabellini, Salvatore Pece, Pier Giuseppe Pelicci

IEO - European Institute of Oncology, Milano, Italy

Chemotherapy remains the most used systemic treatment option for triple-negative breast cancer. Lineage tracing studies demonstrated that the treatment does not significantly alter the clonal composition of primary breast tumors. Nevertheless, the specific transcriptional phenotype(s) conferring chemoresistance at primary tumor (PT) level, as well as at metastatic level, are still largely undetermined, specifically at single-clone resolution.To tackle such unmet clinical issues, we leveraged our recently published *in vivo* single-cell lineage tracing approach under therapeutic pressure, using two chemotherapeutic agents: adriamycin and cyclophosphamide (A+C). Upon administration of A+C we obtained stable disease, as confirmed by histopathological and IHC analyses. Chemoresistance was formally demonstrated by *ex vivo* IC50.By using single-cell lineage tracing on treated and untreated matched PT-lung metastases, we found that chemotherapy shifted clonal hierarchy, with few high-fit clones outcompeting the others, at primary level. Despite this, we noticed the striking increase of pro-metastatic clone frequencies in the chemoresistant model, thus suggesting that chemotherapy selects for few highly-aggressive clones. At metastatic level we found general drop of clonality.Using scRNA-seq, we observed that chemotherapy shapes distinct chemoresistant patterns in the PT and metastases. We then explored the transcriptomics features of the most fit clones within treated tumors, so-called dominant: they were characterized by enrichment of distinctive cancer hallmarks with respect of the whole chemoresistant tumor, including tumor-promoting inflammation and sustained angiogenesis.Chemoresistant/pro-metastatic clones were characterized by the expression of genes that were prognostic for poor survival across patients. Importantly, we observed that the chemoresistant/pro-metastatic phenotype was exacerbated by re-challenge with the same treatment but also stable across multiple re-transplantations. Among the of chemoresistant/pro-metastatic genes, PAEP appeared as the top marker. This was corroborated by GRN analyses, where we observed that the network around PAEP was extremely perturbed by therapeutic pressure. We tested the pro-metastatic effect of PAEP expression in a chemoresistant scenario. We orthotopically injected MDA-MB-231 cells silenced for PAEP expression via shRNA into recipient NSG mice and we performed neoadjuvant and adjuvant chemotherapy. Upon PAEP silencing, we observed slight increase of chemosensitivity at primary tumor level. Strikingly, chemoresistant metastases formation was significantly impaired by PAEP downregulation, as compared to controls.Overall, our data demonstrate that the pro-metastatic phenotype of chemoresistant breast cancer is stable, transcriptionally defined and driven by the expression of PAEP.

#1244 Investigating prostate cancer cellular heterogeneity and treatment response at singe-cell level.

Romuald Parmentier[1], Julien Roux[2], Robin Dolgos[1], Raphaelle Servant[1], Kirsten Mertz[3], Subotic Svetozar[4], Lukas Bubendorf[5], Helge Seifert[6], Cyrill A. Rentsch[6], Clementine Le Magnen[1]

[1]Institute of Pathology, Department of Urology, Department of Biomedicine, University Hospital Basel, Basel, Switzerland,[2]Department of Biomedicine, University of Basel, Basel, Switzerland,[3]Institute of Pathology, Kanton Baselland Liestal Hospital, Liestal, Switzerland,[4]Clinic for Urology, Kanton Baselland Liestal Hospital, Liestal, Switzerland,[5]Institute of Medical Genetics and Pathology, University Hospital Basel, Basel, Switzerland,[6]Department of Urology, University Hospital Basel, Basel, Switzerland

Context and Objectives: Androgen deprivation therapy (ADT) is the mainstay for treatment of advanced castration-sensitive prostate cancer (CSPC); yet most patients eventually relapse and progress to lethal castration-resistant prostate cancer (CRPC). Here we aimed at generating single-cell transcriptomic profiles from advanced CSPC samples and ADT-treated patient-derived organoids (PDOs) to dissect cellular dynamics underlying response to androgen deprivation at single-cell resolution.
Material and Methods: Nine radical prostatectomy or metastasis resection specimens obtained from treatment-naïve high-grade CSPC patients were collected and processed into single-cell suspensions. Single-cell RNA sequencing (scRNA-seq) was carried out on six specimens meeting necessary quality criteria. In parallel, four PDO lines were established, characterized using whole exome sequencing, IHC and immunofluorescence, and cultured in androgen-deficient conditions to mimic ADT. Viability assay confirmed treatment efficacy before scRNA-seq was performed with a lipid-based multiplexing strategy (MULTI-seq).
Results: We generated an atlas encompassing the transcriptomic profiles of more than 20 000 cells originating from rare tissue material. While highlighting patient-specific tumor features and integrating cells from the microenvironment, our single-cell atlas also revealed various epithelial subtypes. This included a subset of intermediate cells, referred to as club/hillock cells, which were previously proposed to play a role in tumor progression. Interestingly, these cells were maintained in PDOs derived from non-metastatic tissues, whereas PDOs derived from metastatic ones were exclusively composed of tumor-like cells. Treated organoids exhibited diverse viability responses and pathway alterations, including changes in the Androgen Response Signature, reflecting their sensitivity to ADT and underscoring their value as clinically relevant *in vitro* models. Simultaneously, scRNA-seq revealed transcriptional shifts within specific subpopulations of treated PDOs, highlighting significant changes in additional pathways that provide insights into the mechanisms driving the onset of castration resistance. Ongoing analyses aim to determine whether these populations pre-exist in the tumor or if this state is gradually acquired during treatment.
Conclusion: PDOs effectively mirror original prostate tumor characteristics and represent functional castration-sensitive models. By leveraging the generated scRNA-seq atlases and transcriptomic shift analyses, we anticipate identifying castration-tolerant cell populations potentially contributing to tumor heterogeneity and relapse.

#1245 Delaying resistance to Gefitinib in EGFR+ NSCLC through optimized treatment scheduling: an integrated experimental-theoretical approach.

Maximilian Strobl[1], Jinling Wu[1], Dagim Tadele[2], Mina Dinh[1], Jacob Scott[1]

[1]Cleveland Clinic, Cleveland, OH,[2]Medical Genetics, Oslo University Hospital, Oslo, Norway

Tyrosine kinase inhibitors (TKI) have revolutionized treatment of oncogene-driven non-small cell lung cancer (NSCLC). However, almost all patients with advanced disease eventually acquire resistance and the disease remains largely incurable. Much research is dedicated to identifying and targeting the molecular mechanisms of resistance. But even when challenged by our most advanced treatments, cancers display a remarkable ability to adapt. We seek to complement the development of new agents by investigating treatment scheduling - the dose and frequency at which drugs are administered - as a means to slow the emergence of therapy resistance. Work by us and others on so-called adaptive therapy has demonstrated that well-timed treatment breaks in prostate can maintain sensitive cells which slow the growth of resistant cells through competition, and they can also induce phenotypic switching of some resistant cells to more sensitive states in melanoma. In this study, we use an integrated experimental-theoretical approach to investigate how treatment scheduling impacts the evolution of resistance to Gefitinib in EGFR+ NSCLC, and we lay the foundation for an adaptive therapy protocol to delay resistance. Using fluorescent, time-lapse microscopy we characterize the evolutionary dynamics of co-cultures of Gefitinib-sensitive and paired resistant cell lines (PC9; PC9+BRAF knock-in; PC9 with evolved resistance to Gefitinib) across four different treatment schedules: i) continuous therapy, ii) intermittent therapy (on/off), iii) intermittent therapy (off/on), iv) continuous therapy at half the full dose. Our results clearly demonstrate that both the dose and the frequency of treatment influence evolutionary dynamics. Intermittent therapy minimizes the final resistant cell and total cell count after six treatment changes (18 days total), across all four dose levels examined (2uM, 200nM, 100nM, 20nM Gefitinib). Moreover, the off/on intermittent schedule outperforms the on/off schedule, suggesting a role for spatial competition in suppressing the outgrowth of resistant cells. Leveraging our in vitro data, we develop a mathematical model of the treatment response dynamics, which reveals an additional subpopulation of drug-tolerant persister cells in the “sensitive” population. Using a step-wise model selection approach we characterize their contribution to the evolution of drug-resistance and investigate how to optimally schedule treatment to balance the three populations. Overall, we provide novel insights into how treatment scheduling impacts resistance evolution in EGFR+ NSCLC and we demonstrate the power of an integrated experimental-theoretical approach for developing more effective treatment schedules.

#1246 Driver and signaling cascade in therapy pressure driven cancer cell plasticity.

Poorvi Subramanian, Sreenidhi Mohanvelu, Sivasubramani Narayanan, Afsana Parveen Jahir Hussain, Sabir Salim, Madhi Oli Ramamurthy, Loganayaki Periyasamy, Natarajan Aravindan

Oklahoma State University, Stillwater, OK

Dynamic adaptations in cancer cell-state due to therapy is a growing concern. Despite multimodal clinical therapy, tumor cells surpass treatment resulting in frequent relapses in rapidly decreasing timeline. We have shown that, in accordance with therapy pressure associated tumor microenvironment, progressive disease (PD) cells exhibit reversible lineage transformation and unparalleled plasticity. However, our understanding of the genetic determinant(s) and/ or mechanism(s) that coordinate cancer cell plasticity is a far reach. Herein, we investigated the crucial cancer cell phenotypic switching in response to therapy pressure. Using a panel of 14 well characterized human neuroblastoma (NB) cells derived during diagnosis (Dx) and PD, we investigated the association of retinal degeneration protein 3 (RD3) status (ELISA) to their migratory and invasive capabilities (transwell assay), clonal expansion (BrDu), EMT (E-CAD, N-CAD, Vimentin, SLUG), metastatic state (Ron-β, MMP-9, MMP-2), stemness maintenance (CD133, CD117, CD114, Oct3/4, Sox2, NANOG, CD54, NOTCH1) and cellular dedifferentiation (β-3 tubulin, NF-L, GFAP, GAP43) (immunoblotting). Further, to investigate the function of RD3 in coordinating cancer cell plasticity, we utilized six select NB cells, three derived during Dx and three derived during PD, representing both primary and metastatic tumors. These patient derived cells were reverse engineered for stable expression of RD3 in $RD3^-$ PD cell lines; stable silencing of RD3 in $RD3^+$ Dx cell lines and were subjected to end-point readouts as above. RD3 loss in PD correlated with the increased cellular migration, invasion, metastatic potential, EMT and stemness maintenance. Directed simulation experiments with stable restoration and silencing systems clearly identified the function of RD3-loss in cancer cell plasticity by regulating stemness (ALDH, CD133), EMT (Vimentin, E-CAD, N-CAD), metastasis (Ron-β, MMP-9), pluripotency maintenance (Oct3/4, Sox2, NANOG), dedifferentiation (β-3 tubulin, NF-H, GFAP, GAP43) and clonal expansion (BrDu). Our findings for the first time identified the defined function and relevance of RD3 in regulating cancer cell EMT, stemness and pluripotency maintenance, metastatic state and prompting differentiation. Crucially, our findings recognized therapy pressure directed RD3 loss dictates cancer cell lineage transformation to cancer stem cells. These outcomes together pave way to develop novel and effective molecular-targeted maintenance therapeutic strategy for patients presented with therapy defying progressive disease.

Funding: This work was partially or in full, funded by Department of Defense CA-210339; OCAST-HR19-04; NIH-P20GM103639 to Dr. Aravindan; and by the NIH-NCI-Cancer center support Grant P30CA225520, NIGMS P30GM154635, Oklahoma Tobacco Settlement Endowment Trust [R23-03] to the OU Health Stephenson Cancer Center.

#1247 Characterizing the evolution of osimertinib resistance in EGFR mutated non-small cell lung cancer .

Mina N. Dinh, Jinling Wu, Masahiro Hitomi, Maximilian Strobl, Jacob G. Scott

Cleveland Clinic Lerner Research Institute, Cleveland, OH

Osimertinib is a 3rd generation EGFR inhibitor and the newest FDA approved adjuvant therapy for non-small cell lung cancer (NSCLC) with EGFR exon 19 deletions or exon 21 L858R mutation. Although the drug can yield impressive initial responses, the majority of patients eventually progress due to the evolution of resistance. While the molecular mechanisms underlying resistance have been well studied, little is known about the ecological and evolutionary laws that govern the creation and subsequent expansion of these mutant populations. How frequently are different evolutionary routes taken and to what degree is evolution repeatable? To study the repeatability of osimertinib resistance evolution, we conducted a large-scale evolution experiment aimed at characterizing the distribution of resistant states that emerge, along with the pathways and vulnerabilities associated with their development. In our experimental design, we evolve PC-9, an EGFR-mutated NSCLC cell line to osimertinib independently 10 times (evolutionary replicates) for 10 cycles (serial passages). We also evolved 6 control replicates that undergo no treatment. At the end of the experiment, we will employ a multi-faceted approach to studying the resistance phenotype: 1) IC50 against osimertinib; 2) bulk RNA-seq; 3) DNA/mutational profiling; 4) a collateral sensitivity screening against 20 FDA approved drugs; and 5) a radiation sensitivity assay against 6 doses. The initial pretreatment dose response curve (DRC) against osimertinib showed a double plateau, suggesting the pre-existence of tolerant or even resistant subpopulation. In our control arms, we see consistent persistence of our double plateau throughout all 10 cycles, indicating this population is maintained in the absence of treatment but cannot expand. Conversely, across all 8 treated replicates we see a large shift in IC50 within the 1st treatment cycle, consistent with rapid selection and outgrowth of this population. More detailed analysis using time-lapse microscopy shows while these cells have a higher IC50, they also have a reduced growth rate, even if treatment is completely removed again (a “resistance cost”). This suggests potential evolutionary vulnerabilities which could be leveraged through strategic treatment breaks or drug sequences.

#1248 Single-cell RNAseq revealed multiple resistance mechanisms in patient-derived xenograft model of rectal cancer during treatment.

Yuwei song[1], Regina K. Irwin[2], Karin M. Hardiman[2], Zechen Chong[1]

[1]Department of Biomedical Informatics and Data Science, University of Alabama at Birmingham, Birmingham, AL,[2]Department of Surgery, University of Alabama at Birmingham, Birmingham, AL

Background: Colorectal cancer (CRC) is the third leading cause of cancer-related deaths in the US, with rectal cancer (RC) accounting for one-third of cases. Only 20% of RC patients have a complete response to chemoradiation therapy (CRT) and may be able to avoid surgery, therefore, therapeutic resistance remains a major barrier to improving outcomes. The genetic and cellular heterogeneity of RC, particularly in treatment-resistant sub-clones, suggests that this diversity could be a harbinger of adverse outcomes. A detailed characterization of treatment responses at the subclonal level is critical to advancing targeted therapeutic strategies.
Methods: Patient-derived xenograft (PDX) models and single-cell RNA sequencing were employed to investigate the cellular and molecular landscape of RC tumors. Two PDX models were established from the primary tumors of pre-treatment RC patients. Mice bearing tumors were subjected to a standard CRT regimen, consisting of 2 Gy daily radiation and 100mg/kg capecitabine daily for two weeks, followed by a week-long rest period. Tumor tissues were collected at baseline and at various post-treatment intervals for sequencing.
Results: Our scRNA-seq analysis of one PDX model identified five distinct tumor clusters with varying responses to treatment. Two clusters demonstrated initial sensitivity to CRT, followed by a resurgence, indicating potential therapy resistance. Pathway analysis revealed cluster-specific activation of critical oncogenic pathways, including HIF-1, p53, and FoxO signaling pathways. These pathways are implicated in tumor progression and therapeutic resistance, and the associated marker genes present potential targets for intervention. Lineage tracing of tumor cells uncovered two differentiation trajectories, with one showing increased resistance. Cell-cell communication analysis highlighted extensive interactions among tumor sub-populations, particularly mediated by CLDN3-CLDN3 adhesion, which may contribute to therapeutic resistance. Additionally, our investigation into copy number variations identified alterations in the chr6p21.31-p21.1 region, which harbors several oncogenes with copy number changes directly implicated in tumor progression. In the second PDX model, scRNA-seq identified two therapy-resistant tumor clusters among five detected clusters. The resistant tumor clusters exhibited enrichment of HIF-1, Hippo, and p53 signaling pathways, similar to the first PDX model but also unique activation of pathways like TGF-β and Hippo signaling.
Conclusion: Our study highlights the dynamic, heterogeneity of RC tumors to CRT, identifying both shared and patient-specific resistance mechanisms in two PDX models. These findings underscore the complexity of RC tumor biology and offer a foundation for developing precision therapies targeting treatment-resistant subclones.

#1249 Chemotherapy enriches one of two pre-existing coordinated cellular programs characterized by inflammatory and stress pathways in high grade serous ovarian cancer.

Nomeda Girnius[1], Muyi Liu[1], Laura M. Selfors[1], Jaeyoung Kang[1], Linna Lahmadi[1], Mayura Thomas[1], Aylin Z. Henstridge[1], Morgan Gruner[2], Jordan Mattson[2], Dennis J. Slamon[3], Gottfried E. Koneckny[4], Andrew C. Nelson[2], Timothy K. Starr[2], Ronny Drapkin[5], Boris J. Winterhoff[2], Joan S. Brugge[1]

[1]Harvard Medical School, Boston, MA,[2]University of Minnesota, Minneapolis, MN,[3]University of California, Los Angeles, CA,[4]University of California, Los Angeles, Los Angeles, CA,[5]University of Pennsylvania, Philadelphia, PA

Women diagnosed with high grade serous ovarian cancer (HGSOC) often respond to initial rounds of neoadjuvant chemotherapy, but then undergo multiple rounds of relapse and regression before acquiring resistance. This pattern of response and relapse suggests that transient, non-genetic resistance mechanisms are engaged by a subpopulation of tumor cells allowing them to survive and re-form a tumor. To elucidate the mechanisms underlying this transient resistance, we performed scRNASeq and cyclic immunofluorescence (CyCIF) on matched pre- and post-treatment tissues from seven patients. Our transcriptomics data revealed that the interferon (IFN) pathways, IFNα and IFNγ, and the tumor necrosis factor alpha (TNFα) pathway were enriched following therapy in six out of the seven patients. The enrichment of the IFN and TNFα pathways following chemotherapy exposure suggested that tumor cells expressing these programs may survive treatment and thus contribute to relapse, prompting us to study this population further. Spatial analysis of three interferon-stimulated genes (ISGs) revealed that a large proportion of ISG+ tumor cells appear to occupy specific niches containing ISG+ immune cells, while other ISG+ tumor cells were not adjacent to ISG+ immune cells, suggesting both intrinsic and extrinsic mechanisms of IFN pathway activation. It has been reported that IFN is activated in the early lesions of HGSOC known as serous tubule intraepithelial carcinomas (STICs). To investigate whether the IFN pathway activity early in tumorigenesis may be associated with reactivation of retroviral DNA, we performed CyCIF on samples containing STICs and found correlated expression of ISGs and the LINE1 protein product, ORF1P. These STIC areas were typically not associated with immune cells. Similarly, we detected correlated expression of ORF1P with ISGs in invasive tumor cells. Together, our findings suggest that endogenous retroviral reactivation may activate the cGAS/STING pathway, turning on IFN and establishing an IFN+ tumor cell population. To further investigate the cellular heterogeneity of HGSOC and identify pathways that are co-expressed with the inflammatory pathways, we sub-clustered individual patients' tumors. This analysis revealed two distinct tumor cell states: one associated with high expression of the inflammatory pathways with additional stress-related programs and EMT, and the other with MYC pathway, cellular proliferation, and metabolic pathways. We were able to confirm this coordinated expression in an additional 40 pre-treatment samples. Using an independent cohort from the TRIO14 ovarian cancer trial, we found the same pattern in HGSOC tumors from 46 patients. Future studies of this population aim to understand the functional importance of the IFN/inflammatory/stress state and to identify targetable vulnerabilities.

#1250 Genetically-defined organoid models reveal early mechanisms of squamous neoplastic progression and therapeutic vulnerabilities in squamous cell carcinoma.

Hua Zhao[1], Young Min Park[2], Yueyuan Zheng[3], Qiong Mao[3], Luda Lin[4], Casey Collet[1], Stephanie Wong[1], Boyan Hu[1], Uttam K. Sinha[1], Alice Soragni[4], **Dechen Lin**[1]

[1]USC - University of Southern California, Los Angeles, CA,[2]Yonsei University College of Medicine, Seoul, Korea, Republic of,[3]Sun Yat-sen University, Shenzhen, China,[4]University of California Los Angeles, Los Angeles, CA

Upper aerodigestive squamous cell carcinoma (UASCC) represents an aggressive and often fatal group of malignancies. Mechanistically, the early steps of neoplastic transformation remain poorly defined. Translationally, despite extensive genomic characterization of UASCC, the application of these findings to clinical practice remains highly limited. We have identified a crucial gap in the field - the lack of viable and valid human models that can: *i)* faithfully recapitulate the stepwise progression from precursor lesions to tumors, and *ii)* rigorously model genotype-phenotype relationships. Here, we developed two complementary, cross-species, genetically-defined organoid systems: (1) a genome-sequenced, patient-derived organoid platform comprising 46 models spanning normal tissue, precursor lesions, and tumors along the oral-esophageal neoplastic continuum; and (2) a CRISPR/Cas9-engineered organoid platform of 16 models, targeting key UASCC genomic drivers to recreate the full spectrum of malignant transformation (hyperplasia → dysplasia → carcinoma). Using these models, we uncovered critical insights into early squamous neoplastic evolution. For example, single-cell RNA sequencing of TP53/CDKN2A double-knockout (DKO) organoids revealed an expanded population of quiescent basal and proliferative squamous cells, alongside a reduction in differentiated squamous cells. This differentiation imbalance was exacerbated by the introduction of additional drivers, such as $PIK3CA^{E545K}$ mutation (DKOP). Furthermore, a senescence-like program, identified as a hallmark of normal squamous epithelium, was markedly attenuated in DKO organoids and further diminished in DKOP organoids, with ANXA1 emerging as a master regulator. We identified a novel ANXA1-SMAD3-p27KIP1 pathway as a critical driver of this senescence-like program, suppressing neoplastic phenotypes in squamous organoid models. Leveraging these genetically-defined organoid systems, we further performed high-throughput drug screening at single-organoid resolution, utilizing bioprinting technology coupled with machine learning. As anticipated, PIK3CA mutations conferred a survival advantage against most therapies tested. However, intriguingly, genetically-engineered organoids with PIK3CA mutations demonstrated heightened sensitivity to mitomycin C and onalespib. These findings were further validated using independent patient-derived HNSCC organoids harboring PIK3CA mutations. In summary, this study provides critical insights into the mechanisms of early squamous neoplastic progression and underscores the utility of genetically-defined organoid platforms for forward genetic studies in cancer. These rigorously validated models offer a powerful platform for identifying genome-guided therapies and advancing precision oncology.

#1251 Decoding tumour evolution and relapse drivers in EGFR mutant NSCLC in never smokers.

Komal Gupta[1], Dawn Lau Pingxi[1], Ngak Leng Sim[2], Kenneth Chow[1], Lan Ying Wang[1], Mengyuan Pang[2], Stephanie Saw[2], Wanjin Hong[3], Anders Martin Jacobsen Skanderup[2], Regina Hoo[1], Daniel Shao Weng Tan[1]

[1]National Cancer Centre, Singapore, Singapore,[2]Genome Institute of Singapore (GIS), Singapore, Singapore,[3]Institute of Molecular and Cell Biology (IMCB), Singapore, Singapore

Non-small cell lung cancer (NSCLC) remains the leading cause of cancer-related deaths worldwide. Unlike smoking-related NSCLC, the incidence of lung cancer among never-smokers in East Asia is rising, often driven by oncogenes such as EGFR. While Tyrosine Kinase Inhibitors (TKIs) have improved outcomes in EGFR-mutated NSCLC, resistance remains a significant challenge. Identifying the genomic and transcriptomic features that underpin tumour progression and relapse in early-stage EGFR-mutated NSCLC in non-smokers is crucial for developing novel therapeutic approaches to improve patient outcomes. Due to the diverse evolutionary trajectories of EGFR mutated NSCLC, our capacity to forecast disease trajectory is still limited. To address this, we performed 578 multi-region whole-exome and transcriptome sequencing from 180 treatment naïve early-stage EGFR-mutated resected NSCLC tumours. Among these, 56 (31%) patients eventually relapsed, while 124 (69%) did not, with a mean follow-up of 5.4 years. Our findings revealed that driver mutations in *EGFR, TP53*, and *RBM10* were predominantly clonal, while a multitude of subclonal driver mutations contributed to increased tumour diversity. Interestingly, mutational signature SBS40a, of unknown origin, was prevalent as clonal alterations, suggesting early involvement in EGFR-mutated tumours. Notably, relapsed tumours exhibited distinct genomic features, including elevated subclonal APOBEC mutational signatures, higher rates of whole-genome doubling, and increased chromosomal aberrations, linking these features to greater mutational burden, intra-tumour heterogeneity and poorer outcomes. Overall, our results demonstrate the diversity of clonal and subclonal mutational processes that shape the clonal architecture of EGFR-mutated NSCLC in non-smokers and identify key features driving relapse. Ongoing efforts are focused on leveraging these features to identify potential prognostic markers for risk stratification and therapeutic targets for early-stage disease.

#1252 Evolution of transcriptomic and epigenomic intra-tumor heterogeneity in high-grade serous ovarian cancer with chemotherapy.

Yuna Landais[1], Juliette Bertorello[1], Marta Puerto[2], Baptiste Simon[3], Amelie Roehrig[3], Adeline Durand[2], Marceau Quatredeniers[3], Constance Lamy[2], Fabrice Lecuru[2], **Christophe Le Tourneau**[2], Celine Vallot[1]

[1]Institut Curie & One Biosciences, Paris, France,[2]Institut Curie, Paris, France,[3]One Biosciences, Paris, France

Background: Intra-tumor heterogeneity (ITH) refers to the presence of diverse cell populations within a single tumor, each with distinct genetic, epigenetic, and phenotypic characteristics. This complexity presents significant challenges in treatment, as different subpopulations may respond variably to therapies, yet the non-genetic component of ITH and its contribution to treatment response remains poorly understood. We mapped transcriptomic and epigenomic ITH to explore evolution of non-genetic ITH in response to therapy in high-grade serous ovarian cancer (HGSOC).
Methods: The SCANDARE study (NCT03017573) included patients with HGSOC that are treated with neo-adjuvant chemotherapy (NAC). Biopsies were performed at baseline, after NAC surgery and at recurrence for single-cell RNA sequencing (scRNA-seq, n=17 samples, 111,946 with 1,773 median gene coverage), single-nuclei RNA sequencing (snRNA-seq, n=35 samples, 151,719 nuclei with 2,843 median gene coverage), and single-cell epigenomics (snCUT&Tag, n=24 samples, 58,892 nuclei with 1,409 unique fragments median coverage). Our snCUT&Tag dataset is one of the first single-cell epigenomic map of cancer patients under treatment, focusing on the histone modification H3K4me1, which accumulates at primed and active enhancers and promoters. We integrated these clinically annotated single-cell/nuclei datasets with publicly available scRNA-seq data of HGSOC.
Results: We constructed a consensus map of functional tumor states in HGSOC, based on scRNA-seq analysis of over 200,000 tumor cells. This analysis revealed 15 recurrent tumor cell phenotypes, ranging from stressed to cycling or inflammatory states. We identified the transcription factors and cell-cell communications specific to each tumor cell state. These tumor states are encoded at the epigenomic level, we could identify in each tumor epigenetic clones - clusters of cells with the same epigenome - each displaying a H3K4me1 landscape characteristic of one tumor state. These results show that tumor states correspond to tumor populations with distinct non-genetic features. Longitudinal sampling by snRNA-seq further showed that tumor state composition consistently evolves upon chemotherapy exposure in patients: tumors lose their cycling, hypoxic and interferon cells while gaining cells with partial mesenchymal and inflammatory characteristics.
Conclusions: We propose a consensus map of transcriptomic and epigenomic tumor states in HGSOC. Our work is a proof of concept that we can monitor functional ITH with high resolution snRNA-seq from frozen biopsies of the standard of care. We show that these consensus tumor states consistently evolve upon treatment in all patients, pinpointing tumor cell states that are not successfully targeted by NAC, that would need to be targeted by adjuvant therapies.

#1253 Single-cell analysis reveals intricate subclonal heterogeneity and evolution in response to standard of care treatments in glioblastoma.

Yueshan Zhao[1], Xiaoyu Lu[2], Verah Nyarige[2], Junfei Zhao[2], Gonzalo Lopez[2], Ann Forslund[2], Maria Ortiz-Estevez[2], Jorge Benitez-Hernandez[2], Wei Zhang[2], Celia Fontanillo[2], Kai Wang[2], **Ha Dang**[2]

[1]University of Pittsburgh, Pittsburgh, PA,[2]Bristol Myers Squibb Company, San Diego, CA

Glioblastoma multiforme (GBM) is the most common and lethal brain cancer. Standard of care treatments (SOC) are limited to surgery, radiation and chemotherapies, providing an average overall survival of only ~16 months. Tumor heterogeneity and cellular plasticity are major contributions to treatment resistance in this disease. We constructed an integrated patient-level and cohort-level framework and analyzed single-nucleotide RNA sequencing data in matched primary and recurrence specimens to understand tumor heterogeneity, evolution, and drug resistance in SOC treated GBM patients (N=23). Patient-level tumor analysis revealed distinct tumor cell transcriptional subclones representing widespread intra-patient heterogeneity. Tumor evolution analysis from primary to recurrence revealed diverse treatment responses representing sensitive, intrinsic resistant, and acquired resistant subclones. Further characterization of tumor subclones and clustering analyses indicated that tumor heterogeneity under SOC treatments was linked to diverse biological mechanisms including neural development and oncogenic pathways, contributing to varying levels of subclonal resistance. Our analysis also highlighted intrinsic resistance was prominent as primary tumors often harbored substantial proportion of resistant tumor cells. This insight on resistant status at cell level then allowed us to compare resistant to sensitive cells and identify both established and potentially novel mechanisms of SOC resistance that were not always obvious in the comparison between primary and recurrence samples. These included activation of PI3K/AKT, MAPK, TGF-β, EMT, and WNT signaling pathways. Taken together, our novel analytical strategy revealed complex tumor heterogeneity and evolution landscape in SOC treated GBM, offering insights on potential mechanisms driving these phenomena and providing a resource for further exploration and development of therapeutic strategies.

#1254 Neural-like progenitor pancreatic cancer cells are enriched in residual disease following neoadjuvant chemoradiotherapy and acquire invasive and stem-like features through inflammatory reprogramming.

Dennis Gong[1], Jimmy A. Guo[2], Jennifer Su[3], Sueda Cetinkaya[3], Peter Wang[4], Ashley Lam[5], Carina Shiau[6], Laleh Abbassi[7], Seema Chugh[8], Andrew J. Aguirre[8], William L. Hwang[4]

[1]Massachusetts Institute of Technology (MIT), Cambridge, MA,[2]School of Medicine, University of California San Francisco, San Francisco, CA,[3]Graduate School of Arts and Sciences, Harvard University, Cambridge, MA,[4]Massachusetts General Hospital, Boston, MA,[5]University College London, London, United Kingdom,[6]Harvard Medical School, Boson, MA,[7]Dana Farber Cancer Institute, Cambridge, MA,[8]Dana Farber Cancer Institute, Boston, MA

Pancreatic ductal adenocarcinoma (PDAC) is notable for a high rate of recurrence and metastasis despite intensive multi-agent chemoradiation. We previously identified a neural-like progenitor (NRP) transcriptional program strongly enriched in PDAC specimens with neoadjuvant chemoradiation and correlated with improved response to therapy. However, the mechanisms for why malignant cells in the post-treatment setting had collectively increased expression of NRP transcriptional signatures remained unclear. In this study, we sought to understand the contexts in which NRP becomes expressed as well as phenotypic effects of NRP genes that may contribute to cancer recurrence. Here, we profile NRP signature expression in human and mouse PDAC model systems, finding that NRP signatures are strongly enriched in regenerative stages of the murine pancreas post-injury, both in oncogene transformed and wild type pancreata. We provide additional evidence that NRP is expressed by a stressed stem-like ductal progenitor population in normal human pancreata and a DCLK1 expressing cancer cell population with undifferentiated cellular morphology in patient tumors. Using 1000-plex whole slide spatial transcriptomic profiling, we identify microenvironmental features associated with NRP cells and propose an inflammatory niche that maintains NRP expression in vivo, that is lost upon in vitro monoculture. Spatial colocalization analysis nominated inflammatory CAFs and IL1B+ macrophage populations as producers of inflammatory factors that upregulate NRP genes. We find that cells utilizing the NRP program have increased reliance on transcription factors involved in pancreatic development and identify Glis3 as a transcription factor highly expressed in NRP cells. We establish isogenic KP ($Kras^{G12D/+}$;$Trp53^{FL/FL}$) murine organoid overexpression models using Gata6 and dNTrp63 for the established classical and basal-like PDAC cell states, as well as Glis3. Notably, we find that Glis3 isogenic organoids have markedly higher metastatic proclivity in the orthotopic transplant setting, greater colony formation capacity, and increased invasive and migratory index relative to Gata6 and dNTrp63 driven isogenic models. Glis3 is enriched in matched human PDAC metastases relative to the primary tumor and is also strongly enriched in human cell lines with high metastatic rate in MetMap. Our work deepens understanding of the neural-like progenitor cell state, and particularly the role of developmental transcription factor GLIS3, which is emergent following cytotoxic chemoradiation. Furthermore, we highlight how treatment induced inflammation and tissue injury in the tumor microenvironment contribute to cellular reprogramming that can lead to cancer recurrence and metastasis in the post-treatment setting.

#1255 Apoptotic DNase DFFB mediates cancer acquired drug resistance by suppressing persister cell intrinsic interferon signaling.

August (Gus) Finley Williams, David A. G. Gervasio, Claire E. Turkal, Anna E. Stuhlfire, Michael X. Wang, Brandon E. Mauch, Ariel H. Nguyen, Michelle H. Paw, Rhea Plawat, Mehrshad Hairani, Cooper P. Lathrop, Sophie H. Harris, Jennifer L. Page, Matthew J. Hangauer

UC San Diego, La Jolla, CA

Tumor adaptation driving cancer acquired drug resistance during oncogene targeted therapy treatment frustrates therapeutic responses and results in patient mortality. Unfortunately, the adaptive mechanisms residual surviving cancer cells utilize to overcome therapeutic stress are not well described. Understanding the processes that drive cancer acquired drug resistance may lead to novel therapeutic strategies to prevent tumor relapses. We used cell culture and mouse xenograft models of chronic, continuous, and toxic oncogene targeted therapy exposure (weeks to months) to study residual surviving “persister” cells and their regrowth into expanded colonies which models acquired drug resistance. Here, we describe how cancer persister cells experience sublethal apoptotic signaling and utilize the caspase-activated DNase DFFB to promote regrowth under drug pressure. We found that while DFFB WT persister cells were able to form colonies in drug, DFFB-deficient cells were severely restricted in their ability to regrow. Mechanistically, DFFB induces persistent DNA damage which both promotes mutagenesis and suppresses growth-arresting interferon signaling. During drug stress, the mitochondrial outer membrane permeabilizes and activates pattern recognition receptors to induce interferon signaling. From single cell RNA sequencing analysis of persister cells, we observed elevated interferon signaling gene sets in DFFB KO cells compared to WT cells. Furthermore, DFFB KO cells also had increased levels of STAT1 and STAT2. This elevated interferon signaling was growth suppressive because colony formation was rescued in DFFB KO cells with a JAK1/2 inhibitor. We then discovered that the stress response transcription factor ATF3 is selectively induced in DFFB WT cells whereas DFFB deficient cells lack ATF3 upregulation. ATF3 is a known modulator of interferon signaling, is induced following DNA damage stress, and has increased expression in patient minimal residual disease tumors on targeted therapy treatment. Consistent with ATF3-mediated interferon suppression, ATF3 depletion in persister cells increased STAT1 levels whereas ATF3 overexpression in DFFB KO persister cells reduced STAT1 levels. This data shows that DFFB-mediated activation of ATF3 promotes residual tumor regrowth by suppressing growth-restricting interferon signaling. This novel tumor adaptation mechanism represents an exciting new therapeutic strategy that could potentially extend the efficacy of targeted therapy treatments and increase patient survival. Importantly, DFFB is a nonessential gene since DFFB knockout mice develop normally. Additionally, increased expression of DFFB and ATF3 correlate with worse patient survival. Therefore, DFFB represents a promising therapeutic target against cancer acquired drug resistance.

#1256 BCL11B predetermines an immature drug-resistant cell state in breast cancer that is vulnerable to TNF-α treatment.

Zhen Qi[1], Gunsagar S. Gulati[2], Angera H. Kuo[1], Shaheen S. Sikandar[3], William Hai Dang Ho[1], Dalong Qian[1], Frederick M. Dirbas[4], Aaron M. Newman[1], Shang Cai[5], Michael F. Clarke[1]

[1]Institute for Stem Cell Biology and Regenerative Medicine, School of Medicine, Stanford University, Stanford, CA,[2]Dana-Farber Cancer Institute, Boston, CA,[3]Department of Molecular Cell and Developmental Biology, University of California, Santa Cruz, CA,[4]Department of General Surgery, Stanford University, Stanford, CA,[5]Westlake Laboratory of Life Sciences and Biomedicine, School of Life Sciences, Westlake University, Hangzhou, China

Background: The development of chemoresistance in many cancer patients poses a significant clinical challenge, while the underlying causes for heterogeneous drug responses remain largely elusive. Increasing evidence suggests that tumors exhibit extensive heterogeneity and harbor cancer subpopulations in less differentiated states that are linked to treatment resistance. Therefore, dissecting immature cancer cell states pre-existing in tumors is essential for identifying biomarkers associated with chemoresistance and establishing targeted therapeutic strategies, particularly in breast cancer, where specific biomarkers are largely lacking.
Methods: We utilized CytoTRACE, a robust computational framework for predicting the cellular differentiation status from single-cell RNA sequencing data, to unveil the immature cancer cell state and its associated markers in single-cell atlases of human breast cancer. The association between the identified immature cell state and patient survival was determined by inferring the proportions of epithelial cancer cells expressing the marker gene in bulk gene expression datasets. The MMTV-PyMT mouse breast cancer model was used to experimentally investigate the role of the immature cancer cell state in conferring chemoresistance. Single-cell profiling was conducted on tumor samples before and after treatment to examine the inherent characteristics of immature cancer cells and their response to drug pressure. The results obtained from mouse tumors were subsequently confirmed using human breast cancer models.
Results and Conclusion: We revealed that BCL11B, a zinc finger transcription factor, defines a distinct immature cancer cell state in human breast cancer that displays basal cell features and demonstrates a heterogeneous expression pattern across breast tumors. Breast cancer patients with higher levels of *BCL11B*$^{+}$ cancer cells face a significantly increased risk of relapse following chemotherapy. This pre-existing tumor subpopulation exhibits intrinsic fitness advantages and demonstrates a remarkable ability to evolve into a drug-resistant state through various BCL11B-dependent pre-existing and transcriptional adaptation mechanisms. Notably, TNF-α was pinpointed as a natural inhibitor of BCL11B, and treatment with TNF-α significantly increases chemotherapy efficacy by inducing the exit of the drug-resistant cell state. In conclusion, our findings identify BCL11B as a valuable biomarker for predicting chemotherapy resistance and suggest TNF-α combination treatment for breast cancer patients at high risk of developing chemoresistance.

#1257 Response to primary therapy predicts tumor evolution dynamics at relapse in ovarian cancer.

Jaana Oikkonen[1], Giulia Micoli[1], Susanna Holmstrom[1], Kari Lavikka[1], Mai T. N. Nguyen[1], Yilin Li[1], Giovanni Marchi[1], Anna Rajavuori[2], Heidi Koskela[2], Johanna Hynninen[2], Sampsa Hautaniemi[1]

[1]University of Helsinki, Helsinki, Finland,[2]Turku University Hospital and University of Turku, Turku, Finland

Chemotherapy resistance is a major contributor of cancer-related deaths. Resistance can either be acquired during treatments or pre-exist at the time of the diagnosis. Previous studies on high-grade serous carcinoma (HGSC) have shown contradictory findings on the role of genetic causes explaining emergence of chemotherapy resistance at relapses, for example selection of resistant clones during chemotherapy. HGSC is often diagnosed at an advanced stage, showing multiple genetically heterogeneous clones present before therapeutic intervention. While most patients respond well to primary therapy, cancer relapses within few years, leading to treatment resistance. To understand genetic impact on emergence of resistance, we used matched pretreatment and treated samples from 214 unselected HGSC patients collected at DECIDER trial (NCT04846933). We constructed phylogenetic trees based on whole-genome sequencing of 804 tumor samples and whole-exome sequencing of 63 circulating tumor DNA (ctDNA) samples from plasma. Cancer evolution analysis identified patients with and without selection during treatments: patients with remained heterogeneity with stalled evolution, and others dominated by subclones originating from single resistant clone. Patients with better primary therapy outcome were linked to re-expansion of a single major clone at relapse, while multiple ancestral clones founded relapse populations in poorer outcome patients. The single-clone expansion pattern also emerged in later relapses, after multiple lines of therapy. Majority of the relapsed cancers evolved further from the ancestral clones already present at pretreatment. The relapse emerging subclonal mutations affected pathways such as carbohydrate metabolism and cAMP signaling. In contrast, sensitive clones eliminated by treatment carried mutations affecting extracellular matrix and NTRK3 signaling. Interestingly, some cancers did not acquire new mutations at relapse, despite becoming chemoresistant, suggesting that non-genetic mechanisms, such as phenotypic plasticity or microenvironmental factors, might underlie their resistance. In summary, we discovered heterogeneous cancer evolution trajectories during treatment which were linked to mutations in key pathways and prior therapy responses. While chemotherapy often selects persistent cancer subclones that seed later relapses, some patients acquire resistance through non-genetic mechanisms, highlighting the complexity of cancer evolution and resistance dynamics. Understanding these dynamics is crucial to define better therapeutic interventions in these poor prognosis patients with advanced HGSC.

#1258 Deciphering evolutionary dynamics and transcriptomic adaptions under therapy in small cell lung cancer.

Laura Constanze Kaiser, Marcel Schmiel, Christian Muller, Maria Cartolano, Martin Peifer, Roman K. Thomas, Julie George

University of Cologne, Cologne, Germany

Introduction: Small cell lung cancer (SCLC) represents a major medical challenge due to its aggressive progression and a dismal 5-year overall survival rate of only 7%. While SCLC initially responds well to chemotherapy, the rapid relapse and emergence of therapy-resistant tumors remain critical barriers to effective treatment. We hypothesized that therapy response is associated with genome evolution, which, in turn, influences transcriptional plasticity. This study aims to decipher the clonal evolution and transcriptomic adaptations in response to chemotherapy in SCLC.

Methods: We established over 100 patient-derived xenotransplant models from clinical SCLC specimens, capturing diverse genetic features and clinical characteristics. A representative subset of 33 models was selected to replicate clinical scenarios in mice, modeling the effects of platinum-based chemotherapy and evaluating tumor responses under controlled conditions. Whole-exome sequencing and single-cell transcriptome profiling were performed to investigate genomic evolutionary trajectories and transcriptional plasticity in therapy-naïve and relapsed tumors.

Results: Xenotransplant models accurately reflect patient responses to chemotherapy. To our surprise, several models from relapsed patients retained sensitivity to treatment. Chemotherapy-resistant models maintain their tumor clones, whereas sensitive models show dynamic shifts from a common ancestral clone with emerging subclones shaped by mutations associated with platinum damage, APOBEC activity, and defective DNA damage repair. The genomic landscape, defined by subclonal mutations and copy number alterations, strongly impacts transcriptomic patterns. Specifically sensitive models post-chemotherapy reveal a strong shift of transcriptional programs reflecting adaptive plasticity as a consequence of genome evolution.

Conclusion: Here we provide an in-depth functional *in vivo* characterization of clonal adaptions in SCLC under therapy. Chemotherapy drives the selection of subclones with favorable genomic features, which in turn impact the broader transcriptomic landscape. Our data provides critical insights into the molecular mechanisms of therapy resistance.

#1259 Studying the role of chromatin accessibility in lineage plasticity of Rb1-deficient EGFR-mutant lung adenocarcinoma.
Bradley Balk, David W. Goodrich

Pharmacology and Therapeutics, Roswell Park Comprehensive Cancer Center, Buffalo, NY

Lung cancer is the leading cause of cancer mortality worldwide. Mutations in the epidermal growth factor receptor (EGFR) account for approximately 27% of all lung adenocarcinoma (LUAD) cases. Osimertinib is a third generation EGFR tyrosine kinase inhibitor (TKI) that is, initially, remarkably effective at treating patients with EGFR mutant LUAD. Acquired resistance to Osimertinib develops in most patients leaving few therapeutic options. This highlights an unmet clinical need to prevent/combat Osimertinib resistance. Lineage plasticity, an epigenetic process characterized by transcriptional heterogeneity and molecular adaptation, has been identified as a mechanism of Osimertinib resistance in the clinic. As more potent targeted therapeutics are deployed for treating cancer patients, plasticity-based resistance will likely increase. The molecular basis of lineage plasticity is not well understood, and greater characterization of lineage plasticity is imperative for developing new therapies aimed at preventing/reversing plasticity-associated resistance. Loss of the major tumor suppressor gene Rb1 has previously been implicated in lineage plasticity of several different cancers including LUAD. How loss of Rb1 contributes towards lineage plasticity remains poorly understood. Rb1 is a known regulator of chromatin accessibility, playing a key role in recruiting epigenetic writers to silence gene expression through repressive methylation of histones and DNA. Whether altered chromatin regulation in the absence of Rb1 contributes to LUAD lineage plasticity has not been examined. To begin examining the chromatin accessibility/plasticity axis *in vitro*, we are developing a PROTAC-based, rapidly inducible Rb1 protein degradation system utilizing the dTAG degron system to characterize the dynamics of molecular changes subsequent to Rb1 loss of function. Changes in chromatin accessibility will be assessed by micrococcal nuclease digestion and a novel nuclear propidium iodide staining technique. Plasticity will be assessed indirectly by *in vitro* colony formation and time to resistance assays. The timing of changes in transcriptional plasticity and chromatin accessibility will be compared. The goal of this project is to gain a greater understanding of how Rb1 loss impacts chromatin structure and subsequently, whether increased chromatin accessibility promotes plasticity-associated Osimertinib resistance.

#1260 Establishment of intratumor heterogeneity models using lung cancer cells.

Tomoko Funazo[1], Hiroaki Ozasa[1], Takahiro Tsuji[2], Yusuke Shima[1], Keiichiro Suminaga[1], Kentaro Hashimoto[1], Hiroshi Yoshida[1], Kazutaka Hosoya[1], Hitomi Ajimizu[1], Takashi Nomizo[1], Hironori Yoshida[1], Toyohiro Hirai[1]

[1]Kyoto Univ. Graduate School of Medicine, Kyoto, Japan,[2]Fred Hutchinson Cancer Center, Seattle, WA

Intratumor heterogeneity has been shown to be associated with treatment resistance in multiple cancers, including lung cancer. Elucidating intratumor heterogeneity is an issue that must be resolved to improve the prognosis of patients. Previous studies of intratumor heterogeneity have mainly involved collecting and analyzing cancer cells from patients, classifying subpopulation with different properties, and conducting comparative analysis. However, there are few studies focusing on the early phase of intratumor heterogeneity. We exposed alectinib to a commercially available ALK-positive lung cancer cell line (H2228) and established alectinib resistant cells (H2228-AR1S). H2228-AR1S was observed two cells with different appearances. In this intratumor heterogeneity model, flow cytometry (FACS) showed that the cell surface CD47 of the parent line was unimodal, whereas that of H2228-AR1S was bimodal, indicating that there were differences in the properties of migration and tumor formation between subpopulation having the CD47 high expression and subpopulation having CD47 low expression. Next, We exposed molecular targeted drugs to patient-derived lung cancer cell lines and evaluated the expression of cell surface markers using FACS. KTOR83-Dab, a cancer cell line derived from a BRAF-positive lung cancer patient that was exposed to dabrafenib for one month, showed bimodal EpCAM expression. The subpopulation of EpCAM low expression had high migration ability and showed reduced epithelial gene expression and increased mesenchymal gene expression, suggesting EMT (epithelial-mesenchymal transition). It was suggested that KTOR83-Dab is a patient-derived intratumor heterogeneity model. Furthermore, experiments are being conducted using these intratumor heterogeneity models to elucidate the mechanism of intratumor heterogeneity. H2228-AR1S is a intratumor heterogeneity model established from commercially available monoclonal cells, and single-cell analysis is performed to extract genes whose expression changes during the process of homogeneous cells becoming heterogeneous. KTOR83-Dab is a patient-derived lung cancer cell line and is therefore polyclonal. We will clone KTOR83-Dab and examine whether there are any cells that become heterogeneous when administered dabrafenib. In addition, based on the results of single-cell analysis of H2228-AR1s, we will also analyze genes involved in heterogeneity in KTOR83-Dab.

#1261 Assessing intratumoral genetic heterogeneity in locally advanced rectal cancer.

Vandeclecio Lira da Silva[1], Ezequias Santos Torres[2], Ramon Torreglosa Do Carmo[2], Franciele Hinterholz Knebel[2], Fabiana Bettoni[2], Guilherme Pagin Sao-Juliao[3], Bruna Vailati[3], Bruna Vailati[3], Leonardo Corbi[3], Natalia Mariana Felicio[3], Angelita Habr Gama[4], Anamaria Camargo[2], Cibele Masotti[2], Rodrigo Oliva Perez[1]

[1]Hospital Beneficencia Portuguesa de Sao Paulo, Sao Paulo, Brazil,[2]Hospital Sirio Libanes, Sao Paulo, Brazil,[3]Hospital Alemao Oswaldo Cruz (HAOC-SP), Sao Paulo, Brazil,[4]Instituto Angelita e Joaquim Gama, Sao Paulo, Brazil

Locally Advanced Rectal Cancer (LARC) is typically treated with neoadjuvant chemoradiotherapy (nCRT), aimed at reducing tumor size to facilitate surgical resection. Patients achieving a complete clinical response (cCR) may be eligible for organ preservation strategies, such as the Watch-and-Wait protocol. However, clinical and radiological evaluations lack precision in predicting treatment response, with tumor regrowth occurring in 30% of cases within two years. We previously demonstrated that rectal tumors present high ITGH and that this heterogeneity increases after nCRT in patients who do not achieve a complete clinical or pathological response. Therefore, ITGH has emerged as a potential biomarker for assessing nCRT response, and we herein evaluate the association between distinct ITGH estimates and nCRT response using local and public genomic datasets. We analyzed a local cohort of 71 LARC patients who underwent long-course nCRT with 5FU-based chemotherapy or Capecitabine (SP cohort). Pre-treatment tumor biopsies were subjected to whole exome sequencing (WES), and patients were classified into two groups: nCRT-R (N=16), nCRT-NR (N=55). Public WES, mRNAseq, and clinical data from 81 patients from Chatila et al. (2022, MSKCC cohort) were also used to ITGH estimates using MATH score (in WES) and DEPTH2 (in mRNAseq). The treatment response was evaluated based on cCR or pathological complete response (pCR) in patients treated with 5FU, Capecitabine or Cisplatin, receiving nCRT alone or combined with induction/consolidation. Low ITGH values correlated with nCRT response in the SP cohort (median MATH score of 28.89 versus 50.97 in nCRT-R and NR, respectively; $p \leq 0.05$, Wilcoxon test). We used ROC curve analysis to determine the best MATH score threshold (26.34, sensitivity=90%, specificity=50%, AUC=0.67). Multivariate analysis with the clinical-radiological features showed a marginal association between MATH-low with nCRT-R (OR:16.05; p=0.003). We observed opposite tendencies for the MSKCC cohort (higher ITGH in nCRT-R): median MATH score of 38.65 (nCRT-R, N=18) versus 33.51 (nCRT-NR, N=63), but the difference was not significant (p=0.2536, Wilcoxon test). Considering MATH score limitations (e.g., number of somatic mutations, WES coverage) we also inferred ITGH from expression data for a subset of these patients. Still, we observed similar, non-significant results: median ITGH of 0.667 versus 0.652 for nCRT-R (N=18) and NR (N=54), respectively (p=0.503, Wilcoxon test). The results from our cohort suggest that ITGH remains a potential biomarker of response. The conflicting data observed may simply reflect the limited statistical power of the analyses. We believe it is necessary to increase the sample size and stratify response groups based on treatment regimens. Therefore, we consider it important to disclose these findings to encourage collaboration and facilitate the expansion of our case series.

#1263 Reconstructing small cell lung cancer evolution using deep whole genome sequencing of circulating tumor DNA.

Benjamin B. Morris, Zhihui Zhang, Andrew R. Lynch, Simon Heeke, Kyle Concannon, C. Allison Stewart, Runsheng Wang, Alexa Halliday, Waree Rinsurongkawong, Vadeerat Rinsurongkawong, J. Jack Lee, Jianjun Zhang, Don L. Gibbons, Ara A. Vaporciyan, Carl M. Gay, Hai T. Tran, John V. Heymach, Lauren A. Byers, Peter Van Loo

UT MD Anderson Cancer Center, Houston, TX

Background: Small cell lung cancer (SCLC) is the most lethal form of lung cancer. This poor prognosis is due in large part to SCLC's recalcitrance to therapy. The standard of care for extensive-stage SCLC (ES-SCLC) is chemotherapy with immunotherapy. While most tumors initially respond, responses are transient and >80% of tumors progress within one year of treatment. Seminal studies have shown that sequencing aggressive tumors before and after therapy identifies drivers of resistance. Unfortunately, the aggressive clinical course of ES-SCLC has precluded collection of patient-matched naïve and recurrent tumor tissue traditionally required to study evolution. In lieu of this, other groups have used circulating tumor DNA (ctDNA) to study SCLC evolution. However, these studies used sequencing panels that analyze <0.004% of the SCLC genome. Using new methods to comprehensively trace how SCLCs evolve to therapy resistance is a significant unmet need.
Methods: Medical records were reviewed to identify treatment naïve ES-SCLC patients with longitudinal liquid biopsies collected pre- and post-therapy using IRB-approved protocols. ctDNA was isolated from plasma using NucleoSnap cfDNA kits. Germline DNA was extracted from buffy coats using NucleoSpin Blood kits. Whole genome sequencing (WGS) libraries were prepared using Twist cfDNA Library Preparation with UMI Adapter kits. Deep WGS of ctDNA (>100X) and germline DNA (>40X) was performed using an Illumina NovaSeqX sequencer. Reads were aligned to the hg38 reference genome with BWA-mem. Somatic mutations were called using a two-of-three consensus approach and Mutect2, Strelka2, and MuSE. Structural variants were called using a consensus approach with GRIDSS, SvABA, and Manta. Subclonal copy number alterations were identified with Battenberg. Pyclone was used to reconstruct clonal populations.
Results: As expected, we identified deleterious genomic alterations impacting TP53 and RB1 tumor suppressor genes, known drivers of SCLC development. COSMIC mutational signature analysis identified high activity of tobacco carcinogen, APOBEC, and replicative-based processes across tumors. We found highly varied patterns of genomic evolution following frontline chemoimmunotherapy across patients. Most ES-SCLC tumors exhibited genomic evolution at recurrence, with evidence of focal amplifications and deletions or whole genome duplication. Some tumors exhibited minimal copy number evolution at relapse, suggesting non-genomic mechanisms driving resistance. We also found that second line therapies forced additional genomic evolution following initial recurrence.
Conclusions: Our data demonstrates that deep WGS of ctDNA recapitulates known genetics of ES-SCLC and enables sensitive characterization of resistant clones. Our data highlights the under-appreciated role of genomic evolution in ES-SCLC frontline therapy resistance.

#1264 Enhanced genomic characterization and clonal profiling in acute myeloid leukemia.

Sanjeeth Rajaram[1], Ruwan Gunaratne[1], Crystal Zhou[1], Emily Yang[1], Charu Tiwari[1], Grace Zhang[1], Ravindra Majeti[1], Asiri Ediriwickrema[1], David Kurtz[2], Tian Yi Zhang[1]

[1]Division of Hematology, Department of Medicine, Stanford University, Stanford, CA,[2]Division of Oncology, Department of Medicine, Stanford University, Stanford, CA

Introduction:
Acute myeloid leukemia (AML) is an aggressive blood cancer characterized by serial acquisition of somatic mutations in hematopoietic stem and progenitor cells which lead to their clonal expansion and differentiation arrest. Current methods for genomically profiling AML are limited by (1) the need for serial invasive bone marrow biopsies and (2) paucity of recurrently shared mutations across AML patients using standard NGS sequencing approaches.
Methods:
We integrate an AML-specific CAPP-Seq (Cancer Personalized Profiling by Deep Sequencing) hybrid capture panel targeting 58 recurrently mutated, 'canonical' AML genes, with personalized panels derived from whole exome sequencing (WES) of flow-sorted AML blasts and T cells (germline) in 56 AML patients. We quantify WES-derived single nucleotide variants (SNVs), indels, and copy number alterations (CNAs) in both canonical and 'noncanonical' genes (non-recurrently mutated) across a median of 5 follow up timepoints from cellular (bone marrow mononuclear cell/BMMC, peripheral blood mononuclear cell/PBMC) and cell-free (PB-ctDNA, BM-ctDNA) compartments. Variant pathogenicity was assessed using CADD, an ensemble model; ESM-1b, a protein language model; PolyPhen2, SIFT and a novel graphical association method. Mutation signatures were extracted using the SigProfiler suite. Clonal hierarchy was modeled using SciClone and latent class analysis.
Results:
We observed a median of 32.5 (IQR 21-42) tumor SNVs per patient compared to only 2.5 (IQR 1-4, $p < 0.0001$) if using the canonical AML panel alone. Overall variant detection was highly concordant across compartments: median of 94% (BMMC vs PBMC), 85% (PB-ctDNA vs BMMC), 94% (PB-ctDNA vs PBMC) and 98% (PB-ctDNA vs BM-ctDNA) respectively. Noncanonical mutations were 99.9% unique across patients and less pathogenic across our ensemble of measures ($p < 0.0001$). C-to-T transitions were enriched in both canonical and noncanonical mutation sets, but other signatures suggest distinct etiology.
Moreover, personalized patient-specific AML-CAPP-Seq enabled better inference of clonal dynamics compared to our institutional standard-of-care bulk NGS assay. Our approach predicted clonal hierarchies supported by single-cell genotyping (Tapestri) and helped predict potential mechanisms of therapy resistance. Finally, we show AML-CAPP-Seq permits monitoring of donor chimerism after allogeneic hematopoietic stem cell transplantation.
Conclusions:
AML-CAPP-seq enables ultrasensitive, WES-scale, non-invasive detection of patient-specific mutations, revealing novel biology in AML pathogenesis and allowing improved clonal profiling for in-depth characterization of therapy resistance mechanisms.

TUMOR BIOLOGY: Human and PDX Models 2
Poster Session

#1268 A matter of TIME: Advancing a humanized mouse model to decode tumor immune microenvironment and uncover mechanisms of resistance in soft tissue sarcoma.

I. Karnik[1], W. Liu[2], M. Liu[2], W. Tan[2], S. Tan[2], S. Fong[2], V. Yang[3], Q. Chen[2];
[1]National University of Singapore, Singapore, Singapore, [2]Institute of Molecular and Cell Biology, Agency for Science, Technology and Research, Singapore, Singapore, [3]National Cancer Centre Singapore, Singapore, Singapore

Background: Humanized mouse models are invaluable tools in cancer research. These immunodeficient mice, engrafted with human hematopoietic stem cells (HSCs) allow the exploration of immune dynamics and therapeutic responses in cancer. However, traditional models underrepresent key immune cells, particularly natural killer (NK) cells, which are crucial for anti-tumor immunity. We developed an improved humanized mouse model with optimized NK cell reconstitution to better reflect human immune responses. Using this model, we studied soft tissue sarcoma (STS), a malignancy with an immunosuppressive tumor immune microenvironment (TIME) that contributes to therapy resistance. Our goal was to better understand immune dynamics and resistance mechanisms within the TIME and explore novel immunotherapy strategies.
Methods: Humanized mice were generated by engrafting immunodeficient mice with HSCs, partially HLA-A matched to the SK-UT1 cell line for better immune representation of STS. NK cell reconstitution was improved with human IL-15 (hIL-15). Mice were engrafted with SK-UT1 STS cells to simulate the TIME. Tumor-infiltrating lymphocytes (TILs) and peripheral immune cells were analyzed by flow cytometry and single-cell sequencing to assess infiltration, activation, and exhaustion. Key immune checkpoints (PD-1, TIM-3, LAG-3, CTLA-4) and cytokines were evaluated to identify differences between TILs and peripheral immune cells.
Results: Diverse immune cell populations were observed within the TIME, including T cells, NK cells, and macrophages. Tumor-infiltrating CD4+ and CD8+ T cells showed higher expression of exhaustion markers compared to circulating T cells, indicating immune suppression and impaired cytotoxic function. The model demonstrated increased infiltration of CD56+ NK cells, offering a more accurate representation of NK cell function in the TIME. The gradient of exhaustion markers across immune cells suggested a highly immunosuppressive environment, contributing to tumor progression and resistance.
Conclusions: Our improved humanized mouse model offers a robust platform for studying immune-tumor interactions, particularly TILs and their role in the TIME. This model holds significant potential for testing novel immunotherapy combinations, including immune checkpoint inhibitors and targeted therapies aimed at reversing immune exhaustion. Ongoing analyses are focused on identifying key pathways involved in immune evasion through single-cell and TCR sequencing, and uncovering distinct resistance mechanisms within the tumor, potentially revealing new strategies to overcome immunotherapy resistance. These insights will reshape our understanding of the TIME and pave the way for innovative treatments to improve outcomes in STS.

#1269 Development of humanized PDX mouse model for high grade serous ovarian cancer.

Tsz Yin (Jacky) Lam[1], Farhia Kabeer[2], Shary Chen[2], Makoto Kishida[3], Yuchen Ding[2], Lorena Zoltan[2], Yvette Drew[4], David Huntsman[2]

[1]Department of Medicine, University of British Columbia, Vancouver, BC, Canada,[2]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada,[3]Centre for Lymphoid Cancer department, BC Cancer, Vancouver, BC, Canada,[4]Department of Gynecology and Obstetrics, University of British Columbia, Vancouver, BC, Canada

Introduction: High-grade serous ovarian cancer (HGSOC) is one the most lethal gynecological cancer, accounting for 70% deaths and with a 5-year survival rate below 50%. Traditionally, HGSOC are considered 'immune-cold' tumors, characterized by low immune infiltration and poor response rates to single agent immune checkpoint blockers (ICB). To improve the therapeutic potential of immunotherapies in HGSOC, combination treatments are being applied such as the Phase 2 MEDIOLA trial, which aims to identify mechanisms of response and resistance. A major challenge in this field remains the lack of suitable preclinical models for ICB-based treatments. Although patient-derived tumor xenografts (PDX) are valuable for developing new therapies, they are limited by the absence of an immunocompetent host. Here we present humanized mouse models incorporating human immune cells.
Methods: Female NRG-W41-3GS mice (ENW) aged 7-8 weeks were used to generate the humanized PDX model. HGSOC tumors originally sourced from human patients and maintained in our laboratory's previous PDX models using NRG mice, were injected subcutaneously into ENW mice. After 3 weeks, $5x10^4$ human hematopoietic CD34+ cells collected from umbilical cord blood of healthy donors were intravenously injected via the tail vein. To monitor human cell engraftment, bone marrow (BM) aspiration was performed at week 6 post-CD34+ cell injection, and peripheral blood (PB) samples were collected from saphenous vein starting from week 7 until the experimental endpoint. BM and PB samples were analyzed by flow cytometry using antibodies against human CD45 and mouse CD45 to determine humanization level. Tumors were harvested at the endpoint, embedded in paraffin, and remaining tumor tissues were cryopreserved for future. To confirm human immune cell infiltration into the tumors, fresh frozen paraffin embedded tumor sections were immunohistochemically stained with rabbit anti-human CD45 antibodies.
Results: We successfully generated sixteen huENW PDX mice. Robust engraftment of human CD34+ cells was confirmed in each mouse, with human CD45+ cells ranging from 35% to 90% (mean ± SD: 76.2 ± 18.5%) in BM samples and from 7% to 35% in PB samples. The huENW PDX model have revealed strong evidence of human immune infiltration with CD45+ cells on immunohistochemistry of the PDX tumors and spleen.
Conclusion: Humanized mouse models offer a valuable resource for advancing HGSOC research. Our humanized PDX HGSOC mouse model demonstrated significant engraftment of human immune infiltration into HGSOC tumors. Future works will focus on characterizing tumor cell heterogeneity, analyzing spatial gene expression patterns and human immune cells distribution using Xenium *in situ* coupled with scRNA seq. We believe our model has the potential to enhance our understanding of HGSOC clonal diversity, microenvironment interactions, and contributing to the development of new therapies.

#1270 An isogeneic Trop-2 expression heterogeneity model for evaluating the bystander killing effect of anti-Trop-2 ADC.

Jingwei Huang[1], Xiangyi Wang[1], **Zhixiang Zhang**[1], Jingxuan Yan[1], Jinyan Ma[2], Man Zhang[1], Qingyang Gu[1]

[1]WuXi AppTec, Shanghai, China,[2]WuXi AppTec, Nantong, China

Antibody-drug conjugates (ADCs) have recently made significant advancements in cancer treatment, particularly for solid tumors. Among the multiple mechanisms of action of ADC drugs, the bystander effect—where the cytotoxic payloads released by the antibodies diffuse into and kill nearby tumor cells—plays a crucial role, especially in tumors with heterogeneous antigen expression. Therefore, constructing an antigen heterogeneity model for evaluating this bystander killing effect is essential for the preclinical assessment of ADC drugs. To establish a stable Trop-2 antigen expression heterogeneity model, we deleted Trop-2 in the Trop-2-positive NCI-H292 cell line and selected a clone with a similar *in vitro* proliferation rate to the parental cells. Additionally, to distinguish Trop-2-negative from Trop-2-positive cells in both *in vivo* and *in vitro* experiments, we introduced luciferase and mCherry into the Trop-2-negative cells. *In vitro*, Trop-2-negative and Trop-2-positive cells were mixed in multiple ratios and treated with graded concentrations of anti-Trop-2 ADC. After five days, flow cytometry quantified Trop-2-negative cells to evaluate the bystander killing effect. *in vivo*, Trop-2-positive and Trop-2-negative cells were mixed in various ratios and implanted subcutaneously in mice, followed by intravenous drug administration. Tumor volume and bioluminescence were measured to assess the efficacy of the anti-Trop-2 ADC and bystander killing activity. The results showed that the anti-Trop-2 ADC exhibited a significant bystander killing effect, significantly inhibiting Trop-2-negative cells both *in vitro* and *in vivo*. In summary, we have successfully established an isogeneic Trop-2 expression heterogeneity model and validated the bystander killing activity of anti-Trop-2 ADCs *in vitro* and *in vivo*. This model provides a robust platform for the pharmacological and efficacy assessment of anti-Trop-2 ADC therapies.

#1271 Generation and optimization of humanized mice models in metastatic uveal melanoma.

S. Deguchi[1], T. Alcaro[1], R. Tanaka[2], S. Koshkin[1], V. Alexeev[1], K. Mccorkell[1], U. Gergis[1], T. Sato[1], M. Terai[1];
[1]Sidney Kimmel Medical College of Thomas Jefferson University, Philadelphia, PA, [2]Osaka Metropolitan University, Osaka, Japan

Background: Uveal melanoma (UM) is the most common primary intraocular malignancy in adults, with poor prognosis in cases of liver metastasis. Tebentafusp, a bispecific T cell engager, has shown survival benefits in patients with metastatic uveal melanoma (MUM); however, the response rate is low (<10%), and there is an unmet need to improve the outcome of immunotherapy against MUM. A major challenge of translational research in MUM immunotherapy is the lack of suitable preclinical models. To address this, we developed a humanized mouse model using human bone marrow (BM)-derived $CD34^+$ hematopoietic stem cells (HSCs).

Method: HSCs were injected into NSG Flt3KO, hFLT3LG Tg mice (NSG-hFLT3) via the spleen or tail vein after 150 cGy whole body irradiation. Blood specimens from the mice were routinely collected to confirm the development of chimerism through human and mouse CD45 staining. Mice were sacrificed at 9 or 20 weeks, and blood specimens and various organs were collected. The distribution and differentiation of human $CD45^+$ cells in BM, spleen, and blood were analyzed by flow cytometry. Furthermore, human $CD45^+$ cells were isolated from the mouse spleen and BM for immune potency assays. For the liver metastasis model in humanized mice, the tdTomato-labeled human MUM cell line, UM001 (HLA-A2), was injected into the mouse liver, 3 or 9 weeks after injection of HLA-A2 matched HSCs into NSG-hFLT3 mice. MUM tumor specimens in the liver were collected 4 weeks after MUM cell line inoculation, and the interactions between human MUM tumors and human $CD45^+$ cells were analyzed.

Result: Three weeks post-injection, human $CD45^+$ cells were confirmed in the blood, indicating the differentiation of injected human $CD34^+$ HSCs. Flow cytometry detected human $CD3^+$, $CD19^+$, $CD14^+$, $CD11c^+$, and $CD56^+$ cells in hematological organs. Monocytes and B cells predominated in the spleen at 9 weeks, while T cells were more abundant in the peripheral blood at 20 weeks. There was no difference in HSC injection routes regarding $CD45^+$ cell differentiation and distribution. Human TNF-α production with LPS was detected from human $CD45^+$ cells obtained from the mouse BM. IFN-γ production with phytohemagglutinin (PHA) was also confirmed in 21.1% of human $CD3^+$ T cells obtained from the mouse BM. In the spleen, B cells and T cells were confirmed, but those cells were rarely seen in other organs. Interestingly, human $CD45^+$ cells infiltrated the MUM tumors in the liver of humanized mice.

Conclusion: NSG-hFLT3 mice successfully adopted human $CD34^+$ HSCs and developed the chimerism with functional human immune cells. This indicates that this mouse model can be utilized to study the interaction between MUM tumors and human immune cells and serve as a platform for investigating the efficacy and resistance mechanisms of cancer immunotherapies. This approach may also be used for an autologous humanized mouse model using $CD34^+$ cells and patient-derived xenograft (PDX) from the same patients.

#1272 The development of PDX models from ADC-resistant breast cancer patient tissue for next-generation therapy evaluation.

Q. Liu, J. Wang, X. Zhang, W. Qian, L. Zhang, L. Bourre, J. Wang;
Crown Bioscience, Inc., San Diego, CA

Introduction: Antibody-drug conjugates (ADCs) are monoclonal antibodies targeting tumor cells linked to cytotoxic drugs via chemical linkers. Trop-2 targeting ADCs like sacituzumab govitecan (SG), datopotamab deruxtecan (Dato-DXd), and SKB-264 have shown success in clinical settings. However, they face challenges such as complex pharmacokinetics, insufficient tumor targeting, suboptimal payload release, side effects and drug resistance. This study developed SG-resistant PDX models to replicate clinical resistance to support new therapy development and understanding of resistance mechanisms.

Methods: Subcutaneous PDX models were established and characterized by histology and sequencing, with Trop-2 expression assessed by immunohistochemistry (IHC) and RNA sequencing (RNA-seq). For *in vivo* efficacy studies, models were treated 3-4 weeks after tumor inoculation with SG (3 or 5 mg/kg, i.v.) and Dato-DXd (5 mg/kg, i.v.) based on clinical SG treatment schedule. Tumor growth inhibition (TGI) was calculated as [1 - (mean treated tumor volume / mean control tumor volume)] * 100%. Homologous recombination deficiency (HRD) scores were calculated (using scarHRD and PureCN from whole exome sequencing, with a cutoff value of 38).

Results A female TNBC patient underwent 8 rounds of chemotherapy and targeted treatment, including 2 cycles of SG in the sixth round. Tissues collected at different treatment stages following SG treatment were used to establish BR9690, BR9806 and BR9801 PDX models. High Trop-2 expression was detected at both gene and protein levels in all three models. To evaluate Trop-2 ADCs efficacy, SG and Dato-DXd were tested in the PDX models. Tumor progression was observed in BR9690 for both SG (3mg/kg TGI=9.40% and 5mg/kg TGI=16.77%) and Dato-DXd (TGI=32.15%). In BR9806, tumor progression was observed in SG with limited TGI (3mg/kg TGI=27.52% and 5mg/kg TGI=25.75%), while Dato-DXd showed a partial response (TGI=59.47%). For BR9801, both SG and Dato-DXd showed limited TGI (SG: 3mg/kg TGI=-2.60% and 5mg/kg TGI=0.21%, Dato-DXd: TGI=24.78%). These results indicate clinical consistency with SG resistance and resistance to other Trop2-ADCs as the disease progresses. Besides, HRD scores for all three PDX models were above the cutoff (BR9690: 42, BR9806: 39, BR9801: 50) indicating genomic instability. In this study, the limited response of the models despite HRD may suggest other factors affecting the drug's efficacy targeting DNA repair mechanism, such as acquired resistance mechanisms or drug efflux activity, overriding the vulnerability provided by HRD.

Conclusion The study successfully established longitudinal breast cancer PDX models from a patient with demonstrated resistance to Trop2-ADCs. These models will aid in evaluating new treatments or combination therapies and understanding drug resistance mechanisms.

#1273 Advancing precision oncology: Development and utilization of breast cancer PDX models for *in vivo* drug discovery.

Erin E. Trachet, Chelsea Holmquist, Stephanie Nagl, Paul M. Gonzales

Translational Drug Development (TD2), Scottsdale, AZ

Breast cancer is a highly heterogeneous disease that poses significant challenges for drug development and personalized treatment. Patient-derived xenograft (PDX) models have emerged as essential tools for preclinical research, faithfully preserving the genetic, transcriptomic, and histopathological features of primary tumors, including intratumoral heterogeneity and the tumor microenvironment. The *Nature* publication, *"A human breast cancer-derived xenograft and organoid platform for drug discovery and precision oncology"*, highlights the utility of breast PDX models in advancing precision medicine and accelerating therapeutic discovery. Through a collaboration with the University of Utah, TD2 has developed three well-characterized in vivo breast cancer PDX models: HCI-015, HCI-043, and HCI-044. HCI-015 and HCI-043 are triple-negative breast cancer (TNBC) models, with HCI-015 harboring BRCA1 and BRCA2 deletions. HCI-044 is an estrogen receptor-positive (ER+), progesterone receptor-positive (PR+), and HER2-negative. These models were selected based on their organoid performance and clinically relevant drug responses. For example, HCI-015 was sensitive to docetaxel and a Notch inhibitor but refractory to navitoclax, while HCI-043 showed high sensitivity to eribulin. The ER+ HCI-044 demonstrated minimal dependence on estrogen supplementation, with growth maintained despite ovariectomy. To preserve tumor heterogeneity and the microenvironment, TD2 developed a low-passage tumor propagation process using orthotopic implantation into the fourth mammary fat pad via a surgical technique. Tumors in HCI-015 and HCI-043 established and grew progressively within 35 days, while HCI-044 required 60 days but maintained reliable growth. Pilot studies confirmed the reproducibility of tumor propagation and response profiles, supporting their use in efficacy testing. For efficacy evaluations, PDX tumors were implanted into female NSG or CIEA NOG mice, and therapeutic regimens were assessed. HCI-015 was highly sensitive to docetaxel, achieving 100% complete regression (CR), while tamoxifen treatment confirmed its ER-negative status, resulting in progressive disease (PD). Similarly, HCI-043 demonstrated 100% CR with carboplatin and eribulin. For the ER+/PR+ HCI-044 model, tamoxifen treatment resulted in PD, but eribulin achieved 100% CR, highlighting subtype-specific therapeutic responses. Despite their transformative potential, breast PDX models face limitations, including cost and incomplete human immune system representation. However, by optimizing these models and integrating patient history, researchers are advancing their fidelity and expanding their applications. These models bridge the gap between preclinical research and clinical outcomes, offering new opportunities to accelerate targeted therapy development and improve patient care.

#1274 Comparative analysis of biological and multi-omics profiling in PDCs and PDOs models.

Ye Jin Moon[1], Woo Sun Kwon[2], Tae Soo Kim[2], Harim Koo[3], Sun Young Rha[4]

[1]Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul, Korea, Republic of,[2]Song-Dang Institute for Cancer Research, Yonsei University College of Medicine, Seoul, Korea, Republic of,[3]Department of Cancer Biomedical Science, Graduate School of Cancer Science and Policy, National Cancer Center, Goyang, Korea, Seoul, Korea, Republic of,[4]Division of Medical Oncology, Department of Internal Medicine, Yonsei Cancer Center, Yonsei University Health System, Seoul, Korea, Republic of

Patient-derived cells (PDCs), cultured in 2D environment, have limitations in reflecting patient's characteristics, while patient-derived organoids (PDOs) better capture heterogeneity and complexity of tumor microenvironment. However, PDOs do not always reflect the patient's condition, and in certain contexts, PDCs may offer distinct advantages. Since most PDCs and PDOs are established from primary tumors, research models from metastatic tumors are necessitated for studying metastatic cancers, like peritoneal metastases. In this study, we aim to compare established PDCs and PDOs models derived from ascites of two advanced gastric cancer patients and to explore the limitations and potential of each model by integrating fundamental characteristics and multi omics analysis.
Morphological features in PDCs and PDOs models of each sample were observed using IHC staining and microscopy. Biological features, including proliferation, cell cycle and HER2 expression were evaluated, while multi-omics was conducted to explore comprehensive biological processes and molecular pathways. Drug sensitivity using Paclitaxel, 5-FU, SN-38 and Oxaliplatin was performed to evaluate the drug responsiveness, and underlying mechanisms were explained by multi-omics.
In PDCs, both samples (YCC-GO10PDCs and YCC-GO30PDCs) exhibited adherent cell morphology, whereas PDOs showed structural variations, presenting compact (YCC-GO10) and grape-like (YCC-GO30) formations. The HER2 expression in YCC-GO10PDCs was consistent with the patient's clinical data. At the stabilized stage of PDCs (24h) and PDOs (72h), the cell cycle showed a normal S-phase distribution (YCC-GO10PDCs 26.4%, YCC-GO30PDCs 14.5% and YCC-GO10 21.62%, YCC-GO30 14.87%). In tumor purity of YCC-GO10, PDCs had 99%, while PDOs had 55%. Similarly in tumor mutation burden, as determined by whole genome sequencing, PDCs (16.27) was higher than PDOs (9.93). In proteomics, genes significantly upregulated in PDOs were associated with microenvironment interactions, such as ECM remodeling and stemness-related pathways, while genes upregulated in PDCs were primarily linked to metabolic and proliferative activities, reflecting the biological differences between two models. Drug response of YCC-GO10 showed similar patterns while YCC-GO30 showed higher sensitivities in PDCs than PDOs. Proteomics revealed that COL4A1 gene, associated with oxaliplatin and enhances cancer cell invasiveness and drug resistance, was upregulated in PDOs.
In conclusion, established PDCs and PDOs models from patient's fluid were used for comparative analysis and provided insights into each model's characteristics. Each research model is expected to serve as representative models reflecting the patient's condition and are anticipated to contribute to the development of personalized treatment.

#1275 Advancing cancer research with PDX-derived organoids: Insights into cancer stem cells and metastasis.

Nicolas Ancellin, Olivier Duchamp, Didier Grillot, Kenny Herry, Nicolas Legrand, **Caroline Mignard**

Oncodesign Services, Dijon, France

Patient-derived xenograft (PDX) organoids (PDXOs) are emerging as powerful tools for studying cancer biology and advancing therapeutic discovery. In this study, we report the generation of PDX-derived organoids from pancreatic ductal adenocarcinoma (PDAC) and colorectal cancer (CRC) PDX models, establishing a translational framework for personalized cancer research.

A key focus was the detection and enrichment of cancer stem cells (CSCs) within colorectal cancer PDXOs (CR PDXOs). Through optimized culture conditions, we achieved a remarkable 30-fold enrichment of CSCs, a pivotal population driving tumor progression, therapeutic resistance, and metastasis. This enrichment highlights the utility of PDXOs in dissecting stem cell biology and targeting CSC-associated mechanisms.

Using the PDAC model, we implemented tricistronic transduction to generate stable PDXOs expressing NanoLuc, mCherry, and EGFP fluorescent proteins. This genetic engineering strategy enables real-time monitoring of organoid dynamics and facilitates downstream applications, including in vivo studies. The reimplantation of these labeled PDXOs into immunocompromised mice successfully reestablished PDX tumors, demonstrating growth curves features nearly identical to those of the original PDX tumors. This PDXO-to-PDX approach underscores the fidelity of PDXOs in recapitulating patient-derived tumor biology.

Further, we investigated metastatic potential using full single-cell dissociation and marker-based detection. Analysis of various tissues from mice bearing reimplanted PDAC PDXOs revealed the presence of metastatic cells in the liver, indicating the ability of PDXOs to model metastatic dissemination. This capability provides a unique platform to study metastatic mechanisms and evaluate anti-metastatic therapies.

In conclusion, our study highlights the versatility and translational relevance of PDXOs in cancer research. By generating PDXOs from diverse models, enriching CSC populations, and employing advanced genetic tools for functional studies, we demonstrate the utility of PDXOs in capturing tumor complexity. The reimplantation experiments and detection of metastasis further validate PDXOs as robust systems bridging in vitro and in vivo research. This integrated approach offers critical insights into tumor biology, therapeutic responses, and metastatic behavior, paving the way for improved drug discovery and personalized treatment strategies.

#1276 UNLOCK, a preclinical platform of PDX models resistant to innovative therapies.

Ludovic Bigot[1], Nobre Nobre[1], Alice Da silva[1], Melissandre Meteau[1], Mathis Delavigne[1], Miguel Soares[1], Francesco Facchinetti[1], Loic Poiraudeau[1], Floriane Braye[1], Inmaculada Inmaculada Alonso Garcia[1], Natalie Ngoi[2], Alexandre Halimi[2], Emlie Natali[2], Damien Vasseur[2], Kristi Beshiri[2], Claudio Claudio Nicotra[2], Maud Ngo-Camus[2], Lambros Tselikas[2], Beatriz Beatriz Alonso de Castro[2], Julieta Rodriguez[2], Maria Fernanda Mosele[2], Maud Kamal[2], Gerome Jules-Clement[2], Karim Fizazi[2], Christophe Massard[2], Benjamin BESSE[2], Luc Friboulet[1], Yohann Loriot[1]

[1]INSERM U981 (Gustave Roussy), Villejuif, France,[2]Gustave Roussy, Villejuif, France

Introduction: Over the past 20 years, advances in precision oncology and cancer genetics have identified numerous actionable oncogenic drivers, leading to new drug development and clinical application. However, tumor cells can adapt and develop resistance mechanisms (MoR), and the mechanisms of action (MoA) of these drugs are only partially understood, highlighting the need for research focused on both MoR and MoA. Following the success of the MATCH-R clinical trial (NCT02517892, 2015-2022), the UNLOCK program was launched in 2022 to better understand the MoA and MoR of innovative drugs (phase 1 to approval) through integrated clinical and translational research. As part of this initiative, we developed preclinical models, including PDX models from patients who developed resistance, to support the development of new therapeutic strategies.
Methods: Fresh tumor biopsy specimens were prospectively collected from patients through the MATCH-R clinical trial (NCT02517892) or the UNLOCK program. PDX models were established in NOD Scid Gamma (NSG) mice, which were then developed and characterized. These models underwent immunohistochemistry, comprehensive molecular profiling (including whole exome sequencing [WES] and bulk RNA sequencing [RNA-seq]), and pharmacological validation.
Results: As of November 2024, 197 PDX models have been successfully generated from 519 biopsies, with a global take rate of 38%. These models are developed across several cohorts, including castration-resistant prostate cancer (22 PDX: 18 AR pathway inhibitors, 1 post-PARPi, and 3 Lu-PSMA), lung cancer (40 PDX with EGFR inhibitors, including 28 post-osimertinib, and 22 PDX with ALK inhibitors, including 2 post-brigatinib, 9 post-lorlatinib, and 6 post-alectinib). Additionally, 34 PDX models have been developed for the FGFR cohorts with FGFR2/FGFR3 alterations across various cancers, including cholangiocarcinomas, bladder carcinomas, gynecological carcinomas, and pancreatic cancers. We also developed 32 PDX models with KRAS mutations (G12C, n=23; G12D, n=9) from various tumors, including lung, pancreatic, gynecological, and colon cancers. Recently, we launched a new UNLOCK cohort focused on antibody-drug conjugate therapy, developing 12 additional PDX models. These PDX models faithfully replicate the genetic, transcriptional, phenotypic, and pharmacologic features of the original biopsies, providing a unique preclinical platform for testing novel drugs and combination therapies. Adaptive treatments with new or combinatorial strategies are being explored to restore drug sensitivity.
Conclusion: The development of 197 PDX models under the UNLOCK program represents a significant resource, enabling the evaluation of novel drugs, adaptive treatment strategies, and combinatorial approaches. This unique collection of PDX models paves the way for advancing precision oncology and overcoming therapeutic resistance.

#1277 *Ex ovo* system for rapid and quantitative modelling of immunotherapy responses in pre-clinical and patient-derived xenografts: PDX*ovo*.

Olivia R. Grafinger, Kabir A. Khan, Esther I. Matus, Sara Mar, Yan Li, David Goertz, Jean Gariepy, Katarzyna J. Jerzak, Robert Kerbel, Hon S. Leong

Sunnybrook Research Institute, Toronto, ON, Canada

Precision oncology for triple-negative breast cancer (TNBC) is urgently needed because not all patients will benefit from PDL1-based immunotherapy. Unfortunately, the expression of PDL1 or abundance of tumor infiltrated lymphocytes (TILs) does not correlate with objective response rates and/or overall survival. We have developed a novel patient-derived xenograft (PDX) model system that offers phenotype-based metrics to predict immunotherapy response prior to receiving treatment. In our PDX model (PDX*ovo*), the patient's tumour is engrafted onto the chorioallantoic membrane (CAM) of chick embryos. Our PDX model offers significant advantages over mouse PDXs which are expensive, time-consuming, and cannot evaluate immunotherapies. To test the efficacy of this model, we implanted immunocompetent mice with a murine mammary cancer cell line (serving as a surrogate for patients), and implanted tumours into the PDX*ovo* system. We found our model to reliably recapitulate immunotherapy responses observed in mice, with anti-PDL1-treated tumours being significantly smaller in volume as compared to IgG-treated tumours. We also found our model to reliably predict patient response to adoptive cell therapy, wherein TILs were FACS-sorted from murine tumour digests, activated and expanded in vitro, and then engrafted together with cultured murine mammary carcinoma cells. We are now testing this model with patient-derived tumours of a variety of origins, in partnership with the Ontario Institute of Cancer Research. To date we have tested tumour responses to Pembrolizumab in five patient-derived samples from various sites, with an 80% success rate in xenograft formation. We have observed varied responses to immunotherapy, which did not correlate with PDL1 expression on TILs in these samples, highlighting the importance of such a qualitative model for precision oncology. This technology has the potential to make a positive impact in the clinic and improve objective response rates, increase progression-free survival intervals, and increase overall survival.

#1278 Tertiary lymphoid structure formation in patient-derived xenograft (PDX) of head and neck paraganglioma: A novel xenogeneic model.

Sara Pagotto[1], Simone De Fabritiis[2], Carola Di Camillo[1], Francesco Del Pizzo[2], Silvia Perconti[2], Diana Liberata Esposito[3], Mario Sanna[4], Pasquale Simeone[5], Paola Lanuti[5], Manuela Iezzi[6], Renato Mariani-Costantini[2], Fabio Verginelli[3], Rosa Visone[1]

[1]Department of Medical, Oral and Biotechnological Sciences, University of Chieti Pescara, Chieti, Italy,[2]University of Chieti Pescara, Chieti, Italy,[3]Department of Sciences, University of Chieti Pescara, Chieti, Italy,[4]"Gruppo Otologico" Piacenza, Piacenza, Italy,[5]Department of Medicine and Aging Science, University of Chieti Pescara, Chieti, Italy,[6]Department of Neurosciences, Imaging and Clinical Sciences, University of Chieti Pescara, Chieti, Italy

Head and neck paragangliomas (HNPGL) are a rare type of neuroendocrine tumor that, although typically slow-growing, can become invasive and deadly. Treatment is primarily surgical, which can be highly invasive and, in some cases, not feasible. Alternative therapies, such as chemotherapy and targeted radiotherapy, have demonstrated limited efficacy. This study aims to address the critical need for suitable in vivo HNPGL models to advance translational research by establishing murine models of immunodeficient NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice engrafted with patient-derived tumor xenografts (PDXs). Interestingly, although HNPGL is not typically classified as a lymphocyte-dominant tumor due to the reduced presence of immune cells in its microenvironment, two consecutive generations of NSG mice engrafted with PDXs derived from a single aggressive HNPGL case in an adolescent patient, exhibited varying degrees of xenogeneic graft-versus-host disease (GVHD). This suggests the co-existence of the patient's tumor-infiltrating lymphocytes (TILs) within the PDXs. In the first generation, 34 days after PDXs implantation, mice exhibited clinical signs of GVHD, including weight loss, severe kyphosis, lethargy, piloerection, and fur loss. *Ex vivo* analysis revealed extensive lymphoid infiltration in multiple organs, including the spleen, lung, liver, and kidney. Immunohistochemical staining confirmed the presence of predominantly human T cell (CD3+) infiltrates, with a minor human B cell (CD20+) component, in both xenografts and the primary tumor. Notably, surgical resection and reimplantation of primary PDX into secondary NSG mice significantly promoted human B-cell expansion and the formation of a well-defined peritumoral tertiary lymphoid structure (TLS). Mice bearing these xenografts exhibited reduced GVHD, and a TLS with a histological organization resembling secondary lymphoid organs at 89 days. This TLS was characterized by a central core of hCD20+ B cells surrounded by a peripheral mantle of hCD3+ T cells, suggesting the formation of a mature TLS with a germinal center that may harbor reactive B cells. Our findings suggest that TLS formation in PDX model may facilitate tumor-specific antibody production and antigen presentation.These insights provide a foundation for leveraging PDX models with TLS as innovative tool for studying HNPGL microenvironment and asses immune signatures that could be used for the development of novel immunotherapeutic strategies.

#1279 Preclinical syngeneic model and xenograft model for evaluating CD3/HER2 bispecific antibody therapy.

Fengqian Chen, Chong Li, Qinjun Wang, Qiang Liu, Baihong Liu, Jiansu Zhang, Xiaofei Zhou

Biocytogen, Waltham, MA

Abnormal expression of HER2 in normal cells and tissues makes them susceptible to carcinogenesis, leading to tumor formation. HER2 gene amplification or protein overexpression has been found in various human tumors, including breast cancer, gastric cancer, and ovarian cancer. The approval of multiple anti-HER2 drugs has greatly improved the prognosis for patients with HER2-positive solid tumors. However, in clinical practice, some HER2-positive patients have developed resistance, possibly due to an immunosuppressive tumor microenvironment (TME). The CD3/HER2 bispecific antibody (BsAb) is considered a strategy to overcome this immunosuppressive TME by redirecting T cells to HER2-expressing tumor cells, thereby killing HER2-positive tumor cells. Biocytogen developed a BsAb Ab1 that targets human CD3 and human HER2, and evaluated its efficacy and mechanism of action in preclinical studies. In vitro cytotoxicity assays showed that CD3/HER2 BsAb Ab1 significantly induced T cell activation and exhibited effective killing effects against B-hHER2 MC38 plus cell line expressing human HER2. In a syngeneic transplantation model using B-hCD3E/hHER2 mice inoculated with the B-hHER2 MC38 plus cell line, BsAb Ab1 effectively inhibited tumor growth. Additionally, CD3/HER2 BsAb also significantly inhibited the growth of human tumor cells NCI-N87 in a PBMC reconstitution model using B-NDG mice. These results indicate that both models are suitable for the preclinical evaluation of the CD3/HER2 BsAb.

#1280 Accessing PDX genomic and treatment response data using the Mouse Models of Human Cancer database (MMHCdb) cBioPortal resource.

Dale A. Begley[1], Debra M. Krupke[1], Emily L. Jocoy[2], Steven B. Neuhauser[1], Carol J. Bult[1]

[1]The Jackson Laboratory, Bar Harbor, ME,[2]The Jackson Laboratory, Sacramento, CA

Mouse models of human cancer are one of the most important sources of information on human disease, and research using mouse models has led to fundamental insights into the genetic and molecular basis of human cancers. Mouse models are also powerful platforms for pre-clinical research into novel therapeutic agents for cancer treatment. Patient Derived Xenografts (PDXs), in particular, have proven to be useful for testing single agent and combination therapies in both adult and pediatric cancers. The Mouse Models of Human Cancer database (MMHCdb) (http://tumor.informatics.jax.org) has implemented a PDX-specific version of cBioPortal that integrates rich genomic characterization data for PDX models with treatment response data obtained from diverse sources, including the Jackson Laboratory, NCI-funded PDXNet consortium, and the Preclinical Pediatric In Vivo Testing (PIVOT) program. The cBioPortal for Cancer Genomics is an open-access, open-source resource that enables web-based exploration, visualization, and analysis of molecular profiles and clinical attributes from large-scale genomics data sets. The MMHCdb PDX specific cBioPortal contains data for 8,231 samples from 1,492 patients representing over 170 different adult and pediatric tumor types. Available genomic data include non-synonymous mutations (682 samples), Copy Number Alteration (CNA) (683 samples), RNA-Seq (4,229 samples), and de-identified patient demographic and clinical data. There is also preclinical efficacy study data available for 415 treatment arms from 100 patients. MMHCdb is an expertly curated, comprehensive knowledgebase of diverse models of human cancer obtained from peer-reviewed scientific publications and direct data submissions from individual investigators and large-scale projects. Information in MMHCdb is standardized using controlled vocabularies and official mouse gene, allele, and strain nomenclature. Information in the knowledgebase is linked to many other cancer model data from other bioinformatics resources, including PathBase, the Mouse Phenome Database (MPD), CancerModels.org, the Gene Expression Omnibus, and ArrayExpress. MMHCdb contains information on over 66,000 mouse models, including Genetically Engineered mice, Diversity Outbred and Collaborative Cross mice, and PDXs. Available data from over 5,400 references includes over 120,000 tumor frequency records, over 9,000 pathology reports, and more than 6,986 histology images. MMHCdb also provides access to over 3,157 histology/immunohistochemistry PDX images. MMHCdb is supported by NCI grant CA089713

#1281 Transfer of a PDX biobank in a humanized setting to enable immune-oncology studies.

Eva Oswald, Lea Grohne, Dorothee Lenhard, Loreen Weichert, **Julia B. Schueler**

Charles River Laboratories, Inc., Freiburg, Germany

The field of cancer immunology is rapidly moving towards immunomodulatory therapeutic strategies, including multi-specific antibodies, immune checkpoint inhibition, as well as potential vaccination strategies. Consequently, large biobanks of patient-derived tumor xenografts (PDX) models can serve as powerful tools. While one well-known major drawback of the PDX platform is the lack of an immunological competent host, the implantation of PDX models in humanized mice compensates for this. The focus of this project was to evaluate subcutaneous implanted PDX models of different tumor types in human immune system (HIS) reconstituted mice for presence of human immune cells. We determined the immune cell infiltration of more than 30 PDX models across different tumor types. PDX were implanted subcutaneous in 1-3 HIS mice per PDX, including models of lung, colon, breast and kidney cancer. Flow cytometry (FC) and immunohistochemistry (IHC) was performed to determine and characterize tumor infiltrating lymphocytes (TIL) in tumor tissue and human immune cells in hematopoietic organs of the mice. In addition, the expression of different surface markers related to immune-oncology pathways such as PDL-1 was determined by IHC in the tumor tissue. Overall, we confirmed good engraftment in the hematopoietic organs of the tumor bearing animals ranging from 40% - 80% in peripheral blood, spleen and bone marrow. The engraftment rate was not influence by the respective PDX model nor the donor of the CD34+ cells used for humanization. In contrast, the TIL rate was variable across the analyzed tumor panel and mainly driven by the tumor model ranging from 0.13% - 45% CD45+ cells. Tumors with a TIL rate >5% were defined as hot vs the cold tumors with a TIL rate < 5%. Up to now, there is no clear pattern of tumor types or molecular subtypes being specifically hot or cold. However, in the group of renal cancer an increased number of hot tumors was determined. The comparison with donor patient samples displayed a high correlation between TIL rate of donor patient tissue and PDX, emphasizing the tumor model as the main driver for this feature. The expression of PDL-1 was influenced by the presence of human immune cells: PDL1 expression was more than threefold upregulated in hot tumors, whereas it was downregulated in cold tumors although this effect was less pronounced. In continuation of the current study, we will examine more PDX models and expand the characterization to the corresponding tumoroids to provide in vitro and in vivo platforms that can robustly translate preclinical results of immunomodulatory drug testing into the clinic.

#1282 Development of a humanized multiple myeloma mouse model using bone-derived MM.1S cells.

Mari I. Suominen[1], Katja M. Fagerlund[1], Mervi Ristola[1], Justyna Zdrojewska[1], Yumei Diao[2], Kristina Witt-Mulder[2], Stefan Svensson Gelius[2], Jukka P. Rissanen[1], Jenni H. E. Maki-Jouppila[1]

[1]Pharmatest Services Ltd., Turku, Finland,[2]Oncopeptides AB, Stockholm, Sweden

Multiple myeloma (MM) is a hematological malignancy characterized by the uncontrolled proliferation of plasma cells in the bone marrow. The symptoms of MM patients include e.g. bone lesions, renal dysfunction, and anaemia. While advancements in treatment have improved outcomes for many patients, MM remains largely incurable. The development of preclinical models is crucial as they provide information on drug efficacy and mechanisms of action. 5 x 10^6 MM.1S cells tagged with GFP and luciferase (BPS Bioscience) were inoculated intravenously to NSG-Tg(huIL-15) mice (The Jackson Laboratory) and harvested for further *in vitro* cell culture at day 77. 20 x 10^6 cells of parental or bone-derived MM.1S cells were later inoculated intravenously to NSG-Tg(huIL-15) mice for comparison of the growth rate. Tumor growth was followed by weekly bioluminescence imaging. *In vivo* micro-CT imaging of the left tibia was performed in each group for evaluation of the bone lesions when the first mouse was sacrificed, at day 35 in the study group with bone-derived cells and at day 60 in the study group with parental cells. The animals with bone-derived cells were euthanized within study days 35-40 based on humane end point criteria. The study groups with parental cells were sacrificed at study day 73, when most mice were at humane end point. The bioluminescence signal was detected in skeletal sites (spine, pelvis, hind limbs, calvaria, ribs) and in soft tissues (spleen, brain, lungs, ovaries). Extensive osteolytic lesions were observed in all mice that had received the bone-derived cells, compared to 40% in the parental cell group. The bone-derived cell line was further characterized for cell surface marker expression. Both parental and bone-derived cells are positive for BCMA, CD138, and SLAMF7, which can be used for tumour cell identification in bone marrow. We conclude that the bone-derived MM cells induce disease progression in this mouse model with shorter study length compared to the parental cell line and that both cell lines express BCMA, CD138, and SLAMF7. The interaction between myeloma cells and their surrounding microenvironment in bone marrow plays a critical role in MM. The bone-derived MM.1S model can be combined with human NK cells or peripheral blood mononucleic cells (PBMCs) to study therapeutics affecting the interaction of cancer and immune cells.

#1283 Targeted genetic profiling of a de novo neuroendocrine patient-derived xenograft (PDX) and organoid model of prostate cancer from patient with African Ancestry.

Diana Rodriguez, Surendra Gulla, Tej Sharma, John Bodkin, Roberto Pili, John E. Tomaszewski, Remi M. Adelaiye-Ogala

Jacobs School of Medicine and Biomedical Sciences at the University at Buffalo, Buffalo, NY

Patient-derived xenograft (PDX) and organoid (PDO) models provide platforms to study prostate cancer (PCa) progression and therapy, especially in underrepresented populations. Here we report the first *de novo* neuroendocrine prostate cancer (NEPC) from a consented patient of African Ancestry with localized disease and no prior treatment before radical prostatectomy. African American patient had a pTN2 tumor, Gleason7 (4+3) and PSA or 3.6. Acquired tissue was collected for PDX and PDO establishment as well as for downstream molecular characterization. To uncover genetic drivers of aggressive phenotypes, we performed whole exome sequencing on DNA from the primary tumor and the established PDX using the Illumina Exome Kit and Twist Biosciences Exome Panel 2.0, followed by bioinformatic analysis. Computational workflows using Nextflow, including nf-core/sarek, identified somatic single nucleotide polymorphisms (SNPs), mutations (Mutect2, Strelka), and copy number variations (CNVs) (CNVkit, Control-FREEC). Variant annotation with Variant Effect Predictor and SnpEff linked variants to cancer progression. Analysis of the tumor samples revealed CNAs and mutations in genes similar to those seen in previously published NEPC. Protein expression by western blot and IHC identified altered protein expression peculiar to the NEPC phenotype further validating our primary tissue and its derived PDX models to have the NEPC phenotype. Furthermore, novel markers identified provide insight into molecular drivers of this prostate cancer phenotype and may inform targeted therapies for high-risk localized PCa.

#1285 PBMC-humanized, MHC-deficient NCG mice support growth of human tumor xenografts, do not develop rapid onset GvHD, and provide enhanced performance in oncology studies.

Steven Bronson[1], Kyle Draheim[2], Christopher Currie[2], Sydney Scatigno[2], Jenny Rowe[1], **David P. Harris**[2]

[1]Charles River Laboratories, Inc., Wilmington, MA,[2]Charles River Laboratories, Inc., Durham-RTP, NC

The application of new in vivo animal models with greater translational relevance is important for successful drug development. Severely immunodeficient mouse strains engineered for enhanced engraftment and persistence of diverse human immune cell types are now widely used in drug discovery across different therapeutic areas. These models allow for testing of immune modulating agents that target specific components of the human immune system, including both lymphoid and myeloid lineages. They have been particularly useful in drug development for oncology and for evaluating cell therapies such as TCR and CAR modified T cells. One approach commonly used to generate humanized mice involves engrafting peripheral blood mononuclear cells (PBMC) into immunodeficient mice. Since PBMC humanization results in efficient engraftment of hCD3+ T cells, xenogeneic GvHD (graft versus host disease) responses can occur with some donors. GvHD responses, which are characterized by progressive weight loss and a decline in animal well-being, can significantly compromise the results of studies in human tumor-bearing mice. In the studies reported here we compared outcomes in three NCG mouse strains. The parental NCG was modified to lack either B2-micoglobulin (NCG-B2m-KO mice deficient in the expression of mouse MHC class I molecules) or a double knock out of both mouse MHC class I and MHC class II molecules (NCG-MHC-dKO mice). RKO colorectal tumor cells were implanted subcutaneously in the three NCG mouse strains (parental NCG, NCG-B2m-KO and NCG-MHC-dKO) and 7 days later mice were engrafted with 10 million PBMCs obtained from two different donors, a Reference Donor and a Rapid Engrafter known to induce GVHD. The growth of RKO tumors and engraftment kinetics of donor PBMCs was evaluated. We observed consistent RKO tumor growth in non PBMC-treated NCG mice. HuPBMC NCG mice engrafted with the Rapid Engrafter showed clear signs of GvHD and reduced tumor growth. Importantly, there was a delay in the onset and significantly reduced severity of GvHD (by body weight loss or general clinical observations) in both MHC-modified strains. The engraftment rate of PBMCs was slightly delayed in both HuPBMC NCG-b2m-KO and NCG-MHC-dKO mice. The lack of GvHD provides a compelling rationale for the use of MHC deficient NCG strains in oncology tumor model studies and cell therapy studies.

#1286 Humanized avian xenograft models: advancing preclinical evaluation of immunotherapies and immune cell therapies with enhanced predictive accuracy.

Marjorie Lacourrege[1], **Romain Teinturier**[1], Paola Bonaventura[2], Clelia Costechareyre[1], Loraine Jarrosson[1], Camille Futelot[1], Mayrone Mongellaz[1], Stephane Depil[2], Celine Delloye-Bourgeois[1], Valerie Castellani[1]

[1]Oncofactory, Lyon, France,[2]ErVimmune, Lyon, France

Despite the success of immunotherapies and immune-cell therapies, clinical attrition remains high due to the limited predictive power of current preclinical models. This underscores the urgent need for innovative tumor-based platforms to facilitate the rapid and reliable evaluation of therapeutic efficacy. To address this challenge, we have developed advanced humanized avian xenograft models, leveraging our proprietary AVI-PDX™ technology. These models involve implanting human cancer cells into avian embryos, enabling the evaluation of therapeutic responses in a matter of days. Our humanized models, IMMUNO-AVI-PDX™ and IMMUNO-AVI-CellDX™, utilize two distinct approaches: (i) co-implantation of human peripheral blood mononuclear cells (PBMCs) and cancer cells, or (ii) implantation of cancer cells followed by intravenous injection of human PBMCs. Using a combination of whole embryo light-sheet microscopy, molecular marker analysis via immunolabelling of cryosections, and flow cytometry on microdissected tumors, we demonstrated efficient PBMC intake, robust immune cell survival, and minimal allogenicity. This approach successfully establishes tumor-immune interactions, with enhanced infiltration and persistence of human T cells, B cells, monocytes, and NK cells within tumors. We validated the functionality of our models by testing the anti-PD1 immune checkpoint inhibitor, pembrolizumab. Intravenous administration of the anti-PD1 monoclonal antibody (mAb) efficiently targeted PD1 receptors on T cells, resulting in their activation and subsequent tumor response. Next, we evaluated anti-PD1 efficacy in a panel of humanized AVI-PDX™ and AVI-CellDX™ models, including colorectal cancer (CRC), breast cancer, and melanoma. Using whole embryo light-sheet imaging and tumor volume quantification, we observed that our models successfully replicated the known response to anti-PD1, showing efficacy in sensitive cell lines and resistance in resistant ones. Notably, our CRC models revealed patient-specific heterogeneity in tumor responses, correlating with molecular features currently used to predict clinical outcomes. Finally, in collaboration with ErVimmune, we extended the application of our models to immune cell therapy by demonstrating the efficacy of a co-implanted HERV-specific T cell clone in an ovarian cancer IMMUNO-AVI-CellDX™ model. Together, these findings highlight the potential of our humanized avian xenograft models to advance the study of immune-based therapies and to identify predictive biomarkers for therapeutic efficacy.

#1287 Clinical relevance and translatability of NSCLC patient-derived xenograft (PDX) models for evaluating cMET-targeted therapies.

Nathlie Leger, Kayla Tyskiewicz, Hanna Koster, Paul Heverly, Mara Gilardi, Stefano Cairo, Frank Smith

Champions Oncology, Inc., Hackensack, NJ

Adoption of tyrosine kinase inhibitors (TKIs) has improved outcomes for Non-Small Cell Lung Cancer (NSCLC) by disrupting tumor-promoting growth factors, but patients develop resistance to these therapies through acquired alterations in other receptors. MET amplification is a common mechanism of resistance to FDA-approved small molecule EGFR inhibitors. Antibody Drug Conjugates (ADCs), such as c-MET targeting telisotuzumab-vedotin, are emerging as a promising therapy for EGFR inhibitor-progressed NSCLC, but understanding ADC efficacy across diverse profiles of advanced tumors remains a challenge. There is a need to develop more translational preclinical models to understand next-generation experimental agent efficacy prior to clinical trial enrollment. Here, we investigate the therapeutic potential of an experimental anti-cMET ADC in a panel of NSCLC low-passage patient-derived xenograft (PDX) models derived from patients pre-treated with EGFR therapies.10 NSCLC PDX models from Champions Oncology's tumor bank were established In Vivo from patient tumors and maintained at a low passage. Genomic and pharmacologic profiling was conducted on established models to characterize EGFR resistance. Models were then treated with 1 or 3 mg/ kg experimental agent every 4 days for 8 doses, with tumor volume and survival data collected over a 4-week treatment period. The experimental anti-cMET ADC demonstrated a range of tumor growth inhibition across the selected models, which is reflective of the heterogeneity seen in NSCLC patients in the clinic. Several cMET-amplified models showed significant tumor regression, while other cMET-amplified models exhibited more variability ranging from partial response to full resistance to treatment. The use of low-passage NSCLC PDX models is essential in preserving the clonal heterogeneity and molecular landscape of patient tumors, making them highly relevant for the preclinical evaluation of novel therapies. Notably, Champions low-passage models preserved the molecular and histological features of the original patient tumors, including mutations and copy number variations, which were critical for understanding the response to therapy. Our study highlights the translational potential of Champions PDX models, serving as a platform for investigating the efficacy and mechanisms of resistance to targeted therapies.

#1289 A preclinical platform of PDX breast cancer models and their cellular counterparts to identify resistance mechanisms and novel therapeutic options.

Emilie Indersie[1], Lea Sinayen[1], Aurore Gorse[1], Marie Tavernier[1], Enora Le Ven[1], Ester Morgado[2], Laurent Pouyet[2], Francois Romagne[2], **Olivier Deas**[1]

[1]XenTech, Evry-Courcouronnes, France,[2]MImAbs, Marseille, France

Despite significant advancements in understanding the biology and genetics of breast cancer, the development of effective therapies requires predictive and physiologically relevant preclinical models for their evaluation. In this context, patient-derived xenografts (PDX) have become a standard tool as they reproduce the biology of the original tumors in terms of histology, genotype, and response to chemotherapy. They have proven their relevance in studying pathways leading to cancer development and progression, mechanisms related to tumor resistance, and identifying new effective therapies. We offer a preclinical platform of over 60 fully characterized breast cancer PDX models and their in vitro cell derivatives for preclinical evaluation of new treatment modalities. Our platform consists of a PDX collection of 43 TNBC models, 9 ER+, 5 HER2+, 3 Luminal B (ER+ HER2+) models, and 14 cellular models derived from a subset of these PDX (PDXDC), representing the diversity of breast cancer. PDX models were obtained by transplanting post-operative tumor samples, either by grafting tumor fragments into the interscapular region of nude mice or by injecting dissociated tumor cells into the mammary fat pad of NOD-Scid mice. PDXDC were obtained from dissociated PDX tumors cultured under various media and matrix conditions. All models (PDX and PDXDC) were characterized at molecular level, including transcriptomic, whole exome sequencing, IHC marker staining and/or cytometry analysis. PDX were characterized pharmacologically for response to standards of care as a single agent or in combination. Our PDX and PDXDC panels primarily reflects the molecular heterogeneity of breast cancer and accurately reproduces the molecular and drug response profile of patient tumors. It is widely used for standard drug evaluations, but also for in vivo "mouse clinical trials" (MCT) which provide more predictive preclinical data on the efficacy of a single agent or drug combination. Additionally, we generated standard-of-care resistant models from our PDX panel to study the resistance mechanisms underlying breast cancer progression and to mimic what is observed in the clinic. Associated to in vitro cell derivatives, our collection provides a powerful preclinical platform for translational and preclinical research to identify mechanisms underlying acquired resistance to current therapies and develop novel treatment strategies against breast Cancers.

#1290 Mice humanized for the immune system as validated tool for therapeutic antibody development.

Lise Pasquet[1], Alice Marchand[1], Marion Mars[1], **Sophie Chabot**[1], Frederique Dol-Gleizes[1], Sebastien Tabruyn[2], Dan Georgess[2], Melanie Pichery[1], Thomas Eden[3], Welbeck Danquah[3], Gerhard Niederfellner[4], Pascale Lejeune[1]

[1]Translational Biology department, Evotec (France) SAS, Toulouse, France,[2]TransCure bioServices, Archamps, France,[3]Evotec International GmbH, Hamburg, Germany,[4]Evotec International GmbH, Gottingen, Germany

Therapeutic antibodies modulating the immune response are frequently specific for human proteins but fail to cross-react with the corresponding rodent homologs. Hence, their preclinical development requires suitable “humanized” mouse models, i.e. mice bearing a human immune system, so that immunotherapies can be evaluated in a more relevant immune context *in vivo*. Such “humanized” mice are developed by reconstituting the immune system of immunodeficient animals with human immune cells from 2 possible origins: $CD34^+$ hematopoietic stem cells ($huCD34^+$ mice) isolated from cord blood, or mature peripheral blood mononuclear cells prepared from buffy coats (huPBMC mice). According to the compound to be tested and the goal of the research, one model may be preferred over the other. $HuCD34^+$ mice exhibit multilineage immune populations allowing the evaluation of immune checkpoint inhibitors. We developed the human A375 melanoma model in $huCD34^+$ NCG mice to evaluate the combination strategy of relatlimab (anti-LAG-3 antibody) and nivolumab (anti-PD-1 antibody) approved at the beginning of 2024 as a first-line treatment for specific melanoma indications. Anti-PD-1 single treatment tended to, albeit without statistical significance, decrease tumor growth compared to isotype control group while anti-LAG-3 alone had no impact on tumor growth. The combination strategy induced a significant decrease in tumor growth compared to single agents in line with the data observed in the RELATIVITY-047 clinical trial (Tawbi H., NEJM, 2022). We also developed huPBMC mice that exhibit exclusively T-cell lineage and are suitable option to evaluate T-cell engagers like the CD3xEpCAM bispecific antibody, solitomab. We observed a dose-dependency between the dose of PBMC injected and the obtained level of human immune cell reconstitution. We observed that the level of reconstitution did not impact engraftment or tumor progression of the A375 melanoma or the BT474-clone5 breast cancer model, and conversely, that the tumor growth did not impact T-cell reconstitution. Finally, in the BT474-Clone5 model, we confirmed a dose-dependent efficacy of solitomab in controlling tumor growth. Moreover, solitomab efficacy was further potentiated by combination treatment with an Evotec proprietary co-stimulatory bispecific antibody. Mice humanized for the immune system exhibiting either multiple lineages (CD34+ huNCG) or just T-cell lineage (huPBMC) proved themselves suitable tools to assess non-mouse cross-reactive anti-tumor immunotherapy assets. Expertise in handling such models and deep knowledge in their benefits and limitations are required to selecting the most valuable model to perform preclinical studies predictive of human therapeutic response.

#1292 Innovative 3D ecm platforms for tumoroid development, drug screening, and personalized treatment.

Navneet Kaur[1], Dazhi Yang[2], Jamel Ali[1]

[1]Florida State University, Tallahassee, FL,[2]Acrogenic Technologies Inc, Rockville, MD

New advances in cancer research require robust *in vitro* and *in vivo* models that mimic the complexity of human tumors. Traditional 2D cell cultures lack the complexity of the tumor microenvironment (TME), leading to unreliable prediction of drug efficacy. Animal models are costly, time-intensive, and fail to fully replicate human tumor biology due to interspecies differences. To overcome these limitations, we present an innovative approach that involves TME-mimetic extracellular matrix (ECM)-based 3D platforms and co-culture systems for the development of tumoroids, circulating tumor cell (CTC) expansion, and PDX models. These 3D systems provide a physiologically relevant TME by mimicking the native cellular and extracellular interactions that drive tumor growth and progression. Tumoroids established through co-culturing a pool of primary cells dissociated from human solid tumors using a solid micro extracellular matrix (smECM) exhibited pathohistological properties, biomarker expression, and tissue architecture closely resembling the original tumors. Incorporating human foreskin fibroblasts (HFFs) in co-culture further enhanced tumor growth and stromal support, making these 3D models highly effective for drug screening and functional studies. Additionally, the use of smECM provided an innovative solution for expanding rare CTCs *ex vivo*. Unlike traditional methods, the 3D smECM platform supported the sustained viability and proliferation of CTCs, preserving their heterogeneity and allowing for rapid generation of CTC-seeded PDX models. These models demonstrated a high degree of clinical relevance by maintaining genomic profiles and chemoresistance characteristics of the original patient tumors. By integrating these advanced 3D platforms, we offer a powerful tool to develop versatile models for cancer research.

#1293 PMed-TRIAL database: an efficient and intelligent data integration platform shaping the future of drug development.

Xinlei Chen[1], Youbing Guo[1], Loc Van[2], Josh Caggiula[2], **Danyi Wen**[1]

[1]Shanghai LIDE Biotech Co., Ltd., Shanghai, China,[2]LIDE Biotech Co., Ltd., Doylestown, PA

With the rapid advancements in artificial intelligence (AI) and big data technologies, the fields of precision medicine and drug development have entered a transformative data-driven era. Leveraging years of accumulation and expertise, LIDE launched the PMed-TRIAL Database in 2023. Through continuous iteration and expansion, PMed-TRIAL has evolved into a comprehensive data integration and query platform, encompassing PDX (Patient-Derived Xenograft), CDX (Cell Line-Derived Xenograft), Syngeneic models, and Conditional Reprogramming Cell Lines. The platform enables efficient data retrieval and intelligent integration, providing indispensable support for clinical and drug research in this new era.

The database currently hosts nearly 1,800 PDX models, making it one of the largest PDX repositories worldwide. These models integrate and standardize a wide range of key datasets, including clinical information, treatment history, growth curves, SOC (standard of care), and H&E (hematoxylin and eosin staining) results, among others, which greatly facilitates the filtering and selection of suitable models for drug research. Furthermore, the associated whole-exome sequencing (WES) and RNA sequencing datasets provide critical evidence for drug mechanisms research. In addition to standard PDX models, the database also includes induced drug-resistance models, providing an essential resource for studying drug resistance mechanisms and screening drugs to overcome resistance.

In addition to PDX models, the PMed-TRIAL Database incorporates information on Patient/PDX-derived conditional reprograming cell lines, PDXO (PDX Derived Organoids), Syngeneic models, and CDX models. This expanded scope addresses the multi-faceted demands of drug development, accelerating research timelines and enhancing the efficiency of the drug discovery process.

Most importantly, the PMed-TRIAL Database standardizes and efficiently integrates diverse model datasets. The accumulation of large-scale, high-quality data not only streamlines current research efforts but also lays the groundwork for advancing AI applications in drug development in the future. By bridging innovative technologies with comprehensive data resources, PMed-TRIAL empowers researchers to unlock new possibilities in precision medicine and therapeutic innovation.

#1294 Primed human immune cell and cancer cell co-inoculated model for immuno-oncology drugs evaluation.

Bin Xie[1], Xin Hou[1], Pengfei Yang[1], Yanbing Zhou[1], Loc Van[2], Diandong Jiang[1], Josh Caggiula[2], **Danyi Wen**[1]

[1]Shanghai LIDE Biotech Co., Ltd., Shanghai, China,[2]LIDE Biotech Co., Ltd., Doylestown, PA

Systemic peripheral blood mononuclear cell (PBMC) humanized model is a straightforward tool to evaluate therapeutic immuno-oncology (IO) drugs, including immune checkpoint blockers (ICB) and bispecific antibodies (BsAb). However, the model has a limited dosing regimen window due to graft-versus-host disease (GVHD), as seen in most immune deficient recipients. There is also a relative lower responsible rate from insufficient antigen specific immune cells infiltration into tumor microenvironment (TME). Thus, application of this PBMC humanized model is restricted in evaluation of multiple ICBs and myeloid cell- *or* lymphocyte-mediated BsAbs. To overcome this, LIDE has developed a unique co-inoculation model with primed human PBMC (or/and its derived immune cell subsets) and cancer cells. Cancer-priming human PBMCs are well mixed with fresh cancer cells in MatriGel, co-inoculated into immune deficient mice to form an immune cell relatively warm tumor tissue for late immunotherapy. This model can significantly delay the graft-versus-host-reaction for another month compared with traditional systemic PBMC humanized model, and achieves maximal therapeutic effect of the testing drugs by pre-activating T cells with specific tumor antigens. Considering minimum innate immune cells in TME, dendritic cells or macrophages are expanded ex vivo, then transferred into this co-inoculated system to work together with T cells with a proper survival rate in the dosing period, mimic clinical biopsy complexity, to help determine the overall effect induced by integrated signals. It seems CD40 agonist or CD47 antagonist could well synergize with anti-PD(L)1 to control melanoma and triple negative breast cancer development, respectively. This optimized immune cell humanized cancer-bearing mice models have been tested for various cancer targets, e.g., PD(L)1, Tim3, Lag3, Tigit, PVRIG, TGFβ1, CD73/CD39, CD47/Sirpa, DLL3/CD3, Claudin18.2/4-1BB/CD3, B7H3/CD3/ADC, GPC3/CD3, GPRC5D/CD3, CSF1R, GITR, (Pro)IL2/IL15, to better understand the interaction and modulation between cancer cells and immune cells.

#1295 A translational approach to ADC evaluation: preclinical single-mouse basket trial with Bayesian modelling analysis.

Diandong Jiang[1], Yuan Ji[2], Jiaxin Liu[2], Bin Xie[1], Hongkui Chen[1], Shizhu Zhao[1], Le Li[1], Yu Di[1], Xinlei Chen[1], Xiaorong Gu[1], Huanhuan Zhang[1], Zhenle Bi[1], Kaimeng Hu[1], Loc Van[3], Josh Caggiula[3], Yulan Tang[1], **Danyi Wen**[1]

[1]Shanghai LIDE Biotech Co., Ltd., Shanghai, China,[2]Bayesoft, Shanghai, China,[3]LIDE Biotech Co., Ltd., Dolyestown, PA

Introduction: Antibody-drug conjugates (ADCs) have revolutionized cancer therapy, exemplified by the clinical success of HER2-targeting ADCs like Trastuzumab-Deruxtecan (T-Dxd), which demonstrates efficacy across a spectrum of HER2 expression levels. However, ADC development remains challenging due to translational gaps between preclinical and clinical findings. This study introduces a robust translational approach for ADC evaluation, combining a single-mouse preclinical model using MiniPDX® technology with Bayesian statistical analysis—a widely recognized and commonly applied statistical approach in clinical research. Bayesian analysis offers the advantage of integrating prior knowledge with experimental data to generate probabilistic predictions, providing a dynamic and nuanced understanding of treatment efficacy. Our study mimics clinical trials of T-Dxd and Trastuzumab-Emtansine (T-DM1) using this robust, translational framework to bridge the gap between preclinical and clinical evaluation.

Methods: We selected 20 PDX models over six cancer types, representing HER2 expression levels (high, medium, low, and negative control). Tumor tissues were dissociated into single-cell suspensions and implanted subcutaneously in mice via MiniPDX capsules. Each mouse received a single 10 mg/kg dose of T-Dxd or T-DM1 over 10 days. Tumor cell viability was assessed using CellTiter-Glo, and tumor relative proliferation rate was calculated as Treatment/Control ratios (T/C%). A T/C ratio <50% indicated significant response. Bayesian modelling was applied to estimate the probability of tumor inhibition across HER2 cohorts.

Results: T-Dxd demonstrated a 100% overall response rate (ORR) in HER2-high models, with significant tumor inhibition. Additionally, T-Dxd exhibited substantial efficacy in HER2-medium (ORR 75%) and HER2-low (ORR 60%) groups. In contrast, T-DM1 displayed strong tumor inhibition in HER2-high models but limited efficacy in HER2-medium and HER2-low groups. In addition, Bayesian analysis yielded posterior probabilities of 83%, 66%, and 54% that T-Dxd slows down tumor growth by 50% when compared to control for the HER2-high, HER2-medium, and HER2-low groups, respectively.

Conclusion: Our study demonstrates the feasibility and promising results of preclinical single-mouse trials using MiniPDX®. The Bayesian statistical analysis provides a rich summary of the effects of T-Dxd and T-DM1. This approach bridges the translational gap, offering a platform to predict ADC efficacy and guide candidate selection in the preclinical setting.

#1296 Development of a novel NCG-hIL15-FcγR-KO model for precise drug efficacy evaluation.

Hongyan Sun[1], Jun Xing[1], Jianming Xu[1], Shiying Guo[1], Xiang Gao[1], Jun Wang[2]

[1]GemPharmatech Co., Ltd, Nanjing, China,[2]GemPharmatech Co., Ltd, San Diego, CA

Humanized mice, particularly immunodeficiency mice reconstituted with human immune systems by the transplantation of human hematopoietic stem cells (HSCs) or human peripheral blood mononuclear cells (PBMCs), have been widely used in tumor immunology. The NCG-hIL15 model has been developed to reconstitute both human T and natural killer (NK) cells, which allows the study of immune checkpoint inhibitors (ICIs) and antibody-dependent cellular cytotoxicity (ADCC)-dependent drugs. However, the remaining murine cells from the myeloid compartment can interfere with the mAb-based drug clearance and efficacy. To precisely evaluate the function of mAb-based drugs, we have successfully developed the NCG-hIL15-FcγR-KO model by disrupting the murine FcγRI, FcγRII, FcγRIII, and FcγRIV genes in NCG-hIL15 mice, resulting in the absence of murine FcγRs. In this model, the interference from mouse FcγRs to induce ADCC was minimal.

Compared to the parental NCG-hIL15 mice, NCG-hIL15-FcγR-KO mice can reconstitute comparable levels of human NK and T cells post-HSC transplantation. Both NCG-hIL15-FcγR-KO and NCG-hIL15 mice showed stable body weight changes with a long survival period. In our study, we found that rituximab could inhibit tumor growth through murine innate cells in the absence of human NK cells in NCG-hIL15 mice bearing Raji tumors, obscuring the interpretation of the results, while it had no inhibitory effect on tumors in NCG-hIL15-FcγR-KO mice bearing Raji tumors. Therefore it was suggested that the NCG-hIL15-FcγR-KO model was able to avoid false positives of ADCC drugs and was able to accurately evaluate the true drug efficacy. Furthermore, compared with the PBMC-NCG-hIL15 mice, nivolumab was able to significantly inhibit the growth of tumors in PBMC-NCG-hIL15-FcγR-KO mice bearing NCI-H1975 tumors. This demonstrated that NCG-hIL15-FcγR-KO mice may provide a good platform to study anti-tumor T cell responses in the absence of murine FcγRs.

Taken together, the NCG-hIL15-FcγR-KO model is the ideal model to accurately reflect the true efficacy. We present this model as a valuable addition to the preclinical research toolkit, promising a deeper understanding of efficacy evaluation for clinical applications.

#1297 Novel mouse models to study bone and brain metastasis.

Hongyan Sun[1], Xue Wu[1], Fang Zhu[1], Jianming Xu[1], Xiang Gao[1], Jun Wang[2]

[1]GemPharmatech Co., Ltd, Nanjing, China,[2]GemPharmatech Co., Ltd, San Diego, CA

Metastasis refers to the dissemination of cancer cells from the primary (original) tumor to distant areas of the body, where they give rise to secondary and more aggressive cancers. This process constitutes a crucial aspect of cancer progression and is frequently linked with poor prognosis. Many cancer patients progress to metastasis at a later stage of tumor development before proper treatment is available. Hence, inhibiting metastasis is an important focus of cancer research. Common tissues where cancer metastasizes include the lung, liver, bone, and brain. Anti-metastatic drugs consist of antibody drugs, small molecule inhibitors, peptides and nucleic acids. According to the different mechanisms of action, they can be categorized into angiogenic factor inhibitors, cell adhesion molecule antagonists, metalloproteinase inhibitors, cellular messenger regulators and so on. Despite significant progress in the therapeutic strategy for treating cancer metastasis, several limitations such as drug resistance and limited druggable pathways remain unsolved.

To better understand the underlying mechanism and support the discovery of novel biomarkers in cancer metastasis, we have developed robust mouse models for bone and brain metastasis. MDA-MB-231-Luc cell line was injected into the left ventricle of the mouse heart using an ultrasound-guided intracardiac injection technique. Through multiple rounds of *in vivo* and *in vitro* screening, we have successfully obtained MDA-MB-231-Luc subclone cell line, which can be colonized in the spine and lower limb bones, recapitulating bone metastasis in clinic. In another approach, we have successfully established a brain metastasis model by injecting the lung cancer cell line LLC1-Luc into the common carotid artery of mice via carotid artery injection. *In vivo* imaging analysis confirmed cancer cells colonization and proliferation in the correspondence tissue.

In conclusion, we have developed preclinical mouse models which recapitulated bone and brain metastasis observed for cancer patients in clinics. Those models can be valuable tools to study metastasis mechanisms as well as for the biomarker discovery.

TUMOR BIOLOGY: Model Systems for Metastasis
Poster Session

#1301 Multiple-channel hybrid neural network to achieve quantitative bioluminescence tomography for cancer research.

B. Deng[1], Z. Tong[1], Y.-c. Tsai[1], J. Kim[1], H. Dehghani[2], **K.-H. Wang**[1];
[1]UT Southwestern Medical Center, Dallas, TX, [2]University of Birmingham, Edgbaston, Birmingham, United Kingdom

Purpose: Bioluminescence tomography(BLT) enhances commonly used 2D bioluminescence imaging by reconstructing 3D distributions of bioluminescence activity, allowing in vivo tumor localization. However, traditional physics model-based BLT struggles with model uncertainties and ill-posedness, limiting its ability to provide quantitative information, such as tumor volume. To address this, we introduce the Multiple-channel Hybrid Neural Network(McHyNN), a machine learning(ML) framework integrating light propagation model, multimodal imaging, and cross-animal data. We demonstrate McHyNN's ability to accurately identify tumors and quantify their volumes, particularly in cases of small and multiple brain metastases(BM), making it highly valuable for cancer research.
Method: McHyNN is a feed-forward neural network(NN) grounded in a light propagation model based on the diffusion equation. It incorporates multi-data channels, including multi-animal and multimodal data. For a given animal set, the data includes BL images emitted from internal tumors and corresponding MRI scan, if tumor is MRI-detectable. The NN outputs n+1 BL tumor distributions, including the *n* existing animal sets and the new subject, whose tumor information is to be determined. McHyNN leverages mutual information from light propagation across multiple animals and multimodal images, and the NN is optimized accordingly using loss function $L = L_{diff} + L_{image}$. L_{diff} and L_{image} minimize the difference between calculated and actual BL tumor distributions based on surface BLI measurement, and MRI data, respectively. We will first present numerical results based on simulated brain metastases in mice with experimentally determined noise, followed by showing the capability of BLT to reconstruct in vivo BMs in SCLC spontaneous animal model.
Results: McHyNN accurately quantifies three small BMs (1.2 to 1.9 mm in length, volume: 0.9 to 1.9 mm^3) within simulated mouse brain at localization accuracy of < 0.5mm and volume errors within 0.8 mm^3, even when the tumors are separated by only 2-2.7 mm. In contrast, the compressive sensing algorithm commonly used in conventional BLT fails to distinguish these tumors. Moreover, we simulated single brain metastasis ranging from 1.0-3.6 mm in length, 0.4-17.2 mm^3 including 10 tumors 1.5<mm or="" 1.75="" mm3. McHyNN successfully localized all cases within 0.5 mm and faithfully reconstructed tumor volumes, showing a linear correlation with ground truth (R^2=0.99). The in vivo BMs study is ongoing. <mm or="" 1.75="" mm
<mm or="" 1.75="" mmConclusions: Our BLT platform incorporating the McHyNN approach has potential to transform qualitative bioluminescence imaging into a 3D quantitative modality, particularly for tumor volume quantification and detecting small malignancies. This innovation will significantly enhance investigators' capabilities in cancer studies.

#1302 A practical method of delivering induced electric field (iEF) therapy.

J. West, C. Barron, **V. Subramaniam**;
EMBioSys Inc., North Andover, MA

Among non-pharmacological treatments, induced electric field (iEF) therapy is the only bioelectric approach that is truly contact-free and non-invasive. In iEF treatment, the permeability of magnetic fields varying in time is exploited to induce electric fields inside cells and tissues without the need for electrodes or adhesive contact with the skin. Induced electric fields are a non-ionizing form of electromagnetic radiation demonstrated to have strong anti-metastasis and anti-tumor activity *in vitro* and *in vivo* against triple negative breast cancer (TNBC), including significant host immune response in a pre-clinical animal model. Moreover, combination treatment of MDA-MB-231 and MDA-MB-468 cells with standard of care chemotherapeutic agents (paclitaxel, capecitabine) plus iEF *in vitro*, significantly improve their anti-viability and anti-proliferation activity (half-maximal inhibitory concentrations or IC_{50} values decreased by ~50% for both paclitaxel+iEF and capecitabine+iEF treatments). EMBioSys has developed technology translating iEF therapy into clinical treatment and delivered in the form of wearable, washable garments such as camisoles. Special conducting threads integrated into the camisole enable a current to be driven through the insulated threads at voltages less than 10 Volts. The time-varying current generates a temporally varying magnetic induction within the space enclosed by the camisole and generates iEFs by Faraday's law. We measured the magnetic inductions, compared with their calculated values, and determined iEF distributions within the space enclosed by the garment. We find that iEF intensities found to have anti-metastasis and anti-tumor effects in animal models of TNBC can be easily attained at human anatomical scales and delivered in a practical manner for use by patients. Our work lays the foundation for first in human studies by extending a treatment developed in the laboratory into a wearable and washable treatment device for cancer patients.

#1303 Tunable ECM interfaces in microfluidic chips for interfacial metastatic studies of breast cancer spheroids.

Hsiang-Pei Chen[1], Hwanseok Jang[2], Seung-Cheol Shin[2], Seok Chung[2]

[1]National Cheng Kung University, Tainan City, Taiwan,[2]Korea University, Seoul, Korea, Republic of

Background: Metastasis remains one of the most challenging aspects of cancer progression to study. Recent research highlights that the physical characteristics of the extracellular matrix (ECM)—such as stiffness, porosity, and microarchitecture—are critical regulators of cancer invasion and metastasis. Tumor cells often encounter heterogeneous microenvironments, including matrix-matrix interfaces (MMIs), which provide unique interfacial cues influencing invasion and dissemination. Despite growing recognition of these interfacial dynamics, the mechanisms by which MMIs modulate metastatic behaviors remain poorly understood. To address this, we developed a microfluidic chip with tunable ECM interfaces, enabling precise modulation of stiffness and structural heterogeneity. This platform facilitates the study of interfacial metastatic behaviors of breast cancer spheroids (BCS) in a controlled and reproducible in vitro setting.
Methods: A tunable microfluidic chip was fabricated using polydimethylsiloxane (PDMS) via photolithography. The chip comprises three distinct channels: a central channel engineered to create defined MMIs with combinations of soft and stiff ECM regions, and two lateral channels designed for nutrient supply. Collagen type I hydrogels were employed as the ECM material, and their physical properties—stiffness and microarchitecture—were characterized using rheometry and scanning electron microscopy (SEM). MDA-MB-231 and MCF7 BCSs were placed near the MMIs formed by juxtaposing ECMs of varying stiffness to model interfacial metastatic behaviors. Spheroid dispersion, invasion, and single-cell migration were observed and recorded using bright-field microscopy after 24 and 48 hours of incubation.
Results: The stiffness of the soft and stiff ECMs measured 5.18 ± 0.19 MPa and 6.58 ± 1.20 MPa, respectively, with porosity differing significantly between the inner regions of soft (6.56 ± 0.22) and stiff (2.71 ± 0.10) ECMs. In homogeneous environments, MDA-MB-231 spheroids exhibited enhanced dispersion and invasive behavior in soft ECM compared to stiff ECM. At MMIs, the presence of a stiff interface further enhanced MDA-MB-231 spheroid invasiveness, compared to soft-soft or homogeneous ECM conditions. In contrast, MCF7 spheroids showed minimal migration across all MMI conditions.
Conclusion: This study underscores the importance of tunable ECM interfaces in understanding the role of physical and structural heterogeneity on the invasive behaviors of BCSs. The results demonstrate how MMIs modulate metastatic dynamics, particularly in response to stiffness variations, and highlight the utility of microfluidic platforms in investigating the interplay between mechanical and biochemical microenvironments.

#1304 A novel organoid-based model to study pediatric tumor metastasis to the lung.

Martha Magdalena Zylka[1], Christoph Hafemeister[1], Lukas Watzke[2], Ulrike Mann[3], Didier Surdez[4], E. Alejandro Sweet-Cordero[5], Bernadette Liegl-Atzwanger[6], Matthias Dettmer[7], Femke Ringnalda[8], Marc van de Wetering[8], Karin Sanders[8], Matthias Farlik[3], Florian Halbritter[1], Martin Metzelder[9], Heinrich Kovar[1], Branka Radic Sarikas[1]

[1]St. Anna Children's Cancer Research Institute, Vienna, Austria,[2]Department of Pathology, Medical University of Vienna, Vienna, Austria,[3]Department of Dermatology, Medical University of Vienna, Vienna, Austria,[4]Faculty of Medicine, Balgrist University Hospital, Zurich, Switzerland,[5]Division of Pediatric Oncology, Department of Pediatrics, University of California San Francisco, San Francisco, CA,[6]Diagnostic and Research Institute of Pathology, Medical University of Graz, Graz, Austria,[7]Institute of Pathology, Klinikum Stuttgart, Stuttgart, Germany,[8]The Princess Maxima Center for Pediatric Oncology, Utrecht, Netherlands,[9]Department of Pediatric Surgery, Medical University of Vienna, Vienna, Austria

Metastasis is the primary cause of mortality in pediatric cancer, with the lung being a common site for secondary tumor growth. A major challenge in treatment is the lack of models that accurately replicate the interactions between tumors and the lung metastatic niche observed in patients. This limitation hinders the investigation of the molecular mechanisms driving lung metastasis in pediatric solid tumors. Furthermore, it impedes the development and testing of potential inhibitors to prevent or treat lung metastases, ultimately delaying progress in improving patient outcomes. We have developed an advanced *in vitro* lung metastasis model using tumor-lung organoid co-cultures. To this end, we first established lung organoids from healthy pediatric lung tissue and characterized them based on the presence of airway, alveolar, and other rare lung epithelial subtypes. Next, we analyzed primary and PDX-derived cells from two types of tumors that frequently metastasize to the lungs: Ewing sarcoma (ES) and osteosarcoma (OS). Our co-culture system revealed two groups of tumor cells with distinct behaviors: The first group of tumor cells actively infiltrated the lung organoids, while the second remained closely associated without invasion. We hypothesized that this behavior *in vitro* corresponds to differences in metastatic potential *in vivo*. In line with this hypothesis, scRNA-seq analysis confirmed a high expression of EMP1, a key driver of metastatic behavior, in infiltrative cells. In contrast, non-infiltrative cells showed no change in EMP1 expression between co- and mono-culture conditions, highlighting EMP1’s specific association with tumor cell infiltration. Furthermore, we noticed phenotypic changes in lung organoids in response to specific tumor co-cultures, suggesting a reactive response to infiltrating tumor cells. Using scRNA-seq, we are currently exploring the underlying molecular mechanisms driving these changes, characterizing overall gene expression changes and signaling pathways. Experiments with conditioned media revealed that lung-organoid secreted factors attract ES and OS cells. Further analysis of the lung organoid secretome identified paracrine signals that direct tumor cell homing, making our model a powerful tool for studying and targeting lung-specific tumor attraction. To further assess the model’s relevance to lung metastases in patients, we performed spatial transcriptomics analysis (10x Visium platform) on paired primary tumors and lung metastases from ES and OS patients. This analysis will both inform the refinement of our organoid model and enhance our understanding of lung metastatic niche spatial organization.In conclusion, our study provides a framework for understanding pediatric tumor lung metastasis and establishes a versatile platform for testing pathway-specific anti-metastatic therapies targeting tumor-lung interactions.

#1305 Brief pulses of high-level fluid shear stress enhance metastatic potential and rapidly alters the metabolism of cancer cells..

Amanda N. Pope[1], Devon L. Moose[1], Sujata Birua[1], Guy O. Hudson[1], Hank Weresh[1], Patrick Breheny[2], **Michael D. Henry**[1]

[1]Molecular Physiology and Biophysics, Univ. of Iowa Holden Comprehensive Cancer Ctr., Iowa City, IA,[2]Biostatistics, Univ. of Iowa College of Public Health, Iowa City, IA

During their journey through the circulation, circulating tumor cells (CTCs) face challenges to their survival including mechanical stresses related to their dynamic fluid microenvironment and oxidative stress related to their detached cell state. Recently, we identified a mechano-adaptive response to transient fluid shear stress (FSS) exposure that enables cancer cells to survive mechanical destruction in the circulation and found that it involves an increase in the activation of RhoA-actomyosin signaling (Moose et al., Cancer Cell 2020). Because RhoA is likely to influence other phenotypes involved in metastasis, such as invasiveness, we hypothesized that activation of RhoA by FSS may promote metastatic behavior in CTCs. Here we find that cancer cells (PC-3, MDA-MB-231, Myc-CaP) exposed to brief pulses of high-level FSS (~1msec, τ =750-6400 dynes/cm^2) exhibit enhanced invasiveness (dependent on RhoA) and anchorage-independent proliferation *in vitro* and enhanced metastatic colonization/tumor formation *in vivo* as compared to static controls. To investigate how exposure to FSS confers these phenotypes, we conducted transcriptomic and metabolomic profiling. Transcriptomic analysis at 3, 6, and 24h post FSS exposure revealed enrichment of gene expression involved in oxidative phosphorylation. Consistent with this, we found elevated oxygen consumption of cells exposed to FSS at 24h. Metabolomic profiling of cells immediately after exposure to FSS demonstrated rapid energy utilization consistent with actin remodeling and contractility as well as depletion of amino acids acids associated with the folate pathway. Cells exposed to FSS exhibit a reduced reactive oxygen species burden as compared to static controls. Consistent with the hypothesis that FSS-dependent activation of the folate pathway reduces oxidative burden in cells exposed to FSS, we found that exposure of cancer cells with the folate inhibitor methotrexate, sensitized those cells destruction by FSS. Taken together our data indicate that cancer cells that survive exposure brief pulses of FSS are primed for metastatic colonization in part through rapid metabolic programming that enables them to withstand oxidative stress associated with cell detachment. Thus, some CTCs may derive an unexpected survival benefit through encountering FSS in the circulation.

#1307 Pericytes promote colorectal cancer metastasis by remodeling perivascular matrix.

Xiaobo Li[1], Jinghua Pan[2], Qun Miao[1], Xiaoyu Wu[1], Zhan Zhao[2], Yihai Cao[3], Minfeng Chen[1], Dongmei Zhang[1], Wencai Ye[1]

[1]State Key Laboratory of Bioactive Molecules and Druggability Assessment, Jinan University, Guangzhou, China,[2]The First Affiliated Hospital of Jinan University, Guangzhou, China,[3]Department of Microbiology, Karolinska Institute, Stockholm, Sweden

Backgrounds: Hematogenous dissemination is a prevalent route of colorectal cancer (CRC) metastasis, whereas the underlying mechanism is unclear. Tumor pericytes (TPCs) are important components of tumor vessels, which are attached to the endothelial cells and embedded within the walls of capillaries. However, as the gatekeeper of vessels, the role of TPCs in hematogenous metastasis remains largely unknown. Here, we aimed to investigate the heterogeneity of TPCs and their effects on CRC metastasis.
Methods: TPCs were first isolated from the primary CRC tissues using microdissection combined with pericyte medium-based approach. Then, TPCs derived from CRC liver-metastatic (CRCLM) and CRC non-metastatic (CRCNM) patients were analyzed by single-cell RNA sequencing (scRNA-seq). Clinical CRC specimens were collected to analyze the association between the molecular profiling of TPCs and CRC metastasis. The mechanisms by which TCF21 regulated perivascular matrix remodeling and integrin α5 modulated *TCF21* DNA hypermethylation were investigated using RNA-sequencing, chromatin immunoprecipitation-sequencing and bisulfite-sequencing. Pericyte-conditional *Tcf21*-knockout mice were constructed to investigate the effects of TCF21 in TPCs on CRC metastasis. Masson staining, atomic force microscopy, second-harmonic generation and two-photon fluorescence microscopy were employed to observe perivascular extracellular matrix (ECM) remodeling. Pericyte TCF21-targeting peptide was designed by AlphaFold and its mechanisms were examined by microscale thermophoresis.
Results: Thirteen TPC subpopulations were identified by scRNA-seq. A novel subpopulation of $TCF21^{high}$ TPCs was found to bed associated with liver metastasis in CRC patients. Genes highly-expressed in $TCF21^{high}$ TPCs were enriched in ECM and ECM remodeling, thus termed as matrix pericytes. TCF21 in TPCs increased perivascular ECM stiffness, collagen rearrangement and basement membrane degradation, establishing a perivascular metastatic microenvironment to instigate CRC liver metastasis. Mechanistic studies indicated that loss of integrin α5 inhibited the FAK/PI3K/AKT/DNMT1 axis to impair *TCF21* DNA hypermethylation in $TCF21^{high}$ TPCs. Conditional deletion of *Tcf21* in TPCs or pharmacological blocking the transcriptional activity of TCF21 in TPCs could mitigate perivascular ECM remodeling and CRC liver metastasis.
Conclusions: This study first uncovers the heterogeneity of TPCs during CRC liver metastasis and reveals a novel mechanism that TPCs drive CRC liver metastasis through remodeling perivascular matrix, which provides a potential diagnostic marker and therapeutic target for CRC metastasis.

#1308 Protein profiling of breast cancer cell-derived extracellular vesicles in breast to bone metastasis.

Guan Huang, Golnaz Morad, Marsha A. Moses

Surgery, Vascular Biology Program, Boston Children's Hospital and Harvard Medical School, Boston, MA

Breast cancer is the most common cancer among women worldwide, with bone being one of the most common sites for breast cancer metastasis. Despite improvements in systemic therapies for breast cancer, the mechanisms underlying breast cancer metastasis to bone remain incompletely understood thereby limiting the development of effective therapeutic options. Emerging evidence suggests that extracellular vesicles (EVs), nanosized particles secreted by cells including cancer cells, play a pivotal role in organ-specific metastasis. However, relatively little is known regarding the contribution of breast cancer cell-derived EVs to bone metastasis. To begin to investigate the potential role(s) of breast cancer cell-derived EVs in breast to bone metastasis, we first analyzed the protein profiles of EVs derived from parental MDA-MB-231 breast cancer cells (P-EVs) and from bone-seeking MDA-MB-231 breast cancer cells (B-EVs) using iTRAQ (isobaric Tags for Relative and Absolute Quantification) quantitative mass spectrometry. Among a total of 126 identified proteins, 13 were upregulated in B-EVs, 8 were upregulated in P-EVs and 105 were shared by both EVs. Gene Ontology (GO) enrichment analyses and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses identified three key biological processes of interest: osteoclastogenesis, EV-cell attachment and bone extracellular matrix-receptor interactions which may contribute to bone-tropism and pre-metastatic niche preparation. Importantly, confocal microscopy studies demonstrated that B-EVs are preferentially internalized by osteoclast precursor cells. This finding was further confirmed by quantitative flow cytometry which demonstrated significantly higher fluorescent signal intensities for B-EVs compared to P-EVs in these osteoclast precursor cells also suggesting enhanced uptake specificity. Moreover, we found that B-EVs can induce the differentiation of osteoclast precursors into multinucleated osteoclasts in long-term *in vitro* studies as confirmed by tartrate-resistant acid phosphatase staining, indicating that EVs derived from a bone-seeking subpopulation of breast cancer cells may promote osteoclastogenesis, a process critical for bone resorption. This, in turn, may contribute to the development of a pre-metastatic microenvironment for bone metastasis. Taken together, these studies begin to enhance our understanding of the role of breast cancer cell-derived EVs in breast to bone metastasis and may ultimately offer potential avenues for therapeutic intervention and early detection of bone metastasis.

(This work was supported by the Breast Cancer Research Foundation, NIH R21 CA253051-01 and the Nile Albright Research Foundation. The authors acknowledge the gift of the MDA-MB-231 parental cell line and its bone-seeking variant from the Massagué Lab.)

#1309 Decellularized extracellular matrix hydrogel for esophageal cancer model.

Yunqing Kevin Kang

Department of Biomedical Engineering, Florida Atlantic University, Boca Raton, FL

Esophageal cancer is currently the sixth most common cancer worldwide and one of the most lethal cancers. The lack of physiologically relevant human esophageal cancer models has hindered the progress of developing effective strategies against esophageal cancer. To address the issue, we developed a decellularized esophageal matrix derived hydrogel. We then characterized the physicochemical properties of the decellularized extracellular matrix (dECM) hydrogel. To bioengineer the cancer model using the dECM hydrogel, we seeded esophageal squamous cell carcinoma KYSE30 on the dECM hydrogel and in the dECM hydrogel. They grew well and spread out in/on the dECM hydrogel. We also casted KYSE30-laden dECM hydrogel on a side of a well to see if KYSE30 will migrate out of the hydrogel. After culturing 3 days, we found that KYSE30 cells grew and started to migrate out of the dECM hydrogel, and migrated more after day 5. We counted the number of migrated cells. We found that cell migrated more at day 5. We then quantified cell proliferation of KYSE30 in the dECM hydrogel. We found that KYSE30 cells proliferated in the dECM hydrogel with time. These findings bring new potential to using the decellularized matrix derived hydrogel for future experiments in studying cancer behavior and practical applications for esophageal cancer models.

#1310 Targeting pro-metastatic stress networks in triple-negative breast cancer using patient-derived models.

Margarite D. Matossian[1], Ali Yesilkanal[1], Michael East[2], Denis Okumu[2], Geetha P. Yerradoddi[1], Rita Nanda[3], Gary Johnson[2], Marsha R. Rosner[1]

[1]The University of Chicago, Chicago, IL,[2]Department of Pharmacology, The University of North Carolina, Chicago, IL,[3]Department of Medicine, The University of Chicago Medicine Comprehensive Cancer Center, Chicago, IL

Metastatic triple-negative breast cancer (mTNBC) remains one of the most challenging forms of breast cancer to treat due to profound heterogeneity; chemotherapy-based regimens continue to be the mainstay of treatment. Kinases are attractive therapeutic targets, but while kinase inhibitors have improved outcomes in many metastatic solid cancers including HER2/Neu-amplified and kinase-driven hormone receptor positive breast cancers kinase inhibitors have not shown durable efficacy in mTNBC and many have failed in clinical trials. Furthermore, the high doses of kinase inhibitors to achieve cytotoxicity have unacceptable side effects for patients limiting real-world use. Preclinical research and secondary analyses from clinical trial data from our group and others demonstrate important differences in how kinase signaling networks, and not individual pathways, drive TNBC metastatic transformation compared to other breast cancer subtypes. Chronic stress states such as those initiated by hypoxia, poor nutrient delivery and higher metabolic demands promote pro-metastatic transcriptional programs in TNBC. Our research focuses on identifying and targeting regulators of the chronic stress response to suppress TNBC metastasis. Our previous work showed that activation of the physiologic metastasis suppressor RKIP effectively reduced TNBC metastasis in cell line models through inhibition of stress MAPK networks (p38, JNK, ERK1/2, MLK) resulting in decreased transcription of the pro-metastatic transcription factor BACH1. We demonstrated that low dose inhibition of multiple kinase nodes of networks, rather than single kinase nodes, more effectively reduces oncogenic signaling output and prevents compensatory signaling activation. Here we take the next step to translate this concept into the clinic. We show that combinations of kinase inhibitors used in early phase clinical trials (Ralimetinib (p38), JNK-in-8 (JNK) or FDA approved (Trametinib (MEK & ERK1/2)) can inhibit stress kinase networks, pro-motility gene expression and TNBC cell migration. Using patient-derived organoids that recapitulate heterogeneous TNBC molecular characteristics we successfully generated stable BACH1-knockout TNBC organoids. We utilized mass spectrometry-based techniques with a kinase inhibitor system (MIB-MS) and proteomics to characterize the activation state of kinase signaling networks in organoids and are investigating the effect of metastatic inhibitors on these pathways. Inhibition of these stress kinase networks should enable effective reprogramming of metastatic or high-risk cancers to more benign states and make tumors more susceptible to approved anti-proliferative therapies such as chemotherapy or immunotherapy while limiting toxicities from kinase inhibitors using a low-dose, multi-drug combination approach.

#1311 CREB-driven tumor cell invasion and ECM remodeling promote metastasis.

Sudhakar Jinka[1], Siddharth Mehra[1], Varunkumar Krishnamoorthy[1], Andrew M. Adams[1], Anna Bianchi[1], Luis N. Alberto[1], Yuguang Ban[2], Jashodeep Datta[1], Nipun Merchant[1], Nagaraj Nagathihalli[1]

[1]Department of Surgery, University of Miami Miller School of Medicine, Sylvester Comprehensive Cancer Center, Miami, FL,[2]Department of Public Health Sciences, University of Miami Miller School of Medicine, Sylvester Comprehensive Cancer Center, Miami, FL

Background: Pancreatic ductal adenocarcinoma (PDAC) is projected to become a leading cause of cancer-related mortality, driven by its late-stage diagnosis, aggressive progression, and propensity for metastasis. Currently, no approved therapeutic effectively targets PDAC metastasis. Emerging evidence implicates extracellular matrix (ECM), and cytoskeletal dynamics are key to invasion, a pertinent contributor to PDAC metastasis. Our previous studies identified cAMP-response element binding protein 1 (CREB) as a key regulator of ECM alterations in PDAC. This study investigates the role of CREB-ROCK in regulating tumor cell invasion and metastasis at the molecular level.

Methods: Expression profiles of *CREB* and invasion-related genes were analyzed using The Cancer Genome Atlas (TCGA) PDAC patient's dataset. CREB expression and localization were examined in PDAC patient's tissue microarrays (TMAs) and patient-derived xenograft (PDX) models. A novel conditional *Creb* knockout ($Creb^{fl/fl}$) model, in an $LSL\text{-}Kras^{G12D/+;}\ Trp53^{R172H/+;}\ Pdx1^{Cre/+}$ (KPC) background ($KPCC^{-/-}$), was generated to delineate CREB's role in PDAC progression and metastasis. Functional studies included lentiviral *creb* overexpression, CRISPR/Cas9-based *Creb* knockout, RNA sequencing (RNA-seq), and chromatin immunoprecipitation sequencing (ChIP-seq). Metastatic lesions were assessed in genetically engineered mouse and orthotopic models (GEMMs). Matrigel invasion assays, immunohistochemistry, immunoblotting, and qPCR were used to validate the findings *in vitro* and *in vivo*.

Results: TCGA analysis revealed elevated *CREB* and invasive gene expression in PDAC compared to normal pancreatic tissues. Immunostaining of TMAs showed increased CREB expression localized to ductal tumor cells. *Creb* knockout in $KPCC^{-/-}$ mice reduced metastatic burden, while *Creb* overexpression induced liver metastasis in orthotopic models. Invasion assays confirmed decreased invasiveness of tumor cells with CREB inhibition or deletion. CREB inhibition showed reduced expression of ROCK1/2, a key mediator of cytoskeletal dynamics and ECM remodeling. RNA-seq, qPCR, and ChIP-Seq analysis identified CREB as a transcriptional regulator of genes essential for invasion and ECM remodeling. CREB deletion or inhibition attenuated the expression of these targets, decreasing tumor cell invasion and metastatic potential.

Conclusion: These findings establish CREB-ROCK as a central mediator of PDAC metastasis and targeting CREB-ROCK represents a promising therapeutic strategy for inhibiting invasive PDAC progression and metastasis.

#1312 FOXF2 expression triggered by endocrine therapy orchestrates therapeutic resistance through reorganization of chromatin architecture in breast cancer.

Rui Zhang, Wen-Jing Jiang, Shuai Zhao, Li-Juan Kang, Qing-Shan Wang, **Yu-Mei Feng**

Tianjin Medical Univ. Cancer Inst. & Hospital, Tianjin, China

Estrogen receptor-positive (ER+) breast cancer accounts for 80% breast cancer cases. The estrogen-ERα signaling pathway plays a pivotal role in driving the progression of ER+ breast cancer cells. Thus, endocrine therapy targeting the estrogen-ERα signaling pathway is a predominant treatment for ER+ breast cancer. Most patients with ER+ breast cancer require long-term treatment with endocrine agents. Although endocrine therapy effectively improves the survival of patients with ER+ breast cancer, bone metastasis frequently occurs in patients receiving long-term endocrine therapy due to acquired resistance. Elucidating the molecular mechanisms underlying endocrine-resistance and developing effective strategies to reverse resistance or treat resistant tumors are crucial unresolved issues. Enhancer reprogramming-mediated lineage-specific transcriptional rewiring systemically regulates the transdifferentiation and plasticity of cancer cells. Enhancers are DNA regulatory elements composed of clusters of transcription factor (TF) binding sites. Enhancer-bound TFs recruit chromatin-modifying enzymes to establish unique histone modifications in enhancer regions, subsequently recruiting cofactors to facilitate or repress the transcription of specific target genes by reorganizing chromatin architecture and altering DNA accessibility. Our previous studies found that forkhead box F2 (FOXF2), a mesenchymal TF, is barely expressed in ER+/luminal breast cancer (LumBC) cells but is commonly expressed in triple-negative breast cancer (TNBC)/basal-like breast cancer (BLBC) cells with mesenchymal characteristics. In the present study, we found that ERα represses *FOXF2* transcription in ER+ breast cancer through H3K27me3 modification, therefore endocrine therapy triggers *FOXF2* transcription by loss of H3K27me3. *FOXF2* transactivation orchestrates endocrine-resistance and bone metastasis. Mechanically, FOXF2 globally activates enhancers of genes involved in epithelial-mesenchymal transition (EMT)/epithelial-osteogenic transition (EOT), as well as super-enhancers of *NCOA3* (a coactivator of FOXF2) and *SP1* (an upstream trans-activator of FOXF2) by recruiting SWI/SNF complex that mediates the reorganization of chromatin architecture. Targeting BRD4, an essential transcriptional coactivator of FOXF2, significantly inhibits FOXF2-orchestrated endocrine-resistance and bone metastases. Our findings uncover a mechanism underlying endocrine-resistance and provide a promising strategy for managing endocrine-resistant breast cancer.

#1314 Enhanced metastasis modeling in mice: impact of primary tumor removal and optimized injection techniques on therapeutic assessment.

Jonas Hummel, Philipp Metzger, Roland Sonntag, Dennis Borrero-Wolff, Gojko Bijelic, Cynthia Obodozie, **Holger Weber**

Reaction Biology Europe GmbH, Freiburg, Germany

The clinical potential of therapies on tumor metastases can be specifically tested on metastases that have naturally developed through the migration of the tumor cells from the primary tumor. Removing the primary tumor allows for a clearer assessment of a substance's efficacy in targeting metastatic sites alone, free from the influence of the primary tumor. If the primary tumor is still present during treatment, it is difficult to determine whether the reduction in metastatic spread is due to the anti-metastatic effect of the treatment or merely to the reduced growth of the primary tumor, which could indirectly limit the progression of metastases. In addition, the rapid growth of the primary tumor reduces the therapeutic window. The intra-mammary implantation approach (also referred to by us as subQperior for non-breast tumor cells) allows for clean surgical removal of the primary tumor, as the tumor remains isolated without adhering to the skin or peritoneum. This uncomplicated removal minimizes local trauma and residual primary tumor and provides a consistent basis for studying metastatic development after tumor resection.

The study of metastasis in mouse models following primary tumor removal provides essential insights into the progression and treatment of metastatic disease. In this context, the luciferase-labelled 4T1 tumor cell line, known for its high metastatic potential, offers a robust model, particularly for investigating lung metastasis. After subQperior implantation of mice, we show that 4T1 cells even at small tumor sizes metastasize rapidly, predominantly to the lungs, making this model highly suitable for evaluating therapeutic strategies against metastatic spread.

Furthermore, experiments with LL-2 tumor cells have shown that subQperior injection and removal of the primary tumor provides significantly better metastasis compared to traditional subcutaneous application. The approach of subQperior application not only increases the homogeneity and growth of the tumor and simplifies the removal of the primary tumor, but also comes closer to an orthotopic implantation with regard to the natural tumor environment and the progression of tumor growth. The advantages of subQperior injection suggest it may be a preferable method for achieving more consistent and representative metastasis in preclinical studies.

Together, the methodological improvement of primary tumor removal and refined injection techniques enable a more precise evaluation of drugs targeting metastasis, avoiding confounding effects associated with the presence of the primary tumor. By isolating the impact of therapies on metastatic sites, researchers can more accurately assess the effectiveness of anti-metastatic agents, advancing our understanding and treatment of metastatic disease.

TUMOR BIOLOGY: Novel Therapeutic Targets for Metastasis
Poster Session

#1317 Lung-specific CRISPR/Cas9 delivery using sulfonium lipid nanoparticles effectively suppresses lung metastases by targeting ATAD3A.

F. Chen[1], D. O. Popoola[2], F. Yang[1], W. Zhen[1], Y. Li[2], **Y. Teng**[1];
[1]Emory University, Atlanta, GA, [2]Upstate Medical University, Syracuse, NY

Metastasis is the leading cause of cancer-related morbidity and mortality. The lung is the second most common site of metastasis. It is estimated that 20 to 54% of malignant tumors that develop elsewhere in the body will have pulmonary metastases. However, limiting metastatic tumor growth and progression in the lung remains a significant bottleneck. ATAD3A, a mitochondrial-localized protein, has been shown to play a critical role in maintaining the growth and survival of metastatic tumors in the lung. The current challenge lies in genetically depleting ATAD3A in lung metastatic tumors. Among various mRNA delivery systems, lipid nanoparticles (LNPs) are the most advanced for clinical use. While clinical trials mainly focus on mRNA/LNP therapies for liver diseases, the potential for gene editing therapies extends far beyond this and remains underexplored. In our previous work, we developed lung-specific sulfonium lipid nanoparticles (sLNPs) for selective mRNA delivery to the lung. Building on this, we have now created CRISPR-based ATAD3A-targeting nanodrugs, termed ltLNP3AKO, which encapsulate Cas9 mRNA and anti-ATAD3A sgRNA in the lung-specific sLNPs. In an experimental mouse model of lung metastasis, intravenous administration of ltLNP3AKO efficiently and specifically delivered Cas9 mRNA and anti-ATAD3A sgRNA to the lung, where it successfully depleted the ATAD3A gene. Most notably, ltLNP3AKO induced significant regression of lung metastatic tumors without causing lung or systemic inflammation or toxicity in major organs. These groundbreaking results not only highlight the safety and efficacy of the lung-targeted sLNP platform, but also provide a novel approach to selectively inhibit ATAD3A in lung metastases. Our study further demonstrates the great potential to advance gene editing therapies for patients with lung metastases.

#1318 Cytotoxic effect of plant-based alkaloid veratridine on patient-derived colorectal cancer spheroid cells.

Kate Schraufnagel, Morgan Eikanger, Khosrow Rezvani

Division of Basic Biomedical Sciences, Sanford School of Medicine, University of South Dakota, Vermillion, SD

Colorectal cancer (CRC) causes approximately 900,000 deaths annually worldwide. The survival rate for patients diagnosed with localized CRC is 90 percent; however, once the cancer metastasizes or spreads to other parts of the body, the five-year survival rate drops to a dismal 15%. There is a clear need for a new generation of targeted therapies that can safely and efficiently target the metastatic features of CRC cells. Our lab has shown that veratridine (VTD), a lipid-soluble plant alkaloid, can enhance the expression of a ubiquitin-like protein called UBXN2A as a colon-specific tumor suppressor protein. UBXN2A binds and negatively regulates the Rictor protein, a key member of the mTORC2 signaling pathway. This results in suppression of mTORC2-AKT's downstream signaling pathway in cancer cells. We hypothesize that the VTD-UBXN2A axis decreases migration in human CRC tumors. Human colon cancer cells and patient-derived xenograft cell lines in 2D and 3D spheroid forms were used to complete this project. Real-time cell migration and invasion analysis using xCELLigence technology and Cytation 1 cell imaging system revealed the VTD-UBXN2A axis can suppress metastatic characteristics of CRC cells, including cell proliferation and migration. Small molecule targeted compounds have been successfully developed in clinical medicine to prolong the survival time of patients with advanced tumors. This ongoing study will provide detailed insight into the therapeutic efficacy of a potential targeted therapy to safely and effectively target CRC cells while normal cells can remain intact.

#1319 Luminal reprogramming, druggable mitogenic pathway activation, and rare outgrowth of occult basal cancers define ER loss in HR+/HER2- MBC.

Nolan Priedigkeit*[1], Stefania Morganti*[1], Alinés Lebrón-Torres[2], Kalie Smith[1], Melissa E. Hughes[1], Busem Binoga Kurt[1], Yvonne Y. Li[1], Hersh Gupta[1], Heather A. Parsons[1], Daniel L. Abravanel[1], Rinath M. Jeselsohn[1], Elizabeth A. Mittendorf[3], Adrian V. Lee[4], Steffi Oesterreich[4], Sara M. Tolaney[1], Andrew D. Cherniack[5], Nancy U. Lin[1], Ana C. Garrido-Castro[1]

[1]Dana-Farber Cancer Institute, Boston, MA,[2]Broad Institute of MIT and Harvard, Boston, MA,[3]Brigham and Women's Hospital, Boston, MA,[4]University of Pittsburgh, Pittsburgh, PA,[5]Broad Institute of MIT and Harvard, and Dana-Farber Cancer Institute, Boston, MA

BACKGROUND: Estrogen Receptor (ER) expression is often lost in treatment-refractory, hormone-receptor (HR) positive metastatic breast cancers (MBC). The biological mechanisms allowing tumors to maintain viability—without the key driver of HR+ disease—are relatively unknown. To better define this subclass of MBCs, we analyzed transcriptome and targeted DNA-sequencing data of HR+/HER2- cases that lost ER at the time of recurrence.

METHODS: A cohort of patients with primary HR+/HER2- breast cancer and subsequent loss of ER in the metastatic setting was identified. Loss of ER was defined as ER<1% in at least one metastatic sample. Transcriptome-capture RNA-sequencing was performed along with differential pathway analysis, PAM50 assignments, and fusion discovery. A subset of cases was analyzed with DNA-sequencing.

RESULTS: We identified 46 patients with ER loss and archival tissue. Of them, 21 patient-matched primary and metastatic pairs were interrogated with RNA-seq. The metastases were collected at a median time of 6 years [range 1-14] from the HR+/HER2- primary diagnosis. In contrast to prior studies of patient-matched tumors, metastases harboring ER loss were more often transcriptionally distinct from their primary tumor—with 15/21 metastases (71.4%) having a discordant PAM50 subtype. The most common discordances were tumors switching from LumA to HER2-Enriched or LumA to LumB (both in 5 of 21 cases, 23.8%). Notably, none of the HER2-E cases harbored *ERBB2* amplification. Luminal to basal switching was observed in two patients, but case review suggested the metastases being from occult primaries. Differential pathway expression analysis showed upregulation in mTOR, G2M checkpoint, and E2F pathways, suggesting selection of bypass mitogenic signaling in the absence of ER. Druggable features driving this transcriptional remodeling were identified, including an acquired in-frame 3' *BRAF* fusion and biallelic loss of *NF1* in two LumA to LumB cases. Further integration with clinicopathological features and DNA-level alterations will be presented.

CONCLUSIONS: Altogether, these results demonstrate significant transcriptomic shifts in HR+/HER2- cancers that lose ER in the metastatic setting. We find ER loss is associated with ER-independent transcriptional reprogramming and multimodal mechanisms of druggable mitogenic pathway activation. In rare cases, an apparent luminal to basal switch occurs—but these events likely stem from occult disease. Importantly, most ER loss cases do not become basal-like, but rather retain their luminal identity in the absence of ER and co-opt other mitogenic pathways to achieve a more proliferative, ER-independent state. We propose that triple-negative disease in the setting of ER loss is biologically unique from canonical basal-like tumors and could benefit from a distinct therapeutic approach.

#1320 Nanotopographical cues enhance glioblastoma migration: Harnessing biomimetic matrices and machine learning to uncover cancer invasion.

Christopher P. Miller[1], **Satvik R. Kethireddy**[2], Seung-Hyuk T. Lee[3], Patrick A. Ting[4], Alex Wu[2], Paul Lazarchuck[3], Raymond J. Monnat[3], Deok-Ho Kim[5], Eun Hyun Ahn[6]

[1]Department of Bioengineering, University of Washington, Seattle, WA,[2]Department of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD,[3]Department of Laboratory Medicine & Pathology, University of Washington, Seattle, WA,[4]Department of Electrical and Computer Engineering, Johns Hopkins University, Baltimore, MD,[5]Departments of Biomedical Engineering and Medicine, Johns Hopkins University, Baltimore, MD,[6]Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD

Glioblastoma (GBM), the most aggressive brain cancer, invariably recurs after surgery and rapidly develops resistance to radiation therapy and chemotherapy. The invasiveness and genetic variation (heterogeneity) of GBM cells are major challenges for developing effective therapies. Furthermore, the study of glioblastoma invasion is particularly challenging due to the lack of robust experimental models that recapitulate the tumor microenvironment. To simulate *in vivo* brain tissue extracellular matrix (ECM), we have established nanotopographically defined extracellular matrix (ECM)-mimetic platforms. Moreover, to preserve the GBM cancer stem cell (GSC) phenotype and mimic tumor microenvironment, GBM cells were cultured on these nanopatterned surfaces in neuro stem cell media under hypoxia. The migration, motility, and morphological changes of the cells were analyzed using live cell microscopy and machine learning (ML). The migratory abilities of GBM cells were enhanced on these nanopatterned surfaces (cells elongate and move along the ridges) in comparison to unpatterned surfaces. Approximately 9 times more number of GBM cells migrated distances exceeding 400 micrometer along the direction of the ridges in the nanopatterned surface than the unpatterned surfaces. The MARS-Net ML analysis demonstrates that stromal nanotopography induces directional migration of GBM cells, and a 64% increase in skewness from rounded to more elongated cell shapes over time when compared to unpatterned surfaces. The genetic landscape of the GBM cells was characterized using whole exome sequencing (WES). About 55% of the single base substitutions (SBS) in GBM cells are missense mutations causing amino acid changes. The most prevalent SBS types are C>T/G>A, followed by T>C/A>G and C>A/G>T. We have developed radiation resistant GBM cells (rGBM) by expanding surviving cells after a series of cytotoxic x-ray treatments. rGBM cells exhibit higher migratory properties and colony formation abilities than normal GBM cells, implying they can be more invasive and oncogenic. Our results underscore the important role of nanotopographical cues in guiding GBM migration and invasiveness. To our knowledge, this study is the first to apply MARS-Net ML analysis to investigate changes in cell motility and morphology within an ECM-mimetic and hypoxic environment. The study has implications in the preclinical screening of anti-migratory chemotherapeutic agents and the molecular classification of glioblastoma.

#1321 Glycosylation dependent metastatic seeding of circulating tumor cells in breast cancer.

Nurmaa Khund Dashzeveg

Pharmacology, Northwestern Univ. Feinberg School of Medicine, Chicago, IL

Chemotherapy has been standard of care for triple negative breast cancer for decades; however, how chemotherapy evasion enables metastasis remains elusive and is one of the biggest challenges impacting patient survival in cancer clinics. Circulating tumor cells (CTC) are pivotal component of longitudinal liquid biopsies. Most of the CTCs are detected as a single cell, whereas small proportion of CTCs are found in multicellular clusters which possess 20-100 times higher metastatic potential than single CTCs and corelates with unfavorable survival in breast cancer patients. Here we observed that chemotherapy evasion is linked to the aggregation of CTCs in breast cancer. Chemo-evasive linked aggregated CTCs show specific loss of ST6GAL1-catalyzed alpha2,6-sialylation. Deficiency of ST6GAL1 promotes CTC cluster formation for metastatic seeding and enables cellular quiescence to evade paclitaxel treatment in metastatic breast cancer. Glycoproteomic analysis identified protein substrates of ST6GAL1, such as adhesion and stemness markers PODXL, ICAM1, ECE1, ALCAM1, CD97, and CD44, contributing to CTC clustering (aggregation) and metastatic seeding. As proof-of-concept, neutralizing antibody against one of the most significant contributors, PODXL, inhibits CTC cluster formation and lung metastasis exacerbated by paclitaxel treatment for breast cancer. This study sheds light in the evaluation of chemotherapy response and developed potential therapy approach against chemotherapy resistance.

#1322 The DNA methylation signature of the driver gene GPRASP1 serves as an early indicator of progression in prostate cancer.

Chengyu Huang[1], Shuo Fang[1], Yuhao Cui[2], Xin Huang[2], Yuhan Lin[3], Yichen Xiao[2], Xinyu Ye[1], **Yi Lu**[2]

[1]School of Medicine, Southern University of Science and Technology (SUSTech Shenzhen), Shenzhen, China,[2]Dept. of Human Cell Biology & Genetics, the Guangdong-Hong Kong Joint Lab, Southern University of Science and Technology (SUSTech Shenzhen), Shenzhen, China,[3]Southern University of Science and Technology (SUSTech Shenzhen), Shenzhen, China

Prostate cancer (PCa) is one of the most common tumors in men in Europe and the United States. Patients with primary localized PCa typically demonstrate long-term survival. However, the disease becomes incurable once it progresses to metastatic PCa. Our research focuses on uncovering the early events in tumorigenesis and progression of PCa using multi-omics strategies. In this study, we identified several methylation driver genes associated with PCa by analyzing RNA sequencing and methylation data from The Cancer Genome Atlas (TCGA) database. The R package *Methymix* was applied to calculate the methylation driver genes. GPRASP1 was selected for further investigation due to its significant role in predicting PCa prognosis. Our analysis of two GEO datasets revealed that GPRASP1 is downregulated in primary PCa, with even lower expression levels observed in metastatic PCa. This finding was supported by immunohistochemistry results from tissue microarray of PCa patients, demonstrating reduced protein levels. Additionally, we identified the downregulation of GPRASP1 due to DNA methylation in its promoter region, as confirmed by targeted DNA methylation sequencing conducted in PCa and normal cell lines. Simultaneously, the use of DNMT inhibitors was found to reverse this downregulation. To investigate the function of GPRASP1 in PCa, we conducted RNA sequencing following the knockdown of GPRASP1 expression to assess changes in the transcriptome. Additionally, we analyzed its interactome using liquid chromatography-mass spectrometry (LC-MS) following immunoprecipitation. The results suggested that GPRASP1 downregulation may promote tumor metastasis through upregulation of CXCR4 and SERPINE1. Moreover, GPRASP1 appears to play a significant role in tumor immunity. To further elucidate this, we performed tumor infiltration analysis by several algorithms. These analyses revealed that GPRASP1 contributes to suppressing anti-tumor immune responses and promotes the remodeling of the tumor microenvironment, thereby facilitating tumor growth. In conclusion, this research identified methylation driver genes by analyzing data from TCGA patients. We specifically focused on GPRASP1 as a pivotal factor for its roles as a driver and its downstream functions in PCa. This investigation provided insights into the early changes associated with PCa and their connections to tumor initiation and progression. These findings enhance our understanding of the genomic landscape in the early stages of PCa and offer a novel approach for this condition's early diagnosis and treatment. This work was supported by the NSFC Project (82173336, 81972766, 81972420), The Science and Technology Project of Shenzhen (JCYJ20210324104214040), the Medical Research Innovation Project (G030410001).

#1323 Monoamine oxidase B: a potential therapeutic target for colorectal cancer.

Unnati Hemant Shah[1], Shivani Soni[1], Lesly Torres-Gonzalez[1], Joshua Millstein[2], Pooja Mittal[1], Francesca Battaglin[1], Karam Ashouri[1], Sandra Algaze[1], Priya Jayachandran[1], Wu Zhang[1], Jean C. Shih[3], Heinz-Josef Lenz[1]

[1]USC Norris Comprehensive Cancer Center, Los Angeles, CA,[2]Department of Population and Public Health Sciences, USC Keck school of Medicine, Los Angeles, CA,[3]Department of Pharmacology and Pharmaceutical Sciences, USC Alfred E. Mann school of Pharmacy, Los Angeles, CA

Background: Colorectal cancer (CRC) is one of the most prevalent cancers globally. Tumor recurrence occurs in approximately 30% of late-stage patients largely due to distant metastases. Angiogenesis plays a pivotal role in CRC progression. Recent studies have reported elevated expression levels of monoamine oxidases (MAO A and MAO B) in several cancers. However, the specific roles of MAO B in CRC progression and prognosis remain largely unexplored. Our previous work demonstrated improved survival outcomes and therapeutic efficacy of MAO A inhibitors in CRC preclinical models. In-silico analyses reveal that MAO B expression is significantly elevated in CRC tissues compared to normal tissues, correlating with higher recurrence rates and mesenchymal-type gene expression patterns.

Objective: This study investigates the therapeutic efficacy of MAO B inhibitors- deprenyl, rasagiline, and safinamide in CRC cell lines, assessing their potential to reduce metastasis through epithelial-mesenchymal transition (EMT) reversal and to study their role in modulating angiogenesis.

Methods: We studied the effects of MAO B Inhibitors on cell viability of CRC cell lines HCT116 and HT29 following treatment by MTS assay. Changes in EMT-related genes were analyzed at transcription and translation level through RT-qPCR, western blot, and immunocytochemistry (ICC). Scratch assays were used to evaluate the metastatic potential of treated cells, and RT-qPCR quantified angiogenesis-related markers.

Results: Our findings confirm that MAO B expression is significantly elevated in CRC cell lines-HCT116 and HT29 compared to normal colon cell - FHC. Treatment with MAO B inhibitors effectively reduced MAO B expression in CRC cells while showing no cytotoxic effect to normal colon cells with the same dose treatment. EMT marker analysis revealed a reduction in mesenchymal characteristics and an increase in epithelial markers, indicating a shift toward less metastatic phenotypes. Scratch assays demonstrated a decrease in metastatic potential in treated cancer cells, further supported by RT-qPCR results showing reduced expression of metastatic markers (N-cadherin, TWIST and Snail) and increased expression of epithelial markers (E-cadherin). We also observed a significant decrease in angiogenesis-related markers (VEGFR2) at transcription level. Western blot and ICC validated these findings at the protein level. Notably, HT29 cells (BRAF-mutant) exhibited greater sensitivity to MAO B inhibitors, showing better reduction in metastatic and angiogenic markers compared to HCT116 cells.

Conclusion: MAO B is a promising biomarker and therapeutic target for CRC. MAO B inhibitors effectively reduce metastatic and angiogenic potential in CRC cells, providing a novel approach for CRC treatment. These findings warrant further investigation to establish their clinical applicability and therapeutic potential.

#1324 MECOM a novel player in AR-driven treatment resistant castration-resistant prostate cancer.

Surendra Gulla[1], Tej K. Sharma[1], Ephraim Gardner[1], Shreya Shyam Sundar[2], Brian Capaldo[3], Jonathan E. Bard[1], Tobi Ogunbowale[1], Abbas Jawadala[1], Min Ma[1], Roberto Pili[1], Jun Qu[1], David J. VanderWeele[4], Remi Adelaiye-Ogala[5]

[1]SUNY at Buffalo, Buffalo, NY,[2]University at Buffalo, Buffalo, NY,[3]National Institutes of Health, Bethesda, MD,[4]Northwestern University Feinberg School of Medicine, Chicago, IL,[5]Medicine, SUNY at Buffalo, Buffalo, NY

The androgen receptor (AR) remains a pivotal target in castration-resistant prostate cancer (CRPC), as its continued activity presents a therapeutic vulnerability. Reprogramming of the AR cistrome, shifting from a canonical (typical or standard) to a non-canonical (atypical or non-standard) form, is associated with disease progression. While canonical AR signatures are prevalent in benign and localized prostate cancer, advanced CRPC exhibits a predominance of non-canonical AR elements. This non-canonical AR cistrome is linked to enzalutamide resistance, a standard AR-targeted therapy in the clinic. In preclinical prostate cancer models, non-canonical AR signatures correlate with enzalutamide resistance, and bulk RNA sequencing and AR-ChIP-seq data suggest an association with the EVI1-target gene set. EVI1, a zinc-finger transcription factor and product of the MECOM locus, is associated with poor prognosis and acts as a co-activator in cancer cell survival. Yet, its role in AR therapy resistance remains unclear. Functional assays demonstrated that MECOM expression, confined to CRPC and enzalutamide-resistant models, interacts with AR and influences cell proliferation. MECOM knockdown in CRPC cells inhibited proliferation in 2D and 3D cell cultures and significantly affected noncanonical AR signature profiles. This suggests a potential re-sensitization to AR inhibitors, a finding that could significantly impact the treatment of CRPC. Poly-ADP ribose polymerase inhibitors (PARPi), particularly Talazoparib, effectively reduced tumor growth in vivo and MECOM protein expression in CRPC cells, organoids derived from PDXs, and tumors. Our findings indicate that MECOM is a key driver of non-canonical AR signaling in CRPC. We propose that patients with MECOM expression may benefit from PARPi therapy, regardless of DNA Damage Repair (DDR) or homologous DNA Repair (HDR) gene mutation status.

TUMOR BIOLOGY: Radiation Sciences 1
Poster Session

#1328 Imaging tumor oxygenation during interstitial photodynamic therapy.

Emily Oakley-Gawrys[1], Irene Canavesi[2], Navin Viswakarma[2], Boris Epel[3], Mrignayani Kotecha[2], **Gal Shafirstein**[1]

[1]Roswell Park Comprehensive Cancer Center, Buffalo, NY,[2]O2M Technologies LLC, Chicago, IL,[3]University of Chicago, Chicago, IL

Background: Interstitial photodynamic therapy (I-PDT) is a promising treatment for patients with large locally advanced or deeply seated tumors. In I-PDT, light is administered to activate a photosensitive drug (photosensitizer), which, in the presence of oxygen, results in the production of reactive oxygen species. In previous preclinical studies, we demonstrated that the light dose rate (irradiance, mW/cm^2) is a main predictor of tumor response to I-PDT. The objective of this investigational study was to perform electron paramagnetic resonance oxygen imaging (EPROI) during I-PDT of locally advanced murine tumors to investigate how changes in the delivered light irradiance, mW/cm^2, correlate to changes in tumor oxygenation.

Methods: C3H mice with locally advanced squamous cell carcinoma (SCC7), between 260-740 mm^3, were treated with I-PDT using the photosensitizer porfimer sodium administered at a dose of 5.0 mg/kg via tail vein injection. Interstitial light was delivered through two 2-cm light diffuser optical fibers placed 6 ± 1 mm apart in the tumor 24 ± 2 hours post the photosensitizer injection. The light intensity delivered from each fiber was either 60 mW/cm, which is associated with low light irradiance and low cure rate (n = 4) or 100 mW/cm, which is associated with high light irradiance and high cure rate (n=3). Partial oxygen pressure (pO_2) maps were acquired prior to and during I-PDT using a 25 mT/720 MHz EPROI instrument and tris(8-carboxyl-2,2,6,6-tetra(2-(1-hydroxy-2,2-d2-ethyl))benzo[1,2-d:4,5-d']bis(1,3)dithiol-4-yl) methyl trityl as the oxygen-sensitive contrast agent. Trityl was delivered to mice via intra-peritoneal injection (300µL, 36.1mM) followed by three intra-tumor injections (50µL, 20mM each). pO_2 maps were derived with following acquisition and reconstruction parameters: pulse sequence - inversion recovery electron spin echo, π/2 and π pulse lengths- 60 ns, spin-echo delay - 400 ns, 16-step phase-cycling, 828 projections, 1.5 G/cm gradient, 9 delays - 204 ns to 40 µs, TR - 45 µs, acquisition time: ~9 min, filtered back projection reconstruction, image resolution 1.32 x 1.32 x 1.32 mm^3, R_1-pO_2 calibration - slope: 111.23 torr/μs^{-1} & intercept: 0.16 μs^{-1}. Results: The mean pre-treatment pO_2 was 9.74 ± 2.37 torr. The average decrease in pO_2 as measured with EPROI was 77.8 ± 17.4% (n = 3) for the 100 mW/cm cohort and 63.0 ± 9.4% (n = 4) for the 60 mW/cm cohort.

Conclusion and Discussion: In conventional PDT, in which the light is administered to the tumor surface, a high irradiance is associated with high oxygen consumption and poor tumor response. In contrast to conventional PDT, the results from this study suggest that a high irradiance, which induces a high oxygen consumption, is required to obtain high cure rates and local tumor response following I-PDT. Future experiments are ongoing to correlate changes in the tissue oxygenation during I-PDT with the delivered light irradiance and overall tumor response.

#1329 Intraoperative light transmission: A translational dosimteric marker for tumor response in interstitial photodynamic therapy.

Cristopher Lawson[1], Emily Oakley-Gawrys[1], Sarah Chamberlain[1], Alan Hutson[1], Nathaniel Ivanick[1], Keith Cengel[2], Theresa Busch[2], **Gal Shafirstein**[1]

[1]Roswell Park Comprehensive Cancer Center, Buffalo, NY,[2]University of Pennsylvania, Philadelphia, PA

Background: Interstitial photodynamic therapy (I-PDT) has shown promising outcomes in controlling locally advanced tumors refractory to standard of care therapies. I-PDT utilizes interstitial laser light delivery to activate a light-sensitive drug (photosensitizer) to produce singlet oxygen within the target tumor. The response to I-PDT is controlled by the intratumoral light dosimetry, photosensitizer concentration and tumor oxygenation. We hypothesize that changes in laser light transmission due to changes in tumor vasculature are a dosimetric marker for response to I-PDT. We test this hypothesis in murine models with locally advanced cancer.
Methods: The relationship between light transmission and tumor response was studied in eight- to twelve-week-old C3H and C57BL/6 female mice with locally advanced (400-600 mm^3) subcutaneous tumors. C3H with squamous cell carcinoma VII (SCCVII) or C57BL/6 with LL/2 (Lewis lung carcinoma) tumors were treated with I-PDT alone or with palliative x-ray radiotherapy (p-XRT) followed by I-PDT at 24 ± 2h after the second fraction of 10 Gy x2 over two days. The I-PDT was conducted with 2 mg/kg verteporfin for injection administered via intravenous tail vein injection 3 h prior to the intratumoral illumination with 689±3 nm laser light planned to generate a minimum of 5 mW/cm^2 and 30 or 37.5 J/cm^2 in the target tumor. The laser light was delivered through a single 2-cm cylindrical diffuser fiber and measured with a light dosimetry fiber at the tumor margins. Photoacoustic imaging was conducted before and after p-XRT and prior to I-PDT. Tumor progression was monitored over 60 days.
Results: In C3H mice with locally advanced SCCVII, the I-PDT produced a 20% (2/10) tumor control rate, while p-XRT followed by the same I-PDT regimen resulted in better ($p<0.05$, log-rank test) tumor control 88% (8/9). In the C57BL/6 mice with LL2, we observed tumor control at day 60 (0/18) in mice treated with I-PDT and 27% (5/18) complete tumor response at day 60 in mice treated with p-XRT followed by I-PDT. There was a significant improvement in light transmission ($p<0.05$, t-test for comparing differences of non-linear regression models) in tumors treated with p-XRT prior to I-PDT in comparison to tumors treated with I-PDT only. The photoacoustic imaging shows a significant increase in tumor blood flow ($p<0.05$, t-test) after the p-XRT and prior to the I-PDT.
Conclusions: Increased light transmission due to increases in tumor blood flow results in better tumor response to I-PDT. Changes in light transmission during I-PDT are a potential dosimetric marker to assess tumor response. This measure can be used in clinical settings to adjust laser power to increase light transmission to improve tumor control in I-PDT of refractory cancerous tumors.

#1330 *In vitro* and *in vivo* YTN16 gastric cancer response to low dose focal SmART+ X-ray irradiation.

Nathaniel Fredette, Yogindra Vedvyas, Yanping Yang, Moonsoo Jin

Houston Methodist Research Institute, Houston, TX

Background: Prior to 2018, no mouse gastric cancer cell line was implantable in C57BL/6 mice. However, now that this YTN16 mouse cancer model has been established, it can be used for evaluating various therapies in an immunocompetent strain. X-ray radiation therapy is a widely used cancer treatment known to cause DNA damage and dimmish a cells ability to proliferate. The Houston Methodist Research Institute has recently acquired a small animal radiation therapy (SmART+) machine developed by Precision X-ray Irradiation (Madison, CT). This machine is capable of image guided radiation delivery through lower energy computed tomography and 225 kV X-ray irradiation. The machine is designed for use with small animals such as mice, rats and rabbits, but is also capable of irradiation of cells in a dish.

Methods: The response of YTN16 cancer cells to SmART+ radiation therapy was studied in a 96-well plate and in both NSG and C57BL/6 mice. For the *in vitro* study, focal plane (at isocenter) X-rays were applied to the plate in six distinct quadrants (four by four well grids). The six quadrants of the plate were exposed to X-ray doses of zero (control), two, five, ten, twenty, and forty Gray. Bioluminescence (Tecan Spark) along with bright field and green fluorescent protein microscopy (EVOS M5000) was used to quantify cell survival. For the *in vivo* study, ten male NSG and ten male C57BL/6 mice were subcutaneously xenografted with one million YTN16 gastric cancer cells per hundred microliters media in a 50% - 50% mixture with Matrigel. Tumors were monitored with a caliper and allowed to grow until they were measured to be one hundred cubic millimeters. Mice were then divided into treatment groups of three mice per cohort with four mice in the control groups. After which, X-ray radiation was applied locally to the tumor in three doses of zero, ten, or twenty Gray. After radiation therapy, one mouse from each cohort was sacrificed and tumors were harvested for tumor microenvironment imaging. The remaining seven mice were monitored for weight loss and tumor shrinkage through scale and caliper measurements.

Results: In the dish, irradiated YTN16 cells showed survival characteristics like other gastric cell cancer lines such as RKO and SW480. In NSG mice, radiation slowed the progression of tumor growth and even caused some shrinkage. In C57BL/6 mice, heightened levels of T-cells and macrophages at the tumor site shown through imaging on the Akoya PhenoCycler show natural immune response to radiation. The level of immune activity was proportional to the radiation dose for the three dose cases explored herein.

Conclusions: Our studies demonstrate that focal SmART+ X-ray radiation can be used to slow or halt YTN16 gastric tumor growth in the petri dish and in immunodeficient and immunocompetent mice. Coupling X-ray radiation with other therapies such a T-cells may have the ability to reduce tumor activity completely.

#1331 Deciphering PSMA7 modulation by RT and TMZ for enhanced GBM treatments.

Christian A. Showalter, Priyani V. Rajasekera, Ramasamy Santhanam, Ashok Kumar, Karnika Singh, Xiaomei Meng, Sagarika Jain, Heather R. Manring, Saikh J. Haque, Arnab Chakravarti

The Ohio State University, Columbus, OH

Objective: Glioblastoma (GBM, WHO grade 4) is one of the most lethal malignancies, with limited advancements since temozolomide (TMZ) and radiation therapy (RT) became the standard of care nearly two decades ago. A critical challenge and overarching goal of our lab is to understand and overcome the resistance mechanisms that undermine these therapies. Using multi-omics profiling, we identified proteasome subunit alpha type-7 (PSMA7), a non-catalytic subunit of the 20S proteasome, as overexpressed in GBM. Elevated PSMA7 correlates with poor survival, and work by our group demonstrated its potential as a therapeutic target. This study investigated how RT and TMZ modulate PSMA7 expression, shedding light on its role in resistance and its feasibility as a target for novel interventions.
Materials/Methods: Patient-derived primary human GBM cells (08-387 and 3359) and normal human astrocytes (NHAs) were used. Protein expression was quantified by Western blot analysis. Cell viability was assessed by ATP assays. TMZ (*in vitro*: Sigma T2577; *in vivo*: SelleckChem S1237). A Rad Source 2000 irradiator was used to treat cells with ionizing radiation *in vitro*. *In vivo*, mice anesthetized with isoflurane were irradiated using a Clinac linear accelerator, with a 1.7-cm x 1.7-cm copper collimator targeting radiation to the tumor. Dosimetry was performed using GafChromic films placed under the irradiated area.
Results: GBM and NHA cell viability decreased with increasing TMZ dose *in vitro*. Western blots showed that PSMA7 expression did not change when GBM or NHA cells were treated once daily for three days with 300 or 600 μM TMZ. However, cleaved PARP expression increased in GBM cells treated with TMZ, but not in NHAs. *In vivo*, PSMA7 expression increased in flank tumors of mice treated with 50 mg/kg TMZ for five days starting at day 7 post-implantation compared to vehicle-treated mice. NHAs treated once daily for three days with 0, 2, 3, or 4 Gy RT had no change in PSMA7 expression *in vitro*, while GBM cells had decreased PSMA7 expression with increasing RT dose. We also performed an *in vivo* study where GBM cells were implanted in the flank of mice and tumors were treated with up to 14.70 Gy RT. Tumors were collected at mouse removal and PSMA7 expression examined by Western blot.
Conclusion: Our findings underscore the selective regulation of PSMA7 by RT and TMZ, highlighting its potential as a therapeutic target. RT's ability to downregulate PSMA7 supports the rationale for integrating PSMA7-targeted small molecule inhibitors into the standard of care following validation in preclinical and clinical trials. Such inhibitors, particularly blood-brain barrier-permeable derivatives actively under development in our lab, offer a promising strategy to synergize with RT and enhance the therapeutic landscape for GBM. By targeting PSMA7, we may overcome the limitations of current treatments and revolutionize GBM therapy by improving outcomes for this aggressive malignancy.

#1332 Major histocompatibility complex class I chain-related gene A and B expression induced by heavy ion beam irradiation in cervical cancer cells.

Sumitaka Hasegawa, Nakako Nakajima

Charged particle therapy research, National Institutes for Quantum Science and Technology, Chiba, Japan

Major histocompatibility complex class I chain-related gene A and B (MIC) are transmembrane glycoproteins to function as a ligand for human NKG2D that is a main activating receptor of NK cells. MIC is minimally expressed on normal cells, but frequently upregulated via viral infection, cellular stress signals, and malignant transformation. NKG2D recognizes MIC, resulting in the activation of NK cells. Recent progress on radiation biophysics has shown that photon or particle radiation can modulate anti-tumor immunity in host irradiated via regulation of immune recognition protein expression on cancer cells. The aim of this study is to investigate the effects of heavy ions beam irradiation on membrane MIC expression in human cervical cancer cells. We used HEK293 cells stably overexpressing HPV-E6 or E7 oncoproteins and 5 human cervical cancer cell lines in this study. Carbon ions beam irradiation (CIR, 290 MeV/n; LET, ~70 keV/μm, mono peak), a beam of heavy ion beams, was performed at the in-house synchrotron accelerator that is able to produce carbon ion beams. Irradiated doses ranged from 0 to 10 Gy of carbon-ions. Cell membrane MIC expression was evaluated by flowcytometry using an anti-MIC antibody. MIC expression was upregulated in HPV-E6 or E7 overexpressing HEK293 cells and 5 human cervical cancer cells in a dose-dependent manner. HPV16-positive human cervical cancer Caski cells showed a remarkable upregulation of MIC expression after CIR. Other cervical cell lines (ME-180, HeLa, SiHa, and C33A) showed a modest MIC upregulation. E6- or E7-overexpressing HEK293 cells exhibited a considerable induction of MIC expression after CIR. We investigated the impact of DNA damage responses on HIR-mediated MIC upregulation using drug inhibitors of proteins that are involved in DNA damage responses. Both pharmacological Chk1 inhibition and ATM inhibition cancelled CIR-mediated MIC upregulation in HPV oncoprotein-positive HEK293 cells. The results indicated that ATM/ATR-Chk1 pathways were involved in MIC upregulation on HPV-positive cells including cervical cancer cells. These findings support the favorable combination of MIC-targeted therapy with heavy ion therapy against cervical cancers.

#1333 Uncovering the mechanisms of action of ^{177}Lu- and ^{212}Pb- based PSMA radioligand therapies.

Feifei Liu, Melissa E. Monterosso, Didier Boucher, **Stelle Shakti**, Kwong Ching Li, Aimee Horsfall, Saawan Kumar, Johannes Koehbach, Kevin Kuan, William Tieu, Stephen Rose, Anna Karmann, Simon Puttick, Gary Li, Thomas Kryza

AdvanCell Isotopes Pty Limited, Sydney, Australia

Introduction: ^{177}Lu-PSMA-617 is the first FDA-approved targeted radioligand therapy (RLT), and in clinical trials, significantly prolonged survival and improved quality of life in patients with metastatic castration resistant prostate cancer (mCRPC). However, approximately 30% of the patients do not respond to the treatment and most initial responders ultimately relapse. Compared to the beta-emitter ^{177}Lu, the alpha therapy isotope ^{212}Pb may prove to be advantageous due to its short half-life, high linear energy transfer, and efficient induction of immunogenic cell death. Here we used *in vitro* and *in vivo* models to elucidate the intrinsic cellular mechanisms of response to ^{177}Lu-PSMA and ^{212}Pb-PSMA treatment.

Methods:^{177}Lu-PSMA-I&T and ^{212}Pb-ADVC001 were used as PSMA-targeted ^{177}Lu- and ^{212}Pb-RLT, respectively. The cytotoxic activity in prostate cancer (PC) cell lines was measured by a cell viability assay. *In vivo* studies were performed in human PC-bearing mice to compare preclinical efficacy of ^{177}Lu- and ^{212}Pb-PSMA therapy. The kinetic mechanisms associated with ^{177}Lu-PSMA-I&T and ^{212}Pb-ADVC001 mediated cell-death were assessed *ex vivo* using proteomics, and their impact on the PC cell cycle by flow cytometry.

Results: ^{212}Pb-ADVC001 displayed potent cytotoxic activity with an average EC50 of 2.7, 7.2 and 3.3 kBq/mL in PC cell lines PC-3-PIP (PSMA^{high}), C4-2 (PSMA^{int}) and LNCaP (PSMA^{int}), respectively, compared to ^{177}Lu-PSMA-I&T (430.7, 434.9 and 814.4 kBq/mL, respectively). In PSMA^{high} xenograft models, ^{212}Pb-ADVC001 (0.43 MBq) significantly improved median survival to 84 days compared to 48 days for ^{177}Lu-PSMA I&T (20 MBq). Multidose efficacy studies demonstrated superior tumor response with two cycles of ^{212}Pb-ADVC001 (0.46 MBq) compared to two cycles of ^{177}Lu-PSMA I&T (15 MBq). ^{212}Pb-ADVC001 administration in ^{177}Lu-PSMA I&T-relapsed mice resulted in improved survival. In PSMA^{int} xenograft models, ^{212}Pb-ADVC001 (0.9 MBq) also suppressed tumor progression more effectively than ^{177}Lu-PSMA-I&T (30 MBq), resulting in prolonged survival. Proteomics analysis of RLT-treated tumors revealed a specific impact of ^{212}Pb-ADVC001 on cell cycle checkpoints and DNA replication. *In vitro* cell cycle experiment further confirmed that ^{212}Pb-ADVC001 caused significant DNA damage, and reduced DNA content in the S-phase of the cell cycle with a concomitant increase in the G1 and G2/M-phase arrest compared to ^{177}Lu-PSMA-I&T ($p<0.05$).

Conclusion: Comparison studies of ^{177}Lu- and ^{212}Pb-PSMA treatment on PC cells showed a higher efficacy of ^{212}Pb-PSMA *in vitro* and *in vivo* supporting the clinical application of ^{212}Pb-PSMA for mCRPC. Mechanistic studies furthered the understanding of PC radiobiology and cellular responses to beta- and alpha-RLT with the identification of a cell cycle arrest in PC specifically induced by ^{212}Pb-PSMA.

#1334 FLASH proton radiotherapy mitigates corneal damage and stimulates wound healing in mice.

Elias El Hoyek[1], Uri Amit[1], Brent A. Bell[2], Vivian Lee[2], Joshua L. Dunaief[2], Costantinos Koumenis[1]

[1]Department of Radiation Oncology, University of Pennsylvania, Philadelphia, PA,[2]Department of Ophthalmology, University of Pennsylvania, Philadelphia, PA

Eye cancers are rare but highly aggressive, with significant risk to vision and quality of life. Worldwide, thousands are affected by ocular cancers, which are challenging to treat due to their proximity to sensitive structures. Proton radiotherapy (PRT) is the current gold standard for intraocular tumors due to its precision in limiting RT exposure to surrounding healthy tissue. However, the delicate location restricts the PRT dose that can be safely administered without risking damage to essential orbital structures. FLASH (FR), a novel approach delivering PRT at ultrahigh dose rates (>40 Gy/s) compared to standard dose rate PRT (SR, <0.003 Gy/s), has shown reduced toxicity in normal tissues like the brain and skin without compromising tumor control. However, its protective effects on eye structures remain unexplored. This study aims to investigate the potential of FR in preserving ocular tissues in a mouse model. Female C57BL/6J mice received 24 Gy of either FR or SR or remained untreated (control) using a "shoot-through" approach, in which both eyes received the same dose. Retinal and corneal damage were monitored using optical coherence tomography and confocal scanning laser ophthalmoscopy, with retinal vascular permeability evaluated by fluorescein angiography and retinal function by electroretinography (ERG). Corneal wound healing was assessed using corneal micro puncture. Evaluations occurred at one week, one, three, and five months post-PRT. Mice treated with SR showed significant corneal thickening and ulceration, leading to visual impairment, while those treated with FR did not. SR mice also showed declining ERG responses, suggesting diminished response to light stimuli, while FR mice demonstrated significantly less ERG abnormalities. Additionally, mice treated with SR showed over 55% reduction in eye surface area compared to both FR and control mice. Both SR and FR reduced retinal thickness, with no significant difference between the PRT treatment groups. Fluoresceine angiography showed no leakage in the retinal microvasculature. To further characterize corneal repair following PRT, we performed a corneal wound healing assay. Notably, FR demonstrated an enhanced wound healing response, with faster recovery of corneal incisions compared to both SR and control mice. This study presents a novel model for evaluating PRT-induced ocular damage, highlighting FR's potential to protect healthy tissues. FR was particularly effective in preserving corneal integrity and promoting wound healing, which are crucial for maintaining both visual function and cosmetic outcomes. Ongoing studies aim to quantify the preservation of stem cells in the corneoscleral junction as a potential mechanism for the observed tissue-sparing effects.

#1335 Comparison of X-ray and gamma-ray irradiators for in vitro and in vivo biological effectiveness.

Caroline Mae Mork[1], Shrey Ramesh[1], Keith Kunugi[2], Kwangok P. Nickel[1], Larry Dewerd[3], Nina Zitzer[4], Randall Kimple[1]

[1]Department of Human Oncology, University of Wisconsin Carbone Cancer Center, Madison, WI,[2]UW Medical Radiation Research Center, University of Wisconsin-Madison, Madison, WI,[3]Department of Medical Physics, University of Wisconsin-Madison, Madison, WI,[4]Department of Pathobiological Sciences, University of Wisconsin-Madison - School of Veterinary Medicine, Madison, WI

Objective: The Cesium Irradiator Replacement Program (CIRP) was established in 2014 to replace radioisotope-based irradiators with non-radioactive alternatives, such as X-rays. As most dosimetry is based on ^{137}Cs irradiators, transitioning to X-ray systems has presented challenges in radiobiological research. A key obstacle stems from the inaccurate assumption of dose as a directly transferable constant across different irradiation types. To mitigate this issue, the University of Wisconsin-Madison, as a CIRP participant, has undertaken a study to establish consistency in dose measurement and biological response, both in vitro and in vivo, with a specific emphasis on the relative biological effectiveness of X-rays compared to cesium.

Methods: Radiation dose was calibrated using experimental phantoms and thermoluminescent dosimeters. To determine dose response in vitro, HeLa cells were treated with 0 to 8 Gy of radiation in 2 Gy increments delivered from a ^{137}Cs, ^{60}Co, or 195 kVp X-ray source. Colony formation and comet assays were used to evaluate clonogenic survival and DNA damage, respectively. For in vivo studies, C57BL/6 mice received a single 8 Gy whole-body radiation dose from a ^{137}Cs, ^{60}Co, 220 kVp, or 300 kVp X-ray source. Lethal Dose$_{50/30}$ ($LD_{50/30}$) was used as the primary end point. Histopathological analysis of the spleen and bone marrow was performed to identify primary radiation-induced organ damage. Quantitative assessment of tissue injury was conducted using Halo image analysis. Hematology and blood chemistry were measured to monitor radiation-induced toxicity and subsequent recovery.

Results: The 195 kVp x-ray source produced the highest number of DNA double-strand breaks, followed by ^{137}Cs and ^{60}Co. This was most evident as the total radiation dose increased and translated to a greater impairment of clonogenic survival, particularly evident at 4, 6, and 8 Gy for 195 kVp X-rays. While ^{137}Cs and ^{60}Co had similar effects at lower doses, ^{137}Cs induced a more pronounced reduction in colony formation at 6 and 8 Gy. In animal studies, ^{60}Co exhibited the highest $LD_{50/30}$ (8.5 Gy), followed by ^{137}Cs (8.1 Gy), 300 kVp (7.3 Gy), and 220 kVp (6.2 Gy), respectively.

Conclusion: The differences were found in the biological effects between radioisotope-based and X-ray irradiators when delivered the identical physical dose. In animal models, radiation from a ^{60}C source demonstrated a weaker biological effect than ^{137}Cs, while 220 kVp X-rays showed the strongest biological effect. Given the differences in the energy spectra of the radiation sources, we will further investigate how dose-energy distribution influences biological effects in radiation-sensitive organs, particularly superficial tissues and hematopoietic systems.

#1336 Combination of gemcitabine and local tumor ablation WST-11 vascular-targeted photodynamic therapy displays superior efficacy compared to either monotherapy in a urothelial cancer model.

Kwanghee Kim[1], Sadna Budhu[2], Fengshen Kuo[1], Lilach Agemy[3], Stephen La Rosa[1], Sylvia Jebiwott[1], Karan Nagar[1], Chris Hughes[1], Jasmine Thomas[1], Laura Alvim[1], Rebecca Dubrovsky[1], Caoimhe Ryan[1], Daniel Rodriguez[1], Abraham Meyerson[1], Philip A. Watson[1], Natalia Kudinova[1], Andreas Aulitzky[1], Lucas M. Nogueira[1], Andrew T. Tracey[1], Wesley Yip[1], Phillip Wong[1], Taha Merghoub[2], Avigdor Scherz[3], Jonathan A. Coleman[1]

[1]Memorial Sloan Kettering Cancer Center, New York, NY,[2]Weil Cornell Medicine, New York, NY,[3]Weizmann Institute of Science, Rehovot, Israel

Vascular-targeted photodynamic therapy (VTP) with the photosensitizing agent padeliporfin (WST-11/TOOKAD Soluble) has demonstrated safety and efficacy for upper tract urothelial cancer (UTUC) in phase 1 (NCT03617003) and ongoing phase 3 clinical trials (NCT0420239). Here, we conducted a correlative study to assess immune modulatory activities of VTP using peripheral blood mononuclear cells (PBMCs) from 19 UTUC patients treated with VTP in the phase 1 trial. We complemented these findings with a preclinical assessment of a potential combination strategy with the chemotherapeutic agent gemcitabine. The frequency of CD8+ T cells among the CD3+ total T cells in patient PBMC was increased immediately after VTP in complete and partial responders (CR and PR), but the increase was more prominent and durable among CR than PR/non-responder (NR), suggesting an association of treatment response with CD8+ T cell driven immune modulation. An analysis of VTP induced effect in ovalbumin (OVA) expressing MB49 bladder tumor model showed an increase in OVA-specific CD8+ T cells in the blood and tumor samples, suggesting an association of treatment response with CD8+ T cell driven tumor antigen specific adaptive response that may be durable. We also observed an immediate increase in the population of myeloid derived suppressor cells (MDSCs) in patient PBMCs up to 24 hours post-VTP among all treated patients. The chemotherapy gemcitabine has been previously shown to reduce MDSCs in many cancer types, which makes it a rational strategy to combine with VTP. Combination of VTP with gemcitabine exhibited statistically significant local tumor control of MB49 and prevented lung metastasis, thereby improving overall survival compared to VTP or gemcitabine alone. Assessment of systemic anti-tumor responses using a bilateral tumor model, re-challenge in surviving mice and host immune effect in $Rag1^{-/-}$ (lacking T-cell and B-cell populations) mice bearing MB49 suggests that there is a long-term systemic immune modulation and antitumor activity enforced by the combination of VTP and gemcitabine. Additional analyses by flow cytometry and immunohistochemistry suggests that the combination of gemcitabine and VTP induces both innate and adaptive immune responses.

#1337 Androgen receptor mediates radioresistance through autophagy and DNA repair in AR-positive prostate cancer.

K.-i. Kudo[1], Q. Feng[2], M. Chosco[2], L. Wenning[2], Z. Huang[2], J. Anzules[2], J. Mohan[2], N. Lin[2], Z. V. Wang[1], J.-W. Rhee[2], Y. Li[2];
[1]City of Hope Beckman Research Institute, Duarte, CA, [2]City of Hope National Medical Center, Duarte, CA

Background: Androgen receptor (AR) is a critical therapeutic target in prostate cancer (PC) and androgen blockade is known to act synergistically with radiotherapy (RT), however, the mechanisms through which AR modulates RT response is complex and remains poorly elucidated.
Aims and Methods: We compared the radiation responses in LNCaP (AR-positive), PC3 (AR-negative), and HEK293T (non-malignant) cells by analyzing DSB induction, cell cycle arrest, and apoptosis. Mechanistic studies, including AR silencing and inhibition, were validated through RNA sequencing and IHC analysis of cell lines and irradiated patient-derived prostate tumors.
Results: At 24-hours post-RT, the apoptotic rate increased by 6-fold in HEK293T and PC3 cells versus LNCaP cells. Cell viability at 48-hours post-RT was decreased to less than 10% in HEK293T, remaining at ~60% in LNCaP cells. Moreover, over 70% of LNCaP cells were arrested in the G_1 phase post-RT, accompanied by rapid p53 activation and p21 induction within 3-hours. AR silencing significantly increased radiosensitivity and reduced cell proliferation in LNCaP but did not alter the expression of p53 downstream genes, eg. *BAX* and p21. Interestingly, AR silencing reduced the phosphorylation of DNA-PKcs, a critical mediator of non-homologous end joining (NHEJ) repair, suggesting that AR facilitates NHEJ. This was further supported by the comet assay results showing that LNCaP repaired RT-induced DSBs more efficiently than PC3 cells.
Moreover, AR regulates autophagy post-RT as AR silencing decreased autophagy-related gene (ATG) expression, and macroautophagy was augmented in LNCaP cells post-RT through LC3-II expression and autophagic vacuole formation. Bulk transcriptome analysis revealed significant upregulation of 11 ATGs, including *ULK1* and *ATG2*, at 24-hours post-RT. Moreover, IHC analysis of patient-derived PC *ex vivo* irradiated tumors consistently showed that RT-induced autophagy supports the survival of AR-positive PC cells.
Discussion and Conclusion: Our results demonstrate that AR plays an essential role in mediating radioresistance in AR-positive PC by enhancing both autophagy and DNA repair, with ongoing spatial transcriptome analysis to further explore these mechanisms in patient-derived tumors.

#1338 System biological approach identifies heterochromatin modification as an important mechanism of acquired cancer radioresistance.

Chaw Yee Beh[1], Sharon Pei Yi Chan[2], Celestia Pei Xuan Yeo[1], Boon Hao Hong[1], Evelyn Mui Cheng Tan[1], Dewi Susanti[1], Pei Ling Tai[1], Eugenia Li Ling Yeo[1], Khee Chee Soo[1], Edward Kai-Hua Chow[2], Melvin Lee Kiang Chua[1]

[1]National Cancer Centre Singapore, Singapore, Singapore,[2]Cancer Science Institute of Singapore, Singapore, Singapore

Radiotherapy (RT) is an integral modality for the treatment of cancer, with >70% patients requiring RT. However, tumor responses to RT are heterogeneous and influenced by several intrinsic and extrinsic factors. Clonal dynamics have identified that in some cancers, radioresistance is dominated by a process of clonal selection and adaptation, thereby suggesting that tumor cells harbor an adaptive pro-survival mechanism to RT. To understand the mechanisms underpinning this process, we generated four radioresistant (RR) cancer models - prostate (22Rv1, DU145) and head and neck (FaDu, HK1) cancer cell lines by exposing them to 90 Gy over 45 fractions delivered over 12 weeks, which mimicked the fractionated radiotherapy schema employed in the clinic. First, WES revealed an enrichment of mismatch repair mutational signatures (SBS6, 14, 15, 20) across our RR models and identified several non-synonymous single nucleotide variants in RAD51AP1, SMC1A, and APC2 genes that are associated with pro-survival pathways. At the transcriptome level, we observed several up- and down-regulated genes across the 4 models, but were able to identify five common overexpressed genes - ATP8A1, BAHD1, CTSD, JAK1, and MYLIP. Over-representation pathway analyses highlighted the association of the BAHD1 gene with an activated heterochromatin formation pathway (rich factor=0.01, adjusted P-value=0.02). This corroborated WB and IF analyses showing an increased expression of heterochromatin markers (H3K9me3 and H3K27me3) in the RR cells post-irradiation, but not in their respective wild-type (WT) parentals. Knockdown of BAHD1 by siBAHD1 treatment consequently reduced the expression of H3K9me3 and H3K27me3 and reversed the RR phenotypes (Clonogeic SF_{2Gy} siBAHD1:control=1.72 [FaDu-RR], SF_{4Gy} siBAHD1:control=1.63 [22Rv1-RR]). Collectively, these findings point to the model that BAHD1-dependent heterochromatin formation is an acquired phenomenon integral to radioresistance in our models. Simultaneously, parallel high-throughput drug screening of our RR and WT cancer cells identified susceptibility to Panobinostat (Pano) - a pan-HDAC inhibitor in vitro and validated in vivo. Combinatorial Pano and AZD7762 - a Chk1 inhibitor exhibited stronger anti-tumor activity against the RR than WT cells (IC_{50}: 0.45 nm [RR] vs 0.60 nm [WT]). Downstream analyses of Pano-treated RR compared with WT tumors further indicated higher apoptosis and reduced proliferation in the former. Finally, knockdown of class I and II HDACs pointed to reversal of HDAC6 expression as the primary target of Pano. Herein, our systems approach of investigations of our RR cancer models uncovered the importance of the heterochromatin in acquired cancer radioresistance, and presented a potential new molecular vulnerability of RR cancer cells to targeting by epigenetic drugs.

#1339 Claudin 18.2-targeted ^{68}Ga-labeled NODAGA-SNA014 for gastric cancer imaging by PET.

Y. Yang, H. Qi, **Z. Qiao**, F. Sun, F. Li, J. Xu, T. Xu;
SmartNuclide Biotech, Suzhou, China

Introduction: Claudin 18.2 is a tight-junction protein with primary expression in nonmalignant gastric epithelium and limited expression in normal tissues. Its upregulation has been identified as the result of the malignant transformation of gastric epithelial tissue. CLDN18.2 therefore becomes a highly selective theranostic target for the development of gastric cancer radiopharmaceuticals. We preclinically evaluated ^{68}Ga-NODAGA-SNA014 as a novel PET imaging agent for gastric cancer.

Methods: SNA014 is a single domain antibody (SdAb) derived from alpaca immunization and screened out by phage display. SNA014 was then conjugated with NODAGA to enable efficient ^{68}Ga labelling. The binding property of ^{68}Ga-NODAGA-SNA014 was characterized by saturation binding assay on claudin 18.2-expressing AGS cells. Screening of renal uptake inhibitors was conducted by *in vivo* biodistribution studies on NDG mice bearing claudin 18.2-expressing tumors. Biodistribution properties, pharmacokinetic profile and competitive blocking effects were determined by *ex vivo* biodistribution studies on AGS 18.2-bearing NDG mice. Organ-adsorbed doses were estimated using OLINDA software to extrapolate to the adult female phantom the *ex vivo* biodistribution data of ^{68}Ga-NODAGA-SNA014.

Results: Radiolabeling was quantitative (>99%) after 15 min of incubation at room temperature. The saturable specific binding of ^{68}Ga-NODAGA-SNA014 indicated a dissociation constant of 2.18 nM. Gleofusine showed effective reduce in the kidney uptake of ^{68}Ga-NODAGA-SNA014 without lowering tumor uptake when compared to other inhibitors. The *ex vivo* biodistribution studies showed the tumor, kidney and stomach uptake of ^{68}Ga-NODAGA-SNA014 were at 15.57 ± 6.06, 6.15 ± 0.90 and 75.27 ± 7.93 %ID/g at 1 h postinjection, respectively. Competitive blocking assay suggested NODAGA-SNA014 was able to block uptake of ^{68}Ga-NODAGA-SNA014 at stomach in a dose-dependent fashion, ranging from 10 to 60 μg, without dramatically altering the tumor and kidney uptake. Pharmacokinetic profile revealed ^{68}Ga-NODAGA-SNA014 had a plasma half-life of 45.23 ± 3.87 min. In addition, the effective dose was estimated at 0.0608 mSv/MBq.

Conclusions: We developed a novel claudin 18.2-targeted PET agent ^{68}Ga-NODAGA-SNA014 for gastric cancer imaging. The ^{68}Ga-NODAGA-SNA014 showed specific *in vitro* binding to claudin 18.2, favorable *ex vivo* biodistribution properties in terms of tumor, kidney and stomach uptake, and safety profile based on dosimetry studies, which prompt us to further develop NODAGA-SNA014-based radiopharmaceuticals for radiotherapy.

#1340 Interstitial photodynamic therapy enhances the efficacy of immune checkpoint inhibitor in estrogen receptor high-expressing breast cancer.

Kohei Chida[1], Emily Oakley-Gawrys[2], Christopher Lawson[2], Amber McKenery[3], Craig M. Brackett[2], John M. L. Ebos[3], Kenichi Hakamada[4], Gal Shafirstein[2], Kazuaki Takabe[1]

[1]Department of Surgical Oncology, Roswell Park Comprehensive Cancer Center, Buffalo, NY,[2]Department of Cell Stress Biology, Roswell Park Comprehensive Cancer Center, Buffalo, NY,[3]Department of Cancer Genetics and Genomics, Roswell Park Comprehensive Cancer Center, Buffalo, NY,[4]Department of Gastroenterological Surgery, Hirosaki University Graduate School of Medicine, Hirosaki, Japan

Introduction: Systemic chemotherapies improve the response to immune checkpoint inhibitors (ICIs) by provoking an immune response in primary breast cancer (BC). However, chemotherapy has systemic side effects, and recent clinical trials in estrogen receptor (ER)-positive BC have shown that higher expression of ER correlates negatively with response to ICI, even when combined with systemic chemotherapy. Photodynamic therapy (PDT) that uses light to activate a photosensitive drug not only ablates primary tumors but also enhances systemic immune response with minimal systemic adverse effects. We hypothesized that ER high-expressing BC will respond to ICI when combined with interstitial PDT (I-PDT), which allows deep light penetration with minimum skin damage by illuminating the tumor with optical fibers inserted directly into the tumor.
Methods: Two syngeneic murine BC cells tagged with luciferase (TS/A-luc: BALB/c background with high ER expression, and E0771: C57BL/6 background with low ER expression) were surgically inoculated into #2 mammary fat pad of 6-8 weeks old female mice. I-PDT (Verteporfin, 2 mg/kg and 689-nm laser light) with/without anti-PD-1 i.p. (200 µg/mouse) was performed when tumors reached 8mm. “Cure” was defined as the absence of detectable tumors by caliper measurement and bioluminescence 60 days after I-PDT.
Results: Response to I-PDT alone was compared between TS/A-luc and E0771-luc models with differing levels of ER expression. I-PDT treatment delayed the growth in all TS/A-luc tumors (6/6), whereas only half of E0771-luc tumors (3/6) were delayed. Among the mice that initially responded to I-PDT, 5 out of 6 mice with TS/A-luc and all 3 mice with E0771-luc demonstrated local recurrence, and the cure rates were 16.7% (1/6) for TS/A-luc and 0% (0/6) for E0771-luc models treated with I-PDT alone. Next, ICI treatment alone did not show any change in ER high-expressing TS/A-luc tumor growth, which is similar to what we see in human patients. On the other hand, the combination of I-PDT and anti-PD-1 demonstrated the cure rate of 43.8% (7/16) in TS/A-luc tumors.
Conclusion: Our study showed that I-PDT delayed the tumor growth in both TS/A-luc and E0771-luc syngeneic murine models. Further, I-PDT enhanced the efficacy of ICIs in TS/A-luc model, which expresses high ER. These findings show the potential of I-PDT as a combinatorial approach with ICIs in ER-positive BC.

#1341 A novel ATR inhibitor combined with radiation sensitizes an immunologically cold tumor to immunotherapy.

Mandira Manandhar[1], **Scott J. Bright**[1], Poliana C. Marinello[1], Jayesh B. Majithiya[2], Desiree Piscitello[2], Marco Ranzani[2], Helen Robinson[2], Graeme Smith[2], Gabriel O. Sawakuchi[1]

[1]Radiation Physics, UT MD Anderson Cancer Center, Houston, TX,[2]Artios Pharma, Cambridge, United Kingdom

ATR is a major DNA damage response (DDR) protein and an attractive target to combine with radiotherapy to amplify radiosensitization and immune-sensitization. ATR has several roles in DDR, including DNA double-strand break (DSB) repair through homologous recombination (HR) and S/G2 cell-cycle checkpoint control. Cancer cells can survive radiation-induced complex DNA lesions by their capacity to repair DSB lesions. The disruption of the G2/M checkpoint by ATR inhibition in cells with elevated DNA damage caused by irradiation increases micronuclei numbers, which activate the cGAS-STING pathway, promoting immune signaling. We hypothesize that inhibition of ATR radiosensitizes cancer cells to photons and protons and amplifies immune activation. To test our hypothesis, we treated H1299, H460 and 4T1 cell lines with a novel ATR inhibitor in clinical development, ART0380, prior to irradiation with 6 MV x-rays (photons) or 3.9 keV/µm protons. We then analyzed clonogenic cell survival [sensitization enhancement ratio (SER) and relative biological effectiveness (RBE)], cell cycle populations at 6 and 24 after irradiation, DNA damage (γH2AX and 53BP1 foci) at 1 and 24 h after irradiation, inhibition of HR (Rad51 foci) at 2 and 6 h after irradiation, micronuclei and cGAS colocalized micronuclei at 24, 48 and 72 h after irradiation, and expression of proteins involved in the cGAS-STING pathway at 24 and 48 h after irradiation. For *in-vivo* studies, syngeneic leg allograft tumors in Balb/c mice with the 4T1 murine breast cancer cell line were irradiated with 6 MV x-rays or 3.9 keV/µm protons (3×8 Gy) in combination with 100 mg/kg ART0380 BID for 3 days on/4 days off for 2 weeks. Tumor volumes were monitored. Compared to radiation alone, radiation+ART0380 decreased the G2 population and increased the mitotic population. Cell lines were also radiosensitized by ART0380 treatment (SER>1) and RBE was modulated. ART0380 treatment increased persistence of the DNA damage caused by radiation. We observed significantly more micronuclei and cGAS colocalized micronuclei in the radiation+ART0380 groups compared to radiation alone, validating our hypothesis of increased immune activation. Interestingly, protons+ART0380 induced a higher effect compared to photons+ART0380. To assess if the increased immune activation induced by the combination of ART0380 with radiation is clinically actionable, we combined in vivo radiation+ART0380 to anti-CTLA4 antibody in a tumor model that is resistant to anti-CTLA4 alone. Remarkably, we detected sensitization to anti-CTLA4 when it was combined with both protons+ART0380 or photons+ART0380. Overall, radiation+ART0380 sensitized an immunologically cold tumor to anti-CTLA4, with protons inducing long-lasting tumor regression. Protons combined with ART0380 may be a novel strategy to sensitize radioresistant and immunologically cold tumors to immunotherapy.

#1342 Double knockdown of RAD51 and RAD54 overcame the radioresistance in bladder cancer.

H. Ide[1], T. Murakami[1], E. Kashiwagi[2], S. Kitaoka[1], T. Shishido[3], M. Yamaguchi[1], M. Katsui[3], S. Funakoshi[3], H. Miyamoto[4], M. Oya[1]; [1]Keio University, Tokyo, Japan, [2]The University of Occupational and Environmental Health, Fukuoka, Japan, [3]Tokyo Saiseikai Central Hospital, Tokyo, Japan, [4]University of Rochester School of Medicine and Dentistry, Rochester, NY

Introduction and Objective: Radical cystectomy remains a main treatment for muscle-invasive bladder cancer (MIBC) because outcomes of radiotherapy (RT) have still been insufficient compared to surgery. Recent evidence indicated the involvement of DNA repair pathways with radio-resistance in MIBC. Previous study showed that RAD51 and RAD54 which promoted DNA repair cooperatively, were significantly associated with radio-sensitivity. More recently, our study demonstrated the significant association between estrogen receptor β signaling which was involved in radio-sensitivity in bladder cancer (BC), and their expression in it. However, there has been no study evaluating the relationship between RAD51/54 and radio-sensitivity in it. Therefore, we evaluated this association in BC cells.
Methods: We compared the inhibitory effect of RT on BC cell viability between control and RAD51 and/or RAD54 knockdown BC cells (5637 and T24) cells. Moreover, we evaluated it on tumor growth in a mouse xenograft model between irradiated control and their knockdown cells. We also compared DNA double-strand breaks (DSB) via γ-H2AX foci formation, and DNA repair gene expressions in these cells after RT.
Results: We then established radio-resistant cells and confirmed considerable elevation of the expressions of RAD51 and RAD54. *In vitro* experiments showed that RT reduced the viability in RAD51 or RAD54 knockdown cells more significantly than control cells. Moreover, double knockdown cells significantly reduced the cell viability more than each knockdown cell after irradiation *in vitro* and *in vivo*. Double knockdown of RAD51 and RAD54 resulted in a delay in DSB repair after RT.
Conclusions: These results suggest that inhibition of both RAD51 and RAD54 has a potential of enhancing the cytotoxic effects of radiation in BC cells.

TUMOR BIOLOGY: Uncovering How Secreted Factors Sculpt the Tumor Microenvironment
Poster Session

#1346 MYC-YTHDF1-IRF2BP2 axis drive tumor metastasis and immune escape in CMS2 colorectal cancer.

Ting Li[1], Yao Shang[2], Dongwen Chen[2], Chong Chen[2], Xinxin Huang[2], Yiran Bie[2], Zhengyu Wei[2], Zhengran Zhou[2], Chuling Hu[2], Xuanhui Liu[2], Yufeng Chen[2], **Peishan Hu**[2]

[1]Department of Gastroenterology and Urology, The Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University/Hunan Cancer Hospital, Changsha, China,[2]Department of Colorectal Surgery, The Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou, China

Colorectal cancer (CRC) has a particularly low 5-year survival rate in patients with metastases. Based on the molecular and pathological characteristics of CRC, the disease can be classified into four consensus molecular subtypes (CMS1-4), with CMS2 being the most prevalent. CMS2 colorectal cancer is characterized by sustained activation of MYC, which drives the elevated expression of the m6A-binding protein YTHDF1. YTHDF1 (YTH m6A RNA-binding protein 1) is an m6A reader that facilitates mRNA translation and plays critical roles in tumor progression. However, its specific mechanisms in CRC remain poorly understood. Investigating the functional role of YTHDF1 could provide valuable insights for developing precise therapeutic approaches and combination treatment strategies for CRC.Interferon regulatory factor 2-binding protein 2 (IRF2BP2) encodes a member of the IRF2BP family of transcriptional regulators. IRF2BP2 is ubiquitously expressed in normal and tumor tissues across various organisms and is involved in regulating numerous cellular processes. However, its specific roles and mechanisms in CRC have not been elucidated. This study aims to systematically investigate the MYC-driven activation of the YTHDF1-IRF2BP2 axis and its impact on distant metastasis and immune evasion in CRC. Our findings aim to provide a foundation for identifying novel prognostic biomarkers and potential therapeutic targets for CRC.We analyzed YTHDF1 expression in CMS2 colorectal cancer using publicly available databases and patient samples from our center. Our findings revealed that CMS2 CRC exhibits elevated YTHDF1 expression, which is positively correlated with MYC expression. To validate the regulatory relationship between MYC and YTHDF1, we knocked down MYC in CRC cell lines. Western blot analysis demonstrated that MYC knockdown significantly suppressed YTHDF1 expression. Given that MYC is a downstream effector of the WNT signaling pathway, we treated CRC cells with the WNT activator Wnt3a, which resulted in increased MYC protein expression alongside enhanced YTHDF1 protein levels. Dual-luciferase reporter assays confirmed that MYC functions as a key transcriptional regulator, directly promoting YTHDF1 transcription.To identify downstream targets of YTHDF1, we performed RIP-Seq and proteomic profiling. Among the identified targets, IRF2BP2 was selected for further validation. Correlation analysis, Western blot, and RIP-PCR assays demonstrated that YTHDF1 regulates IRF2BP2 expression in an m6A-dependent manner. Functional studies and in vivo experiments showed that knocking down YTHDF1 or IRF2BP2 significantly inhibited CRC cell migration. These findings suggest that YTHDF1 promotes distant metastasis and immune evasion in CRC by regulating IRF2BP2, thereby contributing to CRC progression and malignancy.

#1347 The acidic tumor microenvironment drives stemness and chemoresistance in colorectal cancer through DHCR7.

Yiran Bie[1], Zhengyu Wei[2], Dongwen Chen[1], Yao Shang[1], Zhengran Zhou[1], Chong Chen[2], Chuling Hu[1], Xinxin Huang[1], Yufeng Chen[2], **Peishan Hu**[1]

[1]Guangdong Institute of Gastroenterology, The Sixth Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China,[2]Department of Colorectal Surgery, The Sixth Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China

Relapse and chemoresistance are critical determinants of poor prognosis in colorectal cancer (CRC). Cancer stem cells (CSCs) significantly contribute to chemoresistance and relapse in CRC. The acidic tumor microenvironment (TME) not only drives the emergence of CSC phenotypes but also impacts cholesterol metabolism reprogramming. However, whether the acidic TME influences CSC phenotypes and chemoresistance by modulating cholesterol metabolism remains to be elucidated.In this study, we utilized the ARGO and TCGA databases to identify cholesterol metabolism-related genes significantly upregulated under acidic conditions. The gene responsible for the final step in cholesterol biosynthesis, DHCR7(7-dehydrocholesterol reductase), was identified as highly expressed in advanced staging CRC and closely associated with poor prognosis in CRC patients.We confirmed that the acidic TME regulates cholesterol metabolism via DHCR7, thereby modulating cancer stemness and chemoresistance in CRC. Using DHCR7-overexpressing and control cell lines, we conducted subcutaneous tumorigenesis experiments in nude mice. Intratumoral injection of the DHCR7-specific inhibitor AY9944 in combination with oxaliplatin demonstrated that AY9944 enhances the cytotoxic effect of oxaliplatin on CRC by inhibiting DHCR7.To investigate the downstream mechanisms of DHCR7 in regulating the malignant phenotype of CRC, we performed transcriptomic profiling of the overexpressing and knockout cell lines. Differential gene analysis revealed an inverse correlation between DHCR7 and CLCA1 expression. Using CHIP-qPCR and dual-luciferase reporter assays, we confirmed that nuclear DHCR7 binds to the promoter region of CLCA1, suppressing its transcription and activating the Wnt/β-catenin signaling pathway. Additionally, at the cellular and organoid levels, we verified that cytoplasmic DHCR7 enhances cholesterol synthesis, thereby upregulating cancer stemness marker LGR5 expression.The acidic tumor microenvironment promotes DHCR7 expression. DHCR7 functions as a transcription factor in the nucleus to inhibit CLCA1 transcription and activate the Wnt/β-catenin pathway. Concurrently, it enhances cholesterol synthesis in the cytoplasm, leading to increased LGR5 expression. Together, these mechanisms drive the stemness and chemoresistant phenotypes of CRC cells. Our findings suggest that targeting cholesterol metabolism, particularly the key enzyme DHCR7, could inhibit CRC stemness and drug resistance, thereby improving patient prognosis.

#1348 Investigation of sex disparity in hepatocarcinogenesis using a hydrodynamic tail vein injection mouse model.

Araceli Selena Bernal[1], Morgana McLaughlin[1], Ankur Tiwari[2], Brittany Barre[1], Francisco Cigarroa[2], LuZhe Sun[1]

[1]Cell Systems and Anatomy, UT Health Science Center at San Antonio, San Antonio, TX,[2]Department of Surgery, UT Health Science Center at San Antonio, San Antonio, TX

The incidence of hepatocellular carcinoma (HCC), the major form of liver cancer, is two to three times higher in men than in women. Understanding the gender disparity in HCC incidence is challenging due to HCC development being multifactorial, including variations in risk factors, behaviors, and hormones. The liver is a sexually dimorphic organ, and estrogen has been proven to have a protective effect against the development of HCC, although the mechanism is not fully understood. We used the hydrodynamic tail vein injection (HTVI) mouse model of HCC to investigate potential mechanisms underlying the sex disparity of HCC incidence. This model eliminates social and behavioral aspects of gender disparity and allows us to determine if there is biological sex disparity and what role estrogen and androgens play in tumor development. In our preliminary study, the expression vectors of oncogenes, ΔN90-β-catenin and c-MET, to be integrated into the hepatocyte genome via transposons, were co-injected with a Sleeping Beauty (SB) transposase to induce HCC in FVB/NJ mice. Based on plasma alpha-fetoprotein (AFP), an HCC blood biomarker, male FVB/NJ mice had a significantly higher mean AFP trend in comparison to females, indicating that the males developed liver tumors at a faster rate than females. ΔN90-β-catenin and c-MET gene copy number were confirmed to be equally transfected and integrated in both male and female FVB/NJ tumors by quantitative PCR (qPCR). The expression of ΔN90-β-catenin and c-MET transgenes was also shown to be similar between male and female mouse HCC tumors by RT-qPCR and RNA-seq. We plan to repeat the HTVI of ΔN90-β-cat, c-MET, and SB to validate that the males will develop liver tumors at a faster rate than female mice. We will also Western Blot to determine if there is sex disparity in the abundance of the transfected oncogene proteins. In addition, we will compare the abundance of various types of immune cells present in the tumor immune microenvironment (TIME) to interpret potential sex-dependent immune cell differences in the tumor liver. Our research has the potential to uncover mechanisms underlying the sex-dependent disparity in HCC development and progression and the contribution of immune cells to the sex disparity.

#1349 Single-cell transcriptomic architecture deciphering the complexity of tumor microenvironment in ampulla of Vater carcinoma.

Hyemin Kim[1], Young Hoon Choi[1], Hyoung-oh Jeong[2], So Jeong Yoon[1], Yo Han Jeon[1], Hongbeom Kim[1], Sang Hyun Shin[1], Jin Seok Heo[1], In Woong Han[1], Kee-Taek Jang[1], Se-Hoon Lee[1], Kwang Hyuck Lee[1], Kyu Taek Lee[1], Jong Kyun Lee[1], Semin Lee[2], Joo Kyung Park[1]

[1]Samsung Medical Center, Seoul, Korea, Republic of,[2]Ulsan National Institute of Science and Technology, Ulsan, Korea, Republic of

Background: Ampulla of Vater carcinoma (AVC) is a rare gastrointestinal tumor that is associated with a high mortality rate and it's often diagnosed at later stages due to lack of clinical symptoms. Elucidating complex ecosystems and molecular features of AVC is pivotal to proactive cancer prevention and optimal therapeutic intervention.
Methods: We performed single-cell RNA sequencing (scRNA-seq) of treatment-naïve AVC biopsies (N=8), and bulk RNA sequencing of surgically resected tissues (N=62) to investigate the transcriptomic signature in tumor microenvironment (TME) of AVC.
Results: We analyzed the single-cell transcriptome of 34,672 cells and epithelial cells were classified into 4 subtypes by consensus non-negative matrix factorization (cNMF) based on their gene expression profiles. Among them, the $KRAS^{high}$ subtype showed more increased copy number variation than other subtypes and was less differentiated with a high stemness score. Moreover, the $KRAS^{high}$ subtype was reversely related to overall survival (OS) and enriched with granzyme K^{+} CD8 T cells. Deconvolution of bulk RNA-seq data validated the poor OS and progression-free survival (PFS) in the AVC patients with the $KRAS^{high}$ subtype. Also, it was revealed that this subtype was associated with early tumor recurrence in AVC.
Conclusions: We understood the heterogenous TME of AVC and found the prognostic biomarker to predict postoperative recurrence and survival in AVC.

#1350 *ESR1* expression as a predictive biomarker for immune checkpoint inhibitor efficacy in estrogen receptor-positive, HER2-negative breast cancer.

Jun Arima[1], Kohei Chida[2], Rongrong Wu[3], Kohei Taniguchi[4], Mitsuhiko Iwamoto[1], Takashi Ishikawa[3], Kenichi Hakamada[5], Sang-Woong Lee[1], Kazuaki Takabe[2]

[1]Department of General and Gastroenterological Surgery, Osaka Medical and Pharmaceutical University, Takatsuki, Japan,[2]Roswell Park Comprehensive Cancer Center, Buffalo, NY,[3]Department of Breast Surgery and Oncology, Tokyo Medical University, Tokyo, Japan,[4]Center for Medical Research & Development, Division of Translational Research, Osaka Medical and Pharmaceutical University, Takatsuki, Japan,[5]Department of Gastroenterological Surgery, Hirosaki University Graduate School of Medicine, Hirosaki, Japan

Introduction: Immune checkpoint inhibitors (ICIs) hold promise for preventing late recurrence, a major cause of mortality in estrogen receptor-positive/HER2-negative (ER+/HER2-) subtype, which constitutes the majority of breast cancer (BC) cases. The response to ICIs is worse in this subtype compared to triple-negative breast cancer (TNBC). Recent clinical trials, CheckMate 7FL and KEYNOTE-756, have uniformly demonstrated that ER+/HER2- BC with less Estrogen Receptor (ER)-α staining in cancer cells shows a better response to ICIs. Considering the relatively precise quantification achievable through transcriptomic analysis, we hypothesized that ER-α gene (ESR1) expression level is a negative predictive biomarker of ER+/HER2- BC response to ICIs.
Methods: Total of 4138 BC patients from ten cohorts (GSE271080, I-SPY2 [GSE173839], TCGA, METABRIC, SCAN-B [GSE96058], GSE246613, GSE255107/GSE248288, GSE161529, GSE167036) were analyzed. High expression of ESR1 was defined as the top two-thirds of expression levels for each gene.
Results: In the ER+/HER2- subtype, high ESR1 expression was associated with significantly less intratumoral-Tumor Infiltrating Lymphocytes in both TCGA and METABRIC cohorts ($p<0.001$). High ESR1 expression was also associated with less infiltrations of CD8+ T cells, dendritic cells (DCs), cancer-associated fibroblasts (CAFs), a lower CD8/Treg ratio, and a lower cytolytic activity consistently in TCGA, METABRIC, SCAN-B cohorts (all $p<0.01$). Further, the low ESR1 group significantly enriched multiple immune-related gene sets, including IL2 signaling, TNF-α signaling, and interferon-γ response. In the I-SPY2 trial, high ESR1 expression correlated with lower pathological complete response rates after neoadjuvant immunotherapy ($n=50$, $p<0.001$). The predictive accuracy of ESR1 expression (AUC=0.88) exceeded that of PDL1 (AUC=0.60) in HR+/HER2- BC. Single-cell analysis (GSE246613) revealed negative associations between ICI response and ESR1 expression in cancer/normal epithelial cells, endothelial cells, and plasmablasts, as well as activation of estrogen signaling in these cell types. Notably, CAFs showed a positive association between ESR1 expression, estrogen signaling activation, and ICI response.
Conclusion: ESR1 expression is a promising predictive biomarker for ICI response in ER+/HER2- BC. Additionally, CAF infiltration and estrogen signaling activation in CAFs may enhance ICI response, highlighting their potential as therapeutic targets.

#1352 Extracellular adenosine suppress CD8+ T cell attraction resulting in poor prognosis in pancreatic ductal adenocarcinoma.

Eriko Katsuta[1], Yasushi Ichikawa[2], Kazuaki Takabe[3], Subhamoy Dasgupta[3], Daisuke Ban[1]

[1]Institute of Science Tokyo, Tokyo, Japan,[2]Yokohama City University, Yokohama, Japan,[3]Roswell Park Comprehensive Cancer Center, Buffalo, NY

Background: CD73 is a cell surface enzyme. Extracellular ATP and ADP are converted to AMP catalyzed by CD39, then AMP is converted to adenosine by CD73 in the tumor microenvironment (TME). Accumulating extracellular adenosine is known to play an immune suppressive role in TME of various cancers, however, its role in pancreatic ductal adenocarcinoma (PDAC) remains elusive. This study aimed to explore the role of CD73 in PDAC.
Methods: To examine CD73 role in PDAC, we used both bioinformatical and experimental approaches. We employed a publicly available dataset, The Cancer Genome Atlas (TCGA) to analyze clinical relevance by bioinformatic approaches. In order to confirm the findings and explore the mechanisms from the bioinformatical analyses, we conducted in vitro, in vivo experiments. Murine PDAC cell line, Panc02 was used together with overexpression technique.
Results: We found that high expression of CD73 was significantly associated with poor disease-free survival ($P<0.001$) and overall survival ($P=0.002$) in PDAC patients of TCGA cohort. Multivariate analyses revealed that CD73 was an independent prognostic factor in both disease-free ($P=0.001$) and overall survival ($P=0.005$). A deconvolution algorism, xCell showed that the CD73 high expression tumor showed less CD8+ T cell infiltration compared to the counterpart in TCGA cohort ($P=0.009$). There was no significant survival difference between the control and CD73 overexpressing PDAC cell bearing mice of carcinomatosis model when the experiment was conducted in immune deficient mice. On the other hand, when we implanted the cells into immune competent mice, CD73 overexpressing tumor showed significantly shorter survival ($P<0.001$). Further, the CD73 overexpressing tumor had less CD8+ T cell infiltration compared to the control mice ($P=0.041$). Co-culture experiments revealed that CD8+ T cell proliferation was suppressed when they were cultured with CD73 overexpression PDAC cells ($P=0.011$).
Conclusion: We found that high expression of CD73 was associated with poor prognosis along with less CD8+ T cell infiltration in PDAC. These results suggest that accumulated adenosine in the TME suppressed CD8+ T cell infiltration in CD73 high expression tumor.

#1353 Characterizing biological functional changes in cancer tumor microenvironment by integrating histology-based gene imputation with scRNA-seq foundation models.

Paveethran Swaminathan[1], Pengtao Dang[1], Haiqi Zhu[2], Zheng An[1], Xin Lu[1], Zhi Li[1], Yue Fang[1], Min Yang[1], Yuhui Wei[1], Tingbo Guo[1], Xinyu Zhou[2], Xiao Wang[2], Jia Wang[2], Chi Zhang[1], Sha Cao[1]

[1]Center for Biomedical Data Science, Oregon Health & Science University, Portland, OR,[2]Indiana University-Bloomington, Bloomington, IN

Background and Aim: Spatially resolved transcriptomics (SRT) offers insights into tumor microenvironments (TME), mapping gene and cell activities across tissue regions. While systems biology methods utilize SRT data to characterize tissue and cell neighborhood variations, high costs and limited scalability hinder large-scale applications and clinical correlations. In contrast, histological imaging (HI) provides accessible, high-resolution morphological insights with clinical context. Existing HI-based gene expression imputation methods rely on small datasets and cover a limited gene fraction, leaving much of the transcriptome unexplored. This study develops a robust framework for imputing spatially resolved gene expression profiles from HI data by integrating HI-based imputation with scGPT - a foundation models trained on single-cell RNA-seq (scRNA-seq) data. We hypothesize HI data can impute genes linked to TME, tissue structure, and cell types, while scGPT infers additional genes using gene and cell type-specific relationships.
Methods: The proposed framework merges an advanced HI-based gene imputation algorithm with scGPT to predict spatially resolved full-transcriptome profiles. The HI-based model first imputes 500-1,000 tissue structure- and cell type-dependent genes. We enhanced existing methods by (1) incorporating cross-pixel multi-attentions for spatial adjustments, (2) enabling residual connections to preserve early features, and (3) introducing a composite loss function for spatial coherence, gene-gene interaction preservation, and distribution alignment. Tissue annotations provide contextual information that enhances model predictivity. scGPT refines and expands predictions into whole gene profiles, leveraging its robust architecture to capture complex molecular relationships.
Results: The framework demonstrates strong performance across unseen specimens, achieving low mean squared error (0.38) and high correlations (0.79) while mitigating batch effects. Spatial heatmaps capture tissue heterogeneity, with tumor, immune, and stromal boundaries aligning well with annotations. Gene Enrichment analysis shows the model's capability to capture immune, stromal, and tumor compartments, though challenges remain for underrepresented genes. Integrating scGPT generates accurate and meaningful whole-genome profiles. This enables implementation of systems biology models for transcriptomics - including pathway, metabolism, stemness and CNV analysis - into HI data analysis.
Conclusion: This framework bridges histology and transcriptomics by combining advanced imputation algorithms with powerful capabilities of scGPT, enabling scalable, spatially resolved gene expression mapping. Its robust extrapolation transforms understanding of tissue biology and the TME complexity.

#1354 Identification of *GREM-1* and *GAS6* as specific biomarkers for cancer-associated fibroblasts derived from patients with non-small cell lung cancer.

B.-G. Kim[1], K. Park[2], M. Hwang[3], H. Lee[3], K.-M. Park[4], J. Choe[3], S. Shin[3], B.-H. Jeong[3], K. Lee[3], J. Lee[3], Y. Jeon[3], J. Cho[3], H. Kim[3], W.-Y. Park[2], **S.-W. Um**[3];

[1]Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of, [2]Samsung Genome Institute, Seoul, Korea, Republic of, [3]Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of, [4]Kyung-Mi, Samsung Medical Center, Sungkyunkwan University Sc, Korea, Republic of

Background/Aims: Cancer-associated fibroblasts (CAFs) play a crucial role in the tumor stroma. They are primarily associated with tumor maintenance and promotion via diverse mechanisms in cancer. Using RNA sequencing, we conducted an analysis aimed at distinguishing distinct markers indicative of CAFs compared to normal fibroblasts (NFs) within the context of non-small cell lung cancer (NSCLC) across diverse conditions.

Methods: We conducted RNA sequencing analysis on 22 CAFs and 11 NFs from patients with NSCLC. CAFs and NFs were isolated and cultured from tumor tissues (primary tumor or metastatic lymph nodes) and from the most distant non-tumor tissues of the resected lungs, respectively, in NSCLC patients. We performed bulk-RNA sequencing analysis using total RNA from cultured cell pellets of CAFs or NFs. Differential expression, gene set enrichment and CAF subpopulation prediction analyses were performed.

Results: Notably, *COL11A1*, *GREM1*, *CD36*, and *GAS6* showed higher expression in CAFs than in NFs, whereas *TNC* and *CXCL2* were more highly expressed in NFs. *CD36* levels were elevated in CAFs from lymph nodes (LN-CAFs) compared with those from lung specimens (Lung-CAFs) and NFs. *COL11A1* levels in Lung-CAFs surpassed those in LN-CAFs and NFs. Both *GREM1* and *GAS6* showed strong expression in Lung-CAFs and LN-CAFs relative to NFs. CAFs exhibited features of the myofibroblast CAF subpopulation, whereas NFs displayed traits of the antigen-presenting CAF subtype.

Conclusions: In NSCLC, *GREM1* and *GAS6* can be valuable diagnostic and therapeutic targets for CAFs from primary tumors and metastatic sites; they warrant further study.

#1355 Inflammation-related gene signature as a predictor of survival in adrenocortical carcinoma: machine learning and bioinformatics analysis.

Jaber H. Jaradat[1], Lujia Chen[2], Anwaar Saeed[3], Azhar Saeed[4]

[1]Mu'tah University, Al-Karak, Jordan,[2]Department of bioinformatics, University of Pittsburgh, Pittsburgh, PA,[3]Department of Medicine, Division of Hematology & Oncology, University of Pittsburgh Medical Center, Pittsburgh, PA,[4]Department of Pathology and Laboratory Medicine, University of Vermont, Burlington, VT

Background: Adrenocortical carcinoma (ACC) is a rare malignancy with poor outcomes. Inflammation is increasingly recognized to play a significant role in the tumorigenesis of ACC. This study examines the relationship between inflammation-related genes and survival outcomes in patients with ACC.

Methods: We analyzed the RNA-seq and clinical data of 79 patients with ACC obtained from TCGA. Principal component (PC) analysis was performed on the expression data of 222 inflammation-related genes (MSigDB) to categorize patients into high and low inflammation groups (IG) based on the median of the first PC. Differentially expressed genes (DEGs) related to inflammation between the two groups were identified. Stepwise AIC identified the best model based on all DEGs, then genes were fitted to LASSO to mitigate multicollinearity. Uni- and multivariate Cox proportional hazards were used to identify independent predictors of survival. PC analysis was then conducted on the independent predictors of survival genes to compute the inflammation score (IS), with a cutoff point determined based on survival data. The correlation between IS and overall survival (OS) and disease-free survival (DFS) was assessed using the log-rank test. Clinical variables were investigated for possible association with the IS using Welch's t-, chi-square, Fisher's exact, and Wilcoxon tests. Immune infiltration analysis was conducted using CIBERSORT.

Results: Five inflammation-related DEGs associated with survival were identified, namely *ITGB3*, *MARCO*, *MSR1*, *SLAMF1*, and *SELE* with the first 2 identified as independent predictors. *ITGB3* is associated with worse OS (HR= 1.29, P= 0.036) and *MARCO* is linked to worse DFS (HR= 1.17, P= 0.048). Patients were split into high (n=39) and low (n=40) IGs based on inflammation-related DEGs. We calculated IS based on independent predictors of survival genes and the patients were grouped into high and low IS with a cutoff point of - 0.47 determined based on OS data (low IS n= 20, high IS n= 59). Log-rank test revealed significantly lower OS and DFS in the high IS group in comparison to the low IS group. No significant associations were detected between the IS groups and sex, age, T, N, & M stages, or the disease stage. The absolute immune fraction score was significantly higher in the high IS group compared to the low IS group, with means of 26.59 and 11.96, respectively ($P < 0.00001$, Welch's t-test). Gene Ontology (GO) analysis of the DEGs showed overrepresentation in biological processes associated with cell adhesion in high IS group and overrepresentation of processes related to mitochondrial function and energy production in the low IS group.

Conclusions: Our findings underscore the potential link between inflammation and survival outcomes in ACC and support the use of an inflammation-related gene signature as a prognostication tool for survival in this aggressive cancer type.

#1356 The lactylation of SLC26A3 in acidic micro-environment contributes to colorectal carcinoma malignant progression.
Chong Chen, Peishan Hu, Yufeng Chen

The Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou, China

A recently discovered post-translational modification, lactylation, can regulate the structure and function of proteins, influencing cellular functions and disease progression. However, its role in tumor progression remains unclear.This study explored the contribution of lactylation modification of solute carrier family 26 member 3 (SLC26A3) in acidic microenvironments to the malignant progression of colorectal carcinoma (CRC). The findings revealed that the acidic tumor microenvironment reduced the stability and expression of SLC26A3 by promoting its lactylation. co-IP experiment results showed SLC26A3 interacted with RNA-binding proteins HuR and CUGBP1, and when the expression of SLC26A3 was decreased, its binding capacity to HuR/CUGBP1 was weakened. This led to an increased interaction of HuR and CUGBP1 with a subset of oncogenic mRNAs, regulating their stability and expression, ultimately promoting the progression of malignant tumors. The study also indicated that the expression of SLC26A3 was negatively correlated with the expression of tumor stem cell markers, and low expression of SLC26A3 promoted the stemness of CRC cells. Furthermore, the expression of SLC26A3 affected the resistance to oxaliplatin (OXA), invasiveness, and migratory ability of CRC cells. In animal models, normalizing the tumor acidic microenvironment and inducing the expression of SLC26A3 inhibited the development of CRC.These findings highlight the role of SLC26A3 as a potential suppressor of CRC recurrence, drug resistance, and metastasis, providing new insights for improving the clinical treatment and prognosis of CRC.

#1357 miR-16-5p and miR-124-3p block the immunosuppressive phenotype of GBM cells in vitro.

TC Taylor[1], Amanda Johnson[2], Harmon Khela[1], Jack Korleski[3], Sophie Sall[1], Bachchu Lal[1], Karen Smith-Connor[1], Hernando Lopez-Bertoni[1]

[1]Hugo W. Moser Research Institute at Kennedy Krieger., Baltimore, MD,[2]Department of Neurology, Johns Hopkins University School of Medicine., Baltimore, MD,[3]Department of Internal Medicine, Mayo Clinic School of Graduate Medical Education., Baltimore, MD

A hallmark of GBM is its highly-immunosuppressive tumor microenvironment (TME). GBM stem-like cells (GSCs) can evade immune surveillance to allow for continued tumor growth and propagation. GSCs regulate the immune TME through recruitment and production of immunosuppressive cells and factors via tumor cell-intrinsic mechanisms. Blocking these cell-intrinsic immunosuppressive mechanisms is widely viewed as a way to enhance the effects of current immune therapies. We recently showed that TGFβ type II receptor (TGFBR2) promotes a mesenchymal transition in GBM cells characterized by high expression levels of immunosuppressive genes NT5E (CD73), ENTPD1 (CD73), LGALS1 (galectin-1), CD274 (PD-L1), PDCD1LG2 (PD-L2), and TGFB1. miRNA network analysis identified miR-16-5p and miR-124-3p as high-confidence candidates to block the expression of these immunosuppressive signaling axis in GBM cells. Transgenic miR-16-5p and miR-124-3p simultaneously reduced expression of CD274, ENTPD1, NT5E, TGFB1, and LGALS1 and increased tumor cell killing of PBMC-derived CD4+ and CD8+ T cells. We also measured an increase in drivers of the CD8+ effector state and a decrease in expression of exhaustion marker PD1 in PBMC-derived CD8+ T cells. In addition to the effects on CD4+ and CD8+ T cell activation and function, miRNA-mediated knock-down of this immunosuppressive gene signature in tumor cells also resulted in a decrease in CD206 expression and reduced release of anti-inflammatory chemokines in THP1 cells. These findings demonstrate that miRNA-based gene inhibition can block cell-intrinsic immunosuppressive pathways to enhance anti-tumor immune cell function in vitro and predicts that delivering these miRNAs using advanced nanocarriers will cooperate with current immunotherapies in vivo.

#1358 Single nuclei sequencing reveals altered gene expression and immune signaling in tumors from obese endometrioid adenocarcinoma patients.

Jessica L. Long[1], Sophia Agrusa[1], Swornalata Pukhrambam[1], Areebah Qazi[1], Katherine Gurdziel[2], Julie Boerner[1], Michael Wilson[1]

[1]Wayne State University School of Medicine, Detroit, MI,[2]Genome Sciences Core, Wayne State University, Detroit, MI

Over the past several decades, the incidence of endometrial cancer has risen. This trend is largely attributed to the increased prevalence of obesity and is especially pronounced in endometrioid adenocarcinoma, the most common histologic subtype of uterine cancer. These cancers are regarded as hormonally driven tumors and are linked to obesity through the production of aromatase, an enzyme that converts androgens into estrogen, in peripheral adipose tissue. Though the association between obesity and endometrial cancer is well-established, whether excess adiposity has an effect outside of this mechanism—and exactly how that hormonal imbalance perturbs the tumor and its microenvironment—is not well understood. Thus, to examine the impact of obesity on the cellular diversity and transcriptome of endometrial cancer and its microenvironment, we integrated single-nuclei RNA-seq data from seven obese and six normal-weight patients' endometrioid tumor samples. Through evaluation of marker gene expression patterns, we annotated seven clusters distributed across normal-weight and obese tumor samples. To pinpoint major changes in biological networks in tumor cells from obese and normal-weight patients, we performed single-nucleus pathway gene ontology analysis on differentially expressed genes. We then independently conducted Weighted Gene Correlation Network Analysis on the cancer cells in our normal and obese groups and compared the resultant networks. Our analysis revealed a large number of differentially expressed genes in both our cancer cluster and macrophage subcluster. In the cancer cluster, most of the differentially regulated genes were associated with estrogen signaling and epithelial-mesenchymal transition. The results from our Weighted Gene Correlation Network Analysis revealed large differences in the networks containing estrogen receptors. To model obesity in vitro, we treated 12Z endometrioid cells and KLE endometrioid adenocarcinoma cells with adipose-conditioned media derived from human omental tissue, which revealed large differences in cellular morphology. In our macrophage subcluster, our gene ontology analysis yielded differences in macrophage organization and function. Few tumor-infiltrating lymphocytes were detected, which is consistent with the classification of most endometrial cancers as immunologically “cold.” Overall, our results show that obesity influences gene expression in the endometrioid adenocarcinoma tumor microenvironment and tumor itself. Furthermore, the outcomes of our experiments provide additional insights into the impact of obesity on endometrioid adenocarcinoma outside of its role in estrogen signaling.

#1359 iNOS inhibition reprograms the tumor microenvironment enhancing antitumor immunity in obesity associated triple negative breast cancer (TNBC).

Ivonne Uzair[1], Fotis Nikolo[1], Sean Hynes[2], Gianmarco Melone[1], Karina Ortega[1], Jenying Deng[1], Wei Qian[1], Jianying Zhou[1], Liliana Guzman[1], Christoforos Thomas[1], Keith Chan[1], Jenny Chang[1]

[1]Houston Methodist Research Institute, Houston, TX,[2]University of Galway, Galway, Ireland

Purpose of the study: Obesity fosters an adverse tumor microenvironment (TME) characterized by immune suppression, metabolic dysfunction, and pro-tumoral cytokines. Elevated nitric oxide (NO) levels produced by inducible nitic oxide synthase (iNOS) exacerbate vascular dysfunction and metastasis. This study investigates whether combining the NOS inhibitor N(G)-monomethyl-L-arginine (L-NMMA) with docetaxel enhances antitumor efficacy in an obese TNBC model. Experimental Procedures: An orthotopic TNBC model was developed by injecting E0771 cells into the mammary fat pad of C57BL/6 mice fed either a normal diet (ND) or high-fat diet (HFD). Upon tumors reaching 100 mm^3, mice were randomized into vehicle and L-NMMA treatment groups. Immune phenotyping and TME composition were assessed using digital spatial transcriptomics. Results: HFD mice showed significantly accelerated tumor growth and elevated pro-inflammatory cytokines (TNF-α, IL-6, IL-17) and G-CSF levels, alongside reduced T-cell recruiting chemokines (Eotaxin, RANTES), indicative of a pro-tumoral inflammatory state. L-NMMA combined with docetaxel reduced tumor growth and lung metastases compared to single treatments. Heatmap clustering revealed HFD tumors exhibit a distinct immune profile compared to ND or HFD+LNMMA tumors. L-NMMA treatment decreased immunosuppressive macrophages and exhaustion markers, shifting the TME toward an immune-supportive phenotype like ND tumors. L-NMMA-treated HFD tumors displayed decreased expression of proliferation (Ki67) and survival markers (p53, pAktSer473). Immune profiling revealed fewer cancer stem cells (CSCs; $CD44^+CD24^-$), M2 macrophages ($F4/80^+CD206^+$), and exhausted $CD8^+$ T cells ($PD1^+$) in LNMMA-treated tumors, alongside increased $CD4^+$ T cells, dendritic cells, and plasma cells. Moreover, epithelial-mesenchymal transition (EMT) markers shifted, with elevated epithelial cells ($CD71^+$) and reduced mesenchymal cells (Vim^+), further supporting an antitumor shift. Conclusion: L-NMMA effectively remodels the obesity-altered TME, reducing tumor growth and metastasis while promoting an immune-supportive environment. Targeting iNOS with L-NMMA in combination with chemotherapy emerges as a promising therapeutic strategy to enhance TNBC treatment, particularly for obese patients.

#1360 Neutrophil extracellular traps establish the pre-metastatic niche in the omentum by expanding innate-like B cells that express IL-10.

WonJae Lee[1], Song Yi Ko[1], Hironari Akasaka[1], Melanie Weigert[2], Ernst Lengyel[2], Honami Naora[1]

[1]Molecular and Cellular Oncology, UT MD Anderson Cancer Center, Houston, TX,[2]Department of Obstetrics and Gynecology, The University of Chicago, Chicago, IL

Disseminated cancer cells in the peritoneal fluid frequently implant on the omentum, an immune cell-rich fatty tissue that drapes from the stomach, but the underlying mechanisms are poorly understood. In this study, we identified that innate-like B cells accumulate in the uninvolved omentum of mice and women with early-stage ovarian cancer concomitantly with the release of chromatin fibers by neutrophils called neutrophil extracellular traps (NETs). Studies using genetically modified NET-deficient mice, pharmacologic inhibition of NETs, and adoptive B cell transfer showed that NETs induce expression of the chemokine CXCL13 in mesothelial cells in the pre-metastatic omentum, stimulating recruitment of innate-like B cells from the peritoneal cavity. Furthermore, studies using IL-10-reporter mice showed that NETs stimulate innate-like B cells to produce IL-10 that in turn expands regulatory T cells in the pre-metastatic omentum and potentiates metastasis at this site. *Ex vivo* studies showed that NETs elicit IL-10 production in innate-like B cells by generating reactive oxygen species and by inactivating SHP-1, a phosphatase that inhibits B cell activation pathways, through dissociating SHP-1 from CD22. These findings reveal that NETs alter immune cell dynamics in the pre-metastatic omentum and render this niche permissive for cancer cell colonization.

#1361 Severe hyperglycemia and hyperlipidemia alters tumor growth and immune profiles in syngeneic tumor models.

Hao Sun, Ying Zheng, Ryan Strauch, Yi Zhang, Minqi Huang, Luke Tsai

HD Biosciences Co., Ltd., San Diego, CA

Background and Objective: The global prevalence of obesity is increasing rapidly, causing the rise of obesity-associated diseases, such as diabetes and cancers. It has been established that obesity increases the risks for various types of cancers. Over the past decades, studies have proposed that obesity promotes cancer progression through mechanisms like chronic low-grade inflammation, which alters immune responses and the tumor microenvironment, ultimately affecting tumor response to therapies. Previous studies in diet induced obese models showed T cells in the colorectal cancer are reduced in obesity. However, it is not clear how the tumor microenvironment is changed under both severe hyperglycemia and hyperlipidemia in different types of cancers. This poster will highlight the changes of immune cell population in syngeneic tumor bearing mice with various cancer types in genetic obese models characterized by severe hyperglycemia and hyperlipidemia. Our study provides a detailed comparisons of how severe hyperglycemia and hyperlipidemia change the immune cells populations and tumor microenvironment in cancer. Notably, these changes observed in different cancer models provide insights into why the risk of various cancer types increases differently under conditions of severe obesity. In addition, we also provide a platform for studies on cancer therapeutics in hyperglycemia and hyperlipidemia mouse models.

Method and Result: db/db mice and C57BL/6 mice were fed with normal chow diet or high fat diet were used to examine syngeneic tumor growth kinetics. Changes of immune cells populations in different tissues were evaluated by FACS analysis. Both B16F10 melanoma and MC38 colorectal cancer tumors in db/db mice, with severe hyperglycemia and hyperlipidemia, grew significantly faster comparing to lean mice. Changes immune cells populations were observed in both models but differently. Although increased macrophages population is observed in both models, the obesity resulted changes of activated cytotoxic T cells and activated helper T cells were significant in blood and tumors from MC38 bearing mice but not B16F10 bearing mice. The results indicate that MC38 colorectal cancer exhibits more pronounced immune cell changes under obese conditions. RNAseq analysis in the tumor also revealed altered metabolic pathways and oncology pathways in the db/db mice, consistent with the observed faster tumor growth in db/db mice.

Conclusion: Hyperglycemia and hyperlipidemia in obesity promote the tumor growth of many types of cancers. The immune cell population, tumor microenvironment and tumor energy metabolism shift in hyperglycemia and hyperlipidemia condition, leading to an altered tumor growth rate and response to cancer therapies. HD Biosciences, a WuXi AppTec company, provides obese tumor models and have in vivo capability for preclinical cancer drug efficacy study in obese condition.

#1363 Cancer cell-derived exosomal microRNAs enhance immunosurveillance in long-term survivors of high-grade serous ovarian cancer.

Chi Lam Au Yeung, Marina A. Talor, Yadira J. Pacheco, Basant T. Gamal, Sammy Ferri-Borgogno, Samuel C. Mok

UT MD Anderson Cancer Center, Houston, TX

Epithelial ovarian cancer (EOC) is the leading cause of gynecologic cancer-related death in the United States. The major clinical determinant to ovarian patient survival was thought to be the effectiveness of the initial debulking surgery. A more recent study in a large clinical trial, however, showed that the existence of biological differences in advanced stage EOC, including genomic signatures and tumor microenvironment (TME), determined tumor complexity and debulking status and will ultimately affect overall survival. Current studies showed that cells in TME could communicate through the exchange of microRNAs (miRs) via exosomes, which are shed or secreted by almost all cell types, to exert biological functions. As a result, our goal was to investigate the roles of miRs in modulating the activities of various cell types in the TME and identify miRs and their targets that are associated with prolonged survival rates in advanced stage EOC to allow the development of new therapeutic regimens to the disease. To identify exosomal miRs that were transferred from ovarian cancer cells to various cell types in the TME, which modulate the malignant phenotype of HGSC, exosomal miRs were isolated and quantified from nine human HGSC cell lines using miR sequencing analysis. In-situ quantification of miRs identified in exosomes in HGSC tissue sections followed by Kaplan-Meier analysis demonstrated that miR-181c-3p overexpression is associated with improved survival rate in HGSC patients. Further functional studies showed that miR-181c-3p suppresses HGSC cell growth and was correlated with intraepithelial $CD8^+$ cytotoxic T cell (CTL) density. We identified two key immune-regulatory molecules, leukemia inhibitory factor (LIF) and Smad4, as potential direct gene targets of miR-181c-3p using online target prediction algorithms. Altering LIF expression in tumor-associated macrophages (TAMs) was also found to modulate CTL activity in the TME. These findings suggest the role of miR-181c-3p-LIF/Smad4 axis in $CD8^+$ CTL infiltration/activation/function, and subsequently in improving HGSC patients' survival rates. In conclusion, high-grade serous ovarian cancer (HGSC) expresses high levels of miR-181c-3p will secrete and transfer higher levels of exosomal miR-181c-3p to various cell types in the TME. The transfer of miR-181c-3p attenuates the growth promoting effects in cancer cells and endothelial cells and modulates TAMs, which will lead to increased densities of activated $CD8^+$ CTLs in HGSC, thereby improving patients' survival rates.

#1364 Low density lipoprotein cholesterol modulates anti-tumor activity of NK cells within NSCLC tumor microenvironment.

Ying Yang[1], Kai Wang[2], Andreas Lundqvist[1]

[1]Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden,[2]Department of Respiratory and Critical Care Medicine, The Fourth Affiliated Hospital, Zhejiang Univeristy School of Medicine, Zhejiang, China

Background: Non-small cell lung cancer (NSCLC) is a leading cause of cancer-related mortality worldwide. While immunotherapy shows promise for improving survival, reliable treatment response biomarkers are still lacking. Despite evidence showing that body mass index (BMI) link to immunotherapy benefits in NSCLC, the underlying mechanism remains unclear. Natural killer (NK) cells are critical for tumor eradication and hold promise in immunotherapy. This prompts the need for better understanding into the biological link between lipid metabolism and NK cells in tumor microenvironment (TME). Therefore, we sought to elucidate how lipids, particularly low-density lipoprotein (LDL) in the NSCLC TME influence NK function.
Methods: Primary tumor samples from a cohort of 20 patients with NSCLC were analyzed to assess correlations between tissue-resident NK cells and serum lipid profiles. Public TCGA-LUAD and GEO databases were analyzed to assess the clinical significance of LDL receptor-related gene expression in relation to NK cell infiltration in NSCLC. The effect of LDL/oxidized LDL (oxLDL) on NK cell infiltration was investigated in a 3D tumor model, where A549 lung cancer spheroids were co-cultured with NK cells isolated from healthy donor peripheral blood and analyzed via real-time imaging. NK cell cytotoxicity and phenotype were further assessed using flow cytometry.
Results: Analysis of surgically resected NSCLC tumors revealed a positive correlation between increased intra-tumoral NK cell populations and elevated serum levels of total cholesterol, LDL, and apolipoprotein B. *In vitro* co-culture experiments showed that LDL supplementation significantly enhanced NK cell infiltration into lung cancer spheroids ($p<0.001$). Notably, infiltrating NK cells exhibited higher levels of lectin-like oxidized LDL (oxLDL) receptor-1 (LOX-1). Analysis of the TCGA-LUAD cohort showed a positive correlation between LOX1 (*OLR1*) gene expression and NK cell infiltration, which also associated with improved overall survival (HR = 0.734, 95% CI 0.559-0.963, $p<0.05$). Furthermore, *OLR1* expression correlated with enhanced overall survival and progression-free survival in NSCLC patients treated with immune checkpoint inhibitors (ICI). NK cells treated with oxLDL exhibited enhanced killing capacity against A549 cells, potentially driven by an increased presence of $LOX1^{High}$ $CD107a^{+}$ and Granzyme B^{+} NK cell populations ($p<0.05$), along with elevated lipid raft formation ($p<0.05$) on NK cells following oxLDL exposure.
Conclusion: Our findings highlight the interplay between lipid metabolism and NK cell-mediated anti-tumor immunity in NSCLC, suggesting that enhancing LDL/oxLDL uptake by NK cells within the TME could provide a promising therapeutic strategy for advancing NK cell-based immunotherapy in NSCLC.

#1365 Histamine from mast cells protects against breast cancer recurrence.

Claire P. Schane[1], Adam T. Nelczyk[2], Cheng Chen[3], Shruti V. Bendre[1], Erin Weisser[4], Hashni E. V. Gamage[5], Mohammed Kadiri[6], Michael T. McHenry[7], Jiyoung Seo[1], Yu Wang[1], Natalia Krawczynska[1], Dhanya Pradeep[1], Lara I. Kockaya[1], Yifan Fei[1], Shin-Hsuan Hsiao[8], Nicki J. Engeseth[9], Michael K. Wendt[10], Timothy M. Fan[8], William G. Helferich[9], Erik R. Nelson[1]

[1]Molecular and Integrative Physiology, University of Illinois Urbana-Champaign, Urbana, IL,[2]Helixon US Inc, Champaign, IL,[3]University of Pittsburgh Medical Center, Pittsburgh, PA,[4]University of Illinois Chicago, Chicago, IL,[5]Medicine, Dana-Farber Cancer Institute, Boston, MA,[6]Rosalind Franklin University, Chicago, IL,[7]University of Illinois College of Medicine at Chicago, Chicago, IL,[8]College of Veterinary Medicine, University of Illinois Urbana-Champaign, Urbana, IL,[9]Food Science and Human Nutrition, University of Illinois Urbana-Champaign, Urbana, IL,[10]University of Iowa, Iowa City, IA

One in eight women will develop breast cancer in their lifetime. 20-30% of these women develop recurrent metastatic disease, for which clinical treatments have limited efficacy. Metastasis is thought to be a stepwise process, terminating with extravasation into distal tissues. After this distal extravasation, cancer cells do not always immediately proliferate, but remain dormant. These dormant cells may awaken through yet-unknown mechanisms. There is therefore an urgent need to better understand dormancy and reawakening to mitigate the risk of metastatic recurrence. Previous work from our group has shown that elevated circulating cholesterol, and particularly an oxidized metabolite 27-hydroxycholesterol (27HC), has a pro-metastatic effect with breast cancer. Bacon is a high cholesterol food that is often prepared in a highly oxidative pan-frying process. Using mouse models, we demonstrate that consumption of cured, fried bacon promotes metastatic recurrence from dormancy. Assessment of metastatic tissues showed that consumption of cured fried bacon fat dramatically reduced mast cell (MC) numbers. MCs are granulocytic innate immune cells whose function in reemergence from dormancy is not well characterized. Interestingly, depletion of mast cells also stimulated reemergence, suggesting that mast cells might be protective against cancer progression. A major mediator of MC function is histamine. Therefore, we used small molecules to test the effects of histamine. Importantly, histamine 2 receptor antagonism stimulates recurrence. RNA-Sequencing data revealed that H2-receptor antagonism yields a unique transcriptional profile enriched with genes associated with platelet activation. Subsequent work indicates that H2 antagonism primes platelets towards activation which then increases neutrophil NETosis. Thus, we establish a novel metastatic axis: *Diet - Mast cell - H2-receptor - Dormancy.* The ultimate goal is to leverage this axis to develop novel therapeutic targets or lifestyle interventions to prevent recurrence. This work was supported by the American Institute for Cancer Research grant (713063), the National Cancer Institute (R01CA234025) and Department of Defense Breast Cancer Research Program Era of Hope Scholar Award (BC200206). Further support awarded to CPS from the National Institute of Biomedical Imaging and Bioengineering of the National Institutes of Health under Award Number T32EB019944 and ATN from a National Institutes of Environment Health Sciences and Research Training in Toxicology and Environmental Health (T32) Fellowship (ES007326).

#1366 Single-cell gene expression profiling of the tumor immune microenvironment (TIME) and its association with immunotherapy response in syngeneic mouse models.

Jia Wang, Minjuan Deng, Bin Jiang, Han Yan, Yu Jiang, Siyuan Wang, Xiao Ding, Pei Zhang, Wei Jin, Zhirong Shen

BeOne Medicines, Beijing, China

The TIME, comprised of the extracellular matrix and a milieu of both immune and non-immune cells, plays a critical role in tumor development, disease progression, and response and resistance to immunotherapy. Much of our understanding of the TIME's immunological features, cellular heterogeneity, and associations with immunotherapy response have come from the use of syngeneic mouse models representing various cancer cell types. To provide further characterization of the TIME in syngeneic mouse lines commonly used for immunogenic drug discovery, Cd45+ single-cell RNA sequencing was performed in 10 syngeneic mouse models representing 7 distinct cancer types (breast mammary carcinoma: 4T1, EMT6, MMTV-PyMT; colon carcinoma: CT26.WT, MC38; glioma: GL261; renal adenocarcinoma: Renca; lung carcinoma: LL2; melanoma: B16F10; and pancreatic adenocarcinoma: Pan02). Further, to unveil cellular subpopulations that correspond to immunotherapy response, anti-PD-1 therapy efficacy studies were performed in the syngeneic models and correlations with the abundances of distinct immune cell populations were explored.

Across all syngeneic tumor lines examined, seven major immune cell populations were identified and subpopulations within T cells, NK cells, innate lymphoid cells, and distinct myeloid cells were characterized. Efficacy of anti-PD-1 treatment was positively correlated with a unique population of ISGhigh macrophages. Moreover, an over-representation of neutrophils and proliferating/C1Q+ macrophage subsets was observed in models exhibiting resistance to anti-PD-1 treatment.

These findings provide further characterization of these syngeneic lines to aid in rational model selection to facilitate translational relevance. This study also provides further insight into how immune cell composition and abundance within tumors may contribute to immune checkpoint blockade resistance.

#1367 Antigen recognition induces chemokine secretion to promote universal CAR-T cell infiltration.

Ruxuan Li, Youngbin Cho, Matthew Laird, Walter Kaminski, Elisa Ruffo, Jason Lohmueller, Ioannis Zervantonakis

University of Pittsburgh, Pittsburgh, PA

The lack of CAR-T cell infiltration and immune-mediated cancer cell destruction is a dominant mechanism of immunotherapy failure. The characteristics and mechanisms of cognate recognition induced CAR-T cell infiltration have yet to be revealed. In this study, we developed an in-vitro 3D collagen droplet co-culture system to simulate CAR-T cell infiltration in the tumor microenvironment and track T cell motility and interactions with tumor cells in real-time under different micro physiological environments. We employed CAR-T cells overexpressing the SNAP tag (SNAP-CAR T cells), where the contacts between CAR-T cells and HER2+ breast tumor cells can be initiated by adding benzyl guanin-conjugated Herceptin (BG-Herceptin). Quantification of infiltrating cell numbers showed that 3D collagen matrices with HER2+ breast tumor cells significantly recruited a higher number of SNAP CAR-T cells compared to cell-free matrices. Upon initiating the contacts between target and effector cells by adding BG-Herceptin, CAR-T cell infiltration to HER2+ breast cancer matrices were significantly increased. To investigate whether chemokine secretion mediates this process, we employed chemokine arrays. Chemokine secretion such as CCL2 were significantly higher with antigen-specific interactions. Further, CAR-T cell infiltration was inhibited by CCL2 neutralization, which indicates CCL2 plays a major role in cognate induced CAR-T cell infiltration. By analyzing CAR-T cell motility patterns, we found that CAR-T cell infiltration and interaction with tumor cells are dependent on cognate recognition. With BG-Herceptin, CAR-T cell interaction with and swarming around HER2+ breast tumor cells were significantly higher. By inhibiting CCL2, CAR-T cell motility was significantly impaired. Our findings suggest that CAR-T cell infiltration towards the solid tumors is dependent on antigen-specific interactions. Moreover, we demonstrated cognate induced chemokine secretion as a crucial factor in CAR-T cell infiltration and motility.

#1369 Chronic stress drives ovarian cancer progression via MDSC infiltration and notch signaling pathway activation.

Yadiel Alejandro Rivera-Lopez[1], Luinet L. Melendez-Rodriguez[1], Jaydiel A. Casiano-Martinez[1], Alanis P. Torres-Rosado[1], Sofia M. Hernandez-Carrasquillo[1], Melanie Ortiz-Leon[1], Luis M. Rivera-Perez[1], Zuliann S. Galarza-Ruiz[2], Orlando I. Torres-Rodriguez[1], Guillermo N. Armaiz-Pena[1]

[1]Ponce Health Sciences University, Ponce, Puerto Rico,[2]Interamerican University of Puerto Rico, Ponce, Puerto Rico

Ovarian cancer (OC) is the fifth leading cause of cancer-related deaths among women in the U.S. Studies indicate that cancer patients have elevated stress hormones due to various stressors, which can disrupt cancer progression and immune response in the tumor microenvironment (TME). Myeloid-derived suppressor cells (MDSC) are immature, immunosuppressive cells that play a crucial role in the TME and are associated with poor prognoses in cancer patients by disrupting several pathways, including the Notch signaling pathway (NSP). In various cancers, NSP dysregulation contributes to cancer progression. Increasing evidence suggests that chronic stress enhances MDSC infiltration, which promotes immunosuppression in the TME. However, the role of the NSP in stress-mediated MDSC infiltration and OC progression remains poorly understood. This study investigates how chronic stress affects NSP and MDSC biology in the OC TME and its role in disease progression. We hypothesize that chronic stress increases MDSC infiltration in the OC TME, enhancing NSP activity in OC cells. To test this, we inoculated female C57BL/6 mice with $ID8^{Luc}$ or $IG10^{Luc}$ OC cells and subjected them to daily restraint stress. Mice were sacrificed weekly for tumor and bone marrow collection, followed by immunofluorescence and flow cytometry analyses to characterize MDSC based on surface markers. We also isolated bone marrow from naïve C57BL/6 mice to differentiate myeloid cell precursors into MDSC. These cells were then exposed to stress hormones (norepinephrine (NE), epinephrine (EPI), and corticosterone (CC)) for 72 hours to analyze MDSC populations. Our results indicated that chronic restraint stress increased MDSC infiltration and enriched polymorphonuclear-MDSC (PMN-MDSC) in the TME and bone marrow of both OC mouse models. Results from the *ex vivo* experiments showed increased PMN-MDSC enrichment alongside a depletion of monocytic-MDSC in the groups treated with stress hormones. To further investigate the impact of stress hormones on NSP dysregulation, we treated OC cells (ID8 and IG10) *in vitro* with NE, EPI, or CC. Our findings revealed that stress hormones significantly upregulated the expression of the Notch Intracellular Domain (NICD) in OC cells. We also observed increased levels of Notch1, Jagged2, and Hes1 mRNA and protein, which may be mediated by GSK-3 phosphorylation. Additionally, we analyzed the expression of NSP members in tumors from the *in vivo* experiments. The results indicated higher expression levels of Notch1, Jagged2, NICD, and HES1 in tumors from mice subjected to restraint stress. These data suggest that chronic stress may induce MDSC infiltration and activity, possibly mediated by the activation of the NSP, thereby contributing to the immunosuppressive TME and progression of OC.

#1370 Antibodies derived from patient tumors augment response to immune checkpoint blockade in cancer.

Manoj Chelvanambi[1], Monika A. Zelazowska[2], Somnath Paul[2], Joshua B. Plummer[2], Brenda Melendez[3], Sarah B. Johnson[3], Elise F. Nassif Haddad[4], Laurence P. Diggs[1], Rossana N. Lazcano[5], Khalida M. Wani[5], Davis R. Ingram[5], Bharat B. Singh[3], Diana Shamsutdinova[5], Rebecca A. Soto[3], Florentia Dimitriou[1], Michael G. White[6], Matthew J. Lastrapes[7], Golnaz Morad[1], Melissa S. Simper[2], Fabiana J. Veguilla[3], Y. David Seo[8], Beth A. Helmink[1], Russell G. Witt[9], Bin Liu[2], Nadim J. Ajami[3], Tullia C. Bruno[10], Wolf H. Fridman[11], James P. Allison[12], Padmanee Sharma[13], Emily Z. Keung[1], Tina Cascone[14], Christina L. Roland[1], Alexander J. Lazar[5], Kevin M. McBride[2], Jennifer A. Wargo[1]

[1]Department of Surgical Oncology, UT MD Anderson Cancer Center, Houston, TX,[2]Department of Molecular Carcinogenesis, UT MD Anderson Cancer Center, Houston, TX,[3]Department of Genomic Medicine, UT MD Anderson Cancer Center, Houston, TX,[4]Department of Sarcoma Medical Oncology, UT MD Anderson Cancer Center, Houston, TX,[5]Department of Translational Molecular Pathology, UT MD Anderson Cancer Center, Houston, TX,[6]Department of Colon and Rectal Surgery, UT MD Anderson Cancer Center, Houston, TX,[7]Department of Epidemiology, UT MD Anderson Cancer Center, Houston, TX,[8]Department of Surgery, Medical College of Wisconsin, Milwaukee, WI,[9]Department of Surgery, University of Virginia Health Medical Center, Charlottesville, VA,[10]Department of Immunology, University of Pittsburgh, Pittsburgh, PA,[11]Department of Immunology, University of Paris, Paris, France,[12]Department of Immunology, UT MD Anderson Cancer Center, Houston, TX,[13]Department of Genitourinary Medical Oncology, UT MD Anderson Cancer Center, Houston, TX,[14]Department of Thoracic Head and Neck Medical Oncology, UT MD Anderson Cancer Center, Houston, TX

Background:Immune checkpoint blockade (ICB) has revolutionized treatments for cancer; however, only a minority of patients achieve a durable response. Landmark studies have now shown that B-cells and Tertiary Lymphoid Structures (TLS) within the tumor typify exceptional response to ICB. However, the functional role of B-cells and TLS in cancer remains largely unexplored. Here, we produce and evaluate the therapeutic efficacy of tumor-derived antibodies alone or in combination with ICB-based regimen.
Methods and Results:Tumors from high-risk resectable melanoma patients treated with neoadjuvant ICB were analyzed. Major pathologic response (MPR), defined as $\leq$10% viable tumor at the time of surgery, was used to evaluate response (R - responder; NR - non-responder). Formalin-fixed paraffin-embedded (FFPE) surgical resections were analyzed on NanoString GeoMx. TLS+ regions of interest (ROI) were selected based on melanoma (S100B) and immune markers (CD45 and CD20). Principal component (PC) analysis of ~18,000 probes revealed distinct transcriptional profiles between R and NR TLS, with PC1 strongly correlating with response status ($r=0.88$, $p<0.0001$). Differential immune analysis showed that R TLS were enriched for CD8+ T-cells ($p<0.001$), NK cells ($p=0.004$), γδ T-cells ($p=0.017$), and memory B-cells ($p=0.026$). Pathway analysis indicated an upregulation of NK-cell mediated cytotoxicity pathways in R TLS. Targeted BCR sequencing performed on fresh surgical resections from 12 high-risk, resectable melanoma patients (R=9, NR=3) yielded 1277 unique BCR amplicons and 546 unique heavy and light chain-matched antibodies. In silico analysis revealed that BCR clones from R tumors showed higher levels of somatic hypermutation ($p=7.2e-9$) and isotype switching (50.1% in R vs 32.6% in NR). Flow cytometry and immunofluorescence microscopy identified 73 unique antibodies (R=59, NR=14) that bound to A375 human melanoma cells in vitro. Next, an in vitro ADCC assay (NK:tumor ratio of 10:1) was used to identify that 10 of the 73 melanoma-binding antibodies induced significant cell death of both A375 human and BP ($Braf^{V600E}Pten^{-/-}$) murine melanoma cells. In vivo experiments in BP melanoma bearing mice revealed that 4 ADCC+ antibodies (RAPC5-7-156, RAPC5-9-9, RAPC5-9-8, and RAPC5-9-98) homed to the TME within 24 hours of systemic administration. Remarkably, when combined with αPD-L1, RAPC5-7-156 significantly reduced BP melanoma tumor growth compared to mice treated with isotype control ($p=0.0003$) and α-PD-L1 alone ($p=0.005$). Importantly, melanoma derived RAPC5-7-156 did not demonstrate this synergistic effect in ICB-treated MC38 colon carcinoma or LLC1 Lewis lung carcinoma models.
Conclusion:These findings suggest that tumor-resident antibodies are capable of augmenting response to ICB thus presenting new opportunities for target discovery and for improving clinical outcomes with ICB.

#1371 ChemoNETosis is associated with chemotherapy resistance in pancreatic cancer.

Shohei Nogi, Shunsuke Kagawa, Atsuki Taniguchi, Nobuhiko Kanaya, Yoshihiko Kakiuchi, Kazuya Yasui, Satoru Kikuchi, Shinji Kuroda, Hiroshi Tazawa, Toshiyoshi Fujiwara

Gastroenterological surgery, Okayama Univ. Graduate School of Med., Dentistry & Pharm. Sci., Okayama, Japan

Background: Chemotherapy resistance is a major contributor of the poor prognosis of pancreatic cancer, yet its underlying mechanisms remain poorly understood. Neutrophil Extracellular Traps (NETs), a form of neutrophil-specific cell death, have been reported to be associated with poor prognosis in various cancers because they act to suppress tumor immunity and promote metastasis. Recently, chemotherapy-stimulated NETs, termed “ChemoNETosis”, were reported to contribute to chemotherapy resistance in breast cancer (Mousset A, Cancer cell. 2023). Therefore, we investigated the role of NETs in resistance to cytotoxic chemotherapy in pancreatic cancer.
Methods: The abundance of NETs in the tumor microenvironment was analyzed in relation to chemotherapy efficacy using immunohistochemical staining of resected specimens from pancreatic cancer patients treated with neoadjuvant chemotherapy. The ability of pancreatic cancer cells to induce NETs was assessed with and without gemcitabine stimulation by extracellular DNA staining in vitro and by immunohistochemical analysis in vivo. Finally, the efficacy of the combined anti-NETs treatment and gemcitabine was examined in a mouse subcutaneous tumor model.
Results: NETs were more abundant in unresponsive tumors compared to responsive tumors in clinical sample. Gemcitabine-treated pancreatic cancer cells induced significantly higher levels of NETs than untreated cells in vitro as well as in vivo tumor models. This “ChemoNETosis” was effectively inhibited by Navarixin, a CXCR2 inhibitor. Combination therapy with Navarixin and gemcitabine achieved greater tumor reduction compared to gemcitabine alone with, a complete response (CR) rate of 28.6% (2/7) vs 0% (0/7).
Conclusion: NETs may play a key role in chemotherapy resistance in pancreatic cancer. Targeting NETs could provide a novel approach to enhancing the efficacy of chemotherapy in preclinical models and warrants further investigation.

#1372 Development of a multi-omic spatial phenotyping assay for affirmative detection of CAR-T cells in the tumor immune microenvironment of solid pediatric tumors.

Jasmine T. Plummer[1], Felipe Segato-Dezem[1], Yutian Liu[1], Arjumand Wani[1], Hannah Chasteen[1], Arne Christians[2], Jannik Boog[2], Randy Chen[3], Harry Choi[3], Aneesh Acharya[3], Oliver Braubach[2], Giedre Krenciute[1], Jason Chiang[1], Christopher De Renzo[1], Kelsey Bertrand[1]

[1]St. Jude Children's Research Hospital, Memphis, TN,[2]Bruker Spatial Biology, Seattle, WA,[3]Molecular Instruments, Los Angeles, CA

Certain pediatric cancers, like blood leukemia, have shown high cure rates, but effective treatments of solid tumors remain elusive and survival rates for affected children remain low. While CAR-T therapy has demonstrated success in treating acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL), its effectiveness against solid tumors has been limited. Yet, pediatric patients with solid tumors may still receive CAR-T therapy with minimal prior evidence of treatment efficacy. It is imperative to understand why CAR-T treatments succeed or fail in solid pediatric tumors, as such knowledge can guide the development of future treatment options. This study focuses on a pediatric patient enrolled in a Phase 1/2 clinical trial exploring the use of CAR-T therapy for solid tumors. The patient underwent tumor resection before receiving CAR-T therapy (PRE). Following treatment, the tumor did not respond, leading to recurrence and a second resection (POST).Using advanced spatial transcriptomic profiling we compared the tumor immune microenvironment (TiME) between the PRE and POST treatment samples. Our findings revealed significant differences, including changes in immune cell populations and the spatial distribution of T cells in the TiME. However, while we observed alterations in T cell distribution, the precise localization of CAR-T cells relative to other cell types remained unclear. To address this, we developed a novel targeted spatial multi-omics assay that combines next-generation RNA fluorescence *in situ* hybridization (HCR™ Gold RNA-FISH) with multiplex immunofluorescence (mIF). HCR™ Gold RNA-FISH is broadly compatible with protein imaging methods, and therefore enables RNA and protein co-detection on the same tissue sample, which is needed to affirmatively detect and map the putative infiltration of CAR-T cells in the TiME. CAR-T cell detection was enabled by custom-designed HCR™ HiFi probes, targeted against B3-H7. Affirmative identification, phenotyping, and spatial profiling of these cells were enabled via VistaPlex™ mIF assay panels deployed serially on the same formalin-fixed paraffin-embedded (FFPE) tissue samples. All experiments were automated on the CellScape™ spatial biology platform and image data were processed and integrated with a custom image analysis pipeline. Our method enables conclusive follow-up investigations into the efficacy of CAR-T treatments in solid pediatric tumors. With this toolkit in hand, it is possible to (a) confirm the status of the CAR-T cell infiltration, (b) define the phenotype and activation states of these cells, and (c) map the spatial biology and cell-to-cell interactions of the CAR-T cells within the TiME.

#1373 Adenosine-driven immune profile in breast cancer.

Xi Chen, Yong Qin

The University of Texas at El Paso, El Paso, TX

Adenosine accumulation in tumor microenvironment (TME) suppresses T cell activation and effector functions, posing challenges for immune therapies in cancers, including breast cancer (BC). Upregulated adenosine A2A receptor (A2AR) has been observed in BC tumors compared to normal tissues. However, the immune patterns shaped by adenosine signaling across BC subtypes, particularly triple-negative breast cancer (TNBC), remain poorly understood. To examine adenosine-driven immune profile in BC, we analyzed 138 tumor cores from 69 BC patients using multiplex immunofluorescence staining for 12 markers: CD73, A2AR, CD8, CD4, CD56, FOXP3, CD68, CD11b, Ki67, PD-1, Glut-1, and IDO, alongside the nuclear marker DAPI. Our findings revealed elevated levels of A2AR, CD68, CD4, CD8, Ki67, PD-1, and Glut-1 in BC tumors, indicating a TME enriched with CD4+ T helper cells, C8+ cytotoxic T cells, and CD68+ macrophages. Tumors with high A2AR expression exhibited increased PD-1+ immune infiltrates and elevated Ki67 levels, suggesting that A2AR activation impairs T cell function and promotes tumor proliferation. A2AR was predominantly expressed on CD4+ and CD8+ T cells, suggesting the connection among adenosine receptor activation, immunosuppression, and tumor growth. We further explored the role of CD73, an enzyme converting AMP to adenosine, in the BC TME. CD73+ tumors showed significantly higher infiltration of CD4+ cells, CD56+ NK cells, CD68+ macrophages, and PD-1+ immune cells compared to CD73- tumors. CD73 expression, linked to adenosine production, was tied to an adenosine-rich TME increasing immune infiltration but reducing immune functionality, characterized by elevated PD-1 and IDO expression. Additionally, PD-1+ immune infiltrates correlated with higher levels of CD4+, CD8+, CD68+, and CD11b+ cells and increased IDO expression. High CD73 levels correlated with elevated CD68+ macrophage and PD-1 expression, despite greater CD4+ T cell infiltration. These findings suggest that CD73-driven adenosine signaling contributes to an immunosuppressive TME, even with immune cell presence. In TNBC tumors (n=16), Glut-1 expression, a hypoxic marker, was significantly higher than in non-TNBC tumors (n=49), indicating a more hypoxic and adenosine-rich TME. TNBC tumors also showed a higher proportion of CD73+ tumor cells. Ki67 analysis levels revealed higher cancer cell proliferation in TNBC compared to HER2+ tumors (n=17), indicating TNBC's aggressive nature. In conclusion, BC tumors display unique immune profiling linked to adenosine signaling within the TME. High A2AR and CD73+ expressions are associated with tumor proliferation and immunosuppressive infiltrates driven by adenosine and PD-1 signaling. The hypoxic, adenosine-rich TME in TNBC highlights adenosine regulation's critical role in progression. These findings underscore adenosine as a promising therapeutic target for developing anti-tumor strategies, particularly for TNBC patients.

#1374 Targeting lipid-laden macrophage efferocytosis enhances immune response to EGFR-tyrosine kinase inhibitor in EGFR-mutant lung cancer.

Sook-Hee Hong[1], Nahyeon Kang[1], Okran Kim[1], Juyeon Park[1], Soon Auck Hong[2]

[1]Internal Medicine, Seoul St. Mary's Hospital, Seoul, Korea, Republic of,[2]Department of Pathology, College of Medicine, Chung-Ang University, Seoul, Korea, Republic of

During macrophage phagocytosis, efferocytosis is the process of removing senescent or apoptotic cells and, unlike phagocytosis, induces anti-inflammation, angiogenesis, and wound repair. Inhibition of efferocytosis, one of the macrophage responses to apoptosis induced by EGFR-TKIs, may enhance their immunologic effects. We analyzed early immune microenvironment changes following osimertinib treatment in an human EGFR L858R genetically engineered mouse model, focusing on the effect of tumor-associated macrophage (TAM) efferocytosis signals on the immune microenvironment in response to osimertinib-induced tumor cell apoptosis. Methods: Doxycycline-induced transgenic mice (human EGFRL858R) were maintained with doxycyclin-impregnated food pellets to activate rtTA protein phosphorylation. Tumor progression and treatment response were monitored by MRI. Lung tumor development was confirmed, and mouse immune cells were isolated by CD45 MACS for single-cell RNA sequencing. Immediate immune profile changes after osimertinib or osimertinib and MERTK inhibitor (LDC1267) treatment were analyzed (days 1, 5, 15). All animal experiments were approved by the Institutional Animal Care and Use Committee of the Catholic University of Korea. Results: Six macrophage clusters were identified from scRNA-seq of the human EGFR-mutated lung cancer transgenic mouse model. These were categorized as antigen-presenting, alveolar, IFN-responsive, proliferative, and lipid-containing macrophages, with the latter further divided into cluster 2 (*Lepr, Dapk1, MerTK, Olr1, Adarb1*) and cluster 4 (*FSTL1, Fabp5, Gpnmb, S100a1*). Osimertinib treatment increased the proportion of cluster 2 from 8.9% to 13.4%. The top GO terms enriched in cluster 2 included response to oxidative stress, wound healing, immune response activation, tissue remodeling, and response to hypoxia, which demonstrated activated efferocytosis. In vitro macrophage co-culture models showed MerTK-mediated efferocytosis occurring 24 hours post-osimertinib treatment, MerTK inhibition of macrophage significantly decreasing CD206 expression and increasing pSTAT3 in U937 macrophages. Osimertinib and LDC1267 (MERTK inhibitor) combination treatment in the human EGFR mutant lung cancer mouse model increased pSTING expression in macrophages and inducing type 1 IFN response. The combination also enhanced CD8 T cell activation, evidenced by increased IFN-γ, TNF-α, granzyme, and CD25 expression compared to osimertinib alone (days 5 and 15). No change was observed in the CD44+/CD62L-/CD8 T cell population. Conclusions: EGFR-TKI-induced tumor cell death stimulated macrophage efferocytosis. Inhibiting macrophage efferocytosis can increase the type 1 IFN response, potentially inducing of starting immunogenic cell death.

#1375 Active inhibition of chemokine-mediated migration impairs tertiary lymphoid structure formation.

Victoire Boulat[1], Elena Alberts[1], Carin Andrea Brundin[2], Dinis P. Calado[1], Anita Grigoriadis[1]

[1]The Francis Crick Institute, London, United Kingdom,[2]King's College London, London, United Kingdom

The presence of tumor-infiltrating B cells (TIL-B) and tertiary lymphoid structures (TLS) in the primary tumor microenvironment carries prognostic value across cancer types. We have shown that triple-negative breast cancer (TNBC) patients with robust germinal center (GC) responses in their lymph nodes (LN) exhibit higher levels of TILs and more TLS. In this study, we investigated human and mouse immune-cold TNBCs to uncover mechanisms by which tumor-LN crosstalk may be impaired.

In orthotopic TNBC mouse models, we observed robust GC responses in tumor-draining LNs. However, minimal TIL-Bs and no TLS were detected in the primary tumors. The tumor-draining LNs were confirmed as the source of the few TIL-B by using FTY720, a drug that blocks LN exit. Transcriptomic analysis of 124 untreated TNBC patients confirmed a strong association between TIL-B/TLS presence and chemokine-enriched pathways. Notably, in orthotopic TNBC mouse models, multiplex proteomic analyses of the tumor secretome revealed that most TIL-B- and TLS-associated chemokines were absent. Next, we conducted *ex vivo* migration assays and found unexpectedly that supplementing mouse tumor-conditioned medium with chemokines induced significantly less migration compared to chemokine-supplemented culture medium. *In vivo*, co-injection of genetically engineered chemokine-expressing cancer-associated fibroblasts (CAFs) did not elicit TLS formation nor increased TIL-B infiltration in the primary tumors.

These findings suggest the presence of specific inhibitory factors within the tumor secretome that impair chemokine-mediated migration and immune cell infiltration. Using molecular weight-based fractionation and high-throughput analysis, we are narrowing down the identity of these components and conducting functional studies to validate their effects. Preliminary experiments show that removing these factors can restore chemokine activity, permitting B cell migration. In vivo, we are evaluating the combination of genetically engineered chemokine-expressing CAFs and systemic inhibition of these factors to promote TIL-B infiltration and TLS formation. Preliminary data indicate this dual treatment promotes the formation of large peritumoral B cell aggregates, providing critical insights into the factors enabling tumor immune infiltration and organization. Ongoing human transcriptomic analyses on internal and publicly available datasets support a strong association between chemokine expression, reduced inhibitory factor secretion, and TIL-B presence across human cancer types, including TNBC, colon and lung cancer.

This work defines that the absence of TIL-B and TLS in cancer is the consequence of a combination of mechanisms: insufficient chemokine production by the tumor microenvironment to drive immune infiltration from the LN, coupled with the active secretion of factors that impair chemokine functionality.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Apoptosis and BCL2 Family
Poster Session

#1379 Discovering the essential functions of MCL-1 in hematopoiesis using gene-swap mice.

A. Tee[1], A. J. Kueh[2], L. Gibson[1], S. Malelang[1], M. J. Herold[2], G. Kelly[1], A. Strasser[1], K. Brinkmann[1];
[1]WEHI - The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, [2]Olivia Newton-John Cancer Research Institute, Heidelberg, Australia

Pro-survival BCL-2 proteins (e.g., MCL-1, BCL-XL, BCL-2, A1) are over-expressed in human cancers, making them critical targets for anti-cancer therapies [1]. While the BCL-2 inhibitor Venetoclax is highly successful in treating certain blood cancers, inhibitors of BCL-XL and MCL-1 cause toxicity to non-malignant tissues, including hematopoietic cells [2]. These findings from gene targeted mice showed that different pro-survival BCL-2 proteins are critical for the survival of distinct hematopoietic cell subsets. Notably, MCL-1 is essential for implantation, with *Mcl-1*$^{-/-}$ embryos dying at E3.5, the survival of hematopoietic stem and progenitor cells as well as many immature and mature lymphoid and myeloid cell populations[3]. In contrast, the essential roles for BCL-2 and BCL-XL are restricted to the survival of select hematopoietic cell populations. Of note MCL-1 (and to a minor extent BCL-XL) but not BCL-2 have been ascribed some apoptosis unrelated functions in cellular metabolism, such as mitochondrial import of long chain fatty acids. We aim to determine the unique anti-apoptotic and apoptosis unrelated roles of MCL-1 in comparison to the other pro-survival BCL-2 family members in embryogenesis and hematopoiesis.We used CRISPR to produce “gene-swap” mice in which the MCL-1 coding region was replaced with the coding region for BCL-2 (*Mcl-1*$^{Bcl\text{-}2}$), BCL-XL (*Mcl-1*$^{Bcl\text{-}xL}$) or A1. Remarkably, homozygous *Mcl-1*$^{Bcl\text{-}xL/Bcl\text{-}xL}$ and *Mcl-1*$^{Bcl\text{-}2/Bcl\text{-}2}$ embryos could develop until at least E11.5 on a C57BL/6 background. This demonstrates that only the anti-apoptotic function of MCL-1 - but not its apoptosis-unrelated roles - are essential for early embryogenesis.To examine the impact of replacement of MCL-1 by BCL-XL on hematopoiesis, lethally irradiated mice were reconstituted with *Mcl-1*$^{Bcl\text{-}xL/Bcl\text{-}xL}$ hematopoietic stem progenitor cells from E12.5 embryos. Interestingly, the expression of BCL-XL in place of MCL-1 in the hematopoietic system allowed the generation of all blood cell types with a marked increase in platelets and B lymphocytes. Notably, adult heterozygous *Mcl-1*$^{Mcl\text{-}1/Bcl\text{-}2}$ mice showed even more substantial accumulations of B cells and antibody secreting plasma cells in their spleen and bone marrow, and with progressing age they develop SLE-like autoimmune disease similar to what is seen in *vav-bcl-2* transgenic mice that overexpress BCL-2 [4]. These findings show that distinct pro-survival BCL-2 proteins with their differences in protein stability and interactions with the pro-apoptotic members of the BCL-2 family are best suited to safeguard normal hematopoietic cell survival, with too much pro-survival activity causing disease.

References1.Adams, J. M. & Cory, S. *Cell Death Differ* 25, 27-36 (2018).2.Kelly, G. L. & Strasser, A. *Annu Rev Cancer Biol* 4, 299-313 (2020).3.Opferman, J. T. *et al. Nature* 426, 671-676 (2003).4.Ogilvy, S. *et al. Proceedings of the National Academy of Sciences* 96, 14943-14948 (1999).

#1380 Role of cytokine-induced cell death on efficacy of teclistamab-mediated T cell killing of multiple myeloma cells.

Allison Carr[1], Adrian Ting[2]

[1]Mayo Clinic Graduate School of Biomedical Sciences, Rochester, MN,[2]Department of Immunology, Mayo Clinic, Rochester, MN

Multiple myeloma (MM) is the second most common hematologic malignancy among US adults, characterized by high rates of relapse and refractory disease. Anti-Myeloma T cell immunotherapies, such as chimeric antigen receptors (CAR-Ts) and bispecific T cell engagers (BiTEs), have been able to induce deep responses in the clinic and extending survival for many. However, even these responses are not durable and majority of patients still relapse. MM cell signaling in response to cytokines released by these T cells, such as Tumor Necrosis Factor (TNF) Alpha, and Interferon gamma (IFNγ), may offer additional insight into mechanisms of relapse and resistance as well as avenues of therapeutic manipulation. To evaluate the effects of TNF and IFNγ on MM cell death and proliferation, we obtained human MM cell lines (MMCLs) and stably transduced them with a GFP-NLS reporter. We then monitored these cell lines in the presence of TNF, IFNγ, or TNF+IFNγ and tracked their cell death and growth kinetics over time using DiYO-3 incorporation and GFP count, respectively, via IncuCyteS3 live cell imaging. We next utilized Teclistamab, a B cell maturation antigen (BCMA)-directed BiTE, to co-culture MMCLs with healthy donor derived T cells at various effector to target (E:T) ratios and monitored for T cell mediated death again via IncuCyteS3 live cell imaging. While all MMCLs were able to be killed at high E:T ratios (1:1), only cytokine-sensitive lines remained susceptible to T cell killing at lower E:T ratios (1:10). The addition of TNF and/or IFNγ blocking antibodies confirmed that the increased sensitivity of these lines to T cell killing was indeed mediated by the two cytokines. In summary, were able to show an in vitro model of differential MM cell response to Teclistamab mediated T cell killing, and that this difference in response was predicated on the MM cells' baseline sensitivity to cytokine mediated cell death via TNF and IFNγ. Thus, further dissecting mechanisms underlying resistance or sensitivity of MM cells to cytokine induced death could suggest therapeutic targets with the potential to enhance the efficacy of anti-MM T cell immunotherapies.

#1381 BCL-2 selective inhibitor venetoclax combined with topoisomerase I inhibitor irinotecan is effective in small cell lung cancer (SCLC).

Yanli Xing, Krista M. Dalton, Jane L. Roberts, Anthony C. Faber

VCU Philips Institute for Oral Health Research Oral and Craniofacial Molecular Biology, Virginia Commonwealth University - VCU, Richmond, VA

Background: Lung cancer is the third most common cancers globally, and small cell lung cancer (SCLC) accounts for 10-15% of all lung cancer cases. Extensive stage (ES)-SCLC has an overall five-year survival rate of ~5%. Although ES-SCLC initially responds well to platinum-based combination chemotherapy, it almost invariably develops resistance over time. Immunotherapy can induce some responses in ES-SCLC, and arlatamab-dlle, a DLL3 targeted bi-specific T-cell engager, was recently given advanced approval to treat relapsed ES-SCLC. However, ES-SCLC remains a dismal prognosis. Venetoclax is a Bcl-2 selective inhibitor that induces apoptosis by binding to the hydrophobic groove of BCL-2. We previously demonstrated that venetoclax is effective in SCLC with high BCL-2 expression (Lochmann et al., *Clinical Cancer Research* 2018). Irinotecan is an antineoplastic enzyme inhibitor that blocks the topoisomerase I-DNA complex, preventing DNA repair and causing double-strand breaks that lead to cell death. It is often given in the relapsed setting as second-line therapy for ES-SCLC.
Aims: We aimed to determine if venetoclax could enhance responses to irinotecan in SCLC preclinical models, and vice versa.
Methods: Venetoclax was combined with irinotecan and SCLC cell viability was evaluated including CellTiter-Glo (CTG), immunoblotting, and in mouse models, including patient-derived xenografts, in vivo.
Results: Our data showed that combining the BCL-2 selective inhibitor venetoclax with irinotecan demonstrated in many SCLC models, enhanced activity over single-agent alone. In vivo, the combination enhanced survival compared to either single-agent.
Conclusion: We demonstrate that combining the BCL-2 selective inhibitor venetoclax with irinotecan could enhance responses and survival in the refractory SCLC setting.

#1382 The discovery of a novel antibody, 14G6, as a tool for understanding BAK conformational dynamics and their relationship to venetoclax response in leukemia.

Michelle S. Miller, Hema P. Subas-Satish, Sweta Iyer, Melissa X. Shi, Agnes W. Wong, Karla C. Fischer, Ahmad Z. Wardak, Daisy Lio, Rachel T. Uren, Peter E. Czabotar, Ruth M. Kluck

The Walter & Eliza Hall Institute of Medical Research (WEHI), Parkville, Australia

Venetoclax, a BCL2-specific inhibitor, is FDA-approved for the treatment of acute myeloid leukemia and chronic lymphocytic leukemia with varying patient responses. Conformation specific antibodies can help unravel the regulation of BCL2 family proteins and its relation to venetoclax response. We used mouse B cell cloning to identify 14G6, the first antibody that is specific for the non-activated, monomeric BAK conformation. The crystal structure of the 14G6 Fab bound to BAK revealed a binding site encompassing both the α1 helix and α5-α6 hinge regions of BAK, the two sites involved in the transition to the active, dimerisation-ready, conformation of BAK. This makes it a useful tool for determining the levels of non-activated, monomeric BAK in cancer cells by blue native PAGE. We found that 14G6 bound the majority of BAK in several leukemia cell lines, and binding decreased following treatment with venetoclax, indicating only minor levels of activated or sequestered BAK in those cells. 14G6 is thus an important new tool to assess BAK status in response to anti-cancer treatments and understand mechanisms of resistance to venetoclax, potentially aiding in the development of more reliable biomarkers to predict patient response.

#1383 A gated hydrophobic funnel within BAX binds long-chain alkenals to potentiate pro-apoptotic function.

Jerry Edward Chipuk

Oncologial Sciences, Icahn School of Medicine at Mount Sinai, New York, NY

Mitochondria maintain a biochemical environment that cooperates with BH3-only proteins (e.g., BIM) to potentiate BAX activation, the key event to initiate physiological and pharmacological forms of apoptosis. The sphingosine-1-phosphate metabolite 2-trans-hexadecenal (2t-hexadecenal) is one such component described to support BAX activation, but molecular mechanisms remain largely unknown. Here, we utilize complementary biochemical and biophysical techniques to reveal that 2t-hexadecenal non-covalently interacts with BAX, and cooperates with BIM, to stimulate early activation-associated intramolecular rearrangements prior to membrane permeabilization. Integrated structural and computational approaches suggest 2t-hexadecenal binds an undefined region - a hydrophobic cavity formed by core-facing residues of α5, α6, and gated by α8 - we term the "BAX actuating funnel (BAF).” Alkenal length and α8 mobility are critical determinants for the interaction, intramolecular rearrangements, and functional outcome of 2t-hexadecenal binding to the BAF; we also present a first-in-class small molecule that functionally mimics 2t-hexadecenal contributions to apoptosis. Collectively, this work imparts detailed molecular insights into how pro-apoptotic BCL-2 proteins and lipids assemble to initiate the mitochondrial pathway of apoptosis, and reveals the therapeutic potential of targeting the BAF.

#1384 Mitochondrial complex I inhibition enhances chemotherapy efficacy in melanoma.

Semmer Ali[1], Mehrdad Zarei[2], Luke D. Rothermel[2], Jordan M. Winter[2]

[1]Biology, Case Western Reserve University, Cleveland, OH,[2]Department of Surgery, Division of Surgical Oncology, University Hospitals Cleveland Medical Center, Cleveland, OH

Background: Melanoma, characterized by the uncontrolled proliferation of melanocytes, is one of the most common types of cancer in the United States. Despite improvement in therapeutics, it remains a formidable adversary in oncology due to its aggressive resistance to treatment and propensity for metastasis. Metabolic reprogramming is a hallmark of cancers implicated in resistance to therapies. Previous efforts have focused on targeting glycolysis, while mitochondrial oxidative phosphorylation (OXPHOS) and TCA metabolism remain underexplored in melanoma research due to limited understanding of contexts in which OXPHOS is required for cancer cell growth and survival.

Methods: To determine the impact of chemotherapies (Temozolomide and Cisplatin) on cellular metabolism, metabolomics profiling was performed using liquid chromatography-mass spectrometry (LC-MS) and bioenergetics analyses. Therapeutic responses were studied with chemotherapy in combination with a complex I inhibitor (Phenformin and IACS-010759). In vivo studies investigating the combination of complex I inhibitors and Temozolomide were conducted in immunocompetent C57BL/6J mice. Murine YUMM1.7 melanoma cell allografts were established to evaluate tumor growth and therapeutic efficacy through tumor volume analysis.

Results: Our study examined OXPHOS diversity across different melanoma cell lines, identifying those with high and low mitochondrial OXPHOS activity. We found that melanoma cells exhibit significant mitochondrial dependency as evidenced by increased TCA cycle intermediates, ATP production, and oxidative capacity in response to chemotherapy. Additionally, melanoma cells increase mitochondrial electron transport chain (ETC) complex I as an adaptive response to chemotherapy. Pharmacological inhibition of ETC complex I synergizes with conventional anti-melanoma chemotherapy in preclinical models. Furthermore, the combination of complex I inhibitors and Temozolomide proved to be the most effective treatment in vivo, significantly suppressing tumor growth in a murine melanoma model compared to either treatment alone. The combination therapy was well-tolerated, with no observed reduction in body weight, indicating minimal systemic toxicity.

Conclusion: These findings underscore the clinical promise of employing mitochondrial complex I inhibition as part of a combined therapeutic approach to enhance chemosensitivity for individuals with advanced and refractory melanoma.

#1385 Mitochondrial-Nuclear Retrograde Regulator 1 (MNRR1): A novel regulator of apoptosis.

H. Elshenawy, J. Wu, N. Purandare, S. Aras;
Wayne State University School of Medicine, Detroit, MI

Mitochondrial Nuclear Retrograde Regulator 1 (MNRR1, aka CHCHD2) is a bi-organellar regulator protein localized in the mitochondria and the nucleus. In the mitochondria, MNRR1 regulates oxidative phosphorylation (OxPhos) to generate ATP and inhibits intrinsic apoptosis. Nuclear MNRR1 regulates the transcription of ~ 5000 genes, including many cancer-related genes. Reasonably, this explains the upregulated MNRR1 expression in different cancers. Triple-negative breast Cancer (TNBC) is associated with an aggressive phenotype. The lack of hormonal receptors and tumor heterogeneity cause resistance to conventional therapies and the inability to develop targeted treatments. Using TNBC as a model system, we aim to identify the mechanism of MNNR1 function as an anti-apoptotic protein. Intrinsic apoptosis involves disruption of equilibrium between pro- and anti-apoptotic proteins. Anti-apoptotic proteins are translocated upon apoptotic stimuli by pro-apoptotic proteins that accumulate on the outer mitochondrial membrane (OMM), causing its permeabilization. This allows the release of cytochrome *c* from mitochondria to cytosol to activate the caspase pathway. Our findings show that MNRR1 resides in two different forms in the mitochondria based on its Y99 phosphorylation (pY99R1). pY99R1 induces OxPhos, while the non-phosphorylated protein (npR1) interacts preferentially with the anti-apoptotic protein Bcl-xL. Induction of MNRR1 inhibition using knockout cells or chemical inhibitors sensitize cells to an apoptotic stimulus. MNRR1 knockout cells display a pro-apoptotic phenotype as observed by Bax oligomerization. Voltage-dependent anion channel (VDAC) is an OMM protein that interacts with Bcl-xL, preventing the release of cytochrome *c* to the cytosol. Our study shows that npR1 enhances the VDAC-Bcl-xL interaction complex. This anti-apoptotic function is facilitated by N-terminal 52 amino acids constituting the putative Mitochondrial localization Sequence (MLS) in MNRR1 protein. Collectively, the findings of our study highlight the potential use of MNRR1 as a therapeutic target for novel therapeutics or in combination with existing therapies in TNBC.

#1386 The HSP90 Co-chaperone UNC45A is essential for prostate cancer cell proliferation in vitro and tumor growth in vivo.

Taoufik LLBIYI, Asif Elahi, Digvijay Singh, Nada Eisa, Yasmeen Jilani, Ahmed Chadli

Georgia Cancer Center, Augusta, GA

UNC45A is a key co-chaperone supporting cancer cell proliferation and tumorigenesis. Interestingly, UNC45A is essential for cancer cells but not for normal cell proliferation. However, the mechanism underlying UNC45A regulatory function is not well studied. We have previously shown that UNC45A functions as a glucocorticoid receptor (GR) co-activator to drive the mitotic kinase NEK7 gene transcription. NEK7 is essential for centrosome separation, mitotic spindle formation, and cytokinesis. GR has been implicated in promoting aggressive prostate tumor growth and resistance to therapy. Indeed, GR upregulation is an established mechanism to bypass the androgen receptor blockade therapy in metastatic-resistant prostate tumors. Here, we tested the hypothesis that UNC45A promotes GR-mediated resistance to hormonal therapy. To this end, we used shRNA-mediated gene silencing to evaluate the impact of UNC45A loss on PC3 prostate tumor progression. Our results show that silencing UNC45A reduces cell proliferation *in vitro* and tumor growth *in vivo*. TUNEL analysis and Ki67 staining show that loss of UNC45A triggers DNA damage, reduced cell proliferation, and apoptosis in PC3 tumors grown in the NOD SCID mice model. Immunofluorescence analysis shows a remarkable disorganization of centrosomes in tumor cells expressing UNC45A shRNA. The importance of UNC45A in promoting enzalutamide resistance is being tested in tridimensional spheroid culture and *in vivo* and will be discussed in this meeting. Overall, our results support the hypothesis that UNC45A is a promising cancer therapeutic target and highlight the need for developing UNC45A small molecule inhibitors to treat aggressive diseases such as prostate and breast cancers.

#1387 Superoxide induces phosphorylation-mediated Mcl-1 stability to promote vresistance in hematologic malignancies.

Stephen J. F. Chong[1], Jolin X. H. Lai[1], Kartini Iskandar[1], Chuqi Wang[1], Yuhan Wang[1], Romaine Guieze[2], Deepika Raman[1], Rachel H. F. Lim[3], Catherine J. Wu[4], Wee Choo Chng[1], Alice M. S. Cheung[5], Charles T. H. Chuah[3], Matthew S. Davids[4], **Shazib Pervaiz**[1]

[1]National University of Singapore, Singapore, Singapore,[2]Universite Clermont Auvergne, Clermont Ferrand, France,[3]Singapore General Hospital, Singapore, Singapore,[4]Dana Farber Cancer Institute, Boston, MA,[5]Duke-NUS Graduate Medical School, Singapore, Singapore

Acquired resistance to Bcl-2 inhibitor, venetoclax, poses a therapeutic challenge in the management of chronic lymphocytic leukemia (CLL) and acute myeloid leukemia (AML). Venetoclax-resistant cells exhibit increased accumulation of Mcl-1, thereby switching dependence from Bcl-2 to Mcl-1. However, the mechanism behind increased Mcl-1 accumulation upon the acquisition of venetoclax resistance remains elusive. Given that a change in cellular redox state affects cell survival and drug sensitivity, we sought to investigate the interplay between cellular redox state and Mcl-1 accumulation in venetoclax-resistant cells. Our findings reveal that increased Mcl-1 levels in venetoclax-resistant hematologic malignant cells is associated with increased mitochondrial oxygen consumption and intracellular superoxide ($O_2^{\cdot-}$) levels. Corroborating the link between cellular redox state and Mcl-1 accumulation, increased $O_2^{\cdot-}$ levels in venetoclax sensitive cells was accompanied by enhanced threonine-163 phosphorylation of Mcl-1 (T163pMcl-1), thereby reducing Mcl-1 ubiquitination and degradation. Furthermore, $O_2^{\cdot-}$-induced T163pMcl-1 is mediated by AKT, as evidenced by increased serine-473 phosphorylation. Notably, reducing $O_2^{\cdot-}$ levels or inhibiting AKT decreases T163pMcl-1 and Mcl-1 levels, heightens mitochondrial apoptotic-priming, and restores sensitivity to venetoclax-induced apoptosis. Collectively, our findings unravel redox-dependent mechanism of Mcl-1 stability and accumulation upon the acquisition of venetoclax resistance, which could have implications for the design of novel therapeutic strategy targeting cellular pro-oxidant state or AKT in various venetoclax-resistant hematologic malignancies.

#1388 Exploring the apoptosis-inducing potential of *Cannabis sativa* and cannabidiol in pancreatic cancer *in vitro*.

D. J. Tshabalala, X. Sebutsoe, L. R. Motadi;
University of Johannesburg, Johannesburg, South Africa

Pancreatic cancer is deemed one of the most aggressive types of cancer, with a high mortality rate and poor prognosis. It is ranked the 12th most prevalent cancer globally, with 510,992 new cases and 467,409 deaths reported in 2022. In South Africa, it is ranked the 9th most common cancer, with a mortality rate of approximately 84%. Pancreatic cancer is often diagnosed in advanced stages when the disease is too aggressive to manage, rendering the available treatments ineffective. *Cannabis sativa* (*C. sativa*) is a medicinal plant that has been used for centuries based on its health benefits; more recently, it has been explored for its anti-cancer properties. However, knowledge of the impact of *C. sativa* on pancreatic cancer is still limited. This study aimed to explore the apoptosis-inducing potential of *C. sativa* and cannabidiol (CBD) in pancreatic cancer cells. Crude *C. sativa* extracts were obtained by dissolving dried *C. sativa* leaves in absolute ethanol; CBD was isolated from the crude extract using column chromatography. The cytotoxic effects of the crude extract and CBD on pancreatic cancer cells (Mia-PaCa2) and normal lung cells (MRC-5) were determined using the AlamarBlue reagent. Apoptosis was investigated by analysing morphological changes using light microscopy. This was followed by an evaluation of phosphatidylserine externalisation and nuclear condensation, hallmarks of apoptosis, using Annexin V staining and Hoechst staining, respectively. Furthermore, levels of ATP and activation of caspase-3 and -7, key executioners of apoptosis, were measured. DNA fragmentation was analysed using agarose gel electrophoresis. Lastly, gene expression of apoptosis-related genes was assessed using quantitative polymerase chain reaction (qPCR). The results show that the crude and CBD induced substantial cytotoxic effects on Mia-PaCa2 cells but not MRC-5. *C. sativa* and CBD caused significant morphological changes in Mia-PaCa2 cells, including cell detachment and rounding, phosphatidylserine externalisation, and nuclear condensation as seen through light microscopy, Annexin V staining, and Hoechst staining. Furthermore, the crude and CBD extracts reduced ATP levels and activated caspase-3 and -7, with more pronounced effects in the crude extract. DNA fragmentation was also more distinct in cells treated with the crude extract, validating that crude *C. sativa* and CBD induced apoptosis in Mia-PaCa2. This was further supported by the upregulation of BAX, BAK-1, and p53 and the downregulation of BCL-2, indicating the activation of the intrinsic apoptotic pathway. In conclusion, both crude and CBD have apoptosis-inducing potential in pancreatic cancer cells.

#1389 Obesity paradox on ccRCC: A role of fatty acids?.

Isadora Ramos de Andrade

Radiation Oncology, University of Miami Miller School of Medicine, Miami, FL

Introduction: Obesity is a risk factor for clear cell renal cell carcinoma (ccRCC), the most common type of kidney cancer; coincidentally obesity correlates with better outcomes in ccRCC patients, a phenomenon known as the obesity paradox. Obesity is frequently associated with the consumption of diets enriched in lipids. However, how dietary composition, especially the role of different fatty acids (FAs), can affect ccRCC development, as well as how dietary modification could be used in combination with other therapies for better responses is still unclear. We hypothesize that obese individuals with ccRCC respond better to the therapies due to increased amounts of specific FAs in the circulation, which somehow can sensibilize the tumor cells.
Methods: We used two ccRCC cell lines, UOK101 and 786-O, and a proximal tubular cell line derived from normal kidney, HK-2, as a control. Palmitic acid (PA), oleic acid (OA), and linoleic acid (LA), which are saturated, monounsaturated, and polyunsaturated FAs, respectively, were used as stimuli for the cells, mimicking the levels of FAs found in circulation of individuals with obesity [200 μM]. Lipid droplets (LDs) were evaluated by Oil Red O staining; Viability by MTT; Protein expression by Western Blotting; Oxygen Consumption rate (OCR) by Seahorse; Survival by clonogenic assay; mRNA by RT-qPCR, Cell death by Annexin-V/PI kit; ROS by DCFDA/H2DCFDA kit; Mitochondrial potential by MitoTracker®, Gene expression profile by RNAseq.
Results: All FAs increased the amount of LDs compared to the control, but PA was the least potent. PA impaired the growth rate for the tumor cells compared to the control from 24h, which remained in 72h, even at the lowest concentration [50 uM]. LA also decreased the growth rate, but only at 72h. PA, even at a lower concentration, impaired the formation of colonies for both cell lines compared to control and other FAs. LA impaired colony formation only at the higher concentration. PA also decreased cells migration at the time of 12h, induced cell death and increased cleaved caspase3 expression in 24h, a phenomenon observed only for the tumor cell lines. Regarding lipid metabolism, all FAs decreased fatty acid synthase and increased CPT1 expression, suggesting increase in fatty acid oxidation (FAO). In contrast all FAs decreased OCR, and blocking FAO did not rescue cell death induced by PA. PA decreased mitochondrial membrane potential and also DRP1 protein for UOK, although cytochrome C expression or either ROS production were not increased. Gene expression profiling indicated that PA induces endoplasmic reticulum stress.
Conclusion: In summary, we demonstrated that PA decreased malignant characteristics in ccRCC, such as viability, colony formation, migration, and induced cell death. Taken together, our data suggest that PA could be employed as an intervention in ccRCC treatment, although additional studies are required to evaluate how an enriched PA-high fat diet can impart a better outcome for patients.

#1390 Dysregulation of microtubule dynamics caused by *LOXL3* knockout triggers mitosis catastrophe and senescence in U87MG and U251 cells.

Talita de Souza Laurentino[1], Roseli da Silva Soares[1], Ricardo Cesar Cintra[2], Antonio Marcondes Lerario[3], Suely Kazue Nagahashi Marie[1], **Sueli Mieko Oba-Shinjo**[1]

[1]Neurology, University of Sao Paulo, School of Medicine, Sao Paulo, Brazil,[2]Radiology and Oncology, University of Sao Paulo, School of Medicine, Sao Paulo, Brazil,[3]Internal Medicine, Division of Metabolism, Endocrinology, and Diabetes, University of Michigan, Ann Arbor, MI

The amine oxidase LOXL3 has been implicated in tumor development and progression, with elevated expression in glioblastoma (GBM), the most malignant central nervous system tumor. This enzyme influences adhesion, migration, and proliferation of GBM cells. Furthermore, LOXL3 upregulation contributes to poorer GBM patient outcomes, and its expression is correlated with genes encoding tubulins. Microtubules, the major component of the cytoskeleton, are crucial for the cell cycle and mitotic progression. In this study, LOXL3 knockout was performed using CRISPR-Cas9 in two GBM cell lines, U87MG and U251, which resulted in decreased cell viability and migration in both cell lines. Transcriptome enrichment analysis revealed downregulation of genes associated with tubulin acetylation, which was subsequently confirmed by western blot and immunofluorescence analyses. Both U87MG and U251 cells exhibited downregulation of cell cycle- and proliferation-related genes. Functional assays confirmed that *LOXL3*-knockout cells exhibited delayed cell cycle progression and significant alterations in mitotic spindles during the metaphase-anaphase transition. Nuclear analysis revealed morphological changes indicative of mitotic catastrophe in U87MG cells (with wild-type *TP53*) and senescence in U251 cells (with mutated *TP53*). U87MG cells with LOXL3 knocked out presented increased apoptotic cells, which was enhanced after TMZ treatment. Our results suggest that LOXL3 is a potential therapeutic target for GBM treatment, especially in those with higher LOXL3 expression. LOXL3 inhibition can induce tumor cell senescence in GBM with *TP53* mutation and apoptosis in cases with wild-type TP53 boosting TMZ treatment, improving GBM patients´ outcome.

#1391 The role of lysosomal membrane permeabilization in ECM-detached cancer cells.

Hannah M. Tanczos, Lauren Young, Paulina Harnisch, Isabella Gomez, Jianping He, Jordan Cockfield-Iratishe, Zachary T. Schafer

Department of Biological Sciences, University of Notre Dame, Notre Dame, IN

The progression of metastatic cancer invariably leads to a lower 5-year survival rate compared to patients diagnosed with primary tumors alone, so the elimination of metastasizing cells is crucial to improve patient outcomes. A barrier to cancer metastasis is anoikis, an apoptotic form of cell death induced when a cell loses attachment to the extracellular matrix (ECM). However, cells that do successfully metastasize develop anoikis resistance and are able to survive in ECM-detachment. The survival of ECM-detached metastatic cancer cells can result from a variety of changes including elevated antioxidant activity and altered metabolism. Many of these adaptive changes rely on lysosomes, which are acidic, membrane-bound organelles that are responsible for degradation of materials. Therefore, we hypothesize that lysosomal dynamics contribute to anoikis resistance. We have found that lysosomes in non-cancerous mammary epithelial cells grown in ECM-detached conditions exhibit signs of lysosomal membrane permeabilization (LMP) and dysfunction, suggesting that LMP is caused by ECM-detachment and contributes to anoikis. In support of our hypothesis, we have found that some breast cancer cell lines and oncogenically transformed cells are able to maintain intact, functional lysosomes during ECM-detachment. Preliminary data also demonstrate that the downstream effects of LMP, specifically the release of lysosomal protease cathepsin B into the cytosol, may contribute to anoikis signaling. Ongoing work aims to elucidate the mechanism(s) by which ECM-detachment causes LMP in normal cells and understand the changes in this mechanism that confer LMP resistance in cancer cells. In a translational setting, this information could lead to a therapy that prevents metastasis by sensitizing resistant cancer cells to LMP-mediated cell death, thus improving patient outcomes.

#1392 Mechanism of chemoresistance in esophageal squamous cell carcinoma based on mitochondrial DNA copy number.

Koji Tanaka, Yuto Kubo, Kotaro Yamashita, Tomoki Makino, Tsuyoshi Takahashi, Yukinori Kurokawa, Kiyokazu Nakajima, Hidetoshi Eguchi, Yuichiro Doki

Osaka University, Osaka, Japan

Epithelial-to-mesenchymal transition (EMT) has been reported to be associated with metastasis and resistance to therapy in various carcinomas. However, the mechanism of EMT induction and its regulation remain unresolved. Esophageal cancer is one of the most difficult-to-treat cancers that metastasize and are refractory to therapy. In this study, we focused on mitochondrial DNA to elucidate the mechanism of EMT induction in esophageal cancer and to search for therapeutic targets. We established a resistant strain (TE11-R) by long-term exposure of an esophageal cancer cell line (TE11) to chemotherapy (CDDP), and found that TE11-R acquired mesenchymal characteristics. We also observed that the mtDNA copy number was decreased in TE11-R. Next, we established ESCC with reduced mtDNA copy number (TE11-shT) by knockdown of mitochondrial transcription factor A (sh-TFAM). First, mtDNA copy number was reduced to 60% in TE11-shT. Complex IV activity and membrane potential (MMP) were reduced in TE11-shT compared to control TE11. Next, exposure of TE11 to depolarizing agents (CCCP) decreased expression of epithelial markers (CDH1) and increased expression of mesenchymal markers (CDH2, Vimentin, ZEB1) by qPCR and WB. Exposure of TE11-shT and CCCP to DNMT inhibitors increased expression of E-cadherin, decreased expression of N-cadherin and Vimentin, and improved CDDP sensitivity. and CDDP sensitivity were observed. Decreased mtDNA copy number in esophageal squamous cell carcinoma induced EMT via increased DNMT expression, suggesting that DNMT inhibitors may be therapeutic targets for treatment-resistant esophageal cancer cells.

#1393 Functional analysis of mitochondria-targeting Wnt-inhibitors on metabolically distinct breast cancer cell types.

Yoonseok Kam, Lisa Winer, Natalia Romero

Agilent Technologies, Inc., Lexington, MA

Mitochondria are essential organelles responsible for energy production and survival. In cancer cells, mitochondrial function is often altered through metabolic reprogramming, which enhances their growth, survival, and resistance to therapy. Recent findings suggest that bi-directional crosstalk between Wnt signaling and mitochondrial function plays a crucial role in tumorigenesis, as well as in the differentiation and self-renewal of stem cells. This study investigates the potential of targeting the interplay between Wnt signaling and mitochondrial function in breast cancer cells. We screened 71 agents known to alter Wnt signaling and assessed their impact on mitochondrial function and cell viability in BT474 and Hs578T breast cancer cell lines. The Mito Tox Index (MTI) obtained using the Agilent Seahorse XF MitoTox Assay was used as the parameter to evaluate the impact of test compounds on mitochondrial function. The screening identified Pyrvinium, Niclosamide, and Hexachlorophene as the most potent mitochondrial modulators. Pyrvinium and Niclosamide inhibited the mitochondrial electron transport chain, whereas Hexachlorophene acted as an uncoupler when the cells were exposed to the drugs for 24 hours. The mechanisms of mitochondrial perturbation induced by these drugs were further investigated using BT474 cells permeabilized with the Seahorse XF Plasma Membrane Permeabilizer (PMP), which selectively permeabilizes the cellular plasma membrane without affecting mitochondria integrity. This approach enabled detailed characterization of key components in mitochondrial function, including transporters, enzymes, and electron transport chain complexes. Our results indicated that Niclosamide inhibited complex I/II, either directly or indirectly, while Pyrvinium appeared to suppress mitochondrial function indirectly. Despite the different bioenergetic phenotypes of BT474 (highly oxidative) and Hs578T (highly glycolytic) cells, there was no significant difference in mitochondrial susceptibility to the three drugs. However, significant differential effects on ATP production rates were detected using the Agilent Seahorse XF Real-Time ATP Rate, BT474 cells exhibited a significant metabolic energy crisis in the presence of all three compounds, whereas Hs578T cells maintained stable total ATP production despite mitochondrial inhibition. This differential impact on ATP production rates corresponded directly to differences in cell viability, with BT474 cells showing higher susceptibility to these compounds. These findings suggest that the anti-cancer efficacy of mitochondrial modulators can vary depending on the metabolic phenotype of the target cell and should be considered in therapeutic design. Targeting mitochondrial function through Wnt signaling inhibitors presents a promising strategy for developing effective cancer therapies.

#1394 Apoptosis of pancreatic cancer cells after co-treatment with eugenol and tumor necrosis factor-related apoptosis-inducing ligand.

Hyun Hee Kim, Dae-Hee Lee

Department of Marine Bio Food Science, Gangneung-Wonju National University, Gangneung, Korea, Republic of

Pancreatic cancer is a refractory cancer with limited treatment options, and resistance to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) poses a significant therapeutic challenge. This study aimed to investigate whether eugenol, the main component of clove oil with known anticancer, anti-inflammatory, and antioxidant properties, could enhance TRAIL sensitivity by upregulating death receptor (DR) expression levels in pancreatic cancer cells. To explore this, we treated pancreatic cancer cells with eugenol and TRAIL, individually and in combination, and evaluated apoptosis and cell proliferation rates. Expression levels of key proteins, including DR5, FLICE-inhibitory protein (FLIP), and p53, were assessed. Additionally, reactive oxygen species (ROS) generation, endoplasmic reticulum (ER) stress, and C/EBP-homologous protein (CHOP) involvement were analyzed using siRNA knockdown approaches. Our findings revealed that combined treatment with eugenol and TRAIL significantly increased apoptosis and inhibited cell proliferation compared with eugenol alone. Eugenol upregulated DR5 expression, downregulated the anti-apoptotic protein FLIP, and increased levels of the tumor suppressor protein p53. Furthermore, eugenol induced ROS generation and caused ER stress, with CHOP playing a critical role. CHOP knockdown decreased DR5 expression and reduced the synergistic apoptotic effects of eugenol and TRAIL, confirming the ROS-mediated ER stress-CHOP pathway as a key mechanism. These results demonstrate that eugenol enhances TRAIL-induced apoptosis by upregulating DR5 through ROS-mediated ER stress and CHOP activation, which further enhances ER stress via p53 induction and FLIP downregulation. This suggests that eugenol has the potential to serve as a novel TRAIL sensitizer and offers a promising approach to overcoming TRAIL resistance in pancreatic cancer treatment.

#1395 Constitutive photomorphogenesis (COP)-1 leads to apoptosis in B16F10 metastatic melanoma model.

Joanne Kramer Tobacman[1], Insug O-Sullivan[1], Jiyuan Yang[2], Fuming Zhang[2], Sumit Bhattacharyya[1]

[1]Medicine, University of Illinois at Chicago and Jesse Brown VAMC, Chicago, IL,[2]Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, NY

The E3 ubiquitin ligase Constitutive Photomorphogenesis (COP)-1 inhibits UVB-induced light activation in plants by interaction with UV Receptor (UVR)8 and effects on hypocotyl elongation (HY)5-induced growth. UVB is recognized as a causative factor in human melanomas, and this is the first identification of the role of COP1 in inhibition of melanoma growth and the correlation between effects in UVB-stimulated growth in plants and human melanomas. Following tail-vein injection of 200,000 B16F10 cells in 14-week-old female C57BL/6J mice, intravenous treatment with rhARSB (2.0 mg/ml) or saline occurred on days 2 and 7 and mice were euthanized on day 14. Marked reduction in number and size of pulmonary melanomas was evident in the ARSB-treated mice. To determine the mechanisms underlying the inhibition of melanoma growth, assays were performed in the melanoma tissue, in the human A375 melanoma cell line, and in normal human melanocytes. Experiments included: activated caspase-3 by live cell imaging of A375 cells following ARSB and PBMC; ELISA assays of activated caspase-3/7, cytosolic cytochrome c, BCL2, nuclear ETS1, and phospho(Tyr)-ROR1; Western blots of phospho(Ser473)-AKT1; disaccharide analysis of A375 cells following ARSB silencing and exogenous ARSB; chromatin immunoprecipitation of nuclear FOXO3 and COP1 promoter; mRNA expression of COP1; siRNA inhibition of COP1, ARSB, Insulin Growth Factor 2 Receptor, carbohydrate sulfotransferase (CHST)15; treatment by ROR1 inhibitor cirmtuzumab; and treatment by other inhibitors of signaling pathways. Enhanced apoptosis was evident by increase in activated caspase-3. Live imaging of human A375 melanoma cells treated with rhARSB with and without PBMC showed increase in activated caspase 3/7. The apoptotic mechanism was determined to follow declines in nuclear ETS1 and in ETS1-mediated expression of BCL2, with increase in cytosolic cytochrome c and activated caspase-3. COP1 silencing blocked these effects. Expression of COP1 increased following rhARSB-initiated declines in phospho(Tyr)-ROR1 and phospho(Ser473)-AKT1, leading to increased nuclear FOXO3 and COP1 expression, demonstrated by chromatin immunoprecipitation assay of FOXO3 and COP1 promoter sequence. Effect of exogenous ARSB required insulin-like growth factor 2 receptor (IGF2R), a mannose-6-phosphate receptor. Exogenous ARSB reduced carbohydrate sulfotransferase (CHST)15 expression, shown by disaccharide analysis of chondroitin 4,6-sulfate disaccharides. Decline in CHST15, by rhARSB or CHST15 siRNA reduced phospho(Tyr)-ROR1 and phospho(Ser473)-AKT1, leading to increased nuclear FOXO3 and COP1. This series of signaling and transcriptional events in A375 cells and normal human melanocytes provides new insight into how a conserved, inhibitory response to light-stimulated growth can impact on inhibition of human melanoma growth.

#1396 Additional flexibility and choice with Annexin V StarBright^{TM} Dyes.

Michael Blundell[1], Ryan Mendoza[2], Sharon Sanderson[1]

[1]Bio-Rad Laboratories, Kidlington, United Kingdom,[2]Bio-Rad Laboratories, Hercules, CA

Annexin V staining combined with a viability dye is the gold standard to detect apoptotic cells and necrotic cells by flow cytometry due to its quick and reliable detection of externalized phosphatidylserine on cell membranes. Annexin V staining can be readily combined with immunophenotyping using fluorescently labeled antibodies to obtain cell specific apoptosis information. Unfortunately, when it comes to choice of fluorescent label on Annexin V there are limited options, with traditional fluorophores such as FITC, PE and APC being prevalent. This can cause issues with spillover and spreading when multiplexing, with bright fluorescent proteins such as GFP and with viability dyes that have excitation by multiple lasers, leading to suboptimal data and an inability to expand panel size. Here we present data using StarBright Dye Annexin V conjugates. Not only are the dyes bright so you get excellent separation of positive and negative populations, but they are available in a range of eight excitation and emission wavelengths. StarBright Dyes have been selected to have spectral properties that avoid excitation and emission of common viability dyes, such as 7-AAD and propidium iodide, fluorescent proteins like GFP, and are proven to be compatible in large immunophenotyping panels. When combining with immunophenotyping panels you still have the flexibility and choice to apply best practice, even with larger panels, improving the data obtained. Simplify compensation and avoid resolution issues caused by excessive spreading by using Annexin V conjugated to StarBright Dyes in your flow cytometry panels.

#1397 Inhibition of anti-apoptotic Bcl-2 family members leads to synergistic cell death with ER-stress inducers via disruption of autophagy in glioblastoma.

Tianyi Huang[1], Satoshi Takagi[1], Ryohei Katayama[2]

[1]Japanese Foundation for Cancer Research, Tokyo, Japan,[2]Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo, Japan

Glioblastoma (GBM) remains one of the most challenging brain tumors, necessitating the exploration of novel therapeutic strategies. The purpose of this study was to employ the inhibitor library and combination screening to identify potential therapies for GBM. We uncovered a model that shows inhibition of anti-apoptotic Bcl-2 family members disrupts the protective autophagy under ER-stress condition, thus aggravating the stress level, which synergizes with the inhibition of Mcl-1 and Bcl-xL to cause enhanced cell death in GBM. Eight GBM cell lines were treated with our in-house library compounds, revealing that a pan-Bcl-2 family inhibitor, obatoclax, effectively reduced cell viability across all GBM cell lines. siRNA-mediated knockdown of anti-apoptotic Bcl-2 family members confirmed that simultaneous targeting of Mcl-1 and Bcl-xL leads to enhanced apoptosis and growth inhibition, demonstrating their roles as potential survival factors in GBM. To further enhance its efficacy, we combined obatoclax with a large chemical library containing over 350 compounds to evaluate potential combination effects. Significant synergistic effects, including reduced IC_{50} values and enhanced apoptosis induction, were observed between obatoclax and several ER-stress inducers. It was revealed that when GBM cells were undergoing ER stress, inhibition of Mcl-1 and Bcl-xL further exacerbated the stress level and apoptosis, as evidenced by upregulation of ATF-4, CHOP, Bim, and increased caspase-3/7 activity. Such regulation was found to be achieved by the autophagy disruption induced by the presence of obatoclax or the inhibition of anti-apoptotic Bcl-2 family members. The protective autophagy process, serving as a cellular defense mechanism under ER stress, was observed to be terminated in obatoclax-treated GBM cell lines, with decreased autophagic flux, incomplete autophagic degradation, and accumulation of both autophagy markers p62 and LC3-II. Such disruption was proved to directly result in enhanced ER stress and overwhelmed cell homeostasis, leading to significant upregulation of ATF-4, which, on one hand, promotes the translation of pro-apoptotic genes such as CHOP, and, on the other hand, mediates the upregulation of BH3-only proteins like Bim. Bim also cooperates to inhibit anti-apoptotic Bcl-2 family members, especially Mcl-1 and Bcl-xL, contributing to the synergistic cell death in GBM. In conclusion, our study suggests a unique model in which targeting anti-apoptotic Bcl-2 family proteins, such as Mcl-1 and Bcl-xL, coupled with ER-stress induction, demonstrates promising synergism and enhanced cell death in GBM. This highlights the potential of pan-Bcl-2 family inhibitors to overcome current therapeutic limitations in GBM, providing a novel foundation for future therapeutic approaches via the pro-apoptotic targeting method.

#1398 Caspase-1 dependent lytic cell death influences intra-tumoral neutrophil-to-lymphocyte ratio that limits chemotherapy efficacy.

Stephen (Qi Rong) Wong[1], Ethan Subel[1], Kazukuni Hayashi[2], Hongbo Gao[1], Fotis Nikolo[1], Keith Chan[1]

[1]Houston Methodist Research Institute, Houston, TX,[2]Stanford University, San Fransisco, CA

Chemotherapy remains a cornerstone in the treatment of bladder cancer, with most studies emphasizing its direct cytotoxic effects to induce tumor cell death. However, the influence of various cell death modalities—such as apoptosis, pyroptosis, necroptosis, and ferroptosis—on the immune system remains underexplored. These modalities can either promote an immunogenic response or foster immunosuppression, notably through mechanisms such as altering the neutrophil-to-lymphocyte ratio (NLR), which can impact chemotherapy efficacy. In our study, we observed that gemcitabine treatment in a murine bladder cancer model enriched for a caspase-1-dependent lytic cell death that was non-immunogenic and contributed to an immunosuppressive tumor microenvironment by increasing intra-tumoral NLR. Notably, genetic deletion of caspase-1 shifted this non-immunogenic cell death to an immunogenic form. Additionally, pharmacological inhibition of caspase-1 using VX-765 provided therapeutic benefits by reducing NLR, significantly suppressing tumor growth. While lytic or pyroptotic cell death may be advantageous in the context of infections, our findings highlight a paradox: in the context of chemotherapy treatment, caspase-1-dependent lytic cell death/ pyroptosis can play a detrimental role by promoting an immunosuppressive environment that limits efficacy. These results underscore the importance of understanding and modulating cell death pathways to optimize therapeutic outcomes in cancer.

#1399 Selective induction of apoptosis in pancreatic ductal epithelioid carcinoma PANC-1 cells and normal pancreatic ductal epithelial HPDE6c7 cells by Chinese medicinal herbs *Scutellaria barbata* and *Oldenlandia diffusa*.

Abby Shim[1], Jessica Lee[1], Ryan Hayes[2], **Brian Yuen Yau Wong**[1]

[1]Biology, Andrews University, Berrien Springs, MI,[2]Biochemistry, Andrews University, Berrien Springs, MI

Pancreatic cancer is a highly lethal disease, marked by late-stage diagnosis and rapid metastasis. Despite treatment advances, chemoresistance persists, leading to poor survival outcomes. Conventional therapies often lack selectivity, causing damage to healthy cells. *Scutellaria barbata* (SB) and *Oldenlandia diffusa* (OD) are traditional Chinese medicinal herbs used in treatment formulas in Taiwanese hospitals. We previously showed that SB and OD significantly induce apoptosis in PANC-1 cancer cells. To investigate their potential selective ability, aqueous extracts of these herbs were used on pancreatic ductal epithelioid carcinoma PANC-1 cells derived from a 56-year-old male and on normal pancreatic ductal epithelial HPDE6c7 cells obtained from the pancreatic duct of a 63-year-old female. This study used a green/red/blue fluorescent Apoptosis/Necrosis Detection Kit and Human Apoptosis Antibody Array - Membrane (43 Targets) Test (Abcam). Our fluorescent apoptosis data showed that a 2-hour treatment with 1.0 mg/mL aqueous extract of SB induced a statistically significant percentage of apoptosis in PANC-1 cells as compared to the negative control (88.9 ± 5.6% > 2.7 ± 0.9%); $p<0.05$. In contrast, there was no notable induction of apoptosis in the normal HPDE6c7 cells under SB treatment (1.75 ± 0.25%). Similar apoptotic induction was observed under OD treatment in PANC-1 cells (92.0 ± 0.1% > 2.7 ± 0.9%); $p<0.05$, while HPDE6c7 cells remained non-induced (5.25 ± 4.25%). Furthermore, antibody array membrane data revealed significantly higher upregulation of various pro-apoptotic proteins – such as Caspase 8, IGFBP-3, sTNR-R2, and TRAILR-1 – in PANC-1 cells under OD treatment in comparison to normal HPDE6c7 cells. Modulation of pro-apoptotic proteins was also observed under SB treatment. Both OD and SB treatments led to higher expression of anti-apoptotic proteins in HPDE6c7 cells compared to PANC-1 cells. These results suggest that SB and OD contain phytochemicals capable of selectively inducing apoptosis in pancreatic ductal cancer cells PANC-1 while leaving normal HPDE6c7 cells non-induced. Further research could reveal their precise impact on regulating apoptotic pathways, which may uncover their potential as chemopreventive and therapeutic agents for pancreatic cancer.

#1400 Selective induction of apoptosis by aqueous extracts of Chinese medicinal herbs *Oldenlandia diffusa* (OD) and *Scutellaria barbata* (SB) in human pancreatic adenocarcinoma BxPC-3 cells and pancreatic ductal epithelial HPDE6c7 cells.

Jessica Lee[1], Abby Shim[1], Ryan Hayes[2], **Brian Yuen Yau Wong**[1]

[1]Biology, Andrews University, Berrien Springs, MI,[2]Biochemistry, Andrews University, Berrien Springs, MI

Oldenlandia diffusa (OD) and *Scutellaria barbata* (SB) have been used in traditional Chinese medicine to treat liver, lung, and rectal tumors. They are used in most herbal cancer treatment formulas in hospitals in Taiwan. Pancreatic cancer is one of the most lethal and aggressive human cancers with high mortality rates due to late prognosis. BxPC-3 is a human pancreatic cancer cell line derived from a 61-year-old female with adenocarcinoma while HPDE6c7 is a normal ductal epithelial cell line from the pancreatic duct of a 63-year-old female. Apoptosis is the body's programmed cell death process to combat the proliferation of cancer cells. We previously demonstrated that aqueous extracts of OD and SB selectively induced apoptosis in colon HCT-116 cancer cells but not in colon CCD 841 CoN normal epithelial cells by modulating the pro-apoptotic and anti-apoptotic protein expression. Induction of apoptosis and modulation of apoptotic proteins were also observed in BxPC-3 cells with OD and SB treatments. In this study, the effects of SB and OD on the selective induction and modulation of apoptosis in pancreatic adenocarcinoma BxPC-3 cells and HPDE6c7 normal pancreatic cells were assessed using the Apoptosis/Necrosis Detection Kit and Human Apoptosis Antibody Array-Membrane (43 Targets) test (Abcam). Our fluorescent apoptosis data demonstrates that 2 hours of 1.0 mg/mL OD treatment significantly induced apoptosis in BxPC-3 cells as compared to the negative control (79.95 ± 8.95% > 3.6 ± 1.4%); $p<0.05$, while there was no notable induction of apoptosis in normal HPDE6c7 cells (5.25 ± 4.25%). Most HPDE6c7 cells remained non-induced with OD treatment (94.0 ± 4.5%), comparable to the negative water control (98.75 ± 0.25%). A similar selective induction of apoptosis was obtained from the SB treatment in the BxPC-3 cell line as compared to the negative control (25 ± 8.5% > 3.6 ± 1.4%). There was no significant induction of apoptosis in the normal HPDE6c7 cell line treated with SB, similar to the negative control (1.75 ± 0.25% ≃ 1.25± 0.25%). From the antibody array-membrane experiments, modulation of apoptotic markers, such as the up-regulation of pro-apoptotic proteins CD40, CD40L, cytoC, and TRAILR-1 in BxPC-3 cells with OD treatment; and the upregulation of p53, Caspase 8, and HTRA in BxPC-3 cells with SB treatment was observed. Upregulation of various anti-apoptotic proteins was observed in HPDE6c7 cells. These results suggest that OD and SB contain phytochemicals that selectively induce apoptosis in BxPC-3 cancer cells by modulating certain apoptotic markers while not affecting normal HPDE6c7 cells. Further investigation into their specific effects on regulating apoptosis is warranted to uncover their potential as chemopreventive and therapeutic agents against pancreatic and other cancers.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Gene Regulation and Transcription Factors 2
Poster Session

#1404 Coactivator-associated arginine methyltransferase 1 regulates the transcriptional coactivator function of glioblastoma tumor suppressor Quaking.

Kaylene Lu[1], Tanner Wright[2], Seula Shin[1], Mark T. Bedford[2], Jian Hu[1]

[1]Department of Cancer Biology, The University of Texas MD Anderson Cancer Center, Houston, TX,[2]Department of Epigenetics and Molecular Carcinogenesis, The University of Texas MD Anderson Cancer Center, Houston, TX

As the most common and malignant primary brain tumor in adults, glioblastoma (GBM) remains highly refractory to current therapeutic efforts, and patient outcome has not markedly improved in the past decades. Significant intratumor heterogeneity drives aggressive invasion, immune evasion, and tumor recurrence. Novel therapeutic paradigms informed by deeper mechanistic insights of gliomagenesis are urgently needed. Quaking (Qki, encoded by *Qk*) is frequently altered in GBM patients, with the *Qk* gene mutated or deleted in 35% percent of GBM cases and the Qki protein absent in 50-60%. Our lab has established Qki as an important tumor suppressor in GBM, where *Qk* deletion in a genetic mouse model induces the formation of tumors with high penetrance that recapitulate human GBM. Mechanistically, Qki acts as a transcriptional coactivator for the expression of lipid metabolism genes, in which loss of Qki dysregulates lipid homeostasis and compromises the integrity and dynamics of various membrane structures. While several downstream processes governed by Qki have been delineated, upstream regulators of Qki are largely unknown. By examining cancer patient genomic data, we observe recurrent mutations to specific Qki arginine residues that implicate the dysregulation of arginine methylation. This post-translational modification is catalyzed by protein arginine methyltransferases, which play key roles in transcriptional regulation and co-activator function. Through *in vitro* methylation assays, we demonstrate that Qki is selectively methylated by the coactivator-associated arginine methyltransferase 1 (CARM1), also known as PRMT4. This methylation occurs specifically in the C-terminal Quaking regulatory domain (QRD), where cancer mutations have been reported. We observe that Qki directly interacts with known CARM1 substrates including RNA polymerase II and MED12 of the Mediator complex and is co-detected with these binding partners at the promoters of lipid metabolism genes. Through mutagenesis studies, we identified arginine 242 (R242) and arginine 256 (R256) as specific CARM1-mediated methylation sites on Qki. Mutations to these sites in glioma stem cells compromise Qki's activity as a transcriptional coactivator. Moreover, brain-specific deletion of CARM1 in a novel genetic mouse model results in neurological phenotypes reminiscent of those observed previously with Qki deletion. Altogether, we provide evidence that CARM1-mediated arginine methylation represents a novel regulatory mechanism for Qki, an essential tumor suppressor in GBM.

#1407 Alterations of *EIF1AX* and carboplatin treatment induce non-canonical translation initiation in ovarian cancer.

Jermaine Austin, Subin Myoong, Natalia Badnarek, Sridevi Challa

University of Chicago, Chicago, IL

Ovarian cancer (OvCa) is the deadliest gynecologic malignancy with high mortality due to chemoresistance. High grade serous ovarian cancer (HGSOC) is the most common subtype of ovarian cancer. The standard of care for women with HGSOC is cytoreductive surgery and chemotherapy using carboplatin, which causes DNA damage and oxidative stress. Despite aggressive treatment strategies, most patients with high grade HGSOC relapse. We recently demonstrated that post-translational modification of ribosomal proteins affects mRNA translation and protein homeostasis in ovarian cancer cells by regulating polysome formation and stress granule assembly. In this study we aim to understand the role of errors in translation initiation in chemoresistance in ovarian cancers. Eukaryotic Initiation Factor 1A X-linked (*EIF1AX*) is an essential eukaryotic initiation factor (eIF) that is responsible for both scanning and recognizing the canonical start codon on mRNA transcripts: Adenosine-Uracil-Guanine (AUG). Genetic alterations in *EIF1AX* are frequently observed in several cancers including low grade ovarian serous carcinoma (LGSOC) and high grade ovarian serous carcinoma (HGSOC). Using luciferase reporter assays, we have observed that *EIF1AX* ablation and carboplatin treatment in epithelial ovarian cancer cells increases translational initiation from the non-canonical start codon Uracil-Uracil-Guanine (UUG). We have also observed that *EIF1AX* knockdown specifically decreases the growth of carboplatin-resistant ovarian cancer cells, whereas depletion of *EIF1AX* enhances the growth of carboplatin-sensitive ovarian cancer cells. Moreover, EIF1AX knockdown sensitizes ovarian cancer cells to carboplatin treatment. Collectively, our results suggest that *EIF1AX* ablation and carboplatin treatment result in increased non-canonical translation and play a role in chemoresponses in ovarian cancers.

#1408 CSTF2-induced alternative polyadenylation of GPC3 RNA contributes to hepatocellular carcinoma.

Suk Woo Nam, Soyoung Jeon, Sang Yean Kim, Min Jeong Na, Jin Woong Ha

Catholic University of Korea, College of Medicine, Seoul, Korea, Republic of

Alternative polyadenylation (APA) involves the addition of a polyadenylate (poly-A) tail to the 3' end of an mRNA molecule, which is essential for mRNA stability and translation into proteins. This mechanism is critical for gene expression and function, particularly in cancer. Glypican-3 (GPC3) is significantly overexpressed in hepatocellular carcinoma (HCC) and is associated with poor prognosis. APA profiling analysis showed that the 3' UTR of GPC3 is shortened, which strongly correlates with increased GPC3 expression in HCC. Disruption of GPC3 led to inhibited cellular growth and increased apoptotic cell death in HCC cells, while ectopic overexpression of a GPC3 plasmid stimulated growth and proliferation in these cells. Furthermore, Cleavage Stimulation Factor Subunit 2 (CSTF2), a component of the cleavage stimulation factor complex, was found to promote the shortening of GPC3's 3' UTR through APA regulation in HCC cells. CSTF2 expression also correlated with GPC3 levels and the dPDUI (Percentage of Distal PolyA Site Usage Index) of GPC3 in HCC. Knockdown of CSTF2 resulted in decreased GPC3 expression, which inhibited cell growth and proliferation while increasing apoptotic cell death in HCC cells. Additionally, CSTF2 knockdown led to lengthening of the GPC3 3' UTR, whereas overexpression of CSTF2 shows the opposite effect. Using predictive analysis with online tools TargetScan, miRDB, and miRabel, 40 miRNAs were identified as potential binders to the GPC3 3' UTR. *In vitro* functional analysis demonstrated that miR-96-5p and miR-140-5p sustain the normal expression of GPC3 in hepatocytes, and that the shortening of the 3' UTR of GPC3 RNA contributes to hepatocarcinogenesis. Overall, these findings suggest that the aberrant overexpression of GPC3, driven by selective APA regulation mediated by CSTF2, contributes to the malignant transformation and growth of hepatocytes, providing a novel therapeutic target for liver malignancies.

#1409 Reprogramming the alternative polyadenylation of an oncogenic transcription factor as a therapeutic strategy for prostate cancer.

Giulia Pagani[1], Chiara Casirati[1], Stefania Fornezza[1], Roberta Cacioppo[2], **Paolo Gandellini**[1]

[1]University of Milan, Milan, Italy,[2]MRC Laboratory of Molecular Biology, Cambridge, United Kingdom

Alternative polyadenylation (APA) of mRNAs is emerging as a powerful new player in cancer biology, driving aggressive cancer behavior by reshaping how oncogenes are regulated. In particular, altered APA typically fuels cancer growth by shortening the 3'UTR of oncogenes, thus altering mRNA stability and reducing the ability of miRNAs to suppress cancer-promoting genes. This marks APA as a new hallmark of cancer malignancy and a promising novel therapeutic target. We focused on prostate cancer (PCa), the tumor with the highest incidence in male population worldwide, and on *NFYA* oncogene, a driver of aggressive PCa. *NFYA* encodes for the regulatory subunit of the transcription factor NF-Y, a key player in cell cycle regulation and a well-established actor in cancer. We determined that *NFYA* undergoes 3'UTR shortening in PCa cells, thus resulting in increased protein level by means of enhanced mRNA stability, reduced nuclear retention, and attenuation of miRNA-mediated repression. Thus, it was of interest to investigate the effect of APA on the remodeling of the quantity and activity of NF-YA oncoprotein in PCa cells, and how this influences PCa behavior and aggressiveness. To explore this process, we aimed to reprogram *NFYA* APA towards 3'UTR lengthening in PCa cells and evaluated the effects on PCa cell features. Through CRISPR/Cas9 editing and non-degrading antisense oligonucleotides (ASO), we abrogated the usage of the polyadenylation signal (PAS) of the most abundant *NFYA* 3'UTR isoform, which led to a shift towards increased utilization of the longest *NFYA* 3'UTR and decreased NF-YA protein. This, in turn, curbed PCa cell proliferation. Similarly, ASO-treated cells forced to use only the most distal PAS displayed reduced protein level and impaired cell growth. Therefore, these findings highlight APA as a crucial post-transcriptional mechanism regulating NF-YA activity in PCa. Moreover, they provide the proof-of-concept for a possible APA-directed and gene-specific therapy. This is further strengthened by the emerging application of ASOs in the clinics, which opens the possibility of their use to therapeutically manipulate the effects of APA in PCa. Overall, our results build the biological rationale for the development of novel and innovative therapeutic opportunities for aggressive PCa based on *NFYA* APA modulation, possibly combined with standard therapies, which could help treatment optimization and improve patients' survival in the medium-long term.

#1411 ADAR1 enhances ribosome biogenesis and triggers oncogenic translation.

Xuliren Wang, Zhibo Shao, Qi Zhang, Han Zhu, Bingqiu Xiu, Yayun Chi, Jiong Wu

Department of Breast Surgery, Key Laboratory of Breast Cancer in Shanghai,Fudan University Shanghai Cancer Center; Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, China

Background: Ribosome biogenesis (RiBi) is a critical process underpinning cellular protein synthesis, meticulously regulated to maintain homeostasis. Dysregulated RiBi and translational control are integral to the uncontrolled proliferation of tumors, a hallmark of cancer, yet the molecular mechanisms remain poorly understood. Adenosine Deaminase Acting on RNA 1 (ADAR1), a key enzyme mediating RNA editing, has been linked to tumor transcriptomic heterogeneity and resistance to PD-1 immune checkpoint blockade. However, its precise role in tumor ribosome biogenesis and oncogenic translation remains unclear. Here, we uncover a novel connection between ADAR1 and RiBi, revealing its unrecognized role in driving tumorigenesis and therapeutic vulnerability. **Methods**: To identify ADAR1-regulated pathways, RNA sequencing (RNA-seq), ribosome profiling (Ribo-seq), and gene set enrichment analysis (GSEA) were performed in ADAR1-siRNA-treated tumor cells. The association between ADAR1 and RiBi was validated using single-sample GSEA (ssGSEA) in TCGA datasets and the RiBi Alu Editing Index (AEI) in the FUSCC TNBC cohort. Nucleolar morphology was evaluated using immunofluorescence and electron microscopy, while ribosome abundance and translational efficiency were assessed via sucrose density gradient centrifugation, rRNA quantification, and SUnSET assays. RNA pull-down and Sanger sequencing validated downstream targets. Functional and therapeutic potential of ADAR1 inhibition were assessed in mouse xenograft models and patient-derived organoids (PDOs) treated with CX-5461, an RNA polymerase I inhibitor. **Results**: ADAR1 was found to play a pivotal role in driving onco-RiBi, enhancing ribosome production and translation of oncogenic pathways such as MYC, mTOR, and DNA repair. Ribo-seq revealed that ADAR1 knockdown suppressed the translational efficiency of pro-tumorigenic genes, including *MCM2* and *CDC6*. Mechanistically, ADAR1-mediated A-to-I RNA editing of Nucleolar Protein 14 (NOP14) stabilized fibrillarin (FBL), a key nucleolar marker, preserving nucleolar integrity and promoting ribosome assembly. High ADAR1 expression and elevated RiBi AEI scores correlated with poor prognosis in TCGA and FUSCC cohorts. CX-5461 disrupted RiBi, induced nucleolar stress, and suppressed tumor growth in preclinical models. PDOs with high ADAR1 expression demonstrated increased sensitivity to CX-5461, highlighting its therapeutic potential for targeting ADAR1-driven ribosomal dysregulation. **Conclusions**: Our study establishes a critical link between post-transcriptional RNA editing, tumor ribosome biogenesis, and oncogenic translation, addressing a key gap in the field. ADAR1 emerges as a pivotal regulator of onco-RiBi, and targeting ADAR1 in tumors with high expression using CX-5461 represents a promising therapeutic strategy.

#1412 Translational regulation of *MYC* in acute lymphoblastic leukemia.

Sankalp Srivastava[1], Jagannath Misra[1], Rodney Claude[1], Michael Holmes[1], Gang Peng[1], Kirk Staschke[1], Sandeep Batra[1], Utpal Dave[1], Chi Zhang[1], Ronald Wek[1], Jing Fan[2], Ji Zhang[1]

[1]Indiana University, Indianapolis, IN,[2]University of Wisconsin-Madison, Madison, WI

Acute lymphoblastic leukemia (ALL) is the most frequently diagnosed childhood cancer, with a prevalence of almost 25%. ALL cells are auxotrophic for asparagine and have been targeted therapeutically by treatment with bacterial-derived asparaginase. Although asparaginase has been a cornerstone of treatment, ALL can relapse in 10-15% of cases. We previously published that asparagine synthetase (*ASNS*) induction in response to asparagine depletion is a primary resistance mechanism to asparaginase therapy. Using auxotrophic DND-41 cells, we also showed that asparagine starvation suppresses the expression of c-MYC (MYC) protein, a critical oncogenic driver in ALL cells. We did not observe a change in *MYC* transcript levels or protein stability, suggesting the acute decrease in protein level was due to a decrease in translation of *MYC* mRNA. We performed a polysome profiling in DND-41 cells to measure the translation of *MYC* mRNA. Compared to asparagine-replete control, asparagine starvation caused a significant decrease in the amount of heavier polysomes associated with *MYC* mRNA. We further performed a Ribo-seq experiment, where cell lysates were treated with RNAse I, leaving ribosome-protected fragments (RPFs) intact for high throughput sequencing. Our analysis showed that RPFs were distributed throughout the *MYC* coding sequence (CDS) for the control samples but enriched in the N-terminal of the *MYC* CDS when asparagine was limiting. Of note, these RPFs were around closely spaced asparagine codons (+4, +9, +11) near the translation initiating AUG codon, suggesting this is a critical cis-element regulating the translation of *MYC* mRNA. We designed a MYC-translation reporter with the MYC N-terminal region fused to an eYFP-DD construct. The fused protein rapidly degrades upon translation due to the destabilization domain (DD), leading to reduced fluorescence. Treating cells with a small molecule, trimethoprim (TMP), stabilizes the fusion protein, enhancing fluorescence. We defined the translation rate by calculating the ratio of fluorescence intensities with and without TMP. Our results showed that fusing the MYC N-terminal region to the reporter significantly reduced translation compared to the eYFP-DD construct upon asparagine starvation. We also found that deleting the asparagine codons partially rescued the reporter translation, when asparagine is limiting. We will use this system to identify trans-factors regulating *MYC* translation using a dedicated CRISPR library targeting mRNA regulators.

#1413 Dissecting the biology of mutant p53 with the Cellular Thermal Shift Assay (CETSA).

Tomas Friman, Merve Kacal, Laurence Arnold, **Daniel Martinez Molina**

Pelago Bioscience AB, Solna, Sweden

The tumor suppressor protein p53 is one of the most important safeguards against malignant transformation of mammalian cells, but it is also one of the most frequently mutated proteins in cancers. Therefore, p53 constitutes an attractive drug target in oncology. However, there has been little success in finding clinical candidate drugs that directly manipulate the function of p53. The p53 protein offers several challenges for investigators wanting to manipulate its function e.g., the absence of accessible binding pockets, lack of enzymatic activity, and its intracellular location. Moreover, another complication with p53 is the plethora of mutations that alter the protein's characteristics. Most mutational changes in p53 result in a loss of function, but there are also examples of gain of function mutations in p53. We have applied the Cellular Thermal Shift Assay (CETSA) for screening of new chemical hit matter for binding to p53. As CETSA exploits the compound induced changes in protein melting behavior it is important to know the melting characteristics of the protein under investigation. Since p53 displays a wide variety of mutational variants, we decided to investigate three different variants present in three separate breast cancer cell lines: SK-BR-3 ($p53^{R175H)}$, BT-474 ($p53^{E285K}$), and MCF-7 ($p53^{WT}$). The melting behavior of p53 protein was similar irrespective of genomic status of p53 in intact cells. However, in lysed cells there was striking difference in p53 protein melting between MCF-7 cells that carry WT p53 and the two other breast cancer cell lines that carry mutations in the gene coding p53. The melting temperature for WT p53 was nearly the same in both lysate and intact cells, whereas there was 3 to 4°C difference between intact cells and lysates derived from the two cell lines that expressed mutated p53. Both of the mutated forms of p53 found in SK-BR-3 cells and BT-474 cells displayed greater thermal stability in lysate compared to MCF-7. Intrigued by this difference we set out to investigate its cause. It is known that certain mutated forms of p53 are rescued from proteasomal degradation by associating with chaperone proteins like Hsp90. Therefore, the different breast cancer cell lines were treated with inhibitors of Hsp90 function and protein melting of the respective form of p53 was assessed. Hsp90 modulation by Geldanamycin had a much more potent effect on thermal stability of mutant p53 compared to the WT counterpart, which indicates that Hsp90 could be involved in conferring increased thermal stability of mutant p53.

#1415 FraAP, a peptide antagonist against the activator protein 1 transcription factor complex, demonstrates cancer cell cytotoxicity and reduced invasion in vitro and tumor regression in vivo in HNSCC models.

Karen Mendelson, Zachary F. Mattes, Siok Leong, Ricardo Ramirez, Mark Koester, Claudio Scuoppo, Julia Diehl, Erin Gallagher, Binh Lee, Franco Abbate, Lila Ghamsari, Gene Merutka, Barry J. Kappel, Abi Vainstein-Haras, Jim A. Rotolo

Sapience Therapeutics, Inc., Tarrytown, NY

The AP-1 transcription factor complex, comprised of Fra1 and Jun heterodimers, plays a pivotal role in the pathogenesis of head and neck squamous cell carcinoma (HNSCC). This complex significantly contributes to tumor progression and metastasis with its expression positively correlating with poor prognosis. As dimerization of components of the AP-1 complex is required for DNA binding and subsequent transcriptional activity, we designed a peptide (Fra1 antagonizing peptide, FraAP) targeting the basic leucine zipper motif of AP-1 to antagonize AP-1 complex formation and prevent associated activity. Bio-layer interferometry (BLI) and DNA ELISA assays demonstrate target binding and selectivity of FraAP towards AP-1 family members, co-immunoprecipitation experiments reveal that FraAP blocks protein-protein interactions between cJun and Fra1, and reporter assays confirm inhibition of AP-1 transcriptional activity in vitro. Transcriptomic analysis reveals that FraAP suppresses AP-1-driven pathways required for cell cycle progression, proliferation, and invasion and promotes apoptosis. RNAseq data was confirmed by qPCR analysis of relevant target genes. Corresponding functional in vitro assays reveal that FraAP exhibits an inhibitory effect on invasion in Boyden chamber assays and induces a phenotypic mesenchymal to epithelial transition characterized by decreased mesenchymal marker N-cadherin and increased epithelial marker E-cadherin. Annexin V characterization of FraAP treated cells by flow cytometry confirms that FraAP induces dose-dependent apoptosis. Further, in a HNSCC subcutaneous xenograft model, administration of FraAP results in significant tumor growth inhibition, demonstrating the anti-cancer potential of targeting the AP-1 complex. In summary, these data support FraAP as a potent peptide antagonist of the AP-1 transcription factor family that warrants further development as a potential therapeutic option for AP-1 driven tumors.

#1416 Defining the mechanisms of cancer specific transcription factor, tGLI1.

Haddie DeHart[1], Nolan Gregg[2], Julie Heldman[2], Richard Carpenter[1]

[1]Indiana University School of Medicine, Bloomington, IN,[2]Indiana University, Bloomington, IN

Glioblastoma (GBM) is the most common malignant central nervous system tumor in adults with a five-year survival rate of less than 7%. First identified in GBM, truncated GLI1 (tGLI1), an alternative splicing isoform of GLI1, has emerged as a cancer specific GLI1 variant as tGLI1 is highly expressed in both GBM cell lines and primary specimens but not in normal tissue. Despite an in-frame loss of 41 amino acids, the tGLI1 protein has retained all known GLI1 functional domains and appears to respond to sonic hedgehog in a similar manner as GLI1. However, tGLI1 operates as a gain of function transcription factor with the ability to bind to and activate genes unique from GLI1 to promote invasion, migration, angiogenesis, and stemness in both GBM and breast cancer. Despite the advancements in our understanding of tGLI1, a genome wide DNA binding pattern of tGLI1 has not been established and the mechanism by which tGLI1 gains access to these genes is unknown. To establish a more robust understanding of the differential DNA binding patterns of GLI1 and tGLI1, we carried out ChIP-sequencing (ChIP-seq) and found only 14% of GLI1 and tGLI1 binding sites are shared. These results suggest GLI1 and tGLI1 have even more unique binding pattens across the entire genome that previously thought. However, the underlying mechanism for why GLI1 and tGLI1 bind different regions of the genome remains elusive. To determine whether variations in protein interactions of GLI1 and tGLI1 contribute to the unique binding pattern of tGLI1, we carried out immunoprecipitation (IP) followed by mass spectrometry (mass spec). Results identified 45 GLI1 associated proteins and 52 tGLI1 associated proteins with only a 29% overlap between the two. Furthermore, gene ontology analysis revealed 43% of the tGLI1-specific associated proteins are involved in processes related to RNA binding, processing, and metabolism. From these RNA-related proteins, we validated an interaction between tGLI1 and the protein NONO. NONO, which can bind both RNA and DNA, is involved in a range of molecular processes including paraspeckle formation and transcriptional regulation. In GBM, high NONO expression correlates with poor patient outcomes and the mesenchymal subtype. Interestingly, we observed a greater interaction between NONO and tGLI1 as compared to GLI1 and that co-expression of NONO and tGLI1 increased the nuclear localization of both proteins. Overall, these findings enhance our understanding of tGLI1's distinct role in the cell and suggest a potential mechanism underlying its contribution to GBM. Future research will focus on further elucidating the NONO-tGLI1 interaction and investigating the role of NONO in tGLI1-driven phenotypes and GBM progression.

#1417 Defining the mechanism of androgen receptor activation by ABI1.

Kevin Mengen Lin, David M. Solano, Baylee A. Porter-Hansen, Xiang Li, Leszek Kotula

Urology; Biochemistry and Molecular Biology, SUNY Upstate Medical University, Syracuse, NY

Prostate cancer is the most common non-skin cancer among men worldwide and the second leading cause of cancer-related deaths. Prostate tumor development is driven by androgen receptor (AR) signaling, and while early-stage prostate cancer responds well to anti-androgen therapies, resistance frequently emerges. This resistance often involves aberrant reactivation of AR signaling, underscoring the critical need for a deeper understanding of AR regulation to inform new therapeutic strategies.AR activation is mediated by the intrinsically disordered N-terminal domain (NTD), which contains several proline-rich (PXXP) motifs that serve as consensus sequences for SH3 domain binding. Our previous work identified Abelson-interactor 1 (ABI1) as a novel AR-binding protein via ABI1's SH3 domain. ABI1, a key regulator of the actin cytoskeleton as part of the WAVE complex, exhibits dysregulated expression during prostate cancer progression. We hypothesize that ABI1 regulates AR, and its splice variant AR-V7, through multivalent interactions between ABI1's SH3 domain and PXXP motifs in the AR NTD. To investigate this, we used purified AR PXXP peptides and ABI1-SH3 probes in far-western blotting assays, demonstrating that ABI1-SH3 preferentially binds to two of the seven canonical PXXP motifs within AR-NTD. *In vivo* studies with AR NTD-truncation mutants further confirmed that ABI1 regulates AR activity through these interactions. These findings position ABI1 as a regulator of AR activity, potentially linking AR dysregulation with cytoskeletal reorganization and cancer cell metastasis. This work enhances our understanding of ABI1's role in AR-driven tumorigenesis and provides a foundation for future studies aimed at targeting ABI1-AR interactions to develop novel therapies for advanced prostate cancer.

#1418 Mapping the FOXA1 interactome in ER+ breast cancer cells using proximity labeling reveals a novel interaction with NR2C2.

Carla Sofia Rodriguez-Tirado[1], Rosemary Plagens[2], Shen Li[3], Christine Mills[4], Laure Herring[4], Hector Franco[1]

[1]Division of Translational and Clinical Research, University of Puerto Rico Comprehensive Cancer Center, San Juan, PR,[2]Lineberger Comprehensive Cancer Center, Department of Genetics, School of Medicine, University of North Carolina at Chapel Hill, Chapell Hill, NC,[3]Lineberger Comprehensive Cancer Center, Department of Genetics, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC,[4]UNC Proteomics Core Facility, Department of Pharmacology, University of North Carolina at Chapel Hill, Chapell Hill, NC

Approximately 70% of breast cancers are driven by the estrogen receptor alpha (ER), and despite the advent of endocrine therapy to block ER signaling pathways, a significant proportion of women develop resistance to these drugs. The pioneer factor FOXA1 has been shown to facilitate nearly all DNA-binding events of ER in response to estrogen in ER-positive breast cancer (ER+BC). Notably, up-regulation of FOXA1 is a hallmark of endocrine-resistant tumors. Furthermore, FOXA1 has been shown to alter the transcriptome in endocrine therapy resistance. FOXA1 interacting partners, such as ER, androgen receptor (AR), MLL3, YAP1 and GATA-3 mediate transcription activation of genes important for cancer progression. There have been previous studies showing interactors of FOXA1, but none have addressed a comprehensive list of interactors of FOXA1, especially in breast cancer. To address this challenge, we generated MCF-7 cell lines stably expressing the biotin ligase miniTurbo fused to the N- or C-terminus of FOXA1 to classify interactors of FOXA1 in ER+BC. Using proximity labeling (PL) coupled with LC-MS/MS, we aimed to comprehensively catalog interactors of FOXA1. We were able to identify expected proteins such as ER, AR, MLL3, YAP1, and GATA-3. Moreover, we have discovered 157 previously unpublished interactors of FOXA1. Importantly, poorer prognosis for relapse-free survival among ER+BC patients is associated with several of these novel interactors, including NR2C2, an orphan nuclear receptor previously shown to regulate various nuclear receptor signaling but whose roles in breast cancer remain unclear. NR2C2 ChIP-seq in MCF-7 cells under FOXA1-depleted conditions revealed NR2C2 binding loci dependent on FOXA1 levels and binding. By integrating proteomic and genomic approaches, we highlight new mechanisms of FOXA1-mediated transcriptional regulation, with promising potential for endocrine therapy resistance.

#1419 RCZY-711, a novel, highly potent, and orally bioavailable menin-MLL inhibitor with superior efficacy in preclinical acute leukemia models.

Celia X.-J. Chen, Zhengyong Wan, Yu Bai, Xiaohong Liu, Jianming Bao

Rongchang Pharmaceuticals, Ltd., Shanghai, China

Menin, encoded by the MEN1 gene, is a scaffold protein widely expressed in human tissues, regulating diverse functions through interactions in key signaling pathways. The menin-MLL1 (KMT2A) complex plays a critical role in acute leukemias with KMT2A rearrangements or NPM1 mutations, driving aberrant expression of HOX genes and MEIS1, which promote leukemia development. MLL is a histone H3K4 methyltransferase essential for transcription in embryonic development and hematopoiesis and frequently forms oncogenic fusion proteins via chromosomal translocations. Disrupting the menin-MLL interaction is an effective therapeutic strategy for MLL-rearranged and NPM1-mutant leukemias. The FDA-approved menin inhibitor SNDX-5613 treats relapsed/refractory acute leukemia with MLL translocations, while ongoing trials explore its use in KRAS-mutant solid tumors and Wnt-driven cancers. Another menin inhibitor, KO-539, is being tested in advanced gastrointestinal stromal tumors. Developing novel menin inhibitors with enhanced safety and efficacy remains a significant clinical priority.

Herein, we report RCZY-711, a novel, highly potent, and orally bioavailable menin inhibitor with subnanomolar binding affinity, as demonstrated by SPR. Its potency in disrupting the menin-MLL interaction was further validated using a fluorescence polarization assay (subnanomolar IC_{50}). RCZY-711 displayed potent growth inhibition (single-digit nanomolar IC_{50}) in cancer cell lines with KMT2A rearrangements or NPM1 mutations, without cytotoxicity in wild-type MLL cell lines. The compound demonstrated desirable drug-like in vitro ADME characteristics, clean in vitro safety characteristics (hERG IC_{50} > 20 μM), and excellent pharmacokinetic characteristics across multiple preclinical species. Oral treatment with RCZY-711 also showed strong in vivo anti-tumor efficacy in acute myeloid leukemia xenograft mouse models, including the MV-4-11 xenograft model with the MLL-AF4 fusion. The observed superior in vivo efficacy may be attributed to its high cellular potency and improved half-life (mouse: $T_{1/2}$ = 2.0 h). In conclusion, RCZY-711 is a novel, highly potent and orally bioavailable menin-MLL inhibitor with best-in-class clinical potential for treating MLL-rearranged and NPM1-mutant acute leukemias.

#1420 Long-read sequencing of single-stranded DNA of colorectal cancer patients.

Bela Molnar[1], Kristof R. Rada[2], Nikolett Szakallas[2], Barbarad K. Bartak[2], Alexandra Kalmar[2], Istvan Takacs[2]

[1]3DHISTECH Ltd., Budapest, Hungary,[2]Semmelweis University, Dept. of Internal Medicine, Budapest, Hungary

Introduction: The presence of single-stranded (ss) DNA in biological samples has been known for many years, but whole-genome level ssDNA sequencing has only recently become feasible due to advancements in technology.
Aims & Methods: To investigate the potential role of ssDNA further, we measured the ss/dsDNA ratio and fragment length distribution of nearly 200 human biopsy, normal adjacent tissue (NAT) and peripheral buffy coat (BC) samples. This included samples from healthy patients (n=31), as well as individuals suffering from inflammatory bowel disease (n=13), colorectal adenoma (n=53) and carcinoma (n=97).A small subset of these were subjected to a dual enrichment method: negative selection *via* dsDNA digestion is followed by positive selection by AMPure XP beads to yield gDNA enriched in the ss form, which was polyA-tailed and reverse transcribed into an ssDNA-cDNA hybrid, that we subjected to library preparation and whole-genome sequencing on Oxford Nanopore's PromethION platform using various modalities: copy number variations, structural variants, and methylation profiling (5mC and 5hmC). We compared the ds- and ssDNA results of matched samples to highlight key differences between the two forms.
Results: ssDNA concentration was 214 (±14.8) ng/ul, with an integrity (DIN) of 4.45 (±0.13) and length of 14.53 (±1.53) kbs. In the case of dsDNA, this was 152 (±14.6) ng/ul, 7.84 (±0.9) and 59.11 (±3.7) kbps, respectively. Average ss/dsDNA concentration ratio was 2.5 (±0.18), with a few samples having equal amounts, while most of them have at least twice as much single-stranded form. After sequencing, the average coverage was 20x (± 2.14) for dsDNA and 7.33 (±1,17) for ssDNA, with a read length of 4.5 (±0.26) and 2.01 (±0.16) kilobases, consistent with the more fragmented condition of the ss form. CpG 5mC methylation of ssDNA is 43.4% (±2.6) of dsDNA (86.9% when considering one strand is synthetic), which is significant hypomethylation ($p<0.05$). 5hmC methylation ratio is doubling from 4.0% (±1%) on dsDNA to 9.5% (±1%) on ssDNA samples ($p=0.006$). However, we can only detect 1.5% of transversions and transitions, and 0.5% of indels when normalized for coverage differences - strongly uneven representation of the genome in the ssDNA fraction might explain this finding.
Conclusion: Third generation long-read sequencing is a promising and flexible method that can be adapted to examine the ssDNA fraction of the genetic material, providing potential new insights into the background of pathologies.

#1421 RNA-seq for somatic tumor detection reveals synergy between whole transcriptome and targeted RNA library preparation methods.

Chelsea Pinegar, Chen Song, Gautam Naishadham, Jian Sun, Bradley W. Langhorst, Keerthana Krishnan

New England Biolabs, Inc., Ipswich, MA

The interplay between variations in transcriptome profiles and disease progression is an important area of study emerging in cancer diagnostics. Whole-genome or targeted sequencing are conventional methods for somatic tumor assessment, but RNA sequencing has emerged as a powerful tool that can provide additional information on translation events in tumorigenesis. Transcriptome dynamics reflect cell state and can reveal key features in the evolution of disease. RNA sequencing (RNA-seq) has transformed cancer research by providing a more comprehensive view of the tumor especially in comparison to adjacent tissue allowing for advances in characterization and therapeutic options. Using streamlined RNA library preparation workflows in combination with a target enrichment approach can provide quick and cost-effective surveillance of samples, with the addition of sensitivity achieved with targeted sequencing. In this study, we compare bulk RNA-seq datasets with and without target enrichment and assess the power of each data type. They can be used in combination with other DNA based screening methods for biomarker detection in somatic tumors. RNA extracted from paired tumor and normal tissue samples were collected and prepared for Illumina sequencing using the NEBNext UltraExpress RNA Library Prep Kit. Target enrichment panels from Twist Bioscience were utilized to enrich specific transcripts and fusion-gene targets using the same Illumina libraries. Comparison of the whole transcriptome RNA-seq data with the target enrichment dataset for the same libraries reveals the strengths of each approach. The non-enriched RNA-seq library dataset provides a view of transcript levels, and can also be used to call SNVs and short structural variants, as well as splice variants and fusion genes. Target-enriched RNA-seq datasets provide more consistent depth and enhance sensitivity for low-level allele variants in targeted regions. The combination of both methods highlights the synergy between datasets in revealing well-known pathogenic mutations and gene fusions as well as rare or novel events. These sequencing methods, in combination with well validated bioinformatic pipelines, have the power to reveal genetic variations that may otherwise be omitted in more conventional detection methods.

#1422 STAG2-mediated gene expression programs promote invasion in muscle invasive bladder cancer.

S. R. Athans[1], N. Krishnan[2], S. Ramakrishnan[2], A. Stablewski[2], S. Lage-Vickers[3], G. Gueron[3], Z. Kazmierczak[4], A. Woloszynska[2]; [1]University at Buffalo, Roswell Park Comprehensive Cancer Center, Buffalo, NY, [2]Roswell Park Comprehensive Cancer Center, Buffalo, NY, [3]University of Buenos Aires, Buenos Aires, Argentina, [4]University at Buffalo, Buffalo, NY

Stromal antigen 2 (STAG2) is associated with worse overall and progression free survival of muscle invasive bladder cancer (MIBC) patients. Furthermore, STAG2 expression is associated with motility and invasiveness of MIBC cell *in vitro*, suggesting that STAG2 acts as an oncogene in MIBC. In normal cells, STAG2 assists with sister chromatid cohesion, DNA damage repair, and maintenance of genome organization as part of the cohesin complex. However, mechanisms underlying the pro-oncogenic function of STAG2 in MIBC remain unknown. After knockdown of STAG2 in T24 MIBC cells, thousands of genes are differentially expressed. Given this data and the established role of STAG2 genome organization, we hypothesized that STAG2 exerts a pro-oncogenic function through regulating expression of invasion-related genes. To identify target genes, we utilized chromatin immunoprecipitation (ChIP) followed by sequencing and qPCR. From these, we identified the cholesterol efflux pump *ABCA1* as a direct STAG2 target gene, which is significantly downregulated after STAG2 knock out (KO). ABCA1 overexpression is associated with invasion of melanoma and colorectal cancer cells, and therefore we hypothesized that STAG2 mediated *ABCA1* expression may enforce an invasive phenotype in MIBC cells. To assess the mechanism of *ABCA1* regulation, we investigated possible co-regulators and found that in the absence of STAG2, the co-repressor TRIM28 becomes enriched at the *ABCA1* promoter. This suggests that STAG2 and TRIM28 may compete to regulate expression of *ABCA1*, where STAG2 promotes and TRIM28 represses *ABCA1* transcription. In support of this, analysis of patient transcriptome data demonstrates that patients with upper quartile *STAG2* expression or lowest quartile *TRIM28* expression have significantly higher *ABCA1* expression. To further query how loss of STAG2 downregulates *ABCA1,* we analyzed the histone landscape at the *ABCA1* promoter region by ChIP-qPCR. Results show that STAG2 KO is associated with decreased H3K4 and H3K27 acetylation, which are typically associated with active gene promoters. Altogether, these results suggest that STAG2 promotes invasion by enforcing expression of *ABCA1,* which is achieved through displacement of TRIM28 and a subsequent decrease in histone acetylation. This work lays the foundation for investigation of ABCA1 as a targetable molecule in STAG2-high MIBC - for which novel therapies are critically needed.

#1423 Locus-specific proteomics to define transcriptional regulation of GUCY2C hormone expression in colorectal cancer.

Adi Caspi[1], Ariana A. Entezari[1], Jasmine R. Alvarez[1], Jeffrey A. Rappaport[2], Adam E. Snook[1], Scott A. Waldman[1]

[1]Department of Pharmacology, Physiology, and Cancer Biology, Thomas Jefferson University, Philadelphia, PA,[2]Department of Medicine, Johns Hopkins University, Baltimore, MD

The GUCY2C intestinal receptor regulates fluid secretion and epithelial transformation through its hormone ligand guanylin (*GUCA2A*). GUCY2C supports intestinal homeostasis and has been characterized as a tumor suppressor in colorectal cancer. In early tumorigenesis, GUCY2C signaling is silenced through transcriptional repression of guanylin. Guanylin repression is mediated by Wnt/β-catenin/TCF4, a critical signaling pathway aberrantly activated in ~90% of colorectal cancers. Specifically, Wnt signaling regulates expression at a recently characterized 2,683-base pair locus control region (LCR) upstream of the *GUCA2A* gene. This regulation is conferred indirectly, and TCF4, the transcription factor effector of the Wnt cascade, does not interact with the LCR. However, many downstream TCF4 targets are transcription factors canonically implicated in tumorigenesis. Therefore, our goal is to identify factors specifically interacting with the LCR and regulating guanylin expression. Colorectal cancer cell lines expressing inducible Wnt-silencing constructs were used to toggle the expression of guanylin. Using these models, we sought to interrogate Wnt regulation at the LCR by employing C-BERST (d***C***as9-APEX2 ***b***iotinylation at genomic ***e***lements by ***r***estricted ***s***patial ***t***agging), an unbiased proteomics-based approach to profile locus-specific protein interactions. This system relies on peroxidase-mediated biotinylation of proteins by a dCas9-APEX2 fusion protein, driven to a locus of interest by a targeting gRNA. Following localized biotinylation, proteins are enriched by streptavidin pulldown and identified by mass spectrometry analysis. Assessing proteins in the presence and absence of Wnt signaling can reveal Wnt-regulated factors controlling target gene expression. By ChIP-qPCR, we have captured the *GUCA2A* gene locus using targeted gRNAs. Further, dCas9-APEX2 binding does not interfere with the expression of guanylin or its Wnt regulation. Finally, streptavidin-based pulldowns reveal protein interactions at the promoter that can be further characterized by mass spectrometry to elucidate the Wnt regulatory machinery at the *GUCA2A* locus. Identifying the mechanism of Wnt-mediated guanylin silencing, and the transcription factors controlling that regulation, will provide novel insights to inform therapeutic targeting to reconstitute the GUCY2C signaling axis to prevent colorectal cancer.

#1424 Delineating pancreatic ductal adenocarcinoma risk using stem cell derived exocrine pancreas organoids.

K. E. Connelly[1], M. Patel[1], G. Prasad[1], T. Truong Vo[2], H. Arda[2], L. Amundadottir[1];
[1]National Cancer Institute, Rockville, MD, [2]National Cancer Institute, Bethesda, MD

Pancreatic ductal adenocarcinoma (PDAC) is predicted to become the second leading cause of cancer-related deaths by the end of this decade. There is an urgent need to improve risk stratification for prevention and diagnosis and identify novel targets for therapeutic intervention. Both epidemiological factors and genetic variants influence PDAC risk. Epidemiological risk factors include obesity, type 2 diabetes, smoking, and pancreatitis, all of which are associated with inflammation. Genome-wide association studies (GWAS) for PDAC have identified common risk variants with small effect sizes at over 20 loci. Most variants lie in non-coding regions of the genome and thus, the mechanism underlying risk is not obvious. Often enriched in gene regulatory elements, it is thought that the variants influence risk through allele-specific effects on gene regulatory mechanisms, which require extensive follow-up studies. Gene regulatory elements mediate cell-type and stimulus-specific transcriptional programs. Thus, it is crucial to study germline risk variants in models that accurately represent the environment where they exhibit their effects. In an effort to improve our model system for post-GWAS functional studies beyond PDAC-derived cell lines, we have implemented an inducible pluripotent stem cell (iPSC) differentiation protocol to develop exocrine pancreas organoids comprised of acinar and duct-like cells. Preliminary studies demonstrate an enrichment of acinar (*CPA1*, *CTRC*, *AMY2b*) and duct (*SOX9*, *KRT19*, *CA2*) cell markers relative to iPSCs in both the pancreatic progenitor and exocrine organoid cell populations using quantitative PCR. This was further confirmed using immunofluorescence staining of the organoids for CPA1 (acinar), KRT19 (duct) and ongoing flow cytometry analysis. Using Paired-Seq, we are characterizing the chromatin accessibility and transcriptome of the organoids at single-cell resolution to map their gene regulatory elements and compare them to those of PDAC-derived cell lines and FACS-sorted primary exocrine cells. Additionally, we are optimizing different conditions using exogenous cytokine treatment of the organoids to create an *in vitro* system that better resembles an environment conducive to PDAC tumorigenesis. Implementing an iPSC-derived exocrine pancreas model for post-GWAS functional studies opens the door for improved, more in depth functional characterization of risk variants, including how they interact with the cellular environment. This work will help identify risk mechanisms and new therapeutic targets and assist in improving risk stratification of patients.

#1425 Transcriptional and epigenomic landscape of paclitaxel resistant MCF-7 cells.

Julie Hanson Ostrander[1], Thu Truong[2], Marygrace Trousdell[3], Kyla Hagen[2], Nuri Alpay Temiz[4], Michael Ciccone[3], Fatemah Iman Dewji[1], Camila dos Santos[3], Carol A. Lange[1]

[1]Masonic Cancer Center, University of Minnesota, Minneapolis, MN,[2]BMBB, University of Minnesota, Minneapolis, MN,[3]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY,[4]University of Minnesota, Minneapolis, MN

PELP1 and SRC-3 function as essential coregulatory proteins in normal development and cancer. Overexpression of SRC-3 and PELP1 independently influence cancer cell biology through the regulation of 1) proliferation; 2) apoptosis and autophagy; 3) migration, invasion, and metastasis; and 4) therapy resistance. PELP1 and SRC-3 have both nuclear and cytoplasmic functions, and prior reports indicate that cytoplasmic localization of SRC-3 is necessary for SRC-3 phosphorylation that impacts the transcriptional coregulatory function of SRC-3. Cytoplasmic localization of PELP1 promotes tamoxifen and paclitaxel resistance, and breast cancer stem-like cell (CSC) phenotypes. We previously showed that cytoplasmic and nuclear PELP1/SRC-3 complexes are enriched in ER+ therapy resistant breast cancer. Our published data suggests that altered PELP1/SRC-3 signaling leads to altered gene expression that contributes to the expansion of breast CSC population in therapy resistant ER+ breast cancer. To better understand the transcription and epigenomic landscape of this therapy resistant model, we used a combination of RNA-seq and CUT&RUN. Genes previously shown to be associated with paclitaxel resistance were found upregulated in MCF7 TaxR CSC-enriched tumorspheres (i.e. ABCB1, TUBB2B), with ABCB1 being the most differentially expressed gene compared to parental MCF7 cells. RNA-seq analysis suggests that an upregulation of genes involved in interferon and inflammatory response, as well and Ras signaling and EMT in TaxR cells. We also identified 114 overlapping genes from our TaxR model and our previously published cytoplasmic PELP1 model. Pathway analysis indicates that these genes are regulated by nuclear receptors (i.e. ER and PR), which is supported by an increase in PR expression in TaxR CSC-enriched 3D cultures. CUT&RUN data indicates an enrichment in PELP1/SRC-3 overlapping peaks in TaxR cells. Genes associated with overlapping peaks are associated with androgen response, cholesterol homeostasis, notch signaling, protein secretion and TGF-beta signaling MSigDB Hallmark pathways. Together these data support our hypothesis that PELP1/SRC-3 complexes are reprogrammed to promote therapy resistances and CSC phenotypes. We aim to use these studies to identify pathways that can be targeted within CSCs to eliminate this quiescent population and prevent breast cancer metastasis.

#1426 Mechanism study of METTL3/HIF-2α positive feedback loop promoting multidrug resistance in anaplastic thyroid carcinoma.

Junya Ning, Jinpu Yu

Tianjin Medical University Cancer Institute and Hospital, Tianjin, China

Research Background: Anaplastic Thyroid Carcinoma (ATC) is an extremely lethal malignant tumor, with patients having an average survival period of typically less than five months. For ATC patients, conventional treatment methods such as surgery and radioactive iodine therapy are not effective. To date, there is no widely recognized standard treatment protocol, which has resulted in no significant improvement in patient survival rates over the past several decades. In clinical treatment, chemotherapeutic drugs doxorubicin and cisplatin are the primary treatment methods. Additionally, research shows that the multi-targeted tyrosine kinase inhibitor lenvatinib is effective for some ATC patients. However, the reality is that many patients exhibit resistance to these drugs from the early stages of treatment, greatly limiting their potential to benefit from the therapy.

Research Results: Our previous research data found that compared to differentiated thyroid cells and normal thyroid cancer cells, ATC cells have a higher degree of hypoxia, and ATC patients with high tissue hypoxia scores have a worse prognosis compared to those with low hypoxia scores; in vitro experiments demonstrated that hypoxic conditions can induce ATC cell resistance to cisplatin, doxorubicin, and lenvatinib. By comparing tissues with high and low hypoxia scores, we discovered significant enrichment of ABC transport proteins. In ATC tissues, hypoxia scores were significantly negatively correlated with METTL3 expression. Under hypoxic conditions, METTL3 in ATC cells downregulates HIF-2α, thereby reducing ABC transport protein expression. We further found that METTL3 catalyzes m6A methylation modification on HIF-2α RNA, and the "reading protein" YTHDF2 recognizes this modification and participates in RNA degradation, thus reducing HIF-2α RNA levels. Interestingly, we also discovered that HIF-2α can downregulate METTL3 expression through the histone demethylase KDM2B, which removes methylation marks from the 4th lysine of histone H3, thereby suppressing downstream gene transcription.

Research Conclusion: In ATC cells, the hypoxic microenvironment upregulates KDM2B through the HIF-2α pathway. KDM2B downregulates METTL3 through its demethylation action, and METTL3 performs m6A methylation modification on HIF-2α RNA, which is then recognized by YTHDF2, ultimately downregulating HIF-2α expression. This positive feedback loop significantly upregulates the expression of ABC transport proteins involved in multidrug resistance, leading to resistance to drugs such as cisplatin, doxorubicin, and lenvatinib.

#1427 Characterization of epigenetic regulation of MYC in acute myeloid leukemia.

William S. Streitfeld[1], William M. Yashar[1], Itallia V. Pacentine[1], Thai T. Nguyen[1], Samantha J. Worme[2], Gurkan G. Yardimci[2], Andrew C. Adey[3], Theodore P. Braun[1]

[1]Cell, Developmental and Cancer Biology, Oregon Health & Science University, Portland, OR,[2]Cancer Early Detection Advanced Research, Oregon Health & Science University, Portland, OR,[3]Molecular and Medical Genetics, Oregon Health & Science University, Portland, OR

Abnormal epigenetic regulation of *MYC* gene expression is a key driver of aberrant proliferation in Acute Myeloid Leukemia (AML). Using functional and single-cell genomics, we identified enhancer hijacking as a mechanism of *MYC* overexpression in AML cells, describing a new mechanism of oncogenesis. The c-MYC transcription factor is an integral regulator of healthy hematopoiesis and is aberrantly upregulated in multiple malignancies, including AML. The c-MYC protein acts as a fulcrum that balances the differentiation and proliferation of blood cells. Therefore, it represents a target for therapeutic intervention because it acts upstream of a network of additional regulatory factors. However, c-MYC is classically considered undruggable because of its lack of enzymatic function and the high likelihood of treatment-related toxicity owing to its role in nearly all tissues. Recent data have shown that *MYC* transcription depends on interactions with distal enhancer DNA elements, including the *MYC* Blood Enhancer Cluster (BENC). Previous studies have demonstrated that the BENC interacts with the *MYC* promoter to control c-MYC-dependent hematopoiesis and the aberrant upregulation of *MYC* transcription in AML. Additionally, previous characterization of the BENC suggests that eight discrete subregions (BENC modules) independently control the differentiation programs necessary for specific myeloid cell types. Using single-cell ATAC-seq on AML patient samples, we demonstrate that module-specific BENC activation patterns differ between AMLs that display differentiated and primitive phenotypes. However, the functional implications of these patterns remain unknown. We created a map of BENC chromatin activity using CUT&Tag for H3K27ac in human hematopoietic stem and progenitor cell populations isolated by FACS. We also used CRISPR knockout followed by cell culture to demonstrate the functional requirements of each BENC module during healthy human hematopoietic differentiation. Additionally, we used CRISPRi to validate the role of each BENC module in AML cell survival and demonstrated that the proliferative rates of AML cells are sensitive to module-specific BENC inhibition. Our data indicate that BENC usage patterns differ between AML and healthy human hematopoietic cells and suggest that AML cells hijack BENC modules to aberrantly upregulate *MYC* during disease progression. Further characterization of the factors involved in *MYC* regulation by the BENC will provide a framework for identifying and testing new drugs to selectively reverse *MYC* overexpression in AML, with the ultimate goal of improving the treatment outcomes for this difficult-to-treat and highly morbid disease.

#1428 Dissecting a triple-negative breast cancer super enhancer: insights into oncogenic gene regulation.

Natalia Maldonado Vazquez, Nicole Zaragoza Rodriguez, Hector Franco

University of Puerto Rico Comprehensive Cancer Center, San Juan, PR

Triple Negative Breast Cancer (TNBC) is an aggressive and therapeutically challenging subtype of breast cancer, characterized by significant intra-tumoral heterogeneity and the absence of actionable molecular markers. This study addresses the urgent need for targeted therapies by investigating the role of non-coding regulatory elements, specifically super enhancers (SEs), in TNBC pathogenesis. SEs are large clusters of enhancers with high transcription factor occupancy and enhancer RNA (eRNA) production, regulating key genes involved in cell identity and cancer progression. Using Chromatin Immunoprecipitation sequencing (ChIP-seq) and Global Run-On sequencing (GRO-seq) across 13 cancer cell lines, we identified a novel TNBC-specific, transcriptionally active SE on chromosome 17, located upstream of the ITGA3 gene. To elucidate its functional role, we conducted CRISPR-interference (CRISPRi) assays coupled with RNA sequencing in TNBC cells, revealing genome-wide SE target genes. Integration with high-throughput chromosome conformation capture (Hi-C) data established direct chromatin interactions linking the SE to its target genes, which collectively form a gene signature predictive of poor clinical outcomes. Perturbation of this SE via CRISPR significantly impaired tumor cell proliferation, highlighting its role as a potential driver of oncogenic phenotypes. These findings provide new insights into enhancer-target gene interactions in TNBC and identify this SE and its associated gene network as promising therapeutic targets.

#1429 Ubiquitin specific peptidase 37 facilitate prostate cancer oncogenesis by regulation replication stress.

Gunjan Dagar[1], Asha Khuswaha[1], Lakshay Malhotra[2], Ashna Gupta[1], Ajaz Bhat[3], Ammira S Al-Shabeeb Akil[4], Atul Batra[1], Seema Khausal[5], Mayank Singh[1]

[1]Medical Oncology Department, All India Institute of Medical Sciences (AIIMS) New Delhi, New Delhi, India,[2]Department of Biochemistry, Sri Venkateswara College, University of Delhi, New Delhi, India,[3]Sidra Medical and Research Cente, Sidra medicine, Qatar,[4]Department of Human Genetics-Precision Medicine in Diabetes, Sidra Medicine, Doha, Qatar, Doha, Qatar, Qatar,[5]Pathology, All India Institute of Medical Sciences (AIIMS) New Delhi, New Delhi, India

Prostate cancer (PC) is one of the second most common cancer diagnosed and the second most common cause of cancer death in men worldwide. Currently combinations of tumor surveillance, surgery, radiation, hormonal, and chemotherapies are used in the management and treatment of prostate cancer. The molecular basis of disease is complex and mutations in many different oncogenes and tumor suppressor genes have been found including *TP53*, *RB1*, *EZH2*, *BMI*, and *PTEN*. The oncogenesis of prostate cancer appears to be caused by dysregulation of many different cellular signalling pathways especially those involved in cell survival and apoptosis. Protein deubiquitination controls many intracellular processes, including cell cycle progression, transcriptional activation, and signal transduction. Ubiquitin specific peptidases (USPs) remove ubiquitin tags from target proteins to alter protein configuration and function. Recent studies have revealed that Ubiquitin specific peptidase 37 (UPS37) regulates replication stress by regulating important protein involved in several important cellular functions. Analysis of TCGA data indicated that overexpression of USP37 correlated with reduced progression free survival (PFS) in prostate cancer patients. Mass spectrometery analysis of Prostate cancer cells (DU145) indicated that distinct set of genes were altered on overexpression or knockdown of USP37. Our data indicate that USP37 overexpression confers survival advantage while its depletion enhances sensitivity for cell killing in PC cells. USP37 overexpressing cells were able to resolve DNA damage foci much more rapidly than the control cells or cells in which USP37 was depleted in response to genotoxic stress. USP37 depletion results in reduced resolution of γ H2AX and 53BP1 DNA damage foci which indicates the reduced ability of cells to carry out constitutive DNA replication. USP37 was found to interact with different replication factors as also seen in our mass spectrum analysis . We further correlated our data with archived tissue blocks of PC patients by analysing if USP37 overexpression correlated with disease progression. Present data suggests that USP37 is required for tolerance of replication stress in PC and is required to dock additional replication factors and stabilize DNA replication fork. The current data provides new mechanistic details for the regulation of replication stress by USP37 which merits the development of targeting strategies to explore its therapeutic potential in PC by inducing synthetic lethality.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Gene Regulation and Transcription Factors 3
Poster Session

#1432 HMGA1 disrupts 3D genome architecture and chromatin looping to repress MHC-II genes and drive immune evasion.

Linda M. S. Resar, Joseph Kim, Audrey Supreme, Li Z. Luo, Bailey West, Isha Maloo, Jung-Hyun Kim, Leslie Cope, Jerry Spivak

Johns Hopkins University School of Medicine, Baltimore, MD

While targeting immune escape has revolutionized cancer therapy, many tumors, such as acute myeloid leukemia (AML), develop strategies to evade a T-cell mediated immune attack. Myeloproliferative neoplasms (MPN) are clonal hematopoietic stem cell disorders at high risk for transformation to secondary AML (sAML). Leukemic transformation in MPN is almost universally fatal and targetable mechanisms have remained elusive. The *High Mobility Group A1* (*HMGA1*) gene encodes an architectural transcription factor that binds to DNA at AT-rich sequences where it recruits chromatin complexes to modulate gene expression. *HMGA1* is highly expressed during embryogenesis and in adult stem cells, but silenced in differentiated cells. *HMGA1* becomes re-expressed in aggressive cancers where high levels portend adverse outcomes. We previously discovered that *HMGA1* is overexpressed in MPN with highest levels after leukemic transformation. Further, our studies in murine models reveal that HMGA1 is required for MPN progression to sAML. To determine how HMGA1 functions in MPN transformation, we performed multiomic analyses to assess gene expression and chromatin state in MPN AML cells. Here, we uncover a novel role for HMGA1 chromatin regulators as drivers of immune escape by modulating three-dimensional (3D) genome architecture to repress antigen-presenting (AP) genes that trigger a T cell-mediated anti-tumor attack. Mechanistically, HMGA1 binds throughout the class II major histocompatibility complex (MHC-II) locus at key regulatory regions and recruits repressive histones to compact chromatin. HMGA1-mediated chromatin compaction at MHC-II enhancers disrupts binding by the genome organizer, CCCTC binding factor (CTCF), thereby preventing CTCF from orchestrating 3D chromatin loops to activate AP genes and stimulate a T-cell mediated anti-tumor immune attack. Using artificial intelligence, we predicted that the histone deacetylase inhibitor, entinostat, targets HMGA1 networks. Strikingly, *HMGA1* gene silencing or treatment with entinostat restores CTCF-mediated chromatin loops and induces AP genes, resulting in a robust T cell-mediated attack on MPN AML cells. Single cell transcriptomics in patient samples demonstrate that *HMGA1* correlates inversely with MHC-II repression, particularly in AML with bi-allelic *TP*53 mutations, demonstrating the relevance of this HMGA1 network in humans. Together, our data illuminate the HMGA1-MHC-II axis as a promising immunotherapeutic target to activate AP genes and trigger a T cell anti-tumor attack in MPN AML and potentially other cancers overexpressing *HMGA1*.

#1433 Roles of noncoding RNAs in tumor dormancy of breast cancer recurrence.

Noriko Saitoh, Maierdan Palihati, Hiroaki Tachiwana, Tomoko Fujita

The JFCR Cancer Institute, Tokyo, Japan

The vast majority of nuclear long non-coding RNAs (lncRNAs) are expressed in a cell type- or disease-specific manner. They often function as epigenetic factors, and are involved in higher-order chromatin structure [1-3]. Dysregulation of lncRNAs sometimes results in diseases, but the detailed mechanisms remain to be investigated.We have identified ELEANORS (ESR1 locus enhancing and activating non-coding RNAs), a cluster of lncRNAs in a model of endocrine therapy-resistant estrogen receptor (ER)-positive breast cancer, the LTED (long-term estrogen deprivation) cells [4]. ELEANORS are transcribed from a large chromatin domain including the gene encoding ER, *ESR1*. They associate with chromatin, and form a molecular condensate, the ELEANOR cloud, which is required for *ESR1* gene activation. C-technologies, including Hi-C, revealed that ELEANORS define a chromatin domain, TAD (topologically associating domain) of about 0.7 Mb [5]. They also equilibrate cell growth and apoptosis by mediating a long-range chromatin interaction between the *ESR1* and *FOXO3* genes. The active transcription of the *ESR1* locus with ELEANORS may represent frequent RNA polymerase II loading to the locus by a novel transcription initiation mechanism.Analysis of clinicopathological features of ELEANORS using patient tissues revealed that ELEANOR expression in primary tumors positively correlates with late recurrence [6]. Further, cancer stem cell populations that are involved in tumor dormancy for late recurrence were increased in LTED cells. They were decreased upon the ELEANOR knockdown, and the transcription level of the CD44 gene, a stem cell marker, also decreased. The *ELEANOR* and *CD44* genes were frequently co-localized in the LTED cells. Thus, ELEANORS may be involved in stem cell maintenance through chromatin interactions. Our results suggest that nuclear lncRNAs are involved in 3D genome structure and pathological mechanisms.

#1434 Advancing multiomic single-cell ATAC-seq assays by expanding scalability and flexibility using nuclear sample multiplexing and gentle sample preservation techniques.

Hongduan Huang[1], Quyen Bao[1], **Rosary Nguyen**[1], Hye-Won Song[2], Aruna Ayer[1]

[1]BD Biosciences, Milpitas, CA,[2]BD Biosciences, San Diego, CA

Recent advances in multiomic single-cell ATAC-seq assay development have enabled researchers to unravel complex biological questions by simultaneously profiling chromatin accessibility and gene expression in a single nucleus. One major challenge, however, remains in boosting scalability and flexibility while still capturing high-quality multiomic data. Sample preparation and batching could be the key to resolving these issues. Here we demonstrate the feasibility of sample multiplexing utilizing oligo-conjugated nucleoporin antibody for nuclei hashing, allowing higher throughput and more efficient workflows. Up to 6 nuclei samples can be tagged separately and then pooled as one multiplex sample in a single ATAC-seq experiment, which minimizes batch effects and lowers per-sample cost. Additionally, we show the compatibility of sample preservation prior to scATAC-seq workflows using BD® OMICS-Guard Sample Preservation Buffer, providing added flexibility of timing without compromising the health of the cell. Moreover, we have combined these approaches and tested sample multiplexing capabilities on stored samples, further enhancing assays' scalability and flexibility. Our data showed that PBMCs preserved with BD® OMICS-Guard Buffer consistently provided high sensitivity and high specificity ATAC-seq data. High ATAC peak correlation and whole transcriptome (WTA) gene expression correlation indicated that chromatin accessibility, RNA integrity and cell biology were well preserved by BD® OMICS-Guard Buffer. Successful sample multiplexing was observed in both cell line samples and PBMCs. Clear sample de-multiplexing was achieved with > 95% cell identification efficacy and > 95% tagging specificity. No significant impact on scATAC-seq or scRNA-seq data quality was observed, compared to unstained controls. Finally, to understand the usability of both techniques, we tested nuclei tagging of nuclei isolated from cells preserved with OMICS-Guard. The data showed that nuclei tagging and de-multiplexing efficiency remained unaffected by cell preservation. Together, our data support that the applications of BD® OMICS-Guard Sample Preservation Buffer and/or nuclei tagging increase scalability and flexibility to design and perform multiomic ATAC-seq and RNA-seq experiments without compromising data quality, which can profoundly impact experiment costs and overall success.

For Research-Use Only. Not for use in diagnostic or therapeutic procedures.

BD and the BD-Logo are trademarks of Becton, Dickinson and Company. © 2024 BD. All rights reserved. NPM-5011 (v1.0) 1024

#1435 ATRX loss blocks the differentiation of mesenchymal stem cells and advances undifferentiated sarcoma development.

Y. Cai, J. Yao, D. Pan, Y. Jin, J. You, W. Yao, H. Ying;
UT MD Anderson Cancer Center, Houston, TX

Soft tissue sarcomas (STSs) are a collection of rare tumor types originated from the mesenchymal tissues. Among the over 70 histological subtypes of STS, undifferentiated sarcoma (US), which lacks any discernible morphological features of differentiated tissues, accounts for over 20% of all STSs and is correlated with high tumor grade and poor prognosis. Recent genomic profiling in US has identified p53 and ATRX among the most frequently mutated or deleted genes. ATRX is a SWI/SNF2 type of chromatin remodeling factor which functions in a histone variant H3.3-specific chaperone complex to orchestrates replication-independent nucleosome assembly at repetitive heterochromatic DNA regions including telomeres. Loss-of-function mutations occur frequently in many tumor types and is associated with the development of alternative lengthening of telomere (ALT). However, how loss of ATRX function may contribute to tumorigenesis is largely unknown. Here, we demonstrated that ATRX is required for the terminal differentiation of mouse mesenchymal stem cells (MSCs) into multiple lineages, such as adipocytes and osteocytes. Targeted deletion of Atrx in mouse MSCs, but not terminally differentiated mesenchymal lineage cells, accelerated sarcoma initiation induced by loss of p53 and significantly shortened the overall survival. Atrx-deficient tumors express high level of MSC markers such as CD44 and CD29 and exhibit pathological features highly reminiscent of human high-grade US. By analyzing the transcriptome alterations in Atrx null MSC and tumor cells, we identified a cluster of histones, highly involved in stemness maintenance, chromatin structure modifications and cell cycle, are upregulate under Atrx deletion. On the other hand, further assessment in chromatin remodeling reveals abnormal increased chromatin accessibility under Hist1/2 cluster, where most differential expressed histones are located. Transcriptomic analysis under single cell level also indicates that Atrx deficiency is associated with enhanced cell proliferation. Together, our data indicates that ATRX deficiency contributes to undifferentiated sarcoma development by inhibiting mesenchymal stem cell differentiation.

#1436 The nonenzymatic effects of LSD1.

Rachel D. Dreher, Ira Miller, Emily R. Theisen

Nationwide Children's Hospital, Columbus, OH

Ewing sarcoma is an aggressive malignancy and the second most frequent bone tumor afflicting the adolescent and young adult population. Despite knowing the oncologic driving event of Ewing sarcoma for decades, outcomes and treatment options for this disease have not improved. A chromosomal translocation between chromosome 11 and 22, fusing the *EWSR1* gene to the *FLI1* gene (EWSR1::FLI1), is the cause of eighty five percent of Ewing sarcoma cases. Due to the inability to therapeutically target EWSR1::FLI1, the field has instead attempted to target critical co-regulators to disrupt the oncogenic program of EWSR1::FLI1. Histone specific lysine demethylase 1 (LSD1) is overexpressed in Ewing sarcoma and higher levels of LSD1 correlate with poor patient prognosis. Furthermore, LSD1 directly interacts with, colocalizes with, and targets a similar gene set as EWSR1::FLI1, making it a promising target for targeted therapy. Multiple LSD1 inhibitors have been studied with varying effects on the Ewing transcriptome. In particular, OG-L002, a tranylcypromine derivative with potent and specific LSD1 inhibition has little effect on Ewing sarcoma cells, while noncompetitive small molecule inhibitors targeting enzymatic and nonenzymatic functions of LSD1 kill Ewing sarcoma cells at sub micromolar concentrations. As such, we hypothesize that LSD1 has important nonenzymatic functions in Ewing sarcoma. We have curated a suite of molecular tools to deplete LSD1 DNA, mRNA, and protein to define the nonenzymatic effects of LSD1 in Ewing sarcoma. We have knocked out LSD1 via CRISPR genome editing at the DNA level, degraded LSD1 mature RNA via short hairpin RNA interference, degraded LSD1 protein with the UM171 protein degrader, and enzymatically inhibited LSD1 with OG-L002. In every condition we measured the effect on cellular proliferation with Incucyte growth assays, tested the impact on anchorage independent growth with soft agar transformation assays, and found the differentially expressed transcripts with RNA-sequencing. There are only 4 differentially expressed genes when cells are treated with the enzymatic inhibitor OG-L002, compared to hundreds of differentially expressed genes when nonenzymatic functions are depleted as well. In addition, 39 genes that are commonly targeted by depletion at the DNA, RNA, and protein level, corresponding to genes regulated by the nonenzymatic functions of LSD1. We rescued the LSD1 knock out cells with an enzymatically dead mutant (LSD1 K661A) to further specify genes regulated by LSD1 nonenzymatic functions. These genes are related to transcription factor coregulation, axon development, and synaptic organization and signaling. We believe LSD1 serves a scaffolding or recruitment role in various larger chromatin regulatory complexes that are essential for regulation of these genes in Ewing sarcoma oncogenesis that is distinct from LSD1 demethylase activity.

#1437 Enhancer of zeste homologue 2 (EZH2) knockdown reveals molecular drivers of EMT and metastasis in triple negative breast cancer (TNBC).

Maciej Pietrzak[1], Xilal P. Rima[2], Gautam Sarathy[3], Shivani Dhekne[3], Lara Rizzotto[4], Dario Palmieri[4], Sanjay Gupta[5], Daniel Stover[6], Eduardo Reategui[7], Christian Rolfo[8], **Eswar Shankar**[3]

[1]Biomedical Informatics, The Ohio State University College of Medicine, Columbus, OH,[2]William G. Lowrie Department of Chemical and Biomolecular Engineering,, The Ohio State University, Columbus, OH,[3]The Ohio State University Wexner Medical Ctr., Columbus, OH,[4]Cancer Biology and Genetics, The Ohio State University College of Medicine, Columbus, OH,[5]Urology, Case Western Reserve University School of Medicine, Cleveland, OH,[6]Internal, The Ohio State University Wexner Medical Ctr., Columbus, OH,[7]William G. Lowrie Department of Chemical and Biomolecular Engineering, The Ohio State University, Columbus, OH,[8]Internal Medicine, The Ohio State University Wexner Medical Ctr., Columbus, OH

EZH2 drives TNBC metastasis by promoting epithelial-to-mesenchymal transition (EMT), enhancing cancer stem cell traits, and activating pro-metastatic pathways like Wnt/β-catenin and TGF-β signaling. It also represses tumor suppressor genes through H3K27 trimethylation. EZH2 is overexpressed in TNBC, promoting tumor progression via epigenetic silencing, stemness, EMT, and oncogenic pathways, causing drug resistance and reducing the efficacy of therapy. We hypothesized that silencing EZH2 could uncover its target key genes and pathways responsible for driving metastasis in TNBC. EZH2 knockdown was achieved by CRISPR-cas9 in MDA-MB-231 cells. EZH2 knockdown was confirmed by Western blotting. To test the proper formation of spheroid, we employed a 3D culture system of MDA-MB-231 parental and EZH2 KO cells encapsulated in calcium-alginate microgels seeded from a microfluidic droplet generator. The knockdown of EZH2 inhibited tumor spheroid formation and displayed cytoskeleton de-arrangement. The knockdown of EZH2 also attenuated TNBC growth, migration and proliferation in vitro. RNA from parental MDA-MB-231 cells harboring the cas9 control and the EZH2 knockdown (KO) MDA-MB-231 cells was isolated in quadruplets and used for gene expression profiling using RNA sequencing. Differential gene expression (DGE) analysis was performed using edgeR. KO of EZH2 in TNBC and resulted in the upregulation of 1,096 genes and the downregulation of 1,010 genes. Differential gene expression analysis revealed alterations of expression of key regulators of processes such as EMT, migration, and the tumor microenvironment remodeling such as: EGF, IL6, IL6R, JAG1/2, WNT5A, ITGA2/5/V, FN1, and GDF15. Knockdown of EZH2 had a substantial effect on TNBC growth, disrupting the regulation of essential genes and pathways critical for metastasis. Enrichment analysis of the differentially expressed genes identified key biological functions and pathways associated with EZH2 knockdown, including extracellular matrix organization (M610), collagen formation (M631), focal adhesion (MM15913), and degradation of the extracellular matrix (M587). These pathways are crucial for tumor invasion and metastasis, emphasizing the role of EZH2 in regulating the tumor microenvironment. Our findings demonstrate that EZH2 knockdown significantly impairs TNBC growth in vitro and disrupts the regulation of key genes and pathways central to metastasis. (This work is supported by DOD: W81XWH2010065, for Eswar Shankar).

#1438 HMGA2 and protein leucine methylation drive pancreatic cancer lineage plasticity.

S. Kugel[1], S. Dobersch[1], N. Yamamoto[1], A. Schutter[1], S. M. Cavender[1], T. M. Robertson[1], G. Jang[2], A. N. Samraj[3], B. Doron[1], L. A. Poole[1], M. Roudier[1], C. L. Wladyka[1], I. English[1], G. Barreto[4], R. Sears[5], S. Raghavan[6], F. Notta[7], R. N. Eisenman[1], A. C. Hsieh[1]; [1]Fred Hutchinson Cancer Center, Seattle, WA, [2]PanCuRx Research Initiative, Toronto, ON, Canada, [3]University of Washington, Seattle, WA, [4]Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany, [5]Oregon Health and Sciences University, Portland, OR, [6]Dana-Farber Cancer Institute, Boston, MA, [7]University of Toronto, Toronto, ON, Canada

Pancreatic ductal adenocarcinoma (PDAC) is classified into two distinct subtypes, classical and basal. Basal PDACs (~25% of PDACs) have the worst overall survival and are the only class to act as an independent poor prognostic factor. Therefore, there is an urgent need to understand basal PDAC development, identify biomarkers and find its unique vulnerabilities. We found high expression of the chromatin architectural protein, HMGA2 in the basal subtype of pancreatic cancer. This was across patient-derived cell lines, organoids and in RNA-seq and scRNA-seq patient datasets representing hundreds of patients. We also validated by multiplex immunohistochemistry (mIHC) in >500 diverse patient tissue microarray. We introduce a novel genetically engineered mouse model (GEMM) of basal pancreatic cancer. We find that in this model and in human orthotopic xenografts, HMGA2 is required for cancer growth and sufficient to drive a basal subtype. While HMGA2 is a chromatin factor, we have unexpectedly discovered that it plays an essential role in mRNA translation. Our data demonstrate that HMGA2 controls protein synthesis dynamics through reduced methylation of the protein phosphatase PP2A and subsequent activation of S6 Kinase, a key node of translation regulation. HMGA2 accomplishes this through inhibiting the expression of leucine carboxyl methyltransferase 1 (LCMT1). Importantly, loss of LCMT1 in the classical subtype is sufficient to increase mRNA translation and cause the acquisition of basal features. Thus, we describe a new mechanism by which lineage plasticity is regulated in pancreatic cancer. Most importantly, we demonstrate that high levels of HMGA2 in the basal subtype, and high levels of mRNA translation, render basal pancreatic cancer uniquely susceptible to inhibitors of protein synthesis. As such, we describe a new biomarker and subtype-specific treatment for basal pancreatic cancer.

#1439 Gene expression profiling of canine diffuse large B-cell lymphoma: Insights from RNA sequencing.

Nelly Elshafie[1], Ekramy SayedAhmed[2], Michael O. Childress[3], Andrea Pires dos Santos[2]

[1]Purdue University, West Lafayette, IN,[2]Department of Comparative Pathobiology, Purdue University, West Lafayette, IN,[3]Department of Veterinary Clinical Sciences, Purdue University, West Lafayette, IN

Introduction: Diffuse large B-cell lymphoma (DLBCL) is a common and aggressive form of lymphoma in dogs, closely resembling human DLBCL in its clinical behavior and molecular features. While CHOP chemotherapy is the standard treatment, many dogs experience poor responses or early relapse, underscoring the need for a more comprehensive understanding of the disease's molecular underpinnings. This study aimed to investigate the gene expression profiles in canine DLBCL to identify potential biomarkers and therapeutic targets.
Materials and Methods: Fresh frozen lymph node samples were collected from six dogs diagnosed with DLBCL and four healthy controls. Total RNA was extracted, and RNA sequencing (RNA-seq) was performed to profile global gene expression. Differentially expressed genes were identified using DESeq2, and principal component analysis (PCA) was used to distinguish expression patterns between groups. Key findings were validated using quantitative PCR (qPCR) to confirm the dysregulation of selected genes.
Results: RNA-seq analysis revealed 3,156 differentially expressed genes, including 1,418 upregulated and 1,738 downregulated in DLBCL samples. PCA showed distinct clustering between DLBCL and healthy groups, confirming significant gene expression divergence. qPCR analysis validated these results, highlighting the upregulation of genes such as NIT2 (fold change: 3.145; FDR: 0.0074), MYC (fold change: 3.086; FDR: 0.0494), and CDK4 (fold change: 2.151; FDR: 0.0331), suggesting their involvement in tumorigenesis and disease progression. Conversely, genes such as AICDA (fold change: -4.338; FDR: 0.0284), BCL6 (fold change: -2.352; FDR: 0.0469) and CDH1 (fold change: -1.699; FDR: 0.0469) were significantly downregulated, indicating potential suppression of tumor-suppressive pathways.
Conclusion: This study provides valuable insights into the molecular mechanisms underlying canine DLBCL. The identification of differentially expressed genes and pathways highlights novel biomarkers and potential therapeutic targets. Moreover, the findings demonstrate the translational relevance of canine DLBCL as a comparative model for human lymphoma, advancing our understanding of both veterinary and human oncology. *This abstract was edited and formatted with the assistance of generative artificial intelligence (Chatgpt) to enhance clarity and presentation.*

#1440 GEM-X Universal Multiplex: a cost-effective, sample agnostic multiplexing solution for scRNA-seq in cancer research.

Jui-Szu Chen, Sunny Yang, Joe Shuga, Winnia Hui, Ace Santiago, Ivan Akhremichev, Brett Olsen, Daniel Reyes, Mohammad Rahimi, Peter Smibert, Jessica Terry

10x Genomics, Inc., Pleasanton, CA

Resolving heterogeneity within cancer subtypes is critical to better understand the underlying disease mechanisms and to select the most effective treatment options. Single cell RNA sequencing (scRNA-seq) has revolutionized cancer research by providing unprecedented insights into tumor heterogeneity, immune microenvironment, and treatment responses - areas that were previously difficult to explore using bulk RNA-seq. Recently, sample multiplexing has emerged as a significant advancement in scRNA-seq workflows, enhancing throughput, reducing library preparation workload, and lowering per-sample costs. However, traditional multiplexing methods usually require either samples to have distinct genotypes, or the application of cell-specific barcoding before sample pooling, leading to challenges such as high cell input requirements, laborious sample preparation steps, and compatibility limitations across species and sample types.

To address these issues, we present GEM-X Universal Multiplex, a cost-effective, streamlined approach that eliminates the need for upstream tagging, and offers greater flexibility across different sample types, species, and assays. GEM-X Universal Multiplex utilizes a microfluidic chip to co-partition cells from up to four samples, each with a unique gel bead set, simplifying the multiplexing process. After encapsulation, library generation, and sequencing, 10x Barcodes are employed to trace cDNA-derived reads back to their original sample and gel bead set.

We validated GEM-X Universal Multiplex using human kidney dissociated tumor cells derived from a renal cell carcinoma patient and fixed peripheral blood mononuclear cells from patients with acute lymphoblastic leukemia, primary myelofibrosis, multiple myeloma, and squamous cell carcinoma. Our platform demonstrated its ability to uncover the heterogeneity of different sample types and their unique clonotypes. We also compared GEM-X Universal Multiplex with our singleplex product, GEM-X Universal. Both approaches yielded comparable data in terms of library quality and complexity, the number of V(D)J clonotypes detected, and consistent cell type identification and proportions. In conclusion, GEM-X Universal Multiplex provides a powerful and scalable tool for cancer research, offers high accuracy and reduced technical challenges, and enables broader applications in the study of cancers.

#1441 Elevated HOXC gene expression in castration-resistant prostate cancer.

Isaacson B. Adelani, Akira Kurozumi, Tobiloba Owojuyigbe, Sanchit Sanyal, Su Mi Choi, Srinivasan Yegnasubramanian, Shawn E. Lupold

Johns Hopkins University, Baltimore, MD

Background: Androgen deprivation therapy or Androgen receptor-targeted therapies are used to treat metastatic Prostate Cancer (PCa). However, nearly all cases eventually progress to castration-resistant prostate cancer (CRPC). The molecular pathways associated with CRPC development and progression remain incompletely defined. This study aims to identify new drivers and delineate the underlying mechanisms in CRPC.

Methods: Three isogenic castration-sensitive and castration-resistant prostate cancer cell lines were analyzed by miRNA and RNA sequencing. Gene transcripts associated with castration resistance were further characterized by RT-PCR and bioinformatic analyses. Gene knockout and knockdown in CRPC cell lines were carried out using the CRISPR/Cas9 technology and RNA interference (RNAi) technique. In addition, the HOXC gene cluster was overexpressed in castration-sensitive prostate cancer cell lines using predesigned synthetic sgRNAs and the CRISPR activation (CRISPRa) system. Cell growth and survival were assessed using the IncuCyte®-SX5 live cell analysis system.

Results: Differential gene expression analysis identified significantly elevated miR-196a expression in CRPC cell lines. Visualization of RNA sequencing read density peaks in IGV revealed pri-miR-196a-2 as the source of elevated miR-196a expression in CRPC cells. MIR196A2 is located within the HOXC gene cluster. Transcriptional analyses demonstrate that miR-196a-2 is an intronic miRNA co-transcribed within HOXC gene transcripts. Multiple HOXC gene transcripts were more active in CRPC cell lines. Bioinformatic analyses demonstrated significantly higher HOXC gene expression in a subset of metastatic and CRPC tumors. *The elevated* expression was further validated in clinical samples by RT-qPCR. HOXC8 gene over-expression using plasmid gene expression vectors and CRISPRa enhanced prostate cancer cell proliferation, and HOXC8 gene knockout attenuated CRPC cell proliferation.

Conclusions: *HOXC* gene cluster is activated in in a subset of metastatic and castration-resistant prostate cancer with HOXC8 playing a crucial role in regulating cell proliferation.

#1444 Wnt/β-catenin/TCF signaling suppresses GUCY2C hormone transcription by silencing p38.

Ariana A. Entezari[1], Adi Caspi[1], Jasmine R. Alvarez[1], Jeffrey A. Rappaport[2], Adam E. Snook[1], Scott A. Waldman[1]

[1]Pharmacology, Physiology, and Cancer Biology, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA,[2]Department of Medicine, Johns Hopkins University, Baltimore, MD

Sporadic colorectal cancers (CRCs) are initiated by APC mutations inducing aberrant β-catenin/TCF signaling regulating gene transcription. Similarly, the guanylyl cyclase C (GUCY2C) tumor suppressive axis is universally silenced in CRC. While the GUCY2C receptor is retained, transcriptional silencing of its paracrine hormones guanylin and uroguanylin by β-catenin/TCF signaling is among the earliest events in colorectal transformation. Moreover, suppression of the β-catenin/TCF pathway reconstitutes hormone expression. P38 is a tumor suppressor, and its loss promotes tumorigenesis in chemical and genetic mouse models of CRC. We aim to identify mechanisms by which β-catenin/TCF signaling silences the transcription of GUCY2C hormones to enable targeted reconstitution of ligand expression to prevent CRC. Here, we employed four human CRC cell lines with conditional β-catenin/TCF signaling and healthy human and mouse intestinal organoid models to explore the role of p38 in oncogenic transcriptional signaling. Indeed, toggling oncogenic β-catenin/TCF activity regulates p38 phosphorylation and downstream signaling, suggesting that β-catenin/TCF gain-of-function represses the p38 pathway. Further, pharmacological and genetic repression of p38 activity recapitulates β-catenin/TCF gain-of-function and directly eliminates GUCY2C hormone expression. Finally, beyond GUCY2C hormones, regulation of ~80% of a core set of β-catenin/TCF-dependent genes common to human CRC cells is controlled by p38 signaling. Here, we reveal β-catenin/TCF regulation of p38 activity and its role in mediating transcriptional regulation of gene expression underlying nuclear oncogenic signaling in CRC.

#1445 The functional role of promoter germline variants in breast cancer susceptibility genes.

Nelly A. Arroyo[1], Lenin Godoy[1], Doralis De Leon Vazquez[2], Sol V. Perez Martir[2], Julie Dutil[2]

[1]Department of Basic Sciences, Ponce Research Institute, Ponce Health Sciences University, Ponce, Puerto Rico,[2]Clinical and Translational Cancer Research, Comprehensive Cancer Center, San Juan, Puerto Rico

Introduction: Germline variants are associated with breast cancer risk (BC). It is estimated that inherited deleterious or pathogenic variants in high- to moderate-penetrance BC susceptibility genes such as BRCA1, BRCA2, STK11, CDH1, ATM, PALB2, and CHEK2 may explain up to 27% of the BC cases. For the remaining cases, BC develops due to the complex interaction between environmental and lifestyle risk factors and inherited familial predisposition. While low penetrance genetic variants have a limited impact on BC risk when taken individually, their combined effect on polygenic risk scores may provide a risk elevated enough to justify clinical actions. Most of the previous work in hereditary breast cancer research has focused on variants located in the coding region of the genes. and information on regulatory variants remains limited. This study aims to assess germline variants in the promoter region of BC susceptibility genes on gene expression level. We selected the single promoter genes CDH1 and STK11 and the bidirectional promoter genes PALB2/DCTN5 and ATM/NPAT.
Methods: A bioinformatics approach was developed to identify and prioritize promoter variants that could have a functional impact on gene expression levels. Promoters of each gene of interest were cloned into a luciferase reporter expression system. Side-directed mutagenesis was used to introduce the variants of interest in promoter constructs. The reporter constructs were transfected in MCF7 and MCF10A cell lines and the luciferase activity induced by reference promoter was compared to that of the construct carrying the genetic variants All statistical analyses and graphical visualization were conducted in R implemented in R Studio.
Results: A total of 6,427 variants spanning the promoter regions of interest were identified. Of those, thirteen were selected based on conservation level and predicted binding of transcription regulatory proteins. We identified two variants in the promoter region of STK11 that significantly decrease the expression of Luciferase by a factor of 0.83 and -0.04-fold, almost inhibiting the expression of STK11, while the change in CDH1 has a more subtle effect with a variant significantly increase Luciferase expression by a factor of 2.41-fold. Work is on the way to finish characterizing CDH1 variants and the bidirectional promoters ATM/NPAT and PALB2/DCTN5.
Conclusion: This work demonstrates the functional significance of genetic variants located in regulatory regions of BC susceptibility genes. At the clinical level, expanding genetic testing to include regulatory variants could help better define BC risk in women with negative hereditary cancer test results.

#1446 Gene expression characteristics of the young tongue cancer patient.

Bryan Yichong Shi[1], Yi Ren[2], Wei Keat Teo[2], Jerome Wen Jun Bin[2], Joseph W Foley[2], Chor Hiang Siow[2], Kwok Seng Loh[1], Bingcheng Wu[3], Joshua K Tay[1], Donovan Eu[1]

[1]Department of Otolaryngology - Head & Neck Surgery, National University Hospital, Singapore, Singapore, Singapore,[2]Department of Otolaryngology - Head & Neck Surgery, National University of Singapore, Singapore, Singapore,[3]Department of Pathology, National University Hospital, Singapore, Singapore, Singapore

Introduction: Historical risk factors for tongue cancer include age, chronic exposure to tobacco and alcohol use. Smoking-related tongue cancer has declined due to active anti-tobacco campaigns in developed countries, yet the incidence of tongue cancer has increased, particularly in women and patients under in a younger age group. This study aims to investigate differences in the genomic processes driving oncogenesis in young and old patients with tongue cancer.

Methods: We perform laser-capture microdissection on 54 formalin-fixed paraffin-embedded (FFPE) tongue cancer samples collected from glossectomy surgeries performed from 2009 - 2020 at the National University Hospital, Singapore. To ensure purity, tumor epithelial and microenvironment compartments were separately microdissected for each specimen. Gene expression libraries for each compartment were prepared using a specialized RNA-Seq technique optimized for tiny amounts of FFPE material, followed by next generation sequencing. Differential gene expression and geneset enrichment analysis was performed, comparing patients aged ≤ 50 years (Young Group) and > 50 years (Old Group).

Results: We present a large cohort of 149 microdissected gene expression libraries, including 65 tumor epithelial, 61 microenvironment and 23 normal-adjacent regions. Principal component analysis showed distinct clusters of tumor epithelial and microenvironment gene expression profiles, confirming sample purity. Kaplan-Meier survival analysis revealed poorer outcomes in the Young Group compared to the Old Group. Analysis of the tumor epithelial compartment revealed that young and old tongue cancers exhibited similar gene expression profiles. Notably, there were few differentially expressed genes in the Young Group compared to the Old Group. However, in the microenvironment compartment, we observed that genes involved in immune response were downregulated in the Young Group, including cell killing (Normalised Effect Size =-1.80, p-adj=0.00704) and leukocyte-mediated cytotoxicity (NES=-1.77, p-adj=0.0367). Instead, genes involved in muscle cell development (NES=1.93, p-adj=0.000301) were enriched in the Young Group, indicating the presence of tongue muscle tissue instead of an inflamed tumor microenvironment.

Conclusion: The “young tongue cancer” patient has been suggested to be a unique and aggressive biological entity that exhibits distinct epidemiological and clinical characteristics. Our findings indicate that while tumor epithelial compartment in young and old tongue cancers have similar gene expression profiles, differences in the tumor microenvironment, particularly an immune-cold phenotype in young tumors, may explain their poorer survival outcomes and aggressive behavior. Establishing these biological differences will provide critical insights and support the development of treatment strategies to improve survival outcomes for these patients.

#1447 Histone demethylase JMJD1A regulates glutamine metabolism in prostate cancer.

S. Khadka, M. Pornour, H.-Y. Jeon, A. Hussain, J. Qi;
University of Maryland, Baltimore, Baltimore, MD

Epigenetic regulation plays a crucial role in the development and progression of prostate cancer. JMJD1A (also called KDM3A) promotes prostate cancer progression by upregulating gene expression through the removal of repressive H3K9 methylation marks. Here, we found a role for JMJD1A in the regulation of glutamine biosynthesis in prostate cancer cells. JMJD1A knockdown (KD) significantly reduced cellular glutamine levels and inhibited prostate cancer cell growth under glutamine-deprivation conditions. RNA-seq studies showed that JMJD1A KD reduced the levels of GLUL, a key enzyme in *de novo* glutamine synthesis. mRNA levels of JMJD1A and GLUL were positively correlated in human prostate cancer datasets. Re-expression of GLUL in JMJD1A-KD cells significantly restored cellular glutamine levels and partially rescued the growth defect of prostate cancer cells in glutamine-free growth media. Further, ChIP-seq studies showed that JMJD1A interacted with the methyltransferase EZH2 on the GLUL gene promoter, and JMJD1A/EZH2 cooperatively upregulated H3K27ac levels to promote GLUL expression. Our study revealed cooperation between JMJD1A and EZH2 in regulating gene expression and glutamine metabolism and supports the co-targeting of JMJD1A and EZH2 for potential prostate cancer therapy.

#1448 An evolutionarily conserved chromatin remodeling factor, FACT, in regulation of chromatin, gene expression and cancer.

Priyanka Barman, Sukesh Bhaumik

Biochemistry and Molecular Biology, Southern Illinois University, Carbondale, IL

Eukaryotic genome is packed in the form of chromatin that is an array of nucleosomes. Such nucleosomal organization acts as a barrier for gene regulatory factors to access DNA for transcription. Importantly, cells have evolved with mechanisms for nucleosomal disassembly and reassembly for transcription to occur through chromatin. A heterodimeric chromatin remodeling factor, FACT (Facilitates chromatin transcription), plays a key role in nucleosomal disassembly and reassembly to promote transcription. Intriguingly, FACT is upregulated in breast and other cancers, and FACT upregulation induces oncogenesis. Thus, the upstream regulatory pathways/factors of FACT can emerge as cancer biomarkers which can be targeted for an effective cancer therapy. Further, FACT is found to be downregulated and/or mutated in various cancers including breast cancer. Thus, it is crucial to understand regulatory mechanisms lying upstream of FACT with downstream functions in regulation of chromatin to orchestrate gene expression towards understanding cancer pathogenesis with targeted therapeutic potential. In this conference, we will present our recent work on FACT regulation and functions in controlling chromatin and gene expression with implication in cancer.

#1449 The MAPK pathway regulates cellular identity in lung and pancreatic adenocarcinoma.

Sydney N. Larsen, Walter Orellana, Katherine Gillis, Eric Snyder

University of Utah Huntsman Cancer Institute, Salt Lake City, UT

Recently published data from our lab indicates that downstream signaling from oncogenic KRAS can alter the differentiation state of NKX2-1-negative lung adenocarcinoma (LUAD), a subtype of lung cancer with a foregut-like identity similar to classical pancreatic ductal adenocarcinoma (PDAC). In NKX2-1-negative LUAD, inhibition of the MAPK pathway induces a FoxA1/2-dependent lineage switch marked by the down regulation of gastric surface mucosal epithelium markers and induction of genes associated with zymogenic chief cells of the stomach. We used a pharmacological approach to investigate whether MEK/ERK signaling regulates the differentiation state of classical PDAC. Treatment of murine PDAC organoid lines with a MEK inhibitor led to the induction of zymogenic chief cell markers. Further analyses showed that the deletion of either *Foxa1/2* or *Hnf4α* blocked the induction of chief cell transcripts in response to MEK inhibition. Moreover, exogenous expression of FoxA1/2 revealed that the expression of FoxA1/2 alone was sufficient to rescue MEK inhibitor-induced chief cell programming in genetically FoxA1/2-null organoids. This prompted the hypothesis that the genomic localization and activity of FoxA1/2 and HNF4α are regulated by MAPK signaling in PDAC. To elucidate the mechanisms by which FoxA1/2 and HNF4α activate the zymogenic chief cell program in PDAC in response to MAPK pathway inhibition, we treated KRAS-driven, P53-null murine PDAC organoids with various MEK inhibitors. We then performed ChIP-seq for FoxA1/2 and HNF4α on these organoid lines with pharmacological MEK/ERK manipulation to determine whether MEK/ERK activity modulates binding FoxA1/2 and HNF4α to zymogenic chief targets, facilitating this lineage switch. The evaluation of FoxA1/2 binding patterns and differential binding analysis revealed no direct binding of FoxA1/2 and chief cell targets of interest in these PDAC organoids upon MEK/ERK inhibition. This suggests that the induction of the chief cell lineage switch may not be directly orchestrated by FoxA1/2 relocalization but by alternative methods. We are currently investigating potential indirect mechanisms by which FoxA1/2 and HNF4α induce this lineage switch using ChIP-seq and ATAC-seq.

#1450 Differences in expression levels of putative breast cancer risk genes in normal breast tissues between Black and White Americans.

Lili Liu[1], Guochong Jia[2], Jie Ping[2], Xingyi Guo[2], Ran Tao[2], Bingshan Li[2], Qiuyin Cai[2], Wei Zheng[2]

[1]Vanderbilt University, Nashville, TN,[2]Vanderbilt University Medical Center, Nashville, TN

Background: Despite having a similar incidence of breast cancer, Black American women persistently endure higher mortality rates than their white counterparts. Reasons for this disparity are unclear. We evaluated possible racial differences in expression levels of putative breast cancer susceptibility genes identified in recent studies.

Methods: Included in this analysis are 429 lincRNA or protein-coding risk genes identified in our recent transcriptome-wide association and fine-mapping studies using multi-ancestry data. In this study, The RNA sequencing data for these genes were derived from normal breast tissue samples obtained from 161 Black and 287 white participants recruited in the Susan G. Komen Biobank. To address potential batch effects, we used residuals after the probabilistic estimation of expression residuals correction, with standardized gene expression levels as input data after quantile and inverse rank normalization. Generalized linear regressions were used to investigate racial difference in gene expression levels. Models were adjusted for age, body mass index, and menopausal status. The significance threshold was set at 0.05 divided by the number of independent tests.

Results: Out of the 429 genes evaluated in the study, 50 genes showed significant racial differences in expression levels at $P < 4.54e^{-4}$ (0.05/110), the significant threshold after adjusting for multiple comparisons. Of them, 28 genes showed a higher expression level in Black than white participants. The top three genes with highest percent differences were *GSTM1*, *PRSS45*, and *DIRC3*. The *GSTM1* gene showed a 65.86% higher expression level in Black samples (P=2.58e^{-9}), followed by *PRSS45* (percent difference = 37.75%, P = 9.84e^{-9}) and *DIRC3* (15.52%, 2.95e^{-11}). On the other side, the top three genes with lowest percent difference were *LRRC37A*, *ARL17A* and *LRRC37A2*. Gene *LRRC37A* showed a 35.27% lower expression level in Black than white samples (P=2.22e^{-16}), followed by gene *ARL17A* (-25.14%, 1.35e^{-15}) and *LRRC37A2* (-15.38%, 6.51e^{-13}).

Conclusion: We identified 50 genes showing racial differences in expression levels in breast normal tissues. Further research is needed to understand the reasons for these differences.

#1451 *TPM4* isoform expression modulates a cancer stem cell phenotype in human colon cancer cells.

Nia Lowe[1], Giulio Ferrero[2], Barbara Pardini[3], Alessio Naccarati[3], **Michael Papetti**[1]

[1]Touro University, New York, NY,[2]University of Turin, Turin, Italy,[3]Italian Institute for Genomic Medicine, Turin, Italy

Tropomyosin 4 (TPM4) regulates contractility and migration in many cell types and is important in the migration of colon epithelial cells. Several different alternatively-spliced *TPM4* isoforms are predicted to exist in mammalian cells. However, *TPM4.1* and *TPM4.2*, differing only in the inclusion of 1 (*TPM4.2*) or 2 (*TPM4.2*) unique exon(s) at the 5' end, are the only two isoforms experimentally validated. We previously showed that *TPM4* expression may be an early, premalignant molecular event that contributes to adoption of a tumorigenic state and, specifically, that overexpressed *TPM4.2* blocks certain aspects of induced differentiation in colon epithelial cells and promotes a highly proliferative state. Many studies have shown that *in vivo*, tumors are heterogeneous and contain a very small subpopulation of cancer stem cells that not only drive growth of the tumor via robust proliferation but also are highly invasive and resistant to conventional anti-cancer treatments. Identifying molecular characteristics that promote cancer stem cell formation is therefore critical to developing effective strategies to treat and prevent colon cancer. Adherent LS123 cells (LS123 2D), representing minimally-invasive colon cancer cells at an early stage of tumor development, express both *TPM4.1* and *TPM4.2* transcripts, while nonadherent LS123 tumorspheres (LS123 3D), having a highly invasive, robustly proliferating phenotype similar to cancer stem cells, exclusively express *TPM4.2*. Therefore, we hypothesize that *TPM4.2* overexpression, either by itself or in combination with *TPM4.1* suppression, plays a role in transforming colon epithelial cells to a cancer stem cell phenotype. To test this hypothesis, we determined whether knockdown of *TPM4.1* and/or overexpression of *TPM4.2* will induce a gene expression pattern characteristic of cancer stem cells in the LS123 2D cell line. Specifically, we measured the expression of Sox2, Lgr5, epithelial cell adhesion molecule (*EpCAM*), activated leukocyte cell adhesion molecule (*ALCAM*)/*CD166*, aldehyde dehydrogenase I (*ALDH1*), and *CD44* in LS123 2D cells treated with siRNA targeting *TPM4.1* or *TPM4.2* or in these cells in which *TPM4.1* or *TPM4.2* is overexpressed. Our results indicate that favoring *TPM4.2* over *TPM4.1* expression in LS123 cells increases expression of certain stem cell markers, particularly *CD44* and to a lesser extent *EPCAM* and *ALCAM*, whereas favoring *TPM4.1* over *TPM4.2* expression shows the reciprocal effect on cancer stem cell marker expression. These results may identify distinct roles for expression of individual *TPM4* isoforms in colon epithelial cells and may specifically elucidate whether *TPM4.2* overexpression can contribute to a more aggressive tumorigenic phenotype. Ultimately, our studies may identify therapeutic strategies that target perturbations in cell contractility and migration at early stages of colon tumorigenesis.

#1452 DVL1 regulates global transcriptomic changes and associates with the transcription factor ETS1.

Dalia Martinez-Marin[1], Monica Sharma[2], Jenna C. van Wunnik[3], Flavia Sardela de Miranda[2], Geetha P. Boligala[2], Ella C. Jull[4], Grace C. Stroman[4], Rachel L. Babcock[2], Kevin Pruitt[4]

[1]Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC,[2]Cell Biology and Biochemistry, Texas Tech University Health Sciences Center, Lubbock, TX,[3]Immunology and Molecular Microbiology, Texas Tech University Health Sciences Center, Lubbock, TX,[4]Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC

Background: Signaling pathways are integral to the regulation of essential biological processes within cells, and their coordinated control across tissues is critical for numerous vital functions. Dysregulation of these pathways can contribute to the development of various diseases, particularly cancer. In the context of Wnt signaling, aberrations in the pathway can induce chromatin modifications that regulate the epigenome, thereby promoting tumorigenesis and metastasis. A key component of this pathway is Dishevelled (DVL), which, although traditionally considered a cytoplasmic protein, has been shown to translocate to the nucleus. However, the nuclear role of DVL remains perplexing, and little is known about what genomic loci it binds, what regulates its binding, and what DNA binding proteins may be novel interacting partners.

Key Results: Our study utilizes data from our recent DVL ChIP/RNA-seq analyses, publicly available datasets, and extensive genomic and bioinformatics evaluations to investigate the poorly understood role of nuclear DVL1. Our findings uncover novel mechanisms by which DVL regulates downstream signaling. First, we demonstrate that DVL not only binds to promoters of genes linked to tumorigenesis but also targets numerous genes involved in immune system regulation. Second, we identify an interaction between DVL1 and transcription factors such as ETS1. Notably, our discovery that nuclear DVL1 and ETS1 form a complex and co-localize at specific genomic loci is, to our knowledge, the first report of such an interaction. Third, we show that ETS1 is essential for DVL1 binding to several genes not previously associated with Wnt/DVL signaling.

Impact: Our study offers some of the first insights into the novel functions of nuclear DVL, revealing its critical role in regulating ETS1 and a broad range of genes essential for developmental biology, antigen presentation, metabolism, chromatin remodeling, and tumorigenesis. By uncovering these previously uncharacterized functions, our research substantially advances the understanding of DVL's involvement in aberrant Wnt signaling, providing new perspectives on its potential contributions to disease mechanisms. This work not only enriches the current knowledge of DVL's multifaceted role but also opens avenues for targeted therapeutic strategies in diseases driven by dysregulated Wnt signaling.

#1453 Investigating the Role of FOXO4 in glioblastoma.

Megan Keniry[1], David Uribe[2]

[1]University of Texas Rio Grande Valley, Edinburg, TX,[2]SIBCS, University of Texas Rio Grande Valley, Edinburg, TX

Evolutionarily conserved partially redundant Forkhead box transcription factors FOXO -1, -3, and -4 drive stem cell maintenance in embryonic, cancer, mesenchymal, hematopoietic, and neural stem cells. Canonically, high PI3K output leads to cytoplasmic/inactive FOXO factors. Stem cells, however, are fundamentally rewired to have high PI3K Pathway activity and nuclear/active FOXO factors. Our previously published work and the work of others show that FOXO factors directly bind to and activate stem genes in both embryonic stem cells and the poor prognosis of cancer glioblastoma multiforme (GBM) to drive stem cell fate. However, the precise mechanisms utilized by FOXO transcription factors in driving stemness, hindering differentiation, and promoting cancer are incompletely understood. To gain insight into the role of nuclear FOXO4 in glioblastoma, we disrupted the gene encoding this factor using CRISPR Cas9 genome editing and performed RNA-seq in the U87MG background. We identified 2,519 differentially expressed genes in *FOXO4*-deleted U87MG cells compared to unmodified controls, including those that drive invasion. Current work is examining whether FOXO4 promotes invasion of glioblastoma cells.

#1454 Investigating the expression of PTPRN2 and MT1X genes in glioblastoma cell migration using hydrogel-based models.

Ananya Bharadwaj[1], Talia Sanazzaro[2], Stephanie K. Seidlits[3]

[1]College of Natural Sciences, The University of Texas At Austin, Austin, TX,[2]Graduate School; Biomedical Engineering, The University of Texas At Austin, Austin, TX,[3]Associate Professor; Biomedical Engineering, The University of Texas At Austin, Austin, TX

Glioblastoma (GBM) is an aggressive brain tumor originating from glial cells and presents difficulty in treatment. GBM are highly heterogeneous, presenting a challenge in treatment. In addition, cells migrate away often as single cells from primary tumor sites making GBM impossible to be fully resected. GBM cells adapt their behavior based on the mechanical properties of their surrounding microenvironment, making it significant to investigate gene markers associated with these mechanical changes. Our recent efforts to investigate particular gene markers included using an interface hydrogel platform that replicates the local microenvironment from the tumor edge to peritumoral tissue. Using this model we successfully separated migratory and non-migratory cells. Results from single-cell RNA (scRNA) sequencing identified gene targets that were overexpressed within the migratory group. We found the gene PTPRN2, which plays a role in vesicle trafficking and exocytosis, was overexpressed in migratory cells. This could be a promising target for future therapeutic methods to limit glioblastoma cell migration. In addition to PTPRN2, we are exploring the role of MT1X, a gene in the metallothionein family, to validate these findings. We are currently investigating the expression of the PTPRN2 and MT1X genes using GBM xenograft mouse models. We have observed PTPRN2 expression in the GBM mouse models, with the highest expression detected in migratory cells compared to non-migratory cells in soft gel conditions. In the future, we plan to perform western blot analysis to further confirm PTPRN2 and MT1X protein expression and validate their roles as potential target proteins for GBM treatment.

#1455 Impact of ZFHX3 loss on endometrial cancer progression.

Areebah Qazi, Sophia Agrusa, Swornalata Pukhrambam, Juveria Ali, Sanjeev Ganesh, Jessica Back, Mike R. Wilson

Oncology, Wayne State University, Detroit, MI

Endometrial cancer (EC) is the sixth most common cancer among women and ranks as the leading type of gynecological cancer. Although EC is often diagnosed early, current treatment plans significantly limit fertility-sparing options, posing challenges for younger patients. This research focuses on the role of the gene ZFHX3 (ATBF1) to understand its effects on the advancement of endometrial cancer. In order to investigate this, we first transfected 12Z-ESR1 epithelial endometrial cells with ZFHX3 siRNA to create transient gene knockdowns. Following cell collection, western blotting was performed to measure ZFHX3 protein expression levels. Furthermore, we used CRISPR Cas9 plasmid guides tagged with GFP to establish more stable knockdowns. After bacterial transformation, we utilized flow cytometry to sort GFP-expressing cells and confirm successful gene editing. Subsequent western blots were conducted to validate which guide were successful in creating an efficient knockdown of ZFHX3. In vivo studies complemented these cellular experiments. We dissected the uteri of mice with ZFHX3 homozygous and ZFHX3/Pten heterozygous knockdowns. Analysis through H&E staining allowed us to quantify the number of endometrial glands. Our findings revealed that ZFHX3 loss disrupts progesterone signaling, a critical regulator of endometrial function, and leads to increased cell proliferation, which may drive tumor growth. These results highlight ZFHX3 as a potential target for therapeutic strategies aimed at balancing fertility preservation and inhibiting the progression of cancer.

#1456 Tumor lineage plasticity blockade by therapeutic targeting of an epigeneticregulator.

X. Zhang, Sr.[1], Y. Yang[1], E. Corey[2], H. Chen[1];
[1]UC Davis Medical Center, Sacramento, CA, [2]University of Washington, Seattle, WA

Tumor lineage plasticity (LP) is an emerging hallmark of cancer progression in solidtumors, such as prostate cancer, pancreatic cancer and lung cancer. However, theexact molecular mechanisms that drive the aberrant LP programs remain poorlydefined. Here we identified bromodomain protein BRD4 as a crucial player. BRD4knockdown and its inhibitors potently suppressed the LP programs both in vitro and invivo. Integrated ChIP-seq and RNA-seq analysis of tumors revealed that BRD4 drivesLP by increasing local chromatin accessibility and directly activating LP gene programswhich includes neurogenesis, axonogenesis and stem cells and key drivers such asBRN2, ASCL1/2, SOX2, SOX9 and RUNX1/2. Significantly, we demonstrated thatBRD4 inhibitor AZD5153, currently at clinical development, possesses potent activity inalmost complete blockade of tumor growth of both de novo neuroendocrine prostatecancer (NEPC) and treatment-induced NEPC PDXs. Further study revealed thatsuppression of tumor expression of LP programs through reduction of local chromatinaccessibility is a primary mechanism of action (MOA) by AZD5153. Together, ourfindings demonstrated that BRD4 plays a fundamental role in directly activating tumorLP programs and that its inhibitor AZD5153 is highly promising in effective treatment ofthe lethal forms of the diseases.

#1457 YAP tyrosine phosphorylation regulates lineage commitment in liver tumorigenesis.

Muhammed D. Aksu[1], Jayla T. Millender[1], Ryan D. Watkins[1], Jennifer A. Yonkus[2], EeeLN H. Buckarma[1], Nathan W. Werneburg[1], Daniel R. O'Brien[1], Chantal E. McCabe[1], Rondell P. Graham[1], Gregory J. Gores[1], Rory L. Smoot[1], Caitlin B. Conboy[1]

[1]Mayo Clinic, Rochester, MN,[2]University of Texas Health Science Center at Houston, Houston, TX

Background: The transcriptional cofactor Yes-associated protein (YAP) is activated in CCA pathophysiology. While canonical regulation by the Hippo pathway resulting in phosphorylation at serine 127 (S127) is inhibitory, SRC-family kinases (SFKs) phosphorylate YAP at tyrosine 357 (Y357), causing activation. Previously, cholangiocytes, hepatic progenitor cells (HPCs), and even mature hepatocytes were suggested as potential cells of origin of CCA, which highlights the plasticity in the liver. However, the role of YAP activation in selecting the cell of origin and lineage commitment during hepatobiliary oncogenesis is unknown.

Aim: To determine if YAP Y357 phosphorylation is required for CCA tumorigenesis, we utilized an *in vivo* model. Unexpectedly, a shift in tumor phenotype to hepatocellular carcinoma (HCC) was identified. Subsequently, we further explored cell signaling and cell of origin contributions to this phenotypic switch.

Method: C57BL/6 mice underwent biliary transfection with Sleeping Beauty transposase and transposons encoding myr-AKT and either YAP S127A (YAP-S) or YAP S127A/Y357F (YAP-SY), followed by IL-33 treatment. Tumor nodule count, histologic classification, and liver-to-body ratio were assessed at necropsy after 10 weeks. RNA sequencing and ingenuity pathway analysis (IPA) were performed on HCC and CCA nodules. To determine the cell of origin, tumors were generated in lineage tracing mice (Rosa26 mT/mG mice treated with AAV8-TBG-Cre). Finally, two tumor-derived cell lines with YAP-SY and constitutive AKT expression were isolated.

Results: Both YAP-S and YAP-SY models developed similar tumor numbers and bulk. YAP-S mice generated frequent CCA (25.3%) in addition to combined HCC-CCA (cHCC-CCA) tumors (10.7%) and HCC (64%), whereas YAP-SY mice developed predominantly HCC (86.7%), with rare CCA (8.8%) and mixed tumors (4.5%). Transcriptome analysis identified 3,367 differentially expressed genes (DEGs) between HCC from YAP-SY mice and CCA from YAP-S mice. Notably, YAP-regulated genes had decreased expression in the HCC from YAP-SY mice. IPA revealed key upstream regulators, including cytokines and growth factors (e.g., *TGFB1*, *TNF*, *INFG*, *CSF2*, *IL6*, *IL1B*, *IL4*) and transcription factors (e.g., *TP53*, *JUN*, *SP1*, *NFKBIA*, *STAT3*). Lineage tracing demonstrated HCC originated from hepatocytes and CCA from cholangiocytes in both models. Finally, gene expression analysis of novel syngeneic cancer cell lines from YAP-SY mice showed mixed features of both HCC and CCA.

Conclusion: In this murine model, we found that sub-maximal YAP activation is associated with increased formation of HCC from hepatocytes, while a higher level of YAP activation due to YAP Y357 phosphorylation is associated with efficient formation of CCA. We identified cytokines and growth factors that may orchestrate this phenotypic switch. Finally, we developed novel syngeneic cancer cell lines with cHCC-CCA characteristics.

#1458 Tolerance of SOX2 and BRN2 in prostate cancer cell lines: effects on cell cycle and lineage transformation.

Connor Purcell[1], Praveen R. Srinivasan[2], Shengliang Zhang[1], Maximilian Pinho-Schwermann[3], William J. MacDonald[4], Lanlan Zhou[1], Andrea Schmidt[1], Claire Lin[5], Xiaobing Tian[1], Tyler J. Roady[1], Vida Tajiknia[1], Viva Voong[1], Wafik El-Deiry[1]

[1]Legorreta Cancer Center at Brown University, Providence, RI,[2]Weill Cornell Medicine, New York, NY,[3]Brown University Health, Providence, RI,[4]Columbia University Vagelos College of Physicians and Surgeons, New York, NY,[5]Warren Alpert Medical School at Brown University, Providence, RI

Introduction: Prostate cancer (PCa) is the most prevalent malignancy in American men excluding skin cancer. In metastatic disease, resistance to androgen pathway inhibitors (APIs) is a major obstacle to curative treatment. One such resistance mechanism is the histological transformation from adenocarcinoma to a neuroendocrine (NE) phenotype, driven by transcription factors like SOX2 and BRN2. Of note, SOX2 is typically overexpressed in PCa lacking functional Rb and p53 and can regulate cell cycle proteins including Cyclin D1. These findings warrant an investigation into a potential connection between SOX2, the cell cycle, and tumor suppressors to further establish molecular mechanisms of neuroendocrine differentiation (NED). We also plan to investigate the potential role of BRN2 in this process.

Methods: Lentiviral Tet-On® systems were cloned and transduced into the PCa cell lines LNCaP (WT RB1, WT TP53) and DU145 (RB1 -/-, TP53 mutant) for the inducible expression of SOX2 (iSOX2) and BRN2 (iBRN2). In vitro growth assays were performed by plating 5,000 cells/well in a 96 well plate and adding doxycycline the following day (DOX). Western blots were performed to determine protein expression. Cell cycle analysis was performed by PI staining and flow cytometry.

Results: Inducible models showed robust induction of SOX2 or BRN2 after 24h induction. At 4d, iBRN2 cell lines demonstrated NE marker upregulation, specifically synaptophysin. SOX2, but not BRN2, induction decreased Cyclin D1 expression irrespective of Rb and p53 status. Intriguingly, LNCaP iSOX2 cells were less viable following induction and had distinct morphological changes including the loss of their stellate shape. LNCaP iBRN2 cells had less profound, but apparent, changes in morphology. No significant morphological changes were identified in DU145. Cell cycle analysis found a greater percentage of LNCaP iSOX2 cells in the sub-G1 state and a lesser percentage in the G2/M phase versus the empty vector.

Conclusions: These results suggest that SOX2 overexpression (OE) may only be tolerated in an Rb and/or p53 deficient setting. Further research will seek to determine if this phenomenon is due to SOX2-induced repression of Cyclin D1. Subsequent experiments will use RB1 and/orTP53 KO cell lines to generate new SOX2 and BRN2 inducible systems. Novel experiments will seek to determine if RB1 and/or TP53 KO increases tolerance to SOX2 OE. Connecting this knowledge of the SOX2 protein to the cell cycle and hormonal signaling in PCa may offer insight into the interplay between emerging cell cycle-targeting therapeutics and the NED process.

#1460 Clinical relevance of a super-enhancer-associated actin polymerization promoting-factor in endometrial cancer.

Kuniko Horie[1], Erina Iwabuchi[2], Kazuhiro Ikeda[1], Takashi Suzuki[3], Satoshi Inoue[4]

[1]Division of Systems Medicine & Gene Therapy, Saitama Medical University, Saitama, Japan,[2]Institute of Science Tokyo, Tokyo, Japan,[3]Department of Anatomic Pathology, Tohoku University Graduate School of Medicine, Sendai, Japan,[4]Department of Systems Aging Science and Medicine, Tokyo Metropolitan Institute for Geriatrics and Gerontology, Tokyo, Japan

Super-enhancers (SEs) are defined as large clusters of enhancers regulating transcription of genes that exhibit cell-type specific functions. SEs in cancers are often different from those in healthy states and responsible for the development and progression of the disease. We here study the clinical role of a super-enhancer-associated actin polymerization promoting-factor, cordon-bleu WH2 repeat protein-like 1 (COBLL1), in endometrial cancer, since we previously demonstrated its tumor-promoting role in castration-resistant prostate cancer [Proc Natl Acad Sci USA 115:4975, 2018]. In the present study, using multiple patient-derived spheroid cultures established by our group [Endocrinology 160:1895, 2029], endometrial cancer-specific SEs were identified by chromatin immunoprecipitation (ChIP) sequencing for H3K27ac binding sites. Among top-ranked SE-associated genes, we focused on the relevance of COBLL1 in endometrial cancer. Then, we showed that COBLL1 silencing in endometrial cancer cells substantially repressed cell proliferation and reduced the populations of cells in S phase of the cell cycle. Of note, immunohistochemical study of clinical endometrial cancer tissues revealed that COBLL1 immunoreactivity was well correlated with poor prognosis of the patients. Our findings suggest that COBLL1 plays a relevant role in the pathophysiology of endometrial cancer and can be applied as a promising diagnostic and therapeutic target in endometrial cancer management.

#1461 Super-enhancer driven NR3C1 expression promotes 5-FU resistance in gastric cancer.

B. Liu, Junxian Yu, Haorang Feng, Jianfang Li;
Shanghai Jiao Tong Univ., School of Med., Shanghai, China

Background: This study aims to describe the Super-enhancer (SE) landscape in 5-FU resistant gastric cancer (GC), and to illustrate the biological mechanism of SE-driven NR3C1 mediating 5-FU resistance. The study might provide new possibilities of improving chemotherapy efficacy for GC.
Methods: The induced drug-resistant gastric cancer cells were constructed with long-term 5-FU medication. The cChIP-Seq and rank ordering of super-enhancers (ROSE) algorithms were applied to calculate SE in GC cells. The core regulating transcription factor (TF) NR3C1 was determined by siRNA libraries. The correlation between NR3C1 expression and the sensitivity to 5-FU was verified by drug sensitivity curves. In induced resistant cells, NR3C1 expression was detected and was knocked down as a rescue experiment. Confocal microscopy was used to observe the purified NR3C1 fluorescent protein droplets in vitro, and to observe dynamic droplets in the nucleus. Fluorescence recovery after photobleaching (FRAP) verified the droplet fluidity. The CUT&Tag of NR3C1 was applied to analyze the co-binding pattern between NR3C1 and SE on the genome. The SE changes of NR3C1 knockdown cells were detected and were analyzed for downstream target genes. PDX models and GC cells were used to evaluate the inhibitory effect of epigenetic inhibitors JQ1 on NR3C1 and its target genes, and to improve 5-FU responsivity in GC cells.
Results: The SE profiles of 5-FU-sensitive, spontaneously resistant and induced resistant cells were described. SiRNA libraries were used to identify the master TF NR3C1. The 5-FU drug sensitivity curve verified that GC cells with high NR3C1 expression were resistant to 5-FU. Moreover, NR3C1 expression was increased in 5-FU-resistant cells, and knocking down NR3C1 in resistant cells could restore the 5-FU sensitivity. NR3C1 inhibition also improved the sensitivity of GC organoids to 5-FU. NR3C1 protein formed phase separation in vitro, and also formed droplets in the nucleus, together with transcription cofactors. With NR3C1 knockdown, SE profile changed through enhancer reprogramming, and the SE-associated genes transcription was inhibited. Then, NR3C1 downstream target genes, which were associated with 5-FU resistance, were significantly reduced. The use of JQ1 effectively inhibited the expression of downstream target genes, and efficiently improve the tumor inhibition effect of 5-FU in PDX models.
Conclusion: The high expression of NR3C1 driven by SE is positively correlated with 5-FU resistance in GC patients. SE-driven NR3C1 formed phase separation at the SE region in the nucleus, activating the positive feedback loop of TF. NR3C1 inhibition led to remarkable enhancer reconstruction of GC cells. SE-driven NR3C1 strongly promotes the transcription of downstream target genes and mediates the resistance of GC to 5-FU. JQ1 can effectively improve the 5-FU sensitivity of GC

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Genome Maintenance: Mechanisms and Novel Therapeutics
Poster Session

#1465 RAD52 and ERCC6L/PICH have a compensatory relationship for genome stability.
B. Osia, A. Merkell, F. Lopezcolorado, X. Ping, J. M. Stark;
Beckman Research Institute of The City of Hope, Duarte, CA

Human RAD52 plays essential roles in several homology-driven DNA repair pathways, including single strand annealing, transcription-coupled homologous recombination, and mitotic DNA synthesis (MiDAS). Although RAD52 is not essential, RAD52 loss with disruption of either the breast cancer 1 (BRCA1) or breast cancer 2 (BRCA2) genes is synthetic lethal. Thus, RAD52 is an intriguing potential target for treatment of BRCA-deficient cancers. However, the full breadth of pathways and factors that create a state of RAD52-dependence when compromised are not understood. To query RAD52 synthetic lethal relationships, we performed genome-wide CRISPR knock-out screens and identified hundreds of candidate synthetic lethal interactions. We then performed secondary screening and identified genes for which depletion causes reduced viability and elevated genome instability (increased 53BP1 nuclear foci) in RAD52-deficient cells. One such factor was ERCC6L, which marks DNA bridges during anaphase, and hence is important for genome stability in mitosis. Additionally, pan-cancer studies of altered ERCC6L expression have identified increased ERCC6L expression as a marker of poor prognosis in multiple types of cancer, making it a relevant target for cancer therapy. Thus, we investigated the functional interrelationship between RAD52 and ERCC6L. We found that RAD52 deficiency increases ERCC6L-coated anaphase ultrafine bridges, and that ERCC6L depletion causes elevated RAD52 foci in prometaphase and interphase cells. These effects were enhanced with replication stress (i.e. hydroxyurea) and topoisomerase IIα inhibition (ICRF-193), where post-treatment effect timings were consistent with defects in addressing stress in mitosis. Altogether, we suggest that RAD52 and ERCC6L co-compensate to protect genome stability in mitosis.

#1466 Comprehensive analysis of breast and ovarian cancer-identified RAD51D variants.

Kristie Darrah[1], Katherine Oppenheimer[2], Nicole Banks[1], Phoebe Parker[1], Ella Roberts[2], Gayatri Ganesan[1], Kara Bernstein[1]

[1]University of Pennsylvania, Philadelphia, PA,[2]University of Pittsburgh, Pittsburgh, PA

The RAD51 paralogs are essential genes in coordinating the early steps of homologous recombination (HR)-mediated repair of DNA double strand breaks (DSBs). Currently, there are over 300 epidemiology studies linking a single mutation or polymorphism in one of the five canonical RAD51 paralogs to cancer predisposition and tumorigenesis. As one of the most frequently mutated RAD51 paralogs, mutations in RAD51D have been identified in 5% of hereditary ovarian cancers and pathogenic variants are associated with a 25% lifetime risk for breast cancer. We have uncovered more than 160 patient-identified missense variants in *RAD51D*, the majority of which are clinically classified as variants of unknown significance (VUS) and have little to no prior functional analysis. Herein, we have performed a comprehensive screen of all known missense RAD51D VUS identified in breast and ovarian cancer patients, identifying 7 potentially pathogenic variants which disrupt native protein interactions, reduce HR proficiency, and exhibit varying degrees of sensitivity to the typical chemotherapeutics used to treat HR-deficient (HRD) cancers. In defining the functional consequences of RAD51D VUS, we hope to inform formal clinical classification of these VUS in hopes of better estimating cancer risk and leverage precision therapeutic approaches for mutation carriers.

#1468 Chemotherapy-resistant bladder cancer: Involvement of homologous recombination repair.

Nils C. H. van Creij[1], Francisco J. Roa[1], Florian Handle[2], Frederic R. Santer[1], Per Sonne Holm[3], Zoran Culig[1], Renate Pichler[1]

[1]Department of Urology, Medical University of Innsbruck, Innsbruck, Austria,[2]Institute of Pathology, Neuropathology and Molecular Pathology, Medical University of Innsbruck, Innsbruck, Austria,[3]Department of Oral and Maxillofacial Surgery, Medical University of Innsbruck, Innsbruck, Austria

Introduction: Radical cystectomy after neoadjuvant cisplatin-based chemotherapy (NAC) remains the standard of care for localized and locally advanced muscle invasive bladder cancer (MIBC). However, around 65% of patients exhibit resistance to this regimen. HUS1, an important protein in the homologous recombination repair (HRR) pathway, has been associated with NAC resistance in different studies. While the HRR pathway, including proteins such as CHK1 and RAD51, is known to play a role in chemotherapy resistance, the specific mechanisms in MIBC remain underexplored. This study aims to investigate the role of HRR in cisplatin-based chemotherapy resistant bladder cancer.

Materials & Methods: Cisplatin-resistant (CR) HT1197 and UMUC3 cell lines were developed via gradual cisplatin exposure for more than 3 months. To measure HRR in the cells, we developed a DRGFP assay. Using western blot and RNAseq, we compared differences in protein and RNA levels of DNA repair pathway members between the parental and CR cells. Using an Incucyte® imager, we analyzed cell proliferation after silencing or overexpressing HUS1 and RAD51 alone, or in combination with cisplatin treatment. Furthermore, we tested olaparib and radiation therapy in our models.

Results: RAD51 and CHK1 protein levels are respectively 2.69 and 2.07 times higher expressed in our CR cells, underscoring enhanced HRR activity as a potential mechanism of resistance. These elevated RAD51 and CHK1 levels represent promising new biomarkers for predicting cisplatin response. Additionally, our DRGFP assay revealed a 2.5 times increased HRR activity in the CR cell lines compared to their parental lines. We observed a decreased proliferation in the parental (1.3x fold decrease), but not in the CR cells, when combining cisplatin treatment with silencing HUS1. In contrast, decreased proliferation was observed in both parental and CR cells when combining cisplatin with silencing RAD51 (fold decrease of respectively 4.5 and 2.25). Therefore, targeting RAD51 might be a novel approach to overcome chemotherapy resistance. Moreover, western blot data revealed a regulatory interaction between HUS1 and RAD51. Our data also indicated that CR cells exhibit increased resistance to other DNA-damaging agents, such as radiation therapy and the PARP inhibitor olaparib. Finally, RNAseq identified additional signatures potentially involved in chemotherapy resistance, including energy-related pathways and epithelial-mesenchymal transition markers.

Conclusion: To the best of our knowledge, we are the first to show increased HRR in cisplatin-resistant bladder cancer cells and to give initial insights into the role of HRR in platinum-based chemotherapy resistance. Our findings propose promising therapeutic strategies to overcome chemotherapy resistance in bladder cancer.

#1469 Deciphering mechanisms of intrinsic radioresistance in breast cancer: Insights from transcriptomics and CRISPRi screens.

Breanna McBean[1], Priyanka S. Rana[2], Benjamin Hauk[2], Anna R. Michmerhuizen[1], Samuel Lichtman-Mikol[2], Reine Abou Zeidane[2], Vesna Markovic[2], Kari Wilder-Romans[1], Mingfang Tao[2], Alan P. Boyle[1], Corey W. Speers[2]

[1]University of Michigan Medical School, Ann Arbor, MI,[2]Case Western Reserve University, Cleveland, OH

Background: Breast cancer (BC), the most common cancer globally and a leading cause of death in women, often utilizes radiation therapy (RT) as part of treatment, with over 85% of patients receiving RT after breast-conserving surgery. Still, over 15% of women experience local recurrence despite RT, underscoring the need to better understand the molecular mechanisms driving RT response and resistance. We hypothesized that transcriptomic changes that occur after ionizing radiation in intrinsically radiosensitive and resistant BC models would offer mechanistic insight into mediators of this differential response. To test this, we conducted *in vitro* and *in vivo* RNA-seq experiments to characterize transcriptomic changes following RT across a panel of BC cell lines of varying levels of intrinsic radiation sensitivity. Furthermore, we conducted a genome-wide CRISPRi screen to identify genes which are critical to radiation survival and drive radioresistance.

Methods: Eight BC cell lines representing all intrinsic subtypes were treated with 4 Gy RT and RNA was collected 24 hours after treatment for RNA-seq. For 2 cell lines, we also conducted *in vivo* mouse xenograft experiments in which mice received fractionated daily RT (6 fractions of 2 Gy each). Differential gene expression analysis with DeSeq2 was performed, followed by pathway analysis with Advaita Bioinformatics' iPathwayGuide. For the CRISPRi experiments, we used the Dolcetto library in two TNBC cell lines: BT-549 and HCC-1937. Cells were transfected with the library and then treated with 3 fractions of 3 Gy RT. DNA was isolated for sequencing after 7 days and analyzed with MaGeCK to determine sgRNA abundance.

Results: An average of 935 differentially expressed genes were noted in the *in vitro* models (S.D. 906); 142 and 324 were found in the *in vivo* models. Among the most highly enriched pathways from the transcriptomic data in both resistant and sensitive cell lines after RT, we found processes including cell cycle, cellular senescence, and estrogen signaling. Pathways uniquely differentially expressed in the *in vivo* samples for both cell lines include IL-17 signaling and transcriptional misregulation in cancer. From the CRISPRi screen, we identified genes involved in similar pathways, as well as canonical regulators of DNA damage response.

Conclusions: Ionizing radiation induces transcriptomic expression changes that differ between intrinsically radiosensitive and resistant BC models. These data, coupled with results from the CRISPRi radiosensitivity screen, identify pathways that offer potential insight into the mechanisms underlying intrinsic radioresistance and suggest biologic vulnerabilities that may be targeted to more effectively treat women at a high risk of local BC recurrence. Ongoing studies are exploring the potential of therapeutic targeting of these pathways when combined with RT in breast cancer.

#1470 Inhibition of protein myristoylation sensitizes pancreatic cancer cells to DNA damage agents.

Jiaxuan Hu[1], Nho C. Luong[1], Xin Xu[2], John Kim[1], Jinmeng G. Jiang[1], Roshika Roshika[1], Priya Kapoor-Vazirani[1], Sandip K. Rath[1], Ramona Haji-Seyed-Javadi[1], Ronghu Wu[2], David S. Yu[1]

[1]Radiation Oncology, Emory Winship Cancer Institute, Atlanta, GA,[2]School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA

Pancreatic ductal adenocarcinoma (PDAC) has one of the highest cancer mortality rates with a 5-year survival rate of only 12% despite tumor removal and/or chemo-radiotherapy. The poor prognosis is partly due to therapy resistance driven by genes and pathways regulating cell signaling, proliferation, and metastasis. DNA damage response (DDR) pathways contribute to this resistance by enabling cancer cells to repair treatment-induced DNA damage, evade apoptosis, and adapt through alternative repair mechanisms, emphasizing the need for new therapeutic targets. We explored the role of protein myristoylation, which is the addition of a 14-carbon myristic acid to lysine and glycine catalyzed by N-myristoyl transferase (NMT) 1 and 2, in DDR regulation as potential therapeutic targets for PDAC. Analysis of The Cancer Genome Atlas (TCGA) database revealed correlation of lower levels of NMT1 and NMT2 with higher microsatellite instability score, a marker of genomic instability. Inhibition or knockdown of NMT1 and NMT2 in PDAC cells increased γH2AX levels, a marker for genomic instability, suggesting that NMTs may have a role in maintaining cellular genomic stability and regulating the DDR. TCGA and TARGET pan-cancer database analyses showed correlation between lower NMT1/2 expression with better cancer patient outcomes, including overall survival, progression-free survival, and disease-free progression. Gene expression analysis from the TNMplot database revealed elevated NMT1/2 levels in PDAC tumor tissues compared to nontumorigenic tissue. We then confirmed an increased level of NMT1 and NMT2 in PDAC cell lines, including MIA PaCa-2, PANC1, and HPAC, compared to nontumorigenic HPDE pancreatic ductal epithelial cells. Notably, NMT1/2 knockdown with siRNA significantly sensitized MIA PaCa-2 cells to DNA-damaging agents, including ionizing radiation (IR), topoisomerase inhibitor camptothecin (CPT), and poly (ADP-ribose) polymerase (PARP) inhibitor veliparib. Chemical inhibition of NMTs also sensitized MIA PaCa-2 cells to CPT and veliparib. These results suggest that NMTs could govern resistance of PDAC cells to DNA-damaging treatments by regulating DDR and genome stability. To further explore NMTs' role in DDR, we performed affinity purification coupled with quantitative proteomics in HEK293T cells expressing EGFP-tagged NMT1 or NMT2. This approach identified 641 and 678 proteins, respectively, including 32 NMT1-associated proteins and 33 NMT2-associated proteins related to DNA repair. Additionally, global profiling of protein myristoylation using mass spectrometry revealed 576 myristoylated proteins in HCT116 cells, including 14 DNA repair proteins. Thus, NMT-mediated myristoylation may modulate the dynamic and functions of DNA repair proteins, thereby enhancing PDAC cell survival under therapeutic stress.

#1471 Roles of the KU70 C-terminus SAP domain in resilience to ionizing radiation.

Yuan Wang, Michael S. Czap, Hailey Kim, Huimei Lu, Jingmei Liu, Zhiyuan Shen

Rutgers Cancer Institute of New Jersey, New Brunswick, NJ

Ionizing radiation (IR) is a critical modality in cancer therapy but also carries carcinogenic risks, necessitating precise optimization. The KU70 protein is integral to DNA repair, primarily known for its N-terminal domain's role in dimerizing with KU80 to repair DNA double-strand breaks (DSBs). Our study aims to elucidate the function of the less-explored C-terminal SAP domain of KU70. We engineered a variant, KU70-ΔSAP, lacking this domain, to assess its role in cellular responses to radiation. KU70-ΔSAP mice exhibited increased sensitivity to an 8 Gy dose of whole-body irradiation (TBI), significantly affecting hematopoietic stem cells, with marked reductions in long-term and short-term stem cells, as well as in common myeloid and granulocyte-monocyte progenitors post-irradiation. These findings suggest that the SAP domain is crucial for the survival of hematopoietic stem and progenitor populations following IR-induced damage, as well as for the subsequent hematopoiesis necessary to replenish bone marrow and blood. Further analysis using mouse embryonic fibroblast (MEF) clones from our mouse model indicated that deletion of the SAP domain impaired KU70's early recruitment to UV-laser-induced DSB sites, coinciding with decreased Ligase IV levels, an essential enzyme for DNA repair, and increased γH2AX signals, which indicate DNA damage and repair inefficiency. These results highlight the SAP domain's essential role in orchestrating effective DNA repair mechanisms post-IR and enhance our understanding of KU70’s function in improving radiation therapy outcomes and bolstering cellular resilience against radiation-induced damage.

#1473 POLQ facilitates annealing mediated long deletions at chromosomal break in homologous recombination deficient cancers.

Manisha Jalan[1], Erika S. Buechelmaier[1], Jonathan Rafailov[2], Yingjie Zhu[1], Xin Pei[1], Sebastian Brylka[2], Joan Gomez-Aguilar[1], Niamh McDermott[1], Nadeem Riaz[1], Marcin Imielinski[2], Simon N. Powell[1]

[1]Radiation Oncology, Memorial Sloan Kettering Cancer Center, New York, NY,[2]Perlmutter Cancer Center, NYU Grossman School of Medicine, New York, NY

Tumors deficient in homologous recombination (HRD) predominantly rely on alternative DNA repair pathways for maintaining genome stability, especially once end resection has been initiated after a double strand break (DSB). These back up repair mechanisms, especially microhomology-mediated end-joining (MMEJ) and single-strand annealing (SSA), once considered minor contributors to genome stability, have garnered increased attention as recent evidence supports their significant roles in tumorigenesis and their potential as therapeutic targets. Consequently DNA polymerase theta (POLQ), a key regulator of MMEJ, emerges as an attractive target for the selective treatment of HRD cancers, and recent efforts in the development of POLQ inhibitors have demonstrated their potential to enhance the efficacy of existing therapies and overcome resistance mechanisms. In our study, we investigated the genetic drivers of the under-investigated SSA and its connection to the related MMEJ pathway in human cells. A previously unidentified role for POLQ in regulating the frequency of SSA was observed in addition to its role in MMEJ. Beyond its established role as a gap-filling polymerase, we show that the helicase activity of POLQ is critical for homology-mediated annealing in both MMEJ and SSA. POLQ is shown to be a key facilitator of SSA through its ability to process DNA ends and promote annealing of homologous sequences with at least 80% sequence identity (homeology), resulting in a range of deletion sizes. We also provide evidence for these homeology-dependent SSA-like repair scars in tumor genomes and show that they are associated with HRD and elevated POLQ expression, suggesting the utilization of these mechanisms as a critical repair pathway in these tumors. These findings suggest POLQ is the ideal target for therapies of HRD cancer.

#1474 Recurrent PRKCI gene fusions represent a drug target in gastric cancer and esophageal junction cancer.

Shivani Jagannathan Murali, Bharath Kumar Karre, Cristina Ivan, Chao Sima, Asfetaw Abera, David Spetzler, Milan Radovich, Heather O'neill, Ravi Chakra Turaga

Caris Life Sciences, Inc., Tempe, AZ

Background: Protein kinase C (PKC) is a phospholipid-dependent, calcium-independent Ser/Thr protein kinase family with eight members, each involved in processes including apoptosis, migration, and proliferation. PRKCI promotes tumor growth in many types of cancer, including pancreatic, lung, ovarian, and renal cell carcinoma. Structurally, PKCι has an N-terminal regulatory domain and a C-terminal catalytic domain. While PRKCI fusions have been reported, they remain uncharacterized. This study aims to investigate these fusions in gastric cancer and esophageal junction carcinoma, exploring potential mechanisms and co-occurring mutations to inform targeted therapies that could improve patient outcomes.
Methods: We performed a comprehensive analysis of PRKCI fusions identified from whole transcriptome sequencing (WTS) and associated co-occurring pathogenic mutations from whole exome sequencing (WES). We screened 6437 EJC and 3615 gastric adenocarcinoma cancer cases retrospectively and calculated the frequency of occurrence of PRKCI fusions. Additionally, we characterized and mapped the domains of PRKCI fusions and its partners. We further analyzed patient demographics, co-occurring mutations and copy number amplifications.
Results: PRKCI fusions were identified in 0.19% and 0.3% of EEJC and Gastric adenocarcinoma cancer cases. All the PRKCI fusions are formed with other genes on chromosome 3 from intra-chromosomal rearrangement (PHC3, TNIK, SKIL and YES1) proximal to the PRKCI gene. The most common fusions of PRKCI are formed with partner PHC3 and SKIL which account for 11 patient cases. This is followed by 4 cases of TNIK and unique cases of fusions with LRRC31, PCYT1A, ERBB2, VPS13D and YES. Further, the genomic landscape of PRKCI indicated co-occurring mutations in TP53 (46.7%), ARID1A (13.3%), 6.7% inactivating mutations of ATM, FBXW7, CDH1. Expression of PRKCI in PRKCI fusion positive cases was significantly higher as opposed to patient cases that did not harbor gene fusions. PRKCI fusion cases were commonly observed to have PRKCI amplification (46.7%). PRKCI fusions lack the regulatory domain, leading to constitutive kinase activation, which may drive tumor progression by disrupting normal regulation and altering protein localization.
Conclusion: Our studies are the first to identify PRKCI fusions in gastric cancer and EEJC patients, along with associated mutations and amplifications suggesting a novel mechanism. This highlights the importance of personalized molecular diagnostics to guide tailored treatments. PKCι kinase inhibitors may improve survival and outcomes for patients with PRKCI fusions in gastric cancer and EEJC.

#1475 HDAC8 as a critical regulator of cancer cell genome stability: therapeutic implications.

Zih-Yao Yu[1], Wei-Jan Huang[1], Stanton Gerson[2], **Sung-Bau Lee**[1]

[1]Taipei Medical University, Taipei, Taiwan,[2]Case Western Reserve University, Cleveland, OH

In cancer cells, chromatin replication is often misregulated in part due to mutations in oncogenes and tumor suppressor genes, leading to increased replication stress and genomic instability. While this instability drives cancer progression, it also makes cancer cells more dependent on genome maintenance pathways, presenting a therapeutic target. A promising strategy is to exacerbate replication stress, causing lethal genome instability. We have developed a sensitive reporter to monitor replication stress via single-stranded DNA at stressed forks. Our earlier study identified Tousled-like kinases (TLKs) crucial for chromatin assembly during genome replication and discovered TLK inhibitors to treat cancers through increasing replication stress. Additionally, we recently uncovered histone deacetylase 8 (HDAC8) inhibitors that disrupt chromatin replication and compromise genome integrity, particularly when combined with checkpoint kinase inhibitors. In this presentation, I will show you how HDAC8 behaves as caretakers of genetic and epigenetic integrity and how we targeted replication stress to create cancer-specific synergistic vulnerabilities.

#1476 Enhancing radiation sensitivity in CHD5 dysregulated NSCLC by targeting NHEJ repair pathway.

Seohyun Kim, Sandip K. Rath, Gina Cutlip, David S. Yu

Radiation Oncology, Emory University, Atlanta, GA

Lung cancer, particularly non-small cell lung cancer (NSCLC), is a leading cause of cancer deaths, with 5-year survival rates decreasing from 65% for localized cases to 9% for distant metastases. Radiation therapy's effectiveness is often limited due to the upregulation of DNA repair pathways like non-homologous end joining (NHEJ), which helps cancer cells survive DNA damage from radiation. Chromatin remodeling plays a crucial role in regulating DNA repair pathways, including NHEJ. We have identified CHD5, a tumor suppressor involved in chromatin remodeling, as a regulator of DNA double-strand break repair pathway choice. DNA reporter assays show that CHD5 promotes homologous recombination (HR), and its depletion enhances NHEJ activity. Analysis of The Cancer Genome Atlas (TCGA) reveals that CHD5 is downregulated in lung adenocarcinoma (LUAD) patients. This suggests that NSCLC cells may rely more on NHEJ due to reduced CHD5 function. Therefore, targeting CHD5-dysregulated NSCLC cells with NHEJ inhibitors could increase their sensitivity to radiation therapy. Our data indicate that overexpressing CHD5 in H460 NSCLC cells reduces levels of phosphorylated and total DNA-dependent protein kinase catalytic subunits (DNA-PKcs), essential components of the NHEJ pathway. This suggests that CHD5 negatively regulates NHEJ. Cell viability assays confirm that CHD5 depletion via siRNA in H460 NSCLC cells sensitizes them to ionizing radiation (IR) when combined with the DNA-PK inhibitor Nu7441, leading to increased cell death. In summary, CHD5 regulates NHEJ in NSCLC, and its depletion makes cancer cells more dependent on NHEJ for DNA repair. Targeting NHEJ in CHD5-deficient NSCLC cells offers a promising therapeutic strategy for enhancing the efficacy of radiation therapy in NSCLC.

#1477 Do MSH3 mutations really affect colorectal cells neoplastic transformation: an evolution of three African Americans novel mutations within the MSH3 ATPase domains.

Mudasir Rashid[1], Hassan Brim[1], Katherine Casazza[2], Jennifer A. Surtees[2], Shaolei Teng[1], Adebiyi Sobitan[1], Adeyinka O. Laiyemo[1], Zaki Sherif[1], Koi Minoru[3], John Carethers[3], **Hassan Ashktorab**[1]

[1]Howard University, Washington, DC,[2]University of Buffalo, Buffalo, NY,[3]UCSD, San Diego, CA

Background: DNA Mismatch Repair (MMR) genes' (including MSH3) alterations through silencing and/or mutations plays a role in colorectal cancer (CRC) carcinogenesis. MSH3 interacts with MSH2 to form MutSβ, which recognizes and directs repair of insertion/deletion loops during in DNA replication; deficiencies lead to elevated mutation rates. Msh3 alterations are associated with instability at tetranucleotide repeats (termed Elevated Microsatellite Alterations at Selected Tetranucleotide repeats, EMAST), a phenotype elevated frequency among African American (AA). CRCs and associated with poor patient prognosis. Aim: To assess functional impact of 3 out of 4 Exomes (E) E20, E-21, E22, E23 novel deleterious AA variants within the ATPase domain of MSH3. Methods: We used CRISPR-Cas9 knock-in and successfully introduced 3 of these mutations (confirmed by Sanger sequencing) in Msh3 ATPase domain (c.G2926A, c.C3028T, c.G3241A) in SW620 colon cancer cell line (carry Msh3 gene wild-type). In Silico and wet-lab MSH3 functional analysis was performed. We used MTT for cell proliferation, tetranucleotide repeats instability assays, immunofluorescence for MSH3 localization, Western blot for protein expression, co-immunoprecipitation for MSH3 interaction with MSH2 MMR protein, and thin layer chromatography ATPase assays with purified MutSβ complexes.
Results: In silico analysis showed deleterious effect of the 4 novel msh3 mutants. However, biological phenotypic assays revealed no observable changes in cell morphology or proliferation between wild-type and MSH3-variant knocked-in cells, with no EMAST after 7 to 10 passages. Subcellular localization of variant MSH3 was unaffected compared to wild type MSH3, and interaction between mutant MSH3 and MSH2 proteins was not impacted, However, the ATPase activity of two of the variant MutSβ complexes was affected. MSH3 c.G2759T (E20) exhibited overall reduced ATPase activity compared to wildtype and was not affected by the presence of DNA. MSH3 c.C3028T (E22) exhibited elevated ATPase activity compared to wildtype in the presence of specific (loop) or non-specific DNA (homoduplex).
Conclusion: Despite computational deleterious predictions, novel MSH3 variants identified from AA CRCs appeared to show no biological or genetic evidence of pathogenicity, but did reveal altered in vitro biochemical properties. Altered MutSβ ATPase activity, both decreased and increased, has been shown to disrupt proper MMR activity. We propose that disruptions in the ATP hydrolytic cycle contribute to MMR deficiency, resulting in elevated mutation rates with contribution to colorectal cancer pathogenesis. This effect may be enhanced in distinct genetic backgrounds, and directly affect cancer cells' proliferation and cell division.

#1478 A novel HK2 inhibitor HIC-29 exhibits synthetic lethality with PARP inhibitors in ovarian cancer.
Xiu Shi

Xiu Shi (Individual), Suzhou, China

Purpose: In this study, we developed a novel HK2 inhibitor, HIC-29, and investigated its potential to enhance the sensitivity and expand the clinical utility of PARP inhibitors in ovarian cancer.
Methods: The HK2 protein was modeled using a Molecular Operating Environment (MOE), and the most favorable model was selected based on a GBVI/WSA dG scoring function. The Chembridge Compound Library was screened, and the top 50 candidate compounds were tested for their interaction with HK2 and downstream substrates, CHK2-pS19 . HIC-29 emerged as the most promising drug candidate and was further evaluated using multiple assays.
Results: HIC-29 exhibited selective and potent inhibition of HK2. The IC50 of HIC-29 correlated inversely with endogenous HK2 expression in ovarian cancer cell lines. Treatment with HIC-29 significantly inhibited cancer cell growth and sensitized cells to PARP inhibitors in vitro, as well as in ovarian cancer organoids and xenograft models. Mechanistically, HK2 knockdown and HIC-29 treatment reduced CHK2 phosphorylation at Ser19, impaired DNA homologous recombination repair, ultimately inducing apoptosis. These findings highlight the crucial role of HK2 in the DNA HR repair pathway and demonstrate the significant PARP inhibitor sensitization achieved by HIC-29 in ovarian cancer.
Conclusions: HIC-29, a novel HK2 inhibitor, effectively inhibits tumor cell growth in ovarian cancer by interfering with CHK2-mediated DNA HR repair. Furthermore, HIC-29 enhances the efficacy of PARP inhibitors, providing a promising therapeutic strategy to improve outcomes for ovarian cancer patients.

#1479 Defining the interplay between DNAPKcs and shieldin during chromosomal break repair.

K. Makins, M. Cisneros-Aguirre, F. W. Lopezcolorado, J. M. Stark;
City of Hope National Medical Center, Duarte, CA

Chromosomal DNA double strand breaks (DSBs) are the effective lesion of cancer radiotherapy and other cancer therapeutics, such as topoisomerase 2 poisons. Understanding how cancer cells repair DSBs can provide insight into cancer cell resistance to these therapeutics, as well as inform development approaches to improve cancer sensitivity. DSB repair is also significant for cancer biology because aberrant repair of DSBs can cause mutations and chromosomal rearrangements that drive cancer. Repair of DSBs occurs through several pathways, including non-homologous end joining (NHEJ). DNA-PKcs (DNA-dependent protein kinase) promotes NHEJ, inhibits HDR, and is an emerging drug target with kinase inhibitors in oncology clinical trials. DNA-PKcs phosphorylates itself and other DNA damage response proteins and promotes long-range DSB end synapsis to facilitate NHEJ. Another protein complex implicated in long-range DSB end synapsis is Shieldin, which includes the proteins 53BP1, RIF1 (Rap1-interacting factor 1), and other factors. Shieldin is critical for NHEJ to promote class switch recombination, which is a programmed DSB repair event during antibody maturation. However, the role of Shieldin in NHEJ repair outside this context remains poorly understood. Indeed, a specific gap in knowledge is the functional interplay between how DNA-PKcs and Shieldin promote synapsis of DSB ends to favor NHEJ. To study end joining outcomes, I use Cas9 reporter assays to measure end joining frequencies via flow cytometry. These reporter assays are chromosomally integrated into human HEK293 cells. Additionally, I examine end joining junctions using deep sequencing to compare parental HEK293 cell junctions between wild-type and 53BP1-knockout or RIF1-knockout cell lines. This PCR-based approach allows me to categorize junction types into four categories: No Indels, Insertions, Deletions, and Complex Indels. My studies indicate that RIF1 and 53BP1 are genetic modifiers of DNA-PKcs function during DSB repair. I will present our current efforts to address this gap in knowledge.

#1480 Unlocking DNA repair mechanisms with dynamic single-molecule analysis.

Paul Pease, Jack O'Sullivan, Trey Simpson, Josephine Yeh, Andrea Candelli

LUMICKS, Waltham, MA

The maintenance of genomic information through processes such as DNA replication, recombination, and repair is critical to cellular function. Errors in these underlying mechanisms play a major role in diseases such as cancer. As a result, insights into the molecular details are needed to advance our understanding, improve biological models, and develop novel therapeutics. Dynamic single-molecule technologies offer an exciting opportunity to meet these challenges and to study protein function and activity in real time. Here, we present our efforts for enabling discoveries in the field of DNA repair using the combination of fluorescence microscopy with optical tweezers. This technology has been used to study CMG helicase dynamics, the role of BRCA2 in RAD51 nucleation, and the mechanism of the DNA repair complex FANCD2-FANCI. It is also suitable for many applications involving mismatch repair (MMR), base excision repair (BER), and nucleotide excision repair (NER), among other lesions. We present several examples in which our technologies and biochemistry enhanced the understanding of these systems. Importantly, we show that advances in dynamic single-molecule methods can be turned into an easy-to-use and stable instrument that is unlocking new avenues for studying DNA-binding proteins in the field of DNA repair.

#1481 Targeting DNA damage responsive pathways in cancer therapy.

J. Chen;
UT MD Anderson Cancer Center, Houston, TX

DNA double strand breaks (DSBs) result in activation of several key DNA damage response (DDR) kinases including ATM, ATR, and DNA-PK. These protein kinases not only promote DNA damage-induced checkpoint control, but also facilitate DSB repair in humans. Thus, these DDR kinases have become promising drug targets for cancer therapy. However, the benefits of targeting DDR kinases remain to be realized, in part due to the lack of predictive biomarkers. By undertaking CRISPR screens with inhibitors targeting key DDR kinases, we obtained a global and unbiased view of genetic interactions with DDR inhibition. Additionally, we compared the synergistic effects of combining different DDR inhibitors and found that ATM and PARP inhibitors showed remarkable synergy, which depends on the dominant negative function of ATM inhibition. Moreover, to provide comprehensive and unbiased perspective of DDR signaling pathways, we performed 30 fluorescence-activated cell sorting-based genome-wide CRISPR screens with antibodies recognizing distinct endogenous DNA damage-signaling proteins to identify new regulators involved in DNA damage response (DDR). We discovered that proteasome-mediated processing is an early and prerequisite event for cells to trigger camptothecin- and etoposide-induced DDR signaling. Furthermore, we identified PRMT1 and PRMT5 as new modulators that regulate ATM protein level. Additionally, we discovered that GNB1L is a master regulator of DDR signaling via its role as a co-chaperone for PIKK proteins. Collectively, these screens offer a rich resource for further investigation of DDR, which may provide insight into strategies of targeting these DDR pathways to improve therapeutic outcomes. More recently, we have adopted dTAG technology for the investigation of many essential DDR and replication proteins, which provide mechanistic insights into the roles of these essential proteins in genome maintenance. Additionally, we have expanded FACS-based screens for the studies of other cancer-associated pathways and explored key biological processes critical for cancer progression in vivo. These studies will facilitate the development of better therapies for cancer patients.

#1482 Discovery of HW221043, a potent and orally bioavailable KIF18A inhibitor for treating chromosomal instability in cancer.

Qun Li[1], yang zang[1], xin zhao[1], qian wu[1], tiejun bing[2], lifei liu[1], xuejun zhang[1]

[1]Humanwell Healthcare (Group) Co.,Ltd, Wuhan Hubei, China,[2]ICE Bioscience Inc., Beijing, China

Background: KIF18A, a molecular motor protein, is crucial for regulating microtubule length, essential for maintaining proper cell function and division. Chromosomal instability (CIN) significantly contributes to cancer progression and is targeted by emerging therapies such as PARP inhibitors, which exploit synthetic lethality in cancer cells. CIN resulting from whole genome duplication (WGD) is associated with poor outcomes and metastatic disease, with cancers demonstrating sensitivity to spindle assembly checkpoint disruption. This makes KIF18A a promising target in this drug class.
Methods and Results: We have discovered HW221043, a highly potent and orally bioavailable small molecule inhibitor of KIF18A. Biochemical assays revealed that HW221043 potently inhibits the ATPase activity of KIF18A (IC50, 6 nM) without affecting other kinesin family members, even at the highest tested concentration of 10 μM. In cell-based assays, HW221043 markedly inhibited the viability of CIN-positive tumor cell lines at nanomolar concentrations, with no effect on CIN-negative cells even at 10 μM. The compound demonstrated excellent pharmacokinetic (PK) profiles in mice, rats, dogs, and monkeys. In two cell-derived xenograft (CDX) models (OVCAR3 and BT549), oral administration of HW221043 dose-dependently inhibited tumor growth, with nearly complete inhibition observed at 15 mg/kg once daily. Higher doses led to tumor regression. All in vitro and in vivo PK and safety tests yielded favorable results, supporting the preclinical development of HW221043.
Conclusion: These findings provide pharmacological proof-of-concept for the synthetic lethal effect of HW221043 and support KIF18A inhibition as a novel therapeutic strategy in oncology. HW221043 is currently in the Investigational New Drug (IND) enabling stage of development.

#1483 Effect of USP1 inhibitor with or without radiotherapy and chemotherapy on cell viability and biomarkers, independent of BRCA or HRD status.

Mrinal Sivastava[1], Nirjharni Saha[1], Partha Pratim Sarma[2], Prashant Kashinath Bhavar[2], **Uday Kumar Surampudi**[2]

[1]Tata Institute of Fundamendal Research, Hyderabad, India,[2]VeGen Therapeutics, Hyderabad, India

The efficacy of USP1 inhibitors, which target deubiquitinating enzymes critical to DNA damage response pathways, was evaluated in combination with radiotherapy and chemotherapy across different cellular types including BRCA1/2, BRCA null and, homologous proficient BRCA mutations. Here, we investigate the impact of USP1 inhibition, as single agent or in combination with RT or CT, in a panel of three distinct cell lines, UWB1.289, HCT-116 and NCI H-1792 representing diverse gene alterations and tumor types. Our results demonstrate that USP1 inhibition significantly sensitizes BRCA mutant and HRD proficient cell lines to both RT and CT, amplifying DNA damage and enhancing apoptotic responses. Interestingly, the combination of USP1 inhibitors with UV radiation resulted in significant reduction of cell proliferation in HCT-116, known to demonstrate significantly higher HR activity scores compared with the BRCA1/2 deficient cells, suggesting a broader role of USP1 inhibitor beyond HR deficient cell lines. The synergy between USP1 inhibition and radiotherapy was most pronounced in the HRD+ models, underscoring the potential of USP1 inhibitors as a therapeutic strategy in DNA repair deficient cancers. The activity was established using both the cell viability as well by performing immunofluorescence assay using PCNA as a marker. These findings provide novel insights into the role of USP1 in modulating the response to DNA damaging therapies, with implications for personalized treatment strategies in cancers independent of HRD status. Future studies will explore the underlying molecular mechanisms driving the observed therapeutic synergy and validate these results in-vivo.

#1484 USP36-governed p21 homeostasis confers radioresistance in pancreatic cancer.

Haihua Feng, Sindhu Nair, Veronica Castro Aceituno, Linlin Yang, Haiqing Li, Tiantian Cui, Sergio Corrales Guerrero, Ling Gui, Nicholas Eustace, Terence M. Williams

Radiation Oncology, City of Hope National Medical Center, Duarte, CA

Purpose: Pancreatic cancers are highly aggressive tumors that carry a poor prognosis. More than 90% of pancreatic ductal adenocarcinoma (PDAC) harbors mutationally activated KRAS, and oncogenic KRAS signaling has been implicated in contributing to replication stress and PDAC radioresistance. Cell cycle inhibitor $p21^{waf1/cip1}$ (p21) can be transcriptionally induced by oncogenic KRAS and p53 in response to DNA damage. DNA damage can also induce p53-independent p21 nucleolus accumulation. Several studies revealed the antagonistic duality of p21 in oncogenesis depending mainly on its intracellular localization, with a tumor suppressor role in the nucleus and oncogenic role in the cytoplasm. The nucleolar deubiquitinase USP36 is pivotal in some cellular processes such as cell cycle maintenance, ribotoxic stress, and DNA replication stress. However, whether and how USP36 regulates p21 in the nucleolus in response to irradiation-induced DNA double-strand breaks remain unclear.
Experimental Design: We examined p21 protein level in HEK 293T and PDAC cell lines (MIA PaCa-2, PANC-1) by overexpressing wild-type USP36 and its catalytic-inactive mutant (C131A). We monitored the colocalization of GFP-p21 and mcherry-USP36 in both HEK 293T and MIA PaCa-2 cells. We assessed the association of p21 and USP36 by Co-IP. We determined the residues of USP36 that are phosphorylated in response to DNA damage by point mutation, and analyzed cell cycle distribution by flow cytometry. We examined the roles of USP36 and p21 in radiosensitivity with clonogenic assay. β-galactosidase staining was performed to assessed the cell senescence after radiotherapy.
Results: Overexpression of USP36, but not its catalytic-inactive mutant (C131A) induced protein levels of p21 in HEK 293T, MIA PaCa2 and PANC-1 cells. Mcherry-USP36 induced the accumulation of GFP-p21 in nucleolus of HEK 293T cells. In response to DNA damage, p21 translocated to the nucleolus and colocalized with USP36 in cells. Through domain mapping, we determined that the N-terminal of USP36 interacts with p21. Additionally, ATM and ATR phosphorylate USP36 at Ser356 and Ser519. USP36 depleted cells exhibited G1 release and G2/M arrest following DNA damage, and loss of USP36 induced cell senescence in cells following DNA damage. Finally, loss of USP36 sensitizes pancreatic cancer cells to radiation in vitro.
Conclusions: Here we report that loss of USP36 sensitizes pancreatic cancer cells to radiotherapy. Our findings support a model in which, in response to DNA damage, USP36 is hyperphosphorylated and activated by ATM/ATR, resulting in stabilization of p21 through USP36-mediated deubiquitination. Stabilization of p21 alters cell cycle progression, ultimately leading to pancreatic cancer radioresistance through enhanced cell recovery after DNA damage. Together, our data supports targeting USP36 and/or p21 for therapeutic intent.

#1485 BRCA2 haploinsufficiency leads to mitotic DNA synthesis at common fragile sites in human mammary epithelial cells.

Kavya Vipparthi[1], Ariana Bellare[2], **Mihriban Karaayvaz**[1]

[1]Cleveland Clinic Lerner Research Institute, Cleveland, OH,[2]Case Western Reserve University, Cleveland, OH

Germline mutations in BRCA2 confer up to a 70% lifetime risk of developing breast cancer due to BRCA2's critical roles in homologous recombination repair, replication fork stability, and cell cycle regulation. In BRCA2 mutation carriers, mammary epithelial cells are haploinsufficient, meaning a single functional allele is inadequate to maintain genomic stability and suppress tumorigenesis. Genomic instability, a hallmark of cancer, frequently involves fragile site instability, particularly at common fragile sites (CFSs), which are hotspots for chromosomal rearrangements under replication stress. While the association between fragile site instability and cancer has been documented, the specific link between BRCA2 haploinsufficiency and CFS instability remains unexplored. To address this gap, we modeled BRCA2 haploinsufficiency in human mammary epithelial cells using small-interfering RNA (siRNA)-mediated knockdown and induced replication stress with the DNA polymerase inhibitor aphidicolin (APH). Cytogenetic analysis using DAPI banding revealed increased CFS instability, particularly at FRA18B, in BRCA2-deficient cells compared to controls. Furthermore, we observed elevated levels of mitotic DNA synthesis (MiDAS) in BRCA2-deficient cells, a process typically activated at under-replicated regions of CFSs during mitosis to preserve genome integrity. These findings demonstrate that BRCA2 haploinsufficiency promotes CFS instability and aberrant MiDAS, uncovering novel mechanisms of genomic instability in preneoplastic mammary epithelial cells. By elucidating these early molecular events in BRCA2-associated breast cancer, our study provides critical insights into the etiology of tumorigenesis and lays the groundwork for innovative strategies to stabilize the genome in high-risk individuals, potentially improving breast cancer prevention and outcomes.

#1486 Early biomarker dynamics are associated with treatment efficacy in a preclinical model of radiotherapy combined with a DNA damage response agent.

Pablo Binder, Theoni (Tania) Katopodi, Lyndsey Hanson, Charlotte Bell, Eimear Flanagan, Paul Farrington, Nick Moore, Emily Wright, Tobias Bunday, Stewart Brown, Amy Cantrell, Jon Greenall, Shannon Sharman, John Woolley, **Lorraine Mooney**, Jane Kendrew

Sygnature Discovery, Alderley Edge, United Kingdom

Introduction: Radiotherapy is an essential treatment for many types of cancer, with almost half of all cancer patients undergoing it as part of their treatment regimen. It can initiate cytotoxic signaling pathways to induce cancer cell death, but it can also activate cytoprotective mechanisms that respond to cellular damage. These protective mechanisms can be targeted by anticancer agents like DNA damage response (DDR) agents, indicating that radiotherapy might boost the effectiveness of these drugs. Early response biomarkers can be utilized to support preclinical studies to assess the pharmacodynamic effects of a drug, providing insights into its mechanism of action. RNA sequencing technologies provide an opportunity to elucidate gene expression profiles across the breadth of the transcriptome, uncovering disease-specific gene signatures and biomarker candidates.
Methods and Experimental Procedures: The A549 lung cancer cell line was utilized to establish in vitro assays and the in vivo preclinical model for the study. An inhibitor targeting DNA PK (AZD7648) was used as it targets pathways crucial for the repair of radiation induced DNA double strand breaks. The effects on cell viability and DDR biomarkers were assessed after radiotherapy, compound or in combination both in vitro and in vivo. Targeted X rays were delivered to the tumor only using an Xstrahl CIX3 cabinet irradiator. Total RNA was isolated from excised tumors, and RNAseq analysis was performed (Illumina NovaSeq). Differential gene expression and pathway analysis was performed using Qiagen Ingenuity Pathway Analysis software. Protein biomarkers were assessed using the Jess Simple Western platform.
Data: In vitro assays showed increased sensitivity to the AZD7648 inhibitor when combined with radiotherapy, compared to standalone treatments. This combination also resulted in delayed tumor growth in vivo, with radiotherapy being well tolerated. The induction of biomarkers by radiotherapy, either as a monotherapy or in combination with the inhibitor, was assessed. Changes in biomarker expression were consistent with target engagement and treatment efficacy. Notably, γH2AX upregulation was evident in the irradiation monotherapy group but was delayed when combined with the DNA PK inhibitor, demonstrating target engagement as DNA PK inhibition alters the kinetics of γH2AX.
Conclusion: In summary, both in vitro assays and in vivo models have been developed to explore the synergistic effects of combining radiotherapy with DDR agents. The combination of radiotherapy with the AZD7648 inhibitor resulted in delayed tumor growth in vivo. Early changes in protein and gene biomarkers were consistent with target engagement and treatment efficacy. These assays can serve as preclinical tools to investigate compounds or mechanisms that can be effectively combined with radiotherapy.

#1487 MYC inhibition modulates DNA damage response and is capable of enhancing PARPi therapeutic impact.

Fabio Giuntini[1], Andrea Herencia-Ropero[1], Inigo Gonzalez-Larreategui[1], Silvia Casacuberta-Serra[2], Sandra Martinez-Martin[2], Mariano F. Zacarias-Fluck[1], Hugo Thabussot[2], Flaminia Pedretti[1], Laia Foradada[2], Erika Serrano[1], Judit Grueso[1], Sergio Lopez-Estevez[2], Olga Rodriguez[1], Marta Guzman[1], Adela Rodriguez-Hernandez[3], Fara Braso-Maristany[3], Marie-Eve Beaulieu[2], Lara Nonell[1], Violeta Serra[1], Aleix Prat[3], Jonathan Richard Whitfield[1], Daniel Masso-Valles[2], **Laura Soucek**[1]

[1]VHIO Vall D'Hebron Institute of Oncology, Barcelona, Spain,[2]Peptomyc S.L., Barcelona, Spain,[3]IDIBAPS, Barcelona, Spain

Background: The MYC oncogene, amplified or dysregulated in about 70% of human tumors, orchestrates multiple cancer hallmarks. Interestingly, its role in DNA damage response (DDR) has been debated for decades. On one hand, oncogenic MYC has been shown to promote genomic instability, while, on the other hand, it can also enhance DNA repair. We developed Omomyc, the first MYC dominant negative to have successfully completed a Phase I clinical trial, and used it as a research tool to study MYC's role in DDR. We focused on TNBC, where defective DDR is clinically associated with the use of PARP inhibitors (PARPi).
Methods: First, RNAseq, WB, immunofluorescence and flow cytometry were used to test Omomyc's effect on DDR genes in a panel of TNBC cells and its direct contribution to DNA damage. In addition, proliferation, apoptosis and synergy assays were performed to test the combination of Omomyc and PARPi *in vitro*. Then, cell derived xenografts (CDXs) and patient-derived xenografts (PDXs) were treated with the combination *in vivo*. Furthermore, bulk RNAseq from 35 patient derived xenografts (PDXs) was performed to elucidate the role of MYC transcriptional activity in PARPi resistance. Lastly, digital spatial profiling (DSP) of 18 patients' biopsies prior to PARPi treatment is being performed to evaluate if MYC transcriptional signature could be a predictor of patients' response to PARPi.
Results: We found that Omomyc shuts down genes and gene-sets related to homologous recombination repair (HRR) in both BRCA wild-type and PARPi-resistant BRCA-mutated cell lines, by depleting DDR proteins. Also, we observed that Omomyc causes early DNA damage, inducing phosphorylation of H2AX. Importantly, we found that the combination with PARPi, further increases H2AX phosphorylation, resulting in apoptosis in the long term, both *in vitro* and *in vivo*. RNAseq of 35 PARPi-treated PDXs showed that those resistant to PARPi therapy have a significantly increased MYC transcriptional activity, suggesting its role in drug resistance. At the time of submission of this abstract, DSP of 16 patients' biopsies is being performed to evaluate if the MYC transcriptional signature can be used as a predictor for PARPi response.
Conclusions: Overall, we show evidence that MYC inhibition by Omomyc phenocopies HR deficiency and induces DNA damage in TNBC. In addition, our research shows that combining Omomyc with PARPi would be an effective therapy in PARPi-resistant TNBC, where MYC transcriptional activity is over-represented and could potentially be a predictive biomarker of resistance to therapy.

#1488 DNA polymerase kappa slows replication fork speed by promoting fork reversal in glioblastoma.

R. S. Sewilam, M. R. Reed, R. L. Eoff;
University of Arkansas for Medical Sciences, LittleRock, AR

Replication fork stalling in response to replication stress triggers the activation of replication stressresponse (RSR) pathways to facilitate repair and restart replication processes, including fork reversal andtranslesion DNA synthesis (TLS). During fork reversal, the replication fork undergoes a structural rearrangementto form a four-stranded structure, known as a "chicken foot," which helps regulate fork speed by recruitment ofdifferent fork reversal factors, including HLTF, and SMARCAL1. Optimal replication conditions ensure a relativelyconstant and rapid fork progression rate, which is essential for timely genome duplication. TLS requiresspecialized DNA polymerases that assist fork progression by promoting direct bypass of replication blocks. Inglioblastoma, the most aggressive and resistant form of brain cancer, the TLS polymerase kappa (Pol κ) isoverexpressed. This overexpression causes resistance to standard therapeutics and is associated with poorprognosis. A major goal of our study is to understand how Pol κ protects glioma cells from endogenous oncogenicstressors and the DNA-damaging effects of chemotherapeutics. Results from Eoff lab indicate that Pol κ slowsreplication fork speed in glioma cells (T98G, U118-MG) without significantly affecting non-glioma cells (HAP-1,U2OS, RPE). We hypothesized that Pol κ slows replication fork speed by promoting fork reversal in glioma cells.To test this hypothesis, we used a proximity ligation assay (PLA) to verify the colocalization of fork reversalfactors SMARACL1, and HLTF to sites of DNA synthesis in glioma cells. PLA measures the proximity betweenproteins and newly synthesized DNA labeled with nucleotide analog 5-ethynyl-2 -deoxyuridine (EdU), indicatingtheir potential interaction or colocalization, as evidenced by the appearance of fluorescent foci**.** EdU-SMARCAL1,or EdU-HLTF PLA foci were increased in T98G WT without a significant increase in POLK KO T98G cellsfollowing treatment with 50 nM CPT with no considerable increase in POLK KO T98G cells. These resultssuggest that Pol κ has a role in promoting the colocalization of fork reversal proteins to sites of DNA synthesisin T98G (glioma) cells. To support this conclusion and to study how this affects fork speed, we used DNA fiberspreading (DFS) to determine fork speed with/without depletion of the fork reversal factor SMARCAL1 and HLTF.Depletion of SMARCAL1 and HLTF produced longer IdU tracts in T98G WT cells, indicating faster fork speed.The fork acceleration phenotype in POLK-KO T98G cells remained unaffected by the knock-down ofSMARCAL1, suggesting that these proteins work in the same pathway to regulate fork dynamics in glioblastomacells. These results are the first to establish a link between Pol κ and fork reversal, helping to explain how glioma-specific mechanisms tolerate high rates of DNA damage in the tumor microenvironment.

#1489 Targeting the DNA double-strand break repair pathways in MYCN-amplified neuroblastoma increases tumor sensitivity to chemotherapy.

Mahnaz Norouzi, Subin Kim, B. Mark Evers, Eric J. Rellinger, Piotr Rychahou

University of Kentucky, Lexington, KY

Background: DNA double-strand break (DSB) repair is regulated by non-homologous end joining (NHEJ) and homologous recombination (HR). In neuroblastoma (NB), extensive genomic instabilities such as MYCN amplification, 11q and 1p deletions, and 17q gain are linked to tumor progression and high-risk disease. These abnormalities are associated with defective DSB repair and are enriched in high-risk NB. Here we demonstrate the association between DSB repair pathways and MYCN amplification (MNA) in high-risk NB and explore the potential response of MNA NB to therapies targeting these pathways.
Methods: The R2 Genomics Analysis and Visualization Platform was used for gene set enrichment (GSEA) and differential expression analysis in NB cohorts. DNA-PKcs gene knockdown models, along with Peposertib and Olaparib treatment, were used to inhibit DNA repair activity. Western blotting (WB) was performed to verify DNA-PKcs inhibition and measure PARP cleavage in response to DNA-PKcs inhibition in BE(2)-C and SK-N-DZ cells. DNA damage was induced by Doxorubicin and detected using γ-H2AX immunofluorescence staining followed by confocal microscopy. Clonogenic assays were performed to assess the efficacy of DNA repair inhibition on the survival of NB cells.
Results: GSEA using the Kocak cohort study, which contains microarray gene expression data from 649 NB samples from the NB Research Consortium (NRC), revealed that NHEJ was upregulated in MNA high-risk NB (GSE score = 1.183), while the HR pathway showed moderate downregulation (GSE score = -0.428). Additionally, differential analysis revealed that key components of NHEJ, including and PRKDC, XRCC5, NHEJ1 and Rad50, showed significantly higher expression in MNA high-risk NB compared to non-MNA ($p < 0.05$). Confocal imaging demonstrated increased γ-H2AX foci formation in DNA-PKcs knockdown cells, indicating enhanced DSBs in response to DNA damage due to the loss of active NHEJ repair. Increased cleaved PARP was demonstrated in DNA-PKcs siRNA SK-N-DZ and BE(2)-C knockdown cells, indicating increased apoptosis in response to its inhibition in MNA NB cells. Clonogenic analysis showed a significant decrease in NB cell survival in response to Doxorubicin treatment, with a more pronounced decrease in response to the combination with both Peposertib and Olaparib.
Conclusion: High-risk NB is correlated with extensive genomic instability, and here we showed that upregulation of NHEJ is associated MNA high-risk NB. DNA-PKcs knockdown studies revealed that the survival of MNA NB cells depends on the activity of NHEJ pathway, paving the way for more targeted treatments. The significant response of NB cells to the combination of Peposertib and Olaparib highlights a promising strategy to enhance NB cell sensitivity to conventional therapies, particularly Doxorubicin.

#1490 RAD51C-deficient cancer cells require DNA polymerase zeta to bypass cisplatin-induced lesion.

Mohd Azrin Jamalruddin[1], Emmanuel Tawiah[1], Isabela Contreras[1], McKenzie Grundy[2], Kara Bernstein[1]

[1]Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA,[2]University of Pittsburgh, Pittsburgh, PA

RAD51C is a RAD51 paralog protein that mediates RAD51 filament formation on single-stranded DNA (ssDNA) in a canonical homologous recombination (HR) pathway. This step is vital for homology search to repair double strand breaks. RAD51C deficiency is linked to increased risks of developing breast and ovarian cancers. Fortunately, RAD51C-deficient cancer patients are generally responding well to chemotherapeutics drug like cisplatin. HR is one of the important pathways to repair cisplatin-induced lesion. Hence, cancer cells that lack RAD51C are more susceptible to cisplatin treatment. Nonetheless, high grade serous ovarian cancer patients are often relapse even though initial cisplatin treatment were successful. There is still incomplete knowledge on how different DNA repair factors contribute to cisplatin-induced damage repair in HR-deficient cancer cells. Our study is aimed to identify and study these factors to better understand how RAD51 paralog-deficient cancer cells evade cell death upon cisplatin treatment. Apart from HR, there are several DNA damage tolerance mechanisms in which cancer cells can adopt to bypass cisplatin-induced lesions. Based on our synthetic lethality screen in yeast, we observed that translesion synthesis (TLS) factors (*rev1* and *rev3*) deletion, increase the cisplatin sensitivity in RAD51 paralog knockout (KO) cells. Cisplatin often causes the formation of bulky DNA lesions that provide structural barrier to DNA polymerase during DNA replication. TLS DNA polymerases are error-prone DNA polymerases that bypass these lesions to continue DNA replication and evade cell cycle arrest. We also observed the same scenario in human ovarian cancer cells. TLS depletion, that include REV3L of DNA pol zeta, (POL ξ), increase cisplatin sensitivity in RAD51C-deficient cancer cells. Using DNA fiber assay, we discovered that POL ξ limits the replication fork speed in RAD51C-KO cells upon cisplatin treatment. Based on S1 nuclease DNA fiber assay, we showed that POL ξ also prevents the accumulation of ssDNA in cisplatin-treated RAD51C-KO cells. These preliminary data suggest that POL ξ plays a role in DNA replication dynamics in RAD51C-deficient cells to ensure them to survive under cisplatin treatment. Uncharacterized RAD51C point mutations that found in cancer patients are often classified as variants of unknown significance (VUS). While cells that lack RAD51C are generally sensitive to cisplatin, some of these RAD51C VUS variant cells are resistant to it. In our clonogenic assay, we observed that REV3L depletion sensitizes several cisplatin-resistant RAD51C variants to cisplatin. Upon cisplatin treatment and REV3L depletion, we also observed an increase of ssDNA accumulation in RAD51C variant. Our data reveal the potential role of POL ξ in therapeutic avenue for cisplatin-resistant RAD51C variants.

#1491 ATM, CHEK2 and BLM mutant prostate cancers do not have high homologous recombination deficiency scores.

Ekaterina Sh. Kuligina, Aglaya G. Iyevleva, Anna P. Sokolenko, Svetlana N. Aleksakhina, Maria V. Syomina, Ekaterina A. Otradnova, Evgeny N. Imyanitov

N.N. Petrov National Medical Research Center of Oncology, St. Petersburg, Russian Federation

Background: Homologous recombination deficiency (HRD) resulting from inactivating mutations in the BRCA1 and BRCA2 genes promotes increased chromosomal instability and renders tumor cells susceptible to platinum derivatives and PARP inhibitors. Hereditary and/or somatic mutations in BRCA2, ATM, CHEK2 and other loci involved in homologous recombination DNA repair (HRR) have been reported in up to 25% of metastatic prostate cancer (PC) cases. The contribution of alterations in HRR-related genes beyond BRCA1/2 to homologous recombination deficiency remains largely unknown. The aim of the current study was to compare the HRD scores in prostate tumors with alterations in different HRR genes.
Methods: HRD scores were determined as the sum of genomic loss of heterozygosity (LOH), large-scale state transition (LST) and telomeric allelic imbalance (TAI) scores by targeted NGS using HiSNP Ultra Panel v1.0 (Nanodigmbio, China) in two PC groups. The first group consisted of 58 PC cases with germline or somatic mutations in any of 34 HRR genes, while the second group included 214 cases without HRR mutations. The cut-off for the presence of homologous recombination deficiency was set as an HRD score ≥ 42.
Results: HRD was observed more frequently in PCs with HRR mutations (9/58, 16%) than in cases without detectable HRR alterations (10/214, 5%, p = 0.008). In the HRR-mutated subgroup, high HRD scores were found in cases with BRCA2 (n = 4), ATM (n = 2), CDK12, NBN and FANCM mutations. The median HRD scores in PCs with mutations in BRCA2 (n = 9), ATM (n = 12), CHEK2 (n =8), NBN (n = 3), FANCM (n = 3), BRCA1 (n = 3), BLM (n = 3) were 41, 22.5, 7.5, 3, 10, 6, and 3, respectively. The comparison of HRD scores between BRCA2-mutant cases and ATM- (p = 0.046), CHEK2- (p = 0.005), or BLM-mutant PCs (p = 0.042) reached statistical significance. The presence of somatic TP53 mutations was associated with a higher degree of chromosomal instability and elevated HRD scores (p = 0.0001).
Conclusions: A high HRD score is observed in a noticeable proportion of prostate tumors lacking mutations in the HRR genes. Prostate cancer cases with alterations in ATM, CHEK2, BLM, and possibly also NBN, FANCM genes are unlikely to have severe HRD. This work has been supported by the Russian Science Foundation (grant number 23-15-00262).

#1492 Clinical validation on the PAOLA-1/ENGOT-ov25 cohort of HRD calculation performed with the OncoDEEP® kit comprehensive genomic panel.

Marcel Trautmann[1], Simon Lefevre[2], Sebastien Sauvage[2], Jessie Hong[2], Maxime Lienard[2], Karim Ashour Garrido[2], Jean-Francois Vanbellinghen[2], Rapahel Leonard[2]

[1]Gerhard-Domagk-Institute of Pathology, Munster University Hospital, Munster, Germany,[2]OncoDNA, Gosselies, Belgium

Introduction: Homologous Recombination Repair (HRR) pathway repairs DNA double-strand breaks. Loss of HRR capability is known to lead to genomic instability resulting in a phenotype called Homologous Recombination Deficiency (HRD). The utility of HRD biomarker in cancer treatment lies in its ability to predict response to specific types of therapy such as platinum-based chemotherapy and PARP inhibitors (PARPi). HRD tests evaluating scores of allelic imbalances, such as genomic scar (GS) or loss of heterozygosity (LOH), have been shown to identify a subgroup of BRCA wild-type (wt) tumors showing a HRD phenotype. Currently, HRD status is being used as a biomarker in cancer profiling, helping clinicians determine the best treatment option for their patients. In this perspective, OncoDNA® has developed a pan-cancer tumor profiling solution, the OncoDEEP® kit, facilitating the genomic analysis of both tumor DNA and RNA, and supported with Bio-IT analysis and clinical reporting. This end-to-end solution screens for a very wide range of cancer biomarkers and genomic signatures such as HRD. Hence, the OncoDEEP® kit can aid in reducing the costs of testing and deliver faster results for the selection of appropriate cancer treatment.

Methods: OncoDEEP® kit, based on Twist technology, was performed at OncoDNA laboratory facilities on 246 extracted DNA from FFPE ovarian carcinoma of the PAOLA-1/ENGOT-ov25 cohort. Those samples were previously characterized with Myriad Mychoice DX and obtained at Arcagy Gineco®. Sequencing was performed on Illumina Novaseq X. All 638 genes on OncoDEEP® panel, inclusive of BRCA1/2 and other HRR genes, were analyzed *via* OncoDNA proprietary bioIT pipeline and GS was calculated using Loss of Heterozygosity (LOH), Allelic Disparity on Telomere (ADT) and Large-scale Rearrangements (LR), with a positive threshold of 37. Afterwards, results were matched with PFS and treatment.

Results: HRD analysis of OncoDEEP® showed a high correlation with Myriad MyChoice DX, with an overall concordance of 85 %. In addition, OncoDEEP showed to be highly predictive (p-value≤0.001) of tumor response to olaparib+bevacizumab. Tumors harboring a positive HRD phenotype and treated with olaparib+bevacizumab exhibited a median PFS of 36.4 months, while median PFS of placebo-treated tumors was 18.75 months. Finally, OncoDEEP analysis presented a lower failure rate (0.8%) compared to Myriad MyChoice Dx (6.5%).

Conclusion: OncoDEEP® kit is an end-to-end solution allowing the detection of variants in 638 genes and the calculation of genomic signatures such as TMB, MSI and HRD. OncoDEEP® HRD analysis showed to be highly predictive of tumor response treated by olaparib+bevacizumab and is therefore applicable to the identification of ovarian cancer patients eligible to PARPi therapy in oncology routine.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Hypoxia and Oxidative Stress
Poster Session

#1496 Understanding a novel interaction between HSF1 and ERRα in breast cancer.

Sunandan Chakrabarti[1], Yuan Feng[2], David Adelfinsky[3], Jason Tennessen[4], Richard Carpenter[5]

[1]Medical Sciences, Melvin and Bren Simon Comprehensive Cancer Center, Indiana University School of Medicine - Bloomington, Bloomington, IN,[2]Medical Sciences, Indiana University School of Medicine - Bloomington, Bloomington, IN,[3]Medical Sciences, Indiana University Bloomington, Bloomington, IN,[4]Biology, Indiana University Bloomington, Bloomington, IN,[5]Biochemistry and Molecular Biology, Melvin and Bren Simon Comprehensive Cancer Center, Indiana University School of Medicine, Bloomington, IN

Breast Cancer is the most common type of cancer in women with 1 in 8 women being predicted to develop breast cancer during their lifetimes. Even after decades of ground-breaking research, new cases and deaths continue to rise. Oncogenesis can often be attributed to genetic alterations, which inadvertently lead to transcriptional dysregulation, often allowing them to successfully mitigate the effects of oxidative stress, metabolic stress, and others, ultimately giving them survival benefits. ERRα is an orphan nuclear receptor that regulates metabolic gene expression and has been linked to poor prognosis in many cancers, including that of the breast. Inhibiting ERRα has been identified as a potential therapeutic strategy but the underlying mechanism is not fully understood. HSF1, a master transcription regulator involved in heat shock response and protein homeostasis, has been implicated in oncogenesis and promotes cell proliferation and invasion. However, any interaction or cooperation between ERRα and HSF1 has not been investigated. Analyses of ChIP Seq data revealed a substantial number of overlapping binding peaks and shared target genes between HSF1 and ERRα. Gene ontology enrichment revealed these shared target genes are enriched for cellular pathways frequently dysregulated in cancer, such as cell proliferation. Using a novel ERRα transcriptional activity signature, it was observed that ERRα activity was strongly correlated with HSF1 activity in breast cancer patients. Furthermore, patients with high activities of both ERRα and HSF1 were found to have poorer prognosis. Since HSF1 and ERRα are transcription factors, we wanted to check for any cooperativity in their transcriptional activities. Luciferase-based reporter assays revealed ERRα enhanced HSF1 transcriptional activity. To further understand any interaction between ERRα and HSF1, we treated HEK293FT (non-cancer cell line) and HCC1937 (triple negative breast cancer cell line) with an ERRα inhibitor (XCT790) and an HSF1 inhibitor (DTHIB). We observed a reduction in HSF1 protein with ERRα inhibition and a reduction in ERRα with HSF1 inhibition. Moreover, co-immunoprecipitation assay results suggest a physical interaction between HSF1 and ERRα. Taken together, our data suggests a possible protein complex with HSF1 and ERRα that stabilizes both proteins and regulates genes supportive of breast tumor growth. Future studies will be looking at the role of this complex on oncogenesis and progression of breast cancer. These studies will lead to identification of probable therapeutic strategies exploiting this interaction.

#1497 PERK as a biomarker to optimize therapy of GRP78 inhibitor, BOLD-100, in gastric cancer.

Sea Young Choo[1], Ah-Rong Nam[1], Kyoung-Seok Oh[1], Jae-Min Kim[1], Ju-Hee Bang[1], Yoojin Jeong[1], Hyo Jung Kim[1], Jeesun Yoon[2], Tae-Yong Kim[2], Do-Youn Oh[2]

[1]Seoul National University Cancer Research Institute, Seoul, Korea, Republic of,[2]Seoul National University Hospital, Seoul, Korea, Republic of

Background: Targeting endoplasmic reticulum (ER) stress is emerging as a potential therapeutic strategy for developing therapies against solid tumors. GRP78, also known as the master regulator of unfolded protein response (UPR), is an anti-apoptotic ER chaperone that adjusts protein folding capacity to restore ER homeostasis. Moreover, GRP78 is an inhibitory PERK-binding protein that regulates the activation of death-triggering UPR pathways to avoid cell apoptosis. In gastric cancer (GC), GRP78 expression is elevated compared to normal tissue making GRP78 an attractive therapeutic target in GC. However, there have been limited studies on targeting GRP78 in GC. This study investigates the antitumor effects of GRP78 inhibition with BOLD-100 and explore potential biomarkers for therapeutic response in GC.
Methods: A total of 9 gastric cancer cell lines (KATO-III, SNU-601, NCI-N87, MKN-45, SNU-216, SNU-719, SNU-668, AGS, SNU-638) were used. BOLD-100 (GRP78 inhibitor), TUDCA (Tauroursodeoxycholic acid; ER stress inhibitor), and AZD6738 (ATR inhibitor) were used. MTT assay, RT-qPCR, western blot, cell cycle analysis, DCF-DA staining, immunofluorescence, and Annexin V assay were performed to evaluate the antitumor effects of BOLD-100. To evaluate the effect of PERK expression level on the efficacy of BOLD-100, siRNA was used to downregulate PERK and consequent BOLD-100 effects were analyzed by MTT assay, western blot, and flow cytometry.
Results: The antitumor effect of BOLD-100 positively correlated with PERK expression. BOLD-100 suppressed the mRNA level of GRP78 leading to a subsequent increase in unfolded protein load and ROS level in a PERK expression-dependent manner. In PERK-high GC cells (KATO-III, SNU-601), BOLD-100 exerted pronounced activation of PERK-eIF2a-CHOP pathway, resulting in inhibited tumor cell growth and induced apoptosis. PERK knockdown reversed BOLD-100 induced ROS accumulation and UPR activation suggesting that PERK expression level regulates sensitivity to BOLD-100. Conversely, in PERK-low GC cells (AGS, SNU-638), BOLD-100 exerted minimal effects on UPR pathway. Rather, it induced R-loop accumulation and upregulated active forms of ATR and Chk1 - effects not observed in PERK-high cells - suggesting activation of DNA damage repair pathway. A combination of BOLD-100 and AZD6738 showed synergistic effects in PERK-low GC cells by suppressing BOLD-100 induced ATR phosphorylation.
Conclusion: The expression of PERK determines sensitivity to GRP78 inhibition by BOLD-100 in GC. PERK-high GC cells are sensitive to BOLD-100 and show efficacy as monotherapy. In PERK-low GC cells, the combination of BOLD-100 and ATR inhibitor is an optimal therapeutic option for GC treatment.

#1498 SELENOF maintains proteostasis through UPR-IRE1α axis in breast cancer.

An Zhang, Irida Kastrati

Cancer Biology, Loyola University of Chicago, Maywood, IL

Background: SELENOF is an understudied selenoprotein that incorporates selenium in the form of selenocysteine. Our lab's investigation into SELENOF's function in breast cancer indicates that it is likely a tumor suppressor. SELENOF resides in the endoplasmic reticulum (ER) and has been hypothesized to assist in protein folding, specifically disulfide-bond formation. Disruption of protein folding efficiency leads to the accumulation of misfolded proteins, a process referred as EnR stress. In turn, the unfolded protein response (UPR) is triggered to relieve EnR stress and to restore protein processing functions. In cancer, UPR is a double edge sword. To meet the challenges of rapid proliferation, cancer cells exploit this intrinsic adaptive mechanism of UPR. However, with prolonged and unresolved EnR stress, the cell switches UPR from an adaptive response to a terminal death-inducing response. We hypothesize that SELENOF may play a role in UPR stress resolution, and how this affects breast cancer initiation of progression remains to be determined.

Objective:Our study aims to determine the crosstalk between SELENOF and EnR using normal mammary gland tissue and breast cancer models.

Results: SELENOF levels were manipulated by overexpression or knockout in normal breast epithelial cells and in breast cancer cell lines. Loss of SELENOF does not change baseline UPR measured by Thioflavin T staining and EnR stress markers include IRE1a phosphorylation and XBP1 mRNA splicing. Instead, overexpression of SELENOF increased UPR. We also determined that the IRE1-XPB1 axis required, but not the other two, Upon induction of EnR stress using Tunicamycin, Thapsigargin, we find that SELENOF levels is increased via IRE1-XBP1, thus uncovering a crosstalk between UPR and SELENOF. Furthermore, loss of SELENOF reduces the overall UPR. These findings were validated in vivo using wild type and SELENOF knockout mice.

Conclusion: Our findings establish a feed-forward model between UPR and SELENOF; SELENOF level increase upon UPR induction to amplify the UPR response towards terminal UPR. Loss of SELENOF restricts UPR response within adaptive levels to bypass cell death. This may provide a mechanism for cancer cells to survive while coping with higher proteotoxic stress.

#1499 Combination HIF-2alpha inhibition with cholesterol biosynthesis inhibition decreases colorectal carcinoma growth.

Prarthana Dalal[1], Boyan Hu[1], Rashi Singhal[2], Yatrik Shah[1]

[1]University of Michigan, Ann Arbor, MI,[2]Odyssey Therapeutics, Ann Arbor, MI

Hypoxic stress in solid tumors promotes cancer cell growth and survival and confers therapeutic resistance. Hypoxia inducible factors (HIFs) are transcription factors that help cells adapt to hypoxic stress. HIFs are often overexpressed in tumors and are associated with worse clinical outcomes. HIF inhibition is a novel therapeutic strategy and belzutifan is a first-in-class HIF-2α inhibitor. Belzutifan has recently been approved for metastatic renal cell carcinoma treatment. However, despite studies showing that HIF-2α is a critical regulator of colorectal carcinoma, belzutifan is not effective in pre-clinical studies of colorectal carcinoma (CRC) growth. HIF-2α inhibition with a small molecule inhibitor, belzutifan, does not affect proliferation of CRC cells (HCT116, SW480) under normoxic or hypoxic conditions. The primary objective of this study was to identify metabolic vulnerabilities in CRC caused by HIF-2α inhibition that can be synergistically targeted to increase cancer cell death. We first performed an unbiased CRISPR screen and identified the cholesterol biosynthesis pathway as a potential target. Inhibiting the cholesterol biosynthesis pathway with HMG-CoA reductase inhibitors, statins, potentiates cell death and reduces in vitro proliferation of HCT116 and SW480 cells when combined with HIF-2α genetic knockdown or HIF-2α inhibition. This effect is dose-dependent in nature and is preserved across the statins class as tested with pitavastatin, simvastatin, and atorvastatin. Planned future studies include in vivo experimentation with autochthonous mouse models of CRC to assess how combination treatment with statin and HIF-2α inhibition affects tumor progression. Ultimately, statins are a widely used, safe, and affordable treatment for atherosclerosis and our data shows that combining statins with belzutifan may be a synergistic therapeutic strategy for CRC.

#1500 Inhibition of tumor hypoxia response with synthetic transcriptional repressors.

Long Chi Nguyen, Zeyu Qiao, Dongbo Yang, Christopher Dann, Deborah Thomas, Madeline Henn, Andrea Valdespino, Colin Swenson, Scott Oakes, Marsha Rich Rosner, Raymond Moellering

University of Chicago, Chicago, IL

Hypoxia, a hallmark of aggressive solid tumors, promotes metastasis and therapy resistance by activating factors such as hypoxia-inducible factors (HIFs) that support cellular adaptation and tumorigenesis. Under hypoxic stress, transcription factors like HIFs and X-box-binding protein 1 (XBP1) are activated and bind to unfolded protein response element (UPRE) and hypoxia response element (HRE) motifs, regulating oncogenic gene expression. We identified that the core HRE motif (5'-ACGTG-3') is embedded within the canonical UPRE motif (5'-TGACGTGG-3'), and prior research shows overlap in the binding patterns of HIF1α/ARNT and XBP1. We propose a novel strategy to target the tumor hypoxia response by using fully synthetic transcriptional repressor (STR) peptides that mimic the basic leucine zipper DNA-binding domain of XBP1. These peptides effectively bind to UPRE and HRE DNA elements. Our lead peptide, STR22, inhibits the DNA binding of both XBP1 and HIF1A, resulting in global suppression of the hypoxia response transcriptional program in vitro and in triple-negative breast cancer (TNBC) tumor models. STR22 has shown no observable toxicity with either intratumoral or intravenous administration and significantly inhibits tumor growth and metastasis in TNBC-bearing mice. Our findings validate STRs as a safe and promising approach for targeting DNA motifs, including tumor hypoxia response elements, through direct DNA binding competition with oncogenic transcription factors. This strategy offers potential to outperform single-factor therapies, presenting a novel option for clinical applications.

#1501 Ras-dependent activation of BMAL2 regulates hypoxic metabolism in pancreatic cancer.

A. Curiel Garcia[1], C. H. Maurer[2], C. Castillo[3], R. M. Schmid[2], P. Laise[1], L. Tomassoni[1], C. Palermo[1], S. Sastra[1], A. Andren[3], L. Zhang[3], T. Y. S. Le Large[4], I. Sagalovsky[1], D. R. Ross[1], V. Rosario[1], K. Lu[5], E. Ferraiuolo[5], N. Spinosa[5], W. Wong[1], K. Shaw[6], J. A. Chabot[6], J. Genkinger[1], H. Hibshoosh[1], G. A. Manji[1], A. C. Iuga[7], M. A. Badgley[1], K. C. Johnson[5], A. Califano[1], Y. M. Shah[3], C. A. Lyssiotis[3], K. P. Olive[1];

[1]Columbia University Irving Medical Center, New York, NY, [2]Technical University of Munich, Munich, Germany, [3]University of Michigan, Ann Arbor, MI, [4]Dijklander Ziekenhuis Location Hoorn, Hoorn, Netherlands, [5]University of New Hampshire, Manchester, NH, [6]Columbia University, New York, NY, [7]University of North Carolina at Chapel Hill, Chapel Hill, NC

To identify novel drivers of pancreatic ductal adenocarcinoma (PDAC) malignancy, we employed a systems biology approach called regulatory network analysis, which accurately infers the activity of transcription factors and other regulatory proteins based on the integrated expression of their positive and negative target genes. This highly validated approach enables the identification of the most hyper-activated and hyper-repressed regulatory proteins (“master regulators”) that drive phenotypic distinctions. We applied this technique to a set of 200 laser capture microdissected human PDAC samples as well as 45 low-grade precursors for which we had matched histopathological, clinical, and epidemiological annotation. We identified the master regulators associated with 4 malignancy phenotypes: tumor initiation, tumor progression, survival post resection, and association with KRAS activity. Integrating across these phenotypes, the top master regulator was BMAL2, a member of the PAS family. Although BMAL2 is classically associated to the circadian rhythm, gene set enrichment analysis highlighted a potential role in hypoxia response, a key phenotype in PDAC evolution. We previously demonstrated that PDAC in humans and in the genetically engineered “KPC” mouse model is hypovascularized and hypoperfused, and we have since shown that KPC tumors are profoundly hypoxic. Sequence conservation analysis of BMAL2 demonstrated close analogy to hypoxia-related genes, its activity is induced in response to it and inhibited following treatment with multiple RAF, MEK, and ERK inhibitors, validating its computationally inferred association with RAS activity. Strikingly, knockout of BMAL2 in human PDAC cells led to defects in viability and invasion in the setting of hypoxia. Metabolically, loss of BMAL2 impairs the ability to induce glycolysis - key metabolic pathway unregulated during hypoxia response - upon exposure to severe hypoxia. Surprisingly, knockout of BMAL2 in multiple PDAC cell lines led to a complete loss of HIF1A stabilization in response to hypoxia. By contrast, HIF2A was further upregulated under hypoxia in the setting BMAL2 loss. Additionally, metabolomic studies showed an overrepresentation of glycolysis metabolites and related metabolic pathways in hypoxic control conditions but to a lesser degree in BMAL2 knockout cells. Finally, preliminary *in vivo* experiments in cell line derived xenograft models displayed lower tumor growth rates in BMAL2 knock out cells. Together, our data indicates that BMAL2 loss blunts hypoxia response providing also a potential answer to the long-standing question of how these proteins are differentially regulated. Certainly, a connection between RAS signaling and glycolysis has long been apparent, but our findings provide a novel mechanistic understanding of the relationship between RAS and hypoxic metabolism.

#1502 Hypoxia-driven genomic instability and EcDNA formation in lung cancer: insights from TRACERx.

Natasha Sharma[1], Chris Bailey[2], Corentin Richard[1], Jeanette Kittle[1], Charles Swanton[2], Mariam Jamal-Hanjani[1], Nnennaya Kanu[1]

[1]University College London (UCL), London, United Kingdom,[2]The Francis Crick Institute, London, United Kingdom

Tumors exhibit dynamic oxygen gradients, leading to hypoxia, which is linked to poor prognosis and genomic instability. The molecular features of hypoxia and their role in lung cancer outcomes remain unclear. Hypoxia has been linked to the forma on of fragile sites, resulting in chromosomal instability. Recent research has highlighted the generation of extrachromosomal DNA (EcDNA) because breaks. Genes amplified as EcDNA exist acentric circular structures capable driving rapid and significant genomic copy number amplifications. Inherent instability oncogene amplification on EcDNA provides tumor cells with a selective advantage adapting to changing environments. Thus, hypothesize that formation hypoxic tumors provide survivein unfavorable conditions like hypoxia contributes their poor prognosis. This study aims investigate impact disease outcomes formation. Furthermore, can act pressure, we examined its effects mutational burden, positive selection mutations, mutational signatures associated with hypoxia lung TRACERx cohort. TRACERx is multicenter prospective study collects samples from various regions of primary for high-depth next-generation sequencing. Analyzed bulk RNA sequencing data establish levels examine and EcDNA detection. Our preliminary findings reveal variability within between primary tumors, indicating inter- intratumoral hypoxia heterogeneity. High status correlates poorer overall disease-free survival in LUAD, but not LUSC. Additionally, presence significantly associated necrosis. Hypoxia increased circulating tumor (ctDNA) shedding ($p < 0.05$) and elevated whole-genome index (wgii) scores. High-hypoxia exhibit alterations, tumor suppressor b2m, increased expression immunosuppressive chemokines (cxcl8, ccl8, cxcl10). Furthermore, extrachromosomal was detected both LUAD average" hypoxia scores were -0.006 EcDNA-positive compared -0.248 EcDNA-negative samples, highlighting prevalence tumors. In conclusion, shows existence heterogeneity of hypoxia within cohort association clinical outcomes. We highlight link hypoxia, detection, the increased expression contributing a tumor microenvironment.

#1503 Elucidating the role of transcription coregulator MED15 in cancer cell oxidative stress response and proliferation.

Chiaki Shuzenji[1], Xuanjin Cheng[2], Stefan Taubert[1]

[1]Medical Genetics, University of British Columbia Faculty of Medicine, Vancouver, BC, Canada,[2]Canada's Michael Smith Genome Sciences Centre (GSC), Vancouver, BC, Canada

Hyperproliferation is a hallmark of cancer that leads to increased ROS levels, leading to oxidative stress, which causes DNA damage and reinforces cell proliferation. To grow in these conditions, cancer cells activate cellular pathways to rewire the transcriptome to promote oxidative stress resistance. Mapping these response pathways can lead to potential new drugs. Mediator complex subunit MED15 is upregulated in many cancer types and confers oxidative stress resistance in lower animals. To test if MED15 promotes oxidative stress resistance in cancer, we generated three MED15 knockout A549 lung adenocarcinoma cells. Transcriptome, real-time quantitative PCR, and Western Blot analysis showed that MED15 loss downregulates oxidative stress response genes, both at baselines and after stress-inducing compounds. Furthermore, the MED15 knockouts were observed to proliferate slower, so I performed cell cycle analysis using flow cytometry which showed that MED15 knockouts arrest cell cycle more after serum starvation compared to their wild-type counterparts. In sum, our data indicate that MED15 is required for normal cancer cell stress resistance and growth. This has clinical potential, as MED15 can be targeted by small molecules, making it a promising drug target.

#1504 Harnessing the power of mangifera indica: Activation of Nrf2 by leaf-derived oxylipins.

Atif Ali Khan Khalil, Min Seok Woo, **Jung-Hwan Kim**

Gyeongsang National Univ., Jinju, Korea, Republic of

Mangifera indica L., a member of the Anacardiaceae family, is grown all over the world and is known for its therapeutic properties because of its abundance of bioactive chemicals. In the current study, we investigated the effect of mango leaf extract on oxidative stress in HeLa cells. At a concentration of 100 µg/mL, the n-hexane fraction (MLHx) significantly increased antioxidant response element (ARE)-luciferase activity, surpassing other fractions. Furthermore, via raising nuclear NRF2 levels, MLHx enhanced the HO-1 mRNA expression. Increased phosphorylation of ERK1/2 and stability of NRF2 are the fundamental mechanisms. Through bioactivity-guided isolation, six oxylipins were identified: 13(R)-hydroxy-octadeca-(9Z,11E,15Z)-trienoic acid (C-1), 9(R)-hydroxy-octadeca-(10E,12Z,15Z)-trienoic acid (C-2), 13(R)-hydroxy-(9Z,11E)-octadecadienoic acid (C-3), 9(R)-hydroxy-(10E,12Z)-octadecadienoic acid (C-4), 9-oxo-(10E,12E)-octadecadienoic acid (C-5), and 9-oxo-(10E,12Z)-octadecadienoic acid (C-6). Their structures were confirmed by advance spectroscopic methods, such as ^{1}H NMR and MS. Additionally, the structures of compounds C-7 (9-oxo-(10E,12Z,15Z)-octadecatrienoic acid) and C-8 (13-oxo-(9E,11E)-octadecadienoic acid) were characterized via LC-MS/MS mass fragmentation. This work is the first to disclose the isolation of components 1-6 from *M. indica*. In HeLa cells, compounds 3, 5, and 6 showed significant, dose-dependent stimulation of ARE-luciferase activity when tested for their impact on NRF2 activity

#1505 E-cigarette aerosols increase NRF2 expression and enhance cancer stemness in human oral epithelial cells.

Vengatesh Ganapathy[1], Jimmy Manyanga[1], Gautham Chengizkhan[1], Mayilvanan Chinnaiyan[1], Balaji Sadhasivam[2], Ilangovan Ramachandran[3], David A. Rubenstein[4], Lurdes Queimado[1]

[1]Departments of Otolaryngology-Head and Neck Surgery, The University of Oklahoma Health Sciences Center, Oklahoma City, OK,[2]Occupational and Environmental Health, The University of Oklahoma Health Sciences Center, Oklahoma City, OK,[3]Department of Endocrinology, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Chennai, India,[4]Biomedical Engineering, Stony Brook University, Stony Brook, NY

Background: The popularity of e-cigarettes has surged among cigarette and non-cigarette users, in part due to their promotion as a safer choice compared to traditional smoking. Yet, the true health implications of vaping are still uncertain and need to be explored. E-cigarette aerosols contain harmful substances that can cause oxidative stress. We and others have reported that exposure to e-cigarette aerosols increases reactive oxygen species (ROS) and reduces total antioxidant capacity, leading to a significant increase in DNA damage in oral epithelial cells. This raises important questions about the long-term health effects. Nuclear factor erythroid 2-related factor 2 (NRF2), the master regulator of antioxidant signaling, is crucial for managing oxidative stress and regulating cell stemness. In this study, we examined how exposure of oral epithelial cells to e-cigarette aerosols affect the expression of NRF2 and its downstream targets, which play a critical role in stem cell regulation.
Methods: Human oral epithelial cancer (UM-SCC-1) cells were treated with aerosol extracts of e-cigarettes (tobacco flavor) from two brands, every other day for two weeks at 30 ng/ml nicotine. Standard combustible tobacco extracts were used as a positive control. Changes in gene and protein expression were assessed by qRT-PCR and western blotting analysis, respectively. The stemness of cancer cells was evaluated by spheroid assay, and the $ALDH^{+ve}$ cancer stem cell population was measured by the ALDEFLUOR assay. Data analysis was performed using Student's t-test.
Results: Exposure to e-cigarette aerosol extracts significantly increased the mRNA and proteins levels of NRF2, SOD2, CAT, and HMOX1, along with a decrease in KEAP1 protein. We observed enhanced spheroid formation in oral epithelial cells exposed to e-cigarette extracts. Notably, a significant rise in the cancer stem cell population was observed in oral cancer cells exposed to e-cigarette aerosols. Furthermore, we observed a marked increase in the expression levels of specific mRNAs related to stemness factors (e.g., *BMI1*, *OCT4*), which are crucial for maintaining stem cells.
Conclusion: Our findings indicate that exposure to e-cigarette aerosols significantly activates the NRF2 signaling pathway and promotes stemness of oral epithelial cells. The increased expression of NRF2 and its downstream targets suggests an enhanced response to oxidative stress. Additionally, the upregulation of stemness markers highlights the potential of e-cigarette aerosols to enhance cancer stem cell characteristics and tumor progression. These results raise concerns about the role of e-cigarette aerosols in oral carcinogenesis and imply that the perceived safety of e-cigarettes may be misleading, thus warranting further investigation into their long-term effects on human health.
Funding: TSET HPRC (Ganapathy), NIH/NCI (R01CA242168, Queimado).

#1506 Protein acyl-protein thioesterases affect redox homeostasis and ROS signaling through peroxiredoxin.

Tian Qiu, Saara-Anne Azizi, Shubhashree Pani, Bryan C. Dickinson

Department of Chemistry, University of Chicago, Chicago, IL

Peroxiredoxins (PRDXs) are among the most abundant antioxidants and are widely distributed in cancers. They are associated with tumor cell resistance to cell death, such as apoptosis and ferroptosis. Post-translational modifications of PRDX play a critical role in modulating its activity and tuning the balance between reactive oxygen species (ROS) signaling and stress. We previously discovered that mitochondrial localized PRDX3 and PRDX5 are *S*-acylated, with dynamic PRDX5 active site cysteine acylation regulating mitochondrial redox homeostasis. In this investigation, we found that all the PRDX family proteins are subject to *S*-acylation at the nucleophilic cysteine residue in their active site, and that PRDX *S*-acylation responds dynamically to ROS levels in the cell. The PRDX *S*-acylation level decreases under acute ROS stress, and increases under physiological, signaling levels of H_2O_2. Using activity-based fluorescent imaging with DPP-Red, a red-shifted fluorescent indicator for acyl-protein thioesterase (APT) activity, we also discovered that ROS-stress via exogenous H_2O_2 activates both the cytosolic and mitochondrial APTs, whereas EGF-stimulated endogenous H_2O_2 deactivates the cytosolic APTs. These results indicate that APTs help tune H_2O_2 signal transduction and ROS protection through PRDX *S*-deacylation.

#1507 Oxidative stress increases the aggressiveness of colorectal cancer by activating the antioxidant transmembrane chloride intracellular channel 1 (tmCLIC1).

Francesca Cianci[1], Guido Rey[1], Carlotta Tacconi[2], Luca Palloni[1], Matteo Ranucci[3], Giulia Bertolini[4], Luca Roz[4], Alessandro Fantin[1], Michele Mazzanti[1]

[1]University of Milan, Milan, Italy,[2]Libera Universita Vita-Salute San Raffaele, Milan, Italy,[3]Universitaat of Zurich, Zurich, Switzerland,[4]Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy

Colorectal cancer is the third leading cause of death in developed country, especially in the presence of metastases. Metastatic cancer cells depend on antioxidants like glutathione (GSH) to tackle oxidative stress as they adapt to distinctive environments different from those of the primary tumor. The mechanisms that cause oxidative stress in cancer cells are poorly understood, but it was recently discovered that the Chloride Intracellular Channel 1 (CLIC1) protein supports tumor cell Reactive Species of Oxigen (ROS) overproduction. CLIC1 is a ubiquitous protein from the CLICs family, which is unique in its ability to be metamorphic. In normal cells, CLIC1 protein is expressed as a cytosolic form and binds antioxidant glutathione (GSH). Under the chronic stress typical of cancer cells, CLIC1 undergoes a conformational change, translocating to the plasma membrane (tmCLIC1). This transition releases GSH into the cytoplasm while tmCLIC1 facilitates reactive oxygen species (ROS) overproduction, fostering an aggressive cancer phenotype. Studies reveal a contradiction where cancer cells simultaneously exhibit high levels of both ROS and GSH, which are usually inversely related. In this work, we demonstrate that this paradox is linked to tmCLIC1 activity, where GSH release acts as a defense mechanism, while tmCLIC1 overexpression amplifies ROS production, driving cancer cell proliferation and metastasis. Blocking tmCLIC1 activity has shown a reduction of proliferation, migration, and invasion of metastatic colorectal cancer cells (CRC). The study also demonstrated that forcing non-metastatic CRC cells (SW480) to release GSH using the antioxidant N-Acetyl Cysteine (NAC) enriched cytoplasmic GSH and increased tmCLIC1 expression. This effect, however, was absent in cells where CLIC1 was silenced, proving that GSH release depends on tmCLIC1 activity. These cells were converted from non-aggressive cell lines to metastatic ones in vitro and in vivo. These findings challenge the traditional view of antioxidants in cancer treatment, suggesting that tmCLIC1 inhibition could serve as a novel therapeutic strategy against metastatic colorectal cancer, addressing a pressing societal health concern.

#1508 Therapy resistant cancer cells containing stress granules display signatures of oxidative stress.

Louis T. A. Rolle, Luke V. Loftus, Kenneth J. Pienta, Sarah R. Amend

Urology, Johns Hopkins University School of Medicine, Baltimore, MD

Understanding and overcoming therapy resistance in cancer is crucial, as it remains a major driver of lethality. We have shown that following chemotherapy, surviving cancer cells repeatedly skip mitosis, leading to increased genomic content concomitant with an increase in cell size. This survival mechanism has been observed in multiple tumor types and in response to multiple therapy classes, suggesting a convergent phenotype. We hypothesize that this surviving cell phenotype is the actuator of therapy resistance observed in patients with cancer recurrence.

It is widely known that chemotherapies elevate levels of reactive oxygen species (ROS) by indirectly disrupting the electron transport chain, leading to the generation of superoxides. When ROS levels exceed cellular antioxidant capacities, cells experience oxidative stress whereby ROS molecules directly damage important macromolecules, potentially leading to cell death. Using live cell imaging with DCFDA and CellROX dyes, we found that the cells surviving even long after therapy release contain elevated levels of ROS as compared to untreated controls across two cell lines of different tumor types (PC3 prostate cancer and MDA-MB-231 breast cancer) and following treatment with two classes of chemotherapy (cisplatin and docetaxel). In single-cell RNA sequencing datasets, we further find an enrichment for downstream targets of NRF2, a major transcription factor that regulates the response to oxidative stress in cells that survive therapy. This suggests that the cells that survive chemotherapy continue to experience oxidative stress even after therapy is removed and respond by initiating defensive mechanisms to promote survival.

Stress granule formation is a common cellular response to oxidative stress that form immediately following a translational arrest. Stress granules are cytoplasmic aggregates of RNA bound by proteins, such as G3BP1, which form a stable core and recruit binding partners to form a mature granule. Stress granules are transient and offer cells the capacity to resume translation rapidly following recovery from stress. Alternatively, autophagy of stress granules during recovery offers cells a rich pool of biomolecules upon relief from stress.

In both of our cell line models, we find that while stress granules are virtually absent in untreated cells, up to 18% of surviving cells induce stress granule formation, depending on cell type and chemotherapy. We further demonstrated that surviving cells that containing stress granule have enriched nuclear NRF2 levels, suggesting a positive relationship between stress granule formation and cellular response to ROS. Based on these data, we hypothesize that surviving cells experiencing oxidative stress induce stress granule formation. Future work aims to show the pro-survival role of stress granule formation through time-lapse imaging of cells expressing a G3BP1-GFP construct.

#1509 Discovery of choroid plexus targeted gene therapy to protect cancer chemotherapy-related cognitive impairment.

Ahram Jang[1], Boryana Petrova[2], Taek-Chin Cheong[2], Miriam E. Zawadzki[2], Jill K. Jones[2], Andrew J. Culhane[2], Frederick B. Shipley[2], Roberto Chiarle[2], Eric T. Wong[3], Naama Kanarek[2], Maria K. Lehtinen[2]

[1]Division of Endocrinology, Diabetes and Metabolism, Beth Israel Deaconess Medical Center, Boston, MA,[2]Department of Pathology, Boston Children's Hospital, Boston, MA,[3]Brain Tumor Center & Neuro-Oncology Unit, Beth Israel Deaconess Medical Center, Boston, MA

Chemotherapy-induced neurotoxicity in non-cancerous cells can cause lifelong complications including cognitive impairment, also known as chemobrain, which is a devastating side effect for cancer patients and survivors. One mechanism by which chemotherapy damages healthy cells is through oxidative stress. The choroid plexus (ChP)-cerebrospinal fluid (CSF) system delivers important health-promoting factors, such as the antioxidant superoxide dismutase 3 (SOD3), to increase survival in response to oxidative stress, while cell-intrinsic pathways protect neurons from oxidative damage. However, the consequences of chemotherapy on ChP and CSF composition are unknown. Here, we show that the chemotherapeutic drug methotrexate (MTX) induces a global metabolic change in the brain, which is reflected in the CSF. Leveraging metabolomics, we found that MTX caused oxidative stress in the CSF of MTX-treated mice, including a reduction in important antioxidant metabolites. The ChP is the main source of secreted SOD3 in the brain. Following MTX treatment in mice, ChP-SOD3 expression was diminished as well as CSF-SOD3 was decreased. Moreover, overall CSF-antioxidant activity was compromised, indicating that cancer chemotherapy damaged the ChP, resulting in an impoverished CSF with reduced antioxidant capability. MTX-induced oxidative stress may also be tracked to tissues in contact with CSF that are functionally vulnerable to the toxicity of cancer chemotherapy. For example, glutathione peroxidase (GPx) enzyme activity was decreased in the hippocampus, where lipid peroxidation was elevated following MTX treatment. Analyses of CSF samples from primary and secondary central nervous system (CNS) lymphoma patients supported our hypothesis that MTX treatment is associated with diminished antioxidant levels in patient CSF. We employed an intraventricular adeno-associated viral (AAV) gene delivery technique to augment SOD3 production by the ChP. ChP-SOD3 gene therapy inhibited oxidative stress indicators in CSF and mitigated MTX-induced toxicity in the adjacent hippocampus. Furthermore, this gene therapy prevented MTX-induced anxiety as well as short-term learning and memory deficits. Collectively, our findings on AAV-based gene therapy via the ChP-CSF system may represent an underutilized approach for widespread and long-term delivery of health-promoting factors to the brain.

#1510 Glutathione-oxidation of ovarian tumor deubiquitinase-1 promotes lung adenocarcinoma survival through stabilizing system x_c^-.

Reem Aboushousha[1], Jos van der Velden[1], Nicholas Hamilton[2], Cuixia Erickson[1], David J. Seward[1], Jianing Li[3], Yvonne M. W. Janssen-Heininger[1]

[1]Pathology and Laboratory Medicine, University of Vermont, Burlington, VT,[2]Chemistry, University of Vermont, Burlington, VT,[3]Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN

The small thiol antioxidant glutathione (GSH) is critical in maintaining redox homeostasis that is essential in combating oxidative stress. We and others have reported increases of GSH in KRAS-mutant lung adenocarcinoma (LUAD). However, little is known about GSH-dependent protein oxidation (S-glutathionylation- PSSG) in LUAD development and progression. We recently discovered that glutaredoxin (GLRX), the enzyme that reverses PSSG, is decreased in human LUAD, and that $Kras^{G12D}$-induced lung tumorigenesis is enhanced in mice lacking *Glrx*. To elucidate the link between PSSG and GSH levels we conducted a redox proteomics screen and identified ovarian tumor deubiquitinase-1 (OTUB1) as a glutathionylation target. We determined that OTUB1 glutathionylation at Cys23 and Cys204 is a regulator of GSH in LUAD cell lines and organoids. Mechanistically, glutathionylation of OTUB1, specifically at Cys23, promoted interaction with the E2-conjugating enzyme UBCH5A, leading to diminished ubiquitination and proteasomal degradation of the active subunit of system x_C^-, SLC7A11, thereby augmenting GSH. The cystine-glutamate antiporter, System x_C^-, has been linked to aggressive tumors with worse survival. The use of a specific inhibitor of OTUB1-SSG, led to decreased SLC7A11 and GSH levels in LUAD cell lines. These findings provide an intricate link between S-glutathionylation of OTUB1, GSH and system x_C^- and provide a mechanism by which LUAD cells combat oxidative stress. Avenues to interfere with OTUB1-SSG may provide a promising therapeutic approach to overcome LUAD progression and resistance. Funded by: R01 CA273238

#1511 Cancer adaptation to acidic tumor microenvironment.

Manami Hasegawa, Keisuke Maede, Miyuki Nishida, Xu Bo, Sho Aki, Rika Tsuchida, Tsuyoshi Osawa

RCAST, the University of Tokyo, Tokyo, Japan

Cancer cells exhibit a characteristic metabolic pattern known as the Warburg effect, which upregulates glycolysis even in aerobic environments. As a result, cancer cells are exposed to an acidic environment due to enhanced excretion of proton and lactate. We have previously reported that this acidic tumor microenvironment induces the activation of the cholesterol biosynthesis pathway and the accumulation of N1-acetylspermidine. Although the acidic tumor microenvironment is known to suppress cancer cell proliferation, the survival strategies employed by cancer cells under such harsh conditions remain unclear. This study aims to elucidate the cell death pathways triggered by acidic pH using an environment even more acidic than physiological conditions and to reveal how cancer cells evade such cell death under physiological conditions.

Live imaging was performed by exposing the cervical cancer cell line HeLa to an acidic environment (pH 5.6), which confirmed that cell death with membrane rapture was induced. Furthermore, in the pancreatic cancer cell line PANC1, phosphorylation of RIP1 and MLKL was observed, and cell death was suppressed by treatment with Nec-1, an inhibitor of RIP1. These findings identified Necroptosis induced under pH5.6.In addition, PANC1 cells that transitioned to a floating state under acidic conditions (pH 5.6-6.8) regained their adhesion and proliferation abilities when cultured under normal tissue pH conditions (pH 7.4). These findings indicate that some of the floating cells under acidic conditions avoid cell death and adopt certain survival strategies. Then, RNA-seq was performed on cells fractionated into those in suspension and those remaining adherent under pH6.8. Pathways related to the acquisition of stemness such as HDACs deacetylation pathway and PRC2 methylation pathway were observed to be active in the floating cells. Furthermore, the upregulation of EMT transcription factors was also confirmed.

Furthermore, we cultured PANC1 cells under pH6.8, using a genome-wide CRISPR-Cas9 knockout screening system over a 30-day time course and identified key genes essential for the acquisition of tolerance to acidic tumor microenvironment.

We elucidated a mechanism in pancreatic cancer cells whereby Necroptosis is avoided in physiologically acidic environments. This cell death-avoidance pathways under acidic conditions could potentially be applied to the development of novel therapeutic strategies that induce cell death in pancreatic cancer cells, which are highly influenced by the tumor microenvironment, particularly in acidic conditions.

#1512 EGR1 regulates phenotypic plasticity to promote breast cancer progression upon metabolic stress through elaborating HERPUD1-mediated unfolded protein response.

Yi-Zhen Wu[1], Ge-Jyun Liu[1], Shan-Ting Lei[1], Wei-Huan Zeng[1], Mei-Ju Juan[1], Yu-Shu Liu[1], Yong-Han Su[1], David K. Ann[2], Ching-Ying Kuo[1]

[1]Department of Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University, Taipei, Taiwan,[2]Diabetes and Metabolism Research Institute, City of Hope, Duarte, CA

Uncontrolled proliferation is one of the most important hallmarks of cancer. Consequently, cancer cells exhibit a high demand for oxygen and nutrients from the tumor microenvironment. Nonetheless, cancer cells in the core region of a solid tumor often undergo nutrient and oxygen deficiency owing to the restricted vascularization, ultimately leading to the occurrence of metabolic stress. To survive under stress, series of stress response will be activated, providing opportunities for cancer cells to confront unfavorable circumstances and further developing phenotypic plasticity. Without altering genetic sequence, phenotypic plasticity enables cancer cells to adjust their behavior to survive from stress. This critical ability of cancer cells is orchestrated by multiple stress response under different stress stimuli, but its regulatory mechanism upon metabolic stress still requires further investigation. We identified a transcription factor EGR1 that is rapidly induced upon metabolic stress in breast cancer (BC). Known as an immediate early gene, EGR1 can be upregulated by multiple types of stimulation and further regulates tumor progression. Through cell viability and anoikis assay, we found that EGR1 could promote BC cell survival upon metabolic stress under both attach and detach condition. Furthermore, through xenograft tumor model, we noticed the inhibitory effect of EGR1 on tumor growth was more obvious in the late phase of tumor development, when metabolic stress mostly occurred. These results demonstrated the role of EGR1 in regulating phenotypic plasticity to promote BC cell growth upon metabolic stress. To identified genes regulated by EGR1 upon metabolic stress, EGR1-based ChIP-Seq was performed, and further compared with glucose deprivation RNA-Seq data to identify overlapping genes. Being the most upregulated overlapping gene upon glucose deprivation, *HERPUD1* was chosen for further validation. HERPUD1 has been found to be important for degrading unfolded or misfolded protein in unfolded protein response (UPR). However, the role of HERPUD1 upon metabolic stress remains elusive. We found HERPUD1 expression significantly increased upon metabolic stress and was higher in EGR1 knockdown (KD) cells, suggesting EGR1 was a transcriptional suppressor upon metabolic stress. As a critical stress response to promote phenotypic plasticity, UPR was diminished in EGR1 KD cells due to higher expression of HERPUD1, potentially explaining the increased vulnerability of these cells to metabolic stress. Collectively, EGR1 was identified as a transcription factor significantly upregulated upon metabolic stress. By suppressing HERPUD1 expression, EGR1 maintained UPR at an optimal level, which promoted the development of phenotypic plasticity of BC cells and assisted cells to survive and thrive from metabolic stress.

#1513 Morphological analysis of compressed nuclei reveals conserved irregularities of different cell types.

Julian Najera, Bianca Batista, Triston Acher, Meenal Datta

University of Notre Dame, Notre Dame, IN

Solid tumors such as mammary and brain exert compressive forces on the surrounding host tissue over the course of tumor expansion. However, how these forces impact neighboring peritumoral cells remains poorly understood. In this study, we used an *in vitro* transwell compression device to evaluate the effect of compression on mouse fibroblasts and human astrocytes, the most abundant stromal cell types in the breast and brain peritumor, respectively. Strikingly, we noticed that compression altered nuclear morphology in the two cell types. We then utilized an unsupervised machine-learning clustering algorithm to determine the relative abundance of various nuclear geometries present in both groups. We found that compressed nuclei predominantly exhibited diverse morphologies, including circular, elongated, and kidney bean-like shapes. In contrast, uncompressed nuclei were morphologically homogeneous, with most maintaining a canonical circular or elliptical shape. We validated these observations using ImageJ, showing that compressed nuclei possessed significantly lower circularity, roundness, and solidity, while displaying significantly higher eccentricity compared to uncompressed nuclei. Because fibroblasts and astrocytes share analogous roles, we sought to determine whether these observed differences were recapitulated in functionally distinct cell types such as immune cells. Surprisingly, murine macrophages exhibited the same predominant shape modes and similar quantitative differences in shape factors seen in the stromal cells. Furthermore, we performed nuclear morphometric analysis to numerically characterize the relative irregularity of compressed nuclei in astrocytes based on the calculation of a nuclear irregularity index (NII). Our analysis revealed that the NII of compressed nuclei is significantly higher than their uncompressed counterparts while the nuclear area in both groups was similar. Among 7 morphological categories, uncompressed nuclei were classified as “normal” while compressed nuclei were classified as “irregular.” These findings suggest that compressed nuclei may either be undergoing mitotic catastrophe or another nuclear damaging event rather than acquire aberrant morphologies due to mechanically induced senescence or apoptosis. In fact, we were able to demonstrate in preliminary live-dead cell imaging experiments that these morphologies are captured in compressed live cells. Altogether, these results indicate that compression appears to induce morphological changes that are conserved in distinct cell types.

#1514 CRISPRi screening reveals RNA editing as a major determinant of platinum sensitivity in high-grade serous ovarian cancer.

Yi Wen Kong, Erika D. Handly, Robin A. Lu, Molly A. Bird, Jack W. Schaeffer, Samkyu P. Yaffe, Michael B. Yaffe

Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA

Platinum-based chemotherapeutic drugs induce intra- and interstrand purine crosslinks causing DNA damage and tumor cell death. Although these drugs are widely used as front-line treatment for multiple human cancers, particularly high-grade serous ovarian cancer (HGSOC), platinum resistance and tumor recurrence remains a common clinical problem. Here we report results from both a genome-wide CRISPRi screen and an RNA-binding protein-focused CRISPRi screen for modulators of sensitivity and resistance of HGSOC cells to two widely used platinum agents, cisplatin and oxaliplatin, and an microtubule-targeting taxane. Using an *in vitro* approach that mimics clinical treatment protocols, we found that cell lethality after cisplatin or oxaliplatin treatment in p53-defective ovarian cancer cells is markedly enhanced by suppressing genes in the Fanconi anemia (FA) and homologous recombination (HR) pathways, but considerably less enhanced by suppressing genes in the nucleotide excision repair (NER) pathway, despite the well-established importance of NER in reversing platinum-induced intrastrand crosslinks, particularly for cells in G1. Our findings suggest that cell death induced by platinum-DNA crosslinks results from replication stress, which can be further exacerbated by interference with error-free DNA repair mechanisms known to be particularly active during the S- and G2 phases of the cell cycle. These screens further implicated several known and novel RNA-binding proteins (RBPs) as potent sensitizers of HGSOC to both platinum agents and taxanes, including the RNA-modifying enzyme ADAR1, that catalyzes the deamination of adenosine-to-inosine in RNA, altering mRNA coding potential, modifying RNA structure, and regulating RNA localization. Additionally, ADAR1 has been implicated in controlling the integrated stress response (ISR), a signaling network that allows cells to adapt to changes in environment. These findings suggest that activation of an ADAR1-dependent ISR underlies the resistance of HGSOC cells to both platinum-based agents and anti-microtubule drugs that form the current clinical standard of care for HGSOC treatment.

#1515 GCN2 eIF2 kinase promotes polyamine metabolism and progression of prostate cancer.

Noah R. Sommers, Ricardo A. Cordova, Ronald C. Wek, Kirk A. Staschke

Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN

The Integrated Stress Response (ISR) features multiple protein kinases that each sense distinct cellular stresses, phosphorylating eIF2 to direct gene expression that serves to mitigate cell damage and restore homeostasis. Previously, we reported that the eIF2 kinase GCN2 and the ISR are constitutively active in prostate cancer (PCa) and required to maintain amino acid (AA) homeostasis to sustain tumor growth (Cordova et al., 2022, *eLife*, 11:e81083). GCN2 is induced by nutrient limitation, and basal GCN2 activation in PCa enhances the expression of transporters that ensure sufficient AAs are available for PCa proliferation. However, while genetic or pharmacological inhibition of GCN2 sharply reduces AAs and growth of PCa cells in culture and mouse models, there is minimal cell death. We hypothesized that signaling pathways function to support cell survival in GCN2-deficient PCa cells and can be targeted to promote cell death in combination therapies. Indeed, we discovered that loss of GCN2 leads to compensatory activation of p53 signaling that blocks cell growth and lowers nutrient expenditure (Cordova & Sommers et al., 2024, *Science Signaling*, 10.1126/scisignal.adp1375). In this recent report, we also carried out a targeted CRISPR-interference screen in castration-resistant 22Rv1 PCa cells with functional wild type (WT) GCN2 or in cells deleted for GCN2 (GCN2 KO). We discovered that reduced expression of ornithine decarboxylase, the rate-limiting enzyme for polyamine (PA) biosynthesis, was synthetically lethal with loss of GCN2. PAs are polycationic metabolites derived from AAs that are ubiquitous among tissues, but especially abundant in prostate tissue. Given that PA biosynthesis requires AAs and GCN2 maintains AA homeostasis in PCa, we propose that GCN2 regulates PA levels in PCa. Indeed, intracellular levels of both AAs and PAs were decreased in GCN2-inhibited LNCaP cells compared to cells treated with vehicle alone. We sought to determine whether defects in PA metabolism also contribute to the growth defect of GCN2-deficient cells. One of the best characterized functions of PAs involves PA-derived hypusination of the translation factor eIF5A, a post-translational modification of eIF5A that is required for efficient translation of mRNAs encoding polyproline tracts. Interestingly, we found that GCN2 promoted eIF5A hypusination and enhanced the expression of several genes associated with PA metabolism in 22Rv1 cells. To investigate whether GCN2-deficient cells are sensitive to defects in eIF5A hypusination, we cultured WT or GCN2 KO 22Rv1 organoids and treated them with a small molecule inhibitor of eIF5A hypusination. While inhibition of eIF5A hypusination resulted in minimal death of WT cells, there was induced death in GCN2 KO cells. Our study suggests that GCN2 maintains PAs in PCa cells and combined therapies targeting GCN2 and PA processes are an effective strategy for the treatment of PCa.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Metabolic Dependencies
Poster Session

#1519 Therapeutic targeting of phosphoglycerate mutase 1 disrupts cellular redox balance and promotes DNA damage in head and neck cancer.

Md Roman Mogal[1], Krishna Awasthi[1], Ishrat Nourin Khan[2], Andrean Simons-Burnett[1]

[1]Free Radical and Radiation Biology Program, Department of Radiation Oncology, University of Iowa, Iowa City, IA,[2]Interdisciplinary Graduate Program in Human Toxicology, University of Iowa, Iowa City, IA

Head and neck squamous cell carcinomas (HNSCCs) are extremely aggressive cancers that exhibit notable metabolic reprogramming and resistance to therapy. Phosphoglycerate mutase 1 (PGAM1), a glycolytic enzyme, has emerged as an important regulator of cancer cell metabolism and is highly expressed in HNSCCs compared to normal (untransformed) tissues. PGAM1 catalyzes the conversion of 3-phosphoglycerate (3PG) into 2-phosphoglycerate (2PG) which is a highly important step in glycolysis due to the importance of 2PG in serine biosynthesis. In contrast, PGAM1 inhibition results in decreased 2PG production and 3PG accumulation which suppresses both glycolysis and serine metabolism. More importantly, the accumulation of 3PG downregulates the pentose phosphate pathway (PPP) via deceased glucose-6-phosphate dehydrogenase (G6PD) which is necessary for optimal NADP+/NADPH production and cellular redox balance. Given the critical function of PGAM1, this enzyme may be promising as a potential target for treating HNSCC. Here we test if PGAM1 inhibition affects cell viability, NADP+/NADPH ratios, and DNA integrity in HNSCC cells in vitro. Gene expression levels of PGAM1 were compared between control and HNSCC patient groups, and survival analysis based on PGAM1 expression was conducted utilizing data from the TCGA database. PGAM1 was shown to be highly overexpressed in HNSCCs relative to healthy/control patients ($P<0.001$), with its expression linked to poorer survival (HR = 1.43). In addition, the PGAM1 expression level was evaluated based on human papillomavirus (HPV) subtype and found that PGAM1 is expressed significantly higher in HPV-negative patients compared to HPV-positive. The PGAM1 inhibitor - HKB99, was used to detect changes in cell viability via MTT assays in FaDu, Cal-27 and SQ20B HPV-negative HNSCC cell lines. HKB99 over a range of 0.5 - 64 μM for 48 hours decreased cell viability in a dose-dependent manner and IC50 concentrations were established which were 4 μM, 3.4 μM and 6.4 μM for FaDu, Cal-27 and SQ20B respectively. Treatment of the 3 cell lines with HKB99 at respective IC50 doses did not affect PGAM1 mRNA expression level using PCR analyses. However, protein expression of PGAM1 using western blot was decreased in treated cell lines compared to untreated cells. Moreover, HKB99 at respective IC50 doses increased levels of γ-H2AX, a marker for DNA double-strand breaks and significantly increased NADP+/NADPH ratios. Lastly, the antioxidant N-acetylcysteine (NAC) partially rescued the cytotoxic effect of HKB99 in all 3 cell lines using MTT and clonogenic assays. These findings suggest that inhibition of PGAM1 with HKB99 in HNSCC cells increases DNA damage, disrupts redox homeostasis, and triggers cell death. Taken together, our findings indicate that PGAM1 may represent a promising therapeutic target and may potentially sensitize HNSCCs to DNA-damaging therapies.

#1520 Obesity delays melanoma resistance to BRAF/MEKi inhibitors and alters melanoma metabolism.

Mahnoush S. Shafiei, Jacob Sellers, Craig Slingluff, Allan Tsung, Russell Witt

Department of Surgery, University of Virginia School of Medicine, Charlottesville, VA

Background: Obesity is linked to more aggressive melanoma and impaired anti-tumor immunity. However, obese patients show improved survival on BRAF/MEK inhibitors. Obesity induces endoplasmic reticulum stress (ERS), and BRAF/MEK inhibitors shift metabolism from glucose to fatty acid oxidation (FAO), utilizing CPT1A for cell viability. We hypothesize that obesity-induced metabolic reprogramming causes downregulation of CPT1A, impairing melanoma cell metabolism and potentially contributing to longer tumor progression-free survival (PFS) in obese individuals.

Methods: Mice were fed either a standard diet (lean) or a high-fat diet (HFD) to induce obesity. Twelve-week-old mice were subcutaneously injected with 1×10^6 YUMMER 1.7 melanoma cells. Tumors were allowed to grow for 2 weeks, after which the mice were treated with BRAF/MEK inhibitors until tumor resistance was observed.

Results: Obese mice treated with BRAF/MEK inhibitors showed delayed tumor recurrence compared to lean mice with a median of 80 days in obese mice versus 28 days in lean mice (log-rank $p<0.0001$). Tumors from obese mice had elevated DRP1 expression ($P<0.05$) and increased ER stress markers (XBP1, IRE1A, GRP78, PERK). Metabolic reprogramming, including alterations in melanoma state, fatty acid oxidation (FAO), and glycolysis via the BRAF/MEK pathway, resulted in higher levels of CD36, ACADS, and CPT1 in lean mice compared to obese mice ($P<0.05$). Conversely, NGFR, MITF, DUSP59, HK2, and GLUT-1 levels were elevated in tumors from obese mice ($P<0.05$).

Conclusions: Our findings suggest that obesity contributes to delayed tumor progression, potentially due to alterations in endoplasmic reticulum and mitochondrial function, which disrupt the expression of key metabolic enzymes. These results underscore the potential of targeting metabolic pathways as a complementary therapeutic approach in melanoma treatment.

#1521 High-fat diet-induced tumor promotion was abolished by blocking fatty acid oxidation in PDAC.

J. Kang, **W. Ham**, H. Lee, W. Choi, J. Chun, S. Woo, S. Sim, S.-Y. Kim;
National Cancer Center - Korea, Goyang-si, Gyeonggi-do, Korea, Republic of

Purpose: Obesity is well known to promote pancreatic ductal adenocarcinoma (PDAC) tumor development. However, the direct link between obesity and cancer has not been interpreted. We found that ATP production in cancer cells depends on fatty acid oxidation (FAO). Therefore, we propose blocking FAO in cancer may stop obesity from promoting PDAC growth.

Experimental Design: To block FAO, *SLC25A20* (carnitine-acylcarnitine carrier) was knocked down in human PDAC cells, which was implanted for xenograft in mice and treated with a high-fat diet (HFD, 60% fat) or no-fat diet (NFD, calorie-balanced 0% fat). To compare cancer development, survival rates, and histopathological differences were analyzed with KPC mice (*Kras*$^{G12D/+;}$ *Trp53*$^{R172H/+;}$ *Pdx1-Cre*) and KPC/*Slc25a20*$^{+/-}$ mice treated with regular diet (RD, 18% fat).

Results: The median survival of KPC mice with HFD was decreased by 2.0 weeks compared to KPC mice with RD (regular diet). Xenografts of PDAC cells with HFD promoted tumor growth 2-fold compared to the xenograft with regular diet (RD). Xenograft with *SLC25A20* knockdown PDAC cells in NFD reduced a 40-95% of tumor growth, and xenograft with *SLC25A20* knockdown PDAC cells in HFD also resulted in a 65-95% reduction in tumor growth compared to controls.

Conclusion: Blocking FAO reversed HFD-induced tumor growth promotion in pancreatic cancer. Cancer cells take advantage of HFD for ATP production by FAO. IGF or inflammation by HFD is no longer a direct mechanism to understand how HFD promotes tumor development.

Funding source: This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Science and ICT to SK (NRF-2019M3A9G1104345) and by a grant from the National Cancer Center of Korea to SHS (NCC 24H1210-2).

#1522 High-throughput screening identifies HSP-90 inhibitor for pancreatic cancer therapy.

Chiamaka Joy Ezeh, Darren Binder, Rohan Binder, Zeribe C. Nwosu

Molecular Biology and Genetics, Cornell University, Ithaca, NY

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive and lethal malignancies. Currently, it has an overall five-year survival rate of about 13%. This poor prognosis is attributed to the disease's late-stage diagnosis, rapid progression, and high resistance to available therapies. PDAC tumors often exhibit metabolic plasticity and oncogenic signaling dependencies, contributing to their ability to evade treatment, sustain growth under adverse conditions, and develop resistance to the current standard of care. These characteristics highlight the urgent need to identify novel therapeutic targets. In our study, a high-throughput drug screening was conducted to identify novel compounds capable of targeting key survival mechanisms and metabolic pathways in PDAC cells. Among the tested compounds, PU-H71, a heat shock protein 90 (HSP90) inhibitor, emerged as a promising candidate. Functional assays revealed that PU-H71 effectively inhibits the growth and proliferation in a panel of PDAC cell lines. Pathway analysis revealed that PU-H71 acts by disrupting multiple metabolic pathways. Our findings identify PU-H71 as a potent therapeutic candidate for PDAC treatment.

#1523 Discovery and Validation of a novel small molecule metabolic inhibitor targeting glioblastoma growth.

Maheedhara R. Guda, Andrew J. Tsung, Swapna Asuthkar, **Kiran Velpula**

University of Illinois College of Medicine (Peoria), Peoria, IL

Glioblastoma (GBM) is an aggressive brain tumor with poor prognosis and limited treatment options. Pyruvate dehydrogenase kinase 1 (PDK1) plays a critical role in the metabolic reprogramming of GBM cells, making it an attractive therapeutic target. In this study, we investigated the efficacy of a novel PDK1 inhibitor, in inhibiting GBM progression. Using U87 glioblastoma cells, we demonstrated that the UIC novel inhibitor significantly reduced cell viability, induced G2/M cell cycle arrest, inhibited glycolysis, and promoted oxidative phosphorylation. In vitro assays revealed that the UIC novel inhibitor effectively suppressed U87 cell migration and invasion compared to dichloroacetate (DCA), another PDK1 inhibitor. Wound healing and transwell invasion assays showed that the UIC novel inhibitor reduced wound closure and cell invasion by 83%, outperforming DCA. Furthermore, the UIC novel inhibitor treatment increased reactive oxygen species (ROS) production and decreased lactate release, indicating a shift from glycolysis to oxidative metabolism. In vivo, intracranial implantation of U87 cells into mice followed by the UIC novel inhibitor treatment resulted in prolonged survival, delayed tumor-associated weight loss, and reduced tumor burden compared to vehicle controls. Hematoxylin and eosin (H&E) staining confirmed smaller tumor sizes in treated mice, while immunohistochemistry (IHC) revealed decreased expression of PDK1 and phosphorylated EGFR (pEGFR), key oncogenic markers in GBM. These findings suggest that the UIC novel inhibitor is a potent inhibitor of PDK1 with significant anti-tumor activity in GBM models, offering a promising therapeutic strategy for targeting metabolic vulnerabilities in GBM. Further clinical evaluation of the UIC novel inhibitor is warranted to explore its potential as a novel treatment option for glioblastoma patients.

#1524 The cancer specific intramembrane serine protease RHBDL2 serves as a master regulator of glutamine metabolism and chemoresistance in TNBC.

Yosuke Matsushita[1], Kazumasa Okumura[2], Masato Komatsu[2], Tetsuro Yoshimaru[1], Masaya Ono[3], Mitsunori Sasa[4], Yasuo Miyoshi[5], Toyomasa Katagiri[1]

[1]National Institutes of Biomedical Innovation, Health and Nutrition, Ibaraki, Japan,[2]Division of Genome Medicine, Inst. Advanced Med. Sci., Tokushima University, Tokushima, Japan,[3]Dept. Clin. Proteomics, Natl. Cancer Ctr. Res. Inst., Chuo-ku, Japan,[4]Dept. Surg., Tokushima Breast Care Clinic., Tokushima, Japan,[5]Dept. Breast and Endocrine Surg., Hyogo College of Medicine, Nishinomiya, Japan

Glutamine metabolism is critical in cancer, driving cell proliferation and chemoresistance by supporting anabolic processes and energy production. This dependency offers a potential for therapeutic intervention; however, attempts to inhibit glutamine transporters and related enzymes have largely failed due to tumor plasticity, which enables cancer cells to adapt and bypass metabolic blockades. Our studies focus on RHBDL2, a cancer-specific intramembrane serine protease that regulates the glutamine metabolism in triple-negative breast cancer (TNBC).RHBDL2 plays two pivotal roles in glutamine metabolism. First, it binds and stabilizes ASCT2 at the plasma membrane, ensuring efficient glutamine uptake, which is crucial for ATP production and cell proliferation. Second, highlighting RHBDL2 localizes to the endoplasmic reticulum (ER), where it modulates N-glycosylation of ASCT2 by regulating glycosyltransferase activity. This glycosylation is essential for ASCT2 trafficking and stabilization. Collectively, these functions underscore RHBDL2' pivotal roles in maintaining tumor metabolism of TNBC cells. In addition to regulating ASCT2, RHBDL2 forms complexes with other proteins and transporters involved in glutamine metabolism, enhancing cancer cells' adaptability to metabolic stress. Our studies reveal that the RHBDL2-ASCT2 complex correlates with poor prognosis in cancer patients. Moreover, RHBDL2 upregulation has been associated with increased chemoresistance in TNBC, identifying it as a critical factor in therapeutic resistance. We successfully develop the RHBDL2 polyclonal antibody, which inhibits tumor growth by reducing glutamine uptake. Building on this, we are now developing monoclonal antibodies designed to disrupt RHBDL2 function, thereby reducing glutamine uptake and restoring chemotherapy sensitivity. This approach has the potential to impair cancer metabolism, suppress tumor growth, and improve outcomes in TNBC patients.

#1525 Regulation of cellular metabolic stress by aquaporin-7 in breast cancer.

Verodia Charlestin, Chen Dai, Daniel Fulkerson, Carlos E. Arias Matus, Madeline Sheely, Man Wang, Junmin Wu, Zixuan Song, Norman J. Dovichi, Jun Li, Laurie E. Littlepage

University of Notre Dame, South Bend, IN

The reprogramming of metabolism during cancer progression supports metastasis and response to therapies. The underlying relationships between cancer metabolism, gene expression, and oncogenic driver genes have the potential to identify novel biomarkers and targets with prognostic and therapeutic value. To understand these relationships, we used a novel unbiased correlation-based network analysis to integrate metabolomics and gene expression data from breast cancer mouse models to identify driver genes and metabolic pathways of mammary cancer. This approach identified 35 metabolite and 34 gene hubs with the most network correlations. Significantly, these hubs have prognostic value and likely are integral to tumor metabolism and breast cancer. The gene hub Aquaporin-7 (human AQP7/mouse Aqp7), a water and glycerol channel, emerged as a novel regulator of breast cancer. We identified AQP7 as a negative prognostic marker of overall survival and metastasis in breast cancer patients. In vitro and in vivo experiments showed that AQP7 is required for proliferation, primary tumor progression, and lung metastasis. Metabolomics of Aqp7 knockdown mammary cancer cells and tumors further revealed significantly altered lipid levels, redox, and urea/arginine metabolism. Given the prognostic value of AQP7 with breast cancer and its involvement in glycerol transport and lipid homeostasis, we investigated the metabolic reprogramming by AQP7 and evaluated the efficacy of AQP7 inhibition as a cancer therapeutic. We find that AQP7 is a critical regulator of metabolic and signaling responses to environmental cellular stresses and of endocrine therapy efficacy in breast cancer metastasis to lung. Interestingly, in patient data, AQP7 expression is expressed higher in breast tumors that do not respond to endocrine therapy compared to tumors that do respond, which suggests that Aqp7 may be a therapeutic vulnerability in endocrine therapy resistance. We evaluated AQP7 inhibition in combination with endocrine therapy in breast cancer. Interestingly, the aquaporin inhibitor Auphen cooperated with endocrine therapy tamoxifen to reduce the viability of breast cancer cells. This suggests that Auphen treatment makes the cells more susceptible to tamoxifen. Together, this study highlights AQP7 as a potential cancer-specific therapeutic vulnerability and that AQP7 inhibition can be exploited for therapeutic benefit in overcoming endocrine therapy resistance.

#1526 An impaired ability to engage in oxidative phosphorylation as a unifying feature of renal carcinogenesis.

Ziad Bakouny[1], Sonia Boscenco[1], Ruobing Cui[1], Keunwoo Ryu[1], Ritesh R. Kotecha[1], Maria I. Carlo[1], Martin H. Voss[1], A. Ari Hakimi[1], Robert J. Motzer[1], Payam A. Gammage[2], Ed Reznik[1], Craig B. Thompson[1]

[1]Memorial Sloan Kettering Cancer Center, New York, NY,[2]Cancer Research UK Scotland Institute, Glasgow, United Kingdom

Introduction: *VHL* loss is a cardinal feature of renal cell carcinoma (RCC), specifically of the clear cell RCC (ccRCC) subtype, and leads to activation of aerobic glycolysis and suppression of oxidative phosphorylation (OxPhos). In contrast, Non-*VHL* driven forms of RCC are a heterogeneous group of tumors with few known nuclear oncogenic drivers, none of which are shared across these tumors. Recent studies have shown that mitochondrially-encoded electron transport chain (ETC) genes, responsible for OxPhos, can be mutated in a range of cancers.

Methods: Mitochondrial mutations were called using a previously published custom pipeline for variant calling (PMID: 33833465). On-target (whole genome sequencing) and off-target (whole exome and gene panel-based sequencing) reads were used. Only samples with sufficient coverage (>=5 reads across >90% of the mitochondrial genome) were included. This was performed (N with sufficient coverage) in TCGA (N= 3,265; N=324 RCC), the institutional MSK-IMPACT cohort (N= 22,252; N=568 RCC), and CCLE/Depmap (N= 377; N=11 RCC). Mutual exclusivity between each established RCC nuclear driver gene (N=16, PMID: 29617669) and the heteroplasmy of ETC truncating variants was evaluated using pairwise t-tests comparing heteroplasmy between the mutated and wild-type RCC samples for each nuclear driver gene. CERES scores from genome-wide CRISPR screens in Depmap were used to compare gene dependency between cell lines. Benjamini-Hochberg correction was used to control type I error.

Results: Across all cancer types in TCGA, RCC tumors were among the most enriched in ETC truncating mutations. These mutations most commonly affected components of mitochondrial complex I and were enriched to high levels of heteroplasmy. *VHL*-driven ccRCC was relatively depleted in these mutations compared to other subtypes (ccRCC: 8.6%, chRCC: 20.0%, pRCC: 34.0%). Further, the heteroplasmy of truncating mutations was significantly increased in chRCC and pRCC compared to ccRCC ($p<0.05$). Among all established RCC nuclear driver genes, *VHL* was found to be mutually exclusive with high heteroplasmy ETC truncating mutations ($q<0.05$). These results were independently replicated in the MSK-IMPACT cohort. Using data from CCLE/Depmap, we find that while cell lines with high heteroplasmy (>50%; N=13) truncating mitochondrially-encoded ETC mutations have differential dependencies compared to other cell lines, ETC mutations were not synthetically lethal with *VHL* mutations.

Conclusion: We established that mutations in *VHL* and in mitochondrially-encoded ETC genes are mutually exclusive in RCC and that this mutual exclusivity is not accounted for by synthetic lethality. These results suggest that mitochondrial mutations may be phenocopying the effect of *VHL* on OxPhos and form the basis for our planned experimental work evaluating OxPhos dysregulation as a unifying feature of renal carcinogenesis.

#1527 Reversal of the Warburg Effect with BPM31510 treatment in a quinone deficient C6 Glioma resulting in efficacy.

Michael A. Kiebish[1], Abbas Khojasteh[2], Sylwia Stopka[1], Juan J. Aristizabal-Henao[1], Seema Nagpal[2], Lisa Ghiglieri[2], Mustafa Kendirli[2], Maria-D Nastke[1], Stephane Gesta[1], Niven R. Narain[1], Vijay Modur[1], Lawrence Recht[2]

[1]BPGbio, Framingham, MA,[2]Stanford University, Palo Alto, CA

BPM31510-IV is a drug-lipid conjugate nanodispersion comprised of oxidized Coenzyme Q10 (CoQ10) in clinical development for glioblastoma multiforme (GBM). BPM31510 reengineers tumor metabolism, while supporting efficient metabolism of non-tumorigenic tissue. Recent evidence suggests that GBM tumors have a deficiency in quinones, most notable CoQ10, leading to altered mitochondrial function. Additionally, emerging data suggests that the rat C6 glioma also is deficient in quinone metabolites. Herein, we explored the therapeutic MOA of BPM31510 in the treatment of GBM based on twice a day dosing vs intermittent dosing. Survival assessment was performed with untreated, 100 mg/kg BID dosing (IP), 200 mg/kg IV dosing (every other day) and 400 mg/kg IV dosing (every other day) in rats implanted with a C6 glioma. Tumor implantation was confirmed using MRI at day 9 and treatment began at day 11. In a parallel treatment study, rats were treated in a corresponding manner and tissue samples were collected at day 14. Rat brains were immediately frozen and were assessed by spatial metabolomics/lipidomics and quinone assessment (quinomics) to spatially resolve metabolic changes at a single cell level post-treatment. C6 glioma exhibited a deficiency in CoQ9 (dominant CoQ in rats) and treatment with BPM31510 was able to increase CoQ9 in tumors reengineer metabolic activity, thus reversing the Warburg effect. Analysis of BPM31510 treated animals revealed a dose dependent and dosing sequent dependent response impacting survival and tumor resolution as well as an impact on spatial metabolic effect of BPM31510 treatment in brain compared to C6 glioma tissue. Additionally, changes in lactate and citrate were also evident in tumor compared to brain in a dose and cycling dependent manner, which demonstrates reengineering of mitochondrial metabolism in cancer compared to normal brain. These data definitely demonstrate that BPM31510 is capable of entering the brain and tumor, reengineering mitochondrial metabolism resulting in a resolution of the glioma which impacts survival. Both dosing and sequencing of doses impact outcome and metabolic response.

#1528 JAG1-driven macropinocytosis as a critical regulator of nutrient scavenging and tumor progression in pancreatic cancer.

Mofei Huang[1], Bingqing Zou[1], Yimin Ma[1], Wei Xin[2], Lan Zhou[1]

[1]Department of Pathology and Genomic Medicine, Houston Methodist Research Institute, Houston, TX,[2]Department of Pathology, University of South Alabama Hospital, Mobile, AL

Pancreatic cancer (PanCan), known for its aggressive nature, poor prognosis and often resistant to therapeutics, remains a major global health burden (Conroy, Desseigne et al. 2011, Von Hoff, Ervin et al. 2013, Howlader N 2021, Bray, Laversanne et al. 2024). Exploring new therapeutic targets is urgent. Studies have reported that PanCan cells undergo metabolic reprogramming in response to oncogenic signals for survival and tumor progression. Macropinocytosis, as an important nutrient scavenging metabolic pathway, fuels KRAS-driven PanCan cell growth, especially under nutrient-deficient condition (Ramirez, Hauser et al. 2019). The Notch signaling pathway is pivotal in PanCan initiation and development (Miyamoto, Maitra et al. 2003, Thomas, Zhang et al. 2014). Here, we demonstrated that Jagged-1 (JAG1) is the predominant Notch ligand expressed in human PanCan tissues and patient-derived xenografts, but only weakly presented in endothelial cells of normal pancreas. Elevated JAG1 gene expression is associated with worse survival in TCGA PanCan patient cohort, implicating that JAG1 is a prognostic marker for PanCan. However, whether JAG1-driven Notching signaling affects PanCan cellular metabolism remains poorly understood. Preliminary RNA-seq analysis on JAG1-depleting KPC tumor lines shows deactivated pathways activities, including “PI3k-AKT-mTOR”, “positive regulation of endocytosis” and “actin cytoskeleton organization”, all of which are important molecular events for macropinocytosis processes. We further investigated and noticed decreased dextran-TMR uptake capacities in those JAG1-depleting KPC cells, indicating attenuated macropinocytic activity upon JAG1 extinction. Along this line, the widely targeted metabolomics assay revealed peptides and amino acids as the most abundant metabolite family with significant reduction in JAG1-depleting KPC tumor cells. Finally, JAG1 ablation decreased KPC cell proliferation and spheroid forming efficiency in vitro. In addition, JAG1 deletion reduced tumor growth in syngeneic subcutaneous KPC mouse models (6419C5, 7160C2 and 6422C5) as well as in a KPC orthotopic model (6419C5). Collectively, our findings suggest JAG1 as a key driver of macropinocytosis in PanCan cells and imply the potential of JAG1 ablation to inhibit nutrient scavenging and tumor progression.

#1529 Colon cancer cells activate CCL20 and IL10 signaling to combat effects of 5 fluorouracil.

Janani Muralidharan, Shailesh Singh, **Hina Mir**

Microbiology, Biochemistry and Immunology, Morehouse School of Medicine, Atlanta, GA

Advances in screening and therapy have significantly decreased colon cancer mortality rates; however, predictions indicate that by 2030, approximately one million patients may still die from this disease each year. While chemotherapies are effective, cancer cells often develop resistance mechanisms that necessitate higher doses, which can lead to severe toxicities that hinder treatment effectiveness and pose additional health risks for patients. Depending on the stage of cancer, 5FU, an antimetabolite, is typically administered alone or in combination with oxaliplatin. Despite its cytotoxic properties, 5FU can induce inflammation in the colon, which may counteract its effectiveness against cancer. This inflammation, characterized by altered cytokine profile, contributes to the resilience of cancer cells. The focus of our study is on the proinflammatory chemokine CCL20, which is elevated during colon cancer, and plays a significant role in liver metastasis. CCL20 signaling is linked to epithelial to mesenchymal transition and can influence the efficacy of chemotherapy. Our research findings indicate that colon cancer cells treated with cytotoxic doses of 5FU show a notable increase in CCL20 secretion, as well as elevated levels of extracellular fatty acids compared to untreated cells. Furthermore, treatment with palmitic acid enhances CCL20 secretion. After 5FU treatment, there is also an increase in interleukin 10 (IL10). Interestingly, inhibiting CCL20 signaling, either by neutralizing CCL20 or blocking its receptor CCR6, further elevates IL10 levels. This suggests that colon cancer cells increase their tolerance to 5FU by raising CCL20 and IL10.Our study highlights the need for further investigation into these mechanisms to develop more effective treatment strategies that maintain or improve patients' quality of life.

#1530 Elucidating the role of exogenous lipids in ferroptosis in castration resistant prostate cancer.

Jose C. Valentin Lopez[1], Scott M. Dehm[2]

[1]University of Minnesota Medical School, Minneapolis, MN,[2]Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN

Prostate Cancer (PCa) is the leading cause of cancer cases in U.S. men and the second leading cause of male cancer death. An advanced form of the disease, termed castration-resistant PCa (CRPC), is not curable, and accounts for most PCa mortality. Several studies have observed that the use of statins, cholesterol-lowering drugs, leads to a reduced risk of PCa advancing to the metastatic CRPC stage. Additionally, in combination with abiraterone or enzalutamide, statins have been able to prolong time to progression and overall survival in patients with metastatic CRPC. The purpose of this study is to understand the mechanism of how a systemic reduction of cholesterol leads to these outcomes. Ferroptosis is a lipid peroxidation-dependent form of cell death. Several metabolites in the cholesterol synthesis pathway have been observed to protect cells against ferroptosis. We hypothesize that extracellular cholesterol, in conjunction with its precursors, protect CRPC cells from ferroptosis.We used the isogenic LNCaP and LN95 cell line models of CRPC progression as well as PC3 cells, another model of CRPC. We analyzed relative cell number using crystal violet assays and used flow cytometry to assess proliferation, cell death, apoptosis, and lipid peroxidation. Additionally, RNA sequencing and mass spectrometry-based metabolomics were used to analyze changes to the transcriptome and metabolome. We observed that all cell line models exhibit dependence on extracellular lipids for their growth and proliferation. Additionally, removal of extracellular lipids (EL) led to an increase in non-apoptotic forms of cell death. Further experiments showed that the growth reduction caused by removal of EL was rescued in the CRPC models by Ferrostatin-1, a ferroptosis inhibitor, but not the apoptosis inhibitor Q-VD-OpH. Removal of EL also led to an increase in lipid peroxides. Upon gene set enrichment analysis of RNA sequencing data, we identified the Hallmark Cholesterol Homeostasis gene set as the most upregulated across all models cultured in lipid free medium. Additionally, we observed a reduction in cholesteryl ester levels upon the removal of EL across all models. Interestingly, the growth reduction caused by removal of EL was rescued by cholesterol only in the CRPC models but not in the LNCaP model. Cholesterol supplementation after lipid depletion also led to a protection of cells from lipid peroxides and a reduced sensitivity to the ferroptosis inducing agent JKE-1674. Collectively, these data indicate the dependence of PCa models, particularly CRPC models, on extracellular cholesterol for their growth and proliferation. Additionally, CRPC models showed susceptibility to ferroptosis upon removal of EL, which was prevented by adding back cholesterol to the growth medium. These results show cholesterol as an antiferroptotic molecule in cell line models of CRPC.

#1531 The role of HDAC1 in pancreatic cancer metabolism.

Don-Gerard Cavakiah Blanchard Conde[1], Evan J. Zhou[2], Maximillian Farma[2], Chiamaka J. Ezeh[1], Laiba Shiekh[2], Matthew Cheung[2], Zeribe C. Nwosu[2], Nwosu Lab, Cornell University

[1]Cornell University, Ithaca, NY,[2]Molecular Biology and Genetics, Cornell University, Ithaca, NY

Pancreatic cancer is a malignant disease often driven by mutations in the KRAS gene. It is associated with significant metabolic reprogramming driving tumor growth, drug resistance, and adaptation in its dense, nutrient-limited tumor microenvironments. To date, the mediators of PDAC metabolism are not fully understood. Analyzing gene expression changes in PDAC tumors compared to normal tissues identified histone deacetylase 1 (HDAC1) as a consistently upregulated gene. We found that HDAC1-high tumors display strong metabolic alterations, including the upregulation of genes in glycolysis and other metabolic processes, and that treatment with Class I selective HDAC inhibitors (HDACi) selectively blunted growth. Initial experiments altering HDAC1 expression transiently in multiple pancreatic cancer cell lines demonstrate alternate metabolic responses. Consistent with existing literature, we observe that HDACi effectively suppressed pancreatic cancer cell growth, primarily class I selective inhibitors. Furthermore, we recently developed HDAC1 knockout cell lines that demonstrate shifts in metabolic profiles of pancreatic cancer and slowed growth rates in HDACi sensitive cell lines through immunoblotting, metabolomics and Seahorse assay. We expect that our findings will show the significance and prospects of targeting HDAC1 in pancreatic cancer therapy and its potential to overcome resistance with current therapeutic.

#1533 Regulatory mechanism of bile acid-induced hepatokine ORM2 expression and its implication in the liver cancer.

J. Suh[1], **I. Cheon**[1], H.-J. Jung[1], S. Lee[2], M. Heo[1], M. DeBerge[1], C. Wooton-Kee[3], Y.-H. Ahn[4], K. Kim[1];
[1]McGovern Medical School, University of Texas Health Science Center at Houston, Houston, TX, [2]Gwangju Health University, Gwangju, Korea, Republic of, [3]Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX, [4]Ewha Womans University, Seoul, Korea, Republic of

Bile acids (BAs) are increasingly recognized as key signaling molecules with various metabolic effects in diverse pathophysiologic contexts. Uncontrolled BA synthesis or impaired BA flow leads to cholestasis, a severe liver disease, characterized by extensive hepatic damage and progression to liver failure or liver cancer. The nuclear receptors (NRs) farnesoid X receptor (FXR) and small heterodimer partner (SHP) play critical roles in maintaining BA homeostasis; consequently, FXR/SHP double knockout mice (FS-DKO) exhibit juvenile onset cholestasis that ultimately develops liver cancer. We recently identified orosomucoid 2 (ORM2) a novel liver-secreted hormone (i.e., hepatokine), which is highly induced in response to hepatic BA stress. In this study, we identified the molecular mechanism underlying BA-induced *Orm2* transcription, potentially mediated by activation of the xenobiotic NRs, constitutive androstane receptor (CAR) and pregnane X receptor (PXR). BA supplementation directly upregulated *Orm2* expression whereas it was completely abolished in CAR/PXR double knockout livers. Given the diverse roles of BAs and ORM2 in metabolic and carcinogenic processes, our study highlights that BA stress activates CAR and PXR to induce ORM2 expression, which initially exerts a protective function in acute liver injury but eventually promotes liver cancer. These insights suggest a potential link between hepatic BA signaling, CAR/PXR activation, and systemic outcomes in the pathology from cholestasis to liver cancer.

#1534 Targeting oxygen consumption with metformin and phenformin have differential effects on immune cells in the tumor microenvironment.

Sadna Budhu[1], Anais Assouvie[1], Juan Zurita[2], Mamadou A. Bah[1], Svena Verma[1], Mehdi Benzaoui[1], Inna Serganova[3], Jason Koutcher[4], Vladimir Ponomarev[2], Jedd D. Wolchok[3], Taha Merghoub[1]

[1]Pharmacology, Weill Cornell Medicine/Sandra and Edward Meyer Cancer Center, New York, NY,[2]Radiology, Memorial Sloan Kettering Cancer Center, New York, NY,[3]Medicine, Weill Cornell Medicine/Sandra and Edward Meyer Cancer Center, New York, NY,[4]Medical Physics, Memorial Sloan Kettering Cancer Center, New York, NY

Immune checkpoint blockade (ICB) has yielded durable clinical responses which led to its FDA approval as a first-line therapy for melanoma and other malignancies. However, only ~20-40% of patients show benefit when used as monotherapies. This is mainly due to inherent or acquired resistance to these therapies within the tumor microenvironment (TME). There is increasing evidence in pre-clinical studies that tumor metabolism (e.g., glycolysis and oxygen consumption (OXPHOS)) presents a barrier to anti-tumor immunity. High levels of OXPHOS in tumor cells lead to tumor hypoxia. Hypoxia causes altered tumor physiology, increased immunosuppression, and resistance to several therapies such as ICB. We propose that blocking tumor oxygen consumption using drugs that target the mitochondrial complex I (metformin and phenformin) will enhance the efficacy of immunotherapies such as ICB in preclinical models. In this study, we examined how low oxygen levels (hypoxia) influence T cell effector function such as cytotoxic activity and cytokine production in vitro. We co-cultured mouse B16 melanoma cells in the presence of tumor specific T cells and incubated at 37ºC in either normoxia (~21% O2) or hypoxia (3% O2) and assessed killing and intracellular cytokine production 24 - 48 hours later. We show that the ability of tumor antigen-specific T cells to kill tumor cells or make effector cytokine such as IFNγ and TNFα is significantly reduced in hypoxia settings. We also examined the effects of metformin and phenformin on T cell priming and activation. When T cells were activated in the presence of each drug in vitro, there was enhanced expression of T cell activation markers (CD25, Granzyme B) as well as an increase in their differentiation into central memory T cells. We treated mice bearing melanoma and breast cancers with metformin and phenformin and tested whether these drugs could delay tumor growth and improve overall survival in vivo as a monotherapy or in combination with anti-PD1. While both drugs can significantly delay tumor growth as monotherapies, only metformin showed improved anti-tumor efficacy when combined with anti-PD-1 in vivo. We performed flow cytometry on mice bearing melanoma and breast cancers treated with metformin and phenformin in vivo. We found that these drugs have differential effects on the immune system in the tumors and periphery in the two cancer models. These studies show that while both drugs target tumor oxygen consumption (via inhibition of complex I), and can delay tumor growth in vivo, they have differential effects on the TME and will need further considerations when combining with ICBs and other immunotherapies. In addition, these studies will advance our understanding of how tumor metabolism impacts anti-tumor immune responses and could lead to better design of combination therapies for cancer patients treated with diabetes management drugs.

#1535 Glycogen metabolism is critical for cancer-associated fibroblast proliferation and immunosuppressive activity.

Xudong Zhu, Yuning Zhou, Zhijie Yin, Jinpeng Liu, Chi Wang, B.Mark Evers, Qingding Wang

Markey Cancer Center, The University of Kentucky, Lexington, KY

Background: Metabolic reprogramming is a characteristic feature of both cancer cells and their neighboring cells in the immunosuppressive tumor microenvironment (TME). Cancer-associated fibroblasts (CAFs) are the major sources of secreted cytokines contributing to the immunosuppressive activity in the TME. Dysregulation of glycogen metabolism is integral to cancer cell proliferation and metastasis. The purpose of this study was to identify whether glycogen metabolism is important for CAF proliferation and cytokine production.
Methods: CAFs were isolated from colorectal cancer (CRC) patient samples and cultured in conditioned medium (CM) derived from HCT116 human CRC cells. The expression of glycogen phosphorylase liver form (PYGL), glycogen phosphorylase brain form (PYGB), hexokinase 2 (HK2), lactate dehydrogenase A (LDHA), IL6, CLCF1, LIF, and CXCL6 was determined by either real time (RT)-PCR or western blot. Glycogen levels were determined using a Glycogen Analysis Kit. Cell proliferation was determined using WST-1.
Results: (i) Data set analysis identified an elevated glucose metabolism in CAFs compared to normal fibroblasts. (ii) Treatment of CAFs with CM derived from HCT116 cells resulted in activated glycogen metabolism as noted by elevated protein expression of glycogen synthase, PYGL, PYGB, and the reduced level of glycogen in CAFs. In addition, CM increased glucose metabolism in CAFs as noted by significantly increased expression of HK2 and LDHA. Moreover, CM increased the expression of IL6 family cytokines such as IL6, CLCF1, LIF and CXCL6 in CAFs. (iii) Inhibition of glycogen metabolism by treatment with the PYG inhibitor, CP-91149, significantly repressed CAF proliferation and reduced the expression of these cytokines. Consistently, knockdown of PYGB or PYGL significantly decreased the level of these cytokines in CAFs. (iv) Inhibition of glycogen synthase by treatment with the glycogen synthase inhibitor, MZ-101, or knockdown of glycogen synthase 1 decreased the expression of these cytokines in CAFs.
Conclusion: Our results demonstrate that glycogen metabolism is crucial for promoting CAF proliferation and cytokine production. Importantly, our findings suggest that targeting glycogen metabolism in CAFs reshapes the immunosuppressive TME and may enhance the efficacy of immunotherapy for CRC.

#1536 Unsaturated fatty acids promote cell cycle progression and proliferation of platinum resistant ovarian cancer cells.

Ana Maria Isac[1], Andres Valdivia[1], Guangyuan Zhao[1], Yinu Wang[1], Jian-Jun Wei[1], Sandra Orsulic[2], Daniela Matei[1]

[1]Northwestern University, Chicago, IL,[2]University of California, Los Angeles (UCLA), Los Angeles, CA

Introduction: Ovarian cancer (OC) depends on fat as fuel for metastasis and growth. We have previously demonstrated that cisplatin resistant (Pt-R) OC cells uptake higher amounts of fatty acids (FAs) compared to sensitive (Pt-S) cells. Here, we determined the mechanism through which FAs support cancer survival.

Materials and Methods: We used two isogenic OC cell lines pairs, sensitive and resistant to cisplatin: OVCAR5 Pt-S (IC50 = 2.881mM) vs Pt-R (IC50 = 6.831 mM) and PEO1 (IC50 = 4.901 mM) vs PEO4 (IC50 = 8.285 mM). Cells were cultured under low serum conditions, supplemented with 50 µM oleic (OA) or 50 µM palmitic acid (PA), and used for viability assays, RNA-Seq, cell cycle, and cell death analysis. Quantitative RT-PCR measured expression of key FAs transporters in Pt-S and Pt-R cells. Immunohistochemistry (IHC) assessed expression of FABP4 in primary and recurrent human tumors. The FABP inhibitor BMS-309403 was used to pharmacologically inhibit the FA transporters *in vitro* and *in vivo*.

Results: Pt-R cells rely more on unsaturated FAs than Pt-S cells, upregulating transporters for increased FA uptake. Cell viability assays showed Pt-R cells were less viable under serum depletion, with oleic acid (OA, unsaturated) promoting proliferation of both OC cell lines. OA rescued Pt-R cells significantly more than Pt-S cells: 268.4% vs 91.6% for OVCAR-5 and 38.1% vs 12.1% for PEO4 vs PEO1. OA also reduced apoptosis, as shown by Annexin V assays, and decreased Caspase-3 and -8 cleavage. RNA-Seq analysis linked OA-induced proliferation to upregulation of cell cycle-related pathways, including *G2/M checkpoints*, driven by E2F1. OA increased S- and G2/M phase populations (e.g., 6.95% to 11.6%; $p<0.05$), while E2F1 inhibition reduced OA-induced proliferation. Pt-R cells also expressed higher levels of transported proteins FABP4 and CD36. Inhibiting FABPs reduced Pt IC50 values (e.g., from 11.893 µM to 5.0415 µM in OVCAR5) and blocked ovarian cancer progression i*n vivo*. IHC showed FABP4 overexpression in metastatic and recurrent tumors (n=21 paired tumors, $p<0.05$).

Conclusion: Pt-R OC cells are more dependent on unsaturated FAs compared to Pt-S cells and upregulate key transporters to allow increased uptake of FAs. Inhibiting intracellular FA transport reduces OC progression *in vivo*. OA supports proliferation of Pt-R cells by rescuing them from apoptosis and by promoting cell cycle progression from G1 to S- and G2/M phases.

#1537 The extracellular matrix supports pancreatic cancer cell growth under nutrient deprivation.

Ifeoluwa Feyisayo Oyelade, Elena Rainero

Biosciences, University of Sheffield, Sheffield, United Kingdom

Pancreatic cancers have a poorly vascularized and nutrient-deficient microenvironment making them more susceptible to nutrient deprivation. Evidence has shown that Pancreatic Ductal Adenocarcinoma (PDAC) cells survive in a nutrient-deprived and hypovascular microenvironment. Here, we investigate the roles of the Extracellular Matrix (ECM) in supporting the growth of PDAC cells in different nutrient-deprivation conditions. Our cell proliferation, microscopy and targeted Mass Spectroscopy metabolomics data showed that Matrigel, but not collagen I, supports the proliferation of PDAC cells in Glucose and Glutamine deprivation conditions, as well as in Tissue Interstitial Fluid Media (TIFM), which has been reported to mimic the physiological nutrient availability in PDAC tumors. In addition, we found that the role of Matrigel in supporting the growth of pancreatic cancer cells is linked to increasing cell proliferation rather than reducing cell death. Importantly, our Targeted Mass spectrometry metabolomics data showed that the Arginine metabolism is upregulated when the cells are grown under starvation on Matrigel compared to plastic. We have identified some genes that are likely to play a role in the upregulation of this metabolic pathway and we are currently using qPCR and Western blotting techniques to identify whether the ECM and/or nutrient availability affect their expression. Finally, we will determine their role in promoting ECM-dependent PDAC cell proliferation under starvation. Overall, our study has established that the ECM can alter the metabolism of PDAC cells to support their cell growth under nutrient-limiting conditions.

#1538 Impact of MCT1 inhibition on NSCLC metabolism and sensitivity to therapy.

Robert Cameron[1], Massar Alsamraae[2], Maia G. Clare[2], Nia G. Hammond[2], Christine Bestvina[1], Marina Chiara Garassino[1], Brandon Faubert[1]

[1]Department of Medicine, Section of Hematology/Oncology, UChicago Medicine, Chicago, IL,[2]Division of Biomedical Sciences, University of Chicago, Chicago, IL

Introduction: Despite the advent of targeted therapies, prognosis of lung of non-small cell lung cancer (NSCLC) remains poor. Thus, identifying metabolic programs in aggressive disease may allow for the development of novel treatment strategies. Prior work in early-stage NSCLC has demonstrated that lactate import via monocarboxylate transporter 1 (MCT1) is associated with higher recurrence and mortality. However, the potential for targeting MCT1 in treatment efficacy and resistance remains unknown.
Methods: NSCLC cells were cultured in standard RPMI or, to better model in vivo metabolism, human plasma-like media (HPLM). MCT1 was inhibited genetically (CRISPR-KO) or pharmacologically (AZD3965). Isotope tracing experiments were performed using [U-^{13}C]glucose or [U-^{13}C]lactate. DNA damage was assessed via the protein expression of γH2AX. MCT1 inhibition was combined with pemetrexed, and cell viability/cell death was measured using annexin V/propidium iodide. Combinatorial/synergy effects were assessed using synergyfinderplus.org.
Results: H1299 cells incorporate lactate into tricarboxylic acid cycle (TCA) intermediates in both standard and physiologic culture media. Lactate incorporation into the TCA was decreased by treatment with AZD3965. Both genetic and pharmacologic inhibition of MCT1 increased the expression of the DNA damage marker γH2AX. Following 24 hours of pemetrexed treatment, pemetrexed alone significantly decreased cell viability in RPMI but not HPLM. The addition of AZD3965 decreased cell viability in both conditions, but decreases were less pronounced in HPLM cultured cells. In both media conditions, combinatorial dosing of AZD3965 and pemetrexed led to increases in cell death, and these were more pronounced at increasing doses of AZD3965. AZD3965 did not confer additional cytotoxic effect to cells treated with paclitaxel.
Conclusions: MCT1 inhibition decreases lactate import and oxidation in vitro and causes DNA damage to enhance the cytotoxic effects of pemetrexed. Metabolic reprogramming in NSCLC leads to the use of alternate carbon sources, such as lactate, and this can be modeled in both standard and physiologic culture conditions. Notably, this does not appear to be a general sensitization to cell death. Further studies are ongoing to identify the metabolic underpinnings of these effects and how they translate to in vivo systems.

#1539 Lactate induced c-Myc lactylation drives prostate cancer progression by influencing gene transcription.

M. Howlader, A. O. Akinyemi, M. Alam, J. Wang, L. Gu, Z. Li;
University of Kentucky, Lexington, KY

Prostate cancer (PCa) is a leading cause of cancer-related morbidity and mortality, characterized by complex metabolic reprogramming that supports tumor growth and progression. Recently, lactate has emerged as a critical factor in PCa metabolism, produced via aerobic glycolysis and influenced by oncogene/tumor suppressor gene mutations, androgen signaling, and hypoxia. Glycolytic cancer cells and cancer-associated fibroblasts can secrete lactate, fostering "symbiotic" interactions with oxidative cancer cells through lactate shuttling. Lactate accumulation subsequently promoting the lactylation of proteins which is a novel post-translational modification involving proteins that is induced by lactate. The Warburg effect emphasizes the importance of lactylation in cancer biology, influencing gene transcription and supporting rapid tumor growth. Excessive lactate production and rapid lactate transport in cancer cells are mainly driven by the upregulation of c-Myc which is a master regulator of multiple biological programs. Its persistent activation results in abnormal expression of glycolytic enzymes and lactate transporters, contributing to PCa progression. While c-Myc undergoes various post-translational modifications, the potential for c-Myc lactylation and its role in PCa development remain unexplored. Preliminary studies indicate that sodium lactate exposure increases lysine lactylation in PCa cells. We also show that c-Myc oncogene is lactylated. The lactylation of c-Myc not only promotes tumor growth but also contributes to the metabolic flexibility of PCa, enabling them to thrive in hypoxic and nutrient-deprived environments. Understanding the molecular mechanisms underlying c-Myc lactylation provides new insights into PCa biology and opens potential avenues for therapeutic intervention targeting metabolic pathways.

#1540 ADAR1 regulates lipid remodeling through MDM2 to dictate ferroptosis sensitivity.

Che-Pei (Pat) Kung[1], Nick Terzich[2], Michael J. Prinsen[1], Raleigh D. Kladney[3], Alex Mabry[3], Luisangely Soto Torres[3], Jade Weber[4], Ma Xenia G. Ilagen[5], Leonard Maggi[3], Leah P. Shriver[2], Gary J. Patti[2], Jason D. Weber[3]

[1]Washington University in St. Louis, Saint Louis, MO,[2]Chemistry, Washington University in St. Louis, Saint Louis, MO,[3]Medicine, Washington University in St. Louis, Saint Louis, MO,[4]Biomedical Engineering, Washington University in St. Louis, Saint Louis, MO,[5]Biochemistry and Molecular Biophysics, Washington University in St. Louis, Saint Louis, MO

Triple-negative breast cancer (TNBC), lacking expression of estrogen, progesterone, and HER2 receptors, is aggressive and lacks targeted treatment options. An RNA editing enzyme, adenosine deaminase acting on RNA 1 (ADAR1), has been shown to play important roles in TNBC tumorigenesis. We posit that ADAR1 functions as a homeostatic factor protecting TNBC from internal and external pressure, including metabolic stress. We tested the hypothesis that the iron-dependent cell death pathway, ferroptosis, is a ADAR1-protected metabolic vulnerability in TNBC by showing that ADAR1 knockdown sensitizes TNBC cells to GPX4 inhibitors. By performing single-reaction monitoring-based liquid chromatography coupled to mass spectrometry (LC-MS) to measure intracellular lipid contents, we showed that ADAR1 loss increased the abundance of polyunsaturated fatty acid phospholipids (PUFA-PL), of which peroxidation is the primary driver of ferroptosis. Transcriptomic analysis led to the discovery of the proto-oncogene MDM2 contributing to the lipid remodeling in TNBC upon ADAR1 loss. A phenotypic drug screen using a ferroptosis-focused library was performed to identify FDA-approved cobimetinib as a drug-repurposing candidate to synergize with ADAR1 loss to suppress TNBC tumorigenesis. By demonstrating that ADAR1 regulates the metabolic fitness of TNBC through desensitizing ferroptosis, we aim to leverage this metabolic vulnerability to inform basic, pre-clinical, and clinical studies to develop novel therapeutic strategies for TNBC.

#1541 Possible role of ectopic ATP synthase mediating metabolic hypoxic adaptation in retinoblastoma.

Samantha A. McLaughlin[1], Zelia M. Correa[1], Stefan Kurtenbach[1], J. William Harbour[2], Daniel Pelaez[1]

[1]Department of Ophthalmology, Bascom Palmer Eye Institute and Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine, Miami, FL,[2]Department of Ophthalmology Department of Ophthalmology and Harold C. Simmons Comprehensive Cancer C, University of Texas Southwestern Medical Center, Dallas, TX

Altered metabolism is a hallmark of cancer. Yet how metabolic reprogramming benefits tumor growth remains unclear. In retinoblastoma (RB), we have shown that estrogen-related receptor gamma (ESRRG) undergoes continuous positive selection during tumor evolution as evidenced by an ongoing accumulation of genomic aberrations that increase ESRRG activity. Transcriptional analysis revealed that ESRRG is pivotal in mediating metabolic reprogramming, with cells displaying preferential aerobic fermentation and microenvironment acidification. Here, we show that the combination of hypoxic stress and ESRRG deregulation leads to increased ectopic expression of mitochondrial ATP synthase on the plasma membrane, where it is critical to cancer cell proliferation. Translocation of ATP synthase to the plasma membrane was augmented in a time-dependent manner when RB cells were exposed to hypoxia. Pre-treatment of cells with an ESRRG inhibitor eliminated this ATP synthase translocation. Both the F_0 proton channel and F_1 catalytic domains were identified on the outer plasma membrane by confocal microscopy and flow cytometry. Intracellular ATP concentration, measured by luciferase reaction, was significantly increased by media acidification. Inhibition of lactate/proton export through monocarboxylate transporters resulted in media alkalinization and a significant decrease in intracellular ATP, which could be rescued by subsequent exogenous acidification of the media, suggesting that ectopic ATP synthase uses the proton motive force generated by extracellular acidification to synthesize ATP. A novel F_0-targeting ATP synthase inhibitor resulted in near complete cell death of RB cells but showed minimal effect on non-malignant control cells. Interestingly, this drug did not impact the reductive capacity of cancer cell mitochondria compared to control cells, indicating that the drug preferentially affects plasma membrane ATP synthesis at these concentrations. These findings suggest that ATP synthase on the surface of RB cells plays an important role in metabolic adaptation and cell proliferation. These results demonstrate that the use of ATP synthase inhibitors targeting the F_0 domain may represent an innovative new strategy to target cancer cells.

#1542 Metabolic dysregulations of aged bone marrow mesenchymal cells in Acute Myeloid Leukemia reveal novel therapeutic targets..

Mary E. Fuentes[1], Julia K. Christian[1], Amy Kovacs[2], James R. Lerma[3], Liang Niu[4], Alice S. Mims[2], Christopher C. Oaks[2], John C. Byrd[3], Erin K. Hertlein[3], Amina Abdul-Aziz[1]

[1]Fels Cancer Institute for Personalized Medicine, Temple Univ. Lewis Katz School of Medicine, Philadelphia, PA,[2]Internal Medicine, The Ohio State University Comprehensive Cancer Center, Columbus, OH,[3]Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, OH,[4]Environmental and Public Health Sciences, University of Cincinnati College of Medicine, Cincinnati, OH

Background: Despite recent efforts to elucidate the dependence of acute myeloid leukemia (AML) on the bone marrow microenvironment, the contribution of aged bone marrow mesenchymal stem cells (BMMSCs) to AML patient survival and treatment response remains understudied. Deregulated nutrient sensing is an established hallmark of aging and previous works have identified age-dependent metabolic dysregulations in cancer. The dependence of AML on oxidative phosphorylation (OXPHOS) has been well established, as AML adapts to fluctuations of nutrient and oxygen availability in the bone marrow microenvironment. We hypothesized that metabolic pathways, such as oxidative phosphorylation, are critical in aged AML-BMMSCs as they drive pro-tumorigenic properties and drug resistance.

Methods: Our group expanded primary Normal Donor (ND)-BMMSCs and AML-BMMSCs (n=9 CBF-AML, n=11 CK-AML, n=7 ND-BMMSCs) under conditions that mimic the bone marrow microenvironment (91% nitrogen, 4% oxygen, and 5% CO2). We isolated DNA and RNA from early passaged cells to perform methylation analyses using 850k Illumina DNA methylation arrays and gene expression analyses using mRNA-seq. We utilized the Horvath Skin and Blood clock to calculate epigenetic age based on DNA methylation. qPCR assessing mRNA expression of OXPHOS-related genes in ND- and AML-BMMSCs was performed to validate sequencing results. Western blot analyses of Total OXPHOS and its complexes were performed to assess expression levels of OXPHOS-related proteins in ND- and AML-BMMSCs. Seahorse assays were performed to obtain live and repeated metabolic measurements of Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR) in ND- and AML-BMMSCs. Lastly, 2D co-culture experiments were performed to assess cell viability of AML cell lines with BMMSCs treated ± OXPHOS inhibitors (IACS-010759 and Tamoxifen) utilizing AO/PI staining.

Results: Our integrative omics approach showed that with increased epigenetic age, as measured by the Horvath Skin and Blood clock, AML-BMMSCs reveal upregulations in hallmark gene sets for MYC and OXPHOS when compared to ND-BMMSCs. Western blot analyses confirmed upregulation of MYC and OXPHOS complexes in AML-BMMSCs when compared to controls. Results from seahorse assay revealed an increase in OCR and ATP production in AML-BMMSCs when compared to controls. Lastly, our 2D co-culture experiments *in vitro* revealed a decrease in cell viability on AML cells that were co-cultured with AML-BMMSCs treated with IACS-010759 and/or Tamoxifen.

Conclusions: Our results elucidate age-dependent metabolic dysregulations within the bone marrow microenvironment in AML and provide novel targetable approaches. Future portions of testing aim to identify the mechanism of action of AML-BMMSCs and OXPHOS.

#1543 Obesity promotes triple negative breast cancer progression through regulating the plasticity of adipose tissue.

Xi Xu[1], Yang Lin[2], Shalini Bahl[2], Mathieu Lupien[1]

[1]Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada,[2]Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada

The rising global prevalence of overweight and obesity is linked to cancer initiation and poorer overall survival in triple-negative breast cancer (TNBC), making it a modifiable risk factor for both cancer prevention and treatment. The tumor microenvironment (TME) plays a crucial role in tumor development, progression, and response to chemotherapy, and is influenced by both local and systemic factors, such as aging and metabolism. However, most previous studies in this area have relied on animal models or in vitro systems, which provide limited insights into the comprehensive nature of the TME. The mammary gland consists of lobules and ducts with a connective stroma composed of fibro-adipose pockets. Excessive fat deposition in obese patients alters the quantity, function, and plasticity of adipose tissue. It was reported that the adipose tissue undergoes metabolic reprogramming during mammary tumor infiltration into the stromal compartment, which leads to adipose tissue possessing fibroblast/myofibroblast and macrophage-like features, modifying the TME through ECM remodeling and immune response activation, potentially contributing to cancer progression. Adipocyte precursor cells (APCs) are key regulators of adipose tissue plasticity, with the ability to differentiate into either adipogenic or fibrotic cell types. Moreover, fibroblasts are the most abundant stromal cells in TME and strongly associated with breast cancer progression and chemoresistance. Based on the above, I hypothesize that obesity promotes the differentiation of APCs into fibrotic cells, thereby accelerating cancer progression. Single cell multiomics (ATAC + RNA sequencing) has emerged as a powerful tool for studying the TME, as it enables the simultaneous analysis of epigenetic and genetic profiles from the same tumor sample. In this study, we performed single cell multiomics sequencing on primary TNBC tissue samples from 7 lean and 7 obese patients, stratified by body mass index (BMI). Our results revealed that the fibroblasts population in obese patients was more abundant than in lean patients, with significantly increased interactions between fibroblasts and cancer cells. The dominant subtype of fibroblasts in the obese group were identified as myofibroblasts (mCAFs), which were highly enriched in the TGFβ signaling pathway. This enrichment was correlated with a higher epithelial-mesenchymal transition (EMT) signature in cancer cells, observed at both the epigenetic and genetic levels. Furthermore, we leveraged a published human white adipocyte tissue atlas and found that mCAFs shared a high degree of transcriptomic similarity with APCs. This suggests that obesity promotes the differentiation of APCs into mCAFs via the TGFβ signaling pathway, thereby contributing to cancer progression. Our findings provide a novel direction for targeted therapeutic development in the obese patient population.

#1544 Fatty acid oxidation inhibition with anti-cancer agents abrogated tumor growth in a melanoma cancer model.

Jeong Hwan Park, Wonyoung Choi, Woojin Ham, Woosol Hong, Chae-Young Kim, Ho Lee, Soo-Youl Kim

National Cancer Center - Korea, Goyang-si, Gyeonggi-do, Korea, Republic of

Purpose: We found that chemotherapy on melanoma induced fatty acid oxidation (FAO). Therefore, we propose that FAO inhibition may suppress tumor growth synergistically with anti-cancer therapeutics.
Experimental Design: To block FAO, CAC (carnitine-acylcarnitine carrier, gene: SLC25A20) in the mitochondria inner membrane and ACAA1 (acetyl-CoA acyltransferase1, gene: ACAA1) in the peroxisome were targeted for inhibition. KN510 and KN713 were identified through drug screening for their inhibitory effects on CAC, and ACAA1, respectively. Using allograft and xenograft models with mouse and human melanoma cancer cells, we investigated the anti-cancer effects of KN510713 in combination with standard chemotherapy.
Results: Anti-cancer drug treatment such as dabrafenib and dacarbazine induced fatty acid catabolism as well as mitochondrial oxidative phosphorylation for ATP production which is the core metabolic pathway for FAO. Increase of ATP production correlated with mTOR activation. Anti-cancer drug treatment combined with KN510713 induced cell death significantly concomitantly with mTOR inactivation in vitro. Melanoma cancer models of allograft and xenograft showed significant suppression of tumor growth by KN510713 treatment with anti-cancer agents such as dabrafenib or dacarbazine.
Conclusion: FAO inhibition abrogated tumor growth by ATP depletion in melanoma. The changes in molecular signaling upon FAO inhibition revealed that ATP levels are a key regulator of all signaling. Thus, combinations of systemic anti-cancer agents with FAO inhibitors have shown excellent anti-cancer activity in mice. Even if signal transduction is induced by various growth receptors, ATP down-regulation through FAO inhibition may provide a novel therapeutic effect.
Funding source: This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Science and ICT to SK (NRF-2019M3A9G1104345) and by a grant from the National Cancer Center of Korea to WC (NCC 2410891-1).

#1545 IL-9-mediated glutathione synthetase (GSS) expression modulates anti-oxidant response in cutaneous T cell lymphoma (CTCL).

Diksha Attrish[1], Bhavuk Dhamija[1], Ditipriya Mukherjee[1], Vinanti Sawant[1], Neha Sharma[2], Tanuja Shet[3], Hasmukh Jain[2], Rahul Purwar[1]

[1]Biosciences and Bioengineering, Indian Institute of Technology, Bombay, Mumbai, India,[2]Tata Memorial Hospital, Mumbai, India,[3]Pathology, Tata Memorial Hospital, Mumbai, India

CTCL is a rare and clinically heterogeneous blood disorder with an increased incidence. Management of the disease poses a significant challenge, and there is an urgent need for novel therapies that can cure the disease. Since CTCL is a rare disease, the pathophysiology is not completely characterized. Inflammation is a crucial driver of CTCL progression, and IL-9, an inflammatory cytokine, is prevalent in the tumor microenvironment. Previously, our lab reported that the Th9 axis reduces oxidative stress, and in this follow-up study, we deduced the signaling mechanism underlying IL-9-mediated antioxidant response to have a comprehensive understanding of the metabolic rewiring in CTCL.

High-throughput label-free quantitative LC-MS/MS of IL-9-treated lymphoma cells revealed some of the differentially expressed antioxidant enzymes, including peroxiredoxin-4 (fold change = 2.015, $p = 0.018$), glutathione synthetase (fold change = 4.381, $p = 0.001$), and glutathione peroxidase 1 (fold change = 1.88, $p = 0.021$) among which glutathione synthetase was highly expressed. Further, IH NMR spectroscopy identified the metabolic pathways affected by IL-9 signaling. Pathway analysis using MetaboAnalyst 5.0 depicted enhanced glutathione metabolism and elevated glutathione levels. To substantiate the multi-omics predictions, we checked the expression of GSS in multiple CTCL lymphoma lines (MJ, HuT 78, and Jurkat) and activated T-cells using flow cytometry. We found a high intensity of GSS in MJ (4605.75 ± 641 vs. 3921 ± 1187, $p = 0.04$), HuT 78 (11305 ± 530 vs. 8912 ± 603.8, $p = 0.003$), Jurkat (2955 ± 678 vs. 2190 ± 696, $p = 0.001$) and activated T cells (4593 ± 172 vs. 3657 vs. 206, $p = 0.005$) under IL-9 exposure. In agreement, the biochemical assay demonstrated elevated glutathione levels in lymphoma cells treated with IL-9. Immunohistochemical analysis revealed that GSS was widely expressed by infiltrating T-cells in skin biopsy specimens from 5 patients with mycosis fungoides (MF). Subsequently, flow cytometry analysis of liquid biopsy specimens from 11 patients demonstrated its strong expression with a 52% ($p = 0.005$) increase in circulating CD3+ T-cells in patients with MF compared to healthy volunteers. This cumulative analysis asserted its clinical relevance and identified it as a crucial enzyme in IL-9-mediated antioxidant signaling. Further, treating cells with plumbagin depleted GSS levels and increased ROS generation to many folds in all the lymphoma cell lines and activated T cells. In line with this, depleted GSS levels through plumbagin treatment reduced cell growth and potentiated cell death, suggesting that targeting this metabolic pathway sensitizes cancer cells. In conclusion, this study underscores GSS as a critical metabolic vulnerability.

#1547 CXCL17 targets macrophage efferocytosis to modulate cutaneous squamous cell carcinoma (cSCC).

Alok R. Khandelwal[1], Dhananjay Kumar[2], Arif Yurdagul[2], Cherie-Ann O. Nathan[1]

[1]Otolaryngology, Head and Neck Surgery, LSU Health Shreveport, Shreveport, LA,[2]Molecular and Cellular Physiology, LSU Health Shreveport, Shreveport, LA

Cutaneous squamous cell carcinoma (cSCC) is a keratinocyte-derived invasive and metastatic tumor of the skin. It is the second-most commonly diagnosed form of skin cancer, striking 200,000 Americans annually. Excision of cSCC of the head and neck area results in significant facial disfigurement. An increased understanding of the mechanisms involved in the pathogenesis of cSCC could identify means to prevent, inhibit, and reverse this process. Chemokine (C-X-C motif) ligand 17 (CXCL17) is the latest chemokine family member, and an increased CXCL17 protein expression was observed in both mouse and human cSCC cell lines. Further, deletion of CXCL17 was associated with significant inhibition of tumor cell-intrinsic properties such as proliferation, migration, and motility. CXCL17 is identified as a potent chemoattractant for macrophages, dendritic cells and myeloid-derived suppressive cells. Accordingly, utilizing a syngeneic, tumor-cell xenograft cSCC mouse model, we evaluated the effect of CXCL17 deletion on cSCC tumor-immune evasion and elucidated the underlying mechanism. Deletion of CXCL17 was associated with a significant reduction in tumor volume compared to the wild-type counterparts. Further, CXCL17 deleted cSCC tumor cell xenografts exhibited a significant increase in CD8+, cytotoxic T cells in the tumor microenvironment, suggesting an important role of CXCL17 in mediating tumor-immune evasion. Interestingly, treatment with CXCL17 induced macrophage M2 polarization, increased oxidative phosphorylation and promoted macrophage efferocytosis *via* modulating efferocytotic machinery proteins such as MERTK, TIM4, GAS6 and AXL. Our studies have established substantial evidence for the role of CXCL17 in modulating tumor-cell extrinsic properties to affect the progression of cSCC.

#1548 Deletion of peroxisomal 3-ketoacyl-CoA thiolase promoted overall survival in the KPC mice.

Mingyu Kang, **Woosol Hong**, Woojin Ham, Chaeyoung Kim, Ho Lee, Sang Myung Woo, Wonyoung Choi, Sung Hoon Sim, Jung Won Chun, Soo-Youl Kim

National Cancer Center - Korea, Goyang-si, Gyeonggi-do, Korea, Republic of

Background: Fast-growing cancer cells obtained ATP from fatty acid oxidation (FAO). We found that medium-chain acyl-carnitines (MC-AC) were increased by carnitine acyl-carnitine carrier knockdown in pancreatic ductal adenocarcinoma. MC-AC can be produced only in the peroxisome. Therefore, we propose that ATP production in cancer cells may depend on the peroxisomal FAO in addition to mitochondrial FAO.

Methods: We have tested whether peroxisomal FAO is reduced by knockdown of peroxisomal 3-ketoacyl-CoA thiolase (acetyl-CoA acyltransferase 1, *ACAA1*). We have examined effects on ATP level and tumor growth by *ACAA1* knockdown in PDAC cells, and cancer survival by crossbreeding of KPC mice (*Kras*$^{G12D/+}$; *Trp53*$^{R172H/+}$; *P48-Cre*) and *Acaa1*$^{+/-}$ mice.

Findings: Peroxisomal FAO target levels in PDAC patients are higher than in normal individuals. In *in vitro* experiments, *ACAA1* knockdown in PDAC cells reduced 40% of ATP production. In *in vivo* experiments, xenograft mouse models using human PDAC cells with *ACAA1* knockdown showed significantly reduced tumor formation. Crossbreeding of the KPC mice with *Acaa*1$^{+/-}$ mice significantly increased the survival rate.

Interpretation: While normal cells rely on glucose oxidation, ATP production in PDAC cells is critically dependent on FAO in peroxisomes and mitochondria. This suggests that to achieve complete FAO inhibition in cancer, it is necessary to block FAO in both peroxisomes and mitochondria.

Funding source: This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Science and ICT to SK (NRF-2019M3A9G1104345) and by a grant from the National Cancer Center of Korea to SHS (NCC 24H1210-2) and JWC (NCC 2410892-2).

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Antibody-Based Cancer Therapeutics 1
Poster Session

#1552 Novel anti-CD3 heavy chain-only antibodies for use in T-cell engaging therapeutics.

Paul Khalife, Hannah C. Watkins, Elizabeth A. McGurk, Elizabeth R. Parker, Cameron M. R. Henkel, Todd M. Boland, Hailey M. Heston, Whitney E. Renaud, Christin R. Strong, Jingfu Zhou, Carissa Metzger, Michael E. Brown, Jessica P. Dawson, Andrew D. Avery, C. Garrett Rappazzo, Beth H. Sharkey, Morgan B. Morrill, Cory L. Ahonen, Michael B. Battles, Noel T. Pauli

Adimab, Lebanon, NH

T-cell engagers (TCEs) display increasing potential as an immunotherapeutic treatment for both hematologic and solid cancers. These multi-specific antibody-based therapeutics consist of a CD3-targeting arm and one or more tumor associated antigen (TAA) targeting arms. The multimeric nature of these therapeutics can present antibody engineering and manufacturing challenges. Developing an anti-CD3 heavy chain-only antibody (HCAb) reduces the design complexity of the final TCE therapeutic. Functional anti-CD3 HCAbs were discovered by combining llama immunization and a novel yeast-based HCAb presentation and discovery technology. Following several rounds of yeast-based HCAb selection, T-cell stimulating anti-CD3 HCAbs were identified that predominantly competed with UCHT1. Stimulating CD3-specific HCAbs were reformatted into CD3 X CD20 bispecifics, which displayed potent T-cell activation and Raji cell killing in a redirected T-cell cytotoxicity (RTCC) assay. This panel was further validated as a bispecific VHH-TCR fusion using a TCR specific for the gp100 peptide HLA complex; donor T-cell activation and target killing was observed against both SK-MEL-5 and MeWo cancer cell lines. The panel was subsequently humanized while maintaining functional activity and favorable drug developability profiles. These humanized anti-CD3 HCAbs are promising candidates for replacing conventional anti-CD3 IgG Fab arms in next-generation multispecific TCE antibodies.

#1554 Harnessing the Beacon® optofluidic platform for streamlined discovery of internalizing antibodies.

Brittany Benlian, Leqian Liu, Alexander Mastroianni, Minha Park, Vincent Pai, **Alireza Salmanzadeh**

Bruker Cellular Analysis, Emeryville, CA

Antibody drug conjugates (ADCs) are an innovative class of biopharmaceuticals which combine the potency of cytotoxic molecules with the targeted specificity of monoclonal antibodies. The success of a traditional ADC not only hinges on tumor antigen-specific binding but internalization to maximize cytotoxicity from the payload and minimize off-target damage to healthy tissue. The development of an ADC therapy is a complex, multistep process that involves identifying a target antigen, discovering an ideal antibody, selecting the cytotoxic payload and linker, and optimizing the conjugation chemistry - all of which can take years from initial research to therapeutic application. One significant bottleneck within this process is the lack of early phase assays to guide the down-selection of functional antibodies from hundreds of antigen binding clones. Therefore, developing an efficient high-throughput assay for antibody internalization is crucial for accelerating ADC development. The Beacon® optofluidic platform from Bruker Cellular Analysis is an advanced solution for this problem that enables researchers to identify valuable monoclonal antibodies within a week, bypassing the time consuming and laborious process of hybridoma library generation and screening. With this platform, tens of thousands of single primary cells can be isolated using Opto-Electronic Positioning (OEP) technology and assayed for antibody secretion, antigen binding, and receptor-specific internalization in live reporter cells within a single day. This cutting-edge technology is the only platform capable of screening and measuring >10,000 antibodies simultaneously for rapid internalization (1-2 hours). We showcase the ability to streamline early-phase discovery of internalizing antibodies, going beyond mere antigen specificity to enable high-throughput, real-time evaluation of functional antibodies. The Beacon optofluidic platform significantly reduces hands-on time and resource expenditure, focusing efforts on the most promising ADC candidates for downstream characterization and optimization, ultimately expediting the progression of ideal antibodies into clinical applications.

#1556 B7-H7-CD3 bispecific T cell engaging antibodies demonstrate potent anti-tumor activity in B7-H7+ preclinical tumor models.

Karishma Vekaria, Susannah Hewitt, Melissa W. Hayes, Emilien Loeuillard, Jamie Strand, Riale Gilligan, Maja Razlog, Emma Aeschliman, Quangvinh Tran, McKenna Lamb, Bijan Etemad-Gilbertson, Silvia Ferrati, Tatiana Novobrantseva, **Matthew Rausch**

NextPoint Therapeutics, Cambridge, MA

Background: B7-H7 is a B7 family member that suppresses T and NK cell activation through an inhibitory receptor, KIR3DL3, and stimulates immune cell activation through a distinct receptor, TMIGD2. B7-H7 is upregulated on tumor cells of many histological subtypes and is, therefore, explored as a novel tumor targeting antigen. NextPoint has developed two B7-H7-targeted CD3 bispecific antibodies, NPX372 and NPX387, to redirect T cell-mediated immunity toward B7-H7+ tumors. Both antibodies bind B7-H7 with similar avidity and block interaction with KIR3DL3, but induce the formation of distinct immune synapses.
Methods: B7-H7 and CD3 binding were evaluated on-cells expressing human and non-human primate (NHP) versions of the proteins by flow cytometry. Bispecific antibody-mediated T cell activation was assessed using Jurkat reporter assays. Primary T cell activation was evaluated in vitro in co-culture assays with B7-H7+ tumor cell lines by flow cytometry and multiplex cytokine analysis. T cell redirected killing of B7-H7+ tumor cells was assessed using PBMC co-culture assays and flow cytometry. Finally, humanized mouse models were used to test the anti-tumor activity of both NPX372 and NPX387 in B7-H7+ tumor models in vivo.
Results: NPX372 and NPX387 bound to both human and NHP B7-H7 and CD3. While both bispecific antibodies demonstrated similar on-cell avidity to B7-H7, NPX372 demonstrated stronger affinity for human CD3 compared to NPX387. Co-culture assays with Jurkat reporter cells and primary T cells demonstrated that both NPX372 and NPX387 could crosslink B7-H7 on tumor cells to CD3 leading to T cell activation. However, despite having a stronger affinity for CD3, NPX372 induced less cytokine production in primary T cell cultures with B7-H7+ tumor cells compared to NPX387. Both bispecific antibodies also induced potent T cell-mediated killing of multiple B7-H7+ tumor cell lines in vitro with similar potency. Finally, both NPX372 and NPX387 demonstrated anti-tumor activity in a B7-H7+ tumor model in vivo at clinically relevant doses that are tolerated in NHP toxicity studies.
Conclusion: These data demonstrate that B7-H7 represents a novel tumor associated antigen for CD3 bispecific T cell engagement that can induce potent T cell-mediated anti-tumor immunity.

#1558 HDP-201 - a novel multimeric linker exatecan-based ADC targeting Guanylyl cyclase C (GCC) for treatment of gastrointestinal malignancies.

Sarah Jane Neuberth, Christian Orlik, Hendrik Gruss, Christoph Mueller, Kristin Decker, Nick Neubacher, Marisa Schmitt, Aniko Palfi, Irina Dranova, Michael Kulke, Andreas Pahl, Torsten Hechler

Heidelberg Pharma, Ladenburg, Germany

Background: HDP-201 is a novel multimeric linker ADC directed against GCC based on topoisomerase 1 inhibitor exatecan. GCC is highly expressed in gastrointestinal malignancies including gastric and colorectal cancers. The multimeric linker-payload featured with a solubility enhancer facilitates the delivery of more than one hydrophobic payload per conjugation site. HDP-201 employs a proprietary, site-specific conjugation technology based on cysteine-engineered and Fc-silenced antibodies which are devoid of Fc-mediated uptake.
Methods: The cysteine-reactive multimeric exatecan-linker construct was synthesized at Heidelberg Pharma and site-specifically conjugated to engineered cysteine residues of a proprietary anti-GCC antibody, yielding ADCs with a DOL of 2.0 and a DAR of 4.0. HDP-201 potency was tested in cytotoxicity assays using a GCC-overexpressing HEK293-GUCY2C cell line. The anti-tumor efficacy was evaluated in subcutaneous HEK293-GUCY2C tumor models in female NOD SCID mice, and patient-derived xenografts (PDX) treated intravenously with single and multiple doses of HDP-201. Safety and tolerability of HDP-201 was assessed in cynomolgus monkeys.
Results: HDP-201 showed favorable *in vitro* cytotoxicity, with nanomolar activity against the GCC-expressing cell line and no cytotoxicity against target-negative cells. A potent by-stander effect of HDP-201 was demonstrated in co-culture experiments with target-positive and -negative cells, indicating its potential to treat tumors with heterogenous target expression. In mouse xenograft models, intravenous HDP-201 single dose treatment resulted in complete tumor remission in HEK293-GUCY2C xenografts. In gastrointestinal PDX models, HDP-201 significantly inhibited tumor growth. Multiple dosing further enhanced this anti-tumor efficacy without impacting toxicity. In cynomolgus monkeys, a single intravenous dose of 60 mg/kg was well tolerated without severe clinical signs and only transient changes in hematology and serum chemistry parameters.
Conclusion: Targeted delivery of topoisomerase 1 inhibitor to GCC-positive cells was achieved using HDP-201 comprised of an anti-GCC antibody conjugated to a multimeric linker-exatecan. HDP-201 showed target-specific potency, strong anti-tumor efficacy, and high tolerability in preclinical models. The use of HDP-201 in the therapy of GCC-positive gastrointestinal malignancies represents a promising approach in cancer treatment.

#1559 Assessing efficacy of anti-glypican-1antibody-drug conjugate as potential therapeutic approach for gastric cancer.

Y. Suzuki[1], S. Serada[1], K. Kawabata[2], T. Takahashi[2], Y. Doki[2], T. Naka[1];
[1]Iwate Medical University, Yahaba, Japan, [2]Osaka University, Osaka, Japan

Background: Despite significant advancements in cancer treatment, gastric cancer (GC) still has a high incidence and mortality rate. Antibody-drug conjugates (ADCs) are emerging as viable treatment option for GC. We previously identified glypican-1 (GPC1) as a novel cancer antigen in esophageal squamous cell carcinoma using quantitative protein expression profiling analysis focused on plasma membrane proteins, and recognized it as a potential target for ADCs.Glypican-1 (GPC1), a cell surface proteoglycan, has gained interest for its role in tumor growth and as a potential biomarker. This study evaluated the efficacy of an ADC comprising a humanized anti-GPC1 monoclonal antibody with monomethyl auristatin E (GPC1-ADC) to target GPC1 in GC.
Methods: GPC1 expression was assessed in GC cell lines and tissues. The cytotoxic efficacy of GPC1-ADC and its mechanism of action were evaluated using comprehensive *in vitro* assays. Further, *in vivo* efficacy was assessed in xenograft mouse models, focusing on tumor growth inhibition and its performance against peritoneal dissemination.
Results: *In vitro,* GPC1-ADC exhibited significant cytotoxicity against GPC1-positive cell lines by inducing cell cycle arrest and apoptosis. Additionally, GPC1-ADC exhibited promising bystander-killing activity through cancer-associated fibroblasts, underscoring its potential to target the heterogeneous tumor cell microenvironment characteristics of GC. *In vivo*, GPC1-ADC significantly inhibited tumor growth, demonstrating a substantial therapeutic effect in a peritoneal dissemination model. Immunohistochemical analysis of tumor specimens from patients with GC revealed that 90.2% of differentiated types and 66.7% of poorly differentiated types exhibit high GPC1 expression. High GPC1 expression in tumor samples was significantly related to poorer clinical outcomes for progression-free and overall survival.
Conclusions: GPC1-ADC exerts a direct antitumor effect on GPC1-positive GC cells. Additionally, GPC1-ADC can impact GPC1-negative cells via GPC1-positive stromal cells. This mechanism is likely owing to the characteristics of MMAE, which has a bystander effect as the payload of GPC1-ADC. Our results suggest that GPC1-ADC may be a potential therapeutic candidate for GPC1-positive GC.

#1560 SCR-A0011, a novel bispecific ADC targeting cMET and B7H3, exhibits potent anti-tumor efficacy.

Yayuan Fu, Guangcun Cheng, Hui Zheng, Shuai Wang, Lei Jiang, Chunlei Xia, Huipeng Guo, Xiyuan Wang, Renhong Tang

Simcere Zaiming, State Key Laboratory of Neurology and Oncology Drug Development. Simcere Pharmaceutical Group, Shanghai, China

Background: The HGF/cMET signaling pathway plays an important role in the regulation of cell proliferation, differentiation, migration and other normal physiological functions. cMET is expressed in many different normal tissues but is usually expressed at low levels. Abnormal expression and dysfunction of cMET have been found in a variety of tumors. cMET is a well-established target in clinical, but its clinical application is limited, because clinical efficacy was mainly observed in cMET signaling driving tumor or cMET-high expression tumors. To extend the potential therapeutic population and anti-tumor activity, we searching for some new approaches, such as bispecific antibody drug conjugates. Bioinformatics analysis and translational research showed that cMET and B7H3 are co-expressed in multiple types of solid tumors without high expression in normal tissues. Therefore, targeting both B7H3 and cMET could be a new and powerful strategy in pan-tumor therapy.

Methods & Results: We developed a novel B7H3xcMET targeted bispecific antibody-drug conjugate (ADC) called SCR-A0011, which is comprised of B7H3/cMET bispecific antibody conjugated with novel topoisomerase 1 inhibitor (CPT116). SCR-A0011 specifically bind B7H3 and cMET with high affinity, and showed enhanced internalization and binding capacity compared to parental monoclonal antibody ADC separately in the B7H3/cMET co-expressing cell line. SCR-A0011 induced tumor cell lysis in various tumor cell types including NSCLC, CRC, OC, HNSCC, etc. SCR-A0011 showed remarkable anti-tumor efficacy in several CDX models in a dose dependent manner. SCR-A0011 exhibits significantly stronger anti-tumor activity than their parental monoclonal-ADC or clinical ADC in B7H3+cMET+ double positive tumor cells. SCR-A0011 also have very good stability and favorable developability.

Conclusion: These findings illustrated SCR-A011 could be a potential First-in-Class BsADC to conquer the resistance or non-response of current treatment in broad cancer types and may provide a new therapeutic approach for B7H3 and cMET positive cancer patients in the future.

#1561 Antitumor effect of farletuzumab ecteribulin in molecular subtypes of endometrial cancer patient-derived xenograft models.

Shigehiro Yagishita[1], Sho Sato[2], Daisuke Shintani[2], Tadaaki Nishikawa[3], Aiko Ogasawara[2], Masanori Yasuda[4], Hironori Ishii[5], Keiji Furuuchi[6], Toshimitsu Uenaka[6], Hiroshi Yoshida[7], Kosei Hasegawa[2], Akinobu Hamada[1]

[1]National Cancer Center Japan, Tokyo, Japan,[2]Department of Gynecologic Oncology, Saitama Medical University International Medical Center, Saitama, Japan,[3]Department of Medical Oncology, National Cancer Center Japan, Tokyo, Japan,[4]Department of Pathology, Saitama Medical University International Medical Center, Saitama, Japan,[5]Precision Medicine Business Unit, Konica Minolta, Tokyo, Japan,[6]Eisai Inc., Exton, PA,[7]Department of Diagnostic Pathology, National Cancer Center Japan, Tokyo, Japan

Background:Endometrial cancer (EC) represents a significant health burden globally, particularly in postmenopausal women. Current treatment options for advanced-stage EC remain limited, emphasizing the need for novel therapeutic strategies. This study aimed to investigate farletuzumab ecteribulin (FZEC), an antibody-drug conjugate (ADC) targeting folate receptor alpha (FRα), as a potential new therapeutic agent for EC using tumor models. Methods:We utilized a panel of 24 patient-derived xenograft (PDX) models, representing various histological and molecular subtypes of EC with different levels of FRα expression, to evaluate the antitumor effect of FZEC. FZEC was administered intravenously at doses of 5 mg/kg and 12.5 mg/kg. Intratumoral accumulation of eribulin, the payload of FZEC, was visualized using phosphor-integrated dot imaging. Results:FZEC demonstrated dose-dependent antitumor effects across the EC-PDX panel. At 5 mg/kg, the FZEC efficacy was associated with FRα expression, with 100% of FRα 3+ models exhibiting tumor shrinkage compared to 27.2% of FRα-negative models. FZEC also demonstrated broad activity across both histological and molecular subtypes of EC. Intratumoral eribulin accumulation was highly correlated with antitumor effects, even in models with low FRα expression. Follow-up studies confirmed FRα-dependent antitumor effects, while also indicating potential FRα-independent mechanisms of action. Conclusion:FZEC demonstrated robust antitumor effect against the FRα high EC-PDX models. Importantly, significant antitumor effects were observed, even in FRα-low or negative models. Intratumoral eribulin accumulation exhibited a stronger correlation with efficacy than with FRα expression alone. These results support further clinical development of FZEC for EC treatment and highlight the complexity of ADC mechanisms of action.

#1562 Pre-clinical research of ZHB015, an anti-CD26/CD3 bispecific T-cell-engaging antibody.

Chennan Ge, Peipei Cao, Jianhua Hang, Tao Zhang, Yu Zhuang, Na Ding, Bruce Y. Ma

Zonhon Biopharma Institute, Inc., Changzhou, China

PURPOSE: CD26 is a highly expressed transmembrane target in multiple solid tumors such as renal cell carcinoma, prostate cancer and malignant pleural mesothelioma. YS110 is the first reported monoclonal antibody targeting CD26 and has been proved safe in phase I/II clinical trials in Japan and France, but the efficacy is not satisfying. Although FDA approved axitinib in combination with avelumab as the first-line treatment for PD-L1 positive advanced renal cell carcinoma in 2019, the problems still exist such as individual differences and drug tolerance. Consequently, we developed ZHB015, a bispecific T-Cell-Engaging antibody that targets CD26 high-expressing solid tumors, and furtherly to validate its efficacy and safety.

METHODS: ZHB015, which is expressed and manufactured from recombinant CHO cells, has a tandem single chain variable fragment antibody (BiTE) structure. CD26 expression level in tumor cells was detected by FACS. CD26 expression in tumor tissues was detected by immunohistochemistry. The affinity between ZHB015 with human CD26 or CD3 were detected by Octet. The killing effect of ZHB015-mediated PBMC on tumor cells was detected by Calcein-AM release. ELISA was used to detect the cytokine secretion of ZHB015 in the process of mediating the killing effect. The antitumor activity in vivo was studied in mouse xenograft models. Daily intravenous administration dosage of ZHB015 was 1.5mg/kg. Both axitinib in combination with avelumab and YS110 were served as control in vivo.

RESULTS: ZHB015 pilot production process was performed and confirmed good scale-up feasibility. The positive rates of CD26 in 786-0, 0S-RC-2 and A498 renal cancer cells were all over 95%. 99.4% mesothelioma cell NCI-H226 and 93.8% prostate cancer cell PC-3 were CD26 positive. IHC experiments confirmed that CD26 is highly expressed in renal cancer tissues. ZHB015 has an affinity of 0.91nM with CD26 and 0.75nM with human CD3. ZHB015 can bind to both CD26 and CD3 simultaneously. 786-0, OS-RC-2, NCI-H226, and PC-3 CD26 expressing tumor cells were lysed with EC_{50} values in a range of 219.6 to 752.3pg/ml, and the killing activity was significantly higher than homemade YS110. In the process of mediating PBMC to kill target cells, ZHB015 induced a lower cytokines secretion than control molecule YS110-BiTE. In the xenograft models of OS-RC-2 and A498, TGI of ZHB015 was 100%, TGI of different doses of axitinib in combination with avelumab did not exceed 71%, and YS110 had no antitumor effect. The TGI of ZHB015 in PC-3 and NC1-H226 models was 96% and 66%, respectively.

CONCLUSION: ZHB015 simultaneously binds to tumor CD26 and T cell CD3, inducing T cell mediated antitumor effects. ZHB015 has an outstanding anti-tumor activity in vitro and in vivo, which is superior to YS110 and the current first line treatment. Thus, ZHB015 is being further developed as a potential therapy for CD26 positive solid tumor.

#1563 ADRX-0134 as a novel auristatin-based NaPi2b antibody-drug conjugate with widened therapeutic window.

Andrew M. Hau, Kris Zhang, Maria Shahmoradgoli, Mario M.-C. Kuo, Dong Jun Lee, Anna Y. Wang, Peter P. Challita, Oscar Betancourt, Wes Sisson, Erin Nye, Monica Leung, Albert Goldson, Felipe Acosta, Alexander Chu-Kung, Hui Li, Sabine Rottmann, Pia M. Challita-Eid

Adcentrx Therapeutics, Inc., San Diego, CA

Sodium-dependent phosphate transport protein 2B (NaPi2b; SLC34A2) is overexpressed in a variety of cancers, including lung carcinomas, ovarian, and endometrial cancers with restricted normal tissue expression. Based on this differential expression profile, a proof-of-concept for an antibody-drug conjugate (ADC) approach utilizing NaPi2b was achieved with lifastuzumab vedotin and upifitamab rilsodotin. Both ADCs used auristatin tubulin inhibitor payloads but have been discontinued due to adverse toxicities driven by the linker-payload technologies, resulting in reduced drug exposures impacting efficacy. To overcome these limitations in therapeutic window, Adcentrx has developed ADRX-0134, a NaPi2b-targeting next-generation ADC with a drug-antibody ratio (DAR) of 8. ADRX-0134 is conjugated to a novel tubulin inhibitor payload AP052 with a proprietary cleavable linker utilizing Adcentrx' proprietary i-Conjugation® technology, resulting in an ADC with refined biophysical features. The antibody of ADRX-0134 was selected based on its strong nanomolar and highly specific cell binding affinity and similar cross-reactivity to cynomolgus monkey and rodent orthologs. The ADC exhibits sub-nanomolar cytotoxic activity to NaPi2b-expressing tumor cells and has a very stable PK profile with minimal deconjugation. This translates to the observed strong efficacy and durable tumor regression in mouse models of ovarian, endometrial and non-small cell lung cancers with high as well as low/heterogenous NaPi2b expression. Most notably, ADRX-0134 achieved tumor regression in settings where deruxtecan- and vedotin-based NaPi2b ADCs were insufficient. Besides the improvement in efficacy, ADRX-0134 was highly tolerated in a repeat dose non-human primate study at up to 18 mg/kg, the highest tested dose. Here, the stable TK profile ensured minimal and recoverable hematology findings, an unchanged clinical chemistry profile and normal histology of lung, liver, GI and eye, which were key safety liabilities for other NaPi2b ADCs. In summary, ADRX-0134 exhibits excellent biophysical features with highly selective and increased efficacy in NaPi2b-expressing tumors, while being well-tolerated in cynomolgus monkeys at doses that are expected to provide a widened therapeutic window over first-generation NaPi2b ADCs.

#1564 Receptor quantification in the context of human epidermal growth factor receptor 2 (HER2) antibody-drug conjugates.

Mandy W. E. Drossaert-Smeets, Eef F. P. Smits, Milan M. Bolwerk, Janneke J. T. Melis, Dimitri Pappaioannou, **Guido J. R. Zaman**

Oncolines B.V., Oss, Netherlands

Antibody-drug conjugates (ADCs), comprised of cancer cell-specific antibodies linked to cytotoxic drugs, represent an innovative and promising class of anticancer agents. ADCs often rely on high receptor numbers for target binding, followed by internalization and release of the payload to exert a tumor-targeted, intracellular effect. Determination of the exact number of receptors per cell is therefore highly valuable in the context of ADCs. Here, we focused on the quantification of the human epidermal growth factor receptor 2 (HER2), since expression of this target is often amplified in various tumor types. Two HER2-targeting ADCs, *i.e.,* ado-trastuzumab emtansine (T-DM1) and fam-trastuzumab deruxtecan-nxki (T-DXd), have already been approved for clinical use, while roughly twenty HER2-targeting ADCs are currently under investigation. The development and improvement of ADCs could benefit from *in vitro* studies investigating the internalization, cytotoxic effect and possible bystander effect of ADCs related to receptor numbers. The number of HER2 per cell was quantified in a panel of thirteen cancer cell lines originating from different cancer types. The cell lines BxPC-3, CAL-27, COLO 205, HCC1954, HEC-251, K-562, MOLM-13, NCI-H23, RT-112, SK-BR-3, SK-OV-3, SW837 and U-87MG ATCC were cultured for one week in the supplier's recommended medium. Quantification of the number of surface-expressed receptors was performed with flow cytometry using Quantibrite PE beads and PE-conjugated ErbB2/HER2 antibody. The effect of T-DM1 and T-DXd on the viability of HER2 high-, intermediate- and low-expressing cells was determined in proliferation assays in 9-point dose ranges. Three independent flow cytometry experiments revealed that the breast and ovarian cancer cell lines HCC1954, SK-BR-3, and SK-OV-3 expressed the highest number of HER2 per cell, ranging between 20,000 and 53,000 receptors. Intermediate expression was observed on the COLO 205, HEC-251, RT-112 and SW837 cell lines, with a range of 1800 to 3800 receptors per cell. BxPC-3, CAL-27, K-562, MOLM-13, NCI-H23 and U-87MG ATCC belong to the group with the lowest or no expression of HER2, ranging up to 400 receptors per cell. T-DM1 and T-DXd induced efficient cell death in cells expressing high receptor numbers, while little or no effect was observed in cell lines with intermediate, low or no expression of HER2. Since high target expression is correlated with higher efficacy for several ADCs, as shown for T-DM1 and T-DXd is our study, this indicates that HCC1954, SK-BR-3 or SK-OV-3 cells could serve as an attractive model to study HER2 internalization. Moreover, co-cultures of HER2-positive and HER2-negative cells could aid in the investigation of ADC bystander effect. This research demonstrates the importance of receptor number quantification in the context of ADCs and may therefore contribute to future ADC development.

#1565 Design and development of biparatopic antibody-drug conjugates against protein tyrosine kinase 7.

Luying Yang, **Vincent Fung**, Alex Wu, Dunja Urosev, Saki Konomura, Tik Nga Tong, Katina Mak, Elizabeth M. Porter, Diego A. Alonzo, Catrina M. J. Kim, Janice P. H. Tsui, Linglan Fu, Lemlem Degefie, Kaylee Wu, Matthew Bonderud, Jamie R. Rich, Stuart D. Barnscher

Zymeworks Inc., Vancouver, BC, Canada

Background: Biparatopic antibodies and antibody-drug conjugates (ADCs) can provide many functional benefits over their traditional monoparatopic counterparts including increased binding, greater cell surface decoration, superior receptor blockade, and enhanced internalization and payload delivery; however, not all antigens are amenable to biparatopic targeting. We sought to leverage our prior knowledge of biparatopic antibody development and our Azymetric™ technology to develop a novel biparatopic ADC against protein tyrosine kinase 7 (PTK7) that could provide a functional enhancement over monoparatopic ADCs. PTK7 is an attractive ADC target due to preliminary clinical activity demonstrated with cofetuzumab peledotin in non-small cell lung cancer, ovarian cancer, and metastatic breast cancer and evidence that PTK7 is overexpressed in other solid tumor indications including esophageal cancer, colorectal cancer, head and neck cancer, and cervical cancer.

Materials and Methods: Twenty-nine PTK7-targeting paratopes were isolated from a mouse antibody discovery campaign and paratope pairs were combined into a panel of biparatopic antibodies using Azymetric™ Het_Fc mutations. A high-throughput screen was used to assess the functional properties of these antibodies and to identify the top paratope combinations. ADCs were produced by conjugating the top biparatopic and monoparatopic antibodies to ZD06519, a topoisomerase 1 inhibitor payload used in ZW191, an ADC targeting FRα under evaluation in a phase 1 clinical trial (NCT06555744). ADC activity was investigated in a series of in vitro and in vivo experiments using ovarian, lung, and breast cancer models.

Results: Across multiple cancer cell lines with varying PTK7 expression we compared a lead monoparatopic antibody and cofetuzumab with the top biparatopic antibody which demonstrated greater cell surface decoration and better internalization. At clinically relevant doses, the biparatopic ZD06519 ADC was more efficacious than cofetuzumab pelidotin in triple negative breast cancer and lung cancer cell line derived xenograft models. Additional studies of the PTK7 biparatopic ADC in patient-derived xenograft models and a non-human primate tolerability study are planned.

#1566 A novel drug discovery platform identifies epitopes on driver receptors that can be targeted to induce widespread kinase inhibition and cancer cell death.

Rachel S. Welt[1], Virginia Raymond[2], David Kostyal[3], Sydney Welt[1]

[1]Welt Bio-Molecular Pharmaceutical, Briarcliff Manor, NY,[2]Biogent Consultants, Armonk, NY,[3]ARDL, Akron, OH

Welt Bio-Molecular Pharmaceutical (WBMP) has developed a drug discovery platform that identifies critical peptide segments of membrane receptors, which, when targeted with an antibody, block transmembrane signal transmission, leading to widespread kinase inhibition. This platform was first used to generate our lead monoclonal antibody (mAb), WBMP-4, which binds to a region on membrane-bound IgM, a subunit of the B-cell Receptor (BCR), and induces cell growth inhibition (at low doses) and apoptosis (at high doses) in IgM-expressing B-cell malignancies. Here we present the results of kinome and protein expression studies that characterize the mechanism of action of WBMP-4 and validate the algorithm underlying our platform.

We evaluated kinase activity in lymphoma cells treated with WBMP-4 (at 5 and 20 ug/mL, and at 6 and 24 hours) via the PamGene phosphotyrosine kinase assay. This assay measures kinase activity in cell lysates using a microarray chip containing peptide sequences that serve as substrate for 189 phosphotyrosine sites on well-characterized signaling proteins. Additionally, we measured protein expression and phosphorylation of key targets downstream of the BCR with western blot analyses of WBMP-4 treated and untreated IgM-expressing B-cells.

Our detailed kinome analysis reveals widespread kinase modulation following WBMP-4 treatment, with >180 of 189 phosphosites showing a significant change in phosphorylation signal intensity (>153 significantly decreased) upon treatment. Western blot analyses similarly show changes in phosphorylation of kinases (ZAP70, mTOR, AKT, and MEK). However, we also find decreased protein expression of CD79b, ZAP70, BTK, MEK, Myc, and increased LCK following WBMP-4 treatment.

These results show that kinase activity across signaling pathways is altered upon WBMP-4 binding to the BCR's mIgM, with the vast majority exhibiting a downregulation of phosphorylation. We also find that protein expression is impacted in several kinases directly downstream of the BCR. We hypothesize that kinase inhibition induced by WBMP-4 modulates transcription factor activity, and resultantly, protein expression, which maintains growth inhibition signaling and prevents recovery signals, determining the fate of the cells. Further analysis will assess earlier time points and lower dosages to examine the more immediate effect of WBMP-4 inhibition.

WBMP-4's global kinase inhibition encompasses the effects of approved kinase inhibitor drugs (i.e. BTK, PI3K, and Bcl-2 inhibitors), and is designed to be more tumor-restrictive; WBMP-4 only affects IgM-expressing B-cells, sparing healthy B-cells that express other classes of Ig (i.e. IgG, IgA, IgD, or IgE). This tumor specificity and potent inhibitory effects make WBMP-4 a promising clinical candidate.

#1567 GLR1059, next-generation nectin-4-targeted ADC with a novel mechanism-of-action payload, demonstrated significantly potent anti-tumor efficacy and reduced toxicity in preclinical evaluation.

J. Jiao, Y. Sun, J. Wang, L. Che, M. Cui, W. Shao, H. Yuan, Y. Huang, N. Zhai, Y. Tang, X. Zheng, S. Li;
Gan & Lee Pharmaceuticals, Beijing, China

Background: Nectin-4 is a tumor-associated antigen, which was observed in multiple cancer types, including bladder, breast, lung, pancreatic and ovarian cancer. Enfortumab Vedotin (EV) is the first approved nectin-4 antibody-drug conjugate (ADC) with MMAE as its payload for the treatment of urothelial carcinoma (UC). However, beyond UC, EV hasn't been approved for other indications. In addition, severe skin adverse reactions were reported in EV-treated patients. There are several new nectin-4 ADCs in development but haven't entered confirmative clinical studies so far. To overcome the limitations of efficacy and safety, we developed a novel mechanism nectin-4 ADC GLR1059, with a payload that was more effective for potential indications with nectin-4 expression and had lower toxicity, including skin adverse reactions.
Methods: For in vitro studies, binding affinity and specificity, internalization, cancer cell killing, bystander effect and serum stability were performed. For in vivo studies, efficacy studies were conducted on cell line-derived xenografts (CDX) of multiple cancer types, e.g. breast cancer (including triple-negative breast cancer), and urothelial carcinoma. Pharmacokinetic (PK) properties were studied in BALB/c mice and tumor-bearing nude mice, and safety studies were conducted in humanized nectin-4 mice.
Results: GLR1059 is a nectin-4 ADC composed of a novel humanized IgG1 monoclonal antibody, a cleavable linker and a microtubule targeting agent (not auristatin derivatives). Moreover, to generate DAR4 ADCs with higher homogeneity, the payload was site-specifically conjugated to the antibody via a disulfide bond bridge. In surface plasmon resonance (SPR) assays, GLR1059 had strong and specific binding to human nectin-4, and it did not bind to nectin-1, nectin-2, or nectin-3. Furthermore, GLR1059 exhibited efficient internalization and potent activity in nectin-4 expressing cell lines and effective bystander activity. In a panel of CDX models, GLR1059 demonstrated compelling anti-tumor activity across bladder cancer and breast cancer (including TNBC), with tumor growth inhibition rates (TGI) significantly higher than EV (approximately 2-4 times). In the tumor-bearing mice PK study, the Cmax of free payload in tumor tissue was approximately 100-fold higher than that in the blood (131±14ng/g vs 1.04±0.27ng/mL). GLR1059 demonstrated longer survival and less weight loss in the single- and repeated-dose toxicity studies in humanized nectin-4 mice. Notably, GLR1059 significantly reduced the incidence and severity of skin toxicity compared with EV (1/6 vs 6/6).
Conclusion: Preclinical data show that GLR1059 has the potential to achieve better efficacy and safety, and the different drug mechanisms provide new options to overcome drug resistance of current nectin-4 ADCs.

#1568 Humanized PD1 mouse with MC38-hCLDN18.2 cell line provide powerful syngeneic model platform for evaluating Claudin18.2-targeted therapies.

Jane Hong[1], Shuang Li[2], Xiaolei Qiu[1], Hechun Ma[1], Yi Li[1], Ruilin Sun[1]

[1]GenoBioTX, Sugar Land, TX,[2]GenoBioTX, Sugar land, TX

Claudin18.2 is a highly specific tight junction protein that is minimally expressed in normal tissues but aberrantly activated and overexpressed in various malignancies, particularly digestive system tumors such as gastric cancer (approximately 70%) and pancreatic cancer (around 50%). In these tumors, the loss of cell polarity exposes Claudin18.2 on the cell surface, making it accessible to biologics and effector cells. Innovative therapeutic strategies, including monoclonal antibodies, bispecific antibodies, ADCs, and CAR-T cells, leverage this accessibility to enhance tumor-targeting precision and immune activation. Combining Claudin18.2-targeted therapies with immune checkpoint inhibitors, such as PD-1 blockade, holds promising therapeutic potential.To evaluate the efficacy of antibody drugs and ADCs, tumor cell lines overexpressing tumor-associated antigens are critical. We generated MC38-CLDN18.2 murine colon cancer cell lines by overexpressing human CLDN18.2 in MC38 cells. Human CLDN18.2 protein expression was confirmed by flow cytometry. The tumorigenic potential of MC38-CLDN18.2 cells was further validated in vivo.To investigate novel combination therapies or bispecific antibodies targeting CLDN18.2 and PD-1 in an immunocompetent model, we developed a humanized PD-1 (hPD1) mouse model on a C57BL/6 (C57) background. Using CRISPR/Cas9-based gene engineering, the murine *Pdcd1* gene was replaced with the human *PDCD1* gene, ensuring full-length human PD-1 protein expression in the knock-in model. Human PD-1 protein expression was validated by FACS analysis after in vivo anti-CD3 stimulation.The tumor growth inhibition of two CLDN18.2 monoclonal antibodies (IMAB362 and ASKB589) was evaluated in hPD1 mice engrafted with MC38-CLDN18.2 syngeneic tumors. The therapeutic effects of these antibodies were also investigated in combination with anti-PD-1 treatment. Both anti-CLDN18.2 monotherapies demonstrated moderate antitumor efficacy, while their combination with PD-1/PD-L1 immune checkpoint blockade produced significant synergistic effects, resulting in enhanced tumor suppression and increased antitumor immunity with robust T-cell infiltration.In summary, the humanized PD-1 mouse model and MC38-CLDN18.2 cell line provide robust in vivo platforms to evaluate novel therapeutic candidates targeting CLDN18.2.

#1569 Dual action tumor immune agonist (DATIA) ADCs activating STING pathway and subsequently inducing cytotoxicity of tumor cells exhibits potent and lasting in vivo tumor growth inhibition in multiple preclinical tumor antigen positive cancer models.

Yanliang Zhang, Chunlan Dong, Guangming Chen, Qianxiang Zhou, Tony Zhang

Tyligand Bioscience (Shanghai), Ltd., Shanghai, China

Introduction : TSN222, currently in Phase 1 clinical development for solid tumors, is a novel dual action tumor immune agonist (DATIA) via intratumoral administration. Its half-lives inside cells and in plasma were designed to be long enough for it to activate tumor STING pathways, and short enough to limit systemic inflammation. Its active metabolite is a DNA synthesis inhibitor, which subsequently induces apoptosis of the tumor cells and provides neoantigens for the emerging adaptive immune responses. The sequential dual actions of type I interferon production through STING pathway activation and latent cytotoxicity connects the innate and adaptive immune systems.
Method : Antibody drug conjugates (ADCs) employing TSN222 as a payload were characterized for their activities in STING pathway activation and tumor killing by modulating the tumor microenvironment and adaptive immunity against the tumors, through selective tumor targeting by antibody and more convenient administration of i.v. injection.
Results : TSN222 in cell-based assays has shown STING pathway stimulating activity that leads to type I interferon secretion and subsequent cytotoxicity to the cells. TSN222 is well tolerated in preclinical toxicology studies by s.c. administration and Phase 1 clinical trial by i.t. administration. One case of partial response (PR) has been observed during dose escalation at 800μg dosage level. Antibodies against Her2, Trop2 and Claudin18.2, respectively conjugated with TSN222 as a payload, have shown potent tumor growth inhibition (TGI) in antigen specific cancer cell lines and animal models. For example, a single i.v. dose of anti Her2 DATIA ADC at 0.6 mpk in SK-OV-3 her2+ CDX model afforded 109% TGI after 22 days, while the payload TSN222 at 1 mpk i.t. QWx3 gave a TGI of 21%. In EMT6-huHer2+ breast cancer model, a single i.v. dose of the ADC at 3 mpk induced complete remission (CR) at day 20. Upon rechallenging of the CR mice at day 33 with EMT6-huHer2+ and CT26 colorectal tumor cells, TGIs were 64% and -23 % relative to that in naïve mice, respectively, indicating tumor specific immunity was induced in the EMT6-huHer2+ mice.
Conclusion : We have developed a tumor targeting immune stimulating ADC platform with novel mechanism of action that differentiates from other immune stimulating ADC approaches. The dual action of STING pathway activation and subsequent induction of apoptotic signals in tumor cells by the moderately toxic metabolite renders timely presentation of antigens to bridge the innate and adaptive immune system. With the preclinical proof-of-concept studies and ongoing clinical trial data of the payload to inform on safety and tolerability, the DATIA ADC platform is positioned to serve as a distinctly different approach for better safety and efficacy.

#1570 PermaLink®, a stable and scalable bioconjugation platform as an alternative to maleimide-based conjugation.

Adam Lodge, Justyna Mysliwy, **Will A. Stanley**, James Stephenson, Sarah Robinson, Alexander Sanders, Robert Murawski, Bryony Moss, Patrick Higgs, Jutta Deckert, Jenny Thirlway, Robert J. Lutz

Iksuda Therapeutics Ltd., Newcastle Upon Tyne, United Kingdom

IKSUDA Therapeutics' proprietary PermaLink® platform comprises simple, stable, and adaptable conjugation chemistry that overcomes the instabilities associated with the retro-Michael exchange of traditional maleimide-based chemistry. This platform enables ADC generation with a range of drug-to-antibody ratios (DAR) and can be applied to multiple payloads and linker chemistries to improve conjugate properties and maximize the therapeutic index of ADC therapeutics.

ADCs are generated by reducing the hinge region disulfides and/or decapping engineered cysteines with TCEP, conjugation with PL-payload followed by purification. DAR was measured by HPLC. The stability of PL-based ADCs was compared to traditional maleimide-based ADCs in murine and human plasma. In vitro activity and bystander effect were assessed using mono or co-culture experiments by CellTiter-Glo assay. In vivo efficacy studies were conducted in SCID mice with human cancer xenografts. Toxicology and PK studies were conducted in cynomolgus monkeys.

PL-based conjugation followed a simple process of reducing the antibody disulfides with TCEP, conjugation with PL-payload and removal of excess linker toxin by size exclusion chromatography, desalting or filtration. Control of process parameters such as TCEP and linker-payload excesses yielded conjugates with DARs ranging from 4-8 for native wildtype antibodies. Additionally, DAR 10 was achieved for an antibody with two engineered cysteines. The resulting ADCs demonstrated favorable biophysical properties with minimal aggregation by SEC and DAR assessment was consistent by PLRP-HPLC and MS. Typical yields at the research scale ranged from 70-90%.

For preclinical proof-of-concept, MMAE conjugates of Isumab01, targeting FRA, were prepared with a protease-cleavable valine-citrulline (vc) and enzymatically cleavable β-glucuronide (glu) linker. Both PL-based conjugates showed excellent stability in murine and human plasma, whereas FDA-approved maleimide-based ADCs brentuximab vedotin and trastuzumab deruxtecan displayed a significant decrease in DAR. PL-MMAE conjugates were highly potent against JEG-3 cancer cells in vitro, with IC50 values in the pM range. Isumab01-PL-glu-MMAE resulted in complete tumor regression in a JEG-3 xenograft model following a single dose of 2.5 mg/kg and was very well tolerated in cynomolgus monkeys at 12 mg/kg single dose and 10 mg/kg Q3W x2 (highest doses tested), showing improved tolerability over MMAE-based ADCs with maleimide-cathepsin B cleavable linkers. The Isumab01-PL-glu-MMAE conjugate demonstrates a favorable PK profile in monkeys with high stability in circulation consistent with improved bioconjugation.

PermaLink offers a simple, stable alternative to maleimide-based ADC bioconjugation moieties and confers a favorable preclinical anti-tumor activity and safety profile

#1571 Discovery of c-MET degrading bispecific antibodies (EpiTACs) for NSCLC and other c-MET driven tumors.

Jonathan Sitrin, Hai Tran, Lisa Marshall, Kenneth Ng, May Dayao, Jacob Cohen, Kimberly Hoi, Josef Gramespacher, Zhong Huang, Andy Goodrich, Kevin Carlin, Noah Solomon, Shruti Yadav, Aleysha Chen, Katarina Pance, Rami Hannoush, Maia Vinogradova, Isaac J. Rondon, Shyra J. Gardai

EpiBiologics, San Mateo, CA

Extracellular targeted protein degradation (eTPD) has emerged as a promising new drug modality focused on the elimination of pathogenic extracellular and transmembrane proteins. In contrast to intracellular degraders, which require the ubiquitin-proteosome system, extracellular degraders can also harness endosomal-lysosomal protein degradation pathways. EpiTACs are a novel class of bispecific antibody-based eTPD therapeutic, in which one arm binds a target of interest, and the other arm binds a degrading receptor. EpiTACs leverage a novel atlas of disease- and tissue-enriched degrading receptors comprised of transmembrane ubiquitin ligases, cytokine/chemokine receptors, and internalizing receptors, resulting in selective protein degradation. EpiTACs are agnostic of oncogenic mutation status and can deeply antagonize oncogenic signaling through removal of the target and its associated scaffolding function. They also have advantageous pharmacological and drug-like manufacturing properties compared to other degradation technologies. We developed EpiTACs against oncogenic receptor tyrosine kinase family members including cellular mesenchymal-epithelial transition (c-MET) and epidermal growth factor receptor (EGFR). Genomic alterations of c-MET are oncogenic in a variety of cancers, including non-small cell lung cancer (NSCLC), where small molecule inhibitors have been clinically approved for exon 14 skipping mutations. Aberrations in c-MET have also been reported in gastric, colorectal, renal, pancreatic, skin and brain cancers. However, therapeutic benefit to patients has been limited by the broad array of primary and acquired c-MET mutations. Biologics against this target have demonstrated insufficient activity, likely related to antibody-mediated receptor dimerization, and associated pro-oncogenic signaling. A bioinformatics-informed set of cancer-focused degrading receptors was screened in tumor cell-based assays to identify degraders for c-MET. Top hits were enriched in NSCLC and other overexpressed/amplified tumors. c-MET degraders were overlapping and unique compared to previously reported screens conducted against EGFR. They drove internalization and degradation of c-MET in multiple tumor cell-based assays, including those carrying exon 14 skipping mutations and amplifications. Degradation of c-MET robustly inhibited tumor cells in vitro, and strongly suppressed patient-derived xenograft tumor models in vivo. These newly discovered degraders are being paired with novel c-MET binders that lack dimerization-induced signaling capacity, to create safer and more efficacious c-MET degrading therapeutics. In conclusion, eTPD represents a promising new drug modality, and EpiTACs have expanded the toolbox of extracellular targeted protein degraders to address challenging drug targets with a new approach.

#1572 Preclinical activity and safety of STRO-004, a novel ADC targeting tissue factor for solid tumors.

Andrew McGeehan, Helena Kiefel, Brian Vuillemenot, Guifen Xu, Sihong Zhou, Grace Lee, Mark Armanini, Xiaofan Li, Krishna Bajjuri, Miao Wen, Cuong Tran, Jeff Hanson, Daniel Calarese, Amandeep Gakhal, Hanspeter Gerber, Werner Rubas, Gang Yin, **Alice Yam**

Sutro Biopharma, Inc., South San Francisco, CA

Tissue Factor (TF) is a type I transmembrane protein that is aberrantly expressed in multiple solid tumor indications including cervical, head and neck, non-small cell lung, and pancreatic cancers. Its expression is associated with poor prognosis and has been linked to pro-tumorigenic activities such as metastasis, inflammation, and angiogenesis. Under normal physiological conditions, TF plays an important role in blood clotting and, as such, is broadly expressed in many normal tissues, typically in the subendothelium. Upon endothelial damage, TF can combine with clotting factors in circulation to initiate a coagulation cascade, thus constituting a "hemostatic envelope". TF-targeted vedotin ADCs are approved in metastatic cervical cancer and in clinical testing across a range of solid tumor indications. However, toxicities such as peripheral neuropathy, epistasis, and ocular toxicities have been dose-limiting. We have developed STRO-004, a novel TF-targeted ADC with a DAR8 β-glucoronidase-exatecan linker-payload, with enhanced potency and safety in preclinical models. STRO-004 is a TF-targeted ADC, engineered for a wider therapeutic index. The ADC is constructed on an antibody that has been designed to avoid interference in the blood coagulation pathways, thus reducing the potential risk for bleeding. The anti-TF antibody is conjugated to exatecan, a topoisomerase 1 inhibitor, via a β-glucoronidase-cleavable linker (SC3417) at a drug-to-antibody ratio (DAR) of 8. The drug-linker is site-specifically conjugated to the non-natural amino acid p-azidomethylphenylalanine (pAMF) incorporated at optimized sites within the antibody (Ab) sequence using Xpress CF+^{TM}. In preclinical xenograft models, STRO-004 demonstrates potent, dose-dependent activity from 0.25-7.5 mg/kg, including in models with low and heterogeneous levels of TF expression. STRO-004 was evaluated in patient-derived xenograft (PDx) models of cancers with prevalent TF expression and demonstrated 50% overall response rate and 70% disease control rate across all models after single dose treatment. Exploratory toxicology studies show that STRO-004 has a favorable safety profile in cynomolgous monkeys up to 50 mg/kg, the highest dose tested. In circulation, STRO-004 demonstrated extended half-life, low clearance, and stable drug-linkage. Consistent with this finding, only low levels of free exatecan could be detected. Based on these promising preclinical observations, STRO-004 is advancing to IND-enabling studies for the treatment of TF expressing malignancies.

#1573 MW-C1, a novel CLDN1-targeting ADC demonstrates compelling anti-tumor efficacy and favorable safety profile in preclinical studies.

Zhongfei Li[1], Jun Yang[1], Peng Fang[2], Rongrong Hu[1], Cuicui Guo[1], Xiaoding Tan[2], Hai Wu[1], **Xun Gui**[1]

[1]Mabwell (Shanghai) Bioscience Co., Ltd., Shanghai, China,[2]Jiangsu Mabwell Health Pharmaceutical R&D Co., Ltd., Taizhou, China

Background: Claudins are a family of tight junction proteins characterized by four transmembrane domains, with critical roles in epithelial cell junction formation. While claudins localize to tight junctions in healthy tissues, their overexpression in solid tumors leads to aberrant exposure outside of these junctions, making them attractive targets for ADC therapies. CLDN1, in particular, is overexpressed in several solid tumors, including head and neck squamous cell carcinoma (HNSCC), lung squamous cell carcinoma (LUSC), and hepatocellular carcinoma (HCC), where its expression correlates with tumor proliferation, invasion, metastasis, and poor prognosis. Here, we developed a novel CLDN1-targeting ADC, MW-C1, that has shown potent anti-tumor activity across various preclinical models, along with an encouraging safety profile in non-human primates (NHPs).

Methods: MW-C1 was engineered by conjugating monomethyl auristatin E (MMAE) to a CLDN1-targeting monoclonal antibody using site-specific interchain-disulfide conjugation technology and a cleavable linker, resulting in a drug-to-antibody ratio (DAR) of 4. *In vitro*, the internalization and cytotoxicity of MW-C1 were assessed in SCC4, Cal27, Huh7, and OVCAR3 cell lines, which express varying levels of CLDN1. *In vivo* efficacy was evaluated in multiple cell-line-derived xenograft (CDX) and patient-derived xenograft (PDX) models. Toxicology and pharmacokinetic studies were conducted in cynomolgus monkeys.

Results: CLDN1 was found to be overexpressed in 85.7% of HNSCC, 91.7% of HCC, 58% of LUSC, and 77% of cervical cancer samples. MW-C1 demonstrated robust binding, rapid internalization, and potent cytotoxicity in CLDN1-positive cancer cell lines. In *in vivo* studies, MW-C1 induced significant and durable tumor regression in both CDX and PDX models, with varying CLDN1 expression levels. Pharmacokinetic studies revealed favorable properties for MW-C1 in both mice and cynomolgus monkeys, which were attributed to its optimized biophysical characteristics and site-specific conjugation. In a non-GLP NHP toxicology study, MW-C1 was well tolerated, with no significant off-target toxicity or adverse events observed.

Conclusions: MW-C1 demonstrated potent anti-tumor efficacy and a favorable safety profile, highlighting its potential as a transformative therapeutic candidate for the treatment of CLDN1-overexpressing malignancies.

#1576 RenBiologics- a fully human antibody library generated by RenMice facilitates the development of antibody drugs.

Ting Yi, Jun Du, Yue Zhang, Yuting Hu, Shujin Zhang, Xin Ji, Mingyue Gu, Lei Zhang, Yi Yang, **Yong Xie**

Biocytogen, Waltham, MA

Therapeutic antibodies have become important drugs in the field of targeted therapy because of their high specificity to target antigens. Currently, over 100 antibody drugs have been approved for the treatment of various human diseases, yet the targets covered are indeed limited, with approximately half of these drugs targeting only 10 common targets. Approved drugs encompass not only monoclonal antibodies but also antibody-drug conjugates, bispecific antibodies, and antibody fragments. The scarcity of antibody drug targets and subsequent conjugation or engineering modifications pose challenges for the development of novel antibody drugs. In response to the growing demand for fully human antibody drugs, Biocytogen's antibody discovery team has generated a library of antibodies targeting hundreds of antigens using fully human antibody transgenic mice (RenMice). Our RenMice undergo additional gene-editing procedures to knock out designated target genes. Subsequently, antibodies obtained by conventional immunization have a wide range of epitope-binding properties to homologous protein targets, but also exhibit cross-species reactivity. We have completed over 900 fully human antibody target projects and established a library containing over four hundred thousand off-the-shelf fully human antibody sequences. The antibodies generated from RenMice can be used in a variety of applications, and many of the TAA (tumor-associated antigen)-targeting antibodies have been screened for internalization activity to ensure suitability for ADC development. In addition to fully human monoclonal antibody derived from RenMab platform (fully human immunoglobulin variable domains replacing the mouse loci), RenLite-derived common light chain antibodies are easy to assemble into bispecific antibodies (BsAb) with low mismatch rate and ideal physiochemical properties, which are suitable for BsADC, biparatopic antibody (BpAb) and other modalities. Small and flexible fully human nanobodies based on RenNano platform (fully human heavy chain only) also provide solutions for specific antigen epitopes or multi-targets, multi-epitope antigens. Thus, the fully human antibody library can accelerate the development of antibody drugs.

#1577 PF-08046876: A new camptothecin antibody-drug conjugate directed to integrin beta-6 with potent antitumor activity in multiple preclinical solid tumor models.

Rebecca C. Mazahreh, Narayana Yeddula, CB Lim, Gina LoMastro, Nicolas H. Garcia, Vineet Kumar, Christopher Frantz, Lauren Farr, Ryan Lyski, Lori Westendorf, Robert P. Lyon, Vivian H. Trang

Pfizer, Inc., Bothell, WA

Integrins are a large family of cell surface receptors with diverse roles in cellular adhesion, motility, and cytokinesis. Functional integrins exist as heterodimers consisting of single alpha and beta chains. Within this family, integrin beta-6 (IB6), which dimerizes exclusively with integrin alpha-v, is a target of interest in the clinic for its role in cancer. IB6 expression is low in normal adult epithelial tissues and high in solid tumors. In some cases, high IB6 expression has been demonstrated to be associated with poor prognosis such as in non-small cell lung, colorectal, gastric and cervical carcinomas. PF-08046876 is an investigational antibody-drug conjugate (ADC) composed of an anti-IB6 antibody conjugated to a camptothecin-class topoisomerase I (TOPO1) inhibitor, AMDCPT, using a traceless enzyme-cleavable glucuronide linker. The AMDCPT payload was optimized for potency, reduced susceptibility to MDR efflux pumps and enhanced bystander activity. PF-08046876 leverages the same antibody backbone from Sigvotatug Vedotin (SV) and is specific for IB6 but does not bind other alpha-v family members. In preclinical studies, PF-08046876 has demonstrated activity in vitro and in multiple in vivo models spanning a range of antigen expression levels in several solid tumor carcinomas including non-small cell lung, head and neck squamous cell, urothelial, and pancreatic carcinomas. PF-08046876 demonstrates a tolerability profile in non-human primates that is consistent with camptothecin ADCs. These results suggest that PF-08046876 is promising ADC for the treatment of multiple carcinomas and supports further clinical investigation. A Phase 1, first-in-human study is planned to evaluate the safety and anti-tumor activity of PF-08046876.

#1578 Preclinical evaluation of BLB-101, a topoisomerase-inhibitor-based anti-CLDN6/9 antibody-drug conjugate featuring a proprietary hydrophilic linker.

Suk Hyung Lee, Alice Juang, Aastha Jain, Jun Liu, Wen Zhang, Zuoan Yi, Darren Buchwald, **Alice Chen**

Baylink Biosciences, Inc., Palo Alto, CA

Claudin 6 (CLDN6) is a tight junction protein that is highly expressed in various human cancers, including ovarian and endometrial cancers, while it is not found in normal adult tissues. Claudin 9 (CLDN9), which shares significant homology with CLDN6, displays similar expression patterns, being nearly absent in normal tissues but upregulated in ovarian and endometrial cancers. We propose to target both CLDN6 and CLDN9 simultaneously to enhance anti-tumor efficacy and broaden treatment options for more patients. We successfully discovered an antibody that binds with high affinity to CLDN6 and CLDN9 while sparing other claudin family members. The humanized version of this candidate antibody demonstrates stronger binding and higher internalization compared to a benchmark antibody currently in clinical development. BLB-101 is an antibody-drug conjugate (ADC) candidate featuring a topoisomerase-inhibitor-based payload optimized in-house with a proprietary hydrophilic linker. Design features of the linker enable efficient delivery of hydrophobic drug payloads and low likelihood of off-target endocytosis. BLB-101 has drug-to-antibody ratio of 8 (DAR8), demonstrates enhanced potency, favorable in vivo PK, and excellent plasma stability. In vitro studies revealed that BLB-101 ADC quickly internalizes in CLDN6+ or CLDN9+ cancer cells. BLB-101 exhibited strong cytotoxic effects across several cancer cell lines, including PA-1 and JEG-3. In addition, we confirmed the bystander killing effect of BLB-101 under a coculture condition of CLDN6-positive cells and negative cells in vitro. Furthermore, BLB-101 maintains the mAb's capability to induce antibody-dependent cellular cytotoxicity. In xenograft models expressing CLDN6, BLB-101 treatment led to a substantial reduction in tumor size. Additionally, repeated administration of BLB-101 was well tolerated in both mice and in non-human primates. Together, these findings provide strong support for the continued development of BLB-101directed against CLDN6/CLDN9 positive tumors.

#1579 PA289, a prodrug linker-payload with a novel mechanism of action for the development of antibody drug conjugates.

Justyna H. Mysliwy, Jutta Deckert, Adam Lodge, Jenny Thirlway, James Stephenson, Patrick Higgs, Daniel Williamson, Robert J. Lutz

Iksuda Therapeutics Ltd., Newcastle upon Tyne, United Kingdom

Background: PA289, is a novel seco-CBI-based prodrug payload which appears to exert cell-killing activity through the alkylation of one or more cytosolic proteins (Tietze *et al*, 2010). PA289, is the lead linker-payload from our ProAlk payload technology platform and is designed with tumor-selective glucuronide triggers that attenuate the potency of the payload until uptake into target cancer cells and selective release in the lysosome. The glucuronide linker and PermaLink (PL) conjugation chemistry ensure ADC stability. The unique mechanism of action of PA289 compared to currently used chemotherapies and ADC payloads, led us to generate proof-of-concept data for this novel ADC payload.

Methods: The free payload and ADCs were tested for cell-killing activity *in vitro* by CellTiter-Glo assay. The prodrug linker-payload PA289, was conjugated using PermaLink technology to generate ADCs with a drug to antibody ratio (DAR) of approximately 4. Stability was determined in mouse and human plasma and *in vivo* efficacy was performed with subcutaneous human tumor xenografts in nude mice. Bystander effect was analyzed *in vitro* and compared to ADCs composed of MMAE and MMAF payloads. An exploratory toxicology study was conducted in cynomolgus monkeys.

Results: The free payload showed highly potent *in vitro* activity against 248 cancer cell lines representing 15 solid cancer indications with IC50 values in the fM to pM range. Isumab01-PL-PA289, a model ADC targeting FRA, displayed excellent plasma stability and was highly potent against the JEG-3 cell line *in vitro*, with IC50 values in the pM range. A single administration of Isumab01-PL-PA289 in the FRA-positive JEG-3 xenograft model induced complete tumor regression by day 30 when dosed at 2.2 mg/kg, while the Isotype-PL-PA289 non-binding control resulted in weak tumor growth inhibition. Evaluation of bystander killing *in vitro* with a model ADC targeting CA242, Isumab04-PL-PA289, demonstrated stronger bystander cell-killing than benchmark ADCs containing MMAE and MMAF. A pilot toxicity study in cynomolgus monkeys treated with Isumab04-PL-PA289 showed the ADC was well-tolerated with minimal to no test-article related findings at the highest dose tested (7 mg/kg single dose).

Conclusions: ADCs comprised of PA289, a novel protein alkylator prodrug linker-payload, demonstrate 1) strong *in vitro* potency and bystander effect, 2) durable tumor regressions in xenograft models, and 3) excellent tolerability in monkeys. The free payload is highly active against a broad array of cancer cell lines representing a variety of solid tumors suggesting that PA289 is a promising candidate for the development of ADCs with a novel MOA for the treatment of solid tumors.

#1580 HuB14-VA-PL2202, a novel antibody-drug conjugate targeting ASCT2, a novel ADC target over-expressed in both solid and hematological cancers.

Danilo Cucchi, Elizabeth Horsley, Alina Chrzastek, Ben Leatherdale, Simone Sims, Nicole Bledowski, Banusha Rajeenthira, Narinder Janghra, Lara Montolio, Abigail Kimpalu, Christopher Gallagher, Charlie Britten, Lolke de Haan, Paul W. Hogg, Chris Pickford, Patrick H. van Berkel

ADC Therapeutics, London, United Kingdom

SLC1A5 (Solute Carrier family 1 member 5), also known as ASCT2 (Alanine, Serine, Cysteine Transporter 2), is a Na^+ dependent antiporter with its preferred substrate being glutamine. ASCT2 expression is reported in normal tissues, however it has been shown to be dramatically increased in several solid tumors (lung, colorectal) and hematological malignancies (acute myeloid leukemia (AML), multiple myeloma). Moreover, high ASCT2 expression was associated with a worse prognosis in liver and lung cancers. Because of this profile of expression, ASCT2 is an appealing membrane protein for pharmacological targeting. We have developed a new antibody-drug conjugate (ADC) targeting ASCT2 (HuB14-VA-PL2202), composed of a humanized IgG1 directed against human ASCT2, to which PL2202, consisting of a proprietary cleavable linker and exatecan, a topoisomerase I inhibitor, has been site-specifically conjugated with a drug to antibody ratio ~6. FcγR-mediated effector function was abrogated via mutation in the Fc portion of the antibody. The aim of this study was to characterize the *in vitro* cytotoxicity of HuB14-VA-PL2202 in human cancer cell lines, and determine its *in vivo* efficacy. The safety and tolerability of HuB14-VA-PL2202 was also evaluated in cynomolgus monkeys. Membrane expression of ASCT2 was confirmed by IHC in sections from xenograft biopsies and human tissues.*In vitro*, HuB14-VA-PL2202 was readily internalized by ASCT2+ve cancer cells, showed specific binding to CHO cells expressing recombinant human or cynomolgus monkey ASCT2, and displayed potent cytotoxic activity in a range of target positive cancer cell lines, both from solid and hematological tumors. *In vivo*, HuB14-VA-PL2202 dosed once at 6 mg/kg was able to significantly delay tumor growth in the subcutaneous (sc) G-402 human renal leiomyoblastoma cancer xenograft model. Likewise, in the sc MOLM-13 AML xenograft model, HuB14-VA-PL2202 dosed once at 10 mg/kg achieved 5/10 tumor free survivors at the end of the study. In a PK study in human FcRn Tg32 SCID mice, HuB14-VA exhibited a 7.4 day half-life; whilst in a DRF study in cynomolgus monkeys, HuB14-VA-PL2202 was tolerated at 40 mg/kg, indicating there is a favorable therapeutic index. Membrane expression of ASCT2 was confirmed by IHC, in sections of xenograft models and tissue microarray (TMA) of human cancer indications. ASCT2 expression in NSCLC and HNSCC was found to be significantly higher than in the correspondent normal tissue. In conclusion, ASCT2 represents an interesting novel tumor target for an ADC approach. HuB14-VA-PL2202 demonstrated potent and specific *in vitro* cytotoxicity and *in vivo* anti-tumor activity in both hematological and solid tumor models, and was well tolerated in cynomolgus monkeys. Together, the data generated with HuB14-VA-PL2202 warrants its further development into the clinic in cancers over-expressing ASCT2.

#1581 Two novel antibodies targeting different tumor-associated carbohydrate (TACA) antigens for the treatment of solid tumors show promising preclinical activity using different modes of action.

Francesco Muraca, **Wiebke Winkler**, Gustavo M.S.G. Moreira, Fausto G. Gomes, Karla E. G. Soto, Samina Naz, Peter Sondermann, Matthias Ocker

TACALYX GmbH, Berlin, Germany

Tumor-associated carbohydrate antigens (TACAs) are short oligosaccharides that are often overexpressed on the surface of cancer cells. They are derived from changes in glycosylation pathways associated with malignancy, which lead to overproduced or aberrant structures, and represent a still unexplored target class for cancer therapy. We focus on the generation of antibodies against these specific and difficult antigens and were able to obtain two high-affinity monoclonal antibodies (mAbs), TCX-101 and TCX-201, against two distinct TACA structures that are highly expressed across a broad range of cancer indications. Both mAbs show superior affinity and specificity to their target compared to previously generated ones, as assessed by glycan array, flow cytometry, and immunohistochemistry or immunofluorescence. By investigating the characteristics of our glycan targets, we explored different modes of action to tailor the most effective treatment. Previously, our team has shown that TCX-101 can be effectively used as an antibody-drug conjugate (ADC), which showed encouraging results *in vivo* against breast and small cell lung cancer (SCLC). In addition, we here present TCX-101 in a bispecific T-cell engager (TcE) format, showing efficient *in vitro* T-cell activation and killing of cancer cells from different indications endogenously expressing low (SW837), medium (LoVo) to high levels (MCF7) of our TACA target already at an effector:target ratio of 4:1 with antibody concentrations in the pico- to low nanomolar concentration range, i.e. IC50 of 9 nM (SW837), 0.13 nM (LoVo) and 0.0078 nM (MCF7), respectively. *In vivo* evaluation of this TcE is currently investigated using cell-line derived xenograft models in humanized mice. TCX-201, targeting a different TACA, is currently being evaluated as an ADC with two different payloads, monomethyl Auristatin E (MMAE) and Exatecan (XTC). *In vitro* studies show effective killing of various pancreatic (e.g. high expressing HuP-T4 cells with an IC50 for MMAE payload of 0.3 nM) and colon cancer cell lines expressing different levels of the target (e.g. Colo-205 (high expressing) with an IC50 of 0.013 nM or LS180 (medium expressing) with an IC50 of 4.7 nM), and high efficacy for both payloads in a xenograft mouse model using HuP-T4 pancreatic cancer cells. In conclusion, our antibodies against TACAs represent new promising treatment options for different cancer indications and can be effectively employed with different modes of action, depending on the target characteristics.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Apoptosis: Therapeutic Manipulation / Role of Microenvironment in Therapeutic Response
Poster Session

#1586 KH815, a novel dual-payload TROP2-directed antibody-drug conjugate, shows potent antitumor efficacy in pre-clinical tumor model.

Yonghao Zhao, Pengfei Ren, Menglong Guan, Jiao Tang, Lu Qi, Xinzou Fan, Shijun Yin, Gang Lei, Xiao Ke

Kanghong Pharmaceutical Group, Chengdu, China

TROP2 (trophoblast cell surface antigen 2) is expressed at high levels in various human carcinomas and is associated with tumor differentiation and pathological aggression. Several antibody-drug conjugates (ADCs) targeting TROP2, such as sacituzumab govitecan and datopotamab deruxtecan, have shown promising anti-tumor activity in multiple solid tumors, particularly in breast cancer. However, their clinical application remains limited by inadequate efficacy, highlighting the unmet clinical need for improving the efficacy of single payload TROP2-targeting ADCs.This study presents the preclinical evaluation of a novel dual-payload TROP2-targeting ADC, KH815, composed of a humanized IgG1 antibody (hRS7) directed against TROP2 which is conjugated to a topoisomerase I inhibitor(TOP1i) and an RNA polymerase II inhibitor(RNA POL IIi). KH815 achieves an average drug-to-antibody ratio (DAR) of about 7.5 (4+3.5) using cysteine and glycoside antibody coupling technologies with an appropriate DAR, high SEC purity, homogeneous particle size distribution, and favorable thermal stability.The dual mechanism of KH815 enables the simultaneous inhibition of RNA synthesis and the induction of DNA double-strand breaks, resulting in a potent and synergistic anti-tumor effect both in vitro and in vivo xenograft mouse models in comparison to other TOP1i-TROP2-targeting ADCs. In both cell-derived xenograft (CDX) and patient-derived xenograft (PDX) models, KH815 exhibited dose-dependent tumor growth inhibition and demonstrated superior anti-tumor activity compared to the single-TOP1i ADC, at equivalent or even lower doses. Furthermore, KH815 has been shown to be more effective than TOP1i-ADC (sacituzumab govitecan and datopotamab deruxtecan) in tumor inhibition in a PDX model derived from Trodelvy pretreated patients and in a HCT-15 cell CDX model, which expresses P-gp protein, a pump that can extrude DXd. This provides additional evidence that KH815 has the potential to overcome resistance to TOP1i-ADCs. The non-GLP toxicity study in cynomolgus monkeys provided evidence of the favorable safety profile of KH815, the highest non-severely toxic dose (HNSTD) is 40 mg/kg/dose.In summary, KH815, an ADC with dual toxins and unique mechanisms of action, displayed superior anti-tumor effects, the ability to overcome drug resistance, and an acceptable safety profile in preclinical studies. These findings support the potential of KH815 as a promising advanced therapeutic candidate for tumors.

#1587 A novel dual-payload HER3-directed antibody-drug conjugate, shows potent antitumor efficacy in HER3-low *in vivo* tumor models.

Yonghao Zhao, Pengfei Ren, Menglong Guan, Jiao Tang, Lu Qi, Xinzou Fan, Shijun Yin, Gang Lei, Xiao Ke

Kanghong Pharmaceutical Group, Chengdu, China

HER3 (Receptor tyrosine-protein kinase erbB-3) is broadly expressed in several cancers, including breast, lung, pancreatic, colorectal, gastric, prostate, and bladder cancers. Elevated HER3 expression is associated with poor patient outcomes and therapeutic resistance. Several HER3-ADCs have been developed for cancer therapy. To date, patritumab deruxtecan (U3-1402) is the only HER3-targeting therapy that has demonstrated clinical efficacy in both non-small cell lung cancer. But patritumab deruxtecan is ineffective in more challenging tumor type including low HER3 expression tumors.This study presents preclinical evaluation of a novel dual-payload HER3-targeting ADC, KHN922, composed of a humanised IgG1 antibody (patritumab) directed against HER3 which is conjugated to a topoisomerase I inhibitor (TopoIi) and an RNA polymerase II inhibitor (RNA POL IIi). KHN922 achieves an average drug-to-antibody ratio (DAR) of about 7.5 (3.9+3.6) using cystein and glycosite antibody coupling technologies with an appropriate DAR, high SEC purity, homogeneous particle size distribution, and favorable thermal stability.The dual mechanism of KHN922 enables the simultaneous inhibition of RNA synthesis and the induction of DNA double-strand breaks, resulting in a potent and synergistic anti-tumor effect both in vitro and in vivo xenograft mouse models in comparison to other TopoIi-TROP2-targeting ADCs. In low and ultra-low HER3 expression CDX models, the KHN922 demonstrated superior tumor suppression effects compared to single-TopoIi ADCs, patritumab deruxtecan and patritumab exatecan.Furthermore, KHN922 still showed strong activity to DXd-ADC treatment resistance CDX models. The non-GLP toxicity study in cynomolgus monkeys provided evidence of the favorable safety profile of KHN922, the highest non-severely toxic dose (HNSTD) is 50 mg/kg/dose.In summary, KHN922, an ADC with dual toxins and unique mechanisms of action, displayed superior anti-tumor effects, the ability to overcome treatment-resistance and tumour recurrences, and an acceptable safety profile in preclinical studies. These findings support the potential of KHN922 as a promising advanced therapeutic candidate for HER3 tumors.

#1588 The novel trispecific tumor-conditional TRAIL-R agonist M0674 induces tumor cell apoptosis and deepens therapeutic efficacy of systemic and targeted chemotherapies.

Christina Esdar, Doreen Musch, Carolin Sellmann, Ana Hecht, Achim Doerner, Enrico Guarnera, Julian Kreis

the healthcare business of Merck KGaA, Darmstadt, Germany

Background: TRAIL death receptors are upregulated in a wide range of tumors, and their pharmacological activation induces apoptosis. Targeting TRAIL receptors (TRAIL-R) has emerged as a critical therapeutic strategy for cancer treatment. Despite some evidence for preclinical activity with past and present TRAIL-R agonists, only limited clinical efficacy has been reported so far, likely due to insufficient TRAIL-R clustering, decoy receptor binding, and hepatotoxicity. To address these limitations, M0674 has been designed as a trispecific antibody that simultaneously binds to tumor-associated (TA) MUC1, TRAIL-R1 and TRAIL-R2 but not to TRAIL decoy receptors.
Methods: We conducted a detailed pharmacological characterization of M0674 compared to other TRAIL-R agonists through in vitro and in vivo studies. Apoptosis and viability assays were performed on cancer cells and human hepatocytes. The antitumor activity of M0674, both as a monotherapy and in combination with chemotherapy and an anti-CEACAM5 ADC with an exatecan payload (M9140), was evaluated using cell line-derived (CDX) and patient-derived (PDX) xenograft models.
Results: Treatment with M0674 results in potent TA-MUC1-dependent induction of apoptosis and inhibition of viability in cancer cell lines, while human hepatocytes remain unaffected. M0674 demonstrates superior antitumor activity compared to other TRAIL-R agonists in both in vitro and in vivo studies. In vivo models show strong antitumor effects, including regressions, when M0674 is combined with systemic and targeted chemotherapies. MUC1-dependent single-agent activity and combination benefits with docetaxel are observed in three out of five nsqNSCLC PDX models that do not respond to docetaxel alone. Additionally, five out of six PDAC PDX models exhibit a strong combination benefit of M0674 with gemcitabine. Furthermore, the combination of M0674 with the anti-CEACAM5 ADC M9140 induces complete regression in a gastric CDX model.
Conclusion: M0674 induces tumor-specific hyperclustering of TRAIL-R1 and R2 by binding to TA-MUC1, effectively triggering apoptosis in tumor cells without affecting healthy cells. The robust preclinical antitumor activity across various cancer types highlights the potential of M0674 as a safe and effective combination partner for systemic chemotherapies and ADCs.

#1589 Morin activates mitophagy via the PINK1/Parkin pathway, promoting apoptosis and suppressing tumor growth in pancreatic cancer cells.

Vinit Sharma, Mayank Sharma, Ankita Semwal, Ankita Arora, Sakshi Bansal, Anjali Aggarwal

Anatomy, Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India

Background: Pancreatic cancer (PC) is an aggressive malignancy characterized by high rates of invasiveness, chemoresistance, and relapse. Mitochondrial dysfunction, often due to mutations or metabolic imbalances, results in excessive ROS production and DNA damage, enhancing tumor aggressiveness. Additionally, stromal fibrosis in PC hinders drug delivery, complicating treatment outcomes. Therefore, we aim to investigate the effect of Morin, a bioflavonoid with antioxidant and anti-inflammatory properties, in promoting mitophagy in PC cells to inhibit tumor progression.
Methods: Experiments were conducted under control and Morin-treated conditions on PANC-1 cells and normal pancreatic epithelial cells. Cytotoxicity was assessed via the MTT assay, while invasiveness, migration, morphology, and stemness were evaluated using colony-formation, transwell, spheroid-formation assays, and ALDH activity. Mitochondrial membrane potential, functional mitochondria, superoxide production, and ROS were analyzed using JC-1, Mitotracker Green/Red, MitoSOX™ Red, and DCFDA dyes. Apoptosis was quantified with Annexin V/PI staining, and ATP synthase activity was measured using ATP Synthase Profiling Kit. Expression of markers related to mitochondrial function, transport, and apoptosis was analyzed via PCR microarray, qRT-PCR, and immunofluorescence.
Results: Morin exhibited a dose- and time-dependent cytotoxic effect on PANC-1 cells, with an IC50 of 50.018 µM at 24 hours. Treatment reduced spheroid size, made them rounder, and formed pores, indicating apoptosis. Morin also decreased colony formation, migration, and ALDH activity, suggesting reduced cancer stem cell properties. JC-1 staining showed a lower mitochondrial membrane potential, while DCFDA indicated reduced ROS, and MitoSOX Red revealed increased superoxide levels. Mitotracker Red/Green staining indicated fewer dysfunctional mitochondria. Following Morin treatment, Annexin V/PI staining showed significant increases in apoptosis and reduced ATP synthase activity ($p = 0.0286$). Microarray results indicated significant downregulation of genes related to mitochondrial fusion (MFN2, $p = 0.000616$), and apoptosis regulator (SH3GLB1, $p = 0.037$). qRT-PCR and immunofluorescence showed Morin upregulated mitophagy genes (PINK1, Parkin), autophagy markers (LC3b), and apoptosis markers (Cytochrome c), while increasing epithelial markers (E-Cadherin) and decreasing mesenchymal (N-Cadherin, Snail, ZEB1), and stemness markers (Sox2, Nanog, OCT4). *In silico* docking showed stable binding of Morin to mitophagy marker PINK1 (docking score: -8.3 kcal/mol).
Conclusion: Morin targets mitochondrial dysfunction via the PINK1/Parkin pathway, promoting apoptosis and reducing cancer cell survival, highlighting its promise as a potential therapeutic agent.

#1591 Mechanisms of BPM31510-induced cell death in cancer: lipid peroxidation and apoptosis pathways.

Abbas Khojasteh[1], Michael A. Kiebish[2], Lisa Ghiglieri[1], Sylwia Stopka[2], Juan J. A. Aristizabal-Henao[2], Seema Nagpal[1], Maria-D Nastke[2], Stephane Gesta[2], Niven R. Narain[2], Vijay Modur[2], Lawrence Recht[1]

[1]Stanford University, Palo Alto, CA,[2]BPGbio, Framingham, MA

BPM31510, an oxidized Coenzyme Q10 (CoQ10) nanodispersion, has shown potential as an anticancer agent through its ability to induce the generation of reactive oxygen species (ROS) within the mitochondria of cancer cells. Recent studies have highlighted ferroptosis — a form of regulated cell death driven by iron-mediated lipid peroxidation, as a promising mechanism for cancer therapy, particularly for cancers that are highly vulnerable to oxidative stress. Ferroptosis differs from apoptosis in that it involves the accumulation of ROS, leading to lipid peroxidation and cell membrane damage, rather than DNA fragmentation and caspase activation characteristic of apoptosis. Given the strong correlation between BPM31510-induced cytotoxicity and elevated ROS levels, we investigated if BPM31510 induces cancer cell death through ferroptosis. To evaluate lipid peroxidation, we assessed both cancerous (HT1080, U87, GL261 and C6) and non-cancerous cells (NIH 3T3, TNC1, rat astrocytes, and human astrocytes). Lipid peroxidation was quantified using the fluorescent probe BODIPY 581/591. Cells were treated with BPM31510 and Erastin (a ferroptosis inducer) for 3 hours, and fluorescence intensity was measured to assess lipid peroxidation levels. To address whether BPM31510-induced cytotoxicity involves ferroptosis, we performed a cytotoxicity assay with additional conditions including Ferrostatin-1 (a ferroptosis inhibitor) combined with BPM31510 or Erastin. Inhibition of lipid peroxidation by Ferrostatin-1 would suggest ferroptosis involvement. Lastly, we assessed cell death pathways using annexin V and propidium iodide (PI) staining to distinguish apoptotic versus necrotic cell death in cells treated with BPM31510. BPM31510 treatment resulted in significantly elevated lipid peroxidation in cancer cell lines (HT1080, U87, Gl261, C6) compared to healthy cells (NIH 3T3, TNC1, astrocytes), suggesting differential vulnerability of cancer cells to BPM31510-induced oxidative stress. Although Ferrostatin-1 significantly reduced lipid peroxidation induced by Erastin, it did not reverse the lipid peroxidation caused by BPM31510. This resistance to Ferrostatin-1 indicates that BPM31510's cytotoxic effects are independent of ferroptosis, distinguishing its mechanism from that of traditional ferroptosis inducers. Apoptosis assays further confirmed that BPM31510 predominantly activates apoptotic pathways, with limited necrotic cell death observed**.** Our findings demonstrate that BPM31510 differentially induces lipid peroxidation in cancer cells through a mechanism independent of ferroptosis, instead activating apoptosis as the primary pathway of cell death. The ferroptosis-independent cytotoxicity of BPM31510, combined with its selective targeting of cancer cells, highlights its potential as a unique therapeutic agent in glioblastoma.

#1592 Targeting cFLIP to overcome resistance of BRAF V600E melanoma cells with the small molecule CB001.

Claire Lin[1], Matthew J. Hadfield[2], Connor Purcell[3], Shengliang Zhang[3], Vida Tajiknia[3], Wafik S. El-Deiry[3], Benedito A. Carneiro[2]

[1]The Warren Alpert Medical School of Brown University, Providence, RI,[2]Hematology-Oncology Division, Brown University Health, Providence, RI,[3]Department of Pathology and Laboratory Medicine, Brown University, Providence, RI

Background: Over 50% of metastatic melanoma harbor activating BRAF V600E mutations. Vemurafenib selectively inhibits mutant BRAF. However, resistance to vemurafenib frequently develops within a few months of initiating treatment. Vemurafenib-induced apoptosis is inhibited by overexpression of cellular FADD-like IL-1β-converting enzyme-inhibitory protein (cFLIP), an endogenous inhibitor of caspase 8 in the extrinsic apoptosis pathway. Studies have also shown that suppression of cFLIP expression restores the extrinsic pro-apoptotic pathway in melanoma cells. Thus, targeting cFLIP with CB001, a novel cFLIP inhibitor, may represent a novel therapeutic strategy to overcome resistance to BRAF inhibitor in melanoma.
Methods: We investigated the prevalence of cFLIP and BRAF molecular alterations in melanoma and its impact on patient survival using The Cancer Genome Atlas (TCGA) dataset. We investigated cytotoxic effects of CB001 (0-10μM) or the BRAF inhibitor vemurafenib (0-500nM) in BRAF V600E melanoma cell lines (RPMI-7951, SH4, SK-MEL28). Combination indexing for dual treatment with CB001 [0-10μM] and vemurafenib [0-500nM] was performed on SynergyFinder. cFLIP and cPARP expression was investigated after treatment with CB001 (10μM) +/- vemurafenib (400nM) at 24 hours by western blot (WB). Melanoma cells were co-cultured at a ratio of 1:5 with natural killer cells (NKc) in the presence of CB001 (10μM), and fluorescent co-culture images were quantified using the MetaXpress cytoplasmic/nuclear staining software.
Results: cFLIP alterations are frequently enriched in patients with BRAF-mutated tumors (p= 4.049e-3), and amplification of the gene encoding cFLIP is correlated with inferior overall survival compared to wild type tumors. Single-agent CB001 showed variable cytotoxicity across melanoma cell lines, with RPMI-7951 (13.4μM) requiring a higher IC50 than SH4 (3μM) and SK-MEL28 (1.29μM). While interaction between CB001 and vemurafenib was only additive, the combination treatment did overcome apoptosis resistance in vemurafenib-resistant RPMI-7951 (vemurafenib IC50 > 500nM). CB001 with vemurafenib decreased cFLIP expression and increased PARP and caspase 8 cleavage on WB, consistent with increased extrinsic apoptosis pathway activation, in RPMI-7951. CB001 also increased immune-mediated NKc killing of RPMI-7951 (p=6.23e-4), SH4 (p=5.5e-4), and SK-MEL28 (p=5.55e-4) cells in co-culture experiments at a 5:1 NKc:melanoma ratio.
Conclusion: CB001 demonstrates potent cytotoxicity in metastatic melanoma cell lines and may overcome resistance to vemurafenib via downregulation of cFLIP and activation of the extrinsic apoptosis pathway. CB001 also enhances immune-mediated NKc killing of melanoma cell lines, suggesting that targeting cFLIP has an immunomodulatory effect in addition to direct tumor cytotoxicity.

#1594 Lipid-laden macrophages are associated with KRAS therapy resistance in pancreatic cancer.

P. Hou, T.-y. Chu, Y. Huang, D. Deng, H. Begum;
Rutgers New Jersey Medical School, Newark, NJ

Our recent discovery in pancreatic ductal adenocarcinoma (PDAC) demonstrates that tumor-associated macrophages (TAMs) are pivotal in driving resistance to KRAS-targeted therapy. Using a genetically engineered mouse (GEM) model of PDAC with doxycycline-inducible $KRAS^{G12D}$, pancreas-specific p48-Cre, and conditional p53 knockout (known as "iKPC"), we found that myeloid cell remodeling from a neutrophil-rich to a macrophage-rich phenotype occurred after KRAS depletion. TAMs were sufficient and essential for PDAC cells to bypass KRAS dependency. Consistent with our findings, KRAS inhibition (KRASi) rewired the tumor microenvironment (TME) with increased TAM presence in several cancer models, including those of the pancreas, colon, and lung. However, the exact role of TAMs in promoting or impairing KRASi resistance remains controversial. Considering the heterogeneous, plastic, and multi-functional features of TAMs, we performed single-cell RNA sequencing (scRNA-seq) to compare KRAS on and off tumors from iKPC GEM. We identified two major subclusters of TAMs: *Siglecf*+ and *Apoe*+, with only *Apoe*+ TAMs expressing high levels of *Ccr2*, indicating their origin from circulating macrophages. Both subclusters express immune regulatory genes, but genes related to cholesterol homeostasis are upregulated in *Apoe*+ TAMs compared to other cell types. Notably, lipid metabolism regulatory gene signatures are significantly enriched in TAMs from KRAS off tumors versus KRAS on tumors. Specifically, we observed upregulation of genes encoding scavenger receptors, lysosome enzymes, lipid binding proteins, galectins, and complement factors in TAMs from KRAS off tumors versus KRAS on tumors, resembling the characteristics of lipid-laden macrophages. Lipidomic profiling comparing tumor-educated macrophages (TEMs) exposed to conditional media from KRASi pre-treated KPC (*p48*-Cre, $p53^{Lox/+}$, LSL-$KRAS^{G12D}$) PDAC cells (KRASi CM) and DMSO control (DMSO CM) revealed that the most significantly increased lipids were phospholipids, very long chain fatty acids, and ultra-long chain fatty acids. After examination of the expression of rate-limiting enzymes and key receptors involved in fatty acid synthesis, breakdown, and transportation, we found that *Elovl1* and *Abcd1* were significantly upregulated in TEMs exposed to KRASi CM compared to those exposed to DMSO control CM. Consistently, lipid content was higher in KRASi CM-treated TEMs compared to controls, as shown by BODIPY staining. Additionally, we observed an increased number of peroxisomes in KRASi CM-treated TEMs compared to control TEMs. These data collectively indicate that macrophages associated with KRASi-treated tumors exhibit aberrant accumulation of fatty acids.

#1595 Transforming tumor terrain: Effect of datopotamab deruxtecan (Dato-DXd) on the tumor microenvironment in three-dimensional explant lung tumor models.

K. F. Goliwas, S. C. Chabattula, A. Ismail, J. M. Donahue, B. Wei, T. S. Winokur, J. S. Deshane, Y. Boumber, A. Desai;
University of Alabama at Birmingham, Birmingham, AL

Introduction: Antibody-drug conjugates (ADCs) targeting Trop-2 (human trophoblast cell surface antigen 2) are a promising class of therapeutics with potential to enhance therapeutic outcomes for non-small cell lung cancer. Dato-DXd, a Trop-2 targeted ADC, has demonstrated encouraging activity, leading to further interest in testing combinatorial approaches. However, a greater understanding of its effects on the tumor microenvironment (TME) are needed to identify optimal combinations. Herein we examine tumor-stromal interactions in response to Dato-DXd in ex vivo three dimensional-lung tumor models (3D-LTMs).

Methods: 3D-LTMs were generated utilizing freshly isolated, remnant human lung tumor specimen from patients undergoing tumor resection (Institutional Review Board approved protocol) and continuously perfused with culture media to provide nutrient circulation. Starting on day 3 of culture, 4, 6, or 8 mg/kg Dato-DXd or vehicle control was added to circulating media. Dato-DXd used in this study was purchased from a 3rd party commercial vendor (MedChemExpress). On day 14 of culture (day 11 of ADC treatment) histologic and flow cytometry analyses were performed.

Results: An increased trend in the percentage of apoptotic cells (18.5±1.5 vs. 13±1.5; p=0.13) was noted with 6 mg/kg Dato-DXd when compared to vehicle control. Additionally, a significant reduction in the percentage of tumor cells (p=0.025) and a significant increase in non-viable tumor cells (p=0.047) was observed with this dose when compared to vehicle treated tissues. No differences in fibroblasts, $CD45^+$ immune cells, $CD8^+$ T cells, endothelial cells, or non-viable fibroblasts was observed between 6 mg/kg Dato-DXd and control. When an 8 mg/kg dose was utilized a significant increase in total $CD45^+$ immune cells (p=0.0007) and $CD8^+$ T cells (p=0.012) and an increasing trend in endothelial cells (p=0.099) was observed when compared to vehicle control. No significant changes in apoptosis, tumor cells, fibroblasts, or non-viable tumor cells or fibroblasts were observed when comparing this dose to control. When proportions were normalized to reduce variability across tissues, an increase in apoptotic cells was observed in 3 of 5 samples assessed at 4 mg/kg, and 4 of 5 samples at 6 and 8 mg/kg. An increase in immune cells ($CD45^+$) was observed in 3 of 5 samples at 6 and 8 mg/kg. An increase in $CD8^+$ T cells was observed in 3 of 5 samples at 8 mg/kg.

Conclusion: We observed a notable trend towards an increase in immune cell populations (e.g. $CD8^+$ T cells) following Dato-DXd treatment in 3D-LTMs. These preliminary findings indicate an enhanced anti-tumor immune response following treatment with Dato-DXd. Collectively, data suggest that Dato-DXd induces significant alterations in the TME which may facilitate the evaluation of rational drug combinations to amplify the anti-tumor response.

#1596 Tumor-derived RAC1^{A159V} gain-of-function mutation promotes an immunosuppressive tumor microenvironment and represses response to immune checkpoint inhibitors.

M. Cai, M. Adam, F. Guo, Y. Zheng;
Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Immune checkpoint inhibitors (ICIs) specifically target immune checkpoint molecules and activate cytotoxic T lymphocytes (CTLs) to kill cancer cells and have seen great success in various solid tumors. However, the overall response rate of ICI monotherapy in patients is only ~15% due to an immunosuppressive microenvironment of many tumors, and understanding the mechanism of ICI therapy resistance is urgently needed to improve the outcomes. RAC1, a member of Rho GTPase family, regulates multiple cellular processes in cancer, including vesicle trafficking, glycolysis, gene transcription, and exocytosis/endocytosis, all of which are tightly associated with the creation of a tumor immune microenvironment (TIME). RAC1^{A159V} is a gain-of-function mutation found in several cancer types including colon cancer, head and neck cancer, and lung cancer, and is related to poor prognosis. Mammalian target of rapamycin (mTOR) signaling, one of the downstream effectors of RAC1, promotes tumor progression via diverse mechanisms including glycolytic metabolism and tumor cell proliferation. To define the role of RAC1^{A159V} in cancer immune therapy, we have generated RAC1^{A159V} knock-in MC38 colon cancer cells by CRISPR/Cas9 gene editing. PAK-PBD pull-down assay and western-blotting confirmed that RAC1^{A159V} mutation resulted in upregulated RAC1 activity and mTOR signaling *in vitro*. In subcutaneous MC38 tumor xenograft models we found that while MC38 RAC1^{A159V} tumor grew similarly as RAC1^{WT} tumor cells in immunodeficient mouse recipients, it grew faster than RAC1^{WT} tumors and are highly resistant to anti-PD1 therapy in immune competent mice. Flow cytometry analysis revealed that the RAC1^{A159V} tumors have an immunosuppressive TIME with a significantly higher Treg/CTL ratio than RAC1^{WT} tumors. ScRNA-seq of the whole tumors showed that RAC1^{A159V} tumor is a “cold” tumor with suppressed tumor-immune cell interactions. Deep RNA-seq analysis of the tumor cells further indicated that RAC1^{A159V} activates mTORC1 signaling in tumor cells, which in turn increases tumor cell glycolysis and inhibits chemokine production. Rapamycin, a mTORC1 inhibitor, could sensitize RAC1^{A159V} tumors to anti-PD1 treatment by inhibiting tumor glycolysis and promoting chemokine production in tumor cells. RT-qPCR studies further indicated that inhibiting RAC1^{A159V} tumor cell glycolysis by 2-DG also enhanced chemokine production in vitro, similar to the effect observed with rapamycin. Overall, our studies demonstrate that tumor-derived RAC1^{A159V} mutation turns the TIME to “cold” and renders the tumor resistant to ICIs via mTOR mediated changes in glycolysis and chemotaxis. The results provide mechanistic insights of RAC1 mutation-mediated TIME and suggest a combination therapeutic approach for “cold” tumors by combining RAC1 signaling inhibition with ICIs.

#1597 Immune suppression of the lymph node microenvironment causes oligoprogression of metastatic cancer after neoadjuvant immuno-chemotherapy.

P. Ding[1], X. Huang[1], Q. Peng[1], K. Ren[1], L. Gong[2], B. Wang[2], **L. Yang**[1];

[1]Shenzhen People's Hospital（The Second Clinical Medical College，Jinan University；The First Affiliated Hospital, Southern University of Science and Technology）, Shen Zhen, China, [2]Genecast Biotechnology Co., Wuxi, China

Oligometastatic disease is recognized as an intermediate state between localised and systemically metastasised disease. The diagnosis, treatment and prognosis of oligometastatic disease is currently based mainly on imaging examination. Previous clinical studies indicated that standard systemic therapy followed by radical local therapy was beneficial for survival in tumor patients with oligometastatic disease. However, different clinical outcomes (such as oligopersistant and oligoprogression) were found in synchronous and/or metachronous oligometastatic disease after the same systemic therapy. This report explored potential factors of different outcomes in tumor patients with oligometastatic disease after neoadjuvant immuno-chemotherapy. A lymphoepithelioma-like carcinoma (LELC) case with stage IIIa was synchronous oligometastatic disease, which was treated with neoadjuvant immuno-chemotherapy (four courses of Albumin-Bound Paclitaxel (300 mg), Carboplatin (400 mg) and Durvalumab (500 mg)) and subsequent surgery. Lymph nodes 1R and 4R were considered as oligoprogression and oligopersistant, respectively. Whole exome sequencing results showed that single nucleotide variation (SNV), copy number variation (CNV), tumor mutational burden (TMB), microsatellite instability (MSI), human resource development (HRD) and mutant-allele tumor heterogeneity (MATH) were similar between 1R and 4R samples. Tumor-infiltrating immune cells using a multiplex immunoflourescence histochemistry (mIHC) test revealed that immune checkpoints PD1, PD-L1 and CD39 were high expression and there was a low level of CD8+ T cell in the 4R sample. But levels of CD39+CD8+ and PD-L1+CD8+ T cells were higher in the 1R sample than the 4R sample, suggesting a suppressive tumor immune microenvironment in the 1R oligoprogression lymph node. To demonstrate the result, oligometastatic samples from eight non-small-cell lung cancer (NSCLC) cases with neoadjuvant immuno-chemotherapy and surgery were examined using the mIHC. The results indicated that CD39+CD3+/PD-L1+CD3+/TIM3+CD3+ T cells and/or CD39+CD68+ macrophages in the oligometastatic samples with oligoprogression were high levels. Thus, suppression of tumor-infiltrating immune cells might result in one major factor of different outcomes in oligometastatic disease after neoadjuvant immuno-chemotherapy. Funding Sources: This work was supported by the Wu Jieping Medical Foundation (320.6750.2024-16-16).

#1598 Involvement of atypical PKCs in liver cancer.

Abigail Oluwafisayo Olatunji, Mahfuza Marzan, Nuzhat Nowshin Oishee, Mildred Acevedo-Duncan

University of South Florida, Tampa, FL

The liver is an essential organ in the human body, it carries out important biological functions including detoxification, nutrient metabolism, protein synthesis, production of bile and other biochemical reactions necessary for growth. Liver cancer is a disease in which cancer cells form in the tissues of the liver. The death rate for liver cancer was 6.6 per 100,000 men and women per year. HepaRG is an immortalized hepatic cell line that retains many characteristics of primary human hepatocytes while HEPG2 and PLC5/PRF/5 are liver cancer cell lines isolated from liver cancer donors. Atypical Protein Kinase C (aPKC) is a type of protein kinase that does not require Ca2+ or diacylglycerol for their activation, aPKC consists of two isoforms: PKCiota (PKC-ι) and PKCzeta (PKC-ζ). We have previously reported the dose dependent response of HEPG2 and PLC5/PRF/5 cell line to treatment with 5-amino-1-(1R,2S,3S,4R)-2,3-dihydroxy-4-methylcyclopentyl)-1H-imidazole-4-carboxamide (ICA-1S). However, there is no clear understanding of which intracellular chemicals are involved in liver cancer progression and what the involvement of aPKCs is in liver cancer. In this study, we investigated the status and function of aPKC in proliferating and confluent liver cells, our objective was to determine if the expression of PKC-ι and PKC-ζ are altered in liver cancer cells compared to normal liver cells. HepaRG, HEPG2, and PLC5/PRF/5 cells were seeded in 100mm plates and maintained with fresh media. Samples from each cell line were collected in lyses buffer at 50% and approximately 100% confluence. The protein concentration of the prepared samples was quantified with Bradford, Laemmli buffer was added, samples were heated, and Western blot analyses were carried out with equal amounts of protein. Samples were probed for PKC-ι, PKC-ζ, and Caspase3. There was a 32% downregulation of PKC-ι in the HepaRG cell line and a 37% and 9% upregulation were observed in HEPG2 and PLC5/PRF/5 respectively for the expression of PKC-ι as the liver cancer cells gain confluency (N = 3). The results for the expression of PKC-ζ showed a 26% downregulation in HepaRG but a notable 61% upregulation in HEPG2 and a slight 12% upregulation in PLC5/PRF/5 at 100% confluence (N = 3). The unchanged caspase 3 expression confirms that the difference in the expression level of the aPKC is not due to apoptosis. Results demonstrated a relationship between cell confluence and aPKCs protein levels depicting that PKC-ι along with PKC-ζ, play a different role in proliferation of the normal liver cells compared to liver cancer cells. Our ongoing investigation on the effect of aPKCs through the knockdown of PKC-ι resulted in about 30% reduction of HEPG2 proliferation

#1599 CD40L-dependent regulation of BIM leads to lymphoma survival and therapy resistance in protective niches.

Candice Madiot[1], Celine Bellanger[1], Christelle Dousset[1], Salome Decombis[1], Antonin Papin[1], Agnes Moreau-Aubry[1], Benoit Tessoulin[2], Catherine Pellat-Deceunynck[1], David Chiron[1]

[1]INSERM U1232 (CRCINA), Nantes, France,[2]Centre Hospitalier Universitaire de Nantes, Nantes, France

Introduction: Mantle Cell Lymphoma (MCL), an aggressive B-cell lymphoma, is tightly dependent on the microenvironment (TME) for expansion and a dynamic cellular dialogue within lymph node (LN) niches results in ecosystem corruption and tumoral resistance. Within the TME, we identified modulations of the BCL2 family, including a deep inhibition of the pro-apoptotic protein BIM. The aims of this project were to identify the molecular pathways involved in the TME-dependent regulation of BIM and to evaluate the functional consequences in tumor survival and resistance.

Methods: To decipher TME-dependent molecular regulations, we performed RNA-seq and ATAC-seq of MCL and normal B cells (NBC) in culture models previously set up to mimic signals observed in the LN ecosystem (Chiron et al., Blood, 2016). Identified hits/pathways were validated by qPCR and Western Blot. To evaluate the functional consequences of BIM inhibition, we generated BIM-/- MCL cells using CRISPR/Cas9 and performed BH3-profiling, drug testing (chemo, targeted and immune-therapies), serum-free survival studies and *in vivo* tumor growth assays in CAM model.

Results: MCL cells stimulated by CD40L (T-cell signal) recapitulated BIM regulation, which was tumor-specific. Unsupervised analysis of annotated BIM transcription factors, using RNA-seq and TOBIAS (Transcription factor Occupancy prediction By Investigation of ATAC-seq Signal) analysis, highlighted FOXO1 as a candidate for MCL-specific and CD40L-dependent regulation of BIM. FOXO1 nuclear exclusion was further observed upon CD40L stimulation. Downstream of CD40, we demonstrated activation of the AKT, ERK and NFKB pathways. The involvement of AKT and/or ERK pathways in FOXO1/BIM regulation are under investigation. Functional studies showed an increased survival of BIM-/- cells in serum-free condition and an increase tumor growth *in vivo.* While primary MCL cells do not survive without TME support *ex vivo,* BIM-/- cells derived into TME-independent cell lines, reinforcing the association between BIM expression and tumor dependence to their TME. This is also consistent with BIM deletions being enriched in MCL lines. In addition, BIM -/- cells showed reduced mitochondrial priming and resistance to therapies (chemo, targeted). In contrast, T-cell engager efficiently killed both WT and BIM -/- lines, suggesting that modern immunotherapies could overcome BIM downregulation associated resistance.

Conclusions: By integrating high-throughput technologies and cutting-edge functional studies, we have identified BIM inhibition as a TME-dependent source for therapeutic resistance and tumor cell survival. Innovative therapies, such as bispecific T-cell engager, could counteract this TME-induced resistance and provide new strategies for patients with these high-risk hemopathies.

#1600 Tracking adaptation and response to targeted therapies in EGFR-mutant non-small cell lung cancer.

Regina Hoo[1], Aaron Tan[1], Dawn Lau[1], Komal Gupta[2], Kenneth Chow[1], Jia Chi Yeo[3], Lan Ying Wang[1], Poh Sheng Joe Yeong[2], Anders J. Skanderup[3], Daniel S. W. Tan[1]

[1]Division of Medical Oncology, National Cancer Centre Singapore, Singapore, Singapore,[2]Agency for Science, Technology and Research, Institute of Molecular Cell Biology, Singapore, Singapore,[3]Agency for Science, Technology and Research, Genome Institute of Singapore, Singapore, Singapore

Non-small cell lung cancer (NSCLC) accounts for the majority of lung cancer cases, with EGFR-mutant NSCLC among never smokers being particularly prevalent in Asian populations. A major clinical challenge in treating advanced NSCLC is the development of resistance to targeted tyrosine kinase inhibitors (TKIs), highlighting the need to comprehensively profile the cellular mechanisms underlying drug resistance. We propose to tackle this by investigating the adaptive features of residual cancer cells and the role of the tumour microenvironment in sustaining tumour growth, resistance, and disease progression. In this study, we utilise single nucleus and spatial transcriptome profiling to decipher the response of EGFR-mutant tumours in more than 30 NSCLC patients before and during on-TKI treatment, including those that have developed resistance. We present an analytical framework that maps individual residual cancer cell populations that persist after treatment. We then examine the functional phenotypes of neighbouring immune cells including T lymphocytes and mononuclear phagocytes on these populations, and then comparing the immune dynamics to tumours that have fully acquired resistance. Our study reveals a potential mechanism by which metabolic plasticity and paracrine signaling from myeloid cells in the lung tumour microenvironment contribute to the maintenance of cancer stem cell properties, likely driving disease recurrence. We propose to incorporate a wider range of ongoing clinical trial and preclinical data to support our current findings, and with the aim of defining novel therapeutic strategy that can selectively eliminate drug tolerant persister population in EGFR-mutant NSCLC.

#1601 Advancing cancer immunotherapy: Unveiling the potential of PD-L1-targeted IFN-α2a dutaflip molecule through patient-derived tumor explants.

Steffen Dettling[1], Martina Antoniolli[2], Kerstin Patzold[2], Manuela Gies[2], Jakob Rosenhauer[2], Jens Liedke[2], Laura Krumpelmann[2], Marion Lichtenauer[2], David Lamparter[3], Tai-Hsien Ou Yang[4], Petra Schwalie[5], Gabriel Schnetzler[6], Andreas Roller[7], Moritz Rapp[2], Meher Majety[2], Pablo Umana[8], Marina Bacac[8]

[1]pRED oDTA, F. Hoffman-La Roche AG, Basel, Switzerland,[2]pRED oDTA, F. Hoffman-La Roche AG, Penzberg, Germany,[3]Clinical Biomarkers, F. Hoffman-La Roche AG, Basel, Switzerland,[4]Analytics Team II, F. Hoffman-La Roche AG, New York, NY,[5]Predictive Modelling & Data Analytics, F. Hoffman-La Roche AG, Basel, Switzerland,[6]Project Team Leaders, F. Hoffman-La Roche AG, Basel, Switzerland,[7]Biomarker Strategy and Integrative Sciences, F. Hoffman-La Roche AG, Basel, Switzerland,[8]pRED oDTA, F. Hoffman-La Roche AG, Zurich, Switzerland

This study aims to elucidate the mode of action (MoA) of the programmed cell death ligand 1 (PD-L1)-targeted Interferon alpha (IFN-α2a) Dutaflip molecule (RO7694744) in human patient-derived tumor explants (PTEs). PTEs are highly translationally relevant models due to their ability to closely mimic the native tumor microenvironment (TME) and maintain the heterogeneity of patient-specific tumor biology. The goal is to evaluate the specificity and efficacy of immune responses in preclinical development. RO7694744 is a trivalent bispecific antibody-cytokine fusion protein designed to specifically activate IFN-α on PD-L1 positive cells. This targeted activation enhances anti-tumor immune responses while minimizing systemic toxicity and non-specific activity.

Fresh human PTEs from various cancer types (non-small cell lung cancer (NSCLC) n=16 and bladder cancer (BLCA) n=5) were cultivated ex vivo in a dynamic flow 3D cultivation system (MIVO), and treated with RO7694744 for 20 hours. Untreated samples and those treated with recombinant human IFN-α2a (rhIFNAα2a) served as negative and positive controls, respectively. After cultivation, cytokine secretion was analyzed, and single nucleus RNA sequencing (snRNA-seq) was performed to evaluate immune responses and transcriptional changes at the single-cell level. Comprehensive baseline characterization, including clinically relevant histopathological analysis, bulk RNA sequencing, and flow cytometry analyses, was conducted to correlate drug response with tumor-intrinsic features.

RO7694744 treatment induced a consistent cytokine response (IP-10, ISG-15, IFNg) across NSCLC and BLCA samples, comparable to rhIFNAα2a but to a slightly lower extent. Pearson correlation revealed a positive relationship between PD-L1 positive cells and elevated cytokine response levels in samples treated with RO7694744, a specificity not observed with rhIFNAα2a. SnRNA-seq data analysis identified 44 cellular subpopulations, with a preferential activation of PD-L1 positive tumor cells and immune cells by the RO7694744. This activation was characterized by an up-regulation of an inflammatory gene signature in PD-L1 positive cells, not seen in untreated conditions.

In summary, RO7694744 demonstrates a specific immunomodulatory effect on PD-L1 positive tumor and immune cells, inducing a targeted inflammatory response. The use of fresh human PTEs in this study resulted in translationally-relevant findings, underscoring the PTE model as an advanced next-generation tumor model for the precise evaluation of cancer immunotherapies. These findings enhance the understanding of RO7694744's MOA, confirming its PD-L1-dependent tumor targeting and activity within the TME. This supports a patient selection strategy and identifies clinically-relevant pharmacodynamic biomarkers.

#1602 Advancing personalized medicine using precision-cut tumor slices - assessing individual responses to immunotherapy in the tumor microenvironment.

Julia Thiel[1], Jan Schlegel[1], Adrian Kneer[2], Sascha Dreher[3], Annelie Schafer[4], Oliver Seifert[4], Katrin S. Kurz[5], Marc-H Dahlke[6], Georg Sauer[2], Gerhard Preissler[3], Laureano Simon[7], Myriam Fabre[7], Isabel Egana[7], Roland Kontermann[4], Monilola Olayioye[4], German Ott[5], Walter E. Aulitzky[8], Thomas E. Murdter[1], Matthias Schwab[1], Meng Dong[1]

[1]Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany,[2]Department of Gynecology and Obstetrics, Robert Bosch Hospital, Stuttgart, Germany,[3]Department of Thoracic Surgery, Robert Bosch Hospital, Stuttgart, Germany,[4]Institute of Cell Biology and Immunology, University of Stuttgart, Stuttgart, Germany,[5]Department of Clinical Pathology, Robert Bosch Hospital, Stuttgart, Germany,[6]Department of Surgery, Robert Bosch Hospital, Stuttgart, Germany,[7]Oncomatryx Biopharma S.L., Derio, Spain,[8]Department of Oncology, Robert Bosch Hospital, Stuttgart, Germany

Immunotherapies have emerged as a promising pillar for cancer therapy. However, due to their reliance on the individual, complex and dynamic tumor microenvironment, treatment success remains limited to a subset of patients. A deeper understanding of the response of individual tumors and their heterogeneous microenvironment is essential to improve patient stratification and develop novel treatment approaches. This requires suitable model systems which allow tracking of the tumor microenvironment‘s (TME) response to immunotherapy.

In this study, we combined patient-derived precision-cut tumor slices (PCTS) with multiplex immunofluorescence staining and cytokine analyses to trace the treatment response of different cell types in their native TME while preserving their location. PCTS derived from ovarian cancer, lung cancer and colorectal cancer tissue (n=13) were treated with OMTX305, an anti-FAP T-cell engaging bispecific antibody, for up to three days. A co-culture system integrating PCTS with autologous PBMCs was established to mimic the *in vivo* environment, and enable monitoring of immune cell activities. While treatment responses were patient-specific, determination of overall ATP levels of PCTS showed a significant decrease in viability after three days of treatment. Multiplex immunofluorescence stainings were performed to spatially analyze therapeutic effects, including T cell infiltration and distribution, T cell activation (Granzyme B) and cell death induction (Cleaved Caspase-3) in different cell types. T cells were significantly activated after treatment, and tumor slices showed significant cell death induction on the second day of treatment. Some patients showed T cell infiltration into FAP+ regions in response to treatment. Analysis of over 10 cytokines in the culture medium provides deeper insights into how the treatment induced immune cell activation.

By integrating spatial biology and cytokine analysis with our PCTS platform, we can map the relationship between the tumor, stromal, and immune cell components within the TME, visualize inter-patient differences, and predict individual therapy responses to immunotherapy. This makes our platform a valuable tool for personalized cancer medicine.

#1604 Homeobox transcription regulatory gene HOXA10 in pancreatic cancer treatment response.

Addanki Pratap Kumar[1], Amanda Munoz[1], Xiaoyu Yang[1], Meagan Marie Ybarra[1], Joel Michalek[2], Shaye Hagler[3], John Baer[3], Ramya Chengalvala[3], Sukeshi A. Patel[4], Danielle Fritze[5], Glenn Halff[5], Zhao Lai[1], Yuji Ikeno[6], Yidong Chen[2], Rita Ghosh[7]

[1]Molecular Medicine, UT Health Science Center at San Antonio, San Antonio, TX,[2]Epidemiology and Biostatistics, UT Health Science Center at San Antonio, San Antonio, TX,[3]Canopy Biosciences, St. Louis, MO,[4]Medicine, UT Health Science Center at San Antonio, San Antonio, TX,[5]Surgery, UT Health Science Center at San Antonio, San Antonio, TX,[6]Pathology, UT Health Science Center at San Antonio, San Antonio, TX,[7]Urology, UT Health Science Center at San Antonio, San Antonio, TX

Gemcitabine/nab-paclitaxel (GnP) or FOLFIRINOX (FFX) are recommended guidelines for use as first line regimens for pancreatic ductal adenocarcinoma (PDAC) patients. Use of FFX is usually limited to a small subset of younger, fit patients with minimal comorbidities. This underscores the need to focus on efforts to test and develop effective regimens that can be used in a wide range of patients due to potentially minimal toxicity profile and cost. We identified palmatine, a protoberberine alkaloid fulfilling the Lipinski's 5-rule as a potential candidate drug. We show that relative to gemcitabine, palmatine in combination with gemcitabine was more effective against a panel of human PDAC cultured cells, human ex vivo cultured cells, and human PDAC organoid models. Furthermore, repeated measures linear model changes in bioluminescence at the time of termination from baseline (Log_{10}), showed that gemcitabine alone and in combination with palmatine resulted in significant reduction of tumor burden relative to control KPC orthotopic syngeneic mouse model of PDAC. The combination was tolerated well by the animals. Bulk RNA-sequencing from tumors treated with combination showed that expression of *Hoxa10* was significantly reduced. Gene-set enrichment analysis further showed enrichment of B-cell receptor signaling in responding relative to non-responding mice. Spatial transcriptomic analyses of responding and non-responding tumors showed that expression of *Hoxa10* was found in the mesenchymal cell population and that *HoxA10* expression correlated with differentially expressed genes in B cell population. Exploration of tumor immune microenvironment (TIME) using CellScape revealed increased density of B-cells and decreased regulatory T-cells (Tregs) in tumors from responding but not non-responding mice. The *HOXA10*-associated gene signature is reported to be prognostic for survival. Recent evidence shows that *HOXA10* expression correlates differentially with immune cell populations. We found CD274 (PD-L1), IFNGR1, NFκB1 and STAT3 as potential HOXA10 targets based on ChIP-seq data with HOXA10 pull-down from ENCODE project. *In silico* analysis of the pancreatic cancer database shows that HOXA10 expression is elevated in human pancreatic tumors and that high expression is associated with increased survival. *HOXA10*, the homeobox transcription regulatory gene is involved in multiple roles in cancer including cell survival, epithelial mesenchymal transition (EMT), cancer progression, metastasis, and therapeutic resistance and in the context of PDAC it also promotes stromal cell proliferation. Taken together, these findings suggest the potential utility of palmatine in therapeutic sensitization of gemcitabine through deregulation of HOXA10 signaling.
Supported in part through VA Merit Award BX003876 (APK).

#1605 Pretreatment of bone anabolic agent delays or prevents multiple myeloma progression.

Syed Hassan Mehdi[1], Dongjoon Lee[1], Alex Lee[2], Paul Lee[2], **Donghoon Yoon**[1]

[1]Internal Medicine, University of Arkansas for Medical Sciences, Little Rock, AR,[2]Marah Natural Co., Coquitlam, BC, Canada

Multiple myeloma (MM) is the second most common blood cancer. It remains an incurable disease due to relapsing and developing treatment resistance. Treatment resistance and disease progression are not solely due to intrinsic MM characteristics but are also fostered by alterations within the surrounding BM microenvironment. The bone serves as a home for growing myeloma but is also a target organ for myeloma cells. The MM-bone disease (MMBD) is one of the defining features of MM. A previous report showed that myeloma cells colonize the endosteal niche, enter a dormant state, and are activated by bone-lining cells. Many attempts, including ours, were made to test novel or existing bone anabolic drugs for anti-MMBD and found they did not significantly affect myeloma progression, although bone formation has improved. Interestingly, we found that the surviving mice from myeloma transplants become tolerable to subsequent tumor transplantation. *We hypothesized that improving the bone environment may lead to the prevention of myeloma growth.* We pretreated mice with a bone anabolic agent, Sigma Ant-Bonding Calcium Carbonate (SAC), and then tested myeloma growth. It may mimic a treatment in the pre-emerging or -relapsed period of MM. SAC is a chemically/physically modified calcium carbonate with a unique weak bonding and a commercially available dietary supplement. It induces bone formation by increasing the active ionized calcium in the body. We divided the 8-12-week-old NOD SCID gamma mice into three groups: PBS, the SAC-Treated group before Transplant (TbT), and the SAC-Treated group after Transplant (TaT). SAC was gavaged twice daily, five days/week, for four weeks before the transplantation (TbT) or two weeks after transplant (TaT), while PBS was gavaged to the control group when TbT started. The $1x10^6$ luciferase-expressing 5TGM1 cells were injected into the mice *via* the tail vein. Mice were imaged weekly by IVIS imager to assess the myeloma progression and terminated when endpoint criteria were met. All surviving mice were sacrificed one week after all PBS group mice died. We found that the median survival of PBS-treated mice was 39 days, while 45 days in the TaT group. To our surprise, 80% of TbT group mice survived until the end of the study. The bioluminescence image analysis showed that myeloma slowly progressed in both treated group mice. DEXA results demonstrated that bone minerals significantly increased in the spine of SAC-treated mice compared to the control mice. Trabecular thickness and bone volume density were significantly higher in the SAC-treated group at the lumbar spine compared to the PBS group. In conclusion, our results showed that improving the bone microenvironment with SAC pretreatment delays or prevents myeloma cell growth in mice. These results may suggest a novel therapeutic regimen for MM during pre-emerging or -remission of multiple myeloma patients.

#1606 Unraveling the microbiome-iNOS connection in obesity-driven triple-negative breast cancer.

Ivonne Uzair[1], Ann C. Anselme[1], Wei Qian[1], Jianying Zhou[1], Adriana E. Rosato[2], **Roberto R. Rosato**[2], Jenny C. Chang[1]

[1]Houston Methodist Neal Cancer Center, Houston, TX,[2]MaineHealth, Portland, ME

Introduction: A recent Phase 1/2 clinical trial evaluating the efficacy of the inducible nitric oxide synthase (iNOS, NOS2) inhibitor L-NMMA in combination with taxanes for patients with chemorefractory, locally advanced breast cancer or metastatic triple-negative breast cancer (TNBC) reported an overall response rate (ORR) of 45.8%. Notably, stratification by body mass index (BMI) revealed that obese patients had a significantly higher ORR (85.6%) compared to non-obese patients (46.7%), correlating with elevated iNOS expression in tumor biopsies from obese patients. While obesity typically worsens disease-free and overall survival in TNBC, these findings suggest a potential role for iNOS inhibition in overcoming obesity-driven tumor progression. To investigate further, we studied the impact of iNOS inhibition on tumor growth and treatment response in an obese mouse model and examined the role of the gut microbiome. Obesity was induced in C57BL/6 mice using a high-fat diet (HFD) for 10 weeks prior to orthotopic implantation of E0771 TNBC cells in the mammary fat pad of female mice. Control mice were fed a normal diet (ND).
Results: - HFD-fed mice developed larger, more aggressive tumors, with detectable burden occurring ~3 weeks earlier. Tumors in HFD mice reached humane endpoints ~17 days sooner than ND mice, whose tumors grew to 80% of HFD tumor size in the same period. Lung metastasis was significantly higher in HFD mice. - L-NMMA combined with docetaxel delayed tumor growth in both diet groups, though the untreated/treated tumor size ratio was higher in HFD mice (6.5) than ND mice (2.1). - Gut microbiome analysis revealed diet- and tumor-associated compositional changes. FMT (fecal microbiota transplantation) from ND mice to HFD mice reduced tumor size and improved therapeutic response, while FMT from HFD mice to ND mice increased tumor size and impaired response. Immunohistochemistry showed elevated iNOS levels in tumors of HFD mice, which normalized following ND-FMT. Conversely, ND mice receiving HFD-FMT displayed increased iNOS expression. Serum cytokine profiles reflected these tumor and treatment differences.
Conclusion: These findings suggest that HFD-induced gut microbiome alterations create a pro-inflammatory environment driving increased iNOS expression, promoting tumor aggressiveness and influencing therapeutic response. Targeting the gut microbiota, alongside iNOS inhibition, could provide novel strategies for managing obesity-associated TNBC.

#1607 Versican status in the tumor microenvironment heterogeneously impacts immune infiltration and inflammatory pathway expression.

Ruchi Shah[1], Sean G. Kraus[1], Somak Chaudhuri[1], Elizabeth L. Field[1], Cheri A. Pasch[1], Katherine A. Johnson[1], Dustin A. Deming[2]

[1]University of Wisconsin-Madison, Madison, WI,[2]Department of Medicine, University of Wisconsin-Madison, Madison, WI

Background: Versican (VCAN) and its proteolysis product, Versikine (Vkine), have been shown to regulate CD8+ T-cell infiltration in cancers. Here, we evaluate the impact of VCAN proteolysis and accumulation on immune infiltration and immune-related pathway expression across cancer types.

Methods: RNA expression (Z-score) signatures of $VCAN^{hi}/ADAMTS4^{hi}/TIMP3^{lo}$ and $VCAN^{hi}/ADAMTS4^{lo}/TIMP3^{hi}$ were used to identify VCAN proteolytic predominant (VPP) and weak (VPW) cohorts, respectively, from TCGA Pan-cancer Atlas datasets. Gene-set enrichment analysis was performed between VPP and VPW cohorts for each cancer type. 10 tissue microarrays (TMAs, UW Carbone Cancer Center Translational Science Biocore and TissueArray.com) were used to evaluate VCAN, Vkine, and CD8+ tumor infiltrating lymphocytes (TILs) by immunohistochemistry (IHC). VCAN and Vkine stromal intensity was scored from 0-3+. Cancers were categorized by IHC as VPP (VCAN low, Vkine high), VVL (VCAN low, Vkine low), and VPW (all others). CD8+ TILs were quantified per high-powered field (HPF).

Results: Patients (n = 4664) were evaluated across 9 cancer types [breast, esophageal (EsC), gastric, kidney (KC), lung, melanoma, endometrial (EnC), bladder (BlC), and colorectal (CRC)], of which 400 were VPP or VPW. In ≥ 6 cancer types with VPP status, E2F targets and TNFα signaling via NFκB were enriched in ($q < 0.001$). In ≥ 2 cancer types with VPW status, myogenesis, coagulation, IFNα response, and epithelial mesenchymal transition were enriched ($q < 0.05$). *IL6* (average log2 fold change 2.33), *CSF3* (2.26), *CXCL2* (1.76), *CXCL3* (1.71), *CXCL1* (1.47), *CXCL8* (1.42) were upregulated across all VPP groups. *SPP1* (0.96), *PPP1R3C* (0.95), and *COMP* (0.84) were upregulated across most VPW groups. IHC was performed on BlCs, KCs, EsCs, EnCs, triple-negative breast cancers (TNBCs), non-TNBCs, pancreatic ductal adenocarcinomas (PDACs), non-small cell lung cancers, and mismatch repair deficient (dMMR) and proficient (pMMR) CRCs. The most prevalent phenotype was VPP for EnCs (46.3%) and EsCs (52.0%), VPW for TNBCs (63.9%), pMMR CRCs (64.8%), PDACs (80.7%) and non-TNBCs (84.5%), and VVL for BlCs (78.6%) and KCs (85.9%). There were 0 VPP PDACs. CD8+ TILs were higher in VPP than VPW KCs (VPP average 16.3 CD8+ TILs/HPF = 16.3, VPW 13.7), EsC (39.9, 16.8), EnC (13.8, 9.8), dMMR CRC (31.5, 3.1), pMMR CRC (16.4, 2.2), and TNBC (37.0, 7.3). Cancers with < 5 average CD8+ TILs/HPF included VPW dMMR CRCs (3.1), pMMR CRCs (2.2), and PDACs (0.7) as well as VVL PDACs (2.9) and VPP non-TNBCs (4.5).

Conclusion: VCAN proteolysis is associated with CD8+ TIL abundance, especially in EsCs, EmCs, dMMR and pMMR CRCs, and TNBCs, and expression of inflammatory genes and pathways. Further insight into VCAN prevalence and interactions with inflammatory pathways and other TME components is necessary to establish its potential as a pan-cancer biomarker for immunotherapy response.

#1608 Quantifying changes to macrophage recruitment to the breast tumor microenvironment and metastasis in response to Notch4 blockade.

Debdutta Mandal[1], Katherine A. Alexander[1], Jason L. Eng[1], Yu Kato[2], Bhairavi Swaminathan[1], Christie Kang[1], L. A. Naiche[1], Yasuhiro Funahashi[2], Junji Matsui[2], Jan K. Kitajewski[1]

[1]Physiology and Biophysics, University of Illinois at Chicago, Chicago, IL,[2]Eisai Co., Tsukuba, Japan

The Notch signaling pathway is a highly conserved pathway that regulates cell fate and cell differentiation in multicellular organisms. The Notch pathway plays a vital role in tumor angiogenesis. Notch1 is broadly expressed throughout the body, and preclinical studies demonstrate that targeting Notch1 limits tumor vessel perfusion. However, Notch1 blockade has been linked to gastric toxicity and tissue hypoxia, impeding the pharmaceutical development of Notch1 antagonists. In contrast, Notch4 is primarily expressed in the vascular endothelium and is known to be upregulated in human patients with triple-negative breast cancer (TNBC). High levels of Notch4 expression have been associated with poor overall survival in breast cancer. In mice, implantation of mammary tumors into Notch4 null animals reduces mammary tumor angiogenesis, perfusion, and volume. Notch4 inhibition could enable targeting of the Notch signaling pathway in tumor stroma without disrupting normal organ function, making it a valuable potential therapeutic target in the treatment of breast cancer. Multiple inflammatory diseases have been associated with increased Notch4 expression, suggesting that Notch4 might regulate the immune response. Although Notch4 is mainly expressed on the endothelium, some publications have observed Notch4 expression on immune cells, particularly macrophages. We found that treatment with a novel anti-Notch4 neutralizing antibody, E7011, caused a significant increase in the number of tumor-associated macrophages (TAMs). To test the functional importance of TAMs in the mechanism of E7011, we depleted TAMs with an intra-tumoral injection of liposomal chlodronate before administering E7011. Depletion of macrophages via clodronate abrogated the tumor volume reduction of E7011 treatment, suggesting that TAMs are an important player in E7011 efficacy. Therefore, we hypothesize that tumor-associated macrophage Notch4 is responsible for breast cancer metastasis and tumor progression. My project will center on visualizing the effect of Notch4 inhibition on macrophage recruitment to primary mammary carcinoma tumors and dissemination of lung metastasis by immunohistochemistry. I will section cryopreserved primary tumors and lungs from two syngeneic murine mammary carcinoma models, Py8119 and spontaneously metastatic E0771.LMB. Tumor cells from this line can be visualized by red fluorescent protein (RFP). Then, I will perform immunofluorescent staining of frozen primary tumors to quantify changes in total macrophage (CD68+) recruitment and macrophage Notch4 expression in response to E7011 or control IgG treatment. I will also perform immunofluorescent staining of frozen primary lung sections to quantify lung metastatic colonies (RFP+) from tumor-bearing mice treated with E7011 and or control IgG.

#1609 Targeting the oncogenic MAP4K4 regulatory axis in pancreatic cancer tumor microenvironment.

Sunil Singh, Sandeep Kumar, Saket Jha, Harsh Vyas, Piush Srivastava, Rakesh S. Nair, Basabi Rana, Ajay Rana

Surgery, University of Illinois at Chicago, Chicago, IL

Pancreatic Ductal Adenocarcinoma (PDAC) remains remarkably lethal, with a 5-year survival rate of less than 12%. Due to its widespread resistance to conventional therapy, PDAC is expected to be the second most common cause of cancer-related death in the United States by 2030. One of the major impediments to therapeutic failure in PDAC is the presence of an immunosuppressive tumor microenvironment (TME). We examined the function of Mitogen-activated protein kinases (MAPKs) upstream member, MAP4K4, in the PDAC-TME and tested the efficacies of MAP4K4 inhibitor and immunotherapy to overcome PDAC tumor burden. The role of MAP4K4 in regulating innate and adaptive components of PDAC-TME was determined *in vivo* using a preclinical immunocompetent murine model (i.e., in KPC) of PADC. The genes related to T cell dysfunctions were determined by the RT^2 PCR array. Flow cytometry analyses were performed to determine the immune checkpoint proteins and cytotoxic cytokines in T cells. The therapeutic efficacy of MAP4K4 inhibitor and agonists of 4-1BB and OX40 was determined in the murine model of PDAC. The IHC analyses showed that overexpression of MAP4K4 increases tumor infiltration of macrophages and neutrophils and decreases T cells in the murine model of PDAC. Furthermore, treating PDAC mice with MAP4K4 pharmacological inhibitor GNE-495 decreased tumor-infiltrating macrophages and neutrophils and increased T cell counts. However, the GNE-495 treatment increased tumor-infiltrating T cells and was not associated with increased cytotoxicity of T cells. The RT^2 PCR array analysis showed that treatment of GNE-495 decreased 4-1BB gene expression in peripheral and tumor-infiltrating T cells. The combined therapy of GNE-495 and 4-1BB agonistic mAb showed noticeable tumor regression and increased survival in the preclinical model of PDAC. Our study suggests that MAP4K4 regulates innate and adaptive immune cells in pancreatic-TME, and pharmacological inhibition of MAP4K4 decreases tumor macrophages and neutrophil counts. Moreover, the rationalized approach of combining a MAP4K4 inhibitor and a 4-1BB agonist mAb induced a T cell-mediated antitumor response, and this combination could serve as a viable treatment for PDAC.

#1610 Notch4 plays distinct roles in different cell types of the breast cancer microenvironment.

Katherine A. Alexander[1], Jason W. L. Eng[2], Yu Kato[3], Bhairavi Swaminathan[2], Debdutta Mandal[4], L. A. Naiche[2], Yasuhiro Funahashi[3], Junji Matsui[5], Jan Kitajewski[2]

[1]Department of Pharmaceutical Sciences, University of Illinois Chicago Retzky College of Pharmacy, Chicago, IL,[2]Department of Physiology and Biophysics, University of Illinois College of Medicine, Chicago, IL,[3]Eisai Co., Ltd., Ibaraki, Japan,[4]University of Illinois Chicago, Chicago, IL,[5]Eisai Inc., Woodcliff, NJ

The Notch developmental pathway regulates cell fate in tumor angiogenesis and influences immune cell phenotypes in the tumor microenvironment (TME). While Notch receptors Notch1-3 have been extensively studied, the role of Notch4 in the tumor microenvironment is poorly understood. Previous efforts to target Notch signaling and tumor angiogenesis have been limited by broad expression of Notch receptors and their cognate ligands, leading to significant gastrointestinal toxicity. However, Notch4 is thought to be expressed primarily in the endothelium, making it a highly specific and, therefore, more attractive candidate to attenuate Notch signaling in cancer. To this end, we collaborated to develop a first-in-class Notch4 neutralizing antibody, E7011. We demonstrated that E7011 treatment caused reduced tumor growth in multiple tumor types, including mammary tumors, that express Notch4 in tumor endothelium. To specifically examine the role of endothelial Notch4 on breast cancer growth, we used a Notch4 conditional endothelial-specific knockout (ECKO) mouse and implanted orthotopic murine breast cancer cells. We found that tumors implanted into Notch4 ECKO mice were consistently and significantly larger in two distinct models of murine mammary carcinoma, Py8119 and E0771.LMB, suggesting that endothelial Notch4 has an anti-tumor role. These results indicate that the anti-tumor effect of blocking Notch4 with E7011 is mediated by Notch4 in other cell type(s). Single-cell RNA sequencing of Py8119 tumors treated with E7011 revealed that although Notch4 transcripts were found primarily in endothelial cells, the most notable effect of E7011 was a dramatic increase in the number of tissue-resident macrophages (TRMs). Confirmatory immunohistochemistry showed that E7011 alters macrophage populations during multiple stages of tumor development. Analysis of the macrophage transcriptome showed decreased expression of markers associated with anti-inflammatory, “pro-tumor” macrophage populations. To examine whether macrophages are required for treatment efficacy, we performed clodronate-based intratumoral depletion of macrophages. While treatment with E7011 or clodronate reduced tumor volume as expected, co-administration of both E7011 and clodronate was not significantly different from control tumors. This finding suggests that macrophages potentiate the anti-tumor effect of E7011. These findings support distinct and opposing roles for Notch4 on endothelial and macrophage populations in the breast cancer microenvironment. Further studies of the contrasting roles of Notch4 in breast cancer can guide considerations for tailoring anti-Notch therapies.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Cell Death Pathways and Treatment
Poster Session

#1614 Ferroptosis mediated cell death in colorectal cancer cells through oxidative stress.

Mohamed A. Eltokhy, Shreyas R. Gaikwad, Morgan Moore, Sanjay K. Srivastava

Texas Tech University Health Sciences Center, Abilene, TX

Ferroptosis, a regulated form of non-apoptotic cell death, occurs due to lipid peroxide accumulation resulting from impaired cellular antioxidant defenses, particularly the inactivation of glutathione peroxidase 4 (GPX4). This process has gained attention as a novel therapeutic target, particularly for colorectal cancer (CRC), a malignancy often resistant to conventional apoptosis-based therapies. Our study investigated the anticancer potential of HLN, an antimicrobial agent. The cytotoxicity of HLN was evaluated in CT26, HCT116 & HT29 cell lines by SRB assay. To confirm HLN-induced ferroptosis, we employed ferro-orange staining to detect lipid peroxidation, GSH and malondialdehyde (MDA) assays to quantify oxidative damage. Mitochondrial membrane potential and ROS generation were determined by flow cytometry, Western blotting was used to determine GPX4, GPX1, NRF2, KEAP1 and SLC7A11/xCT expression in CRC cells. HLN exhibited significant cytotoxic effects across all CRC cell lines, with IC50 values ranging from 1-4 μM. Ferro-orange staining and MDA assays confirmed extensive lipid peroxidation by HLN treatment. HLN treatment also significantly depleted intracellular GSH levels and depolarized mitochondrial membrane, whereas increased ROS production, indicating oxidative stress. Western blot results showed reduced GPX4 and SLC7A11/xCT as well as modulation in ferroptotic markers, such as GPX1, NRF2, KEAP1, by HLN treatment. In conclusion our data suggests that HLN induces ferroptosis in CRC cells by promoting lipid peroxidation, mitochondrial dysfunction, and oxidative stress

#1615 Targeting PGK1 to enhance ferroptosis in breast cancer.

F. F. Oyelami[1], A. T. Shinkle[1], C. M. Ngule[1], X. Ren[1], F. A. Oyelami[2], O. Obaleye[1], J.-M. Yang[1];
[1]University of Kentucky, Lexington, KY, [2]University of Ilorin, Ilorin, Nigeria

A key feature common to triple-negative breast cancer is metabolic reprogramming that contributes to the malignant phenotype. Dysregulation of phosphoglycerate kinase 1 (PGK1), an essential player in glycolysis, is involved in several oncogenic pathways. In the current study, we aimed to determine whether PGK1-mediated glucose metabolism impacts the induction of ferroptosis in breast cancer. We found that expression of PGK1 varies in TNBC cell lines, with MDA-MB-231, MDA-MB-458 and MDAMB-453 lines showing significantly higher PGK1 expression than BT-549 and HCC70, and than the luminal breast tumor cell lines MCF7 and T47D. Depletion of PGK1 by siRNA or inhibition of the kinase by the small molecule inhibitor NG52 resulted in elevations of the core ferroptosis markers, ACSL4, CD71 and TFRC, but led to a decreased level of GPX4 - a key enzyme in the regulation of ferroptosis via detoxifying lipid peroxides to their corresponding alcohols using glutathione as a co-factor. Induction of ferroptosis was glucose-dependent, as the higher glucose-containing culture caused increased cell death. Notably, depletion of PGK1 sensitized TNBC cells to ferroptotic cell death induced by erastin, an activator of ferroptosis, and decreased colony formation and attenuated migratory ability of tumor cells. Similar observation was made in the murine TNBC cell line, mammary 4T1 adenocarcinoma. Furthermore, we demonstrated that the glycolytic inhibitor, sodium dichloroacetate (DCA), enhanced ferroptosis, while the glycolysis inducers such as hypoxia and oligomycin reduced ferroptotic cell death, in the tumor cells subjected to PGK1 depletion. Immunofluorescence assay demonstrated an interaction of PGK1 with GPX4, the anti-ferroptosis peroxidase, and the pulse-chase experiment showed that the proteasomal turnover of GPX4 protein was faster in the PGK1-depleted tumor cells than that in the tumor cells without depletion of PGK1. Moreover, the tumor cells with depletion of PGK1 showed an upregulation of pyruvate dehydrogenase as compared with the cells without PGK1 depletion, suggesting that PGK1 mediated-down-regulation of pyruvate dehydrogenase plays a role in ferroptosis resistance in TNBC cells. Animal studies are ongoing to recapitulate our in vitro findings on the role of PGK1 in ferroptosis resistance using 4T1 TNBC mouse model. This study demonstrates that PGK1 inhibition can attenuates GPX4, elevate pyruvate dehydrogenase expression, and cause a metabolic shift, thereby sensitizing tumor cells to ferroptosis induction. Thus, PGK1 may be exploited as a therapeutic target for overcoming ferroptosis resistance in breast cancer.

#1616 Artesunate-induced ferroptosis: A novel strategy to overcome chemoresistance in ovarian cancer.

Y. Chan, O. Wong, C. Cheung, E. Wong, X. Cong, A. Cheung;
The University of Hong Kong, Hong Kong, Hong Kong

The high propensity of ovarian cancer to develop chemoresistance contributes to its generally poor prognosis. Therefore, novel therapeutic approaches tackling this issue are highly sought after. Ferroptosis is a form of cell death characterized by iron dependency, increased lipid peroxidation, and disrupted cellular membranes. Many studies suggest that ferroptosis plays a crucial role in tumor suppression, thus providing new opportunities for cancer therapy. Artesunate is a well-known anti-malarial agent that can induce ferroptosis in multiple cancer types. Here in this study, we aim to investigate the potential of artesunate to induce ferroptosis and to attenuate cisplatin resistance in ovarian cancer cells. Cell viability assays indicated that artesunate effectively induced cell death. Cell death caused by artesunate was partially mitigated by the ferroptosis inhibitor ferrostatin-1 and the autophagy inhibitor chloroquine, suggesting the cytotoxic effects of artesunate are mediated through induction of both ferroptosis and autophagic cell death. Moreover, artesunate treatment significantly increased levels of reactive oxygen species (ROS) and lipid peroxidation (LPO), which are key hallmarks of ferroptosis. Western blot analysis also demonstrated that artesunate altered the expression of protein markers associated with increased ferroptosis, autophagy, and apoptosis in a time-dependent manner, suggesting a complex interplay between different cell death pathways. Co-treatment of artesunate with sub-cytotoxic concentrations of cisplatin significantly reduced cell viability after 48 hours, whereas ferrostatin-1 co-treatment partially rescued cell death. The combined treatment also led to an upregulation of ROS and LPO levels. Results suggested artesunate may enhance the cytotoxic effects of cisplatin by promoting ferroptotic cell death. These findings highlight the potential of artesunate as a novel agent to combat ovarian cancer chemoresistance through induction of ferroptosis, thereby enhancing therapeutic efficacy in clinical settings.

#1617 Targeting CDK11 in high-risk B cell acute lymphoblastic leukemia.

Yuki Murakami[1], Elizabeth Helmke[1], Kamhung Lam[1], John McPherson[2], Noriko Satake[1]

[1]Department of Pediatrics, University of California Davis School of Medicine, Sacramento, CA,[2]Department of Biochemistry and Molecular Medicine, University of California Davis School of Medicine, Sacramento, CA

The outcome for children with high-risk B-cell acute lymphoblastic leukemia (B-ALL) is poor. Disease relapse is speculated to be due to leukemia cells escaping treatment. Cyclin-dependent kinases (CDKs) have been demonstrated to be therapeutic targets in many cancers, and inhibitors of some CDKs have already been developed in the clinic. For example, CDK4/6 inhibitors have been used for treating patients with breast cancer, and a CDK2 inhibitor is being evaluated in a clinical trial for solid cancers. CDK11, known as a direct splicing regulator in CDKs, is overexpressed in several types of tumors, such as osteosarcoma and multiple myeloma and is shown to be a therapeutic target in many cancers. In B-ALL, dysregulated RNA splicing is linked to drug resistance and disease relapse. In this study, we investigated the therapeutic potential of CDK11 inhibition in B-ALL. CDK11 protein expression was significantly higher in B-ALL cell lines (Reh and JM1) and 26 primary B-ALL samples (13 each for standard-risk and high-risk), regardless of the risk group, than in normal B-cells and hematopoietic stem cells. A CDK11 inhibitor, OTS964, showed significant dose-dependent cytotoxicity in the cell lines with IC50 of 22nM and 14nM. OTS964 also showed significant cytotoxicity in three harvested high-risk patient-derived xenograft (PDX) samples with high CDK11 expression. OTS964 did not show cytotoxicity in normal B-cells at the same tested concentrations. OTS964 is known to regulate RNA splicing by the deactivation of SF3B1, a core component of the spliceosome. We confirmed OTS964 rapidly inhibited the phosphorylation of SF3B1, which is highly activated in B-ALL. G2/M cell cycle arrest and apoptosis induction were observed 24 hours after treatment in B-ALL cell lines. OTS964 induced p53 expression, which regulates cell cycle and apoptosis, as early as 1 hour after treatment. Furthermore, OTS964 induced short pro-apoptotic spliced isoforms, instead of anti-apoptotic forms, in *MCL-1,* a gene within the BCL-2 family, as early as 3 hours after treatment. The combination of splicing modulators and BCL-2 inhibitors shows synergism by each drug inhibiting different BCL-2 family genes. Therefore, we tested the combination with OTS964 and navitoclax, a BCL-2 inhibitor inhibiting BCL-2, BCL-x, and BCL-w in BCL-2 family genes. As expected, the combination showed strong synergisms in B-ALL cell lines and in the three harvested high-risk PDX samples. It was also effective in the sample with low CDK11 expression *in vitro*. Our *in vivo* study of the combination therapy is ongoing.In conclusion, these data demonstrated the therapeutic potential of CDK11 inhibition in high-risk B-ALL. OTS964 rapidly deactivated SF3B1 and induced the pro-apoptotic forms of MCL-1, leading to cell apoptosis. Additionally, the new combination with OTS964 and navitoclax showed synergism. In future studies, we will investigate how CDK11 inhibition regulates splicing factors other than SF3B1.

#1618 Blocking MDM2 induces downregulation of PARP and enhances apoptosis in SK-N-SH neuroblastoma cells.

Shyam Sundar Jaganathan, Umamaheswari Natarajan, Appu Rathinavelu

Rumbaugh-Goodwin Institute for Cancer Research, Barry & Judy Silverman College of Pharmacy, Nova Southeastern University, Davie, FL

Introduction: Neuroblastoma is a pediatric cancer originating from neural crest cells often characterized by poor prognosis and resistance to standard therapies. RG-7388 is a potent MDM2 inhibitor that disrupts the interaction between p53-MDM2. However, the impact of RG-7388 on cellular pathways, particularly its role in post-translational modifications of cancer cells, remains unexplored. PARP is a critical protein in DNA repair and apoptosis, is often targeted for degradation in cancer therapies. However, the mechanisms underlying PARP degradation in response to RG-7388 treatment in neuroblastoma cells largely remain unclear. We hypothesize that MDM2 re-activation by the rebound mechanism of RG-7388 induces PARP degradation in SK-N-SH neuroblastoma cells, possibly through a E3 ligase mediated ubiquitination mechanism that is eventually contributing to enhanced apoptosis.
Methods: To test this hypothesis, SK-N-SH cells were treated with RG-7388 (2 µM), MDM2 specific E3 ligase inhibitor (10 µM), and iRucaparib-AP6 (10 µM), alone or in combination with RG-7388. Cell viability was evaluated using the Trypan Blue Dye Exclusion (TBDE) assay method, while Caspase-3/7 activation was quantified through cleavage of DEVD-*Amc* substrate. qRT-PCR, Western blot, and Immunofluorescence analyses were conducted to assess expression levels of the cell cycle and apoptotic markers, such as MDM2, p53, p21, and PARP.
Results: Treatment with RG-7388 significantly reduced the viability of SK-N-SH cells compared to the control. The decrease in PARP levels seen after RG-7388 treatment is more significant than the levels observed in iRucaparib-AP6. In addition, the Caspase-3/7 activity, that was measured *via* the DEVD-*Amc* substrate cleavage assay, was markedly higher in RG-7388 treated cells compared to control. qRT-PCR analysis revealed upregulation of p53 and p21 in RG-7388 treated cells and a significant downregulation in PARP expression. Western blot analysis also confirmed a reduction of PARP levels in RG7388-treated cells, which was greater than the decrease seen with iRucaparib-AP6 treatment. Reversal of the RG-7388 and iRupacarib-AP6 induced downregulation of PARP by E3 Ligase inhibitor suggested that degradation may be major contributor to the downregulation in SK-N-SH cells.
Conclusion: Our study results indicate that RG-7388 induces significant downregulation of PARP in the SK-N-SH cells, which was leading to enhanced apoptosis. Furthermore, the results of RG-7388 treatment obtained from SK-N-SH cells confirmed its ability to induce cell death through both p53 activation and downregulation of PARP.
Acknowledgment: This project was supported by the National Pediatric Cancer Foundation (NPCF) and also by the Royal Dames of Cancer Research Inc. Ft. Lauderdale, Florida

#1619 The HIV protease inhibitor nelfinavir increases the sensitivity of epithelial ovarian cancer cells to cisplatin.

Benjamin Forgie[1], Sarah T. Ferrier[2], Farah H. Abdalbari[1], Edith Zorychta[1], Alicia A. Goyeneche[2], Julia V. Burnier[2], Carlos M. Telleria[1]

[1]McGill University, Montreal, QC, Canada,[2]Research Institute of the McGill University Health Centre, Montreal, QC, Canada

Chemotherapy resistance is a major challenge in epithelial ovarian cancer (EOC) treatment. Standard treatment involves cytoreductive surgery in combination with platinum (Pt)-based chemotherapy. While initial treatment is often successful, over 80% of patients develop recurrent Pt-resistant disease. Therefore, therapeutics which increase sensitivity to platinating agents such as CDDP are essential to kill EOC cells. One candidate drug that may increase the sensitivity of CDDP to resistant EOC cells is the HIV protease inhibitor nelfinavir (NFV), which has previously shown anti-cancer activity against EOC *in vitro*. The goal of this study was to determine if the combination of NFV and CDDP is effective against EOC cells. Four EOC cell lines, with varying degrees of CDDP resistance, were exposed to increasing concentrations of CDDP in the presence or absence of NFV. In all cell lines, the addition of NFV significantly enhanced the cytotoxicity and decreased the IC_{50} of CDDP. Furthermore, the combination of NFV and CDDP was determined to be synergistic using the Loewe model of drug interaction. Interestingly, EOC cells treated with NFV and CDDP displayed characteristics of lytic cell death, such as an increased proportion of annexin-V and 7-AAD co-staining, and the release of LDH into the culture supernatant. Additionally, morphological assessment with brightfield and fluorescent microscopy revealed significant cellular swelling and cytoplasmic vacuolization, signs of inflammatory cell death. Immunoblotting revealed cleavage of the pyroptotic effector proteins gasdermin D and gasdermin E, suggesting a shift from apoptosis to pyroptosis with NFV and CDDP combination. Further evidence for pyroptosis include cleavage of the inflammatory caspase-1, as well as release of the inflammatory cytokine IL-1β into the supernatant as determined through ELISA. Interestingly, the combination of CDDP and NFV resulted in decreased expression of mature lamin A, leading to severe nuclear deformation and increased DNA damage. Furthermore, the loss of lamin A was associated with the leakage of nuclear DNA into the cytosol, as analyzed through droplet digital PCR (ddPCR) after cellular fractionation. Cytosolic dsDNA is an activation signal for the AIM2 inflammasome; to confirm its involvement, EOC cells were incubated with CDDP and NFV in the presence or absence of the AIM2 inhibitor suramin. Addition of suramin to the CDDP and NFV treated EOC cells rescued cell viability, and simultaneously decreased IL-1β. Overall, these results demonstrate a transition from apoptotic to pyroptotic cell death when EOC are treated with CDDP and NFV. Resistance to apoptosis is a major mechanism by which EOC cells become Pt-resistant. Therefore, NFV and CDDP together, may be a feasible therapeutic option for these patients through the induction of pyroptosis.

#1620 Vulnerability to selective CTPS1 inhibition in cancers lacking CTPS2.

Philip A. Beer[1], John P. Thomson[2], Charlie Gourley[2], Alison Meynert[2], Andrew E. Parker[1], Brian Schwartz[1], Patricia Roxburgh[3]

[1]Step Pharma, Saint-Genis-Pouilly, France,[2]University of Edinburgh, Edinburgh, United Kingdom,[3]University of Glasgow, Glasgow, United Kingdom

Background
De novo pyrimidine synthesis is a universal requirement for cancer cell proliferation; however, targeting this pathway results in toxicity to all dividing cells. The rate limiting conversion of UTP to CTP is catalysed by two isoforms with the same enzymatic activity (CTP synthase 1 and 2; CTPS1 & CTPS2). CTPS1 plays an essential role in lymphoid cell proliferation; differential targeting of CTPS1, sparing the paralog CTPS2, is a therapeutic strategy in lymphoid cancers that can potentially avoid toxicity. In epithelial tissues (and cancers derived from them), CTPS2 is also active and may compensate for CTPS1 inhibition. Somatic loss of CTPS2 would expose a synthetic lethal vulnerability to CTPS1 inhibition. A highly selective, orally available CTPS1 inhibitor has entered clinical development.
Methods
Pan-Cancer Analysis of Whole Genomes data were accessed via cBioPortal. Ovarian cancer tumour microarrays (n=117 tumours) were analysed by immunohistochemistry (IHC) using a monoclonal CTPS2 antibody. CTPS2 was disrupted in cancer cell lines by clustered regularly interspaced short palindromic repeats (CRISPR); protein loss was confirmed in clonal progeny by western blotting. STP-B is a potent and highly selective inhibitor of CTPS1 (PMID: 37008165); IC50 values for isogenic cell line pairs (n=5) and primary ovarian cancers (n=10) were assessed using CellTiter-Glo. Immunodeficient mice were transplanted subcutaneously with a CTPS2 positive or negative primary ovarian cancer (n=1 each) and treated with STP-B at 10-50 mg/kg/day or vehicle control (n=10 mice per arm).
Results
Hemi/homozygous loss of CTPS2 (chrX gene) was most prevalent in ovarian, lung, bladder and gastroesophageal cancer (17 to 19%). IHC analysis of tumour microarrays confirmed lack of CTPS2 protein in 24% of ovarian cancers, as well as in other cancer types at prevalences broadly commensurate with the genomic data. CRISPR-induced CTPS2 loss in cancer cell lines resulted in an 11-fold mean increase in sensitivity to CTPS1 inhibition by a highly selective small molecule (STP-B). CTPS2 null primary ovarian cancers showed uniformly greater sensitivity to STP-B in vitro and underwent tumour regression with STP-B treatment in vivo. CTPS2 loss was largely mutually exclusive with BRCA1/2 mutation in two independent ovarian cancer cohorts.
Conclusions
In vitro and in vivo studies demonstrate that loss of CTPS2 exposes a synthetic lethal vulnerability to selective CTPS1 inhibition. The prevalence of CTPS2 loss is >10% in a number of common cancer types. A highly selective oral CTPS1 inhibitor is currently in clinical development for the treatment of lymphoma (NCT05463263). Dose escalation in solid tumours commenced September 2024, and will be followed by a safety expansion cohort for patients with CTPS2 null ovarian cancer (NCT06297525).

#1621 Investigate PARP1-dependent cell death in hepatocellular carcinoma.

Chun Yin YU, Hoi Tang MA

The University of Hong Kong, Hong Kong, China

Hepatocellular carcinoma (HCC) is one of the most prevalent types of liver cancer, representing the third-leading cause of cancer-related mortality worldwide. It is typically characterized by an environment of high oxidative stress and persistent DNA damage. The persistent DNA damage in HCC triggers and initiates the ADP-ribosylation signalling pathway, which locates the DNA lesion sites for the recruitment of proteins involved in DNA damage repair. While ADP-ribosylation is critical for DNA damage repair, the impact of disrupting the homeostasis of ADP-ribosylation on HCC remains poorly understood. To explore the impact of dysregulated ADP-ribosylation in HCC, a degron-mediated gene silencing system was employed in HCC cell lines with PARG and ARH3 knockout (KO). With the addition of doxycycline (Dox) and Auxin (IAA), the degron-tagged PARG was degraded, establishing the PARG and ARH3 KO environment. Acute loss of PARG and ARH3 resulted in PARP1 hyperactivation, leading to PAR accumulation in the cells, followed by massive cell death in a time-dependent manner. The PARP1-dependent cell death induced by PARG and ARH3 KO was confirmed using the PARP1 KO model and the supplementation of a PARP1 inhibitor to cells. While activation of apoptosis and parthanatos (canonical PARP1-dependent cell death pathways) was observed, targeting the key enzymes in these pathways failed to suppress PARG and ARH3 KO-mediated cell death, indicating that the underlying mechanism is yet to be identified. Nevertheless, an interconnection between the NAD cycle and PARP1-dependent cell death was also examined. Supplementation of the metabolite in the NAD cycle suppresses PARP1-dependent cell death. Interestingly, targeting the rate-limiting enzyme in the NAD salvage pathway using CRISPR-Cas9-mediated KO or NAMPT inhibitor could suppress cell death. Altogether, these results extend the understanding of PARP1-dependent cell death mediated by the loss of PARG and ARH3 and provide insights into the therapeutic development for targeting the homeostasis of ADP-ribosylation in HCC.

#1622 Inhibition of bone morphogenetic protein receptor 2 increases mitochondrial bioenergetics and synergistically enhances cell death induced by BCL 2 inhibitors in lung cancer and leukemia cells.

Ashley Toussaint, Manohar Singh, Arindam Mondal, Dongxuan Jia, John Langenfeld

RWJMS, New Brunswick, NJ

Background: Bone morphogenic proteins (BMP) proteins are highly overexpressed in non-small cell lung carcinomas (NSCLC) and leukemia cells. Studies have shown that BMP signaling is growth promoting and enhances survival of cancer cells. The mechanism by which BMP signaling promotes tumorigenesis has not been fully elucidated. Our prior studies revealed that BMP signaling suppresses AMP kinase activation in lung cancer cells, which is conserved in C elegans. We also found that the BMP2 ligand significantly suppressed tricarboxylic acid (TCA) cycle intermediates in lung cancer cells. These studies suggest that BMP signaling suppresses mitochondrial bioenergetics. To better understand the role of BMP signaling in cancer, our team developed specific small molecules inhibiting the BMP type 2 receptor (BMPR2). In this study, the BMPR2 inhibitor JL189 and genetically engineered BMPR2 knockout (KO) cells were used to examine the effects of BMP signaling on mitochondrial bioenergetics. We also examined if altering mitochondrial bioenergetics increased cytotoxicity of BCL-2 inhibitors, which promote mitochondrial induced apoptosis. Mitochondrial metabolic metabolites were measured by liquid chromatography mass spectrometry. Mitochondrial mass was determined by MitoTracker Green labeling quantified by flow cytometry. Metabolomic studies demonstrated that JL189 significantly increased TCA intermediates in lung cancer cells. Knockout of BMPR2 in A549 cells also significantly increased TCA cycle intermediates. Agilent Seahorse analyzer demonstrated that BMPR2 inhibition increased oxidative phosphorylation in NSLC cells. Inhibition of BMPR2 signaling with JL189 or KO cells increased mitochondrial mass in NSCLC and leukemia cell lines. JL189 synergistically enhanced cell death induced by the BCL-2 inhibitors ABT-263 (navitoclax) and ABT-199 (venetoclax) in both NSCLC (A549, H1299, Calu-1) and leukemia cell lines (Jurkat, Kasumi-1, THP-1). By Western blot analysis, the activation of caspase-3 was higher in cancer cells treated with JL189 combined with ABT-263 compared to each compound alone. Cell death induced by BMPR2 inhibition alone and in combination with BCL-2 inhibitors was more pronounced in leukemia cells lines compared to NSCLC cell lines.
Conclusion: Inhibition of BMPR2 signaling increased mitochondrial biogenesis in NSCLC and leukemia cell lines, suggesting that the cancer cells are more susceptible to mitochondrial induced cell death. BMPR2 inhibition also significantly increased cancer cell death when combined with a BCL-2 inhibitor. Priming the mitochondria with a BMPR2 inhibitor may represent a novel strategy to enhance cancer therapeutics that promote mitochondrial induced apoptosis. Our data suggests that BMPR2 inhibition may be more effective in leukemia compared to NSLC.

#1623 Mechanistic insights into low-frequency ultrasound-induced cancer cell apoptosis.

Aditi Singh, Felix Margadant, Michael Sheetz

University of Texas Medical Branch (UTMB), Galveston, TX

Cancer cells undergo rigidity independent growth due to low expression of mechanosensory cytoskeletal protein known as tropomyosin 2.1, acting as rigidity sensing module. This enables their survival on soft matrices by inciting distinct response to the mechanical cues, in contrast to non-transformed cells. We previously found that treatment of cancer cells with low-frequency ultrasound results in calcium influx through activation of piezo1 channels, microtubule depolymerization through calpain proteases, activation of RhoA-ROCK pathway and increased myosin IIA-mediated actomyosin contractility, ultimately causing cell death in calcium-induced calcium release manner. However, the underlying processes connecting the initiation of apoptosis upon mechanical stimuli remain inadequately understood. Our research focus involves ER-mitochondrial stress pathway comprising of mitochondria-associated ER membranes (MAMs), that might be involved in causing apoptosis in cancer cells exhibiting variations in their mechanoresponse via differential cell killing, while leaving the normal cells unaffected. Recently, we found lowering of mitochondrial membrane potential in cancer cells post-ultrasound treatment. Mitochondrial distribution of normal and cancer cells after ultrasound treatment varied dramatically, leading to increased punctate forms in cancer cells. Inhibition of mitochondrial fission showed significant decrease in apoptosis of cancer cells after ultrasound treatment. We conclude crucial role of mitochondrial fission, ROS and electron transport chain in low-frequency ultrasound-mediated apoptosis in cancer cells along with the cytoskeletal proteins. Tuning of desired parameters involving frequency, pressure and pulse duration could aid in the development of non-invasive cancer therapeutics.

#1624 Stat1 promotes lesion growth and tumor immunity of tuberin-deficient cells in lymphangioleiomyomatosis.

Tasnim Olatoke, Jane Yu

University of Cincinnati, Cincinnati, OH

Lymphangioleiomyomatosis (LAM) is a cystic lung disease that primarily affects women, with an estimated global prevalence of 19 per million women. LAM is caused by the invasion of metastatic smooth muscle-like cells into the lung parenchyma, leading to abnormal cell proliferation and progressive respiratory failure. LAM cells have TSC gene mutations, which occur sporadically or in people with Tuberous Sclerosis Complex. Although it is known that hyperactivation of the mechanistic target of rapamycin complex 1 (mTORC1) due to TSC2 mutations contributes to aberrant cell growth in LAM lung, tumor origin and invasive mechanism remain unclear. Integrative single-cell omics and validation analyses revealed the activation of a novel uterine-similar HOX/PBX transcriptional network in the LAM lung compared to other lung cells, suggesting that the uterus is one of the origins of LAM cells. Further omics studies indicated that STAT1 and STAT3 interact with PBX1 to increase LAM cell survival. The objective of this study is to delineate mechanisms by which STAT signaling may regulate LAM pathogenesis, with hopes to find potential therapeutic options for LAM and TSC-associated disorders. We hypothesize that STAT1 suppression attenuates LAM patient-derived (TSC2-null) cell viability and tumor growth in LAM. We first confirmed our network prediction analysis by showing that aberrant PBX1 activation upregulates STAT1 in LAM cells and tissues. Our cell viability assays show that STAT1 suppression induced TSC2-null cell apoptosis compared to controls. Similarly, our mouse models show that STAT1 suppression attenuates lung lesion and uterine tumor growth in TSC2-deficient LAM models. We also show that STAT1 activation induced PD-L1 upregulation, indicating that STAT1 helps tumor cells to evade immune surveillance in LAM. Our findings provide proof of concept that STAT1 mediates LAM tumor proliferation through two mechanisms. (1) by promoting lesion growth and (2) by promoting immune evasion via upregulation of PD-L1. STAT1 signaling is critical for LAM cell survival and could be targeted to treat LAM and TSC-associated disorders.

#1625 Redox homeostasis is a therapeutic vulnerability in lethal neuroendocrine prostate cancer.

Mu-En Wang[1], Wei-Ling Tu[1], Ji-Hoon Kim[1], Yi Lu[1], Alyssa Bawcom[1], Andrew J. Armstrong[1], Qianben Wang[1], Yuzhuo Wang[2], Jiaoti Huang[1], Ming Chen[1]

[1]Duke University School of Medicine, Durham, NC,[2]University of British Columbia, Vancouver, NC, Canada

Background: Neuroendocrine prostate cancer (NEPC) is an aggressive and lethal subtype of prostate cancer that often develops after second-generation anti-androgen therapies. The current treatment options for NEPC are limited and only produce disappointing results, highlighting the urgent need for novel therapies. Previously, we have found that NEPC cells are vulnerable to BCL2 and GPX4 inhibitors compared to their counterparts, prostate adenocarcinoma (PADC). Here, we aim to determine whether and how combining BCL2 and GPX4 inhibitors would enable optimal therapeutic effects in NEPC.

Methods: We conducted drug synergism analyses of BCL2 and GPX4 inhibitors using NEPC cell lines and determined their impacts on the major cell death pathways by performing rescuing experiments. To further determine the molecular mechanisms of BCL2 and GPX4 inhibitors-induced cell death in NEPC, we measured the intracellular reactive oxygen species (ROS) levels and tested the rescuing effects of various antioxidants and reducing agents on cell death. Finally, we determined the *in vivo* therapeutic efficacy of BCL2 and GPX4 inhibitors in an NCI-H660 cell line-derived xenograft (CDX) tumor model.

Results: Our results demonstrate that the BCL2 inhibitor ABT-199 and GPX4 inhibitor C18 synergistically induced apoptosis in NEPC cell lines in a ROS-independent manner. Although N-acetylcysteine (NAC) rescued ABT-199 and C18 cotreatment-induced apoptosis, the CellROX Green assay revealed no significant changes in ROS levels in ABT-199 and C18-cotreated cells. Surprisingly, reducing agents including tris(2-carboxyethyl)phosphine (TCEP) and Dithiothreitol (DTT) strongly rescued cell death induced by ABT-199 and C18 cotreatment. Our findings therefore suggested that the cell-killing effects of ABT-199 and C18 were due to ROS-independent disruption of redox homeostasis. Lastly, our preclinical tumor study showed that the ABT-199 and C18 combinatorial therapy significantly suppressed the tumor growth of NEPC.

Conclusions: Our study validated the combination of BCL2 and GPX4 inhibitors as a feasible approach for the treatment of NEPC and suggested that redox homeostasis is a therapeutic vulnerability in NEPC.

#1626 CDK9 inhibition enhances apoptosis of TP53 mutated AML when combined with standard chemotherapy.

E. C. Trull, D. B. Sykes, P. C. Amrein;
Massachusetts General Hospital, Boston, MA

Background: The prognosis of patients with acute myeloid leukemia (AML) remains poor for those with mutations or deletions of the TP53 gene. Inhibiting CDK9 is a strategy to trigger apoptosis downstream of p53 with the goal of overcoming the poor prognostic nature of mutant p53. Here we test dinaciclib, a multi-CDK and potent CDK9 inhibitor, alone and with azacitidine and venetoclax to demonstrate that CDK9 inhibition as monotherapy and in combination leads to apoptosis in TP53 mutated AML.
Methods: To determine the effect of inhibiting CDK9 on the cytotoxicity of AML and normal cells, we treated p53 mutated AML cell lines (THP1, NOMO1, and U937), wild type (WT) p53 AML cell lines (MV4-11 and HL-60) and peripheral blood mononuclear cells (PBMCs) from healthy donors with azacitidine, venetoclax, and dinaciclib. We performed viability assays to examine cell death and determine the IC_{50} and optimal exposure time of these drugs. Apoptosis was measured through flow cytometry with Annexin-V and 7-AAD staining, and through the quantification of apoptotic proteins with immunoblotting.
Results: Treatment of mutant and WT p53 AML cell lines with azacitidine, venetoclax, and dinaciclib as monotherapy demonstrated a dose-dependent decrease in cell viability. After testing various concentrations and exposure times of these drugs, we determined concentrations of 200nM azacitidine, 6000nM venetoclax, and 10nM dinaciclib at an exposure time of 8-hours were highly effective with dinaciclib monotherapy and in the triple-drug combination. When used in the mutant p53 cell line, THP1, and WT p53 cell line, MV4-11, the addition of dinaciclib to azacitidine and venetoclax showed increased synergy (scores of 29.6 and 12.9, respectively, using the HSA reference model) compared to azacitidine and venetoclax alone (synergy scores of -0.98 and 1.4, respectively). The addition of dinaciclib to THP1 cells resulted in increased apoptosis (55.2%) when compared to the two-drug combination of azacitidine and venetoclax (17.0%) as measured by the quantification of c-PARP expression normalized to beta-actin through immunoblotting. When this drug combination was tested in PBMCs of healthy donors, they were notably less sensitive and had lower synergy scores (1.2) than the AML cell lines.
Conclusions: We have shown that the addition of a CDK9 inhibitor to azacitidine and venetoclax results in enhanced cytotoxicity of mutant p53 and WT p53 AML cell lines at low nanomolar concentrations. These results suggest that inhibition of CDK9 sensitizes AML cells independently of TP53, with less pronounced cytotoxicity in normal PBMCs of healthy donors. We plan to further examine the effect of CDK9 inhibition using a highly selective CDK9 inhibitor and validate these results with primary AML and normal bone marrow samples. Ultimately, we hope these experiments will guide the development of human clinical trials to benefit those patients with p53 mutated AML.

#1627 Switching cell fate from senescence to apoptosis by the combination of a p53 corrector with the MDM2 antagonist ASTX295.

George Ward, Judit Espana-Agusti, Keisha Hearn, Mark Wade, Lynsey Fazal, Hugh Walton, Andrea Biondo, John Lyons, Martin Sims, Simone Jueliger, Jessica Brothwood, Andrew Woodhead, Louise Walsh, Tomoko Smyth, Nicola Wilsher, **Maria Ahn**

Astex Pharmaceuticals, Cambridge, United Kingdom

Background: ASTX295 is a potent MDM2 antagonist designed to have a shorter half-life ($t_{1/2}$ 4-6 hours in plasma), which leads to an improved safety profile (bone-marrow sparing), making it more amenable to combinations compared to other MDM2 antagonists. The recent success of targeting *TP53:Y220C* mutant cancers with a p53 corrector molecule holds great promise for treatment of all *Y220C TP53* mutant cancers. However, for effective therapy, a high level of p53 signalling will be required to overcome the elevated apoptotic thresholds seen in some cancer cells with co-mutations in pro-survival signalling pathways. We rationalised that this could be achieved by removing the MDM2-driven negative feedback loop that is triggered following *Y220C* correction.

Materials and Methods: Corrected p53 signalling was determined in two *TP53:Y220C* mutant pancreatic cancer cell lines with additional MAPK mutations: BxPC3 (*BRAF:L485-P490del*) and T3M-4 (*KRAS:Q61H*). Two small-molecule TP53:Y220C correctors were tested alone and in combination with ASTX295 using real-time microscopy, western blotting, and qPCR. Markers of p53 signalling (at the protein and mRNA level), senescence (β-galactosidase), apoptosis (Annexin V), and DNA damage (phospho-H2A.X) were measured after treatment with compounds. A mouse xenograft model was established in male CB17 SCID mice inoculated subcutaneously with BxPC3 cells, allowing PK/PD and efficacy evaluation.

Results: The combination of ASTX295 and the *Y220C* corrector led to increased cell death in both BxPC3 and T3M-4 cell lines *in vitro*, with elevated p53 signalling (p21), apoptosis (PUMA), and DNA damage (phospho-H2A.X) when MDM2 is antagonised. The *KRAS* mutant line, T3M-4, was less sensitive to the combination, but could be driven to apoptosis at higher concentrations of the corrector. PK/PD evaluation in BxPC3-xenograft-bearing mice demonstrated prolonged p53 signalling following combination treatment up to 16 h post dose, with a subsequent reduction at 24 h consistent with the ASTX295 exposure profile.

Conclusions: We have shown that MDM2 antagonism leads to sustained corrected p53 signalling over time, which drives cell death, even in tumor cells harbouring co-mutations conferring higher apoptotic resistance. Indeed, our study provides proof of concept that efficacy could be achieved in multiple tumor types in which the p53 pathway can be targeted therapeutically through combination with ASTX295, which would drive and maintain p53 signalling levels above the apoptotic threshold.

#1629 Targeting HSBP1 Cys137 residue by 4-Iodobutyl acetate selectively kills triple-negative breast cancer.

Ruei-Chen Hung[1], Chiou-Feng Lin[1], Yuan-Feng Lin[2]

[1]Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, Taipei City, Taiwan,[2]Graduate Institute of Clinical Medicine, College of Medicine, Taipei Medical University, Taipei City, Taiwan

Background: Triple-negative breast cancer (TNBC) accounts for 15-20% of breast cancers and has a higher mortality rate because of metastatic character as compared to the non-TNBC subtypes. Chemotherapy is a non-selective anti-cancer treatment and currently remains as a first-line regimen for TNBC patients. As a result, identifying a more effective and selective drug target is urgently needed for TNBC. The aim of this study was made to discover the iodoacetamide derivative-targetable cysteine (Cys) residues of protein which is involved in the specific protein-protein interactions (PPIs) that are critical for TNBC survival.
Methods: MTT assay was used to discover the iodoacetamide derivative that has a cytotoxic effectiveness on TNBC cell line HCC1806 better than normal mammary cell line MCF10A. Mass spectrometric analysis was utilized to identify the iodoacetamide derivative-targetable proteins and Cys residues. Site-directed mutagenesis was employed to validate the specific incorporation of iodoacetamide derivative candidate into the identified protein Cys residue. Cell-based assays and mouse models were established to determine the anti-TNBC effectiveness of iodoacetamide derivative candidate. Pulldown assay was performed to determine the specific PPIs of iodoacetamide derivative candidate-targeted protein in TNBC cells.
Results: MTT assays revealed that 4-Iodobutyl Acetate (IBA) is able to selectively kills HCC1806 cells with half maximal inhibitory concentration (IC_{50}) at 0.01 μM which is much lower than that of MCF10A cells (IC_{50} = 7.91 μM). Mass spectrometric analysis indicated that IBA incorporates into the Cys137 residue of HSPB1 protein and thereby induces cell apoptosis in HCC1806 cells. Either the removal of iodo-group from IBA or the mutation of Cys137 to Ser significantly abolished its cytotoxic effects on HCC1806 cells both *in vitro* and *in vivo*. Pulldown assay demonstrated that the incorporation of IBA into Cys 137 leads to the disruption of HSPB1 PPIs with Akt, Cytochrome c and Caspase-3, which ultimately results in the loss of metastatic ability and induction of cell apoptosis in HCC1806 cells.
Conclusion: Our results provide a novel, selective and even more safe therapeutic strategy for combating TNBC via targeting the pocket surrounding the Cys137 of HSPB1 protein.
Keywords: Triple-negative breast cancer; iodoacetamide derivative; protein-protein interactions; HSPB1; 4-Iodobutyl Acetate

#1630 Targeting the LIF/LIFR axis reduces the progression of inflammatory breast cancer by promoting ferroptosis.

Bianca A. Romo[1], Zenaida Fuentes[2], Lois Randolph[1], Megharani Mahajan[1], Emily J. Aller[1], Behnam Ebrahimi[1], Bindu Santhamma[3], Hareesh B. Nair[3], Christoforos Thomas[4], Ratna K. Vadlamudi[1], Suryavathi Viswanadhapalli[1]

[1]UT Health Science Center at San Antonio, San Antonio, TX,[2]Vanderbuilt University, Nashville, TN,[3]Evestra Inc., Shertz, TX,[4]Houston Methodist, Houston, TX

Background: Inflammatory breast cancer (IBC) is a rare subtype of breast cancer affecting 2-4% of the U.S. population. With the incidence being quite low, IBC is responsible for 7% of breast cancer related mortality often affecting younger women. The only currently approved options for treatment are surgical resection, chemotherapy, and radiation. Novel therapeutic targets are desperately needed for the generation of new therapeutics. IBC progression has been linked to growth factor and cytokine signaling abnormalities. In previous studies, the cytokine LIF/LIFR signaling axis has been identified as a crucial pathway in the progression of triple negative breast cancer. However, the role of LIF/LIFR in IBC progression remains unknown. The objective of this study is to test the utility of LIFR and test the effectiveness of LIFR inhibitor at treating IBC.

Methods: The utility of using LIFR inhibition as a treatment strategy in IBC was tested using cell survival, apoptosis, and colony formation assays using established IBC cell models (SUM149, SUM190PT, and KPL-4). We have reduced LIFR levels by 70% in IBC cells utilizing two different siRNAs that target LIFR. Western blotting, luciferase reporter assays, RT-qPCR, and lipid peroxidation assays were used to establish the mechanism of EC359 mediated growth suppression of IBC. *In vivo* treatment of cell-derived xenografts (KPL-4) was used to assess the utility of EC359.

Results: All three IBC models exhibited detectable expressions of LIF and LIFR. Reduction of LIFR using siRNA decreased cell survival, colony formation, and invasion capacity of IBC cells relative to cells transfected with control siRNAs. Pharmacological inhibition of LIFR with EC359 effectively reduced cell survival and clonogenic capacity of IBC cells in the low nanomolar range. RT-qPCR tests revealed EC359 markedly decreased the expression of LIFR target genes. Western analyses confirmed that EC359 treatment suppressed downstream LIF/LIFR signaling pathways, including STAT3, AKT, and pS6, and promoted apoptosis. Treatment of cells with the ferroptosis inhibitor Ferrostatin 1 negated the capacity of EC359 to induce cell death. The evaluation of lipid peroxidation in cells via flow cytometry, utilizing the oxidation of BODIPY™ reagent, further confirmed EC359 induction of ferroptosis. Mechanistic investigations demonstrated that EC359 predominantly triggered ferroptosis by inhibiting the glutathione antioxidant defense system through the downregulation of SLC7A11 and GPX4 levels. EC359 (5 mg/kg/day) was effective in reducing the growth of the IBC KPL4 xenograft tumors. Immunohistochemical examinations of tumors validated reduced cell proliferation as assessed by Ki67 staining.

Conclusion: Collectively, our research suggests that the inhibition of LIFR promotes ferroptosis-mediated cell death in IBC, and LIFR inhibitors represent a novel therapeutic option for IBC.

#1631 Targeting ER stress for treating hepatocellular carcinoma (HCC).

Adriana Baker[1], Xue Yang[2], Uday P. Pratap[1], William C. Arnold[1], Alyssa D. Friudenberg[3], Gaurav Sharma[1], Chia-Yuan Chen[4], Scott Elmore[4], Sukeshi Patel Arora[1], LuZhe Sun[1], Suryavathi Viswanadhapalli[1], Ganesh V. Raj[5], Jung-Mo Ahn[4], Ratna K. Vadlamudi[1]

[1]The University of Texas Health Science Center at San Antonio, San Antonio, TX,[2]The University of Texas MD Anderson Cancer Center, Houston, TX,[3]Texas State University at San Marcos, San Marcos, TX,[4]The University of Texas at Dallas, Dallas, TX,[5]EtiraRx, Dallas, TX

Background: Hepatocellular carcinoma (HCC) accounts for more than 90% of instances of liver cancer, which ranks as the fifth most frequent cancer in the United States. Furthermore, Texas leads the nation in the age-adjusted incidence of HCC. Most patients are diagnosed with advanced, unresectable HCC, and their 5-year survival rate is less than 5% when using current systemic treatments. Therefore, there is an immediate unmet need for new treatment approaches for HCC. Recently, the unfolded protein response (UPR) and endoplasmic reticulum (ER) stress have been recognized as targetable vulnerabilities. From a curated screen, we have previously identified that a synthetic oligo-benzamide, ERX-315, that targets protein encoded by lysosomal acid lipase A (*LIPA*), causes ER stress and cancer cell death without affecting normal cells. This study aims to evaluate the utility of ERX-315 for treating HCC by targeting ER stress

Methods: Tissue micro arrays (TMAs) and Immunohistochemistry (IHC) were used to confirm the expression of LIPA in HCC. LIPA expression in HCC tumors was also confirmed using TNM database. The impact of ERX-315 on 6 well-established HCC cell lines was evaluated using the MTT and colony formation assays. The specificity of ERX-315 targeting activity was confirmed by CRISPR-KO of *LIPA* in one of the HCC cell lines. For mechanistic investigations, Western blotting, RT-qPCR, and splicing assays were employed. Huh7 organoids generated from xenografts were utilized to evaluate the effects of ERX-315 *ex vivo*. Huh7 cell-based xenografts and patient-derived xenograft (PDX) models were used to validate the efficacy of ERX-315 in vivo.

Results: TNM plot analysis revealed that HCC tumors exhibited elevated levels of LIPA expression in comparison to normal tissue. Analysis of TMA samples showed that compared to normal tissue, HCC samples exhibit increased levels of LIPA expression. ERX-315 treatment significantly decreased both colony formation and cell viability (IC_{50} between 30-150nM) and promoted apoptosis in HCC cells. In contrast, ERX-315 did not cause apoptosis in normal liver epithelial cells. Compared to wild type cells, KO of LIPA dramatically attenuated the effect of ERX-315 on colony formation and cell viability in HCC cells. Mechanistic studies using splicing assay, RT-qPCR, and Western blotting demonstrated elevated levels of ER stress indicators upon treatment with ERX-315 in a dose dependent manner. Xenograft-derived Huh7 organoids' cell viability was considerably reduced by ERX-315. ERX-315 therapy dramatically decreased the tumor volume in Huh7 xenograft and PDX models in both male and female mice.

Conclusion: Collectively, our results suggest that ERX-315 promotes ER stress and cell death in HCC *in vitro*, *ex vivo*, and *in vivo*. ERX-315 represents a promising novel therapeutic option for HCC. Since ERX-315 is in clinical trials, these data strongly support evaluation of ERX-315 in patients with liver cancer.

#1632 EGFR-TKIs facilitate cuproptosis via regulating SLC31A1 in cancer cells.

Xiyi Wei[1], Weiyu Kong[1], Zijie Yu[1], Chao Qin[1], Bing Yao[2], Zhou Yang[1]

[1]Department of Urology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China,[2]School of Basic Medical Sciences, Nanjing Medical University, Nanjing, China

Cuproptosis, a novel cellular demise pathway linked to copper homeostasis and copper ionophores, offers promising therapeutic avenues across diverse human ailments. However, the effectiveness of copper ionophores in cancer treatment is not that satisfactory. Tyrosine kinase inhibitors (TKIs) have revolutionized the approach to combating various types of cancer by regulating different cell death manners. In this research, we identified EGFR-TKIs and MEK-TKIs significantly sensitized cuproptosis. Our investigation delineates that the EGFR/MEK signaling cascade phosphorylates JUND, augmenting its transcriptional prowess as a key repressor of SLC31A1. Interestingly, EGFR-TKIs enhance the interaction between EGFR and SLC31A1, impeding UBE3C-mediated degradation, thereby stabilizing SLC31A1 levels. Moreover, copper binding to the extracellular domain of EGFR promotes its dimerization and downstream phosphorylation, orchestrating a protective mechanism under copper-induced stress, whereas EGFR-TKIs interrupt EGFR-SLC31A1 cascade to sensitize cuproptosis. Overall, our findings shed light on the dual roles of EGFR-TKIs and MEK-TKIs in sensitizing cuproptosis within cancer contexts through intricate transcriptional and post-transcriptional regulation of SLC31A1. The prospective combination of EGFR/MEK-TKIs with copper emerges as a promising strategy for cuproptosis-targeted therapies across multiple malignancies with or without EGFR mutation, underscoring the innovative contributions of our study.

#1634 The role of the deubiquitinase USP7 and the E3/E4 ubiquitin ligase complex ITCH/UBE4B in the regulation of cell death in neuroblastoma.

Christophe Le Clorennec, Carla Sampaio, Peter E. Zage

UCSD Moores Cancer Center, La Jolla, CA

Dysregulation of the Ubiquitin Proteasome System has been linked to many human diseases, including cancer. Expression of UBE4B ubiquitin ligase is associated with neuroblastoma patient outcomes and its functional roles in neuroblastoma pathogenesis are not known. We have recently identified an ubiquitin ligase complex ITCH/UBE4B which mediates ubiquitination of Ku70 and c-FLIPL proteins for proteasomal degradation allowing HDAC inhibitor-mediated caspase-8 dependent apoptosis. We also confirmed that the deubiquitinase USP7, critical player in tumor suppression and DNA repair, can be a potential therapeutic target for neuroblastoma. Highly selective USP7 inhibitors have demonstrated significant antitumor activity in preclinical models of adult cancer, and we reported that these inhibitors alone can be more effective against neuroblastoma tumor growth. Our hypothesis is that USP7 inhibition will be more effective against neuroblastoma tumors through destabilization of ITCH/UBE4B complex protein targets allowing a stronger induction of cell death in response to treatment for the children. To evaluate efficacy of USP7 inhibitors in combination with HDAC inhibitors or chemotherapy against neuroblastoma tumor growth, neuroblastoma cell lines depleted for UBE4B or USP7 were treated with increasing concentrations of USP7 inhibitors alone or in combination with chemotherapy or with HDAC inhibitors. Cell proliferation was measured using continuous live cell imaging and apoptosis by caspase cleavage and PARP cleavage detection by western blot. We have evaluated also, whether ubiquitination/deubiquitination and degradation rates of p53, Ku70 and c-FLIPL proteins could modulate induction of apoptosis and necroptosis. USP7 inhibition resulted in decreases in cell viability in p53 wild-type neuroblastoma cell lines via the induction of apoptosis and necroptosis by increasing phosphorylation of MLKL, RIPK3 and RIPK1. In UBE4B or USP7 depleted cells, a huge induction of necroptosis and a small rate of apoptosis were observed. We also identified USP7 as a deubiquitinase of Ku70 and c-FLIPL, stabilizing the Ku70/c-FLIPL/Bax “FLIPosome” complex. USP7 inhibition destabilizes ku70 and c-FLIPL for protein degradation leading to induction of apoptosis as well as necroptosis. UBE4B depletion in neuroblastoma inhibits HDAC inhibitors mediated caspase-8 mediated apoptosis but enhances necroptosis, notably because the stabilization of the FLIPosome Ku70-c-FLIPL can allow the blockade of caspase-8 activation and can trigger the activation of the necroptosome. Our data suggests that USP7 and ITCH/UBE4B can control the switch between apoptosis and necroptosis in response to USP7 and HDAC inhibitors. USP7 inhibition and ITCH/UBE4B activation by HDAC inhibitors could be a promising therapeutic strategy for children with high-risk and relapsed neuroblastoma.

#1635 Targeting ferroptosis in CCDC88A-overexpressing metastatic triple-negative breast cancer.

Wei-Ling Tu, Mu-En Wang, Ji-Hoon Kim, Alyssa Bawcom, Yi Lu, Ming Chen

Pathology, Duke University, Durham, NC

Triple-negative breast cancer (TNBC) is a form of breast cancer that does not express estrogen receptor (ER), progesterone receptors (PR) or human epidermal growth factor receptor 2 (HER2). It accounts for 15-20% of all breast carcinomas and preferentially affects women who are African American, younger than age 40, or have a *BRCA1* mutation. The TNBC subtype is highly aggressive and heterogeneous and has a poor prognosis relative to other breast cancer subtypes due to a lack of highly recurrent and actionable biomarkers that can be used to direct therapy. Although standard chemotherapies show a high initial response rate in TNBC, tumor relapses and metastasis often occur rapidly. Moreover, for patients who have recurrent disease or progressive disease during chemotherapy, treatment options are limited. Therefore, there is an unmet need to identify effective therapies targeting TNBC. By analyzing public human datasets, we found that CCDC88A (also known as Girdin or GIV), a putative oncogene, is highly expressed in TNBC compared to other breast cancer subtypes. CCDC88A has been found to be involved in key cancer development events such as tumor angiogenesis, invasion, wound healing, and metastasis. Interestingly, we found that, although the high expression of CCDC88A conferred the ability of lung metastasis, it increased the susceptibility of TNBC to ferroptosis, a type of cell death induced by iron-dependent lipid peroxidation. Mechanistically, CCDC88A sensitized TNBC cells to ferroptosis by limiting intracellular glutathione (GSH) levels. shRNA-mediated CCDC88A knockdown in TNBC cells significantly increased GSH levels and in turn blunted cellular sensitivity to ferroptosis. On the other hand, inhibition of GSH synthesis with a glutamate-cysteine ligase (GCL) inhibitor, BSO, restored the ferroptosis sensitivity in CCDC88A knocked-down TNBC cells. Thus, our findings uncover CCDC88A as a novel ferroptosis regulator and also suggest that ferroptosis is a therapeutically tractable target for metastatic TNBC that overexpresses CCDC88A.

#1636 Novel CDK12/13 inhibitor CTX-439 downregulates MCL1 via transcriptional mRNA read-through and synergistically acts with BCL-XL inhibitors for cancer therapy.

Hiroko Yamakawa[1], Raghda Khatab[2], Yusuke Tarumoto[2], Mariko Takano[2], Seiichi Sugino[2], Shafiqul Islam[2], Shunsuke Ebara[1], Kosuke Kawaguchi[3], Masakazu Toi[3], Akio Mizutani[1], Midori Sugiyama[1], Misaki Yoshida[1], **Daisuke Morishita**[1], Kosuke Yusa[2]

[1]Chordia Therapeutics Inc., Fujisawa, Japan,[2]Stem Cell Genetics, Institute for Life and Frontier Sciences, Kyoto University, Kyoto, Japan,[3]Department of Breast Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Combination therapeutic strategies for cancer use multiple agents targeting distinct pathways to enhance the treatment efficacy and minimize resistance. By simultaneously disrupting key cancer cell survival mechanisms, these strategies improve the outcomes better than monotherapies, effectively combating cancer complexity and adaptability. Cyclin-dependent kinase (CDK)-12/13 regulates RNA transcription by phosphorylating RNA polymerase II, promoting the elongation of transcripts involved in the DNA damage response, such as BRCA1/2. As an emerging drug target, the potential of CDK12 to enhance the poly (ADP ribose) polymerase (PARP) inhibitor effects has been re-evaluated. CDK12/13 and PARP inhibitors exert synergistic effects. CDK12/13 inhibitor is currently undergoing clinical trial. However, therapeutic potential of CDK12/13 inhibitor combinations with other factors beyond PARP remains unclear, necessitating further investigations to expand their clinical applications. We successfully developed CTX-439, a novel orally bioavailable ATP-competitive CDK12/13 small-molecule inhibitor (Yamakawa *et al*., AACR2022/2024). CTX-439 exhibited anti-tumor activity alone and in combination with PARP inhibitors. To identify other factors synergistic with CTX-439, we performed a CRISPR activation screening using the SUM149 breast cancer cell line and identified four genes, whose overexpression conferred CTX-439 resistance. Anti-apoptotic factors BCL-XL and BCL2 were among the top candidates, whose inhibition enhanced CTX-439 efficacy. Moreover, dual inhibition of CTX-439 and AZD4320, which targets BCL-XL, synergistically induced rapid apoptosis *in vitro* and significantly suppressed tumor growth *in vivo*. Mechanistically, CTX-439 downregulated the protein levels of anti-apoptotic factor MCL1, but not BCL-XL, thereby shifting the dependency to BCL-XL for cell survival, suggesting BCL-XL inhibition as a critical apoptosis mechanism in CTX-439 combination strategies. Notably, CTX-439 significantly increased the read-through transcription of the *MCL1* mRNA, leading to its nuclear retention. Furthermore, lack of mature mRNA supply resulted in the rapid turnover of cytosolic *MCL1* mRNA due to its short half-life, leading to the downregulation of MCL1 protein levels. In summary, previously reported combination strategies with CDK12/13 inhibitors focused on regulating long mRNAs, particularly those associated with the DNA damage response. Interestingly, our study revealed a novel CDK12/13 inhibition mechanism targeting short mRNAs, such as *MCL1*, that can be used to establish new therapeutic combination strategies with BCL-XL inhibitors. Additionally, our study provides a new avenue to improve cancer therapy outcomes.

#1637 GPX4-mediated Pathway Regulates Iron Efflux to Prevent Ferroptotic Cell Death and Promote Immunosuppressive Signals in Osteosarcoma Cells.

Md Abdullah, Donghee Lee, Rong Li, Eslenur Nipa, Jong Hyuk Kim

University of Florida, Gainesville, FL

Osteosarcoma is a malignant bone sarcoma prevalent in children, adolescents, and young adults, with limited treatment options. Targeting ferroptosis, an iron-dependent form of cell death driven by lipid peroxidation, presents a potential therapeutic strategy for cancers. However, the molecular mechanisms that regulate iron metabolism in ferroptosis remain unclear. In this study, we explored cell defense mechanisms against ferroptosis mediated by intracellular iron regulation and immune signaling pathway in osteosarcoma cells. First, we found that xCT and GPX4 protein were detected with variable expressions across five osteosarcoma cell lines. Erastin, an xCT inhibitor, and RSL3, a GPX4 inhibitor, induced ferroptotic cell death in these cells. Specifically, higher inhibitory dose of RSL3 induced a significant reduction in GPX4 levels in U2OS and MG-63 cells in a dose- and time-dependent manner. In contrast, xCT expression increased in these cells, suggesting an adaptive mechanism to counteract oxidative stress following GPX4 depletion. RSL3 treatment elevated lipid peroxidation levels in U2OS and MG-63 cells, which were restored by Ferostatin-1. Our RNA-seq analysis identified 18 differentially expressed genes in response to RSL3 treatment in U2OS cells, with the highest expression in AKR1C1 gene, which plays a significant role in oxidative stress. We confirmed that GPX4 inhibition upregulated AKR1C1 gene expression as well as other oxidative stress markers, including NRF2, HMOX1, and NQO1.Our data also showed that osteosarcoma cells modulated intracellular iron homeostasis in response to GPX4-mediated ferroptotic stress. GPX4 inhibition significantly enhanced FPN and FTH1 expression, which are essential for iron efflux and storage, in U2OS cells, while MG-63 cells only increased FTH1 with minimal change in FPN. Furthermore, macrophage stimulating protein receptor (MSPR) protein expression was significantly increased by GPX4 inhibitor using Proteome Profiler analysis. We observed that combination treatment with dual MSPR and MET inhibitor, BMS-777607, showed synergistic effects with GPX4 inhibition. Altogether, our findings demonstrate that ferroptotic cell death is inducible in osteosarcoma cells, and the potential resistance mechanism to ferroptosis is mediated by oxidative stress mechanism and intracellular iron regulation. We also identified novel target, MSPR, regulated by GPX4 inhibition, which potentially contribute to the activation of immunosuppressive signals from osteosarcoma. In conclusion, this study underscores that targeting the ferroptotic pathway holds a great promise for therapeutic potential in osteosarcoma by regulating both intracellular pathway and the immunosuppressive tumor microenvironment.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Degraders and Glues 2
Poster Session

#1642 Next trip: a promising CK1α-selective molecular glue degrader in MSS colorectal cancer.

Hyunsun Jo, Hoon Choi, Hyemin Kim, Sohyun Park, Yelim Shin, Hyewon Shin, Yeonju Yoo, Dohee Yoon, Cheongbi Kim, Jinuk Kong, Goun Choi, Hadong Kim

Pin Therapeutics, Seongnam-si, Korea, Republic of

Casein kinase 1α (CK1α) is a serine/threonine kinase that plays a critical role in cellular homeostasis. In previous studies, CK1α promotes p53 inactivation by regulating MDM2 and MDMX, and this mechanism may represent a promising anticancer target Indeed, many studies have been reported recently on the development of CK1α inhibitors and degraders as anticancer agents. We recently developed PIN5018, a selective CK1α inhibitor, and reported that it exhibits promising anticancer activity in acute myeloid leukemia (AML) and adenoid cystic carcinoma (ACC). Interestingly, PIN5018 showed anti-tumor activities in aggressive subtypes of AML patient xenograft (PDX) and ACC patient-derived xenograft (PDX) model. Strikingly, our CK1α MGD not only achieved complete remission in the ACC PDX model during drug administration but also maintained this effect for more than 20 days after discontinuation of the treatment. We conducted 2D or 3D *in vitro* activity assessments to screen for anticancer activity in additional candidate solid tumors. Based on these results, we focused our research on colorectal cancer (CRC). CRC is the third most prevalent cancer and a leading cause of cancer-related deaths worldwide. Over 80% of CRC patients are of the microsatellite stable (MSS) type, which, unlike the MSI group, represents a population with high unmet needs. For example, while chemotherapy and certain targeted therapies are available, they are applicable to only a subset of MSS patients, resulting in limited treatment options. From this perspective, we aimed to investigate the correlation between specific biomarkers and the *in vitro* and *in vivo* efficacy of PIN5018 in MSS CRC. As a result, we identified promising anticancer efficacy of PIN5018-mediated CK1α degradation under specific combinations of biomarker mutation conditions. Furthermore, we observed synergistic effects with PIN5018 when combined with standard of care (SoC) drugs. Taken together, these findings demonstrate the potential of our CK1α-selective MGD as a promising therapeutic candidate not only for AML and ACC but also for MSS CRC. Lastly, we are aiming for clinical entry by 2025 Q1.

#1643 Establishing rational combination strategies with selective CBP degraders in solid tumor indications.

Molly M. Wilson, Hafiz Ahmad, Benjamin Adams, Ketaki Adhikari, Wesley Austin, Breanna Bullock, Mike Collins, Danette Daniels, Julie Di Bernardo, Thomas Dixon, Claudia Dominici, Brian Ethell, Anais Gervais, Md Imran Hossain, Dave Lahr, Laura La Bonte, Michael Lehrke, Mei Yun Lin, David Mayhew, Karolina Mizeracka, Paige Monsen, Solymar Negretti, John Pulice, Abira Ramakrishnan, Darshan Sappal, Shawn Schiller, Samantha Schultz, Gromek Smolen, Nihan Ucisik, Grace Werosta, Elizabeth Wittenborn, Xiaohuan Wu, Qianhe Zhou, Mark Zimmerman

Foghorn Therapeutics, Cambridge, MA

The paralog lysine acetyltransferases CREB binding protein (CBP) and E1A binding protein P300 (EP300) function as transcriptional coactivators that regulate diverse cellular programs. Mutations in *CBP* and *EP300* have been implicated in the biology of various cancer types. The bidirectional synthetic lethal relationship between these paralogs in tumor cells highlights a therapeutic opportunity in targeting CBP selectively in *EP300*-mutant cancers. We have previously demonstrated that our selective CBP degraders have potent antiproliferative activity in *EP300*-mutant cancers through both in vitro assays and cell line-derived xenograft models when used as a single agent. We have now expanded our studies to explore strategies for combination treatment with approved chemotherapies to enhance tumor growth inhibition.

Here, we demonstrate that our selective and potent CBP degrader compounds provide combinatorial benefit when used with approved chemotherapeutic and targeted agents. Through a large-scale in vitro combination screen, we show that CBP degrader treatment synergizes with multiple standard-of-care chemotherapies, including paclitaxel, as well as targeted agents, including CDK4/6 inhibitors. Furthermore, by genome-wide CRISPR knockout screening across four cell line models of different solid tumor types, we establish genetic determinants of resistance and sensitivity to CBP degradation in an *EP300*-mutant background, providing further mechanistic insight into combination therapy approaches. Collectively, we have defined cellular processes that present therapeutic vulnerabilities that can be leveraged for rational combination strategies with selective CBP degraders in solid tumor indications.

#1644 Novel CDK9 degrader XY0597 potently inhibits gastroesophageal cancer growth and overcome resistance by increasing chromatin accessibility and down-regulating YAP/TEAD signaling.

Yan-ting Yann Zhang[1], Mikel Ghelfi[1], Yu Xue[2], Haiying Chen[2], Gennaro Calendo[1], Dipti Athavale[1], Curt Balch[1], Xiaodan Yao[1], Woonbok Chung[1], Luke Chen[1], Francis Spitz[3], Generosa Grana[4], Jia Zhou[2], Jean-Pierre Issa[1], Shumei Song[1]

[1]Coriell Institute for Medical Research, Camden, NJ,[2]Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX,[3]Departments of Surgery, Cooper University Hospital, Camden, NJ,[4]MD Anderson Cancer Center at Cooper, Cooper University Hospital, Camden, NJ

Gastroesophageal cancer (GEAC) consists of upper GI malignancies of the esophagus, gastroesophageal junction, or stomach. Despite recent advancements in treatment modalities, GEAC remains a major health burden and listed as the second leading cause of cancer-related deaths globally. GEAC poses a significant challenge with short survival and lack of effective therapeutics. Thus, uncovering novel targets is urgent need. Cyclin-dependent kinase 9 (CDK9), one of the transcription-associated CDKs, has been implicated in regulation of transcription via RNA Pol II pausing/release, and epigenetic modification of genes that contribute to tumorigenesis. However, the functional role of CDK9 and its potential targeting in GEAC remain largely unknown. In this study, we demonstrated that nuclear CDK9 expression was significantly elevated in primary GEAC tumor tissues including PDXs compared to normal tissues, in association with poor survival. Moreover, we developed a novel CDK9 degrader, YX0597 and showed that YX0597 strongly suppresses CDK9 expression, inhibits RNA Pol II Serine 2 phosphorylation and dramatically decrease its target MCL-1 that accompanied by potently inhibition of GEAC cell growth, decreased tumor cell invasion and tumor sphere formation especially in radiation resistant tumor cells. Mechanistically, YX0597 strongly enhanced chromatin accessibility and activated epigenetically silenced genes. Further, both CDK9 and YAP1 colocalized in tumor tissues and significantly correlated in GEAC; and RNAseq revealed YAP1, TEAD4 and its targets (CTGF, Cyr61) were dramatically reduced upon XY0597 treatment that correspond to CDK9 inhibition. Correspondingly, XY0597 strongly suppress expression of CDK9, MCL-1, YAP1, YAP1 transcription factor TEAD4 in radiation resistant cells compared to parental cells and showed more potently suppressed radiation resistant tumor cell growth *in vitro* and *in vivo*. Most importantly, the combination of CDK9 degrader XY0597 and a novel YAP1/TEAD inhibitor, VT00278, synergistically attenuated resistant tumor growth and progression *in vitro* and in the PDX model. Taken together, our studies open a new avenue for targeting CDK9 hyperactivated GEAC tumors especially in combination with YAP1/TEAD inhibition.

#1645 Developing first-in-class AR-V7/AR-fl molecular glue degrader to revolutionize prostate cancer therapeutics.

CheukMan Cherie Au[1], Michelle Naidoo[1], Catrina Estrella[1], Prerna Vatsa[1], Ying Wang[2], Zhao Wang[2], David M. Nanus[3], Urko del Castillo[1], Paraskevi Giannakakou[3]

[1]Department of Medicine, Weill Cornell Medicine, New York, NY,[2]Baylor College of Medicine, Houston, TX,[3]Meyer Cancer Center, Weill Cornell Medicine, New York, NY

Metastatic castration resistance prostate cancer (mCRPC) is a lethal disease due to the resistance of standard-of-care (SoC) treatments, including androgen receptor (AR) pathway inhibitors and taxanes. Treatment resistance occurs partly due to the expression of constitutively active AR splice variants, that hijack AR signaling even after castration. AR-V7 is the most prevalent variant and is expressed in ~75% of patients with mCRPC, which confers resistance to SoC treatment. Currently, there is no AR-V7 pharmacologic inhibitor, limiting patients' therapeutic options. Thus, AR-V7 inhibition remains an urgent clinically unmet need. To address this gap, we performed a robust high-throughput phenotypic screen of ~170K compounds to identify the first AR-V7 protein glue degraders. In addition to degrading AR-V7, our hit compound also degraded full-length AR (AR-fl), having a unique chemotype compared to all existing AR modulators. Hit-to-lead optimization through medicinal chemistry and SAR studies has yielded more potent lead AR-V7/AR-fl degraders (IC50 of <100 nM). Mechanistically, our lead compounds promoted Cullin-RING E3 ligase-mediated proteasomal degradation of AR-V7 and AR-fl within 4 hours, which was blocked by the proteasome inhibitor, bortezomib. Currently, while several AR-fl-PROTAC degraders are in preclinical and early clinical development, no AR-V7 degrader exists. Our lead compounds are the only degraders that target both AR-fl and AR-V7. They bind to the TAU1 and AF-1 functional subdomains within the AR N-terminus (NTD), which is shared by both AR isoforms. Preliminary molecular docking experiments using RoseTTAFold2 further supported this binding. Additionally, Cryo-EM analysis identified conformational changes of AR-fl in the presence of our compounds indicating direct binding, which was also confirmed by cellular thermal shift assays. Importantly, our lead compounds effectively reversed SoC treatment-resistance in preclinical models and demonstrated higher potency than enzalutamide or ARV-110 (AR-fl-PROTAC). Our lead compounds showed excellent on-target activity, favorable drug-likeness, and promising pharmacokinetics, with strong stability, oral bioavailability, and efficacy in enzalutamide-resistant mCRPC xenografts expressing both AR-V7 and AR-fl. We are also conducting an E3-ligase CRISPR screen to nominate the E3 ligase that mediates the degradation activity and using global proteomics to assess potential off-target effects. Collectively, these data support a first-in-class molecular glue degrader mechanism, enabling rapid degradation of both AR-fl and AR-V7 through targeting the AR NTD. In summary, our compounds offer a dual-targeting approach in a single treatment and have the potential to dramatically improve survival for patients with mCRPC and prevent the emergence of AR-V7 expression in patients with earlier-stage disease.

#1646 Elucidating the molecular mechanism of action of the first in human SMARCA2 selective degrader PRT3789.

Min Wang, Miles Cowart, Joy Cote, Justin Kurian, Stephanie Rodgers, Chaoyi Xu, Corey Basch, Philip Pitis, Klare Bersch, Raul Leal, Liang Lu, Michael Hulse, Andrew Moore, Alexander Grego, Joseph Rager, Tom Emm, Koichi Ito, Neha Bhagwat, Andrew Combs, Sandy Geeganage, Peggy Scherle

Prelude Therapeutics, Wilmington, DE

Mammalian SWI/SNF (BAF) complexes play an important role in controlling gene expression by remodeling chromatin. SMARCA2 (BRM) and SMARCA4 (BRG1) are the core catalytic subunits of the SWI/SNF complexes and regulate the composition of nucleosomes via their ATPase activity. Approximately 10-12% of non-small cell lung cancer (NSCLC) patients harbor SMARCA4 mutations, resulting in loss of SMARCA4 protein or loss of its function (LOF). These SMARCA4-deficient cancer cells are highly dependent on the paralog gene SMARCA2 for their growth and survival. We have identified PRT3789, a first-in-class SMARCA2 selective targeted protein degrader currently in clinical trials (NCT05639751). PRT3789 promoted the formation of ternary complexes between the bromodomain of SMARCA2 or SMARCA4 with the VHL E3 ligase. Although SMARCA2 and SMARCA4 share 87% identity in their bromodomain, the structural differences between PRT3789 induced SMARCA2-VHL and SMARCA4 -VHL ternary complexes lead to more productive ubiquitination on SMARCA2 compared to SMARCA4. Proteomics and mutagenesis studies identified specific lysine residues that were ubiquitinated on the bromodomain of SMARCA2 and SMARCA4, and a unique loop on SMARCA2 that contributed to ternary complex stabilization and ubiquitination. In summary, we describe the systematic characterization of PRT3789 mediated SMARCA2 degradation and illustrate the molecular mechanism of action for how PRT3789 achieves degradation selectivity against SMARCA4.

#1647 CC2000199, a potent and selective androgen receptor ligand-directed degrader (AR LDD) with a dual mechanism of action, exhibits anti-tumor activity in multiple enzalutamide-resistant preclinical models for metastatic castration-resistant prostate cancer.

S. Nayak[1], **M. Ammirante**[1], E. Rychak[1], D. Liao[1], J. D. Norris[2], S. E. Wardell[2], S. Kasibhatla[1], V. Plantevin-Krenitsky[1], E. Horn[1], J. R. Piccotti[1], S. Norris[1], R. Kandimalla[1], J. Meiring[1], S. Reiss[1], J. M. Baughman[1], M. Khater[1], D. S. Mortensen[1], B. Cathers[1], N. Bence[1], D. W. Pierce[1], J. D. Hansen[1], L. G. Hamann[1], D. P. McDonnell[2], R. K. Narla[1], M. Rolfe[1], S. Xu[1];

[1]Bristol Myers Squibb, San Diego, CA, [2]Duke University School of Medicine, Durham, NC

The androgen receptor (AR) is a key driver of the cellular processes that contribute to the pathology of prostate cancers at most stages, and drugs that inhibit the activity of this receptor or which interfere with androgen biosynthesis have become the cornerstone of treatments for prostate cancer. Nevertheless, *de novo* and acquired resistance remains an impediment to a durable clinical response in patients with metastatic castration-resistant prostate cancer (mCRPC), and overcoming resistance to AR pathway inhibitors (ARPIs) has emerged as a major challenge in the treatment of patients with mCRPC. CC2000199 (also called CC-199) is a heterobifunctional ligand-directed degrader (LDD) that enables $CRL4^{CRBN}$ E3 ligase-dependent ubiquitination and degradation of AR and a close analog of BMS-986365. It is a highly potent and selective AR degrader that induces rapid and deep degradation of both wildtype and mutant forms of the receptor. In addition to AR degradation, CC-199 displays AR-inhibitory activity as a direct receptor antagonist with a potency similar to enzalutamide (ENZ) for wildtype AR. The drug is ~100-fold more potent than ENZ at inhibiting androgen-stimulated transcription of AR target gene FKBP5, and ~10- to 40-fold more potent than ENZ at inhibiting AR-dependent proliferation of multiple prostate cancer cell lines *in vitro*. In animal models of advanced prostate cancer, CC-199 demonstrates on-target activity, inducing deep AR degradation, suppressing AR signaling, and inhibiting tumor growth. Indeed, significant tumor volume reductions were achieved by CC-199 in validated models of advanced CRPC and therapy resistant patient-derived xenografts, including those with acquired resistance to ENZ. Collectively, our preclinical data suggest that the AR degrader CC-199 is superior to standard-of-care AR antagonists, such as ENZ, in disease-relevant animal models and ENZ-resistant mCRPC that remain dependent on AR.

#1648 LT-010366, a first-in-class orally bioavailable KRAS G12D degrader in the treatment of multiple KRAS G12D-mutated cancer models *in vitro* and *in vivo*.

Shuang Yan, Zhengqing Ye, Haiji Chen, Chenli Ding, Juan Xu, Yan Feng

Leadingtac Pharmaceutical (Shaoxing) Co., Ltd, Shanghai, China

Recent successes in targeting $KRAS^{G12C}$ have provided both insight and inspiration for targeting alternative KRAS mutants. Among KRAS mutations, KRAS G12D is the most frequent driver mutated isoform and is found in approximately 34% of patients with pancreatic ductal adenocarcinoma (PDAC), 12% of patients with colorectal cancer (CRC), 4% of patients with lung adenocarcinoma, and in a subset of patients with other solid tumors. We have developed LT-010366 as a first-in-class orally bioavailable KRAS G12D degrader with high potency and selectivity. In *In vitro* degradation assays, LT-010366 demonstrated extraordinary degradation activities with DC_{50} of 0.96-2.55 nM and efficiency (D_{max}) over 95% against KRAS protein in multiple cancer cell lines harboring KRAS G12D mutation, as well as remarkable inhibition of p-ERK with IC_{50} of 0.52-26.06 nM in those cell lines. In cell proliferation assays, LT-010366 showed a strong antiproliferative potency with IC_{50s} of 0.12-15.77 nM and max inhibition of 74.42-98.4% in tested KRAS G12D mutant cancer cell lines. Moreover, LT-010366 selectively degrades KRAS, sparing HRAS and NRAS. In CDX tumor, daily oral administration of LT-010366 exhibited potent antitumor activities in both PDAC (PK-59) and CRC (GP2D) models with KRAS G12D mutation, resulting in profound tumor regression without body weight loss. In GP2D xenograft model, LT-010366 demonstrated dose-dependent tumor inhibition at the dose of 10 mg/kg, 30 mg/kg and 100 mg/kg PO, QD with TGI of 95.7%, 114.4% and 116.5% after 14-day treatment, respectively. In PK-59 model, LT-010366 demonstrated dose-dependent tumor inhibition at the dose of 3 mg/kg, 10 mg/kg and 30 mg/kg PO, QD with TGI of 69.3%, 116% and 126.8% after 15-day treatment, respectively. In GP2D model, LT-010366 also exhibited dose-dependent degradation of KRAS protein and demonstrated marked inhibition of downstream p-ERK and p-S6. In addition, LT-010366 also displays a high-quality drug-like profile. In summary, our results demonstrated that LT-010366 induced degradation of KRAS G12D protein, inhibition of KRAS downstream signaling mediators and exhibited antitumor activity in multiple KRAS G12D-mutated cancer cells *in vitro* and *in vivo*. LT-010366 is a promising therapeutic agent to be advanced to clinical development for patients with tumors harboring KRAS G12D mutation.

#1649 Discovery of first-in-class potent and selective oral degraders of KAT6A that demonstrate anti-cancer activity in pre-clinical models.

Monisha Sivakumar, Sarah Pawley, Anthony Reichelderfer, Corey Basch, Jimin Park, Jessica Burtell, Chaoyi Xu, Min Wang, Chun Chen, Justin Kurian, Joy Cote, Miles Cowart, Stephanie Rodgers, Amy Crossan, Joseph Rager, Koichi Ito, Sandy Geeganage, SangHyun Lee, Andrew Combs, Peggy Scherle, Andrew Buesking, **Jack Carter**

Prelude Therapeutics Incorporated, Wilmington, DE

MYST proteins (e.g. KAT6A, KAT6B, KAT7) are histone acetyltransferases that epigenetically regulate chromatin accessibility. KAT6 expression is associated with cancer growth and *KAT6A* is recurrently amplified in several cancer types, including breast and lung. KAT6A complexes with multiple proteins, such as bromodomain and PHD finger containing 1 (BRPF1), enhancing its regulation of cell cycle, estrogenic, and other oncogenic genes. Recent first-in-human clinical data with a dual KAT6A/B inhibitor demonstrated promising efficacy in heavily pre-treated patients with ER+/HER2- breast cancer and provided insight into on-target safety considerations like neutropenia. Reports describing the role of KAT proteins in hematopoietic cells, and non-catalytic functions for KAT6A, led us to hypothesize that a targeted protein degradation approach to selectively degrade KAT6A may improve the anti-cancer activity and safety profile for KAT6 therapies. We report the discovery of potent (<1 nM DC_{50}) oral KAT6A degraders with excellent selectivity over KAT6B and KAT7. Degradation occurred via the proteosome and was dependent on target and E3-ligase binding. KAT6A degraders suppressed breast cancer cell proliferation, enriched in ER+/HER2- models expressing KAT6A, and retained activity in endocrine therapy resistant cells. To assess the differential biology of KAT6A degradation, we compared an inhibitor with a degrader. Strikingly, KAT6A degradation had a deeper anti-proliferative effect in breast cancer cell lines compared to the inhibitor. The superior response was reversible by competition with the binder warhead and an E3-inactive degrader displayed limited activity, suggesting that KAT6A degradation drove this effect. Encouragingly, KAT6A degraders also demonstrated superior anti-proliferative activity in multiple lung cancer cell lines. Mechanistically, RNA-seq revealed degraders induced a more profound global effect on gene expression, enriched in estrogenic, cell cycle, DNA replication and repair, and MYC signaling pathways. Consistent with the gene expression changes, KAT6A degraders induced a deeper reduction in estrogen receptor (ERα) expression. Notably, only KAT6A degraders reduced BRPF1 expression, a subunit of the KAT6 complex, suggesting that degradation of KAT6A may destabilize the protein complex. *In vivo*, we show a KAT6A degrader robustly inhibits tumor growth, while degrading KAT6A and reducing ERα expression, in a breast cancer xenograft. Lastly, we utilized a predictive assay for neutropenia (CFU-GM), where we found KAT6A-selective degraders were inactive, in contrast to KAT6A/B inhibitors. Our first-in-class KAT6A degraders demonstrated proof-of-concept for a differentiated strategy with potential to improve the depth of response and safety profile for a clinically active anti-cancer target.

#1651 NRX-0305: a pan-mutant BRAF degrader with broad preclinical efficacy, brain penetrance, and synergistic potential with MEKi across class 1/2/3 BRAF-mutant cancers.

Alexandra Borodovsky, Ya-Wen Lu, Ge Peng, Karthik Arumugam, Paul Auger, Robert Cass, Stefan Gajewski, Scott Kimura, Daniel Medina-Cleghorn, Mariah Mesner, Davorka Messmer, Madeleine Nemchek, Prabu Raman, Ryan Rountree, Rusha Sardhara, Sangita Sridharan, Jennifer Stokes, Leslie Tong, Jennifer Tung, Ge Wei, Jeffrey Wu, Jordan Ye, Gwenn M. Hansen

Nurix Therapeutics, Inc., San Francisco, CA

Mutations in BRAF, a key component of the mitogen-activated protein kinase (MAPK) pathway, drive constitutive pathway activation and oncogenic transformation and are commonly found in a variety of cancers including melanoma, NSCLC and CRC. BRAF mutations are categorized into three classes: Class 1 (e.g., V600X), which are RAS-independent and targetable with currently approved BRAF inhibitors (BRAFi); Class 2, which require dimerization; and Class 3, which are kinase-impaired and rely on upstream RAS activation. While approved BRAFi provide significant survival benefit to Class 1 patients, drug durability and efficacy are limited by the emergence of primary and acquired resistance that often involves RAF dimerization and BRAF amplification. Furthermore, patients who have progressed on BRAFi, especially in melanoma, frequently present with brain metastases, for which there are limited treatment options due to poor CNS penetrance of available drugs. To address this, we developed NRX-0305, a pan-mutant BRAF degrader that selectively degrades mutant BRAF across all classes while sparing wildtype BRAF. In vitro, NRX-0305 potently degrades mutant BRAF protein and suppresses downstream pERK1/2 signaling. NRX-0305 exhibits strong anti-proliferative effects across a panel of Class 1/2/3 BRAF-mutant cell lines including those expressing BRAF fusion proteins. In vivo, daily oral dosing with NRX-0305 induces robust BRAF degradation and exhibits single agent efficacy in several cell line and patient-derived xenograft models of Class 1/2/3 BRAF mutant cancers. Notably, NRX-0305 demonstrates robust single agent activity in BRAFi resistant Class 1 PDX models and a melanoma brain metastasis model, highlighting its brain penetrant properties. Furthermore, NRX-0305 in combination with MEK inhibition achieves complete tumor regression in a Class 3 bladder cancer PDX model, demonstrating its synergistic potential. These findings establish mutant-specific BRAF degradation as a promising therapeutic strategy, displaying activity across a broad range of mutations and addressing the limitations of BRAFi in Class 1/2/3 BRAF-mutant cancers.

#1653 Preclinical characterization of PLX-61639, a potent and orally bioavailable SMARCA2-selective monovalent direct degrader.

Gregory Parker, Geoffray Leriche, Aleksandar Jamborcic, Taylor Kampert, Linette Yang, Julia Toth, Kenneth Steadman, Jay Chung, Duc Tran, Luis Lopez, Farhana Barmare, Kyohei Hayashi, Gang Liu, Jianguo Ma, Alex Campos, Meg McCarrick, Kevin Freeman-Cook, Peggy Thompson

Plexium, San Diego, CA

SMARCA2 and SMARCA4 are essential, yet redundant, catalytic subunits of human BAF complexes, which are involved in controlling gene expression through the remodeling of chromatin structure. In a subset of solid tumors, SMARCA4 is commonly mutated, rendering SMARCA4-deficient cancer cells highly dependent on SMARCA2 for viability. Therefore, selectively targeting SMARCA2 in patients with SMARCA4-deficient tumors may provide an effective and safe treatment option. Targeted protein degradation (TPD) using the endogenous Ubiquitin Proteasome System (UPS) is a drug discovery strategy that may be well suited for exploiting this SMARCA2/4 synthetic lethal relationship. Here we describe the discovery and characterization of PLX-61639, a potent, selective, and orally bioavailable SMARCA2 degrader. To enable degrader discovery, we utilized a monovalent direct degrader strategy where small molecules are designed to bind the target protein and induce its degradation through the recruitment of an E3 ligase complex. Rational design of compound libraries from known family VIII bromodomain binders resulted in the identification of monovalent direct degraders of SMARCA2/4. Subsequent chemical series optimization dramatically improved degradation selectivity and ultimately resulted in our development candidate, PLX-61639. PLX-61639 induces potent and selective degradation of SMARCA2 across a panel of tumor cell lines, eliciting anti-proliferative activities supportive of the synthetic lethal dependency in $SMARCA4^{MUT}$ tumors. A UPS focused CRISPR screen identified a specific E3 ligase complex involved in the degradation mechanism, which follow up mechanistic studies confirmed. Omics studies identified PLX-61639-induced changes to chromatin mapping and SMARCA2-dependent transcriptionally regulated gene sets. *In vivo*, oral administration of PLX-61639 in $SMARCA4^{MUT}$ xenograft models exhibited sustained target degradation and dose-dependent tumor growth inhibition at well tolerated doses. PLX-61639 had no impact on tumor growth using a $SMARCA4^{WT}$ xenograft model, supporting the dependency of SMARCA4 mutational status for SMARCA2-selective degrader efficacy. These results highlight the efficient discovery and development of potent and selective SMARCA2 monovalent direct degraders. Further, the preclinical characterization of our development candidate, PLX-61639, demonstrates its activity and potential utility in the treatment of $SMARCA4^{MUT}$ solid tumors.

#1654 Discovery of the first-in-class DOT1L PROTAC degrader.

H. Yim, Z. Xu, H. Kaniskan, J. Jin;
Icahn School of Medicine at Mount Sinai, New York, NY

The histone methyltransferase, DOT1L is often dysregulated in MLL-rearranged (MLL-r) leukemia, which leads to hyperactivation of MLL target genes and poor prognosis. This dysregulation is particularly common in infants, who are highly vulnerable to treatment-related toxicity. However, current treatment options remain limited, with intensive chemotherapy as the standard approach. Given these circumstances, targeting DOT1L with PROteolysis TArgeting Chimeras (PROTACs) could represent a promising alternative therapeutic strategy. Here, we report the discovery of the first-in-class DOT1L PROTAC degrader, MS2133, which induces DOT1L degradation in a dose- and time-dependent manner via the ubiquitin-proteasome system, without affecting DOT1L mRNA expression. MS2133 shows selectivity for DOT1L over other methyltransferases and effectively inhibits the growth of MLL-r leukemia cells while having no toxic effect on normal cells. Overall, MS2133 is a valuable chemical biology tool for studying DOT1L functions and offers promise as a therapeutic option for MLL-r leukemia treatment.

#1655 ARV-393, a PROTAC B-cell lymphoma 6 (BCL6) degrader, combined with biologics or small molecule inhibitors (SMIs) induces tumor regressions in diffuse large B-cell lymphoma (DLBCL) models.

Anna Van Acker, Lynn DeCarr, Sarah Eaton, Dan Sherman, Elizabeth Bortolon, Mark Bookbinder, Jennifer Pizzano, Morena Scopel, William Corwin, Ignacio Juncadella, Yongqing Huang, Ram Lingamaneni, Stephanie Renzullo, Jennifer Cantley, XiaoZhe (Janet) Wang, Ian Taylor, Sean Landrette, Sheryl M. Gough

Arvinas Operations, Inc., New Haven, CT

BCL6 is a preclinically validated oncogenic driver of DLBCL historically considered to be undruggable. ARV-393, a PROteolysis TArgeting Chimera (PROTAC) degrader, directly binds an E3 ubiquitin ligase and BCL6 to induce ubiquitination of BCL6 and its subsequent proteasomal degradation. ARV-393's iterative activity overcomes rapid resynthesis of BCL6 resulting in potent, single-agent activity and tumor regressions in many cell- and patient-derived xenograft (CDX and PDX) DLBCL models. ARV-393 monotherapy is being evaluated in a phase 1 trial (NCT06393738) in patients with non-Hodgkin lymphoma, including DLBCL.

Given the heterogeneity and multiple resistance mechanisms of DLBCL and that BCL6 regulates hundreds of genes linked to oncogenesis and resistance, BCL6 degradation has the potential for broad drug combinability. For example, CREBBP/EP300 and BCL6 have opposing transcriptional activities and BCL6 degradation may prevent CD20 downregulation by restoring CREBBP/EP300 chromatin access, supporting combination with anti-CD20 biologics; cooperation of EZH2 with the BCL6 repressor complex supports combining with tazemetostat (taz). Thus, we assessed the activity of ARV-393 in combination with biologics or SMIs based on potential complementary mechanistic roles.

ARV-393 was administered in combination with clinically relevant doses of the standard of care (SOC) biologics rituximab, tafasitamab (tafa) and polatuzumab to mice bearing SU-DHL-4 CDX, representing a high-grade B-cell lymphoma (HGBCL, with *MYC, BCL2* and *BCL6* rearrangements). ARV-393 was also combined with investigational SMIs of EZH2 (taz), BCL2 (venetoclax) and BTK (acalabrutinib), in HGBCL or aggressive CDX models SU-DHL-6 (*EZH2*^{mut}), OCI-Ly1 (*BCL2+*) and activated B-cell (ABC)-like OCI-Ly10 (*MYD88*^{mut}), respectively.

ARV-393 combined with SOC biologics resulted in superior tumor growth inhibition (TGI) compared with monotherapy, with complete tumor regressions in 9/9 mice when combined with rituximab, 4/10 with polatuzumab, and 10/10 with tafa (vs 55% TGI tafa + lenalidomide). ARV-393 also produced tumor regressions in 10/10 mice when combined with taz, 10/10 with venetoclax, and 10/10 with acalabrutinib. In a SU-DHL-6 model, MYC, EZH2 and BCL2 protein levels were increased 56%, 66% and 12%, respectively, with ARV-393 alone vs vehicle, but were decreased 75%, 80% and 96% vs vehicle, with greater BCL6 degradation (65% vs 87%) when combined with taz. Studies to examine the molecular mechanisms underlying the combination synergies are ongoing.

ARV-393 demonstrates synergistic antitumor activity, including complete regressions, in combination with SOC agents and select SMIs in high grade and aggressive DLBCL models. These findings support future clinical investigation of ARV-393 in combination with other therapies for DLBCL.

#1656 TGRX-3544 is a highly potent and mutant-selective degrader of oncogenic KIT with oral activity.

Yanxia Shi, Linxin Li, Xiangyu Hong, Yingying Zuo, Zhen Dong, Yixin Ai, **Yihan Wang**

Shenzhen TargetRx, Inc., Shenzhen, China

Oncogenic mutations of the receptor tyrosine kinase KIT drive cancers such as gastrointestinal stroma tumors (GIST) and systemic mastocytosis (SM). The mutations in the juxtamembrane domain (Exon 11, E11) and extracellular domain (E9) are most commonly found in GIST, and the A-Loop D816V mutation (E17) in over 90% SM cases. Currently approved KIT inhibitors either lacked efficacy against certain mutants, like imatinib, or lacked selectivity such as the multi-kinase targeting sunitinib and regorafenib. Primary or acquired resistance or intolerance is common and poses a great clinical challenge. Non-kinase scaffold functions of oncoproteins are being revealed, as exemplified by EGFR, BTK, and BCR-ABL1. Since all the currently approved TKIs only inhibit the catalytic activity, the scaffold functions may constitute residual oncogenic signaling and eventually lead to acquired resistance and disease progression. We reasoned that a degrader, rather than an inhibitor, may potentially provide more benefit for patients with oncogenic KIT. TGRX-3544 is a heterobifunctional degrader which promotes ubiquitination and proteasomal degradation of oncogenic KIT. In GIST T1 cells, TGRX-3544 potently induced degradation of Del560-578 (E11) mutant KIT (DC_{50} = 1.6 nM at 24h). The degradation activity was also robustly observed in Ba/F3 isogenic cells expressing KIT Del557-558 (E11), InsAY502-503 (E9), D816V (E17), and Del557-558/D816V (E11/E17) mutants, but not wildtype KIT. TGRX-3544 potently inhibited proliferation of GIST T1 (KIT E11) cells and Kasumi-1 (KIT N822K, E17) cells. It also inhibited proliferation of Ba/F3-KIT cells expressing KIT E11, E17, and E11/E17 mutants (IC_{50} 0.9 to 3.9 nM), as well as the E9 mutant. The cellular activity of TGRX-3544 against the E11, E17 and E11/E17 mutants was superior to that of all the currently approved KIT inhibitors imatinib, sunitinib, regorafenib, avapritinib and ripretinib. Unlike other KIT inhibitors, TGRX-3544 had little or no activity against the closely related PDGFRA or FLT3, nor did it show cytotoxicity in a panel of normal cell lines (IC_{50} > 10000 nM), suggesting its high selectivity. *In vivo*, TGRX-3544 led to dose-dependent tumor regression in xenograft models expressing E11 or E11/E17 mutant KIT. In the E11/E17 model, TGRX-3544 was much more efficacious than the corresponding warhead. TGRX-3544 rapidly reduced the protein levels of E11 or E11/E17 mutant KIT by over 50% 4h after oral dosing. It also diminished the downstream signaling pathways of tumor samples as indicated by reduced phosphorylation of KIT, STAT5, AKT and ERK1/2. In summary, we have developed a highly mutant-selective, event-driven, scaffold-eliminating KIT degrader with superior cellular potency and *in vivo* oral activity. The balanced preclinical profile of TGRX-3544 supports its further clinical investigation in KIT-driven malignancies such as GIST and SM.

#1657 HZ-S109, a potent and selective HPK1 degrader with enhanced T cell modulation efficacy and improved bioavailability.

Yizhe Wu, Jiangfeng Xie, Jinglai Huang, Mengchao Zheng, Xinglu Zhou

HealZen Therapeutics Co., Ltd., Hangzhou, China

Background: Hematopoietic Progenitor Kinase 1 (HPK1/MAP4K1) has been demonstrated to restrain T cell activation through phosphorylating SLP-76 at Serine 376 during the T-cell activation process. Given its irreplaceable negative regulation role in TCR pathway, HPK1 is considered as a promising target in immuno-oncology. Recently, HPK1 has been shown to function as scaffolding protein and bind to adaptor proteins to promote JNK signaling and actin cytoskeleton rearrangement involved in lymphocyte adhesion. Additionally, the turnover rate of HPK1 is low. These evidences suggest that HPK1 is a suitable target to develop protein-degrader.
Objective: Accordingly, we described our state-of-the-art work in discovery and identification of HPK1 degrader HZ-S109. We asked if this degrader could exhibit stronger engagement with the HPK1 target, leading to the restoration of suppressed T-cell function and inhibition of tumor growth.
Methods: HZ-S109 was identified based on our in-house unique DaTProD® platform. CCK-8, ELISA and WB detection were performed by PEG2, NECA and TGF-β induced depleted cell model in vitro.PK/PD detection and tumor measurement were performed by oral gavage of CT-26 subcutaneously transplanted tumor models in C57BL/c mice.
Results: HZ-S109 exhibits potent HPK1 degradation with DC_{50} values lower than 10 nM across various cell types, including Jurkat, Ramos, PBMC, and CAR-T cells. Importantly, HZ-S109 demonstrated no significant effect on other family members, such as GCK, GLK, HGK, or KHS. HZ-S109 can significantly enhance the activation of primary T cell at nanomolar level concentrations, with the maximum activation fold exceeding 15-fold. Furthermore, HZ-S109 restores T cell function in the presence of multiple immune-suppressed conditions including NECA/ PGE2/ TGF-β or their combination. Despite its higher molecular weight, HZ-S109 boasts superior oral bioavailability. In in-*vivo* anti-tumor efficacy evaluation, oral administration of HZ-S109, either as a single agent or in combination with PD-L1 antibody, robustly suppressed the tumor growth in CT26, A20 and MC38 syngenic mice model, achieving a tumor growth inhibition (TGI) of over 90%. These results suggest that HZ-S109 stands out as a potent and distinct HPK1 modulator, exhibiting enhanced potency and oral bioavailability. At present, HZ-S109 is in the IND-enabling study stage.

#1658 REM-422, a first-in-class mRNA degrader of the MYB oncogene, demonstrates anti-tumor activity in xenograft models of adenoid cystic carcinoma and acute myeloid leukemia.

Samantha Levin-Furtney, Michael P. Thomas, Alycen Harney, Alexander Ivliev, Jesper Maag, Sudeep Prajapati, Michael Seiler, Dominic Reynolds, Silvia Buonamici, **Charles Kung**

Remix Therapeutics, Inc., Watertown, MA

The c-MYB (MYB) oncogenic transcription factor is a key regulator of hematopoietic cell differentiation and proliferation. Recurrent genetic lesions and dysregulation of MYB have been identified in a variety of cancers, including adenoid cystic carcinoma (ACC), acute myeloid leukemia (AML), colorectal cancer (CRC), and others.

We describe the mechanism of action and biological activity of REM-422, a first-in-class, potent, selective, oral small molecule messenger RNA (mRNA) degrader of MYB being developed for the treatment of ACC (NCT06118086) as well as AML and high-risk myelodysplastic syndrome (HR-MDS) (NCT06297941).

Using biophysical and cellular assays, REM-422 is shown to act by selectively facilitating the binding of the U1 snRNP spliceosome complex, which governs exon usage by recognizing 5' splice sites, to a normally unused 'poison exon' (PE), which contains a premature termination codon, in the MYB pre-mRNA transcript. Cells treated with REM-422 demonstrated increased MYB poison exon inclusion, leading to degradation of MYB mRNA by nonsense-mediated decay and subsequent reduction in MYB protein levels. REM-422 phenocopied gene expression changes observed with shRNA hairpin-mediated knockdown of MYB and demonstrated preferential anti-proliferative activity in cell lines with high MYB expression and known MYB genetic dependency.

To understand the pharmacokinetic, pharmacodynamic, and anti-tumor effects of REM-422 in vivo, ACC patient-derived xenograft (PDX) models harboring MYB translocations were established in nude mice. QD PO administration of REM-422 demonstrated strong and dose-dependent reductions in MYB mRNA and protein levels. Furthermore, REM-422 treatment led to dose-dependent inhibition of tumor growth and tumor regressions at well tolerated doses. RNAseq and immunohistochemistry analysis confirmed modulation of MYB target genes in tumors from mice exposed to REM-422.

To evaluate the activity of REM-422 in a disseminated PDX model of AML, patient cells were engrafted by tail vein injection into NOG mice and mice were dosed PO QD with REM-422. After 24 days of dosing, REM-422 led to the eradication of human leukemia cells from both compartments and prevented AML cell engraftment-induced body weight loss. Immunohistochemistry staining confirmed the reduction of MYB protein in the bone marrow of treated animals. Flow cytometry analysis of bone marrow and whole blood demonstrated that REM-422 treatment led to an increase in blasts expressing CD14 and CD11b, known myeloid differentiation markers, as well as an increased frequency of Annexin V+ blasts, a marker of early apoptosis.

These data illustrate the potential for REM-422 as an investigational agent for the treatment of a variety of solid tumor and hematological malignancies.

#1659 ETX-636, a novel allosteric pan-mutant-selective PI3Kα dual inhibitor and degrader with best-in-class potential.

Robert F. Koncar, Mingzong Li, Jingyan Gao, Fei Pang, Ying Lin, Raj Nagaraja, Yong Tang, Hannah Szeto, Zipeng Fan, Karan Kapoor, Robbie Chen, Eric Simone, Minghong Hao, Shengfang Jin, Tao Liu, Meghana Kulkarni, Jeffery Kutok

Ensem Therapeutics, Inc., Waltham, MA

PIK3CA, which encodes p110α, the catalytic subunit of phosphatidylinositol 3-kinase alpha (PI3Kα), is one of the most frequently mutated oncogenes and dysregulated PI3Kα activity is important for tumor growth. Up to 40% of hormone receptor (HR)-positive breast cancer (BrCA) tumors and 13% of all solid tumors harbor *PIK3CA* mutations, with the majority of mutations occurring within p110α kinase and helical domains. Orthosteric ATP-competitive inhibitors, alpelisib and inavolisib, which inhibit both Wild-type (WT) and mutant PI3Kα, are approved in combination regimens for treating *PIK3CA*-mutant, HR+/HER2-, advanced or metastatic BrCA. However, because PI3Kα is critical for insulin signaling, inhibition of WT PI3Kα results in hyperglycemia, and limits the clinical utility of these inhibitors. To overcome this limitation, ETX-636 was designed as an allosteric, pan-mutant-selective PI3Kα inhibitor and degrader by leveraging our Kinetic Ensemble® platform for optimal binding to a unique metastable state of p110α. In addition to greater biochemical selectivity for mutant PI3Kα over WT PI3Kα, ETX-636 has stronger target binding affinity, better on-target potency in biochemical and cellular pharmacodynamic assays, and demonstrates superior anti-tumor activity *in vivo* when compared to other allosteric, pan-mutant-selective PI3Kα inhibitors (i.e. RLY-2608 and STX-478). In viability assays run on a panel of 65 cancer cell lines, the presence of *PIK3CA* mutations was a strong predictor of ETX-636 sensitivity and ETX-636 potency was superior to both RLY-2608 and STX-478. *In vitro* and *in vivo* mechanistic studies revealed ETX-636 induces significant proteasome-dependent degradation of mutant p110α, while sparing WT protein (a feature not seen with other pan-mutant allosteric inhibitors), resulting in more durable pathway inhibition. ETX-636 dosed orally, once daily, results in deep and durable pathway inhibition and induces tumor regression in kinase and helical domain *PIK3CA*-mutant xenografts. In an ER-positive, HER2-negative, PI3Kα-mutant BrCA xenograft, ETX-636 is efficacious as a single agent and shows synergistic activity with fulvestrant, inducing tumor regression while being well-tolerated. Blood glucose levels after dosing in multiple species indicate that ETX-636 does not disrupt glucose homeostasis at efficacious exposures, even under non-fasting conditions. In addition, based on pharmacokinetic, pharmacodynamic, efficacy, and toxicology studies, predicted human efficacious doses of ETX-636 are not projected to cause hyperglycemia. The preclinical profile of ETX-636 underscores its best-in-class potential as an allosteric pan-mutant-selective PI3Kα dual inhibitor and degrader, and supports its clinical development both in single agent and combination clinical trials in patients with *PIK3CA*-mutant tumors, including HR+/*PIK3CA*-mutant BrCA.

#1660 Seeking new glues - Toward an E3 ligase-agnostic molecular glue degrader identification platform.

Allan M. Jordan[1], Kuhulika Bhalla[1], Helen Burston[2], Francesca Cavallo[1], Prasamit Baruah[2], Martin Soste[3], Polina Scichkova[3], Roland Bruderer[3], Daniel Redfern[3], Matevz Stefancic[3], Yuehan Feng[3], Stuart Thomson[1], Roland Hjerpe[1]

[1]Sygnature Discovery, Nottingham, United Kingdom,[2]Sygnature Discovery, Saint-Laurent, QC, Canada,[3]Biognosys AG, Schlieren, Switzerland

Targeted Protein Degradation (TPD) is a rapidly growing modality in oncology and is broadly driven by development of bifunctional (PROTAC-type) or monovalent degraders. Whilst the latter group largely consists of molecular glue degraders (MGDs), it also encompasses compounds that act as degraders via allosteric or other mechanisms. Screening for compounds with MGD properties can be undertaken via biophysical methods, where proximity of an effector and target protein is measured, limiting the effect of the compound to a known, selected effector, such as a particular ubiquitin-ligase of interest. Whereas this approach benefits from full control over the target and ligase pairing, cell-based systems offer the advantage of screening for target degradation against the entire complement of expressed ubiquitin ligases, potentially increasing the probability of identifying a hit compound. Conversely, a key challenge with cell-based screening approaches is to understand the mechanism by which the target is degraded, relying on the identification of the ubiquitin ligase or adaptor protein that is mediating the effect of the compound.

Here, we demonstrate the utility of our cell-based screening platform by developing degrader assays for two separate targets, Cyclin K and GSPT1, both therapeutically relevant for oncology, and with respective MGDs in clinical trials (CT7439 for cyclin K by Carrick Therapeutics, and MRT-2359 and Eragidomide from Monte Rosa Therapeutics and Cellgene/BMS, respectively).

Further, to address the challenge of deconvoluting the mechanistic basis for monovalent degrader hits from cell-based assays, we have utilized limited proteolysis coupled with mass spectrometry (LiP-MS). LiP-MS has become a powerful, label-free technique for identifying small molecule and peptide targets directly in cell lysates. This methodology allows the identification of the proteins involved in mediating degradation of a target, such as a ubiquitin ligase recruited to a target via an MGD.

Our approach integrates flexible cell-based assays for degrader screening with a powerful method of mechanistic deconvolution, allowing identification of the specific ligase driving target degradation. This knowledge can then facilitate the development of ligase- and target-specific molecular glue assays to drive hit optimisation and, ultimately, the delivery of novel MGD chemotypes against new oncology targets of choice.

#1661 Discovery of an SHP2 degrader with potent anti-cancer activity as a monotherapy and exceptional combination effects in KRAS mutant cancers.

Soohyun Lee, Jiyoung Kim, Ji Youn Park, Beomseon Suh, Han Wool Kim, So Hyuk Kim, Hyun-Hwi Kim, Onnuri Bae, Jeonghwa Han, Ju Young Kang, Jihye Lee, Je Ho Ryu, **Song Hee Lee**

Ubix Therapeutics Co., Ltd., Seoul, Korea, Republic of

Background: SHP2 is a key regulator of the RTK/RAS/MAPK signaling pathway, which is often disrupted in various cancers. Numerous allosteric SHP2 inhibitors are being actively developed to treat cancers driven by this pathway. However, these inhibitors face certain challenges, including their inability to target the active form of SHP2 and a narrow therapeutic window that requires intermittent dosing to manage toxicity. To address these limitations, we have developed SHP2 protein degraders as next-generation therapeutic agents targeting SHP2. In this study, we present the compelling characteristics of our SHP2 degraders.
Methods: For inhibitor combination assay, we did clonogenic assay in NCI-H358 KRAS G12C mutant NSCLC and 3D proliferation in various cell lines. For in vivo assessment of pharmacological profile, we utilized an NCI-H358 xenograft. SHP2 protein levels in cell lysates and tumors were analyzed to assess SHP2 degradation efficiency and DUSP6 mRNA levels were analyzed to evaluate downstream effects. For selectivity analysis, we analyzed phosphatase panel profiling and proteomics.
Results: Our compounds showed superior degradation activity achieving sub-nanomolar DC_{50} levels and significant cell growth inhibition exhibiting single digit nanomolar IC_{50} levels in NCI-H358 NSCLC (non-small cell lung cancer). In clonogenic and 3D cell proliferation assay, compound 5 showed significant growth inhibition with various inhibitors such as EGFR, MEK, ALK, RAF and KRAS G12C inhibitors. Phosphatase panel profiling and proteomics demonstrated compound 5 degraded SHP2 selectively. In particular, the superior anti-cancer effects of combination treatments with other drugs were confirmed across various cancer cell lines. In vivo experiments using a KRAS mutant CDX model revealed compelling in vivo profiles. Once-weekly intravenous administration achieved strong tumor growth inhibition without inducing the hematological and liver toxicities that were issues in the clinical studies of SHP2 inhibitors.
Conclusion: We have successfully identified new SHP2 degraders with exceptional activity. Our findings demonstrate that, by leveraging the characteristics of these degraders as a strategy to overcome toxicity, they have the potential to address the limitations of allosteric SHP2 inhibitors, offering a promising therapeutic approach for patients with RTK/RAS/MAPK-dependent cancers.

#1662 Preclinical studies of RP04340: an orally available and potent PROTAC compound targeting KRAS G12C/D/V mutant tumors.

Xiang Ji, Chao Deng, Gang Wu, Qiguo Zhang, Xiaolin He, Yanpeng Wu, Bing Zong, Xiaojin Xu, Chao Liang, Beibei Wang, Yuwei Zhang, Qingyao Hu, Huanping Li, Bing Bai, Lin Wang, Jinchao Ai, Leduo Zhang, Honggui Zhou, Shihao Sun, Yijie Wang, Youhong Wang, Xianqi Kong, Dawei Chen, **Tianlun Zhou**, Jiasheng Lu

Risen Pharmaceuticals Private Ltd., Shanghai, China

Mutations in the RAS oncogene are highly prevalent in pancreatic ductal adenocarcinoma (PDAC), colorectal cancer (CRC), and lung adenocarcinoma (LUAD), with codon 12 of the *KRAS* gene being the most frequently altered site. Substitutions of glycine at codon 12 to cysteine (G12C), glutamate (G12D), or valine (G12V) occur in 66.3%, 23.7%, and 24.4% of PDAC, CRC, and LUAD patients, respectively. While recently approved drugs targeting KRAS G12C mutation have shown clinical benefit, there remains a significant unmet need for therapies addressing the highly prevalent G12D and G12V mutations. Proteolysis-targeting chimeras (PROTACs) represent a novel therapeutic strategy for the targeted degradation of oncogenic proteins, potentially offering superior efficacy and mitigating resistance. In this study, we present RP04340, a potent and orally bioavailable PROTAC compound that selectively degrades KRAS G12C/D/V proteins and demonstrates robust anti-tumor activity. RP04340 induced significant degradation of KRAS G12C, KRAS G12D, and KRAS G12V proteins in cell lines Miapaca-2, PK-59, and NCI-H727, respectively, with a 50% degradation concentration (DC50) in the low nanomolar range. Within 12 hours of treatment, RP04340 reduced KRAS G12C/D/V protein levels by over 80% and effectively suppressed downstream signaling pathways, including pERK and DUSP6. Additionally, RP04340 inhibited cell proliferation in multiple KRAS G12C/D/V mutant cell lines, surpassing the anti-tumor efficacy of its enzymatic inhibitor warhead. In vivo studies using mouse xenograft models (Miapaca-2, PK-59, and NCI-H727) demonstrated significant tumor growth inhibition following daily oral administration of RP04340 at doses of 25, 50, and 100 mg/kg, with a clear dose-response relationship. Pharmacokinetic analyses revealed that RP04340 accumulated preferentially in tumor tissues, achieving high intratumoral concentrations with repeated dosing. Furthermore, tissue distribution studies in mouse showed significant accumulation of RP04340 in the lungs and pancreas after 15 days of consecutive dosing, supporting its suitability for treating cancers in these organs. In conclusion, RP04340, a PROTAC compound with compelling preclinical efficacy and favorable pharmacokinetic properties, represents a promising therapeutic candidate for KRAS G12C/D/V-driven cancers.

#1663 Discovery of novel, potent and orally bioavailable SMARCA2 PROTACs with synergistic anti-tumor activity in combination with KRAS G12C inhibitors.

Y. Lissanu, S. Kotagiri, N. Blazanin, Y. Wang, Y. Han, X. Liang;
UT MD Anderson Cancer Center, Houston, TX

Cancer genomic studies have identified frequent mutations in subunits of the SWI/SNF chromatin remodeling complex including SMARCA4 in non-small cell lung cancer with a frequency of up to 33% in advanced stage disease, making it the most frequently mutated complex in lung cancer. We and others have identified SMARCA2 to be synthetic lethal to SMARCA4, indicating SMARCA2 is a high value therapeutic target. Here, we disclose the discovery and characterization of potent, selective and orally bioavailable Cereblon-based SMARCA2 PROTACs. Biochemically, YDR1 and YD54 are potent SMARCA2 degraders with an average DC50 of 7.7nM and 3.5nM respectively in SMARCA4 mutant lung cancer cells. Phenotypically, both YDR1 and YD54 selectively inhibited growth of SMARCA4 mutant cancer cells. Further, we showed anti-tumor growth inhibitory activity of YDR1 and YD54 in SMARCA4 mutant xenograft models of lung cancer. Finally, we show that YDR1 and YD54 synergize with the KRAS G12C inhibitor sotorasib to inhibit growth of SMARCA4 and KRAS G12C co-mutant lung cancer cells. These findings provide additional evidence for the utility of single agent or combination regimens containing SMARCA2 PROTACs as synthetic lethal therapeutics against SMARCA4 mutant cancers.

#1664 Survivin degrader 7I14 synergizes with docetaxel in castration-resistant prostate cancer by disrupting survivin dimerization and inducing its proteasome-dependent degradation.

Qingbin Cui[1], Caoqinglong Huang[1], Xunzhen Zheng[1], Zizheng Dong[1], Jing-Yuan Liu[2], Jian-Ting Zhang[1]

[1]Department of Cell and Cancer Biology, University of Toledo College of Medicine and Life Sciences, Toledo, OH,[2]Department of Medicine, University of Toledo College of Medicine and Life Sciences, Toledo, OH

Survivin, the smallest member of the Inhibitor of Apoptosis Protein (IAP) family, forms homodimers and coordinates with other IAPs to inhibit apoptosis by suppressing pro-apoptotic caspase 3/9 activation, contributing to drug resistance. Due to its overexpression in cancer cells and lack of expression in normal adult tissues, survivin is an attractive cancer target. However, developing survivin-selective inhibitors has been challenging due to the absence of suitable active sites for targeting. Strategies have been pursued to inhibit survivin expression by targeting its upstream transcriptional regulators, notably leading to discovery of YM155, which unexpectedly suffers from poor pharmacokinetics and multiple targets, including the ubiquitous transcription factor Sp1. We recently developed a novel approach to directly target survivin for discovery of survivin degraders by disrupting its dimerization, exposing the hydrophobic interface, and promoting proteasome-mediated degradation. The compound 7I, a quinoxaline-based survivin degrader, likely interacts with key residues for survivin dimerization via π-π interactions. We hypothesized that electron-withdrawing groups on 7I enhance its binding to survivin. To test this hypothesis, we synthesized a series of 7I analogs and identified two improved leads, 7I10 and 7I14, with up to 20-fold greater activity, selectively degrading survivin in castration-resistant prostate cancer (CRPC) cell lines C4-2 and PC-3. Both 7I10 and 7I14 dose-dependently induced spontaneous apoptosis and synergized with docetaxel, a first-line therapy for metastatic CRPC. In a PC-3 xenograft model, 7I14 at 15 mg/kg administered via IP twice/week effectively suppressed tumor growth and enhanced docetaxel’s effects by degrading survivin, with no added toxicity, as assessed by body weight, white blood cell counts, and hemoglobin levels. Thus, targeting survivin's dimerization domain is a promising strategy for its degradation, and 7I10 and 7I14 have potential as single agents or in combination with docetaxel for treating metastatic CRPC.

#1665 A comprehensive platform for the screening and assessment of IRAK4-targeting PROTACs.

Zhaoxia Yin, Xiaojian Wang, **Tj (Tiejun) Bing**

ICE Bioscience, Inc., Beijing, China

Interleukin-1 receptor-associated kinase 4 (IRAK4) serves as a pivotal mediator in the signaling cascades of both the interleukin 1 receptor (IL-1R) and toll-like receptor (TLR) pathways. It is crucial for myddosome signaling, as its kinase and scaffolding activities are indispensable for the proper formation and signaling of the myddosome complex. This complex, in turn, initiates the activation of key downstream pathways, including NFkB and interferon-responsive factors 5/7 (IRF 5/7), culminating in the release of inflammatory cytokines and chemokines such as TNF-α, IL-6, IL-1β, and IL-23. These molecules are associated with the development of various autoimmune diseases, such as pyogenic tonsillitis, psoriasis, and atopic dermatitis, positioning IRAK4 as a promising therapeutic target for dampening inflammation mediated by TLR/IL-1R.

In this context, we have developed a comprehensive screening and evaluation platform for IRAK4 that spans from in vitro to in vivo studies. The platform encompasses an array of assays at both the biochemical and cellular levels. It features tests for binary and ternary complexes, intracellular detection of ternary complex formation, and a suite of methods for detecting target protein degradation, including flow cytometry, enzyme-linked immunosorbent assay (ELISA), and HiBiT. The platform also offers cellular function assays, such as cytokine release assays utilizing peripheral blood mononuclear cells (PBMCs) and whole blood, as well as assays focused on proteins associated with signal transduction pathways. It also includes off-target and selectivity testing, and extends to in vivo efficacy assessments within the IMQ-induced psoriasis model. Collectively, these assays are designed to facilitate a swift and thorough discovery and evaluation process for IRAK4-targeting PROTACs.

#1666 Novel endoxifen-based proteolysis targeting chimera (PROTAC) small molecules synergize with olaparib and exhibit potent antitumor activity in triple negative breast cancer through induction of DNA damage.

Aamod S. Dekhne[1], Swaathi Jayaraman[1], Lucy Rai Thulung[1], Becky Bruinsma[1], Baustin M. Welch[1], Samuel Wyatt[1], Esther P. B. Rodman[1], Sayantani Bhattacharya[1], Thomas R. Caulfield[2], John R. Hawse[1], Matthew J. Schellenberg[1], Matthew P. Goetz[1]

[1]Mayo Clinic, Rochester, MN,[2]Mayo Clinic, Jacksonville, FL

Breast cancer (BC) is the most common cancer diagnosed in American women. The majority of breast cancers express estrogen receptor (ER), progesterone receptor (PR), or human epidermal growth factor receptor 2 (HER2). However, 15-20% lack expression of all three and are called triple-negative breast cancer (TNBC). TNBC accounts for a disproportionately large amount of morbidity and mortality due to its aggressive nature and the paucity of effective adjuvant maintenance therapy, driving an urgent need for novel therapeutics. Z-endoxifen (ENDX) is a potent tamoxifen metabolite that dually targets ERα and protein kinase C beta I (PKCβI). We recently described next-generation molecules, the first-in-class endoxifen-PROTAC (ENDX-PROTAC) compounds which incorporate ENDX as the warhead and cereblon (CRBN) as the E3 ubiquitin ligase recruitment moiety (Jayaraman 2024, SABCS). We used the PRISM screen of over 900 cancer cell lines to determine that ENDX-PROTACs have broad antitumor activity in both TNBC and ER+ breast cancer in addition to, leukemia, pancreatic, lymphoma, lung, melanoma, and ovarian cancer cells, among others. ENDX-PROTACs primarily target a key translation termination factor, G to S phase transition 1 (GSPT1). Here, we demonstrate for the first time that these compounds also cause marked DNA damage. The lead compounds of this series, EPrC-007 and EPrC-009 demonstrated potent anti-proliferative effects in MDA-MB-231, BT549, and MDA-MB-436 TNBC cell lines. EPrC-007 induced substantial DNA double strand breaks in MDA-MB-231 cells as assessed by γ-H2AX Western blotting. Interrogation of DNA repair pathways revealed activation of ATM/CHK2 and downregulation of ATR/CHK1, suggesting inhibition of DNA repair as a potential mechanism. Additionally, EPrC-007 also induced γ-H2AX foci in MDA-MB-231 and BT549 cells, but not in the MCF10A normal breast tissue cell line, suggesting selective anti-tumor activity. Since we suspect that EPrC-007 blocks DNA repair machinery, we next sought to determine if these molecules would synergize with DNA damaging agents or other DNA repair inhibitors. Promisingly, EPrC-007 demonstrated remarkable synergy with olaparib (poly-(ADP-ribose) polymerase (PARP) inhibitor), hydroxyurea (ribonucleotide reductase inhibitor), and cisplatin (direct DNA damaging agent), but not camptothecin (topoisomerase I inhibitor). Importantly, only olaparib co-treatment demonstrated synergy in the PARP- and PROTAC-resistant HCC1143 TNBC cell line. Furthermore, the combination of EPrC-007 with olaparib induced markedly more γ-H2AX foci than either agent alone, confirming synergy at the DNA damage level. Taken together, our studies demonstrate the remarkable potential of ENDX-PROTACs in the single-agent setting as well as in combination with PARP inhibitors.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Drug Combination Strategies for Cancer Treatment
Poster Session

#1670 The SHMT inhibitor SHIN1 augments the antitumor efficacy of 5-Fuin preclinical models of gastric cancer.

Huan Deng, Yajie Wang, Lin Xiao, Yisheng Pan

Peking University First Hospital, Beijing, China

Advanced gastric cancer (GC) is a malignant tumor with poor prognosis, which requires in-depth exploration of its malignant progression mechanism and the development of efficient therapeutic protocols. Serine hydroxymethyltransferase (SHMT), an important hub in serine-glycine-nucleotide metabolic pathways, plays a crucial role in the occurrence and progression of GC. Bioinformatics analysis and cytological experiments preliminary identified the important role of SHMTs in GC. Drug synergistic screening assays were used to build the therapeutic model in the study. The transcriptomic analysis was performed to clarify the underlying mechanism of combination treatment. Here, we found that the SHMT1 and SHMT2 promoted malignant progression and 5-Fu resistance on GC, and their targeted inhibitor SHIN1 is a promising antitumor drug for GC. The synergistic screening analysis showed that SHIN1 was an efficient synergist for 5-Fu, and combination therapy, alleviated 5-Fu resistance to GC. Mechanistically, The combination treatment induces cell cycle arrest, DNA damage and cellular senescence through the activation of the P53 signaling pathway. These unique characteristics of cell growth inhibition, which are induced via interfering with nucleotide synthesis, were validated by substantial in vitro and in vivo assays. The regimen of SHIN1 combination with 5-Fu may be an efficient preclinical model for the treatment of GC.

#1671 Targeting histone H3K27 demethylase for anti-tumor activity: Monotherapy and combination with ceralasertib in non-small cell lung carcinoma.

Pony Yu-Ling Lee[1], Kun-Yuan Lin[1], Chao-Di Chang[1], Shan-Yun Cheng[1], Ya-Wen Hung[1], Chih-Chieh Yang[1], Yu-Hsien Chang[1], Chien-Chang Shen[1], Shu-Ping Wang[2]

[1]Pharmacology Discovery Services Taiwan, Ltd., New Taipei City, Taiwan,[2]Institute of Biomedical Sciences, Academia Sinica, Taipei City, Taiwan

Lung cancer is the leading cause of cancer-related deaths worldwide, with non-small cell lung carcinoma (NSCLC) accounting for 85% of cases and associated with poor prognosis. The lysine-specific demethylase KDM6A, responsible for removing H3K27 trimethylation at gene promoters and enhancers, is frequently mutated in various cancers. While KDM6A is predominantly considered as a tumor suppressor gene, recent studies suggusted it may support oncogenic transcriptional regulation. To investigate the role of KDM6A in tumorigenesis, we utilized CRISPR-Cas9/sgRNA to knock out (KO) KDM6A in NCI-H1975 NSCLC cells. Phenotypic assessments demonstrated cell cycle arrest with inhibitory effects in cell proliferation with KDM6A-depleted NCI-H1975 cells. RNA sequencing in conjunction with CUT&RUN revealed KDM6A target genes involved in cell cycle regulation, DNA replication, and DNA damage repair pathways, which were further validated using RT-qPCR and CUT&RUN-qPCR. Immunofluorescence confirmed the effects of KDM6A loss on DNA replication and DNA damage-induced repair mechanisms. These findings support our hypothesis that KDM6A is a highly pleiotropic factor in tumorigenesis, playing a critical role in regulating DNA replication-associated gene activation and facilitating the repair of replication stress-induced DNA damage. Notably, loss of KDM6A induced hyperactivation of checkpoint signaling pathways such as the ATR-CHK1 axis, suggesting KDM6A-depleted tumor cells attemption to alleviate replication stress to avoid apoptosis. To evaluate the therapeutic potential of KDM6A along with inhibition of the KDM6A-depletion-induced ATR-CHK1 pathway, ceralasertib (formerly AZD6738), an oral selective ATR inhibitor, was combined with KDM6A depletion for in vivo evaluation. Introducing KDM6A depletion significantly reduced NCI-H1975 tumor volume compared to the control group, demonstrating anti-tumor activity. Consistent with in vitro cell-based assays, the combination therapy of ceralasertib with KDM6A depletion showed enhanced tumor growth inhibition and regression in the NCI-H1975 xenograft model. Our findings reveal a pro-oncogenic role of KDM6A in sustaining tumor maintenance through engagement in DNA replication and repair pathways. Targeting KDM6A alone emerges as a promising therapeutic strategy for NSCLC. Furthermore, combining KDM6A depletion with ceralasertib significantly enhances anti-tumor activity by disrupting the ATR-CHK1 checkpoint signaling pathway, leading to an enhanced anti-tumor activity.

#1672 Synergistic efficacy of FGFR4 inhibitor (H3B-6527) and oxaliplatin combination therapy in gastric cancer models.

Nadeem Bhat, Mohammed Soutto, Ahmed Gomaa, Shoumin Zhu, Selma Maacha, Marwah Al-Mathkour, Mohammed Chwihne, Ahmed El-Rifai, Oliver McDonald, Wael El-Rifai

University of Miami, Miami, FL

Background: Resistance to chemotherapy remains a significant challenge in the treatment of gastric cancer. This study evaluates the therapeutic potential of a combination of the FGFR4 inhibitor H3B-6527 and Oxaliplatin in preclinical models, focusing on their synergistic effects on tumor suppression and cell death mechanisms.
Material and Methods: To evaluate the efficacy of H3B-6527 and oxaliplatin combination, we employed IC50 analyses, colony formation assays, patient-derived xenograft (PDX) models, immunofluorescence (IF), immunohistochemistry (IHC), western blotting, and caspase-3/7 luminescence assays. These techniques were used to assess the impact of the drug combination on tumor cell proliferation, DNA damage, and apoptosis in gastric cancer cell lines (MKN28 and HGC27) and tumor tissues.
Results: IC50 analyses demonstrated significantly enhanced sensitivity to the combination therapy in both MKN28 and HGC27 cell lines compared to either agent alone. Colony formation assays revealed that the combination therapy drastically reduced colony counts relative to monotherapies, highlighting its superior anti-proliferative effects. In PDX mouse models, the combination therapy led to pronounced tumor growth inhibition and significantly prolonged survival compared to controls. IF and IHC analyses showed marked increases in DNA damage, as evidenced by γH2AX expression, along with reduced proliferation (Ki67) and elevated apoptosis (cleaved caspase-3). Caspase-3/7 luminescence assays confirmed heightened apoptotic activity in treated cell lines. Western blot analysis revealed elevated levels of cleaved PARP and γH2AX in both gastric cancer cell lines and PDX tissues, corroborating the enhanced DNA damage and apoptotic response induced by the combination therapy.
Conclusions: This study demonstrates the synergistic efficacy of H3B-6527 and oxaliplatin in promoting apoptosis, enhancing DNA damage, and inhibiting tumor proliferation in gastric cancer models. These findings provide compelling evidence for the potential clinical application of this drug combination as a promising therapeutic strategy for gastric cancer patients, especially those with chemoresistance.

#1673 Advancing bladder cancer treatment with live attenuated salmonella ZH9: from monotherapy to combination strategies.

Nicholas Glanville[1], Claudia Prevosto[1], Sonia Domingos-Pereira[2], Lenka Polak[2], Victoria Morel[3], Marine Leblond[3], Gregory Verdeil[3], Denise Nardelli-Haefliger[2], Livija Deban[1]

[1]Prokarium Ltd., London, United Kingdom,[2]Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland,[3]University of Lausanne, Lausanne, Switzerland

For over 30 years, microbial immunotherapy with Bacillus Calmette-Guérin (BCG) has been the standard-of-care for non-muscle invasive bladder cancer (NMIBC). Global demand for BCG however exceeds supply and BCG therapy is associated with disease recurrence and high treatment burden. Most novel bladder cancer treatments in development either target BCG unresponsive patients as second line or are administered in combination with BCG and thus share its shortcomings. There remains a significant need for innovative therapies to replace BCG in bladder cancer treatment, and live-attenuated Salmonella Typhi ZH9 presents a promising alternative, both as a monotherapy and as a combination partner. Efficacy of ZH9 was investigated in syngeneic mouse MB49 and *p53*$^{-}$/*Pten*$^{-}$ GEMM orthotopic tumor models in which animals were treated in the bladder via catheterization, with or without combination intraperitoneal anti-PD-L1 or anti-PD-1 treatment. Immune responses were characterized by flow cytometry and histology analyses of bladder tissue. Intravesical ZH9 monotherapy following orthotopic MB49 tumor inoculation demonstrated significant survival benefit compared to both vehicle control and BCG treatments. A single administration of ZH9 was sufficient for efficacy, inducing far greater magnitude and duration of immune responses than standard-of-care BCG, and resulting in systemic anti-tumor immunity that protected against both local bladder and distant MB49 tumors. Combination of local ZH9 treatment with regimens of checkpoint inhibitor antibodies anti-PD-L1 (MB49 model) and anti-PD-1 (GEMM model) that were otherwise ineffective, led to significant efficacy, suggesting local *Salmonella* ZH9 treatment can synergize with systemic immunotherapies. These data support Salmonella ZH9 as an effective first-line monotherapy and checkpoint inhibitor combination therapy for bladder cancer, with potential to reduce treatment burden on patients, lower associated healthcare costs and to ease microbial therapy supply constraints due to a scalable manufacturing process. Clinical evaluation of ZH9 in a Phase 1b study evaluating safety, pharmacology, and early efficacy in NMIBC is ongoing.

#1674 Combinatorial screen with standard of care and selected anticancer agents identifies multiple combinations with activity against a panel of patient-derived colorectal organoids.

Nathan P. Coussens[1], Thomas S. Dexheimer[1], Phillip R. Sanchez[1], Thomas Silvers[1], Eric M. Jones[1], Bryce M. N. Butler[1], John R. Britt[1], Carmen Allegra[2], James H. Doroshow[2], Beverly A. Teicher[2]

[1]Applied and Developmental Research Directorate, Frederick National Laboratory for Cancer Research, Frederick, MD, [2]Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, MD

Thirty-two patient-derived colorectal organoid models were selected from the NCI's Patient-Derived Models Repository (https://pdmr.cancer.gov) to serve in a high-throughput screening panel. The models were derived from primary or metastatic tumors of patients with varied ancestries, ranging in age from 32–90 yrs, that were either previously treated or treatment naïve. Variations were determined in microsatellite stability, consensus molecular subtype, and genetics, including clinically relevant oncogenic variants of RAS, BRAF and PIK3CA. A screen was conducted with twenty-two combinations of agents, including SOC and investigational oncology drugs. Organoids were exposed to single agents or combinations at four concentrations covering 2 logs up to the clinical Cmax, if known. Following compound addition, organoid growth was measured by live bright field imaging every 24 h and cell viability was determined by CellTiter-Glo 3D at 48 h and 168 h. Modest responses were observed in most models from the combination of leucovorin with 5-FU; however, >1 log of cytotoxicity was observed in only several models. The panel was more responsive to FOLFOX, which was consistent with the sensitivity of models to oxaliplatin. Responses to TAS-102 varied and >1 log of cytotoxicity was observed in several models. The MEK inhibitor cobimetinib was active in models with wild type KRAS and generally more active in models with oncogenic KRAS. Combination of the BCL-2/BCL-xL inhibitor pelcitoclax plus cobimetinib achieved >1 log of cytotoxicity in most organoid models. Combinations of cobimetinib with the EGFR inhibitors erlotinib, afatinib or cetuximab demonstrated synergy in many organoids according to the Bliss independence model and frequently achieved ≥1 log of cytotoxicity. As a single agent, the KRAS G12D inhibitor MRTX1133 was active against organoid models harboring KRAS G12D. The combination of cobimetinib and MRTX1133 had synergistic activity by Bliss independence in multiple organoid models and frequently achieved >1 log of cytotoxicity. Additionally, combinations of MRTX1133 with an EGFR inhibitor had additive and greater-than-additive cytotoxicity in a range of organoids. The pan-RAS(ON) inhibitor RMC-6236 had substantial single-agent activity against organoid models with wild type KRAS and most models with an oncogenic KRAS variant. In combination with cobimetinib, RMC-6236 demonstrated additive and synergistic cytotoxicity, frequently ≥1 log, in organoid models with BRAF V600E and harboring wild type KRAS or an oncogenic variant. When combined with the pan-PI3K inhibitor copanlisib, RMC-6236 demonstrated substantial synergy, achieving ≥2 logs of cytotoxicity in several organoid models with wild type KRAS. Complimentary PDX studies are in progress. Funded by NCI Contract No. 75N91019D00024 & 75N91020F00032.

#1675 Evaluation of combination strategies with RAS-targeted inhibitors in multi-cell type spheroids.

Thomas S. Dexheimer[1], Nathan P. Coussens[1], Thomas E. Silvers[1], Eric M. Jones[1], Naoko Takebe[2], James H. Doroshow[2], Beverly A. Teicher[2]

[1]Molecular Pharmacology Laboratory, Frederick National Laboratory for Cancer Research, Frederick, MD,[2]Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, MD

RAS genes are among the most frequently mutated oncogenes in cancer. Though previously considered undruggable, agents have recently been developed that specifically target RAS. The agents can be categorized as (1) those that selectively target specific RAS oncoproteins and (2) pan-RAS inhibitors that target a wide range of RAS alterations, including oncogenic variants and amplifications. This study evaluated the activity of four novel RAS-targeted inhibitors in combination with other targeted agents in thirty solid tumor cell lines of diverse cancer types. These included twenty-six patient-derived cancer cell lines from the NCI Patient-Derived Models Repository (https://pdmr.cancer.gov/) and four established cell lines. The cells were grown as multi-cell type (mct) tumor spheroids from 60% malignant cells, 25% endothelial cells, and 15% mesenchymal stem cells. All agents were tested at concentrations up to the clinical C_{max} value, if known or 10 μM. As single agents, the KRAS-G12C inhibitors RMC-6291 and divarasib, along with the KRAS-G12D inhibitor MRTX-1133, demonstrated selective activity against the mct tumor spheroids harboring the targeted variants. In contrast, the pan-RAS(ON) inhibitor RMC-6236 demonstrated activity against the mct tumor spheroids harboring a range of KRAS variants or wild-type KRAS. When used in combination, each of the four RAS-targeted inhibitors exhibited strong synergistic effects with SHP2 inhibitors as determined by the Bliss independence model and reductions in cell viability and spheroid volume. In tumor models harboring different KRAS variants, the pan-RAS(ON) inhibitor showed increased cytotoxicity with the MEK inhibitor cobimetinib, as well as PI3K-AKT-mTOR pathway inhibitors, such as inavolisib (PI3Kα), ipatasertib (AKT), and sapanisertib (mTORC1/2). When the same agents were combined with variant-specific KRAS inhibitors similar effects were observed in mct tumor spheroids carrying the corresponding RAS variant. These preclinical findings might provide guidance for the selection of combination regimens with KRAS inhibitors to improve clinical efficacy. Funded by NCI Contract No. 75N91019D00024.

#1677 Navitoclax, a Bcl-2/xL inhibitor, and YM155, a survivin inhibitor, in combination with carboplatin effectively inhibit ovarian cancer tumor growth.

Tejas Samantaray[1], Hilary Kenny[1], Lucy Kelliher[1], Ernst R. Lengyel[1], Carman K.M. Ip[2], Kasjusz Kordylewicz[1], Rachael Hoffmann[1], Sarah Rauch[1], Beatrice Malacrida[3], Sophie Skingsley[3], Frances R. Balkwill[3], Chiara Battistini[4], Ugo Cavallaro[5], Wolf R. Wiedemeyer[6]

[1]Obstetrics and Gynecology, University of Chicago, Chicago, IL,[2]Cellular Screening Department, University of Chicago, Chicago, IL,[3]Queen Mary University of London, London, United Kingdom,[4]Unit of Gynecological Oncology Research, European Institute of Oncology IRCCS, Milan, Italy,[5]Department of Experimental Oncology, European Institute of Oncology IRCCS, Milan, Italy,[6]Abbvie Inc., Chicago, IL

Introduction: High-grade serous ovarian cancer (OvCa) is generally treated with upfront chemotherapy, including carboplatin. Recurrent disease is driven by the persistence of platinum-resistance cells.
Experimental Procedures: A high throughput screen (HTS), using a 3D organotypic culture assembled with extracellular matrix, primary human fibroblasts, and mesothelial cells, was established and validated. Using a library of FDA approved drugs, the 3D HTS was performed with the goal of identifying a combination of drugs that synergistically target two populations of OvCa, aldehyde dehydrogenase (ALDH) high and ALDH low enzyme activity cells, that are less sensitive to carboplatin treatment than the bulk OvCa cells. Summary of Results. Initial results showed that Omipalisib, Verteporfin, CA3, Mitoxantrone, Navitoclax, Venetoclax and YM155 had significant single drug activity in either the ALDH low or both the ALDH low/high cell populations. Synergistic drug activity was identified with three drug combinations, Navitoclax/Omipalisib, Navitoclax/YM155, and YM155/Omipalisib. *In vitro* the combination of Navitoclax/YM155 was most efficient at blocking primary human OvCa sphere formation and the proliferation of four different OvCa cell lines in the 3D organotypic culture. *In vivo* the combination of Navitoclax/YM155/carboplatin decreased the total number and weight of metastases, decreased the percentage of ALDH high OvCa cells in tumors, and increased survival when compared to carboplatin treatment alone in xenograft models.
Conclusion: Our results suggest that the combination of Navitoclax/YM155/carboplatin has promise as a therapy for the treatment of OvCa.

#1679 APG-2449, a novel focal adhesion kinase (FAK) inhibitor, enhances the antitumor activity of chemotherapy in preclinical models of small-cell lung cancer (SCLC) with activated FAK.

Zhou Yu[1], Zhiyan Liang[2], Xinyi Yao[1], Daojie Liu[1], Dajun Yang[1], Yifan Zhai[1]

[1]Ascentage Pharma (Suzhou) Co., Ltd., Suzhou, China,[2]Ascentage Pharma Group Inc., Rockville, MD

Background: SCLC is a genetically heterogeneous disease with no standardized targeted therapy options. Despite the recent advancement of immune checkpoint inhibitors, improvements in overall survival have been modest, and platinum-based chemotherapy combined with topoisomerase inhibitors remains the standard of care in SCLC. FAK, a non-receptor tyrosine kinase, has been reported to regulate cellular proliferation, migration, invasion, and DNA-damage repair. Previous studies have shown that FAK is amplified and overexpressed in SCLC tumors. Although non-small-cell lung cancer cells harboring $FAK^{6,7}$ (splicing isoforms of FAK that increase phosphorylation of FAK [pFAK]) are more sensitive to FAK inhibition than wild-type FAK (FAK^{WT}), the expression and implications of $FAK^{6,7}$ expression in SCLC remain unclear. Given the role of FAK in cancer progression, we hypothesized that inhibition of FAK would augment the antitumor effects of chemotherapy in SCLC. The aim of this study was to evaluate the antitumor activity of investigational agent APG-2449 alone and combined with chemotherapy in SCLC.

Methods: Cellular proliferation was measured by CellTiter-Glo® assays and apoptosis assessed via flow cytometry. Protein expression levels were examined via western blot. An H446 xenograft model was used to evaluate antitumor effects *in vivo*.

Results: SCLC cell lines harboring $FAK^{6,7}$ (H446, H69, and H526) showed increased sensitivity to APG-2449 in a dose-dependent manner compared to a FAK^{WT} cell line (DMS-114). When combined with chemotherapy (topotecan or etoposide plus carboplatin), APG-2449 synergistically decreased cell viability and increased apoptosis in H446 and H526 cell lines. Mechanistically, APG-2449 combined with topotecan and etoposide plus carboplatin enhanced DNA damage and apoptosis compared to either therapy alone in western blot assays. APG-2449 synergistically enhanced the antitumor effects of topotecan (synergy ratio: 22.08) and etoposide plus carboplatin (synergy ratio: 3.81) in the H446 xenograft model.

Conclusions: Novel FAK inhibitor APG-2449 demonstrated a synergistic enhancement when combined with first- and second-line chemotherapies in SCLC, significantly increasing antitumor activity. These encouraging findings support further clinical development of APG-2449 for the treatment of patients with SCLC.

#1680 Therapeutically targeting genes and pathways activated by high-fat diet in high-risk neuroblastoma.

Md Jahidul Hasan[1], Ritu Mittra[1], Marie Joseph[1], Mukesh Raipuria[1], Nisitha Jayatilleke[1], Daniel R Carter[1], Margaret J Morris[2], Belamy B Cheung[1], Glenn M Marshall[1]

[1]Children's Cancer Institute, Lowy Cancer Research Centre, UNSW, Sydney, New South Wales, Australia,[2]Faculty of Medicine and Health, UNSW, Sydney, New South Wales, Australia

MYCN-amplified neuroblastoma (NB) is one of the most aggressive and treatment refractory cancers in children. NB has a prenatal origin, and therefore environmental factors *in utero* may play a critical role in driving NB tumorigenesis. A recent epidemiological study showed that children born to mothers who gained weight more than 30 kg during pregnancy had a 2-fold higher risk of developing NB. Using the *Th-MYCN* NB transgenic mouse model, we found that high-fat diet (HFD)-induced maternal obesity resulted in accelerated NB tumorigenesis in the Th-MYCN homozygous offspring. Transcriptomic patterns in tumour tissues from Th-MYCN homozygous offspring of HFD-fed mothers showed upregulation of fatty acid (FA) metabolism and mitochondrial oxidative phosphorylation (OXPHOS) pathways. We then used human NB cell lines exposed to excess FA to study mechanisms and therapeutics. *In vitro* studies showed that excess FA increased growth, proliferation, and colony formation in human NB cell lines, which was reversed by Fatostatin, a FA metabolism inhibitor, with an effect which was partially MYCN-dependent. Fatostatin showed significant synergy in combination with doxorubicin and vincristine in reversing FA-accelerated growth, proliferation, and colony formation in human NB cell lines, with a wide therapeutic window. ATP5G1 and ATP5G2 (genes encoding human mitochondrial ATP synthase membrane c-subunit) were identified as candidate genes in the HFD-activated OXPHOS pathways. siRNA knockdown of both genes significantly reversed FA-accelerated growth and proliferation in human NB cell lines. Chromatin immunoprecipitation (ChIP)-PCR showed that ATP5G1 and ATP5G2 are transcriptional targets of MYCN, and their transcription is significantly increased in the presence of FA. Further, the overall ATP production was significantly increased in the presence of FA in human NB cell lines, but not in human normal fibroblasts. siRNA knockdown of ATP5G1 and ATP5G2 significantly decreased this effect. Together, our data strongly support a role for OXPHOS and FA metabolism pathways in HFD-accelerated NB tumorigenesis and highlight a key role of the mitochondrial ATP synthase membrane c-subunit genes, ATP5G1 and ATP5G2 in HFD-accelerated NB tumorigenesis.

#1681 Strong synergistic activity of an EZH1/2 dual inhibitor, HM97662, in combination with standard chemotherapy in preclinical models of advanced solid tumors.

Seon Yeong Han, Gunwoo Lee, Aran Park, **Seung Hyun Jung**, Jooyun Byun, Young Gil Ahn

Hanmi Pharmaceutical Co., Ltd., Hwaseong-si, Korea, Republic of

Many cancer patients eventually develop resistance to chemotherapy drugs after treatment. For instance, platinum-based drugs are commonly used as first-line treatments for ovarian cancer. While many patients initially respond well to this therapy, approximately 80% experience tumor recurrence within two years and ultimately acquire platinum-resistance. Recent studies have shown that EZH2 plays a role in regulating cisplatin resistance in ovarian cancer. As a similar example, small cell lung cancer (SCLC) is aggressive with poor prognosis, particularly in relapsed or refractory cases. While irinotecan is commonly used as 2nd-line therapy due to its manageable toxicity and efficacy, its effectiveness as a monotherapy is limited by chemotherapy resistance. Preclinical studies suggest that combining EZH2 inhibitors with chemotherapy may enhance anticancer efficacy by modulating the epigenetic environment and disrupting DNA repair mechanisms. This combination holds promise for overcoming resistance and improving outcomes in SCLC and other cancers. EZH2, an enzymatic subunit of the polycomb repressive complex 2 (PRC2), catalyzes the tri-methylation of histone H3 at lysine 27 (H3K27me3), repressing target genes involved in cell cycle regulation, differentiation, and tumor suppression. EZH2 and SWI/SNF have opposing roles in chromatin regulation, and EZH2 overexpression is linked to SWI/SNF mutations. This interaction affects cancer cell survival, highlighting EZH2 as a critical therapeutic target. Dual inhibition of EZH1 and EZH2 may offer greater anti-tumor efficacy, as EZH1 can compensate for the loss of EZH2 activity. Herein, we introduce a novel EZH1/2 dual inhibitor, HM97662, which concurrently suppressed the methyltransferase activity not only wild-type EZH1, but also wild-type and gain of function mutant EZH2 at nanomolar concentrations. HM97662 potently repressed tri-methylation of H3K27me3 in cancer cell lines. HM97662 also showed a wide and strong growth inhibitory effect in various solid cancer cell lines. Moreover, HM97662 exhibited potent antitumor activities in xenograft mouse models with various solid cancer cells including ovarian, bladder, small cell lung cancer, and gastric cancers. Additionally, HM97662 showed synergistic effects with chemotherapies such as topoisomerase inhibitors and platinum-based standard treatment in those xenograft models. These results suggest that concurrent treatment with HM97662 and chemotherapy enhances in vivo tumor regression at well-tolerated doses. In conclusion, this preclinical study demonstrated that HM97662, an EZH1/2 dual inhibitor, has promising therapeutic potential against advanced solid tumors, either alone or in combination with standard-of-care cytotoxic agents. It is urgent to assess the effectiveness of HM97662 in further clinical trials.

#1682 Novel CDK2/CDK9 inhibitor fadraciclib targets cell survival and DNA damage pathways and synergizes with encorafenib in human colorectal cancer cells with BRAF(V600E).

Md Mohiuddin[1], Vanda Povoa[2], Rita Fior[2], Frank A. Sinicrope[1]

[1]Department of Medicine, Gastrointestinal Research Unit, Mayo Clinic Alix School of Medicine, Rochester, MN,[2]Champalimaud Centre for the Unknown, Champalimaud Research, Champalimaud Foundation, Lisbon, Portugal

Background: Mutant *BRAF(V600E)* is present in 8-12% of human colorectal cancers (CRCs) and is associated with treatment resistance and poor survival. Targeted therapy with inhibitors of BRAF and EGFR is associated with only an incremental survival benefit.
Methods: We studied the novel CDK2/CDK9 inhibitor, fadraciclib, alone and in combination with the BRAF inhibitor encorafenib on isogenic CRC cells with two, one, or no $BRAF^{V600E}$ alleles ($RKO^{+/+}$, $A19^{+/-}$, $T29^{-/-}$). Analysis of apoptosis, colony formation, and the cell cycle was performed. The effect of drug treatment on growth of tumor xenografts was determined in a zebrafish model.
Results: Fadraciclib induced apoptosis and inhibited colony formation in a gene dose-dependent manner. Its growth inhibitory effect when combined with encorafenib was synergistic. Mechanistically, fadraciclib suppressed the expression of pro-survival MCL-1 and decreased the phosphorylation of RNA polymerase II indicating suppression of RNA transcription. Fadraciclib also impaired Rb phosphorylation and promoted a DNA damage response indicated by the cleavage of PARP, pH2AX (ser139), and activation of p53. Drug treatment was associated with a greater suppression of Rb phosphorylation, cell cycle inhibition, and apoptosis in $RKO^{+/+}$ versus $A19^{+/-}$ and $T29^{-/-}$ cells. In a zebrafish tumor xenograft model, fadraciclib plus encorafenib significantly reduced the tumor xenograft size concurrent with increased caspase-3 activation.
Conclusion: Fadraciclib plus encorafenib potently induced apoptosis and synergistically suppressed tumor cell growth in a gene dose-dependent manner in $BRAF^{V600E}$ CRC cells, and exerted anti-tumor effects in a tumor xenograft model. These data suggest a novel therapeutic strategy in this CRC subtype.

#1683 Combining annamycin, a non-cardiotoxic potent topo II poison, with azacitidine, cytarabine, gemcitabine, ifosfamide, trabectedin, or vincristine to synergize anticancer effects and identify potential clinical applications.

Rafal J. Zielinski, Krzysztof Grela, Stanislaw Skora, Izabela Fokt, Roberto Cardenaz-Zuniga, Natalia Baran, **Waldemar Priebe**

UT MD Anderson Cancer Center, Houston, TX

Background: Annamycin (ANN) is a potent topoisomerase II poison structurally related to doxorubicin (DOX) but displaying superior biological properties, such as high *in vivo* efficacy, reduced toxic side effects, and activity against cancers resistant to common therapeutic agents. ANN is currently clinically evaluated as a liposome formulated drug product, L-ANN. Importantly, L-ANN preclinical and clinical data clearly indicate a lack of cardiotoxic activity, which is a common dose-limiting factor in this class of therapeutics. In addition, ANN and L-ANN display unique organotropism that significantly differs from that of DOX. This finding further increased our interest to study the potential synergistic or additive effects of combining ANN with different anticancer agents. The efficacy of L-ANN has been established in multiple tumor models covering both liquid cancers and solid tumors, further strengthening the rationale of this study. Objective The objective of the study was to assess the efficacy of ANN in combination with different anticancer agents in order to identify the potential clinical application of ANN alone and with a therapeutic partner.

Methods: The activity of ANN in combination with selected FDA approved drugs was assessed *in vitro* after 72 hours of treatment using a cytotoxicity assay followed by determination of the combination indexes. In separate *in vitro* experiments, ANN activity was tested against drug resistant cell lines, including cells resistant to cytarabine and venetoclax. The most efficacious drug combinations from the *in vitro* studies were then tested using *in vivo* models of leukemia and solid tumors, including sarcoma and pancreatic cancer.

Results: The *in vitro* screening performed in set of cancer cell lines led to selection of the following agents combined with ANN for testing efficacy in clinically relevant animal models: 1) Cytarabine, 2) Azacitidine, 3) Venetoclax, 4) Vincristine in AML model; 5) Ifosfamide in fibrosarcoma; 6) Irinotecan and 7) Gemcitabine in PATC53 pancreatic cancer model. All combinations listed above showed improved survival when compared to single agent control groups. Importantly, our preclinical studies led to the clinical development of L-ANN plus cytarabine regimen, a combination that demonstrated clinical anticancer activity in Phase I/II studies in AML patients. Phase III clinical studies of L-ANN in a combination with cytarabine is expected to be initiated in early 2025.

Conclusion: ANN appears to be a versatile drug forming efficacious combinations with numerous mechanistically different anticancer agents. Our studies are working toward identifying new clinical applications for ANN with a long-term goal of developing novel therapeutic strategies for drug-resistant cancers.

#1684 Combination of L-NMMA and sacituzumab govitecan as a novel targeted therapy for inflammatory breast cancer.

Karina A. Ortega Martinez, Maria F. Chervo, Wei Qian, Jianying Zhou, Liliana Guzman-Rojas, Ivonne D. Uzair, Gianmarco Melone, Mailin Li, Christoforos Thomas, Jenny C. Chang

Breast Oncology Department Cancer Center of Excellence, Houston Methodist Research Institute, Houston, TX

Background: Inflammatory breast cancer (IBC) is a rare and aggressive form of breast cancer characterized by rapid progression, early metastatic potential, and distinct clinical and pathological features. The main treatment includes systemic cytotoxic therapy, surgery, and radiation. There is a need for novel targeted therapies to improve patients'outcomes. Inducible nitric oxide synthase (iNOS) is a mediator of various cancers including breast cancer. The pan-NOS inhibitor L-NMMA has been studied in preclinical and clinical settings, primarily focusing on cancers with high iNOS expression. Our preclinical studies provided the rationale for a recently completed phase I/II clinical trial of L-NMMA plus taxane for treating patients with triple-negative breast cancer (TNBC), with favorable toxicity and robust responses. Exploring the interaction between iNOS and IBC could identify novel therapeutic strategies, improving management approaches for this aggressive and highly lethal breast cancer subtype.
Hypothesis: we hypothesize that, in combination with sacituzumab govitecan, the inhibition of iNOS by L-NMMA will significantly reduce tumor progression and survival in preclinical models of IBC. Sacituzumab govitecan is an antibody-drug conjugate where SN-38, a topoisomerase I inhibitor, is coupled to an anti-trophoblast cell-surface antigen 2 (Trop-2) monoclonal antibody, currently used in the treatment of TNBC.
Experimental Procedures: The IBC xenograft SUM149 was orthotopically established in the mammary fat pad of female NSG mice by injection of $2x10^6$ cells/mouse. Once tumors reached 200 mm^3 in size, mice were randomly assigned into the following groups (n=7 mice per group): 1) Control (Saline, administered via i.p. injection); 2) Sacituzumab (administered at 5 mg/kg via i.v. injection weekly); 3) L-NMMA plus the calcium channel blocker amlodipine (LNMMA administered at 400 mg/kg on day 1 followed by 200 mg/kg per subsequent day by daily oral gavage; amlodipine administered at 10 mg/kg via i.p. injection daily); 4) Combination of Sacituzumab, L-NMMA, and amlodipine (Combo). Animals were treated as indicated for 21 days. Tumor size was monitored twice per week using caliper measurements.
Results: The Combo treatment was more effective at reducing tumor volume than each individual drug (adj p value = 0.0010 by one-way ANOVA).
Conclusions: Treatment with the Combo significantly reduced tumor growth compared to single drugs and control group. There was no statistically significant difference between the L-NMMA group and sacituzumab govitecan-treated mice. Overall, our findings demonstrate the synergism between L-NMMA and sacituzumab govitecan, highlighting the potential use of these drugs in future clinical trials.

#1685 Evaluation of antitumor and molecular responses to abiraterone acetate plus capivasertib in mouse PTEN-deficient prostate cancer.

Marco A. De Velasco[1], Yurie Kura[1], Kazuko Sakai[1], Simon T. Barry[2], Cath Eberlein[2], Claire Rooney[3], Kazuto Nishio[1], Kazutoshi Fujita[1], Hirotsugu Uemura[1]

[1]Kindai University, Faculty of Medicine, Osaka-Sayama, Japan,[2]Bioscience Early Oncology, AstraZeneca, Cambridge, United Kingdom,[3]Translational Medicine, AstraZeneca, Cambridge, United Kingdom

Inhibition of the androgen receptor (AR) is the primary treatment for metastatic hormone-sensitive prostate cancer (mHSPC). The addition of antiandrogen therapies to androgen deprivation therapy (ADT) has greatly improved patient outcomes. However, loss of the tumor suppressor PTEN is common in prostate cancer (PCa), and the resulting increase in AKT signaling contributes to PCa progression. Capivasertib (AZD5363) is currently being investigated as an oral pan-AKT inhibitor in combination with abiraterone (Abi) and ADT for treating mHSPC in patients with PTEN loss (CAPItello-281). We previously demonstrated the efficacy of targeted AKT inhibition using capivasertib in preclinical mouse models of PTEN-deficient PCa (PMID: 26910118). In this study, we evaluated the antitumor and molecular responses to the combination of Abi and capivasertib in vivo using conditional knockout mouse models with targeted deletions of *Pten* (KO) and both *Pten* and *Trp53* (DKO). In DKO mice, four weeks of ADT (via surgical castration) followed by four weeks of Abi resulted in a 9.4% tumor growth inhibition (TGI) compared to ADT alone. Additionally, 3/8 (37.5%) mice treated with Abi had tumor burden reductions >20% compared to ADT alone. In a hormone-sensitive model using DKO mice, four-week treatment with ADT/Abi resulted in a 7.4% TGI based on genitourinary tract weight compared with ADT alone. The combination of ADT and Abi+capivasertib further improved the TGI to 21.8%. DKO mice with advanced PCa also exhibited improved antitumor activity with the combination of ADT/Abi+capivasertib compared with ADT/Abi. In survival studies involving DKO mice with advanced PCa, the addition of capivasertib to ADT/Abi reduced metastasis, extended tumor doubling time, and improved OS compared with ADT/Abi alone. Immunohistochemical analysis revealed that mice treated with ADT/Abi+capivasertib exhibited significantly lower proliferation and increased apoptotic rates. Molecular profiling studies revealed that mice treated with Abi exhibited increased AKT signaling, as indicated by increased PRAS40 and S6 phosphorylation, but this enhancement was inhibited by capivasertib. Syngeneic allograft transplantation models using PTEN-expressing TRAMP-C1 and *Pten*-null-derived PCa cells demonstrated that, while both models responded to capivasertib, *the Pten*-null allografts exhibited greater sensitivity to the ADT/Abi+capivasertib combination. In conclusion, we provide preclinical evidence that abiraterone-resistant tumors with hyperactivation of AKT due to loss of PTEN are sensitive to AKT inhibition.

#1686 Enhancing chemotherapy efficacy in PTEN-deficient prostate tumors with targeted AKT inhibition.

Marco A. De Velasco[1], Yurie Kura[1], Kazuko Sakai[1], Simon T. Barry[2], Cath Eberlein[2], Claire Rooney[3], Kazuto Nishio[1], Kazutoshi Fujita[1], Hirotsugu Uemura[1]

[1]Kindai University, Faculty of Medicine, Osaka-Sayama, Japan,[2]Bioscience Early Oncology, AstraZeneca, Cambridge, United Kingdom,[3]Translational Medicine, AstraZeneca, Cambridge, United Kingdom

Taxane chemotherapy is the standard treatment for metastatic castration-resistant prostate cancer (mCRPC). The tumor suppressor PTEN is frequently deleted in advanced prostate cancer (PCa), contributing to cancer progression and poor clinical outcomes. Hyperactivation of AKT resulting from PTEN loss and reciprocal crosstalk between AR and PI3K/AKT signaling may impair the efficacy of taxane therapy. Capivasertib (AZD5363) is a potent oral pan-AKT inhibitor that has shown efficacy against PCa in preclinical and early phase clinical studies. Capivasertib is currently being evaluated in a Phase 3 trial (CAPItello-280) along with docetaxel (Dtx) for the treatment of patients with mCRPC. Here, we assessed the in vivo preclinical efficacy of Dtx plus capivasertib using transgenic mouse models of *Pten*-null PCa. In an early-stage model of PCa using double knockout mice with conditional inactivation of *Pten* and *Trp53*, four weeks of treatment with Dtx resulted in tumor growth inhibition (TGI) of approximately 25% and 7% compared with vehicle-treated control mice in hormone-sensitive PCa (HSPC) and CRPC settings, respectively. Adding capivasertib to Dtx further increased tumor TGI by ~14% in both settings. More robust improvements were observed with the combination therapy in CRPC compared with HSPC. Namely, 7/10 (70%) and 9/10 (90%) of HSPC mice treated with Dtx and Dtx+capivasertib, respectively, exhibited a >25% reduction relative to the control group. In contrast, 2/15 (13.3%) and 8/18 (44.4%) of CRPC mice treated with Dtx and Dtx+capivasertib, respectively, showed similar reductions. After one week of treatment, capivasertib suppressed the phosphorylation of PRAS40 and S6 downstream molecules of AKT, and this suppression was sustained for four weeks. In HSPC, Dtx+capivasertib resulted in a greater decrease in cancer cell proliferation compared with DTx alone and control mice. One week of administration of Dtx with or without capivasertib in CRPC mice increased cleaved caspase-3 levels to levels higher than those in control mice, whereas both Ki67 and cleaved caspase-3 levels plateaued in atrophic acini during week four suggesting M-phase cell cycle arrest. The therapeutic benefits of Dtx+ capivasertib were further evaluated in a late-stage CRPC model. Mice with tumors treated with Dtx+ capivasertib after progression to androgen deprivation therapy (surgical castration) and apalutamide had longer survival and longer tumor doubling times than those treated with Dtx alone. In conclusion, PTEN-deficient CRPC is less sensitive to Dtx than HSPC. However, our model showed that adding capivasertib to Dtx suppressed tumor growth and improved the therapeutic efficacy of CRPC.

#1687 Estrogen-related receptor gamma (ERRγ) as a therapeutic target and the synergetic anti-tumor effect of DN200434 (ERRγ inverse-agonist) and MK2206 (Akt inhibitor) in non-small cell lung carcinoma (NSCLC).

T. Singh;
All India Institute of Medical Sciences (AIIMS) New Delhi, New Delhi, India

Introduction: Non-small cell lung cancer (NSCLC) accounts for over 85% of all lung cancer cases and has the greatest mortality rates among both men and women, making it the primary cause of cancer-related fatalities globally. Estrogen Related Receptor Gamma (ERRγ), a member of the NR3B nuclear receptor subfamily, holds therapeutic promise in various cancer treatments. This study delves into the mechanistic understanding of ERRγ expression in NSCLC cells to gauge its therapeutic potential and examine the anti-tumor impact of combining DN200434 (ERRγ inverse agonist) with MK2206 (Akt inhibitor) in vitro.
Experimental procedures: Expression of ERRγ were investigated in different NSCLC cell lines (A-549, H-1299, H-460) by western blot. Different dose of DN200434 was treatment to study the proliferation effect in NSCLC cells. CCK-assay was used to measure the IC50 value post DN200434 or/and MK2206 in NSCLC cells after 24 hours. NSCLC cells were treated with single drug or in combination to assess their inhibitory effect, apoptosis, cell cycle arrest, colony formation efficiency, migration potential, and spheroid forming ability and the markers of the corresponding events was validated by western blot.
Summary of unpublished findings (Results): ERRγ was found to be overexpressed in the NSCLC cells. Treatment of DN200434 in NSCLC cells inhibit cell proliferation via apoptosis and cell cycle arrest in dose dependent manner. DN200434 in combination with MK-2206 demonstrate synergetic killing effect in NSCLC cells. Combination treatment inhibits cell proliferation via apoptosis and cell cycle-arrest, colony formation efficiency, migration potential, and sphere forming ability of NSCLC cells. This combination not only reduced the toxic concentration of both the drug but also target multiple key driving factors of NSCLC progression. Further, DN200434/MK-2206 reduced the stemness property of NSCLC cell-derived spheroids.
Conclusions: Our present findings thus demonstrate that ERRγ functions as a positive regulator of NSCLC progression and a potential therapeutic target. DN200434, inverse agonist of ERRγ, along with MK-2206 suppresses cancer growth and represses the expression of ERRγ mediated signalling. This combination is certainly providing a new strategy to proceed towards clinical trial.

#1688 CRISPR-Drug combinatorial screening identifies effective combination treatments for *MTAP* null cancer.

Nikola Knoll[1], Sarah Masser[1], Devishi Kesar[2], Eliana Destefanis[1], Guangyan Li[3], Blanka Bordas[1], Jayu Jen[3], Yao He[3], Srivatsan Raghavan[3], William Sellers[3], Kathleen M. Mulvaney[1]

[1]Virginia Polytechnic Institute & State University, Washington, DC,[2]Heidelberg University, Heidelberg, Germany,[3]Broad Institute, Cambridge, MA

Homozygous deletion of *CDKN2A/MTAP* occurs frequently in non-small cell lung cancer and other solid tumors including glioblastoma and pancreatic ductal adenocarcinoma. Lung cancer remains the leading cause of cancer-related mortalities and fewer than 15% of glioblastoma or pancreatic cancer patients survive 5 years underscoring the need for more effective therapies. PRMT5 is a synthetic-lethal dependency in *MTAP* null tumors and an attractive therapeutic target for *CDKN2A/MTAP* deleted cancers. A new revolutionary class of inhibitors, referred to as MTA-cooperative PRMT5 inhibitors, has shown promising results in ongoing early phase clinical trials. Nonetheless, effective cancer treatment typically requires therapeutic combinations to improve response rates and defeat emergent resistant clones. Thus, we sought to determine whether perturbation of other pathways could improve the efficacy of MTA-cooperative PRMT5 inhibitors. Using a paralog and single gene targeting CRISPR library we screened *MTAP* deleted cancers in the presence or absence of PRMT5 inhibitors. We identified several genes sensitizing to PRMT5 inhibition including members of the MAP kinase pathway. We demonstrate that genetic depletion or chemical inhibition of MAP kinase pathway members using KRAS, MEK, ERK, and RAF inhibitors synergize with PRMT5 inhibition to kill *CDKN2A/MTAP* null, RAS-active tumors. Further, MTA-cooperative PRMT5 inhibitors combined with either KRAS or RAF inhibitors led to durable *in vivo* complete responses, emphasizing the potential benefit for patients.

#1689 Targeting PARP to enhance sensitivity to MEK inhibitors in low-grade serous ovarian cancer.

Gamze Bildik[1], Rumeysa Ozyurt[1], Weiqun Mao[1], Marta Llaurado-Fernandez[2], Hannah Kim[2], Olivier De Wever[3], Kwong K. Wong[4], Mark S. Carey[2], **Robert C. Bast**[1], Zhen Lu[1]

[1]Department of Experimental Therapeutics, The University of Texas MD Anderson Cancer Center, Houston, TX,[2]Department of Obstetrics and Gynecology, University of British Columbia, Vancouver, BC, Canada,[3]Department of Human Structure and Repair, Ghent University, Ghent, Belgium,[4]Department of Gynecologic Oncology and Reproductive Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX

Low-grade serous ovarian cancer (LGSOC) presents therapeutic challenges due to its unique characteristics including a distinct genetic profile and high chemoresistance. Up to 40% of cases involve RAS-MAPK pathway mutations, driving cell growth and metabolic dysregulation. MEK inhibitors, such as trametinib and selumetinib, hold therapeutic potential, but their efficacy is often limited by inherent or acquired resistance linked to dysregulated lipid metabolism and ferroptosis. PARP1 has also been implicated in detoxifying phospholipid peroxidation, independent of its DNA repair function. This study explores the combination of MEK inhibitors with olaparib, an FDA-approved PARP inhibitor, to enhance therapeutic efficacy by co-targeting lipid metabolism and ferroptosis, leveraging their complementary mechanisms. We tested a panel of LGSOC cell lines with diverse mutations in KRAS (PM-LGSOC-01, HCC5075, HOC-7, VOA-7681), NRAS (VOA-6406), or with WT RAS (VOA3448). We also used a murine LGSOC cell line (PKOSE10) isolated from a mouse with the $Pten^{fl/fl}$ -$Kras^{G12D/+}$Amhr2-Cre genotype which had developed an LGSOC with peritoneal metastases. Cells were treated with MEKi ± PARPi and drug sensitivity was measured via ATP-based bioluminescence. Synergy was quantified using SynergyFinder Plus (Loewe additivity synergy scoring model). Reverse Phase Protein Array (RPPA) was conducted to confirm ferroptosis and explore whether additional pathways might be involved. The combination of trametinib and olaparib displayed robust synergy, enhancing trametinib efficacy in 5 of 7 cell lines, increasing cytotoxicity, reducing colony formation, and potentiating cell death. Effects were observed regardless of RAS mutation status, though the synergistic magnitude varied. In contrast, selumetinib combined with olaparib showed only additive cytotoxicity in 3 of 7 LGSOC cell lines. Trametinib and olaparib combination treatment induced ferroptosis, as evidenced by the downregulation of GPX4, SLC7A11, and NRF2, alongside increased iron-dependent phospholipid peroxidation. Elevated ROS levels and mitochondrial iron accumulation further indicated a transition to a ferroptosis-sensitive state, driven by reduced expression of antioxidant enzymes. RPPA analysis reinforced these findings, revealing changes in key signaling proteins, including 4EBP-1, FTO, WTAP, Vimentin, Yap, LRP6, Connexin-43, and YTHFD3, highlighting the interactions between epithelial-mesenchymal transition (EMT) pathways and ferroptosis. In conclusion, treatment with the PARP inhibitor olaparib substantially enhances the efficacy of MEK inhibition in LGSOC by inducing ferroptosis, with synergy between olaparib and trametinib. This approach offers a promising strategy to extend clinical benefits, potentially address treatment resistance, and broaden the therapeutic landscape for LGSOC patients with limited options.

#1690 Sulforaphane enhances cytototoxic effects of novel non-thermal plasma and Tirapazamine combined therapy in pancreatic adenocarcinoma cells.

Ishfar Shaan[1], Brandon Gulledge[2], Maksym Poplavskyi[2], Michelle Eubank[1], Dilbar Alseid[2], Anya Weinrieb[3], Gamal Rayan[2], **Shoshanna N. Zucker**[2]

[1]University at Buffalo, Buffalo, NY,[2]D'Youville College School of Pharmacy, Buffalo, NY,[3]Ithaca College, Ithaca, NY

Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal cancers, with a five-year survival rate of only 12% due to late detection and limited treatment options. A promising novel combination therapy of Non-Thermal Plasma (NTP) and Tirapazamine (TPZ) has shown success in treating cancers such as melanomas and glioblastoma. This study investigates the efficacy of the novel NTP+TPZ therapy on premetastatic BxPC-3 and metastatic AsPC-1 PDAC cells in vitro and the effects of adding sulforaphane (SF) as a sensitizing agent to enhance the effectiveness of the combined therapy. NTP produces reactive oxygen and nitrogen species (RONS), which induce oxidative stress and apoptosis, while TPZ, a hypoxia-activated prodrug, causes further DNA damage in tumor cells by acting as a topoisomerase II inhibitor. SF was chosen to act as a sensitizing agent since previous studies suggest SF can upregulate Connexin 43 (Cx43) gap junctions, which is an indicator of reversing EMT phenotype, so we hypothesized SF could enhance the therapeutic efficacy of NTP+TPZ. The cytotoxicity of TPZ was determined for both cell lines, yielding IC50 values of 33μM for BxPC-3 and 101μM for AsPC-1. Cell viability assays showed that NTP+TPZ reduced viability by 68.4% in BxPC-3 and 70.1% in AsPC-1 cells, indicating a synergistic effect. Additional trials with SF added at 10 μM in combination with NTP+TPZ resulted in viability decreasing by 86.7% in BxPC-3 and 97.6% in AsPC-1 cells which is an average additional reduction of 32% in BxPC-3 and 40% in AsPC-1 cells compared to treatments without SF. Western blot analyses of EMT markers vimentin and E-cadherin revealed that while SF alone reduced vimentin expression in AsPC-1 cells, it also decreased E-cadherin expression and did not upregulate Cx43 in any SF concentration. In addition, adding SF to the NTP+TPZ therapy unexpectedly stabilized vimentin and decreased E-cadherin expression across multiple conditions, neither of which suggest reversal of the EMT phenotype to explain the increased cytotoxicity of NTP+TPZ. Overall, the data shows that SF increases the cytotoxicity of NTP+TPZ. However, the Western blots show inconsistent loss of vimentin, consistent loss of E-cadherin, and no upregulation of Cx43. While these results suggest SF has potential as an adjunct agent, further investigation is needed to understand the causes of increased cytotoxicity between SF and NTP+TPZ in pancreatic adenocarcinomas.

#1691 Tumor treating fields (TTFields) sensitize BRCA wild type pancreatic cancer cells to treatment with FOLFIRINOX.

H. Ene, K. Ben Meir, H. Gabay, **R. Engelman**, E. Dor-On, A. Haber, M. Giladi, Y. Palti;
Novocure Ltd, Haifa, Israel

Background: FOLFIRINOX is a first-line chemotherapy treatment for patients with advanced pancreatic cancer and good performance status. This combination regimen of fluorouracil, oxaliplatin, irinotecan and leucovorin seems to be more effective in BRCA-mutated tumors due to the platinum-based component, acting by the induction of DNA damage. Tumor treating fields (TTFields) are electric fields that disrupt cellular processes crucial for cancer cell viability. TTFields have shown efficacy in preclinical pancreatic cancer models, and to reduce expression of proteins from the BRCA-dependent DNA repair pathway in various cancer types. The current study examined the potential use of TTFields to sensitize BRCA wild-type pancreatic cancer cells to treatment with FOLFIRINOX.
Methods: Human pancreatic BRCA wild-type cancer cells BxPC3 and AsPC1 were treated with TTFields, using the inovitro device (Frequency: 150 kHz; Intensity: 0.7 V/cm RMS for BxPC3 cells; 1 V/cm RMS for AsPC1 cells). FOLFIRINOX was administered to the cells at increasing concentrations, with or without co-treatment with TTFields. After 72h of treatment, cell count, colony formation, and apoptosis were measured. For mechanistical insight, RNA was isolated from TTFields-treated and control samples following 24- and 48-hours treatment and examined by RNA-sequencing and real-time PCR. Additionally, protein was isolated from 72-hours treated samples and examined by Western blot.
Results: TTFields application to the pancreatic cells together with FOLFIRINOX reduced cell count and colony formation and induced apoptosis relative to FOLFIRINOX or TTFields alone. TTFields downregulated DNA damage repair, DNA replication, and cell cycle related processes, with reduced expression of several central players in the BRCA DNA damage repair pathway.
Conclusions: Application of TTFields together with FOLFIRINOX exhibits potential improvement relative to FOLFIRINOX alone in treatment of pancreatic cancer cells lacking background BRCA mutations. This outcome may be rationalized based on downregulation of key DNA repair pathways.

#1692 PRMT5 inhibitor displays synergy with PARP inhibitor in bladder urothelial carcinoma models.

Maryam Ghandali[1], Connor Purcell[1], Leiqing Zhang[1], Lanlan Zhou[1], Dinara Ryspayeva[1], Maximilian Pinho-Schwermann[1], Brian K. Baker III[2], Paola Gyuliane[1], Benedito A. Carneiro[1], Wafik S. El-Deiry[1]

[1]Legorreta Cancer Center at Brown University, Providence, RI,[2]Morehouse School of Medicine, Atlanta, GA

Introduction: Approximately 30% of bladder cancers exhibit loss of methylthioadenosine phosphorylase (MTAP), a key enzyme in the methionine salvage pathway. This loss leads to an increase in its substrate, MTA, a metabolite that acts as an endogenous inhibitor of Protein arginine methyltransferase 5 (PRMT5) activity. PRMT5 uses S-adenosylmethionine (SAM) as a methyl donor to methylate various cellular substrates, including transcription factors, thereby regulating gene expression and DNA damage response (DDR) pathway. MTAP-deletion are suggested to be sensitive to PRMT5 inhibitors. We investigated the potential synergy between PRMT5 and PARP inhibitors in models of bladder cancer (BCa) harboring MTAP deletion.
Methods: We investigated the effect of PRMT5 inhibitor AZ14209703 on viability of bladder cancer cell lines (MTAP-deficient: Umuc-3, RT4; MTAP-proficient: 5637, j82) and a patient-derived MTAP-deleted bladder cancer organoid model. Viability assays were performed after 72 hours and 10 days of treatment using the CellTiter-Glo and colony formation assays. Additionally, the synergy between the AZ14209703 and the PARP inhibitor (PARPi) olaparib was assessed through viability testing.
Results: Cell viability assays conducted after 10 days of treatment revealed that MTAP-deficient cell lines (UMUC-3, RT4) are more sensitive to the PRMT5 inhibitor compared to MTAP-proficient cell lines (5637, J82). The 10-day IC50 values, measured by CellTiter-Glo, were as follows: MTAP-deficient cell lines RT4 (100 nM) and UMUC-3 (260 nM), and MTAP-proficient cell lines 5637 (2.24 μM). J82 exhibited even lower sensitivity, with treatment up to 10 μM failing to reduce viability by 50%. Similar results were obtained from colony formation assays, where the maximum dose of 4 μM for 10 days showed comparable outcomes. Additionally, the 10-day IC50 value for patient-derived MTAP-deficient organoids was 90 nM, as determined by CellTiter-Glo. The combination of PRMT5 inhibitor and olaparib demonstrated synergy in both MTAP-deficient and MTAP-proficient cell lines, as well as in patient-derived MTAP-deficient organoid models. This synergy was also observed in MTAP-proficient GBM cell lines (SNB19, LN229), an MTAP-proficient prostate cancer cell line (22RV1), an MTAP-deficient pancreatic cancer cell line (Capan-1), and an MTAP-proficient colorectal cancer cell line (HCT116).
Conclusion: Our results highlight a promising new therapeutic approach for bladder cancer with potential application in the treatment of other cancers. The PRMT5 inhibitor AZ14209703 combined with olaparib offers a novel therapeutic strategy for MTAP-deficient tumors. Our ongoing studies are exploring the mechanisms of synergy and effects of PRMT5 inhibitors and combinations on immune-mediated killing of cancer cells.

#1693 Elraglusib (9-ING-41), a glycogen synthase kinase-3β inhibitor in combination with imipridones for treatment of solid cancer.

Maryam Ghandali, leiqing Zhang, Benedito A. Carneiro, Wafik S. El-Deiry

Legorreta Cancer Center at Brown University, Providence, RI

Glycogen Synthase Kinase-3 (GSK-3) is a serine/threonine kinase with multiple biological roles, including cancer progression, therapeutic resistance and immune regulation. The GSK3β inhibitor elraglusib has shown promising preclinical and clinical activity in multiple tumor types. Previous studies demonstrated that GSK3 inhibitors can sensitize cancer cells to TRAIL-mediated apoptosis and downregulate the NF-κB pathway. Imipridones are a family of small molecule compounds that induce Death Receptor 5 and tumor necrosis factor (TNF)-related-apoptosis inducing ligand (TRAIL), causing cell death in multiple cancer models; the compounds can also inhibit the NF-κB pathway. We hypothesized that combining these two compounds would enhance anti-tumor activity. Ovarian, GBM, pancreatic, and colorectal cancer cell lines were treated with the novel drug combination of elraglusib and imipridone ONC206. CellTiter-Glo viability assays were performed after 72 hours to investigate the combinational treatment synergy. The combination treatment showed synergy in ovarian cancer (TOV21G, A2780, SKOV3), glioblastoma (SNB19, U251, LN229), pancreatic cancer (Capan-1), and colorectal cancer (HCT116) cell lines. In pancreatic and colorectal cancer cell lines, synergy was also investigated and observed between elraglusib and imipridone ONC212. Western blot analysis was performed to investigate the combinatorial effects. Single and combination treatments showed decreased expression of NF-κB p65 , anti-apoptotic proteins MCL-1 and survivin in cancer cells. Our ongoing studies are exploring other potential mechanisms of synergy and effects on immune-mediated killing of cancer cells. Our results show a potentially effective new combination of Elraglusib and Imipridones that can be further developed for cancer treatment.

#1694 Combination of inhibitors of RAD51 and FLT-3 and other tyrosine kinase inhibitors synergistically inhibits proliferation of cultured human leukemia cell lines.

Mark D. Vincent, Peter Ferguson, **Morgan Black**, Jenny Ho, James Koropatnick

London Health Sciences Centre, London, ON, Canada

Ten to 20% of acute myeloid leukemias (AML) are driven by a mutation in FMS-like tyrosine kinase 3 (FLT3), a membrane-based ligand-inducible signal transducer. These FLT-3-mutant AMLs have a poor prognosis and high rate of relapse in spite of the use of inhibitors that specifically target this protein (Cancer Sci. 2019;111: 312-322. doi: 10.1111/cas.14274). However, FLT-3 remains an attractive therapeutic target if new iterations of these drugs are able to circumvent acquired mechanisms of resistance as well as target the wild-type (wt) FLT-3 protein to inhibit tumor growth. Also, chronic myeloid leukemia (CML) in blast crisis continues to have a poor prognosis. IBR2, an inhibitor of the DNA repair protein RAD51, was previously demonstrated to enhance the antiproliferative activity of imatinib against the CML blastoid cell line K562 (EMBO Mol Med 5: 353-365, 2013. DOI 10.1002/emmm.201201760). In our studies, IBR2 enhanced the antiproliferative activity of regorafenib, an inhibitor of multiple tyrosine kinases, in a concentration-dependent manner by up to 80% against K562 cells (J Pharmacol Expt Ther, 364: 46-54, 2018. doi.org/10.1124/jpet.117.241661). JKYN-1-mesylate, a derivative of IBR2, works synergistically with numerous anticancer agents to inhibit proliferation of cell lines derived from a broad range of tumor types (Proc Amer Assoc Cancer Res, 63: Abst. 346, 2022). In an effort to develop a potential drug combination to treat acute leukemias with either mutated or wt FLT-3 we tested combinations of JKYN-1-mesylate and FLT-3 inhibitors against FLT-3-wt HL-60 and FLT-3-mutant MV-4-11 AML cell lines. MV-4-11 cells were 100- to 1000-fold more sensitive to quizartinib and gilteritinib than HL-60. Proliferation of both cell lines (4 days, alamarBlue™ assay) was inhibited by monotherapy JKYN-1-mesylate with IC_{50} values in the range of 3 to 5 μM. The combination of JKYN-1-mesylate and quizartinib synergistically inhibited proliferation of HL-60 cells with an apparent decrease in the IC_{50} value of quizartinib by up to 80%. However, inhibition by the combination of JKYN-1-mesylate and gilteritinib was only additive in this FLT-3-wt cell line. As well, JKYN-1-mesylate was at best additive with quizartinib or gilteritinib against FLT-3-mutant MV-4-11. These findings suggest that: (1) the combination of quizartinib and a RAD51-inhibitor could be a useful treatment against FLT-3-wt AML; (2) quizartinib and gilteritinib may not act in exactly the same way against the FLT-3 target or other related targets; (3) novel combinations of a RAD51 inhibitor with non-traditional treatments for CML may provide a potential improvement in outcome for CML blast crisis; (4) RAD51 inhibitors may be active as monotherapy in myeloid leukemias. The combination of JKYN-1-mesylate with FLT-3 inhibitors under current development is being tested.

#1695 Paricalcitol plus hydroxychloroquine enhances gemcitabine activity and induces mesenchymal to epithelial transition in pancreatic ductal adenocarcinoma.

G. P. Nagaraju[1], M. S. Saddala[2], S. Sarvesh[1], D. R. Bandi[1], A. M. Khaliq[3], A. Masood[3], M. Akce[1], B. F. El-Rayes[1];
[1]University of Alabama at Birmingham, Birmingham, AL, [2]University of California at Irvine, Irvine, CA, [3]Indiana University, Bloomington, IN

Epithelial mesenchymal transition (EMT) describes a process by which the epithelial cells acquire mesenchymal properties associated with increased migration, invasion, and resistance to therapy. Targeting the intercellular communications and molecular changes that enable EMT is a rational therapeutic approach in PDAC. Our group has previously reported on KPC-Luc PDAC orthotopic mouse models treated with 1. Sham, 2. paricalcitol plus hydroxychloroquine (PH), 3. gemcitabine (G), and 4. GPH. Single cell RNA sequence (scRNASeq) was performed and analyzed using hdWGCNA (high dimensional Weighted Gene Co-expression Network Analysis) and GSEA (Gene Set Enrichment Analysis) to plot and determine intracellular interactions as well as gene expression changes in various treatment conditions. GPH treatment reduces EMT, which is associated with the downregulation of the essential gene fibronectin (*Fn1)*. *Collagen* and *Fn1* pathways co-expression decreases in GPH-treated KPC-Luc tumors. Cancer-associated fibroblasts (CAFs) appear dominant in Collagen signaling, whereas macrophages mediate Fn1 signaling. GPH treatment reduces the expression interaction strength between ligands and receptors (Collagen-Integrin and Fn1-Cd44 or Fn1-Sdc4) compared to sham, PH, and G. This comprehensive study of cell-cell communication and molecular changes during therapy offers a deep understanding of the molecular underpinnings of PDAC-EMT and metastasis and identifies possible targets for GPH.

#1696 Combination therapy of cell cycle inhibitor and HDAC inhibitor induces metabolic alterations in pancreatic cancer.

Shraddha P. Bhutkar, Anjali Yadav, Vikas V. Dukhande

St. John's University, Queens, NY

Pancreatic ductal adenocarcinoma (PDAC), characterized by several multigene mutations, is one of the lethal solid cancers accounting for several drug treatment challenges. NCI statistics show that the overall 5-year survival rate of PDAC remains at 12.8%. The tumor aggressiveness and invasive-metastatic potential of the cancer, along with drug resistance issues, demand the development of novel and effective multi-drug therapies. Our initial results show that combination therapy of a cell cycle inhibitor Abemaciclib (Abe), and histone deacetylase inhibitor Panobinostat (Pan) effectively decreases pancreatic cancer cell proliferation and growth and causes apoptotic cell death. Cell cycle and epigenetic changes drive tumor progression by rewiring metabolic signaling pathways of pancreatic cancer cells. Therefore, we hypothesized that co-treatment of pancreatic cancer with Abe and Pan modulates cancer cell metabolism, developing energy imbalance leading to cell death. Our Seahorse metabolic experiments depicted a reduced oxygen consumption rate upon combination treatment in pancreatic cancer cells with upregulation of extracellular acidification rate. We also performed metabolomic profiling upon combination treatment in pancreatic cancer cells, revealing alterations in the levels of metabolites involved in carbohydrate, lipid, and amino acid metabolism in the combination treatment group compared to control pancreatic cells. We also observed a moderate increase in mitochondrial membrane potential upon combination treatment, suggesting altered mitochondrial metabolic signatures compared to control and individual drugs along with combination treatments. These results suggest that mitochondrial dysfunction may underlie the Abe and Pan combination treatment, impairing metabolic homeostasis and energy balance. Our future studies include deciphering the in-depth mechanism of alterations in mitochondrial dynamics and metabolic signaling pathways upon combining cell cycle and HDAC inhibitor treatment in pancreatic cancer cells by performing transcriptomic profiling on PDAC cells and *in vivo* xenograft studies.

This study is funded by the College of Pharmacy and Health Sciences at St. John’s University, New York, and is also supported by the National Institute of General Medical Sciences (Award Number R16GM145557).

#1697 Chemotherapy combined with fatty acid oxidation inhibition completely regressed tumor growth in a pancreatic cancer model.

S. Woo, **C. Kim**, W. Ham, J. Kang, M. Kang, W. Hong, J. Park, W. Choi, S. Kim;
National Cancer Center - Korea, Goyang-si, Gyeonggi-do, Korea, Republic of

Purpose: We found that most ATP production in cancer cells depends on fatty acid oxidation (FAO). Most chemotherapeutic reagents including FOLFIRINOX regimen targeted anabolic metabolism of cancer cells such as DNA replication. All synthesis mechanisms require ATP for chemical reactions. Therefore, we proposed that blocking FAO together with chemotherapeutics may synergize anti-cancer effect by suppressing cancer growth metabolism.

Experimental Design: To block FAO, CAC (carnitine-acylcarnitine carrier, gene: *SLC25A20*) in the mitochondria inner membrane and ACAA1 (acetyl-CoA acyltransferase1, gene: *ACAA1*) in the peroxisome were targeted for inhibition. KN510 was selected for CAC inhibition, and KN713 was selected for ACAA1 inhibition. To achieve maximum anti-cancer effect of FAO inhibition, KN510 and KN713 were combined for the treatment as KN510713. Anti-cancer effect was examined by xenograft model using human pancreatic cancer cells. Synergy of KN510713 with chemotherapeutic drugs also tested using a xenograft model.

Results: Chemotherapy combined with KN510713 showed synergistic anti-cancer effect on 11 cancer types in in vitro analysis. Furthermore, xenograft pancreatic cancer showed completely suppressed effect of KN510713 treated with chemotherapeutics such as irinotecan only or FOLFIRINOX regimen.

Conclusion: FAO inhibition stopped tumor growth by ATP depletion in pancreatic cancer. The changes in molecular signaling upon FAO inhibition revealed that ATP levels are a key regulator of all signaling. This suggests that whatever the signaling induced by the growth receptor, ATP down-regulation through FAO inhibition may provide a novel therapeutic effect. Thus, combinations of chemotherapeutic agents with FAO inhibitors have shown excellent anti-cancer activity in mice.

#1698 Synergy with decitabine expands the utility of talazoparib: A novel therapeutic strategy for BRCA-proficient pancreatic cancer.

Zachery Keepers[1], Brandon Carter-Cooper[2], Hurley Ryan[1], Stephen B. Baylin[3], Rena Lapidus[2], Feyruz V. Rassool[1], Hem D. Shukla[1], Aaron T. Ciner[2]

[1]Radiation Oncology, Univ. of Maryland School of Medicine, Baltimore, MD,[2]Medicine, Univ. of Maryland School of Medicine, Baltimore, MD,[3]Oncology, The Johns Hopkins School of Medicine, The Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD

Background: Patients with pancreatic ductal adenocarcinoma (PDAC) have a 5-year survival rate of 13%. In those with unresectable disease, standard chemotherapy offers limited benefit, with a median survival between 8 and 12 months. Approximately 17% of patients with PDAC carry a germline or somatic mutation in the BRCA or PALB2 genes. These patients may benefit from poly (ADP-ribose) polymerase inhibitors (PARPi), which induce synthetic lethality in tumors with homologous recombination deficiency. The POLO trial showed that maintenance olaparib - a PARPi - doubled progression-free survival in patients with germline BRCA mutations and metastatic PDAC. We've previously reported that DNA methyltransferase inhibitors (DNMTi) can induce a "BRCAness" phenotype in other types of cancer, thus sensitizing BRCA-proficient cancer cells to PARPi. Here we explore the synergistic potential of PARPi talazoparib (TAL) and DNMTi decitabine (DAC) in pancreatic cancer cell lines and patient-derived pancreatic tumor organoids (PTOs).
Methods: Synergy studies with TAL and DAC were performed in two murine (Panc02 and KPC) and three human (Capan1, SW1990, and MIA PaCa-2) pancreatic cancer cell lines with diverse genetic profiles. Of note, Capan1 harbored a BRCA2 mutation. Combination TAL+DAC treatment was performed in a dose-dependent and fixed ratio scheme, and the various degrees of synergy were determined using Chou-Talalay mathematical modeling (Compusyn). Clonogenic assays were performed with murine cell lines. Three patient-derived PTOs were treated with single agent (TAL or DAC) or combination (TAL+DAC) regimens and response was determined by MTS assay. Methylomic profiles including gene level methylation and hydroxymethylation fraction in treated and untreated PTOs were acquired using Biomodal's Duet Multiomics Assay. Statistical analyses for clonogenic and MTS assays were performed using GraphPad Prism.
Results: A synergistic inhibitory effect was observed in all pancreatic cancer cell lines when treated with TAL+DAC. The Capan1 cell line was more sensitive to TAL, as expected. Clonogenic survival assays showed a statistically significant decrease in colony numbers after treatment with the combination regimen compared to single-agent treatment. All PTOs exhibited growth inhibition when treated with DAC alone, whereas TAL alone had limited effects. The combination of TAL and DAC at low doses was more effective than either agent alone. Methylomic analysis revealed decreased methylation of key genes in DAC-treated PTOs, validating DAC's mechanistic effects.
Conclusions**:** The combination of TAL and DAC demonstrates synergistic cytotoxicity in pancreatic cancer cell lines and tumor organoids, suggesting potential for this treatment strategy in BRCA-mutated and BRCA-proficient PDAC.

#1699 A study on prediction model of drug sensitivity using patient-derived organoids.

Rie Hayashi, Norikatsu Miyoshi, Rie Mizumoto, Kengo Haruna, Shiki Fujino, Mitsunobu Takeda, Yuki Sekido, Tsuyoshi Hata, Atsushi Hamabe, Takayuki Ogino, Mamoru Uemura, Yuichiro Doki, Hidetoshi Eguchi

Department of Gastroenterological Surgery, Osaka University, Graduate School of Medicine, Suita, Japan

Background: Cancer treatment is increasingly based on precision medicine, which tailors therapies to the genetic and biological characteristics of a patient's tumor. PDOs offer a promising platform for predicting drug sensitivity, helping to identify effective therapies. However, existing models still face limitations in replicating clinical tumor heterogeneity and predicting drug responses accurately. In this study, we developed a novel two-dimensional (2D) culture system (2DO) for CRC PDOs that improves reproducibility and better mirrors the tumor microenvironment compared to traditional methods.
Purpose: The goal of this study is to evaluate the effectiveness of PDO-based drug sensitivity assays in predicting clinical outcomes for CRC patients. We aim to explore the potential of PDO assays in personalized treatment strategies, improving CRC treatment outcomes.
Methods: Tumor samples from CRC patients were obtained via surgery or endoscopic biopsy. These tumors were washed, dissociated, and filtered to isolate viable tumor cells, which were then cultured as PDOs under optimized conditions that preserve the tumor's genetic and phenotypic characteristics. Drug sensitivity assays were performed by treating PDOs with different chemotherapeutic agents and measuring cell proliferation using a method: the Incucyte system (SARTRIUS, Germany) for live-cell imaging.
Results: Drug sensitivity assays using PDOs showed promising results. The Incucyte system had a positive predictive value (PPV) of 75% and a negative predictive value (NPV) of 33% when compared to clinical outcomes. This result suggests that PDOs are useful for predicting tumor response to chemotherapy, though improving the NPV is necessary for more reliable predictions. The combination of imaging and biochemical assays offered a comprehensive evaluation of drug effects, and the PDOs closely mimicked patient-specific treatment responses.
Conclusion: This study demonstrates the potential of PDO-based drug sensitivity assays as a tool for personalized treatment in colorectal cancer. While promising, further refinement is needed, particularly in improving the negative predictive value, to optimize PDO models for clinical use. PDO-based assays may help bridge the gap between laboratory findings and real-world therapeutic outcomes, contributing to more individualized cancer treatments in the future.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Drug Resistance in Molecular Targeted Therapies 2
Poster Session

#1700 Valproic acid potentiates the efficacy of anti-EGFR monoclonal antibodies in sensitive and resistant RAS/BRAF wild type colorectal cancer models.

Roberta Affatato[1], Elena Di Gennaro[1], Alessandra Leone[1], Biagio Pucci[1], Susan Costantini[1], Rita Lombardi[2], Eleonora Perfetto[1], Maria Serena Roca[1], Francesca Bruzzese[2], Francesca Collina[3], Claudia Cardone[4], Tania Moccia[1], Elena Sinopoli[5], Antonio Avallone[4], Alfredo Budillon[6]

[1]Dept of Translational research-Experimental Pharmacology Unit, National Cancer Institute-IRCCS-Fondazione Pascale, Naples, Italy,[2]Dept of Translational research-Experimental Animal Unit, National Cancer Institute-IRCCS-Fondazione Pascale, Naples, Italy,[3]Pathology Unit, National Cancer Institute-IRCCS-Fondazione Pascale, Naples, Italy,[4]Experimental Clinical Abdominal Oncology, National Cancer Institute-IRCCS-Fondazione Pascale, Naples, Italy,[5]Scientific Directorate-Monitoring and quality assurance of research Unit, National Cancer Institute-IRCCS-Fondazione Pascale, Naples, Italy,[6]Scientific Directorate, National Cancer Institute-IRCCS-Fondazione Pascale, Naples, Italy

Background.Colorectal cancer (CRC) is a major global health issue, with fewer than 20% of metastatic CRC (mCRC) patients surviving beyond 5 years. Although, monoclonal antibodies (MoAbs) targeting EGFR, such as cetuximab or panitumumab (PAN), combined with chemotherapy, are standard treatments in RAS/BRAF wild type (wt) mCRC patients, their efficacy is limited by intrinsic and acquired resistance mechanisms. Here, we evaluated a novel therapeutic strategy, based on the repurposing of valproic acid (VPA), an anticonvulsant drug with histone deacetylase inhibitor and anticancer properties, in combination with anti-EGFR MoAbs, with or without irinotecan (IRI)-based chemotherapy, in CRC models.
Methods.The antitumor effect was assessed in a panel of RAS/BRAF wt CRC cell lines, sensitive, intrinsically or acquired resistant to anti-EGFR MoAbs, by evaluating clonogenic growth, apoptosis and tumor spheroids formation. Acquired resistant models were characterized using biological assays and multi-omics approaches (proteomics, phosphoproteomics and metabolomics).
Results.PAN-resistant models exhibited significantly higher proliferation rates in both 2D and 3D cultures, as well as increased migration compared to parental cells. An integrated multi-omics analysis revealed that secondary resistance to PAN was associated with metabolic alterations, extracellular matrix remodeling, impaired DNA damage repair pathways and alterations of growth factor receptors signaling. Interestingly, the combination of VPA and PAN with or without IRI-based chemotherapy induced synergistic antiproliferative and pro-apoptotic effects across all tested cell lines, including PAN-acquired resistant models. Mechanistically, VPA/PAN treatment led to a significant downregulation of pEGFR (Tyr1068) and its downstream signaling molecules, including pMAPK and pAKT, key drivers of anti-EGFR resistance, confirming multiomic data. *In vitro* and *in vivo* experiments are underway to confirm the ability of VPA to enhance anti-EGFR efficacy, overcome, delay or prevent resistance and provide further insights into the underlying molecular mechanisms. Based on these evidences, we launched the randomized phase 2 trial in mCRC patients in a third-line rechallenge setting (VICTORIA trial). In details, RAS/BRAF ctDNA wt patients are randomized to receive either standard rechallenge with IRI and PAN alone (ARM A) or in combination with VPA (ARM B). In patients in ARM A, at time of disease progression, a second study will explore if the addition of VPA to IRI and PAN might revert the resistance occurrence.
Conclusions.Overall, our findings propose a novel therapeutic strategy for the management of mCRC patients that could optimize the treatment of RAS/BRAF wt mCRC patients and expand the continuum of care in later treatments lines.

#1701 Investigating trastuzumab-deruxtecan (DS-8201) resistance mechanisms using clinically relevant PDX models and combination strategies with anti-PD-1 antibody.

Diandong Jiang[1], Bin Xie[1], Yulan Tang[1], Hongkui Chen[1], Xin Hou[1], Cui Xu[1], Dan Shao[1], Xinlei Chen[1], Xiaorong Gu[1], Loc Van[2], Josh Caggiula[2], **Danyi Wen**[1]

[1]Shanghai LIDE Biotech Co., Ltd., Shanghai, China,[2]LIDE Biotech Co., Ltd., Doylestown, PA

Background: Trastuzumab-Deruxtecan (T-Dxd, DS-8201), an antibody-drug conjugate (ADC) targeting HER2, is clinically approved for cancer patients with high and low HER2 expression levels; however, the response rate to DS-8201 are modest, especially in gastric cancer and the mechanisms of resistance remain elusive. In this study, we aimed to address this gap by developing clinically DS-8201 resistant patient derived xenograft (PDX) models in gastric and breast cancer. Our objectives were to elucidate possible mechanisms of resistance with this topoisomerase inhibitor-linked HER2 ADC and explore whether combining DS-8201 with an anti-PD-1 antibody could overcome resistance by using a humanized mouse model.

Method: Humanized mice were established by injecting human CD34 positive hematopoietic stem cells into NCG mice, followed by inoculation with PDX tissues from DS-8201-resisatnt gastric or breast cancer patients. Once tumors reached approx.150-200mm^3, the mice were administered a single dose of DS-8201, pembrolizumab (twice weekly for 4 weeks) and a combination. Tumor samples were harvested 24h after the final dose, fixed with 10% NFB overnight, and subjected to IHC staining with antibodies targeting CD8, CD4 and HER2 to assess changes in the tumor microenvironment. Furthermore, DNA and RNA were extracted from tumor samples using the K-Cell® kit for subsequent whole-exome sequencing (WES) and RNA sequencing (RNAseq) analysis, respectively.

Results: the data revealed limited tumor inhibition in DS-8201 treated group, consistent with clinical findings. Elevated expression of ATP-binding cassette (ABC) transporters, known as drug efflux pumps, was observed in this group, suggesting a potential strategy for overcoming resistance by targeting these transporters. Concurrent administration of pembrolizumab enhanced the efficacy of DS-8201, leading a significant inhibition in tumor growth compared to both the vehicle and monotherapy arms. An increase in CD3+ T cells in blood and CD8+/CD4+ T cell ratio was observed in the combination therapy group, suggesting modulation of the tumor microenvironment.

In conclusion, the establishment of novel gastric and breast PDX cancer models, derived from patients exhibiting resistance to DS-8201 in clinical settings, provided a valuable approach for investigating mechanisms of resistance in HER2 targeted ADCs. Our findings suggest that combining pembrolizumab with ADC may represent a promising strategy for overcoming resistance by modulation of tumor-infiltrating lymphocytes.

#1702 OBI-992, a novel TROP2 antibody-drug conjugate, exhibits distinct resistance profiles compared to other TROP2 targeting ADCs.

Lifen Shen, Tzer-Min Kuo, Yu-Hsuan Tsao, **Ya-Chi Chen**, Ming-Tain Lai

Biology Discovery, OBI Pharma, Inc, Taipei, Taiwan

BACKGROUND/AIM: Trophoblast cell surface antigen-2 (TROP2), a transmembrane glycoprotein often overexpressed in a variety of cancers, has been proposed as a potential cancer driver in colorectal cancer (CRC) cells. As a result, several antibody-drug conjugates (ADC) have been developed targeting TROP2, such as Sacituzumab Govitecan (SG) and Datopotamab Deruxtecan (Dato-Dxd). One of the major challenges in the development of ADC is the emergence of resistance via different mechanism of actions. This study aims to characterize the potential resistant mechanisms of TROP2 ADCs, including a novel TROP2 ADC OBI-992 in CRC cells.
METHODS: DLD1 CRC cells resistant to OBI-992, SG, and Dato-Dxd analogs were established with sequential increase of respective ADC concentrations. Cytotoxicity of three ADCs and their payloads, including exatecan, SN-38, and Dxd, was evaluated to assess the fold of resistance. Cell surface and total TROP2 protein were measured by flow cytometry and western blotting. DNA mutation and gene expression profiling were conducted by whole exome sequencing (WES) and RNA sequencing (RNA-seq).
RESULTS: TROP2 reduction and multidrug resistance proteins (MRPs) enhancement were not detected in OBI-992-induced resistant cells. Notable different DNA mutations in ATRX, BRCA2, and PALB2 of OBI-992 resistant cells were detected compared to those found with Dato-Dxd and SG-selected resistant cells. A TOP1 inhibitor-resistant mutation, TOP1 R364H, was identified as 100% variant allele frequency (VAF) in Dato-Dxd and SG resistant cells, whereas lower percentage of VAF was observed in OBI-992 resistant cell lines (29 % and 48.6 % in 0.5 uM and 2uM of OBI-992, respectively). OBI-992 resistant cells showed a clear divergence in differential expression gene (DEG) profiles, whereas Dato-Dxd and SG resistant cells were similar. c-MET and REG4 levels were greatly increased in cells with Dato-Dxd and SG resistance but not with OBI-992. Extracellular matrix (ECM) receptor interaction pathway was enriched in OBI-992 resistant cells.
CONCLUSION: OBI-992, a novel TROP2 targeting ADC, emerges as a potential promising therapeutic option for patients who are resistant to other TROP2 targeting ADCs such as SG and Dato-Dxd. OBI-992 exhibits distinct resistance profiles compared to other TROP2 targeting ADCs. Understanding of potential mechanisms of resistance can provide potential combination options for TROP2-targeting ADCs to combat the drug resistance.
Keywords: OBI-992, TROP2, antibody-drug conjugate, Colon cancer, resistance

#1703 Molecular drivers of enzalutamide resistance in prostate cancer: insights into CYP3A5 and associated pathways.

Imran Khan Mohammed, Ranjana Mitra

Roseman Univ. of Health Sciences, Las vegas, NV

Prostate cancer (PC) disproportionately affects Black/African American (AA) men, who experience higher incidence rates, more aggressive disease, and worse outcomes compared to non-Hispanic White Americans (NHWA). CYP3A5, a cytochrome P450 enzyme, is expressed at higher levels in individuals carrying the *1 variant, prevalent in AAs, compared to the *3 variant, more common in NHWA. Elevated CYP3A5 expression in AAs has been associated with aggressive prostate cancer phenotypes, potentially due to its role in activating androgen receptor (AR) signaling and promoting crosstalk with Wnt signaling pathways. Resistance to AR-targeted therapies, such as enzalutamide, remains a major challenge in metastatic castration-resistant prostate cancer (mCRPC), particularly in aggressive disease subtypes.

To investigate mechanisms underlying enzalutamide resistance, we generated resistant PC cell lines of NHWA origin (low CYP3A5-LNCaP) and AA origin (high CYP3A5-MDAPCa2b). In resistant MDAPCa2b cells, RT2 androgen receptor profiler arrays revealed upregulation of MME, SGK1, SNAI, and STEAP, genes implicated in Wnt pathway regulation (SGK1), epithelial-to-mesenchymal transition (SNAI), and enhanced proliferation and migration (STEAP). Additionally, tumor suppressors and regulatory genes, including ADAMTS1, KLK4, MT2A, NDRG1, and TIPARP, were significantly downregulated, suggesting reduced ECM remodeling, oxidative stress responses, and DNA repair capacity in resistant cells. Importantly, CYP3A5 expressions were significantly elevated in resistant MDAPCa2b cells but remained unchanged in resistant LNCaP cells, highlighting a potential link between high CYP3A5 expressions and resistance mechanisms.

The upregulation of SGK1, SNAI, and STEAP, alongside the downregulation of ADAMTS1, KLK4, MT2A, NDRG1, and TIPARP, suggests that resistance to enzalutamide involves AR and Wnt signaling, EMT, and loss of tumor suppressive functions. These findings support the hypothesis that the *1 CYP3A5 variant in AAs drives aggressive disease and therapeutic resistance, providing a molecular basis for observed clinical disparities and potential pathways for targeted intervention.

#1704 Overcoming resistance in HER2-positive gastric and breast cancers: Efficacy of disitamab vedotin in preclinical models resistant to trastuzumab emtansine and trastuzumab deruxtecan.

Negar Pourjamal[1], Vadim Le Joncour[1], Gyorgy Vereb[2], Cilla Honkamaki[1], Jorma Isola[3], Jeffrey V Leyton[4], Pirjo Laakkonen[1], Heikki Joensuu[5], Mark Barok[1]

[1]Faculty of Medicine, University of Helsinki, Helsinki, Finland,[2]Faculty of Medicine, University of Debrecen, Debrecen, Hungary,[3]Faculty of Medicine, University of Tampere, Tampere, Finland,[4]Faculty of Medicine, University of Ottawa, Ottawa, QC, Canada,[5]Oncology, Helsinki University Hospital, Helsinki, Finland

BACKGROUND: HER2-positive gastric and breast cancers often develop resistance to the approved antibody-drug conjugates (ADCs) trastuzumab emtansine (T-DM1) and trastuzumab deruxtecan (T-DXd). Disitamab vedotin (DV), a novel anti-HER2 ADC, binds to a distinct epitope on HER2 compared to trastuzumab. This study investigated the therapeutic potential of DV in preclinical models of gastric and breast cancer, including those resistant to T-DM1 and T-DXd and explored whether combining DV with these ADCs could enhance anti-tumor efficacy.

METHODS: We evaluated the efficacy of DV, T-DM1, and T-DXd individually and in combination using AlamarBlue cell proliferation assays in HER2-positive gastric and breast cancer cell lines, including models resistant to T-DM1 and T-DXd. We assessed the in vivo efficacy of DV in SCID mouse xenograft models of gastric and breast cancer that had progressed on T-DM1 and/or T-DXd. We examined dual combinations of the three ADCs in xenograft models to evaluate potential synergistic effects.

RESULTS: DV demonstrated significant anti-tumor activity in both in vitro and in vivo models, including cell lines and xenografts that were resistant to T-DM1 and T-DXd. In xenograft models, DV effectively inhibited tumor growth in cancers that had progressed following treatment with T-DM1 and/or T-DXd. Notably, combining DV with either T-DM1 or T-DXd resulted in superior anti-tumor effects compared to the use of single agents alone, both in cell culture and in xenograft studies.

CONCLUSIONS: This study highlights the effectiveness of DV in overcoming resistance to T-DM1 and T-DXd in preclinical models of HER2-positive gastric and breast cancer. The combination of DV with these ADCs demonstrated enhanced efficacy, underscoring the potential of dual ADC strategies to improve outcomes in HER2-positive cancers.

#1705 MUC1-C is a target for antibody-drug conjugate treatment of MRTX1133-resistant KRAS G12D mutant pancreatic ductal adenocarcinomas.

Hiroki Ozawa, Keisuke Shigeta, Shinkichi Takamori, Atrayee Bhattacharya, Donald Kufe

Dana-Farber Cancer Institute, Boston, MA

Background: The *MUCIN 1* (MUC1) gene evolved in mammals to protect barrier tissues from biotic and abiotic insults. *MUC1* encodes a transmembrane MUC1-C subunit that is activated in response to the loss of homeostasis and promotes proliferative, inflammatory, and remodeling responses necessary for wound repair. However, in contrast to its protective function, prolonged activation of MUC1-C by chronic inflammation drives cancer progression. In cancer cells, MUC1-C forms complexes with receptor tyrosine kinases (RTKs), such as EGFR, at the cell membrane, contributing to downstream RTK→RAS signaling pathways. Previous research identified MUC1-C addiction in KRAS G12D mutant pancreatic ductal adenocarcinoma (PDAC) cells, against which there are limited therapeutic options. Treatment of KRAS G12D mutant PDACs has been advanced by development of the MRTX1133 inhibitor; however, resistance to this agent invariably occurs by unclear mechanisms.

Results: We report that treatment of PDAC KRAS G12D cells with the MRTX1133 inhibitor is associated with marked induction of cell surface MUC1-C expression. Our results demonstrate that MRTX1133 activates an inflammatory MUC1-C/NF-κB p65 auto-inductive pathway. We further demonstrate that MRTX1133-induced MUC1-C/NF-κB signaling (i) regulates the gasdermin E (GASDME) pore-forming protein that paradoxically protects PDAC cells from pyroptosis, (ii) drives ZEB1 and the epithelial-mesenchymal transition (EMT), and (iii) is necessary for MRTX1133 resistance. These results indicate that MUC1-C is a target for the treatment of MRTX1133 resistant PDAC tumors. There are presently no effective antibody-drug conjugates (ADCs) for PDAC treatment. In extending our results, we show that an anti-MUC1-C ADC is highly effective against MRTX1133-resistant PDAC KRAS G12D cells and that targeting MUC1-C with the ADC is synergistic with MRTX1133 in vitro. We also demonstrate that the anti-MUC1-C ADC inhibits growth of PDAC MRTX1133 resistant tumor xenografts and PDX models.

Conclusions: These findings indicate that MUC1-C confers resistance of PDAC KRAS G12D mutant cells to MRTX1133 and identify MUC1-C as a target for ADC treatment of patients refractory to MRTX1133 treatment.

#1706 Establishment and characterization of a panel of ovarian XPDX models from patients with clinical resistance to anti-folate receptor therapies.

Alyssa Simonson[1], Johnnie Flores[1], Anna Stackpole[1], Jennifer Garcia[1], Crystal Moreno[1], Gabriella Graciano[1], Erika Mata[1], Danielle Trevino[1], Rodolfo Castellanos[1], Sarah Lopez[1], Leatrice Wilson[1], Maria Jose de Miguel[2], Sreenivasa Chandana[3], Andrew Cunningham[1], Michael J. Wick[1]

[1]XenoSTART, San Antonio, TX,[2]START Madrid, Madrid, Spain,[3]START Midwest, Grand Rapids, MI

Background: Folate receptor α is an actionable therapeutic target in cancer. Mirvetuximab (MIRV) is an antibody-drug conjugate (ADC) consisting of a humanized anti-folate receptor alpha (FRα) IgG1 antibody conjugated to soravtansine (DM4) recently approved for treatment of FRα-positive, platinum refractory ovarian cancer. MIRV has been shown effective; however, patients that progress on this therapy often have few remaining treatment options. To better understand mechanisms of resistance to FRα-targeting therapies, we established four XPDX models from three ovarian cancer patients who progressed clinically on MIRV or farletuzumab, a humanized anti-FRα antibody. These models were characterized by DNA and RNA sequencing, folate receptor expression, and in vivo sensitivity to various therapies including MIRV.

Methods: Four post-FRα ovarian XPDX models were established and characterized: ST409 was from a 59-year-old Hispanic female pretreated with chemotherapy and eleven months of farletuzumab; ST6205B and ST6205D were collected two months apart from a 74-year-old Hispanic female pretreated with chemotherapy, four months of niraparib, and seven months of MIRV; STM476 was from a 65-year-old Caucasian female pretreated with chemotherapy, eleven months of rucaparib, and two months of MIRV. Models were passaged, banked, and profiled using genomic analysis, including WES and RNA_{seq}, receptor expression, and in vivo drug sensitivity studies. For in vivo studies, each model was tested against cisplatin, a PARP inhibitor, and MIRV. In vivo study endpoints included tumor volume and time from treatment initiation with %T/C values and tumor regression reported at study completion; a %T/C of ≤ 20% versus control was considered sensitive.

Results: All models were positive for FRα staining with values of 2+ or 3+. Genomic characterization identified a $BRCA1^{L1254X}$ truncation in ST409 and an RB1 frameshift in ST6205B and ST6205D; TP53 mutations were identified in all models. In vivo, ST409 was found sensitive to single agent cisplatin (%T/C<0%) but resistant to MIRV (%T/C=73%), while STM476 was resistant to cisplatin (%T/C=41%) and MIRV (%T/C=78%). ST6205B and ST6205D were resistant to cisplatin and MIRV (%T/C>30%); no model was sensitive to PARPi at the evaluated treatment regimens.

Conclusion: We have established and characterized a panel of ovarian XPDX models from patients with clinical resistance to anti-folate receptor therapies. These models can be utilized as a valuable tool in better understanding mechanisms of resistance to FRα-targeting therapies and in developing novel therapies for patients who progress clinically on MIRV or similar agents.

#1707 Characterization of egfr ectodomain mutation in acquired resistance to cetuximab in colorectal cancer.

Neeraj Joshi, Hidenori Tanaka, Galina Bogatcheva, Ramona Graves Deal, Claudia Wahoski, Sarah Harmych, Claiborne Tydings, Marisol Ramirez, James Higginbotham, Maria J. Irudayam, Jeffrey L. Franklin, Qi Liu, Jens Meiler, Robert J. Coffey, Eben L. Rosenthal, Bhuminder Singh

Vanderbilt University Medical Center, Nashville, TN

Colorectal cancer (CRC) remains the second leading cause of cancer-related deaths in the United States (US), and individuals with surgically unresectable stage four CRC have a five-year overall survival of 13%. Targeting of the receptor tyrosine kinase (RTK), EGF receptor (EGFR) with therapeutic monoclonal antibodies (cetuximab, panitumumab) is approved for use in wild-type KRAS/NRAS/BRAF (RAS/RAF) advanced CRC. However, these antibodies as a single agent have response rates of 12-17% and expected progression-free survival of only 3-4 months. Accordingly, resistance may be present at the outset (de novo resistance) or develop during treatment (acquired resistance). To model and understand drug resistance, we cultured CRC lines in a defined 3D matrix (type 1 collagen) with anti-epidermal growth factor receptor (EGFR) antibody cetuximab. Here, we report the identification of an EGFR ectodomain missense mutation acquired *in vitro* in response to cetuximab treatment. Using 2D and 3D drug sensitivity assays, we demonstrate that this EGFR ectodomain mutation, in addition to being resistant to cetuximab and panitumumab, also could not be targeted by the oligoclonal anti-EGFR antibody cocktail MM-151. To determine whether this EGFR ectodomain mutation is necessary and sufficient to impart cetuximab resistance, (i) we engineered stable cell lines that inducibly overexpress mutant EGFR and (ii) demonstrated the sufficiency of mutant EGFR overexpression in conferring cetuximab resistance under both *in vitro* and in nude mouse xenografts. Further, our structural, functional, and biochemical studies implicate reduced binding of EGFR mutant to cetuximab and sustained downstream signaling activity as a cause of cetuximab resistance. Collectively, this mutation necessitates additional treatment strategies for a subset of subjects with advanced CRC who do not respond to treatment with current anti-EGFR biologics.

#1708 CKD-702, a novel bispecific antibody, enhances anti-tumor effect of combination with EGFR TKIs in NSCLC preclinical model.

Mi Jin Yoon, Soon-Ki Hong, Gun-Woo Park, Kyung-Woo Lee, Sunhong Kim, Young-Shin Kwak, Ju-Hee Lee

CKD Research Institute, Yongin-si, Korea, Republic of

The development of drug resistance is a major cause of poor prognosis and therapeutic failure in NSCLC. The mutual synergism between cMET and EGFR is known to promote acquired drug resistance, which complicates effective treatment strategies. In NSCLC, cMET gene amplification is reported in 2-4% of previously untreated patients and in 5-20% of patients with EGFR mutations and acquired resistance to EGFR TKIs. This crosstalk between the cMET and EGFR pathways underscores the need for dual inhibition as a promising treatment for NSCLC patients, especially those with acquired cMET activation. cMET, a proto-oncogene encoding the RTK HGFR, plays a crucial role in cell proliferation, motility, and survival. Aberrant activation of cMET through gene amplification, mutation, or overexpression has been implicated in the development of various cancers, including NSCLC. EGFR, another critical RTK, is frequently mutated in NSCLC, leading to uncontrolled cell growth and survival. The interplay between cMET and EGFR signaling pathways facilitates the emergence of resistance to EGFR TKIs, a common occurrence in NSCLC patients undergoing treatment. This resistance often results from secondary mutations in the EGFR gene, such as T790M and C797S, or from activation of alternative signaling pathways, including cMET amplification. CKD-702 is a bispecific antibody specifically designed to target both cMET and EGFR. It is a tetravalent IgG1-like bispecific antibody, in which a single-chain variable fragment specific to EGFR is genetically fused to the C-terminus of a conventional IgG1 of cMET. This innovative design allows CKD-702 to bind with high affinity to the extracellular domains of both cMET and EGFR, effectively inhibiting their signaling by blocking ligand binding. Additionally, CKD-702 induces the internalization and degradation of both receptors, thereby further inhibiting their activity and downstream signaling pathways. This study aims to evaluate the efficacy of combination therapy with EGFR TKIs and CKD-702 for overcoming resistance in NSCLC, particularly in EGFR exon 20 insertion mutant models and triple mutant models, including the C797S mutation. The combination therapy demonstrates superior efficacy compared to monotherapy in both cellular and animal models, effectively inhibiting signaling pathways and tumor growth. These findings are promising for addressing the significant challenge posed by the development of resistance to EGFR TKIs in the treatment of NSCLC. A Phase I study of CKD-702, which began in 2020, is currently ongoing. Current clinical trials of CKD-702 monotherapy show promising results, with combination therapy being considered to address resistance mechanisms in NSCLC.

#1709 Cyanobacteria-derived RNA aptamer supports prostate cancer hormone sensitivity.

Carlos David Cruz-Hernandez, Bethany Smith, Sandrine Billet, Manish Thiruvalluvan, Gabrielle Gonzalez, Neil Bhowmick

Medicine, Cedars-Sinai Medical Center, Los Angeles, CA

Prostate cancer (PCa) hormone therapy resistance is the second leading cause of cancer death among American men. Multiple mechanisms inherent to the disease can lead to castrate-resistance, including those that mediate lineage plasticity. We have reported that inhibiting AR signaling can increase glutamine (L-Gln) secretion by cancer-associated fibroblasts (CAF). L-Gln-free media significantly limits PCa cell growth. However, targeting L-Gln metabolism or transporters provides limited benefit. On the other hand, non-metabolizable L-Gln analogues like 6-Diazo-5-oxo-l-norleucine (DON) and DRP104 have shown promising antitumor effects. We reasoned that extracellular interruption of L-Gln uptake could be a therapeutic strategy that bypasses metabolic adaptation of a specific enzyme or transporter, especially if it could be tumor-selective. To target L-Gln, we explored cyanobacteria-structured non-coding RNA riboswitches, which are typically associated with metabolite-binding for gene regulation. Riboswitches in cyanobacteria that sense L-Gln through an aptamer motif, glnA, were chosen due to their stability and high specificity. Since L-Gln concentrations in bacteria can be higher than those in the tumor microenvironment, we optimized an RNA aptamer binding affinity through structural analysis, computer modeling, and in vitro and in vivo testing. The optimized aptamer depleted L-Gln from PCa cells by both L-Gln sequestration and the recruitment of extracellular glutaminase activity, serving as an allosteric activator. Glutamine depletion reduced FOXM1 transcriptional occupancy on the FGF8 promoter, known mediator of PCa castrate resistance. Further tumor targeting was achieved by functionalizing gold nanoparticles with the aptamer. The L-Gln-depleting aptamer, with demonstrated serum stability, limited the proliferation, and lineage plasticity of the castrate resistant prostate tumors alone and in combination therapy with AR antagonists, enzalutamide and apalutamide in subcutaneous and orthotopic mouse models. A point mutation in the binding pocket of the 56mer rendered the aptamer ineffective in L-Gln binding, FGF8 regulation, and had a limited impact on tumor growth. Superiority of the functionalized nanoparticle targeting was demonstrated in an orthotopic PCa model over the un-targeted aptamer in reducing tumor weight. The studies demonstrated elevated L-Gln can be necessary and sufficient for castrate resistance, as its depletion sensitizes prostatic tumors to AR inhibition.

#1710 Advancing HER2-targeted ADC drug discovery through resistant cell line generation and cancer cell panel analysis.

Lili Chai, Taotao Cao, Xue Yang, Wei Liu, Zhengtai Li, Xiaojian Wang, **Tj (Tiejun) Bing**

ICE Bioscience, Inc., Beijing, China

Antibody-drug conjugates (ADCs) represent a cutting-edge class of targeted cancer therapeutics, seamlessly integrating the high specificity of monoclonal antibodies with the cytotoxic power of chemotherapy agents. This strategic union facilitates the precise delivery of a potent cytotoxic payload to HER2-expressing cancer cells, where HER2 is a well-established therapeutic target. Despite the promising efficacy of HER2-directed ADCs in treating HER2-positive breast cancer, the emergence of resistance in tumor cells poses a significant obstacle to their therapeutic potential.

The present study is designed to meticulously assess the anti-tumor efficacy of HER2-targeted ADCs across a diverse panel of cancer cell lines exhibiting varying levels of HER2 expression. Furthermore, to delve into the complexities of resistance, we have developed in vitro models of resistance by subjecting cells to cyclical exposure to DS-8201, a leading HER2-targeted ADC, and its payload, Dxd. Our findings reveal a pronounced inhibitory effect of HER2-targeted ADCs on HER2-positive cancer cell lines, underscoring their selective cytotoxicity. A critical insight from our research is the observation of a significant reduction in HER2 protein levels in cell lines that have developed resistance. This decrease is hypothesized to be a key factor in the resistance mechanism, potentially by limiting the availability of the target for ADC binding. The elucidation of these resistance mechanisms is pivotal, as it informs the strategic development of next-generation HER2-targeted therapies. By addressing the issue of resistance, we aim to enhance the efficacy of HER2-targeted treatments and ultimately improve patient outcomes in the battle against breast cancer.

#1711 Genomic and transcriptomic mediators of resistance to antibody-drug conjugates (ADCs) in metastatic breast cancer (MBC): A comprehensive multi-center study.

Samer Alkassis[1], Marla Lipsyc-Sharf[1], Catherine Hegarty-Traverso[2], Jacob Mercer[2], Binyam Yilma[2], Stamatina Fragkogianni[2], Yuan Yuan[3], Funda Meric-Bernstam[4], Hope Rugo[5], Joyce O'Shaughnessy[6], Mothaffar Rimawi[7], Rachel Schiff[7], David Elashoff[1], John Glaspy[1], Martina McDermott[1], Amy Cummings[1], Calvin Chao[2], Aditya Bardia[1]

[1]UCLA Health Jonsson Comprehensive Cancer Center, Los Angeles, CA,[2]Tempus AI, Inc., Chicago, IL,[3]Cedars Sinai Medical Center, Los Angeles, CA,[4]MD Anderson Cancer Center, Houston, TX,[5]UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA,[6]Baylor University Medical Center, Texas Oncology, Sarah Cannon Research Institute, Dallas, TX,[7]Baylor College of Medicine, Baylor College of Medicine, Lester and Sue Smith Breast Center and Dan L Duncan Comprehensive Cancer Center, Houston, TX

ADCs have revolutionized the therapeutic landscape in oncology. Three ADCs are US FDA-approved in MBC: sacituzumab govitecan (SG), trastuzumab deruxtecan (T-DXd) and trastuzumab emtansine (T-DM1), with many others in development. Despite these advances, ADC resistance mechanisms remain unknown. To discern biomarkers of therapeutic resistance, we evaluated genomic and transcriptomic differences in MBC before and after ADC treatment (tx).

We analyzed next-generation sequencing (NGS) data from patients (pts) in the Tempus Database that received SG, T-DXd or T-DM1 pre or post biopsy (bx) (n_{SG}=157, $n_{T\text{-}Dxd}$=120, $n_{T\text{-}DM1}$=84; 32.4% TNBC, 27.1% HR+/HER2-, 20.2% HER2+, 20.2% NOS). Pre-tx groups included pts with bx collected < 1 year before or < 15 days after the ADC start date. Post-tx groups included pts who had bx collected < 3 months (mo) after ADC end date. Acquired resistance (AcqRes) was analyzed by comparison of pre- and post-tx bx for pts treated with ADC for > 3 mo. Primary resistance (PrRes) was defined as pre- and post-tx bx for pts treated with ADC for < 3 mo. We evaluated resistance based on ADC mechanisms of action (MoA), including genes related to antigen expression, processing mechanism, payload effect and efflux pumps. Somatic differences were compared using Pearson's Chi-squared test or Fisher's exact test, as appropriate. In this exploratory analysis, all p-values are reported as uncorrected. The median log2(TPM) gene expression was compared between groups using the Wilcoxon rank sum exact test.

In the AcqRes cohort, there were no significant differences in mutation frequency or expression of the target antigen for pre/post-SG or T-DXd bx, though in the pre- vs post-T-DM1 bx there was a decrease in *ERBB2* alterations (69% vs 40%, p=0.047) and expression (10.9 vs. 8.4, p=0.024). A trend toward higher expression across efflux pump genes was seen in pts with AcqRes for SG (*ABCB1*: 2.7 vs 3.4, p=0.08; *ABCC2*: 2.4 vs 3.4, p=0.2) and T-DXd (*ABCC1*: 6.3 vs. 6.7, p=0.016; *ABCB1*: 2.77 vs. 3.44, p=0.4), with mixed changes in T-DM1 (*ABCB1*: 3.3 vs. 2.7, p=0.08; *ABCC1*: 6.3 vs. 6.8, p=0.05; *ABCC2*: 3.19 vs. 3.18, p=0.2). A trend of higher efflux pump gene expression was associated with PrRes to SG and T-DXd. Overall, there were no significant differences in expression of genes associated with ADC processing or DNA damage repair, though rare mutations in *TOP1* and *ATM* were associated with PrRes to T-DXd.

Genomic and transcriptomic analysis identified potential mechanisms of AcqRes and PrRes to SG and T-DXd, including higher drug efflux pump expression. This might be related to the ADC MoA, particularly the payload release and bystander effect. Additional research is needed to validate these novel findings and the molecular underpinnings mediating resistance to ADCs.

#1712 CD36 enrichment in HER2+ mesenchymal stem cells potentiates therapy refractoriness in HER2+ breast cancer.

Lorenzo Castagnoli[1], Valeria Cancila[2], Martina Bigliardi[1], Matteo Dugo[3], Paola A. Corsetto[4], Claudia Chiodoni[1], Viola Regondi[1], Michela Francesconi[1], Antonino Belfiore[5], Andrea Vingiani[6], Giancarlo Pruneri[7], Francesca Ligorio[8], Elda Tagliabue[1], Claudio Tripodo[9], Claudio Vernieri[10], Tiziana Triulzi[1], **Serenella M. Pupa**[1]

[1]Department of Experimental Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy,[2]Tumor Immunology Unit - Department PROMISE, Universita di Palermo, Palermo, Italy,[3]Department of Medical Oncology, IRCCS Ospedale San Raffaele, Milan, Italy,[4]Department of Pharmacological and Biomolecular Science "Rodolfo Paoletti", Universita degli Studi di Milano, Milan, Italy,[5]Department of Diagnostic Pathology and Laboratory Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy,[6]Department of Diagnostic Pathology and Laboratory Medicine/ Department of Oncology and Hemato-Oncolo, Fondazione IRCCS Istituto Nazionale dei Tumori/ Universita degli Studi di Milano, Milan, Italy,[7]Department of Diagnostic Pathology and Laboratory Medicine/ Department of Oncology and Hemato-Oncolo, Fondazione IRCCS Istituto Nazionale dei Tumori/ Universita degli Studi di Mialano, Milan, Italy,[8]Medical Oncology Department/the AIRC Istitute of Molecular Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori/IFOM ETS, Milan, Italy,[9]The AIRC Institute of Molecular Oncology, IFOM ETS, Milan, Italy,[10]Medical Oncology Department/The AIRC Istitute of Molecular Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori/IFOM ETS, Milan, Italy

Approximately 15-20% of breast cancers (BCs) are characterized by amplification/overexpression of HER2. Although the introduction of anti-HER2 drugs has improved the prognosis of HER2-positive (HER2+) BC patients, a considerable number of tumors fail to respond to or acquire resistance to targeted therapies, highlighting the need for new strategies. Emerging evidence shows that the reprogramming of fatty acid (FA) metabolism plays a key role in the aggressiveness and therapeutic resistance of HER2+ BC. HER2+ BC has been defined as a "lipogenic disease" due to the functional reciprocal crosstalk occurring between HER2-mediated oncogenic signaling and fatty acid (FA) biosynthesis via fatty acid synthase activity. Once lipid biosynthesis is inhibited by anti-HER2 agents, cancer cells could enhance their ability to uptake extracellular FAs to guarantee their supply. In this context, the functional role of the reprogramming of CD36-mediated FA uptake in HER2+ BC progression remains unclear. Our study provides an in-depth molecular, biological and functional characterization of the transmembrane FA transporter CD36 in epithelial (MET-like)- and mesenchymal (EMT-like) HER2+ breast cancer stem cell (CSC) subsets, selected HER2+ BC cell lines and patients treated with neoadjuvant trastuzumab. We reveal consistent enrichment of CD36 in HER2+ EMT-like CSCs from all tested preclinical and clinical therapy-refractory BC models. Notably, we demonstrate the functional interplay occurring between Wnt signaling regulation/activation and CD36 enrichment in HER2+ BC cells. Dual blockade of CD36 and HER2 increases the anti-CSC efficacy of anti-HER2 drugs. Additionally, expression of CD36 in intratumor HER2+ mesenchymal CSCs is significantly associated with resistance to trastuzumab in HER2+ BC patients. These results support the critical role of CD36-mediated FA uptake in HER2+ therapy-refractory BC. Our study provides evidence that targeting CD36 might be an effective therapeutic strategy for this malignancy. (Supported by AIRC)

#1713 Disrupting ER-AR crosstalk using AR degraders: a novel approach to treating endocrine resistant breast cancer.

Matt Konda, Obdulia Covarrubias-Zambrano, Stefan H. Bossmann, Joan Lewis-Wambi

University of Kansas Medical Center, Kansas City, KS

Background: Estrogen receptor positive (ER+) breast cancer accounts for approximately 70% of all breast cancer cases and is the most diagnosed subtype amongst women. Current treatments include aromatase inhibitors (AIs), which block the biosynthesis of estrogens, tamoxifen (ER antagonist), and fulvestrant (ER degrader) (FUL). Despite therapeutic effectiveness, 30% of patients develop resistance, necessitating a deeper understanding of the underling mechanisms. Recent evidence points to the upregulation of androgen receptor (AR) signaling pathway as a potential mechanism of acquired resistance in breast cancer. To address the role of ER/AR crosstalk in acquired endocrine resistance, we generated an AI-resistant breast cancer cell line, MCF-7:5C (5C), mimicking the resistance seen in patients undergoing AI treatment.
Methods: The inhibitory effect of FUL and ARCC4 (AR PROTAC degrader) on AI-resistant 5C and parental MCF-7 cells was examined using CellTiter-Blue® Cell Viability assay and trypan blue cell counting. Western blot, immunofluorescence, and nuclear fractionation assays were performed to determine ER and AR protein expression and cellular localization in response to FUL and ARCC4 treatments. siRNAs were used to knockdown ER and AR expression in resistant 5C cells to assess functional significance. Additionally, flow cytometry analysis using the RhodB-PTP (10mg/mL) was used to probe for surface expression of plectin in resistant cells.
Results: AI-resistant 5C cells expressed markedly elevated levels of ER and AR and were more sensitive to the inhibitory effects of FUL and ARCC4 compared to parental MCF-7 cells. Immunofluorescence and nuclear fractionation assays revealed predominant nuclear localization of ER and AR in 5C cells compared to MCF-7 cells, and both proteins were markedly reduced (>95%) by FUL and ARCC4 treatments and siRNAs targeting both ER and AR. Notably, flow cytometry data revealed a strong correlation between ER, AR, and surface Plectin in 5C cells, thus identifying an additional target in these cells.
Conclusions: Plectin is a cytoskeleton linker protein found in non-transformed cells, however, evidence suggests that its cancer-specific mislocalization to the cell surface contributes to its oncogenic function. As such, therapeutic targeting of surface plectin, along with ER/AR using FUL and ARCC4, represents an attractive opportunity to impede tumorigenesis and possible reverse endocrine resistance in breast cancer.

#1714 Understanding ADC resistance using preclinical enhertu-induced resistant models.

Hongyan Sun[1], Yuan Fang[1], Fang Zhu[1], Jianming Xu[1], Xiang Gao[1], Jun Wang[2]

[1]GemPharmatech Co., Ltd, Nanjing, China,[2]GemPharmatech Co., Ltd, San Diego, CA

Antibody-drug conjugates (ADCs) combine the specificity of monoclonal antibodies (mAbs) with potent cytotoxic payloads, offering a promising approach for targeted cancer therapy. Enhertu (trastuzumab deruxtecan, DS-8201), an ADC targeting HER2-positive cancers, has demonstrated remarkable efficacy in clinical settings, setting new benchmarks for anti-HER2 ADCs. However, drug resistance remains a significant challenge, underscoring the need to elucidate resistance mechanisms and develop strategies to overcome them. To address this important question, we established a robust preclinical platform to study Enhertu resistance by subjecting HER2-positive JIMT-1 xenograft tumors to a series of *in vivo* passaging and drug induction. Massive parallel sequencing analyses, including RNA sequencing (RNA-Seq) and whole-exome sequencing (WES), revealed multiple resistance-associated pathways, with a particular emphasis on payload-resistance mechanisms. Flow cytometry and IHC analyses confirmed no significant changes in HER2 expression levels between resistant and parental tumors, ruling out target downregulation as the main resistance mechanism. Notably, the resistant tumor provided a valuable platform for studying interactions within the tumor microenvironment (TME), including stromal and immune components that may contribute to resistance development.

The establishment of these preclinical Enhertu-resistant models offers a unique opportunity to investigate resistance mechanisms that may not yet be observed in clinics. Furthermore, these models provide a foundation for testing innovative therapeutic strategies, such as combination treatments targeting multiple pathways or the development of next-generation ADCs with improved payload efficacy. By enhancing our understanding of ADC resistance, these findings contribute to the advancement of personalized cancer therapies and may ultimately improve outcomes for patients with HER2-positive malignancies.

#1715 Novel patient-derived organoids (PDOs) models for studying HER2-positive endometrial cancer.

Natalia Bednarek[1], Anh Q. Nguyen[2], Julieta Barroeta[3], Benedikt Nagele[4], Subin Myong[1], Katherine Kurnit[2], Diane Yamada[2], Gini Fleming[2], Sridevi Challa[2]

[1]The University of Chicago Medicine Comprehensive Cancer Center, Chicago, IL,[2]Obstetrics and Gynecology, The University of Chicago Medicine, Chicago, IL,[3]Pathology, The University of Chicago Medicine, Chicago, IL,[4]The University of Chicago, Chicago, IL

Endometrial carcinoma (EC) is the most common cancer of the reproductive organs in women with 66,200 new cases and 13,030 deaths in United States in 2023. Recent clinical trials are evaluating the use of antibody-drug conjugates (ADCs), such as trastuzumab-deruxtecan (T-Dxd) for the treatment of solid tumors, including EC. T-Dxd consists of anti-HER2 antibody (trastuzumab) and topoisomerase I inhibitor (deruxtecan). The mechanism of action of T-Dxd involves the HER2-directed cytotoxic effect induced by deruxtecan, as well as the bystander killing effect when deruxtecan is released into neighboring cells. This dual mode of action makes T-Dxd a suitable candidate for the treatment of both HER2-high and HER2-low cancers.

Patient-derived organoids (PDOs) have emerged as models for studying tumor biology and screening for new drugs. As opposed to regular 2D cultures, organoids provide a more accurate representation of the key features and therapeutic response of patient tumors. The primary goal of our project is to generate endometrial cancer PDOs to test the efficacy of new combination treatments including T-DXd.

We have successfully generated several organoid lines from endometrioid and clear cell cancer tissues using patient samples obtained from surgeries. Established organoids were submitted for pathological review and genetic testing which demonstrated that tissue morphology and genetic alterations of PDOs strongly correlate with patient tumors. Immunohistochemical analysis has shown that T-DXd treatment of the organoids causes a decrease in HER2 levels and an increase in pERK1/2 levels. We also treated the HER2-positive organoids with the combination of T-DXd and MEK inhibitors and monitored changes in growth. We observed decreased growth of organoids after combination treatment compared to untreated control.

In conclusion, we successfully generated PDOs models of endometrial tumors and confirmed the response to single-agent and combination treatments. Obtained organoid lines can be further utilized to study tumor biology and test new combinations of drugs.

#1716 Acquired cell adhesion molecule 1 (CADM1) ADC resistance model for optimizing payload efficacy and toxicity in osteosarcoma preclinical testing.

Zhongting Zhang, Wendong Zhang, Xin Zhou, Caterina Longo, Yifei Wang, Dongxue Mao, Yanhua Yi, Zhaohui Xu, Zhao Li, Simon Olivares, Sylvester Jusu, Harjeet Singh, Michael Roth, Jonathan Gill, Bissan Al-Lazikani, Richard Gorlick

UT MD Anderson Cancer Center, Houston, TX

Instruction: Antibody-drug conjugates (ADCs) are a rapidly advancing therapeutic approach. A proof of principle CADM1 ADC has shown significant efficacy in osteosarcoma preclinical studies; however, its activity may be compromised by inherent or acquired resistance during prolonged treatment. In this study we generated osteosarcoma models resistant to a CADM1 ADC to define the underlying molecular mechanisms.

Methods: The antitumor efficacy of CADM1 ADC was validated in vivo in six osteosarcoma patient-derived xenografts (PDXs) at a 3 mg/kg dose. To model acquired resistance, osteosarcoma PDXs were subjected to three cycles of treatment with a low-dose (0.5 mg/kg) CADM1 ADC. Tumor samples were harvested from pre-treated controls and post-treatment resistant PDXs. These samples were analyzed using immunohistochemistry (IHC), flow cytometry, whole-genome sequencing (WGS), and bulk RNA sequencing (RNA-seq). Resistant PDXs were further tested with two alternative ADCs: one using the same payload but targeting a different antigen and another using the same target antigen but with a different payload. IC_{50} values were determined in pre-treated and resistant PDX-derived cells to evaluate cytotoxicity.

Results: After three cycles of low-dose CADM1 ADC treatment, PDX models exhibited a gradual decline in tumor regression, indicating the development of resistance. IHC and flow cytometry analyses demonstrated variable CADM1 expression between pre-treatment and resistant samples, with only some tumors exhibiting decreased target expression. Whole-genome sequencing (WGS) revealed increased gene copy number gains in resistant tumors compared to controls. RNA-seq analysis identified significant changes in gene expression, including reduced CADM1 expression in some samples and upregulation of resistance-associated pathways. Notably, the multidrug resistance-associated protein (MRP) transporter *ABCC3* was significantly overexpressed in resistant tumors compared to pre-treatment samples. Cytotoxicity assays further confirmed resistance, with resistant PDX-derived cells exhibiting higher IC_{50} values compared to pre-treated cells, indicating reduced sensitivity to CADM1 ADC.

Conclusions: CADM1 ADC demonstrates potent antitumor activity in osteosarcoma PDX models but resistance can develop. Future studies will delve deeper into protein localization and post-translational modifications as well as single cell genomics to better understand the mechanisms of resistance.

#1717 Establishment and characterization of enzalutamide resistant prostate CDX models.

Haiting Dai, Jie Yang, Xiu Fan, **Wen-Jen Yu**

ICE Bioscience INC, Beijing, China

Prostate cancer (PCa) is common and mostly androgen dependent cancer occurred in men and ranked fifth of the causes of death over the world, reduction the serum level of androgen become the priority therapy method for treating PCa patients. Although the first generation of anti-androgen receptor (AR) new drugs such as flutamide, nilutamide, and bicalutamide were launched few years ago and lighted new hopes for treatment of androgen dependent PCa, drug resistance was developed quickly and push the second generation of anti-AR drugs such as enzalutamide, apalutamide, and darolutamide to the market by their high specific binding to AR and better drug efficacy. Despite new anti-AR drugs exerted better efficacy, drug resistance still hampered the treatment of PCa patients, new challenge was burst for anti-PCa drugs through AR pathway. In this study, we developed an enzalutamide resistant C4-2B cell line in vitro by escalated the enzalutamide concentration gradually. The phenotype and genotype of C4-2B cell line were checked while enzalutamide concentration was increased. After 6 months of induction and identification, C4-2B enzalutamide resistant cell line was established and showed 140 times of drug-resistant index compare to that of parental cell. The in vivo drug-resistant efficacy was also confirmed in C4-2B enzalutamide resistant (C4-2B Enz-R) CDX models using 30 mpk enzalutamide through PO administration for 28 days (TGI=8.37%, T/C=93.22%) compared to the enzalutamide efficacies on LNCAP and 22RV1 CDX models. Western blot for androgen receptor expression level in tumor tissues and ELISA assay for prostate specific androgen (PSA) level in serum are highly correlated to the tumor volume of C4-2B Enz-R CDX models. The well characterized novel C4-2B Enz-R cell line and CDX model were appropriate for new drug discovery of anti-PCa drugs through AR pathway in vitro and in vivo.

#1718 Role of IKKε in resistance to second-generation hormone therapy in prostate cancer.

Fayrouz Annab, Mariana Llasera, Benjamin Peant, Anne-Marie Mes-Masson, Francis Rodier, Fred Saad

Centre de Recherche du Centre Hospitalier de L'universite de Montreal, Montreal, QC, Canada

Introduction: Prostate cancer is the most commonly diagnosed cancer and the third leading cause of cancer-related death among men in Canada. Patients with castration-resistant prostate cancer (CRPC) are treated with various agents, including Enzalutamide, a second-generation hormone therapy that improves overall survival. However, in the long term, all patients eventually develop resistance. This highlights the critical need to better understand the mechanisms of Enzalutamide resistance in mCRPC and to identify new therapeutic strategies to improve patient outcomes. IκB kinase-epsilon (IKKε), a member of the IKK protein family, is involved in the inflammatory response and the inflammation phenotype of rheumatoid arthritis. IKKε has been identified as an oncogene, and deregulation of its expression has been associated with prostate cancer progression. Preliminary experiments conducted in our laboratory have shown an increased expression level of IKKε in Enzalutamide-resistant cell lines. We previously demonstrated that IKKε inhibitors, such as Amlexanox, reduce the proliferation of CRPC cells.
Hypothesis: We hypothesize that inhibiting IKKε using Amlexanox restores sensitivity to Enzalutamide in Enzalutamide-resistant prostate cancer cells. Our overarching goal is to characterize the role of IKKε in the development of Enzalutamide resistance and to evaluate the therapeutic potential of the Amlexanox-Enzalutamide combination to treat CRPC patients.
Methodology and Results: We obtained Enzalutamide-resistant and -sensitive CR cell lines derived from LNCaP. Cells have been treated with an increased dose of Amlexanox and Enzalutamide for 7 days to calculate the IC_{50} of each drug for each cell line. Based on these IC50, we performed real-time imaging assays using different dose combinations to determine the additive or synergistic effect of Amlexanox and Enzalutamide cotreatment. In addition, we confirmed IKKe and AR inhibition using Amlexanox and Enzalutamide, by Western blot assays. We also looked for the expression of apoptosis and senescence markers to determine the behavior of each studied PC cell line in response to treatments. Finally, we started Bulk RNA barcoding and sequencing (BRB-seq) assays on all LNCaP-derived cell lines treated with Enzalutamide and Amlexanox, alone or in combination.
Conclusion: We aim to develop a combination therapy that can restore sensitivity to Enzalutamide, improve patients' quality of life, and more effectively control disease progression.

#1719 Constitutive aryl hydrocarbon activity promotes resistance to bortezomib which is exacerbated by leptin and potentially involves Src phosphorylation by JAK2 in prostate cancer cells.

Kofi K. Khamit-Kush[1], Daniel J. Walters[2], Nathan J. Bowen[1], Jeffrey A. Handy[3], Joann B. Powell[1]

[1]Biological Sciences, Clark Atlanta University, Atlanta, GA,[2]Biological Sciences, Morehouse School of Medicine, Atlanta, GA,[3]Biological Sciences, Morehouse College, Atlanta, GA

The aryl hydrocarbon receptor (AhR) plays a role in regulating diet-induced obesity and is a nuclear transcription factor involved in the transcriptional regulation of target genes, such as enzymes of xenobiotic metabolism (CYP1, UGTs) or genes involved in the transcriptional regulation. Induced enzymes facilitate the metabolism of various endogenous compounds and drugs, such as bortezomib. We report that the constitutive activity of AhR promotes drug resistance which is exacerbated in the presence of leptin and potentially involves AhR-mediated Src phosphorylation by JAK2 in a prostate cancer in vitro model. Leptin is known to contribute to cancer progression and has an emerging role in chemoresistance. Of note, both leptin receptor and aryl hydrocarbon receptor are overexpressed in prostate cancer cell lines. Additionally, our lab previously reported an integrative bioinformatics analysis using TCGA data (n=498) which revealed a positive correlation in AHR and LEPR mRNA expression in patients with prostate cancer. Our in vitro results also show AhR protects androgen-insensitive C4-2 cells but not LNCaP cells from apoptosis induced by anti-androgens, suggesting AhR activity leads to androgen independence and potentially multi-class chemoresistance. Further investigation into AhR as a drug target in obesity-associated conditions for the treatment of advanced prostate cancer upon entering the phase of drug resistance coupled with failure of first-line androgen deprivation therapy.

#1720 IGFBP3 signaling promotes resistance to next-generation antiandrogens therapeutics in advanced prostate cancer.

Amy Rose Leslie[1], Shu Ning[1], Masuda Sharifi[1], Zachary A. Schaaf[1], James P. Maine[1], Wei Lou[1], Chengfei Liu[1], Alan P. Lombard[2], Allen C. Gao[1]

[1]Urologic Surgery, UC Davis, Davis, CA,[2]Biochemistry and Molecular Medicine, UC Davis, Davis, CA

Background: Next-generation antiandrogen therapeutics (NGATs) have irrefutably improved the survival of prostate cancer patients. However, treatment resistance to NGATs remains a clinical challenge for men with castration-resistant prostate cancer (CRPC). The molecular mechanisms underlying resistance to NGAT remain confounding. Insulin growth factor binding protein 3 (IGFBP3) is associated with prostate cancer tumorigenesis and progression. It is known that IGFBP3 induces sphingosine kinase (SphK1) activation subsequently inducing sphingosine-1-phosphate (S1P) production. Previously, we found that IGFBP3 is necessary for PARP inhibitor resistance in acquired resistant models (OlapR cells). We hypothesize that IGFBP3 is required for acquired and intrinsic NGAT resistance.

Methods: Enzalutamide-resistant (MDVR), Abiraterone-resistant (AbiR), Apalutamide-resistant (ApalR), and Darolutamide-resistant (DaroR) C4-2B subline cells exhibit stout NGAT resistance compared to C4-2B parental cells. In addition, this study also utilized 22Rv1 cells that display intrinsic resistance to Enzalutamide. We performed transcriptomic analysis on enzalutamide-treated patients' cohorts and the enzalutamide-resistant prostate cancer cell lines. RNAseq, real time qPCR, immunoblot, and ELISA were used to determine IGFBP3 expression and secretion levels. Cell viability was determined in resistant cell lines after silencing IGFBP3 and SphK1 using siRNA or inhibiting SphK1 using SphK1 inhibitor, SKI-II.

Results: This study found that IGFBP3 was upregulated in NGAT resistant models (MDVR, AbiR, ApalR, DaroR, and 22Rv1 cells) using transcriptomics. We found that NGAT resistant models highly express IGFBP3 and SphK1 using RNA-seq, qPCR, and immunoblot. SphK1 is a lipid kinase that phosphorylates sphingosine to form S1P metabolite which promotes tumor survival, proliferation, and resistance. We discovered high levels of IGFBP3 and S1P secretion in the resistant models. We found that inhibition of IGFBP3/SphK1 decreased both SphK1 expression and S1P secretion. Mechanistically, IGFBP3 activated the Sphingolipids pathway to promote cell survival and subsequently enforce the growth factor signaling to enhance cell proliferation. Ablating the expression of IGFBP3 promotes the formation of ceramide which induces the apoptotic cascade.

Conclusion: We demonstrated that increased IGFBP3 expression by NGAT treatment leads to increased sphingolipid metabolism by simultaneously demoting ceramide-induced apoptosis and fostering S1P production through SphK1 activation. Thus, IGFBP3 induces the activation of SphK1 to hijack the sphingosine metabolism to fuel NGAT resistance. Our findings demonstrate that targeting the IGFBP3/SphK1 signaling axis enhances our ability to resensitizes resistant CRPC to NGAT therapeutics.

#1721 AXL signaling promotes adaptive resistance to HER2 tyrosine kinase inhibitors in HER2 aberrant tumor cells.

M. Ishida[1], T. Yamada[1], Y. Katayama[1], K. Yasumoto[2], K. Takayama[1];

[1]Department of Pulmonary Medicine, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan, [2]Department of Medical Oncology, Kanazawa Medical University, Uchinada, Ishikawa, Japan, Kanazawa, Japan

Introduction:Human epidermal growth factor receptor 2 (HER2) is an oncogene target in various cancers, including lung, stomach, and breast cancers. Several HER2-tyrosine kinase inhibitors (TKIs) have been developed for clinical use in patients with tumors exhibiting HER2 aberrations. However, achieving complete tumor remission remains challenging. Therefore, identifying optimal combined therapeutic approaches with HER2-TKIs is crucial for improving clinical outcomes. This study focuses on elucidating the adaptive resistance mechanisms to HER2-TKIs and the therapeutic strategies needed to overcome this resistance.
Methods:We conducted in vitro and in vivo experiments using HER2-aberrant tumor cell lines to evaluate the adaptive responses to HER2-TKIs. The involvement of AXL signaling was examined using MTT assays, western blotting, qPCR, and flow cytometer.
Results:We showed that adaptive resistance to HER2-TKIs in HER2-aberrant tumors is mediated by AXL signaling, activation of which is induced by its ligand GAS6 production. Inhibition of AXL reduced the viability of HER2-aberrant tumor cells that overexpress AXL by enhancing apoptosis and cell cycle arrest induced by HER2 inhibition. This process contributed to adaptive resistance to HER2-TKIs in HER2-aberrant cancers through the Shc1BP-Shc1 axis, one of the adaptor proteins. HER2-TKIs induced the dissociation of ShcBP1 from Shc1, promoted the binding of Shc1 to AXL, and triggered the translocation of ShcBP1 to the nucleus, which is associated with the cell cycle. In xenograft models of AXL-overexpressing HER2-aberrant tumors treated with HER2-TKIs, initial combination therapy with an AXL inhibitor significantly reduced tumor size and delayed tumor regrowth compared to HER2-TKI monotherapy.
Conclusion:These findings highlight the critical role of the GAS6-AXL signaling axis in intrinsic resistance to HER2-TKIs and the potential benefits of its inhibition in overcoming this resistance.

#1722 Targeting synaptotagmin 4 to overcome enzalutamide and abiraterone resistance in castration resistant prostate cancer.

Masuda Sharifi, Shu Ning, Amy Rose Leslie, Zachary A. Schaaf, James Patrick Maine, Wei Lou, Allen C. Gao

Urologic Surgery, UC Davis, Sacramento, CA

Background: Castration-resistant prostate cancers (CRPCs) are predominantly driven by androgen receptor (AR) signaling. Although next-generation anti-androgen therapies (NGATs), such as enzalutamide (Enza) and abiraterone (Abi), have been shown to extend patient survival, resistance to these therapies often develops. The underlying mechanisms driving resistance to these treatments remains unclear. Synaptogamin 4 (SYT4), a membrane protein involved in vesicle trafficking, is highly expressed in our established Enza and Abi resistant CRPC cells. Here, we explore the contribution of SYT4 in disease progression and NGATs resistance in CRPC.
Methods: Differences in SYT4 expression were determined in Enza-resistant C4-2B MDVR (MDVR) and Abi-resistant C4-2B AbiR (AbiR) cells versus parental C4-2B cells by RNA-sequencing, real time PCR, western blot and immunofluorescence microscopy. SYT4 expression was analyzed in GEO and cBioPortal databases from patients with prostate cancer. Specific siRNAs were used to inhibit SYT4 expression. The effect of SYT4 knock down on global gene expression, cell growth and sensitivity to Enza and Abi was assessed in our resistant cell lines.
Results: SYT4 gene expression was found to be markedly elevated in MDVR and AbiR cells compared to their parental C4-2B counterparts. Analysis of clinical data further revealed that SYT4 expression is significantly upregulated in patients with advanced prostate cancer; with high SYT4 levels being associated with poorer overall survival outcomes. Transcriptomic profiling identified enrichment of genes involved in calcium-mediated cell adhesion in MDVR cells, which is suppressed upon transfection with SYT4-specific siRNA. Additionally, silencing SYT4 expression via siRNA not only inhibited prostate cancer cell growth but also restored sensitivity of MDVR and AbiR cells to their respective treatments, underscoring SYT4 as a promising therapeutic target for treatment-resistant prostate cancer.
Conclusions: Our findings indicate that elevated expression of SYT4 promotes resistance to Enza and Abi; correlating with poorer outcomes in prostate cancer patients. Targeting SYT4 reduces the expression of genes involved in calcium-dependent cell adhesion and reduces cellular viability, thereby restoring sensitivity to NGATs like Enza and Abi, offering potential new strategies for overcoming treatment-resistant prostate cancer.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Kinase and Phosphatase Inhibitors 1
Poster Session

#1726 Preclinical development of APL-045: A selective PERK inhibitor targeting ER stress pathways in cancer.

Krishna Kolluri[1], Clara Stead[1], Susanne Wright[1], Paul Glossop[2], Emmanuel Demont[1], Richard Butt[1], Nadine Clemo[1]

[1]Apollo Therapeutics Limited, Cambridge, United Kingdom,[2]Sandexis, Sandwich, United Kingdom

The unfolded protein response (UPR) is a critical component of the integrated stress response (ISR) pathway, activated in response to the accumulation of unfolded or misfolded proteins within the endoplasmic reticulum (ER). A key mediator in this pathway is protein kinase RNA-like ER kinase (PERK), which becomes active under conditions of ER stress. Upon activation, PERK phosphorylates eIF2α, temporarily halting general protein translation to reduce the burden on the ER. Cancer cells can exploit PERK signalling to enhance their survival and proliferation under stress, leveraging this pathway to adapt to UPR triggered by hypoxic, nutrient-deprived tumour microenvironments, or as a response to anticancer therapies. Inhibition of PERK is therefore a promising therapeutic target in oncology.
APL-045 is a novel, selective, ATP-competitive inhibitor of PERK. APL-045 demonstrated potent inhibition of PERK, with a Ki of 4.6 nM and IC50 for eIF2α phosphorylation inhibition of 20 nM. Selectivity screening showed high specificity, with very limited off-target effects among 468 kinases tested in a Kinome Scan®.
In vitro studies demonstrated that APL-045 effectively inhibited downstream effectors of the PERK-mediated UPR pathway, ATF4 and CHOP, in a dose-dependent manner with the IC50 values ranged from 50 to 250 nM across renal cell carcinoma (RCC) and colorectal cancer cell lines. In vivo, APL-045 significantly reduced tumour volume in 786-O RCC xenograft models, achieving over 95% tumour growth inhibition after three weeks of treatment. These findings underscore the potential of APL-045 as a promising therapeutic candidate for cancers driven by PERK-dependent pathways.
APL-045 has a highly favourable pharmacokinetic profile, with good solubility, high permeability, and robust metabolic stability in both microsomal and hepatic assays in rodent, dog and human in vitro systems. These properties have translated to high bioavailability. This favourable profile is projected to represent a best-in-class PERK inhibitor.
These data describe the efficacy and drug-like properties of APL-045, a candidate stage, best-in-class PERK inhibitor poised to progress into IND-enabling and clinical studies.

#1727 Sequencing MEK inhibitor therapy after tovorafenib in *BRAF* fusion-driven cancers: Preclinical evidence of sustained tumor regression.

S. Perino[1], Y. Wang[1], S. Rastogi[1], N. P. Damayanti[2], K. E. Pollok[2], S. G. Raju[1], M. W. Kieran[1], S. C. Blackman[1], E. Venetsanakos[1]; [1]Day One Biopharmaceuticals, Brisbane, CA, [2]Indiana University School of Medicine, Indianapolis, IN

BRAF fusions and BRAF V600 point mutations are oncogenic drivers found in many pediatric and adult cancers, and constitutively activate the downstream MAPK pathway (Jones et al 2009, Yaeger and Corcoran 2019). Tovorafenib is the first and only type II RAF inhibitor approved for the treatment of *BRAF*-altered relapsed/refractory pediatric low-grade glioma (pLGG) in patients ≥ 6 months of age. In the ongoing ph 2 study, FIREFLY-1, tovorafenib demonstrated robust clinical activity in patients with *BRAF*-altered pLGG who progressed on systemic therapy, which may have included MEK inhibitors (Kilburn et al 2024). This preclinical study evaluated if tovorafenib-sensitive *BRAF* fusion tumors that have regrown during a drug holiday would also respond to a MEK inhibitor. The potential emergence of resistance with long-term exposure to tovorafenib was also explored. Due to the lack of availability of pLGG *KIAA1549*::*BRAF* fusion (the most commonly occurring *BRAF* fusion in pLGG) PDX models, in vivo experiments were conducted using a *AGK*::*BRAF* fusion melanoma PDX model. Mice bearing *AGK*::*BRAF* fusion melanoma tumors were treated with a clinically relevant dose (25 mg/kg oral, once daily) of tovorafenib for 14 days or until complete regression of tumors was observed. An off-treatment period was then initiated, during which tumors regrew. Once individual tumors regrew to 100-200 mm^3 size (within approximately 2 weeks), mice were treated for 14 days with a MEK inhibitor (trametinib; 0.3 mg/kg oral, once daily), or tovorafenib was resumed. Complete tumor regression was observed in all tumors in response to either trametinib or tovorafenib retreatment. Further evaluation of other MEK inhibitors and different dosing sequences of MAPK inhibitors is ongoing. In addition, the efficacy of tovorafenib in a *CDC42SE2*::*BRAF* anaplastic pleomorphic xanthoastrocytoma (APXA) PDX model is also being investigated. To explore potential mechanisms of acquired resistance, we also evaluated whether resistant tumors would emerge with continuous dosing of tovorafenib. Tovorafenib was administered (25 mg/kg oral, once daily) to mice bearing *AGK*::*BRAF* fusion tumors for 90 days. Over the entire course of treatment, significant tumor regression was maintained with no emergent tumors. In conclusion, our study provides evidence for the efficacy of sequencing MEK inhibitor treatment after tovorafenib in a PDX model of *AGK*::*BRAF* fusion melanoma. Furthermore, long-term efficacy was maintained with continuous dosing of tovorafenib in vivo, with no signs of emerging resistance preclinically, supporting the hypothesis that acquired resistance may be less common in *BRAF* fusion driven tumors treated with tovorafenib.

#1729 Mutagenesis screens support potential best-in-class profile for neladalkib (NVL-655), a brain-penetrant and TRK-sparing ALK inhibitor.

Anupong Tangpeerachaikul, Henry E. Pelish

Nuvalent, Cambridge, MA

Introduction. ALK fusions drive ~5% of non-small cell lung cancer (NSCLC). Three generations of ALK tyrosine kinase inhibitors (1G, 2G, and 3G TKIs) have been approved for the treatment of ALK-positive NSCLC. However, response to these TKIs can be limited by acquired drug resistance. Single ALK mutations have been reported in >50% of patients progressing on a 2G ALK TKI, whereas compound (i.e., ≥2 cis-allelic) ALK mutations have been observed after sequential treatment with 2G-3G ALK TKIs. NVL-655 is an investigational ALK TKI designed to be brain-penetrant, spare TRK, and maintain broad activity against single and compound ALK mutations that confer resistance to previous-generation therapies. Here, in vitro N-ethyl-N-nitrosourea (ENU) mutagenesis screens further support NVL-655 as more effective than previous-generation therapies at suppressing single and compound ALK mutations.
Methods. Cells were treated with ENU for 1 day followed by alectinib (2G), lorlatinib (3G), or NVL-655 for 28 days. Wells with proliferating cells were counted on day 28, indicating TKI-resistant clones. Mutations in the ALK kinase domain were identified by Sanger sequencing. Some resistant clones harboring ALK kinase-domain mutations were isolated and used to profile the potency of ALK TKIs.
Results. ENU screens were performed on Ba/F3 EML4-ALK, to simulate a first-line treatment setting, and on Ba/F3 EML4-ALK G1202R, the most common point mutation observed at progression on 2G ALK TKIs, to simulate a later-line treatment setting. NVL-655 unbound concentrations in the two screens were 8.0 and 3.2 nM, respectively, both below its 14 nM average clinical exposure at the recommended phase 2 dose (RP2D). Resistant clones emerged in only 9 (<2%) and 3 (<1%) of 480 wells, respectively, suggesting that resistance to NVL-655 would be unlikely in both treatment settings. By contrast, all 480 out of 480 wells became resistant to alectinib (in Ba/F3 EML4-ALK) or lorlatinib (in Ba/F3 EML4-ALK G1202R) at clinically relevant exposures, indicating lower effectiveness than NVL-655 at suppressing resistance in these screens.
Conclusion. NVL-655 was highly effective at suppressing resistance in ENU mutagenesis screens simulating first-line (EML4-ALK) and later-line (EML4-ALK G1202R) treatment. At clinically relevant concentrations, <2% of all wells became resistant to NVL-655, compared to 100% for alectinib and lorlatinib. This preclinical study indicates the potential for NVL-655 to prevent acquisition of on-target resistance and thereby provide deeper and more durable target inhibition. The broad preclinical activity against ALK and diverse ALK mutations, together with its brain penetrance and TRK-sparing design, suggests NVL-655 as a potential best-in-class ALK therapy.

#1730 Onvansertib in combination with carboplatin demonstrates enhanced anti-tumorigenic effects in pre-clinical models of serous endometrial cancer.

Glenn P. Boyles[1], Haomeng Zhang[1], Chelsey Vranes[1], Michael Emanuele[2], Chunxiao Zhou[1], Victoria Bae-Jump[1]

[1]Obstetrics and Gynecology, Division of Gynecologic Oncology, University of North Carolina at Chapel Hill, Chapel Hill, NC,[2]Pharmacology and Cancer Cell Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC

Objectives: Polo-like kinase 1 (PLK1) has key roles in regulating cell division and DNA damage responses, among other critical cellular functions. It is overexpressed in many cancers including endometrial cancer (EC). Onvansertib is a highly selective PLK1 inhibitor that has shown synergistic effects combined with chemotherapy in preclinical models of multiple solid tumors, and combination therapy is currently being evaluated in phase I/II clinical trials of breast and colon cancer. Serous endometrial cancer is an aggressive subtype of EC with a poor prognosis. The backbone adjuvant treatment consists of a platinum-based doublet. We aimed to investigate the effects of onvansertib in combination with carboplatin on cell proliferation, cellular stress, apoptosis, and cellular adhesion and migration in serous EC cell lines.

Methods: The human serous EC cell lines, ARK1 and SPEC2, were treated with both onvansertib and carboplatin, alone and in combination. Cell proliferation was evaluated by MTT and colony formation assays. Cellular stress was evaluated by measuring levels of reactive oxygen species (ROS) via DCFH-DA assay and change in mitochondrial membrane potential via JC-1 assay. Apoptosis was evaluated using cleaved caspace-3 assay. Cell adhesion was evaluated using laminin-1 assay, and cell migration was assessed by wound healing assay. Western immunoblotting was used to measure downstream protein expression related to cell cycle progression, cellular invasion, and apoptosis.

Results: Onvansertib and carboplatin both inhibited the proliferation and colony formation of ARK1 and SPEC2 cells, and combined treatment produced a synergistic response (CI<1). Combination treatment with onvansertib and carboplatin of serous endometrial cancer cells resulted in significantly higher levels of ROS production ($p<0.05$) and decreased mitochondrial membrane potential ($p<0.01$) relative to single-agent treatment. Onvansertib combined with carboplatin also significantly increased the activity of cleaved caspase-3 compared with single-agent treatment ($p<0.05$). Importantly, compared to each single-drug treatment, combination treatment impeded cell migration (scratch closure) and significantly decreased cell adhesion (laminin assay) in the ARK1 and SPEC2 cell lines (all $p<0.05$). Western immunoblotting supported these results, showing combination treatment resulted in downregulation of cell cycle regulatory proteins CDK2, CDK4, and cyclin D1 and the EMT markers SLUG and beta-catenin, and upregulation of the pro-apoptotic protein Bax.

Conclusions: The combination of onvansertib and carboplatin demonstrated potent anti-tumorigenic effects in serous EC cells compared with onvansertib or carboplatin alone. Further studies are warranted to evaluate if this combination may translate into a novel clinical therapeutic option for serous EC.

#1731 Combination therapy with onvansertib and carboplatin demonstrate anti-tumorigenic effects in endometrioid endometrial cancer cell lines.

Chelsey Vranes, Glenn Boyles, Haomeng Zhang, Michael Emanuele, Chunxiao Zhou, Victoria Bae-Jump

University of North Carolina, Chapel Hill, NC

Objectives: Polo-like kinase 1 (PLK1) is a serine/threonine kinase that plays a key role in cell cycle regulation, tumor cell proliferation, and DNA damage repair. PLK1 has arose as a potential cancer therapeutic target, since it is overexpressed in many tumor types including endometrial cancer (EC). Onvansertib is a highly specific PLK1 inhibitor and has shown promising results when combined with chemotherapy in pre-clinical models of several solid tumor types. Onvansertib is being tested in phase 1/2 trials for breast and colon cancer. PLK1 is also a downstream product of the mTOR pathway, a pathway often altered in EC, supporting that ovansertib may be a logical drug for EC treatment. We aimed to investigate the effects of onvansertib combined with carboplatin on cell proliferation, adhesion, apoptosis, and cellular stress in endometrioid EC cells.
Methods: Human endometrioid EC cell lines, HEC1B and KLE, were treated with onvansertib and carboplatin, both alone and in combination. Cell proliferation was assessed by colony formation and MTT assays. Cellular stress was evaluated by quantifying levels of reactive oxygen species (ROS) via DCFH-DA assay and changes in mitochondrial membrane potential (MMP) via the JC-1 assay. Apoptosis was measured using cleaved caspace-3 assays. Cell adhesion was evaluated using laminin-1 assay, and cell migration by wound healing assay. Western immunoblotting was used to measure downstream protein expression related to cell cycle progression, cellular invasion, and apoptosis.
Results: Onvansertib and carboplatin both inhibited cell colony formation in endometrioid EC cells, and when combined, showed a synergistic effect (Combination Index or CI<1). Combination therapy in both cell lines exhibited increased levels of ROS formation ($p<0.05$) and decreased MMP ($p<0.05$) compared to single-drug treatment. Combination therapy was found to significantly increase caspase-3 activity relative to either drug alone ($p<0.05$). Combination therapy also decreased cellular adhesion and inhibited migration (scratch closure) of the endometrioid EC cells ($p<0.05$). These results were confirmed by western immunoblotting demonstrating that combined therapy upregulated the cellular stress protein Bax, and downregulated the cell cycle regulatory proteins CKD2, CDK4, and cyclin D1. Proteins important for cellular adhesion and migration, SLUG and beta-catenin, were also downregulated with combination therapy.
Conclusion: Combination therapy with onvansertib and carboplatin demonstrate compelling anti-tumorigenic effects in endometrioid EC cell lines. Ongoing investigation is merited for pursuit of possible translation into clinical treatment options of this novel drug combination in EC.

#1732 Mastering metastases by novel selective CDK9 Inhibitor ORI-15412.

G. Galabova, M. Almstetter, N. Brusis, C. Gilch, R. Koestler, J. Kondrotas, J. Kubitzki, H. Kuhn, F. Medda, P. Perez-Galan, J. Tassel, N. Traube, A. Treml, D. Wifling, N. Yehia, P. Zegar, **M. Thormann**;
Origenis GmbH, Planegg, Germany

Metastases, the leading cause of cancer-related morbidity and deaths, remain a major therapeutic challenge, highlighting the critical need for novel treatments. Our study evaluates the efficacy of ORI-15412, an oral and fully brain-penetrant CDK9 inhibitor developed by Origenis, designed to meet this need through selective inhibition of CDK9 and modulation of key pathways involved in cancer progression and metastasis. ORI-15412 was engineered using Origenis' proprietary technologies - MOREsystem®, MolMind®, BRAINstorm™, and Cippix® - for optimized blood-brain barrier penetration, selectivity, and potency. By targeting the pTEFb complex and downstream effectors, including phospho-RNA Pol II, MCL-1, and c-Myc, ORI-15412 induces apoptosis and suppresses osteoclast differentiation in cancer models. It also demonstrates dose-dependent reduction in NF-κB-dependent p52 expression, suggesting potential immune modulatory effects. In vivo studies using the aggressive intracardial MV4-11_luc metastatic mouse model showed that ORI-15412 achieved 95% tumor growth inhibition (TGI) and up to 99.99% reduction in metastasis across the brain, bones, bone-marrow, lymph nodes, and lungs. The drug was well-tolerated with no observed toxicity, and efficacy was dose-dependent, underscoring its potential for treating metastatic cancers. Having completed all IND-enabling safety and toxicity studies with NOAEL at efficacious doses, ORI-15412 is positioned as a clinical-ready candidate, offering a promising new approach for patients with metastatic cancers, particularly those with brain and bone involvement.

#1733 XL495 is a potent, selective, and orally bioavailable inhibitor of PKMYT1.

Dana Gwinn[1], Jimmy Lai[1], Alice Rico[1], Christopher Kochansky[1], Nina M. Munoz[1], Ragadeepthi Tunduguru[1], Aditya Kumar[1], Pragalath Sundararajan[1], Akhil Choppa[1], Donna Fong[1], Mi Chen[1], Amy Hildebrand[1], Jeremy Murray[1], Christopher Szeto[1], Matthew G. Rees[2], Melissa M. Ronan[2], Jennifer A. Roth[2], Wei Xu[1], Paul Gillespie[1], Paige Mahaney[1], Dana T. Aftab[1], Lisa D. Belmont[1], Jae H. Chang[1]

[1]Exelixis, Alameda, CA,[2]Broad Institute of MIT and Harvard, Cambridge, MA

Background: Protein kinase, membrane-associated tyrosine/threonine-1 (PKMYT1) phosphorylates cyclin-dependent kinase 1 (CDK1) at threonine-14 (Thr14), inhibiting the catalytic activity of the CDK1/cyclin B kinase complex, which blocks cells in G2 from entering M phase. Disruption of this PKMYT1-mediated G2/M checkpoint in cancer cells allows mitotic events to occur while DNA synthesis is still ongoing, resulting in catastrophic levels of DNA damage and cell death. We report preclinical data for XL495, a potent and selective orally bioavailable small molecule inhibitor of PKMYT1.

Methods: XL495 selectivity was assessed using a KinomeProfiler™ assay. Viability across hundreds of cancer lines was assessed using the PRISM screen. Viability of cell lines treated with a combination dose response matrix was measured using CellTiterGlo®. Antitumor activity of XL495 orally administered as a single agent was evaluated in a colorectal cancer and a breast cancer cell line-derived xenograft (CDX) model. Antitumor activity of XL495 in combination with irinotecan or gemcitabine was evaluated in a colorectal cancer CDX model. Phosphorylation of CDK1 at Thr14 and phosphorylation of KAP1 at Ser824 from tumors was measured by AlphaLISA™. Drug-like properties were assessed and pharmacokinetic (PK) parameters were determined following administration of XL495 in rat, dog, and monkey.

Results: XL495 is a potent, selective inhibitor of PKMYT1 in biochemical and cellular assays. Cancer cell lines with *CCNE1* amplification were more sensitive to XL495 than other models. XL495 demonstrated dose-dependent antitumor activity, inhibition of phosphorylation of CDK1 at Thr14, and induction of phosphorylation of KAP1 at Ser824 in CDX tumors. When XL495 was dosed in combination with gemcitabine or irinotecan in the CDX model, tumor regression was achieved. XL495 showed favorable drug-like properties including high solubility and moderate permeability in Caco-2 cells. XL495 exhibited low clearance (CL) in rat and monkey and moderate CL in dog. Bioavailability was high in rat and moderate in dog and monkey. Preliminary PK assessments predict low CL and a long half-life for XL495 in humans.

Conclusions: XL495 is potentially a best-in-class PKMYT1 inhibitor. PK assessments in conjunction with preclinical antitumor activity suggest that once-daily oral administration of XL495 may achieve clinical activity alone or in combination with DNA-damaging agents. XL495 is currently under investigation in a phase 1 first-in-human study in patients with advanced solid tumors (NCT06630247).

#1734 RP-1664: A potent and selective PLK4 inhibitor causing tumor regressions in TRIM37-high xenograft models of solid tumors.

Elliot Goodfellow[1], Frederic Vallee[1], Matias Casas-Selves[1], Monica Bubenik[1], Martin Duplessis[1], Boubacar Sow[1], Catalina Suarez[1], Li Li[1], Cathy Caron[1], Robert Papp[1], Marie-Eve Leclaire[1], Bingcan Liu[1], Simon Surprenant[1], Philippe Mochirian[1], Shou Yun Yin[1], Parham Nejad[2], Joseph D. Schonhoft[2], Rino Stocco[1], Claude Godbout[1], Prasamit Baruah[1], Alexanne Bonneau-Fortin[1], **Anne Roulston**[1], C. Gary Marshall[2], Michal Zimmermann[1], Michel Gallant[1], W. Cameron Black[1], Stephen J. Morris[1], Marc Hyer[2]

[1]Repare Therapeutics Inc., Saint-Laurent, QC, Canada,[2]Repare Therapeutics Inc., Cambridge, MA

Introduction: Polo-like kinase 4 (PLK4) is a Ser/Thr mitotic kinase crucial for centriole duplication prior to mitosis. During PLK4 inhibition centrioles are lost, however microtubules can still be nucleated from within the pericentriolar material (PCM), allowing cell division. Overexpression of the E3 ligase TRIM37, often driven by gene amplification in cancer cells, results in degradation of the PCM, rendering these cancer cells hypersensitive to PLK4 inhibition due to increased dependence on centriole duplication. TRIM37 amplification occurs at high frequency in neuroblastoma, and in several solid tumors including HER2+ and HR+/HER2- breast cancers, making PLK4 inhibition a compelling cancer drug target.

Methods: The identification of RP-1664 was assisted by biochemical & cell-based target engagement assays, standard ADME and PK assays and selectivity determined by kinome profiling. Functional activity of RP-1664 was measured in cell growth assays and by monitoring PLK4 and p21 levels in cells by IHC and western blot. In vivo efficacy was evaluated in several breast and solid tumor xenograft models.

Results: RP-1664 is a potent and selective PLK4 inhibitor with excellent oral bioavailability. In vitro, TRIM37 high cell lines across multiple lineages are more sensitive than non-TRIM37 amplified lines to RP-1664 mediated PLK4 inhibition, confirming the synthetic lethality phenotype. RP-1664 treated cells exhibit the expected loss of centrioles, and an accumulation of PLK4 and p21 protein, consistent with PLK4 inhibition results observed by others. In vivo, RP-1664 is highly efficacious, resulting in >80% tumor growth inhibition and sustained regressions in multiple cell and patient-derived xenograft models of breast cancer at well tolerated doses and on various schedules. PK/efficacy relationships demonstrate a steep dose response suggesting a threshold of PLK4 inhibition is required for anti-tumor activity and that efficacy correlates better with extent of drug exposure (AUC) rather than maximum blood concentration (C_{max}) or duration of target inhibition.

Conclusions: The highly selective, orally bioavailable PLK4 inhibitor RP-1664 demonstrates overall profound efficacy in pre-clinical in vitro and in vivo xenograft models and activation of the appropriate biomarkers indicating the expected on-target activity. These data suggest that further clinical investigation of RP-1664's anti-tumor activity in breast and other solid tumors is warranted (NCT06232408).

#1735 The discovery of PKMYT1 inhibitors to exploit synthetic lethality caused by replication stress.

Richard M. Marais, Joanna Grabarek, Myria Ioannou, Leo Leung, Ali Raoof, Mike Brown, Deborah Smithen, Chris Fitzpatrick, Chris Chester, Jacob Leigh, Mohammed Aljarah, Monika Lisauskaite, Oleksandr Grytsai, Ryan Burgin, Dan Niculescu-Duvaz, Graeme Thomson, Caroline Springer

ONCODRUG LTD, Manchester, United Kingdom

Background: Replication stress is a feature of the hyper-proliferation experienced by many cancers, leading to genome instability and accumulation of potentially lethal DNA damage. During their evolution, many cancer cells inactivate specific DNA-damage response pathways, placing high dependence on the pathways that remain, one of which is regulated by PKMYT1. This protein kinase prevents unscheduled G2 to M transition to allow cells the time needed for DNA repair before division. In cells that rely on PKMYT1, its inhibition forces cells to divide when they are not ready, leading to synthetic lethal killing. PKMYT1 is important when replication stress is driven by Cyclin E amplification and other processes. Thus, PKMYT1 inhibitors offer therapeutic opportunities that can be exploited in combination with DNA damaging agents across a range of cancers.
Materials and Methods: We synthesized over 1500 novel PKMYT1 inhibitors and validated their activity in biochemical, biophysical, cell biology and tumor growth analyses. Target engagement and cellular responses were monitored with validated target engagement and cell response biomarkers.
Results: We have discovered low nM PKMYT1 inhibitors that are exquisitely selective when assayed against a range of other protein kinases, which includes >1000-fold selective over the closely related protein kinase WEE1. Our inhibitors show extremely slow dissociation kinetics from the target in cells and selectively inhibit proliferation in cells with the genetic features of replication stress. Extensive biomarker analyses confirm target engagement in cells and demonstrate that our inhibitors cooperate with DNA damaging agents to increase accumulation of DNA damage in sensitive cells, but not in insensitive cells. The inhibitors are orally bioavailable, and we confirm target engagement in human tumor xenografts grown in immunocompromised mice. Critically, our inhibitors cooperate with DNA damaging agents to achieve regressions of human tumor xenografts.
Conclusions: We have discovered novel orally bioavailable well tolerated PKMYT1 inhibitors. Our inhibitors are exquisitely selective for PKMYT1 over a range of protein kinases, including the closely related protein kinase WEE1. We confirm PKMYT1 engagement in cells and show that our inhibitors display extremely long dissociation kinetics from the target. We show that our PKMYT1 inhibitors cooperate with DNA damaging agents to increase accumulation of DNA damage in cancer cells with high levels of replication stress but not in healthy cells. In human cancer xenografts, our well tolerated inhibitors enhance the anti-tumor efficacy of DNA damaging agents achieving significant tumor growth delay benefits compared to chemotherapy alone.

#1736 Novel fourth-generation ALK inhibitors targeting compound mutations in *ALK*-positive cancers.

Yuhao Gao[1], Jinghan Wang[2], Kai Zhang[1], Chang Lu[1], Yun Bai[1], Tianxia Liu[1], Miao Zhang[1], Sanxu Chen[2], He Wang[3], Xijie Liu[2], Hong Luo[3], **Yinghui Sun**[1]

[1]Department of Discovery Biology, Shouyao Holdings (Beijing) Co., Ltd, Beijing, China,[2]Department of Medicinal Chemistry, Shouyao Holdings (Beijing) Co., Ltd, Beijing, China,[3]Department of Drug Metabolism and Pharmacokinetics, Shouyao Holdings (Beijing) Co., Ltd, Beijing, China

The emergence of compound mutations in the ALK tyrosine kinase domain presents a significant challenge in treating *ALK*-positive cancers, especially in patients who have received sequential therapy with second- and third-generation ALK tyrosine kinase inhibitors (TKIs). Key compound mutations, such as G1202R/L1196M, I1171N/D1203N, single mutations (G1202R and I1171N) induce conformational changes that hinder drug binding and severely limit the efficacy of existing therapies, highlighting the urgent need for advanced fourth-generation (4G) ALK TKIs with enhanced potency, selectivity, and increased central nervous system (CNS) penetration. In this study, we developed a series of novel 4G ALK TKIs targeting ALK compound mutations, demonstrating potent activity *in vitro* and *in vivo*. Enzymatic assays revealed robust inhibition of ALK compound mutations, with IC_{50} values below 1 nM and high selectivity against off-target kinase NTRK. Strong cellular antiproliferative effects were observed in series of Ba/F3 EML4-ALK compound-mutant cell lines with low-nanomolar IC_{50} values. These compounds significantly inhibited ALK autophosphorylation and downstream signaling pathway in a dose-dependent manner, resulting in G0/G1 cell cycle arrest and cell apoptosis. *In vivo* efficacy studies using subcutaneous xenograft models with EML4-ALK wild-type and compound-mutant cell lines (Ba/F3 and NCI-H3122) demonstrated dose-dependent tumor growth inhibition, with some cases achieving complete tumor regression. Notably, these 4G ALK TKIs exhibited effective transport across the blood-brain barrier (BBB) with a notable Kp,uu in rats. In intracranial xenograft models harbouring EML4-ALK (wild-type or compound-mutant), these 4G ALK compounds substantially inhibited intracranial tumor growth, underscoring the potent CNS antitumor activity. And the treatments were well tolerated across all animal models, with no significant body weight loss, supporting a favorable safety profile. Meanwhile, pharmacokinetic studies in animals also revealed favorable properties of these compounds. In conclusion, these selectivity 4G ALK compounds demonstrate significant *in vitro* and *in vivo* efficacy, effective CNS penetration, and a favorable safety profile. They represent a promising advancement in the treatment of *ALK*-driven cancers, providing potential benefits for refractory cases following second- and third-generation ALK TKI sequential therapy and as viable front-line options. Comprehensive preclinical evaluations are warranted to support further clinical investigation.

#1738 Pan-inhibition of super-enhancer-driven oncogenic transcription by novel synthetic ecteinascidins yields potent anti-cancer activity.

J. Obid, M. Cigrang, M. Nogaret, F. Coin;
IGBMC - Univ. of Strasbourg, Illkirch CEDEX, France

Transcriptional addiction is a cancer hallmark, defined as the strong dependence of cancer cells on the high transcriptional activity of specific oncogenes promoting their proliferation and survival. This phenomenon often involves the acquisition of large clusters of enhancers known as super-enhancers (SE), which are bound by extremely abundant levels of ubiquitous transcription factors that sustain the robust oncogenic expression. Despite recent efforts to develop compounds disrupting SE-driven oncogenic expression, such as CDK7 and BRD4 inhibitors, their clinical success has been limited due to poor pharmacokinetics and short half-lives. Skin cutaneous melanoma (SKCM) serves as a valuable model for studying transcriptional addiction, as melanoma cells can dynamically shift between various cellular states through transcriptional and epigenetic reprogramming, posing a challenge for current therapeutic strategies. To address this, we developed two novel synthetic ecteinascidin derivatives of lurbinectedin, a drug approved for the treatment of advanced small cell lung cancer. Our results demonstrate that these newly developed ecteinascidins, along with the parent compound lurbinectedin, exhibit potent anti-tumor effects against melanoma, both *in vitro* and in mouse xenografts models resistant to current therapies, leading to reduced tumor growth and prolonged animal survival. Mechanistically, our extensive RNA-seq and chem-map assays reveal that synthetic ecteinascidins significantly suppress SE-mediated oncogene expression in melanoma cells by directly binding to and inhibiting the promoters of genes encoding ubiquitous transcription factors and coactivators that are highly enriched at oncogenic SE sites. Overall, our study provides the first evidence that the clinically approved drug, lurbinectedin, as well as its derivatives, can target and disrupt SE-dependent oncogenes, highlighting their potential as therapeutic options for cancers with transcriptionally heterogeneous landscapes where conventional therapies face significant challenges.

#1739 Identification of a potent and selective KAT6A/6B inhibitor: HW321005 exhibits strong antitumor activity in KAT6A-high ER+/HER2– breast cancer.

Xin Zhao[1], qun li[1], qian wu[1], zexin tong[1], yang zang[1], tiejun bing[2], lifei liu[1], xuejun zhang[1]

[1]Humanwell Healthcare (Group) Co.,Ltd, Hubei Wuhan, China,[2]ICE Bioscience, Beijing, China

KAT6A and its paralog KAT6B are histone lysine acetyltransferases (HATs) that regulate gene transcription via the acetylation of histones. Dysregulation of KAT6 leads to lineage-specific gene expression changes in cancer. Overexpression of KAT6 is associated with poorer clinical outcomes in ER+/HER2– breast cancers. Dysregulation of KAT6 activity, including gene amplification, overexpression, fusion, and mutation, is observed in various cancers, including breast cancer. We have developed potent and selective small molecule inhibitors targeting KAT6A/6B. Our proprietary compound, HW321005, inhibits KAT6A/6B with single-digit nanomolar IC50 values, demonstrating high selectivity over other KAT family members. The efficacy of HW321005 was evaluated in multiple cancer cell lines, including ZR-751 (IC50 = 1.7 nM). Treatment with HW321005 led to a marked downregulation of KAT6A-mediated histone acetylation and estrogen receptor signaling in KAT6A-high ER+ breast cancer models. Consistent with in vitro findings, HW321005 showed significant anti-tumor efficacy and a robust pharmacodynamic response (H3K23Ac, acetylation marker; ERα, estrogen receptor signaling marker) in ZR-751 xenograft model in a dose-dependent manner. HW321005 demonstrates substantial biological potency and high selectivity, with favorable drug-like properties, including desirable in vitro ADME characteristics, excellent in vivo exposure, clearance, and good oral bioavailability across multiple preclinical species. These findings highlight the potential of HW321005 as a novel and highly effective KAT6A/6B inhibitor for the treatment of KAT6A-high ER+ breast cancer. IND-enabling studies for HW321005 are planned for early 2025.

#1740 Generation of a best-in-class AR-NTD inhibitor targeting a unique binding pocket identified by computational modeling.

Jeffrey N. Lindquist[1], Sumana Ghosh[2], Naveen Naganaboina[3], Nishan Shettigar[4], Sanjita Banerjee[1], Michael Green[1], Ravi K. Muttineni[5], Hirdesh Uppal[1]

[1]Capulus Therapeutics LLC, San Francisco, CA,[2]Capulus Therapeutics LLC, New Delhi, India,[3]Capulus Therapeutics LLC, Hyderabad, India,[4]Capulus Therapeutics LLC, Ashburn, VA,[5]Immunocure Discovery Solutions Pvt Ltd, Hyderabad, India

Background: Current prostate cancer (PC) therapies for both mCRPC and nmCRPC patients are limited to targeting the ligand-binding domain (LBD). But due to the emergence of LBD lacking variants, and mutations in the LBD of androgen receptor (AR), these therapies become ineffective over time. Thus, there is an unmet need to develop treatments targeting N-terminal Domain (NTD) of the AR to potentially inactivate AR. Although the AR-NTD is a promising target but the structural aspects such as conformational flexibility and lack of a stable secondary structure without defined binding pocket make it technically challenging for designing NTD specific inhibitors. Thus, our efforts are being focused on generating consensus structure and developing small molecules antagonists that bind to AR-NTD.
Methods: For NTD targeting we generated a consensus AR-NTD structure by proprietary and integrated drug discovery AI (Artificial Intelligence)-based platform. Multiple MDS (T= 100 ns) together with deep learning neural networks identified two druggable sub-pockets in the consensus NTD structure model (M5). Fragment and structure based virtual screening against one of the deep sub pockets of AR-NTD combined with novel protein motion evaluation methods generated novel chemical matters by cell based assay that resulted nano-molar inhibitors over several SAR rounds.
Results: To validate the binding of NTD binding small molecules to the AR-NTD protein in real time, surface plasmon resonance (SPR) analysis was performed. This allowed for the measurement of binding kinetics and affinity, providing robust evidence of the direct interaction between the lead CAP-molecules and AR-NTD with K_d 5-10 micromolar/sec. Research Lead molecules, selected based on their favorable in vitro potency, ADME, pharmacokinetic, and tolerability profiles, were advanced to 28-day AR/ARV7 driven VCaP xenograft model to test for in vivo efficacy. One molecule showed outstanding efficacy and was well tolerated and moved to an ARV7 driven model, 22RV1, a model known for its expression of AR variants & resistance to standard AR-targeted therapies, making it ideal for evaluating the potential of NTD inhibitors. The study results showed significant tumor growth inhibition and impressive synergistic effect when combined with enzalutamide while maintaining stable animal weight. This promising effect of an NTD targeted inhibitor towards 22RV1 xenograft model validates the computer modelling, AI driven structure prediction and virtual screening for generating IP protected allosteric small molecule NTD inhibitor with novel druggable pockets in AR. The novel AR-NTD inhibitor has been further incorporated into two chemistry strategies: AR-NTD targeting PROTACs, AR-NTD & cellular essential protein targeting heterobifunctional molecules; pharmacological activity evaluation is being pursued for these two approaches.

#1741 STAT3 Orchestrates common and divergent mechanisms in different cell types to promote pancreatic cancer development and progression.

Monday O. Ogese[1], Lihong Huo[2], Yue Chen[1], Benjamin Jackel[3], Francisco Lopez-Tapia[1], Richard T. Waldron[3], Aurelia Lugea[3], Stephen J. Pandol[3], **James Turkson**[1]

[1]Department of Medicine, Division of Medical Oncology; Cancer Biology Program, Cedars-Sinai Cancer, Cedars-Sinai Medical Center, Los Angeles, CA,[2]Department of Medicine, Division of Gastroenterology, Cedars-Sinai Medical Center, Los Angeles, CA,[3]Cancer Biology Program, Cedars-Sinai Cancer; Department of Medicine, Division of Gastroenterology, Cedars-Sinai Medical Center, Los Angeles, CA

Pancreatic adenocarcinoma (PDAC) is a major healthcare challenge. Currently, there are no good treatments for PDAC, and patient survival is dismal. *In vitro* and *in vivo* models to study PDAC pathogenesis have shed some light on the molecular and cellular landscape of the disease, including the prevalence of altered signal transducer and activator of transcription 3 (STAT3) functions. While constitutively-active STAT3 is implicated in PDAC progression, its functional role in driving PDAC development is not fully understood. Using human PDAC (hPDAC) cell lines, hPDAC-derived cancer-associated fibroblasts (hCAFs), and high-fat diet (HFD)-fed mutant *KrasG^{12D}* (KC) mice, we interrogated aberrantly-active STAT3 and its cell-type specificity to delineate STAT3's role in PDAC development and therapeutic response. Human PDAC cell lines (AsPC-1, Mia-PaCa-2, and PANC-1) and hCAFs harbor constitutively-active STAT3, and their stimulation with IL-6, EGF or PDGF further enhanced the phospho-tyrosine-STAT3 (pYSTAT3) levels. In KC mice, feeding a HFD led to constitutive pYSTAT3 induction in the pancreas in parallel with the development of precancerous pancreatic intraepithelial neoplasia (PanIN) lesions. Induction of pYSTAT3 occurred across diverse cell types, including acinar-ductal-metaplasia (ADM) elements, neoplastic ducts, stromal and immune cells, but not in acinar cells. Treatment with the STAT3 inhibitor, H182, blocked the pYSTAT3 levels in the hCAFs and hPDAC cells *in vitro.* Significant profile changes for IL-6-stimulated cytokine and chemokine secretion were also observed, including decreased IL-8, SERPINE1, CXCL1, CXCL12, CCL2 and MIF secretion. Mia-PaCa-2 cells exposed to H182 for 48 h showed a repression of genes involved in immune evasion (HLA-E, AGRN), angiogenesis (LOXL2), cell proliferation and migration (IL-8, UBC, JAG1), oxidative stress (NQO1, SRXN1, TXNRD1) and tumor progression (BCL10, HMOX1, HMGA1, SERPINE1), in parallel with the upregulation of TP53 and NBPF1 tumor suppressor genes. H182 treatment of the hPDAC lines further repressed mitochondrial oxygen consumption rate and viable cell numbers and induced apoptosis. Administration of H182 to HFD-fed KC mice prevented acinar cell differentiation into ADM/PanINs and suppressed pro-fibroinflammatory genes in the pancreas, including CXCL12, CCL2 and CCL5, while inhibiting the growth of subcutaneous hPDAC xenografts in mice. The decreased proinflammatory cytokine and chemokine profiles across diverse cell types, associated with the enrichment of acinar cells in the pancreas of KC mice in response to STAT3 inhibition underscores declining STAT3-dependent pro-tumorigenic mechanisms that are orchestrated through a HFD-induced chronic inflammation. The translational significance is that the blockade of aberrant STAT3 functions suppresses growth of PanINs and cancer cells in PDAC.

#1742 Discovery and preclinical profiling of a novel PKMYT1 inhibitor, EVT-0003023, with high efficacy in late-stage, post-treatment non-small cell lung cancer models.

Daniel Miller, Alfie Brennan, Jan Lanz, Oliver Vipond, Noah Harrison, James Aaronson, Anna Hercot

Evariste Ltd, London, United Kingdom

Protein kinase membrane-associated tyrosine/threonine 1 (PKMYT1) is a negative regulator of CDK1 activity and has been identified as a putative synthetic lethal target in cancers which up-regulate cyclin E (CCNE1) or harbor mutations in genes such as FBXW7. We used our internal Frobenius platform to identify highly potent and differentiated inhibitors of PKMYT1 with exceptional selectivity over key off targets, including the Raf family and WEE1. Machine learning-driven optimization delivered a preclinical candidate compound, EVT-0003023, with best-in-class selectivity and a significantly improved pharmacokinetic profile in relevant animal models, and in vivo efficacy in CCNE1-high and FBXW7-mutated xenograft models.

Compounds from this series were used to validate a differentiated biomarker for sensitivity to PKMYT1 inhibition in cellular and PDX-derived models, expanding the patient population treatable with PKMYT1 inhibitors into non-small cell lung cancer. Using clinical data, we demonstrated that this biomarker is significantly up-regulated in patients who have progressed on existing standard-of-care treatment, and demonstrated exceptional monotherapy activity for EVT-0003023 in PDX models reflecting this patient population, as well as tumor regression when combined with low doses of chemotherapy.

#1743 QLS1209, A highly potent and selective PKMYT1 inhibitor demonstrates activity in tumors with CCNE1^{Amp} and/ or FBXW7^{LoF} mutations.

Yuxing Zhang[1], Jiasheng Fu[1], Rongyan Wang[1], Jianping Chen[1], Changliang He[1], Junguo Hao[1], Xinrui Shao[1], Ling Li[1], Hua Qin[1], Guangyue Lei[1], Ping Chen[1], Dong Yang[1], Xinghua Cheng[1], Qingmei Zheng[2], Zheng Wang[1], Guqin Shi[1], Wenyuan Qian[1], Daqing Sun[1], Weimei Sun[1], Weikang Tao[1]

[1]Shanghai Qilu Pharmaceutical Research and Development Center Ltd., Shanghai, China,[2]Qilu Pharmaceutical Co., Ltd., Jinan, China

Cell cycle checkpoints serve as cellular critical safeguard mechanisms that ensure orderly progression through the cell cycle. PKMYT1, a membrane-associated kinase, functions as a negative regulator of CDK1 activity by phosphorylating CDK1 at the Thr14 residue. This enzyme is frequently overexpressed in a variety of cancers. Recent studies suggest that cancer cells harboring CCNE1 amplification (CCNE1^{Amp}) and/or FBXW7 loss-of-function (FBXW7^{LoF}) mutations are particularly relying on PKMYT1 for orderly entry to mitosis. As a result, inhibition of PKMYT1 can lead to premature mitotic entry, inducing DNA damage, and triggering mitotic catastrophe. CCNE1^{Amp} is a common genetic alteration found across a spectrum of cancer types, with particular prevalence in gynecological malignancies. Elevated levels of cyclin E are linked to genomic instability, whole-genome doubling, and resistance to existing therapeutic interventions. FBXW7, a tumor suppressor gene, is frequently mutated in various cancers, including colorectal and ovarian serous carcinoma. Loss of FBXW7 function is recognized as an independent prognostic factor, significantly correlating with tumor cell resistance to chemotherapy and unfavorable disease outcomes. Moreover, FBXW7 acts as an E3 ubiquitin ligase for cyclin E, potentially leading to increased levels of cyclin E through post-translational mechanisms. QLS1209, an orally bioavailable, potent, and selective inhibitor of PKMYT1 was discovered and evaluated preclinically. QLS1209 exhibited single-digit nanomolar potency in inhibiting PKMYT1's enzymatic activity and demonstrated approximately 30-fold selectivity over its paralogue Wee1. QLS1209 potently suppressed phosphorylation of CDK1 at Thr14, with a minimal impact on Tyr15. QLS1209 was well tolerated and demonstrated anti-tumor activity in CCNE1^{Amp} HCC1569 and OVCAR3 xenograft models in vivo, it also showed enhanced efficacy when combined with Gemcitabine. In a gastric patient-derived xenograft (PDX) model harboring an FBXW7^{LoF} mutation, QLS1209 dose-dependently inhibited tumor growth, with higher dose leading to tumor regression. QLS1209 exhibits excellent pharmacokinetic profile, and promising in vitro ADME properties, along with a favorable safety profile. IND enabling studies are currently underway and are expected to be completed by the end of 2024. Collectively, these findings highlight the promising potential of QLS1209 as a therapeutic strategy, either as monotherapy or in combination with other anti-cancer therapies, for a variety of cancer types characterized by CCNE1^{Amp} and/ or FBXW7^{LoF} mutations.

#1744 Preclinical efficacy of a selective, orally bioavailable HER2 small molecule inhibitor targeting HER2 alterations and Enhertu-resistant cancers.

Jiyoung Jeon, Sun Young Jang, Ho Yeon Nam, HyungSeok Yoo, Jooyun Byun, Gunwoo Lee, Soye Jeon, Young Gil Ahn

Hanmi Pharmaceutical Co., Ltd., Hwaseong-si, Korea, Republic of

HER2 alterations, including overexpression, amplification, and other mutations are found in various solid tumors and have a significant impact on cancer cell growth and metastasis. To address this issue, HER2-targeted therapeutics are being developed and evaluated across multiple tumor types, including breast cancer, gastric cancer, HER2-positive cholangiocarcinoma, colorectal cancer, non-small cell lung cancer, and bladder cancer. Existing HER2 inhibitors inhibit not only HER2 alteration but also EGFRWT, causing issues with EGFR-related toxicity (skin rash, diarrhea, etc.). This highlights the need to develop selective HER2 inhibitors that specifically target HER2 and its mutations without affecting EGFRWT. The Hanmi compound selectively targets the HER2 receptor tyrosine domain via covalent binding, sparing EGFRWT and minimizing EGFR-related toxicity. The Hanmi compound inhibited both HER2 mutant and HER2WT cells and enzymes, had a DMPK profile compatible with oral administration. It also demonstrated potent antitumor activity as a single agent in various HER2 mutant xenograft models. Despite the good efficacy of Enhertu (trastuzumab deruxtecan), which is approved for the treatment of all HER2 cancers, resistance that develops within 12 months of drug administration remains a major clinical challenge. To address this unmet need, we evaluated a novel HER2 inhibitor in a preclinical model that acquired resistance to Enhertu. The Hanmi compound effectively inhibited tumor growth in Enhertu resistant NCI-N87 mouse models. In conclusion, Hanmi compound, an orally bioavailable small molecule inhibitor, showed high efficacy against both HER2 mutant and HER2WT tumors, while preserving EGFRWT and minimizing EGFR-related toxicities. Furthermore, it demonstrated the potential to overcome resistance to Enhertu by effectively inhibiting tumor growth in Enhertu-induced resistant xenograft mouse model, and these preclinical results support Hanmi compound as a promising treatment for HER2 altered cancers.

#1745 Combining HRI/PERK/GCN2 inhibitor CRD-799 with proteasome inhibitors provides a novel approach to overcoming resistance in multiple myeloma treatment.

Yasumichi Inoue[1], Koki Hikami[2], Shunsuke Ebara[2], Midori Sugiyama[2], Robert Oda[2], Akio Mizutani[2], Misaki Yoshida[2], Hiroko Yamakawa[2], Shigehiro Yagishita[3], Akinobu Hamada[3], Hidetoshi Hayashi[1], Takaomi Sanda[4], Masaki Ri[4], Shinsuke Iida[4], **Daisuke Morishita**[2]

[1]Department of Cell Signaling, Graduate School of Pharmaceutical Sciences, Nagoya City University, Nagoya, Japan,[2]Chordia Therapeutics Inc., Fujisawa, Japan,[3]Division of Molecular Pharmacology, National Cancer Center Research Institute, Tokyo, Japan,[4]Department of Hematology and Oncology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

The introduction of novel treatments for multiple myeloma (MM), particularly proteasome inhibitors (PIs) such as bortezomib (BTZ), has significantly improved patient outcomes. However, many patients eventually relapse, underscoring the urgent need for new therapeutic strategies to overcome PI resistance. The integrated stress response (ISR) is a conserved intracellular signaling network activated by various intrinsic and extrinsic stressors. This response is mediated by four specialized kinases, HRI, PERK, PKR, and GCN2, which converge on the phosphorylation of eIF2α. Phosphorylated eIF2α promotes the translation of ATF4, a critical regulator of cellular survival and homeostasis. Our prior studies demonstrated that PI treatment induces mitochondrial stress, endoplasmic reticulum stress, and amino acid starvation, triggering the activation of HRI, PERK, and GCN2, respectively. Targeting this triple kinase axis has shown potential in overcoming PI resistance. Building on these findings, we developed CRD-799, an orally bioavailable small-molecule inhibitor designed to target HRI, PERK, and GCN2. CRD-799 effectively inhibited ISR kinase activity, leading to complete suppression of eIF2α phosphorylation and ATF4 activation under PI treatment conditions. When combined with BTZ, CRD-799 exhibited potent anti-proliferative effects both *in vitro* and *in vivo* (AACR 2024 #1233, Inoue *et al.*). Further investigation revealed a novel mechanism of cell death induced by the combination of CRD-799 and PIs. Apoptosis inducers, including NOXA and CHOP, were significantly upregulated during combination treatment, yet this process appeared independent of the canonical ATF4-mediated transcriptional pathway. Comprehensive RNA-seq analysis revealed suppression of the canonical ATF4-driven stress response, while ATF3 induction and NOXA-dependent apoptosis were preserved. Notably, the combination treatment activated the IRE1-TRAF2-JNK1/2-c-Jun/ATF3 signaling axis, suggesting its role in driving NOXA-mediated apoptosis independently of the classical ISR-ATF4 pathway. This mechanism may underlie the variability in treatment sensitivity observed across MM models. In summary, the combination of the HRI/PERK/GCN2 inhibitor CRD-799 with PIs offers a promising strategy to overcome PI resistance in MM. These findings provide critical mechanistic insights into the heterogeneity of treatment responses, potentially guiding the personalized application of this combination therapy in clinical settings.

#1746 FGFR3 inhibition is a highly effective therapy in MTAP-deficient, FGFR3 altered urothelial carcinoma .

Yu-Yi Chu[1], Jianfeng Chen[1], Cindy Y. Jiang[2], Ying Mei[1], Yuxue Zhang[1], Arlene Siefker-Radtke[1], Jianjun Gao[1]

[1]Genitourinary Medical Oncology, UT MD Anderson Cancer Center, Houston, TX,[2]Hematology/Oncology Fellowship program, UT MD Anderson Cancer Center, Houston, TX

FGFR3 alterations are observed in more than 15% of metastatic urothelial cancers. In 2019, erdafitinib was the first FGFR inhibitor approved by the FDA for treatment of FGFR3-altered metastatic urothelial cancer with an objective response rate of 40%. However, a high percentage of patients with FGFR3 alterations still do not respond to erdafitinib. Thus, it is critical to identify additional biomarkers to stratify patients and increase the therapeutic efficacy of FGFR inhibitors. Our previous study identified that FGFR3 was more frequently altered in methylthioadenosine phosphorylase (MTAP)-deficient tumors (MTAP-deficient: 31% vs MTAP-proficient: 8%), which raises our interest to investigate whether MTAP deficiency leads to enhanced dependency on FGFR3 activity and increases tumor treatment response to FGFR inhibitors. Here, we report that IC_{50} of FGFR inhibitors is significantly lower in MTAP-deficient tumors compared to MTAP-proficient tumors. Notably, an MTAP enzyme, which was developed to deplete its substrate, MTA [a known inhibitor of protein arginine methyltransferase 5 (PRMT5)], significantly increases the PRMT5 activity and results in resistance to FGFR inhibitors in MTAP-deficient tumors. Moreover, our data demonstrate that PRMT5 inhibition sensitizes MTAP-proficient tumors to FGFR inhibitors, suggesting that inhibition of PRMT5 activity by MTA contributes to tumor response to FGFR inhibitors in MTAP-deficient tumors. Taken together, our current findings suggest that: 1) MTAP deficiency could serve as a rational biomarker to predict response of FGFR inhibitors and; 2) FGFR inhibitors may be highly effective therapies for patients with MTAP-deficient, FGFR3 altered urothelial cancer.

#1747 Targeting NRF2-responsive kinases for the treatment of esophageal squamous cell carcinoma.

Boopathi Subramaniyan[1], Zhaohui Xiong[2], Yahui Li[1], Chorlada Paiboonrungruang[2], Francis Spitz[1], M. Ben Major[3], Xiaoxin Chen[1]

[1]Cooper University Health Care, Camden, NJ,[2]Coriell Institute for Medical Research, Camden, NJ,[3]Washington University in St. Louis, St. Louis, MO

Esophageal squamous cell carcinoma (ESCC) ranks as the 8th most commonly diagnosed cancer and the 6th leading cause of cancer-related deaths worldwide. NRF2 has been identified as a *bona fide* cancer driver in human ESCC, with mutation rates ranging from 10% to 22%. Hyperactivation of NRF2 promotes tumor growth, metastasis, and resistance to conventional therapies in ESCC. Evidence suggests that NRF2 also regulates kinases influencing ESCC phenotypes, though the specific kinases involved are yet to be identified. NRF2 ChIP-seq analysis revealed enrichment of 42 kinases, including *Akt2* and *Pkm*, and 31 phosphatases, such as *Pten* and *Dusp16*, in the *Keap1*$^{-/-}$ mouse esophagus compared to wild-type tissue. We confirmed NRF2 binding at the promoter regions of *Akt2* and *Pte*n. mRNA expression profiling revealed enrichment of the PI3K/AKT, RAS, ERK, and GSK3β signaling pathways in the *Keap1*$^{-/-}$ esophagus relative to *Nrf2*$^{-/-}$*;Keap1*$^{-/-}$ esophagus. Immunohistochemical staining showed overexpression of pAKT, p-mTOR, pS6, and PKM2 in the esophagus of both *Keap1*$^{-/-}$ and *Sox2CreER;LSL-Nrf2*$^{E79Q/+}$ mice. Using PhosphoExplorer and PamChip arrays, we identified several kinases, including PI3K, as NRF2-responsive kinases in *NRF2*^{W24C}-KYSE70 cells compared to isogenic *NRF2*^{null}-KYSE70 cells, as well as in *Sox2CreER;LSL-Nrf2*$^{E79Q/+}$ mouse esophageal epithelium versus wild-type tissue. In *NRF2*^{null}-KYSE70 cells, expression of pAKT, pS6, p-PTEN, PIK3CA, AREG, and pEGFR was significantly downregulated compared to *NRF2*^{W24C}-KYSE70 cells. Similar results were seen in *NRF2*^{D77V}-KYSE180 cells treated with NRF2 siRNA. Furthermore, *NRF2*^{null}-KYSE70 cells showed increased sensitivity to EGFR inhibitor (Gefitinib), PIK3CA inhibitor (Alpelisib), and AKT inhibitor (Capivasertib), compared to *NRF2*^{W24C}-KYSE70 cells. Notably, Alpelisib and an NRF2 inhibitor (Pyrimethamine) synergistically suppressed *NRF2*^{W24C}-KYSE70 cell growth. Consistent with these findings, pNRF2 expression correlated with PI3K signaling proteins, including pAKT and p-mTOR, in human ESCC tissues. These studies highlight the potential of combining an NRF2 inibitor with a PI3K pathway inhibitor as a promising therapeutic strategy for ESCC.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Novel Antitumor Agents 1
Poster Session

#1749 Discovery of MOMA-313, a potent and selective inhibitor of the Polθ DNA helicase domain for the treatment of HR-deficient tumors.

Jordan A. Krall, Michael Reutershan, Vinny Motwani, Kelly McGlynn, Yonghong Bai, Christopher Quinn, Manaswni Dhingra, Cen Gao, David Waller, Salah Nabhan, Marc Ballas, Timothy Guzi, Peter Hammerman, Erica Evans

MOMA Therapeutics, Cambridge, MA

Loss of homologous recombination (HR), a core DNA damage repair pathway, is common in many types of cancer that are caused by inactivating mutations in BRCA1, BRCA2 and functionally related proteins. HR-deficient tumors are widely treated with PARP inhibitors, but the duration of response and survival benefit is often limited by tolerability issues and by the emergence of clinical resistance mediated by secondary mutations that restore HR activity. MOMA-313 is a novel, potent and selective small molecule inhibitor of DNA polymerase theta (Polθ) in development with a PARP inhibitor to drive deeper and more durable responses in HR-deficient tumors. Polθ is a DNA repair enzyme composed of both a helicase and a polymerase domain that work in tandem to repair DSBs through a process called theta-mediated end-joining (TMEJ). Polθ functions as a back-up DNA repair pathway in most contexts, and genetic ablation of Polθ is well-tolerated in mouse models and in HR-proficient cell lines. However, when HR is disabled, cells rely heavily on Polθ activity for DSB repair. Loss of Polθ activity may also prevent the emergence of clinical PARP inhibitor resistance caused by BRCA reversion mutations which have been linked to Polθ DNA repair activity. MOMA-313 is an inhibitor of the Polθ helicase domain that inhibits cellular TMEJ activity in vitro and in vivo with single-digit nM potency. MOMA-313 synergizes with PARP inhibitors against HR-deficient cell lines in vitro, with no combination impact on HR-proficient cells. Low doses of MOMA-313 drive the regression of HR-deficient tumor xenografts when combined with the PARP inhibitor olaparib, including in models with limited response to olaparib alone. A PK/PD/efficacy relationship directly links exposures required for tumor regressions to target inhibition, as determined by measuring TMEJ inhibition within tumor xenografts. Mechanistically, cryo-EM studies demonstrate MOMA-313 binds to the DNA channel, preventing DNA translocation and synapse formation between opposing ends of the broken DNA. By inhibiting the first of the two sequential Polθ DNA repair activities, MOMA-313 likely prevents the engagement of either Polθ functional domain. MOMA-313 has been optimized to achieve high target coverage at a low human dose, a strong safety profile, and broad combinability especially with PARP inhibitors, where mechanistic synergy may drive deeper responses and prevent clinical resistance in HR-deficient tumors. Clinical evaluation of MOMA-313 is ongoing (NCT06545942), with monotherapy and PARP inhibitor combination arms.

#1751 Beta catenin c-mods are orally bioavailable small-molecules targeting Wnt-driven tumors.

Isaac Klein, Kip West, Doug Baumann, Adam Talbot, Thomas Durand-Reville, Karl Hsu, Ann Boija

Dewpoint Therapeutics, Inc., Boston, MA

Constitutive activation of the Wnt/beta catenin pathway drives malignancy in a wide range of cancers. The oncogenic transcription factor beta catenin has been notoriously difficult to target due to its disordered nature and lack of defined drug-binding pockets. Using Dewpoint's proprietary AI/ML-powered discovery platform, we have identified and optimized small molecule condensate modulators (c-mods) that act via a novel mechanism of action, sequestering beta catenin into inactive condensate depots. Sequestering beta catenin into nuclear condensates selectively inhibits beta catenin-driven transcription and induces robust cancer cell death. The development candidate DPTX3186 is an orally bioavailable small molecule that demonstrates strong anti-tumor activity across multiple tumor types driven by various defects along the Wnt/beta catenin pathway and modulates Wnt pathway activity in vivo. Profound pharmacological efficacy, including regressions and complete responses, has been observed in murine models of gastric cancer. Collectively, these findings highlight the potential of condensate biology to target previously undruggable, high-value oncology targets, paving the way for novel treatments for patients with significant unmet medical needs.

#1752 SY-13380, a novel menin-MLL inhibitor, exhibits efficacy against resistant mutations and anti-tumor activity for MLL-rearranged and NPM1-mutant acute myeloid leukemia in pre-clinical models.

Xiaopeng Li[1], Tiantian Hao[2], Hongjuan Li[1], Chang Lu[1], Shikang Cheng[1], Xinhe Xu[2], Huiyan Luo[3], Xijie Liu[2], Hong Luo[3], **Yinghui Sun**[1]

[1]Department of Discovery Biology, Shouyao Holdings Co., Ltd, Beijing, China,[2]Department of Medicinal Chemistry, Shouyao Holdings Co., Ltd, Beijing, China,[3]Department of DMPK, Shouyao Holdings Co., Ltd, Beijing, China

The menin-KMT2A interaction is a critical epigenetic regulator for leukemogenesis in many acute leukemias, including KMT2A-rearranged (KMT2Ar) acute leukemia and NPM1-mutant (NPM1m) acute myeloid leukemia (AML). Patients with these alterations always have dismal response rates and poor prognosis. Some selective inhibitors of the menin-KMT2A complex have demonstrated encouraging activity in patients with R/R AML. However, early trials with revumenib have reported the rapid acquisition of resistance somatic mutations of MEN1 gene in about 40% patients which can impair treatment success. Herein, we introduce SY-13380, a novel menin-MLL inhibitor, exhibits potent binding affinity against wild type and resistant mutations and displayed excellent anti-tumor activity in pre-clinical models. Biochemical assay revealed that SY-13380 potently blocked menin-MLL binding with an IC_{50} under 10.0 nM, and it also displayed very strong potency on series of resistant mutants (M327I, M327V, G331R and T349M) similar to that on WT proteins. In cellular assays, SY-13380 also strongly inhibited cell proliferation in multiple cancer cell lines carrying KMT2Ar or NPM1m, especially in M327I mutant MOLM13 cells which was insensitive to approved Menin inhibitor SNDX-5613. In cell functional studies, SY-13380 dose-dependently down-regulated menin-MLL target gene HOXA9 and MEIS1, and induced the expression of differentiation marker CD11b. *In vivo* studies showed that treatment of SY-13380 achieved excellent tumor growth inhibition in various xenograft tumor models including MV-4-11, RS4;11, OCI AML3 and MOLM13 harboring M327I mutant menin. Notably, tumor regression was found in MV-4-11 xenograft tumor models after treatment with SY-13380 at dosage of 10mpk. In addition, SY-13380 displayed good PK parameters and tolerability *in vitro* and *in vivo* models. These data indicate that SY-13380 is a novel and potent menin-MLL inhibitor with robust anti-tumor activities, favorable PK and safety profiles making it promising for further clinical development and efficacy against resistant mutations warranting it long-term response for treatment of MLL-r and NPM1m acute leukemias.

#1753 A novel FOXM1 inhibitor inhibits FOXM1 cell proliferation, migration, and invasion tumor growth in triple negative breast cancer.

Sayra Dilmac[1], Nermin Kahraman[1], Ogun Ali Gul[1], Ferah Comert Onder[2], Bulent Ozpolat[1]

[1]Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX,[2]Department of Medical Biology, Canakkale Onsekiz Mart University, Canakkale, Turkey

Triple-negative breast cancer (TNBC) accounts for ~15-20% of breast cancer cases (52,000 women in the US only), is characterized by lack of estrogen, progesterone, and HER2 receptor expression; aggressive phenotype, high rates of early relapse and metastasis and the worst mortality rates among all breast cancer subtypes. TNBC is insensitive to the available targeted therapies, including anti-estrogens (i.e, tamoxifen, aromatase inhibitors), anti-HER2 and EGFR antibodies due to lack of actionable targets. Unfortunately, about 50-70% of the patients cannot achieve complete pathological remission in response to chemotherapy and die within the first 5 years after diagnosis. Therefore, there is a critical need for the development of highly effective and novel therapies to improve patient survival. We previously validated for the first time FOXM1 protooncogenic transcription factor as a potential molecular target in TNBC through genetic knockdown studies and demonstrated that genetic inhibition of FOXM1 completely blocks TNBC tumor growth in multiple tumor models in mice with no detected toxicity. We also found that FOXM1 expression is associated with dramatically shorter patient survival and identified it as a poor prognostic marker. We demonstrated that FOXM1 drives tumorigenesis, progression by inducing cell proliferation, invasion/metastasis, drug resistance by transactivating expression of multiple oncogenes. We found that FOXM1 is broadly overexpressed in TNBC tumors due to widespread *p53* mutations which are detected in about 84% of TNBC patients, suggesting that *TP*53 mutations are the major driver of FOXM1 overexpression. Overall data suggest that FOXM1 is an excellent potential molecular target for TNBC. However, currently, there is no FDA approved inhibitor of FOXM1**.** To identify potential inhibitors, utilizing silico docking and molecular dynamics studies we screened the chemical libraries and found identified a novel which interacted with FOXM1 protein based on in silico molecular dynamics studies and demonstrated that this inhibitor suppresses FOXM1 expression and cell proliferation, migration, invasion, and induces apoptosis in TNBC cells and more potent that well known FOXM1 inhibitor (FDI-6). *In vivo* treatment mice bearing MDA-MB-231 tumors with novel FOXM1 inhibitor (20mg/kg, twice a week) incorporated in single-lipid nanoparticles suppressed growth of TNBC tumor xenografts in mice with no toxicity. In conclusion, the findings indicate that the novel FOXM1 inhibitor represents a potent and safe therapeutic agent with significant potential for the treatment of TNBC and other FOXM1-driven cancers. This discovery offers new hope for improving outcomes in a disease that currently has limited treatment options.

#1754 Evaluating the synergism between circadian clock compounds with chemoradiation against GBM.

Priscilla Chan[1], Yoshiko Nagai[2], Qiulian Wu[3], Jiarui Wang[1], Theodore Kamenecka[4], Laura A. Solt[5], Jamie Cope[6], Tsuyoshi Hirota[2], Jeremy N. Rich[3], Steve A. Kay[1]

[1]Neurology, University of Southern California, Los Angeles, CA,[2]Institute of Transformative Bio-Molecules, Nagoya University, Nagoya, Japan,[3]University of Pittsburgh School of Medicine, Pittsburgh, PA,[4]Molecular Medicine, University of Florida, Jupiter, FL,[5]Immunology and Microbiology, University of Florida, Jupiter, FL,[6]Synchronicity Pharma, Inc., San Jose, CA

The circadian clock has been associated with numerous diseases and, in recent years, been linked to every known cancer hallmark. In the case of glioblastoma (GBM), high expression of positive regulators of the clock, such as Brain and Muscle ARNT-Like 1 (BMAL1), has been linked to shorter survival time while high expression of the negative regulators of the clock confers longer survival time. Furthermore, BMAL1 and Circadian Locomotor Output Cycles Kaput (CLOCK) were found to be essential for GBM stem cell (GSC) proliferation, stemness, and metabolism. GSCs are typically resistant to chemoradiation and secrete factors that drive neoangiogenesis and immune suppression in the tumor microenvironment (TME). However, we found that GSCs are specifically sensitive to genetic and pharmacological perturbation of these core clock components. Consequently, in this study, we sought to explore the anti-GBM effects of chemoradiation in combination with the novel Cryptochrome (CRY) stabilizer SHP1705, which inhibits BMAL1 and CLOCK transcriptional activity, and the novel REV-ERB agonist SR29065, which inhibits *BMAL1* transcription. SHP1705 has proven to be safe and well-tolerated in Phase I clinical trials and SR29065 is well-tolerated in mice without evidence of cytotoxicity. Our findings highlight the potential of small molecule circadian clock modulating compounds as a novel treatment paradigm that sensitizes resistant GBM cell populations to chemoradiation.

#1755 Discovery of a novel, selective WRN inhibitor, DAT-5000A, in the treatment of microsatellite-unstable tumors.

Jingxi Zhang, Huixian Chen, Dan Yan, Xi Chen, Jincong Zhuo, **Kevin Zhou**

Danatlas Pharmaceuticals Co., Ltd., Beijing, China

Werner syndrome ATP-dependent helicase (WRN) is a member of RecQ helicase involved in DNA replication and repair and is identified as a synthetic lethal target in microsatellite instability-high (MSI-H) tumors. MSI-H feature has been found in a variety of tumor types including colorectal, gastric and endometrial cancers. The clinical benefits of immune checkpoint inhibitor (ICI) such as anti-PD-1 or anti-PD-L1 antibodies on MSI-H cancers have been well documented; however, nearly 20-50% of these cancer patients are intrinsically resistant to PD-(L)1 therapies or develop acquired resistance with prolonged treatment. Thus, agents targeting MSI-H tumors are of great interest. Here we described the discovery of a novel non-covalent WRN inhibitor, DAT-5000A, with excellent potency and PK profile. DAT-5000A potently inhibited WRN *in vitro* activity both on ATPase and unwinding assays and showed good selectivity over BLM, another member of RecQ helicase. DAT-5000A also showed selective killing of MSI-H SW48 but not MSS (microsatellite stability) SW620 tumor cell line. In addition, DAT-5000A demonstrated good drug-like properties including high cell permeability and microsomal metabolic stability, as well as favorable PK profile with low *in vivo* clearance and optimal oral bioavailability, across multiple species. DAT-5000A exhibited single-agent activity with tumor regression on MSI-H cell-line derived xenograft (CDX) tumor models, meanwhile DAT-5000A did not cause any abnormality on these mice. Our data demonstrated that DAT5000A is a potent, selective WRN inhibitor with sufficient *in vivo* exposure and acceptable preliminary safety profile, which supports DAT-5000A to be further evaluated in human clinical trials.

#1756 A fully characterized KIF18A inhibitor with superior potency and good druggability ready for IND application.

Congcong Cai[1], Shiya Zhu[1], Chunlin Chen[2], Jian Liu[2], Hongkun Li[1], Shu Cai[1], Linjun Wang[1], Min Wen[1], Mingxin Qian[1], Shifeng Liu[1], **Bin Shi**[1], Hancheng Zhang[1]

[1]Hangzhou Innogate Pharma Co., Ltd, Hangzhou, China,[2]Shanghai Medicilon Inc, Shanghai, China

About ~90% of the solid tumors and ~75% of the hematologic malignancies are aneuploidy. The process that gives rise to aneuploidy is chromosomal instability (CIN), characterized by chromosome mis-segregation and other mitotic aberrations. KIF18A (kinesin family member 18A), part of the kinesin family of motor proteins, is expressed in the G2/M phase of the cell cycle and regulates chromosome positioning during cell division. Genetic depletion of KIF18A impacts the chromosome alignment, elongation of mitotic spindles, and traps chromosomally unstable tumor cells in mitosis, while having negligible impact on normal cells. KIF18A and CIN or whole genome doubling (WGD+, or aneuploidy) have been tested and are synthetic lethal in tumor cells. Thus, KIF18A inhibition has become an attractive approach for the treatment of the vulnerable CIN/WGD+ related tumors. Here we report the discovery and development of small-molecule KIF18A inhibitors, leading to the nomination of a preclinical candidate compound (PCC). The PCC molecule had superior *in vitro* potency, *in vivo* efficacy in cancer models, and good druggability. Thereafter we completed IND-enabling studies and submitted the pre-IND application in November 2024, with a scheduled IND filing in early 2025.

The PCC molecule exhibited potent target engagement both in enzymes and cell-based assays with IC_{50} in double digit nM in multiple cancer cell lines including breast cancer, ovarian cancer, NSCLC, and colorectal cancer. The enzyme activity is highly selective within the kinesin superfamily members (CENP-E, Eg5, Chromokinesin, KIF3C, KIFC3, MCAK and MKLP) with IC_{50} all >30 μM. In the 435-kinase panel screening, 1 μM of the PCC molecule did not significantly inhibit any of the 435 kinases (<25% inhibition in all tested kinases), demonstrating great selectivity in the kinase panel. The PCC molecule also exhibited a good in vitro safety profile in hERG assay and in the 44-enzyme/receptor/ion-channel safety panel profiling. The PCC molecule proved desirable in vitro ADME characteristics and excellent in vivo pharmacokinetic profile with low clearance, long half-life, as well as good oral bioavailability in mice, rats, beagle dogs and non-human primates. Furthermore, The PCC molecule exhibited excellent in vivo efficacy in CIN+ ovarian cancer OVCAR3 CDX model, and two CIN+ triple negative breast cancer (MDA-MB-231 and HCC1806) models, without body weight loss and other safety issues observed. These anti-tumor efficacies were dose-dependent and well correlated with a robust pharmacodynamic (PD) western blot detection using phosphor-H3, a mitotic marker. In non-GLP and GLP-tox studies in rats and beagle dogs, the PCC molecule was well tolerated, providing a potential good therapeutic window. Overall, a novel and highly potent KIF18A inhibitor is ready to enter phase 1 clinical trials in 2025 for the treatment of CIN/WGD+ cancers.

#1758 ATX-559, a first in class DHX9 inhibitor, and targeted therapeutic for molecularly defined tumors with genomic instability and replicative stress.

Jennifer Castro, Matthew H. Daniels, Sunaina Nayak, David Brennan, Cindy Collins, Sophie A. Shen, Monique Laidlaw, Jie Wu, Anugraha Raman, Deepali Gotur, Kevin Knockenhauer, Shihua Yao, Simina Grigoriu, Gordon J. Lockbaum, Kate Newberry, Stephen J. Blakemore, P. Ann Boriack-Sjodin, Kenneth W. Duncan, Stuart Ince, Jason A. Sager, Robert A. Copeland, Serena J. Silver

Accent Therapeutics, Lexington, MA

DHX9, a multi-functional RNA helicase, contributes to genome stability by unwinding nucleic acid secondary structures, including DNA/RNA hybrids (R-loops), circular RNA and G-quadruplexes. Multiple cancer types exhibit increased expression of DHX9, and selective dependance to DHX9 has been observed in molecular defined tumors that exhibit genomic instability. These tumors often have elevated replicative stress due to rapid unregulated proliferation and defective DNA damage repair (DDRD) pathways; DHX9 inhibition in these tumors further increases replicative stress and DNA damage to catastrophic levels. We previously demonstrated that DHX9 genetic loss or inhibition by the DHX9 tool compound ATX968 was efficacious in tumor types that exhibit DDRD and/or genomic instability; these include tumors with defective mismatch repair and/or high microsatellite instability (dMMR/MSI-H) or alterations in the DDR genes BRCA1 and/or BRCA2 (BRCA).

Here we describe ATX-559, a potent, selective, orally bioavailable, small-molecule inhibitor of DHX9 helicase activity that is currently in clinical development. In preclinical studies, ATX-559 treatment leads to increased accumulation of R-loops and G-quadruplexes resulting in increased replicative stress and stalling, subsequent DNA damage, and cell death selectively in cancer models with high genomic instability. In a panel of 36 colorectal (CRC) and endometrial cancer (EC) cell lines, approximately 55% were sensitive ($IC_{50} \leq 1$ μM) to ATX-559 treatment, with enriched antiproliferation activity (80%) in dMMR/MSI-H cells. ATX-559 sensitivity was also enriched in breast cancer cell lines, including 76% of BRCA altered triple negative breast cancer (TNBC) models.

ATX-559 is well tolerated *in vivo* across multiple cell line-derived xenograft (CDX) and patient-derived xenograft (PDX) models, when dosed orally BID for periods out to 28 days or more. Robust dose-dependent tumor growth inhibition (TGI) and regression are selectively observed in CRC and EC dMMR/MSI-H and BRCA altered TNBC CDX models. TGI is correlative with plasma and tumor drug exposure, as well as with induction of the target engagement PD biomarker, circBRIP1, in both tumors and mouse PBMCs. ATX-559 was also profiled in a PDX mouse clinical trial design *in vivo* study of 46 models including TNBC, CRC, EC, and gastric cancers. Oral treatment of 45 mg/kg BID demonstrated enriched sensitivity in BRCA altered TNBC (75%) and dMMR/MSI-H (68%) PDX models.

Together, these findings represent preclinical pharmacological validation of DHX9 as a selective therapeutic target in cancers that exhibit genomic instability and replication stress. ATX-559 is currently in a first-in-human, Phase 1, open-label, dose-escalation and expansion study in patients with locally advanced or metastatic solid tumors and molecularly defined cancers (NCT06625515).

#1761 SYNB011128: A novel bispecific peptide-drug-conjugate targeting EGFR and CAIX.

Caihong Zhou[1], Michael Poss[2], Cheng Lu[1], Ruochi Zhang[3], Yu Wang[3], Yuanpeng Xiong[3], Xin Gao[3], Xiao Zhang[3], **Yan Degenhardt**[2]

[1]Syneron Tech, Shanghai, China,[2]Syneron Tech, Lawrenceville, NJ,[3]Syneron Tech, Beijing, China

Peptide-drug-conjugates (PDCs) are emerging as promising alternatives to antibody-drug-conjugates (ADCs) in oncology. PDCs, with their smaller size (<10 kDa), can offer improved tumor penetration. Additionally, PDCs can potentially exhibit a better safety profile due to their shorter blood PK, absence of an Fc domain, and reduced immunogenicity. Furthermore, PDCs can be entirely chemically synthesized, providing a homogeneous chemical entity. Epidermal Growth Factor Receptor (EGFR) is a key target that is overexpressed in many tumor types. Current EGFR inhibitors primarily focus on mutant forms, creating a treatment gap for tumors with only wild-type overexpression. Carbonic Anhydrase IX (CAIX) represents another cancer-specific marker that supports tumor survival in hypoxic or acidic conditions and is frequently co-overexpressed with EGFR in cancers such as non-small cell lung cancer (NSCLC), head and neck cancers, and pancreatic cancers. SYNB011128, a bispecific PDC, exhibits high affinity and specificity for binding both EGFR and CAIX. Upon internalization, SYNB011128 releases MMAE, a potent tubulin inhibitor, through linker cleavage by tumor-specific proteases. In total, SYNB011128 employs three primary anti-cancer mechanisms: 1) Competing with EGF to inhibit the EGFR pathway. 2) Inhibiting CAIX enzyme function, crucial for cancer cell survival in hypoxic environments and resistance clone development. 3) Delivering the cytotoxic MMAE payload. Preclinical studies showed that SYNB011128 achieved complete tumor regression in multiple pancreatic and colon cancer models, outperforming EGFR-antibody-drug conjugates (EGFR-ADCs). Additionally, SYNB011128 showed efficacy in models of Osimertinib-resistant non-small cell lung cancer (NSCLC). With a molecular weight of 4 kDa, SYNB011128 exhibited rapid and deep tumor penetration. PDC induced biomarker was detected in both the periphery and core of the tumor within 6 hours post-dosing. Despite its relatively short blood half-life, SYNB011128 ensured sustained accumulation of the payload within tumors. This pharmacokinetic profile may reduce on-target toxicity and enhance safety. In mouse studies, SYNB011128 demonstrated no adverse effects, such as weight loss or liver/kidney damage, after 28 days of treatment at doses that induced tumor regression. Chemically, SYNB011128 is synthesized as a single enantiomer, without diastereomeric isomers. In summary, PDC SYNB011128 effectively targets both EGFR and CAIX, utilizing multiple anti-cancer mechanisms and achieving deep tumor penetration, which may lead to robust clinical efficacy. By binding to two cancer antigens to enhance tumor selectivity over normal tissues, and a pharmacokinetic profile of sustained tumor accumulation vs a short blood half-life, SYNB011128 may also have an improved safety margin.

#1762 Preclinical and clinical studies of PEP07, a novel brain penetrating oral Chk1 inhibitor, on AML and MCL treatments.

Hui-Ling Chen, Chieh-Fang Cheng, Feng-Yu Lee, Cheng-Hao Liu, Hong-Ren Wang

PharmaEngine Inc., Taipei, Taiwan

PEP07 is an orally available and brain penetrating selective Chk1 inhibitor. Chk1 is involved in the DNA damage response and cell cycle regulation and helps maintain the integrity of the genome during cell division, especially in response to DNA damage or replication stress. Acute myeloid leukemia (AML) is characterized by an impaired DNA damage response (DDR) pathway and elevated Chk1 expression, which correlates with poor patient outcomes. Likewise, mantle cell lymphoma (MCL), a rare and aggressive form of Non-Hodgkin lymphoma, features a chromosomal translocation that leads to cyclin D1 overexpression. Since Chk1 regulates cdk1/cyclin activity, Chk1 inhibitors have been proposed as a novel therapeutic approach in this cancer.

In addition to *in vitro* inhibitory effect on multiple hematology cancer cell lines, PEP07 has been demonstrated as monotherapy to show tumor growth inhibition and tumor regression in the AML and MCL xenograft models, respectively. It also showed enhanced tumor growth inhibition in the cytarabine-resistant AML xenograft model when combined with cytarabine.

PEP07 is currently being evaluated in clinical studies for both hematologic and solid cancers. PEP07-101 (NCT05659732), a Phase 1b, open-label, multi-center PK/PD and dose-escalating study, employs an accelerated titration design combined with a traditional 3+3 approach. This study is ongoing in multiple countries and evaluates oral PEP07 as monotherapy for AML and MCL. With its ability to penetrate the blood-brain barrier (BBB), PEP07 is also being considered for investigating treatment options for brain cancer in clinical settings.

#1763 Arsenoplatin-7, a family member of arsenoplatins, as a novel therapeutic strategy to overcome platinum-resistance in ovarian cancer.

Wenan Qiang[1], Denana Miodragovic[2], Yi Yang[1], Bong Jin Hong[3], Chi-Hao Luan[1], David Fang[4], Daniela E. Matei[5], Thomas V. O'Halloran[3]

[1]Chemistry Life of Processes Institute, Northwestern University - Evanston, Evanston, IL,[2]Chemistry, Northeastern Illinois University, Chicago, IL,[3]Microbiology, Genetics & Immunology; Chemistry, Michigan State University, East Lansing, MI,[4]Chemistry, Northwestern University - Evanston, Evanston, IL,[5]Obstetrics & Gynecology, Northwestern University -Chicago, Chicago, IL

Ovarian cancer (OC) is the fifth leading cause of cancer death in women. OC inevitably acquires resistance to platinum-based chemotherapy, leading to treatment failure and disease progression. There is an unmet need to overcome platinum OC resistance. Arsenic trioxide (ATO) is an approved drug for treating acute promyelocytic leukemia, but not in solid tumors. Arsenoplatins (APs), a family of novel anticancer dual-pharmacophore agents containing ATO in the form of arsenous acid attached to a platinum center, can deliver ATO into the tumor cells. APs have previously shown in vitro potency in leukemia, breast, and ovarian cell lines in NCI-60 screens. It was hypothesized that APs (AP-1 and AP-7) could have therapeutic effects in cisplatin (CDDP) resistant OC due to the unique nature of the drug delivery. Three paired CDDP-sensitive and resistant OC cell lines (A2780, SKOV3, and OVCAR5) were used to test the potency of AP-1 and AP-7 compared to CDDP, ATO, or a combination of both. In addition, a single dose of maximum tolerated dose (MTD) was determined in mice, and the efficacy of AP-1 and AP-7 was evaluated in clinically translational platinum-resistant OC PDX tumor models. RNA-seq analysis of tumor tissue and in silico molecular docking was further studied to explore the mechanism of action to identify potential biomarkers for AP-7 therapy. AP-1 and AP-7 demonstrated great potency and showed similar cell growth inhibition in all tested CDDP-resistant cell lines. AUC, a parameter of combined measurement of both Emax and IC_{50}, was used to rank the effectiveness of APs in these cell lines, with ATO being the most potent, followed by a combination of ATO and CDDP, then AP-1, AP-7, and CDDP was the least potent. Compared to the MTD for CDDP and ATO in mice, APs have much lower toxicity(higher MTD). AP-7, but not AP-1, demonstrated efficacy in the platinum-resistant OC PDX tumor study. AP-7 showed significant tumor volume reduction starting from week 3 ($P<0.01$); at the end of 5 weeks, there was a significant reduction of 60.78% ($P<0.001$) in tumor volume and 42.80% ($P<0.01$) in tumor weight over control. No body weight change was observed throughout the study. Molecular docking of AP-7 with GLI1 revealed that AP-7 binds to a pocket in proximity to the Zn-binding site of GLI1, potentially disrupting Zn coordination by interacting with key cysteine and histidine residues. The RNA-seq sequencing from AP-7 treated PDX tumor tissues was completed, and data analysis is ongoing for differential gene expressions and alternative splicing. Our preliminary results indicate that AP-7 is a potential therapeutic agent for overcoming platinum resistance in OC by delivering arsenous acid to tumor cells with greater efficacy and less toxicity. However, the prediction of potential target GLI1 warrants further validation.

#1764 A highly potent novel STAT5 and STAT3 direct dual inhibitor in acute myeloid leukemia.

Eunhye Hong, Minjung Kong, Hyeryung Kang, Minhee Kim, So Jung Son, Sungjae Choi, Kyeong Eun Kim, Woon-Ki Cho, Chae Won Kang, gijeong Seong, A Young Ji, Seon Ho Koh, Sehee Cha, Sangyeop Hyun, Ji Min Shin, Eori Nam, Yeon Ju Hong, Chan-Hee Park

C&C Research Laboratories, Gwacheon-si, Korea, Republic of

Background: Acute myeloid leukemia (AML) is a heterogeneous hematologic malignancy caused by abnormal differentiation and uncontrolled proliferation of myeloid cells, along with various mutations. FLT3-ITD mutation, found in approximately 25-30% of AML cases, is known to be associated with poor prognosis, including a high risk of relapse and shorter overall survival. Furthermore, acquired point mutations in tyrosine kinase domain (TKD), such as the 'gatekeeper' mutation (F691L), is considered as a main culprit of acquired resistance to FLT3 inhibitors in clinic. Different mechanisms of resistance have been reported in FLT3 inhibitor treated patients in clinic and novel strategies have been tried to overcome the resistance issue. Here we report novel direct STAT5 and STAT3 dual inhibitor, CNC-01 would be promising option for FLT3mt AML patients having acquired resistance to FLT3 inhibitors.
Methods: We developed CNC-01, a selective dual inhibitor targeting STAT5 and STAT3, which is an orally available small molecule capable of overcoming drug-resistant FLT3-TKD mutations. We established FLT3 inhibitor-resistant models using OCI-AML5 cell lines, incorporating the gatekeeper point mutation (F691) and continuously interleukin 3 (IL3)-expressing cells to mimic the bone marrow microenvironment. The direct binding to STAT5 and STAT3 was confirmed using SPR assay and cryo-EM analysis. We evaluated anti-tumor effects and target gene expression inhibition compared to Gilteritinib, and also assessed its efficacy in Venetoclax non-responsive models.
Results: CNC-01 directly binds to STAT5 and STAT3 proteins, which are implicated in resistance to FLT3 inhibitors. By inhibiting pY694-STAT5 and pY705-STAT3, CNC-01 abrogates pro-tumorigenic transcriptional activities mediated by these proteins. Furthermore, CNC-01 demonstrated potent anti-tumor effects and inhibited target gene expression more effectively than Gilteritinib. Synergistic effects were observed when CNC-01 was combined with Venetoclax, a major treatment option for AML. Additionally, CNC-01 showed high anti-tumor effects in Venetoclax non-responsive models.
Conclusion: CNC-01, a novel selective direct STAT5 and STAT3 inhibitor, shows promising results in overcoming FLT3 inhibitor resistance and demonstrates efficacy in Venetoclax-resistant models, too. CNC-01 is under preclinical stage and is expected to be highly promising treatment option for AML patients. This program is to submit Phase 1 IND next year.

#1765 RPT1G, a first-in-class hyperbolic NAMPT inhibitor that avoids on-target toxicity.

Gregory Thomas Crimmins[1], Min Wu[1], Timothy Jarvela[1], Yushma Bhurruth-Alcor[1], James Digel[1], Francis Roushar[1], Claudia Ramos Aguilar[1], Dexter Davis[1], Caroline M. Robb[1], Michael Schelle[1], Banumathi Cole[1], Frank McCormick[2], Dennise A. De Jesus-Diaz[1]

[1]Remedy Plan Therapeutics, Gaithersburg, MD,[2]UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA

Therapeutic inhibition of NAD^+ synthesis has been challenging to achieve in the clinic due to severe on-target toxicity stemming from the critical role of NAD^+ in living systems. The elevated energy requirements of cancer cells necessitate increased NAD^+ recycling through the nicotinamide phosphoribosyltransferase (NAMPT) salvage pathway. Drug candidates have attempted to reduce NAD^+ in cancer cells by complete inhibition of NAMPT, resulting in systemic, dose-limiting toxicity. RPT1G is a first-in-class hyperbolic inhibitor that significantly reduces, but does not eliminate, NAMPT activity. This unique mechanism-of-action reduces NAD^+ below survival thresholds in cancer cells while allowing survival of healthy tissues, manifest by limited on-target toxicity in GLP studies and robust therapeutic efficacy in multiple oncological models. RPT1G has been approved to start clinical testing in healthy volunteers. This first-in-human study will determine the safety and pharmacokinetics of hyperbolic NAMPT inhibition while measuring metabolic changes resulting from reduced NAMPT activity. RPT1G represents the first hyperbolic NAMPT inhibitor to be tested in humans, opening opportunities to therapeutically modulate NAD^+ synthesis and providing a roadmap for the development of hyperbolic inhibitors of other essential enzyme targets.

#1766 PE-0260, an oral brain-penetrant MTA-cooperative PRMT5 inhibitor, drives tumor regressions in MTAP-deleted solid tumors.

Cheng-Hao Liu, Hui-Ling Chen, Chieh-Fang Cheng, Feng-Yu Lee, Long-Zhi Lin, Hong-Ren Wang

PharmaEngine Inc., Taipei, Taiwan

PE-0260 is a potent and selective oral methylthioadenosine (MTA)-cooperative protein arginine methyltransferase 5 (PRMT5) inhibitor leveraging the synthetic lethal relationship of PRMT5 with methylthioadenosine phosphorylase (MTAP) gene deletion. The well-studied synthetic lethality is an attractive approach for multiple cancer treatments due to the 10-15% incidence rate of *MTAP* deletion in all cancers. PE-0260 shows good potency of MTAP del cell viability (<5 nM IC_{50}) and >200X selectivity compared with isogenic MTAP WT cells. In a diverse cell line panel, PE-0260 selectively inhibits MTAP del cell lines over MTAP WT cell lines with median IC_{50} < 30 nM and >50X selectivity across NSCLC, GBM, pancreatic and breast cancers. This outstanding potency was successfully translated to dose-dependent anti-tumor activities observed in xenograft models with tumor regressions, and significant inhibition of symmetric dimethylarginine (SDMA) modified protein formation in tumors. Through the PK-PD analysis, on-target driven efficacy has been demonstrated, suggesting a low likelihood of off-target toxicity. PE-0260 also shows balanced *in vitro* ADME properties with low risk for drug-drug interactions and the capability to penetrate the blood-brain barrier. In conclusion, PE-0260 is a potential best-in-class MTA-cooperative PRMT5 inhibitor and a strong candidate for further clinical development.

#1767 Discovery of novel STING allosteric activators for targeted cancer immunotherapy.

J. Lee[1], E. Kim[2], S. Y. Baek[1], W. Park[2], J. Lee[1], Y. S. Kim[1], **E. Kim**[2], S. Lee[1];
[1]Korea Institute of Science and Technology, Seoul, Korea, Republic of, [2]Ajou University, Suwon, Korea, Republic of

The Stimulator of Interferon Genes (STING) serves as a pivotal regulator of innate immunity, but the therapeutic modulation of this pathway poses a risk of triggering hyperimmune responses. To address this challenge, our study explores a natural STING agonist (cGAMP)-dependent STING allosteric activator as a potential solution. This novel allosteric activator demonstrates synergistic efficacy specifically under the low cGAMP conditions across various rodent and human cell lines. The compound induces structural modification in STING, enhancing its sensitivity to minimal cGAMP levels and thereby elevating type-1 interferon production.
In vivo studies reveal that the compound enhances type I interferon induction, promotes a shift from innate to adaptive immune responses, and increases lymphocyte infiltration into tumors, leading to significant tumor regression with tumor-specific immune memory in a mouse model. In addition, co-administration with an immune check point inhibitor markedly enhances immuno-oncological efficacy. These findings underscore the potential of allosteric STING activators to expand the therapeutic index of cancer immunotherapy, offering new insights into the development of safer and more effective agents in cancer immunotherapy.

#1768 Pharmacological inhibition of METTL1 as a novel therapeutic strategy against aggressive malignancies.

Eliza Yankova[1], Alexandra Sapetschnig[2], Beth Thomas[2], Sian Evans[1], Maria Eleftheriou[1], James Russell[1], Vidur Tandon[1], Natalie Webster[2], Harry Fischl[2], Sarah Bucknell[2], George S. Vassiliou[1], Walid T. Khaled[1], Maria P. Alcolea[1], Oliver Rausch[2], Konstantinos Tzelepis[1]

[1]University of Cambridge, Cambridge, United Kingdom,[2]Storm Therapeutics, Cambridge, United Kingdom

METTL1 catalyzes N7-methylation of guanine (m^7G) in a subset of transfer RNAs (tRNAs). We have shown that METTL1 is a promising therapeutic target for various malignancies, including acute myeloid leukemia (AML), melanoma, and pancreatic ductal adenocarcinoma (PDAC). Here, we present characterization of STM9005, a novel small-molecule inhibitor of METTL1, as an effective therapeutic strategy against cancer.**Aims:** To evaluate the therapeutic potential of STM9005, a novel and bioavailable METTL1 tRNA methyltransferase inhibitor, in aggressive malignancies.

Methods: High-throughput screening and subsequent chemical optimization identified STM9005 as a potent small-molecule inhibitor of the catalytic activity of METTL1. STM9005 was tested *in vitro* to assess its impact on cancer models, including effects on growth, differentiation, and cell cycle regulation. RNA sequencing, m^7G profiling of tRNAs, and proteome analysis identified molecular pathways affected by the inhibition of METTL1. *In vivo* experiments assessed therapeutic efficacy in solid and hematological cancer models.

Results: The impact of STM9005 was measured by cellular mechanistic assays and modification-induced misincorporation tRNA sequencing, revealing decreased m^7G methylation and lower levels of target tRNAs. The efficacy of STM9005 was determined in a panel of cancer models, including AML, melanoma, and PDAC. METTL1 inhibition impaired cell proliferation and viability in multiple cancer models, with no effects in non-cancerous cells, suggesting a cancer-specific mechanism of action. *In vitro* treatment with STM9005 led to significant differentiation of AML, suggesting an important connection between m^7G and status of oncogenesis. STM9005 treatment also reduced cell cycle progression and downregulated cell cycle oncogenes. Mechanistic interrogation using m^7G profiling of tRNAs, RNA sequencing, and proteome analysis identified drug-specific changes at both RNA and protein levels. *In vivo* characterization of STM9005 demonstrated excellent bioavailability in mice with no detectable toxicity. We evaluated its anti-cancer effects across a range of aggressive cancer models, including melanoma, PDAC, and AML patient-derived xenografts (PDX). Daily administration of STM9005 significantly inhibited disease progression, indicating potent anti-cancer activity. Target engagement was confirmed by reduced m^7G levels on METTL1-dependent tRNA substrates.

Summary We describe STM9005 as a first-in-class inhibitor targeting the tRNA methyltransferase METTL1. Small-molecule inhibition of METTL1 demonstrates potent anti-cancer effects *in vitro* and *in vivo* using physiologically and clinically relevant models of advanced malignancies. These findings provide proof-of-concept that pharmacological inhibition of METTL1 offers a novel therapeutic strategy for aggressive cancers with unmet clinical needs.

#1769 A selective BRM (SMARCA2) inhibitor for the treatment of BRG1 (SMARCA4) mutant cancers.

D. Zhou, Z. Wang, Y. Liu, T. Fu, Z. Cheng;
Zion Pharma Limited, Shanghai, China

Background: ATP-dependent BRG1/BRM associated factor complexes play a key role in regulating gene expression by remodeling chromatin. BRG1 is mutated in ~5% of all tumors, and in ~7-10% of NSCLC tumors. BRM is essential for the growth of tumors that harbor loss of function BRG1 mutations. BRM and BRG1 ATPase share high protein-sequence homology (76% similarity), particularly in the ATPase domain (92% similarity). The Phase 1 clinical trial results of a BRG1/BRM dual inhibitor, in patients with BRG1 mutated cancers, showed potential safety risks. Improved safety margins may be achieved through the selective suppression of BRM activity. We identified a selective and potent BRM inhibitor (ZN-7035) with an excellent safety margin.
Methods: The inhibition potency of ZN-7035 against isolated recombinant full-length BRM or BRG-1 ATPase activity was assessed by ADP-Glo assay. BRM-dependent KRT80 gene-expression in a BIG1-mutant NCI-H838 cell line was measured by Real-Time PCR. In vivo pharmacology was conducted to evaluate the efficacy of ZN-7035 oral administration monotherapy and in combination with a MCL1 inhibitor or chemotherapy in NCI-H838 NSCLC xenograft models. The pharmacokinetics of ZN-7035 was evaluated in mice, rats and dogs. The toxicity of ZN-7035 was assessed in 28-day orally administered repeat-dose studies in Sprague Dawley rats. Safety margins were determined based on the ratio of unbound AUC obtained from 28-day toxicology studies and predicted human PK at efficacious dose.
Results: ZN-7035 exhibited strong inhibition (IC_{50}=3.3 nM) against BRM ATPase as well as potent inhibition (IC_{50}=8.3 nM) of KRT80 mRNA expression, a PD biomarker of BRM. ZN-7035 monotherapy demonstrated anti-tumor activity and synergistic tumor growth inhibition in combination with AMG176, a MCL1 inhibitor, in an NCI-H838 xenograft model carrying BRG1 loss-of-function mutation. KRT80 PD biomarker modulation in NCI-H838 established an exposure-response relationship. Further, ZN-7035 (20mg/kg) free concentration in plasma covers KRT80 IC_{90} for ~17-19h and achieved 80% tumor inhibition. In addition, ZN-7035 demonstrated dose dependent anti-tumor activity and synergistic tumor growth inhibition in combination with chemotherapy in NSCLC xenograft models. In these studies, ZN-7035 demonstrated excellent pharmacokinetic and safety properties. Due to good BRG1 selectivity, ZN-7035 was well tolerated in rats at 120 mg/kg/day following repeat dosing for 28 days. Based on DMPK and rat DRF data, the predicted human efficacious dose is 115 mg BID, and the predicted human safety margin is >30-fold.
Conclusion: ZN-7035 is a highly potent, selective, orally available, small-molecule, enzymatic inhibitor of BRM. Preclinical findings highlight that ZN-7035-based combination therapies have the potential to be effective and safe treatments for patients with tumors harboring BRG1 mutations.

#1770 HM100760, a potent and selective MAT2A inhibitor, demonstrates a synergistic anticancer effect when combined with a PRMT5 inhibitor in MTAP-deleted cancer.

Minjeong Kim, Eun Joo Kwak, Miyoung Lee, Soonki Park, Hobin Lim, Soyeon Kim, Hye Sun Han, Seung Hyun Jung, Jooyun Byun, Sang Hyun Lee, Young Gil Ahn

Hanmi Pharmaceutical Co., Ltd., Hwaseong-si, Korea, Republic of

Methionine adenosyltransferase 2A (MAT2A) is the primary enzyme responsible for the synthesis of S-adenosylmethionine (SAM) from methionine and ATP. SAM serves as a universal methyl donor in various transmethylation and biosynthetic pathways. Recently, MAT2A has emerged as a synthetic lethal target in cancers harboring deletions of the methylthioadenosine phosphorylase (MTAP) gene, which occurs in approximately 15% of all cancers.

MTAP deletion results in the accumulation of methylthioadenosine (MTA), which partially inhibits protein arginine methyltransferase 5 (PRMT5). Inhibition of MAT2A in MTAP-deficient cancers leads to reduced intracellular SAM levels, which sensitizes PRMT5 to further inhibition. Likewise, this interplay offers a strong rationale for the combinational use of MAT2A inhibitors, which block SAM synthesis, with MTA-cooperative PRMT5 inhibitors.

Here, the biochemical activity of HM100760, a potent and selective MAT2A inhibitor, was evaluated in terms of its effect on MAT2A enzymatic function, cellular SAM levels, and symmetric dimethylarginine (SDMA) regulation. HM100760 exhibited strong inhibition of MAT2A catalytic activity, leading to significant reductions in SAM and SDMA levels in HCT116 MTAP-null cells. In addition, HM100760 displayed robust anti-proliferative effects across a range of MTAP-deficient cancer cell lines, including those derived from pancreatic, bladder, and non-small cell lung cancer (NSCLC).

In preclinical in vivo studies, HM100760 demonstrated dose-dependent tumor growth inhibition in multiple MTAP-null xenograft models, including HCT116, KP-4, and LU99. Moreover, combining HM100760 with a PRMT5 inhibitor in the endogenous MTAP-null LU99 xenograft model resulted in a synergistic antitumor effect, outperforming monotherapy treatments. Notably, HM100760 achieved this therapeutic efficacy without causing significant body weight loss or observable clinical symptoms, underscoring its favorable safety profile.

In conclusion, HM100760 is considered a promising therapeutic candidate for MTAP-deleted cancers. It exhibits potent antitumor activity as a monotherapy and demonstrates enhanced efficacy when combined with a PRMT5 inhibitor. Ongoing preclinical studies aim to further characterize HM100760’s therapeutic potential and broaden its application in MTAP-deficient cancer treatment.

#1771 Ecubectedin's role in targeting transcriptional regulators in neuroendocrine prostate cancer.

Laura Almale[1], Maria Jose Guillen[2], Paula Diaz[3], Ismael Fernandez-Miranda[2], Paula Iglesias[1], Marcelo Lima Ribeiro[2], Maria Ovejero-Sanchez[3], Elena Castro[1], Pedro P. Lopez-Casas[1], Carmen Cuevas[2], Pablo M. Aviles[2], David Olmos[1]

[1]Prostate Cancer Genomics and Therapuetics Research Group, Instituto de Investigacion Hospital 12 de Octubre, Madrid, Spain,[2]Pharma Mar, S.A., Madrid, Spain,[3]Translational Research in Cancer Genetics Group, Instituto de Investigacion Hospital 12 de Octubre, Madrid, Spain

Introduction: Neuroendocrine prostate cancer (NEPC) is a highly aggressive variant of prostate cancer with poor clinical outcomes and limited treatment options, highlighting the urgent need for therapies targeting its unique molecular profile of ecubectedin, a novel inhibitor of transcription from the ecteinascidin's family, in both *in vitro* and *in vivo* models of NEPC. **Methodology:** Human NEPC cells, NCI-H660, along with eight NE-SCLC cell lines (as the well-known NE model) were treated with ecubectedin for 72 hours. Cell viability was assessed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) short-term assay. Treatment-induced changes in gene expression profiles were analyzed by bulk RNA sequencing. *In vivo* antitumoral activity of ecubectedin was evaluated in in two human-derived NEPC models: H660 (cell-derived xenograft or CDX) and CP-IBI-034 (a transformed NEPC patient-derived xenograft or PDX obtained from a liver metastasis, confirmed histologically). Both H660 and CPI-IBI-034 were implanted subcutaneously (s.c.) in athymic *nu/nu* mice. Ecubectedin/placebo was intravenously (i.v.) administered (at 0.75 mg/kg) on days 0, 7 and 14 in n=6-8 mice per arm and model. **Results:** Ecubectedin markedly reduced NCI-H660 cell viability in similar proportions to NE-SCLC, with an average IC_{50} of 2.3 nM. At a transcriptional level, ecubectedin induced significant downregulation of key NE regulators, including ASCL1, NEUROD1, POU3F2, MYCN, EZH2 and SOX2, in both NEPC and NE-SCLC. In CP-IBI-034 tumor bearing mice, ecubectedin significantly reduced (*vs* placebo) the tumor volume during the experimental phase (up to Day 35) with a 22% of ecubectedin-treated mice resulting in complete tumor remissions. In H660 xenografts, a significantly antitumor activity (*vs* placebo) was also observed with complete tumor remissions in 100% of mice that lasted until the end of the experiment (Day 91). **Conclusions:** Ecubectedin may impact transcriptional networks driving the cell plasticity and NE transformation in NEPC tumors. More importantly, a significant *in vitro* and *in vivo* antitumor activity of ecubectedin was observed in NEPC models, suggesting a significant potential as a promising novel therapeutic for this indication associated to very poor outcomes.

#1772 A novel small molecule inhibitor, HO-4200, modulates macrophage polarization and suppresses MDSCs to enhance antitumor immune responses in ovarian cancer.

Ganesh Yadaigiri, Kalpana Deepa Priya Dorayappan, Lakshmi Narasimhan Chakrapani, Shyam Sundaram, Jessica Velasquez, Casey Cosgrove, David M. O'Malley, David E. Cohn, Selvendiran Karuppaiyah

Obstetrics and Gynecology, The Ohio State University Wexner Medical Center, Columbus, OH

Introduction: Ovarian cancer (OC) remains the deadliest gynecologic malignancy, with minimal improvement in the 5-year survival rate over the past three decades despite advancements in treatment. High-grade serous ovarian cancer (HGSOC) is characterized by aggressive progression, driven by elevated secretion of extracellular vesicles (EVs), tumor-associated macrophage (TAM) activation, and increased myeloid-derived suppressor cell (MDSC) populations. These factors contribute to immune suppression, metastasis, and poor patient outcomes. Here, we report on a novel small-molecule inhibitor, HO-4200, which modulates macrophage polarization and suppresses MDSCs within the ovarian tumor microenvironment.
Methods: EV quantification and size characterization were performed using nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM). In vivo studies using immunocompetent mice evaluated HO-4200's anticancer efficacy. Macrophage polarization and MDSC populations were analyzed via flow cytometry. The effects of HO-4200 on ovarian cancer and primary ascites-derived cell lines were examined in vitro. Additionally, expression levels of EV secretion pathway proteins TMEM205 and Rab27a were measured by ELISA and RT-PCR.
Results: HO-4200 demonstrated significant antiproliferative effects, reducing ovarian cancer cell viability by 60-75% at concentrations of 2-5 μM after 48 hours. HO-4200 also inhibited the expression of EV secretion pathway proteins TMEM205 and Rab27a in ovarian cancer cells. Mechanistic studies revealed that HO-4200 promoted M1 macrophage polarization and reduced MDSC populations in vitro and in vivo immunocompetent mouse models. These findings suggest that HO-4200 suppresses EV secretion, thereby enhancing antitumor immune responses and reducing tumor progression and metastasis in ovarian cancer.
Conclusion: This study provides the first evidence that HO-4200 inhibits ovarian cancer cell proliferation, promotes M1 macrophage polarization, and reduces MDSC populations by targeting EV secretion pathway proteins. These results highlight the therapeutic potential of HO-4200 in modulating the immune microenvironment and combating ovarian cancer progression and metastasis.

#1773 PM54, a novel transcription inhibitor with promising broad-spectrum antitumor activity.

Marta M. Diez[1], Gema Santamaria Nunez[1], Tommy Darriere[2], Federico M. Ruiz[2], Carlos Fernandez-Tornero[2], Ismael Fernandez-Miranda[1], Maria Jose Guillen[1], Pablo M. Aviles[1], **Marcelo Lima Ribeiro**[1], Carmen Cuevas[1]

[1]Pharma Mar, S.A., Madrid, Spain,[2]Centro de Investigaciones Biologicas Margarita Salas, CSIC, Madrid, Spain

PM54, a novel synthetic member of the ecteinascidin family, is derived from lurbinectedin, a marine-derived compound recognized for its potential in cancer therapy. This study aimed to characterize the *in vitro* antitumor activity of PM54, elucidate its mechanism of action through transcriptomic profiling and validate its antitumor efficacy *in vivo*. The antineoplastic activity of PM54 was assessed using a panel of 37 cancer cell lines representative of breast, gastric, melanoma, ovary, small cell lung cancer (SCLC), and prostate cancers through MTT assays. Cell cycle analysis, as well as apoptosis assessment, were both performed after a 24-h incubation period with PM54 at 50 nM by FACS. DNA binding capacity was assessed using electrophoretic mobility shift, DNase I footprinting and FRET-based assays, with further interaction mode evaluation by X-ray analysis. ^{3}H-uridine incorporation assays were conducted to measure mRNA synthesis. RNA-seq was used to analyze gene signatures associated with 6-h of incubation with 50 nM of PM54. The *in vivo* antitumor efficacy of PM54 was characterized in relevant xenograft models of breast, gastric, melanoma, ovary, SCLC, and prostate cancers. PM54 exhibits potent *in vitro* antitumor activity, with mean GI_{50} values in the low nanomolar range. It exhibits a strong covalent binding affinity for specific DNA triplets, particularly those with guanine in the second position and either guanine or cytosine in the third position. Mechanistically, PM54 inhibits mRNA synthesis by causing the stalling and proteasomal degradation of RNA Polymerase II. This disruption results in double-strand DNA breaks, S-phase cell cycle arrest, and ultimately culminates in apoptotic cell death. RNA-seq analysis revealed that PM54 robustly downregulates genes associated with cell cycle regulation, key signaling pathways (WNT, MAPK, NOTCH, ERBB) and DNA damage response, including homologous recombination repair. Weekly intravenous (i.v.) administration of PM54 at its maximum tolerated dose (MTD) demonstrated significant antitumor activity and survival benefits across multiple preclinical tumor models, including breast, melanoma, gastric, ovarian, SCLC, and prostate. Tumor reduction and survival improvements were statistically significant in all models, with p-values ranging from 0.0002 to <0.0001. Median overall survival (OS) times in PM54-treated groups were markedly longer than in controls, highlighting PM54's potential as an effective therapeutic agent. In conclusion, PM54 demonstrates robust antitumor effects across various cancers by impairing mRNA synthesis and inducing DNA damage, underscoring its therapeutic promise.

#1774 A novel role of SNORA71D in the regulation of proteasome inhibitor induced apoptosis.

Alexei F. Kisselev, Olasubomi A. Akintola, Addison M. Wilson

Auburn University, Auburn, AL

Small nucleolar RNAs (snoRNA) are 60-300 nucleotide long RNAs that guide modification of rRNAs during ribosome biogenesis. Many of them have non-canonical functions, such as regulation of splicing of specific mRNAs. We have made a serendipitous discovery that treatment of cells with proteasome inhibitors (PIs) induces expression of a poorly characterized snoRNA, SNORA71D. Two other agents that induce production of misfolded proteins, tunicamycin and thapsigargin, also induced SNORA71D, but DNA-damaging agent doxorubicin did not. Knocking SNORA71D down reduced PI-induced apoptosis. The mechanisms of SNORA71D involvement in this process is under investigation. These findings indicate a novel and unexpected role of SNORA71D in cellular response to proteotoxic stress.

#1775 Oncogenic reduction after exposure to capsaicin in oral squamous cell carcinoma.

Samira B. F. Abdullah-Vargas[1], Riseilly Ramos-Nieves[2], Angel J. Amaral-Maisonet[1], Amanda Prats-Marrero[1], Jeannette L. Salgado-Montilla[1], Gabriel Borges-Velez[1], Josue Perez-Santiago[1]

[1]University of Puerto Rico Comprehensive Cancer Center, San Juan, PR,[2]University of Puerto Rico Medical Sciences Campus, San Juan, PR

Background: Oral Squamous Cell Carcinoma (OSCC) is the sixth most common type of cancer worldwide with an increasing death rate of 0.6% each year from 2009 through 2021. Although there have been advances in the treatment, survival rates for oral cancer patients remain low, highlighting the need for alternatives. Capsaicin, an alkaloid compound that acts as a metabolite, could be a potential alternative for cancer treatment due to its anti-proliferative and apoptotic effect on cancer cells. Additionally, previous data from our lab has demonstrated an increased cell viability in OECM-1 cells when treated with Lipopolysaccharides (LPS) from *P. gingivalis*. Therefore, in this study, we evaluated the effects of capsaicin on the viability, proliferation, and migration of the OSCC cell line OECM-1 with and without *P. gingivalis* LPS treatment.

Methodology: Cell viability was measured by AlamarBlue after 24 and 48 hours (h) of treatment with capsaicin using serial dilutions starting at 1000µM and calculated capsaicin's half-maximal inhibitory concentration (IC50). Cell proliferation was measured by seeding 150,000 cells per well and counting with TrypanBlue the change in cell number after treatment with capsaicin (100µM and 200µM) for 24h and 48h. Cell migration was assessed by wound healing assays, in which the length of the wound was measured before and after exposure to treatment by calculating the percentage of wound closure after 24h and 48h. We also measured the impact of capsaicin in the proliferation and migration of OSCC cells using a combination of capsaicin (100µM and 200µM) and LPS derived from *P. gingivalis* (4ng/mL and 8ng/mL).

Results: Higher concentrations of capsaicin reduced cell viability at both timepoints with IC50s of 470uM and 398uM at 24h and 48h, respectively. While cell proliferation increased at 24h of exposure to capsaicin at only 200µM ($p=0.010$), cell proliferation significantly decreased at both 100µM and 200µM of capsaicin after 48h ($p=0.004$ and $p=0.005$, respectively). Higher concentrations of capsaicin decreased the percentage of closure of the wound at both timepoints. On the other hand, cell proliferation decreased when treated with *P. gingivalis* LPS (4ng/mL) and 100µM ($p=0.016$) and 200µM ($p=0.029$) of capsaicin at 48h. In the wound healing assay, both concentrations of capsaicin with *P. gingivalis* LPS (8ng/mL) presented a decreased percentage of closure of the wound.

Conclusion: Capsaicin was able to reduce oncogenic phenotypes in OSCC and counteract the proliferative effects of *P. gingivalis* LPS. Understanding the molecular impact of capsaicin in OSCC may lead to a novel treatment strategy.

#1776 Investigation into the enhancement of 20S proteasome-mediated proteolysis as a therapeutic for HPV-associated cervical cancer.

Shannon M. Cartwright, Linh Le, Kate Hartnett, Jetze J. Tepe

Chemistry, University of Virginia, Charlottesville, VA

Human papillomaviruses (HPVs) are one of the most common sexually transmitted infections (STIs) in the world. HPVs can be divided into two main categories, low-risk and high-risk. Low-risk HPVs do not cause cancer and can often be cleared by the infected person's immune system. However, high-risk HPVs are responsible for approximately 5% of cancers worldwide - most commonly cervical cancer, and there is currently no cure once infection occurs. While prophylactic vaccines exist that can successfully prevent the infection of many high-risk HPVs, vaccination rates worldwide are low. After infection, the virus incorporates its genome into the host cell genome, and the cell begins to produce HPV proteins. In high-risk HPVs, two of these proteins, HPV E6 and E7, are oncoproteins with partially disordered structures. E6 and E7 proteins have many functions impacting cancer development, including inducing the degradation of tumor suppressor proteins p52 and pRB, respectively, through the ubiquitin-26S proteasome system (UPS). The removal of these cell cycle progression checkpoints allows for uncontrolled cell growth, which can promote cancer. The Tepe lab works on the development of small molecules which can increase the protein degradation capacity of the 20S proteasome, a protease that specifically degrades disordered proteins in a ubiquitin-independent manner. This project works to establish if the 20S proteasome can degrade HPV E6 and E7 proteins, and if the use of 20S proteasome enhancing small molecules can deplete levels in HPV+ cervical cancer cells. We have discovered that treatment with two of these compounds decreases E6 and E7 levels in a HPV18+ cervical cancer cell line and work is ongoing to determine if this impact is through 20S proteasome activation. Additionally, we are investigating if this decrease is enough to inhibit the out-of-control cell growth caused by E6 and E7. The goal of this project is to understand if 20S proteasome activators can be used as a therapy for HPV-mediated cervical cancer.

#1777 OST11098, an orally available and selective Cyp11A1 inhibitor for the treatment of castration-resistant prostate cancer (CRPC) with next-generation hormone agents (NHAs) resistance.

F. Wu, L. Deng, z. Ma, Z. Lu, G. Deng;
Open Source Therapeutics, Suzhou, China

Next-generation hormone agents (NHAs) such as abiraterone and enzalutamide have become standard treatments for castration-resistant prostate cancer (CRPC), significantly prolonging patient survival. However, resistance to NHAs inevitably develops, presenting a critical and unmet clinical challenge. Inhibition of CYP11A1 disrupts the entire biosynthesis pathway of androgens and other steroids by blocking the formation of pregnenolone. This mechanism halts the activation of the androgen receptor (AR) axis, thereby preventing further cancer progression and potentially overcoming NHAs resistance in heavily pretreated CRPC patients.In this study, we introduce OST11098, a newly identified, orally bioavailable, selective, and potent CYP11A1 inhibitor with demonstrated anti-tumor efficacy against NHA resistance. OST11098 displays favorable pharmacokinetic and safety profiles. In vitro, it effectively inhibited pregnenolone biosynthesis at low nanomolar concentrations in the H295R adrenocortical carcinoma cell line, leading to a rapid and complete blockade of all measured downstream steroid hormones. When administered orally, OST11098 showed consistent, dose-dependent pharmacokinetic/pharmacodynamic (PK/PD) correlations in adult non-castrated male mice, successfully inhibiting the biosynthesis of androgen receptor (AR) activators. In a VCaP CRPC xenograft mouse model, OST11098 demonstrated significant anti-tumor efficacy. Moreover, an engineered LNCaP CRPC xenograft mouse model with NHA resistance was successfully established and evaluated for its response to OST11098 treatment, providing compelling preclinical evidence supporting the use of CYP11A1 inhibitors as a promising strategy for managing NHA-resistance.Overall, our studies demonstrated OST11098 as a highly potent and selective CYP11A1 inhibitor with great PK properties, clean safety profiles, and significant anti-tumor activity for the treatment of CRPC. These promising preclinical results strongly support the potential of OST11098 to address unmet needs in CRPC treatment, justifying its further clinical development.

#1778 MALAT1 and TUG1 long non-coding RNAs signalling mechanisms in response to SRPK1 inhibitor in prostate cancer.

Linomtha Gabada[1], Zodwa Dlamini[1], **Rahaba Makgotso Marima**[2]

[1]University of Pretoria, Pretoria, South Africa,[2]University of Pretoria, Johannesburg, South Africa

Introduction: Prostate cancer (PCa) is a leading cause of cancer death, and there are significant racial disparities in patient outcomes. Non-coding RNAs (lncRNAs) MALAT1 and TUG1 are recognised for their roles in oncogenic pathways. Their precise interacting mechanisms with splicing factors and splicing factor kinases is understudied.
Aim: This study aims to map the biological pathways influenced by TUG1, MALAT1 in response to SRPK1 inhibitor prostate cancer cells
Materials and Methods: Bioinformatics tools LncSEA 2.0, STRING, DAVID and Reactome were used to analyse the interactions between MALAT1, TUG1 and relevant miRNAs and their target genes. Resozuring cell viability assay was used to measure PCa cell viability (PC-3 cells vs HEK) in response to Sphinx31 at 24hrs and 48hrs. Next, RT-qPCR was used to measure gene expression levels of the lncRNAs.
Results and Discussion: MALAT1 and TUG1 act as competing endogenous RNAs (ceRNAs) to sponge miRNAs that regulate critical tumour proliferation and migration genes, including PTEN and BCL2. SRPK1 regulates splicing factors to enhance tumour aggressiveness. The MALAT1-miR-125b-BCL2 and TUG1-miR-29b-PTEN axes are critical pathways for PCa progression, especially in advanced stages. Sphinx31 is cytotoxic in a time dependent manner to the PCa cells, while the 2 lncRNAs expression is elevated in PCa cells prior to Sphinx31 treatment.
Conclusion: Targeting lncRNA-miRNA networks and splicing factor interactions, especially MALAT1 and SRPK1, is a potential therapeutic target for PCa.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Novel Drug Delivery Technologies
Poster Session

#1782 Preclinical development of iSAP-0909: A novel self-assembled fluorescent contrast agent targeting XIAP for image-guided surgery in bladder cancer.

Min Han[1], Gantian Lyu[1], Yuzhen Qian[1], Hongwei An[2], Yingbin Zhang[1], Lin Fu[1], Hao Wang[1]

[1]Isap (Beijing) Biotechnology Co., Ltd., Beijing, China,[2]National Center for Nanoscience and Technology, Beijing, China

Background: Fluorescence-guided surgery as an intraoperative optical imaging technique offers surgeons real-time guidance on for tumor delineation. However, there is still a pressing clinical need for next-generation tumor-targeted fluorescent imaging agents that exhibit enhanced specificity, sensitivity, and photostability. iSAP-0909 is developed as an experimental peptide-based tumor-targeting fluorescent agent employing an *in vivo* self-assembly approach for adjunctive diagnosis and intraoperative localization in non-muscle-invasive bladder and other solid tumors.

Method: This research involved extensive preclinical studies, comprising *in vitro* assays on human bladder cancer cells, *Ex vivo* near-infrared (NIR) imaging on clinical bladder cancer specimens, *in vivo* imaging evaluation on mouse orthotopic bladder cancer model, and comprehensive toxicity studies on rats and dogs.

Results: iSAP-0909 is a novel peptide-conjugated NIR fluorescent cyanine dye targeting X-linked inhibitor of apoptosis protein (XIAP). The peptide binding activates downstream caspase enzyme, promoting cleavage of iSAP-0909 to initiate self-assembly process. Hereby, formation of β-sheet structure-enriched nanofibers within tumor cells is triggered, resulting in enhanced accumulation and retention of the cyanine dye. *In vitro* uptake and retention assays indicated that iSAP-0909 has notably higher tumor cell selectivity greater than HAL (by 5.4 fold), and showcases prolonged intracellular retention. *Ex vivo* imaging studies on human bladder revealed that iSAP-0909 distinctly delineates tumor boundaries and minor lesions through fluorescence imaging, with specificity of 97.96% and sensitivity of 99.58% derived from 338 clinical specimens. In an orthotopic tumor model derived from KU19-19 (human bladder cancer cell), iSAP-0909 achieved detection rate of 83.3% within 4 hours post-administration at a pharmacologically active dose, and the resulting signal-to-noise ratio was 7.53. Repeat-dose toxicology and safety pharmacology studies in rats and dogs indicated no adverse effects for iSAP-0909, suggesting favorable tolerability and no-observed genotoxic risk. Toxicokinetic analysis also reflected minimal plasma drug exposure (≤0.5%/BQL).

Conclusion: iSAP-0909 represents a novel self-assembled fluorescent contrast agent, demonstrating high specificity and sensitivity for bladder tumor tracing. This probe offers potential clinical advantages in localizing malignant lesions in patients with bladder cancer. The FDA has granted clinical approval of iSAP-0909 (IND 172343). Concurrently, an investigator initiated trial (IIT) study is underway to assess the safety and feasibility of imaging following intravenous administration of iSAP-0909 in cancer patients.

#1783 Harnessing macrophage-drug conjugates for allogeneic cell-based therapy of solid tumors.

Bartłomiej Taciak[1], Maciej Białasek[1], Malgorzata Kubiak[2], Ilona Marszalek[1], Paulina Kucharzewska Siembieda[2], Tomasz Rygiel[3], **Magdalena Krol**[1]

[1]Cellis AG | Warsaw University of Life Sciences, Zurich, Switzerland,[2]Warsaw University of Life Sciences, Warsaw, Poland,[3]Cellis AG, Zurich, Switzerland

The treatment of solid tumors remains a significant challenge, necessitating the ongoing development of innovative therapeutic strategies. Tumor-infiltrating macrophages play a crucial role in the tumor microenvironment, making them an attractive target for drug delivery systems. Here, we introduce the Macrophage-Drug Conjugate (MDC) platform, a pioneering approach that leverages macrophages as delivery vehicles for ferritin-drug complexes. We demonstrate that macrophages actively internalize human heavy chain ferritin (HFt) via macrophage scavenger receptor 1 (MSR1) in vitro. These drug-loaded macrophages transfer ferritin to neighboring cancer cells through a novel mechanism termed **TRAnsfer of Iron-binding proteiN (TRAIN)**. This process requires direct cell-to-cell contact facilitated by an immune synapse-like structure, enabling precise drug delivery. MDCs were formulated with various anti-cancer drugs and evaluated for safety and efficacy in multiple preclinical models, including syngeneic mouse and orthotopic human tumor models. MDC-250 exhibited potent anti-tumor activity in an orthotopic human pancreatic cancer model (BxPC3). MDC-735 demonstrated remarkable efficacy across diverse cancer models, including pancreatic cancer (BxPC3), ovarian cancer (SK-OV-3), breast cancer lung metastases (EMT6), bladder cancer (MB49), and head and neck cancer (SCC7). These effects were observed both as a monotherapy and in combination with immune checkpoint inhibitors. Significantly, MDCs were prepared as off-the-shelf products, maintaining their activity upon thawing, highlighting their potential for clinical translation. This MDC platform represents a breakthrough in solid tumor therapy, offering a targeted, scalable, and effective treatment approach with broad applicability across multiple cancer types.

#1784 Dynamic control of cerebrospinal fluid to achieve personalized drug delivery in the central nervous system.

Priya Kumthekar[1], Marcie Glicksman[2], Margaret Schwartz[1], Seema Nagpal[3], Isabella Glitza,[4], Peter Forsyth[5], Ernesto Ruiz[2], Adam Sonabend[1]

[1]Northwestern University, Chicago, IL,[2]EnClear Therapies, Newburyport, MA,[3]Stanford University, San Francisco, CA,[4]University of Texas MD Anderson, Houston, TX,[5]Moffitt Cancer Center, Tampa, FL

Background: Systemically delivered therapeutics have limited access to the brain due to the tightly regulated blood brain barrier. There is a fine balance between production (20 mL/h), volume (150 mL), and removal/turnover (every 5h) of cerebrospinal fluid (CSF). The high turnover of CSF and the active efflux makes CNS therapeutics particularly challenging. Direct delivery of therapeutics to the CSF intrathecally is increasingly utilized in clinical practice but penetration deep into the brain parenchyma is poor. This poses a significant challenge for the efficacy of intrathecally delivered small molecules, antibodies and gene therapies. Ideally, a CSF delivery system would have the capability to deliver therapeutics broadly within the CSF compartment, while simultaneously also improving parenchymal delivery. We show with our device how we can move dye between the two ventricles in a controlled fashion resulting in improved distribution of methotrexate.

Methods: Sheep (n=8) were used as a model, which have a brain size and geometry closer to humans when compared to rodents. EnClear's drug delivery system utilizes two CSF access points; Group 1 accessed the right and left lateral ventricle (V2V) and Group 2 accessed the right or left lateral ventricle and lumbar thecal sac (V2L). An extracorporeal pump was used to control the speed and direction of CSF flow at 50 µL/mL. Sensors continuously monitored pressure, respiration, and heart rate. Imaging studies with Omnipaque dye determined the best parameters for controlling CSF flow. Administration of methotrexate was followed by a protocol to move CSF between the two access points. Methotrexate levels were measured with LC/MS in CSF, blood, brain regions, spinal cord, kidney, and liver.

Results: Omnipaque dye in V2V demonstrated the dye was observed to move between the two ventricles, whereas, in V2L, the dye disappeared within minutes from the ventricles. In Group 1 (V2V), safe pressures (< 25 mmHg or 34 cm water) were sustained in all subjects. In both groups, effective levels of >1 ug/mL methotrexate were measured in all brain tissues, including deeper brain tissues. There was a three-fold higher level of methotrexate in striatum with the V2V compared to V2L subjects. Consistently, CSF levels were two-fold higher at 2h. Importantly, methotrexate levels were significantly lower in lumbar spinal cord, kidney, and liver-potential sources of toxicity.

Conclusions: In this large animal study, EnClear's system delivered high(er) levels of methotrexate in the parenchyma of deep brain structures than traditional IT injection. To the authors knowledge, this first intraCSF drug delivery device to show improved distribution throughout the CNS including deep brain structures, while keeping lumbar spinal cord, liver, and kidney levels very low. Clinical trials with the device are currently being planned.

#1785 Development of dual-payload anti-GPC3 antibody-drug conjugate by dual-payload antibody conjugation (AD2C) platform for hepatocellular carcinoma treatment.

Hao-Kang Li, Cheng-Wei Lai, Chien-Yu Lin, Yi-Jang Chen, Pei-Ju Leng, Chao-Min Cheng, **Sai-Wen Tang**, Shih-Chia Hsiao

Acepodia Biotech Inc., Alameda, CA

Glypican-3 (GPC3) has been reported as a promising target for the treatment of hepatocellular carcinoma (HCC). Although current therapies are available, tumor heterogeneity and the development of drug resistance continue to pose significant challenges to effective treatment. Therefore, combination therapy has emerged as a promising strategy to address these challenges. Acepodia successfully established a Dual-Payload Antibody Conjugation (AD2C) platform utilizing our proprietary bioorthogonal chemistry, which allows conjugating two (or multiple) different payloads to the antibody without requiring antibody engineering. In this study, we developed an anti-GPC3 antibody with dual payloads (anti-GPC3 AD2C) through our AD2C platform to enhance treatment efficacy of HCC. The anti-GPC3 AD2C exhibits comparable GPC3 binding affinity and internalization to the parental anti-GPC3 antibody. No significant degradation or aggregation was observed after long-term storage, demonstrating the stability of anti-GPC3 AD2C. In the pharmacokinetics study, anti-GPC3 AD2C showed a similar terminal half-life ($T_{1/2}$) to the parental antibody, and its volume of distribution (Vss) was equivalent to the blood volume, indicating primary distribution in the blood. These findings suggest that the dual-payload conjugation has a limited impact on the *in vivo* stability and safety of the anti-GPC3 AD2C. The in vitro efficacy studies show that anti-GPC3 AD2C exhibits enhanced cytotoxicity compared to the mono-payload ADCs and parental antibody in GPC3-expressing cancer cell lines but not in the GPC3 negative cell lines and primary normal cells. The HCC xenograft study further demonstrated that the anti-GPC3 AD2C completely suppressed tumor growth at doses of up to 10 mg/kg, with no significant body weight change observed. In conclusion, the dual-payload ADC produced by AD2C platform offers a potential strategy to overcome tumor heterogeneity and drug resistance while maintaining an ideal safety profile, supporting its potential clinical application in HCC treatment.

#1786 AST-203, a novel Trop2-targeting aptamer-drug conjugate, exhibits potent antitumor efficacy for the treatment of solid tumors.

Ju-hyung Kang, Daekyun Lee, Seo-young Kwak, Jieon Lee

Aptamer Sciences Inc., Seongnam-si, Korea, Republic of

Background: Trophoblast cell surface antigen 2 (Trop2), a transmembrane glycoprotein overexpressed in various epithelial cancers, including pancreatic ductal adenocarcinoma (PDAC), breast, lung, and bile duct cancers, represents an attractive target for cancer therapy. Trodelvy, a Trop2-targeting antibody-drug conjugate (ADC) linked to the topoisomerase I inhibitor SN-38, has been approved for the treatment of triple-negative breast cancer. This study focuses on the development of a novel Trop2 aptamer-MMAE conjugate (AST-203) and its characterization in comparison to the ADC.
Methods: AST-203 was designed by attaching a Trop2-targeting aptamer to three MMAE molecules (a microtubule inhibitor) using a proprietary branched linker (Blinker). Binding affinity (kd) to rhTrop2 was measured by BLI, with specific Trop2 targeting confirmed by FACS and ICC. The internalization rate of the aptamer was determined by FACS. In vitro cytotoxicity was evaluated in 2D and 3D cultures using CellTiter-Glo assays. The penetration capability was assessed through ICC in BxPC3 spheroid models. For in vivo studies, AST-203 was administered intravenously at doses of 2, 3, and 4 mpk, and Trodelvy (6 mpk) was used as a comparator in a PDAC xenograft model.
Results: The Trop2 aptamer binds to human Trop2 with an affinity of 8.6 nM and specifically targets Trop2-positive cells (BxPC3, Capan1) but not Trop2-negative PANC1 cells. It internalizes with a half-time of 15.23 minutes (95% CI: 11.30-23.34 minutes), localizing to lysosomes as confirmed by ICC. The IC50 of AST-203 was 20 nM in BxPC3 cells and 99 nM in Capan-1, while >1 μM in PANC1 cells. Additionally, studies on the anticancer effects in various cancer cells revealed a correlation with Trop2 expression levels. Notably, the Trop2 aptamer showed 6-10 times better penetration in spheroid cultures than a Trop2 antibody. AST-203 treatment significantly reduced Ki-67 expression in spheroid interiors compared to Trodelvy, consistent with its superior penetration. In addition, conditioned media from AST-203-treated cells also elicited a bystander effect on Trop2-negative PANC1 cells. Cell cycle arrest and the activation of apoptotic signal cascade was demonstrated as the mechanism of cell death. AST-203 showed minimal cytotoxicity in PBMCs and megakaryocyte (platelets precursor cell) compared to Trodelvy. In vivo, AST-203 effectively inhibited tumor growth in a BxPC xenograft model, showing superior efficacy at doses of 3 and 4 mpk compared to Trodelvy (6mpk).
Conclusion: AST-203 shows the potential of a promising next-generation therapeutic for Trop2-positive solid tumors. Its superior efficacy and safety compared to traditional antibody-drug conjugates highlight its potential to improve therapeutic outcomes for Trop2-expressing cancers. These findings underscore AST-203 as a significant advancement in aptamer-based therapeutics.

#1787 Novel strategy for producing ovarian cancer-targeting antibodies using cancer-derived small extracellular vesicles.

Maryam Firouzi, Changsun Kang, Xiaoyu Ren, Seonghyun Ryu, Dongin Kim

Pharmaceutical Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK

Introduction: Despite advancements in tumor-targeting agents, meta-analyses reveal that only 0.7% (mean value) of the injected therapeutic dose reaches the tumor site, leading to significant off-target adverse effects. This highlights the need for innovative targeting agents to enhance tumor-targeting efficacy. We developed a novel tumor-targeting antibody production method using cancer-derived small extracellular vesicles (sEVs). Since sEVs inherit their structure and function from their parent cells, the antibodies produced against sEVs could target both cancer cells and sEVs. Using this approach, antibody-decorated drug delivery systems may achieve enhanced tumor-targeting efficiency, overcoming the suboptimal drug concentration in the tumor. **Methods:** sEVs were isolated from ovarian cancer cells (OVCAR-8) and injected into mice. After confirming the specificity of the collected plasma to OVCAR-8 cells, hybridoma cells were generated by fusing the mice's B cells (antibody-producing cells) with myeloma cells. Hybridomas were screened to produce monoclonal antibodies (mAbs) with the highest cytotoxicity against OVCAR-8 cells (AB1, based on CCK8 assay) and the best OVCAR-8-targeting efficacy (AB2, determined through immunocytochemistry). The antibodies (AB1 and AB2) were purified and conjugated to paclitaxel-loaded CD8+ T cell sEVs (AB-TSEV/P). The formulations (AB1-TSEV/P and AB2-TSEV/P) were injected into OVCAR-8-bearing mice, and tumor volume and body weight were monitored over 80 days. An in vivo biodistribution study was performed using an IVIS Spectrum Imaging System based on fluorescence intensity measurement of IR-780 loaded AB-TSEV (AB-TSEV/IR) to assess tumor-specific accumulation. **Results:** Characterization showed that both OVCAR-8 and CD8+ T cell-derived sEVs were ~150 nm in size. Plasma from mice injected with OVCAR-8-derived sEVs demonstrated significantly higher cytotoxicity and targeting specificity against OVCAR-8 cells than non-cancerous HOSE cells. Based on the results from antibody screening, AB1 exhibited the highest cytotoxicity to OVCAR-8 cells, while AB2 showed the best OVCAR-8 cell-targeting efficacy compared to HOSE cells. Tumor volume measurements indicated that AB1-TSEV/P and AB2-TSEV/P significantly reduced tumor growth ($p<0.0001$) without affecting body weight, suggesting good tolerability. Biodistribution studies showed that antibody-decorated sEVs accumulated significantly ($p<0.0001$) at the tumor site. **Conclusions:** We developed an innovative strategy for producing tumor-targeting antibodies using cancer-derived sEVs, significantly enhancing tumor-specific drug delivery. Although focused on ovarian cancer, this approach could be applied to other cancers and diseases, offering a promising method for targeted therapies and addressing the limitations of current treatments.

#1788 Exosomal formulation of cannabidiol (CBD) inhibits lung cancer proliferation and enhances uptake and tumor targeting.

Disha Nagesh Moholkar[1], Raghuram Kandimalla[1], Jeyaprakash Jeyabalan[2], Ramesh C. Gupta[1], Farrukh Aqil[1]

[1]Department of Pharmacology and Toxicology, University of Louisville, Louisville, KY,[2]3P Biotechnologies, Inc, Louisville, KY

Lung cancer remains a leading cause of cancer-related deaths, with treatment specificity posing a major challenge. Thus, we aim to repurpose cannabidiol (CBD) as an antitumor agent, delivered via tumor-targeted exosomes (Exo). We demonstrated that oral CBD administration inhibited orthotopic lung tumor growth by ~60% ($p < 0.01$), with enhanced efficacy (~80%; $p < 0.001$) achieved using folic acid-functionalized exosomes loaded with CBD. We hypothesized that the enhanced antitumor efficacy is due to 1) increased accumulation at the tumor site due to FA-targeting; 2) improved cellular uptake of CBD via exosomal delivery; and 3) modulation of mechanistic pathways by CBD. To evaluate tumor targeting, FA-Exo labeled with Alexa Fluor-750 (AF750) were administered orally to tumor-bearing mice. Single doses of free AF750, Exo-AF750, and FA-Exo-AF750 were compared (n=3 per group). After 12 hr, mice were euthanized and imaged *ex vivo* using Odyssey LiCor Imager. For bioavailability studies, female C57BL/6 mice were given a single oral dose of 40 mg/kg CBD or ExoCBD, and tissue CBD levels were quantified 4 hr post-administration. Additionally, ER stress modulation by CBD was assessed in A549 lung cancer cells through analysis of key markers. FA-Exo-AF750 exhibited 3-fold higher signal in tumor tissues compared to non-functionalized exosomes ($p<0.01$). In bioavailability studies, ExoCBD demonstrated significantly higher CBD levels in the liver (5-fold) and mammary pads (10-fold) compared to free CBD ($p<0.001$). Notably, free CBD was undetectable in plasma, whereas ExoCBD achieved plasma CBD levels of 500 ng/mL, suggesting enhanced bioavailability and prolonged circulation time. In A549 lung cancer cells, CBD downregulated key ER stress markers, including PERK, IRE1α, BiP, CHOP, Calnexin, and PDI, anti-inflammatory marker NFκB and oncogenic CB2. Furthermore, combination of CBD with paclitaxel (PAC) substantially reduced the IC50 value of PAC against both drug-sensitive (12.5 to 5 nM) and drug-resistant (325 to 80 nM) A549 cells compared to PAC alone. In fact, the activity of PAC and CBD was synergistic against A549 cells, as shown by the combination index (CI = 0.62) calculated using Calcusyn software. Importantly, CBD reversed PAC resistance by downregulating MDR-1 in drug-resistant A549 cells. Overall, tumor-targeted exosomal formulations significantly enhance CBD's bioavailability, tumor accumulation, and therapeutic efficacy. Combined with its ability to overcome chemotherapy resistance, these findings highlight the promise of CBD-loaded exosomes as a novel therapeutic against lung cancer.

Funding: Supported by Agnes Brown Duggan Endowment Funds, the Jewish Heritage Fund for Excellence Research Enhancement Grant and IPIBS Fellowship at the University of Louisville.

#1789 Preclinical characterization of BGB-B3227, a MUC1 x CD16A bispecific engager, for the treatment of MUC1-expressing solid tumors.

Hengrui Zhu[1], Hui Li[2], Zhuo Li[1], Qi Liu[2], Liu Xue[2], Liang Qu[2], Ming Jiang[2], Jie Li[1], Xin Chen[2], Wenjie Wang[2], Yun Chen[1], Jie Pan[1], Yuanyuan Xie[2], Ting Shao[2], Peiqi Li[1], Renke Li[1], Caizhi Liu[2], Xiaoliang Chen[2], Lei Jiang[2], jingyuan Sun[2], Lingmin Zheng[2], Xiangli Ma[2], Juan Yang[1], Haoxuan Song[1], Fuyun Sun[1], Jiyuan Zhang[1], Xi Wu[2], Xianyan Tang[2], Qiansheng Ren[2], Chichi Huang[2], Mingchao Kang[2], Xiaomin Song[2], Zhirong Shen[2], Lai Wang[2]

[1]BeiGene Ltd., Shanghai, China,[2]BeiGene Ltd., Beijing, China

Background: MUC1 is frequently overexpressed in various human epithelial cancers, including NSCLC, gastrointestinal, breast, pancreatic, ovarian, and colon carcinomas. Despite the development of MUC1 targeted therapies, none have shown significant clinical efficacy. BGB-B3227, a novel bispecific antibody targeting MUC1 and CD16A, is designed to induce NK cell activation and subsequent cytotoxicity against MUC1-expressing tumor cells.
Methods: The target binding activity of BGB-B3227 was characterized using biochemical and cell-based assays. The ability of BGB-B3227 to avoid the interference from soluble MUC1 was assessed by a competitive FACS assay. The ADCC and ADCP activities of BGB-B3227 were evaluated in co-culture systems consisting of effector cells and tumor cells expressing varying levels of MUC1, both in the presence and absence of human IgG proteins. Furthermore, the in vivo anti-tumor efficacy of BGB-B3227, both as a monotherapy and in combination with anti-PD-1 antibody, was evaluated in the MC-38/hMUC1X model in human CD16A knock-in mice.
Results: BGB-B3227 demonstrated high binding affinity to recombinant human CD16A protein and CD16A expressing cells, with comparable binding affinities to both CD16A-158V and 158F variants. BGB-B3227 displayed no detectable binding to either human CD16B NA1 (A) and NA2 variants, underscoring its high selectivity for CD16A. Additionally, BGB-3227 demonstrated high binding affinity to the recombinant SEA domain of human MUC1 protein and MUC1 expressing tumor cells. Compared to HMFG1, which targets MUC1-N, the BGB-B3227's binding to MUC1 expressing cells was less interfered by soluble MUC1. In cellular assays, BGB-B3227 induced potent ADCC and ADCP activity against MUC1-expressing cells in a dose-dependent manner, which was greater than that of Fc-enhanced mAb and was less affected by human IgG. No activity was observed in MUC1-negative tumor cells, indicating the effect of BGB-B3227 is MUC1 dependent. In mouse models, BGB-B3227 monotherapy demonstrated a dose-dependent anti-tumor efficacy, which was further enhanced when combining with an anti-PD-1 antibody. Importantly, no significant changes in animal body weight were observed across all treatments, suggesting good tolerability of BGB-B3227 in mouse models.
Conclusion: BGB-B3227 is a bispecific MUC1xCD16A NK engager demonstrating potent ADCC and ADCP activity, along with notable in-vivo anti-tumor efficacy when used as a monotherapy or in combination with anti-PD-1 therapy. BGB-B3227 represents a promise therapeutic option for MUC1-expressing cancers, with the potential to overcome the limitations of MUC1-N antibodies by mitigating the sink effect from soluble MUC1. Currently, BGB-B3227 alone and in combination with Tislelizumab is under clinical investigation in participants with advanced or metastatic solid tumors (NCT06540066).

#1790 Enhancing ADC efficacy through Fc-binding peptide engineered with ECM binding moiety: A versatile platform targeting various tumors and other malignancies.

Dae Young Kim, Yujin Park, Dahye Sim, Hana Kang, Sung Min Kim, Yejin Bang, Hee Young Kang, Hoil Choi

Peptron Inc., Daejeon, Korea, Republic of

Antibody-drug conjugates (ADCs) are an emerging class of immunotherapeutics permitting the delivery of therapeutics with sufficient potency and specificity by doing so systemic toxicity typical of conventional chemotherapy is minimized. However, the challenges in controlling their internalization have posed a hurdle in maximizing the therapeutic potential of ADCs. Here we present a novel FcBP-based modality called FcBC17, an FcBP coupled with an extracellular matrix (ECM)-binding moiety, which plays an ADC enhancer by improving the cell binding and internalization of ADCs through a biologically active mechanism. FcBC17 comprises an FcBP, a linker, and an ECM-binding peptide and tightly associates with IgG antibodies. The efficacy of FcBC17 was demonstrated using PAb001, a humanized IgG1 antibody targeting MUC1-C developed by Peptron. Due to its reversible binding to the Fc-region of PAb001, the structural and functional integrity of PAb001 remains and also additional effort to modify PAb001 is unnecessary, representing a simple, broadly applicable strategy to enable antibody delivery into cells. We show that FcBC17 improved cell binding of PAb001 in diverse cancer cells with different levels of MUC1. In addition, PAb001-FcBC17 conjugate showed a better internalization in cancer cells and intriguingly, a biologically active mechanism such as endocytosis seemed to mediate the internalization, suggesting that FcBC17 might enhance the cytotoxicity of PAb001-ADC (PAb001-MMAE or PAb001-DXd) through enhancing its cell binding and internalization actively. Indeed, the cytotoxicity of PAb001-ADC was greatly improved with the conjugation of FcBC17 against cancer cells that otherwise showed a poor IC50 with the treatment of PAb001-ADC, and no cytotoxicity was observed with the treatment of FcBC17 alone, indicating that the ADC enhancement was likely due to the improved internalization of PAb001-ADC through the conjugation of FcBC17. Moreover, due to the nature of FcBP whose binding is separated on acidic pH conditions, PAb001-FcBC17 conjugate remains stable under normal physiological conditions, ensuring that the efficacy of PAb001-ADC is not compromised upon clinical treatment. In conclusion, the binding and internalization of PAb001-ADC on cancer cells could be greatly enhanced with the conjugation of FcBC17 which in turn has resulted in the dramatic improvement of the cytotoxicity mediated with PAb001-ADC, implying that the critical limitation of conventional ADCs in controlling cellular internalization may be ameliorated through our FcBP-based approach. The versatility of our approach as an ADC enhancer will be further demonstrated across various applications, not only in targeting MUC1-expressing tumors but also in treating other types of cancers and malignancies through diverse therapeutic antibody formats.

#1791 An injectable depot technology for sustained locoregional delivery improves the efficacy and safety of immunotherapies and adoptive cell therapies.

John H. Klich, **Anthony C. Yu**, Eric A. Appel

Materials Science & Engineering, Stanford University, Stanford, CA

Advances in immunotherapy have revolutionized care for cancer patients. Unfortunately, many approaches fail to mount a robust anti-cancer effect, in part due to the highly immunosuppressive tumor microenvironment in solid tumors. Similarly, while adoptive cell therapies (ACT) like CAR-T therapies have demonstrated immense potential in treating various B cell malignancies, they have been less successful in treating solid tumors on account of the immunosuppressive environment. To address these challenges, the field has turned to either combination immunotherapies or locoregional delivery of therapies as systemic administration of potent therapies can result in severe immune related adverse effects. Enabling potent combination immunotherapies requires administering these immune agonists in a way that more closely resembles the endogenous cancer immunity cycle, a tightly regulated sequence of cues in both space and time. Here, we will discuss the development of an injectable hydrogel depot technology for rational localization and prolonged exposure of potent immunotherapeutic cytokines (IL-12, IL-2), antibodies (OX40a, PD1), and ACTs to improve these therapies. Specifically, we have developed a self-assembled polymer-nanoparticle (PNP) hydrogel system that is both highly shear-thinning and rapidly self-healing, enabling minimally invasive implantation though direct injection or catheter delivery to tissues. First we will discuss how these hydrogels enable targeted delivery of immunomodulatory cargo either intratumorally (IT) or peritumorally (PT). We hypothesized that selectively altering biodistribution would more effectively augment the immune response more than sustained locoregional delivery alone. Using *in vivo* imaging, we demonstrated that site of administration is critical to redistributing cargo to either the tumor or tumor draining lymph node (tdLN). In this way we demonstrated that targeted localization of cytokine and antibody therapies synergistically improved treatment efficacy in the B16F10 and MC38 tumor models and altered cellular phenotypes in these microenvironments. Second we will discuss how these hydrogels enable the controlled co-delivery of stimulatory cytokines and adoptive cells to improve treatment of solid tumors. The unique architecture of these materials simultaneously enables prolonged retention of entrapped cytokines while maintaining active motility of entrapped cells. Upon administration this hydrogel depot forms a transient inflammatory niche that induces more tumor-reactive phenotypes and sustains exposure of activated adoptive cells, thereby improving treatment efficacy. Overall, this presentation will discuss how PNP hydrogels represent a crucial new tool for improving therapeutic efficacy of immunotherapies and adoptive cell therapies for cancer.

#1792 A novel lipid nanoparticle formulation transfects acute myeloid leukemia cells and primary AML samples with high efficiency and low toxicity.

L. Zhang[1], Y. Yan[1], J. Chen[2], R. Hurren[1], J. Bu[1], J. Chen[1], D. Ling[1], N. Duong[1], A. Arruda[1], H. He[1], M. D. Minden[1], R. Bremner[3], G. Zheng[1], B. Li[2], A. D. Schimmer[1];

[1]UHN Princess Margaret Cancer Centre, Toronto, ON, Canada, [2]Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada, [3]Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada

RNA interference is a powerful research tool for studying gene function in cancer cells. However, functional genomic studies in Acute Myeloid Leukemia (AML) remain challenging. Classic lipid-based transfection reagents are ineffective in delivering siRNA to AML cells and lentiviral delivery of shRNA is complex and time-consuming. Lipid Nanoparticles (LNPs) are a novel approach to deliver genetic material to cells that are difficult to transfect. However, a formulation of LNP capable of transfecting AML cells and primary AML samples with high efficiency and low toxicity is lacking. Here, we developed an LNP formulation that delivers siRNA to AML cell lines and primary samples without impacting their growth and viability.

To identify LNPs able to transfect AML cell lines, we generated a library of LNPs that differed in lipid compositions, aqueous phase pH, and RNA-to-ionizable lipid weight ratio through the Design of Experiment (DOE). We synthesized 54 unique LNPs formulations encapsulating siRNA targeting luciferase. We then screened this library of LNPs against OCI-AML2 leukemia cells over-expressing luciferase. From this screen and subsequent validation studies, we identified a novel formulation, LNP 1-28, as the top hit based on transfection efficiency and safety. LNP1-28 also suppressed luciferase expression in 3 AML cell lines (OCI-AML2, OCI-AML3, MOLM13) with > 80% target knockdown with 100nM of siRNA 3 days post-transfection.

Next, we asked if LNP 1-28 could deliver siRNA to knockdown an endogenous protein, CD45. AML cell lines (OCI-AML2, OCI-AML3, MOLM13) were treated with LNP 1-28 carrying siRNA targeting CD45 or control sequences. Knockdown of CD45 protein was observed by flow cytometry in all tested AML cells with knockdown being time and concentration dependent. CD45 knockdown was observed as early as 1-day post-transfection and persisted for 7 days before gradually returning towards baseline. Greater than 82.5% knockdown was observed 4 days after 100nM siRNA-LNP treatment in all tested cell lines. LNP 1-28 had no effect on cell growth and viability. Using the low passage primary AML culture model 8227 cells, we demonstrated that LNP 1-28 could deliver siRNA to both bulk and CD34+/CD38- stem populations of AML. Finally, LNP 1-28 efficiently transfected primary AML patient samples and delivered siRNA to knock down CD45.

In summary, we developed a novel LNP formulation capable of transfecting AML cells and primary AML samples with siRNA at high efficiency and without toxicity. Thus, we have developed a nanocarrier system for *in vitro* transfection that will aid in functional genomics studies of AML.

#1793 Pre-targeting with in vivo click chemistry enables preferential drug exposure in tumors versus normal tissues.

Travis L. Biechele, Masa Aleckovic, Stefanie Wagner, George Coricor, Sangeetha Srinivasan, Tri-Hung Nguyen, Jesse M. McFarland, Nathan A. Yee, Michael Zakharian, Jose M. M. Oneto

Shasqi, San Francisco, CA

Off-target toxicity has long been a dose-limiting factor for antibody-drug conjugates (ADCs) and targeted radiopharmaceutical therapies (TRT). This fundamental limitation primarily arises due to the catabolic elimination process of both therapeutic modalities which exposes healthy tissues to cytotoxic agents. Pre-targeting offers a transformative solution to overcome these challenges as it separates the tumor-targeting and payload-delivery steps, thereby minimizing systemic exposure and maximizing on-target effects. Leveraging this concept, we present Click Activated Protodrugs Against Cancer (CAPAC®) technology as the ideal pre-targeting reunion platform.

CAPAC uses in vivo click chemistry to selectively capture drug payloads at tumors with minimal systemic exposure, thus extending the therapeutic window of targeted therapies. CAPAC employs a two-component system: 1) a clickable binder infusion targeting a tumor antigen, and 2) a subsequent infusion of a clickable payload ("protodrug"). These two components "click" at tumors, enabling delivery of high therapeutic payload to the target while sparing normal tissues.

We have generated proof-of-concept data on CAPAC using clickable binders against HER2, TROP2, and CEACAM5, which we paired with different clickable payloads, including a *trans*-cyclooctene (TCO)-modified biotin probe and a protodrug of monomethyl auristatin E (MMAE). We evaluated the anti-tumor efficacy, pharmacokinetics (PK), and biodistribution across CDX models or in tumor-free mice. Payload capture was antigen-dependent and markedly higher in tumors vs plasma and normal tissues, with tumor levels exceeding systemic levels by more than 10-fold at 8.5 hours post-infusion. Tumor payload exposure was proportional with receptor density, as the HER2-binder captured more biotin than the TROP2-binder. Slower antigen internalization (HER2, CEACAM5) extended the duration of payload exposure in tumor vs plasma compared to faster internalization (TROP2). These PK and biodistribution results validate the highly selective tumor targeting of CAPAC. Importantly, enhanced tumor payload exposure translated into significant anti-tumor efficacy with the MMAE protodrug, which achieved dose-dependent tumor growth inhibition across various CDX models. These findings underscore CAPAC's potential to overcome the limitations of off-target toxicities and extend the applicability of cytotoxic agents to poorly internalizing targets.

Our preclinical data confirm in vivo click chemistry as a powerful pre-targeting reunion technology, offering modularity to accommodate various antigen targets and payloads. By delivering targeted therapies with unprecedented precision, CAPAC fulfills Paul Erlich's vision of a truly targeted delivery system. CAPAC opens new avenues for safer and more effective cancer treatments, addressing needs are yet unmet by ADCs and TRTs.

#1794 Preclinical development of TGW101, a first in class click-cleavable ADC with MMAE against non-internalizing pan-carcinoma marker TAG72.

Raffaella Rossin[1], Martijn H. den Brok[1], Lieke W. M. Wouters[1], Joep Houkes[1], Ron M. Versteegen[2], Marleen H. M. van Stevendaal[1], Luc H. M. Zijlmans[1], Maria Cincotta[1], Jay M. Feingold[1], laurens H. J. Kleijn[1], **Marc S. Robillard**[1]

[1]Tagworks Pharmaceuticals B.V., Nijmegen, Netherlands,[2]SyMO-Chem, Eindhoven, Netherlands

Current ADCs typically rely on intracellular drug release. However, the number of suitable internalizing receptors in solid cancers is limited while there are suitable non-internalizing tumor markers, provided effective extracellular cleavage can be achieved. To expand the ADC target scope, Tagworks is pioneering a radically new approach to ADCs based on its Click-to-Release platform, which centers on selective payload release from a *trans*-cyclooctene (TCO) linker upon click reaction with a tetrazine molecule (the trigger). In this approach, following tumor accumulation and blood clearance of the click-cleavable ADC, a tetrazine trigger molecule is administered i.v. that rapidly releases the payload from the extracellular ADC, allowing it to distribute into surrounding cells, maximizing the bystander effect. The first in class click-cleavable ADC therapy TGW101 combines a tetrazine trigger (TRG001) with a click-cleavable ADC, TGW101-ADC, targeting non-internalizing tumor marker TAG72, expressed by many adenocarcinomas. The ADC comprises a 60 kD diabody, site specifically conjugated with TCO-linked MMAE at a drug to antibody ratio of 3.8. TGW101 uses a new TCO / tetrazine combination with 100-fold higher reactivity than in previous versions, allowing markedly lower trigger doses, as low as 3 mg/kg, for complete ADC conversion in mice. The purpose of this study was to characterize the anticancer activity of TGW101, and its tolerability and pharmacokinetics (PK) in the rat and non-human primate (NHP). In vitro, TGW101-ADC showed fast and quantitative release of MMAE upon reaction with TRG001. Cell killing was dependent on binding to TAG72 on the cell and extracellular reaction with TRG001. In vivo, TGW101 gave high and stable free intratumor MMAE concentrations (300 nM at 2 mg/kg ADC) and showed strong dose dependent activity in several TAG72-positive xenografts in single and repeated cycles, using a 2-day interval between TGW101-ADC and TRG001, without TGW101-related toxicity. TGW101-ADC alone was stable and very well tolerated in NHPs at repeated doses at 50 mg/kg (NOAEL) with a clearance half-life of 25h. Similarly, TRG001 was very well tolerated, with HNSTD/NOAEL of 700 mg/kg and 300 mg/kg in rat and NHP, respectively, with a half-life of 0.6h in NHP. When used in combination in NHPs, biweekly cycles of ADC followed 4 days later by a high dose of trigger (75 mg/kg) afforded an HNSTD of 5 mg/kg for TGW101-ADC (which equates to 12.5 mg/kg for mAb size), with reversible and non-adverse effects consistent with free MMAE released from residual circulating ADC by Trigger. In conclusion, TGW101 demonstrated potent and specific in vitro and in vivo anti-tumor activity, while it was well tolerated in rats and NHPs at doses at or higher than current MMAE ADCs. This warrants clinical development of this promising click-cleavable ADC with the aim to expand the ADC target scope.

#1795 Targeting the blood-brain tumor barrier with engineered tumor necrosis factor synergizes with temozolomide antitumor activity against glioblastoma.

Giulia Taie[1], Anna Maria Gasparri[1], Federica Pozzi[1], Barbara Colombo[1], Federico Rossari[2], Nadia Coltella[2], Elisa Sangiovanni[3], Giulia Anderluzzi[1], Matteo Monieri[1], Tamara Canu[4], Elisa Bruno[5], Antonello Spinelli[4], Anna Palmisano[5], Antonio Esposito[5], Andres J. M. Ferreri[6], Imane Nafia[7], Corti Angelo[5], **Flavio Curnis**[1]

[1]Division of Experimental Oncology, IRCCS Ospedale San Raffaele, Milan, Italy,[2]San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), IRCCS Ospedale San Raffaele, Milan, Italy,[3]Division of Immunology, Transplantation and Infectious Diseases, IRCCS Ospedale San Raffaele, Milan, Italy,[4]Preclinical Imaging Facility, IRCCS Ospedale San Raffaele, Milan, Italy,[5]Vita-Salute San Raffaele University, Milan, Italy,[6]Department of Onco-Hematology, IRCCS Ospedale San Raffaele, Milan, Italy,[7]Explicyte Immuno-Oncology, Bordeaux, France

Background: The blood-brain tumor barrier (BBTB) is an obstacle in delivering anticancer drugs to brain tumors. Therefore, novel strategies aimed at targeting and breaching this structure are of high experimental and clinical interest. We developed a derivative of tumor necrosis factor-α (TNF) that can target and alter the BBTB. This drug, called NGR-TNF, consists of a TNF molecule fused to the Cys-Asn-Gly-Arg-Cys-Gly peptide (called NGR), a ligand of aminopeptidase N (CD13)-positive tumor blood vessels. Administration of low-dose NGR-TNF to patients with primary central nervous system lymphomas promotes BBTB alteration, increases the efficacy of R-CHOP (a chemo-immunotherapy regimen), and improves patient survival. Unfortunately, the inherent instability and heterogeneity of NGR-TNF have limited the further development of this drug. We have shown that fusing a serine to its N-terminus generates a new drug (called S-NGR-TNF) with increased stability, reduced heterogeneity and improved antitumor activity in preclinical lymphoma and sarcoma models, alone or in combination with chemotherapy.

Methods: We evaluated the antitumor activity of murine S-NGR-TNF (5 ng/kg) alone or with temozolomide (TMZ, 20 mg/kg, administered 48 h and 96 h post S-NGR-TNF) in 2 glioblastoma (GBM) mouse models. These models involved intracranial implantation of GL261 cells engineered to express Luc2 firefly luciferase (GL261-Luc2) or Luc2 firefly luciferase along with the truncated human nerve growth factor-receptor (GL261/SR-Luc2 cells). Tumor growth was monitored using bioluminescent imaging, with pharmacological treatments starting 7 d after tumor implantation and repeated every 11-13 d for 2-4 cycles. Major immune cell populations infiltrating the tumors were assessed by FACS 48 h after S-NGR-TNF administration.

Results: In the GL261-Luc2 model, S-NGR-TNF-treated mice showed a significant antitumor response compared to controls, with complete tumor regression in 4 out of 10 treated mice, while none in the control group showed a similar response. No treatment-related toxicity was observed. Cumulative results from 3 independent experiments using the GL261/SR-Luc2 model also showed significant antitumor effects. The S-NGR-TNF/TMZ combination group showed stronger antitumor effects than the groups treated with TMZ, S-NGR-TNF, or vehicle (19/30, 12/28, 12/29, and 6/28 cured mice, respectively). Analysis of GL261-Luc2 tumors after S-NGR-TNF treatment showed reduced tumor-infiltrating B lymphocytes, CD8+ T cells, Tregs and M2 macrophages.

Conclusion: S-NGR-TNF synergizes with TMZ in GBM models, potentially by decreasing tumor-infiltrating immunosuppressive cells. Further preclinical studies combining S-NGR-TNF with TMZ and radiotherapy, the current standard of care for GBM, are warranted to evaluate its clinical application potential.

#1796 Development of a CSF-1Ri liposomal formulation using a continuous manufacturing approach and design of experiments (DOE).

Asma M. AlTerawi[1], Raneem R. Aldaqqa[1], Fatemah S. Sunbul[2], David Edwards[3], Sandro R. P. da Rocha[1]

[1]Department of Pharmaceutics and Center for Pharmaceutical Engineering - School of Pharmacy, Virginia Commonwealth University, Richmond, VA,[2]Department of Respiratory Therapy, A-Mana College for Medical Sciences, Saudi Arabia, Dammam, Saudi Arabia,[3]Department of Mathematical Sciences, The Military College of South Carolina, Charleston, SC

Osteosarcoma (OS) predominantly metastasizes to the lungs, with approximately 20% of patients presenting detectable osteosarcoma lung metastases (OSLM) at first diagnosis, while most of the other patients are expected to have undetectable lung micrometastases. OSLM is associated with a 5-year survival rate of only 20%, which not improved over the past four decades. Current OSLM pharmacotherapies are not curative, and experimental immune checkpoint inhibitor therapies have shown minimal efficacy. PLX-3397 (PLX), an FDA-approved colony-stimulating factor-1 receptor inhibitor (CSF-1Ri), targets tumor-associated macrophages (TAMs) in solid tumors, offering a promising strategy to support standard of care in OSLM. However, systemic administration of PLX is limited due to poor tissue distribution and serious off-target toxicities. This study aimed to develop a clinically translatable liposomal PLX (L-PLX) suspension for localized lung delivery via nebulization. A scale-independent continuous manufacturing process and a Design of Experiments (DOE) approach were employed to optimize L-PLX formulations to meet the Quality Target Product Profile (QTPP). Critical quality attributes (CQAs), including hydrodynamic diameter (HD), polydispersity index (PDI), zeta potential (ζ), drug encapsulation efficiency (EE%), and drug loading (DL%), were evaluated. Optimization was performed using A-Optimal DOE with 24 runs, assessing material attributes (MAs) such as Lipid-to-PLX (L:D) ratio and aqueous medium composition, alongside process parameters (PPs) including total flow rate, temperature, and flow rate ratio (FRR).The optimized formulation showed excellent model adequacy with R-squared and adjusted R-squared values of 0.995493 and 0.990575, respectively, confirming its predictive accuracy for particle characteristics. Key factors identified were FRR, L:D ratio, and heating. The optimal formulation, with a L:D ratio of 5, PBS as the aqueous phase, FRR of 5, and room temperature, was validated experimentally, aligning predicted and observed outcomes for particle size, PDI, EE%, and DL%. After additional purification via Tangential Flow Filtration (TFF) to reduce ethanol content to 0.5%, and sterilization, the final product remained stable for over three months at 2-8 ºC, showing consistent HD, PDI, and zeta potential. Aerosol characteristics, tested with a hand-held nebulizer, maintained HD/PDI, suggesting compatibility with pulmonary delivery. Particle size distribution was similar to Arikayce®, the only FDA approved inhaled nanoformulation for lung administration. *In vitro* experiments with RAW 264.7 macrophages polarized to an M2-like phenotype showed that L-PLX reduced M2-like phenotype, confirming its potential to modulate TAM phenotypes while retaining therapeutic activity. These results highlight L-PLX as a promising candidate for OSLM therapy.

#1797 Dendrimer cisplatin and salinomycin complexes as a new combination therapy for ovarian cancer.

Sridharan Jayamohan[1], Sushesh Srivatsa Palakurthi[2], Jessica D. Johnson[1], Salvador C. Alejo[1], Prabhakar P. Venkata[1], Diksha Jamwal[1], Suryavathi Viswanadhapalli[1], Rajeshwar R. Tekmal[1], Edward Kost[1], Ratna K. Vadlamudi[1], Srinath Palakurthi[2], **Gangadhara R. Sareddy**[1]

[1]Obstetrics & Gynecology, UT Health Science Center at San Antonio, San Antonio, TX,[2]Department of Pharmaceutical Sciences, Texas A&M University, Kingsville, TX

Background: Ovarian cancer (OC) is the most lethal gynecologic cancer that initially responds to platinum-based therapy but often becomes resistant, leading to high mortality rates. Given the heterogeneity of OC, it is critical to develop a safe and effective drug combination that targets different tumor cell populations, including chemosensitive, resistant, and cancer stem cells (CSCs). In this study, we evaluated the efficacy of nanocarriers designed to specifically target CD44-expressing OC cells utilizing hyaluronic acid (HA)-conjugated poly(amidoamine) (PAMAM) dendrimer complexes of cisplatin and salinomycin to treat OC. Methods: The efficacy of HA-dendrimer-cisplatin (HA-CIS) and HA-dendrimer-salinomycin (HA-SAL) nanoformulations on cell viability, survival, and apoptosis of established and patient-derived OC cells were examined by Cell Titer Glo, colony formation, and Annexin V assays, respectively. Stemness and self-renewal of CSCs were examined using sphere formation and extreme limiting dilution assays. Mechanistic studies employed RNA-sequencing, RT-qPCR, Western blotting, and immunohistochemistry. The effect of combination therapy on DNA double-strand break (DSB) repair was determined using gH2AX, RAD51 foci, and comet assays. Orthotopic, PDX, and PDEX models were used for the preclinical evaluation.
Results: The combination of HA-CIS and HA-SAL synergistically reduced the cell viability, survival, and self-renewal and promoted apoptosis of OC cells. RNA-seq studies showed that HA-CIS upregulates the DNA-DSB repair pathway, specifically homologous recombination (HR). However, combination treatment resulted in the attenuation of HR. Accordingly, combination therapy significantly decreased the DNA repair ability of OC cells, which was evident from increased gH2AX foci formation and reduced RAD51 foci formation. Further, comet assays demonstrated enhanced DNA damage with combination treatment compared to monotherapy. Importantly, the combination treatment of HA-dendrimer-CIS and HA-dendrimer-SAL significantly reduced tumor progression in orthotopic intrabursal models and in patient-derived xenograft (PDX) and explant (PDEX) models.
Conclusions: Our results establish that HA-dendrimer-CIS and HA-dendrimer-SAL combination therapy may represent a novel therapeutic combination for treating OC.

#1798 K-679: A novel, ultra-high-DAR antibody drug-loaded unimicelle conjugate (ADUC) enabling more effective treatment in EGFR-expressing solid tumors compared to general ADCs.

H. Yoshida, K. Suzuki, K. Hosono, N. Fujimaki, M. Tamiya, M. Shiraishi, H. Higashi, M. Mori;
Kowa Company, Ltd., Tokyo, Japan

Background: Conventional antibody-drug conjugates (ADCs) face significant challenges in treating solid tumors due to heterogeneous antigen expression or low antigen levels, resulting in suboptimal targeting and reduced therapeutic efficacy. While increasing the drug-to-antibody ratio (DAR) could enhance efficacy, traditional high-DAR approaches often compromise pharmacokinetics due to increased hydrophobicity and molecular size. To address these limitations, we engineered a novel platform technology: a compact, 20-nm antibody drug-loaded unimicelle (unimer-micelle) conjugate (ADUC) utilizing single-chain polymer nanoparticles. This platform achieves an unprecedented DAR of approximately 40 while maintaining favorable physicochemical properties. Using this platform, we developed K-679, which combines cetuximab with DM1-loaded unimicelles.
Methods: Antigen-binding specificity and cellular internalization of K-679 were evaluated using surface plasmon resonance, flow cytometry, and fluorescence microscopy. In vitro cytotoxicity, in vivo anti-tumor activities, pharmacokinetics, and biodistribution were investigated in multiple human cancer cell lines and xenograft models. The bystander killing effect was quantified using an in vivo imaging system in mice co-inoculated with EGFR-positive and luciferase-expressing EGFR-negative cells. The benchmark ADCs were prepared by conventional conjugation of cetuximab with DM1.
Results: K-679 exhibited EGFR-binding affinity equivalent to that of unconjugated cetuximab, efficient cellular internalization, and triggered cell-cycle arrest, apoptosis, and cell-growth inhibition in EGFR-positive cells, even in chemotherapeutic drug-resistant lines. In vivo studies demonstrated superior tumor-targeted delivery compared to conventional antibodies, and efficient payload release in tumors with minimal DM1 levels in circulation. This resulted in potent anti-tumor effects across multiple EGFR-positive tumor xenograft models, while showing no activity in EGFR-negative models. These observations reveal that K-679 mediates its anti-tumor effects in an EGFR-dependent manner through DM1-mediated cytotoxicity. Notably, K-679 showed significant bystander killing of EGFR-negative cells within heterogeneous tumors and exhibited efficacy in EGFR-low tumor models in which benchmark ADCs proved ineffective. Comparative studies further highlighted that K-679 outperformed lower-DAR variants at equivalent payload doses.
Conclusion: Our findings establish K-679 as a promising therapeutic candidate for heterogeneous EGFR-expressing solid tumors, characterized by a more potent anti-tumor effect through an exceptionally high DAR, enhanced drug accumulation into tumors and significant bystander effect.

#1799 Harnessing the GlycOBI® enabling technologies: next-generation site-specific glycan ADCs with versatile DAR to enhance therapeutic index.

Teng-Yi Huang, Ka-Shu Fung, Yu-Chao Huang, Chi-Sheng Shia, Chi-Huan Lu, Nan-Hsuan Wang, Ya-Chi Chen, Ming-Tain Lai

OBI Pharma, Inc, Taipei, Taiwan

The GlycOBI® enabling technologies represent a novel approach for the development of antibody-drug conjugate (ADC) to achieve site-specific glycan conjugation through the proprietary dual-action enzyme EndoSymeOBI®. This innovative technology is capable of attaining homogeneous ADC at specific drug-to-antibody ratio (DAR) while maintaining antibody integrity and function. A key feature of the technology is its exclusive linker, which has a unique design to enhance payload stability and enable selective release in the tumor microenvironment, thereby potentially broadening the therapeutic index. The ADCs that use the GlycOBI® enabling technologies are shown to be highly homogeneous while achieving uniform DAR configurations. These next generation ADCs, combined with optimized and novel linker designs, exhibit superior pharmacokinetics, stability, and antitumor activity compared to conventional ADCs in various animal models. The technology supports mono-specific and multi-specific antibodies, expanding its versatility for various therapeutic modalities. The flexibility of DAR configurations and proprietary linker design further demonstrate the technologies' adaptability across diverse payload classes, including cytotoxic agents, immunomodulators, and degraders. In preclinical models, OBI-902 (TROP2 GlycOBI® DAR4 ADC) and OBI-904 (Nectin-4 GlycOBI® DAR8 ADC), both using Exatecan, a potent topoisomerase 1 inhibitor as the payload, have demonstrated promising antitumor activities. Notably, OBI-904, with its DAR8 format, exhibits superior activities in animal models, including in low antigen expressing tumor models, compared to approved and other benchmark Nectin-4 ADCs. Further, both ADCs maintained their stability and avoided antibody aggregation. Both candidates are targeted for IND submissions in 2025, representing an important milestone in advancing the GlycOBI® ADCs to the clinics. The integration of glycan engineering, EndoSymeOBI®, and a novel linker design establishes the GlycOBI® suite of technologies as a robust and adaptable solution to address the current ADC limitations. These advancements underscore its transformative potential in driving the next generation of therapeutics.

#1800 BL0175: An intramuscular nano-conjugate of fulvestrant, exhibits superior tumor distribution and antitumor efficacy compared to fulvestrant injection in MCF-7 xenograft mouse model.

Haoyuan Jiang, Xianjie Chen, Yuxiang Shao, Fuyao Zhang

Shanghai Best-Link Bioscience, LLC, Shanghai, China

Background: Breast cancer (BC) was the most commonly diagnosed cancer and the second leading cause of cancer death among women worldwide. Fulvestrant injection was the first approved SERD (selective estrogen receptor degrader) for metastatic/advanced HR positive (HR+) breast cancer. Although the clinical efficacy of fulvestrant has been defined, its higher efficacy has not been achieved due to several inherent disadvantages, such as low solubility, poor pharmacokinetics (PK), injection site pain with oil-based concentrate formulation. To overcome these drawbacks, we developed a novel nano-conjugate candidate BL0175 for targeted delivery of fulvestrant. BL0175 is highly water-soluble and has a low viscosity intramuscular formulation with high drug loading, which can result in improved efficacy along patient compliance compared to original formulation of fulvestrant. In this preclinical study, we evaluated the efficacy as well as PK/PD properties of BL0175 in MCF-7 xenograft model.
Methods: PK/PD study was performed in MCF-7 xenograft model in a 336 h-period. The drug of fulvestrant concentration was determined in plasma and tumor tissue at different time points. MCF-7 (human BC) tumor fragments were implanted subcutaneously in NOD/SCID mice. Three doses of BL0175 (containing 25, 50 and 100 mg/kg of fulvestrant) or fulvestrant injection(100 mg/kg) were administered subcutaneously (s.c.) weekly for 3 weeks.
Results: In the PK/PD experiments, BL0175 showed excellent pharmacokinetic properties. The 336-h tumor AUC of released fulvestrant from BL0175 was 140 times higher than fulvestrant injection in tumor tissue. The tumor-accumulation efficiency (AUC_{Tumor}/AUC_{Plasma}) of fulvestrant released from BL0175 was 210, which was 21 times higher than that of fulvestrant injection. In the MCF-7 xenograft model, BL0175 had much better efficacy than fulvestrant injection as monotherapy. All three treatment groups of BL0175 showed strong efficacy (TGI 75.1% at 25 mg/kg, 81.0% at 50 mg/kg, 87.3% at 100 mg/kg, $p < 0.001$) compared to fulvestrant injection (TGI 36.7% at 100 mg/kg, $p < 0.001$) on day 28. Furthermore, BL0175 was safe and well tolerated without sign of clinical toxicity and body weight loss.
Conclusions: BL0175 is a unique preclinical nano-drug candidate that exhibited remarkedly enhanced tumor retention and excellent pharmacokinetic properties compared to fulvestrant injection. Furthermore, BL0175 demonstrated superior anti-tumor efficacy against fulvestrant injection in breast cancer. The pre-clinical efficacy and safety studies supported its further development in the clinical stage for HR+ breast cancer therapy.

#1801 Development and evaluation of KMD111: A HER2-targeted novel drug delivery system for gastric and breast cancer therapy.

Jong Kook Rho, Gyeong Hee Lee, Seung Min Byun, Hyo Kyoung Park, JinBeam Kim, YoungKuk Yun, Myung Hoon Kim

KMD Bio Co., Ltd., Daejeon, Korea, Republic of

Background: HER2-low expressing cancers, including gastric and breast cancers, have historically been categorized as HER2-negative, limiting targeted therapy options. To address this, we developed KMD111, a novel HER2-targeted drug delivery platform using mesoporous silica nanoparticles (MSNs) loaded with a potent anticancer drug and coated with HER2-targeting affibody proteins via a glutathione-sensitive GST-GSH bio-linker. KMD111 demonstrated high tumor-targeting efficiency, superior anti-tumor efficacy, and a favorable safety profile in preclinical models, providing stability in the bloodstream and selective drug release regulated by GSH concentrations in the tumor microenvironment.
Methods: KMD111 was synthesized by conjugating HER2-targeting affibody proteins to drug-loaded MSNs. Binding affinity and internalization were assessed in HER2-expressing cancer cell models. Anti-tumor efficacy was evaluated in gastric and breast cancer xenografts, while mechanistic studies examined cell cycle arrest, apoptosis activation, and HER2 signaling inhibition. Safety was evaluated through macrophage uptake, histopathological analysis, and toxicity testing.
Results: KMD111 demonstrated efficient drug loading and GSH-responsive drug release in vitro, with high tumor-targeting efficiency and strong internalization in HER2-expressing cancer cells. It exhibited superior anti-tumor efficacy compared to Enhertu®, Kadcyla®, and Cyramza® and effectively triggered cell cycle arrest and apoptosis activation through a potent anticancer drug. KMD111 also showed a favorable safety profile, including minimal macrophage uptake, negligible myelosuppression, and no significant off-target toxicity, with a therapeutic index exceeding five times the effective dose.
Conclusion: KMD111 demonstrates robust targeting, high therapeutic efficacy, and a favorable safety profile in HER2-expressing gastric and breast cancer models. This innovative approach, leveraging a glutathione-regulated bio-linker for enhanced delivery and tumor specificity, represents a promising foundation for next-generation drug delivery systems targeting HER2-expressing malignancies.

#1802 Exosomes-mediated oral gene delivery to treat lung cancer.

Raghuram Kandimalla[1], Margaret Wallen[2], Disha N. Moholkar[1], Jeyaprakash Jeyabalan[2], Wendy Spencer[2], Farrukh Aqil[3], Ramesh C. Gupta[4]

[1]Brown Cancer Center, Department of Pharmacology & Toxicology, University of Louisville, Louisville, KY,[2]3P Biotechnologies Inc., Louisville, KY,[3]Brown Cancer Center, Department of Medicine, University of Louisville, Louisville, KY,[4]Brown Cancer Center, Department of Pharmacology & Toxicology, University of Louisville, 3P Biotechnologies Inc., Louisville, KY

Gene therapy is an emerging field in cancer therapeutics that targets specific proteins to control cancer cell proliferation. In recent years, several attempts to treat lung cancer using gene therapy have shown promise, but clinical translatability remains a significant challenge. Factors such as immunogenicity, toxicity, inflammatory responses, and cost-effectiveness hinder the widespread use of gene delivery vectors. Oral delivery of genes could potentially address these challenges; however, achieving therapeutic dosages at the targeted site is difficult due to the degradation of nucleic acids in the gastrointestinal tract before reaching systemic circulation. In the current study, we report a novel exosome-polyethyleneimine (PEI) matrix (EPM) for the oral delivery of nucleic acid therapeutics (siRNA) to treat lung cancer. Exosomes were isolated from bovine colostrum powder by rehydration and differential centrifugation. The tumor-targeting ligand folic acid (FA) was covalently attached to the exosome surface via click chemistry, while PEI was conjugated through ionic interaction to form FA-EPM. The siRNA entrapment efficiency by EPM was found to be more than 90% of the supplied siRNA (up to 20 µg). The transfection efficiency of EPM-siRNA was assessed *in vitro* via western blot analysis, determining KRAS and NRF2 knockdown in human lung cancer cells (A549). Results demonstrated that EPM-si*KRAS* and EPM-si*NRF2* suppressed KRAS and NRF2 expression by 70-80% in A549 cells in a dose-dependent manner. The therapeutic efficacy of orally administered EPM-si*NRF2* and FA-EPM-si*KRAS* was tested in subcutaneous and orthotopic lung tumor xenografts generated in nude and nod scid mice, respectively. The EPM-si*NRF2* formulation inhibited subcutaneous lung tumor growth by 59% compared to control animals ($p < 0.01$), matching the tumor inhibition by the same formulation administered intravenously. Additionally, orally administered FA-EPM-si*KRAS* inhibited orthotopic lung tumor growth by 74% ($p < 0.01$) accompanied with >80% downregulation of the tumor KRAS expression level. This study demonstrated that colostrum exosome-based EPM technology effectively delivers siRNA to tumor sites when administered orally, achieving significant gene knockdown and lung tumor suppression. These findings suggest a simple and effective exosome-based oral gene delivery strategy for cancer therapeutics.

#1803 Exosome-encapsulated PD-L1 siRNA reduces tumor growth by modulating the tumor-associated macrophages.

Raghuram Kandimalla[1], Omar S. Sarkar[2], Disha N. Moholkar[3], Margaret Wallen[4], Chuanlin Ding[5], Kavitha Yaddanapudi[6], Ramesh C. Gupta[7], Farrukh Aqil[8]

[1]Brown Cancer Center, Department of Pharmacology & Toxicology, University of Louisville, Louisville, KY,[2]Department of Microbiology and Immunology, University of Louisville, Louisville, KY,[3]Brown Cancer Center, Department of Pharmacology & Toxicology, University of Louisville, Louisville, KY,[4]3P Biotechnologies Inc., Louisville, KY,[5]Brown Cancer Center, Department of Surgery, University of Louisville, Louisville, KY,[6]Brown Cancer Center, Department of Microbiology and Immunology, Department of Surgery, University of Louisville, Louisville, KY,[7]Brown Cancer Center, Department of Pharmacology & Toxicology, 3P Biotechnologies Inc.; University of Louisville, Louisville, KY,[8]Brown Cancer Center, Department of Medicine, University of Louisville, Louisville, KY

Immunotherapy, a rapidly advancing cancer treatment, influences the patient's immune system to combat tumors. However, cancer cells evade T-cell immunosurveillance by overexpressing PD-L1 on their surfaces. Additionally, tumor-associated macrophages (TAMs) further complicate the therapeutic efficacy of immune checkpoint inhibitors (ICIs) within the tumor microenvironment. The success of immunotherapy in solid tumors is limited to 20-45%, primarily due to tumor heterogeneity, an immunosuppressive tumor microenvironment, and inefficient tumor-specific trafficking of therapeutics. In this study, we developed an exosome-polyethyleneimine matrix (EPM) nanoplatform functionalized with a tumor-targeting ligand, folic acid (FA-EPM), for the targeted delivery of PD-L1 siRNA to the lung tumor. This approach efficiently silences PD-L1 expression on both tumor cells and TAMs. Exosomes were isolated from bovine colostrum through rehydration and differential centrifugation processes, resulting in exosomes with an average particle size of 66±2.5 nm, a zeta potential of -9.6 mV, and a polydispersity index (PDI) of 0.28. The entrapment efficiency of siPD-L1 within the EPM matrix exceeded 90%. siPD-L1 sequences with specificity for mouse and human PD-L1 were screened *in vitro* against LLC and A549 cells, respectively. Protein lysate analysis revealed that EPM-siPD-L1 significantly downregulated PD-L1 expression in LLC-1 cells (up to 80%) and in A549 cells (>80%). The most effective siPD-L1 candidate from the *in vitro* studies was tested *in vivo* in C57BL/6 mice bearing orthotopic lung tumor xenografts established with LL/2-Luc2 cells (50 × 10^3 cells/mouse). Tumor-bearing mice were divided into three groups (n = 7-9) and treated intravenously with vehicle, FA-EPM-scrambled siRNA, or FA-EPM-siPD-L1 (15 µg/dose). Tumor growth was monitored twice weekly using bioluminescence imaging. FA-EPM-siPD-L1 treatment significantly reduced tumor growth (>70%; $p < 0.01$) compared to vehicle or scrambled siRNA-treated mice. Additionally, a significant reduction in tumorous lung weight (~60%; $p < 0.01$) was observed in the FA-EPM-siPD-L1 group. At euthanasia, tumorous lungs were processed into single-cell suspensions and analyzed for myeloid and T-cell markers using flow cytometry. The tumor reduction was found to be associated with the marked reduction in PD-L1 expression, a significant depletion of immunosuppressive TAMs and increased levels of anti-tumoral gamma delta T cells. In summary, these results demonstrate that EPM-mediated delivery of siPD-L1 enhances immune surveillance, effectively inhibits lung tumor progression, and establishes EPM as a promising nanoplatform for delivering siRNA-based therapeutics.
Funding: Supported by Immunotherapy CCII CoBRE pilot project (P20GM135004) and the Agnes Brown Duggan Endowment.

#1804 An innovative dual-payload ADC combining topoisomerase 1 inhibitor and a tubulin inhibitor efficiently overcomes drug resistance.

Ailin Shan, Chun Deng, Peng Zou, Shuai Song, **Jiaqiang Cai**, Tongtong Xue

MediLink Therapeutics (Suzhou) Co., Ltd., Suzhou, China

Antibody Drug Conjugates (ADC) have achieved great success as anti-cancer therapies, with 15 ADC drugs approved in global market. Despite the initial response to ADC treatment, drug resistance and tumor relapse inevitably happen due to the high heterogeneity in advanced tumors. The mechanism of resistance can be due to downregulation of drug targets, upregulation of multi-drug resistance mechanisms, such as ABCB1/ABCG2 and others. Overcoming these resistance mechanisms are a big challenge to next-generation ADC development for cancer therapy. To address these challenges, MediLink has developed an innovative dual-payload ADC with two payloads of different MOAs: a topoisomerase 1 inhibitor and a tubulin inhibitor. Two conjugation methods were developed: (1) Tubulin inhibitor payload-linkers were conjugated at specific lysine sites of antibody while Topo1i payload-linker were conjugated with free cysteine thiols via TMALIN® Technology, resulting in a dual-payload ADC with DAR of 2 and 8 for each payload-linker, respectively. (2) Reduced antibody conjugates with pre-mixed or step wisely added Topo1i payload linker and Tubulin payload linker in different proportions to obtain any desired ratio of the two different payloads. ADC characterization showed good stability, homogeneity and precise DAR values for the conjugation of both payload-linkers to Trastuzumab. In vitro, the dual-payload Her2 ADC demonstrated strong cytotoxicity against tumor cells with different levels of Her2 expression, especially in cells overexpressing ABCB1 and ABCG2, and Enhertu-resistant tumor cells. In vivo, the dual-payload ADC exhibited superior efficacy in Enhertu-resistance CDX model and Her2 low CDX models. In summary, we developed dual-payload ADCs with intention for resolving topo1 inhibitor induced tumor resistance issues. The dual-payload Her2 ADC demonstrated potent in vitro and in vivo anti-tumor activities, showing the potential to overcome the multidrug resistant mechanism and Enhertu resistance.

#1805 Dual-payload TME-activated ADC platform.

Cheng Liu, Yuan Liu, Rui Zhang

Affinity Biopharmaceutical Co., Ltd., Shanghai, China

The combination of Antibody-Drug Conjugates (ADCs) and Immuno-Oncology (IO) agents is regarded as one of the most exciting and promising trends in the rapidly evolving field of cancer treatment. Affinity Biopharma has developed the Tumor MicroEnviroment Activated (TMEA) platform, with proprietary TMEA linkers applicable in both Small Molecule Drug Conjugates (SMDCs), ADCs and dual-payload ADCs. The TMEA linkers could achieve extracellular activation of payloads in the Tumor MicroEnviroment (TME) by a tumor-specific protease Legumain (AEP), which is an acid endopeptidase with tumor site over-expression and a strict specificity for one particular amino acid (Asn), making it a first-in-class, highly specific and pan-solid tumor target in novel drug design. Compared to GGFG linkers cleaved by lysosomal enzymes such as cathepsins B and L, the TMEA linkers exhibited superior stability in human plasma at 37℃, and demonstrated higher cleavage efficiency under identical condition in tumor homogenates (pH=6.5), generating a remarkably clean cleavage profile with minimal to no inactive intermediates. Such advantageous characteristics of TMEA linkers could be supported by a TMEA-SMDC drug Legubicin in clinical trial that releases payload Doxorubicin. For dual-payload TMEA-ADCs, each hydrophilic TMEA linker is paired with two insoluble payloads such as TLR7/8 agonists and topoisomerase I inhibitors, which show similar maximum tolerated doses (MTD) in mice as linker-single-payload. Dual-payload TMEA-ADCs have effectively addressed the CMC challenges and enabled homogenous formation of 8-DAR dual-payload design. In various PBMCs-CDX models, dual-payload TMEA-ADCs such as IMD526 (HER2-ADC), IMD2126 (PD-L1-ADC) and IMD2113 (EGFR&TROP2-ADC) have displayed significant dose-dependent antitumor activities and induced complete tumor regressions with no observable toxicities. Compared to mono- or combo treatments with single-payload ADCs, IMD526 validated its superiority in the *in-vivo* CT26-HER+ syngeneic mouse model, and long-term elimination of detectable tumors was achieved with IMD526 both alone and combined with IMD101 (a TMEA-Cytokine releasing IL-2). In tumor re-challenge studies, mice that were cured exhibited immune memory and were resistant to tumor re-implantation. In non-human primate (NHP) studies, dual-payload TMEA-ADCs showed excellent stability in circulation and a strong safety profile, indicating a greatly expanded therapeutic window. And different from the tumor cell endocytosis activation by GGFG-DXd ADCs, the bio-distribution of TMEA-ADCs resulted in faster and higher levels of payload accumulation in the tumor local tissues. In summary, by integrating antibody targeting, TME activation, cytotoxic therapy, and I/O therapy into one single molecule, the dual-payload TMEA-ADCs has demonstrated the synergistic effects of ADCs conjugated with 16 insoluble payloads from 2 distinct mechanisms of action in tumor models.

#1806 Cymirafen: A protein-drug conjugate that target LGR4/LGR5/LGR6-positive cancer stem cells.

M. Mulero Roig, S. A. Jadhav, W. Pi, O. Tai, S. B. Howell;
UCSD Moores Cancer Center, La Jolla, CA

INTRODUCTION: Cancer stem cells (CSC) are often drug-resistant and the source of recurrence; their destruction is a major therapeutic goal. LGR4/LGR5/LGR6 mark active stem cells and highly expressed in many solid cancers. Cymirafen consists of a human IgG1 Fc domain fused to the receptor-binding domains of RSPO1. The sortase reaction is used to site-specifically conjugate cleavable Gly_3-val/cit-PAB-MMAE linkers to the C-terminal ends of the two chains through the formation of a highly stable peptide bond. We report here pre-IND studies that support the further development of cymirafen.

METHODS: The protein component of cymirafen is constitutively produced by a stable CHO-K1 clone. Kd values were determined by ELISA using recombinant human and cynomolgus LGR5-His. In vitro growth rate inhibition assays used the CCK8 reagent. CDX models used SC implantation. Plasma cymirafen was measured by ELISA using antibodies to different RSPO1 epitopes for capture and detection. Free MMAE in plasma was determined by LC-MS/MS.

RESULTS: Cymirafen binds to human and cynomolgus LGR5 with Kd of 0.27 and 0.38 nM, respectively**.** Its DAR is in the range 1.8-2.0. Across a panel of human cancer cell lines of multiple types, 76% had an IC_{50} of <10 nM, and in 36% IC_{50} was <5 nM. Against pairs of isogenic LGR5-low and LGR5-high OVCAR8 and HEK293 cells, cymirafen exhibited a 1.5 log differential IC_{50} in vitro. When grown as CDX, cymirafen was >2-fold more effective against OVCAR8/LGR5 than OVCAR8/EV. On a dose schedule of 85 mg/kg q7dx4, cymirafen was well tolerated in mice and active in CDX models of multiple types of cancer (colon, gastric, ovarian, neuroblastoma). Only 0.001, 0.002 and 0.013% of the MMAE was released from cymirafen in human, rat and cynomolgus plasma during 96 h at 37°C. MTD in mice is ~128 mg/kg. A dose-ranging study in rats identified a single dose STD_{10} of 75 mg/kg. Serum chemistries disclosed no organ dysfunction. Adverse events were limited to dose-related neutropenia > thrombocytopenia with recovery by two weeks at all doses. Initial and terminal plasma half-lives were 0.8 and 21.5 h; PK was close to linear over the range of 30 - 75 mg/kg. In SKNAS CDX tumors, cymirafen peaked at 4 h and remained above the IC_{50} value for this tumor for ~24 h.

CONCLUSIONS: Cymirafen targets tumors with increased levels of LGR4, LGR5 and potentially LGR6, and co-receptors RNF43 and ZNRF3; an ADC can target only one receptor at a time. This favors greater control of drug resistance and recurrence. Unlike MMAE ADCs with long half-lives and cumulative toxicity, cymirafen delivers MMAE to tumors at high concentrations over a shorter period of time. It has a much higher normalized MMAE content than MMAE-containing ADCs. This permits administration of much higher doses on a well-tolerated weekly schedule. These features distinguish cymirafen from all MMAE ADCs and support its further development as a stem cell-targeted therapeutic.

#1807 Distribution, fate, inhalation exposure of nanoparticles: advancing noninvasive strategies for lung cancer treatment.

Rahul Tiwari, Meghana Kolli, Neeraj Chauhan, Eswara N Ghali, Iris Enriquez, Vivek Kumar Kashyap, Subhash Chauhan, Murali Yallapu

The University of Texas Rio Grande Valley, McAllen, TX

Background: Lung cancer remains the leading cause of cancer-related morbidity and mortality worldwide, including in the United States. Noninvasive nebulized inhalation represents a promising therapeutic strategy for lung cancer, offering enhanced nanoparticle targeting efficiency and prolonged retention within lung tissue. Gambogic acid is an experimental chemotherapeutic agent for lung cancer. Suboptimal biodistribution, and multi-targeting properties can lead to significant systemic toxicity challenges. To mitigate these undesirable side effects and improve clinical translation, nanotechnology-based strategies offer a promising solution. This study aims to develop inhalable nanoparticles (INPs) for effective delivery of therapeutic agents in lung cancer cell lines and *in-vivo* models.
Methods: INPs were synthesized via solvent evaporation and self-assembly and characterized for particle size, drug loading efficiency, and drug release using various analytical methods. Their cellular uptake, intracellular distribution, and colocalization efficacy of INPs were assessed in 2D and 3D lung cancer cell line (A549 and NCI-H1299) models using flow cytometry and confocal microscopy. The fate and biodistribution of INPs were monitored using live animal imager at 24 and 48 hrs post-treatment. The accumulation of INPs in lung tissue was evaluated using in vivo, ex vivo, and microscopy methods. The therapeutic efficacy evaluation of gambogic acid encapsulated INPs was evaluated through in vitro assays, using cell proliferation, clonogenicity, migration, and apoptosis assays.
Results: The developed INPs exhibited an average size of approximately 110 nm based on dynamic light scattering measurements. These formulations exhibited notable mucoadhesion and mucopenetration potential in-vitro models. Drug release experiments displayed a sustained and higher drug release at lysosomal pH (pH 5.0) compared to pH environment (pH 7.4), attributed to the pH-sensitive bonding. The gambogic loaded INPs were confirmed to have superior inhibitory effects on cellular proliferation, colony formation, and migratory characteristics of lung cancer cells. Biodistribution analysis of INPs in mice demonstrated that the particles were predominantly concentrated in lung tissues, with minimal distribution to other organs. Immunohistochemistry and blood chemistry analyses indicate that the INPs possess biologically safe properties.
Conclusions: This study confirms the potential of INPs a novel therapeutic modality for lung cancer treatment. The INPs formulation demonstrated superior therapeutic benefits, including improved drug delivery, enhanced cellular uptake, and increased anti-cancer efficacy across various lung cancer models. These findings suggest that INPs could serve as an innovative therapeutic modality for the treatment of lung cancer.

#1808 Engineering lipid-polymer nanoparticles for siRNA delivery to breast cancer cells.

Abdulelah Alhazza[1], Arthur Manda[1], Hamidreza Montazeri Aliabadi[1], Hasan Uludag[2]

[1]Chapman University, Irvine, CA,[2]University of Alberta, Edmonton, AB, Canada

RNA interference (RNAi) is a powerful tool that can specifically target the expression of virtually any protein without the expensive and time-consuming drug development studies. Despite the initial excitement and extensive efforts, the potential impact of RNAi approaches is yet to be materialized fully in clinical settings. This is mainly due to the challenges in delivering RNA molecules. Lipid nanoparticles (LNPs) have been the leading delivery system for nucleic acids, an achievement established by introducing the first FDA-approved small interfering RNA (siRNA) drug and COVID-19 vaccine to clinics. However, targeted delivery to a solid tumor still eludes the developed LNPs. On the other hand, polymers are among the oldest delivery systems for nucleic acids, and polyethyleneimine (PEI) was once considered the gold standard in nucleic acid delivery. In this study, we introduce a novel lipid-polymer nanoparticle (LPNP) platform, meticulously engineered for targeted delivery of siRNA to cells implicated in breast cancer. We hypothesized that specially designed low molecular weight PEIs can partially or completely replace the ionizable lipids for a more accommodating structure for additional moieties, which could lead to a safer and more efficient nucleic acid delivery. We first optimized the LNP formulations as a point of reference for cellular uptake, cytotoxicity, and protein silencing efficiency, employing sophisticated designs facilitated by the Design-Expert software. Leveraging the optimal LNP formulation, we integrated specifically designed cationic polymers as partial or complete replacements for the ionizable lipid. This methodological approach, incorporating optimal combined designs and response surface methodologies, refined the LPNPs to an optimal efficiency. Our results indicate that these refined LPNPs enhance the delivery of siRNA, leading to efficient gene silencing in targeted cancer cells. The improved delivery efficiency not only underscores the potential for specific therapeutic applications, but also suggests a broader utility for this platform in various cancer treatments.

#1809 The role of HyDIFFUZE^{TM} in co-formulation with subcutaneous infliximab.

Younseo Oh, Hyungip Kim, Heeyoun Kim, Donghwan Kim, Boyoung Park, Juyoung Byun, Chaeyoung Lim

Huonslab Co., Ltd, 17, Changeop-ro, Sujeong-gu, Seongnam-si, Gyeonggi, Korea, Republic of

Background : Recombinant human Hyaluronidase PH20 (rHuPH20) is an enzyme that degrades subcutaneous (SC) hyaluronan and has been used as a drug dispersion agent to optimize the SC delivery of co-formulated therapeutic antibodies. HyDIFFUZE^{TM}, a stand-alone rHuPH20 is under development as a dispersion agent and tissue permeation modifier for co-formulated drugs in the stage of the registration study(N=243) in S. Korea. Recently, SC formulation of Infliximab, a tumor necrosis factor inhibitor, has been approved for the treatment of autoimmune diseases. Although the therapeutic response to infliximab is strongly associated with drug exposure, the effect of HyDIFFUZE^{TM} on absorption and therapeutic response of co-formulated infliximab remains unclear. The purpose of the study was to demonstrate the applicability of HyDIFFUZE^{TM} as an effective drug dispersion agent by evaluating the effects of HyDIFFUZE^{TM} on the pharmacokinetics (PK) of co-formulated infliximab, compared to SC infliximab alone.

Methods : To investigate whether HyDIFFUZE^{TM} enhances dispersion of infliximab, PK of infliximab was analyzed after SC injection along with HyDIFFUZE^{TM} and compared with that of infliximab without HyDIFFUZE^{TM}. A single dose was administered to SD rats (n=5/group), and blood samples were collected at 12 time points over 28 days post injection. Plasma concentrations of infliximab were determined using ELISA and PK parameters were obtained by Phoenix WinNolin.

Results : In vivo PK studies showed that formulation of 84 mg/kg Infliximab combined with 1400 unit/kg HyDIFFUZE^{TM} resulted in AUC_t 127.2% and C_{max} 119.9%, compared to 84 mg/kg infliximab without HyDIFFUZE^{TM}. Furthermore, PK profiles of 63 mg/kg infliximab with HyDIFFUZE^{TM} similar to that of 84 mg/kg infliximab alone with AUC_t 105.5% and C_{max} 95.1%. These results suggest that HyDIFFUZE^{TM} might reduce the dose of infliximab required to generate equivalent therapeutic effects. Increasing the dose of Infliximab/HyDIFFUZE^{TM} resulted in a greater-than-dose-proportional increase in AUC_t, C_{max}, and $T_{1/2}$, indicating enhanced systemic drug exposure. Additionally, SC formulated of 168 mg/kg infliximab with HyDIFFUZE^{TM} showed an AUC_t approximately 90% of that achieved with IV injection of 60 mg/kg infliximab.

Conclusions : This study demonstrated that the HyDIFFUZE^{TM} significantly enhanced intravascular uptake of infliximab, with sustained therapeutic plasma concentrations of Infliximab observed over an extended duration as the dose increased. These findings suggest that combining infliximab with HyDIFFUZE^{TM} may allow enhanced systemic drug exposure even at lower dose sand potentially extend dosing intervals. Furthermore, the results presented the possibility that SC infliximab/HyDIFFUZE^{TM} may potentially replace intravenously (IV) infliximab in the induction regimen resulting in an entire treatment of induction and maintenance with SC infliximab/HyDIFFUZE^{TM} only.

#1810 Development miR-205-loaded tannic acid-stabilized liposomes for enhanced therapeutic delivery.

Victoria Herrera, Rahul Tiwari, Bridgette Lozano, Neeraj Chauhan, E N Kumar Ghali, Subhash Chauhan, Murali Yallapu

University of Texas Rio Grande Valley, Edinburg, TX

Background: The therapeutic potential of microRNAs (miRNAs) in cancer treatment has garnered significant research interest. miR-205 emerging as a promising therapeutic candidate for cancer treatment due to its tumor-suppressive properties. Despite its potential, the clinical translation of miR-205 is hindered by the lack of efficient and scalable delivery systems. Liposomal formulations offer a versatile platform for miRNA delivery, with broad applications in vaccines and anticancer therapies (e.g., mRNA, small molecule drugs). However, challenges such as large-scale production, precise size control, and unform size distribution of lipid-based drug delivery systems remain significant barriers to the widespread use in the pharmaceutical industry. This study aims to develop stable miR-205 liposome formulations for cancer therapeutics.

Methods: A microfluidics chip-based platform, leveraging commercially available microfluidic device technology, was employed to synthesize miR-205-loaded liposomes. Liposomes were produced at a total flow rate (TFR) of 10 ml/min and a flow rate ratio (FRR) of 1:3. The effect of key formulation parameters, including TFR, FRR, and total lipid concentration, on particle size and size distribution, was systematically investigated. Liposomes were also produced using a conventional method for comparative analysis. The formulations were characterized for their physiochemical properties using dynamic light scattering (DLS) and Fourier-transform infrared spectroscopy (FTIR), and their stability. The cell viability of endometrial cell lines treated with different concentrations of liposome were assessed by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and cell counting. Cell migration was evaluated using wound-healing assay.

Results: All liposomal formulations displayed an average particle size below 200 nm with acceptable physicochemical stability and a low polydispersity index (PDI). The microfluidic approach demonstrated superior control over liposome size and PDI compared to traditional bulk methods, highlighting its potential for scalable and reproducible production. Furthermore, miR-205-loaded liposomes produced via microfluidics exhibited robust encapsulation efficiency and effective intracellular delivery of miR-205 in cancer cell models. The in vitro functional assays confirm superior anti-cancer potential in cancer cells.

Conclusions: This study highlights the potential of microfluidic platforms as a scalable and efficient technology for manufacturing of liposomal formulation. The miR-205-loaded liposomes developed in this study showed significant promise in delivering therapeutic miRNAs to cancer cells, demonstrating the feasibility of this platform for clinical translation.

#1811 Use of lipid nanoparticles to generate CAR T cells targeting colon cancer.

Pedro Pires Goulart Guimaraes, Walison Nunes da Silva, Marco Tullio Rodrigues Alves, Pedro Henrique Dias Moura Prazeres, Gabriel Henrique Costa da Silva, Gabriel Vieira Azevedo, Maria Luiza Gomes Viana

Physiology and Biophysics, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brazil

Introduction: Chimeric Antigen Receptor (CAR) T cells represent a breakthrough in cancer immunotherapy. However, current methods for engineering T cells to express CARs are expensive and typically rely on viral vectors, which are integrative and have been linked to severe adverse effects resulting from the continuous expression of CARs. In contrast, non-viral vectors, particularly lipid nanoparticles (LNPs), have emerged as a promising alternative for generating CAR T cells with transient CAR expression. In this study, we developed a nanoparticle-based mRNA platform to generate CAR T cells targeting Claudin 6, a marker for various solid tumors.
Methods: mRNA encoding the CAR targeting Claudin 6 was synthesized via in vitro transcription and subsequently loaded into LNPs. The LNPs were synthesized using microfluidic mixing, incorporating an ionizable lipid and excipient lipids at different molar ratios. To assess transfection efficiency, Jurkat cells and primary human T cells were transfected with CAR mRNA encapsulated in LNPs (LNP-CAR) and analyzed by flow cytometry. T cell activation was evaluated by co-culturing CAR-expressing cells with colorectal cancer cells (Colo 205) for 24 hours, followed by measurement of CD69 expression in CAR+ cells. Primary human T cells were transfected with an optimized LNP formulation and co-cultured with colorectal cancer cells at varying effector-to-target (E:T) ratios to assess specific killing by flow cytometry.
Results: We developed an LNP formulation to deliver mRNA encoding the CAR to T cells. The LNPs had a size of 98 nm, a polydispersity index of 0.19, a slightly negative zeta potential of -3.4 mV, and an encapsulation efficiency of 82%. Enhanced CAR expression was observed in both Jurkat and primary human T cells treated with LNP-CAR, compared to control cells, with peak CAR expression occurring 24 hours after treatment. When co-cultured with colorectal cancer cells at a 1:1 ratio, Jurkat and primary human T cells transfected with LNP-CAR exhibited increased CD69 expression, indicating T cell activation. Importantly, primary T lymphocytes transfected with the optimized LNP-CAR formulation effectively killed colorectal cancer cells in vitro.
Conclusion: In this study, we identified a lead LNP formulation that induced CAR expression in primary T lymphocytes, which were subsequently activated and capable of specific killing of colorectal cancer cells. This non-viral delivery method using LNPs offers a promising alternative for producing CAR T cells. Furthermore, this approach can be adapted to other CAR constructs, potentially broadening its applicability to the treatment of solid tumors, an area where immunotherapies currently face significant challenges.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Radiation Treatment Combinations for Tumors, Normal Tissue Poster Session

#1815 Targeting the serine metabolism-NRF2 axis to overcome radiation resistance in pancreatic cancer.

Prince Kumar, Huseyin Ayhan, Lobna Abdulrahman, Justine Bailleul, Jonathan Ng, Erina Vlashi

Radiation Oncology, UCLA - University of California Los Angeles, Los Angeles, CA

Pancreatic ductal adenocarcinomas (PDAC) have very poor prognosis largely due to extreme resistance to existing therapies, including radiation therapy (RT). Metabolic reprogramming has been shown to promote therapy resistance in PDAC, but little in known in the context of RT. Here, we present findings of a critical intersection between serine metabolism and antioxidant defenses following RT of PDAC that place serine metabolism in a radio*protective* role. We find that this is enabled, in part, by the upregulation of the NRF2 (nuclear factor (erythroid-derived 2)-like 2) pathway following RT, beyond basal, mKRAS-induced levels. Our discovery is in line with the role that the serine metabolism coupled with one-carbon metabolism (1CM) plays in generating antioxidants for offsetting oxidative damage, as well as generating nucleotide precursors for DNA repair. Our TCGA analysis on patient PDAC shows a significant correlation between tumor NRF2 expression and SLC1A5 serine transporter, PSPH (the last enzyme in serine synthesis) and NADPH-producing enzymes of 1CM. Moreover, co-overexpression of PHGDH and NRF2 correlates with poorer overall survival, underscoring the intersection of serine metabolism and NRF2-regulated antioxidant responses in human PDACs. In pre-clinical models we show that RT enhances PDAC cellular glucose consumption in excess of anabolic basal levels, and this is accompanied by increased gene expression levels of enzymes that drive the *de novo* serine biosynthesis pathway (SBP), a branch of glycolysis. PDAC radiosensitization can be achieved by interfering with serine metabolism, be it through inhibiting the first SBP enzyme, phosphoglycerate dehydrogenase, or by removing exogenous serine. The radiation-induced antioxidant pathway involving NRF2 is also implicated in metabolic PDAC reprogramming in part by promoting upregulation of glucose consumption following RT and driving flux of glucose carbons into the SBP. In keeping with this, PDAC radiosensitization can also be achieved by inhibiting the NRF2 pathway (*in vitro* and *in vivo).*
Our findings suggest that NRF2-serine-metabolism axis generates a synchronized metabolic response that protects PDAC from lethality in the aftermath of RT and constitutes a promising and novel radiotherapeutic target.

#1816 BRD4 inhibitor I-BET151 sensitizes glioblastoma to radiotherapy by suppressing super-enhancer-driven COL1A1.

Xichen Fan, Xuenan Li, Ziheng Wang, Weichen Duan, Yafei Wang, **Jiajia Chen**

Department of Oncology, Shengjing Hospital of China Medical University, Shenyang, China

Glioblastoma (GBM) is a highly aggressive and fatal brain tumor typically treated with high-dose radiation (RT) and chemotherapy. However, the limited RT efficacy has spurred interest in radiosensitizing strategies. BRD4, a BET protein, promotes tumor progression by regulating oncogenes through super-enhancers (SEs). Consequently, BRD4 inhibitors, such as I-BET151, are being investigated as potential therapeutic agents. This study investigates the synergistic efficacy of I-BET151 in combination with RT for GBM therapy. Analysis of public databases revealed elevated BRD4 expression in recurrent GBM compared to newly diagnosed cases, correlating with poor prognosis. Immunofluorescence analysis showed increased BRD4 expression in human (T98G and U87) and murine (GL261) GBM cells 48- and 96- hours post-RT (5 Gy). Pretreatment with I-BET151 (1μM or higher) sensitized GBM cells to RT (2.5 Gy or higher), enhancing RT-induced suppression of clonogenic survival, reducing proliferation and invasion, and significantly increasing apoptosis [e.g., GL261: 63.3% ± 0.5% vs. 10.9% ± 0.6% with RT alone ($p < 0.001$), 11.6% ± 0.6% with I-BET151 ($p < 0.001$)]. Co-treatment also amplified RT-induced DNA damage (γH2AX foci) 24 hours post-RT. In a subcutaneous GBM GL261 model in C57BL/6 mice, I-BET151 (10 mg/kg/day, intraperitoneally) administered concurrently with fractionated RT (2 Gy daily for 5 days) significantly reduced tumor growth compared to either treatment alone [e.g., tumor volume at 4 weeks: 259 ± 92.9 mm^3 vs. 574.8 ± 126.5 mm^3 with RT alone ($p < 0.001$), 705.8 ± 168.3 mm^3 with I-BET151 ($p < 0.001$)]. Tumor tissues from the combination group consistently showed the highest levels of apoptosis (TUNEL staining), and lowest Ki67 index, confirming improved therapeutic efficacy. Mechanistically, BRD4 drives activation of SEs marked by H3K27ac. ChIP-seq analysis for H3K27ac in GL261 cells revealed that RT significantly increased SEs, which were reduced with I-BET151 co-treatment. RNA-seq identified 75 RT-upregulated genes that were downregulated with I-BET151 co-treatment. Pathway analysis linked these BRD4-regulated, RT-responsive genes primarily to extracellular matrix pathways. Integrated ChIP-seq and RNA-seq analysis identified COL1A1 as a key BRD4-dependent SE target post RT. I-BET151 suppressed COL1A1 expression both in vitro and in vivo. Moreover, RNAi-mediated COL1A1 silencing reduced proliferation, increased apoptosis, and enhanced RT-induced DNA damage, underscoring its pivotal role in BRD4-mediated radioresistance. In conclusion, I-BET151 enhances radiosensitivity in GBM cells by blocking RT-induced SE-activation and COL1A1 expression, thereby amplifying DNA damage and apoptosis. Further validation in orthotopic GBM models is essential to advance the clinical translation of this promising combination therapy.

#1817 Overcoming tumor hypoxia in MPNSTs using an implantable micro-catheter.

Samual Hatfield, Ryan Courtney, Emily Witt, Jianling Bi, James Byrne

Carver College of Medicine, University of Iowa, Iowa City, IA

Malignant peripheral nerve sheath tumors (MPNSTs) are a devastating and rare type of tumor, accounting for 5-10% of all soft-tissue sarcomas, with a 5-year overall survival rate of only 50%. A key contributing factor to the poor outcomes is treatment resistance due to tumor hypoxia. Despite efforts to mitigate tumor hypoxia as a therapeutic strategy, significant challenges remain, including the kinetics of oxygenation, peak partial pressures achieved, and the safety of existing methods. Here, we report on an implantable system for direct intratumoral oxygen injection that synergizes with radiotherapy. We demonstrate that tumor oxygenation using this system slows tumor growth and extends survival in a mouse model of MPNST. Six-to-eight-week-old female C57BL/6J mice bearing subcutaneous right-flank MPNST allograft tumors were separated into four treatment cohorts: oxygen infusion + radiation, radiation, oxygen infusion, and no treatment. For mice receiving oxygen infusion, the center of the tumor was catheterized once tumors reached a volume of 200 mm^3. Intratumoral oxygen infusion was achieved using a syringe pump to deliver oxygen at a partial pressure of 50 mmHg. The microcatheters were then removed, and a single 15 Gy radiation dose was administered using a small animal radiation research platform. Following treatment, tumor growth was monitored and measured three times weekly using calipers. Tumor volume was approximated using the formula $V = 0.5 \times \text{length (L)} \times \text{width (W)}^2$, where length and width correspond to the x and y measurements of the tumor in mm. Animals were euthanized, and tumors were harvested once their volume reached 2000 mm^3. In a separate cohort of mice, tumors were resected one hour after irradiation, formalin-fixed, paraffin-embedded, and stained for γ-H2AX foci. Intratumoral oxygen infusion combined with radiation inhibited tumor growth and extended the median survival of mice with MPNSTs from 13 days (no treatment) to 25 days (oxygen infusion + radiation). Additionally, there was a significant increase in γ-H2AX foci per nucleus in tumors treated with oxygen infusion + radiation compared to radiation alone. We demonstrate that direct intratumoral oxygenation can radiosensitize MPNSTs in a mouse model. The significant reduction in tumor growth observed in mice receiving oxygen infusion + radiation, compared to radiation alone, suggests that tumor oxygenation may improve outcomes when combined with current standard-of-care therapies.

#1818 Enhancing double-strand break induction in hypoxic glioblastoma cells through dNTP modulation and its potential therapeutic implications.

Edidiong R. Usoro, Dominique Monroe, Kristen Carver, Emily Steinbeck, Arilyn Williams, Waaqo Daddacha

Augusta University, Augusta, GA

Glioblastoma (GBM), the most lethal primary brain tumor, remains resistant to current treatment modalities that include surgical resection, chemotherapy, and radiotherapy (RT). A critical challenge in GBM therapy is the presence of severe hypoxic tumor regions caused by the rapid growth of solid tumors and poor vascularization. Hypoxia drives therapeutic resistance by impairing DNA damage induction and repair mechanisms, particularly double-strand breaks (DSBs), which are the most cytotoxic lesions induced by RT and chemotherapeutic agents.Cellular deoxyribonucleoside triphosphate (dNTP) pools, tightly regulated by ribonucleotide reductase (RNR) and SAM- and HD-domain-containing protein (SAMHD1), play a pivotal role in DNA replication and repair. Hypoxia disrupts this balance by reducing dNTP synthesis due to the oxygen dependency of RNR, a rate-limiting enzyme, leading to diminished replication and cell cycle arrest. These metabolic adaptations hinder the efficacy of DSB-inducing agents in hypoxic GBM cells. Notably, rapidly replicating cells are more sensitive to cancer therapeutic agents, partly due to replication-induced conversion of single-strand breaks and other manageable damages into highly lethal DSBs.To address this resistance mechanism, we investigated the role of dNTP modulation in enhancing DNA damage and therapeutic outcomes in hypoxic GBM cells. Our study reveals that pharmacologically increasing dNTP levels in hypoxic GBM cells restored cell cycle progression and DNA replication, thereby sensitizing cells to DSB-inducing therapies. Specifically, we found that:

1. Elevated dNTP pools reversed hypoxia-induced cell cycle arrest: Flow cytometry analysis demonstrated a marked increase in S-phase entry upon dNTP modulation under hypoxic conditions.
2. Increased DSB formation following therapy: Single-cell microscopic analysis revealed enhanced DSB induction in hypoxic GBM cells treated with ionizing radiation (IR) or bleomycin after dNTP elevation.
3. Enhanced apoptotic response: Pharmacological dNTP modulation amplified apoptosis activation following DSB induction, indicating improved therapeutic efficacy.

This study establishes the critical link between dNTP availability, DNA replication, and hypoxia-induced therapeutic resistance in GBM. By leveraging dNTP modulation, we provide a novel preclinical strategy to overcome hypoxia-mediated resistance to DSB-inducing therapies. Our findings support further exploration of this approach as an adjunctive treatment to improve outcomes in patients with hypoxic GBM.

#1819 Inhibition of the cell-scaffolding protein DLG5 sensitizes glioblastoma stem cells to radiotherapy.

Somanath Kundu[1], Soham Mitra[1], Abigail Venskus[2], Harish Babu[2], Jason Horton[3], Mariano Viapiano[4]

[1]Neuroscience & Physiology, SUNY Upstate Medical University, Syracuse, NY,[2]Neurosurgery, SUNY Upstate Medical University, Syracuse, NY,[3]Neuroscience & Physiology and Orthopedics, SUNY Upstate Medical University, Syracuse, NY,[4]Neuroscience & Physiology and Neurosurgery, SUNY Upstate Medical University, Syracuse, NY

Glioblastoma (GBM) is a fatal CNS tumor that responds poorly to conventional chemo- and radiotherapy. Although radiation achieves a significant adjuvant effect after surgical debulking, radioresistant tumor cells repopulate the tumor bed, resulting in unavoidable recurrence. GBM radioresistance has been largely associated with a population of GBM stem cells (GSCs) driven by Sonic Hedgehog (SHH) and Hippo pathways that promote metabolic reprogramming, DNA repair, and cell proliferation. We have previously described that GBM stemness pathways such as SHH are regulated by a cell-scaffolding member of the Scribble/DLG family, DLG5, which is a "high hierarchy" target that maintains a persistent GSC population. Given the related identity of stemness and post-treatment resistance in GSCs, we have investigated here if DLG5 can be targeted to increase the sensitivity of GSCs to conventional radiotherapy. Our results in cultured GSCs confirmed that DLG5 not only regulates the SHH pathway -as we have previously shown- but also the Hippo pathway. Specifically, DLG5 knockdown increased the expression of the Hippo-repressor kinases, MST-1/MST-2, and phosphorylation of the Hippo effector YAP; these alterations were accompanied by downregulation of Hippo-downstream genes (CTGF, BIRC5) as well as genes involved in DNA-damage repair (BRCA1, RAD51) and stemness (OCT4, NANOG). In agreement with these results, DLG5-deficient GSCs subjected to different irradiation regimes (2 - 8 Gy) showed decreased cell viability, reduced survival fraction in clonogenic assays, and reduced self-renewal ability compared to control GSCs. Irradiated DLG5-deficient cells had elevated cleaved Caspase-3 and PARP1 compared to control cells, increased long-term DNA damage, and accumulation in G2/M phase suggesting mitotic catastrophe. The sensitizing effects of DLG5 deficiency were also tested in intracranial GSC-derived tumors subjected to fractionated radiotherapy (5 x 2Gy q24h). Radiotherapy significantly increased the survival of animals implanted with DLG5-deficient tumor cells compared to controls. Overall, our work suggests that targeting DLG5 increases the efficacy of radiotherapy in GBM by disabling the ability of this cell-scaffolding protein to regulate signaling pathways that drive tumor stemness. Work in progress is currently focused on identifying critical functional domains of DLG5 and developing inhibitors to block DLG5-driven mechanisms in GBM tumors.

#1820 Investigating whether FLASH radiotherapy spares late-responding organs using a rat spinal cord model.

Banghao Zhou[1], Lixiang Guo[1], Yi-Chun Tsai[1], Albert van der Kogel[2], John Wong[3], Iulian Iordachita[3], Rongxiao Zhang[4], Varghese Anto Chirayath[5], Robert Timmerman[1], Weiguo Lu[1], Kai Jiang[1], Chul Ahn[1], Paul Medin[1], **Ken Kang-Hsin Wang**[1]

[1]UT Southwestern Medical Center, Dallas, TX,[2]University of Wisconsin-Madison, Madison, WI,[3]Johns Hopkins University, Baltimore, MD,[4]University of Missouri, Columbia, MO,[5]University of Texas at Arlington, Fort Worth, TX

Purpose: FLASH radiotherapy (FLASH-RT) delivers curative dose to tumors at ultra-high dose rates (UHDR, >40 Gy/s) while offering normal tissue protection via the FLASH effect. Most supporting evidence relies on acute toxicity, but data on late-responding tissues are limited. Inadequate understanding of dose-response relationships has led to severe late toxicity, such as bone necrosis, halting a FLASH trial in cats. To establish FLASH-RT as a clinical modality, determining the dose-response for late-responding organs is essential. The spinal cord, with its steep dose-response curve and risk of myelopathy, serves as an ideal model. This study investigates whether FLASH-RT mitigates late toxicity using the rat spinal cord, a critical step for clinical translation.

Methods: The cervical-thoracic spine (C1-T2) of rats was irradiated with an 18 MeV LINAC-based FLASH beam with a posterior-anterior $2\times1cm^2$ field in a single fraction. A pulse control and monitoring system was developed to ensure precise delivery at single-pulse resolution required to establish the dose response curve. A scintillation detector was placed within the C1-T2 region of a rat carcass for validating Monte Carlo (MC) dose calculations. A custom immobilization device, combined with portable X-ray imaging, was used for precise C1-T2 localization for irradiation. An ion chamber was placed beneath the electron cone to monitor Bremsstrahlung as the surrogate for real-time UHDR output measurement. Dose groups were based on the reported median effective dose (ED_{50}) for conventional dose rate (CONV) treatments (20.4-21.5 Gy) and dose-modifying factors (DMF) for FLASH (1-1.4). Sprague Dawley rats were irradiated with FLASH doses ranging from 15.3 to 28.4 Gy. The dose-related paresis incidence was assessed over a 7-month follow-up period to determine the dose-response curve and whether FLASH enhances spinal cord tolerance compared to CONV-RT.

Results: MC calculations demonstrated that the 18 MeV FLASH achieved profile non-uniformity of <5% along the 12 mm length of the C1-T2, effectively mitigating the dose-volume effect, and was reproducible across animals. The dose rate achieved 390 Gy/s, exceeding the threshold of 40 Gy/s. Radiation-induced skin damage healed spontaneously by 10 weeks post-irradiation. The animals showed steady weight gain after irradiation, suggesting that no significant radiation-induced esophagitis was observed. Preliminary results indicated that 3 out of 8 rats in both 21.8 and 28.4 Gy FLASH groups exhibited paralysis. The data collection is still ongoing, and the full dose-response curves for both UHDR and CONV irradiation will be established.

Conclusions: Our project addresses a critical knowledge gap in FLASH-RT by elucidating the FLASH dose-response relationships for a key late-responding organ, the spinal cord. The findings will have significant implications for the use of FLASH-RT in clinical setting.

#1821 *In vivo* synergy is observed with AMG-232 and radiotherapy in endometrial cancer.

Aaron P. Petty, Yvonne Parker, Daniel Lindner, Roberto Vargas

Cleveland Clinic, Cleveland, OH

Background: Approximately 15% of high-grade *TP53*-mutated endometrial cancers (EC) are heterozygous and retain wild-type (WT) signaling. Our previous *in vitro* findings nominated MDM2 inhibitors as potential radio-sensitizing strategies that leverage *TP53* WT signaling. Here, we further explore this radio-sensitizing role for one particular inhibitor, AMG-232, in an *in vivo* xenograft model with a *TP53* WT EC cell line.

Methods: To evaluate the radio-sensitizing potential of AMG-232 *in vivo*, we utilized a xenograft tumor model with the JHUEM-2 (*TP53* WT) cell line. Cells ($2x10^6$) were injected subcutaneously in the bilateral flanks of 6-8 week old female NSG mice. Mice were separated into 4 treatment groups and 6 mice (n= 12 tumors) were included in each group: Vehicle, Radiation (RT), AMG-232, and Combination. Once the largest tumors were 200-300mm^3 in size, mice were given either Vehicle or AMG-232 (30 mg/kg) by gavage for 9 days. In the RT and Combination groups, a single dose of 1 Gy of radiation was given 24 hours following first drug dose. Assessment of treatment synergy was carried out using both the Highest Single Agent (HSA) and Bliss Independence models with bootstrap analysis at either single timepoints, or over the entire growth curve based on area under the curve (AUC). Synergy scores were calculated as a measure of deviation from additivity, with positive values indicating synergy. Synergy analyses were carried out using the invivoSyn package in R software.

Results: AMG-232 and RT together significantly suppressed tumor growth at treatment days 7 and 9 compared to either RT alone (P= 0.004 and P= 0.0014, respectively) or AMG-232 alone (P= 0.003 and P= 0.0061, respectively). Synergy analysis of growth inhibition at treatment day 9 using both the HSA and Bliss models showed significant synergy in the combination group, with mean synergy scores of 27 (P= 0) and 25 (P= 0.003), respectively. Entire growth curve AUC analysis also showed synergy only in the less stringent HSA model, with synergy score of 37.5 (P= 0.033).

Conclusions: Overall, these findings further support the use of MDM2 inhibitors to improve radiotherapy response in EC. The improved tumor response that we observed at clinically relevant doses further highlights the potential of these agents to either enhance responses to current therapeutic strategies, or to lower the radiotherapy dose necessary for complete response.

#1822 O-GlcNAcylation of NDRG1 mediates radiation resistance in prostate cancer.

Manish Thiruvalluvan, Sandrine Billet, Neil A. Bhowmick

Cedars-Sinai Cancer, Cedars-Sinai Medical Center, Los Angeles, CA

Prostate cancer (PCa) remains one of the most prevalent malignancies worldwide, with localized cases commonly treated through surgical resection and radiation therapy (RT). Both RT and androgen-targeted therapies can stimulate glutamine (L-Gln) secretion by the PCa-adjacent stroma, promoting tumor adaptation and resistance. Beyond its metabolic role, our findings revealed that L-Gln critically influences protein O-GlcNAcylation, a key process for DNA repair and therapy resistance. We hypothesized that targeting L-Gln signaling could enhance PCa sensitivity to RT. Our studies demonstrated that L-Gln starvation significantly increased RT sensitivity in both parental and radio-resistant PCa cell lines, a phenotype recapitulated by silencing OGT, the key enzyme mediating O-GlcNAcylation. Conditioned media from stromal fibroblasts elevated global O-GlcNAcylation and enhanced cell viability in PCa cells, highlighting the role of the tumor microenvironment. Sodium phenylbutyrate (SPB), a clinically available compound that sequesters L-Gln, effectively mimicked the effects of L-Gln depletion, contrasting with metabolic inhibitors of L-Gln that have failed to show clinical benefit. In subcutaneous xenograft models of PCa epithelia co-implanted with cancer-associated fibroblasts, the combination of SPB and RT markedly reduced tumor size compared to either treatment alone. Further, SPB significantly decreased plasma and intratumoral L-Gln concentrations. Mass spectrometry analysis of tumor tissues identified NDRG1 as a protein highly O-GlcNAcylated in response to RT, a modification suppressed by SPB. CRISPR/Cas9-mediated substitution of key O-GlcNAcylated serine residues in NDRG1 significantly enhanced RT sensitivity by destabilizing the protein and impairing its nuclear translocation. Mechanistically, NDRG1 dysfunction was associated with elevated endoplasmic reticulum (ER) stress, evidenced by increased CHOP and NRF2 expression and reduced Akt phosphorylation. RNA sequencing further revealed that SPB disrupted PCa cell metabolism by inhibiting fatty acid oxidation and reducing amino acid transporter activity, leading to diminished nucleotide pools and energy availability. This dual disruption of metabolic and O-GlcNAcylation processes compromised DNA repair and the detoxification of radiation-induced reactive oxygen species, thereby enhancing radiosensitivity. These findings suggest that targeting L-Gln bioavailability is a clinically viable strategy to overcome therapy resistance and improve therapeutic outcomes in PCa patients.

#1823 Spatial transcriptome profiling shows a decrease in heterogeneity of GBM xenografts that regrow after irradiation and suggests novel therapeutic targets.

C. M. Degorre;
National Cancer Institute, Bethesda, MD

Radiation remains a primary treatment for glioblastoma, yet tumors frequently recur within two years. As a model for investigating the biology of recurrent GBMs, we used orthotopic xenografts initiated of GBM-stem like cells (GSCs) implanted into the right striatum of nude mice. Thirty-five days post-implant, mice bearing brain tumor xenografts were locally irradiated (10Gy) and brains collected 14 and 21 days later. In untreated mice, tumors were highly diffuse with tumor cells invading from the injection site in the right hemisphere (RH) to the anterior part of the brain, invading the corpus callosum, cortex, and olfactory bulb (OB). A different growth pattern was detected in tumors that regrew after irradiation. At 14 days post-10Gy, tumors were located primarily in the OB with an increased cell density and a clearly demarcated border. 21 days post-10Gy tumor cell density increased with the demarcated border extending further into the hemisphere, i.e., farther away from the OB. This growth pattern suggests that tumor regrowth after irradiation is initiated in the OB, consistent with previous results showing that GBM cells in the OB are radioresistant compared to those in the RH. To compare transcriptomes of untreated and recurrent tumors we used digital spatial profiling (DSP). Tumor cells were identified by Sox2 staining, and regions of interest (ROIs) were selected in various locations within the OB and the RH of untreated tumors, and throughout recurrent tumors (D21 post-10Gy). Cluster analysis showed that ROIs across the OB and RH of untreated tumors were more similar to each other as compared to ROIs from the tumors that regrew after irradiation. Variance in the expression of individual genes across the ROIs was reduced in the recurrent tumors compared to the untreated tumors. These results suggest a reduction in phenotypic heterogeneity among the cells of the regrown tumor. Comparative gene expression analysis identified 463 differentially expressed genes with 319 downregulated and 144 upregulated in the recurrent versus untreated tumors. GSEA revealed a significant enrichment of pathways related to cell cycle regulation in the regrown tumors as compared to non-irradiated tumors. Further examination of the cell cycle control related pathways indicated an enrichment in proteasome genes. Specifically, 22 genes encoding proteasome proteins were significantly upregulated in the recurrent tumors compared to the untreated group, suggesting that the proteasome may provide a therapeutic target unique to the recurrent GBM. Altogether, these results suggest that this xenograft model of GBM regrowth after irradiation, characterized by an altered growth pattern and a distinct molecular profile may be useful for investigations into the biology and treatment of recurrent GBM.

#1824 Harnessing a radiation-resistant organoid platform to propose novel strategies for precision treatment of advanced oral cancer.

Sumin Kang[1], Mi Rim Lee[2], Jonghyun Lee[2], Dongkwan Shin[2], Sun-Young Kong[2], Jong-Ho Lee[2], Ikjae Kwon[3], Dohyun Kwon[3], Soung-Min Kim[3], Joo Yong Park[2], Wonyoung Choi[2], Jiyoung Lee[2], Yu-Sun Lee[2], Hye Won Shon[2], Gyeongmin Kang[1], Sung Weon Choi[2], Yun-Hee Kim[2]

[1]Graduate School of Cancer Science and Policy, National Cancer Center, Goyang, Korea, Republic of,[2]Research Institute and Hospital, National Cancer Center, Goyang, Korea, Republic of,[3]Oral and Maxillofacial Surgery, Oral Cancer Center, School of Dentistry, Seoul National University, Seoul, Korea, Republic of

Oral squamous cell carcinoma (OSCC) has high recurrence rates and limited treatment options, with 40-60% of patients diagnosed at advanced stages (III/IV). Despite surgery following radiotherapy, over 40% of patients with advanced OSCC experience recurrence, with 70% being locoregional. Postoperative radiotherapy reduces recurrence but causes significant side effects, such as xerostomia and osteoradionecrosis. The challenge of accurately reflecting the clinical characteristics and tumor heterogeneity of oral cancer hinders clinical application. Therefore, research models that preserve patient traits are essential for developing tailored treatments, reducing side effects, and improving outcomes. In this study, we used a radiotherapy-relapse mimicking patient-derived organoid model to identify intrinsic radiation resistance and overcome it in advanced oral cancer. Radiation-resistant (RR) organoids were successfully developed by gradually increasing radiation intensity over 10 passages, with a total dose of 60 Gy. Of the 23 cases, 14 successfully generated RR organoids. These RR organoids exhibited enhanced radiation resistance and proliferation. Evaluation with multiple radiation fractions proved more reliable than single-fraction assessments, as it minimized interference from proliferation-related factors. By linking patient recurrence status with the establishment of RR organoids, it was observed that organoids derived from recurrent patients had a higher success rate. This suggests that radiation resistance is driven by pre-existing subclones. The characteristics of subclones that survived radiation exposure were examined through differential gene expression (DEG) analysis, revealing upregulation of genes related to the cell cycle, DNA damage repair, and inflammatory response pathways, while genes associated with apoptosis and differentiation were downregulated. Inhibitors targeting the altered pathways were selected, and combination treatments with radiation were performed. The ATR inhibitor, BAY1895344, demonstrated synergistic effects. Finally, genes with sequentially altered mRNA levels based on radiation responsiveness were selected to develop the radiation resistant index (RRI). This study aims to address the limitations of existing preclinical models by utilizing patient-derived organoids that preserve tumor heterogeneity, thereby providing a platform and treatment prediction for clinically relevant research. These findings are anticipated to contribute significantly to the development of precision medicine-based therapeutic strategies for advanced oral cancer.

#1825 Pharmacological evaluation of bioconjugated trastuzumab using the AbYlink™ regio-selective conjugation technology in gastric cancer expressing HER2+.

Celine Mothes[1], Peggy Provent[1], Marie Ruch[2], Mathieu Moreau[2], Michael Claron[2], Alex Helbling[3], Viktoriia Postupalenko[4], Leo Marx[4], Patrick Garrouste[4], **Eftychia Koumarianou**[1]

[1]Oncodesign Services, Dijon, France,[2]Institut de Chimie Moleculaire de l'Universite de Bourgogne, Dijon, France,[3]Centre Georges Francois Leclerc, Dijon, France,[4]Debiopharm Research & Manufacturing SA, Martigny, Switzerland

Purpose/introduction

Bioconjugation is a critical step in the development of Antibody Drug Conjugates with two main strategies dominating the field. One approach employs covalent chemistry to randomly bind a drug to the accessible Lysines or Cysteines on an antibody. The other strategy is site-specific, using covalent chemistry to bind a drug ligand at defined amino acid positions within the antibody. AbYlink™ is a novel regio-selective labeling method for antibodies, enabling a single-step covalent conjugation of payloads such as radionuclide chelators to the Fc domain of an antibody. Given the selectivity of this reaction for the Fc region of the immunoglobulin, the affinity of the antibody to the antigen isn't compromised, unlike randomly labelled antibodies. The chelators that are covalently attached to the antibody enable radiolabeling with radioisotopes such as Gallium-68, Lutetium-177 and Indium-111 for molecular radiotherapy or molecular imaging purposes.

Experimental design

The commercially available trastuzumab was labeled via regio-selective conjugation with a DOTA-GA chelating agent using the AbYlink™ technology, then radiolabeled with Indium-111. The in vivo biodistribution of the HER2 radiolabeled bioconjugate was evaluated in mice bearing NCI-N87 gastric cancer tumors.

Results

Trastuzumab conjugation resulted in a homogeneous labeling of antibodies with a Degree of Conjugation (DoC) close to 2, conducted in a regio-selective manner. The biodistribution and imaging experiments demonstrated successful accumulation of the antibody within the NCI-N87 tumour implanted in mice. The radiolabeled conjugate remained stable in vitro and in vivo, demonstrating the AbYlink™ as a valuable tool for biologics conjugation.

Conclusion

We have successfully validated in vivo the use of AbYlink™ technology in the context of Antibody Radio Conjugates. This regio-selective bioconjugation strategy holds potential for wider application across molecular radiotherapies or pharmacodynamic assessment of antibodies.

#1827 Investigating the efficacy of 177-Lu therapeutics in a series of castrate resistant prostate cancer PDX models.

Stefano Cairo[1], Benedetta Arno[2], Mara Gilardi[1], Hsiu-Wen Tsai[1], Juliana Maynard[2], Marianna Zipeto[1], Michael Ritchie[1]

[1]Champions Oncology, Inc., Hackensack, NJ,[2]Medicines Discovery Catapult, Cheshire, United Kingdom

Recent advancements in alpha and beta particle-emitting radiopharmaceuticals underscore the therapeutic potential of targeted radionuclide therapy, presenting a promising alternative for patients with advanced or metastatic disease where conventional treatments fall short. This field is pushing the boundaries of personalized medicine, allowing clinicians to tailor treatments to individual patients' genetic and molecular profiles, thereby improving outcomes. As translational research in radiopharmaceutical development progresses, it holds significant promise for redefining therapeutic options and setting new standards in patient care. Targeting and maintaining high specificity to chosen targets, such as PSMA, is crucial for the clinical success of novel radiopharmaceuticals. The development and translation of these therapies require a series of steps through the drug discovery pipeline, including selecting the right radionuclide, chemistry, model, and biomarkers for patient selection. Historically, radiopharmaceutical development has relied on cell lines. While cell-derived xenografts are cost-effective and essential for initial characterization, screening, biodistribution, and efficacy, clinically relevant pre-clinical models predictive of patient outcomes are indispensable for de-risking radiopharmaceutical development. Unfortunately, these models have been underutilized due to the limited availability of combined PDX and radiopharmaceutical capabilities. Here, we present an end-to-end approach for testing 177-Lu therapeutics, from dosimetry and biodistribution in cell lines to efficacy in metastatic castration-resistant PDX models. When treated with 177-Lu based prostate therapeutics, PDX models exhibited a diverse range of responses. RNA-seq and proteomics analyses confirmed that Champions Oncology metastatic castration-resistant prostate cancer PDX models expressed varying levels of PSMA, reflecting the patient population in clinical settings. Our results highlight the importance of integrating robust radiochemistry and biodistribution workflows with clinically relevant patient-derived models to derive translational insights and accelerate the development of radiopharmaceuticals. This comprehensive approach not only enhances the predictive power of pre-clinical models but also ensures that the therapeutic strategies developed are closely aligned with the clinical realities faced by patients.

#1828 Efficacy and mechanism of radiotherapy combined with fruquintinib and tirelizumab in mCRC.

Mingsheng Zhang, **Qingqing Yu**, Huiying Hou, Xiaoting Su, Qin Huang, Hong Qiu, Le Huang, Liang Zhuang, Qiang Fu, Yanmei Zou, Li Sun, Liu Huang, Shunfang Liu, Fei Liu, Xianglin Yuan

Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Background: Immune checkpoint inhibitors (ICIs) targeting programmed cell death protein 1 (PD-1) or programmed death ligand 1(PD-L1) respond well to deficient-microsatellite(dMMR) colorectal cancer and poorly to proficient-microsatellite (pMMR) CRC. Anti-vascular therapy is the standard backline treatment regimen for advanced metastatic colorectal cancer and also potentiates the immunotherapeutic efficacy of CRC by promoting immune cell infiltration and remodeling the tumor immune microenvironment (TIME). However, it is not clear whether combining radiotherapy, anti-vascular and anti-PD-1 can affect the efficacy of pMMR CRC. In this experiment, we investigated the antitumor efficacy of radiotherapy combined with fruquintinib and tirelizumab in pMMR CRC mice.
Methods: CT26 cellular hormonal tumor corresponding to pMMR CRC. A mouse model with subcutaneous transplanted tumors is established, divided into control, radiotherapy (IR), fruquintinib + tirelizumab (F+T), and radiotherapy + fruquintinib + tirelizumab (IR+F+T) groups. Immunofluorescence (IF) experiments were conducted to investigate the number and function of tumor vessels. Immunohistochemistry (IHC) and flow cytometry (FC) were utilized to examine immune cell infiltration and alterations within the TIME.
Results: Compared to the control group, tumor growth was significantly inhibited after treatment. When compared to IR and F+T, IR+F+T demonstrated a remarkable suppression of tumor growth. The quantitative analysis results showed a significant decrease in Ki-67 positive cells in the target tumors with IR+F+T compared to IR and F+T. The TUNEL results indicated that treatment promoted tumor cell apoptosis, and the effect was further enhanced with triple combinational therapy. Both F+T and IR+F+T repressed CD31 expression and improved the ratio of α-SMA$^+$/CD31$^+$ in tumor tissue, yet there was no significant difference between the two groups. The immunohistochemistry and flow cytometry results demonstrated that triple combinational therapy increased tumor-infiltrating CD8$^+$ T cells and significantly elevated the proportions of CD8, CD69, and CD86. Both F+T and IR+F+T boosted PD-L1 expression, with no significant difference between them.
Conclusions: Combined irradiation on top of fruquintinib and tirelizumab treatment enhanced the efficiency in CT26 murine CRC syngeneic tumor model. There was no significant effect of radiotherapy on the anti-angiogenesis of fruquintinib and the promotion of normal vascular function. Irradiation promoted CD8$^+$ T cell and dendritic cell infiltration and activation.

#1829 Potentiating antitumoral type 1 interferon immune signaling in triple-negative breast cancer (TNBC) via combination treatment of Aurora kinase B (AURKB) inhibition and radiation therapy.

Kassidy M. Jungles[1], Caroline R. Bishop[1], Cydnee Wilson[1], Meilan Liu[1], Corey W. Speers[2], Lori Pierce[1], James Rae[1]

[1]University of Michigan, Ann Arbor, MI,[2]Case Comprehensive Cancer Center, Cleveland, OH

Purpose: Triple-negative breast cancer (TNBC) is an aggressive BC subset that is less responsive to standard therapies resulting in the need for novel treatment options. Radiotherapy (RT) is common for treating TNBC patients; however, locoregional recurrence remains a clinical issue. Modulation of the antitumoral immune response may improve tumor control in TNBC. Type 1 interferon (T1IFN) is a crucial regulator of antitumoral immunity that can activate cytotoxic immune cells. AURKB is upregulated in TNBC patients, yet it is unknown whether AURKB modulates T1IFN signaling. While our preliminary data suggests that AURKB inhibition makes TNBC cells more sensitive to RT, the effects on T1IFN are unknown. Here, we examined the effects of combined RT and AURKB inhibition on T1IFN signaling in TNBC.

Methods: The half-maximal inhibitory concentrations (IC_{50}) were calculated using cell viability assays with the treatment of AURKB inhibitors Barasertib-HQPA and SP-96 in human and murine TNBC cells. Micronuclei formation was assessed using DAPI stain and immunofluorescent microscopy following treatment with AURKB inhibitors and RT. T1IFN signaling was assessed via a T1IFNβ-GFP reporter transfected into human TNBC cells, rt-qPCR for T1IFN response genes, and flow surface staining for T1IFN markers. For these studies, TNBC cells were pretreated with AURKB inhibitor 24 hours prior to RT and harvested five days later.

Results: Aurora kinase B inhibitors (Barasertib-HQPA and SP-96) reduced cell viability in human and murine TNBC cells (IC_{50} values: 500 nm - 5 μM). AURKB inhibition delivered before RT induced micronuclei—a known ligand of T1IFN signaling—in TNBC cells. Further, sub-IC_{50} concentrations of AURKB inhibitors (25 nM Barasertib-HQPA, 100 nM SP-96) and RT (6 Gy in 1 fraction) induced T1IFN signaling in the human Cal-51 T1IFN reporter system ($p<0.0001$). This was also observed in combination with fractionated RT (2 Gy in 3 fractions), suggesting that combined AURKB inhibition and RT potentiate T1IFN ($p<0.0001$). Combined treatment (6 Gy RT + 10 nM Barasertib-HQPA) also induced expression of the T1IFN response genes *Cxcl10* and *PD-L1* in human TNBC BT549 cells compared to vehicle control ($p<0.001$). Further, AURKB inhibition and RT also potentiated the T1IFN markers PD-L1 and MHC1 in human and murine TNBC cells.

Conclusion: AURKB inhibition and RT induces T1IFN signaling in human and murine TNBC cells. Combination treatment also induces micronuclei formation, suggesting an upstream mechanism for the observed T1IFN signaling. These data suggest that AURKB inhibition and RT may be a promising strategy for the treatment of TNBC and can modulate the tumor immune microenvironment. Further studies are warranted to examine the extended effects of this treatment in vivo and further implications on the antitumoral immune response.

#1830 A chemoradiation platform for evaluating multimodal therapy effects on tumor viability and invasion ex vivo.

Luda Lin, Krishna K. Bommakanti, Bowen Wang, Yazeed Alhiyari, Christian Wooten, Andreanne Sannajust, Lauran K. Evans, Peyton Tebon, Maie A. St. John, Alice Soragni

UCLA - University of California Los Angeles, Los Angeles, CA

Treatment of advanced head and neck squamous cell carcinoma (HNSCC) involves radiotherapy combined with chemotherapy, targeted therapy or immunotherapy. We have developed a high-throughput organoid-based drug screening platform to assess the combined effects of radiotherapy and chemotherapy or targeted therapies on HNSCC viability and invasion. To validate the platform, we used HNSCC cell lines establishing bioprinted 3D models in mini-ring format (Phan et al, 2019; Tebon et al, 2023; Al Shihabi et al 2024; Nguyen et al, 2024). Cells are exposed to X-ray radiation in combination with various small molecules and biologics. We quantified viability via ATP-release assays and extracellular matrix (ECM) invasion using a machine-learning-based brightfield image analysis pipeline (Al Shihabi et al, 2022). Proof-of-concept experiments with HPV-negative HNSCC lines (HN30 and HN31, established from primary and metastatic disease from the same patient) and HPV-positive HNSCC cells (SCC154) revealed that treatment with cetuximab and ibrutinib enhances radiosensitivity in HN31 cells. The PI3K inhibitor copanlisib radio-sensitized both HN30 and HN31, while alpelisib sensitized SCC154 cells. Additionally, copanlisib, afatinib, and ibrutinib were shown to limit ECM invasion of HN31, while the AKT inhibitor ipatasertib and the mTOR-targeting rapamycin promote invasion of HN30 cells, as observed in previous studies (Brolih et al., 2018). Our pilot experiments using bioprinted patient-derived HNSCC organoids confirmed the feasibility of performing sensitivity screening by integrating bioprinting, conventional viability assays, and advanced image analysis techniques. Our goal is to establish a personalized therapeutic pipeline for patients with advanced HNSCC, maximizing responses to radiotherapy and targeted agents to improve outcomes, and avoiding modulators that may promote tumor invasion.

#1831 Development and characterization of a radiation resistant GBM cell line from PDX for the screening of novel treatment modalities.

Sigrid Cold[1], Jeremy Ariey-Bonnet[2], Monica Brandt[1], Rikke Norgaard Boge[1], Emma Papin[1], Ida Thoroe Michler[1], Lotte Kellemann Kristensen[1], Jacob Hald[1], Michael Wick[3], Andreas Kjar[1], Krister Wennerberg[2], Carsten Hagen Nielsen[1], **Sebastian Gnosa**[1]

[1]Minerva Imaging ApS, Olstykke, Denmark,[2]Biotech Research and Innovation Centre (BRIC), University of Copenhagen, Copenhagen, Denmark,[3]Xenostart, San Antonio, TX

Glioblastoma (GBM) remains one of the most lethal brain tumors, with standard-of-care therapies such as radiation and temozolomide offering limited survival benefit. Patient-derived xenograft (PDX) models have become critical tools for studying treatment resistance and identifying novel therapeutic strategies. Here, we report the establishment and characterization of a novel GBM cell line, ST146c, derived from a radiation-resistant PDX model (ST146). This cell line retains the therapy-resistant phenotype observed in the patient, making it a valuable resource for preclinical studies. To uncover vulnerabilities, ST146c was subjected to a mid-throughput drug screen, identifying SIK as a promising target. Subsequent in vitro studies confirmed that SIK inhibition significantly impairs ST146c cell proliferation and survival. To validate these findings, we conducted in vivo experiments using the parental PDX model. SIK inhibition demonstrated robust anti-tumor activity, corroborating the in vitro results and underscoring its potential as a therapeutic target in radiation-resistant GBM. Building upon these findings, we performed a focused synergy screen, testing 40 handpicked drugs in combination with radiation. Among these, SIK3 inhibition emerged as a top candidate, showing evidence of synergistic effects with radiation in vitro. We are now extending these investigations to in vivo models to further evaluate the potential for SIK3 inhibitors to enhance the efficacy of radiation therapy. This study highlights the translational potential of the ST146c model for identifying and validating novel therapies targeting radiation-resistant GBM. The discovery of SIK3 as both a monotherapy target and a radiation-sensitizing agent opens new avenues for overcoming resistance in this challenging disease.

#1832 Identifying GSK3b inhibitor as a radiation induced type I interferon stimulator in lung cancer.

Chen Braun, Nan Li, Steven H. Lin

Department of Radiation Oncology, University of Texas MD Anderson Cancer Center, Houston, TX

Radiation therapy (RT) is commonly used to treat multiple tumor types to reduce the risk of tumor recurrence. Despite impressive clinical responses only a subset of patients responds to RT, due to the lack of an adequate immune response within lung tumors. There is a strong motivation to develop novel approaches to overcome immunosuppression in tumors after radiation therapy. Here, we aim to identify pathways that could specifically augment type I interferon (IFN) response with RT to bypass the immune suppressive effects. We combined radiation and >700 pharmacological inhibitors in a high throughput screen to identify agents that induced type I IFN response. We revealed a GSK3β inhibitor (GSK3βi), Kenpaullone, as a drug hit that induced synergistic response with RT to enhance type I IFN expression. The selected GSK3βi was validated for its IFNβ1 induction in the mRNA and protein levels in multiple lung cancer cell lines, and by examining the associated AKT/GSK3β/IRF3 pathway. The effectiveness of this unique GSK3βi-RT combination to enhance IFNβ1 immunologic stimulation was tested in syngeneic tumor bearing animals and significantly reduced tumors size compared to radiation or drug alone. COMET analysis from mice tumors showed that combination therapy increased T cells and M1 macrophages at the tumor microenvironment (TME), while M2 macrophages and PDL-1 decreased. This pharmacological-RT combination approach has the potential to enhance the immunologic response to RT in lung cancer patients to reduce tumor burden in those that already exhausted standard of care therapies.

#1833 Assessment of shifts in tumor-specific frequencies on the proliferation of hepatocellular carcinoma cells.

Callum T. McGrath[1], Preeya F. Achari[1], Carl Blackman[1], Janaka P. Liyanage[2], **Hugo Jimenez**[2], Boris C. Pasche[2]

[1]Wake Forest University School of Medicine, Winston Salem, NC,[2]Department of Oncology, Wayne State University School of Medicine, Detroit, MI

Background: Radiofrequency Electromagnetic Fields Amplitude-Modulated at tumor-specific frequencies (AM RF EMF) are FDA approved for the treatment of advanced hepatocellular carcinoma (HCC) and have been shown to inhibit cancer growth *in vitro*, *in vivo*, and in clinical trials. The mechanism behind AM RF EMF induced proliferative inhibition is intracellular Ca^{2+} influx via the $Ca_v3.2$ T-type calcium ion channel exclusively within malignant cells. However, it is unknown how AM RF EMF opens the $Ca_v3.2$ channel. We hypothesize that the tumor specific frequencies are the natural frequencies of the malignant $Ca_v3.2$ channels and thus AM RF EMF is a resonance phenomenon. To test this, we first assessed the impact on proliferative inhibition from the RF carrier wave and ambient magnetic fields. We then shifted base HCC treatment frequencies from +0.1 Hz to +0.0001 Hz logarithmically and compared proliferative inhibition to base treatment frequencies.

Methods: Huh7, Hep3B and SNU-423 HCC cell lines were treated with HCC-specific AM RF EMF under two conditions: normal incubator conditions, and within a magnetically shielded metal box. Growth inhibition relative to control was measured between the two groups via thymidine incorporation assay. Next, Huh7 and Hep3B cells were exposed to the unmodulated 27.12 MHz RF carrier wave and growth inhibition compared to control via thymidine incorporation assay. Lastly, Huh7 and Hep3B cells were treated with HCC AM RF EMF frequencies shifted by either +0.1 Hz, +0.01 Hz, +0.001 Hz, or +0.0001 Hz. Growth inhibition relative to standard treatment was measured via thymidine incorporation assay.

Results: There was no change in growth for Huh7 and Hep3B cells exposed to the unmodulated carrier wave compared to their respective controls. There was no change in proliferative inhibition between normal and magnetically shielded conditions for Huh7, Hep3B, and SNU-423 cells. All frequency shifts resulted in significantly decreased treatment efficacy relative to base frequencies (+0.0001 Hz: $p = 0.0021$, +0.001 Hz: $p = 0.0172$, +0.01 Hz: $p = 0.0145$, and +0.1 Hz: $p = 0.0279$)

Conclusion: Neither the unmodulated carrier wave nor ambient magnetic fields contribute to AM RF EMF induced proliferative inhibition. AM RF EMF is not a resonance phenomenon but is highly sensitive to small fluctuations in treatment frequencies, highlighting the specificity required for optimal treatment.

#1834 Tumor treating fields (TTFields) plus chemotherapy abrogates pancreatic ductal adenocarcinoma (PDAC) proliferation and alters the tumor microenvironment.

Hani M. Babiker, Santanu Bhattacharya, Douaa Albelal, Shamit Kumar Dutta, Enfeng wang, Debabrata Mukhopadhyay

Mayo Clinic Florida, Jacksonville, FL

Background: TTFields are non-invasive and novel treatment modality that utilizes low intensity, alternating, and intermediate frequency electrical fields to inhibit cancer cell proliferation through a multitude of mechanisms. These include inhibition of mitosis, disruption of cancer cell membranes, metabolomic dysregulation of cancer cells, and immunogenic activation of the tumor microenvironment (TME). TTFields are approved in glioblastoma multiforme, non-small cell lung cancer and malignant mesothelioma. The PANOVA2 trial demonstrated promising efficacy for TTFields when applied together with chemotherapy in patients with PDAC. Mechanisms of TTFields in PDAC need further delineation. We sought to investigate the efficacy of TTFields *in vitro* and *in vivo* in PDAC cell lines and mouse models. Preclinical studies were conducted in concert with an going clinical trial (NCT04605913).

Experimental Procedures: Utilizing the *inovitro* apparatus we studied the effect of TTFields (150kHz, intensity 2.9+0.2 V cm RMS, 37ċ, over 72 hrs) in an array of PDAC (Panc1, KPC, Panc2, and AsPC1) and human pancreatic stellate cells (hPSC) cell lines, alone or together with cisplatin (Cis, 0.1µM) or gemcitabine (Gem, 0.01µM), compared to control. Cell viability analysis was conducted in each of the groups. In addition, cell lysates from Panc1 cells from concomitant treatment of TTFields plus Gem were probed for STAT3 and α-SMA to analyze stromal proteins and RAS pathway components (KRAS, P38, P42, and others) were performed via Westen Blot. We also performed RNA sequencing for the different groups to analyze gene expression levels. We further assessed the effect of TTFields (150kHz, intensity 1.8-2 V/cm RMS, over 2 weeks) using the *inovivo system* with and without chemotherapy (Cis, 0.25 mg/Kg per dose (i.p.) 3 times a week) compared to control in C57BL6 orthotopic murine models (6-7 weeks old C57BL6 mice).

Summary of Results: Our analysis revealed statistically significant abrogation of PDAC cancer cell lines for the different groups and hPSC cell viability when compared to control for TTFields monotherapy ($p < 0.001$) and TTFields concomitantly with Gem or Cis ($p < 0.0001$). Western blot analysis demonstrated that TTFields alone and when combined with chemotherapy alters both the stroma and RAS pathways with confirmed downregulation of pSTAT3 and α-SMA and KRAS, P-P38, and P-P42. *In vivo* analysis revealed significant decrease in tumor volume and weight for the concomitant effect of TTFields + Cis.

Conclusion: Our preclinical studies demonstrated the efficacy of TTFields as monotherapy and concomitantly with chemotherapy in PDAC and hPSC cell lines and mouse models. Mechanistic insights from the pre-clinical studies will inform the design of future TTFields studies in PDAC.

#1835 The impact of artemin depletion on acute radiation-associated oral pain.

Faihaa A. Ahmed, Thitsana Ingkasri, Sophi Schofield, Elizabeth Wright, B. Duncan X. Lascelles, Santosh K. Mishra, Michael W. Nolan

North Carolina State University, Raleigh, NC

A common complication of therapeutic radiotherapy for head and neck cancer is the development of severe pain. The biological mechanisms underlying acute orofacial radiation-associated pain (RAP) are unknown but may involve cold-sensing and cold-signaling pathways. A key cold receptor is TRPM8 (Transient Receptor Potential Melastatin family member 8), whose activation might be mediated by the neurotrophic factor artemin (ARTN) acting on its primary receptor, GFRα3. To test the hypothesis that radiation triggers release of ARTN, which results in activation of TRPM8 to cause RAP, we performed tongue-irradiation in mice that had been treated with either monoclonal anti-ARTN-neutralizing antibody or control (IgG). Lingual ARTN expression (IHC) increased after lingual irradiation, and this was mitigated by anti-ARTN treatment. Assessments of glossitis severity, body weight, and behavioral pain measures indicated that irradiation caused severe pain, which ARTN depletion failed to mitigate. However, after topical ophthalmic application of cold saline solution, tongue-irradiated anti-ARTN treated mice wiped their eyes less than their IgG-treated counterparts, and as assessed via ex vivo calcium imaging of trigeminal ganglia neurons, their afferent sensory nerves were less responsive to cold buffer. We therefore conclude that at the molecular level, ARTN depletion reduced radiation-induced cold sensitivity, but was insufficient to reduce behavioral indicators of RAP. Ongoing work in tissues from these mice is exploring whether changes in cold sensitivity correlated with TRPM8 expression, and future experiments are planned to assess: (1) whether genetic deletion of ARTN reduces RAP, and (2) if modulation of ARTN leads to upregulation of alternative TRPM8 activators.

#1836 Radiation effects in normal oral tissues caused by boron neutron capture therapy.

Lin Ma[1], Yupeng Luo[2], Jianyue Liu[2], Yonghui Luo[2], Zhiyi Wang[2], Zexi He[2], Haotian Tang[2], **Qi Liu**[3]

[1]Shenzhen University General Hospital, Shenzhen University, Shen Zhen, China,[2]Shenzhen University, Shen Zhen, China,[3]International Cancer Center, Shenzhen University, Shen Zhen, China

As a promising modality of binary radiation therapy, boron neutron capture therapy (BNCT) combines the advantages of boron-10 delivery agents for tumor-targeting and heavy charged particles for robust cell killing. BNCT has been approved for clinical treatment of head and neck cancer in Japan. However, the heavy ion rays generated from ^{10}B (n,α) ^{7}Li reactions as well as the concurrent gamma ray and thermal neutron radiation during BNCT can not only destroy cancer cells but also inevitably damage normal oral tissues, which constitutes the primary concern for head and neck cancer patients who are suitable for BNCT. Unfortunately, our knowledge about the radiobiology of BNCT-caused oral tissue damage is very limited to date. Therefore, we set out this study to explore the potential mechanisms in oral tissue reactions following BNCT. We collected various oral tissues from a mouse tumor model treated with BNCT and extracted total RNA from tongue tissues of 11 mice (4 controls without BNCT and 7 BNCT-treated samples with 2.5 mM/kg boron-10 delivery agents) for transcriptome sequencing. By comparing the gene expression profiles between the BNCT-treated group and the control group, we identified a total of 308 differentially expressed genes. Notably, these genes were enriched in cell junctions and cell membrane pathways. We further analyzed the interactome network of the 308 genes by common topological algorithms, i.e., Maximal Clique Centrality (MCC), Maximum Neighborhood Component (MNC), and Degree, where we identified a subset of 11 genes based on the shortest paths with the algorithm Stress. Among the 11 genes, both β-Catenin (CTNNB1) and Annexin A2 (ANXA2) were significantly downregulated in BNCT-treated group ($P<0.05$). Further RT-qPCR analyses confirmed the results, while immunohistochemistry analyses demonstrated consistent BNCT effects in protein levels of these two genes. The encoded protein from CTNNB1 constitutes adherens junctions and anchors the actin cytoskeleton for maintenance of epithelial cell layers. In addition, ANXA2 plays an important role in tissue inflammation and vascular integrity. Thus, we speculate that BNCT induces cell-cell junction disorder through CTNNB1 and ANXA2, leading to damaged cell junctions in blood vessels and normal tissue inflammation. Indeed, disrupted epithelial structure as well as increased interferon-γ expression was detected in oral tissue microenvironment following BNCT. These findings set a basis for developing strategies of normal tissue protection and improving quality of life of head and neck cancer patients that were treated with BNCT.

CHEMISTRY: Drug Delivery
Poster Session

#1840 Potent antitumor efficacy of Dato-DXd compared with sacituzumab govitecan in brain metastases mouse model of triple negative breast cancer.

Chikako Funasaka[1], Hiroki Takashima[2], Daisuke Okajima[3], Tsuyoshi Karibe[3], Takanori Maejima[3], Satoru Yasuda[3], Toru Mukohara[4], Toshihiko Doi[1], Masahiro Yasunaga[2]

[1]Experimental therapeutics, National Cancer Center Hospital East, Kashiwa City, Japan,[2]Developmental Therapeutics, Exploratory Oncology Research & Clinical Trial Center, Kashiwa City, Japan,[3]Daiichi Sankyo Company, Limited, Tokyo, Japan,[4]Medical Oncology, National Cancer Center Hospital East, Kashiwa City, Japan

Background: Metastatic brain tumors are common, especially in patients with triple-negative breast cancer (TNBC) and lead to poor prognosis. Current drug therapies are unsatisfactory, and the development of new drugs is needed. TROP2 is overexpressed in various cancers and an attractive target molecule in breast cancer. Datopotamab deruxtecan (Dato-DXd) is an antibody-drug conjugate (ADC) targeting TROP2 and designed to selectively release the payload in the tumor cell via a cleavable tetrapeptide-based linker. Dato-DXd has demonstrated preliminary intracranial activity in patients, but its pharmacodynamics in brain metastases is not yet clarified. Here, we evaluated the efficacy and payload distribution of Dato-DXd and sacituzumab govitecan (SG), another TROP2-directed ADC that was designed to be cleaved by hydrolysis which may result in gradual payload release in blood circulation and the tumor, in a mouse brain metastasis model of TNBC.
Methods: We prepared a brain metastasis model by transplanting luciferase-expressing TROP2-positive TNBC cells into the brains of nude mice. To characterize ADC in treatment against intracranially transplanted tumors, the antitumor activity by measuring chemiluminescence and the concentration of the payload in tumors after intravenous administration of Dato-DXd were compared with those of SG.
Results: Chemiluminescence showed that Dato-DXd administered at 10 mg/kg on Day 1 significantly reduced tumor size in the brain metastasis model compared with SG administered at 10 mg/kg on Days 1 (average -99.5% vs. -1.8%, p=0.02). Median survival was 18 days (95% confidence interval [CI]; 14-not assessed [NA] days) in control group, 48 days (15-NA days) in SG group, and 83 days (49-NA days) in Dato-DXd group. The DXd payload from Dato-DXd was rarely detected in the serum (Lower limit of quantification [LLOQ] of DXd: 0.01 ng/mL), while the SN-38 payload from SG was gradually increased (LLOQ of SN38: 0.015ng/mL). After administration of Dato-DXd, the payload in the tumor was accumulated and maintained, whereas the payload concentration gradually decreased in SG group (the area under the concentration-time curve [AUC] of the payload: 320.9 vs. 161.8 ng*hr/g). In contrast, the AUC of the payload in normal brain after administration of Dato-DXd was significantly lower than that after administration of SG (13.7 vs. 48.5 ng*hr/g).
Conclusion: In this mouse model of brain metastases, Dato-DXd showed more potent antitumor activity against intracranially transplanted tumors of TNBC with higher accumulation of the payload in tumors compared with SG. The dosing utilized in this model does not directly correlate with human dosages, necessitating caution in interpreting the study results. This study warrants further clinical evaluation of Dato-DXd in TNBC patients with brain metastases.

#1841 NanoGhost - A drug targeting system for treatment of solid tumors.

Haim Tsubery[1], Ziv Azoulay[1], Shir Weber[1], Michal Lulu[1], Limor Matzrafi[1], Yonatan Malca[1], Marcell Machluf[2], **Osnat Bohana-Kashtan**[1]

[1]NanoGhost, Natanya, Israel,[2]Technion - Israel Institute of Technology, Haifa, Israel

Introduction: NanoGhosts (NGs) are nanovesicles derived from the cell membrane of human bone marrow mesenchymal stem cells (hBM-MSCs). NGs preserve MSCs cell membrane architecture and characteristics, thus harnessing MSCs' innate targeting capability towards tumors. SN38 (a topoisomerase 1 inhibitor) is a highly potent anti-cancer drug with poor water solubility, low plasmatic stability, high systemic toxicity, and low bioavailability. SN38 is approved for colorectal cancer (CRC), and pancreatic cancer as the prodrug Irinotecan or as liposomal Irinotecan, respectively. It is also approved as the antibody drug conjugate sacituzumab govitecan-hziy for triple negative and HR+HER2- breast cancer and metastatic urothelial cancer. We aim to target SN38 to various solid tumors by utilizing the inherent tumor targeting capability of MSCs derived NGs while minimizing SN38 systemic exposure.
Methods: MSCs derived NGs were loaded with SN38 using an NHS ester-activated linker. NGs bearing SN38 were tested for anti-cancer activity in vitro and in-vivo.
Results: SN38-NHS linker reacted in excess of 20 equivalents to protein content in NGs suspension resulted in average loading of ~0.08 µg SN38 per 1 µg NGs lipid. SN38 loaded NGs demonstrated relatively low leakage (of up to 34%) after up to 6 hr at 37°C in mouse serum. In vitro, SN38 loaded NGs inhibited proliferation of H460 lung cancer cells (IC50 of 1.6 µg/mL after 6 hr exposure). In vivo, IV administration of SN38 loaded NGs at a dose level of 5 mg/kg NGs lipid (~0.4 mg/kg SN38), resulted in tumor growth inhibition of HCT116 CRC in mice, similar to the positive control given at a dose level that was ~25 fold higher. Biodistribution analysis showed concentration of Cy7 labeled SN38-NGs at the tumor site while minimal-no retention in infiltrating organs. Maximal tolerated dose of SN38 loaded NGs was not yet achieved following administrating of up to 10 mg/kg, supporting use of higher levels in follow on studies.
Summary: Our data support the use of NGs for targeting highly potent and toxic drugs with draggability barriers (such as SN38) to tumors.

#1843 Therapeutic implication of pH/hypoxic responsive nanoencapsulated tunicamycin on pancreatic cancer by targeting K-Ras^{G12D}.

Axel H. Breier[1], Debasmita Dutta[2], Anjali Kambhampati[1], Inamul Haque[3], Archana De[1], Sunil P. Upadhyay[1], Sushanta K. Banerjee[4], Mohiuddin Quadir[5], Snigdha Banerjee[6]

[1]Cancer Research Unit, Kansas City VA Medical Center, Kansas City, MO,[2]Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA,[3]Kansas City VA Medical Center, Kansas City, MO,[4]University of Kansas Medical Center, Kansas City, MO,[5]Coatings and Polymeric Materials, North Dakota State University, Kansas City, MO,[6]Cancer Research Unit, University of Kansas Medical Center and VA Medical Center, Kansas City, MO

Pancreatic ductal adenocarcinoma (PDAC) remains one of the leading sources of cancer mortality worldwide, including US Veterans. An initial response to chemotherapy, such as Gemcitabine (GEM) alone or in combination with other chemotherapies, is often followed by emergent resistance, reflecting an urgent need for targeted therapies. PDAC cells are highly addicted to oncogenic K-RAS mutations for their growth, progression, immunosuppression, and drug resistance, but mutant K-RAS in PDAC is still tricky to target. A glycosylation inhibitor, Tunicamycin (TM), is a potent killer of PDAC cells. However, free-form TM is found to be very toxic in *in vivo* implication as a therapy. To repeal the limitation of TM, we have synthesized a pH/Hypoxic-responsive iRGD-tagged biodegradable nano-encapsulated TM (^{NP}TM) that was found to inhibit PDAC cell growth parallel with blocking K-RAS^{G12D} expression and its downstream signaling pathways associated with desmoplasia, and program cell death ligand 1 (PD-L1) expression. Moreover, ^{NP}TM markedly reduced the xenograft tumor growth, increased intratumoral CD8+ T cells, and increased in overall survival of treated mice. Furthermore, ^{NP}TM treatment synergizes with GEM to further regress PDAC growth in a PDAC xenograft model. These studies provide insights into ^{NP}TM treatment with a potential clinical application.

#1844 From barrier to bullet: Genetically engineered CAF-derived exosomes for PDAC therapy.

Leyla Escalante Gonzalez[1], Subin Yu[1], Aya Jaros[1], Alaa Bakr[1], Cuiyan Xin[2], Luke Lee[1], Ahmed Elzoghby[1]

[1]Engineering in Medicine Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA,[2]Renal Medicine Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal cancers, with a five-year survival rate of less than 10%. This poor prognosis is due in part to the tumor's dense desmoplastic stroma, which impedes nanoparticle penetration and immune cell infiltration, rendering conventional therapies ineffective. The current therapeutic paradigm, which directly targets PDAC cells with nanoparticles or relies on immune modulation, often fails due to the "immune cold" PDAC tumor microenvironment (TME) characterized by low infiltration of cytotoxic T lymphocytes (CTLs) and abundance of cancer-associated fibroblasts (CAFs) that create a formidable stromal barrier. Therefore, there is a critical need for a paradigm shift in PDAC treatment—moving beyond traditional strategies that directly target cancer cells or modulate the immune system, and instead, focusing on harnessing the unique properties of the TME itself to combat the tumor. Therefore, we transformed the inherent limitations of the PDAC TME into therapeutic opportunities by reprogramming CAFs to act as endogenous factories of cytotoxic exosomes capable of selectively targeting and killing cancer cells. By leveraging the natural abundance and strategic location of CAFs within the TME, we employed exosome fusion protein technology to engineer CAFs to produce exosomes loaded with cytotoxic proteins, such as granzyme B (GrB), or pro-melittin, fused to one of the exosomal components e.g., CD63 on gene level, and specific targeting motifs e.g., B7-H3 single-chain variable fragment (scFv) to ensure selective uptake by PDAC cells inducing their apoptosis. These cytotoxic exosomes bypass the need for CTL infiltration, thereby overcoming the challenges posed by the "immune cold" TME. We engineered CAF-targeted lipid nanoparticles (LNPs) for delivering plasmid DNA (pDNA) encoding the fusion proteins into PDAC patient-derived CAFs. The cells could be successfully transfected in vitro and subsequently produced the desired cytotoxic exosomes. The expression of fusion proteins in the exosomes was confirmed in vitro via western blot and proteomic analysis. The GFP-expressing exosomes showed efficient internalization into PDAC cells as evidenced by immunofluorescence imaging and flow cytometry. The genetically engineered CAF-derived exosomes induced efficient apoptosis of patient-derived PDAC cells in vitro revealing their cytotoxic power. Moreover, intravenous administration of the pDNA-loaded lipid nanoparticles into pancreatic tumor mouse model significantly suppressed tumor growth. Histopathological studies and biochemical analysis showed the safety of our engineered nanoparticles. This approach leverages the unique properties of the TME to provide a new paradigm in cancer therapy that avoids the need for direct targeting of cancer cells or enhancement of immune cell infiltration.

#1845 Increased antitumor activity and reduced organ toxicity of doxorubicin administered using RRx-001 modified RBCs.

Ana Sanchez, Garett Soapes, Chris Larson, **Tony Reid**, Bryan Oronsky

EpicentRx, Inc., La Jolla, CA

RRx-001 is currently in a phase III clinical trial for small cell lung cancer and showed activity in a phase II trial in colorectal cancer. It has negligible side effects so could be used in combination regimens with low risk of additive toxicity. It has multiple mechanisms of action including binding to hemoglobin to alter red blood cell (RBC) properties, increasing RBC adhesion to the endothelium and increasing membrane phosphatidyl serine exposure. Importantly, RRx-001-treated RBCs are known to preferentially localize to solid tumors. Existing therapeutics that partition into RBCs could therefore be localized to cancers and have a greater therapeutic index by combining with RRx-001. Doxorubicin has been a cornerstone of treatment for many cancer types for decades and has well-known limiting side effects including cardiotoxicity and cytopenias. It partitions into RBCs at equilibrium with approximately 60% of doxorubicin becoming intracellular. Therefore, treatment with RBCs loaded with doxorubicin and modified with RRx-001 to promote localization to tumors may improve its activity and reduce its potential side effects. Previous research in mice carrying H22 tumors found that biodistribution to the tumor was higher after treatment with RBCs loaded with doxorubicin and treated with RRx-001 than with free doxorubicin or RBCs loaded with doxorubicin without RRx-001, while biodistribution to the heart and skin was decreased. H22 tumor growth was lower after treatment with RBCs loaded with doxorubicin and treated with RRx-001 than with doxorubicin alone or RBCs loaded with doxorubicin without RRx-001. Further experiments identified an approach for loading RBCs with doxorubicin and treating with RRx-001 that could be implemented clinically while achieving high doxorubicin loading efficiency given its rate of equilibration into RBCs, avoiding hemolysis, and minimizing doxorubicin precipitation. The final chosen procedure would be to draw blood with citrate anticoagulation while mixing with RRx-001 to reach 5 μM, add 2 mg/mL doxorubicin in normal saline to reach a final concentration of 0.3 mg/mL, mix for 1 hour at 37°C, and re-infuse the treated blood. For a clinical trial with a starting dose of 20 mg/m^2 doxorubicin weekly, a patient with a BSA of 2 m^2 would require 101 mL of blood to be drawn for mixing and re-infused 1 hour later, which would be practical even for patients with advanced cancer who are candidates for doxorubicin. For reference, a typical blood donation is approximately 450 mL. In conclusion, this describes the pre-clinical rationale and establishes a clinical procedure for administering RBCs loaded with doxorubicin and treated with RRx-001 as a way to improve its activity and reduce side effects.

#1846 Enhancing chemoimmunotherapy of metastatic TNBC through potent and immunomodulatory drug combination nanoparticles.

Caroline B. Linde, Xiaolin Xu, Rodney J. Y. Ho, Mary L. Disis, **Qingxin Mu**

University of Washington, Seattle, WA

Breast cancer is the most common cancer in women worldwide, and triple-negative breast cancer (TNBC) represents the most challenging subtype to treat due to the lack of hormone or HER2 receptors. Current guidelines for metastatic TNBC recommend a combination of pembrolizumab with chemotherapy for patients with PD-L1 expression (CPS ≥ 10). To enhance the efficacy of existing regimens, we previously reported that water soluble gemcitabine (G) and insoluble paclitaxel (T) can be stabilized with lipid excipients and a novel process into drug combination nanoparticle suspension (GT-in-DcNPs, ~60 nm) capable of co-delivering both drugs to the tumor. We demonstrated that a single IV dosing of GT-in-DcNP, but not soluble form, inhibited lung metastasis of 4T1 in mouse by nearly 100%. In this study, we hypothesized that GT-in-DCNPs not only improve tumor inhibition compared to free GT, but also increase PD-L1 expression in the primary tumor and reduce myeloid-derived suppressor cells (MDSCs), thereby sensitizing tumors to anti-PD-1/PD-L1 immune checkpoint inhibitor therapy. Consequently, combining antibody therapy with GT-in-DcNPs could further improve therapeutic outcomes. Our *in vitro* studies showed that free G or T increased PD-L1 mRNA and protein expression in 4T1 TNBC cells in a dose- and time-dependent manner. When the drugs were combined in G-to-T molar ratios of 30:1 to 1:1, PD-L1 mRNA levels were upregulated by 4- to 5-fold after 24 hours. Under static cellular incubations, GT-in-DcNPs and free GT counterpart produced a similar impact on PD-L1 upregulation. We further investigated orthotopic 4T1 tumoral PD-L1 upregulation in BALB/c mice. Six days after a single IV dose of 35/3.5 mg/kg GT-in-DcNP (~30:1 molar ratio) but not free GT elevated PD-L1 mRNA expression in tumor by ~70%. In a preliminary efficacy study with the orthotopic 4T1 model (~100 mm^3), a single GT-in-DcNP (35/3.5 mg GT, IV, day 0) and anti-PD-L1 antibody (100 µg, IP, day 6) combination exhibited significant tumor growth reduction (168%) than the GT-in-DcNP or free drug treatment (248% and 265%, respectively; $n = 8$, $p < 0.05$), by day 12. Flow cytometry analysis of tumor and spleen cell suspensions revealed that MDSC populations (Gr-1+ CD11b+) in both tissues were reduced by GT-in-DcNPs on day 6 post-treatment, whereas no changes were observed in the free drug groups. Furthermore, the spleen CD8+ T cell population on day 12 was increased by 65% in DcNP-antibody combination group compared to free GT-antibody combination group ($p < 0.05$). Overall, these data suggest that GT-in-DcNP treatment elevates PD-L1 expression in 4T1 TNBC cells *in vitro* and *in vivo* and reduces MDSC populations *in vivo*, and its combination with PD-L1 antibody enhances tumor inhibition beyond that achievable by GT in conventional dosage form. Thus inclusion of GT-in-DcNP with immune checkpoint inhibitor against PD-L1 may provide additional benefits.

#1847 Closed-loop drug delivery system to personalize chemotherapy dosing.

L. DeRidder[1], K. Hare[1], T. Smierciak[1], M. Chao[1], A. Lopes[1], J. Jenkins[1], N. Fitzgerald[1], E. MacPherson[1], N. Fabian[1], J. Morimoto[1], J. Chu[1], A. Kirtane[1], W. Madani[1], K. Ishida[1], J. Kuosmanen[1], N. Zecharias[1], C. Colangelo[2], H.-W. Huang[1], M. Chilekwa[1], N. Lal[1], S. Srinivasan[1], A. Hayward[1], B. Wolpin[3], D. Trumper[1], T. Quast[4], D. Rubinson[3], R. Langer[1], G. Traverso[1];
[1]Massachusetts Institute of Technology (MIT), Cambridge, MA, [2]Agilent Technologies, Santa Clara, CA, [3]Dana-Farber Cancer Institute, Boston, MA, [4]University of South Florida, Tampa, FL

While most oncologists know that many chemotherapies are dosed on a body surface area (BSA) basis, fewer know that the equations used to estimate BSA are based on a study done over 100 years ago using data from just 9 people. Moreover, two patients with the same BSA can have many factors, such as body composition and genetics, which can impact a drug's pharmacokinetics and pharmacodynamics, resulting in worse therapeutic outcomes. To solve this problem, we developed a medical device that can personalize the dose of chemotherapy to the patient during an infusion center visit. Our medical device, which we call CLAUDIA (the Closed-Loop AUtomated Drug Infusion regulator) is a closed-loop drug delivery system that measures the concentration of drug and changes the infusion rate in real time to keep the concentration of drug within the therapeutic window. CLAUDIA uses high performance liquid chromatography-mass spectrometry (HPLC-MS) as the sensor, which allows it to be relatively easily translated to control many different drugs, either individually or in a combination chemotherapy regimen. We demonstrate CLAUDIA can control the concentration of 5-fluorouracil (5-FU) in rabbits, which were manipulated to capture the pharmacokinetic variability observed clinically. We then adapted CLAUDIA to also control the concentration of irinotecan, demonstrating its ability to be rapidly translated to additional chemotherapies. Finally, we perform a cost-effectiveness analysis that demonstrates that CLAUDIA is cost-effective compared to the current standard of care for drug dosing of 5-FU (i.e., BSA). Overall, CLAUDIA presents promising medical device that can personalize the dosing of chemotherapy, which may be able to improve the therapeutic outcomes for multiple chemotherapies.

#1848 Layered zirconium phosphate nanoparticles as anticancer drug carrier.

Aleannette Lopez-Cubero[1], Getsemary Cabrera[1], Armando Santiago[2], Yairisis Rivera[2], Maria M. Rivera[3], Alianys Rosario[4], Magaly Martinez-Ferrer[5], Jorge L. Colon[1]

[1]Chemistry, University of Puerto Rico, San Juan, Puerto Rico,[2]Biology, University of Puerto Rico, San Juan, Puerto Rico,[3]Division of Cancer Clinical & Translational Research, University of Puerto Rico Comprehensive Cancer Center, San Juan, Puerto Rico,[4]Program SEED, American Chemical Society, San Juan, Puerto Rico,[5]2Division of Cancer Clinical & Translational Research, School of Pharmacy, University of Puerto Rico Comprehensive Cancer Center, University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico

Layered zirconium phosphate nanoparticles (ZrP), inorganic structures with a layered composition of zirconium atoms linked by phosphate groups, exhibit no cytotoxic effects on healthy or cancer cells, indicating a strong safety profile. We are investigating ZrP as a carrier for mitoxantrone (MTX), an anticancer drug that inhibits topoisomerase II and disrupts DNA synthesis but lacks selectivity between healthy and cancer cells. MTX shows prolonged retention in the body, with potential systemic toxicity affecting thyroid, hepatic, and cardiac tissues. This study aims to reduce systemic toxicity and protect healthy tissues from MTX by intercalating MTX into ZrP (MTX-ZrP) via ion exchange, determining the conditions that affect MTX's release, and evaluating the cytotoxicity of MTX-ZrP on prostate cancer cells.

MTX was intercalated with ZrP nanoparticles, forming MTX-ZrP, which was then characterized by Fourier Transformed infrared spectroscopy, scanning electron microscopy/energy dispersive X-ray spectroscopy, and X-ray powder diffraction at different molar ratios. A drug release study to determine conditions affecting MTX liberation from ZrP at different pHs using simulated body fluid and lysosomal fluid showed that ZrP nanoparticles maintain stable and intercalated MTX at biological pH 7.4. However, at pH 4.5, which simulates the extracellular environment of the tumor, MTX is released, implying that this process is pH-regulated. Cell viability studies with androgen receptor-negative prostate cancer cell line PC3 showed that MTX-ZrP produced a cytotoxic effect. These findings represent the initial phase of a possible drug delivery method that may greatly minimize adverse effects and harm to healthy tissues, offering promise for a more bearable cancer treatment. In the future, we expect to further improve the drug carrier system's efficacy by modifying the surface of MTX-ZrP, which could significantly enhance its selectivity and specificity.

#1849 Developing a formulation of MDM2 inhibitor: Idasanutlin for enhanced stability, solubility, and anticancer activity in p53 sensitive lung cancer.

Bhoomi Dholariya, Akanksha Patel, Henis Patel, Ketan Patel

St. John's University, Queens, NY

Lung cancer is generally categorized into two major class, non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Both types chiefly disrupt the lung's primary function of supplying oxygen to the body due to uncontrolled cell growth. Lung cancer metastasis is a major obstacle in controlling the prevalence of the tumor. One contributing factor is the over expression of MDM2 protein which inhibits the tumor suppressive effect of p53 protein, thereby fostering the proliferation and metastasis of NSCLC. To address this, we employed a second generation MDM2 inhibitor - Idasanutlin (IDA) to develop and characterize self-emulsifying nanocomplex of idasanutlin-ionizable lipid (IDLIN). IDA has demonstrated promising anticancer activity *in vitro* in p53 sensitive lung cancer cell lines. However, as a brick-dust molecule with poor solubility in both water and oil, it poses a significant challenge in development of lipid based nano formulation. We hypothesized that forming a hydrophobic complex between the carboxylic group of anionic IDA and the tertiary amine of DLin-DMA would improve the stability of IDA in water. The anticancer efficacy of this formulation was tested using various 2D and 3D cell culture assays in wild type p53 cells (A549 and H460). To formulate IDLIN, we used IDA along with Dimethyl Acetamide (DMA) as a cosolvent, DLin-DMA as the ionizable lipid, and 1:1 preconcentrate of oil and non-ionic surfactant. Medium Chain Triglycerides (MCT) served as oil, while Solutol HS 15 was used as surfactant. The inclusion of DMA and DLin-DMA significantly enhanced drug loading, achieving 3%w/w compared to 0.5%w/v in the conventional self-nanoemulsifying system. The observed particle size and zeta potential for IDLI (formulated with IDA, DMA, and MCT:HS15 without ionizable lipid) were 63.98nm and -30.7mV, respectively, whereas for IDLIN, the particle size and zeta potential were 71.69nm and -23.1mV, respectively. The *in vitro* anticancer efficacy of IDLIN was assessed against IDA dissolved in dimethyl sulfoxide at a concentration of 1 µM using a multicellular 3D spheroid assay. Both treatments resulted in a significant reduction in spheroid diameter and area compared to control group. By the end of the treatment, both groups also exhibited higher levels of red fluorescence compared to the control group, indicating a substantial increase in dead cells across both cell lines. In A549 cells, the treatment groups showed a decrease in spheroid area by 45.70% for IDA and 46.18% for IDLIN. In H460 cells, the decrease was 57.64% for IDA and 59.23% for IDLIN. The observed IC_{50} of IDA and IDLIN in A549 cells were 3.615µM and 4.766µM respectively and in H460 cells, 0.641µM and 0.707µM was respectively. This is the very first research in use of ionizable lipid in stabilizing anionic hydrophobic molecules and testing nano-formulation of MDMD2 inhibitor in lung cancer.

#1850 MUC13-targeted PPNPs efficiently inhibited pancreatic tumor growth and influenced tumor immune surveillance.

Vivek Kumar Kashyap, Neeraj Chauhan, Mohammed Sikander, Eswara N. HK Ghali, Fnu Shabnam, Rahul Tiwari, Sheema Khan, Bilal B. Hafeez, Murali M. Yallapu, Meena Jaggi, Subhash C. Chauhan

University of Texas Rio Grande Valley, McAllen, TX

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal types of cancer, characterized by a dismal 5-year overall survival rate, primarily due to lack of early detection and limited effective treatment. Gemcitabine (GEM) is most effective therapy for pancreatic cancer (PanCa), but it only offers a marginal survival benefit of 6 months. A combination of Paclitaxel (PTX) albumin nano-formulation (Abraxane) and GEM has shown marginal improvement in PanCa treatment. This poor treatment response has been associated with desmoplastic stroma, microbiome effects, altered tumor microenvironment (TME), suppressive immune cells and PDAC-specific signaling pathways. Our research has demonstrated that MUC13 is highly overexpressed in PanCa and is associated with cancer progression and metastases. Considering these facts, we have generated an MUC13 antibody targeted nanoparticle formulation for tumor specific targeted delivery of paclitaxel using a multi-layered Pluronic F127 and polyvinyl alcohol stabilized and poly-L-lysine coated poly(lactic-co-glycolic acid) nanoparticles (PPNPs) to improve therapeutic outcome of PanCa therapy. Our MUC13-targeted PPNPs (MUC13-PPNPs) showed enhanced cellular uptake of MUC13-PPNPs formulation in HPAF-II (MUC13+) compared with Panc-1 (MUC13) PanCa cells as determined by immunofluorescence. Further, our novel formulation effectively inhibits growth and metastatic phenotypes in MUC13+ cells as compared with MUC13- cells of PanCa *in vitro.* In our PanCa xenograft mouse model (HPAF-II + stromal cells), we found that PPNPs alone significantly inhibited growth of tumor ($P < 0.05$ when compared with control (PLGA) and treatment group PTX. Furthermore, the tumor volume of MUC13-PPNPs group was not only highly significantly lower than control PLGA and PTX ($P < 0.05$), but also lower than PPNPs alone group ($P < 0.05$) or control ($P < 0.05$). Our findings also demonstrated that PPNPs/MUC13-PPNPs synergize GEM therapy response *in vitro* and *in vivo* mouse models. Further, tumor tissue treated with PPNPs/MUC13-PPNPs demonstrate the downregulated expression of sonic hedgehog and other oncogenic signaling axis determined by qPCR, Western blots, and immunofluorescence analyses. Additionally, our nano formulation effectively reduced tumor volume in syngeneic orthotopic xenograft mouse model involving injection of KPC-Luc in C57Black/6 mice and target tumor-associated macrophages (TAM) by repolarizing M2 into M1 phenotype *via* inhibiting expression of M2 markers and an increase in M1 markers. In conclusion, we observed that our novel formulation has high therapeutic ability for achieving pancreatic tumor specific delivery PTX, synergize GEM therapy response and altered the tumor immune surveillance mechanisms. Thus, this unique approach can effectively enhance therapeutic efficacy main line chemotherapies and immune checkpoint immunotherapies.

#1851 Development of a smart nanocarrier system for prostate cancer immunotherapy.

Imani Roundtree[1], Angelo Altenor[1], Rodney Valdivia[1], Tovah St. Ignac[1], Alexandra Esposito[1], Karelys DiazDavila[2], Richard Lamptey[2], **John Fetse**[3]

[1]Larkin University College of Pharmacy, Miami, FL,[2]Palm Beach Atlantic University, West Palm Beach, FL,[3]School of Pharmacy and Pharmaceutical Sciences, Binghamton University, Binghamton, NY

Prostate cancer (PCa) is characterized by the overexpression of monoamine oxidase-A (MAO-A), a driver of tumor growth, metastasis, and treatment resistance. Monoamine oxidase inhibitors (MAOIs), such as phenelzine, have shown promise as repurposed agents for PCa therapy. However, systemic administration of phenelzine at effective doses carries significant risks of neuropsychiatric side effects, including aggression, seizures, and suicidal ideation, highlighting the need for a targeted delivery approach. This study aims to develop a smart nanocarrier system for the co-delivery of phenelzine (PHE) and the anti-programmed death ligand 1 (PD-L1) peptide (C7), enhancing therapeutic efficacy while reducing off-target effects. The nanocarrier employs a pH-sensitive hydrazone bond and a matrix metalloproteinase-2 (MMP-2) cleavable linker to achieve site-specific drug release. The system integrates polyethylene glycol-hydrazone-phenelzine (PEG-HZ-PHE) and an MMP-2cleavable linker tethered to the anti-PD-L1 peptide (C7-MMP2), into a lipid nanocarrier platform. Characterization using dynamic light scattering (DLS) revealed a particle size of 190.0 ± 3.9 nm, a polydispersity index (PDI) of 0.17 ± 0.02, and a zeta potential of -33.6 ± 1.3 mV. pH-dependent release studies showed that phenelzine release was highest at pH 5.4 (74.7 ± 0.6%) and lowest at pH 7.4 (11.3 ± 0.1%) after 18 hours, with a 42.3 ± 0.6% release at pH 6.4. Enzyme cleavage studies demonstrated that 73.8 ± 14.5 % of C7 was released within 4 hours of incubation with MMP-2 enzyme, with only 20.3 ± 2.1% of C7-MMP2 remaining after the same period. Cytocompatibility assessment using a red blood cell (RBC) hemolysis assay showed no significant hemolytic activity compared to the negative control (PBS buffer), while the positive control (0.5% Triton X-100) caused significant hemolysis. In conclusion, the developed dual-responsive nanocarrier is stable at physiological pH (7.4) but efficiently releases therapeutic agents in acidic and MMP-2 enzyme-rich environments, offering potential for improved PCa therapy by minimizing off-target effects.

#1852 Uncovering regulatory mechanisms and therapeutic targets in EV-mediated nanoparticle transfer: A proteomics and high-throughput screening approach.

Xian Wu, Hong-Bo Pang

University of Minnesota, College of Pharmacy, Minneapolis, MN

Extracellular vesicles (EVs) are naturally occurring vesicles that facilitate the transfer of biomolecules between cells, playing a vital role in cellular communication and regulation. Recently, EVs have been discovered participating active transport of nanomaterials between cells, suggesting a novel method to enhance the delivery of nanoparticles. A 3D intercellular exchange assay has been developed by our group to monitor and quantify EV-mediated transfer of silver nanoparticles (AgNPs) across different cell types. Furthermore, distinct EV subpopulations, those containing AgNPs (AgNP-containing) and those without (AgNP-free), were isolated from AgNP-exposed cells using density gradient ultracentrifugation. In this study, we leverage proteomics and high-throughput screening (HTS) to reveal the regulatory mechanisms of EV-mediated intercellular communication and identify compounds that regulate this process. Using mass spectrometry and advanced bioinformatics, unique protein signatures associated with each EV type were identified, uncovering biomarkers that indicate origin and functional relevance in EV-mediated transport process. To investigate potential regulators of EV-mediated intercellular exchange, we combined HTS with our 3D intercellular exchange assay to identify compounds that modulate EV-mediated transfer of nanomaterials. Integrating HTS with quantitative proteomics enabled us to identify key compounds that specifically impact the formation and secretion of AgNP-free and AgNP-containing EV subpopulations. These findings not only provide new insights into the regulatory mechanisms controlling nanomaterial-sorting and EV genesis but also highlight potential therapeutic targets for modulating EV-mediated intercellular exchange, which may further improve nanomedicine delivery. This work underscores the utility of combining proteomics and HTS for the discovery of small molecules capable of regulating EV-mediated transfer, with implications for therapeutic development and biomarker discovery.

#1853 BIK-E715E, an integrin-targeting ligand-drug conjugate equipped with a novel drug delivery system, demonstrates enhanced antitumor efficacy in glioblastoma.

K. Shin, H. Sung, J. Jung, S. Kim;
BIK Therapeutics Inc., Seongnam, Korea, Republic of

We developed a novel drug delivery system ELiTE-DDS, designed to enhance target-selective intracellular delivery of therapeutic agents, where ELiTE stands for enhancing ligand targeting and endocytosis. Integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$, highly expressed in glioblastoma tumor cells, are molecular targets for glioblastoma treatment. In this study, we applied the ELiTE-DDS platform to a conjugate consisting of a high-affinity integrin $\alpha_v\beta_3/\alpha_v\beta_5$-binding ligand, D-[c(RGDfK)]$_2$, and a topoisomerase I inhibitor exatecan, yielding BIK-E715E as a drug candidate for glioblastoma. BIK-E715E exhibited high selectivity and binding affinities for integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ and was internalized through an Arf6-dependent, dynamin-independent endocytic pathway. Cellular treatment with BIK-E715E produced PARP cleavage and phosphorylation of Chk1 and histone H2AX, indicating drug-induced apoptosis and DNA damage, respectively. There was significant translocation of BIK-E715E in a hCMEC/D3 in vitro blood-brain barrier (BBB) model, suggesting its receptor-mediated transcytosis across the BBB. In U87MG subcutaneous and orthotopic xenograft mouse models, BIK-E715E accumulated in tumors with high efficiency and resulted in marked inhibition of tumor growth, which was greater than exatecan-induced growth inhibition. These data demonstrate that BIK-E715E is an attractive drug candidate for glioblastoma, equipped with a novel drug delivery system. Further, they also suggest that our ELiTE-DDS platform has great potential for enhancing target-selective intracellular delivery of therapeutic agents across the BBB.

#1854 Neurotransmitter-derived lipid nanoparticles (LNPs) for brain-targeted drug delivery.

Shuyu Wang

Cancer Molecular Diagnostics Core, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China

The treatment of central nervous system (CNS) diseases, such as neurodegenerative disorders, brain tumors, brain infections, and stroke, is severely constrained by the blood-brain barrier (BBB) because it prevents the transfer of most of small-molecule drugs and macromolecules (e.g., peptides, gene drugs, and protein drugs) into the brain. We have developed a previously unknown class of neurotransmitter-derived lipidoids (NT-lipidoids) as simple and effective carriers for enhanced brain delivery of several BBB-impermeable cargos. Particularly, doping the NT-lipidoids into BBB-impermeable lipid nanoparticles (LNPs) gave the LNPs the ability to cross the BBB. Using this novel brain delivery platform, we successfully delivered small molecular drug, genes and proteins into the mouse brain via systemic intravenous administration. For example, the BBB-impermeable paclitaxel was delivered to the brain to treat glioma, and genome-editing fusion protein (–27)GFP-Cre recombinase was delivered for effective genome editing in neuronal cells of the Ai14 mouse brain through intravenous injection. Our recent results demonstrate that by doping the NT-lipidoids into BBB-impermeable clinical used liposome nanomedicines can give the nanomedicines the ability to cross the BBB to treat brain tumors and brain infections. We believe this NT-derived LNPs is a new, simple, universal, and effective brain delivery system, which may find wide applications in treating CNS diseases including brain tumors and providing a tool to study the brain function.

#1855 Insights from drug development experts: Surveying challenges and outcomes for large-volume subcutaneous oncology drugs.

Mehul Desai[1], Omar Rahman[1], Beate Bittner[2], Wei Chen[3], Maxwell Koobatian[4], Deepa Rajagopalan[5]

[1]Medical Affairs, Enable Injections, LLC, Cincinnati, OH,[2]Global Product Strategy, Roche, Basel, Switzerland,[3]Wuxi Biologics (US), Worcester, MA,[4]Corporate Development, Crinetics Pharmaceuticals, San Diego, CA,[5]45 Management LLC, New York, NY

With several well-known large-volume subcutaneous (SC) oncology drugs, such as daratumumab, trastuzumab, rituximab, nivolumab, and pembrolizumab, either approved or in trials, understanding how administration volume impacts drug development is critical. Patients generally prefer SC over intravenous administration. However, SC adoption is often limited by the perception that delivery volumes must stay below 3 milliliters without a permeation enhancer. This misconception hampers SC oncology drug prioritization and stifles innovation. To better understand these challenges, a survey was conducted to investigate decision-making processes surrounding large-volume SC oncology drug candidates. The online survey, conducted November 20-26, 2024, targeted 80 drug development professionals with SC drug development experience equally representing new product planning, drug formulation, combination products, and clinical development. Of the participants, 43 primarily worked in oncology. The 23-item survey explored perceptions of SC volume thresholds, factors influencing the deprioritization of oncology drug candidates, the potential of large-volume delivery solutions in oncology, and how organizations track and revisit deprioritized candidates. The oncology respondents stated that 32% of drugs are abandoned due to challenges with drug volume. The most common reason for abandonment of the asset was lack of experience with large-volume formulations or devices. Similarly, the respondents stated that 29.5% of drugs are deprioritized due to difficulties in reducing subcutaneous drug volumes. When it came to deprioritizing assets, the primary reason was that large-volume drugs are only pursued if they address a unique unmet need regardless of the competitive landscape. Participants rated the commercial potential of these abandoned or deprioritized drugs at a 3 on a scale of 1-4 (1 = low potential, 4 = high potential) indicating that these drugs had worthwhile commercial potential. Fortunately, participants ranked the organizations willingness to revisit these drug candidates at a 3 on a scale of 1-4 (1 = not open at all, 4 = extremely open). The results suggest that oncology molecules with worthwhile commercial potential remain unexplored within companies due to misconceptions around large-volume SC delivery, with a high willingness to revisit these candidates.

#1856 Synergistic combination of PROTACs degrading oncogenic proteins: In vitro studies and formulating a long-acting injectable for in vivo testing in lung tumor-bearing mice.

Akanksha Patel, Mukti Vats, Ketan Patel

St. John's University, Queens, NY

Introduction: Lung cancer remains a leading cause of cancer-related mortality worldwide, with accountability of more than 85% of cases of non-small cell lung cancer (NSCLC). Current therapies, such as EGFR inhibitors (e.g., erlotinib, gefitinib), face significant challenges, including severe side effects, drug resistance, and financial burdens, leading to patient noncompliance. In recent years, Protein-Targeting Chimeras (PROTACs), a class of protein degraders, is a groundbreaking strategy that degrades, rather than merely inhibits, targets proteins. This work investigates a combination of PROTACs degrading key oncogenic proteins of lung cancer. BRD4 degrading PROTAC (BPRO) and an EGFR degrading PROTAC (EPRO) were combined to simultaneously degrade BRD4 and EGFR to suppress c-MYC, EGFR and K-RAS, offering therapeutic potential for NSCLC with diverse genetic profiles.

Objectives: Our study objectives include (a) evaluating BE-PRO synergism in various NSCLC cell line i.e. wild-type EGFR (H460 cells), EGFR TKI-sensitive (PC9 cells), and EGFR TKI-resistant (H4006/GR) cells; (b) assessing anticancer efficacy of BE-PRO in 2D and 3D NSCLC models; and (c) optimizing a self-injectable biodegradable subcutaneous depot formulation (BERD) for sustained delivery; (d) to analyze the *in vivo* anticancer efficacy of BERD in NSCLC cells tumor xenograft model.

Methods: *In vitro* assays - IC_{50} and combination index (CI), invasion assay, clonogenic assay, and 3D spheroids growth were carried out to identify the synergistic ratio of BPRO and EPRO in different NSCLC cell lines. 3D-cell viability and apoptosis were studied using Cell Titer-Glo® 3D and Live/Dead Cell imaging assays. Further, depot formulation was developed using a biodegradable polymer and tested using a subcutaneous tissue-mimicking phantom using IVIS imaging. To determine the *in vivo* efficacy of BERD, a subcutaneously engrafted HCC827 cells xenograft model has been developed, and currently, we are measuring tumor growth using ultrasound and vernier caliper methods.

Results: The combination index and cytotoxicity interaction graph demonstrated of BPRO and EPRO (CI = 0.25±0.026 in H460; 0.151±0.047 in PC9, 0.693±0.013 in H4006/GR) and significant inhibition of invasion and clonogenic activity. The combination showed minimal toxicity in healthy cells, HEK 293, and lung fibroblast. In 3D spheroids, BE-PRO achieved ~74.5% and ~53.3% growth inhibition under chronic and acute dose regimens, respectively. BERD ensured sustained drug release over 72 hrs, with minimal precipitation and reduced initial burst effects compared to the individual drug solution.

Conclusion: A sustained-release combination of BRD4 and EGFR degrading PROTACs could be a promising approach, tackling key oncogenic drives while minimizing dosing frequency and off-target toxicities.

#1858 Nanoformulation strategy for oral delivery of KRAS inhibitor (sotorasib): pH-independent dissolution to mitigate PPI drug interaction.

Henis Patel, Akanksha Patel, **Ketan Patel**

St. John's University, Queens, NY

Biovariability poses a significant challenge in the oral administration of sotorasib (AMG 510), an FDA-approved KRAS G12C inhibitor for non-small cell lung cancer (NSCLC). Sotorasib, a weakly basic drug with limited aqueous and pH-dependent solubility, necessitates a high daily dose (960 mg; 8 tablets). In general, high doses, pill burdens, and poor solubility often result in poor patient compliance and therapeutic outcomes. Co-administration with proton pump inhibitors (PPIs) in cancer patients significantly reduces the bioavailability of weakly basic drugs including sotorasib. To address these limitations, we developed a pH-independent nanocrystalline formulation through wet media milling, aiming to achieve a stable formulation with enhanced bioavailability, improved redispersibility, and minimized fasted/fed variability. The nanosuspension, formulated using anionic surfactant and non-ionic polymer as stabilizers, achieved a high drug load (45% w/w) with uniformly distributed nanocrystals (173 ± 21.54 nm) and a negative zeta potential (-36.0 ± 0.57 mV). Cryoprotectants hydroxypropyl-β-cyclodextrin (HPβ-CD) and trehalose were effective in preventing aggregation, ensuring excellent redispersibility across pH conditions in both simulated gastric (pH 4.0) and intestinal (pH 6.8) environments. *In vitro* dissolution studies demonstrated > 95% drug release within 5 minutes at both pH levels, indicating rapid dissolution and robust stability. Solid State Characterization studies, including X-ray diffraction (XRPD), differential scanning calorimetry (DSC), scanning electron microscopy (SEM), and cryo-TEM, confirmed the preserved crystallinity, reduced particle size, and improved homogeneity. Additionally, BET surface area analysis indicated an increased surface area for the lyophilized nanosuspension, supporting enhanced dissolution. The *in-silico* model predicted increased absorption in the intestinal region, particularly the duodenum and jejunum due to improved saturation solubility and minimized drug precipitation. *In vivo* pharmacokinetic studies in SD rats revealed superior systemic exposure, with a significant increase in C_{max} (3529 ± 564.57 ng/mL) and AUC_{last} (8294.5 ± 1346.66 h*ng/mL) compared to the physical mixture. It may address the challenges of high dosing requirements and drug interactions with acid-reducing agents, offering a promising solution to enhance therapeutic outcomes for lung cancer patients.

#1860 Development of novel AXL inhibitor-loaded PLGA nanoparticles for targeted therapy in triple negative breast cancer.
Fatma Kazdal, Nermin Kahraman, Bulent Ozpolat

Nanomedicine, Houston Methodist Research Institute, Houston, TX

Triple Negative Breast Cancer (TNBC) is the most aggressive and heterogeneous subtype of breast cancer with the highest mortality rates. TNBC is characterized by a lack of Estrogen (ER), PR, and HER2 receptors and currently, there is no effective targeted therapy for TNBC patients. AXL, a receptor tyrosine kinase (AXL-RTK), is overexpressed in TNBC cells and patient tumors and has been linked to poor prognosis. Despite its potential as a therapeutic target, there are currently no FDA-approved inhibitors for AXL in TNBC. In this study, we aimed to develop AXL- inhibitor-based targeted therapy and evaluate its therapeutic potential for TNBC using a PLGA (poly-lactic-co-glycolic acid)) nanoparticle delivery system. We formulated it into PLGA nanoparticles to improve bioavailability and enable targeted delivery to TNBC tumors. Various dosages of AXL inhibitor-loaded PLGA nanoparticles were tested on MDA-MB-231 and MDA-MB-436 human TNBC cell lines, and significant inhibition of cell growth and colony formation was observed, with a half-maximal inhibitory concentration (IC50) of 5 μM. The size and morphology of the nanoparticles were characterized using Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM), which revealed nanoparticle sizes ranging from 63-99.9 nm. Western blot analysis showed that the AXL inhibitor-loaded PLGA nanoparticles effectively inhibited AXL protein expression and its downstream signaling pathways, leading to superior anti-proliferative effects compared to free AXL inhibitors. These results suggest that AXL inhibitor-loaded PLGA nanoparticles offer a promising strategy for enhancing the therapeutic efficacy of AXL inhibitors in TNBC. In vivo, efficacy studies are currently underway to evaluate their potential for targeted therapy in both primary and metastatic TNBC models.

#1861 A study of the effects of sodium alginate concentration on the physical properties of injectable alginate scaffolds for the treatment of glioblastoma.

Serenade N. Trevino[1], Dorina A. Madrid[1], Samantha Davila[2], Marco A. Arriaga[3], Sue Anne Chew[1]

[1]Health & Biomedical Sciences, University of Texas - Rio Grande Valley, Brownsville, TX,[2]UTRGV Mathematics and Science Academy, Brownsville, TX,[3]School of Medicine Division of Human Genetics, University of Texas - Rio Grande Valley, Brownsville, TX

Glioblastoma Multiforme (GBM) is a Grade IV malignant brain cancer with a high recurrence and low survival rate. Despite advances in various therapeutic methods, prognosis has not improved much, thus, alternative treatment methods are needed. Minocycline (MINO), an antibiotic, has been shown to have anti-angiogenic properties useful for GBM treatment. Alginate is a biodegradable polysaccharide that can be used for the fabrication of injectable hydrogels for drug delivery. The objective of this study was to investigate the effects of sodium alginate (SA) concentrations (i.e., 1.00, 1.50, and 2.00 wt./vol.%) on scaffold pH, gelation time, dimensions, weight, and cell viability. Scaffolds were fabricated by dissolving SA and calcium carbonate (CaCO3) in water, homogenizing with glucono-delta lactone (GDL), then injecting the solution into a 24-well plate to form uniform scaffolds. Scaffold properties were evaluated by determining pH values with a pH meter, gelation time using a tilt test with a digital timer, dimensions with a digital caliper and wet and dry weight (after lyophilization) using an analytical scale. To determine cell viability, U87 GBM cells were treated with 24 hr scaffold release solution, and an MTT assay was performed after 48 hrs. Scaffolds made with the different SA concentrations reached similar pH values after 60 min. (~6.0). They had workable gelation time (~15-36 min.) and were able to conform to the topography of the mold. The increase in SA concentration resulted in a decrease in gelation time and a significant increase in diameter, height, dry weight, and overall stability of the scaffold. The increase in SA polymer chains (with higher concentration of SA), allowed for more cross-linking which could have led to the sturdier scaffolds with a significant increase in scaffold height. As expected, as the concentration of SA increases, more components make up the scaffold structure, resulting in a significant increase in scaffold dry weight. The wet weights were not significantly different, probably due to the high-water content of the hydrogels masking the difference in the weight of the scaffold components. Increasing the concentration of SA had no significant effect on the cell viability of the blank scaffolds. The blank and minocycline-loaded scaffolds resulted in 65-70% and 40-45% cell viability, respectively. The drug-loaded scaffolds were able to enhance cell death compared to the control and blank scaffolds. In conclusion, the concentration of SA can be optimized to tune the physical properties of the scaffolds (which may lead to different scaffold degradation rates and drug release kinetics). The alginate scaffolds fabricated in this study may be a promising adjuvant therapy for GBM treatment and future studies will focus on evaluating the effects of the SA concentration on scaffold degradation rate and drug release kinetics.

#1862 Biomimetic poly lactic acid based nanoparticles for tumor targeted delivery of chemotherapeutic drugs.

Harshdeep Kaur, Harpal Singh

Center for biomedical engineering, Indian Institute of Technology Delhi (IIT Delhi), New Delhi, India

Global cancer burden is increasing day by day and to mitigate this situation, chemotherapy is widely used as systemic treatment modality. Along with many pros, chemotherapy does have various cons such as: systemic toxicity, faster degradation in physiological conditions hence low bioavailability and poor tumor accumulation. To overcome these shortcomings, nano-drug delivery systems are used. The nanoparticles can be tailor made according to physiological conditions of the tumor microenvironment. Herein, the redox responsive polymer has been synthesized by introducing the disulphide linkage in the polymer. The chemo-drug Pirarubicin has been encapsulated by double emulsion method in the nanoparticles prepared with redox-responsive polymer. The drug release profile indicated the release of the drug is more efficient in presence of 10mM Dithiothreitol (reducing agent for disulphide linkage) when compared to 0mM dithiothreitol, hence suggesting the successful synthesis of stimulus responsive drug delivery system. In this context, this study investigates the therapeutic efficacy of Pirarubicin encapsulated in polylactic acid based redox responsive nanoparticle(NP) whose surface have been modified with tumor and macrophage cell membranes. The coated cell membrane provides advantages such as immune escape, long blood circulation time, specific molecular recognition and tumor cell targeting. The prepared nanoparticles have a hydrodynamic size in nano-range with high negative zeta potential. The cellular internalization studies of free drug vs the cell membrane coated NPs (CMNPs) were done and it was confirmed with fluorescence microscopy as well as flow cytometry that CMNPs were internalized more efficiently in one hour drug incubation study. The same was confirmed when cancer cell proliferation inhibition assay was done, and half maximal inhibitory concentration was highest in case of uncoated NP, then that of free a drug, and lowest in case of CMNPs. This study provides the synthesis and characterization of redox responsive polymer, preparation of cell membrane coated nanoparticles and in-vitro studies for the same. This study has a future scope in the field of personalized medicine at much cheaper price when compared to active targeting methods by antibodies, CART, proteins etc.

#1863 A cytotoxic peptide designed for tumor-targeted delivery of co-injected molecules.

Norio Miyamura[1], Chisato M. Yamazaki[2], Kyoji Tsuchikama[2], Kazuki N. Sugahara[1]

[1]Department of Surgery, Columbia University, New York, NY,[2]McGovern Medical School, The University of Texas Health Center at Houston, Houston, TX

Clinical trials have highlighted its promise as a tumor-targeted delivery system and enhancer of anti-cancer therapies. In this study, we developed and analyzed an iRGD, tumor specific homing peptide, conjugate linked to monomethyl auristatin F (MMAF), a highly potent antimitotic agent. This conjugate demonstrated dual functionality as a tumor-targeted cytotoxic agent and a delivery platform for co-administered drugs. The iRGD-MMAF conjugate selectively internalized and killed tumor cells in vitro through αv integrin-dependent pathways. In vivo, systemic injection of iRGD-MMAF resulted in tumor-specific localization and widespread distribution within the extravascular tumor tissue, consistent with iRGD's tumor-penetrating properties. Additionally, iRGD-MMAF significantly improved tumor-specific uptake of a co-injected drug, confirming its efficacy as a delivery scaffold. These findings suggest that chemically modified iRGD peptides with added therapeutic properties maintain their ability to enhance co-administered drug delivery to tumors.

#1864 Folate-mediated delivery of tumor-suppressive miRNA-34a to treat osteosarcoma.

Iman T. Mevaa[1], Ahmed M. Abdelaal[1], Ikjot S. Sohal[1], Shreyas G. Iyer[1], Kasireddy Sudarshan[2], Harish Kothandaraman[3], Nadia A. Landman[4], Philip S. Low[2], Andrea L. Kasinski[1]

[1]Biological Sciences, Purdue University, West Lafayette, IN,[2]Chemistry, Purdue University, West Lafayette, IN,[3]Purdue University Institute of Cancer Research, West Lafayette, IN,[4]Comparative Pathobiology, Purdue University, West Lafayette, IN

Osteosarcoma is a common type of bone cancer primarily observed in children, teenagers, and young adults. Current treatments (including surgery, chemotherapy, and radiotherapy) are invasive and induce aggressive side effects. Alternative approaches such as using microRNAs (miRNA) are emerging as promising anticancer agents. miRNA modulates tumorigenesis via tumor-suppressive and oncogenic miRNAs. Tumor-suppressive and oncogenic miRNAs respectively downregulate oncogenic and tumor-suppressive mRNA transcripts. Hence, leveraging the anticancer activity of tumor-suppressive miRNAs appears as a viable anticancer treatment approach. The overarching challenges with miRNA therapy are 1) achieving specific non-toxic delivery of tumor-suppressive miRNA to cancer cells, and 2) enhancing the stability of the miRNA, which can be degraded by nucleases. To circumvent these obstacles, we developed a folate-conjugated fully modified miRNA-34a that specifically targets osteosarcoma cells, which often overexpress folate receptor-α on their surface. The fully modified miRNA-34a bears phosphorothioate linkages at the 3’ and 5’ ends of the sense and antisense strands which significantly reduces nucleases attack. The substitution of the 2’ hydroxyl with 2’ methoxy and 2’ fluoro groups in an alternative fashion further enhances stability. Overall, the fully modified miRNA-34a shows continuous downregulation of target genes 120 hours post-delivery in breast cancer in-vivo models. Here, we present preliminary results for folate-mediated delivery of fully modified miRNA-34a in osteosarcoma samples. This study highlights the potential of miRNA-34a as a safe and effective anticancer agent for osteosarcoma treatment.

#1865 Optimization of miRNA-6883 lipid nanoparticles for targeted CDK4/6 and HIF1a inhibition in colorectal and breast cancer cells.

Connor Purcell[1], Kadhambari Rajendran[2], Wafik S. El-Deiry[1], Theresa M. Raimondo[2]

[1]Legorreta Cancer Center at Brown University, Providence, RI,[2]School of Engineering, Brown University, Providence, RI

The design of delivery vehicles to protect microRNAs (miRNAs) from degradation, enable tissue-specific targeting, and avoid potential off-target effects is a central challenge for their therapeutic application. miRNA encapsulation by lipid nanoparticles (LNPs) has been used to extend in vivo half-life, and LNPs may also be conjugated to an antigen targeting moiety to guide delivery to tumors. In vitro studies have identified miR-6883 as a promising therapeutic agent which targets CDK4/6, induces apoptosis, and reduces HIF1α levels in colorectal and breast cancer. In the present study, we employ a partial factorial design of experiments (DOE) to determine the optimal molar ratios of LNP components for on-target tumor potency, and use the miRNA-LNPs to further explore the potential therapeutic application of miR-6883. Methods: LNPs were formulated with MC3 or Lipid 331, DOTAP, DSPC, cholesterol, and DMG-PEG2k. Aqueous and lipid phases were combined a 3:1 ratio, followed by rapid pipetting and overnight dialysis. Encapsulation and RNA concentration were determined by Ribogreen assay. Polydispersity index (PDI) and diameter were determined by dynamic light scattering. Cancer cell lines were reverse transfected with 50nM of LNP-encapsulated RNA (miR-6883 or control). Viability was determined via CellTiter-Glo assay and protein levels by western blot. Fluorescent imaging and flow cytometry were performed to evaluate uptake of fluorescently-labeled LNPs. Partial factorial DOE was used to survey a design space consisting of a 4-part mixture (ionizable lipid, phospholipid, cholesterol, and PEG molar ratios) and a binary categorical variable to include 5% cationic lipid (JMP software). Results: Initial dosing with fluorescently labeled LNPs showed a dose-dependent increase in fluorescence, at lipid doses as low as 1μg/mL. Following formation of 12 LNP formulations based on the DOE screening design, LNPs had a maximum RNA encapsulation of 94.32%, PDIs from 0.07-.279 and diameters from 98.85-240.3nm. Multiple formulations showed potent anticancer activity, with a maximal 93.52% reduction in viability observed in HCT116 after 72h of 50nM miR-6883 compared to an untreated control. Protein level modulation of CDK4 and CDK6 was observed in all LNPs tested, as well as a reduction of HIF1α after 4 days. Notably, multiple samples exhibited an *increase* in CDK6, warranting further investigation. Conclusions: Our DOE identified candidate miR-6883-LNP formulations for further optimization. Future work will assess the optimized miR-LNP therapeutic in murine models of colorectal and breast cancer. Currently, we are generating antibody-conjugated LNPs, with the aim of increasing potency and mitigating off target toxicity, and are continuing LNP optimization for in vivo studies.

#1866 EphA2 siRNA-loaded lipid nanoparticles decrease tumor burden in breast cancer liver metastases models by modulating the tumor microenvironment.

Anjana Tiwari[1], Eric Chau[1], Jenna Carr[2], LaMargaret Temukisa Johnson[3], Dylan Goodin[4], Hermann Frieboes[3], Biana Godin[1]

[1]Houston Methodist Research Institute, Houston, TX,[2]Texas A&M, College Station, TX,[3]Department of Bioengineering, University of Louisville, Louisville, KY,[4]Department of Bioengineering,, University of Louisville, Louisville, KY

Breast cancer is the leading cause of cancer-related mortality in women, with distant metastasis being the primary reason. The liver is the third most frequent site of breast cancer distant metastasis. Unlike primary tumors and metastatic lesions in other organs, breast cancer liver metastasis (BCLM) growth relies heavily on the organ vasculature, leading to lesions that are hypovascularized. This prevents systemically administered agents from efficiently reaching the cancer cells in these lesions, causing transport-related resistance to therapy. Macrophages are the most abundant immune cell type in the cancer tumor microenvironment (TME), playing important roles in tissue remodeling, homeostasis, angiogenesis, progression, and response to therapy. These phagocytic cells remain in close proximity to BCLM and have been clinically utilized for imaging with nanomaterials. Transport barriers can be overcome using macrophages as carriers for nanotherapeutics, leading to efficient therapy for BCLM. siRNA therapeutics are nucleic acid-based macromolecules that hold promise for tumor therapy. Challenges related to siRNA delivery and clinical translation include poor stability and limited transport across biological barriers, which necessitate using nanoparticles as carriers. Ephrin type-A receptor 2 (EphA2) tyrosine kinase regulates important tumor-driving processes and is overexpressed in breast cancer. In the present study, we designed, characterized and evaluated the effect of siRNA EphA2 loaded lipid nanoparticles (siEphA2-LNP) in combination with albumin bound paclitaxel (nAb-PTX) on macrophage phenotype, transport and anti-cancer efficacy in *in vitro* and *in vivo* BCLM models. The siEphA2-LNP and nAb-PTX treated macrophages shifted the TME towards the pro-inflammatory, anti-tumorigenic milieu by polarizing the macrophages from the M2 to M1 phenotype. This resulted in increased apoptosis and decreased proliferation in MCF7 and MDA-MB-231 human breast cancer cells in treated groups as compared to controls. Studies in the 3D *in vitro* model that mimics clinically observed hypovascularized BCLM surrounded by macrophages, suggested that a balance between M1 and M2 macrophage phenotypes in the TME is required to optimize nanotherapeutic efficiency. *In vivo* studies in a syngeneic model of primary triple negative breast cancer and experimental BCLM showed decreased tumor volume when treated with siEphA2-LNP. Additionally, there was an increase in CD163 (M2 marker), F4/80 (pan-macrophage) and Ki67 (apoptosis) in the lesions. These findings were further utilized to computationally simulate BCLM therapeutic response to siEphA2-LNP and evaluate clinically relevant dosing strategies. Altogether, this study shows that EphA2 siRNA-loaded lipid nanoparticles can decrease BCLM tumor burden by modulating the tumor microenvironment.

#1867 Development and validation of fluorescent lipid nanoparticles for RNA delivery applicable to personalized in vitro oncology models.

Xavier Warnet, Celia Bosso-Lefevre, Erica Cirri, Eric Mennesson, Marie Morin, Melanie Sapateiro, Maxime Rochet, Laetitia Edmond, Alexandra Foucher, Armelle Vindrios, Alzbeta Komarkova, Flavien Carpenier, Romain Cordonnier-Goulay

TebuBio, Le Perray-en-Yvelines, France

Tebubio offers contract services in RNA-based therapeutic discovery, in vitro modeling, biomarker mapping, and data analysis. Leveraging these platforms, we have developed innovative lipid nanoparticles (LNPs) designed to encapsulate and deliver RNA. This advancement features a fluorescent lipid, created by Echelon Inc., which enables imaging-based tracking of the LNPs. Using this fluorescent lipid, we formulated LNPs encapsulating a mRNA Coding for a fluorescent protein. We validated the distribution of these LNPs within 2D mammalian cell line model and confirmed RNA expression in these cells through imaging techniques. This technology is applicable to in vitro oncology models, including patient-derived spheroids and tumoroids, which allow for a personalized approach to therapeutic testing. Ultimately, Tebubio's platform provides end-to-end support, from RNA optimization and custom LNP formulation to efficacy validation in vitro, enabling personalized medicine models for RNA-based therapeutics.

CHEMISTRY: Proteomic Signatures in Cancer
Poster Session

#1872 MSH6 modulates PARP inhibitor sensitivity in BRCA-proficient high-grade serous ovarian cancer.
O. Deng, T. Nepomuceno, B. Fang, E. A. Welsh, R. H. Martin, E. M. George, A. N. Monteiro, U. Rix;
Moffitt Cancer Center, Tampa, FL

Ovarian cancer is the leading cause of gynecologic cancer-related deaths, with high-grade serous ovarian cancer (HGSOC) being the most common subtype. While BRCA1/2 deficiencies are established biomarkers to predict PARP inhibitor (PARPi) sensitivity, some HGSOC patients with BRCA-proficient tumors also respond to PARPi therapy. However, the mechanisms driving PARPi sensitivity in BRCA-proficient HGSOC remain poorly understood. We hypothesized that PARP1 protein complex composition and PARylation-mediated signaling regulate PARPi sensitivity or resistance in BRCA-proficient HGSOC. Through viability screening of BRCA-proficient ovarian cancer cell lines treated with PARPis we identified three sensitive and three resistant lines. Chemical proteomics with rucaparib showed enrichment of PARP1, PARP2, and associated binding proteins, with notably higher MSH6 levels in sensitive cell lines compared to resistant ones. Co-immunoprecipitation further revealed differences in PARP1-MSH6-PARP2 complex compositions between sensitive and resistant cells lines. Genetic knockout or knockdown of MSH6 via CRISPR and siRNA, respectively, conferred resistance to rucaparib, with a more pronounced effect in sensitive cells. To investigate downstream signaling, we performed ADP-ribosylation proteomics using clickable NAD+ analogs, uncovering distinct PARylation profiles between sensitive and resistant lines. CHAF1A, a known MSH6 binder and PARP1 substrate, was identified in PARPi-sensitive cells. More importantly, MSH6 knockdown elevated CHAF1A levels, while rucaparib treatment reduced CHAF1A expression, particularly in sensitive cells. These findings suggest that CHAF1A upregulation through MSH6 inhibition may rescue PARPi-induced cell death. This study integrates chemical proteomics and ADP-ribosylation analysis to delineate the interplay between PARP1 complex composition and signaling dynamics in PARPi response. Our findings highlight MSH6 and CHAF1A as critical modulators of PARPi sensitivity, providing new insights into resistance mechanisms and identifying potential biomarkers to improve PARPi efficacy in BRCA-proficient HGSOC.

#1873 Deep plasma proteome profiling to discover drug treatment related novel biomarkers in non-small cell lung cancer.

Cui Liu[1], Dongsheng Yue[2], Bin Zhang[2], Ruiqi Huang[3], Wenting Du[3], Xiaopeng Ma[1], Cunjing Yu[1], **Wenhao Li**[1], Xikun Wu[3], Yongchun Tong[1], Lanjun Xu[1], Si Mou[1], Changli Wang[2], Zhirong Shen[1]

[1]BeiGene (Beijing) Co., Ltd., Beijing, China,[2]Department of Lung Cancer, Tianjin Lung Cancer Center, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China,[3]BeiGene (Shanghai) Biotechnology Co., Ltd., Shanghai, China

Background: Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of various cancers. A better understanding of ICI induced dynamics and response / resistance mechanisms may contribute to the optimization of treatment strategies. In early-stage non-small cell lung cancer (NSCLC) cohort with neoadjuvant chemoimmunotherapy, we utilized the plasma proteomics platform to deepen the understanding of disease biology and explore potential response and resistance biomarkers. **Methods:** Plasma samples collected at various time points during neoadjuvant chemoimmunotherapy were analyzed using an unbiased mass spectrometry-based plasma proteomics platform. Taking advantage of the protein corona formed on the surface of nanoparticles, low-abundant proteins related to immunomodulation and signal transduction were enriched and identified. A comprehensive statistical analysis framework was adapted to reveal mechanism and explore predictive and prognostic biomarkers. Immunotherapy-induced changes of blood proteins were estimated by linear mixed model. Molecular insights were studied through mediation analysis. In the association analysis with clinical outcomes, classic logistic regression was applied for binary clinical outcomes, while cox proportional hazard model for survival outcomes. **Results:** 180 plasma samples from 92 individuals were profiled, resulting in the quantification of 2303 proteins in peripheral blood. The consistency between quantitative proteomics and established techniques were demonstrated by known biomarker CA125, CRP and gender related proteins. A total of 541 upregulated proteins and 504 downregulated proteins after neoadjuvant chemoimmunotherapy were identified. Pathway enrichment analysis revealed that neoadjuvant chemoimmunotherapy promoted immune activation. **Conclusion:** Deep plasma proteome profiling enables a deeper understanding of the dynamics and mechanisms induced by ICI treatment.

#1874 Glycoproteome and surfaceome mapping of epithelial-to-mesenchymal transition reveals key ion channel regulation and novel biomarkers in cancer metastasis.

Xing Xu, Kejun Yin, Ronghu Wu

Department of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA

The epithelial-to-mesenchymal transition (EMT) is a crucial process driving cancer metastasis. In this transition, cell morphologies and properties are dramatically changed, but the underlying molecular mechanisms remain to be explored. In this work, we performed a comprehensive analysis of the proteome, the glycoproteome, and the surfaceome during the EMT process induced in A549 lung carcinoma cells using mass spectrometry-based proteomics. We identified five distinct EMT states characterized by alterations in protein expression and N-glycosylation patterns. We characterized 8,891 proteins, including 1,366 N-glycoproteins and 635 surface glycoproteins, and further bioinformatic analyses provide valuable insights into the mechanisms of EMT. Notably, we observed significant regulation of sodium, chloride, and calcium ion channels during EMT, underscoring their importance in cancer metastasis. Surface glycoproteins such as MET and SLC2A1 emerged as potential biomarkers for partial EMT, paving the way for advancements in early cancer diagnosis and targeted therapies. Furthermore, our findings reveal that EMT-associated N-glycosylation occurs in specific protein domains and aggregation-prone regions (APRs), affecting protein stability and trafficking. This work advances our understanding of the molecular mechanisms of EMT and underscores the therapeutic potential of targeting glycoproteins and ion channels in metastatic cancer.

#1875 PBK expression promotes the aggressive phenotypes of mesothelioma.

Kazumi Hori[1], Ichidai Tanaka[1], Tatsuhiro Sato[2], Mika Sato[1], Yuta Kodama[1], Hideyuki Itoigawa[1], Yuichi Abe[3], Taketo Kato[4], Ayumu Taguchi[5], Mitsuo Sato[6], Yoshitaka Sekido[7], Toyofumi Fengshi Chen Yoshikawa[4], Makoto Ishii[1]

[1]Respiratory Medicine, Nagoya University, Nagoya, Japan,[2]Division of Cancer Biology, Aichi Cancer Center Research Institute, Nagoya, Japan,[3]Division of Molecular Diagnostics, Aichi Cancer Center Research Institute, Nagoya, Japan,[4]Thoracic Surgery, Nagoya University, Nagoya, Japan,[5]Molecular Diagnostics, Aichi Cancer Center Research Institute, Nagoya, Japan,[6]Integrated Health Sciences, Nagoya University, Nagoya, Japan,[7]Cancer Biology, Aichi Cancer Center Research Institute, Nagoya, Japan

Mesothelioma is one of the most aggressive neoplasms worldwide that has a particularly poor prognosis. Novel therapeutic strategies to effectively treat patients with mesothelioma are urgently needed. We have previously discovered that oxytocin receptors (OXTR) are highly expressed in mesothelioma and that *OXTR* knockdown significantly decreases the proliferation of mesothelioma cells with high *OXTR* expression by disturbing the tumor cell cycle but targeted therapies utilizing the OXTR signaling system have not emerged. In this study, we performed quantitative proteomic profiling of two mesothelioma cell lines with high *OXTR* expression using mass spectrometry to investigate the downstream signals of OXTR in mesothelioma cells. We found that *OXTR* knockdown significantly downregulated PDZ-binding kinase (PBK)—a serine/threonine protein kinase belonging to the bispecific MAPKK family which is a crucial factor in cell mitosis, cell proliferation, migration, and anti-apoptosis and support of testicular functions. *PBK* knockdown significantly suppressed proliferation, migration, and colony formation in mesothelioma cells with high *PBK* expression, and decreased Akt and MAPK phosphorylation levels. We also found that a PBK inhibitor, OTS-514, suppressed the proliferation of these cell lines at low concentration. Furthermore, immunohistochemical analysis of PBK in surgical specimens obtained from patients with mesothelioma showed that high PBK expression was strongly associated with poor overall survival (log-rank test *P*=0.0031; hazard ratio 3.339 and 95% confidence interval 1.12-10.00) and recurrence-free survival (log-rank test *P*=0.0024; hazard ratio 3.355 and 95% confidence interval 1.25-9.04). In addition, the clinical significance of PBK expression was validated in mesotheliomausing datasets from TCGA. Multivariable Cox regression analysis, incorporating stage and *OXTR* mRNA expression, demonstrated that *PBK* mRNA expression was the strongest independent predictor of overall survival and the hazard ratio for *PBK* was higher than that for *OXT*R. Our findings indicate that PBK plays a crucial role in the aggressiveness of mesothelioma, making it a promising therapeutic target and potential prognostic biomarker for mesothelioma.

#1876 Distinct kinase signatures define therapeutically relevant subtypes in colorectal cancer.

Gitanjali Dharmadhikari, Simar Pal Singh, Monique Mommersteeg, Liesbeth Houkes, Martijn Dankers

PamGene, 's-Hertogenbosch, Netherlands

Introduction: Colorectal cancer (CRC) is heterogenous disease and second leading cause of cancer-related mortality worldwide. Despite advances in understanding its pathophysiology, molecular mechanisms underlying this heterogeneity remain poorly understood. Identifying actionable molecular markers is crucial to improve patient stratification and therapeutic decision-making. Recent studies have highlighted the kinome—a dynamic network of kinase signaling pathways—as a promising avenue to uncover molecular signatures associated with cancer progression and treatment response. We hypothesized that profiling global kinase activity in CRC patient-derived xenografts (PDXs) could provide valuable insights into subtype-specific signaling alterations.
Methods: Kinase activity profiling was conducted using PamGene's KinomePro platform on 12 CRC-PDX models. Frozen tissue sample were lysed with M-PER Mammalian Extraction Buffer supplemented with Halt Phosphatase and Protease Inhibitor Cocktail and protein quantification was determined using Pierce Coomassie Plus (Bradford) Assay Kit. Tyrosine and Serine/threonine kinase activity profiles were generated using PamChip® comprising 340 different phosphosites that are the known downstream substrates. Peptide phosphorylation and Upstream Kinase Analysis was performed with PamGene's Tercen analysis pipeline.
Results: Kinase activity profiling revealed two distinct molecular subtypes of CRC based on EPH and SRC kinase activity. The EPH-high subgroup showed elevated JAK kinase activity, whereas EPH-low tumors exhibited heightened SRC kinase activity. Functional validation was conducted using dose-response assays with specific inhibitors: Ruxolitinib (JAK inhibitor) and Dasatinib (SRC inhibitor). These experiments demonstrated strong correlation between kinase activity status and inhibitor sensitivity, with the EPH/JAK-high group exhibiting a higher IC50 for Ruxolitinib compared to the low subgroup. To further elucidate signaling perturbations in these subtypes, we integrated transcriptomic data with kinase activity profiles. This in silico analysis confirmed their distinct RNA expression signatures, underscoring the heterogeneity in molecular signaling across the cohort.
Conclusions: This study highlights the utility of kinase activity profiling as a robust approach for molecular stratification of colorectal cancer, identifying EPH and SRC kinase activity as potential biomarkers. The validation of differential inhibitor sensitivity supports the potential clinical utility of these markers in guiding personalized therapies. By integrating kinome analysis with transcriptomics, we provide a comprehensive framework for understanding the molecular underpinnings of CRC heterogeneity. PamGene's KinomePro technology emerges as a promising tool for biomarker discovery and patient stratification in CRC.

#1877 Decoding cancer prevention: Exercise, dark proteome signatures, and spatial proteomics.

Parthiban Periasamy[1], Patrick Sitjar[2], Xinru Lim[1], Jiangfeng Ye[1], Meng Jia[1], Jorming Goh[2], Yap Yoon Sim[3], Darren Wan-Teck Lim Wan- Teck Lim[3], Roger Ho[4], Joe Yeong[1]

[1]Cancer Signalling & Therapy Division, A*STAR - Inst of Molecular and Cell Biology (IMCB), Singapore, Singapore,[2]Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore (NUS), Singapore, Singapore,[3]Medical Oncology, National Cancer Centre Singapore, Singapore, Singapore,[4]Department of Psychological Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore

With rising cancer rates and widespread physical inactivity—a known risk factor for non-communicable diseases like cancer—exercise is increasingly recognized for its potential in cancer prevention and management. Despite strong evidence supporting its benefits, exercise remains underutilized in clinical oncology. Physical activity may reduce cancer risk and progression through mechanisms such as hormone regulation, reduced inflammation, improved insulin sensitivity, antioxidant enhancement, immune support, and direct tumor effects. Studies suggest that regular exercise could lower breast, colon, and lung cancer incidence by up to 40%. Exercise is generally safe for cancer patients, enhancing physical function, mental health, and potentially slowing tumor progression. Given that one-third of adults globally are inactive, incorporating exercise into cancer care offers a holistic approach to improve patient outcomes alongside conventional treatments. In our study, we used mass spectrometry-based serum proteomics to investigate exercise's molecular effects on both the known and "Dark Matter" proteome—regions of proteins lacking structural characterization and potentially holding novel cancer biomarkers. We compared protein profiles between healthy individuals and pre-cancer patients, identifying early cancer signatures. Notably, Factor Xa (FA10), a vitamin K-dependent glycoprotein with pro-inflammatory effects, and fibulin-1 (FBLN1), a potential tumor suppressor involved in cell adhesion, were upregulated in pre-cancer patients. In an exercise intervention cohort, we observed that FA10 levels, initially elevated in pre-cancer patients, could be restored to baseline through exercise. This finding suggests that exercise may mitigate early cancer risk by reversing specific molecular changes associated with cancer progression. Ongoing work includes determining if unique proteins within the dark proteome can serve as distinctive cancer markers. We are currently performing spatial proteomics to determine the spatial distributions of selected dark proteome-derived proteins and to functionally characterize their roles in cancer prevention and progression.

#1878 Profiling protein-protein interactions to predict sensitivity to drugs targeting BCL2 family members in lymphoma models.

Alberto J. Arribas[1], Byungsan Choi[2], Elisa Civanelli[1], Eleonora Cannas[1], Camila Scalise[1], Saem Hong[2], Hongwon Lee[2], Francesco Bertoni[1]

[1]IOR - Institute of Oncology Research, Bellinzona, Switzerland,[2]PROTEINA Co., Ltd., Seoul, Korea, Republic of

Introduction: Pharmacological inhibition of anti-apoptotic proteins, especially with BCL2 inhibitors (i), has proven its clinical efficacy for treating patients with hematological cancers. However, there are not yet widely available tools to predict the sensitivity of individual patients to the different drugs targeting BCL2, MCL1, or BCLXL. A new technology, Single-molecule Protein Interaction Detection (SPID), based on immunoprecipitation of anti-apoptotic proteins followed by the addition of fluorescently labeled probes and detection at single-molecule accuracy, has shown promising results in predicting the sensitivity of acute myeloid leukemia cells to the BCL2i venetoclax (Chun et al., 2024). Here, we explored SPID to perform extended profiling of protein-protein interactions of BCL2, BIM, BAX, BAK, BCLXL and MCL1 in models derived from mantle cell lymphoma (MCL), marginal zone lymphoma (MZL) including models of secondary resistance to BTK, PI3K and BCL2i.
Methods: Cell viability was assessed by MTT assay after 3 days of exposure to increasing concentrations of drugs or DMSO. Sensitivity parameters included IC25, IC50, IC90, area under the curve, cell viability at 1μM of drug, or at the median of the concentration range used in the experiment. BCL2 family members' levels as single proteins or in complexes were measured by SPID. Drug sensitivity parameters were integrated with protein data using Spearman Correlation and Multiple Linear Regression (MLR, Lasso).
Results: We exposed MCL (n=10, JVM2, REC1, JEKO1, UPN1, SP49, SP53, MAVER1, GRANTA519, Z138, MINO), MZL (n=6, VL51, SSK41, Karpas1718, HC1, HAIRM, ESKOL) cell lines and MZL models of secondary resistance (MZL-RES) to idelalisib (n=2), copanlisib (n=2), ibrutinib (n=2), venetoclax (n=1) and copanlisib/venetoclax (n=2), to BCL2i (venetoclax, sonrotoclax), BCL2/BCXLi (navitoclax), and MCL1i (S63845). MCL models showed increased sensitivity to BCL2i, while MZL cells were more sensitive to MCL1i and BCLxLi. Elevated BCL2, BCL2-BAX, and, to a lesser extent, BCL2-BIM were associated with sensitivity to BCL2i, while high levels of MCL1-BIM, BCLxL and BCLxL-BIM were associated with BCL2i resistance. Expression of BCLxL, BCLxL-BAK and BCLxL-BIM predicted sensitivity to navitoclax, while resistance was observed with elevated MCL1-BIM. The response to the MCL1i did not correlate with MCL1 or MCL1-BIM levels, but an MLR model combining BCL2-BIM, BCLxL-BIM and MCL1 levels significantly enhanced the prediction of response.
Conclusions: Anti-apoptotic inhibitors show variable heterogeneous activity in MCL and MZL models, including those resistant to PI3Ki and BTKi, which could be predicted quantifying the BCL2-family members levels and interactions using the SPID. Such an approach might have a clinical potential to predict the sensitivity of patients with mature B cell lymphomas to this class of agents.

#1879 Serum biomarker candidates for predicting radiotherapy response in glioblastoma.

Paulo Roberto Del Valle, Janette Herr, Miah Elizabeth Nicolls, Kaylie Cullison, Alessandro Valderrama, Gregory A. Azzam, Jessica J. Meshman, Scott Welford, Macarena I. De La Fuente, Eric A. Mellon

Univ. of Miami Sylvester Comprehensive Cancer Ctr., Miami, FL

Introduction: Glioblastoma remains an incurable disease with limited treatment options. Imaging remains the primary diagnostic tool, but it has limitations in distinguish tumor progression from benign events like pseudoprogression. Currently, no biomarkers exist to differentiate these conditions. This study aimed to identify predictive biomarkers of glioblastoma progression by profiling serum proteins, seeking candidates for future validation.
Methods: Blood samples were collected from patients pre-therapy, and during the first and fourth weeks of chemoradiotherapy. We selected 11 samples for non-progression (NP), 12 for progression (P), and 6 for pseudoprogression (PS) based on availability and clinical classification. Proteomic analysis was performed using a proximity extension assay platform targeting more than 5400 serum proteins. Differential protein expression was assessed among groups and correlated with clinical outcomes.
Results: Pre-therapy analysis (NP vs P vs PS) identified 185 different proteins (Kruskal-Wallis, $p<0.05$). Enrichment analysis linked these to glutamatergic synapse, cell proliferation, and neuronal activity. Comparing responders (P) vs. non-responders (NP+PS) revealed 86 proteins (Wilcoxon, $p<0.05$), associated with interferon regulation and calcium homeostasis and clustered samples accordingly. In PS vs. NP+P, 87 proteins (Wilcoxon, $p<0.05$) were enriched for homologous recombination and neuronal apoptosis and clustered samples accordingly. At the first therapy week, NP vs. P vs. PS comparisons revealed 301 proteins (Kruskal-Wallis, $p<0.05$), linked to innate immunity and apoptosis. P vs. NP+PS revealed 95 proteins (Wilcoxon, $p<0.05$) associated with T cell activation. PS vs. NP+P identified 172 proteins that clustered samples accordingly (Wilcoxon, $p<0.05$), linked to receptor activity and carbohydrate binding. By week four, protein differences decreased. NP vs. P vs. PS comparisons identified 197 proteins (Kruskal-Wallis, $p<0.05$) related to nervous system development and wound healing. P vs. NP+PS identified 94 proteins (Wilcoxon, $p<0.05$) linked to cell adhesion. PS vs. NP+P revealed 75 proteins (Wilcoxon, $p<0.05$) that discriminated and clustered all PS samples on the same group and enriched for defense response and TRAIL production.
Conclusion: This study demonstrates that a sensitive proteomic approach can point to candidate biomarkers predictive of glioblastoma progression. These findings offer insights into radiotherapy response and warrant further mechanistic studies and validation in larger cohorts.

#1880 Advancing spatial discovery multiomics: Integration of a novel 1,000+ plex discovery proteome atlas with an 18,000+ plex whole transcriptome atlas for same-slide investigation of multiple cancer pathologies.

Ashley Heck, Hiromi Sato, Christine Kang, Brian Filanoski, Lori Hamanashi, Giang Ong, Terence Theisen, Erin Piazza, Kyla Teplitz, Margaret Hoang, Michael D. Rhodes, Joseph Beechem

Bruker Spatial Biology, Seattle, WA

Spatial multiomics at high plex represents a transformative approach to understanding complex biological systems. Whereas high plex spatial transcriptomics have transformed tissue analyses, spatial proteomics have been limited by low plex and lacking coverage of major biological pathways. Proteins, which represent the functional units of cellular response and activity, are essential for studying the heterogeneity of cancer and immune pathology. Furthermore, cellular responses to intrinsic and extrinsic stimuli are often driven by post-translational modifications of proteins. Enabling simultaneous analyses of proteins and RNA in FFPE tissue sections in spatial context with a sequencing readout and theoretically unlimited plex, the GeoMx® Digital Spatial Profiler (DSP) platform is uniquely positioned to support high plex proteomics and multiomics. In prior work, we demonstrated a same slide spatial multiomics workflow using the GeoMx DSP platform with the GeoMx Whole Transcriptome Atlas (18,000+ genes) and the GeoMx Human Immuno Oncology Proteome Atlas (570+ proteins). In the present study, we expand this platform by adding 500 additional protein targets, increasing the spatial proteomic atlas to over 1,000 targets. This expanded content allows for the discovery of novel biomarkers and potential therapeutic targets across multiple domains, including immune-oncology, immunology, oncology, neuroscience, signaling pathway regulation, cell death, metabolism, and epigenetics. We introduce the Discovery Proteome Atlas, a 1,000+ plex antibody-based proteomic assay and validate its performance in terms of specificity, sensitivity and reproducibility of each antibody in multiplex. When applied on the same-slide with the WTA assay to a tumor tissue microarray containing 50+ cases from various organs (e.g., skin, lung, testis, prostate, pancreas), we observe distinct multiomic profiles of cells with specific tumor regions and assess the abundance of multiple phosphorylated targets. Phosphorylation, like other post translational modifications, can only be studied through proteomics. By combining proteomic and transcriptomic atlases with spatial context in the same cell populations, this approach provides comprehensive insights into heterogenous tissues with complex disease mechanisms, offering a discovery portal for novel therapeutic or biomarker identification.
-Bruker Confidential-

#1881 Bimodality in pan-cancer proteomics reveals new biomarker discovery opportunities.

Wen Jiang[1], Damian Bikiel[2], Jan Zaucha[3], Jixin Wang[1], Sanddhya Jayabalan[4], Yongjin Li[4], Matthew Sung[4], James Harper[3], Jimmy Zhao[2], Krishna Bulusu[3], Wenyan Zhong[2]

[1]AstraZeneca US, Gaithersburg, MD,[2]AstraZeneca US, New York, NY,[3]AstraZeneca UK, Cambridge, United Kingdom,[4]AstraZeneca US, Waltham, MA

Bimodal protein expression, characterized by a protein distribution with two modes, is linked to phenotypic variation across various biological systems. While previous studies focused on RNA expression data, we developed a bimodality model tailored for proteomics to enhance the identification of cancer-associated biomarkers and targets, facilitating precision oncology. We analyzed proteomics data from various cancer types and identified 2,401 tumor-associated bimodal proteins. These tumor-associated bimodal proteins were evaluated for pathway enrichment, revealing significant associations with critical cancer pathways, such as metabolism of non-essential amino acids, interaction between the extracellular matrix and its receptors on the surface of cells and central carbon metabolism in cancer. Utilizing an AI-enhanced knowledge graph, we further delineated common patterns among pan-cancer tumor-associated bimodal proteins. A case study on the bimodal expression of TROP2 in colon adenocarcinoma highlighted up-regulation of MYC and WNT-beta-catenin signaling pathways and down-regulation of inflammatory and interferon-related pathways in the TROP2-high group. The biological difference between TROP2-high and TROP2-low groups underscored its significance in determining cancer severity and informing potential treatment approaches. Our findings show the utility of proteomics in uncovering novel biomarkers and advancing precision medicine, setting a precedent for further multi-omics integration and clinical validation.

#1882 Development of hit compounds targeting STAT3 for autoimmune diseases and cancers using a chemoproteomics-driven approach.

Heejung Yang[1], Hojin Yoo[1], Sang-Jun Han[2], Sijin Kim[2], Donggyun Hong[1], Jeongyeon Kang[1], Birang Jeong[3], Go-Yeon Jung[2], Namgil Lee[1], Hoguen Kwak[1], Byung-Seok Kim[2]

[1]Bionsight Inc., Chuncheon, Korea, Republic of,[2]Incheon National University, Incheon, Korea, Republic of,[3]Kangwon National University, Chuncheon, Korea, Republic of

Signal transducer and activator of transcription 3 (STAT3) is a critical regulator of immune responses, cellular proliferation, and survival, positioning it as a promising therapeutic target in autoimmune diseases and cancer. In this study, we present a chemoproteomics-based approach for the discovery and optimization of small-molecule inhibitors targeting STAT3. A series of novel small molecules capable of selectively modulating STAT3 activity were identified. By integrating structure-based design with our chemoproteomics platform, we not only validated the interactions of these compounds with STAT3 but also elucidated their downstream signaling effects in disease-relevant autoimmune models. Our chemoproteomics platform facilitates the unbiased identification of drug targets and off-target effects at a proteome-wide scale, significantly accelerating the lead optimization process. These findings highlight the potential of our STAT3 inhibitors as a novel class of therapeutics for autoimmune diseases. Ongoing studies are focused on optimizing these compounds for clinical trials by using the chemoproteomics platform to identify their molecular mechanisms of action and to predict potential off-target effects.

#1883 Large-scale proteomic analysis of glioblastoma tissues reveals novel molecular insights and potential therapeutic targets.

Juanjuan Wang[1], Simone Maarup[2], Vincent Fougner[2], Annelaura B. Nielsen[3], Ulrik Lassen[2], Luca Robinson[3], Frederik O. Bagger[4], **Dorte S. Noeroexe**[2]

[1]Center for protein research, Copenhagen University, Copenhagen, Denmark,[2]Department of oncology, Copenhagen University Hospital, Copenhagen, Denmark,[3]Center for genomic medicine, Copenhagen University Hospital, Copenhagen, Denmark,[4]Center for genomic medicine, Copenhagen University hospital, Copenhagen, Denmark

Introduction: Glioblastoma (GBM), classified as WHO grade 4, is a highly aggressive and incurable brain tumor with limited treatment options. Standard treatment consists of maximum safe surgery followed by concurrent chemo/radiation and adjuvant chemotherapy, the Stupp protocol. Despite extensive molecular characterization through DNA sequencing and RNA expression studies, the complex heterogeneity of GBM has yet to translate into improved standard treatments. A key challenge lies in predicting the impact of genetic and transcriptomic alterations at the protein level.
Aim: To identify promising protein biomarkers in GBM and trace these back to DNA and RNA data to determine whether they can be detected at earlier stages. We seek to uncover mechanisms of relapse and identify proteomic alterations that may be therapeutically targeted, integrating our findings with clinical data to discover potential prognostic and predictive biomarkers.
Experimental Procedures: We collected tumor tissue from 360 newly diagnosed GBM patients between 2016 and 2023, who provided informed consent for whole-exome/genome and RNA sequencing. In total, 514 protein samples were analyzed, including a subset of 58 samples from primary tumors and their matched first relapse. High-throughput proteomic analysis was performed using an Evosep system coupled with a high-sensitivity Orbitrap Astral mass spectrometer. Concurrently, we are analyzing the corresponding RNA and DNA data and plan to analyze 150 plasma samples from 50 of the included patients.
Results: Proteomics analysis of 514 GBM tissue samples identified 12,446 proteins, establishing this study as one of the most extensive proteomic investigations of GBM to date. Notably, O6-methylguanine-DNA methyltransferase (MGMT) exhibited significant regulation in MGMT-methylated patients. Furthermore, in patients with Neurofibromatosis type 1 (NF1) mutations, NF1 was significantly downregulated. Functional analysis revealed that proteins involved in glycosaminoglycan metabolism were upregulated in patients who responded to the STUPP protocol, suggesting key alterations in extracellular matrix composition and signaling pathways. Hedgehog signaling was markedly downregulated in relapse samples, a finding associated with increased cancer aggressiveness, poor prognosis, and elevated mortality. Additionally, the KRAS oncogenic signaling pathway was downregulated, pointing to potential changes in the tumor microenvironment and growth dynamics in relapsed GBM.
Conclusion: Our findings provide new insights into GBM proteomics and highlight potential therapeutic targets that could lead to more effective treatment strategies. By linking proteomic data to genetic and transcriptomic profiles, this study offers a valuable resource for improving patient stratification and developing targeted therapies for GBM.

#1884 Pancreatic cyst fluid proteins distinguish dysplasia grade of intraductal papillary mucinous neoplasm.

Chunliang Liu[1], Amber Mosley[1], Ehsan Irajizad[2], Michele Yip-Schneider[1], Huangbing Wu[1], Whitney R. Smith-Kinnaman[1], Thoa Tran[1], James P. Long[2], Kim-Anh Do[2], Johannes Fahrmann[2], John M. DeWitt[1], Samir Hanash[2], C. Max Schmidt[1], **Jianjun Zhang**[1]

[1]Indiana University, Indianapolis, IN,[2]The University of Texas MD Anderson Cancer Center, Houston, TX

Pancreatic cancer is the third leading cause of cancer-related death and is projected to be the second by 2030 in the US. The 5-year relative survival rate of pancreatic cancer is only 12.8% as over 80% of patients have a late diagnosis. To improve survival, it is vital to detect early-stage pancreatic cancer and its precursors. One such precursor is intraductal papillary mucinous neoplasm (IPMN), the most common type of pancreatic cyst often incidentally detected in asymptomatic patients. The current consensus guidelines, largely based on imaging features, have high sensitivity but low specificity in differentiating benign from malignant IPMNs, leading to widespread overtreatment of presumed “high-risk” cysts that have a low risk of malignancy on surgical pathology. Therefore, it is highly warranted to discover and validate biomarkers for improving the preoperative risk stratification of IPMN. Pancreatic cyst fluid is proximal to the tumor microenvironment and may thus harbor enhanced tumor-derived predictive biomarkers. Pancreatic cyst fluid samples were obtained from patients with pathologically confirmed low-grade (n=73) or high-grade/invasive (n=18) IPMN resected at Indiana University from 1992 to 2019. Global proteome quantitation was performed using liquid chromatography-tandem mass spectrometry. Differentially expressed proteins (DEPs) between the two groups were analyzed using Wilcoxon rank-sum test. We discovered 152 upregulated and 74 downregulated DEPs by comparing low-grade IPMN with high-grade/invasive IPMN (all $p < 0.05$). The enriched upstream regulators of these DEPs included let-7, miR-122, IL15, and FLT1 ($p = 2.45 \times 10^{-5}$ - 5.97×10^{-3}). Five discriminatory biomarkers with the largest LASSO coefficients and each with the area under the receiver operating characteristic curve (AUCs) of >0.75 (FAHD2A, TCEAL3, TWF1, MMUT, and NTPCR) were identified. The combined five-protein model achieved an AUC of 0.96 (95% CI: 0.92, 1.00). The AUCs of cyst fluid and serum CA19-9 measured in the same patients (Spearman's rank correlation coefficient: 0.65) were 0.67 and 0.70, respectively, both of which were smaller than the AUCs of each of the top five proteins identified. Combining the five best-performing proteins with cyst fluid or serum CA19-9 yielded a near complete separation of malignant from benign IPMN (AUC: 0.981 for the cyst fluid panel and 0.997 for the serum panel). A combined analysis of TCGA and GTEx databases revealed TWF1 overexpression in pancreatic cancer ($p < 0.0001$) that was associated with poor prognosis ($p = 0.0029$). In conclusion, the present study identified a panel of cyst fluid proteins (particularly TWF1) that are predictive of malignant pancreatic cysts. If validated in other patient populations, these biomarkers could increase the accuracy of the preoperative detection of high-risk IPMN and thereby improve patient outcomes.

#1885 Identification of the novel immune synapse-localized proteome for immuno-oncology using Microscoop®-induced targeted photo-biotinylation.

Chantal Hoi Yin Cheung[1], **Weng Man Chong**[1], Chun-Kai Huang[1], Hsiao-Jen Chang[1], Chia-Wen Chung[1], Jung-Chi Liao[1], Joseph Breier[2]

[1]Syncell Inc., Taipei City, Taiwan,[2]Syncell Inc., Watertown, MA

Although numerous immune synapse (IS) protein species have been identified, many IS-localized protein species remain unknown. Understanding the proteome of the IS between a target cell and a lymphocyte is crucial for advancing immuno-oncology. However, the low abundance of ISs and the absence of a definitive enrichment marker have hindered efficient proteomic profiling. In this study, we utilized Microscoop®, an innovative system that integrates microscopy, machine learning, and photochemical labeling to enable precise and spatially specific enrichment of IS proteins, thereby facilitating proteomic discovery for the IS. We employed Raji B cells as antigen-presenting cells (APCs) and induced IS formation with Jurkat T cells. The system first employed immunofluorescence imaging of CD3, a common IS marker in Jurkat T cells, and utilized a convolutional neural network-based deep learning algorithm to recognize IS formation from CMTPX-stained Raji B cells. Our automated system successfully achieved spatially targeted biotin-tagging of proteins at ISs through multiple rounds of imaging, deep learning-driven pattern generation, and photochemical labeling. Subsequent streptavidin pull-down and mass spectrometry analysis enabled the identification of IS-specific proteins. Remarkably, our spatial proteomic approach led to the isolation and identification of hundreds of different species at the IS interface, including proteins associated with key components of T-cell receptor (TCR) signaling pathways such as the TCR/CD3 complex, Src and Tec family tyrosine kinases, and pivotal NF-kB signaling proteins. Additionally, we identified a significant enrichment of proteins not previously associated with the IS. Our study not only illuminates previously unknown aspects of immune regulation at the IS interface but also has significant implications for cancer research, particularly in understanding and manipulating immune responses for therapeutic purposes.

#1886 Proteogenomic insights into chemotherapy resistance and therapeutic strategies in triple-negative breast cancer.

Dong Ki Lee[1], Min Hwan Kim[2], Yumi Hwang[3], Seul-Gi Kim[2], Gun Min Kim[2], Kyung-Hee Kim[4], Jong Bae Park[4], Min Jung Kim[5], Jee Ye Kim[6], Hyung Seok Park[6], Seho Park[6], Seung Il Kim[6], Heon Yung Gee[1], Joohyuk Sohn[2]

[1]Department of Pharmacology, Yonsei University College of Medicine, Seoul, Korea, Republic of,[2]Division of Medical Oncology, Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea, Republic of,[3]Avison Biomedical Research Center, Yonsei University College of Medicine, Seoul, Korea, Republic of,[4]Department of Cancer Biomedical Science, Graduate School of Cancer Science and Policy, National Cancer Center, Goyang, Korea, Republic of,[5]Department of Radiology, Yonsei University College of Medicine, Seoul, Korea, Republic of,[6]Division of Breast Surgery, Department of Surgery, Yonsei University College of Medicine, Seoul, Korea, Republic of

Background: Triple-negative breast cancer (TNBC) is an aggressive subtype associated with high mortality and frequent chemotherapy resistance, necessitating the identification of biomarkers to predict treatment outcomes and elucidate resistance mechanisms. To address this challenge, we conducted a comprehensive proteogenomic analysis to uncover molecular determinants of chemotherapy response in TNBC.
Methods: Pretreatment tumor samples from stage II or III TNBC patients (n=50) treated with adriamycin, cyclophosphamide, and taxane-based neoadjuvant chemotherapy, along with post-treatment samples from patients (n=31) who did not achieve a pathologic complete response (pCR), were analyzed. Multiomics integration of exome sequencing, RNA-seq, and TMT-based proteomics and phosphoproteomics identified key pathways and molecular subtypes.
Results: Non-negative matrix factorization (NMF) clustering identified five molecular subtypes: “immune-enriched” (n=9), “xenobiotic metabolism” (n=2), “epithelial-mesenchymal transition (EMT)” (n=7), “Myc target” (n=12), and “myogenesis” (n=11). The “immune-enriched” subtype exhibited the highest pCR rate (5/9, 55.6%), whereas no pCRs were observed in the “xenobiotic metabolism” or “EMT” subtypes. Gene set enrichment analysis (GSEA) revealed immune-related pathways enriched in the pCR group across RNA and protein data, while EMT pathways were predominant in the non-pCR group. Notably, the estrogen response pathway was enriched in the non-pCR group exclusively in the protein GSEA data, emphasizing the greater clinical relevance of proteomics over transcriptomics in this context. Differentially expressed genes in the immunomodulatory subtype, based on the Lehmann classification, demonstrated upregulation of doxorubicin metabolism (*ARK1C2*) and efflux (*ABCA13*) genes in non-pCR patients, contributing to chemotherapy resistance. Cytoband GSEA identified ITGB8, a membrane protein on chromosome 7p21, whose loss correlated with a significantly higher pCR rate (48.3% vs. 14.3%, p = 0.031). Phosphoproteomics-based PTM-SEA highlighted the GRK2 pathway as enriched in non-pCR tumors and tumors without ITGB8 loss, suggesting its role as a resistance mechanism. In vitro experiments confirmed that GRK2 silencing enhanced the efficacy of taxane-based chemotherapy. Comparison of pre-treatment and post-treatment tumor phosphoproteomes unveiled enrichment of the AURKB pathway in post-treatment samples, with functional validation demonstrating a synergistic effect of AURKB inhibition with paclitaxel, suggesting a promising combination therapy.
Conclusion: This integrative proteogenomic analysis identifies proteomic estrogen signature, ITGB8, GRK2, and AURKB as promising biomarkers and therapeutic targets, providing new insights into chemotherapy resistance and response in TNBC.

#1887 Proteomics unveil characteristic proteins of patients with benign and malignant pulmonary nodules.

Ximin Gao, Zhangyan Lyu, Guojin Si, **Fengju Song**

Tianjin Medical Univ. Cancer Inst. & Hospital, Tianjin, China

Background: LDCT screening can significantly lower the mortality rate of lung cancer among high-risk individuals. Nevertheless, the limitations of CT might lead to frequent follow-up examinations and false positive outcomes, thereby causing unnecessary interventions and overtreatment. Therefore, the development of reliable and convenient biomarkers to accurately differentiate between benign and malignant nodules and to assess the likelihood of cancerous transformation is essential. We attempted to provide meaningful biomarkers based on plasma proteomic studies.

Methods: The participants in this study were chosen from individuals aged 40 to 74 years in the Chinese Colorectal, Breast, Lung, Liver, And Stomach Cancer Screening Trial (C-BLAST). We selected 10 patients with malignant lung nodules and 10 with benign lung nodules, matched for age and sex. Malignant lung nodules were defined as those with a LUNG-RADS diagnostic category of 4A, 4B, or 4X, accompanied by a biopsy confirming malignancy; benign nodules were those with a diagnostic category not exceeding 3. Plasma samples from two groups were collected and subjected to proteomic analysis using the Somascan Assay 11k detection platform. Paired t-tests were employed to identify the differential proteins between malignant and benign pulmonary nodules. The functional pathways enriched by these proteins were determined based on Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. Finally, the STRING was utilized to construct a protein-protein interaction network (PPI) and determine the key proteins related to malignant nodules.

Results: The average age of both groups was 61.4 years. A comparison of the proteomics between the malignant pulmonary nodule group and the benign pulmonary nodule group identified 188 differentially expressed proteins ($P < 0.05$), among which 102 were up-regulated proteins and 86 were down-regulated proteins. GO analysis of the differential proteins indicated functional enrichment in pathways such as chemical carcinogenesis, fluid shear stress and atherosclerosis, and biosynthesis of cofactors. According to KEGG analysis, they were mainly enriched in pathways like chemical carcinogenesis-reactive oxygen species, fluid shear stress and atherosclerosis, and metabolism of xenobiotics by cytochrome P450. Through PPI analysis, ten key proteins were determined, including CRP, FCGR3B, CCL2, CYP3A5, GSTA3, GSTM1, GSTM3, GSTM5, CD163, and GSTM4. These molecules possess anti-atherosclerotic and anti-inflammatory activities, as well as chemotactic activity for monocytes and basophils, and play roles in hydrolyzing nucleotides and host defense.

Conclusions: Our research results provide ten potential plasma protein biomarkers for the discrimination of benign and malignant pulmonary nodules, which might broaden our understanding of their characteristics.

#1889 Proteomic profiling using high-dimensional multiplex platforms to identify prospective risk factors for lymphoid malignancies across multiple cohorts.

Pieter Martijn Kolijn[1], Karl Smith-Byrne[2], Vivian Viallon[3], Matthew Lee[4], Keren Papier[2], Anton W. Langerak[5], Florentin Spath[6], Arjan Diepstra[7], Raul Zamora-Ros[8], Alessandra Macciotta[9], Amaia Aizpurua[10], Rosario Tumino[11], Ruth C. Travis[2], Elio Riboli[12], Marc J. Gunter[12], James McKay[13], **Roel C. H. Vermeulen**[1]

[1]Population Health Sciences, Utrecht University, Utrecht, Netherlands,[2]University of Oxford, Oxford, United Kingdom,[3]Population Health Sciences, International Agency for Research on Cancer, Lyon, France,[4]University of Bristol, Bristol, United Kingdom,[5]Erasmus MC, Rotterdam, Netherlands,[6]Umea University, Umea, Sweden,[7]University of Groningen, Groningen, Netherlands,[8]Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain,[9]University of Turin, Turin, Italy,[10]Biodonostia Health Research Institute, San Sebastian, Spain,[11]Iblea Association - Epidemiological Research (AIRE-ONLUS), Ragusa, Italy,[12]Imperial College London, London, United Kingdom,[13]International Agency for Research on Cancer (IARC), Lyon, France

Introduction: Circulating proteins play a central role in the development of lymphoid malignancies and the immune system's response to these cancers.
Methods: In this study, we measured 6,412 unique proteins in prediagnostic plasma samples using the SomaScan 7K panel from a total of 4,565 participants of a case-cohort study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC)—484 who later developed a lymphoid malignancy (median follow-up 9 years) and 4,081 who did not. We conducted subtype-specific analyses for non-Hodgkin lymphoma (NHL, n=348), chronic lymphocytic leukemia (CLL, n=80), multiple myeloma (MM, n=116), diffuse large B-cell lymphoma (DLBCL, n=80), follicular lymphoma (FL, n=51) and Hodgkin lymphoma (HL, n=20) using Prentice-weighted Cox regression with Benjamini-Hochberg correction for multiple testing.
Results: We observed significantly altered plasma levels prior to NHL diagnosis for 147 unique proteins (161 aptamers), of which 131 were upregulated and 16 were downregulated. Increased plasma levels of members of several major immunomodulatory protein families, including the FC-receptor family and the semaphorin family, were associated with increased risk of NHL. Alongside established biomarkers such as sCD23 for CLL, CXCL13 for DLBCL, and sBCMA for MM, we identified potent novel markers, including FCMR and FDCSP for NHL. Time-stratified analyses revealed that a subset of these protein-lymphoma associations are evident over a decade before diagnosis, underscoring the protracted preclinical phase of lymphoid malignancies. Pathway analyses revealed dysregulation in immune modulation, epigenetic regulation, cytokine and chemokine signaling, B-cell receptor signaling, the NF-κB signaling pathway, alternative splicing and N-glycan biosynthesis. In total, 2136 out of 6412 proteins measured in our current study overlapped with those measured in a recent UK biobank study. The significant protein-cancer associations observed in our current analyses within EPIC showed high concordance in the UK biobank in terms of both FDR-adjusted significance (58%-82%) and the direction of the association (89%-92%), meaning positively or negatively associated with cancer risk.
Conclusion: In conclusion, we identified hundreds of proteins associated with lymphoid malignancy risk, revealing a robust set of markers detectable many years prior to diagnosis, and advancing our understanding of lymphoid malignancy pathogenesis.

#1890 Proteomic analysis of colorectal cancer risk across two prospective cohorts.

Matthew A. Lee[1], Vivian Viallon[1], David C. Muller[2], Ziqiao Wang[3], Paula Jakszyn[4], Pietro Ferrari[1], Giovanna Masala[5], Domenico Pali[5], Salvatore Panico[6], Carlotta Sacerdote[7], Karl Smith-Byrne[8], Ruth C. Travis[8], Rosario Tumino[9], P. Martijn Kolijn[10], Roel Vermeulen[11], Monique Verschuren[11], Nicholas Wareham[12], Elizabeth A. Platz[3], Nilanjan Chatterjee[3], Elio Riboli[2], Marc J. Gunter[13]

[1]International Agency for Research on Cancer, Lyon, France,[2]Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, United Kingdom,[3]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD,[4]Cancer and Nutrition Unit, Cancer Epidemiology Research Program, Catalan Institute of Oncology-IDIBELL, Bareclona, Spain,[5]Institute for Cancer Research, Prevention and Clinical Network, Fiorentina, Italy,[6]University of Naples Federico II, Naples, Italy,[7]University of Turin and CPO Piemonte, Turin, Italy,[8]Nuffield Department of Population Health, University of Oxford, Oxford, United Kingdom,[9]Provincial Health Authority (ASP), Ragusa, Italy,[10]Erasmus MC, Rotterdam, Netherlands,[11]UMC Utrecht, Utrecht, Netherlands,[12]University of Cambridge, Cambridge, United Kingdom,[13]Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, UK, London, United Kingdom

High-throughput proteomic profiling in prospective cohorts has the potential to uncover biomarkers of cancer risk and biology of cancer development. We investigated prospective associations of up-to 7,335 aptamers with overall and site-specific colorectal cancer in a case-cohort study within the European Prospective Investigation into Cancer and Nutrition (EPIC) and then evaluated associations in the Atherosclerosis Risk in Communities (ARIC) study.

Hazard ratios and 95% confidence intervals were estimated using Prentice weighted Cox-proportional hazard models (stratified by recruitment center, sex, and 5 year age group and adjusted for fasting status, day of blood draw, body mass index, smoking status, daily alcohol consumption, physical activity level, and highest level of education) in 977 incident colorectal (colon, 658; rectum, 319) cancer cases and 5,057 non-cases under a case-cohort design. The identified associations were then evaluated in ARIC in a cohort design including 271 colorectal (colon, 235; rectum, 36) cancers in 9,495 individuals using multivariate Cox-proportional hazard models.

Overall, 37 aptamers were associated (false discovery rate[FDR] p-value < 0.05) with colorectal cancer in EPIC; data on 27 of these were available in ARIC, 7 of which had consistent directions of association in both studies and met a nominal p-value (0.05) threshold in ARIC (positive associations: ACAA1, ASL, GDF15, IQCF1, TFF3; inverse associations: CLEC3B, COMP). Associations did not change after 5-year follow-up exclusion. In site-specific analyses in EPIC, 66 and 6 aptamers were specifically associated with colon and rectal cancer, respectively at FDR<0.05. Four of these aptamers were nominally (P<0.05) associated with colon cancer in ARIC (inverse association: CLEC3B; positive association: ACAA1, GDF15, TFF3) while a further 29 showed associations directionally consistent with those in EPIC.

These large-scale proteomic analyses identified a set of proteins associated with colorectal cancer risk across two independent cohorts. These proteins reflect pathways involved in cell migration and adhesion, colorectal mucosal integrity and growth and differentiation, among others, and may represent novel biomarkers of colorectal cancer risk.

#1891 Identification of highly accurate early-stage specific NSCLC biomarkers from extracellular vesicles identifies immune response and metabolic biomarkers associated with cancer.

Zack Opheim[1], Cheryl Bandoski[1], Poorva Mudgal[1], Valesca Anschau[2], Issa Isaac[2], Martin Mehnert[2], Alan Ezrin[1], **Todd A. Hembrough**[1]

[1]Nexosome Oncology, Durham, NC,[2]Biognosys, Schleiren, Switzerland

Lung cancer is one of the leading causes of cancer-related deaths worldwide, with non-small cell lung cancer (NSCLC) accounting for approximately 85% of cases. Early-stage lung cancer is often asymptomatic, leading to delayed diagnosis. Challenges in early detection underscore the need for more reliable, non-invasive diagnostic biomarkers. Plasma extracellular vesicles (EVs) represent a promising target for tumor detection and monitoring as they provide an abundant, stable, an easily accessible source of biomarkers. The purpose of this study is to utilize EVs to identify novel cancer related biomarkers and translate these findings into a clinical/immunoassay. To identify novel biomarkers, we enriched EVs using size exclusion chromatography and a proprietary buffer system. TrueDiscovery™ Data-independent acquisition (DIA) mass spectrometry (MS) analysis was conducted on EVs from 74 NSCLC patients (IA/B (32), IIA/B (8); III (17); IV (17)) and 50 normal plasma samples. An in-house machine learning (ML) pipeline was developed to identify differentially expressed proteins and model candidate multiplexes to identify those with extremely high diagnostic accuracy (> 0.98). Additionally, candidate multiplex targets were tested by ELISA to assess mass spectrometry translation to a clinical platform.

Comparing tumor vs normal samples (avg=2735 protein IDs) using our ML pipeline we identified 595 Pan Lung cancer differentially expressed proteins (absolute Log2FC>0.5; FDR adjusted p-value <0.01; AUC >0.5). When individual stages were compared to control plasma, a large number of differentially expressed proteins were also seen: Stage I – 549, Stage II – 120, Stage III – 355, and Stage IV – 373 proteins (absolute Log2FC>0.5; FDR adjusted p-value <0.01; AUC >0.5). Significant proteomic differences were also seen between early-stage and late-stage NSCLC, suggesting Stage specific biomarkers may be necessary for maximal diagnostic accuracy. Using the best individual biomarkers, we used Support Vector Machine to test all possible 3-Plexes and identify 3-Plexes with nearly perfect accuracy (>0.99) for each NSCLC Stage: Stage I (140 plexes), Stage II (92), Stage III (67), and Stage IV (50). Early-stage 3-Plexes consist largely of immune, inflammatory, and metabolic pathway proteins. Differential expression of key biomarkers was confirmed using ELISAs, and the performance of multiple highly accurate 3-Plexes is currently being assessed by ELISA on independent intended use case/control cohorts, for potential clinical development.

The potential of these highly accurate 3-Plexes to detect even the smallest Stage Ia1 tumors suggests that multiplexed proteomics of EV-associated biomarkers may be an extremely sensitive and specific platform for detection of very small early-stage and recurrent tumors.

#1892 Proteomic profiling identifies and validates a prognostic survival biomarker and pathways associated with novel subtypes of esophogeal adenocarcinoma.

Adel T. Aref[1], Daniel Bucio-Noble[1], Ahilya Singh[1], Jerresa Jabson[1], Myra Cheung[1], George Craft[1], Daniella Jaber[1], Rebecca Poulos[1], Erin K. Sykes[1], Jennifer M. Koh[1], Erin M. Humphries[1], Dylan Xavier[1], Peter G. Hains[1], Phillip J. Robinson[1], Thejaani Udumanne[2], Sarah J. Lord[2], Yi Jin Liew[3], Jason Ross[3], Dan Falkenback[4], Jan Johansson[4], Duncan McLeod[5], David Whiteman[6], Reginald V. Lord[2], **Roger R. Reddel**[1]

[1]ProCan, Children's Medical Research Institute (CMRI), Sydney, Australia,[2]St. Vincent's Centre for Applied Medical Research, Sydney, Australia,[3]CSIRO Health and Biosecurity, Sydney, Australia,[4]Lund University, Lund, Sweden,[5]Institute of Clinical Pathology and Medical Research, Sydney, Australia,[6]QIMR Berghofer Medical Research Institute, QLD, Australia

Introduction: Esophageal adenocarcinoma (EAC) is an aggressive malignancy with poor survival rates. Current management relies on tumor stage and grade, with neoadjuvant therapy (NT) showing improved outcomes over surgery alone. However, robust biomarkers are still required to improve staging and patient selection for therapy, including NT.

Methods: A training cohort of fresh frozen samples from 64 patients with EAC, not treated with NT, was analyzed by data-independent acquisition mass spectrometry. Formalin-fixed paraffin-embedded samples from an independent cohort of 188 patients with EAC treated with NT were used as validation. Unsupervised consensus clustering (CC) was performed followed by differential abundance (DA) analyses (DAA) and pathway enrichment analyses (PEA) to identify the DA proteins and enriched pathways in each cluster. Survival analyses using Cox regression were performed to assess the prognostic power of the proteomic clusters for overall survival (OS). Finally, a prognostic signature for OS was built on the training cohort using multivariable Cox regression analyses with LASSO regularization followed by StepAIC to select the most prognostic proteomic combination. The predictive power of the proteomic signature was tested using the Area Under the Receiver Operating Characteristic (AUROC) analysis and via inclusion in a nomogram with other prognostic variables.

Results: Proteomic analyses identified 8,730 and 5,214 proteins in the training and validation cohorts, respectively. The CC analyses revealed five clusters in the training cohort. Clusters 2 and 3 showed shorter OS compared to the other clusters ($p=0.0025$). Independent DAA and PEA revealed upregulation in extracellular matrix-related pathways in the poor prognostic clusters within both cohorts. A five-protein signature was developed in the training cohort and was associated with OS ($p<0.001$). This signature was independent of other prognostic variables, including N stage, T stage, grade, and age. The five-protein signature was also associated with higher odds of having positive nodes at the time of resection (Odd Ratio 6.3, $p=0.009$). The proteomic signature demonstrated an AUROC of 0.81 and was the most prognostic variable in a nomogram for survival risk. Finally, the proteomic signature maintained its prognostic power in the validation cohort ($p=0.016$) and was independent of other clinical variables, showing the highest prognostic value in the nomogram.

Conclusion: Using two independent cohorts of EAC surgical samples, we were able to identify a prognostic proteomic-based classification and develop a proteomic-based signature that was prognostic for OS and independent of other clinical variables. This signature may be investigated further as a criterion to tailor treatment and select patients who can benefit from treatment de-escalation.

#1893 Proteomic profiling of advanced hepatocellular carcinoma identifies predictive signatures of response to treatments.

Adele Delamarre[1], Marie Decraecker[1], Sylvaine Di-Tommaso[2], Cyril Dourthe[1], Jean-William Dupuy[3], Melanie Moreau[1], Nathalie Allain[1], Isabelle Mahouche[1], Julie Giraud[1], Giovanni Benard[4], Claude Lalou[5], Paulette Bioulac-Sage[1], Jerome Boursier[6], Jean-Frederic Blanc[7], Brigitte LeBail[1], Anne-Aurelie Raymond[1], **Frederic Saltel**[1]

[1]U1312 (BoRdeaux Insitute of onCology), Inserm, Bordeaux, France,[2]TBMcore, Inserm, Bordeaux, France,[3]TBMcore, Bordeaux University, Bordeaux, France,[4]Rare Diseases Laboratory: Genetics and Metabolism, Inserm U1211, Inserm, Bordeaux, France,[5]U1211 MRGM, Inserm, Bordeaux, France,[6]Department of Hepatology and Oncology,, Angers University Hospital, Bordeaux, France,[7]CHU Bordeaux, Bordeaux, France

Background and Aims: Hepatocellular carcinoma (HCC) is the most common form of liver cancer but, due to late diagnosis, the prognosis is extremely poor and the majority of cases are advanced HCC, which are only eligible for palliative systemic therapies. After a decade of exclusive sorafenib monotherapy, with a response rate of < 10%, the advent of new combinations including immunotherapies represents a revolution in the management of HCC. The combination of atezolizumab/bevacizumab is recommended as the first-line systemic treatment for HCC, with a response rate of no more than 30%. Additionally, less than 25% of non-responding patients are eligible for second-line treatment. However, there are currently no predictive factors for response to these different treatment options.
Method: We profiled, by high-resolution mass spectrometry-based proteomics combined with machine learning analysis, a selected cohort of formalin-fixed, paraffin-embedded biopsies of advanced HCC adenomas with clinical follow-up collected from several French hospitals. We grouped subject according to their objective response to treatments, corresponded to a tumor regression > 30% (G1) and tumor progression > 20% (G2) at 6 months after the beginning for atezolizumab/bevacizumab and sorafenib treatments.
Results: We generated a proteome database of 50 selected HCC samples. We compared the relative protein abundance between tumoral and non-tumoral liver tissues from advanced HCC patients treated with atezolizumab/bevacizumab or sorafenib. The clear distinction of these two groups for each treatment using principal component analysis with a deregulation for 141 protein or 87 for atezolizumab /bevacizumab and sorafenib treatment, respectively. These signatures were sufficient to predict the response. Moreover, these signatures reveal the biological pathways involved in the resistance to treatments. Particularly, we revealed and validated a shift in tumor cell energy metabolism inducing an immunosuppressive environment involved in the resistance to atezolizumab/bevacizumab.
Conclusion: We performed an in-depth analysis of quantitative proteomic data from HCC biopsies using statistical packages and found that their proteome includes information to predict the treatment response to advanced HCC giving the ability to optimize patient management.

#1894 Correlative multiscale 3D spatial proteomics.

Jingtian Zheng, Seung Young Lee, Yi-Chien Wu

Pharmaceutical Sciences, University of Illinois at Chicago, Chicago, IL

Tissues and organisms are complex systems, characterized by 3D structures, interconnected distinct functional subregions, and cellular heterogeneity, particularly in diseased states. To comprehensively understand the cellular and molecular mechanisms underlying disease, like tumor or neurodegenerative disease, it is crucial to construct a complete 3D map of tissues that integrates microanatomical structures, spatial cell composition, and molecular expression landscape, providing a holistic view of tissue pathophysiology. Existing tissue imaging methods can provide 2D spatial cellular and molecular information in tissue slices; however, only a fraction of the full 3D tissue architecture, leaving critical spatial and contextual information about the tissue microenvironment unresolved. To address this gap, we developed a novel methodology that integrates spatial proteomics with multiscale 3D optical tissue microscopy. The workflow begins with an overall 3D imaging of the tissue by light-sheet microscopy (LSFM), utilizing immunofluorescence (IF) or autofluorescence (AF) to visualize whole tissue morphology, cells, and molecules, providing a preview to identify Regions of Interest (ROIs). Using a tracking-based approach, macrosections containing these ROIs are physically isolated for subsequent 3D multiplex IF staining by cyclic staining and confocal microscopy (CLSM), allowing detailed, high-resolution cellular-level image and analysis. These sections are then subjected to thin-slicing and Super-Resolution Microscopy or Laser Capture Microdissection (LCM), which precisely extracts tissue regions for spatial proteomics analysis. Finally, the spatial proteomics data are aligned with multiscale 3D imaging data to provide a comprehensive, integrated analysis of the tissue. This integrated pipeline not only bridges 3D tissue imaging with molecular profiling but also enables selective analysis of ROIs, significantly saving time and resources while maintaining spatial and molecular fidelity, offering new insights into complex biological systems and disease mechanisms.

#1895 Improving capability for biomarker discovery of glioblastoma-enriched poly-ligand profiling library using aptamer capture microarrays.

Xixi Wei, Justin Saul, Daniel Martin, Alexis Rodin, Anna Walton, Danielle Richard, Tassilo Hornung

Caris Life Sciences, Inc., phoenix, AZ

Adaptive dynamic artificial poly-ligand targeting (ADAPT) biotargeting system is an innovative aptamer-based poly-ligand profiling and therapeutic target discovery platform employed to uncover the complexity of cancer in various sample types including Formalin-fixed paraffin-embedded tissue (FFPE). The enrichment process, selective evolution of ligands by exponential enrichment (SELEX), selects single-stranded oligodeoxynucleotides (ssODNs) from a diverse starting pool of ssODNs, which are supposed to preferentially bind to markers expressed in cancer tissues. In practice, the biological and technical complexity of sequence selection leads to an enriched library of ssODNs that contains many persistent sequences with poor differential binding between cancer and normal control tissues. Traditionally SELEX is performed to enrich a library from which the top performing candidates are synthesized and characterized, leading to indistinctive sequences being screened out through this time-consuming and expensive process. ADAPT, on the other hand, is predicated on many thousands of unknown interactions between the sequences in the enriched library and their cognate markers in the FFPE tissues. Consequently, the performance of an enriched library for ADAPT is susceptible to the abundance of indiscriminate ssODN sequences. In this work, after identifying potential binder sequences from an enriched library against glioblastoma (GBM) FFPE tissue, we proposed using Aptamer Capture Microarrays (ACM) to isolate sub-pools of sequences of interest and remove indiscriminate ssODNs from the library. Clinical GBM patient and normal control FFPE tissues were stained with a GBM-enriched ADAPT library. The bound libraries were recovered and sequenced with next generation sequencing (NGS). Different analytical strategies were used to identify sub-pools of sequences that potentially bind preferentially to GBM tissues. By designing microarray probes to capture selected sequences, and then eluting these targeted sequences from the microarray, the percentage of sample reads of the selected sequences were increased from a less than 5% in original enriched library to more than 80% in final cleaned up library. Our results show enhanced discrimination of GBM and normal tissues through FFPE tissue staining. This novel application of microarray capture is a highly cost-effective alternative to oligo pool synthesis, affording multiple partitions of a library to test varied ssODN selection methodologies. Libraries optimized by this method is expected to improve our ability to identify biomarkers.

#1896 Spatial proteomics and tumor-immune phenotyping identifies a diverse immune landscape in syngeneic murine tumor models.

A. Bose[1], E. Q. Alonzo[2], S. Struble[1], J. Fisher[2], R. A. Heil-Chapdelaine[1], N. F. Diaz Granados[1], V. Agrawal[1];
[1]Leica Microsystems, Inc., Waltham, MA, [2]Cell Signaling Technology, Danvers, MA

Syngeneic mouse models have a functionally intact immune system, and therefore, serve as a crucial tool for evaluation of the immune response to tumors and pre-clinical evaluation of immunotherapeutic strategies. However, the immune response varies between different syngeneic models. Identification and selection of appropriate models for pre-clinical evaluation is challenging. To understand the dynamic interplay between the tumor and immune system, we implemented multiplexed spatial proteomics across various syngeneic tumor models. Spatial mapping of multiple tumor and immune markers in untreated tumor provides an overview of the tumor immune architecture. This information can be applied to identify optimal models for preclinical immunotherapy approaches, to understand the response to therapies at the preclinical stage, and to identify potential biomarkers for clinical evaluation. The Cell DIVE Multiplexed Imaging Solution, in combination with IHC-validated antibody conjugates from Cell Signaling Technology (CST), enables profiling of immune cell populations in the context of human and mouse tumor tissue. Here, we demonstrate multiplexed Cell DIVE imaging of syngeneic mouse tumor models using a novel CST® panel of mouse-reactive antibodies. We apply segmentation and clustering analysis to identify spatially co-localized populations of cells and discuss the complexities of immune cells and their interactions. Cell type specific markers combined with multiplexed tissue imaging and spatial mapping is an approach that enables a better understanding of the anti-tumor immune response, which can be applied to address challenges associated with developing immune-based therapies. The study highlights the importance of simultaneously observing multiple immune cell types and tumor characteristics in spatial context.

CLINICAL RESEARCH: Diagnostic Biomarkers 3: Early Detection Biomarkers
Poster Session

#1901 Applications of silicon quantum dots as biomarkers for cancer detection and treatment, emphasizing biocompatibility studies.

Dorra Gargouri, Artjima Ounkaew, Xuyang Liu, David Antoniuk, Jonathan Veinot

Applied Quantum Materials Inc., Edmonton, AB, Canada

Silicon quantum dots (SiQDs) are emerging as advanced nanomaterials for cancer treatment and detection. SiQDs are an attractive alternative to organic dyes for biological labeling, offering tunable photoluminescence response and high resistance to photobleaching. In our hands, SiQDs possess tailorable surface chemistry, as well as stable size and surface chemistry dependent luminescent response spanning visible and near-infrared spectral regions with high photoluminescence quantum efficiencies (approaching 85%). SiQDs are an enticing heavy-metal-free, non-toxic and biocompatible alternative to status quo QDs. Applied Quantum Materials Inc. (AQM) possesses world leading expertise to produce high-purity narrow size distribution SiQDs with custom surface modifications, including biomolecules (8 kDa to 150 kDa) for biology applications. We have performed a biocompatibility study that shows AQM SiQDs have achieved over 90% cell viability, even at concentrations up to 500 µg/mL after 24 h of incubation. In addition, we modified SiQDs to improve cancer cell uptake to enhance photodynamic therapy (PDT) efficacy under low-level laser therapy (LLLT). Using NIR laser irradiation, AQM SiQDs significantly reduced cancer cell viability, enhancing reactive oxygen species (ROS) generation and establishing SiQDs as effective photosensitizers for cancer therapy. SiQDs exhibited excellent photostability and minimal toxicity, outperforming organic dyes. Bioconjugation of SiQDs with biomolecules are proving to be powerful tools for cancer cell detection. This opens new possibilities for oncologists to select the most suitable therapy based on a comprehensive understanding of the cancer type and its progression. We are currently developing a liquid biopsy system for precision cancer management. Powered by SiQDs, this advanced diagnostic tool will enable automated blood analysis to identify and quantify rare circulating tumor cells (CTCs) with high sensitivity in blood patients. Working with our academic collaborators, we are addressing the complexities of brain tumor resection in glioblastoma. We synthesized a novel nanoscale material combining SiQDs with anti-cancer DNA aptamers for fluorescence-guided surgery. These SiQDs labeled aptamers will be validated on resected brain tumor samples and compared with pathological margin assessments. Additionally, the imaging potential of AptaSiQDs for primary and metastatic brain tumors will be evaluated in preclinical in vivo models. As we progress to in vivo studies, we will assess their cytotoxicity and biocompatibility in mice, aiming to expand their use in targeted drug delivery, imaging, and theranostics for personalized medicine. SiQDs are at the forefront of life sciences, offering diverse benefits, making them ideal for cancer diagnosis.

#1902 Novel monoclonal antibodies specific for cancer-associated proliferating cellular nuclear antigen for tumor monitoring and diagnosis.

Malaney C. Young[1], Caroline M. Li[1], Robert G. Lingeman[1], Chun Li[1], Aimin Li[2], Linda H. Malkas[1], Robert J. Hickey[1]

[1]Beckman Research Institute of The City of Hope, City of Hope, CA,[2]City of Hope, City of Hope, CA

Proliferating cellular nuclear antigen (PCNA) is a biomarker associated with cancer progression and is expressed in all cancer types. Its expression is increased before DNA synthesis with the transition of cells from G1 to S phase, making it a useful diagnostic marker for tumor cell proliferation. Our lab has identified an acidic and basic isoform of PCNA that can be resolved using 2-dimensional gel electrophoresis (2DE). Cancer cells express the acidic, cancer-associated isoform of PCNA (caPCNA), whereas healthy cells only express the basic, non-malignant isoform of PCNA (nmPCNA). Thus, caPCNA is an important biomarker that can be used for identifying early-stage cancer and monitoring tumor growth, metastasis, and response to treatment. Immunohistochemical staining (IHC) of tissue biopsies is an essential method for tumor detection and monitoring of cancer progression. Antibodies are used either individually or in panels and typically require markers that are specific to each tumor's origin. Because caPCNA is not specific to a single cancer type, it is a universal biomarker which can be used to monitor cancer of any origin. Commercially available antibodies for PCNA do not distinguish between the cancer isoform and the non-cancer associated normal isoform. We have developed a series of monoclonal antibodies that are capable of distinguishing caPCNA from nmPCNA and have characterized these antibodies using 2DE and IHC. These novel monoclonal antibodies targeting the caPCNA isoform could enable pathologists to distinguish the presence of cancer cells in difficult to diagnose biopsy specimens, more efficiently monitor surgical margins, and potentially identify the presence of circulating tumor cells in blood samples.

#1903 Actionable mutational landscape in solid tumors: a case study of ovarian and colon cancer.

Amit Kumar[1], Samir Agarwal[2], Pankaj Khanna[1], Shobit Gupta[1], Rama Chandran[1], **Ananth S. Bommakanti**[1]

[1]Genes2Me, Gurugram, India,[2]Army Hospital Research And Referral, New Delhi, India

Background: Solid tumors are abnormal tissue masses that can be benign or malignant. They are named after the type of cells that form them, and can be categorized by where they originate such as breast, colon, bladder, ovary, and lung.
Methods: Genomic profiling was done with sequencing of FFPE tumor samples 40 using Genes2Me PanCancer assay with 681 genes and the target size of 1.7 Mb on Illumina platform. Bioinformatics analysis was performed using standard GATK.v4.1.2 somatic variant calling pipeline. Mutational plots were generated using MAFtools and inhouse python script for post data analysis.
Results: The number of mutant allele frequency (MAF) of the most frequently mutated genes varied significantly across tumor types, with the highest MAF observed in this cancer type. Significantly mutated cancer-related genes in solid tumors found the most frequently altered genes to be TP53(45%), KRAS(18%), TERT(14%), CDKN2A(12%), PIK3CA(11%), APC(36%), EGFR(10%), ESR1(20%), KIT(9%), AR(64%), MYC(44%), FGFR1(21%), BRCA1(22%), BRCA2(63%), CCND1(46%) in colon and ovarian carcinoma. Tumormutational burden (TMB) varied significantly across tumor types, and patients with high MAF had a significantly higher TMB score with one of the investigated platforms.
Conclusion: These findings describe the similarities and differences between mutational signature of different solid tumors from cancer patients, providing valuable information for personalized medicine and also an important implication for the selection of patients for clinical trials with molecularly targeted therapies.

#1904 Cell-based reference standards for the development and validation of assays for the assessment of minimal residual disease in acute myeloid leukemia.

Colt W. Nash, Matthew G. Butler, Jayanthi Ramprakash, Ojaswee Dahal, Andrew T. Anfora, David E. Merriam, Russell K. Garlick, **Yves Konigshofer**

LGC Clinical Diagnostics, Inc., Gaithersburg, MD

Here, we describe the generation of novel cell-based reference standards for the development and validation of molecular diagnostic assays that assess measurable residual disease (MRD) in acute myeloid leukemia (AML). Unlike other hematological malignancies that are characterized by a defined genetic abnormality - such as the BCR::ABL1 translocation in chronic myelogenous leukemia - AML is associated with numerous different mutations that vary between patients. While about third of AML patients harbor a mutation in NPM1 and/or an internal tandem duplication (ITD) in FLT3, this is not the case for the majority of patients. This complicates the monitoring of the presence or absence of MRD during and after treatment because assays need to be very sensitive but inexpensive enough to allow for repeated measurements over time. For example, in the absence of an NPM1 or FLT3-ITD mutation, it may be beneficial to design patient-specific assays that focus on only the mutations found in that patient's AML.

As an initial proof of concept, we engineered an AML cell line to add NPM1 and/or FLT3-ITD mutations. The number of mutations per cell was quantified by digital PCR. This allowed for the generation of clones that represented AML cells with only a defined NPM1 or FLT3-ITD mutation, as well as AML clones with both. These were combined with peripheral blood mononuclear cells to dilutions as low as 1:100,000 and analyzed by flow cytometry (a traditional way of monitoring for MRD in AML), electrophoresis (FLT3-ITD), digital PCR, and NGS. Compatibility was observed with all methods, but digital PCR and NGS appeared to be more sensitive.

We then expanded the NPM1 variants beyond type A (c.860_863dup) to include types B, D, and J, and added additional FLT3-ITDs to include duplicated lengths as long as 228 bp. In order to increase the information obtained from a single sample in NGS, which is capable of independently measuring multiple variants, we combined these into a pool that was quantified by digital PCR and analyzed by NGS but also retained compatibility with flow cytometry to enable sorting and single-cell analyses.

In conclusion, we have generated reference standards that incorporate multiple variants in NPM1 and FLT3, which are designed for the development and validation of molecular biology-based assays. In order to expand beyond the mutations found in only a third of AML patients, we are applying the same method to add mutations in additional genes such as IDH1, IDH2, and TP53.

#1905 Deep learning-based histomorphological classification to identify novel causal genes in gastric cancer.

Moonyoung Lee[1], Kwangil Yim[1], Sangjeong Ahn[2], Hyun-Jong Jang[3], Sung Hak Lee[1]

[1]Department of Hospital Pathology, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea, Republic of,[2]Department of Pathology, Korea University Anam Hospital, College of Medicine, Korea University, Seoul, Korea, Republic of,[3]Catholic Big Data Integration Center, Department of Physiology, College of Medicine, The Catholic University of Korea, Seoul, Korea, Republic of

Introduction: Gastric cancer (GC) has high mortality and limited treatment options, with histomorphological features predicting outcomes and treatment response. Stricter criteria for undifferentiated GC in Japanese guidelines underscore its poor prognosis. This study developed a deep learning (DL) model to classify GC as differentiated or undifferentiated, reducing diagnostic variability. The model revealed genetic differences aligned with or distinct from pathologist classifications, providing precise spatial insights into GC phenotypes to enhance molecular understanding, diagnosis, and therapy.

Methods: A fully automated method was utilized to classify gastric cancer tissue whole-slide images (WSIs) from The Cancer Genome Atlas stomach adenocarcinoma dataset (TCGA-STAD) into differentiated and undifferentiated tumor types. To focus on the analysis of differentiated and undifferentiated types, GC samples of Epstein-Barr virus-positive, microsatellite-unstable/instability types were excluded from the study. For SNV variants, the downstream analysis focused on splice sites, nonsense mutations, and frameshift insertions/deletions within protein-coding regions that are directly related to the phenotype and likely to cause the disease. In addition to SNV analysis, CNV analysis was also performed to identify novel causal genes. All experiments were analyzed based on three classification outcomes: classifications made by expert pathologists, classifications made by the DL model, and the overlapping classifications agreed upon by both expert pathologists and the DL model.

Results: SNV analysis identified *TP53, CDH1, CNGA4,* and *SPTA1* as causal genes for undifferentiated GC, with recurrent variants across all three classification methods. The identification of *TP53, CDH1,* and *CNGA4,* known causal genes in gastric cancer, validates our findings. Additionally, *SPTA1* was identified as novel causal gene of undifferentiated GC. In addition, in the results of mutation signature analysis, we confirmed that SBS1, SBS10b, and SBS6, which are commonly related to carcinogens, stood out in the results classified by three criteria. We also confirmed that the TMB and CNV results were similar among subgroups.

Conclusion: This study demonstrated the potential of a DL-based approach in the task of subclassification of GC using WSIs, highlighting its utility in identifying genes associated with GC subtypes. In this study, in addition to previously well-known genes, a novel gene, *SPTA1,* was identified as causative genes. This information may enhance our understanding of the prognostic significance of tissue slides and provide additional insights to guide therapeutic decision-making.

#1906 Comprehensive genomic profiling for solid tumors.

Shobit Gupta[1], Amit Kumar[1], Pankaj Khanna[1], Rama Chandran[1], Samir Agarwal[2]

[1]Genes2Me, Gurugram, India,[2]Army Hospital Research And Referral, New Delhi, India

Background: Next-generation sequencing technologies provides deep knowledge into the foundation of tumors and can advance the field of precision oncology. It can be used to effectively select the treatment based on druggable genomic alterations. In the present study, we prospectively investigated the clinical utility of a CGP assay in patients with metastatic tumors of the lung, breast, gynecologic, and colon etc.

Methods: Genomic profiling was done on FFPE tumor samples using Genes2Me PanCancer assay. This assay, specifically adapted for the present molecular screening, is validated for clinical use and accredited by CEIVD and based on the guidelines published by the Association for Molecular Pathology, College of American Pathologists. It is a NGS-based product especially designed to identify genomic alterations, based on Food and Drug Administration, National Comprehensive Cancer Network guideline references, European Medicine Agency approvals, European Society for Medical Oncology (ESMO), updated every 3-6 months. It Targets around 681 genes with the size of 1.7 Mb containing different biomarkers such as DNA/RNA fusions, TMB, MSI and HPV related genes. It also incudes homologous recombinational repair (HRR) related genes. This panel was also designed to identify large scale rearrangements and special biomarkers such as MET exon 14skipping. Sequencing was done on Illumina platform. Bioinformatics analysis was performed using standard GATK.v4.1.2 somatic variant calling pipeline.

Results: Most of the samples were sequenced with average depth of >1000X. On target ration ranged between 82-94% with coverage % at 10X and 30X was more than 99%. In all the samples mutations were identifies with VAF ranges from 8-28%. mutations More than 50% samples have identified mutations with actionable FDA approved drugs.

Conclusion: Although further studies are needed to support, this CGP assay is a noninvasive assay to guide therapeutic choice in oncology. It provides tumor heterogeneity profile that could be incorporated in routinely clinical practice. These findings may support the modification of the primary treatment in patients with metastatic solid tumors thus be the first step for future advances in precision oncology

#1907 A novel DNA library prep method enables high sensitivity variant detection from matched fresh frozen and FFPE patient tissues.

Chen Song, Margaret R. Heider, Jian Sun, Adrian Reich, Brittany S. Sexton, Bradley W. Langhorst, Laura Blum, Keerthana Krishnan

New England Biolabs, Inc., Ipswich, MA

In cancer genomics, a common source of DNA is formalin-fixed, paraffin-embedded (FFPE) tissue from patient surgical samples, where in most cases high quality fresh or frozen tissue samples are not available. FFPE DNA poses many notable challenges for preparing NGS libraries, including low input amounts and highly variable damage from fixation, storage, and extraction methods. It is difficult to obtain libraries with sufficient coverage and the sequencing artifacts arising from damaged DNA bases confound somatic variant detection. Additionally, many laboratories process FFPE tumor samples alongside matched, high quality, normal DNA and many library prep workflows are not readily compatible with both sample types.

We developed a novel NGS library prep method compatible with both high quality and very low quality FFPE DNA samples, employing a novel enzymatic DNA repair mix, enzymatic fragmentation mix, and PCR master mix. To validate this workflow on real patient sample sets, we obtained DNA extracted from matched tumor and normal tissue of various tissue types preserved by both fresh frozen and FFPE methods. The FFPE DNA samples ranged in quality from DNA integrity number (DIN) 1.5 to 6.8 and fresh frozen DNA was DIN 8 to 9. We prepared libraries using this method compared against other library prep workflows and sequenced them by WGS and target capture. We assessed the performance of these workflows by library yield, library quality metrics, depth and evenness of coverage, and somatic variant calling with fresh frozen-extracted DNA providing the gold standard for library quality and mutation content.

This new enzymatic fragmentation-based library prep workflow not only reduced the false positive rate in somatic variant detection by repairing damage-derived mutations in FFPE DNA samples but also improved the library yield, library quality metrics, library complexity, coverage depth and uniformity, and hybrid capture library quality metrics. Comparing the variant calls from matched FFPE and frozen tissues revealed an improved sensitivity and accuracy of variant calling using this library prep method compared to mechanical shearing and other enzymatic fragmentation library prep approaches. Furthermore, library quality and variant detection sensitivity were equivalent when using this FFPE workflow compared to gold standard mechanically sheared libraries.

This new suite of enzyme mixes allows even highly damaged FFPE samples to achieve high quality libraries with a greater sensitivity for somatic variant identification. The workflow is robust and flexible, compatible with both FFPE DNA and matched high quality DNA samples as well as automation-friendly for convenience in sample processing.

#1909 Extracellular vesicle-derived biomarkers surpass cfDNA performance in a next-generation early detection strategy for colorectal cancer.

Sudipto Kumar Chakrabortty, J. Christian J. Ray, Dulaney Miller, Kyle Manning, Yang Yang, Shuran Xing, T. Jeffrey Cole, Christopher J. Benway, Sinead Nguyen, Allan George, Julia Doo, Yiyuan Yao, Pawan Upadhyay, Joe Johnson, Sonia Kumar, Sivakumar Gowrisankar, Brian Haynes, Johan Skog

Exosome Diagnostics, a Bio-Techne brand., Waltham, MA

Introduction: Colorectal cancer (CRC) increasingly impacts younger individuals, emphasizing the urgent need for earlier, more accurate, and minimally invasive screening methods. Current plasma-based liquid biopsy tests, typically relying on cell-free DNA (cfDNA) and/or free proteins, effectively detect CRC but fall short in identifying early pre-cancerous lesions such as advanced adenomas (AAs). We hypothesized that a multi-omic approach combining cfDNA and extracellular vesicle (EV)-derived biomarkers could enhance early detection of CRC, including AAs, which for current cfDNA-based assays have poor performance of detection.
Methods: A predefined statistical analysis plan guided the enrollment of approximately 230 individuals undergoing colonoscopy across three independent clinical sites. Plasma cfDNA methylation levels were analyzed using a combination of enzymatic conversion and enrichment of a panel covering over 4 million CpG sites. Comprehensive profiling of EV-derived long RNAs to measure gene expression and splice variants was performed using a hybrid capture panel covering 20,000 protein coding RNAs and 20,000 lncRNAs. Unbiased EV small RNA profiling was performed to measure all small non-coding RNAs between 18-200nts in length. Levels for up to 552 cancer associated proteins in EVs were measured using the Olink platform. Stringent quality control ensured reproducibility and no significant batch effects were seen across sites. Feature selection and model development employed a stratified, 5-fold cross-validation approach with a support vector machine (SVM) model. Sensitivities were evaluated using a Clopper-Pearson estimator at a target specificity of 91%, and prevalence-adjusted.
Results: Among the omics channels, EV-derived RNA demonstrated the highest sensitivity for detecting both CRC and pre-cancerous AAs at 87% (±6%) and 34% (±7%) respectively, significantly outperforming cfDNA methylation (76% ±7% and 10% ±4% respectively), EV-derived protein (85% ±7% and 24% ±7% respectively), and EV-derived miRNA (49% ± 9% and 21% ±7% respectively). Cross-validation identified key features relevant to CRC biology, with pathway enrichment analysis of EV-derived gene expression revealing significant activation of immune and inflammatory pathways.
Conclusion: While most EV derived analyte surpassed the performance achieved by cfDNA methylation, EV long RNAs emerged as the most sensitive analyte for early detection of CRC and pre-cancerous AAs. This study highlights the potential of EV-derived multi-omic biomarkers to address critical gaps in CRC screening, offering a transformative approach to improving early detection and patient outcomes. Combining EV RNA with cfDNA may give synergistic improvements in performance when applied in a larger cohort.

#1910 Allelic imbalance for early detection of breast cancer in high depth whole genome sequencing.

Laura Andersen[1], Konner Nelson[2], Julia Ah-Reum An[2], Pedram Razavi[2], Mark Robson[2], Dan Landau[3], Nicolai Birkbak[1], Adam Widman[2]

[1]Aarhus University Hospital, Aarhus, Denmark,[2]Memorial Sloan Kettering Cancer Center, New York, NY,[3]Weill Cornell Medicine, New York, NY

Liquid biopsy for circulating tumor DNA (ctDNA) offers potential for noninvasive early cancer detection. However, existing mutation- and methylation-based multi-cancer early detection tests have poor sensitivity in low-shedding, high-aneuploidy tumor types like breast cancer.

We previously developed an ultrasensitive tumor-informed liquid biopsy approach that used B-allele frequency (BAF) to detect cancer aneuploidy. Our BAF classifier, which measures allelic imbalance from the millions of single nucleotide polymorphisms (SNPs) across the genome, demonstrated high sensitivity for minimal residual disease in triple-negative breast cancer (TNBC) using only standard whole genome sequencing (WGS). To expand our BAF approach to the tumor-naïve early detection context, we sought to enhance aneuploidy signal through (i) high-depth plasma WGS (150-200x) using Ultima Genomics to better interrogate allelic imbalance at individual SNP sites and (ii) long-read sequencing of normal tissue to phase A and B haplotypes, allowing for the aggregation of signal from large regions of neighboring SNPs. We characterized two metrics of allelic imbalance: BAF and a fragment length score (FRAG) characterized by cancer-specific differences in fragment lengths between phased alleles.

To assess the performance of integrated BAF and FRAG signals for ctDNA detection, we collected a pilot cohort of BRCA-mutant, early-stage breast cancer patients with 7 pre-treatment and 28 post-surgery plasma samples from patients with no documented recurrence. ctDNA was overtly detected in pre-treatment samples from a stage III HR+/HER2- breast cancer and a stage II TNBC. We further sought to evaluate performance in stage I breast cancer, a particularly challenging setting for ctDNA detection through existing early detection approaches. We detected a stage I pT1c HR+/HER2- breast cancer and a stage I pT1b (<1 cm) TNBC at 100% specificity against post-surgery samples, demonstrating the potential of our classifier for non-invasive early cancer detection. Overall area under the curve (AUC) between pre-treatment (n=7) and post-surgery (n=28) samples was 0.86 in this challenging cohort that included 3 HR+/HER2- stage I (2 pT1a, 1pT1b) breast cancer samples. Longitudinal analysis of serial plasma samples throughout neoadjuvant treatment and following surgery demonstrated the ability of our tumor-naïve classifier to track therapeutic response.

Our method demonstrates promising potential for early cancer detection and tumor-naïve monitoring of treatment response in breast cancer patients, including those with very small tumors. We expect this method to extend beyond breast cancer to the many highly aneuploid cancer types where ctDNA detection through conventional methods has been limited. Additional samples will further validate our approach.

#1911 Platform agnostic lung cancer plasma signature identified using multidimensional proteomics and explainable machine learning.

P. J. Liu[1], H. R. Ferguson[1], L. Hankey[1], H. Huang[1], J. Syed[1], I. Kaneva[1], E. Mi[1], D. A. Szulc[1], L. J. Zhan[2], D. Patel[2], M.-S. Tsao[3], R. Fischer[4], B. M. Kessler[4], G. Liu[3], N. I. Gushterov[1], A. J. Halner[1];

[1]Oxford Cancer Analytics, Oxford, United Kingdom, [2]Princess Margaret Cancer Centre, Toronto, ON, Canada, [3]University of Toronto, Toronto, ON, Canada, [4]University of Oxford, Oxford, United Kingdom

Introduction: Lung cancer is the leading cause of cancer mortality worldwide with 70% diagnosed at the late stage. Low-dose CT (LDCT) is available for screening but its uptake remains low. A blood test providing a molecular signature that identifies those at highest risk of lung cancer can integrate with existing screening pathways to increase uptake and enable earlier detection. The blood proteome harbors promising information for minimally invasive biomarker discovery especially for early stage and low tumor-burden lung cancer with limited circulating tumor DNAs.

Methods: We combined data-independent acquisition mass spectrometry (MS) as an unbiased approach with a targeted proximity extension assay (PEA, Olink) for biomarker discovery in 614 patients: 490 lung cancer (180 stage I/II, 310 stage III/IV, all major histologies) and 124 matched control (from a LDCT screening program) plasma samples collected under standardized protocol (PMCC, Toronto, 2008-2019). To identify a lung cancer molecular signature, we used a 80/20% discovery/holdout testing approach and incorporated explainability in a tailored machine learning (ML) platform (iScience, 2023) to provide ranking and rationale for feature selection decision making. Finally, aptamer based proteomic profiling (SomaScan) validated a focused molecular signature panel.

Results: In total 5,403 distinct protein groups were identified, 3,655 by MS, 2,884 by PEA, with 1,136 overlapping. Both approaches identified proteins significantly enriched for inflammation, chemotaxis/migration, humoral immune response, and complement activation in lung cancer. MS identified additional proteins distinctly enriched for epithelial cell migration and ROS metabolism. ML directed feature selection identified focused panels of 17 and 20 proteins from MS and PEA datasets, each achieving 92% and 98% area under the ROC curve (AUC) respectively in hold-out validation, demonstrating sensitivity/specificity for overall lung cancer detection at 88/90% and 94/90% using ≤20 biomarkers. Importantly, each panel accurately detects early stage, late stage, small cell, adenocarcinoma, and squamous cell carcinoma of the lung, with AUCs of 88%-98% (MS) and 95%-99% (PEA) for each subtype. Further evaluation of the signatures using an aptamer platform confirmed a biomarker panel with strong concordance across the three distinct proteomic approaches, creating a platform agnostic lung cancer plasma signature for disease detection.

Conclusion: We identified a platform agnostic plasma molecular signature for lung cancer using three distinct proteomic approaches and explainable ML. This molecular signature can be highly scalable across different protein detection platforms, with prospective studies underway to assess its impact enhancing existing screening programs, enabling earlier detection, and improving survivorship.

#1912 Immune and molecular markers in benign breast disease predict risk of node-positive breast cancer.

Matilde Rossi[1], Nicole Cruz-Reyes[1], Trista Navarro[1], Melody L. Stallings-Mann[1], Bryan M. McCauley[2], Robert A. Vierkant[2], Stacey J. Winham[2], Mark E. Sherman[3], Amy C. Degnim[4], Derek C. Radisky[1]

[1]Department of Cancer Biology, Mayo Clinic, Jacksonville, FL,[2]Department of Qualitative Health Sciences, Mayo Clinic, Rochester, MN,[3]Department of Epidemiology, Mayo Clinic, Jacksonville, FL,[4]Department of Surgery, Mayo Clinic, Rochester, MN

Background: Breast cancer (BC) remains a leading health concern worldwide and is often undetected until it metastasizes, limiting treatment options and worsening prognosis. Early detection of molecular and immune markers in benign breast disease (BBD) could identify women at elevated risk of developing invasive, node-positive BC, enabling targeted surveillance and prevention efforts. Building on this premise, we investigated the immune and molecular landscape of BBD biopsies preceding BC development to enhance risk stratification and inform preventive strategies.

Methods: Utilizing the Mayo Clinic BBD cohort, we conducted a case-control study comparing women who developed node-positive BC following their BBD biopsy (cases; n=8) to those who remained cancer-free (controls; n=11), matched on patient age and biopsy date. Differential gene expression was assessed using the NanoString nCounter system to identify differentially expressed genes between cases and controls. We developed a multiplex immunofluorescence (MxIF) approach using the Opal system by Akoya Biosciences for comprehensive marker detection in formalin-fixed, paraffin-embedded (FFPE) tissue samples. Digitized images were segmented for cell phenotyping, and spatial relationships of immune cells were analyzed and compared between cases and controls.

Results: Our findings revealed significantly increased expression levels of immunosuppressive genes in the case group, including CD48, CD84, IL-32, and LAIR1. Immunohistochemical analyses showed that controls exhibited higher frequencies of $CD68^+$ (macrophages), $CD11c^+$ (M1 macrophages), and $CD206^+$ (M2 macrophages) cells compared to cases. Notably, controls had a significant increase in $CD68^+CD11c^+$ cells, indicative of a pro-inflammatory, antitumorigenic M1 macrophage polarization. Furthermore, our MxIF analyses provided novel insights into spatial biomarker localization, enhancing prognostic accuracy.

Conclusions: The decreased numbers of immune cells and increased expression of immunosuppressive genes in BBD biopsies may be associated with an elevated risk of developing axillary node-positive BC. These findings emphasize the importance of dissecting molecular and immune interactions in BBD for risk assessment. Our results have the potential to improve individualized risk stratification, leading to more targeted surveillance and informed screening. This research not only contributes to our understanding of BC pathogenesis but also opens avenues for developing novel preventive strategies, potentially reducing BC incidence and mortality through early intervention.

#1913 Cystic fluid acetylated polyamines predict risk of malignancy of intraductal papillary mucinous neoplasms of the pancreas.

R. A. Leon Letelier[1], **R. Ballaro**[1], Y. Chen[1], R. Dou[1], E. Irajizad[1], M. Yip-Schneider[2], R. Wu[1], R. Ejaz[1], J. Vykoukal[1], M. Hurd[1], A. Maitra[1], J. Dennison[1], K.-A. Do[1], J. Zhang[3], S. Hanash[1], C. Smith[2], J. F. Fahrmann[1];

[1]The University of Texas MD Anderson Cancer Center, Houston, TX, [2]Indiana University School of Medicine, Indianapolis, IN, [3]Richard M. Fairbanks School of Public Health, Indianapolis, IN

Background: Intraductal papillary mucinous neoplasms are recognized precursor lesions to pancreatic ductal adenocarcinoma (PDAC). There remains an unmet need to establish biomarkers for identifying IPMN at high-risk of malignant progression for early interception. Previously, we reported that acetylated polyamines inform upon presence of PDAC. Here, we assessed to what extent cystic fluid polyamines predict risk of malignancy for patients with IPMN.

Methods: Using mass spectrometry, we screened for polyamines in cystic fluid from 19 patients with either high-grade dysplasia (HG; N=12) or HG with a concurrent PDAC (IPMN/PDAC; N=7) and 70 patients with low-grade (LG) dysplasia). A total of 8 polyamines were detected and quantified, consisting of six that are host derived (spermidine, spermine, N-acetylputrescine (NAcPut), acetylspermidine (AcSpmd), diacetylspermidine (DiAcSpmd), and diacetylspermine (DAS) and two of microbial origin (acetycadaverine (AcCad) and diacetylcadaverine (DiAcCad). Predictive performance of individual polyamines for identifying HG or PDAC/IPMN was determined and complementarity with cystic fluid CA19-9 assessed. Findings were compared to metabolic profiles generated on 9 IPMN tumors (N= 3 LG; 3 HG, and 3 IPMN/PDAC) as well as cell lysates and conditioned medium from doxycycline-activated mutant *Kras;Gnas* IPMN cell lines. Publicly available spatial and single cell transcriptomic datasets of IPMN tumors were used to evaluate for spatial and cell-type specific differences in transcript levels of cognate enzymes involved in polyamine metabolism.

Results: Of the 8 quantified polyamines, spermine, AcSpmd, DAS, and DiAcCad were statistically significantly elevated in cystic fluid of patients with HG or PDAC/IPMN compared to those with LG IPMN. DAS had the highest predictive performance for HG or PDAC/IPMN with an overall AUC of 0.73 (95% CI: 0.58-0.88). An OR-rule based on CA19-9, DAS, and AcSpmd achieved sensitivity of 52.6% and specificity of 91% for detecting HG and PDAC/IPMN. Polyamines were elevated in PDAC/IPMN tissues compared to LG IPMN tissues and polyamine levels accumulated in condition medium of mutant *Kras;Gnas* IPMN cells. Spatial and single cell transcriptomic data revealed transcript levels of polyamine-metabolizing enzymes to be preferentially elevated in neoplastic epithelium and in tumor-associated macrophages.

Conclusion: Cystic fluid polyamines offer potential utility for determining risk of malignancy of IPMN, capable of complementing CA19-9. The polyamine signature may aid clinical management of patients with IPMN.

#1914 Highly accurate detection of early-stage colorectal cancer using tumor and immune extracellular vesicles biomarkers.

Poorva Mudgal[1], Zack Opheim[1], Cheryl Bandoski[1], Valesca Anschau[2], Martin Mehnert[2], Issa Isaac[2], Alan Ezrin[1], **Todd A. Hembrough**[1]

[1]Nexosome Oncology, Durham, NC,[2]Biognosys, Schleiren, Switzerland

Colorectal cancer (CRC) is one of the most common cancers worldwide, and early detection is critical for treatment and improved survival rates. Precancerous and early-stage CRC present significant diagnostic challenges due to the small size of lesions and the very low expression of tumor-specific biomarkers in plasma. Current genomics-based diagnostic methods struggle to detect these lesions, making it imperative to develop more sensitive and specific approaches. Circulating extracellular vesicles (EVs) are emerging as a promising solution for early-stage CRC detection, since EVs are produced by tumor, microenviroment and host cells. Plasma EVs may offer a novel means to detect and monitor small early-stage tumors through detection of tumor-associated biomarkers, tumor specific host response, and microenvironment biomarkers To identify novel early-stage CRC specific biomarkers, we performed proteomic analysis on EVs purified from patient plasma using size exclusion chromatography and a proprietary buffer system that enhances EV and corona protein recovery. TrueDiscovery™ Data-independent acquisition (DIA) mass spectrometry (MS) analysis was conducted on 24 pre-cancer/stage 0 and 25 stage 1 CRC and 75 normal patient plasma samples. An in-house machine learning (ML) pipeline was developed to identify differentially expressed proteins and model multiplexes to identify those with extremely high diagnostic accuracy (> 0.99).An average of ~2,500 QCed proteins were identified per sample and included in bioinformatics analysis. Comparative analysis of control patient EVs and either pre-cancerous/Stage 0, or Stage 1 colon cancer EVs using our ML pipeline identified 336 and 493 differentially expressed proteins for each group respectively (absolute Log2FC>0.5; FDR adjusted p-value <0.001; AUC>0.5). The 17 best pre/stage 0 proteins and 53 stage 1 proteins were trained and tested using Support Vector Machine to assess all potential 3-plexes to identify those with mean diagnostic accuracy > 99%. Our analysis yielded five 3-plexes in precancer/stage 0 and thirty-four 3-plexes in stage 1 with near perfect diagnostic accuracy in the discovery cohort. 3-Plexes were translated to ELISAs and initial testing retained very high accuracy (0.953), sensitivity (0.948) and specificity (0.958) classifying cancer vs control samples. The performance of highly accurate ELISA multiplexes is currently being assessed on an independent intended use case/control cohorts. Key biomarkers from CRC patient EVs indicate the potential to detect and diagnose early-stage and low tumor burden cancers using our methods. Interestingly, the most accurate 3-plexes consist of proteins from immune, inflammatory and metabolic processes, suggesting that for the detection of early-stage cancer it may be critical to include both tumor and host specific biomarkers.

#1915 A plasma biomarker panel of microRNAs miR-133-3p and miR-153-3p for diagnosis of prostate cancer.

S. Verma, C. L. Thompson, P. Fu, G. T. MacLennan, **S. Gupta**;
Case Western Reserve University School of Medicine, Cleveland, OH

Prostate cancer is highly prevalent and the second-leading cause of cancer-related death among men in the United States. Its incidence rate has been rising by 3% per year overall and by about 5% per year for advanced-stage disease. Current diagnostic methods, particularly the PSA test, remain controversial due to variable sensitivity and limited specificity, highlighting the need for more precise and minimally invasive diagnostic tools. MicroRNAs (miRNAs)—19-24-nucleotide, non-coding RNAs—have emerged as critical regulators of gene expression and are frequently deregulated in cancer. These miRNAs exhibit cancer-specific dysregulation, high sensitivity, and stability, positioning them as strong candidates for blood-based biomarkers in early cancer detection and prognosis. Using computational miRNA target prediction analysis, we identified several miRNAs—namely, miR-133a/b, miR-144, miR-153, and miR-590—that could serve as highly sensitive and specific biomarkers for early prostate cancer detection. Our bioinformatics analysis further revealed that these miRNAs regulate glutathione S-transferase P1 (GSTP1), a gene often epigenetically silenced in early prostate cancer stages, suggesting their potential diagnostic value. To evaluate this, we analyzed miRNA levels in 84 human plasma samples from 56 prostate cancer patients and 28 non-cancer individuals served as controls using quantitative real-time PCR. Sensitivity and specificity were assessed through Receiver Operating Characteristic (ROC) curve analysis. The expression of miR-133-3p and miR-153-3p were significantly elevated in the plasma of prostate cancer patients compared to the control group, with p-values of 0.021 and 0.0006, respectively. Binary logistic regression and ROC analyses comparing these miRNAs to PSA showed significantly higher sensitivity and specificity, with p-values of 0.0001 and 0.0003. This two-miRNA panel exhibited near-perfect diagnostic accuracy, with an area under the curve (AUC) ranging from 0.98 to 1.0. Compared with previously identified miRNA biomarkers, this 2-miRNA panel demonstrated greater diagnostic stability and superior performance across public dataset cohorts. Our findings suggest that miR-133-3p and miR-153-3p could serve as reliable blood-based biomarkers for diagnosis of prostate cancer. Their association with GSTP1—a gene implicated in early prostate cancer through epigenetic silencing—underscores their biological relevance to the disease. The high sensitivity and specificity of this panel represent a significant improvement over PSA-based screening and indicate its promise for clinical application in prostate cancer diagnostics. Overall, this study supports the development of miRNA-based diagnostic tests to enable earlier detection, reduce unnecessary treatments, and ultimately improve outcomes for prostate cancer patients.

#1916 Oncogenic fusion detection for leukemia patients using droplet digital PCR.

Hannah C. Davis, Ellie R. Wilson, Xiaojuan Cao, Maggie J. Cox, Francesca Ferraro, David H. Spencer, Stephen T. Oh, Grant A. Challen, Andrew L. Young

Washington University in St. Louis, St. Louis, MO

Accurate quantification of residual disease burden in acute myeloid leukemia (AML) patients is of paramount importance for treatment decision-making and long-term survival. Currently, measurable residual disease (MRD) detection is assessed using multiparameter flow cytometry, RT-qPCR or sequencing. Unfortunately, these techniques are limited by high costs, utility in only a subset of patients, and slow turnaround.

In half of leukemia patients, gene fusions drive their disease. Despite oncogenic fusions representing the ideal biomarker for MRD monitoring in these patients, molecular assays to detect these fusions are available in only a limited subset of cases (e.g. *BCR-ABL*, *RUNX1-RUNX1T1*). This is largely due to qPCR-based assays not being scalable to cover all the potential fusions, reliance on standard curves, and relative quantification. To address this unmet need, we have developed a sensitive, specific, rapid and scalable droplet-digital PCR (ddPCR)-based assay for personalized gene fusion detection. This assay uses fusion break-point information obtained from clinical sequencing to inform a personalized ddPCR assay targeting each individual's disease-defining fusion. As proof of principle, we applied our assay to *KMT2A* fusion detection. We selected *KMT2A* fusions because they are challenging to target for MRD detection given the diverse spectrum of fusion partners and the clinical need to assess response to therapy with novel Menin inhibitors that target these fusions.

In this proof-of-principle study at Washington University, we banked serial specimens from 19 patients with *KMT2A* fusion driven acute leukemia. From sequencing obtained at diagnosis, we determined each patient's fusion breakpoint and designed their personalized fusion detection assay. In diagnostic specimens, these assays reliably detected each patient's oncogenic fusion. We subsequently banked 1-17 serial peripheral blood or bone marrow specimens per patients for longitudinal monitoring. For four patients with treatment failure, we were able to detect persistent/increasing fusion abundance in the peripheral blood up to 100 days before detection by standard of care bone marrow assessment. For the remainder of patients who achieved a remission, we observed clearance of their fusions to a limit of detection of 1:100,000.

This study highlights the utility of our approach for sensitive, personalized fusion MRD detection. This assay has the potential to change the paradigm for MRD monitoring in acute leukemia from discrete testing of bone marrow samples at the end of induction or consolidation to serial peripheral blood monitoring throughout treatment. This is possible because our assay is inexpensive, rapid to turnaround, and highly sensitive in peripheral blood. Long-term this could improve real-time disease burden assessment to improve treatment decision-making and outcomes for patients with any fusion driven leukemia.

#1917 Validation of Oncuria for the early detection of bladder cancer.

Lynne Wilkens[1], Loic Le Marchand[1], Gong Yang[2], Jian-Min Yuan[3], Martha Shrubsole[2], Michael Luu[4], Ian Pagano[1], Sunao Tanaka[4], **Hideki Furuya**[4], Charles J. Rosser[4]

[1]University of Hawaii Cancer Center, Honolulu, HI,[2]Vanderbilt University, Nashville, TN,[3]UPMC Hillman Cancer Center, Pittsburgh, PA,[4]Cedars-Sinai Medical Center, Los Angeles, CA

Objectives: Longitudinal multi-marker combinations have the potential to improve sensitivity while maintaining the high specificity required for early detection of bladder cancer, i.e. before the development of symptoms. Our objective was to identify a multi-marker panel suitable for bladder cancer, where each individual marker has its own baseline, permitting longitudinal algorithm development.
Materials and Methods: In this multicenter case-control study, we measured the 10 protein biomarkers associated with Oncuria (ANG, APE, A1AT, CA9, IL8, MMP9, MMP10, PAI1, SDC1 and VEGF) in mid-stream spot voided urine samples from 247 subjects who would go on to develop bladder cancer in the ensuring 3-60 months and 247 matched controls. Statistical analyses included: reporting mean/median of the biomarkers, bootstrapping analysis, regression analysis with the biomarkers as explanatory variables, logistic regression analysis to generate the nonparametric receiver operating characteristic (ROC) curve for each biomarker and using a generalized estimating equation (GEE) model to depict difference between biomarkers from cancer and control subjects at the varying time points.
Results: Urinary concentrations of all 10 biomarkers were elevated in patients who went on to develop bladder cancer compared with non-bladder cancer with significance approached in all biomarkers except MMP10. Furthermore, the following biomarkers were significant elevated in bladder cancer subjects vs controls at 12 months prior to bladder cancer presentation; angiogenin at 12 months ($p=0.008$), APOE at 12 months ($p<0.001$), CA9 at 12 months ($p=0.010$), MMP10 at 12 months ($p=0.002$), MMP9 at 12 months ($p=0.011$), PAI1 at 12 months ($p=0.005$), SDC1 at 12 months ($p=0.003$), and VEGF at 12 months ($p=<0.001$). IL8 approached significance at 12 months ($p=0.067$) while A1AT approached significance at 24 months ($p=0.070$).
Conclusions: In this case-control multicenter study assessing 10 protein biomarkers in voided urine, we found clinically relevant differences in 9 of the 10 biomarkers 12-60 months prior to bladder cancer subjects developing cancer compared to matched controls. Therefore, this multiplex urine-based immunoassay test holds promise for facilitating the early detection of bladder cancer in at risk patients, i.e., heavy smokers (>20 pack-year history) over the age of 50 years and help to identify early stage cancers when they are more amenable to our current treatment strategies.

#1918 Investigation of early dissemination in ductal carcinoma in situ patients using circulating tumor cells.

N. Nagpal[1], B. Rupp[1], Y. Hong[2], F. Behbod[2], M. Wicha[1], S. Nagrath[1];
[1]University of Michigan, Ann Arbor, MI, [2]University of Kansas Medical Center, Kansas City, KS

In this study, circulating tumor cells are enriched from blood samples of ductal carcinoma in situ (DCIS) patients and investigated as a potential prognostic biomarker. Raised awareness of the importance of early diagnostics in breast cancer is leading to an increased incidence of DCIS detection through mammographic screening. DCIS is widely defined as a 'Stage 0' carcinoma with neoplastic cells still confined to the basement membrane. However, the 20-year breast cancer mortality rate following a DCIS diagnosis, with or without treatment, remains at 3.3%. Ultimately, the goal of the project is to better inform DCIS patient risk stratification via the isolation and analysis of circulating biomarkers.

We establish a workflow that targets the gaps in traditional tissue-based diagnostics with liquid biopsies. Our study builds on emerging literature on the early dissemination of breast cancer cells by investigating the value of circulating tumor cells (CTCs) as a biomarker in DCIS. To achieve a more holistic understanding of a patient's carcinoma, we supplement our study with the Mouse INtraDuctal (MIND) models - an animal model that recapitulates the tumor microenvironment of DCIS in the mammary ducts of mice.

Methods: We employed a high throughput, label-free microfluidic platform to enrich CTCs from DCIS patient samples. Enriched CTCs underwent single cell RNA sequencing (scRNA-seq) along with matched tissue samples to understand the clonal origin of these cells. Further, to correlate findings in patient samples to an animal model wherein the timeline of progression to invasive disease can be shortened to 9 to 12 months (vs. 10+ years in patients), we analyzed blood samples from 3 MIND models longitudinally.

Results: Applying our workflow to 34 patient samples and 10 healthy controls, we identified CTCs in 76% of the patient samples. The median concentration of CTCs per mL of blood in DCIS patients (6.1 CTCs/mL) was significantly greater than that in healthy controls (0.92 CTCs/mL; $p < 0.0001$, Mann-Whitney U Test). Via scRNA-seq, inferred CNVs were identified in CTCs. Distinct, dominant subclones were also identified in the tissue epithelial cells as potential contributors to the CTC population. In addition, during the MIND model time course study, CTCs were found in circulation as early as 4 months after formation of the models and while the models were still pre-invasive.

Overall, our study enables the assessment of CTCs as a prognostic biomarker for DCIS patients. Not only have we successfully enumerated CTCs in patient samples, but we have also identified distinct subclones in the tissue epithelial cells from which the CTCs could originate, supporting our overarching goal of demystifying early dissemination. Ultimately, we hope to investigate the value of CTCs as a clinical biomarker to inform personalized risk stratification for DCIS patients.

#1919 Development of a diagnostic method for early-stage pancreatic cancer through exosome-derived microRNA profiling.

Takahiko Sakaue, Hironori Koga, Hideki Iwamoto, Toru Nakamura, Atsutaka Masuda, Toshimitsu Tanaka, Hiroyuki Suzuki, Yohei Hara, Kyoyoshi Saito, Yutaka Shimamatsu, Sohei Yoshimura, Shingo Hirai, Hiroya Terabe, Hidetoshi Takedatsu, Takumi Kawaguchi

Kurume University, Kurume, Japan

[Background] Pancreatic cancer has an extremely poor prognosis, making early detection and treatment crucial. We have previously identified pancreatic cancer-specific exosomal microRNAs (Ex-miRs) from pancreatic juice in close contact with pancreatic cancer (Patent pending: PCT/JP2021/026597). [Purpose] To compare the diagnostic capability of pancreatic cancer-specific Ex-miRs in pancreatic juice and serum with existing tumor markers, and to evaluate their potential as non-invasive early diagnostic tools by investigating their detectability in stool samples.
[Methods] We initially performed miRNA array analysis using pancreatic juice-derived Ex-miRs and identified pancreatic cancer-specific Ex-miRs through our proprietary methodology. qPCR was conducted using pancreatic juice from 15 pancreatic cancer patients (Stage 0/I/II/III/IV: 0/0/6/3/6) and 11 chronic pancreatitis patients. Subsequently, we analyzed serum samples from 4 pancreatic cancer patients and 5 chronic pancreatitis patients who provided both pancreatic juice and serum. We compared Ex-miR expression levels before and after curative resection in two Stage II cases and performed in situ hybridization (ISH) on one resected specimen. Further qPCR analysis was conducted on serum samples from 75 cases, including 40 pancreatic cancer patients (Stage 0/I/II/III/IV: 0/6/8/4/22) and patients with other gastrointestinal cancers. Finally, we analyzed Ex-miR expression in stool samples.
[Results] We identified novel pancreatic cancer-specific Ex-miR-4516 and Ex-miR-4674 in pancreatic juice. In pancreatic juice diagnosis, Ex-miR-4516 and Ex-miR-4674 showed sensitivity, specificity, and accuracy of 80% and 73.3%, 81.8% and 90.9%, and 80.8% and 80.8%, respectively. Combining these Ex-miRs with cytology improved diagnostic accuracy (sensitivity/specificity/accuracy: 93.3/81.8/88.5%). In serum diagnosis, Ex-miR-4516 demonstrated high sensitivity (97.5%) for pancreatic cancer detection, with positive results in all six Stage 0/I cases. Ex-miR-4516 expression significantly decreased after curative resection in two Stage II cases ($p<0.001$). ISH analysis revealed high expression of miR-4516 and miR-4674 exclusively in pancreatic cancer cells. Stool analysis confirmed the feasibility of Ex-miR-4516 extraction and quantitative measurement, showing varying expression levels across samples.
[Conclusions] These two pancreatic cancer-specific Ex-miRs were quantifiable in serum from early-stage pancreatic cancer patients. Serum Ex-miR-4516 showed promise for treatment response assessment and disease monitoring. Furthermore, Ex-miR-4516's detectability in stool suggests potential clinical application as a non-invasive early diagnostic tool for pancreatic cancer.

#1920 XAGE1 cancer-testis antigen is a diagnostic and therapeutic target in lung adenocarcinomas.

Mikio Oka[1], Koji Kurose[2], Yuji Nojima[3], Shinsuke Saisho[3], Katsuhiko Shimizu[3], Toru Oga[2], Masao Nakata[3]

[1]Immuno-Oncology, Kawasaki Medical School, Kurashiki, Japan,[2]Respiratory Medicine, Kawasaki Medical School, Kurashiki, Japan,[3]General Thoracic Surgery, Kawasaki Medical School, Kurashiki, Japan

Introduction: Lung cancer is the leading cause of cancer deaths worldwide, and lung adenocarcinomas (LUAD) occupy approximately 50% among lung cancers. For LUAD, novel target therapies, immunotherapies, and ADCs have been extensively developed. TTF-1 has been widely used in pathological diagnosis of LUAD, although it is generally positive in normal alveolar and bronchial epithelial cells. There have never been LUAD-specific molecules and cancer antigens. On the other hand, we have reported that XAGE1 cancer-testis antigen expression is comparatively specific in LUAD and XAGE1 elicits spontaneous humoral and cellular immune responses in LUAD patients. Additionally, serum XAGE1 antibody (Ab) predicted clinical response to immune checkpoint therapy for non-small cell lung cancer (NSCLC), regardless of tumor PD-L1 status. Here, XAGE1 expression and serum XAGE1 Ab were examined in LUAD including adenosquamous carcinomas.

Patients and Methods: We analyzed 415 (57% [415/727]) LUAD patients among 636 NSCLC and 91 small-cell lung cancer (SCLC) between 2015 and 2024 at Kawasaki Medical School Hospital. XAGE1 was stained in LUAD surgical specimens, using USO9-13 anti-XAGE1 Ab. The patients' sera were obtained at diagnosis, and serum XAGE1 Ab was quantitatively measured by ELISA. The cutoff value of the positive Abs was defined as ≥ 100 titer.

Results: XAGE1 Ab was detected in 20% (84/415) of overall LUAD patients, and 6% (8/134) of stage I, 15% (6/39) of II, 23% (16/69) of III, and 31% (53/169) of IV. XAGE1 protein was positive in 97 (42%) of 231 NSCLC surgical specimens, 93 (52%) of 179 LUAD, and 4 (8%) of 52 non-LUAD. Ninety-three (97%) of 97 XAGE1-positive NSCLC was LUAD, resulted in LUAD diagnostic accuracy of 52% sensitivity and 97% specificity. According to pathological stages of LUAD, XAGE1 protein was positive in 50% (61/123) of stage I, 50% (16/32) of II, and 64% (14/22) of III, regardless of driver gene status. Additionally, there were no differences in positive rates of XAGE1 cells across stages. Serum XAGE1 Ab was positive in 8% (5/61) of XAGE-positive stage I LUAD, 25% (4/16) of II, and 29% (4/14) of III. In analyses of tumor immune microenvironment, LUAD with XAGE1 protein and Ab showed abundant CD8 T-cell and few Treg infiltrations, conversely, LUAD without XAGE1 Ab did few CD8 T-cell and abundant Treg infiltrations.

Conclusions: Our study demonstrated that XAGE1 protein was specifically expressed in approximately 50% or more of LUAD across disease stages but XAGE1 Ab was detected only in 10 to 30% of LUAD patients. Thus, XAGE1 is promising diagnostic marker in LUAD and novel therapeutic target especially in immunotherapy of LUAD.

#1922 Selective microfluidic capture and molecular analysis of plasma extracellular vesicles from meningiomas.

Abha Kumari[1], Harika Lingamarla[2], Mateo Gomez[3], Scott Smith[1], Kathleen McCortney[3], James Chandler[3], Craig Horbinski[3], David Raleigh[4], Adam Sonabend[3], Stephen Magill[3], Mark Youngblood[3], Sunitha Nagrath[5]

[1]Chemical Engineering and Biointerfaces Institute, University of Michigan, Ann Arbor, MI,[2]Biomedical Engineering and Biointerfaces Institute, University of Michigan, Ann Arbor, MI,[3]Feinberg school of medicine, Northwestern University, Chicago, IL,[4]Radiation Oncology, University of California-San Francisco, San Francisco, CA,[5]Chemical and Biomedical Engineering, Biointerfaces Institute, Rogel Cancer Institute, University of Michigan, Ann Arbor, MI

Abstract: Meningioma (MGM) comprises 39% of all primary intracranial tumors and is classified by the WHO into three grades depending on potential for recurrence. Risk stratification plays a crucial role in determining post-operative therapy and treatment response. Current classifications depend on tumor histology and a small number of genomic markers, though molecular profiling (DNA methylation, copy number, gene expression) may carry higher prognostic value. Minimally invasive liquid biopsy approaches could improve clinical management of MGM by identifying high-risk patients prior to surgery, as well as tumors that have transformed post-operatively. Extracellular vesicles (EVs) are nanometer sized membrane-bound capsules that carry cellular cargo and are abundant in plasma. In this work, we demonstrate the use of a microfluidic based technology (MGMExoChip) to isolate EVs specifically from MGM tissues using a specific protein (MGM-p). Using various characterization methods, we confirmed high capture efficiency and abundant proteins and RNA from 100 µL of plasma. These EVs were isolated from patients with high- and low-grade meningiomas, and then studied using proteomics and mRNA sequencing to identify features that associate with tumor aggression.

Methods: MGMExoChip has a polydimethylsiloxane top and a glass bottom. Experiments were performed using MGM cell-line CH157, as well as primary cell cultures and plasma samples collected from MGM patients. Isolated EVs were characterized using nanoparticle tracking analysis, scanning electron microscopy, western blot, and proteomics. RNA extraction was performed using Norgen Biotek kit, sequenced in Illumina platform at 40M raw reads. Data analyses were done using R.

Results: MGMExoChip was optimized for flow and capture conditions using EVs harvested from CH157 and two patient-derived primary cell cultures. Using MGM-p-based capture, the capture efficiency of EVs was significantly higher in CH157 relative to lung cancer (A549) (80.5±0.9% vs 6.2±4.2%). Among three antibodies tested for capture (anti-CD63, anti-MGM-p, and annexin-V), western blot confirmed that MGM-p had the highest specificity to isolate MGM EVs. We determined that only 100 µL of plasma was needed to extract an average of 7 µg of proteins and 5 ng of RNA without saturating the device. We further utilized proteomics, and mRNA-seq to explore molecular features that distinguish EVs in low and high grade meningiomas from normal controls among a cohort of 19 plasma samples.

Conclusions: The use of microfluidics for liquid biopsy is a novel approach to study meningioma. ^{MGM}ExoChip isolates MGM-specific EVs from patient plasma that allows extraction of molecular cargo for downstream characterization. Investigation of MGM EVs in a larger cohort could identify promising biomarkers for risk stratification of meningioma and determination of post-operative therapy.

#1923 A tract of homozygosity analysis reveals methylation-driven *CSMD1* expression in non-small cell lung cancers.

Jing Jin[1], Yasir Rahmatallah[2], Horacio Gomez-Acevedo[2], Yong-Moon Mark Park[3], David W. Ussery[2], Mohammed S. Orloff[3]

[1]Biostatistics, University of Arkansas for Medical Sciences, Little Rock, AR,[2]Biomedical Informatics, University of Arkansas for Medical Sciences, Little Rock, AR,[3]Epidemiology, University of Arkansas for Medical Sciences, Little Rock, AR

INTRODUCTION: The genetic and epigenetic alterations associated with smoking remain incompletely defined in Non-Small Cell Lung Cancer (NSCLC) carcinogenesis. This study investigates methylation-driven gene expression differences by smoking behaviors in lung adenocarcinoma (LUAD) and squamous cell carcinoma (LUSC) using the Tracts of Homozygosity (TOH) approach.
METHODS: Genetic, epigenetic, and environmental factors were examined to identify potential methylation-driven genes in NSCLC by subtype and smoking status. The analysis incorporated data from a genome-wide association study (GWAS), the Cancer Genome Atlas Program (TCGA), including methylation arrays/RNA-Seq, and clinical information among European Americans. A TOH block is defined as a string of SNP genotypes that harbor both common and rare variants. Multivariable logistic regression models were used to identify significant genes that also overlapped significant TOH clusters and contained differentially methylated regions (DMRs). A refined analysis was performed to assess methylation status at the probe level for all differentially expressed genes in the overlapping TOH regions and DMRs. Further, the multivariable Cox proportional hazards regression model was used to evaluate the association between gene expression levels and mortality risk.
RESULTS: Analysis of differentially expressed genes in TOH regions and DMRs showed hypermethylation at a CpG promoter site (cg08383399) upstream of *CSMD1* in smokers with LUAD and negatively correlated with *CSMD1* expression ($r=-0.18$; $p=0.004$). LUAD smokers with high *CSMD1* expression showed better survival probability than those with low *CSMD1* expression, especially among those with > 30 pack-years of smoking history (Hazard Ratio: 0.57; 95% Confidence Interval: 0.32, 0.99). In addition, we identified several genes related to smoking-induced inflammation from the list of differentially expressed genes overlapping DMRs and differentially methylated probes in TOH regions.
CONCLUSION: The TOH approach identified smoking-associated methylation patterns linked to differentially expressed genes in NSCLC by subtypes. These findings contribute to the identification of smoking-induced biomarkers for NSCLC facilitating personalized screening and suggesting intervention strategies to reduce smoking-related risks.

#1924 Urine extracellular vesicles derived CA9 and CIZ1 as non-invasive biomarkers for early diagnosis of clear cell renal cell carcinoma.

Xiyi Wei[1], Xi Zhang[1], Xinghan Yan[1], Zijie Yu[1], Weiyu Kong[1], Yize Li[2], Chao Qin[1], Hongwei Liang[3]

[1]Department of Urology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China,[2]Division of Oncology, Department of Medicine, School of Medicine, Washington University in St. Louis, St. Louis, MO,[3]School of Life Science and Technology, China Pharmaceutical University, Nanjing, China

Renal cell carcinoma (RCC) is a common malignant tumor of the urinary system, with clear cell renal cell carcinoma (ccRCC) being the most prevalent pathological subtype. Metastasis is observed in 20-30% of patients at initial diagnosis, and 20% of cases develop metastasis even after surgery, resulting in poor prognosis due to insensitivity to radiotherapy and chemotherapy. Early and accurate diagnosis is critical to improving outcomes and survival rates. However, specific humoral markers for non-invasive early screening and diagnosis of RCC are currently lacking. Urinary extracellular vesicles (EVs) have emerged as promising biomarkers for urinary system diseases due to their stability and ease of collection. In this study, we conducted a comprehensive analysis of urine EVs to identify specific biomarkers for ccRCC. Urine samples were collected from 100 healthy donors, 100 renal cyst patients, and 300 RCC patients (200 ccRCC, 50 papillary RCC [pRCC], and 50 chromophobe RCC [chRCC]). Agglutinin-coupled magnetic beads were employed to enrich exosomes, followed by proteomic analysis to identify differential protein expression. Results revealed significant upregulation of CA9 and CIZ1 proteins in urine EVs from ccRCC patients compared to controls. Importantly, these proteins were elevated in early-stage and small-sized ccRCC cases. Stability testing demonstrated that CA9 and CIZ1 proteins in EVs maintained stability over time and increased with urine volume. Diagnostic efficacy was evaluated in a larger cohort (1,500 cases) using receiver operating characteristic (ROC) curve analysis. CA9 and CIZ1 achieved area under the curve (AUC) values of 0.8917 and 0.8843, respectively, in distinguishing ccRCC from normal and other RCC cases. A combined model of the two markers achieved the highest discriminative power, with an AUC of 0.9633, sensitivity of 96.52%, and specificity of 86.69%. This study highlights the potential of CA9 and CIZ1 in urine-derived EVs as non-invasive biomarkers for ccRCC diagnosis. These findings provide a foundation for novel early screening methods and lay the groundwork for future research to validate these biomarkers in larger cohorts and investigate their stage-specific expression, ultimately facilitating personalized diagnosis and treatment of ccRCC.

#1925 Development of a multi-omics diagnostic approach for the early detection of ovarian cancer in asymptomatic women.

Rachel Culp-Hill[1], Charles Nichols[1], Collin Hill[1], Brendan Giles[1], Robert A. Law[1], Enkhtuya Radnaa[1], Kian Behbakht[2], Benjamin G. Bitler[2], Maria Wong[1], Connor Hansen[1], Mattie Goldberg[1], Vuna Fa[1], Violeta Guthrie[1], James R. White[3], Anna Jeter[1], Abigail McElhinny[1]

[1]AOA Dx, Inc., Denver, CO,[2]University of Colorado Anschutz Medical Campus, Aurora, CO,[3]Resphera Biosciences, Baltimore, MD

Objectives: Ovarian cancer (OC) is the fifth leading cause of cancer-related deaths among women. Unfortunately, for most patients, detection of OC occurs at late stages (III/IV) when the five-year survival rate is <30%. To change this paradigm, screening methods must be developed that are minimally invasive, highly sensitive, and disease specific. We have determined that utilizing multi-omics, which include novel classes of lipid and protein biomarkers with machine-learning, enables the robust detection of ovarian cancer across stages and subtypes, while requiring <500µL of serum.

Methods: We utilized a multi-omics approach to characterize a clinically annotated cohort comprising serum samples from individuals with ovarian cancer (N=235) and normal donors (N=82). The cohort was obtained from the University of Colorado Gynecologic Tissue and Fluid Bank (IRB #07-935 and 21-4787) and commercial vendors. UHPLC-MS data were generated from 20µL of extracted serum to profile lipids and fatty acids, while manual immunoassays were performed for a panel of protein biomarkers using unextracted serum. Machine learning (ML) model training was performed using the biomarker classes separately and in combination to identify top-performing models, using 20-fold cross validation.

Results: We profiled a total of 611 features of the lipidome by UHPLC-MS and protein biomarkers by immunoassay. The main molecular drivers contributing to best performing initial OC-specific signatures included a combination of lipids, fatty acids, and proteins together: multi-omic model consistently exhibited highest AUC when compared to individual biomarker classes. The AUC for High Grade Serous OC (HGSOC) was 0.98. At 98.2% specificity, sensitivity was 93.1% for all stages and subtypes of OC (93.8% sensitivity for all subtypes of early-stage (I/II) OC). The sensitivity for early stage HGSOC was 92.6%.

Conclusions: Early detection of OC is critical to improve patient outcomes, but current screening tools for OC lack early-stage sensitivity and specificity. The application of a multi-omics, machine learning approach for the screening of post-menopausal asymptomatic women offers significantly improved performance over CA125 for the detection of early-stage HGSOC. Future research will validate the performance of this approach in a large prospective cohort.

#1927 High-sensitivity screening of a large number of samples for KRAS and PIK#CA mutations using digital PCR.

J. Qin;
QIAGEN, Inc., Germantown, MD

Colorectal cancer (CRC) is one of the top commonly occurring cancers with 1.9 million new cases and 935,000 deaths worldwide in 2020 according to CDC. KRAS plays an important role in colorectal metastasis and progression, affects the tumor microenvironment and has been implicated in angiogenesis. KRAS mutations are the most common, presenting in approximately 25% of all tumors and is one of the most common gene mutations linked to cancers. It drives 40% of colorectal cancers, 32% of lung cancers, and 85% of pancreatic cancer cases. Phosphatidylinositol 3-kinases (PI3Ks) participate in signaling pathways essential for cell proliferation, adhesion, survival and motility; and are the initiators of carcinogenic process. PI3K mutations produce an altered p110a subunit, which allows PI3K to signal without regulation and initiates broad spectrum carcinogenic process. (Cell Cycle. 2009 May 1; 8(9): 1352-1358). Studies have shown a strong association of simultaneous mutations in PIK3CA exon 9 and KRAS exon 2 were the most common (67.2% of CRC cases) (Oncol.Rep.25(6) (2011) 1691-1697). When that happens, tumors with the bi-mutations were found to be significantly associated with aggressive pathological features compared with WT tumors, for example, increased proportions of proximal colon cancer that were prone to metastasize to distal organs at initial surgery. (Q. Luo et. al. Elsevier Translational Oncology 13 (2020) 100874). There is an increasing need for simple and robust tools to frequently screen and monitor such activating mutations with a sensitivity of 0.05-0.5%, which is suitable for testing of FFPE DNA samples. (I. Oscorbin et. al. Cellpress Heliyon 8 (2022) e11804). Here we introduce a novel digital PCR instrumentation and nanoplate technology that allows the screening of a large number of samples within 2 hours. We selected KRAS/G12S and PIK3CA/H1047R LNA mutational assays from the QIAGEN Geneglobe assay database and tested them against mutants from 2 germline cell models (Horizon Discovery) under various amount of WT gDNA backgrounds. Data shows good detection sensitivity at the 0.05-0.1% level.

#1929 Metabolomic insights into androgen deprivation therapy, and saliva as a non-invasive surrogate for serum to enable community-based biomarker studies.

Alireza Abdshah[1], Mohammad Alyamani[1], Marijo Bilusic[2], Dipen J. Parekh[1], Benjamin Spieler[3], Rakesh Singal[2], Brandon A. Mahal[3], Erin Kobetz[2], Sanoj Punnen[1], Matthew C. Abramowitz[3], Jaime R. Merchan[2], Nima Sharifi[1]

[1]Desai Sethi Urology Institute, University of Miami, Miller School of Medicine, Miami, FL,[2]Sylvester Comprehensive Cancer Center, University of Miami, Miller School of Medicine, Miami, FL,[3]Department of Radiation Oncology, University of Miami, Miller School of Medicine, Miami, FL

Introduction: Androgen deprivation therapy (ADT) significantly alters metabolism in prostate cancer patients. Serum metabolomics are the gold standard for studying these effects; however, saliva offers a non-invasive alternative for biomarker assessment. This study evaluates the impact of ADT on serum and saliva metabolites and the correlation between the two matrices. By evaluating saliva as a potential surrogate for serum, this work explores its application in biomarker analysis of chronic risk factors within community health programs.

Methods: Blood and saliva were collected from 20 eugonadal men and 20 men receiving hormonal therapy for prostate cancer, and were processed per standard protocols and stored at -80°C. Untargeted metabolomics using UPLC-MS analysis of polar and non-polar metabolites were performed; and glucocorticoids were quantified using targeted LC-MS/MS. Data were analyzed using MassHunter, METLIN database, and MetaboAnalyst, with log-transformation and by Mann-Whitney tests (False Discovery Rate (FDR)-adjusted P-values < 0.05).

Results: In untargeted metabolomics, 1,198 metabolites were identified across serum and saliva. Comparing ADT to eugonadal serum, 35 metabolites had fold-changes (FC)>2 and 35 had FC<0.5, with 248 metabolites reaching statistical significance (adjusted-P<0.05). Similarly, in saliva, 54 metabolites had FC>2 and 69 had FC<0.5, with 82 metabolites showing significant differences (P<0.05). Comparing serum to saliva across all participants, 248 metabolites had FC>2 and 268 had FC<0.5, with 697 reaching statistical significance (P<0.05). When integrating fold changes (FC>2 or FC<0.5) and significance thresholds (adjusted-p<0.1), we identified 14 upregulated and 20 downregulated metabolites in serum, and 14 upregulated and 28 downregulated metabolites in saliva of ADT patients compared to eugonadal patients; and 247 upregulated and 268 downregulated metabolites in saliva compared to serum among participants. Targeted metabolomics also confirmed strong correlations between serum and salivary cortisol ($r=0.612$, $p<0.001$) and cortisone ($r=0.618$, $p<0.001$). These findings underscore the potential of glucocorticoids as biomarkers for stress-related metabolic alterations, suggesting that saliva potentially reflects the systemic metabolomic profile. This highlights saliva's promise for non-invasive biomarker studies in stress and broader community health applications.

Conclusion: ADT is associated with significant changes in serum and salivary metabolome, highlighting its systemic and local impact on metabolism. Moreover, targeted metabolomic analysis revealed strong correlations between glucocorticoids in serum and saliva, suggesting saliva may serve as a non-invasive surrogate for serum in biomarker studies to facilitate research in community health programs.

#1930 Advancing cervical cancer early detection: A novel lateral flow immunoassay targeting p16INK4a.

Samrin F. Habbani[1], Sayeh J. Dowlatshahi[2], Scott C. Bolton[2], Lucy T. Tecle[2], Lisa Flowers[3], Jacqueline C. Linnes[2], Sulma I. Mohammed[1]

[1]Comparative Pathobiology, Purdue University, West Lafayette, IN,[2]Weldon School of Biomedical Engineering, Purdue University, West Lafayette, IN,[3]Emory University School of Medicine, Atlanta, GA

Cervical cancer ranks as the fourth most prevalent cancer among women globally, with a stark disparity in disease burden and mortality between high-income and low- to middle-income countries (LMICs). Sub-Saharan Africa faces the highest incidence and mortality rates, primarily due to limited access to preventive measures such as vaccines, screening, early detection, and treatment. With approximately 90% of cervical cancer cases occurring in LMICs, there is an urgent need for effective, affordable screening solutions. This study addresses this critical gap by developing a cost-effective, easy-to-use point-of-care (POC) screening test. Specifically, we aim to create a lateral flow immunoassay (LFIA) to detect the overexpression of cyclin-dependent kinase inhibitor 2A (CDKN2A or p16INK4a), a key tumor suppressor protein overexpressed in cervical cancer. The LFIA is designed to be capable of detecting p16INK4a in precancerous and cancerous cells from cervical swab samples, thereby providing an accessible and efficient tool for cervical cancer screening in resource-limited settings. The developed POC LFIA utilizes antigen-antibody interactions. It has four key components: a sample pad, a conjugate pad, a reaction membrane, and an absorbent pad. Cervical swab samples applied to the sample pad rehydrate the dried conjugates, allowing them to interact with the analyte and migrate to the reaction zone, where a gold nanoparticle (AuNP)-conjugated antibody facilitates detection. Initial validation of p16INK4a levels in clinical swab samples was performed using an enzyme-linked immunosorbent assay, which revealed significant overexpression of p16INK4a in high-grade squamous intraepithelial lesions (HSIL), confirming its role as a biomarker for cervical precancerous conditions. Biolayer interferometry was used to select antibodies with optimal nanomolar to picomolar binding affinities. The chosen antibody was conjugated with AuNPs through passive adsorption, achieving a stable 100:1 molar antibody-to-AuNP ratio over seven days. The test and control lines were optimized using in-house dipsticks, with 1.5 mg/mL anti-mouse immunoglobulin G proving ideal for the control line. The current test can reliably detect p16INK4a concentrations as low as 0.5 µg/mL, with further refinements underway to lower the detection limit for clinical use. This simple and cost-effective LFIA offers robust performance, addressing the critical need for affordable and accessible cervical cancer screening, particularly in LMICs. With continued optimization, this tool holds significant promise for improving early detection and treatment outcomes in resource-limited settings. Keywords: point-of-care test, cervical cancer, p16INK4a, early detection, prevention, LMICs

CLINICAL RESEARCH: Liquid Biopsy: Circulating Nucleic Acids 5 / Circulating Tumor Cells 2
Poster Session

#1933 Improving detection of colorectal cancer recurrence using serial ctDNA measurements: A systematic literature review.

Ning Yan Gu[1], Christine Waters-Banker[2], Gebra Cuyun Carter[1], Danielle Badgley[2], Aleksandra (Sasha) Vidisheva[2], Melanie Palomares[1], Heather Johnson[1], Nathaniel J. Smith[2]

[1]Exact Sciences, Madison, WI,[2]Maple Health Group, LLC, New York City, NY

Introduction: Detecting molecular residual disease (MRD) in solid tumors via circulating tumor DNA (ctDNA) is an emerging practice to effectively assess curative treatment success and inform risk for future recurrence.
Methods: A systematic literature review (SLR) search was executed in PubMed and Embase from 01/01/2018 to 11/01/2023 to identify studies detecting MRD via ctDNA in adult patients with solid tumors after surgical resection. Studies providing diagnostic sensitivity and specificity for recurrence, and clinical lead times for colorectal cancer (CRC) are reported.
Results: A total of 4,308 non-duplicate records were screened by title and abstract; 224 were screened as full texts; 19 of the included studies reported the outcomes of interest in CRC. There was notable heterogeneity related to sample timing and frequency, length of follow-up time, and ctDNA assay methodology. Most patients were stage II/III; median follow-up time ranged from 12.5-49 months. Overall sensitivity for detecting recurrence ranged from 27.0%-100%; specificity from 80.1%-100%. Sensitivity for recurrence for single post-surgical or post-adjuvant samples ranged from 27.0%-67.0% (n=5). Sensitivity was higher for studies employing serial sampling (e.g., every 3-6 months), ranging from 66.7%-100%; with most studies (n=4) reporting values ranging from 82.6%-100%. Importantly, ctDNA detected recurrence several months earlier than radiological imaging for all studies reporting lead time (i.e., difference between ctDNA and radiological detection) (Table).
Conclusion: Variability of ctDNA sensitivity in detecting recurrence may be due to factors related to the heterogeneity of sample timing, follow-up duration, population, and assays. Regardless of the variation in sensitivity, ctDNA appears to be a valuable tool for assessing MRD and detecting recurrence earlier than radiological imaging, especially with repeated sampling.

Table: Diagnostic performance and lead time for included CRC studies

	Sensitivity for Recurrence (Range %)	**Specificity for Recurrence (Range %)**	**Median Lead Time**	**Mean Lead Time**
Overall (All Studies)	27.0%-100% (13 studies, n=1,917)	80.1%-100% (10 studies, n=1,688)	1-11.5 months (6 studies, n=88)	2.6-8.7 months (3 studies, n=36)
Single Sample	27.0%-67.0% (5 studies, n=746)	86.5%-100% (5 studies, n=746)	3 months (1 study, n=9)	2.6 months (1 study, n=3)
Serial Sampling (Predetermined Intervals)	66.7%-100% (5 studies, n=452)	84.0%-100% (4 studies, n=394)	5.3-11.5 months (2 studies, n=23)	5.01-8.7 months (2 studies, n=33)

#1934 Gene expression profiling of circulating tumor cells provides prognostic insights in oral squamous cell carcinoma.

Anshika Chauhan[1], Geeta S. Boora[1], Sushmita Ghoshal[2], Arindam Maitra[3], Arnab Pal[1]

[1]Biochemistry, Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India,[2]Radiotherapy, Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India,[3]BRICS-National Institute of Biomedical Genomics, Kalyani, West Bengal, India

Background: Oral Squamous Cell Carcinoma (OSCC) often presents with bad prognosis due to high frequency of loco-regional metastasis and relapse. Circulating Tumour Cells (CTCs) are precursors for metastasis, can be identified through liquid biopsy, but their molecular analysis has been challenging due to limitations in current isolation and downstream analysis techniques. To address this, we developed an innovative FACS-based method for CTC isolation and explored the prognostic potential of CTC gene expression in OSCC.

Methods: We developed an innovative technique that isolates CTCs by depleting CD45+ hematogenous cells and positively selecting CTCs using four markers— EpCAM, EGFR, CK, and Vimentin (Chauhan et al., 2024) using FACS. Next, we performed whole transcriptome analysis of isolated CTCs and paired primary tumours from 24 OSCC patients. Gene expression signatures in CTCs were validated in separate cohort of 60 patients.

Results: Data analysis revealed distinct gene expression profiles between CTCs and primary tumours, with 521 differentially expressed genes. Interestingly, PADI4 gene, an enzyme that is involved in the citrullination of various proteins was significantly upregulated in CTCs ($p<0.0001$). Validation of this finding in the separate patient cohort showed that patients with detectable PADI4 levels in CTCs had significantly poorer disease-free survival ($p=0.038$, HR=2.571). Interestingly, PADI4 expression was more in CTCs which having EMT signatures. Upon unsupervised hierarchical clustering of CTC transcriptome data, two subclusters of CTCs (namely C1A and C1B) with a total of 372 differentially- expressed between genes between them were found. Patients in CTC cluster C1B had significantly poorer overall survival ($p=0.0028$) and disease-free survival ($p=0.0185$), with a higher incidence of loco-regional metastasis ($p=0.0082$). We also validated topmost upregulated genes in separate cohort of patients and identified that TNFAIP2, NFKBIZ, CTSS and PTGS2 are significantly associated with increased probability of cancer relapse. Multiple logistic regression modelling using these genes identified that cumulative expression of baseline TNFAIP2, NFKBIZ, CTSS and PTGS2 expression can successfully predict recurrence in our cases. The developed model was validated using calibration plot analysis.

Conclusion: Our study demonstrates that the gene expression profiles of CTCs can serve as prognostic indicators in OSCC and we successfully developed a four-gene panel model for predicting relapse in OSCC patients.

#1935 New emerging patients-derived circulating tumor cells (CTCs) as promising tools for cancer research.

Paul Lovell, Karlie Wysong, Fang Tian

American Type Culture Collection (ATCC), Manassas, VA

Circulating tumor cells (CTCs) play a significant role in understanding the mechanisms of cancer biology and, more specifically, metastatic tumors. CTCs are cells from a primary tumor that break away to circulate through the bloodstream and metastasize in other areas of the body. CTCs have been proposed as valuable tools for the early diagnosis, monitoring, and identification of tumor progression as they provide possible new therapeutic targets. However, CTCs have relatively low numbers in the bloodstream as compared to other cell types, which makes isolating and establishing a CTC line difficult. Further, while researchers are able to identify and isolate these cells with new techniques, there is a lack of established CTC lines that are widely available to the research community for basic mechanism studies and translational research. Collaborating with leading research institutions, ATCC® is putting effort into developing methods and protocols for the expansion and characterization of human CTC lines isolated from clinical patient samples. In this study, we demonstrated the propagation and characterization of a melanoma CTC line MEL 167 (ATCC® CRL-3651™), which originated from a metastatic melanoma patient sample. Here, we evaluated the MEL 167 cell line at the genetic and protein levels and assessed its bio-functional aspects. Genetic profiling of MEL 167 by sequencing analysis revealed various oncogenes that may enable the progression of metastasis. Immunofluorescence staining was performed to assess the presence of a distinct melanoma molecular marker panel. Additionally, the drug response to BRAF-inhibitors of MEL 167 was evaluated. We also compared the drug profile of this new CTC model to the established melanoma cell line model A375 and two other drug-resistant melanoma cell lines that were created through CRISPR gene editing. We found that melanoma metastases are highly malignant with a low response rate to immunotherapies and high potential for relapse from drug-resistance stemming from tumor heterogeneity. As a newly emerging CTC model, MEL 167 (ATCC® CRL-3651™) offers a promising tool for pre-clinical studies and for evaluating CTCs in the early diagnosis and monitoring of metastatic melanoma.

#1936 Circulating epithelial tumor cells as a biomarker to predict treatment failure of radiotherapy in breast and prostate cancer.

Dorothea Schott[1], Matthias Maurer[2], Monika Pizon[1], Katharina Pachmann[1]

[1]Laboratory Dr. Pachmann, Bayreuth, Germany,[2]Department of Radiotherapy and Radiation Oncology, University Hospital Jena, Jena, Germany

Background: In primary breast and prostate cancer, radiotherapy is an integral part of the treatment toolbox. However, despite the significant reduction in local relapses in both breast and prostate cancer, distant relapses still occur in a considerable number of patients. Therefore, biomarkers are urgently needed to identify patients at increased risk of developing metastatic disease. Distant metastases arise from cells released from the primary tumor that are capable of forming metastatic tumors in distant organs, ultimately leading to fatal outcomes.
Methods: We have therefore used the maintrac® approach to monitor and characterize circulating epithelial tumor cells (CETC/CTC) numbers during radiotherapy and to correlate them with treatment outcomes.
Results: In breast cancer, an increase in CETC/CTC numbers during the course of radiotherapy was highly predictive of relapse, particularly in the neoadjuvant setting, with a hazard ratio of 4.886 ($p<0.01$). This finding suggests that an increase in CETC/CTC during radiotherapy in breast cancer patients previously treated with neoadjuvant chemotherapy indicates the need for additional treatment following radiotherapy.In prostate cancer, where radiotherapy is increasingly used without surgery due to the advanced age and frailty of patients and the considerable side effects of prostatectomy, adjuvant radiotherapy was administered to 12 patients. Among these, two patients showed an increase in CETC/CTC numbers from mid-treatment to the end of radiotherapy, and both experienced relapse.In patients receiving definitive radiotherapy without surgery, cell counts were available for 27 individuals. Of these, 8 showed an increase in CETC/CTC numbers. Three of the 8 patients experienced relapse, while 5 have remained in complete remission for up to 8 years despite increasing cell numbers. Additionally, 1 out of 19 patients with decreasing cell numbers experienced a local relapse.Although patients with increasing CETC/CTC counts from mid-treatment to the end of radiotherapy had a significantly higher risk of relapse, not all of them experienced relapse. Other cell characteristics, such as PD-L1 expression, might also play a crucial role. In fact, the fraction of PD-L1-positive cells increased from before to after radiotherapy in patients who experienced relapse during adjuvant radiotherapy but decreased in patients who remained in complete remission. This pattern was similarly observed in patients undergoing definitive radiotherapy.
Conclusion: These results highlight the potential of monitoring both CETC/CTC numbers and their characteristics as a prognostic tool. This approach could help identify patients who require more intensive adjuvant treatment, which may contribute to improved treatment outcomes in the future.

#1937 How circulating tumor cells disguise.

Dorothea Schott, Monika Pizon, Andreas Riess, Katharina Pachmann

Laboratory Dr. Pachmann, Bayreuth, Germany

Background: The most threatening aspect of malignant tumors is their ability to develop metastases in distant organs eventually leading to fatal outcome. These metastases must be derived from cells seeded into the circulation from the primary tumor. It is, however, well known that the majority of metastases do not develop during growth of the primary tumor but only months or even years after the removal of the primary tumor. Circulating tumor cells have been claimed to rapidly disappear from the blood after surgical removal of the primary tumor leading to the assumption that they are eradicated with a half- life of few hours. Therefore, already the presence of circulating tumor cells has been (over) interpreted as a sign of metastatic disease. Indeed, initially, we, like most other approaches for circulating tumor cell detection, did not find any tumor cells right after surgery. However, using a standardized approach (1) we were able to detect increased numbers of tumor cells in blood days after surgery not only in lung (2) but also in breast cancer (3). Rapid disappearance of cells shed from the tumor is not necessarily due to eradication of these cells by the immune system but dormant cells obviously can survive due to recirculation into foreign tissues or encryption. The question is: How can cancer cells conceal in the circulation?
Methods: Using the maintrac® approach including resting the samples for 24 hours at room temperature on the shelf lead to detection of cells previously concealed from detection with anti-EpCAM antibody. In addition the presence of platelets was monitored using red fluorescing anti CD36 or anti-CD41/CD61 antibody.
Results: The maintrac® approach allowed detection of live tumor cells seeded at any time into the circulation. When, accidentally, we stained cells and platelets right after blood draw we found aggregations of red fluorescing platelets which upon inspection in transmitted light were shown to be clustered on cells. In time lapse photography on individual cells for 24 hrs it was observed that platelets were increasingly released from the cells and anti-EpCAM staining became more and more apparent. This indicates that platelets initially clustered around epithelial tumor cells prevent anti-EpCAM from binding to the cells and only if platelets are released the cells can be identified as positive cells. Most possibly hypoxic conditions induce release of the platelets.
Conclusion: Rapid disappearance of cells shed from the tumor may be due to occultation of the cells by platelets. Since platelets express PD-L1 this may contribute to suppress anti-cancer immune cell activity.

#1938 Immune regulatory molecule expression on circulating tumor cells and circulating cancer stem cells in breast cancer patients.

Monika Pizon, Dorothea Schott, Erika Schill, Ulrich Pachmann, Katharina pachmann

Laboratory Dr. Pachmann, Bayreuth, Germany

Background: Circulating Epithelial Tumor Cells (CETCs/CTCs) are known to evade host immune surveillance by modulating the expression of immune regulatory molecules such as PD-L1 and CTLA-4 in cancer patients. Circulating Cancer Stem Cells (cCSCs) play a critical role in cancer recurrence and metastasis. Although the exact mechanisms underlying immune evasion by cCSCs remain unclear, recent studies suggest that the increased expression of specific immune checkpoint molecules on these cells may facilitate immune evasion and enhance their self-renewal capacity. In this study, we analyzed CETCs/CTCs and cCSCs for the expression of immune checkpoint molecules, particularly PD-L1 and CTLA-4, as non-invasive biomarkers of immune evasion and potential predictors of immunotherapy efficacy.
Methods: The study included 80 patients with breast cancer at various stages of the disease. CETCs/CTCs and cCSCs determination was performed using maintrac and the sphere formation assay, respectively. Additional staining with anti-PD-L1 and anti-CTLA-4 antibodies was used to assess the expression of PD-L1 and CTLA-4 on both CETC/CTCs and breast tumorspheres.
Results: CETCs/CTCs were detected in all patients, with counts ranging from 100 to 1150/ml of peripheral blood. The proportion of PD-L1-positive CETCs/CTCs ranged from 25% to 89%, with significantly higher PD-L1 expression observed in patients with triple-negative breast cancer (TNBC) compared to those with other biological subtypes. The proportion of CTLA-4-positive CETCs/CTCs ranged from 10% to 66%, which was significantly lower than PD-L1 expression levels. The expression of immune checkpoint molecules on CETCs/CTCs exhibited heterogeneity both within and between patients. The number of tumorspheres increased significantly with tumor progression and the aggressiveness of the primary tumor. All tumorspheres in all patients expressed both immunoregulatory molecules, PD-L1 and CTLA-4, with substantial heterogeneity in their expression levels.
Conclusion: The findings indicate that evaluating PD-L1 and CTLA-4 expression on CETCs/CTCs and cCSCs from breast cancer patients is both feasible and informative. The quantity of tumorspheres cultured from peripheral blood serves as a direct marker of the primary tumor's aggressiveness and proliferative capacity. Tumorspheres with elevated PD-L1 and CTLA-4 expression may play a key role in immune regulation. Gaining deeper insights into the interplay between cCSCs and tumor immunology could pave the way for strategies to target this small, therapy-resistant subpopulation more effectively.

#1939 The potentiality of capillary electrophoresis as a novel detection method for circulating tumor cells.

T. Yamamoto, H. Fukita, R. Nishizawa, K. Mitamura, A. Taga;
Kindai University, Osaka Prefecture, Japan

Background: Circulating tumor cells (CTCs) are involved in metastasis, and detection of CTC has clinical utility as a biomarker for diagnosis of metastatic cancer patients. Therefore, detection of CTCs from blood is one of the most important approaches to identify metastatic cancer. However, the number of CTCs present in blood is considered less than a few hundred per mL of blood. Therefore, development of sensitive analytical method is required for CTC detection. We previously described a direct cell analysis method based on differences in cell surface charge using capillary electrophoresis (CE). In the present study, we investigate whether this analytical method can detect cancer cells in blood.
Methods: We used the two human colorectal cancer cell lines (DLD-1 and SW480) and one pancreatic cancer cell line (BxPC-3), and blood sample from healthy donor as model samples. To prepare CTC model blood, cancer cells solution was added to blood sample. The stability of CTCs in blood was determined to analyze the model CTC samples after repetition of freeze-thawing. Capillary electrophoresis was performed at room temperature (ca. 25°C) using a linear polyacrylamide-coated fused silica capillary tube (50 cm × 75 μm i.d., effective length 9.5 cm), and 50 mM phosphate buffer (pH 6.8) was used as running buffer. The sample solution was introduced to the anodic end of the capillary with the hydrostatic introducing method (10 cm × 10 s).
Results: Analysis of each sample revealed that peaks corresponding to cancer cells and blood cells were detected at different migration times. To clarify whether cancer cells in blood can be detected, we prepared CTC model blood by mixing cancer cell line and blood. We could successfully detect peaks corresponding to cancer cells in the blood separately from peaks corresponding to blood cells. These findings suggested that cancer cells can be distinguished from blood cells as cancer cell surfaces are more charged than blood cells. On the other hand, peak pattern of CTC model sample which repeated freeze-thawing was not different that of CTC model sample with non-freezing. This finding suggested that stored frozen blood samples might be useful for blood test to detect CTCs using this analytical method by CE.
Conclusion: Therefore, direct cell analysis method using CE might be a potentially novel detection method for CTCs in blood.

#1940 EpCAM and L1CAM expression signatures in circulating tumor cells of advanced colorectal cancer.

G. Felix, J. Gong, F. Aguirre, L. Zhou, R. Ramirez, M. P. Hitchins;
Cedars-Sinai Medical Center, Los Angeles, CA

Background: Colorectal cancer (CRC) is the third most common deadliest cancer among both men and women in the United States. Hence, to improve CRC clinical management, there is an unmet need for affordable and personalized approaches, such as non-invasive blood tests that analyze circulating tumor cells (CTCs). However, there is limited data about the expression of markers associated with epithelial-mesenchymal transition (EMT) of CTCs in advanced CRC. In this study, we sought to evaluate EMT markers signatures in CTCs of advanced cases of CRC.
Method: Peripheral blood samples (10-20 mL) were collected from five patients with metastatic CRC. CTCs were isolated using the Single-cell CellSelect Slides™ enrichment or in-house protocol, followed by FACS (Fluorescence-Activated Cell Sorting). The following markers were analyzed: Ghost Dye™, CD45, CD133, LGR5, L1CAM, CD326 (EpCAM), CD325 (CDH2), CD274 (PD-L1), CD87 (VIM), and Cytokeratin-7/-8. FACS data analysis was done in FlowJo and plugins needed.
Results: The average age of diagnosis was 51.6 years. At the time of blood collection, all CRC patients had stage IV disease and were progressing. Self-organizing map clustering and dimensionality reduction algorithm analysis of 10,197 sorted CD45 negative living cells showed that LGR5 was the least expressed marker. The highest expression of CD133 was associated with highest expression of either EpCAM or L1CAM, which were both inversely correlated with LGR5. Co-expression of EpCAM and L1CAM were negatively associated with CD133, but positively associated with LGR5. EpCAM and L1CAM expression had significant association with CDH2, VIM, Cytokeratin-7/-8 and PD-L1.
Conclusion: In advanced CRC, the mutually exclusive expression of EpCAM and L1CAM in the presence of CD133, and absence of LGR5 could be linked to increased plasticity leading to EMT, and increased risk of metastasis and recurrence. In addition, it is important to consider other markers alongside EpCAM for the identification of CTCs in advanced CRC.

#1941 Development of a multiomic approach for the detection of actionable biomarkers in metastatic breast cancer.

Martin Blankfard[1], David Bourdon[1], Raj Srikrishnan[1], Alessandra Cunsolo[1], Kenneth Valkenburg[2], Cynthia Maddox[2], Renee Sears[3], Taylor Jensen[2]

[1]Epic Sciences, San Diego, CA,[2]Labcorp, Baltimore, MD,[3]Velsera, Boston, MA

Background: Thirty percent of all primary breast cancer patients develop metastatic disease with a 5-year survival rate of 22%. At the forefront of treatment are molecular diagnostic tests that guide treatment options for patients. The multiomic approaches of utilizing protein biomarker detection and next generation genomic analysis has improved blood-based diagnostics. We have coupled the characterization of circulating tumor cells (CTC) by immunofluorescence (IF) assays and single cell measurements of chromosomal instability with next-generation sequencing (NGS) of ctDNA in this multiomic approach. The combined assays identify actionable genomic variants, CTC chromosomal instability and protein biomarkers to characterize MBC.

Methods: Contrived samples were created with cell line cells (CLCs) spiked into noncancerous blood from healthy donors for analytical validation. 46 patients with complete pathology and tissue biopsies were included in the clinical validation. Whole blood was collected in Streck® tubes and centrifuged for the isolation of plasma. cfDNA was extracted from plasma, and actionable variants were identified with PGDx elio plasma complete™ (EPC) NGS assay. EPC is a 521-gene NGS test, validated in a CAP/CLIA laboratory, that detects SNVs, Indels, fusions, CNVs, MSI, and bTMB. Red blood cells were lysed, and all nucleated cells were deposited on glass slides at a density of 3M cells per slide. CTC protein biomarkers were analyzed on the glass slides with IF assays. CTCs were identified as cytokeratin-positive and CD45/CD31-negative cells. CTCs were probed with HER2 and ER IF assays to determine biomarker status. CTCs were isolated and DNA was extracted for single cell sequencing and analysis for chromosomal instability and ERBB2 amplification.

Results: EPC exhibited greater than 93% analytical sensitivity, and specificity. Overall precision was greater than 85%. An LOD of 1 CTC in 6 million cells and range of 1 - 300 cells/slide was reported. Sensitivity, specificity, accuracy and precision of the CTC assays was greater than 90%. 100% of the healthy donors exhibited no presence of CTCs and exhibited no actionable variants by the NGS ctDNA assays. Conversely, 19 (59%) cancer cases were found to have actionable alterations. CTCs were identified in 28 (88%) of the cases. Chromosomally unstable CTCs were found in the 24 (75%) of the patients. HER2-overexpressing CTC were found in 14 (43%) of cases, and concordant with 59% of matched tissue cases by HER2 (IHC). ER-overexpressing CTCs were found in 23 (71%) of the cases and concordant with 68% of matched tissue cases by IHC.

Conclusion: This multiomic approach allowed for the identification of biomarker status in patients with MBC and for identification of actionable variants by NGS ctDNA. These data show the value of using a multiomic approach in liquid biopsy to provide an informed decision for further treatment in the metastatic setting.

#1942 Copy number aberrations in the ctDNA uncover clinical and biological heterogeneity in aggressive B-cell lymphomas.

Maare Arffman[1], Leo Meriranta[1], Judit Jorgensen[2], Marja-Liisa Karjalainen-Lindsberg[3], Klaus Beiske[4], Mette Pedersen[5], Kristina Drott[6], Oystein Fluge[7], Sirkku Jyrkkio[8], Peter Brown[9], Harald Holte[10], Sirpa Leppa[1]

[1]Research Programs Unit and Department of Oncology, University of Helsinki and Helsinki University Hospital Comprehensive Cancer Centre, Helsinki, Finland,[2]Department of Hematology, Aarhus University Hospital, Aarhus, Denmark,[3]Department of Pathology, Helsinki University Hospital, Helsinki, Finland,[4]Department of Pathology, Oslo University Hospital, Oslo, Norway,[5]Department of Pathology and Department of Clinical Medicine, Zealand University Hospital and Faculty of Health and Medical Sciences, University of Copenhagen, Roskilde, Denmark,[6]Department of Oncology, Skane University Hospital, Lund, Sweden,[7]Department of Oncology, Haukeland University Hospital, Bergen, Norway,[8]Department of Oncology, Turku University Hospital, Turku, Finland,[9]Department of Hematology, Rigshospitalet, Copenhagen, Denmark,[10]Department of Oncology, Oslo University Hospital and KG Jebsen Centre for B-cell malignancies, Oslo, Norway

Introduction: Circulating tumor DNA (ctDNA) has emerged as a valuable tool in revealing molecular heterogeneity and risk factors of large B-cell lymphoma (LBCL). Several ctDNA estimates, such as variant profiling and minimal residual disease kinetics, have been thoroughly dissected, while multiple other molecular aspects accessible in the liquid biopsy (LB), such as DNA copy number aberrations (CNAs) and changes in serum proteome, remain to be disclosed. We studied CNAs from the ctDNA of LBCL patients treated in a Nordic phase II trial to expand the clinically applicable LB repertoire.

Materials and methods: The patients (n=123) included in the study were young (18-64 years), had clinically high-risk LBCL and were treated in a Nordic LBC-06 trial with dose-dense immunochemotherapy. A duplex adapter informed 750 kb gene panel was applied to pretherapeutic plasma ctDNA samples, and variants and CNAs were called using Illumina's DRAGEN pipeline. Clinical data, ctDNA variants (n=123), and serum protein levels, including inflammatory (PD-L1, IL10, IL18, and CXCL9) and subtype-specific (TARC and TACI) markers measured with Luminex assay (n=122) were explored in the context of CNAs.

Results: Profiling of ctDNA revealed CNAs (range 1-38) in 80 (65%) patients. The detection of CNAs was affected by ctDNA variant allele frequency. The most recurrently altered regions included gains in the *REL* (2:p16) and *BCL6* (3:q27) genes and losses in the *CDKN2A/B* (9p:21) and histone (6p:22) loci. Comparison of *TP53* status (loss in 11 (9%) patients) with clinical data uncovered that *TP53* assessment from the LB was more predictive than routinely performed FISH-informed *TP53* analysis for overall survival (OS; HR 7.23, $p = 0.001$ vs HR 2.84, $p = 0.09$). Moreover, loss of *TP53* correlated with P53 immunohistochemistry positivity ($p < 0.001$) and remained prognostic for OS in multivariable analysis together with age-adjusted International Prognostic Index (HR 8.49, $p < 0.001$). Correlation with serum protein profiles showed associations between high levels of inflammatory proteins and CNAs in key oncogenic pathways. These included gains in the NFκB pathway genes *MALT1* ($p < 0.001$) and *REL ($p < 0.01$)*, immune evasion genes, such as *CD274 ($p < 0.05$)*, and losses in the tumor suppressor genes *PRDM1* ($p < 0.05$) and *CDKN2A ($p < 0.05$)*. Furthermore, extended LB analysis of serum TARC levels, ctDNA variants, and CNAs revealed a subgroup of patients initially diagnosed as diffuse LBCL with a primary mediastinal B-cell lymphoma-like molecular fingerprint.

Conclusions: Together, we show that minimally invasive CNA profiling improves risk stratification and molecular characterization of LBCL subtypes. By integrating several LB approaches, we present CNA landscapes that reflect immune evasion and a more aggressive clinical course of the disease and provide potential actionable vulnerabilities for clinical decision-making.

#1943 Promoter methylation as a cancer biomarker: insights from ctDNA-based targeted methylation data.

Margaret Antonio, Eran Mick, Roham Razaghi, Chun Zhang

GRAIL Inc., Menlo Park, CA

Introduction: Promoter methylation is a hallmark of cancer and holds promise as a biomarker for early cancer detection, prognostication, treatment guidance, and response monitoring. This study explores the utility of GRAIL's ctDNA-based targeted methylation assay in detecting gene-specific promoter methylation signals and assessing their potential as cancer biomarkers.
Methods: A comprehensive literature review was conducted to identify genes with promoter methylation proposed as a cancer biomarker, focusing on those covered by GRAIL's ctDNA targeted methylation assay. We then prioritized genes implicated in multiple cancer types across several studies. As a proof-of-concept, four genes (*EYA4*, *SFRP1*, *BNIP3*, *ASCL1*) were evaluated using the ctDNA targeted methylation data from the Circulating Cell-free Genome Atlas (CCGA; NCT02889978) study. Contrived samples with known methylation fractions were utilized to establish the limit of detection (LoD) and linearity. Our analysis included 6127 CCGA cancer samples, spanning 21 cancer types, and 2323 non-cancer samples. We estimated the fraction of methylated fragments in each promoter region for all samples and the prevalence of promoter methylation for each cancer type. A Cox proportional hazard model was employed to evaluate the association of promoter methylation for a given gene with 5-year overall survival, adjusting for cancer stage.
Results: *EYA4* promoter methylation was prevalent in colorectal (39%,108/279, 95% CI: 32%-44%) and liver/bile duct cancer (68%,47/69, 95% CI: 56%-79%). Significant association of *EYA4* promoter methylation with survival was observed in colorectal cancer (HR=2.79, p<0.001) and in the pan-cancer setting (HR=1.71, p<0.001). *SFRP1* promoter methylation was significantly associated with poorer survival in lung (HR=1.62, p=0.006), colorectal (HR=1.96,p=0.036) and stomach cancers (HR=2.79, p=0.048). *BNIP3* promoter methylation, reportedly associated with chemoresistance, was prevalent in colorectal (37%, 104/279, 95% CI: 32%-43%) and pancreatic (34%, 70/205, 95% CI: 23%-41%) cancer, with significant association with worse survival (pancreatic: HR=2.72, p<0.001; colorectal: HR=1.98, p=0.009). *ASCL1* promoter methylation correlated with clinical HPV status of HPV-associated cancers, with higher fraction of methylated fragments in HPV-positive head and neck and cervical cancers (p=0.015).
Conclusion: This proof-of-concept study underscores the potential of GRAIL's ctDNA-based targeted methylation assay in detecting clinically informative promoter methylation signals. It also demonstrates the value of the CCGA cohort in facilitating biomarker discovery in plasma samples across multiple cancer types. Further validation and investigation in therapeutic settings are necessary to fully leverage promoter methylation as a biomarker in precision oncology.

#1944 Longitudinal circulating tumor DNA (ctDNA) analysis and acquired mechanisms of resistance to osimertinib (osi) in the China cohort of the FLAURA study.

Ying Cheng[1], **Dan Wang**[2], Jacqulyne Robichaux[3], Yong He[4], Wei Li[5], He-long Zhang[6], Qing Zhou[7], Buhai Wang[8], Chunling Liu[9], Yao Wang[10], Xiangning Huang[11], Zhanjian Dong[10], Rui Mao[12], Tianyuan Zhou[2], Ryan Hartmaier[3]

[1]Department of Medical Oncology, Jilin Provincial Cancer Hospital, Changchun, Jilin, China,[2]Translational Medicine, Oncology R&D, AstraZeneca, Shanghai, China,[3]Translational Medicine, Oncology R&D, AstraZeneca, Boston, MA,[4]Respiratory Disease, Daping Hospital, Chongqing, China,[5]The First Hospital of Jilin University, Changchun, Jilin, China,[6]Tang du Hospital of Fourth Military Medical University, Xi'an, Shaanxi, China,[7]Guangdong Lung Cancer Institute, Guangdong Provincial People's Hospital and Guangdong Academy of Medical Sciences, Guangzhou, China,[8]Department of Oncology of Northern Jiangsu People's Hospital Affiliated to Yangzhou University, Yangzhou, China,[9]Tumor Hospital, Xinjiang Medical University, Urumqi, Xinjiang, China,[10]Biometrics, Oncology R&D, AstraZeneca, Shanghai, China,[11]Global Medicines Development, GMED Oncology, AstraZeneca, Cambridge, United Kingdom,[12]Clinical Science, Oncology R&D, AstraZeneca, Shanghai, China

Background: In the China cohort of the FLAURA study (NCT02296125), first-line (1L) osi demonstrated clinically meaningful progression-free survival (PFS) and overall survival benefits vs comparator EGFR-TKI in Chinese patients (pts) with EGFR-mutated (EGFRm) advanced NSCLC. This exploratory longitudinal ctDNA analysis from the FLAURA China cohort assessed if EGFRm alone vs 168-gene panel (including EGFRm) ctDNA testing identified PD before RECIST PD, the correlation of baseline (BL) plasma EGFRm detection and its clearance with PFS and investigated acquired resistance mechanisms to osi.

Methods: Pts ≥18 yrs with untreated, EGFRm (Ex19del or L858R) locally advanced or metastatic NSCLC were randomized 1:1 to osi 80 mg once daily (QD; n=71) or comparator EGFR-TKI (gefitinib or erlotinib; all sites selected gefitinib 250 mg QD, n=65). ctDNA samples were collected at BL, throughout the osi treatment period, and after investigator-assessed RECIST PD and were sequenced by a capture-based 168-gene panel (Burning Rock OncoCompass^{TM}). ctDNA profiling was assessed for acquired resistance and vs PFS for molecular response and ctDNA PD.

Results: Overall, 2426 ctDNA samples from 127 pts in China were analyzed. At BL, pts with undetected plasma EGFRm (n=17, 13%) had longer median PFS (mPFS) vs pts with detected EGFRm (n=110); 20.6 mo vs 13.8 mo, respectively (HR 0.68; 95% CI 0.33, 1.25). At Week 3, 64% (38/59) and 72% (36/50) of pts in the osi and comparator arms, respectively, had EGFRm clearance. In both arms, pts with EGFRm clearance had longer mPFS than pts with non-clearance (osi: HR 0.38; 95% CI 0.19, 0.77; comparator: HR 0.23; 95% CI 0.12, 0.48); a positive association with mPFS was also seen at Week 6. When analyzing all mutations (mut) in the 168-gene panel at Week 3, the results were consistent; those pts with EGFRm clearance also had clearance of other BL detected mut. For pts with samples at Week 3 and RECIST PD, 52/81 (64%) had EGFRm ctDNA PD (by predefined criteria) that was equivalent (within 1 week) or earlier than RECIST PD, with median lead times of 2.4 and 2.1 mo for the osi and comparator arms, respectively. Similarly, using the entire 168-gene panel, 56/81 pts (69%) had total ctDNA PD equivalent/earlier than RECIST PD, with median lead times of 2.7 and 2.8 mo for the osi and comparator arms, respectively. In 35 pts in the osi arm with detected EGFRm at BL and PFS events, the most common acquired mut in the last 3 samples up to RECIST PD were MET amplification (6%), EGFR L718Q (6%), PIK3CA mut (6%) and CDKN1B/CDKN2A (6%).

Conclusions: BL undetected or early clearance of EGFRm ctDNA were associated with longer mPFS. Longitudinal ctDNA analysis could detect molecular PD before RECIST PD. Analysis of EGFRm was as informative as a 168-gene panel in its association with PFS and detection of molecular PD lead time. Acquired resistance mut profiles to 1L osi were consistent with prior reports.

#1945 High-sensitive gastrointestinal cancers early detection by cell-free DNA targeted methylation sequencing with an innovative modeling approach.

Ping Lan[1], Zhihua Liu[2], Hong Xu[3], Xiaosheng He[1], Zhigang Li[4], Lei Lian[1], Yin Li[2], Songbing He[5], Xiaofeng Guo[6], Qun Zhao[7], Jianhong Lian[8], Zhijian Xu[2], Nan Lin[9], Jiandong Tai[3], Quan Wang[3], Feng Li[8], Jian Li[10], Lulu Zheng[10], Zhihui Xu[10], Yanzhan Yang[10], Yonghui Li[10], Baoliang Zhu[10], Xiaohui Wu[10]

[1]The Sixth Affiliated Hospital, Sun Yat-sen University, Guangzhou, China,[2]Cancer Hospital, Chinese Academy of Medical Sciences, Beijing, China,[3]The First Hospital of Jilin University, Changchun, China,[4]Shanghai Chest Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China,[5]The First Affiliated Hospital of Soochow University, Suzhou, China,[6]Anyang Cancer Hospital, Anyang, China,[7]The Fourth Hospital of Hebei Medical University, Shijiazhuang, China,[8]Shanxi Cancer Hospital, Taiyuan, China,[9]The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, China,[10]Shanghai Weihe Medical Laboratory Co., Ltd, Shanghai, China

Background: Gastrointestinal (GI) cancers, including esophageal squamous cell carcinoma (ESCC), colorectal cancer (CRC) and stomach adenocarcinoma (STAD), contribute to nearly one-third of cancer-related deaths in China. The late-stage diagnosis of these cancers is largely due to limited access to endoscopic procedures and low screening adherence. A blood-based multi-cancer early detection (MCED) test offers a more convenient alternative; however, the efficacy in detecting early-stage cancers remains unsatisfactory. This study presents an MCED assay utilizing targeted DNA methylation sequencing to detect GI cancers by implementing an innovative modeling approach integrating synthesized early-stage cancer samples.

Methods: A targeted sequencing panel was developed to identify tumor-specific hyper- and hypo-methylation markers. A cohort consisting of 1,618 plasma samples (238 ESCC, 262 CRC, 309 STAD, 809 non-cancers controls; stage: I 15%, II 29%, III 40%, IV 16%) was collected from a prospective, multicenter study (the PROFUTURE study, NCT05874648) and randomly split into training and validation sets (4:1) with 20 iterations of splitting. A novel Methylation-Guided Synthetic Sample (MGSS) model was trained through a combination of real and synthetic samples using a machine learning XGBoost algorithm, validated by real samples. In each iteration, synthetic samples were generated by blending late-stage cancer plasma samples with a pool of 20 independent healthy plasma samples, optimized to closely mimic authentic early-stage methylation patterns. And the number of synthetic samples was set to half the number of cancer samples in the training set.

Results: The MGSS model demonstrated high performance, achieving an area under the curve (AUC) of 0.934 (95% CI: 0.912-0.956), with a sensitivity of 69.7% (95% CI: 64.0%-75.5%) at a specificity of 98.8% (95% CI: 96.4%-100%). CRC exhibited the highest sensitivity at 83.8% (I 47.9%; II 81.4%, III 88.7%, IV 100.0%), followed by ESCC with a sensitivity of 77.6% (I 36.9%; II 67.6%; III 95.0%; IV 100.0%), and STAD at 53.7% (I 21.1%; II 46.9%, III 69.2%, IV 90.3%). Additionally, the sensitivity for stage I-III GI cancers was 68.1% (CRC: 82.8%; ESCC: 76.6%; STAD: 50.5%).

Conclusions: The MGSS model, which leverages synthetic sample methodologies, represents a cost-effective advancement and performance improvement in GI cancers. This innovative approach holds promise for enhancing the performance of MCED for other cancers potentially improving early diagnosis and outcomes.

#1946 Postoperative lymphatic exudate in HPV-negative head and neck cancer detects recurrence prior to adjuvant treatment decisions.

Seka Lazare[1], Zhuosheng Gu[1], Noah Earland[2], Adam Harmon[1], Maciej Pacula[1], Megan Long[1], Ashley Tellis[1], Damion Whitfield[1], Adam Benson[1], Lucien Khalil[3], Robert L. Ferris[3], Marra Francis[1], Aadel A. Chaudhuri[4], Jose P. Zevallos[3], **Wendy Winckler**[1]

[1]Droplet Biosciences, Inc., Cambridge, MA,[2]Washington University School of Medicine, St. Louis, MO,[3]University of Pittsburgh Medical Center, Pittsburgh, PA,[4]Mayo Clinic, Rochester, MN

Recurrence after surgery remains a major cause of failure in head and neck squamous cell carcinoma (HNSCC), particularly for HPV-negative patients whose 2-year failure rate is > 50%. There is unmet need for an accurate diagnostic test that predicts risk of recurrence prior to adjuvant therapy selection. We evaluated ctDNA in lymphatic exudate ("lymph") collected via surgical drains at 24 hours to detect molecular residual disease (MRD) and compared its performance to plasma and adverse pathology features.
Lymph, plasma and blood were collected from 76 HPV- HNSCC patients 24 hours after surgery. Tumor tissue was collected at surgery. 73 patients passed QC. Samples were sequenced using a 699-gene custom panel to a median of 6322x (lymph and plasma cfDNA) or 533x (tumor and blood genomic DNA). Tumor variants were directly genotyped in lymph and plasma using a tumor-informed pipeline. MRD status was determined using a calibrated cutoff based on mean variant allele fraction. Wilcoxon signed-rank test was used for comparisons. Kaplan-Meier estimator with log-rank test and Cox proportional hazards model were used for survival analysis.
We previously demonstrated that 24 hour postoperative lymph ctDNA detects recurrence in HPV(-) HNSCC across two independent multi-site cohorts and by orthogonal sequencing approaches (sensitivity (SN) = 72%, specificity (SP) = 65%, p = 0.003, hazard ratio (HR) = 3.1, n = 73). When compared to other data available at this early time point (pathology and 24 hour postoperative plasma), lymph ctDNA outperformed each for recurrence detection (extranodal extension (ENE, p = 0.30), lymphovascular invasion (p = 0.02), perineural invasion (p = 0.38), positive lymph nodes (p = 0.03), plasma (p = 0.8)).
We next evaluated lymph ctDNA in a critical subpopulation: patients considered intermediate risk by pathology (negative for ENE or margins but with one or more other adverse pathologic feature). Current NCCN guidelines recommend radiotherapy and to "consider systemic therapy" for these patients. Lymph ctDNA accurately stratified recurrence in this group with high sensitivity (SN = 88%, SP = 69%; P = 0.013, HR = 9.1, n = 24). Within this cohort, 77% of patients who received RT alone and later recurred were positive for lymph ctDNA at 24 hours, suggesting an opportunity to use molecular data in concert with pathology to identify patients who may benefit from adjuvant treatment intensification.
Postoperative lymph represents a novel proximal analyte for MRD detection in HPV- HNSCC designed specifically for use in the immediate post-surgical window when adjuvant therapy decisions must be made. Lymph ctDNA is significantly associated with recurrence and outperforms both time-matched plasma and pathology. Accurate MRD identification at this early timepoint has the potential to augment traditional pathology and personalize adjuvant treatment paradigms in HPV-negative HNSCC.

#1947 Assessment of cancer subtypes across multiple cancer types using a circulating tumor DNA (ctDNA)-based targeted methylation assay.

Joerg Bredno[1], Rita Shaknovich[1], Alexis Thornton[2], Susanne Tilk[2], **Oliver Venn**[2], Chun Zhang[2]

[1]GRAIL, Inc. (Affiliation at time of study), Menlo Park, CA,[2]GRAIL, Inc., Menlo Park, CA

Background: Accurate identification of cancer subtypes is essential for effective diagnosis, prognosis, and treatment selection. Traditionally, histologic type, cell of origin, and underlying pathogenesis are determined using tissue histology, requiring invasive biopsies. This study evaluates the use of a ctDNA-based targeted methylation sequencing assay to identify cancer subtypes across multiple cancer types through a tissue-free blood test.
Methods: Within the Circulating Cell-free Genome Atlas (CCGA, NCT02889978) study, clinical data were recorded and plasma samples processed using the targeted DNA methylation assay. We defined a set of 14 cancer subtypes spanning histologic types (adenocarcinoma, squamous cell carcinoma, neuroendocrine cancer, urothelial carcinoma), lineages of the cell of origin (lymphoid, myeloid, plasma cell, hepatocellular, mesenchymal, melanocytic, mesothelial, Mullerian), or an oncogenic process (human papillomavirus-mediated). Pathology reports were used to assign a subtype to each sample. An “other” category was used for less frequent subtypes not captured by these subtypes. A machine learning classifier was trained using ctDNA methylation data from 3,340 non-cancer and 3,239 cancer participants with informative pathology to predict subtype. GRAIL’s Cancer Research Solution is a tissue-free, Research Use Only (RUO) multi-cancer detection test. To ensure a reliable assessment of the subtypes, cancer patients found positive by the RUO test in the held-out test set were shedding sufficient cell-free DNA and were reported for performance evaluation.
Results: 1,404 out of the 2,669 cancer participants in the held-out test set were detected as cancer by the RUO test. This included 165 stage I, 293 stage II, 416 stage III, 493 stage IV and 37 non-informative stage across 54 different AJCC-defined cancer types. Classifier prediction accuracy was 92.7% (95% CI 91.3-94%, 1,302/1,404) across all 14 subtypes. In the subtypes with more than 50 samples in the data, high accuracy was observed in histologic types (e.g. adenocarcinoma: 730/749 = 97.5%), lineage of the cell of origin (e.g. Mullerian: 91/103 = 88.3%) and oncogenic process (e.g. HPV associated carcinoma: 92/96 = 95.8%).
Conclusions: A ctDNA-based targeted methylation platform based on a previously validated assay demonstrated the ability to accurately predict cancer subtypes in a wide range of cancers using only a single blood draw. It offers potential in clinical decision-making, especially when tissue biopsies are unavailable, by supporting early cancer detection, identifying cancer types with unknown primaries, informing treatment selection, and monitoring resistance mechanisms (e.g. lineage switching). Further validation is needed to enhance personalized cancer diagnosis and therapy in precision medicine.

#1948 Real-world insights into the accuracy and precision of the decentralized MSK-ACCESS powered with SOPHiA DDM solution.

Florian Klemm[1], Alex Tuck[1], Fuad Mohammad[1], Xiaobin Xing[1], Anne Pradines[2], Laura Keller[2], Daniel Kazdal[3], Markus Ball[3], Morten Lapin[4], Oddmund Nordgard[4], Persephone Du-Parcq[5], Alasdair Heasman[5], Sasha Hansel[5], Julia Stevenson[6], Gareth Gerrard[7], Justin Wheelan[8], Eric Antoniou[8], Rehan Faridi[9], Faisal Khan[9], Leah Thompson[10], Thomas Stricker[10], Cristovam Scapulatempo-Neto[11], Marie Piecyk[12], Lea Payen[12], Paul Hofmann[13], Zhenyu Xu[1]

[1]SOPHiA GENETICS, Rolle, Switzerland,[2]Laboratoire de Biologie Medicale Oncologique, Institut Universitaire du Cancer de Toulouse Oncopole, Toulouse, France,[3]Center for Molecular Pathology/Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany,[4]Department of Haematology and Oncology, Stavanger University Hospital, Stavanger, Norway,[5]Cancer Genetics Laboratory at Guy's Hospital, Synnovis, London, United Kingdom,[6]King's Health Partners Cancer Biobank, Guy's Cancer Centre, Guy's Hospital, London, United Kingdom,[7]South East Genomic Laboratory Hub, Guy's & St. Thomas' NHS Trust, London, United Kingdom,[8]Oncology Genomics, BioReference Laboratories, Elmwood Park, NJ,[9]Molecular Diagnostics, OncoHelix, Calgary, AB, Canada,[10]Center for Molecular and Genomic Studies, Tennessee Oncology, Nashville, TN,[11]Diagnosticos da America S.A. (DASA), Sao Paulo, Brazil,[12]LBMMS du CHU de Lyon, Service de Biochimie et Biologie Moleculaire, Hospices Civils de Lyon, Lyon, France,[13]IHU RespirERA, Cote d'Azur University, Nice, France

Objectives: Liquid biopsy (LBx) is a powerful, non-invasive approach to identify treatment selection or resistance markers, assess treatment efficacy, monitor disease, and predict relapse. To facilitate genomic profiling of cell-free DNA (cfDNA) in cancer SOPHiA GENETICS has developed a decentralized version of MSK-ACCESS (Memorial Sloan Kettering Cancer Center - Analysis of Circulating cfDNA to Evaluate Somatic Status). The MSK-ACCESS powered with SOPHiA DDM solution interrogates 146 cancer-associated genes for somatic variants and exhibits a high concordance to the original single-site test. It incorporates white blood cell (WBC) sequencing to reduce the risk of including germline and clonal hematopoiesis (CH) alterations in the tumor variant set. To evaluate the real-world performance of MSK-ACCESS powered with SOPHiA DDM an observational, multi-center study was initiated to evaluate accuracy (concordance to orthogonal tests and tumor tissue analyses) and precision (repeatability & reproducibility).
Methods: In participating laboratories sequencing libraries were generated from peripheral blood plasma cfDNA and WBC DNA samples and a common set of reference materials using MSK-ACCESS powered with SOPHiA DDM and sequenced on Illumina NovaSeq 6000 or NextSeq 2000. Variant detection and annotation of germline and CH variants were performed with the SOPHiA DDM platform. Accuracy was determined in the clinical samples through the concordance to orthogonally reported variants, tumor tissue genomic profiling results and to the reference cfDNA variants. Repeatability and reproducibility were measured through reference material variant allele frequency (VAF) variability.
Results: In clinical samples 94.1% of reported SNVs/Indels with an expected VAF ≥ 0.5% were detected (159/169, positive percent agreement per site: 71.4% - 100%). Across all externally reported variants (n = 246), 58 were not enriched in cfDNA compared to WBC DNA and annotated as germline or CH. The average percent agreement in the 0.5% VAF reference samples was 99.1%. The coefficient of variation of the 0.5% VAF samples had the same magnitude within intra-lab repeatability (0.29, mean VAF 0.48, SD 0.14) as for inter-lab reproducibility comparisons (0.29, mean VAF 0.48, SD 0.14).
Conclusion: Interim real-world results of the MSK-ACCESS powered with SOPHiA DDM solution illustrate its accuracy in clinical samples. By distinguishing cfDNA from CH/germline origin variants, potential biological false positives were removed. Further analyses of patient-matched tissue will be used to establish concordance between cfDNA and tumor tissue genomic profiles and investigate causes of discordance, including biological, technical and protocol factors. The assay’s observed precision demonstrates that site-to-site discordance, a major barrier to the adoption of LBx testing, has been addressed by this solution.

#1949 *ESR1* mutations in circulating tumor DNA (ctDNA) are significant predictors of chemotherapy resistance to paclitaxel and carboplatin in metastatic breast cancer (MBC).

Q. Zhang[1], J. W. Zhang[2], A. Davis[3], J. Jiao[4], P. D'Amico[5], L. Gerratana[6], W. Qin[4], X. Wang[1], A. Chawla[1], L. Flaum[1], L. C. Platanias[1], W. Gradishar[1];

[1]Robert H. Lurie Cancer Center, Feinberg School of Medicine, Northwestern University - Evanston, Chicago, IL, [2]Northwestern University and Whitney Young High School, Chicago, IL, [3]Washington University in St. Louis, St. Louis, MO, [4]Xijing Hospital, Innovation Center for Tumor Immunocytology Therapy Technology, Xijing Innovation Research Institute, Fourth Military Medical University, Chicago, China, [5]Merck, Rahway, NJ, [6]University of Udine, Udine, Italy

Background: *ESR1* (estrogen receptor 1) mutations play a significant role on resistance to endocrine therapy in estrogen receptor-positive (ER^+) MBC. Our previous work demonstrated that monitoring ctDNA ESR1 mutations serves as a predictive tool for poor prognosis in MBC (ASCO 2022 #1057, SABCS 2024 #1421). Here, we report new findings indicating that increasing ctDNA *ESR1* mutations are associated with chemotherapy resistance, an area that has been scarcely explored.

Methods: The study included 158 ER^+ MBC patients enrolled under IRB-approved trial (NU16B06) who received treatments in 2016-2022 at Robert H. Lurie Cancer Center, Northwestern University. The median follow-up period was 126.3 months. Baseline ctDNA was analyzed by Guardant 360 NGS panel. Only patients with *ESR1* mutations were included for statistical analysis (Causal Inference) of correlation between the ESR1 mutations with the chemotherapy resistance.

Results: *ESR1* mutations were identified in 10 hotspots among 40 out of 158 patients. The ctDNA *ESR1* mutations including 8 E380Q (mean 6.3%), 12 Y537S (mean 5.6%), 13 D538G (mean 11.9%), 1 V392I (0.1%), 1 Y537N (1.7%), 1 L536P (3.4%), 1 L536R (11.0%), 1 L536H (23.9%), 1 K520K (0.6%) and 1 K362 (15.8%). The criterion for chemotherapy resistance is defined as a change in the drug following treatment or disease progression observed by the last visit after this treatment. The numbers of cases sensitive vs resistant to chemotherapy along with the corresponding drugs are as follows: Doxorubicin (22 vs 18), Epirubicin (33 vs 7), Pegylated Liposomal doxorubicin (37 vs 3), Carboplatin (25 vs 15), Paclitaxel (21 vs 19), Docetaxel (23 vs 17), Vinorelbine (29 vs 11) , Ixabepilone (34 vs 6), Eribulin (32 vs 8), Capecitabine (17 vs 23), Cyclophosphamide (16 vs 24), Gemcitabine (36 vs 4), and Nab paclitaxel (35 vs 5). Significant differences were observed in rising ctDNA ESR1 mutation levels with a cut-off above 2.9% between the Paclitaxel-resistant group (19 patients) and the Paclitaxel-sensitive group (21 patients), with a chi-square statistic of 6.352 ($P=0.011$). Similarly, significant differences were found in ESR1 mutation levels with a cut-off above 2.1% between the Carboplatin-resistant group (15 patients) and the Carboplatin-sensitive group (25 patients), with a chi-square statistic of 4.177 ($P=0.041$). No significant associations were identified between *ESR1* mutations and resistance to other drugs.

Conclusions: These findings demonstrate for the first time that ctDNA *ESR1* mutations are associated with chemotherapy resistance to both Carboplatin and Paclitaxel. Such mutations contribute to tumor heterogeneity, enabling mutated receptors to support tumor survival despite cytotoxic treatment. Detecting ESR1 mutations in MBC may serve as a consideration for guiding chemotherapy decisions and exploring alternative therapeutics.

#1950 Enhanced detection of pancreatic cancer through methylation-based liquid biopsy: insights into tumor heterogeneity, early alterations, and stromal contributions.

Rui Wang[1], Diego Almanza[1], Mari Olsen[2], Xiaoman Kang[1], Daniel King[3], Maximilian Diehn[1], Ash Alizadeh[1]

[1]School of Medicine, Stanford University, Palo Alto, CA,[2]Stanford University, Palo Alto, CA,[3]Zucker School of Medicine, Hofstra University, Hempstead, NY

Pancreatic cancer is one of the deadliest malignancies, with a high mortality rate attributed to late-stage diagnosis and limited treatment options. Recent advancements in liquid biopsy, particularly through cell-free DNA (cfDNA) analyses, offer hope for early detection and prognosis. Specifically, cfDNA methylation profiling shows promise, outperforming traditional mutation-based methods such as single nucleotide variants (SNVs) and copy number variations (CNVs) in terms of sensitivity and specificity. In this study, we conducted paired CAPP-seq and EM-seq analyses on 29 pancreatic cfDNA samples. Our results indicated that methylation-based detection achieved a detection rate of 62.10% with 95% specificity, significantly higher than the 44.82% detection rate observed with CAPP-seq. Following the application of JEEPERS (Jagged-End Error Polishing of Enzymatically misRepaired Sequences) to mitigate methylation errors from jagged ends, the detection rate further improved to 72.41%. This aligns with existing literature highlighting the superior sensitivity of methylation profiling. We propose three hypotheses to explain this enhanced sensitivity: (1) Some pancreatic tumors exhibit CNVs prior to SNVs or contain subclonal KRAS mutations that are not detectable via SNV-based methods; (2) Methylation alterations may occur early during the PanIN stage, allowing the release of both normal and tumor-derived epithelial cells into circulation, identifiable by tissue-specific methylation markers; (3) PDAC tumors characterized by increased fibroblast content and epithelial-to-mesenchymal transition engage unique methylation signatures. To validate these hypotheses, we analyzed both pancreatic tumor tissues and cfDNA samples using CAPP-seq, LPWGS, emWGS, and single-cell nuclear sequencing data. Our findings confirm that CNVs frequently precede SNVs and highlight that pancreatic cfDNA comprises a notable proportion of epithelial cells. Importantly, methylation-based methodologies are capable of detecting signals from both normal and tumor-derived epithelial cells, leveraging cell-type-specific patterns. In contrast, SNV-based approaches are restricted to tumor-only signals. Furthermore, fibroblast methylation markers elucidate stromal contributions to the overall methylation landscape. These findings underscore the potential of methylation-based cfDNA analysis as a robust method for pancreatic cancer detection. We advocate for further validation through the development of a targeted panel that integrates SNVs, CNVs, and cell-type-specific markers to enhance diagnostic accuracy in clinical settings.

#1951 Inferring immune and tissue cell type contributions to cell-free DNA (cfDNA) with a DNA methylation assay.

M. Gloudemans[1], E. Zotenko[1], T. Banh[1], K. Zhu[1], G. Martovetsky[1], S. Wienke[1], M. Sun[1], A. Selewa[1], S. Liang[2], J. Connolly[2], S. Mortimer[1], N. Vardi[1], E. Tsang[1], D. Kennedy[1], M. Singer[1];
[1]Guardant Health, Palo Alto, CA, [2]Parker Institute for Cancer Immunotherapy, San Francisco, CA

Background:
In tissue-localized disease, dying cells release DNA into the bloodstream, enabling disease detection and characterization via blood draws. Circulating tumor DNA (ctDNA) is a key biomarker for cancer screening and monitoring. Quantification of immune cell- and tissue-derived DNA in plasma can expand the range of conditions identifiable through liquid biopsies. Here, we demonstrate the ability to infer cell type contributions to plasma cell-free DNA (cfDNA) from a clinically validated differential methylation-based cfDNA assay.
Methods:
We first implemented and validated a non-negative least squares cell type deconvolution method that uses single-site methylation (SSM) data. Accuracy was assessed by comparisons to gold-standard CyTOF and RNA-seq-based deconvolution on blood samples, as well as *in silico* simulations in blood and plasma.
Next, we developed a model to estimate immune and tissue cell types contributing to plasma based on data derived from a clinically validated differential methylation-based cfDNA assay. Using molecule counts from >2700 regions, we trained a model to optimize alignment with our validated SSM deconvolution method run on the same samples. Training and evaluation was done using leave-one-out cross validation.
Results:
Within the cellular blood compartment, SSM deconvolution accurately estimated T cell, B cell, and neutrophil proportions compared to CyTOF ($r \geq 0.82$; N=13) and RNA-seq-based deconvolution ($r \geq 0.78$; N=70).
Within the plasma compartment, we used SSM deconvolution to infer, in 106 healthy plasma samples, the frequencies of cell types commonly contributing to cfDNA: T cells, B cells, neutrophils, monocytes, megakaryocytes, erythroid cells, hepatocytes, and endothelial cells. *In silico* simulations showed the limit of detection (LOD90) for these cell types in plasma to be <=1.1% (median=0.28%). Inferred abundances aligned with distributions in published literature (Loyfer et al., Nature 2023).
We compared the hypermethylation assay-derived cell type estimates to our validated SSM deconvolution approach on 106 healthy plasma samples run on both assays. We observed Pearson correlations of 0.66-0.98 for all evaluated cell types except for monocytes ($r = 0.3$; $P<0.003$), indicating accurate estimation of the main cell types contributing to cfDNA in plasma.
Conclusions:
We developed an algorithm using a clinically validated cfDNA assay to accurately infer immune and tissue cell type contributions to plasma cfDNA. This technology has the potential to improve disease detection and management by identifying biological processes involving immune and/or tissue shedding into plasma. Further development of this technology will include application and evaluation on additional sample cohorts, including various disease states.

#1952 Circulating tumor DNA for assessing neoadjuvant treatment response and recurrence risk in rectal cancer patients.

Malene Krabbe Ostergaard[1], Christina Demuth[1], Tenna Vesterman Henriksen[1], Nadia Ogaard[1], Per Vadgaard Andersen[2], Kare Andersson Gotschalck[3], Lene Hjerrild Iversen[4], Claudia Jaensch[5], Mads Falk Klein[6], Jeppe Kildsig[6], Nis Hallundbak Schlesinger[7], Ole Thorlacius-Ussing[8], Claus Lindbjerg Andersen[1]

[1]Department of Molecular Medicine, Aarhus University Hospital, Aarhus N, Denmark,[2]Department of Surgery, Odense University Hospital, Odense, Denmark,[3]Department of Surgery, Regional Hospital Horsens, Horsens, Denmark,[4]Department of Surgery, Aarhus University Hospital, Aarhus N, Denmark,[5]Department of Surgery, Regional Hospital Godstrup, Herning, Denmark,[6]Department of Surgery, Copenhagen University Hospital - Herlev & Gentofte, Herlev, Denmark,[7]Department of Surgery, Copenhagen University Hospital - Bispebjerg and Frederiksberg Hospital, Bispebjerg, Denmark,[8]Department of Surgery, Aalborg University Hospital, Aalborg, Denmark

Introduction: Disease recurrence after surgery is the main factor affecting survival in rectal cancer patients. Hence, identifying high-risk patients is important to optimize postsurgical treatment. In recent years, effective use of neoadjuvant therapy (NAT) has led to increased focus on non-operative “watch-and-wait” strategies. However, clinical modalities for assessing complete response following NAT are not sufficiently accurate for selecting patients suitable for non-operative management. Novel biomarkers such as circulating tumor DNA (ctDNA) offer great potential for predicting neoadjuvant treatment response and identifying patients at risk of recurrence.
Materials and
Methods: The Danish IMPROVE trial (NCT04084249) recruited 2750 stage I-III colorectal cancer patients between 2018 and 2023 with the purpose of evaluating the clinical utility of ctDNA analyses. Of these, the present study includes 114 patients with locally advanced rectal cancer, who were treated with NAT prior to curative-intent surgery in accordance with national guidelines. Plasma samples were collected before NAT, after NAT, and after surgery (n=319). Analysis of ctDNA was performed using a tumor-agnostic droplet digital PCR test targeting three methylation markers. Recurrence-free survival (RFS) was calculated as the time between surgery and radiological recurrence (local or distant), with censoring at the end of radiological follow-up. Overall survival (OS) was calculated as the time between surgery and death from any cause, with censoring at end of follow-up. The median follow-up was 21.9 months (Interquartile Range (IQR) 11.8-36.0) for recurrence and 34.7 months (IQR 22.6-47.0) for survival.
Results: The ctDNA detection rates were 86.8% (n=79/91) pre-treatment, 28.9% (n=33/114) after NAT, and 22.8% (n=26/114) after surgery. Post-NAT ctDNA status was significantly associated with pathologic complete response (pCR; p=0.03, Fisher’s exact). None of the 11 patients with pCR had ctDNA detected following NAT and the sensitivity for detecting residual disease was 32%. Detectable ctDNA was associated with worse RFS and OS, both after NAT (RFS HR 3.2, 95%CI 1.5-6.8, p=0.004; OS HR 3.0, 95%CI 1.1-8.4, p=0.03) and after surgery (RFS HR 8.3, 95%CI 3.7-18.9, p<0.001; OS HR 10.6, 95%CI 3.4-33.4, p<0.001).
Conclusions: In conclusion, we showed that ctDNA status after NAT was significantly associated with tumor response to preoperative therapy and could potentially aid in patient selection for nonoperative management. Moreover, our study demonstrated a strong prognostic value of ctDNA analysis in predicting recurrence and survival outcomes, highlighting its potential to improve postoperative management through risk stratification.

#1953 Detection of clinically actionable somatic variants in post-operative samples from patients with molecular residual disease.

Thereasa A. Rich, Jun Zhao, Christopher Pretz, Yupeng He, Derek Dustin, Katie Quinn, Tingting Jiang, Errin Lagow, Krysten Shipley, Kristin Price, Leslie Bucheit, Kimberly Banks

Guardant Health, Palo Alto, CA

Introduction: Molecular residual disease (MRD) detection via ctDNA analysis refines recurrence risk estimation. Patients with MRD have substantial risk for metastatic disease and may have genomic biomarkers that could inform treatment. We describe detection rates of clinically actionable genomic biomarkers in plasma based on ctDNA fraction (TF) in the post-operative MRD setting.
Methods: Positive Guardant Reveal samples were tested using Guardant360. Reveal is a tissue-free epigenomic assay for post-operative MRD detection and quantitation in stage II-III colorectal (CRC), breast, and lung cancers; Guardant360 is intended for comprehensive epigenomic and genomic profiling (739 genes). Actionable variants were defined based on associations with targeted or immunotherapy benefit referenced in guidelines and those suggestive of another primary cancer (e.g., ESR1 resistance mutations in lung or CRC). We evaluated the association between TF and likelihood of actionable variant detection.
Results: 4,203 Reveal positive samples (median TF 0.17%, IQR 0.05-1%) from 4,165 patients were sequenced on Guardant360. Actionable variants were detected in 28% (1% were suggestive for another primary cancer). Increasing TF was associated with higher likelihood of clinically actionable variant detection. Across all three cancer types, TFs >0.05% had high yield of actionable genomic findings (~36%) and 97% of all actionable variants found were above this threshold (Table 1). Samples <0.05% TF rarely had detectable actionable variants.
Conclusion: These data suggest profiling with Guardant360 is reasonable in MRD-positive samples with TF >0.05%. Reflex testing may have utility for rapid return of genomic data to facilitate treatment planning in these high-risk patients. This paradigm may be particularly beneficial for patients with limited tumor specimens or who acquired resistance mutations to targeted therapy in the (neo)adjuvant setting.

Table 1. Rate of actionable variant detection by MRD TF and cancer type

	CRC	**Breast**	**Lung**	**Overall**
No. samples	2,769	781	653	4,203
% with actionable variant	29%	29%	24%	8%
% >0.05% TF	77%	79%	63%	75%
% with actionable finding by TF				
<0.05%	2%	6%	5%	3%
>0.05%	37%	35%	36%	36%
Proportion of all detected actionable variants by TF				
<0.05%	2%	4%	8%	3%
>0.05%	98%	96%	92%	97%
Top five actionable genes/variants detected in samples with >0.05% TF				
	KRAS (26%), *BRAF* V600E (5%), MSI-H (4%), *NRAS* (2%), *ESR1* (1%)	*PIK3CA* (18%), *ESR1* (12%), TMB-H (7%), *PTEN* (3%), *AKT1* (2%)	*KRAS* (16%), *EGFR* (10%), *ERBB2* (3%), *ESR1* (3%), *MET* (2%)	

#1955 Blood-based mapping of the personalized tumor epigenomic landscape.

Elena Zotenko, Rachel Gittelman, Alan Selewa, Noam Vardi, Tam Banh, Gleb Martovetsky, Oscar Westesson, Emily Tsang, Sara Wienke, Drew Kennedy, **Meromit Singer**, Stefanie Mortimer, Darya Chudova

Guardant Health, Palo Alto, CA

Background: Mapping the epigenetic landscape of tumors provides insights into regulatory and functional aspects beyond somatic mutations, offering valuable information that can influence clinical decisions and benefit the patient journey. Given the challenges of obtaining tissue biopsies, especially in the longitudinal monitoring or progression setting as tumors evolve due to selective pressure from treatment, the advantages of non-invasive liquid biopsies to characterize tumors through blood are highly beneficial. Here, we demonstrate that the epigenetic landscape of a patient's tumor can be evaluated in plasma using an epigenetics circulating tumor DNA assay, providing a promising approach for non-invasive tumor profiling.

Methods: We collected paired tumor and plasma samples from lung, breast and colorectal cancer patients, and applied an analytically validated next-generation sequencing epigenomics hybrid capture assay to measure DNA methylation across over 1.3 million CpG loci. A total of 44 patients (10 colorectal, 13 breast, 21 lung) with circulating tumor DNA (ctDNA) detected at a level of 0.5% or greater were included in the analysis. For each patient, we identified personalized tissue differentially methylated regions (DMRs) as the set of regions that were highly methylated in tissue but showed low methylation in an independent set of 197 cancer-free plasma samples. For each region from a personalized tissue DMR list, we computed the expected count in plasma as a function of tumor fraction and assay efficiency.

Results: Across the 44 analyzed tissue samples, the median number of personalized tissue DMRs identified was 1,133, with a range of 136 to 4,220 and a standard deviation of 850. For paired tissue and plasma samples from the same patient the Spearman correlation between the expected and observed counts in plasma was high across all samples (Mean=0.687, SD=0.120). The correlation between expected and observed signals at personalized tissue DMRs was calculated for all possible plasma and tissue sample pairs, regardless of whether the samples were from the same patient. Notably, for 43 out of 44 plasma samples, the highest correlation between expected and observed plasma counts occurred with the plasma's matched tissue sample (Mean difference=0.196, SD=0.096).

Conclusions: These data demonstrate a strong concordance between a tumor tissue's unique epigenetic landscape and the epigenetic signal in plasma. Furthermore, the finding that each plasma sample correlated most with its paired tissue sample over other pairings indicates that the assay can reliably measure personalized tumor epigenetic features in plasma. This supports the use of an epigenetics-only ctDNA assay for monitoring patients and guiding treatment decisions.

#1956 Comparison of somatic variations between circulating tumor DNA and paired tissue in 1,111 pan-cancer patients.

Meng Zhang[1], Yi Feng[1], Xue Du[1], Changda Qu[1], Meizhu Meng[1], Meiying Ye[1], Min Liang[1], Ziran Yang[1], Wenjuan Gong[1], Xingyu Ma[1], Jialiang Guo[1], Wenmei Li[1], Chenxuan Wang[2], Qiuxiang Ou[2], Sha Wang[2], **Haimeng Tang**[2], Shuqin Jia[1]

[1]Peking University Cancer Hospital & Institute, Beijing, China,[2]Geneseeq Technology Inc, Nanjing, China

Circulating tumor DNA (ctDNA) offers a noninvasive method to assess tumor-specific mutations. This retrospective study investigated the concordance of genomic variations between ctDNA and tissue and its influence factors. Paired blood and tissue samples were collected from 1,111 patients with ten tumor types and underwent sequencing of 425 genes. We evaluated the relationship between the concentration of cell-free DNA (cfDNA), sensitivity of ctDNA, mutation landscape, variant allele frequency, and various clinical parameters. The median concentration of cfDNA and overall sensitivity of ctDNA was 16.8 ng/ml and 61.5%, which correlated positively with clinical stage and tumor diameter and negatively with treatment response. The overall concordance rate of genomic alterations between ctDNA and tissue was 24.6%. The top five altered genes and pathway-based disruption signatures identified by ctDNA profiling were similar to tissue. For mutations with a normalized allele frequency less than 0.2, the proportion of ctDNA-specific mutations was higher than concordant mutations (61.5% and 9.7%). Advanced clinical stage, large tumor diameter, poor treatment response, the presence of liver metastasis, and impaired liver function lead to a high concordance rate. Patients with ctDNA-positive status, subclonal mutations, and more ctDNA-specific mutations had shorter overall survival than those with ctDNA-negative status, no subclonal mutations, and fewer ctDNA-specific mutations, respectively. In conclusion, a higher concordance rate of somatic variations between ctDNA and tissue indicated an advanced clinical stage, larger tumor size, worse treatment response, and presence of liver metastasis. Subclonal or more ctDNA-specific mutations detected were associated with poor prognosis.

#1957 MORIOKA study: Tumor-informed frequent ctDNA monitoring for quantitative tumor burden assessment using digital PCR across 23 cancer types during treatment.

S. S. Nishizuka[1], H. Hiraki[1], A. Yashima-Abo[1], T. Iwaya[2];

[1]Iwate Medical University Institute for Biomedical Sciences, Yahaba, Japan, [2]Iwate Medical University School of Medicine, Yahaba, Japan

Background: The ultimate goal of post-treatment surveillance in advanced cancer is the quantification of tumor burden, represented by somatic mutations identified in the treatment-targeted tumor. However, as most circulating tumor DNA (ctDNA) variant allele frequencies (VAFs) during treatment are below 1%, it has been challenging to measure ctDNA without mutation-specific probes for target sequences. To address this, we developed a digital PCR (dPCR) primer/probe library, OTS-Probes, comprising over 1,000 probes targeting human cancer-related mutations. This study investigates the clinical validity of frequent ctDNA monitoring across 23 types of advanced cancers.

Patients and Methods: From May 2019 to May 2023, 314 patients were enrolled in the MORIOKA study, representing 23 tumor types, including lung (n=57), colorectal (53), esophagus (26), uterus (25), liver (22), stomach (17), ovary (13), brain (12), bone and soft tissue (10), prostate (10), bile duct (10), urothelium (9), renal (8), breast (7), head and neck (7), pancreas (6), bladder (5), malignant melanoma (5), cervix (3), pediatric (3), gallbladder (2), multiple myeloma (2), and unknown primary (2). Tumor-specific somatic mutations were identified and then selected for ctDNA monitoring. Blood samples were collected approximately every three months until patients were censored or observed for up to 730 days. ctDNA VAFs were quantified using dPCR with the OTS-Probes, and ctDNA dynamics were analyzed in conjunction with clinical and imaging data.

Results: As of November 20, 2024, analyzable patients had received at least one treatment regimen across 585 treatment opportunities. A total of 1,568 imaging studies were conducted, averaging 5.6 per patient. Panel sequencing identified somatic mutations for ctDNA monitoring in 89.7% of tumors. During treatment, approximately 90% of ctDNA VAFs from 2,474 time points were below 1%. Among ctDNA-analyzable cases, the median pre-treatment ctDNA VAF was 0.12%, with ctDNA undetectable in 27% of cases. Using OTS-Probes, nearly 80% of somatic mutations were promptly monitored via dPCR without the need for additional validation. Changes in ctDNA VAFs before and after treatment correlated with imaging results: VAFs increased in progressive disease (PD), decreased in partial response (PR), and decreased in many cases of stable disease (SD). Further analyses of ctDNA monitoring by tumor type, treatment regimen, and metastasis pattern are ongoing.

Conclusion: Tumor-informed frequent ctDNA monitoring enabled timely quantitative tumor burden estimation across a broad range of cancer types. ctDNA dynamics were particularly useful in clarifying ambiguous imaging findings, such as decreased ctDNA levels in SD. The timely assessment of ctDNA demonstrated robust clinical validity, supporting imaging diagnoses and treatment decisions.

#1958 Chromatin accessibility landscapes of colorectal cancer development and genome-wide cell-free DNA fragmentation.

Nicholas A. Vulpescu[1], Zachariah H. Foda[1], Pieter H. A. Wisse[2], Christopher Cherry[1], Jamie E. Medina[1], Stephen B. Baylin[1], Kasper D. Hansen[1], Vilmos Adleff[1], Remond J. A. Fijneman[2], Michael A. Beer[1], Robert B. Scharpf[1], Gerrit A. Meijer[2], Beatriz Carvalho[2], Victor E. Velculescu[1]

[1]Johns Hopkins University School of Medicine, Baltimore, MD,[2]Netherlands Cancer Institute, Amsterdam, Netherlands

Introduction: The stepwise progression of colorectal cancer (CRC) from healthy epithelium, to premalignant adenoma, to cancer is accompanied by genome-wide epigenetic reprogramming which may be reflected in circulating cell-free DNA (cfDNA). However, current analyses of these processes have been hampered by the complex mixture of cells associated with colonic epithelial tissue, as well as the absence of suitable adenoma model systems. Cultured colorectal organoids, comprised of pure epithelial cells, could enable insights into the epigenetic changes leading to progression from healthy tissue to cancer. Such features could be instrumental in early cancer detection using genome-wide analyses of cfDNA fragmentation.
Methods: Using 43 patient-derived colorectal organoids derived from healthy, adenoma, and cancer tissues, as well as 10 freshly collected peripheral blood mononuclear cell (PBMC) control samples, we examined the chromatin landscape of tumor development using transposase accessible chromatin analyses with next-generation sequencing. We analyzed enrichment for gapped-kmer abundance to reveal gene regulatory networks underlying differences between tissue types. We defined a consensus set of nucleosome depleted regions (NDRs) for each disease state and identified loci with differential accessibility between PBMCs, colon healthy, adenoma, and cancer tissues. We analyzed these differential loci for fragmentation characteristics in the circulating cfDNA of 250 healthy individuals and 51 patients with metastatic CRC.
Results: Across all organoid samples, we identified a consensus set of 62,890 NDRs. Associating these regions with nearby genes, we identified unique CRC-related signatures comprising genes of known and novel pathways, including those involved in WNT, hippo, and RAS signaling. Additionally, we discovered a gene regulatory network which drives chromatin accessibility differences between the healthy, adenoma, and carcinoma tissue in colon. While most chromatin accessibility differences were shared between adenomas and cancers, we identified 895 chromatin changes which were unique to adenoma. In healthy individuals, we found that cfDNA coverage was negatively correlated with accessibility at white blood cell NDRs (Spearman ρ= -0.56), while in individuals with cancer, changes in NDRs were associated with independent circulating tumor DNA abundance by droplet digital PCR (R^2=0.69, $p<0.001$). Consequently, genome-wide cfDNA fragmentation features could be used to distinguish healthy individuals from those with CRC (AUC=0.94).
Conclusions: These analyses reveal chromatin accessibility landscapes that were unique to different pathologic stages of CRC development, highlighting important genes and networks regulated by chromatin remodeling and providing an avenue for evaluating these through genome-wide analyses of cfDNA fragmentation.

#1959 Variant Origin Prediction (VOP) determines the tumor-somatic, germline, or clonal hematopoiesis (CH) origin of short variants (SVs) detected in liquid biopsy (LBx).

Derek W. Brown, Alexander D. Fine, Daokun Sun, Hanna Tukachinsky, Michael McDevitt, Kerriann Pontbriand, Eliana Polisecki, Angela Kou, Mingyue Li, Shumeng Zhang, Zheng Kuang, Aparna Aiyer, Bahar Yilmazel, Brennan Decker, Jason Hughes, Chang Xu

Foundation Medicine, Inc., Boston, MA

Introduction: Distinguishing the origin of variants detected by LBx is essential as tumor-somatic and germline, but not CH variants, can provide prognostic insights and inform treatment decisions. We describe a machine learning model that generates variant origin predictions for SVs (including base substitutions and short indels) detected by LBx.
Method: For all SVs detected in the FoundationOne®Liquid CDx (F1LCDx) assay, VOP generates a 3-way probability that the variant originated as germline, CH, or tumor-somatic. Tumor-somatic and germline probabilities can be summed to predict that the SV is in the tumor. This model was trained using 1977 F1LCDx results for which matched buffy coat (BC) DNA was also sequenced to equal depth. A variety of features, including fragmentomics and variant allele frequency, but no patient demographics, were used to train the classification model. Probability cutoffs were applied to pathogenic SVs and validated for accuracy from paired BC (658) and reproducibility using LBx replicates.
Results: VOP made accurate best call predictions (>50% probability cutoff) for pathogenic SVs across all origin types, with high sensitivity and high positive predictive value of tumor and CH (PPA > 93%, PPV > 91%). Critically, VOP performs well for SVs with VAFs ≤ 1% (PPV > 90%), as well as in genes known to harbor both CH and tumor-somatic SVs, such as *TP53* (PPV > 88%). For a use case requiring higher confidence in tumor origin, an 80% tumor cutoff maintained high PPA (90%) while increasing PPV overall (99%), including low VAF and *TP53* SVs (PPV > 96%). These cutoffs were assessed in 31 unique samples across 546 replicates and showed high reproducibility (Table).
Conclusion: VOP is a highly accurate and reproducible method to predict the origin of SVs from LBx alone. VOP performs well even when VAF ≤ 1% and in heavily confounded genes such as *TP53*. Increased confidence in variant origin is valuable for improving clinical care.

Table. VOP validation accuracy and reproducibility performance in pathogenic SVs

	Positive Percent Agreement (PPA)	Positive Predictive Value (PPV)	Positive Reproducibility	Negative Reproducibility	PPA (VAF ≤1%)	PPV (VAF≤1%)	PPA (*TP53*)	PPV (*TP53*)
High confidence tumor (germline + tumor-somatic)	90.11% (1458/1618)	98.51% (1458/1480)	98.02% (4909/5008)	90.11% (565/627)	78.80% (461/585)	96.85% (461/476)	84.19% (245/291)	98.79% (245/248)
Best call tumor (germline + tumor-somatic)	94.81% (1534/1618)	96.12% (1534/1596)	98.67% (5250/5321)	94.27% (296/314)	89.23% (522/585)	91.74% (522/569)	93.13% (271/291)	96.44% (271/281)
Best call CH	93.81% (940/1002)	91.80% (940/1024)	94.27% (296/314)	98.67% (5250/5321)	92.54% (583/630)	90.25% (583/646)	94.01% (157/167)	88.70% (157/177)
Best call tumor-somatic	93.61% (1420/1517)	95.82% (1420/1482)	97.13% (4780/4921)	92.02% (657/714)	88.89% (520/585)	91.71% (520/567)	92.71% (267/288)	96.39% (267/277)
Best call germline	99.01% (100/101)	89.29% (100/112)	86.82% (349/402)	98.97% (5179/5233)	NA (n < 5)	NA (n < 5)	NA (n < 5)	NA (n < 5)

#1960 Evaluation of mono- and di-nucleosomal cell-free DNA in fragmentome analyses.

Sarah Short[1], Shashikant Koul[1], Akshaya V. Annapragada[1], Dimitrios Mathios[2], Jamie E. Medina[1], Zachariah H. Foda[1], Carolyn Hruban[1], Elaine J. Chiao[1], Vilmos Adleff[1], Robert B. Scharpf[1], Victor E. Velculescu[1], Jillian Phallen[1]

[1]Johns Hopkins University, Baltimore, MD,[2]Washington University School of Medicine, St. Louis, MO

INTRODUCTION: Liquid biopsies are promising, noninvasive tools for cancer detection. Genome-wide fragmentomes reflect the nucleosomal origin of cell-free DNA (cfDNA) in the blood. Variations in cfDNA concentrations and characteristics of mono-nucleosomal (mono) compared to di-nucleosomal (di) cfDNA have not been well described.

METHODS: We evaluated liquid biopsies from 356 individuals in the LUCAS cohort (Mathios et al, 2021) including 146 without cancer, 85 with benign lung lesions, and 125 with lung cancer. Plasma (2.2-4 mL) was isolated from whole blood, cfDNA was extracted, and genomic libraries for whole-genome sequencing (WGS) were prepared. WGS was performed on the Illumina NovaSeq6000 S4 at 10X coverage. Mono and di cfDNA concentrations were quantified post-extraction and post-library preparation using the Agilent Bioanalyzer at each peak, and post-sequencing using fragment sizes of 0-250bp (mono) or 251-500 bp (di). Chromosomal representation and fragmentation patterns in cfDNA were analyzed using z-scores and short-to-long fragment ratios, respectively. The DNA Evaluation of Fragments for early Interception (DELFI) approach for lung cancer detection in high-risk individuals was used to evaluate total extracted cfDNA concentration (ng/mL) as an additional feature.

RESULTS: The fraction of mono to total cfDNA per mL of plasma increased significantly from cfDNA extraction (a), to library preparation (b), to sequencing (c) in individuals without cancer (median=0.77(a), 0.87(b), 0.92(c); IQR=0.71-0.83(a), 0.85-0.90(b), 0.91-0.94(c); $p<0.0001$ (a vs b and a vs c)), in those with benign lung nodules (median=0.78(a), 0.88(b), 0.93(c); IQR=0.72-0.85(a), 0.84-0.90(b), 0.92-0.94(c); $p<0.0001$ (a vs b and a vs c)), or in patients with lung cancer (median=0.76(a), 0.87(b), 0.92(c); IQR=0.71-0.82(a), 0.85-0.91(b), 0.91-0.94(c); $p<0.0001$ (a vs b and a vs c)). Changes appeared to be driven by decreases in di cfDNA across experimental steps in all individuals. Mono fragment sequences more accurately reflected copy number changes in individuals with or without cancer compared to di fragments. Inclusion of cfDNA concentration (ng/mL), reflective of both mono and di cfDNA, as an additional DELFI feature did not improve lung cancer detection (AUC=0.93, 95% CI: 0.88-0.97) compared to fragmentation and copy number features alone (AUC=0.93, 95% CI: 0.89-0.97).

CONCLUSION: We observed that mono cfDNA was retained, while di cfDNA was largely lost throughout experimental steps. Copy number changes were more apparent from sequenced mono cfDNA compared to di cfDNA. Including total cfDNA concentration as an additional feature to DELFI did not enhance performance.

#1961 Analysis of lung cancer clinical characteristics using cell-free DNA fragmentomes.

Milou Schuurbiers[1], Zachary L. Skidmore[2], Paul van der Leest[3], Jamie E. Medina[2], Garrett Graham[2], Tony Wu[2], Jacob Carey[2], Alessandro Leal[4], Bryan Chesnick[2], Kim Monkhorst[3], Nicholas Dracopoli[2], Robert B. Scharpf[5], Victor E. Velculescu[5], Daan van den Broek[3], Michel van den Heuvel[1], **Lorenzo Rinaldi**[2]

[1]Department of Pulmonary Diseases, Radboud University Medical Center, Amsterdam, Netherlands,[2]Delfi Diagnostics, Baltimore, MD,[3]Netherlands Cancer Institute, Amsterdam, Netherlands,[4]Comprehensive Cancer Center, NYU Langone Health Perlmutter, New York, NY,[5]The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD

Liquid biopsy approaches provide an opportunity for both cancer detection and clinical assessment in a non-invasive manner. Here we apply a blood-based lung cancer screening test to 578 individuals diagnosed with lung cancer through a real-world diagnostic prospective trial (LEMA, NCT02894853). Pre-treatment plasma samples were processed through the DELFI assay, a validated cell-free DNA (cfDNA) test based on a locked machine learning analysis of genome-wide fragmentation patterns. Clinical data, including tumor stage (I=165, II=60, III=147, IV=206), histologic subtypes, lymph node invasion, comorbidities, medications, smoking history, treatment type and overall-survival data were collected from all patients. Tissue molecular profiling was performed to identify actionable alterations in driver oncogenes.

Genome-wide cell-free DNA fragmentome analyses revealed that LEMA patients had similar chromosomal gains (1q, 3q, 5p, 8q) and losses (3p, 4q, 5q, 10q, 13q) to lung cancer patients collected in TCGA (LUAD and LUSC) and L101 trial (NCT04825834), suggesting the landscape of cfDNA alterations are consistent with other lung cancer studies in Europe and the United States.

DELFI fragmentation scores were significantly different amongst both tumor stage ($p<0.0001$, kruskal-wallis) and lymph node invasion ($p<0.0001$, kruskal-wallis), exhibiting progressively higher scores with increasing disease burden. Lung adenocarcinoma displayed lower DELFI scores compared to squamous cell carcinomas, while small-cell lung cancer cases presented the highest scores among all lung cancer subtypes.

Stage III patients not eligible for surgery with curative intent (n=96) had significantly higher DELFI scores compared to surgery-eligible stage III patients ($p=0.02$, Wilcox rank-sum).Localized cancer cases (I-III) experiencing cancer relapse after complete surgical resection also showed significantly higher DELFI scores compared to patients who did not experience relapse ($p<0.01$, log-rank). Metastatic cancer patients, treated with chemotherapy, immunotherapy or targeted therapy had a median overall survival of 8.7 months. For all these treatment types, patients with low DELFI score at baseline (below the median) had longer overall-survival (OS) compared to patients with high DELFI score (above the median $p<0.01$, log-rank).

Neither systemic comorbidities nor medications were found to significantly alter DELFI scores amongst groups ($q>0.05$, Wilcox rank-sum) and cancer cases driven by oncogenic alterations showed equivalent DELFI scores to cases without any driver mutation detected by tissue profiling.

Overall, this study reveals that DELFI fragmentomes can be used as prognostic biomarkers, similarly in lung cancer patients from US and European populations. DELFI scores increase with stage and tumor burden, predict survival, and are not altered by mutational status, comorbidities, or medications.

#1962 Detection of plasma circulating tumor DNA is not affected by kidney and liver function in patients with muscle-invasive bladder cancer..

Deema Radif[1], Sia V. Lindskrog[1], Iver Nordentoft[1], Karin Birkenkamp-Demtroder[1], Mads Agerbak[2], Jorgen B. Jensen[3], Lars Dyrskjot[1]

[1]Department of Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark,[2]Department of Oncology, Aarhus University Hospital, Aarhus, Denmark,[3]Department of Urology, Aarhus University Hospital, Aarhus, Denmark

Introduction: Circulating tumor DNA (ctDNA) has demonstrated significant potential for early relapse detection and therapeutic monitoring in patients with muscle-invasive bladder cancer (MIBC). Advancing its clinical utility requires examining factors that may affect plasma ctDNA levels, including those influencing clearance. Here, we evaluated the association between plasma ctDNA detection and kidney and liver function markers in MIBC patients. Secondly, we explored the association between plasma ctDNA detection and immune markers, as well as the prognostic potential of the evaluated biochemical markers.
Methods: Tumor-informed plasma ctDNA analysis was available for 276 MIBC patients undergoing radical cystectomy (RC), of which 174 patients received neoadjuvant chemotherapy (NAC). Biochemical measurements collected within 10 days of an available ctDNA test (median: 0 days [IQR: 0-2 days]) and clinical data were retrieved from the patients' electronic health records. Immune marker measurements were confined to the pretreatment period. The median levels of all biochemical markers were within normal reference ranges, except for the neutrophil-to-lymphocyte ratio (NLR) which was above normal range in ctDNA positive patients. Wilcoxon rank-sum test, Spearman's correlation and Bonferroni correction for multiple testing were applied.
Results: The primary analysis indicated no correlation between kidney (plasma creatinine, eGFR) and liver (albumin, lactate dehydrogenase, alanine aminotransferase (ALAT), bilirubine, alkaline phosphatase) function markers in MIBC patients and plasma ctDNA detection or ctDNA variant allele frequencies (VAFs). Subgroup analyses of NAC-treated and NAC-naïve patients showed similar results, with no association observed, except for a weak positive correlation between ALAT and plasma ctDNA detection in NAC-naïve patients (P=0.036, n=72; not significant after multiple test correction). Levels of leukocytes (P=0.00098, n=146), neutrophils (P=0.0004, n=134), NLR (P=0.00016, n=77) and C-reactive protein (P=0.04, n=241), demonstrated positive correlations with plasma ctDNA detection. Multivariable logistic regression including tumor stage at diagnosis confirmed significant correlations between ctDNA detection and leukocytes (P=0.00322, n=146), neutrophils (P=0.00152, n=134) and NLR (P=0.00185, n=77). None of the biochemical parameters were associated with pathological downstaging after NAC. Reduced pretreatment plasma lymphocyte levels correlated with recurrence in the NAC-treated patients (P=0.012; not significant after multiple test correction).
Conclusion: Clinical application of ctDNA can be implemented with minimal risk of detection being influenced by fluctuations in the patients' kidney and liver function. Finally, biochemical parameters alone showed no strong prognostic value.

CLINICAL RESEARCH: Liquid Biopsy: Circulating Tumor Cells 1
Poster Session

#1966 Evaluation of circulating tumor cells as a real-time biomarker in metastatic urothelial carcinoma.

Wadih Issa[1], Navneet Kaur[1], Qinhan Zhou[1], Andrew DeVilbiss[1], Ze Yu[2], Damla Gunenc[1], Michael Poellmann[3], Changchuan Jiang[1], Qian Qin[1], Jue Wang[1], Suzanne Cole[1], Seungpyo Hong[3], Chao Xing[4], Andrew Z. Wang[5], Tian Zhang[1]

[1]Division of Hematology and Oncology, Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX,[2]Eugene McDermott Center for Human Growth and Development, University of Texas Southwestern Medical Center, Dallas, TX,[3]Pharmaceutical Sciences Division, School of Pharmacy, University of Wisconsin-Madison, Madison, WI,[4]Eugene McDermott Center for Human Growth and Development; Lyda Hill Department of Bioinformatics, University of Texas Southwestern Medical Center, Dallas, TX,[5]Radiation Oncology, University of Texas Southwestern Medical Center, Dallas, TX

Background: Patients with metastatic urothelial carcinoma (mUC) have poor prognosis and need biomarkers to guide treatment and monitor treatment responses. Circulating tumor cells (CTCs) offer a minimally invasive approach to capture tumor dynamics in real-time. This study aim to correlate mUC CTCs with treatment response and evaluate therapy-induced molecular alterations.
Methods: Patients with mUC were prospectively enrolled at the University of Texas Southwestern Medical Center. Peripheral blood samples were collected at baseline, 4 weeks, and 12 weeks of treatment. CTCs were isolated using a dendrimer-based assay functionalized with antibodies against EpCAM, Trop-2, Nectin-4, and FGFR3, identifying nucleated CTCs (DAPI+/CK+, CD45-). Single cell proteomic profiling of CTCs was performed via the Nanostring GeoMx Immuno-Oncology (IO) Proteome Atlas panel. The primary objective was to assess CTC variation as a biomarker for treatment response. CTC changes were considered concordant if they decreased in patients with complete or partial response (CR or PR), remained stable in those with stable disease (SD), or increased in patients with progressive disease (PD), as defined by RECIST 1.1.
Results: Eighteen patients with mUC were enrolled with median age of 76 years-old, 89% male and 61% Caucasian. Among the cohort, 78% (n=14) received enfortumab vedotin (EV) and pembrolizumab (P), 17% (n=3) received chemotherapy, and 5% (n=1) received sacituzumab govitecan. The median CTC counts (cells/ml) were assessed at baseline (39.3, IQR 26.5-64.4), week 4 (31.05, IQR 7.6-43.6), and week 12 (32.5, IQR 23.25-44.98). 13 patients had sufficient radiographic and clinical follow up. CTC changes were concordant with treatment responses in 77% (10/13) of patients, including 6/7 patients with PR, 1/2 with SD, and 3/4 with PD. Preliminary proteomic profiling of three patients who responded to 1L EV-P showed 58 upregulated and 70 downregulated proteins from baseline to week 4. We found that post-therapy, proteins related to antigen presentation (HLA-A, HLA-C, HLA-E), metabolism (beta glucuronidase), GLB1/Beta-galactosidase (Hexokinase 1), cell death (pro-apoptotic protein Bax, Smac/Diablo), and epithelial-mesenchymal transition (c-MET, N-cadherin, and vimentin) were upregulated, while proliferation signals (Ki67, Her2/ERBB2), metastasis (E-Cadherin, Phospho S431 Cytokeratin 8, c-Jun), and DNA damage repair (BRCA1, γH2A, ChK1, cleaved PARP1) were downregulated.
Conclusion and Future Directions: Novel capture of CTCs in patients with mUC can be potential biomarkers for monitoring treatment response and detecting therapy-induced molecular alterations. Some on treatment molecular expression changes may be further targeted with targeted treatments. We continue to characterize CTCs to identify key molecular pathways that contribute to treatment sensitivity and resistance.

#1967 Plexin-B2 mediates tumor cell clustering with monocyte interactions in breast cancer metastasis.

Emma Schuster[1], Nurmaa Dashzeveg[1], **Fangjia Tong**[1], Yuzhi Jia[1], Lamiaa El-Shennawy[1], Tong Zhang[2], Andrew Hoffmann[1], Kibria Golam[1], Youbin Zhang[3], Joshua Squires[1], Erika Ramos[1], Rokana Taftaf[1], David Scholten[1], Hannah Almubarak[1], Valery Adorno-Cruz[1], David Sullivan[4], Carolina Reduzzi[5], Allegra Minor[6], William Purev-Ochir[6], Rong Xu[7], Kalliopi Siziopikou[6], Leonidas Plantanias[6], Ami Shah[6], William Muller[6], William Gradishar[6], Chia-Feng Tsai[2], Tujin Shi[2], Huiping Liu[1]

[1]Pharmacology, Northwestern University, Chicago, IL,[2]Biological Sciences Division, Pacific Northwest National Laboratories, Richland, WA,[3]Department of Medicine, Northwestern University, Chicago, IL,[4]Pathology, Northwestern University, Chicago, IL,[5]Weill Cornell School of Medicine, New York, NY,[6]Northwestern University, Chicago, IL,[7]Case Western Reserve University, Cleveland, OH

The dogma of single tumor cell-mediated metastasis has been challenged by the discovery of multicellular circulating tumor cells (CTCs) clusters which are 20-50 times more likely to produce viable metastasis than single CTCs. However, the molecular mechanisms underlying CTC clusters and CTC interactions with immune cells are largely unknown. Combining integrated proteomic ranking, targeted analyses, and functional determination, we identified the transmembrane protein Plexin-B2 (PLXNB2) as one of the top hits whose expression in human primary tumors is associated with unfavorable distant metastasis-free survival. PLXNB2 expression is also enriched in CTC clusters compared to single CTCs from patients with advanced breast cancer. Loss of PLXNB2 reduces the formation of homotypic tumor cell clusters and heterotypic tumor-myeloid cell clusters in triple-negative breast cancer. Interactions between PLXNB2 and its ligand Sema4C on tumor cells mediate homotypic cluster formation, and PLXNB2 binding with Sema4A on myeloid cells (monocytes) promotes heterotypic CTC cluster formation, thereby driving lung metastasis in breast cancer. Depletion of *Plxnb2* in mouse breast tumor cells also blocked CTC-myeloid cell clustering and spontaneous metastasis in immune-competent mice. Additionally, using global proteomic analysis, we identified novel downstream effectors of the PLXNB2 pathway associated with tumor cell clustering in breast cancer. Thus, PLXNB2 is a novel therapeutic target for preventing new metastasis in triple-negative breast cancer.

#1968 Comparable detection of large tumor-derived vesicles (oncosomes) by known CTC platforms: Potential new analyte for biomarker monitoring?.

Eszter Papp[1], Pieter Mestdagh[2], Luc Y. Dirix[3], Peter Vermeulen[4], Mark Kockx[2]

[1]CellCarta, Montreal, QC, Canada,[2]CellCarta, Antwerp, Belgium,[3]Medical Oncology, ZAS St-Augustinus Hospital, Antwerp, Belgium,[4]Translational Oncology Research Unity, ZAS St-Augustinus Hospital, Antwerp, Belgium

Background: Monitoring tumors and detecting biomarkers through blood analysis remains challenging. Large oncosomes (LOs) are tumor-derived extracellular vesicles that offer a promising avenue for understanding tumor biology and biomarker status, potentially surpassing circulating tumor cells (CTCs) in accuracy. Validated LO detection methods are required to unlock LOs analytical potential. We evaluated two LO detection methods using known CTC platforms, the FDA-approved CellSearch, and RareCyte to compare their performance. In addition, we assessed the potential of LOs for HER2 detection in metastatic breast cancer (MBC) patients.
Methods: LO counts were analyzed in parallel blood samples from 96 MBC patients using either the CellSearch/ACCESS tdEV analysis package, or the RareCyte method with in-house LO analysis. Overall survival (OS) was defined as the time from baseline blood draw to death or last follow-up. HER2 scores from primary tumor biopsies were retrieved from patient charts. HER2 overexpression in LOs and CTCs was determined using the RarePlex HER2/ER CTC panel kit (n=9). Correlations between LO and CTC counts were assessed using Spearman's Rho test, and comparability of LO counts between platforms was evaluated with the Wilcoxon Signed Ranks test. Cutoffs for favorable vs. unfavorable LO counts were determined using an online tool. Survival curves for these groups were compared using the log-rank test. Correlation of HER2+ analytes with tissue biopsy HER2 scores was also analyzed using Spearman's Rho test.
Results: A strong positive correlation was observed between LO counts from the two platforms ($r = 0.49$, $p < 0.001$). LO counts also correlated with their respective CTC counts (CellSearch: $r = 0.88$, $p < 0.001$; RareCyte: $r = 0.93$, $p = 0.001$). Higher LO counts were associated with worse survival outcomes (CellSearch: $r = 0.35$, $p < 0.001$; RareCyte: $r = 0.39$, $p = 0.006$). Patients with unfavorable LO counts had significantly shorter survival compared to patients with favourable LO counts (CellSearch: HR = 3.5, $p < 0.001$; RareCyte: HR = 3.6, $p = 0.02$). RareCyte detected significantly more LOs than CellSearch (median LO count: 132 vs. 41, $p = 0.01$).
HER2 signal was detected in 5/9 patients with CTCs and in 8/9 patients with LOs. Notably, the proportion of HER2+ LOs positively correlated with tissue biopsy HER2 score ($r=0.799$, $p=0.01$). In contrast, the proportion of HER2+ CTCs did not correlate with tissue biopsy.
Conclusions: While RareCyte captured more LOs than CellSearch, LO counts were highly concordant across platforms, validating both methods. The association of LO counts with overall survival suggests that LOs reflect tumor progression, and may complement CTC-based monitoring. Furthermore, the correlation between LO HER2 status and tumor biopsy HER2 scores underscores the potential of LO analysis for biomarker monitoring in metastatic breast cancer.

#1969 Advancing metastatic prostate cancer diagnostics: STEAP1-based imaging flow cytometry for circulating tumor cell detection and characterization.

Minzhi Sheng[1], Shuyang Feng[1], Omar Alawamry[1], Urban Emmenegger[2], Kristin Cimolini[1], Danny Vesprini[3], Andrew Loblaw[3], Laurence Klotz[4], Christopher S. Lim[5], Stanley K. Liu[6], Hon S. Leong[1]

[1]Sunnybrook Research Institute, Toronto, ON, Canada,[2]Department of Hematology and Oncology, Sunnybrook Health Sciences Centre, Toronto, ON, Canada,[3]Department of Radiation Oncology, University of Toronto, Toronto, ON, Canada,[4]Department of Urology, Sunnybrook Health Sciences Centre, Toronto, ON, Canada,[5]Department of Radiology, University of Toronto, Toronto, ON, Canada,[6]Biological Sciences Platform, Sunnybrook Research Institute, Toronto, ON, Canada

Prostate cancer (PCa) is the most common cancer among Canadian men, with metastatic PCa (mPCa) patients experiencing a low 5-year survival rate of just 30%. Liquid biopsies using circulating tumor cells (CTCs) offer a non-invasive approach to diagnosing and monitoring treatment efficacy. Six transmembrane epithelial antigens of the prostate 1 (STEAP1) have been identified as a promising biomarker due to its significant overexpression in most PCa cases compared to normal tissues. We have developed monoclonal antibodies specifically targeting STEAP1 and propose their application in imaging flow cytometry (imFC) for identifying CTCs in mPCa patients. Our CTC staining and imFC protocols were validated and optimized using benign prostate hyperplasia and PCa cell lines. We also compared two CTC isolation techniques: density-based separation using Ficoll-Paque and microfluidic isolation with the BioRad Genesis Cell Isolation System for the best CTC purity. Isolated peripheral blood mononuclear cells (PBMCs) were labelled with our STEAP1 antibodies, DAPI for nuclear staining, CD45 antibodies for hematopoietic cells, and EpCAM antibodies, a widely used marker for CTCs. Additionally, we developed analytical pipelines leveraging imaging software with built-in machine-learning capabilities to classify probe binding patterns and identify subtypes of CTCs and cancer-derived extracellular vesicles (EVs) in immune cells. These optimized protocols were then tested on blood samples from patients with localized and metastatic PCa. CTCs were successfully detected in mPCa patients based on their expression of PCa biomarkers and lack of immune cell markers. Furthermore, STEAP1-positive vesicle-like signals were observed in association with hematopoietic cells, suggesting the uptake of PCa EVs by immune cells. Our findings demonstrate that STEAP1 antibodies in imFC can effectively detect and characterize CTCs. This approach represents a next-generation liquid biopsy for mPCa, offering improved diagnostic accuracy and the potential to enhance long-term outcomes for mPCa patients.

#1970 Quantitative analysis of HER2, HER3, and ɣ-H2AX expression on circulating tumor cells for clinical trials.

Erin G. Bayer, Brock Bartels, Rachel Ponting, Casey Helmicki, Arturo B. Ramirez

RareCyte, Inc., Seattle, WA

Background: Circulating Tumor Cells (CTCs) are an attractive target for pharmacodynamic measurements in clinical trials, providing quantitative biomarker expression of the tumor, independent of tissue biopsy. The increasing challenge to obtain tissue samples continues to focus attention on non-invasive liquid biopsy approaches. The RareCyte platform offers a highly sensitive method for CTC enumeration and protein expression on CTCs. In this study, we describe the development of assays to quantify HER2, HER3, and ɣ-H2AX protein expression on CTCs. Each of the single biomarker assays allow for the inclusion of an additional biomarker of interest to be measured on each CTC.

Methods: Cell lines with known positive or negative expression for each biomarker were spiked into healthy donor blood to generate model CTCs. Nucleated cells were collected from these samples using the AccuCyte® sample preparation kit and spread onto microscope slides. Slides were fixed and stained with antibodies targeting cytokeratin, EpCAM, and CD45 to detect CTCs and exclude white blood cells (WBCs), as well as primary antibodies to HER2, HER3 or ɣ-H2AX to evaluate protein expression on identified CTCs. Slides were imaged with the CyteFinder® automated immunofluorescence scanning microscope, to quantitatively measure Mean Fluorescence Intensity (MFI) of biomarkers to assess protein expression on individual CTCs.

Results: Assays for the detection of HER2, HER3, and ɣ-H2AX on CTCs were designed and optimized to maximize the signal-to-background ratio. The final assays were run on replicate slides of positive and negative cell lines over 3 independent days to evaluate the analytic performance of each assay. Thresholds for biomarker positivity were established based on MFI measurements from positive and negative control slides. The accuracy of all three biomarker assays was >90%. Both repeatability (intra-run precision) and inter-run precision, assessed by the Coefficient of Variations (CV), were <20% for all three assays. The HER2 biomarker assay was tested on 16 cell lines with varying levels of HER2 expression, demonstrating its ability to confirm HER2 protein levels and distinguish between cell lines with known HER2 expression (0, 1+, 2+, 3+).

Conclusions: We developed biomarker assays for quantifying protein expression on individual CTCs, that demonstrate high accuracy and reproducibility for assessing biomarker expression of tumors through liquid biopsy and are currently being utilized in clinical studies. Recent findings suggest that patients may respond to targeted therapies even when their tumors have low or ultra-low HER2 expression, if HER2 expression is detected on CTCs. Quantifying target protein expression on CTCs offers a promising approach for patient selection and longitudinal, non-invasive monitoring of cancer patients, generating valuable data for clinical trial sponsors.

#1971 Leveraging CK and CD45 dual positive cells as cancer diagnostic markers in liquid biopsy.

Sumin Lee[1], Amos Chungwon Lee[1], Wooseok Lee[2], Han-Byoel Lee[3], Sunghoon Kwon[2]

[1]Meteor Biotech, Seoul, Korea, Republic of,[2]Seoul National University, Seoul, Korea, Republic of,[3]Department of Surgery, Seoul National University College of Medicine, Seoul, Korea, Republic of

Liquid biopsy has emerged as a revolutionary, non-invasive diagnostic tool, enabling real-time monitoring of tumor dynamics. Circulating tumor cells (CTCs), shed from primary or metastatic tumors into the bloodstream, represent a critical component of liquid biopsy with immense potential for early cancer detection, prognosis, and therapeutic monitoring [1]. While identification of CTCs as cytokeratin (CK) positive and CD45 negative is standard of immunofluorescence assay among cells in blood sample, curious cases have been reported that the presence of CK and CD45 dual positive (DP) cells [2]. However, neither the identity of DP cells nor the roles of them had been understood. Rather, their presences were neglected so far. This presentation highlights recent findings of the DP cells about their identity and their potential roles as biomarkers, especially cancer diagnostic markers. Our state-of-the-art technologies [3] enabled detection and isolation of the DP cells, streamlined to single cell RNA-seq and the downstream analysis with high quality of data in spite of heterogeneity. Our studies focus on the molecular profiling of CTCs and DP cells to identify transcriptional signatures that correlate with tumor metastasis mechanisms. Additionally, emerging evidence underscores the potential edges of the prognostic value of DP cell enumeration over CTCs. By bridging the gap between liquid biopsy innovations and clinical application, DP-cell-based diagnostics hold promise for transforming precision oncology. Future directions include integrating DP analysis with other biomarkers, such as CTC, circulating tumor DNA and extracellular vesicles, to construct a comprehensive liquid biopsy platform. This work emphasizes the role of DP cells as a novel target of liquid biopsy, paving the way for earlier interventions and more personalized therapeutic strategies in cancer care.

References[1] Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, Reuben JM, Doyle GV, Allard WJ, Terstappen LW and Hayes DF: Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med. 351:781-791. 2004.[2] Carolina Reduzzi, Marta Vismara, Lorenzo Gerratana, Marco Silvestri, Filippo De Braud, Francesco Raspagliesi, Elena Verzoni, Serena Di Cosimo, Laura D. Locati, Massimo Cristofanilli, Maria Grazia Daidone, Vera Cappelletti, The curious phenomenon of dual-positive circulating cells: Longtime overlooked tumor cells, Seminars in Cancer Biology, Volume 60, 2020,[3] Lee, A.C., Lee, Y., Choi, A. et al. Spatial epitranscriptomics reveals A-to-I editome specific to cancer stem cell microniches. Nat Commun 13, 2540 (2022).

#1972 Utilization of flow cytometry for the characterization of mesenchymal and embryonic properties of circulating tumor cells.

Tamas Ricard Linkner[1], Zsofia Brigitta Nagy[1], Nikolett Szakallas[2], Eszter Farkas[1], Fruzsina Banyai[1], Istvan Takacs[1], Bela Molnar[1]

[1]Semmelweis University, Budapest, Hungary,[2]Eotvos Lorand Tudomanyegyetem, Budapest, Hungary

Background: Circulating tumor cells break off from the primary cancer tissue and enter into the bloodstream, where they reach the distant parts of the body where they can form metastases. The tumor cell could lose its adhesive properties and takes on a mobile, mesenchymal phenotype and they undergo epithelial-mesenchymal transition (EMT). The detection of these circulating tumor cells carries great diagnostic potential. Their identification is based on specific cellular markers such as cytokeratin or vimentin expression, which are key molecules in EMT, or SOX9 which plays a prominent role in the development of stem cell-like properties of tumor cells and carries therapy resistance potential.
Aims: The aim of our study was to develop and optimize a technique suitable for the detection of circulating tumor cells and cells undergoing epithelial-mesenchymal or embryonic transition from blood.
Materials and Methods: Magnetic cell separation technique (autoMACS Pro, Miltenyi) was performed in order to deplete CD45 immune cells from blood. Additionally, flow cytometry technique was used to detect fluorescent antibody labeled circulating tumor cells. CD45-AF700, CD15-PeVio770, CK-FITC, Vimentin-APC, SOX9-PE labeled antibodies were utilized. Peripheral whole blood of colorectal carcinoma patients (CRC) were analyzed, four of the patients were classified with grade II and grade III. Data was compared to healthy controls.
Results: After the depletion of CD45 positive immune cells from the blood 5CTC/100000 counted cell were characterized as cytokeratin positive cell. Moreover, 50CTC/100000 counted cell was observed as vimentin positive cells. Additionally, SOX9 positive cells (6CTC/100000) were also detected from patients. These results indicates the presence of cancer cells with mesenchymal and stem cell characteristics in the circulation of cancer patients.
Conclusion: Overall, magnetic separation is suitable technique for the enrichment of circulating tumor cells from whole blood. Moreover, flow cytometry is a key method for the detection of circulating tumor cells and cancer stem cells that have undergone embryonic transformation. With the sorting of these cells we hope that we can create the base of future diagnostic analysis of cancer patients.

#1973 The expression of PD-L1 in circulating tumor cells is correlated with positive estrogen receptor status and higher CTC counts in HER2-negative metastatic breast cancer.

Qiang Zhang[1], Justin W. Zhang[2], Andrew A. Davis[3], Lorenzo Gerratana[4], Jian Jiao[5], Paolo D'Amico[6], Weijun Qin[5], Xinkun Wang[7], Akhil Chawla[8], Lisa Flaum[9], Leonidas C. Platanias[10], William J. Gradishar[1]

[1]Department of Medicine, Division of Hematology/Oncology, Circulating Tumor Cell (CTC) core facility, Robert H. Lurie Cancer Center, Feinberg School of Medicine, Northwestern University - Evanston, Chicago, IL,[2]Department of Surgery, Division of Surgical Oncology, Northwestern University and Whitney Young High School, Chicago, IL,[3]Department of Medicine, Washington University in St. Louis, St. Louis, MO,[4]Department of Medicine, University of Udine, Udine, Italy,[5]Department of Urology, Xijing Hospital, Innovation Center for Tumor Immunocytology Therapy Technology, Xijing Innovation Research Institute, Fourth Military Medical University, Xi'an, China,[6]Merck, Rahway, NJ,[7]Department of Cell and Developmental Biology, Robert H. Lurie Cancer Center, Feinberg School of Medicine, Northwestern University - Evanston, Chicago, IL,[8]Department of Surgery, Division of Surgical Oncology, Robert H. Lurie Cancer Center, Feinberg School of Medicine, Northwestern University - Evanston, Chicago, IL,[9]Department of Medicine, Division of Hematology/Oncology, Robert H. Lurie Cancer Center, Feinberg School of Medicine, Northwestern University - Evanston, Chicago, IL,[10]Department of Medicine, Division of Hematology/Oncology, Robert H. Lurie Cancer Center, Feinberg School of Medicine, Northwestern University-Evanston, Chicago, IL

Background: Hormone therapies alter the tumor microenvironment with the potential to sensitize estrogen receptor (ER)-positive metastatic breast cancer (MBC) to immunotherapy. Current trials are ongoing investigating the combination of hormone therapies, including aromatase inhibitors or selective ER modulators, with immune checkpoint inhibitors that target PD-1/PD-L1. We previously reported a significant correlation between tumor markers (e.g., *ESR1*) and circulating tumor cells (CTCs) regarding therapy resistance in MBC (2023 ASCO, 2024 SABCS). Herein, we sought to evaluate the if PD-L1 expression is associated with ER status, and CTC enumeration in patients with HER2-negative MBC.

Methods: This study included 23 HER2-negative MBC patients enrolled between 2017 and 2022 at Robert H. Lurie Comprehensive Cancer Center of Northwestern University. Whole blood sample (7.5 mL/each) was collected from each patient. CTC enumeration and PD-L1 detection was conducted using the FDA-approved CellSearch® System (Menarini). CTCs were identified as $CK^+/DAPI^+/CD45^-$. We assessed the association between PD-L1 expression, ER status, and CTC enumeration using Kruskal-Wallis test for statistics.

Results: Among 23 patients, 12 patients were ER-positive and 11 were ER-negative, as determined through pathological identification. CTC counts of ≥5 were detected in 16 patients, while 7 patients had CTC counts <5. In the ER-positive group, the mean total CTC count was 68.9, with a mean of 25.1 $PD\text{-}L1^+$ CTCs and 10 CTC clusters. In contrast, the ER-negative group had a significantly lower mean total CTC count of 48.5, with an average of 2.4 $PD\text{-}L1^+$ CTCs and no CTC clusters ($p < 0.0001$). These findings indicate that PD-L1 expression in CTCs is strongly associated with ER-positive status in MBC patients. Additionally, PD-L1+ CTCs were found in 16 patients, who exhibited a mean total CTC count of 61.4 and 6.5 CTC clusters, significantly higher than the 7 PD-L1-negative patients who had a mean total CTC count of 49.1 and no CTC clusters ($p<0.05$). Among patients with CTC counts ≥5, four had a PD-L1+ CTCs/total CTCs ratio above 50%, with an average of 87 total CTCs per patient, which was significantly higher than the average of 80.5 total CTCs seen in the other 12 patients with a ratio below 50% ($p < 0.05$).

Conclusions: Our findings indicate that ER-positive, HER2-negative MBC is associated with significantly higher PD-L1 expression in CTCs compared to ER-negative, HER2-negative MBC. This increased PD-L1 expression in CTCs may contribute to immune evasion by affecting immune cell infiltration and response, potentially reducing the effectiveness of immunotherapy in ER-positive cases and predicting a more aggressive metastatic potential. Adding ER status and CTC enumeration when assessing PD-L1 expression in CTCs could be considered for treatment stratification in MBC.

#1974 Prostate cancer lineage states detected by circulating tumor cell RNA sequencing are prognostic biomarkers for overall survival and resistance to androgen receptor pathway inhibitors and177Lutetium-PSMA-617.

Joshua M. Lang[1], Marina Sharifi[1], Jamie Sperger[1], Amy K. Taylor[1], David Kosoff[1], Hamid Emamekhoo[1], Christos Kyriakopolous[1], Andrew Armstrong[2], Xiao Wei[3], Rana McKay[4]

[1]University of Wisconsin-Madison, Madison, WI,[2]Duke University, Durham, NC,[3]Dana Farber Cancer Institute, Boston, MA,[4]University of California-San Diego, San Diego, CA

Background: The development of treatment resistance remains universal for metastatic prostate cancer patients, driven by AR alterations and lineage state transitions. Identifying the evolution of molecular alterations during treatment has been limited by the challenges of serial tumor biopsies, which can be overcome by blood-based liquid biopsies. However, standard cell-free DNA approaches cannot directly assess changes in gene expression, crucial to understanding resistance.

Patients and Methods: We developed a novel circulating tumor cell (CTC) isolation approach with purity comparable to tissue biopsies and performed CTC RNA sequencing of 273 blood samples from a prospective multi-institutional cohort of 117 metastatic prostate cancer patients. We examined prostate cancer lineage states associated with prognosis and response to 177Lu-PSMA-617 in men with metastatic prostate cancer.

Results: Single sample pathway analysis of high CTC purity samples identified four transcriptional phenotypes: luminal A-like (LumA), luminal B-like (LumB), low proliferation (LP), and neuroendocrine (NE). Compared to patients with low CTC burden/purity (median OS not reached), patients with LumA and LP phenotypes showed similar survival, while patients with LumB and NE CTC phenotypes had shorter survival (LumB: median OS 6m, HR 9.1[3.8-21.8], p<0.0001, NE: median OS 3.7m, HR 11.8[2.4-57.2], p=0.0019). We isolated CTCs longitudinally from 37 patients receiving 177Lu-PSMA-617. LumA/LP/Low burden samples had significantly greater benefit from ARPIs compared to LumB and NEPC, as hypothesized. Pretreatment LumA/LP/Low burden CTC phenotypes had a significantly higher clinical benefit rate with 177Lu-PSMA-617 than the LumB phenotype (64% vs 14%, Fisher's Exact p=0.033), which was associated with early progression on 177Lu-PSMA-617 (Fisher's Exact p=0.038).

Conclusion: We report the largest CTC RNA-seq cohort of patients with metastatic prostate cancer and demonstrate that high-purity CTC RNA-seq identifies prostate cancer lineage states mirroring those in tissue, including an NE phenotype concordant with small cell/neuroendocrine histology. We also identified a more common LumB phenotype defined by persistent AR signaling and high proliferation and associated with poor prognosis and poor response to 177Lu-PSMA-617.

#1975 The use of circulating tumor DNA (ctDNA) in HER2+ metastatic breast cancer (MBC) for prognostication and management.

Surbhi Warrior[1], Diana Jaber[1], Natalie Heater[1], Sarah Marini[1], Yangruijue Ma[1], Zequn Sun[1], Lisa Flaum[1], Patricia Robinson[1], Regina Stein[1], Regina Stein[1], Massimo Cristofanilli[2], William Gradishar[1], Janice Lu[1]

[1]Northwestern Memorial Hospital, Chicago, IL,[2]Weill Cornell, New York, NY

Background: HER2+ breast cancers account for 20% of all breast cancers, and survival rates in the metastatic setting remain low. There have been advances in treatment with HER2 targeted therapies, but progression free survival (PFS) decreases after each subsequent line of therapy. Understanding patients' mutational profile with the use of ctDNA may identify resistance to HER2 targeted therapies, aid in prognostication, and guide the addition of further targeted therapies to improve outcomes.
Methods: Patients with HER2+ MBC were enrolled from 2016-2024 under an IRB approved clinical trial (NU16B06) at Northwestern University were retrospectively analyzed. Plasma ctDNA was analyzed by Guardant 360 and tissue NGS was performed using commercially available tests such as FoundationOne or TempusX. Mutations that developed as well as additional clinical, pathologic, treatment, and response data were collected for analysis.
Results: There were 67 patients with HER2+ MBC included in the analysis. The mean initial PFS following HER2 targeted therapy was 29.8 months, the mean second PFS was 12 months, and the overall mortality was 63%. The overall second PFS was significantly shorter than the initial PFS (HR 4.00, $p < 0.001$). ESR1+ patients had a significantly shorter initial PFS compared to ESR1 wildtype (wt) patients (14 vs 22 months, $p=0.038$). There was no significant difference between second PFS for ESR1+ patients compared to ESR1wt patients (5.5 vs 6.8 months, $p=0.28$). There was a significant difference in initial PFS for patients FGFR+ patients and FGFRwt patients (12 vs 22 months, $p=0.002$) but not for second PFS (6 vs 8 months, $p=0.07$). The initial PFS for PIK3CA+ patients was shorter compared to PIK3CAwt (12 vs 22 months, $p=0.087$) but was similar for the second PFS (6 vs 6 months, $p=0.52$).
Conclusion: The use of ctDNA in HER2+ MBC can aid in understanding a patient's genomic landscape and prognosis. There is overall shorter PFS in patients with identified mutations and that significantly worsens with progression of disease. The initial PFS in patients with ESR1, FGFR, and PIK3CA mutations is significantly less than in respective wildtype patients. While targeted therapies for these mutations currently exist in the hormone positive breast cancer setting, our findings demonstrate that HER2+ patients may also benefit from additional targeted therapy in adjunct to HER2 directed therapy in the early metastatic setting. This therapeutic benefit should be prospectively studied in a larger cohort to understand if there exists a clinically significant improvement in outcomes.

#1976 Circulating tumor cell (CTC) RNA sequencing and single cell protein phenotyping reveal heterogeneity of cell surface target expression in metastatic prostate cancer CTCs.

Alex H. Chang[1], Jamie M. Sperger[1], Viridiana Carreno[1], Katherine R. Kaufmann[2], Matthew L. Bootsma[3], Shannon R. Reese[2], Luke A. Nunamaker[2], Charlotte Linebarger[2], Amy K. Taylor[1], Katharine E. Tippins[1], Kyle T. Helzer[3], David Kosoff[4], Shuang G. Zhao[3], Joshua M. Lang[1], Marina N. Sharifi[1]

[1]Department of Medicine, Carbone Cancer Center, Univ. of Wisconsin Madison Sch. of Med. & Public Health, Madison, WI,[2]Carbone Cancer Center, Univ. of Wisconsin Madison Sch. of Med. & Public Health, Madison, WI,[3]Department of Human Oncology, Univ. of Wisconsin Madison Sch. of Med. & Public Health, Madison, WI,[4]William S Middleton Memorial Veterans Hospital, Madison, WI

Background: Metastatic prostate cancer is an androgen receptor (AR) driven disease. While AR targeted therapies (ARPIs) have led to improvements in patient outcomes, development of treatment resistance remains universal. ARPI resistance can occur through lineage state transitions that bypass AR signaling and culminate in neuroendocrine prostate cancer (NEPC). Cell surface targeted therapies are a new therapeutic class that has shown promise for the treatment of ARPI-resistant metastatic prostate cancer. However, heterogeneity in cell surface target expression during lineage state transitions could impact the efficacy of these approaches. CTCs are uniquely suited to longitudinal profiling to understand the evolution of cell surface target expression during the lineage state transition from AR-driven adenocarcinoma to NEPC.

Methods: CTCs were isolated from peripheral blood from patients with ARPI-resistant metastatic prostate adenocarcinoma utilizing our automated microfluidic platform integrating immunomagnetic negative and positive selection, followed by protein staining on chip for single CTC fluorescent quantification of prostate adenocarcinoma (PSMA) and NEPC (DLL3) cell surface target expression (n=29) or RNA isolation on chip and bulk RNA sequencing (n=146).

Results: In the CTC RNA-seq cohort, 112/146 (76.7%) samples had expression of FOLH1 (PSMA) only, 5/146 (3.4%) samples had expression of DLL3 only and 9/146 (6.2%) samples had expression of both DLL3 and FOLH1. Median expression of DLL3 in the double positive group was similar to that in the DLL3 only group (4.34 v 6.67 log2(tpm+1), p = 0.076), while median expression of FOLH1 in the double positive group was similar to that of the FOLH1 only group (6.80 v 7.26 log2(tpm+1), p = 0.316). In the single CTC protein phenotyping cohort, 27/29 (93%) of samples had detectable CTCs, with a mean of 18 CTCs per patient (range 1-172). 19/29 (65.5%) samples had PSMA positive CTCs, 1/29 (3.4%) samples had DLL3 positive CTCs, and 2/29 (6.9%) samples had both PSMA positive and DLL3 positive CTCs, but no single CTCs with co-expression of both proteins were identified.

Conclusion: CTC RNA sequencing identifies a subset of ARPI-resistant metastatic prostate cancers with cell surface target expression associated with small cell/neuroendocrine differentiation (DLL3), including samples with co-occurring gene expression of adenocarcinoma (PSMA) and small cell (DLL3) markers. Single CTC protein phenotyping also identified patients with simultaneous detection of both PSMA positive and DLL3 positive CTCs, however no PSMA/DLL3 double positive CTCs. The presence of mixed lineage state populations with potentially mutually exclusive cell surface target expression suggests consideration of co-targeting approaches to improve therapeutic efficacy in the ARPI-resistant setting.

#1977 Implementation of a comprehensive clinical cfDNA analysis platform for aqueous humor liquid biopsy in retinoblastoma.

Laura A. T. Kagami[1], Eirini Christodoulou[2], Venkata Yellapantula[2], Dong Xu[2], Cindy Fong[2], Dejerianne Ostrow[2], Liya Xu[3], Jesse Berry[3], Jaclyn A. Biegel[2]

[1]Cancer and Blood Disease Institute, Children's Hospital Los Angeles, Los Angeles, CA,[2]Department of Pathology and Laboratory Medicine, Children's Hospital Los Angeles, Los Angeles, CA,[3]The Vision Center, Children's Hospital Los Angeles, Los Angeles, CA

Introduction: The development of liquid biopsy testing based on sequencing analysis of cell-free DNA (cfDNA) has the potential to transform cancer care in pediatric tumors, which are characterized by a wide array of genomic alterations including copy number alterations (CNAs), sequence variants, epigenetic alterations, and structural rearrangements in tumor-associated genes. In patients with retinoblastoma where direct tumor biopsy is not possible due to the risk of tumor spread, aqueous humor (AH) is a rich source of cfDNA. Comprehensive cfDNA testing, combining a low pass whole genome sequencing (LP-WGS) assay to detect CNAs and a custom targeted sequencing panel (TSP) to detect mutations, can provide a highly effective platform to inform diagnosis, risk stratification, response to therapy, and surveillance in patients with retinoblastoma.

LP-WGS Results: We validated a liquid biopsy LP-WGS assay, LBSeq4Kids, to detect CNAs in cfDNA from plasma, cerebrospinal fluid, or AH. Clinical testing has been performed for the past two years for diagnostic evaluation and monitoring response to therapy using AH from retinoblastoma patients.

Thirty-six children with unilateral or bilateral retinoblastoma (44 eyes) underwent LBSeq4Kids LP-WGS analysis. CNAs were detected in 30/32 (94%) samples at diagnosis or recurrence. Serial sampling of 22/44 (50%) eyes was performed using a range of 1-10 samples per patient. Seventeen of those 22 (77%) eyes demonstrated clearance of CNAs with treatment. Five eyes showed persistence of abnormal profiles, necessitating enucleation of 2 eyes. Treatment and evaluation are ongoing for the remaining three eyes.

Targeted Sequencing Panel Results: We are validating a custom cfDNA TSP to detect single nucleotide variants and small indels in the coding sequence of 136 genes and fusions involving *EWSR1, FOXO1*, and *BRAF* for patients with ocular disease, solid tumors and brain tumors.

To date, 17 children (21 eyes) diagnosed with retinoblastoma have had TSP testing using AH. All eleven (100%) patients with germline variants demonstrated the same *RB1* alterations in the AH using the panel. Six of these patients (55%) had a second somatic alteration in *RB1* detected with the panel. Two of 11 (18%) with known germline *RB1* mutations also demonstrated mutations in *BCOR, ARID1A,* or *MSH6*. Somatic variants in *RB1, ARID1A* and *BCOR* were detected in the remaining six patients with sporadic retinoblastoma. Targeted sequencing of the AH surveillance samples from these patients is in progress.

Conclusion: Here we demonstrate clinical utility for aqueous humor liquid biopsy molecular testing as a complementary means of diagnosis and monitoring treatment response in patients with retinoblastoma. Continued surveillance of these patients is ongoing to monitor disease status and rule out recurrence, as well as identify novel prognostic biomarkers.

#1978 Circulating rare events as a biomarker of anti-tumoral effect of ketamine therapy in non-small cell lung cancer.

Anya Shah[1], Stephanie Shishido[1], Jeremy Mason[1], George W. Fischer[2], David Amar[2], Dawn Desiderio[2], Joseph R. Scarpa[3], David R. Jones[2], Amitabh Gulati[2], Alessia Pedoto[2], Alessandro De Camilli[2], Cosmin Guaran[2], Jacob C. Jackson[2], Alexandra Lewis[2], Michelle Zanone[2], Lucy Nystrom[2], Howard Scher[2], Ethan Barnett[2], Joshua Mincer[2], Peter Kuhn[1]

[1]USC Convergent Science Institute in Cancer, Los Angeles, CA,[2]Memorial Sloan Kettering Cancer Center, New York, NY,[3]Weill Cornell Medicine, New York, NY

Non-small cell lung cancer (NSCLC) remains one of the leading causes of cancer-related mortality worldwide, with surgical resection (with or without adjuvant chemotherapy) often being the recommended treatment for resectable localized disease. However, even with curative-intent resections, 30% to 55% of localized NSCLC patients experience recurrence and will die from the disease. Tumor-associated cells detected in peripheral blood, referred to here as circulating rare events, are believed to play a key role in metastatic dissemination, underscoring their potential as indicators of treatment response and recurrence risk. Recent retrospective analyses have revealed that the use of intraoperative Ketamine (Ket) during primary tumor resection surgery is associated with a lower recurrence rate, suggesting a potential utility in cancer treatment beyond its approved indication as an adjunct anesthetic. The study presented here investigates the effects of different anesthetic protocols on circulating rare events in NSCLC patients, specifically comparing Ket and dexmedetomidine (Dex) (also commonly employed as an adjunct anesthetic), to assess whether Ket exhibits anti-tumor effects and modulates the systemic tumor environment. Peripheral blood samples (n=60) were collected from 22 early-stage NSCLC patients undergoing primary tumor resection, with samples taken preoperatively (before surgical incision and after surgical closure) and at a three-week follow-up. Circulating rare events were identified through advanced imaging and phenotypic analysis and validated by comparison to blood samples from normal donors, which served as controls. Results indicated no significant differences between the anesthetic agents in pre- and post-surgery samples; however, at the three-week follow-up, Ket was associated with a higher incidence of circulating rare events and an increase in a specific cellular phenotype consistent with endothelial cells. These findings suggest that Ket may influence tumor vasculature and enhance tumor shedding, which could lead to a better prognosis after tumor resection in lung cancer patients. This highlights the potential of Ket as a perioperative agent that modulates tumor biology, as demonstrated by increased rare cell detection, and supports further investigation into a possibly expanded role in surgical oncology for potential to improve long-term outcomes in NSCLC patients.

#1979 Low CD47 expression in specific populations of tumor macrophage fusion cells in blood correlates with progressive disease in metastatic cancers.

Sonia Muthuraj[1], Massimo Cristofanilli[2], Saranya Chumsri[3], Susan Tsai[4], Raymond C. Bergan[5], Cha-Mei Tang[6], Daniel L. Adams[7]

[1]Rutgers University, New Brunswick, NJ,[2]Weill Cornell Medicine, New York, NY,[3]Mayo Clinic Cancer Center, Jacksonville, FL,[4]Ohio State University, Columbus, OH,[5]Fred & Pamela Buffett Cancer Center, University of Nebraska Medical Center, Omaha, NE,[6]Creatv MicroTech, Inc., Rockville, MD,[7]Creatv MicroTech, Inc., Monmouth Junction, NJ

Tumor associated macrophages are known to fuse with cancer cells resulting in the formation of a cellular phenomenon called tumor-macrophage fusion cells (TMFCs). TMFCs are a highly aggressive cell subtype that may move into circulation, evade immune surveillance, and possibly aid in seeding of metastatic sites. The formation of TMFCs involves CD47, a ubiquitous surface receptor that acts as a “don’t eat me” signal, interacting with the macrophage protein SIRPa, a down regulator of phagocytosis, and syncytin-1, a mediator of hyperploidy cancer fusion. In this analysis, we isolated circulating TMFCs from the blood of n=39 metastatic cancer patients (pts) to evaluate the expressions of CD47, SIRPa and syncytin-1 as it relates to stable or progressive disease via 30 day imaging by PET scan. We conducted a prospective pilot study of n=39 pts with pathologically confirmed metastatic breast (n=17), prostate (n=7), pancreas (n=7), or colon (n=8) cancer receiving standard of care systemic therapy. We isolated TMFCs from 7.5ml peripheral blood using a CellSieve microfilter, and stained with CD47, SIRPa, & syncytin-1. TMFCs were identified based on large cell size (>30 µm) & multinucleated structure, and were categorized into 4 distinct subtypes: A) >2 distinct non-contacting nuclei within a TMFC, B) 2 distinct non-contacting nuclei, C) 2 contacting nuclei, D) a single multinucleated nuclei. CD47, SIRPa, and syncytin-1 expressions were categorized as negative, low, or high. TMFCs subtypes and marker expressions were compared to pts’ clinical status (i.e. progressive, stable or responding) at first available PET/CT scans after blood draw (~45 days). TMFCs were identified in 72% of pts (n=28/39), average 8 cells/sample, and ranged 1-77 cells. Of the TMFCs, 12% were subtype A, 11% as B, 11% as C, and 66% as D. CD47 expression was seen in 100% of TMFs, with low expression in 39% of subtype A cells, 58% subtype B, 39% subtype C, & 62% subtype D. Notably, CD47 was significantly lower ($p=0.010$) in subtype B than other groups. Further, the expression of syncytin-1 in subtype B cells was significantly lower ($p=0.02$) than in other subtypes, and subtype D cells had 75% higher syncytin-1 localized to the nuclear regions. No correlations were found in the SIRPa expression and subtypes. Interestingly, progressing pts had 200% more TMFCs, more subtype B (83%), and significantly lower ($p<0.001$) CD47 expressing TMFCs than non-progressing pts. In this pilot study on the expression profiles of circulating TMFCs, it appears that specific subtypes of TMFC with low CD47 expression may be associated with progressive disease. Interestingly, this contrasts existing studies on primary tumors which often correlate higher CD47 with more aggressive cancers. This finding suggests that low CD47 cancer cells circulate blood in some pts with progressing disease and may be pertinent to efforts targeting CD47 in cancer.

#1980 The significance of circulating tumor cells and circulating tumor DNA as prognostic markers in human papillomavirus-positive head and neck squamous cell carcinoma.

Hiroe Tada, Hideyuki Takahashi, Kazuaki Chikamatsu

Gunma University School of Medicine, Maebashi, Japan

Patients with human papillomavirus (HPV)-positive head and neck squamous cell carcinoma (HNSCC) respond favorably to treatment; however, some are resistant to treatment. Liquid biopsy is a potential prognostic biomarker for various types of cancer. In this study, we comprehensively investigated circulating tumor cells (CTCs), cell-free DNA (cfDNA), and circulating tumor DNA (ctDNA) and compared them to determine their potential as predictive biomarkers for HPV-positive HNSCC. First, we isolated CTCs and cfDNA from the peripheral blood of 42 HPV-positive HNSCC patients before treatment. CTCs were obtained using a size-based separation method with a microfilter or the CD45 depletion method. The expression of three epithelial-related genes (*EPCAM*, *MET*, and *EGFR*) in the isolated CTCs was determined by RT-qPCR. When at least one of the three epithelial-related genes was detected, the sample was defined as positive for CTCs. cfDNA was isolated from serum samples using the QIAamp Circulating Nucleic Acid Kit (QIAGEN) and quantified using an Agilent 4200 TapeStation. We then determined the level of cfDNA per 1ml of serum. Next, we examined ctDNA to detect E7 genes specific to HPV in cfDNA as ctDNA using droplet digital PCR. Finally, we analyzed the clinical significance of CTCs, cfDNA and ctDNA. CTCs were isolated in 21patients (50.0%) and the HPV E7sequence was detected in 17 patients (40.1%) of 42 HPV-positive HNSCC patients. Among the clinical factors, only N stage and ctDNA positivity were significantly associated(p=0.03). There were no associations between the presence of CTCs and clinical factors. We further investigated whether CTCs and ctDNA correlated with prognosis using multivariate analysis. CTC positivity was most strongly associated with progression-free survival (PFS) (Hazard Ratio=5.549, p=0.037); however, the presence of ctDNA was not correlated with PFS. The presence of CTCs was found to be the most prognostic factor in HPV-positive HNSCC patients compared to the presence of ctDNA. Moreover, the CTC-positive/ctDNA-negative group showed the most unfavorable PFS(p=0.02). The cfDNA levels were significantly lower in the CTC-positive/ctDNA-negative group (p=0.01). Previous studies have reported that cfDNA is primarily released by tumor apoptosis. The low levels of cfDNA in CTC-positive/ctDNA-negative patients suggest that CTCs may be shed into the peripheral bloodstream in a state of survival.Thus, our study provides new insights into predictive biomarkers of treatment resistance in patients with HPV-positive HNSCC.

#1981 Analytical validation of ANGLE's combined IF and FISH assay for assessment of HER2 in circulating tumor cells.

Mariacristina Ciccioli[1], Alex Young[1], Laila Vlietinck[1], Bethan Edwards[1], Chassidy Johnson[2], Anne-Sophie Pailhes-Jimenez[1]

[1]ANGLE Europe Limited, Guildford, United Kingdom,[2]BioView Inc., Billerica, MA

Background: Evaluation of HER2 status in tissue biopsies, through immunohistochemistry (IHC) and, in equivocal cases, fluorescence in-situ hybridization (FISH), is routinely performed to guide diagnosis and treatment decisions for breast cancer (BC) patients. Tissue biopsy is an invasive procedure for which re-examination is difficult and not commonly performed. Conversely, liquid biopsy is minimally invasive, with unbounded opportunity for recurrent sample collection, allowing monitoring of circulating tumor cells (CTCs). This study aimed to validate a Research Use Only assay by which HER2 immunofluorescence (IF) and FISH are performed on the same CTCs in each sample.
Method: Analytical performance of the test was established using contrived samples consisting of HER2+ (SKBR3) and HER2- (MCF-7 and Hs 578T) cultured cancer cells spiked into healthy volunteer blood samples, drawn in Streck Cell-Free DNA tubes. Blood was separated within 144 hours post-draw on ANGLE's Parsortix® instrument, a marker-independent microfluidic device which isolates CTCs based on size and deformability. Harvests were spun onto CellKeep™ slides and stained with ANGLE's HER2 IF panel, comprising a nuclear dye, epithelial, mesenchymal and leukocyte markers and an anti-HER2 antibody. Slides were imaged on a BioView Allegro Plus, a platform equipped with artificial intelligence for automated fluorescence imaging, quantitative cellular measurements, CTC candidate identification and reporting. IF stained slides were destained and probed for ERBB2 and CEP17 genes by FISH. Using the Allegro Plus single cell relocation capabilities, CTCs identified by IF were relocated and imaged at 40× to capture HER2 and CEP17 foci. Results. The workflow was performed on a total of 186 contrived samples to determine analytical performance of the assay. Linearity, specificity, sensitivity, repeatability, reproducibility (inter-donor, operators, days and reagents) and endogenous interference (Albumin, Haematocrit and Triglycerides) were assessed. The IF assay was observed to be linear over the range of 0 - 1000 cells, with R2 of 0.96 and degree of non-linearity of 0.004. Analytical specificity and sensitivity of all IF markers were >96%. Analytical specificity and sensitivity of the FISH assay were 100%. The assay was shown to be repeatable and reproducible across spiked donors, operators, days and reagents. Endogenous substances investigated did not affect qualitative results of the assay.
Conclusion: This study demonstrated that ANGLE's combined IF and FISH assay is a robust method for determination of HER2 protein expression and concomitant interrogation of HER2 gene amplification on the cancer cells. This workflow will be utilized to analyze CTCs isolated from a cohort of ~100 metastatic BC patients to evaluate its clinical feasibility and validity and these results will be reported at the AACR annual meeting.

#1982 Transcriptomic subtype identification of circulating tumor cells by label-free capture and single-cell RNA sequencing.

Yoon-Tae Kang, Patricia Schnepp, Rashika Shrestha, Rain Yu Hong, Errile Pusod, Will Chow, Floyd Watkins, Kyle Thurston, Dominique Winston, Marta Gonzalez-Plasky, Angelica Olcott, David Coe, Michelle Racey, Elizabeth Dreskin

Bio-Rad Laboratories, Hercules, CA

Introduction: Single-cell RNA sequencing (scRNA-Seq) of rare cells, such as circulating tumor cells (CTCs), is critical for identifying various cell subtypes and understanding intercellular heterogeneity at the genetic level. Despite recent advances in scRNA-Seq, transcriptomic profiling of single CTCs has been technically demanding due to its limited cell utilization and background noise from unwanted cells during sequencing. In addition, conventional CTC isolation methods often miss important rare cell subtypes due to their low abundance and reliance on specific surface markers for detection. This limitation hinders comprehensive transcriptomic analysis, especially for CTCs undergoing the critical epithelial-mesenchymal transition (EMT), which may not express typical epithelial markers. To address these challenges, we present an optimized workflow that integrates high-purity label-free CTC capture with in-droplet barcoding-based scRNA-seq to achieve higher cell utilization and lower background sequencing noise.
Methods: To model heterogeneous CTC subpopulations at different EMT status, samples were prepared by spiking in two types of cancer cells, MCF7 (epithelial CTCs) and HeLa (mesenchymal CTCs), into a total of 7.5 ml of whole blood from a healthy donor at a concentration of 100 cells/ml. CTCs were captured using the Genesis Cell Isolation System and the enriched CTCs were further purified to reduce leukocyte contamination or red blood cell (RBC) contamination using CD45 pre-conjugated magnetic beads or RBC lysis reagent, respectively. The processed CTC sample was then used to create scRNA-Seq libraries following the Bio-Rad ddSEQ Single-Cell 3' RNA-Seq Kit protocol, which includes single-cell in-droplet barcoding, reverse transcription, and cDNA synthesis.
Results: This Genesis System allowed for capturing both epithelial and mesenchymal CTCs to be sequenced. After sequencing and Omnition Software Analysis, individual cells were visualized using a Uniform Manifold Approximation and Projection (UMAP) plot and clustering analysis showed a distinct separation between epithelial and mesenchymal CTCs while each different cell population revealed expression of representative genes for each cell type.
Discussion and Conclusions: By combining rare single cell capture with scRNA-seq library prep and analysis, our workflow provides an easy, highly sensitive and cost-effective solution for CTC single-cell transcriptomic studies, compatible with commercially available instruments and kits. This scRNA-seq workflow can be integrated into a broad spectrum of applications in liquid biopsy across many different cancer types and empowering researchers to gain deeper insights into tumor heterogeneity and gene expression patterns with greater efficiency and within the same budget.

#1983 Phase II study of rucaparib and nivolumab in patients with leiomyosarcoma.

Carmelina Charalambous, Ronak H. Shah, Michael F. Berger, Sujana Movva

Memorial Sloan Kettering Cancer Center, New York, NY

Introduction: Rucaparib, a small molecule inhibitor of PARP, is approved for treating patients with refractory deleterious BRCA mutations, while nivolumab, a human monoclonal antibody targeting PD-1, is approved for various cancers. This phase II study aimed to explore whether rucaparib could enhance the immune response of nivolumab in patients with leiomyosarcoma (LMS). Here, we present an exploratory analysis of circulating tumor DNA (ctDNA) dynamics and imaging-based responses.
Methods: Twenty patients with LMS were enrolled in the trial and received rucaparib (600 mg twice daily for 28 days) in combination with nivolumab (480 mg day 1 of every 4-week cycle). Plasma samples were collected for ctDNA analysis at baseline and at weeks 5, 9, 17, 25, and the end of treatment, utilizing the MSK-ACCESS cfDNA assay to detect alterations across 129 genes. Matched tumor tissue was sequenced using MSK-IMPACT. Treatment continued until disease progression, unacceptable toxicity, or patient withdrawal. The primary endpoint was efficacy, measured by best response by week 24, according to RECIST 1.1 criteria.
Results: In this exploratory analysis in LMS patients, ctDNA was detected at baseline in 100% of samples. Concordance analysis between MSK-ACCESS and MSK-IMPACT revealed a baseline concordance of 63.6% in terms of the specific mutations detected, which increased to 72.7% when considering all plasma samples. The ctDNA dynamics were compared to RECIST imaging. Among the patients, eight exhibited RECIST-confirmed progression at the first imaging assessment, seven of whom had increasing ctDNA levels. Additionally, five patients discontinued the study due to clinical progression despite RECIST indicating stable disease, with 80% (4 of 5) showing rising ctDNA levels. This study highlights the limitations of using RECIST criteria and suggests that ctDNA dynamics may be a more sensitive indicator of disease progression. Baseline and post-chemotherapy cycle-2 ctDNA levels have been previously associated with survival outcomes in LMS. Consistently, our analysis revealed that ctDNA levels at week 5 predicted radiologic response, with 90% of patients experiencing disease progression showing increases in ctDNA. Notably, one patient who achieved a partial response demonstrated undetectable ctDNA at week 5, which remained so until treatment discontinuation due to toxicity.
Conclusion: The combination of ctDNA dynamics with RECIST imaging shows potential for improving the monitoring of patient response and predicting disease progression in LMS. Overall, while the study underscores the limitations of the rucaparib and nivolumab combination, ctDNA appeared to predict early progression and response to therapy and was a useful adjunctive test in cases where the treatment benefit was unclear. Further study is needed to validate these findings.

#1984 Correlating circulating tumor cell dynamics with clinical outcomes in patients with advanced stage melanoma.

Vincent T. Ma[1], Yeonhee Park[2], Matthew C. Mannino[1], **Jennifer L. Schehr**[1], Alexander Birbrair[3], Joshua M. Lang[1], George S. Zhao[4]

[1]Medicine; Division of Hematology, Medical Oncology, and Palliative Care, University of Wisconsin Carbone Cancer Center, Madison, WI,[2]Biostatistics and Medical Informatics, University of Wisconsin Carbone Cancer Center, Madison, WI,[3]Dermatology, University of Wisconsin Carbone Cancer Center, Madison, WI,[4]Human Oncology, University of Wisconsin Carbone Cancer Center, Madison, WI

Background: Liquid biopsies in advanced solid tumors can provide a unique noninvasive approach to monitor disease burden over time. Detection and capturing circulating tumor cells (CTCs) in melanoma patients have faced many challenges and limitations. In our study, we use our novel, high-sensitivity, CTC capturing technology to assess numerical heterogeneity of serially collected CTCs in advanced stage melanoma patients undergoing systemic treatment and correlate CTC dynamics with tumor response and risk of disease progression.

Methods: We utilize a unique microfluidic exclusion-based sample preparation (ESP) technology at the University of Wisconsin (Madison) to isolate CTCs from patients with stage III/IV melanoma. Capture specificity of antibodies against melanoma associated proteins, NG2 (MCSP) and CD146 (MCAM) were used. We prospectively collected plasma samples from patients and evaluated the quantitative and qualitative change in numerical CTCs at baseline to 1-3 months after the start of systemic therapy. Based on CTC dynamics, a logistic regression model was used to evaluate the odds of an objective response (partial response and complete response per RECIST v1.1) and a cox proportional hazard model was used to investigate its effect on progression free survival (PFS).

Results: 35 patients were evaluated and longitudinally followed. Patients were treated with immune checkpoint inhibitors (29), BRAF/MEK targeted therapy (4), or tebentafusp-tbn (2). Median follow up was 13.1 months. A range of 0 and 531 CTCs were detected at baseline (i.e. before start of treatment). Patients with an objective response had a mean slope of -22.2 CTCs/month and patients with stable disease or progressive disease had a mean slope of +10.4 CTCs/month (Wilcox p=0.0008). A qualitative decrease in CTC count was associated with an objective response (OR 6.50, 95% CI 1.80-41.57, p=0.0004) and a longer PFS (HR 0.38, 95% CI 0.15-0.91, p=0.031) compared to an increase in CTC count from baseline to 1-3 months after starting systemic therapy.

Discussion: Our study demonstrates the prognostic value of CTC enumeration in advanced stage melanoma patients. Using our novel ESP technology, we are able to successfully capture CTCs and correlate CTC dynamics with clinical outcomes, including objective response and PFS. A larger sample cohort is planned to validate its utility in predicting treatment outcomes in clinical practice.

#1985 5hmC-sequencing of matched cfDNA and tissue from men with mCRPC is concordant and identifies loss of AR signaling in NEPC and DNPC.

Rensheng Wan[1], Raunak Shrestha[1], Gulfem Guler[2], Yuhong Ning[2], Aishwarya Subramanian[1], Adam Foye[3], Meng Zhang[1], Xiaolin Zhu[3], Thaidy Moreno-Rodriguez[4], Haolong Li[1], Shuang G. Zhao[5], SU2C/PCF West Coast Prostate Cancer Dream Team, Joshi J. Alumkal[6], Rahul Aggarwal[3], Alexander W. Wyatt[7], David Quigley[4], Samuel Levy[2], Eric Small[3], Felix Feng[1], Martin Sjostrom[1]

[1]Radiation Oncology, UCSF - University of California San Francisco, San Francisco, CA,[2]Clearnote Health, San Diego, CA,[3]Medicine, UCSF - University of California San Francisco, San Francisco, CA,[4]Urology, UCSF - University of California San Francisco, San Francisco, CA,[5]Human Oncology, University of Wisconsin-Madison, Madison, WI,[6]Hematology and Oncology, University of Michigan Rogel Cancer Center, Ann Arbor, MI,[7]Urologic Sciences, University of British Columbia, Vancouver, BC, Canada

Purpose: In this study, we aimed to study whether 5-hydroxymethylcytosine sequencing (5hmC-seq) of circulating cell-free DNA (cfDNA) predicts gene expression in tumor tissue and can distinguish tumor subtypes defined in tissue in metastatic castration-resistant prostate cancer (mCRPC).

Methods: We performed 5hmC-seq on cfDNA samples from 86 mCRPC patients with matched tumor tissue profiled with 5hmC-seq (N=49) and RNA-sequencing (N=86) and we compared cfDNA 5hmC levels with matched tissue 5hmC levels and tissue gene expression. We developed a 5hmC-seq-based circulating tumor-DNA fraction (ctDNA-fraction) classifier and assessed if gene-level and pathway-level differences between mCRPC subtypes could be detected in cfDNA using a differential 5hmC-analysis adjusting for ctDNA-fraction.

Results: Patients with androgen receptor (AR)-positive prostate cancer (ARPC) exhibited lower ctDNA-fraction, whereas patients with neuroendocrine (NE) prostate cancer (NEPC) and double-negative prostate cancer (DNPC) tumors displayed higher ctDNA-fraction (median ctDNA-fraction across subtypes 0.09 [95% confidence interval (CI), 0.04-0.14] (ARPC), 0.41 [95% CI, 0.18-0.64] (NEPC) and 0.37 [95% CI, 0.30-0.44] (DNPC), pairwise t-test $P = 5.5 \times 10^{-2}$ (ARPC vs. NEPC) and $P = 8.8 \times 10^{-4}$ (ARPC vs. DNPC) respectively). Nearly 30% of all protein-coding genes showed significant concordance between cfDNA 5hmC and tissue RNA-seq, after adjusting for ctDNA-fraction (adjusted $p<0.05$ in linear model), which were enriched in the androgen response, EGFR, and ERBB signaling pathways. Compared to ARPC, NEPC displayed upregulated 5hmC enrichment in NE-related genes, and downregulated androgen response and MYC target pathways in cfDNA. As expected, an NE pathway score calculated from cfDNA 5hmC was significantly different between tissue-confirmed ARPC and NEPC ($p=0.03$). Double-negative prostate cancer (DNPC) showed downregulated androgen response and MYC targets and upregulated epithelial cell pathways compared to ARPC in cfDNA. Furthermore, DNPC indicated an aggressive phenotype with cell proliferation pathways even more upregulated when compared to NEPC.

Conclusions: We created a cohort of 86 matched tissue and cfDNA samples and demonstrated concordance for a significant number of transcribed protein-coding genes. Expected biological differences previously seen in tissue between NEPC and ARPC were readily detected via 5hmC profiles in cfDNA. Furthermore, DNPC showed a clear downregulation of androgen response signaling in cfDNA, indicating the possibility to identify a group of patients in addition to classical NEPC that may have reduced response to standard androgen-targeting agents. Future work will aim to develop single-sample multi-class subtype classifiers and evaluate differences in prognosis and treatment response based on cfDNA-based subtyping.

#1986 A novel single-cell CTC platform enables first liquid biopsy for ADC target expression profiling.

Antoine Herzog[1], Xiaokang Li[1], Mattia Lazzaroni[1], Salome Thuler[1], Sylvie Andre[2], Marina Alexandre Gaveta[2], Stefano Varricchio[1], Olivier Dubey[1], Bastien Vergnet[1], Krisztian Homicsko[2]

[1]Parithera SA, Epalinges, Switzerland,[2]Department of Oncology, CHUV, Lausanne, Switzerland

Introduction: Despite the expanding landscape of antibody-drug conjugates (ADCs), patient selection remains challenging due to limited liquid biopsy methods for target assessment. Current tissue approaches lack longitudinal assessments and provide insufficient resolution of target expression heterogeneity that could inform ADC selection. We present the first liquid biopsy platform specifically designed for ADC therapy, designed to inform treatment selection through single-cell RNA sequencing of circulating tumor cells (CTCs). This platform provides comprehensive profiling of target antigen expression across multiple ADC targets, establishing the foundation for target expression-based ADC selection. Beyond initial target profiling, this approach could provide a foundation for studying target dynamics and potential resistance mechanisms.

Methods: 10 patients with various solid tumors were consented for research from the observational clinical study CTC-M. Blood samples (3x10mL) were collected and processed using either our proprietary platform or a combination of existing commercial instruments. Individual CTCs were barcoded and analyzed via single-cell RNA sequencing to profile expression of multiple ADC targets (including HER2, TROP2, Nectin-4, and c-MET) along with global transcriptome patterns. Patient demographic and disease characteristics were collected in a de-identified database.

Results: Single-cell RNA sequencing of isolated CTCs revealed detection of ADC target expression across various cancers. Our fully automated platform delivers barcoded CTCs ready for single-cell sequencing in under 1 hour compared to 6+ hours for existing workflows, integrating both isolation and molecular barcoding steps. The novel isolation technology achieves a 10-100X improvement in CTC purity versus current methods, overcoming a key technological hurdle compared to other systems. The platform maintains plasma integrity throughout processing, enabling simultaneous CTC and ctDNA analysis from a single blood draw.

Conclusions: We have developed the first integrated platform enabling rapid, automated preparation of CTCs for single-cell ADC target profiling. Our workflow demonstrates significant advantages in speed, purity, and cost-effectiveness, while enabling concurrent CTC and ctDNA analysis from a single blood draw. This technology establishes the foundation for comprehensive liquid biopsy-based ADC target assessment, addressing a critical unmet need in precision oncology.

#1987 Circulating tumor cell-based molecular responses stratify EGFR-TKI efficacy in EGFR-mutant lung cancer patients.

Seoyoung Lee[1], Chaeyeon Kim[2], Chang Gon Kim[3], Min Hee Hong[3], Mina Han[2], Wonrak Son[2], Gamin Kim[2], Minseok Kim[4], Hye Ryun Kim[3]

[1]Gangnam Severance Hospital, Seoul, Korea, Republic of,[2]Yonsei university college of medicine, Seoul, Korea, Republic of,[3]Yonsei cancer center, Seoul, Korea, Republic of,[4]CTCELLS Inc., Seoul, Korea, Republic of

Background: Liquid biopsy offers a minimally invasive alternative to tissue biopsy, enabling therapeutic monitoring, precision medicine-based treatment decisions, and detection of tumor heterogeneity. Circulating tumor cell (CTC) analysis is a promising minimally invasive biomarker for monitoring treatment response and stratifying prognosis in *EGFR*-mutant non-small cell lung cancer (NSCLC). Meanwhile, detection of CTCs in peripheral blood is challenging due to their rarity and the limitations of both marker-dependent and marker-independent isolation techniques, which may compromise sensitivity and specificity. Continuous Centrifugal Microfluidics - Circulating Tumor Cell Disc (CCM-CTCD) enriches heterogeneous CTCs through automated microfluidic extraction and leukocyte depletion without interrupting centrifugation.

Method: In this study, 77 *EGFR*-mutant NSCLC patients treated with EGFR-TKIs were enrolled. CTCs were isolated using CCM-CTCD before and after EGFR-TKI treatment and compared with plasma cfDNA and tissue biopsy for *EGFR* mutation analysis. CK-positive, CD45-negative CTCs were isolated and visualized using immunofluorescence staining. Patients were categorized as CTC molecular responders or non-responders based on a ≥30% reduction in CTC count from baseline. Progression-free survival (PFS) and tumor burden changes were evaluated.

Results: The CCM-CTCD method showed concordance with Cobas® liquid biopsy but demonstrated superior sensitivity in detecting *EGFR* mutations. Mutational discordance between tissue, plasma cfDNA, and CTCs highlighted tumor heterogeneity. CTC molecular responders had significantly longer median PFS (46.3 vs. 12.2 months) and greater tumor burden reduction (-39.2% vs. -32.2%, $p = 0.025$) compared to non-responders. Multivariable analysis revealed that CTC molecular response was an independent predictor of improved PFS (HR 0.45, $p = 0.023$). Additionally, a non-significant trend was observed toward shorter PFS in patients receiving later-line treatments compared to first-line (HR 1.30, $p = 0.523$), first- or second-generation EGFR-TKIs compared to third-generation EGFR-TKIs (HR 1.59, $p = 0.196$), and in those with a history of smoking (HR 1.45, $p = 0.300$). CTC profiling was found to complement traditional methods for identifying genomic alterations and predicting early progression.

Conclusion: CTC analysis using CCM-CTCD demonstrates potential as a reliable tool for predicting treatment response and stratifying prognosis in *EGFR*-mutant NSCLC. Stratifying patients based on CTC molecular response may guide treatment intensification strategies, offering a pathway for risk-adapted precision oncology. Prospective studies are warranted to validate these findings and optimize therapeutic decision-making.

#1988 Detection of minimal residual disease using cell free plasma DNA to guide maintenance therapy in high grade serous ovarian cancer.

Pamela Soberanis Pina[1], Stephenie D. Prokopec (co-primary author)[1], Eduardo Gonzalez Ochoa[1], Derek Wong[1], Tracy Stockley[1], Amit Oza[1], Neesha C. Dhani[1], Robert C. Grant[1], Nitthusha Singaravelan[1], Alexander Fortuna[2], Bernard Lam[2], Ilinca Lungu[2], Madhuran Thiagarajah[2], Faridah Mbabaali[2], Sharanjit Singh[2], Danny Xie[3], Steven Siman[3], Judy Quintos[3], Valerie Bowering[3], Lisa Wang[3], Stephanie Lheureux (co-corresponding author)[3], Trevor Pugh (co-corresponding author)[3]

[1]Medical Oncology, UHN Princess Margaret Cancer Centre, Toronto, ON, Canada,[2]Ontario Institute for Cancer Research, Toronto, ON, Canada,[3]Medical Oncology, Princess Margaret Hospital, Toronto, ON, Canada

Purpose: Optimal maintenance strategy following re-challenge with platinum-based chemotherapy in patients with relapsed high grade serous ovarian cancer (HGSOC) after PARP inhibitor (PARPi) therapy remains to be established. Use of cell-free DNA (cfDNA) sequencing to detect minimal residual disease (MRD) has emerged as a promising strategy to identify patients at higher risk of relapse who might benefit from more timely, additional treatment.
Methods: Nineteen patients with histologically confirmed TP53 mutant HGSOC with platinum sensitive relapse were enrolled into an interventional cfDNA study selecting for patients with confirmed MRD (NCT04510584). Each patient provided a blood sample at the end of standard-of-care platinum treatment (pre or post cycle 6) from which cfDNA was analyzed using targeted sequencing (10,000X coverage) using a 10 gene panel. As a comparator, 30X cell-free whole genome sequencing (cfWGS) was performed for tumour-informed genome-wide mutational sampling benchmarked against 13 platinum-sensitive HGSOC patients and 25 healthy controls.
Results: The targeted panel approach detected a somatic TP53 mutation in 1/19 patients above the limit of detection of 1%. In contrast, 30X cfWGS identified evidence of MRD in 8/19 patients (47%) with detection of 319-2,327 known somatic mutations sampled genome-wide (including single-read evidence for 4 somatic TP53 mutations). Genome-wide fragmentomics approaches further identified MRD in 17/19 platinum-resistant patients (89%).
Conclusions: cfWGS can be used to examine numerous features capable of identifying patients likely to experience rapid relapse of high grade serous ovarian cancer.

#1989 Heterogeneity of epithelial, mesenchymal, progenitor, and stem cell phenotypes on CTCs correlates to clinical outcomes in metastatic cancer patients.

Alexis B. Duffy[1], Massimo Cristofanilli[2], Carolina Reduzzi[2], Cha-Mei Tang[3], Saranya Chumsri[4], Susan Tsai[5], Mohammed Aldakkak[6], Daniel L. Adams[1]

[1]Creatv MicroTech, Inc., Monmouth Junction, NJ,[2]Weill Cornell Medicine, New York, NY,[3]Creatv MicroTech, Inc., Potomac, MD,[4]Mayo Clinic Cancer Center, Jacksonville, FL,[5]Ohio State University, Columbus, OH,[6]Medical College of Wisconsin, Milwaukee, WI

Circulating tumor cells (CTCs) are cancer cells that detach from tumors, enter the blood and seed distant sites. CTCs spread as single cells, or cluster aggregates, and can be heterogenous with differing biological phenotypes, i.e. epithelial, mesenchymal, progenitor, & stem cell. Three clinically relevant CTC subtypes are often described in patients (pts) 1) CTCs with normal epithelial traits (strong filamentous cytokeratin (CK+ & CD45-/CD14-), 2) Epithelial-mesenchymal CTCs (EMTs), cancer cells with downregulated epithelial & upregulated mesenchymal traits (low diffused CK+, CD45-/CD14-) and 3) tumor-macrophage fusion cells (TMFCs), cancer cells that fuse with macrophages (polynucleated DAPI+, diffuse CK+ and CD14+/CD45+). To better elucidate the biological and clinical importance of the 3 CTC subtypes, we isolated CTCs by a marker agnostic method from the blood of n=49 metastatic cancer pts. We then enumerated and phenotypically subtyped CTCs comparing clinical utility of clustering and CTC heterogeneity over 2 years. Peripheral blood samples were collected from 49 metastatic cancer pts, breast (n=38), pancreatic (n=9), colon (n=3), lung (n=1), and prostate (n=1). Whole blood (7.5mL) was filtered by size exclusion using CellSieve microfilters and fluorescently stained for CD45, CD14, CK, and DAPI to identify CTCs, EMTs and TMFCs. Cells were imaged, enumerated and fluors quenched by the QUAS-R technique; followed by re-stained for CD44, CD24, CD133, CD144, N-Cadherin, EpCAM, and Vimentin. Markers were categorized as negative, low, medium, or high. A heterogeneity biomarker score was developed as high, 2-3 log deviations between marker expression, or low 0-1. Pt progressive free survival (PFS) and overall survival (OS) were analyzed over 2 years. CTCs were found in 40% of pts, EMTs in 51%, & TMFCs in ~93%. For subtyping, CD44 was in CTCs 88%, EMTs 59%, & TMFCs 91%; CD24 in CTCs 40%, EMTs 41%, & TMFCs 6%; CD133 in CTCs 100%, EMTs 8%, & TMFCs 100%; CD144 in CTCs 100%, EMTs 77%, & TMFCs 82%; N-cadherin in CTCs 100%, EMTs 0%, & TMFCs 45%; Vimentin in CTCs 51%, EMTs 100%, & TMFCs 83%; EpCAM in CTCs 74%, EMTs 12%, & TMFCs 20%. CTCs and EMTs were found to have high heterogeneity in 30% of samples in both individual cells and clusters, with a high biomarker heterogeneity significantly correlated with poorer outcomes (PFS: HR=15.4, p=0.0014, CI=3.4-68.9; OS: HR=12.4, p=0.0056, CI=2.6-59.1). TMFCs were found to have high heterogeneity, 40% of samples, but this was not correlated with outcomes. In this pilot study, it appeared that isolated CTC, EMTs and TMFCs subtypes have differing heterogenous expressions of epithelial, mesenchymal, progenitor, and stem cell markers. Further, high rates biomarker heterogeneity in CTCs and EMTs, but not TMFCs, correlated with faster rates of progression & death. Larger validation experiments with biomarker stratification is ongoing.

#1990 New diagnostic technology to detect myeloma cells in the blood of multiple myeloma patients.

Jihyun Lee, Hoin Kang, Soee Kim, Ju Bi Lee, Min-Ji Song, Jung Won Kim

Cytogen, Inc., Seoul, Korea, Republic of

Introduction: Androgen-receptor splice variant 7 (AR-V7) is constitutively activated isoform of AR and has been associated with the resistance towards AR targeting therapies and development into metastatic castration-resistant prostate cancer (mCRPC). Analysis of AR-V7 expression is an important step in prostate cancer diagnosis and determining the therapeutic drug, but the detection of AR-V7 with tissues has limitations such as clinical challenge to poorly accessible tumor tissues before and after treatment. Here, we developed the method of AR-V7 detection in circulating tumor cells (CTC) after isolation with CytoGen's. Smart Biopsy CTC isolator by droplet digital polymerase chain reaction (ddPCR) with high sensitivity and specificity, overcoming the limitations of tissue biopsy.
Method: Sensitivity and specificity were tested using AR-V7 positive cells (VCaP or 22Rv1) and negative cells (PBMC). Limit of detection (LOD) test of AR-V7 expression was performed that AR-V7 positive cells (10 and 5 cells) were spiked into human blood sample (5mL, healthy donor) to mimic CTC after isolation by CytoGen's Smart Biopsy CTC isolator, subsequently AR-V7 expression was tested with ddPCR. All ddPCR processes included droplet making and reading for each assay were performed on the QX200 ddPCR System (Bio-Rad). For the clinical evaluation, blood samples (each 20mL) of metastatic prostate cancer patients (n=5) were tested with IF assay (5mL) to count CTC number and ddPCR (15ml) to analyze the expression of AR-V7.
Results: In in vitro spiking test, AR-V7 expression was clearly detected in the LOD test of AR-V7 positive cells spiked into human blood and was not detected at all in the negative controls. In clinical evaluation study, isolation of CTC from the blood of prostate cancer patients using CytoGen's Smart Biopsy CTC isolator was confirmed from the result of IF assay, and the expression of AR-V7 in CTC was successfully detected by ddPCR. IF analysis identified 3 or more CTCs in 5 ml of blood from all patients, and expression of AR-V7 was verified by ddPCR in 4 out of 5 mCRPC patients.. These results demonstrate that it is possible to clearly detect the presence of RNA variants is related with drug resistance in mCRPC patients using liquid biopsy, utilizing CTCs, which exist in only very small quantities in the blood.
Conclusions: AR-V7 expresses only a part of cancer tissue, so it is difficult to accurately diagnose with traditional histological assays. Detection of AR-V7 (RNA signature) in intact live CTC might overcome limitations of tissue biopsy. In this study, we developed the method to detect AR-V7 in CTC, and presented the results of verifying AR-V7 expression using in vitro models and blood of mCRPC patients. Expanded clinical validation study with more patients' samples and clinical correlation between AR-V7 expression in CTC and clinical outcome will be performed and analyzed.

#1991 Circulating tumor cells migrate beyond the central nervous system in glioblastoma multiforme.

J. Srovnal[1], P. Stejskal[1], A. Rehulkova[1], A. Srovnalova[1], O. Kalita[2], M. Slachta[2], M. Hajduch[1];
[1]Palacky University. Faculty of Medicine, Olomouc, Czech Republic, [2]Olomouc University Hospital, Olomouc, Czech Republic

Introduction: The concept of liquid biopsy (LB) has garnered significant attention over the past decade, offering the potential to transform cancer care. However, its clinical utility requires further validation through rigorous trials. This study focuses on glioblastoma multiforme (GBM), an aggressive and fatal subtype of central nervous system malignancies. Circulating tumor cells (CTCs), which originate from primary tumors and migrate via peripheral blood to distant sites, are closely linked to tumor spread and recurrence. In GBM, systemic dissemination is rare due to challenges like the blood-brain barrier (BBB), lack of lymphatic drainage, and the disease's short survival timeframe. Despite these challenges, noninvasive detection of CTCs holds promise for diagnostic, predictive, and prognostic applications.
Materials and Methods: Peripheral blood samples from 110 curatively resected GBM patients were collected preoperatively using Cell-Free DNA BCT® tubes (Streck, Inc.) to preserve cell stability. CTC identification was conducted with the CytoTrack CT11TM (2/C) system, a semi-automated immunofluorescence microscopy platform employing glial fibrillary acidic protein (GFAP), vimentin, CD45 immunostaining, and DAPI counterstaining. The tumor origin of CTCs was confirmed through aCGH analysis using DNA extracted from single CTCs and matched FFPE tumor tissues in 10 GBM patients. A preliminary Kaplan-Meier survival analysis was performed on 55 patients with a follow-up period exceeding two years, stratified by CTC presence.
Results: We evaluated peripheral blood samples from 110 GBM patients, identifying CTCs or cancer cell clusters in 35% of cases. The aCGH genomic profiling verified the tumor origin of CTCs in 90% of cases (9/10). Kaplan-Meier survival analysis revealed no significant correlation between CTC presence and overall survival of GGM patients. This outcome may be attributed to the generally short survival associated with GBM, which could improve with advancements in oncology and palliative care. Another possibility is the suppression of tumor cell growth outside the CNS.
Conclusions: This study confirms the presence of CTCs in the peripheral blood of 35% of GBM patients, although currently without prognostic significance. Patient enrollment and the collection of clinical data are ongoing. Acknowledgments: This work was supported by the European Union - Next Generation EU (LX22NPO5102), Palacký University Olomouc (LF UP 2024_007), and the SALVAGE project (CZ.02.01.01/00/22_008/0004644).

#1992 Detection rate for *ESR1* mutations is higher in CTC-derived genomic DNA than in paired plasma cfDNA samples as revealed by ddPCR.

S. Smilkou[1], A. Ntzifa[1], V. Tserpeli[1], I. Balgkouranidou[2], A. Papatheodoridi[3], E. Razis[4], H. Linardou[5], C. Papadimitriou[6], A. Psyrri[7], F. Zagouri[3], S. Kakolyris[2], **E. Lianidou**[1];

[1]Analysis of Circulating Tumor Cells, Laboratory of Analytical Chemistry, Department of Chemistry, University of Athens, Athens, Greece, [2]University General Hospital of Alexandroupolis, Department of Medical Oncology, Alexandroupolis, Greece, [3]Alexandra Hospital, Department of Clinical Therapeutics, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece, [4]Third Department of Medical Oncology, Hygeia Hospital, Athens, Greece, [5]Metropolitan Hospital, Oncology Unit, Athens, Greece, [6]Oncology Unit, Aretaieion University Hospital, National and Kapodistrian University of Athens, Athens, Greece, [7]Department of Medical Oncology, Second Department of Internal Medicine, "Attikon" University General Hospital, Athens Medical School, National and Kapodistrian University of Athens, Athens, Greece

Plasma cell-free DNA (cfDNA) analysis to track estrogen receptor 1 (*ESR1*) mutations is highly beneficial for the identification of tumor molecular dynamics and the improvement of personalized treatments for patients with metastatic breast cancer (MBC). Plasma cfDNA is, up to now, the most frequent liquid biopsy analyte used to evaluate *ESR1* mutational status. Circulating tumor cell (CTC) enumeration and molecular characterization analysis provides important clinical information in patients with MBC. In this study, we investigated whether analysis of CTCs and circulating tumor DNA (ctDNA) provide similar or complementary information for the analysis of *ESR1* mutations. We analyzed both plasma cfDNA (*n*=90) and paired CTC-derived genomic DNA (gDNA; *n*=42) from 90 MBC patients for seven *ESR1* mutations. Eight out of 90 (8.9%) plasma cfDNA samples tested using the ddPLEX Mutation Detection Assay (Bio-Rad), were found positive for one *ESR1* mutation, whereas 11/42 (26.2%) CTC-derived gDNA samples were found positive for at least one *ESR1* mutation. Direct comparison of paired samples (*n*=42) revealed that the *ESR1* mutation rate was higher in CTC-derived gDNA (11/42, 26.2%) than in plasma cfDNA (6/42, 14.3%) samples. Our results, using this highly sensitive ddPLEX assay, reveal a higher percentage of mutations in CTC-derived gDNAs than in paired ctDNA in patients with MBC. CTC-derived gDNA analysis should be further evaluated as an important and complementary tool to ctDNA for identifying patients with *ESR1* mutations and for guiding individualized therapy.

#1993 A translational circulating tumor cell drug sensitivity screening platform for integrated drug development and precision medicine: Results from Antrodia cinnamomea profiling.

Chia-Liang Yen, Pei Yu Chen, **Shih Pei Wu**

CancerFree Biotech, Jersey City, NJ

Reliable preclinical drug sensitivity screening platforms are crucial for advancing precision medicine and drug development. We have established a circulating tumor cell (CTC) drug sensitivity screening platform that enables comprehensive applications, including treatment response prediction and molecular profiling. To demonstrate the platform's utility, we evaluated multiple compounds including Antrodia cinnamomea (AC) and standard chemotherapy across various cancer types.

For the AC study, CTCs were isolated from patients (n=9) with brain tumors (n=3), osteosarcoma (n=2), renal cell carcinoma (n=1), small cell lung cancer (n=1), breast cancer (n=1), and colon adenocarcinoma (n=1). CTCs were expanded using the E.V.A. Select platform with biomimetic 3D surface structure. Cells were treated with AC (0-125 μg/mL) and responses were compared with platinum-based chemotherapy (cisplatin/carboplatin). Cell viability was assessed using CellTiter-Glo® luminescent assay after 72 hours. Our platform has also demonstrated capability for next-generation sequencing in separate drug discovery cohorts. Data analysis was assisted by artificial intelligence tools (Claude, Anthropic).

AC demonstrated consistent dose-dependent cytotoxicity, with survival rates at 125 μg/mL ranging from 5.7-13.8% across cancer types. Compared to platinum drugs (survival rates 22-67%), AC showed stronger or comparable efficacy. Brain tumor samples showed particular sensitivity to AC (125 μg/mL: 5.7-13.8% survival vs. cisplatin: 66.5% survival). Osteosarcoma samples demonstrated consistent AC response (125 μg/mL: 6.1-10.0% survival vs. carboplatin: 31.4-43.5% survival). The platform has successfully supported both drug sensitivity screening and molecular profiling across multiple drug development programs.

This study showcases our platform's versatility in drug sensitivity screening and comprehensive drug development applications. The E.V.A. Select platform supports both drug response profiling and molecular characterization, making it a valuable tool for drug discovery and precision medicine applications. These capabilities enable systematic evaluation of novel compounds while supporting mechanistic understanding through molecular analyses.

#1994 Performance evaluation of the Smart biopsy™ CTC isolator for circulating tumor cells' isolation in liquid biopsy.

Soee Kim, Jihyun Lee, Hoin Kang, Ju Bi Lee, Minseon Hwang, Keoung Sun Son, Jung Won Kim

Cytogen, Inc., Seoul, Korea, Republic of

Introduction: Current cancer diagnostic methods primarily involve tissue biopsies, which are invasive and may not fully reflect tumor heterogeneity. Liquid biopsy, a minimally invasive alternative, has emerged as a promising solution to overcome the limitations of tissue biopsies, offering advantages such as real-time monitoring and reduced patient discomfort for cancer diagnosis. Therefore, the development of reliable, non-invasive diagnostic platforms is crucial for effective cancer management. CytoGen has developed the Smart biopsy™ CTC isolator, a liquid biopsy system designed to isolate circulating tumor cells (CTCs) in blood.. However, since CTCs are present in very low quantities in the blood, it is essential to consider key metrics such as the limit of detection (LoD) and cell recovery rates when developing liquid biopsy-based cancer diagnostic platforms. This study aims to evaluate the performance of the Smart biopsy™ CTC isolator in the isolation of CTCs by assessing LOD and cell recovery rates.

Method: To evaluate the performance of CytoGen's Smart Biopsy™ CTC Isolator, recovery rate and limit of detection (LOD) tests were performed using GFP-tagged cancer cell lines. MCF-7, H358, H1975, and RPMI8226 cells (each 200 cells) were spiked into a 5 mL blood sample from a healthy donor to simulate CTCs in patient blood. CTCs were isolated from these spiked samples using CytoGen's Smart Biopsy™ CTC Isolator. For the LOD test, H358 GFP-tagged cells (spiked at quantities of 20, 10, 5, 3, and 1 cell) were introduced into 5 mL of blood, and the recovery rate was assessed using CytoGen's Smart Biopsy™ CTC Isolator. Recovery rate and detection precision were analyzed across multiple trials to ensure reproducibility.

Results: The recovery rate of Cytogen's Smart Biopsy TM CTC isolator device demonstrated an average cell recovery rate of over 90% across the four cell lines tested. As a result of LoD test, H358-GFP cells were clearly isolated even in the small number of cells (3 cells in 5mL blood) were spiked into blood. These results suggest that the Cytogen's Smart Biopsy TM CTC isolator can isolate very small number of CTCs in a blood with high recovery rate, supporting its reliable application in liquid biopsy.

Conclusions: Cytogen's Smart Biopsy TM CTC isolator demonstrated high recovery rates and detection precision, establishing it as a promising tool for CTC isolation in liquid biopsy applications. Its consistent performance in CTC isolation highlights its potential as a non-invasive diagnostic platform, effectively addressing the limitations of traditional tissue biopsies. This study emphasizes the capability of Smart Biopsy TM CTC isolator to facilitate early cancer diagnosis and monitoring through liquid biopsy, contributing to significant advancements in non-invasive cancer diagnostics.

#1995 Clinical evaluation of new diagnostic system for the detection of androgen-receptor splice variant 7 in circulating tumor cells of mCRPC.

H. Kang, J. Lee, M. Hwang, K. Son, S. Pak, J. Kim;
Cytogen, Inc., Seoul, Korea, Republic of

Introduction: Androgen-receptor splice variant 7 (AR-V7) is constitutively activated isoform of AR and has been associated with the resistance towards AR targeting therapies and development into metastatic castration-resistant prostate cancer (mCRPC). Analysis of AR-V7 expression is an important step in prostate cancer diagnosis and determining the therapeutic drug, but the detection of AR-V7 with tissues has limitations such as clinical challenge to poorly accessible tumor tissues before and after treatment. Here, we developed the method of AR-V7 detection in circulating tumor cells (CTC) after isolation with CytoGen's. Smart Biopsy CTC isolator by droplet digital polymerase chain reaction (ddPCR) with high sensitivity and specificity, overcoming the limitations of tissue biopsy.

Method: Sensitivity and specificity were tested using AR-V7 positive cells (VCaP or 22Rv1) and negative cells (PBMC). Limit of detection (LOD) test of AR-V7 expression was performed that AR-V7 positive cells (10 and 5 cells) were spiked into human blood sample (5mL, healthy donor) to mimic CTC after isolation by CytoGen's Smart Biopsy CTC isolator, subsequently AR-V7 expression was tested with ddPCR. All ddPCR processes included droplet making and reading for each assay were performed on the QX200 ddPCR System (Bio-Rad). For the clinical evaluation, blood samples (each 20mL) of metastatic prostate cancer patients (n=5) were tested with IF assay (5mL) to count CTC number and ddPCR (15ml) to analyze the expression of AR-V7.

Results: In in vitro spiking test, AR-V7 expression was clearly detected in the LOD test of AR-V7 positive cells spiked into human blood and was not detected at all in the negative controls. In clinical evaluation study, isolation of CTC from the blood of prostate cancer patients using CytoGen's Smart Biopsy CTC isolator was confirmed from the result of IF assay, and the expression of AR-V7 in CTC was successfully detected by ddPCR. IF analysis identified 3 or more CTCs in 5 ml of blood from all patients, and expression of AR-V7 was verified by ddPCR in 4 out of 5 mCRPC patients.. These results demonstrate that it is possible to clearly detect the presence of RNA variants is related with drug resistance in mCRPC patients using liquid biopsy, utilizing CTCs, which exist in only very small quantities in the blood.

Conclusions: AR-V7 expresses only a part of cancer tissue, so it is difficult to accurately diagnose with traditional histological assays. Detection of AR-V7 (RNA signature) in intact live CTC might overcome limitations of tissue biopsy. In this study, we developed the method to detect AR-V7 in CTC, and presented the results of verifying AR-V7 expression using in vitro models and blood of mCRPC patients. Expanded clinical validation study with more patients' samples and clinical correlation between AR-V7 expression in CTC and clinical outcome will be performed and analyzed.

CLINICAL RESEARCH: Metastasis Biomarkers / Prognostic Biomarkers 1
Poster Session

#1999 Defining the distinct molecular landscapes of primary tumors and metastases in Merkel cell carcinoma.

Y. Yang[1], S. Kilpinen[2], H. Sihto[3], L. Janska[1], X. Wang[1], C. Larsson[1], W.-O. Lui[1];
[1]Karolinska Institutet, Stockholm, Sweden, [2]University of Helsinki, Helsinki, Finland, [3]University of Helsinki and Helsinki University Hospital, Helsinki, Finland

Merkel cell carcinoma (MCC) is an aggressive skin cancer characterized by high metastatic potential and poor survival rates. Identifying biomarkers for early detection of metastatic MCC is crucial for improving clinical outcomes. In this study, we analyzed bulk RNA-sequencing data from the GEO dataset GSE235092, identifying 481 differentially expressed genes (DEGs) between primary tumors (n=43) and metastases (n=28) (fold change >2, *adjusted p* <0.01). Among these, 66 genes were upregulated and 415 were downregulated in metastases. Downregulated DEGs were enriched in processes and pathways associated with metastasis, such as cell-cell adhesion, focal adhesion, and epithelial-mesenchymal transition. To further investigate, we performed pseudobulk differential expression analysis on tumor cells from primary (n=13) and metastasis tumors (n=10) using single-cell RNA-sequencing (scRNA-seq) data (GSE235090). This analysis revealed 43 DEGs (fold change >1.5, *adjusted p* <0.05), with 3 overlapping with the bulk RNA-seq findings. Subsequent subdivision of scRNA-seq metastasis samples into regional and distant metastases identified 6 DEGs specific to regional metastases compared to primary tumors, 5 to distant metastases, and 1 common to both. Validation of these DEG candidates in our MCC cohort of 102 primary tumors, based on RNA sequencing, identified *SLC4A7, CPVL, ENG, MYLIP,* and *MXRA8* as strong candidates linked to tumor metastasis, survival outcome, Merkel cell polyomavirus (MCPyV) status and other clinical characteristics. Additionally, scRNA-seq analysis highlighted distinct tumor microenvironment compositions among primary tumors, regional metastases, and distant metastases. Considering the role of MCPyV in MCC progression, we examined DEGs between primary and metastasis under MCPyV-positive and MCPyV-negative statuses using bulk RNA data. Gene set enrichment analysis uncovered 55 MYC-targeted genes and 52 E2F-targeted genes that were suppressed in MCPyV-positive patients but activated in MCPyV-negative patients. These findings provide new insights into MCC metastasis and highlight potential biomarkers for tumor progression and targeted therapies.

#2001 Proteomic analysis of plasma exosomes identifies biomarkers for liver and lung metastasis of colorectal cancer patients.

Boyeon Kim[1], Jwa Hoon Kim[2], Jiwon Lee[2], Soohyeon Lee[2]

[1]Korea University, Seoul, Korea, Republic of,[2]Korea University Anam Hospital, Seoul, Korea, Republic of

Metastasis is the leading cause of mortality in colorectal cancer (CRC), with liver and lung Metastasis being the most common sites of progression. Although early detection of metastasis is crucial for improving patient outcomes, non-invasive predictive strategies remain limited. Exosomes, a class of extracellular vesicles encapsulating cell-derived proteins, DNA, mRNA, and miRNA within a lipid bilayer, closely reflect the molecular characteristics of their originating cells and exhibit exceptional stability in biological samples. These unique features position exosomes as promising candidates for biomarker discovery. In this study, we utilized label-free liquid chromatography-tandem mass spectrometry (LC-MS/MS) to analyze plasma samples from 19 individuals: 5 healthy controls, 7 CRC patients with liver metastasis, and 7 CRC patients with lung metastasis. Blood samples were collected between March 2017 and April 2021, with only plasma from patients who developed metastasis repeatedly and exclusively in the liver or lung during the observation period included in the analysis. From 500 µL of plasma, we extracted an average of 149.9 µg of protein (173.8 µg from healthy controls, 145.9 µg from liver metastasis samples, and 136.8 µg from lung metastasis samples) and identified 851 proteins for further investigation. Our findings revealed that exosomes from CRC patients with metastasis contained elevated levels of proteins associated with angiogenesis, cell differentiation, and cell adhesion—such as ANGPTL6, COL1A1, LAMA2, and RENL—compared to healthy controls. Specifically, VCAN and ANGPTL6 were significantly upregulated in liver metastasis, while CFD and CRTAC1 were highly expressed in lung metastasis. These proteins are known to play critical roles in tumor progression and metastasis across various cancer types. Kaplan-Meier survival analysis based on the mRNA expression levels of these proteins indicated that CRC patients with higher expression exhibited worse overall survival (OS) and relapse-free survival (RFS) compared to those with lower expression levels. Future research will focus on the functional characterization of these identified proteins, the isolation of liver- and lung-specific exosomes, and the identification of small RNA biomarkers. This study represents a significant step toward the development of liquid biopsy-based approaches, particularly leveraging exosomes, for the early prediction of liver and lung metastasis in CRC patients.

#2002 A blood-based microbial metabolites signature for associated with metastatic duodenopancreatic neuroendocrine tumors in patients with multiple endocrine neoplasia type 1.

R. Ballaro[1], A. R. Wasylishen[2], C. R. C. Pieterman[3], C. Olsen[1], E. Irajizad[1], R. Wu[1], H. Katayama[1], H. Liu[1], Y. Cai[1], J. Dennison[1], S. Waguespack[1], K.-A. Do[1], S. Agarwal[4], M. Walter[5], J. Welch[4], L. Weinstein[4], J. E. Blau[4], S. Jha[4], N. Nilubol[4], M. R. Vriens[3], R. S. van Leeuwaarde[3], M. J. C. van Treijen[3], G. D. Valk[3], N. D. Perrier[1], S. M. Hanash[1], J. F. Fahrmann[1];
[1]UT MD Anderson Cancer Center, Houston, TX, [2]University of Cincinnati College of Medicine, Cincinnati, OH, [3]University Medical Center Utrecht, Utrecht, Netherlands, [4]National Institutes of Health, Bethesda, MD, [5]National Institutes of Health, Bethesda, TX

Metastatic duodenopancreatic neuroendocrine tumors (dpNETs) are the primary cause of mortality among patients with Multiple Endocrine Neoplasia Type 1 (MEN1). Emerging evidence implicates the microbiome and microbial-derived secreted factors in promoting cancer development and progression. In the current study, we report that circulating microbial-associated uremic toxins trimethylamine N-oxide (TMAO), indoxyl sulfate (IS), cresol sulfate (CS), cresol glucuronide (CG), and phenol sulfate (PS) are elevated in MEN1 patients with metastatic dpNETs. Proteomic and metabolomic-based analysis of resected dpNET tissues from MEN1 patients revealed detectable levels of uremic toxins that positively correlated with peptide-based signatures corresponding to *Faecalibacterium prausnitzii, Faecalibacterium nucleatum,* and *Klebsiella pneumoniae* and negatively correlated with *Streptococcus pneumoniae* and *Streptococcus thermophilus*. A microbial-associated uremic toxin panel (MUTP) was developed and the panel yielded an area under the receiver operating characteristic curve (AUC) of 0.94 (95% CI: 0.85-1.00) with 67% sensitivity at 95% specificity for identifying MEN1 patients with metastatic dpNETS in an independent validation cohort. Increases in circulating microbial-associated uremic toxins during early stages of neoplasia were also found to be associated with poor overall survival in a $Men1^{fl/fl}Pdx1\text{-}Cre^{Tg}$ mouse model of MEN1 pancreatic neuroendocrine tumors. Our findings suggest that microbial dysbiosis is associated with disease aggressiveness and that increases in circulating microbial-associated uremic toxins may identify individuals who are at risk of having metastatic dpNETs to better guide clinical management and intervention.

#2003 Targeting the human adrenomenulin 2 hormone inhibits cancer cell intravasation.

S. Karimian, J. Lewis, K. Stoletov;
University of Alberta, Edmonton, AB, Canada

Cancer cell intravasation is the crucial step of cancer metastasis, and no therapeutic agents thattarget this process exist. Our laboratory uses high-resolution multidimensional intravital confocalimaging to visualize intratumoral cancer cell invasion and metastasis. Using this techniquecombined with fluorescent reporters, we discovered that in the live tumours, cancer cells withelevated ATF2 transcription factor (ATF+) are lining up the intratumoral vasculature, activelyinvading the perivascular space and responsible for 100% of cancer cell intravasation events. Incontrast, cancer cells low in ATF2 activity (ATF2-) fail to interact with the intratumoralvasculature and do not intravasate. Most cancer cell intravasation events occur in the large(~50µm) veins deep within the tumour mass (intravasation ports). Isolation of ATF2+ and ATF2-cells followed by their comparative RNAseq analysis revealed that ADM2 (Adrenomennulin 2)peptide hormone is significantly (~3x) overexpressed in ATF2+ compared to ATF2- tumour cells.shRNA-mediated knockdown of ADM2 expression in cancer cells decreased ATF2 activity,revealing a possible positive feedback loop. We found that shRNA knockdown of ADM2expression drastically inhibited the intratumoral cancer cell invasion and formation of the sizeableintratumoral vasculature. Notably, cancer cells deficient in ADM2 expression fail to invadeintratumoral perivascular space and intravasate. Our findings reveal Adrennomenulin 2 as a noveland potent anti-cancer metastasis target that can specifically inhibit cancer cell intravasation.

#2004 Integrative bioinformatics approach reveals potential liquid biopsy-based biomarkers for breast cancer metastasis monitoring.

Jina Kim[1], Cynthia Jinno[1], Hyoyoung Kim[1], Sunao Tanaka[1], Takeo Fujii[2], Sungyong You[1], **Hideki Furuya**[1]

[1]Cedars-Sinai Medical Center, Los Angeles, CA,[2]National Cancer Institute, Bethesda, MD

Background: Despite recent advances in screening and therapeutic strategies, breast cancer remains the first leading cause of cancer death among females. The 5-year survival rate is high as 95% at localized stages but drops significantly to 31% once the cancer metastasizes. Therefore, developing predictive tools of metastatic recurrence is an unmet clinical need to design preventive clinical trials.
Methods: This study used a public dataset (GEO ID: GSE102484), which is a microarray dataset that contains primary tumor samples (n = 683) sourced from subjects with (n = 95) and without (n = 582) distant recurrence (metastasis) of breast cancer (met and non-met, respectively). Our approach began with two sets of differentially expressed genes (DEGs). The first set of DEGs are from all the samples in the dataset regardless of breast cancer subtypes, while the second set of DEGs are from triple negative breast cancer (TNBC) samples only. To identify subjects with TNBC, basal-like breast cancer samples were selected using PAM50 algorithm with genefu R package. To select potential blood biomarker candidates, we developed an integrative bioinformatics pipeline. Briefly, 1) Differential expression analysis between met and non-met were performed using limma R package. We then selected DEGs with log2 fold change ≥ 0.58 and false discovery rate (FDR) < 0.1, 2) Genes with housekeeping/hematopoietic markers were removed, and 3) The genes that can be detectable in human bloods were selected using the human secretome database from the Human Protein Atlas (HPA).
Results: The differential expression analysis of all breast cancer samples identified 134 significantly upregulated genes in met and the selection process with the pipeline resulted in 13 marker candidates including COL10A1, CD93, HPRT1, MMP1, EPHB4, HSPD1, MAP4, CCDC80, MMP11, SAFB2, CALD1, CRISP3, and GAPDH. Subsequent GO enrichment analysis identified functions associated with these markers including angiogenesis, calcium ion binding, and extracellular matrix and structure organizations. Nucleotide metabolism was observed significance in pathway. We then focused on the TNBC samples from the dataset and identified 162 significantly upregulated genes in met from TNBC. Out of the 13 marker candidates from the 134 upregulated genes, 8 marker candidates including CALD1, CD93, EPHB4, SAFB2, HSPD1, MAP4, CCDC80, and HPRT1 were also found in TNBC.
Conclusion: Through an integrative computational analysis, we identified 13 markers associated with breast cancer metastasis, of which 8 were specifically linked to metastasis in TNBC. Future studies will focus on validating these biomarkers using liquid biopsy samples from breast cancer patients, potentially offering a less invasive diagnostic approach.

#2005 ROR1-specific extracellular vesicle tetraspanin-8 levels predict distant metastasis in triple-negative breast cancer.

Yinchao Zhang, Linze Xu, Jin Zhang, Dandan Wu, Yang Liu

Tianjin Medical University Cancer Institute & Hospital, National Clinical Research Center for Cancer, Key Laboratory of Cancer Prevention and Therapy, Tianjin's Clinical Research Center for Cancer, Tianjin, China

Background: Triple-negative breast cancer (TNBC), comprising 15-20% of all breast cancer cases, is the most aggressive subtype, with limited efficacy from targeted therapies and immunotherapies. Metastasis drives TNBC-related mortality, emphasizing the urgent need for predictive methods to identify high-risk cases early, guide clinical management, and facilitate timely interventions. Extracellular vesicles (EVs) play a pivotal role in cancer progression and metastasis, offering a promising avenue for metastasis research. However, isolating tumor-derived EVs from the total EV population remains a significant clinical challenge, underscoring the necessity for precise detection strategies.

Methods: EVs were isolated from MCF-10A cells, MCF-7 cells, and MDA-MB-231 cells using ultracentrifugation, followed by morphological characterization and surface marker protein validation. Mass spectrometry and UniProt gene ontology analyses were conducted to identify TNBC-specific EV proteins, classified by molecular functions or biological processes. TSPAN8 expression in breast cancer cells was analyzed using the CCLE and TCGA databases. Cholesterol-PEG-Biotin and Streptavidin-APC (Cholesterol-PEG-APC) were employed to anchor EV membranes, enabling concurrent detection of quantum dot-labeled EV proteins and membrane signals.

Results: ROR1, a transmembrane protein expressed in TNBC cells and EVs, was identified as a specific capture protein for TNBC-derived EVs. Database analysis revealed elevated TSPAN8 expression in highly metastatic TNBC MDA-MB-231 cells and in the tissue of TNBC-LAR subtype, where TSPAN8 plays a decisive role in metastasis. Using ROR1 to capture MDA-MB-231 EVs demonstrated that the fluorescence intensity from quantum dot-labeled QD605 anti-TSPAN8 and APC increased with EV concentration. Furthermore, higher average EV-TSPAN8/APC levels were associated with greater metastatic risk in patients. Correlation analysis using the TCGA database showed a positive relationship between ROR1 and TSPAN8 expression. VCAM1 was identified within the interaction network of ROR1 and TSPAN8, with positive correlations to both, providing a theoretical basis for leveraging ROR1-specific EV-TSPAN8 detection in metastasis research.

Conclusions: ROR1 effectively captures tumor-derived EVs and holds significant potential for enhancing EV-TSPAN8 detection, providing a promising strategy for predicting metastasis in TNBC. Evaluating ROR1-specific EV-TSPAN8 levels in TNBC blood samples and its link to disease progression warrants further investigation. Additionally, exploring its clinical utility across diverse TNBC subtypes and treatment regimens could refine its application, ultimately improving the precision of early metastasis prediction in TNBC.

Research Sponsor: National Natural Science Foundation of China (No. 82202603).

#2006 Exploring a novel crosstalk between PTK7 and EPHA2 tyrosine kinase receptors in metastatic colorectal cancer.

Charlotte Dessaux[1], Constantin Semenchenko[1], Luc Camoin[2], Stephane Audebert[2], Emilie Baudelet[2], Avais Daulat[1], Flavio Maina[1], Jean-Paul Borg[1]

[1]Centre de Recherche en Cancerologie de Marseille (CRCM), Marseille, France,[2]Centre de Recherche en Cancerologie de Marseille (CRCM), Marseille Proteomics platform, Marseille, France

Colorectal cancer (CRC) remains a significant public health challenge, with an urgent need of novel prognostic biomarkers and therapeutic targets. PTK7, a membrane tyrosine pseudokinase and Wnt receptor, is overexpressed in CRC and linked to poor prognosis. In CRC, PTK7 expression correlates with metastatic progression and decreased survival in non-metastatic patients, positioning PTK7 as both a potential biomarker and a promising drug target. Notably, a targeted antibody-drug conjugate (ADC) against PTK7 has recently shown efficacy in inducing tumor regression in patient-derived xenograft (PDX) models of solid tumors and has progressed to a Phase I clinical trial with promising results. Other PTK7-targeting ADCs and anti-PTK7 CAR-T cells recently entered into preclinical development.

At the plasma membrane, PTK7 can interact with a range of membrane receptors, including VEGFR, EGFR, ROR2, and Plexins, enhancing their signaling capabilities. Using biotin proximity labeling in HCT116 CRC cells, we identified a novel network of PTK7-associated membrane proteins. Among these, we focused on EPHA2, an active tyrosine kinase receptor also implicated in cancer. Our findings indicate that PTK7 and EPHA2 interact via their extracellular domains (ECDs), and that PTK7 depletion significantly upregulates EPHA2 expression and signaling in response to its ligand EFNA-1 in HCT116 cells. Additionally, PTK7 regulates EPHA2's endosomal sorting through a Rab11-dependent mechanism, as well as its K63-linked ubiquitination and subsequent lysosomal degradation. Mechanistically, PTK7 does not appear to participate directly in EFNA-1 binding but instead promotes multimerization of active EPHA2, a crucial step in amplifying EPHA2 signaling.

This study aims to elucidate the functional role of the interaction between PTK7 and EPHA2 in colorectal cancer progression. By mapping the PTK7:EPHA2 interface using AlphaFold3 predictions and introducing targeted deletions and mutations within the ECDs of both receptors, we seek to understand how disrupting this interaction influences tumorigenic and metastatic processes. Our findings indicate that PTK7 depletion triggers cell rounding and detachment, with a marked increase in total and activated EPHA2 levels, which may drive pro-metastatic activity *in vivo*. Outcomes may significantly impact the use of anti-PTK7-ADCs or CAR-T cells and orient new therapeutic strategies to design agents interfering with PTK7 and EPHA2 interaction and function.

#2008 Intra-tumoral NK cell number is associated with immune response, and combination of it and clinical features is a strong prognostic biomarker in gastric cancer.

Masanori Oshi[1], Yuko Tamua[2], Rongrong Wu[3], Li Yan[1], Colin J. Rog[1], Kizuki Yuza[2], Takashi Ishikawa[3], Itaru Endo[2], Kazuaki Takabe[1]

[1]Roswell Park Comprehensive Cancer Center, Buffalo, NY,[2]Yokohama City University Graduate School of Medicine, Yokohama, Japan,[3]Tokyo Medical University, Tokyo, Japan

Background: The number of Natural Killer (NK) cell infiltration has been implicated for prognosis of gastric cancer patients. However, tumor immune microenvironment of gastric cancer patients with high NK cell infiltration has not yet been fully investigated.
Methods: NK cell infiltration fraction of gastric cancer in total 831 patients was quantified by using xCell algorithm, and its clinical relevance was investigated in gastric cancer patients at our institution (Yokohama City University Hospital (YCU), and publicly available cohorts with transcriptome (TCGA, GSE84437 and GSE167036), and single cell sequence cohort (GSE150290).
Results: In the single cell sequencing cohort, the distribution of NK cells was similar to that of NK cell-related gene expression, including *PRF1, NKG7, NCR1, GZMB, KLR-B1/C1/D1,* and *IFNG*. High NK cell infiltration in gastric cancer correlated with enriched immune gene sets, such as interferon (IFN)-α and IFN-γ responses (all normalized enrichment score (NES) > 1.50, and all false discovery rate (FDR) < 0.20), and also linked to increased cytolytic activity and low stromal cell infiltration, including fibroblasts, lymphatic/microvascular endothelial cells, pericytes, and adipocytes (all $p < 0.05$), consistently in both TCGA and GSE84437 cohort. Gastric cancer with high NK cell infiltration was also significantly associated with higher mutation rates ($p < 0.01$). However, they did not enrich with several pro-cancerous hallmark cancer gene sets, such as angiogenesis, epithelial mesenchymal transition, hypoxia, nor apoptosis. Clinically, high NK cell infiltration was associated with better overall survival ($p = 0.023$ in GSE84437, and $p = 0.015$ in TCGA). Interestingly, as the treatment response of chemotherapy improved, the infiltrating fraction of NK cell increased ($p = 0.012$ in YCU cohort). Furthermore, the NK cell score was independent factor for patient survival. Additionally, combining NK cell score with clinicopathological factors, including age at a diagnosis, AJCC T- and N-category, provided a powerful prognostic score for gastric cancer patients, consistently in multiple cohorts ($p < 0.001$, hazard ratio (HR) = 1.96 in TCGA, and $p = 0.017$, HR = 2.73 in the YCU cohort).
Conclusions: Gastric cancer with high NK cell infiltration is associated with increased immune activity and lower stromal cell infiltration. Combining clinicopathological factors with NK cell score may provide a powerful tool as prognostic predictor for gastric cancer patients.

#2009 Co-expression of interleukin-4 receptor alpha and KRAS correlates with disease severity and prognosis in pancreatic cancer patients.

Ashley Lea[1], Toshio Fujisawa[2], Hiroyuki Isayama[2], Supreet Khanal[1], Santosh Panthi[1], Mona Mansouri[1], Samir Lababidi[3], Steven R. Bauer[4], Kyung Sung[1], Nirjal Bhattarai[1], **Bharat H. Joshi**[1]

[1]FDA-CBER (Center for Biologics Evaluation and Research), Silver Spring, MD,[2]Juntendo University, Tokyo, Japan,[3]Mathematical Statics, FDA-Office of Commissioner, Silver Spring, MD,[4]Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC

Introduction: There will be 66,400 new cases of pancreatic cancer identified and 51,750 will die by end of 2024 in the United States. KRAS is the most common oncogenic driver occurring in ≥85% of pancreatic cancer patients. Previously, we identified an overexpression of high affinity binding protein Interleukin-4 Receptor alpha (α) of Interleukin 4 (IL-4) in numerous solid human cancers including pancreatic cancer. Herein, we investigated if IL-4Rα and KRAS co-expression can act as a biomarker of disease severity in pancreatic cancer patients.

Experimental: One hundred and seven archival tumor specimens were examined by six investigators for IL-4Rα and KRAS expression by IHC and ISH techniques in a blinded manner. The data were stratified into pathological grades and stages of the patients. The results were analyzed statistically if individual/combined expression of IL-4Rα or KRAS can serve as biomarker/s of disease severity. Kaplan-Meier survival analysis was performed to investigate if these gene-expression had any influence on overall survival of the patients.

Results: Our IHC results demonstrate that ≥68% samples overexpressed IL-4Rα alone in moderately to poorly differentiated grade tumors and in samples from clinical stage II + III patients with ≥2+ immunostaining intensity. Interestingly, ≥80 % of the specimens with moderately to poorly differentiated grades showed a co-expression for KRAS and IL-4Rα (≥2+). In contrast, patients with well differentiated grade and clinical stage I revealed ≤ 73% co-expression of these genes. We observed a similar pattern of mRNA expression in these patients by ISH. Further, Kaplan-Meier analysis revealed a significantly shorter survival ($P \leq 0.001$) in patients with ≥2+ positive expression of IL-4Rα plus KRAS in moderately to poorly differentiated grades and advanced clinical stages. In contrast, the patients overexpressing either of these genes alone, regardless of grades and stages, survived relatively longer compared to those expressing both IL-4Rα and KRAS .

Summary and Conclusion: Our data show that IL-4Rα and KRAS co-expression, correlate with disease severity in pancreatic cancer patients. Thus our study uncovers a novel role of combined expression of IL-4Rα and KRAS as a biomarker of prognosis and driver of oncogenesis in pancreatic cancer patients and may serve a unique druggable target/s for developing targeted cellular or gene therapy-based cancer immunotherapies.

#2010 A spatial profiling approach to evaluating the prognostic impact of heterogeneity in the triple-negative breast cancer immune microenvironment.

Prerana Sensharma[1], Huidan Zuo[2], Melanie Dawe[3], Megan Hopkins[4], Zeynep Baskurt[5], Osvaldo Espin-Garcia[5], Philippe L. Bedard[6], Melanie Spears[4], Susan J. Done[3]

[1]Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada,[2]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada,[3]Princess Margaret Cancer Center, University Health Network, Toronto, ON, Canada,[4]Department of Medical Biophysics, Ontario Institute of Cancer Research, Toronto, ON, Canada,[5]Department of Biostatistics, Princess Margaret Cancer Center, University Health Network, Toronto, ON, Canada,[6]Department of Medicine, University Health Network, Division of Medical Oncology & Hematology, Toronto, ON, Canada

Triple negative breast cancer (TNBC) is the most aggressive breast cancer subtype with the poorest patient outcomes. The composition and active immune pathways of the immune microenvironment play a critical role in tumor progression. TNBC is frequently characterized by a high and heterogeneous immune infiltration in the tumor stroma. The degree of immune infiltration impacts current management: neoadjuvant chemotherapy and anti-PD1 agent pembrolizumab.[1,2,3] Previously, our lab studied a treatment-naive cohort of 192 patients diagnosed with TNBC and found that patients with higher B cell infiltration in the stromal regions had better outcomes (Kanwar and Balde et. al, Cancer Res 2021)[4]. While previous studies employed low-plex methods of analysis to study archival formalin-fixed paraffin-embedded tissue samples, the emergence of high-plex in situ protein detection methods like the GeoMx Digital Spatial profiler (DSP) has drastically enhanced the resolution of the tumor microenvironment that can be assessed.[5] Using the GeoMx DSP, we are quantifying a panel of 40 immune proteins, to expand our understanding of the immune microenvironment of the pre-treatment cohort previously studied. The protein panel enables the detection of multiple immune cell types and subtypes, certain immune pathways like T cell activation and exhaustion, and the expression of immunotherapeutic targets. Preliminary results show that patients with high CD163 expression, associated with immunosuppressive macrophages had poorer outcomes ($p= 0.035$). The immune microenvironment for larger tumors expressed higher T cell activation markers ($p=0.016$). While the overall heterogeneity in immune cells did not have an impact on patient outcomes, a high heterogeneity in T cell activation markers was significantly associated with patient outcomes ($p=0.029$) as well as tumor size ($p=0.024$). We have characterized the stromal immune cell infiltrates and their spatial heterogeneity, finding immune markers that may significantly impact patient outcomes. Our findings aid in the identification of therapeutic targets prevalent in the TNBC stroma as well as potentially informing therapy decisions based on the tumor immune microenvironment composition.

1. Won KA, Spruck C. (2020). Int J Oncol, 57(6), 1245-1261. DOI:10.3892/ijo.2020.5135

2. Marra A et al. (2020). NPJ Breast Cancer, 6, 54. DOI:10.1038/s41523-020-00197-2

3. Obidiro O et al. (2023). Pharmaceutics, 15(7), 1796. DOI:10.3390/pharmaceutics15071796

4. Kanwar N et al. (2021). Cancer Res, 81(24), 6196-6206. DOI:10.1158/0008-5472.CAN-21-1079

5. Bergholtz H et al. (2021). Cancers, 13(17), 4456. DOI:10.3390/cancers13174456

#2011 Attenuated levels of surface TREM1 on circulating neutrophils impair NETosis and increase infection risk in DLBCL patients.

Hao-Yuan Wang[1], Po-Chun Liu[2], Fu-Chen Yang[2], Ching-Fen Yang[3], Chun-Kuang Tsai[1], Po-Shen Ko[1], Yao-Chung Liu[1], Nien-Jung Chen[2]

[1]Division of Hematology and Oncology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan,[2]Institute of Microbiology and Immunology, National Yang Ming Chiao Tung University, Taipei, Taiwan,[3]Department of Pathology and Laboratory Medicine, Taipei Veterans General Hospital, Taipei, Taiwan

Neutrophils can undergo NETosis, but their roles in lymphoma remain unexplored. The triggering receptor expressed on myeloid cells-1 (TREM1) amplifies inflammation and may regulate NETosis. This study investigates surface TREM1's role in NETosis regulation and predicting progression-free survival (PFS), overall survival (OS), and infection risk in untreated DLBCL patients. This prospective study enrolled 118 untreated DLBCL patients. Circulating neutrophils were isolated, identified as CD45+ CD15+ using flow cytometry, and stimulated with PMA to assess NETosis by SYTOX Green or confocal microscopy. Neutrophils from healthy donors (HDs) were paired with patients to calculate normalized TREM1 or NETosis levels: (Patient - HD) / (HD) x 100%. Among 118 DLBCL patients (median age 66, 61.9% male), 39.8% were stage IV, 33.1% high-risk IPI, 23.7% had bone marrow (BM) involvement, 72.9% were non-GCB subtype, 40.7% were double expressor lymphoma (DEL), and 9.3% were Epstein-Barr virus (EBV) positive. Clinically significant infections occurred in 26 patients, with typhlitis as the most common infection focus (n = 7) and *Escherichia coli* as the most commonly identified pathogen (n = 10). Most patients had lower neutrophil surface-TREM1 levels than HDs (median -40.1%). High-risk IPI and non-GCB patients had significantly lower normalized TREM1 levels than their counterparts (P = 0.036 and P = 0.031, respectively). Trends were also observed for lower TREM1 levels in patients with BM involvement, DEL, and age ≥75. Patients were classified into high (≥ -43.8%; n = 66) and low (< -43.8%; n = 52) TREM1 level groups using ROC and AUC analysis. After 25.3 months of follow-up, low TREM1 patients had worse PFS (2-year PFS rate: 57.8% vs. 79.3%, log-rank P = 0.001), OS (2-year OS rate: 62.9% vs. 90.6%, log-rank P < 0.001), and a higher infection risk (cumulative incidence rate of clinically significant infection within 180 days post-DLBCL diagnosis: 36.5% vs. 10.6%, log-rank P = 0.001). Multivariate analysis showed TREM1 as an independent predictor for PFS, OS, and infection risk. Neutrophils from all 118 patients were treated *ex vivo* with PMA (100 nM), and the supernatant was collected to measure MPO. Patients with low TREM1 had significantly impaired NETosis (-54.6% vs. -16.8%, P < 0.001) and reduced MPO levels (61.5 vs. 82.0 ng/mL, P = 0.006). Further treatment with PMA or *E. coli*, followed by immunofluorescent staining and confocal microscopy, showed that neutrophils from high TREM1 patients had mildly impaired NETosis, further inhibited by the TREM1 inhibitor LP17. Neutrophils from low TREM1 patients had significantly impaired NETosis, which was restored by the TREM1 agonist PY159.
In conclusion, low surface TREM1 levels on circulating neutrophils predict poorer PFS and OS, higher infection risk, and impaired NETosis in untreated DLBCL patients. These findings warrant further clinical investigation.

#2012 Prognostic role of CD3+and CD8+cells density in resected stage IIIAN2 non-small cell lung cancer (NSCLC): A study from the randomized Lung ART IFCT 0503 trial.

Victor Albarran-Artahona[1], Maria-Rosa Ghigna[2], Wael Zrafi[3], Nicolas Signolle[4], Guillaume Granier[5], Nathalie Cozic[6], Julien Adam[7], Ludovic Lacroix[8], Benjamin Besse[1], Cecile Le Pechoux[9], Antonin Levy[9]

[1]Department of Cancer Medicine, INSERM U848 (Institut Gustave Roussy), Villejuif, France,[2]Department of biology and pathology, INSERM U848 (Institut Gustave Roussy), Villejuif, France,[3]Radiation Epidemiology Team, Centre for Epidemiology and Population Health, INSERM U848 (Institut Gustave Roussy), Villejuif, France,[4]UMS AMMICa PETRA CNRS UAR3655/ INSERM US23, Gustave Roussy, Villejuif, France,[5]Department of pathology, Centre Hospitalier Henri-Duffaut, Avignon, France,[6]Department de biostatistique et d'Epidemiologie, INSERM U848 (Institut Gustave Roussy), Villejuif, France,[7]INSERM U1186 GR/Universite Paris Saclay, Paris, France,[8]Department of Cancer Medicine, INSERM U848 (Institut Gustave Roussy), Villejuif, France, Villejuif, France,[9]Radiation Oncology, INSERM U848 (Institut Gustave Roussy), Villejuif, France

BACKGROUND: Immune cells (IC) density has a prognostic role in NSCLC[1]. Most results come from small cohorts with limited representation of stage III samples and show heterogeneity[2-4].
METHODS: The LungART trial (NCT00410683)[5] randomized 501 patients (pts) with resected stage IIIAN2 NSCLC (AJCC 7th ed.) to adjuvant radiotherapy (ART) or observation. Up to 96% had (Neo)adjuvant chemotherapy (ChT). No disease-free survival (DFS) benefit was found for ART. For pts who consented, a primary tumor blocs was collected; $CD3^+/CD8^+$ cells were assessed by immunohistochemistry (DAKO/Roche). Automatic quantitative analysis of selected regions of interest was performed using the QuPath v.0.5.1 Bioimage software for assessment of IC density and correlated with outcomes.
RESULTS: 272/501 pts had available samples; 47% received ART. Baseline characteristics were well balanced (table 1). A higher $CD3^+$ cells density was found in non-squamous histology (p=0.003) and in pts receiving neoadjuvant ChT (p=0.03). Higher density of $CD3^+$ and $CD8^+$ cells correlated with the incidence of new events after 6 (p=0.0004 and 0.04, respectively) and 24 months (m) (p=0.003 and 0.04 respectively). High $CD3^+$ cells density correlated with longer median DFS: 52 m (95% CI: 26.1 m-NR) *vs* 22 m (95% CI: 22.5-31.2 m,) as well as high $CD8^+$ cells density (52.1 m; 95% CI: 34-NR *vs* 20.9 m; 95% CI: 16.9-28.8 m). A multivariate Cox model confirmed both high $CD3^+$ and $CD8^+$ cell density as independent prognostic factors for DFS (HR=0.68 [95%CI: 0.53-0.83], p=0.01 and HR=0.57 [95%CI: 0.38-0.86], p=0.07, respectively) and overall survival (HR=0.66; 95%CI: 0.49-0.88; p=0.005 and HR=0.5; 95%CI: 0,30-0.85; p=0.10, respectively).
CONCLUSIONS: Our study is the largest cohort showing a prognostic role of IC density in resected stage IIIAN2 NSCLC, composed solely by surgical samples, which may identify a subset of poor prognosis pts who could benefit of an intensive therapeutic approach.

Baseline characteristics of study's population

Characteristic	**RT, N=143**	**Observation, N=129**
Age	61 (55- 66)	61 (56- 67)
Female	45 (31%)	40 (31%)
Male	98 (69%)	89 (69%)
Smoking history		
Yes	117 (89%)	108 (91.5%)
No	16 (11%)	16 (11%)
Histology		
Non-squamous	112 (78%)	109 (84%)
Squamous	31 (22%)	20 (16%)
pT		
1	42 (29%)	39 (30%)
2	70 (49%)	66 (51%)
3	26 (18%)	22 (17%)
4	5 (3.5%)	2 (1.6%)
pN		
0	3 (2.1%)	1 (0.8%)
1	2 (1.4%)	2 (1.6%)
2	138 (97%)	126 (98%)
Chemotherapy (ChT)		
Preop. ChT	16 (11%)	13 (10%)
Postor. ChT	115 (80%)	107 (83%)
Pre and post ChT	7 (4.9%)	5 (3.9%)
Median $CD3^+$ density	970 (598-1615)	1100 (677-1691)
Median $CD8^+$ density	366 (186-674)	487 (267-756)

#2013 Serum immune markers are associated with pathological complete response and immune-related adverse events in immuno-oncology arms of the I-SPY2 breast cancer trial.

S. Alkhafaji[1], K. Chow[1], D. Wolf[1], R. W. Sayaman[1], A. Basu[1], L. Brown-Swigart[1], R. Nanda[2], J. Chien[1], M. Liu[3], G. Hirst[1], L. J. Esserman[1], L. van 't Veer[1], M. J. Campbell[1];

[1]UCSF - University of California San Francisco, San Francisco, CA, [2]The University of Chicago Medicine, Chicago, IL, [3]Natera, San Carlos, CA

Background: Predicting early response and immune-related adverse events (irAEs) is crucial for therapies with immune checkpoint inhibitors (ICI) in early-stage breast cancer (BC). This study evaluated serum immune markers at pre-treatment and early on-treatment, and changes between timepoints in women with BC treated with neoadjuvant chemotherapy plus ICI (anti-PD-1) or ICI plus SD-101 (an immunomodulatory toll-like receptor 9 agonist) in the I-SPY2 trial.

Methods: Serum samples from women with HER2-negative BC (N=114) in the I-SPY2 trial were collected at baseline (T0) and three weeks post-treatment initiation (T1). Patients received paclitaxel with anti-PD-1 (N=49) or anti-PD-1 plus SD-101 (N=65), followed by anthracycline/cyclophosphamide. Serum was analyzed for 18 immune markers, including checkpoint molecules (sPD-L1, LAG-3), cytokines (IL-6, IL-8, IL-10, sCD25, G-CSF, IFNγ, TNFα), chemokines (CXCL9, CXCL10, CXCL11, CCL2, CXCL13), angiogenesis markers (VEGF-C), proteins and macrophages with inflammatory roles (CRP, CD163), and molecules that induce cell apoptosis (Granzyme B). Associations between serum markers and pathologic complete response (pCR) or irAEs were assessed using the Mann-Whitney test, and significance was defined at $P<0.05$. Associations were also assessed within hormone receptor (HR+/HER2- N=68, HR-/HER2- N=46) and I-SPY2 immune positive (ImPrint+, N=54) subtypes in both treatment arms. ImPrint is a 53-gene signature that was independently validated to predict the likelihood of a pCR with PD-1/PD-L1 ICIs with high sensitivity and specificity.

Results: Among all HER2-negative patients, baseline CRP, CXCL10, and sCD25 levels were positively associated with pCR. In HR+/HER2- and HR-/HER2- patients, baseline CRP levels were also positively associated with pCR. In ImPrint+ patients, baseline G-CSF levels were positively associated with pCR, even after adjusting for HR status and treatment arm. T1 IFNγ levels were positively associated with pCR in HER2-negative patients, and within HR-/HER2- and ImPrint+ groups. Immune-related AEs were reported in 9 of 49 patients in the anti-PD-1 arm. VEGF-C increase between T0 and T1 was associated with irAEs in HR+/HER2- patients, while granzyme B increase was associated with irAEs in HR-/HER2- patients.

Conclusions: Serum immune markers may influence tumor immune cell infiltration, potentially affecting the likelihood of achieving pCR and systemic immune activity linked to irAEs. Serum levels of CRP, CXCL10, sCD25, G-CSF, and IFNγ could be used to monitor and predict clinical benefit from anti-PD-1 and immunomodulatory agents. This study also suggests that VEGF-C and granzyme B can be possible candidate prognostic biomarkers for irAEs in patients receiving anti-PD-1 therapy. Validation in a larger sample size is crucial to confirm the observed trends.

#2014 Immune exclusion signatures identify patients at high risk of relapse despite adjuvant chemotherapy in stage 2 colon cancer in the TOSCA phase 3 trial: PROSIT study.

Luca Mazzarella[1], Alberto Puccini[2], Lorenzo Ferrando[3], Gianmaria Frige'[1], Eliana Rulli[4], Anna Maria Pessino[5], Alessandro Pastorino[6], Valentino Martelli[7], Anna Garuti[8], Davide Bedognetti[9], Alberto Ballestrero[8], Valter Torri[10], Roberto Labianca[11], Matteo Fassan[12], Sara Lonardi[13], Gabriele Zoppoli[14], Alberto Sobrero[15]

[1]IEO - European Institute of Oncology, Milano, Italy,[2]Humanitas Cancer Center, Rozzano, Italy,[3]Internal Medicine Dept., IRCCS Ospedale Policlinico San Martino, Milano, Italy,[4]Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milano, Italy,[5]Hemato-Oncology Department, IRCCS Ospedale Policlinico San Martino, Genoa, Italy,[6]Medical Oncology Dept., IRCCS Ospedale Policlinico San Martino, Genoa, Italy,[7]Dipartimento di Oncologia Medica 1, IRCCS Ospedale Policlinico San Martino, Genoa, Italy,[8]Internal Medicine Dept., IRCCS Ospedale Policlinico San Martino, Genoa, Italy,[9]University of Genoa, Genoa, Italy,[10]Istituto di Ricerche Farmacologiche Mario Negri - IRCCS, Milano, Italy,[11]Province Department of Oncology, ASST Papa Giovanni XXIII,, Bergamo, Italy,[12]Azienda Universitaria Ospedaliera di Padova, Padua, Italy,[13]Oncology Department, IOV - Istituto Oncologico Veneto IRCCS, Padua, Italy,[14]Department of Internal Medicine, Universita degli Studi di Genova e Ospedale Policlinico San Martino IRCCS - DiMI, Genoa, Italy,[15]Department of Medical Oncology, IRCCS Ospedale Policlinico San Martino, Genoa, Italy

Background: The decision to administer adjuvant chemotherapy (ACT) in stage II colorectal cancer (CRC) remains challenging. Clinical and histopathological criteria, even when integrated with ctDNA-based assessment of residual disease, are still suboptimal ACT benefit predictors. Immune cell infiltration has been repeatedly associated with better outcome, but the prognostic value of transcriptional immune signatures has not been assessed in prospective trial cohorts in a systematic way. We aimed to identify a prognostic immune transcriptional signature for high-risk stage II CRC patients undergoing ACT in the TOSCA trial. TOSCA tested the noninferiority of 3 vs 6 months of FOLFOX/CAPOX, and, with a median follow-up of more than 6 years, is an ideal patient set to test outcome-associated variables.

Methods: We built a case-control cohort nested in the TOSCA trial database, matching 62 patients with high-risk stage II CRC who relapsed (R) with 62 who did not (NR). Matching criteria included recruitment center, pT stage, ACT type and duration. Whole transcriptome sequencing (WTS) was conducted on formalin-fixed, paraffin-embedded (FFPE) blocks using stranded ribodepletion library prep. MMR status was derived from WTS by majority report of three well-established methods. Immune gene signatures were assessed by the Miracle and ssGSEA packages. Differentially expressed signatures between R and NR were evaluated with the Log Rank test. Covariates included in the multivariable Cox regression models included MMR status, ACT duration (3 vs 6 months) and type (FOLFOX vs CAPOX).

Results: Out of 124 stage II CRC samples, 72 were of sufficient quality for RNAseq and were included in the analysis (“final cohort”). No key matching variable was differentially represented in the final cohort. Median follow-up was 70.9 months. The median age was 62.5, 40% of cases were right-sided, 60% received FOLFOX, 54% received 3 months of ACT, and 19% were MSI-high. We tested 67 different signatures. Immune constant of rejection and immune-exclusion signatures (IES) were the only ones with significant adjusted p-values. IES-high patients (~27%) had significantly shorter disease-free survival (41 months vs median not reached, HR 2.5, p-value=0.02). IES signature remained significant after adjusting for known covariates. Longer (6 months) ACT duration did not show any advantage over 3 month ACT in neither IES-high nor -low patients.

Conclusion: IES signature identifies patients at high risk of relapse despite receiving standard ACT, irrespective of ACT duration. IES can be exploited to design novel adjuvant trials for treatment intensification in specific patient subgroups. Funding: Italian Ministry of Health Ricerca Corrente di Rete (ACCORD) 2022-2024, AIRC GRANT IG 2018

#2015 Single-cell atlas of tumor and immune cells in disease progression from MGUS to MM.

Romanos Sklavenitis-Pistofidis[1], Ting Wu[2], Elizabeth Lightbody[1], Yoshinobu Konishi[1], Mahshid Rahmat[1], Michael Agius[1], Junko Tsuji[2], Jean-Baptiste Alberge[1], Ankit Dutta[1], Michelle Aranha[1], Nayda Bidikian[1], Hadley Barr[1], Nicholas Haradhvala[2], Gad Getz[2], Irene Ghobrial[1]

[1]Dana-Farber Cancer Institute, Boston, MA,[2]Broad Institute of MIT and Harvard, Cambridge, MA

Introduction: Multiple Myeloma (MM) is a bone marrow (BM) plasma cell malignancy that is preceded by asymptomatic stages; Monoclonal Gammopathy of Undetermined Significance (MGUS) and Smoldering Multiple Myeloma (SMM). Despite their early stage, patients with MGUS/SMM exhibit immune dysregulation. Understanding how immune dysregulation evolves and how tumor and immune cells interact to promote progression to MM may help predict and intercept it.
Methods: We performed single-cell RNA/BCR/TCR-seq (10X Genomics) on ~6 million tumor and immune cells from 877 samples, 533 BM/blood specimens, and 365 patients with MGUS/SMM/MM and healthy donors. For 241 individuals, both tumor and immune cells were sequenced to enable integrative analyses of tumor and immune biology.
Results: We observed a significant increase in the proportion of GZMB+ CD8+ effector memory T cells (TEMs) in MM compared to MGUS. This was due to increased clonal expansion and a differentiation bias in CD8+ TEMs, which also showed higher proliferative capacity and IFN-gamma expression. Despite these seemingly advantageous changes, GZMB+ CD8+ TEMs showed decreased activity of an expression signature marked by *SLC7A5* and *PIK3R1*, genes critical for T cell functionality. Patients with lower activity of this signature showed higher risk of progression. To characterize its impact on tumor cells, we split patients into high and low T cell functionality groups and compared their tumor cell expression profiles. The top upregulated gene in tumor cells from patients with high T cell functionality was *SERPINB9*, which neutralizes granzymes and protects tumor cells from cytotoxic T cells. From this analysis, we derived the transcriptional footprint of T cell functionality on tumor cells, which we termed the “immune control” signature. We found that it, too, decreases with progression, and validated it in two external cohorts. To test whether this observed decrease could be the result of tumor-intrinsic genomic evolution or, alternatively, reflects the receding immune pressure by progressively more dysfunctional T cells, we constructed tumor phylogenetic trees and compared the activity of the immune control signature across ordered subclones within tumors. We observed stable expression of the immune control signature across subclones in our trees, suggesting that this signature may reflect external immune pressure, rather than tumor genomic evolution. Independent of the patient’s disease stage, tumors with higher immune control showed lower cycling capacity, demonstrating that high T cell functionality is associated with disease control.
Conclusion: This study may have implications for: (i) the risk stratification of patients with MGUS/SMM/MM, (ii) patient selection for immunotherapy administration and interception of progression, (iii) preemptive T cell banking for future CAR-T cell therapy, and (iv) the development of novel immunotherapeutics.

#2016 Prediction of prognosis in tumor-associated macrophage using immunohistochemistry with artificial intelligence.

Rie Mizumoto, Ayumi Nagae, Norikatsu Miyoshi, Kengo Haruna, Rie Hayashi, Mitsunobu Takeda, Yuki Sekido, Tsuyoshi Hata, Atsushi Hamabe, Takayuki Ogino, Mamoru Uemura, Yuichiro Doki, Hidetoshi Eguchi

Gastroenterological Surgery, Osaka University, Suita, Japan

Tumor microenvironment (TME) is a cellular environment around the tumor. The quantification of lymphocyte populations called immunoscore has been developed and proven to have prognostic value. The impact of the degree of macrophage infiltration on the prognosis differs according to the type of cancer, and good prognosis with higher infiltration of macrophage is reported in colorectal cancer. Additionally, Image recognition technology based on deep learning is developing. There has been significant focus on classification by AI on immunohistochemistry (IHC) images. In this study, we evaluate correlation between tumor associated macrophage (TAM) distribution and prognosis of colorectal cancer, and we analyzed the data using artificial intelligence and deep learning techniques for predicting prognosis. Data from 159 patients who underwent R0 resection of primary colorectal cancer between April 2011 and December 2012 were reviewed. The 159 patients were divided into the following two cohorts: a training set of previous 100 patients and a validation set of 59 patients. Using the Anti-Iba-1 monoclonal antibody (NCNP27), we quantified TAM populations at the tumor invasive front in resected specimens and categorized by percentage of stained cytoplasm. A 0-75% density was assessed as low IM (invasive margin) score and a density between 75% and 100% was assessed as high IM score. In the training set, low IM score was also associated with poorer overall survival. The risk analysis of each factor for overall survival revealed significant difference in IM score. For the validation set, we employed MATLAB (Mathworks, R2021a) to analyze TAM distribution using a convolutional neural network (CNN). The sensitivity and specificity of the CNN mode for diagnosis were found to be 0.459 and 0.909, respectively. Consistent with the training results, a low IM score in the validation cohort was linked to the worst prognosis (P = 0.037). The findings emphasize the correlation between TAM distribution and patient prognosis in colorectal cancer, suggesting that IHC quantification of macrophages, when analyzed through AI techniques, offers valuable prognostic insights. This study highlights the potential of integrating AI with histopathological analysis to enhance prognostic prediction in oncology.

#2018 The co-expression of HER family members, EGFRvIII, putative stem cell marker CD44, and their prognostic significance in patients with glioblastoma.

Ermira Mulliqi[1], Said Khelwatty[1], Izhar Bagwan[2], Ahmad Kamaludin[3], Anna Morgan[1], Natalie Long[3], Keyoumars Ashkan[3], Helmout Modjtahedi[1]

[1]Kingston Univ. London School of Life Sci., Kingston upon Thames, United Kingdom,[2]Berkshire Surrey Pathology Services, Royal Surrey County Hospital, Kingston upon Thames, United Kingdom,[3]King's College Hospital and King's College London, London, United Kingdom

The aberrant expression and activation of HER family members is a known major oncogenic pathway for the proliferation, progression, and metastasis of a wide range of human malignancies including brain cancer. Despite numerous efforts to target EGFR, commonly dysregulated in glioblastoma, approaches directed against EGFR have not achieved the same degree of success as seen in other tumour types. The tumour heterogeneity and other redundant cell signalling pathways could be some of contributing factors to the treatment with the EGFR inhibitors. In this study, our aim was to examine the relative expression and prognostic significance of all members of the HER family, and the type III EGFR mutant (EGFRvIII) as well as the putative stem cell marker CD44, in patients with glioblastoma. The expression levels of wild-type EGFR (wtEGFR), HER2, HER3, HER4, EGFRvIII and CD44 were determined in tumour specimens from 80 patients by immunohistochemistry. The staining was scored at cut off value of >5%, 10%, 20% and 50% with positive staining, the intensity and the cellular location of immunostaining. The association between the expression level of the biomarkers and patient overall survival was evaluated using Chi-squared, Kaplan-Meier survival curves and log rank test. At cut-off value of >5% with positive staining, there was moderate to high expression of wtEGFR, HER2, HER3, HER4, EGFRvIII, and CD44 in 46%, 75%, 19%, 71%, 85%, and 95% of the cases examined, respectively. The cellular location of wtEGFR was found to be membranous and cytoplasmic, HER2 and HER3 predominantly cytoplasmic, whereas HER4 was membranous, cytoplasmic and nuclear. The expression of EGFRvIII was mainly cytoplasmic and nuclear and CD44 membranous. Interestingly, at a cut off value of >5% of tumour cells with staining, the expression of wtEGFR was accompanied with co-expression with HER2 (35%), HER3 (0%), HER4 (30%), EGFRvIII (36%), CD44 (44%), HER2/EGFRvIII (28%), HER2/CD44 (31%), EGFRvIII/CD44 (36%) of the cases examined. In 83% and 55% of the cases examined, there was co-expression of EGFRvIII with CD44 and HER2/HER4/CD44 respectively. With the exception of HER2 expression, at cut off values of >5% of tumour cells with staining, which was associated with better overall survival [HR=0.57 (p=0.038) in univariate , HR=0.56 (p=0.034) multivariate], there was no significant association between the expression of other members of the HER family, EGFRvIII and CD44 and overall survival in both univariate and multivariate analysis. Taken together, our results suggest that the co-expression of different members of the HER family, with EGFRvIII and CD44 occurs in patients with glioblastoma and warrants further investigation on the predictive value of these biomarkers and their potential as targets for therapeutic interventions with drugs targeting these receptors.

#2019 The relationship between pSMAD3 expression, clinicopathological features, tumor microenvironment and survival in operable colorectal cancer patients.

Chia Yew Kong[1], Natthamon Sonthi[1], Leonor Schubert Santana[1], Lily V. S. Hillson[1], Kathryn Pennel[1], Peter Alexander[1], Noori Maka[2], Joanne Edwards[1], Colin W. Steele[1], Campbell S. Roxburgh[1]

[1]University of Glasgow - School of Cancer Sciences, Glasgow, United Kingdom,[2]Department of Pathology, Queen Elizabeth University Hospital, Glasgow, United Kingdom

Background: Consensus molecular subtype 4 (CMS4) colorectal cancer (CRC) is associated with prominent Transforming Growth Factor-β (TGF-β) dysregulation & poorer prognosis. The TGF-β pathway has context-dependent tumor suppressor & promoting activity regulated by SMAD transcriptional factors. Increasingly, SMAD4 loss is shown to lead to tumor suppression loss in CRC (1). Evidence around other SMADs is lacking. We examined the relationship between phosphorylated SMAD3 (pSMAD3) expression, clinicopathological features, gene expression & survival in a large cohort of CRC patients.
Methods: Patients who underwent curative resection of primary CRC from 1997 to 2012 at 3 hospitals in Glasgow, UK, were included. pSMAD3 protein immunohistochemistry (abcam Ab52903) was performed on a tissue microarray. Tumor/stromal & subcellular pSMAD3 expression histoscores were quantified on Qupath. High/low expression thresholds were determined by maximally selected ranked statistics. Expression was correlated with clinicopathological factors & survival. Gene set enrichment analyses (MSigDB Hallmarks) were performed on bulk transcriptomic (TempO-Seq, Bioclavis) data in 318 patients (2).
Results: 1236 patients were eligible for analysis. High tumor cytoplasmic, nuclear & stromal cytoplasmic & nuclear pSMAD3 groups comprised 77.8%, 50.6%, 34.9% & 54.6% of the cohort respectively. High pSMAD3 expression across all compartments was associated with favourable tumor features: high tumor cytoplasmic, & stromal cytoplasmic & nuclear scores related to lower T-stage (all $p<0.05$). High tumor cytoplasmic & nuclear, & stromal cytoplasmic scores related to absence of venous invasion (all $p<0.01$). High tumor nuclear & stromal cytoplasmic scores also related to a strong Klintrup-Makinen immune microenvironment score (both $p<0.01$). High pSMAD3 was associated with TGF-β pathway gene set enrichment in CMS4 tumors alone. Within CMS4, high pSMAD3 was also associated enriched MYC & epithelial mesenchymal transition (EMT) pathways. Conversely, in non-CMS4 tumors, high pSMAD3 relates to suppressed EMT. High pSMAD3 across all compartments relates to better cancer specific survival (CSS) on univariate analyses (all $p<0.05$). On multivariate analysis, high stromal nuclear pSMAD3 retained independent prognostic significance (HR0.69, 95%CI 0.5-0.95, $p=0.023$). This survival relationship persisted in T2-3 & stromal poor tumors but not in T4 or stromal rich tumors.
Conclusion: High pSMAD3 expression relates to favourable tumor features & stage-independent improved CSS, indicative of preserved TGF- β tumor suppression. In contrast, within CMS4 tumors, high pSMAD3 relates to enriched tumor promoter pathways, suggesting contextual TGF-β pleiotropy.
References 1 Voorneveld et al. Transl Oncol 2015;8(1):18-24 **2**Liberzon et al. Cell Syst 2015;1(6):417-425

#2020 Fibroblast derived type III collagen pro-peptides (PRO-C3) in plasma is associated with outcome for patients with NSCLC treated with anti-PD1 plus chemotherapy.

Nicholas Willumsen[1], Lola Lecru[2], Thibaut Brugat[2], John Stagg[3], Bertrand Routy[4], Morten A. Karsdal[1]

[1]Nordic Bioscience, Herlev, Denmark,[2]Domain Therapeutics, Illkirch, France,[3]Centre de Recherche du Centre Hospitalier de l'Universite de Montreal, Montreal, QC, Canada,[4]Axe Cancer, Centre de Recherche du Centre Hospitalier de l'Universite de Montreal, Montreal, QC, Canada

Background: Tumor fibrosis is essential for defining outcome of patients with various solid tumors including lung cancer. The associated cancer associated fibroblasts (CAFs) activation and increased deposition of type III collagen leads to immune exclusion and high interstitial pressure in the tumor microenvironment. Recently, the USA FDA issued a Letter of Support to encourage use of the non-invasive fibrosis biomarker PRO-C3 (type III collagen pro-peptides) in patients with solid tumors (https://www.fda.gov/media/169332/download). Here we investigate PRO-C3 as a prognostic biomarker in patients with non-small cell lung cancer (NSCLC).

Methods: First, we investigated the PRO-C3 production (measured by ELISA) and total collagen deposition (Sirius red staining) in primary lung CAFs activated with TGF-beta, by use of the validated scar-in-a-jar in vitro model. Second, we measured PRO-C3 in pre-treatment plasma samples from 40 patients with stage IIIa to IVb NSCLC. Patients were treated with Pembrolizumab (anti-PD1) plus platinum-doublet chemotherapy (carboplatin, cisplatin, pemetrexed, or paclitaxel). PRO-C3 was correlated to overall survival outcome (OS) by Kaplan Meier analysis and Cox regression analysis after allocating patients into PRO-C3 subgroups (below/above the median of 51.5 ng/mL).

Results: PRO-C3 was produced by lung CAFs after activation with TGF-beta in vitro and PRO-C3 correlated to increased net-collagen deposition. Among the 40 patients with NSCLC, the median follow-up was 13.2 months. Patients with high PRO-C3 had a median OS of 9.0 months compared to 31.5 months in patients with low PRO-C3 (log-rank p-value=0.0023). In support, patients with high PRO-C3 had a Hazard Ratio (HR) for OS of 3.8 (95%CI: 1.5-9.7, p=0.004).

Conclusions: Type III collagen pro-peptides (PRO-C3) was produced by activated lung CAFs and is a surrogate of fibrogenesis. High PRO-C3 levels in pre-treatment plasma was associated with poor outcome for patients with NSCLC treated with anti-PD1 plus chemotherapy. These data suggest that quantifying tumor fibrosis is important for prognostication of patients with lung cancer.

#2021 Impact of corticosteroids on predictive and prognostic value of neutrophil-to-lymphocyte ratio in patients with non-small cell lung cancer treated with immune checkpoint inhibitor therapy.

A. Lim[1], A. Meyer[1], L. Polyakov[1], R. Cooper[2], A. Mades[2], S. Ye[3], J. Ni[1], H. Chen[4], L. Contreras[1], J. Sarkar[4], R. Sharma[5], B. H. Segal[4], R. Hsu[2], J. Nieva[2], G. K. Dy[4], F. Ito[1];

[1]University of Southern California, Norris Comprehensive Cancer Center, Los Angeles, CA, [2]Keck School of Medicine, University of Southern California, Los Angeles, CA, [3]USC/Los Angeles General Medical Center, University of Southern California, Los Angeles, CA, [4]Roswell Park Comprehensive Cancer Center, Buffalo, NY, [5]University at Buffalo Jacobs School of Medicine and Biomedical Sciences, the State University of New York, Buffalo, NY

Introduction: An elevated baseline neutrophil-to-lymphocyte ratio (NLR) can signal an imbalance between pro-tumor and anti-tumor inflammatory responses and has been associated with worse overall survival (OS) and lower response rates in patients with non-small cell lung cancer (NSCLC) treated with immune checkpoint inhibitors (ICIs). Corticosteroids, known to cause neutrophilia and lymphopenia, are widely used in NSCLC, but their impact on biomarker performance remains elusive. This study investigates how baseline steroid use affects biomarker performance in NSCLC patients on ICIs.

Methods: We retrospectively evaluated 252 patients with NSCLC treated with ICIs at the Roswell Park Comprehensive Cancer Center (RPCCC) (n=81) (discovery cohort) and the University of Southern California (USC) (n=171) (validation cohort). We analyzed the association between baseline NLR at ICI initiation with overall response rate (ORR), progression free survival (PFS), and OS. NLR was defined as absolute neutrophil count divided by absolute lymphocyte count. Patients were stratified by NLR: NLR ≥ 5 (high) and NLR < 5 (low). ORR was estimated using Fisher's exact test, and Kaplan-Meier survival distributions for PFS and OS were generated.

Results: In our cohort, 159 (63.1%) of 252 patients had an NLR < 5 and 93 (33.9%) had NLR ≥ 5. NLR < 5 at baseline was correlated with longer PFS (RPCCC: $p<0.0005$; USC: $p<0.005$) and OS (RPCCC: $p<0.0005$; USC: $p<0.05$) although there was no significant relationship between NLR and treatment response rates in RPCCC or USC cohorts. We identified 19 patients who were on systemic steroids at the time of initiation of ICI therapy. Indications for corticosteroids were brain metastases or comorbid lung conditions such as COPD, and all 19 patients remained on steroids for at least 12 weeks from the initiation of ICI therapy. When examining the predictive value of NLR by steroid and non-steroid subgroups in both cohorts, we found no significant association between NLR and treatment response rates for patients taking steroids and patients not taking steroids. Patients not taking steroids with low NLR showed markedly longer PFS (RPCCC: $p<0.005$; USC: $p<0.05$) and OS (RPCCC: $p<0.001$; USC: $p<0.05$). However, patients taking steroids showed no significant correlation of NLR with PFS or OS.

Conclusions: Baseline NLR was associated with prognosis of NSCLC patients undergoing ICI therapy who do not receive steroids at the initiation of ICI therapy but was not correlated with response to ICI therapy. The results of NLR can be obtained via routine bloodwork and readily available for prompt feedback to oncologists and patients; however, clinicians must take care in the prognostic value of NLR in patients who were on steroids at the initiation of ICI therapy.

#2023 AccuScan: an ultra-sensitive and robust MRD detection using WGS with single-read error correction.

Xinxing Li[1], Lui Ng[2], George Yeung[3], Yingyu Wang[3], **Paul Tang**[3], Tobias Wittkop[3], Malek Faham[3], Li Weng[3], William Cho[4], Dominic, Chi-chung Foo[2], Zhiqian Hu[1]

[1]Tongji Hospital, Shanghai, China,[2]University of Hong Kong, Hong Kong, Hong Kong,[3]AccuraGen, Inc., San Jose, CA,[4]Queen Elizabeth Hospital, Hong Kong, Hong Kong

Whole-genome sequencing (WGS) of cell-free DNA (cfDNA) holds tremendous potential for detecting molecular residual disease (MRD), but its accuracy has been constrained by errors introduced during library preparation and sequencing. To overcome these limitations, we introduced AccuScan, a novel and robust cfDNA WGS technology that enables genome-wide error correction at the single-read level, achieving an error rate of 4.2×10^{-7}, which is orders of magnitude improvement over conventional read-centric de-noising approaches. AccuScan has demonstrated analytical sensitivity down to parts per million (ppm) circulating variant allele frequency (cVAF), while maintaining 99% specificity at the sample level. By combining the convenience of WGS with the sensitivity and specificity of tumor-informed approaches, AccuScan enables precise detection of MRD in a timely manner, addressing critical unmet needs in colorectal cancer (CRC) adjuvant therapy decision-making. In the current clinical study involving 56 CRC patients who went through curative intended surgery (stages I: 7, 12.5%; stage II: 17, 30%; stage III: 26, 46.4%; stage IV: 6, 10.7%), AccuScan achieved ~88% sensitivity for MRD detection from a single sample collected within six weeks post-surgery. The test showed a strong correlation with recurrence risks over a three-year follow-up period while delivering 100% specificity. Notably, AccuScan captured MRD at <100 ppm cVAF, demonstrating its ultra-sensitive detection capabilities at the landmark timepoint when adjuvant therapy decisions need to be made. With a simple and distributable workflow, AccuScan demonstrated high performance and a high success rate in processing cfDNA samples across a broad range of input DNA quantities. Its current performance is powered by a straightforward statistical model that leverages single nucleotide variant (SNV) information. Future advancements in performance are anticipated with expanded cfDNA WGS datasets and the incorporation of cutting-edge machine learning strategies.

#2024 Circulating tumor DNA detectability and targetability in patients undergoing neoadjuvant therapy for localized breast cancer.

Arielle J. Medford[1], Elyssa Denault[2], Veerle Bossuyt[3], Michelle Hutchinson[4], Emily Westheimer[4], Jianhua Zhao[4], Bob Daber[4], Steven J. Isakoff[1], Beverly Moy[1], Dejan Juric[1], Laura M. Spring[1], Ryan Corcoran[1], Aditya Bardia[5]

[1]Mass General Cancer Center / Harvard Medical School, Boston, MA,[2]Mass General Cancer Center, Boston, MA,[3]Pathology, Mass General Cancer Center / Harvard Medical School, Boston, MA,[4]Labcorp Genetics, Burlington, NC,[5]UCLA Health Jonsson Comprehensive Cancer Center, Los Angeles, CA

Background: Circulating tumor DNA (ctDNA) detection can identify patients (pts) at higher risk for cancer recurrence. Hormone receptor positive (HR+)/HER2- breast cancer (BC) is typically less responsive to chemotherapy, and standard indicators of tumor response to neoadjuvant therapy (NAT) are less informative of prognosis. In this study, we analyzed banked plasma samples for ctDNA in pts receiving neoadjuvant BC treatment to determine the associations between ctDNA detectability and post-NAT surgical pathology and overall outcomes.

Methods: Plasma samples from pts with high-risk localized HR+/HER2- BC treated with NAT were retrospectively analyzed for ctDNA using the Invitae Personalized Cancer Monitoring™ assay. Primary tumors underwent whole exome sequencing (WES), and personalized primer sets were designed for up to 50 tumor-specific variants. High throughput next generation sequencing was then used to evaluate plasma samples for ctDNA at up to 3 timepoints: prior to, during, and after NAT. Levels of residual disease were determined by residual cancer burden index (RCB). WES data was interrogated for variants with approved matched targeted therapies.

Results: Retrospective plasma samples from twelve patients with high-risk early BC (9 stage II, 3 stage III) underwent ctDNA analysis. Five pts had +ctDNA pre-NAT; 3 experienced ctDNA clearance on NAT (all chemotherapy), and 2 of these pts showed sustained post-NAT ctDNA clearance. One of these pts had RCB-0 and the other RCB-2 disease. To date, these 2 pts have not had recurrence of their disease (f/u 45.7 & 47.1 months). Among the 2 pts with +ctDNA on NAT, 1 was receiving chemotherapy and 1 endocrine therapy. The other 3 pts with pre-NAT +ctDNA who did not have documented post-NAT ctDNA clearance experienced recurrences: 14.4, 68.4, & 72 months after surgery. The 7 pts with -ctDNA pre-NAT have not experienced recurrence to date (median f/u 62.7 months). Overall, 7/12 pts (58%) had targetable variants detected on tissue WES: 4 *PIK3CA* (N345K x2, Q546K, H1047R) and 3 *AKT1*^{E17K}. Three of these pts had +ctDNA, and all had activating *PIK3CA* mutations. One pt with *PIK3CA*^{N345K} experienced sustained ctDNA clearance.

Conclusions: Persistence of ctDNA despite NAT appears to be associated with disease recurrence, though the number of patients in this retrospective analysis was small. These hypothesis generating findings require validation in larger datasets. If validated, results could guide development of interception clinical trials with PI3K inhibitors for pts with ctDNA+ *PIK3CA*-mutant breast cancer.

#2025 Regulatory role of CDC20 phosphorylation in the onset and progression of endometrial carcinoma.

Wanbao Ding[1], Jiuqin Lu[1], Yanfang Zhao[1], Hui Dai[1], Feilong Zhao[2], Ding Zhang[2], Huan Guo[1]

[1]Kunming Yan'an Hospital., Kunming, China,[2]3D Medicine, Shanghai, China

Background: Phosphorylation is a critical post-translational modification regulating key biological processes. CDC20 is a key regulator in cell cycle control, but its phosphorylation's role in Uterine Corpus Endometrial Carcinoma (UCEC) is not well understood.
Methods: We used two datasets from the Clinical Proteomic Tumor Analysis Consortium (CPTAC). PDC000126 was the training set, and PDC000441 was used for validation. Both datasets contained proteomic and phosphoproteomic data. Differentially expressed phosphorylation (DEP) sites were identified. Kinase prediction was performed using a kinase-recognition motif library, and functional enrichment analysis was carried out using GSEA.
Results: We identified 7223 phosphorylation sites after filtering samples for data quality. Principal component analysis (PCA) distinguished normal from tumor samples but did not separate early- and late-stage tumors. CDC20(T106) and TRA2A(S100) phosphorylation (pCDC20 and pTRA2A for shot) were significantly upregulated in both early and late-stage tumors compared to normal tissues. No significant differences were found between early- and late-stage tumor phosphorylation levels, suggesting these sites are involved in tumor initiation and remain elevated during progression. pCDC20 was associated with worse survival outcomes, particularly when dichotomized using the 70th percentile as a threshold (HR 7.32, P = 0.022). pTRA2A was not significantly related to survival. Multivariate Cox regression, adjusted for tumor grade, confirmed pCDC20 as an independent prognostic factor. CDC20 protein expression alone was not significantly associated with prognosis, but pCDC20 remained significant after adjusting for protein levels (HR = 37.18, P = 0.025). Kinase prediction identified CDK1 and CDK2 as potential regulators of pCDC20. CDK1/2 expression was positively correlated with pCDC20, and protein interaction analysis confirmed strong interactions between CDC20 and CDK proteins. GSEA showed activation of epithelial-mesenchymal transition (EMT), vasculature development, and cell morphogenesis pathways in the pCDC20 high-phosphorylation group. pCDC20 was validated in the PDC000126 dataset, showing similar trends in early- and late-stage tumors. Correlation between pCDC20 and CDC20 protein was confirmed, as was the association between CDK1/2 expression and phosphorylation. Survival analysis in the validation set indicated poorer prognosis in patients with high pCDC20, which became significant after adjusting for tumor grade (HR 3.28, P = 0.04).
Conclusions: CDC20 phosphorylation is a prognostic marker in UCEC, possibly functioning by promotion of vasculature development and cell morphogenesis. Targeting pCDC20 may offer therapeutic potential in UCEC treatment.

#2026 Comprehensive molecular and immunological characterization of CLDN18.2 in pancreatic cancer.

M. Malla[1], S. Deshmukh[2], T. Samec[2], S. Wu[2], J. Xiu[2], M. Akce[1], G. Gupta[1], Q. Hussaini[1], D. Outlaw[1], R. Arrend[1], A. Desai[1], A. Basu[1], P. N. Ganji[1], M. Evans[2], H.-J. Lenz[3], E. Lou[4], D. Castillo[2], R. Schroff[5], D. Spetzler[2], B. El-Rayes[1];
[1]University of Alabama at Birmingham, Birmingham, AL, [2]Caris Life Sciences, Phoenix, AZ, [3]University of South California, Los Angeles, CA, [4]University of Minnesotta, Minnesotta, MN, [5]University of Arizona, Tucson, AZ

Introduction: Claudin 18 (*CLDN18*) is an emerging biomarker and transmembrane protein that maintains a tight junction between cells in pancreatic cancer (PC). Due to the high expression of *CLDN18.2,* an isoform of *CLDN18*, genomic profiling has demonstrated *CLDN18.2* as a potential target with prognostic impact; we aimed to characterize the molecular and immunological features associated with *CLDN18.2* expression in PC.
Methods: 9,837 PC samples were tested by NGS (592, NextSeq; WES, NovaSeq) and WTS (NovaSeq; Caris Life Sciences, Phoenix, AZ). PD-L1 expression was assessed using immunohistochemistry (SP142 clone). Immune cell fractions were calculated by deconvolution of WTS: Quantiseq. Tumors with *CLDN18.2*-high(H) and *CLDN18.2*-low(L) RNA expression were classified by top and bottom quartile, respectively. Real world overall survival (OS) was obtained from insurance claims and calculated from tissue collection to last contact using Kaplan-Meier estimates. Statistical significance was determined by chi-square and Mann-Whitney U test with *p*-values adjusted for multiple comparisons (q<0.05).
Results: Median *CLDN18.2* expression was higher in primary compared to metastatic PC (3-fold, *q*< 0.05). *CLDN18.2*-H primary and metastatic PC had higher frequency of *KRAS* (95.2% vs 83.8%, 91.7% vs. 82.8%) and *SMAD4* (28.4% vs 15.3%, 23.1% vs 14.9%), but lower frequency of *ARID1A* (6.6% vs 11.1%, 8.9% vs 13.2%) mutation (all q<0.05). *CLDN18.2*-H primary and metastatic PC had lower PD-L1 positivity (4.1% vs 27.9%, 5.3% vs 29.9%, q<0.05). *CLDN18.2*-H primary and metastatic tumors had higher infiltration of B cells (5.1% vs 3.8%, 4.4% vs 3.3%), M2 Mφ (3.6% vs 3.3%, 3.7% vs 3.1%), and NK cells (2.8% vs 2.5%, 2.5% vs 2.3%), all q<0.05. *CLDN18.2*-H primary and metastatic PC had differential expression of immune checkpoint genes (upregulation: *CTLA4*, *HAVCR2, FOXP3, CD47, BTLA, TIGIT, CD160*; fold change (FC): 1.1 - 1.46; downregulation: *CD274*, *LAG3, IDO1, CD276*, FC: 1.1 - 1.3, all q<0.05). *CLDN18.2*-H primary and metastatic PC had higher T-cell inflamed score (25 vs -28, 21 vs 18.5, q<0.05). *CLDN18.2*-H was associated with better OS compared to *CLDN18.2*-L in primary (mOS: 20 vs 13.2 months; HR: 0.73, 95% CI 0.64 - 0.83, p < .00001) and metastatic (mOS: 9.0 vs 5.9 months; HR: 0.76, 95% CI 0.7 - 0.83, p <.00001) PC. A multivariate Cox analysis revealed that above median values of NK cells were strongly associated with better survival in *CLDN18.2*-H primary (HR: 0.75, 95% CI 0.66 - 0.85, p <.00001) and metastatic (HR: 0.73, 95% CI 0.67 - 0.8, p <.00001) PC.
Conclusions: *CLDN18.2* expression is associated with distinct genomic alterations, a differentially modulated immune microenvironment and PC patients' survival. Prospective clinical trials using novel approaches to target *CLDN18.2* in PC are underway. Further studies are required to study the impact of *CLDN18.2* expression and targeted strategies on survival outcomes of patients with PC.

#2027 Developing an image analysis algorithm to evaluate immunofluorescence intensity for Rb and phospho-Rb expression in circulating tumor cells in HR+/HER2- breast cancer.

Shivaani Suresh Kanna[1], Mantasha Tabassum[2], Meghana V. Trivedi[1]

[1]Department of Pharmacy Practice and Translational Research, University of Houston, College of Pharmacy, Houston, TX,[2]Department of Pharmacological and Pharmaceutical Science, University of Houston, College of Pharmacy, Houston, TX

Introduction: Our lab is developing a multiplex immunofluorescence (IF) assay that can be used on circulating tumor cells (CTCs) to assess Rb and pRb expression to predict efficacy of CDK4/6 inhibitors. In this study, we aimed to develop an image processing algorithm for analyzing Rb and pRb expression.

Methods: Positive control (MCF7 and T47D) and negative control (MDA-MB-468 and T47D palbociclib-resistant) cell lines were stained with anti-Rb-AF594, anti-pRb-AF488, and DAPI. The IF-stained cells were imaged using a Leica SP8 STED confocal microscope and processed with the ImageJ Fiji software. An image analysis pipeline was developed by comparing various parameters for each condition for optimized z project (Max intensity vs. Sum slices), background subtraction (Rolling ball radius vs. Mean), and threshold settings (Default vs. Otsu). Mean fluorescence intensities (MFI) between various parameters were compared using the Mann-Whitney U test. A p-value of <0.05 was considered statistically significant. Signal-to-noise ratio (SNR) was calculated as (mean signal from positive control cells - mean background from negative control cells) / background standard deviation. This pipeline was further validated on mixed cell populations of MCF7 P + leukocytes, which were stained with anti-CK-AF647, anti-CD45-AF555, anti-Rb-AF594, anti-pRb-AF488, and DAPI. At least 3 independent experiments were included. MFI was determined for DAPI+/CK+/CD45- cells.

Results: A systematic approach was employed, beginning with standardizing image acquisition parameters, including laser intensity and gain settings, to ensure consistency between samples within an experiment using 2 positive and 2 negative control cell lines. For initial optimization with z-projection settings, significantly higher MFI was obtained with Sum slices compared to Max intensity for both positive and negative control cells. For background subtraction, Rolling ball radius versus Mean background subtraction showed no significant differences between the two settings. For threshold comparisons Otsu method introduced significant background noise in negative controls, compromising intensity accuracy, leading to the selection of Default method. Therefore, proposed pipeline included Sum slices, Rolling ball radius, and Default method optimized using internal controls with an SNR of 16 for Rb and 170 for pRb. Validation with MCF7 P + leukocyte samples produced MFIs (median (range)) of 103,802 (5,484 - 627,148) for Rb and 149,274 (8,379 - 791,202) for pRb for the DAPI+/CK+/CD45- cells.

Conclusion: We have established the IF image processing pipeline for Rb and pRb expression assessment, which will be further refined to assess the sensitivity and specificity of the IF assay, laying a foundation for its future application to patients' isolated CTC samples.

#2028 Development of a miRNA-based prognostic signature for clear cell renal cell carcinoma.

Minghui Zhang, Brock C. Christensen, Lucas A. Salas

Dartmouth Geisel School of Medicine, Hanover, NH

Background: Clear cell renal cell carcinoma (ccRCC) represents about 80% of renal cell carcinoma cases. MicroRNAs (miRNAs) play a critical role in RNA silencing and post-transcriptional regulation of gene expression, impacting tumorigenesis and cancer progression. This study aimed to develop a miRNA-based prognostic model to predict overall survival in ccRCC patients by leveraging genome-wide miRNA expression profiling.
Methods: Sequencing data from tumor kidney samples were obtained from the Dartmouth Renal Tumor Biobank. Data was processed using the Nextflow smrnaseq pipeline and analyzed in R. Log-transformed Transcripts Per Million (logTPM) values were used for downstream analysis. After quality control, survival-associated miRNAs were identified using Cox proportional hazards (CoxPH) models, controlling for batch effects and tumor stage. miRNAs with a p-value < 0.01 were further refined through the LASSO method, yielding an optimal set of miRNAs for prognostic modeling. The prognostic risk score was calculated as: Risk score = $\sum$(logTPM×coefficient). Patients were stratified into high and low risk groups based on the median risk score. Kaplan-Meier survival analyses and CoxPH models were used to assessed survival differences between risk groups. Covariates, including batch, cell proportion, grade, stage, age, and sex, were included in adjusted analyses. Predictive accuracy was assessed by calculating the area under the curve (AUC) for 1, 3, and 5 years using the survivalROC package with the nearest neighbor estimation.
Results: 147 tumor samples with complete covariates passed quality control. For the initial analysis, 37 miRNAs were associated with survival, and 19 miRNAs were retained using the LASSO method. Of these, 15 miRNAs were previously reported to have tumor-related functions. Notably, miR-466, -4437, and -524-3p were associated with prognosis in other cancers. The risk score, derived from the expression levels and coefficients of the 19 miRNAs, followed a normal distribution (Shapiro-Wilk normality test p-value = 0.15) with a median value of -4.29. Patients in the high-risk group had a significantly higher hazard for death compared to those in the low-risk group, with a hazard ratio (HR) of 6.21 (95% CI: 3.94-9.78, p-value = 3.28 E-15). After adjusting for relevant covariates, the high-risk group remained an independent predictor of poor survival (HR: 9.06, 95% CI: 4.99-16.46 , p-value = 4.66 E-13). The predictive performance was robust, with AUCs of 0.85, 0.86, and 0.81 for 1, 3, and 5 years, respectively.
Conclusion: The miRNA-based risk score effectively predicts survival in ccRCC patients, offering a potential prognostic biomarker for ccRCC. It may provide valuable insights for predicting survival outcomes and guiding clinical decision-making. Future studies should validate these findings in external cohorts and explore the underlying biological mechanisms of the identified miRNAs.

CLINICAL RESEARCH: Predictive Biomarkers 3
Poster Session

#2032 Interleukin-8 expression inversely predicts the therapeutic effectiveness of doxorubicin and paclitaxel on triple-negative breast cancer.

Hui-Yu Lin[1], Yuan-Feng Lin[2], Hui-Wen Chiu[2], Ling-Ming Tseng[3]

[1]School of Medicine, Division of Breast Surgery and General Surgery, Department of Surgery, Cardinal Tien Hospital, Fu-Jen Catholic University, New Taipei City, Taiwan,[2]Graduate Institute of Clinical Medicine, Taipei Medical Universtiy, Taipei City, Taiwan,[3]Comprehensive Breast Health Center, Surgery, Taipei Veterans General Hospital, Taipei, Taiwan

Background: Triple negative breast cancer is a subtype of breast cancer and exhibits highly metastatic potentials. Neoadjuvant or adjuvant chemotherapy, e.g. Anthracycline and Taxane, remains as a main regimen to treat TNBC currently. However, TNBC patients usually suffered from chemotherapy, e.g. doxorubicin and paclitaxel, owing to the lack of useful biomarkers to predict their therapeutic effectiveness. The aim of this study was made to estimate the usefulness of pro-inflammatory cytokine interleukin-8 (IL-8) for predicting the therapeutic efficacy of doxorubicin and paclitaxel in TNBC.

Methods: The *in silico* analysis was performed to the potential biomarkers for predicting the therapeutic effectiveness on TNBC patients. Cell-based cytotoxicity assay was established to measure the half maximal inhibitory concentration (IC_{50}) of doxorubicin and paclitaxel in the tested TNBC cells with IL-8 supplement or knockdown. Animal model was established to evaluate the therapeutic effectiveness of doxorubicin and paclitaxel on the xenograft tumors derived from TNBC cells without or with IL-8 overexpression or knockdown. Gene Set Enrichment Analysis (GSEA) was employed to predict the potential pathways associated with IL-8 upregulation in TNBC.

Results: The *in silico* analysis demonstrated that IL-8 is a potential biomarker to predict the therapeutic outcome of TNBC patients who received doxorubicin or paclitaxel. The IL-8 supplement desensitized TNBC cell line HCC1937 that harbors a lower endogenous IL-8 expression to doxorubicin treatment but enhances its sensitivity to paclitaxel; Accordingly, the administration of doxorubicin failed to but paclitaxel effectively suppressed tumor growth of the xenograft tumors derived IL-8-overexpressing HCC1937 cells compared to the control group. In contrast, the treatment with doxorubicin dramatically inhibited but paclitaxel did not affect tumor growth of the xenograft tumors derived IL-8-silenced MDA-MB231 cells compared to the non-silencing control. GSEA results indicated that IL-8 upregulation in primary tumor of TNBC patients highly associates with the activation of E2F and NF-κB pathway. The inclusion of IL-8 protein in HCC1937 cells dramatically promoted but its knockdown in MDA-MB231 cells blocked RB phosphorylation, E2F activity and NF-κB activation. Pharmaceutical inhibition of IL-8 receptor by Reparixin or NF-κB by SN50 and BAY 11-7082 significantly reversed the IL-8-triggered doxorubicin resistant/paclitaxel sensitive responsiveness in HCC1937 cells.

Conclusion: These findings demonstrated that IL-8 expression could be a useful indicator to predict the responsiveness of TNBC patients who are deciding to receive doxorubicin or paclitaxel therapy.

#2033 Identification of biomarkers for drug candidate LP-184 in precision oncology applications.

Aditya Kulkarni, Jianli Zhou, Giulia Agnello, Reggie Ewesuedo, Marc Chamberlain, Kishor Bhatia

Lantern Pharma Inc., Dallas, TX

LP-184, Lantern Pharma's acylfulvene small-molecule alkylator currently being evaluated in a Phase 1a trial, exhibits potent preclinical activity against multiple solid tumors. LP-184 is a prodrug bioactivated intracellularly by the enzyme prostaglandin reductase 1 (PTGR1), often elevated in cancer. In cell line experiments, PTGR1 expression by RNAseq was found to be highly concordant with that by RT-PCR ($r = -0.975$, $p < 0.0001$). The assay identified a ΔCt value of 7.5 as the PTGR1 (normalized to housekeeping genes ACTB and GAPDH) expression threshold associated with LP-184 sensitivity (IC50 < 300 nM) and with average peak plasma exposure from preliminary clinical pharmacokinetics. We then established an RT-PCR assay for PTGR1 quantification in FFPE tissue samples. In the ongoing Phase 1a trial, 80% of analyzed tumor samples showed PTGR1 expression greater than this threshold. In silico analysis of 20 solid tumor TCGA histologies showed that 78% of samples expressed PTGR1 levels greater than the corresponding RNAseq expression threshold (4.5 log2TPM+1) associated with LP-184 sensitivity. In cancer cell lines, LP-184 induced DNA double strand breaks (DSBs) and triggered DNA repair as measured by the SensiTive Recognition of Individual DNA Ends (STRIDE) assay. 24-hour LP-184 treatment in parental and engineered DLD1 colon cancer cell lines led to 2-fold elevated levels of DSBs and γH2AX (phosphorylated H2AX, DNA damage marker) in BRCA2 knockout cells compared to wild type. LP-184 demonstrated 3-fold higher in vitro sensitivity in DNA Damage Repair (DDR) deficient tumor models compared to non-DDR altered cell lines, along with complete, durable tumor regressions in multiple xenograft DDR deficient tumor models. Preclinical data on LP-184 anti-tumor activity and biomarker status indicate that the inactivation of BRCA1/2, ATM/ATR, and ERCC3/4, and the activation of EGFR are key biomarker candidates predicting response to LP-184. In addition, a genome-wide CRISPR chemogenetic screen of LP-184 in the TNBC cell line MDAMB231, suggested that ATRX, EZH2, HSP90AA1, CDKN2B, MAD2L2, PSMA6, NDUFA1, and BRAF are other potential predictive biomarkers of LP-184 activity. Drug-gene associations derived from preclinical studies have identified a panel of biomarkers associated with LP-184 bioactivation (PTGR1:RT-PCR/tissue), pharmacodynamics (DNA DSB, γH2AX: Multiplexed STRIDE or IF assay/tissue), and response prediction (BRCA1/2 etc.: liquid biopsy or tissue/ TSO500 NGS panel). A retrospective biomarker validation will be performed using established NGS assays to analyze liquid biopsy and/or tissue samples from the ongoing Phase 1a LP-184 trial. Future directions include determining associations between biomarker expression or mutation and clinical outcomes, refining PTGR1 expression threshold, and utilizing emerging biomarkers to guide patient selection in subsequent expansion studies.

#2034 Investigation of TROP2 expression and immune microenvironment in ovarian clear cell carcinoma.

Toru Imai[1], Yuki Kojima[1], Hiroshi Yoshida[2], Rui Kitadai[1], Mai Hoshino[1], Ayumi Saito[1], Munehiro Ito[1], Shosuke Kita[1], Aiko Maejima[1], Kazuki Sudo[1], Masaya Uno[3], Tatsunori Shimoi[1], Mitsuya Ishikawa[3], Kan Yonemori[1]

[1]Department of Medical Oncology, National Cancer Center Hospital, Japan, Tokyo, Japan,[2]Department of Diagnostic Pathology, National Cancer Center Hospital, Japan, Tokyo, Japan,[3]Department of Gynecology, National Cancer Center Hospital, Japan, Tokyo, Japan

Background: Ovarian clear cell carcinoma (OCCC) is a rare subtype of ovarian cancer, with a higher incidence in East Asia compared to Western countries and a tendency to affect younger individuals. OCCC has poor prognosis due to its low sensitivity to chemotherapy, presenting a significant unmet medical need. However, therapeutic development has been limited due to its rarity. Recently, advancements in antibody-drug conjugates (ADCs) targeting Trophoblastic cell-surface marker (TROP2) have shown promise, but the expression of these therapeutic targets in OCCC remains unclear. This study aimed to explore new therapeutic targets for OCCC by analyzing TROP2 expression and the immune microenvironment.
Methods: Clinical data were collected from OCCC patients who underwent surgery at the National Cancer Center Hospital between 2007 and 2024. TROP2 (MAB650), ARID1A (polyclonal), and PD-L1(E1L3N) were evaluated. PD-L1 expression was evaluated using both Tumor Proportion Score (TPS) and Combined Positive Score (CPS). TROP2 expression was assessed using the H-score, with a score of ≥10 defined as positive. Pathological features, including stromal characteristics, cytoplasmic features, and tissue architecture, were also analyzed.
Results: A total of 125 OCCC patients were included. TROP2 expression was positive in 90 patients (72.0%) and negative in 35 patients (28.0%). ARID1A was retained in 52 patients (41.6%) and lost in 73 patients (58.4%). TPS values were <1 in 44 patients (35.2%), 1-49 in 73 patients (58.4%), and ≥50 in 8 patients (6.4%). CPS values were <1 in 33 patients (26.4%), 1-9 in 30 patients (24.0%), and ≥10 in 62 patients (49.6%). No significant differences were observed in TROP2 expression across these variables. Overall survival did not significantly differ between TROP2-positive and TROP2-negative patients (HR=1.22, 95%CI: 0.48-3.09, p=0.67). ARID1A status was also not associated with survival (HR=1.48, 95%CI: 0.62-3.49, p=0.37). However, CPS ≥10 was associated with a trend toward worse prognosis (HR=2.92, 95%CI: 0.95-8.41, p=0.06). Poor prognosis was also observed in cases with high stromal inflammation (HR=2.47, 95%CI: 1.09-5.60, p=0.03) and solid tissue architecture (HR=2.89, 95%CI: 0.99-8.46, p=0.05). Patients with both CPS ≥10 and TROP2 positivity accounted for 47 individuals (37.6%) and showed a trend toward worse prognosis compared to CPS-negative and TROP2-negative patients (HR=3.17, 95%CI: 0.71-14.06, p=0.13).
Conclusions: In OCCC, 72% of patients exhibited TROP2 positivity, and 49.6% had CPS ≥10. Patients with CPS positivity tended to have worse prognosis, and those with both TROP2 and CPS positivity represented 37.6% of the cohort. These findings suggest that TROP2-ADC and combination immunotherapy may represent promising therapeutic strategies for this patient subgroup.

#2035 Clinical-immunological correlates in platinum resistant or refractory ovarian cancer patients treated with chimeric oncolytic adenovirus encoding TNFa and IL-2 (TILT-123) in combination with pembrolizumab.

James H. A. Clubb[1], Matthew Block[2], Johanna Maenpaa[3], Victor Arias[4], Mirte van der Heijden[4], Santeri Pakola[4], Dafne C. A. Quixabeira[1], Tatiana Kudling[4], Elise Jirovec[4], Lyna Haybout[1], Tuomo Alanko[3], Daniel Adamo[2], Susan Ramadan[3], Jorma Sormunen[3], Juha Kononen[3], Michael J. Chisamore[5], Julia W. Cohen[5], Nadena Singh[5], John Goldfinch[6], Suvi Sorsa[1], Riikka Havunen[1], Claudia Kistler[1], Joao M. Santos[1], Victor Cervera-Carrascon[1], Akseli Hemminki[1]

[1]TILT Biotherapeutics Oy, Helsinki, Finland,[2]Mayo clinic, Rochester, MN,[3]Docrates Cancer Center, Helsinki, Finland,[4]Translational Immunology Research Program, University of Helsinki, Helsinki, Finland,[5]Merck & Co., Inc, Rahway, NJ,[6]TILT Biotherapeutics Oy, Orchard Park, NY

Background: Ad5/3-E2F-D24-hTNFa-IRES-hIL-2 (TILT-123) is an oncolytic adenovirus armed with TNFa and IL-2. The cytokine pairing demonstrated superior tumor immunomodulation in preclinical studies. Subsequent studies supported evaluation of TILT-123 in PROTA, a phase 1 study of TILT-123 in combination with pembrolizumab in platinum resistant or refractory ovarian cancer patients. Biomarker analysis is ongoing.

Experimental procedures: In this phase 1a dose escalation trial (NCT05271318), 15 patients received an initial single intravenous dose of TILT-123 ranging from $3x10^{11}$ to $4x10^{12}$ VPs , followed by intratumoural and/or intraperitoneal injections ranging from $1x10^{11}$ to $5x10^{11}$ VPs. Patients also received i.v. pembrolizumab (200mg) every three weeks starting on day 36. The primary endpoint was safety by day 92, while efficacy by RECIST1.1 and overall survival (OS) were secondary endpoints. Collection of tumour biopsies and blood, including PBMCs and serum, enabled evaluation of MoA and identification of preliminary biomarkers.

Summary of data: ORR was 7.1% in evaluable patients (1/14). Disease control rate was 64% in evaluable patients (n = 14) and median OS was 190 days (n =15). Patients with disease control and no disease control by 92 achieved a median OS of 571 days and 138 days respectively. Correlations of complete blood count with differential to OS (n =15) and imaging response (n = 14) revealed a greater drop in lymphocyte count after treatment, as well as baseline lymphocyte count positively correlated with OS and disease control (PR/SD vs PD). Correlation of prothrombin INR to OS revealed patients with a slower blood-clotting rate at baseline and post-treatment positively correlated with OS. Analysis of anti-adenovirus neutralizing antibody (nAb) titer revealed 77% of all patients were positive for nAbs prior to treatment. All patients developed nAbs in blood and ascites (ascites n = 3). Patients presenting with nAbs at baseline achieved longer OS than those not positive. Additionally, a higher titer post-treatment positively correlated with OS and disease control. Percentage and fold change increase in IgM expression on plasma cells on day 36 positively correlated with OS. In addition, a higher expression of IgM on the surface of plasma cells at baseline was observed in patients achieving disease control.

Conclusion: These preliminary results identify a key role of circulating lymphocyte re-distribution and humoral immune response as markers of treatment benefit. Initial phenotyping implicates IgM producing plasma cells as a potentially relevant immune cell population. Additionally we observed a correlation between the coagulation cascade and OS.

#2036 Transcriptomic immune signatures and reconstructed immune cell types associated with pathological complete response to neoadjuvant chemotherapy in triple-negative breast cancer.

T. Oshino[1], T. Noguchi[1], N. Tomioka[2], K. Hatanaka[1], A. Evdokimova[3], P. Turova[3], V. Kushnarev[3], N. Kotlov[3], K. Chernyshov[3], Z. Polyakova[4], K. Kryukov[3], A. Makarova[3], M. Hosoda[1], S. Ariga[1], R. Moku[1], Y. Shiotsu[4], Y. Hatanaka[1], I. Kinoshita[1], M. Takahashi[1]; [1]Hokkaido University Hospital, Sapporo, Japan, [2]Hokkaido Cancer Center, Sapporo, Japan, [3]BostonGene, Waltham, MA, [4]BostonGene Japan, Tokyo, Japan

Background: Pathological complete response (pCR) following neoadjuvant chemotherapy (NAC) is positively correlated with long-term event-free survival and overall survival in patients with triple-negative breast cancer (TNBC). However, the molecular subtypes of TNBC associated with pCR remain to be elucidated.

Methods: We conducted a transcriptomic and clinical outcome analysis of 28 TNBC patients who underwent surgery following NAC at Hokkaido University Hospital and Hokkaido Cancer Center in Japan between 2017 and 2022. The research protocol was approved by the Internal Review Board at the medical institutes and BostonGene (023-0109). We used an internally developed algorithm (Breast Cancer Classifier, BCC) to classify tumors into 5 intrinsic subtypes: Basal, Luminal A (LumA), Luminal B (LumB), HER2 high, and HER2-low. Using methods described by Bagaev et. al. (*Cancer Cell, 2021*), we selected 21 functional gene expression signatures to identify tumor microenvironment (TME) subtypes. Next, we used deconvolution by Kassandra algorithm for precise reconstruction of TME. The Mann-Whitney U test and chi-square test with Benjamini-Hochberg correction were used for statistical analysis.

Results: Twelve patients achieved pCR, with a median age of 53 years, while 16 were non-pCR with a median age of 63 years. The clinical stages at diagnosis in the pCR group were stage I (n=3), stage II (n=7), and stage III (n=2), while the non-pCR group included stage II (n=7), and stage III (n=9). Anthracycline followed by taxane regimens were administered to 8 pCR and 13 non-pCR patients, and the remainder received investigational regimens as clinical trials. HER2 scores of 0, 1+ and 2+ were observed in 14, 5, and 9 patients, respectively. BCC revealed 54% (15/28) of patients had basal-like and 25% (7/28) had HER2-low TNBC. It also classified 12% (3/28) as having LumA and another 12% (3/28) as having LumB TNBC. The pCR rates across these subtypes were 53%, 43%, 33%, and 66%, respectively. Kassandra identified 4 TME subtypes: Immune-Enriched, Fibrotic (IE/F); Immune-Enriched, Non-Fibrotic (IE); Fibrotic (F); and Immune-Desert (D). Notably, 11 of 12 pCR patients exhibited the IE subtype, while the non-pCR group displayed a heterogeneous distribution of TME subtypes. Gene signatures of tertiary lymphoid structures were upregulated in pCR patients. Kassandra revealed increased infiltration of T cells, B cells, and NK cells in the TME of pCR patients, corroborated by the presence of lymphocyte clusters in hematoxylin and eosin staining.

Conclusion: Our study demonstrates that BostonGene's TME subtyping and Kassandra can accurately identify TNBC patients likely to achieve pCR after NAC. These findings warrant further prospective evaluation to establish the utility of these approaches in predicting treatment response in TNBC.

#2037 Early identification of long-term survivors on tremelimumab plus durvalumab and chemotherapy in POSEIDON from pre-treatment CT images.

Kedar Patwardhan[1], Harish RaviPrakash[1], Qin Li[2], Ross Stewart[3]

[1]Oncology Data Science, Oncology Research & Development, AstraZeneca, Waltham, MA,[2]Cancer Biomarker Development, Oncology Research & Development, AstraZeneca, Waltham, MA,[3]Translational Medicine, Oncology Research & Development, AstraZeneca, Cambridge, United Kingdom

Background: The combination of CTLA-4 and PD-(L)1 inhibitors either with or without chemotherapy is an approved treatment for metastatic non-small-cell lung cancer (mNSCLC). Identifying which patients are most likely to derive additional benefit from these regimens, as compared anti-PD-1 alone or in combination with chemotherapy (CTx) would help optimize clinical management of these patients.

Methods: Pre-treatment baseline Computed Tomography (CT) images of the thorax from patients treated with durvalumab plus tremelimulab (D+T; n=647) as part of the MYSTIC and NEPTUNE studies were split into a training (n=557) and a validation cohort (n=90). A 3D Deep Learning model with a *DenseNet* architecture was trained to predict patients with overall survival (OS) longer or shorter than the median (~1 year), and then optimized using the validation set. The final model (named RaDx) was then applied *de-novo* to the non-squamous mNSCLC cohort from POSEIDON.

Results: All reported hazard ratios (HR) are comparing RaDx+ and RaDx- groups unless specified otherwise. In the validation cohort, the median OS (mOS) in the RaDx+ (n=38) and the RaDx- (n=52) groups was 21 and 6.3 months respectively with HR=0.44 (0.27-0.73). In the anti-CTLA-4 containing arm of POSEIDON (D+T+CTx; n=187), the mOS for RaDx+ (n=77) and RaDx- (n=110) groups was 26.6 and 10.8 months respectively with HR = 0.44 (0.3-0.64). In the durvalumab and chemotherapy arm (D+CTx; n=182), the mOS for the RaDx+ (n=71) and RaDx- (n=111) groups was 17.2 and 11.2 months respectively with HR = 0.68 (0.48-0.97). In the CTx only arm (n=178), the mOS for the RaDx+ (n=73) and RaDx- (n=105) groups was 15.1 and 11.6 months respectively with HR = 0.82 (0.59-1.13). Within the RaDx+ sub-group a comparison of D+T+CTx to D+CTx patients favoured D+T+CTx with HR = 0.63 (0.42-0.96).

Conclusions: We demonstrated feasibility that a Deep Learning model using baseline CT images may be used to identify a sub-group of patients for whom the addition of tremelimumab to chemo-immunotherapy may bring additional benefit. Such a biomarker could have the potential to guide optimal treatment selection in this 1L mNSCLC population.

#2038 Antibody Drug Conjugates Treatment Response Score (ADC TRS) for predicting ADC treatment response.

S. P. Thomas[1], L. A. Habel[2], J. M. Suga[1], N. Achacoso[2], J. R. Nugent[2], C. Jiang[2], E. Chung[2], S. Bush[3], M. D. Nitchie[3], K. M. Robinson[3], J. Inman[3], R. White[3], K. Plouffe[3], D. R. Rhodes[3], S. A. Tomlins[3], B. Johnson[3];
[1]Kaiser Permanente, Vallejo, CA, [2]Kaiser Permanente Division of Research, Pleasanton, CA, [3]Strata Oncology, Ann Arbor, MI

Background: Predictive biomarkers for multiple ADCs/tumor types (beyond 3+ HER2 IHC) are lacking. We previously reported the discovery of ADC TRS—an algorithm combining individual ADC target expression, proliferation, and adhesion by RNAseq to predict ADC clinical benefit across tumor types/targets—and association with OS after ADC treatment (Abstract 3140, ASCO 2024). Here we evaluated the final ADC TRS biomarker (ADC-specific; tuned on >100 tumor type/ADC RRs) by a qRT-PCR based clinical trial assay (CTA) for predicting ADC clinical benefit.
Methods: Adults in the Kaiser Permanente Northern California (KPNC) system with advanced bladder or breast cancer in an observational trial (NCT03061305) who were treated with an approved ADC (enfortumab vedotin [EV], sacituzumab govitecan [SG] or trastuzumab deruxtecan [T-DXd]; first dose by Apr. 21, 2023) with valid CTA TRS results from FFPE tissue (collected prior to 1st ADC treatment) were eligible; patients with prior trastuzumab or hormonal (between tissue collection/ ADC start) treatment were excluded. KPNC oncologists (blinded to ADC TRS status) determined best response to therapy from chart review. ADC TRS status (+ vs. -) / response data were merged by the KPNC statistician and analyzed per a prespecified plan.
Results: A total of 117 KPNC patients with advanced bladder (n=58 [50%]) or breast (n=59 [50%]) cancer treated with an approved ADC (n= 56 [48%], 47 [40%], and 14 [12%] EV, SG, and T-DXd, respectively) were evaluable for TRS and RR. As shown in the Table, TRS+ status was associated with significantly higher RR and longer PFS and OS for approved ADC treatment; results were generally similar across ADCs and tumor type.
Conclusions: By a qRT-PCR based CTA, TRS+ status was associated with improved RR, PFS and OS for approved ADCs in an observational cohort of patients with advanced bladder or breast cancer. Prospective validation for approved and/or investigational ADCs is warranted.

Analysis	**TRS +**		**TRS-**		**Odds/Hazard Ratio**	**p-value[#]**
	n	**Outcome**	**n**	**Outcome**		
RR (95% CI)*	37	51% (34-68%)	68	32% (22-45%)	2.21 (0.95-5.14)	0.032
PFS (mo; 95% CI)^	37	6.5 (5.0-8.0)	68	3.5 (2.7-4.8)	0.64 (0.42-0.98)	0.02
OS (mo; 95% CI)^	40	15.5 (9.9-18.4)	77	8.3 (5.5-12.0)	0.51 (0.32-0.79)	0.0013
The primary objective evaluated whether ADC TRS status (Pos [+] vs. Neg. [-]) is associated with best response rate (RR) to ADC treatment ([partial response + complete response] / all patients) in those with at least one post ADC follow-up imaging. Exploratory analyses evaluated subgroups, progression free survival (PFS) and overall survival (OS).						
*Odds ratio and p value from logistic model with age, Charlson Comorbidity Index (CCI), and indication.						
^Cox proportional hazard ratios adjusted for age, CCI, and indication.						
[#]1-sided directional hypothesis test.						

#2039 High mesothelin expressing triple negative breast cancer is associated with enhanced cell proliferation but without impact on survival outcomes.

Takumi Sato[1], Brendan Hagerty[2], Rongrong Wu[3], Takashi Ishikawa[3], Kazuaki Takabe[4]

[1]The University of Tokyo Hospital, Tokyo, Japan,[2]Department of Surgical Oncology, Roswell Park Comprehensive Cancer Center, Buffalo, NY,[3]Department of Breast Surgery and Oncology, Tokyo Medical University, Tokyo, Japan,[4]Department of Surgery, University at Buffalo Jacobs School of Medicine and Biomedical Sciences, State University of New York, Buffalo, NY

Introduction: Mesothelin (MSLN) is a cell surface glycoprotein commonly expressed on several types of cancer including breast cancer (BC). Despite lacking an intracellular signaling domain or known physiologic function, MSLN is implicated in aggressive tumor behavior. Although known for high MSLN prevalence, its biological role in triple-negative breast cancer (TNBC) remains largely unexplored. We utilized multiple independent large patient cohorts with comprehensive gene expression profile and clinical variables to unravel the functional and clinical implications of MSLN expression in TNBC.

Methods: Clinical variables and transcriptome of a total of 628 TNBC patients were gathered from three independent cohorts: The Cancer Genome Atlas (TCGA, n=160), the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC, n=313), and The Sweden Cancerome Analysis Network - Breast (SCAN-B, GSE96058, n=155). Four neoadjuvant chemotherapy (NAC) cohorts; GSE32603, GSE25066, GSE163882, and GSE123845 were also analyzed.

Results: Using single-cell sequence cohorts, SCP1039 and SCP1106, we found that MSLN is exclusively expressed in cancer epithelial cells, and its expression is associated with less stromal cells (adipocytes and fibroblasts) in the TNBC tumor microenvironment. High expression of MSLN was associated with the TNBC subtype and advanced lymph node metastasis in BC of TCGA, METABRIC, and SCAN-B cohorts (all $p < 0.001$). Unexpectedly, MSLN expression was not associated with Disease-Free, Disease-Specific, nor Overall Survival in any of the TNBC cohorts analyzed (all $p > 0.05$). MSLN expression was significantly higher in TNBC that achieved pathological complete response after neoadjuvant chemotherapy in the GSE25066 cohort ($p < 0.001$), although there was no survival difference by MSLN expression in this cohort, either. Consistent with its immunological role, MSLN-high tumors exhibited a pro-inflammatory microenvironment, including higher Cancer-Testis Antigen (CTA) scores and increased activity of immune cells, notably immature dendritic cells (iDCs) and M1 macrophages (all $p < 0.05$). Biologically, MSLN expression was linked to elevated cellular proliferation, as evidenced by correlation with Ki67 gene expressions and histological grade consistently in TCGA, METABRIC and SCAN-B. Hallmark cell proliferation-related gene sets, G2M checkpoint, E2F targets, and Mitotic Spindle were significantly enriched in MSLN-high TNBC in METABRIC and SCAN-B (all FDR < 0.25).

Conclusion: MSLN-high TNBC is characterized by enhanced cell proliferation and a pro-inflammatory microenvironment, showing better response to neoadjuvant chemotherapy without significant
impact on long-term survival outcomes. These data may be useful for future studies in MSLN-targeted therapies.

#2040 βAlt score provides independent and significant clinical value in multiple tumor types.

Mary Helen Barcellos-Hoff[1], Hang Chang[2]

[1]UCSF - University of California San Francisco, Berkeley, CA,[2]Lawrence Berkeley National Lab, Berkeley, CA

We reported that a functional relationship between loss of TGFβ signaling and gain of error-prone alternative end-joining DNA repair in cancer cells is evident in RNA sequencing data as a negative correlation between the transcriptomic signatures of TGFβ targets and genes necessary for alternative end-joining DNA repair. A score, βAlt, was designed to represent this relationship, and was shown to predict response to genotoxic chemotherapy and radiotherapy in multiple cancers. High βAlt, indicative of loss of TGFβ signaling competency and gain of error-prone repair, is associated with better response to genotoxic therapy (DOI;10.1126/scitranslmed.abc4465). As previously reported (DOI: 10.1158/1078-0432.ccr-21-2846), we used quartiles to analyze human papilloma virus (HPV)-positive versus HPV-negative head and neck squamous cell carcinoma (HNSCC) using specimens from TCGA and the HPV-negative HNSCC GSE41613 data. The βAlt score predicted response in HNSCC TCGA specimens (p=0.0015) and HPV-negative HNSCC GSE41613 patients (p=0.0033). To demonstrate the independent and significant clinical value of βAlt score and to justify the potential clinical utility of βAlt, we optimized the cut-off of βAlt score. We defined the maximized overall survival difference between patient stratification (survminer package in R, version 0.4.9), and thereafter partitioned patients in each cohort of study into βAlt Low and βAlt High groups. We determined that the value of βAlt score was independent from other clinical factors revealed by multivariate Cox-proportional hazard regression analysis (survival package in R, version 3.3.1) in all cohorts: HPV-positive HNSCC TCGA, p=0.006, HR 5.586 (1.655, 18.851); HPV-negative HNSCC TCGA, p=0.029, HR 1.654 (1.052, 2.601); HPV-negative HNSCC GSE41613 patients, p=0.019, HR 2.068 (1.125, 3.803). The C-Index predictive power calculated using a bootstrapping strategy at an 80% sampling rate with 1,000 iterations of βAlt combined with these clinical factors was significantly improved (p<2.22e-16). We also analyzed βAlt using the TCGA glioblastoma (GBM) cohort. High βAlt in GBM also predicts longer survival (p<0.0001), remains significant after adjusting for important clinical factors, i.e., stage, age, and gender (p<0.001), and significantly improves C-Index predictive power of overall survival (p<2.22e-16). These data underscore that loss of TGFβ signaling, which underlies the marked sensitivity of HPV-positive HNSCC to standard of care chemoradiation, is predictive in non-HPV associated cancers. These results provide robust support that βAlt is a high-quality biomarker for the precision management of cancer patients.

#2041 Microsatellite instability as a biomarker for predicting chemoradiotherapy response in rectal cancer: insights from pathological and radiomic analyses.

Euna Jeong[1], **Hyowon Hong**[2], Jaemin Jo[3], Shin Kim[4], SangJun Byun[5], Incheol Seo[6], Woon Kyung Jeong[7], Seong Kyu Baek[7], Hye Won Lee[3]

[1]Department of anatomy, Keimyung University School of Medicine, Daegu, Korea, Republic of,[2]Research Affairs, Keimyung University Dongsan Medical Center, Daegu, Korea, Republic of,[3]Department of Pathology, Keimyung University School of Medicine, Daegu, Korea, Republic of,[4]Department of Immunology, Keimyung University School of Medicine, Daegu, Korea, Republic of,[5]Department of Radiation Oncology, Keimyung University School of Medicine, Daegu, Korea, Republic of,[6]Department of Immunology, Kyungpook National University School of Medicine, Daegu, Korea, Republic of,[7]Department of Surgery, Keimyung University Dongsan Hospital, Daegu, Korea, Republic of

Background: Colorectal cancer (CRC) is one of the common causes of cancer-related deaths around the world. Rectal cancer accounts for approximately 30% of all colon cancers, half of which are diagnosed as locally advanced rectal cancer. Microsatellite instability (MSI) is a key biomarker in colorectal cancer (CRC), impacting immunotherapy outcomes and potentially influencing chemoradiotherapy (CRT) responses. In this study, we investigate the correlation between treatment response to CCRT according to MSI status.
Methods: Tissue specimens were obtained by preoperative biopsy from 87 patients with rectal adenocarcinoma who underwent CRT. Patients received fluorouracil-based long-course neoadjuvant therapy. MSI analysis was performed on DNA extracted from tumors and matched normal tissue. The extracted DNA was tested by PCR targeting five microsatellite loci: BAT25, BAT26, D5S346, D2S123, and D17S250. Clinical and pathological parameters, including lymphovascular invasion and T/N stage, were compared between CRT responders and non-responders. In vitro, 3 MSI-H and 6 MSS colorectal cancer cell lines underwent gradient radiation exposure. Radiosensitivity was evaluated through XTT cell viability assays and apoptotic rates quantified via flow cytometry (FACS).
Results: Preoperative MSI status was observed divided into MSI (MSI-H+MSI-L) and MSS. In the responder group (n=27), there were 5 people (18.52%) with MSI and 22 people (81.48%) with MSS, and in the non-responder group (n=60), 0 (0.0%) MSI and 60 (100%) MSS were observed (P = <0.005). Additionally, there were significant differences in ypT stage (P<0.001), ypN stage (P=0.003), lymphovascular (P=0.043) and perineural invasion (P=0.035). MSI-H cell lines demonstrated greater radiosensitivity, with significantly reduced viability in XTT assays and higher apoptosis rates in FACS analysis compared to MSS cell lines.
Conclusion: Our findings suggest that MSI status is a significant predictor of response to CCRT, with patients exhibiting MSI tumors showing a higher likelihood of treatment response compared to those with MSS tumors. Enhanced radiosensitivity in MSI-H cell lines further supports the predictive value of MSI testing. Future studies should confirm these findings in larger cohorts.

#2042 ROS1 single nucleotide variants predict favorable survival outcomes on immunotherapy regimens in non-small cell lung cancer.

Yue Wu[1], Jaclyn LoPiccolo[2], Sebastian Carrasco Pro[3], Krishna C. Bulusu[1], Etai Jacob[1], Alexander Gusev[2], Gerald J. Sun[1]

[1]AstraZeneca, Waltham, MA,[2]Medical Oncology, Dana-Farber Cancer Institute, Boston, MA,[3]Tempus AI, Inc, Chicago, IL

Background: Only a fraction of non-small cell lung cancer patients who receive immune checkpoint inhibitor (ICI) treatment will respond, largely because current FDA-approved biomarkers still leave room for improvement to identify more responders. We hypothesized that modeling the time-dependent interaction between treatment and tumor genetics may reveal novel associations. We further hypothesized that real-world data (RWD), as opposed to clinical trial data, may contain these associations, as RWD possesses a potentially more diverse patient population and time-to-event treatment history across the diverse regimens used in routine clinical practice.

Methods: We examined the relation between tumor genetics, treatment, and real-world progression-free survival (PFS) in two RWD cohorts (Tempus discovery cohort, n = 1458; Dana Farber Cancer Institute Profile independent validation cohort, n = 466) by utilizing a Cox proportional hazards model with a time-dependent covariate for whether a patient was receiving an ICI-containing treatment regimen. Specifically, we included an indicator variable that denoted whether an individual was receiving an ICI regimen (=1) or not (=0), and enumerated the time intervals associated with each treatment status. We performed a univariate analysis for each mutated gene from tumor sequencing, and included a gene-treatment interaction term to permit identification of genes that were predictive (i.e. specific to a treatment) rather than prognostic. Our model included additional covariates to control for known predictive (tumor mutation burden [TMB]) and prognostic factors (age, sex).

Results: In the discovery cohort, we identified a significant association between ROS1 single nucleotide variants (SNV; prevalence = 5%) and favorable PFS on ICI therapy (interaction HR = 0.34, 95% CI = 0.18-0.64, p = 7.8e-4, q = 0.08) versus non-ICI treatment. This association was not explained by ROS1 rearrangement or ROS1 TKI therapy. ROS1 SNV associated with worse PFS when not on ICI treatment (marginal HR = 2.3, 95% CI = 1.4-3.6, p = 4e-4, q = 0.044). This ROS1 SNV association was replicated in the validation cohort, which exhibited a favorable interaction with ICI treatment (interaction HR = 0.11, 95% CI = 0.04-0.33, p = 7.46e-5), and poor PFS on non-ICI treatment (marginal HR = 5.39, 95% CI = 2.5-11.6, p = 1.96e-5). ROS1 SNV treatment interaction effect was also not explained by TMB or PD-L1 status. Finally, we observed the ROS1 SNV ICI-specific protective effect was specific to patients ICI-treated in the first line setting.

Conclusions: We identified an association between ROS1 SNVs and favorable PFS specific to ICI treatment, independent of common pre-existing predictive biomarkers for ICIs. Because this association was specific to the first line ICI setting, our finding may clarify ICI usage where clinical practice for ICI treatment is still evolving.

#2043 Circulating tumor DNA (ctDNA) mutation analysis at baseline (BSL) and end of treatment (EOT) with sacituzumab govitecan (SG) and clinical impact on efficacy in patients (pts) with HR+/HER2- metastatic breast cancer (mBC): Biomarker results from TROPiCS-02.

Hope S. Rugo[1], Yi Zhang[2], Shan Tang[2], Frederik Marme[3], Peter Schmid[4], Javier Cortes[5], Sara M. Tolaney[6], Oh Kyu Yoon[2], Wendy Verret[2], Meghna Das Thakur[2], Aditya Bardia[7]

[1]UCSF Helen Diller Family Comprehensive Cancer Ctr., San Francisco, CA,[2]Gilead Sciences, Inc, Foster City, CA,[3]Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany,[4]Centre for Experimental Cancer Medicine, Barts Cancer Institute, Queen Mary University of London, London, United Kingdom,[5]International Breast Cancer Center (IBCC), Pangaea Oncology, Quironsalud Group, Barcelona, Spain,[6]Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA,[7]David Geffen School of Medicine, University of California, Los Angeles, Jonsson Comprehensive Cancer Center, Los Angeles, CA

Background: SG is approved in pretreated HR+/HER2- mBC based on the TROPiCS-02 study (NCT03901339). Mutations in specific genes may affect efficacy of SG, including *PIK3CA*/*TP53* (common in BC, may impact outcomes) and *TACSTD2*/*TOP1* (may confer resistance to Trop-2-directed ADCs like SG). We assessed specific mutations at BSL and EOT in TROPiCS-02 to identify genes that may be involved in acquired SG resistance.

Methods: Randomized pts received SG or chemotherapy treatment of physician's choice (TPC). Plasma samples were collected at BSL and EOT, and ctDNA was analyzed using Guardant Health's Infinity RUO ctDNA panel. Specific genes were analyzed to determine association with clinical outcomes (progression-free survival [PFS] and overall survival [OS]). Unbiased analysis at EOT was used to identify genes associated with SG resistance.

Results: Pt characteristics, PFS, and OS were similar between the intent-to-treat (ITT; N=543) and ctDNA (46% of ITT) populations. PFS and OS benefit was observed with SG vs TPC regardless of *PIK3CA* or *TP53* mutation status (Table). At BSL and EOT, no *TACSTD2* mutations were observed in the SG arm. At BSL, 3 *TOP1* mutations (I435V, D122H, M319I) were detected in 2 pts, and at EOT, 1 new *TOP1* mutation (G717V) was detected in the SG arm (184 days treatment). At BSL 64% of pts had ≥1 deleterious variant in the 69-gene DDR panel. No clear expansion or clearance of individual DDR genes or variants was observed at EOT.

Conclusions: Survival benefit was observed with SG vs TPC in pts with and without *PIK3CA* and *TP53* mutations, similar to the ITT population. Our matched BSL to EOT analyses did not identify any genes that were clear drivers of SG resistance in pretreated HR+/HER2- mBC. Future analyses would benefit from paired biopsies at BSL and at disease progression to identify potential drivers of SG resistance.

Median survival, Mo	**ITT**		**ctDNA**		***PIK3CA* WT**		***PIK3CA* Mut**		***TP53* WT**		***TP53* Mut**	
	SG (n = 272)	**TPC (n = 271)**	**SG (n = 132)**	**TPC (n = 118)**	**SG (n = 84)**	**TPC (n = 76)**	**SG (n = 47)**	**TPC (n = 41)**	**SG (n = 67)**	**TPC (n = 59)**	**SG (n = 64)**	**TPC (n = 58)**
PFS	5.5	4.0	5.3	4.2	5.5	4.2	4.3	4.3	4.4	4.3	5.5	3.1
95% CI	4.2-7.0	3.1-4.4	4.1-6.9	3.0-5.6	4.1-7.1	2.8-5.5	2.7-7.2	2.1-5.8	4.0-7.2	3.4-5.6	3.7-7.1	1.6-5.6
HR	0.66		0.71		0.69		0.71		0.77		0.65	
95% CI	0.53-0.83		0.52-0.96		0.47-1.00		0.42-1.20		0.50-1.19		0.42-0.99	
OS	14.4	11.2	15.0	12.4	15.0	12.2	15.4	12.8	16.0	11.9	14.5	12.7
95% CI	13.0-15.7	10.1-12.7	12.3-17.5	10.7-14.9	12.7-17.5	10.0-15.2	10.9-20.8	11.0-17.5	12.3-20.8	10.1-20.1	10.5-17.5	10.0-15.2
HR	0.79		0.89		0.87		0.84		0.87		0.82	
95% CI	0.65-0.96		0.66-1.19		0.60-1.26		0.52-1.36		0.56-1.34		0.55-1.24	

ctDNA, circulating tumor DNA; HR, hazard ratio; ITT, intent-to-treat; mo, months; Mut, mutant; OS, overall survival; PFS, progression-free survival; SG, sacituzumab govitecan; TPC, treatment of physician's choice; WT, wild type.

#2044 High TLR3 transcript levels correlate with high TIM3 and VISTA checkpoints, but not with outcome after immune checkpoint blockade.

Marvin Hsieh[1], Daisuke Nishizaki[2], Taylor J. Jensen[3], Sarabjot Pabla[3], Jeffrey M. Conroy[3], Paul DePietro[3], Shumei Kato[2], Razelle Kurzrock[1]

[1]Medical College of Wisconsin, Milwaukee, WI,[2]University of California, San Diego, La Jolla, CA,[3]Labcorp, Burlington, NC

Introduction: The Nobel Prize-winning discovery of Toll-like Receptors (TLR) in 2011 sparked the exploration of TLR3 as a key target in cancer pathogenesis and immunotherapy treatment. Upon activation by double-stranded RNAs, TLR3 stimulates the production of cytokines and chemokines that augment inflammation and cell death, which are risk-factors in several cancers including colorectal cancer (CRC). Despite the growing interest in TLR3, its specific role in influencing patient outcomes and interactions with other immune checkpoints remains inadequately understood. In this study, we evaluated the association between TLR3 RNA expression levels and clinical outcomes to determine its potential role as a biomarker in cancer prognosis and immune checkpoint inhibitor (ICI) treatment response.

Methods: RNA expression levels of TLR3 and other immune checkpoints were evaluated in 514 tumors (489 patients with full clinical outcome annotation) in a clinical-grade laboratory and categorized as “High” (75-100 percentile) or “Not-High” (0-74 percentile). Univariate logistic regression assessed the association between high TLR3 expression, patient characteristics, immune markers, and cancer subtypes, followed by multivariable logistic regression for variables with $p \leq 0.05$. Survival analyses was conducted through the Kaplan-Meier method on patients with available survival data. Overall survival (OS) and progression-free survival (PFS) were stratified by TLR3 expression (“High” vs “Not-High”) and compared using the log-rank test.

Results: High expression of TLR3 RNA was seen in 90/514 (17%) among all patients and 11/140 (8%) in CRC patients. The highest proportion of high TLR3 expressors were seen in liver and bile duct cancers (7/19, 36.8%), pancreatic cancer (16/55, 29.1%), and ovarian cancer (12/43, 27.9%). High TLR3 RNA expression was significantly correlated with high VISTA (OR = 2.12, p = 0.004) and TIM3 (OR = 2.10, p = 0.026) RNA levels, and was lower in CRC (OR = 0.39, p = 0.008). CRC was the only cancer subtype in logistic regression analyses with a statistically significant correlation with high TLR3 RNA expression. In survival analyses of all cancer patients and CRC patients, high TLR3 RNA expression did not significantly correlate with OS and PFS, regardless of ICI treatment status.

Conclusion: High TLR3 RNA expression was associated with higher levels of VISTA and TIM3 RNA; CRC had lower TLR3 levels than other cancers. However, TLR3 expression was not a statistically significant predictive biomarker for better outcomes (OS and PFS) in cancer patients, regardless of ICI treatment.

#2045 High-dose intervention with atorvastatin affects serum micro-RNAs in men with localized prostate cancer - an exploratory sub-analysis of ESTO1 placebo-controlled clinical trial.

Reetta Natkin[1], Tiina Jokela[2], Aino Siltari[1], Tia-Marje K. Korhonen[2], Eija Laakkonen[2], **Teemu J. Murtola**[1]

[1]University of Tampere, Tampere, Finland,[2]University of Jyvaskyla, Jyvaskyla, Finland

Introduction: Prostate cancer (PCa) is associated with increased cholesterol production, and statins, which inhibit the mevalonate pathway, can reduce PCa cell proliferation. This pathway regulates key cellular processes, including inflammation and growth. Previous trials have shown that statins lower PCa growth in men with localized PCa. Thus, further understanding the effect of statin intervention on PCa is crucial. MicroRNAs (miRNAs) are involved in the post-translational regulation of gene expression and have been linked to PCa risk and outcomes. This study aims to assess the impact of atorvastatin on the serum miRNA profile in men with localized PCa.
Materials & Methods: The ESTO1 trial enrolled 158 men with localized PCa who were scheduled for radical prostatectomy. Participants were randomly assigned to receive either 80 mg of atorvastatin or a placebo daily for 21 days before surgery. Serum samples were collected at baseline and the day before surgery. This exploratory sub-analysis included samples from 48 men, representing both high-grade (ISUP grade 3 or higher) and low-grade (ISUP 1-2) cases, with both atorvastatin and placebo groups. RNA was extracted from serum samples, pooled by trial arm and ISUP grade, and analyzed using small RNA sequencing on Illumina equipment. Differential expression was analyzed using DESeq2 in R Studio. MiRNAs showing the most significant changes were confirmed via dPCR analysis in individual samples.
Results: In pooled samples, the expression of several miRNAs shifted during atorvastatin treatment in comparison to the placebo group. Most of these miRNAs have been previously linked to PCa, including miRNA-205-5p, miRNA-21-3p, and miRNA-210-3p. Individual sample analysis confirmed atorvastatin-induced upregulation of miRNA-205-5p in high-grade cases ($p = 0.03$). Atorvastatin also altered the expression of certain miRNAs that have not been previously studied in the context of PCa, such as miRNA-10b-3p, miRNA-2355-3p, and miRNA-502-3p.
Conclusions: Atorvastatin treatment induced expression changes in several miRNAs, particularly increasing miRNA-205-5p in high-grade cases, which was confirmed through individual sample analysis. This suggests that atorvastatin's tumor growth-inhibiting effects in PCa may be partially mediated by its influence on miRNA expression and epigenetic regulation.

#2046 Coefficient variation of glycosylphosphatidylinositol-anchored 80 kDa protein (GPI-80) on neutrophil status in metastatic renal cell carcinoma (MRCC) patients treated with combination therapy of nivolumab plus ipilimumab.

Sei Naito[1], Yuji Takeda[2], Sabrina Saima[2], Hiromi Ito[1], Tomoyuki Kato[1], Takafumi Narisawa[1], Yuki Takai[1], Shinta Suenaga[1], Mayu Yagi[1], Hisanobu Asao[1], Norihiko Tsuchiya[1]

[1]Urology, Yamagata University, Yamagata, Japan,[2]Immunology, Yamagata University, Yamagata, Japan

Introduction: Immature neutrophils suppress T cell immunity as myeloid-derived suppressor cells. However, recent studies have demonstrated that neutrophils are essential for eradicating antigenically heterogenous tumors via T cell immunotherapies, and CTLA-4 antibodies can recruit neutrophils into tumors. Our previous research identified GPI-80 as a marker of neutrophil maturation, where the coefficient variation of GPI-80 (GPI-80 CV) inversely correlated with the T cell proliferation. We also demonstrated that MRCC patients with high GPI-80 CV had a poor prognosis before the advent of immune-checkpoint inhibitors (ICI). The aim of this study is to investigate the association between GPI-80 CV and the inflammatory marker C-reactive protein (CRP) status and prognosis in mRCC patients treated with the combination therapy of anti-PD-1 nivolumab plus anti-CTLA-4 ipilimumab (NI). Methods: Prior to ICI treatment, peripheral blood samples were collected from 25 patients treated with NI and 10 patients treated with nivolumab monotherapy. GPI-80 was measured using flow cytometry. NI response was categorized as follows: EPD (early progressive disease; disease progressed within 60 days), LPD (late progressive disease; disease progressed between 60 days and 2 years), DR (durable response; disease did not progress beyond 2 years). Results: In NI, GPI-80 CV was significantly higher in durable responder DR patients (P = 0.03, Steel-Dwass test) and marginally higher in EPD patients (P = 0.08) compared to LPD patients. Serum CRP was significantly higher in EPD compared to DR (P = 0.03). When patients were divided into groups 1-3 according to GPI-80 CV and CRP levels: group 1, >10 mg/dl CRP; group 2, <10 mg/dl CRP and <36 GPI-80 CV; group 3, <10 mg/dl CRP and >36 GPI-80 CV. Group 3 had significantly better time-to-treatment-failure (TTF) and overall survival (OS) compared to Group 1 (HR = 0.36 and 95% CI = 0.17-0.73 in TTF, HR = 0.29 and 95% CI = 0.12-0.67 in OS) and Group 2 (HR = 0.15 and 95% CI = 0.03-0.62 in TTF, HR = 0.18 and 95% CI = 0.03-0.93 in OS). In nivolumab monotherapy, TTF OS did not differ between these groups (HR = 1.85 and 95% CI = 0.41-8.16 in group 3 vs. group 2). Conclusions: In patients receiving NI therapy, high GPI-80 CV—a marker of immature neutrophils—was associated with improved TTF and OS. This association was not observed with nivolumab monotherapy, suggesting a unique role for neutrophil immaturity in the efficacy of combination immunotherapy including CTLA-4 blockade. The combination of GPI-80 and CRP is a candidate for predictive biomarker for NI efficacy in MRCC.

#2047 Homologous recombination deficiency in lung cancer in the INSPIRE study.

Katherine G. Roth[1], Kevin J. McDonnell[1], Ernest Nadal[2], Joseph D. Bonner[1], Arman Seuylemezian[1], Suravi Nahar[1], Lawrence Shaktah[3], Jonathan Salazar[1], Sidney Lindsey[1], Xiaoyu Xia[1], Sara A. Byron[4], Jonathan J. Keats[4], Allen Mao[1], Adrien Larsen[1], Bryce Turner[4], Ravi Salgia[1], Stacy W. Gray[1], Stephen B. Gruber[1]

[1]City of Hope National Medical Center, Duarte, CA,[2]Catalan Institute of Oncology, Barcelona, Spain,[3]Technical University of Dresden, Dresden, Germany,[4]Translational Genomics Research Institute, Phoenix, AZ

Introduction: Evaluating genomic signatures may improve prognostic information and outcomes for patients with lung cancer.
Objective: To determine if homologous recombination deficiency (HRD) is associated with clinical features or overall survival in patients with lung cancer.
Methods: This is a retrospective cohort study derived from City of Hope's Implementing Next-Generation Sequencing for Precision Intervention and Risk Evaluation (INSPIRE) study. Study participants with histologically confirmed lung cancer and adequate tissue for comprehensive paired tumor-normal whole exome sequencing were eligible for analysis. 197 patients were sequenced, excluding 14 with rare histologies. Clinical and treatment variables were obtained from cancer registries and the electronic medical record and were recorded by experienced data curation and abstraction specialists. HRD scores were calculated using the scarHRD R package. Mutational signatures were analyzed using SigProfiler and SigEstimation. Associations between clinical variables and HRD status were measured using univariate and multivariate methods. Unsupervised analysis was performed to identify genes in HRD-high (sum score greater than or equal to 42) and HRD-low samples (sum score less than 42). Overall survival was quantified using non-parametric Kaplan-Meier statistics and multivariate Cox-proportional hazards models.
Results: Of the 183 lung cancer samples analyzed, 63 (34.4%) samples were HRD-high. Of these, 43 had a *TP53* alteration and 20 did not; 120 (65.6%) samples were HRD-low, 50 with a *TP53* alteration and 70 without (OR 3.01 (95%CI 1.58, 5.72), p=0.0008).
Conclusion: *TP53* alterations were strongly associated with high HRD scores. Overall survival was improved in patients with HRD-high tumors treated with platinum therapy, although this difference did not reach statistical significance.

#2048 Extracellular vesicle-derived MASP2 as a prognostic indicator in pancreatic cancer.

Shatovisha Dey[1], Deep Pandya[1], Tammy Lo[2], Ryan Narbutas[3], Bhavna Khandpur[4], Pramila Krumholtz[2], Mohammadreza Shervinrad[4], Kiyoe Sullivan[4], Deborah August[4], Sarah Evans[2], Saraswathi Nair[4], Nader Okby[5], Gregory Niland[4], Richard C. Frank[2]

[1]Rudy Ruggles Biomedical Research Institute, Nuvance Health, Danbury, CT,[2]Department of Medicine, Nuvance Health, Norwalk, CT,[3]Department of Radiology, Nuvance Health, Norwalk, CT,[4]Department of Pathology, Nuvance Health, Norwalk, CT,[5]Department of Pathology, Nuvance Health, Danbury, CT

Advanced pancreatic cancer (APC) has an overall survival (OS) of 10 months with chemotherapy, yet some patients live only a few weeks whereas others up to two years. Novel early biomarkers are needed to guide prognostication and first-line chemotherapy efficacy, prior to tumor progression or treatment toxicity, which often preclude further therapy. In this study, serially collected serum extracellular vesicles (EVs) from chemotherapy-treated advanced pancreatic cancer (APC) patients were analyzed using mass spectrometry at three critical timepoints: pre-treatment, best response, and treatment resistance. Differentially expressed candidate proteins were identified, validated by ELISA, and correlated with treatment response and prognosis. Functional association studies were conducted using in-vitro assays with primary and metastatic pancreatic cancer (PC) cell lines, while immunohistochemistry (IHC) on tumor biopsies was performed for protein localization. Mass spectrometry analysis identified nine candidate proteins, with MASP2 emerging as the most promising candidate for further investigation. A >20% decline in EV-MASP2 levels after 2 months (M2) of chemotherapy predicted treatment response in 80% of responders (n=25), whereas a >20% increase predicted treatment resistance in 73% of non-responders (n=15). Analysis of a larger cohort (n=75) showed that a decrease vs increase at M2 was prognostic for EV-MASP2 (OS 11 vs. 8 months; p=0.0037) but not for CA19-9 (OS 11 vs. 12 months respectively, p=0.70). In situations where CA19-9 is not evaluable, EV-MASP2 retained prognostic significance. In-vitro studies indicate that systemic changes in EV-MASP2 levels are not directly driven by primary or metastatic PC cells, consistent with IHC staining patterns showing minimal or no MASP2 expression in tumor cells and moderate expression in stromal regions of both primary tumors and liver metastatic sites. In conclusion, our work indicates that serial analysis of serum EV proteins is a promising approach to identifying novel biomarkers for treatment response and prognosis in APC. Findings from this study suggest that changes in circulating EV-MASP2 levels soon after chemotherapy initiation may help guide treatment decisions and improve patient outcomes.

#2049 The 27-gene IO Score on the Ion Torrent Platform for classification of the tumor immune microenvironment.

Tyler J. Nielsen, Jim Fee, Geoff Bien, Brock Schweitzer

Oncocyte Inc., Nashville, TN

Background: The tumor immune microenvironment (TIME) plays a critical role in modulating cancer progression and therapeutic response, especially for immune checkpoint inhibitors (ICIs). DetermaIO, a 27-gene signature assay, has demonstrated clinical utility in predicting immune checkpoint inhibitor responsiveness through classification of the TIME. This study focuses on adapting DetermaIO to the Ion Torrent platform as a research-use-only (RUO) kit, OncoTIME. This assay enables precise and reproducible gene expression profiling of the immune system, tumor biology, and surrounding stromal biology in solid tumors, using this to derive the OncoTIME Score.
Method: OncoTIME was designed as a multiplexed targeted RNA sequencing assay using Ion AmpliSeq technology to quantify the expression of 27 immune-related genes and 5 housekeeping (HK) genes. Target regions for the 27-gene panel guided by prior design of the DetermaIO assay. HK genes were identified through a composite approach, combining three methodologies:

1. Expression Stability: Evaluated consistency across tissue types and tumor states using datasets from The Cancer Genome Atlas (TCGA) and internal NGS datasets.
2. Variance Filtering: Selected genes with minimal inter-sample variance within experimental datasets.
3. Biological Context: Ensured HK genes were biologically non-variable and minimally involved in immune-related pathways.

These methods ensured robustness of normalization across diverse sample types and RNA input qualities. The assay design used Thermo Fisher's Ion Torrent GeneStudio platform, leveraging its efficient library preparation and sequencing workflow.
Results: The OncoTIME panel demonstrated:

- High specificity and coverage of target regions for all 27 immune-related genes.
- Reproducibility assessed across intra- and inter-run precision studies.
- Consistent normalization using the 5 HK genes, enabling accurate quantification of immune signatures across a dynamic range of tumor microenvironment profiles. Preliminary studies confirmed that OncoTIME recapitulates the DetermaIO Score.

Conclusion: OncoTIME democratizes access to TIME classification for research use and provides a scalable, reproducible, and cost-effective assay for the Ion Torrent platform. This RUO kit recapitulates DetermaIO while enabling broader utility in characterizing the TIME and its potential biological associations.

#2050 The impact of cell cycle inhibitors on bladder cancer with low NECTIN-4 expression.

Jee Soo Park, Ji Eun Lee, Myung Eun Lee, Jongchan Kim, Won Sik Ham

Yonsei University College of Medicine, Seoul, Korea, Republic of

Background: Antibody-drug conjugates (ADC) combine a cytotoxic agent with a monoclonal antibody for targeted delivery. Enfortumab-vedotin (EV) links a monoclonal antibody targeting NECTIN-4 with the cytotoxic agent monomethyl auristatin E (MMAE). Given the near-ubiquitous expression of NECTIN-4 in urothelial cancer (UC) tissues, EV has shown efficacy in UC and is approved for metastatic urothelial carcinoma (mUC). However, significant heterogeneity in NECTIN-4 expression within UC limits therapeutic efficacy in cases with low NECTIN-4 expression. This study evaluated cell cycle inhibitors' efficacy in UC cells with low NECTIN-4 expression.
Methods: We measured NECTIN-4 and cell cycle-related molecules (Cyclin D1 and PLK1) in bladder cancer cell lines (253J, 253J-BV, 5637, RT4, T24, J82, HT1197, HT1376) and correlated these levels with sensitivity to the PLK1 inhibitor. Drug sensitivity was assessed by a CCK-8 assay over 72 hours following treatment with the PLK1 inhibitor (onvansertib). An ex vivo model using bladder tumor samples from patients validated the in vitro findings. NECTIN-4 levels in bladder cancer cell lines (HT1376 and RT4) were modulated by siNECTIN-4 transfection for 48 hours. Cell cycle analysis and the expression levels of cyclin D1 and PLK1 were examined in NECTIN-4 knockdown cell lines.
Results: The therapeutic efficacy of the PLK1 inhibitor was significantly enhanced in bladder cancer cell lines with reduced NECTIN-4 expression (253J, 253J-BV, 5637, T24, and J82), as indicated by an IC50 <0.1 μM. In contrast, cell lines with high NECTIN-4 expression (RT4, HT1197, and HT1376) were resistant to onvansertib (IC50 >1 μM). NECTIN-4 expression correlated with cell cycle-related molecule levels. Consistently, bladder tumor samples showed a negative correlation between NECTIN-4 expression and PLK1 inhibitor sensitivity. PLK1 inhibitor-resistant tumors (IC50 >40 μM) had lower NECTIN-4 levels than sensitive samples (20 μM < IC50 < 40 μM). NECTIN-4 knockdown via siRNA increased G0/G1 phase cells and reduced cyclin D1 and PLK1 levels. Furthermore, siNECTIN-4-transfected cells were more sensitive to the PLK1 inhibitor than control siRNA-transfected cells.
Conclusion: This study underscores the therapeutic potential of cell cycle inhibitors, notably PLK1 inhibitors, in bladder cancer cells with low NECTIN-4 expression, showing an inverse relationship between NECTIN-4 expression and sensitivity to PLK1 inhibitors. NECTIN-4 levels could predict PLK1 inhibitor treatment response. NECTIN-4 knockdown experiments confirmed that reduced NECTIN-4 expression disrupted cell cycle regulation and increased PLK1 inhibition efficacy. These findings advocate for further investigation of combination therapies involving ADCs and cell cycle inhibitors to counter resistance and improve outcomes in bladder cancer patients.

#2051 Baseline and adaptive activated kinome profiling identifies PTK6 as a mediator of resistance to vemurafenib and dabrafenib but not encorafenib in *BRAFV600*-mutant metastatic melanoma.

Steve P. Angus[1], Naim U. Rashid[2], Andrew J. Walther[2], David L. Corcoran[3], Steven D. Rhodes[4], Frances A. Collichio[5], C. Paige Jones[6], Mikaela J. Bauer[7], Joel S. Parker[3], Noah Sciaky[1], Alisha R. Coffey[3], Samantha M. Bevill[1], Brian T. Golitz[1], Timothy J. Stulhmiller[1], Nancy E. Thomas[8], David W. Ollila[6], Norman E. Sharpless[5], Carrie B. Lee[5], Gary L. Johnson[1], **Stergios J. Moschos**[5]

[1]Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC,[2]Biostatistics, University of North Carolina at Chapel Hill, Chapel Hill, NC,[3]Genetics, University of North Carolina at Chapel Hill, Chapel Hill, NC,[4]Pediatrics Medicine and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN,[5]Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC,[6]Surgery, University of North Carolina at Chapel Hill, Chapel Hill, NC,[7]University of North Carolina at Chapel Hill, Chapel Hill, NC,[8]Dermatology, University of North Carolina at Chapel Hill, Chapel Hill, NC

To investigate the landscape of the activated kinome in MM, we applied a proteomic approach that interrogates the activation status of more than half of the entire kinome (~300), termed multiplexed kinase inhibitor bead affinity chromatography coupled with mass spectrometry (MIB/MS) on 45 MM patient (pt) tumors (*BRAFV600*, n=25; *NRASQ61*, n=5, *NF1* loss-of-function, n=4; Triple wild-type n=11) in conjunction with whole exome sequencing and RNA sequencing (RNAseq). We performed exploratory analyses (Wilcoxon rank sum test for each kinase) on the untreated tumors to identify differentially activated kinases in *BRAFV600*-mutant MM vs. all others. While no activated kinases were differentially expressed in *BRAFV600*-mutant MM after adjustment for multiple testing, AKT1, PDGFRA, and MAP3K1 kinases exhibited higher MIB binding (i.e., more activated) in *BRAFV600E*-mutant pts (unadjusted p-value < 0.05). Spearman's rank correlation tests between the activated kinase and its gene expression by RNAseq and adjusted for multiple testing did not identify a significant correlation for any kinase, suggesting a complex mechanism of kinase activation other than sole gene expression. K-means clustering analysis to classify melanomas into 4 subtypes using a 6-gene discriminant expression signature showed that *BRAFV600*-mutant tumors were more frequently classified as melanocytic (9/24, *MLANA*/*MITF*/*SOX10*^{high}, *AXL*^{low}). In contrast, triple wild-type tumors were more frequently classified as transitory (5/10, *MITF*/*SOX10*/*ETV4*^{high}, *AXL*^{low}). We then performed similar exploratory analyses on the baseline (untreated) tumors from the subset of the 20 *BRAFV600E*-mutant pts who received D+T as part of a prospective clinical trial (NCT01726738; median follow-up 30.5 months, range 2.0-116.1 months) to identify differentially activated kinases that would predict shorter (<12 months) vs. longer progression-free survival. Again, while no kinases survived multiple comparison testing, the bromodomain protein TRIM28, known to regulate melanoma plasticity, exhibited a more consistent loss of MIB binding in *BRAFV600E*-mutant pts who progressed shorter than in pts who progressed longer than 12 months on D+T (unadjusted p-value < 0.05). 7 baseline-progression tumor pairs were evaluated for MIB/MS, and 6 were for RNAseq. Although diverse resistance mechanisms were identified, as previously described, we also identified melanoma subtype switch in 4/6 pt tumors and a consistent loss of MIB binding of the non-receptor tyrosine kinase PTK6 in response to D+T, an off-target of D and vemurafenib but not encorafenib. Our in vitro findings revealed that off-target PTK6 inhibition by D could activate beta-catenin signaling and contribute to the development of resistance.

#2052 Genomic and transcriptomic correlates of response to neoadjuvant chemotherapy and survival outcomes in breast cancer.

M. Dong[1], J. Chen[1], N. Lu[1], S. Wang[2], W. Wei[1], Z. Wang[1], J. Wang[1], J. Zhang[1], X. Han[1], F. Wang[2], Q. Ou[2], **H. Tang**[2], X. Ma[1], B. Shan[1], Y. Pan[1];

[1]The First Affiliated Hospital of University of Science and Technology of China (USTC), Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, China, [2]Nanjing Geneseeq Technology Inc., Nanjing, China

Background: Neoadjuvant chemotherapy (NAC) is standard for breast cancer treatment, aimed at downgrading tumors and enhancing the feasibility of radical surgery. Pathological complete response (pCR), confirmed through histopathologic examination, is a potential surrogate marker of long-term survival. However, the absence of reliable biomarkers to predict NAC response and prognosis poses a significant challenge, hindering timely treatment adjustments and optimal adjuvant therapy selection.

Methods: This study included 73 stage II/III breast cancer patients who underwent NAC followed by curative-intent surgery. We analyzed whole-exome and transcriptome sequencing data from baseline and surgical tumors to identify biomarkers associated with pCR and survival outcomes. Plasma samples were collected at three time points: before NAC (P1), during NAC (P2), and after NAC (P3), to evaluate the predictive value of undetectable circulating tumor DNA (ctDNA) for pCR.

Results: *TP53*, *PIK3CA*, and *TTN* mutations were common in breast cancer but were exclusive to surgical samples from non-pCR patients. pCR was associated with baseline mutations in *ADGRL3*, *IFT172*, *DNHD1*, *MUC16*, *TPTE*, *ABCA13*, *SULF1*, *TRANK1*, *PLEC*, and genetic alterations in the HIPPO pathway. At the transcriptional level, pCR tumors exhibited heightened immune activation, characterized by higher fractions of memory B cells and resting NK cells, alongside fewer activated mast cells and cancer-associated fibroblasts. Responding tumors also showed significantly downregulation of pathways related to extracellular matrix organization, PI3K-Akt, and AGE-RAGE signalling compared to non-pCR tumors. Surgical samples from non-pCR tumors exhibited deminished leukocyte-mediated immunity and immune receptor activity, suggesting a potential resistance mechanism to NAC. Undetectable ctDNA at P2 and P3 time points was strongly correlated with pCR (P=0.03 and P=0.001, respectively), with P3 demonstrating slightly better sensitivity (0.90; 95% confidence interval [CI]: 0.55-1.00 vs. 0.80; 95% CI: 0.44-0.97) and specificity (0.63; 95% CI: 0.44-0.80 vs. 0.62; 95% CI: 0.44-0.79). Additionally, baseline *USH2A* mutations (hazard ratio [HR]: 11.9; 95% CI: 2.8-50.8; P<0.001) and chromosome 8q amplifications in surgical samples (HR: 4.3; 95% CI: 1.1-16.0; P=0.02) were predictive of shorter disease-free survival. Furthermore, elevated *NHSL1* expression, while indicative of initial response, was associated with tumor relapse and poor overall survival (P=0.026 and P=0.023, respectively).

Conclusions: This study identified key predictive and prognostic biomarkers associated with NAC response and long-term survival in breast cancer, providing valuable insights for developing individualized therapeutic strategies tailored to patient-specific molecular profiles.

#2053 Hemizygous focal deletions in *MACROD2* alter tumor biology and genomic instability in colorectal cancer.

Carmen Rubio Alarcon*[1], Soufyan Lakbir*[2], Elisabeth J. van Bree[1], Bram van den Broek[1], Amber Hondema[1], Petur Snaebjornsson[1], Steven L. C. Ketelaars[1], Marianne Tijssen[1], Anne Bolijn[1], Pien Delis-van Diemen[1], Margriet Lemmens[1], Maartje Alkemade[1], Mette Kalager[3], Magnus Loberg Loberg[3], Miriam Koopman[4], Jeanine M. L. Roodhart[4], Geraldine R. Vink[5], PLCRC-MEDOCC group, Ellen Stelloo[6], Jaap Heringa[7], Gerrit A. Meijer[1], Harma Feitsma[6], Beatriz Carvalho[1], Sanne Abeln[8], **Remond J. A. Fijneman**[1]

[1]Netherlands Cancer Institute, Amsterdam, Netherlands,[2]Netherlands Cancer Institute / Vrije Universiteit Amsterdam / Utrecht University /, Amsterdam/Utrecht, Netherlands,[3]University of Oslo / Oslo University Hospital, Oslo, Norway,[4]University Medical Center Utrecht / Utrecht University, Utrecht, Netherlands,[5]University Medical Center Utrecht / Utrecht University / Netherlands Comprehensive Cancer Organisation, Utrecht, Netherlands,[6]Cergentis B.V., a Solvias company, Utrecht, Netherlands,[7]Vrije Universiteit Amsterdam, Amsterdam, Netherlands,[8]Vrije Universiteit Amsterdam / Utrecht University, Amsterdam/Utrecht, Netherlands

Introduction: *MACROD2* is a large gene located within a common fragile site (CFS), a genomic region that is vulnerable to acquiring focal deletions under replicative stress. Structural variants (SVs) in *MACROD2* are highly prevalent in patients with colorectal cancer (CRC), but their putative role in tumor initiation and progression remains to be established.

Aim of this study*: In silico* and *in vitro* characterization of the effects of somatic SVs in *MACROD2* in CRC initiation and evolution.

Methods: Five datasets were used for *in silico* characterization of SVs in *MACROD2*: HMF (metastatic CRC, n=610), PCAWG (stage I-IV CRC, n=60), PLCRC-PROVENC3 (stage III CRC, n=234), PLCRC-MEDOCC (stage II-III CRC, n=214) and IntEnd (adenomas, n=634). The system-wide impact of intragenic SVs in *MACROD2* on gene expression profiles was evaluated using the tool CIBRA. SVs in *MACROD2* were associated with features of genomic instability, including tumor mutational burden (TMB), fraction of genome altered (FGA), tumor break load (TBL) and length of somatic SVs. The most frequently occurring SVs in *MACROD2* were mimicked in vitro by CRISPR-Cas9 genetic engineering of a colorectal cancer cell line and an adenoma organoid. Their effect on tumor biology was determined by evaluating mRNA sequencing data, cell viability, mono-ADP-ribose levels, DNA damage repair and drug testing.

Results: Focal deletions in *MACROD2* were absent in adenomas, and present in 56% primary non-metastatic tumors and 71% of the metastatic CRC lesions evaluated. Focal deletions affecting the MACRO domain, in particular *MACROD2* exons 5 or 6, were associated with system-wide expression changes (CIBRA score 0.37; FDR < 0.01), increased TBL ($p < 10^{-4}$) and an increased number of SVs genome wide, specifically deletions of approximately 100kb ($p < 10^{-15}$). *In vitro*, the models containing hemizygous or homozygous focal deletions showed lower levels of mono-ADP-ribose in the nucleus, and higher levels of DNA damage accumulation in the cancer cell line. Drug testing revealed that hemizygous deletions of *MACROD2* made the cells more susceptible to treatment with 5-fluorouracil compared to WT (GR50: 17.6 µM vs 62.1 µM, P= 0.004). A similar trend was observed for the homozygous deletion (GR50: 30.3 µM vs 62.1 µM, ns)

Conclusions: This study evaluated the effect of the most prevalent SV in CRC. Hemizygous intragenic focal deletions in *MACROD2* that affect exon 5 and/or 6 are sufficient to alter system-wide expression profiles, nuclear mADPr levels, DNA damage repair and response to 5-fluorouracil.

#2055 Molecular profiling of the PI3Kδ inhibitor roginolisib in metastatic uveal melanoma and its correlation with clinical outcomes.

Alessio Bevilacqua[1], Tan Ziyang[2], Lakshmikanth Tadepally[2], Petter Brodin[2], Anna Maria Di Giacomo[3], Michele Maio[3], Jeff Evans[4], Armando Santoro[5], Tracey Hammett[1], Paramijt Kaur[1], Lars van der Veen[6], Michael Lahn[1], **Giusy Di Conza**[1]

[1]iOnctura SA, Geneva, Switzerland,[2]Karolinska institutet, Stockholm, Sweden,[3]University hospital of Siena, Siena, Italy,[4]Beatson Cancer center, Glasgow, United Kingdom,[5]Humanitas Cancer Center, Rozzano-Milan, Italy,[6]iOnctura BV, Amsterdam, Netherlands

Introduction: Uveal melanoma (UM) is a rare orphan disease with significant unmet clinical need. Unlike cutaneous melanoma, UM has a low tumor mutational burden and a highly immunosuppressive microenvironment, rendering it largely unresponsive to checkpoint inhibitor therapies. PI3Kδ is involved in the pathophysiology of the disease, by enhancing regulatory T cell activity and supporting tumor cell survival. Roginolisib, a PI3Kδ inhibitor, was evaluated in 23 UM patients at an 80 mg biological effective dose (BED). Here, we report data from a comprehensive translational analyses of genomic, transcriptomic, proteomic and cellular data from UM patients, supporting PI3Kδ as a suitable therapeutic target for UM patients.

Methods: IOA-244-101 (NCT04328844) was conducted in patients with advanced cancer, which included metastatic uveal melanoma patients. Baseline and on-treatment blood and plasma samples were analyzed to evaluate circulating immune cells using mass cytometry, circulating tumor DNA (ctDNA) via whole exome sequencing, and circulating proteins through targeted proteomics. Additionally, biopsies at baseline and Day28 of treatment were analyzed for tumor transcriptomic and genomic profiles using whole transcriptome and whole exome sequencing.

Results: Roginolisib treatment induced progressive decrease of circulating regulatory T cells and increased CD39+CD8T cells, accompanied by reduction of soluble PD1, CCL22 and increase of IL15 and IFNGR2. We identified a subpopulation of mUM patients that particularly benefited from roginolisib treatment. Patients with RECIST 1.1-defined stable disease (SD) at 16 weeks experienced significantly longer median overall survival (28.5 months) compared to those with progressive disease (PD) (10.9 months). SD patients had enhanced $CD8^+$ T cell/Treg ratios and downregulation of PI3K/mTOR-associated proteins in plasma. Conversely, PD patients showed upregulation of these proteins after8 weeks of treatment , suggesting a potential resistance mechanism. Transcriptomic analysis of tumor biopsies revealed that SD patients had higher baseline expression of 325 genes, including 61 GPCR (G-protein coupled receptors), which were downregulated post-treatment. Longitudinal ctDNA profiling further demonstrated dynamic tumor evolution, with specific variants emerging or disappearing over time.

Conclusion: These findings highlight the importance of immune modulation, PI3K/mTOR signaling, and GPCR gene expression in potentially driving treatment outcomes, and encourage further evaluation of Roginolisib in a Phase 2 randomized study.

#2056 Development of a response prediction Model for venetoclax-based therapy utilizing molecular interactions among BCL2 family members.

Byungsan Choi[1], Yunseo Lee[1], Lilly Gu[2], Keonho Lee[1], Eunyoung Kim[2], Martha L. Arellano[2], William G. Blum[2], **Hongwon Lee**[1], Janghee Woo[2]

[1]PROTEINA Co., Ltd., Seoul, Korea, Republic of,[2]Winship Cancer Institute of Emory University, Atlanta, GA

Venetoclax (VEN), in combination with hypomethylating agents (HMAs) such as azacitidine or decitabine, is a widely used treatment for AML patients, yet the molecular biomarkers contributing to sensitivity to the VEN+HMA regimen remain incompletely elucidated, particularly with regard to the role of BCL2-family proteins.

In this study, we retrospectively collected BMMCs or PBMCs from AML patients prior to initiation of VEN+HMA therapy at Winship Cancer Institute of Emory University. A total of 47 specimens were analyzed, including 33 from newly diagnosed AML patients and 14 from relapsed or refractory AML cases at the time of sample collection. Clinical outcomes were categorized based on the best response to VEN+HMA therapy, with refractory cases labeled as "NR" (non-responders; 17 patients) and all other cases labeled as "R" (responders; 26 patients). To investigate protein interactions between the BCL2 family, we employed the Single-molecule Protein Interaction Detection (SPID) platform (Chun et al., Nat. Biomed. Eng., 8, 2024). 12 distinct protein biomarkers were assessed based on three anti-apoptotic proteins: BCL2, BCLxl, and MCL1. ROC analysis was performed to evaluate the association between individual biomarkers and clinical outcomes of VEN+HMA therapy. To develop a predictive model incorporating BCL2-family biomarkers, we utilized a linear model rectified with a ReLU activation function. The trained model was interpreted using SHAP value analysis.

To enhance the variability of biomarkers, we calculated normalized protein complex levels relative to protein levels. For example, the BCL2-BAX complex level was divided by the BCL2 protein level to assess the fraction of occupied BCL2 across samples. Among the 21 biomarkers analyzed, the normalized BCLxl-BAK complex level demonstrated the strongest predictive performance, with an AUC of 0.734 (95% CI: 0.536-0.859) for distinguishing responders to VEN+HMA therapy. We developed a response prediction model for VEN+HMA therapy with 12 biomarkers, and achieved performance with 96.2% sensitivity and 100% specificity. Interpretation of the model using SHAP analysis revealed key contributors to sensitivity and resistance to the VEN+HMA regimen. Specifically, Higher levels of the BCL2-BAX and BCLXL-BAK complexes were associated with sensitivity to VEN+HMA therapy. Conversely, lower levels of the BCL2-BIM and MCL1-BIM complexes were linked to resistance to the treatment. Notably, the expression level of MCL1 (measured after heat denaturation) significantly influenced sensitivity to VEN+HMA therapy.

This study highlights the potential of BCL2-family interaction profiles in developing a response prediction model for VEN+HMA sensitivity. Further model refinement and validation in independent cohorts are essential to improve its predictive performance and reliability.

#2057 CG>TG mutation frequency as negative predictor of homologous recombination deficiency in ovarian and breast cancer.

Jan Budczies, Eva Romanovsky, Michael Menzel, Klaus Kluck, Susanne Beck, Markus Ball, Peter Schirmacher, Daniel Kazdal, Albrecht Stenzinger

Heidelberg University Hospital (UKHD), Heidelberg, Germany

Homologous recombination deficiency (HRD) is a predictive biomarker for PARP inhibition and platinum-based chemotherapy. Copy number alteration-based scores such as HRDsum =LST+TAI+LOH are included in therapy approvals of PARP inhibition, but have limited sensitivity for HRD detection in samples of low tumor purity. Biomarkers based on single base substitutions (SBS) beyond Mutational Signatures are underinvestigated as predictors of HRD. WES data of the TCGA pan-cancer cohort and an in-house ovarian cancer cohort were annotated by genetic and epigenetic alterations of BRCA1/2 and additional genes causative of HRD. Using this reference, the new biomarker f_{deam} defined as frequency of C>T transitions at CpG sites in relation to all SBS and HRDsum were compared for the detection of HRD. In the TCGA ovarian cancer, the in-house ovarian cancer, and the TCGA breast cancer cohorts, f_{deam} performed non-inferior to HRDsum (AUC=0.84, AUC=0.85, and AUC=0.88). The cutpoint f_{deam}=13.1% maximized the balanced accuracy in the TCGA ovarian cancer cohort and resulted in sensitivity=89% and specificity=77% in the in-house cohort. In a simulation study, f_{deam} retained high sensitivity for HRD detection and outperformed HRDsum for tumor purity 40%, 20%, and 10%. Thus, the new biomarker overcomes the limited robustness of copy number alteration-based biomarkers against low tumor purity and offers the opportunity of a more sensitive HRD detection in clinical tumor samples. Further studies are warranted to confirm its clinical validity and utility and explore its potential for liquid biopsies.

#2058 Clinical and biological implications of plasma proteomic patterns in NSCLC patients treated with immune checkpoint inhibitors.

M. Harel[1], N. Dahan[1], C. Lahav[1], E. Jacob[1], Y. Elon[1], I. Puzanov[2], R. J. Kelly[3], Y. Shaked[4], R. Leibowitz[5], D. P. Carbone[6], D. R. Gandara[7], A. P. Dicker[8];

[1]OncoHost, Binyamina, Israel, [2]Roswell Park Comprehensive Cancer Center, Buffalo, NY, [3]Baylor University Medical Center, Dallas, TX, [4]Technion - Israel Institute of Technology, Haifa, Israel, [5]Shamir Medical Center, Zerifin, Israel, [6]The Ohio State University, Columbus, OH, [7]University of California Davis Comprehensive Cancer Center, Sacramento, CA, [8]Thomas Jefferson University, Philadelphia, PA

Introduction: Immune checkpoint inhibitors (ICIs) offer significant benefits for patients with advanced non-small cell lung cancer (NSCLC). However, resistance to ICIs poses a major clinical hurdle. Recently, we described PROphet, a treatment guidance tool that predicts clinical benefit from ICI-based treatment using baseline plasma proteins termed ‘resistance-associated proteins’ (RAPs). Here, we conducted an in-depth bioinformatic analysis of RAPs to uncover the biological mechanisms underlying ICI resistance and their clinical relevance.

Methods: 388 RAPs were analyzed using multiple bioinformatic and statistical tools. Putative tissue origins of the RAPs were explored using publicly available datasets. Enrichment analyses were performed to investigate RAP-related biological processes. Plasma proteomic data from healthy subjects (n=50) and NSCLC patients displaying clinical benefit (CB; n=76) or no CB (NCB; n=196) were compared to identify differential expression patterns between the three subpopulations.

Results: RAPs were found to originate from both host and tumor tissues, with significant enrichment of lung cancer- and liver-associated proteins. A comparison of RAP expression levels in healthy, CB and NCB populations revealed five distinct expression patterns that provide mechanistic insights into treatment resistance. One such pattern characterized by higher RAP expression levels in healthy subjects compared to NSCLC patients included proteins associated with antitumor activities. Another pattern characterized by elevated RAP expression levels in the NCB population included multiple proteins associated with treatment resistance mechanisms such as angiogenesis, cell proliferation, immune modulation, and chemoresistance. Patient-specific scores based on these four resistance mechanisms revealed inter-patient diversity, highlighting the potential of RAP profiles for guiding personalized combination therapies. A protein-drug database screen showed that 17.5% of the RAPs could be targeted therapeutically, demonstrating that the RAP set is a rich source of additional potential therapeutic targets.

Conclusions: The study provides insights into the biological processes associated with ICI resistance in patients with NSCLC and highlights the potential clinical value of RAP profiles for developing personalized therapies addressing resistance.

#2059 High intratumoral heterogeneity and altered immune microenvironment associated with 11q13 amplification in Chinese NSCLC implications for hyperprogressive disease.

Zuoqing Song[1], Yifan Yifan Zhang[2], Hao Zhang[2]

[1]Department of Lung Cancer Surgery, Tianjin Medical University General Hospital ; Tianjin Medical University General Hospital Airport Hospital, Tianjin, China,[2]Department of Lung Cancer Surgery, Tianjin Medical University General Hospital, Tianjin, China

Background: Immune checkpoint inhibitors have revolutionized the treatment landscape for non-small cell lung cancer (NSCLC), but a subset of patients experiences hyperprogressive disease (HPD) following immunotherapy. Previous studies indicate a potential link between specific genetic alterations and HPD. This study aims to investigate the role of the 11q13 chromosomal amplification, encompassing genes CCND1, FGF3, FGF4, and FGF19, in contributing to immunotherapy resistance and the occurrence of HPD in NSCLC patients.

Methods: We performed comprehensive genomic analyses on tumor specimens from 12,682 NSCLC patients using a next-generation sequencing (NGS) panel of 733 genes, alongside multiplex immunofluorescence assessments to characterize the immune microenvironment. Gene mutation profiles, tumor mutation burden (TMB), intratumoral heterogeneity (ITH), and immune cell infiltration profiles of 11q13 amplified and non-amplified patients were analyzed.

Results: Among these patients, we identified 208 cases with 11q13 amplification. Analysis revealed that patients without 11q13 amplification exhibited higher mutation frequencies in key DNA damage repair (DDR) pathway genes, including ATR (10.1%), ATM (8.65%), MSH3 (7.21%), and others, compared to patients with 11q13 amplification. Notably, TMB was significantly elevated in 11q13-amplified patients ($P<0.001$), contradicting the expected positive correlation between high TMB and better immunotherapy outcomes. Furthermore, ITH was significantly higher in the 11q13-amplified cohort ($P<0.001$), indicating greater tumor heterogeneity, which has been associated with poorer immunotherapeutic responses. Immune microenvironment analysis demonstrated a significantly decreased presence of CD68+ HLA-DR+ M1 tumor-associated macrophages (TAM) in the tumors of 11q13-amplified patients. In contrast, there was an elevated frequency of CD3+CD4+FOXP3+ regulatory T cells (Treg) in the tumor stroma of these patients, aligning with prior findings suggesting Treg involvement in HPD.

Conclusion: Our findings underscore that the 11q13 chromosomal amplification is associated with a unique genomic and immunological profile in NSCLC patients, characterized by high ITH and altered immune microenvironments, which may contribute to the development of HPD following immunotherapy. These insights highlight the potential need for tailored therapeutic strategies that address the specific challenges posed by 11q13 amplification in NSCLC.

#2060 Independent validation of endogenous retrotransposable elements as predictive biomarkers of immune checkpoint blockade response.

Mercedes Herrera[1], Sajid A. Marhon[1], Farnoosh Abbas-Aghababazadeh[1], David Chen[2], Amy Liu[1], Helen LooYau[1], Emily Van de Laar[1], Jeffrey Bruce[1], Helen Chow[1], Philippe L. Bedard[1], Albiruni Razak[1], Anna Spreafico[1], Aaron R. Hansen[1], Marcus O. Butler[1], Stephanie Lheureux[1], Trevor J. Pugh[1], Benjamin Haibe-Kains[1], Daniel D. De Carvalho[1], Lillian L. Siu[1], Pavlina Spiliopoulou[3]

[1]UHN Princess Margaret Cancer Centre, Toronto, ON, Canada,[2]University of Toronto, Toronto, ON, Canada,[3]School of Cancer Sciences, University of Glasgow, Glasgow, United Kingdom

Background: Endogenous retrotransposable elements (EREs) can modulate antitumor immune responses via viral mimicry response. We previously described an association between ERE upregulation and radiological response to immune checkpoint blockade (ICB) in advanced solid tumors in the phase II INSPIRE trial (NCT02644369) (ASCO 2024). Here, we validate the association of EREs and ICB response in two independent cohorts of patients (pts) with melanoma and non-small cell lung cancer (NSCLC) treated with ICB.
Methods: Publicly available datasets were screened to identify cohorts of pts with solid tumors receiving ICB with accessible clinical outcomes (responders, RP; or non-responders, NR) and total RNA-sequencing data. Two independent datasets (*Riaz et al*., PMID 29033130; *Jung et al.,* PMID 31537801) were examined. ERE expression was analyzed in total RNA-seq from anonymized raw data in baseline (B) and on-treatment (T) samples of pts with melanoma and B samples of NSCLC. Differentially expressed EREs between RP and NR were quantified by the TPM*score* (mean of normalized transcript per million values for upregulated EREs in a sample comparison) and its standardized Z*score*. Immune cell infiltration was inferred from RNA-seq-based deconvolution. The cytolytic activity (CYT) immune score, derived from the mRNA expression of *GZMA* and *PRF1*, was reported in the melanoma cohort, while tumor mutational burden (TMB) data was available for both cohorts.
Results: A total of 43 pts (14 RP and 29 NR) in the melanoma cohort and 27 pts (8 RP and 19 NR) in the NSCLC cohort were identified for analysis. Baseline upregulated ERE classes included LINE (27%), SINE (30%), LTR (23%), simple repeats (10%), and others (10%) in melanoma, and LINE (34%), SINE (21%), LTR (29%), simple repeats (8%), and others (8%) in NSCLC. Differential ERE expression was observed in RP compared to NR across B samples in both cohorts (melanoma: RP vs NR Z*score* 0.50 vs -0.23, $p< 0.001$; NSCLC: RP vs NR Z*score* 0.89 vs -0.27, $p= 0.001$). In the melanoma cohort, on-treatment samples confirmed higher ERE expression in RP vs NR (Z*score* 0.77 vs -0.32, $p< 0.001$). A moderate positive correlation between TPM*score* of RP and $CD8^+$ T cell expression vs NR was seen in the melanoma pts in B samples (Pearson correlation $R = 0.54$, $p = 0.001$) and on-treatment ($R= 0.62$, $p< 0.001$), but no correlation was seen in B samples of the NSCLC cohort ($R= 0.2$, $p= 0.31$). Z*score* did not correlate with TMB in melanoma or NSCLC pts. In melanoma pts, the Z*score* showed a moderate positive correlation with CYT immune score ($R= 0.41$, $p= 0.017$).
Conclusion: This validation study on melanoma and NSCLC pts adds to the previously observed association of ERE upregulation and clinical outcomes during ICB treatment in a pan-cancer cohort. The association with $CD8^+$ T cell infiltration and CYT immune score, but not with TMB, suggests an immune activation independent of other biomarkers such as TMB.

#2061 Monitoring PD-L1 expression in cancer associated macrophage-like cells in blood predicts clinical outcomes in metastatic cancer patients treated with PD-L1 immunotherapies.

Dimpal M. Kasabwala[1], Steven H. Lin[2], Massimo Cristofanilli[3], Carolina Reduzzi[3], Cha-Mei Tang[4], Thai H. Ho5 H. Ho[5], Daniel L. Adams[6]

[1]Rutgers University, New Brunswick, NJ,[2]MD Anderson Cancer Center, Houston, TX,[3]Weill Cornell Medicine, New York, NY,[4]Creatv MicroTech, Inc., Potomac, MD,[5]Medical University of South Carolina, Charleston, SC,[6]Creatv MicroTech, Inc., Monmouth Junction, NJ

Studies have described the efficacy of immunotherapies (IMT) utilizing programmed death 1 receptor and its ligand (PD-L1) for treating solid tumors. However, many patients (pts) fail to respond to IMT, underscoring the need for better predictive biomarkers for improved pt identification and stratification. Poor IMT responses are often attributed to the dynamic nature of PD-L1 which may change after chemotherapy or radiation and is quantified by static immunostaining. Recently, studies have described PD-L1 upregulation in giant phagocytic stromal cells, i.e. Cancer associated macrophage-like cells (CAML), circulating macrophages that have engulfed tumors before entering circulation and may predict IMT responses. We conducted a pilot study to monitor the peripheral blood of n=46 metastatic cancer pts undergoing systemic treatment with IMT in combination with other therapies, to evaluate CAML PD-L1 prior to and post IMT induction with clinical correlation at 2 years. In a prospective pilot study of n=46 metastatic cancer pts, breast (n=14), non-small cell lung (n=24), and renal cell (n=8), all starting new lines of systemic chemotherapy in combination with IMT (pembrolizumab [n=30], nivolumab [n=13], Durva [n=1], or atezolizumab [n=3]) for newly diagnosed recurrent metastasis (n=24) or with previously treated progressive metastatic disease (n=22). We isolated CAMLs from 7.5 ml baseline (T0) blood using the LifeTracDx® PD-L1 test and scored PD-L1 as high or low. If possible, a follow-up sample (T1) was taken (~48 days) after IMT induction. Pts' progressive free survival (PFS) and overall survival (OS) hazard ratios (HRs) were analyzed by censored univariate analysis based on RECIST v1.1 over 2 years. T0 PD-L1 CAML data was available for 89% (n=41/46) of pts, with 39% (n=16/41) having high CAML PD-L1 which was not correlated with improved PFS (HR=0.9, p=0.985, CI=0.5-1.9) or OS (HR=1.0, p=0.899, CI=0.4-2.2). T1 PD-L1 CAML data was available for 74% (n=34/46) of pts, with 53% (n=18/34) having high CAML PD-L1, which significantly correlated with improved PFS (HR=2.9, p=0.012, CI=1.3-6.4) and OS (HR=5.5, p=0.0005, CI=2.2-13.6). In comparing CAML PD-L1 change post IMT, it was found that consistently low PD-L1 at T0 & T1 had the poorest responses, median PFS (mPFS)=2.3 months and median OS (mOS)=5.5 months. In contrast, consistently high PD-L1 at T0 & T1 had better responses, mPFS=6.1 months and mOS=16.7 months. Further, pts who increased in CAML PD-L1 after IMT had the best response rates, mPFS=10.3 months and mOS=14.8 months. Here, we describe a cancer agnostic blood-based biopsy to monitor dynamic PD-L1 changes in circulating tumor immune cells and predict clinical benefit to PD-L1 IMTs in several cancer types. While this initial pilot study appears to stratify pts with better IMT responses, larger validation studies are needed.

CLINICAL RESEARCH: Spatial Proteomics and Transcriptomics 2
Poster Session

#2065 A deep-learning approach for tissue mapping and cell phenotyping in imaging mass cytometry data analysis.

Smriti Kala[1], Katherine Hales[2], David Mason[2], Hiroyuki Suzuki[3], James Robert Mansfield[1], Regan Baird[4]

[1]Standard BioTools, Markham, ON, Canada,[2]Visiopharm A/S, Horsholm, Denmark,[3]Fukushima Medical University, Fukushima, Japan,[4]Visiopharm A/S, Horsholm, Denmark

Imaging Mass Cytometry™ (IMC™) technology is a multiplexed imaging technique that generates high-dimensional spatial data at subcellular resolution without the complications of autofluorescence and cyclic imaging. IMC technology has two distinct whole slide imaging (WSI) modes: Preview Mode (PM) and Tissue Mode (TM). PM rapidly scans stained tissue to provide a comprehensive overview within minutes, while TM provides fast acquisition of the entire tissue at 5-micron resolution, mapping out the distribution of over 40 markers and revealing tissue heterogeneity. Both WSI modes enable researchers to make informed decisions about selecting tissue areas that warrant closer examination at single-cell resolution. Following PM, regions of interest (ROIs) are selected on the same slide for high-resolution imaging using Cell Mode (CM). This facilitates single-cell analysis of the ROIs identified during PM. These imaging modes together with an automated slide loader function support non-stop acquisition of tissue samples all week.
Tissue sections of lung cancer were stained with a 40-marker IMC panel by combining the Human Immuno-Oncology IMC Panel with the Human Immune Cell Expansion IMC Panel to study spatial organization and cellular interactions in the tissue. Images were acquired on the Hyperion™ XTi Imaging System (Standard BioTools), first in PM and then in CM with automatic selection of ROIs using Phenoplex™ software (Visiopharm®). ROIs were automatically selected based on three criteria: 1) tertiary lymphoid structures (TLS) expressing CD20 and CD3; 2) Granzyme B-rich areas and 3) areas with high number of CD68 and Vimentin double-positive cell clusters. An adjacent serial section was acquired in TM for a whole slide morphologic segmentation comparison.
Tissue segmentation for all modes was performed using Phenoplex by training a deep-learning algorithm to recognize morphological features of the images, including the TLS. Single-cell analysis of the images generated in CM was performed using Phenoplex; cell segmentation was based on iridium DNA channels, and phenotyping was performed using the guided workflow. This data was used to compare the immune contexture through a series of t-SNE plots partitioned by spatial region and clinical variables.
Many mature TLS with proliferating germinal centers were present in the tissue. A high number of granzyme B-expressing neutrophils were found. Vimentin-positive macrophage-like cells were associated with the TLS. This work demonstrates that the Hyperion XTi Imaging System with its three acquisition modes (PM, TM and CM) can greatly advance the ability of IMC users to obtain answers from complex samples. The interactive capabilities of Phenoplex allow the user to quickly identify pertinent cell types, find them within a tissue map, define their spatial relationship and analyze their neighbors, leading to valuable biological insights.

#2066 Magnifying glass on sinonasal NUT carcinoma heterogeneity using spatial transcriptomics.

Diana Bell[1], Garret Choby[2], Michelle Afkhami[3], Ellie Maghami[4], Ehab Hanna[5], Raja Seethala[1]

[1]Pathology, University of Pittsburgh, Pittsburgh, PA,[2]Head and Neck Surgery, University of Pittsburgh, Pittsburgh, PA,[3]Molecular Pathology & Therapy Biomarkers, City of Hope Cancer Center, Duarte, CA,[4]Head and Neck Surgery, City of Hope Cancer Center, Duarte, CA,[5]Head and Neck Surgery, University of Texas MD Anderson Cancer Center, Pittsburgh, PA

Introduction: Nuclear protein in testis (NUT) carcinomas (NCs) are clinically aggressive, with signature translocation *NUTM1::BRD4* genes (70%), *BRD3::NUTM1* (15%), *NSD3::NUTM1* (6%). Histologically, NCs depict undifferentiated basaloid cells with squamous differentiation; broad lineage immunomarkers lack in NCs. Demonstration of translocation is imperative for NC diagnosis; NUT immunostaining is a surrogate. NC treatment faces obstacles (drug resistance, metastasis). Spatial transcriptomics (ST) technology emerged as ideal approach to elucidate cancer cell heterogeneity and spatial distributions within tissues.
Methods: 3 cases of sinonasal NC carcinoma with FFPE formed the study material. Next generation sequencing confirmed NC diagnosis: *BRD3::NUTM1* in NC #1, *BRD4::NUTM1* in NC # 2, NC # 3. A representative region of each of NCs was subjected to 10X Genomics Visium Spatial Gene Expression analysis. Ingenuity Pathway Analysis (IPA) employed for pathways and network analyses. Resulting data were visualized/ analyzed in 10X Genomics Loupe Browser.
Results: All cases demonstrated statistically distinct (graph-neural network based) transcriptomic clusters. These clusters were correlated with histologic characteristics by parsing all spots by region types based on tissue distribution including tumor, limited stroma and normal epithelium. Cluster constitution and transcriptomic pattern were consistent with the morphologic features of each tissue region. NC #3 had relatively low number of tumor clusters in comparison to NC #1, NC#2. Table 1- summary of results.
Conclusions: Spatial transcriptomic profiling corresponds to histopathologic features in NC and yet highlights cell type diversity across tumors and in the case of NC#2, within a given tumor. Behavior correlates are speculative, but NC#3 for which the patient had a rapidly lethal outcome showed a preponderance of neural gene expression which may be of interest in tumor cell progression/ evolution.

Table 1

Case	**NC #1**	**NC #2**	**NC #3**
Age (yo)	63	53	28
Sex	F	M	M
Outcome	NED	AWD	DOD
Fusion type	*BRD3::NUTM1*	*BRD4::NUTM1*	*BRD4::NUTM1*
#Tumor Cell Clusters	5	3	1
Shared within top 100 of all 3 cases	Rank		
NTS	28	30	9
HOXB3	41	21	10
HOXB6	59	62	21
PRSS21	55	39	38
NUTM1	48	33	49
KRT14	40	41	99
Top 10 Genes	LOR, SPRR2A, DLK1, IV/involucrin, SPINK5, SPRR1B, SPINK6, SPINK7, SPRR2F, HMGCS2	SPINK6, SPINK5,CA9, CA2, EPHA7, TRPC6, GPR174, HDAC9, PIK3C2G, PTPRD	OPN5, NRN1, NTS, DSG1, DSC1, NKX2-8, HOXB3, MMP12, AFP, ALB

#2067 Single-cell spatial whole transcriptome reveals tumor heterogeneity and stromal dynamics in invasive ductal carcinoma of the breast.

Liang Zhang, Shanshan He, Michael Patrick, Stefan Rogers, Haiyan Zhai, John Lyssand, Michael Rhodes, Joseph Beechem

Bruker Spatial Biology, Seattle, WA

Invasive ductal carcinoma (IDC) is the most common and aggressive form of breast cancer, characterized by tissue invasion and metastasis, contributing to significant morbidity and mortality. The tumor microenvironment (TME), composed of stromal and immune cells, plays a critical role in IDC progression and therapy resistance. However, the spatial organization and molecular heterogeneity within the IDC TME remain underexplored. Spatial transcriptomics enables the preservation of tissue architecture while revealing molecular signatures at single-cell resolution, offering new insights into cancer biology. We utilized the CosMx® Spatial Molecular Imager (SMI) platform with the CosMx Whole Transcriptome panel (approximately 19,000 genes) to analyze a formalin-fixed, paraffin-embedded (FFPE) IDC tumor sample. The platform provides high-resolution, multiplexed RNA detection within its native tissue context. Hematoxylin and eosin (H&E) staining of the same slide provided a histological reference for spatial transcriptomics. Transcriptomic data were analyzed using Uniform Manifold Approximation and Projection (UMAP) for dimensionality reduction and Leiden clustering to identify cell clusters and spatial patterns. Marker genes for each cluster were used to annotate cell types and assess their roles within the TME. Analysis identified two distinct tumor regions, each subdivided into subregions with unique and shared gene expression profiles. These tumor regions aligned with histological features observed in H&E staining, validating the spatial transcriptomic findings. We observed region-specific stromal compositions, with fibroblast populations exhibiting distinct gene expression patterns. For example, one region was enriched for PIGR, CLU, and LTF, markers linked to immune response, tumor progression, and tumor-suppressive properties. These results highlight the molecular and spatial heterogeneity of IDC and the complex interactions within the TME. This study demonstrates the potential of spatial transcriptomics to uncover the molecular and spatial complexity of IDC. Integrating transcriptomic data with histological validation revealed novel insights into the TME, highlighting key gene signatures and stromal dynamics. The CosMx platform is a powerful tool for advancing precision oncology and guiding future therapeutic strategies in IDC.

-Bruker Confidential-

#2068 Mapping the spatial whole transcriptome from normal to tumor tissue in renal clear cell carcinoma: tumorigenesis and microenvironmental shifts at single-cell resolution.

Shanshan He, Liang Zhang, Michael Patrick, Sierra McKenzie, Haiyan Zhai, Christine Kang, Michael Deryck Rhodes, Joseph Beechem

Bruker Spatial Biology, Seattle, WA

Renal clear cell carcinoma (ccRCC) develops through significant molecular and spatial reprogramming, transforming normal kidney tissue into a malignant state and reshaping the tumor microenvironment. To investigate the transition from normal to cancerous tissue, we utilized the CosMx® Whole Transcriptome (WTX) panel to perform high-resolution spatial transcriptomic imaging of FFPE sections containing ccRCC and adjacent normal kidney. H&E staining on the same tissue sections enabled direct correlation of molecular profiles with histopathological features, uncovering novel insights into tumorigenesis. FFPE tissue sections comprising equal parts ccRCC and adjacent normal kidney tissue underwent CosMx WTX imaging to analyze approximately 19,000 genes at single-cell resolution. The same sections were stained with H&E, providing histopathological context for tumor boundaries, tumor-normal interfaces, and regions of vascularization or necrosis. Custom software aligned transcriptomic and histopathological images, enabling the integration of molecular and morphological data for downstream analyses and AI model development. CosMx WTX detected thousands of transcripts per cell with exceptional sensitivity and accuracy. Spatial transcriptomics revealed distinct transcriptional programs and cell-type-specific changes across normal and tumor regions. Ligand-receptor analysis identified critical interactions driving tumorigenesis, while comparative analysis between transcriptome profile and H&E stain highlighted molecular shifts in tumor-adjacent normal areas, preserving renal tubule morphology but exhibiting subtle transcriptomic alterations. The tumor-normal interface displayed unique gene expression signatures, identifying spatially restricted therapeutic targets. This study presents a high-resolution spatial atlas of ccRCC, detailing molecular and cellular transitions from normal to tumor tissue. Integrating CosMx WTX with H&E-guided annotation not only identifies the tumor-normal interface as critical for understanding progression but also highlights the potential for AI-powered diagnostic tools. By linking transcriptomic signatures to routine histopathological images, this work sets the stage for future advancements in automated cell typing and transcriptome prediction, transforming research in clinical workflows and precision oncology.

-Bruker Confidential-

#2069 Simultaneous, high-throughput detection of RNA and protein biomarkers using HCR™ Gold and the Orion™ imaging system for comprehensive analysis of tumor samples in clinical trials.

Jon J. Ladd[1], Adam Maddox[2], Aneesh Acharya[2], Arturo Ramirez[1]

[1]RareCyte, Inc., Seattle, WA,[2]Molecular Instruments, Inc., Los Angeles, CA

Background: Understanding the tumor microenvironment (TME) during cancer progression and treatment has been aided by multiomic analysis of tissues collected at initial patient screening and after treatment. Cyclic staining methods have recently demonstrated multiomic capabilities on a single tissue, but with drawbacks that can include tissue degradation and processing times up to several days. The Orion™ imaging system overcomes these limitations through single round staining and scanning of whole tissue samples of up to 18 biomarkers simultaneously. When paired with Molecular Instruments' HCR™ Gold RNA-FISH, a novel product featuring a truly protease-free workflow with next-generation amplification technology, transcriptomic and proteomic multiplex immunofluorescence (mIF) analysis can be carried out in parallel on a single tissue section in a straightforward, high-throughput approach to provide comprehensive insight into tumor biology and immune response.

Methods: A 16-plex multiomic assay featuring 5 RNA markers and 11 protein markers was developed to interrogate formalin-fixed paraffin-embedded (FFPE) tissue samples. Following dewaxing and antigen retrieval, HCR™ HiFi Probes against RNA targets of interest were incubated on the tissue sample followed by fluorescently-labeled HCR™ Gold amplifiers for signal generation. Subsequently, ArgoFluor™-conjugated antibodies directed to proteins of interest were incubated in a single cocktail on the same tissue, streamlining the staining procedure and decreasing downsides inherent to some amplification-based IF methods. Following coverslipping, whole tissues were scanned on Orion™ in one pass. Images were processed and interrogated with a custom analysis pipeline.

Results: HCR™ Gold RNA-FISH and mIF protein data for 16 biomarkers were obtained from human colon, lung, gastric, and pancreatic cancer tissues after a single round of staining and scanning. Immune cells were characterized within the TME based on biomarker expression. HCR™ Gold RNA-FISH was used to probe secreted factors, such as the cytokines IL-6, TNFα, and IFNγ , which were found to be colocalized with tumor-associated cells identified by protein mIF. Colocalization of RNA and protein markers differed across tissues.

Conclusions: The speed and versatility of Orion™ combined with the 1-step protease- and enzyme-free HCR™ Gold amplification enables development of custom assays to detect both protein and RNA markers on the same tissue in one cycle of staining and scanning. This powerful combination overcomes the limitations of low-plex cyclic staining and long protocol RNA-ISH methods to provide researchers and clinicians with a high-throughput method of translating basic research discoveries into assays for use in clinical trials and diagnostics.

#2070 Subcellular mapping of immune repertoire profiles with *in situ* sequencing on G4X.

Michael J. Lawson, Tung T. Le, Zane Hiatt, Ryan Shultzaberger, Kenneth Gouin III, Daan Witters, Eli N. Glezer

Singular Genomics, San Diego, CA

Detailed spatial analysis of immunology and pathology, such as immune repertoire profiling in tissue, requires *in situ* sequencing of variable gene regions. We introduce Direct-Seq, a novel technology using the G4X platform for *in situ* RNA sequencing at sub-cellular resolution, demonstrated by sequencing IgH and TCRB receptors in PBMCs and FFPE tissue.

We developed a probe panel that targets the conserved V and J-regions that flank CDR3 on IgH transcripts and a second panel that targets the V and C-regions that flank CDR3 and J regions on TCRB transcripts. PBMCs were seeded onto a glass substrate, followed by fixation. The diverse gap sequence was reverse transcribed into the probes, which were then clonally amplified. Clonal amplification products were sequenced *in situ* using Singular Genomics' SBS chemistry. We also applied the technique to tissue samples by transferring 5-µm thick FFPE tonsil sections to G4X slides, followed by deparaffinization and antigen retrieval. Amplicon preparation and sequencing were conducted in the same way as for PBMCs.

Direct-Seq successfully profiled ~ 60% of all B-cells (relative to CD19-positive fraction) and ~ 20% of all T-cells (relative to CD3-positive fraction) in PBMC populations. We observed the expected high diversity of clonotypes, with 92-98% of B and T-cells having distinct CDR3 sequences. Comparing multiple 50 bp reads within the same cell, we calculate the sequencing accuracy to be ~ 98%. For cells that had at least 1 read, we detected on average ~ 6 reads/cell for B-cells and ~ 2 reads/cell for T-cells. For FFPE tonsil samples we observed similar levels of diversity (94-99%) and accuracy (97-99%) as we did in PBMCs, but the detection efficiency was significantly lower, likely due to reduced RNA accessibility and degradation resulting from fixation.

In conclusion, Direct-Seq has demonstrated its unique capability for *in situ* sequencing of variable regions of IgH and TCRB transcripts in both PBMCs and FFPE tissues. This technology enables detailed mapping of immune cell clonality and diversity, providing a powerful tool for advancing our understanding of immunity and disease.

#2071 Spatial tracking of the pre-metastatic niche in colorectal cancer.

Chun Hua Hung[1], Chung-Ta Lee[2], Yu-Min Yeh[3], Chun-Hui Lee[3], Wen-Tai Liu[4], Meng-Ru Shen[5], Peng-Chan Lin[6]

[1]Department of Bioengineering, National Cheng Kung University, Tainan, Taiwan,[2]Department of Pathology, National Cheng Kung University, Tainan, Taiwan,[3]Department of Oncology, National Cheng Kung University Hospital, Tainan, Taiwan,[4]Department of Bioengineering, College of Engineering, National Cheng Kung University, Tainan, Taiwan,[5]Department of Pharmacology, National Cheng Kung University, Tainan, Taiwan,[6]Department of Genomic Medicine, National Cheng Kung University Hospital, Tainan, Taiwan

Lymph node metastatic positively correlates with poor prognosis of colorectal cancer. Thus, it is urgent to uncover the molecular mechanisms and develop better prediction criteria of the metastasis. Tumor budding refers to the presence of a cluster of tumor cells at the leading edge of tumor invasion and metastasis. This cluster is composed of either a single tumor cell or multiple tumor cells. Tumor budding is commonly observed in various types of cancer, including colorectal cancer (CRC), on histopathological slides. It is recognized as an independent predictive factor for lymph node metastasis and prognosis. In this study, the NanoString GeoMx equipment was applied to establish the expression profile of cancer transcriptomic atlas (CTA) in CRC specimens. According to the morphology of CRC tissues determined by markers including pancytokaretin, CD45, and alpha-smooth muscle actin, the regions of interest (ROIs) were categorized into four groups, including normal, deep tumor, superficial tumor, and budding-enriched area. With digital spatial analysis, we compared the CTA expression among the four groups of regions of interests (ROIs). We characterized a marker panel for tumor budding and poorly-differentiated clusters in CRC, which tracks the progression of CRC from normal part to tumor invasive front. Our data show that the tumor budding-enriched gene panel is primarily composed of genes regulating cytoskeleton, extracellular matrix (ECM), and some secreted factors. Furthermore, our data also indicate that a morphology-based gene panel better delineates the invasion progression. Those results allow us tracking the evolution of signaling transduction for metastasis from the primary tumor to tumor budding and subsequent distant metastasis, facilitating the development of targeted drugs aimed at preventing metastasis in colorectal cancer.

#2072 Spatial genomic ISH predicts prognostic significance of retinal degeneration protein 3 (RD3) transcriptional loss in colon cancer.

Natarajan Aravindan[1], Poorvi Subramanaian[1], Sreenidhi Mohanvelu[1], Sheeja Aravindan[2]

[1]Physiological Sciences, Oklahoma State University, Stillwater, OK,[2]OU Health Stephenson Cancer Center, Oklahoma City, OK

Our studies documented remarkable expression of Retinal degeneration protein 3 (RD3) in adult and fetal colon tissues and a significant loss in colon cancers. Further, in other solid cancers we recognized the function of RD3 in the disease pathogenesis. Here, we investigated the prognostic relevance of RD3 transcriptional loss in colorectal cancer, the second most common cause of cancer deaths. Additionally, we explored the real-time translational benefit of the spatial genomic ISH (RNAScope) as a tool for predicting the prognostic relevance and significance of RD3-loss in clinical outcomes. For this, tumor specimens (n=491) representing stage, primary/metastasis, diagnosis (Dx) or progressive disease (PD) from a test cohort of colon cancer patients (n=267) were assessed for RD3 status. Tissues in triplicate were custom archived in tissue microarrays (TMAs), and the spatiotemporal profiling of RD3 mRNA was performed using the chromogenic in situ hybridization (CISH) utilizing RNAScope with a custom designed target probe to human RD3 with PPIB and DapB (positive & negative) controls. Slides were scanned (40X, Zeiss Axio Scan) and group-analyzed for RD3 mRNA-specific positivity. Spatiotemporal distribution and quantitation of RD3 mRNA were performed using Halo image analysis configured to report copies per region of interest and probe copies per cell. RNAScope revealed significant fluctuations in RD3 transcription to cell and tissue-type, disease status (normal adjacent tissue to the tumor tissue), disease progression etc. Assessing the predictive relevance of RD3 transcription, our findings indicated a clear ‘low-is-worse’ association and RD3 profiling could serve as the independent variable (MANOVA) to predict disease aggression. The outcomes critically recognized that RD3 loss in cancers is significantly associated with the development of metastatic disease and could be designated as a prognostic marker for metastatic disease. Crucially, profiling RD3 transcription prior to and after therapy significantly recognized the anew loss of RD3 after the current first-line intensive multimodal clinical therapy. More importantly, a sizable low-is-worse correlation to the tumor recurrence, death, etc., Evidently, high levels of RD3 transcription exclusively displayed a significant association with better overall survival and showed that RD3 loss is an independent predictor of OS. Together, these results recognize transcriptional loss RD3 as an independent predictive biomarker for colon cancer progression and clinical outcomes. Further the results clearly imply that spatiotemporal RD3 RNAScope could serve as a clinical tool to predict disease evolution, that could lead to defined treatment stratification and better clinical outcomes in deadly colon cancer.

#2073 Technological benchmarking of integrated multiome analysis using multiplex immunofluorescence and spatial transcriptomics across cancer types.

Jonathan Chui[1], Asier Antoranz[1], Jon Pey[2], Nikolina Dubroja[1], Madhavi Dipak Andhari[3], Chiara Caprioli[4], Katy Vandereyken[1], Carmen Bravo Gonzalez-Blas[4], Julie Morscio[4], Alexandre Arnould[5], Pouya Nazari[4], Gautam Shankar[1], Bart de Moor[5], Thierry Voet[1], Francesca M. Bosisio[1], Frederik De Smet[1]

[1]KU Leuven, Leuven Institute for Single-Cell Omics (LISCO), Leuven, Belgium,[2]Intelligent Biodata, San Sebastian, Spain,[3]Department of Imaging and Pathology, KU Leuven, Translational Cell and Tissue Research Unit, Leuven, Belgium,[4]Department of Imaging and Pathology, KU Leuven, Laboratory for Precision Cancer Medicine (LPCM), Leuven, Belgium,[5]Dept. of electrical engineering, KU Leuven, Dynamical Systems, Signal Processing and Data Analytics (STADIUS),, Leuven, Belgium

Spatially resolved technologies are transforming cancer research by enabling the simultaneous visualization of molecular and cellular processes within tissue contexts. We performed a comprehensive benchmarking of various multiplex immunofluorescence (IF) platforms, including COMET™ and MILAN, and spatial transcriptomics technologies, including RNAscope™ and Xenium, and their combinations to evaluate their performance, compatibility, and potential for deep phenotypic profiling. Tissues from multiple cancer types were subjected to multiplex IF using the COMET and MILAN platforms. Complementary spatial transcriptomics analyses were performed using RNAscope and Xenium on the same sections. Performance metrics and cross-platform reproducibility were systematically assessed and the added value of evaluating protein and RNA markers simultaneously was also evaluated. To facilitate data processing and integration, we developed a novel bioinformatics software, DISSCOVERY, specifically designed for the integrated analysis of high-dimensional multiplex IF and spatial transcriptomics data. Each multiplex IF and spatial transcriptomics platform demonstrated unique strengths and limitations, both individually and in combination. Initial evaluations focused on the performance of each platform independently, followed by assessments of their combined application on the same tissue section. Notably, the combination of the Xenium and MILAN platforms provided unparalleled flexibility and scalability, enabling high-throughput analysis of both large marker panels and extensive sample sets. The COMET platform, which integrates RNAscope with multiplex IF, also proved highly valuable for its speed and automation, particularly in validating newly discovered mechanisms. Integration of these data through the DISSCOVERY software enabled seamless cross-platform analysis, uncovering spatial co-localization patterns and gene-protein associations. This approach allowed us to identify novel cellular niches and functional interactions across various cancer types, highlighting the powerful synergy of multiplex IF, IHC and spatial transcriptomics for advancing our understanding of cancer biology. Overall, our benchmarking underscores the complementary capabilities of multiplex IF and spatial transcriptomics technologies in dissecting the tumor microenvironment. The DISSCOVERY pipeline streamlines data integration, facilitating multi-omic insights into cancer biology. These findings provide a roadmap for selecting and combining spatial technologies to advance cancer research.

#2074 MALDI-ICC: a new tool for high-plex, multiomic profiling of single cells for cancer research and cancer therapy.

Mark J. Lim, Gargey Yagnik, Leonardo G. Dettori, Ziying Liu, Jonathan Bell, Kenneth J. Rothschild

AmberGen, Billerica, MA

Introduction: High-plex, multiomic profiling of single cells in cancer research and therapy is essential for understanding basic cancer mechanisms, discovery of new biomarkers and monitoring disease progression. Current profiling methods rely mainly on immunocytochemistry (ICC), multiparameter flow cytometry (MFC) and single cell RNA seq. However, these methods are limited by the number and/or classes of biomarkers that can be detected and/or inability to profile small molecular species such as lipids and metabolites. In contrast, a new method for high-plex, multiomic spatial imaging of single cells known as MALDI Immunocytochemistry (MALDI-ICC), based on novel photocleavable mass-tagged (PCMT) affinity probes in conjunction with single cell MALDI mass spectrometric imaging (MSI), facilitates the rapid profiling of individual cells at the proteomic, metabolomic, post-translational, and transcriptomic level in one integrated workflow on the same instrument.
Methods: A variety of cell types such as peripheral blood mononuclear cell (PBMC) suspensions spiked with HeLa and MCF7 cancer cells, and chimeric antigen receptor (CAR) expressing cells were profiled using MALDI-ICC. Cells were processed by randomly depositing and desiccating them on glass or conductive IntelliSlides followed by a modified immunostaining protocol that combined PCMT-antibody and PCMT-oligo probes. Label-free, untargeted MSI of lipids/metabolites was first performed using a Bruker timsTOF fleX followed by a second MSI scan after binding a high-plex panel of PCMT-probes. A dual-labeled fluorophore/PCMT histone-targeted antibody was also included for high resolution microscopy.
Results: High-plex PCMT-oligo and PCMT-antibody probe panels were utilized to target a variety of plasma membrane, cytoplasmic, and nuclear biomarkers at the mRNA and protein level, for several individual cell types, using the MALDI-ICC workflow. For example, MCF7 cells exhibited the epithelial marker E-cadherin, whereas HeLa did not but were detected with the mesenchymal marker vimentin, in full agreement with the Human Protein Atlas cell line database. Conversely, the lineage markers CD20 characteristic of B-cells and CD3 characteristic of T-cells were detected at the expected ratios for the cellular composition of the spiked PBMC. This method was further enhanced to detect >100 lipids/metabolites by performing an initial label-free, untargeted MSI scan before next implementing the MALDI-ICC protocol. AI clustering algorithms of all ‘omic’ layers revealed several distinct cell types.
Conclusions: These results demonstrate that MALDI-ICC can provide a new method for rapid, high-plex and multiomic profiling of the same cell population on the same instrument. Potential uses include profiling of cancer cells in the field of hematology/oncology and of CAR T-cells both during manufacture and after infusion into patients.

#2075 Combining Vizgen's MERSCOPE spatial transcriptomics profiling and InSituPlex (ISP) multiplex immunofluorescence (mIF) to unravel the complexity of the tumor-immune microenvironment (TiME).

Angela Vasaturo, Cassandra Kysilovsky, Lauren Duro, Sudhir Tattikota, Renchao Chen, Justin He, Manisha Ray, Jiang He He, George Emmanuel

Vizgen, Cambridge, MA

Despite remarkable results for many patients, cancer therapies have still a poor response rate. Biomarkers used to select patients for treatment are few and only single-biomarker assays are clinically approved. Such single-biomarker assays are unable to capture the complexity and dynamic nature of the TiME and fail to improve patients' clinical response rate. Here we aim to enable a comprehensive understanding of the molecular, morphological and functional landscape of tumors using a multi-omics approach combining spatial transcriptomics and proteomics assays. Vizgen's MERSCOPE® platform allows for high-resolution, spatially resolved analysis of up to 1000 genes, enabling the study of molecular and cellular signatures associated with the TiME while Vizgen's spatial proteomics ISP assays allow for ultrasensitive and high-throughput detection of tumor and immune cell interactions, functions and spatial distribution. We used these approaches to generate datasets from multiple tumors and evaluated the complementary nature of analytical spatial detection methods to elucidate how local microenvironment influence gene expression and protein activity. Formalin-fixed paraffin-embedded (FFPE) specimens from multiple tumors were cut into 5µm sections for both technologies. For mIF experiments, slides were stained with a cocktail of primary antibodies using Leica Biosystems BOND RX autostainer, amplified simultaneously, and imaged on the Zeiss AxioScan.Z1 using multiple rounds of detection. Images were co-registered and exported for analysis using Ultivue's STARVUETM image data science platform. Serial sections were profiled with Vizgen's MERSCOPE Platform and the 500 Gene Immune-Oncology (IO) Panel with MERFISH 2.0 chemistry. The resulting spatial transcriptomic data was segmented using Cellpose2 and analyzed using both UMAP and spatial projections for visualization. Concordance of RNA and protein results were established by evaluating the expression of markers present in both the 500-gene IO panel and the ISP probes. We then evaluated immune responses measured by gene signatures for multiple functions and the corresponding protein markers. We similarly examined changes between the tumor boundary and within the tumor mass. We demonstrate the ability to orthogonally validate findings and expand on the biomarker-driven protein data with pathway-level data from the transcriptomic data. The integration of gene expression profiling enabling screening of a broad range of targets, and mIF assays for target validation, allows for a holistic understanding of spatial and molecular insights of the TiME. These complementary technologies can be used in parallel for biomarker discovery and IO pathways interrogation providing synergistic insights into the complex and dynamic TiME.

#2076 Spatiotemporal mapping of colitis-associated colorectal cancer evolution.

Yoonho Lee[1], Yongjae Kim[1], Minseok Lee[2], Do Kyung Yoon[1], Jiwon Baek[1], Sung Wook Hwang[3], Jong Lyul Lee[4], Sang Hyoung Park[3], Dong-Hoon Yang[3], Jihun Kim[5], Suk-Kyun Yang[3], Buhm Han[2], Kyuyoung Song[1], Yong Sik Yoon[4], Byong Duk Ye[3], Ho-Su Lee[1]

[1]Department of Biochemistry and Molecular Biology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, Republic of,[2]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Korea, Republic of,[3]Department of Gastroenterology, Inflammatory Bowel Disease Center, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, Republic of,[4]Division of Colon and Rectal Surgery, Department of Surgery, Inflammatory Bowel Disease Center, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, Republic of,[5]Department of Pathology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, Republic of

Colitis-associated colorectal cancer (CAC) is a severe complication of inflammatory bowel disease, particularly ulcerative colitis. Despite its clinical significance, the spatiotemporal dynamics of neoplastic progression in CAC remain poorly understood. This study aimed to elucidate the evolutionary trajectory of CAC using spatial transcriptomics. We employed spatial transcriptomics to analyze four tissue samples from three patients with ulcerative colitis at various stages of neoplastic progression. Whole-slide spatial transcriptomics RNA sequencing was performed, enabling simultaneous analysis of gene expression and tissue architecture. Integrated spatial transcriptomic data revealed recurring epithelial programs along the progression of copy number variation (CNV). Our analysis using CNV score and hematoxylin and eosin staining image revealed progressive changes in gene expression patterns as tissue transitioned from non-tumor to pre-tumor and finally to tumor states. This progression was quantified using beta coefficient values, allowing us to track gradual alterations in gene expression across the disease continuum. We identified a core set of 412 genes that exhibited consistent changes in at least three of the analyzed samples. Among these, 349 genes showed a progressive decrease in expression, while 63 genes, including *CLDN1, E2F1,* and *RECQL4*, demonstrated a gradual increase in expression as the tissue advanced towards a tumorous state. These upregulated genes pointed to potential oncogenic drivers or compensatory mechanisms activated during tumor development. Pathway analysis revealed that downregulated genes were enriched in processes such as KRAS signaling, hypoxia, and inflammatory response, while upregulated genes were enriched in the MYC targets pathway. This study provides high-resolution mapping of evolutionary trajectory of CRC, revealing key molecular events and spatial features associated with neoplastic progression. Our findings enhance the understanding of CAC pathogenesis and highlight potential targets for early intervention and personalized treatment strategies, contributing significantly to the field and potentially informing future therapeutic approaches.

#2077 Elevated PI16 expression in fibroadenoma fibroblasts is associated with subsequent breast cancer development.

Pria Westerman[1], Nicole Cruz-Reyes[1], Camila Pacheco Erak[1], Robert Vierkant[2], Bryan McCauley[2], Stacey Winham[2], Mark Sherman[3], Amy Degnim[4], Derek Radisky[1]

[1]Department of Cancer Biology, Mayo Clinic College of Medicine and Science, Jacksonville, FL,[2]Department of Qualitative Health Sciences, Mayo Clinic College of Medicine and Science, Rochester, MN,[3]Department of Epidemiology, Mayo Clinic College of Medicine and Science, Jacksonville, FL,[4]Department of Surgery, Mayo Clinic College of Medicine and Science, Rochester, MN

Introduction: Fibroadenoma is the most common type of benign breast lesion and is not traditionally considered an indicator of increased breast cancer risk. This study aimed to identify biomarkers within fibroadenoma associated with subsequent breast cancer development using patient samples from the Mayo Clinic Benign Breast Disease (BBD) cohort.
Methods: We analyzed formalin-fixed paraffin-embedded (FFPE) breast biopsy samples from patients diagnosed with non-proliferative fibroadenoma who either developed breast cancer later (n=10) or remained cancer-free (n=5). NanoString RNA profiling identified differentially expressed genes in fibroblasts associated with subsequent cancer development. Immunohistochemistry was first done to compare overall expression of PI16 in this cohort. Opal multiplex immunofluorescence staining (mIF) was then performed to evaluate the expression of PI16 in fibroblasts (Vim^+PanCK^-) and assess immune cell infiltration ($CD45^+$) in different tissue compartments. Fibroblasts were identified by vimentin positivity (Vim^+), and epithelial cells were marked by pan-cytokeratin positivity ($PanCK^+$).
Results: Compared to patients who remained cancer-free, those who developed breast cancer showed significantly increased expression of PI16 in fibroblasts ($PI16^+Vim^+PanCK^-$, 3.25-fold increase, p=0.0011). Additionally, the number of immune cells ($CD45^+$) was significantly decreased (0.52-fold, p=0.03) in the fibroadenoma lesions of patients who later developed cancer.
Conclusions: Our findings suggest that elevated expression of PI16 in fibroblasts within fibroadenoma may serve as potential biomarkers for predicting breast cancer risk for women with fibroadenoma. PI16 encodes Peptidase Inhibitor 16, which is implicated in modulating fibroblast activity and extracellular matrix remodeling—processes that can contribute to a tumor-supportive microenvironment. Elevated levels of this protein in fibroblasts suggest an altered microenvironment that may foster tumor initiation and progression. The decreased immune cell infiltration further implies an immunosuppressive microenvironment that may facilitate cancer progression. As these markers can be detected in FFPE samples, our results could lead to the development of a readily applicable clinical test to identify fibroadenoma patients at increased risk for breast cancer, enabling earlier intervention and surveillance.

#2078 Single-cell spatial transcriptomics in colon adenocarcinoma reveals tumor heterogeneity and immune microenvironmental shifts.

Margaret Hoang, Yi Cui, Shanshen He, Michael Patrick, Megan Vandenberg, Stefan Rogers, Evelyn Metzger, Haiyan Zhai, Martin Shelton, Michael Rhodes, Joseph Beechem

Bruker Spatial Biology, Seattle, WA

Colon adenocarcinoma (CRC) features complex molecular changes and a dynamic remodeling of the tumor microenvironment (TME). Understanding spatial organization of gene expression in tumor and adjacent normal tissues is crucial for insights into tumorigenesis, immune modulation, and therapeutic resistance. Traditional methods like single-cell RNA sequencing (scRNA-seq) provide cellular insights but lack spatial context. Spatial transcriptomics addresses this gap, offering high-resolution, whole transcriptome analysis within tissue architecture. This study applies spatial transcriptomics to CRC, correlating molecular data with histopathological features to explore tumor heterogeneity, immune dynamics, and therapeutic targets. FFPE tissue sections (5 µm) from colon adenocarcinoma and adjacent normal tissues were analyzed using the CosMx® Spatial Molecular Imager (SMI) to create a spatially resolved whole transcriptome, single-cell map. The sections were stained with H&E for histopathological context, and spatial gene expression data was aligned with H&E-stained sections using custom software. Additionally, scRNA-seq was performed on a thicker curl (25 µm) from the same tissue block for comparative analysis. Spatial transcriptomic analysis revealed distinct gene expression patterns between tumor and normal regions, highlighting tumor heterogeneity with subpopulations of cancer cells showing varying differentiation states. Immune cell infiltration varied spatially, indicating diverse immune responses across the tumor. While both platforms identified similar transcriptional profiles, the CosMx Whole Transcriptome (WTX) panel preserved tissue architecture, allowing exploration of gene expression in its native context. CosMx WTX showed a more accurate cell composition ratio than scRNA-seq, which may introduce bias due to tissue dissociation. CosMx WTX also captured rare cell populations, such as tumor-infiltrating lymphocytes, often underrepresented in scRNA-seq due to sample size limitations. The spatial context enabled pathway activity analysis and ligand-receptor interaction mapping, providing deeper insights into tumor biology. This study highlights the power of spatial transcriptomics in colon adenocarcinoma, offering insights into tumor heterogeneity, immune infiltration, and the TME. Integrating spatial gene expression with histopathological features enhances understanding of tumor progression and immune modulation. The comparison with scRNA-seq underscores the unique benefits of spatially resolved profiling, presenting a more complete picture of cellular functions within their native tissue architecture. These findings emphasize the potential of spatial transcriptomics to advance cancer research, guide therapeutic strategies, and reveal novel insights into tumor biology.

#2079 Spatially guided single-cell analysis integrating spatial transcriptomics and spatial cell sorting for in-depth profiling.

Sumin Lee, Amos Lee

Meteor Biotech, Seoul, Korea, Republic of

Spatial transcriptomics technologies, such as Xenium, provide detailed spatial mapping of gene expression at the single-cell level, revealing intricate tissue organization and distinct cell populations. However, translating these spatial insights into actionable molecular information often requires further downstream analysis of specific cells of interest. To address this, we introduce an integrated workflow combining SLACS (Spatially-resolved Laser-Activated Cell Sorting) with Xenium-derived spatial data, enabling targeted isolation and in-depth transcriptomic analysis of defined cell populations. SLACS technology is a novel cell sorting method that utilizes laser-based activation for precise, spatially guided isolation of individual cells. In this study, we applied SLACS to samples previously analyzed using Xenium spatial transcriptomics, focusing on cell populations identified as functionally relevant or rare based on their spatial gene expression profiles. The targeted cells were isolated using SLACS, followed by high-resolution RNA-seq analysis to further characterize their unique transcriptomic signatures. Utilizing two distinct breast cancer samples analyzed via Xenium, we demonstrate the effective identification of regions of interest (ROIs) for SLACS-based isolation and subsequent high-resolution transcriptomic analysis. The integration of SLACS with Xenium spatial data significantly enhanced the resolution of single-cell transcriptomic analysis. In the luminal A sample, SLACS successfully isolated rare cell populations, including luminal B stem cells and CAFs, which exhibited distinct gene expression profiles. RNA-seq analysis revealed differential expression of key markers, such as ESR1 and PGR, which are crucial for understanding hormone receptor signaling pathways in luminal A breast cancer. In contrast, the TNBC sample displayed higher heterogeneity, with a prominent presence of EMT-transitioning cells and cancer cycling cells. These findings suggest active paracrine signaling between cancer cells and the surrounding stromal cells, contributing to the aggressive phenotype observed in TNBC. Our study demonstrates the powerful synergy of combining SLACS with Xenium spatial transcriptomics for targeted single-cell analysis. By leveraging spatial information to guide cell sorting, we achieved high-resolution characterization of rare and functionally distinct cell populations. This integrated approach provides new insights into the cellular and molecular mechanisms underlying breast cancer, highlighting key differences between luminal A and triple-negative subtypes.

#2080 Whole transcriptome single cell spatial imaging of tumor vs normal tissue microarray.

Wei Yang, Isabel Lee, Michael Patrick, Yi Cui, Shanshan He, Martin Shelton, Haiyan Zhai, Michael Rhodes, **Joseph Beechem**

Bruker Spatial Biology, Seattle, WA

Tissue microarrays (TMAs) are widely used for high-throughput research but present challenges due to variations in tissue fixation, morphology, and cellular composition, which can impact staining, RNA/protein preservation, and segmentation accuracy. Analyzing diverse tissue types, especially when comparing normal and tumor samples, requires advanced imaging and computational tools. The CosMx® Spatial Molecular Imager (SMI) addresses these challenges by enabling accurate definition of single cells with multi-tissue AI segmentation models and high-resolution data extraction from complex TMA samples. We employed the CosMx SMI with the CosMx Human Whole Transcriptome (WTX) panel, which targets approximately 19,000 RNA targets, to analyze human FFPE tissue microarrays. Two FFPE TMAs—one with normal and the other with cancerous tissue—were constructed with cores from multiple organs including breast, skin, lung, colon, liver, prostate, and brain. RNA was detected in its native tissue context, and hematoxylin and eosin (H&E) staining provided histological reference for spatial transcriptomics. Uniform Manifold Approximation and Projection (UMAP) and Leiden clustering were used for dimensionality reduction and cell type identification based on marker gene expression. Differential expression (DE) and pathway analyses identified molecular signatures and biological processes across tissue types. CosMx SMI achieved high cell counts per sample and precise segmentation, enabling reliable analysis of diverse tissue types. H&E staining on the same slide enhanced AI algorithm training, improving tissue structure identification and segmentation accuracy. We identified distinct cell types and tissue-specific biomarkers. DE and pathway analysis revealed potential therapeutic targets and biomarkers, such as PSA, PSMA, AR, PTEN, and ERG fusion in prostate cancer, along with key pathways like AR signaling and DNA damage repair. Transcript profiles localized to specific cell types, demonstrating the platform's robustness in spatial transcriptomics. CosMx SMI significantly enhances single-cell transcriptome studies, providing detailed molecular insights across diverse tissue types. This platform is a powerful tool for tissue analysis, biomarker discovery, and spatial genomics, supporting a wide range of molecular and cellular research applications.

-Bruker Confidential-

#2081 Tissue-niche-based and cell-type-selective proteomics.

Yi-Chien Wu[1], Elie Abi Khalil[1], Aditi Upadhye[2], Jalees Rehman[2], Nermin Kady[3], Ryan Wilcox[3], Steve Lee[1]

[1]Pharmaceutical Sciences, University of Illinois at Chicago, Chicago, IL,[2]Biochemistry and Molecular Genetics, University of Illinois at Chicago, Chicago, IL,[3]Internal Medicine, University of Michigan, Chicago, MI

Background: The spatial organization of cell populations within tissues is crucial for maintaining physiological function and understanding the mechanisms underlying various diseases. Proteins are the functional components that drive essential processes such as tissue homeostasis, disease progression, and therapeutic response. Spatial proteomics integrates molecular profiling with cellular location data, offering a powerful approach to decode these mechanisms. Here, we present an innovative spatial proteomics (SP) tool that combines widely adopted methodologies in biological research, enabling cell type-specific and comprehensive proteomic insights within tissue niches.

Method: Our approach utilizes thick tissue macrosections (≥300 µm) to precisely select regions of interest (ROIs) based on 3D multiplexed microscopy images. We perform microscopic photobleaching on the immunofluorescence-stained cells in ROIs to create a distinguishable fluorescent label for capturing their spatial context. These labeled cells are subsequently dissociated and collected via flow sorting. Lastly, we conducted LC-MS/MS analysis for a comprehensive spatial proteomic analysis of the collected cells.

Result: We confirmed that our SP technology does not significantly alter the protein expression in the samples. No significant changes in protein expression were observed in the heatmap and the volcano plot compared to the control samples. Using this technology, we investigated dendritic cell (DC) subsets in mouse spleen during inflammation. The result reveals significant proteome differences among three splenic DC subsets, categorized by their locations (“inside” or “outside” spleen T-cell zones) and control DCs. Our results align with published proteome data on inflammatory splenic DCs, demonstrating the feasibility and reproducibility of our technology. Importantly, we identified 50 proteins, including cytoskeletal proteins FSCN1 and SH3BP1, with differential expression between the “outside” and “inside” DC subsets in the inflamed spleen. These findings suggest varying migration capabilities between these subsets, providing potential therapeutic targets for inflammatory diseases. Additionally, we adapted this technology to study T cell-macrophage interaction in mouse peripheral T cell lymphoma and explore tumor cell heterogeneity in human tumors.

Conclusion: Our SP technology provides extensive protein profiling tailored to cell-type-specific spatial proteomics. It also demonstrates a broad applicability across diverse tissue specimens in various diseases. These findings hold promise for identifying novel cell types and potential drug targets to enhance treatment strategies for inflammatory diseases and cancers.

#2082 Spatial deconvolution of non-small-cell lung cancer tissues prior to immune checkpoint inhibitor therapy.

James Monkman[1], Chin Wee Tan[2], Rafael Tubelleza[1], Ken OByrne[3], **Arutha Kulasinghe**[1]

[1]University of Queensland, Woolloongabba, Australia,[2]WEHI, Melbourne, Australia,[3]Princess Alexandra Hospital, Woolloongabba, Australia

Introduction. Immune checkpoint immunotherapy (ICI) has shaped disease management for patients with non-small cell lung cancer (NSCLC). The blockade of the interaction between PD-1 and PD-L1 elicits pro-immunogenic tumour clearance in a subset of patients, however current methods to stratify patients likely to benefit from ICIs is lacking. This study leverages full tissue sections from first-line nivolumab treatment in stage IV or recurrent NSCLC to understand the properties of tissues from patients that benefit from ICI compared to those with refractory disease.

Methods. Pre-treatment tissues from patients that received first-line nivolumab in advanced stage setting were processed by high-plex immunofluorescence on the Akoya PhenoCycler platform. Patient response to ICI was grouped by RECIST 1.1 category (CR=1, PR=4, PD=4, SD=1) Supervised clustering was performed to phenotype broad cell lineages, and gaussian mixture models were fit to assign positive expression of functional markers. Spatial K-means clustering on tumour or non-tumour cells allowed tumour, non-tumour, and tumour-interface regions to be defined. Additionally, contours of tumour density were mapped as discrete regions. Cell frequencies and cell-cell interactions were considered in each of these spatial compartments to delineate the features that aligned with clinical outcomes.

Results. The staining panel allowed for deep phenotyping of $3.3x10^6$ tumour cells and $3.4x10^6$ non-tumour cells. Of immune cell lineages, we identify the most abundant macrophage populations express discrete mixtures of LAMP-1, HLA-DR, PD-L1, MMP9 and VISTA. CD8 T cells most commonly express granzyme-B and PD-1, with discrete populations of LAMP-1^+/HLA-DR^+, while CD4 T cells and Tregs express discrete mixtures of PD-1 and ICOS. Interestingly, both CD8 and CD4 T cells display activated $CD38^+$/$IFNg^+$ populations. Spatially, we observe that higher vasculature as well as macrophages positive for LAMP-1 and HLA-DR in both tumour and tumour-interface regions are associated with refractory disease. Additionally, activated $CD38^+$ CD4 cells are enriched in the stroma of ICI responsive patients. Higher levels of tumour cells positive for both PD-L1 and FOXP3 were also associated with poorer ICI response.

Conclusion. Our study showcases deep cellular phenotyping of large tissue specimens and utilises unbiased and automated methods to identify tissue regions for which to measure cellular features. We identify a number of properties that define the characteristics of NSCLC tissue in relation to therapy outcomes.

#2083 Activated fibroblasts in benign breast tissue are associated with subsequent breast cancer development.

Nicole Cruz-Reyes[1], Pria Westerman[1], Matilde Rossi[1], Camila Pacheco Erak[1], Melody Stallings Mann[1], Bryan McCauley[2], Robert Vierkant[2], Stacey Winham[2], Mark Sherman[3], Amy Degnim[4], Derek Radisky[1]

[1]Department of Cancer Biology, Mayo Clinic, Jacksonville, FL,[2]Department of Qualitative Health Sciences, Mayo Clinic, Rochester, MN,[3]Department of Epidemiology, Mayo Clinic, Jacksonville, FL,[4]Department of Surgery, Mayo Clinic, Rochester, MN

Introduction: Pseudoangiomatous stromal hyperplasia (PASH) is a benign breast lesion characterized by stromal myofibroblast accumulation. While most women with PASH do not develop breast cancer, a subset eventually does, often in the same breast where PASH was originally diagnosed, even after surgical removal of the lesion. This pattern suggests the presence of a field effect, where alterations in the PASH microenvironment extend beyond the lesion itself, potentially influencing neighboring breast tissue. This study aims to identify specific fibroblasts within PASH-containing tissues that may create a microenvironment conducive to cancer initiation.
Methods: We conducted a retrospective analysis of formalin-fixed, paraffin-embedded breast tissue samples from women diagnosed with PASH, comparing those who later developed breast cancer (cases) to those who remained cancer-free (controls). Using transcriptional profiling with NanoString IO360/BC360 panels, multiplex immunofluorescence (MxIF), and spatial transcriptomics via NanoString CosMX SMI, we investigated molecular and histopathological differences. We focused on the expression of fibroblast activation markers (ITGB1, PDGFRβ) and their spatial relationships with proliferating epithelial cells, as well as changes in extracellular matrix (ECM) composition and stiffness.
Results: Transcriptional profiling revealed significant upregulation of proliferation-associated genes in epithelial cells from cases. MxIF showed higher Ki-67 levels in epithelial cells from cases, with proliferating cells forming clustered regions. Activated fibroblasts expressing ITGB1 and PDGFRβ were markedly increased in cases and were located closer to proliferating epithelial cells. Spatial transcriptomics uncovered gene expression changes in fibroblasts indicative of an activated phenotype, and in epithelial cells, consistent with responses to increased tissue stiffness and mechanotransduction signaling. Polarized light imaging with picrosirius red staining revealed enhanced collagen cross-linking and thick fibril formation in cases, indicating greater ECM stiffness.
Conclusions: Our findings suggest that a subset of activated fibroblasts in benign breast tissue creates a pro-tumorigenic microenvironment through ECM remodeling and increased tissue stiffness, promoting epithelial proliferation and potentially driving cancer development. The spatial proximity of these fibroblasts to proliferating epithelial cells underscores their possible role in early carcinogenesis. This study introduces the novel concept of cancer-predisposing fibroblasts in benign breast tissue, providing new insights into microenvironmental changes that precede cancer and offering potential targets for early detection and intervention.

#2084 Development of a novel tri-omic spatial biology assay that provides in-depth immune profiling of the tumor immune microenvironment.

Arne Christians[1], Jannik Boog[1], Daniel Jimenez Sanchez[1], Sara Bodbin[2], Agata Wicher[2], Subham Basu[2], Aneesh Acharya[3], Randy Chen[3], Hong Liang[4], Shruti Shridar[4], Tan Char Loo[4], Ng Siok Bian[4], Michelle Poon[4], Anand Jeyasekharan[4], Oliver Braubach[1]

[1]Bruker Spatial Biology, St. Louis, MO,[2]Navinci Diagnostics, Uppsala, Sweden,[3]Molecular Instruments, Los Angeles, CA,[4]National University Cancer Institute Singapore (NCIS), Singapore, Singapore

Spatial biology has advanced our understanding of cancer biology and shed light on the immense transcriptomic and proteomic heterogeneity that exists within the tumor-immune microenvironment (TiME). To date, most analytical and diagnostic approaches only provide one type of biological readout per tissue section, and the generation of true multi-omic data has remained challenging. We developed a workflow that allows the integration of multiple omic modalities on a single section. The workflow is centered around a new signal removal strategy, EpicIF™ technology, which effectively and rapidly removes fluorophores while leaving tissue and probe chemistries unaffected. Using this workflow on the CellScape™ platform, we iteratively performed spatial proteomic, transcriptomic, and interactomic assays all on the same tissue sections. Formalin-fixed, paraffin-embedded tissue sections were subjected to standard histological processing and then incubated with primary antibodies against PD1 and PD-L1, which were then treated with oligonucleotide-modified secondary probes designed for an in situ Proximity Ligation Assay (isPLA) to detect PD1 and PD-L1 interactions. Next, the detection of RNA targets was accomplished by adding 12 HCR™ HiFi RNA-ISH probes, which were visualized by addition of target-specific fluorescently labeled HCR™ Gold amplifiers (RNA-FISH). Lastly, spatial proteomic labeling was conducted with VistaPlex™ multiplex immunofluorescence (mIF) panels targeting 30+ biomarkers. We deployed this tri-omic assay on samples of primary CNS lymphoma (PCNSL), a rare and aggressive form of B-cell lymphoma that remains an area of unmet clinical need, with over half of diagnosed patients succumbing to the disease. Unlike diffuse large b-cell lymphoma (DLBCL), its morphologically identical systemic counterpart, PCNSL shows sensitivity to PD1 checkpoint inhibition. We investigated if our assay could describe unique interactions between the PD1-PDL1 axis and the lymphoma TiME in PCNSL (n=24), in comparison to DLBCL (n=152) cases. High-resolution imaging with the CellScape platform allowed for precise detection and quantification of isPLA, RNA-FISH and mIF signals on the same slide. The combined assay showed differential biomarker expression and interaction profiles associated with immune-regulatory processes in the interrogated tumor samples, revealing remarkable inter- and intra-tumoral heterogeneity. This spatial multi-omics approach allows for a more comprehensive insight into the complex interplay of immune and non-immune cell populations in the TiME.

#2085 Visium HD combined with deep-learning-based cell segmentation on H&E images yield accurate cell annotation at single-cell resolution.

Tianyou Luo, Mario G. Rosasco, Christine M. Hoeman, Sonal Khare, Daniel J. Gorski, Tim A. Rand, Richard A. Klinghoffer, Justin Guinney, Chi-Sing Ho

Tempus AI, Inc., Chicago, IL

Background: Bulk and single-cell next-generation sequencing (NGS) have been instrumental tools for characterizing gene expression profiles of tumor samples. However, the lack of spatial and cellular context limits their utility in investigating tissue architecture and cellular interactions in the tumor microenvironment (TME). NGS-based Spatial Transcriptomics (ST) technologies have gained increasing attention for their ability to provide spatial context of gene expression, but they have been constrained by low resolutions until the recent launch of 10X Genomics Visium HD platform. This platform achieves whole-transcriptome profiling at 2 µm resolution. However, there are complications in downstream biological interpretations with the default binning of Visium HD at 8 µm resolution.
Methods: Primary tumor samples were collected from 2 patients with non-small cell lung cancer (NSCLC). The 10X Genomics Visium HD platform was used to generate high-resolution ST data. To study spatial expression at the single-cell level, we trained a cell-segmentation neural network and applied it to H&E images, generating cell segmentation masks. These masks were used to summarize raw Visium HD data at 2 µm resolution into single-cell-level gene counts. Clustering was performed on the generated single-cell level data and a large language model-based (LLM-based) cell type annotator was used to infer the cell types of each cluster.
Results: The LLM-annotated cell clusters from Visium HD were highly consistent with pathologist's annotations based on H&E images. Specifically, in the immune-active sample, 93.1% of cells in annotated lymphocyte regions were classified as lymphocytes based on cell-level ST counts, and 88.8% of cells identified in annotated tumor regions were classified as cancer cells. Cell-level ST clustering also identified a benign epithelium cell cluster that is confirmed by pathologist's annotations. Similarly, in the immune-inactive sample, 72.8% of cells in annotated tumor regions and 95.8% of cells in annotated stroma regions were classified as cancer cells and stromal cells respectively.
Conclusions: Our findings showcase the feasibility and advantages of analyzing Visium HD data at single-cell resolution using deep-learning-based segmentation models applied to H&E images, empowering clinical biomarker discovery and new mechanistic insights.

#2086 Multiplex spatial bioimaging and AI-based image analysis for precision cancer therapeutics.

A. Bose[1], E. Q. Alonzo[2], S. Struble[1], J. Fisher[2], R. A. Heil-Chapdelaine[1], N. F. Diaz Granados[1], V. Agrawal[1];
[1]Leica Microsystems, Inc., Waltham, MA, [2]Cell Signaling Technology, Danvers, MA

Breast cancer (BC) is a heterogeneous disease both at the molecular and clinical level. Worldwide, BC is the leading cause of cancer-related deaths in women. Besides conventional chemo/radio therapy, immunotherapy is one of the most promising choices for a small subset of responding individuals. However, these treatments often fail to prevent recurrence and metastasis. Disease heterogeneity in BC poses challenges for survival prediction, as patients with similar diagnoses often respond differently to treatment. In this study, we apply multiplexed spatial proteomics analysis on breast cancer tissue paired with normal tissue and observe multiple immune cell types and biomarkers that could provide insight into features of the tumor microenvironment (TME) that influence response to immunotherapy. Our findings show that systematic mapping of the TME by multiplexed spatial proteomics, could empower precision immuno-oncology. The Cell DIVE Multiplex Imaging Solution allows probing and imaging of dozens of biomarkers on a whole single tissue section with an iterative staining and dye inactivation workflow. At its core, Cell DIVE is a precise and adaptable open multiplexing solution that enables flexibility in antibody selection of biomarker panels used in a multiplexed imaging study. Cell Signaling Technology (CST) has a broad portfolio of IHC-validated antibodies to detect key proteins in the TME, enabling immune cell detection and phenotyping in tissue. CST offers off the shelf (OTS), ready-to-ship antibody conjugates that have been verified to work on Cell DIVE. CST employs a rigorous approach to IHC validation, followed by verification on the Cell DIVE platform to ensure successful detection of proteins. Here, we demonstrate multiplexed Cell DIVE imaging using a novel panel of dozens of CST biomarkers across human breast cancer tissue samples. In summary, multiplexed whole slide imaging using validated antibodies and Aivia, AI-based image processing software enables spatially resolved tissue analysis of the tumor microenvironment.

#2089 Spatial profiling of exhausted T cells using high-plex imaging mass cytometry.

Thomas D. Pfister, Jyh Yun Chwee, Qanber Raza, Nikesh Parsotam, David Howell, Liang Lim, Christina Loh

Standard BioTools, Markham, ON, Canada

The spatial organization and cellular composition of the tumor microenvironment (TME) has the potential to inform clinical and translational researchers about mechanisms of disease progression and therapeutic success. Of particular interest are immune cells, especially T cells, which can become exhausted due to chronic stimulation. This poses a significant challenge in cancer therapy as exhausted T cells have reduced effector functions and sustained expression of inhibitory receptors such as PD-1 and CTLA-4, resulting in failure to effectively eliminate tumor cells.

Imaging Mass Cytometry™ (IMC™) technology is a high-plex imaging technique that enables deep characterization of the heterogeneity and complexity of the TME. The Hyperion™ XTi Imaging System utilizes IMC technology to provide signal intensities over a wide dynamic range and entails one-step detection of 40-plus markers without issues of tissue autofluorescence, making it ideally suited for spatial biology applications. Whole slide imaging modes and an automated slide loader function enable a streamlined, versatile scalable workflow for high-throughput analysis.

We used IMC technology to characterize immune cell populations and the spatial distribution of T cell exhaustion markers across various cancer types. We applied a 40-plus-marker IMC panel by integrating the Human Immuno-Oncology IMC Panel with the Human T Cell Exhaustion IMC Panel to study the TME of multiple human tissues.

IMC analysis revealed striking heterogeneity with distinct tumor and immune-rich niches in the TME. In colon adenocarcinoma, we detected functional effector T cells and multiple tertiary lymphoid structures (TLSs). In contrast, we observed fewer TLSs and more T cells expressing PD-1 and TIM-3 at the tumor periphery in the urothelial carcinoma tissue, suggesting that they are in advanced exhaustion stages. We also observed expression of PD-L1, IDO and VISTA in the tumor cells. t-SNE and PhenoGraph clustering analysis of Cell Mode data segregated and spatially resolved the T cells into effector and exhausted T cell subpopulations, which were mapped back to the segmented cell masks. Unsupervised pixel clustering and analyses of Tissue Mode data further delineated distinct tumor areas based on the presence of infiltrating immune cells, tumor replicative activity and spatial proximity to stromal components.

This work characterizing T cell exhaustion markers in multiple cancers showcases the capabilities of IMC technology and establishes it as a reliable high-plex, high-throughput spatial biology imaging platform. IMC technology is ideally suited for developing future translational and clinical applications and has the potential to help guide personalized therapeutic strategies for cancer treatment.

For Research Use Only. Not for use in diagnostic procedures.

#2090 Integrative spatial and single-cell analysis elucidates tumor microenvironment heterogeneity in NSCLC.

Qiming Zhou[1], Jiadong Zhou[2], Beixi Wang[2], Jingwen Shi[2], Han Yan[2], **Xin Chen**[3], Yun Zhang[2], Pei Zhang[2], Zhirong Shen[1]

[1]BeiGene (Shanghai) Co., Ltd., Shanghai, China,[2]BeiGene (Beijing) Co., Ltd., Beijing, China,[3]BeiGene USA, Inc, Ridgefield Park, NJ

Background: Tumor microenvironment (TME) heterogeneity plays a crucial role in cancer progression and therapeutic response. Advanced multi-omics spatial and single-cell technologies offer unprecedented resolution for dissecting TME complexity. However, the application of these technologies to formalin-fixed paraffin-embedded (FFPE) samples remains underexplored. This study aims to demonstrate the capabilities of these technologies in analyzing FFPE samples, as well as to benchmark and cross-validate different platforms and assays, and deepen our understanding of TME heterogeneity at the single-cell level and its spatial context.
Methods: We performed following assays including 1) High-plex multiple IHC platform: PhenoCycler Fusion (PCF) protein-level spatial analysis (n=10), 2) FFPE Single-Cell RNA Sequencing (FFPE scRNA-seq) (n=4) and 3) Bulk RNAseq RNA-level analysis with FFPE NSCLC samples.
Results: Firstly, to evaluate the PCF marker expression pattern, cell density of PanCK+PD-L1+ cell ratio (%) was compared to PD-L1 IHC TC score, which showed a high correlation. Further, By image co-registration between H&E with ML-based tumor/stromal classification and PCF PanCK-PD-L1 staining, PD-L1+ cell density in tumor cells specifically can be precisely calculated. To further evaluate the marker gene expression consistency, high gene expression concordance was observed across PhenoCycler Fusion (PCF), formalin-fixed paraffin-embedded (FFPE) single-cell RNA sequencing (scRNA-seq), and bulk RNA sequencing (bulk RNA-seq) data. This high concordance (average pearson r=0.84) validates the consistency and reliability of our multi-omics approach, ensuring that the data obtained from different sample types are comparable and robust. A diverse TME was identified, highlighting the complexity and heterogeneity of TME components. This diversity was evident in the range of immune cell subsets and cancer-associated stromal cells detected, underscoring the importance of comprehensive TME analysis even in small cohorts. In addition, FFPE scRNAseq enabled a deeper profiling at transcriptome level with a median gene recovery rate at 1130 genes per cell, which contribute to a more detailed cell lineage identification including small populations like Mast cells and neutrophils.
Conclusions: Overall, the study demonstrates the effectiveness and reliability of PCF in evaluating PD-L1 expression and the TME. The high concordance across multi-omics approaches and the detailed transcriptome profiling provided by FFPE scRNAseq highlight the robustness of these latest methodology on FFPE samples. Despite the limited sample size, the diverse TME identified emphasizes the need for comprehensive and personalized TME analysis. These findings provide a foundation for further investigations into the TME.

#2091 Integrating single-cell spatial whole transcriptome and histopathology to uncover drivers of tumor heterogeneity in lung adenocarcinoma and squamous cell carcinoma.

Michael Patrick, Liang Zhang, Patrick Danaher, Shanshan He, Sierra Mckinzie, Haiyan Zhai, Michael Rhodes, Joseph Beechem

Bruker Spatial Biology, Seattle, WA

Lung cancer, the leading cause of cancer-related deaths worldwide, primarily consists of non-small cell lung cancer (NSCLC), including subtypes like lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). These subtypes exhibit distinct histopathological features but share overlapping molecular characteristics. Understanding the spatial and molecular heterogeneity of these tumors is critical for identifying novel biomarkers and therapeutic targets. This study utilizes the CosMx® Spatial Molecular Imager (SMI) platform to profile LUAD and LUSC, aiming to uncover shared and unique gene signatures, tumor heterogeneity, and rare tumor cell subtypes, while integrating histopathological insights from H&E staining. Two formalin-fixed, paraffin-embedded (FFPE) lung tumor samples—one LUAD and one LUSC—were profiled using the CosMx Whole Transcriptome (WTX) panel, which profiles approximately 19,000 genes with single-cell subcellular resolution. Spatial transcriptomic data were analyzed using Uniform Manifold Approximation and Projection (UMAP) for dimensionality reduction and Leiden clustering to identify cell clusters. Marker genes were used to annotate cell types and generate a high-resolution cellular atlas of the tumor microenvironment (TME). Hematoxylin and eosin (H&E) staining was performed on the same slides, providing histopathological context and validating CosMx-derived cell types, including immune and tumor hotspots. The alignment of H&E and CosMx SMI data highlights their potential for AI model training that integrates spatial transcriptomics and histopathology. Our analysis revealed shared and unique gene signatures between LUAD and LUSC. Shared signatures included common tumorigenic processes such as cell proliferation, immune modulation, and angiogenesis. Unique signatures distinguished each subtype, with LUAD enriched in epithelial-to-mesenchymal transition pathways, while LUSC showed upregulation of squamous differentiation markers. UMAP and Leiden clustering identified distinct tumor and immune cell subpopulations, demonstrating spatial and molecular heterogeneity with clear immune and tumor hotspots. CosMx SMI data and H&E staining corresponded well, suggesting their potential for AI model integration. Integrating spatial transcriptomics and histopathology using CosMx SMI provided valuable insights into the molecular and spatial complexity of LUAD and LUSC. This study identified rare tumor cell subtypes and mechanisms of tumor progression, underscoring the
-Bruker Confidential-potential for AI-driven biomarker discovery and therapeutic strategies in NSCLC and beyond.
-Bruker Confidential-

#2092 Multiomic fluorescent co-detection of biomarkers in the tumor microenvironment using a new RNAscope™ assay on Roche DISCOVERY™ ULTRA.

Renzo Adilardi[1], Rose Delvillar[1], Wendy French[2], Qi Shen[1], Emerald Doolittle[1], Anushka Dikshit[1], Henry Lamparski[1], Maithreyan Srinivasan[1]

[1]Advanced Cell Diagnostics, Inc., Newark, CA,[2]Roche Tissue Diagnostics, Tucson, AZ

Understanding the interactions between different cell types in the tumor microenvironment (TME) is crucial for advancing cancer and immuno-oncology research. New spatial transcriptomic and proteomic technologies enable a context-based study of the TME. Automated RNAscope™ assays allow high throughput RNA *in situ* hybridization (ISH) and can be coupled with immunolocalization of proteins within the same tissue section. However, visualization of multiple biomarkers with standard chromogenic detection becomes challenging as the number of targets increases, due to the lack of color contrast for resolving different signals. Using fluorescent dyes enables imaging of each target in an independent channel and overlaying of each channel for the combined interpretation. Fluorescent analysis allows for greater confidence of signal identity when analyzing and quantifying complex cellular interactions. This is especially relevant in cancer research, where ISH can be used to visualize expression of multiple tumor biomarkers and combined with immunofluorescence (IF) to provide comprehensive information about the interaction between a tumor, infiltrating cells, and the surrounding environment. We developed a new assay enabling fluorescent detection of multiple RNAs that expands the current RNAscope™ assay capabilities on the Roche DISCOVERY™ ULTRA System. Here, we introduce a new, fully automated protease-free workflow to enable the fluorescent co-detection of multiple RNAs and proteins. This workflow includes harmonized cell conditioning for effective combined RNA and antigen retrieval, and a new pretreatment reagent, the protease-free VS PretreatPro, allowing for RNA detection without disruption of protease-sensitive epitopes. Following tissue pretreatment, new RNAscope™ Duplex Fluorescent reagents were used to detect two target RNA species with fluorophore-conjugated tyramide dyes, followed by sequential detection of multiple proteins by IF in the same sample. To demonstrate the capabilities of this new assay, we analyzed the interaction of tumors with their microenvironment by co-detecting tumor expression of hypoxia markers as *HIF1A* and *VEGFA*, and chemokines as *CXCL9*, *CXCL10* by ISH, followed by immunofluorescent detection of cell-surface markers to identify cells within the TME, including CD163+ macrophages, CD3+ T cells, as well as protease-sensitive targets, including CD45 (pan-hematopoietic) and CD20 for identification of B cells. Further, we analyzed the anti-tumor response and immune checkpoints identifying the expression of *IFNG*, *GZMB*, *LAG3*, and *CTLA4* by ISH, together with PD-1 and PD-L1 by IF. The combination of multiple dyes produced high-intensity signals and negligible background. This new automated multiomic assay demonstrated high sensitivity and specificity for the detection of diverse targets in tumor tissue.

#2093 Evaluation of IDH1, EGFR, IGF1R and Ki67 biomarkers in glioblastoma using Lunaphore COMET platform.

Jennifer C. Jones[1], Ashley Oliver[2], Jodi Hagen[2], Kristine Trueman[2], Grace Leland[2], Alexander Kalyuzhny[2]

[1]Bio-Techne Denver, Centennial, CO,[2]Bio-Techne Corporation, Minneapolis, MN

Glioblastoma (GBM) is the most aggressive primary neuroepithelial tumor diagnosed in the United States with approximately 14,000 diagnoses each year. Patients with GBM have a poor prognosis and only 5% of these patients survive for more than 5 years. Diagnosis of GBM requires tumor biopsies with consideration of histopathological and molecular characteristics. Typically, IHC is done using a single-biomarker HRP-DAB detection technique which is not suitable for the simultaneous spatial analysis of multiple biomarkers. Specific markers involved with the histopathology of glioblastomas include Isocitrate Dehydrogenase 1 (IDH1), Epidermal Growth Factor Receptor (EGFR), Insulin Like Growth Factor 1 Receptor (IGF-1R), and a cell proliferating marker, Ki67.

A deeper understanding of the pathology of GBM has the potential to improve early diagnosis and treatment options for patients. The complex nature of the tumor microenvironment (TME) makes spatial multiplex immunohistochemistry (IHC) and immunofluorescence (IF) ideal for visualizing biomarker expression and cellular localization. Emerging automated staining and imaging platforms rely on validated antibodies for accurate marker detection. Lunaphore COMET™ is a fully-automated, high-throughput, hyperplex platform for spatial analysis of Formalin Fixed Paraffin Embedded (FFPE) and frozen tissues. It performs sequential immunofluorescence (seqIF™) assays through cycles of staining, imaging, and elution.

Antibody specificity, sensitivity, and availability for multiplex IF is a critical success factor for TME characterization. We use a systematic process to qualify antibodies for use on COMET™ which includes specificity on a positive tissue sample, sensitivity using different antibody dilutions and elution efficiency using secondary antibody only incubation. In this study, we evaluated IDH1, EGFR, IGF-1R and Ki67 expression in GBM on COMET™. This will streamline multiplex panel development for spatial analysis of GBM to understand biomarker expression at the single-cell level.

#2094 Development of a multiomic workflow using Oncore Pro X to stain tissues for high performance Dual Channel™ Validation of RNA expression with downstream quantitation using AI-based image analysis with Oncotopix Discovery™..

Randy Chen[1], Aneesh Acharya[1], Daniel Winkowski[2], Jason Ramos[3], Harry Choi[1], **Regan Baird**[2]

[1]Molecular Instruments, Los Angeles, CA,[2]Visiopharm, Arvada, CO,[3]Biocare, Pacheco, CA

Accurate quantification of RNA expression remains a critical challenge in advancing RNA-ISH multiomic technologies for translational and clinical applications. Current barriers include lack of guidelines for assay validation and difficulties in correlating RNA abundance with spatial localization. In this study, we introduce a robust pipeline leveraging Dual Channel™ Validation (DCV) of HCR™ HiFi Probes and AI-enabled analysis to enable relative quantification of RNA abundance.

The DCV approach uses two spectrally distinct fluorescent channels to confirm the specificity and sensitivity of RNA detection. This dual-channel strategy minimizes false positives while providing a reliable proxy for RNA transcript abundance through fluorescent intensity correlations. To enhance RNA quantitation, our pipeline integrates automated tissue staining for consistent and high-quality labeling plus advanced AI-based algorithms trained on combined cellular nuclear plus membrane segmentation markers to precisely delineate cell boundaries. This dual-marker framework addresses limitations of rule based nuclear expansion-based segmentation methods and improves the accuracy of RNA spot assignment.

Our results demonstrate that DCV, combined with autostaining and AI-enabled cellular segmentation, achieves superior quantification fidelity. This approach not only validates RNA-ISH assay performance but also provides a scalable, reproducible method for relative RNA quantitation across diverse tissue types. By coupling high-performance probes with optimized automated tissue staining (on Oncore Pro X) and advanced computational analysis, our pipeline bridges critical gaps in RNA quantification, facilitating reliable, clinically meaningful insights into RNA expression within complex tissue environments.

CLINICAL RESEARCH: Spatial Proteomics and Transcriptomics 3: Other Topics/Biomarkers
Poster Session

#2098 Development and testing of specimen-protecting microscopy consumables that enable the automation of cyclic immunofluorescence methodology.

Michael Craft[1], **Noemi Kedei**[2], Nels Wedin[1], Geoffrey K. Feld[3], Nikolay Samusik[1]

[1]Parhelia BioSciences, Austin, TX,[2]National Cancer Institute, Bethesda, MD,[3]Geocyte LLC, Columbus, OH

Introduction: Spatial biology provides unparalleled proteomic and transcriptomic information on the organization of the tumor microenvironment. However, the manual handling and processing of microscope slides hinder the clinical impact of cutting-edge techniques.
Experiment and Data: We introduce and test “image-ready” coverpads to automate sample preparation workflows for multiplex immunofluorescence and immunohistochemistry methods on the Parhelia Spatial Station™ system to acquire single cell level spatial information on whole sides. These “permanent” coverpads with 0.07 mm spacers and thickness No. 1.5 simultaneously provide a reaction chamber for capillary-gap application of staining and washing reagents while protecting specimens from damage generally occurring during manual handling, and allow for imaging without the need for coverslip exchange. Image-ready coverpads were applied to different cyclic immunofluorescence (CycIF) assays using automation on the robot, whereby reagents (multiple antibodies or reporters) are cyclically applied, imaged, stripped, and re-applied to build a larger multiplex target panel. The final step in each cycle of these methods is to immerse the specimen in mounting medium and apply coverslip for imaging. Image-ready coverpads combined with Parhelia’s mounting medium that is fluid enough to be applied by the Spatial Station, allowed multi-cycle imaging on multiple microscopes/scanners (Zeiss Axioscanner, Phenocycler Fusion using adapted settings) without fading and tissue damage, and also saving significant amount of time by not having to remove coverslips. These experiments demonstrate the coverpad utility in efficient and consistent antibody staining in cyclic methods. Ongoing studies with assay manufacturers and academic collaborators will further extend and optimize automated protocols to other assays, expanding the utility of these coverpads to a menu of technologies.
Conclusion: Overall, Image Ready coverpads provide a facile mechanism enabling the standardization of cyclic immunofluorescence and immunohistochemistry techniques, an essential milestone in translating spatial biology from research to clinical settings.

#2099 Leveraging spatial transcriptomics and proteomics to assess the Immune response and DNA damage in high grade serous ovarian cancer (HGSOC) treated with neoadjuvant chemotherapy.

Dhruva Dave[1], Natalie R. Gassman[2], Emily O'brein[1], Fibiana Oladipo[1], Rebecca Arend[1]

[1]Obstetrics and Gynecology, University of Alabama at Birmingham, Birmingham, AL,[2]Pathology, University of Alabama at Birmingham, Birmingham, AL

Ovarian cancer is the sixth leading cause of cancer-related deaths among women in the United States. Many women (>80%) with OC are diagnosed at an advanced stage and a 5-year survival rate of less than 30%. Newer therapies, such as antibody-drug conjugates (ADCs), have revolutionized the treatment landscape for solid tumors, including ovarian cancer. While platinum-based chemotherapy stays the stalwart in systemic therapy for OC treatment, most of these patients experience recurrence (>50%) despite receiving standard of care therapy. Hence, significant efforts are made to target candidates for a personalized approach to therapy. 9 eligible patients with biopsy confirmed, newly diagnosed, advanced-stage serous EOC deemed appropriate for NACT seen at the University of Alabama at Birmingham Gynecologic Oncology clinic were recruited. EMR was reviewed and demographic, survival, surgery, and tumor response data were collected. Target receptor expression pre and post ADC treatment was also evaluated using IHC staining of the target protein. Actively, we are using spatial transcriptomics and proteomics to assess DNA damage and tumor immune microenvironment (TIME) pre and post NACT. Repair assisted damage detection (RADD) assay, The Lunaphore COMET and Nanostring GeoMx platform will all be utilized to perform this analysis. The RADD assay gives a DNA damage score that will be compared pre- and post-treatment. The Lunaphore COMET antibody staining panel will include antibodies against the surface and intracellular proteins pan-cytokeratin, CD3, CD4, CD5, CD8, CD20, CD25, CD31, CD68, DAPI, Granz B, FOXP3, CXCR3, CXCR5, CCR6, CTLA4, PD-1, PDL1, TIM3, and pan-cytokeratin, allowing us to evaluate changes in subpopulations within the tumor immune microenvironment in response to ADCs in combination with platinum therapy. NanoString GeoMX platform will use targeted transcriptomic sequencing to further evaluate PanCK+ tumor cells, CD20+ B cells, and CD8+ T cells, or subpopulations identified from COMET results. Results Median age of this cohort was 68.5 (52-81), most patients were white (n=7), all patients had stage III or IV disease (n=9). Based on RECIST 1.1 criteria, median change in tumor burden from baseline was -19.31% (+9.81% to -85.64%), showing the impact of NACT. The secondary outcomes of this study are to identify predictive biomarkers as potential therapeutic targets through analysis proteomics and transcriptomics. ADCs have demonstrated potential to monitor change in tumor burden, thereby validating the efficacy of targeted therapy based on protein or receptor expression in OC. Utilizing technologies such as COMET and Nano-string, our results will help us understand the intricacies of NACT therapy in the context of ADCs and contribute to pave the way for a more tailored therapeutic approach.

#2100 Integrative spatial analysis reveals tumor heterogeneity and immune colony niche related to clinical-outcomes in small cell lung cancer.

Chaoqiang Deng[1], Changbin Zhu[2], Tao Hu[2], Jing Wang[2], Haiquan Chen[1]

[1]Fudan University Shanghai Cancer Center, Shanghai, China,[2]Amoy Diagnostics Co., Ltd., Shanghai, China

Recent advances have shed light on the molecular heterogeneity of small cell lung cancer (SCLC), yet the spatial organizations and cellular interactions in tumor immune microenvironment remain to be elucidated. Here, we employed co-detection by indexing (CODEX) and multi-omics profiling to delineate the spatial landscape for 165 SCLC patients, generating 267 high-dimensional images encompassing over 9.3 million cells. Integrating CODEX and genomic data revealed a multi-positive tumor cell neighborhood within $ASCL1^+$ (SCLC-A) subtype, characterized by high SLFN11 expression and associated with poor prognosis. We further developed a cell colony detection algorithm (ColonyMap) and revealed a spatially assembled immune niche consisting of antitumoral macrophages, $CD8^+$ T cells and natural killer T cells (MT^2) which highly correlated with superior survival, and predicted improving immunotherapy response in an independent cohort. This study serves as a valuable resource to study SCLC spatial heterogeneity and offers insights into potential patient stratification and personalized treatments.

#2101 Absolute lymphocyte count after CAR-T therapy has different kinetics and predicts better outcomes in B-cell malignancies.

Juan Esteban Velez-Hernandez[1], Theresia Akhlagi[2], Caitlin Unkenholz[3], Juan Harvey Martinez[1], Juan Jose Fernandez-Rengifo[2], Christian A. Gordillo[4], Hei Ton Chan[4], Markus Mapara[4], Giorgio Inghirami[2], Ran Reshef[4], Samuel Yamshon[2], Mateo Mejia Saldarriaga[2], **Mark Bustoros**[2]

[1]Hospital Alma Mater, Medellin, Colombia,[2]Weill Cornell Medicine, New York, NY,[3]Weill Cornell Medical College, New York, NY,[4]Columbia University Irving Medical Center, New York, NY

Introduction: Chimeric antigen receptor (CAR) T-cell therapy targeting CD19 and BCMA antigens are now a valuable option for patients with relapsed or refractory (R/R) B-Cell malignancies Commercially available products consist of an antigen binding domain against CD19 or BCMA and one of two main co-stimulatory molecules, CD28 (Axi-Cel , Brexu-Cel) and 41-BB (Tisa-Cel, Liso-Cel, Cilta-cel, and Ide-cel). In this analysis, we studied the prognostic impact of absolute lymphocyte count (ALC) in patients with B-Cell malignancies treated with CAR-T therapy and investigated the differences in ALC kinetics between the products and co-stimulatory molecules.
Methods: We analyzed data from patients with R/R B-Cell malignancies treated with CAR-T therapy from 2017 to 2024 at our institutions. All relevant baseline characteristics were collected. ALC_{max} was defined as the highest ALC from day 0 through 15, not attributable to leukemic disease. The ALC values and kinetics were compared among products and co-stimulatory molecules. Survival and Cox proportional hazards analyses were performed with PFS as an outcome.
Results: We identified 245 patients with B cell malignancies (DLBCL, MCL, FL, CLL, and MM). The most common malignancy was DLBCL (62%) followed by MM (26%). Most used product was Axi-cel 119 (49%) in the lymphoma cohort in comparison to cilta-cel in the MM cohort. ALC_{max} was significantly higher in Liso-Cel and Tisa-Cel compared with Axi-cel and Brexu-Cel treated patients ($p < 0.001$). ALC_{max} was also higher in CD19 and BCMA 4-1BB CAR- T products compared to CD19-CD28 products (0.76 vs 1.28 vs 0.59 x 10^3/uL, respectively) ($p < 0.001$). In Univariate analysis of DLBCL patients treated with any of the CD19 CAR-T products, higher ALC_{max} was associated with improved PFS (HR: 0.71, CI: 0.5- 0.9), while an $ALC_{max} < 0.5$ x 10^3/uL was associated with worse PFS (Hazard ratio (HR) 2.2, CI 1.13 - 3.18). This ALC_{max} cutoff was still associated with worse PFS in CD19 CAR-T products with either CD28 or 41-BB co-stimulatory molecules. Similarly, ALC_{max} was associated with better PFS after accounting for lines of therapy and high-risk cytogenetics in MM patients treated with the BCMA CAR-T products (HR: 0.42, CI: 0.26 - 0.78, $p = 0.001$). In the DLBCL cohort, $ALC_{max} < 0.5$ x 10^3/uL and high LDH were independent risk factors of worse PFS after accounting for age, stage, ferritin levels. Flow cytometry data showed the ALC_{max} was positively correlated with CAR-T cells expansion in both MM and DLBCL.
Conclusions: ALC_{max} was associated with better PFS in DLBCL patients treated with both types of CD19 products and in MM patients treated with BCMA-41-BB CAR-T products. This data suggests that ALC_{max} is a good marker for response to CAR-T therapy and encourages better understanding of the factors influencing ALC levels between the CAR-T products and disease subtypes.

#2102 A comparative study of tissue and blood samples reveals the frequency and characteristics of BRCA and PALB2 mutations in Chinese pancreatic cancer.

Yue Zhu, Yanli Wang, Wenfen Lu, Zhihui Lu, Guihe Huang

Guizhou Aviation Group No.302 Hospital, Guizhou, China

Background and Purpose: Pancreatic cancer is a highly lethal malignancy often associated with genetic mutations, particularly in the BRCA1, BRCA2, and PALB2 genes. Identifying mutations in these genes is crucial for understanding the genetic basis of the disease, guiding treatment decisions, and providing genetic counseling. This study aims to evaluate the mutation frequency and types of BRCA1, BRCA2, and PALB2 in pancreatic cancer patients using both tissue and blood samples through next-generation sequencing (NGS) with a 733-gene panel.
Methods: A total of 1425 pancreatic cancer patients were included in this study, with 1228 patients undergoing tissue-based NGS and 197 patients undergoing blood-based NGS. The mutation frequencies, along with the proportions of germline and somatic mutations for BRCA1, BRCA2, and PALB2, were calculated and analyzed.
Results: The overall mutation frequency for BRCA1 was (772/1425) 54.18%, with (762/772) 98.7% being germline mutations and (10/772) 1.3% being somatic mutations. In tissue samples, the BRCA1 mutation detection rate was (769/1228) 62.62% (germline: 98.83%; somatic: 1.17%). However, the blood sample analysis yielded a significantly lower detection rate of (3/197) 1.52%, comprising 66.67% germline and 33.33% somatic mutations. For BRCA2, the mutation frequency was (136/1425) 9.54%, with 79.41% being germline alterations; the rates were (132/1228) 10.75% in tissue (germline accounted for 78.79%) and (4/197)2.03% in blood samples, the latter consisting entirely of germline mutations. PALB2 mutations had a frequency of (61/1425) 4.28%, with germline alterations accounting for 93.44%. Tissue analysis showed a PLAB2 mutation detection rate of (59/1228) 4.8% (germline accounted for 93.22%), whereas blood samples showed a rate of (2/197) 1.02%, all being germline mutations. Notably, nonsynonymous mutations were the most prevalent mutation type across all genes in both tissue and blood analyses.
Conclusion: The study reveals significant differences in the mutation frequencies and types of BRCA1, BRCA2, and PALB2 detected in pancreatic cancer patients, highlighting higher rates in tissue samples compared to blood samples. The predominance of germline mutations, particularly in BRCA1 and PALB2, emphasizes the importance of genetic testing for personalized treatment strategies and risk assessment in this patient population. These findings contribute valuable insights into the genetic landscape of pancreatic cancer and underscore the need for further investigation into the clinical implications of these mutations.

#2103 Pharmacogenomic biomarkers of oncology drugs approved by the US FDA classified on the basis of BEST (Biomarkers, EndpointS, and other Tools) resource: systematic review.

Tariq H. M. Alqurayshah, SFDA Oncology and Hematology Group

Oncology and Hematology, Saudi Food and Drug Authority, Riyadh, Saudi Arabia

Objective: To characterize pharmacogenomic biomarkers of oncology indications approved by the US FDA classified on the basis of BEST (Biomarkers, EndpointS, and other Tools).
Resource Design: Systematic review. Data sources: FDA oncology/hematologic malignancies approvals and databases, BEST (Biomarkers, EndpointS, and other Tools) Resource, and CDER / CBER's DDT Qualification Project Search database: Stage 1: Letter of Intent (LOI), Stage 2: Qualification Plan (QP), and Stage 3: Full Qualification Package (FQP).
Study Inclusion: All Biomarkers include germline or somatic gene variants (polymorphisms, mutations), functional deficiencies with a genetic etiology, gene expression differences, chromosomal abnormalities; and selected protein biomarkers in all approved labeling sections published by the FDA.
Results: BEST Resource defines seven biomarker categories: susceptibility/risk, diagnostic, monitoring, prognostic, predictive, pharmacodynamic/response, and safety. The biomarkers are more linked to three cancers: breast, lung and colorectal cancers.

#2104 Epigenetic, transcriptomic and genetic analyses of HR+/HER2- primary and metastatic breast cancer results in cohort stratification by aggressiveness and reveals heterogeneous mechanisms of endocrine therapy resistance.

Jomar Sangalang[1], Tony Sourisseau[2], Veronique Scott[1], Diep Tran[1], Nusaibah Ibrahimi[3], Alicia Tran-Dien[4], Hakim Benkirane[3], Nicolas Servant[5], Fabrice Andre[1], Sergey Nikolaev[1]

[1]U981, Institut Gustave Roussy, Villejuif, France,[2]Equipe 9 - Sarcotarget, Institut Bergonie, Bordeaux, France,[3]Oncostat, Institut Gustave Roussy, Villejuif, France,[4]Plate-forme de Bioinformatique, Institut Gustave Roussy, Villejuif, France,[5]CurieCoreTech Bioinformatics (CUBIC), Institut Curie, Paris, France

Endocrine therapy (ET) is typically the first-line treatment in hormone receptor-positive, human epidermal growth receptor 2-negative (HR+/HER2) breast cancer (BC) patients; however, almost half of the patients exhibit ET resistance after initial response. Mechanisms of ET resistance are not fully understood, and require an integrated epigenetic, transcriptomic and genetic approach. We conducted ATAC, RNA, and whole-exome sequencing on primary and metastatic BC (pBC and mBC) from 459 HR+/HER2- patients. pBC samples were derived from tumor resections of patients, which were then treated with adjuvant ET and experienced either a relapse or sustained remission over a five-year period. Samples of mBC were obtained from metastatic lesions of ET-resistant patients. We performed analysis of chromatin accessibility, gene expression, and mutational data aiming to understand relapse mechanisms in pBC and resistance mechanisms in mBC. PAM50 probability scoring of our cohort of HR+/HER2- BC revealed subgroups within pBC and mBC, which resembled PAM50 subtypes of BC. In pBC, the “Basal” and “Her2” subgroups displayed the highest percentage of relapse, compared to the “LumA” and “LumB” subgroups. TP53 alterations were enriched in “Her2” subgroups, while MAP3K1 alterations were more frequent in “LumA” subgroup. In mBC, ESR1 mutations characterized “LumB” tumors, "Her2” subgroup exhibited ERBB2 alterations, and “LumA” subgroup was enriched with FOXA1 mutations. In both pBC and mBC, the subgroups were enriched with gene sets pertaining to their associated PAM50 subtypes. “LumB” subgroup was associated with higher estrogen response. “Basal” and “Her2” subgroups showed enrichment of gene sets related to true Basal and Her2 subtypes, and transcription factor motifs of AP-1 and TFAP2. “Basal” and “Her2” subgroups was characterized by poor survival. PAM50 probability scoring was useful to dissect heterogeneity and to unveil insights into molecular mechanisms associated with the risk of relapse and ET resistance within HR+/HER2- BC. These findings may improve prognostic accuracy and patient stratification in HR+/HER2- BC cohorts.

#2105 Characterization of oral carcinoma risk in patients with oral epithelial lesions employing an AI-assisted cytopathology-on-a-chip platform.

Kritika Srinivasan Rajsri[1], Michael McRae[1], Nancy Ruel[1], A. Ross Kerr[2], Nadarajah Vigneswaran[3], Adam Jacobson[4], Jonathan Shum[5], Rachelle Wolk[2], Nicolaos Christodoulides[1], John T. McDevitt[1]

[1]Molecular Pathobiology, NYU College of Dentistry, New York, NY,[2]Oral and Maxillofacial Pathology, Radiology and Medicine, NYU College of Dentistry, New York, NY,[3]UT Health School of Dentistry, Houston, TX,[4]Otolaryngology-Head and Neck Surgery, NYU Grossman School of Medicine, New York, NY,[5]Oral and Maxillofacial Surgery, UT Health, Houston, TX

Oral squamous cell carcinoma (OSCC) accounts for >350,000 new cases and >177,000 deaths worldwide. Oral potentially malignant disorders (OPMD), harboring oral epithelial dysplasia (OED) are 15 times more common than OSCC - a significant proportion transforming to malignancy. Furthermore, field cancerization may contribute to multifocal disease-risk comprising other oral sites. Oral epithelia lesions (OEL) are often challenging to diagnose for front-line clinicians and process of monitoring patients for new/evolving local disease lacks consensus - challenging opportunities for triage, surveillance and early detection. To address these gaps there is a compelling need to complete accurate surveillance testing of OEL for disease evolution in at-risk patients chairside/point-of-care, aiding clinical decision making. This study leverages an NIH-funded, multisite time-course investigation of patients with a history of OSCC or OED, utilizing AI-linked cytopathology capabilities derived from a microfluidics engine analyzed with a three-color fluorescence analyzer. Minimally invasive oral brush cytology specimens were collected from suspicious lesions and contralateral sites, with clinical and histopathological diagnoses blinded until patient exit. The study employs a chairside-ready, AI-assisted *cytopathology-on-a-chip* (COC) platform. This platform integrates microfluidics-facilitated immunoassays, cytomic measurements, automated analysis of cellular and molecular signatures, and result generation. The output includes a numerical index that profiles disease severity and progression, linked to the patient's clinical and histopathological data, all delivered in approximately 40 minutes. Initial results from this study highlight the strong potential of a disease severity numerical index for assessing risk and distinguishing between oral cavity cancer patients who experience recurrence and those who do not. Similarly, OED patients at risk of malignant transformation exhibit higher numerical indexes. Moreover, contralateral lesions provide an advanced perspective on patients with a stronger predisposition to field cancerization. This noninvasive approach aligns well with findings obtained through more invasive biopsy and histological analysis. The AI-assisted chairside-ready COC minimally-invasive platform has strong potential to aid oral lesion characterization, for early-identification of disease risk and evolution. These capabilities include use of static numerical indexes and associated time-course changes. Measurement of contralateral-site specimen also offers new insights for referral or routine-surveillance, towards achieving better outcomes, disease prevention and management.

#2106 Comparing the methylation profiles between lung adenocarcinoma and squamous cell carcinoma.

Hyeyeun Lim[1], Christopher I. Amos[2], Jinyoung Byun[2], Aaron P. Aaron[1]

[1]Baylor College of Medicine, Houston, TX,[2]University of New Mexico, Albuquerque, NM

Background: Lung cancer is a leading cause of death. Despite advancements in early diagnosis and treatment, most patients are diagnosed at later stages, and the average 5-year survival rate is < 30%. Lung adenocarcinoma (LUAD) is the most common non-small cell lung cancer (NSCLC), representing about 40% of cases, followed by lung squamous cell carcinoma (LUSC) at 25%. The biological patterns and molecular characteristics of LUAD and LUSC exhibit differences. Changes in DNA methylation levels of various genes have been observed in lung cancer subtypes, yet research on differences in DNA methylation patterns between LUAD and LUSC is limited.

Methods: The methylation microarray dataset (GSE39279, Illumina HumanMethylation450 BeadChip) from Gene Expression Omnibus at the National Center for Biotechnology Information was used. The dataset comprised 444 NSCLC samples derived from tumor tissues, of which 322 were LUAD and 122 were LUSC. We used the Champ (Chip Analysis Methylation Pipeline) package to identify differentially methylated probes (DMPs, Benjamini-Hochberg adjusted p-value < 0.05) and genes representing specific biological pathways between LUAD and LUSC. Singular Value Decomposition regression analysis was used to estimate the impact of age, sex, and smoking status and then adjusted for the covariates with p-values < 0.05. We used the eFORGE TF database to calculate the Find Individual Motif Occurrences (FIMO) p-values for the overlap with transcription factor binding sites. The Gene Expression Profiling Interactive Analysis database was used to measure the gene expression levels of LUAD and LUSC samples in the Genotype-Tissue Expression and the Cancer Genome Atlas Program portals.

Results: In total, 223,007 DMPs were identified by comparing LUAD and LUAC, with 130,610 hypomethylated and 92,467 hypermethylated. Using the absolute difference of β values between groups ≥ 0.3, we identified 15 DMPs, 11 hypomethylated and four hypermethylated. The DMPs on promoter regions were all hypomethylated in LUSC compared to LUAD (cg00415665 and cg22997040 in *ZHX2*, cg27649037 in *ST18*, cg20691436 in *CALML3*, and cg24580076 in *C7orf20*). We also identified DMPs based on the importance scores (> 0.1) from a 5-fold cross-validation random forest analysis. The two DMPs in the *ZHX2* gene had the highest importance scores (> 8.0). Among those, cg00415665 was covered by the Zfp161_secondary motif (FIMO p-value: < 10^{-5}). The average expression level of *ZHX2* in LUSC (transcripts per million, TPM=9.7) was higher than in LUAD (TPM=8.2).

Conclusions: We characterized the methylation landscape of NSCLCs by histological subtypes and identified that the methylation pattern of cg00415665 in *ZHX2,* a tumor suppressor gene, was significantly associated with the histological subtype of NSCLC. Further investigation of these findings will provide additional insight into the biology and genetics of LUAD and LUSC.

#2107 Insights into inherited genetic variations and genetic ancestry of patients with high-risk melanoma.

Ahmad A. Tarhini[1], Zhihua Chen[1], Sandra J. Lee[2], F. Stephen Hodi[3], Islam Eljilany[1], Tingyi Li[1], Howard Streicher[4], Vernon K. Sondak[1], Xuefeng Wang[1], Peter A. Kanetsky[1], John M. Kirkwood[5]

[1]H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL,[2]Dana Farber Cancer Institute, Harvard Medical School, Boston, MA,[3]Dana-Farber Cancer Institute, Boston, MA,[4]National Cancer Institute, Investigational Drug Branch, Cancer Therapy Evaluation Program, Bethesda, MD,[5]UPMC Hillman Cancer Center, Pittsburgh, PA

Introduction. For melanoma patients (pts) with resectable regional or distant metastases, there is paucity of data related to inherited genetic variations and genetic ancestry in the North American patient population.
Methods. We conducted genome-wide genotyping on samples from 744 consenting pts enrolled in E1609 adjuvant trial that tested ipilimumab vs interferon-α in high-risk melanoma including sites across the U. S. and Canada. We used Illumina Infinium Global Screening Array v.3.0. After imputation, pruning, and incorporating genotypes from 1KGP3 as reference, genetic ancestry was estimated using admixture v1.3.0 in the unsupervised mode. Population structure was visualized through a UMAP dimensionality reduction (R package umap_0.2.9.0). Genetic ancestry proportions were visualized using python package PONG v1.5.
Results. In UMAP reduction, most (728) cases clustered with the 1KGP3 European (EUR) reference, with small subsets (12 and 14, respectively) clustering with the admixed American (AMR) and East Asian (EAS) references. Considering potential ancestral origins for the study pts, we assumed between 4 to 8 ancestral populations in order to capture a wider range of ancestral contributions. Unsupervised admixture inference on the combined genetic data from our cohort and the 1000 Genomes reference identified ancestral axes clearly along continental geographical lines. K=5 was deemed most optimal in broadly capturing the potential complexity of genetic contributions in U.S. context. The ancestry populations identified were Africa (AFR), AMR, EAS, EUR, South Asia (SAS). For most (734) samples, the estimated EUR proportion was >50%, while 4 had >50% EAS ancestry, 4 >50% AMR ancestry, and 2 >40% EUR and AMR ancestry. Most (725) participants self-reported their race as White, 4 as Asian, and 1 as multi-race; 14 missing information on race. For the 725 White participants, 13 identified as Hispanic and 24 missing information on ethnicity; and for these participants, there was a high degree of EUR ancestry (>95% in 22), with only 2 having between 4-6% combined AMR and AFR contributions. The 4 Asian participants had a high degree of EAS ancestry, and the multi-race participant had a high degree of EUR ancestry with minor degrees of AMR and EAS ancestry. Participants with missing information on self-reported race had either a high degree of EUR ancestry or various combinations of significant AMR, EUR, and AFR contributions. The 17 participants reporting Hispanic ethnicity had a significant AMR ancestral contribution. The vast majority of participants not reporting (699) or missing (28) information on Hispanic ethnicity did not have AMR ancestry.
Conclusions. Our analysis in the context of a U.S. Intergroup phase 3 trial revealed important insights into genetic ancestry of patients with high-risk melanoma. Ongoing analyses are investigating associations with survival outcomes and the risk of irAEs.

#2108 Comparative analysis of fusion gene detection rates in tissue versus blood samples from Chinese diverse solid tumors.

Jun-Ling Wang, Hong-Wei Li, Hong-Xia Liu, Xiu-Ping Li, Jian-Peng Hu, Qian Xiong, Jin-Si Yang, Zong-Sheng Wu, Ting Ji

Clinical Laboratory, Qujing First People's Hospital of Yunnan Province, Qu Jing Shi, China

Background: The landscape of genetic alterations, particularly fusion genes, plays a critical role in the diagnosis and treatment of various solid tumors. As liquid biopsy methods evolve, understanding the differences in mutation frequencies between tissue and blood samples becomes essential for precision oncology. This study aimed to evaluate the detection rates of specific fusion genes in both tumor tissue and blood samples from patients with diverse solid tumors using a 733-gene panel through Next-Generation Sequencing (NGS).

Methods: We conducted a comprehensive analysis of 23 types of solid tumors (including bladder cancer (BLCA), breast cancer (BRCA), biliary tract cancer (BTC), cervical cancer (CESC), colorectal cancer (COAD), esophageal cancer (ESCA), gastrointestinal stromal tumors (GIST), head and neck squamous cell carcinoma (HNSC), intestine cancer, kidney renal clear cell carcinoma (KIRC), liver hepatocellular carcinoma (LIHC), melanoma, neuroendocrine tumors, non-small cell lung cancer (NSCLC), ovarian cancer (OV), pancreatic adenocarcinoma (PAAD), prostate cancer (PRAD), sarcoma (SARC), small cell lung cancer (SCLC), stomach cancer (STAD), thyroid carcinoma (THCA), thymoma (THYM), and uterine corpus endometrial carcinoma (UCEC)). The frequencies of fusion genes (including ALK, BRAF, ESR1, FGFR1, FGFR2, FGFR3, MET, NRG1, NTRK1, NTRK2, NTRK3, PDGFB, PDGFRA, RET, ROS1) were calculated from NGS results of tissue and blood samples.

Results: Significant differences in fusion gene detection rates were observed between tissue and blood samples for several cancers. ALK fusions were significantly more prevalent in tissue samples from NSCLC (2.4% vs 0.9%, $P<0.05$), COAD (0.1% vs 0.5%, $P<0.05$), and THCA (1.6% vs 9.8%, $P=0.04$). BRCA fusions showed similar trends in NSCLC (0.1% vs 0.3%, $P<0.05$), HNSC (0.1% vs 1.5%, $P=0.03$), STAD (0.1% vs 0.4%, $P=0.01$), and COAD (0.1% vs 0.7%, $P<0.05$). Furthermore, FGFR2 fusions were detected significantly more in blood than tissue in STAD (0.5% vs 1.1%, $P=0.03$). Other fusion genes did not exhibit significant differences in detection rates between the two sample types. Notably, in blood tests, ALK (9.8% in THCA and 5.0% in THYM), BRAF (2.3% in melanoma), NTRK1 (2.4% in THCA), and NTRK3 (2.1% in intestine) had detection rates exceeding 2%. In tissue specimens, ALK (2.4% in NSCLC), FGFR2 (2.0% in BTC), and RET (3.1% in THCA) were the only fusion genes with detection rates over 2%.

Conclusion: This study highlights significant discrepancies in the detection rates of fusion genes between tissue and blood samples across various solid tumors, emphasizing the potential of blood-based biomarkers in clinical practice. These findings suggest the need for integrated approaches in managing and monitoring the genetic landscape of solid tumors, enhancing personalized treatment strategies.

#2109 Comparison of four non-enzymatic, mechanical dissociation methods for formalin-fixed tissue in flow cytometric analysis and single-cell workflows.

Hannah L. B. Veloz, Samantha M. Hill, Gloria Asantewaa, Lisa L. Gallegos, Nelson R. Alexander

Roche Tissue Diagnostics, Oro Valley, AZ

Introduction: Formalin-fixed tissue dissociation is a common method to isolate single cells from tissues that can be used for various downstream applications such as flow cytometry or other single-cell workflows. Common dissociation methods often use enzymes to break down tissue; however, this approach can inconsistently damage cell membranes, limiting the expression of biomarkers detectable by flow cytometric analysis. Non-enzymatic tissue dissociation preserves cell epitopes for optimal antibody recognition. In this study, we provide a comparison of four different mechanical dissociation methods and determine how each method affects cell integrity.
Methods: Surgically resected, formalin-fixed breast and colon tumors were processed using the representative sampling method and the non-tumor tissue was further homogenized into single cells using four different mechanical dissociation methods. The mechanical methods used in this comparison were the IKA tube mill, Miltenyi C tube, hand-held cordless homogenizer, and a pestle. Prior to mechanical dissociation, the tissue was incubated in a cell conditioning buffer. Single cell suspensions were quantified using the Countess II, and equal cell inputs were stained in suspension for Cytokeratin 8/18, CD45, CD68, CD163. DNA content was assessed via DAPI staining. Samples were analyzed on BD FACSMelody and data analysis was performed using FCS Express 7 software.
Results: The four non-enzymatic, mechanical tissue dissociation methods were successful at generating single cell suspensions with no significant difference in the total cell counts. Flow cytometric analysis showed no significant difference in singlet generation visualized by DAPI. There was no significant difference in the percentage of CD68 and CD163 positive populations seen via flow. A statistically non-significant difference was observed in the percentage of CD45 positive and Cytokeratin 8/18 positive populations, indicating that certain methods might be better at liberating immune vs tumor cells.
Conclusion: We demonstrate that four non-enzymatic, mechanical tissue dissociation methods are capable of liberating single cells from formalin-fixed tissues. Preservation of cell surface biomarkers is a significant improvement from isolation of nuclei that enables single-cell suspensions to be analyzed using fluorescently labeled antibodies and the enrichment of cell populations of interest via fluorescence activated cell sorting (FACS). Single cell generation and analysis of intact single cells from formalin fixed tissue is novel, and increases downstream applications that can be useful in the research and translational space, and may have clinical applications in the future.

#2111 In vivo expansion of autologous GD2-CAR-T cells in patients with aggressive primary tumors may be impaired by previous alkylator therapy.

Michael P. Brown[1], Sidra Khan[2], Jesikah Logan[1], Nga T. H. Truong[2], David S. Ziegler[3], Tessa Gargett[2], Abbey LeBlanc[1]

[1]RAH Cancer Clinical Trials Unit, Royal Adelaide Hospital, Adelaide, Australia,[2]Translational Oncology Laboratory, Centre for Cancer Biology, Adelaide, Australia,[3]Kids Cancer Centre, Sydney Children's Hospital, Sydney, Australia

GD2 is a validated cancer immunotherapy target and has a highly restricted normal tissue expression. Clinically activity of GD2-CAR-T cell therapy has been observed in the high GD2-expressing tumor types of diffuse midline glioma and neuroblastoma. Recently, we initiated two phase 1 clinical trials of escalating doses of intravenously administered autologous GD2-CAR-T cells either in pediatric patients with diffuse midline glioma at Sydney Children's Hospital (LEVI'S-CATCH; www.anzctr.org.au - ACTRN12622000675729) or adult patients with recurrent glioblastoma at Royal Adelaide Hospital (KARPOS; ACTRN12622001514796). Our ex vivo CAR-T cell manufacturing protocol was changed from the original method to a modified method, which permitted CAR-T cells of memory phenotype to dominate in vitro. Our published 12-patient GD2-CAR-T cell trial in solid tumor patients showed that in vivo expansion of GD2-CAR-T cells was significantly greater among patients whose cells were prepared using the modified over the original method. Hence, we adopted the modified method for our current brain tumors trials. In the KARPOS study, six patients have been enrolled without experiencing DLTs. In the first dose cohort of three patients, none received lymphodepletion chemotherapy whereas in the second dose cohort of three patients, all received lymphodepletion chemotherapy. Clinical histories, which recorded the extent of previous alkylator therapy used for the treatment of primary and recurrent glioblastoma in adult patients, led us to hypothesize that cumulative exposure to alkylator drugs limited CAR-T cell expansion in vivo.The presence of CAR-T cells in serial peripheral blood samples was measured using quantitative DNA-PCR. The extent of CAR-T cell expansion in peripheral blood samples was described using two measures: (i) area under the curve (AUC), and (ii) peak expansion or Cmax. From the concentration of the CAR transgene in total genomic DNA (ng/µg gDNA) in blood plasma as a function of time, AUC values were calculated using GraphPad Prism according to the trapezoid rule. We found correlation coefficients of -0.90 and -0.83 for the relationship between cumulative dose of prior alkylator therapy and AUC and Cmax, respectively. These data are consistent with previous clinical data that indicate temozolomide has deleterious effects on human lymphocyte number and function. Our data suggest that patient selection may be an important parameter to consider when enrolling glioblastoma patients to clinical trials of CAR-T cell therapy. For example, randomized controlled trial evidence indicates that patients who are considering such trials and whose tumors lack MGMT promoter methylation may reasonably be spared exposure to six months of consolidation temozolomide chemotherapy as part of the conventional treatment protocol.

#2112 Association between visceral fat obesity and perioperative complications of undernutrition in laparoscopic surgery for colorectal cancer.

Kengo Haruna, Norikatsu Miyoshi, Shiki Fujino, Rie Mizumoto, Rie Hayashi, Mitsunobu Takeda, Yuki Sekido, Tsuyoshi Hata, Atsushi Hamabe, Takayuki Ogino, Mamoru Uemura, Hirofumi Yamamoto, Yuichiro Doki, Hidetoshi Eguchi

Department of Gastroenterological Surgery, Graduate School of Medicine, Osaka University, Osaka, Japan

Background: Visceral obesity and malnutrition are each associated with poor prognosis in gastrointestinal cancers. This study investigates the impact of undernutrition combined with visceral obesity on perioperative complications in laparoscopic colorectal cancer surgery.
Methods: We retrospectively analyzed 223 colorectal cancer patients who underwent curative laparoscopic resection at our institution between January 2010 and December 2011. Undernutrition was defined as the Geriatric Nutritional Risk Index (GNRI), calculated using the ideal body weight ratio and serum albumin level, with GNRI < 107 indicating undernutrition. Visceral obesity was defined as a visceral fat area (VFA) >100 cm^2 at the umbilical level. Patients were divided into two groups: those with both undernutrition and visceral obesity (n=40) and others (n=183). Associations with perioperative complications were analyzed, along with background factors including age, gender, BMI, albumin (Alb), CRP, tumor markers, and other nutritional indices. Additionally, a new nutritional status score for predicting postoperative complications was developed using ROC curve analysis based on easily measurable indicators from blood tests, CT scans, and physical exams.
Results: The malnutrition and visceral obesity group showed a significantly higher incidence of Grade 2 or higher perioperative complications compared to the other group (25.0% vs. 10.9%; $p = 0.037$). Analysis of nutritional and oncological indicators identified age, BMI, VFA, CRP, lymphocyte count, and CA19-9 as associated with postoperative complications. A novel nutritional status score incorporating these factors predicted Grade 2 or higher complications with a sensitivity of 43.0% and a specificity of 83.3%.
Conclusions: Undernutrition combined with visceral obesity significantly increased the risk of perioperative complications. Preoperative nutritional interventions to improve patient status may reduce these risks and should be further investigated.

CLINICAL RESEARCH: Therapeutic Antibodies, Including Engineered Antibodies 1
Poster Session

#2115 SIM0680, a potent antibody-drug conjugate for the treatment of solid tumors expressing ENPP3.

Abdullah Emran[1], Yutian Zhang[2], Yi Yang[1], Liting Xue[2], Lu Gong[1], Amin Al-Shami[1], Yayuan Fu[2], Renhong Tang[2]

[1]Simcere Zaiming, Cambridge, MA,[2]Simcere Zaiming, Shanghai, China

Introduction: The ATP- and cGAMP-hydrolysing ecto-nucleotide pyrophosphatase/phosphodiesterase 3 (ENPP3 or CD203c) is an extracellular enzyme which has been implicated in checkpoint regulation of innate immune responses. Analysis of data in The Cancer Genome Atlas (TCGA) and Cancer Cell Line Encyclopedia (CCLE) indicates elevated expression of ENPP3 in multiple solid tumors compared to normal tissues. Moreover, immunohistochemistry studies suggest that the expression of ENPP3 on healthy epithelial lining is polarized toward the lumen of internal organs but becomes disorganized and non-apical after malignant transformation. This spatial distribution of expression could make ENPP3 an ideal target for antibody therapies, which may have access to CD203c exclusively on tumor cells and not on healthy tissues.
Results: We have developed an antibody-drug conjugate, SIM0680, which is a fully humanized antibody that binds a membrane-proximal epitope on ENPP3 with high affinity and is coupled to a payload through a proprietary linker. Immunohistochemistry confirmed strong expression of ENPP3 in liver, renal, and colorectal carcinomas. SIM0680 had potent and specific cytotoxic activity against cell lines originating from these tissues and displaying various levels of ENPP3 expression. Consistent with the observed in vitro efficacy, SIM0680 demonstrated strong single-dose and dose-dependent anti-tumor activity in vivo against multiple cell line- and patient-derived renal cell and hepatocellular carcinoma xenografts in mice, without causing significant weight loss. Similar potent anti-tumor efficacy was observed in human myeloid and megakaryoblastic leukemia models.
Conclusion: Our findings underscore the potential of targeting the apically restricted tumor antigen ENPP3 with antibody therapy and support clinical development of SIM0680 as a novel therapeutic modality for treatment of ENPP3-positive solid tumors.

#2116 2MW7061, a novel LILRB4xCD3 bispecific T-cell engager targeting monocytic acute myeloid leukemia.

Yu Pang, **Cuicui Guo**, Zhen Han, Fenglin Yun, Ao Li, Hai Wu, Xun Gui

Mabwell (Shanghai) Bioscience Co., Ltd., Shanghai, China

Background: Acute myeloid leukemia (AML) is a highly aggressive hematologic malignancy with a high recurrence rate, highlighting the urgent need for therapies that induce durable responses and improve survival outcomes. LILRB4 is highly expressed in FAB M4 and M5 subtypes of AML, while absent in normal hematopoietic stem and progenitor cells, making it an appealing target for selective immunotherapy. We developed 2MW7061, a bispecific LILRB4xCD3 T-cell engager, designed to target and eliminate LILRB4-expressing tumor cells by selectively activating T cells.

Methods: 2MW7061 was generated through the screening and optimization of bispecific antibodies with varying geometries, formats, and CD3-binding affinities. The final construct utilizes a 1+2 format, with two Fab arms targeting LILRB4 and one single-chain variable fragment (scFv) arm targeting CD3. The affinity of 2MW7061 was measured using Octet, and its binding to T cells and AML cell lines was assessed. Functional activity was evaluated using Jurkat NF-AT reporter assays, T-cell activation assays, and tumor-directed cytotoxicity assays (TDCC). *In vivo* anti-tumor efficacy was tested in B-NDG/hu-PBMC and B-NCG/hu-PBMC mouse models implanted with THP-1-luc and MOLM-13 tumors, respectively. Safety and tolerability were evaluated in cynomolgus monkeys.

Results: 2MW7061 demonstrated reduced binding to T cells in the absence of tumor cells, effectively minimizing off-target T-cell activation and mitigating the risk of cytokine release syndrome (CRS). *In vitro*, 2MW7061 exhibited tumor-associated antigen (TAA)-dependent binding and induced potent cytotoxicity. *In vivo*, 2MW7061 significantly inhibited tumor growth in both THP-1 (high LILRB4 expression) and MOLM-13 (low LILRB4 expression) xenograft models, indicating that its anti-tumor activity is LILRB4-dependent. In non-human primates, 2MW7061 administration resulted in minimal and transient increases in serum cytokines and C-reactive protein, with no significant signs of toxicity observed.

Conclusion: 2MW7061 is a bispecific T-cell engager with potent LILRB4-dependent anti-tumor activity in preclinical models of monocytic AML. Its low nonspecific T-cell binding and favorable safety profile in non-human primates support its continued development as a promising therapeutic candidate for clinical evaluation in AML.

#2117 GenSciP107, a MICA/B-directed and Fc-enhanced antibody, potently inhibits tumor growth via ADCC and stimulation of immune response.

Ming Lei, Jiuhe Sun, Hanxi Yu, Li Tong, Yihui Lin, Chunsheng Zhang, Lei Song, Yanshuang Liu, Yanhou Liu, Jiyuan Zhao, Yingxin Bi, Weiqing Li, Yunna Gao, Yuanyuan Yuan, Bo Guo, Fu Li, John L. Xu

Changchun GeneScience Pharmaceutical Co., Ltd., Changchun, China

Background: The expression of major histocompatibility complex class I polypeptide-related sequence A and B (MICA/B) is induced in normal cells under stress and in tumor cells including hepatocellular carcinoma (HCC), non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), and urothelial cancer (UC), etc. Membrane-bound MICA/B (mMICA/B) interacts and activates NKG2D, a stimulatory receptor on NK cells, γδ T cells and a subset of $CD8^+$ T cell. Shed MICA/B (sMICA/B), however, inhibits the function of NK and other $NKG2D^+$ immune cells. Thus, an anti-MICA/B antibody can be an intriguing immuno-oncology therapy for cancer, by inhibiting MICA/B shedding from tumor cells and by stimulating NK and $CD8^+$ T cell functions, resulting in potent killing of tumor cells.
Methods: GenSciP107 is an anti-MICA/B monoclonal antibody isolated from immunized mice and then humanized and affinity matured. The Fc region of GenSciP107 was mutated for enhanced effector functions and for extended half-life. A set of *in vitro* assays were conducted to examine the impact of GenSciP107 on the MICA/B-NKG2d axis in immune cells. *In vivo* anti-tumor activity of GenSciP107 was evaluated in CDX models of NSCLC and HCC in mice. Pharmacokinetic properties of GenSciP107 were assessed following an intravenous administration in cynomolgus monkeys.
Results: GenSciP107 bound to recombinant human MICA/B proteins with single digit nanomolar affinity, as analyzed using SPR technology. It stimulated NK cells and macrophages to lyse $MICA/B^+$ tumor cells with an EC_{50} in the double digit picomolar range in the LDH release-based ADCC assay and in the single digit nanomolar range in the ADCP assay, respectively. GenSciP107 also increased the apparent expression levels of MICA/B on tumor cells by preventing their shedding, resulting in excellent stimulation of NK cells and subsequent cytotoxicity of tumor cells. Furthermore, GenSciP107 reversed the inhibitory effect of sMICA/B on NK cells and on PBMCs in an *in vitro* ADCC assay. GenSciP107 significantly inhibited tumor growth in the CDX models of human HCC and NSCLC. Half-life of GenSciP107 was determined to be about 377 hours following an intravenous injection at the dose of 10 mg/kg in non-human primates.
Conclusion: These data highlight the potential of GenSciP107 as a novel cancer therapy with multiple mechanisms of action and support its further evaluation in IND-enabling studies.

#2118 ABN501: A CLDN3-targeted monoclonal antibody with proven safety, pharmacokinetics, and synergistic anti-tumor efficacy.

Saehyung Lee[1], **Jae Choi**[1], Sunghyun Hong[1], Ha Yeon Park[1], Ho Jin Lee[1], Jaemun Kim[1], Sangin Lee[1], Seung Hwa Jung[1], Myeung Ryun Seo[2], Hobin Yang[3], Sungyoul Hong[4], Jun Young Choi[1], Na Young Kim[1], Young Kee Shin[2]

[1]ABION Inc., Seoul, Korea, Republic of,[2]Department of Molecular Medicine and Biopharmaceutical Sciences, Graduate School of Convergence Science and Technology, Seoul National University, Seoul, Korea, Republic of,[3]College of Pharmacy, Kyungsung University, Busan, Korea, Republic of,[4]College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul, Korea, Republic of

ABN501 is an afucosylated monoclonal antibody specifically designed to target claudin-3 (CLDN3), a protein frequently overexpressed in various solid tumors, including small-cell lung cancer (SCLC). Given the critical role of CLDN3 in cancer cell proliferation and tumorigenesis, it is recognized as a promising therapeutic target. In this study, we evaluated the preclinical safety and efficacy of ABN501, focusing on its potential for clinical development as a targeted cancer therapy. To identify suitable animal models for toxicity assessment, we investigated the cross-species reactivity of ABN501 with CLDN3. Our results confirmed that ABN501 binds to CLDN3 across human, mouse, rat, and cynomolgus monkey species. Based on these findings, rats and cynomolgus monkeys were selected for comprehensive toxicity evaluations, including single-dose and repeated-dose studies. Notably, even at the highest administered doses, ABN501 exhibited no significant changes in key toxicity markers, demonstrating its exceptional safety profile. We further assessed the therapeutic potential of ABN501 as both monotherapy and in combination with chemotherapy. Chemotherapy was shown to upregulate CLDN3 expression in lung cancer cell lines, thereby enhancing the antitumor activity of ABN501 and confirming a synergistic effect. In conclusion, our preclinical studies demonstrate that ABN501 is a safe and effective CLDN3-targeting monoclonal antibody for treating CLDN3-overexpressing cancers. Its excellent safety profile and synergistic effects in combination with chemotherapy present a promising strategy for improving treatment outcomes, particularly in cancers with limited targeted treatment options. These findings pave the way for further clinical investigation, particularly in lung cancer and other CLDN3-expressing malignancies.

#2119 Targeting KIT in chromophobe renal cell carcinoma.

M. Alchoueiry[1], H. Mansour[1], D. Khabibullin[1], T. Han[1], S. Chaturantabut[2], J. F. Williams[1], M. S. Hirsch[1], C. Priolo[1], W. R. Sellers[2], E. P. Henske[1];

[1]Brigham and Women's Hospital, Harvard Medical School, Boston, MA, [2]Broad Institute of MIT and Harvard, Cambridge, MA

Background: Chromophobe renal cell carcinoma (ChRCC) arises from the specialized intercalated cells of the distal nephron. There are no proven therapies for patients with metastatic ChRCC. In the metastatic setting median survival is estimated to be 27 months. KIT (CD117) is a membrane-associated tyrosine kinase receptor. Binding of the ligand stem cell factor (SCF) to KIT results in kinase activation and internalization. Antibody-drug conjugates (ADC) targeting KIT were previously found to be effective in preclinical models and safe in primates, but have not been tested in ChRCC.

Results: In The Cancer Genome Atlas (TCGA), KIT (CD117) mRNA expression is higher in ChRCC than any other tumor type with the mean expression 12 times higher than matched normal kidney. In contrast, in clear cell RCC (ccRCC), KIT (CD117) is 5 times lower than matched normal kidney. Immunohistochemistry demonstrates KIT protein expression on the membrane of ChRCC tumor cells. Of the 14 metastatic ChRCC specimens stained for KIT at our institution, 85% (12) were positive. In the TCGA, mRNA levels of SCF (KITLG), the KIT ligand, in ChRCC are the second highest compared to other tumors, and in published single-cell RNA sequencing data, KIT and SCF are co-expressed in ChRCC tumor cells. We found that KIT (CD117) mRNA expression is significantly higher in ChRCC-derived cells compared to ccRCC-derived cells and HK cells (derived from normal human kidney). Western blot analysis confirmed KIT expression in five ChRCC cell lines. Despite the high KIT expression, knockdown of KIT or treatment with Imatinib, a tyrosine kinase inhibitor that targets KIT, did not decrease the proliferation of ChRCC-derived cells. This indicates that KIT is not driving the proliferation of ChRCC cells. LOP628 is a KIT ADC consisting of the anti-KIT antibody LMJ729 conjugated to the microtubule destabilizing maytansinoid DM1. The two ChRCC cell lines with the highest expression of KIT (RCJ T1 and RCJT2) and ccRCC-derived 786-0 cells (with low KIT expression, as a control) were treated with 20nM of LOP628 for 24 hours. LOP628 decreased the viability of the ChRCC-derived cells by ~60% with no effect on the ccRCC cells.

Conclusions: KIT and its ligand SCF are highly expressed by ChRCC cells and LOP628, a KIT ADC, decreases ChRCC cell proliferation.

#2120 A novel engineered IL-21-based immunocytokine as a therapeutic approach for tumor targeting in renal cancer.

Anna Rastorgueva, Nicholas A. Maskalenko, Ilia Fedorov, Ekaterina Davydova, Ashot Nazaretian, Kerry S. Campbell, **Peter Makhov**

Cancer Signaling and Microenvironment Program, Fox Chase Cancer Center, Philadelphia, PA

RCC is a lethal disease whose incidence is on the rise. Clear cell renal cell cancer (ccRCC), the most aggressive and the deadliest variant of this cancer, accounts for ~ 85% of all RCC cases. In the past decade, the landscape of immunotherapeutic approaches with the focus on use of cytokines for advanced ccRCC has expanded rapidly. The therapeutic potential of IL-21 as a novel treatment option for patients with advanced ccRCC has been evaluated in phase 1 and 2 clinical trials. Despite promising anti-tumor effects, severe dose-limiting toxicities of systemic administration of IL-21 were frequently reported in clinical trials. CD70, a TNF-ligand family member, is transiently expressed and restricted to a subset of highly activated T, B, and dendritic cells, but is highly overexpressed in all ccRCC tumors. In RCC, tumor-expressed CD70 results in exhaustion of immune cells, thereby promoting immune escape. Therefore, targeting CD70 has been thought to be promising therapeutic strategy for RCC. Thus, a variety of CD70-targeting approaches, focused on antibody dependent cellular cytotoxicity (ADCC) or antibody-drug conjugates, have been evaluated in multiple trials. Despite some studies demonstrated limited response rate, frequently related to dose-limiting off-target cytotoxicity, the successes indicated generation of an effective immune response toward RCC. To achieve our goals aiming to take an advantage to combine the benefits of anti-CD70 therapy with tumor restricted targeting of IL21, we have designed and generated for the first time a novel humanized anti-CD70-IL-21 fusion antibody. This fusion antibody simultaneously binds CD70, a ligand that is highly over-expressed in ccRCC, and induces IL-21 receptor (IL-21R) mediated downstream signaling. We also disrupted heparin binding activity by site-directed mutagenesis of IL-21 portion of the immunocytokine. This resulted in significant improvement of target specificity, PK/PD properties, and ADCC-inducing activity of the immunocytokine. Importantly, our novel immunocytokine has an advantage over existing anti-CD70 targeting approaches, as it markedly alleviates ADCC-mediated exhaustion of NK cells. Finally, our animal experiments indicate that our novel immunocytokine has a significant anti-tumor potential against ccRCC in humanized mouse model reconstituted with NK-92.CD16 cells. Taken together, our data demonstrate the superior efficacy of the novel immunocytokine against ccRCC. Furthermore, this successful immunotherapeutic approach can be expanded for the management of other CD70-positive tumors.

#2121 MAIT engagers: Providing a large therapeutic window for the treatment of solid tumors.

S. E. Plyte[1], M. Fraudeau[1], D. Winterberg[2], C. Germain[1], C. Rouseau[1], G. Sodaro[1], M. Audin[1], A. Ivagnes[1], H.-H. Oberg[2], P.-E. Gerard[1], M. Peipp[2], D. Wesch[2], J. Prigent[1];

[1]Biomunex Pharmaceuticals, paris, France, [2]Universitats Klinikum Schleswig-Holstein, Kiel, Germany

Background: MAIT cells are an abundant subset of non-conventional T-cells with potent cytotoxic capacity that are resident in most tissues and solid tumors. MAIT cell engagers are bispecific antibodies that are able to simultaneously bind the MAIT cell TCR and a tumor associated antigen, creating an immunological synapse, resulting in efficient elimination of cancer cells. These MAIT engagers only bind and activate MAIT cells and, at difference to classical CD3 engagers, do not cause cytokine release or activate regulatory T cells offering the promise of better safety and efficacy than CD3 engagers.

Methods: Using the Biomunex proprietary BiXAb platform, bispecific, tetravalent antibodies were generated that target the MAIT iTCR (invariant T cell receptor) and the HER2 tumor associated antigen. MAIT-cell activation, proliferation and degranulation were followed by gating on MAIT cells within a purified CD8 cell population. Tumor cell lines (varying [HER2]) were co-cultured with MAIT cells and the BiXAbs in several cytotoxic assays (evaluated by Chromium release). Cytokine release was assessed by Legend Plex assays or ELISA. Regulatory T cells were enriched from PBMCs. Cytotoxicity of tumor-resident MAITs, from fresh patient samples, was determined by impedance measurements and in patient derived 3D organoids by imaging (The Hub organoids). Cytokine levels in serum of Cynomolgus monkeys was measured by Legend plex.

Results: MAIT engagers induce the rapid activation, proliferation and degranulation of MAIT cells leading to efficient killing of engaged HER2+ cancer cells with a potency similar to that of a classical HER2-directed CD3-T cell engager (in clinical development). The MAIT engager did not activate the regulatory T cells or other CD4/CD8 subsets. In addition, using PBMCs, total cytokine release was 1000 fold less with the MAIT engager compared to a classical TCE, opening up a large therapeutic window, which should permit effective treatment of solid tumors. Strong signs of cytokine release syndrome and high blood cytokine levels were observed in cynomolgus monkeys treated with a CD3 engager whilst no effect was observed with the MAIT engager. *Ex vivo* cytotoxicity of tumor-resident MAIT cells using dissociated Ovarian cancer samples at “real E:T ratios” and MAIT engager-mediated cytotoxicity of patient derived organoids (CRC) demonstrate the antitumor capability of MAIT engagers in solid tumors.

Conclusions: MAIT cell engagers are a promising approach for the treatment of solid tumors that have the potency and efficacy of a classical TCE but with a significantly improved cytokine safety profile leading to a larger therapeutic window.

#2122 FS-8002, a bifunctional antibody fusion protein of anti-VEGF and anti-TGF-β, exhibits significant anti-tumor activity in preclinical studies.

Libo Zhou, Qiran Guo, Ruxia Zhou, Hua Jiang, Chaoyi Xu, **Xinzhong Jon Wang**

Fusion Bioscience, Inc., Shanghai, China

Background: FS-8002 is a bifunctional antibody fusion protein targeting vascular endothelial growth factor (VEGF) and transforming growth factor β (TGF-β), which is generated by fusing a humanized IgG1 antibody against VEGF and the extracellular domain of TGF-β receptor II (TGF-βRII). FS-8002 can simultaneously bind to and neutralize VEGF and TGF-β, thus achieving anti-tumor effects through the following proposed mechanisms: 1) inhibiting tumor neovascularization and normalizing tumor blood vessels; 2) relieving the suppression of anti-tumor immune cells in the tumor microenvironment (TME); 3) inhibiting the formation of the immune barrier or extracellular matrix (ECM) and promoting cytotoxic T cell infiltration into tumors, and 4) blocking the invasion and metastasis of tumor cells.

Methods: The mechanism of action (MOA) and anti-tumor effects of FS-8002 were evaluated in a series of studies including *in vitro* target binding, biological activity assays, and *in vivo* efficacy study in xenograft tumor models.

Results: *in vitro*, FS-8002 binds to VEGF and TGF-β simultaneously. FS-8002 bound to VEGF165 and VEGF121 and thus inhibited VEGF-induced human umbilical vein endothelial cell (HUVEC) proliferation. FS-8002 also specifically bound to TGF-β1 and TGF-β3, but only had weak affinity to TGF-β2. By neutralizing TGF-β, FS-8002 could block TGF-β/Smad signaling pathway, inhibit TGF-β1-induced tumor cell metastasis, and relieve immunosuppression in the TME by activating NK cells and inhibiting Treg proliferation. FS-8002 exhibited significant anti-tumor effect in two "cold tumor" xenograft models (human PBMC reconstituted MDA-MB-231 model and HT-29 cells model) as single agent or in combination with anti-PD-1 antibody (Keytruda®). IHC analysis of the tumors from the two mouse xenograft models revealed that FS-8002 treatment could inhibit the tumor neovascularization, inhibit the formation of the immune barrier by reducing fibrosis formation in tumor tissues, and promote T cells infiltration into tumor tissue to kill tumor cells, therefore exhibit anti-tumor effect. The results of GLP repeat-dose toxicity study in cynomolgus monkeys combined with safety pharmacology assessment indicated that FS-8002 was well tolerated under the doses ranged from 15 to 135 mg/kg/dose (HNSTD) after four weeks of weekly intravenous infusion administration, and the adverse effects were within expectations and can be managed in clinical trials. FS-8002 is currently under clinical evaluation for safety and activity in both US (IND approved) and China.

Conclusion: All the preclinical data supported FS-8002 to be studied in clinical trials.

#2123 KJ-103: First-in-class naked anti-TROP2 HCAb with immune modulatory mechanisms and tumor regression potential in clinical trials.

Amit Subedi, Hiba A. Zahreddine, Sophie E. Cousineau, Richard Wargachuk, Xiaowei Wang, Lucy Lai, Dominic Hou, Elijus Undzys, Gordon Ngan, Luis da Cruz, David Young

KisoJi Biotechnology, Montreal, QC, Canada

Trophoblast cell surface antigen-2 (TROP2) is a membrane protein highly expressed in a wide range of advanced epithelial cancers and is an attractive target for cancer therapies. TROP2-positive cancers have been targeted by antibody-drug conjugates (ADCs). Here we employed our proprietary heavy chain-only antibody (HCAb) transgenic mouse platform to discover and develop KJ-103, a novel naked anti-TROP2 HCAb.KJ-103 induced regression and eradication of large breast and colon cancer xenografts. The anti-tumor efficacy of KJ-103 was completely abolished when using an IgG1-LALAPG variant which lacks Fc gamma receptor (FcγR) interaction. Furthermore, the in vivo efficacy of KJ-103 was also abolished in a mouse model that lacks all FcγRs. These results underscored the importance of FcγR engagement in the therapeutic action of KJ-103. Although the precise immune cell responsible for this efficacy has yet to be determined, these results showed that, in immunodeficient mice, the anti-tumor efficacy of KJ-103 is mediated by effector function cells present in the tumor microenvironment. Further analysis of the tumor microenvironment via bulk RNA sequencing revealed that KJ-103 treatment reduced the content of immunosuppressive macrophages and activated genes associated with phagocytosis and cytotoxicity which may contribute to its potent antitumor effects. Additionally, KJ-103 treatment activated antigen-presenting genes, suggesting its potential to stimulate T-cell responses, which may amplify the immune-mediated anti-tumor response of KJ-103. These findings highlight the multifaceted immune response induced by KJ-103, making it a promising candidate for enhancing immune system-mediated tumor control. Surface plasmon resonance (SPR) analysis confirmed that KJ-103 strongly binds to human and cynomolgus TROP2 but has no interaction with murine and rat orthologs. KJ-103 was well tolerated in a five-week, weekly-dosing dose range finding (DRF) study in non-human primates, at levels up to 100mg/kg, with no clinical or lab-changes, and had a favorable PK profile. KJ-103 binds to the human and cynomolgus normal tissues where TROP2 is present, both by FFPE and frozen IHC highlighting its applicability for biomarker testing. Epitope mapping and co-crystal analysis revealed that KJ-103 targets a unique epitope on TROP2, which could not be competed by other known anti-TROP2 antibodies. This work highlights the feasibility of developing functional, payload-free anti-TROP2 antibodies for clinical use, addressing current challenges associated with ADCs and expanding therapeutic options for patients with TROP2-positive cancers. KJ-103, as the first naked anti-TROP2 HCAb is under development for clinical trials and represents a groundbreaking advancement in cancer immunotherapy.

#2124 A potent therapeutic antibody BGM-2121 targeting parathyroid hormone-related peptide (PTHrP) for pancreatic cancer.
C. Ho, C.-F. Tseng, C.-J. Huang;
BioGate Precision Medicine Corp., Taipei, Taiwan

Although approximately 35% of approved drugs target G-protein-coupled receptors (GPCRs), cancer-specific GPCR-targeted therapies remain limited. Notably, several GPCRs and their ligands, including parathyroid hormone-related protein (PTHrP), are overexpressed in various solid tumors, including pancreatic cancer. In about 30% of pancreatic cancer cases, the *PTHLH* gene is co-amplified with the *KRAS* oncogene on chromosome 12, a phenomenon also observed in lung and colorectal cancers. Furthermore, the *PTHLH* gene can drive cancer progression through self-amplification. To address this, BioGate has developed BGM-2121, a potent humanized IgG1 monoclonal antibody targeting the N-terminal region (a.a. 1-34) of PTHrP, aiming to treat pancreatic cancer by targeting the PTHrP/PTH1R pathway. Preclinical studies have confirmed the specificity and antitumor activity of BGM-2121. In vitro assays demonstrated the antibody's high affinity and specificity for PTHrP, while in vivo animal studies using human pancreatic cancer cell models in mice showed significant antitumor activity. Notably, BGM-2121 effectively reduced tumor growth, halted disease progression in mouse models, and significantly extended survival. Additionally, in lung squamous cell carcinoma models, BGM-2121 successfully prevented and even reversed hypercalcemia and weight loss—key contributors to humoral hypercalcemia of malignancy (HHM) and cancer cachexia in human patients. Comprehensive safety and pharmacokinetic (PK) studies, including toxicokinetics (TK) and GLP-compliant repeated-dose toxicity studies, have thoroughly evaluated the safety profile of BGM-2121. In conclusion, this potent PTHrP-targeting antibody shows considerable promise as a therapeutic agent for cancers with PTHrP overexpression (e.g., pancreatic and lung cancers), humoral hypercalcemia of malignancy, and PTHrP-mediated cancer cachexia. We are currently preparing to submit an Investigational New Drug (IND) application to the TFDA and plan to initiate clinical trials to evaluate its potential as a novel biologic treatment for hard-to-treat metastatic cancers in Taiwan.

#2125 The trispecific T cell engager platform enhances T cell function and therapeutic efficacy via integrated 4-1BB VHH antibody co-stimulation.

Kaiqing Zhang, Baihong Liu, Liu Yang, Saina Ma, Jiawei Wu, Jinhua Zhao, Yang Chen, Xueyuan Jiang, Peiyan Pan, Jing Zhang, Yi Yang

Biocytogen, Waltham, MA

CD3 T cell engagers (TCEs) have gained significant clinical interest, with 9 approvals in the past decade. Advances in solid tumor-targeting TCEs, such as DLL3xCD3, GP100xCD3, and STEAP1xCD3, indicate the potential to overcome the challenges posed by solid tumor barriers. However, issues like cytokine release syndrome (CRS), low T cell infiltration, treatment resistance, and limited patient response continue to pose hurdles. To optimize efficacy and minimize adverse effects, CD3 TCEs need further development.
The inclusion of the co-stimulatory molecule 4-1BB, which promotes T cell proliferation and upregulates T cell survival genes expression and activation, in the design of trispecific TCE presents an attractive approach. CAR-T cell therapies suggest that combining 4-1BB signaling achieves more robust and durable anti-tumor responses. Therefore, we developed a tumor antigen - dependent CD3×4-1BB trispecific antibody (TriAb) platform by integrating 4-1BB nanobody generated from RenNano^{TM} mice to maximize T cell activation and sustain the T cell response.
Compared with the canonical CD3 TCE, Our TriAb platform features a weaker binding activity to the CD3 receptor through steric hindrance, reducing safety risk relative to canonical CD3 TCEs. We validated the TriAb platform with three targets: Claudin18.2, DLL3 and CDH17. In vitro, TriAbs demonstrated robust antigen-dependent cytotoxic activity against tumor cell lines, surpassing bispecifc antibodies and benchmark counterparts in T cell activation and cytokine production. Importantly, TriAb did not induce cytotoxicity in tumor antigen-negative cells or non-specific cytokine release.
In xenograft model with established tumors, TriAbs showed significant tumor growth inhibition. Additionally, TriAbs exhibited an antibody-like PK profile in CD3ε×4-1BB humanized mice, supporting intermittent dosing in human. Taken together, these data indicate our TriAb platform holds promise as a next-generation therapeutic option for clinical development.

#2126 LY6G6D targeting with a T cell engager is a promising therapeutic for MSS colorectal cancer.

B. Yigit, P. Paluru, E. Jurgaityte, K. Nuno, J. Chen, N. Fisher, A. Martinko, M. Mumbach, A. Easton, R. Guerrero, M. O'Huallachain, P. Ram, J. Verboon, M. Lu, J. Granja, D. Rock, D. Kugler;
Cartography Biosciences, Inc., South San Francisco, CA

T cell engagers (TCEs) are proving to be a potent therapeutic strategy for the treatment of solid tumors. Clinical data have shown that TCEs can drive T cell activity in "cold" tumors, including colorectal cancer. However, identifying highly specific tumor antigen targets is crucial for delivering doses that achieve optimal tumor exposure and maximize therapeutic efficacy.
Analysis of primary patient samples indicated enrichment of LY6G6D in microsatellite stable (MSS) and instability-low (MSI-L) colorectal cancer (CRC). A highly potent, stable, and manufacturable CB21 TCE was designed to optimally engage LY6G6D and CD3. Strong binding affinity to the LY6G6D target antigen, and an optimized CD3 affinity, maximized T cell activation and cytokine production yielding significant cytotoxic effects across multiple cell lines with differing physiologically relevant levels of LY6G6D. Additionally, in endogenous LY6G6D expressing xenograft models the CB21 TCE displayed robust tumor growth inhibition in a dose-dependent manner. A pharmacokinetics (PK) and tolerability study with the cynomolgus cross-reactive CB21 TCE was administered to nonhuman primates. Exposure levels multiple times higher than the predicted clinical exposures were achieved with no significant cytokine release or meaningful alterations in clinical blood chemistry observed. Human PK were predicted to have antibody-like characteristics. Combined with the PK and PD data, human dose predicts efficacy with a favorable therapeutic window. An accelerated phase I clinical plan is presented.
Utilizing our ATLAS platform, we identified LY6G6D as the most promising target in MSS CRC due to its specific expression in malignant cells, high antigen surface stability, and persistence in both primary and metastatic CRC. The data presented support that the CB21 TCE has been meticulously engineered to be a potentially first and best-in-class TCE targeting LY6G6D.

#2127 Efficacy of monoclonal antibodies against pathogenic S100A8/9 in myelodysplasia and leukemia models.

Rahul Sanawar[1], Ariel Fromowitz[2], Srinivas Aluri[1], Sarah Aminov[1], Srabani Sahu[1], Leah Kravets[1], Shanisha Gordon Mitchell[1], Mani Mohindru[3], Aditi Shastri[1], Alan List[3], Eric Padron[4], Rongbao Zhao[1], Amit Verma[1]

[1]Department of Oncology, Montefiore Einstein Cancer Center, Albert Einstein College of Medicine, Albert Einstein College of Medicine, Bronx, NY,[2]Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas MD Anderson Cancer Center, Houston, TX,[3]Roshon Therapeutics, Bronx, NY,[4]Malignant Hematology, Moffitt Cancer Center, Tampa, FL

Introduction: Myelodysplastic syndromes (MDS); chronic myelomonocytic leukemias (CMML) and acute myeloid leukemia (AML) are characterized by a block in normal differentiation of stem and progenitor cells that leads to cytopenias. The diseased marrow microenvironment is characterized by expansion of immunosuppressive myeloid derived suppressor cells (MDSCs) that produce S100A8 and S100A9 proteins. These proteins form heterodimers that bind multiple receptors including TLR4/MD2, CD33 and RAGE, and lead to leukocyte recruitment, inflammatory cytokine secretion and formation of MDSCs. Even though S100A8/9 heterodimers (also known as DAMPs, alarmins, calprotectin) are known drivers of disease pathogenesis, no specific targeting therapeutic approaches have been tested in MDS/CMML.

Results: Analysis of a large dataset of MDS patient derived CD34+ hematopoietic stem and progenitors (N=183) showed increased expression of S100A8/9 receptors TLR4/MD2, CD33 and RAGE when compared to healthy controls. Furthermore, within the MDS patient cohort, those with higher S100A8/9 expression had more severe anemia and required more RBC transfusions. Additionally, in a CMML dataset (N=39), scRNA-seq profiles showed significantly higher expression of both S100A8 and S100A9 in the disease initiating, inflammatory (cluster 2) cells. Distinct neutralizing monoclonal antibodies (mAbs) targeting human S100A8/9 homo/heterodimers were generated by single B cell cloning from immunized rabbits. Selected rabbit derived, chimeric and subsequently humanized antibodies demonstrated nanomolar neutralizing activity, effectively inhibiting interaction of S100A8/A9 with RAGE, Siglec-3, Siglec-9, CD147, CD146, CD300ld and CD69. In functional assays, these neutralizing mAbs also suppressed the formation of MDSCs and T-regulatory cells from human PBMC cultures. Primary MDS, CMML and AML mononuclear cells (N=11, with numerous mutations) were treated with monoclonal S100A8/9 neutralizing antibodies and grown in clonogenic assays. Neutralization of S100A8/9 led to increased erythroid colony formation and increased erythroid differentiation as evident from Glycophorin A and CD71 expression by FACS analysis.

Conclusion: We demonstrate overexpression of S100A8/9 and its receptors in MDS and leukemia samples and have developed potent neutralizing monoclonal antibodies against the S100A8/9 heterodimer that can deplete MDSCs and reduce T-reg cell formation. Additionally, antibody neutralization of S100A8/9 stimulates erythropoiesis in a broad range of primary MDS and myeloid leukemia patient specimens. These results provide compelling pre-clinical rationale for clinical development of S100A8/9 antibodies for the treatment of myeloid malignancies.

#2128 Uncovering the multiple therapeutic mechanisms of trastuzumab deruxtecan (T-DXd): effective extracellular payload release and immunomodulatory stimulation.

Li-Chung Tsao[1], John S. Wang[2], Xingru Ma[3], Sirajbir Sodhi[4], Joey V. Ragusa[3], Bushangqing Liu[1], Jason McBane[1], Tao Wang[1], Junping Wei[1], Cong-Xiao Liu[1], Xiao-Yi Yang[1], Gang-jun Lei[1], Ivan Spasojevic[5], Ping Fan[5], Timothy N. Trotter[1], Michael A. Morse[4], Herbert K. Lyerly[1], **Zachary C. Hartman**[1]

[1]Surgery, Duke University Medical Center, Durham, NC,[2]MSTP Program, Duke University Medical Center, Durham, NC,[3]Pathology, Duke University Medical Center, Durham, NC,[4]Medicine, Duke University Medical Center, Durham, NC,[5]PK/PD Core Laboratory, Duke University Medical Center, Durham, NC

Trastuzumab deruxtecan (T-DXd), also known as fam-trastuzumab-deruxtecan-nxki, is a novel HER2-targeting antibody-drug conjugate (ADC) that has shown remarkable clinical efficacy in breast cancer (BC) and other cancers with varying levels of HER2 expression. However, the mechanisms underlying its activity in HER2-low and HER2-ultralow BC, and the role of immune activation, remain poorly understood. Here, we demonstrate that T-DXd's efficacy in HER2-low/ultralow BC is predominantly driven by the extracellular tumor-specific protease Cathepsin L (CTSL), but not Ccathepsin B (CTSB). Our in vitro and in vivo studies reveal that extracellular CTSL cleaves T-DXd's linker, releasing the DXd payload (validated by mass spectrometry) and inducing cytotoxicity within HER2-low/ultralow tumor microenvironments. This mechanism mediates potent anti-tumor effects across multiple HER2-negative cancers. Importantly, analysis of BC biopsies and tissue microarrays from T-DXd-treated patients revealed high CTSL expression in tumor and stromal compartments, independent of HER2 levels, suggesting broad therapeutic potential. In addition to extracellular cleavage, we also found that T-DXd elicits immune activation as an anti-tumor mechanism in HER2-positive BC. The DXd payload induces immunogenic cell death, activating nearby myeloid immune cells via TLR4 and STING pathways, while its antibody backbone engages Fcγ-receptors to stimulate Antibody-Dependent Cellular Phagocytosis (ADCP). This process enhances tumor antigen uptake and drives the expansion of antigen-specific CD8+ T cells. Furthermore, DXd upregulates CD47 on tumor cells, making them more susceptible to immune clearance. Combining CD47 blockade with T-DXd significantly enhanced anti-tumor efficacy in an immune suppressive HER2-transgenic BC mouse model, promoting CD8+ T cell memory and preventing tumor recurrence post-treatment. These studies suggest that strategies targeting the CD47-SIRPa axis may enhance the ability of ADCs to elicit tumor-specific immunity, thus extending the efficacy of these agents and generating long term memory responses against non-immunogenic cancers.

#2129 An innovative multi-specific Fc fusion protein simultaneously targeting PD-L1, VEGF-A, and PLGF for pancreatic cancer treatment via reshaping the tumor microenvironment.

Hyungul (David) Yang, Jin A Shin, Hyeseong Lim, Eunbyul Cho, Daeseok Oh

Panolos Bioscience, Inc., Hwaseong, Korea, Republic of

Introduction: Pancreatic cancer is a notoriously cold tumor characterized by a dense extracellular matrix and an immunosuppressive microenvironment, resulting in the limited efficacy of many immune-oncology therapies. Here, we investigated the therapeutic potential of PB203, a novel multi-specific Fc fusion protein that simultaneously blocks PD-L1, VEGF-A, and PLGF, for the treatment of pancreatic cancer.
Methods: PB203 was engineered and assessed for its binding affinities to the target molecules and its inhibitory capacity against ligand-mediated signaling in vitro at the cellular level. Its therapeutic efficacy was further evaluated in an orthotopic human pancreatic cancer model using humanized mice. Endpoints included anti-tumor effects, fibrosis modulation, and immune profile remodeling within the tumor microenvironment (TME).
Results: PB203 demonstrated picomolar binding affinities to VEGF-A and PLGF and sub-nanomolar binding affinity to PD-L1. Remarkably, it maintained its binding affinities under conditions mimicking simultaneous target engagement, showing comparable efficacy to single-target interactions, regardless of binding sequence or target occupancy. The Fc fusion protein effectively inhibited ligand-mediated signaling without compromising the potency of its parental molecules. In an orthotopic pancreatic cancer model using humanized mice treated with gemcitabine, PB203 achieved significant anti-tumor efficacy, including cases of complete regression. Notably, gemcitabine treatment alone increased fibrosis within the TME; however, co-treatment with PB203 significantly reversed this effect. PB203-mediated fibrosis reduction correlated with a decreased abundance of several cancer-associated fibroblast (CAF) subsets, highlighting its potential to remodel the TME and enhance therapeutic synergy in pancreatic cancer.
Conclusions: PB203 demonstrated substantial anti-tumor activity in an orthotopic pancreatic cancer model featuring an immunosuppressive TME, closely mirroring patient conditions. Its ability to inhibit CAFs and reduce fibrosis within the TME, likely driven by PLGF blockade, represents a unique mechanism of action. These findings suggest that beyond emerging PD-(L)1/VEGF-A bi-specific strategies, triple blockade of PD-(L)1, VEGF-A, and PLGF with PB203 could offer a novel therapeutic option for highly desmoplastic and hard-to-treat cancers like pancreatic cancer. Further clinical investigations are warranted to validate these findings.

#2130 Optimization of tarlatamab step-up dosing regimen to mitigate cytokine release syndrome using quantitative systems pharmacology modeling.

Oleg Demin Jr, Galina Kolesova, Dmitry Shchelokov

InSysBio CY, Paphos, Cyprus

Tarlatamab is a DLL3/CD3 bispecific T-cell engager (TCE) recently approved by the FDA for the treatment of extensive-stage small cell lung cancer (ES-SCLC). The dosing follows a step-up regimen for the first cycle, with 1 mg given on day 1, and 10 mg given on days 8 and 15. A similar step-up dosing regimen, but with 100 mg instead of 10 mg, was also tested in a pivotal phase 2 trial (Ahn et al. *N Engl J Med*. 2023 Nov 30;389(22):2063-2075). The alternative step-up dose or step-up regimen were not investigated in the study. The aim of our work was to explore the alternative step-up regimens of tarlatamab, predicting trimer (complex of TCE with target receptors) kinetics in the tumor using quantitative systems pharmacology (QSP) modeling.

The QSP model integrated pharmacokinetics of tarlatamab, its distribution into the tumor of patients with ES-SCLC, binding with CD3 expressed by T-cells and DLL3 expressed by cancer cells, formation of trimer in the immunological synapse between T-cell and cancer cell, turnover of free and bound receptors, trimer internalization. Predicted peak trimer levels in the tumor are associated with cytokine release syndrome (CRS) incidence following tarlatamab administration. Higher peak levels of trimer are predicted for the doses and regimens causing more frequent CRS events.

The model showed that 1 mg is the optimal step-up dose for the regimens tested in clinical trials since lower doses led to a higher peak trimer level after second dose (10 mg given on day 8). However, split dosing of the 1st or 2nd doses and a longer step-up schedule decreased the peak trimer levels following tarlatamab administration. Splitting the first dose in the approved regimen resulted in a reduction of peak trimer by 30-40%, thus mitigating CRS incidence. Administration of the 0.5 mg on day 1 and 0.5 mg on day 4 also caused a minor decrease of peak trimer level after administration of 10 mg on day 8. A substantial reduction of the maximal level of the trimer after the second dose was predicted for the step-up regimen with 100 mg if the second dose given on day 8 is 3 mg instead of 100 mg. This dose optimization might lower the probability of CRS grade 3 events observed in patients treated with 100 mg.

Split-dose regimens and regimens with more than one step-up dose allow for minimizing CRS incidence without compromising the efficacy of TCEs. QSP modeling is a useful tool to investigate various doses and regimens of bispecific and multispecific antibodies.

#2131 Glycosylated protein targets open access to multiple tumor indications for different therapeutic modalities .

Aaron C. Groen[1], Nisha Shrestha[1], Boris Klebanov[1], Constantine Theodoropulos Constantine Theodoropulos[1], **Hans H. Wandall**[2]

[1]GO-therapeutics, Cambridge, MA,[2]University of Copenhagen, Copenhagen, Denmark

Using advanced O-glycoproteomics we pinpointed relevant glycopeptide epitopes and developed a highly cancer-specific, humanized monoclonal antibody (R800). This antibody binds to truncated O-glycans in the context of a peptide backbone with low nanomolar affinity. The crystal structure of R800 provides insights into its binding specificity showing direct interaction with both peptide and the truncated glycan. Development of a companion diagnostic assay based on immunohistochemistry with the humanized therapeutic antibody confirmed the specificity of R800 across multiple epithelial cancers. This included selective binding to 20-30% of both primary and metastatic triple negative breast, lung, pancreatic, colorectal, and ovarian cancers. There was no binding to healthy tissues. The versatility of R800 allows the application of the mAb in multiple therapeutic modalities, including chimeric antigen receptor T-cell therapies (CAR-T cells) and antibody-drug conjugates (ADCs). In CAR-T cell applications using R800's antigen-binding domain demonstrated potent, selective cytotoxicity against R800-target expressing cancer cells in vitro in vivo in CDX models. In ADC applications, R800 formulated with either the anti-mitotic agent, monomethyl auristatin E payload (R800-MMAE) or with the topoisomerase 1 inhibitor payload, exatecan (R800- exatecan) show potent activity in vitro (EC50 ~1nM) across multiple cell lines. Both R800 ADCs effectively eliminated tumors in preclinical PDX models, including lung, ovarian, and TN breast PDx models with a MED of 2mg/kg. By targeting the cancer-specific truncated O-glycans recognised by R800, we exploit a novel target space offering unparalleled cancer selectivity. This strategy holds significant potential for enhancing the efficacy and safety of treatments for solid tumors.

#2132 KA-3123-LD38: a novel bispecific antibody-drug conjugate (ADC) targeting EGFR and B7H3 with a DNA topoisomerase I inhibitor payload.

Guojin Wu[1], Chuanying Xu[2], Weihong Nian[2], Zhengcheng Guo[1], Jiabei Liang[1], Feng He[2], Qing Zhou[2]

[1]Kyinno Biotechnology Co., Ltd, Beijing, China,[2]Shanghai Escugen Biotechnology Co., Ltd, Shanghai, China

KA-3123-LD38 is a novel bispecific antibody-drug conjugate (ADC) designed to target both EGFR and B7H3, two markers that are highly expressed in various tumor cells but have limited expression in normal tissues. The co-expression of EGFR and B7H3 in tumor cells is particularly high, making them promising therapeutic targets. By targeting both of these markers simultaneously, KA-3123-LD38 aims to reduce the risk of "on-target/off-tumor" cytotoxicity, a common side effect in cancer therapies. This ADC is a IgG1 with a common light chain, conjugated with LD-38, an Escugen proprietary ADC platform composed of Topli payload and highly hydrophilic stable cleavable linker. KA-3123-LD38 binds to B7H3 with high affinity, with a Kd of less than 0.01 nM, while its affinity for EGFR is weaker, with a Kd greater than 10 nM. When compared to monoclonal antibodies targeting B7H3, KA-3123-LD38 exhibits a significantly faster internalization rate in tumor cells that co-express EGFR and B7H3. In vitro assays show that KA-3123-LD38 effectively induces cytotoxicity in tumor cells expressing either EGFR and B7H3 together or B7H3 alone. However, the cytotoxic effect is notably stronger in cells co-expressing both targets. Furthermore, KA-3123-LD38 demonstrates strong bystander killing activity, further enhancing its therapeutic potential. In xenograft mouse models, KA-3123-LD38 strongly inhibits the growth of a variety of tumor types, including NSCLC, esophageal squamous cell carcinoma, colorectal cancer, and gastric cancer. Ongoing toxicity studies suggest that KA-3123-LD38 has a favorable safety profile, with low toxicity observed. These promising results highlight KA-3123-LD38 as a potentially powerful treatment for a broad range of solid tumors.

#2133 KA-2887-LD38: a novel bispecific antibody-drug conjugate (ADC) targeting both EGFR and PD-L1.

Guojin Wu[1], Chuanying Xu[2], Weihong Nian[2], Zhengcheng Guo[1], Jiabei Liang[1], Feng He[2], Qing Zhou[2], Jinying Ning[1]

[1]Kyinno Biotechnology Co., Ltd, Beijing, China,[2]Shanghai Escugen Biotechnology Co., Ltd, Shanghai, China

PD-L1 is widely expressed across various cancer types and suppresses immune responses against tumor cells. EGFR is also highly expressed in many solid tumors and plays a key role in driving tumor growth. Targeting the PD-1/PD-L1 signaling pathway and the EGF/EGFR signaling pathway using antagonistic antibodies or ADCs has shown promising results in cancer therapy. However, both approaches can lead to undesirable "on-target/off-tumor" effects. Co-expression of PD-L1 and EGFR is rare in normal tissues but is commonly observed in most PD-L1-expressing tumor cells. In this study, we developed a bispecific antibody, KA-2887, using our common light chain antibody discovery platform to target both EGFR and PD-L1. This bispecific antibody was then used to construct the ADC KA-2887-LD38. KA-2887-LD38 has a conventional IgG1 structure, conjugated with LD-38, an Escugen proprietary ADC platform composed of Topli payload and highly hydrophilic stable cleavable linker. It binds to EGFR with an affinity of 3.7 nM and to PD-L1 with an affinity of 0.6 nM. KA-2887-LD38 effectively blocks both the interaction between EGF and EGFR, and the interaction between PD-1 and PD-L1.The expression of EGFR on tumor cells enhances the inhibitory effect of KA-2887-LD38 on the PD-1/PD-L1 interaction, and vice versa. Compared to monoclonal antibodies targeting PD-L1, KA-2887-LD38 shows a much faster internalization rate when binding to tumor cells co-expressing EGFR and PD-L1. In vitro, KA-2887-LD38 demonstrates cytotoxicity against tumor cells expressing either EGFR and PD-L1 together or EGFR alone, but has a weaker effect on tumor cells expressing only PD-L1. Tumor cells co-expressing EGFR and PD-L1 are more sensitive to KA-2887-LD38. Additionally, KA-2887-LD38 exhibits strong bystander killing activity. Xenograft mouse tumor models show that KA-2887-LD38 strongly inhibits the growth of various tumor types, including NSCLC, head and neck squamous cell carcinoma, and esophageal squamous cell carcinoma. Toxicity studies on KA-2887-LD38 are ongoing. The results suggest that KA-2887-LD38 holds significant potential for the treatment of a variety of solid tumors.

#2134 KA-2886-LD38: a bispecific antibody-drug conjugate (ADC) targeting both EGFR and MET, incorporating a novel linker-payload that inhibits DNA topoisomerase I.

Guojin Wu[1], Chuanying Xu[2], Weihong Nian[2], Zhengcheng Guo[1], Jiabei Liang[1], Feng He[2], Qing Zhou[2], Jinying Ning[1]

[1]Kyinno Biotechnology Co., Ltd, Beijing, China,[2]Shanghai Escugen Biotechnology Co., Ltd, Shanghai, China

Epidermal growth factor receptor (EGFR) and mesenchymal-epithelial transition factor (MET) are frequently co-expressed in multiple solid cancers, and over-activation of MET signaling is one of the major mechanisms of resistance to EGFR tyrosine kinase inhibitors (TKIs). Antagonistic bispecific antibodies targeting both EGFR and MET have been shown to overcome this resistance. KA-2886-LD38 is a bispecific ADC targeting both EGFR and MET. It is a bispecific common light chain IgG1 , conjugated with LD-38, an Escugen proprietary ADC platform composed of Topli payload and highly hydrophilic stable cleavable linker. It binds to MET with an affinity of 0.02 nM and to EGFR with an affinity of 1 nM. KA-2886-LD38 blocks the binding of HGF to MET with an IC50 of about 0.3 nM and the interaction of EGF with EGFR with an IC50 of about 2 nM. Compared to the benchmark ADC AZD9592, KA-2886-LD38 exhibits a similar internalization rate when binding to tumor cells but demonstrates significantly stronger cytotoxicity and bystander killing activity across different tumor cell lines in vitro. Additionally, in xenograft mouse tumor models with various tumor types, including NSCLC, head and neck squamous cell carcinoma, colorectal cancer, esophageal squamous cell carcinoma, and triple-negative breast cancer, KA-2886-LD38 showed much stronger in vivo efficacy compared to AZD9592. Moreover, KA-2886-LD38, when combined with osimertinib, also demonstrated a combination inhibitory effect on H1975 tumor growth. KA-2886-LD38 is well-tolerated at doses up to 30 mg/kg in EGFR and MET humanized genetically modified mice, and toxicity studies in monkeys are ongoing. These results suggest that KA-2886-LD38 has remarkable potential in the landscape of anti-EGFR/cMet therapies.

#2135 QLS4131, a proprietary T cell engager targeting both BCMA and GPRC5D for the treatment of multiple myeloma.

Liuqing Yang[1], Hongliang Qian[1], Juan Luo[1], Ke Liu[2], **Ling Zhang**[1], Shuyong Zhao[2], Hua Ying[1], Weikang Tao[1]

[1]Shanghai Qilu Pharmaceutical Research and Development Center LTD., Shanghai, China,[2]Qilu Pharmaceutical Co., Ltd., Jinan, China

Multiple myeloma (MM) is a genetically complex and heterogeneous malignancy with poor prognosis. Despite recent advances in the development of new therapeutics, clinical management of MM remains a big challenge largely due to quick emergence of drug resistance. Thus, novel therapies which can cause deeper response or overcome drug resistance are highly desired. It has been found that both BCMA and GPRC5D are overexpressed in MM in a manner of either co-expression or mono-expression. In fact, T cell engagers (TCE) targeting either BCMA or GPRC5D alone have been approved by FDA for MM treatment. Hence, TCE target both BCMA and GPRC5D have a great potential to demonstrate superior efficacy and overcome resistance to TCE only targeting a single antigen. QLS4131 demonstrated a higher affinity for MM tumor cell lines when compared to JNJ-79635322, which is a clinical stage TCE targeting both BCMA and GPRC5D developed by Janssen Research & Development. QLS4131 was capable of effectively depleting both dual (BCMA and GPRC5D) and single (BCMA or GPRC5D) target-expressing cells, and its cytotoxicity was substantially more potent than JNJ-79635322. Meanwhile, the level of IL-6 cytokine release was comparable to that of JNJ-79635322. Additionally, QLS4131 maintained robust TDCC activity even in the presence of 2.5 μg/mL soluble BCMA. The single-dose PK evaluation revealed linear pharmacokinetic characteristics with a half-life (T1/2) of approximately 120 hours, it also demonstrated high bioavailability (79.83%) when SC dosing. In in vivo pharmacology studies, QLS4131 could inhibit tumor growth in a dose-dependent manner and even induce tumor regression and exhibited superior antitumor efficacy related to JNJ-79635322. QLS4131 was well tolerated in a cyno monkey QW x 4 GLP toxicity study. Skin and nail toxicity (related to the GPRC5D target) was not observed. The HNSTD was 2 mpk for both intravenous and subcutaneous injections. In conclusion, the excellent efficacy, favorable PK, and safety profile support the potential of QLS4131 as a novel therapeutic agent to address the significant unmet needs in multiple myeloma. A Phase 1 dose-escalating study of QLS4131 in multiple myeloma is currently underway (NCT06500507).

#2136 ADORA2A specific antibodies show enhancement of anti-tumour immunity.

Paule Hermet[1], Siret Tahk[1], Gaily Kivi[1], Joan Teyra[1], Madis Jakobson[1], Ana Shahpazir[1], Liis Siigur[1], Robin Loving[2], Reet Kurg[3], Mart Ustav[1], Andres Mannik[1], Mart Ustav[1]

[1]Icosagen Cell Factory OU, Tartu, Estonia,[2]Salipro Biotech, Stockholm, Sweden,[3]University of Tartu, Tartu, Estonia

Background: ADORA2A (A2AR), is an adenosine receptor that suppresses immune cell toxicity through sensing extracellular adenosine. A2AR belongs to a group of seven-transmembrane receptor proteins called G protein-coupled receptors (GPCRs) which act as detectors to various signals from outside the cell. We aim to reduce the immunosuppressive properties of adenosine in the tumor microenvironment (TME) by targeting A2AR with monoclonal antibodies (mAbs). mAbs are an attractive modality to therapeutically target complex multi-pass membrane proteins, but mAb development is hindered by many challenges. Here we report the discovery and functional characterization of 23 A2AR-specific monoclonal antibodies developed using Icosagen's proprietary antigen displaying Virus-Like-Particle (VLP) based chicken immunization and mAb screening platforms.

Methods: Monoclonal antibodies against A2AR were generated using a DNA or mRNA and VLP-based immunization strategy. VLPs were generated using a proprietary technology ensuring high density of display of A2AR on VLPs. Chickens were selected as immunization hosts due to being evolutionally more divergent from mammals increasing the chance of immunogenicity and obtaining antibody candidates against conserved membrane proteins. Antibody discovery was performed with Icosagen's proprietary HybriFree B-cell cloning technology, complemented with chicken derived phage display library screening platform. Antibody specificity was confirmed using VLP-based immunoassays and overexpression cell lines. Antagonistic function of A2AR-specific antibodies was confirmed on immune cells and reporter cell line-based assays.

Results: Immunization of chicken and dynamic antibody discovery methods resulted in discovery of 23 unique A2AR-specific antibody clones. These antibodies exhibit high affinity binding to A2AR, as indicated by low nanomolar EC50 values determined through cell-based assays, complemented with SPR analysis using A2AR-expressing nanoparticles. Antibody clones with A2AR-antagonistic function show enhancement of T cell activation upon A2AR agonist treatment, highlighting their potential to boost anti-tumor immune response. These antibodies can further translate into boosting CAR-T efficacy in overcoming suppressive TME, thereby expanding it for treatment for solid tumors.

Conclusions: This study presents highly specific monoclonal antibodies targeting the adenosine receptor ADORA2A. The lead antibody clones exhibiting antagonistic function *in vitro* will undergo further optimization using Icosagen's phage display-based affinity maturation platform. This process aims to enhance their properties for development into clinical candidates, advancing therapeutic interventions targeting ADORA2A and supporting their potential in immunotherapy applications.

#2137 EVOLVE205, a dual (2:1) CD20 targeted CD3 trispecific T cell engager with CD2 costimulation for the treatment of B cell malignancies and B cell autoimmune disorders.

Xingyue An, Mohosin Sarkar, Guixian Jin, Tiffany Liang, Afsana Sabrin, Tracy Lichter, Danielle Klaskin, Evelyn Teran, Abliz Abduqadir, Hayden Karp, Julio Rodriguez, Hemachander Capiralla, Zengzu Lai, Louis Matis, Stella Martomo, Agnes Meade, Martin Preyer, Jay S. Fine, Jeremy S. Myers

EvolveImmune Therapeutics, Inc, Branford, CT

T cell engager (TCE) CD3-bispecifics have improved the treatment of patients with B cell lymphomas leading to recent approvals of the CD20-targeted CD3 bispecifics including Glofitamab, Mosunetuzumab, and Epcoritamab for patients with relapsed, refractory diseases. Recently, combinations of CD3-bispecifics and tumor-targeted costimulatory agonists have demonstrated enhanced efficacy in preclinical models. These combination approaches are currently being investigated in the clinic but may have challenges in establishing optimal dose scheduling and dose levels to ensure simultaneous CD3 and costimulatory pathway activation. T cell costimulation using CD2 is an alternative strategy that may provide significant benefits compared to 4-1BB and CD28 due to sustained CD2 surface expression on T cells found in the tumor microenvironment.

EVOLVE205 molecules are dual (2:1) CD20-targeting TCE with integrated CD2 agonism to redirect optimized T cell effector function through CD2 co-stimulation, increasing avidity-driven binding to CD20, to achieve potential superior performance to clinical benchmarks.

EVOLVE205 molecules show superior cytotoxicity in diffuse large B-cell lymphoma cell line models and primary tissue B cells. In a PBMC-HT ($CD20^{low}$) co-culture assay, EVOLVE205 displays improved T cell expansion and superior tumor cell killing without a substantial increase in cytokine release compared to 1:1 and 2:1 CD20-targeted clinical benchmark bispecifics. Importantly, in a B-cell depleted PBMC-HT tumor co-culture assay, the tumor-killing potency of EVOLVE205 was improved by up to 70-fold compared to Glofitamab, and by over 1,000-fold compared to anti-CD20 mAbs such as Rituximab. EVOLVE205 also demonstrated superiority in depleting primary tissue B cells over approved CD20 or CD19 targeting clinical benchmarks. These data suggest the potential for EVOLVE205 to establish an improved therapeutic index relative to these clinical benchmarks. In a PBMC-engrafted xenograft NSG mouse model with $CD20^{high}$ WSU-DLCL2 cell line, EVOLVE205 showed significantly enhanced tumor growth inhibition efficacy at the tumor growth permissible dosing level of Glofitamab.

CD20-targeted EVOLVE205 shows high tumor-killing potency without inducing exaggerated cytokine production in vitro. It also demonstrates significant in vivo efficacy and a favorable developability profile. Our data indicated that EVOLVE205 with integrated CD2 co-stimulation offers greater efficacy and safety advantages compared to clinically available CD20-targeted TCE therapies and anti-CD20 mAbs for the treatment of B cell lymphomas and for B cell-mediated autoimmune diseases.

#2138 ALG01, a next-generation anti-PD-L1×TROP-2 bispecific antibody-drug conjugate (BiADC) with STAR technology, demonstrated excellent anti-tumor efficacy & safety in preclinical studies.

J. Xiang, W. Gao;
Allygen Biologics, Shanghai, China

The combination of anti-TROP2 ADCs with PD-(L)1 inhibitors represents a promising therapeutic strategy, merging targeted therapy with immunotherapy to improve treatment outcomes. Clinical trials are underway to explore its safety and efficacy in various cancer types. However, challenges remain, including accumulative toxicity, dosing difficulties, and tumor resistance. Bispecific ADCs (BiADCs), which target two antigens simultaneously, offer a promising advancement by enhancing tumor specificity, improving therapeutic efficacy, and reducing damage to healthy tissues. BiADCs are emerging as a pivotal innovation in oncology, particularly for difficult-to-treat cancers.Here, we present preclinical findings for ALG01, a novel anti-PD-L1×TROP2 BiADC with STAR (Smart TME (Tumor Microenvironment) Activated Release) technology and incorporated with targeted therapy and immunotherapy in one molecule, has shown remarkable anti-tumor activity and a favorable safety profile. ALG01 comprises a humanized PD-L1-targeting IgG1 fused with TROP2-targeting biparatopic VHHs at the C-terminal of the heavy chain, linked to exatecan via transglutaminase-mediated site-specific conjugation. In vitro studies demonstrated that ALG01 exhibits potent cytotoxicity against various TROP2 and/or PD-L1-positive cancer cell lines. Ex vivo, ALG01 was selectively and efficiently cleaved by proteases as designed within tumor tissues, compared to plasma and normal tissues (e.g., liver, spleen, lung), resulting in the release of anti-PD-L1 IgG1 and small-size anti-TROP2 VHH-drug conjugates. In vivo, ALG01 significantly regressed tumors in a TROP2-positive cell line-derived xenograft (CDX) model. Furthermore, in a MC38-PDL1-TROP2 syngeneic model, ALG01 treatment led to complete tumor eradication in 7 of 8 mice, showing significantly superior efficacy compared to reference anti-TROP2 ADC and its combination with reference anti-PD-L1 antibody.A rechallenge study further supported long-lasting ALG01's anti-tumor efficacy. After complete (8 of 8 mice) tumor eradication following a single dose of ALG01, mice were re-inoculated with tumor cells without additional treatment. Remarkably, the newly implanted tumors in 7 of 8 mice were once again fully eliminated, revealing a lasting immune activated memory effect. In toxicology studies, ALG01 was well tolerated in cynomolgus monkeys, with the highest non-severely toxic dose (HNSTD) observed at ≥45 mg/kg for DAR6 or ≥65 mg/kg for DAR4.These preclinical findings indicate that ALG01 offers a wide therapeutic window and may benefit patients with challenging-to-treat cancers. An IND application is planned for submission in 2025.

#2139 Determination of first in human dose of the T cell-redirecting bispecific antibody CT-95 targeting mesothelin.

Stanley A. Roberts, Kelly Byrnes-Blake, **Eric Butz**

Context Therapeutics, Philadelphia, PA

CT-95 is a mesothelin (MSLN) x CD3 bispecific T cell-redirecting antibody that is entering phase 1 clinical development. It is affinity tuned to avoid the soluble MSLN sink that has interfered with earlier MSLN-targeting programs. The first-in-human dose of CT-95 was determined using the minimal anticipated biological effect level (MABEL) approach, as required for immune agonist drugs. This is the dose level expected to lead to a minimal biological effect level in humans. The most sensitive and pharmacologically relevant measure of CT-95 activity was determined to be induction of CD25 expression on human T cells using a panel of six human MSLN expressing tumor cell lines. The median EC50 for this panel was 9.94 pM and resulted in an estimated target serum concentration of 1.98 ng/mL. Based on nonclinical PK data, the predicted volume of distribution is 123.5 mL/kg, which suggests a starting dose of 0.24 µg/kg. An additional safety factor was applied to obtain a proposed starting dose of 0.1 µg/kg. From the results of the GLP monkey study, the highest non-severely toxic dose (HNSTD) was considered to be 0.01 mg/kg, and the no-observed-adverse-effect level (NOAEL) was 0.003 mg/kg. Relative to doses in the one-month repeat dose GLP-compliant toxicology study, the proposed starting dose for CT-95 (0.1 µg/kg) is 30-fold lower than the NOAEL (3 µg/kg) and 100-fold lower than the HNSTD (10 µg/kg). The weight of evidence of these data supports the safety of the proposed clinical starting dose of 0.1 µg/kg for CT-95.

#2140 Beyond antibodies and CAR-T: CC312, a first-in-class, anti-CD19, anti-CD3, anti-CD28 trispecific antibody in treatment with relapsed and/or refractory B-cell malignancies.

Yingfeng Huang, Xiaofang Zhang, Ruixia Zhang, Zhen Jing, Caizhi Zhao, Fangfang Pan, Beibei Zheng, Ruixue Dai, Yingying Yang, **Li Zeng**

CytoCares (Shanghai) Inc., Shanghai, China

Background: Depletion of B cells has resulted in major therapeutic benefits in hematological malignancies. Several FDA-approved B cell-depleting therapies are available, including CAR-T and T cell engagers (TCE). Blinatumomab, the only CD19-targeting bispecific T cell engager approved for B cell malignancies, has disadvantages in clinical setting including sub-optimal pharmacokinetics, neurotoxicity, CRS, and lack of long-term efficacy. In this study we present CC312, a trispecific antibody engineered for better efficacy by leveraging “Two Signal” co-stimulation, as well as improved safety via target-dependent activation and high potency / low dosage leading to low off-target effects.

Methods: Effects of CC312 on T cell activation, proliferation, central memory T cell differentiation/proliferation and T cell killing activity were compared with blinatumomab in *ex vivo* assays. *In vivo* efficacy for CC312 was investigated in a Raji-luc s.c. engraftment CD34+ HSC-NCG mouse model. Moreover, a Phase Ia, open-label study is currently ongoing, and is designed to characterize the safety and tolerability of CC312 in patients with R/R B-NHL and R/R lymphocytic leukemia, to determine recommended dose(s), schedule(s), and route(s) of administration for future studies, and to estimate the preliminary anti-tumor activity of CC312.

Results: *In vitro*, CC312 exhibited more robust T cell activation, higher proliferation and ~10-fold greater potency versus blinatumomab. Additionally, CC312 induced a higher proportion of central memory T cells, and resulted in longer target cell killing relative to blinatumomab. *In vivo*, CC312 demonstrated similar tumor growth inhibition versus blinatumomab when dosed twice-weekly and more potent tumor growth inhibition when dosed once-weekly. In the ongoing Ph1a trial, CC312 has completed five levels of priming dose escalation as well as upper levels of treatment dose escalation, and has exhibited a favorable clinical safety profile to date with no DLTs or grade 3+ CRS/ICANS. CC312 achieved multi-fold depletion of peripheral blood B cells across dosages as well as ignited CD4 and CD8 T cell expansion and activation in B cell lymphoma patients.

Conclusion: In this study, CC312 exhibited key advantages over leading CD3xCD19 TCE blinatumomab in multiple clinically relevant *in vitro* functional assays, demonstrated robust tumor growth inhibition in B cell lymphoma mouse models and achieved thorough depletion of CD19+ B cells and potent activation of T cells in B cell lymphoma patients across dosages while maintaining a favorable safety/tolerability profile. Therefore, via its “Two Signal” co-stimulation approach, CC312 represents a promising candidate for treatment of B cell lymphoma. CC312’s clinical benefit will continue to be explored at higher doses as part of ongoing Ph1a dose escalation study.

#2141 Exploration of FGL-1 and LAG-3 expression and nivolumab resistance mechanisms in esophageal cancer.

Hiroki Matsuda, Tomoki Makino, Takafumi Hagi, Kota Momose, Kotaro Yamashita, Takuro Saito, Koji Tanaka, Tsuyoshi Takahashi, Yukinori Kurokawa, Hidetoshi Eguchi, Yuichiro Doki

Osaka University, Osaka, Japan

[Background] Anti-PD-1 antibody therapy for esophageal squamous cell carcinoma (ESCC) faces challenges such as low response rates, emphasizing the urgent need to develop novel regimens or predictive biomarkers. Lymphocyte Activation Gene 3 (LAG-3) is an inhibitory immune checkpoint molecule primarily expressed on T cells. It has been demonstrated that combination therapies (anti-LAG-3 and anti-PD-1 antibodies) are more effective than monotherapy (anti-PD-1 antibody) in melanoma. Recently, Fibrinogen-like protein 1 (FGL-1) has been identified as a major ligand for LAG-3. While FGL-1 is secreted from normal hepatocytes, it is known to be secreted in greater quantities by tumor cells in several cancers. FGL-1 that is detectable in peripheral blood holds potential as a biomarker for predicting ICI efficacy and prognosis. However, there are few reports on LAG-3 and FGL-1 in ESCC.
[Objectives] To clarify relationship between LAG-3/FGL-1 expression and patient survival or expression of other immune cells in ESCC. To investigate the significance of FGL-1 levels of peripheral blood as a biomarker for ICIs.
[Methods] In 316 preoperatively untreated ESCC patients who underwent curative resection, immunohistochemical staining was performed to confirm FGL-1 expression and localization. The relationship between FGL-1 expression, prognosis, and expression of CD3 and CD8 was evaluated.Serum FGL-1 levels were measured using the ELISA method in blood samples before start of treatment of 53 ESCC patients that underwent radical resection. The expression levels were compared with those of healthy controls. In patients with unresectable advanced or recurrent ESCC, plasma FGL-1 levels were measured using the ELISA method before administering anti-PD-1 antibody and evaluated its relationship with treatment efficacy and prognosis.
[Results] FGL-1 was localized in tumor cells in ESCC. 121 patients were defined as high FGL-1 expression, which was identified as a poor prognostic factor. However, no correlation was found between FGL-1 expression in tumor cells and the number of T cells. Serum FGL-1 levels were higher in ESCC patients compared to healthy individuals. No association was found between FGL-1 expression in serum and tumor tissue. Plasma FGL-1 levels were significantly higher in the non-responders compared with responders before anti-PD-1 antibody administration.
[Conclusions] Using pre-treatment biopsy specimens, FACS analysis will confirm LAG-3+CD8+ T cell expression among tumor-infiltrating lymphocytes, and the relationship with T cell subsets and FGL-1 expression will be evaluated.

#2143 Sequential anti-folate (pemetrexed) and anti-PD-L1 (avelumab) combination therapy in patients with previously treated MTAP-deficient advanced urothelial cancer: Clinical outcomes and immune correlative analyses.

Amishi Y. Shah[1], **Xinmiao Yan**[2], Rebecca S. Tidwell[3], Jianfeng Chen[1], Arlene O. Siefker-Radtke[1], Sangeeta Goswami[1], Omar Alhalabi[1], Yuxue Zhang[1], Ying Mei[1], Charles Guo[4], Miao Zhang[4], Ana C. Adriazola[1], Abu Hena Mostafa Kamal[5], Nagireddy Putluri[6], Mark A. Titus[1], Bogdan Czerniak[4], Matthew T. Campbell[1], Linghua Wang[2], Jianjun Gao[1]

[1]Department of Genitourinary Medical Oncology, UT MD Anderson Cancer Center, Houston, TX,[2]UT MD Anderson Cancer Center, Houston, TX,[3]Department of Biostatistics, UT MD Anderson Cancer Center, Houston, TX,[4]Department of Anatomical Pathology, UT MD Anderson Cancer Center, Houston, TX,[5]Metabolomics Core, Advanced Technology Cores, Baylor College of Medicine, Houston, TX,[6]Baylor College of Medicine, Houston, TX

MTAP-deficiency in urothelial cancer is associated with poor clinical outcomes but correlates with sensitivity to antifolate chemotherapy. This single-arm phase 2 trial (N=15) assessed the clinical and biological effects of a sequential combination therapy using the immune modulating antifolate agent pemetrexed and anti-PD-L1 agent avelumab in patients with advanced urothelial cancer resistant to prior chemotherapy and/or immunotherapy. The combination regimen treatment resulted in an overall response rate (ORR) of 20% (95% CI: 5%, 42%), a disease control rate (DCR) of 47% (95% CI: 23%, 71%), a median progression-free survival (PFS) of 2.3 months (95% CI: 1.9, 5.5), and a median overall survival (OS) of 13.5 months (95% CI: 3.2. 22.4). To investigate the relationship between clinical outcomes and tumor microenvironment (TME), single-cell RNA sequencing was performed on tumor samples collected from 15 patients at three time points: baseline, after pemetrexed monotherapy (cycle 2, day 1), and following combined treatment (cycle 4, day 1). This analysis profiled 49,161 cells spanning nine major cell types, including T cells, B cells, plasma cells, myeloid cells, epithelial cells, fibroblasts, and endothelial cells. Correlating TME composition with clinical outcomes revealed that higher proportions of T and NK cells at baseline were positively associated with PFS and clinical benefit. Conversely, elevated levels of proliferating myeloid cells and macrophages correlated with poorer responses to therapy. Pemetrexed monotherapy notably reshape the immune landscape, promoting increased infiltration of type-2 conventional dendritic cells (cDC2). Furthermore, patients with augmented B and plasma cell infiltration following pemetrexed treatment exhibited better clinical outcomes. Resistance to treatment was associated with increased adenosine signaling, suggesting a potentially targetable pathway to enhance treatment efficacy. Preclinical data suggest that targeting adenosine signaling pathway may further enhance the therapeutic effects of pemetrexed and anti-PD-L1 for MTAP-deficient bladder cancer. Additionally, urine methylthioadenosine (MTA), which accumulates due to MTAP deficiency, emerged as a promising biomarker for treatment monitoring. These discoveries unveil the link between TME of MTAP-deficient tumors and response for this clinical trial, suggest potential targetable mechanism to improve efficacy, which better the understanding of MTAP-deficient tumors and design promising strategies to improv clinical translation. These findings underscore the intricate link between the TME and treatment response in MTAP-deficient tumors, offering insights into resistance mechanisms to optimize clinical outcomes in MTAP-deficient urothelial cancer.

IMMUNOLOGY: Interplay between Immune System and Radio-, Chemo- and Targeted Therapies 1
Poster Session

#2147 Radiosensitization via ATM inhibition in small cell lung cancer leads to increased immunogenicity and tumor infiltration.

Bell Wu[1], Xiaozhuo Ran[2], Mary Shi[2], Lifang Song[2], Vivek Philip[2], Ming-Sound Tsao[2], Benjamin Lok[2]

[1]University of Toronto, Toronto, ON, Canada,[2]University Health Network, Toronto, ON, Canada

Purpose: Small cell lung cancer (SCLC) is an aggressive form of lung cancer with a dismal survival. PD-1/PD-L1 inhibitors have improved survival outcomes, but many patients fail to respond possibly due to a lack of T cell infiltration. Ionizing radiation (IR) activates innate regulatory genes through micronuclei formation and cGAS-STING upregulation. A previous *in vitro* dropout CRISPR screen has identified ATM as a top-ranked determinant of radiosensitization in SCLC cell-lines. Evaluation of ATM inhibition (ATMi) in combination with IR increases tumour T cell immunogenicity through a cGAS-STING-mediated mechanism.
Methods and Materials: We investigated the combination of ATMi (AZD1390) and IR in SCLC cell-lines SBC5 and SHP77, to determine the synergism of cGAS-STING activation via immunofluorescence. Chemokine and cytokine expression following combination treatment was probed by qPCR and surface expression of PDL1 and HLA class I was quantified through flow cytometry. In the SCLC syngeneic mice, we evaluated the efficacy and immune-sensitizing effects of a triple combination of ATMi, RT and anti-PDL1 (ATMi+IR+ICB).
Results: ATMi plus IR (ATMi+IR) lead to a significant ($p < 0.001$) increase of micronuclei frequency and cGAS micronuclei translocation relative to RT alone. ATMi+IR also resulted in a significant increase in CCL5 and CXCL10 expression compared to IR alone in SBC5 (FC = 6 and 1.5, respectively) and SHP77 (FC = 1.5 and 2, respectively). This phenotype was partially abrogated in cGAS knockout in SBC5 and H1048 cell-lines. Surface PDL1 increased by a FC of 3 in H82 in ATMi+IR relative to IR, while no change in surface HLA was observed. Within the KP1 syngeneic SCLC murine model, ATMi+IR+ICB led to a significant increase (p-value < 0.001) in T cell ($CD45^{+}CD3^{+}$) infiltration at day 12 relative to ATMi+IR. Interestingly, ATMi+IR had no change in T cell infiltration relative to both IR alone and IR plus anti-PDL1. Although an increase in T cell infiltration was observed, there was no additional benefit in tumour control of ATMi+IR+ICB compared to ATMi+IR.
Conclusions: In this study, we demonstrated pharmacological inhibition of ATM enhanced SCLC chemokine expression in a cGAS-STING-dependent manner. We further validated this phenotype in a syngeneic SCLC murine model. Despite this marked improvement in SCLC T cell infiltration no further benefits was observed. Further studies are required to understand this lack of benefit.

#2148 HDAC6 inhibitors modulate macrophage functional activity in the post-radiotherapy tumor microenvironment.

Xintang Li[1], Satish Noonepalle[1], Karen Tan[1], Sonia Sebaoui[1], Manasa Suresh[1], Nithya Gajendran[1], Marie Durr[1], David Quiceno-Torres[1], Bryan Weselman[1], Vidhi Sharma[1], Abishek Sehgal[1], Scott Grindrod[2], Anatoly Dritschilo[2], Alejandro Villagra[1]

[1]Oncology, Georgetown University, Washington, DC,[2]Shuttle Pharmaceuticals, Gaithersburg, MD

Introduction: In recent decades, radiation therapy (RT) has been a significant therapeutic tool for treating multiple cancer types. Primarily, radiation-induced immunogenic tumor cell death re-modulates the tumor microenvironment (TME) with the potential to enhance the efficacy of immunotherapies. Tumor-associated macrophages (TAMs) play a critical role in the anti-tumor innate immune response. However, post-RT, the phenotype of TAMs can easily convert from the pro-inflammatory M1-like to the immunosuppressive and tumor-promoting M2-like due to their plasticity. M2-like macrophages cause relapse and metastasis of the tumor. Therefore, reprogramming TAMs is critical. Our lab has reported that targeting Histone Deacetylase 6 (HDAC6) in macrophages can disrupt their phenotype shifting from M1-like to M2-like in TME.

Methods: We demonstrated the suppressive effect of HDAC6 inhibitors (HDAC6i) on M2-like macrophage phenotype markers by flow cytometry and qRT-PCR. Furthermore, in-vitro assays were performed, including trans-well migration assays, phagocytosis assays, and in-vivo syngeneic tumor models against different tumor cell types, including SM1 murine melanoma, 4T1 breast cancer, and TRAMP-C2 prostate cancer.

Results: The trans-well-based migration assay indicated an increment in M2-like macrophage migration to irradiated cancer cells compared to M1-like macrophages. This increment was suppressed when culturing M2 macrophages with HDAC6i. Furthermore, the co-culture assay between macrophages and cancer cells indicated that M1 macrophages showed a higher phagocytosis rate when co-culture with irradiated cells and HDAC6i treatment increased tumor cell phagocytosis. Meanwhile, an in-vivo study based on the 4T1 and SM1 tumor models model suggested that RT combined with HDAC6i treatment has better efficacy than the monotherapy treatment arms due to increased M1/M2 macrophage ratio. These results demonstrate that targeting macrophages with HDAC6is could enhance the overall therapeutic outcome of radiation therapy.

#2149 Copper-67 based targeted radioligand therapy to the somatostatin receptor 2 (SSTR2) provides added efficacy and may prime small cell lung cancer for immunotherapy.

Jaclyn L. Lange[1], Lachlan E. McInnes[1], Kurt R. Gehlsen[1], Jessica Van Zuylekom[2], Benjamin Blyth[2], Stacey E. Rudd[3], Paul S. Donnelly[3], **Matthew Harris**[1]

[1]Clarity Pharmaceuticals, Sydney, Australia,[2]Peter MacCallum Cancer Centre, Melbourne, Australia,[3]School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Melbourne, Australia

Background and Purpose: Immune checkpoint therapy (ICT) has revolutionized cancer treatment; however, efficacy remains poor for some cancers, including small cell lung cancer (SCLC). Strategies to enhance immune responses include combining ICT with other existing cancer therapies. Targeted radioligand therapy uses a radiolabeled cancer-targeting vector, allowing for specific delivery of radiation to all tumor sites while minimizing radiation exposure to healthy tissues. Targeted Copper Theranostics (TCTs) is a targeted radioligand platform utilizing copper-64/67. We evaluated [^{67}Cu]Cu-SARTATE in combination with ICT in a murine animal model using RP116 tumor cells.

Methods: We administrated ~5 MBq [^{64}Cu]Cu-SARTATE to RP116 (a murine SCLC cell line expressing SSTR2) tumor-bearing immunocompetent C57BL/6 mice and assessed biodistribution and tumor uptake via PET imaging at 1, 4 and 24 h post IV injection. After completion of a dose escalation study, an efficacy study using [^{67}Cu]Cu-SARTATE, mouse ICT analogues and the combination of [^{67}Cu]Cu-SARTATE and ICT treatment groups in the same animal model was performed to evaluate therapeutic efficacy of copper-67-based TCT.

Results: Tumor uptake of [^{64}Cu]Cu-SARTATE was visualized by PET imaging over the first 24 h post-injection with high tumor uptake, consistent with multiple studies previously published showing uptake in human xenograft models with the same product. Tumor uptake of [^{67}Cu]Cu-SARTATE was confirmed by *ex vivo* biodistribution and Cherenkov imaging, with no significant radiotoxicity observed via body condition and body weight measurements in mice receiving injected activities up to the maximum tested dose of 30 MBq. The combination of 30 MBq [^{67}Cu]Cu-SARTATE, with both anti-PD-L1 and anti-CTLA4, improved median survival by 3, 7, or 13 days, compared to ICT-only (anti-PD-L1 plus anti-CTLA4), [^{67}Cu]Cu-SARTATE-only or saline-only treated groups respectively.

Conclusion: Biodistribution studies demonstrated high tumor-specific uptake for [64Cu]- and [67Cu]-Cu-SARTATE in this mouse syngeneic SCLC model. A dose escalation study demonstrated copper-67-based TCT could be used to effectively inhibit tumor growth with minimal radiotoxicity. The combination of TCT with ICTs improved overall survival compared to single-treatment control groups. Collectively, our results demonstrate that [67Cu]Cu-SARTATE in combination with ICTs improves overall survival. [67Cu]Cu-SARTATE may prime immunologically “cold” SCLC tumors to improve responsiveness to ICTs through a synergistic response. Tumor biomarkers are being investigated to understand how immune infiltration differs depending on treatment regime.

#2151 Combining PD1 inhibition with radiotherapy enhances anti tumor responses in the CT26 colorectal mouse model.

Charlotte Bell, Theoni (Tania) Katopodi, Pablo Binder, **Tobias Bunday**, Emily Wright, Amy Cantrell, Nick Moore, Stewart Brown, Paul Farrington, Shannon Sharman, Lyndsey Hanson, Lorraine Mooney, Jane Kendrew,

Translational Oncology, Sygnature Discovery, Alderley Edge, United Kingdom

Introduction In recent years, therapies that enhance anti cancer immune responses, like immune checkpoint inhibitors (ICIs), have transformed cancer treatment. Although these therapies can induce lasting responses across various cancer types, not all patients benefit from them. To maximize the potential of immuno oncology (IO) agents, researchers are exploring combinations with other treatments, such as radiotherapy, in both preclinical models and clinical trials. Understanding how IO therapies interact with radiotherapy to influence the tumor immune microenvironment (TIME), and other biomarkers is crucial for advancing drug discovery research. Methods and experimental procedures CT26 cells were exposed to various irradiation doses *in vitro*, and DNA damage response biomarkers were assessed using the Jess automated western blot system. Additionally, to establish a tolerated radiotherapy dosing regimen *in vivo*, different irradiation doses were tested in Balbc CT26 tumor bearing mice. The most effective and tolerated dose regimen was then combined with PD1 dosing. The response of the CT26 cancer model to PD1, with or without radiotherapy, was evaluated. Mice receiving radiotherapy were administered targeted X rays localized only to the tumor area. Changes to the tumor immune microenvironment (TIME) following these therapies were assessed using flow cytometry, while changes in splenic immune populations were analyzed by flow cytometry and ELISPOT. Serum cytokines at study termination were assessed using the Luminex x map technology. Data Treatment with radiotherapy or PD1 as monotherapies led to a mixed response, with tumor regressions obtained in a fraction of the animals treated. The combination of PD1 with radiotherapy significantly increased therapeutic efficacy. Characterizing the TIME following treatment with PD1 in combination with radiotherapy revealed modulation of the intra-tumoral immune infiltrate that reflected the increased therapeutic efficacy. Notably, a significant decrease in the frequency of tumor associated macrophages and an increase in T, NK, and B cells, was observed in tumors treated with the combination of anti PD1 and radiotherapy. Conclusion We have identified and established effective dosing regimens for radiotherapy in vivo in combination with PD1 therapy. Additionally, we have assessed changes to the tumor and spleen immune populations in a syngeneic mouse cancer model. These findings highlight the utility of these models in immuno-oncology (IO) drug discovery research.

#2152 NT-I7 increases lymphocytes and enhances chemotherapy and immunotherapy efficacy in HNSCC.

Zhibin Cui[1], Jacqueline L. Yee[2], Hua Li[1], Yan Zeng[1], Liam Woerner[1], Alexandra A. Wolfarth[3], Sara Ferrando-Martinez[3], Hyunseok Kang[4], Jennifer R. Grandis[1], Matthew H. Spitzer[5], Daniel E. Johnson[1]

[1]Department of Otolaryngology - Head and Neck Surgery, University of California, San Francisco, San Francisco, CA,[2]Department of Microbiology and Immunology, University of California, San Francisco, San Francisco, CA,[3]NeoImmuneTech, Inc., Rockville, MD,[4]Division of Hematology and Oncology, Department of Medicine, University of California, San Francisco, San Francisco, CA,[5]Department of Microbiology and Immunology; Department of Otolaryngology - Head and Neck Surgery, University of California, San Francisco, San Francisco, CA

Background: Lymphopenia is associated with poor outcomes and is exacerbated by conventional chemoradiation therapies in head and neck squamous cell carcinoma (HNSCC). NT-I7 (rhIL-7-hyFc, efineptakin alfa; NeoImmuneTech, Inc.), a long-acting human IL-7, has the potential to restore lymphocyte populations, modulate immune responses, and enhance anti-tumor activity. This study investigated NT-I7's effects on lymphocyte populations and its synergy with cisplatin and immune checkpoint inhibitors (ICIs) in syngeneic, immunocompetent HNSCC mouse models.

Methods: The impact of NT-I7 was studied in two syngeneic tumor models, MOC1 and MOC22. Flow cytometry and Cytometry by Time of Flight (CyTOF) analyses were used to evaluate NT-I7 effects on peripheral and intratumoral immune cells. Anti-tumor efficacy was assessed following NT-I7 treatment alone, in combination with cisplatin, or with anti-PD-1 therapy.

Results: Mice bearing syngeneic MOC1 and MOC22 tumors exhibited significant lymphopenia, characterized by reductions in $CD4^+$ and $CD8^+$ T-cells, Tregs, NK cells, and NKT cells, resembling clinical observations in HNSCC patients. NT-I7 treatment significantly increased lymphocyte levels in both spleen and tumor, with notable induction of $CD8^+$ T-cells and NKT cells, peaking at day 7 and day 4 post-treatment, respectively, and minimal impact on intratumoral Treg levels. NT-I7 modestly inhibited MOC1 tumor growth and potently suppressed MOC22 tumor growth. Combining NT-I7 with cisplatin significantly enhanced anti-tumor activity in MOC1 tumors, accompanied by increased numbers of peripheral and intratumoral CD4+, CD8+, and NKT cells. Furthermore, NT-I7 augmented the efficacy of anti-PD-1 therapy in MOC1 tumors, resulting in greater tumor growth inhibition compared to either treatment alone. CyTOF analysis revealed preferential expansion of CD8+ Tpex cells, a key subset of tumor-specific cells that expand in response to ICIs, highlighting NT-I7's role in overcoming lymphopenia and potentiating anti-tumor immunity.

Conclusions: NT-I7 effectively increases lymphocyte populations in HNSCC preclinical models that demonstrate tumor-associated lymphopenia, and enhances the efficacy of cisplatin and anti-PD-1 in HNSCC preclinical tumor models.

#2153 Preliminary analysis of immune cell profiling to neoadjuvant chemotherapy response in pancreatic cancer patients.

Byeong-Gon Kim, Sung Jun Kim, Gyuryang Park, Tae Young Kim, Sung-Sik Han, Sang-Jae Park, Woo Jun Lee, Sun-Young Kong, Yun-Hee Kim, Jung Won Chun, Sang Myung Woo

National Cancer Center, Goyang, Korea, Republic of

Background: Neoadjuvant chemotherapy (NAT) is used in the clinical setting to improve the chance of surgery for pancreatic cancer (PC) patients. Immune profiling shows great promise for improved prediction of response to chemotherapy, But the role of the immune system of PC remain unclear. This study aimed to identify immune biomarkers for improved prediction of response to chemotherapy.
Materials and
Methods: Blood samples were collected from 23 pancreatic cancer patients undergoing neoadjuvant chemotherapy (NAT) before (pre, n=23) and during the 2-month follow-up after treatment (post 2M, n=15). Peripheral blood mononuclear cells (PBMC) were isolated from whole blood samples using Ficoll buffer and flow cytometry analyzed using 18 colors-LSRFortessa for immune cell profile. The following fluorescent-labeled antibodies were used: Fixable viability stain 700, Fluorescein isothiocyanate anti-CD3, Brilliant violet (BV) 605 anti CD14, BV510 anti-HLA-DR, BV655 anti-CD123, BV421 anti-CD11c, Brilliant ultraviolet (BUV) anti-CD4, and BV711 anti-CD8. The immune cell profiling in peripheral blood mononuclear cells were analyzed by flow cytometry. Patients were classified as responders (compete response, partial response) or non-responders (stable disease, progressive disease) base on their response to NAT, and their immune cell profiles were compared.
Results: The baseline characteristics of the two groups were similar. The median number of NAT cycles was 8 (range, 4-9) in responders and 8 (4-12) cycles in non-responders, respectively. Among various immune cell types, the myeloid dendritic cells (mDC) levels were significantly increased in responder of the NAT group (P = 0.025); however, the levels of CD4, CD8, monocyte, overall DC, and plasmacytoid DC were not significantly different. After NAT 2-month, a decrease in mDC levels was frequently observed in the non-responder group (75% vs. 43%), but the difference was not significant.
Conclusion: Our study suggests that mDC in blood may be a useful biomarker for predicting NAT response and improving treatment responses in pancreatic cancer patients. Further research is required to fully assess the role of mDC in predicting NAT response.Funding: This work was supported in part by the National Cancer Center, Korea (No. 2410680).

#2154 Inhibition of CD39 by AB598 enhances the effects of chemotherapy and anti-PD-1 therapy to promote myeloid cell and T cell anti-tumor immunity.

K. Jin, J. Clor, L. Stetson, R. Fuchs, S. Cho, J. Singh, A. Kaplan, M. Peterson, M. Walters, E. Fernandez-Salas, C. Bowman; Arcus Biosciences Inc., Hayward, CA

Extracellular ATP (eATP) is a potent immunostimulatory signal in the tumor microenvironment (TME). Halting the degradation of eATP by inhibiting CD39 (gene: *ENTPD1*), the dominant extracellular ATPase in the TME, may lead to an immunostimulatory environment resulting in anti-tumor immunity. AB598 is a novel, humanized, Fc-silent anti-CD39 therapeutic antibody that inhibits CD39 enzymatic activity with sub-nanomolar potency and is currently being evaluated in ARC-25, a Phase 1/1b clinical trial in subjects with gastric cancer, in combination with FOLFOX and zimberelimab, an anti-PD-1 antibody. Analysis of publicly available gastric cancer single-cell RNA sequencing datasets demonstrates that *ENTPD1* is expressed predominately on endothelial cells, fibroblasts, and immune cell subsets in gastric tumors. Examination of CD39 expression at the protein level confirmed high levels of intratumoral CD39 present in the vasculature, stroma, and immune cells, but not cancer cells, in gastric tumors. Intratumoral ATP levels are modulated by CD39 regardless of the cellular origin, but the immunostimulatory effect of ATP results from signaling through purinergic receptors on the surface of immune cells. To fully understand the ability of AB598 to preserve eATP and prompt immune responses, we performed *in vitro* and *in vivo* studies to assess the effect of enhanced ATP levels on immune cell activation. Co-culture of macrophages and NK cells with the addition of ATP and AB598 resulted in NK cell activation, reflected in increased production of pro-inflammatory and cytotoxic cytokines. A triple co-culture system including HGC-27 gastric cancer cells, monocyte-derived dendritic cells (moDCs), and T cells was utilized to exemplify the therapeutic hypothesis. In this model, no exogenous ATP is added, instead, ATP is generated upon oxaliplatin-induced cell death of the HGC-27 cancer cells. The ATP produced by the HGC-27 cancer cells is preserved by AB598 to promote moDC activation and ultimately T cell cytokine production, providing evidence for the ability of AB598 to promote adaptive anti-tumor immunity. In a human CD39 knock-in mouse model, which retains the natural distribution of CD39 and a fully competent immune system, treatment of mice bearing MC38 tumors with the combination of murinized AB598 and PD-1 antibodies led to a reduction in tumor growth. Immunophenotyping of both tumor and tumor-draining lymph nodes demonstrated that myeloid cells and T cells were activated as a result of AB598 treatment. In conclusion, the co-culture *in vitro* and murine *in vivo* data support the combination of AB598 with the standard of care agents oxaliplatin and anti-PD-1 to enhance myeloid cell and T cell activation to drive anti-tumor immunity.

#2155 Phage display discovery of Trop-2 peptide for targeted liposomal chemo-immunotherapy in lung cancer.

B. S. Mustafa, B. Nagalo, M. Tesfay, U. Ferdous, A. Cios;
University of Arkansas for Medical Sciences, Little Rock, AR

Background: Lung cancer remains a leading cause of cancer-related mortality worldwide. Targeted therapies offer a promising approach to improve treatment efficacy and reduce systemic toxicity. Trophoblast cell surface antigen 2 (Trop-2) is a transmembrane glycoprotein that is overexpressed in multiple cancers including lung cancer. Trop-2 is an ideal candidate for targeted therapy of NSCLC as it is highly expressed in NSCLC in comparison to normal cells and it is a transmembrane protein with an extracellular domain that can be targeted by therapies. Recently various Trop-2-targeted therapies have been investigated in clinical trials, including anti-Trop-2 antibodies and Trop-2-targeted antibody-drug conjugates (ADC). This study aims to discover a Trop-2 specific peptide using phage display biopanning and develop a liposomal formulation surface-modified with the identified peptide for targeted chemo-immunotherapy.
Methods: A peptide phage display library was screened to identify peptides with high affinity for Trop-2 protein. The selected Trop-2 peptide was conjugated to the surface of liposomes encapsulating cisplatin and anti-PDL1 single-chain variable fragment (scFv). The targeting efficiency, cytotoxicity, and immunomodulatory effects of the Trop-2 peptide-modified liposomes were evaluated in vitro using A549 lung cancer cell lines and in vivo in Calu-3 NSCLC xenograft model.
Results: The phage display biopanning successfully identified a high-affinity Trop-2 peptide. Liposomes modified with this peptide demonstrated enhanced binding and uptake by A549 lung cancer cells compared to non-targeted liposomes. In vivo, the targeted liposomal formulation exhibited superior tumor growth inhibition and prolonged survival in the Calu-3 NSCLC xenograft model.
Conclusion: The discovery of a Trop-2 specific peptide via phage display and its application in targeted liposomal delivery of cisplatin and anti-PDL1 scFv represents a novel and effective strategy for lung cancer chemo-immunotherapy. This approach holds potential for improving therapeutic outcomes and minimizing adverse effects in lung cancer patients.

#2156 Combination therapy of platinum chemotherapy and HIF1α/c-MET peptide vaccine suppresses tumor growth and intraperitoneal metastasis by modulating tumor microenvironment in high grade serous ovarian carcinoma.

Soon Young Lim, Jinhwa Hong, DongGeRaMi Moon, Hye Yeon Roh, Ju Won Kim, Jiwon Lee, Kyong Hwa Park

Korea University, Seuol, Korea, Republic of

Ovarian cancer is the most lethal form of gynecological cancer. Approximately 75% of patients are diagnosed at stage 3 or 4, where the cancer has a high metastasis and mortality rate. Platinum chemotherapy is the standard first line treatment for ovarian cancer. However, 60% of patients with advanced stage disease experience drug resistance and side effects, with recurrence occurring within a few years. Resultantly, the five year survival rate for patients with advanced stage ovarian cancer is only 20-30%. Among the mechanisms proposed to explain platinum resistance, tumor hypoxia is known to contribute to the acquisition of several aggressive tumor characteristics, including increased cellular proliferation, inhibition of apoptosis, enhanced invasion and metastasis, chemoresistance, and immune evasion. Thus, immune checkpoint inhibitors are not the current central treatment in ovarian cancer. In this study, we aimed to investigate the role of tumor hypoxia in the development of platinum resistance and to explore the effect of a strong Type I immunity in overcoming platinum resistance in ovarian cancer. In ovarian cancer cell line, we evaluated the effect of tumor hypoxia on resistance to carboplatin and related proteins. We observed in hypoxia, the expression of relevant proteins was increased compared to normoxia, and the IC50 of carboplatin was also elevated in ovarian cancer cell line. On the contrary, treatment with IFN-γ in vitro induced a reduction in the expression of these proteins in hypoxia, and showed a synergy with carboplatin in cell death. Based on these findings, we identified HIF-1α and c-MET as potential therapeutic targets and developed a HIF1α/MET derived peptide vaccine to enhance local IFN-γ production via helper T-cell immunity. We evaluated the efficacy of a HIF1α/MET peptide vaccine in a high grade serous ovarian carcinoma mouse model (C57BL/6 mice). The mice were divided into four groups: control, carboplatin, vaccine, and combination of carboplatin and vaccine. After three vaccinations, tumor cells were injected, followed by carboplatin treatment, and the tumor suppression effect was evaluated. Tumor growth was significantly suppressed in both monotherapy compared to the control, with the combination demonstrating the most pronounced tumor inhibition. Immunohistochemical analysis revealed reduced expression of HIF-1α & c-MET and an increased infiltration of CD8+ T cells in both monotherapy, with the combination showing the greatest effect. In conclusion, immunotherapy using a HIF-1α/c-MET-derived peptide vaccine, a key player of tumor hypoxia, synergy with carboplatin by modulating the tumor microenvironment and enhancing antitumor immunity. This approach represents a promising strategy for overcoming hypoxia driven resistance in ovarian cancer.

#2157 Rational combination of chemotherapeutic agents with immune checkpoint inhibitors in orthotopic metastatic breast cancer models.

Balaji Ramachandran, Satheeshkumar Rajendiran, Rajendran Venkidusamy, Kowshik Karmakar, Raghu Patil, Krishnappa Haladasappa

Pharmacology, Adgyl Lifesciences Private Limited, Bangalore, India

Breast cancer is broadly diagnosed clinically and deadly among women. Due to heterogeneous nature of cancer and resistance, standalone treatments often fail. Consequently, combination therapy is essential as it targets multiple pathways and enhances efficacy and reduce resistance. Clinically relevant orthotopic mammary fat pads (MFP) implanted models are used for evaluating combination therapies. In the present study, we have evaluated efficacy of anti-PD1, anti-CTLA-4 antibody and Doxorubicin (DOX) as standalone and combination regimen in orthotopic breast cancer models [4T1, EMT6]. 0.5x10^6 cells were implanted orthotopically into fourth mammary fat pad. Mice were randomized based on tumor volume (~100mm^3) and treatment was initiated with anti-PD1 and anti-CTLA-4 at 10mg/kg, i.p; Q4D x 4 doses and DOX 4mg/kg, i.v; Q4Dx 4 doses as standalone or in combination. Tumor volume, changes in body weight, clinical signs, mortality, distant organ metastasis were monitored upto 4 weeks. At the end, lungs were harvested weighed, perfused with Indian ink, and fixed in 10% neutral buffered formalin for histopathology evaluation. In 4T1 tumor model, treatment with standalone anti-PD1, anti-CTLA-4, and DOX resulted in non-significant tumor growth inhibition (TGI). Whereas combination of anti-PD1 and anti-CTLA-4 with Doxorubicin demonstrated moderate TGI and marginally reduced distant organ metastasis compared to the standalone regimen. In EMT6 tumor model, standalone IO agents anti-PD1, anti-CTLA-4, and Doxorubicin treatment exhibited moderate TGI. However, combination of anti-PD1 and anti-CTLA-4 with DOX achieved synergistic significant tumor growth inhibition which was evident from absence of distant organ metastasis and resulted in prolonged survival. Overall, outcome of this study suggests combination therapy with conventional chemoagents, and immune checkpoint blockade can prevent progression to metastatic disease and may limit to overcome resistance. Such combination regimen represents a multimodal translational approach to enhance chemotherapeutic efficacy for the treatment of metastatic breast cancer.

#2158 Developing therapeutic strategy to address chemoresistance in cervical cancer using 3D *ex vivo* sensitivity testing drug.

L. R. Motadi;
University of Johannesburg, Johannesburg, South Africa

Cervical cancer (CC) is ranked the fourth most common cause of cancer related deaths amongst women. The situation is particularly dire in low to lower middle-income countries. It continues to affect these countries due to poor vaccine coverage and screening. Cervical cancer is mostly detected in the advanced stages leading to poor outcomes. Many different chemotherapy drugs are used to treat recurrent CC, including cisplatin, ifostamide, carboplatin and gemcitabine. These drugs may be used alone or in combination. However, in a country like South Africa limited in resources this has become a challenge. In this study we aimed to develop a new therapuetic strategy that include repurposing current HPV vaccine through immune targeting strategy. The 3D ex vivo cancer cells were used and cells were targeted with neutralizing antibodies from HPV targed with cannabidiol. Following treatement, Cell viability, qPCR, Annexin V, immunocytochemistry and DNA fragmantation was performed. The IC50 was observed to be at 26ug/ml. The DNA fragmentation showed positive apoptosis induction while genes like p53, bax and bak1 were all increased. immunocheckpoint were all down in all treatments. The staining for protein showed increase in proteins associated with apoptosis. The results suggest apoptosis induced mechanism.

#2159 B7-H3 as a strategic target for CAR-CIK immunotherapy to eliminate chemoresistant cancer stem cells across diverse tumor histotypes.

Letizia Vitali[1], **Alessia Proment**[1], Annamaria Massa[2], Chiara Donini[1], Alessandra Merlini[1], Federica Galvagno[2], Andrea Maria Lombardi[1], Elisa Vigna[1], Lucilla Minori[1], Elisa Landoni[3], Gianpietro Dotti[3], Claudia Chiodoni[4], Patrizia Gasparini[4], Silvia Novello[1], Valeria Leuci[2], Sandro Pasquali[4], Dario Sangiolo[1]

[1]Universita degli Studi di Torino, Orbassano, Italy,[2]Istituto di Candiolo FPO-IRCCS, Candiolo, Italy,[3]Lineberger Comprehensive Cancer Center University of North Carolina, Chapel Hill, NC,[4]Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy

Purpose. The objective of our study is the translational exploration of a cellular immunotherapy strategy with CAR-engineered lymphocytes to target solid tumors resistant to conventional treatments. Specifically, we focus on attacking the tumor stem-like component, considered responsible for relapses and resistance to therapies. We have chosen B7-H3 as the target molecule due to its broad expression across multiple tumor types and its association with stemness and tumor aggressiveness. As effectors we exploited Cytokine-induced Killer lymphocytes(CIK) that, given their T-NK function and intrinsic HLA-independent antitumor activity, could be advantageous against tumors with heterogeneous CAR target expression.

Experimental Design. Our preclinical platform is based on tumor cell lines from multiple histotypes (lung and ovarian cancer, osteosarcoma, soft tissue sarcomas) and patient-derived CIK engineered with a second-generation anti-B7-H3 CAR, including CD28 as costimulatory molecule. To visualize putative cancer stem cells (CSCs) within tumor cultures, we employed a strategy previously developed and published by our group. This involves transducing tumor cultures with a lentiviral vector (CSC-detector) encoding GFP under the control of the OCT4 stemness gene promoter, enabling the identification and tracking of CSCs as “GFP-positive”.

Results. Across all the various tumors studied, we confirmed homogeneous and intense expression of B7-H3 (80-100%), as well as key NKG2D ligands recognized by CIK (MIC A/B and ULBPs, > 70%). The rate of CSCs (GFP+) varied among tumor types (mean 24%±4). As expected, CSCs were more quiescent, with reduced proliferative potential *in vitro* compared to their differentiated counterparts, showing an extended G2 phase (2.5fold) in the cell cycle suggestive of a potential enhanced apoptotic resistance. Furthermore, GFP+CSC subsets across diverse histotypes were relatively resistant *in vitro* to reference chemotherapeutics (doxorubicin, cisplatin, PARPi). Functionally, anti-B7-H3 CAR-CIK exhibited potent killing activity against all tumor types, even at low effector-to-target (E:T) ratios (70-85% at E:T 1/1: >60% E:T 1/2). Unlike observed with chemotherapeutic drugs, anti-B7-H3 CAR-CIK effectively eliminated the CSC tumor component, with enhanced activity (2.5 fold) against this subset compared to surrounding tumor cells.

Conclusions. B7-H3 is a valuable and relevant target across diverse solid tumors, allowing to eliminate cell subset with stemness features, resistant to conventional treatments and potentially capable to promote disease relapse. CIK are a suitable platform for CAR-based approaches and our data provide rationale to promote clinical trials with anti-B7-H3 CAR-CIK for relapsed or refractory tumors.

#2160 Trastuzumab deruxtecan induces immunogenic cell death, immune cell activation and -migration in viable human breast cancer slices.

Nina Schulte[1], **Misa Ikuta**[2], Anne Mertes[1], Ivan Kanchev[1], Tsuneo Deguchi[2], Gernot Polier[1]

[1]Daiichi Sankyo Europe GmbH, Martinsried, Germany,[2]Daiichi Sankyo Co., Ltd., Tokyo, Japan

Trastuzumab deruxtecan (T-DXd) is an antibody-drug conjugate composed of a humanized immunoglobulin G1 monoclonal antibody specifically targeting HER2, a tetrapeptide-based cleavable linker, and a potent topoisomerase I inhibitor payload (DXd). It is approved for several indications including adult patients with HER2-positive and HER2-low breast cancers. While immune activation by T-DXd was reported in mouse models, it has not yet been shown in patient samples. We hypothesize that generation of an anti-tumor immune response could exist in patients' tumors treated with T-DXd.

In this preclinical research study we used primary human breast cancer tissue to evaluate the cell types which can internalize T-DXd in culture, induction of immunogenic cell death within tumor cells, intra-tumoral immune cell activation and autologous immune cell migration.

Viable human tumor slices (VHTS) were prepared from surgically removed, primary tumors from breast cancer patients using a vibratome. For each readout, we analyzed three to seven samples from an overall pool of five HER2-positive and 16 HER2-low patient samples (three neoadjuvantly treated). To analyze internalization, VHTS were cultured overnight in presence of fluorescently labeled T-DXd or control IgG (10-15 µg/ml). For functional assays, VHTS were cultured up to five days in the presence of unlabeled T-DXd (15 or 150 µg/ml), DXd (30 or 100 nM) or left untreated. After that the culture supernatants were collected for cytokine analyses and the slices were dissociated and submitted to flow cytometry analyses.

As a result, it was observed that T-DXd was internalized into tumor cells in a target-dependent manner. Compared to untreated slices, T-DXd-treated VHTS showed significantly enhanced levels of the apoptosis marker active/cleaved caspase-3 within tumor cells, significantly reduced HER2-harbouring tumor cell counts, and in some patient samples increased release of HMGB1 in culture supernatants indicating the induction of immunogenic cell death. In addition, we observed increased immune cell activation upon T-DXd incubation manifested by upregulation of CD86 levels, potentiated antigen presentation demonstrated by elevation of MHC class II on macrophages and MHC class I on tumor cells as well as increases of granzyme B+ cytotoxic T cell frequencies. In line with this, cytokine analysis of supernatants revealed higher levels of pro-inflammatory cytokines including GM-CSF, TNFα and IFN-γ. Finally, we also observed enhanced immune cell infiltration of autologous PBMCs into T-DXd-treated VHTS. These results suggest that T-DXd has immune activating effects in human HER2-positive and HER2-low breast cancer tissues. Further studies are needed to assess the use of T-DXd as a complementary treatment to immuno-oncology drugs in patients.

#2161 Improving the tumor microenvironment with liposomal gemcitabine, FF-10832, enhances the effects of immune checkpoint blockade.

Tadaaki Ioroi[1], Michie Morimoto[1], Hitomi Nishinakamura[2], Keisuke Watanabe[2], Shohei Koyama[2], Hiroyoshi Nishikawa[2], Mikinaga Mori[1], **Susumu Shimoyama**[3]

[1]FUJIFILM Corporation, Kanagawa, Japan,[2]National Cancer Center Japan, Tokyo, Japan,[3]FUJIFILM Pharmaceuticals, USA, Cambridge, MA

Cancer immunotherapy, including immune checkpoint blockade (ICB), is a type of cancer treatment that achieves tumor regression in various types of cancer. However, more than half of patients are resistant to ICB therapy. As the tumor microenvironment (TME) is different in each cancer patient, it is important to understand the state of immune cells in the tumor and develop strategies, including combination therapies. In the TME, immunosuppressive cells such as regulatory T cells (Treg), tumor associated macrophages (TAMs) and myeloid derived suppressor cells (MDSCs) work in correlation with ICB resistance. These immunosuppressive cells are promising targets for ameliorating resistance to ICB treatment and a lot of drugs targeting these cells have been developed. In particular, the therapy targeting TAMs have proved effective. We previously developed liposomal nanoparticle, FF-10832, encapsulating the cytotoxic anti-tumor drug, gemcitabine. Non-clinical studies demonstrated that FF-10832 was more stable than unencapsulated gemcitabine and preferentially distributed to solid tumor sites, resulting in an improved therapeutic index. A phase I clinical trial indicated that FF-10832 was well-tolerated in heavily pre-treated patients with advanced solid tumors at the recommended phase 2 dose (NCT03440450). Therefore, this study investigated the application of FF-10832 as an immunomodulator in combination therapy with ICB. Treatment of mouse models of solid tumor and in vitro analysis showed that FF-10832 is engulfed into the cytoplasm of TAMs in the tumor by phagocytosis. Combination therapy with FF-10832 and anti-PD-1 antibody resulted in significant tumor regression compared to gemcitabine monotherapy, altering the balance immunosuppressive cells in the TME. These results suggest that FF-10832 is a promising immunomodulator for TAM-targeted cancer immunotherapy. Combination therapy of FF-10832 and pembrolizumab is under investigation for non-small cell lung cancer and urothelial cancer in a phase 2a clinical trial (NCT05318573).

#2162 A potent and highly selective HPK1 inhibitor as a combination strategy to improve the clinical response of the immune checkpoint inhibitor.

J. Byun, J. Lee, S. Jo, D. Kim, H. Shin, J. Choi, S. Ji, H.-J. Kim, D. Kim, B. Kim;
HK inno.N Corporation, Seoul, Korea, Republic of

Combining the HPK1 inhibitor with the anti-PD-1 antibody has demonstrated clinical benefit by restoring suppressed immune response. Here, we present our novel HPK1 inhibitor IN-122517 exhibiting good HPK1 inhibition potency and selectivity against 468 kinases including immune-related off-target kinases. Consistently, it potently reduced SLP76 phosphorylation and induced IL-2 production in human peripheral blood mononuclear cells (PBMCs).
Preclinical studies have shown beneficial tumor response to IN-122517 as a monotherapy and also improved efficacy when combined with anti-PD-1 monoclonal therapy. In the combination group, complete responses (CRs) were observed in a subset of animals, and re-challenge experiments confirmed the absence of tumor regrowth, indicating the establishment of an immune memory response. Tumor-infiltrating lymphocytes showed increased infiltration of T cells, natural killer (NK) cells, and dendritic cells (DCs), demonstrating the compound's potency in activating and recruiting immune effectors within the tumor microenvironment (TME).
These data supported the nomination of IN-122517 into IND-enabling studies as a combination partner with Immune checkpoint inhibitors (ICIs).

#2163 Inhibition of HIF2A increases efficacy of immune checkpoint blockade in renal cell carcinoma.

K. Frazier[1], E. Muise[1], J. Stolz[1], L. Fischer[1], M. Buchanan[1], C. Schneider[2], B. Chan[2], R. Carr[3], A. Palmieri[1], R. Perini[1], B. Long[1], **D. Linn**[1];

[1]Merck & Co., Inc., Boston, MA, [2]Merck & Co., Inc., Cambridge, MA, [3]Merck & Co., Inc., West Point, PA

Hypoxia Inducible Factors (HIFs) are transcription factors implicated in cellular processes that are key to survival, proliferation, and function, particularly for cancer cells. In the context of clear cell renal cell carcinoma (ccRCC), HIF2A is an oncogenic driver of tumor growth via up-regulation of angiogenesis (VEGFA), cell cycling (CCND1), vascularization (EPO), and metabolism (GLUT1). HIF2A can be targeted by belzutifan (MK-6482), a first-in-class HIF2A inhibitor that is FDA-approved for VHL-associated and advanced ccRCC, significantly reducing tumor burden in patients. Both HIF1A and HIF2A are expressed by all cells in the hypoxic tumor microenvironment, including infiltrating immune cells, and HIFs have previously been implicated in T cell anti-tumor effector function. Several ongoing clinical trials are evaluating MK-6482 in combination with immune checkpoint blockade therapy for advanced ccRCC, however the potential impact that MK-6482 may directly have on the adaptive immune response remains poorly understood. Considering the functional link between HIF signaling and T cell function, we explored whether HIF-2A inhibition by MK-6482 could impact activation and/or anti-tumor action of T cells supported by anti-PD-1 and anti-CTLA-4 immune checkpoint blockade. Interrogation of primary T cell activation by mixed lymphocyte reaction revealed that MK-6482 did not induce significant changes in cytokine secretion (IFNg, IL-2) or cell proliferation with or without immune checkpoint blockade, despite evidence of HIF2A protein reduction in T cells. MK-6482 combined with immune checkpoint blockade resulted in almost complete tumor regression compared to either monotherapy in a ccRCC humanized mouse model. Histological analysis of tumors revealed a significant reduction in HIF2A protein following combined treatment, potentially driving the drastic tumor regression. By multiplexed IMC analysis, we found that tumors exhibited increased infiltration of CD4+ and CD8+ T cells with elevated markers of activation, proliferation, antigen-interaction, and cytotoxicity. mRNA sequencing of tumors also revealed increased expression of T cell activation pathways, further suggesting that the combination treatment induced greater anti-tumor activity of infiltrating T cells. Together, our data support ongoing clinical evaluation of belzutifan and checkpoint blockade combination treatment for ccRCC, and we continue to explore the potential mechanistic effects by both in vivo and in vitro follow-up studies.

#2164 BAL0891 as a dual kinase inhibitor inducing anti-tumor immunity: A promising partner for immune checkpoint inhibitors.

MinKyung Kang[1], Sanghee Yoo[2], Seunghyun Ma[3]

[1]Sillajen Inc, Seoul, Korea, Republic of,[2]Qureator, Inc., San Diego, CA,[3]Medical Affair, Sillajen Bio, San Francisco, CA

BAL0891 is a first-in-class dual inhibitor targeting threonine tyrosine kinase (TTK) and polo-like kinase 1 (PLK1). These kinases collaborate to activate the mitotic spindle assembly checkpoint (SAC) at the kinetochore, ensuring proper chromosome alignment and segregation prior to mitotic exit. Dual inhibition of TTK and PLK1 by BAL0891 rapidly disrupts the SAC, leaving tumor cells insufficient time for proper chromosome segregation, thereby exerting potent anti-cancer effects. Recent studies have underscored the potential of cell cycle inhibitors, including TTK and PLK1 inhibitors, as therapeutic partners for immune checkpoint inhibitors in various cancers. Given these findings, we investigated whether BAL0891 induces anti-cancer immune responses and evaluated its potential for combination therapy with immune checkpoint inhibitors.Our results demonstrate that BAL0891 induces aberrant cell cycle progression in cancer cells, leading to elevated levels of cytosolic DNA, a well-known activator of the cGAS/STING signaling pathway. Activation of this pathway by BAL0891 promotes cellular senescence and enhances anti-tumor immunity through the secretion of pro-inflammatory cytokines and chemokines. Notably, BAL0891 treatment increases pro-inflammatory factors such as IFNβ1, CXCL10, and CXCL11. Among these, IFNβ1 plays a pivotal role in upregulating PD-L1 expression on cancer cells, potentially enhancing the efficacy of immune checkpoint inhibitors like anti-PD-1/PD-L1 agents. Furthermore, CXCL10 and CXCL11 contribute to recruiting immune cells to the tumor microenvironment, further amplifying immune responses. In an in vivo syngeneic mouse model, BAL0891 treatment increased the presence of CD4+ T cells, CD11c+ dendritic cells (DCs), and NK1.1+ NK/NKT cells, while reducing immunosuppressive populations such as regulatory T cells (CD25+ FoxP3+ CD4+) and myeloid-derived suppressor cells (Gr-1+ CD11b+) in tumors and tumor-draining lymph nodes. These results demonstrate BAL0891's potential to reshape the tumor microenvironment in favor of anti-tumor immunity.This study highlights the potential of BAL0891 as a combination partner for immune checkpoint inhibitors in cancer therapy. By dual inhibition of TTK and PLK1, BAL0891 not only exerts potent anti-cancer activity but also enhances anti-tumor immunity through activation of the cGAS/STING pathway and modulation of the tumor microenvironment. These results support further investigation of BAL0891 in preclinical and clinical studies, particularly in combination immunotherapy strategies.

#2166 PTPN1/2 dual inhibition sensitizes solid tumors to checkpoint blockade immunotherapy by enhancing T cell cytotoxicity and by decreasing cancer cell immune evasion.

Alexandre Poirier[1], Luis-Alberto Perez-Quintero[2], Erika Walback[3], Rui Su[4], Chenyue Wu[3], Michel L. Tremblay[5]

[1]Goodman Cancer Institute, McGill University, Montreal, QC, Canada,[2]Charles River Laboratories, Montreal, QC, Canada,[3]Microbiology and Immunology, McGill University, Montreal, QC, Canada,[4]Cornell University, Ithaca, NY,[5]Biochemistry, Goodman Cancer Institute, McGill University, Montreal, QC, Canada

Immunotherapy has revolutionized the way we treat patients suffering from cancer. However, many patients are still refractory to immunotherapy, which highlights the importance of identifying new genetic targets that can achieve therapeutic outcomes. Recent work has revealed that protein tyrosine phosphatase non-receptor 2 (PTPN2) can act as such a target by enhancing T-cell and interferon responses against cancer. Development of PTPN2 inhibitors paves the way for a new therapeutic modality to fight solid malignancies. However, current inhibitors also inhibit PTPN1, a closely related phosphatase with largely different biological roles. There remains limited evidence, however, whether dual inhibitors are truly optimal in terms of efficacy, safety and biological activity, or whether PTPN2 specific inhibitors should be pursued. Our aim was thus to systematically investigate the role of these two phosphatases in T-cell responses and tumor immune escape, to further refine the use of PTPN1/2 inhibitors as a therapeutic modality against cancer. We found that in CD8+ T cells, PTPN2 is the major player in limiting cytotoxic activity against cancer cells. However, PTPN2 deletion alone shows only a modest increase in cytotoxicity and is accompanied by a compensatory enhancement of PTPN1 protein levels. Additional deletion of PTPN1 in PTPN2 deficient T cells (dKO) is required for maximal cytotoxic activity and for the establishment of a distinct T cell memory subcluster capable of resisting canonical exhaustion. On the tumor side, the deletion of both phosphatases results in the synergistic augmentation of CXCL9, a potent CD8+ T cell chemoattractant. Using various cancer models, we demonstrate that cancers insensitive to checkpoint blockade are made vulnerable to immunotherapy by PTPN1/2 inhibition. Dual PTPN1/2 inhibitors achieve this effect through distinct cancer cell and T cell-specific effects that act in a complementary manner to checkpoint blockade. We are now pursuing the development of our lead PTPN1/2 inhibitor in clinical trials for the treatment of solid malignancies.

#2167 AKT inhibition enhances antitumor efficacy of radiation and immune checkpoint blockade in triple-negative breast cancer by modulating tumor-infiltrating myeloid cells.

Nawon Park[1], Seung Hyuck Jeon[2], Yoomin Kim[1], Seongmin Kim[1], In Ah Kim[1]

[1]Seoul National University, Seoul, Korea, Republic of,[2]Seoul National University Bundang Hospital, Seoul, Korea, Republic of

Although radiotherapy (RT) and immune checkpoint blockade (ICB) are effective for treatment of triple-negative breast cancer (TNBC), significant portion of patients do not experience a clinical benefit, partly due to activation of immunosuppressive cells. Capivasertib, an AKT inhibitor, is approved for use in advanced luminal breast cancer, but its immunomodulatory role in breast cancer remains still unclear. Given the ability of PI3K/AKT/mTOR inhibition to regulate immunosuppressive myeloid cells, we hypothesized that capivasertib could enhance the efficacy of RT and ICB in TNBC. In this study, we investigated the antitumor effects and phenotypes of immune cells in 4T1 syngeneic mouse TNBC model treated with RT, dual ICB (PD-L1 and CTLA-4 blockade), and capivasertib. A dual-tumor model was used to examine abscopal effects, with one tumor receiving RT and the other remaining unirradiated. We found that the quadruple therapy (i.e. RT + anti-PD-L1 + anti-CTLA-4 + capivasertib) resulted in greatest growth delay of both irradiated (primary) and unirradiated (secondary) tumors, along with the lowest number of metastatic lung nodules. Notably, compared to the RT + anti-PD-L1 + anti-CTLA-4 treatment, the quadruple therapy significantly reduced tumor-infiltrating macrophages, with an increase in inflammatory M1 macrophages and a decrease in immunosuppressive M2 macrophages. Moreover, $CD86^+$ mature dendritic cells within tumor microenvironment were increased by addition of capivasertib to RT + anti-PD-L1 + anti-CTLA-4 treatment. These changes were not observed within the spleen and tumor-draining lymph node. Proportion and phenotypes of myeloid-derived suppressor cells (MDSCs) and T cells in tumor were not significantly changed by the quadruple therapy. These findings suggest that capivasertib, when combined with RT and ICB, led to less immunosuppressive tumor microenvironment by modulating tumor-infiltrating myeloid cells. Taken together, this combination strategy could be a viable approach to overcome therapeutic resistance of ICB in TNBC patients.
(Work supported by grants from KHIDI & AstraZeneca, and the National Research Foundation of Korea #2023R1A2C3003782)

#2168 Targeting HDAC6 to modulate the tumor microenvironment and enhance anti-PD-L1 immunotherapy in MSS colorectal cancer.

Ahran Yu[1], Jeong Eun Kang[2], Ju Yeon Park[2], Seijong Kim[2], Hye Kyung Hong[2], Yong Beom Cho[3]

[1]Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of,[2]Institute for Future Medicine, Samsung Medical Center, Seoul, Korea, Republic of,[3]Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Department of Health Sciences and Technology, SAIHST, Sungkyunkwan University, Department of Biopharmaceutical Convergence, Sungkyunkwan University, Seoul, Korea, Republic of

Colon cancer is one of the most prevalent malignancies of the digestive system, characterized by high morbidity and mortality rates. Its development is driven by a complex interplay of factors within cancer cells and the tumor microenvironment (TME). Microsatellite-stable (MSS) colorectal cancer, often referred to as a “cold tumor,” is characterized by chromosomal stability and a lack of neoantigen production. As a result, MSS tumors are generally unresponsive to immunotherapy. Post-translational modifications of proteins are critical in tumor remodeling and progression. Histone deacetylase 6 (HDAC6), a member of the HDAC family, plays a key role in regulating the deacetylation of both histone and non-histone substrates. Overexpression of HDAC6 has been reported in multiple tumor types and is associated with tumor growth and metastasis. While immune checkpoint blockade (ICB) therapies, such as those targeting programmed cell death protein 1 (PD-1) and its ligand programmed cell death ligand 1 (PD-L1), have achieved clinical success in several malignancies, the majority of colorectal cancer patients particularly those with MSS tumors fail to respond to these treatments. Recent evidence indicates that the intratumoral immune context strongly influences the efficacy of ICB therapies. Patients with high levels of intratumoral CD8+ T cell infiltration, elevated tumor mutational burden (TMB), and increased PD-L1 expression within the TME are more likely to respond favorably to immunotherapy. In this study, we focused on enhancing the immunogenicity of the TME and upregulating PD-L1 expression through selective HDAC6 inhibition to improve the efficacy of anti-PD-L1 immunotherapy. Analysis of The Cancer Genome Atlas (TCGA) data revealed that HDAC6 expression is inversely correlated with several immune-related genes, including PD-L1. This inverse relationship suggests a potential role for HDAC6 in regulating immune responses in MSS colorectal cancer. These findings imply that HDAC6 may influence the TME and immune evasion mechanisms, particularly by modulating immune checkpoint molecules such as PD-L1. In the CT26 syngeneic mouse model, inhibition of HDAC6 led to an enhanced immune response characterized by increased PD-L1 expression, improved antigen presentation, and upregulation of MHC-II. This was accompanied by a polarization toward M1 macrophages and elevated levels of pro-inflammatory cytokines, including TNF-α and IFN-γ, which are crucial for T cell activation. Furthermore, HDAC6 inhibition resulted in a reduction of TGF-β, a key immunosuppressive cytokine, thereby moderating immune suppression within the tumor microenvironment. These results highlight the potential of HDAC6 inhibitors to reprogram the tumor immune landscape and sensitize MSS colorectal cancer to immune checkpoint therapies.

#2169 TTK/PLK1 dual antagonist BAL0891 synergizes with pembrolizumab in a vascularized 3D tumor microenvironment model.

Junfeng Wang[1], MinKyung Kang[2], Byungjun Lee[1], Delaney Donnelly[1], Jihye Baek[1], Bushra Rajput[1], Tsung-Li Liu[1], Hyeon-geun Park[1], Yunha Shin[1], Aneesh Sathe[1], Yangji Kim[1], Kyusuk Baek[1], Sanghee Yoo[1], Salman Ahmed[3], Jae Jung Lee[2], Seunghyun Ma[3]

[1]Qureator, Inc., San Diego, CA,[2]SillaJen, Inc., Seoul, Korea, Republic of,[3]Medical Affair, SillaJen Bio, San Francisco, CA

Introduction: BAL0891, a first-in-class dual TTK/PLK1 inhibitor, activates the cGAS/STING pathway and induces a senescence-associated secretory phenotype (SASP), resulting in increased PD-L1 expression and pro-inflammatory cytokine production in cancer cells. These changes enhance tumor susceptibility to immunotherapies. However, evaluating the synergy between BAL0891 and immune checkpoint inhibitors like pembrolizumab (PD-1 inhibitor) is challenging in traditional preclinical models, which lack human immune cells and key features of the tumor microenvironment. To address these limitations, Qureator's advanced 3D tumor microenvironment (TME) model, incorporating perfusable vessels, human primary cells, and patient-derived organoids (PDOs) was utilized. This physiologically relevant system enabled a detailed investigation of BAL0891's mechanism of action (MOA) and its combination with pembrolizumab across multiple cancer types, aiming to guide clinical trial design and patient stratification.
Methods: Vascularized TME models were developed by co-culturing endothelial cells, fibroblasts, and PDOs derived from renal cell carcinoma, triple-negative breast cancer, gastric cancer, and colorectal cancer within Qureator's Microphysiological System (MPS). Following vasculature formation, models were primed with BAL0891 for two days and then treated with PBMCs and pembrolizumab or isotype control antibodies. Tumor growth, immune infiltration, and tumor cell killing were assessed via live cell imaging and immunofluorescence staining. Post-treatment immuno-profiling included flow cytometry to analyze macrophage polarization and T cell activation/exhaustion. Multiplex secretome analysis was performed to characterize chemokine and cytokine profiles associated with BAL0891 and the combination therapy.
Results: BAL0891 priming sensitized a subset of PDOs to pembrolizumab, demonstrating synergistic anti-tumor activity in the vascularized TME model. Responsive PDOs exhibited significant organoid shrinkage, increased immune cell infiltration, enhanced T cell activation, and macrophage polarization towards an anti-tumoral phenotype. Cytokine and chemokine analysis revealed a shift to a pro-inflammatory microenvironment. Conversely, non-responders showed limited immune infiltration and minimal activation.
Conclusion: Qureator's vascularized TME model provided a robust platform to evaluate the synergy between BAL0891 and pembrolizumab. The study demonstrated BAL0891's ability to reshape the tumor immune landscape and enhance the efficacy of the immune checkpoint inhibitor. These findings offer valuable insights into the MOA of BAL0891 within this highly representative tumor model, supporting its further clinical development in combination with checkpoint inhibitors.

#2170 S100a6-Lars2 oncogenic signaling shaping immunosuppressive environment contributes to platinum-based resistance.

Yuqing Wang, Chuxia Deng

University of Macau, Macau, China

Chemotherapy-induced drug resistance is a very complex process. About 20% of cancer patients have received effective therapy with platinum (Pt) medicines, and drug resistance has since emerged through several mechanisms. To investigate the complicated heterotypic interactions between cancer cells and immune cells during the resistant tumor outgrowth processes under Pt treatment, we dissected the crosstalk signals with single-cell RNA sequencing and performed functional studies on Pt-induced drug resistance using overexpression, knockout, and DOX-inducible technologies. In this study, we have identified that: 1) S100a6-Lars2 oncogenic signaling could promote TGF-β production from tumor and immune cells and induce expressions of PD1, Tim3, and Selplg on T cells, which could contribute to resistant tumor outgrowth under Pt treatment; 2) Pt drugs could promote the survival of cancer stem cells (CSCs) with elevated hyaluronan-mediated motility receptor (Hmmr) and mTOR oncogenic pathway; 3) we validated Lars2 oncogenic action in resistant tumor outgrowth in FVB immunocompetent mice and the dynamic changes of the immune cell population; 4) combinatory treatment involving the inhibition of TGF-β signaling, the mTOR pathway, and Hmmr action, along with PD1 antibody, shows a significant effect in inhibiting tumor growth. This study advances the understanding of the mechanism of Pt drug-induced resistant tumor outgrowth, which may allow us to better manage the tumor immune microenvironment (TIME) and postpone the onset of resistance.

#2171 Amezalpat, a peroxisome proliferator-activated receptor alpha (PPARα) antagonist, inhibits suppressive macrophage development, activation and function.

Mandy I. Cheng[1], Ademola Esomojumi[2], Dara Burdette[1], Valerie Chen[1], Jorg H. Fritz[2], Sam Whiting[1], Nathan Standifer[1]

[1]Tempest Therapeutics, Brisbane, CA,[2]Microbiology and Immunology, McGill University Research Center on Complex Traits (MRCCT), Dahdaleh Institute of Genomic Medicine (DIgM), Montreal, QC, Canada

Amezalpat (TPST-1120) is a first-in-class, small molecule competitive antagonist of peroxisome proliferator-activated receptor alpha (PPARα), the master transcriptional regulator of fatty acid oxidation (FAO). Analysis of biomarker data from a previous Phase I study demonstrated amezalpat dose-dependent changes in immune genes associated with myeloid populations. Suppressive M2 macrophages are a prominent myeloid population associated with poor prognosis in many cancers. As M2 macrophages are reliant on FAO, we sought to assess the effects of amezalpat on development, activation and function of these cells. Flow cytometry analysis of M2 macrophages polarized in the presence of amezalpat displayed concentration-dependent reductions in canonical M2 macrophage markers CD163, CD206, and Arg-1 ($p<0.05$ by Wilcoxon's test). ELISA measurement of cytokine production by amezalpat-treated M2 macrophages demonstrated a statistically significant reduction in the suppressive cytokines IL-10 ($n=8$; $p<0.05$ by Wilcoxon's test) and TGF-β ($n=3$ by One-way ANOVA), suggesting amezalpat-induced functional impairment of this population. Additionally, amezalpat treatment of M2 macrophages caused significant reductions in mitochondrial mass, measured with a mitochondria-specific dye by flow cytometry ($p<0.01$ by two-sample T test). Finally, in co-cultures of M2 macrophages with autologous activated CD8+ T cells and HCC tumor cells (SNU-449), amezalpat induced tumor cell cytotoxicity and M2 cell death commensurate with cell-type specific expression levels of its target PPARα. Together, these data suggest amezalpat modulates suppressive macrophage development and function and supports the contribution of an immune-mediated mechanism to anti-tumor activity reported in clinical studies.

#2172 NLRP3 inflammasome targeting to overcome platinum and PARPi resistance by modulating cancer related inflammation in ovarian cancer.

Shaheen Mahira[1], Mackenzy Radolec[2], Philip Jones[2], Santiago Panesso[2], Siddharth Krishnan[1], Fernando Munguia[3], Sibel Yilmaz[2], Robert P. Edwards[1], Alexander B. Olawaiye[1], Haider Mahdi[1]

[1]University of Pittsburgh, Pittsburgh, PA,[2]University of Pittsburgh Medical Center, Pittsburgh, PA,[3]Augustana College, Rock Island, IL

Background: Under chronic conditions, NLRP3 inflammasome acts as instigator of pathological inflammation causing inflammation-induced carcinogenesis. Despite killing the cancer cells and being primary treatment modalities, platinum and PARPi regimen activate NLRP3 inflammasome that drives the cancer-promoting inflammatory and immunosuppressive environment and promotes inflammation-associated tumor progression ultimately contributing to resistance and therapy failure. We hypothesize that combining NLRP3 inhibitor (NLRP3i)/IL-1β inhibitor (IL-1βi) to platinum agent and PARPi can improve their anti-tumor activity by modulating inflammation associated ovarian cancer (OvCa) and overcoming resistance in pre-clinical OvCa models.
Methods: The cytotoxicity of the drugs, platinum agent (cisplatin), PARPi (olaparib), NLRP3i (MCC950) and IL-1βi (AS101) as single agent and in combination against human OvCa cells (UWB1.289.BRCA1, OVCAR-3, PEO-1 and PEO-4) was analyzed by MTT assay and drug synergism was evaluated by combination index. qPCR, flow cytometry, multiplex immunoassay and immunoblot analysis were performed in human OvCa cells co-cultured with primary human blood monocytes and the human monocytic cell line (THP-1). Statistical significance was determined by two-tailed Student's t test. $p<0.05$ were considered statistically significant.
Results: The drug combination showed synergistic enhanced anticancer activity by apoptosis induction and cancer cell proliferation inhibition. Platinum agent and PARPi was associated with upregulation of inflammasome downstream genes, IL-1β, IL-6 and CCL2 in OvCa cells indicating NLRP3 activation. There was a significant decrease in NLRP3, IL-1β, IL-6 and CCL2 levels in combination groups indicating that NLRP3 blockade can delay OvCa cell growth by alleviating immunosuppressive and inflammatory microenvironment. Combination treatment polarized M2 macrophages by downregulating M2-related markers, CCR2, ARG1, CD163 and CD206 towards the M1 phenotype by upregulating M1-related markers, iNOS, CD86 and CXCL10 suggesting that NLRP3 regulates OvCa cell function by modulating recruitment and polarization of tumor associated macrophages. Enhanced anti-tumor immunity was evidenced by increased IFN-β level in combination groups.
Conclusion: Our study evaluated the effects of inflammasome modulation on anti-tumor efficacy of platinum agent and PARPi highlighting the interconnection between inflammatory mechanisms and carcinogenesis. Targeting NLRP3 inflammasome synergized and enhanced the anti-tumor activity of platinum agent and PARPi potentially by reshaping the tumor microenvironment (TME) and increasing the anti-tumor immunity and apoptosis. Overall, NLRP3 blockade can be a novel strategy for overcoming therapy resistance and for modulating TME in OvCa.

#2173 Targeting USP7 with MEK1/2 inhibition synergizes to induce pyroptosis and enhance immunotherapy in *NRAS* mutant melanoma.

L. Su[1], D. Wang[2], T. J. Purwin[3], S. Ran[1], Q. Yang[4], Q. Zhang[2], **W. Cai**[1];

[1]Simmons Cancer Institute at Southern Illinois University, Springfield, IL, [2]University of Calgary, Calgary, AB, Canada, [3]Thomas Jefferson University, Philadelphia, PA, [4]Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ

Introduction: There is an urgent need for targeted therapies in metastatic melanoma patients with NRAS mutations, who comprise more than 25% of this population. This study investigates the therapeutic potential of targeting Ubiquitin-Specific Peptidase 7 (USP7) with a highly selective inhibitor, FT671, in NRAS mutant melanoma. In addition, the synergistic effects of the combination of FT671 with MEK1/2 inhibitor (MEKi) on pyroptosis induction, tumor suppression and immune activation were evaluated. Moreover, the potency of triple combination of FT671, MEKi and anti-PD-1 antibody was assessed.

Experimental Procedures: We employed a combination of genetic knockout models and pharmacological inhibition to validate the selectivity of FT671 in vitro. Cell proliferation assays were used to determine IC50s and the synergistic effects. Flow cytometry assays were performed to monitor cell cycle arrest and cell death and analyze intratumoral immune cells. Western blot assays were used to examine if the combinations markedly induced the cleavage of GSDME. A CRISPR based screen was performed to explore the mechanism of action of FT671. We also utilize isograft melanoma models in immunocompetent mice to assess tumor growth, immune cell infiltration, and therapeutic outcomes.

Results: As a validated USP7 selective inhibitor (USP7i), FT671 treatment effectively induced cell cycle arrest, reduced cell proliferation in vitro, and delayed tumor growth in NRAS mutant melanoma models in vivo. Knockout of TP53BP1, TP53 or CDKN1A conferred resistance to FT671, indicating activation of a functional p53 pathway is a major mechanism of action for the efficacy of USP7i in melanoma. The combination of FT671 with MEKi resulted in synergistic effects, and induced pyroptosis in vitro. In vivo, the combined treatment significantly suppressed tumor growth, prolonged survival, and enhanced anti-tumor immunity, evidenced by increased infiltration of $CD8^+$ T cells and mature dendritic cells in tumors. Notably, the triple therapy with FT671, MEKi, and anti-PD-1 antibody led to tumor regression and durable responses in an isograft melanoma model.

Conclusions: These findings highlight the potential of USP7 inhibition, combined with MEKi, as a promising strategy for treating NRAS mutant melanoma, an 'undruggable' target. Moreover, this combinatorial approach is able to enhance the efficacy of anti-PD-1 antibody, providing a compelling rationale for its clinical application as an adjuvant for immune checkpoint inhibitors.

#2174 Diacylglycerol kinase inhibitors in MHC-I-induced ICB-resistant triple negative breast cancer.

Jacey L. Marshall[1], Justin M. Balko[2]

[1]Vanderbilt University, Nashville, TN,[2]Department of Medicine, Vanderbilt University Medical Center, Nashville, TN

This study is designed to assess how diacylglycerol kinase alpha/zeta inhibitors (DGKis) can be used to improve response to immune checkpoint blockade (ICB) in patients with downregulated MHC-I expression. Antigen presentation by MHC-I is required for CD8 T cell-mediated tumor death and response to ICB, e.g. a-PD-1/a-PD-L1 therapy. Triple negative breast cancers (TNBC) downregulate MHC-I to suppress anti-tumor immunity and patients with decreased antigen presentation have worse outcomes to ICB. Thus, loss of MHC-I expression may be a candidate biomarker for lack of ICB response, and therapeutic strategies to overcome MHC-I downregulation are an area of high translational relevance and interest. DGKis increase T cell activation by increasing concentrations of diacylglycerol (DAG) in T cells via inhibition of the conversion of DAG into phosphatidic acid and enhancing downstream DAG signaling. This putatively amplifies T cell responses to decreased levels of antigen presentation and low-affinity stimulation in vitro and enhances response to ICB in semi-responsive models. We hypothesized that DGKis will improve response to ICB in TNBC with low MHC-I expression by improving CD8 T cell response to decreased antigen presentation. Modeling low MHC-I expression, as opposed to CRISPR-mediated total KO, presents a technical challenge. Thus, we created a novel model of MHC-I post-transcriptional downregulation, leveraging miRNA binding element mutations to titrate MHC-I expression - miSFITs. We used isogenic miSFIT cell lines in the orthotopic EMT6 model of TNBC. We observed trends of increasingly aggressive tumor growth with decreased MHC-I expression. Coordinately, when treated with a-PD-L1, there was decreasing benefit of ICB treatment with decreasing MHC-I expression (IgG v aPDL1, day 14 tumor volume, n = 10 mice/group; 100% expression p = 0.002, 60% p = 0.02, 30% p = 0.02, 10% p = 0.19, 0% p = 0.2). In the moderately a-PD-L1 responsive parental EMT6 cell line, DGKi/a-PD-L1 dual therapy further improved response through decreased tumor growth (IgG v. DGKi/a-PD-L1, p=0.0023, n=20) and doubled survival rates. However, in non-ICB responsive models of TNBC (4T1 and E0771) the same benefit of combination therapy was not observed. Treatment of the MHC-I downregulated miSFIT cell lines with DGKi + a-PD-L1 revealed a trending benefit of single agent DGKi therapy which was only slightly enhanced by the addition of a-PD-L1 therapy and did not reach significance. This is in contrast with recent in vitro findings in which decreased T cell activation in response to low levels of antigen presentation was overcome with DGKi treatment, implying further nuance in vivo that remains to be understood. Thus far, our data shows that DGKis are beneficial in deepening responses to ICB in already response-prone patients but may not extend benefit to patients who are not responsive to ICB due to highly decreased tumor-specific MHC-I levels.

#2175 Combined anti-CSF-1R and anti-TIM-3 overcome immune-mediated mechanisms of PARP inhibitor (PARPi) resistance in BRCA1-associated triple negative breast cancer.

Adam Nelson[1], Anita K. Mehta[1], Madeline G. Townsend[1], Daniel E. Michaud[1], Madisson Oliwa[1], Kelly F. Zheng[1], Carlos Wanderley[1], Patrice A. Lee[2], Nicholas A. Saccomano[2], Filipa Lynce[3], Nabihah Tayob[4], Geoffrey I. Shapiro[3], Jennifer L. Guerriero[1]

[1]Department of Surgery, Brigham and Women's Hospital, Boston, MA,[2]Pfizer, Boulder, CO,[3]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA,[4]Department of Data Science, Dana-Farber Cancer Institute, Boston, MA

Background: Poly (ADP-Ribose) polymerase inhibitors (PARPi) have improved outcomes for *BRCA*-associated triple negative breast cancer (TNBC); however, resistance develops, resulting in lack of durable responses. Our previous work demonstrated that PARPi activates the cGAS/STING pathway inducing recruitment of anti-tumor CD8+ T-cells, which are required for tumor clearance. These studies led to clinical trials testing PARPi plus immune checkpoint blockade (ICB). However, clinical trials demonstrated that PARPi + ICB is not superior to PARPi monotherapy, indicating T-cells in the tumor microenvironment (TME) are inhibited. We previously showed that PARPi induce CSF-1R+ suppressive tumor associated macrophages (TAMs) that restrict anti-tumor immune responses, contributing to PARPi resistance. Removing TAMs with anti-CSF-1R therapy significantly enhanced overall survival (OS) when combined with PARPi in naïve tumors. Here, we test if targeting CSF-1R can overcome PARPi resistance and if ICB can further enhance PARPi + anti-CSF-1R therapy in both naïve and PARPi-resistant tumors.

Methods: Mice bearing naïve or PARPi resistant BRCA1-deficient TNBC (*K14-Cre;Brca1$^{f/f}$;Trp53$^{f/f}$*) tumors were treated with PARPi ± anti-CSF-1R ± ICB and followed for tumor size and OS. Flow cytometry was employed to determine alterations in TME composition.
Results: In PARPi-resistant tumors, TAMs expressed higher levels of CSF-1R and PD-L1 and lower levels of antigen presentation machinery compared to PARPi-naïve tumors, indicating increased immunosuppression. Additionally, in PARPi-resistant tumors, CD4+ and CD8+ T-cells expressed higher levels of PD-1 compared to naïve tumors, indicating increased exhaustion. PARPi + anti-CSF-1R therapy significantly restored PARPi efficacy and increased overall survival in PARPi-resistant tumors. The combination of PARPi + anti-CSF-1R + anti-TIM-3 significantly increased OS in PARPi-resistant tumors, whereas combination of PARPi + anti-CSF-1R + anti-CTLA-4 did not improve therapy efficacy. While the combination of PARPi + anti-CSF-1R with anti-PD-1 improved therapy in PARPi-naïve tumors, it did not improve responses in PARPi-resistant tumors. PARPi + CSF-1R + anti-TIM-3 significantly increased CD8+ T-cell infiltration and granzyme B production indicating an increased anti-tumor immune response. Additionally, anti-TIM-3 increased TRAIL production by TAMs indicating a more pro-inflammatory phenotype.
Conclusion: We demonstrate that PARPi-resistant tumors have increased T-cell exhaustion and infiltration of immunosuppressive TAMs. Importantly, our data show that targeting TAMs can overcome acquired PARPi resistance, which select ICB can further enhance defining a novel strategy addressing a critical unmet medical need for PARPi-treated patients with BRCA-associated breast cancer.

#2176 Tumor treating fields (TTFields) together with gemcitabine and nab-paclitaxel (Gem/NabP) are effective against pancreatic ductal adenocarcinoma (PDAC), in vitro and in vivo.

Tal Kan, Tharwat Haj Khalil, Yiftah Barsheshet, **Tali Voloshin**, Avital Vorontsov, Boris Brant, Simona Zisman-Rozen, Lilach Koren, Roni Blatt, Shay Cahal, Catherine Tempel Brami, Sara Jacobovitch, Adi Haber, Moshe Giladi, Uri Weinberg, Yoram Palti

Novocure Ltd, Haifa, Israel

Background: Tumor treating fields (TTFields) are a cancer therapy, approved for the treatment of glioblastoma, pleural mesothelioma, and non-small cell lung cancer (NSCLC). The efficacy and safety of TTFields concomitant with standard-of-care gemcitabine and nab-paclitaxel (Gem/NabP) are currently being tested in a phase 3 trial as front-line treatment for locally advanced pancreatic ductal adenocarcinoma (PDAC). TTFields are electric fields that disrupt critical cellular processes and induce immunogenic cell death (ICD) in cancer cells. They have also been shown to elicit a systemic anti-cancer immune response in animal models and to provide clinical benefit in patients with NSCLC when applied together with immune checkpoint inhibitors (ICI). The current study aimed to investigate the involvement of a TTFields-induced immune response in PDAC and the therapeutic potential of applying TTFields together with standard-of-care chemotherapies in preclinical models.
Methods: Human BxPC-3 and AsPC-1, and mouse Panc02 (with stable luciferase expression) pancreatic cancer cells were treated with TTFields (150 kHz, 1.62 V/cm RMS) and examined for induction of apoptosis and ICD. The markers used to test ICD included ATP secretion, calreticulin surface exposure, and CD47expression. Panc02 cells were inoculated orthotopically into immunocompetent C57Bl/6 mice. The mice were randomized into four groups: control, TTFields, Gem/NabP, and Gem/NabP + TTFields. TTFields were applied continuously for 10 days, and the drugs were injected i.p. once a week for a total of two administrations at a concentration of 50 mg/kg for each drug. At the end of the treatment, tumor volume was measured using a caliper, after which the tumor was processed as a single-cell suspension for flow cytometry-based immunophenotyping. Blood was collected for immunophenotyping and examination of circulating levels of the ICD marker high mobility group box 1 protein (HMGB1).
Results: TTFields application induced apoptosis and elicited ICD in all tested pancreatic cell lines. The latter was identified by ATP depletion, increased calreticulin surface exposure, and decreased CD47 expression. In vivo, TTFields concomitant with Gem/NabP significantly reduced tumor volume compared to all other treatment groups. TTFields treatment elevated the levels of HMGB1 and of effector CD8+ T cells in the peripheral blood.
Conclusions: TTFields induce ICD and an antitumor immune response in PDAC and enhance the efficacy of standard-of-care chemotherapy for the treatment of PDAC in preclinical models. A phase 2 study evaluating the potential efficacy of TTFields together with Gem/NabP and ICI in metastatic PDAC is underway.

IMMUNOLOGY: Local and Systemic Immunity against Cancer: New Analytic and Prognostic Tools
Poster Session

#2177 Spatial transcriptomic and functional analysis of HLA/PD-1/CTLA4 multi-gene edited CD19 CAR-iNK cells targeting pericytes in the glioblastoma tumor microenvironment to inhibit tumor migration and growth.

Kyung-Sun Kang

Seoul National University, Seoul, Korea, Republic of

Targeting glioblastoma (GBM) stem cell-derived pericytes in the perivascular niche offers a promising strategy to overcome intratumoral heterogeneity and inhibit tumor migration. By targeting CD19-positive pericytes, CD19 chimeric antigen receptor (CAR)-expressing natural killer (NK) cells can mitigate off-tumor toxicity; however, their interactions within the GBM tumor microenvironment (TME) require further investigation. In this study, we developed a GBM TME xenograft model by transplanting GBM-blood vessel assembloids (GBVA) into immunodeficient mice to study the effects of CD19 CAR-expressing iPSC-derived NK (CAR-iNK) cells on pericytes and their interactions within the TME. Additionally, we enhanced the efficacy of CD19 CAR-iNK cells by knocking out PD1 and CTLA4, enabling these cells to better target GBM within an immunosuppressive TME. As a result, GBVA xenografts recapitulated the GBM TME including human pericytes and macrophages, providing a robust in vivo platform for validating CD19 CAR-iNK cell efficacy against GBM. Optimization of CAR-iNK cell dosing and efficacy resulted in significant antitumor effects. Spatial transcriptome analysis using SLACS (Spatially Linked Antibody and cDNA Sequencing) revealed that CD19 CAR-iNK cell infiltration altered gene expression in the TME, elucidating mechanisms of GBM lysis, migration inhibition, and evasion of immunosuppressive influences. These findings underscore the therapeutic potential of CD19 CAR-iNK cells for GBM treatment through precise targeting of pericytes and modulation of the TME.

#2178 Multimodal analysis of the PDAC tumor microenvironment in response to radiation and immunotherapy reveals insights into treatment resistance.

Sarah Kang[1], Aparna R. Parikh[2], Milan Parikh[1], Julie L. Koenig[2], Leon Pappas[2], Moshe Sade-Feldman[1], Lynn Bi[1], Nicole Carzo[2], Tarin M. Grillo[2], Islam Baiev[2], Olanike Asupoto[3], Irena Gushterova[2], Tom LaSalle[2], Anna Gonye[2], David P. Ryan[2], David T. Ting[2], Dana Pe'er[4], Theodore S. Hong[2], Nir Hacohen[1], Ryan J. Park[5], Arnav Mehta[1]

[1]Broad Institute of MIT and Harvard, Cambridge, MA,[2]Massachusetts General Hospital, Boston, MA,[3]Dana Farber Cancer Institute, Boston, MA,[4]Memorial Sloan Kettering Cancer Center, New York, NY,[5]MD Anderson Cancer Center, Houston, TX

Pancreatic ductal adenocarcinoma (PDAC) is a deadly disease with few treatment options and poor prognosis. Immunotherapy is effective in other cancers but shows worse outcomes in PDAC due to a desmoplastic stroma, an abundance of immunosuppressive cells, and a lack of antigen-presenting dendritic cells, which result in low immune infiltration. We previously reported a study combining dual immune checkpoint blockade (ICB) and radiation therapy in metastatic PDAC patients who progressed on at least one line of chemotherapy and showed an 18% overall response rate (ORR) and 29% disease control rate (DCR). Here, we performed a follow-up phase 2 study on previously treated PDAC patients and observed 3.6% ORR, 10.7% DCR, 2.3 months median progression-free survival, and one complete response. To uncover mechanisms of treatment resistance, we profiled 33 samples from 23 patients with 13 paired pre and on treatment biopsies across distinct tissue sites including liver metastases and primary pancreas. Using single nucleus sequencing (>240k cells), single-cell RNA sequencing (>160k cells) with TCR (scTCR) sequencing (>25k cells), and Visium spatial transcriptomics of a subset of samples (n=15), we comprehensively characterized the cell states and gene programs in the TME of metastatic PDAC prior to and after radiation and ICB. Post radiation, tumor cells were enriched for classical states, exhausted CD8 and proliferating T cells were expanded, and interferon-related programs were upregulated in epithelial subtypes, C1QC+ and MHCII+ macrophages, and CD14+/CD16+ monocytes, suggesting cell type-specific responses to treatment. Expanded T cell clonotypes showed phenotypic shifts toward CD8 exhausted and cytotoxic activated states. Using a published gene signature, we identified tumor-reactive T cells, which correlated with known proxies for tumor reactivity including CXCL13 expression. Only 2.2% of T cells were tumor-reactive, and 71% of these came from the single patient who displayed complete response to treatment. Moreover, bulk TCR sequencing of serial peripheral blood samples showed long-term persistence of tumor-reactive clonotypes in this patient over a year post-treatment. Spatial transcriptomics demonstrated co-localization of CXCL13+ T cell, dendritic cell, and inflammatory chemokine gene programs, and separate co-localization of macrophage, CCL8+ CAF, Th17/Tc17, Class II MHC antigen presentation, and interferon stimulated gene programs. Together, our multimodal dataset offers valuable insight into radiation and immunotherapy-induced shifts in the TME.

#2179 Multiomic co-detection of proteins, mRNA and protein-protein interactions reveal PD1-PDL1 interactions in the tumor microenvironment of bladder cancer patients treated with anti-PD-L1.

Ge-Ah Kim[1], Sonali A. Deshpande[1], Li-Chong Wang[1], Julia Yu[1], Hamidreza Sepasizangabadi[1], Ji Zhang[1], Jason Au[1], Kathryn Allaire[2], Junyan He[2], Maithreyan Srinivasan[1], David Y. Oh[2], Lawrence Fong[3]

[1]Advanced Cell Diagnostics, a Bio-Techne brand, Newark, CA,[2]Division of Hematology/Oncology, Department of Medicine, University of California, San Francisco, San Francisco, CA,[3]Immunotherapy Integrated Research Center, Fred Hutchinson Cancer Center, Seattle, WA

Protein-protein interaction (PPI) is one of the mechanisms where individual cells communicate with nearby cells or the extracellular matrix through autocrine, juxtacrine and paracrine mechanisms. The network of interactions and the underlying stoichiometry both within a tissue and with the immune repertoire is adversely impacted in many diseases, including cancer. While screening technologies to predict and map the protein interactome are advancing our understanding of intercellular/intracellular protein-protein interactions (PPIs), no orthogonal technologies exist that can validate inferred PPIs in the context of known mRNA/protein markers. To address this need, we have developed a technology that uses modifications to RNAscope^{TM} - a validation technology known for its high sensitivity and specificity - to visualize PPIs, proteins, and mRNA *in situ* on a single tissue section. Oligonucleotides that bind RNAscope signal generation complexes were conjugated to antibodies and protein targets were visualized *in situ* along with PPIs/mRNAs using a fully automated, protease-free RNAscope Multiomic LS workflow on a BOND RX instrument followed by whole slide scanning and analysis. The assay, which interrogate one PPI and any combination of up to 5 mRNA and protein targets, was deployed on samples from patients with localized, muscle-invasive bladder cancer who received neoadjuvant anti-PD-L1 checkpoint inhibitor therapy prior to planned surgical cystectomy on a clinical trial (NCT02451423). To characterize tumor immune microenvironment of these patients, both responders and non-responders to the treatment, PD-1/PD-L1 interactions were assessed using a panel of targets to include cell phenotyping protein markers for both immune and tumor cells (CD3, CD4, CD8, FoxP3, and PanCK) and mRNA markers for effector molecules (*IFNG*, *GZMB*, *GZMK*). We visualized PD-1/PD-L1 interactions, and changes in cellular interactions between cytotoxic CD8+ T cells or regulatory CD4+ T cells and CK+ tumor cells before and after immunotherapy treatment. We will present data to illustrate the novelty of the assay as well as secondary analysis to characterize the importance of PPIs for more granular dissection of immune modulation in the tumor microenvironment.

#2180 Utilization of multiparameter flow cytometry for immune monitoring of tumor infiltrating lymphocytes isolated from fresh tumor tissue.

Rachel Owen, Angelina Bisconte, Deborah Phippard

Precision for Medicine, Frederick, MD

A multiparameter flow cytometry panel was designed to assess immune populations infiltrating solid tumors. Obtaining viable immune cells from fresh tissue for analysis can be challenging, however, using optimized shipping conditions and isolation procedures developed by Precision for Medicine (PfM) for fresh solid tumor biopsy pieces or fine needle aspirates, a variety of solid tumor indications, including melanoma, colorectal adenocarcinoma, sarcoma, triple negative breast cancer, renal cancer, head & neck cancer, lung cancer and stomach cancer, were analyzed by flow cytometry. Following overnight shipment from collection sites to PfM's laboratory, a real time sample processing procedure using a mechanical dissociation protocol followed by multiparametric flow cytometric analysis was performed. Post dissociation of tumor samples, cell viabilities ranged between 50-90%, and the sample quality was sufficient for flow cytometric analysis of tumor infiltrating lymphocytes (TILs). This analysis revealed that the frequency and phenotype of infiltrating immune cells could be assessed successfully. It was observed that variations in the frequency and phenotype of infiltrating cells were present in the tumor tissues. This study shows that real time multiparametric flow cytometric analysis of fresh tumor tissues, within 48 hours of collection, can be implemented in clinical trials with careful planning and consideration of clinical operations. This is of value for early phase clinical studies evaluating the impact of immune therapies on immune cell infiltration within solid tumors, and provides an additional immune monitoring tool.

#2181 Establishing a multi-modal, cell therapy characterization workflow to identify cell subsets with heightened cytotoxicity.

Richard Yau[1], Shreya Deshmukh[1], JangKeun Kim[2], Jiwoon Park[2], **Yanping Yang**[3], Makenzie Sacca[1], Shan Sabri[1], Teresa Ai[1], Pier Federico Gherardini[1], Gary Schroth[1], Christopher E. Mason[2], Moonsoo M. Jin[3]

[1]Cellanome, Inc., Foster City, CA,[2]Weill Cornell Medicine, New York, NY,[3]Houston Methodist Research Institute, Houston, TX

Cell therapies have been groundbreaking for B-cell lymphomas but optimizing efficacy for solid tumors remains challenging. Lack of consensus on potency measures, unknowns about novel engineering strategies, and product heterogeneity drive extensive characterization to discover product-specific mechanisms of action (MoAs). This process is costly and limited by variable results from simple molecular assays and need to infer relationships between data from disparate technologies. Thus, we created a multi-modal technology with which to establish a multi-functional, longitudinal cellular characterization workflow. With a single sample input, this workflow evaluates multiple live phenotypes from the same individual cells across tens of thousands of cells at once. We leveraged TALL-104 cells before validating compatibility with clinical-stage cell therapies with AIC100 - a Phase 1 CAR-T product targeting thyroid cancer (NCT04420754). Cellanome's technology enables multi-modal evaluation of live, cellular behavior at the resolution of single cells or single-cell interactions. Within an 8-lane flow cell, tens of thousands of single TALL-104 cells were enclosed with K562 target cells in bio-compatible hydrogel compartments called CellCage™ enclosures (CCEs). These CCEs enable longitudinal monitoring of single TALL-104 cells interacting with K562 cells for hours to days with time-lapse imaging. During this period, imaging-based evaluation of surface markers, cytokine secretion, and cytotoxicity are integrated from the same single T-cells. Our analysis pipeline links target cell death to single T cells in CCEs, enabling cell killing to be studied with unprecedented resolution. Similar to flow cytometry via CD107 as a surrogate for killing, with Cellanome's technology, we could identify cytotoxic T cells (~85%). Uniquely, we can directly evaluate killing by single TALL-104 cells and further segment them into groups. Group 1 consists of strong killers (killing 100% of targets), Group 2 of medium killers (50-99%), and Group 3 of weaker killers (0-49%). We next characterized single AIC100 cells enclosed with HeLa as targets and observed that, unlike TALL-104 cells where Group 2 was the largest, for AIC100, Group 3 was the largest, suggesting distinct killing mechanisms. Thus, we asked whether AIC100's heterogeneous composition of CD4 / 8 cells impacts product efficacy. We configured this workflow to test killing by ~100 combinations of CD4:CD8 AIC100 cells in a single experiment. Preliminarily, more killing occurred in CCEs containing a mixture of CD4 and CD8 T cells relative to CCEs containing only CD4 or CD8 cells. We are continuing to identify the optimal CD4:CD8 compositions for greater potency. We envision multi-modal characterization of clinical products with our platform, pre and post-patient infusion, will inform efforts to advance cell therapies for solid tumors.

#2182 Optimizing immunolabeling strategies in preparation for magnetic activated cell sorting for chimeric antigen receptor-natural killer and multiple myeloma cells.

Kaitlyn Bali[1], Yukiko Higa[2], Long Huynh[2], Lei Tian[3], Jianhua Yu[3], Nicholas Graham[2]

[1]Rosalind Franklin University of Medicine and Science, North Chicago, IL,[2]Mork Family Department of Chemical Engineering and Materials Science, University of Southern California, Los Angeles, CA,[3]Division of Hematology/Oncology, Department of Medicine, School of Medicine, University of California, Irvine, Irvine, CA

Chimeric antigen receptor (CAR) natural killer (NK) cells are of increased interest compared to CAR-T cell therapy because CAR-NK cells can circumvent the challenges of graft-versus-host disease, serve as an off-the-shelf therapy, and avoid the neurotoxic side effects and cytokine release syndrome that accompany CAR-T cell immunotherapy. However, CARs are currently optimized for use in T cells and are not optimized for NK cell mechanisms. There are currently no comprehensive studies that characterize CAR-NK cellular pathways for CAR-NK optimization. In preparation for a comprehensive CAR-NK phospho-proteomic pathway analysis, we evaluated strategies for magnetic-activated cell sorting (MACS) to isolate CAR-NK cells prior to phospho-proteomics including alternating formalin fixation and immunolabeling.

To identify the most optimal immunolabeling strategy, NK cells transduced with a viral construct that included a truncated CD34 component (tCD34) were tested for an ability to optimally bind Human CD34 Biotinylated Antibody followed by Streptavidin-Allophycocyanin (SAv-APC) with varying formalin fixation sequences. Cells were either formalin fixed before antibody and SAv-APC labeling, after antibody and SAv-APC labeling, or after antibody and before SAv-APC labeling. Human CD34 APC-conjugated Antibodies, NK cells without tCD34, and OPM-2 multiple myeloma cells were used as controls for understanding SAv-APC background binding. Lastly, CAR-NK and control NK cell labeling effectiveness was also tested against the cells' incubation periods.

There were no consistent notable differences in CAR-NK labeling efficiency with alternating fixation and immunolabeling patterns. However, increased labeling was consistently found in unfixed samples. Formalin fixation also leads to unintentional background binding as demonstrated by labeled control NK and OPM-2 cell numbers. Furthermore, incubation time for CAR-NK cell samples proves advantageous in labeling CAR-NK cells irrespective of fixation and staining patterns. By analyzing control NK and CAR-NK samples over four weeks, we found that control NK cells were dying at a faster rate while CAR-NK cells remained at a consistently high viability. This increased death rate of the control NKs in the CAR-NK samples allowed for a greater proportion of CAR-NK detection with reduced noise from background control NK binding.

Formalin fixation poses a challenge for optimal CAR-NK labeling due to background binding and a decrease in labeling levels when compared to non-fixed samples. However, incubation time of CAR-NK cells can potentially circumvent these difficulties and serve as a tool for cell sample preparation due to biological differences in CAR-NK and control NK proliferation. This can be a novel strategy for optimizing MACS for phosphoproteomic analysis of CAR-NK cells.

#2183 *DNAH* mutations as a potential biomarker for immune checkpoint inhibitor response and survival.

S. Wang, **H. Tang**, X. Wu, Q. Ou;
Nanjing Geneseeq Technology Inc., Nanjing, China

Introduction: The integration of immune checkpoint inhibitors (ICIs) with precision medicine offers a promising avenue for personalized therapies through the identification of biomarkers that predict clinical response and prognosis. However, existing biomarkers have shown limited predictive power, underscoring the need for continued research.

Methods: We analyzed whole-exome sequencing data from 342 melanoma patients treated with ICIs to identify genetic determinants of ICI response and survival. Two external cohorts were used to validate findings and explore underlying mechanisms: a non-small cell lung cancer cohort (N=91) and The Cancer Genomic Atlas pan-cancer cohort (N=942).

Results: Responders, defined as patients who achieved a complete or partial response to ICIs, were more likely to have received anti-PD-1 therapy compared to anti-CTLA-4 and had higher tumor mutation burden (both $P<0.001$). All 13 dynein axonemal heavy chain (*DNAH*) family genes were detected, with *DNAH2* ($P=0.03$), *DNAH6* ($P<0.001$), and *DNAH9* ($P<0.01$) more prevalent in responders. Five *DNAH* mutations were markedly associated with overall survival (OS), including *DNAH2* (hazard ratio [HR]: 0.66; 95% confidence interval [CI]: 0.44-0.98; $P=0.039$), *DNAH6* (HR: 0.48; 95% CI: 0.32-0.73; $P<0.001$), *DNAH9* (HR: 0.64; 95% CI: 0.46-0.90; $P=0.009$), *DNAH5* (HR: 0.67; 95% CI: 0.51-0.90; $P=0.006$), and *DNAH12* (HR: 0.57; 95% CI: 0.36-0.92; $P=0.019$). Only *DNAH6* mutations were associated with longer progression-free survival (PFS) (HR: 0.54; 95% CI: 0.36-0.80; $P=0.002$). Notably, *DNAH2*, *DNAH6*, and *DNAH9* mutations (collectively referred to as *DNAH*-altered) were more frequent in responders and significantly associated with longer PFS (HR: 0.69; 95% CI: 0.51-0.92; $P=0.013$) and OS (HR: 0.58; 95% CI: 0.43-0.78; $P<0.001$). These prognostic associations were consistently observed in the validation cohort (HR: 0.28; 95% CI: 0.12-0.61; $P<0.001$). Multivariate analysis identified *DNAH* mutations as the most significant predictor of improved OS (HR: 0.56; 95% CI: 0.42-0.76; $P<0.001$), independent of sex, distant metastases, treatment regimen, and *BRAF* V600E mutations. Functional analysis showed upregulation of chemokine signaling, cytokine-cytokine receptor interaction, cell cycle regulation, and p53 signaling in *DNAH*-altered melanomas, which were independently validated in the TCGA cohort. Immune-related signatures, including those implicated in interferon signaling, cytolytic activity, T-cell function, and immune checkpoint regulation, were also enriched in *DNAH*-altered tumors.

Conclusions: This study is the first to identify *DNAH* mutations as a potential biomarker for predicting ICI response and patient survival. These findings, initially observed in melanoma, may have broader implications for guiding personalized immunotherapy in other cancers.

#2184 Dynamics between germline immune checkpoint variants and susceptibility for cancer and autoimmunity.

Zade Akras[1], Benjamin M. Neale[1], Nina Mars[2]

[1]Broad Institute of MIT and Harvard, Cambridge, MA,[2]University of Helsinki, Helsinki, Finland

Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of cancer and are approved for a wide range of malignancies. ICIs block the interactions of checkpoint proteins that limit T cell-mediated immune responses, thereby boosting cytotoxic activity against cancers. The most widely used ICIs act through CTLA-4, PD-1, or PD-L1 pathway blockade, and numerous agents are at various stages of development. Due to increased T cell activity, ICIs are associated with immune-related adverse events, and little is known about their long-term impacts. Individual-level genetic variation mimics the effects that are seen with larger magnitude when the same pathways are perturbed through checkpoint modulation. Leveraging large-scale biobanks with genomics data, proteomics data, and rich health registries can enable us to understand the broad impacts of checkpoint modulation on adverse events and its potential for treatment of common diseases. We utilize FinnGen, a biobank study of 520,210 Finnish individuals, to investigate germline variants affecting immune checkpoint proteins and their associations with cancer and autoimmunity. The Finnish population, being a genetic isolate, has an unusual enrichment of disease-associated variants compared to many other ancestry groups, enabling variant-disease associations not detectable in many other populations. Using phenome-wide association analysis, we identify several coding variants in checkpoint protein-encoding genes with opposite associations to autoimmune diseases and cancer or other tumors. Such associations are supported by Olink proteomic quantitative trait loci for respective proteins (*cis*-pQTLs). For example, rs149722682 (*LAG3* missense variant; 440x enriched in Finns) is protective of colon adenomas (OR 0.26, $p=8.4x10^{-5}$) but increases the risk of asthma (OR 1.5, $p=1.1x10^{-4}$) and hypothyroidism (OR 1.9, $p=1.7x10^{-9}$). We also identify multiple variants with associations to a range of conditions beyond classical autoimmune diseases, pointing to potentially new or rare adverse events of ICIs. For instance, rs781221749 (*LAG3* missense; 2x enrichment) increases the risk of manic episode (OR 23.3, $p=1.6x10^{-4}$) and COVID-19 infection (OR 4.9, $p=1.2x10^{-4}$). Finally, we develop checkpoint protein-specific genome-wide polygenic risk scores to quantify the cumulative impacts of *cis* and *trans* checkpoint protein-associated variants on different cancer- and autoimmune-related endpoints. Our findings offer insight into the short- and long-term toxicities associated with checkpoint inhibition and provide a framework for detecting common and rare adverse events in current and emerging therapies.

#2185 Advancing macrophage-directed therapies: bioluminescent assays for macrophage effector function.

Jonathan Mitchell, Denise Garvin, Rich Moravec, Julia Gilden, **Adityarup Chakravorty**, Jim Hartnett, Pete Stecha, Mei Cong, Jamison Grailer

Promega, Madison, WI

Macrophages play a pivotal role in anti-cancer immunity through phagocytosis and presentation of tumor antigens to T cells. Antibody-dependent cellular phagocytosis (ADCP), mediated by macrophages and other myeloid cells, is a key mechanism-of-action (MoA) for antibody-based cancer immunotherapies. Strategies that enhance ADCP and other macrophage effector functions not only promote direct tumor destruction but also facilitate broader anti-tumor immunity. ADCP is initiated by the binding of antibody Fc domains to Fc gamma receptors (FcγRs), which triggers receptor crosslinking and signal transduction, leading to inflammatory cytokine production and phagocytosis. These effector functions are further regulated by immune checkpoints, and therapeutic interventions that block inhibitory checkpoints (e.g., SIRPα, ILT4, ILT2) or activate stimulatory pathways (e.g., TLR signaling) can enhance macrophage activity. However, current methods to measure macrophage functions are often low-throughput, labor-intensive, and highly variable. To address these challenges, we developed a suite of robust, cell-based bioluminescent bioassays to assess macrophage effector functions. These include: **1) ADCP**: Using monocytic cells with endogenous FcγR expression and a FcγR-activation-dependent luminescent reporter. **2) Direct Phagocytosis Detection**: Utilizing the HiBiT split luciferase system for sensitive and quantitative measurements. **3) Immune Checkpoint Modulation**: Bioassays to measure SIRPα/CD47, ILT2/HLA-G, and ILT4/HLA-G interactions. **4) TLR Activation**: Reporter bioassays for TLR activation. These assays are robust, easy-to-use, and pre-qualified according to ICH guidelines. Together, this comprehensive toolbox enables screening and the accelerated development of immunotherapies that target macrophage effector functions, paving the way for innovative cancer treatment strategies.

#2186 Deciphering the regulation and function of lipid associated macrophages in immunotherapy resistance of hepatocellular carcinoma.

Xiaohang Long[1], Stephen Lam Chan[2], Siyuan Huang[1], Joaquim Si Long Vong[1], Xiaoyu Zhan[1], Yalin Tu[1], Joseph, JY Sung[1], Alfred Sze Lok Cheng[1]

[1]School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, Hong Kong,[2]Department of Clinical Oncology, The Chinese University of Hong Kong, Hong Kong, Hong Kong

Background: Hepatocellular carcinoma (HCC) is a significant global health concern, ranking as the sixth leading cause of cancer-related deaths worldwide. While immune-checkpoint blockade (ICB) has revolutionized cancer treatment, its effectiveness is limited by the immunosuppressive tumor microenvironment (TME) and the diverse etiologies of HCC, resulting in response rates below 30%. We have previously identified key transcriptional and epigenetic regulators that promote immune exclusion and myeloid-mediated immunosuppression underlying ICB resistance in preclinical models (*Gut* 2018, 2020, 2023, *Sci Transl Med* 2021). Identifying the central nodes of TME landscapes from ICB-resistant patients is instrumental for developing precise immunotherapy strategies and improving clinical outcomes.
Methods: We performed single-cell RNA sequencing (scRNA-seq), single-cell Assay for Transposase-Accessible Chromatin using sequencing (scATAC-seq), and spatial transcriptomics to investigate the TME of HCC patients resistant to ICBs (NCT05873244) and treatment naïve-patients as controls.
Results:Our multi-omics analysis revealed a subcluster of tumor-associated macrophages (TAMs) that express high levels of lipid-associated genes. In our syngeneic and orthotopic ICB-resistant mouse models, which recapitulate human immune-excluded HCCs, we also found a subset of lipid-associated macrophages (LAMs) is enriched in concordance with the clinical data. Bioinformatics analysis of the open chromatin regions uncovered a transcription factor (TF) that is significantly up-regulated in the TAMs in ICB-resistant HCCs compared to the treatment-naïve tumors. Moreover, chromatin immunoprecipitation-sequencing showed that this TF occupancy in THP-1 cells was enriched in phagocytosis.
Conclusions: Our study provides insights into how this TF drives macrophage lipid phagocytosis to promote ICB resistance of HCC. This investigation elucidates the underlying epigenetic and transcriptional changes and identifies potential therapeutic targets for ICB resistance. Our findings may offer valuable insights into the molecular dysregulation of TAM within the immunosuppressive myeloid landscape, forming the basis for developing new strategies against the immunometabolic axis to avert ICB resistance.
Acknowledgment: This study is supported by the General Research Fund (14120621 and 14119023) and the Li Ka Shing Foundation.

#2187 Functional classification of tumor-associated macrophages and changes in TAM subpopulations in response to various immunotherapies.

Bryan T. Weselman, Satish K. R. Noonepalle, Manasa Suresh, Marie Durr, Nithya Gajendran, Alejandro Villagra, Xintang Li

Georgetown Lombardi Comprehensive Cancer Ctr., Washington, DC

Despite single-cell omics ushering in many attempts at reclassifying tumor-associated macrophages (TAMs), there remains a lack of a consensus for a widely applicable model that accurately describes their biological functions. Based on single-cell RNA seq data from both mouse and human melanoma tumors, we propose a new model for categorizing TAMs by their function in the tumor microenvironment (TME). We identified eight clusters with various functions including a distinct population of proliferating macrophages with tissue-resident characteristics. Samples from mice with SM1 melanoma tumors that have been treated with α-PD-1 immune checkpoint blockade, the novel HDAC6 inhibitor SS208, combination SS208 + α-PD-1, and adoptive cell therapy (ACT) using HDAC6 inhibition show changes in the proportions of putative subpopulations, most notably a significant decrease in “Pro-Angiogenic/Arginine Metabolism” macrophages within tumors after all treatments, an increase in “Antigen Presenting/Phagocytosis” macrophages in mice treated with α-PD-1 therapy, and an increase in proliferating macrophages in mice treated with ACT. We also identified heterogeneity in the expression of certain markers historically categorized as “M1” or “M2,” indicating that using specific markers interchangeably may be inaccurate in measuring different subpopulations within the tumor. In an attempt to move away from artificial polarization of macrophages *in vitro* via cytokines, TAMs were cultured in tumor-conditioned media and M-CSF to more accurately retain TME phenotype. The proposed functions of these subpopulations have been further confirmed by *in vitro* and *ex vivo* functional assays from isolated TAMs. Additionally, we outline an efficient method for applying the proposed subpopulation gene signature to bulk RNA seq data of macrophages, which allows for more accurate analysis of changes in TAM subpopulation in response to therapy.

#2188 Enabling sensitivity, repeatability and reproducibility required for immuno-oncology research on the CyTOF XT Pro mass cytometer.

Stephen Li, Rita Straus, Michael Sullivan, Andrea McEwan, **Ling Wang**, Christina Loh, Alexander Loboda

Standard BioTools, Markham, ON, Canada

Understanding the functional phenotype of immune cells from patients with cancer is critical for identifying mechanism of action, determining disease prognoses and monitoring the clinical efficacy of immunotherapies. The ability to reproducibly identify functional markers with low expression and use functional markers to identify rare cell populations can be challenging with fluorescence flow cytometry due to spectral spillover and autofluorescence. Mass cytometry overcomes many challenges of fluorescence-based cytometry, as low signal spillover and the absence of autofluorescence negate the need for spectral compensation and unmixing. To minimize technical variation, metal-tagged antibody cocktails and stained samples can be frozen for later use and acquisition. Antibody panels that detect both surface and intracellular markers can be rapidly designed, conveniently stained and acquired in a single tube to provide streamlined workflows with high consistency. In this study, human whole blood and PBMC samples were stained with antibody panels containing up to 50 surface and cytoplasmic targets. Stained samples were frozen and acquired on a later date using CyTOF™ XT PRO and CyTOF XT systems to assess repeatability and reproducibility, and to ensure data quality was not compromised when samples were acquired at 4x speed with the CyTOF XT PRO system. Population frequencies and resolution indices for markers were assessed by manual gating. There was no significant difference between population frequencies analyzed between the two CyTOF systems. Both the CyTOF XT and CyTOF XT PRO system had high sensitivity and dynamic range for both abundant and low-abundance markers. The cell population frequency and staining intensity results generated from the CyTOF XT and CyTOF XT PRO system were highly repeatable and reproducible across multiple instruments. Overall, these studies find that the CyTOF XT PRO system generates comparable signal resolution to the CyTOF XT system with up to 4x increased event-rate acquisition. Automated acquisition by the CyTOF XT PRO system enables researchers to accurately and reproducibly streamline human immunophenotyping and functional profiling, leading to accelerated discoveries.

#2189 A spectral flow cytometry panel and workflow for the reproducible assessment of colorectral tumor immunophenotype in the context of immunotherapies.

Joseph P. Kolb, Kathryn R. Meshaw, Timothy Hutchinson, Karsten Fynboe, Jacob Hauser, William D. Culp, Beth A. Hollister

Powered Research, LLC, Durham, NC

Multiparameter flow cytometry analysis of immune cells within solid tumors has provided insight into the mechanism of action of novel immunotherapies, but challenges exist regarding the interpretation of data stemming from variation in sample processing, selection of optimal combinations of fluorochrome-conjugated antibodies, and population gating strategy. Here we present a 20-color spectral flow cytometry backbone panel and gating scheme that can be applied to syngeneic colorectal tumor samples from different mouse strains with minimal manual adjustment. Using the CT26 and MC38 subcutaneous implant models in BALB/c and C57BL/6 mice, respectively, we tested the ability of our panel and workflow to identify changes in tumor immune cell populations after treatment with the immune checkpoint inhibitors (ICIs) anti-PD-1 and/or anti-CTLA-4 in two independent experiments. Our panel identified differences between the models, largely confined to protein surface expression within the monocyte, macrophage, and dendritic cell compartments. In addition, the panel provided readouts of T cell and NK cell function that correlated with ICI efficacy and with increases in tumor cytokines, including IFNγ, CXCL10, and CCL5, without the need for lengthy *ex vivo* stimulation and intracellular cytokine staining. The use of autofluorescence extraction and the designation of autofluorescence tags, which is possible on spectral flow cytometers, was essential to properly identify populations. We demonstrate the ability of modern spectral flow cytometers to produce remarkably consistent data over time, without the need for manual detector gain adjustments, which could improve data interpretation between investigators and facilitate the development of novel therapies.

#2190 Genomic scarring score is associated with T cell exhaustion in early stage non small cell lung cancer.

Ioannis Pateras[1], Konstantinos Ntostoglou[2], Georgios Christodoulopoulos[3], Katerina Tsilingiri[4], Anna Chalari[4], Giannis Vatsellas[5], Georgia Christopoulou[6], Pantelis Constantoulakis[6], Eleni Patsea[2], Konstantinos Potaris[7], Ioannis Vamvakaris[8], Dora Chatzidaki[2], Georgina Zachou[2], Athanasios Kotsakis[9], Apostolos Klinakis[10], Vassilis Georgoulias[2]

[1]2nd department of pathology, Attikon University Hospital, Athens, Greece,[2]Hellenic Oncology Research Group, Athens, Greece,[3]Department of Medical Oncology, University General Hospital, Larissa, Greece,[4]Department of Basic Research, Biomedical Research Foundation of the Academy of Athens, Athens, Greece,[5]Greek Genome Center, Biomedical Research Foundation of the Academy of Athens, Athens, Greece,[6]Genotypos S.A., Athens, Greece,[7]Department of Thoracic Surgery, Sotiria General Hospital, Athens, Greece,[8]Department of Pathology, Sotiria General Hospital, Athens, Greece,[9]Department of Medical Oncology, University General Hospital, Athens, Greece,[10]2nd Department of Pathology, Biomedical Research Foundation of the Academy of Athens, Athens, Greece

Introduction: We have recently reported that almost a third of the patients in an early stage NSCLC cohort harbor a high genomic scarring score (GSS) indicating HRD. Immunotherapy is an attractive partner of PARP inhibitors (PARPi), especially in case of PARPi resistance. Immunotherapy-based regimens are shown to be highly efficient and provide significant clinical benefit in the pre- and peri-operative setting in early-stage NSCLC suggesting that the TME is permissive for the immunotherapy effect in this tumor type.
Purpose: We wished to characterize the different subpopulations of tumor infiltrating lymphocytes (TILs) in early-stage NSCLC and correlate them with the clinical outcome.
Methods: Next generation sequencing was used to evaluate the GSS of primary tumors from 136 early-stage NSCLC based on a commercially available sequencing kit which also provides information on the genetic status of relevant HRR and other genes (Amoy Dx) and patients were stratified as low (<50, l-GSS) or high (>50, h-GSS) GSS. Analysis of the tumor microenvironment (TME) immune infiltrate was performed by single and double staining ICH using monoclonal antibodies against T cells and B cells as well as PD1 and TCF1.
Results: 39 (28.7%) and 97 (71.3%) tumors were h-GSS and l-GSS respectively. There was a marginal enrichment of somatic HRR gene mutations in the h-GSS compared to the l-GSS group but this was not statistically significant. There were no significant differences regarding the occurrence of co-mutations in the two groups except for *TP53* which was significantly higher in h-GSS (94.4%) compared to l-GSS samples (51.5%; p<0.001). TIL immunophenotyping did not show significant differences regarding the CD8+, CD4+ and FOXP3+ T cells and CD79+ B cells. Expression of $PD1^{+}TCF1^{+}$ and $PD1^{+}TCF1^{-}$, namely progenitor and terminally exhausted T cells respectively, was significantly higher in the h-GSS (5.1 ± 5.1; 15.0 ± 12.3) compared to l-GSS (2.5 ± 2.2; 8.8 ± 7.2; p=0.037 and p=0.040) group. In the subgroup of patients who received adjuvant platinum-based chemotherapy the increased infiltration of tumor center with both $PD1^{+}TCF1^{-}$ and $PD1^{-}TCF1^{+}$ cells was independently associated with DFS [HR=3.1 (p=0.040) and in these patients both DFS and OS was significantly improved in patients co-harboring a h-GSS with pathogenic/likely pathogenic TP53 mutations compared to patients with l-GSS/TP53wt (p=0.012 and p=0.032, respectively).
Conclusions: Our data demonstrate for the first time that a significant percentage of early-stage NSCLC patients exhibit genomic instability (h-GSS) which is associated with a unique *TP53* status and the immune profile of the exhaustion state. The latter highlights the heterogeneity of the different subpopulations of exhausted TILs in the TME of NSCLC, which seems to have substantial prognostic and predictive clinical relevance especially in patients with h-GSS.

#2191 A 50-marker mass cytometry intracellular cytokine staining panel: unprecedented resolution enables unrivaled detection of functional diversity present among human immune cell subsets.

Laura Polanco[1], Michael J. Cohen[2], Erika L. Smith-Mahoney[1], Lauren Tracey[2], Christina Loh[2], Amedeo J. Cappione[2], Anna C. Belkina[1], David King[2], Jennifer E. Snyder-Cappione[1]

[1]Boston University Chobanian & Avedisian School of Medicine, Boston, MA,[2]Standard BioTools, Markham, ON, Canada

Understanding mechanisms of immune evasion to therapeutics or during responses to cancer, autoimmunity and infectious diseases requires high-dimensional functional profiling at the single-cell level. Measurement of functional immune cell signatures that include both inflammation and immunosuppression provides key insights into several facets of cancer research and therapy. If single-cell detection of cytokines spanning many cell lineages was possible, 1) determining mechanisms underlying success/failure of checkpoint blockade, 2) defining immunosuppressive activity of the tumor-resident cell subsets and 3) identifying immunological biomarkers that predict clinical outcomes would be within reach. We used CyTOF™ technology to achieve this goal of high-parameter cross-functional profiling to avoid the limitations of fluorescence-based cytometry, such as signal spillover, autofluorescence, compensation errors and spectral unmixing complications.

A 50-marker CyTOF panel that achieves comprehensive profiling of immune subpopulations with detection of 20-plus intracellular cytokines spanning Th1, Th2, Th17 and Treg lineages was developed. PBMC were cultured with an array of stimulation conditions, stained and acquired on the CyTOF XT system. Datasets were analyzed using PhenoGraph clustering and visualized with opt-SNE to determine cellular functional diversity. Our findings show clear detection of IL-5-, IL-10- and IL-13 producing cells, found in surprising co-expression patterns with pro-inflammatory cytokines such as IFNγ. More significantly, we were able to detect expression of a number of secretory analytes that are historically difficult to identify by flow cytometry at a single-cell level, including the immunosuppressive cytokine TGF-β.

Mass cytometry, in concert with this new expanded cytokine panel, provides a far wider lens of visualization of the functional diversity of human immune cells than has been achieved previously. We predict that through the use of this panel, novel immune regulatory mechanisms that abate/prevent cellular responses in tumors will be revealed. In sum, our findings indicate that the CyTOF XT platform is well positioned as a catalyst for seminal discoveries in immune profiling to drive therapeutic design and advanced disease monitoring in cancer.

#2192 Enhancing veterinary cancer drug development with innovative bioanalytical assays.

Jun Wang, **Ildiko Kasza**, Denise Garvin, Trish Trish, Rod Flemming, Jeff Nelson, Ngan Lam, Marjeta Urh, Mei Cong, Jamison Grailer

Promega Corporation, Fitchburg, WI

The development of biologic drugs for veterinary health requires precise and reliable bioanalytical tools. To that end, we have developed several new assays, extending our industry-leading portfolio of human bioassays to emerging veterinary therapeutic targets. Featured here are cell-based reporter bioassays: Canine ADCC Reporter Bioassay and Canine PD-1/PD-L1 Blockade Bioassay. Additionally, we present two new Lumit Immunoassays for characterizing binding to the FcRn receptor: Canine FcRn Binding Immunoassay and Feline FcRn Binding Immunoassay.

Utilizing luminescence detection, these assays offer sensitive and specific measurement of target interactions. Our assay development process involves rigorous testing for accuracy, precision, linearity, and specificity, adhering to ICH guidelines. The assays demonstrated high sensitivity and specificity, as well as robust performance in detecting and quantifying the potency of target molecules. Our cell-based bioassays are available in convenient thaw-and-use kit formats, allowing for easy adoption and highly reproducible results. These assays can be utilized from early development through lot release, supporting every stage of the drug development process.

Promega's advanced bioassays and immunoassays provide a comprehensive platform for the development and characterization of veterinary biologic drugs. These tools support the entire drug development pipeline, enhancing the efficacy and safety of veterinary therapeutics through high sensitivity, specificity, and reproducibility. Additionally, our Lumit Immunoassays significantly reduce assay time compared to traditional ELISAs, providing faster and more efficient workflows for the characterization of binding interactions to the FcRn receptor.

#2193 Patient-derived colorectal microtumors predict response to anti-PD1 therapy.

Duy T. Nguyen[1], Matthew A. Schaller[2], Krista P. Terracina[2], Xia Xu[3], Diego I. Pedro[1], Alfonso Pepe[1], Juan M. Uruena[2], Zadia Dupee[2], Nickolas Diodati[2], Ryan Smolchek[4], Jack E. Famiglietti[4], Tran Yen Nhi Nguyen[1], Gerik Tushoski[2], Kuoyuan Cheng[3], Lan Chen[3], Doug Linn[3], Vania Vidimar[3], Aquila Fatima[3], Soon Woo Kwon[3], Dongyu Sun[3], Hongmin Chen[3], Haiyan Xu[3], Brian Long[3], Lily Y. Moy[3], Bonnie J. Howell[3], George H. Addona[3], W. Gregory Sawyer[1]

[1]H. Lee Moffitt Cancer Center, Tampa, FL,[2]University of Florida, Gainesville, FL,[3]Merck & Co., Inc., Rahway, NJ,[4]Aurita Bioscience, Gainesville, FL

Immune checkpoint inhibitors have made remarkable impacts in treating various cancers, including colorectal cancer (CRC). However, CRC still remains a leading cause of cancer-related deaths. While microsatellite instability (MSI) colorectal cancer (CRC) has shown positive responses to anti-PD1-therapy, this subgroup represents a minority of all CRC patients. Extensive research has focused on identifying predictive biomarkers to understand treatment response in CRC. Interestingly, a growing number of clinical cases have reported favorable outcomes from a subtype of supposedly non-responder microsatellite stable (MSS) CRC, characterized by DNA polymerase ε (POLE) proofreading domain mutations with high tumor mutational burden (TMB). This subtype has shown a notable response, either partial or complete, to pembrolizumab as salvage treatment, often following significant disease progression. To improve efficiency, cost-effectiveness, and clinical outcomes, there is an essential need for a testing platform capable of promptly identifying evidence of anti-PD-1 response to inform treatment strategies. Here, we established a novel *ex vivo* immunotherapy model using patient-derived tumor microexplants (or microtumors <1 mm) co-cultured with autologous peripheral blood mononuclear cells (PBMCs) from treatment-naïve CRC patients. We demonstrate here that long-term *ex vivo* treatment with pembrolizumab induced a heterogeneous but appreciable interferon-gamma (IFN-gamma) secretion, accompanied by infiltrating PBMCs. Intriguingly, a case study involving an MSS CRC phenotype harboring POLE mutation and associated ultrahigh TMB demonstrated a response to PD-1 blockade, potentially from the intratumoral immune cell population. Ultimately, this novel model could serve as a valuable tool in complementing clinical diagnostics and guiding personalized treatment plans for CRC patients, particularly those with specific phenotypes and mutational profiles.

#2194 Patient-derived triple negative breast cancer organoids show heterogeneous radiation-induced immune signaling.

Diego Sialer[1], Xiaoxue Deng[1], Maud Charpentier[1], Neil Rupchand[1], Marvin Campos[1], Jenna Moyer[2], Wen Shen[1], Olivier Elemento[2], Doron Betel[3], Silvia Formenti[1], Sandra Demaria[1]

[1]Radiation Oncology, Weill Cornell Medicine, New York, NY,[2]Englander Institute for Precision Medicine, Weill Cornell Medicine, New York, NY,[3]Institute for Computational Biomedicine, Weill Cornell Medicine, New York, NY

Only a minority of triple-negative breast cancer (TNBC) patients respond to immune checkpoint inhibitors (ICI). Radiation therapy (RT) increases ICI responses in preclinical TNBC models and in some patients[1,2], but there are no biomarkers to predict RT benefit. TNBC comprises multiple subtypes and we hypothesized that patient-derived tumor organoids (PDOs) representative of such heterogeneity could be used to study RT-induced cancer cell-intrinsic responses.Two PDOs molecularly classified as Luminal Androgen Receptor (LAR) and Basal-Like 1 (BL1) TNBC subtypes[3] were mock-treated or irradiated with 3 daily doses of 8Gy and harvested 24 hours post-RT. Differential gene expression analysis (DESeq2), Gene Set Enrichment Analysis (GSEA), and Qiagen's Ingenuity Pathway Analysis® were performed. Protein expression was assessed by western blot and flow cytometry. Healthy donors CD14+ monocytes were matured in M-CSF for one day prior to culture with PDO. RT upregulated multiple pro-inflammatory pathways (padj < 0.05), including TNFα via NF-κβ, antigen presentation via MHC-I and type I interferon (IFN-I), in the LAR PDO. In contrast, in the BL1 PDO only TNFα via NF-κβ was upregulated by RT, while IFN-I and antigen presentation pathways were not. RT also upregulated genes with immunosuppressive functions (*CD73* and *TGFB1*) in the LAR PDO, and Pathway Analysis identified *TGFB1* as a key upstream regulator ($p = 7.33 \times 10^{-17}$). Epithelial to mesenchymal transition (EMT) pathway was induced by RT in both PDOs. Flow cytometry confirmed RT-induced increase in MHC-I and CD73 expression in the LAR PDO, while only CD73 was upregulated in the BL1 PDO. Given the central role of IFN-I induction via cGAS/STING in RT-induced anti-tumor immune responses[4], we investigated cGAS and STING expression by the two PDOs. Western blot revealed suppressed expression of STING in the BL1 PDO. STING repression induces resistance to ICI in mouse models of TNBC and was shown to be mediated by MYC amplification in human TNBC[5]. Consistently, the BL1 PDO was derived from a tumor with MYC amplification. Finally, macrophages co-cultured with the LAR PDO showed reduced MHC-II and CD86 expression, an effect that was reversed when the PDO was treated with RT before co-culture. Our data support the use of PDOs that reflect the heterogeneity of human TNBC as models to study the immunomodulatory effects of RT. Findings show differential induction of key pro-immunogenic mediators by RT in different PDOs and suggest that CD73 is an actionable target to improve RT-induced ICI responses[6]. Understanding the multifaceted effects of RT using a PDO-based platform may enable a precision use of RT that is tailored to the specific characteristics of a given patient TNBC. *Supported by BCRF 23-053 & DOD W81XWH-21-2-0034. DS is the recipient of a Burroughs Welcome Physician-Scientist Award. PMID: 1. 37620372 2. 38194915 3. 27310713 4. 32161398 5. 35561311 6. 32047024*

#2195 Development of a faster potency assay for evaluation of immune cell-mediated cytotoxicity in vitro.

Danielle Califano, Ben Streeter, Sierra Barnes, **Skylar Dewees**, Denise Sullivan, Daniel Millard, Stacie Chvatal

Axion BioSystems, Atlanta, GA

Assessment of the efficacy of CAR T cell therapy relies on robust *in vitro* potency assays that evaluate the interaction between CAR T cells and cancer cells. Traditional methods require extended incubation periods of target cells of over 24 hours, limiting throughput and efficiency in assay development. In this study, we aimed to significantly reduce assay time by utilizing an impedance-based system to monitor the cytotoxic activity of CAR T cells against SKOV3 cancer cells. By comparing the standard protocol with an expedited approach that allows for a shorter resting period of target cells, we aim to establish a more efficient framework for potency testing that maintains accuracy, while facilitating faster advancements in CAR T cell therapies. In addition to shortening the overall assay time, adding CAR T cells at an earlier timepoint also ensures that the true Effector to Target (E:T) ratios are maintained by reducing the time for target cell doubling. Here, we describe an *in vitro* potency assay that shortens the assay time course from 72+ hours to 24 hours for immune cell-mediated cytotoxicity. SKOV3 target cells were seeded at 10k cells per well into a 96-well microplate with embedded electrodes in the substrate that detect the attachment and proliferation of target cells. HER-2 CAR T cells were added at either 4 or 24 hours post SKOV3 cell seeding at multiple E:T ratios (1:10, 1:5, 1:2, 1:1, and 5:1). The killing kinetics among each group were directly compared by calculating the Kill Time 50 (KT50), or the time required for 50% of the target cells to be killed. The KT50 for target cells treated at 4 hours was 91.6 ± 3.7 (1:10), 47.8 ± 22.7 (1:5), 19.8 ± 1.8 (1:2), 16.6 ± 0.9 (1:1), and 9.1 ± 0.7 (5:1) hours, while the KT50 for target cells treated at 24 hours was 150.0 ± 3.6 (1:10), 115.6 ± 7.1 (1:5), 83.8 ± 4.5 (1:2), 48.7 ± 2.5 (1:1), and 29.2 ± 0.8 (5:1) hours. As expected, the well groups treated with CAR T cells at 4 hours demonstrated faster KT50 times due to less SKOV3 cell proliferation and, therefore, more accurate E:T ratios. At 24 hours post CAR T addition, a dose response curve was generated and the EC50 was determined to be an E:T ratio of 1:2.4 for target cells treated 4 hours post seeding and E:T ratio of 1:1 for target cells treated 24 hours post seeding. Overall, the data described illustrates a label-free, *in vitro* workflow to assess potency of CAR T cells in a quicker, more efficient manner using the Maestro Z impedance assay.

#2196 Characterizing the impact of SOCS1 and Regnase-1 inactivation on tumor reactive T cells present in CRISPR/Cas9-engineered TIL (eTIL®) therapies derived from melanoma and NSCLC tumors through functional and single cell analyses.

David Monteiro[1], Meghan Travers[1], Tyler G. Searles[2], Gustavo J. Martinez[1], Sharon Lin[1], Ashish Yeri[1], Alex Smashnov[1], Mitali Ghose[1], Seamus McKenney[1], Paul Dunbar[1], Charles H. Yoon[3], Dipen Sangurdekar[1], Karrie Wong[1], Joseph D. Phillips[4], Mary Jo Turk[2], Micah J. Benson[1]

[1]KSQ Therapeutics, Inc., Lexington, MA,[2]Dartmouth Cancer Center and the Department of Microbiology and Immunology, The Geisel School of Medicine at Dartmouth, Lebanon, NH,[3]Brigham and Women's Hospital, Harvard Medical School, Boston, MA,[4]Department of Surgery, The Geisel School of Medicine at Dartmouth, Lebanon, NH

Tumor Infiltrating Lymphocyte (TIL) therapy involves the isolation and *ex vivo* expansion of TIL for the treatment of solid tumors. The T cell receptor (TCR) repertoires present within TIL drug products (DPs) are highly patient unique and include TCRs specific against tumor antigens at variable frequencies. The clinical activity of TIL is thought to be mediated in part by the anti-tumor functionality profile of TIL and the frequency of tumor reactive T cells (TRTs) within the TIL DP. Seeking to improve the clinical activity of TIL therapy, we developed KSQ-001EX and KSQ-004EX, two CRISPR/Cas9-engineered TIL (eTIL®) therapies wherein the genes encoding SOCS1 or SOCS1 in combination with Regnase-1 are inactivated, respectively. SOCS1 is a negative regulator of cytokine signaling while Regnase-1 is an RNase that degrades transcripts encoding proteins involved in regulating T cell anti-tumor function. We have shown in preclinical models that inactivation of SOCS1 and SOCS1/Regnase-1 enhance the anti-tumor functionality of TIL; however, the incidence of TRTs in eTIL DP and how inactivation of SOCS1 and Regnase-1 impact their functionality remains unclear.To identify and characterize TRT clones in eTIL, we generated KSQ-001EX and KSQ-004EX using TIL derived from patients with melanoma or non-small cell lung cancer (NSCLC) and analyzed them with distinct but complimentary methods. We functionally characterized melanoma-derived DPs via coculture with paired autologous melanoma tumor cell lines, with samples exhibiting cognate reactivity against tumor through induction of CD69 and CD137. Consistent with its biology, we observed strong SOCS1-driven enhancement of IFNγ and CD25 in this setting. We extended these insights to NSCLC using single cell RNA/TCR sequencing performed on DPs and tumor starting material. In tumor starting material, we identified $CD8^+$ T cell clusters aligning to *IL7R*$^+$ circulating/stem, *GZMB*$^+$ effector, and *CXCL13*$^+$ resident memory (T_{rm}) states. Predicted TRT (predTRT) clones were identified using a custom gene set constructed from published TRT signatures and cells from these clones were overrepresented in T_{rm}. The majority of predTRT clones were also detected in DPs, where they preferentially aligned to effector memory and proliferating states. Further demonstrating the impact of SOCS1 and Regnase-1 edits on predTRT clones, we observed enrichment of an activated interferon-stimulated gene signature in eTIL DPs, with predTRT clones in dual-edited KSQ-004EX uniquely enriched for heightened expression of a Regnase-1 transcriptional signature.To conclude, we observe that eTIL possess reactivity against tumor through retention of TRT clones, with SOCS1 and Regnase-1 inactivation enhancing the anti-tumor functionality of TRT clones within eTIL DP.

#2197 Uncovering immune evasion in EGFR-mutant and treatment resistant NSCLC: insights from a novel mouse model.

Anchit Bhagat, Cong-Xiao Liu, Xiao Yang, Melissa Gajda, Jason McBane, Herbert K. Lyerly, Zachary C. Hartman

Duke University, Durham, NC

Lung cancer remains one of the most prevalent and deadly malignancies globally, with non-small cell lung cancer (NSCLC) comprising approximately 84% of all cases. Despite advances in treatment, the overall 5-year survival rate for NSCLC remains at 15%. Among NSCLC cases, 15-20% are driven by activating mutations (such as L858R) in the epidermal growth factor receptor (EGFR), which are treated with EGFR tyrosine kinase inhibitors (TKIs). Although three generations of TKIs have been developed, a number of genetic mutations (T790M and C797S) have been shown to evolve that confer resistance in most patients. This has spurred interest in combination therapies, particularly with PD-1 and PD-L1 immune checkpoint inhibitors (ICIs), however, these have limited efficacy in patients with EGFR-mutant NSCLC. Therefore, understanding the immune landscape of these tumors is critical. We developed a mouse model of EGFR-mutant lung cancer using a transgenic mouse model expressing an EGFR carrying L858R activating, as well as T790M and C797S resistance mutations. We demonstrate the expression of this gene after intranasal delivery of an adenovirus carrying GFP-Cre (Ad-GFP-Cre) from a CAG promoter, along with expression of Td-tomato and Luciferase to allow for tumor tracking and visualization. In a time-course experiment, we monitored tumor progression and immune infiltration. By 4-6 weeks post-Ad-GFP-Cre injection, we observed consistent and palpable tumor growth, establishing a critical window for therapeutic intervention. At 2 weeks post-injection, lung tissues showed robust infiltration of CD8+ T cells, highlighting a strong initial immune response. However, levels of regulatory T cells (Tregs) increased significantly over time. This accumulation of Tregs coincided with tumor growth and suppression of cytotoxic T cell activity, suggesting a shift toward an immunosuppressive microenvironment. CD4+ T cells remained relatively constant, underscoring the specific role of Tregs in facilitating immune evasion. Systemic adaptive immunity against EGFR was detected in all tumor bearing mice, demonstrating the potency of local immune suppression in EGFR+ tumor evolution. To evaluate the therapeutic relevance of these findings, we treated mice with a PD-1 inhibitor starting at 4 weeks post-injection. Tumor response was minimal, mirroring clinical observations of limited efficacy for ICIs in EGFR-mutant NSCLC. The early wave of cytotoxic immune activity followed by Treg-mediated suppression offers insights into potential therapeutic targets. This will be a useful model to study the impact of different EGFR TKIs, as well as the combination of these agents with different immunotherapeutic modalities to identify strategies to optimize combination therapies. This work has the potential to inform innovative approaches for treating EGFR-mutant NSCLC and improving patient outcomes.

#2198 Platform for purifying antigen-presenting myeloid cells based on frequency of interactions with T cells.

Eric Ferguson[1], Zhixuan Huang[2], John M. Beierle[1], **Behrad Azimi**[1], Kenneth H. Hu[2]

[1]Narwhal Bio Inc., San Diego, CA,[2]Department of Immunology, The University of Texas MD Anderson Cancer Center, Houston, TX

Cell-cell interactions' underlying biology drives many promising therapeutic opportunities in treating cancer. However, studying cell-cell interactions is challenging because the cells are alive and remain motile, and the complexity of downstream analysis increases with every observed interaction event. Today, researchers study cell-cell interactions using a variety of commercially available spatial approaches on fixed tissue samples. While these approaches can help study regions of interest or groups of cells based on their location within the tissue sample, they don’t offer single-cell resolution and fail to provide information on the more complex behavior of said cells, including the frequency and temporal nature of their interactions. Narwhal Bio Inc.’s A1 instrument, software, and consumable platform allow for real-time cell identification, tagging, and downstream characterization of live cells in cell mixtures. The live-cell-in, live-cell-out process enables the purification of live cells based on cell behaviors for use in downstream multiomics analyses. Here, we apply this platform to study the clinically relevant T-cell activating interactions between myeloid and T cells. We screen myeloid cells presenting various concentrations and types of antigens and monitor interactions with T cells over days. Myeloid cells are tagged by the platform when in contact with T cells at multiple time intervals resulting in the ability to stratify highly involved antigen-presenting cells (APCs) from less involved APCs. The cells are then isolated live, stratified based on their level of T-cell interactions, and then subjected to further downstream characterization and assay. At the core of the presented application is the tenet that a better understanding of cell-cell interactions will expose important underlying biological pathways, thereby increasing the chance of discovering targets for therapeutic intervention.

#2199 Ready-to-use oxygen gradient microplates for customized in vitro assays for the phenotypic and functional characterization of primary macrophages in immuno-oncology drug development.

Christoph Schifflers[1], Martijn Vlaming[1], Emmanuelle Sidot[1], Jezabel Lefevre[1], Elise Pepermans[2], Ellen Boelen[1], Enrico Grassilli[3], Sofie Pattyn[1]

[1]IQVIA, Gosselies, Belgium,[2]Immunespec, Niel, Belgium,[3]insimili, Bologna, Italy

Ready-to-use oxygen gradient microplates for customized *in vitro* assays for the phenotypic and functionalcharacterization of primary macrophages in immuno-oncology drug developmentChristoph Schifflers[1], Martijn Vlaming[1], Emmanuelle Sidot[1], Jezabel Lefèvre[1], Elise Pepermans[2], EllenBoelen[1], Enrico Grassili[2] and Sofie Pattyn[1]1IQVIA Laboratories, In Vitro Immunology (formerly known as ImmunXperts), Rue Clément Ader 10, 6041Gosselies Belgium[2]ImmuneSpec, Galileilaan 18 bus 6, 2845 Niel, Belgium[3]InSimili srl, Viale Giuseppe Fanin 48, Bologna, 40050, ItalyOxygen level fluctuations in tumor micro-environment (TME) can alter the phenotype and function ofcancer cells as well as tumor-infiltrating immune cells, such as macrophages. For the functional evaluationof new immuno-oncology drug candidates, it can be key to take into consideration the effects of varyingO2 concentrations. In fact, it is well established that varying oxygen concentrations and tumor hypoxia candrive treatment resistance and cancer recurrence. Modeling this layer of functional complexity of thetumor microenvironment, typically requires the use of animal models or tumoroid systems which can becomplex to establish, time-consuming and expensive. InSimili developed a new technology that providesready-to-use microplates that model spatially controlled O2 levels at fixed O2 concentrations or O2 gradients(adaptable for individual wells). Combining this technology with highly optimized *in vitro* assays using highquality primary immune cells can provide a useful tool with improved predictive value for the cost-effectivefunctional characterization and lead selection of new immuno-oncology drug candidates.In this study, the impact of varying O2 concentrations (ranging from 20% to 1% O2) was assessed usingcustomized microplates on the phenotype of macrophages derived from healthy donor PBMCs.Furthermore, the impact of different O2 concentration on the capacity of M2-like macrophages to suppressT cell activation was explored. Finally, the impact of several oxygen concentrations on the reprogrammingof M2-like macrophages was evaluated.Taken together, this study showed that InSimili's plates provide a ready-to-use tool that can be used incustomized *in vitro* assays to model varying O2 concentrations. Integrating this technology with highlycustomized *in vitro* bioassays can serve as a valuable tool for immuno-oncology drug development whiletaking into account an additional degree of the complexity of the TME in 2D and 3D systems.

#2200 Development and optimization of cost-effective non-human primate in vitro assays for immunogenicity screening and functional evaluation of immuno-oncology drug candidates.

C. Schifflers[1], V. Martijn[1], E. Sidot[1], J. Lefevre[1], E. Pepermans[2], E. Boelen[1], S. Pattyn[1];
[1]IQVIA, Gosselies, Belgium, [2]ImmuneSpec, Niel, Belgium

A surge in demand and a decrease in supply of non-human primate (NHP) test subjects have caused acritical shortage and a major price hike for biomedical research. Therefore, researchers are actively lookinginto refinement and/or alternatives to studies using NHP. The development of cost-effective NHP *in vitro*assays with predictive value for the assessment of unwanted immunogenicity as well as the functionalityof drug candidates can provide useful tools in this context. For instance, PBMCs isolated from blood,sourced from Cynomolgus Macaques, can be cryopreserved and thawed thereafter on multiple separateoccasions to be used for isolation of immune cell subsets of interest.The development of customized *in vitro* assays based on cynomolgus monkey PBMCs can be used for thepre-selection of drug candidates and therefore reduce the number of NHP that are required for *in vivo*studies. For instance, cynomolgus PBMCs can be used to evaluate the unwanted immunogenicity of testcompounds *in vitro*. Here, it was shown that ATR-107, an anti-IL21R antibody and Bococizumab triggeredsignificant proliferation of cynomolgus PBMCs. Furthermore, using immunopeptidomics (in collaborationwith ImmuneSpec) Infliximab-derived peptide fragments that are known to be immunogenic in humanwere found to be presented at the surface of cynomolgus dendritic cells after their incubation withInfliximab during their maturation.We previously reported the development of cynomolgus monkey PBMC-derived *in vitro* assays that canbe used for the functional evaluation of new drug candidates (*e.g., macrophage reprogramming,* cytokinerelease, M2-suppression assay, ADCP etc.), target binding studies as well as Cynomolgus Macaque PBMCx human dendritic cell (DC) mixed lymphocyte reactions (MLR). Continuous efforts to optimize these assayscan additionally minimize the requirements of cynomolgus monkey material. For instance, it was shownthat by adapting the cell culture conditions for the generation of cynomolgus macrophages, cell yields canbe substantially improved, reducing the number of required PBMC samples, and allowing to perform largerassays for a given amount of NHP material.With our expanding portfolio and continuous optimization of Cynomolgus Macaque assays we aim to aidbiomedical drug development and to refine/reduce NHP usage.

IMMUNOLOGY: Microbiome, Inflammation, and Cancer
Poster Session

#2204 Probiotic *Lactobacilli* and their secreted metabolites exert anti-inflammatory and anti-genotoxic effects in Barrett's esophagus associated tumorigenesis *in vitro* and *in vivo*.

Maanya Pradeep, Divya Joshi, Joshua N. Bernard, Thomas Andl, **Claudia D. Andl**

Burnett School of Biomedical Sciences, University of Central Florida, Orlando, FL

Gastroesophageal reflux disease (GERD) leads to the accumulation of bile-induced reactive oxygen species and oxidative stress in esophageal tissues, causing inflammation and DNA damage. As GERD progresses to Barrett's esophagus (BE), the only known precursor lesion for esophageal adenocarcinoma, probiotic bacteria with anti-inflammatory and anti-genotoxic function are of significant interest in the prevention and treatment of Barrett's esophagus (BE) and esophageal adenocarcinoma. To assess the outcome of probiotic *Lactobacillus* administration *in vivo,* we used the model of L2-driven expression of IL1b for inflammation-associated Barrett's tumorigenesis. We administered 10^9 CFU/ml (colony-forming unit) *L. acidophilus* and *L. plantarum* by oral gavage starting at 6 weeks of age until the IL-1β mice were 6 months old (prevention model) or initiated treatments when the mice were 4 months old until 6 months of age (treatment group). At the end of the study, mice were sacrificed, and esophageal and forestomach tissues were harvested for DNA and RNA extraction and FFPE. Histopathological analysis following *Lactobacillus* spp. administration in the murine study showed a significant reduction in inflammation ($p<0.07$) and tumor incidence ($p<0.05$) compared to untreated sham mice in the prevention and treatment groups. Assessing mucin production at the GEJ visualized by Alcian Blue/PAS, we observed 80% of the sham mice had extended glands compared to significantly less when *Lactobacillus* spp. was administered ($p<0.03$).Multiplex immunofluorescence staining was used to evaluate immune cell recruitment and BE marker expression. *In vitro* assays utilized immortalized normal esophageal cells (STR), and the BE cell lines, CP-B and BAR-T and cell-free Lactobacillus supernatant. Upon DNA damage induction using deoxycholic acid or ox bile, cell lines were incubated with live Lactobacilli or cell-free supernatant secreted by *L. fermentum*, *L. acidophilus* and *L. plantarum.* To identify if inflammation and DNA damage can also be reduced using potential Lactobacillus-secreted bioactive metabolites, we detected 8-oxo-dG, pH2AX and RAD51 *in vitro* using immunofluorescence. We show a reduction in DNA damage and NF-KB-mediated inflammation in the presence of Lactobacillus cell-free supernatants similar to the effect of live bacteria.Our data demonstrate the feasibility of utilizing probiotic *Lactobacillus* supplementation in mouse models of BE, as well as support the hypothesis that *Lactobacillus* spp. aid in the prevention of disease progression and its treatment in experimental models of gastroesophageal reflux disease.

#2205 Exploring the presence and role of causative viruses in glioblastoma using a multi-omics approach.

Bavani Gunasegaran[1], Shamini Ayyadhury[2], Shivani Krishnamurthy[1], Seong Beom Ahn[1], Benjamin Heng[1]

[1]Macquarie University, Sydney, Australia,[2]The Donnelly Centre, University of Toronto, Toronto, ON, Canada

Glioblastoma (GB) is the most aggressive brain cancer with a poor survival rate. While molecular markers have been established to improve treatment response with modest outcomes, the cause of this cancer remains unknown. Viruses were proposed as potential contributing factors to glioblastoma, particularly given that approximately 20% of all human cancers are virus-induced. While previous studies have detected viral presence in glioblastoma tissues, these investigations were limited by small sample sizes and often focused exclusively on either viral DNA or proteins, restricting the scope of their findings. To address these shortcomings, we explored the role of viruses in glioblastoma by utilising advanced analytical chemistry and high-throughput technologies to study viral proteins and genes and their interaction with tumour signalling pathway(s). We analysed 233 publicly accessible mass spectrometry metaproteome datasets encompassing 196 glioblastoma, 21 adjacent, and 16 normal brain tissues (PMID: 31331834, 31154438, 36720864), using the Trans-Proteomic Pipeline (PMID: 36648445) which converts raw data files, identifies, validates and quantifies peptides, and protein inference. To expand our analysis, we incorporated metaproteomic data from 100 samples (52 glioblastoma, 48 normal) and metagenomic data from seven glioblastoma tumours sourced from Hunter Cancer Biobank. Our comprehensive analysis, integrating meta-proteomics and meta-genomics, revealed a higher prevalence of multiple herpesvirus species, including human Herpes Simplex Virus-1, Herpes Simplex Virus-2, and Herpesvirus 8 within tumour tissues compared to control and adjacent tissues. To further elucidate the role of viral infections in tumour progression, we are currently investigating meta-transcriptomic data from 15 tumours.We are also investigating human proteins within a cohort of 100 samples to identify potential associations with our detected viruses. We aim to explore whether viral infections may influence human proteins involved in critical biological processes in glioblastoma. In a preliminary analysis, we quantified 3,991 human proteins, of which 1,155 were found to be deregulated. Gene enrichment analysis revealed up-regulated gene ontology biological processes associated with interspecies interactions and defence responses to other organisms. This suggests that these genes may potentially play a role in the host's reaction to viral infections. Additionally, Ingenuity Pathway Analysis identified 182 deregulated pathways, including several that may be implicated in viral responses, warranting further investigation.Collectively, our findings indicate a potential role of viruses in tumour development, possibly having the capacity to redefine the stratification of tumour types in GB patients.

#2207 Intratumoral bacteria predict survival in clear cell renal cell carcinoma (ccRCC).

Alice C. Martin[1], Anne-Laure Cattin[1], Irene Lobon[1], Zayd Tippu[1], Fiona Byrne[1], Charlotte Spencer[1], Clara Becker[2], Martha Zepeda Rivera[3], Krupa Thakkar[4], Hongui Cha[4], Angel Fernandez-Sanroman[1], Annika Fendler[1], Taja Barber[1], Leo Bickley[1], Daqi Deng[1], Scott Shepherd[1], Parise Lockwood[1], Maximiliano Gutierrez[1], Susan Bullman[3], Kevin Litchfield[4], Samra Turajlic[1]

[1]Francis Crick Institute, London, United Kingdom,[2]Fred Hutchinson Cancer Center, Seattle, WA,[3]MD Anderson Cancer Center, Houston, TX,[4]UCL Cancer Institute, London, United Kingdom

Background: ccRCC is marked by niches of immune evasion in late-stage disease, that are associated with resistance to immune checkpoint inhibitors (CPI). Intratumoral microbes are emerging as key modulators of the tumor immune microenvironment and may therefore play a yet unrecognised role in ccRCC disease progression and therapy resistance, marking them as potential biomarkers, and novel therapeutic targets.

Methods: We extracted bacterial reads from three cohorts of treatment-naïve primary tumors and one cohort of pre- and post-CPI treated tumors: Genomics England Renal (GEL) (636 patients, WGS), TRACERx Renal (154 patients, 629 samples, WGS); TCGA Renal (494 patients, RNA) & ADAPTeR (15 patients, 56 samples, RNA). Bespoke denoising, and decontamination were applied. Live presence of intratumoral bacteria was confirmed through culture and RNAscope. Genera associated with survival were identified using ElasticNet feature selection.

Results: We observed significant heterogeneity in bacterial abundance within and across tumors, driven by differences in *Cutibacterium* abundance. *Cutibacterium* makes up ~90% of bacteria in each tumor sample and is enriched in tumors compared to adjacent normal tissue (p=7.1e-10). 9 of 11 colonies grown from two positive tumors were genotyped as *Cutibacterium acnes,* confirming its live presence and relative abundance in ccRCC tumors. *Cutibacterium* is higher in late-stage tumors (III&IV) than early-stage tumors (I&II) (p=7.8e-3). Its abundance also separates patients by progression free survival (PFS) and overall survival (OS) (p=9.9e-3, p=0.016) and is higher in CPI non-responders than responders (p=0.028). Association with survival is confirmed in the TCGA cohort independently of stage (PFS = 0.031, OS = 7.3e-4). While other genera associate with survival in either TRACERx or TCGA, only *Cutibacterium* is prognostic in both.

Enrichment analyses of bulk RNA (TRACERx & TCGA), show upregulation of leukocyte taxis and innate immune response with *Cutibacterium* abundance. To further probe this interaction with the immune microenvironment we are performing single cell spatial transcriptomics, and *in vitro* co-cultures. Results of these ongoing experiments will be presented at the conference.

Conclusions: *Cutibacterium* either creates or exploits a disease- and CPI-resistance-promoting environment in ccRCC tumors and can therefore act as a prognostic and predictive biomarker. The genus is known for causing chronic inflammation in sebaceous skin follicles, induces Nf-kB signalling, and M2-macrophage differentiation *in vitro*, and associates with myeloid response in ccRCC tumors. Together this supports a compelling hypothesis that *Cutibacterium* reduces survival in ccRCC by creating immunosuppressive niches, thereby fostering disease progression and CPI-resistance.

#2208 *Phocaeicola vulgatus* mediates immunotherapy resistance in hepatocellular carcinoma via reducing indoleacetic acid production.

Caining Zhao[1], Shanshan Li[1], Thomas Yau[2], Wenqi Chen[1], Ren Ji[3], Xinyuan Guan[4], Fengming Spring Kong[4]

[1]Clinical Oncology, University of Hong Kong-Shenzhen Hospital, Shenzhen, China,[2]Medicine, University of Hong Kong, Hong Kong, Hong Kong,[3]Surgery, University of Hong Kong-Shenzhen Hospital, Shenzhen, China,[4]Clinical Oncology, University of Hong Kong, Hong Kong, Hong Kong

Background: Gut microbiota has been widely reported to influence anti-PD-1 efficacy in melanoma and lung cancer, in both clinical specimens and animal models. However, the role of gut microbiota in hepatocellular carcinoma (HCC) remains less explored, with limited understanding of the underlying mechanisms. Method: Advanced HCC patients treated with anti-PD-1 with/without anti-CTLA-4 were prospectively enrolled, and categorized into responders and non-responders groups based on their radiological response after 6 months of immunotherapy according to modified Response Evaluation Criteria for Solid Tumors (mRECIST) guideline. The fecal metagenomics and plasma metabolome data were integrated to reveal the profiles of gut microbiota. Meanwhile, fecal microbiota transplantation (FMT), single strain and metabolite intervention animal models, as well as in vitro CD8+T cell culture were employed to study its mechanism of modulating immunotherapy efficacy in HCC. Results: In the metagenomic analysis of 65 patients, the responders and non-responders exhibited distinct gut microbiota profiles, which were replicated in mice via FMT. Lefse analysis revealed a higher abundance of *Phocaeicola vulgatus* (*P. vulgatus*) in non-responders compared to responders. Moreover, patients with higher *P. vulgatus* had a mean progression free survival (PFS) of 11.1 months and overall survival (OS) of 22.9 months, which were significantly shorter than those with lower *P. vulgatus* (PFS: 30.8 months, $p = 0.047$; OS: 63.2 months, $p = 0.037$). Notably, *P. vulgatus* enriched in non-responders restrained anti-PD-1 efficacy in syngeneic and orthotopic tumor models and inhibited the CD8+T cell cytotoxicity, evidenced by decreased IFN-γ+ and GzmB+ populations. Mechanistically, *P. vulgatus* reduced gut microbiota-derived indoleacetic acid (IAA), weakening CD8+ T cell effector functions and impairing immune response. Supplementation with IAA restored CD8+ T cell cytotoxicity and counteracted the immune-suppressive effects of *P. vulgatus*. Conclusion: *P. vulgatus* impaired anti-PD-1 efficacy in HCC via reducing IAA production, which in turn diminishes the effector functions of CD8+ T cells. This study establishes a causal link between *P. vulgatus* and anti-PD-1 resistance in HCC, highlighting gut microbiota-derived IAA as a potential therapeutic target.

#2209 MaaT034, a new co-cultured microbiome ecosystem therapy candidate, is capable to safely colonize the gastro-intestinal tract of germ-free mice to restore a healthy gut physiology and to stimulate immunity.

Bastien Laperrousaz, Julie Reygner, Charlotte Petitjean, Aurore Duquenoy, Cyrielle Gasc, Diane Plouchart, Sophie Declomesnil, Carole Schwintner, Nathalie Corvaia

MaaT Pharma, Lyon, France

Background: Increasing evidence suggests that gut microbiome composition modulates tumor response to therapies, including immune checkpoint inhibitors (ICI). Clinical proofs of concept were obtained in pilot studies using healthy or ICI-responder donor-derived fecal matter transplants to modulate the gut microbiome of non-responding cancer patients and improve their response to ICI. We have developed Microbiome Ecosystem Therapies^{TM} (MET) replicating at large industrial scale the effects of ICI-responders gut microbiome. MaaT034, the first-in-class co-cultured microbiome product, has been selected through artificial intelligence and *in vitro* screening assays to optimize intestinal homeostasis and to activate immune functions alone and in combination with an ICI treatment.
Method: We used germ-free mice to evaluate MaaT034 engraftment and safety, as well as its impact on metabolite production, immunity as well as gut morphology.
Results: Metagenomic analysis demonstrates that MaaT034 is capable of engrafting in the gut of germ-free mice, as evidenced by the presence of the four major phyla of the human product in mice. KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis revealed the presence of genes involved in the synthesis of key metabolites such as short chain fatty acids, indoles, as well as secondary biliary acids. In line, metabolite quantification in the feces of mice further confirmed the capability of MaaT034 microbes to produce these metabolites. MaaT034 was able to improve the gastrointestinal physiology and to stimulate immunity in germ-free mice (reduction of the size of cecum, increase in the depth of intestinal crypts, increase in the percentage of Goblet cells, increase of spleen weight). All mice remained healthy throughout the experiment and no adverse event was observed.
Conclusion: Altogether, these findings underscore MaaT034's potential to safely colonize the gastro-intestinal tract of mice and to produce key metabolites allowing to restore a healthy gut physiology and to stimulate both local and systemic immunity. As such, MaaT034 could offer a promising therapeutic benefit for the treatment of solid tumors in combination with ICI therapy.

#2210 Microbiome profiling reveals that fecal microbiota transplantation (FMT) modulates response and toxicity when combined with immunotherapy in patients with lung cancer and melanoma (FMT-LUMINate NCT04951583).

Sreya Duttagupta[1], Meriem Messaoudene[1], Rahima Jamal[1], Catalin Mihalcioiu[2], Nicolas Marcoux[3], Sebastian Hunter[1], Gianmarco Piccinno[4], Michal Puncochar[4], Karl Belanger[1], Mustapha Tehfe[1], Normand Blais[1], Scott Owen[1], Jacques Raphael[5], Jade Maillou[1], Yongjia Hu[1], Myriam Benlaifaoui[1], Somayeh Nilli[1], John Lenehan[5], Wiam Belkaid[1], Seema Nair Parvathy[5], Diogjena Katerina Prifti[1], Marine Fidelle[6], Seunghee Kim-schulze[7], Thomas Marron[7], Sylvere Durand[6], Guido Kroemer[6], Nicola Segata[8], Lisa Derosa[6], Laurence Zitvogel[6], Antoine Desilets[1], Michael Silverman[5], Saman MalekiVareki[5], Bertrand Routy[1], Arielle Elkrief[1]

[1]Research Center of the Centre Hospitalier de l'Universite de Montreal, Montreal, QC, Canada,[2]Mcgill University Health Centre, Montreal, QC, Canada,[3]Centre Hospitalier de l'Universite de Quebec, Quebec City, QC, Canada,[4]University of Trento, Trento, Italy,[5]Western University, London, ON, Canada,[6]Institut Gustave Roussy, Paris, France,[7]Mount Sinai, New York, NY,[8]IEO, European Institute of Oncology, IRCCS, Milan, Italy

Background: Trials have shown that FMT plus immune checkpoint inhibitors (ICI) could improve αPD-1 response in melanoma. The microbiome and immune shift, along with the clinical impact of FMT with αPD-1 in non-small cell lung cancer (NSCLC) and in combination with αPD-1+αCTLA-4 (dual ICI) in melanoma is unknown.
Methods: 20 patients (pts) with advanced NSCLC treated with first-line (1L) αPD-1 and 20 with advanced melanoma treated with 1L dual ICI were recruited. FMT from 1 healthy donor (HD) was administered orally to each pt post bowel prep. 1 week later ICI was initiated. A total of 11 different HD were used. The primary endpoint was objective response rate (ORR). The gut microbiome was profiled using shotgun metagenomics. Metabolomic profiling was performed using HPLC. Olink proteomics (96 markers) on plasma and flow cytometry (40 markers) on PBMCs were assessed. FMT was performed in germ-free (GF) mice to recapitulate engraftment.
Results: In NSCLC, ORR was 80% (16 PR) with no grade ≥3 immune-related adverse events (irAE). In melanoma, ORR was 75% (5 CR), with 65% (13 pts) developing grade ≥3 irAE, including 3 (15%) myocarditis. The irAE occurred earlier than previously reported (median onset of 42 days). Pre-FMT patient microbiome composition was distinct from HD. Post-FMT, the responders (R) and non-responders (NR) clustered separately (p=0.03). Post-FMT, we observed increased polyamines and bile salts in R. Elevation of exhausted T central and T effector memory cells were noted in R. Conversely, proteomics analyses revealed elevated markers of immune exhaustion including CD28 and IL10 in NR. Addressing the link between microbiome and irAE, in melanoma, 6 out of the 13 pts (46%) that developed grade ≥3 irAE, including 2/3 of the myocarditis received FMT from the same donor (D5). D5 was not associated with irAE in NSCLC treated with αPD-1 alone. Microbiome analysis revealed that D5 clustered separately ($p<0.01$) from other HD with a high relative abundance of *Prevotella copri*. We observed a signal for the development of irAE in pts that engrafted *P. copri*. Pts with grade≥3 irAE exhibited higher levels of leaky gut markers such as sST2 post-FMT (p=0.002). In GF mice, oral gavage of baseline stool of R, only when followed by the stool of the respective donor, led to a significant increase in α diversity and number of species engrafted over time ($p<0.01$), while those that received baseline stool from NR with or without donor had no change in α diversity or species engrafted.
Conclusions: This study validates the role of FMT to improve ICI activity in NSCLC and melanoma, while differentially modulating irAE according to ICI backbone. We demonstrated that the microbiome shift is distinct among R while engraftment of *P. copri* was associated with dual ICI irAE. Our results directly inform future microbiome-centered trials.

#2211 A novel combined immunotherapy of gut microbial metabolite butyrate and oncolytic adenovirus in colorectal cancer.

Tetsuya Katayama[1], Shinji Kuroda[1], Masaki Sakamoto Sakamoto[1], Eri Takeda[1], Yu Mikane[1], Shunya Hanzawa[1], Daisuke Kadowaki[1], Yusuke Yoshida Yoshida[1], Masashi Hashimoto[1], Nobuhiko Kanaya[1], Yoshihiko Kakiuchi[1], Satoru Kikuchi[1], Shunsuke Kagawa[1], Hiroshi Tazawa[1], Yasuo Urata[2], Toshiyoshi Fujiwara[1]

[1]Okayama University Medical School, Okayama city, Japan,[2]Oncolys BioPharma, Inc., Tokyo city, Japan

Butyrate, a short-chain fatty acid (SCFA) produced by gut bacteria in the colon, serves as a critical energy source for colonocytes. Gut microbiota and its primary metabolite, butyrate, have been reported to potentially modulate tumor sensitivity to chemotherapy or immunotherapy, although the underlying mechanisms remain unclear. Oncolytic virotherapy is a promising antitumor treatment that selectively induces oncolytic cell death in tumor cells. Previously, we developed OBP-702, a telomerase-specific oncolytic adenovirus armed with the p53 tumor suppressor gene, which demonstrated potent antitumor effects across various cancer types. Oncolytic virotherapy is also known to stimulate antitumor immunity. In this study, we investigated the combined effects of OBP-702 and butyrate on colorectal cancer and elucidated the underlying mechanisms. In vitro, the combination of OBP-702 and butyrate exhibited synergistic cytotoxicity against various human and murine colorectal cancer cell lines. In vivo, the combination therapy significantly suppressed tumor growth in HCT116 and CT26 subcutaneous tumor models compared to monotherapy with either OBP-702 or butyrate alone. These synergistic effects were mediated by butyrate enhancing OBP-702's efficacy through the upregulation of coxsackievirus and adenovirus receptor (CAR) and integrin expression on tumor cells, improving viral infection efficiency. Butyrate also increased MHC-I expression on tumor cells through activation of the cGAS-STING pathway, leading to enhanced CXCL10 expression. This, in turn, activated antitumor immunity by recruiting CD8-positive T cells to the tumor microenvironment. The synergistic effects of butyrate and OBP-702 were further confirmed in an orthotopic colorectal tumor model with liver metastases using luciferase-expressing CT26 cells. In this model, the combination therapy not only reduced tumor growth but also improved survival by enhancing antitumor immunity mediated by CD8-positive T cells. To translate these findings into a more clinically relevant approach, butyrate-producing bacteria, Clostridium butyricum Miyairi 588 (CBM588), were used in place of butyrate. The combination therapy of CBM588 and OBP-702 demonstrated similar synergistic antitumor effects in mouse subcutaneous and rectal tumor models. In conclusion, the combination of OBP-702 and butyrate shows promise as a synergistic antitumor strategy and may represent a novel therapeutic approach for colorectal cancer.

#2212 Microbial metabolic pathways guide response to immune checkpoint blockade therapy.

Iris L. Mimpen[1], Thomas W. Battaglia[1], Miguel Parra Martinez[1], Laurien J. Zeverijn[1], Birgit S. Geurts[1], Louisa R. Hoes[1], Vincent van der Noort[2], Hans Gelderblom[3], Krijn K. Dijkstra[1], Lodewyk F. A. Wessels[4], Emile E. Voest[1]

[1]Molecular Oncology and Immunology, The Netherlands Cancer Institute, Amsterdam, Netherlands,[2]Department of Biometrics, The Netherlands Cancer Institute, Amsterdam, Netherlands,[3]Leiden University Medical Center, Leiden, Netherlands,[4]Molecular Carcinogenesis, The Netherlands Cancer Institute, Amsterdam, Netherlands

Recent studies have identified a link between specific microbiome-derived bacteria and immune checkpoint blockade (ICB) efficacy. However, these species lack consistency across studies and their immunomodulatory mechanisms remain elusive. We hypothesized that community-level metabolic pathways across microbes are more suitable to predict ICB response. Using a curated resource of pan-cancer metagenomics data of ICB-treated patients, we identified several microbial metabolic processes significantly associated with response. Methylerythritol phosphate (MEP) pathway activity was associated with response and intratumoral Vδ2 T cell infiltration and induced Vδ2 T cell-mediated anti-tumor responses in patient-derived tumor organoids. In contrast, the riboflavin pathway was associated with ICB resistance, and its intermediates led to mucosal-associated invariant T (MAIT) cell-mediated immune suppression. Collectively, our results highlight the relevance and complexity of metabolite-mediated microbiome-immune crosstalk.

#2213 Obesity alters cancer-related microbial pathways in women with benign and malignant breast disease.

Jack W. Sample[1], Matteo Redaelli[1], Stephen Johnson[2], Tanya L. Hoskin[3], Marina R. Walther-Antonio[4], Jun Chen[2], Tina J. Hieken[1]

[1]Surgery, Mayo Clinic, Rochester, MN,[2]Quantitative Health Sciences, Mayo Clinic, Rochester, MN,[3]Health Sciences Research, Mayo Clinic, Rochester, MN,[4]Obstetrics and Gynecology, Mayo Clinic, Rochester, MN

Breast cancer is the leading cause of cancer death among women worldwide. Most cases occur in those without identifiable modifiable risk factors, highlighting critical gaps in our understanding of breast cancer biology including the influence of the gut microbiome. Here we studied the gut microbiome with shallow shotgun metagenomic sequencing from preoperative stool samples in a well-characterized clinical cohort of 131 women, median age 59 years, undergoing breast-conserving surgery for benign (n=23), high-risk (n=47), or malignant (n=61) breast disease. Taxonomic profiling was performed with Sourmasth 4.2.4 with GTDBv202 as reference database; functional profiling of metagenomic data employed HUMAnN 3.6 with identified gene families mapped to MetaCyc pathways. Differences in alpha diversity in terms of species richness were observed across diagnosis groups (benign, high-risk, malignant) and was lowest for high-risk lesions (p=0.031). Decreased alpha diversity was also associated with higher body mass index (BMI) (Shannon p=0.12, Inverse Simpson p=0.025). Significant beta diversity differences across diagnosis groups also were seen (Bray-Curtis p=0.044, Jaccard p=0.013) as were differences in association with BMI (Bray-Curtis, Jaccard, both p=0.002). No significant diversity differences associated with age, mammographic breast density or menopausal status were observed. Functional pathway profiling stratified by BMI showed a correlation between increasing BMI and greater relative abundance of genes in nicotinamide adenine dinucleotide (NAD) biosynthesis, folate metabolism, and aromatic amino acid metabolism pathways (p=0.0279, 0.0667 and 0.0860, respectively) - pathways implicated in breast cancer detection, survival and prognosis. Cross-referencing our significantly (FDR <5%) differentially abundant taxonomic findings with these functional pathway data identified enrichment of several *Blautia* species, linked to NAD biosynthesis via tryptophan metabolism, in patients with higher BMI. We also noted enrichment of *Dorea formicigenerans,* a species associated with increased breast cancer risk in obese women due to its pro-inflammatory effects, in individuals with a higher BMI. Here we report significant differences in gut microbiome diversity, taxonomy and functional pathway signals in association with BMI in women with breast disease. Differentially abundant species seen with rising BMI may contribute to functional pathways that drive increased breast cancer risk and may provide an actionable target for risk reduction independent of conventional risk reduction strategies.

#2214 Inhibiting the mevalonate pathway with atorvastatin alters gut microbiota and has potential as an anti-cancer treatment for ovarian cancer.

Juliane M. Liberto[1], Emily Ke[2], Daniel Labbe[2], Sabrene Mechergui[2], Kala Visvanathan[3], Ie-Ming Shih[1], Tian-Li Wang[1]

[1]Pathology, Johns Hopkins School of Medicine, Baltimore, MD,[2]Johns Hopkins University, Baltimore, MD,[3]Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

Statins, a mevalonate (MVA) pathway antagonist, are widely used to treat and prevent hypercholesterolemia by blocking cellular production of cholesterol. However, new research has uncovered that statins exhibit pleotropic anti-tumor and immune modulating effects, as downstream metabolites from the MVA pathway have been linked to several cellular systems commonly dysregulated in cancer patients. Furthermore, statins have been shown to induce significant changes in intestinal enterotypes in both humans and mice. This is uniquely important as studies on the intestinal microbiota have shown a strong correlation between gut health and cancer outcomes. Therefore, we hypothesized that statins could be utilized to therapeutically target pre-clinical models of ovarian cancer by modulating the systemic and tumor immune landscape as well as the intestinal microbiome. Female C57BL/6 mice injected with $5x10^5$ ID8-$BRCA1^{null}$ cells received low-dose atorvastatin (5mg/kg/day, n=14) or DMSO (<2%(v/v)/day, n=14) control for 5 weeks. Histopathology was performed on necropsy tissues, and immune profiling via flow cytometry was performed on splenocytes and cells isolated from ascites to examine the impact of statins on immune cell populations. In a separate study, we administered to female C57BL/6 mice atorvastatin (5mg/kg/day, n=5) or DMSO (<2%(v/v)/day, n=5) control for 4 weeks and sequenced 16S ribosomal RNA in their stool to determine how statin affected intestinal flora. Lastly, to determine if host genetics affect statin-mediate microbial changes, we simultaneously administered atorvastatin (5mg/kg/day) or DMSO (<2%(v/v)/day) control to three commonly used inbred mice strains (C3H/He, n=3; BALB/c, n=3; C57BL/6, n=5) and compared 16S sequencing results across all three strains.
Conclusion: We found that statins, in the absence of standard of care regimens, are able to elicit immune modulatory effects in an aggressive ascitic model of ovarian high-grade serous carcinoma thereby offering moderate improvements to tumor outcomes. Furthermore, we found that statins favorably alter the intestinal microbiome, increasing microbial diversity within the gut and increasing the abundance of beneficial microbiota. Importantly, our data emphasize the role of host genetics in shaping microbiome responses to statin therapy, which could have implications for patient-specific treatment strategies. Overall, these results support further research to evaluate statins' use as a therapeutic or preventive agent for ovarian cancer.

#2215 Age and indoleamine 2,3-dioxygenase 1 (IDO1) interact to affect gut microbiome homeostasis in subjects with glioblastoma.

Lijie Zhai[1], Akriti Shrestha[2], Kristen L. Lauing[1], Manon Penco-Campillo[1], Oluwatomilayo Odum[1], Prashant Bommi[1], Taylor Koch[1], Robert H. McCusker[3], Douglas E. Anderson[4], Anand Germanwala[4], Vikram C. Prabhu[4], Jigisha P. Thakkar[4], Jacob M. Allen[2], Rimas V. Lukas[5], Derek A. Wainwright[6]

[1]Cancer Biology, Cardinal Bernardin Cancer Center, Loyola University Medical Center, Maywood, IL,[2]Health and Kinesiology, Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL,[3]Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL,[4]Neurological Surgery, Loyola University Medical Center, Maywood, IL,[5]Neurology, Northwestern University Feinberg School of Medicine, Chicago, IL,[6]Cancer Biology, Neurological Surgery, Cardinal Bernardin Cancer Center, Loyola University Medical Center, Maywood, IL

Objective: This study aimed to determine how age and IDO1 interact to affect the gut microbiome, microbial metabolite levels, and survival outcomes in subjects undergoing IDO1-targeted immunotherapy against glioblastoma (GBM).
Methods: Serum and colon contents from younger 16-20- or older 92-118-week-old wild-type (WT), IDO1 knockout (IDO1-KO), and IDO1 enzyme null (IDO1-H350A) mice were analyzed for 16S rRNA gut microbiome composition and microbial-derived aromatic amino acid metabolites via LC/MS/MS. Fecal samples from younger 7-8 or older 81-85 week-old WT mice with intracranial syngeneic GL261 GBM cells treated with or without brain radiation (RT) and PD-1 mAb were also analyzed. Fecal and plasma samples from 12 newly diagnosed IDHwt/MGMT promoter unmethylated GBM patients who received RT plus PD-1 mAb and IDO enzyme inhibitor were also analyzed.
Results: Young and old IDO1-KO and IDO1-H350A mice demonstrated a unique gut microbiome signature with an elevated abundance of *Bifidobacterium* and *Helicobacter* ($p<0.05$) and a reduced abundance of *Ventriosum* and *Sireaum* compared to WT mice ($p<0.001$). *Prevotellaceae*, *Muribaculum*, *Alistipes*, *Enterohabdus*, *Clostridia*, and *Akkermansia* were different in IDO1-KO and IDO1-H350A mice compared to WT - but only in older mice. Serum microbiome-derived aryl-lactates phenyllactate (PLA), indolelactate (ILA), and 4-hydroxyphenyllactate (4HPLA) were increased in IDO1-KO mice compared to WT and IDO1-H350A mice ($p<0.001$) - but only in older mice. Older WT mice with GL261 GBM showed lower fecal aryl-lactates (PLA, 4HPLA, and ILA) compared to younger counterparts. Treatment with RT + PD-1 mAb also showed decreased serum indole propionic acid (IPA) levels in young but not old mice with GL261 GBM ($p<0.05$). Analyses from GBM patient baseline fecal samples revealed that higher abundance of *Massilioclostridium coli*, G*GB3819-SGB5184*, *Dysosmobacter welbionis*, and *Phocaeicola plebeius* was associated with longer survival ($p<0.004$), while higher levels of fecal ILA and lower levels of plasma IPA also correlated with elongated survival ($p<0.04$ and $p<0.01$ respectively). Combined treatment of RT + PD-1 mAb + IDO1 inhibitor decreased plasma IPA when compared to baseline ($p<0.01$).
Conclusions: IDO1 possesses both enzyme- and non-enzyme-dependent effects that change gut microbiota composition and bioactive microbial aromatic amino acid metabolite levels, which is also influenced by aging. Certain gut microbiota species and metabolites may serve as prognostic markers for predicting outcomes to immunotherapy for GBM. These findings warrant further investigation into how IDO1 interacts with age to affect immunotherapeutic efficacy in subjects with GBM.

#2216 Modulating gut microbiota to enhance the efficacy of immunotherapy in triple negative breast cancer.

Samarpan Majumder[1], Manisha Poudel[1], Fokhrul Hossain[1], Luis Del Ville[1], Lucio Miele[1], Justin Brown[2]

[1]LSU Health New Orleans, New Orleans, LA,[2]Pennington Biomedical Research Center, Baton Rogue, LA

Background: Triple-negative breast cancer (TNBC) is the most aggressive form of breast cancer and is diagnosed more frequently in younger, premenopausal women. Obesity is a known risk factor for increased TNBC incidence. The association of gut dysbiosis with obesity is now established. Immune checkpoint blockade with anti-PD-1 is currently the standard of care for early TNBC. Evidence suggests modulating the gut microbiome enhances cancer immunotherapy efficacy in a few cancer types. However, there are no studies on early TNBC.
Overarching Challenge: The number of patients with early TNBC who benefit from anti-PD-1 therapy remains suboptimal.
Objective: Our study aims to enhance the efficacy of anti-PD-1 therapy by modulating the gut microbiota in a mouse model of obesity and TNBC. We hypothesize that a probiotic supplement will enhance the efficacy of anti-PD-1 therapy.
Methods: We determined the efficacy and safety of two weeks of probiotic supplementation before anti-PD-1 therapy on objective response rates in an obese mouse model of triple-negative breast cancer. This was evaluated by measuring the objective response rate (tumor burden) after two weeks of anti-PD-1 treatment. FVB female obese mouse model and authenticated TNBC C0321 claudin low mouse tumor cells (In Vitro Technologies) were used for engrafting tumor. We used a probiotic formulation from Creative Enzyme which is a blend of 13 human probiotic strains (Cat # PRBT-035, NY).
Results: One way ANOVA comparing three groups of mice (anti-PD1, anti-PD-1+ serotype and probiotics + anti-PD1) produced a statistically significant outcome confirming our prediction that probiotics positively affect anti-PD1 therapy. A Kaplan-Meier survival plot based on our record of mouse survival over the course of the treatment demonstrates that only probiotics plus anti-PD1 therapy could provoke statistically significant survival benefit over isotype control ($p=0.046$) in our study. Analysis of cytokine profile revealed elevated level of interferon-γ and downregulation of proinflammatory cytokines in plasma from mice treated with probiotics only. Furthermore, we observed increased CD8+T and CD4 +T cells in the tumor tissue of probiotics treated mice when compared to untreated mice in our Immunohistochemistry analysis. For feasibility study in human subjects, our pilot study is recruiting volunteers (NCT06318507) to confirm whether the fecal microbiome differs between patients with and without obesity and early TNBC and who achieve pCR from preoperative anti-PD-1 therapy.
Impact: The study provides critical experimental data to demonstrate causality and feasibility to justify larger-scale interrogations to modulate the gut microbiome in women with early TNBC who plan to begin anti-PD-1 therapy.
Innovation: This study is the first to determine how a probiotic supplement can enhance anti-PD-1 efficacy in early TNBC.

#2217 Commensal bacteria are internal triggers of chronic inflammation and B-cell lymphoma development in myeloid cell-specific TRAF3-deficient mice.

Jaeyong Jung, Sining Zhu, Almin I. Lalani, Judith Shakarchi, Brygida Matracz, Guojun G. Wu, Wei-Xing Zong, Liping Zhao, **Ping Xie**

Rutgers University - New Brunswick, Piscataway, NJ

Myeloid cells are central players in innate immunity and inflammation. Their function is regulated by the adaptor protein TRAF3. We previously reported that aging myeloid cell-specific TRAF3-deficient (M-*Traf3*$^{-/-}$) mice spontaneously develop chronic inflammation and B-cell lymphoma (BCL). In the present study, we aimed to identify the internal trigger of this disease phenotype in these mice. We first detected gut dysbiosis and transmigration of commensal bacteria to the liver in aging M-*Traf3*$^{-/-}$ mice. Interestingly, depletion of commensal bacteria using antibiotics effectively prevented chronic inflammation and BCL development in these mice. Systemic IgG responses against commensal bacteria were induced and the IgH CDR3 sequences of malignant B-cell clones of M-*Traf3*$^{-/-}$ mice showed high homology to prevalent bacteria-reactive Ig clonotypes. Furthermore, M-*Traf3*$^{-/-}$ mice with BCL exhibited high titers of antibodies against commensal bacteria in their serum. Together, our findings suggest that commensal bacteria dysbiosis and transmigration can promote chronic inflammation and BCL development in individuals with compromised innate immunity. Accordingly, targeting relevant commensal bacteria may serve as a therapeutic strategy for preventing and treating chronic inflammation and BCL in these patients.

This study was supported by the National Cancer Institute Grant R01CA293055, the National Institute of Allergy and Infectious Diseases Grant R21AI128264, a HealthAdvance Award, the Grossman Innovation Prize, and a New Jersey Commission on Cancer Research (NJCCR) grant DCHS19CRF005.

#2218 Prognostic relevance of immune phenotyping of the tumor immune microenvironment and its interactions with the mammary microbiota in Ethiopian breast cancer patients.

Meron Yohannes Nigussie[1], Zelalem Desalegn[2], Marcus Bauer[3], Kathrin Stuckrath[4], Endale Anberbir1[5], Yonas Bekuretsion[6], Mathewos Assefa[7], Tariku Wakuma[8], Yasin Worku[9], Pablo S. C. Santos[10], Lesley Taylor[11], Adamu Adissie[12], Claudia Wickenhauser[3], Chiara Massa[13], Martina Vetter[14], Eva J. Kantelhardt[15], Barbara Seliger[13], Tamrat Abebe[16]

[1]Department of Medical Laboratory Science, Addis Abada University, Addis Ababa, Ethiopia,[2]Department of Microbiology Immunology and Paraistology, Addis Abada University, Addis Ababa, Ethiopia,[3]Institute of Pathology, Marthin Luther University, Halle, Germany,[4]5University Clinic and Polyclinic for Gynecology, Marthin Luther University, Halle, Germany,[5]Department of Surgery, Addis Abada University, Addis Ababa, Ethiopia,[6]Department of Pathology, Addis Abada University, Addis Ababa, Ethiopia,[7]Department of Oncology, Addis Abada University, Addis Ababa, Ethiopia,[8]Aira Hospital, Aira, Ethiopia,[9]Department of Oncology, Wollo Univeristy, Dessie, Ethiopia,[10]Global and Planetary Health Working Group, Institute of Medical Epidemiology, Biometrics, Marthin Luther University, Halle, Germany,[11]Department of Medical Laboratory Science, City of Hope National Medical Center, Duarte, CA,[12]School of Public health, Addis Abada University, Addis Ababa, Ethiopia,[13]Institute of Medical Immunology, Marthin Luther University, Halle, Germany,[14]University Clinic and Polyclinic for Gynecology, Marthin Luther University, Halle, Germany,[15]Global and Planetary Health Working Group, Institute of Medical Epidemiology, Biometrics and Inform, Marthin Luther University, Halle, Germany,[16]Department of microbiology Immunology and Parasitology, Addis Abada University, Addis Ababa, Ethiopia

Background: Breast cancer (BC) is a highly heterogeneous disease, and refining additional clinical parameters is crucial for its effective management. The tumor immune microenvironment, influenced by factors such as infiltrating intratumoral microbiota, has been shown to play a key role in cancer prognosis and treatment response.
Methods: RNA was isolated from formalin-fixed paraffin-embedded (FFPE) tissue from 82 women with BC. Expression of 54 immune genes was analysed using Nanostring and differentially expressed genes (DEGs) were determined using ROSALIND®. Immune cell populations were profiled using Nanostring's cell type profiling module, while tumour infiltrating lymphocytes (TILs) were assessed via haematoxylin and eosin (H&E) staining. For microbiome profiling, DNA was isolated from 29 biopsy samples using MoBio PowerMag Soil DNA Isolation kit and sequenced with an Illumina MiSeq. In addition, the PIK3CA gene was genotyped for three hotspot mutations using qPCR. Log-rank test was performed to evaluate the prognostic significance of immune phenotypes.
Results: Hierarchical clustering of the gene expression profiles revealed four discrete immune phenotypes (IP1-4) characterized by differential expression of immune-activating and immune-regulatory genes. An immunologically inert phenotype (IP2) was identified, marked by consistent downregulation of both immune suppressive and immune activating genes, alongside the lowest levels of immune cell infiltration. In contrast, IP4 exhibited an immunologically active TME, characterized by upregulation of key cytotoxic genes and the highest abundance of immune cells. IP1 and IP3 had intermediate characteristics. A significant association was found between IPs and BC subtypes: luminal A tumors predominated in IP2, while basal BC tumors were most common in IP3. No significant differences in PIK3CA mutations were observed across the IPs. Importantly,
IPs with an active TME were associated with improved overall survival compared to those with an inert immune environment.Microbiota diversity and abundance did not show significant differences across the IPs. However, microbiota composition differed significantly between TIL groups. Notably, the *Firmicutes* and *Verrucomicrobia* phyla, as well as the *Lactobacillaceae, Akkermansiaceae* and *Caulobacteraceae* families, were more abundant in intermediate TIL groups. At the genus level, *Delftia* was significantly enriched in the high TIL group ($P < 0.0001$), while *Akkermansia* and *Cutibacterium* were more abundant in the intermediate TIL groups.
Conclusion: Immune phenotyping of the TME identified distinct immune subgroups that are associated with prognosis and could guide treatment selection. Additionally, potential immune-microbiome interactions were observed, suggesting a role in modulating anti-tumor immunity.

#2219 Clinical response to immune checkpoint inhibitors in melanoma is associated with distinct gut bacterial species that promote anti-tumor immunity by different mechanisms.

Matthew James Robinson[1], Laure Castan[1], Fernanda Schreiber[1], Clelia Villemin[1], Chiara Berlato[1], David J. Adams[2], Pippa Corrie[3], Tim Sharpington[1], Trevor D. Lawley[4], Ghaith Bakdash[1]

[1]Microbiotica, Cambridge, United Kingdom,[2]Experimental Cancer Genetics, Wellcome Sanger Institute, Cambridge, United Kingdom,[3]Oncology Department, Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom,[4]Host-Microbiota Interactions, Wellcome Sanger Institute, Cambridge, United Kingdom

The gut microbiome of cancer patients impacts the response to immune checkpoint inhibitor (CPI) therapy. Using high precision microbiome profiling, we have recently identified 9 diverse species of gut commensal bacteria that are all consistently and strongly associated with CPI response across 4 independent cohorts of melanoma patients. These 9 species represent all 3 of the major phyla in the human intestinal microbiome, and include 4 novel species. A consortium of 9 strains, each representing 1 of these species, is being developed as a live biotherapeutic product (LBP), MB097. In the MCA-205 syngeneic tumor model, MB097 demonstrated synergistic tumor growth inhibition in combination with anti-PD1. The mechanisms by which each of the 9 species exert their anti-tumor effects were investigated, using in vitro assays with primary human immune cells. The bacteria strongly activate dendritic cells (DCs) inducing interleukin (IL)-12. These DCs stimulated by the bacterial strains very potently triggered $CD8^+$ Cytotoxic T Lymphocytes (CTLs) to upregulate Granzyme B and interferon (IFN)γ and kill tumor cells in vitro. Similarly, bacteria-stimulated DCs prompted natural killer (NK) cell activation, as demonstrated by increased expression of IFNγ and elevated capacity for killing tumor cells. DC stimulation by 3 of the 9 strains markedly skewed the cytokine profile to higher IL-12 and lower IL-10, and also led to particularly potent CTL and NK cell activation. These strains represent 3 different families of Firmicutes. Blocking IL-12 reversed the activation and tumor killing effect of DCs stimulated with these strains of Firmicutes. Moreover, the bacterial metabolites derived from 4 different strains triggered CTLs to upregulate the expression of granzyme B and IFNγ and kill tumor cells in vitro. In summary, we have identified 9 gut commensal bacteria as being consistently associated with response to CPI in metastatic melanoma. These bacteria can interact directly with DCs to potently activate CTLs and NK Cells, and some strains of Firmicutes do this via induction of IL-12. Other strains also release metabolites that directly enhance CTL activation. These data begin to identify the different mechanisms by which gut commensal bacteria drive immunotherapy response in human systems. MELODY-1 (NCT06540391) is an ongoing interventional phase 1b study to evaluate MB097 in combination with pembrolizumab in advanced melanoma patients.

#2220 Gut microbiome impacts the tumor microenvironment and enhances the effectiveness of anti-PD1 therapy in head and neck cancer.

Po-Han Lin[1], Ya-Hui Chen[1], Chi-Maw Lin[1], Jessica Tien Yu Ho[2], Chih-Hao Yu[1], Chen-Yi Wang[1], Yuan-Chia Chu[1], Chun-Yu Liu[3], Ying-Cheng Chiang[1], Ruey-Long Hong[1]

[1]National Taiwan University Hospital, Taipei City, Taiwan,[2]National Taiwan University, Taipei City, Taiwan,[3]Taipei Veterans General Hospital, Taipei City, Taiwan

Although the Food and Drug Administration has approved anti-PD1 as an effective therapy for recurrent head-and-neck squamous cell carcinoma (HNSCC), its response rate remains limited to approximately 10-30%, and the prognosis for patients is still poor. In this study, we seek to enhance the therapeutic efficacy of anti-PD1 for recurrent HNSCC. First, we compared the pathology of responders and non-responders to pembrolizumab treatment for HNSCC. Immunohistochemical staining revealed a significantly higher abundance of CD8 T cells in responders, and RNA sequencing, through Gene Set Enrichment Analysis (GSEA), demonstrated a significant activation of CD8 T cell pathways in responders compared to non-responders. Second, 16S full-length sequencing of stool DNA identified an enrichment of Eggerthella lenta (E. lenta) in responders. Furthermore, the presence of E. lenta was positively correlated with CD8 T cell activation and cytotoxic activity. Third, to assess the impact of E. lenta on the anti-tumor response to anti-PD1 therapy in vivo, we implanted HNSCC tumors subcutaneously in antibiotic-induced microbiome depletion mice. The mice were treated with anti-PD1 monoclonal antibody and oral E. lenta administration, resulting in significant tumor regression compared to that of the controls (no treatment), anti-PD1 alone, or E. lenta alone. The excised xenograft HNSCC tumors were analyzed by flow cytometry, revealing that the combination of anti-PD1 therapy and oral E. lenta synergistically increased tumor-infiltrating CD8 T cells and enhanced their activation, as evidenced by elevated interferon-gamma and TNF-alpha expression. Metabolomic analysis identified changes in fatty acid pathways linked to CD8 T cell activation, E. lenta administration, and responders. Our results suggest that E. lenta enhances the response to anti-PD1 therapy, offering new insights into the connection between the microbiome and cancer immunotherapy.

#2221 Obesity influences the tissue resident breast microbiota impacting complement signaling and tumorigenesis.

Mohamed Gaber[1], Adam S. Wilson[1], Adam J. Katz[2], Karen Haas[3], Katherine L. Cook[1]

[1]Department of Cancer Biology, Wake Forest University School of Medicine, Winston-Salem, NC,[2]Department of Plastic and Reconstructive Surgery, Wake Forest University School of Medicine, Winston-Salem, NC,[3]Department of Microbiology and Immunology, Wake Forest University School of Medicine, Winston-Salem, NC

Obesity is a risk factor for breast cancer, influencing both the incidence and prognosis of the disease. Obesity alters the breast microbiome in ways that may promote breast carcinogenesis, and microbiome perturbations are evident in breast cancer patients. Yet, it is unclear if these microbial perturbations are drivers of breast cancer. Our group previously showed that diet and anti-cancer therapies modify the breast tissue microbiome. We further showed that certain commensal bacterial species in the mammary gland reduce tumorigenesis in high-fat diet-fed MMTV-PyMT mice, implicating tissue-resident microbiota in modifying breast cancer risk. Thus, we investigated the impact of obesity-induced microbiome alterations on genomic instability in mammary epithelial cells, as a hallmark of breast cancer risk. Our preliminary studies showed that obesity caused elevated systemic and breast tissue levels of lipopolysaccharide (LPS) and flagellin. *In vitro* and *in vivo* experiments demonstrated their ability to induce DNA damage in the mammary epithelium. 16S rRNA sequencing on DNA isolated from non-cancerous breast tissue revealed an enrichment of Proteobacteria in obese premenopausal subjects. Bacteria from this phylum harbors highly immunogenic LPS and the majority of them are flagellated. Importantly, Proteobacteria proportional abundance was associated with breast tissue levels of DNA damage. Intriguingly, these associations were absent in postmenopausal women. One key difference between breast tissues of the two menopausal groups was in their complement activity and immune responses to LPS and flagellin. Redox proteomics analysis showed that obesity caused an enrichment in the oxidation of several complement C3 peptides in both menopausal groups. Normally, oxidation of complement C3 causes its activation through the generation of a C3 convertase that cleaves C3 into the active fragments; C3a and C3b. However, this was only observed in premenopausal women where the C3 oxidized peptides showed significant correlations with C3a levels. Complement system dysfunction in postmenopausal women was also manifested by having lower C3a levels, higher B-cell trafficking and lower anti-LPS IgA levels in their breast tissues than premenopausal women. Ongoing experiments are being performed to determine if there are breast tissue differences in complement regulatory proteins such as factor H and CD59. To demonstrate causality, we performed a carcinogen-induced mammary tumorigenesis model in wild-type (WT) and C3 knockout (KO) mice fed a low-fat or high-fat diet. C3 KO mice showed increased tumor-incidence compared to WT in the context of a high-fat diet, but not a low-fat diet, suggesting a role of complement signaling in anti-tumor immunity in the context of obesity. Overall, this work identifies a novel immunity-microbiome signaling axis potentiating obesity-mediated breast cancer risk.

#2222 Trimethylamine-N-oxide (TMAO) mediates immunotherapy resistance in advanced HCC.

Mari Nakazawa, Vivian L. Raj, NV Rajeshkumar, Noushin Rastkari, Michael Davis, Elizabeth De Oliveria, Madelena Brancati, Ervin Griffin, Kabeer Munjal, James Leatherman, Sarah Mitchell, Marina Baretti, Won Jin Ho, Mark Yarchoan, Chi V. Dang

Oncology, Johns Hopkins University School of Medicine, Baltimore, MD

Recent approvals of immune checkpoint inhibitor (ICI) based regimens have transformed the clinical landscape of advanced hepatocellular carcinoma (HCC), however, clinical responses are only observed in a minority of patients. Trimethylamine-N-oxide (TMAO) is an amine oxide biosynthesized from trimethylamine (TMA), generated when gut bacteria metabolize dietary choline via the enzyme CutC. While prior studies have shown that TMAO concentrations positively correlate with the risk of several gastrointestinal malignancies, newer studies have demonstrated that TMAO may enhance antitumor immunity in some tumor types. TMAO may therefore play an important role in modulating the tumor immune response, but its significance in HCC is unknown.

We prospectively collected whole blood from patients with HCC prior to initiating standard-of-care ICI-based therapy at Johns Hopkins (IRB #00267960). Clinical records were reviewed for demographic characteristics and clinical outcomes. Additionally, we studied the impact of acute and chronic choline supplementation on anti-PDL1 efficacy in a syngeneic murine model of HCC (Hep53.4 in C57BL/6 mice). Baseline choline, TMA, and TMAO plasma concentrations in clinical and preclinical specimens were quantified via liquid chromatography-mass spectrometry (LC-MS) utilizing the SCIEX Triple Quad 6500+ system.

The clinical cohort included 42 patients with advanced HCC. Patients were predominantly male (81.0%) with Barcelona Clinic Liver Cancer (BCLC) stage C disease (73.8%) who underwent treatment with atezolizumab + bevacizumab (52.4%) or durvalumab + tremelimumab (31.0%). In a multivariable Cox model, higher plasma TMAO concentration at baseline was associated with inferior progression-free survival (PFS; HR 1.36 per interquartile range [IQR] increase, $p=0.032$) and overall survival (OS; HR 1.63 per IQR increase, $p<0.001$) after controlling for BCLC stage, albumin-bilirubin index, alpha fetoprotein (AFP) concentration, and baseline choline and TMA concentrations. In our preclinical model, mice given chronic choline supplementation (1% choline diet for 14 days prior to treatment with anti-PDL1 therapy) had increased plasma TMAO concentrations ($p<0.001$) and exhibited no response to anti-PDL1 therapy, compared to mice given standard and acute choline (1% choline diet starting concurrently with anti-PDL1 treatment) diets.

Our clinical and preclinical results suggest that high plasma TMAO concentration is associated with inferior outcomes on ICI therapies in HCC. These findings contrast with existing preclinical studies that suggest TMAO may potentiate responses to ICI therapy. We hypothesize that chronic TMAO exposure may lead to immune cell exhaustion via promotion of an inflammatory microenvironment and oxidative stress. Studies examining the effect of CutC inhibition are ongoing in our laboratory and may provide further rationale for targeting TMAO in HCC.

#2223 Phase I clinical trial combining camu camu prebiotic enriched with castalagin modulates bile acids and metabolites in combination with cancer immunotherapy in patients with treatment-naïve lung cancer and PD-1 refractory melanoma (NCT05303493).

Jade Maillou[1], Reilly Pidgeon[2], Diogjena Katerina Prifti[1], Meriem Messaoudene[1], Sreya Duttagupta[1], Myriam Benlaifaoui[1], Yongjia Hu[1], Alyse Filin[1], Mayra Ponce[1], Wiam Belkaid[1], Julie Malo[1], Guido Kroemer[3], Sylvere Durand[3], Olivier Barbier[4], Nathalie Daaboul[5], Normand Blais[1], Mustapha Thefe[1], Marie Florescu[1], Rahima Jamal[1], Wilson Miller[6], Antoine Desilets[1], Bastien Castagner[2], Arielle Elkrief[1], Bertrand Routy[1]

[1]CHUM Research Centre, Montreal, QC, Canada,[2]McGill University, Montreal, QC, Canada,[3]Institut Gustave Roussy, Paris, France,[4]CHUQ Research Centre, Laval University, Quebec, QC, Canada,[5]Charles Lemoyne Hospital, Sherbrooke University, Montreal, QC, Canada,[6]Jewish General Hospital, Montreal, QC, Canada

Introduction: Several strategies to improve immune checkpoint inhibitors (ICI) activity are under evaluation. Pre-clinical murine studies have shown the potential of castalagin, a prebiotic polyphenol extracted from Camu Camu (CC) berry to beneficially shift the microbiome and metabolites leading to enhanced ICI response. We tested CC, an approved natural product, in a trial combined with ICI in melanoma (MM) and non-small cell lung cancer (NSCLC).

Methods: We completed a phase I trial combining 1.5g daily of CC in oral capsules, started 7 days prior ICI initiation and continued for 3 months. The study included 2 arms: (1) advanced treatment-naïve NSCLC patients (pts) with PD-L1 <50% receiving anti-PD-1 and platinum doublet chemotherapy (chemo), and (2) ICI-refractory MM pts rechallenged with ICI. The primary endpoint was safety, the secondary endpoint included disease control rate (DCR; rate of partial response (PR), complete response, and stable disease (SD)). Exploratory analyses focused on CC's impact on the microbiome composition and HPLC analysis for metabolites with particular focus on bile acid (BA) production. In parallel, murine experiments with supplementation of castalagin were conducted and *in vitro* assays were performed to explore its link to the BA pathway.

Results: Of the 29 pts enrolled, none experienced AE related to CC alone. Among 14 NSCLC pts receiving CC+ICI+chemo, incidence of grade 3 adverse events (AE) was 29%, the DCR reached 43% (all PR), with 0 patient experiencing primary progressive disease. In the refractory MM cohort, incidence of grade 3 AE related to CC+ICI was 16%. Among the 12 evaluable pts, the DCR was 35% with 1 achieving a PR, while 2 had durable SD over an 18-month period. We measured BA concentrations in the feces of NSCLC pts and observed a rise in both primary and conjugated BA post-CC. In addition, metabolomic analysis revealed higher phenylbutyric acid level in the plasma of NSCLC. In MM, conjugated BA were enriched in the plasma post-CC. Similarly, administration of castalagin in a murine model resulted in elevated levels of DCA and UDCA in feces and serum. In mice, the modulation of FXR (main BA receptor) inhibited castalagin antitumor activity, highlighting its reliance on BA pathway. Moreover, stimulation of liver cells with urolithins metabolites of castalagin, induced the upregulation of key BA transporters *Ostα/β*, *Tgr5* receptor and *Cyp8b1* enzyme.

Conclusion: Administration of CC showed no increase in toxicities with encouraging preliminary efficacy to ICI in pts mainly in the setting of refractory MM. Ongoing analyses aim to identify how microbial signature can shift BA pool and their functional role in clinical responses. These results position castalagin as a novel experimental therapeutic target in immuno-oncology.

#2224 Impact of NT/NTR1 signaling on metabolic pathways and tumor-associated microbiomes in colorectal cancer.

Haoming Wu, Yang Wang, Moumita Banerjee, Jinpeng Liu, Chi Wang, Dahe He, Therese Bocklage, Jing Li, B.Mark Evers

Markey Cancer Center, Lexington, KY

Background: Colorectal cancer (CRC) is a leading cause of cancer-related deaths worldwide with substantial heterogeneity in patient outcomes. Neurotensin (NT) and its high-affinity receptor (NTR1) have been implicated in CRC progression, particularly in modulating tumor metabolism and immune responses. NT/NTR1 expression has been linked to CRC progression and outcomes, while gut microbiota is increasingly recognized for its role in cancer development and therapy. The purpose of current study was to explore the relationship between NT/NTR1 expression, microbial communities, and transcriptomic changes in tumor versus adjacent normal tissues, focusing on their role in shaping tumor-associated microbiomes and influencing cancer progression.
Methods: We performed: (i) RNA sequencing of tumor samples from 96 Appalachian Kentucky CRC patients to assess NT/NTR1 expression and its association with transcriptomic pathways; (ii) paired RNA sequencing and 16S rRNA-based microbiome profiling from tumor and adjacent normal tissues of 15 patients to evaluate transcriptomic and microbial differences. Functional gene prediction was performed using Picrust2 based on 16S rRNA profiles, and differential expression analysis was conducted using DESeq2. NT/NTR1 expression and bacterial abundance were validated by qPCR.
Results: (i) RNA sequencing of the 96-patient cohort showed that high NT/NTR1 expression was associated with dysregulation of multiple metabolic pathways (e.g., lipid, carbohydrate, and amino acid metabolism) and immune-related pathways (e.g., "immune disease" and "infection disease: bacteria"). (ii) In paired tumor and adjacent normal tissues, NT/NTR1 expression was significantly elevated in tumor tissues. Microbial profiling revealed higher microbial abundance and diversity in tumor tissues, with notable enrichment of *Campylobacter* and *Chlamydia* species. Functional gene prediction using Picrust2 identified significant upregulation of flagellar assembly-related genes, such as K13626, in tumor microbiomes compared to normal tissues ($P = 3.66 \times 10^{-3}$). These findings suggest enhanced microbial motility and interaction in the tumor microenvironment facilitated by NT/NTR1-driven metabolic changes.
Conclusions: NT/NTR1 signaling remodels metabolic pathways in CRCs, enhances nutrient absorption and creates a more favorable environment for microbial colonization. The observed enrichment of motility-related bacterial species and functional genes highlights the role of the NT/NTR1 signaling pathway in promoting tumor-microbe interactions. Importantly, these findings provide potential avenues for CRC diagnosis and therapeutic strategies targeting the NT/NTR1 axis.

#2225 Diet with high soluble fiber induces favorable gut microbiota and metabolomic profiles, increases anti-tumor immunity, and suppresses lung cancer progression.

Manvi Sharma[1], Yuejiang Liu[1], Altai Enkhbayar[2], Sujuan Yang[1], Inti C. T. Reischle[1], Mohamed A. Jamal[1], Tieling Zhou[1], Zahraa Rahal[1], Lorena I. Gomez B.[1], Hannah Moreno[3], Matthew C. Ross[3], Lin Tan[1], Chidera O. Chukwuocha[1], Eleeka Nia[4], Anusha S. Thomas[1], Jennifer A. Wargo[1], Nadim J. Ajami[1], Philip L. Lorenzi[1], Robert R. Jenq[2], Seyed Javad Moghaddam[1], Kristi L. Hoffman[3], Humam Kadara[1]

[1]UT MD Anderson Cancer Center, Houston, TX,[2]City of Hope, Duarte, CA,[3]Baylor College of Medicine, Houston, TX,[4]University of Houston, Houston, TX

Accumulating evidence suggests that taxonomic composition and phenotypic traits of the gut microbiome, which acts as a central regulator of cancer progression and response to immunotherapy, are influenced by diet. Prior studies have demonstrated that dietary constituents, notably soluble fibers, stimulate probiotic proliferation in the gut, maintain integrity of the intestinal barrier, and modulate immune responses. Yet, mechanisms by which a high soluble fiber diet (HSFD) impacts the gut-immune axis and pathogenesis of cancers external to the gut are poorly defined. Here, we employed a human-relevant mouse model in which knockout of G-protein coupled receptor 5A (*Gprc5a*$^{-/-}$) and exposure to tobacco lead to development of preneoplasia and lung adenocarcinoma. Mice were randomized in dietary treatments: no fiber, high insoluble fiber, or HSFD, administered either post-weaning (early life) or after transplant of lung cancer cells (prophylactic intervention). Tumor growth was most significantly reduced in HSFD-fed mice, with notable anti-tumor effects observed in both early life and prophylactic intervention settings. These effects were recapitulated in an independent syngeneic model and tobacco carcinogen-exposed *Gprc5a*$^{-/-}$ mice. 16S rRNA-Seq analysis revealed HSFD-induced enrichment of beneficial taxa including *Bacteroides*, *Faecalibaculum*, *Akkermansia,* and *Bifidobacterium,* alongside evident decrease in tumor-promoting genera such as *Odoribacter* and *Alistipes*, with some of these microbial changes correlated with dietary fiber intake in cancer-free individuals. These microbial shifts corresponded with elevated levels of key gut metabolites, notably butyrate. Flow cytometry of colonic lamina propria revealed changes in immune profiles, characterized by reduced pro-IL-1β^{+} macrophages and dendritic cells, with parallel systemic effects evidenced by decreased inflammatory cytokines in sera. HSFD also skewed the tumor microenvironment toward an antitumorigenic milieu, marked by expanded B lineage cells, cDC1s and M1-like macrophages and, conversely, decreased levels of activated cDC2s and M2 macrophages. Fecal microbiota transplant models showed that effects of HSFD were largely mediated by the gut microbiome as mice that received fecal microbiota from syngeneic HSFD-fed donors showed significant tumor inhibition compared to littermates receiving microbiota from no-fiber-fed donors. Moreover, butyrate supplementation in a fiber-free diet mimicked the anti-tumor effects of HSFD, as reflected in gut immune populations, tumor metabolomics and systemic cytokines. Our findings inform on novel mechanisms by which HSFD robustly impacts gut consortia and immune milieu in the inhibition of lung cancer, suggesting that incorporation of HSFD in cancer treatments could improve outcomes for patients with lung cancer.

IMMUNOLOGY: Modulation of Tumor Microenvironment: Enhancing Immunogenicity and Counteracting Suppression Poster Session

#2230 Persistent elimination of tumors via balanced regulation of immune signals.

Xiaodong Xiao[1], Lei Han[2], Yuan Yuan[3], Hua Jiang[1], Yueqing Xie[1], Jianwei Zhu[1]

[1]Jecho Laboratories, Frederick, MD,[2]Jecho Institute, Shanghai, China,[3]School of Pharmacy, Shanghai Jiao Tong University, Shanghai, China

Despite its success, there are still numerous unmet clinical needs in cancer immunotherapy. Many patients failed to respond due to the lack of effective and properly regulated T cell stimulation. Molecules capable of inhibiting multiple immune checkpoints, enhancing the co-stimulatory signals on T cells to “revive” the tumor antigen-specific T cell response, as well as sustaining the responses to prevent the recurrence of the tumor are highly desirable. Here we report a Fc-based, tripartite, multifunctional fusion protein JLM019 that has been rationally designed to integrate the multiple mechanisms of PD-1/PD-Ligands checkpoint inhibition and T cell co-stimulations through CD80-mediated signaling. Fused at the C-terminus of the Fc fragment, structure-guided mutations A132L and Loop15 were introduced into the human PD-1 ectodomain to improve affinity with its ligands. Fused at the N-terminus, the wild-type CD80 provided a canonical co-stimulatory signal compared to hyperreactive anti-CD28 agonistic antibodies. Combined effect of PD-1 signaling blockade and balanced T cell activation through CD80 binding with CTLA4/CD28 by JLM019 led to persistent elimination of wide spectrum of tumors, including tumors with no PD-L1 expression, or those resistant to conventional PD-1 checkpoint inhibitors in CDX models. JLM019 acts not only on the tumor but also through activating tumor-specific T cells that depend on other APCs and/or through PD-L2 signaling. Significantly, JLM019 treatment induced effective immune memory, which was confirmed by experiments using transferred splenocytes from cured mice that not only prevented tumor initiation but also eliminated tumors in the established tumor-bearing mice. The sophisticated mechanism of the action by JLM019 is explored and discussed. In summary, we developed an immune signal regulator JLM019 that can modulate immune signaling against advanced tumors in a potent yet well-balanced manner. Pre-clinical toxicity and PK/PD studies in cynomolgus monkey have shown a promising safety and toxicity profile, with a dose up to 100mg/kg being tolerated. These encouraging results prompted us to advance JLM019 for clinical studies in humans.

#2231 CY-101 inhibits the tumor growth and improves anti-PD-1 immunotherapy in beta-catenin-driven tumors.

M. Poussard[1], M. Noman[1], K. Van Moer[1], J. Hossain[2], R. Gao[1], E. Solel[2], F. Grego[3], L. Prestegarden[3], R. Bjerkvig[2], **B. Janji**[1];
[1]Luxembourg Institute of Health, Luxembourg City, Luxembourg, [2]University of Bergen, Bergen, Norway, [3]Cytovation AS, Bergen, Norway

CY-101 is a bi-functional peptide with a dual mechanism of action. It selectively targets cancer cell membranes, forming pores that release neoantigens to the tumor microenvironment, thereby triggering a robust anti-tumor immune response. In addition, CY-101 inhibits the oncogenic Wnt/beta-catenin signaling pathway, which is frequently dysregulated in several cancers such as colorectal carcinoma (CRC), adrenocortical carcinoma (ACC), and melanoma. Given the high prevalence of beta-catenin mutations in these tumors, targeting this pathway offers significant therapeutic potential.
We show that CY-101 induces a dose-dependent cell death across mouse and human beta-catenin-driven CRC, ACC, and melanoma cell lines *in vitro* following suppression of the Wnt/beta-catenin pathway. In preclinical animal models, CY-101 elicited a potent anti-tumor activity by completely eradicating ACC tumors and significantly enhancing the efficacy of anti-PD-1 therapy in CRC and melanoma models. Notably, CY-101 treatment reshaped the tumor immune landscape by modulating cytokine expression, leading to increased infiltration of cytotoxic CD8+ T cells. Mechanistically, CY-101-treated tumors exhibited downregulation of Wnt/beta-catenin downstream target genes, emphasizing its role as a potent Wnt/beta-catenin inhibitor.
These preclinical findings support the observations seen in the first in human CICILIA Phase 1/2a trial, which reported clinical activity in ACC and melanoma patients. Collectively, our study demonstrates the therapeutic potential of CY-101 in beta-catenin-driven tumors, both as monotherapy and in combination with anti-PD1 checkpoint inhibitors.

#2232 BGB-R046, an IL-15 pro-drug demonstrates robust pharmacodynamic effects and immune activation within tumor microenvironment in a mouse syngeneic model.

Yu Jiang[1], Amin Zhang[2], Minjuan Deng[1], Feihong Li[1], Qinyi Kong[2], Silu Liu[1], Xiaoyu Li[1], Li Luo[2], Yue Wu[1], Mengjia Wang[1], Xudong Luan[1], Huixia Hu[1], **Xin Chen**[3], Wei Jin[1], Zhirong Shen[1]

[1]BeiGene (Beijing) Co., Ltd., Beijing, China,[2]BeiGene (Shanghai) Biotechnology Co., Ltd., Shanghai, China,[3]BeiGene USA, Inc., Ridgefield Park, NJ

Background: Interleukin-15 (IL-15) is crucial for the proliferation and survival of NK and memory T cells, and has great potential in cancer immunotherapy. Rapid plasma clearance of conventional IL-15 and therefore insufficient tumor exposure and immune activation pose a significant limitation in its development as an immuno-oncology therapeutics. BGB-R046 is developed as an IL-15 pro-drug, which masks free drug release in circulation and is activated in tumors by utilizing tumor enriched proteases to release active IL-15Rα-sushi-IL-15, an IL-15 super-agonist(SITC#950). BGB-R046 induced pharmacodynamic effects and immune activation was evaluated in this study, in immune competent mice bearing syngeneic tumor model, to study its pharmacodynamic effects in both circulation and tumor.

Method: Anti-tumor efficacy was evaluated in the MC38 syngeneic model in IL-15 and IL-15 receptors humanized mice. Active IL-15 released from pro-drug induced downstream signaling events were evaluated in human primary CD8+T and NK cells. The pharmacokinetics (PK) and pharmacodynamic(PD) biomarkers were evaluated in humanized IL-15/IL-15 receptor mice bearing MC38 syngeneic tumor after BGB-R046 administration.

Results: BGB-R046 exhibits protease dependent activation and shows significant anti-tumor efficacy in MC38 syngeneic model. Active pro-IL-15 robustly engages the IL-15 pathway, demonstrated by STAT5 phosphorylation and CD122 downregulation in vitro, which are two target engagement PD markers induced by IL-15. Following BGB-R046 administration in vivo, minimum active drug release was detected in the circulation while active drug release was more sustained in tumor. Consistently, STAT5 phosphorylation was significantly increased in tumor, while undetectable in circulation on day 7 after BGB-R046 treatment. In addition, surface CD122 level on NK cells was downregulated in tumor while maintained in the circulation, suggesting robust IL-15R target engagement within tumor microenvironment by pro-drug design. Accordingly, BGB-R046 treatment induced significant immune activation in tumor, demonstrated by CD8+ T and NK cell proliferation and activation, consistent with its robust anti-tumor activity in this model.

Conclusions: BGB-R046 is an IL-15 prodrug with minimum free drug release in the peripheral and induced robust pharmacodynamic effects and immune activation within tumor microenvironment in the MC38 mouse syngeneic model. A clinical study evaluating BGB-R046 as monotherapy and in combination with tislelizumab (anti-PD-1 antibody) in patients with advanced or metastatic tumors is ongoing(NCT06487858).

#2234 SOF10, a novel selective anti-latent TGF-β1 antibody, augments the efficacy of anti-PD-L1 antibody via modulation of the tumor microenvironment.

Kenji Nakagawa[1], Izumi Sato[1], Masakazu Kanamori[1], Silvia Sun[2], Kentaro Asanuma[1], Hiroki Kawauchi[1], Chisako Kanamaru[1], Yoko Kayukawa[1], Saori Matsuo[1], Meiri Shida-Kawazoe[3], Takehisa Kitazawa[1], Tomoyuki Igawa[1], Hideaki Shimada[2]

[1]Chugai Pharmaceutical Co., Ltd., Yokohama, Kanagawa, Japan,[2]Chugai Pharmabody Research Pte. Ltd., Synapse, Singapore,[3]Chugai Pharmaceutical Co., Ltd., Chuo-ku, Tokyo, Japan

Background: TGF-β is a highly pleiotropic cytokine, consisting of three isoforms: TGF-β1, TGF-β2, and TGF-β3. The TGF-β signaling is known to be associated with a poor response to immune checkpoint inhibitors. Multiple agents targeting TGF-β have been developed with focus on the physiological effects of TGF-β in the tumor microenvironment; however, inhibition of multiple TGF-β isoforms have exhibited serious cardiac toxicity and bleeding in nonclinical studies. TGF-β1 is expressed as inactive latent complex and reported to be most highly expressed among all the isoforms in tumors. Selective inhibition of latent TGF-β1 activation may address the potential safety risk and provide an effective approach for targeting the TGF-β pathway in tumors.
Methods: We generated SOF10, a novel anti-latent TGF-β1 antibody, which selectively inhibits latent TGF-β1 activation. To assess the inhibitory mechanism of SOF10 for latent TGF-β1 activation, we evaluated multiple proteases-mediated TGF-β1 activation, integrin-mediated TGF-β1 activation, and determined the structure of the SOF10 Fab and latent TGF-β1 complex. We then tested combinational efficacy of SOF10 with anti-PD-L1 antibody in EMT6 murine breast cancer model, and CT26 murine colon cancer model. To analyze the effects on tumor microenvironment, we evaluated CD8 T cells and cancer-associated fibroblasts (CAFs). Finally, we conducted 3-month repeat-dose toxicity studies (GLP) in mice and cynomolgus monkey.
Results: SOF10 inhibited multiple proteases- and αvβ8-mediated latent TGF-β1 activation. Structural analysis of SOF10 showed the unique binding mode in which SOF10 inserted into the dimer surface of the LAP domain of TGF-β1. In vivo studies demonstrated combinatorial effect of SOF10 and anti-PD-L1 antibody in tumor growth inhibition for the treatment of EMT-6 and CT26 murine cancer models. Evaluation of CD8 T cells showed increased influx and enhanced activation of CD8 T cells in the combination group. RNA analysis of CAFs also revealed downregulation of ECM synthesis by SOF10 treatment. Lastly, no toxicologically relevant changes were observed in 3-month repeat-dose toxicity studies (GLP).
Conclusions: Our data suggest that SOF10, a selective anti-latent TGF-β1 antibody, can exert antitumor efficacy in combination with anti-PD-L1 antibody by modulating CAF dynamics and overcomes immune exclusion with preferable safety profile. Clinical studies of SOF10 are currently on-going for patients with solid cancer (NCT05867121)

#2236 A novel SIRPαxPD-L1 bispecific antibody remodels the innate and adaptive tumor microenvironment, and enhances tumor control.

Mariela Navarrete[1], Cristobal Costoya[1], Erik van Buijtenen[2], Simone Wouters[2], Joost Kreijtz[2], Paul Vink[2], Hans van Eenennaam[2], Sergio A. Quezada[1]

[1]Cancer Immunology Unit, Research department of Haematology, University College London (UCL) Cancer Institute, London, United Kingdom,[2]Sairopa, Rotterdam, Netherlands

Checkpoint blockade immunotherapy has transformed cancer treatment. However, many patients fail to respond to these therapies, underscoring the urgent need for new therapeutic strategies. Targeting alternative pathways and immune cell subsets within the tumor microenvironment (TME) holds promise for improving outcomes in these patients. One key mechanism of anti-tumour immunity is the phagocytosis of tumor cells by myeloid cells, which can be suppressed by the interaction between the SIRPα receptor on myeloid cells and its ligand CD47, a "do not eat me" signal, on tumor cells. Inhibition of the SIRPα/CD47 axis restores myeloid cell activity, promotes antigen presentation and enables tumor cell elimination. In this study, we evaluated a novel bispecific antibody that simultaneously blocks SIRPα and PD-L1 (SIRPαxPDL1), critical immune checkpoint molecules frequently overexpressed in the TME. SIRPαxPDL1 demonstrated enhanced macrophage-mediated phagocytosis of tumour cells *in vitro*. Moreover, in the MC38 murine tumor model, SIRPαxPDL1 significantly improved tumor control and survival, compared to monotherapies targeting either PD-L1 or SIRPα alone or in combination. High-dimensional flow cytometry revealed the unique ability of SIRPαxPDL1 to promote monocyte-to-macrophage differentiation whilst enhancing $CD8^+$ T cell infiltration and activation within the TME compared to the combination of SIRPα and PDL1 antibodies. Remarkably, SIRPαxPDL1 synergized with a tumour-targeting antibody, further augmenting tumor control and survival. These findings highlight, for the first time, the potential of SIRPαxPDL1 to remodel the myeloid compartment and activate antitumor immunity, offering a promising novel avenue in cancer immunotherapy and combination approaches.

#2237 IOMX-0675, a LILRB1/LILRB2 cross-specific antibody that repolarizes the tumor microenvironment to drive potent anti-tumor activity.

Kristina Heinig, Christina A. Hartl, Marisa Stebegg-Wagner, Eugenia Korotkova, Michail Maraslis, Carmen Ginzel, Thomas Jaquin, Bettina Langer, Jonas Schilz, Alina Huth, Maximilian Aigner, Alexander N. Marziale, **Stefan Bissinger**

iOmx Therapeutics AG, Martinsried / Planegg, Germany

IOMX-0675 is a monoclonal immunoglobulin G1 (IgG1) antibody targeting LILRB1 (ILT2) and LILRB2 (ILT4), key immunosuppressive receptors of the leukocyte immunoglobulin-like receptor (LILR) family, that play a pivotal role in tumor immune evasion. LILRB1 and LILRB2 recognize both classical and non-classical MHC-I molecules and are highly expressed on tumor-infiltrating myeloid cells, with LILRB1 also found on lymphoid cells. Their frequently observed upregulation in patients non-responsive to T cell checkpoint blockade, implicate a role in driving tumor resistance. Expression profiling revealed co-expression of immune-activating LILR members, LILRA1 and LILRA3, with LILRB1 and LILRB2 in specific cancer indications, underscoring the importance of selective receptor targeting to achieve effective therapeutic outcomes. IOMX-0675, identified through iOmx's proprietary phage display library, exhibits a highly differentiated binding profile with selective, high-affinity binding to the inhibitory receptors LILRB1 and LILRB2, while showing only weak affinity to the closely related immune-activating LILR family members LILRA1 and LILRA3. In autologous immune cell co-cultures, IOMX-0675 effectively reprograms immunosuppressive macrophages in a dose-dependent manner, restoring lymphoid immune cell function. Importantly, IOMX-0675 retains its high selectivity for LILRB1 and LILRB2 and its pharmacodynamic activity, even in environments dominated by LILRA1 and LILRA3. IOMX-0675 enhances phagocytic activity, stimulates pro-inflammatory functions across various macrophage subtypes and drives robust pro-inflammatory cytokine secretion in stimulated PBMCs, both *in vitro* and in *ex vivo* whole blood assays. In a $CD34^+$ stem cell-engrafted humanized mouse melanoma model, IOMX-0675 exhibited potent anti-tumor activity and repolarized tumor-associated macrophages, closely aligning with *in vitro* findings and underscoring its therapeutic promise. In summary, we developed IOMX-0675, a highly selective cross-specific antibody that effectively antagonizes both LILRB1 and LILRB2 while exhibiting negligible binding to immune-activating LILR family members. IOMX-0675 demonstrates robust reprogramming of the immunosuppressive myeloid compartment and restores cytotoxic T cell activity within the tumor microenvironment, both *in vitro* and *in vivo*. Its differentiated binding profile positions IOMX-0675 as a best-in-class therapeutic candidate with the potential to maximize anti-tumor efficacy and address significant unmet medical needs for patients.

#2238 NOX2 plays a key role in M2 polarization of macrophages associated with cancer-associated fibroblast in tumor microenvironment.

H. Jang, J. Um, E. Lee, S. Moon, S. Lee;
Aptabio Therapeutics Inc., Yongin, Korea, Republic of

The tumor microenvironment (TME) is a dynamic ecosystem surrounding a tumor. The TME consists of various components such as cancer cells, stromal tissue, immune cells and the extracellular matrix (ECM). In particular, the interaction between cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs) is a key regulator of immunosuppression in the TME, subsequently contributing to tumor progression, resistance and metastasis. CAFs stimulate TAMs to differentiate into a M2-like macrophage phenotype, supporting immune evasion and tumor growth. It has been found that reactive oxygen species (ROS) produced by NADPH oxidase (NOX) enzymes are involved in process. ROS have been reported to stimulate CAF activity and TAM differentiation, but the role of NOX in the interaction between CAF and TAM has not yet been elucidated. Herein, we aimed to investigate the role of NOX2 in M2 polarization of macrophages, especially CAF-mediated TAM differentiation, and evaluate the therapeutic potential of targeting NOX2 in the TME. In this study, we utilized THP-1 monocytic cell lines and human pancreatic CAFs (pCAFs) to examine the functional significance of NOX2. NOX2 was predominantly expressed in THP-1 cells compared to other NOX isozymes when polarized into M2-like macrophages and knockdown of NOX2 effectively inhibited M2-like polarization of THP-1-derived macrophages. In addition, knockdown of NOX2 significantly reduced the secretion of M-CSF by pCAFs. These findings suggest that NOX2 plays a crucial role in CAF-mediated M2 differentiation of macrophages. Based on these findings, we confirmed the effects of NOX2 inhibition using com-19, a selective NOX inhibiting molecule. Com-19 modulated M2 polarization of macrophages and downregulated NOX2 mRNA levels in the cells. Moreover, com-19 also significantly reduced ROS production and the secretion of cytokines/chemokines in macrophages under M-CSF-induced conditions. Importantly, the results also demonstrated that com-19 inhibited pCAF-induced M2-like polarization of THP-1. Taken together, we confirmed that CAFs and TAMs interact closely and NOX2 inhibition could be a potential therapeutic target for CAF- and TAM-mediated immunosuppression, especially for M2-polarization of macrophages in the TME. Furthermore, com-19 can provide a novel approach to overcome immunosuppressive barriers by interrupting their interaction in the TME, offering the potential for more effective combination therapies in cancer treatment.

#2239 FB849, a highly selective HPK1 inhibitor, reshapes the tumor immune microenvironment by modulating various immune cells.

Seungmook Lim[1], A Yeong Park[1], Hyekyoung Kim[1], Seung Hyuck Jeon[2], Yong Joon Lee[2], Jinhwa Lee[1], Eui-Cheol Shin[2], Jamie Jae Eun Kim[1]

[1]1ST Biotherapeutics, Yongin-si, Korea, Republic of,[2]Korea Advanced Institute of Science and Technology, Daejeon, Korea, Republic of

A highly unmet medical need exists for immuno-oncology agents to modulate the tumor microenvironment through broad-spectrum immune cell modulation by promoting crosstalk among T cells, B cells, and APCs, to exert a strong and durable anti-cancer immunity. Beyond T cells, other immune cells can play a crucial role in driving clinical activity, particularly in combination with current checkpoint inhibitors or patients with T cell dysfunction. We have discovered and developed FB849, a potent, highly selective, and orally bioavailable HPK1 inhibitor. FB849 has demonstrated robust anti-tumor activity across multiple preclinical models and is currently being evaluated in a Phase I/II clinical trial for advanced solid tumors (NCT05761223). To elucidate its immune-modulatory mechanisms and impact on both T cells and non-T cells, we extended our research to human primary immune cells and tumor-infiltrating lymphocytes (TILs). FB849 effectively activated T cells increasing IL-2 and IFNγ production, and stimulated B cell secreting IgG, IL-6 and IL-8. In monocyte-derived DCs, FB849 also significantly enhanced the secretion of IL-12 and IL-1β, and CD80 and CD86 expression. In vivo mouse TIL analyses revealed that FB849 increased T cell activation while re-shaped the myeloid compartment within the tumors by increasing dendritic cell infiltration with elevated cDC1, promoting tumor-associated macrophage (TAM) depletion with M1 polarization, and increasing NK cell infiltration. These create a tumor microenvironment favorable to anti-cancer immunity. Notably, FB849 retained anti-tumor activity even after CD8 T cell depletion, highlighting the pivotal role of non-T cells. To further delineate FB849's effects on TILs, we conducted single-cell RNA sequencing and TCR sequencing with the single cell suspension samples from RCC, HCC, HNC and BRC patients. FB849 prominently expanded multiple types of T cell clones, especially the CXCL13+ CD8 TIL cluster enriched with neoantigen signature genes. Furthermore, TCR sequencing revealed a considerable overlap in between CXCL13+ CD8 and MKI67+ CD8 T cells when stimulated with autologous EpCAM+ tumor cells or NY-ESO-1 overlapping peptides, validating that CXCL13+ CD8 TILs are tumor-reactive. We hypothesize that the intra-tumoral expansion of CXCL13+ CD8+ T cells enhance interactions between T cells and various non-T cells including B cells and dendritic cells, potentially driving tertiary lymphoid structure (TLS) formation via the CXCL13 chemokine secretion, which warrants further validation in preclinical and clinical research. Altogether, our work represents a promising approach to modulate a broad spectrum of immune cells simultaneously and enhance their crosstalk, paving the way for durable clinical activity and supporting further development in the clinic.

#2240 High dose acetaminophen re-polarizes CD11B+ cells in the tumor microenvironment towards an activated macrophage phenotype.

A. Bryan[1], P. Pingali[1], B. Patel[1], W. Lee[1], J. Landry[2], **A. J. Neuwelt**[1];
[1]Richmond VA Medical Center, Richmond, VA, [2]VCU/Medical College of Virginia, Richmond, VA

Background: High dose acetaminophen (AAP) has demonstrated promising results in early phase clinical trials. When administered concurrently with the CYP2E1 inhibitor fomepizole, AAP can be dose-escalated 100-fold relative to standard dosing without liver toxicity and without compromising anti-tumor activity. The present study aims to characterize the effects of high dose AAP on the tumor immune microenvironment.

Methods: The current study used a 23-plex flow cytometry panel to study AAP induced changes in the tumor immune microenvironment of syngeneic mouse breast tumors. AAP-induced changes in macrophage phenotype were studied in vitro using phagocytosis and antigen presentation assays, both alone and in combination with PD-1 antibody therapy.

Results: Flow cytometric analysis of the tumor immune micro-environment revealed that the vast majority of CD45+ cells in triple negative breast cancer model are CD11B+ cells of the innate immune system. The CD11B+ cells in the tumor micro-environment of vehicle-treated mice were mostly comprised of GR1+ myeloid derived suppressor cells. On the other hand, the CD11B+ cells in the high dose AAP-treated mice were mostly of the activated macrophage phenotype (F4/80+CD80+MHCII+). Other observed changes within the tumor of AAP-treated mice include a large influx of B-cells, NK-cells and T-cells. Given the increased number of activated macrophages observed in the AAP-treated tumors, in vitro studies were performed to better understand AAP effects on macrophage function. It was demonstrated that AAP enhances phagocytosis as well as antigen presentation by macrophages to antigen-reactive T-cells. These effects are amplified when PD-1 antibodies are combined with AAP in the in vitro antigen presentation assay. Furthermore, AAP has synergistic anti-tumor activity in vivo when combined with PD-1 antibody therapy, an effect that is macrophage dependent.

Conclusion: High dose AAP can be administered safely when combined with fomepizole-based rescue in mouse models. The present study demonstrates profound AAP-induced changes in the tumor immune microenvironment, particularly within the phenotype of CD11B+ cells of the innate immune system leading to increased “M1” type macrophages in the tumor immune microenvironment.

#2241 Immune-modulatory therapeutic cancer vaccines against IDO1 and PD-L1 control tumor growth through target specific changes in the tumor microenvironment.

Marion Chapellier[1], Anne Madsen[1], Rasmus Agerholm-Nielsen[1], Lea Svendsen[1], Ines Lecoq[1], Evelina Martinenaite[1], Shih-Chun Shen[2], Erika Sutanto-Ward[2], James DuHadaway[2], Souvik Dey[2], Dema Ghaban[2], Mads Hald Andersen[3], Alexander J. Muller[2], Ayako Wakatsuki Pedersen[1]

[1]IO Biotech, Copenhagen, Denmark,[2]Lankenau Institute for Medical Research, Wynnewood, PA,[3]Center for Cancer Immune Therapy, Copenhagen, Denmark

Despite the established clinical efficacy of checkpoint inhibitors such as blocking antibodies against Programmed Death protein (PD)-1 and Programmed Death Ligand (PD-L)1, drug resistance remains a significant obstacle in various cancer types. Indoleamine 2,3-dioxygenase 1 (IDO1) and PD-L1 are critical immunosuppressive drivers in the tumor microenvironment (TME) that favor cancer progression. The development of immune-modulatory vaccines against IDO1$^+$ and PD-L1$^+$ immune suppressive and/or tumor cells in the TME (IO102-IO103) has revealed promising clinical results[1] and is now being investigated in a Phase 3 clinical trial in advanced melanoma. Using preclinical models, the present study aims to further characterize the mode of action of these vaccines and compare it with conventional checkpoint inhibitors.

Anti-tumor efficacy of the vaccines was evaluated by monitoring tumor growth in syngeneic murine models after treatment with IDO1 or PD-L1 peptides in Montanide adjuvant. Tumor tissues, spleens and lymph nodes were collected and processed for flow cytometry or functional readouts. T cell activity was assessed using IFNγ Elispot assay. Gene expression analysis was performed using the Nanostring nCounter PanCancer IO360 Panel on bulk tumors from animals treated with IDO1 or PD-L1 vaccines; or anti-PD1/PD-L1.

Vaccination against IDO1 and PD-L1 induced specific T-cell expansion in splenocytes, associated with reduced tumor growth. In particular, PD-L1 specific T cells were also detected in lymph nodes and tumor infiltrating lymphocytes from MC38 tumor-bearing animals. Target specific activation of T cells was confirmed *ex vivo* upon restimulation of cells by the detection of IFNγ and TNFα within CD8$^+$ cells. Gene expression analysis of tumors revealed distinct immune signatures for each treatment group, either supporting cytotoxic T-cell activity (PD-L1 vaccine) or reducing the immune suppression within the TME (IDO1 vaccine). Comparison with samples from animals treated with anti-PD-1 or anti-PD-L1 showed non-overlapping gene expression changes induced by PD-L1 vaccine.

Our results from murine models show that IDO1 and PD-L1 peptide vaccines each contribute to tumor growth control through the activation of target specific T cells and their recruitment at tumor site. Gene expression analysis suggests that this is achieved through changes in the expression of immune-suppressive markers and enhanced T cell activation and cytotoxic activity. The gene expression changes observed were different from the signatures of anti-PD-L1 and anti-PD1, suggesting a complementary mode of action to those therapeutics. Altogether, our data support the clinical potential of IDO1 and PD-L1 immune-modulatory vaccines to improve existing therapeutics in cancer.

1.Kjeldsen et al. Nat Med 2021 DOI:10.1038/s41591-021-01544-x

#2242 Inhibition of the tumor cell TCA cycle suppresses ER stress-associated THBS2 to selectively potentiate antitumor response of CD36^{+} effector T cells.

J. Yang, F. Chen, Z. Fu, **Y. Teng**;
Emory University, Atlanta, GA

The tricarboxylic acid (TCA) cycle, occurring within the mitochondrial matrix, plays a pivotal role in mitochondrial metabolism, which is essential for generating energy to support tumor cell proliferation and survival. CPI-613 (devimistat) is a novel systemic therapeutic that targets the TCA cycle by inhibiting pyruvate dehydrogenase (PDH) through hyperactivation of its regulatory kinases and by inducing reactive oxygen species (ROS)-mediated inhibition of ketoglutarate dehydrogenase (KGDH). Here we report a novel mechanism by which CPI-613 potentiates the antitumor response of CD8^{+} T cells to head and neck squamous cell carcinoma (HNSCC). Using proximity biotinylation and LC/MS analysis, we found that CPI-613 inhibits the secretion of thrombospondin-2 (THBS2), a glycoprotein linked to poor prognosis and immune evasion in HNSCC patients. Mechanistically, CPI-613 blocks the TCA cycle in HNSCC cell mitochondria, leading to endoplasmic reticulum (ER) stress and upregulation of XBP1s, which transcriptionally suppresses THBS2 expression. As a result, CPI-613-treated HNSCC cells secrete reduced levels of THBS2, which leads to the inactivation of its receptor, CD36, on CD8+ T cells. This inactivation subsequently triggers the activation of the downstream AKT/mTOR signaling pathway, enhancing the proliferation and cytotoxic potential of CD8+ T cells. These results highlight the immunomodulatory effects of CPI-613 and provide a strong rationale for developing it as a promising treatment strategy for HNSCC.

#2243 Preclinical studies of BB-1709, a CD73-targeting ADC potentially suppresses tumor growth through dual mechanisms.

Bing Xia, Lixia Cao, Jianfeng Yang, Cui Feng, Fangdun Jiang, Chen Li, Lixia Gu, Yifan Yang, Jing Tian, Jiasheng Geng, Ziping Wei, Yuhong Zhou

Bliss Biopharmaceutical (Hangzhou) Co., Ltd., Hangzhou, China

CD73, also known as ecto-5'-nucleotidase, is a cell surface enzyme that catalyzes the conversion of AMP to adenosine. It is highly expressed in various types of cancers, including gastric, glioblastoma, non-small cell lung, and colorectal cancers. Elevated CD73 expression is highly correlated with poor prognosis, increased metastasis, and resistance to chemotherapy. In the tumor microenvironment, CD73-derived adenosine exerts multiple immunosuppressive effects, promoting tumor growth and metastasis. Currently, there are no approved antibody-drug conjugates (ADCs) targeting CD73. In this study, we introduce BB-1709, a CD73-targeting ADC that incorporates MMAE as its cytotoxic payload, demonstrating its antitumor activity through direct cytotoxicity against CD73-expressing tumor cells and its ability to inhibit CD73 enzyme activity.
A comprehensive set of studies was conducted to assess its in vitro cytotoxicity, in vivo antitumor activity as well as its ability to inhibit CD73 enzyme activity.
In vitro studies demonstrated that BB-1709 exhibited potent and selective cytotoxicity against various CD73-positive cancer cells and significantly inhibited CD73 enzyme activity, effectively blocking the conversion of AMP into adenosine. In vivo studies using xenograft models showed that a single dose of BB-1709 resulted in substantial antitumor activity. Pharmacokinetic and toxicology assessments in non-human primates revealed a favorable safety profile for BB-1709 at clinically relevant dosages and administration schedules.
In summary, these results suggest that BB-1709 not only exhibits good preclinical efficacy and safety, but also has the potential to promote anti-tumor immune responses by inhibiting CD73 enzymatic activity. A phase I clinical trial of BB-1709 is currently underway to further explore its therapeutic potential in cancer treatment.

#2244 ASO-mediated STAT3 knockdown relieves immunosuppression sensitizing tumors to immunotherapies.

Veronica Finisguerra[1], **Marie-Aline C. Neveu**[1], Joppe Lochten[1], Dido Van de Velde[1], Natalie De Geest[1], Marie Wallays[1], Marijke Pellens[1], Michel Janicot[2], Andrew Denker[1], Tiziana Girardi[1]

[1]Flamingo Therapeutics, Leuven, Belgium,[2]JMi ONConsulting, Brussels, Belgium

To improve the clinical outcome of cancer immunotherapies, strategies that modulate the immunosuppressive tumor microenvironment (TME) are currently under evaluation. Our study aims to dissect the immune-modulatory effects of danvatirsen, an antisense oligonucleotide (ASO) targeting the transcription factor Signal Transducer and Activator of Transcription 3 (STAT3) currently being studied in combination with pembrolizumab in the PEMDA-HN trial, a randomized Phase 2 trial in recurrent/metastatic head and neck squamous cell carcinoma (NCT05814666). In response to cytokines and growth factors, STAT3 regulates genes involved in cell differentiation, survival, inflammation, and angiogenesis. In tumors STAT3 is often hyperactivated, both in tumor and TME cells, and correlates with poor prognosis. Danvatirsen is a 16-mer generation 2.5 ASO designed by Ionis Pharmaceuticals to specifically target human STAT3 mRNA for degradation. Previous preclinical and clinical data have demonstrated tumor uptake of danvatirsen and STAT3 protein reduction in post-treatment biopsies. To better elucidate the mechanism, we performed *in vitro* and *in vivo* studies to assess STAT3 knockdown (KD) and effects on immune cell phenotype. Danvatirsen strongly reduces STAT3 mRNA and protein *in vitro*, both in human cancer cells and Peripheral Blood Mononuclear Cells (PBMCs), as well as *in vivo*, with a protein KD of 75% in human tumor xenografts implanted in immunodeficient mice. Interestingly, we found that danvatirsen increases the killing activity of PBMCs co-cultured with lung or liver human cancer cell lines, in line with the immunostimulatory effects of STAT3 KD. Moreover, we confirmed that danvatirsen affects the polarization of *in vitro*-differentiated pro-tumoral M2 macrophages towards an anti-tumoral M1 phenotype. A mouse surrogate STAT3 ASO administered *in vivo* reduced tumor STAT3 mRNA and protein levels by 60% and 75%, respectively, in the mouse breast cancers 4T1 and EMT6, and in the mouse lung cancer LL2. STAT3 KD was also observed in immune cells in circulation or infiltrating 4T1 tumors, with 33% and 55% KD in tumor-infiltrating T cells and macrophages, respectively. STAT3 ASO treatment *in vivo* remodels the TME composition, leading to pro-tumoral M2 macrophage repolarization towards an anti-tumoral M1 phenotype, among other effects. Those changes were associated with tumor growth inhibition (TGI) of 30% in 4T1-tumor bearing mice. When combined with anti-PD-1, STAT3 ASO reduced tumor growth by 55% compared to the control, and showed an additive effect to anti-PD-1 treatment alone, where TGI was 25%, supporting the combination of danvatirsen and pembrolizumab. Similar results were obtained in the EMT6 model. In conclusion, these data showed that ASO-mediated STAT3 KD relieves immunosuppression thereby sensitizing tumors to immunotherapies.

#2245 TFF-2-mediated CXCR4 partial agonism outperforms CXCR4 antagonism in reducing murine gastric cancer by suppressing PMN-MDSC generation.

Jin Qian[1], Chenkai Ma[2], Quin T. Waterbury[1], Xiaofei Zhi[1], Christine S. Moon[1], Ruhong Tu[1], Hiroki Kobayashi[1], Feijing Wu[1], Biyun Zheng[1], Yi Zeng[1], Hualong Zheng[1], Yosuke Ochiai[1], Ruth A. White[1], David W. Harle[1], Jonathan S. LaBella[1], Leah B. Zamechek[1], Lucas ZhongMing Hu[1], Ryan H. Moy[1], Arnold S. Han[1], Bruce L. Daugherty[3], Seth Lederman[3], Timothy C. Wang[1]

[1]Columbia University Irving Medical Center, New York, NY,[2]CSIRO, Westmead, Australia,[3]Tonix Pharmaceuticals, Inc., Chatham, NJ

Introduction: Polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) are pathologically activated neutrophils that impair immunotherapy responses. The chemokine receptor CXCR4, a central regulator of hematopoiesis, represents an attractive PMN-MDSC target.

Methods: We generated a novel peptide, TFF2-MSA, as a CXCR4 partial agonist by fusing Trefoil Factor 2 (TFF2) with murine serum albumin (MSA), and compared its treatment efficacy in combination with anti-PD-1 antibody to that of the clinically available CXCR4 antagonist plerixafor (AMD3100). Murine syngeneic gastric cancer cells were subcutaneously implanted into the Hdc-GFP transgenic mice, which enabled tracking of PMN-MDSCs via histidine decarboxylase (Hdc) expression across multiple tissues. Treatments were then initiated every three days. To elucidate mechanisms, myeloid progenitors were cultured with or without TFF2-MSA *in vitro*. Bone marrow $Lin^{-}c\text{-}Kit^{+}$ hematopoietic progenitors from tumor-bearing Hdc-GFP (CD45.2) mice following vehicle or TFF2-MSA treatments were transferred into CD45.1 mice to validate TFF2-MSA's effects on PMN-MDSC generation. In tumor-free mice, we compared the short-term bone marrow cell mobilization effects of TFF2-MSA and AMD3100. Additionally, we analyzed human blood and single-cell RNA sequencing (scRNA-seq) data from gastric cancer tissues for immune profiles.

Results: The combination of TFF2-MSA plus anti-PD-1 antibody showed significant efficacy in reducing/eradicating primary and metastatic tumors and extending mouse survival, in contrast to the CXCR4 antagonist AMD3100, which did not enhance anti-PD-1 response. Mechanistically, TFF2-MSA inhibited the expansion of CXCR4-high granulocyte-monocyte progenitors (GMPs) and the generation of Hdc-GFP^{+} PMN-MDSCs in culture, which underlies its systemic reduction of Hdc-GFP^{+} PMN-MDSCs in tumor, blood, and spleen and GMPs in bone marrow *in vivo*. In contrast, AMD3100 induced peripheral accumulation of Hdc-GFP^{+} PMN-MDSCs and compensatory elevation of GMP levels in bone marrow. Congenic adoptive transfer experiments confirmed that TFF2-MSA suppressed Hdc-GFP^{+} PMN-MDSC generation *in vivo*. Consistently, in tumor-free mice, TFF2-MSA did not significantly mobilize granulocytes from bone marrow, unlike AMD3100, thus better preserving bone marrow homeostasis. In gastric cancer patients, a $CXCR4^{+}LOX\text{-}1^{+}$ PMN-MDSC population expanded in both blood and tumor, exhibiting immunosuppressive potential, and associated with reduced TFF2 plasma level compared with healthy donors.

Conclusions: The novel CXCR4 partial agonist exhibited superiority in tumor control and effective PMN-MDSC reduction, and preservation of bone marrow homeostasis compared to the CXCR4 antagonist AMD3100. CXCR4 partial agonism may represent a promising strategy for targeting PMN-MDSCs in gastric cancer patients.

#2246 VISTA-induced tumor suppression and immunosuppression by shared cell-intrinsic mechanisms.

Yan Zhao, Tina Andoh, Ishrat Durdana, Joshua Gruber

University of Texas Southwestern Medical Center, Dallas, TX

VISTA is a key immune checkpoint receptor under investigation for cancer immunotherapy; however, its signaling mechanisms remain unclear. Here we identify a four amino acid (NPGF) intracellular motif in VISTA that suppresses cell proliferation by constraining cell-intrinsic growth receptor signaling. The NPGF motif binds to the adapter protein NUMB and recruits Rab11 endosomal recycling machinery. In tumor cells with high VISTA levels, the NPGF motif sequesters NUMB at endosomes, which interferes with epidermal growth factor receptor (EGFR) trafficking and signaling to suppress tumor growth, even without a functioning immune system. To understand if similar principles underlie VISTA-mediated immunosuppression we engineered mice with targeted mutations in the VISTA NPGF motif. These animals had increased T cell signaling and proliferation leading to improved control of tumor growth. These results define a generalizable mechanism by which VISTA represses NUMB to control cell signaling across disparate cell types that could be exploited for immunotherapies.

#2247 Adenosine production suppression and PD-1 immune check point inhibitor for treatment of malignant pleural mesothelioma. (MPM).

luciano Mutti[1], Antonio Giordano[2], Pierpaolo Correale[3], Caterina Costa[4]

[1]University of L'Aquila, L' Aquila, Italy,[2]Temple University, Philadelphia, PA,[3]GOM, Reggio Calabria, Italy,[4]Pascale, Napoli, Italy

Background: Although immune-checkpoint inhibitors (ICIs) have positively impacted the treatment of some tumors, several evidences clearly suggest that the actual relevance of these treatments has not been fully unraveled yet Hypoxic tumors activate the purinergic pathway, which acts as a major immunosuppressive mechanism. CD39 and CD73 are enzymes involved into ATP degradation to AMP and AMP degradation to the immune-suppressive adenosine, respectively thus the inhibitors of these pathways are progressively attracting the attention as actionable targets for immunotherapy in many solid tumors MPM is a highly aggressive malignancy whose treatment challenging for different reasons, including among others acidic and hypoxic tumor microenvironment (TME). Hypoxic TME and the expression of purinergic receptors and enzymes on tumor cells are considered criteria to select patients for immunotherapy with adenosine pathway inhibitor. We have already shown the expression of adenosine receptor 2 B (ADORA2b) and CD73 enzyme on MMP cells is inversely related to patients' survival and that MMP cells degrade AMP to adenosine via CD73. Aim of our study was assessing if ADORA2B and or CD73 with or without PD-1 ICi were able to curb tumor cells growth in a syngeneic mouse MPM model.
Methods: For orthotopic mouse models of MPM, CD73 ADORA2B positive 105 AB1B-LUC cells were injected intraperitoneally (i.p.) in immunocompetent BALB/C mice. After three weeks we started monitoring tumor growth and the animals were randomized into five groups of three mice each for treatment with PBS 100 µl (control), Pembro (anti murine PD-1; MCE) 4.4 mg/Kg, TT-4 (ADORA2 Inhibitor Portage), 3 mg/Kg, Pembro+TT-4, AB-680 (CD73 inhibitor, MCE, HY-125286) 20 mg kg) and Pembro+AB-680 were injected every two days for three weeks. Disease was measured with IVIS® Lumina XR in vivo imaging system, thus facilitating non-invasive longitudinal monitoring of disease progression or regression.
Results: after 21 days AB680 and TT4 resulted to be the most effective single treatments (fluorescence reduction vs control $p<0.01$) whereas Pembro+TT4 Pembro+AB680 did not differ significantly from the single treatments. IHC of murine samples from mice treated as above revealed infiltration of CD8 T cells mostly with AB680 single agent or in combo compared to the cold pattern of the control samples. No relevant toxicity was recorded.
Conclusions: Inhibition of the purinergic pathway exerts a significant immune-mediated MPM growth inhibition

#2248 Inhibiting NNMT in cancer-associated fibroblasts reduces immunosuppression.

Janna Heide[1], Agnes J. Bilecz[1], Maria F. Allega[1], Samarjit Patnaik[2], Gordon M. Stott[3], Matthew D. Hall[2], Andras Piffko[4], Ernst Lengyel[1]

[1]University of Chicago, Chicago, IL,[2]National Center for Advancing Translational Sciences (NCATS), National Institutes of Health (NIH), Rockville, MD,[3]Leidos Biomedical Research, Frederick National Laboratory for Cancer Research, Frederick, MD,[4]Department of Radiation and Cellular Oncology, University of Chicago, Chicago, IL

Cancer-associated fibroblasts (CAFs) are critical in supporting cancer progression, yet effective CAF-targeted therapies are lacking. Through spatial transcriptomics and single-cell RNA sequencing, we identified nicotinamide N-methyltransferase (NNMT) upregulation and the resulting histone hypomethylation as a global regulator across various CAF subtypes. NNMT-expressing CAFs activate the complement system and recruit myeloid-derived suppressor cells (MDSCs), driving an immunosuppressive tumor microenvironment. Using high-throughput screening, we developed a potent NNMT inhibitor (NNMTi) that significantly reduces tumor burden in murine cancer models by decreasing MDSC infiltration and enhancing $CD8^+$ T cell activation, thereby restoring the efficacy of immune checkpoint inhibitors. These findings establish NNMT as a key regulator of the CAF landscape and present a novel therapeutic strategy for reprogramming the tumor microenvironment.

#2249 Selective degradation of Helios induces Treg cell reprogramming and enhanced antitumor immunity.

Michael J. Barnes

Research, Cancer Immunology and Cell Therapy, Bristol Meyers Squib, Seattle, WA

Regulatory T (Treg) cell suppression of antitumor immunity can limit responses to immunotherapy in multiple solid tumor indications. The transcription factors Helios (IKZF2) and Eos (IKZF4) are abundantly expressed by Treg cells in the tumor microenvironment and act as critical nodes in a transcriptional network that enforces a stable immunosuppressive Treg cell phenotype. In preclinical models, inducible genetic loss of Helios in Foxp3-expressing Treg cells alone or with anti-PD-1 immune checkpoint blockade yields improved or complete responses to subcutaneous MC38 tumors, respectively, highlighting Helios as a druggable target for immunotherapy. BMS-986449 is a CELMoD molecular glue that redirects the E3 ubiquitin ligase Cereblon to interact with and induce the degradation of Helios and Eos. It was optimized to induce rapid and deep degradation of Helios and Eos in primary Treg cells with selectivity over other CELMoD neosubstrates, as assessed by global proteomic analysis of primary T cells. When primary human Treg cells were cultured under activating conditions with BMS-986449, a reprogrammed transcriptional phenotype emerged by 8 days in culture, including increased protein expression of effector molecules, such as Granzyme B, and abundant expression of inflammatory gene transcripts. In tumor bearing hCRBN-KI mice, BMS-986449 daily dosing induced monotherapy activity in the CT26, MB49, and MC38 tumor models with TGI values of up to 75%, 62%, and 42%, respectively; in combination with anti-PD-1 immune checkpoint blockade more robust efficacy was achieved with TGI values of 95%, 97%, and 92%, respectively. Additionally, in cynomolgus monkeys, daily dosing of BMS-986449 (0.1 mg/kg) maintained greater than 80% degradation of Helios in circulating Treg cells, supporting the advancement of BMS-986449 into Phase 1/2 clinical trials for patients with advanced solid tumors.

#2250 UCHL1 hijacks tolerogenic DC maturation and promotes mregDC-Treg crosstalk to nullify anti-PD-L1 therapy.

Yufei Zhao[1], Peiyi Xie[1], Qinghai Ye[1], Mien-Chie Hung[2], Hui Li[1]

[1]Zhongshan Hospital, Fudan University, Shanghai, China,[2]China Medical University, Taiwan, China

Background: Immune-checkpoint blockade (ICB) therapies have revolutionized cancer treatment, but such immunotherapy regimens fail in a subset of patients. Dendritic cells (DCs) are a heterogeneous group of professional antigen-presenting innate immune cells that activate adaptive immunity and determine the efficacy of immunotherapies. While they can also be hijacked by tumor-mediated factors to contribute to immune tolerance and tumor progression. However, little is known about the molecular mechanisms that drive the tolerance maturation of DCs in the tumor microenvironment (TME).

Methods: We enrolled 85 patients with advanced hepatocellular carcinoma (HCC) exhibiting varying response to immunotherapy, and profiled their tumor ecosystems using a single-cell transcriptomes sequencing (scRNA-seq) and mass cytometry by time of flight (CyTOF) for 10 patients, and plasma protein level quantification, conducted both pre-treatment and post-treatment across all patients. We integrated our in-house data and 6 additional published scRNA-seq cohorts of 83 donors to generate a comprehensive landscape of cellular dynamics underlying different responses to immunotherapy. We verified the prognostic value in our in-house tumor microarray of 342 patients.

Results: UCHL1 overexpression nullifies anti-PD-L1 therapy by driving conventional DC transformation into mature DCs enriched in immunoregulatory molecule (mregDC) via tolerogenic maturation and promoting mregDC and regulatory T (Treg) cell crosstalk, thereby restrains $CD8^+$ T anti-tumor immunity. Mechanistically, UCHL1 enhances glycolysis by stabilizing HIF1α and lactate accumulation in TME, which further promotes SREBP2 activation and nuclear translocation in DCs. We verified the positive correlations of UCHL1 with HIF1α/VEGFα/LAMP3/FOXP3 in 342 patients with HCC. Genetic ablation or pharmacological inhibition of UCHL1 all reduce the mregDC and Treg accumulation, restore the immuno-surveillance of tumor-infiltrating lymphocytes, and safeguard anti-tumor immunity and efficacy of anti-PD-L1 therapy in mouse models.

Conclusions: UCHL1 hijacks tolerogenic DC maturation and promotes mregDC-Treg crosstalk to nullify anti-PD-L1 therapy. Genetic ablation or pharmacological inhibition of UCHL1 unleash the immuno-surveillance of tumor-infiltrating lymphocytes, and safeguard the anti-tumor immunity. Plasma level of UCHL1 predicts the efficacy of anti-PD-L1 therapy.

#2251 Non-depleting anti-OX40 antibodies boosts tumor-infiltrating T cells and impairs Tregs without effector T cell depletion.

Cristobal Costoya, Mariela Navarrete, Aniruddh Tyagi, Dafne Franz Demane, Maria Vila de Mucha, Sergio A. Quezada

Cancer Immunology Unit, Research Department of Haematology, University College London (UCL) Cancer Institute, London, United Kingdom

While antagonistic antibodies targeting CTLA-4 and PD-1 have demonstrated significant success in cancer immunotherapy, agonistic antibodies such as anti-OX40 have shown limited efficacy. This disparity is potentially due to a lack of understanding of how antibody Fc regions interact with FcγRs on innate immune cells. Anti-OX40 antibodies currently in clinical trials are predominantly human IgG1, a strong FcγR engager. This design assumes differential surface OX40 expression will result in regulatory T cell (Treg) depletion and CD4+ effector T cell (Teff) activation, yet the therapeutic outcomes remain suboptimal. Using transplantable murine cancer models, we compared the efficacy of anti-OX40 isotypes with distinct FcγR-binding affinities. Weak FcγR-binding mouse IgG1 triggered robust pan-T cell activation and expansion within the tumor microenvironment while reducing Treg suppressive capacity and destabilizing their transcriptional program. Conversely, strong FcγR-binding mouse IgG2a resulted in the depletion of both Tregs and Teffs from tumors, compromising therapeutic efficacy. Notably, combining anti-OX40 mouse IgG1 with anti-PD-1 resulted in synergistic responses, achieving nearly 100% complete tumor rejection, while anti-OX40 mouse IgG2a failed to show such synergy. These findings indicate that anti-OX40 antibodies with strong FcγR-binding may hinder optimal anti-tumor activity. A more effective approach could involve using weak FcγR-binding isotypes, which preserve effector T cells while activating agonistic signaling and impairs Treg function, thereby enhancing therapeutic efficacy without off-target effects. Future studies will validate these results in patient-derived tumor models to inform the development of anti-OX40-based therapies.

#2252 ASO targeting of intratumoral human FOXP3+ Tregs in humanized SGM3-IL15 mice.

Wayne W. Hancock[1], Tatiana Akimova[1], Liqing Wang[2], Zhanna Bartosh[2], Lanette M. Christensen[1], Pali Kaur[3], Veenu Aishwarya[4]

[1]Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA,[2]Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, Philadelphia, PA,[3]The Jackson Laboratory, Bar Harbor, ME,[4]AUM Biotech, LLC, Philadelphia, PA

Introduction: Humanized mice are an indispensable tool for pre-clinical cancer immunotherapy research, but there are limited data regarding human antitumor T cell immunity in such models, especially regarding the development of intratumoral T cell exhaustion.
Methods: We wanted to develop a Treg-dependent tumor model in humanized mice to study the effect of AUMsilence FOXP3 antisense oligonucleotides (ASO) targeting of intratumoral human CD4+ FOXP3+ regulatory T cells (Tregs) in vivo. The ideal model would support development and function of human Tregs, CD4+ and CD8+ T cells, allow tumor growth without apparent allogeneic reactivity, demonstrate the development of a suppressive tumor microenvironment, including with exhaustion of tumor-infiltrating T cells, and prove to be a Treg dependent tumor model. To do so, we evaluated two novel JAX strains of humanized CD34+ engrafted mice, NSG-SGM3 and NSG-SGM3-IL15.
Results: Both strains of mice had high (>80%) reconstitution by human CD45+ cells, no signs of GVHD, and increased proportions of FOXP3+ Tregs in their spleens, blood and livers (23-35% of CD4+ cells). Suppressive function of isolated liver and splenic Tregs were comparable with healthy donor blood Tregs. Both strains of mice supported similar growth of A549 lung tumor cells without rejection, and tumors were infiltrated by human CD45+ cells (8-20%), including 15-35% of FOXP3+ Tregs. Expression of T cell exhaustion markers was observed in tumors, but not in spleens, livers or blood. FOXP3 ASO treatment resulted in a decrease in tumor growth, while scramble treated mice developed multiple lung metastases and larger tumors. FOXP3 expression was also decreased in tumors (2.7 times, $p = 0.02$) but not in the spleens and livers of ASO treated mice. Of note, targeting of intratumoral Tregs led to decreased expression of most T cell exhaustion markers in tumors, confirming our previous report that intratumoral Treg may promote T cell exhaustion.
Conclusions: NSG-SGM3 and NSG-SGM3-IL15 mice provide a platform for preclinical drug testing involving an immunosuppressive tumor microenvironment and accumulation of exhausted T cells and fully functional Tregs. AUM*silence* FOXP3 ASO therapy has powerful anti-tumoral effects in vivo, decreases metastases and reverses features of T cell exhaustion.

#2253 HDAC6 inhibitors reprogram macrophages in antitumor-adoptive cell therapy.

Satish Kumar Reddy Noonepalle[1], Maria Gracia Hernandez[2], Nima Aghdam[3], Nithya Gajendran[1], Manasa Suresh[1], Xintang Li[1], Abishek Sehgal[1], David Quiceno[1], Michael Berrigen[4], Tessa Knox[1], Karen Tan[1], Eduardo Sotomayor[5], Katherine Chiappinelli[6], Duncan Wardrop[7], Anelia Horvath[8], Anatoly Dritschilo[9], Rohan Fernandes[10], Karthik Musunuri[11], Alejandro Villagra[1]

[1]Oncology, Georgetown University, Washington, DC,[2]George Washington University, Washington, DC,[3]Radiation Oncology, Beth Israel Deaconess Medical Center, Boston, MA,[4]School of Medicine, George Washington University, Washington, DC,[5]Cancer Institute, Tampa General Hospital, Tampa, FL,[6]Department of Microbiology, Immunology, and Tropical Medicine, George Washington University, Washington, DC,[7]Chemistry, University of Illinois, Chicago, IL,[8]Biochemistry and Molecular Medicine, George Washington University, Washington, DC,[9]Radiation Medicine, Georgetown University, Washington, DC,[10]Cancer Center, George Washington University, Washington, DC,[11]Avsetra Therapeutics, Malvern, PA

Tumor-associated macrophages (TAMs) exhibit hybrid phenotypes ranging between M1-like (anti-tumor) and M2-like (pro-tumor) macrophages. In most tumor types, a higher M1/M2 ratio favors antitumor immunity. Therefore, strategies to enhance the M1/M2 ratio can potentially alter the tumor microenvironment (TME) towards antitumor immunity. These strategies include reprogramming resident TAMs, altering the fate of tumor-infiltrating monocytes towards M1-like macrophages, and finally, adoptive cell therapy (ACT) with M1-like macrophages. A significant challenge with the above strategies is to retain the antitumor properties of M1-like macrophages in an overwhelmingly immunosuppressive TME. In this study, we demonstrate a macrophage-based ACT where treatment with HDAC6 inhibitors (HDA6is) retains the M1-like characteristics while significantly suppressing the tumor growth in syngeneic SM1 murine melanoma and with radiation therapy (RT). We reprogrammed GFP-expressing antitumor M1 macrophages ex-vivo with HDAC6 inhibitors to lock into the M1 phenotype and administered intratumorally as ACT in the syngeneic SM1 murine melanoma model and humanized NSG-SGM3 melanoma model. We performed histological analysis of tumors for macrophage markers, immune phenotyping of infiltrated immune cells, single-cell secretome analysis of tumor macrophages, and single-cell RNA-seq analysis of CD45+ immune cell populations. Initially, tail vein injected (i.v) administration of luciferase-expressing monocytes in combination with HDAC6i reached the tumors and diminished SM1 tumor growth compared to the control group. On the contrary, luciferase-expressing M1 macrophages failed to reach the tumor as visualized by IVIS imaging, suggesting that i.v is not an effective route for macrophage therapy. Whereas, intratumor HDAC6i treated M1 macrophage ACT resulted in diminished tumor growth. Single-cell (Sc) RNA-seq analysis of CD45+ sorted immune cells revealed a significant increase in the M1/M2 macrophage ratio in concurrence with flow cytometry data. NicheNet cell-cell interaction analysis indicated that M1-like TAMs activated infiltrating T-cells and monocytes through ligand-receptor interactions. Cell fate analysis of infiltrated monocytes indicated differentiation toward M1-like macrophages in ACT tumors. Flow cytometry analysis indicated an increase in CD8 effector T-cells. Sc-secretome analysis of F4/80 sorted tumor macrophages by the Isoplexis platform revealed polyfunctionality of HDAC6-treated M1 macrophages. Histological examination of tumor sections for macrophage phenotypic markers suggested that transplanted macrophages retained the M1 phenotype post-ACT in both SM1 murine and NSG-SGM3 melanoma tumor models. Our study comprehensively demonstrated the potential of reprogramming macrophages with HDAC6 inhibitors as a viable macrophage cell therapy for treating solid tumors.

#2254 Therapeutic depletion of FR-β-positive M2 macrophages in solid tumor indications: A novel IO strategy.

Michael Adam[1], R. Paul Wilson[1], Helen Sanderson[2], Chris Hopson[1], Julie Krueger[1], Veronica Herrera[3], Heidi Emerich[3], Andrew Sanderson[2], Carol Harris[2], James Smothers[4]

[1]GSK, Upper Providence, PA,[2]GSK, Stevenage, United Kingdom,[3]Johnson & Johnson, Springhouse, PA,[4]SpringWorks Therapeutics, Durham, NC

Traditional IO therapy has primarily focused on T cell checkpoints, however macrophages typically represent the largest immune population present within tumors and can constitute up to 50% of total tumor mass. Macrophages are implicated in both immune mediated control of tumors as well as tumor progression depending on the phenotype of macrophages in the tumor microenvironment (TME). Immune suppressive M2-like tumor associated macrophages (TAMs) release immuno-suppressive cytokines such as IL-10 and TGF-β which promote regulatory T cells, angiogenesis, metastasis, and tumor progression correlating with a poor prognosis in several solid tumor indications including breast, bladder, ovarian, gastric, prostate, RCC, melanoma, and lung cancers. Folate Receptor beta (FRβ) expression is associated specifically with M2-like immune suppressive macrophages and is not observed on proinflammatory M1-like macrophages. FRβ expression correlates with poor prognosis across lung cancer indications. Depletion of FRβ macrophages by natural killer (NK) cells using an Fc enhanced monoclonal antibody (mAb) to drive antibody-dependent cellular cytotoxicity (ADCC), is proposed as a therapeutic modality, by removing M2-like immunosuppressive macrophages from the TME, while preserving anti-tumorigenic M1-like macrophages. Modified Fc enhanced human FRβ targeting tool mAb M909 results in M2 specific ADCC activity in human monocyte derived macrophage populations *in vitro*. A single dose of modified Fc enhanced murine FRβ targeting tool mAb CL10 results in depletion of murine FRβ+ myeloid cells from MC38 tumors *in vivo* for up to 6 days. To guide patient stratification in lieu of highly invasive biopsy sampling, a soluble biomarker that can be assessed in peripheral blood and correlates to tumor FRβ expression is highly desirable. In patient matched peripheral blood and non-small cell lung cancer (NSCLC) tumor biopsy samples, peripheral blood FRβ+ nonclassical monocytes correlated with FRβ+ TAMs and CX3CR1+ cells which are known to drive macrophage recruitment to the TME. Interestingly, prevalence of NK cell marker NKG2D in peripheral blood also correlated to FRβ+ TAMs. Taken together, the data suggest further exploration of a depleting anti-FRβ+ Fc enhanced mAb as a therapeutic strategy for certain solid tumor indications is merited.
All studies conducted according to GSK's Policy on the Care, Welfare and Treatment of Lab Animals and reviewed by the Institutional Animal Care and Use Committee at GSK or by the ethical review process at the institution where the work was performed.

#2255 Pro-inflammatory macrophage phenotype skewing induced by tumor treating fields (TTFields).

Tal Kan, Yiftah Barsheshet, Boris Brant, Tharwat Haj Khalil, **Tali Voloshin**, Lilach Koren, Simona Zisman-Rozen, Adi Haber, Moshe Giladi, Uri Weinberg, Yoram Palti

Novocure Ltd, Haifa, Israel

Introduction: Tumor treating fields (TTFields) therapy is an approved cancer treatment based on the delivery of electric fields that disrupt cellular processes critical for cancer cell viability. TTFields have been shown to induce immunogenic cell death in cancer cells, elicit a systemic anti-cancer immune response in preclinical models, and are approved for concomitant treatment with PD-1/PD-L1 inhibitors or docetaxel in patients with metastatic non-small cell lung carcinoma after platinum-based therapy. The current study examined the possible effects of TTFields on macrophage phenotype modulation.
Methods: Bone marrow-derived macrophages (BMDMs), flushed from femurs and tibias of 8-10-week-old Balb\C or C57bl/6 mice, were cultured in the presence of granulocyte-colony stimulating factor (GM-CSF) for 7 days. BMDMs were then exposed to either IFN-γ alone or IFN-γ with LPS for M1 polarization or IL-4 for M2 polarization. The polarized BMDMs were next treated with TTFields (150 kHz) for 24 h, followed by flow cytometry examination of surface expression of the F4/80 macrophage marker and activation markers including CD80, major histocompatibility complex class II (MHC-II), inducible nitric oxide synthase (iNOS), CD206, and ARG-1. Lysates of TTFields-treated macrophages were examined for RhoA activation using an appropriate kit and by western blot for phosphorylation of GEF-H1, c-Jun, and the NF-κB subunit p65. iNOS expression was also examined in cells treated with IFN-γ in the presence of TJ-M2010-5, an inhibitor of the TLR component myeloid differentiation primary response 88 (MyD88).
Results: BMDMs exposed to TTFields demonstrated elevated expression of the pro-inflammatory M1 markers CD80 and MHC-II and decreased expression of the anti-inflammatory M2 markers CD206 and ARG-1. BMDMs treatment with TTFields also induced RhoA activation and GEF-H1, c-Jun, and p65 phosphorylation. TTFields treatment of BMDMs that were exposed to IFN-γ markedly elevated iNOS expression to a level similar to that of BMDMs that were exposed to IFN-γ together with LPS, while blockage of TLR signaling substantially reduced iNOS expression.
Conclusions: TTFields demonstrate immunomodulatory effects, promoting pro-inflammatory macrophage polarization and activation in vitro. TTFields exhibit LPS-like activity by acting as a second signal to polarize M1 macrophages, possibly via involvement of the TLR4 signaling pathway and the transcriptional response driven by the GEF-H1/RhoA/ROCK/NF-κB pathway.

#2257 A TGFβ-directed immune-modulatory vaccine induces T cell activation and drives antitumor activity by modulating the tumor microenvironment.

Justin Vareecal Joseph, Matteo Bocci, Ulla Kring Hansen, Marcos Iglesias, Ayako Wakatsuki Pedersen

IO Biotech, Copenhagen, Denmark

Activation of the Transforming Growth Factor (TGF)β signaling cascade plays an essential role in a wide range of tumors and other diseases. Indeed, cancer cells and components of the tumor microenvironment (*e.g.* fibroblasts, myeloid cells, and blood vessels) exploit this pathway to support disease progression during tumor evolution. Nonetheless, global inhibition strategies targeting TGFβ pathway in clinical studies thus far fell short of the anticipated success. These clinical observations suggest that TGFβ modulation - rather than its inhibition - could be the key in sustaining meaningful antitumoral activity in the clinical setting. We report here an alternative approach, where TGFβ-expressing cells are targeted *via* a peptide vaccine directed against TGFβ-specific T cell epitopes. This is based on earlier studies reporting the existence of intrinsic T cell immunity against TGFβ in human[1,2], thus supporting the rationale for targeting these T cells through a vaccine encoding TGFβ epitopes.

To test this approach, we employed a range of murine platforms including the syngeneic Pan02 model of pancreatic adenocarcinoma (PDAC), and a CRISPR/Cas9-driven model of prostate cancer[3]. Collectively, treatment with the TGFβ vaccine significantly reduced the total tumor burden, without affecting the body weight and overall welfare of the animals. *Ex vivo* T-cell responses evaluated through interferon (IFN)-γ ELISpot confirmed expansion and activation of TGF-β-specific T cells upon immunization. Compared to overt malignant masses in the control cohort, TGFβ-directed immunization slowed down the progression of prostate tumors in the CRISPR/Cas9 model, as evinced by the numerous premalignant-like hyperplastic glands and resembling more physiological structures. Furthermore, smooth muscle actin (α-SMA) staining in the Pan02 model uncovered that the treatment ameliorated the fibrotic response - a hallmark of PDAC in the clinical setting - in this experimental setup, with an increased infiltration of CD8+ lymphocytes and F4/80+ macrophages into the TME. Currently, to clarify the mechanism of action, we are implementing the 4T1 model of mammary carcinogenesis to model metastatic disease and exploring spatial relationships to identify which cell types and tissue architectures are pivotal for meaningful responses.In conclusion, our data indicate the efficacy of TGFβ peptide vaccine in eliciting an immune response and tumor growth inhibition in mouse models of cancer. These observations warrant further preclinical investigation to better understand the efficacy and the safety profile of this novel TGFβ-targeting strategy. References: 1. Holmström *et al*., 2021; DOI: 10.1038/s41423-020-00593-5 2. Mortensen *et al*., 2021; DOI: 10.1038/s41423-021-00770-0 3. Cai *et al*., 2024; DOI: 10.1038/s41467-024-46370-0

POPULATION SCIENCES: Advancing Genomic and Epigenetic Insights in Cancer Across Diverse Populations: Genetics, Genomics, and Epigenetics
Poster Session

#2260 Social vulnerability and epigenetic signatures in adult survivors of childhood cancer with African ancestry: An epigenome-wide association study.

Yoonji Kim, Xiaoxi Meng, Jaesung Choi, Tiffany Eulalio, Noel-Marie Plonski, Heather Mulder, John Easton, Emily Walker, Geoff Neale, Kirsten Ness, Melissa M. Hudson, Gregory T. Armstrong, I-Chan Huang, Zhaoming Wang

Epidemiology and Cancer Control, St. Jude Children's Research Hospital, Memphis, TN

Background: Social determinants of health (SDOH) are increasingly recognized as critical factors in shaping long-term adverse health outcomes among childhood cancer survivors (survivors) because long-lasting toxicities from cancer treatment may be exacerbated by social adversity. Our previous epigenetic study of SDOH primarily focused on survivors of European ancestry, using measures such as educational attainment, personal income, and the Area Deprivation Index. This study aims to enhance understanding of the biological embedding of social adversity in survivors of African ancestry using the Social Vulnerability Index (SVI). The SVI provides a more comprehensive representation of SDOH, capturing neighborhood-level social vulnerabilities and aligning with the objectives of Healthy People 2030.
Methods: Survivors of African ancestry from the St. Jude Lifetime Cohort were included. DNA methylation (DNAm) profiles were generated with EPIC BeadChip v1 using blood-derived DNA. SDOH factors included social vulnerability (four domains from the SVI: socioeconomic, household composition/disability, minority status/language, and housing/transportation), educational attainment, and personal income. An epigenome-wide association study (EWAS) was used to evaluate the association between DNAm at each CpG and SDOH factors, adjusting for attained age, sex, cancer treatment exposures, and leukocyte composition.
Results: The study included 367 survivors (50.0% males; median age at blood draw for DNAm = 32.75 and interquartile range = 27.9-38.5 years). EWAS identified an epigenome-wide significant association between cg25793377 and overall SVI (beta=0.18, SD=0.033, $p=8.73\times10^{-8}$). Specifically, this association was significant for the socioeconomic domain of the SVI (beta=0.14, SD=0.035, $p=8.11\times10^{-5}$), but not significant for three other domains of SVI). Moreover, this CpG was not associated with personal-level SDOH factors, including educational attainment and personal income. This CpG maps to FFAR*4*, which encodes a G protein-coupled receptor (GPR) belonging to the rhodopsin family of GPRs and is a receptor for free fatty acids, including omega-3s, indicated in suppression of anti-inflammatory responses and insulin sensitizing; cg25793377 is also associated with protein levels in whole blood, including GZMK and TNFRSF17, both implicated in immune function and pro-inflammation.
Conclusion: This study highlights the potential impact of neighborhood-level socioeconomic status in epigenome alteration among childhood cancer survivors with African ancestry, suggesting that socioeconomic vulnerability has molecular effects. These findings support the hypothesis that distal social factors become biologically embedded through epigenetic modulation, potentially influencing health trajectories in childhood cancer survivors.

#2261 Distinct genomic and high-risk cytogenetic features in black multiple myeloma patients: Insights from the PE-CGS Network.

Yizhe Song[1], Reyka G. Jayasinghe[1], Mark Fiala[1], Michael J. Slade[1], Song Cao[1], Fernanda Martins Rodrigues[1], R. Jay Mashl[1], Erin Linnenbringer[2], Bailey Martin-Giacalone[2], Yingduo Song[1], Erik Storrs[1], Chien-Wei Peng[1], Fankun Zeng[1], Hasani Bland[2], Danielle Grasso[2], Julie Fortier[1], Zachary Crees[1], Robert S. Fulton[3], Shelly O'Laughlin[3], Tracie L. Deluca[3], Eric J. Duncavage[4], David H. Spencer[1], Ravi Vij[1], Kiran Vij[4], Bettina F. Drake[2], Ryan C. Fields[2], Graham A. Colditz[2], Li Ding[1]

[1]Department of Medicine, Washington University School of Medicine, St. Louis, MO,[2]Department of Surgery, Washington University School of Medicine, St. Louis, MO,[3]Department of Genetics, Washington University School of Medicine, St. Louis, MO,[4]Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO

Introduction: Landmark cancer genome sequencing programs have significantly advanced cancer research, leading to novel therapies and preventive strategies. However, the underrepresentation of racial minority groups in these studies limits the applicability of findings and leaves critical gaps in understanding cancer disparities. The Participant Engagement and Cancer Genome Sequencing (PE-CGS) Network, established under the National Cancer Institute's Cancer Moonshot, seeks to address these gaps by engaging with understudied populations. Multiple myeloma (MM), which disproportionately affects Black patients with higher incidence, exemplifies the need for inclusive research. High-risk cytogenetic events are strongly associated with poor outcomes in MM but are not well characterized in Black patients due to limited data. To address these disparities, we analyzed the genomic landscape of Black MM patients recruited through the PE-CGS Network.

Methods: A total of 191 self-reported Black MM patients (59% female, aged 41-98 years, median: 66) were recruited. Whole-exome sequencing was performed on 124 tumor-normal paired samples, including 83 newly diagnosed MM (NDMM), 20 plasmacytoma, and 21 relapsed/refractory MM (RRMM) cases. We conducted a 1:2 matched case-control study comparing Black NDMM patients from the PE-CGS study with White NDMM patients from MMRF CoMMpass study, matched by age and sex, to identify genomic differences between two groups. Three high-risk cytogenetic events, del(17p), del(1p) and gain(1q), were assessed in both groups. Copy number variants (CNVs) were categorized based on the tumor-to-normal copy number ratio as high amplification (≥4 copies), gain (>2.5 & <4 copies), deletion (<1.5 copies), or neutral (1.5-2.5 copies).

Results: Among 124 tumor samples from Black MM patients, 36.4% harbored at least one high-risk cytogenetic event. In the 83 tumor samples from Black NDMM patients, del(17p) occurred in 22.3%. High amplification of 1q was present in 25.30% of Black patients, higher than 8.04% in White patients. Del(1p) was observed in 18.18% of tumors from Black patients. Somatic mutation analysis revealed that KRAS (22.1%) and NRAS (15.6%) were the most frequently mutated genes among Black patients. TP53 mutations were observed in 5.7% of Black patients, exceeding previously reported frequencies. TP53 CNVs varied by self-identified race, with high amplification more frequent in White (30.36%) than Black patients (1.61%).

Conclusions: In this study, we highlight the distinct genomic landscape in Black MM patients, including higher rate of high-risk cytogenetic events and unique mutational patterns compared to Whites. These findings underscore the importance of engaging underrepresented populations in cancer research, as exemplified by the PE-CGS Network, to improve risk stratification and advance equitable healthcare outcomes.

#2262 A case-control analysis of germline pathogenic variants among patients with sarcoma.

Adela Rodriguez Hernandez[1], Miki Horiguchi[2], Carolyn Horton[3], Linda M Polfus[3], Brittany L. Bychkovsky[1], Colin Young[3], Suzanne George George[4], Priscilla Merriam[4], Judy E Garber[1], Huma Q Rana[1]

[1]Division of Cancer Genetics and Prevention, Dana-Farber Cancer Institute, Boston, MA, USA, Dana-Farber Cancer Institute, Boston, MA,[2]Department of Data Science, Dana-Farber Cancer Institute,Department of Biostatistics, Harvard T.H. C, Dana-Farber Cancer Institute, Boston, MA,[3]Ambry Genetics, Aliso Viejo, CA,[4]Dana-Farber Cancer Institute, Boston, MA

Introduction: Sarcomas are heterogeneous malignancies with diverse subtypes and treatments. The utility of multi-gene germline testing in patients (pts) with sarcoma is unclear. This study examines the prevalence and distribution of germline pathogenic variants (PVs) in pts with sarcoma compared to controls to refine genetic testing recommendations.

Methods: This retrospective case-control study included pts with sarcoma who underwent germline testing of 37 cancer-related genes from January 2016 to January 2024 at Ambry Genetics (Aliso Viejo, CA). A control group of 1464 cancer-free individuals matched 1:3 by age, sex, and ethnicity, served as the comparator. The prevalence of PVs was compared between cases and matched controls using Fisher's exact test for each selected gene. The degree of association was summarized with odds ratios (ORs) and corresponding 95% exact confidence intervals (CIs). In the case-only cohort, clinical characteristics were evaluated using logistic regression to assess associations with the presence of PVs in any gene.

Results: Among 488 pts with sarcoma, 67.8% (n=331) were female and 32.2% (n=157) male, with a median age at sarcoma diagnosis of 47 years (IQR: 0.5-87.5). PVs were found in 26.2% (n=128) of the pts. The prevalence of PV in pts with sarcoma and no other cancer diagnosis compared to pts with sarcoma and multiple cancer diagnoses was 24.0% (n=61) and 28.6% (n=67) (p=0.141) respectively. PV burden was increased among cases compared to the control group (26.2% vs 12.0%) (OR 2.62, CI: 2.01-3.41; p <0.001). A higher frequency of PV was observed in the cases compared to the control group in the following genes: *BRCA2* (4.1% vs 2.1%; p= 0.028), *CHEK2* (2.8% vs 1.0%; p=0.012), *NF1* (2.7% vs 0.1%; p<0.001), *POT1* (0.9% vs 0.1%; p=0.047), *SDHA* (1.4% vs 0.4%; p= 0.043) and *TP53* (5.5% vs 0.1%; p < 0.001). Among *CHEK2* PV, 4/13 were not founder mutations, and the low-risk variants (*p.I157T*, *p.S428F*, *p.T476M)* were excluded from the PV count. There was no significant difference in age at sarcoma diagnosis (45.5 vs. 47.0 years; p=0.172) between PV carriers and non-carriers. Multivariable analysis identified male sex (OR 1.63; p=0.039) and younger age at testing (OR 0.99; p=0.037) as significant factors associated with PV presence.

Conclusions: This study reveals a prevalence of germline PVs exceeding 25% among pts with sarcoma, reinforcing associations with *BRCA2*, *POT1,* and *TP53* and uncovering potential new signals in *CHEK2*. Our findings underscore the importance of comprehensive genetic testing in adult pts with sarcoma, as age at diagnosis alone is insufficient to predict PV status. The lack of family history data in this study introduces potential bias related to referral patterns for germline testing. These results contribute to refining germline testing criteria and advancing personalized prevention strategies. Validation in future, unbiased cohorts is essential to confirm these findings.

#2263 DNA methylation-estimated C-reactive protein levels and lung cancer risk and mortality in individuals with a heavy smoking history.

L. Grieshober[1], S. Graw[2], M. J. Barnett[3], G. E. Goodman[3], C. Chen[3], D. C. Koestler[4], C. J. Marsit[5], J. A. Doherty[1];
[1]Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, [2]Everest Clinical Research Corporation, Markham, ON, Canada, [3]Fred Hutchinson Cancer Center, Seattle, WA, [4]University of Kansas Medical Center, Kansas City, KS, [5]Rollins School of Public Health, Emory University, Atlanta, GA

Inflammation, a hallmark of cancer and chronic disease, is an established risk factor for lung cancer and is also associated with worse survival in lung cancer cases. C-reactive protein (CRP) is one circulating biomarker of inflammation that has been shown to be associated with DNA methylation (DNAm) in epigenome-wide association studies (EWAS). Several epigenetic CRP scores have been proposed using results from these studies, offering the unique opportunity to approximate CRP in studies without directly measured CRP. A validated DNAm CRP score was recently published by Hillary et al., which explains 18% of CRP variation beyond age, sex, and CRP-based genotype. This score, mCRP, includes 1,468 CpGs obtained from elastic net regression on EWAS results for measured CRP in a training set of nearly 18k individuals. Using an additive linear combination of the published CpGs and EWAS regression weights, we estimated pre-diagnosis mCRP in individuals with a heavy smoking history from the β-Carotene and Retinol Efficacy Trial (CARET) and assessed associations with both lung cancer risk and mortality for all cases and by histotype. Analytic samples included: 316 lung cancer case-control pairs through 2005, matched on age (±5 years), sex, race and ethnicity, enrollment year (±2 years), smoking status (ever/never), asbestos exposure, and follow-up time, (mean 57 pack years, range 11-185) for risk; and 372 lung cancer cases diagnosed through 2013 (mean 59 pack years, range 20-162) for mortality. DNAm data was assayed on the Illumina EPIC array in blood collected on average 4.3 years before diagnosis in cases for risk and 4.9 years for mortality. Conditional logistic regression models adjusted for age and pack years revealed no strong evidence of increased lung cancer risk with increasing mCRP. Cox proportional hazards models adjusted for age, sex, smoking status (current/former), pack years, and time from blood draw to diagnosis with stage as a stratification variable (early I/II, advanced III/IV, unknown) showed a greater hazard of death per standard deviation increase in mCRP among all lung cancer cases (Hazard Ratio (HR) 1.13, CI: 1.02-1.25), adenocarcinoma (HR 1.21, CI: 1.02-1.43), and small cell carcinoma (SCLC; HR 1.33, CI: 1.06-1.68). No associations were observed for squamous cell carcinoma, and results were similar for lung cancer-specific mortality among each case group. While these results do not provide support that pre-diagnosis mCRP is associated with lung cancer risk in individuals with a heavy smoking history, we observed that greater pre-diagnosis mCRP may be an indicator of mortality in members of this population. This work highlights the possible importance of pre-diagnosis inflammation via mCRP and mortality in heavy smokers who later develop lung cancer, particularly adenocarcinoma and SCLC.

#2264 Disparities in perceptions and uptake of genetic testing among the United States population: trends from 2011-2022.

Ariana Naaseh, Mengyao Shi, Steven Tohmasi, Erin L. Linnenbringer, Julie A. Margenthaler, Graham A. Colditz, Yin Cao

Department of Surgery, Washington University School of Medicine in St. Louis, St. Louis, MO

Background: Over the last decade, genetic testing has become more readily available, but it is not known whether this has translated to greater public awareness and usage over time. We aimed to evaluate patterns and temporal trends in genetic testing awareness and usage, along with their sociodemographic and cancer history correlates, in the United States (US) population.
Methods: We performed a serial, cross-sectional analysis of nationally representative data from the National Cancer Institute's Health Information National Trends Survey (HINTS). We analyzed survey responses from adults aged 18 or older from HINTS 4 Cycle 1 (2011), 4 Cycle 2 (2012), 4 Cycle 3 (2013), 4 Cycle 4 (2014), 5 Cycle 1 (2017), 5 Cycle 4 (2020), 6 (2022). Crude linear trend was evaluated using a linear regression model and multivariable logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CIs).
Results: Data on 22,256 individuals (mean [SD] age, 46.7 [17.0] years; 13,208 (50.7%) female; non-Hispanic White 13,559 (65.2%)) were analyzed from 2011-2022 HINTS data. Awareness of genetic testing increased from 36.6% in 2011 to 81.7% in 2022 ($p<0.001$). Older adults, men, non-Hispanic Black individuals, non-Hispanic Asian individuals, Hispanic individuals, those with an education level less than some college, and individuals with annual household incomes less than $75,000 were significantly less likely to be aware of genetic testing. Uptake of genetic testing, however, lagged behind awareness with rates of 21.4% in 2020 and 35.6% in 2022. Uptake of genetic testing was significantly lower among non-Hispanic Asian individuals, those with household incomes under $35,000, and those without a personal history of cancer.
Conclusion: Despite high levels of awareness, genetic testing uptake remains disproportionately low, with notable disparities across sociodemographic groups. Targeted educational interventions focused on genetic testing are necessary for marginalized populations and the providers caring for them. Further study is needed to understand genetic testing uptake patterns in the US.

#2265 Identifying disparities in prostate cancer treatment and genomic data availability utilizing the All of Us Research Program.

Dhruv G. Pillai[1], Benjamin C. Brim[1], Nicholas G. Micheletti[2], Hyoyoung Choo-Wosoba[2], Laura M. Yee[2], Cindy H. Chau[1], William D. Figg[1]

[1]Genitourinary Malignancies Branch, National Cancer Institute, Bethesda, MD,[2]Office of Collaborative Biostatistics, National Cancer Institute, Bethesda, MD

The All of Us Research Program (AoURP) is a clinical and genomic database established by the National Institutes of Health that aims to encapsulate the diversity of the United States population. AoURP intends to enroll 1 million participants of diverse backgrounds through its robust outreach efforts, which selectively engage communities under-represented in biomedical research. AoURP may help elucidate disparities in prostate cancer (PCa) treatment and clinical research participation. This study aimed to explore whether PCa patients of various demographic backgrounds receive novel cancer treatments and participate in genomic research at different rates. To obtain a diverse cohort of participants, we identified every PCa patient in AoURP (n = 6271). We determined whether these patients opted for genetic testing by AoURP and extracted electronic health record data on commonly prescribed PCa treatments, including androgen deprivation therapy, second-generation anti-androgens, chemotherapy, prostatectomy, radiation, and bone-targeting agents. We stratified patients who obtained genetic testing and standard PCa treatments by demographic factors of household income, educational attainment, race, ethnicity, and distance from a National Cancer Institute (NCI)-designated comprehensive cancer center (NCIDCCC). To establish distance from an NCIDCCC as a categorical variable, we determined whether PCa patients resided within forty- and eighty-mile radii of one of these facilities. The study revealed that Black/African American patients received bone-targeting agents less frequently than their White/Caucasian counterparts. We also observed that more patients inside forty- and eighty-mile radii of an NCIDCCC obtained second-generation anti-androgens and bone-targeting agents than those outside these thresholds. Upon stratifying patients with history of prostatectomy or radiation based on additional thresholds (40, 50, 60, 70, 80, 90, and 100 miles), we found that more patients received prostatectomy than radiation at greater distances from NCIDCCCs. This result indicates that geographic factors may influence treatment of localized disease, with prostatectomy being more common for patients farther from major clinical centers. Additionally, we detected no variability in the rates at which different demographic groups underwent genetic testing. Overall, our study suggests that distance from an NCIDCCC and race may represent risk factors for treatment underuse in PCa patients, highlighting that additional outreach efforts by NCIDCCCs are necessary to encourage equitable usage of cancer treatments.

#2266 Evaluations of more than 1500 blood metabolite biomarkers with risk of eight common cancers and their subtypes using genetic instruments.

Zheng Guo, Guochong Jia, Fangcheng Yuan, Lili Liu, Shuai Xu, Jirong Long, Wei Zheng

Division of Epidemiology, Vanderbilt University Medical Center, Nashville, TN

Background: Many epidemiologic studies have measured blood metabolites and evaluated their associations with risk of cancers. However, conventional epidemiologic studies may suffer from various biases, limiting their ability for causal inferences. We performed a large Mendelian randomization (MR) study to systematically evaluate blood metabolite biomarkers in relation to cancer risk, including large numbers of associations not previously investigated in either convention epidemiologic or MR studies.
Methods: We systematically searched the literature and obtained data from 29 genome-wide association studies (GWAS) to identify blood metabolite-associated genetic loci (mQTLs) among 324,771 individuals of European ancestry. After systematically evaluating all metabolites and mQTLs evaluated in these studies, we selected 1630 metabolite biomarkers for MR analyses using summary statistic data generated from GWAS conducted among 2,068,121 individuals of European ancestry for eight common cancers (breast, colorectal, endometrial, kidney, lung, ovarian, pancreatic, and prostate) and 19 subtypes of breast, kidney, lung, and ovarian cancers. The inverse variance weighted (IVW) method was used as the primary approach for MR analyses. Other methods, including MR-Egger, and MR-PRESSO, were used to evaluate possible pleiotropic effects and robustness of study findings. Statistical significance was judged using Bonferroni and FDR corrected P-value to reduce type 1 errors.
Results: We identified 106 biomarker-cancer associations under the significant threshold after Bonferroni correction. Among these, 68 were associated with an increased risk of cancer, while 38 were associated with a decreased risk of cancer. Under an FDR-corrected $P < 0.05$ but not reaching the Bonferroni-adjusted significant threshold, we identified 119 additional associations, including 65 metabolite biomarkers as potential risk factors and 54 as potential protective factors.
Conclusion: Our MR analysis uncovered large numbers of novel associations and provided strong evidence for causal inferences of blood metabolite biomarkers with cancer risk.

#2267 Mapping genetic risk for Barrett's esophagus and esophageal adenocarcinoma to key tissues and transcriptional regulators.

Elnaz Naderi[1], Xu Zhang[1], Kathryn Graz[1], Carlos Maj[2], Johannes Schumacher[2], Li Yan[3], Matthew F. Buas[1]

[1]Memorial Sloan Kettering Cancer Center, New York, NY,[2]University of Marburg, Marburg, Germany,[3]Roswell Park Comprehensive Cancer Center, Buffalo, NY

Background: Esophageal adenocarcinoma (EAC), the most common subtype of esophageal cancer in Western countries, typically arises from Barrett's esophagus (BE), a pre-neoplastic condition marked by replacement of the normal esophageal epithelium with intestinal metaplasia. Genome-wide association studies (GWAS) have revealed a substantial heritable component of BE/EAC susceptibility, but mechanistic understanding remains limited. Identifying tissues, cell types, and transcriptional regulators mediating genetic risk is essential for elucidating etiologic pathways and defining novel molecular targets for translation.
Methods: We employed a permutation-based approach to evaluate the enrichment of BE/EAC-associated variants in open chromatin regions (OCRs) across >50 diverse tissues and cell types. For a subset of implicated tissues, we further assessed enrichment in the binding regions of multiple transcription factors (TFs) using ChIP-seq profiles, with complementary analyses based on DNAse-I footprinting data and motif scanning. Multiple testing correction was implemented using the false discovery rate (FDR<0.05).
Results: BE/EAC variants were significantly enriched in OCRs of gastrointestinal tissues, particularly fetal stomach (OR = 1.6, P = 6e-04) and fetal large intestine (OR = 1.5, P = 1.5e-03). Enrichments were also observed in muscle (OR = 1.5, P = 9e-04) and fibroblast (OR = 1.4, P = 8.8e-03) cells, suggesting a broader multi-lineage regulatory landscape. Key TFs implicated in subsequent testing included GATA6 (OR = 3.0, P <1e-04) and GATA4 (OR = 2.2, P = 2e-04) in GI tissues; EZH2 in muscle cells (OR = 5.7, P = 2e-04); and EZH2 (OR = 3.5, P <1e-04) and AR (OR = 1.4, P = 1.2e-03) in fibroblast cells.
Conclusions: This study indicates that BE/EAC risk variants act through regulatory elements across diverse tissues, with prominent roles suggested in gastrointestinal, muscle, and stromal cells. The significant enrichment signals for transcription factors such as GATA6, GATA4, and EZH2 underscore their regulatory importance in GI-tract development and BE/EAC pathogenesis. These insights enhance our understanding of the cellular/molecular origins and genetic underpinnings of BE/EAC, providing a promising foundation for experimental and translational studies targeting specific transcriptional regulatory networks.

#2268 Prospective and genetic analyses implicate lower immunosurveillance in the aetiology of prostate cancer.

Mahboubeh Parsaeian[1], Wing Ching Chan[1], Joshua Atkins[1], Keren Papier[1], Trishna Desai[1], Zhe Huang[1], David Conti[2], David Bogumil[3], Jiayi Shen[3], Malcolm Sim[4], Konstantinos Tsilidis[5], James Yarmolinsky[5], Sabina Rinaldi[6], Rudolf Kaaks[7], Verena Katzke[7], Matthias Schulze[8], Catarina Schiborn[9], Saverio Caini[10], Lorenzo Milani[11], Raul Zamora-Ros[12], Marcela Guevara[13], Maria-Jose Sanchez[14], Maria-Dolores Chirlaque[15], Pilar Amiano[16], The PRACTICAL Consortium, Timothy Elliott[4], Ian Mills[17], Elio Riboli[5], Loic Le Marchand[18], Christopher Haiman[2], Tim Key[1], Karl Smith-Byrne[1], Ruth Travis[1]

[1]Cancer Epidemiology Unit, University of Oxford, Oxford, United Kingdom,[2]Department of Preventive Medicine, University of Southern California, Los Angeles, CA,[3]Department of Population and Public Health Science, University of Southern California, Los Angeles, CA,[4]Oxford Cancer Centre for Immuno-oncology, University of Oxford, Oxford, United Kingdom,[5]Department of Epidemiology and Biostatistics, Imperial College London, London, United Kingdom,[6]Nutrition and Metabolism Branch, International Agency for Research on Cancer, Lyon, France,[7]Division of Cancer Epidemiology, German Cancer Research Center, Heidelberg, Germany,[8]Institute of Nutritional Science, University of Potsdam, Potsdam, Germany,[9]Department of Molecular Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany,[10]Cancer Risk Factors and Lifestyle Epidemiology Unit, Institute for Cancer Research, Prevention, and Clinical Network, Florence, Italy,[11]Department of Clinical and Biological Sciences, University of Turin, Turin, Italy,[12]Unit of Nutrition and Cancer, Bellvitge Biomedical Research Institute, Barcelona, Spain,[13]Instituto de Salud Publica y Laboral de Navarra, Pamplona, Spain,[14]Escuela Andaluza de Salud Publica (EASP), Granada, Spain,[15]Department of Epidemiology, Murcia University, Murcia, Spain,[16]Ministry of Health of the Basque Government, Sub Directorate for Public Health and Addictions of Gipuzkoa, San Sebastian, Spain,[17]Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom,[18]University of Hawaii Cancer Center, Honolulu, HI

Tumor promoting inflammation and the ability to evade immune destruction are two of the hallmarks of cancer, but there is limited prospective evidence for the role of specific inflammation and immune-related pathways and protein markers in the development of prostate cancer. This study used data on the circulating levels of 368 inflammation and immune-related proteins (Olink Explore Inflammation I panel) from a nested case-control design within the European Prospective Investigation into Cancer and Nutrition (EPIC), including 1,434 men who developed prostate cancer and 1,434 matched controls - 488 of the cases also had clinically aggressive disease. Conditional logistic regression was used to estimate protein associations with cancer risk in EPIC, per standard deviation and the effective number of tests (ENT) was used to control for multiple testing. We additionally performed a fixed-effect meta-analysis combining associations for these proteins estimated in EPIC with those in 21,481 men from UK Biobank (UKBB), of whom 1,147 developed prostate cancer. Logistic regression was used to estimate exome protein score association with cancer risk in UKBB and replication analyses were performed in the Multiethnic Cohort (MEC). Mendelian randomisation and colocalisation analyses using data on 79,148 overall prostate cancer cases from the PRACTICAL consortium were also conducted to aid triangulation. In EPIC, no inflammatory proteins were associated with prostate cancer risk overall or with more clinically aggressive subtypes after correction for multiple testing. In the EPIC+UKBB meta-analyses, FLT3LG and CNTNAP2 were significantly associated with overall prostate cancer risk (RR_{Meta}: 0.88, 95% CI: 0.84-0.92 and RR_{Meta}: 1.10, 95% CI: 1.05-1.16, respectively), and in the analyses stratified by time to diagnosis, IL15 was associated with risk of prostate cancer diagnosed more than seven years after blood draw (RR_{Meta}: 0.86, 95% CI: 0.81-0.93), along with five other proteins (FLT3LG, BCL2L11, PGF, CKAP4, and TNFRSF11A). Exome protein scores in UKBB identified PARP1 as associated with an increased risk of prostate cancer (OR: 1.05, 95% CI: 1.02-1.07), which replicated in MEC (OR: 1.04, 95% CI: 1.01-1.08). MR and colocalisation analyses also identified SPINT2 (OR: 2.11, 95% CI: 1.70-2.63) and NME3 (OR: 1.25, 95% CI: 1.11-1.41) as being associated with an increased risk of prostate cancer overall. Our findings suggest higher levels of proteins involved in immunosurveillance pathways, including the recruitment and activation of natural killer and T cells, may be linked to a lower risk of developing prostate cancer, while PARP1 inhibition may be relevant for prostate cancer prevention.

#2269 Factors associated with declining to participate in cancer genetic and genomic studies among Hispanic/Latine patients with colorectal cancer: findings from a Cancer Moonshot[SM] project.

Diego I. Alvarez-Lopez[1], Joel Sanchez Mendez[1], Jessica Sanchez[2], Fibi Berhane[1], Carmen Chavez[3], Lucia Enriquez[3], Vernon S. Pankratz[2], Andrew L. Sussman[2], Ursa A. Brown-Glaberman[2], Stephanie Solomon Cargill[4], Heinz-Josef Lenz[3], John D. Carpten[5], Shiraz I. Mishra[2], Mariana C. Stern[1]

[1]Department of Population and Public Health Sciences, Keck School of Medicine, University of Southern California, Los Angeles, CA,[2]University of New Mexico Health Sciences Center, University of New Mexico Comprehensive Cancer Center, Albuquerque, NM,[3]Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, CA,[4]Alden March Bioethics Institute, Albany Medical College, Los Angeles, NY,[5]City of Hope Comprehensive Cancer Center, Duarte, CA

Introduction: Colorectal cancer (CRC) is the third leading cause of cancer-related mortality among Hispanic/Latine (H/L) men and women in the United States (US), yet H/L patients are underrepresented in cancer genetic/genomic studies. As part of the Cancer Moonshot[SM] initiative, the Center for Optimizing Engagement of Hispanic Colorectal Cancer Patients in Cancer Genomic Characterization Studies (COPECC) launched the ENLACE study. This study engages H/L patients with CRC to characterize germline genetics and tumor somatic mutations and gathers information on participation determinants. We examined factors influencing the decision to decline participation in cancer genetic/genomic research.

Methods: We compared characteristics of patients with CRC diagnosed between February 2014 and November 2024 who accepted to participate in the ENLACE study to those who declined and wanted no further contact. We descriptively summarized sociodemographic characteristics and made between-group comparisons using Student's t-tests for continuous variables and Fisher's exact tests for categorical variables. An open-ended question was included to capture reasons for declining participation, which were analyzed using inductive thematic analysis. To further explore these barriers, we initiated a scoping review on reasons for declining participation in cancer genetics/genomic studies in the US. We deployed a piloted search strategy across MEDLINE (PubMed), Embase (Ovid), and Scopus and have identified 12,726 peer-reviewed references in English (MEDLINE: 5,970; Embase: 1,657; Scopus: 5,099). Independent reviewers will assess full texts for inclusion, and findings will be summarized numerically and narratively.

Results: We included data from 249 patients in the analysis; 40 (16.1%) had declined to participate. Decliners were, on average, 5 years older than participants ($p<0.05$), less likely to be Catholic (52.5% vs. 71.8%; $p<0.05$), and more likely to be Christian (40.0% vs. 19.1%; $p<0.05$). The proportion of patients diagnosed with stage 3 or 4 CRC was similar (decliners = 45.0% vs. participants = 47.1%; $p=0.86$). The three most common reasons for declining were: 1) lack of energy due to treatment; 2) prior genetic testing; and 3) concerns about adding further stress to their health situation.

Conclusions: Despite the high burden of CRC among the H/L population, participation in cancer genetic/genomic studies remains low. Our findings highlight factors influencing engagement and offer insights for culturally tailored strategies to enhance participation in cancer research. Clarifying to patients that the testing offered by ENLACE is more comprehensive than prior genetic testing (e.g., standard commercial testing) could address this specific reason for declining participation and improve engagement in the study.

#2271 Real-World data of germline variants in 5,893 Korean hereditary cancer patients.

Ha-Eun Lee[1], Sang-Yoon Park[2], Seeyoun Lee[3], So-Youn Jung[3], Myong Cheol Lim[2], Han-Sung Kang[3], Jun-Ha Jang[1], Sun-Young Kong[1]

[1]Targeted Therapy Branch, National Cancer Center - Korea, Goyang, Korea, Republic of,[2]Center for Gynecologic Cancer, National Cancer Center - Korea, Goyang, Korea, Republic of,[3]Center for Breast Cancer, National Cancer Center - Korea, Goyang, Korea, Republic of

Background: The advent of next-generation sequencing (NGS) has significantly enhanced hereditary cancer comprehensive germline genetic testing. While initial efforts targeted BRCA1 and BRCA2, NGS now reveals a broader range of cancer susceptibility genes, highlighting diverse genetic variations. The variability of pathogenic variants (PVs) across ethnic groups underscores the critical importance of population-specific studies. This study evaluates PVs frequency and their clinical relevance in Korean hereditary cancer patients, providing detailed insights into the genetic characteristics in this population.
Methods: We analyzed real-world data from 5,893 patients who underwent genetic testing at the National Cancer Center in Korea between 2006 and 2023. Genetic testing included either *BRCA1/2* test (n=2,342) or NGS panel testing (n=3,551), with the type of test determined by the physician's clinical decision and insurance reimbursement criteria. We collected demographic characteristics, personal and family history and analyzed the impact of these factors on PV carrier status.
Results: The median age of the patients was 51.3 years (range: 4-92), with 96.3% female. The distribution of diagnosed cancers was as follows: breast cancer (55.8%), ovarian cancer (37.2%), thyroid cancer (2.4%), pancreatic cancer (2.4%), endometrial cancer (2.4%), colorectal cancer (1.9%), prostate cancer (1.7%), lung cancer (0.8%), gastric cancer (0.8%), other cancers (2.8%) and multi-organ cancers (n=677). Among 43.6% patients with a family history of cancer, 87.1% reported a first-degree relative (FDR), 40.5% a second-degree relative (SDR), and 5.2% a third-degree relative (TDR).PVs were observed in 184/1,612 (11.4%) breast cancer, 202/732 (27.5%) ovarian cancer in *BRCA1/2* test and 221/1,681(13.1%) breast cancer, 333/1,463 (22.8%) ovarian cancer in NGS panel testing. In patients who underwent NGS panel testing, 606 individuals represented 626 PVs. The genes most commonly affected included *BRCA1* (35.8%), *BRCA2* (27.3%), *RAD51D* (4.2%), *ATM* (3.8%), *PALB2* (3.5%), *MUTYH* (3.0%), *CHEK2* (2.4%), *MSH2* (2.4%), *BRIP1* (2.2%), *MLH1* (2.1%), *MSH6* (1.9%) and *PMS2* (1.8%).In breast and ovarian cancer, multiple organ involvement emerged as a significant risk factor for suspected germline PVs ($p < 0.01$). PV of breast cancer patients was significantly associated with family history of 50.9% with FDR, 13.8% with SDR, and 1.7% with TDR.
Conclusion: Our study identified overall 16.6% prevalence of germline PVs in Korean patients and there is a notable finding involving genes other than BRCA1/2, which highlights the broader spectrum of genetic alterations, suggesting the need for greater awareness and preparedness in hereditary cancer.This study was supported by grant from the National Cancer Center. (grant number NCC-2410821).

#2273 The spectrum of germline cancer gene mutations in Central American pediatric cancer.

Jesica M. Godinez Paredes[1], Claudia Garrido[2], Patricia Calderon[3], Isabel Rodriguez[1], Yi Xie[1], Jia Liu[4], Wen Luo[4], Herbert Higson[4], Kristine Jones[4], Hong Lou[4], Lisa Garland[1], Dulilio Leyton[3], Lesly Chapman[1], Edmundo Torres-Gonzalez[1], Julie Sawitzke[4], Matthew Gianferante[1], Sandra Luna-Fineman[2], Veronica Giron[2], Mauricio Castellanos[2], Federico Antillon-Klussman[5], Lisa Mirabello[1], Gerardo Mejia[6], Michael Dean[1]

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD,[2]Unidad Nacional de Oncologia Pediatrica, Guatemala City, Guatemala,[3]Department of Hematology/Oncology, Children's Hospital Manuel de Jesus Rivera, Managua, Nicaragua,[4]Frederick National Laboratory for Cancer Research, National Cancer Institute, Frederick, MD,[5]Unidad Nacional de Oncologia Pediatrica and Universidad Francisco Marroquin, Guatemala City, Guatemala,[6]Department of Genetics, MINSA, Managua, Nicaragua

Pediatric cancers are a diverse set of both hematologic and solid malignancies. Approximately 5% of pediatric cancer cases are caused by known genetic conditions, but little is known about genetic susceptibility in Central American children. Most pediatric leukemia and lymphoma cases have high response and cure rates, and many solid malignancies have better outcomes than similar adult cancers. We evaluated the germline genetic variation of pediatric solid tumor cases in Guatemala and Nicaragua in reference hospitals, capturing most of the cancer cases nationwide. We performed exome sequencing on 1360 subjects (957 with solid tumors and 405 with leukemias) and classified variants using an automated pipeline using ClinVar and InterVar and by manual review. The most prevalent cancer types were acute lymphocytic leukemia (357), Hodgkin's lymphoma (229), osteosarcoma (119), and retinoblastoma (93). Pathogenic mutations were identified in 25 pediatric cancer susceptibility genes. Excluding retinoblastoma, 6.5% of cases had a Pathogenic or Likely Pathogenic germline mutation in a cancer-predisposing gene. As expected, *RB1* mutations were frequent in retinoblastoma patients, with 81% of confirmed bilateral cases having a Pathogenic or Likely Pathogenic mutation. Mutations in the *NF1* gene were observed in neurofibromatosis cases and non-rhabdomyosarcoma. *TP53* mutations were observed in osteosarcoma and rhabdomyosarcoma cases, and *WT1* mutations were observed in Wilms tumor and renal cancer cases. A single case of pheochromocytoma was found to have a novel *VHL* gene mutation. Higher rates of pediatric Hodgkin's lymphoma (HL) are found in Central American countries, and the cause of this is unknown. We did not find a significantly mutated gene in the HL cases, suggesting that a common germline mutation is not a significant factor. In conclusion, 6.5% of Central American solid tumor cases have a germline mutation in genes known or suspected to influence cancer risk.

#2274 Implications of multiple myeloma polygenic risk scores (PRS) for MGUS.

Angelica Macauda[1], Alyssa Clay-Gilmour[2], Cristine Allmer[1], Danelle Moonen[1], Aaron D. Normann[1], Nicholas J. Boddicker[1], Joselle M. Cook[1], Linda Baughn[1], Elizabeth E. Brown[3], Vincent S. Rajkumar[1], Esteban Braggio[4], David L. Murray[1], Susan L. Slager[1], Shaji Kumar[1], Celine M. Vachon[1]

[1]Mayo Clinic, Rochester, MN,[2]University of South Carolina, Greenville, SC,[3]University of Alabama, Birmingham, AL,[4]Mayo Clinic, Phoenix, AZ

Genome-wide association studies (GWAS) of multiple myeloma (MM) in individuals of genetic European ancestry (EA) have identified 35 susceptibility loci. Co-heritability analyses have revealed a strong genetic correlation between MM and its precursor condition, monoclonal gammopathy of undetermined significance (MGUS). Previous research validated the association between a polygenic risk score (PRS) based on 23 MM risk loci and the risk of MGUS. Went and colleagues recently reported 12 new MM risk loci. We constructed a MM-PRS using all 35 MM risk variants and assessed its association with MGUS. Additionally, among individuals with MGUS, we assessed associations of MM-PRS with established prognostic factors [M-protein (<0.2 g/dL; 0.2-1.5 g/dL; ≥1.5 g/dL); isotype (IgG, IgA, IgM); free light chain (FLC) ratio (Normal (0.26-1.65), Abnormal (<0.26 or >1.65))]. Our study population included 1,723 individuals with MGUS seen at Mayo Clinic, Rochester, MN and 14,175 controls from the Mayo Clinic Biobank who lived in the 27-county region around Rochester, MN, and screened negative for MGUS using MALDI-TOF MS. The majority of MGUS patients and controls were of EA. We used logistic or multinomial regression to calculate odds ratios (ORs) and 95% confidence intervals (CIs). All models were adjusted for age, sex, and the first three principal components. The MM-PRS was significantly associated with MGUS when assessed continuously (OR, 1.28 per standard deviation (SD); 95% CI, 1.22-1.35). When comparing to the middle quintile of the MM-PRS distribution, individuals in the highest quintile had 1.41-fold increased risk of MGUS (95% CI, 1.21-1.64), and individuals in the lowest quintile had 0.68 decreased risk of MGUS (95% CI, 0.57-0.81). Of the 35 SNPs evaluated, 27 demonstrated consistent directions of effect for risk of MGUS with published risk estimates for MM. The effect of MM-PRS differed by isotype ($P = 2.2 \times 10^{5}$), with higher MM-PRS associated with IgA MGUS (OR, 1.40 per SD; 95% CI, 1.08-1.81) and a lower MM-PRS associated with IgM (OR, 0.66 per SD; 95% CI, 0.52-0.84) compared to IgG MGUS. The MM-PRS was higher in MGUS patients with M-protein ≥ 1.5 g/dL compared to M-protein of size 0.2-1.5 g/dL, although not statistically significant (OR, 1.41 per SD; 95% CI, 0.95-2.08); there were no differences in the MM-PRS between MGUS patients with M-protein size <0.2 g/dL compared to 0.2-1.5 g/dL (OR, 1.01 per SD;95% CI, 0.81-1.26). There were also no differences in MM-PRS by FLC ratio, (OR, 0.95 per SD; 95% CI, 0.852-1.10 for abnormal vs. normal). Our findings of an association of the MM-PRS with risk of MGUS provides further evidence for shared heritability between MGUS and MM. Future studies are needed to examine whether the expanded MM-PRS adds to existing models for MGUS progression.

#2275 Exploring the genetic underpinnings of cancer-related fatigue in colorectal cancer patients.

Elham Kazemian[1], Qianxing Mo[2], Xiaoyin Li[2], Aasha I. Hoogland[2], Sylvia L. Crowder[2], Brian D. Gonzalez[2], Laura B. Oswald[2], Alix G. Sleight[1], Nathalie Nguyen[1], Nicole C. Lorona[1], Victoria Damerell[3], Khaled R. Komrokji[2], Kathi Mooney[4], Mary C. Playdon[4], Cornelia M. Ulrich[4], Christopher I. Li[5], David Shibata[6], Adetunji T. Toriola[7], Jennifer Ose[4], Anita R. Peoples[4], Erin M. Siegel[2], Julienne E. Bower[8], Biljana Gigic[3], Heather S. L. Jim[2], Jane C. Figueiredo[1]

[1]Cedars-Sinai Medical Center, West Hollywood, CA,[2]H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL,[3]Heidelberg University Hospital, Heidelberg, Germany,[4]University of Utah, Salt Lake City, UT,[5]Fred Hutchinson Cancer Research Center, Seattle, WA,[6], University of Tennessee Health Science Center, Memphis, TN,[7]Washington University St. Louis, St. Louis, MO,[8]University of California, Los Angeles, Los Angeles, CA

Background: Cancer-related fatigue (CRF) is a prevalent and debilitating symptom among colorectal cancer (CRC) patients. This study aimed to identify genetic variants associated with CRF trajectories and assess their relationships with clinical and demographic factors.
Methods: Participants (N=1,219) were recruited from the ColoCare Study across six U.S. sites and the University of Heidelberg in Germany. Eligible participants were adults (≥18 years) with a new diagnosis of primary colon or rectal cancer. CRF was assessed at five timepoints (baseline, 3, 6, 12, and 24 months) using the European Organization for Research and Treatment of Cancer (EORTC QLQ-C30) fatigue subscale. Longitudinal analysis of CRF patterns was performed using piecewise growth mixture models (GMMs), which identified three distinct trajectories: high, moderate, and low fatigue. Fatigue outcomes were assessed in three ways: as a binary variable (high vs. moderate/low trajectories), as a continuous measure (mean fatigue scores), and through an extreme phenotype approach (highest fatigue observed across all timepoints). Genome-wide association analyses (GWAS) were conducted using PLINK. Analyses were adjusted for potential confounders, including age, sex, cancer stage, smoking status, and population stratification (top two principal components). We performed fine-mapping analyses to identify independent secondary signals in known loci, focusing on SNPs previously associated with CRF and regions with p-values $<5 \times 10^{-6}$ in at least two of our GWAS models.
Results: Among 1,219 CRC patients, 378 (31.0%) reported high fatigue, while 651 (53.4%) were categorized as having low/moderate fatigue. Significant differences in cancer-related fatigue were observed by race/ethnicity, smoking status, cancer stage, recurrence status, and receipt of neoadjuvant treatment. When examining **fatigue as a binary outcome**, a locus on chromosome 4 near *LINC02505* was associated with higher risk of fatigue (rs6531463, OR = 3.25, 95% CI: 2.83-3.67, $p=3.88 \times 10^{-8}$). Several variants near *NEK10* and *SLC4A7* on chromosome 3 also showed suggestive associations (p-value range: $5\times10^{-8}<p<5\times10^{-6}$). Similar results were observed when examining **average fatigue as the outcome**. The most significant locus was on chromosome 3p24.1 near *NEK10* and *SLC4A7* (rs7617094, OR = 0.57, 95% CI: 0.47-0.69, $p=2.21 \times 10^{-8}$). For **extreme fatigue**, loci on chromosomes 3p24.1 and 5q13.1 also were associated with elevated risk (p-value range: $5\times10^{-8}<p<5\times10^{-6}$). Fine-mapping analyses identified 3 credible sets across 3 loci including 5q13.1, 3p24.1 and 5q13.1.

Conclusion: **This study investigates the genetic basis of CRF in CRC patients, identifying novel loci and refining known susceptibility regions.** Key loci associated with CRF include *LINC02505*, *NEK10*, and *SLC4A7*. These findings if validated offer potential targets for interventions focused on CRF prevention and management.</p<5×10</p<5×10

#2276 Whole exome sequencing of immune-mediated colitis in cancer patients treated with immune checkpoint inhibitors.

Pooja Middha[1], Zoe Quandt[2], Karmugi Balaratnam[3], Eduardo Cardenas[1], Christina J. Falcon[4], Princess Margaret Lung Group, Matthew A. Gubens[1], Scott Huntsman[1], Khaleeq Khan[3], Min Li[1], Christine M. Lovly[5], Devalben Patel[3], Luna Jia Zhan[3], Melinda C. Aldrich[6], Geoffrey Liu[7], Adam J. Schoenfeld[8], Elad Ziv[1]

[1]UCSF Helen Diller Family Comprehensive Cancer Ctr., San Francisco, CA,[2]Division of Endocrinology and Metabolism, Department of Medicine, University of California San Francisco, San Francisco, CA,[3]Princess Margaret Cancer Centre, Toronto, ON, Canada,[4]Fiona and Stanley Druckenmiller Center for Lung Cancer Research, Memorial Sloan Kettering Cancer Center, New York, NY,[5]Department of Medicine, Division of Hematology and Oncology, Vanderbilt University Medical Center and Vanderbilt Ingram Cancer Center, Nashville, TN,[6]Vanderbilt University Medical Center, Nashville, TN,[7]Princess Margaret Cancer Centre, Temerty School of Medicine, Dalla Lana School of Public Health, University of Toronto, Toronto, ON, Canada,[8]Memorial Sloan Kettering Cancer Center, New York, NY

Background: Immune checkpoint inhibitors (ICIs) have transformed cancer treatment, providing survival benefits across multiple cancer types. Despite this remarkable advancement, ~15-30% of patients develop immune-related adverse events (irAEs). One of the common irAEs is immune-mediated colitis, and the incidence ranges from 1% to 25% by type of therapy. Previously, we have shown that polygenic risk scores of ulcerative colitis predict colitis irAE. However, the role of rare coding variants on the development of colitis irAE remains largely unknown.

Methods: We conducted whole exome sequencing for colitis irAE in 1,215 cancer patients treated with ICIs. Association analyses were carried out for each gene separately for protein-truncating missense variants, predicted splice (Sp) variants, and non-synonymous predicted deleterious (deN; CADD≥20 or VEST≥0.8) or any non-synonymous (N) variants. The main association analyses were burden tests in which genotypes were collapsed to a 0/1 variable based on whether samples carried a variant of the given class. All models were adjusted for age at diagnosis, sex, type of ICI therapy, recruitment site, and five ancestry-informative principal components. The exome-wide threshold for significance was set at 2.5×10^{-6}, with genes beyond this considered significant.

Results: Among 1,215 patients treated with immune checkpoint inhibitors (ICIs), 60 (4.9%) developed colitis. We identified rare coding variants in two genes associated with an increased risk of colitis at the exome-wide significance level: PT+Sp+N variants in *LRRC47* (OR = 5.5, P = 2.4×10^{-6}) and PT+S+deN variants in *PFKP* (OR = 65.9, P = 1.2×10^{-6}). Additionally, PT+S+deN variants in *PTPRH* showed a suggestive association with colitis irAEs (OR = 8.8, P = 7.1×10^{-5}).

Conclusions: To our knowledge, this is the first study to assess the role of rare coding variants in colitis immune-related adverse events (irAEs) among cancer patients treated with ICIs. Our findings highlight two exome-wide significant associations, implicating variants in *LRRC47* and *PFKP* in the development of colitis irAEs. *LRRC47* has previously been linked to inflammatory bowel disease through differential methylation patterns. Furthermore, genes involved in glycolysis, including *PFKFB3*, *PKM*, and *PFKP*, have been shown to be upregulated in patients with ulcerative colitis. Abnormal activity of *PTPRH* has also been reported to disrupt the regulatory mechanisms of the intestinal mucosa, potentially contributing to inflammation. These findings provide novel insights into the genetic underpinnings of colitis irAEs in cancer immunotherapy.

#2277 Validation and context-dependent effects of a prostate cancer polygenic risk score in the All of Us Research Platform.

Shuyan Cheng[1], Austin Hammermeister Suger[1], Louisa B. Goss[2], Boya Guo[2], Sara Lindstrom[1], Burcu F. Darst[2]

[1]University of Washington, Seattle, WA,[2]Fred Hutchinson Cancer Center, Seattle, WA

Background: Polygenic risk scores (PRS) are valuable tools for assessing genetic susceptibility to prostate and other cancers. The All of Us (AoU) Research Program, with its diverse and inclusive cohort, provides a unique opportunity to validate and further investigate context-dependent associations of cancer PRS, offering insights into their applicability in diverse populations.
Methods: A previously established multi-ancestry PRS of 451 genetic variants associated with prostate cancer risk was calculated in AoU. Logistic regression models were employed to evaluate associations between the standardized continuous PRS and prostate cancer risk, adjusting for age and the top 10 principal components to account for population stratification. Comparisons were also made between individuals in the top PRS decile (90-100%) and those in the average range (40-60%). Analyses were stratified by genetic ancestry groups, age, BMI, and smoking status explored potential demographic and lifestyle factors affecting PRS predictive power.
Results: PRS validation included 4,668 prostate cancer cases and 71,644 controls across six genetic ancestry groups defined by AoU. Odds ratios (ORs) per unit increase in PRS were 2.23 (95% CI = 2.14-2.33) in European, 1.83 (95% CI = 1.67-2.00) in African, 2.16 (95% CI = 1.86-2.51) in American, 2.67 (95% CI = 1.53-4.67) in East Asian, 2.46 (95% CI = 1.14-5.33) in Middle Eastern, and 1.67 (95% CI = 1.00-2.77) in South Asian populations. Individuals in the top PRS decile (90-100% of the PRS) had ORs of 3.97 (95% CI = 3.42-4.61) in European, 3.16 (95% CI = 2.41-4.14) in African, and 3.51 (95% CI = 2.31-5.36) in American populations compared to the average 40-60% PRS category. Age-stratified analyses showed diminishing PRS effect sizes with advancing age, with ORs declining from 2.44 (≤55 years, 95% CI = 2.10-2.83) to 2.05 (>70 years, 95% CI = 1.91-2.20) in European, and from 1.92 (≤55 years, 95% CI = 1.57-2.36) to 1.48 (>70 years, 95% CI = 1.19-1.85) in African populations.
Moreover, BMI-stratified analyses showed slightly increased predictive performance in higher BMI categories, with ORs of 1.87 (95% CI = 1.22-2.88) in underweight, 2.06 (95% CI = 1.91-2.23) in normal weight, 2.12 (95% CI = 2.00-2.24) in overweight, and 2.26 (95% CI = 2.12-2.41) in obese individuals. Smoking stratified analyses demonstrated slightly higher PRS performance in never smokers, with ORs of 1.90 (95% CI = 1.68-2.16) in current smokers and 2.16 (95% CI = 2.03-2.29) in never smokers.
Conclusion: This study validates the multi-ancestry PRS as an effective tool for stratifying prostate cancer risk across diverse populations. The findings emphasize the importance of ancestry-, age-, and lifestyle-specific interpretations in PRS applications. Analyses are underway for breast, colorectal, and lung cancers to evaluate the broader utility of PRS for cancer risk stratification and screening strategies within AoU.

#2278 Correlation between DNA-methylation predicted and measured immune-cell type proportions by age in ARIC.

Z. Lin[1], D. S. Michaud[2], N. Zhao[2], V. A. Burk[1], E. Boerwinkle[3], J. Bressler[3], D. C. Koestler[4], K. T. Kelsey[5], E. A. Platz[1];
[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, [2]Tufts University School of Medicine, Boston, MA, [3]The University of Texas Health Science Center at Houston, Houston, TX, [4]University of Kansas Medical Center, Kansas City, KS, [5]Brown University, Providence, RI

Background: DNA methylation (DNAm)-predicted deconvolution uses cell-specific methylation markers of leukocytes in peripheral blood to estimate immune cell type proportions. Validity is high for predicted proportions of immune cell types. Given known age-related immune system changes and changes in predicted immune cell proportions by age, we assessed whether the correlations between predicted and measured proportions differ across age. Such information is needed for cancer studies on immunoaging in cohorts without CBC differential or stored whole blood.
Methods: Lymphocyte, neutrophil, monocyte, eosinophil, and basophil proportions were previously estimated using DNAm deconvolution (Salas et al., PMID: 35140201) and measured by CBC among Black participants (N=151; 62% female) without cancer in the Atherosclerosis Risk in Communities study. Pearson correlation coefficients were calculated in age tertiles (T1: 47-53, T2: 54-60, T3: 61-67 years). To minimize confounding by health factors, we stratified by more (no diabetes, BMI <30 kg/m^2, and non-smoker) and fewer (≤ 2 of the factors) positive health factors. Correlations in age T1 and T3 were compared after Fisher's z-transformation.
Results: Correlations between predicted and measured proportion were similar across age tertiles, albeit slightly weaker in the oldest tertile (Table, except for basophils). This same pattern was present stratifying by health factors, although the correlations appeared slightly stronger for more vs. fewer positive health factors (e.g., age T1, T3 - neutrophils: more 0.81, 0.77; fewer 0.78, 0.66). However, all p-values were > 0.1 comparing correlations in the youngest and oldest age tertiles.
Conclusion: For each common immune cell type, correlations between DNAm-predicted and measured proportion were generally similar across mid- to older adulthood, supporting use of the algorithm in immunoaging studies. Studies with participant diversity, including ages ≥ 70 yrs are needed.

Correlation between DNAm predicted and measured immune cell proportions by age tertile, ARIC Visit 2

Immune Cell Type	Overall	Age at Blood Draw, Tertiles			p-value
		T1	T2	T3	
N	151	47	53	51	
Lymphocyte	0.78(0.71, 0.83)	0.83(0.71, 0.90)	0.76(0.61, 0.85)	0.72(0.56, 0.83)	0.2
Neutrophil	0.75(0.68, 0.82)	0.79(0.65, 0.88)	0.76(0.62, 0.86)	0.68(0.5, 0.81)	0.3
Monocyte	0.42(0.28, 0.55)	0.51(0.26, 0.69)	0.41(0.16, 0.62)	0.36(0.09, 0.58)	0.4
Eosinophil	0.43(0.29, 0.55)	0.44(0.17, 0.64)	0.45(0.21, 0.64)	0.18(-0.11, 0.43)	0.2
Basophil	0.13(-0.03, 0.29)	0.02(-0.27, 0.30)	0.18(-0.1, 0.43)	0.13(-0.15, 0.4)	0.6

#2279 Real-world data on germline pathogenic variants using GenMineTOP, a tumor/normal paired comprehensive genomic profiling test, in Japan.

Eri Habano[1], Miho Ogawa[2], Kousuke Watanabe[2], Nana Akiyama[3], Hyangri Chang[3], Aya Shinozaki-Ushiku[1], Masakazu Akahori[4], Toshimitsu Ichijo[4], Shuichi Tsutsumi[5], Kenji Tatsuno[5], Hiroyuki Aburatani[5], Hidenori Kage[6], Katsutoshi Oda[1]

[1]Division of Integrative Genomics Graduate School of Medicine, The University of Tokyo, Tokyo, Japan,[2]Next-Generation Precision Medicine Development Laboratory, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan,[3]Department of Clinical Genomics, Hospital, The University of Tokyo, Tokyo, Japan,[4]KONICA MINOLTA, INC., Tokyo, Japan,[5]Research Center for Advanced Science and Technology, The University of Tokyo, Tokyo, Japan,[6]Department of Respiratory Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

[Background] Comprehensive genomic profiling (CGP) expands treatment options for solid tumor patients and identifies hereditary cancers. However, confirmatory tests have been done in only 31.4% of patients who were found to have presumed germline pathogenic variants (GPVs) with a tumor-only test in Japan. Consequently, many GPVs are likely undiagnosed. Analysis of paired tumor and normal specimens enables distinction of somatic and GPVs. GenMineTOP, reimbursed by Japan's universal national health insurance in August 2023, analyzes tumor/normal paired samples and reports GPVs in 40 genes (updated to 59 genes, based on the ACMG SF v3.2). This study clarifies the landscape of GPVs by using real-world data from GenMineTOP.
[Methods] We analyzed 1,356 patients with solid tumors who underwent GenMineTOP testing in the Center for Cancer Genomics and Advanced Therapeutics (C-CAT) database between August 2023 and July 2024. C-CAT aggregates nationwide CGP data and allows combined analysis with clinical information. The study focused on GPV detection rates, distribution of targeted genes, and features of genes included in secondary findings. Additionally, GPVs were categorized as "On-Tumor" (associated with the tumor type) or "Off-Tumor" (not associated with the tumor type) according to NCCN Guidelines® and other various guidelines, and the proportion of GPVs detected as Off-Tumor was calculated.
[Results] The median age of patients was 61 years (range: 0-90), with equal gender distribution. The most common cancer types were colorectal (15%), soft tissue sarcoma (12%), and brain tumors (11%). Seventy-nine GPVs were detected in 76 patients (5.6%). Patients with detected GPVs had a median age of 56 years (range: 1-87) with an equal gender distribution. However, the age distribution showed that the most common age group was the 70s for males (24.3%, 9/37) and the 40s for females (30.8%, 12/39). The most frequently detected gene was *BRCA2* (n = 19), followed by *BRCA1* (n = 9), *RAD51D* (n = 8), and *NF1* (n = 8). Cancer types with GPV detection rates exceeding 10% included peripheral nervous system tumors (20%), prostate cancer (12.5%), and ovarian cancer (11.9%).Of the GPVs detected, 44.3% (36/79) were Off-Tumor, including 47.4% (9/19) of *BRCA2* patients. In addition, patients with homologous recombination deficiency (HRD)-related genes (*ATM, BRCA1, BRCA2, BRIP1, PALB2, RAD51C, RAD51D*) detected included 19 males (median age: 69 years) and 24 females (median age: 54 years). Among males, 57.9% (11/19) were aged 65 years or older.
[Discussion] GPVs were identified in 5.6% of patients using GenMineTOP. The high prevalence of Off-Tumor GPVs and elderly male patients highlights the under-recognition of hereditary cancer in this demographic. These findings support the use of paired CGP to improve the diagnosis of hereditary cancers that might otherwise remain undetected.

#2280 Whole-exome sequencing analysis of early-onset soft tissue sarcoma in a multiethnic family-based population study.

Naying Zhou, Joseph L. Wiemels

USC - University of Southern California, Los Angeles, CA

Background: Sarcomas are a rare group of cancers originating in bones and soft tissues, and represent approximately 1% of adult cancers and 15-20% of pediatric cancers. The development of sarcomas is often driven by inherited or de novo mutations in genes involved in critical processes such as cell growth, development, and signaling pathways, which play central roles in their pathogenesis. Notably, the risk of early-onset cancer (diagnosed before the age of 29) is significantly elevated—68 times higher—in siblings of individuals with soft tissue sarcomas, suggesting a strong contribution of inherited germline variants to the observed familial cancer risk.

Methods: Cancer patients included in this study were identified using a linked dataset integrating information from the California Cancer Registry and the California Birth Statistical Master File. To explore rare genetic predispositions associated with soft tissue sarcomas, a cohort of 54 patients from 43 families was analyzed. This cohort included 11 sibling pairs, each with at least one individual diagnosed with soft tissue sarcoma, ICDO-3 histology code 8900-8991. Additionally, 32 unrelated individuals diagnosed with multiple cancers, including soft tissue sarcoma, were included in the analysis. Whole-exome sequencing (WES) was performed on neonatal dried blood samples from these 54 participants. Germline short variant discovery, including single nucleotide variants and insertions/deletions (indels), was conducted using the Genome Analysis Toolkit pipeline. Variants were annotated according to guidelines from the American College of Medical Genetics and Genomics.

Results: A total of 603 cancer-associated variants were identified in this study, with 74 variants detected in multiple individuals. Among these, 12 variants were classified as "Pathogenic/Likely Pathogenic, " two of which were found in two children each. Notably, a mutation in the NF1 gene, previously linked to Neurofibromatosis Type 1 syndrome, was confirmed in a Latino-White family, where one child presented with mixed-type rhabdomyosarcoma and another with neurofibroma. Additionally, a novel association was observed between the USP6 gene mutation and rhabdomyosarcoma diagnosis in two unrelated Southeast Asian individuals, one of Filipino descent and the other Guamanian. Furthermore, a homozygous mutation in the AR gene on the X chromosome was identified in a single patient.

Conclusion: Our study employs WES analysis to investigate predisposition genes in individuals diagnosed with soft tissue sarcoma, alongside their cancer-affected siblings. Through this analysis, we have identified novel genetic mutations linked to soft tissue sarcoma diagnosis. Additionally, certain variants appear to exhibit ancestry-specific patterns in this multiethnic population.

#2281 The interrelationship between maternal periconception folate nutritional intake and child's genetic liability in the etiology of childhood acute lymphoblastic leukemia.

Yijin Xiang[1], Catherine Metayer[2], Scott Kogan[3], Xiaomei Ma[4], Joseph Wiemels[1]

[1]Department of Population and Public Health Sciences, USC - University of Southern California, Los Angeles, CA,[2]University of California, Berkeley, Berkeley, CA,[3]University of California, San Francisco, San Francisco, CA,[4]Yale University, New Haven, CT

Introduction Maternal prenatal folate intake is associated with a decreased risk of acute lymphoblastic leukemia (ALL) in children. Genome-wide association studies (GWAS) show that variations in genes regulating folate metabolism impact serum folate level irrespective of nutritional intake. Polygenic risk scores (PRS) summarize the cumulative genetic liability associated with specific phenotypes. This study aimed to examine if the child PRS related to folate level interacts with maternal periconception folate intake from food and supplements, contributing to ALL risk.

Methods Data on periconceptional folate intake spanning the year prior to pregnancy were obtained from the California Childhood Leukemia Study (CCLS). Folate intake from diet and supplement was measured in dietary folate equivalents (DFE). A PRS related to folate level was constructed using summary statistics from a GWAS of serum folate levels among Europeans. After assigning genetically predicted folate levels, odds ratios (ORs) and 95% confidence intervals (CIs) per 0.05 unit decrease of the folate PRS and per risk-associated T allele (at rs1801133 in the *MTHFR* gene) were estimated using logistic regression comparing ALL cases with controls, adjusted for child's age, sex, potential confounders, and ancestry principal components. Analyses were stratified by Latino and non-Latino Whites.

Results We identified significant interactions between folate PRS/ rs1801133 and total folate from food in the Latino population, with interaction p-values of 0.008 and 0.005. The positive association between lower genetically predicted folate and ALL diminished with the increase in total folate intake from food. For folate PRS, ORs of ALL decreased from 1.32 (95% CI: 1.02-1.70) at 0 DFE, to 1.05 (95% CI: 0.93-1.20) at median intake (517.4 DFE), and to 0.54 (95% CI: 0.31-0.93) at maximum intake (2018.4 DFE). For the number of T allele at rs1801133, ORs decreased from 2.09 (95% CI: 1.19-3.68), to 1.05 (95% CI: 0.80-1.36), and to 0.14 (95% CI: 0.04-0.56). No interaction was found with folate from supplements. Additionally, lower folate PRS and T allele were shown to be positively associated with an increased risk of ALL among non-Latino Whites, regardless of folate intake from food or supplements, with ORs of 1.23 (95% CI: 1.08, 1.43) and 1.41 (95% CI: 1.04, 1.91), respectively.

Conclusion Our findings underscore the critical role of dietary folate intake against ALL development within the Latino population, where increased folate intake appeared to mitigate the impact of genetic liability. In contrast, in CCLS non-Latino population, the risk of ALL associated with child genetic liability was independent of maternal folate intake. These results suggest that folate interventions tailored to genetic profiles may be effective for motivating improvements to nutrition status.

#2282 Genetic architecture of breast cancer across diverse populations: Assessing heritability, genetic correlation, and polygenicity.

James L. Li[1], Maria Zanti[2], Jacob Williams[3], Om Jahagirdar[3], Guochong Jia[4], Qiang Hu[5], Jean-Tristan Brandenburg[6], Li Yan[5], Weang-Kee Ho[7], Jingmei Li[8], Jose P. Miranda[9], Devika Godbole[3], Julie-Alexia Dias[10], Leila Dorling[11], Xiaomeng Zhang[12], Wenlong C. Chen[6], Nicholas Boddicker[13], Ying Wang[14], Alicia Martin[14], Martin J. Zhang[15], Yan Zhang[16], Joe Dennis[11], Esther M. John[17], Gabriela Torres-Mejia[18], Larry Kushi[19], Jeffrey Weitzel[20], Susan L. Neuhausen[21], Luis Carvajal-Carmona[22], Christopher Haiman[23], Elad Ziv[24], Laura Fejerman[22], Wei Zheng[4], Dezheng Huo[1], Douglas Easton[11], Nilanjan Chatterjee[25], Peter Kraft[3], Montserrat Garcia-Closas[26], Wendy Wong[3], Kyriaki Michailidou[2], Qianqian Zhu[5], Diptavo Dutta[3], Thomas U. Ahearn[3], Haoyu Zhang[3]

[1]University of Chicago, Chicago, IL,[2]Cyprus Institute of Neurology & Genetics, Cyprus, Engomi, Cyprus,[3]National Cancer Institute, Division of Cancer Genetics & Epidemiology, Rockville, MD,[4]Vanderbilt University, Nashville, TN,[5]Roswell Park Comprehensive Cancer Center, Buffalo, NY,[6]University of the Witwatersrand, Johannesburg, South Africa,[7]University of Nottingham Malaysia, Semenyih, Malaysia,[8]The Genome Institute of Singapor, One North, Singapore,[9]Pontificia Universidad Catolica, Santiago, Chile,[10]Harvard T.H. Chan School of Public Health, Boston, MA,[11]University of Cambridge, Cambridge, United Kingdom,[12]Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom,[13]Mayo Clinic, Rochester, MN,[14]Broad Institute, Cambridge, MA,[15]Carnegie Mellon University, Pittsburgh, PA,[16]The University of Hong Kong, Pokfulam, Hong Kong,[17]Stanford University, Stanford, CA,[18]Research Institute in Cuernavaca, Mexico, Cuernavaca, Mexico,[19]Kaiser Permanente, Oakland, CA,[20]Kansas Medical Center, University of Kansas, Kansas City, KS,[21]City of Hope, Duarte, CA,[22]University of California, Davis, Davis, CA,[23]University of Southern California, Los Angeles, CA,[24]University of California, San Francisco, San Francisco, CA,[25]Johns Hopkins University, Baltimore, MD,[26]The Institute of Cancer Research, Sutton, United Kingdom

Introduction: Breast cancer genome-wide association studies (GWAS) have identified over 200 susceptibility loci, many replicated in diverse populations. However, cross-ancestry evaluation of breast cancer genetic architecture remains limited. We examined breast cancer genetic architecture using GWAS summary results from European (EUR; cases (ca) = 188,474, controls (co) = 96,201), East Asian (EAS; ca = 20,393, co = 86,329), African American (AA; ca = 9,235, co = 10,184), and US Hispanic/Latina and Latin American (H/L; ca = 2,396, co =7,468) studies.

Methods: GWAS results were derived from the Breast Cancer Association Consortium, the African Ancestry Breast Cancer Genetics Consortium (AABCG), Biobank Japan, and a meta-analysis of five studies of H/L women with breast cancer. Linkage disequilibrium (LD) scores were generated for EUR, EAS, and AMR using the 1000 Genomes Project, while AA LD scores were derived from a subset of AABCG controls. Heritability was estimated for each ancestry group using LD score regression. Genetic correlations between populations were assessed via Popcorn. Polygenicity was analyzed using GENESIS; however, due to the limited sample size in the H/L studies, these estimates were restricted to AA, EAS, and EUR. Enrichment of heritability across regulatory elements was evaluated using stratified LD score regression across all populations.

Results: The logit-scale heritability and corresponding standard error (SE) were 0.466 (0.066) for EAS, 0.501 (0.050) for EUR, 0.588 (0.360) for H/L, and 0.614 (0.095) for AA. The estimated number of independent susceptibility loci was 4,446 for EAS, 5,235 for EUR, and 8,308 for AA. Using a clumping and thresholding approach, an optimal set of common variants were projected to explain 38.6% (EUR), 39.4% (EAS), and 26.2% (AA) of genetic variance for samples of 100,000 cases and 100,000 controls with AUCs from corresponding estimated polygenic risk scores (PRSs) of 0.621 (EUR), 0.634 (EAS), and 0.611 (AA). Genetic correlations were strongest between EUR and EAS ($\rho = 0.79$, SE = 0.08) and EUR and H/L ($\rho = 0.68$, SE = 0.21), and weakest between AA and EAS ($\rho = 0.42$, SE = 0.14) and AA and H/L ($\rho = 0.26$, SE = 0.24). Among 73 genomic features, we found significant ($P < 0.05/73$) enrichment in heritability in EUR for ‘ancient promoter’ regions, transcription factor binding sites, H3K4me3, ‘super-enhancers’, H3K4me1, H3K27ac; in EAS for ‘super-enhancers’; and in AA for H3K27ac. No genomic features were significantly enriched in H/L, likely due to limited power.

Conclusion: These findings suggest a shared breast cancer genetic architecture across diverse populations, as well as the potential for a similar level of breast cancer risk stratification by PRS in these populations. Expanding GWAS in underrepresented populations is essential to improve genetic risk predictions and foster equitable cancer prevention.

#2283 Ancestral and genetic determinants of pathologic tumor infiltrating lymphocytes in a large multi-ethnic population of women with breast cancer.

Peter N. Fiorica[1], Lucas Mendicino[1], Li Yan[1], Angela R. Omilian[1], Elisa V. Bandera[2], Scott I. Abrams[1], Lawrence H. Kushi[3], Thaer Khoury[1], Christine B. Ambrosone[1], Song Yao[1]

[1]Roswell Park Comprehensive Cancer Center, Buffalo, NY,[2]Cancer Epidemiology and Health Outcomes, Rutgers Cancer Institute of New Jersey, New Brunswick, NJ,[3]Division of Research, Kaiser Permanente Northern California, Pleasanton, CA

Background: There are well-documented racial and ethnic disparities in breast cancer, with Black women having the highest mortality and Asian women having the lowest mortality. While reasons for this disparity are multi-factorial, differences in tumor biology and tumor microenvironment may contribute. Over the past two decades, pathologic tumor-infiltrating lymphocytes (TILs) have emerged as a histologic biomarker of prognosis, where higher TIL levels are associated with better outcomes and overall survival, and a predictive biomarker for cancer treatment. In recent years, there is increasing evidence of genetic determinants of immune response in the tumor microenvironment, which is known to vary by ancestral background and may explain differences in patient survival. We hypothesize that genetic selection and adaptation to distinct pathogenic environments during the evolution of diverse human populations led to ancestral differences in host immunity.

Methods: To investigate this hypothesis, we performed a local ancestry-informed GWAS of pathologic TILs using Tractor, a statistical framework and software package. With data from 4,728 women of European (n=2,740), African (n=1235), Amerindian (n=318), and East Asian (n=435) ancestry from the Women's Circle of Health Study (WCHS) and the Pathways Study, we estimated four-way local ancestry with the tool FLARE using reference genomes from 1000 Genomes Project and Human Genome Diversity Project. Given the non-normal distribution of TIL scores, we applied linear regression to the inverse normal transformation of TIL scores to assess genotype-local ancestry interaction effects.

Results: Global genetic similarity to African reference populations was significantly associated with increased TILs ($P=3.8x10^{-22}$). We identified rs17132663 on African haplotype ($P=1.7x10^{-8}$) and rs6812740 on Amerindian haplotype ($P=3.8x10^{-8}$) as genome-wide significant SNPs in our analyses. rs17132663 is an intronic variant of *AMZ1* and *GNA12*. rs6812740 is located 2 KB upstream of *HPGD* and is significantly associated ($P=1.6x10^{-12}$) with expression of the gene in GTEx version 10. To our knowledge, neither of these SNPs previously have been significantly associated with the tumor immune response. In addition, 20 other loci were significant at $P<1x10^{-6}$.

Conclusion: These findings underscore ancestral differences in breast tumor immune response and highlight the critical need to expand datasets with matched genotype and histology data to further explore these associations.

#2284 Linkage disequilibrium score regression identifies genetic correlations between hepatocellular carcinoma and clinically relevant traits.

Younghun Han[1], Vikram Shaw[2], Jinyoung Byun[1], Rikita Hatia[3], Katherine McGlynn[4], Lewis Roberts[5], Manal Hassan[3], Christopher I. Amos[1]

[1]The University of New Mexico Comprehensive Cancer Center, Albuquerque, NM,[2]Baylor College of Medicine, Houston, TX,[3]The University of Texas MD Anderson Cancer Center, Houston, TX,[4]National Cancer Institute, Rockville, MD,[5]Mayo Clinic, Rochester, MN

Hepatocellular carcinoma (HCC) mortality is increasing globally, partly due to the growing prevalence of non-viral liver diseases. Genome-wide association studies (GWAS) have identified genetic variants associated with HCC development. Leveraging GWAS summary statistics and linkage disequilibrium score regression (LDSR), we investigated disease co-development with non-viral HCC to provide unique insights into HCC etiology and prioritize relationships for further causal inquiry. We utilized the LDSR statistical framework to estimate the genetic correlation and heritability between non-viral HCC with 923 epidemiologic, behavioral, and clinical traits from the United Kingdom Biobank (UKBB) cohort. We observed a SNP-array heritability (h_2 obs) of 0.20 for non-viral HCC. Next, we estimated the pairwise genetic correlation between non-viral HCC and heritable (h_2 obs > 0.02) UKBB traits, identifying significant positive genetic correlations between non-viral HCC and blood-based biomarkers of liver injury (ALT, GGT) and allostatic load (including glycated hemoglobin, blood pressure, and total albumin). We also identified a positive genetic correlation between non-viral HCC and diseases associated with metabolic dysfunction-associated steatotic liver disease (MASLD), including diabetes, hypertension, chronic ischemic heart disease, and others. Non-viral HCC was negatively associated with filtered coffee intake but positively associated with instant coffee intake. In addition, smoking behavior and the amount of alcohol consumption in the UKBB cohort showed a positive association with non-viral HCC. Our study provides an atlas of cross-trait genetic correlations in non-viral HCC. Taken together, our results demonstrate possible polygenic pleiotropy between non-viral HCC and different phenotypic traits. The results support further exploration of the predictive power of blood-based biomarkers and selected behaviors for inferring HCC development among non-viral individuals.

#2285 Utility of ctDNA to detect minimal residual disease in colorectal cancer: A systematic review and network meta-analysis.

Tung Hoang[1], Moon Ki Choi[2], Jae Hwan Oh[2], Jeongseon Kim[3]

[1]University of Health Sciences, Vietnam National University Ho Chi Minh City, Binh Duong, Viet Nam,[2]Center for Colorectal Cancer, National Cancer Center, Goyang, Korea, Republic of,[3]Department of Cancer AI & Digital Health, National Cancer Center Graduate School of Cancer Science and Policy, Goyang, Korea, Republic of

Importance: Circulating tumor DNA (ctDNA) has shown promise as a biomarker for predicting minimal residual disease (MRD) in non-metastatic resected colorectal cancer (CRC) patients and guiding treatment decisions. A comprehensive review of clinical settings and optimal timing for ctDNA use in resected CRC patients is needed.
Objective: To examine the study designs and settings of ongoing clinical trials that use ctDNA to guide treatment decisions and determine the best timing for detecting MRD in non-metastiatic CRC.
Data sources: A search of studies published before October 2024 was conducted using PubMed, Embase, Web of Science, Cochrane Library, and clinicaltrials.gov for records in English, including clinical trials and prospective studies.
Study selection: We included studies of non-metastatic resected CRC patients with ctDNA testing done post-surgery (for clinical trials) or at any time (for prospective studies), reporting recurrence, relapse, or survival outcomes.
Data extraction and synthesis: Data on CRC stage, intervention, comparison, and endpoints for clinical trials, as well as the timing of ctDNA testing and effect size of the association with CRC prognosis for prospective studies, were extracted.
Main outcomes and measures: The ctDNA settings from clinical trials were categorized by randomization to ctDNA testing, treatment options based on ctDNA results, and the timing of ctDNA testing relative to adjuvant therapy. A network meta-analysis using a frequentist approach was conducted to examine pairwise associations of different ctDNA timing strategies with MRD, defined as recurrence, relapse, and progression.
Results: The main approaches in ctDNA-based interventional trial designs were categorized as ctDNA-guided treatment, ctDNA-by-treatment, ctDNA-guided surveillance, and ctDNA-enriched adjuvant therapy for guiding treatment decisions, including both escalation and de-escalation strategies, and surveillance. Overall, both preoperative and postoperative ctDNA detection were linked to higher risks of progression, with pooled HRs (95% CIs) of 5.23 (2.10-13.00) and 7.95 (5.30-11.91), respectively. Among timing strategies, ctDNA testing after adjuvant therapy was the most effective for identifying high-risk patients, strongly suggesting the presence of residual disease.
Conclusions and relevance: This study comprehensively reviewed clinical settings of ctDNA in ongoing trials and provided evidence supporting the selection of post adjuvant therapy as optimal timing for ctDNA testing.

#2286 Integrating multi-ancestry genomic and proteomic data to identify blood risk biomarkers and target proteins for breast cancer genetic risk loci.

Guochong Jia[1], Jie Ping[1], Ran Tao[2], Jirong Long[1], Lili Liu[1], Shuai Xu[1], Stefan Ambs[3], Mollie E. Barnard[4], Yu Chen[5], Ji-Yeob Choi[6], Yu-Tang Gao[7], Montserrat Garcia-Closas[8], Jian Gu[9], Jennifer J. Hu[10], Motoki Iwasaki[11], Esther M. John[12], Sun-Seog Kweon[13], Koichi Matsuda[14], Keitaro Matsuo[15], Katherine L. Nathanson[16], Barbara Nemesure[17], Olufunmilayo I. Olopade[18], Tuya Pal[19], Sue K. Park[20], Boyoung Park[21], Michael F. Press[22], Maureen Sanderson[23], Dale P. Sandler[24], Song Yao[25], Ying Zheng[26], Prisca O. Adejumo[27], Thomas Ahearn[8], Abenaa M. Brewster[28], Anselm J. M. Hennis[29], Hidemi Ito[30], Michiaki Kubo[31], Eun-Sook Lee[32], Siew-Kee Low[31], Timothy Makumbi[33], Paul Ndom[34], Dong-Young Noh[35], Katie M. O'Brien[24], Andrew F. Olshan[36], Mojisola M. Oluwasanu[37], Min-Ho Park[38], Sonya Reid[39], Taiki Yamaji[11], Gary Zirpoli[4], Ebonee N. Butler[36], Wei Zheng[1]

[1]Vanderbilt Epidemiology Center, Vanderbilt University Medical Center, Nashville, TN,[2]Department of Biostatistics, Vanderbilt University Medical Center, Nashville, TN,[3]National Institutes of Health, Bethesda, MA,[4]Slone Epidemiology Center, Boston University, Boston, MA,[5]NYU Grossman School of Medicine, New York, NY,[6]Seoul National University Graduate School, Seoul, Korea, Republic of,[7]Shanghai Jiaotong University School of Medicine, Shanghai, China,[8]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD,[9]Department of Epidemiology, The University of Texas MD Anderson Cancer Center, Houston, TX,[10]Department of Public Health Sciences, University of Miami School of Medicine, Miami, FL,[11]Division of Epidemiology, National Cancer Center Institute for Cancer Control, Tokyo, Japan,[12]Departments of Epidemiology and Population Health and of Medicine, Stanford University School of Medicine, Stanford, CA,[13]Department of Preventive Medicine, Chonnam National University Medical School, Hwasun, Korea, Republic of,[14]Laboratory of Clinical Genome Sequencing, Graduate School of Frontier Sciences, The University of Tokyo, Tokyo, Japan,[15]Division of Cancer Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan,[16]Division of Hematology/Oncology, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA,[17]Department of Family, Population and Preventive Medicine, Renaissance School of Medicine, Stony Brook University, Stony Brook, NY,[18]Center for Clinical Cancer Genetics and Global Health, Department of Medicine, The University of Chicago, Chicago, IL,[19]Division of Genetic Medicine, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN,[20]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Korea, Republic of,[21]Department of Medicine, Hanyang University College of Medicine, Seoul, Korea, Republic of,[22]Department of Pathology, Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA,[23]Department of Family and Community Medicine, Meharry Medical College, Nashville, TN,[24]Epidemiology Branch, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC,[25]Department of Cancer Prevention and Control, Roswell Park Comprehensive Cancer Center, Buffalo, NY,[26]Shanghai Municipal Center for Disease Control and Prevention, Shanghai, China,[27]Department of Nursing, College of Medicine, University of Ibadan, Ibadan, Nigeria,[28]Department of Clinical Cancer Prevention, The University of Texas MD Anderson Cancer Center, Houston, TX,[29]George Alleyne Chronic Disease Research Centre, University of the West Indies, Bridgetown, Barbados,[30]Division of Cancer Information and Control, Aichi Cancer Center Research Institute, Nagoya, Japan,[31]RIKEN Center for Integrative Medical Sciences, Yokohama, Japan,[32]National Cancer Center, Seoul, Korea, Republic of,[33]Department of Surgery, Mulago Hospital, Kampala, Uganda,[34]Yaounde General Hospital, Yaounde, Cameroon,[35]College of Medicine, Cancer Research Institute, Seoul National University, Seoul, Korea, Republic of,[36]Department of Epidemiology and Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC,[37]Department of Health Promotion and Education, College of Medicine, University of Ibadan, Ibadan, Nigeria,[38]Department of Surgery, Chonnam National University Medical School, Gwangju, Korea, Republic of,[39]Division of Hematology and Oncology, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN

Genome-wide association studies (GWAS) have identified more than 200 risk loci for breast cancer. However, target genes and their encoded proteins in these loci remain largely unknown. In this study, we utilized genetic prediction models for 1,350 circulating proteins derived from individuals of African (n= 1,871) and European (n= 7,213) ancestry to investigate genetically predicted protein levels in association with breast cancer risk among females of African (n=40,138), Asian (n= 137,677), and European (n=247,173) ancestry. We identified 70 blood protein biomarkers associated with breast cancer risk, overall or by subtypes, at a false discovery rate (FDR) <0.05, including 37 proteins encoded by genes located at least 500kb away from any of the known risk loci identified in GWAS. Of the 33 proteins located at GWAS-identified risk loci, associations for 14 proteins were significantly attenuated after adjustment for the index risk variant of each respective locus, suggesting that these proteins may be target proteins for the risk loci. Encoding gene expression levels in normal breast tissue could be genetically predicted for 35 of the 70 identified proteins, and 17 encoding genes were associated with breast cancer risk in the same direction (P <0.05). Our study identified potential protein targets of GWAS risk loci and biomarkers for breast cancer risk and provided additional insights into breast cancer genetics and etiology.

#2288 APOBEC dominant mutational signature defines distinct oral cavity cancer subgroup.

Benjamin M. Wahle[1], Angela L. Mazul[2], D. Neil Hayes[3], Jose P. Zevallos[2], Sidharth V. Puram[4]

[1]Otolaryngology/Head and Neck Surgery, Indiana University School of Medicine, Indianapolis, IN,[2]Otolaryngology/Head and Neck Surgery, University of Pittsburgh, Pittsburgh, PA,[3]Department of Medicine, Division of Med Hematology, University of Tennessee Health Science Center, Memphis, TN,[4]Otolaryngology/Head and Neck Surgery, Washington University School of Medicine, St. Louis, MO

Background: Up to 25% of oral cavity squamous cell carcinoma (OCSCC) cases have no significant exposure to tobacco and alcohol. Compared to the rest of the OCSCC population, these cases tend to be older, more female, and display distinct patterns of oral cavity subsite dominance. The underlying molecular pathophysiology in atypical OCSCC cases remains poorly defined. We hypothesized that analysis of OCSCC genomes using single base substitution (SBS) mutational signatures, which computationally discern patterns associated with discrete mutational processes, would reveal discrete subgroups within the OCSCC population.
Methods: We performed an exploratory genomics study using data from The Cancer Genome Atlas (TCGA). Cases included primary oral cavity tumors with whole exome sequencing data. Cases were excluded if oral cavity subsite was not specified or if the tumor had fewer than fifty single bases substitutions. SBS signatures were generated using python packages SigProfilerMatrixGenerator and SigProfilerAssignment with COSMIC SBS Signatures V3.4 as the reference. APOBEC-dominant cases were defined as those where SBS2 and SBS13 accounted for the greatest proportion of mutations relative to the tumor's total mutational burden.
Results: A total of 217 OCSCC cases were included. Of these, 135 (62.2%) showed some degree of ABPOEC-related mutational burden, and in 62 (28.6%) cases APOBEC was the dominant mutational process. APOBEC-dominant cases showed greater SBS mutational burden (median difference 2.65 mutations/MB, Wilcoxon P = 2.22e-09). Compared to the rest of the OCSCC population, APOBEC-dominant cases were older in age (mean difference 4.08 years, 95% CI 0.35 - 7.81, T-test P 0.032), more female (46.8% vs 27.8%, OR 2.28, 95% CI 1.18 - 4.40), and had a significantly lower rate of tobacco use at diagnosis (OR 0.47, 95% CI 0.22 - 0.94). APOBEC mutational burden varied by oral cavity subsite (Kruskal-Wallis P = 1.66e-05), with the majority of hard palate and buccal mucosal tumors being APOBEC-dominant (85.7% and 66.7%, respectively) and floor of mouth tumors showing the lowest rate of APOBEC-dominance (16.1%). APOBEC-dominant cases showed a greater degree of mutations in RAC1, EP300, CASP8, and FAT1 (FDR corrected P <0.05). APOBEC dominant cases showed a trend toward a lower rate of TP53 mutations (OR 0.48, 95% CI 0.23 - 1.01).
Conclusions: In TCGA, ABPOEC-dominant OCSCC cases resemble these atypical populations by being enriched for female sex, older age, lower rates of tobacco use, and certain oral cavity subsites. However, APOBEC-dominance was not exclusive to any clinical characteristic, highlighting the potential importance of molecular class labels in understanding population heterogeneity in OCSCC. Further investigation focused on induction of APOBEC in OCSCC is warranted and may elucidate the pathophysiology of oral cavity cancer beyond tobacco and alcohol exposure.

#2289 *DDX41* - more than meets the eye?.

Jaclyn Zamzow, Jenna Gedminas, **Moon Ley Tung**

University of Iowa, Iowa City, IA

Introduction: Germline variants in the *DDX41* gene (OMIM: 608170) have been mainly associated with late onset myelodysplastic syndrome (MDA) and acute myeloid leukemia (AML). Based on a study of healthy adults within the UK Biobank sample, approximately 1 in 430 adults carry a germline pathogenic variant in *DDX41*. The odds ratio of these individuals developing MDS/ AML is between 7.5 t0 15.1 compared to individuals without any germline pathogenic variants in *DDX41*. Although some of the reported individuals diagnosed with DDX41-related hematological malignancies had received chemotherapy or radiation therapy for solid tumors prior to their diagnosis of MDS/AML, there has been no systematic analysis of *DDX41* as a tumor suppressor gene in childhood cancers. We describe a novel finding of a germline, likely pathogenic variant in the *DDX41* gene in a 2-year-old male who was diagnosed with a Stage 1, group 1, very low risk embryonal rhabdomyosarcoma of the oral commissure.

Case study: A 2-year-old, previously healthy male was found to have a growth in the right oral commissure at around 20 months of age. Excisional biopsy of this exophytic mass returned as an embryonal rhabdomyosarcoma on pathology. As part of the Children's Oncology Group evaluation, he underwent a paired tumor-germline genomic testing which detected a heterozygous, likely pathogenic *DDX41* variant (NM_016222.4: c.771del, p. Tyr259MetfsTer45). Tumor allele fraction was at 46% and the germline allele frequency was at 47%. He was subsequently referred to the Division of Medical Genetics and Genomics for further evaluation and post-test genetic counseling. Physical examination was unremarkable with no dysmorphic features except for two hyperpigmented nevus on his left shin and lower back. Birth and developmental history were normal. Interestingly, family history revealed that the proband's father had a personal history of a left parotid acinar cell carcinoma which was diagnosed at the age of 11. He underwent surgical resection but relapsed at the age of 27 and received surgery and consolidative radiotherapy. There was a history of a paternal great uncle diagnosed with leukemia in his sixties.

Results: Targeted parental testing for this *DDX41* variant was completed and revealed that this was a de novo variant in the proband. In view of the absence of evidence of presence of *DDX41* germline variants in childhood cancers, we searched the St Jude Cloud PeCan database and found 33 reports of embryonal rhabdomyosarcoma but no evidence of either somatic or germline *DDX41* variants described.

Conclusion: This is the first description of a pediatric patient with a de novo germline, heterozygous, likely pathogenic variant in *DDX41* and presents with a non-hematological malignancy. Increased access to paired tumor-germline genetic testing will likely uncover more patients with an underlying germline *DDX41* variant and will help decode the underlying pathology and risks of development of both solid and hematological malignancies.

POPULATION SCIENCES: Cancer Disparities in Behavioral, Psychological, Psychosocial, and Molecular Factors Poster Session

#2293 *KLRC2* gene deletion is associated with active cytomegalovirus infection and risk of head and neck cancer.

Heather H. Nelson[1], DeVon Hunter-Schlichting[1], Rondi Butler[2], Anna Prizment[1], Bharat Thyagarajan[1], Karl Kelsey[2]

[1]Masonic Cancer Center, Minneapolis, MN,[2]Brown Unversity, Providence, RI

Human cytomegalovirus (CMV) is a common infection that can have a profound impact on the immune system which, in turn, has the potential to impact cancer risk. Natural Killer (NK) cells are the front-line defense against viral infection and CMV seropositive individuals develop a specialized subset of NK cells, the adaptive NK (aNK), in response to CMV reactivation. A defining feature of aNK is surface expression of the NKG2C receptor, encoded by the *KLRC2* gene. There is a polymorphic 15.5 kb deletion of the *KLRC2* gene and approximately 5% of the Caucasian population completely lacks the NKG2C receptor due to this polymorphism. *KLRC2* gene copy number has been shown to influence steady-state levels of aNK cells in CMV seropositive individuals, and deletion of the gene is associated with both early life CMV infection and CMV viremia after transplantation. Here, we have evaluated whether the polymorphic *KLRC2* gene deletion is associated with active CMV infection and risk of head and neck squamous cell carcinoma (HNSCC) using the resources of the CoHANDS epidemiology study. *KLRC2* deletion was measured using PCR with gel visualization in 1,096 cases and 1,179 controls. Among a subset of the controls (n=207) we measured CMV DNAemia, the presence of cell free CMV DNA in serum, which is an established biomarker of active CMV infection. *KLRC2* deletion was associated with the presence of CMV DNAemia: among those with one copy of *KLRC2* the risk of active infection was 2.74 (95% CI 1.30 - 5.78), and among those with homozygous deletion of *KLRC2* the risk was 5.17 (95% CI 1.56 - 17.16). These results from a healthy population are consistent with prior reports that the *KLRC2* gene is important in the control of CMV reactivation. We then evaluated *KLRC2* as a risk factor for HNSCC. Homozygous deletion of the *KLRC2* gene was strongly associated with increased risk of HNSCC after adjusting for age and sex (OR 3.99; 95% CI 2.62 - 6.10). Collectively, these data support the hypothesis that active CMV infection may be a risk factor for HNSCC.

#2294 Association of analgesic use with risk of renal cell cancer in the Nurses' Health Studies.

Jinhui Li[1], Minji Jung[1], Benjamin Chung[1], Mark Preston[2], Marvin Langston[1], Rebecca Graff[3]

[1]Stanford University, Palo Alto, CA,[2]Brigham and Women's Hospital, Boston, CA,[3]University of California, San Francisco, San Francisco, CA

Objectives: This study aims to investigate the association between commonly utilized analgesics—aspirin, non-aspirin nonsteroidal anti-inflammatory medications (NSAIDs), and acetaminophen and the risk of renal cell cancer (RCC) in women.
Methods: In 1980, the Nurses' Health Study (NHS) collected data on aspirin usage from 92,125 women. Additional non-aspirin NSAIDs and acetaminophen were included in 1990. In 1989, 112,292 women were collected data in the NHS II and followed up since 1989. The association was also further investigated by stratification of histological subtypes, pathologic stage, and differentiation subtypes for RCC. Cox proportional hazards models were employed to generate cohort-specific, multivariable hazard ratios (HRs) and 95% confidence intervals (CIs) for each analgesic. Results from both cohorts were aggregated by random-effects meta-analyses.
Results: For the over 20-year follow-up period, there are 11,978, 22,838, and 28,350 women currently use acetaminophen, aspirin, and NSAIDs from the NHS study, and 25,416, 12,958, and 22,407 women currently use these analgesic medications individually. 575 RCC and 142 fatal RCC cases were identified across the two cohorts. For RCC, in NHS II. We observed a significantly increased risk of RCC among the current acetaminophen users (Relative risk (RR): 1.56, 95%CI:1.13,2.15). Pooled results showed positive associations: acetaminophen (RR:1.02, 95%CI: 0.75,1.95); aspirin (RR:1.11, 95%CI: 0.91,1.31); non-aspirin NSAIDs (RR:1.29, 95%CI:0.90, 1.83). Similar trends were observed for fatal RCC. When stratified the RCC subgroups, aspirin use shows a significantly positive association with the increased risk of different pathologic states (pooled RR: 1.29, 95%CI: 1.02, 1.63), and non-aspirin NSAID use was also associated with increased risk of different histological, pathological and differentiated RCC risk significantly.
Conclusions: These data illustrated that the use of analgesics in NHS and NHS II cohorts is potentially associated with an increased risk of RCC among women.

#2295 Molecular epidemiology of high-risk HPV circulating in women living with HIV in Nigeria: Protocol for a multi-center cross-sectional study.

I. M. Ifeorah[1], B. O. Olakunde[2], U. E. George[3], S. C. Eneh[1], U. U. Akpan[4], N. N. A. Awolola[5], A. A. F. Banjo[5], C. O. Chigbu[1], E. E. Ezeanolue[1], L. Hou[6], O. M. Adewumi[7];

[1]IVAN Research Institute, University of Nigeria Nsukka, Enugu, Nigeria, [2]College of Public Health University of North Texas Health Science Center, Forth Worth, Texas Houston, TX, [3]African Centre of Excellence for Genomics of Infectious Diseases, Redeemer's University, Ede Osun State, Nigeria, [4]Excellence Community Education Welfare Scheme (ECEWS), Abuja, Nigeria, [5]Lagos University Teaching Hospital, Lagos, Nigeria, [6]Feinberg School of Medicine, Northwestern University, Chicago, IL, [7]College of Medicine, University of Ibadan, Ibadan Oyo State, Nigeria

Introduction: Cervical cancer (CCa) is the second leading cause of CCa-related deaths among women in Nigeria. Specifically, women living with HIV (WLHIV) face a significantly higher risk of developing CCa. Despite this, comprehensive data on circulating high-risk HPV (hrHPV) strains among WLHIV in Nigeria remains scarce. This study aims to determine the distribution of hrHPV among WLHIV in Nigeria to inform and optimize interventions geared at CCa prevention.

Methods: This cross-sectional study will examine fifteen hrHPV in 1,320 consenting WLHIV in six health facilities from the Nigeria Implementation Science Alliance Model Innovation and Research Centers across Nigeria's six geopolitical zones. WLHIV will be eligible for participation if they are between 25-60 years; have not been diagnosed with CCa, have not undergone hysterectomy, and not pregnant. Clinical and demographic data will be collected using a pretested questionnaire. Cervical swab samples will be obtained with a cytobrush and Ayre spatula. The cytobrush will be placed immediately into a sterile specimen tube containing virus transport medium. The cervical specimen collected with ayre spatula will be used to make duplicate cytological smears, fixed in ethanol, and subsequently stained by papanicolaou method. Human Papilloma Virus genotyping assay will be used to detect hrHPV in the study population. For hrHPV-positive samples, papanicolaou staining will classify cytology results using the Bethesda system. Statistical analyses will include descriptive statistics of hrHPV prevalence and distribution. Associations between hrHPV and clinical as well as demographic factors will first be evaluated using Fisher's exact or Pearson's chi-squared tests. Multivariable logistic regression will further be used to identify factors associated with hrHPV. Cytological outcomes will be descriptively categorized by hrHPV genotype and participant characteristics.

Expected Results: This study will provide reliable and representative data on the prevalent circulating hrHPV and the geographical variation among WLHIV in Nigeria. It will also provide insights into the possible risk factors associated with hrHPV among WLHIV in Nigeria.

Conclusion: Findings from this study will be critical to effective CCa prevention and control among WLHIV in Nigeria by bridging the knowledge gap and providing evidence to inform optimization of HPV-DNA screening to target prevalent hrHPV circulating among WLHIV. In addition, it will elicit robust scientific engagement on the potential impact of the HPV vaccination program in this high-risk population.

#2296 Ascertainment of uterine cancer surgery in the Surveillance, Epidemiology, End Results (SEER)-Medicare linked database: Comparison of SEER and claims data.

Ashley S. Felix[1], Caitlin E. Meade[1], Qinhan Hu[1], Anusha M. Vable[2], Tasleem J. Padamsee[1], Marcarius Donneyong[3], Kemi M. Doll[4], Jennifer A. Sinnott[5]

[1]The Ohio State University College of Public Health, Columbus, OH,[2]University of California, San Francisco, San Francisco, CA,[3]The Ohio State University College of Pharmacy, Columbus, OH,[4]University of Washington School of Medicine, Seattle, WA,[5]The Ohio State University College of Arts and Sciences, Columbus, OH

Purpose: Hysterectomy with bilateral salpingo-oophorectomy (BSO) is the cornerstone of uterine cancer treatment. The Surveillance, Epidemiology, and End Results (SEER)-Medicare linked database provides two sources of surgery information: medical record abstraction (SEER) and billing claims (Medicare). Concordance between these two sources has not been evaluated among uterine cancer patients. Given the increasing use of SEER-Medicare for assessment of treatment-related uterine cancer survival effects, an understanding of the concordance of these two sources is needed.
Methods: Patients diagnosed with uterine cancer between 2000 and 2019 were identified using the SEER-Medicare linked database. We calculated sensitivity, specificity, positive predictive value, negative predictive value, and Kappa statistics for receipt of hysterectomy with or without BSO and receipt of hysterectomy with BSO using Medicare claims as the presumed gold standard. Analyses were repeated stratified by race, histology, and stage.
Results: A total of 34,904 uterine cancer patients [9% Black, mean (standard deviation) age at diagnosis: 74.6 (6.7) years] were included in the comparison of surgery derived in SEER vs. Medicare claims, after excluding 39.7% with unknown surgery status from SEER. A total of 30,943 patients were identified as having hysterectomy with or without BSO in both the claims and SEER, resulting in a moderately strong sensitivity [78.4%, 95% confidence interval (CI)= 77.1%,79.8%]. A total of 29,733 patients were identified as having hysterectomy with BSO in both the claims and SEER, resulting in a sensitivity of 69.5% (95% CI= 68.1%, 70.8%). For both procedures, lower sensitivity was noted for non-Hispanic Whites, those diagnosed with grade 1 endometrioid, or those diagnosed with stage 1 disease. Specificity was high for both treatment types and across race, histology, and stage categories (>96.9%). In cases where there was discordance between claims and SEER, claims were more likely to report a corresponding insurance claim when there was no corresponding surgery indication in SEER.
Conclusions: In the subset of uterine cancer patients with known SEER surgery, SEER data reliably identified receipt of hysterectomy with or without BSO and hysterectomy with BSO; however, variations according to patient characteristics were observed. Of concern is the large proportion of unknown surgery data in SEER, which has implications for research aimed at estimating the population prevalence of surgery and investigations comparing outcomes between surgically and non-surgically treated patients. Investigations of the origins of unknown SEER surgery data are warranted.

#2297 Racial and ethnic disparities in incidence of Langerhans cell histiocytosis differ across age groups.

J. Velazquez, K. L. McClain, P. J. Lupo, C. E. Allen, M. E. Scheurer, E. C. Peckham-Gregory;
Baylor College of Medicine, Houston, TX

Background: Langerhans cell histiocytosis (LCH) is an inflammatory myeloid neoplasia with a median diagnosis age of 30 months, though it can occur at any age. The estimated annual incidence is 5 cases per million children under the age of 15 and 1-2 cases per million adults. We previously reported an increased risk of LCH among children of Hispanic parents using data from the Texas Cancer Registry. However, less is known about differences in underlying biology between children and adults, and it is unclear whether incidence profiles differ across age groups. Therefore, this study aimed to compare the incidence of LCH by race/ethnicity in the United States (US) across the age spectrum using data from the US Cancer Statistics (USCS) database.

Methods: LCH incidence data were obtained from the USCS database for the period 2001-2021, representing 99.6% of the US population, and stratified by age at diagnosis and race/ethnicity. Age was defined as childhood (0-14 years), AYA (15-39 years), and adult (40+ years). Race/ethnicity was categorized as non-Hispanic White (NHW), non-Hispanic Black (NHB), non-Hispanic American Indian or Alaska Native (NHAIAN), non-Hispanic Asian or Pacific Islander (NHAPI), and Hispanic. Age-adjusted incidence rate-ratios (IRRs) and 95% confidence intervals (CIs) were calculated using SEER*Stat (v8.4.4) to assess differences in LCH incidence across these groups.

Results: We identified 3,825 childhood, 1,306 AYA, and 1,718 adult LCH cases in the USCS database. Hispanic children showed significantly higher LCH IRRs ($IRR_{Hispanic_0\text{-}14}$=1.17, 95% CI: 1.09-1.26) compared to NHW children. However, this trend was not observed in Hispanic AYAs or adults, who exhibited significantly lower IRRs than NHWs. In a sub-analysis of childhood cases, Hispanic children aged 0-4 and 5-9 showed significantly higher LCH IRRs compared to NHW children ($IRR_{Hispanic_children_0\text{-}4}$=1.26, 95% CI: 1.14-1.39; $IRR_{Hispanic_children_5\text{-}9}$=1.16, 95% CI: 1.01-1.34), while this trend attenuated among Hispanics aged 10-14 ($IRR_{Hispanic_children_10\text{-}14}$=0.94, 95% CI: 0.79-1.12). NHBs showed significantly lower LCH IRRs across all age groups compared to NHWs as well as in sub-analyses of NHB children aged 0-4, 5-9, and 10-14 ($IRR_{NHB_0\text{-}4}$=0.37, 95% CI: 0.31-0.45; $IRR_{NHB_5\text{-}9}$=0.37, 95% CI: 0.27-0.48; $IRR_{NHB_10\text{-}14}$=0.24, 95% CI: 0.16-0.35).

Conclusions: This study reveals significant differences in LCH incidence by racial/ethnic group across ages. Hispanic pediatric cases show higher incidence compared to NHWs, particularly in children aged 9 and below, a trend not seen in Hispanic AYAs and adults. In contrast, NHBs exhibit a decreased incidence across all age groups compared to NHWs. These disparities in LCH incidence by race/ethnicity may suggest differences in underlying LCH etiology across the age spectrum. Future studies are warranted to characterize the molecular components of LCH in adults compared to children.

#2298 Endometrial cancer incidence by age across Asian/Pacific Islander ethnic subgroups.

Minh Tung Phung[1], Elizabeth Gehrig[2], **Alice W. Lee**[2]

[1]University of Wisconsin-Madison, Madison, WI,[2]California State University, Fullerton, Fullerton, CA

Introduction: Endometrial cancer is the most common gynecologic cancer in the U.S. Although late-onset endometrial cancer (LOEC, diagnosed age 50+) is more common, the incidence of early-onset endometrial cancer (EOEC, age <50) is rising, with recent data showing EOEC incidence increasing twice as fast as LOEC incidence during 1995-2017. Interestingly, when these findings were looked at by race/ethnicity, only Asian/Pacific Islander (APIs) showed incidence of LOEC increasing faster than that observed for EOEC. However, because APIs constitute unique ethnic subgroups that differ in culture, socioeconomic status, and access to care, we have examined EOEC and LOEC incidence for the seven largest API subgroups.

Methods: The Surveillance, Epidemiology, and End Results Program (SEER-9) specialized database with Asian population denominators was used to examine EOEC and LOEC incidence from 1995 to 2014 for the following API subgroups: Asian Indian/Pakistani, Chinese, Filipino, Hawaiian/Pacific Islander, Japanese, Korean, and Vietnamese. Incidence rate ratios (IRRs) were calculated for each API subgroup, with NHW as the reference. Joinpoint Regression Program version 5.2.0.0 was used to calculate the average percent change (APC) of incidence rates in 4-year groups. All statistical tests were 2-sided, and statistical significance was defined as $P<0.05$.

Results: The analysis included 14,803 EOEC (2,365 API; 12,438 NHW) and 119,637 LOEC cases (8,213 API; 111,424 NHW). The proportion of EOEC was higher in all API subgroups than in NHW (10%), with the highest among Hawaiian/Pacific Islander (35%) and the lowest among Chinese (15%). EOEC incidence was 6-20 times lower than LOEC incidence. EOEC incidence rates among API subgroups range from 2.11 (Asian Indian/Pakistani) to 16.72 per 100,000 (Hawaiian/Pacific Islander). LOEC incidence rate ranged from 0.24 (Korean) to 93.94 per 100,000 (Hawaiian/Pacific Islander). Hawaiian/Pacific Islander is the only subgroup with both EOEC and LOEC incidence rates higher than NHWs (IRRs=3.64 and 1.18, respectively). There was an increasing trend in EOEC and LOEC incidence during 1995-2014 for most API subgroups. The APCs for EOEC were lower than LOEC in most API subgroups, except for Hawaiian/Pacific Islander. In contrast, the APC for EOEC was greater than LOEC among NHW. Among the API subgroups, the APCs for EOEC ranged from -0.80 (Japanese) to 3.73 (Hawaiian/Pacific Islander). The APCs for LOEC ranged from 1.46 (Japanese) to 3.72 (Asian Indian/Pakistani).

Conclusions: There was heterogeneity in incidence rates and trends of EOEC and LOEC by API subgroups. Both EOEC and LOEC incidence rates increased across nearly all API subgroups, and the increases were greater for LOEC, which is different from NHW. These differential increases underscore the need to monitor risk factor trends and develop tailored interventions to reduce endometrial cancer burden by age and API subgroups.

#2299 Risk assessment and development of a machine learning-based prediction model for survival in patients with medulloblastoma.

Yu Su[1], Kaiwen Deng[2], Tao Jiang[2], Xiaoguang Qiu[2], Jiao Zhang[3], Hailong Liu[2], Yu Tian[1]

[1]Capital Medical University, Beijing, China,[2]Beijing Tiantan Hospital, Capital Medical University, Beijing, China,[3]Texas Children's Hospital, Houston, TX

Background: While clinical risk factors have long guided medulloblastoma (MB) treatment, the significance of genetic events in therapeutic strategies has not been fully elucidated. We aimed to explore whether revised molecular risk categories could inform management by analyzing long-term follow-up profiles, and concurrently to establish and validate an explainable prediction model for MB based on machine learning (ML) approaches, and to assess its prognostic implications in patients.
Methods: A multicenter cohort of 729 patients from 2001 to 2023 was enrolled as a discovery cohort. Multivariate survival analyses assessed the associations between radiotherapy dose and survival by different revised molecular risk stratifications which integrated clinical and genetic events, followed by utilizing this cohort for training and internal validation of the model. The area under the receiver-operating-characteristic curve (AUROC) and decision curve analysis (DCA) were used to compare the predictive performance. The SHapley Additive exPlanation method was used to explain the final model.
Results: Fifteen-year OS and DFS rates were 64.1% (95% CI, 57.9 to 71.0) and 59.1% (95% CI, 53.2 to 65.5) for clinical average-risk group (n = 554), and 55.9% (95% CI, 44.4 to 70.2) and 60.6% (95% CI, 51.9 to 70.7) for clinical high-risk group (n = 175), respectively. Within this revised high-risk stratification of SHH-MB, higher PF dose (≥ 55.8 Gy) combined with reduced CSI dose (23.4 ~ < 30.6 Gy) was associated with better prognoses. In Gr.3-MB, MYC amplification (activation), as a high-risk marker of dissemination, indicated that CSI dose should be escalated to high intensity (36.0 Gy, p = 0.03) for survival benefits. In the revised model of Gr.4-MB, CDK6 activation well stratified the patients, with 10-year OS of 71.0% (95% CI, 54.0 to 93.5) for average-risk and 52.1% (95% CI, 39.5 to 68.8) for high-risk group. Furthermore, Clinical and molecular information from discovery and validation cohort was involved into model training. The Random survival forest method demonstrated the most optimal predictive efficacy among the six ML models, as evidenced by the training set AUROC of 0.73 (95% CI 0.68-0.78) for 5-year survival rates, 0.76 (0.70-0.82) for 10-year survival rates, and 0.86 (0.81-0.92) for 15-year survival rates in all patients. The final model could accurately predict survival rates in both internal and external validations, and has been deployed through a free, publicly available online software interface.
Conclusion: The revised molecular risk stratification was suggested to potentially guide risk-adapted radiotherapy. Except that 36.0 Gy CSI dose is warranted for Gr.3-MB with MYC amplification, CSI reduction is recommended. Furthermore, this study, based on clinical and molecular information, constructs an available survival prediction tools for MB patients.

#2300 Blood microbiome, circulating tumor cells, and survival in Black and White women with metastatic breast cancer.

Tengteng Wang[1], Prachi Trevedi[1], Mouadh Barbirou[2], Maysa Abu-Khalaf[2], Zheng Sun[3], Yang-Yu Liu[3], Mridula George[1], Elisa V. Bandera[1], Hushan Yang[2]

[1]Rutgers Cancer Institute, New Brunswick, NJ,[2]Sidney Kimmel Comprehensive Cancer Center, Thomas Jefferson University, Philadelphia, PA,[3]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

Background: Metastatic breast cancer (MBC) is a devastating disease, and recent evidence suggests that the human microbiome may play a critical role in cancer metastasis. However, the role of the blood microbiome in MBC prognosis has not been explored.
Methods: This study analyzed sixteen blood samples collected from women diagnosed with MBC who participated in the hospital-based cohort at Thomas Jefferson University. Plasma DNA samples were extracted from these samples collected before initiating new lines of therapy. We utilized 2bRAD sequencing for Microbiome (2bRAD-M), a reduced sequencing technology capable of decoding the blood microbiome (bacteria, fungi, archaea, and viruses) with high precision and resolution. Circulating tumor cells (CTCs) were enriched from blood samples using CellSearch®. To investigate blood microbiome differences by CTC levels and race, both conventional statistical analysis and machine learning are performed to examine the variations in blood microbial diversity, taxonomic structures, and functional pathways. Cox proportional hazard regression models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for associations between microbial diversity index and MBC overall survival.
Results: We identified 265 microbial species in the plasma samples. Lower blood microbial alpha diversity was significantly correlated with higher CTC counts (R = -0.66, p = 0.01), a marker associated with poorer breast cancer outcomes. Random forest analysis identified fifteen key species (e.g., *Sphingopyxis_sp001468225, Malassezia restricta*) that can differentiate between CTC groups, with an area under the curve of 0.67 to 0.75. Pathway analysis revealed the bacterial Type VI Secretion System (T6SS) as the top pathway associated with differential microbes across CTC levels; women with a CTC count >5 showed over 1000-fold higher abundance of the T6SS pathway. For survival prediction, patients with low microbial alpha diversity (≤ median of 2.04) exhibited a nearly threefold increased risk of death (HR = 2.62, 95% CI=0.74 -17.26), with even higher risk after adjusting for tumor ER status (HR = 4.43), age at diagnosis (HR = 4.86), and CTC count (HR = 6.83). These findings indicate a strong negative association between blood microbial diversity and key prognostic factors in MBC. Lastly, we also observed that Black MBC patients had a lower microbial richness than White participants (median 27 vs. 42), and had a significantly lower abundance of *Klebsiella pneumoniae* than White women (p*<0.001*).
Conclusion: Our compelling pilot findings suggest that 1) blood samples may serve as a novel sample type for microbiome assessment in a diverse cohort of women with MBC, and 2) blood microbiome features differ by CTC levels and race and may significantly influence MBC survival. Further studies with larger cohorts are warranted as the next step.

#2301 CA125 levels as a proxy for residual disease in a diverse cohort of epithelial ovarian cancer patients: results from the Kaiser Permanente Research on Ovarian Cancer Survival (KPROCS) study.

Lindsay Jane Collin[1], Farzin Khosrow-Khavar[2], Valerie S. Lee[3], Juraj Kavecansky[3], Christine Garcia[3], Scarlett L. Gomez[4], Jennifer A. Doherty[5], Lawrence H. Kushi[3], Elisa V. Bandera[2]

[1]Emory University, Rollins School of Public Health, Atlanta, GA,[2]Rutgers Cancer Institute, New Brunswick, NJ,[3]Kaiser Permanente Northern California, Oakland, CA,[4]University of California at San Francisco, San Francisco, CA,[5]Huntsman Cancer Institute, Salt Lake City, UT

Background: Ovarian cancer has a poor 50% five-year survival rate. The presence of residual disease at the time of surgery is a key prognostic indicator; however, it is often not available or challenging to obtain. Cancer antigen (CA)125 is a well-established biomarker for monitoring ovarian cancer progression, but its utility as a biomarker to identify women with residual disease after surgery have not been evaluated.

Methods: We included 1218 epithelial ovarian cancer patients from the Kaiser Permanente Research on Ovarian Cancer Survival (KPROCS) cohort who had both information on residual disease from surgical notes (*i.e.,* gold standard) and CA125 measurements within 6 months of surgery. Residual disease from surgical notes was determined using natural language processing to classify as optimal (<1cm) or suboptimal debulking (≥1cm). For each patient, we obtained the lowest CA125 value during the period starting 2 weeks after surgery and up to 6 months post-surgery (if they did not have chemotherapy) or up to the date of the first chemotherapy cycle. CA125 values were then dichotomized (>100 vs ≤100 ug/mL) to evaluate utility in identifying presence or absence of residual disease by estimating sensitivity and specificity, as well as positive and negative predictive values (PPV and NPV, respectively), with corresponding 95% confidence intervals (CIs). We evaluated differences by race and ethnicity and time since surgery (≥30 days vs <30 days).

Results: In this validation study of 1218 epithelial ovarian cancer patients, 229 (19%) were Asian American/Pacific Islander, 66 (5.4%) were non-Hispanic Black, 171 (14%) were Hispanic, 738 (61%) were non-Hispanic white, and 14 (1.0%) were of other racial and ethnic groups. The average time since surgery to the lowest CA125 value was 33 days (interquartile range 25, 46 days). According to the surgical notes, 5% were classified as having suboptimal debulking status, whereas 80% were classified as having CA125 >100 ug/mL, which corresponded to sensitivity of 70%, specificity of 82%, PPV of 17%, and NPV of 98%. The sensitivity and specificity were 78% and 87% among Asian American/Pacific Islander individuals, 43% and 95% among non-Hispanic Black individuals, 63% and 83% among Hispanic individuals, and 77% and 79% among non-Hispanic White individuals. Finally, the sensitivity and specificity were 72% and 50% for those who had their lowest CA125 value <30 days after surgery and 56% and 72% for ≥30 days after surgery.

Conclusions: In our initial results, we demonstrate that elevated CA125 serves as a modest proxy to identify residual disease, with some variation by race and ethnicity and time since surgery. Future work will seek to explore CA125 cut points and variation by patient characteristics to optimize performance.

#2302 Overall survival and treatment patterns in elderly patients with stage IV colorectal cancer in the United States: SEER-medicare analysis, 2007-2020.

Alicia C. McDonald, Xinyue Liu, Ruixuan Jiang, Jae Min, Pierre Leconte, Anne C. Deitz, Changxia Shao

Merck & Co., Inc., Rahway, NJ

Introduction: Traditional therapeutic options in metastatic colorectal cancer (mCRC) are limited and associated with severe toxicity. This limitation may be greater in populations where cancer treatment disparities have been reported. To better understand survival outcomes and patterns of treatment in these populations, we conducted an observational study to examine overall survival (OS) and lines of therapy (LOT) by sex, age, and race/ethnicity in elderly patients with stage IV CRC in the United States using the Surveillance, Epidemiology, and End Results (SEER) Medicare database.

Methods: Patients aged ≥65 years with stage IV CRC were identified in the SEER-Medicare database between January 1, 2007, and December 31, 2020. The frequency of the first, second, and third LOT (1L-3L) and the regimens within each line were calculated. Kaplan-Meier curves were generated to examine OS. All analyses were conducted for the total population and by sex, age group, and race/ethnicity.

Results: There were 9,042 CRC cases identified with a median age of 76 years. Most patients were female (55.0%), non-Hispanic White (72.5%), and had a proximal site of initial diagnosis (44.8%). Overall, the median OS was 7.8 months (95% CI, 7.5-8.2). Older age (≥85 years), female, and non-Hispanic Black and non-Hispanic American Indian/Alaska Native (AI/AN) patients had worse OS compared with younger age groups, male, and other racial/ethnic patients, respectively. Of the 9,042 patients, 4923 (54.4%), 2761 (30.5%), and 1556 (17.2%) patients received 1L, 2L, and 3L, respectively. The most common regimens were FOLFOX ± anti-VEGF (47.7%) for 1L, FOLFIRI ± anti-VEGF (27.0%) for 2L, and FOLFIRI ± anti-VEGF (18.7%) and other (17.5%) for 3L. The most common regimens by LOT were similar by sex and race/ethnicity. However, different treatment patterns were observed by age group. Compared with younger age groups, older age groups received fewer 2L and 3L and more capecitabine-based regimens or anti-VEGF monotherapy. As for OS, compared to patients aged 65-74 years, worse OS was observed for patients aged 75-84 years within each LOT and patients aged ≥85 years within 1L (P values < 0.01). Compared to non-Hispanic White patients, worse OS was observed for non-Hispanic Black patients within each LOT and non-Hispanic AI/AN patients within 3L (*P* values < 0.01). OS was similar between male and female patients within each LOT.

Conclusion: Compared with younger patients, older patients received fewer 2L and 3L, received less intensive therapy regimens, and had worse OS. Although therapy regimens across LOT were comparable by sex and race/ethnicity, worse OS was observed in female, non-Hispanic Black, and non-Hispanic AI/AN. This study may provide insight on identifying needed treatment among patients with mCRC.

#2303 The impact of adherence to cancer prevention guidelines on cancer risk, cancer incidence and mortality in a large cohort of Canadian adults.

S. S. Fiati-Kenston, R. A. Murphy, J. M. Spiegel, T. J. B. Dummer;
University of British Columbia, School of Population and Public Health, Faculty of Medicine, Vancouver, BC, Canada

Background: The World Cancer Research Fund (WCRF) and the American Institute of Cancer Research (AICR) publish behavioral guidelines for cancer prevention. The guidelines provide standards on body weight, physical activity, diet, alcohol consumption, and smoking. This study examined the impact of adherence to these guidelines on cancer incidence and mortality in a large cohort of Canadian adults.
Methods: Data were obtained from the British Columbia Generations Project (BCGP), a longitudinal cohort of 30,000 adults aged 35-69 years recruited between 2009 and 2015, with a median follow-up of 10 years. Participants with a baseline cancer diagnosis were excluded, leaving 23,482 eligible individuals. A 3-level adherence score was constructed for each guideline. Cox proportional hazard models were employed to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for cancer incidence and mortality, adjusting for sociodemographic and health covariates. Propensity score matching was conducted to assess the robustness of the results.
Results: 3,068 new cancer cases and 769 cancer deaths were recorded during follow-up. Full adherence to the WCRF/AICR guidelines was achieved by only 19% of men and 31% of women. High adherence was associated with a significant reduction in all cancer incidence, with HRs of 0.81 (95% CI: 0.68-0.92) for women and 0.82 (95% CI: 0.73-0.95) for men. Cancer mortality was also lower among adherent individuals, with HRs of 0.83 (95% CI: 0.66-1.09) for women and 0.86 (95% CI: 0.70-0.96) for men. Additionally, total mortality decreased by 14% in women (HR = 0.86; 95% CI: 0.69-0.95) and 11% in men (HR = 0.89; 95% CI: 0.72-0.93). These associations remained robust after adjusting for sociodemographic and health covariates and were confirmed through propensity score matching.
Conclusions: Adherence to cancer prevention guidelines was associated with lower cancer incidence and mortality in both men and women. These findings highlight the importance of lifestyle modifications to reduce cancer risk among Canadian adults.
Keywords: cancer incidence, cancer prevention, physical activity, diet, body weight, smoking, alcohol

#2304 Plant-based dietary pattern and risk of pancreatic cancer: Findings from the Singapore Chinese Health Study.

Yen Thi-Hai Pham[1], Renwei Wang[1], Randall E. Brand[2], Tin C. Nguyen[3], Jian-Min Yuan[1], **Hung N. Luu**[1]

[1]UPMC Hillman Cancer Center, University of Pittsburgh, Pittsburgh, PA,[2]Division of Gastroenterology, Hepatology, and Nutrition, Department of Medicin, University of Pittsburgh, Pittsburgh, PA,[3]UPMC Hillman Cancer Center, Auburn University, Department of Computer Science and Software Engine, AL

Background: Pancreatic cancer is one of the leading causes of cancer-related death worldwide, with approximately 460,000 new cases and more than 430,000 deaths yearly. While most common cancers showed a declined trend in incidence, the incidence rate of pancreatic cancer has increased steadily by 1.5% per year during the past three decades. Established risk factors for pancreatic cancer are tobacco smoking, obesity, type 2 diabetes, and chronic pancreatitis. It is imperative to note that these established risk factors collectively account for less than 50% of pancreatic cancer burden. Recently, plant-based diets have received a significant attention due to their potential benefits to health outcomes, including cancer. The plant-based dietary indices (PDI) include total and healthful PDI (hPDI) and unhealthy PDI (uPDI), which were based on overall intake and sub-categories of food items that deem to be “healthfulness” and/or “unhealthfulness” to better account for the quality of plant foods, Limited number of prospective epidemiological studies have examined the association between PDI and pancreatic cancer risk. We, therefore, prospectively evaluated the associations for PDI, hPDI and uPDI scores with the risk of developing pancreatic cancer.

Methods: The current analysis used data from the Singapore Chinese Health Study, a prospective cohort study of 63,275 participants aged 45-74 living in Singapore who were recruited during 1993-1998 period. The three plant-based dietary scores (i.e., PDI, hPDI and uPDI) were derived from the semi-quantitative food frequency questionnaire at baseline. The incident cases of pancreatic cancer were identified through record linkage analysis with the nationwide Singapore Cancer Registry. Cox proportional hazard regression method was used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for pancreatic cancer in relation with PDI, hPDI and uPDI scores, adjusted for potential confounding factors.

Results: After 17.6 years of follow-up, we identified 316 incident cases of pancreatic cancer. While there was a null association for total PDI or hPDI with risk of pancreatic cancer, uPDI was found to be associated with increased risk of pancreatic cancer. The respective HRs (95% CIs) for quartiles 2, 3, and 4 (the highest quartile) relative to quartile 1 of uPDI were 1.07 (0.75-1.52), 0.86 (0.63-1.24), and 1.61 (1.13-2.28), respectively (P_{trend}=0.02).

Conclusions: Our findings showed that higher level of unhealthy plant-based dietary index was associated with increased risk of pancreatic cancer. Our results have public health implications for primary prevention against the development of pancreatic cancer, a malignancy among a few with unimproved survival rate during the past four decades.

#2305 Plant-based diets and subsequent risk of gastrointestinal cancers in Black and low-income Americans: Findings from a large prospective cohort study.

Fangcheng Yuan, Lei Wang, Danxia Yu, Wei Zheng

Division of Epidemiology, Department of Medicine, Vanderbilt Epidemiology Center, Vanderbilt University Medical Center, Nashville, TN

Background: Plant-based diets have been promoted for their health benefits. However, no study has specifically evaluated plant-based diets in relation to risk of gastrointestinal cancers in Black and low-income populations who disproportionately experience poor health outcomes. We aimed to investigate associations of plant-based dietary patterns with incident colorectal, liver, and pancreatic cancers in 70,255 participants of the Southern Community Cohort Study, a population-based prospective cohort conducted among predominantly Black and low-income Americans.
Methods: At baseline (2002-2009), dietary intakes were collected by a validated food frequency questionnaire from which adherences to overall, healthy, and unhealthy plant-based diets were assessed using established indices (i.e., PDI, hPDI, and uPDI). Incident cancer cases were ascertained via linkage to state cancer registries and the National Death Index. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated from Cox proportional hazards models after adjusting for potential confounders. In a subset of participants, we also examined associations of the three indices with shotgun metagenomic sequencing-profiled gut microbiome from stool samples (n=417) collected between 2018-2021 and mass spectrometry-measured metabolites from baseline plasma samples (n=1,581). Cancer cases diagnosed before stool or blood collection were excluded.
Results: During a median follow-up time of 12.6 years, 544, 370, and 276 incident primary colorectal, liver, and pancreatic cancer cases were identified after excluding participants with less than 2 years of follow-up. Compared with the lowest quartile, the highest quartile of PDI was associated with lower colorectal cancer (CRC) risk (HR=0.72, 95% CI=0.55-0.94, P_{trend}=0.01), whereas that of uPDI was associated with elevated CRC risk (HR=1.41, 95% CI=1.07-1.86, P_{trend}=0.02). A high hPDI was linked to reduced liver cancer risk (HR=0.60, 95% CI=0.42-0.87 for the highest versus lowest quartile, P_{trend}=0.006). No index was associated with pancreatic cancer risk. After adjusting for multiple testing (FDR-adjusted P<0.10), PDI was inversely associated with species *MGYG-HGUT.02013* within genus *Collinsella*. PDI, hPDI, and uPDI were associated with 201, 107, and 47 circulating metabolites, respectively. Over-representation analysis suggested that caffeine and branched-chain amino acids metabolism pathways were significantly enriched for PDI-associated metabolites.
Conclusion: Adherence to a diet characterized by healthy plant foods and low animal foods may lower colorectal and liver cancer risk.

#2306 Estrogen metabolism-related lifestyle scores and breast cancer risk among 62,000 Chinese and American postmenopausal women: Findings from two large prospective cohort studies.

Fangcheng Yuan[1], Wanqing Wen[1], Steven C. Moore[2], Wei Zheng[1]

[1]Division of Epidemiology, Department of Medicine, Vanderbilt Epidemiology Center, Vanderbilt University Medical Center, Nashville, TN,[2]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD

Background: An increased 2-hydroxylation (2-OH) of estrogen has been linked to reduced risk of breast cancer (BC). We hypothesized that lifestyle patterns related to an elevated estrogen 2-OH metabolism were associated with lower BC risk and tested this hypothesis among post-menopausal women in two prospective cohorts.

Methods: Estrogens and estrogen metabolites (EMs) were profiled by liquid chromatography-mass spectrometry in a subset of post-menopausal women in the Shanghai Women's Health Study (SWHS, *n*=804) and Prostate, Lung, Colorectal and Ovarian Screening Trial (PLCO, *n*=842), respectively. Blood (PLCO) or urine (SWHS) samples used in these assays were collected at baseline prior to any cancer diagnosis. We applied elastic net regularized regression to derive two lifestyle scores that characterize relative abundance of 2-OH pathway EMs, estimated separately by the ratio of 2-OH EMs to parent estrogens and the ratio of 2-OH EMs to parent estrogens and all EMs combined. Intakes of foods and nutrients assessed by baseline food frequency questionnaires, and measures of body fat, alcohol intake, cigarette smoking, and leisure-time physical activity were included as predictors. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for associations of derived lifestyle scores with post-menopausal BC risk in the larger SWHS (*N*=35,453) and PLCO (*N*=26,758) cohorts, after adjusting for potential confounders.

Results: The lifestyle scores explained 10-12% of the total variance in the 2-OH EM ratios. A high lifestyle score for the ratio of 2-OH EMs to parent estrogens was associated with decreased BC risk in the SWHS (HR=0.73, 95% CI=0.59-0.90 for the highest versus lowest quartile, P_{trend}=0.001). This inverse association was seen only among estrogen receptor (ER)-positive BC cases ($P_{heterogeneity}$=0.03). A similar association was observed for the lifestyle score for the ratio of 2-OH EMs to the combined parent estrogens and EMs. In the PLCO, the score for the ratio of 2-OH EMs to parent estrogens was inversely related to BC risk (HR=0.83, 95% CI=0.70-0.99, P_{trend}=0.06), whereas no significant association was found for the other lifestyle score.

Conclusion: A lifestyle pattern reflecting elevated estrogen 2-OH metabolism may lower post-menopausal BC risk. Women with a low 2-OH of estrogen may benefit from diet and lifestyle modifications.

#2308 Sustained adherence to the American Cancer Society guideline for diet and physical activity for cancer prevention and 8-year all cancer risk: an emulated target trial in the Cancer Prevention Study-II Nutrition Cohort.

Valeria Elahy[1], Marjorie L. McCullough[1], Yu-Han Chiu[2], Alpa V. Patel[1], Erika Rees-Punia[1], Clara Bodelon[1], Anita R. Peoples[1], Ying Wang[1]

[1]American Cancer Society, Atlanta, GA,[2]Penn State College of Medicine, Hershey, PA

Background: The 2020 American Cancer Society (ACS) Guideline for Cancer Prevention recommends maintaining a healthy body weight, engaging in regular physical activity, following a healthy diet, and abstaining from alcohol. While evidence of the cancer preventive effects of individual guideline components is well-established, few studies have assessed the long-term impact of adhering to multiple recommendations jointly using an emulated target trial design. An emulated target trial design mimics a hypothetical pragmatic randomized trial to answer causal questions using observational data.
Objectives: To examine 8-year cancer risk under sustained adherence (intervention) to the physical activity, diet, and alcohol components of the ACS Guideline, both jointly and individually, compared to the behavior reported in the cohort (no intervention), stratified by body mass index (BMI) categories.
Methods: Data from cancer-free, nonsmoking participants aged 47-80 years at baseline (1999) in the Cancer Prevention Study-II Nutrition Cohort were analyzed, including 30,111 individuals with normal weight (BMI 18.5 - <25 kg/m^2), 27,986 with overweight (BMI 25 - <30 kg/m$^{2)}$ and 11,631 with obesity (BMI ≥30 kg/m^2). Using an emulated target trial design, we assessed the effect of sustained adherence to the ACS Guideline components on cancer risk. Sustained adherence over 8 years was defined as meeting recommendations for physical activity (≥15 MET-hrs/wk), diet (≥6 serv/d and ≥21 unique fruits and vegetables per month, ≥2 serv/d of whole grains, ≤0.5 serv/d of red/processed meat, 0 serv/wk of sugar-sweetened beverages, ≤25% of total energy from processed foods and refined grains), and alcohol abstinence (0 serv/wk). The g-formula estimated absolute 8-year all cancer risk, adjusting for factors related to adherence and loss to follow-up.
Results: The estimated 8-year risk of all cancers under no intervention was 13.5% for those with normal weight, 15.7% for those with overweight, and 14.4% for those with obesity. Compared to no intervention, sustained adherence to the joint ACS Guideline showed a 1.3% (95% CI: -2.3, -0.1), 1.0% (95% CI: -2.5, 0.4), and 0.7% (95% CI: (-2.7, 1.6) lower cancer risk for those with normal weight, overweight, and obesity, respectively. Among individual components, alcohol abstinence had the most pronounced mean risk reduction, ranging from 0.7% (95% CI: -1.7, 0.2) for those with overweight to 1.1% (95% CI: -1.9, -0.3) and 1.1 (95% CI: -2.2, 0.1) for those with normal weight and obesity, respectively.
Conclusions: Sustained adherence to the ACS Guideline modestly reduced cancer risk, particularly for individuals with normal weight. Alcohol abstinence was a key contributor to the observed risk reductions.

#2309 An ecological study on environmental exposure to PFAS and thyroid cancer incidence rates by races/ethnicities in Florida.

Ming S. Lee, Laura Wild, Niko DiStefano, Garrett Forman, Elizabeth Franzmann, Natasha Solle, Alberto Caban-Martinez, Erin Kobetz

University of Miami Miller School of Medicine, Miami, FL

Background: The incidence rate of thyroid cancer in the US has increased significantly over the last few decades. However, besides radiation exposure and obesity, no modifiable risk factors have been identified for thyroid cancer. Although theories suggest exposure to per- and polyfluoroalkyl substances (PFAS) could contribute to the development of papillary thyroid cancer, existing studies on the effects of PFAS on thyroid cancer risk do not yield consensus results. Using data for the state of Florida, this ecological study aims to determine whether incidence rates of thyroid cancer vary with respect to residential proximity to industrial facilities handling PFAS materials as well as living in areas where drinking water containing detectable levels of PFAS.
Methods: Florida cancer registry data from 2011 to 2020 was used to calculate the incidence rates of papillary thyroid cancer by race/ethnicity (Hispanic, non-Hispanic White (NHW), and non-Hispanic Black (NHB)) for all census tracts in Florida. Environmental Protection Agency data was used to identify the locations of industrial facilities handling PFAS as well as ZIP codes with public drinking water testing positive for PFAS above reportable levels. A geographic information system was used to calculate the number of PFAS facilities within 10KM of a census tract. Correlation, linear regression, and t-tests for sample mean comparison were used to assess the associations between PFAS exposure and thyroid cancer rates at the census tract level.
Results: A total of 14,994 cases (9,213 in NHWs, 3,995 in Hispanics, and 989 in NHB) of thyroid cancers were diagnosed in Florida from 2011 to 2020. Correlation analyses show a positive correlation between residential proximity to PFAS facilities and greater Hispanic and NHB population compositions by higher percentages of ($r=0.36$, $p<0.001$) with lower income ($r=-0.192$, $p<0.001$)) and higher obesity rate ($r=0.197$, $p<0.001$)). Regression analyses show that proximity to PFAS facilities is a significant factor for thyroid cancer rates in Hispanic ($p=0.006$) and NHW ($p<0.001$). Hispanic and NHW living are in IPZIP code areas with reportable PFAS in drinking water also have higher average thyroid cancer rates than those in areas with no reportable PFAS (t-test $p=0.036$ for Hispanic and $p=0.039$ for NHW). With a lower average incidence rate than Hispanic and NHW, thyroid cancer rates in NHB show no statistically significant associations to PFAS exposure.
Conclusions: ecological Our findings suggest potential effects of environmental PFAS exposure on thyroid cancer disparities. set up trials to validate these effects by sampling volunteers for blood-level PFAS measurement from neighborhoods with different levels of PFAS exposure (facility proximity and drinking water).

#2310 Food environment index is inversely associated with cancer survival in North Carolina.

S. Wu[1], S. Ulrich[1], T. Feng[1], Y. Liu[2], L. Tseng[1];

[1]Appalachian State University, Boone, NC, [2]The University of North Carolina at Chapel Hill, Chapel Hill, NC

Background: Exploring the potential role of the food environment on cancer survival could facilitate prevention and control approaches to improve cancer survivorship. This study examined the associations between food environment index (FEI) and survival for overall cancer in North Carolina.

Methods: This retrospective cohort study included 1,292,991 patients diagnosed with overall cancer from January 1, 2000 to December 31, 2022 from the North Carolina Cancer Central Registry. The individual-level demographic, tumor, and treatment information were provided by the North Carolina Cancer Central Registry. FEI and other county-level exposures were obtained from the County Health Rankings & Roadmaps databases. The county-level food environment index is an indicator of access to healthy foods (0 is worst, 10 is best). Cox regression was used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for the associations between FEI and cancer survival after adjusting for individual-level and county-level covariates.

Results: Higher levels of FEI were associated with a statistically significant better survival for overall cancer (fully adjusted HR for every score increase=0.983, 95% CI 0.979, 0.988; P <0.0001; adjusted HR for the 3rd quartile vs. 1st quartile = 0.98, 95% CI 0.94, 0.99; and adjusted HR for the 4th quartile vs. 1st quartile = 0.95, 95% CI 0.94, 0.96).

Conclusion: These results suggest that a healthy food environment, as measured by FEI, may be a protective factor for cancer survival in North Carolina. To improve cancer survival, further strategies to improve food environment at the county level are warranted.

#2311 Temporal trends and HPV attributability in oropharyngeal cancer: A cross-sectional study in multi-racial Singapore.

Yew Mun Lee[1], Nhi Thi Mai Nguyen[2], Zhi Yong Lim[1], Zi Ting Corrina Tan[3], Jia Qi Gabie Chua[3], Yirong Dai[2], Xin Xiu Sam[4], Jia Hui Isabelle Jang[1], Eriko Yamada[5], Kok Hing Lim[4], **Chwee Ming Lim**[6]

[1]Department of Otolaryngology-Head and Neck Surgery, Singapore General Hospital, Singapore, Singapore,[2]Duke-NUS Medical School, Singapore, Singapore,[3]Department of Clinical Translational Research, Singapore General Hospital, Singapore, Singapore,[4]Department of Pathology, Singapore General Hospital, Singapore, Singapore,[5]MSD Pharma (Singapore), Singapore, Singapore,[6]Department of Otolaryngology-Head and Neck Surgery and Department of Clinical Translational Research, Singapore General Hospital, Singapore, Singapore

Introduction: The purpose of this study is to estimate the proportion of oropharyngeal squamous cell carcinoma (OPSCC) attributable to Human Papillomavirus (HPV) in Singapore across two different time periods (2008-2011 and 2018-2021).
Experimental procedures: This non-interventional, cross-sectional study involves the retrospective analysis of pre-treatment OPSCC tissue samples under Singapore General Hospital (SGH) after obtaining institutional ethics approval. Retrospective biobank records of primary OPSCC patients with archival pre-treatment tissue samples from 2008-2011 (cohort 1, n=60) and 2018-2021 (cohort 2, n=60) were retrieved. Both $p16^{INK4a}$ immunohistochemistry staining (IHC) and HPV-DNA testing to determine HPV genotypes for up to 32 HPV genotypes using INNO-LiPA® HPV Genotyping (Fujirebio, Japan) were performed. Data on patient demographics, tumor characteristics, and risk factors were collected from medical records and entered a case report form (CRF). Descriptive statistical analyses were performed to determine the HPV-attributable fractions (HPV-AFs) and their corresponding patient characteristics. The current abstract described the results from the cohort 1.
Results: The primary outcome of this study is to determine the proportion of OPSCC cases attributable to HPV, defined by the presence of both HPV-DNA and $p16^{INK4a}$ positivity. The secondary outcome is to identify the distribution of HPV genotypes within the HPV-AFs of the two cohorts. In cohort 1, approximately 70% of diagnosed OPSCC cases were men, with HPV-attributable cases making up 20% of the total. Among the HPV-AFs, more than half of the HPV-positive cases were male. HPV16 (83.3%) was the predominant genotype in the HPV-AFs, followed by HPV26 (8.3%) and HPV58 (8.3%). The mean age of cohort 1 was 63.1 years (±11.0). Reflecting Singapore's multi-racial demographic, 68% of cohort 1 were Chinese, and 17% were Indian.
Conclusion: Our study fills a critical evidence gap regarding the burden of HPV-related OPSCCs in a multi-ethnic Singaporean community. By comparing the burden of HPVs in OPSCC across the two distinct time periods, this study offers insights into the temporal changes in trends of HPV-attributable OPSCCs.

#2312 Analysis of mortality and survival in colon cancer at ISSSTECALI from 2017 to 2022.

Fernando Ramirez-Perales[1], Angelica Pon-Mendez[2], Diana Evelyn Villa-Guillen[3], Diego Fernando Ovalle-Marroquin[4]

[1]Urgent Care, Hospital General del Estado de Sonora, Hermosillo, Mexico,[2]ISSSTECALI, Mexicali, Mexico,[3]Research and Teaching, Hospital General del Estado de Sonora, Hermosillo, Mexico,[4]Universidad Autonoma de Baja California, Mexicali, Mexico

Introduction: Colorectal cancer (CRC) is a leading cause of cancer-related deaths worldwide, including 2.68% of malignancies in Mexico. In 2018, it caused 880,000 deaths, primarily in low- and middle-income countries. In Mexico, the lack of organized screening programs leads to late-stage diagnoses and poor outcomes. ISSSTECALI, serving government workers in Baja California, faces challenges in CRC detection and management due to healthcare disparities.
Objective: This study aims to evaluate survival rates and identify factors associated with colon cancer mortality among patients treated at ISSSTECALI in Baja California, Mexico, from 2017 to 2022.
Methods: A retrospective cohort study was conducted, including patients diagnosed with colon cancer between January 2017 and September 2022. Survival functions were estimated using the Kaplan-Meier method, and differences between survival curves were assessed with the log-rank test. Mortality risk factors were analyzed using a multivariable Cox proportional hazards regression model. The dependent variable was survival, while age, sex, municipality, histology, metastasis, cancer stage, and treatment type were included as independent variables.
Results: A total of 118 colon cancer cases were analyzed, of which 42.3% (50) resulted in mortality. Survival rates were 80.9%, 70.8%, 54.6%, and 46.8% at 1, 2, 3, and 4 years, respectively. The overall survival rate at the median follow-up (1.58 years) was 76.5%. Significant differences in survival curves were observed based on age group, general syndrome, metastasis, and cancer stage ($p < 0.05$). In the Cox regression model, metastasis was the strongest predictor of a poor prognosis (HR = 4.32, 95% CI: 1.92-9.68). Although the stage at diagnosis showed significant differences in the log-rank test, it was not a significant predictor after adjustment in the Cox model (HR = 2.30, 95% CI: 0.46-11.35).
Conclusions: This study emphasizes the importance of early diagnosis in improving survival rates and reducing mortality from colon cancer. It highlights the need for organized and accessible screening programs, particularly in healthcare systems like ISSSTECALI, to ensure timely detection and treatment. Implementing non-invasive screening methods—such as Fecal Occult Blood Testing at age 25 and colonoscopies at age 45 for average-risk individuals—could enhance early detection. The identification of metastasis as a key predictor of poor prognosis highlights the need for targeted interventions for advanced disease. Addressing disparities in cancer detection and management is essential to improve survival rates and reduce the burden of colon cancer in Mexico.

#2313 Trends in use of neoadjuvant systemic therapy for head and neck squamous cell carcinoma.

Wesley L. Cai[1], **Vanessa Helou**[1], Angela L. Mazul[1], Dan P. Zandberg[2], Matthew E. Spector[1], Jose P. Zevallos[1], Robert L. Ferris[3], Kevin J. Contrera[1]

[1]Department of Otolaryngology-Head and Neck Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA,[2]UPMC Hillman Cancer Center, University of Pittsburgh Medical Center, Pittsburgh, PA,[3]UNC Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC

Importance: Neoadjuvant systemic therapy, including chemotherapy and immune checkpoint inhibitors, have potential benefits in head and neck squamous cell carcinoma (HNSCC). These potentially include reducing distant metastases, organ preservation, and treatment personalization. KEYNOTE 689 has the potential to change the treatment paradigm; however, trends in the use of neoadjuvant therapy leading up to the publication of this study remain unclear.
Objective: To evaluate trends in the use of neoadjuvant systemic therapy in patients with HNSCC from 2015 to 2022.
Design, Setting, and Participants: This retrospective, population-based cohort study utilized data from the National Cancer Database (2015-2021) to analyze neoadjuvant systemic and immunotherapy trends in HNSCC. Neoadjuvant treatment was defined as therapy administered at least 8 weeks prior to definitive surgery. Clinical staging and p16 status were assessed using AJCC 8th edition staging for patients diagnosed after 2018. Associations were analyzed using chi-square tests.
Main Outcomes and Measures: The primary outcomes were trends in neoadjuvant therapy use and associations with tumor stage, anatomic site, and p16 status.
Results: Among 292,181 patients (216,048 [74%] male; 76,133 [26%] female), 922 received neoadjuvant systemic therapy, and 603 received neoadjuvant immunotherapy. Neoadjuvant therapy use began to rise in 2015 with an immunotherapy use rate of 0.078%, peaking at 0.325% in 2019 (a 4-fold increase) and stabilizing at 0.165% in 2022 (a 2-fold increase from 2015). Similarly, overall neoadjuvant chemotherapy use was 0.22% in 2015, 0.44% in 2019, and 0.26% in 2022. Sites with the largest increases in neoadjuvant immunotherapy use were the floor of mouth (3-fold), gums (2.4-fold), and tongue (2-fold). Patients receiving neoadjuvant therapy were significantly more likely to present with stage 4 disease compared to stages 1-3 (58% vs. 27%; $P = .0005$). p16 positivity rates were comparable between groups (84% vs. 80%; $P = .68$).
Conclusions and Relevance: The use of neoadjuvant immunotherapy increased by 112% from 2015 to 2022, while overall neoadjuvant systemic therapy use rose by 18%. Patients undergoing neoadjuvant therapy were more likely to present with advanced-stage disease. With the upcoming release of KEYNOTE 689, understanding the current trends and treatment approaches for neoadjuvant therapy are crucial.

#2314 The epidemiology and impact of aspiration pneumonia in patients admitted with esophageal cancer: a nationwide data analysis.

Abdu Mohammed[1], Adamsegd I. Gebremedhen[2], Yifang Song[1], Mamdouh Souleymane[2], Ibrahim Shanti[2], Malik Samardali[3], Toni O. Pacioles[4]

[1]Internal Medicine, Trinity Health System, Steubenville, OH,[2]Internal Medicine, Marshall University Joan C. Edwards School of Medicine, Huntington, WV,[3]Internal Medicine, St Elizabeth Youngstown Hospital, Youngstown, OH,[4]Hematology and Oncology, Marshall University Joan C. Edwards School of Medicine, Huntington, WV

Background: Esophageal cancer (EC) is frequently associated with dysphagia, arising from tumor invasion or treatment-related adverse effects, such as surgical resection or chemoradiation. Dysphagia substantially increases the risk of aspiration pneumonia (AP), a frequent complication in this population. Despite the established link between AP and head and neck cancers, its epidemiology and clinical impact in patients with EC remain underexplored. This study aims to delineate the burden of AP in EC hospitalizations and its effect on inpatient outcomes, addressing a critical gap in oncological and infectious disease literature.
Methods: Hospitalizations with a primary diagnosis of EC were extracted from the National Inpatient Sample (NIS) from 2019 to 2021, using ICD-10 codes. The cohort was split by the presence or absence of AP. Sociodemographic variables, comorbidities and the presence of concurrent COVID-19 infection were assessed. The primary outcome was all-cause in-hospital mortality, with secondary outcomes including rates of complications, resource utilization, length of stay (LOS), and hospitalization costs. Multivariate regression analyses were conducted with statistical significance set at $p < 0.05$.
Results: Among 42,059 EC hospitalizations, 3,550 (8.4%) were complicated by AP. The AP cohort was older (mean age 67.9 vs. 66.6 years; $p < 0.05$), predominantly from the southern United States (36.6%; $p < 0.05$), and exhibited a higher prevalence of severe comorbidities (Charlson Comorbidity Index ≥ 3: 79.1% vs. 75.4%; $p < 0.05$). AP was independently associated with a significantly elevated risk of in-hospital mortality (adjusted odds ratio [aOR] 3.28; 95% CI, 2.60-4.13). Furthermore, patients with AP demonstrated increased odds of respiratory failure (aOR 7.50; 95% CI, 6.35-8.86), mechanical ventilation (aOR 4.64; 95% CI, 3.68-5.86), severe sepsis (aOR 10.65; 95% CI, 8.15-13.92), and vasopressor use (aOR 3.11; 95% CI, 2.08-4.64). Additionally higher odds of shock (aOR 5.18; 95% CI, 4.10-6.52), acute kidney injury (aOR 2.11; 95% CI, 1.74-2.55), and deep venous thrombosis (aOR 2.13; 95% CI, 1.56-2.92) were seen. Hospitalizations involving AP were associated with significantly prolonged LOS (16.03 vs. 8.05 days; adjusted incidence rate ratio [aIRR] 1.89; 95% CI, 1.76-2.05) and higher total costs ($232,449 vs. $122,488; aIRR 1.84; 95% CI, 1.63-2.07).
Conclusions: Hospitalizations of EC patients complicated by AP are associated with markedly increased odds of in-hospital mortality, severe complications, prolonged LOS, and higher healthcare costs. These findings underscore the necessity for targeted interventions to mitigate AP-associated morbidity and mortality in this high-risk patient population. Further research should focus on optimizing preventative and therapeutic strategies to improve clinical outcomes in this subset of oncology patients.

#2315 Projecting lung cancer incidence in Guam: the impact of smoking prevalence, ethnic disparities, and aging population.

Cabrini Cruz Aguon[1], Grazyna Badowski[1], Aurienne Cruz[1], Grace G. Bordallo[2]

[1]University of Guam, Hagatna, Guam,[2]Guam Department of Public Health and Social Services, Hagatna, Guam

Lung cancer incidence in Guam is slightly lower than in the U.S. overall (50.9 vs. 52.6 per 100,000), but CHamoru and Micronesian populations have significantly higher rates (69.4 and 78.6 respectively). Given the established link between smoking and lung cancer, this study aims to project lung cancer incidence in Guam considering smoking rates across sex, age, and ethnic groups, accounting for the island's aging population (median age increased from 30.4 in 2010 to 33.7 in 2020). Data on 1,157 invasive lung cancer cases reported to the Guam Cancer Registry from 2001 to 2020 for individuals aged 34 and older were analyzed. Variables included age, sex, and ethnicity including CHamoru (52.3%), Filipino (20.9%), Micronesian (9.4%), Asian (6.4%), and White (7%). Smoking prevalence data from the 2011-2020 Behavioral Risk Factor Surveillance System (BRFSS) were used to estimate smoking rates for age × sex × ethnicity strata. A Poisson regression model was used to examine relationships between lung cancer incidence rates and demographic factors, including strata-level smoking prevalence. Temporal trends were evaluated across two periods: 2001-2010 and 2011-2020. Predicted rates were applied to 2020 Guam Census population counts to estimate lung cancer cases for 2021-2030 and the annual percent change (APC). From 2011 to 2020, smoking prevalence in Guam declined from 30.2% to 19% (APC = -3.7), with variation among ethnic groups. CHamoru and Asian males had the highest smoking rates. Micronesians showed the largest decrease (APC = -7.3%, from 35% to 10%), while CHamoru males had the smallest reduction (APC = -2.8%, from 46% to 35%). Lung cancer incidence was significantly higher for individuals aged 75+ compared to those aged 35-44 (IRR = 38, 95% CI: 26.9, 55). Males had over twice the rate of females (IRR = 2.3, 95% CI: 2.0, 2.7). Micronesians and CHamorus had significantly higher rates than Filipinos (IRR = 3.5 and 2.4, respectively). Each unit increase in smoking prevalence was associated with a 2% rise in lung cancer incidence for Micronesians and CHamorus. Projected lung cancer cases over 10 years are highest among CHamoru males (246) and females (130). The model predicts annual increases of 1.2% for males and 0.5% for females, with the largest increases among Asian males (2.6%) and Micronesian females (3.3%). Significant differences in lung cancer and smoking rates exist, with the highest burden among CHamorus and Micronesians. Despite a decrease, Guam's 2020 smoking prevalence remained significantly higher than the U.S. average of 12.5%, indicating persistent disparities. This study highlights the need for targeted tobacco control interventions and screening programs to address the evolving cancer burden. This research was funded by the National Cancer Institute Grant: U54CA143728 (University of Guam)/Grant U54CA143727 (University of Hawaii Cancer Center).

#2317 Historical trends in age of menarche and implications for breast cancer risk in the US population.

Lin Yang[1], Adetunji Torioa[2]

[1]CancerControl Alberta-Alberta Health Services, Calgary, AB, Canada,[2]Surgery, Washington University School of Medicine, St. Louis, MO

Background: Breast cancer incidence is increasing, especially among premenopausal women. Age of menarche is a critical marker of female pubertal development and a known risk factor for breast cancer. We, therefore, examined trends in the age of menarche among females born between 1910 and 2000 as well as the differences by race/ethnicity and country of birth.
Methods: This serial, cross-sectional study analyzed the age of menarche from the nationally representative National Health and Nutrition Examination Survey (NHANES). Participants included noninstitutionalized US women from ten NHANES study cycles (1999-2000 to 2017-March 2020 pre-pandemic). Age of menarche was assessed as the age of first menstrual period by participants responding to the question “How old when you had your first menstrual period?” (range 0-22 years old). Participants who responded with an age of first menstrual period > 16 years were excluded from the current analysis. The year of birth was categorized into eight birth cohorts from 1910-1930, then every 10-year interval to 1990-2000. We calculated the weighted mean age and standard error of age of menarche overall and by birth cohort, race/ethnicity, and country of birth. We calculated weighted prevalences of the age of menarche by ages ≤ 10, 11, and 12 years, overall, and by birth cohort, race/ethnicity, and country of birth.
Results: Data on 24,391 US women were analyzed. For women born between 1910-1930 to those born between 1990-2000, the mean age of menarche declined from 12.9 (95% CI: 12.8 to 10.3) to 12.4 (95% CI: 12.3 to 12.6) years (difference=0.5, 95% CI: 0.3 to 0.7, p for trend<0.001). The significant decline in age of menarche was observed in women of all racial/ethnic groups (non-Hispanic White: 12.9 [95% CI: 12.8 to 13.0] to 12.6 [95% CI: 12.4 to 12.8], difference=0.3 [95% CI:0.01 to 0.5], non-Hispanic Black: 13.1 [95% CI: 12.8 to 13.3] to 12.2 [95% CI: 11.9 to 12.4], difference=0.9 [95% CI: 0.5 to 1.2]; Hispanic: 13.2 [95% CI: 12.7 to 13.8] to 12.1 [95% CI: 11.9 to 12.3], difference=1.1 [95% CI: 0.5 to 1.7]; Others: 13.0 [95% CI: 12.4 to 13.6] to 12.3 [95% CI: 11.9 to 12.6], difference=0.8 [95% CI: 0.03 to 1.5]). The decline in age of menarche was observed in both US (12.9 [95% CI: 12.8 to 13.0] to 12.4 [95% CI: 12.3 to 12.6]) and non-US born (13.3 [95% CI: 13.0 to 13.7] to 12.4 [95% CI: 12.1 to 12.8]) women. Among women in the 1990-2000 birth cohort, 11.1%, 27.9%, and 53.9% of women attained their age of menarche at ages younger than 10, 11, and 12 years, respectively.
Conclusions: Women born in the 1990-2000 birth cohort experienced half a year younger age of menarche compared to those born between 1910-1930, with shaper declines in women of racial/ethnical minorities and those who were non-US born. These shifts suggest a growing proportion of women experiencing menarche earlier, likely contributing to the rising incidence of breast cancer.

#2318 Waist circumference and the risk of cancer: Two-sample mendelian randomization.

Su Hyun Lee[1], Sun Ha Jee[2], Yoon-Jung Choi[1]

[1]National Cancer Center - Korea, Goyong, Korea, Republic of,[2]Yonsei University, Seoul, Korea, Republic of

Background: The normal range of waist circumference for Asians is less than 90 cm for men and less than 85 cm for women, according to the Korean Society for the Study of Obesity (KSSO) guidelines. While observational studies have suggested an association between obesity and cancer, the causality of obesity in cancer development is not fully established. A two-sample Mendelian randomization (MR) analysis was used to assess whether a genetic predisposition index for waist circumference (WC) was associated with overall cancer and 10 site-specific cancer risk.While observational studies have suggested an association between obesity and cancer, the causality of obesity in cancer development is not fully established. A two-sample Mendelian randomization (MR) analysis was used to assess whether a genetic predisposition index for waist circumference (WC) was associated with overall cancer and 10 site-specific cancer risk.

Methods: The MR method can assess potential causal relationships in various diseases. Summary-level data for WC were extracted from The Korean Cancer Prevention Study-II (KCPS-II) Biobank, and summary-level data for cancer were extracted from The Korean Genome Epidemiology Study (KoGES).

Results: There was no causal relationship between WC and total cancer, but a positive correlation was found in colorectal cancer and thyroid cancer (total cancer OR=1.009, 95% CI=0.995-1.023; colorectal cancer OR=1.051, 95% CI=1.011-1.091; thyroid cancer OR=1.037, 95% CI=1.006-1.068). In cervical cancer and prostate cancer, protective results were shown for WC (cervical cancer OR=0.943, 95% CI=0.891-0.998; prostate cancer OR=0.874, 95% CI=0.766-0.998).

Conclusion: The evidence supporting a causal relationship between WC and cancer was limited. Genetically predicted WC was positively correlated with colorectal cancer and thyroid cancer. The current study found causal detrimental effects of WC on several cancers. We propose to strengthen cancer screening in patients with large WC to enable early detection of cancer.

#2319 Anti-diabetes medication use and risk of colorectal polyps: a colonoscopy-based study using natural language processing.

Jessica van Onselen[1], Ryzen Benson[2], Stephanie Richardson[3], Maci Winn[4], Candace Winterton[3], Bejamin Krick[5], Svenja Pauleck[4], Ainhoa Gomez-Lumbreras[4], Polly Newcomb[6], Neli Ulrich[1], John Inadomi[7], Sheetal Hardikar[1]

[1]Population Health Sciences, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT,[2]Department of Biomedical Informatics, University of Utah, Salt Lake City, UT,[3]Huntsman Cancer Institute, Salt Lake City, UT,[4]Population Health Sciences, University of Utah, Salt Lake City, UT,[5]Department of Political Science, Duke University, Durham, NC,[6]Public Health Sciences, Fred Hutch Cancer Center, Salt Lake City, UT,[7]Internal Medicine, University of Utah, Salt Lake City, UT

Purpose: Type-2 diabetes (T2D) has been previously associated with an increased risk of colorectal polyps, but the effect of anti-diabetes medication use on polyp risk remains unclear.
Methods: Using pathology reports from the University of Utah (UU) Enterprise Data Warehouse (EDW), we developed a rule-based natural language processing (NLP) pipeline to extract colorectal polyp diagnoses and features (site, shape, number, size) on 15,679 patients who underwent a colonoscopy at the UU Gastroenterology clinic from 2011-2020. The NLP pipeline, validated by manual abstraction of 350 pathology reports, demonstrated excellent performance (accuracy 91%). We identified 6,556 T2D patients using ICD codes and extracted patient characteristics from the EDW, including age, sex, race, smoking, BMI, and active anti-diabetes medication prescriptions (insulin, metformin, other). Adjusted odds ratios (OR) and 95% confidence limits (95%CI) were calculated for medication use using multivariable polytomous logistic regression models.
Results: A total of 73% of patients with T2D used anti-diabetes medications and were, on average, 62 years old, 76% White, and 50% female, with a mean BMI of 33 kg/m2. Patients taking insulin had a statistically significant increased risk of colorectal polyps compared to patients not taking anti-diabetes medications or those who were taking medications other than insulin [OR(95% CI)= 1.44(1.22,1.70) and 1.36(1.17,1.60)], respectively. Metformin use was not associated with an increased risk of polyps when compared to patients not on anti-diabetes medications [OR(95% CI)= 1.16(1.00,1.34)]. When compared to patients taking medications other than metformin, those on metformin had a decreased risk for polyps [OR(95% CI)= 0.63(0.54,0.74)]. When evaluated by polyp subtype, insulin use was associated with an increased risk for adenoma and serrated polyps [OR(95%CI)= 1.28(1.08,1.53) and 1.76(1.44, 2.16), respectively], compared to no anti-diabetes medication use. Patients taking metformin had an increased risk for serrated polyps [OR95%CI= 1.36(1.11,1.67)], compared to non-users of metformin. When evaluating polyp severity, risks were higher for patients with advanced pathology (advanced adenoma and sessile serrated polyps) compared to those with non-advanced pathology.
Conclusion: Insulin use was associated with an increased risk, while metformin use was associated with a decreased risk for colorectal polyps, compared to patients taking other medications. These results are consistent with some published literature for colorectal cancer, suggesting that these pathways may be acting at the pre-neoplasia stage rather than during neoplastic conversion from polyps to colorectal cancer. Further research is needed to understand how T2D and its treatment contribute to colorectal neoplasia.

#2320 Cardiovascular disease mortality among breast cancer survivors in Puerto Rico: 2004-2019.

Carola T. Sanchez-Diaz[1], **Genesis Rodriguez-Ortiz**[1], Liliana Castro-JImenez[1], Barbara Segarra-Vazquez[2], Francisco Cordova[3], Vivian Colon-Lopez[1], Cynthia Perez-Cardona[4]

[1]University of Puerto Rico Comprehensive Cancer Center, San Juan, Puerto Rico,[2]Clinical Laboratory Science Program, School of Health Professions, San Juan, Puerto Rico,[3]Section of Cargiology, Veterans Affairs Medical Center, San Juan, Puerto Rico,[4]Epidemiology and Biostatistics, University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico

Purpose: Cancer and Cardiovascular disease (CVD) are the two leading causes of death in both the United States and Puerto Rico (PR). Breast cancer (BC) survivors, considered since the time at diagnosis, face an elevated risk of CVD mortality, largely due to the cardiotoxic effects of cancer treatments and the presence of shared cardiometabolic risk factors with BC. However, there is a need for studies examining the risk of CVD mortality specifically among BC survivors in PR, one of the most rapidly aging populations in the world. This study aims to assess and compare the risk of CVD mortality among BC survivors and the general population in PR from 2004 to 2019.

Methods: Data on BC survivors were obtained from the PR Central Cancer Registry for the period 2004 to 2019. Follow-up started approximately 12 months after diagnosis to account for treatment completion and extended through 2021. Standardized Mortality Ratios (SMRs) were calculated to compare CVD mortality risk between BC survivors and of the general population of women in PR aged 18 and older. The expected number of deaths was determined using age-, sex- and calendar year-specific mortality rates from the general population, obtained from the National Vital Statistics System.

Results: The overall SMR for CVD mortality among BC survivors was 2.18 (95% CI: 1.99-2.38), with 470 observed, compared to 215.89 expected CVD deaths during the study period. The total person-years at risk amounted to 66,388. A significant positive trend in increased risk of CVD mortality was observed by calendar year ($p < 0.001$), starting with a SMR of 1.51 (95% CI: 1.13-2.03) in 2004 and peaking in 2019 (SMR: 8.39; 95% CI: 4.64-15.15). Women under 60 years of age faced a particularly elevated risk (SMR: 8.48; 95% CI: 5.82-12.37), with SMRs declining to 1.73 (95% CI: 1.53-1.95) in women over 80 years of age.

Conclusion: Our findings reveal a significantly elevated CVD mortality risk among BC survivors in PR, particularly in younger survivors, compared to the general population. Increasing SMRs over time highlight the importance of understanding cardiometabolic risk factors and stress-related social drivers throughout survivorship to guide targeted interventions for improving cardiovascular health.

#2321 Longitudinal evidence of healthy lifestyles slowing biological aging and reducing cancer risk.

J. Jia[1], C. P. Wen[2], H. Tu[1], J. Pan[1], W. Lu[1], M. Yang[1], A. Xu[1], S. Wang[1], W. Li[1], X. Wu[1];
[1]Zhejiang University, Hangzhou, China, [2]National Institute for Data Science in Health and Medicine, Hangzhou, China

Background: Aging is an important risk factor of cancer, and a healthy lifestyle is one possible measure to delay aging. Limited research has investigated the relationship between a healthy lifestyle, biological aging, and cancer incidence.
Methods: This prospective study enrolled 122,023 individuals from the Taiwan MJ cohort. The multivariable logistic regression model was utilized to examine the associations between the healthy lifestyle scores (HLS, integrating never or quitted smoking, non-harmful drinking, active physical activity, healthy body fat percentage, and healthy diet) and biological aging acceleration alteration, which was measured by changes in multi-dimensional aging measure acceleration (ΔMDAgeAccel). The Cox regression model was used to evaluated the joint association of HLS and ΔMDAgeAccel with cancer risk.
Results: Compared to individuals with HLS of 0, increasing HLS was associated with decreased odds of MDAgeAccel increase, with the adjusted odds ratio (aOR) of 0.684 (95% CI 0.557 to 0.841), 0.589 (0.483 to 0.718), 0.500 (0.411 to 0.610), 0.426 (0.349 to 0.519), and 0.379 (0.310 to 0.464) for HLS of 1, 2, 3, 4, and 5, respectively. These results remained consistent across stratifications by baseline aging acceleration status, age group, and sex. Individuals with accelerated aging at baseline and higher HLS exhibited lower odds ratio of persistent aging acceleration. The hazard ratio (HR) for cancer incidence was 0.774 (0.665 to 0.900) for individuals with a higher HLS and decreased MDAgeAccel, 0.932 (0.779 to 1.116) for individuals with a higher HLS and increased MDAgeAccel, compared with those with a lower HLS and increased MDAgeAccel.
Conclusions: Adherence to healthy lifestyle may lower aging acceleration, and reducing cancer risk.

PREVENTION / EARLY DETECTION / INTERCEPTION: Biology of Precancers, Development of Early Detection Methods and Biomarkers, and Cancer Interception
Poster Session

#2325 Anatomical genomic and transcriptomic analyses of Helicobacter pylori-driven gastric transformation.

Yosuke Tanaka, Fumishi Kishigami, Toshihide Ueno, Shinya Kojima, Taiki Hashimoto, Yukinori Yamagata, Takaki Yoshikawa, Shigeki Sekine, Hiroyuki Mano

National Cancer Center Japan, Tokyo, Japan

Helicobacter pylori (HP) drives a chronic inflammation in the gastric mucosa, leading to atrophic gastritis, intestinal metaplasia (IM), and further culminating in gastric cancer. Oncogenesis by HP infection has been mainly attributed to epigenetic dysregulation, and recent advances in the understanding of clonal expansion have shed light on the mutational architecture of IM. However, these analyses have been performed separately, leaving our understanding fragmented. Furthermore, analyses on small-sized tissue cannot determine the spatial events in the entire stomach. These limitations of previous studies highlight the necessity for integrative genomic and transcriptomic analyses using the entire stomach samples. Here, we conducted exome sequencing on 295 crypts isolated from the gastric mucosa and 13 tumors of 13 individuals. In addition, we performed an extensive investigation of the spatial distribution of cell clones by characterizing crypts at 5 mm intervals from 360 areas in the pyloric region to the body regions throughout the stomach in 2 patients, which underwent RNA and exome sequencing. Mutational analyses revealed that the expansion of cell clones with gene mutations was accelerated by HP infection and smoking habits. Mutations in cancer-related genes, including *MUC6*, were positively selected in the crypts, specifically in the pylorus region. However, such gene sets mutated in the crypts were different from those in gastric cancer. Macroscopic spatial genomic analyses revealed the distribution profiles of these clones, and spatial transcriptomic examination further clarified an IM-specific expression profile with oncogenic potential. These results suggest a coordinated role for mutation acquisition and transcriptomic activation of the AP-1 signaling pathway or stem-like expression in HP-infected mucosa to drive oncogenesis. Our study elucidated the previously unknown coordinated relationship between clone formation and transcriptomic remodeling of HP-infected gastric mucosa. Given the oncogenic transcriptional circuit of IM, early Helicobacter pylori eradication, smoking cessation, and close monitoring of IM may be beneficial to reduce the opportunity of additional hazardous mutation acquisition.

#2326 Nicotinamide adenine dinucleotide precursors delay pancreatic cancer initiation and progression by promoting DNA repair and improving mitochondrial function.

Faith Nakazzi[1], Mehrdad Zarei[2], Omid Hajihassani[1], Hallie J. Graor[2], Priyashree Sunita[2], Goutam Dey[2], Akram S. Shalaby[3], Jordan M. Winter[3]

[1]Department of Biochemistry, Case Western Reserve University, Cleveland, OH,[2]Department of Surgery, Case Western Reserve University, Cleveland, OH,[3]Division of Surgical Oncology,, University Hospitals Cleveland Medical Center, Cleveland, OH

Background: Pancreatic cancer (PC) is the third leading cause of cancer-related death in the United States, with a dismal 5-year survival rate of just 13%. Because PC has no screening biomarkers and is aggressive and resistant to most chemotherapy, focus should be put on discovering PC preventative alternatives. Cancer progression is driven primarily by increased cellular reactive oxygen species and mitochondrial dysfunction in cells, which promote somatic DNA mutations and, eventually, cancer initiation. Nicotinamide adenine dinucleotide (NAD+) precursors have shown promise in maintaining redox balance, cellular signaling, DNA repair, and mitochondrial function in aging-related non-malignant conditions. We investigated the impact of NAD+ supplementation on stressed pancreatic ductal epithelial cells and PC progression in an autochthonous pancreatic cancer mouse model.
Methods: We evaluated three NAD+ salvage pathway precursors Nicotinamide mononucleotide (NMN), Nicotinamide (NAM), and Nicotinamide riboside (NR) on non-transformed pancreatic ductal epithelial cell lines (HPDE, HPNE) under various stressors: phenformin (to simulate mitochondrial dysfunction), oxaliplatin (to induce DNA damage), and hydrogen peroxide (to induce oxidative stress). We assessed cell viability, mitochondrial function, reactive oxygen species (ROS) levels, DNA repair, and mutation rates. Additionally, we treated $p48^{Cre/+}$-$LSL\text{-}KRAS^{G12D/+}$ mice with either vehicle or NAM (400mg/kg for 6 months starting at 8 weeks), sacrificed the mice, and obtained pancreases to asses pancreatic intraepithelial neoplasm (PanIN) progression, DNA damage, and immunological markers.
Results: NAM-treated mice showed predominantly focal low-grade PanINs, while vehicle-treated mice developed primarily diffuse high-grade PanINs, indicating delayed cancer progression. There were increased H2AX foci, increased inflammation markers (Cox-2 and pSTAT3), and increased lipid peroxidation in vehicle-treated mice's pancreases compared to those treated with NAM. In vitro, NMN enhanced cell survival under stress due to improved oxygen consumption rate, increased ATP production, and reduced cellular reactive oxygen species levels; NMN treatment also promoted Nrf-2 nuclear translocation and increased expression of superoxide dismutase and glutathione. NMN supplementation also enhanced DNA repair through increased PARylation. These effects resulted in reduced mutation rates in NMN-treated cells.
Conclusions: NMN protects pancreatic epithelial cells from stress by enhancing DNA repair, improving mitochondrial function, and maintaining redox homeostasis. These mechanisms likely contribute to the delayed cancer progression observed in NAM-treated mice, suggesting that NAD+ precursors are promising candidates for pancreatic cancer prevention.

#2327 Boosting cancer vaccine efficacy with Vitamin E for immunoprevention of ER- breast cancer.

Yi Xiao, Yuan Zhang, Yunhai Li, dihua Yu

UT MD Anderson Cancer Center, Houston, TX

Estrogen receptor negative (ER-) breast cancers have worse prognoses with no effective prevention strategies. Developing safe and effective tactics for immunoprevention of poorly immunogenic breast cancers is highly challenging. Recently, we found that vitamin E (VitE) stimulates dendritic cell (DC) functions and potentiates anti-tumor immunity of immunotherapies. Here, we explored whether VitE can enhance anti-tumor immunity of cancer vaccines for immunoprevention of ER- breast cancer. Indeed, VitE, delivered as a vaccine adjuvant, significantly increases dendritic cell antigen presentation and boosts potent antigen-specific T cell responses. We noted that local injection of VitE with vaccines yielded substantially higher efficacy than oral VitE administration Moreover, compared to the vehicle-treated and vaccine only groups, VitE, given as vaccine adjuvants, significantly delayed tumor outgrowth and extended tumor-free survival in multiple mammary tumor transplantation models and in spontaneous mammary tumor model of MMTV-neu transgenic mice, whereas anti-PD1 administration showed a marginal effect in these tumor models. Mechanistically, in addition to targeting DC checkpoint SHP1, VitE fosters antigen uptake, increases co-stimulatory molecules CD80/CD86/CD40 expression and downregulates PD-L1 in DCs, that ultimately boosts antigen-specific effector/memory T cells. Furthermore, VitE administration altered landscapes of CD8+ effector T cells and fostered their transition to memory T cells. Together, our study demonstrates that VitE potentiates cancer vaccine efficacy via DCs and memory T cells, thus can be given as a safe and effective adjuvant of cancer vaccine for immunoprevention of ER- breast cancer.

#2328 Predictive effect of IGFBP-3 on low-dose tamoxifen efficacy in non-invasive breast cancer in the phase III Tam-01 trial.

Harriet Johansson[1], Debora Macis[1], Martino Oliva[2], Matteo Puntoni[3], Eva Blondeaux[4], Aliana Guerrieri-Gonzaga[1], Valentina Aristarco[1], Irene Maria Briata[2], Tania Buttiron-Webber[2], Alessio Carbone[2], Luca Boni[4], Matteo Lazzeroni[1], Davide Serrano[1], Livia Giordano[5], Maria Digennaro[6], Laura Cortesi[7], Francesco Millo[8], Katia Cagossi[9], Giuseppe Aprile[10], Fabio Falcini[11], Elisa Gallerani[12], Bernardo Bonanni[1], **Andrea De Censi**[2]

[1]European Institute of Oncology IRCCS, Milan, Italy,[2]Medical Oncology, Galliera Hospital, Genova, Italy,[3]Clinical & Epidemiological Research Unit, University Hospital of Parma, Parma, Italy,[4]IRCCS Ospedale Policlinico San Martino, Genova, Italy,[5]Azienda Ospedaliera-Universitaria Citta della Salute e della Scienza, Torino, Italy,[6]IRCCS- Istituto Tumori "Giovanni Paolo II", Bari, Italy,[7]Azienda Ospedaliera-Universitaria Policlinico di Modena, Modena, Italy,[8]Ospedali Riuniti ASL AL-Ospedale SS. Antonio e Margherita, Tortona, Italy,[9]Division of Medical Oncology, "B.Ramazzini" Hospital, Carpi, Italy,[10]Department of Oncology, San Bortolo General Hospital, ULSS8 Berica, Vicenza, Italy,[11]IRCCS Istituto Romagnolo per lo Studio dei Tumori "Dino Amadori"-IRST S.r.l., Meldola, Italy,[12]Aziende Socio Sanitarie Territoriale dei Sette Laghi, Varese, Italy

Tamoxifen 5 mg/d (Babytam) decreases the incidence of new breast cancer events by 42% in women with breast intraepithelial neoplasia with limited toxicity, providing a new treatment option in these disorders. However, predictive biomarkers of Babytam efficacy are lacking. We studied whether baseline IGF-I, insulin-like growth factor binding protein-3 (IGFBP-3), estradiol, sex hormone-binding globulin (SHBG), and their ratios predict Babytam efficacy on breast cancer events in a pre-planned secondary analysis.Within a 1:1 placebo-controlled, randomized trial of Babytam or placebo given for 3 years after surgery in women with ADH or LCIS or DCIS, 406/500 participants consented to blood sampling at baseline, at 1 and 3 years. Serum IGF-I, IGFBP-3, estradiol, SHBG levels and their ratios were measured by chemiluminescent immune assays. Biomarker changes were estimated using mixed-effects models, and Incidence Rate Ratios (IRR) were calculated after 10 years of follow-up with Poisson regression. Subgroup analyses were performed using an interaction test and subpopulation treatment effect pattern plot.Baseline levels of IGFBP-3 in the three top quartiles (≥3.44 ug/mL), but not in the lower quartile, predicted greater Babytam efficacy compared to placebo (p-interaction=0.006, Table). Baseline IGF-I, estradiol, or SHBG were not predictive of Babytam efficacy, whereas the IGF-I/IGFBP-3 ratio was borderline significant (p-interaction=0.067).High baseline levels of IGFBP-3 (≥3.44 ug/mL) predicted Babytam efficacy and may help differentiate which women benefit most from this treatment.

Table. Mean event rate (x1000 person-years) by treatment arm and baseline IGF-I, IGFBP-3, E2, SHBG, and IGF-I/IGFBP-3 and E2/SHBG ratio at 10 year follow-up.

10y follow-up	**BabyTam**	**Placebo**	**IRR (95% CI)**
IGF-I†, ng/mL			
≤median (127.3)	11.8 (6.3-21.9)	20.9 (13.2-33.2)	0.49 (0.22-1.11)
>median	11.0 (5.9-20.5)	21.5 (13.5-34.0)	0.52 (0.23-1.15)
IRR (95% CI)	0.90 (0.35-2.31)	1.09 (0.53-2.22)	$P_{interaction}$=0.967
IGFBP-3†, ug/mL			
Q1 (≤3.43)	10.6 (4.0-28.3)	8.8 (3.3-23.4)	1.24 (0.33-4.66)
Q2 (3.44 to 4.21)	21.4 (11.5-39.8)	25.5 (13.3-49.0)	0.75 (0.30-1.85)
Q3 (4.22 to 4.91)	11.6 (4.8-27.8)	20.6 (10.3-41.3)	0.59 (0.18-1.98)
Q4 (≥4.92)	2.2 (0.3-15.9)	30.7 (18.2-51.8)	0.07 (0.01-0.52)**
IRR (95% CI)	0.71 (0.50-1.01)	1.36 (1.02-1.82)*	$P_{interaction}$=0.006
IGF-I/IGFBP-3†			
≤median (0.167)	7.7 (3.7-16.2)	26.3 (17.2-40.4)	0.29 (0.13-0.69)**
>median	15.9 (9.2-27.4)	16.4 (9.7-27.7)	0.89 (0.41-1.94)
IRR (95% CI)	1.72 (0.67-4.40)	0.60 (0.30-1.20)	$P_{interaction}$=0.067
E2†, pg/mL			
≤median (15.8)	7.9 (3.7-16.5)	21.1 (13.3-33.5)	0.38 (0.16-0.91)*
>median	15.0 (8.7-25.8)	21.3 (13.4-33.8)	0.62 (0.29-1.34)
IRR (95% CI)	1.47 (0.54-3.96)	0.90 (0.39-2.11)	$P_{interaction}$=0.390
SHBG†, nMol/L			
≤median (60.2)	13.2 (7.3-23.8)	25.2 (16.4-36.8)	0.50 (0.24-1.05)
>median	9.7 (5.1-18.7)	17.3 (10.5-28.8)	0.54 (0.23-1.30)
IRR (95%CI)	0.67 (0.24-1.90)	0.77 (0.32-1.85)	$P_{interaction}$=0.801
E2/SHBG†			
≤median (1.37)	9.4 (4.7-18.9)	19.3 (12.0-31.1)	0.49 (0.21-1.16)
>median	13.2 (7.5-23.2)	23.2 (14.8-36.4)	0.52 (0.24-1.10)
IRR (95% CI)	0.91 (0.33-2.50)	1.09 (0.51-2.35)	$P_{interaction}$=0.977

IRR, incidence rate ratio; *p<0.05, **p<0.01. P-values were calculated using Poisson regression adjusted for age and body mass index at baseline; † All women; E2, estradiol.

#2329 Understanding epigenetic-driven transposable re-expression and its impact on immune activation in the serrated pathway of colorectal tumorigenesis.

Zhengyi Chen[1], Sojung Lee[2], Alan J. Simmons[1], Robert J. Coffey[3], Martha J. Shrubsole[3], Jason W. H. Wong[2], Ken S. Lau[1]

[1]Vanderbilt University School of Medicine, Nashville, TN,[2]University of Hong Kong, Hong Kong, China,[3]Vanderbilt University Medical Center, Nashville, TN

An active immune system plays a central role in preventing the progression from precancerous lesions to invasive cancers. Identifying the molecular markers that dictate effective immune surveillance is essential for advancing cancer prevention strategies. A higher level of immune cell infiltration is observed in serrated lesions, a subtype of colorectal premalignant lesions, in comparison to the other common subtype, conventional adenoma. However, the mechanism underlying the elevated immune response in serrated lesions remains poorly understood because hypermutation, which is considered as the source of immunogenicity conventionally, is absent in serrated lesions. One alternative source of immunogenicity can be transposable elements (TEs), which are mobile DNA sequences typically epigenetically silenced in mature tissues but expressed in fetal developmental stage and cancers. We hypothesize that aberrant expression of TEs drives immune activation in serrated lesions. Notably, only serrated lesions exhibit a fetal gene expression program, suggesting a potential link to epigenetically deregulated TE expression mediated by the PIWI-piRNA pathway. We quantify and compare TE expression and methylation levels across subtypes of colorectal precancers and normal tissues, integrating these data with spatial transcriptomics to elucidate the relationship between TE expression and immune cell distribution. Our results reveal a significantly higher number of differentially expressed TEs in serrated lesions compared to conventional adenomas and suggest a positive association between TE expression and the immunogenic landscape in serrated lesions. These findings inform strategies to reprogram colorectal precancers toward an anti-tumor immune environment, offering potential benefits for cancer prevention and early immuno-intervention strategies in colorectal cancer.

#2330 The Jekyll and Hyde of DARC: Balancing prognosis and metastasis in breast cancer.

J. Talukdar, T. Srivastava, R. Dhar, S. Karmakar;
All India Institute of Medical Sciences (AIIMS) New Delhi, Delhi, India

Background: DARC, a decoy receptor for chemokines, is implicated in breast cancer progression. Understanding its interplay with key molecular markers and its impact on tumor behaviour is crucial for developing targeted therapies.
Methodology: This study employed a multi-faceted approach, including immunohistochemistry, quantitative PCR, and bioinformatical analysis of publicly available datasets (TCGA and METABRIC). The DARC, CCL8, c-MYC, ALDH1, and CHEK2 expression patterns were analyzed in breast cancer tissues. Correlation analyses and survival analyses were performed to assess the prognostic significance of these markers.
Results: Our findings revealed a positive correlation between DARC and CCL8 expression, suggesting a potential role of DARC in promoting tumor cell migration and invasion through CCL8 signalling. Additionally, elevated DARC expression was associated with increased c-MYC, ALDH1, and CHEK2 levels, indicating its potential involvement in tumor cell proliferation, stemness, and chemoresistance. Bioinformatical analysis further corroborated these findings and identified DARC as a potential prognostic biomarker.
Conclusion: These results highlight the complex interplay between DARC and key molecular markers in breast cancer. Targeting DARC may offer a novel therapeutic strategy to inhibit tumor progression and improve patient outcomes. Further validation studies are warranted to elucidate the precise mechanisms underlying DARC-mediated tumorigenesis.

IHC expression levels of ACKR1, CHEK2, c-MYC, ALDH1, and CCL8 in breast cancer patients

Patient	**Non-TNBC**	**DARC**	**CCL-8**	**c-MYC**	**ALDH1**
1	Non-TNBC	Low	Low	Moderate	High
2	Non-TNBC	Moderate	Low	Low	High
3	Non-TNBC	High	Moderate	High	High
4	Non-TNBC	High	High	High	High
5	Non-TNBC(Post-Chemo)	High	Moderate	Low	Moderate
6	Non-TNBC (Recurrent)	Low	Low	Moderate	Moderate
7	Non-TNBC	High	High	Moderate	High
Patient	TNBC	**DARC**	**CCL-8**	**c-MYC**	**ALDH1**
1	TNBC	High	Moderate	Moderate	Moderate
2	TNBC	High	High	Moderate	High
3	TNBC	High	High	Moderate	Moderate
4	TNBC	High	High	Moderate	High
5	TNBC	High	High	Moderate	Moderate
6	TNBC	High	Moderate	Moderate	Moderate
7	TNBC	High	Moderate	Low	Low

#2331 WNT/β-catenin modulation by activation of the farnesoid X receptor for interception of familial adenomatous polyposis-associated colorectal cancer.

Abel Martel-Martel, Nan Deng, Fahriye Duzagac, Jacklyn V. Thompson, Mahira Zaheer, Araceli Garcia-Gonzalez, Krishna M. Sinha, Eduardo Vilar

Clinical Cancer Prevention, The University of Texas MD Anderson Cancer Center, Houston, TX

Introduction: Familial adenomatous polyposis (FAP) is a hereditary syndrome caused by germline alterations in *APC* and characterized by the presence of multiple colorectal adenomas that are associated to high risk of colorectal cancer (CRC) development. *APC* mutations dysregulate WNT signaling leading to aberrant β-catenin activity. The Farnesoid X Receptor (FXR) is a key regulator of bile acid homeostasis and has been identified as a suppressor of intestinal tumorigenesis. FXR downregulation has been shown to exacerbate β-catenin activity, further promoting malignant transformation of intestinal stem cells. Therefore, pharmacologic FXR activation represents a promising preventive strategy for the FAP population. In this study, we investigate the ability of the FXR agonist obeticholic acid (OCA) to restore FXR function and stabilize WNT/β-Catenin signaling for CRC interception in FAP.

Methods: The effect of OCA was evaluated on colonic organoids generated from a FAP mouse model (*Cdx2-Cre;Apc*$^{LoxP/+}$), 4 different human CRC cell lines, and patient-derived intestinal (duodenal and colonic) organoids from FAP patients. Organoids and cell lines were treated with OCA at different doses and time points followed by analyses of cell viability, apoptosis, and gene expression. Co-culture assays with patient-derived organoids and matched peripheral blood mononuclear cells (PBMCs) were performed to assess immune-related FXR regulation after OCA treatment.

Results: OCA treatment led to a time- and dose-dependent decrease in cell viability in both mouse- and patient-derived organoids as well as in human CRC cell lines. In mouse-derived organoids, OCA significantly restored the gene expression levels of *FXR* (*Nrh14*) and downstream targets (*Nr0b2* and *Fabp6*), and increased *Epcam* levels while reducing intestinal stem cells (*Lgr5* and *Wnt3a*), cancer stem cells (*Ascl2*, *Myc*, *Axin2*, and *Acox2*), proliferation (*Ki67*), and invasion (*Vimentin*) markers. Further, FXR restoration by OCA also promoted apoptosis in all CRC cell lines tested in a dose-dependent manner. To further assess the effects of OCA on anti-tumor immune responses, a co-culture experiment using colonic and duodenal organoids from FAP patients and their matched PBMCs, showed that the combination of 10μM OCA with PBMCs significantly reduced cell viability after 48 hours of co-culture. Low and high doses of OCA are currently being analyzed *in vivo*, alongside single-cell RNA sequencing from co-culture assay to identify epithelial cell clusters affected by OCA exposure.

Conclusions: Taken together, our findings reveal that OCA has promising potential to intercept CRC progression in FAP patients, thus providing further support for ongoing clinical trials (NCT05223036) with the FXR agonist OCA in FAP patients.

#2332 Ductal carcinoma in situ: Potential to metastasize? A nationwide cancer registry-based study.

Merle van Leeuwen, Sandra van den Belt- Dusebout, Petra Kristel, Lennart Mulder, Joyce Sanders, Carmen Vlahu, **Esther H. Lips**, Jelle Wesseling

Netherlands Cancer Institute, Amsterdam, Netherlands

Background: The current clinical paradigm around Ductal Carcinoma in Situ (DCIS) is that it consists of malignant cells confined to the breast ducts, and therefore cannot metastasize. Nonetheless, several studies have reported DCIS with metastasis in the sentinel lymph node (SN+). For accurate risk communication and management, we aimed to assess to what extent registered “metastatic spread” in DCIS could be explained by limitations in registration or missed invasive breast cancer at time of diagnosis.
Methods: Data from the nationwide cancer registry and national pathology database on women diagnosed with DCIS SN+ in the Netherlands, spanning from 2005 to 2020, was curated and reviewed, taking into account their histories of prior DCIS, invasive breast cancer, or other malignancies. Cases were excluded from further analysis if pathology data indicated registration errors, DCIS mixed with other lesion types, or diagnostic uncertainties. Next, hematoxylin and eosin-stained tissue slides of eligible DCIS SN+ cases were independently reviewed by two pathologists to assess the presence of invasive breast cancer and SN status. Additional immunohistochemical staining (CK 5/6 or CK 8/18) was performed when findings were unclear. Inter-observer agreement was evaluated using the linearly weighted Kappa statistic.
Results: A total of 30,863 patients were identified with a DCIS diagnosis between 2005 and 2020, of which 16,070 (52%) underwent SN biopsy according to cancer registry data. SN+ was registered in 454 (3 %) patients: 47 (10%) had macrometastases (>2 mm), 78 (17%) had micrometastases (>0.2 to <= 2 mm), and 329 (73%) were positive for isolated tumor cells (<= 0.2 mm). Out of the 454 registered DCIS SN+ cases, 273 (60%) were excluded from further investigation based on pathology data, including registration errors (n=44), DCIS mixed with other lesions (n=147), and diagnostic uncertainties (n=82). Tissue material of 46 (37%) out of 125 registered cases with macro- and micrometastases was reviewed. Observer variability in assessing the presence of invasive breast cancer was high (k= 0.14; 95% CI 0.08 - 0.34; $p = 0.12$) and additional CK 5/6 staining was requested for 38 cases. Two cases were classified as primary invasive breast cancer, 25 as pure DCIS, while 19 remained inconclusive, due to variation in tissue sections or suboptimal tissue quality. In six cases the SN was scored as negative by pathology revision, likely due to tissue section variability.
Conclusions: Our study indicates that DCIS in itself has minimal to no metastatic potential. Ongoing clonality analysis of 9 cases with macrometastases aims to determine whether the SN metastases are clonally related to the DCIS lesions.

#2333 TRAIL-inducing imipridones ONC201, ONC206, and ONC212 demonstrate anti-neoplastic effects in colonic adenoma-derived organoids.

Alexis J. Lannigan[1], Jasper Chan[1], Lanlan Zhou[1], Varun V. Prabhu[2], Michael Dame[3], Dean Brenner[3], Jason Spence[3], Wafik S. El-Deiry[1], Alexander G. Raufi[1]

[1]Legorreta Cancer Center at Brown University, Providence, RI,[2]Chimerix Inc., Durham, NC,[3]University of Michigan Medical School, Ann Arbor, MI

Background: Colorectal cancer (CRC) is the second leading cause of cancer-related death in the United States. Currently, screening is the most effective method to reduce mortality from CRC. Chemoprevention, which aims to delay or even prevent the onset of CRC through eradication or prevention of precursor colonic adenomas, is an alternative strategy to address this problem. The imipridone Dordaviprone/ONC201 is a potential chemopreventive agent that has been shown to induce apoptosis in cancer cells through both upregulation of tumor necrosis factor-related apoptosis-inducing ligand (*TRAIL*) and the integrated stress response. More recently, we have shown that ONC201 induces *TRAIL* and apoptosis in a dose-dependent manner in adenoma-derived organoids and reduces adenoma formation in the $Apc^{min/+}$ CRC mouse model. We hypothesized that ONC201's analogues, ONC206 and ONC212, would similarly enhance human colonic adenoma cell death through both *TRAIL*-dependent and *TRAIL*-independent mechanisms. We therefore evaluated the effects of ONC206 and ONC212 in two adenoma-derived organoid lines: one developed from an adenoma from an individual with familial adenomatous polyposis (FAP) and the second from a sessile serrated adenoma (SSA).

Methods: Sensitivity of the FAP and SSA organoid lines to ONC206 and ONC212 was assessed using CellTiter-Glo® luminescent cell viability assay. Organoids were plated in triplicates and treated with ONC206 and ONC212 (concentration ranging from 0 uM to 10 uM). Results were analyzed after 72 hours of incubation. Western blotting was performed on lysates generated from both FAP and SSA organoids treated with either DMSO, ONC206, or ONC212 for 24, 48, or 72 hours. Co-culture experiments were performed with the adenoma-derived organoids (dyed using blue CMAC dye) and human NK-92MI cells (dyed with green CMFDA dye) treated with either DMSO, ONC201, ONC206, or ONC212. Fluorescent images following administration of red live/dead dye were performed using DAPI, FITC, and cherry red channels on ImageXpress® at 0, 24, 48, and 72 hours.

Results: ONC206 and ONC212 demonstrated antineoplastic effects on adenoma-derived FAP and SSA organoids, with half maximal inhibitory concentrations (IC50) less than that of ONC201 in FAP and SSA organoids. As was noted in ONC201 studies, the mechanism of action of ONC206 and ONC212 appears to be mediated through modulation of multiple pathways including the *TRAIL* pathway (*TRAIL* and *DR5* upregulation) and the integrated stress responses (*ATF4* upregulation). Co-culture with NK cells revealed a significant increase in NK-mediated organoid cell death. The ability of ONC206 and ONC212 to enhance NK-mediated killing is being evaluated. Further analysis of the effects of ONC206 and ONC212 on stemness markers are currently underway and may reveal other potential biomarkers and chemopreventive strategies for ONC206 and ONC212.

#2334 Immunologic and proteomic biomarkers of progression risk in Barrett's esophagus.

H. Park[1], C. Lewis[1], A. Omstead[1], E. Grayhack[1], C. Sherry[1], K. Xiao[1], G. Kochhar[1], M. Landau[1], D. Stairs[2], A. H. Zaidi[1], P. L. Wagner[1];

[1]Allegheny Health Network, Pittsburgh, PA, [2]The Pennsylvania State University, Hershey, PA

Barrett's esophagus (BE) is an established risk factor for the development of high-grade dysplasia (HGD) or esophageal adenocarcinoma (EC), but clinical predictors of progression remain poorly defined. Biomarkers of progression risk are therefore in high demand, and in this study we examined the utility of immune cell density and proteomic profiling to identify progression risk indicators in biopsy samples from patients in surveillance for BE. Formalin-fixed paraffin embedded tissue samples (n=58) from patients with non-dysplastic BE undergoing surveillance biopsy were examined. During follow-up, 25 patients were diagnosed with HGD or EC ("progressors"), while 33 patients did not during at least 5 years of subsequent surveillance ("non-progressors"). To analyze immune markers for cell densities, samples were stained for CD3 and CD8 positive lymphocytes, CD68/CD86 co-positive ("M1- like") macrophages, and CD68/CD206 co-positive ("M2-like") macrophages. Regions of interest (ROI) were selected from the lamina propria of the gastroesophageal junction. Subgroup comparisons of cell density were carried out using the Wilcoxon rank sum test and logistic regression for the progression outcome. Proteomic profiling was assessed using label-free, liquid chromatography-tandem mass spectroscopy (LC-MS/MS), using data-independent acquisition. The raw LC-MS/MS data were searched using Spectronaut against a spectral library generated from 6 progressor and 6 non-progressor samples. Differential protein expression analysis was conducted using Perseus to compare the progressor and non-progressor groups. A total of 728 ROIs were examined for lymphocytes. lymphocyte cell densities were lower in progressors vs. non-progressors (CD3+: $p=0.03$; CD8+: $p=0.003$), as was the CD8:CD3 cell density ratio ($\bar{x}$: 0.66 vs 0.84, $p<0.0001$). On logistic regression, CD8+ cell density ($p=0.003$) and CD8:CD3 ratio ($p=0.007$) were significantly associated with progression. A total of 587 and 489 ROIs were examined for M1 and M2 cell densities, respectively. M1 like macrophages were significantly increased in progressors vs non-progressors ($\bar{x}$: 93.4 vs 35.4, $p=0.0004$). A total of 7,209 proteins were identified across the samples, among which 163 were differentially-expressed based on progression risk (false positive rate<0.05). 4 major GO processes and KEGG pathways were associated with progressors: rRNA processing, Wnt signaling, bacterial invasion of epithelial cells, and G protein-coupled receptor signaling. In this study, we present immunologic and proteomic biomarkers of progression risk in BE. More comprehensive assays for immune microenvironment and mechanistic studies are planned in a prospective BE surveillance cohort obtained from an ongoing registry-repository study. The enhanced understanding of malignant progression in BE could provide opportunities to improve surveillance or prevent progression in the future.

#2335 Predicting cirrhosis risk in patients with metabolic dysfunction-associated steatotic liver disease.

P. Sherpa[1], S. Armasu[2], J. Frank[2], I. Yan[1], K. Wangensteen[3], T. Patel[1], S. Antwi[1];
[1]Mayo Clinic Florida, Jacksonville, FL, [2]Mayo Clinic Rochester, Rochester, MN, [3]Mayo Clinic, Rochester, MN

Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) affects about 32% of adults worldwide and is projected to increase in many parts of the world. Liver cancer is a recognized complication of end-stage MASLD. An estimated 20% of patients with MASLD will progress to cirrhosis, a premalignant state in liver cancer evolution. Because MASLD is usually asymptomatic, its progression to cirrhosis can be difficult to detect early for timely intervention. There are currently no known biological markers for predicting cirrhosis risk in patients with MASLD, making it challenging to identify patients at risk of disease progression. The aim of this study was to identify plasma protein biomarkers that can predict future cirrhosis development in patients with MASLD.
Methods: We performed a nested case-control study among patients with MASLD at baseline who later progressed to cirrhosis and control patients with MASLD at baseline who never progressed to cirrhosis, matched on age at MASLD diagnosis (± 1-year), sex, race, year of blood sample collection, and length of follow-up (± 1-year). An aptamer-based array (SOMAscan™) platform was used to measure over 7000 proteins in baseline plasma samples obtained from the participants. The study sample was split 70/30 for training and validation analyses. We identified plasma protein biomarkers significantly differentially expressed in the training sample, adjusting for multiple testing with Bonferroni-corrected p-values. The significant set of proteins from the training set was used for prediction modeling in the validation sample, computing prediction metrics (area under the curve [AUC], sensitivity, specificity, and accuracy).
Results: A total of 64 MASLD to cirrhosis cases matched to 64 MASLD controls were included in the analysis. Six protein biomarkers (*PTPRU, ATL2, MFAP4, C7, CSTN2*, and *COLEC11*) were overexpressed in cases, compared to controls, in the training data. Prediction modeling of the six-protein biomarker panel in the validation sample yielded an AUC = 0.85, sensitivity = 0.72, specificity = 0.78, and accuracy = 0.75.
Conclusions: We have identified a panel of six plasma protein biomarkers that predict risk of cirrhosis development in patients with MASLD. These proteins could serve as candidates for future mechanistic studies on MASLD progression to advanced liver disease. If validated in larger studies and with assays suitable for clinical laboratory testing (e.g., ELISA), these protein biomarkers could be useful clinically for cirrhosis risk prediction in MASLD patients, and hence those at risk of liver cancer, enabling timely surveillance or interventions to prevent disease progression.

#2336 Discovering plasma proteins associated with common solid cancer incidence in ARIC.

Ziqiao Wang[1], Vernon A. Burk[2], Nilanjan Chatterjee[1], Elizabeth Platz[2]

[1]Department of Biostatistics, Johns Hopkins University, Baltimore, MD,[2]Department of Epidemiology, Johns Hopkins University, Baltimore, MD

Background: Plasma proteins, reflecting both exogenous and endogenous factors, could serve as the basis for risk stratification to guide decision-making for primary and secondary prevention of cancer in adults. Published studies have investigated the prospective risk of cancers and other chronic diseases associated with Olink-based proteomic data available in the UK Biobank Study. There is a need for further investigations based on alternative measurement platforms, longer follow-up data, and diverse populations.
Aim: Identify proteins measured by SomaScan assay® 5K that are associated with risk of solid cancers in the Atherosclerosis Risk in Communities (ARIC) study, a prospective cohort of middle-aged and older Black and White men and women.
Methods: The study was conducted based on a cohort of 9,495 individuals for which a total of 590, 416, 22, 97, 271, 88, 136 incident cancers of prostate, lung, liver, kidney, colorectal, pancreatic, and bladder were observed over a maximum follow-up period of 25.9 years. Log_2-transformed relative fluorescence unit of 4,955 SOMAmers measured in plasma collected at Visit 2 were adjusted in a linear regression model including proteomic PEER factors, study sites, and ten genetic principal components (PCs). Corrected protein quantifications based on rank-inverse normalization of the residuals were used in the analysis. Incident cancers were ascertained primarily by cancer registry linkage. The Cox regression model and time-dependent coefficient Cox model for age at Visit 2 were applied to estimate associations between individual proteins and each solid cancer risk, adjusting for potential environmental risk factors, race, genetic PCs, and PEER factors. Sensitivity analyses examined associations with extended lag-time (diagnosis more than 5 years after Visit 2) and minimally adjusted Cox model for cancer risk.
Results: We identified 32 proteins (19 positively, 13 inversely) for liver cancer risk, 5 proteins (4 positively, 1 inversely) for lung cancer risk in both main and sensitivity analyses at false discovery rate (FDR) <0.05. 2 known proteins (MMP7 and HAVCR1) were identified to be positively associated with kidney cancer, and 2 (ACP3 and KLK3) were identified for prostate cancer. We further identified GSTM1 associated with bladder cancer risk [HR (95% CI): 0.68 (0.56, 0.82)] and found evidence of enrichment of signals for proteomics association with colorectal cancer at nominal threshold $P < 0.05$ (371 proteins for colorectal cancer, 401 proteins for colon cancer only, and 425 proteins for rectal cancer).
Conclusion: We discovered multiple circulating proteins associated with lung and liver cancer risks, and confirmed previously established circulating proteins for kidney and prostate cancer risks. Additional evaluations in independent and large cohort studies and laboratory testing will be necessary to validate our results. Support: NHGRI, NHLBI, NCI, NPCR.

#2337 Prediagnostic circulating proteins and risk of bladder cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC).

Alison Dillman[1], Zhe Huang[1], Joshua Atkins[1], Marc Gunter[2], Pietro Ferrari[3], Antonio Agudo[4], Carlotta Sacerdote[5], David C. Muller[2], Domenico Pali[6], Giovanna Masala[6], Monique Verschuren[7], Nicholas Wareham[8], Raul Zamora-Ros[9], Roel C. H. Zamora-Ros[10], Rosario Tumino[11], Salvatore Panico[12], Vivian Vallon[3], Elio Riboli[2], Karl Smith-Byrne[1], Ruth Travis[1]

[1]University of Oxford, Oxford, UK, United Kingdom,[2]Imperial College London, London, United Kingdom,[3]International Agency for Research on Cancer, Lyon, France,[4]Institut Catala d'Oncologia, Barcelona, Spain,[5]University of Turin, Turin, Italy,[6]Institute for Cancer Research, Prevention and Clinical Network, Florence, Italy,[7]UMC Utrecht, Utrecht, Netherlands,[8]University of Cambridge, Cambridge, United Kingdom,[9]Unit of Nutrition and Cancer, Cancer Epidemiology Research Program, Catalan Institute of Oncology (ICO), Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain,[10]Division of Environmental Epidemiology and Veterinary Public Health, Institute for Risk Assessment S, UMC Utrecht, Utrecht, Netherlands,[11]Iblea Association, Epidemiological Research (AIRE-ONLUS), Ragusa, Italy,[12]University of Naples Federico II, Naples, Italy

Background*:* Bladder cancer is the tenth most common cancer globally. Circulating proteins are key regulators of cellular functions, and proteomic analyses allow for a comprehensive investigation of proteins that may have causal roles in cancer development.

Methods: We investigated the association between plasma proteins and bladder cancer risk in a case-cohort study within EPIC using a SomaLogic assay, comprising 4,047 sub-cohort members (15.3 years mean follow-up) and 254 bladder cancer cases (9.8 years mean follow-up), including 160 urothelial cell carcinoma (UCC) cases and 118 aggressive tumors. Prentice-weighted Cox regression models, with adjustment for smoking and other covariates, were conducted to estimate hazard ratios (HRs) and 95% confidence intervals (CIs), with false discovery rate correction. Covariates included smoking status (never; former; current: ≤15 cigarettes/day, occasional or cigar smoker; current: >15 cigarettes/day), alcohol consumption (mL/day), BMI (kg/m2), diabetes (yes, no), physical activity (inactive; moderately inactive; moderately active; active), education (primary/none, secondary, university degree).

Results*:* Of 7,363 aptamers, we identified 31 significant associations with bladder cancer risk after false discovery rate correction. All HRs changed less than 15% when comparing the multivariable-adjusted to the minimally-adjusted model, and 11 proteins were also conventionally significant among never-smokers. Subgroup analyses identified nine proteins associated with UCC and 31 proteins associated with aggressive tumors. Twenty-five proteins were significant ≥10 years from blood collection. Notably, positive associations for I3L1E1 were observed for bladder cancer overall (HR: 1.23, 95% CI: 1.11-1.36), in never-smokers, and for cases diagnosed ≥10 years from blood collection, for WFIKKN1 in relation to UCC (HR: 1.33, 95% CI: 1.18-1.51), and for IRF4 in relation to aggressive tumors (HR: 1.29, 95% CI: 1.16-1.45).

Conclusions: Evidence for several protein-cancer associations may suggest these could be used as novel biomarkers and provides insight into bladder cancer etiology.

#2338 Plasma proteomics associated with ovarian cancer risk in the Nurses' Health Studies.

Nan Lin[1], Allison F. Vitonis[1], Simon T. Dillion[2], Ngo Long[2], Shelley S. Tworoger[3], Towia A. Libermann[2], Kathryn L. Terry[1], Naoko Sasamoto[1]

[1]Department of Obstetrics and Gynecology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA,[2]Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA,[3]Knight Cancer Institute, Oregon Health & Science University, Portland, OR

Background: While inflammation and gonadotropin levels are hypothesized to contribute to ovarian cancer initiation, specific proteomic pathways and other potential mechanisms are unclear. Thus, we aimed to identify novel blood-based non-invasive proteomic risk biomarkers for invasive epithelial ovarian cancer.

Method: We measured 7,310 pre-diagnostic plasma proteins using SomaScan v4.1 in 169 incident ovarian cancer cases [140 high-grade serous (HGS) cases] and 169 matched controls nested within the Nurses' Health Studies. We excluded proteins with coefficient of variation ≥ 25% and influenced by delayed processing, resulting in 3,131 proteins included in this analysis. We transformed protein levels to probit scores to achieve normality and used conditional logistic regression modeling to calculate the odds ratios (ORs) and 95% confidence intervals (CIs) per one standard deviation increase in protein levels. We used Gene Set Enrichment Analysis to identify biological pathways and STRING to conduct protein-protein interaction network and functional enrichment analysis. We also conducted analyses restricted to HGS histotype.

Results: All ovarian cancer cases included in the current analysis had blood samples collected years prior to diagnosis (median=14 years). Average age at blood collection was 56.3 years in both ovarian cancer cases and controls. When examining individual proteins associated with ovarian cancer risk, 94 proteins were significantly associated with ovarian cancer risk with nominal p-value<0.05. Biological pathway analysis revealed complement cascade (NES=2.0; FDR p=0.049) and innate immune system (NES=1.5; FDR p=0.049) pathways being upregulated in ovarian cancer cases compared to controls. STRING analysis showed protein-protein interactions were enriched in pathways such as tissue remodeling (e.g., COL6A1, COL18A1, IGFBP2), lipid metabolism (e.g., NCOA2, ME1), and B cell function (e.g., IGLL5, MZB1) in ovarian cancer cases compared to controls. Similar results were observed for HGS ovarian cancer.

Discussion: We conducted a comprehensive examination of the plasma proteins associated with ovarian cancer risk in a prospective cohort study and observed upregulation of pathways related to innate immune system and complement cascade being associated with ovarian cancer risk.

#2339 Development of a blood cell analysis-based AI model for detecting early ovarian cancer signals.

Eunyong Ahn[1], Sungmin Park[1], Sarah Kim[1], Se Ik Kim[2], Hyejin lee[1], Heeyeon Kim[3], Seong Eun Kang[4], Ji Won Park[4], TaeJin Ahn[1], Yong-Sang Song[5]

[1]ForetellMyHealth, Inc., Pohang-si, Gyeongsangbuk-do, Korea, Republic of,[2]Department of Obstetrics and Gynecology, Seoul National University Hospital, Seoul, Korea, Republic of,[3]Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea, Republic of,[4]Division of Colorectal Surgery Department of Surgery, Seoul National University Hospital, Seoul, Korea, Republic of,[5]Department of Obstetrics and Gynecology, Myongji Hospital, Gyeonggi-do, Korea, Republic of

Ovarian cancer (OC) remains one of the most lethal gynecologic malignancies, primarily due to delayed detection. Blood cell analysis presents a promising non-invasive diagnostic approach, capable of detecting systemic signals associated with early OC. This study focuses on developing and validating an artificial intelligence (AI)-based diagnostic model to detect OC risk signals using comprehensive blood cell data. To minimize confounding factors, samples from male participants, symptomatic controls, drug-treated individuals, and pre-anesthesia collections were excluded. Additionally, samples with missing data were removed. A total of 185 samples, including 85 for training and 100 for testing, were analyzed, comprising OC, benign tumor, and control groups. The model utilized 24 features: 18 directly measured blood cell parameters (via Sysmex) and 6 derived composite indices, including the systemic immune-inflammation index (SII: [NEUT×PLT]/LYM). A Random Forest algorithm, optimized through 5-fold GridSearch cross-validation and soft voting, was trained and validated with balanced accuracy (BA) as the performance metric. The optimal cutoff values were determined based on the training set's best BA. In the training set, the model achieved a sensitivity of 100.0%, specificity of 92.6%, a positive predictive value (PPV) of 77.3%, and a negative predictive value (NPV) of 100.0%, with an area under the curve (AUC) of 0.987. For the test dataset, the model demonstrated a sensitivity of 75.0%, specificity of 92.9%, PPV of 66.7%, and NPV of 95.1%, with an AUC of 0.923. The platelet-to-lymphocyte ratio (PLT/LYM) and SII emerged as the most influential features, contributing significantly to model performance. The specificity for control samples, excluding benign tumors, was 1 in the training set, and validation confirmed a specificity of 0.984, demonstrating clear separation between the OC and control groups. Composite indices further improved the model's reliability in detecting OC risk signals. This AI-driven blood cell analysis model exhibited strong performance in identifying early ovarian cancer signals, achieving high sensitivity and specificity. By leveraging non-invasive biomarkers, it holds significant promise for clinical implementation in OC screening. Future efforts will focus on expanding the diversity of analyzed samples to better distinguish cancer-specific signals from those associated with other diseases and incorporating molecular markers to enhance the accuracy and precision of the diagnostic algorithm.

#2340 Proteomic profiling of breast tissue: identifying protein associations with breast density in premenopausal women.

Ghazaleh Pourali[1], Chao Li[2], Minsoo Son[3], Young Ah Goo[3], Graham A. Colditz[1], Xingyi Guo[2], Adetunji T. Toriola[1]

[1]Division of Public Health Sciences, Department of Surgery, Washington University School of Medicine, Saint Louis, MO,[2]Division of Epidemiology, Department of Medicine, Vanderbilt Epidemiology Center, Vanderbilt University Medical Center, Nashville, TN,[3]Mass Spectrometry Technology Access Center at the McDonnell Genome Institute, Washington University School of Medicine, Saint Louis, MO

Introduction: Mammographic breast density (MBD) is a well-established risk factor for breast cancer. However, the underlying molecular pathways that lead to breast cancer development in women with dense breasts remain less understood. Proteomics is a robust method of understanding these mechanisms by directly analyzing the functional state of cells. However, data on tissue proteomics of premenopausal breast cancer is limited. To address this, we performed proteomic profiling of breast tissues from premenopausal women with breast cancer and examined the associations between protein expression and MBD.
Methods: We performed proteomic profiling of adjacent normal breast tissues in 50 premenopausal women with breast cancer archived at St. Louis Breast Tumor Registry. Samples were analyzed with Orbitrap Eclipse Tribrid coupled with Vanquish Neo LC system (Thermo Fisher Scientific). Data were processed using MaxQuant (v2.5.0.0) with default settings. The Andromeda search engine scored MS2 spectra against tryptic peptides from the UniProt human reference proteome (20,419 entries, release 2023/11). Label-free quantitation was used for relative protein quantification, with precursor mass tolerance set at 4.5 ppm and fragment ion mass tolerance at 20 ppm. A target-decoy approach was used to ensure a false discovery rate of <1%. We performed imputation and quantile normalization in the Perseus framework. We investigated associations of protein expression with MBD (dense vs. non-dense) using logistic regression models, adjusted for confounders. We corrected for multiple testing by the Benjamini-Hochberg procedure. Statistical analyses were conducted in R (v4.3.2).
Results: Out of 3376 proteins, we identified 63 proteins positively associated with MBD, and 84 proteins inversely associated with MBD (adjusted-p <0.05). Top three proteins positively associated with MBD were MBD3 (β=3.49, SE=1.17; p=2.96×10^{-3}), involved in chromatin remodeling, KAT7 (β=2.95, SE=1.07; p=5.75×10^{-3}), a histone acetyltransferase that regulates gene expression, and ERBIN (β=3.54, SE=1.32; p=7.20×10^{-3}), which interacts with ERBB2/HER2. Conversely, top three proteins inversely associated with MBD were GPX4 (β=-1.67, SE=0.58; p=4.12×10^{-3}), an antioxidant enzyme, NDRG1 (β=-1.20, SE=0.42; p=4.31×10^{-3}), involved in stress responses, and CDKN2A (β=-3.02, SE=1.10; p=6.12×10^{-3}), a tumor suppressor that regulates the cell cycle by inhibiting cyclin-dependent kinases.
Conclusions: Our findings reveal several proteins associated with MBD in premenopausal women, some of which are targets of approved drugs, highlighting the potential for targeting them in premenopausal breast cancer prevention. Larger studies are needed to validate these findings. Additionally, these proteins and their downstream pathways can be investigated in preclinical studies to confirm their translational potential.

#2341 Impact of delayed processing on cell-free (cf) DNA quantity and quality using different cfDNA-stabilizing blood tubes: a Connect for Cancer Prevention Pilot Study Cohort.

Jazmyn Bess[1], Domonique Hopkins[1], Norma Diaz Mayoral[2], Heather Cooley[3], Angela de Palatis[2], David Wong[4], David Elashoff[4], Fang Wei[4], Feng Li[4], Mohammad Aziz[4], Chris Karlovich[2], Eric Greenbank[2], Stephanie Weinstein[1], Christian Abnet[1], Marie-Josephe Horner[1], Amanda Black[1], Michelle Brotzman[1], Kathleen Wyatt[1], Lynn Sorbara[1], Matthew R. Young[1], Mia Gaudet[1], Nicolas Wentzensen[1]

[1]National Cancer Institute, Rockville, MD,[2]Frederick National Laboratory for Cancer Research, Frederick, MD,[3]Frederick National Laboratory for Cancer Research, Rockville, MD,[4]University of California, Los Angeles, CA

Background: Prospective evaluations of cfDNA/liquid biopsy assays are critical to assess their potential for improved early detection of cancer. Important knowledge gaps related to blood collection, preservation, and processing practices must be addressed for adequate assay execution.[2] The NCI Connect for Cancer Prevention Cohort Study (Connect) aims to study new approaches in early detection. Connect conducted pilot studies evaluating the STRECK Cell-Free DNA BCT (DNA), Streck Nucleic Acid (NA), and PAXgene tubes to determine the impact of processing time on cfDNA quantity and quality utilizing quality control assays.
Methods: Plasma samples were collected from 40 healthy doners using Streck DNA, Streck NA, and PAXgene tubes through the NCI Frederick Research Donor Program. Half of the samples from each tube set were processed at room temperature immediately and the other half after five days. Three assays were performed to compare cfDNA quantity and quality: (1) Qubit/Spectramax assay measuring total DNA concentration (ng/uL); (2)TapeStation measuring %cfDNA and average fragment size (bp); and (1) an EFIRM Alu assay measuring Alu element concentration (copies/mL). Descriptive analyses were performed to compare metric concentrations between processing time points, with and without samples identified as outliers.
Results: After five-day processing, hemolysis for the PAXgene tubes (50.0%) and the Streck DNA tubes (40.0%), but not the Streck NA tubes (0.0%). No hemolysis was observed before immediate processing. Qubit/Spectramax and TapeStation assays showed that all three tube types had samples with normal fragment size between 50-700bp, over 90% of with normal total DNA concentration of less than 1.00ng/uL, and a majority with more than 60% of cfDNA. Streck DNA had the most outliers with less than 60% cfDNA (n=10) compared to PAXgene (n=9) and Streck NA (n=1). Streck DNA also had more outliers with 1.00 or greater total DNA concentration (n=4) compared to PAXgene (n=2) and Streck NA (n=0). EFIRM Alu results will be available during conference presentation.
Conclusions: Streck DNA, Streck NA, and PAXgene showed variable cfDNA quality and quantity performance. The Streck NA tube had the lowest number of samples with hemolysis and the lowest number of outliers for %cfDNA and total DNA concentration. With respect to preanalytical variables of prolonged processing time and tube type, the Streck Nucleic acid seems most suited at maintaining cfDNA quantity and quality.

#2342 Profiling microRNAs in fecal immunochemical test leftover buffer to improve colorectal cancer screening.

Barbara Pardini[1], Giulio Ferrero[2], Sara Bulfamante[3], Sonia Tarallo[1], Giulia Francescato[1], Davide Ricupero[1], Gianluigi Ferrante[3], Carlo Senore[3], Alessio Naccarati[1]

[1]Italian Institute for Genomic Medicine (IIGM), Candiolo, Italy,[2]Department of Clinical and Biological Sciences, University of Torino, Turin, Italy,[3]Epidemiology and Screening Unit-CPO, University Hospital Citta della Salute e della Scienza, Turin, Italy

Colorectal cancer (CRC) is one of the leading causes of cancer death worldwide, but large screening programs can effectively reduce both incidence and mortality. Fecal immunochemical test (FIT) is widely used in CRC screening programs and cost-effective. However, FIT test shows a low sensitivity for advanced adenomas (AA) and is also prone to false positive results, resulting in unnecessary total colonoscopies (TC). Leftover from FIT samples after hemoglobin measurement may offer unique opportunity to study alternative biomarkers, which may allow adopting a tailored screening approach. Altered miRNA profiles can be detected non-invasively in fecal samples. Our group studying the whole miRNome in stool recently contributed to the identification of a miRNA signature that accurately discriminates sporadic CRC at diagnosis (Pardini et al, Gastroenterology 2023) and demonstrated the feasibility of miRNA profiling in FIT leftovers (Birkeland et al. Mol Cancer 2023). However, a potential implementation of miRNA markers in FIT-based screening has not been fully explored. The aim of the study was to investigate the association of miRNA expression profiles with CRC or adenomas as well as the predictivity of miRNA signatures for outcomes at the subsequent screening round among screens examined within the population-based screening program in Turin, Italy. Small RNA sequencing was performed on leftover FIT samples of 438 subjects: 183 FIT positive (FIT+) detected with colorectal neoplasm (45 early adenomas, 110 AA, and 28 CRC), 93 with FIT+ and negative TC and 162 FIT negative (FIT-) controls. We compared the miRNA expression profiles of FIT+ screens with a negative TC and of those detected with neoplasia. For a subset of 213 subjects with a negative FIT at the previous round, we compared the miRNA profile at the first sample among screens with persistent negative results with those with a positive FIT and neoplasms. An average of 691 miRNAs were detected and 198 among them identified in more than 90% of the samples. Differential expression analyses between FIT+ and FIT- samples showed 379 miRNAs with significantly different levels (adj. $p<0.05$), mainly in AA or CRC. Notably, the previously identified 5-miRNA signature in stool was confirmed able to distinguish in FIT+ subjects with AA or CRC with respect to FIT-. miR-1246 was able to distinguish also subjects with neoplasm from those with negative TC. An increase of miR-1246 levels was observed in leftover samples of subjects at first round of screening who were detected AA or CRC following a positive FIT result at second round with respect to those with FIT- test. The previously identified miRNA signature shows a good performance in discriminating subject with neoplasm within FIT+ subjects. Further analyses to investigate the performance of various miRNA sets according to different FIT levels or screening rounds are ongoing.

#2343 CancerSpot: a multi-cancer early detection test using targeted methylation sequencing.

Urvashi Bahadur[1], Divya Vishwanath[1], Prakash Hiremath[1], Nihesh Rathod[1], Swaraj Basu[1], Sampuran Chakraborty[1], Aastha Tripathi[1], Preetha R T[1], Aditi Chatterjee[1], Arunima Ghosh[1], Sameer Phalke[1], Subuhi Yete[1], Ankita Khan[1], Sweta Sanguri[1], Mohammed Rahman[1], Sewanti Limaye[2], Charles Cantor[3], Vamsi Veeramachaneni[1], **Ramesh Hariharan**[1]

[1]Strand Life Sciences, Bengaluru, India,[2]Sir H N Reliance Foundation Hospital and Research Centre, Mumbai, India,[3]Strand Life Sciences Scientific Advisory Board, New York, NY

Background: Liquid biopsy-based cancer detection is a noninvasive method to identify cell-free DNA (cfDNA) fragment based signatures in the bloodstream characterized by distinct methylation patterns. Tumor-derived fragments (ctDNA) form a tiny fraction of all cfDNA fragments in the bloodstream. ctDNA originating from some specific genomic regions can have altered methylation patterns. Reliable and cost-effective detection of altered methylation pattern in these regions requires a broad targeted sequencing approach.
Methods: We developed CancerSpot, a multi-cancer early detection (MCED) test based on cfDNA methylation signatures in a cohort of 688 cancer patients and 326 healthy controls representing 10 cancer types. A custom genomics workflow prioritizes high-quality reads by rejecting poorly aligned or imperfectly converted data while rescuing reliable reads to calculate methylation scores across genomic regions at the read level accounting for detection of a weak signal from tumor derived DNA. Machine learning (ML) models were developed using methylation signatures from ~7000 genomic markers. A gradient-boosted trees approach with repeated cross-validation incorporated data perturbations to account for variability in lab protocols and sequencing platforms ensuring robust prediction accuracy and error tolerance.
Results: CancerSpot achieved 79% sensitivity and 97% specificity for detecting Stage I-III tumors across 10 cancer types. It also demonstrated a 77% accuracy in identifying the tumor tissue of origin within the top two predictions. The robust workflow enhances detection of tumor-derived cfDNA signals even in samples with low conversion efficiency while mitigating background noise from normal cfDNA. The regions used by the test show strong differential methylation in tumor-normal data from a diverse population in TCGA indicating its wide applicability.
Conclusions: CancerSpot provides a cost-effective and accurate MCED solution tailored to diverse cohorts.

Type	**Control**	**Benign**	**I**	**II**	**III**	**IV**	**Total**
Control	63 / 65	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	63 / 65
Colorectal	0 / 0	1 / 2	2 / 5	4 / 6	4 / 9	4 / 6	15 / 28
Esophagus	0 / 0	1 / 2	1 / 2	4 / 5	3 / 3	4 / 4	13 / 16
Gall Bladder	0 / 0	1 / 1	1 / 1	2 / 2	3 / 3	3 / 3	10 / 10
Liver	0 / 0	1 / 1	1 / 1	1 / 1	2 / 2	2 / 2	7 / 7
Lung	0 / 0	1 / 1	3 / 3	3 / 3	3 / 3	4 / 4	14 / 14
Pancreas	0 / 0	0 / 1	2 / 2	3 / 4	2 / 2	3 / 3	10 / 12
Stomach	0 / 0	0 / 0	4 / 4	2 / 4	4 / 4	2 / 4	12 / 16
Breast	0 / 0	2 / 3	3 / 3	4 / 4	2 / 3	4 / 4	15 / 17
Cervix	0 / 0	0 / 2	3 / 4	3 / 4	2 / 4	3 / 3	11 / 17
Ovary	0 / 0	1 / 2	3 / 4	3 / 4	4 / 4	4 / 5	15 / 19
Total	**63 / 65**	**8 / 15**	**23 / 29**	**29 / 37**	**29 / 37**	**33 / 38**	**122 / 156**
	96.92%	**53.33%**	**79.31%**	**78.38%**	**78.38%**	**86.84%**	**78.20%**

Performance of CancerSpot on a leave-out set.

#2344 Novel multiplex paired-antibody amplified detection (MPAD) assay panel in combination with CA19-9 for early detection of pancreatic cancer using plasma samples.

Malcolm MacKenzie, Matthew Preimesberger, Shu Feng Zhou, Ilya Alexandrov

ActivSignal, Natick, MA

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is an aggressive malignancy with one of the lowest five-year survival rates among major cancers, due to asymptomatic progression and the lack of reliable biomarkers for early detection. CA19-9, the only FDA-approved biomarker related to PDAC, is significantly elevated in many individuals with PDAC, but falls short as a single marker early-detection screen due to its high levels of false negatives and positives. Therefore, identifying a novel biomarker panel to orthogonally buttress CA19-9's sensitivity and specificity is critical for enhancing early PDAC detection.
Objectives: This study is aimed to identify and test protein biomarkers and develop a non-invasive, high performing, affordable diagnostic screen for early-stage PDAC.
Methods: The study cohort included matched healthy controls, patients with benign pancreatic conditions, and early- and late-stage PDAC patients. A Multiplex Paired-Antibody Amplified Detection (MPAD) platform uses paired antibodies conjugated with unique DNA barcodes and optimized epitope selection. Specific read-out is done using NGS. This approach enables the simultaneous detection of multiple biomarkers in a single well. Signal amplification techniques enhance the sensitivity and accuracy of detecting low-abundance biomarkers frequently associated with PDAC.
Results: Using rigorous selection criteria, we designed a novel MPAD panel containing multiple protein targets as biomarkers for PDAC detection. These biomarkers demonstrated significant effect size for differentiating PDAC from non-cancer samples, and demonstrating low correlation with CA19-9, underscoring their complementary nature for this diagnostic application. The combination panel increased sensitivity from 58% (CA19-9 alone) to 89% for All PDAC vs. non-cancer samples, at a specificity of 96% and an AUC of 95.8%. While, for early-stage PDAC vs. non-cancer samples, the sensitivity was increased from 50% (CA19-9 alone) to 90% in the MPAD panel + CA19-9, suggesting that the MPAD panel can effectively buttress CA19-9 and that the combination shows potential as the basis for an early-detection diagnostic. In PDAC samples with low levels of CA19-9, the MPAD panel without CA19-9 showed strong discrimination between PDAC vs. both benign pancreatic and healthy control samples, with sensitivity of 80% and AUC of 86%, suggesting that the MPAD panel has potential to effectively salvage false negative samples missed by CA19-9 alone.
Conclusions: The MPAD panel complements CA19-9 with minimal correlation, providing an independent diagnostic advantage. It significantly enhances the sensitivity of early-stage PDAC detection, particularly in patients with low CA19-9, making it a promising basis for early diagnosis and surveillance.
Disclosure: AI was used for abstract text editing.

#2345 Trans-renal DNA: Urine liquid biopsies methodology to increase compliance, convenience, and yield of systemic ctDNA.

Floyd E. Taub, Lindsey Ray, Charles Preston Neff, Elijah Barstis, Charles Henry, Kirsten Arnold

aiGENE, Inc., Golden, CO

Liquid biopsies promise monitoring residual disease, monitoring for minimal recurrence, evaluating the immediate effects of therapy, and even screening healthy subjects for cancer. Access and patient considerations should be built into the system from ground up. The success of all existing screening programs relies more on participation rates than incremental gains in sensitivity and specificity. Resistance to blood draws, especially for underserved patients is high and COVID, immunosuppression, and personal beliefs decrease participation when needle puncture is required. Venipuncture increases costs, biohazard waste and is sufficiently burdensome to lower participation and medical benefit. Urine is truly noninvasive. While long known to contain trans-renal cfDNA (tr-cfDNA), less effort has gone into specifically isolating this subset of urine DNA and using it as a substitute for blood cfDNA in cancer studies. Striking success has been seen but kits modified from blood kits typically isolate predominantly urinary DNA. Methods designed to handle shorter tr-cfDNA fragments are needed. The glomerular filtration limit of ~10 nm permits cfDNA of ≤100 bp to transit into the urine. Nucleosomes exceed 100 nm. Thus, we have designed a system to capture short non-nucleosomal tr-cfDNA. Urine is available in many hundreds of mls, compared to blood typically providing ≤10 mls of plasma. The latter is often insufficient to have the sequence of interest. Even with urine, data suggests that the more tested, the more likely rare sequences, like those from cancer, are detected. Further detailed understanding of diurnal, biological, daily, and weekly longitudinal ctDNA dynamics, in an outpatient setting, is facilitated by urine sampling. Herein we present a patient focused device that allows self-collection of urine voids (up to 500 mls) with immediate stabilization and separation, within minutes, of trans-renal and urothelial DNA onto separate filters contained within a filter cartridge. The tightly captured DNA excludes DNase binding, the DNA is thus stable at ambient temperature. A single filter cartridge can capture multiple voids and can then be sent by standard shipping. Even from small voids ≥2 ug rather than 10-20 ng amounts of cfDNA are collected. This allows higher sensitivity, methods requiring more DNA, as well as reuse should an initial assay fail, and banking for subsequent studies. The system specifically purifies tr-cfDNA based on interaction of the anionic free DNA with cationic filter bound polymer that does not bind urothelial nucleosomal DNA with positively charged histones. In the lab DNA is washed and eluted with simple syringe-based methods resulting in a purified preparation in 10 minutes. This generates a single peak of the expected trans renal size (80-100 bp) on capillary electrophoresis. Included data demonstrate single copy PCR and high quality (95% Q30s) in NGS.

#2346 Association of serum creatinine- and cystatin c-based diagnostic indices for sarcopenia with major chronic diseases.

Fangfang Song, Huijun Zhou, Fubin Liu, Yu Peng, Peng Wang, Jianxiao Gong, Jiale Gu, Ailing Qin

Tianjin Medical Univ. Cancer Inst. & Hospital, Tianjin, China

Background: Sarcopenia is a risk factor for chronic non-communicable diseases. The serum creatinine (Cr)/cystatin C (CysC) ratio (CCR) and the sarcopenia index (SI) have attracted attention as a surrogate marker for sarcopenia but have not been adequately studied in the general population. We aimed to explore the relationships of CCR and SI with major chronic diseases [cancer, cardiovascular disease (CVD), respiratory disease], investigate the validity of CCR and SI as predictors of sarcopenia and propose statistical cut-off values.
Methods: We utilized two large-scale cohorts: the UK Biobank (UKB) and the National Health and Nutrition Examination Survey (NHANES). Serum creatinine and cystatin C levels were measured at baseline to calculate the CCR and SI. Cox regression models were applied to evaluate the impact of CCR and SI on major chronic diseases' incidence and mortality risks. Diagnostic value was analyzed through receiver operating characteristic (ROC) curves. The Youden index was calculated to determine the optimal cut-off points. Sarcopenia was then defined separately utilizing CCR, SI, and the European Working Group of Sarcopenia in Older People 2 criteria.
Results: CCR and SI had negative associations with incidence and mortality risks of major chronic diseases as well as all-cause mortality. For the UK general population, the optimal cut-off values of CCR and SI for sarcopenia were 95.246 and 82.018 in men and 79.326 and 68.516 in women, respectively, with areas under the ROC curves (AUCs) of 0.717 and 0.709 in men, and 0.693 and 0.682 in women, respectively. For the USA general population, the optimal cut-off values of CCR and SI for sarcopenia were 94.521 and 85.296 in men and 90.993 and 76.904 in women, respectively, with AUCs of 0.755 and 0.752 in men and 0.595 and 0.588 in women, respectively. Furthermore, in the UKB database, CCR- and SI-defined sarcopenia were associated with a higher risk of pan-cancer and CVD incidence, as well as an increased risk of mortality from major diseases. In addition, CCR- and SI- defined sarcopenia in the NHANES database were positively associated with pan-cancer prevalence in women, and CCR-defined sarcopenia was dominantly correlated with an increased risk of mortality from major diseases in men. It is notable that CCR- and SI-defined sarcopenia were more strongly associated with the risks of major chronic diseases compared to EWGSOP2-defined sarcopenia.
Conclusions: CCR and SI are both negatively associated with the incidence and mortality risks of major chronic diseases in the general population and may serve as surrogate markers for sarcopenia. Considering serum Cr and CysC commonly assessed in clinical practice, the application of Cr and CysC-derived CCR and SI may facilitate the early detection of sarcopenia and the prevention of chronic diseases in the general population.

#2347 A genetic test for skin cancer shows potential for accelerating early personalized chemoprevention in ultra-high risk transplant patients.

Mathias Seviiri, Nirmala Pandeya, Catherine M. Olsen, Matthew H. Law, David C. Whiteman, Stuart MacGregor

Population Health, QIMR Berghofer Medical Research Institute, Brisbane, Australia

Background: Transplant patients are at extreme risk of multiple invasive skin cancers. Chemopreventive medication can reduce this risk by ~50%, but clinicians are unable to identify high-risk patients before cancer onset.
Objective: To assess the efficacy of a genetic test for skin cancer to identify ultra-high risk transplant patients who might benefit from chemoprevention or develop skin cancers with poor prognostic features.
Methods: We developed a polygenic risk test for skin cancer using data from 1.2 million people using a Bayesian approach that models genetic information from the whole genome. We applied the genetic test to a cohort of transplant patients (STAR n=357) from a high UV environment in Australia to assess whether it could predict ultra-high risk individuals suitable for chemoprevention.
Results: In the STAR cohort, higher genetic risk patients were more likely to: develop skin cancer (OR=2.04, 95%CI=1.54-2.71, P=8.72e-07), be treated with cancer-preventive medication (e.g. acitretin: OR=2.55, 95%CI=1.31 - 4.95, P=5.62e-03), have skin cancers with poor prognostic features, have poorly differentiated tumors (OR=2.29, 95%CI=1.39-3.77, P=1.18e-03), have tumors with unclear margins (OR=2.09, 95%CI =1.03-4.25, P=0.041), have large tumors >2cm (OR=4.09,95%CI=1.88-8.90, P=3.92e-04), and have highly invasive disease (OR=1.95, 95%CI=1.18-3.23, P=9.06e-03). We are now conducting a comparative analysis in a large cohort (QSkin) of non-transplantees.
Conclusion: Our genetic test for skin cancer has the potential to identify ultra-high risk transplant patients who should be prioritized by clinicians for personalized early chemoprevention.

#2348 Unveiling dichotomies between liver cancer and adjacent normal tissues in Wilson's disease model.

R. Adhikari[1], C. Dash[2], R. Roy[2];
[1]The Quarry Lane School, Dublin, CA, [2]Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC

Objective: The Long-Evans Cinnamon (LEC) rat is a model for Wilson's disease (WD) and is associated with chronic inflammation and hepatocellular carcinoma. This study performed a gene expression microarray using total RNA extracted from LEC liver tissue at 84 weeks late chronic hepatitis, tumor-adjacent normal (84 weeks N), and 84 weeks tumor (84 weeks T). Understanding the difference between a tumor and adjacent normal within the same animal can be very intriguing as both are in the same environment, and one becomes and sustains a tumor status, but the other still maintains its normalcy.
Experiment: Total RNA was extracted from liver tissue of three LEC rats at 84 weeks N and 84 weeks T and hybridized to the Affymetrix GeneChip Rat Genome 230 2.0 Array (Affymetrix Inc, Santa Clara). GeneGO Pathways software was used to analyze the differentially expressed genes using a cutoff of P-value ≤0.05 and FDR <0.25% and a fold change +/- 2. A volcano plot was generated to visualize significant gene change expression between 84 weeks N vs 84 weeks T. Agglomerative Hierarchical and K-means clustering were used to group similarly expressed genes into clusters and build a hierarchy of clusters. Each cluster obtained from K-means clustering analysis within the up or down-regulated groups were checked for pathway analysis to understand the interplay between biological pathways by QIAGEN Ingenuity® Pathway Analysis (QIAGEN IPA). In addition – log(p) > 1.3, a z-score cut-off ≥ 2 for activation and a z-score cutoff ≤ -2 for inhibition was used to call a pathway activation, and inhibition is significant.
Result: The seven most significant pathways that were activated are the S100 Family Signaling Pathway, Extracellular Matrix Organization, Response to Elevated platelet cytosolic Ca2+, HMGB1 Signaling, Sertoli Cell-Sertoli Cell Junction Signaling, Cachexia Sunaina Pathway, and Nicotine Degradation II. Except for Nicotine Degradation II pathway, all other pathways are shown activated in non-alcoholic fatty liver disease (NAFLD) and hepatocellular carcinoma (HCC) patients previously but shown in the WD model for the first itme. The Nicotine Degradation II pathway is shown to be activated in the NAFLD patient before.
Conclusion: To our knowledge, this is the first report where gene expression analysis was done between tumor-adjacent normal and tumor within the same animal in the WD model. Our results identified multiple pathways in the model that could lead us to new diagnostics and therapeutic targets when validated.

#2349 Implementation of a novel immune monitoring strategy for multicenter clinical trials to enable equity in cancer prognosis for individuals from remote settings of Australia.

Natalie Smith[1], Michael Cohen[2], Julie Alipaz[2], Christina Loh[2], David King[2], Steven Kao[3], Sandra Taylor[4], Rajat Rai[4], Barbara Fazekas de St Groth[1], **Helen McGuire**[1]

[1]The University of Sydney, Sydney, Australia,[2]Standard BioTools, San Francisco, CA,[3]Chris O'Brien Lifehouse, Sydney, Australia,[4]Dubbo Base Hospital, Dubbo, Australia

Improved cancer prognosis begins with the collection of high-quality data from clinical trials. However, the execution of longitudinal immune phenotyping studies by flow cytometry is complex, primarily due to logistical challenges that could affect data quality. The challenges of immune monitoring are exacerbated when considering remote and rural communities in Australia, which are burdened by both an increased prevalence and worse prognosis of cancer. Paradoxically, these communities are often underrepresented in clinical trials. Our group has developed a novel blood-based “immune signature” that robustly predicts failure to make a clinical response to checkpoint therapies targeting the PD-1/PD-L1 pathway in melanoma and lung cancer. These groundbreaking findings were achieved through applying a comprehensive 38-parameter immunophenotyping CyTOF™ panel to biobanked peripheral immune cells from cancer patients. We now aim to increase our clinical implementation in remote settings, with an expanded CyTOF panel of 50-plus-parameter analysis. Recent developments in CyTOF technology facilitate a highly simplified workflow of asynchronous sample collection and staining, compatible with current hospital laboratories that typically run on unpredictable schedules, to remote settings. This is uniquely enabled by using a dried-down cocktail of CyTOF antibodies that are stable, simple to use and yield reproducible staining while minimizing sample required. Furthermore, this workflow, unique to mass cytometry, provides flexibility and minimizes technical variation via sample barcoding and freezing of stained samples for shipment to a central site for batch acquisition. As such, this study is designed to ultimately demonstrate both clinical impact of the large panel and utility of CyTOF technology for a highly simplified and robust workflow for multi-site clinical trials. Here we present findings from the implementation of this novel workflow through a 100-sample pilot study. Remote asynchronous sample collection and surface staining was performed at five sites across Australia. Stained samples were then shipped to a central lab for multiplex barcoding and intracellular staining of several key functional markers. A combination of manual gating and automated high-dimensional clustering was performed in CellEngine, with principal component analysis of all samples demonstrating harmony across the sites. Overall, this workflow enables access to underserved communities, facilitating for the first time equity and scalability of immune phenotyping studies to harness truly geographically dispersed clinical centers.

#2350 Protein biomarkers of HCC development in patients with metabolic liver disease.

A. Siaw[1], S. M. Armasu[2], J. A. Frank[2], I. K. Yan[1], K. J. Wangensteen[2], T. Patel[3], **S. O. Antwi**[1];
[1]Mayo Clinic, Jacksonville, FL, [2]Mayo Clinic, Rochester, MN, [3]Mayo Clinic Florida, Jacksonville, FL

Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) is a fast-rising global public health problem. About 10% of patients with MASLD will develop hepatocellular carcinoma (HCC), but molecular markers for predicting future risk of HCC development in patients with MASLD are lacking. We investigated whether protein biomarkers in pre-malignant plasma samples can predict susceptibility to HCC in the setting of MASLD.

Methods: A nested case-control study was performed among patients with MASLD who later developed HCC (cases) and MASLD patients who never progressed to HCC (controls), matched on age at MASLD diagnosis (± 1-year), sex, the year of blood sample collection, and length of follow-up (± 1-year). MASLD at baseline was determined by the presence of hepatic steatosis (assessed by imaging or biopsy) plus at least one cardiometabolic condition (obesity, diabetes mellitus, hypertension, or dyslipidemia). Archival baseline plasma samples stored at -80°C were used to perform the SomaScan 7k v4.0 assay. We also performed genotyping of the well-established HCC susceptibility variant, *PNPLA3*-rs738409 (I148M), among the cases and controls. The study sample was split into discovery (70%) and validation (30%) sets, and we selected promising protein biomarkers in the discovery set using differential expression analysis with volcano plots and Bonferroni-corrected p-values. A final set of significant proteins was used for prediction modeling in the validation sample, calculating area under the receiver operating characteristic curve (AUC-ROC), sensitivity, specificity, and accuracy.

Results: The study sample included 54 MASLD to HCC cases and 54 MASLD controls, split into discovery and validation sets. We identified five significantly differentially expressed proteins in the discovery set, four overexpressed (*TSP2, GPR56, MIC-1, TIMP-1*) and one under-expressed (*IGFALS*) in the cases *versus* controls. Fitting the five proteins in the validation sample yielded an AUC=0.88, sensitivity=0.87, specificity=0.75, and accuracy=0.81. Adding *PNPLA3*-rs738409 to the model improved the model's specificity and accuracy (AUC=0.88, sensitivity=0.87; specificity=0.81, accuracy=0.84).

Conclusions: Our findings show that a five-protein biomarker panel can robustly predict future HCC development in patients with MASLD. Adding a well-established HCC genetic risk variant, *PNPLA3*-rs738409, slightly improved the model's performance. These initial findings provide evidence of HCC prediction with baseline plasma biomarkers and genetic risk variants, toward improving risk stratification and enhancing early detection of HCC in the setting of metabolic perturbations.

#2351 Association of germline genetic variants with CAR T-cell therapy outcomes.

Salem Werdyani[1], Xiaotong Sun[2], Yuanyuan Zha[1], Peter Riedell[1], Justin Kline[1], Xin He[2], Aly A. Khan[1]

[1]Biological Sciences Division (BSD), University of Chicago, Chicago, IL,[2]Department of Human Genetics, University of Chicago, Chicago, IL

Purpose: CAR T-cell therapy has shown promise in treating B-cell malignancies, but patient responses vary significantly. We hypothesize that germline genetics influence this variability by impacting T-cell programming and consequently CAR T-cell efficacy. This study aims to investigate the association between germline genetics and CAR T-cell therapy outcomes.
Materials and Methods: We assembled a retrospective cohort of 108 B-cell lymphoid malignancies patients who were treated with CAR T-cell therapy at the University of Chicago Cell Therapy Biobank from May 2018 to Nov 2023 (IRB approval: IRB23-1114). A comprehensive clinical dataset including demographics, disease characteristics, and post-treatment outcomes was obtained from patients' electronic health records and clinical notes. DNA samples were extracted from 105 patients' peripheral blood and underwent low-pass whole-genome sequencing (WGS). Genotype imputation and quality control filtering identified ~ 11 million genetic variants across the study participants. Polygenic risk scores (PGSs) from the PGS Catalog were calculated to test association of PGSs of major immune diseases with the CAR T-cell treatment response. We used 6-month progression-free survival (PFS) based on clinical evaluation as our primary measure of treatment outcome.
Results: Of 98 patients with comprehensive clinical and sequencing data, 55 responded (mean age 61.48 ±12.21 years; 30.91% female) to treatment, while 43 did not respond (mean age 61.60 ±11.28 years; 30.23% female) to treatment. Among clinical covariates, we reproduced a significant association between lactate dehydrogenase (LDH) concentrations and 6-month PFS (adjusted $P < .02$). We conducted multivariate logistic regression including LDH levels to explore correlations between computed PGSs and CAR T-cell treatment outcomes. PGSs related to type 1 diabetes (T1D) were associated with the CAR T-cell treatment response combining multiple PGSs with Aggregate Cauchy Association Test (ACAT $p = 0.016$), in contrast to type 2 diabetes (T2D) PGSs that did not show an association. These results suggest the immune component of T1D are specifically associated with CAR T-cell treatment response. Further analysis in larger T1D GWAS is required to substantiate these findings.
Conclusion: The association between T1D genetics and the CAR T-cell therapy outcomes suggests germline genetics, particularly autoimmune-related variants, modulate therapy efficacy. These genetic variants may elevate T-cell activation, contributing to autoimmunity but leading to increased CAR T-cell exhaustion and reduced effectiveness. Further research will explore these mechanisms. Integrating genetic insights into patient evaluation and CAR T-cell engineering could improve therapy personalization and clinical outcomes.

#2352 Evaluation of alcohol, tobacco, and HPV's synergistic regulation of HNSCC's potential treatment response to PD-L1 checkpoint inhibitor treatment.

Ruomin Xin, Omar Mokhashi, Miski Abdi, Riya Chhabra, Ling Duan, Alanna Sun, Daniel John, Wei Tse Li, Jessica Wang-Rodriguez, Weg M. Ongkeko

UC San Diego, La Jolla, CA

Background: Enhanced PD-L1 (Programmed Death-Ligand 1) expression in tumor cells aids the evasion of immune surveillance. Nivolumab and pembrolizumab, PD-L1 blockade agents, have been applied in clinical settings to treat Head and Neck Squamous Cell Carcinoma (HNSCC), yet low response rates, unimproved prognosis, and treatment resistance necessitate further research into the underlying molecular mechanism. While alcohol, tobacco, and HPV exposure are the most prominent risk factors responsible for HNSCC pathogenesis, their synergistic influence on PD-L1 blockade treatment responses is largely unknown. In this study, we characterized how different combinations of the three etiologic factors alters the molecular landscape of HNSCC, influencing either favorable or adverse PD-L1 blockade treatment outcomes.

Methods: HPV abundance was characterized in 498 primary HNSCC tumor samples. Together with alcohol and tobacco exposure history, samples were classified into 8 subgroups reflecting all possible combinations of the three exposure factors. Differential expression analysis was performed to examine both mRNA and miRNA regulation in relation to key PD-L1 signaling pathways (NF-κB, PI3K/Akt, and Ras/MEK/ERK). Tumor mutation burden (TMB) calculation and subgroup immune landscape profiling were performed to assess the potential response to PD-L1 blockade treatment. Gene Ontology (GO) and KEGG pathway enrichment analysis were then conducted to investigate genetic pathway and cellular processes associated with differential PD-L1 blockade treatment outcomes.

Results: PD-L1 was significantly downregulated in the triple-exposure group (1.51 fold reduction, $p < 0.05$). Dysregulation of PD-L1-related genes, including JAK2 (triple-exposure group: 1.44 fold reduction, $p < 0.05$) and BRAF and ATR, was observed in multi-exposure subgroups (cigarette + HPV group: BRAF 0.781 fold reduction, $p < 0.05$; ATR 0.722 fold reduction, $p < 0.05$). miR-106, implicated in HPV pathogenesis, was most dysregulated in triple-exposure cases. miRNAs including miR-374b-5, miR-16, miR-29b, and miR-143, associated with poor prognosis, and key PD-L1 regulating pathways (PI3K/Akt and P53), were significantly altered in triple-exposure group. Pathway disruptions, including those related to histone regulation and chromosomal structure, increased with additional exposure factors. Tobacco-HPV dual exposure group showed the highest TMB with a 1.94-fold increase ($p < 0.01$) compared to the unexposed subgroup. The triple-exposure group exhibited the highest M2 macrophage enrichment (1.59 fold increase, $p < 0.05$) and CD8+ T-cell reduction (2.03 fold reduction, $p < 0.05$). Further studies are required to identify specified immunotherapeutic administration techniques to address the differential molecular landscapes in different exposure subgroups.

#2353 CRHBP, a novel multiple cancer biomarker connected with better prognosis and anti-tumorigenicity.

Wonbeak Yoo, Yeeun Lee, Kyung Chan Park, Hyunji Choi

Korea Research Institute of Bioscience & Biotechnology (KRIBB), Daejeon, Korea, Republic of

Corticotropin-releasing hormone-binding protein (CRHBP) is a key modulator of the hypothalamic-pituitary-adrenal (HPA) axis and plays a crucial role in stress responses, immune regulation, and neuroendocrine functions. Despite its importance in physiological processes, the role of CRHBP in tumor biology and its clinical relevance remain largely unknown. This study aims to comprehensively investigate CRHBP expression across diverse cancer types, its prognostic significance, and its potential molecular mechanisms in tumor progression. We show that CRHBP expression was consistently downregulated in the majority of solid tumors, with the most significant reductions observed in LUAD, LIHC, and KIRC. Low CRHBP expression was strongly correlated with poor prognosis, including shorter OS and DFS, in multiple tumor types. Methylation analysis revealed that hypermethylation of the CRHBP promoter region was associated with its downregulation in several cancers. Further, we identified a set of miRNAs (miR-21, miR-155, miR-29a, and miR-181a) that were upregulated in these tumors and may contribute to the suppression of CRHBP expression. Gene enrichment analysis revealed that CRHBP is involved in key cellular processes, including intracellular signal transduction, metabolic regulation, and hormone-related pathways. CRHBP expression also showed significant correlations with the immune microenvironment, particularly with the infiltration of regulatory T cells (Tregs), macrophages, and dendritic cells. PPI network analysis indicated that CRHBP interacts with several proteins involved in the stress response, immune regulation, and cellular metabolism. Functionally, overexpression of CRHBP inhibited tumor cell proliferation in LUAD, LIHC, and KIRC cell lines, as measured by MTT and colony formation assays. Furthermore, CRHBP overexpression significantly reduced cell migration and invasion in KIRC and hepatocellular carcinoma (HCC) cell lines. Together, these data demonstrated that CRHBP expression is associated with tumor progression, immune cell infiltration, and key molecular pathways. Overexpression of CRHBP inhibits cell proliferation and mobility in multiple cancers, suggesting its potential as a biomarker and therapeutic target in cancer.

#2354 Developing a gene signature from circulating biomarkers to forecast Lu-PSMA response in advanced prostate cancer.

Srinivas V. Koduru[1], Mark Kidd[1], Alin Chirindel[2], Nicolas Guillaume[2], Abdel Halim[1]

[1]Wren Laboratories, LLC, Branford, CT,[2]Clinic for Radiology and Nuclear Medicine, University Hospital Basel, Basel, Switzerland

Introduction: ^{177}Lu-PSMA-targeted radioligand therapy (RLT) for metastatic castration-resistant prostate cancer (mCRPC) leverages the high PSMA expression on cancer cells. The radiolabeled molecule binds to PSMA, delivering beta radiation to selectively destroy cancer cells while sparing surrounding healthy tissue. Despite its sophistication, not all mCRPC patients with adequate PSMA expression respond to this therapy. Currently, there is no mean to predict response or optimize patient selection. We performed transcriptomic profiling on patients undergoing Lu-PSMA therapy, aiming to identify a gene signature that can predict treatment response and help select the most suitable patient population.
Methods: Whole blood samples were collected from mCRPC patients receiving Lu-PSMA-based RLT and categorized into three groups based on PCWG3 criteria: Responders (R, n=30), Partial Responders (RR, n=10), and Non-Responders (NR, n=26). Total RNA was isolated and subjected to RNA sequencing using the NovaSeq 6000 platform. Differentially expressed genes were identified through data analysis with Partek Flow, and pathway analysis was performed to investigate associated biological processes.
Results: RNA sequencing of the three groups (R, NR, and PR) revealed 825 statistically significant genes ($FC > 1$; $P < 0.05$) in the R vs. NR comparison, 1,807 genes in R vs. PR, and 1,548 genes in PR vs. NR. Furthermore, over 90% of responders exhibited prolonged survival, while more than 95% of non-responders and over 80% of partial responders were recorded as deceased.
Conclusion: Our findings suggest that the identified gene signature could serve as a predictive biomarker for clinical response prior to initiating ^{177}Lu-PSMA treatment. Additionally, the pronounced expression of certain genes in non-responders presents an opportunity for the development of novel therapeutic strategies.

PREVENTION / EARLY DETECTION / INTERCEPTION: Biomarkers and Molecular Targets for Prevention
Poster Session

#2358 XPO1 inhibition serves as an effective chemoprevention strategy in colorectal cancer mouse models.

Andrew E. Evans[1], Alexa N. Magstadt[1], Dan A. Dixon[2]

[1]University of Kansas, Lawrence, KS,[2]University of Arkansas for Medical Sciences, Little Rock, AR

Background: Familial Adenomatous Polyposis (FAP) is a hereditary condition resulting in a 100% chance of developing colorectal cancer (CRC) if left untreated. Individuals with FAP often undergo colectomies in their 20s. Thus, there is a critical need for novel chemopreventive approaches to reduce CRC risk in FAP patients. XPO1 is a nuclear export protein overexpressed in multiple cancer types, including CRC. The overexpression of XPO1 has been shown to promote tumorigenesis by excessively removing numerous tumor suppressors from the nucleus. To combat XPO1 overexpression, Selective inhibitors of Nuclear Export (SINE) compounds have been developed. One of those compounds, Eltanexor (KPT-8602), is a second-generation SINE compound currently in Phase I/II clinical trials. Eltanexor has been shown to exhibit fewer side effects compared to its predecessors.

Methods and Results: Our research shows that XPO1 is overexpressed in multiple different stages of CRC and in *Apc*$^{min/+}$ mice, a FAP mouse model, adenomas. In xenograft studies with HCT116 cells, Eltanexor significantly inhibits CRC tumorigenesis. Further analysis revealed that Eltanexor reduced cyclooxygenase-2 (COX-2) expression, a key target in chemoprevention, by downregulating Wnt/β-catenin signaling. Deletion mapping and TOPFlash luciferase assays confirmed Eltanexor's modulation of Wnt/β-catenin activity. Notably, Eltanexor enhanced nuclear retention of Forkhead Box O3a (FOXO3a), a known XPO1 cargo and Wnt/β-catenin regulator. Moreover, organoid drug sensitivity assays we performed indicated that *Apc*$^{min/+}$ adenoma organoids are more sensitive to XPO1 inhibition compared to wild-type intestinal organoids. To explore Eltanexor as a chemopreventive compound, we treated *Apc*$^{min/+}$ mice with Eltanexor for 6 weeks, leading to a significant 62% decrease in tumor burden and a reduction in tumor size. Moreover, to further evaluate Eltanexor's potential role in chemoprevention, Eltanexor was administered to *Apc*$^{min/+}$ mice with celecoxib, a COX-2 inhibitor shown previously to effectively limit *Apc*$^{min/+}$ mice tumorigenesis. Our results revealed mice administered the combined treatment experienced a significant >80% reduction in tumor burden and diminished tumor size. Importantly, both Eltanexor as a monotherapy and the combination treatment were well-tolerated, with no observed toxicity in the treated mice.
Conclusions: Our findings show XPO1 inhibition using the small molecule Eltanexor results in inhibition of the Wnt/ β-catenin signaling pathway, a crucial pathway in CRC tumorigenesis. Furthermore, we show that Eltanexor treatment is an effective chemoprevention strategy in *Apc*$^{min/+}$ mice, a FAP mouse model. These findings highlight a promising chemopreventive strategy for high-risk CRC individuals.

#2359 Age-dependent transcriptional changes in colonic fibroblasts revealed through single-cell RNA sequencing.

Jonathan M. Downie[1], Ryan J. Musich[2], Saleh Khawaled[3], Alexander Caraballo[1], Kylor Lachut[1], Shijie He[1], Marina Magicheva-Gupta[1], Ingrid Crumpton[1], Liza Hote[1], Karen Kirunda[1], Perry Labelle[1], Omer H. Yilmaz[3], Thaddeus Stappenbeck[2], Andrew T. Chan[1], David A. Drew[1]

[1]Gastroenterology, Harvard Medical School/Massachusetts General Hospital, Boston, MA,[2]Inflammation and Immunity, Cleveland Clinic, Cleveland, OH,[3]MIT Koch Institute for Integrative Cancer Research, Cambridge, MA

Introduction: Fibroblasts are key enhancers of colorectal cancer (CRC) development. Cancer-associated fibroblasts (CAF)s promote oncogenesis via extracellular matrix remodeling and the secretion of inflammatory cytokines. CAFs likely arise from normal tissue-resident fibroblast populations that are altered with aging/senescence and environmental exposures. Thus, fibroblasts may mediate CRC development by amplifying etiologic exposures or age-related mucosal changes. This study examines age-related transcriptional changes in fibroblasts from intestinal biopsies to assess the effects of aging.

Methods: Using 10X Genomics Chromium single-cell RNA sequencing, we profiled cells derived from rectosigmoid biopsies of 48 individuals with a history of colorectal adenoma (Young: 24, aged <55; Older: 24, aged >65). *Cellranger*, *Cellbender, and Seurat* were used to derive gene counts, cell types, and clusters. Analyses focused on the stromal cell compartment. *Milo* and *Nebula* identified age-related differences in cell abundance and differentially expressed genes (DEGs).

Results: Among 61,605 stromal cells, 39,074 fibroblasts (*ADAMDEC1*^{high}) were subtyped as crypt top fibroblasts (CTFs; *PDGFRA*^{high}), colon crypt fibroblasts type-1 (cCF1, *PDGFRA*^{low}/*GREM1*$^{-}$), colon crypt fibroblasts type-2 (cCF2, *GREM1*$^{+}$/*RSPO3*$^{+}$), and fibroblastic reticular-like cells (FRLCs, *CCL19*$^{+}$). We did not observe changes in the abundance of fibroblasts between the age groups. However, we found 181 ccF1, 68 ccF2, and 44 CTF specific age-associated DEGs. FRLCs had one significant DEG: *PLAU* (LogFC = 1.96; FDR = 0.021), a factor that converts fibroblasts into CAFs. In cCF1, *GDF15* (LogFC = 1.16; FDR = 5.73 x 10^{-5}), a marker of senescence and CRC risk, was the most upregulated age-associated gene. Gene set enrichment analysis revealed several inflammaging pathways were upregulated in aged fibroblasts, including interleukin-2, interleukin-6, tumor necrosis factor-α, and interferon-γ pathways. Additionally, *KRAS* signaling (all fibroblasts), p53 (cCF1), and epithelial-mesenchymal transition (cCF2) were upregulated with aging, while Myc targets (all fibroblasts) were downregulated.

Conclusions: Fibroblasts in individuals at CRC risk acquire a senescent phenotype with aging, promoting a chronic inflammatory microenvironment. These changes may alter colon crypt regeneration and increase susceptibility to CRC risk factors. Further studies may clarify fibroblast roles in early- and typical-onset CRC.

#2360 Targeting of microRNA let-7b-5p-mediated aberrant AR signaling inhibits the growth and proliferation of preneoplastic and preinvasive stages of triple-negative breast cancer.

Leslie Faye T. Cando[1], Zhenlin Ju[2], Leslie Michelle M. Dalmacio[3], Preethi H. Gunaratne[4], Jing Wang[2], Anjana Bhardwaj[1], Isabelle Bedrosian[1]

[1]Department of Breast Surgical Oncology, UT MD Anderson Cancer Center, Houston, TX,[2]Department of Bioinformatics and Computational Biology, UT MD Anderson Cancer Center, Houston, TX,[3]Department of Biochemistry and Molecular Biology, College of Medicine, University of the Philippines Manila, Manila, Philippines,[4]Department of Biology and Biochemistry, University of Houston, Houston, TX

Background: In search for targets for triple-negative breast cancer (TNBC) prevention, we analyzed molecular changes that occur early in the tumorigenic process. We hypothesize that the early loss of microRNA let-7b-5p, and subsequent upregulation of its target gene androgen receptor (AR), promotes the growth and proliferation of preneoplastic and preinvasive lesions. And while targeting AR has been studied in the therapeutic setting for breast cancer, it may also have potential use in preventing disease progression.
Methods: TNBC patient samples included tumors and histologically normal tissue adjacent (<2 cm) and distant (>2 cm) from tumor. Sublines of MCF10A TNBC progression model corresponding to preneoplastic lesions MCF10.NeoT (hyperplasia) and MCF10.AT1 (atypical hyperplasia), and preinvasive tumor MCF10.DCIS (ductal carcinoma *in situ*) were used to study the effect of let-7b rescue and AR inhibition on proliferation, survival, or migration as measured by MTT, colony formation, and scratch assays. Let-7b gene targets were identified by cross-referencing the RNA sequencing data of MCF10A panel for upregulated genes that are predicted targets of let-7b. QPCR and RNA sequencing were used to measure AR and let-7b expression.
Results: In TNBC patients, let-7b expression is reduced by 68-74% in tumors compared to adjacent ($p=0.015$) and distant normal tissues ($p=0.048$)—confirming previous results in the MCF10A model, where let-7b expression is reduced from normal-like to AT1 by 35% and to DCIS by 65% ($p<0.05$). Let-7b rescue results in 15-32% lower proliferation ($p<0.0001$), 6-9% reduction in migration ($p<0.003$), and a 25% ($p=0.076$), 66% ($p<0.0001$), and 55% ($p=0.030$) decrease in colony formation compared to scramble control in NeoT, AT1, and DCIS cells, respectively. After screening potential let-7b targets, we selected AR for validation. As expected of the inverse regulation by let-7b, AR increases in the MCF10A model, with a notable 23-fold increase in DCIS as compared to normal-like ($p<0.0001$), and similarly in TNBC patient tumors compared to normal tissue controls ($p=0.018$). Furthermore, we validated AR as a direct target with let-7b overexpression causing AR downregulation by 67% in NeoT ($p=0.001$), 86% in AT1 ($p<0.0001$), and 15% in DCIS ($p=0.011$). Finally, treatment with AR inhibitor enzalutamide reduced DHT-dependent AR mediated proliferation by 29-41% ($p<0.0001$) and colony formation by 82-95% ($p<0.0001$) as compared to DHT only control at a minimal concentration of 25μM in AT1 and DCIS.
Conclusions: Let-7b-5p, which acts as a tumor suppressor microRNA, is lost early in tumorigenesis of TNBC, with resulting upregulation of AR. Inhibiting the AR signaling pathway impacts cellular growth and proliferation of preneoplastic and preinvasive cancer cells, suggesting a potential opportunity for TNBC prevention.

#2361 An *ex vivo* organoid study to identify biomarkers of sensitivity to ST316, a clinical-stage β-catenin antagonist peptide.

F. Abbate, L. Ghamsari, C. Scuoppo, Z. Mattes, R. Ramirez, M. Koester, S. Leong, B. Kappel, A. Vainstein-Haras, J. Rotolo; Sapience Therapeutics, Inc., Tarrytown, NY

Background. The WNT/β-catenin pathway, a key oncogenic driver in various cancers, was long deemed undruggable. Historically, attempts at targeting its components resulted in toxicity due to β-catenin's role in homeostatic functions such as intestinal stem cell renewal and bone osteogenesis. ST316 is a β-catenin antagonist peptide that disrupts its interaction with co-activator BCL9, targeting oncogenic signaling and immune exclusion while preserving homeostatic functions. ST316 reduces β-catenin transcriptional activity and tumor growth *in vitro* and *in vivo,* yet GLP-toxicology studies and Phase 1 clinical trial data confirm no WNT-inhibition-associated toxicity. ST316 has now progressed to a Phase 2 trial in patients with colorectal cancer (CRC), an indication with a high prevalence of WNT-related mutations. Here, we evaluated the anti-tumor activity of ST316 in 60 tumor-derived organoids with diverse genetic backgrounds to identify pathways associated with treatment responses. RNA sequencing and qRT-PCR identified transcriptional signatures of cell lines treated with ST316 or vehicle.

Results. The biomarker study of 60 organoids identified genetic backgrounds linked to ST316 responses, with TP53 WT models showing the highest sensitivity. Further analysis revealed higher ST316 sensitivity in models with BRAF, NRAS, and NF1 mutations, suggesting co-dependency between MAPK and WNT/β-catenin pathways. Given the importance of MAPK signaling in cancer, we evaluated dual inhibition of β-catenin and BRAF by combining ST316 with the BRAF inhibitor encorafenib in BRAF-mutant organoids. This combination showed additive/synergistic anti-tumor effects, highlighting new therapeutic opportunities for BRAFV600E-mutant CRC, a subgroup with limited treatment options.

Conclusions. ST316, a novel β-catenin antagonist, preserves WNT/β-catenin homeostasis while targeting oncogenic and immune-suppressive β-catenin/BCL9 signaling. Analysis on 60 patient-derived tumor organoids revealed that tumors with TP53 WT and mutations in the MAPK pathway significantly correlate with sensitivity to ST316. Additionally, combining ST316 with encorafenib showed additive/synergistic effects in BRAFV600E-mutant CRC organoids. β-catenin has been linked to resistance to BRAF inhibitors, and combination therapies targeting both β-catenin and BRAF are proposed to overcome resistance. Given the poor prognosis and limited treatment options for BRAFV600E-mutant CRC, ST316 offers a promising new therapeutic approach for this difficult-to-treat cancer. Our findings support rational combinations of ST316 with clinically relevant MAPK inhibitors.

#2362 Bitter taste receptors detect oral dysbiosis and trigger oral cancer cell death via β-arrestin-biased NLRP3 activation.

Sungmin Joo, Seong Yu, Jeong-Mu Shin, Yeshin Kwon, Na-Young Song

Yonsei University College of Dentistry, Seoul, Korea, Republic of

Bitter taste receptors (taste 2 receptors, T2Rs) are a family of G protein-coupled receptors (GPCRs) that transduce bitter taste signals in the taste buds via the gustatory G protein α-gustducin. Recently, T2Rs have emerged as novel regulators in various extraoral physiological processes, including microbial interactions and carcinogenesis. Notably, T2R4 and T2R14 can recognize bacterial quorum sensing (QS) molecules, such as N-(3-oxododecanoyl)-L-homoserine lactone (oxo-C12). Reanalysis of oral microbiome datasets from oral squamous cell carcinoma (OSCC) patients, a major subtype of head and neck squamous cell carcinoma (HNSC), revealed an upregulation of bacterial QS pathways associated with oxo-C12 synthesis. Lower expression levels of T2R4 or T2R14 correlated with poor outcomes and impaired bacterial defense responses in OSCC/HNSC patients. We hypothesized that T2R4 and T2R14 mediate the interplay between oral microbial dysbiosis and OSCC in response to the bacterial QS molecule oxo-C12. Our results showed that oxo-C12 induces pyroptosis and apoptosis in human OSCC cells, unlike other T2R agonists such as quinine and apigenin. Unexpectedly, T2R4 and T2R14 mediated oxo-C12-induced cell death independently of α-gustducin, instead activating the NLRP3 inflammasome via β-arrestin-1/2 (β-ARR1/2). This indicates that oxo-C12 acts as a β-ARR1/2-biased ligand, triggering OSCC cell death through the T2R-β-ARR1/2-NLRP3 axis. A positive correlation between β-ARR2 and T2R4, T2R14, or NLRP3 in HNSC patient datasets supports this signaling pathway. Taken together, T2R4 and T2R14 function as crucial interkingdom sensors that detect oral microbiome dysbiosis and trigger defensive cell death mechanisms in OSCC via β-ARR1/2-biased NLRP3 activation. These findings suggest the potential of T2Rs as chemotherapeutic targets, particularly through ligand-specific biased pathways involving either G protein- or β-ARR1/2-dependent signaling.

#2363 PRMT5 as a target in CRC interception: Synthetic lethal dependency identified in APC-LOF preclinical models of FAP disease.

Dana S. Gaffney[1], Jan Willem Thuring[2], Fabian Hulpia[2], Eileen Vesely[1], Edward Retzbach[1], Zienab Etwebi[1], Zhengyu Jiang[1], Albi Francis[1], Bethany Mattson[1], Victoria Wong[1], Mariana Silva[3], Gerben ten Hag[3], Rene Overmeer[3], Carla Verissimo[3], Sylvia F. Boj[3], Johan Nicolai[2], Mark Hixon[4], Gerry Chu[1], Andrea DiSandro[1], Kathleen Clancy[1], Alexandra Liddane[1], Kurtis Bachman[1], David Pocalyko[1]

[1]Johnson & Johnson Innovative Medicine, US, Springhouse, PA,[2]Janssen Pharmaceutica NV, Beerse, Belgium,[3]HUB Organoids BV, Utrecht, Netherlands,[4]Johnson & Johnson Innovative Medicine, US, La Jolla, CA

Colorectal cancer (CRC) is one of the most common causes of cancer-related deaths in the world and loss of the Adenomatous Polyposis Coli (*APC*) gene represents the disease-initiating event in the majority of CRC cases. Strategies for targeting *APC*-loss of function (*APC*-LOF) may provide an opportunity to intercept tumorigenesis in high-risk CRC patients, such as those with Familial Adenomatous Polyposis (FAP). To discover targets for therapeutic intervention in *APC*-LOF-driven disease, we conducted a high-throughput screen in isogenic *APC* knockout (APC^{KO}) colorectal organoids of >6000 compounds with known clinical relevance. From this screen, we uncovered a novel synthetic lethal dependency of *APC*-LOF on protein arginine methyltransferase 5 (PRMT5). JNJ-64619178 (JNJ-9178), a potent clinical PRMT5 inhibitor, demonstrated an >800-fold window in growth inhibition of engineered APC^{KO} organoids compared to wild-type (WT). Pharmacodynamic assessments showed a dose-dependent decrease in SmD3, an established biomarker for PRMT5 target engagement, with JNJ-9178 treatment, while no decrease was observed with a weakly active compound, providing evidence that the antiproliferative activity observed in the APC^{KO} organoids is a direct consequence of on-target PRMT5 inhibition. We further validated this PRMT5/APC dependency by testing other S-adenosylmethionine (SAM)-competitive PRMT5 inhibitors which show a similar trend in selectively inhibiting growth of the APC^{KO} organoids vs WT. Furthermore, we assessed the impact of JNJ-9178 on colon polyp burden in the $Apc^{min/+}$-DSS mouse model, an in vivo model of FAP, where we started treatment either before (early interception) or after (late interception) significant polyp burden had been established. A 72% and 66% reduction in polyp burden was observed, respectively, compared to vehicle-treated mice after 3 weeks of dosing (BID). Taken together, these findings showcase the impact of PRMT5 inhibition in both human and mouse models of FAP disease. Orally bioavailable PRMT5 inhibitors, including JNJ-9178, present challenges as therapeutics for CRC interception due to on-target cytopenias observed with systemic exposure. To address these dose-limiting toxicities, we have developed a GI-restricted approach and discovered new compounds that only inhibit PRMT5 activity locally in the intestinal tract. This strategy is detailed in a second abstract.

#2364 The role of β1 and β5 integrins as biomarkers in anti-motility therapy treatment response.

W. Chen, N. Woods, H. Law, F. Qiao, R. C. Bergan;
UNMC Eppley Institute, Fred & Pamela Buffett Cancer Center, Omaha, NE

Introduction. Metastasis is the major cause of mortality in patients with solid tumors. Our group has identified precision therapeutic inhibitors of cell motility and shown their ability to prevent metastasis. In striving to move novel first-in-class therapy into humans, it is important to identify biomarkers of therapeutic efficacy. As β1 and β5 integrins mediate prostate cancer cell migration, they are strong biomarker candidates. While our novel anti-motility agent, KBU2046, inhibits cell motility, its effects on β1 and β5 integrin expression and dynamics are not fully understood. We, therefore, investigated the impact of KBU2046 on β1 and β5 integrin patterns in cells and their potential as predictive biomarkers of therapeutic response.

Experimental procedures. Initial screening studies consisted of orthogonal protein expression measures, including mass spectrometry and Western blot-based analytics, in the context of time- and concentration-based treatment anti-motility therapeutics. This was then followed by immunofluorescence (IF) analysis by confocal microscopy of a panel of human prostate primary and metastatic cells, examining patterns of expression under conditions of static as well as synchronized cell attachment.

Results. Evaluation of the proteome through bioinformatic analytics identified changes in cell adhesion proteins as high-potential biomarkers. From this, we specifically investigated the dynamics of β1 and β5 integrins in response to antimotility treatment with KBU2046. In this manner, treatment induced significant alterations in cell morphology. This was associated with a dynamic, time-dependent redistribution of β1 and β5 integrins. The pattern of this change demonstrates disruption in normal integrin trafficking and supports a basis for disruption of normal integrin function. These findings provide orthogonal support for the established role of KBU2046 in inhibiting cell motility and identify β1 and β5 integrin expression patterns as high-potential biomarkers of therapeutic efficacy for anti-motility agents.

Conclusions. Our investigation reveals that treatment with KBU2046 induces significant changes in the dynamics of β1 and β5 integrins in prostate cancer cells, disrupting their normal function and trafficking. Using IF and protein expression analyses, we identified β1 and β5 integrins as potential biomarkers indicative of therapeutic efficacy in response to anti-motility treatment. These findings underscore the significance of β1 and β5 integrin expression patterns as key indicators for assessing the effectiveness of future anti-motility strategies. Further investigation of these biomarkers in clinical settings could offer valuable insights to inform therapeutic decisions in the treatment of prostate cancer.

#2365 Determining the role of pregnancy-induced IgG antibodies in parity-associated mammary oncoprotection.

Dhivyaa Anandan[1], Marygrace Trousdell[2], Amritha Varshini Hanasoge Somasundara[2], Johannes T.-H. Yeh[2], Camila O. dos Santos[2]

[1]Stony Brook University, Renaissance School of Medicine, New York, NY,[2]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

An early age of first pregnancy reduces the lifelong risk of developing breast cancer (BC) by ~30%. The dos Santos Lab has shown that pregnancy-induced alterations to the mammary immune microenvironment, including adaptive immune cells, are crucial for the oncoprotective effect of pregnancy. While plasma cells and memory B cells in the pregnant/lactating mammary gland (MG) are largely appreciated for their role in secreting antibodies into breastmilk, B cells may also encounter and secrete antibodies against self-antigens that arise during pregnancy-associated MG development and milk synthesis. Several antigens expressed in the pregnant/lactating MG are also expressed in BC. As a result, we hypothesize that pregnancy induces IgG production against antigens associated with pregnancy and mammary tumorigenesis, contributing to parity-associated mammary oncoprotection.

To obtain an unbiased profile of pregnancy-induced alterations to the mammary immune microenvironment, we performed single cell RNA-sequencing on virgin and parous non-tumor-bearing mammary tissue from a mouse model of pregnancy-induced mammary oncoprotection (Brca1 loss of function). Our analysis reveals an enrichment of plasma cells and memory B cells in the parous MG, raising the question of whether these cells and their antibody production may play a role in the inhibition of mammary tumorigenesis in parous mice in this model. To understand whether pregnancy induces alterations to the antigens recognized by B cells, we performed BCR-sequencing of MGs from virgin and parous wildtype mice. Using clonotype abundance analysis, we identified five IgG clonotypes that were most abundant and unique to the parous MGs. We used the BCR sequences of these clonotypes to synthesize five recombinant pregnancy-induced IgG antibodies. Through immunofluorescent staining analysis of mammary tissue from various stages of development and tumorigenesis, we have identified three clones that bind to pregnancy-induced mammary epithelial antigens that are also present in virgin pre-neoplastic mammary tissue. These results suggest that pregnancy may influence the BCR repertoire and enable IgG production against antigens expressed by pre-neoplastic cells in the MG. We are testing whether these recombinant pregnancy-induced IgG antibodies can be administered *in vivo* and inhibit mammary tumorigenesis in mouse models of BC.

#2366 Dynamic crosstalk between endothelial and epithelial cells drives tumor progression in lung adenocarcinoma.

Hong Chen[1], Bo Zhu[1], Junya Fujimoto[1], Yanhua Tian[1], Jian-Rong Li[2], Chenyang Li[1], Pingjun Chen[1], Alexandre Reuben[1], Monique Nilsson[1], Xiuning Le[1], Alissa Poteete[1], Shawna M. Hubert[1], Don L. Gibbons[1], Ignacio I. Wistuba[1], Jia Wu[1], Chao Cheng[2], John V. Heymach[1], Jianjun Zhang[1]

[1]UT MD Anderson Cancer Center, Houston, TX,[2]Baylor College of Medicine, Houston, TX

Background: Lung adenocarcinoma (LUAD) is a leading cause of cancer-related deaths, but the mechanisms driving its initiation and progression are not fully understood. This study investigates the interactions between epithelial and endothelial cells in LUAD and their roles in tumor progression.
Methods: Single-cell RNA sequencing and spatial transcriptomics (Visium) were employed to explore LUAD carcinogenesis in two mouse models: a genetically engineered KrasG12D mouse model (GEMM) and a urethane-induced carcinogen model (CITM), both in the 129S4 background. LUAD and its precursors (normal lung, hyperplasia, adenoma, and adenocarcinoma) were classified by pathologists. Lung tissues collected at multiple time points were analyzed to characterize epithelial-endothelial crosstalk during LUAD initiation and progression.
Results: As LUAD progressed, the proportion of epithelial cells gradually increased. In GEMM, tumor cells exhibited higher scores in KRAS signaling, hypoxia, apoptosis, and the P53 pathway compared to normal epithelial cells, indicating activation of these pathways during tumorigenesis. In endothelial cells (ECs), tip ECs were significantly upregulated during tumor progression, highly expressing Angpt2, Cxcr4, Pgf, Col4a1, Col4a2 and Insr, which are associated with EC migration, matrix remodeling, and vascular endothelial growth factor (VEGF) signaling. These cells were predominantly localized within tumor regions, as demonstrated by spatial transcriptomics, indicating their role in tumor angiogenesis. In contrast, aerocytes (aCap) decreased significantly over time, suggesting a shift from a quiescent to a more tumor-supportive phenotype. The upregulation of tip ECs and the downregulation of aCap cells suggest that tumor progression is accompanied by a dynamic reshaping of the endothelial compartment, which promotes the formation of a more permissive tumor microenvironment. We also observed enhanced crosstalk between tip ECs and epithelial tumor cells, particularly via the Col4a1-Cd44 and Mif-(Cd74+Cxcr4) ligand-receptor pairs, facilitating tumor cell adhesion, migration, and invasion. Notably, Cd44 and Mif expression in epithelial cells progressively increased, supporting further epithelial-endothelial interactions, and contributing to tumor cell survival and immune evasion. Furthermore, we observed a significant increase in BATF+ Tregs within the tumor microenvironment as LUAD progressed, indicating an evolving tumor-supportive immune environment.
Conclusions: This study highlights the critical role of tip ECs and endothelial-epithelial interactions in LUAD progression. The expansion of BATF+ Tregs underscores the complex immune interplay involved in this process. Targeting endothelial-epithelial crosstalk could provide a promising strategy to treat LUAD precancers and prevent the development of invasive disease.

#2367 Changes in breast hormone signaling and inflammation underlie radiologic features of cancer risk.

Nadine Goldhammer[1], Marin Bont[2], Michael Choi[1], Shruti Warhadpande[1], Heather Greenwood[1], Soumi Gottipati[1], Tam Binh Bui[1], Michael Bruck[1], Shoko Emily Abe[1], Michael Alvarado[1], Cheryl Ewing[1], Karen Goodwin[1], Rita Mukhtar[1], Jasmine Wong[1], Laura van't Veer[1], Nola Hylton[1], Laura J. Esserman[1], Jennifer M. Rosenbluth[1]

[1]UCSF - University of California San Francisco, San Francisco, CA,[2]University Medical Center Utrecht, Utrecht, Netherlands

Background: Image-based features of the normal breast such as mammographic density and background parenchymal enhancement (BPE) on contrast-enhanced breast magnetic resonance imaging (MRI) are independent breast cancer risk factors. Both are associated with estrogen signaling and respond to endocrine therapy. Yet, molecular and cellular mechanisms underlying these risk factors remain unclear. We aimed to investigate such mechanisms with the goal of identifying novel approaches for cancer risk stratification and personalized prevention.

Methods: Single-nucleus RNA sequencing on a 10x Chromium platform was performed on normal human breast tissues from 28 women undergoing prophylactic mastectomies (n = 11), lumpectomies (n = 10), or mastectomies for breast cancer (n = 7) who consented to our biobanking protocol. Differences in cell composition and signaling pathways between individuals with different breast densities and BPE levels were determined. Additionally we assessed estrogen responsiveness in patient-derived organoid (PDOs) from women with different levels of BPE using qRT-PCR. Evaluation of immune infiltration as potential mediator of BPE is underway using immunofluorescence and spatial transcriptomics.

Results: 12,377 nuclei from 28 women were analyzed, including 16 with low (minimal or mild) and 12 with high (moderate or marked) BPE levels on MRI, and 5 cases with not dense and 23 with dense breasts on mammography. All main breast epithelial and stromal cell types were represented in our data. We observed great heterogeneity in cell composition between patients. Samples from BPE-high patients contained luminal secretory cells with reduced expression of differentiation markers and luminal hormone sensing cells with higher expression of estrogen response genes like *PGR* and *GREB1*. In agreement with this, we observed increased response to estrogen signaling in PDOs from high (n = 5) versus low BPE cases (n = 5). Moreover, luminal cells from BPE-high samples expressed significantly higher levels of *CD74* which has been correlated with immune infiltration in cancer. Supporting this, interactions between luminal cells with macrophages via *CD74* and *APP*/*COPA* were predicted in BPE-high but not in low samples. Comparing samples with distinct mammographic densities, we observed a higher proportion of luminal secretory cells, basal cells, and fibroblasts in patients with high mammographic densities suggesting that distinct mechanisms mediate BPE levels and breast density.

Conclusions: Our findings indicate that BPE is influenced by two distinct mechanisms: estrogen-mediated changes in cellular composition and chronic inflammation, both potentially increasing breast cancer susceptibility. Understanding the basis of modifiable breast cancer risk factors will enable risk stratification and personalized treatment, ultimately reducing breast cancer incidence in high-risk populations.

#2368 The vesicle component released from renal cell carcinoma cells transforms tumor blood vessels into PSMA-positive and enhances angiogenic potential.

R. Watanabe[1], K. Kagimoto[1], M. Chosei[1], H. Arai[1], T. Sakaue[1], M. Kurata[2], N. Miura[1], R. Kitazawa[3], T. Kikugawa[1], S. Higashiyama[4], T. Saika[1];

[1]Ehime University Graduate School of Medicine, Toon, Japan, [2]Ehime University Proteo-Science Center, Toon, Japan, [3]Ehime University Hospital, Toon, Japan, [4]Osaka International Cancer Institute, Osaka, Japan

Background: Prostate-specific membrane antigen (PSMA) expression rises as prostate cancer progresses and metastasizes. Recently, 68Ga-labeled PSMA PET imaging has been utilized in prostate cancer diagnosis. Previous studies have shown that PSMA-positive vesicles secreted by prostate cancer cells increase angiogenic activity in vascular endothelial cells, suggesting a role for PSMA in tumor-related angiogenesis. This study aims to explore the relationship between PSMA expression in peritumoral vessels and patient outcomes, particularly focusing on tumor recurrence in surgically resected renal cell carcinoma samples.

Methods: Immunohistochemistry was performed on renal cell carcinoma tissue from 45 patients who underwent nephrectomy or partial nephrectomy at our institution between January 2018 and December 2022. The intensity of PSMA expression in tumor vessels was compared with recurrence rates. Furthermore, PSMA levels in human umbilical vein endothelial cells (HUVECs) treated with conditioned media (CM) from the renal cancer cell lines Caki1 and ACHN were assessed via Western blotting (WB) and immunofluorescence (IF). Angiogenic activity was measured using a tube formation assay, while RNA sequencing (RNA-seq) was employed to examine gene expression changes in endothelial cells induced by vesicles from renal cancer cells. Spatial gene expression analysis was performed to compare differences in gene expression between tumor vessels near and vessels away from the tumor.

Results: Immunohistochemical staining for PSMA and CD31 demonstrated that only the vessels surrounding the tumors were positive for PSMA. The intensity of PSMA expression was classified as weak (18 cases), moderate (11 cases), or strong (16 cases), and was found to correlate with the recurrence rates. When the 10,000 g pellet fraction of CM from the renal cancer cell lines was applied to HUVECs, the cells became PSMA-positive, and tube formation was enhanced. RNA-seq analysis revealed that HUVECs treated with CM from renal cancer cell lines showed upregulation of pathways linked to angiogenesis.

Conclusions: Vesicles secreted by renal cancer cells stimulate PSMA expression in vascular endothelial cells and promote angiogenic activity. Future studies will investigate the mechanisms leading to PSMA expression in normal vascular endothelial cells, with the goal of developing novel anti-angiogenic treatments.

#2369 Targeting peroxiredoxin 2 prevents hepatocellular carcinoma development in metabolic liver disease.

Emilie Crouchet[1], Eugenie Schaeffer[1], Frank Juhling[1], Hussein El Saghire[1], Anouk Charlot[2], Naoto Fujiwara[3], Shija Zhu[3], Fahmida Akter Rasha[3], Julien Moehlin[1], Marine A. Oudot[1], Clara Ponsolles[1], Romain Martin[1], Nicolas Brignon[1], Sarah C. Durand[1], Marie Parnot[1], Nourdine Hamdane[1], Danijela Heide[4], Jenny Hetzer[4], Mathias Heikenwalder[4], Emanuele Felli[5], Patrick Pessaux[6], Joffrey Zoll[2], Nathalie Pochet[7], Laurent Mailly[1], Yujin Hoshida[3], Thomas F. Baumert[1], Catherine Schuster[1]

[1]University of Strasbourg, Inserm, Institute for Translational Medicine and Liver Disease UMR_S1110, Strasbourg, France,[2]University of Strasbourg, Biomedicine Research Center of Strasbourg (CRBS), UR 3072 "Mitochondrie, Stress oxydant et Plasticite musculaire", Strasbourg, France,[3]Division of Digestive and Liver Diseases, Department of Internal Medicine, Liver Tumor Translational Research Program, Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center, Dallas, TX,[4]Division of Chronic Inflammation and Cancer, German Cancer Research Center, Heidelberg, Germany,[5]Hospital Group Saint Vincent, Strasbourg, France,[6]Pole des Pathologies Hepatiques et Digestives, Strasbourg University Hospitals, Strasbourg, France,[7]Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge, MA

Background: HCC is the third most common cause of cancer-related death and the leading cause of death among cirrhotic patients. While several treatments for HCC have been approved, chemopreventive strategies for HCC are lacking. Aiming to discover novel therapeutic targets, we combined genome wide transcriptomic analysis of liver tissues from patients with advanced liver disease and HCC and a cell-based system predicting liver disease progression and HCC risk. Computational analysis identified peroxiredoxin 2 (PRDX2) as a candidate driver of hepatocarcinogenesis.

Methods: The role of PRDX2 in liver disease progression and HCC development was investigated by *Prdx2* KO in a state-of-art CRIPSR/Cas9 mouse model for MASH-induced hepatocarcinogenesis. The mechanism of action was explored using RNA-Seq analyses of mouse liver tissues and validated by perturbation studies in human liver cell-based models. The translatability of our results was confirmed by targeting *Prdx2* using GalNac siRNA in a mouse model of MASH, CDX mouse model, as well as perturbation studies in patient-derived HCC tumorspheroids.

Results: In vivo perturbation studies in mouse models for MASH driven hepatocarcinogenesis showed that *Prdx2* KO improves steatosis and robuslty prevents HCC development. RNA-Seq analyses of mouse liver tissues with validation in human cells showed that loss of *Prdx2* function suppresses oncogenic signaling (i. e. p38/MAPK, PI3K/AKT signaling) and improves metabolic liver functions (i.e cholesterol, fatty acid and bile acid metabolism) through activation of the AMPK pathways. Finally, PRDX2 loss-of-function studies in hepatoma cell-based models, a CDX mouse model and patient tumorspheroids unraveled that targeting PRDX2 sensitizes cancer cell to oxidative stress and triggers apoptosis.

Conclusion: Our findings demonstrate an important functional role of PRDX2 in hepatocarcinogenesis. Targeting PRDX2 is a previously undiscovered therapeutic opportunity for prevention of MASH-induced HCC.

#2370 Microbiome alterations of colorectal cancer according to age of onset, stage, anatomic location, and mismatch repair deficiency.

Gianmarco Piccinno[1], Kelsey N. Thompson[2], Paolo Manghi[1], Luca Lazzari[3], Chiara Pozzi[4], Andrew R. Ghazi[2], Andrew M. Thomas[1], Aitor Blanco-Miguez[1], Francesco Asnicar[1], Katarina Mladenovic[1], Federica Pinto[1], Federica Armanini[1], Giulio Ferrero[5], Sonia Tarallo[6], Long H. Nguyen[7], Yan Yan[2], Mingyang Song[7], Veronika Vymetalkova[8], Mario Trompetto[9], Vaclav Liska[10], Tomas Hucl[11], Pavel Vodicka[8], Beatrix Bencsikova[12], Martina Čarnogurska[13], Vlad Popovici[13], Salvatore Siena[14], Andrea Sartore-Bianchi[14], Michela Lizier[4], Carmen Correale[4], Alessandra Raimondi[15], Filippo Pietrantonio[15], Federica Marmorino[16], Chiara Cremolini[16], Barbara Pardini[6], Francesca Cordero[17], Lisa Derosa[18], Silvia Marsoni[3], Alberto Bardelli[3], Maria Rescigno[4], Yin Cao[19], Andrew T. Chan[7], Laurence Zitvogel[18], Curtis Huttenhower[2], Alessio Naccarati[6], Eva Budinska[20], Nicola Segata[1]

[1]Department CIBIO, University of Trento, Trento, Italy,[2]Department of Biostatistics, Harvard T. H. Chan School of Public Health, Boston, MA,[3]IFOM ETS - The AIRC Institute of Molecular Oncology, Milano, Italy,[4]IRCCS Humanitas Research Hospital, Rozzano, Milano, Italy,[5]Department of Clinical and Biological Sciences, University of Torino, Torino, Italy,[6]Italian Institute for Genomic Medicine (IIGM), c/o IRCCS, Candiolo, Italy,[7]Harvard Chan Microbiome in Public Health Center, Harvard T. H. Chan School of Public Health, Boston, MA,[8]Department of Molecular Biology of Cancer, Institute of Experimental Medicine of the Czech Academy of Sciences, Prague, Czech Republic,[9]Department of Colorectal Surgery, Clinica S. Rita, Vercelli, Italy,[10]Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic,[11]Department of Hepatogastroenterology, Institute for Clinical and Experimental Medicine, Videnska, Prague, Czech Republic,[12]Masaryk Memorial Cancer Institute, Brno, Czech Republic,[13]Research Centre for Toxic Compounds in the Environment (RECETOX), Masaryk University, Kamenice, Brno, Czech Republic,[14]Dipartimento di Oncologia ed Emato-Oncologia, Universita degli Studi di Milano, Milano, Italy,[15]Medical Oncology Department, Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy,[16]Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy,[17]Department of Computer Science, University of Torino, Torino, Italy,[18]INSERM U1015, Equipe Labellisee-Ligue Nationale contre le Cancer, Gustave Roussy Cancer Campus, Universite Paris-Saclay, Villejuif, France,[19]Division of Public Health Sciences, Department of Surgery, Washington University School of Medicine, St Louis, MO,[20]Research Centre for Toxic Compounds in the Environment (RECETOX), Masaryk University, Brno, Czech Republic

Colorectal cancer (CRC) is the third most common and lethal cancer type worldwide. Although CRC incidence in the older population has decreased in many countries, early-onset CRC cases (< 50 years age, EOCRC) have been rapidly increasing globally. The fecal microbiome has been reproducibly associated with CRC. However, microbiome differences according to age of onset, stage, anatomic location, and genomic instability remain only minimally explored. Here, we integrated data generated from the ONCOBIOME consortium, Cancer Grand Challenges PROSPECT project and the ARETHUSA study, providing seven new cohorts of gut microbiome samples from healthy, adenoma- and CRC individuals (n total = 1,668) combined with 12 public studies, for a total of 3,784 metagenomic samples, including 190 EOCRC and 270 age-matched control samples. Information on tumor stage, anatomic location, microsatellite stability/instability-high (MSS/MSI-H) has been curated and harmonized across datasets. Metagenomes were analyzed using the bioBakery suite for community-, species-, strain-level, and gene-level profiling, and downstream integrative analysis was performed via meta-analysis and leave-one-dataset-out learning tasks. We obtained increased microbiome-based CRC prediction capabilities (average leave-one-dataset-out AUC = 0.84) and accurate species- and strain-level characterization of CRC stages (e.g., Slackia exigua and Ruminococcus bicirculans, respectively, between stage 0-II and III/IV), while the microbiome in adenoma samples did not differ substantially from controls (average leave-one-dataset-out AUC < 0.6). The fecal microbiome of right-sided CRC cases presented lower species and higher oral commensals richness than left-sided cases. The gut microbiome composition of MSI-H CRC was significantly different than MSS, as reflected by machine learning discrimination performances (average cross-validation AUC = 0.71), and characterized by reduced alpha-diversity and fewer biomarkers than in MSS cases, such as Ligilactobacillus salivarius and Limosilactobacillus fermentum. We confirmed that EOCRC microbiome was significantly different from age-matched controls (average leave-one-dataset-out AUC = 0.84), harboring typical CRC-associated bacteria, such as F. nucleatum and D. pneumosintes. Overall, we defined cross-cohort reproducible microbiome associations across tumor stages, primary location, genetic instability, and EOCRC, providing improved resolution within the CRC microbiome and more refined targets for diagnosis and therapeutic stratification.

#2372 Circulating pre-diagnosis immune markers and breast cancer risk: findings from the Cancer Prevention Study-3.

Shamim Shahi[1], James Hodge[1], Mia Gaudet[2], Alpa V. Patel[1], Laure Dossus[3], Sabina Rinaldi[3], Lauren Teras[1]

[1]Department of Population Science, American Cancer Society, Atlanta, GA,[2]Department of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD,[3]Hormones and Metabolism team, Nutrition and Metabolism Branch, International Agency for Research on Cancer, Lyon, France

Background: Breast cancer is recognized as potentially immunogenic with early evidence suggesting patients may benefit from immunotherapy. Cytokines including tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), and those from the interleukin (IL) family (IL-6, IL-8, and IL-10) play a critical role in immune system regulation. However, the association of pre-diagnostic cytokines levels with breast cancer risk remains unclear with few studies reporting inconsistent findings.
Objective: The goal of this study was to examine associations between circulating cytokines (IL-6, IL-8, and IL-10, TNF-α and IFN-γ) and subsequent breast cancer risk.
Methods: For this study, we utilized data from the Cancer Prevention Study 3 (CPS-3), a prospective cohort study initiated by the American Cancer Society (ACS), which fully enrolled 198,632 women aged 30-65 years from 2006-2013, across 35 states, Washington D.C., and Puerto Rico. A nested case-control design was used for this study. The analytic sample included 606 primary invasive breast cancer cases, matched to 606 controls based on age at study enrollment, race, blood draw date, and menopausal status. Blood collected at CPS-3 enrollment was used to quantify five immune markers of inflammation and immune regulation: IL-6, IL-8, IL-10, IFN-γ, and TNF-α using the V-PLEX multiplex immunoassay at the International Agency for Research on Cancer (IARC). Each immune marker was analyzed both as a continuous and a categorical (quartiles) variable. Conditional logistic regression was used to estimate odds ratios for the association between each immune marker and overall breast cancer risk.
Results: The median age at diagnosis was 54 years (interquartile range (IQR): 48,59). Cases and controls were fairly comparable across key demographic, clinical and behavioral variables. For every one-unit increase in log-transformed IFN-γ, the odds of breast cancer decreased by 16% (OR=0.84, 95% CI: 0.72-0.99). All other immune markers were not significantly associated with overall breast cancer risk.
Conclusion: With the exception of IFN-γ, the findings do not provide evidence that these selected immune markers are associated with breast cancer risk. Given the heterogenous nature of breast cancer, future studies should consider breast cancer molecular and histologic subtypes and potential modifications by other clinical and tumor characteristics.

#2373 Colonic polyp preventive effect of rLON protease in FAP-rat intestinal polyposis model.

Venkateshwar Madka[1], Karthikkumar Venkatachalam[1], Gopal Pathuri[1], Nicole Stratton[1], Anil Singh[1], Shizuko Sei[2], Mark S. Miller[2], Vignesh Gunasekharan[2], Chinthalapally V. Rao[1]

[1]Center for Cancer Prevention and Drug Development, Department of Medicine, OU Health Stephenson Cancer Center, Oklahoma City, OK,[2]Division of Cancer Prevention, National Cancer Institute, Rockville, MD

Hereditary Familial Adenomatous Polyposis (FAP) patients develop large number of colonic polyps that can progress to aggressive carcinomas. In the FAP patients, Apc or β-catenin mutations lead to c-Myc overexpression, which in turn drives the metabolic and immune alterations leading to colorectal cancer, making it a potential target for CRC prevention. Unfortunately, finding c-Myc inhibitors has been difficult and only recently studies show that uropathogenic *E. Coli* rLON protease (rLONP) can degrade the c-Myc in tumor tissues. In this study, PIRC rats (10- or 12- weeks age) were treated with rLONP (0.375mg/rat/day dose) in PBS by oral gavage twice daily for either 2- or 4- weeks and assessed for toxicity and intestinal tumor burden. Organ morphology and weight were not significantly different from control PIRC rats. Also, hematological profile was very similar in control and rLONP treated male rats, however in female PIRC rats, rLONP treatment for 4 weeks showed significant differences in WBC, neutrophils, and lymphocytes count compared to control. Serum analysis indicated a non-significant ($p>0.05$) increase in AST (297.6±74.3 u/L) and ALT (365.3±86.8 u/L) levels with 4-weeks rLONP treatment when compared to control group (133.9±27.5 u/L & 184.5±30.3 u/L respectively). ALKP levels were slightly lower (187.6±14.4 u/L) in rLONP treated rats compared to control (221.3±16.7 u/L). BUN (17.6±0.7 mg/dL) and CREA (0.15±0.03 mg/dL) of the rLONP treated rats were non-significantly different from control group (16.6±1.1 mg/dL & 0.15±0.02 mg/dL respectively). Histopathology of all major organs of rLONP treatment appeared normal, with no significant differences as compared to untreated groups. Colonic and small intestinal polyps were compared between control and rLONP treated rats. Average colonic polyps were less in rLONP treated male rats at both 2- and 4- week endpoints (40% reduction) compared to their respective control rats while this effect was not distinguishable in the females due to low polyp number and slow rate of polyp and tumor development. Small intestinal polyps also appeared to be less in number in rLONP treated male and female rats as compared to control. Total intestinal polyps showed ≥50% reduction of multiplicity, male ($p<0.012$; $p<0.009$) and female ($p=0.06$; $p<0.022$) with 2- and 4- week rLONP treatment, respectively, as compared to control group. Protein expression analysis of control and treated colonic polyp tissues suggested reduction of c-MYC expression with rLONP treatment. Also, IHC analysis showed reduction in Ki-67, Cyclin D1, and c-Myc expression in the rLONP treated rat colonic polyps when compared with control polyps. Based on these observations, it is concluded that rLONP treatment did not cause any overt-toxicities and reduced intestinal polyps in PIRC rats in a short-term study. (*Project funded in whole with Federal funds from the NCI, NIH, DHHS, under Contract 75N91019D00020_75N91022F00001*).

#2374 Identifying ZNF469 as a biomarker in cancer of the colon and stomach.

Aaron Shaykevich[1], Isaac Silverman[2], Radhashree Maitra[2]

[1]Public Health and Preventative Medicine, SUNY Upstate Medical University, Syracuse, NY,[2]Biology, Yeshiva University, New York, NY

Introduction: Zinc Finger Protein 469 (ZNF469) is a protein involved in the formation of the extracellular matrix. While the non-coding RNA linc-ZNF469-3 has previously been identified to be overexpressed in certain cancers and promote metastasis, there have yet to be any studies attempting to establish a link between the protein ZNF469 and cancer formation or growth. In this study, we attempt to determine whether ZNF469 may act as a biomarker in colon and stomach cancers.
Methods: A systems biology approach was used to explore the impact of varying ZNF469 expression and mutations. Patient data was retrieved from the TCGA database. TIMER2.0 software was utilized for statistical analysis along with visualization.
Results: The expression of ZNF469 was found to increase in cancer compared to non-tumor control in the colon ($p = 2.4e-11$) and stomach ($p = 2.2e-17$). Furthermore, Kaplan-Meier plots indicate that higher expression of ZNF469 resulted in worse probability of survival in colon (HR = 1.70, CI = 1.35-2.15) and stomach (HR =1.68, CI = 1.34-2.10). We also found an association between immune infiltration and ZNF469. In colon cancer, ZNF469 expression moderately correlated with the immune infiltration of macrophages (partial correlation = 0.50, $p = 6.2e-27$), neutrophils (partial correlation = 0.57, $p = 3.9e-35$), and dendritic cells (partial correlation = 0.56, $p = 3.9e-34$). In stomach cancer, these correlations were present but weaker in macrophages (partial correlation = 0.23, $p = 6.5e-6$), neutrophils (partial correlation = 0.25, $p = 8.5e-7$), and dendritic cells (partial correlation = 0.29, $p = 1.6e-8$). In the colon, arm level gain mutations of ZNF469 were found to correlate with altered immune infiltration in CD8+ T cells ($p < 0.001$), neutrophils ($p < 0.001$), and dendritic cells ($p < 0.01$) with a few additional mutations found to be significant as well. Conversely, in the stomach, arm level deletions were found to correlate with altered immune infiltration in B cells ($p < 0.01$), CD8+ T cells ($p < 0.001$), neutrophils ($p < 0.05$), and dendritic cells ($p < 0.001$) with additional mutations found to be significant.
Conclusion: The increased expression of ZNF469 in stomach and colon cancer suggests that it may facilitate in oncogenesis and may be induced by rapid tumor growth. The expression correlating with worse outcomes further supports ZNF469 as a pro-oncogenic protein in these cancers. High ZNF469 levels were also correlated with higher immune infiltration, especially in colon cancer, and arm level gain or arm level deletion mutations were associated with altered immune infiltration. Overall, the findings suggest that ZNF469 can serve as a biomarker in colon and stomach cancers. ZNF469 is upregulated in these cancers, and the increase of ZNF469 correlates with both worse outcomes as well as increased immune infiltration. This data suggests that further research into ZNF469 may be beneficial in stomach and colon cancers.

#2375 Investigating airway epithelial TP53 somatic mutation prevalence as a common risk factor for COPD and lung cancer.

Andrew Boring[1], Erin L. Crawford[1], Dan J. Craig[1], Heidi Chen[2], Rami Ahmad[1], Mohamed Omballi[1], Eric Grogan[2], Stephen Deppen[2], James C. Willey[1]

[1]University of Toledo College of Medicine and Life Sciences, Toledo, OH,[2]Vanderbilt University Medical Center, Nashville, TN

Background: Lung cancer (LC) and Chronic Obstructive Pulmonary Disease (COPD) are leading causes of morbidity and mortality both in the United States and worldwide. There is a high degree of association between COPD and LC, which is not well understood. Smoking is a risk factor for COPD, while both cigarette smoke and COPD are risk factors for LC. Approximately 10% of current smokers are at risk for developing COPD and every year an estimated 1% of COPD patients develop LC. We recently reported that in the airway epithelium of smokers, there is an increased prevalence of somatic TP53 mutations. Further, among heavy smokers TP53 mutation prevalence was associated with LC. Due to the known association between COPD and LC risk we investigated whether TP53 mutation prevalence in airway epithelium may be a common factor.

Methods: Patients undergoing bronchoscopy were recruited into an IRB-approved research study wherein soft brushings of the large airways were collected. Bronchial brush specimens from 59 subjects were sequenced by PCR-amplicon library NGS. The TP53 biomarker was measured from 59 subjects, 30 LC and 29 non-LC. The non-LC group comprised 7 COPD and 22 non-COPD subjects. Using specialized internal standards to compensate for sequencing error, VAF was observable down to 0.01%.

Results: The TP53 biomarker was associated ($p<0.05$) with COPD among all 59 subjects with 76% specificity, 62% sensitivity, positive predictive value (PPV) 59% and negative predictive value (NPV) 78%. When plotted on a receiver operator characteristic (ROC) curve the area under the curve (AUC) was 0.82. Notably, the TP53 biomarker was significantly associated with COPD among non-LC subjects, with 95% specificity, 57% sensitivity, PPV 80% and NPV 87.5%. When fit to an ROC curve, the AUC for the COPD cohort was 0.84

Conclusions: TP53 prevalence was associated with COPD diagnosis among both LC and non-LC patients in this small discovery set. We plan to validate the association in larger case-control studies. If validated, this biomarker may enable early detection of those at risk for COPD before the obstructive defect develops as well as those who should be prioritized for LC screening. We will also further investigate interindividual variation between which patients develop lung cancer and or without the background of COPD through the lens of the TP53 biomarker.

#2376 Transitional phenotype of epithelial progenitor cells in human lung adenocarcinoma premalignancy.

Khosbayar Lkhagvadorj[1], Bridget K Greuel[1], Alexis S Assante[1], Amy B Frey[2], Simran Randhawa[2], Adrie VanBokheven[3], York E Miller[1], Moumita Ghosh[1]

[1]Pulmonary Sciences and Critical Care Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO,[2]Rocky Mountain Regional Veteran Affairs Medical Center, Aurora, CO,[3]Cancer Center Biorepository, Department of Pathology, University of Colorado Anschutz Medical Campus, Aurora, CO

Introduction: Lung adenocarcinoma (ADC) is the most common subtype of non-small cell lung cancer with poor 5-year survival. Interventions during the premalignant stage of carcinogenesis can improve outcomes. Epithelial progenitors are critical cells that maintain and repair the epithelium due to their proliferation and multipotent differentiation. Previously, we discovered diminished function of progenitor cells in the premalignant lung containing ADC. In the current study, we tested whether progenitor dysfunction in premalignancy involves changes in progenitor proliferation and/or differentiation.
Methods: Lung tissues resected for early-stage ADC (N=14) were used, and progenitor function was determined using a 3D organoid culture. Lung organoids were grown from the uninvolved (UN, 5-15 cm) and tumor-adjacent lungs (TA, 0.2-2 cm) from the tumor. We hypothesized that processes active in these spatially distinct areas from the tumor-bearing lungs represent oncogenic changes in premalignancy. Organoids were cultured under either proliferation or differentiation conditions, and molecular profiles of organoids were examined by single-cell RNA-sequencing (ScRNA-seq).
Results: Analysis of proliferation and differentiation showed an opposing outcome for the UN and TA tissues. Under proliferation conditions, greater numbers of organoids were made by the cells from TA, while under differentiation, UN areas gave rise to higher numbers of organoids (p<0.05 both). Furthermore, proliferative organoids from UN tissues expressed higher numbers of $KRT5^+/P63^+$ basal stem cells compared to organoids from TA, while higher numbers of $Ki67^+$ proliferative cells were present in TA organoids (p<0.05 both). In contrast, there was no difference in epithelial cell types present in organoids grown from UN and TA tissues under differentiation conditions. ScRNA-seq comparing transcriptomic profiles of organoids from TA and UN tissues showed that under proliferation, TA organoids contained a specific cluster of basal cells that was enriched for genes belonging to EGFR signaling with higher expression of amphiregulin (AREG), a canonical ligand for EGFR. Immunostaining of organoids from TA and UN tissues confirmed significantly higher numbers of $AREG^+$ cells in TA organoids. The addition of exogenous AREG in proliferative media with or without EGFR inhibitor showed that AREG can drive progenitor cell proliferation independent of EGFR.
Conclusions: Our results suggest that progenitor dysfunction in ADC premalignancy involves increased proliferation and decreased differentiation. AREG has been described as a marker of transitional cells involved in cancer development. Thus, our findings indicate that progenitors within the TA tissue express transitional cell phenotype. Future analysis will focus on the AREG-mediated signaling in progenitor cell proliferation during premalignancy.

#2377 Gut microbiome modulation by a black raspberry nectar for improving anti-PD1 therapy effectiveness in pre-clinical cancer models.

Shiva Jahanbakhshi[1], Amna Bibi[2], Rebecca Hoyd[2], Caroline Dravillas[2], Nyelia Williams[2], Shiqi Zhang[3], Aaditya Pallerla[2], Shankar Suman[2], Joseph Amann[2], Mounika Goruganthu[2], Tamio Okimoto[2], Yangyang Liu[4], Marisa A. Bittoni[2], Ni Shi[2], Bailey Conrad[5], Lane Nevers[4], Kristen Heitman[6], Maxine Webb[2], Elizabeth M. Grainger[2], Madison Grogan[2], Christian Quiles[7], Tong Chen[2], Carolyn Presley[2], Lang Li[8], Patrick Bradley[7], Yael Vodovotz[1], David P. Carbone[2], Steve K. Clinton[2], Jiangjiang Zhu[5], Daniel Spakowicz[2]

[1]Department of Food Science and Technology, College of Food, Agriculture, and Environmental Sciences, The Ohio State University, Columbus, OH,[2]Division of Medical Oncology, Department of Internal Medicine, The Ohio State University Comprehensive Cancer Center, Columbus, OH,[3]Department of Human Sciences and Comprehensive Cancer Center, The Ohio State University College of Medicine, Columbus, OH,[4]Pelotonia Institute for Immuno-Oncology, The Ohio State University Comprehensive Cancer Center, Columbus, OH,[5]The Ohio State University College of Medicine, Columbus, OH,[6]Department of Health Sciences, The Ohio State University College of Medicine, Columbus, OH,[7]Department of Microbiology, The Ohio State University, Columbus, OH,[8]Department of Biomedical Informatics, The Ohio State University College of Medicine, Columbus, OH

Immune checkpoint inhibitors (ICIs) have improved cancer survival rates, but only a small percentage of patients experience significant and sustained tumor responses. Recent evidence points to the gut microbiome as a modifiable factor that may improve the efficacy of ICIs. This study utilized a human clinical trial and an experimental model to assess whether a dietary intervention could enhance ICI therapeutic outcomes. Specifically, we investigated the effects of black raspberry nectar (BRB) consumption on the microbiome and subsequently in murine models of anti-PD1 cancer therapy. The BEWELL clinical trial was a randomized, placebo-controlled, cross-over dietary intervention trial where 40 individuals received 80 mL of BRB for 4 weeks or placebo for 4 weeks. The BRB altered the participants' microbiomes, significantly enriching microbes in the family *Lachnospiraceae*. Mice were gavaged with microbiomes from human participants who exhibited significant *Lachnospiraceae* enrichment post-BRB dietary intervention showed substantial reductions in tumor volume following anti-PD1 therapy. These results suggest that *Lachnospiraceae* may be key in enhancing ICI response. To further isolate the impact of specific microbes, we focused on *Blautia*, a representative *Lachnospiraceae* genus, to determine whether its presence could individually improve ICI therapeutic outcomes. Mice gavaged with *Blautia*-enriched microbiomes and provided anti-PD1 therapy demonstrated significantly lower tumor growth rates (p-value = 0.012) and a higher frequency of tumor-free status than controls (78% vs60%). A metabolomic analysis revealed distinct metabolic changes, with L-carnitine levels elevated in mice showing smaller tumors (p-value = 0.013). No significant changes were observed in butyrate, indole-derivatives, or inosine, which were previously shown to alter ICI response, suggesting a novel mechanism. Our findings underscore the potential of *Lachnospiraceae*, particularly *Blautia*, in improving cancer treatment outcomes. By extending human dietary modifications into preclinical models, this study reveals a promising strategy for using microbiome composition as a tool to enhance ICIefficacy.

#2378 Developing new potent therapeutic strategies for neuroendocrine carcinomas.

Tanya Ivanova Stoyanova

UCLA - University of California Los Angeles, Los Angeles, CA

Neuroendocrine carcinomas, such as neuroendocrine prostate cancer (NEPC), small cell lung cancer (SCLC), and neuroblastoma, share common histological features, including expression of neuroendocrine markers, rapid relapse after treatment, and poor prognosis. Due to the poor clinical outcome of patients with neuroendocrine carcinomas, there is a critical need to identify new drivers, therapeutic targets, and effective therapeutic strategies for these malignancies. Ubiquitin carboxy-terminal hydrolase L1 (UCHL1) is a protease that has a dual function in regulating protein stability. We demonstrated that UCHL1 is significantly elevated in tissues from patients with NEPC, SCLC, and neuroblastoma. Loss of UCHL1 decreases tumor growth and inhibits metastasis of these malignancies. UCHL1 maintains neuroendocrine differentiation and promotes cancer progression by regulating nucleoporin, POM121, and p53. UCHL1 stabilizes POM121 to regulate POM121-associated nuclear transport of key transcriptional factors, E2F1 and c-MYC. Here, we developed novel and more potent UCHL1 small molecule inhibitors as a new therapeutic strategy to target neuroendocrine carcinomas. Our new compounds demonstrate high potency with safety profile in vivo for neuroendocrine carcinomas. Importantly, treatment with UCHL1 inhibitors significantly reduces tumor growth of neuroendocrine carcinomas in pre-clinical settings. Our study reveals new mechanisms of UCHL1 function in neuroendocrine carcinomas tumorigenesis, identifies UCHL1 as a therapeutic target and potential molecular indicator for diagnosis and monitoring treatment responses in neuroendocrine carcinomas and develops new inhibitors to target UCHL1.

#2379 AptBCis2 plus pemetrexed for lung cancer leptomeningeal carcinomatosis and cancer-associated cachexia.

Emily Pei-Ying Lin, Bo-Tsang Huang

Medicine, Taipei Medical University, Taipei, Taiwan

Introduction: Cancer-associated cachexia (CAC) is a multifactorial syndrome characterized by anorexia and body weight loss. CAC is associated with poor survival outcomes and is common in patients with advanced lung cancer, such as leptomeningeal carcinomatosis (LM). Treatment for lung cancer CAC and LM is challenging, and remains an unmet clinical need worthy of investigation. Aptamers are small synthetic oligonucleotides considered as antibody surrogates for targeted delivery. Here we report a novel aptamer-cisplatin conjugate, AptBCis2, which suppressed tumor progression and restored body weights in the mouse LM models. The effects were even more pronounced when combination regimens with AptBCis2 plus pemetrexed were used.

Methods: The aptamer was identified via *in vivo* SELEX using a lung cancer LM orthotopic mouse model. The aptamer was conjugated with cisplatin to form AptBCis2. The tumor suppressive effects were investigated using mouse models. The AptBCis2 was administered through the tail vein; pemetrexed was injected intraperitoneally, and tumor burden was monitored *via* In Vivo Imaging System. The plasma and the CSF cisplatin concentrations were measured via Inductively Coupled Plasma Mass Spectrometry.

Results: AptBCis2 1 mg/kg (equivalent to cisplatin 0.35 mg/kg) alone or in combination with pemetrexed 100 mg/kg both showed superior tumor suppressive effects than cisplatin 2 mg/kg or pemetrexed 100 mg/kg monotherapy, as well as cisplatin 2 mg/kg plus pemetrexed 100 mg/kg combination therapy in lung cancer LM orthotopic mouse models. The treatments were given on day 2, 3, 5, 7, and 9 post tumor inoculation. On day 14 post tumor inoculation, percent of body weight change was about negative 20% in the cisplatin or cisplatin plus pemetrexed groups, positive 3% in the AptBCis2 group, and positive 20% in the AptBCis2 plus pemetrexed group (n = 8 in each group). The mice were sacrificed at day 14 for biochemical and image studies. Early mortality was observed on day 7 in the pemetrexed group with a negative body weight change of 20%.

Conclusion: AptBCis2 in combination with pemetrexed showed not only tumor suppressive effects in lung cancer LM orthotopic mouse models but also restored mouse body weights during the treatment course. The data suggested potential roles of AptBCis2 plus pemetrexed in lung cancer LM and CAC.

BIOINFORMATICS / COMPUTATIONAL BIOLOGY / SYSTEMS BIOLOGY / CONVERGENT SCIENCE: Application of Bioinformatics to Cancer Biology
Poster Session

#2384 Improved non-invasive gene expression inference using advanced machine learning.

Jurik A. Mutter[1], **Amirsaman Ashtari**[2], Nick Phillips[1], Benoit Tessoulin[3], Jan Boegeholz[4], Cedric Rossi[5], Steven Le Gouill[6], Maximilian Diehn[7], Mohammad Shahrokh Esfahani[2], Ash A. Alizadeh[8]

[1]Division of Oncology, Stanford University, Stanford, CA,[2]Division Oncology, Department of Radiation Oncology, Stanford University, Stanford, CA,[3]Department of Hematology, University Hosptial Nantes, Nantes, France,[4]Department of Medical Oncology and Hematology, University Hospital Zurich, Zurich, Switzerland,[5]Department of Hematology, Central Hospital University (CHU) Dijon Bourgogne, Dijon, France,[6]Hematology Department, Institut Curie, Paris, France,[7]Department of Radiation Oncology, Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA,[8]Division Oncology, Stanford Cancer Institute, Division of Hematology, Stanford University, Stanford, CA

Background: Gene expression profiling is crucial for understanding cancer dynamics and development, aiding in the identification of molecular subtypes, predicting patient outcomes, and discovering therapeutic biomarkers. Traditional profiling methods rely on invasive tissue specimens analyzed through RNA-seq or microarrays. These approaches can delay diagnosis due to scheduling challenges, pose risks of complications during and after surgery, and may be inaccessible to high-risk or frail patients. Recently, non-invasive gene expression inference from blood plasma has emerged as a promising alternative that analyzes fragmentation patterns of cell-free DNA molecules to infer gene expression (EPIC-Seq, Esfahani, Nat Biotechnol 2022). Further, we demonstrated that EPIC-Seq data can be effectively applied to lymphoma signatures derived from RNA-seq, yielding robust results (Mutter, ASH 2023). However, despite these advances, EPIC-Seq results cannot be directly compared to RNA-seq profiles on a single gene level. To address this challenge, we built a machine learning model that maps EPIC-Seq data into an RNA-seq-compatible format.
Methods: We developed an advanced, generative AI framework to learn mappings between non-invasive EPIC-Seq from blood plasma and tumor RNA-seq from invasive tissue biopsies. The model leverages advanced AI techniques to identify patterns in the EPIC-Seq domain and translate them into equivalent representations in the tumor RNA-seq domain and is designed handle complex, high-dimensional data while preserving key biological and functional features. For this, we sequenced a total of 304 formalin-fixed paraffin embedded (FFPE) and blood plasma samples from 227 diffuse large B-cell lymphoma (DLBCL) (n=110) and mantle-cell lymphoma (MCL) (n=117) patients using both paired (DLBCL: n=33; MCL: n=44) and unpaired data (DLBCL: n=52 FFPE, 25 plasma; MCL: n=56 FFPE, 17 plasma).
Results: Using a combination of paired and unpaired data we were able to robustly predict gene expression in RNA-seq space from non-invasive EPIC-Seq data. By splitting the cohorts in 80% training and 20% validation (n=88 patients, n=115 samples), we observed a high concordance of predicted gene expression and RNA-seq data in DLBCL samples (R=0.91). Similar accuracy was achieved in MCL (n=93 patients, n=129 samples) further confirming the model performance (R=0.94). In addition, applying the model trained on DLBCL data yielded high concordances in MCL data (R=0.68), emphasizing the robustness and applicability of the model to different lymphoma subtypes. Consistently, the model trained on MCL data achieved high concordance on DLBCL data (R=0.65).
Conclusion: These findings suggest that EPIC-Seq can serve as a reliable surrogate for RNA-seq data, offering comparable gene expression values for enhanced diagnosis and personalized treatment strategies in lymphoma patients.

#2385 Discovery of non-coding somatic driver mutations in single-cell chromatin accessibility data.

Song Cao[1], Liting Zhang[1], Alla Karpova[1], Fernanda M. Rodrigues[1], Yingduo Song[1], Nadezhda V. Terekhanova[1], Benjamin J. Raphael[2], Ryan C. Fields[1], Feng Chen[1], Li Ding[1]

[1]Washington University in St. Louis, St. Louis, MO,[2]Princeton University, Princeton, NJ

scATAC-seq data presents unprecedented opportunities to identify somatic mutations in regulatory regions. However, existing tools struggle to reliably detect somatic mutations from scATAC-seq data due to the unbalanced coverage between tumor and normal cells. To address this, we developed scVar, a pipeline designed to detect somatic mutations from scATAC-seq data. Applying scVar to snATAC-Seq data from about 200 tumor samples spanning 10 cancer types, we identified over 70,000 somatic mutations, with 95% located in non-coding regions. Comparison with bulk WGS and WXS data demonstrated that scVar achieves high accuracy in detecting somatic mutations. Notably, scVar effectively recaptured the UV mutational signature in skin cancer. Variant allele frequency (VAF) analysis revealed an increase in VAF in snATAC-seq data compared to WXS data. For tumor suppressor genes such as TP53, VHL, BAP1, CDH1, CBFB, TGIF1, and ZNF750, homozygous mutations with VAFs approaching 1 were consistently detected in snATAC-seq data, emphasizing the critical role of biallelic variants in tumor initiation. We also observed recurrent TERT promoter mutations across 20 samples, with VAFs close to 1, which were associated with increased chromatin accessibility and expression in glioblastoma, but not in skin cancer. Additionally, novel promoter mutations, such as in CDK12, were linked to increased gene expression. These findings highlight the utility of scVar in uncovering somatic mutations and their functional effects in non-coding regions from scATAC-seq data.

#2386 Preclinical model atlas to enable clinical translatability for precision oncology.

Anlu Chen[1], Thanh Bui[1], Weilong Zhao[1], Shaobo Qin[1], Vivian Prindle[2], Kimberly R. Sher[2], Adam Richardson[2], Joshua P. Plotnik[2], Aloma L. D'Souza[1], Marybeth A. Pysz[1], Josue A. Samayoa[1], Sarah Kongpachith[1], Xi Zhao[1]

[1]AbbVie Inc., South San Francisco, CA,[2]AbbVie Inc., North Chicago, IL

Preclinical models, such as 2D cultures, 3D organoids, and patient-derived xenograft (PDX) models, play a crucial role in drug development pipelines. However, their respective suitability for various research questions remains unclear. For example, RSL3-induced ferroptosis in 2D models is abolished in 3D models and *in vivo*. Similarly, some compounds targeting KRAS pathways are potent in 2D but not effective *in vivo*. Comprehensive understanding of the strengths and limitations of these models is paramount for drawing meaningful scientific conclusions. Identifying the models best suited to represent patient populations and forecast treatment response with precision is essential in enabling reverse translational research for hypothesis testing and target validation. Building upon our previous work which focused on 133 3D culture models from DEPMAP, we have expanded our efforts to encompass a larger, more comprehensive array of preclinical models. This includes DEPMAP models (207 3D and 2099 2D), 48 paired 2D-3D organoids from AbbVie, as well as around 800 internally generated and 4582 externally acquired PDX models, giving a grand total of roughly 7800 preclinical models with genomics and transcriptomics data. We employed CellTindeRX, a model-free and likelihood-based tool, to enable precision oncology by forecasting effective treatments for individuals. Briefly, we identify the tumor model most representative of a given human sample by their transcriptomics similarity, then we use the matching tumor model to further link the human sample to corresponding drug sensitivity measurements. Our approach preserves the transcriptomic signals from immune and stroma components of patient tumors, so that model comparisons consider both intrinsic and extrinsic aspects of the diseases. In general, we observe agreement for most human cancer types in TCGA between pre-clinical models and their corresponding patient diseases; however, some indications, such as stomach adenocarcinoma (STAD), show poor agreement, underscoring the biological gaps between preclinical models and patient samples. We further align preclinical models to patient samples from real world data and clinical trials to demonstrate a synthetic drug screen framework where we forecast patients' treatment response using PRISM drug sensitivity profiles from matched cell lines. Using this screening platform, TOP1i is identified as the top efficacious drug class for small cell lung cancer (SCLC). This observation is aligned with clinical treatment guidelines for this disease.These findings offer valuable guidance for selecting preclinical models that faithfully represent the patient population for reverse translational efforts, such as compound efficacy testing or MOA/target validation, thereby enhancing the accuracy and relevance of our drug development efforts.

#2387 Characterizing HERV expression patterns across breast cancer subtypes: Implications for tumor biology and therapeutic targets.

Nathalia Da Roz D'Alessandre[1], Gabriela Der Agopian Guardia[2], Helena Beatriz da Conceicao[2], Rafael Loch Batista[3], Pedro Alexandre Favoretto Galante[2]

[1]Hospital Sírio-Libanês, Sao Paulo, Brazil,[2]Hospital Sirio-Libanes, Sao Paulo, Brazil,[3]University of Sao Paulo, Sao Paulo, Brazil

Endogenous Retroviruses (ERVs) are remnants of ancient retroviral infections that have permanently integrated into host genomes, including humans. Human Endogenous Retroviruses (HERVs) are ‘fossil’ viral sequences, having lost their ability to generate infectious viruses due to mutations and deletions accumulated over millions of years. Although most HERVs are deemed non-functional, they constitute up to 8% of the human genome. Some HERVs have roles in gene expression regulation, and others are transcribed under specific conditions, such as in cancer. HERV-K is the most recently integrated class within our genome, with some members retaining relatively intact open reading frames (ORFs), suggesting their potential to encode proteins. In cancer, reactivation of HERV expression may impact gene expression, genomic stability, and cell signaling pathways.In this study, we investigated the expression of HERVs in breast cancer (BC) and non-tumor breast tissue using RNA sequencing (RNA-Seq) data from The Cancer Genome Atlas (TCGA). Specifically, we analyzed the expression profiles of HERVs across BC subgroups and their association with protein-coding gene expression profiles. We identified differentially expressed HERVs in BC subgroups (HR-/HER2-, HR+/HER2+, HR-/HER2+, HR+/HER2-) in comparison to non-tumor samples. Our analysis revealed 98 differentially expressed HERVs in HR-/HER2-, 51 in HR+/HER2+, 81 in HR-/HER2+, and 48 in HR+/HER2-. Distinct expression profiles across all subtypes were evident. Furthermore, we identified 45 differentially expressed protein-coding genes with transcription start sites within 5 kb of differentially expressed HERVs. This proximity suggests a potential regulatory influence of HERVs on these genes, indicating the need for further research to clarify the underlying mechanisms. In conclusion, the unique HERV expression profiles across BC subtypes suggest specific molecular characteristics and gene regulation patterns. These findings may aid in identifying new therapeutic targets and treatment strategies for breast cancer, emphasizing HERVs' importance in tumor biology.

#2388 Enhancing clonal hematopoiesis variant detection in tumor-normal matched sequencing using machine learning.

Anne Sonnenschein, Timothy Baker, Singer Ma, Christine Lo, Robert Tell, Brett Mahon, Nirali M. Patel, Jerod Parsons

Tempus AI, Chicago, IL

Introduction: The xT tumor-normal matched assay, which sequences solid tumor biopsy paired with matched buffy coat, has enabled the accumulation of large amounts of clonal hematopoiesis (CH) data. Although buffy-coat matched sequencing is the gold standard for distinguishing tumor from non-tumor variants, accurately identifying CH variants and distinguishing them from germline or artifactual variants presents unique algorithmic challenges. The buffy coat is sequenced at lower depth than the tumor, potentially impacting the accuracy of variant calling at low variant allele fractions (VAFs). For CH variants with high VAFs, which are the most clinically relevant, distinguishing germline variants is computationally challenging. Due to immune infiltration, CH may be found in both the normal and tumor samples, and copy number variants and loss of heterozygosity in tumor samples can substantially bias VAFs. Here, we demonstrate methods for identifying CH with high accuracy accounting for these challenges.
Methods: We developed an algorithm that calculates the expected VAF for a heterozygous germline variant in a tumor sample by integrating copy number variations, the balance between major and minor alleles, and tumor purity. This algorithm was incorporated into a machine learning model trained on common germline variants and canonical CH mutations sourced from xT data. We validated the accuracy of the xT CH pipeline against higher depth sequencing in xF+ liquid biopsy using 3,000 unique samples.
Results: Our algorithm substantially improves the separation between CH and germline variants. In a representative distribution of variants with VAFs over 20% held out for testing, sensitivity for CH variants improved from 77% to 91%, precision from 80% to 97%, and specificity from 99.5% to 99.9% (ROC-AUC of 0.995) compared to a heuristic logic that does not incorporate copy number. For canonical CHIP variants detected in xF+ with a VAF above 5%, 92% are detected as CH in xT. Variants identified as CH in xT with VAF above 2% are validated as true CH variants by xF+ with 90% precision. CH variants detected in both plasma and buffy coat have highly correlated VAFs (R^2=0.79, m=0.94), and 3.6% of the samples assessed contain a high VAF (>=20%) CH variant.
Conclusions: We demonstrate that the xT platform can distinguish between CH, germline, and artifactual variants with high sensitivity and a low false positive rate. Although CH at high VAF is present in only a minority of samples (3.6%), it is regularly observed in high throughput clinical sequencing. These variants are likely to be clinically significant and may represent secondary hematologic malignancies, which are more common in cancer patients than in healthy populations. Therefore, accuracy-driven approaches for identifying CH in buffy coat matched data are important for improving clinical outcomes and understanding the underlying biology of these variants.

#2389 The role of pyroptosis in the tumor microenvironment of head and neck squamous cell carcinoma (HNSCC).

Hani Samarah[1], Julian Jackson[1], Hannah L. Kenny[1], Zachary Kaplan[2], Parvesh Kumar[2], Zhao Lin[3], Adam Luginbuhl[1], Eric Mastrolonardo[1], Alban Linnenbach[4], Kelly Bridgham[1], Teresa Alnemri[5], Joseph Curry[1]

[1]Department of Otolaryngology, Thomas Jefferson University, Philadelphia, PA,[2]Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA,[3]Medical Oncology, Thomas Jefferson University, Philadelphia, PA,[4]Sidney Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA,[5]Biochemistry & Molecular Biology, Thomas Jefferson University, Philadelphia, PA

Background: HNSCC exhibits diverse tumor microenvironments (TME), influencing progression and therapeutic responses. Pyroptosis, a form of inflammatory programmed cell death may be associated with TME's and anti-tumor immunity. This study investigates pyroptosis-related gene expression in HNSCC TMEs, classified into immune-enriched (IE), immune-enriched fibrotic (IE/F), immune-depleted (ID), and fibrotic (F).

Methods: RNA-seq data from TCGA HNSCC cohort were stratified into two TME subtypes. Tumors were grouped into IE (IE & IE/F) and ID (ID & F) for comparative analysis. Pyroptosis-related genes were curated from 838 gene sets using the MSigDB and validated through STRING protein-protein interaction analysis. Pathway enrichment was evaluated via supervised and unsupervised analyses using Gene Set Enrichment Analysis (GSEA) and Gene Set Variation Analysis (GSVA). Key contributors were identified through leading-edge analysis, with survival associations assessed using Cox proportional hazards modeling. Preliminary analysis using EcoTyper and CIBERSORTx has identified cell states and cancer ecotypes associated with TME.

Results: Unsupervised GSEA identified 31 enriched pathways in HPV-negative samples (FDR q-value <0.05), including Regulation of Cell Killing, Interferon Responsive Genes, and Pyroptosis. Leading-edge analysis revealed overlapping immune-regulatory pathways, such as Positive Regulation of Lymphocyte Apoptotic Process and Role of Phospholipids in Phagocytosis, with shared signals from genes including CD2, PDCD1, and TIGIT. Reactome Pyroptosis (FDR=2.04e-25) highlighted molecular interactions involving GSDME and CASP3. Core genes in IE phenotypes included CD3E, IL12RB1, and FASLG. Supervised analysis of pyroptosis pathways (NES=1.71, FDR q-value=0.069) identified 35 enriched genes such as CASP1, NLRC4, and GSDMD. STRING analysis highlighted pathways including cytotoxic T-cell pyroptosis (FDR=1.03e-03) and immune-associated pyroptosis (FDR=1.2e-14). GSVA revealed 424 significant pathway differences between immune-enriched and immune-depleted phenotypes, including Pyroptosis (p=0.0004) and Cell Killing (p=0.0021). IE phenotypes upregulated pathways including Interferon Gamma Signaling (p=0.0008) and Positive Regulation of Leukocyte Activation (p=0.0013). Cox analysis linked 82 pathways including Reactome Mitophagy (HR 3.56, p=3.13e-05) and Reactome Autophagy (HR 5.69, p=0.0003) to improved OS and PFS.

Conclusions: Pyroptosis and immune-regulatory pathways are more strongly associated with IE TME phenotypes and correlate with improved OS and PFS. These findings support pyroptosis as a modulator of prognosis in HNSCC, which might inform therapeutic mechanisms in overcoming treatment-resistant disease. Future study will apply this analysis to clinical trial RNA-seq data, among patients who underwent anti-PD-1 therapy.

#2390 Noninvasive early detection of colorectal cancer by a deep read-level model for accurate identification of methylation-specific circulating tumor DNA.

Ping Lan[1], Xiaosheng He[1], Hong Xu[2], Zhijian Xu[3], Nan Lin[4], Jingjing Hou[5], Xiaowen He[1], Yanzhen Cao[6], Jiandong Tai[2], Liyuan Zhao[7], Yiran Cai[7], Yonghui Li[7], Yanzhan Yang[7], Min Li[7], Yang Wang[7], Fang Liu[7], Hui Yu[7], Baoliang Zhu[7], Xiaohui Wu[7]

[1]The Sixth Affiliated Hospital, Sun Yat-sen University, Guangzhou, China,[2]The First Hospital of Jilin University, Changchun, China,[3]Cancer Hospital, Chinese Academy of Medical Sciences, Beijing, China,[4]The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, China,[5]Zhongshan Hospital of Xiamen University, Xiamen, China,[6]The Third Clinical Medical College/Affiliated Cancer Hospital of Xinjiang Medical University, Urumqi, China,[7]Shanghai Weihe Medical Laboratory Co., Ltd, Shanghai, China

Background: Liquid biopsy enables noninvasive cancer screening through the detection of plasma circulating tumor DNA (ctDNA), yet faces challenges due to the low ctDNA abundance in plasma, especially in early-stage cancer. Precise discrimination of ctDNA fragment can significantly enrich tumor signals and enhance detection accuracy, while having difficulties owing to the ambiguous labelling and insufficient characterization of tumor-derived reads. Here, we propose DREAM (a Deep Read-level model for accurate Identification of Methylation-specific ctDNA) for early colorectal cancer (CRC) detection, which can robustly identify ctDNA through simultaneous capture of DNA sequence and methylation information of tumor-derived reads.
Methods: The read-level model DREAM was trained on a well-labelled dataset derived from whole-genome bisulfite sequencing (WGBS) reads of 55 CRC tissues and 200 healthy plasma samples, which located in CRC-specific hyper- and hypo-methylated regions. The CRC tissue reads were firstly denoised based on the methylation continuity of healthy samples in these regions to filter non-tumor signal noise. The sequence and methylation information of the processed CRC reads and healthy plasma reads were then encoded as training set for a convolutional neural network (CNN)-based model to predict the tumor likelihood of each read. To validate the classification capability and subsequent CRC detection performance of DREAM, a plasma cohort including 208 CRC and 686 healthy plasmas with targeted bisulfite sequencing (TBS) was collected for model training. With DREAM, the regional ctDNA fraction of each sample was estimated and then utilized in an XGBoost classifier to predict sample-level cancer probabilities. The classifier was subsequently validated in the test set including 54 CRC, 170 healthy and 11 colon benign individuals.
Results: DREAM reached a remarkable read-level performance enhancement by the refined denoising procedure, with an accuracy increase from 0.80 to 0.96. For the test plasma cohort, DREAM achieves 85.2% sensitivity (95% CI: 84.1%-88.5%) at 95.6% specificity (95% CI: 95.1%-96.9%) for CRC detection. Specifically, the sensitivities for stage I to IV were 60.0% (3 / 5), 81.3% (13 / 16), 88.9% (24 / 27), and 100% (6 / 6). Notably, among the 11 benign samples in the non-cancer group, DREAM successfully differentiated them from malignant CRC with an AUC of 0.966, showing the effectiveness and accuracy of capturing tumor-derived signals.
Conclusions: In conclusion, DREAM showcases a significant advancement in early CRC detection through its precision in enriching tumor signals, which may contribute to more effective cancer screening and be further broadened in a wider range of cancer types.

#2391 Comprehensive quantification and annotation of viral gene integration and expression across thousands of human cancer models.

Sowmya Iyer, Moore van Tienen, Phillipp Trollman, Yao He, William R. Sellers, Catarina D. Campbell

Broad Institute of MIT and Harvard, Cambridge, MA

Viral infections have been demonstrated to influence host gene expression and drive tumorigenesis. This study aims to systematically annotate genome integration and expression of ~3000 exogenous and human-endogenous viral species across all public DepMap cancer cell lines, representing a diverse set of cancer types and lineages. Our preliminary results from this analysis pipeline successfully recapitulated known Human Papillomavirus (HpV strains 16, 18, 30 and 53) signatures in 12 cervical, 2 head and neck, and 4 engineered prostate cancer cell lines, demonstrating sensitivity at both the strain and gene levels. Associating these with genome-wide CRISPR screening data, we identified dependencies that were more correlated with viral presence rather than their respective lineages. Additionally, we observed expression of Epstein-Barr Virus(EBV) in multiple cancer types, a majority of which were of lymphoid lineage. We also found expression of the env gene of Human Endogenous Retrovirus-K (HERV-K113) in multiple lineages, with the highest average expression in skin cancers. By analyzing whole-genome sequencing we seek to identify viral integration sites in the cell lines' genomes, to complement viral gene expression. From this large-scale multimodal analysis of features derived from genomic and transcriptomic data combined with gene dependencies, we aim to uncover potential viral contributions to oncogenesis and biomarker or target discovery. This will provide a foundational resource for understanding the role of viruses in cancer, offering new insights into viral-driven mechanisms of disease.

#2392 The use of transcriptomic analysis to identify novel drug targets for DSA drug combination studies in acute myeloid leukemia.

William A. Chen[1], Valery Filippov[2], Jie Fang[3], Pedro Ochoa[4], Carlos A. Casiano[4], Kristopher E. Boyle[1], Sinisa Dovat[5], Hongjian Jin[6], Jun Yang[3], Olivia L. Francis-Boyle[7]

[1]Department of Pharmaceutical Science, Loma Linda University, Loma Linda, CA,[2]Department of Basic Sciences, Loma Linda University, Loma Linda, CA,[3]Department of Surgery, St. Jude Children's Research Hospital, Memphis, TN,[4]Center for Health Disparities and Molecular Medicine, Loma Linda University, Loma Linda, CA,[5]Departments of Pediatrics and Biochemistry & Molecular Biology, Pennsylvania State University, Hershey, PA,[6]Center for Applied Bioinformatics, St. Jude Children's Research Hospital, Memphis, TN,[7]Departments of Pharmaceutical Science; Basic Sciences; Pathology & Human Anatomy, Loma Linda University, Loma Linda, CA

Acute myeloid leukemia (AML) is a heterogeneous hematological malignancy that is commonly characterized by uncontrolled proliferation of myeloid blasts that overwhelm the bone marrow, leading to hematopoietic failure. AML is the most common form of acute leukemia in adults and its incidence increases with age. Despite recent advances, the overall prognosis of AML patients remains poor and relapse rates are high. Thus, there remains a need to identify potential therapeutic candidates that can improve patient survival outcomes and reduce the risk of relapse in AML patients. The objective of this study was to utilize transcriptomics to identify potential genes that can be targeted by drug candidates for future use in Duocarmycin SA (DSA)-targeted therapy combination studies in AML. DSA is a DNA alkylating agent that binds to the minor groove of DNA forming DNA adducts that lead to DNA damage. Previously, our lab showed that DSA reduced proliferation, induced cell cycle arrest at the G2/M phase, and triggered apoptosis in AML cells in a dose- and time-dependent manner. Therefore, we hypothesized that RNA sequencing analysis of genes obtained from DSA-treated AML cells will identify novel genes that can targeted by potential drug candidates. To test our hypothesis, human AML cell lines (Molm-14 and HL-60) were treated with high (Molm-14: 100 pM; HL-60: 500 pM) and low (Molm-14: 11.12 pM; HL-60: 112.7 pM) concentrations of DSA for 36 hours, cells were harvested, RNA was isolated and RNA sequencing and gene expression profiling analysis were performed by the Hartwell Center for Biotechnology and the Center for Applied Bioinformatics at St. Jude Children's Research Hospital, respectively. Pathway analysis was performed using the NIH's Database for Annotation, Visualization and Integrated Discovery (DAVID) tool to identify the top DSA-induced pathways and their associated genes. Differentially expressed genes (DEGs) for all four DSA treatment groups were identified using the cutoffs: P-value <0.05, Log2Fold Change >0.5 and <-0.5, and then the overlapping DEGs were visualized using Venny 2.1.0. Our results showed that the top pathways that were activated in all four DSA treatment groups included oxidative phosphorylation, chemical carcinogenesis - reactive oxygen species, and metabolic pathways. Genes that were upregulated within these pathways primarily included mitochondrial associated genes that play a role primarily in apoptosis (MT-*ATP6*, MT-CO2, and MT-*ND5*). Additionally, some of the pathway genes play a role in proliferation (*MT-ND2* and *TPM2*) and chemoresistance (*MT-ND2, MT-ND4* and *TPM2*). Our findings suggest that the genes associated with proliferation and chemoresistance can serve as potential therapeutic targets that can be used to develop novel targeted therapies to be evaluated in combination with DSA for the treatment of AML.

#2393 Gene regulatory networks of enzalutamide resistance in prostate cancer.

Sini Hakkola, Anni Peramaki, Karishma Sajnani, Antti Kiviaho, Alfonso Urbanucci, Matti Nykter, Mikael Marttinen

Prostate Cancer Research Center, Faculty of Medicine and Health Technology, Tampere University and Tays Cancer Centre, Tampere University Hospital, Tampere, Finland

Introduction: Castration-resistant prostate cancer (CPRC) is typically managed with antiandrogens, such as enzalutamide (ENZA), which can delay disease progression but are not curative, as resistance to these treatments eventually develops. This resistance is likely a consequence of significant remodeling of gene regulatory landscape of cells adapting to an androgen-deprived, stressful environment. Understanding the aberrancies in gene regulation underlying ENZA resistance is essential for improving the therapeutic strategies for CRPC.
Methods: Using single-cell chromatin accessibility (ATAC) and transcriptome sequencing data sourced from the parental and ENZA-resistant LNCaP prostate cancer cells, we have applied knowledge graph-based neural networks to model transcription factor (TF) regulons and their associated molecular processes underlying the cell states from initial ENZA response towards ENZA resistance.
Results: We have identified TF regulons associated with stress responses, cell cycle progression and androgen signaling, altered in ENZA-resistant cells. Specifically, the ENZA-resistant cells are characterized by the gain of SOX4 regulon, likely to contribute to the metabolic and proliferative reprogramming of the resistant cells. Cell cycle analysis revealed that while the cell proportion is largest in G1 phase in each treatment, the proportion of cells in S phase is increased in resistant samples, suggesting enhanced S-phase transition in resistant cells in contrast to G1 arrest caused by ENZA in treatment-responsive cells. We validate our findings in independent transcriptomic studies of ENZA-resistant VCaP and DuCaP cell lines indicating the robustness of the results. Moreover, utilizing spatial transcriptomic data from a longitudinal patient cohort, we pinpoint that the SOX4 regulon target genes are enriched in the epithelium of CRPC patients, compared to treatment-naïve and neoadjuvant-treated disease states.
Conclusion: In summary, we determine gene regulatory alterations associated with ENZA resistance in preclinical models, providing valuable insights into uncovering the mechanisms of resistance to antiandrogens in CRPC.

#2394 Survival and response modeling using diverse mouse mammary tumor models.

Matthew D. Sutcliffe[1], Kevin R. Mott[1], Denis O. Okumu[1], Michael P. East[2], Gary L. Johnson[2], Shawn M. Gomez[2], Timothy C. Elston[2], Charles M. Perou[1]

[1]Department of Genetics, University of North Carolina, Chapel Hill, NC,[2]Department of Pharmacology, University of North Carolina, Chapel Hill, NC

Triple-negative breast cancer (TNBC) is a clinically heterogeneous subtype of breast cancer with poor outcomes and limited molecular predictors of survival. To better understand TNBC tumor growth dynamics and to identify biomarkers of treatment response, we developed a diverse panel of 26 mouse mammary tumor models (syngeneic transplants (n = 23) and autochthonous (n = 3)) that are immune-competent and reflect the molecular subtypes seen in human TNBC. These 26 tumor models exhibit distinct baseline tumor growth rates, drug sensitivities, and molecular profiles, which we have extensively characterized through bulk RNA-seq, scRNA-seq, DNA exome sequencing, and mass spectrometry-based kinase proteomics. This comprehensive, multi-platform data set provides a unique resource to perform machine learning analyses for response and survival predictions.

Using this murine dataset, we first identified survival-associated gene expression features through Cox proportional hazards modeling. Murine tumors with high expression of basal-like subtype gene signatures were associated with shorter survival while luminal subtype and immune cell features correlated with longer survival; these findings closely align with patterns observed in human breast cancer. Second, we trained an Elastic Net-based survival predictor using untreated tumor RNA-seq data from each of the 26 mouse models and validated its prognostic utility using human breast cancer datasets such as SCAN-B (human set c-index = 0.63).

Additionally, our mouse tumor models dataset includes efficacy testing for immune checkpoint inhibitors (ICI; anti-PD1/anti-CTLA4) and MEK inhibitor therapy (trametinib). Pre-treatment tumor RNA-seq analyses identified features associated with greater survival benefit in ICI-treated models, including high PDL1 mRNA and multiple T-cell and B-cell associated gene expression signatures. Similar analyses for MEK inhibition revealed key resistance-associated genes, such as Akt1, whose high baseline expression correlated with MEKi resistance. DNA copy number analyses of the murine tumor data identified distinct chromosomal regions linked to MEKi sensitivity (i.e. chr 12q) and resistance (i.e. chr 2q). These genomic alterations overlapped the gene expression associations, suggesting potential biomarkers for MEKi response. Ongoing work involves developing predictive Elastic Net models for both ICI and MEKi sensitivity, which will be validated using available human clinical trial datasets. Overall, this work demonstrates the translational potential of this diverse mouse models dataset as a platform for biomarker discovery and therapy optimization.

#2395 The role of exonized mobile elements in chimeric transcript emergence in glioblastoma.

Rafael L. V. Mercuri[1], Thiago L. A. Miller[1], Filipe F. dos Santos[1], Matheus de Lima[2], Aline Rangel-Pozzo[2], Pedro A. F. Galante[1]

[1]Institudo de Ensino e Pesquisa do Hospital Sirio-Libanes, Sao Paulo, Brazil,[2]University of Manitoba, Winnipeg, MB, Canada

Background: Glioblastoma (GBM) is the most aggressive type of primary brain tumor in adults, with a median survival of approximately 15 months. Unfortunately, the prognosis for GBM has remained unfavorable for decades. The genetic and transcriptomic heterogeneity of GBM complicates treatment, underscoring the need for a more comprehensive understanding of its molecular basis. Recently, retrotransposons have been shown to influence gene expression and the formation of chimeric transcripts. Although they are associated with various cancers, their precise role in GBM remains unclear. In this study, using paired-end RNA-Seq data from The Cancer Genome Atlas (TCGA) and the GBM (U251) cell line, we examined the exonization of mobile elements and the generation of GBM-specific chimeric transcripts. For retroelement references, we utilized RepeatMasker annotations. To detect, characterize, and quantify the expression of chimeric transcripts, we developed FREDY, a tool designed to identify the exonization of retrotransposable elements in RNA-seq data. Initially, we identified 1,012 chimeric transcripts linked to the exonization of intragenic (intronic) mobile elements. Specifically, Alu element exonization was the most common (~45%) and occurred primarily in the 5' regions of transcripts. As expected, the majority (83%) of these exonizations involved Alu elements located in the opposite transcriptional direction of their host genes. In contrast, LINE1 elements and retrocopies of protein-coding genes were predominantly exonized (46% and 56.7%, respectively) in the 3' regions of host genes and in the same transcriptional direction as their host genes. Notably, we identified two candidate chimeric transcripts impacting the tumor suppressor gene ROS1. Furthermore, when comparing potential protein domains, we found that the coding sequences of these chimeric transcripts lacked two ROS1 domains, suggesting a premature cessation of transcription and potentially translation. Additionally, we quantified that these chimeric transcripts represent 84% of the total expression of the ROS1 gene in the identified GBM samples.
Conclusions: We have established a framework for detecting chimeric transcript formation in cancer through the exonization of mobile elements. We applied this approach to GBM samples and cell lines, identifying chimeric transcripts of the tumor suppressor gene ROS1 that may play a significant role in GBM carcinogenesis.
Sponsor: FAPESP 20/02413-4

#2396 Gene fusion and variant-aware isoform detection with functional prediction from long-read RNA sequencing.

Colette Felton[1], Andrea Galvez[1], Leanne Sayles[2], Christopher Vollmers[1], Alejandro Sweet-Cordero[2], Angela Brooks[1]

[1]Biomolecular Engineering and Bioinformatics, University of California, Santa Cruz, Santa Cruz, CA,[2]Department of Pediatrics, University of California, San Francisco, San Francisco, CA

Although most cancer variant profiling is done with short-read-based methods, many cancers are driven by structural variants that are difficult to detect with these methods. Our current approach to understanding driver mutations is also limited to single variants and rarely considers the context in which they are expressed. Alterations in the expression and splicing of genes containing variants can both impact their tumorigenicity and allow them to develop resistance to therapies. We present FLAIR3, which generates a custom transcriptome from long-read RNA sequencing and identifies SNVs, insertions, deletions, and gene fusions from alignment to this transcriptome. We show that this improves accuracy above alignment to the genome or annotated transcripts. FLAIR3 then integrates these variants with the transcripts to predict functional changes to the amino acid sequence. We apply this approach to patient-derived osteosarcoma cell lines, a cancer whose pathology is driven by complex structural variation. In these samples, we identify more complex changes to previously identified amplified drivers such as alternative splicing of MYC and alternative splicing of a gene fusion in CCNE1. We also identify a novel set of actionable cancer gene alterations not previously detected by short-read methods. This includes a large deletion in KEAP1 and a number of novel gene fusions, including TP53 and other genes fused with intergenic regions and a complex 4-locus fusion in NOTCH1 not detected by any preexisting tools. We then validated the protein sequence of novel fusions with Quantum-Si protein sequencing. This analysis shows that long-read RNA sequencing can detect novel variants in actionable cancer genes and that integrating splicing and variant alterations provides the most complete picture of gene alterations in cancer.

#2397 Order parameter methods for interpretable comparisons across tumors and cell lines.

Lauren Golden[1], Naima Abdirahman[2], Xiaomeng Zhang[1], Maria Yampolskaya[3], Barbara DeKegel[1], Pankaj Mehta[3], Francisca Vazquez[1], Catarina D. Campbell[1]

[1]The Broad Institute of MIT and Harvard, Cambridge, MA,[2]Wellesley University, Wellesley, MA,[3]Boston University, Boston, MA

As precision cancer medicine develops, new bioinformatic tools that are both more granular and interpretable become imperative. In particular, when working with cancer cell lines to identify and validate potential treatment targets in cohorts of interest it is necessary to understand whether and precisely how cancer cell lines recapitulate tumor biology. As part of the Cancer Dependency Map project we are working to develop approaches for relating cell lines to patient tumors.

Here we demonstrate a simple supervised classifier inspired by order-parameter methods from spin-glass physics that uses a pre-determined set of tissue type labels to produce scores per-gene and per-type that can be evaluated on both tumors and cell lines. The model described here complements this previous work, producing scores that qualitatively agree with the unsupervised model, while also enabling a quantitative, gene-level understanding of the origin of the classification result. Further, this method allows multiple cohorts of tumors and cell lines to be compared directly, relative to a common basis.

We describe both the method itself, a variation of which has been deployed in single-cell developmental biology, as well as the metadata processing necessary for its effective use in comparing models to tumors. We developed an algorithm that defines our scope of tissue types maximizing granularity while minimizing collinearity and accounting for gaps and overlaps in annotation between tumor and cell line datasets, with over 35 total disease types assessed. We show using several examples from cell lines that this simple method can identify not only which type of tumor each cell lines most closely resembles, but also extract the most important genes involved in that determination. Our model, using 75 samples per tissue type, achieves >95% top-3 accuracy on a test dataset of over 10k tumor samples. The matchup between annotated type and predicted type is weaker in cell lines and also highly type-specific, ranging from 95% in some cohorts of 20% in others, and we explore the reasons for this. In one example of lung cell line models our method identifies cell line cohorts that match up to adenoid as well as squamous tumors, and another cohort that doesn't match with either set of tumors. Based on the genes identified by the model as important for classifying these lines we describe their phenotype as mesenchymal. Additionally, we show how this method can be augmented with normal tissue data from GTEx to foreground signals that are specific to cancer relative to related normal tissues.

#2398 Pregnancy-specific glycoproteins in tumors are strong predictors of outcome in female lung adenocarcinoma patients.

Jung Hun Oh[1], Gabrielle Rizzuto[1], **Rena Elkin**[1], Corey Weistuch[1], Larry Norton[1], Gabriela Dveksler[2], Joseph O. Deasy[1]

[1]Memorial Sloan Kettering Cancer Center, New York, NY,[2]Uniformed Services University of Health Sciences, Bethesda, MD

Pregnancy-specific glycoproteins (PSGs) are normally critical regulators of maternal immune tolerance during pregnancy, preventing fetal rejection by modulating innate and adaptive immune responses. Recent studies have identified that mRNA expression of PSG genes in tumors is associated with poor prognosis in lung, mesothelioma, breast, and ovarian cancers. Given their pregnancy-related function, we hypothesized that PSGs could exert sex-specific effects in some cancers. An analysis of sex-differential effects of PSGs as prognostic markers was conducted using two datasets from The Cancer Genome Atlas (TCGA) including 235 male and 271 female patients and Clinical Proteomic Tumor Analysis Consortium (CPTAC) including 70 male and 36 female patients. To assess the predictive power of PSGs, we evaluated whether incorporating PSG expression levels improved machine learning models for survival prediction. Lung adenocarcinoma (LUAD) was identified as showing a significant sex-specific prognostic difference. Female LUAD patients expressing PSG genes had notably worse survival outcomes compared to those without PSG expression. Male patients did not show similar effects. Combinatorial analysis showed that a combination of PSG3, PSG7, and PSG8 expression resulted in particularly poor prognosis in the female group (30% of the female patients) with $p = 8.67E-06$. This finding was independently validated in the smaller CPTAC dataset with $p = 0.0382$. Further analysis of correlated pathway changes revealed that female LUAD patients with PSG expression exhibited enrichment of the "KRAS Signaling Down" pathway, suggesting a potential mechanism underlying the observed sex-specific effects. Including the KRAS pathway activity into the PSG model demonstrated robust performance in predicting survival outcomes for female patients (validated C-index = 0.78 using the CPTAC dataset) but did not improve prediction for male patients. These findings highlight a unique role for PSGs in influencing LUAD progression and survival in a sex-dependent manner. Targeting PSG-related pathways may offer a tailored treatment strategy for female LUAD patients, potentially improving outcomes. Further research is needed to unravel the relationship between PSG expression and KRAS pathway activation. We also plan to examine the sex-specific prognostic difference, looking into pregnancy history and the influence of hormone-related genes. Since PSGs are not typically expressed in the absence of pregnancy, they could be a promising drug target.

#2399 Genomic and transcriptomic profiling of advanced early-onset colorectal cancer.

Erika Zuljan[1], Shuba Varshini Alampalli[1], Eric Blanc[2], Bruno Kohler[3], Rene-Filip Jackstadt[4], Ulrich Keller[1], Sebastian Stintzing[1], Ulrich Keilholz[1], Hanno Glimm[5], Stefan Frohling[6], Dieter Beule[2], Ivan Jelas[1], Damian Rieke[1], Manuela Benary[1]

[1]Charite - University Medicine Berlin, Berlin, Germany,[2]Berlin Institute of Health (BIH), Berlin, Germany,[3]Heidelberg University Hospital, Heidelberg, Germany,[4]Deutsches Krebsforschungszentrum Heidelberg (DKFZ), Heidelberg, Germany,[5]Nationales Centrum fur Tumorerkrankungen Dresden (NCT), Dresden, Germany,[6]Nationales Centrum fur Tumorerkrankungen Heidelberg (NCT), Heidelberg, Germany

Background: Incidence rates of early-onset colorectal cancer (EO-CRC) are increasing in high-income countries, yet the underlying causes and optimal clinical management strategies remain unclear.
Methods: Comprehensive molecular profiling was conducted on colorectal cancer patients enrolled in the DKTK MASTER program, which includes patients with fresh tumor material, age <50 years, and/or rare cancer types. Profiling utilized RNA sequencing (RNA-seq) and whole-exome (WES) or whole-genome sequencing (WGS). WES data was analyzed for genomic variants, tumor mutational burden (TMB), and mutational signatures. WGS underwent metagenomic analysis, mapping unmapped reads to a metagenome assembled genomes (MAGs) of German CRC patients to identify CRC-associated microbiome. Transcriptomic data were analyzed for consensus molecular subtypes (CMS) and inflammation signatures. A small cohort of late-onset CRC patients (>50 years, n=10) and TCGA-COAD data were used as reference cohorts.
Results: The cohort included 168 patients, 154 aged <50 years (median age: 43; range: 19-50) and 10 aged >50 years (4 age data unavailable). Primary tumors were located in the right colon (n=32) or left colon (n=127) (9 NA).
Common molecular alterations included TP53 (77%), APC (70%), and KRAS (48%) mutations. The median TMB was 3.5 mutations/megabase and was comparable to TCGA-COAD (3.8 mutations/megabase). TMB>10 Mut/Mb was only identified in 2 samples. Age was not associated with inflammation levels. CMS subtypes I-IV were identified in 15%, 26%, 9%, and 34% of cases, resembling TCGA-COAD but with slightly higher CMS IV and lower CMS III. Findings suggest a molecular profile similar to late-onset CRC (LO-CRC).
Mutational signatures in WES analysis revealed a prevalence of clock-like and defective DNA damage repair signatures (SBS1, SBS3, SBS5, SBS6). Clock-like signatures (SBS1, SBS5) showed no significant age-related differences.
Metagenomic analysis revealed distinct prevalence patterns and notable dissimilarity in gut-associated microbes between early- and late-onset cohorts. Significant differences in abundance were observed in Firmicutes such as Blautia wexlerae, Coprococcus comes, Veillonella parvula, and Bacteroidetes such as Prevotella copri.
Conclusion: Comprehensive molecular profiling revealed no significant differences between sporadic EO-CRC and LO-CRC, thus not suggesting different clinical management in this setting. The identification of distinct microbiome compositions warrants further investigation.

#2400 Utilizing machine learning to predict clinical staging with head and neck cancer gene expression datasets.

Nathan DeMichaelis[1], Amrit Menon[2], Dalton Schutte[3], Frank G. Ondrey[4]

[1]Dartmouth College, Hanover, NH,[2]University of Chicago, Chicago, IL,[3]Department of Surgery, University of Minnesota Medical School, Minneapolis, MN,[4]Department of Otolaryngology - Head & Neck Surgery, University of Minnesota Medical School, Minneapolis, MN

Introduction: Head and neck cancer is a deadly disease with relatively stagnant cure rates. Predictive oncology might allow treatment stratification for surgery, radiation, and chemotherapy combinations based on both stage and other risks. As more high dimensional data from genomic expression datasets become publicly available, machine learning algorithms could be used for staging/treatment refinement. In the present study, we applied machine learning algorithms to our previously published smaller U133A Affymetrix gene expression dataset and the NCI Cancer Genome Atlas (TCGA).

Methods: Models were trained to classify T and N staging data for TCGA and Affymetrix chip datasets, by early (T1/2) versus advanced T stage (T3+), and for N0 versus N1+, respectively, for TCGA and Affymetrix data separately. TCGA data was split according to HPV status into two datasets. Models to predict early vs. advanced stage were trained and tested on randomly (proportional with respect to staging) split data: large dataset (HPV- T and HPV- N, n>=350) models were trained on 80% and tested on the unseen 20% samples, while for the remainder (n<=50), data were split 70%-30% for sufficient testing. Scikit-learn models utilized were: Bagged (aggregated) Support Vector Classifiers (BSVCs), Random Forest (RF), Gradient Boosting (GB), and Linear Regression (LR). LR was added unaltered as a baseline prediction, while the other three models were subjected to hyperparameter tuning (assessed for all combinations of selected settings) and feature selection: models were trained and tested on whole expression data, iteratively reduced to the top half important genes down to <=100 genes. Models were assessed by test accuracy and area under receiver operator characteristic curve (AUC-ROC), then hyperparameter-tuned models and respective gene lists were assessed over 50 trials (random initializations and data splits).

Results: Hyperparameter-tuned RF and BSVCs models with selected gene lists significantly outperformed LR in 6/6 tasks, while GB outperformed LR in 2/6 tasks. Example highest performing models (test accuracy [95% CI], AUC-ROC [95% CI]) were: For Affymetrix N, BSVCs (0.893 [0.872-0.915], 0.95 [0.931-0.969] outperformed LR (0.49 [0.453-0.527], 0.476 [0.435-0.517]). For TCGA HPV- N, BSVCs (0.832 [0.818-0.847], 0.892 [0.877-0.907]) outperformed LR (0.581 [0.568-0.595], 0.615 [0.6-0.631]). For TCGA HPV+ T, RF (0.835 [0.815-0.854], 0.954 [0.942-0.965]) outperformed LR (0.669 [0.645-0.694], 0.76 [0.732-0.789]).

Conclusions: Utilizing this hyperparameter tuning and feature selection approach, we have identified machine learning techniques that can be used to analyze high dimensional data of small and large genomic expression datasets to predict clinical features and identify predictive genes for further analysis, applicable to head and neck cancer, a relatively rare malignancy (about 3% of total cancers).

#2402 Comparative genomics at prostate cancer risk loci to identify human-specific risk variants predisposing the human male to prostate cancer.

Chidi N. Molokwu

Bradford Teaching Hospitals NHS Trust, Bradford, United Kingdom

Introduction: Prostate cancer (PCa) is the most common cancer in the human male but is absent in other primates, including our closest extant relative the chimpanzee. Predisposition to PCa is multifactorial and includes both genetic and environmental factors. Genome wide association studies (GWAS) have identified hundreds of PCa associated single nucleotide polymorphisms (SNPs), but it is not clear which SNPs are the most relevant to carcinogenesis and disease progression. PhyloP scores are a measure of conservation between species at each position in the aligned genome, with positive scores indicating higher conservation. Human Accelerated Regions (HARs) are regions in the genome that show conservation between mammalian species but unexpected divergence in humans. A comparative genomics approach was used to identify SNPs and candidate genes that confer PCa risk on the human male.

Methods: PCa associated SNPs were obtained from studies submitted to the GWAS Catalog (https://www.ebi.ac.uk/gwas/home), and the GWAS Diversity Monitor Database (https://gwasdiversitymonitor.com). Risk Allele Frequencies (RAFs) were obtained from Ensembl (https://www.ensembl.org/), using regional classifications and RAF data from the 1000 Genomes. Human and chimpanzee genomes were aligned using the Cactus genome alignment tool (https://cglgenomics.ucsc.edu/data/cactus/). PhyloP scores were determined for each SNP position and the closest HAR was identified using the Bedtools package. The allele for each specie at the PCa SNP position was compared to determine positions where the PCa risk allele was divergent from the ancestral primate allele.

Results: 520 PCa associated SNPs were identified. Eleven SNPs occurred at positions with PhyloP score>1.0, and where the PCa risk allele was different from the chimpanzee allele. Two of the 11 SNPs had the risk allele occurring in more than half of humans (rs6062509 at 71%, and rs8064454 at 58%). Two SNPs with the highest PhyloP scores had RAFs of 11% for rs76832527 (PhyloP score 8.616) and 17% for rs2277283 (PhyloP score 3.842). A non-coding region of chromosome 8 containing rs870167 had RAF of 17% in humans. ZGPAT, HNF1B, ANO7, INCENPS, GATA2-AS1 and CLND23 were identified as candidate genes for prostate cancer carcinogenesis and/or progression. There was no overlap of PCa associated SNPs with HAR regions.

Conclusions: Six candidate genes harbored PCa risk SNPs that are in loci that show high conservation between mammalian species, but a divergent PCa risk allele in human males. ZGPAT, HNF1B, ANO7 and GATA2-AS1 are relevant to PCa progression and are candidate genes for human-specific predisposition to PCa. Further studies on these genes and non-coding loci are needed to determine their role in PCa predisposition in the human male.

#2403 Efficient detection of whole genome duplication: a targeted sequencing approach.

Hua Bao, **Haimeng Tang**, Rui Liu, Ningyou Li, Min Wu, Xunbiao Liu, Zhili Chang, Xue Wu

Nanjing Geneseeq Technology Inc., Nanjing, China

Objective: Whole Genome Duplication (WGD) involves the replication of an organism's entire genome and is linked to increased evolutionary complexity and cancer progression. In tumors, WGD contributes to heterogeneity and therapy resistance, making its identification crucial for targeting genomic instabilities and informing treatment strategies. Current methods for detecting WGD are often expensive and time-consuming, highlighting the need for a cost-effective and efficient detection panel to improve therapeutic strategies, patient outcomes, and resource allocation in clinical settings.
Methods: We retrospectively analyzed 4,941 tissue samples from patients spanning 14 different cancer types, including ovarian, uterine, breast, pancreatic, prostate, cervical, liver, lung, colorectal, and gastric cancers. Using a targeted sequencing panel (GENESEEQ GENAVISTA) focused on 437 cancer-related genes, we performed comprehensive mutational profiling (~1,000X). WGD was determined by analyzing read depth, allele frequency, and employing variant calling algorithms to detect gene duplications and genomic rearrangements. Additionally, a prospective cohort of 33 cancer patients was evaluated to compare the performance of our panel with whole exome sequencing (WES, ~200X) and whole genome sequencing (WGS, ~30X).
Results: Our analysis revealed varying prevalence of WGD across cancer types, with significant rates observed in ovarian (50.93%) and breast cancers (52.32%). Pelvic cancer (60.61%) and melanoma (60.00%) also showed high WGD-positive rates. Conversely, endometrial cancer exhibited a notably lower rate of 19.41%. Pancreatic and prostate cancers showed moderate WGD frequencies of 27.46% and 21.51%, respectively, while liver and bile duct cancers had an incidence of 41.30%. In the prospective cohort, our panel demonstrated higher sensitivity compared to WES (100% vs. 84.62%) but slightly lower specificity (90% vs. 95%). The overall concordance with the gold standard WGS was 93.9%, surpassing that of WES (90.9%).
Conclusion: Our study highlights the significant variability of WGD across cancer types, with a notably high prevalence in ovarian and breast cancers. This underscores the potential of WGD as a biomarker for therapeutic targeting. Our targeted sequencing panel offers an efficient and cost-effective detection method, demonstrating substantial clinical potential for informing personalized treatment strategies and improving patient outcomes.

#2404 Change in expression of microRNAs following limb amputation in canine osteosarcoma patients predicts survival time after chemotherapy.

Heather Treleaven[1], Michael Edson[2], Latasha Ludwig[3], Alicia Viloria-Petit[4], R. Darren Wood[1], R. Ayesha Ali[5], Geoffrey A. Wood[1]

[1]Pathobiology, University of Guelph, Guelph, ON, Canada,[2]MaRS Centre, University Health Network, Toronto, ON, Canada,[3]Population Medicine and Diagnostic Sciences, Cornell Univeristy, Ithaca, NY,[4]Biomedical Sciences, University of Guelph, Guelph, ON, Canada,[5]Mathematics and Statistics, University of Guelph, Guelph, ON, Canada

Osteosarcoma (OS) is the most common primary bone tumour in dogs and humans. There are an estimated 8,000 new cases of canine OS per year in the United States compared to only 1,000 in humans. The most common primary location for both species is the appendicular skeleton, with metastases forming mostly in the lungs and bones. Appendicular OS is not only more common in dogs, but also more aggressive, making dogs a valuable spontaneously metastasizing animal model of aggressive human OS. Median survival after amputation and adjuvant chemotherapy in dogs is 1 year, less than 30% survive 2 years, and an estimated 90% of dogs develop metastases after therapy. In contrast, humans have a 76% 5-year survival rate and 15-25% will develop metastases. Predicting clinical outcomes after treatment is difficult in both species, particularly for those without clinically detectable metastatic disease at diagnosis. MicroRNAs (miRNAs) are small, non-coding RNA that control gene expression and are often dysregulated in cancer. MiRNAs share entirely or nearly identical sequences between dogs and humans and are present in blood, making them good potential biomarkers. Recently, we identified multi-miRNA models which could reliably predict survival using pre-amputation canine OS plasma samples. Tumour cells can release miRNAs into the blood via active and passive mechanisms; therefore, with the primary tumour removed after amputation, miRNA expression levels in the blood should be altered. We hypothesize these changes in miRNA expression can be used to prognosticate canine OS patients better. MiRNA expression was measured using real-time quantitative PCR in matched pre- and post-amputation plasma samples from canine OS patients (n=25) at the Ontario Veterinary College. Change in expression was calculated for each miRNA as [(post-amputation) - (pre-amputation)] for each matched pair of samples. For each dog, survival was measured as overall survival and disease-free interval (DFI). Overall survival is the time from diagnosis to death and DFI is the time from diagnosis to clinically detectable metastasis. Dogs were censored if they died from non-OS events, were lost during follow-up, or were still alive. Prognostic ability was evaluated for individual miRNAs by identifying an optimal expression cutoff point using the survminer package in R and visualized using Kaplan-Meier curves. Decision trees were grown using the rpart package in R to evaluate whether multi-miRNA models offered improved prognostic ability compared to single miRNAs. We found that a model combining miRNA-144 and miRNA-23a, for both overall survival and DFI metrics, was able to identify 3 distinct groups which reflected survival time worse, on par, and better than the median survival time reported in literature. These models offer a simple prognostic test that may have clinical applications for veterinary and human OS patients.

#2405 NGS-based molecular profiling reveals remarkable anticancer synergy of EZH1/2 dual inhibitor HM97662 with standard-of-care therapy on small cell lung cancer.

Gunwoo Lee, Seungheon Baek, Jungwha Park, Aran Park, Seung Hyun Jung, **Jooyun Byun**, Young Gil Ahn

Hanmi Pharmaceutical Co., Ltd., Gyeonggi-do, Korea, Republic of

The development of EZH1/2 inhibitors is crucial in targeting tumors due to their central role in epigenetic regulation. These EZH1/2 enzymes contribute to tumorigenesis by promoting transcriptional repression of tumor suppressor genes. Studies have shown that inhibiting EZH1/2 can suppress tumor growth and improve clinical outcomes, particularly in hematological malignancies. However, the selective potency of EZH1/2 inhibitors in a limited range of solid tumors highlights the need for precise selection of indications based on molecular characteristics. Recent research has discovered that cancers with a small cell neuroendocrine (SCN) phenotype, characterized by high aggressiveness and limited treatment options, share molecular characteristics and drug susceptibility profiles with hematological malignancies. Moreover, the role of EZH2 in the cancer with SCN feature, such as small cell lung cancer (SCLC) and neuroendocrine prostate cancer (NEPC), has been increasingly recognized. Notably, EZH2 contributes to chemo-resistance in SCLC by repressing the transcription of Schlafen 11 (SLFN11), a biomarker of sensitivity to DNA-damaging chemotherapies. Inhibition of EZH2 upregulates SLFN11 expression and restores chemosensitivity in chemo-resistant SCLC models, suggesting EZH2 blockade as a promising therapeutic strategy. Previously, we evaluated antiproliferative properties of HM97662 on diverse solid cancers and demonstrated its great potential in various SCLCs. Therefore, we explored to determine the molecular factors that influence the sensitivity to HM97662 and synergism with standard-of-care (SoC) in SCLC. Using next generation sequencing (NGS), we analyzed the transcriptomic profiles of HM97662-sensitive and non-sensitive SCLC cell lines, verifying the molecular determinants of the sensitivity. We also identified the transcriptomic changes when EZH1/2 were inhibited through HM97662 in the SCLC cell lines at the gene as well as pathway level. We found that HM97662 treatment selectively increased the expression of genes involved in cell cycle regulation including CDKN1A (p21) in sensitive SCLC cell lines. Similarly, SLFN11 level was also dose-dependently increased. Remarkably, HM97662 exhibited super-synergistic effect with SoC in SCLC xenograft models, outperforming to both an EZH2 inhibitor and another EZH1/2 dual inhibitor. This study highlights the transformative potential of combining epigenetic modulation with conventional treatment regimens to overcome therapeutic limitations. Leveraging NGS, we identified molecular signatures predictive of enhanced response to the EZH1/2 dual inhibitor HM97662. These findings pave the way for personalized therapeutic strategies, offering renewed hope for patients with small cell lung cancer and other cancers with SCN feature as well.

#2406 LossFinder: a novel algorithm for detecting PTEN homozygous deletions in cancer.

Ting Kuang, Chenglin Liu, Zhihong Zhang, Zhou Zhang, Fujun Qiu, Yuzi Zhang

Burning Rock Biotech, Guangzhou, China

Introduction: The PTEN gene, located at 10q23.31, is a tumor suppressor that regulates the PI3K/AKT signaling pathway. PTEN deletions and/or loss of protein are common in cancers, with The Cancer Genome Atlas (TCGA) reporting a prevalence of 10-85%, about half of which are due to copy number loss. PTEN homozygous deletions, more significant than hemizygous deletions, are key markers in cancer treatment, especially with the approval of the AKT inhibitor capivasertib. Homozygous PTEN deletions occurs in 4.28% of cancers, 5.08% in breast cancer, and 17.42% in prostate cancer. Detecting exon-level deletions in the PTEN gene is particularly challenging, given the short coding regions, the influence of tumor heterogeneity, and the effects of sample quality on Next-Generation Sequencing (NGS) data. To overcome these challenges, we developed LossFinder, a novel algorithm for detecting homozygous PTEN deletions.

Methods: LossFinder is designed to detect homozygous gene copy number deletions in tumor NGS sequencing. The algorithm: 1) employs multi-depth correction and noise reduction to enhance the signal-to-noise ratio, mitigating the effects of tumor heterogeneity and experimental noise; 2) uses the circular binary segmentation (CBS) algorithm for precise breakpoint localization, improving detection of small exon-level copy number alterations; 3) assesses tumor purity and ploidy through mutation frequency and copy number alterations, restoring absolute copy numbers to distinguish homozygous from hemizygous deletions. We evaluated limits of detection (LoD) and precision using mixed tumor cell lines from ATCC. Additionally, clinical tumor samples were analyzed for PTEN deletion prevalence, with IHC used as the gold standard for comparison.

Results: In cell line simulations, the algorithm demonstrated 100% specificity and 96.21% sensitivity when tumor purity was ≥30%. The LoD was achieved with 30% tumor purity, and precision with 35% tumor purity was 100%. In a cohort of 5,944 clinical FFPE tissue samples from over 20 cancer types, the prevalence of PTEN homozygous deletions was 4.32% overall, 6.56% (12/183) in breast cancer, 6.52% (3/46) in prostate cancer, and 3.77% (116/3078) in lung cancer, matching TCGA data for breast cancer but with a lower prevalence in prostate cancer, likely due to sample size limitations. Whole PTEN gene homozygous deletions accounted for 40% of PTEN homozygous deletion-positive samples. IHC validation in 97 clinical samples showed 85.71% consistency with NGS.

Conclusions**:** The LossFinder algorithm offers high sensitivity and specificity in detecting PTEN homozygous deletions. It accurately identifies both whole and partial homozygous deletions, showing a high level of consistency with IHC.

#2408 Bioinformatics analysis of genes that confers apoptosis resistance to EGFR-PI3K co-targeting.

Adeoluwa Adeluola[1], Humaira Noor[2], **A.R.M. Ruhul Amin**[1]

[1]Pharmaceutical Sciences, Marshall University, Huntington, OH,[2]Stanford University, Palo Alto, CA

Background: Large scale genomic studies have identified the overexpression of epidermal growth factor receptor (EGFR) and constitutive activation of the phosphoinositide-3-kinase/protein kinase B (PI3K/AKT) pathway as common genetic aberrations in head and neck cancer. Previously, we reported that the combination of EGFR inhibitor erlotinib with PI3K inhibitor BKM120 had synergistic effects and induced efficient apoptosis in a panel of head and neck cancer cell lines, but some cells were still resistant. The purpose of the current study is to identify molecular pathways that confer resistance to EGFR and PI3-K co-targeting through RNA-Seq and bioinformatics analysis.

Methods: JHU022, a cell line resistant to EGFR and PI3K co-targeting was treated with the combination of erlotinib and BKM120 ± dasatinib (Src inhibitor) or crizotinib (Met inhibitor) for 24h. Total RNA was isolated and used for RNASeq analysis. In order to identify major pathways that were associated with resistance to EGFR and PI3K co-targeting, bioinformatics pathway analysis was conducted using 2-folds differentially expressed genes. The mRNA expression levels of selected differentially expressed genes were confirmed by qPCR.

Results: FDR of ≥0.1% and fold-change of ≥2.0 were used as cut-off for selecting differentially expressed genes. A total of 961 genes were modulated by the combination of erlotinib and BKM120, 2205 by the combination of erlotinib, BKM120 and dasatinib, and 2251 by the combination of erlotinib, BKM120 and crizotinib when compared with untreated control. The addition of crizotinib to the combination of erlotinib and BKM120 resulted in the modulation of 624 genes, while the addition of dasatinib to erlotinib and BKM120 modulated 555 genes. KEGG pathway analysis identified DNA replication, cell cycle, mismatch repair, base excision repair, homologous recombination etc. as the major pathways affected by double and/or triple drug treatment. E2F targets, G2-M checkpoint, Myc targets were the common pathways identified by MSigDB analysis using differentially expressed genes. These pathways were also identified as major affected pathways in GO_Biological_Processes_2021 analysis.

Conclusions: SRC-Met signaling confers resistance to EGFR and PI3K co-targeting by modulating genes associated with cell cycle, DNA replication, and cellular response to DNA damage.

Grant Support: R15DE032063 and P20GM103434

#2409 Examining APOBEC3B in TCGA and ICGC breast cancer datasets reveals altered drug metabolism pathways.

Joel Pardo, Nuri Temiz, Douglas Yee

University of Minnesota, Minneapolis, MN

Despite advancements in screening and treatment, breast cancer mortality remains significant, with nearly one-third of patients worldwide succumbing to the disease. APOBECs, a large family of cytosine deaminase enzymes, function endogenously to restrict viral replication. However, their upregulation in many cancers has been linked to a distinct mutational signature characterized by cytosine-to-uracil conversions, leading to C-to-T transitions. Among these enzymes, APOBEC3B (A3B) is notably overexpressed in breast cancer. It remains uncertain, however, whether tumors with an enriched A3B mutational signature consistently exhibit elevated A3B expression across different breast cancer subtypes. Studies estimate the A3B mutational signature is present in 30-50% of breast cancer cases, with higher prevalence in triple-negative breast cancer (TNBC) and HER2-enriched subtypes. Understanding the pathways influencing A3B expression is an area of growing research interest. Using DNA and RNA sequencing data from breast cancer patients in The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC), we observed that over 64% of tumors with enriched A3B genomic mutational signatures exhibited above-median A3B mRNA expression compared to non-enriched tumors ($p < 0.001$). Pathway analyses, including KEGG, Gene Ontology (GO), and Reactome, of high versus low A3B-expressing tumors revealed associations with pathways involved in tumor drug metabolism. Notably, the tamoxifen regulatory pathway was altered in high A3B-expressing tumors, which showed reduced expression of *CYP2D6* and *CYP3A10*. This downregulation was consistent in luminal-A-like and luminal-B-like samples. Previous studies in mouse model systems have demonstrated diminished responses to tamoxifen in cells with high A3B expression; our findings suggest a potential relationship between high A3B expression and altered tamoxifen metabolism through *CYP2D6*. Additionally, pathways related to uracil synthesis, involving drugs such as 5-fluorouracil and 6-mercaptopurine, were downregulated in tumors with high A3B expression. Downregulated genes included *IMPDH1*, *NME1*, *TK1*, and *DPYS*, many of which are directly associated with pyrimidine synthesis. This pattern of downregulation was consistent across luminal-A-like, luminal-B-like, and basal-like breast cancer subtypes. These findings suggest that tumors with elevated A3B expression may have a heightened ability to develop resistance to therapies such as tamoxifen. Conversely, such tumors might exhibit increased susceptibility to treatments like 5-fluorouracil. Evaluating the functional consequences of A3B expression in breast cancers could pave the way for strategies to modulate A3B activity, enhancing the efficacy of existing therapies and mitigating drug resistance.

#2410 Copy number-based instability in breast cancer.

H. Wong[1], A. Korsakova[2], A. Wu[1], A. Perera[1], C. K. Patro[3], S. Chen[4], S. Sundararaman[1], V. Warrier[1], D. G. Tenen[1], J. J. Pitt[1]; [1]Cancer Science Institute of Singapore, Singapore, Singapore, [2]Calico Life Sciences LLC, San Francisco, CA, [3]National University of Singapore, Singapore, Singapore, [4]Yong Loo Lin School of Medicine, Singapore, Singapore

Copy number alterations (CNAs), involving the acquisition and loss of DNA segments, are common genetic changes associated with cancer initiation and genomic instability (GI). Similar to single base substitutions, CNAs in cancer genomes often accumulate non-randomly, linked to aberrant DNA damage and repair mechanisms. Investigating higher-order CNA patterns, especially in CNA-prone malignancies like breast cancer, offers insights into GI's nature, causes, and clinical implications. While GI is historically described using predefined scores or de novo CNA signature extraction, these approaches haven't been comprehensively combined and profiled in genomically diverse diseases such as breast cancer. Fourteen pre-existing CNA scores depicting diverse GI phenomena were reintegrated within a unified computational framework. Applied to a large cohort of breast cancer patients (n = 2,763) from TCGA and METABRIC, this framework, along with de novo CNA signature extraction, identified eight robust signatures. CNA scores and signature activities were associated with genomic features and clinical information, including subtype and patient outcomes. Breast cancer oncogenes such as ESR1, CCND1, and IKBKE were preferentially amplified by tandem duplication in a subtype-dependent manner. Additionally, two CNA signatures were characterized as harboring relatively few CNAs ("CNA quiet") within diploid and tetraploid genomes, respectively, with the latter representing an unreported subset of whole genome doubled tumors. Samples with a combination of CNA quiet genomes and low macrophage infiltration exhibited significantly better survival outcomes, independent of standard clinical risk factors. Intriguingly, several signatures coincided with the inactivation of BRCA1/2, genes critical for proficient homologous recombination (HR). These signatures showed differential activity in samples lacking BRCA1 and BRCA2, indicating a potential gene-specific CN pattern of HR deficiency. Segments assigned to another signature exhibited substantial overlap with chromothripsis-like patterns and extrachromosomal DNA (ecDNA), suggesting a common process underlying the generation of these complex GI phenomena. These findings emphasize how exploring scores and signatures can elucidate diverse mechanisms underlying CNA-driven GI in breast cancer. The relative activities of these signatures may hold notable prognostic potential, especially when integrated with other clinically relevant data modalities such as immune cell infiltration. Overall, comprehensive profiling of higher-order CNA patterns in breast cancer can not only uncover the diversity and sources of GI but also provides translational impact.

#2411 Identification of loss of heterozygosity-associated genes linked to loss of function in Korean population through in-silico analysis.

Sun-Woo Lee, Jae-Yoon Kim, Hyojin Heo, Seung-Woo Baek, Jee-Woo Seo, Jong-Lyul Park, Seon-Young Kim

Korea Research Institute of Bioscience & Biotechnology (KRIBB), Daejeon, Korea, Republic of

Loss of heterozygosity (LOH) is a common event in cancer development, disrupting gene and protein translation and resulting in loss of function (LOF). In terms of additional vulnerability, recent studies have suggested that investigating LOF due to LOH can be beneficial for cancer treatment. Additionally, genetic regions, including single nucleotide polymorphisms (SNP) linked to LOF caused by LOH, vary across cancer types. From this perspective, we conducted an in-silico analysis of Korean genomic data, cancer cell line data, and public genomic databases to identify genes associated with SNPs leading to LOF due to LOH. We analyzed a total of 10 million SNPs from a large cohort of 1,496 Koreans using whole genome sequencing (WGS) data, as well as up to 9 million SNPs from 1,007 cancer cell lines (including 129 lung, 21 stomach, and 28 colon samples) based on whole exome sequencing (WES). After filtering out rare variants, we identified 453 functional SNPs affecting protein translation in both Korean and cancer cell line data. Of these, 194 SNPs with heterozygous genotype frequencies between 0.1 and 0.9 were selected. Using copy number loss regions from the COSMIC database, we identified 39 genes associated with potential LOH-related SNPs. Finally, through the analysis of gene expression data from The Human Protein Atlas and previous studies, six LOH-associated genes linked to LOF in Koreans were identified: *ADAMTS1, ANXA2, IL17RB, LPL, TUBA8,* and *ZNF117*. Based on these six genes, we propose a list of drugs that, when used in combination, enhance the efficacy of conventional anticancer treatments. We plan to validate the effectiveness of these drugs in addressing additional vulnerabilities through in vitro experiments. While experimental validation is ongoing, we anticipate that our study will serve as a valuable resource for researchers aiming to identify population-specific LOH-associated genes using in-silico approaches.

#2412 Adenine AI: Advancing germline and somatic evidence discovery for variant assessment and gene validation.

Ronak Y. Patel

Nucleati LLC, Houston, TX

Technological advancements have significantly reduced the cost of panel assays, exome, and genome sequencing, yet the classification and interpretation of germline and somatic variants for pathogenicity and oncogenicity remain challenging. Irrespective of the guidelines used, genetic and functional evidence plays a crucial role in supporting variant classification and interpretation. For instance, at least eight evidence codes (PP1, PM6, PS2, PS3, PP4, BS3, BS4, and BP3) from ACMG guidelines and another eight evidence codes (OS1, OS2, OS3, OM4, OM3, OP3, OP4, and SBS2) from joint ClinGen/CGC/VICC guidelines rely on genetic or functional evidence. Published case reports, multi-patient studies, and functional assessments are essential in strengthening or refuting variant classification and gene-disease associations in both somatic and constitutional cancer contexts.However, the ever-expanding body of scientific literature—now exceeding 40 million articles—makes finding, collecting, and curating relevant evidence a daunting task. Manual efforts by expert professionals, while accurate, are labor-intensive, costly, and inherently unscalable. These limitations impede timely variant interpretation and indirectly affect patient care and diagnosis in both germline and somatic contexts.To address these challenges, we have developed an automated pipeline leveraging artificial intelligence (AI) and workflow automation to streamline literature collection and curation. Our system continuously scans newly published evidence, evaluates relevance, categorizes it into germline or somatic contexts (e.g., single case, multi-case, or functional assessments), and extracts critical details such as gene, disease, patient demographics, cohort size, study type, interventions, variant identity, and assay type. The extracted data is normalized, mapped to ontologies, and made available through our user-facing platform, Adenine AI (accessible at https://adenine.ai).Adenine AI simplifies evidence discovery through an intuitive interface while offering powerful, documented web APIs for seamless integration with electronic health records (EHR) and variant classification/interpretation systems. Beyond ease of access, our platform enables secondary analyses, confirming and extending findings on somatic hotspot mutations while revealing significant gaps in widely used resources such as ClinVar, where evidence for many genes remains absent.This study introduces our automated pipeline and Adenine AI, available for free trial, and demonstrates its potential to improve evidence accessibility and streamline variant classification workflows, ultimately advancing both research and clinical practice.

BIOINFORMATICS / COMPUTATIONAL BIOLOGY / SYSTEMS BIOLOGY / CONVERGENT SCIENCE: Artificial Intelligence for Digital Pathology and Spatial Molecular Technologies
Poster Session

#2416 CELLama: cell embedding leveraging language model abilities for analyzing cell types and spatial relationship in tumor.

Jiwon Kim, Jeongbin Park, Sumin Kim, Dongjoo Lee, Sungwoo Bae, Haenara Shin, Daeseung Lee, Hongyoon Choi

Portrai, Inc., Seoul, Korea, Republic of

Background: Single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics (ST) have opened up new avenues that will expand our knowledge on cellular heterogeneity and spatial tissue architecture in the tumor microenvironment. However, the mere volume of data as well as different error profiles pose significant challenges in comprehensive data integration and subsequent analysis, particularly for robust cell type identification and spatial context analysis. Here, we developed CELLama (Cell Embeddings Leverage Language Model Abilities), a foundation model that relies on natural language processing (NLP) methods to embed the information from the cell and space.
Methods: CELLama converts scRNA-seq data into natural language-like sentences of input, with major focus on gene expression and including metadata on tissue of origin and disease status. These will be projected to high-dimensional vectors for each cell by a pre-trained sentence transformer. This embedding was also fine-tuned with sentence pairs of the generated sets labeled for cosine similarity that utilized gene expression profiles. In testing the performances of CELLama and the other existing models on multitissue and disease datasets, zero-shot cell typing and spatial context analysis were performed. To analyze the spatial architecture of the tumor microenvironment, neighborhood cell type patterns were generated, and cell subtypes within the tumor microenvironment were clustered based on these patterns.
Results: CELLama demonstrated better integration and annotation of cells across multiple datasets. It showed better performance than other foundation cell embedding models including scGPT in terms of accuracy, precision, and recall, especially after the addition of metadata like tissue of origin. As an example of leveraging CELLama for
spatial analysis of tumor microenvironment architecture, it has been applied to identify five distinct fibroblast subpopulations in lung cancer, differentiated by their spatial context.
Conclusions: CELLama represents a unique cellular data analysis leveraging NLP, which changes more complicated transcriptomic data into highly interpretable language-like formats, integrates various metadata and spatial context, and will further extend to atlas-level integrations and disease-specific tissue analyses. It simplifies complex analyses by providing a thorough understanding of intricate cell types within the tumor microenvironment and accelerating the study of their spatial architectural patterns.

#2417 N-Orbit: An interpretable, distance-based framework for comparison of tumor microenvironments and beyond.

Barbara Xiong[1], Yuxuan Hu[2], Kai Tan[1]

[1]University of Pennsylvania, Philadelphia, PA,[2]Xidian University, Xi'an, China

The tumor microenvironment (TME) plays a major role in cancer progression and treatment resistance. Advances in single-cell resolution spatial omics technologies have facilitated the exploration of the TME and its role in these processes. Recent developments in quantitative methods for tissue cellular neighborhood (TCN) detection have provided further insights into the organization of spatial heterogeneity within individual tissue samples. Yet a theoretical framework for modeling and comparing tissue architecture across time points and clinical subtypes remains lacking. We introduce, N-Orbit, a mathematical model that captures both cell type composition and spatial relationships within tissue cellular neighborhoods, encoding this information in a vector format for computationally efficient distance metric calculations. We benchmark our N-Orbit-based neighborhood distance metric on spatial omics datasets with manually annotated ground truth anatomical regions and on patient datasets with corresponding clinical variables. We also demonstrate that N-Orbit neighborhood distances recapitulate transitions across treatment phases and can be used to identify resistance-enriched neighborhood clusters in triple-negative breast cancer. Additionally, N-Orbit enhances model interpretability by allowing these neighborhood clusters to be traced back to enriched cell-cell interactions. N-Orbit holds significant potential for deepening our understanding of how TMEs, as well as tissue microenvironments more broadly, evolve across treatment timelines and clinical subtypes.

#2418 CellClique: Dissecting tumor microenvironments at the single cell level using generative AI and spatial transcriptomics.

Sachini Weerasekara[1], Natasha Darras[2], Nicolas Fernandez[3], Melinda Chen[2], Alina Ainbinder[2], **Colles Price**[2]

[1]Northeastern University, Boston, MA,[2]Takeda Pharmaceutical Company Ltd. (U.S.), Cambridge, MA,[3]Broad Institute, Cambridge, MA

Novel cellular therapy has the potential to change cancer treatment. However, to design new therapies requires a better understanding of the tumor microenvironment (TME) and the cellular dynamics that occur within the tumor. This requires a high resolution and subcellular view of cancer cells, immune cells while identifying the neighborhood architecture that exists between them. Spatial transcriptomics and spatial proteomics show subcellular information and can be used to identify cellular cell types and find spatial deviation between cells. However, there are a lack of tools to investigate cellular behaviors on an individual cell basis while integrating pathological information, limiting the future of spatial biology. We introduce CellClique, a tool that assists in dissecting TMEs using spatial transcriptomics with the Xenium platform and Hematoxylin and Eosin (H&E) stains. CellClique integrates both generative and analytical capabilities to provide a comprehensive view of TMEs. On the generative end, CellClique uses generative AI to model both gene expressions and cell-neighborhood interactions for cell annotation, gene expression prediction, and gene imputation. On the analytical end, CellClique offers a comprehensive four-way spatial analysis scheme aimed at 1) understanding the effects of neighboring cells on a target cell, 2) dissecting cancer-normal cell boundaries, 3) observing immune invasion mechanisms, and 4) exploring cellular subtypes with the TME. We applied CellClique to study the cellular landscape in lung and colon cancers using multiple different neighborhood analyses. We generated proximal (10 microns), local (60 microns), and distal neighborhoods (120 microns) for each cell within each tissue and found distinct expression profiles of tumor-infiltrated lymphocytes, which are functionally different from lymphocytes excluded from the tumor. Additionally, we discovered expression variation patterns in cancer-normal boundary cell communities with various cell compositions. These compositions changed significantly as distance from the boundary changed. We also compared lymphocytes at different stages of life by analyzing expression differences among lymphocytes classified with over 80% confidence, those with a 50% probability of being lymphocytes, and those with below 20% probability. We further compared how these neighborhoods changed between lung cancer and colon cancer and showed cancer specific spatial deviations and expression. Together, these methods paint a complex picture of the TME but also identifies numerous avenues for future discovery and molecular investigation. These new approaches will guide future cellular therapy and the development of new treatments. The future integration of AI and machine learning will continue to make a tremendous impact on spatial transcriptomics and our approach to treating disease.

#2419 Impact of immune contexture on immunotherapy response in NSCLC: insights from multiplex IHC and machine learning-based phenotyping.

Jean-Philippe Guegan[1], Imane Nafia[1], Florent Petraud[2], Lucile Vanhersecke[3], Christophe Rey[1], Alban Bessede[1], Antoine Italiano[2]

[1]Explicyte Immuno-Oncology, Bordeaux, France,[2]Department of Medicine, Institut Bergonie, Bordeaux, France,[3]Department of Pathology, Institut Bergonie, Bordeaux, France

Background: The relationship between immune phenotypes (infiltrated, excluded, and desert) and response to immunotherapy in non-small cell lung cancer (NSCLC) is an area of active investigation. Understanding the tumor immune contexture can indeed provide valuable insights into the tumor microenvironment (TME) and immune dynamics, potentially guiding personalized therapeutic strategies.
Methods: We analyzed a discovery cohort of 148 NSCLC patients using the CD8/PanCK multiplex immunohistochemistry (mIHC) panel to define the immune phenotype. Tumors with <2% of CD8+ cells were classified as "desert." Tumors with >2% CD8+ cells within the tumor area were categorized as "infiltrated," while those with >2% CD8+ cells restricted to the periphery were labeled "excluded." Correlations between immune phenotypes and immunotherapy responses (PFS, OS and response rates) were assessed. Validation was performed on an independent cohort of 117 patients.
Results: In the discovery cohort, tumors with an "infiltrated" phenotype demonstrated significantly better responses to immunotherapy compared to "excluded" and "desert" tumors (p = 0.009 “infiltrated” vs “desert”, p=0.045 “infiltrated” vs “excluded”). Immune exclusion was associated with lack of response. These findings were confirmed in the validation cohort, reinforcing the prognostic value of immune phenotyping in NSCLC. Additionally, tertiary lymphoid structures (TLS) and macrophage infiltration, known predictors of response to immunotherapy in NSCLC, were analyzed using an ISO-compliant multiplex immunohistofluorescence (mIHF) panel. Machine learning algorithms were employed to automate immune phenotyping and produce rapid summaries of TME characteristics. The multiplex IHF panel reliably assessed immune phenotypes, TLS presence, and macrophage infiltration with high reproducibility. Automated phenotyping using machine learning enabled rapid and accurate characterization of TME features, facilitating the integration of immune phenotyping into clinical workflows.
Conclusions: Tumor immune contexture significantly influences the outcomes of immunotherapy in NSCLC. "Infiltrated" tumors are associated with favorable responses, while immune exclusion predicts resistance. Our ISO-certified multiplex IHF panel, combined with machine learning automation, offers a robust and scalable approach to immune phenotyping, supporting its implementation in precision oncology.
Keywords: NSCLC, immune phenotyping, multiplex IHC, immune exclusion, infiltrated tumors, tertiary lymphoid structures, macrophage infiltration, machine learning, immunotherapy response.

#2420 Deep learning enables automated detection of circulating tumor cell-immune cell interactions with prognostic insights in cancer.

Yuanfei Sun[1], Joshua R. Squires[1], Andrew Hoffmann[1], Youbin Zhang[2], Allegra Minor[3], Anmol Singh[3], David Scholten[3], Chengsheng Mao[3], Yuan Luo[4], Deyu Fang[5], William J. Gradishar[2], Massimo Cristofanilli[2], Carsen Stringer[6], Huiping Liu[7]

[1]Department of Pharmacology, Northwestern Univ. Feinberg School of Medicine, Chicago, IL,[2]Department of Hematology and Oncology, Northwestern Univ. Feinberg School of Medicine, Chicago, IL,[3]Northwestern Univ. Feinberg School of Medicine, Chicago, IL,[4]Northwestern Univ. Institute for Artificial Intelligence in Medicine, Chicago, IL,[5]Department of Pathology, Northwestern Univ., Chicago, IL,[6]HHMI at Janelia Research Campus, Ashburn, VA,[7]Northwestern Univ. Feinberg School of Medicine; Chan Zuckerberg Biohub Chicago, Chicago, IL

Background: Circulating tumor cells (CTCs) are critical biomarkers for predicting therapy response and survival in breast cancer patients. Multicellular CTC clusters exhibit enhanced metastatic potential, yet their detection and characterization are constrained by low frequency in blood samples and reliance on labor-intensive manual analysis. Advancing these methods could significantly improve prognostic evaluation and therapeutic strategies.

Methods: Leveraging FDA-approved CellSearch technology and single-cell sequencing, we analyzed 2,853 blood specimens, longitudinally collected from 1358 patients with advanced cancer (breast, prostate, etc) and other diseases. Integrating machine learning and deep learning tools, we developed a novel *CTCpose* platform to automate detection and analysis of CTCs, immune cells, and their interactions. Using artificial intelligence (AI)-driven image analysis, we extracted over 270 cellular and nuclear features including intensity, morphometry, fourier shape, gradient/edge, and haralick of cytokeratin, CD45, and DAPI expression patterns, enabling precise characterization of CTCs, white blood cells (WBCs), CTC clusters, and their interactions with immune cells (WBCs).

Results: The *CTCpose* platform enabled automated identification of CTCs, WBCs, homotypic CTC clusters, heterogenous CTC-WBC clusters, and immune cell clusters, providing comprehensive insights into cell morphology, biomarker expression, and spatial organization. These features correlated with patient survival, disease progression, and treatment response. Our findings highlight the clinical significance of CTC-immune cell interactions and dynamic alterations of CTCs (singles and clusters) and underscore their potential in stratifying patients into distinct risk categories.

Conclusions: This study demonstrates the transformative potential of deep learning in overcoming limitations of traditional CTC detection methods and integrating imaging data with large cohorts of patient data. By automating and enhancing the analysis of CTC-immune cell interactions, we present a robust framework for developing predictive models with direct clinical relevance. This work opens avenues for personalized treatment strategies, underscoring the impact of AI in advancing precision oncology.

#2421 Development and validation of an AI-based model for lymphocyte identification in NSCLC H&E image using spatial transcriptomics.

Seo Hye Park[1], Haenara Shin[1], Hosub Park[2], Jaemoon Koh[3], Kwon Joong Na[1], Hongyoon Choi[1]

[1]Portrai, Inc., Seoul, Korea, Republic of,[2]Department of Pathology, Hanyang University Seoul Hospital, Seoul, Korea, Republic of,[3]Department of Pathology, Seoul National University Hospital, Seoul, Korea, Republic of

Background: Spatial transcriptomics (ST) enables the integration of gene expression data with spatial context in tissue samples, providing high-resolution insights without requiring single-cell dissociation. With subcellular resolution, ST offers a precise method for annotating cell types, surpassing traditional approaches based on manual pathologic annotation. Conventional AI models for pathology images typically rely on annotations from pathologists to label cellular compositions in hematoxylin and eosin (H&E)-stained slides. In contrast, our AI model leverages training data generated from subcellular resolution ST, providing a distinct advantage by achieving higher accuracy and objectivity in identifying specific cell types. In this study, we focus on lymphocyte identification in non-small cell lung cancer (NSCLC) pathology images to demonstrate the capabilities of this approach.
Methods: This model analyzes H&E slide images obtained from surgical specimens of NSCLC patients. The training dataset consisted of ST data (Xenium, 10X genomics) and H&E images from 90 NSCLC samples collected from a single institution. After aligning ST data with H&E images, cell-type masks were generated for lymphocyte labeling. For performance evaluation, 456 patches (256 × 256 pixels, 0.45 μm/pixel) were randomly selected from 30 NSCLC H&E slide images. Two pathologists independently annotated lymphocytes in these patches. Of the 456 patches, 355 with consistent annotations between the two pathologists were selected for model evaluation. The consensus annotations served as the reference standard to assess the model's performance in lymphocyte identification.
Results: This model achieved an AUROC of 0.8411, indicating high diagnostic accuracy. The optional threshold was determined to be 0.3870, with specificity and sensitivity of 72.76% and 78.92%. Compared to two pathologists' consensus annotations, the model showed a sensitivity of 81.66%, specificity of 69.70%, and accuracy of 77.29% for lymphocyte identification.
Conclusion: This AI model trained from subcellular ST provides a robust performance for analyzing the distribution of lymphocytes in NSCLC H&E images. This model offers an innovative approach to analyzing the composition of cells from H&E images, demonstrating its potential contribution to TME research and the development of precision medicine.

#2422 Fine-grained inflammatory cell segmentation in histopathology with deep learning.

Pierre-Antoine Bannier, Benjamin Adjadj, Sebastien Mandela, Guillaume Horent, Thomas Mathieu, Ulysse Marteau, Valentin Gaury, Laura Dumont, Aurore Lyon, Reda Belbahri, Benoit Schmauch, Kathryn Schutte, Lucie Gillet, Katharina Von Loga, Caroline Hoffmann

Owkin, Paris, France

Introduction: Deep learning can advance clinical research by improving patient subtyping and uncovering biological insights. However, many models have limited interpretability, reducing clinical utility. To address this, we developed a model that detects, segments, and classifies cells on hematoxylin and eosin (H&E) slides, including understudied types like neutrophils, eosinophils, and plasmocytes, enabling novel biomarkers related to the tumor microenvironment (TME).

Methods: We collected a novel training set composed of 1,479 patches of size 112µm x 112µm from H&E-stained TCGA slides across five indications: bladder cancer (n=400), colon adenocarcinoma (n=242), lung adenocarcinoma (n=231), lung squamous cell carcinoma (n=220), and mesothelioma (n=386). We collected two external validation sets including patches from muscle invasive bladder cancer (MIBC) (n=79) and mesothelioma (n=48) cohorts. For both training and validation, three expert pathologists annotated cells into seven classes: cancer cells, lymphocytes, fibroblasts, neutrophils, eosinophils, plasmocytes, and others. We used a consensus rule to resolve discrepancies at the cell level between pathologists. In total, 151,404 nuclei were annotated in the training set and 9,565 in the validation set.

We trained a model in 3-fold cross-validation on the training set. We independently evaluated its performance on our validation sets and on the Lizard dataset (colorectal cancer). For each cell type we report the panoptic quality (PQ), which combines detection accuracy and segmentation quality.

Results:

Class-wise panoptic quality metrics across training and validation cohorts

Class-wise PQ metrics	Cancer Cells	Lymphocytes	Fibroblasts	Neutrophils	Eosinophils	Plasmocytes
Cross-validation performance from literature for comparison:Lizard dataset. Source: https://arxiv.org/pdf/2108.11195	N/A	0.58 (0.05)	**0.50 (0.01)**	0.16 (0.04)	0.22 (0.04)	0.37 (0.04)
Cross-validation performance: our model (training set)	**0.61(0.02)**	**0.66(0.02)**	**0.50(0.01)**	**0.51(0.01)**	**0.53(0.04)**	**0.66(0.01)**
External transfer: Lizard dataset	N/A	0.42[0.41 - 0.42]	0.39[0.39 - 0.40]	0.40[0.38 - 0.41]	0.31[0.30 - 32]	0.42[0.41 - 0.43]
External transfer: MIBC	0.53[0.49 - 0.57]	0.49[0.40 - 0.60]	0.40[0.35 - 0.44]	0.27[0.19 - 0.49]	0.42[0.21 - 0.59]	0.52[0.26 - 0.65]
External transfer: Mesothelioma	0.56[0.53 - 0.59]	0.55[0.46 - 0.61]	0.43[0.39 - 0.48]	0.23[0.05 - 0.41]	0.46[0.33 - 0.76]	0.48[0.22 - 0.89]

Conclusion: Our model demonstrates consistent performance across multiple validation cohorts, including understudied cell types for which it even outperforms models trained in-domain on Lizard. This reliability highlights its potential for broader clinical applications. By enabling the identification of immune cells, the model paves the way for designing novel interpretable TME-related biomarkers.

#2423 Automated classification of pediatric sarcoma using digital histopathology.

Adam Thiesen[1], Sergii Domanskyi[1], Ali Foroughi pour[2], Jingyan Zhang[3], Todd B. Sheridan[4], Steven B. Neuhauser[5], Alyssa Stetson[6], Katelyn Dannheim[6], Danielle B. Cameron[6], Shawn Ahn[7], Hao Wu[7], Emily R. Christison-Lagay[7], Carol J. Bult[5], Jeffrey H. Chuang[1], Jill C. Rubinstein[1]

[1]The Jackson Laboratory for Genomic Medicine, Farmington, CT,[2]St. Jude Children's Research Hospital, Memphis, TN,[3]Johns Hopkins University, Baltimore, MD,[4]Hartford Healthcare, Hartford, CT,[5]The Jackson Laboratory, Bar Harbor, ME,[6]Massachusetts General Hospital, Boston, MA,[7]Yale University School of Medicine, New Haven, CT

Introduction: Pediatric sarcomas are challenging to accurately classify due to their rarity and the wide diversity of subtypes. The process requires highly specialized pathologists as well as molecular and genetic testing that is expensive, takes time, and is not universally available. Deep neural network models (DNNs) trained on histopathology slides can reduce the time and cost to diagnosis and attenuate disparities in care based on geographical location and socioeconomic status. Here, we demonstrate the efficacy of automated image analysis for assigning sarcoma diagnoses across centers by identifying Ewing Sarcoma (ES), distinguishing rhabdomyosarcoma (RMS) vs non-rhabdomyosarcoma soft tissue sarcomas (NRSTS), as well as classifying alveolar, embryonal, and spindle cell RMS subtypes.

Methods: Images were collected from Massachusetts General Hospital, Yale School of Medicine, St Jude Children's, and the Children's Oncology Group. A total of 691 images were collected across all centers, including 9 different sarcoma subtypes. Images were harmonized using our published STQ pipeline including focus checking, resolution standardization, and stain normalization. Image tiling and feature extraction was performed comparing multiple deep learning backbones (CTransPath, UNI, CONCH). Tile-level features were transposed to whole slide-level representations using our published SAMPLER method in which each feature is represented as the vector of decile values of its distribution across all tiles. Resulting feature sets were fed into logistic regression models for sarcoma classification tasks. We benchmark this approach against transformer based multi-head self-attention models trained on a V100 GPU.

Results: We are able to distinguish ES from all other sarcoma types with an AUROC of 0.966. In the task of NRSTS v. alveolar RMS v. embryonal RMS we achieve an AUROC of 0.939. Restricting to RMS subtypes, we distinguish alveolar from embryonal with an AUROC of 0.95. Also, despite uneven sample representation, we obtain an AUROC of 0.88 for alveolar v. embryonal v. spindle type RMS. Finally, we have developed spatially-resolved attention maps, which provide interpretability for the regions of a slide that contain malignant cells.

Conclusion: To our knowledge, we have amassed the largest multicenter pediatric sarcoma imaging dataset with broad representation across subtypes, anatomical locations, race, and sex. Our pipeline allows for further integration of images from any center, which may be co-analyzed to attenuate center-specific batch effects. Our classification accuracies are state of the art for multiple tasks fundamental to clinical sarcoma pathology, including novel multiclass distinction among three different RMS subtypes. Importantly, our pipeline and SAMPLER model can be run with minimal computational requirements, allowing for broad accessibility.

#2424 Integrating digital spatial profiling and H&E images to develop predictive biomarkers for immunotherapy outcomes in triple-negative breast cancer from imputed spatial gene expression.

Thazin Nwe Aung[1], Tianci Song[2], Lajos Pusztai[3], Mark Gerstein[4], David L. Rimm[1], Jonathan H. Warrell[5]

[1]Pathology, Yale School of Medicine, New Haven, CT,[2]Machine Learning Department, NEC Laboratories America, Princeton, NJ,[3]Medical Oncology, Yale School of Medicine, New Haven, CT,[4]Molecular Biophysics and Biochemistry, Yale School of Medicine, New Haven, CT,[5]Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT

Introduction: Triple-negative breast cancer (TNBC) is an aggressive subtype with poor prognoses and limited biomarkers for predicting treatment outcomes [1]. Tumor-infiltrating lymphocytes and PD-L1 expression, while associated with immune checkpoint inhibitor (ICI) efficacy, fail to predict responses reliably [2, 3]. Spatial transcriptomics is a cutting-edge technology that enables the precise mapping of gene expression within tissue samples. However, the high cost of sequencing limits its clinical utility. This study aims to develop cost-effective predictive biomarkers by integrating spatial transcriptomic data and machine learning to impute gene expression from widely available Hematoxylin and Eosin (H&E) images.

Methods: This study involves two cohorts of TNBC patients treated with pembrolizumab (N=75) and durvalumab (N=57) in the neoadjuvant setting. Digital Spatial Profiling (DSP) was used to generate Spatial transcriptomics data. Gene signatures predicting ICI outcomes were developed and validated using Least Absolute Shrinkage and Selection Operator (LASSO) regression models. Imputed gene expression was derived from H&E images, employing adaptive spatial graph neural networks (asGNN [4]), and evaluated against DSP data on a subset of patients. Signatures were trained using imputed transcriptomics directly and in combination with a hold-out set of H&E images. Prediction accuracies were evaluated against spatial transcriptomics and clinical outcomes using mean squared error and AUC.

Results: Imputed gene expression from H&E images closely mirrors spatial transcriptomic profiles. Imputed spatial gene expression using asGNN achieved better prediction of DSP expression than competing methods (HE2RNA [5] and Sequoia [6]) and non-adaptive GNN methods (p=0.031). Signatures trained directly using H&E imputed spatial expression achieved accurate prediction of treatment outcomes (AUC=0.75±0.24). Integrating the DSP transcriptomics further increased the accuracy of the predictions; the signatures trained using imputed transcriptomics combined with DSP data achieved higher prediction accuracy (AUC=0.85±0.08), which significantly outperformed the non-DSP trained models (p=0.021).

Conclusion: This study demonstrates the feasibility of leveraging H&E images for gene expression imputation in TNBC. Future work will optimize models across diverse cancer types and expand validation with independent datasets. This innovation advances personalized medicine by bridging the gap between cutting-edge spatial transcriptomics and routine clinical diagnostics.

#2425 AI-powered approaches accurately predict t(11;14) positive multiple myeloma from H&E-stained histologic sections by identifying regions demonstrating lymphoplasmacytic cytology.

Neel Patel[1], Raymond Biju[1], Syed Ashar Javed[1], Sandrine Degryse[1], **Lara Murray**[1], Xiao Zhou[2], Jeremy A. Ross[2], William Wijaya[2], Pedro Munoz[2], Aditee Shrotre[2], Joann Palma[2], Patrick Calpazi[2], Yan Li[2], Pok Fai Wong[2], Francine Chen[2], Kenneth Emancipator[2], Kevin Kolahi[2]

[1]PathAI, Boston, MA,[2]AbbVie, Inc., North Chicago, IL

Venetoclax, a highly selective oral BCL-2 inhibitor, has demonstrated efficacy in patients with t(11;14) multiple myeloma (MM). Fluorescence *in situ* hybridization (FISH) is the mainstay methodology to determine t(11;14) status, but requires significant quantities of bone marrow tissue or aspirate and is low-throughput. Herein, we: (i) describe an artificial intelligence (AI)-based model that accurately predicts t(11;14) status from routine H&E-stained MM whole slide images (WSIs) and (ii) demonstrate that the biological signal underlying the model predictions is driven by regions containing the lymphoplasmacytic phenotype of t(11;14) positive MM cells.

H&E-stained MM WSIs (n=231) with known t(11;14) status were split into training/validation/test sets (60/20/20), and CNN-based tissue and cell segmentation models were trained from expert pathologist annotations to identify high density regions of MM cells. Additive multiple instance learning (aMIL) models with 5-fold cross validation were then trained using embeddings from pathology universal transformer (PLUTO) foundation model backbone [1], to predict t(11;14) status. To interpret the biological signal underlying aMIL model predictions, nuclear features [2] for MM cells and sparse autoencoder (SAE) dimensions [3] (based on PLUTO embeddings) were extracted from the model-predicted t(11;14) positive and negative regions. Mann-Whitney U test was used to compare distribution of nuclear features between t(11;14) positive and negative regions. Activation frequency (AF) delta (AF in positive images - AF in negative images) was used to find the SAE dimensions most active in t(11;14) positive regions.

Validation and held-out test sets were leveraged to evaluate the aMIL model's performance for predicting t(11;14) status, resulting in AUROC of 0.813 and 0.849, respectively. Upon investigating the regions predicted as t(11;14) positive, analyses revealed that these contained MM cell nuclei with higher circularity, solidity and less variability in shape and size (p-values<0.001 for all comparisons) compared to those predicted as t(11;14) negative. Additionally, SAE dimensions capturing circular cells (SAE-2202) and lymphocytes (SAE-1567, SAE-1355) were significantly more active in model-predicted t(11;14) positive images compared to the predicted negative images (AF delta- SAE-2202: 0.73, SAE-1567: 0.61, SAE-1355: 0.57).

t(11;14) status can be predicted with high accuracy using routine H&E WSI. Model predictions rely on biologically meaningful signals based on regions containing MM cells with lymphoplasmacytic morphology. This technology has the potential to facilitate screening for t(11;14)-directed MM clinical trials.

References

[1] *arXiv:2405.07905* (2024). [2] *npj Precis. Onc.* 8, 134 (2024). [3] *arXiv:2407.10785* (2024).

#2426 TMEseg: Connecting histopathology with spatial transcriptomics through tumor microenvironment segmentation for lung cancer.

Xiaoxi Pan[1], Maria E. Salvatierra[2], Caner Ercan[2], Lakshmi Kakarala[2], Wei Lu[2], Ou Shi[2], Idania C. Lubo Julio[2], Ignacio I. Wistuba[2], Luisa M. Solis Soto[2], Yinyin Yuan[1]

[1]Department of Translational Molecular Pathology, and The Institute for Data Science in Oncology, UT MD Anderson Cancer Center, Houston, TX,[2]Department of Translational Molecular Pathology, UT MD Anderson Cancer Center, Houston, TX

The tumor microenvironment (TME) plays a key role in lung cancer progression. Spatial transcriptomics offers insights into tumor heterogeneity but relies on integration with histological features to decode tumor transcriptomic programs, which is challenging due to TME complexity. To overcome this, we developed AI models that accurately identify 10 tissue types from histology images, streamlining TME analysis.

We used public annotations to train AI models, DeepLabV3+ and Segformer, for TME segmentation. The training set included hematoxylin&eosin-stained slides from breast and lung cancers in TCGA, combined with newly curated annotations from TCGA and four internal slides of surgically resected tumors, totaling 3403 patches (breast: 3110; lung: 293) at 0.44 μm/pixel. The testing set included 2000 patches from lung cancer slides at 1 μm/pixel. The models were trained to classify 10 tissue types: tumor, stroma, inflammatory, necrosis/hemorrhage, adipose, bronchi epithelium, vessel, macrophage area, alveoli, and muscle. For comparison, predictions of adipose, vessel, and muscle were grouped into stroma, while inflammatory and macrophage areas were grouped as immune. Model performance was evaluated using the Dice score. We further validated the models using spot-level manual annotations from Visium data for four lung cancer samples. Pathologists labeled spots as tumor, stroma, immune aggregates, macrophage, bronchi epithelium, vessel, and alveoli using Loupe Browser. The type with the highest number of predicted pixels by AI within a spot-based patch was assigned as the predicted label. Concordance was assessed using Fleiss's kappa.

At pixel level, Segformer outperformed DeepLabV3+, achieving an average Dice score of 0.856 and 0.809. At spot level, kappa index between pathologist and Segformer was 0.28-0.66, showing fair (n=2) to good (n=1) agreement, consistent with the concordance between pathologist and DeepLabV3+ (0.34-0.722). Kappa index between Segformer and DeepLabV3+ showed good (n=3) to perfect (n=1) agreements (0.677-0.842). Interestingly, the first annotated sample showed good agreement between the pathologist and AI models (0.66-0.722), whereas the last annotated sample demonstrated only fair agreement (0.28-0.343). Notably, this discrepancy was not observed when comparing Segformer and DeepLabV3+ directly, implying a high reproducibility for AI models. In terms of efficiency, the pathologist required about five hours in theory to annotate a slide containing around 12000 spots, while AI models, running on an A100 GPU, completed the segmentation in about 20 minutes.

Our study shows the effectiveness and efficiency of AI models in recognizing the TME from histology images in lung cancer. These models streamline the integration of tissue histopathological features with Visium assay and facilitate the discovery of image-derived biomarkers.

#2427 Optimization of preprocessing strategies for developing AI-based disease diagnosis model using whole transcriptomic data.

Ki Wook Lee, Hye Jung Min, Hyun Woo Park, Balachandran Manavalan, Young-Jun Jeon

Sungkyunkwan University, Suwon, Korea, Republic of

Current research in medicine and biology predominantly focuses on applying artificial intelligence (AI) to analyze patient-specific imaging data, such as CT and MRI scans. However, the application of AI to next-generation sequencing (NGS) data, particularly transcriptomics, remains underutilized. RNA sequencing data is prone to biological and technical variability during generation, and the choice of error-correction and preprocessing methods can significantly influence downstream analyses. These challenges underscore the need for clear and systematic guidelines to optimize transcriptome data for AI-based analyses. In this study, we aim to identify optimal preprocessing strategies for developing AI models leveraging transcriptomic data. We utilized RNA sequencing datasets from 25 independent cohorts, comprising over 5,800 patients with conditions such as lung adenocarcinoma, colorectal cancer, diabetes, and other diseases. A total of 18 combinatorial preprocessing pipelines were systematically assessed, encompassing 6 normalization methods (Raw, CPMTMM, RLE, UQ, RPKM, TPM) and 3 scaling methods (None, MinMax, Z-score). Over 20,000 transcripts were processed using each combination of normalization and scaling techniques. We then developed disease diagnosis models using 13 machine learning and deep learning algorithms. The performance of these models was evaluated to identify the most effective preprocessing strategies for transcriptomic data. We identified algorithm-specific optimal preprocessing strategies to develop robust AI models utilizing transcriptomic data. Performance variations driven by normalization and scaling methods were observed for each algorithm, with these differences being particularly pronounced in datasets characterized by inherently lower model performance (difficult tasks). Our findings underscore the critical role of preprocessing in shaping model outcomes and provide a foundation for the development of tailored AI frameworks for disease diagnosis. This study offers a systematic approach to optimize transcriptomics-based predictive modeling, advancing the integration of AI into transcriptomic data analysis for clinical applications.

#2428 Artificial intelligence for predicting spatial proteomics using high-plex digital spatial profiling in carcinomas.

Yasin Shokrollahi[1], Tanishq Gautam[1], Alejandra Serrano[2], Simon P. Castillo[1], Pingjun Chen[1], Karina Pinao[1], Maria Esther Salvatierra[2], B. Leticia Rodriguez[1], Patient Mosaic Team[3], Luisa M. Solis Soto[2], Yinyin Yuan[1], Xiaoxi Pan[1]

[1]Department of Translational Molecular Pathology, and The Institute for Data Science in Oncology, UT MD Anderson Cancer Center, Houston, TX,[2]Department of Translational Molecular Pathology, Division of Pathology and Laboratory Medicine, UT MD Anderson Cancer Center, Houston, TX,[3]UT MD Anderson Cancer Center, Houston, TX

Background: High-plex digital spatial profiling (DSP) enables mapping protein expression onto tissue context to study heterogeneity of tumor microenvironment (TME). However, current techniques rely on manual annotations to select representative regions for profiling, introducing subjectivity and uncertainty. We present a deep-learning approach to predict spatial proteomics associated with immune response and proliferation in carcinomas from tissue space to high-throughput protein expression analysis across the full sections while preserving spatial context.

Methodology: Our study utilized a diverse dataset of 62 carcinomas and adenocarcinomas cases, with major types including Papillary Urothelial Carcinoma (25) and Colorectal Adenocarcinoma (5) among others, totaling 721 regions of interest (ROIs). Using morphology markers (panCK, CD45) for mIF images and 12 proteins for profiling, we conducted a two-phase study. First, YOLOv8 identified epithelial (panCK+) and immune cells (CD45+) from mIF images and correlated their abundance with protein expression. Second, we developed a multi-instance learning framework using Swin Transformer to predict protein expression from mIF images, processing 256×256 pixel adjacent patches (average 1,947 per slide, total 120,729) with attention mechanisms. The dataset was split into training (47), validation (9), and test (6) sets.

Results: We observed strong correlations between cell abundance from mIF images and protein expression, (panCK: rho=0.624, p<0.001; CD45: rho=0.676, p<0.001), validating the effectiveness of the YOLOv8 model in cell recognition. The multi-instance model demonstrated promising prediction performance, marginally improving the correlations for panCK (rho=0.701, p<0.001) and CD45 (rho=0.699, p<0.001) when compared with YOLOv8. For markers included in the protein profiling DSP panel but not for the morphology, our model captured the signals for immune checkpoint and proliferation markers: PD-L1 (rho=0.741, p<0.001), Ki-67 (rho=0.730, p<0.001). Moderate correlations were observed for stromal and immune cell markers (FAP-alpha: rho=0.569, p<0.001; CD163: rho=0.465, p=1.45e-03; CD3: rho=0.409, p=2.02e-02; CD4: rho=0.381, p=7.46e-03; FOXP3: rho=0.344, p=3.24e-02) while a weak correlation for CD8 (rho=0.210, p=4.83e-02). These findings suggest that cellular morphology and spatially encoded information in the TME can be learned by our model to predict immune checkpoint expression and proliferation.

Conclusion: Our integration of mIF images with spatial proteomics enables high-throughput protein expression prediction from limited markers, potentially extending to H&E images. This could reduce sequential biopsies, enabling real-time treatment monitoring. The model's PD-L1 prediction capabilities provide insights into tumor-immune interactions to guide immunotherapy decisions.

#2429 A multi-modal learning framework to integrate digital pathology image and multi-omics data in breast cancer.

Naresh Prodduturi[1], **Xiaojia Tang**[1], Hamid R. Tizhoosh[2], Kevin J. Thompson[1], Richard Weinshilboum[3], Karthik V. Giridhar[4], Judy C. Boughey[5], Eric W. Klee[1], Liewei Wang[3], Matthew P. Goetz[4], Vera Suman[1], Krishna R. Kalari[1]

[1]Department of Quantitative Health Sciences, Mayo Clinic, Rochester, MN,[2]Department of Artificial Intelligence and Informatics, Mayo Clinic, Rochester, MN,[3]Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN,[4]Department of Oncology, Mayo Clinic, Rochester, MN,[5]Department of Surgery, Mayo Clinic, Rochester, MN

Deep learning has advanced digital pathology and multi-omics data analysis. However, there is still a critical need to integrate these modalities to better understand complex diseases. Deep learning models, such as Prov-Gigapath, a state-of-the-art pathology foundation model, have been developed for analyzing whole-slide images (WSI). Our previously published deep-learning framework, OmicsFootPrint (OFP), transforms high-dimensional multi-omics data into intuitive genomically-arranged circular images and utilizes transfer learning to overcome the challenges of high-dimensional, low-sample-size data. In this study, we aimed to combine WSI and multi-omics data to develop a framework capable of distinguishing between invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC), which is clinically significant as it may help explain differential responses to therapies, such as tamoxifen versus letrozole. We enhanced the performance of our OFP model by dividing each multi-omics circular image (1024x1024x3 pixels) into 16 equal-sized patches (256x256x3 pixels). Each patch was trained using a separate EfficientNetV2 architecture with pre-trained ImageNet weights. The probability outcomes from these 16 models were subsequently combined through a meta-learner model using the AutoGluon framework. Concurrently, WSI images corresponding to the same patients were independently trained, maintaining consistent train/validation/test splits. The Prov-Gigapath model, which was trained using a dataset from the Providence Health System, was employed to extract patch-level embeddings from the WSI data. These embeddings were fine-tuned using a LongNet-based masked autoencoder to predict outcomes. Finally, predictions from the OFP model and the WSI-based Prov-Gigapath model were integrated through a meta-learner, preserving the original train/validation/test split. Model performances (AUC) were evaluated using the held-out test set. The gold standard used for the analysis was histological identification of IDC versus ILC, against which the performance of omics data alone, WSI data alone, and the multi-modal integration approach were compared. We evaluated our integrated model as a proof-of-concept using The Cancer Genome Atlas (TCGA) breast cancer cohort, comprising 92 ILC and 391 IDC samples. Multi-omics data, including gene expression, microRNA, copy number variation, and RPPA, were analyzed using the OFP framework, while WSI data were processed using the Prov-Gigapath method. The results demonstrated that the multi-modal integration approach significantly outperformed individual modalities, achieving an AUC of 0.98 compared to 0.87 for OFP (original), 0.95 for OFP (16-patches), and 0.92 for WSI. Integrating WSI and multi-omics data improves predictive accuracy, significantly improving the classification of breast cancer subtypes over histology alone.

#2430 Artificial intelligence enables prediction of MET amplification & associated morphologic features from H&E-stained NSCLC specimens.

Nhat Le[1], **Ylaine Gerardin**[1], Syed Ashar Javed[1], Jennifer Hipp[1], Jacqueline Brosnan-Cashman[1], Miles Markey[1], Lara Murray[1], Bahar Rahsepar[1], Aditee Shrotre[2], Adam Luo[3], William Wijaya[2], Dawn Spelke[2], Pok Fai Wong[2], Joshua Hernandez[2], Sheila Bheddah[2], Ramya Seshadri[4], Steven Chirieleison[2], Patrick Caplazi[2], Yan Sun[2], Athan Vasilopoulos[4], Amita Mistry[4], Yan Li[2], Francine Chen[2], Peter Ansell[4], Kenneth Emancipator[4], Kevin Kolahi[2]

[1]PathAI, Inc., Boston, MA,[2]Precision Medicine, AbbVie, South San Francisco, CA,[3]Exploratory Statistics, AbbVie, North Chicago, IL,[4]Precision Medicine, AbbVie, North Chicago, IL

Background: MET receptor tyrosine kinase, when upregulated by mutation, fusion, or gene amplification, acts as an oncogenic driver in many cancers, including non-small cell lung cancer (NSCLC). Tumors with MET gene amplification (MET-amp) exhibit distinct clinical behavior. Thus, distinguishing MET-amp from non-MET-amp cases, typically determined by fluorescent in situ hybridization (FISH), is crucial. Here, we introduce an artificial intelligence (AI) model to predict MET-amp status directly from whole slide images (WSIs) of routine hematoxylin and eosin (H&E)-stained histologic sections and reveal elevated densities of highly pleomorphic (HP) tumor cells in MET-amp NSCLC.
Methods: Additive multiple instance learning (aMIL) models were trained with 5-fold cross-validation to predict MET-amp, as determined by FISH, from H&E-stained WSIs from multiple cancers (Ntotal=1,384, Namp=545) using embeddings from the pathology universal transformer (PLUTO) foundation model backbone1,2. Model performance was assessed on a held-out test set of NSCLC H&E-stained WSIs (N=170) using the area under the receiver operating characteristic curve (AUROC) for FISH status comparison. PLUTO embeddings were also used to train a logistic regression classifier to identify HP tumor cells in WSIs. These embeddings were extracted from pathologist-annotated HP tumor cells (n=1,725) and non-HP tumor cells (n=4,872) in NSCLC WSIs. The trained model was deployed on embeddings extracted from all tumor cells in each WSI, and probability scores were converted to HP/non-HP cell predictions using a cutoff of 0.5. The proportion of predicted HP cells to total tumor cells was computed for each NSCLC WSI (Ntotal=262, Namp=131).
Results: The aMIL MET-amp prediction model achieved an AUROC of 0.74 when deployed on the NSCLC test set. The HP cell classifier consistently and accurately identified HP tumor cells on a test set for this task (n=340 HP tumor cells and n=778 non-HP tumor cells), achieving sensitivity=98% and specificity=84%. When deployed on NSCLC H&E-stained WSIs, the model-predicted proportion of HP cells was significantly elevated in MET-amp cases (p=0.0004, Mann-Whitney U test). The proportion of HP cells was also predictive of MET-amp in cases with adenocarcinoma (AUROC=0.65).
Conclusions: We demonstrate that an aMIL model using embeddings from a pathology foundation model accurately predicts MET-amp directly from H&E. MET-amp was further shown to be associated with an increased number of HP cells and is consistent with the report that MET-amp tumors are more likely to be poorly differentiated3. This work may enable a screening method to identify patients likely to have MET-amp from routinely collected H&E slides.

1. Juyal, D, et al. (2024) arXiv:2405.07905

2. Javed, SA et al. (2022) arXiv:2206.01794

3. Overbeck, Y, et al. (2020) Transl Lung Cancer Res. 9:603-16

#2431 Enhanced cancer detection using TransUnet for low-resolution histopathology images across multiple cancer types.

Muhammad Shaiq Paracha, Faisal F. Khan, Arsalan Riaz, Madina Shirdel

Precision Medicine Lab, Peshawar, Pakistan

Digital histopathology has become an indispensable tool for cancer diagnosis and prognosis, enabling pathologists to analyze tumor morphology using Whole Slide Images (WSI). However, the high cost and limited availability of high-resolution imaging equipment in underdeveloped regions pose significant challenges. This study investigates the use of Low-Cost Low-Resolution (LCLR) histopathology images for detecting four cancer sites: Oral, Gastrointestinal, Colorectal and Breast cancers.
We compared the performance of four models: AlexNet, EfficientNet, Vision Transformer and TransUnet. These models were trained on two datasets: the Tabassum et al. (2020) OSCC dataset (1,223 images, 10X and 40X magnification) used for selecting the right model and HistoVault v1 (17,500 images, 10X and 40X magnification) for training the selected model for all cancer sites. TransUnet, which integrates U-Net's encoder-decoder architecture with Transformers, was fine-tuned with hyperparameters including a learning rate of 1e-5, batch size of 32 and a dropout rate of 20%. The evaluation metrics included accuracy, sparse categorical cross-entropy loss and confusion matrices to quantify the models' robustness.
TransUnet achieved the highest accuracy of 96.8% with a loss of 3% over other models on Tabassum et al. (2020) OSCC dataset. Individual accuracies for Oral, Gastrointestinal, Colorectal and Breast cancers were 98.7%, 95.9%, 96.1% and 97.2% on Histovault v1 dataset. Its coarse-to-fine attention refinement enabled precise segmentation in noisy, low-resolution images, outperforming other models. Notably, the model exhibited robust generalization for multi-class classification, achieving a test accuracy of 90.5% in differentiating eight classes (Non Cancerous and Cancerous with each cancer site).
TransUnet possesses significant promises for LCLR image-based cancer detection, especially in environments with limited resources. Its hybrid design improves accuracy and segmentation precision in challenging datasets by combining the advantages of transformers and convolutional neural networks. With the objective to improve precision diagnosis, future research will investigate cancer grading and use explainable AI algorithms.

#2432 Automated detection of genome-doubled tumors in H&E whole-slide images: towards a novel visual biomarker for predicting overall survival.

Bojan Karlas[1], Marc Glettig[2], Giuseppe Tarantino[3], Jiajia Chen[3], Guihong Wan[1], Tyler J. Aprati[3], Haitham A. Elmarakeby[3], Christine G. Lian[4], Yevgeniy R. Semenov[1], David Liu[3]

[1]MGH/Harvard Medical School, Boston, MA,[2]ETH Zurich, Zurich, Switzerland,[3]Dana-Farber Cancer Institute, Boston, MA,[4]Brigham and Women's Hospital, Boston, MA

Background: Several studies have outlined whole genome-doubled (WGD) tumor tissue characterized by elevated genome ploidy as an important biomarker positively associated with immunotherapy response and overall survival of cutaneous melanoma patients (Liu NatMed2019, Tarantino BioRXiv2022), as well as conferring therapeutic vulnerabilities (Quinton Nat2021). A robust and cost-effective method for detecting this biomarker would significantly improve patient risk assessment and treatment optimization. Unfortunately, current approaches depend on whole-exome sequencing of both tumor and normal tissue - a costly process rarely performed in standard clinical practice.

Methods: We focus on H&E-stained whole-slide images (WSIs) routinely collected to diagnose cancer patients. We use publicly available melanoma WSIs (TCGA SKCM) and ploidy calls (Conway NatGen2020) to train a machine learning pipeline for classifying WSIs as diploid or WGD. After splitting the WSI into tiles we apply a wide array of recently released deep-learning foundation models that have been pre-trained on vast amounts of WSIs and were shown to be effective at extracting biologically meaningful features. To demonstrate the utility of these features for predicting WGD, we use tile-level features to train several types of slide-level classifiers. To evaluate the prognostication potential of H&E-inferred WGD, we use it to fit a univariate Cox survival model using publicly available survival data (PanCanAtlas). We perform a cluster analysis on tile-level features and model-assigned importance scores leveraging the help of a board-certified pathologist to identify visually interpretable morphological features significantly correlated with WGD.

Results: We performed both an internal cross-validation using our training cohort and an external validation cohort collected at Dana Farber Cancer Institute. We evaluate 100s of deep learning pipelines, each characterized by a unique combination of foundation model, slide classifier, and various other hyperparameters. Best-performing pipelines predict WGD with AUC >0.7 on both internal and external validation cohorts, demonstrating a robust association between tissue morphology and ploidy. Furthermore, in univariate Cox survival analyses, best-performing pipelines achieve a hazard ratio <0.7 with a p-value <0.05, underlining the ability of WGD to serve as a visually identifiable biomarker for predicting melanoma outcomes.

Conclusion: Our study demonstrates the potential of H&E-based deep learning pipelines to robustly predict WGD in melanoma tissue with reliable performance across internal and external cohorts. Furthermore, WGD inferred from tissue morphology shows significant prognostic value for overall survival, offering a cost-effective and clinically feasible biomarker for personalized treatment strategies.

#2433 Uncovering molecular insights in breast cancer prognostics utilizing foundation model and digital pathology.

Shlomit Strulov Shachar[1], Tal Etan[2], Didi Feldman[2], Ariella Deutsch-Lukatsky[1], Lee Ben-Itzhak[2], Inbar Gilboa[3], Inbal Gazy[3], Ron Sinai[3], **Addie Dvir**[4], Reva Basho[5], Amir Sonnenblick[1], Dov Hershkovitz[6]

[1]Oncology Division, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel,[2]Tel Aviv Sourasky Medical Center, Tel Aviv, Israel,[3]Imagene AI, Tel Aviv, Israel,[4]Imagene AI, Miami, FL,[5]Ellison Medical Institute, Los Angeles, CA,[6]Institute of Pathology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel

Despite widespread use of clinicopathologic features and multigene assays, such as the Oncotype DX test, to guide clinical decisions, Breast Cancer (BC) remains a leading cause of mortality. There is a need to identify novel biomarkers to better prognosticate patients and ultimately influence treatment strategies to improve patient outcomes. In this study we developed an AI model for the prediction of BC distant disease recurrence (DDR) risk and applied it on the TCGA cohort to extract insights from gene expression data. Whole slide images (WSIs) of early HR+ HER2- BC cases were collected from Sourasky Medical Center. The median age was 61 years (range 25-75), median follow-up time 122.8 months (range 22.5-197.4) and median time to DDR 36.2 months (range 2.5-55.2). Utilizing a version of our foundation model, CanvOI, embeddings were extracted from WSIs, and a 5-fold cross-validation scheme was applied to develop the recurrence risk model. The model was then applied on the TCGA BC cohort WSIs, which included 98.8% females (n=1027), 55.3% HR+ HER2- (n=575) cases, and a median age of 59 years (range 26-90). Analysis of the WSIs of the TCGA BC cohort using the established model assigned a recurrence prediction score for each sample. The cohort was then divided into high-risk samples with the highest prediction scores and low-risk samples with the lowest scores followed by analysis of differentially expressed genes between the groups. The model development cohort included 291 H&E WSIs of female patients with early DDR (n=19, 6.5%) or no recurrence. 1039 TCGA WSIs were then analyzed with the established BC DDR risk model. Analysis of differentially expressed genes between the high AI risk group (n=100) and low AI risk group (n=124) was conducted. Notably, genes linked to estrogen receptor (ER) signaling pathways showed lower expression levels in the high-risk group, consistent with the established association of ER with more favorable outcomes, thus supporting the concept of this method. Further comparative analysis identified PTPRT as the second most differentially expressed gene between the groups, with lower expression in the high-risk group. Previous studies have associated PTPRT with tumor growth suppression, suggesting it may be an interesting candidate for further investigation. This proof-of-concept study highlights the potential of this approach of developing an AI model using a clinical cohort and applying it to an external cohort to extract insights from expression data. This enables the use of small clinical datasets with known outcomes to train models that can then be applied to larger datasets, such as the TCGA, which contain rich expression data, not accessible in the clinical data, to extract meaningful insights. This method effectively bridges the gap between limited clinical cohorts and expansive multimodal datasets, creating opportunities for broader and more precise discoveries.

#2434 Preliminary feasibility of co-registering tissue autofluorescence with 3D spatial transcriptomics for multimodal analysis of destructively profiled colorectal tumor sections.

Zarif Azher[1], Gokul Srinivasan[1], Minh-Khang Le[1], Alos Diallo[2], Harsimran Kaur[3], Fred Kolling[2], Laurent Perreard[2], Scott Palisoul[4], Keluo Yao[1], Ken S. Lau[3], Louis J. Vaickus[4], Xiaoying Liu[4], Joshua Levy[1]

[1]Cedars-Sinai Medical Center, West Hollywood, CA,[2]Dartmouth College Geisel School of Medicine, Hanover, NH,[3]Vanderbilt University, Nashville, TN,[4]Dartmouth Health, Lebanon, NH

Spatial multiomics, combining spatial transcriptomics (ST), which maps gene expression across tissue sections, with assays like LA-ICPTOF-MS (1-micron resolution elemental profiling), can uncover nuanced biomolecular interactions within the tumor microenvironment (TME) linked to tumor progression. However, many spatial assays are destructive, often requiring co-registering serial or non-adjacent tissue sections, introducing discrepancies that hinder spatial multimodal association studies. To assess the impact of profiling distant tissue layers on data analysis from destructively profiled sections, we co-registered H&E staining, autofluorescence (AF) imaging, and ST data across 16 serial sections from two T4 N0/1 colon cancer patients (A & B). AF data were acquired using DAPI and GFP channels on the EVOS™ M7000 Imaging System. VALIS software aligned AF imaging with 40X H&E whole-slide images (Leica Aperio GT450) to create a 3D model of serial sections, applying transformations to same-section Visium CytAssist ST data. Co-registration accuracy across sections was assessed using target registration error (TRE) from QuPath annotations of matching points across 3D serial section inflammation and vascular TME components. ST data were analyzed using 3D and 2D spatial domain analyses with Leiden clustering to identify biologically relevant gene expression patterns. Enrichr gene set enrichment identified pathways (Reactome) associated with 3D domains present in all slices within patients. Correlations of the top 35 spatially variable genes' (Moran's I) expression across sections were assessed, with hierarchical regression modeling used to examine how gene expression correlations changed with increasing section separation (random intercept by gene). AF co-registered with ST across sections with high accuracy, with minor losses due to architectural changes (TRE- A: 9.5±1.0μm, B: 10.4±1.1μm). The number of 2D spatial domains varied across sections (A: 6.4±1.3, B: 5.1±1.5), while 3D ST identified more domains (A: 8, B: 8). Persistent 3D domains were linked to pathways associated with colorectal cancer progression (e.g., A: Extracellular Matrix Degradation, $p=0.001$, B: CD163 Mediating Anti-Inflammatory Response, $p=0.003$). Gene expression correlations across sections for spatially variable genes decreased with greater section separation (A: $\beta=-0.03$, $p<0.001$; B: $\beta=-0.02$, $p<0.001$). Our work demonstrates successful 3D AF-ST co-registration providing non-destructive capture of persistent histological features across sections, crucial for destructively profiled samples. The diminishing correlation across sections highlights the need to explore information loss and biases from serial sectioning, including imputing ST data to improve spatial multiomics associations tied to tumor progression.

#2435 Automatic detection of prostate cancer in 3D pathology datasets based on synthetic immunolabeling of cytokeratins.

Robert B. Serafin[1], Jennifer Salguero-Lopez[2], Rui Wang[1], Sarah Chow[1], Elena Baraznenok[3], Lydia Lan[3], Kevin W. Bishop[3], Michelle Downes[4], Xavier Farre[5], Lawrence D. True[6], Anant Madabhushi[2], Jonathan T. C. Liu[1]

[1]Mechanical Engineering, University of Washington, Seattle, WA,[2]Biomedical Engineering, Emory University, Atlanta, GA,[3]Bioengineering, University of Washington, Seattle, WA,[4]Laboratory Medicine & Pathobiology, University of Toronto, Toronto, ON, Canada,[5]Public Health Agency of Catalona, Lleida, Spain,[6]Laboratory Medicine & Pathology, University of Washington, Seattle, WA

For AI-analysis of 3D pathology datasets, delineating benign and cancerous tissue regions is often a critical first step. For prostate cancer (PCa) this can be challenging due to the complex intermixing of benign and cancerous glands in 3D. Automated detection of cancerous glands could improve the efficiency and accuracy of downstream computational tasks, such as risk stratification using 3D histomorphometric features. For example, we previously developed machine classifiers based on 3D glandular and nuclear morphologies in PCa biopsies but relied on pathologists to manually identify cancer-enriched volumes for analysis, which was tedious and could introduce bias. Here, we present an approach to automatically identify cancer-enriched regions in 3D pathology datasets of prostate tissue. The tissues are labeled with inexpensive small-molecule fluorescence analogs of H&E staining and are imaged with open-top light-sheet (OTLS) microscopy. Generative adversarial networks were used to train separate models to convert our H&E-analog datasets into synthetic 3D immunofluorescence datasets of two distinct biomarkers: a low-molecular weight cytokeratin (CK8) expressed by luminal epithelial cells found in all prostate glands, and a high-molecular weight cytokeratin (CK5) expressed by basal epithelial cells that are only found in benign prostate glands. Each 3D image-translation model was trained on OTLS microscopy images of prostate tissues that were tri-labeled with fluorescent analogs of H&E plus the antibody of interest (i.e. CK5 or CK8). The models achieved high accuracy for synthetic immunolabeling in held-out validation datasets (Dice scores of 0.72 and 0.83 respectively). By training these models to predict the expression of each CK target directly from H&E-analog datasets, we can avoid the time and cost of immunolabeling large intact tissues. The 3D image-translation models were applied to 3D pathology datasets of 3-mm diameter punch biopsies extracted from archived prostatectomies from 100 PCa patients with known biochemical recurrence outcomes. Using the predicted expression of CK5 and CK8, we developed a simple algorithm to generate a spatial heatmap of cancer-enriched regions throughout each biopsy. For validation, we compared these 3D heatmaps against annotations from 3 GU pathologists of 40 regions of interest, yielding an average Dice score of 0.82. We plan to show improved prognostication with classifiers trained on 3D histomorphometric features derived from cancer-enriched regions (identified by our method) vs. all regions of a prostate specimen.

#2436 Prostate cancer risk stratification based on 3D histomorphometric features related to perineural invasion (PNI).

Sarah S. L. Chow[1], Rui Wang[1], Yujie Zhao[1], Robert Serafin[1], Elena Baraznenok[1], Lydia Lan[1], Xavier Farre[2], Kevin Bishop[1], Gan Gao[1], Lawrence D. True[1], Anant Madabhushi[3], Jonathan T. C. Liu[4]

[1]University of Washington, Seattle, WA,[2]Public Health Agency of Catalonia, Lleida, Spain,[3]Georgia Institute of Technology and Emory University, Atlanta, GA,[4]Mechanical Engineering, Bioengineering, Laboratory Medicine and Pathology, University of Washington, Seattle, WA

Prostate cancer (PCa) treatment decisions rely heavily on the examination of 2D histology sections (i.e. Gleason grading). However, the limited sampling of specimens afforded by 2D histopathology, and the ambiguities of viewing cross-sections of complex structures such as prostate glands, can cause high interpathologist variability and nonoptimal treatment decisions, especially for low- to intermediate-grade PCa. Our lab has previously shown that computational analysis of 3D histomorphometric features, such as those derived from gland and nuclear segmentation, can improve PCa risk assessment compared to analogous 2D features. Here, we expand on these findings by exploring the prognostic value of 3D features from nerves. These structures are critical as prostate cancer cells follow them to migrate and metastasize, i.e. perineural invasion (PNI), which is correlated with worse patient outcomes. We therefore aim to show the analysis of 3D features related to PNI can provide additional prognostic value.

To analyze 3D nerve structures, we utilized a 3D deep learning-based segmentation model, nnU-Net, trained on 3D data from punch biopsies extracted from archived formalin-fixed paraffin-embedded (FFPE) prostatectomy specimens and imaged with a 4th-generation open-top light-sheet (OTLS) microscope. To train a segmentation model without requiring tedious manual annotations, prostate specimens were triple labeled with a fluorescent analog of H&E (nuclear and cytoplasmic stains) plus an antibody targeting PGP9.5, which labels nerves. The H&E-analog channels serve as inputs for the nnU-Net model. To train the model, ground-truth 3D segmentation masks were generated from the PGP9.5 immunofluorescence channel. The trained model allows us to generate 3D segmentation masks of nerves directly from specimens labeled with small-molecule (quickly diffusing) fluorescent analog of H&E, which is fast and inexpensive compared with thick-tissue immunolabeling.

The trained 3D nerve segmentation model achieved an average Dice score of 0.86 on held-out validation datasets of tri-labeled specimens (n=10). The segmentation performance was also evaluated on 2D regions (n=53) extracted from 3D pathology datasets of 8 prostatectomy specimens, yielding an average Dice score of 0.64 compared against annotations of nerves generated by a board-certified pathologist.

The trained model has been applied to 3D pathology datasets of 120 archived prostatectomy specimens from patients with known biochemical recurrence (BCR) outcomes. We are extracting 3D histomorphometric features (i.e. spatial features) from the resulting nerve segmentations and are integrating them with 3D features from previously developed segmentations of glands, nuclei, and/or cancer-enriched tissue regions. We aim to show that 3D features related to PNI are superior to analogous 2D features for PCs prognostication.

#2437 Deep-learning triage of 3D pathology datasets for accurate and efficient pathologist assessments.

G. Gao[1], A. H. Song[2], L. E. Barner[3], F. Wang[1], R. Yan[1], H.-C. Hsieh[1], D. Brenes[1], R. Wang[1], S. S. L. Chow[1], K. W. Bishop[3], L. D. True[1], D. M. Reddi[1], W. M. Grady[4], F. Mahmood[2], J. T. C. Liu[1];
[1]University of Washington, Seattle, WA, [2]Mass General Brigham, Boston, MA, [3]Broad Institute of MIT and Harvard, Boston, MA, [4]Fred Hutchinson Cancer Research Center, Seattle, WA

Introduction: Human tissue exhibits heterogeneous morphologies and microenvironments that are readily appreciated in 3D. In the current standard of clinical care, pathologists assess only a few thin 2D sections, typically sampled arbitrarily from one side of a tissue volume/biopsy, in which diagnostically important tissue structures may be missed or misinterpreted. Recent advances in non-destructive 3D pathology enabled by open-top light-sheet (OTLS) microscopy allow for comprehensive imaging of large clinical specimens (e.g. whole biopsies), thus offering the potential to improve the accuracy of diagnostic determinations. While fully automated computational analysis methods are being developed by us and others, a potential early route towards clinical adoption of 3D pathology is to rely upon pathologists for final diagnostic determinations based on viewing familiar 2D H&E-analog cross-sections from the 3D datasets. Because it is not practical for pathologists to manually review massive 3D pathology datasets (equivalent to hundreds of sections per specimen), we present a deep-learning-based triage method to automatically identify the highest-risk 2D cross-sections within 3D pathology datasets for time-efficient pathologist review.
Methods: For each 2D cross-section in a 3D pathology dataset, 2D tissue patches are converted into representative low-dimensional feature vectors using the pre-trained CONCH model. We perform attention-based aggregation of the patches within each 2D cross-section and then integrate neighboring 2D sections across depths to predict a context-aware 2.5D risk score. The ground truth labels for model training are based on consensus pathologist diagnoses.
Results: We implemented the AI-triaged 3D pathology workflow for two clinical scenarios. For prostate cancer risk stratification based on Gleason grading, our 2.5D method achieved an area under the curve (AUC) of 0.93 for triaging low vs. higher-grade prostate cancer cross-sections on around 110 specimens, outperforming AI analysis on individual 2D cross-sections without depth context (AUC 0.87). In another clinical scenario, where endoscopic biopsies from Barrett's esophagus patients were screened for neoplasia (dysplasia or adenocarcinoma), the 2.5D method similarly surpassed its 2D counterpart (AUC 0.92 vs. 0.89) for identifying benign vs. neoplastic cross-sections on around 95 specimens.
Conclusion: These preliminary results indicate that the integration of additional spatial context along the depth dimension enhances the model's discriminative capabilities to triage high-risk cross-sections from 3D pathology datasets. Finally, preliminary clinical validation studies comparing diagnoses based on AI-triaged 3D pathology vs. conventional 2D histopathology are underway to validate that our methods can improve diagnostic accuracy while minimizing pathologist workloads.

#2438 Bridging whole slide images and large language model for slide-level question answering.

Z. Gao[1], K. He[2], W. Su[3], W. McGough[1], I. Machado[1], M. Jimenez-Linan[4], B. Rous[4], R. Wray[1], C. Li[1], X. Pang[3], T. Gong[3], M. Feng[2], C. Li[3], M. Crispin-Ortuzar[1];

[1]University of Cambridge, Cambridge, United Kingdom, [2]National University of Singapore, Singapore, Singapore, [3]Xi'an Jiaotong University, Xi'an, China, [4]Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom

Background: Vision Language Models (VLMs), integrating large language models (LLMs) with vision encoders to enable better understanding and interaction with visual data, have broken entirely new ground in computational pathology. Recent studies have demonstrated that employing a simple linear layer as an 'adaptor' to transform image embeddings, extracted by pathology-specific vision encoders, into LLM-recognizable tokens is effective for developing patch-level VLMs in pathology. However, the gigapixel-scale size of whole-slide images (WSIs) and their pyramidal structure pose significant challenges in designing effective adaptors for slide-level analysis. In particular, different diagnostic questions and tasks at the slide level require focusing on various sub-regions at different magnifications or combining multiple regions and magnifications. Here, we aim to assess adaptors that meet these challenging demands and develop a slide-level VLM to enable slide-level question answering.
Methods: We develop Llama-Path, the first general-purpose slide-level VLM, using WSIs paired with descriptions and question-answer (Q&A) pairs. First, we collect 35,943 WSIs from public databases such as TCGA and GTEx, along with corresponding pathological reports or notes, covering a wide range of tumor and tissue types. Based on these reports and notes, we curate a WSI description dataset and a WSI Q&A dataset (337,000 Q&A pairs) using GPT-4. Then, we extract hierarchical patch embeddings from each WSI by the pathology foundation model, Conch. Next, we propose and evaluate six distinct slide-level adaptors: four vision-only, and two vision-text interactive. These adaptors are finetuned with Llama-3.1-8B across two stages to develop various versions of Llama-Path: The first stage uses the WSI description dataset to provide Llama-Path with a slide-level vision understanding ability. The second stage enhances the slide-level instruction-following capability of Llama-Path with the WSI Q&A dataset. Finally, we evaluate different versions of Llama-Path on a hold-out dataset consisting of 367 tumor and non-tumor cases with 4,440 Q&A pairs.
Results: All versions of Llama-Path demonstrated exceptional performance, each achieving over 90% accuracy in close-ended Q&A pairs. GPT-4 assessed open-ended performances with high accuracy, showing 36% of responses correct, 27% partially correct, and 3% incorrect. The remaining 34% could not be evaluated without access to the corresponding WSIs. The LongNet-based vision-text interactive adaptor outperformed others, likely due to its targeted capture of visual information and simultaneous optimisation of WSI embeddings.
Conclusions: Our findings highlight the potential of VLMs to enhance slide-level computer-aided diagnosis systems. Results from ongoing analyses, based on pathologists' evaluations, will be presented shortly.

#2439 Spatial transcriptomics-informed latent diffusion models generate histopathological images reflective of tumor microenvironment.

Gokul Srinivasan[1], **Minh-Khang Le**[1], Zarif Azher[1], Harsimran Kaur[2], Fred Kolling[3], Keluo Yao[1], Ken S. Lau[2], Xiaoying Liu[4], Louis J. Vaickus[4], Joshua J. Levy[1]

[1]Cedars-Sinai Medical Center, West Hollywood, CA,[2]Vanderbilt University, Nashville, TN,[3]Dartmouth College Geisel School of Medicine, Lebanon, NH,[4]Dartmouth Health, Lebanon, NH

While digitized whole slide images (WSIs) can improve cancer prognosis algorithms, limited data curation given high image complexity/size can hinder performance in underrepresented populations. Synthetic WSI generation offers a solution by simulating tissue heterogeneity and anonymizing data. However, current methods for histopathology cannot reproduce tumor microenvironment (TME) and other biomolecular features with high fidelity at gigapixel scale, limiting further application. Spatial transcriptomics (ST) paired with H&E-stained WSIs can provide this missing context. We present a novel method for using ST to guide image generation by conditioning latent diffusion models (LDM) on 55-micron resolution gene expression data from Visium ST. Preliminary evaluation demonstrates that LDMs can reproduce realistic TME histological features of primary colon tumors. We profiled 41 colon tumors with paired 40X H&E WSIs and Visium ST. A total of 255,744 WSI patches (512-pixel) were matched with 55-micron ST spots for subsequent LDM training. Inspired by single-sample gene set enrichment analysis methods, spot-level ST data were encoded into pathway representations via a CLIP text encoder using the top 50 genes, ranked through gene-wise comparison to non-zero median expression. A modified UNET was fine-tuned for 100,000 iterations (learning rate: 1e-5) to denoise CLIP-encoded ST data into histology images. Two pathologists evaluated 90 held-out randomly selected tissue patches, comparing original and ST-generated images at the same tissue locations. Pathologists- blinded to image origin (real/generated)- assessed the abundance of tumor, stromal, immune, and fat features on a four-point scale and determined whether the original and generated images contained similar histology. Hierarchical bivariate ordinal regression assessed correlations between original/generated features and calculated intraclass correlation coefficients (ICC) as a measure of interrater agreement. Hierarchical logistic regression determined proportion of images with similar histology, with image/pathologist random intercepts. Pathologic examination showed strong correlations for tumor (ρ=0.71; $p<0.001$) and immune (ρ=0.67; $p<0.001$) TME features. Excluding artifacts, stromal (ρ=0.68; $p<0.001$) and fat (ρ=0.57; $p<0.001$) features also correlated. Inter-rater agreement was high/comparable for most features (Real/Generated ICC- Tumor: 0.95/0.96, Stroma: 0.92/0.88, Immune: 0.63/0.69, Fat: 0.57/0.38; $p>0.05$). Pathologists found 86% of images reflected similar histologies (95% CI: [69%-95%]). This study is the first to generate realistic histology from spatially co-registered transcriptomic data. Further refinement is needed to scale the approach to whole tissue slides for prognostic modeling in cohorts with limited data.

#2440 Correlation analysis between AI-based H-score and clinical data in MET IHC stained WSIs.

Hyeon Seok Yang[1], Yunseob Hwang[1], DoKyung Kim[1], Yoon-La Choi[2], Kyungsoo Jung[2], Minjung Sung[2], Young Kee Shin[3], Ji-Hye Nam[4], **Tae-Yeong Kwak**[1], Sun Woo Kim[1], Hyeyoon Chang[1]

[1]Deep Bio Inc., Seoul, Korea, Republic of,[2]Samsung Medical Center, Seoul, Korea, Republic of,[3]Seoul National University, Seoul, Korea, Republic of,[4]LOGONE Bio-Convergence Research Foundation, Seoul, Korea, Republic of

Background: MET protein expression is considered a potential biomarker in lung cancer, but there is no guideline for analysis, and its clinical significance has not been sufficiently analyzed. In order to explore its potential as a biomarker, it is necessary to analyze the relationship between the expression level of the biomarker and clinical data. In this study, we analyze the relationship between H-score, which is automatically measured based on cell detection detected by a deep learning model, and clinical data.
Design: The dataset included 270 whole slide images (WSIs) of non-small cell lung cancer (NSCLC) immunohistochemically stained with a MET antibody (SP44), obtained through resection, biopsy, or endobronchial ultrasound (EBUS). For each of these WSIs, pathologists estimated the proportions for 0, 1+, 2+, and 3+ according to duty, and calculated the H-score based on these values. In addition, the analysis areas were manually specified to exclude the internal controls. We developed analysis models based on deep learning. The cancer area localization model identified the cancer areas for each patch, and the cell analysis model identified the cell membrane, cytoplasm, and nucleus of each cell. Additionally, the MET expression intensity of each cell was classified as 0, 1+, 2+, and 3+ based on the mean diaminobenzidine (DAB) optical density (OD) measured in the cell membrane. The H-score was calculated accordingly.
Result: For each WSI, the correlation between the H-score estimated by AI and the pathologist's H-score was calculated as Pearson's correlation coefficient = 0.6909 ($p<0.0001$). In addition, when analyzing the H-score estimated by AI by tumor subtype of clinical data, the mean H-score of adenocarcinoma samples was estimated to be 177, which was significantly higher than the mean H-score of 116 estimated for squamous cell carcinoma samples (Mann-Whitney test, $p < 0.0001$).
Conclusion: For MET IHC stained WSIs, H-scores were estimated through cell analysis using deep learning and image analysis, and the correlation with pathologist's H-scores was confirmed, and H-score differences between tumor subtypes were confirmed. Semi-automated AI analysis can be used to analyze the correlation between clinical data and MET staining expression.

#2441 Artificial intelligence-based quantification of PD-L1 staining intensity in non-small cell lung cancer: Beyond binary assessment.

Yunseob Hwang[1], Gui Young Kwon[2], Jeongwon Kim[3], Jiyoon Jung[3], DoKyung Kim[1], **Tae-Yeong Kwak**[1], Sun Woo Kim[1], Hyeyoon Chang[1]

[1]Deep Bio Inc., Seoul, Korea, Republic of,[2]Seoul Clinical Laboratories, Yongin-si, Korea, Republic of,[3]Kangnam Sacred Heart Hospital, Hallym University, Seoul, Korea, Republic of

Background: Determining the non-small cell lung cancer (NSCLC) patients likely to benefit from treatment based on the proportion of PD-L1-positive cells in immunohistochemistry (IHC)-stained slides, such as the Tumor Proportion Score (TPS), is effective but suboptimal. Manual PD-L1 quantification is labor-intensive, subjective, and restricted to binary assessment (positive/negative). To address these challenges, we developed an artificial intelligence (AI)-powered IHC image analysis pipeline that identifies cancer cells and their components while quantifying the staining intensity of the cancer cell membrane where PD-L1 is bound, on a scale from 0 to 100. We validated the AI-generated PD-L1 expression metrics by comparing them with clinically assessed PD-L1 TPS in NSCLC.
Methods: The AI pipeline consists of two deep learning components: cancer area localization and cancer cell staining analysis. The first component extracts patch images from whole-slide images (WSIs) and identifies cancer areas using a segmentation model for cancer localization. The second component classifies pixels of the tile image into the cell nucleus, cytoplasm, or membrane using a cellular compartment segmentation model. This component measures the optical density of Diaminobenzidine (DAB) staining at the target component corresponding to the target antibody for each cancer cell (e.g., PD-L1 on the cell membrane). We collected 366 PD-L1-stained NSCLC WSIs, which were clinically assessed for TPS by expert pathologists and used as the independent set for evaluation. Pearson's correlation coefficient (r) was used to compare clinically assessed TPS (C-TPS) values with PD-L1 staining metrics generated from the AI pipeline, such as TPS (AI-TPS), H-score, and the mean PD-L1 intensity of all tumor cells (Mean PD-L1 Intensity). The Mann-Whitney test was employed to compare the distribution of staining intensity values across NSCLC subtypes.
Results: The C-TPS values showed a strong correlation with AI-generated PD-L1 staining intensity metrics: Mean PD-L1 Intensity ($r = 0.9042$, $p < 1e-6$), H-score ($r = 0.8995$, $p < 1e-6$), and AI-TPS ($r = 0.8896$, $p < 1e-6$). Additionally, the C-TPS values exhibited a strong correlation with PD-L1 intensity heterogeneity metrics: the standard deviation of PD-L1 intensity ($r = 0.7261$, $p < 1e-6$), interquartile range of PD-L1 intensity ($r = 0.6125$, $p < 1e-6$). The Mean PD-L1 Intensity in lung adenocarcinoma was significantly higher than that in squamous cell carcinoma (Mann-Whitney test, $p < 0.0001$).
Conclusion: We demonstrated that the AI-driven PD-L1 staining intensity metrics show a high correlation with clinically assessed TPS values. This AI pipeline extends beyond binary PD-L1 assessment and can be applied to novel targets for PD-L1 evaluation.

#2442 STHD: Probabilistic cell typing of single spots in whole transcriptome spatial data with high definition.

Chuhanwen Sun[1], **Yi Zhang**[2]

[1]Department of Neurosurgery, Duke University, Durham, NC,[2]Department of Neurosurgery, Department of Biostatistics and Bioinformatics, Duke University, Durham, NC

Understanding of the cellular heterogeneity of tumor has been essential for uncovering cancer progression mechanisms and developing effective cancer immunotherapies. Emerging spatial transcriptomics (ST) technologies that enable single- and sub-cellular resolution profiling of gene expression across the whole transcriptome have recently become compatible with cancer samples. However, the transition to high-definition ST significantly increased data sparsity and dimensionality, posing significant computational challenges. For example, identifying cell types, deciphering neighborhood structure, and detecting differential expression are all crucial steps to interpret cancer ST samples. Here we present STHD, a novel machine learning method for probabilistic cell typing of single spots in whole-transcriptome, high-resolution ST data. Unlike the current binning-aggregation-deconvolution strategy, STHD directly models gene expression at single-spot level to infer cell type identities without cell segmentation or spot aggregation. STHD addresses sparsity by modeling count statistics, incorporating neighbor similarities, and leveraging reference single-cell RNA-seq data. We show in VisiumHD data that STHD accurately predicts cell type identities at single-spot level, which achieves precise segmentation of both global tissue architecture and local multicellular neighborhoods. The high-resolution labels facilitate various downstream analyses, including cell type-stratified bin aggregation, spatial compositional comparisons, and cell type-specific differential expression analyses. We applied STHD to analyze the newest colon cancer VisiumHD data, which identified patterns like spatial regions of cancer cell-specific stemness, spatially heterogenous cytokine communication activities, confined hypoxia macrophages, and distinct T cell-myeloid cell interactions and cytotoxicity activities located in different immune hubs. Moreover, STHD labels further reveal frontlines of inter-cell type interactions at immune hubs. STHD is scalable and generalizable across diverse samples, tissues, diseases, and cancer types, facilitating genome-wide analyses in various spatial organization contexts. Overall, STHD maps cell type of cancer ST samples with ultra-resolution and facilitates discovery of novel spatial biomarkers and molecular mechanism of cancer progression and treatment responses. The STHD software is publicly available at https://github.com/yi-zhang-compbio-lab/STHD.

#2443 High-resolution AI-based spatial biology tool for lung cancer trained by image-based spatial transcriptomics data to analyze tumor microenvironmnet.

Haenara Shin, Dongjoo Lee, Yooeun Kim, Daeseung Lee, Kwon Joong Na, Hongyoon Choi

Portrai, Inc., Seoul, Korea, Republic of

Background: Spatial biology enables the analysis of tumor microenvironments by revealing cellular relationships within the heterogeneous tumor landscape, which are closely associated with tumor characteristics and immunology. In this study, we utilized high-resolution H&E images and image-based spatial transcriptomics (ST) data from lung cancer to develop a highly accurate deep learning model for mapping and AI-based workflow analyzing spatial relationships in the tumor microenvironment using only H&E images.
Methods: A total of 164 NSCLC samples with high-resolution ST data (Xenium) were used for model training. Tissue microarrays (TMAs) were prepared, and image and ST data were registered using manual keypoints followed by affine transformation. Refined and major cell types were mapped using label transfer based on ST data aligned with scRNA-seq references. Segmented cell-type maps served as ground truth for training the deep learning model to predict detailed cell-type distributions from H&E images. Model performance was evaluated using the area under the ROC curve (AUROC) for each cell type. External validation was conducted using independent H&E-ST datasets (6 TMA cores and 4 whole-slide images). The trained models were integrated with a customized StarDist-based nucleus segmentation tool for H&E images.
Results: In the external validation datasets, the model achieved mean AUROC values of 0.96, 0.92, 0.95, 0.94, 0.95, and 0.94 for epithelial cells, myeloid cells, fibroblasts, T-cells, endothelial cells, and B-cells, respectively. For refined immune cell types, including CD4+ T-cells, CD8+ T-cells, dendritic cells, and NK cells, the AUROC values were 0.94, 0.97, 0.94, and 0.97, respectively. The segmented cell-type maps were integrated with nucleus segmentation to generate spatial data formats comprising cell types, enrichment scores, and spatial coordinates. This integrated deep learning model provided an H&E-based AI tool for spatial biology, enabling the analysis of spatial relationships in the tumor microenvironment, such as cell type density in tumor epithelial niches.
Conclusion: Large-scale image-based ST data with precisely registered H&E images can achieve highly accurate cell-type definitions, even for refined immune cell types like CD4+ and CD8+ T-cells and dendritic cells. This integrated workflow offers a powerful tool for analyzing spatial features using only H&E images in tumor biology.

#2444 A deep learning framework for the reconstruction of gene expression profiles derived from formalin-fixed paraffin-embedded samples.

Lingli He[1], Kai Song[1], Ying Li[1], Celia Yujuan Dong[1], Cherry Y. N. Wong[1], Lin Qi[1], Xianrui Zhang[1], **Feng Gao**[2], Yufeng Chen[3], Louis Vermeulen[4], Shentao Zhou[5], Simon Siu Man Ng[1], Xin Wang[1]

[1]The Chinese University of Hong Kong, Hong Kong, Hong Kong,[2]Department of General Surgery (Department of Colorectal Surgery), The Sixth Affiliated Hospital, Sun Yat-sen University, Guangzhou, China,[3]The Sixth Affiliated Hospital, Sun Yat-sen University, Guangzhou, China,[4]Amsterdam University Medical Centers, Amsterdam, Netherlands,[5]West China Second Hospital, Sichuan University, Chengdu, China

Formalin-fixed paraffin-embedded (FFPE) tissue samples often suffer from RNA degradation due to fixation, embedding, and storage processes, affecting the integrity of transcripts for RNA sequencing (RNA-seq). Herein, we developed a novel deep learning model to reconstruct gene expression profiles from FFPE-derived RNA-seq data. We first compared the transcript integrity numbers (TINs) of two in-house datasets derived from paired fresh frozen (FF) and FFPE tissues, with different fixation durations and library preparation strategies. We found that tissue samples fixed for 24 hours and subjected to the rRNA depletion-based library preparation strategy exhibited higher TINs. Using RNA-seq data for 832 FFPE samples across seven cancer types, we identified genes with consistently low TINs on the genome-wide scale. Interestingly, the signature genes of immune cells are more prone to degradation, compared to other cell types such as tumor epithelial cells. To reconstruct gene expression profiles, we developed a deep learning framework, based on 9568 FF tumor samples across 28 cancer types in the TCGA database. The framework demonstrated remarkable performance on both simulated data (672 transcriptomes) and in-house cancer cohorts with RNA-seq data, including one FFPE and three FF-FFPE-matched cohorts. On the simulated data with noise levels ranging from 10% to 60%, the reconstructed data achieved consistently high correlation coefficients (> 0.85) with the ‘clean’ data without noise. For the FF-FFPE-matched datasets, compared to the original FFPE-derived data, the reconstructed gene expression profiles significantly improved the correlation with matched FF profiles, even for immune cell signature genes (all $P < 0.05$). In our application to cancer subtyping, the gene expression profiles reconstructed improved the classification accuracy from 67% to 92%. Furthermore, the reconstructed profiles recapitulated the subtype-specific biological properties lost in the FFPE-derived data and showed more significant association with patient survival. As the first deep learning model to rectify FFPE-derived RNA-seq data, our framework enables more efficient use of archived FFPE tissue samples that are widely available in the clinic.

#2445 Extending ENPP3 expression inference from tissue microarrays to whole tissue sections: building a case for automated patient enrichment and IHC scoring.

Erik A. Burlingame, Fatemeh Koochaki, Tsun-wen Sheena Yao, Shajo Kunnath-Velayudhan, Chaitanya Parmar, Laurie Lenox, Tommaso Mansi, Joel Greshock, Kristopher Standish, Albert Juan Ramon

Johnson & Johnson, New Brunswick, NJ

Enrollment of patients with target-positive tumors is thought to be a factor of success in CD3-redirection targeted therapy trials. This process can incur significant testing costs for assays like immunohistochemistry (IHC) target expression assessment and requires laborious pathologist scoring. An ongoing Phase I study (NCT06178614) is evaluating the safety and preliminary anti-tumor activity of ENPP3xCD3 (JNJ-87890387) in ENPP3-unselected, advanced-stage solid tumors with a high prevalence of ENPP3 expression. This includes lung adenocarcinoma (LUAD) and colon adenocarcinoma (COAD) where ENPP3 cell surface expression is seen in >50% of patients. To help mitigate potential future IHC testing costs, we propose (1) an AI-based patient enrichment model that uses hematoxylin and eosin (H&E)-stained whole tissue sections (WTS) to infer ENPP3 expression and rule out LUAD and COAD patients likely to be ENPP3 negative. We also propose (2) a complementary model to infer H-scores from ENPP3 IHC-stained tissues as an alternative to pathologist scoring to help reduce workload. Training such models typically requires a large collection of WTS, which can be challenging to score, and may not be available in early clinical studies. Alternatively, tissue microarrays (TMAs) can incorporate hundreds of cases on a single slide and are readily available through commercial vendors, reducing the number of slides needed for model development. As TMA cores are typically orders of magnitude smaller than WTS, we (3) test the hypothesis that models trained on TMA cores can be deployed on WTS. To enable model training we developed a TMA preprocessing workflow that aligns paired serial H&E and IHC core images with their ground truth pathologist-derived H-scores. For our model backbone we use a vision transformer foundation model pre-trained on 55,000 H&E WTS spanning tissue types, which conditions the model for tasks in histopathology. We then train separate fully supervised models to achieve two goals: (1) infer whether an H&E core's paired ENPP3 H-score is greater than zero, i.e., H-score > 0 classification for patient enrichment, and (2) infer an IHC core's H-score directly, i.e., H-score regression for automated H-scoring. The H&E-based COAD H-score > 0 classification model achieved AUC=0.79 on a held-out test set of 29 WTS. The IHC-based H-score regression models for COAD and LUAD achieved intraclass correlation coefficients of 0.76 and 0.88 on their respective held-out test sets (n=29 WTS for COAD and n=46 cores for LUAD). This approach could (1) help reduce the number of patients requiring IHC assessment and (2) minimize pathologists' scoring workload, thus reducing trial costs and expediting patient enrollment. Our results suggest that (3) TMA cores may contain sufficient signal to train models for deployment on WTS for some tasks, which could help minimize modeling costs.

BIOINFORMATICS / COMPUTATIONAL BIOLOGY / SYSTEMS BIOLOGY / CONVERGENT SCIENCE: Digital Pathology Poster Session

#2449 Using digital pathology to differentiate survival phenotypes in high-grade serous ovarian cancer.

Kelsey C. Goon, Leonhard Donle, Ernst Lengyel

University of Chicago Pritzker School of Medicine, Chicago, IL

Background: High-grade serous ovarian cancer (HGSOC) has a median survival < 5 years. Yet approximately 10% of patients become long-term survivors (LTS), living > 10 years. Predicting extremes of survival may reveal insights into HGSOC biology. Machine learning (ML) can predict survival outcome in other malignancies from hematoxylin and eosin (H&E) whole slide images (WSIs) though no published models address overall survival (OS) in HGSOC. This project uses ML to predict OS in HGSOC H&E WSIs, comparing tumor and stromal regions.
Methods: Short-term survivors (STS) were defined as OS < 2 years and LTS as OS > 7 years. The model was trained on 138 H&E WSIs (56 LTS, 82 STS) at 20x magnification with 5-fold cross-validation repeated 3x and validated on The Cancer Genome Atlas-Ovary (TCGA) dataset (34 WSIs; 6 LTS, 28 STS). After converting WSIs to tiles, features were extracted using the UNI foundation model. A linear discriminant analysis predicted tile association with survival, a pretrained tumor segmentation model separated tumor and stroma, and a logistic regression classifier predicted survival based on the ratio of STS to LTS. Performance was compared across regions: whole slide, tumor alone, stroma alone, a 500-µm band at the tumor-stroma interface, and a combination of tumor-based and stroma-based predictions.
Conclusions: Models using both stroma and tumor outperformed compartmentalized models. This trend was consistent in the TCGA dataset though performance dropped significantly, proving the feasibility of using ML on H&E WSIs to predict survival in HGSOC. Our results identify a need to optimize generalizability for this relatively data-scarce malignancy and suggest that successful prediction of OS should acknowledge tumor-stromal interactions.

	Internal Test Set		**TCGA Validation**	
	AUC	**Balanced Accuracy**	**AUC**	**Balanced Accuracy**
WSI without segmentation	0.746 ± 0.163	0.701 ± 0.097	0.557 ± 0.034	0.558 ± 0.069
Tumor only	0.716 ± 0.166	0.614 ± 0.091	0.550 ± 0.012	0.547 ± 0.085
Stroma only	0.729 ± 0.162	0.642 ± 0.119	0.537 ± 0.037	0.515 ± 0.031
Tumor prediction + Stroma prediction	0.754 ± 0.171	0.699 ± 0.106	0.563 ± 0.022	0.533 ± 0.058
Tumor-stroma interface only	0.769 ± 0.143	0.716 ± 0.088	0.587 ± 0.030	0.511 ± 0.070

#2450 Slide-level foundation models exhibit effective diagnostic and prognostic performance in H&E-stained histopathology.

P. Chen, A. G. Serrano, T. Gautam, **S. Ranjbar**, B. Rodriguez, P. Team, L. M. Solis Soto, Y. Yuan;
The University of Texas MD Anderson Cancer Center, Houston, TX

Introduction: Computational pathology has progressed from tile-level to slide-level foundation models, eliminating the need to train separate tile aggregation networks. Slide-level foundation models are trained on extensive datasets of whole-slide images. For instance, Prism is trained on 587,196 slides from 17 tissue origins, while GigaPath is trained on 171,189 slides covering 31 tissue types, both sourced from private data cohorts. By leveraging vast amounts of data to capture intricate tissue patterns, slide-level models promise to extract clinically relevant features. However, currently there is limited independent research evaluating the performance of these models in more complex tasks, such as multiclass sub-typing diagnosis and survival predictions.
Methods: We curated FFPE H&E slides from both the public TCGA and our in-house cohort. From TCGA, we collected slides from 5 cancer types: 344 BLCA, 505 KIRC, 348 LIHC, 350 STAD, and 500 THCA cases. The in-house cohort consisted of rare cancer cases, categorized into 54carcinoma, 67 sarcoma, and 18 melanoma cases. We applied slide-level foundation models, Prism and GigaPath, to embed the slides into feature representations. For the diagnosis task, we used a support vector machine (SVM) model to classify TCGA slides into 5 categories and the in-house slides into 3 categories. For the prognosis task, we trained a multi-layer perceptron (MLP) model to predict the hazard risk. We conducted 5-fold cross-validation for both diagnosis and prognosis tasks.
Results: On TCGA slides, we achieved a classification accuracy of 0.973±0.008 using Prism and 0.944±0.022 using GigaPath. For the in-house slides, the classification accuracy was 0.885±0.042 with Prism and 0.848±0.056 with GigaPath. These results demonstrate the strong discriminative capabilities of the feature representations extracted by both slide-level foundation models. Notably, Prism outperformed GigaPath on both the TCGA and in-house datasets. For survival prediction, we applied the Prism model to five TCGA cancer types and the in-house cases. The C-index values were 0.595±0.029 for BLCA, 0.719±0.039 for KIRC, 0.655±0.058 for LIHC, 0.591±0.046 for STAD, 0.720±0.114 for THCA, and 0.592±0.025 for all in-house cases combined. These varying levels of survival prediction performance reflect the challenges posed by different tasks, while the lowest value being above 0.59 suggests prognostic information was adequately extracted.
Conclusion: By evaluating the diagnostic and prognostic capabilities of slide-level foundational models, we demonstrated their effectiveness on both public and in-house datasets. Our findings corroborate that these models can distill clinically relevant feature representations even without fine-tuning, underscoring their potential for broad computational pathology applications.

#2451 H&E whole-slide image (WSI) based artificial intelligence (AI) model to detect EGFR and ALK mutation in non-small cell lung cancer (NSCLC).

Chan Mi Jung[1], Brandon Park[2], Hannah Son[1], Liam IL-YOUNG Chung[1], Young Kwang Chae[1]

[1]Northwestern University, Chicago, IL,[2]University of Missouri-Kansas City School of Medicine, Kansas City, MO

Detection of mutated epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) are essential to guide therapeutic decisions for patients with NSCLC. Although traditional molecular testing methods-immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH)-and comprehensive genomic profiling (CGP) can provide extensive molecular insights, they are limited by prolonged turnaround time, higher tissue requirements, and elevated costs. To address this challenge, we tested a deep learning model that leverages digital pathology to analyze scanned H&E-stained WSIs for identifying EGFR and ALK mutations in NSCLC patients.

The study utilized a commercial assay to predict EGFR and ALK genomic alterations directly from H&E-stained WSIs through a cloud-based platform. The assay was developed by Imagene Inc. using multiple instance learning (MIL) methods, trained with embeddings that were extracted by the commercial CanvOITM Foundation Model (FM). The FM ensured robust generalizability and computational power by leveraging 632,608 digitized H&E-stained tissue samples. Thresholds were optimized on a held-out dataset to achieve positive predictive values (PPVs) of 50% and 40% for EGFR and ALK, respectively, while maintaining a 99% negative predictive value (NPV) for both genes.

We tested the predictions in a blind retrospective evaluation including 164 digitized H&E images with EGFR molecular information and 158 images with ALK fusion data. In this study, 29 patients had EGFR-positive and 3 had ALK-positive mutations detected by commercial CGP providers. For EGFR mutation prediction, our model achieved a sensitivity of 48.3%, a specificity of 88.3%, a PPV of 66.7%, and an NPV of 100%. For ALK mutation prediction, the model achieved a sensitivity and NPV of 100%, specificity of 99.1%, and a PPV of 75%. Additionally, 54.88% (90/164) of EGFR and 28.48% (45/158) of ALK cases were classified as "intermediate," indicating uncertain results.

Our study highlighted the potential of our prediction model for identifying EGFR and ALK mutations to guide targeted therapies in NSCLC patients. The model's NPV of 100% demonstrates its strong ability to accurately rule out both EGFR and ALK mutations, ensuring that patients without these mutations are not subjected to unnecessary treatments. Furthermore, the ALK mutation prediction was promising with a sensitivity of 100% and specificity of 99.1%, demonstrating the model's ability to detect all true positive cases while effectively minimizing false positive results. The presence of intermediate results suggests the need for additional diagnostic steps to confirm or exclude mutations and ensure accurate clinical decisions. Excluding EGFR and ALK alterations directly from the diagnostic H&E image addresses the demand for rapid genomic analysis driven by the rising use of neoadjuvant immunotherapy.

#2454 Spatial profiling of arsenic-responsive gene expression in bladder cancer models via multiplex in situ hybridization.

Sonalika Singhal[1], Kevin Gardner[2], Michael L. Miller[2], Anushka Dixit[3], Samarth Singhal[1], Donald Sens[1], Sandeep Singhal[1]

[1]University of North Dakota, Grand Forks, ND,[2]Columbia University Medical Center, New York, NY,[3]Bio-Techne, Newark, CA

Multiplex Fluorescent In Situ Hybridization (mFISH) enables precise identification of distinct cell phenotypes and facilitates the assessment of spatial interactions within the tumor microenvironment. Analyzing images generated from mFISH studies allows for detailed characterization and comparison of the spatial distribution of tumor cells and tumor-associated immune cells. In our prior study, we developed a gene expression-based model for predicting bladder cancer risk under arsenic exposure, identifying three key genes - NKIRAS2, AKTIP, and HLA-DQA1 that demonstrated high predictive accuracy (AUC: 94 for training and 75 for testing) (PMCID: PMC8760535). The current study employs an image-based digital Pathology approach to further validate this model, exploring the role of these potential biomarkers at single-cell resolution.Bladder cancer biopsy samples contain RNA of sufficient integrity and quality for molecular analysis (RNA Integrity Number > 7), and confirmed through positive and negative control assessments, were selected for accurate and reliable gene expression analysis in downstream. Five mFISH images were generated by ACD Bio-Techne, and hematoxylin and eosin (H&E) images were annotated by the Department of Pathology and Cell Biology at Columbia University Medical Center. Using Warpy, the mF and H&E images were overlaid to delineate tumor and non-tumor regions on whole slide images (WSI). Quantification of the WSI was conducted with QuPath, where a custom data column containing the weighted 3-gene module score for each cell was appended to the sample data. Spatial distribution of cells was analyzed in python using Sklearn, padas, numpy and matplotlib libraries.The analysis demonstrated that the previously established three-gene risk prediction model exhibited a significantly higher signal density within tumor regions across all mFISH bladder cancer samples. Tumor regions displayed a markedly closer spatial proximity between cells compared to non-tumor regions. When comparing the genomic risk prediction equation for individual cells in tumor versus non-tumor regions, the model achieved a training AUC score of 0.92 and a test accuracy of 0.85. Notably, this trend was independent of tumor grade, whereas expression intensity declined with increasing patient age.In conclusion, this study reinforces the predictive significance of the previously identified gene set comprising NKIRAS2, AKTIP, and HLA-DQA1 in assessing bladder cancer risk. The methodologies utilized highlight the potential of low-complexity, single-cell gene expression profiling through multiplex mFISH, coupled with the integration of AI-driven digital pathology tools. However, the study is limited by its small sample size and the narrow range of clinical parameters evaluated, underscoring the need for further research to validate and expand these findings.

#2455 AI-based robust testicular cancer triaging, a promise for an efficient and cost-effective diagnostic/predictive modality.

Siddhesh Thakur[1], Ujjwal Baid[2], Andres Martin Acosta[1], Muhammad T. Idrees[1], Michael Feldman[1], Spyridon Bakas[1]

[1]Indiana University School of Medicine, Indianapolis, IN,[2]Emory University, Atlanta, GA

Testicular cancer is one of the most common malignancies in males aged 20-44, with its two major subtypes being Seminoma and Non-Seminomatous Germ Cell Tumors (NSGCT). Accurate classification is essential for patient management and treatment, after initial histologic diagnosis on radical orchiectomy specimens or biopsies of metastases. Histopathological diagnosis, though effective, is time-consuming, costly, and subject to inter-observer variability. Digital pathology combined with artificial intelligence (AI) promises to improve diagnostic consistency and efficiency while benefitting settings without expertise in these tumors. We seek rapid and robust triaging of these subtypes through a foundation model interrogating H&E-stained tissue sections and an attention-based Multiple Instance Learning (abMIL) approach providing interpretable predictions highlighting decision-driving regions. Retrospective multi-institutional data from 144 patients (254 slides) were used to develop our AI model. 118 patients (202 slides) were used for model training, 14 (30 slides) for validation, and 14 (22 slides) as an independent hold-out set. The UNI foundation model was pre-trained on diverse datasets and fine-tuned using abMIL, identifying discriminative features from the training data without requiring pixel-level annotations. Independent hold-out set evaluation revealed Area Under the Curve=96%, Accuracy=94.54%, Specificity=92%, and importantly, Sensitivity=100%. Specifically, all predicted NSGCT are correctly identified as NSGCT, and all Seminomas are always detected. In other words, no Seminoma case is overlooked, while 8% of the actual NSGCT may be misclassified as Seminoma. A sensitivity of 100% supports our model as a triaging tool, enabling faster decision-making for high-risk patients. Our proposed abMIL approach leveraging the foundation model UNI, provides a perfectly sensitive and scalable solution for clinical triaging between Seminoma and NSGCT testicular tumors in digitized tissue sections, ensuring subtype detection and supporting pathologists in improving diagnostic accuracy and efficiency. Furthermore, the model's focus on highlighting AI decision-driving regions of the tissue section, renders it well-suited for large-scale clinical data, where it can make further data-driven contributions in extending our current disease knowledge.

#2456 Artificial intelligence-powered spatial analysis of tumor-infiltrating lymphocytes predicts prognosis in gallbladder cancer after resection.

Hyemin Kim, Young Hoon Choi, So Jeong Yoon Yoon, Cheolyong Joe, Se-Hoon Lee, Sang Hyun Shin, In Woong Han, Jin Seok Heo, Yo Han Jeon, Kee-Taek Jang, Kwang Hyuck Lee, Jong Kyun Lee, Kyu Taek Lee, Hongbeom Kim, Jookyung Sophie Park

Samsung Medical Center, Seoul, Korea, Republic of

Background: Tumor-infiltrating lymphocytes (TIL) play an essential role in various cancer types. However, their prognostic significance in resected gallbladder cancer remains largely unknown.
Methods: This study included 225 patients who underwent curative resection for gallbladder cancer. H&E-stained whole slide images were analyzed using the Lunit SCOPE IO, an artificial intelligence (AI)-powered spatial TIL analyzer. Based on the density of intratumoral TILs in the cancer epithelium and stromal TILs in the cancer stroma, three immune phenotypes (IP) were defined: immune-inflamed phenotype (IIP), immune-excluded phenotype (IEP), and immune-desert phenotype (IDP). Survival outcomes were analyzed, with subgroup analysis conducted for early-stage (stage 0-II) and advanced-stage (stage III-IV) disease.
Results: Of the 225 patients, 58 (25.8%) had IIP, 151 (67.1%) had IEP, and 16 (7.1%) had IDP. During a median follow-up of 71 months, 82 deaths (36.4%) were recorded. IIP and IEP were significantly associated with more prolonged disease-free survival (DFS) and overall survival (OS) compared to IDP.
Conclusions: Immune phenotypes identified through AI-powered spatial TIL analysis were associated with DFS and OS in resected gallbladder cancer, particularly in late-stage disease. These findings highlight the prognostic potential of immune phenotyping, especially in advanced disease.

Univariate and Multivariate Cox Regression Analyses for DFS and OS

	DFS					**OS**			
	Univariable		**Multivariable**			**Univariable**		**Multivariable**	
	HR(95% CI)	***P***	**HR(95% CI)**	***P***		**HR(95% CI)**	***P***	**HR(95% CI)**	***P***
Age, years									
< 66	1.00		1.00			1.00		1.00	
≥ 66	1.15(0.77–1.72)	0.506	1.52(0.98–2.36)	0.062		1.38(0.89–2.15)	0.155	1.72(1.05–2.80)	0.030
Sex									
Male	1.00		1.00			1.00		1.00	
Female	0.58(0.39–0.86)	0.008	0.71(0.47–1.07)	0.099		0.57(0.37–0.89)	0.013	0.75(0.48–1.18)	0.216
Stage									
0, I, II	1.00		1.00			1.00		1.00	
III, IV	5.20(3.30–8.22)	<0.001	5.63(3.38–9.38)	<0.001		5.51(3.31–9.18)	<0.001	6.13(3.50–10.73)	<0.001
Adjuvant Tx									
No	1.00		1.00			1.00		1.00	
Yes	2.05(1.36–3.07)	<0.001	1.05(0.66–1.69)	0.833		1.81(1.16–2.83)	0.010	1.00(0.60–1.68)	0.999
IP		0.081		0.006			0.017		0.002
IDP	1.00		1.00			1.00		1.00	
IEP	0.51(0.26–0.99)	0.048	0.38(0.19–0.75)	0.006		0.41(0.21–0.81)	0.010	0.32(0.16–0.65)	0.002
IIP	0.43(0.20–0.91)	0.028	0.28(0.13–0.61)	0.001		0.34(0.15–0.74)	0.007	0.26(0.12–0.57)	0.001

DFS, disease-free survival; OS, overall survival; HR, hazard ratio; Tx, therapy; IP, immune phenotype; IDP, immune-desert phenotype; IEP, immune-excluded phenotype; IIP**,** immune-inflamed phenotype.

#2457 Prognostic value of CD8+ T-cells at the invasive margin is comparable to immune score in non-metastatic colorectal cancer: A prospective multicentric cohort study.

Durgesh Wankhede[1], Niels Halama[1], Matthias Kloor[2], Dominic Edelmann[1], Hermann Brenner[1], Michael Hoffmeister[1]

[1]German Cancer Research Center, Heidelberg, Germany,[2]Institute of Pathology University of Heidelberg, Heidelberg, Germany

Background: Immunoscore predicts colorectal cancer prognosis but faces adoption barriers due to complex software and reimbursement issues. This study used open-source methods to explore a simplified prognostic model in non-metastatic CRC by focusing on single T-cell markers.

Methods: A multicentric prospective cohort study in non-metastatic CRC patients assessed CD3+ and CD8+ tumor infiltrating lymphocytes (TILs) in the invasive margin (IM) and tumor core (TC) using QuPath. An immune cell score, based on TIL densities (CD3-IM, CD8-IM, CD3-TC, CD8-TC), was calculated similarly to the Immunoscore®. A split sample approach (70:30) estimated adjusted hazard ratios for cancer-specific survival (CSS) in training and validation sets. CART analysis identified the most prognostic TIL, and its model was compared to an immune cell score model for performance (Brier score) and discrimination (Concordance Probability Estimate, CPE).

Results: Over a median follow-up of 9.0 years, 203 CRC-specific deaths occurred among 1,260 patients. CART-selected CD8-IM was the most prognostic TIL at a cut-off of 231 cells/mm2. Patients with CD8-IMHi had better CSS than CD8-IMLow in both training (HR 0.58, 95% CI 0.40-0.84) and validation sets (HR 0.35, 95% CI 0.21-0.60). Brier scores of CD-8IM and immune cell score survival models were comparable in both training and validation cohort, whereas the survival discrimination of CD8-IM slightly outperformed the immune cell score in the validation set (CPE: CD8-IM 0.748, IS 0.730).

Conclusion: CD8-IM alone provided prognostic information comparable to the immune cell score. Simplified, cost-effective TIL assessments could improve clinical translation and guide adjuvant therapy in early-stage CRC.

#2458 Prediction of atezolizumab benefit in NSCLC by computational pathology: Retrospective validation in a phase III trial.

Patrick Leo[1], Niha Beig[1], Barzin Y. Nabet[1], Chang Ho Ahn[2], Soohyun Hwang[2], Sanghoon Song[2], Chan-Young Ock[2], Daniel Ruderman[1]

[1]Genentech, Inc., South San Francisco, CA,[2]Lunit, Seoul, Korea, Republic of

Background: Anti-PD-(L)1 therapies are widely used to treat non-small cell lung cancer (NSCLC). However, suboptimal disease response motivates development of biomarkers to identify patients likely to benefit from these therapies. Here, we demonstrate an artificial intelligence (AI) powered model that analyzes the tumor microenvironment (TME) of hematoxylin and eosin (H&E) images to predict patient benefit from atezolizumab (anti-PD-L1) in NSCLC.
Methods: 1,243 digitized H&E slides were collected from two trials of atezolizumab versus docetaxel in second-line advanced NSCLC. POPLAR, a phase II trial, was used for training (n=162) and OAK, its phase III follow-on, was used as validation (n=1,081). Lunit Scope IO, a previously developed deep learning model specialized for TME analysis, was deployed on each image to label tissue and cells. Then, the densities of tumor cells, lymphocytes, fibroblasts, macrophages, endothelial cells, and mitotic figures were calculated in the tumor epithelium and intra-tumoral stroma. An elastic-net penalized Cox regression model using these density values was fit on the atezolizumab-treated patients of the training set to predict overall survival (OS) and applied to the entire dataset. To divide patients into model-low/high groups, the risk score threshold maximizing between-arm median OS difference in model-high patients was identified on the training set. Model performance was evaluated by concordance index (c-index) in each treatment arm, by hazard ratio (HR) between arms in the model-low/high groups, and by a Cox regression model with treatment arm, model-low/high category, and their interaction in the validation set.
Results: In the tumor epithelium, lymphocytes and tumor cell densities were positively correlated with OS in atezolizumab-treated patients, while mitotic cell and macrophage densities were negatively correlated. In the intra-tumoral stroma, densities of fibroblasts, macrophages, lymphocytes, and endothelial cells were positively correlated with OS. Model risk score was more strongly associated with OS in the atezolizumab arm (c-index=0.58 95% confidence interval: [0.55, 0.61]) than in the docetaxel arm (c-index=0.54 [0.51, 0.57]). Among the n=464 model-high validation set patients, atezolizumab-treated patients had significantly longer OS (HR=0.65 [0.53, 0.80]) with no significant difference between arms in model-low patients (n=617, HR=0.92 [0.78,1.10]). Model low/high category had a significant interaction (p=0.02) with treatment in survival prediction.
Conclusions: We applied AI-driven analysis of histology to large randomized trials to quantify how TME cellular composition correlates with benefit from immunotherapy. Our results suggest that digital pathology-based approaches in combination with AI analysis could yield biomarkers for patient stratification and treatment selection strategies.

#2459 Membrane stain-enhanced quantification of HCR™ Pro RNA-ISH using HALO image analysis software.

Randy Chen[1], Anne Hellebust[2], Aneesh Acharya[1], Harry Choi[1]

[1]Molecular Instruments, Inc., Los Angeles, CA,[2]Indica Labs, Albuquerque, NM

Accurate quantification of RNA in situ hybridization (RNA-ISH) signals is critical for understanding spatial gene expression, particularly at the single-cell level. Traditional methods using Hematoxylin or DAPI staining often fall short due to their inability to clearly define cell boundaries, leading to inaccurate dot counting. In the context of digital pathology, this challenge can hinder precise interpretation of spatial gene expression data. Here, we demonstrate how the incorporation of membrane stains enhances RNA-ISH signal quantification by delineating cell borders, enabling precise dot counting with HALO image analysis software.

Membrane stains were applied across both chromogenic and fluorescent HCR™ Pro RNA-ISH assays, facilitating robust signal detection in brightfield and fluorescent imaging modalities. This approach also enabled the integration of cell-type specific markers (e.g., CD3 and CD4) to provide insights into gene expression dynamics within distinct cell subpopulations relevant to pathology workflows.

Comparative analysis revealed superior accuracy in dot quantification when using membrane-enhanced staining compared to traditional Hematoxylin/DAPI-expanded methods. These findings underscore the importance of combining membrane stains with advanced imaging analysis tools like HALO in digital pathology for precise and reproducible quantification of RNA-ISH signals. This advancement paves the way for more detailed studies of differential gene expression and cellular heterogeneity within clinical and research pathology settings.

#2460 Judith: An agentic AI system for biomedical image analysis and scientific discovery in precision oncology.

Ming Y. Lu[1], Faisal Mahmood[2], **Richard J. Chen**[1]

[1]Modella AI, Boston, MA,[2]Pathology, Brighman and Women's Hospital, Boston, MA

The rapidly increasing volume of multimodal biomedical data presents unprecedented opportunities in precision oncology but poses significant challenges for researchers analyzing complex modalities such as gigapixel pathology slides. To address these challenges, we developed Judith, an AI-driven research assistant that leverages foundation models, multimodal generative AI, and other advanced computational tools for streamlining complex workflows in biomedical image analysis, predictive modeling, and biomarker discovery. Judith enables researchers to execute computational tasks through natural language commands, automating tasks such as response prediction, multimodal cancer prognosis, and histopathology image retrieval. We demonstrate Judith's capabilities in several precision oncology use cases. (1) Predictive modeling and biomarker discovery: Using a retrospective dataset of pretreatment biopsies from invasive breast carcinoma patients treated with Trastuzumab (n=85 patients), Judith trained an AI model that predicts pathologic complete response with an AUROC of 0.855. Judith could also interpret the model, identifying key cellular features linked to tumor proliferation and finding histologic biomarkers that explain cohort-level differences between responders and non-responders. (2) Multimodal data integration: Judith orchestrated the development of a survival prediction model integrating histology, radiology, and multi-omics data for progression-free survival prediction in low-grade glioma patients (n=127), achieving a C-Index of 0.684. Using an integrative analysis, Judith further stratified patients into high- and low-risk groups and identified unfavorable prognostic features for cancer progression such as high tumor cellularity and PSPH overexpression. (3) Histology slide retrieval: Judith automated the development of a histology slide search system (n=138), enabling pathologists to retrieve slides with similar cancer diagnoses and morphologies. Judith was then able to validate the performance of histology slide retrieval, with successful retrieval of rare cancer types such as leiomyosarcoma. Overall, Judith represents a major advancement in biomedical research by automating AI workflows and enhancing data-driven discovery. Its ability to analyze diverse data modalities, identify meaningful biomarkers, and generate interpretable insights empowers researchers to maximize the scientific insights in their data. Future developments will focus on expanding multimodal capabilities, refining interpretability tools, and integrating real-time collaboration features.

#2461 Federated learning enables multi-institution collaboration without sharing Hand E slides.

Kodi Taraszka[1], Oded Rosolio[2], Intae Moon[1], Yevgeniy Semenov[3], Alexander Gusev[1]

[1]Dana-Farber Cancer Institute, Boston, MA,[2]Duality Technologies, Tel Aviv, Israel,[3]Massachusetts General Hospital, Boston, MA

Hematoxylin and eosin slides provide vital visual information on a collected tissue sample and are a key component of cancer diagnostics. Previous work in computational pathology has utilized deep learning architectures to accurately and efficiently tackle complex tasks such as tumor classification. However, the generalizability of these algorithms is currently limited due to the inherent difficulty of cross institution collaboration. Here, we propose leveraging federated learning (FL) which aggregates model level information between institutions to enable collaboration without exposing sensitive data. This allows the deep learning model to incorporate diverse patient populations and heterogeneous data sources which are needed for generalizability while preserving patient privacy.

As a proof-of-concept, we used attention based multiple instance learning within the FL framework to perform cancer subtype classification between lung adenocarcinoma and lung squamous cell carcinoma with three distinct cohorts: Dana-Farber Cancer Institute (DFCI), Massachusetts General Hospital (MGH), and The Cancer Genome Atlas (TCGA). We present comparisons using three approaches: FL, institution specific analyses, and a centralized model where data is combined at one site (gold standard). We find all three approaches have comparable overall AUC. Additionally, we see that there is significant heterogeneity in AUC between cohorts which may indicate FL is more robust to overfitting and more generalizable. Comparing features generated using the foundation pathology model (UNI) to those generated from a ResNet50 model pre-trained on ImageNet (ResNet), we observed more institutional level heterogeneity in the UNI results than in ResNet which may indicate UNI is learning institution specific features that limit its generalizability. Overall our work shows FL enables generalizable deep learning across institutions without risking patient privacy.

Cohort	Total Sample Size	Sample Size Per Site	Number of Sites	ResNet: ResNet50 pretrained on ImageNet				UNI: Foundation Pathology Model			
				Overall AUC	DFCI AUC	MGH AUC	TCGA AUC	Overall AUC	DFCI AUC	MGH AUC	TCGA AUC
DFCI, MGH + TCGA	540	180	3	0.85	0.843	0.869	0.841	0.859	0.864	0.89	0.851
DFCI, MGH + TCGA	270	90	3	0.796	0.825	0.819	0.744	0.775	0.750	0.891	0.781
DFCI, MGH + TCGA	180	60	3	0.847	0.844	0.867	0.767	0.788	0.778	0.878	0.611
DFCI + MGH	180	90	2	0.855	0.938	0.806	---	0.808	0.644	0.900	---
DFCI + TCGA	180	90	2	0.817	0.844	---	0.800	0.872	0.856	---	0.869
MGH + TCGA	180	90	2	0.782	---	0.781	0.775	0.780	---	0.894	0.613
MGH + TCGA (combined)	180	180	1	0.840	---	---	---	0.946	---	---	---
DFCI	180	180	1	0.848	0.848	---	---	0.848	0.848	---	---
MGH	180	180	1	0.875	---	0.875	---	0.914	---	0.914	---
Average	---	---	---	0.834	0.857	0.836	0.785	0.843	0.790	0.894	0.745

#2462 Novel digital pathology algorithms to explore the role of tumor microenvironment in next generation immuno-oncology clinical trials utilizing 17 plex fluorescent immunohistochemistry.

Richard Van Krieken, Jaspreet Kaur, James Santos, Naveen Dakappagari, Jennifer Bordeaux

Navigate BioPharma Services, Carlsbad, CA

Purpose: A deeper understanding of the intricacies between tumor microenvironments (TMEs) and immune cell infiltrates is critical for creation of next generation immune modulating therapies. We previously validated a clinical grade, 17 plex tumor immune landscape assay which characterizes T cells, B cells, myeloid cells, plasma cells, and tumor cells across five major cancer types. This assay was developed on the Orion platform, which images up to 18 markers concurrently and allows for same slide hematoxylin and eosin (H and E). Using the Orion platform, we characterized the TME through two approaches: cellular neighborhoods and clustering based on H and E and segmented fluorescent immunohistochemistry data. Our findings elucidate innovative strategies for leveraging high dimensional data from Orion to uncover clinically useful insights, opening new avenues for understanding mechanism of response or resistance in next generation immune oncology (IO) clinical trials
Study Design: We designed a mFIHC assay (Granzyme B, Ki67, CD3e, CD20, CD4, CD163, CD8a, CD56, PDL1, PD1, LAG3, FOXP3, CD11b, CD138, SOX10, Cytokeratin and Hoechst) to evaluate expression of immune markers on non small cell lung cancer, breast carcinoma, colorectal cancer, prostate cancer and melanoma. After the initial evaluation, we validated the mFIHC assay for clinical trial use with automated staining (IntelliPath FLX) and imaging (Orion). Image analysis was conducted using HALO technologies.
Results: HALO analysis revealed distinct tumor immune microenvironments in the examined tissues. Microenvironment data were assessed by analyzing immune cell infiltration alongside immune activation markers. Our analysis revealed unique cell clusters in these microenvironments.
Conclusion: Our clinically validated 17 plex mFIHC assay, combined with advanced spatial algorithms, offers improved characterization of the TME. We expect these novel algorithms to contribute to gaining novel insights into tumor biology and development of next generation IO therapies.

#2463 AI-based EGFR-mutation prediction from haematoxylin and eosin (H&E) images in non-small cell lung cancer (NSCLC): A global multi-cohort validation study.

Jongchan Park[1], Biagio Brattoli[1], Jack Shi[1], Michael Senior[2], Talha Qaiser[2], Jack Rawson[2], Woochan Hwang[1], Sangwon Shin[1], Taebum Lee[1], Chan-Young Ock[1], Sergio Pereira[1], Huw Bannister[2], Elia Riboni-Verri[2], Siraj Ali[1], **Luiza Moore**[2]

[1]Lunit, Seoul, Korea, Republic of,[2]Astrazeneca, Cambridge, United Kingdom

Background: Despite guideline recommendations for EGFR testing in NSCLC patients, many are under-tested due to resource constraints and long turnaround times. Existing AI-based genotype prediction models using H&E whole slide images (WSIs) have mostly been validated in limited settings, resulting in barriers to real-world applicability. To address these challenges, we developed and validated a deep learning model that demonstrates robust and consistent performance across various clinical settings and independent patient cohorts.

Method: Our study utilized WSIs from 4,684 EGFR-mutated and 7,576 wild-type NSCLC cases from multiple sites, including USA, China, and Republic of Korea, to train convolutional neural networks (CNN) and ViT-based models to predict EGFR mutations from H&E WSIs. To improve generalization performance, data-centric approaches, including augmentations, oversampling, and weighted losses, were adopted, and the ensemble of prediction scores was used as the final prediction. We report validation performance in three cohorts: an independent internal validation cohort; a multi-scanner cohort of paired images scanned by six different scanners and with two different magnifications; and independent external validation cohorts from AstraZeneca.

Results:The internal validation cohort (n=625) and the multi-scanner cohort (n=2,261; 323 in each subgroup) included 206 (33.0%) and 168 (52.0%) EGFR-mutated samples, respectively. Our methods showed significant improvement in performance, achieving an area under the ROC curve (AUC) of 0.880 compared to 0.723 from a model we previously presented. The model demonstrated generalized performance across mutation subtypes (AUC 0.843 to 0.894), specimen types (0.823 to 0.897), scanners (0.822 to 0.855), and magnifications (0.824 to 0.832). The model was further validated on independent external cohorts which include additional EGFR-mutated samples.

Conclusion:An AI model demonstrated robust validation performance across diverse settings for EGFR mutation prediction from H&E WSIs in NSCLC, representing a vital step toward real-world clinical impact.

#2464 Inferring genomic properties from H&E whole-slide images of >60,000 solid tumors.

Kevin M. Boehm[1], Arfath Pasha[1], Armaan Kohli[1], Raymond Lim[1], Tom Pollard[1], Darin Moore[1], JianJiong Gao[1], Nikolaos M. Dimitriou[1], Madison Darmofal[1], Meera Hameed[2], Ahmet Dogan[2], Natasha Rekhtman[2], Klaus J. Busam[2], Lora H. Ellenson[2], Jie-Fu Chen[2], Fresia Pareja[2], Daniel Gomez[3], Hikmat Al-Ahmadie[2], Nancy Lee[3], Eric Sherman[4], Jason Chang[2], Himanshu Nagar[3], Britta Weigelt[2], James Fagin[4], Susan Fu[5], Jonathan Alarcon[5], Neelraj Patil[1], Areej Alsaafin[1], Georgios Asimomitis[1], Anika Begum[1], Andrew Aukerman[1], Justin Jee[1], Christopher Fong[1], Michele Waters[1], Lior Z. Braunstein[3], Michael F. Berger[6], Luke R. G. Pike[3], Pedram Razavi[4], Paul Romesser[3], Walid K. Chatila[1], Aasiya Islam[1], Elana Sverdlik[7], Ino de Brujin[1], Karl Pichotta[1], Nikolaus Schultz[1], Sohrab P. Shah[1], Francisco Sanchez-Vega[1]

[1]Halvorsen Center for Computational Oncology, Memorial Sloan Kettering Cancer Center, New York, NY,[2]Department of Pathology & Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, NY,[3]Department of Radiation Oncology, Memorial Sloan Kettering Cancer Center, New York, NY,[4]Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY,[5]Digital Informatics & Technology Solutions, Memorial Sloan Kettering Cancer Center, New York, NY,[6]Marie-Josee & Henry R. Kravis Center for Molecular Oncology, Memorial Sloan Kettering Cancer Center, New York, NY,[7]Gerstner Sloan Kettering Graduate School of Biomedical Sciences, Memorial Sloan Kettering Cancer Center, New York, NY

Background: Clinical tumor-normal panel sequencing has enabled personalized approaches to targeted therapies. However, the impact of somatic mutations on tissue morphology remains incompletely understood, and financial hurdles limit universal adoption of personalized approaches.

Methods: We developed machine learning tools to infer genomic properties from hematoxylin and eosin (H&E) whole-slide images (WSIs). We assembled a pan-cancer cohort of 62,266 sequenced tumor samples matched to 315,383 H&E WSIs from 54,446 patients, the largest to date. Using these data, we developed transformer models to aggregate embeddings of tissue by part to infer genomic features. We then tested performance on 172 OncoKB-annotated predictive or prognostic biomarkers across 127 histologies and analyzed how the models could be used to identify indicated targeted therapies from H&E WSIs alone.

Results: We highlight four putative use cases of our models, two each for predictive and prognostic biomarkers. First, *BRAF* mutational status, inferred with AUROC 0.85 (precision 0.95, recall 0.63) in papillary thyroid cancer has level two evidence for dabrafenib and trametinib in metastatic differentiated thyroid cancer. Second, *FGFR3* mutational status, identified with AUROC 0.92 (precision 0.95, recall 0.42) in bladder cancer indicates erdafitinib, surpassing the prior state of the art of AUROC 0.73. Prognostically, the models accurately identify unfavorable *STK11* mutations in lung adenocarcinoma (AUROC 0.92) and favorable *POLE* exonuclease domain mutations in uterine endometrioid carcinoma (UEC) (AUROC 0.90), which could guide shared decision making around adjuvant therapy.

Furthermore, disaggregating disease subtypes reveals that phenome-genome relations cited by previous works vary significantly by subtype. For example In UEC, *WNT* pathway aberrations are detected with AUROC 0.87 (95% C.I. 0.82-0.91) in microsatellite-stable UEC, whereas in MSI UEC, there is no detectable phenome-genome relation (AUROC 0.61, 95% C.I. 0.44-0.76). MSI status itself can be inferred accurately from both colorectal adenocarcinoma (AUROC 0.99, 95% C.I. 0.98-0.99) and UEC (AUROC 0.91, 95% C.I. 0.87-0.95).

Conclusions: Taken together, our work uncovers phenome-genome relations for granular subtypes lumped together in prior studies. Such approaches advance our knowledge of how somatic mutations affect tissue morphology and have the potential to improve access to precision oncology.

#2465 Aperio manual scan software: beta testing for challenging slide imaging in research.

Erica Pawlak, Shubham Dayal, Elizabeth Sullivan, **Jacob Persky**, Diane Robirds, Timothy Murphy-Stevens, Duff Pickering

Leica Biosystems, Deer Park, IL

Purpose: The Aperio Manual Scan feature was developed to improve whole slide imaging for challenging samples, including faintly stained, sparse, or artifact-laden slides. This study evaluated the feature's usability and its ability to address imaging challenges.
Experimental Procedures: Fifteen challenging slides, including cytospins, wet tissue touch preps, immunohistochemistry (IHC), and in situ hybridization (ISH) samples, were scanned during a beta test at a pharmaceutical research facility. Laboratory scientists and technicians used the manual scan feature to adjust scan areas, focus points, and calibration points. Participants provided feedback through a structured questionnaire.
Results: Of 15 slides, 6 were successfully scanned using the automated workflow, while 9 required manual adjustments. The manual scan feature allowed successful imaging of sparse or faintly stained specimens, precise exclusion of artifacts, and focus optimization for multi-plane samples such as cell pellet arrays. Users highlighted the feature's intuitive interface and its ability to generate high-quality images for previously unscannable slides.
Conclusions: The Aperio Manual Scan software enhances flexibility and precision in whole slide imaging, complementing the automated workflow. It allows researchers to overcome imaging challenges efficiently and is anticipated to benefit research facilities significantly.

#2466 Pathomics prediction of thymic epithelial tumors histological subtypes: the AI-TET study.

Lodovica Zullo[1], Mathilde Bateson[2], Jose Carlos Benitez[3], Audrey Lupo[4], Damien Sizaret[5], Alvaro Lopez-Gutierrez[1], Daniela Miliziano[1], Kathryn Schutte[2], Juan David Florez-Arango[6], Pascale Missy[7], Vincent Thomas De Montpreville[8], Mercier Olaf[8], Jordi Remon[1], David Planchard[1], Nicolas Girard[9], Thierry Molina[10], Benjamin Besse[1]

[1]Gustave Roussy, Villejuif, France,[2]Owkin, Paris, France,[3]Biomedical Research Institute of Malaga (IBIMA Plataforma Bionand), Malaga, Spain,[4]Cochin Hospital, APHP Centre, Paris, France,[5]CHRU Tours - Hopital Trousseau, Chambray-les-Tours, France,[6]Instituto de Cancerologia - AUNA, Medellin, Colombia,[7]Intergroupe Francophone de Cancerologie Thoracique, Paris, France,[8]Hopital Marie-Lannelongue, Le Plessis-Robinson, France,[9]Institut Curie, Paris, France,[10]Hopital Necker-Enfants Malades, Paris, France

Background: Diagnosis of thymic epithelial tumors (TETs) is challenging due to multiple primary classes (A, AB, B1, B2, B3, C), based on tumoral and non-tumoral components, and their rarity. We aim to train and test a multiple-instance learning (MIL) model to classify the histological subtypes using hematoxylin-eosin+/-safran (HE/HES)-stained slides collected in the French RYTHMIC network.
Patients and Methods: HE/HES slides from TET (A, AB, B1, B2, B3, C) cases addressed to RYTHMIC between 2012 and 2016 were retrieved and digitized. All pathological diagnoses were revised by central pathologists within the network. A MIL model was trained on digitized images, with no adjoint clinical features; internally validated using 3-repeated 2-fold cross-validation; further tested on a set of prospectively digitized cases addressed to RYTHMIC (and centrally revised) between 2022 and 2024, to classify the main TET types. Class predictions were evaluated by AUC scores and ROC curves. Interpretability was performed via Shapley value and heatmaps.
Results: The training set comprised 456 slides from unique histological samples, with 243 (53%) obtained through thymectomy. Internal 3-repeated 2-fold cross-validation showed a mean AUC of 0.94 [std 0.005] for the overall prediction of subtypes. High-attention regions across slides contain both epithelial and lymphocytes, in different percentages according to subtype, showing the biological consistency of the prediction. In the test set (*n*=43), class prediction had a mean AUC of 0.96 [0.92-0.98]. Sensitivity/specificity for each class in the two sets are shown in the table.
Conclusion: Our model holds promise as a diagnostic tool for TET, highlighting the potential of digital pathology in diagnosing rare and challenging entities. These results will be validated on a larger scale, and mixed components will be integrated.

Internal cross-validation subtype*(n)*	Sensitivity	Specificity	Test set subtype*(n)*	Sensitivity	Specificity
A (33)	0.76	0.95	A (4)	0.25	1.00
AB (129)	0.74	0.95	AB (20)	0.85	0.87
B1 (26)	0.81	0.91	B1 (3)	0.33	1.00
B2 (110)	0.65	0.97	B2 (11)	1.00	0.81
B3 (60)	0.73	0.97	B3 (2)	1.00	1.00
C (98)	0.88	0.96	C (3)	0.67	1.00

#2467 Advancing multimodal machine learning techniques to predict platinum resistance in high-grade serous ovarian cancer.

Leonhard Donle, Kelsey C. Goon, Ernst Lengyel

University of Chicago Pritzker School of Medicine, Chicago, IL

Background: Median survival in high-grade serous ovarian cancer (HGSOC) drops to <2 years when it advances rapidly and is declared platinum-resistant (PR). Prior efforts using machine learning to predict PR from hematoxylin and eosin (H&E) whole slide images (WSIs) have shown modest success with an area under the curve (AUC) ~0.6. Current Computational Pathology (CPath) methods frequently overfit due to relative data scarcity and dimensionality mismatches between gigapixel-resolution WSIs and a binary outcome. This project aims to improve CPath performance in predicting PR in newly diagnosed HGSOC by optimizing multimodal fusion of clinical data elements.

Methods: We defined PR as progression or recurrence within 12 months of completing platinum-based therapy, and platinum sensitivity (PS) as recurrence > 12 months after completing treatment. The model was trained on 243 H&E WSIs from 127 patients (62 PR, 65 PS) with repeated 5-fold cross-validation and validated on The Cancer Genome Atlas-Ovary (TCGA) dataset comprising 1156 WSIs from 578 patients (188 PR, 390 PS). H&E WSIs at 20x magnification were tiled, with features extracted via the UNI foundation model and analyzed using attention-based multiple-instance learning to predict PR. FIGO stage and largest residual at primary cytoreduction were used as clinical parameters and fused into the model using different strategies - early, intermediate via concatenation, intermediate via Kronecker product (KP), and late - and performance compared across techniques.

Results: On the internal test set, intermediate-fusion via KP achieved the highest AUC and balanced accuracy (bAcc) (AUC 0.806, bAcc 0.744), followed by early (AUC 0.726, bAcc 0.661), intermediate via concatenation (AUC 0.680, bAcc 0.617), late (AUC 0.672, bAcc 0.628) and the model without clinical data (AUC 0.629, bAcc 0.593). On the TCGA dataset, the best-performing model had an AUC of 0.622 and bACC of 0.566, compared to published baseline AUC of 0.602 (Ahn et al, May 2024) which was trained on 754 WSIs of 394 patients (132 PR, 262 PS).

Conclusions: Despite a smaller training set, we surpass recently published models for predicting PR in HGSOC from H&E WSIs by adding simple clinical data points, favoring an interwoven intermediate fusion technique. Multimodal fusion via KP thus represents a means to expand the use of CPath to predict clinical outcomes in diseases with relative data scarcity. These results suggest progress towards better matching cases of PR HGSOC to non-platinum treatment.

#2468 Automated co-registration of histology and mass spectrometry imaging for enhanced prostate cancer pathomics.

Jader Giraldo-Guzman[1], Cameron A. Czerpak[1], Cody Weimholdt[2], Lewis J. Thomas[3], Sheng-Kwei Song[1], Peggi M. Angel[4], Richard R. Drake[4], Joseph E. Ippolito[1], **Jose Marcio Luna**[1]

[1]Department of Radiology, Washington University School of Medicine in St. Louis, St. Louis, MO,[2]Department of Pathology and Immunology, Washington University School of Medicine in St. Louis, St. Louis, MO,[3]Department of Surgery, Washington University School of Medicine in St. Louis, St. Louis, MO,[4]Department of Pharmacology and Immunology, Medical University of South Carolina, Charleston, SC

Background: Prostate cancer (PCa) progression has been shown to be influenced by the relationship of extracellular matrix (ECM) proteins within the stroma, and immune and tumor cells. However, the current standard of care for PCa patients does not take these factors into account. Previously, our team identified sets of ECM glycans using matrix-assisted laser desorption/ionization (MALDI) imaging that were associated with PCa. We also found preliminary evidence that MALDI imaging can guide pathomic textural analysis in H&E images. However, our approach required manual registration between the MALDI and hematoxylin and eosin stain (H&E) images to derive pathomic textural features. In this study, we aim to fully automate the image co-registration and segmentation to guide the identification of pathomic textural features, and to demonstrate that glycan expression in MALDI images can identify regions of PCa in H&E images as tumor.

Methods: We identified a cohort of PCa patients (n=10) who had undergone radical prostatectomy treatment (grade groups GG3-GG5), and with available H&E images, as well as five MALDI images per patient representing the distribution of N-glycan species at 1419, 2539, 2686, 1663, and 1809 m/z. The H&E and MALDI images were co-registered using feature-based deformable registration methods based on mutual information, and segmentations were automatically calculated using a computational imaging framework based on rolling ball, erosion, dilation, Otsu's thresholding, and large connected component analysis. This created 6 regions of interest on the H&E image (5 N-glycans, 1 Pathologist) for analysis of pathomic textural features. Dice scores were used to measure the overlap between the glycan mask and the pathologist segmentation in H&E. We measured 70 pathomic textural features using PyRadiomics on the H&E images for the 5 glycan regions and compared every combination of textural features using Wilcoxon signed rank tests.

Results: The median Dice scores between the N-glycan mask and pathology tumor segmentation were: 1419 (0.48 [0.39–0.65]), 2539 (0.64 [0.44–0.76]), 1663 (0.27 [0.15–0.37]), 1809 (0.38 [0.14–0.42]), and 2686 m/z (0.29 [0.09–0.35]). This indicates the glycans with the largest overlap with tumors were 1419 and 2539 m/z, while glycans 1663, 2686 and 1809 m/z overlap most with non-tumor regions. Out of the 70 pathomic textural features, 55 were significantly different ($p<0.05$) between the masks for 1419 and 1663 m/z, 48 between 1419 and 1809 m/z, 47 between 2539 and 1663 m/z, and 50 between 2539 and 1809 m/z. In addition, there were only 10 significantly different features between 1419 and 2539 m/z, and 4 features between 1663 and 1809 m/z.

Conclusions: We provided further evidence that tissue properties and pathomic textural analysis in H&E images can be guided by MALDI imaging, and preliminary evidence that the process can be fully automated.

#2469 Enhancing pathology workflows with chemical imaging and AI-driven generative models.

Kianoush Falahkheirkhah[1], Sudipta Mukherjee[1], John Cheville[2], Rohit Bhargava[1]

[1]University of Illinois at Urbana-Champaign, Champaign, IL,[2]Mayo Clinic, Rochester, MN

Histopathology has been a cornerstone of biomedical tissue assessment for decades, involving a labor-intensive and multi-step process that includes biopsy, gross examination, sampling, and subsequent tissue processing into snap-frozen or formalin-fixed paraffin-embedded (FFPE) blocks. These blocks are then sectioned, stained, and examined microscopically to diagnose and grade tissues. Despite its effectiveness, the traditional workflow is time-consuming, resource-intensive, and reliant on chemical dyes and expert interpretation. Recently, innovative chemical imaging techniques, such as Stimulated Raman Scattering (SRS) microscopy, have begun to redefine this field by enabling direct measurement of the molecular composition of tissues, thereby bypassing conventional tissue processing and staining protocols. This study demonstrates the integration of SRS microscopy into pathology workflows to rapidly acquire high-resolution chemical data from minimally processed fresh-frozen tissues. Unlike traditional methods that rely on thin-sectioned slides, this approach collects data from intact thick tissues using "optical sectioning," enabling the generation of three-dimensional datasets across multiple planes. These chemical images are processed through a generative adversarial network (GAN) pipeline to produce virtual hematoxylin and eosin (H&E) images that are visually and diagnostically comparable to traditionally stained specimens. Furthermore, the generative model is extended to simulate the morphology and staining patterns of FFPE-processed tissues, offering a versatile tool for creating archival-quality images. This method significantly enhances diagnostic capabilities by preserving lipids and small molecules that are often lost during conventional processing. Within minutes, it generates high-quality stained images that demonstrated high inter-pathologist agreement in tissue grading, proving diagnostically reliable for clinical use. We evaluate our approach through a comprehensive survey of pathologists, assessing its diagnostic utility and reliability. Our results demonstrate that the generated images are comparable in quality to real histological images, showing no discernible inferiority in diagnostic utility. In prostate pathology, the integration of SRS microscopy and AI significantly streamlines traditional workflows, reducing complexity and processing time while preserving diagnostic precision.

#2470 Transforming cancer diagnosis with digital chemical pathology.

Kianoush Falahkheirkhah[1], Kevin Yeh[1], Ji-Hun Oh[1], Yen-Ting Liu[1], Ruo-Jing Ho[1], Ishaan Sharma[1], Matthew Confer[1], Andres Orr[1], Mykolas Tamonis[1], John Cheville[2], Rohit Bhargava[1]

[1]University of Illinois at Urbana-Champaign, Champaign, IL,[2]Mayo Clinic, Rochester, MN

Pathology remains a labor-intensive discipline, relying on workflows rooted in practices established over a century ago. Despite incremental technological advancements, the diagnostic process—from tissue preparation to interpretation—still heavily depends on thinly sliced tissue sections stained and examined under brightfield microscopy by skilled pathologists. Difficult cases often necessitate iterative staining or complex immunohistochemistry (IHC) analyses. However, the recent emergence of machine learning (ML) presents an opportunity to revolutionize traditional diagnostic workflows. One promising advancement is Digital Chemical Pathology (DCP), a paradigm that combines modern ML algorithms with infrared (IR) imaging to streamline pathology and enhance diagnostic accuracy. Infrared wavelengths inherently align with molecular vibrational frequencies, providing intrinsic contrast based on the chemical composition of samples. Mid-IR spectroscopic microscopes leverage this principle to spatially resolve molecular distributions at the pixel level, creating "chemical fingerprints" that ML algorithms can correlate with cellular identities and disease states. Recent advancements, particularly the development of broadly tunable quantum cascade lasers (QCLs), have enabled faster IR imaging, paving the way for clinical applications of IR spectroscopic microscopes. In this research, we demonstrate the potential of DCP using frozen tissue samples. Our approach focuses on two core tasks: (1) virtual staining, translating IR images into realistic, stained images interpretable by pathologists, and (2) tissue segmentation, enabling the automated detection of cancer and the distinction between normal and cancerous tissues. Furthermore, we incorporate statistical safety algorithms to generate uncertainty maps, ensuring the reliability of predictions. Our findings highlight that the integration of IR imaging and ML can facilitate automated diagnostics, providing pathologists with powerful tools for enhanced healthcare delivery. By leveraging DCP, we aim to modernize pathology workflows, reduce diagnostic turnaround times, and improve patient outcomes through precise and efficient analysis.

#2471 Interpretable HPV detection in head and neck cancer using foundation models and synthetic digital pathology.

Hanna M. Hieromnimon[1], Anna Trzcinska[2], Frank Wen[3], Frederick M. Howard[3], James M. Dolezal[4], Emma Dyer[3], Sara Kochanny[3], Jefree Schulte[5], Cindy Wang[6], Heather Chen[7], Jeffrey Chin[3], Elizabeth Blair[3], Nishant Agrawal[3], Ari Rosenberg[3], Everett Vokes[3], Rohan Katipally[3], Aditya Juloori[3], Evgeny Izumchenko[3], Mark W. Lingen[3], Nicole Cipriani[2], Jalal B. Jalaly[8], Devraj Basu[9], Samantha J. Riesenfeld[10], Alexander T. Pearson[3]

[1]Graduate Program in Biophysical Sciences, University of Chicago, Chicago, IL,[2]Department of Pathology, University of Chicago, Chicago, IL,[3]Department of Medicine, University of Chicago, Chicago, IL,[4]Geisinger Cancer Institute, Danville, PA,[5]Department of Pathology and Laboratory Medicine, University of Wisconsin, Madison, WI,[6]Department of Pathology, Stanford University, Palo Alto, CA,[7]Department of Pathology, University of Michigan, Ann Arbor, MI,[8]Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA,[9]Department of Otorhinolaryngology-Head and Neck Surgery, University of Pennsylvania, Philadelphia, PA,[10]Pritzker School of Molecular Engineering, University of Chicago, Chicago, IL

Introduction: Detecting human papillomavirus (HPV) status is crucial for treating Head and Neck Squamous Cell Carcinomas (HNSCCs). While p16 immunohistochemistry is the current standard for HPV detection, its moderate sensitivity and complex implementation limit its global utility. The ability to diagnose HPV status in HNSCC has become increasingly critical worldwide, as rising HPV-positive HNSCC rates observed in high-income countries may signal a global trend, and HPV status remains essential for treatment selection. Although hematoxylin and eosin (H&E) stained slides are clinically ubiquitous, artificial intelligence (AI) methods applied to these images have not matched molecular assays' performance nor provided needed clinical interpretability. Recent advances in vision transformer-based foundation models for computational pathology offer a promising approach to address this unmet need.

Methods: We analyzed H&E images from 981 HNSCC patients across four datasets: TCGA (n=HPV+ 33/total 401), CPTAC (n=1/109), UCH (n=159/364), and PENN (n=106/106). Fifty percent of the patients were used for validation. Using UNI, a foundation self-supervised learning (SSL) model, we extracted feature vectors from 10x effective magnification tile images across each whole slide. We identified an HPV feature axis using recursive support vector machine and principal component analysis to isolate SSL features that differentiated HPV tumors, then interpreted the relevant histologic features using HistoXGAN to generate synthetic histology images. This approach enabled the isolation of histologic features specific to HPV+ tumors, whereas real histology images contain multiple sources of variation. An expert pathologist validated the biological relevance of the identified HPV-axis features. We developed a predictive model for HPV status by varying the percentages of tiles within each slide required to exceed a binary threshold on the HPV-axis. This model was robust across threshold choices.

Results: Our method identified an HPV-axis that robust HPV detection performance (sensitivity 0.83, specificity 0.88) across all datasets (balanced accuracy - TCGA 0.77, CPTAC 0.98, UCH 0.78, PENN 1.00). Using synthetic histology images and pathologist validation, we identified key, morphological features aligning with established HPV-associated histology, including nuclei size, color, and cell borders. Together with our Grundium slide scanning pipeline the time-to-prediction for a slide is less than 3 minutes.

Conclusions: Foundation models and synthetic digital pathology enabled HPV detection from histology with accuracy comparable to current diagnostic standards, while providing pathologist-interpretable predictions. This accessible, rapid, and explainable method holds promise for expanding testing of HPV and potentially other molecular features in resource-limited settings.

#2472 HER2 protein localization in super-resolution and anti-HER2 therapy response in breast cancer.

Vicente Peg[1], **Yuko Saruta**[2], Yun Li[2], Santiago Ramon y Cajal[1]

[1]Vall d'Hebron University Hospital, Barcelona, Spain,[2]Sysmex R&D Center Europe, Hamburg, Germany

Introduction: Human epidermal growth factor receptor 2 (HER2) in breast cancer (BC) is an important biomarker for a better decision making of anti-HER2 therapy. Trastuzumab has been the standard of care treatment for HER2 positive patients, but recently new antibody-drug conjugates (ADCs) have given an alternative option in clinical practice. Since ADCs efficacy is due to HER2 antibody localization and internalization in the cell, we hypothesize that cellular sensitivity to the drug may be linked to cellular vesicle trafficking systems and membrane protrusions which indicate the resistance of HER2 internalization. Super resolution fluorescence microscope named HM-1000 achieves a resolution less than 20 nm. With this new tool, we aim to study the intracellular distribution pattern of HER2 including vesicle-like structures and membrane protrusions to better identify responders of anti-HER2 therapies, especially ADCs.
Method: A retrospective unicentric study is designed to recruit BC patients who have received ADCs treatment that have formalin fixed paraffin-embedded tissue samples stored for routine diagnosis. 5 baseline tumor samples were stained by commercialized HER2 in situ immunofluorescence, and O2 scavenging buffer was applied and sealed to capture the fluorescent blinking every 30 milliseconds by HM-1000. The 50,000 image frames per microscopic field were processed using ImageJ software and created super-resolution images.
Results: We observed that HER2 signals from ADCs responding patients exhibit predominant membrane localization, especially in those with 3+ immunohistochemistry staining status, compared to ADCs non-responding HER2 3+ cases which show low membrane localization of HER2 and more intracellular distribution. Additionally, this finding is highlighted by intense membrane protrusions in HER2 3+ tumor which present very good response of ADCs.
Conclusion: We found different HER2 localization in ADC responding and non-responding patients. The presence of membrane protrusions may facilitate HER2 targeted antibody binding and induce both cellular cytotoxicity from immune effector cells and endocytosis of cytotoxic drug, which explains ADCs response. Further studies are needed to support this finding and better predict the effectiveness of ADCs therapies in HER2 positive tumors.

#2473 Generative AI improves breast cancer genomic subtype prediction from histology.

Brennan G. Simon[1], Clemens L. Weiss[2], Christina Curtis[3]

[1]Cancer Biology, Stanford University, Stanford, CA,[2]Stanford Cancer Institute, Stanford University, Stanford, CA,[3]Genetics, Stanford University, Stanford, CA

Despite advancements in prognostic biomarker identification in cancer, the clinical infeasibility of testing for many biomarkers limits their adoption into clinical practice. Computational pathology has demonstrated remarkable success in efficiently predicting a variety of biomarkers from routine histology images that would otherwise require expensive sequencing or spatial profiling. However, current approaches are limited for data-poor and challenging tasks such as the prediction of genomic subtype in breast cancer. Here, we show that generative AI improves state-of-the-art computational pathology methods by leveraging the power of synthetic data in the task of predicting breast cancer genomic subtype from histology images. Incorporating generative AI to enrich the training dataset improves model AUC, overall accuracy, and accuracy on the underrepresented high risk subtype. Finally, we show that model interpretation using explainable AI techniques on the tile and pixel levels corroborates expected phenotypes as well as identifies previously unknown histologic features associated with genomic subtype. Our results present an improved framework for computational pathology for the enhanced clinical detection and translation of biomarkers.

#2474 Deep learning-based tissue segmentation enables quantitative assessment and identifies dynamic markers of response from routine staining in glioblastoma.

Georges Ayoub[1], Dina Elharouni[1], Connor Bossi[1], Seth Malinowski[1], Prem Prabhakar[1], Constantin Moller[1], Muhammad Shaban[2], Anurag Jayant Vaidya[3], Julia Japo[1], Andrew Zhang[3], Andres Santos[2], Drew F.K. Williamson[4], David Meredith[2], Faisal Mahmood[2], Keith L. Ligon[1]

[1]Dana-Farber Cancer Institute, Boston, MA,[2]Brigham and Women's Hospital, Boston, MA,[3]Harvard-MIT Health Sciences & Technology, Cambridge, MA,[4]Emory University School of Medicine, Atlanta, GA

Glioblastoma (GBM), the most common and aggressive primary brain tumor in adults, has a highly heterogeneous histopathologic profile on routine H&E staining with challenging traditional morphology assessment. The reliability of morphological features in reflecting response to therapy is debated, as current assessment approaches remain inherently qualitative with a lack of dynamic quantifications after treatment. To address this, we aimed to (1) train an interpretable deep convolutional neural network classifier for semantic segmentation of tissue regions in GBM whole-slide images (WSIs) from routine H&E staining, (2) establish a quantitative baseline of morphological variations after standard-of-care treatment (radiotherapy/temozolomide) in a paired clinical cohort, and (3) assess this approach's utility in identifying markers of response. Therefore, we annotated 3,545 region-level ground truth labels in a supervised approach and trained a DenseNet classifier on the HALO AI platform to segment seven tissue classes in GBM: TC1 (Geographic necrosis), TC2 (Palisading necrosis), TC3 (Mesenchymal tissue), TC4 (Hemorrhage), and TC5 (Parenchyma), subdivided into TC5.1 (Heavy infiltration) and TC5.2 (Mild infiltration). The classifier achieved high performance on an independent set of 828 annotations (precision = 0.88, recall = 0.84, F1-score = 0.86), and digital quantifications strongly correlated with manual estimations extracted from clinical pathology reports ($p < 0.001$). We then identified a retrospective longitudinal cohort of 142 IDH-wildtype GBM patients and scanned the corresponding 912 whole-slide images of tissue from diagnostic resection paired with tissue from recurrence after standard-of-care treatment. Between the two time points, quantifications by the applied classifier showed significant increases in the classes of mild infiltration (+87%) and geographic necrosis (+75%), coupled with parallel decreases in heavy infiltration (-31%) and palisading necrosis (-45%) ($p < 0.01$). On recurrence, the ratio of heavy to mild infiltration (TC5.1:TC5.2) allowed significant stratification of overall survival, and rapid progressors had significantly larger areas of geographic necrosis. On multivariable analysis, dynamic increase in mild infiltration and decrease in mesenchymal tissue were independently associated with reduced risk of poor outcome. Taken together, our AI-based tissue segmentation classifier enables an automated quantitative assessment and allows interpretable clinical response stratification from routine staining, providing a potential enhancement of GBM diagnostic reporting.

#2477 Accurate, high-throughput spatial profiling of whole slide samples with the NeoLYTX pipeline.

K. Gallagher, J. Kuo, J. Lock, J. Fei, M. Rohafza, C. Todorov, E. Parnell, M. Lazare, Q. Au, H. Nunns;
Neogenomics Laboratories, Inc., Aliso Viejo, CA

Multiplexed immunofluorescence (mIF) enables spatially resolved single-cell phenotyping within the tumor microenvironment (TME), which is being increasingly recognized as an important predictor of patient response to immunotherapies. MultiOmyx^{TM} is a mIF platform provided by NeoGenomics Laboratories, Inc capable of detecting up to 60 proteins on a single formalin-fixed paraffin-embedded tissue section, with an image-able area of 10 square centimeters. The platform enables profiling of millions of individual cells in a single slide, providing unbiased spatial phenotyping at scale.

Here we present an improved NeoLYTX^{TM} image analysis pipeline that has been optimized for whole-slide analysis. We demonstrate the pipeline's capabilities using a 16-marker TME panel to spatially characterize a set of 20 non-small cell lung cancer (NSCLC) samples. Accuracy is benchmarked against clinically validated immunohistochemistry (IHC) for a subset of representative immune phenotypes. Cell density and intensity data generated by the NeoLYTX pipeline demonstrated strong concordance with IHC assays for all markers evaluated.

To achieve high throughput whole slide processing while maintaining high accuracy, we use an automated pipelined backed by 100% human quality control (QC). Automation begins with image acquisition, where QC algorithms monitor image collection in real-time to detect imaging failures and trigger corrective action. In post-processing, tissue and stain QC algorithms then identify regions of tissue artifacts such as tissue loss, tissue folding, and necrotic regions to be excluded from downstream analysis.

We next transform our mIF images into a single-cell map of the TME by segmenting the tissue into spatial regions and phenotyping on a single cell level. Region segmentation delineates the tissue from surrounding glass, and then further segments the tissue into different regions such as intratumoral, stromal, vasculature, and leading-edge. Single cells and subcellular compartments are segmented and intensity is quantified over the relevant expression patterns of each marker. Cells are phenotyped using an in-house library of AI algorithms trained on millions of annotated cells - the models integrate morphological and intensity features to accurately distinguish target populations from artifacts and identify phenotypes across diverse tissue types and qualities.

Finally, we demonstrate our comprehensive spatial profiling on the cohort of 20 NSCLC samples. Cell distributions are quantified through a combination of unsupervised neighborhood clustering, nearest neighbor distances, and tissue region profiling. By mapping critical cell populations—such as tumor-infiltrating lymphocytes, cancer-associated fibroblasts, and other cellular signatures—across distinct tissue regions, we extract salient spatial information that provides biological insight into the TME.

BIOINFORMATICS / COMPUTATIONAL BIOLOGY / SYSTEMS BIOLOGY / CONVERGENT SCIENCE: Quantitative Methods and Pipelines
Poster Session

#2480 DeMixT 2.0: A deconvolution framework for sparse sequencing data using embedded negative binomial distribution.

H. Yan[1], M. Montierth[2], L. Xie[3], P. Yang[1], S. Guo[1], R. Li[1], X. Pan[1], C. Ercan[1], Y. Yuan[1], K. Nemeth[1], G. A. Calin[1], W. Wang[1];
[1]The University of Texas MD Anderson Cancer Center, Houston, TX, [2]Baylor College of Medicine, Houston, TX, [3]The University of Texas at Austin, Austin, TX

Background: Deconvolution methods have traditionally focused on estimating cell-type proportions in bulk tissues with mRNA profiles. They often overlook the cell transcriptional proportion (i.e., transcriptional activity), which reflects the cell functional states and could be important for understanding disease progression (e.g., tumor). Current methods face additional challenges when analyzing other RNA species, such as miRNAs, due to their low abundance and inherent sparsity. Similar limitations also affect spatial transcriptomics (ST) technologies, which enable *in situ* gene expression profiling but suffer from high data sparsity and limited resolution, with each spot mimicking a mini-bulk sample. Besides, methods for estimating transcriptional activity in ST data remain underdeveloped.
Methods: To fill this gap, we extended our semi-reference-based DeMixT framework by incorporating a negative binomial (NB) distribution to account for sparse profiling data, namely DeMixNB. DeMixNB models the observed mixed expression Y_{ig} for gene g in sample i as the sum of tumor (T_{ig}) and non-tumor (N_{ig}) components, with each component following an NB distribution parameterized by gene-specific means, dispersion parameters, and sample-specific tumor transcriptomic proportions. The NB framework naturally accommodates the sparsity and overdispersion. DeMixNB runs in two tiers: it first leverages non-tumor reference to estimate the non-tumor component parameters, then uses an Iterated Conditional Modes (ICM) to jointly estimate tumor-specific parameters and tumor proportions in mixed samples.
Results: We comprehensively validated DeMixNB from three aspects. First, we simulated data under various settings, including sample sizes, degrees of similarity between tumor and non-tumor components, and the number of distributions mixed in tumors. Across all scenarios, DeMixNB consistently achieved higher accuracy compared to existing methods. Then, to create a controlled experimental benchmark for miRNA analysis, we generated artificial mixtures using HS-5 fibroblasts combined with either wild-type or *Dicer1* knockout HCT116 colorectal cancer cells at varying proportions. This design represents real tumor samples with known ground truth. DeMixNB maintained robust performance on sparse miRNA expression. To assess its broader utility, we applied DeMixNB to ST data from multiple cancer types. The estimated transcript proportions showed strong concordance with pathological annotations and revealed cell differentiation patterns not captured by conventional cell-type deconvolution methods.
Conclusions: The DeMixT 2.0 framework, featuring DeMixNB, provides a robust solution for deconvolving sparse transcriptomic data. By accurately estimating tumor-specific transcriptional proportions, it enables deeper insight tumor heterogeneity at molecular and spatial levels.

#2481 Employing joint modeling to support clinical interpretation of ctDNA dynamics during the treatment of advanced colorectal cancer.

Christopher R. Pretz, Jiemin Liao, Aaron Hardin, Amar Das

Real World Evidence, Guardant Health, Palo Alto, CA

Background: Recent evidence suggests dynamic changes in cell-free circulating tumor DNA (ctDNA), defined as the maximum variant allele fraction (maxVAF), is predictive of overall survival (OS) in colorectal cancer (CRC) patents. As such, we leverage an advanced statistical approach, joint modeling of longitudinal and time-to-event data (JM), to associate ctDNA dynamics and OS. Contrasting with traditional methods, a unique advantage of JM is results assume the form of patient-specific adaptive dynamic predictions, where utilizing these predictions can enhance clinical decision-making in longitudinal monitoring.
Methods: We applied JM to a retrospective cohort of patients with advanced CRC who received chemotherapy (chemo). The cohort was extracted from GuardantINFORM, a real-world clinico-genomics database. Patients analyzed had baseline Guardant360 (G360) ctDNA tests within 14 days before chemo initiation and ≥2 G360 tests on treatment. JMs are comprised of two sub-models: a hierarchical cubic spline random effects sub-model evaluated the serial ctDNA measures and a Cox-regression sub-model examined OS. Covariates incorporated into each sub-model included: age, gender, smoking status, and the Charlson Comorbidity Index. JMs, based on different association structures (AS) designed to connect information between sub-models, were investigated. ASs included the biomarker's estimated value, instantaneous rate of change, and cumulative effect.
Results : In total, 137 patients with 759 ctDNA time points were analyzed using three different JMs, one for each AS. After controlling for confounders, each AS demonstrated a significant relationship between ctDNA progression and OS (respective p-values < 0.005). As results are best interpreted graphically, various dynamic predictions are provided, each accentuating how the AS modifies the relationship between ctDNA and OS.
Conclusions : To our knowledge, using JM to link dynamic ctDNA changes and OS in CRC patients is novel. In this study we demonstrate that a biomarker's updated estimated value, instantaneous rate of change, or cumulative effect can forecast OS. As such, JM may afford healthcare providers different options to monitor patient progression and update prognosis as new ctDNA information becomes available on clinical reports. In sum, these results establish a foundation for integrating serial ctDNA measures for prognostic clinical interpretation.

#2482 Inferring cancer type-specific patterns of metastatic spread using Metient.

Divya Koyyalagunta[1], Karuna Ganesh[2], Quaid Morris[2]

[1]Weill Cornell Medicine, Memorial Sloan Kettering Cancer Center, New York City, NY,[2]Memorial Sloan Kettering Cancer Center, New York City, NY

Reconstructing the metastatic spread of a cancer from molecular sequencing of primary and metastatic tumors is a challenging problem with broad applications in clinical cancer research. In particular, this problem often yields multiple solutions, which current methods resolve with ad hoc criteria that assume the most likely patterns of metastatic spread - thus sidestepping a core question of metastasis research. Here we introduce Metient, a statistical method which proposes multiple possible hypotheses of metastatic spread in a cohort of patients and rescores these hypotheses using independent data on genetic distance of metastasizing clones and organotropism. Metient is more accurate and is up to 50x faster than the current state-of-the-art, making it the first method capable of scaling to lineage tracing datasets with thousands of cells while providing a multi-hypothesis approach to metastatic spread. In a reanalysis of metastasis in 169 patients based on 490 tumors, Metient automatically identifies cancer type-specific trends of metastatic dissemination in melanoma, high-risk neuroblastoma, and non-small cell lung cancer. Its reconstructions often align with semi-manual expert analyses but frequently reveal more plausible migration histories, including those with metastasis-to-metastasis seeding. Furthermore, Metient's heightened sensitivity suggests that polyclonal seeding is far more common than previously reported. Together, these findings challenge existing assumptions about metastatic spread, enhance our understanding of cancer type-specific metastasis, and offer insights that could inform future clinical treatment strategies of metastasis.

#2483 DirectHRD enables sensitive scar-based classification of homologous recombination deficiency (HRD).

Ruolin Liu[1], Eugenia Roberts[2], Heather Parsons[3], Elizabeth Stover[3], Atish Choudhury[3], Justin Rhoades[1], Timothy Blewett[1], David Yang[3], Joyce Liu[3], Erica Mayer[3], Viktor Adalsteinsson[1]

[1]Broad Institute of MIT and Harvard, Cambridge, MA,[2]Yale University, New Haven, CT,[3]Dana-Farber Cancer Institute, Boston, MA

Introduction: Homologous recombination deficiency (HRD) is a predictive biomarker for response to PARP inhibition and platinum chemotherapy but remains challenging to detect. The discovery of patients without pathogenic mutations in known HR genes but exhibiting genomic scars indicative of HRD led to the FDA approval of the first scar-based HRD test. Despite advancements in whole genome sequencing (WGS) combined with machine learning models, current methods lack the sensitivity required for detecting HRD scars in low tumor purity samples such as liquid biopsies.

Method: We developed DirectHRD, a WGS-based HRD scar classifier. Compared to other HRD scar classifiers, DirectHRD exclusively utilizes a highly specific HRD scar—deletion with microhomology—and its associated signatures (COSMIC ID6 signature) in a probabilistic framework to detect HRD scars with higher sensitivity.

Results: We first applied DirectHRD to 474 tumor biopsy samples from the PCAWG (Pan-Cancer Analysis of Whole Genomes) project, focusing on the four cancer types with the highest HRD prevalences (breast, ovary, pancreas, and prostate). We achieved 100% detection of patients with bi-allelic BRCA1/2 losses with high specificity (>90%) across all four cancer types. Next, we applied DirectHRD to WGS of tumor biopsies and in-silico dilutions from 27 patients with stage II-III triple-negative breast cancer from the TBCRC030 trial. DirectHRD achieved an AUC of 0.99 at 5% tumor fraction, compared to an AUC of 0.62 and 0.48 for CHORD and HRDetect, respectively. Finally, we applied DirectHRD to 90 ctDNA (circulating tumor DNA) samples across three cancer types (breast, ovarian, and prostate). The ground truth HRD statuses were determined from tumor biopsies using scar-based methods for breast (Myriad MyChoice, scarHRD and SigMA) and ovarian cancers (scarHRD and SigMA) and by identifying germline and somatic pathogenic mutations in HR genes for prostate cancers. DirectHRD achieved an AUC of 0.86 in stage II-III breast cancers and 0.9 in metastatic breast cancers. For ovarian cancers and metastatic prostate cancers, DirectHRD achieved AUCs of 0.9 and 0.87, respectively.

Conclusion: Our findings suggest that DirectHRD can reliably detect HRD scars in low tumor purity samples, such as liquid biopsies, using moderate depths (e.g., 50x) of WGS. This could broaden access to HRD testing including when tissue biopsies are insufficient or infeasible.

#2484 A novel algorithm to expand mechanistic computational models of biochemical reaction networks to heterogeneous tumor cell communities.

Amy Gill, Feilim Mac Gabhann

Biomedical Engineering, Johns Hopkins University, Baltimore, MD

Background: Tumors are complex ecosystems of interacting cell types, and the effects of heterogeneous environments on cell signaling are challenging to study by experiment alone. Mechanistic computational modeling of biochemical reaction networks using ordinary differential equations (ODEs) is a powerful approach for analyzing cell signaling, yet most existing ODE-based models of cell signaling describe a population of homogeneous cells. We introduce Mechanistic Modeling of Cell Communities (M2C2), a novel algorithm to expand a biochemical reaction network model described in one cell type to a community of interacting cell types.
Summary: M2C2 represents a cell community as multiple intracellular compartments, each representing a pool of cells with similar properties (“cell types”), interacting through a common extracellular compartment. M2C2 algorithmically expands a one-cell-type model to describe an interacting community of any number of distinct cell types. The expansion assumes all compartments share the same set of possible reactions and rate equations, though the rate constants can be adjusted on a per-cell basis, e.g. for cell-specific mutations or inhibitors. Compartments have independent reactant concentrations, reflecting cell-specific protein expression and interventions. In the context of mass-action kinetics, this leads to different network dynamics in each cell type.
Algorithm: The M2C2 function takes two inputs: (1) an integer n representing the desired number of interacting cell types and (2) a single-cell-type model encoded in a vector-matrix form inspired by chemical master equation approaches for stochastic modeling.1 The matrices are arranged into blocks using location to group the molecular species (extracellular, cellular) and reactions (extracellular, interface, cellular). M2C2 expands the original matrix by replicating n copies of cellular and interface blocks while leaving the extracellular block intact. Because this expansion is algorithmic, it works for any integer n;;; without manual correction. M2C2 outputs the expanded model in vector-matrix form, which can be numerically solved using standard methods. Simulated molecular dynamics in each cell type can be analyzed with statistical and visualization methods.
Conclusions: Mechanistic Modeling of Cell Communities (M2C2) is a novel approach for mesoscale modeling of cell signaling within multicellular systems such as tumor microenvironments. Cell type proportions and cell-type-specific expression patterns of M2C2-expanded models could be parameterized with multi-omic single-cell data for in silico simulation of personalized tumors and treatments.
References: 1. Gillespie DT. Stochastic simulation of chemical kinetics. Annu Rev Phys Chem. 2007;58:35-55. doi: 10.1146/annurev.physchem.58.032806.104637. PMID: 17037977.

#2485 A Bayesian framework for unraveling disease biology from spatially resolved single-cell omics datasets by combining unbiased approaches with biological priors for cell-based microdomain discovery.

Brian Falkenstein[1], Raymond Yan[1], Shannon Quinn[1], Akif Burak Tosun[1], **S. Chakra Chennubhotla**[1], Arutha Kulasinghe[2], Filippo Pullara[1]

[1]PredxBio, Inc., Pittsburgh, PA,[2]Frazier Institute, Faculty of Medicine, The University of Queensland, Brisbane, Australia

Background: The emergence of single-cell spatial omics platforms generating high-plex proteomic and transcriptomic measurements sets the foundation for accurate downstream biologically interpretable analyses. This includes biased and unbiased approaches for cell typing, cell-cell interactions and the discovery of microdomains, complex multicellular microenvironments, underlying disease progression.

Problem: There is a lack of a computational framework for optimizing unbiased approaches with biological priors for unraveling a deeper understanding of disease mechanisms. This requires innovative integrational methods that assign confidence and robustness to data-driven biological hypotheses, e.g., presence of transition cells and other rare cell types, emergent microdomains and spatially modulated network biology.

Solution: We present a Bayesian computational approach, SpaceIQ™, that not only elucidates potential novel hypotheses driving disease mechanisms, but also cross-references existing working hypotheses from alternative methods. SpaceIQ is agnostic to imaging platforms with the ability to ingest any combinatorial forms of spatial data (e.g., transmitted light, proteomics and transcriptomics).

Results: To illustrate our approach, we compare the results from SpaceIQ to the interpretative analyses presented in He et al. Nat Biotech 2022, a publicly available 960-plex RNA data from CosMx platform on NSCLC samples. We aim to provide additional mechanistic insights underlying Stage II/III progression that can augment the biological annotations reported. We have identified 16 unbiased cell types with probabilistic representation of a priori phenotypes annotated as myeloid, lymphocytes, endothelial, epithelial/tumor, and fibroblast. IFI27, SOX4, MALAT1, TYK2, CD74, HLADRB1, and COL3A1 were among the highly-expressed discriminatory genes among cell types. 28 significant spatial interactions ($p<0.1$) were found resulting in 7 microdomains. Comparison of microdomains to niche neighborhoods defined in He et al. shows that macrophages in the stroma and TLS play a role in tumor progression. Ligand-Receptor analysis on microdomains identified IL-2 signaling, GPCR ligand binding, TNF activity, and TGF regulation to be significant cell-cell communication channels in NSCLC progression ($p<1e^{-04}$).

Conclusions: We proposed a Bayesian integration framework to further the biological interpretations of the spatially-resolved high-plex single-cell CosMx dataset on NSCLC patients presented in He et al. We've found unbiased cell types linked to known cell phenotypes in addition to potentially novel gene signatures. These unbiased cell types formulate microdomains with identified key signaling events that are associated with cancer progression.

#2486 Decoding tumor evolution through fitness landscapes of aneuploid cells.

Richard J. Beck, Noemi Andor

H. Lee Moffitt Cancer Center, Tampa, FL

Aneuploidy, prevalent in most solid tumors, significantly alters cellular phenotype and fitness. Despite its critical role in tumor evolution, a detailed quantitative understanding of its evolutionary dynamics remains elusive. To address this gap, we developed ALFA-K, a novel method to infer the fitness landscape of chromosome-level karyotypes. Utilizing longitudinal single-cell karyotype data from evolving cell populations, our method estimates the fitness of thousands of karyotypes proximal to the input data in karyotype space, enabling predictions of unseen karyotypes. We validated our approach using both synthetic data from an agent-based model (ABM) and empirical data from in vitro and in vivo passaged cell lines. The empirical data comprised over 40,000 sequenced cells across five cell lines, each sequenced longitudinally at 5-10 timepoints per lineage. Analysis of the fitted landscapes yielded several key insights: 1) WGD alters the distribution of fitness effects of copy number alterations (CNAs) (K.S. test: $P < 10^{-8}$). Specifically, karyotypes with WGD exhibit more mutations with positive selection coefficients compared to diploid karyotypes; 2) Mutations in cisplatin-treated lineages showed changes in fitness effects on average 2.3 times larger than those in untreated lineages (t-test: $P < 2.2 \times 10^{-16}$); 3) The fitness impact of a CNA varies depending on the parental karyotype; 4) The rate of chromosome missegregation can dictate the predominant karyotypes in an evolving population.

To further investigate how the tumor microenvironment shapes karyotype evolution, we are integrating these inferred fitness landscapes into an existing spatial ABM. This modeling simulates the spatially heterogeneous tumor microenvironment and its influence on karyotypic selection and adaptation. By leveraging this ABM, we aim to capture the effects of spatial constraints—such as resource availability, competition, and cellular interactions—on the fitness and distribution of karyotypes. Complementing these simulations, we are developing a bioinformatics pipeline for spatial transcriptomics data from glioblastoma clinical samples. This dataset includes samples from various regions (normal, tumor, and tumor-infiltrating) across 31 samples from 19 patients, using 10X Visium with an average of 3,100 spots per sample. The pipeline is designed to provide detailed information about the spatial organization of karyotypic diversity, elucidating how aneuploid karyotypes are distributed and interact within the tumor microenvironment. By integrating this spatially resolved data with the ABM, we aim to generate a comprehensive framework to explore how microenvironmental pressures in glioblastoma tumors shape the evolution of karyotypic diversity and drive the emergence of therapeutic resistance in patient-specific contexts.

#2487 LINMAP: A framework for comprehensive cell division lineage reconstruction via molecular barcoding and machine learning.

Lin Wang[1], Zheng Yin[1], Wenjuan Dong[1], Jianting Sheng[1], Li Yang[1], Kun Han[1], Dingcheng Gao[2], Stephen T. C. Wong[1]

[1]Houston Methodist Neal Cancer Center, Houston, TX,[2]Weill Cornell Medicine, New York, NY

Purpose: Reconstructing cell division lineages across generations is central to understanding developmental biology, cancer progression, cell differentiation, drug resistance, and tissue aging. By integrating CRISPR-Cas9 induced gene editing with single cell RNA-Seq, high-resolution lineage tracing through molecular barcoding becomes feasible. However, tracking mutation evolution across successive guide RNA edits, especially with homing guide RNA (hgRNA) technology, poses challenges due to ongoing dynamic retargeting and genetic modifications that complicate lineage reconstruction.
Methods: To overcome these challenges, we developed LINMAP, a computational framework that applies machine learning to trace and map evolving mutations across cell divisions, reconstructing intermediate histories. LINMAP calculates mutation transition probabilities at each division to identify potential paths for mutation accumulation and defines cell-cell distances based on these transition probabilities, enabling precise division count measurements between cell pairs.
Results: We created an MMTV-PyMT (mouse mammary tumor virus-polyoma middle tumor-antigen)-Cas9 tracing cell line with dual hgRNAs to investigate mutation evolution networks. LINMAP accurately inferred mutation evolution and predicted allele frequencies, aligning well with both in vitro data from MMTV-PyMT cell lines and computational simulations. Moreover, in a separate in silico experiment simulating lineage history from a progenitor, LINMAP reconstructed the cell division lineage with high precision. Applying LINMAP to a CRISPR-Cas9 lineage-traced lung cancer mouse model, we successfully traced tumors of varying clonality within a single mouse. Based on lineage characteristics, LINMAP differentiated tumors, reconstructed their complete lineage, and identified each tumor’s monoclonal or polyclonal origins. It recreated a timeline of tumor emergence and tracked cell state transitions, capturing cell state shifts during cancer progression. These findings aligned with gene expression-based cell state trajectories from the original study, further validating LINMAP's accuracy.
Conclusions: LINMAP integrates mutation ratios and transition probabilities at each cell division, surpassing precision than existing methods. It enables comprehensive single cell lineage tracing, providing insights into tumor evolution and complexity, tracking tumor development, distinguishing metastatic sites from primary tumors, and studying drug resistance through subclonal selection over time.

#2488 Employing RNA base editors to engineer personalized tumor-specific neoantigens.

Rona Merdler-Rabinowicz[1], Netanel Landesman[1], Eytan Ruppin[2], Erez Levanon[1]

[1]Bar-Ilan University, Ramat-Gan, Israel,[2]NCI, NIH, Bethesda, MD

Enhancing the quantity and effectiveness of neoantigens in tumors is essential for advancing immunotherapy and developing targeted cancer vaccines. Crafting neoantigens presents a challenge due to the need to specifically modify peptide translation in tumor cells while avoiding effects on normal cells. To tackle this challenge, we introduce a novel method that enables the editing of selected nucleotides in the mRNA of cancer cells in order to produce potent neoantigens. The method is built on the use of Endogenous-ADAR base-editors, an established technology that allows for precise RNA-level edits, which is safer than DNA-level modifications and eliminates the need for external peptides by leveraging the native ADAR enzyme. The algorithm designs a guide RNA tailored to each tumor unique genetic profile and the patient's HLA type, ensuring a precise and personalized approach to induce edits that are predicted to generate potent neoantigens in the cell. By targeting tumor-specific somatic mutations, our method ensures that edits are confined to tumor cells, sparing normal cells, thus offering a safer, more effective strategy for generating personalized neoantigens. We tested our computational algorithm on thousands of tumor samples available in The Cancer Genome Atlas (TCGA) database, demonstrating its capability to produce neoantigens across a broad spectrum of cases and cancer-types. As Endogenous-ADAR technology advances and delivery methods improve, the algorithm we present shows great promise as a valuable complement to immunotherapy and cancer vaccines.

#2489 The new approach for measuring nonuniformity of read coverages reveals the quality of RNA-seq data.

Wonyoung Choi, Miyeon Yeon, Jay Lee, Hyo Young Choi, David Neil Hayes

University of Tennessee Health Science Center, Memphis, TN

High-quality RNA is crucial for obtaining reliable RNA sequencing (RNA-seq) data, with metrics like RNA Integrity Number (RIN). However, these metrics, while effective for evaluating RNA integrity, do not always correlate with RNA-seq data quality, especially at the transcript level. This gap is particularly evident in total RNA-seq, where existing measures such as the coefficient of variation (CV) for read coverage fail to fully capture data quality and are influenced by confounding factors like read coverage depth. To address this, we developed a novel method to assess RNA-seq data quality by quantifying nonuniformity in read coverage while minimizing the influence of read coverage depth. In this study we invented new matric called windowCV (wCV) and applied to a diverse range of RNA-seq datasets, including fresh frozen (FF) and FFPE total RNA-seq data, as well as poly(A)-enriched mRNA-seq data. In mRNA-seq, our method captured 3' read coverage bias, a hallmark of RNA degradation, particularly in longer transcripts. For total RNA-seq data, we identified noisy coverage patterns associated with poor data quality, even in samples with high RIN values. By fitting regression lines between wCV and mean coverage depth (MCD) and calculating the area under the curve (wCVAUC), we refined the assessment to account for RNA quality variability. Using the TCGA pilot study and our own datasets, we demonstrated that wCVAUC reliably identified low quality RNA-seq data and highlighted its impact on downstream analyses, including gene expression quantification and clustering. Importantly, we observed that low RIN values do not always predict poor RNA-seq data quality, as some samples with RIN values below 7 exhibited high-quality RNA-seq data based on wCVAUC. It means that our analyses showed that wCVAUC effectively distinguished high-quality from low-quality samples, including cases where traditional metrics like RIN and CV were insufficient. Additionally, our investigation into the relationships between nonuniformity of read coverage, exon GC content, and RNA localization revealed that the transcript-level RNA-seq data quality of lncRNA genes in FFPE samples is influenced by low exon GC content and nuclear localization. In conclusion, our method provides robust, transcript-level metrics for assessing RNA-seq data quality across platforms, enabling more accurate identification of low-quality data and minimizing biases in downstream analyses. This approach offers a new standard for integrating RNA-seq data quality with sample variability, particularly for challenging datasets such as FFPE and total RNA-seq.

#2490 Empirical Bayesian batch effect correction for single-cell RNA-seq data.

Maximilien Colange, Guillaume Appe, Lea Meunier, Solene Weill, Akpeli Nordor, Abdelkader Behdenna

Epigene Labs, Paris, France

We introduce pycombat_sc, a Bayesian method to assess and correct batch effects in single-cell RNA-Seq data (scRNA-Seq).scRNA-Seq data is similar to bulk RNA-Seq data, except for a higher dropout rate, due to the much lower quantity of genetic material, which may drop below the detection threshold. Bayesian approaches for RNA-Seq data analysis (ComBat-Seq, DESeq2, edgeR) model RNA-Seq expression data with a negative binomial distribution. As suggested earlier (ZINB-WaVe) we propose to model scRNA-Seq data with a zero-inflated negative binomial distribution, where the inflation of zeroes accounts for the higher dropout rate.
Our approach is the continuation of ComBat and ComBat-Seq, which model microarray and RNA-Seq data with normal and negative binomial distributions, respectively. Our approach extends the ComBat-Seq model with a zero-inflation parameter. We propose two variants of the algorithm:• one where the zero-inflation component is batch-dependent - i.e. the drop-out rate differs from one batch to another;• one where the zero-inflation component is batch-independent - i.e. the drop-out rate is the same for all batches.
Parametric Bayesian approaches, such as ComBat or ComBat-Seq, are recognized for their versatility (through their ability to account for user-provided covariates in the model) and for their robustness to small-size cohorts. pycombat_sc retains the versatility of its predecessors with the ability to account for arbitrary covariates. This is in contrast to recent approaches based on deep learning, which typically require training data which incurs a dual limitations:• these methods are sensitive to any bias present in the training data. Combined with the lack of interpretability of the deep-learning models used, this shortcoming requires a thorough validation, to properly detect any bias in the training data.• training of deep-learning typically requires large amounts of data. While scRNA-Seq cohorts typically feature hundreds or thousands of cells, the stake here is to properly capture inter-cohort variability, or risk over-fitting to intra-cohort variability.
To properly assess the potential of our approach, we compare it to state-of-the-art tools, either based on clustering (Seurat, harmony) or on deep-learning models (scvi, scanvi, scanorama). For the sake of completeness, we also include ComBat and ComBat-Seq in our benchmark. To our knowledge, our study is the first to evaluate ComBat-Seq for batch effect correction in scRNA-Seq data, although its model is a priori more adapted to scRNA-Seq than the one of ComBat.

#2491 Conditional generation of mutational profiles in cancer.

Andy Wu, Hannan Wong, Aakansha Narain, Vedant Sandhu, Jason Pitt

Cancer Science Institute of Singapore, Singapore, Singapore

The accumulation of DNA damage on the cancer genome, often summarized as mutational profiles, is influenced by a complex interplay of environmental and cellular mutagenic conditions. Despite cancer genomics having some of the largest biological datasets in existence collected by pan-cancer consortiums, a significant portion of the diverse spectrum of cancer etiologies is still underrepresented and understudied. We leveraged the power of generative artificial intelligence (Gen AI) to develop MutaGen, a conditional variational autoencoder and generative adversarial network hybrid model (CVAE-GAN), to generate realistic mutational profiles conditioned on various clinical and genetic features. Trained on the PCAWG cohort, MutaGen can generate pan-cancer mutational profiles across four mutation types: single base substitutions (SBS96), small insertions and deletions (ID83), structural variations (SV32), and copy number alterations (CN48). MutaGen's cancer-conditioned generations show high similarities to the real PCAWG samples, with median cosine similarities of 0.98, 0.97, 0.92, and 0.96 for SBS96, ID83, SV32, and CN48 respectively. When conditioned on homologous recombination deficiency (HRD) status, MutaGen can generate zero-shot (i.e. cases not seen in training set) HRD lung cancers with high similarity to a real BRCA2-null lung cancer patient in both SBS96 and ID83 (0.93 and 0.78 cosine similarities respectively). The generation is significantly more similar than non-HRD lung cancer or non-lung HRD cancer in the PCAWG training set. Inspection of the generated profiles revealed the generations harbouring both lung cancer-associated and HRD-associated mutational patterns, showing that MutaGen can generate novel and biologically plausible profiles. By providing a versatile tool for generating synthetic mutational data, MutaGen can facilitate the exploration of understudied cancer subtypes and enable the training and benchmarking of predictive models.

#2492 Diagnostic algorithm for salivary gland oncocytic lesions.

Bacem Khalele, Alena Skalova

Charles University In Prague, Pilsen, Czech Republic

Aim: Diagnosing salivary gland lesions with oncocytic differentiation is challenging as it encompassing 21 reactive and neoplastic lesions. With the extensive differentiation, morphological overlapping is possible especially that some tumors can be purely oncocytic. We propose a diagnostic algorithm based on analyzing oncocytic detection in the salivary gland lesion in AS' registry (501 out of 6383 SGL) to propose a differential diagnosis based on morphology, immunohistochemistry, and molecular findings.
Methods: We developed a weighted probabilistic scoring algorithm incorporating dimensional reduction and hierarchical clustering analysis of morphological features, immunohistochemical profiles, and molecular labelling that represents the greatest weighted values. If molecular information is missing, the algorithm computes the values corresponding to morphology and immunohistochemical vectors respectively. The algorithm uses supervised machine learning with backpropagation for continuous refinement of classification boundaries.
Results: Salivary gland lesions with oncocytic differentiation comprise malignant neoplasms (e.g., acinic cell carcinoma, salivary duct carcinoma, myoepithelial carcinoma), benign neoplasms (e.g., pleomorphic adenoma, Warthin tumor, oncocytoma), cystic lesions and reactive process (e.g. multifocal oncocytic metaplasia), with different extent of oncocytic differentiation, either extensive, focal, or wall-lining, in which oncocytes are positive for MIA. Principal component analysis revealed three distinct oncocytic architectural patterns with eigenvalues >1.0: extensive (λ_1=2.84), focal (λ_2=1.92), and wall-lining (λ_3=1.37). Hierarchical clustering demonstrated discrete entity groupings with a cophenetic correlation coefficient of 0.89. The algorithm achieved a primary diagnostic accuracy of 92% (95% CI: 88.3-95.7%), while maintaining differential diagnostic sensitivity of 85% at a lower boundary condition of 0.3. Nevertheless, the algorithm is designed to propose a diagnosis for well-investigated cases only. Otherwise, the differentials are provided. A user-friendly interface will be made available at https://www.biopticka.cz/en/publications/tools/Oncocytic/
Conclusions: The algorithm demonstrates acceptable performance metrics in differential diagnosis generation. Although oncocytic differentiation can theoretically occur in any lesion, the inclusion of the salivary oncocytic lesions was confined to real data with adequate molecular investigation. Coverage could be expanded if reliable inputs show oncocytic differentiation, for example, in secretory carcinoma, polymorphous adenocarcinoma (and its cribriform subtype), microsecretory carcinoma, or palisading adenocarcinoma.

#2493 SCANs: SNP-based contamination assessment with nuanced signal in targeted methylation sequencing data.

Shaohua Ma[1], Chaoyang Liang[2], Shuliang Guo[3], Luyan Shen[1], XiaoSheng He[4], Zhihui Xu[5], Jing Zhao[5], Yonghui Li[5], Shiqing Chen[5], Baoliang Zhu[5], Xiaohui Wu[5]

[1]Peking University Cancer Hospital and Institute, Beijing, China,[2]China-Japan Friendship Hospital, Beijing, China,[3]The First Affiliated Hospital of Chongqing Medical University, Chongqing, China,[4]The Sixth Affiliated Hospital, Sun Yat-sen University, Guangzhou, China,[5]Shanghai Weihe Medical Laboratory Co., Ltd, Shanghai, China

Background: Multi-Cancer Early Detection (MCED) represents a promising approach with the potential for high sensitivity in detecting rare tumor-derived signals in the blood. However, contamination from adjacent samples or from environmental sources poses significant challenges that can compromise the accuracy of these results. Current contamination detection methods primarily designed for tissue samples, leaving a notable gap in methodologies for assessing low-level contamination in cfDNA targeted methylation sequencing data in blood. The challenges include the absent of matched control samples, low sequencing depth, and probe capture bias. To address these limitations, we have developed a machine learning-based method aimed at improving contamination detection accuracy in MCED datasets.

Methods: We first identified single nucleotide polymorphisms (SNPs) with high prevalence in the East-Asian population. We calculated the allele frequencies of SNPs using in-silico synthetic contaminated samples and fitted the distributions of allele frequencies at varying contamination levels to identify informative SNPs. To enhance signal-to-noise value, we constructed features based on reads containing alternative bases of these informative SNPs at each tier, which was categorized according to its coverage and assumed contamination levels. This comprehensive feature matrix was used to train an XGBoost algorithm on 185 normal samples and their corresponding in-silico synthetic contaminated samples. The performance of our approach was validated using two distinct datasets: the first set comprised in-silico simulated contamination samples with contamination levels ranging from 0.05% to 10%, while the second was built by titrating three healthy cfDNA samples into three other healthy cfDNA backgrounds, with titration levels ranging from 0.05% to 2%.

Results: This method effectively identifies contamination, particularly in scenarios where coverage at SNPs is relatively low, ranging from 50X to 300X. It demonstrated the ability to detect contamination levels as low as 0.2% in the titrated samples, with an in-silico limit of detection (LoD) established at 0.1%.

Conclusions: We have developed SCANs, a tool designed to address the challenges posed by low sequencing depth and capture bias-induced abnormal allele signals in methylation data. SCANs enables the detection of low-level contamination, thereby facilitating the precise analysis of cfDNA methylation data in MCED.

#2494 Elucidating developmental methylation programs and cell type compositions in glioblastoma using combinatorial optimization.

X. C. Li[1], A. Hari[1], Y. Liu[1], O. Singh[1], Z. Wu[1], S. M. Mount[2], K. D. Aldape[1], E. Ruppin[1], S. Sahinalp[1], A. A. Schaffer[1];
[1]National Cancer Institute, Bethesda, MD, [2]University of Maryland, College Park, College Park, MD

Glioblastoma (GBM) is one of the most aggressive forms of brain cancer. Previous research has shown that GBM tumors may comprise one or more developed brain cell types, including astrocytes, oligodendrocytes, oligodendrocyte precursor cells, and neural stem cells. However, it is not yet known whether GBM tumors originate from mature or progenitor states of these brain cell types. To explore the interplay between the developmental program of the brain cells and GBM tumor progression, we leverage methylation, an epigenetic marker that has been established as a reliable signal for clinical subtyping of brain cancers and understanding tumor evolution and brain development. While methylation profiles for developed brain cell types are available, little is known about the methylation profiles of their progenitors. To simultaneously determine cell type composition in bulk GBM samples and infer the methylation profiles of progenitor brain cells, we developed a novel computational framework, Qombucha (Quadratic prOgraMming Based tUmor deConvolution with cell HierArchy).

Qombucha uses a tree hierarchy depicting the developmental progression of mature brain cells, with leaves representing established mature brain cell types and internal nodes representing unobserved progenitors. Based on the premise that the value of a specific progenitor methylation feature equals those of its mature descendants, provided the descendants are concordant, Qombucha infers the values of the remaining unknown progenitor methylation features by using bulk methylation profiles of tumor samples from GBM patients. Qombucha implements a combinatorial optimization formulation that simultaneously infers the cell type composition of each tumor sample. The objective of Qombucha is to minimize the differences between the inferred and observed methylation profiles across the entire cohort of tumor samples. The inferred cell type compositions for tumors can then be used to investigate differences between GBM subtypes.

We applied Qombucha to real GBM patient data we compiled, representing three most common methylation-based subtypes: receptor tyrosine kinase (RTK) I, RTK II, and mesenchymal typical (MES TYP). Qombucha successfully recapitulated the known finding that GBM MES TYP patients exhibit higher immune infiltration, as indicated by the increased Qombucha-imputed microglia fractions. We also identified GBM subtype-specific brain development processes. Interestingly, the Qombucha-imputed cell compositions demonstrate comparable or better performance in clustering GBM subtypes than state-of-the-art brain tumor classifying methylation sites used for clinical decision-making. Overall, Qombucha provides a novel framework for understanding the crosstalk between brain development and tumor progression and offers valuable insights into the heterogeneity among GBM subtypes.

#2495 Does single-cell gene expression reflect RNA abundance?.

Christie Jay Lau[1], Liliane Cabral-Fernandes[2], Yadong Wang[3], Sumaiyah K. Rehman[3], Geoffrey Liu[3], Catherine A. O'Brien[3], Gregory W. Schwartz[3]

[1]Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada,[2]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada,[3]Princess Margaret Cancer Centre, Toronto, ON, Canada

Transcriptional dysregulation is a hallmark of cancer that is often studied using single-cell RNA sequencing (scRNA-seq) to capture the transcriptomic profile of individual cells. However, these measurements are not directly representative of gene expression levels due to biased transcript detection which can lead to dramatic misinterpretations of the underlying biology. To demonstrate this phenomenon, we profiled baseline and drug-tolerant persister cells from a patient-derived colorectal cancer xenograft model. Although persister cells are known to be globally hypotranscriptive and exhibit lower total RNA and housekeeping expression by cell number-normalized (CNN) quantitative PCR (qPCR), significantly more transcripts and genes were detected by single-nuclei RNA-seq as compared to baseline. Additionally, we identified this trend in two independent lung adenocarcinoma persister scRNA-seq experiments and noticed an association with higher sequencing read depth per transcript. In all three cases, transcript over-detection could not be ameliorated through filtering, read subsampling, batch correction, or normalizations specifically developed to address read depth bias. As such, we developed a novel computational approach to correct the effects of sequencing read depth bias on scRNA-seq data and recover the relative expression of cells across samples. To benchmark the validity of our approach, we identified five genes predicted to be significantly upregulated from raw and conventionally-normalized counts but downregulated from our depth-normalized counts. Using CNN qPCR, we found decreased abundance of all five targets in colorectal cancer persister cells, in agreement with the depth-normalized analysis approach and results. Beyond the context of persister models, we also examined single-cell ATAC+RNA-seq and spatial transcriptomic data from lymphoma and prostate cancer specimens and demonstrate how depth normalization can be helpful for addressing technical anomalies, such as transcript under-detection due to tissue edge effects. Altogether, sequencing read depth normalization allows us to move beyond the current approach of scaling all cells to the same expression level and achieve more biologically accurate inference from scRNA-seq data.

#2496 GOALS: a novel approach for gene ontology analysis using layered shell to enhance functional insight mining in cancer genomics.

Zongliang Yue[1], Christopher D. Willey[2], Robert S. Welner[3], Rajesh Amin[4], Jake Y. Chen[5]

[1]Department of Health Outcomes Research and Policy, Auburn University, Auburn, AL,[2]Department of Radiation Oncology, the University of Alabama at Birmingham, Birmingham, AL,[3]Department of Hematology and Oncology, the University of Alabama at Birmingham, Birmingham, AL,[4]Department of Drug Discovery and Development, Auburn University, Auburn, AL,[5]Department of Biomedical Informatics and Data Science, the University of Alabama at Birmingham, Birmingham, AL

Gene Ontologies (GOs) are standardized descriptions of gene functions in terms of biological processes, molecular functions, and cellular components, capturing their Parent-Child relationships in a structured framework and advancing cancer biological modeling to provide consistent and meaningful insights into functional genomics analysis. The conventional GO hierarchical structure is defined by human curation experts, with levels determined by the shortest path to the root term. However, grouping GOs poses challenges due to the uneven distribution of gene members within GO terms and inconsistencies in the level of detail across terms at the same GO level. In this work, we introduce Gene Ontology Analysis using Layered Shells (GOALS), a novel tool that discretizes GOAs into optimal layers. GOALS creates a scalable number of GO layers (typically within 10) while maintaining a balanced number of genes across GOs in each layer. Unlike existing tools, GOALS organizes GO terms using a bottom-up approach based on their co-membership network, discreting GOs to achieve an exponential fit with GO's gene member size. Meanwhile, GOALS reveals clusters or supersets reflecting biological relevance by unsupervised clustering of GO's latent projections. In the glioblastoma (GBM) and chronic myeloid leukemia (CML) case studies, GOALS identified distinct GO functional clusters with multi-GO layers to reveal multiple levels of detail—from specific to abstract contexts—to maximize signal discovery and uncover those signal's association with drug resistance. More importantly, GOALS outperformed existing enrichment analysis tools by effectively classifying functional differences with varying treatment outcomes. GOALS offers a robust and innovative framework for exploring disordered GO clusters, mining GO activities, and analyzing potential GO-GO interplays. By addressing critical challenges in functional genomics, GOALS provides a powerful tool for advancing our understanding of cancer heterogeneity and uncovering actionable insights for therapeutic development.

#2497 Modeling the hidden danger of invisible tumor growth in the clinic.

Bryan Hawickhorst[1], Amy H. Tang[2]

[1]Old Dominion University, Norfolk, VA,[2]Department of Biomedical and Translational Sciences, Macon & Joan Brock Virginia Health Sciences at Old Dominion University, Norfolk, VA

Tumor dormancy, relapse, and resistance are not fully understood. Residual and resistant tumor cells often persist and remain invisible for months or years, despite clean MRI/CT scans following neoadjuvant and/or adjuvant systemic therapies. In current literature reviews we see that residual tumor sizes and tumor detection thresholds can vary widely, further complicating patient prognosis and risk stratification. To address this unmet need, we developed an algorithm to model the timing, frequency, and imaging detection limits of tumor measurements to augment tumor relapse prediction with improved accuracy for clinical application, especially when tumors are below the current detection limits in the clinic. To visualize how long it takes an invisible residual tumor to recur, we generated multiple tumor growth curves to model the remission and relapse time course of 1, 10^1, 10^2, 10^3, and 10^4 tumor cells, and calculated the time for these therapy-refractory invisible tumors to develop into large $3cm^3$ MRI/CT-visible tumors. Firstly, we used simple exponential growth curves to model an unimpeded cancer cell doubling time ranging from 2 days to 30 days. Secondly, we used the Spratt model, a modified logistic model, to model the complex tumor growth curves *in vivo*. Tumor doubling times were varied from 5 to 30 days, indicative of tumor aggressiveness, dynamic tumor/tumor microenvironment interaction, and other tempo-spatial-context-dependency. Treatment frequency and duration were varied as biweekly, monthly, and 6-month office visits to simulate the time course of tumor resistance development following multiple rounds of therapeutic interventions. Additionally, tumor remission and relapse were modeled utilizing smaller residual tumors with longer remission periods. Using these parameters, we generated multiple growth curves to model how invisible and resistant residual tumor sizes, ranging from 10^{-2} mm^3 to 10^{-6} mm^3, will determine the time of diagnosis to time of relapse. Our dynamic tumor progression models demonstrate a rapid tumor relapse time course to reach the detection threshold, highlighting the need for invisible residual tumor modeling for diagnosis and relapse prediction in the clinic. This work highlights the importance of minimizing residual tumor size and reducing drug resistance. We will develop an invisible tumor model as a companion prognostic machine learning tool to identify and quantify therapeutic efficacy, risk stratify patients, and improve outcome and survival in the future.

#2498 PharmaRonNet: integrating multi-omics data to reveal key biological pathways.

Kaiqiang Hu, Wanyu Tao, Yunyang Zhang, Pengwei Pan, Fang He

IVB, Pharmaron Beijing Co., Ltd., Beijing, China

In this study, we present PharmaRonNet, a new bioinformatics tool based on the ResponseNet algorithm. PharmaRonNet constructs weighted networks from either single-omics or multi-omics data, revealing key pathways and mediators involved in specific biological or pathological processes. To validate PharmaRonNet's functionality, a case study was conducted using a public transcriptomics dataset of the liver cancer cell line HepG2 treated with insulin. PharmaRonNet successfully identified several insulin-response-related genes as key factors, outperforming standard methods such as differentially expressed genes (DEGs) analysis and other gene network-building tools. Subsequently, PharmaRonNet was used to investigate the mechanisms leading to resistance to the selective CDK4/6 inhibitor, Palbociclib, in hormone receptor (HR)-positive breast cancer. With WES, transcriptome, and proteome data from the Palbociclib-resistant MCF7 breast cancer cell line, PharmaRonNet revealed the involvement of tyrosine phosphorylation and cyclic nucleotide-mediated signaling along with cell growth regulation. Within these pathways, certain "core" genes were identified with the most linkages within the PharmaRonNet. Synergy effect tests were then performed to validate the roles of these key factors in mediating Palbociclib resistance. Overall, PharmaRonNet helps link different layers of omics data, enabling the discovery of hidden mechanisms often overlooked by canonical bioinformatics analysis, and enhancing our understanding of complex biological phenomena. These insights pave the way for developing novel strategies to combat drug resistance in cancers.

#2499 Precision oncology empowered: A web platform for population pharmacokinetic modeling.

Ahmed Fadiel

University of Chicago, Chicago, IL

Background: More than 85% of oncology clinical trial failures are due to problems in pharmacokinetics, pharmacodynamics, and inter-patient variability. In cancer research, therapeutic windows are narrow, and inability to predict drug responses across genetically and clinically diverse populations further complicates the problem. Population pharmacokinetics thus provides a solution by characterizing variability and optimizing dosing; however, it is not yet widely adopted in translational oncology because of technical complexity. Herein, we describe an advanced web-based platform that streamlines PPK model creation, validation, and simulation to optimize trial design and therapy selection in oncology.

Methods: Advanced computational approaches tailored for cancer drug development are integrated into the platform. Hierarchical parameterizations for fixed effects, random effects, and residual error structures enable precise modeling of drug behavior in oncology settings. Hybrid estimation techniques combine stochastic approximation and nonlinear mixed-effects optimization for robust parameter convergence in high-dimensional cancer datasets. Real-time diagnostic plots and multi-dimensional parameter exploration refine models iteratively. Virtual trial simulations incorporate demographic and genetic heterogeneity to predict drug responses and optimize dosing strategies.

Results: The platform validated, with high accuracy, a one-compartment PK model for an anti-cancer agent. Virtual trial simulations demonstrated variability in dose-response across subgroups of cancer patients and identified strategies to optimize therapeutic windows. The platform reduced computational complexity, improved precision, and enabled rapid iteration of complex models. Simulations of alternative trial scenarios identified opportunities to refine inclusion criteria and dosing strategies, offering potential to reduce oncology trial failure rates.

Conclusion: This platform now offers sophisticated tools to tackle the most relevant challenges in drug development against cancer, thus allowing more successful clinical trials and personalized treatments. It is a quantum leap in precision oncology through the in-silico simulation of drug responses and optimization of trial parameters. Currently available internally, the tool will be broadly distributed following further validation.

#2500 An automated and scalable pipeline for high-dimensional immune cell phenotyping in mass cytometry datasets.

B. Waked[1], G. Bellan[1], X. Durand[1], A. Maillard[1], F. Verdonk[2], B. Gaudilliere[3], H. McGuire[4], N. Smith[4], C. Loh[5], D. King[5], D. Blanchard[1], J. Hedou[1];

[1]SurgeCare, SAS, Paris, France, [2]Saint-Antoine and Tenon Hospitals, Assistance Publique-Hopitaux de Paris, Paris, France, [3]School of Medicine, Stanford University, Stanford, CA, [4]University of Sydney, Sydney, Australia, [5]Standard BioTools Canada Inc, Markham, ON, Canada

Context: With the increasing use of spectral flow and mass cytometry technologies, efficient single-cell phenotyping has become essential for the identification of complex cell populations. Traditionally, these populations are identified through manual gating, a time-consuming process that is also subject to variability and a lack of reproducibility. Here, we adapted a deep learning model [1] to devise an automated gating pipeline with the goal of enhancing the accuracy, speed, and reproducibility of immune cell gating.
Methods: Our pipeline was evaluated on two mass-cytometry datasets [1], [2] (>5M cells, 35 markers), that had been previously gated by experts for comparison purposes. Phenotypic markers were used for identification, while the median expression of intracellular markers was used to determine the functional properties of populations. Accuracy and F1-score were used to compare classified populations against manually gated populations. A third dataset (15M events from 57 patients diagnosed with lung cancer) was used to compare the prediction of lung cancer progression after immunotherapy using properties based on manually vs. automatically gated populations.
Results: The automated gating pipeline achieved high overall accuracy comparable to expert manual gating (n=30 cell populations) (0.917 and 0.921 for dataset 1 and 2, respectively). Processing time was significantly reduced: <12min for both datasets (8CPUs, 8GB of RAM). High-level populations (n=10) were identified with excellent accuracy (e.g. Tcells, Bcells with f1-score of 0.993, 0.937 on the first dataset and 0.996, 0.934 on the second dataset). However, rare populations (n=20) showed higher discrepancies (e.g. intermediate monocytes, DC with f1-scores of 0.864, 0.816 and 0.678, 0.552 on dataset 1 and 2, respectively). Differences in identification scores had low effect on the functional properties of populations , as 91.4% of the functional properties defined from our pipeline were highly correlated (r>0.75) with those derived from manual gating. Further, the prediction of lung cancer progression after immunotherapy showed similar or improved results using functional properties based on automated identified populations (AUC=0.82) compared to manually gated populations (AUC=0.70).
Conclusion: This automated approach eliminates operator biases and handles multiple markers simultaneously, offering reliable and efficient analyses for research and clinical applications. To explore discrepancies, future work will incorporate datasets annotated by multiple experts to assess inter-expert variability.

1. Blampey, Q. et al, *A biology-driven deep generative model for cell-type annotation in cytometry*. Brief Bioinform. 2023 Sep 20;. doi: 10.1093/bib/bbad260.
2. https://clinicaltrials.gov/study/NCT05523713
3. Ina A. Stelzer et al, Sci.Transl.Med.13, (2021). DOI:10.1126/scitranslmed.abd9898

#2501 pyVIPER: Enabling scalable and rapid insights from single-cell RNASeq data in cancer research.

Alexander L. E. Wang[1], Zizhao Lin[1], Luca Zanella[1], Lukas Vlahos[1], Miquel Anglada Girotto[2], Aziz Ur Rehman Zafar[1], Heeju Noh[1], Andrea Califano[1], **Alessandro Vasciaveo**[3]

[1]Department of Systems Biology, Columbia University, New York City, NY,[2]Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain,[3]NCI-Designated Cancer Center, Sanford Burnham Prebys Med. Discovery Inst., San Diego, CA

Single-cell sequencing has transformed cancer research by enabling the study of tumor heterogeneity and the complex interactions within the tumor microenvironment at an unprecedented resolution. However, the low signal-to-noise ratio in single-cell RNA sequencing (scRNASeq) poses challenges for quantitatively deciphering key cancer processes. We have demonstrated that gene regulatory network (GRN) analysis can address this by identifying Master Regulator (MR) proteins, using the VIPER algorithm, to pinpoint critical drivers of cancer and its microenvironment. As scRNASeq datasets from patient-derived tumors grow in size and complexity, there is a pressing need for scalable tools capable of analyzing datasets with hundreds of thousands of cells. To meet this need, we introduce pyVIPER, a highly efficient and scalable Python toolkit designed to assess protein activity in large-scale scRNASeq datasets. pyVIPER facilitates tumor and microenvironment analysis by supporting multiple enrichment algorithms, advanced data transformations, and a novel data structure for GRN manipulation. With seamless integration into publicly available state-of-art scRNASeq tools, as well as machine learning libraries, pyVIPER provides the ideal platform for analyzing tumor-derived samples. Benchmarking shows that pyVIPER reduces runtime from hours to minutes for large datasets, compared to previous tools, enabling a fast and comprehensive analysis of cancer and tumor microenvironment dynamics at single-cell resolution. This advancement makes VIPER-based analysis accessible for virtually any large-scale single-cell dataset from patient tumors, offering new insights into cancer biology and therapeutic targets.

#2502 Biologically informed cell typing in the tumor microenvironment with lightweight LLMs.

Yooeun Kim, Jiwon Kim, Hongyoon Choi

Portrai, Inc., Seoul, Korea, Republic of

Background: The tumor microenvironment (TME) is a complex ecosystem of diverse cell types that interact dynamically, influencing tumor development and therapy response. Accurate cell type annotation in the TME is challenging due to partial overlap among marker genes, scarcity of representative reference datasets, and emergence of novel cell phenotypes. Our approach uses open-source Large Language Models (LLMs) including Llama 3.2 to provide a reference-free, efficient, and controllable method for cell type classification based on marker genes. Unlike tools that depend on curated reference datasets, this method demonstrates consistent performance across a wide range of cell types. By generating rationale for predicted cell types, our method offers a practical and interpretable framework for annotating diverse cellular populations while offering adaptability to the complexities of the TME.
Methods: LangChain is a “Swiss-knife” platform for LLM applications. We developed a framework identifying cell types in the TME leveraging marker gene contexts. This approach combines structured outputs with predefined constraints to ensure biological plausibility and marker-based reasoning for interpretable cell type identification. Prompt engineering and schema-enforced validation ensure robust, biologically informed outputs, offering flexibility in ambiguous contexts while minimizing hallucinations.
Results: Our method successfully identifies cell types in several cancer datasets, demonstrating high interpretability and reduced misclassification compared to competing methods, such as ScType and GPTCelltype. By employing a de novo cell typing approach, the model effectively resolves ambiguities in overlapping marker genes and adapts well to complex cell populations. The method also achieves superior performance in terms of agreement scores, TTFT (time-to-first-token), and TPOT (time-per-output-token), highlighting its efficiency in time-critical tasks. Explanations provided by the LLM support biological relevance and enhance user confidence in cell typing tasks.
Conclusion: This study highlights the potential of light-weighted LLM for heterogeneous cell typing in complex environments like the TME. By circumventing the limitations of other cell typing methods, including those of reference-dependent and independent, this approach offers a flexible, robust, and interpretable solution for identifying diverse cell types in challenging biological contexts.

#2503 Analytic platform for optimal target pair selection in bispecific antibody-drug conjugates using scRNA-seq and spatial transcriptomics data.

Minki Choi, Sungwoo Bae, Dongjoo Lee, Hongyoon Choi

Portrai, Inc., Seoul, Korea, Republic of

Background: Bispecific antibody-drug conjugates (BiADCs) are innovative anticancer therapeutics that simultaneously bind to two targets. The limitations of single-target antibody-drug conjugates(ADCs) have become evident due to tumor heterogeneity. BiADCs are expected to overcome the limitations of single-target ADCs by broadening the targetable regions within the tumor. Here, we have developed a platform based on single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics (ST) data to identify the optimal pair of targets that maximizes the therapeutic efficacy and minimizes the systemic toxicity of BiADCs.
Methods: We created an integrated cell atlas by combining the human single-cell transcriptomics atlas from 'Tabula Sapiens' and the cancer scRNA-seq datasets from the 'Curated Cancer Cell Atlas'. to calculate the 'Organ-Level Specificity' (expression fold-change between cancer and matched normal tissue) and the 'Critical Organ Toxicity' (average target expression in critical organs). And the ST data are used to calculate the 'Intratumoral target expression', 'Microenvironment-Level Target Specificity', defined by the expression of the target in malignant cells relative to normal cells. We utilized Visium data from 96 breast cancer based on the tissue microarray. These checkpoints were calculated for BiADCs by simultaneously analyzing two selected targets and computed for all possible target pairs. We focused on 13 targets that are actively being investigated in clinical trials for ADCs in solid tumors.
Results: After calculating the checkpoints for each target pair, we identified target pairs that are expected to perform better than single targets based on the following criteria. First, we selected pairs that showed higher checkpoint values compared to their corresponding single targets. Then, from these selected pairs, we chose those that outperformed their corresponding single targets in at least three checkpoints. Applying the criteria, we identified target pairs for BiADCs that are likely to achieve superior therapeutic outcomes compared to single targets. The pairs involving CEACAM5 or VTCN1 generally achieve a high therapeutic index. And the target pairs with VTCN1 exhibit lower critical organ toxicity compared to other targets for breast cancer.
Conclusion: The platform presented in this study utilizes scRNAseq and ST data to systematize the complex target selection process for BiADCs. We developed checkpoints to evaluate target efficacy at the systemic level using scRNAseq and at the tumor microenvironment level using ST data. Using breast cancer as an example case and the list of 13 ADC targets, we identified CEACAM5 or VTCN1-based target pairs as optimal partners for BiADCs. Consequently, our pipeline demonstrated its ability to identify target pairs that are expected to perform better than specific single-target ADCs.

#2504 SNAP: Streamlined Nextflow Analysis Pipeline for profiling circulating histone modifications identifies tumor epigenomic signatures in cancer plasma.

Ze Zhang, Paulo Cordeiro, Brad Fortunato, Karl Semaan, Razane El Hajj Chehade, Gunsagar Gulati, Garyoung Lee, Shahabeddin Sotudian, Ji-Heui Seo, Matthew Freedman, Sylvan Baca

DFCI/Harvard Medical School, Boston, MA

Background: Cell-free chromatin immunoprecipitation sequencing (cfChIP-seq) and methylated DNA immunoprecipitation sequencing (cfMeDIP-seq) are increasingly being used to profile cancer epigenomes from blood. The data these assays generate require specialized analytical approaches that are not met by conventional pipelines for ChIP-seq. ChIP-seq analysis focuses on calling “peaks” to identify where a protein of interest binds DNA, while cfChIP-seq often requires quantification of low-abundance signals from cancer chromatin. Additionally, input cell-free chromatin amounts are generally small and variable, and conventional indicators of data quality like peak number are not optimized for cfChIP-seq and cfMeDIP-seq. To address these challenges, we developed SNAP—a streamlined, containerized, Nextflow pipeline optimized for cell-free histone modification and DNA methylation profiling.
Methods: We implemented key steps in cfChIP-seq and cfMeDIP-seq data processing using Nextflow and Docker containerization to create a reproducible and portable workflow. Through reanalysis of > 800 datasets spanning H3K4me3, H3K27ac, and DNA methylation, we established quality metrics that identify datasets with acceptable quality for downstream analyses. Optimum thresholds were established for the total number of DNA fragments and enrichment of fragments mapping to “on-target” versus “off-target” regions. We tested strategies for signal normalization to optimize comparability between samples. SNAP performs quality control assessment and normalization, as well as genotype fingerprinting to rule out sample swaps and confirm that epigenomic profiles derived from the same individual where expected.
Results: Fragment count cut-offs were set at 2,100,000 for H3K4me3 and H3K27ac ChIP-seq, and 7,700,000 for MeDIP-seq. Enrichment score thresholds were established at 10.9, 10.3, and 3.2 for H3K4me3, H3K27ac, and MeDIP-seq, respectively. Filtering our cohort based on these thresholds maximized the ability to distinguish cancer vs. healthy plasma based on the signal at tumor-type-specific regulatory elements. Sample clustering based on SNP fingerprints reliably grouped samples derived from the same individual. SNAP has been successfully deployed on HPC clusters and is compatible with cloud computing platforms, ensuring accessibility for diverse research environments.
Conclusion: We developed SNAP, a Nextflow-based pipeline specifically optimized for plasma cell-free chromatin epigenomic profiling, including H3K4me3 ChIP-seq, H3K27ac ChIP-seq, and MeDIP-seq. Customized quality control metrics enhanced the detection of tumor-associated epigenomic signatures in cancer plasma samples compared to healthy controls. SNAP provides a robust, reproducible, and portable solution for high-sensitivity plasma epigenomic profiling.

#2505 Semi-automated image registration and cell typing integrates multiplexed imaging data to investigate the tumor microenvironment in clinical biopsies.

Cadence Chang[1], Egmidio Medina[1], Sarah Samordnitsky[2], Alan Zelin[1], Qin Zhou[3], Siwen Hu-Lieskovan[3], Michael C. Wu[2], Antoni Ribas[1], Katie M. Campbell[1]

[1]UCLA - University of California Los Angeles, Los Angeles, CA,[2]Fred Hutchinson Cancer Center, Seattle, WA,[3]University of Utah, Salt Lake City, UT

Introduction: Spatial profiling technologies are individually limited by the number of proteins evaluated, making it beneficial to integrate imaging analysis across multiple slides from a single biopsy. Here, we describe a semi-automated workflow for the scaled image registration and downstream analysis of clinical melanoma biopsies treated with immunotherapy and evaluated across multiplexed protein platforms.

Methods: Clinical biopsies (n=119) from patients with melanoma (N=39) were assayed on the Vectra Polaris platform. Two sequential slides from each biopsy were individually processed under pre-specified custom named panels, being: PD1 panel (DAPI, CD8, PDL1, Ki67, PD1, SOX10, and CD68) or the L panel (DAPI, CTLA4, CD8, FOXP3, CD4, CD20, and PD1). The VALIS program was used to perform semi-automated image registration on these sequential slides. A bootstrapping approach determined cell marker positivity to identify cell types including melanoma cells (SOX10), CD8 T cells (CD8), CD4 T cells (CD4, FOXP3-), regulatory T cells (Tregs; CD4, FOXP3), B cells (CD20), and macrophages (CD68). Following image registration, cell type relationships were evaluated for regional annotation of tumor content, T-cell infiltration, and T-cell activation.

Results: There were 9,376,260 cell segments detected across biopsies (average n=42,235 segments per biopsy, range 1,611-214,050). Melanoma cells were detected based upon nuclear SOX10 staining. Biopsies had varied levels of tumor cellularity (average 8.9%, range 2.1-24.0%). Image registration was performed for samples that had corresponding PD1 and L panels (n=112). Automated registration was achieved for 86 of 112 paired samples (76.8%); 8 of the 86 (9.3%) required manual changes in parameters for accurate registration, and 18 samples were unable to be registered with VALIS due to imaging noise or poor-quality biopsies. This successful registration in the majority of cases led to the automated annotation across images, transferring of the PD1-panel image on to the registered L-panel image.

Conclusions: Semi-automated image registration can help to overcome protein expression measurement limitations in spatial profiling technologies, enabling the expanded knowledge transfer across these data modalities. This workflow allows the effective integration of data measurements from multiple stainings for more comprehensive analysis using these technologies to study the tumor microenvironment.

#2506 Microhunt: an integrated pipeline for precise microbial quantification in low-biomass clinical samples.

Gengwen Deng[1], Lingxuan Zhu[1], Weiming Mou[1], Liling Xu[1], Shujia Lin[1], Keren Wu[1], Hao Li[1], Zhixing Deng[1], Hongsen Huang[1], Zeji Guo[1], Qiheng Liang[1], Aimin Jiang[2], TIng Wei[1], Jiang Zhang[1], Quan Cheng[3], **Anqi Lin**[4], Peng Luo[1]

[1]Department of Oncology, Zhujiang Hospital, Southern Medical University, Guangzhou, China,[2]Department of Urology, Changhai Hospital, Naval Medical University (Second Military Medical University), Shanghai, China,[3]Department of Neurosurgery, Xiangya Hospital, Central South University, Changsha, China,[4]University of Macau, Guangzhou, China

Accurately quantifying microbial abundance from high-throughput sequencing data is challenging, especially in low-biomass samples where host DNA/RNA dominates microbial content. Host or environmental contaminants may obscure true microbial signals, leading to false positives. Here, we introduce MicroHunt, an advanced pipeline integrating quality control, host sequence depletion, microbial classification, and contaminant exclusion.MicroHunt incorporates comprehensive human reference genomes (hg38, T2T-CHM13, Gencode) to efficiently remove host-derived reads and eliminates contaminant sequences source, such as vectors, phages, and plasmids. It supports multiple microbial classification tools and utilizes a curated reference database encompassing archaea, fungi, bacteria, viruses, and protozoa. By incorporating host genome references into the classification process, MicroHunt clearly distinguishes host from microbial sequences, significantly improving classification accuracy and reducing false positives. MicroHunt also provides integrated contaminant control strategies effective even without negative controls, enabling reliable microbial quantification from low-biomass samples. Using ribosomal RNA-depleted whole-transcriptome sequencing data from 807 colorectal cancer (CRC) tumor samples in the AVANT Phase III trial, MicroHunt effectively characterized the dominant microbial phyla present within the tumor microbiome of CRC patients, with Pseudomonadota and Bacillota being the most prevalent. Further analysis revealed that CRC patients with high abundance of Lactobacillus iners (L. iners) in tumor tissues were significantly associated with poor prognosis following chemotherapy combined with bevacizumab treatment (Hazard Ratio = 2.21, 95% CI = 1.14-4.32, p = 0.017). Sequence alignment analysis confirmed that the reads identified as L. iners were not derived from potential contamination sources. Moreover, integrating microbiome data generated by MicroHunt with patient transcriptomic data uncovered complex interactions between the gut microbiome and host gene expression in CRC patients. Pathway enrichment analysis revealed a significant association between L. iners and the fatty acid oxidation pathway (Spearman's correlation coefficient r = 0.094, p = 0.0079), suggesting that microbial metabolism may influence patient prognosis in CRC. Finally, we validated the utility of MicroHunt using sequencing data from other tumor types, including non-small cell lung cancer and bladder cancer, successfully identifying intratumoral microbes and elucidating their potential biological significance.

#2507 Comparative assessment of data analysis methods to enable robust 10-second diagnosis of tissue pathologies with picosecond infrared laser mass spectrometry.

Darah Vlaminck[1], Alexa Fiorante[1], Lan Anna Ye[2], David Munoz[3], Sunit Das[3], Gelareh Zadeh[2], Howard Ginsberg[3], Arash Zarrine-Afsar[2]

[1]Medical Biophysics, University of Toronto, Toronto, ON, Canada,[2]University Health Network, Toronto, ON, Canada,[3]Unity Health Toronto, Toronto, ON, Canada

A survey of clinical outcome data suggests surgical approaches of the central nervous system cancers must vary based on type of cancer, its aggressiveness, chance of recurrence and associated risks. This is usually informed by feedback from a neuropathologist on call wherein the accuracy of diagnosis depends highly on the experience and training level of the pathologist. As such, to deliver robust personalized resections, the subjectivity of intraoperative diagnosis must be reduced.Picosecond infrared laser mass spectrometry (PIRL-MS) generates a fingerprint of cancer-specific tissue lipids that can form the basis for non-subjective diagnosis in only 10 seconds. Here, a differential diagnosis model must be incorporated with finer data analytics methods to help tease out cancer-specific spectral features in complex datasets.A hierarchical differential diagnosis (decision tree) model optimizing evidence-based adjustment of the extent of resection for n=62 lymphoma (no resection), n=96 metastatic carcinomas (aggressive resection) and n=216 gliomas (maximum safe margin resection), the latter comprised of n=93 isocitrate dehydrogenase-1 (IDH1) positive and n=132 negative mutational status was created. This tree flows in a logical order based on what a neuropathologist would use for diagnosis, albeit utilized for automated non-subjective diagnosis based on 10-second PIRL-MS lipid analysis. The goal was to optimize the best machine learning method at each discrimination point from a list of principal component analysis-linear discriminant analysis (PCA-LDA), partial-least squares discriminant analysis, lasso regression, random forest, XGBoost, and support vector machine.The hierarchical approach enabled simplification of class comparisons to only those in differential diagnosis, as such, eliminating the misclassification between classes at finer branches of tree. This allowed performance metrics to be generated at each discrimination point not distinguished in simultaneous prediction of all classes using a single multiclass model.Within all nodes, PCA-LDA (a linear method) showed best performance, especially compared to ensemble approaches. Using this approach, the hierarchical method delivered diagnosis of lymphoma, glioma and metastatic tumours in node 1 with sensitivity and specificity of 87% and 93%, respectively and was able to differentiate between IDH1 mutant and WT gliomas with the sensitivity and specificity of >93%, the latter not being possible in current intraoperative diagnosis workflows due to long analysis times.The utility of this optimized hierarchical approach with PIRL-MS data is manifested in the users' ability to adjust the diagnosis depth commensurate with desired performance criteria, enabling incorporation of the clinical 'cost' of total resection of one tumour class compared to another.

#2508 A multiscale framework combining mechanistic models and LSTM-GCN networks for predicting drug sensitivity.

Chenhui Ma, Evren Gurkan-Cavusoglu

Case Western Reserve University, Cleveland, OH

Understanding tumor evolution and treatment resistance requires an integrative approach that combines cellular dynamics with molecular interactions. While mechanistic models excel in capturing physiological processes and predicting behaviors beyond the original data, their specificity limits their generalizability. Conversely, deep learning enables universal predictions but often lacks interpretability, posing challenges for application in cancer research. To address these limitations, we propose a novel computational framework that integrates mechanistic simulations, graph-based modeling, Long Short-Term Memory (LSTM) networks, and Bayesian inference to predict the sensitivity of six cancer types to targeted therapies, chemotherapy agents, immunotherapies, and their combinations. In this framework, mechanistic models provide an individualized baseline simulation for different cancer types, while deep learning integrates these outputs with drug-related information to generate robust and interpretable predictions tailored to specific types. Our hybrid framework leverages a mechanistic pan-cancer model that simulates molecular signaling pathways, cell cycle dynamics, and apoptosis across four biological compartments. The model is parameterized using published gene expression profiles and mutation data from xenograft models, including key mutations such as *KRAS*, *EGFR*, and *TP53*. Simulations are performed for six cancer types, including breast carcinoma, colorectal cancer, and pancreatic carcinoma, to approximate patient-specific tumor biology. Tumor cell populations generated from these simulations are represented as graphs, with each node corresponding to an individual cell characterized by proliferation status and dynamic molecular states (e.g., protein concentrations and phosphorylation levels). Edges are weighted and defined based on molecular similarity between cells. The graph is processed using a Graph Convolutional Network (GCN), which generates embeddings that capture tumor population heterogeneity. These embeddings are then fed into an LSTM to extract the temporal dynamics of the tumor population. The final hidden states computed by the LSTM are further combined with embeddings of drug chemical structure and drug-target interactions to make drug sensitivity predictions. To enhance the robustness of predictions, Bayesian inference is incorporated to quantify uncertainty in estimated drug responses. This framework bridges the gap between mechanistic modeling and machine learning, offering a scalable and interpretable approach to studying tumor evolution under the selective power of drug treatment. By integrating the physiological insights of mechanistic models with the predictive power of deep learning, it enables the identification of molecular drivers of resistance and informs the development of personalized therapies.

REGULATORY SCIENCE AND POLICY: Regulatory Science and Policy
Poster Session

#2512 History and summary of current practices for assessment of drug-induced QT-prolongation in oncology drug development.

Louis-Guillaume Marquier[1], Frederique Lemaux-Duvillier[1], Stephanie Le Goaller[2], Philippe L'Hostis[3], Eric Delpy[4], Mathieu Grace[1], Jean-Louis Pinquier[1]

[1]Biotrial, Rennes, France,[2]Biometrics, Biotrial, Rennes, France,[3]Neuroscience, Biotrial, Rennes, France,[4]Pharmacology, Biotrial, Rennes, France

The ICH E14 guideline issued in 2005 made the assessment of drug-induced QT-prolongation a mandatory step in the development of all new therapeutics. The guidance provided a clear requirement, however the means to achieve this requirement within the specific space of oncology clinical development remained challenging. Several updates to the guidelines, mainly in the form of Q&As published in 2012, 2015 and 2022 helped to clarify regulator expectations and thus determine the strategies available in a field where a straightforward investigation performed in Healthy Subjects (i.e. the dedicated TQT trial) is not feasible for reasons of drug tolerability ; a dedicated trial in the patient population also poses significant ethical questions in addition to logistical hurdles. Today, following the different updates to ICH E14 we feel that a clear strategy may be implemented by sponsors developing new treatments in the field of oncology. These strategies, which include in vitro and in vivo testing and a specific approach for ECG recordings in phase I, are followed by several companies, but are not yet universally included in development plans from their start. We thus believe that a simplified roadmap should hold value for professionals looking to develop novel oncology therapeutics. The focused and precise assessment of drug-induced QT prolongation also meets the needs of clinicians looking to assess this risk in complex clinical setting with multiple risk factors[1,2].
The purpose of our abstract will be to provide a clear history of the evolution of the ICH E14 guideline and illustrate what could be the current practice implemented for new therapeutics in the field of oncology. Our main interest will be for NCEs who cannot be safely administered to Healthy Participants however approaches for NBEs will also be described, with illustrations of results. This will include a description of the in vivo preclinical and clinical strategies which can be followed.

#2513 Factors influencing participant attrition in Phase 0 hybrid clinical trials for patients with high-grade glioma.

Jocelyn L. Harmon[1], Amy Hong[1], Artak Tovmasyan[1], An-Chi Tien[1], Kristin Hendrickson[1], Jennifer Eschbacher[1], Wonsuk Yoo[1], Igor Barani[2], William Kennedy[2], Zaman Mirzadeh[2], Yoshie Umemura[1], Shwetal Mehta[1], Nader Sanai[1]

[1]Ivy Brain Tumor Center at Barrow Neurological Institute, Phoenix, AZ,[2]Ivy Brain Tumor Center, Barrow Neurological Institute, Phoenix, AZ

Background: Phase 0 hybrid trial designs administer a subtherapeutic experimental agent pre-surgically and then use predetermined pharmacokinetic (PK) and/or pharmacodynamic (PD) thresholds to qualify patients for follow-on therapeutic dosing. While such protocols are rare, they are increasingly prevalent within neuro-oncology, necessitating a better understanding of factors influencing participation. Here, we investigate participant enrollment in Phase 0 hybrid trials for high-grade glioma patients at a single center.
Methods: This retrospective study examined characteristics of high-grade glioma patients enrolled in Phase 0 hybrid clinical trials at the Ivy Brain Tumor Center. Clinical, pathological and demographic data, as well as PK/PD profiles, were analyzed and related to participant enrollment and attrition.
Results: A total of 284 newly-diagnosed and recurrent high-grade glioma patients were analyzed and 276 (97.2%) participants completed the Phase 0 component of their study. Of these, 176 (63.8%) Phase 0 participant tumors did not meet criteria for therapeutic dosing, including 63 (35.8%) due to histological diagnosis, 62 (35.2%) due to PK/PD results, 39 (22.2%) due to clinical status decline, 7 (4.0%) due to patients' decision to not proceed, and 5 (2.8%) for unknown reasons. Six (2.1%) participants did not complete Phase 0 and 2 (0.7%) participants were screen failures.
Conclusions: In the upfront and recurrent settings, tumor histopathology and drug PK/PD performance are the principal determinants of participant suitability for the therapeutic dosing component of a Phase 0 hybrid study. In this single-center analysis, the graduation rate from Phase 0 to therapeutic dosing was 36.2%. This retrospective study, the largest to date in high-grade glioma patients, highlights participant- and protocol design-related features that will help refine future Phase 0 hybrid protocols in neuro-oncology.

#2514 Refining oncology dose selection: A framework for tailored dose optimization in the era of targeted and immunotherapies.

N. Dharia[1], J. Zhu[1], A. Schroeder[1], S. Frank[2], C. Boetsch[2], C. Jamois[2], N. Kassir[1], **K. Korfi**[2], E. Punnoose[1], A. Vaze[1], P. Trask[1], P. Gosai[3], J. Fridlyand[1], C. Li[1];

[1]Genentech, Inc., South San Francisco, CA, [2]F. Hoffmann - La Roche Ltd, Basel, Switzerland, [3]Hoffmann-La Roche Limited, Mississauga, ON, Canada

Historically, oncology dose selection was driven by the identification of the maximum tolerated dose, often resulting in significant toxicity. The advent of molecular targeted therapies and immunotherapies has created opportunities to achieve similar efficacy at a lower, more tolerable dose. Recent FDA draft guidance highlights the importance of dose optimization in drug development. Building on this, we present a novel framework categorizing drugs into four molecular classes based on their mechanisms of action: (1) small molecule targeted therapies and antibody-drug conjugates, (2) large molecule antagonists, (3) cancer immunotherapy agonists, and (4) molecules with limited or no single-agent activity.

Unique considerations and strategies for dose optimization are detailed for each class, supported by case examples. We propose integrating proof of activity as a gating criterion for dose expansion to inform robust dose decisions and maximize the use of patient resources. This perspective underscores the integration of preclinical and clinical data—including pharmacokinetics, pharmacodynamics, and patient-reported outcomes—combined with quantitative pharmacology and statistical modeling, to optimize doses effectively while balancing efficacy and safety.

While no one-size-fits-all approach exists, this tailored guidance for specific mechanistic classes provides balanced approaches to dose optimization that ultimately enhance the probability of success in drug development.

#2515 Enhancing oncology data collection and submission: The value of the national cancer institute (NCI) oncology codetable mapping file.

Karine Provost[1], **Stephanie Le Goaller**[1], Mathieu Grace[1], Pierre Montagne[2]

[1]Biotrial Biometrics, Rennes, France,[2]Biotrial Pharmacology, Rennes, France

Data collection for efficacy endpoints in oncology clinical trials present various challenges and thus require adapted strategies and tools. Use of the Guidances to follow for surrogate endpoint analysis and reporting, such as RECIST, Lugano, RANO or others are straightforward, as this depends on the target malignancies and the type of compound being studied. It is however challenging to ensure that, once the endpoints for a trial are selected, the method for their collection and identification be clearly defined. This paper will focus on the use of the tools developed by the NCI and CDISC communities to ensure that data be efficiently collected and organized, in line with regulatory requirements. We will be explaining the role of the following tools which are used throughout a trial, starting with the protocol design and all the way until the final reporting:- CDASH Implementation Guide (CDASHIG) : this refers to the guidances for data collection- SDTM Implementation Guide (SDTMIG) : this refers to the guidances for data submission- Therapeutic Area User Guides (TAUGs) : these include disease-specific metadata, examples and guidances on implementing CDISC standards for a variety of uses, including global regulatory submissions (ie : Breast Cancer, Prostate Cancer, Colorectal Cancer, Lung Cancer)
- RECIST 1.1 Disease Response Supplement to SDTMIG
- Controlled Terminology (i e: Tumor or Lesion Identification Test Code/Name Codelists Tumor or Lesion Properties Test Code/Name Codelists , Category of Oncology Response Assessment, Oncology Response Assessment Test Code/Name Codelists, Oncology Response Assessment Result)
- Newly Oncology Codetable Mapping File which shows relationships between terms (i.e : Modalities for Target/Non-Target Response for RECIST1.1, iRECIST or LUGANO)
It is of particular interest to evaluate the value added of the NCI Oncology Codetable Mapping File in oncology clinical trials, as demonstrated through its use in EDC and database design. We will describe the approach taken to integrate this new tool into EDC and Database design and this enables further alignment with guidelines (e.g. RECIST, Lugano), controlled terminologies and Regulator expectations. We will also investigate the value added for investigator sites, who can benefit from this further standardization of data collection practices across clinical trials, regardless of sponsor or CRO, to simplify their working practices.As a conclusion, we will summarize the value added by the NCI Oncology Codetable Mapping File to the existing landscape (CDISC tools, Surrogate Endpoint Guidances) of oncology clinical trials. We will emphasize its potential to simplify complex data requirements for topics specific to the field of oncology.

#2516 A qualitative research study on hematology and oncology consultants' experiences and opinions on using minimal residual disease (MRD) as a fast-track approval endpoint in early-phase clinical trials in multiple myeloma in Saudi Arabia.

Albandari A. Alnasser, Tariq H. M. Alqurayshah

Oncology and Hematology, Saudi Food and Drug Authority, Riyadh, Saudi Arabia

Introduction: The US FDA has recently considered minimal residual disease (MRD) as an acceptable accelerated approval endpoint in multiple myeloma (MM). Meta-analysis studies have shown the clinical benefit of MRD negativity in MM.
Objective: This qualitative research study aims to assess the opinions and experiences of hematology and oncology consultants in Saudi Arabia on MRD as an intermediate endpoint for fast-track drug approvals by the Saudi Food and Drug Authority in MM.
Study Design: Interviewing 30 registered physicians with more than one year of experience. The interviews will be conducted through a single focus group and several face-to-face interviews or performed online. The predetermined set of semi-structured questions will cover topics such as the definition of MRD, its clinical and research significance, MRD detection thresholds, duration of MRD negativity, and its correlation with other clinical endpoints, such as progression free survival (PFS) and overall survival (OS). The interviews will be conducted by scientific oncology and hematology evaluation experts at the Saudi Food and Drug Authority. Before starting any interview, participants will complete consent forms, including the study's goals and who will conduct the study.
Results: First focus group included seven consultants and highlighted MRD's value as a surrogate endpoint, emphasizing its strong correlation with PFS and OS. MRD negativity is associated with improved PFS and OS across all disease stages, including newly diagnosed and relapse/refractory multiple myeloma. It is particularly valuable in early phase trials for evaluating novel therapies and in maintenance therapy for monitoring sustained remission. However, the use of MRD as a surrogate endpoint for drug approval remains debated. While it is considered as a good endpoint for expedited approvals of MM new medicinal products and indications but not a perfect surrogate for overall survival. The focus group emphasized that while MRD is a valuable indicator, it does not replace the need for collecting and analyzing OS and PFS data to confirm long term benefit for patients. In Saudi Arabia, methods for detecting MRD include flow cytometry and next-generation sequencing (NGS). Flow cytometry is the most accessible and cost-effective method, widely available in many hospitals. The threshold for MRD negativity depends significantly on the detection method used, as each method offering varying level of sensitivity. Flow cytometry typically achieves a sensitive threshold 10-5 . Also, next-generation sequencing (NGS) can achieve a higher sensitivity of up 10-6.
Conclusion: MRD could be a promising surrogate endpoint in multiple myeloma due to its strong association with PFS and OS with the need for the long-term clinical data.

#2517 Meaningful change in the ability to perform physical activities for patients with cachexia and cancer.

Carrie Northcott[1], Abigail Newell[2], Soohyun Hwang[3], Josh Coulter[4], Lewis Kopenhafer[3], Mar Santamaria[5], Isik Karahanoglu[5], Martine C. Maculaitis[3], Joseph C. Cappelleri[1], Kathleen Beusterien[3], Maria Gonzalo[2], Erica Fortune[2], Claire Saxton[2], Elif Andac-Jones[6], Brett Hauber[4]

[1]Pfizer Inc, Groton, CT,[2]Cancer Support Community, Washington, DC,[3]Oracle Life Sciences, Austin, TX,[4]Pfizer Inc, New York, NY,[5]Pfizer Inc, Cambridge, MA,[6]Tigerlily Foundation, Stone Ridge, VA

Digital health technologies (DHTs) can quantify changes in physical activity in cachexia clinical studies. But what is a meaningful change? We estimated the meaningfulness to patients with cachexia and cancer of incremental changes in ability to perform physical activities of different intensity and time spent each day doing non-sedentary activity that can be measured with DHTs. An online survey was conducted with US adults with cachexia and cancer. Meaningfulness of 14 changes in ability to perform physical activities and 3 increases in daily time spent in non-sedentary activity were assessed on a 5-point rating scale (not at all meaningful to extremely meaningful). Subgroups (tumor type, cancer stage, ECOG performance status) were compared using chi-square (or Fisher's exact) tests. Overall, 181 patients participated. All changes in physical activity intensity, and both a 14-min and a 30-min increase in time spent in non-sedentary activity/day, were at least moderately meaningful to most patients, regardless of tumor type, cancer stage, or ECOG performance status (Table). Some meaningfulness ratings differed significantly by tumor type (both walking pace and distance were less meaningful in NSCLC and sitting to standing was less meaningful in pancreatic) and performance status (walking pace was less meaningful in patients with ECOG 3-4). Majorities of patients, except for those with colorectal, rated a 4-min increase/day in non-sedentary activity as at least moderately meaningful. Patients with cancer and cachexia perceived even small changes in physical activities and time spent in non-sedentary activity as being at least moderately meaningful, suggesting that DHTs that measure changes in movement duration can be used to capture important patient-centric endpoints in clinical studies.

Table

Change in Ability to do Physicial Activities	Proportion of Patients Rating Each Change as at Least Moderately Meaningful									
	All Patients	Tumor Type					Cancer Stage		Performance Status	
		NSCLC	Breast	Colorectal	Pancreatic	Other	Stage I-III	Stage IV	Higher	Lower
	N=181	N=20	N=69	N=30	N=15	N=47	N=123	N=52	N=147	N=34
Cannot go walking outside to Able to walk a short distance	93.9%	95.0%	94.2%	93.3%	93.3%	93.6%	92.7%	98.1%	93.9%	94.1%
Able to walk a short distance to Able to walk a long distance	90.6%	**65.0%***	**91.3%***	**96.7%***	**100.0%***	**93.6%***	91.9%	88.5%	91.8%	85.3%
Cannot walk, even at a slow pace to Able to walk at a slow pace	94.5%	100.0%	95.7%	93.3%	93.3%	91.5%	93.5%	98.1%	95.9%	88.2%
Able to walk at a slow pace to Able to walk at an average walking pace	90.6%	**70.0%***	**92.8%***	**93.3%***	**100.0%***	**91.5%***	89.4%	94.2%	**93.2%***	**79.4%***
Able to walk at an average walking pace to Able to walk at a fast pace	79.6%	70.0%	78.3%	93.3%	93.3%	72.3%	79.7%	78.8%	80.3%	76.5%
Cannot be up and about to Able to be up and about for <1 hour a day	93.9%	100.0%	92.8%	93.3%	100.0%	91.5%	94.3%	94.2%	94.6%	91.2%
Able to be up and about for <1 hour a day to Able to be up and about 3 hours a day	96.1%	100.0%	94.2%	90.0%	100.0%	100.0%	95.1%	100.0%	95.9%	97.1%
Able to be up and about 3 hours a day to Able to be up and about 6 hours a day	92.3%	80.0%	89.9%	93.3%	100.0%	97.9%	90.2%	98.1%	92.5%	91.2%
Cannot move from sitting to standing to Able to move from sitting to standing with effort	89.0%	**95.0%***	**91.3%***	**100.0%***	**60.0%***	**85.1%***	88.6%	92.3%	89.8%	85.3%
Able to move from sitting to standing with effort to Move from sitting to standing easily	95.0%	100.0%	94.2%	100.0%	100.0%	89.4%	95.1%	96.2%	95.9%	91.2%
Cannot do any activities, even while sitting to Able to do activities while sitting	95.0%	95.0%	92.8%	100.0%	100.0%	93.6%	94.3%	98.1%	95.2%	94.1%
Able to do activities while sitting to Able to do light activities	96.1%	100.0%	94.2%	93.3%	100.0%	97.9%	97.6%	94.2%	97.3%	91.2%
Able to do light activities to Able to do moderate activities	91.2%	95.0%	87.0%	93.3%	93.3%	93.6%	91.1%	92.3%	92.5%	85.3%

Able to do moderate activities to Able to do vigorous activities	75.7%	60.0%	75.4%	80.0%	86.7%	76.6%	78.9%	67.3%	78.2%	64.7%
Able to do non-sedentary activities for an additional 4 minutes per day	65.2%	**65.0%***	**75.0%***	**48.1%***	**86.7%***	**60.9%***	70.0%	62.7%	66.4%	69.7%
Able to do non-sedentary activities for an additional 14 minutes per day	76.2%	80.0%	77.9%	66.7%	93.3%	80.4%	79.2%	76.5%	79.0%	75.8%
Able to do non-sedentary activities for an additional 30 minutes per day	85.6%	90.0%	89.7%	85.2%	100.0%	82.6%	88.3%	88.2%	88.8%	84.8%
* indicates statistically significant differences (P<0.05) in proportion among subgroups										

#2518 Implementing an AI triage platform for identification and management of patients suspected of cancer risk to improve elective recovery post pandemic.

Miles Payling[1], Seema Dadhania[2], Michael Moss[1], Judith Gordon[1], Bea Bakshi[1]

[1]C the Signs Limited, London, United Kingdom,[2]Imperial College London, London, United Kingdom

Background: The COVID-19 pandemic caused a significant backlog in the UK National Health Service (NHS), with 7.6 million patients awaiting consultant appointments as of August 2024. A major consequence of this was a surge in urgent suspected cancer (USC) referrals, which rose by 27% compared to pre-pandemic levels without a proportional increase in cancer detection rates. General Practitioners (GPs) often default to USC referrals, bypassing initial investigations, which strains secondary care services.
C the Signs, an AI-led cancer prediction platform, supports GPs to improve cancer triage by identifying high-risk patients for appropriate pathways (such as direct access diagnostics or non-urgent referrals) without increasing referral volumes. This system optimizes USC referrals, reserving them for patients truly requiring intensive diagnostic resources, reducing pressure on secondary care.
Methods: From January 2020 to September 2024, data from 1,084 GP practices using C the Signs were analyzed, covering 235,662 risk assessments. The platform stratified patients into categories: urgent admission, USC referral, non-urgent referral, and direct access diagnostics (e.g. blood tests, FIT, X-ray). Patients not at risk were safely excluded from cancer pathways.
Results: C the Signs redirected 21.7% of patients away from the USC pathway, safely excluding 21,449 (9.1%), triaging 18,063 (7.7%) to tests and diagnostics, and 11,587 (4.9%) to non-urgent referrals—reducing USC pathway pressure by 61,099 patients. Meanwhile, 184,487 patients (78.3%) were appropriately referred for USC, ensuring focus on high-risk cases. Only 1.5% of assessments resulted in multiple USC referrals, and just 76 patients (0.0%) required urgent admission.
A total of 13,585 cancers were diagnosed, yielding a 7.3% conversion rate—20.9% higher than the NHS England average of 6.0%.
Conclusion: C the Signs effectively stratifies suspected cancer patients, excluding low-risk individuals from USC pathways and directing others to appropriate care. This enhances NHS resource efficiency and supports elective recovery while maintaining diagnostic safety. If implemented nationally, C the Signs could reduce USC referrals by 505,196 (17.5%) without compromising safety or outcomes. Longitudinal studies could further assess the platform's impact on healthcare outcomes and efficiency.

CLINICAL RESEARCH: Liquid Biopsy: Circulating Nucleic Acids 5 / Circulating Tumor Cells 2
Poster Session

#6357 A liquid biopsy-based targeted proteomic assay to identify druggable proteins from circulating tumor cells (CTCs).

Alec Horrmann[1], Ali T. Arafa[2], Kaylee Kamalanathan[1], Alexa Hesch[1], Grant Schaap[1], Mahdi Ahmadi[1], Nathaniel Bristow[1], Badrinath Konety[1], Howard Scher[3], Emmanuel S. Antonarakis[4], Jiarong Hong[1], **Justin M. Drake**[4]

[1]Astrin Biosciences, St Paul, MN,[2]Department of Medicine, University of Minnesota, Minneapolis, MN,[3]Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY,[4]University of Minnesota, Minneapolis, MN

Background: While cancers often arise from a specific tissue the drugs designed to fight them are largely tissue agnostic. Drugs most commonly target mutant or amplified proteins such as transcription factors, kinases, or other cell surface proteins. An ever-growing body of research shows that these well characterized protein targets are commonly found across many cancer types and can serve as potential targets for pan-cancer therapies. Despite this growing consensus, enrollment criteria for clinical trials often do not include any test to detect or quantify the presence of the drug's target protein. However, patient-specific protein profiles should be utilized as a tool to not only minimize patient harm but also to categorize patients so drug trials can be designed to identify only the patients most likely to benefit in mind. To that end, we developed A liquid biopsy-based proteomic assay to identify druggable targets from CTCs from cancer patients' blood.
Methods: CTCs were isolated using Astrin Bioscience's proprietary enrichment system yielding matched CTC and white blood cell (WBC) fractions. Following enrichment, samples were processed in parallel using a custom developed protein aggregation capture protocol followed by protein digestion to yield peptide samples ready for bottom-up targeted peptide analysis on a FAIMS equipped Exploris 480 instrument with internal normalization to heavy labeled peptide standards.
Results: We prospectively enrolled 20 heavily-pretreated metastatic castration resistant prostate cancer (mCRPC) patients from whom matched CTC and WBC profiles were analyzed. The mean CTC count was 10.4 cells/mL with a median of 4.9 CTCs/mL. The median age of these patients was 74 years, 80% had Gleason GG4-5, 30% had visceral metastases, median number of prior systemic therapies was 5, and median PSA level was 135 ng/mL. Following CTC enrichment, we observed vastly diverse inter- and intra-patient proteomic profiles. Some of the highly relevant proteins to mCRPC identified and quantified from CTCs in 100% of patients for androgen receptor (AR) and B7 homolog 3 (B7-H3), 57% of patients for trophoblast cell surface antigen-2 (TROP2), 43% of patients for prostate specific membrane antigen (PSMA), 28% of patients for programmed cell death antigen 1 (PD-L1), and 14% of patients for neuroendocrine markers chromogranin A (CHGA) and delta like ligand 3 (DLL3) with significantly less or no detection of these proteins in the WBC fraction.
Conclusions: With the clear differences seen between the CTC and WBC fractions, combined with expected proteins observed in the CTC fraction, this demonstrates the successful isolation of CTCs. Together, this shows a first of its kind assay able to probe multiple highly relevant proteins from a patient's own CTCs that could be crucial to help minimizing patient harm and designing better precision therapeutics.

CHEMISTRY: Drug Delivery
Poster Session

#6893 Synthesis and characterization of IL-4R ligand mimic (AP1)-functionalized elastin-like polypeptide-based nanopolymers as selective delivery vehicles for paclitaxel in ovarian cancer.

Ridhima Goel, Deepak Gulwani, Thoudam Debraj Singh

All India Institute of Medical Sciences (AIIMS) New Delhi, New Delhi, India

Introduction- Elastin-like polypeptides (ELPs) have emerged as promising nanocarriers for drug administration, owing to their advantageous properties, including biocompatibility and distinctive biophysical behavior. These polymers can be tailored to incorporate functional motifs that allow for accurate drug loading and targeted delivery. In cancer therapy, ELP-based nanocarriers have shown potential by increasing drug solubility, stability, and allowing for tumor-specific targeting. This study aims to develop and evaluate AP1-functionalized ELP-based nanopolymers as vehicles for the “selective” delivery of paclitaxel (PTX) in ovarian cancer (OC).
Materials and methods- Maleimide-reactive cysteine residues were introduced into the ELP/AP1-ELP sequence. Paclitaxel, activated by N-ε-maleimidocaproic acid (EMCA), was coupled to ELP/AP1-ELP by a Michael addition reaction. Chemical conjugation was assessed using MALDI-TOF mass spectrometry, transmission electron microscopy (TEM), and dynamic light scattering (DLS). SKOV-3 and OVCAR-3 cell lines were analyzed for IL-4R expression followed by assessment of differential binding of Alexa-488 labelled ELP/AP1-ELP using flow cytometry (FACS). IC50 values of ELP/AP1-ELP-PTX were measured using the CCK-8 assay. Additionally, cell cycle progression and apoptosis were examined by FACS. Furthermore, an agarose-based 3D spheroid model of OC cells was used to establish the selective targeting efficacy of PTX-loaded AP1-ELP.
Results- Sanger sequencing confirmed the successful incorporation of maleimide-reactive cysteine residues into ELP polymers. MALDI-TOF, TEM, and DLS analyses demonstrated stable conjugation of the drug to the polymer. IL-4R expression in SKOV-3 and OVCAR-3 via FACS showed positivity for the receptor. Binding assays with Alexa-488-labelled ELP/AP1-ELP showed enhanced binding to IL-4R-expressing cells for the AP1-functionalized polymers compared to the non-functionalized ELP. Cytotoxicity assays revealed that AP1-ELP-PTX exhibited relatively lower IC50 values, indicating stronger anti-cancer activity compared to ELP-PTX. FACS-based analysis of the cell cycle and apoptosis further confirmed the higher cytotoxicity of AP1-ELP-PTX. Moreover, 3D spheroid cultures of OC cell lines established the significance of AP1-based targeting, displaying superior cytotoxicity in comparison to ELP-PTX.
Conclusion- In this study, we demonstrate the potential of AP1-functionalized ELP in the selective delivery of PTX in ovarian cancer. AP1-ELP exhibits superior cellular association, facilitating improved cytotoxic ability for the appended drug, PTX. Further evaluation of the AP1-ELP-PTX selective delivery ability *in vivo* is required for full realization of the AP1-ELP polymer as a delivery vehicle.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Antibody-Based Cancer Therapeutics 1
Poster Session

#7337 Development of novel anti BCMA as a low cost adoptive cellular therapy candidate in multiple myeloma.

Ashna Gupta, Gunjan Dagar, Ravi Chauhan, Kalpana Luthra, Mayank Singh

Medical Oncology, All India Institute of Medical Sciences, New Delhi, India

Background: Multiple myeloma (MM) involves the proliferation of malignant plasma cells (PCs) in the bone marrow (BM), leading to excessive monoclonal immunoglobulins in patients' blood and urine. The use of monoclonal antibodies (MoAbs) as immunotherapies in MM treatment has significantly improved outcomes. However, MM remains incurable for most patients due to the emergence and evolution of drug-resistant clones. BCMA (B Cell Maturation Antigen), overexpressed in MM, is being explored as a target for antibody-based and cellular therapies.
Method: BCMA expression in MM patients was quantified via RQ-PCR, and soluble BCMA levels were measured using ELISA to analyze BCMA dynamics in this cohort. The extracellular domain (ECD) of BCMA was cloned, sequenced, and expressed in the Expi293 system, with characterization confirmed through SDS-PAGE. To generate anti-BCMA ECD antibodies, mice were immunized with the purified ECD protein , leading to the development of hybridoma cells. These were purified and validated through western blotting and other assays to identify top-binding clones. The highest-affinity clone was purified using a protein A IgG purification kit. Molecular docking and dynamic simulations were then conducted to determine the BCMA ECD epitope/paratope targeted by our antibody.
Results: We analyzed BCMA expression in multiple myeloma (MM) patients and observed elevated mRNA levels and increased serum BCMA, both linked to poor treatment outcomes. BCMA was validated as a promising therapeutic target, and the BCMA ECD protein was successfully expressed in a eukaryotic system, facilitating hybridoma generation. From this, 20 unique clones were developed and screened via ELISA for optimal binding to the BCMA ECD protein, with the clone 9C4 emerging as the lead candidate. Molecular docking revealed four novel epitopes (S, Q, N, E) distinct from previously identified ones. Our antibody demonstrated superior binding, interacting with 23 BCMA residues compared to 12-16 residues targeted by existing antibodies. The ScFv sequence of the 9C4 clone was incorporated into a second-generation CAR construct. This novel Anti-BCMA CAR was successfully expressed on T cells, enabling the development of CAR-T cells for advanced therapeutic applications.
Conclusion: Our findings confirm BCMA as a key target in multiple myeloma, demonstrated by the successful development of the high-affinity 9C4 antibody. Incorporating the 9C4 ScFv into a second-generation CAR construct establishes a foundation for developing a novel academic Anti-BCMA CAR-T cell therapy for multiple myeloma.

: 2025 AACR Undergraduate Scholars and Margaret Foti Undergraduate Prize Recipients
Poster Session

#FOTI01 Margaret Foti Foundation Prizes.

Margaret Foti

American Association for Cancer Research, Philadelphia, PA

The Margaret Foti Foundation was founded by the AACR Chief Executive Officer Margaret Foti. The Margaret Foti Foundation Undergraduate Prizes for Cancer Research were established in 2018 and are presented to meritorious participants at the AACR Undergraduate Student Caucus and Poster Competition during the AACR Annual Meetings. These prizes reward excellence in research conducted and presented by undergraduate students.

CLINICAL RESEARCH: Real-World Data and Real-World Evidence: Clinico-Genomics
Poster Session

#1156 Real-world clinical multi-omics analyses reveal bifurcation of ER-independent and ER-dependent drug resistance to CDK4/6 inhibitors.

Z. Kan[1], J. Wen[1], V. Bonato[1], J. Webster[2], W. Yang[1], V. Ivanov[3], K. Kim[1], W. Roh[1], C. Liu[1], X. Mu[1], J. Lapira-Miller[1], J. Oyer[1], T. VanArsdale[1], P. A. Rejto[1], J. Bienkowska[1];
[1]Pfizer, Inc., San Diego, CA, [2]Pfizer, Inc., New York, NY, [3]Pfizer, Inc., Cambridge, MA

To better understand the molecular mechanisms of drug resistance to CDK4/6 inhibitors (CDK4/6i) and inform precision medicine strategies, we retrospectively analyzed a multi-omics dataset of 400 HR+/HER2- mBC patients who received CDK4/6i plus endocrine therapies in routine clinical practice, including 200 pre-treatment (Pre), 227 post-progression (Post) samples. Integrative clustering analysis identified three subgroups of tumors harboring different drug resistance mechanisms: ER driven, ER co-driven and ER independent. The ER independent subgroup increased the most in prevalence from 4% pre-treatment to 23% post-progression and is characterized by down-regulation of estrogen signaling and enrichment of drug resistance markers including *TP53* and *RB1* mutations, Basal-like and Her2-like subtypes and *CCNE1* over-expression. We deconvoluted bulk tumor expression profiles to derive cancer-specific expression (CSE) profiles for investigating tumor intrinsic mechanisms that give rise to ER independent drug resistance. We then performed trajectory inference analyses often used in single-cell transcriptomics on CSEs and identified a latent pseudotime variable strongly correlated with ER independence and disease progression. Elastic principal graph (EPG) analysis further revealed bifurcated evolutionary trajectories for drug resistance that correspond to ER-dependent vs. ER-independent mechanisms. Using elastic net models trained on in vitro CRISPR knockout screen data, we predicted a shift in therapeutic dependency on *ESR1* and *CDK4* among ER-dependent tumors to *CDK2* among ER-independent tumors. These predictions were experimentally validated using genetically modified isogenic models.

TUMOR BIOLOGY: Advanced Nanotechnology and Imaging
Poster Session

#2522 N-heterocyclic carbene (NHC)-protected gold nanoclusters for cancer imaging and therapy.

J. Chen[1], E. Zeinizade[1], V. K. Kulkarni[2], A. I. Sullivan[2], B. C. Wilson[1], M. Koritzinsky[1], C. M. Crudden[2], G. Zheng[1];
[1]University Health Network, Toronto, ON, Canada, [2]Queen's University, Kingston, ON, Canada

Gold nanoclusters (AuNCs), commonly stabilized by thiol groups, hold significant promise for cancer theranostics, where their small size enables efficient renal clearance, and their atomically precise structures offer potential for drug-like properties [1-2]. N-heterocyclic carbene (NHC) ligands further enhance the versatility of AuNCs, introducing unique cluster compositions and improved stability [3]. Recently, NHC-protected AuNCs have emerged as tunable, emissive platforms with exceptional stability and photoluminescence, making them ideal candidates for applications in fluorescence imaging and photodynamic therapy [4]. In our work, we synthesized ultra-small bis-NHC-protected Au13 nanoclusters with π-extended aromatic systems, observing a correlation between increased structural rigidity and both high fluorescence quantum yield (57%) and reactive oxygen species (ROS) production efficiency (7%). Extensive stability and photophysical characterizations confirmed long emission lifetimes and high optical purity, with these nanoclusters maintaining robust stability and showing low toxicity under physiological conditions. Confocal microscopy and ICP-MS analyses verified effective cellular uptake across various cancer cell lines, and upon light activation, the nanoclusters functioned as potent photodynamic therapy agents, selectively inducing cancer cell destruction. PEGylation significantly improved water solubility and biocompatibility while maintaining photonic properties. Additionally, azide functionalization on cluster wings enabled targeted ligand attachment *via* click chemistry, demonstrated with folate receptor-mediated targeting for cancer imaging. With high stability, superior photoluminescence quantum yield, efficient cellular uptake, and clickable framework, these NHC-AuNCs provide a versatile and powerful nanoplatform for advanced cancer imaging and targeted therapy.References1. Huang, Y etc., *Nat. Nanotechnol.* 2023, *18* (6), 637-646. 2. Zhang, X.-D. etc., Enhanced Tumor Accumulation of Sub-2 Nm Gold Nanoclusters for Cancer Radiation Therapy. *Advanced Healthcare Materials* 2014, *3* (1), 133-141.3. Shen, H.; Tian, G.; Xu, Z.; Wang, L.; Wu, Q.; Zhang, Y.; Teo, B. K.; Zheng, N. etc., N-Heterocyclic Carbene Coordinated Metal Nanoparticles and Nanoclusters. *Coordination Chemistry Reviews* 2022, *458*, 214425. 4. Albright, E. L. etc., N-Heterocyclic Carbene-Stabilized Atomically Precise Metal Nanoclusters. *J. Am. Chem. Soc.* 2024, *146* (9), 5759-5780.

#2523 Porphyrin-lipid Nanoparticle-Based Multifunctional Cancer Imaging and Therapy: From Cell to Patient.

Robert A. Weersink[1], Theo Husby[1], Ivan Kosik[1], Michael Daly[1], Jason Townson[1], Ben Motx[1], Alessandra Ruaro[1], Juan Chen[1], Christine Demore[2], Stephanie Lheureux[1], Amit Oza[1], Jonathon Irish[1], Gang Zheng[1], Brian C. Wilson[1]

[1]University Health Network, Toronto, ON, Canada,[2]University of Toronto, Toronto, ON, Canada

Background: Porphysomes (PS) are first-in-class organic porphyrin-lipid nanoparticles with unprecedented intrinsic multifunctionality that enables a range of complementary imaging, image-guided interventions and therapies. Examples are presented in a range of preclinical tumor models, including head & neck, lung and gynecological cancers, demonstrating progress towards first-in-human trials.
Methods & Results: High-density packing of the porphyrin-lipid monomers in the nanoparticle bilayer enables efficient light absorption and conversion to heat for enhanced photoacoustic imaging (PAI) and photothermal therapy (PTT). Using wide-band acoustic detection at low frequencies multispectral PAI can quantify PS concentration in tumors that span sub-mucosal disease to several cm depth. A novel method has also been developed for direct temperature mapping to monitor PS-mediated PTT delivery.
Upon structural dissociation following cell uptake, the fluorescence (FL) and photodynamic therapy (PDT) activity of the porphyrin monomers are restored. Corresponding diffuse optical spectroscopy (DOS) has measured longitudinal PS pharmacokinetics *in vivo*. This will allow identification of residual tumor during surgical resection and also serve as a component of PDT dosimetry. FL imaging of tumor-targeted PS has been demonstrated in veterinary clinical trials of oral cancer, showing efficacy in tumor localization and surgical guidance. AI-assisted spatial-frequency-domain FL imaging in particular shows promise in measuring tumor depth prior to resection and tumor extent during resection.
Attaching gadolinium to the PS lipid chains enables combined intra-operative surgical planning and guidance, using Gd-contrast MRI for pre-resection planning and higher-resolution FGS during resection. We are evaluating MR-FGS in pre-clinical models, comparing PS localization using MRI and DOS to pathology and surgical resection outcomes.
Finally, PS chelated with ^{64}Cu for PET imaging of tumors has been demonstrated in several large animal models and serves as the key modality to assess PS pharmacokinetics in first-in-human studies in advanced ovarian cancer, leading to first therapeutic trials. ^{64}CuPS imaging will also be used for planning and dosimetry of ^{67}CuPS radioisotope therapy of disseminated disease.
Conclusions: Porphysomes provide unique multifunctional imaging and theranostic capabilities for cancer detection, localization and personalized therapy, both stand-alone and combined with standard modalities.

This work is supported by the Terry Fox Research Institute (Grant 1137-04).

#2524 Lipid-based nanoparticles for the theranostic treatment of brain tumors using focused ultrasound.

Paul Cressey[1], Chris Payne[2], Amelia Claxton[1], Benedetta Arno[3], Christopher Ireson[4], Maral Amrahli[5], David Thurston[1], Antonios Pouliopoulos[2], Maya Thanou[1]

[1]Institute of Pharmaceutical Science, King's College London, London, United Kingdom,[2]School of Biomedical Engineering & Imaging Sciences, King's College London, London, United Kingdom,[3]Medicines Discovery Catapult, Macclesfield, United Kingdom,[4]Pharmidex, London, United Kingdom,[5]Apeikon, London, United Kingdom

Glioblastoma is an extremely aggressive brain tumor and currently has limited effective treatment options, with a median survival of 14 months. This low survival is partially attributed to the blood-brain barrier (BBB), especially in early-stage glioblastoma. An emerging clinically approved technology to non-invasively and transiently open the BBB is focused ultrasound (FUS) in combination with microbubble cavitation. In this study, we formulated a dual-imageable drug-loaded lipid-based nanoparticle (ActNano) and tested their efficacy against U87-MG in vitro and in vivo (subcutaneous). In addition, we performed focused ultrasound (FUS) induced BBB opening to deliver ActNano into both healthy mice brains and mice bearing orthotopic brain tumors (U87-MG). ActNano uptake was determined and quantified in vivo and ex vivo using MRI and near-infrared fluorescence (NIRF) imaging, respectively. In addition, drug uptake enhancement following FUS BBB opening in orthotopic tumours was also determined using HPLC (SN-38) and ICP MS (carboplatin). ActNano was loaded with SN-38 (1.11 ± 0.08 mg/ml) and carboplatin (0.84 ± 0.14 mg/ml). The ability of ActNano to supress tumor growth was investigated with single treatments against a U87-MG subcutaneous flank xenograft model (10 mg/kg SN-38 & carboplatin). FUS-induced BBB opening was performed in mice (peak-negative pressure = 320 kPa, number of cycles = 500, pulse repetition frequency = 5 Hz, number of pulses = 600). SonoVue microbubbles (2 ml/kg) and ActNano (8 ml/kg, 10 mg/kg SN-38) were administered intravenously during FUS treatment. T_1-weighted MRI was performed in a 9.4-T MRI 4 hours after BBB opening, to allow time for ActNano to accumulate in the brain/tumors for optimal T_1 contrast. NIRF imaging, H&E staining and drug quantification was performed on perfused and excised brains after the final time point, to confirm successful liposome delivery into the sonicated volume. ActNano was successfully formulated with both MRI and NIRF contrast. ActNano (10 mg/kg SN-38) treatment caused an increase in survival of mice with xenograft tumours when administered alone and a significant increase in survival and tumor uptake when combined with FUS. MRI and NIRF imaging confirmed localized increased uptake of ActNano where FUS-induced BBB opening was performed compared to the contralateral control side. Quantification of Gd, carboplatin and SN-38 confirmed a significant increase in drug concentration in the tumor following BBB opening. These results suggest that MRI/NIRF-tagged drug loaded lipid-nanoparticles are suitable drug carriers for the treatment of glioblastoma in combination with FUS-induced BBB opening. Future work includes evaluation of the survival benefit of ActNano in tumor-bearing mice.

#2525 Translating clinically validated antibodies into a multiplexed immunofluorescent panel for the spatial profiling of lymphoid malignancies.

Tania Pannellini[1], Fabio Iannelli[1], Valentina Tabanelli[1], Carmen De Simone[1], Roberto Chiarle[1], Samuel Aubert[2], Pedro Machado Almeida[2], Maria Giuseppina Procopio[2], Saska Brajkovic[2]

[1]Haematopathology Division, IEO - European Institute of Oncology, Milan, Italy,[2]Lunaphore, Tolochenaz, Switzerland

Lymphoid malignancies present significant diagnostic challenges due to overlapping immunophenotypic features between tumor cells and reactive background cells, complicating the distinction between indolent lymphomas and benign lymph node hyperplasia [Nicolae A, Hemato, 2024, DOI:10.3390/hemato5030026]. Moreover, some Hodgkin lymphoma (HL) subtypes, such as lymphocyte-rich (LRHL) and nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) and certain variants of diffuse large B-cell lymphoma (DLBCL), exhibit similar phenotypic profiles despite having different therapeutic implications [PMID: 35741318]. This study aims to leverage sequential immunofluorescence (seqIF™, PMID: 37813886) for the spatial profiling of lymphoma to improve differential diagnosis and to uncover spatial patterns unique to each lymphoma subtype, providing insights into potential predictors of immunotherapy response. Here, we optimized a seqIF™ panel of 21 markers, using antibodies approved for clinical immunohistochemistry (IHC), to phenotype mature B-cell lymphomas on the COMET™ platform. This panel was applied to tissue microarrays prepared from representative cases of DLBCL, HL, indolent lymphomas, and benign lymph node hyperplasia. Each marker of the optimized panel was visually validated by certified pathologists, comparing seqIF™ results against corresponding IHC images. Computation of spatial matrix successfully identified tumor cells and reactive cells using thresholding of fluorescence intensity and cell diameter, particularly in HL and DLBCL cases that exhibited similar immunophenotypes at first glance. This enabled us to map classified cells events back to the tissue sections, allowing direct spatial correlation with stained images for a comprehensive analysis of cell distribution. Ongoing work aims at refining our lymphoma spatial profiles through the integration of spatial transcriptomics readout on the same sample cohort. Together these results highlight the potential of multiplex immunofluorescence (mIF) for the diagnostic phenotyping of lymphoma, transferring clinically validated antibodies into a mIF panel. The ability to assess both phenotypic and spatial characteristics of tumor and reactive cells will enhance our understanding of lymphoma biology and provide new insights into predictive markers for immunotherapy responses.

#2526 Development of ultrahigh sensitivity magnetic particle imaging for stem cell tracking.

Yimeng Li[1], Yu An[1], Yang Du[2], Lin Shen[3], Jie Tian[1]

[1]Beihang University, Beijing, China,[2]Chinese Academy of Sciences, Beijing, China,[3]Peking University Cancer Hospital, Beijing, China

In vivo tracking of stem cells is critical to stem cell transplantation and regenerative medicine research. Achieving long-term, non-invasive imaging of transplanted cells demands highly sensitive imaging technology. Magnetic particle imaging (MPI) is an emerging modality capable of visualizing the distribution of superparamagnetic iron oxide nanoparticles (SPIOs). Previous studies have reported that MPI can detect clusters of approximately 2500 SPIO-labeled stem cells. However, more than this sensitivity level is needed to monitor the survival, migration, differentiation, and regeneration of stem cells. To address this limitation, we developed an ultrahigh-sensitivity magnetic particle imaging (US-MPI) device capable of detecting stem cells at scales of hundreds of cells.

Through full-chain design and optimization, US-MPI maximizes the capture of SPIO signals from stem cells. Using a commercial SPIO (VivoTrax), mouse mesenchymal stem cells were labeled, and samples with varying cell numbers (ranging from 50 to 1×10^7) were tested using US-MPI and 9.4 T MRI (uMR 9.4T, United Imaging Life Science Instrument, Wuhan, China) for comparison. US-MPI demonstrated the ability to detect as few as 50 labeled stem cells in vitro and 150 cells in vivo, whereas 9.4 T MRI required at least 1×10^5 cells for detection in vitro. Thus, US-MPI exhibited a sensitivity 2,000 times higher than that of 9.4 T MRI.

The efficacy of US-MPI for stem cell tracking was further validated in a lipopolysaccharide (LPS)-induced mouse hind limb inflammation model. Eight hours after SPIO-labeled stem cells were injected intravenously, US-MPI detected strong signals on the inflamed limb, which remained significantly higher than those of the healthy limb during a 48-hour monitoring period. Post-mortem Prussian blue staining of inflamed tissues confirmed the presence of blue-stained SPIO particles, verifying that the US-MPI signals originated from labeled stem cells. These results highlight the ultrahigh sensitivity of US-MPI for stem cell tracking, providing a valuable tool for advancing stem cell research.

#2528 Bioinspired multispectral imaging for intraoperative detection of metastatic lymph nodes: enhancing precision through UV and NIR fluorescence.

Zhongmin Zhu, Yifei Jin, Brianna Hajek, Shuming Nie, Viktor Gruev

Electrical and Computer Engineering, University of Illinois at Urbana-Champaign, Urbana, IL

Intraoperative identification of metastatic lymph nodes (LNs) remains a clinical challenge, with current techniques often leading to unnecessary removal of healthy tissue or retention of cancer-positive nodes. To address this issue, we developed a bioinspired multispectral imaging system that integrates ultraviolet (UV) autofluorescence and near-infrared (NIR) fluorescence detection. This platform mimics the mantis shrimp's unique visual system, leveraging vertically stacked photodiodes and pixelated spectral filters to achieve simultaneous imaging across the UV, visible, and NIR spectral ranges without spatial misalignment or loss of resolution. The system uses indocyanine green (ICG) as an FDA-approved NIR fluorescent probe for LN localization and UV excitation to evaluate LN status via autofluorescence. The UV fluorescence leverages endogenous biomarkers, such as tryptophan, commonly enriched in tumor-associated tissues. This dual-modality imaging approach enables real-time identification of both the anatomical location and pathological status of LNs, reducing dependence on postoperative histopathological analysis. In a clinical study involving 33 breast cancer patients, the imaging system achieved a positive predictive value of 96% and a receiver operating characteristic (ROC) area under the curve (AUC) of 89% in distinguishing metastatic from non-metastatic lymph nodes. These results were corroborated by pathology, which served as the ground truth. By providing immediate and accurate feedback to surgeons, this system has the potential to improve surgical precision, reduce unnecessary excisions, and minimize the need for follow-up procedures. Unlike traditional multispectral imaging systems reliant on mechanically rotating filters or dichroic beamsplitters, our bioinspired sensor eliminates co-registration errors and significantly reduces system complexity. Its compact design and capability to perform high-resolution imaging under challenging surgical lighting conditions make it well-suited for widespread clinical adoption. Further studies are underway to validate the system’s efficacy across different cancer types and expand its applications to other surgical scenarios. This novel imaging platform offers a significant step forward in integrating real-time molecular and anatomical imaging into intraoperative workflows, addressing critical gaps in precision oncology.

#2529 Targeted light-activable multi-agent nanoparticle for enhanced photoimmunotherapy treatment of peritoneal metastasis.

Sumiao Pang[1], Carla Arnau Del Valle[2], Robert Perttila[3], Nada Fadul[1], Dana Roque[4], Tayyaba Hasan[5], Petteri Uusimaa[3], Huang Chiao` Huang[1]

[1]University of Maryland, College Park, MD,[2]Instituto Interuniversitario de Investigacion de Reconocimiento Molecular y Desarrollo Tecnologico, Valencia, Spain,[3]Modulight Corporation, Tampere, Finland,[4]University of Maryland Baltimore, Baltimore, MD,[5]Massachusetts General Hospital and Harvard Medical School, Boston, MA

Background: Peritoneal metastasis is the result of direct shedding of tumor cells from ovarian, colorectal, and other cancers that lead to studding and growth on the peritoneal surfaces and abdominal organs. Standard chemotherapy and surgery can transiently improve symptoms, but long-term disease control and survival extension remain rare due to residual disease and treatment resistance. In the past 40 years, tremendous progress in photoimmunotherapy (PIT) has been made to treat metastasis. However, many are lost in translation due to heterogeneity in photosensitizer drug uptake and treatment response. Thus, we hypothesize that the delivery of targeted therapies with a multimodal approach for PIT and integrates well with current standards of care strategies can help overcome heterogeneous delivery and improved treatment efficacy. Our novel targeted photoactivable multi-agent liposome (TPMAL) combined with fluorescence-guided PIT was demonstrated in this newly reported study.
Methods: TPMAL consists of antibody drug conjugates (photosensitizer drug and anti-EGFR antibody) conjugated onto active-loaded liposomal surface. Female athymic nude mice (18-20 g and 4-6 weeks old) were injected intraperitoneally with 3 x 10^7 OVCAR-5 human ovarian cancer cells suspended in 2 mL of PBS. Post tumor implantation, mice were i.p. injected with TPMAL or ADC alone on Day 10. Post 24h, tumor nodules were collected and frozen with Tissue-Tek O.C.T compound at -80°C until slicing. Fluorescence and bright field imaging of whole tumor nodule slices were performed with a Lionheart™ FX Automated Microscope. The center of each tumor nodule was selected for further image analysis using ImageJ. Lastly, intraperitoneal fluorescence-guided light delivery PIT with TPMAL was performed using the ML7710 medial laser device and Modulight cloud software for survival studies.
Results: TPMAL active-loaded formulation demonstrated batch-to-batch consistency and stability was observed for up to one year. This formulation also demonstrated enhanced photosensitizer drug delivery into peritoneal tumor nodules by 1.6X and up to 616.4 µm penetration depth compared to antibody drug conjugates alone. TPMAL FGLD PIT combination therapy with adjuvant chemotherapy also demonstrated enhanced long-term survival by 240X compared to no treatment and chemotherapy alone groups.
Conclusions: Our novel targeted photoactivable multi-agent liposome (TPMAL) combined with fluorescence-guided PIT was investigated for its translational potential and therapeutic efficacy by (1) developing an active-loaded liposomal formulation, (2) studying tumor nodule penetration depth of TPMAL, (3) understanding tissue optical property via oxygenation and how it may affect PIT using the ML7710, and (4) enhancing long term therapeutic efficacy compared to monotherapy treated groups in a mouse peritoneal carcinomatosis model.

#2530 Multispectral imaging system for intraoperative molecular imaging with two near-infrared fluorescent probes.

Brianna Hajek, Zhongmin Zhu, Viktor Gruev

Electrical and Computer Engineering, University of Illinois at Urbana-Champaign, Urbana, IL

Image-guided surgery using near-infrared (NIR) fluorescent probes offers surgeons real-time guidance to locate tumors and sentinel lymph nodes. The past decade has seen a proliferation in NIR fluorophore development, with options for targeting different tumor markers and tissue types. Using multiple fluorophores could improve tumor detection sensitivity and specificity, allow simultaneous tumor imaging and lymph node mapping, or distinguish tumors from vital anatomical structures. Current imaging systems, however, are limited to detecting a single NIR probe. This has motivated the development of a novel multispectral camera, capable of simultaneously imaging multiple NIR fluorophores with similar emission spectra that are excited with a single excitation source. The camera features a unique spectral filter array, with filters to capture both visible light and nine overlapping spectral bands in the NIR, monolithically integrated with three vertically stacked photodiodes. The result is a compact instrument that can produce overlaid visible and NIR images without co-registration error, while sampling the NIR spectrum in a nuanced manner to distinguish similar NIR fluorophores. The pixelated filters are arranged in a 2x2 “super-pixel” pattern, comprising a visible filter and three NIR filters with several passbands. The NIR filters produce nine spectral features, which are used to classify fluorescent spectra using machine learning. Initial experiments were performed to demonstrate the camera’s ability to differentiate two NIR fluorophores in vials. Combinations of indocyanine green (ICG), pafolacianine, and JAS239 in 10% FBS solutions were mixed with a range of volumetric ratios. The fluorophore concentrations were chosen to have similar overall fluorescence intensity, indistinguishable by a traditional NIR camera. Gradient boosting classifiers were trained to predict the dye or combination of dyes that produced the fluorescence at each pixel in an image. Pure ICG and pafolacianine were classified with 97% accuracy and their mixtures with 77% accuracy. ICG and JAS239 were discriminated with 99% accuracy, and mixtures of these two dyes with 81% accuracy. The results demonstrate that our single-chip multispectral imager captures sufficient information to distinguish two NIR fluorescent probes, a task unachievable by current instruments. Future steps will test the discrimination capabilities in more complex samples, moving closer to clinical applications.

#2531 Novel staining method for visualization, isolation and processing of intracranial brain tumors.

Sonia Jain[1], Anders Herfindahl-Quint[1], Caroline D. Doherty[2], Wenjuan Zhang[3], Danielle M. Burgenske[1], Jann N. Sarkaria[1]

[1]Department of Radiation Oncology, Mayo Clinic Cancer Center Minnesota, Rochester, MN,[2]Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic Cancer Center Minnesota, Rochester, MN,[3]Department of Pharmaceutics, University of Minnesota, Minneapolis, MN

Background and Objective: Glioblastoma (GBM) is most common primary brain tumor with dire need of novel therapeutics. Studies with intracranial tumors are required for robust characterization and development of novel drugs for brain cancer. Tumor harvest and processing is an important aspect to study pharmacokinetics, pharmacodynamics, drug delivery, and tumor evolution. Typically, delineating and dissecting tumor from surrounding brain for varied downstream processes is dependent on the distinct morphology of the tumor and/or expression of fluorescent marker. However, genetic modification of the tumor cells for the expression of fluorescent protein is not always possible, especially for patient-derived xenograft (PDX) tumor models. Thus, we have developed a dye-based staining method to confidently visualize tumor in the brain and assessed usability of isolated tumors for drug and DNA analysis.

Methodology: Whole brains were harvested from mice with intracranial Mayo GBM PDX tumors and stored at -80°C freezer. Few days prior to staining, brains were transferred to -20°C freezer. Brain was cut into 1 mm thick coronal sections and transferred onto glass slides. Slices were covered with toluidine blue (TB) solution (0.05 to 10 mg/mL) for 45 s. Excess stain solution was removed, and slices were observed under white light. Regions of tumor became more transparent, while normal brain remained opaque. This enabled dissection of tumor from normal brain for downstream analyses.

Results: TB concentration (0.05 to 10 mg/mL) was optimized to stain 1 mm thick sections of fresh frozen brain, and the best contrast between tumor to normal brain was observed at 0.1-0.3 mg/mL. TB aided differential visualization of tumors in several PDX models - GBM12, GBM43, GBM39 and GBM108 - suggesting broad applicability. To evaluate the accuracy of TB staining, brain with PDX tumor transduced with pSINeGFP/fLuc2 was observed by both TB staining and eGFP fluorescence. This analysis demonstrated excellent concordance across 6 animals with three distinct PDX models. Genomic DNA was isolated from tumor dissected under TB guidance and analyzed by PCR for primers specific for murine vs human PTGER2. 50-70% of the DNA in tumor samples was detected to be of human origin while normal brain tissue had 90-95% of the mouse DNA highlighting the specificity of TB stain for tumor detection. Tumors from drug treated GBM108 PDX were isolated under TB guidance and comparable drug levels were detected by LC-MS/MS as analyzed in GFP-assisted tumor samples.

Conclusion: Staining fresh frozen thick brain sections with toluidine blue enables visualization of tumor from normal brain regions that is compatible with varied downstream processes. Development of such staining assays can help faster and easier tumor processing and is not dependent on genetic modification of tumor cells prior to experimentation.

#2532 Utilization of longitudinal ultrasound imaging as a novel pharmacodynamic marker of graft versus host disease progression in mice.

B. John[1], T. Kierski[2], T. Czernuszewicz[2], F. Zhao[1], D. Repetto[1], J. Neil[1], **D. Harris**[1];
[1]Charles River Laboratories, Inc., Durham, NC, [2]Revvity, Waltham, MA

Graft versus host disease (GvHD) is a potentially life-threatening condition that occurs most commonly after allogeneic transplantation of bone marrow or stem cells, and less frequently after organ/tissue transplantation. GvHD can present as an acute disease, or it can develop more slowly, resulting in a chronic condition affecting multiple tissues and organs. Allogeneic (acute and chronic) and xenogeneic GvHD models have been developed in mice that aim to replicate some of the clinical manifestations seen in human disease. However, endpoints such as GvHD scoring which are commonly used to track disease progression are subjective and difficult to replicate consistently. In addition, terminal sampling as well as longitudinal blood sampling for biomarker analysis often requires the use of satellite groups which is costly and at odds with the principles of 3R animal usage. To overcome these challenges, we describe the novel use of a Vega ultrasound imaging system (Revvity, Waltham, MA, USA), to monitor longitudinal changes in splenic volume, a well-known biomarker associated with GvHD and other diseases. To determine the feasibility of this workflow, a two-phase pilot study was conducted. The first phase of the pilot study utilized N=15 mice split into 3 groups of 5 mice each. Group 1 mice were controls while groups 2-3 were affected by chronic GvHD. Each mouse was imaged with 3-D high-frequency B-mode ultrasound using the Vega on day 100, 106, and 114 post transfer of splenocytes and bone marrow cells into irradiated recipient mice. Spleens were manually segmented from each image to measure spleen volume. The second phase of the pilot study was focused on tracking changes in an acute model of GvHD. For this phase, N=8 mice were split into 4 groups of 2 mice each. Group 1 were controls, while groups 2-4 received 3e6 to 5e6 dose of splenocytes and 1e7 bone marrow cells. Mice were imaged on day 10, 21, and 31 using the Vega, and spleen volumes were again determined via manual segmentation. In the chronic model, spleen volumes in affected animals were significantly reduced (37.9 ± 11.5 mm3) compared to control animals (84.8 ± 15.3 mm3). In the acute model, we observed an initial phase of splenomegaly followed by a drastic shrinkage of the spleens to roughly 50% of the control volumes. In summary, this study suggests the use of 3D non-invasive ultrasound imaging for assessing organ size and structure differences in GvHD model that will facilitate the discovery of new therapies.

#2533 Image-guided surgery with a new optical probe for breast cancer.

Sunam Mander, Duy Binh Tran, Goto Masahide, Konstantin Christov, Albert Green, Tapas K. Das Gupta, **Tohru Yamada**

Surgery, University of Illinois at Chicago, Chicago, IL

Accurate surgical removal of tumors significantly impacts patient outcomes. However, distinguishing between breast tumors and normal tissue during surgery is often challenging or even impossible. Given the lack of visual discrepancy between malignant and surrounding normal tissue, current breast-conserving surgery (BCS) is associated with a high re-excision rate. Due to this clinical challenge, a new method of complete tumor removal at the initial surgery is needed. To address this, we developed a new fluorescent imaging agent, ICG-p28, for intraoperative imaging guidance to better aid surgeons in achieving negative margins in BCS. ICG-p28 consists of the clinically nontoxic tumor-targeting peptide p28 and the FDA-approved near-infrared (NIR) dye indocyanine green. Here, we evaluated our imaging approach with ICG-p28 *in vitro* and in breast cancer animal models. First, we determined the detection limit of the objective size and depth using phantoms incorporating tumor-simulating inclusions. Even with the smallest objective (1 mm), NIR fluorescence imaging of ICG-p28 at nanomolar concentrations was able to detect the specific signals. We estimated the detection limit of the cell number in vitro. NIR-specific signals were detected from breast cancer cells as low as 200 cells in 1 mm size. Next, we evaluated ICG-p28 in multiple animal models. Athymic mice xenografted with MDA-MB-231 and IOWA-1T human breast cancer cells received a single injection of ICG-p28 i.v. at 0.5 mg/kg (=125 nmole/kg). Twenty-four hours after ICG-p28 injection, we observed the preferential uptake of ICG-p28 by tumors and a clear contrast between the tumor and the surrounding tissue. The histological analyses with H&E and Ki-67 staining on the set of tumor margin samples revealed that image-guided surgery with ICG-p28 could result in a clear negative margin at the edges of mammary tissues. Moreover, our imaging approach can also visually detect LN metastases (metastatic breast cancer in lymph nodes) through the preferential uptake of ICG-p28 concentrated over a period of time in small (~1 mm) LN metastases. Overall, this nontoxic, and cost-effective method can offer a new approach that enables surgeons to identify tumor margins intraoperatively and potentially improves overall outcomes for breast cancer patients.

#2534 A multimodal animal cradle inlay system to facilitate optical, PET, µCT, and MRI co-registrations of a metastatic, colorectal cancer syngeneic mouse model.

Andrew D. G. Van Praagh[1], Paul Ballieu[1], Ali Bahadur[1], Kristin Granlund[1], Saaussan Madi[1], Mark Mattingly[1], Dianne Begemann[2], Fallon Noto[2], Todd Sasser[1]

[1]Preclinical Imaging Lab, Bruker BioSpin Corporation, Billerica, MA,[2]Hera BioLabs, Lexington, KY

INTRODUCTION: Multimodal imaging can dramatically enhance the diagnostic capabilities of pre-clinical animal research models. Investigators in the fields of oncology and immuno-oncology have sought to add optical imaging to their PET/µCT/MRI data sets because optical adds a sensitive, low-cost, high-throughput imaging capability that can predict the biodistributions of high-cost, low throughput PET/µCT/MRI data sets. In this poster, we demonstrate a proof-of-concept multimodal animal cradle system for optical/PET/µCT/MRI co-registrations.

METHODS: Mouse Model: A CT26-luc mouse colorectal carcinoma cell line (derived from ATCC CRL-2638), expressing Red Firefly Luciferase, was injected into 12 adult Balb/C female mice, at dose levels of either $1x10^5$ (n=6) or $5x10^5$ (n=6) by tail vein IV injection. Optical/PET Reporters Used to Detect Cancer Cells: CT26-luc cells were detected *in vivo* either by Red Firefly Luciferase bioluminescent signal, and/or by the FDG PET/µCT/MRI at various points over the study time course. Mouse Multimodal Cradle Inlay System: A novel multimodal cradle inlay system was designed to be used in a range of preclinical imagers including the Lago X (optical), SkyScan (µCT), PET/CT Si78 (PET/µCT) and BioSpec (MRI) systems. All optical/PET/µCT/MRI multimodal co-registrations were performed in the PMOD software platform (Bruker BioSpin, Billerica, MA).

RESULTS and DISCUSSION: Early, Predictive Detection of Metastatic Colorectal Cancer in the Mouse Lung by Optical Imaging. Metastatic colorectal cancer engraftment was achieved in the pulmonary cavity of 10 challenged female, Balb/C mice. The presence of CT26-luc cancer cells in mouse lungs was first detected as early as 24-hours post challenge, by Red Firefly Luciferase bioluminescent imaging (BLI). Soon thereafter, CT26-luc cancer cells were also detected by FDG PET. Optical BLI was found to be predictive of FDG PET biodistributions. Additionally, the quantitative tomographic, anatomical details of FDG PET biodistributions were confirmed by PET/µCT/MRI co-registrations.

The ability of optical BLI to provide sensitive, high-throughput, early predictions of FDG PET biodistributions highlighted the value of the novel cradle inlay system tested here. Specifically, this cradle inlay system proved to be a vital hardware component that facilitated the process of acquiring multimodal optical/PET/µCT/MRI imaging data sets that could then be co-registered in PMOD.

CONCLUSIONS:

• *In vivo* Red FireFly BLI of metastatic colorectal cancer cells in a syngeneic mouse model successfully predicted observed *in vivo* FDG PET biodistributions.

• A proof-of-concept cradle inlay system successfully facilitated the process of acquiring diagnostic, multimodal optical/PET/µCT/MRI data sets that were then readily co-registered in PMOD.

#2535 Uncovering bias in AI-based digital pathology analysis.

Erika M. Skaggs[1], Daniel R. Plaugher[1], Sally R. Ellingson[2], Christine F. Brainson[3]

[1]Toxicology and Cancer Biology, University of Kentucky, Lexington, KY,[2]Division of Biomedical Informatics and Markey Cancer Center, University of Kentucky, Lexington, KY,[3]Toxicology and Cancer Biology; Markey Cancer Center, University of Kentucky, Lexington, KY

As the most transformative technology in a generation, Artificial Intelligence (AI) is a tool that brings tremendous promise and significant risks, particularly in regard to healthcare. Our project aims to address two challenges faced by researchers utilizing AI in the analysis of immunohistochemical (IHC) images: the potential for covert bias and the opaque nature of “black box” systems. We have trained an AI-driven model to detect tumor and stromal regions in non-small cell lung cancer (NSCLC) tissues stained with a variety of IHC markers, including the histone methyltransferase EZH2, the histone mark H3K27me3, the antigen presentation components B2M, HLA-DR,DQ,DP, the immune evasion molecular PD-L1 and the enzyme CBS. By measuring stain intensity in each region, we observed that EZH2 and PD-L1 staining are significantly higher in squamous cell carcinoma tumor cells than in adenocarcinoma tumor cells, and that HLA-DR,DQ,DP has an inverse relationship with EZH2. These data implicate a reason why some squamous cell carcinomas fail anti-PD1 immunotherapy. However, to ensure that the system was not creating or perpetuating existing disparities, we next endeavored to assess the algorithm’s accuracy across multiple demographic bounds. Using the Cohen’s Kappa of Inter-Rater Reliability, we were able to determine that our algorithm has near perfect agreement with trained human observers in each of the six stains tested (EZH2, H3K27me3, B2M, HLA-DR,DQ,DP, PD-L1, and CBS). This was true regardless of race (Black vs Caucasian) or geographical origin (Appalachian Kentucky vs Non-Appalachian Kentucky). However, when comparing performance by gender, one category fell below the threshold for near perfect agreement (CBS in males). Next, we sought to explore what factors might drive the AI’s decision-making process by comparing its accuracy in various histologic contexts. We analyzed the size of the nuclei, the number of nuclei per mm^2, the degree of immune infiltration, the width of the stroma, and the degree of randomness in the arrangement of the tumor and stroma. While the size of the nuclei was not found to have a significant impact on accuracy, the other variables did have the potential to “confuse” the AI, which could lead to less reliable results. By tackling these challenges head-on, we hope that our findings will help set a standard for the equitable and transparent application of artificial intelligence in healthcare. Work supported by Markey STRONG Scholars Program through the American Cancer Society IRG-22-152-34 (EMS), AIML Hub pilot (EMS), T32 CA165990 (DRP), R01 CA237643 (CFB), R01 HL170193 (CFB), P30 CA177558 (Markey Shared Resources)

#2536 *In vivo* label-free imaging reveals distinct CD8 T cell metabolic changes during effective radio-immunotherapy in cold murine melanoma versus hot colon carcinoma.

Alexa R. Heaton[1], Anna Hoefges[2], Nathaniel J. Burkard[2], Arika S. Feils[2], Noah W. Tsarovsky[2], Garrett M. Lublin[2], Alina A. Hampton[2], Samantha K. Burkard[2], Aurora D'Amato[2], Mildred A. Felder[2], Anqi Gao[2], Huy Q. Dinh[2], Amy K. Erbe-Gurel[2], Alexander L. Rakhmilevich[2], Paul M. Sondel[2], Melissa C. Skala[1]

[1]Morgridge Institute for Research, Madison, WI,[2]University of Wisconsin, Madison, WI

In vivo multiphoton autofluorescence microscopy provides label-free, single-cell imaging of metabolic changes. These metabolic changes are quantified via the metabolic coenzymes NAD(P)H and FAD which are autofluorescent molecules endogenous to all cells. Metabolic reprogramming is a hallmark of cancer. Immune cells also change their metabolism during cancer progression and this metabolic switch correlates to immune cell phenotype and function. Here, we study metabolic changes during administration of a potent radio-immunotherapy regimen in murine melanoma and colon carcinoma. This therapy includes external beam radiation, hu14.18-IL2 immunocytokine (anti-GD2 mAb fused to IL2) or free IL2, and anti-CTLA-4, leading to *in situ* vaccination and cure of established murine tumors. We created a transgenic mCherry-labeled T cell mouse to study the critical T cell response. We implanted syngeneic B78 (GD2+) melanoma or MC38 (GD2-) colon carcinoma cells into the flanks of mCherry-labeled CD8 T cell reporter mice to induce tumors. Under anesthesia, skin flap surgery was performed, and tumors were imaged at multiple timepoints during therapy. Multiphoton imaging was performed to collect NAD(P)H, FAD, and mCherry signal. Fluorescence lifetime images were collected using time correlated single photon counting electronics. Tissues were also harvested and analyzed via flow cytometry, single-cell RNA sequencing, and multiplex immunofluorescence to corroborate imaging findings and characterize the immune infiltrate. Our *in vivo* label-free imaging revealed that CD8 T cells from treated versus control tumors exhibit different metabolic phenotypes including changes in NAD(P)H and FAD protein binding and redox balance across these two solid tumors. In cold B78 melanoma tumors, *in vivo* metabolic imaging revealed that CD8 T cells are activated early but maintain an oxidative metabolic state consistent with exhaustion or a memory phenotype. In hot MC38 colon carcinoma, *in vivo* metabolic imaging revealed that CD8 T cells are activated early and then quickly transition to a glycolytic state that fuels their cytotoxic function. scRNAseq confirmed the metabolic changes we observed within our B78 model and provided additional insight into CD8 T and tumor cell changes during therapy. Flow cytometry illustrated significant changes in the immune infiltrates of both tumor types over time and treatment. These results show that label-free *in vivo* imaging enables single-cell quantification of metabolic changes during therapy - across multiple solid tumors. Combined with other traditional assays, we can elucidate key immune cell populations and the crucial timepoints during therapy where changes are occurring. This imaging platform may be leveraged to help develop new immunotherapy combinations and study other cancer types where cures have not been realized.

#2537 *In vivo* advanced molecular imaging of the probiotic *L. lactis* expressing fluorescent proteins to monitor its transit through the gastrointestinal tract.

Nestor Rubio-Infante[1], Martin Ramos-Gonzalez[1], James Amos-Landgraf[2], Satyanarayana Rachagani[3], Lacey R. McNally[4], Jorge Gomez-Gutierrez[5]

[1]NextGen Precision Health, University of Missouri - Columbia, Columbia, MO,[2]Department of Veterinary Pathobiology, University of Missouri - Columbia, Columbia, MO,[3]Department of Veterinary Medicine and Surgery, University of Missouri - Columbia, Columbia, MO,[4]Department of Surgery, University of Oklahoma, Oklahoma, OK,[5]Department of Pediatrics, School of Medicine, University of Missouri - Columbia, Columbia, MO

Introduction: The real-time imaging of probiotic bacteria expressing a reporter protein represents an important area for gastrointestinal (GI) research. Fluorescent probiotic bacteria could be useful to evaluate the interaction of probiotics with the gut microbiome, either under normal conditions or dysbiosis. Also, they could be employed as diagnostic tools or in treatments preferentially directed to the harsh hypoxic, and/or acidic microenvironment of digestive diseases such as inflammatory bowel diseases (IBD), adenomatous polyps of the colon or colorectal cancer (CRC).

Methods: Recombinant strains of probiotic bacterium *Lactococcus lactis* expressing different fluorescent proteins were orally administered to C57BL/6 and Balb/c mice, their transit through the GI tract was monitored by *in vivo* advanced molecular imaging and multispectral optoacoustic tomography at different time points after oral gavage inoculation. The *L. lactis*-iRFP intensity signal in live animals was quantified using the software Aura 4.0.7.

Results: We found that the green channel to detect *L. lactis*-GFP results in a strong tissue autofluorescence in both C57BL/6 and Balb/c mice and the signal from *L. lactis*-mCherry could not be detected in neither black nor withe mice because the red wavelength lacks enough deep tissue penetrance to pass through the intestines and abdominal wall. In contrast, *L. lactis*-iRFP bacterium produce a strong, specific and well-localized signal, that can be detected in real-time for up to 24 h after oral gavage administration in Balb/c (white) mice, whereas in C57BL6 (black) mice it could be detected as an unspecific signal which suggests that melanin excess may interfere with the detection of *L. lactis*-iRFP. On the other hand, the pilot optoacoustic tomography imaging revealed that biliverdin, the iRFP substrate generated a strong signal which made it difficult to distinguish between the negative controls and *L. lactis*-iRFP. This result suggests that additional optimization of optoacoustic tomography imaging for iRFP is needed.

Conclusions: These results suggest that *L. lactis*-iRFP in Balb/c mice has the desired features to serve as a fluorescent reporter for *in vivo* imaging that can be used to monitoring the bacterial presence, permanence and interactions with the anatomical structures while transiting through the GI tract. Additionally, optoacoustic tomography imaging requires further optimization and improvement.

#2539 Imaging heterogeneity in pre-clinical models of soft-tissue sarcoma with multi-parametric MRI.

Ellen O'Sullivan, Clementine Lesbats, Paul H. Huang, James P. B. O'Connor, Simon P. Robinson

Radiotherapy and Imaging, The Institute of Cancer Research, London, United Kingdom

Soft tissue sarcomas (STS) are highly aggressive and heterogeneous cancers. There is a clinical need for techniques to image inter- and intratumoral heterogeneity of STS for personalized adaptation of treatment and to improve diagnostic accuracy. Multiparametric MRI (mpMRI) quantitatively maps spatial variations of functional and structural features of tumors *in vivo*. mpMRI data was acquired from mice bearing subcutaneous ICR-LMS-1, SK-UT-1 (both leiomyosarcoma, LMS) or HS-SY-II (synovial sarcoma, SS) xenografts. Biomarkers included quantification of the tumor apparent diffusion coefficient (ADC), native T_1 and T_2 relaxation times, magnetisation transfer ratio (MTR), the transverse relaxation rate R_2*, and the complex shear modulus |G*| using MR elastography to assess tumor stiffness. Pathology correlates were sought from MRI-aligned histological sections. Frequency histograms visualized the distribution of values to assess statistical dispersion. Median ADC and T_2 were significantly higher in the two LMS models compared to the more cellular dense HS-SY-II tumors (table 1). Significantly higher MTR in the HS-SY-II tumors suggests greater macromolecule (e.g protein) content. Tumor R_2*, a proxy for blood vessels, was slowest in the SK-UT-1 cohort, consistent with their low CD31 positivity. All three models exhibited a relatively soft biomechanical phenotype. HS-SY-II tumors displayed unimodal histograms, suggesting homogeneously distributed MRI biomarkers, and had little inter-tumor variation. In contrast, both LMS models exhibited bi- or trimodal distributions and higher interquartile range values, particularly for ADC, indicating pronounced intratumor heterogeneity and marked inter-tumour variation. mpMRI can inform on and assess differences in tissue heterogeneity within STS subtypes. Quantification of tumor ADC may provide a sensitive MRI biomarker to assess differential response of LMS and SS to therapy, which has clear translational potential.

Table 1

	ICR-LMS-1 (n=7)	SK-UT-1 (n=8)	HS-SY-II (n=10)
Tumor volume (mm^3)	374 ± 116	343 ± 26	405 ± 52
ADC ($x10^{-6}mm^3/s$)	1303 ± 139	1083 ± 91	629 ± 30 ***
T_1 (ms)	2549 ± 42	2607 ± 49	2496 ± 33
T_2 (ms)	110 ± 14	96 ± 4.6	73 ± 2 ***
MTR (%)	16.5 ± 6	20 ± 0.46	22 ± 0.41 *
R_2* (s^{-1})	31.7 ± 14.5	26 ± 1.5	43.7 ± 3.4 *
G* (kPa)	3.46 ± 0.45	2.87 ± 1.02	4.06 ± 0.22

#2540 Biodistribution and dosimetry of FAPI-46 in mouse model of colorectal carcinoma with PET and SPECT imaging.

Johanna Rokka, Henrik Vaherto, Susanne Back, **Jussi Rytkonen**

Charles River Laboratories, Inc., Kuopio, Finland

Fibroblast Activation Protein (FAP) is overexpressed in cancer-associated fibroblasts of many epithelial cancers. In nonmalignant conditions FAP is overexpressed in wounds healing, inflammatory and fibrotic tissues, whereas in healthy adult tissue FAP expression is low. Radiolabeled FAP Inhibitors (FAPIs) are used in clinic for imaging and/or radiotherapy. FAPIs are usually conjugated to a DOTA chelating site which allows the use of both diagnostic and therapeutic radionuclides. In this study we investigated the radiochemical properties, pharmacokinetics and dosimetry of FAPI-46 in after Lu-177 or Ga-68 labelling in preclinical mouse model of colorectal carcinoma.

FAPI-46 (non-GMP grade) was radiolabeled with Ga-68 and Lu-177 using previous described synthesis with slight modifications (Loktev et al., J Nucl Med 2019). Radiochemical purity was determined with radio-HPLC (LabLogic). A colon cancer mouse model was created by inoculating $5x10^6$ HCT116 cells in 100 µl 50% matrigel subcutaneously (s.c.) in the interscapular area of NMRI-Foxn1^{nu} male mice. Approximately two weeks post inoculation, animals were randomized into groups based on tumor volume (n = 2-4 / timepoint). Animals were dosed with ^{177}Lu-FAPI-46 or ^{68}Ga-FAPI-46, scanned with SPECT (Mediso) or PET (Sedecal) up to 90 minutes. Simultaneously satellite animals were terminated and sampled for gamma counting (Perkin Elmer) at 30-, 60- and 90-minutes post injection. Image analysis was performed with PMOD software. Dosimetry calculations will be performed with OLINDA 2.0.

^{68}Ga- and ^{177}Lu-FAPI-46 were produced with high radiochemical yield and purity. Nuclear imaging of radiolabeled FAPI-46 with both radiolabels shows fast kinetics and excretion via kidney as shown in literature. The dosimetry calculations are still ongoing. FAPI is suitable for multipurpose imaging and there is a great interest towards this molecule in the fields of imaging and oncology. The same precursor structure can be labeled with more than one radionuclide with high radiochemical yield and purity.

#2541 Imaging mass cytometry detects the true dynamic range of low-abundance biomarker expression in human tumors.

Smriti Kala, Qanber Raza, Jyh Yun Chwee, **Thomas D. Pfister**, Liang Lim, David King, Christina Loh

Standard BioTools, Markham, ON, Canada

Detecting clinically relevant biomarkers in cancer tissues provides key insights into the unique tumor characteristics of patients, allowing for more personalized and effective therapies. Immunohistochemistry (IHC) is the gold-standard technique for biomarker detection and is widely used by pathologists to score low-abundance biomarkers (LABs) in tissues. Limitations related to plexity, quantitation and false signal detection are frequently observed using IHC and day-to-day variability due to multiple steps of signal amplification and pigment mistaken for true signal can misinform pathologists about LAB expression.

Imaging Mass Cytometry™ (IMC™) technology is a multiplexed imaging technique that incorporates quantitative assessment of 40-plus biomarkers simultaneously on the same slide. The Hyperion™ XTi Imaging System, in association with an automated slide loader, permits 24/7 data acquisition and provides biological insights critical for assessment of the tumor microenvironment. We strove to determine whether IMC can be used for pathological evaluation of LABs and provides additional key biological insights for clinical evaluation offered through multiplexed analysis.

We performed a comparison of IHC and IMC technology to detect clinically relevant LABs (PD-1, PD-L1, CTLA-4 and LAG-3) on human tumor tissue microarray and whole tissue samples. For IMC technology, we detected single cells using the Human Immuno-Oncology IMC Panel, which offers cell phenotyping of tumor and immune cell subtypes and their functional states. We stained serial sections of tissues using the same antibody clone and generated IHC and IMC data, which was assessed by a board-certified pathologist. We conducted quantitative image analysis to detect LAB expression on single cells.

Our results demonstrate that while IMC and IHC are similar in detecting LABs, IMC technology can accomplish this without signal amplification, offering an opportunity to evaluate LABs in their true dynamic signal ranges. Quantitative analysis of IHC and IMC data further demonstrated the equivalent performance of both platforms. Multiplexed single-cell analysis using IMC data provided insights about LAB expression on specific immune and tumor cells. While IHC is semi-quantitative and cannot reliably determine the high abundance of a target, IMC technology offers the opportunity of signal quantitation as it displays the complete dynamic range of signal. Clinical assessment of tissues using IMC technology offers an advantage over traditional IHC methods by providing true biological context due to multiplexing capabilities. The ability of IMC to provide high-dimensional spatially resolved data makes it a powerful tool for clinical and translational applications and shows that it is poised to significantly contribute to biomarker discovery and drug development.

For Research Use Only. Not for use in diagnostic procedures.

#2542 Artificial Intelligence(AI)-powered delineation of the prostate cancer surfaceome through subcellular-level expression profiling from immunohistochemistry (IHC) images.

Sukjun Kim, **Jimin Moon**, Sanghoon Song, Hosik Kim, Biagio Brattoli, Jeongun Ryu, Donggeun Yoo, Sergio Pereira, Taebum Lee, Soohyun Hwang, Siraj Ali, Chan-Young Ock

Lunit Inc., Seoul, Korea, Republic of

Background: To assess the expressed surfaceome, we developed an AI-powered analyzer capable of scalably measuring for a given immunostained target cellular and subcellular level expression. We applied this analyzer to assess millions of prostate cancer cells.
Methods: A total of 8,464 prostate cancer and normal IHC images for 74 genes from Human Protein Atlas were analyzed. The 74 genes were the most frequently amplified genes (frequency > 7.8%) in prostate cancer, along with known prostate-specific targets, PSMA (*FOLH1*), *KLK2* and *KLK3*. The AI model, trained on pathologist-annotated histology images from 30+ cancer types and 15+ different IHC stainings, took the IHC images as input to predict cell types and subcellular compartments (nucleus, cytoplasm, and membrane) along with respective intensity scores. Immunostained proteins were evaluated by 1)TCS as the ratio of positive tumor cells to the total number of positive cells, normalized by the proportion of tumor cells, 2) membrane intensity score (MIS), and 3) MBS as the ratio of the membrane intensity score to the sum of intensity scores from all three subcellular compartments.
Results: The IHC analyzer detected and assessed 21.5M immunostained cells including 1.6M prostate cancer cells and 19.9M normal cells in non-prostate samples. The 71 amplified prostate targets had a mean TCS of 0.817 (range 0.181-1.415), a MIS of 0.132 (0.090-0.193), and a MBS of 0.271 (0.221-0.349). The three genes with the highest TCS values were the known therapeutic targets *KLK2* or *KLK3* (KLK2/3, 52.3) and PSMA (33.5), as well as the lesser known *NCALD* (3.97).The lower MBS(x0.63, PSMA: 0.40, KLK2/3: 0.25) and MIS (x0.25, PSMA: 0.08, KLK2/3: 0.02) values suggest that KLK2/3 expression may be less specific to the plasma membrane. Importantly, despite *NCALD* having a lower TCS relative to PSMA, it exhibits higher MIS (0.15) and MBS (0.45) values indicating high proportionate and specific membrane expression.
Conclusion: We developed a pipeline leveraging AI-powered and big-data-driven approaches to assess both the expression and membrane localizations of immunostained targets. We identified *NCALD* as having high membrane specificity which could enable the development of highly selective cell surface targeted therapeutics such as ADCs and bispecifics for prostate cancer.

#2543 Dynamic whole-body PET/CT can predict the treatment response to chemoradiotherapy of esophageal cancer patients.

Yoshihiro Kurata[1], Moe Iwata[1], Koichi Hayano[2], Yasunori Matsumoto[1], Ryota Otsuka[1], Akira Nakano[1], Tadashi Shiraishi[1], Nobufumi Sekino[1], Toyozumi Takeshi[1], Masaya Uesato[1], Gaku Ohira[1], Hiisahiro Matsubara[1]

[1]Department of Frontier Surgery, Chiba Univ. School of Medicine, Chiba, Japan,[2]Chiba Univ. School of Medicine, Chiba, Japan

Background: Majority of positron emission tomography-computed tomography (PET/CT) studies are based on static images. However, dynamic whole-body PET-CT (DW-PET/CT) enables quantification of tissue glucose metabolism dynamics. The purpose of this study is to investigate whether DW-PET/CT can predict the treatment response in esophageal squamous cell carcinoma (ESCC) patients treated with chemoradiotherapy (CRT).
Methods: Fifty ESCC patients who underwent pretreatment DW-PET/CT were evaluated. DW-PET/CT parameters of the main tumor including Patlak slope (PS, absolute rate of tracer consumption) and Patlak intercept (PI, degree of tracer perfusion in the tumor) were calculated using 4 whole-body PET/CT image series acquired at every 5 minutes from 60 minute after injection of 18F-fluorodeoxyglucose. The maximum standardized uptake value (SUVmax) of the tumor was also measured at 60 minute of the tracer injection. The relationships of these PET derived parameters with treatment response was analyzed.
Results: Treatment responders were 34 patients, while non-responders were 16 according to the RECIST criteria. Tumors of responders showed significantly higher SUVmax, PS and PI than non-responders (P=0.03, 0.0003, 0.001, respectively). PS and PI showed a good performance to predict the treatment response with AUC of 0.75 and 0.79, whereas SUVmax showed a poor performance with AUC of 0.69. Tumor contraction rate during CRT showed significant positive correlation with PS and PI (P=0.005, 0.03, respectively).
Conclusions: DW-PET/CT might enable pretreatment prediction for the treatment response to CRT in ESCC patients.

TUMOR BIOLOGY: Aging and Senescence in the Tumor Microenvironment
Poster Session

#2547 The impact of aging on YAP activity in melanoma cells: nuclear localization and condensate formation.

Ethan Black, Danfeng Cai, Ashani Weeraratna

BMB, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD

Melanoma is a highly lethal form of skin cancer which disproportionately impacts older patients, leading to increased rates of melanoma diagnosis and worse prognostic outcomes in this population. This is driven, in part, by higher rates of invasion and therapy resistance caused by age-related changes to the tumor microenvironment. One mechanism this occurs through is the increased senescence of fibroblasts which disrupts extracellular matrix (ECM) deposition, resulting in a more aligned and stiffer matrix. The Hippo pathway is known to shut off in response to matrix stiffness which allows for increased YAP nuclear localization. YAP is a known marker for increased invasion in melanoma. While the importance of YAP in melanoma is known, it is understudied in the context of an aging tumor microenvironment. Additionally, YAP's ability to form transcriptionally active condensates and whether this activity is vital to its function has yet to be elucidated in melanoma. Our lab has previously shown that the increased stiffness of cell-derived matrices (CDMs) created with aged fibroblast cell lines when compared to CDMs from young fibroblasts is sufficient to increase YAP nuclear localization in HUVEC cells. We hypothesize that age-associated increases in ECM stiffness will also increase YAP nuclear localization in melanoma cells, resulting in increased YAP target gene activation, YAP condensate formation, and downstream pro-invasive melanoma cell behavior. To investigate this we employed several techniques, including the creation of CDMs from aged/young fibroblasts, immunofluorescence (IF) staining, and Airyscan super-resolution microscopy. Our data suggests that melanoma cells grown on cell-derived matrices (CDMs) created by aged dermal fibroblasts have increased nuclear YAP localization when compared to cells grown on CDMs created with young dermal fibroblasts. However, the downstream effects of this increased YAP activity on invasion and therapy resistance in melanoma cells as well as whether melanoma cells being plated on aged CDMs increases YAP condensate formation are still being examined. Our IF data from these melanoma cells also suggests that YAP condensates assemble endogenously in melanoma when plated on these CDMs, and the importance of their role in YAP target gene activation warrants further study. As we continue this work, it will allow us to better understand how the aging tumor microenvironment changes YAP activity and the downstream effect this has on the invasive behavior that is characteristic of melanoma in older patients.

#2548 Elucidating the significance of senescent tumor microenvironment in progression of pancreatic adenocarcinoma.

Ankita Semwal[1], Anjali Aggarwal[2], Vinit Sharma[2], Mayank Sharma[2], Rajesh Gupta[3], Ritambhra Nadda[4]

[1]Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India,[2]Anatomy, Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India,[3]GI Surgery, HPB and Liver Transplantation, Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India,[4]Histopathology, Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India

Background: Oncogenes, oxidative stress, DNA damage response are typical stimulators for cellular senescence in tumor microenvironment (TME). The recent literature emphasizes on the dual nature of senescence where its tumor-promoting effects can outweigh the initial tumor-suppressive effects, especially when senescent cells accumulate and are not efficiently cleared. This study aims to see whether senescence in TME plays any role as contributing factor for the progression and relapse in pancreatic adenocarcinoma.
Methodology: In 30 pancreatic adenocarcinoma tissue specimens, senescence was assessed by lysosomal mass quantification, immuno-staining of DNA damage foci, gene expression of cell cycle arrest markers and SASP factors profiling by ELISA. The relevant transcripts of metastasis, stemness and Wnt signaling were evaluated by gene and protein expression. In-vitro Doxorubicin induced senescence model (Panc-1 cells) was created and evaluated for validation of results.
Results: The senescence was markedly elevated in tumor tissue, demonstrated by increased positivity of SA-β-gal, ɣ-H2AX foci, cell cycle arrest markers (p16, p53, p21), pro-inflammatory cytokine (IL-10>0.0547*). The accumulated senescence was found significantly correlated with increased expression of invasiveness markers (Vimentin, and N-cadherin) stemness (Sox2, Oct4, Nanog). The co-localization of ɣ-H2AX and Sox2 as seen in immunofluorescence microscopy concludes the presence of senescence along with stemness. Genes of Wnt signaling cascade (Wnt2<0.0039**; c-Myc< 0.0039**) which are involved in both senescence and stemness were significantly raised in pancreatic adenocarcinoma.
Conclusion: Our findings highlight the critical role of metabolically active senescent cells in remodeling tumor microenvironment through secretory factors and presence of active stemness within. The senescence has positive influence on tumor promoting features like invasion, metastasis and tumor proliferation. Selective elimination of senescence can be a promising therapeutic implication considering the balance between beneficial and harmful effects of senescence in cancer treatment.

#2549 The aged tumor microenvironment limits T cell control of breast cancer.

Jia Meng[1], Denise Goh[1], Kai Soon Ng[1], Xinru Lim[1], Jeffrey Lim[1], Lee Hong Song[2], Jabed Iqbal[2], Darren Wan-Teck Lim[3], Elaine Hsuen Lim[3], Joe Yeong[1]

[1]A*STAR - Inst of Molecular and Cell Biology (IMCB), Singapore, Singapore,[2]Department of Anatomical Pathology, Division of Pathology, Singapore General Hospital, Singapore, Singapore,[3]Division of Medical Oncology, National Cancer Centre Singapore, Singapore, Singapore

Introduction: Breast cancer is the leading cause of cancer-related death among women worldwide and the most commonly diagnosed invasive cancer. Given that the risk of developing cancer increases dramatically with age and is often paralleled with a progressive decline in immune function, breast cancer presents unique challenges for understanding how aging influences tumor development. Amid a globally aging population, the etiology and effect of age-related immune dysfunction in cancer remains unclear.
Methods: To address this, we investigated the major cellular determinants of aging in an orthotopic mouse model using EO771 breast carcinoma cells on young (2-month-old) and old (27-month-old) BALB/c mice separately. 21 days post inoculation, tumors were immuno-profiled with extensive flow cytometry and multiplex immunohistochemistry. Young and aged human breast biopsies were processed to generate tumor-infiltrating lymphocytes, which were subsequently analyzed using single-cell RNA sequencing.
Results: We observed that tumor size increased with mice age, while the frequencies infiltrating CD4 and CD8 T cell decreased. Specifically, CD8 T cell exhaustion is significantly induced in aged breast tumors: quantification by flow cytometry and multiplex immunohistochemistry shows a consistent upward trend in $CD39^{+}$CD8 T, $PD\text{-}1^{+}$CD8 T, and TOX^{+}CD8 T cells in 27-month-old mice. Complemented with a detailed single-cell RNA sequencing analysis of over 5,000 T cells from young and aged human breast biopsies, we verified the presence of exhausted CD8 T cell subsets in the aged tumor microenvironment, characterized by *Pdcd1*, *Havcr2* and *Entpd1* high expression.
Conclusions: Our findings demonstrate that limited priming of $CD8^{+}$T cells in the aged tumor microenvironment within breast cancer, primarily restricts tumor control in aging. Further studies are ongoing to expand the cohort and investigate age-related responses to immunotherapy in immunopathological context with advance single-cell spatial technologies.

#2550 Chemotherapy-induced senescence of cancer-associated fibroblasts promotes gastric cancer progression and resistance to chemotherapy.

In-Hye Ham, Yulim Lee, Hur Hoon

Ajou University School of Medicine, Suwon, Korea, Republic of

Purpose: Chemotherapies for patients with gastric cancers (GCs) has been shown disappointing results in the clinics. The cancer-associated fibroblasts (CAFs) could be considered one cause of the resistance to chemotherapy in GCs. However, the chemotherapy-induced reprogramming of CAFs and its effects on GC progression and the resistance to chemotherapy has not been elucidated.
Methods: IncuCyte and transcriptome were used to identify chemotherapy-induced senescence of CAFs. Screening for drugs to inhibit senescence of CAFs was performed. The cell proliferation and migration assays and phospho-protein array were performed to investigate the effects of senescent CAFs on GC cells. Inhibitory effects of screened drugs on CAFs were also evaluated in experimental models. Senescent CAFs and their clinical meanings were also investigated in the human GC tissues undergoing preoperative chemotherapies.
Results: Chemotherapy could alleviate proliferation and widen cytoplasm of CAFs. Some secretory molecules like *TNF-α* were upregulated in the CAFs with chemotherapy. Chemotherapy-treated CAFs increased the resistance to chemotherapy and the migratory potentials of GC cells. The COX2 inhibitor, one of the screened drugs, has inhibitory effect on chemotherapy-induced senescence CAFs. Consequently, it could suppress GC progression and resistance to chemotherapy. The GC patients with senescent CAFs were significantly correlated with poor histologic response to chemotherapy.
Conclusions: In this study, we are convinced that the chemotherapy-induced senescence of CAFs could have a significant effect on progression and resistance to chemotherapy in GC. Inhibitory drugs for chemotherapy-induced senescence of CAFs could be used to overcome the CAF-induced resistance to the chemotherapy in GCs.

#2551 Aged microenvironments promote liver metastasis in pancreatic ductal adenocarcinoma.

Emma Kartalia, James M. Leatherman, Jae W. Lee, Sara E. Young, Elizabeth M. Jaffee, Won Jin Ho, **Daniel J. Zabransky**

Johns Hopkins University, Baltimore, MD

Advancing age is the strongest independent risk factor for developing pancreatic ductal adenocarcinoma (PDAC). Older patients are more likely to present with advanced stage disease and have inferior overall survival when liver metastases are present. There is a critical need to understand the molecular factors that promote PDAC metastasis in order to guide development of more effective therapies. Despite the strong association of PDAC incidence and liver metastasis with aging, mechanisms through which aging impacts this process are not understood.

Our prior work identified age-related changes in the PDAC tumor microenvironment (TME) that promote orthotopic PDAC tumor growth in aged (>12-month-old) compared to young (8-week-old) mice. GDF-15, a divergent TGFβ superfamily member, is a critical factor released by aged fibroblasts in the TME that promotes PDAC growth in an age-dependent fashion. We have recently identified that liver metastases are significantly more frequent in aged hosts, consistent with clinical observations.

To determine how aging promotes PDAC metastases, we performed studies using novel human and mouse models that incorporate age as a variable. Primary tumors from aged mice have increased angiogenesis and decreased CD8+ T cell infiltration, findings associated with metastasis. To determine if GDF-15, released at high levels by aged but not young pancreatic fibroblasts, is involved in priming tumors for metastasis, we performed recombinant GDF-15 treatment of young mice harboring orthotopic KPC tumors. We found that GDF-15 significantly increases angiogenesis and decreases CD8+ T cell infiltration, changes associated with increased likelihood of metastasis. Tumors in aged GDF-15 knock out mice give rise to fewer metastases and have decreased angiogenesis and CD8+ T cell infiltration compared to aged wild type mice. Treatment of both human and mouse pancreatic cancer cells *in vitro* with aged fibroblast conditioned media or recombinant GDF-15 results in increased migration and invasion driven by GDF-15 specific activation of AKT signaling relative to controls.

We next tested if the aged liver, independently of effects in a primary tumor, contributes to metastasis. Portal vein injection of KPC and KPCY clones into aged mice resulted in increased and larger metastases compared to young mice. Using imaging mass cytometry, we identified that liver metastases in aged mice were immune excluded with low CD8+ T cell infiltration and increased spatial interactions between immune cells and fibroblast populations with immunosuppressive phenotypes.

Together, these data show that aging alters the primary tumor and metastatic liver microenvironments to promote more efficient metastatic spread of PDAC cells. We will present this and other ongoing work targeting the aging-driven GDF-15 signaling for the treatment and prevention of PDAC metastasis.

#2552 *TET2*-mutant clonal hematopoiesis enrichment in the tumor microenvironment is an actionable driver of treatment resistance in solid tumors: Thyroid cancer as a paradigm.

P. Sanchez Vela[1], V. Tiedje[1], J. L. Yang[1], B. R. Untch[1], L. Boucai[1], A. J. Stonestrom[1], A. Bueno Costa[2], S. Franch Exposito[1], S. Franch Exposito[1], A. Srivastava[3], M. Kerpelev[1], J. Greenberg[1], M. Wereski[1], A. Kulick[1], K. Chen[1], T. Qin[1], S.-Y. Im[1], A. R. Martinez Benitez[1], R. Pluvinet[2], M. Sahin[1], K. Menghrajani[1], G. P. Krishnamoorthy[1], E. de Stanchina[1], A. Zehir[1], R. Satija[4], J. Knauf[1], R. L. Bowman[1], M. Esteller[2], S. Devlin[1], M. F. Berger[1], R. P. Koche[1], R. L. Levine[1], J. A. Fagin[1];

[1]Memorial Sloan Kettering Cancer Center, New York, NY, [2]Josep Carreras Leukaemia Research Institute, Barcelona, Spain, [3]The Wistar Institute, Philadelphia, PA, [4]New York University, New York, NY

Recurrent mosaic somatic mutations in circulating leukocytes can be frequently found in the aging population. These mutations frequently arise in epigenetic modifier genes like *TET2.* In the absence of signs of hematologic malignancies this condition is termed clonal hematopoiesis (CH). Although CH has been associated with increased incidence and adverse outcomes in patients with solid tumors, the disease-specific effects and mechanisms by which CH alters solid tumor biology have not been delineated. To develop insights into the interplay between mutant CH clones and epithelial tumor cells we analyzed a cohort of over forty-seven thousand patients who underwent paired blood and tumor sequencing. After correcting for age, sex, ancestry, stage, smoking, and previous treatment history, we identify unique patterns of poor survival associated with specific CH genotypes and solid tumor histologies. Given that the detection of tumor-infiltrating *TET2*-CH clones is especially associated with poorer outcomes in thyroid cancer patients (HR 2.18, 95%CI 1.18-4.05, p=0.013), we focused on studying the role of CH in thyroid cancer biology. Compared to other CH alleles, *TET2*-mutant CH is enriched in the tumor microenvironment (TME) across solid tumors and is associated with adverse prognosis specifically in Anaplastic thyroid cancer (ATC) patients. ATC is a clinically aggressive malignancy with a dismal prognosis. Combined BRAF/MEK inhibition offers significant therapeutic benefit in patients with $BRAF^{V600E}$-mutant ATCs. However, relapses are common and overall survival remains poor. A hallmark of ATC is significant infiltration with myeloid cells, particularly macrophages. ATCs are most common in the aging population, which also has an increased incidence of *TET2*-mutant CH. These mutant macrophages have been shown to accelerate CH-associated pathophysiology including atherosclerosis. However, the clinical and mechanistic contribution of *TET2*-mutant clones to the prognosis and/or treatment response in solid tumors has not been elucidated. Using subclonal murine models of *Tet2*-mutant CH we confirm that mutant macrophages selectively infiltrate mouse $Braf^{V600E}$-mutant ATC models (58% higher enrichment of $Tet2^{-/-}$ cells in the TME compared to the WT counterparts) and confer resistance to BRAF/MEK inhibition (68-days median survival compared to no mortality after 147 days). Using single-cell CITEseq, we identify that the overexpression of Tgfβ-family ligands by CH macrophages is the driver of this resistance. Importantly, inhibition of Tgfβ signaling or the depletion of mutant-macrophages completely restored the sensitivity to MAPK pathway inhibition. In summary, this work identified a novel actionable resistance mechanism mediated by a clonally driven process within the tumor-immune microenvironment.

#2553 Hypoxia-associated lncRNA-induced glycolysis and lactylation-driven senescence-associated secretory phenotype in cancer-associated fibroblasts enhances peritoneal dissemination.

J. Zhang;
Liaoning Cancer Hospital & Institute, Shenyang, China

Peritoneal Dissemination is the main site of diffuse gastric cancer (DGC) distant metastasis and indicates an extremely poor prognosis and survival. Our previous research revealed that HIF-1a can initiate the expression of LINC01355, promoting the progression of DGC. Here, in DGC orthotopic transplantation model, we observed that overexpression of LINC01355 could accelerate peritoneal dissemination. We found that LINC01355 could directly bind to Pyruvate Kinase M1/2 (PKM), inhibiting its degradation, thereby facilitating glycolysis in GC and creating a microenvironment with accumulated lactate. Single-cell analysis reveals the presence of a hypoxia-associated subtype of cancer-associated fibroblasts (hypoxiaCAF) within TME of DGC. This distinct subtype is independent of myoCAF, iCAF, and apCAF, representing a novel CAF population. HypoxiaCAFs exhibit a slow proliferation rate and display a senescence-associated secretory phenotype (SASP). Mass spectrometry and related experimental data suggest that lactate accumulation in the TME downregulates the specific histone lysine delactylase HDAC3, promoting histone H4 lysine 12 lactylation (H4K12la). H4K12la is enriched in the promoters of SASP-related genes, driving SASP transcription and exacerbating the senescence of CAFs. Inhibition of HDAC3 blocks the increased viability of DGC cells induced by senescent CAFs and peritoneal tumor formation. Single-cell mass cytometry reveals the presence of fibroblasts in the ascites of patients with peritoneally disseminated DGC, with fibroblast populations displaying high levels of p16 expression and SASP factors. These findings provide insights into the role of histone lactylation in sustaining inflammation-related SASP and the involvement of senescent CAFs in DGC peritoneal dissemination.

#2554 Caloric restriction ameliorates aging and tumor progression via tumor immunity in syngeneic mice model.

T. Horino, H. Horiguchi, Y. Miyamoto, M. Iwatsuki, Y. Oike;
Kumamoto University, Kumamoto City, Japan

Background: Caloric restriction (CR) without malnutrition reportedly improves life span and ameliorates aging in preclinical models. Decreased expressions of aging-related genes and inflammation-related genes have been observed in CR mice. Moreover, CR decreases cancer risk and inhibits tumor progression, but the underlying mechanisms are not fully understood.
Methods: In this study, we developed a syngeneic model using C57BL/6N male mice, and verified the tumor-suppressing mechanisms of CR. Mice were randomized into 2 groups: normal diet (ND) group fed with ad lib diet, and CR group with 30% reduced feeding compared with the ND group, started from 2 months age. MC38, murine colorectal adenocarcinoma cells, were implanted subcutaneously. We compared tumor growth and survival between the CR group and the ND group. Moreover, to verify the contribution of tumor immunity, we compared the tumor-suppressing effects of anti-PD-1 antibody between the CR group and the ND group. To clarify the impacts of CD8+ cytotoxic T cells, we also conducted CD8 depletion by anti-CD8 antibody.
Results: Firstly, we observed significantly restricted tumor progression of the CR group in aged mice, but it was relatively modest in young mice. Secondly, the tumor-suppressing effect of anti-PD-1 was equivalent between the CR group and the ND group in aged mice, which suggests tumor immunity is essential to demonstrate anti-tumor effects in CR mice. However, CD8 depletion in CR mice showed only partial tumor-promoting effects, which suggests immune function excluding CD8+ T cells contributes to suppressing tumor progression in the CR group.
Conclusion: Our findings suggest that CR ameliorates aging and tumor progression. Promoted immune function, especially immune cells excluding CD8+ T cells, might play a role in the tumor-suppressing effects of CR. CD4 depletion and comprehensive analyses of intratumoral T cells are currently underway to elucidate the underlying mechanisms of tumor suppression induced by CR.

#2555 Multimodal atlas of cellular senescence in human bone marrow reveals insights into aging and multiple myeloma.

Wen-hung Chou[1], Julia T. Wang[1], Xiang Li[1], Kapur B. Dhami[1], Daniel Rapp[2], Andre Luiz N. Targino da Costa[1], Andrew Houston[1], Xiyi Wei[1], Yuting Zhao[1], Maede Shahin[1], Yizhe Song[1], Xiangwei Fang[1], Julie Fortier[1], Yuwei Zhang[1], Matthew Wyczalkowski[1], Reyka G. Jayasinghe[1], Kelsey Gallant[1], Michael Slade[1], Karolyn A. Oetjen[1], Kiran Vij[1], Daniel C. Link[1], Ryan Nunley[3], Ravi Vij[1], Feng Chen[4], Ryan C. Fields[2], Li Ding[1]

[1]Department of Medicine, Division of Oncology, Washington University School of Medicine in St. Louis, St. Louis, MO,[2]Department of Surgery, Surgical Oncology, Washington University School of Medicine in St. Louis, St. Louis, MO,[3]Department of Orthopedic Surgery, Washington University School of Medicine in St. Louis, St. Louis, MO,[4]Department of Medicine, Division of Nephrology, Washington University School of Medicine in St. Louis, St. Louis, MO

Hematologic malignancies, such as multiple myeloma, exhibit a markedly increased incidence with age and are profoundly influenced by the bone marrow microenvironment. The aged microenvironment can lead to the accumulation of senescent cells, characterized by permanent cell cycle arrest and a pro-inflammatory secretory phenotype. Despite its significance in age-related diseases, the role of senescent cells in the bone marrow microenvironment and their impact on aging and hematologic malignancies remain poorly understood.As part of the Cellular Senescence Network (SenNet), we created a multimodal atlas to characterize senescent cells in the human bone marrow using single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics. Analysis of 55 scRNA-seq samples from healthy donors (aged 25-84) revealed that mesenchymal cells exhibited the strongest senescence signatures, which were elevated in aged individuals. While monocytes also displayed senescence signatures, they did not show an age-associated increase. Hematopoietic stem cell (HSC) subpopulations showed senescent profiles, suggesting that senescence spans multiple cell types in the bone marrow.Using the Xenium spatial transcriptomics platform, we profiled the bone marrow microenvironment in 17 samples from 15 healthy donors across a similar age range. We identified 32 distinct cell types and uncovered putative senescent subpopulations in plasma cells and vascular smooth muscle cells. Our analysis revealed 9 unique cellular neighborhoods, some anchored around established niches like the endosteum and arteriole, while others suggested novel niche candidates such as adipocytes, early myeloid progenitors, and megakaryocytes. Aging was associated with significant microenvironment remodeling, including increased mature myeloid cell types and lymphoid aggregates, along with a decline in lymphoid and progenitor populations.In 22 multiple myeloma samples from 15 untreated and 4 relapsed patients, we found significant microenvironment changes, such as the formation of plasma cell-rich pockets. These neighborhoods were enriched with monocytes and CD8 T cells, the latter showing elevated expression of exhaustion markers. In agreement with our neighborhood analysis, monocytes and CD8 T cells were found in closer proximity to plasma cells in samples with high tumor burden. Mesenchymal stem cells near plasma cells also showed increased senescence signatures compared to age-matched controls.Overall, our findings illuminate the senescent landscape of the bone marrow and provide a resource for understanding its role in aging and multiple myeloma.

#2556 Therapy-induced senescent cancer cells facilitate immune evasion in cancer by promoting PD-L1 upregulation through a ribophorin 1-dependent pathway.

H. Hwang[1], D. Kang[1], **J. Shin**[1], J. Jung[1], S. Ko[1], K. Jung[1], S.-S. Hong[1], J. Park[2], M. Oh[2], H. An[2], W.-H. Yang[3], Y.-G. Ko[4], M. Choi[1], J.-H. Cha[1], J.-S. Lee[1];

[1]Inha University, Incheon, Korea, Republic of, [2]Chungnam National University, Daejeon, Korea, Republic of, [3]China Medical University, Taiwan, Taiwan, [4]Korea University, Seoul, Korea, Republic of

Cancer senescence induced by conventional chemotherapy and radiotherapy, characterized by limited proliferation, drug resistance, and a senescence-associated secretory phenotype, is increasingly recognized as a contributor to cancer relapse and the development of an immunosuppressive tumor microenvironment. However, the relationship between cancer senescence and anti-tumor immunity remains poorly understood. In this study, we demonstrate that senescent cancer cells upregulate PD-L1 expression by enhancing its transcription and glycosylation. We identify ribophorin 1 as a key regulator of PD-L1 glycosylation during cancer senescence. Ribophorin 1 depletion significantly reduces PD-L1 levels through the ER-lysosome-associated degradation pathway, rendering senescent cancer cells more susceptible to T-cell-mediated killing. Furthermore, ribophorin 1 depletion suppresses tumor growth by decreasing PD-L1 levels and enhancing cytotoxic T lymphocyte (CTL) activity. Additionally, targeting ribophorin 1 or using anti-PD-1 therapy effectively reduces the number of senescent cancer cells in irradiated tumors and suppresses cancer recurrence by activating CTLs. These findings clarify how senescent cancer cells evade T-cell immunity following treatment, contributing to cancer recurrence. Our results highlight the therapeutic potential of targeting senescent cancer cells as a strategy in cancer treatment.

#2557 Age of host and its impact on progression and treatment response in tumors derived from early or late onset Hispanic colorectal cancer patients.

Lesly Torres-Gonzalez[1], Shivani Soni[1], Goar Symbatyan[1], Jae Ho Lo[1], Pooja Mittal[1], Unnati Hemant Shah[1], Francesca Battaglin[1], Karam Ashouri[1], Sandra Algaze[1], Priya Jayachandran[1], Wu Zhang[1], David W. Craig[2], Julie Culver[1], Mariana C. Stren[3], John D. Carpten[2], Bodour Salhia[1], Heinz-Josef Lenz[1]

[1]Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, CA,[2]Beckman Research Institute, City of Hope, Duarte, CA,[3]Department of Population and Public Health Sciences, Keck School of Medicine, University of Southern California, Los Angeles, CA

Background: Colorectal cancer (CRC) is one of the most commonly diagnosed cancers and causes of cancer-related death in the Hispanic/Latino (H&L) population. Rates of early onset (<50, EO) CRC have increased more rapidly in H&L than other ethnic and racial groups. EO CRCs are often diagnosed at advanced stages, suggesting a more aggressive tumor biology. Investigations on the effect of age within both the tumor and host environments could provide insight into key differences between EO and late onset (>50, LO) CRCs. Our study (Cancer Moonshot Program), explores how both age of the host and tumor intrinsic factors influence growth, progression, and treatment response.

Methods: CRC patient derived xenograft (PDX) models were selected from our biorepository based on race and age. Tumor samples from an EO 36 year old (young patient, YP) and a LO 66 year old (old patient, OP) H&L male were expanded using 2 month old NSG mice. Following expansion, each tumor was then implanted into both young (2 months, YM) and old male NSG mice (13-14 months, OM) to establish four PDX models: YP in YM, OP in YM, YP in OM, and OP in OM. Each model was treated with oxaliplatin (6mg/kg), 5-fluorouracil (5-FU, 50mg/kg), or vehicle (control) twice a week for four weeks and monitored for survival. Tumor volume was measured twice per week until an endpoint of 15mm at any diameter was reached. Tumor tissue and blood samples were collected and used for metabolomic and epigenomic analysis.

Results: Initial patient tumor expansion showed differing tumor growth rates between YP and OP tumors, with each being ready for mouse implantation at 63 and 90 days respectively. In all models, control mice had significantly higher tumor growth rates compared to treatment groups ($p<0.01$), except in the 5-FU treated OP in OM. Survival analysis showed significantly increased survival in treatment groups for YP in YM and YP in OM ($p<0.05$). Within each treatment group, YP in YM showed significantly increased tumor growth when compared to other models ($p<0.01$). Additionally, OP in YM also showed significantly reduced growth in the 5-FU treatment group compared to other groups ($p<0.05$).

Conclusion: Our current findings showed that all patient tumor-mouse host age combinations showed significant tumor growth reduction when treated with oxaliplatin or 5-FU, except in 5-FU treated OP in OM. The YP in YM model displays significantly more aggressive tumor growth regardless of the treatment given. However, comparison of treatments to control in YP in YM show significant tumor growth reduction and increased survival, suggesting a more effective response to treatments. The similarity in growth rates of the three other models, regardless of treatment, may suggest that the aggressive characteristics of YP in YM are influenced by both the host and tumor. Further studies are warranted to understand the modifications that influence the observed differences.

#2558 Ferroptosis modulates melanoma progression in an age-specific manner through changes in the tumor microenvironment.

Murilo Ramos Rocha[1], Yash Chhabra[2], Alexis E. Carey[1], Cheyenne Palm[1], Kevin Zhang[3], Edwin Kumah[1], Vania Wang[1], Laura Huser[1], Elizabeth I. Harper[1], Fan Huang[1], Ashani Weeraratna[1]

[1]Biochemistry and Molecular Biology, Johns Hopkins Bloomberg Sch. of Public Health, Baltimore, MD,[2]Fox Chase Cancer Center, Philadelphia, PA,[3]Department of Pathology, Johns Hopkins University, Baltimore, MD

Aging is an independent prognostic factor for melanoma, the most aggressive form of skin cancer. Although immune therapy has shown a dramatic improvement in melanoma treatment, resistance to it still occurs in some patients. Higher oxidative damage in tumor cells and lipid and iron accumulation are singular characteristics of the aged tumor microenvironment (TME). Here, we investigate the impact of these characteristics of the aged TME in promoting ferroptosis, a caspase-independent iron-mediated type of cell death, and as a possible therapeutic opportunity for older patients. Lipid peroxidation levels, resistance to ferroptosis inducers, and the impact of the aged TME secretome were assessed through in vitro and in vivo modeling. Initially, correlation analysis between transcriptomic data (TCGA-SKCM) and ferroptosis sensitivity (CTD^2) was performed to identify common denominators among transcripts associated with the aged TME and ferroptosis pathway. Phenotype-switching markers, such as AXL and Wnt5a, showed a strong correlation with several ferroptosis-related transcripts and an inverse correlation with ferroptosis resistance. Using a panel of melanoma cell lines with distinct Wnt5a levels, we confirmed this association and observed that three critical regulators of lipid peroxidation are downregulated in Wnt5a high lines: GPX4, AIFM2, and GCH1. The comparison through IHC of young and aged mouse skin samples highlighted a pro-ferroptosis signature increasing with age. Senescence markers such as p16 and p21 could be modulated in dermal fibroblasts through induction or inhibition of lipid peroxidation. When associating conditioned media (CM) derived from these dermal fibroblasts (capable of inducing phenotype switching), RSL-3, a GPX4 inhibitor, induced a higher lipid peroxidation and cell death in melanoma cells exposed to aged CM. To understand how ferroptosis impacts tumor growth in young and aged mice, we used a syngeneic tumor model injecting Yumm1.7 cells intra-dermally and the treatment with a lipid peroxidation inducer (imidazole ketone erastin, IKE) or an inhibitor (Liproxstatin-1). Tumors from aged mice showed stronger staining with 4-HNE antibody when compared to young, correlating with AXL and Wnt5a staining. Interestingly, both IKE and Liproxstatin-1 lead to age-specific reduced tumor growth, followed by changes in immune cell populations such as monocytes, macrophages and NK cells. We are currently exploring how these treatments impacted metastatic dissemination, stromal architecture and sensitivity in BRAF/MEK-resistant tumors. These results indicate that ferroptosis modulation is a promising avenue for combined treatment with existing clinical strategies.

#2559 The impact of aging on the brain microenvironment and melanoma brain metastasis: Vasculature dysfunction, inflammation, and tumor colonization.

Cheyenne M. Palm[1], Alexis Carey[2], Murilo Ramos Rocha[1], Kevin Zhang[1], Ashani Weeraratna[1]

[1]Biochemistry and Molecular Biology, Johns Hopkins Bloomberg Sch. of Public Health, Baltimore, MD,[2]Cellular and Molecular Biology, Johns Hopkins School of Medicine, Baltimore, MD

Melanoma is the third most common source of brain metastases after lung and breast cancer; however, melanoma has the highest risk of spreading to the central nervous system (CNS). Previous studies have shown that age is an independent negative prognostic factor for early-stage and metastatic melanoma. Our lab has shown differences in the skin, lung, and pancreatic aging microenvironment that affect older patients' prognosis and response to therapy and emphasizes the need to uncover other molecular mechanisms of cancer progression in older patients. Currently, the role of the aged brain microenvironment in the development of melanoma brain metastases (MBM) is poorly understood due to limited data available. We hypothesize that changes in the aged brain microenvironment create a more tumor-permissive environment for melanoma metastasis to the brain. Previous studies have shown that the native brain environment, particularly glial cells such as astrocytes and microglia, can directly interact with melanoma in both a pro- and anti-tumorigenic manner. Additionally, melanoma cells have been shown to disrupt the blood-brain barrier (BBB) during migration; however, whether the aging microenvironment makes the BBB more susceptible to this disruption is not clearly understood. To understand these factors, we employed various techniques, including in vitro primary culture of brain cells from young and aged mice, ex vivo brain co-cultures with melanoma, and in vivo mouse models to characterize the aging brain microenvironment. We have shown that the young and aged brain endothelium impacts melanoma migration and have observed differences in vascular integrity and vasculature changes with aging and tumor presence. Additionally, aged mice that are more prone to vasculature dysfunction have a higher rate of melanoma colonization in the brain and higher activation of pro-inflammatory factors from the native brain microenvironment. These results give insight into differences in melanoma migration and colonization in the brain between aged and young mice. However, whether this results from astrocyte involvement in melanoma recruitment or disruption of the blood-brain barrier remains to be elucidated. The knowledge from this study will be pivotal to shaping future work by analyzing molecular changes in young and aged patients with malignant melanoma. It may provide new therapeutic targets for effectively preventing and treating this disease.

#2560 Age-dependent differences in breast tumor microenvironment: Challenges and opportunities for efficacy studies in preclinical models.

Paolo Falvo[1], Stephan Gruener[2], Stefania Orecchioni[1], Giovanna Talarico[1], Giulia Mitola[1], Giulia Bravetti[1], Federica Pisati[3], Juliane Winkler[2], Iros Barozzi[2], **Francesco Bertolini**[1]

[1]IEO - European Institute of Oncology, Milan, Italy,[2]Medical University of Vienna, Vienna, Austria,[3]Cogentech, Milan, Italy

Immunity declines with age, leading to functional deficits and an increased incidence of cancer in the elderly. Despite this, most cancer models utilize young (6-8 week old) mice, which are not representative of adult cancer patients. Triple negative breast cancer (TNBC) typically occurs around 30-50 years of age, which can be approximated to 12 months in mice. We previously demonstrated that a Triple-Therapy (TT) including APC activation via vinorelbine and the generation of TCF1+ stem-cell-like T cells (scTs) by cyclophosphamide (CTX), significantly enhances anti-PD-1 efficacy in TNBC and lymphoma models resistant to anti-PD1 treatment by promoting T-cell-mediated tumor abrogation. In this study, we examine TT effects on TNBC growth and the tumor microenvironment (TME) in young (6-8 weeks) vs adult (12 months) mice. We investigated two models, one (4T1) with predominantly lymphoid TME and the other (EMT6) with a predominant myeloid TME. Regular-dose-TT efficacy was similar across age groups, as - notably - CD8+ scTs were only slightly reduced in adults. At variance, low-dose TT and/or metronomic CTX were significantly less effective in adult mice. This might explain why encouraging preclinical results of metronomic CTX where sometimes difficult to replicate with adult cancer patients. Single-cell analyses revealed substantial differences in the TME: adults exhibited fewer CD4+ scTs, naïve B cells, and NK cells, but more memory B cells. Cancer-associated fibroblasts (CAFs) with an extracellular matrix (ECM) deposition signature (Matrix-CAFs) were more prevalent in young mice, whereas pro-inflammatory stromal populations and myofibroblasts were more frequent in adults. In adult mice, Matrix-CAFs displayed reduced ECM-remodeling capacity, decreased collagen deposition, and distinct interaction patterns with other cells in the TME. To gain further insight, non-epithelial cells from the mammary gland and scRNA data of Tabula Muris Senis were contextualised with the TME age-related signature. Age-related transcriptional differences observed in the TME already show an age-related trend in the stroma of the normal mammary gland, including a reduction of T cells, and an increase in the ECM remodeling ability of fibroblasts. Nevertheless, the extent of these differences is larger in the TME as compared to normal ageing breast, suggesting the tumor cells can leverage and exacerbate these differences, potentially to the tumor’s advantage. These findings underscore the importance of considering age-dependent TME variations when designing preclinical studies.

#2561 Immune mediated expansion of p53 mutant clonal hematopoiesis following carboplatin treatment.

Priyanka Khanna, Dexin Yang, Muharrem Muftuoglu, Lauren Ostermann, Shayaun Khazaei, Ran Zhao, Mahesh Basyal, Michael Andreeff, Mihai Gagea, Ruoyan Li, Koichi Takahashi, **Rasoul Pourebrahim**

UT MD Anderson Cancer Center, Houston, TX

Therapy-related myeloid neoplasms (t-MNs) are devastating complications arising from cancer chemotherapy, particularly affecting patients treated with agents like alkylating agents and platinum compounds. *TP53* mutations are the most frequent genetic alterations in t-MNs, present in 25-40% of cases. While the selective expansion of p53-mutant HSCs under chemotherapeutic pressure, known as clonal hematopoiesis (CH), has been implicated in t-MN development, the transition from clonal expansion to malignant transformation remains poorly understood.

To elucidate the mechanisms driving the transformation of p53 mutant HSCs following chemotherapy, we established a mouse model of p53-mutated CH. Using a chimeric bone marrow transplantation approach, we co-transplanted GFP-labeled *Trp53*^{R172H} mutant BM cells and RFP-labeled wild-type BM cells into irradiated recipients. Six weeks post-transplantation, mice were treated with weekly intraperitoneal injections of carboplatin or no treatment for four weeks. Flow cytometry revealed a significant expansion of p53 mutant cells in the carboplatin-treated group compared to both baseline and controls. All carboplatin-treated mice developed t-MNs within four months, while none of the control group did. Bone marrow analysis using CyTOF identified a distinct population of proliferative erythroid progenitors expressing c-Kit, Ki-67, mutant p53, and CD71 exclusively in the carboplatin group. Whole exome sequencing of sorted GFP-positive p53 mutant cells revealed recurrent deletions in chromosomal regions 1q, 4q, and chromosome 10. Frequent mutations in RAS pathway genes were also identified, with *Ptpn11* mutations in six out of eight mice and *Kras* and *Nf1* mutations in the remaining two. Single-cell RNA sequencing of GFP+c-Kit+ cells from t-MN mice uncovered proliferative progenitor clusters with upregulated DNA damage response and repair pathways. Importantly, a recurrent deletion on chromosome 4 included genes encoding the interferon alpha (Ifn-α) family was identified. In NSG mice lacking functional immune cells, p53 mutant clones did not expand or progress to AML following carboplatin, suggesting a non-cell-autonomous immune role in clonal expansion.

Our findings reveal a critical role of immune-mediated mechanisms, particularly through IFN-α signaling, in driving the expansion and transformation of p53 mutant HSCs. Modulating immune interactions may offer a strategy to prevent t-MNs. Future studies should explore combining chemotherapy with immunosuppressive therapies to mitigate the risk of t-MNs and improve patient outcomes.

#2562 Cell populations in human breast cancers are molecularly and biologically distinct with age.

Adrienne Parsons[1], Esther Sauras Colon[2], Milos Spasic[1], Busem Binboga Kurt[3], Beyza Koca[4], Meghana Manjunath[1], Dimitry Lineker[1], Ashka Patel[4], Craig Snow[4], Alexander Swarbrick[5], Rachel Freedman[4], Elizabeth Mittendorf[4], Peter van Galen[1], Sandra McAllister[1]

[1]Hematology, Brigham and Women's Hospital, Boston, MA,[2]Universitat Rovira i Virgili, Tortosa, Tarragona, Spain,[3]Massachusetts General Hospital, Boston, MA,[4]Dana-Farber Cancer Institute, Boston, MA,[5]Garvan Institute of Medical Research, Darlinghurst, Australia

Aging is associated with increased breast cancer risk and outcomes are worse for the oldest and youngest patients, yet it is unknown how cells in the breast tumor microenvironment (TME) are impacted by age or how age contributes to disease pathology. Here, we discover age-associated differences in cell states and interactions in human estrogen receptor-positive (ER+) and triple-negative breast cancers (TNBC) using computational analyses of existing bulk and single-cell gene expression data.

We developed the age-specific program enrichment (ASPEN) pipeline, revealing age associations by correlating gene expression and pathway enrichment to age using single cell RNA sequencing (scRNAseq) data. Using the scRNAseq and spatially resolved breast cancer atlas (Wu et al., *Nature Genetics*, 2021), we identified cell-specific, age-related transcriptional programs in TNBC and ER+ breast cancer. ASPEN revealed increased tumor cell epithelial-mesenchymal transition (EMT), cancer-associated fibroblast (CAF) inflammatory responses, and T cell stress responses with age in TNBC. ER+ breast cancer is dominated by increased luminal cell activity and reduced immune cell metabolism with age.

Analysis of differential gene expression between younger and older patients followed by GSEA using the METABRIC bulk gene expression dataset (n = 63 TNBC >65 years, n= 50 TNBC <45 years, n = 386 ER+ >65 years, n = 86 ER+ <45 years) confirmed enrichment of immune pathways (e.g., antigen processing and presentation, immune response) with increased age in TNBC, and pathways related to oncogenic signaling (e.g., TP53 and RB1 targets) and breast biology (e.g., mammary stem cell, luminal v basal) enriched in younger donors in ER+ breast cancer.

Cell interactome analysis revealed candidate signaling pathways that could drive these cell states. Expression of the immune-inhibitory protein, Galectin-9 (*LGALS9),* was observed in both age cohorts in TNBC, yet interactions with the receptor TIM-3 (*HAVCR2*) were elevated in the younger cohort and those with the receptor prolyl 4-hydroxylase beta (*P4HB*) were exclusive to the older cohort. Interactions between the EMT-inducing factor, *PLAU*, expressed by myCAFs and monocyte/macrophages, and its receptor, *PLAUR,* on cancer cells, were increased with age in TNBC, supporting the finding of increased EMT signatures with age. In ER+ breast cancer, adhesion proteins and extracellular matrix (ECM) engagement comprised most signaling interactions in both age cohorts. Nearly all adhesion/ECM signaling involving either cancer luminal A cells, differentiated perivascular-like cells, or macrophages were enriched in the older cohort; those involving iCAFs, myCAFs, and ACKR1+ endothelial cells were enriched in the younger cohort.

This work reveals key biological differences with age at cell type resolution and lays a foundation for discovery of new, age-adapted therapeutic interventions for breast cancer.

TUMOR BIOLOGY: CAFs and Beyond: A Central Role for the Tumor Microenvironment in Cancer
Poster Session

#2566 Dissecting PDAC cell-microenvironment interplay in liver metastasis by single cell analysis.

Claudia Fioravanti[1], Arianna Vallerga[2], Giulia Garattini[1], Ilaria Craparotta[2], Federica Di Leva[1], Chiara Grasselli[2], Fabio Sangalli[1], Andrea Resovi[1], Raffaella Giavazzi[2], Marco Bolis[2], Giulia Taraboletti[1], **Dorina Belotti**[1]

[1]Mario Negri Institute for Pharmacological Research, Bergamo, Italy,[2]Mario Negri Institute for Pharmacological Research, Milano, Italy

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal tumors. Because most PDAC patients are diagnosed with an already advanced and metastatic stage of the disease, only 15% are suitable for surgical intervention. Thus, an improved understanding of the metastatic mechanism is critical to improve the outcome of these patients. The most common target for PDAC metastasis is the liver. Outgrowth of metastases requires acquisition of tumor genetic alterations in conjunction with alterations in the pancreas and liver microenvironment. The aim of the study was to investigate the role of the tumor microenvironment and the molecular alterations in tumor and stroma cells leading to PDAC liver metastasis formation. To this purpose a metastatic cell variant was established from the FC1199 pancreatic cancer cell line, derived from tumors arisen in LSL-Kras$^{G12D/+}$;LSL-Trp53$^{R172H/+}$;Pdx-1-Cre mice and growing orthotopically in C57BL/6 mice. The FC199LV metastatic variant was obtained by successive rounds of *in vivo* selection of cells implanted in the pancreas, isolated from liver metastases and re-implanted in the pancreas for a total of 5 rounds. IHC and *in vivo* MicroCT analysis of livers at different time points after tumor cell injection highlighted the presence of metastases only in mice transplanted with FC1199LV. Single cell RNA-sequencing analysis of dissociated primary tumors and livers of mice transplanted with FC1199 and FC1199LV highlighted differences in the microenvironmental transcription profile between the two tumor variants. The spontaneous metastatic model of PDAC is an important tool to study the interplay between tumor and stroma in PDAC metastatic process and to identify a tumor and tumor microenvironment gene expression signature associated with PDAC liver metastasis.
Supported by AIRC IG 2019 ID23443

#2567 CX3CR1 surface expression on metastatic prostate cancer cells is increased by the target organs and is associated with a distinct metabolic phenotype.

Ruxu Zhai, Jieyi Zhang, Olimpia Meucci, Alessandro Fatatis

Pharmacology & Physiology, Drexel University College of Medicine, Philadelphia, PA

Studies from us and others have shown that the chemokine receptor CX3CR1 drives cancer cells to the skeleton, activates pro-survival signaling pathways and bears unique therapeutic potential. We have recently reported that the small subsets of prostate cancer cells that expose CX3CR1 on their surface ($CX3CR1^{Surf+}$) are endowed with stemness features and behave as metastasis-initiating cells (MICs). Interestingly, their $CX3CR1^{Surf-}$ counterparts can re-express CX3CR1 on the cell surface while undergoing partial EMT, thus re-acquiring MIC features. This phenotypic plasticity can be temporally monitored *in vitro*, with cultures of 100% $CX3CR1^{Surf-}$ cells reaching a phenotypic equilibrium (5-7% of $CX3CR1^{Surf+}$) in ~ 8 days. However, when cells at phenotypic equilibrium are grafted in mice via systemic blood circulation, the resulting tumors in the skeleton and soft tissues show a dramatic increase in the $CX3CR1^{Surf+}$ fraction. This suggests that the stroma of target organs promotes CX3CR1 plasticity, an effect not observed when the same cells are grafted subcutaneously. We also show that FX-68, a potent and specific antagonist of CX3CR1, dramatically reduces the number of disseminated tumors and total tumor burden in mice. FX-68 does not alter the percentage of $CX3CR1^{Surf+}$ cancer cells in disseminated tumors, suggesting that its anti-tumor effects are exerted by functional inactivation of CX3CR1. Notably, we found a strong association between the $CX3CR1^{Surf+}$ status and a metabolic shift away from the OXPHOS pathway and towards glycolysis. Based on these findings, we conducted further studies by assessing the short- and long-term effects of CX3CR1 activation on the metabolic profile of prostate cancer cells. Furthermore, *in vivo* studies with tumor-bearing mice treated with FX-68 revealed the effects of this compound on the metabolic profiles of cancer cells harvested from tumors in the skeleton and soft tissues. Our work reports for the first time the association of CX3CR1 with the metabolic status of prostate cancer cells. We also provide evidence that interfering with CX3CR1 signaling could impede a metabolic switch promoted by target organs and sustaining metastatic progression.

#2568 Cancer-associated fibroblasts-derived periostin promotes lymph node metastasis of cholangiocarcinoma through lymphatic endothelial reprogramming.

Z. Song, X. Li, H. Zhang, C. Liu;
Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, China

Purpose: Cholangiocarcinoma (CCA) is a bile duct malignancy with poor outcomes, especially in patients with lymph node metastasis (LNM). However, the mechanism of LNM in CCA as well as its therapeutic options remain undefined. Understanding crosstalk between the cellular components in the complex tumor microenvironment (TME) of CCA, and how it influence LNM, may help identifying novel therapeutic targets in LNM of CCA.
Method: Freshly resected tumors from 10 CCA patients were collected for single-cell RNA sequencing (scRNA-seq), aiming to identify the differences between CCA with or without LNM. Immunohistochemistry (IHC) and enzyme-linked immune sorbent assay (ELISA) were applied to validate the identified targets. In vivo study was conducted using foot pad-popliteal LNM mouse model to find out main factors affect LNM. Co-culture system was established to interrogate cellular crosstalk between human lymphatic endothelial cells (HLECs) and patient-derived CAFs in TME of CCA. Other phenotypic and mechanistic experiments include tube formation assay, trans-endothelial migration assay, cell adhesion assay, pull down assay, etc.
Results: Our scRNA-seq revealed the enrichment of a distinguish subtype of CAFs that express periostin (POSTN) in CCA tumors with LNM. IHC and immunofluorescence staining in CCA sections validated that the CAF-specific expressing protein, POSTN, expresses significantly higher in CCA with LNM, which is also associated with higher circulating tumor cells (CTCs), and worse prognosis. In vivo study shows that CAFs highly expressing POSTN (POSTN+CAFs) lead to higher incidence of LNM. In vitro study indicates that either with co-culture of POSTN+CAFs or treatment of recombinant human POSTN (rhPOSTN), HLECs developed increased capacity of tube formation and migration, while CCA cells also show increased capacity of migration in trans-endothelial assay but not traditional migration assay. Meanwhile, POSTN also impairs integrity of lymphatic vessels, providing routes for CCA cells to disseminate. Pull down assay identifies the binding of POSTN to its receptor, αVβ3 integrin, on HLECs. We demonstrate that the effects of POSTN aforementioned can be abrogated by knockdown of ITGB3 in HLECs, suggesting that POSTN exerts its effects in an αVβ3-dependent manner. We further show that POSTN's effects are attributed to the activation of integrin-FAK/Src pathway. Moreover, in vivo application of Cilengitide, an αVβ3/5 inhibitor, decreases the incidence of LNM in CCA, indicating its therapeutic efficacy in mitigating LNM burden in CCA patients with higher expression of POSTN.
Conclusion: This study uncovers the novel role of CAF-derived POSTN in promoting LNM of CCA, presenting the potential therapeutic promise of the FDA-approved αVβ3/5 inhibitor, Cilengitide, in inhibiting LNM in CCA.

#2569 Cancer associated fibroblast dependency in a model of muscle invasive bladder cancer.

Henkel Valentine, Uttam Satyal, Alexander Metz, Rhea Arya, Tahmina Hague, Laura Bukavina, Philip H. Abbosh

Fox Chase Cancer Center, Philadelphia, PA

Introduction: The relationship between genotype and phenotype in targeted therapy is crucial, especially in diseases like bladder cancer where the PI3K/AKT/mTOR pathway is widely activated but untargeted. We have developed an immunocompetent, CRISPR-edited bladder cancer mouse model that recapitulates common PI3K/AKT/mTOR mutations. Through targeting generated PI3K/AKT/mTOR mutations, we hope to establish rationale for clinical investigation of PI3K/AKT/mTOR-directed therapy. Our mouse model displays a dependency on Cancer Associated Fibroblasts (CAFs) to create a pro-tumorigenic environment *in vivo*, facilitating engraftment and tumor progression. Given the observed *in vivo* CAF dependency, we aim to understand the mechanisms of immune modulation between CAFs and the tumor microenvironment.

Methods and Results: Urothelial cells were isolated from the bladder of Rosa26 LSL-Cas9/GFP transgenic mice to develop organoids. These organoids were co-edited for *Trp53* knockout and either *Rb1 knockout* or *9p21* (homologous to mouse 4q) large deletion. Further editing of the *Pten* gene resulted in a nine-fold increase in sensitivity to the kinase inhibitor ipatasertib, while a Pikc3a H1047R hotspot mutation sensitized organoids up to 2000-fold to the Pik3 inhibitor pictilisib. Despite successful engraftment into mouse bladder, these edited cells regressed before tumor formation. We have since demonstrated that co-injecting edited organoids with skin cancer or bladder cancer derived-CAFs yielded palpable subcutaneous and orthotopic tumors. To further understand the mechanisms of immune suppression *in vivo*, we performed a medium throughput cytokine array with a panel of 16 mouse adaptive immune markers. Cytokine analysis revealed that CAFs secreted interlukin-6 in the presence of organoids which themselves secreted GM-CSF. This combination is well-known to be pro-tumorigenic, facilitating recruitment of M2 macrophages which we also observe in *in vivo* tumors. Further, CAFs activate TGF-β pathway as demonstrated by phosphorylation of SMAD2/3. Taken together we believe that CAFs, through GM-CSF/IL-6 secretion along with TGF Beta pathway activation, regulate the TME to create a suitable environment for tumor engraftment.

Conclusions: Our research demonstrates the importance of CAFs in bladder cancer progression. Through further examining the mechanistic interplays between CAFs and immunoregulation, we can gain a better understanding of the main drivers and potential targets in bladder tumorigenesis.

#2570 Deciphering local immunosuppression in pancreatic cancer: targeting CAF-myeloid cell crosstalk to enhance immune response.

Marie C. Hasselluhn[1], Lukas Vlahos[1], Yuanqing Xue[1], Dafydd Thomas[2], Quin T. Waterbury[1], Allison C. Hess[1], Tanner C. Dalton[1], Amanda R. Decker-Farrell[3], Alvaro Curiel Garcia[1], Stephen A. Sastra[1], Carmine F. Palermo[1], Timothy C. Wang[1], Andrea Califano[1], Kenneth P. Olive[1]

[1]Columbia University Irving Medical Center, New York, NY,[2]PMV Pharmaceuticals, Inc., Princeton, NJ,[3]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Immunotherapy has revolutionized cancer treatment but remains largely ineffective in pancreatic ductal adenocarcinoma (PDAC). A hallmark of PDAC is local immunosuppression, driving cytotoxic T cell exclusion and deactivation, undermining immune-mediated tumor control. Better understanding of the cellular crosstalk in the tumor microenvironment (TME) and identifying stromal and immune populations shaping this immunosuppressive landscape is critically needed. Oncogenic KRAS activation in PDAC cells promotes recruitment and proliferation of tumor-supporting stromal cells while excluding cancer-targeted cytotoxic T cells. Local immunosuppression is driven by interactions between subtypes of cancer-associated fibroblasts (CAFs) and resident myeloid cells. Previous efforts to reverse immunosuppression by targeting individual stromal populations have largely failed, highlighting the complexity of stromal interactions in the TME. The stromal diversity of PDAC complicates investigating paracrine signaling involving multiple cell types. To address this, we use in vivo mouse models that recapitulate human disease, alongside a novel tumor explant system enabling short-term culture of intact human or murine PDAC tissue. These explants retain histopathological architecture and cellular diversity, providing a medium-throughput platform for testing drugs and mechanistic hypotheses in the native TME. Preliminary data show that Smoothened inhibition (SMOi) reduces myCAF proliferation and activity but drives CD11b-positive myeloid cell expansion in vivo. We hypothesize local immunosuppression in PDAC is sustained by a delicate balance between myCAFs and myeloid cells, collectively hindering T cell infiltration. Single-cell RNA sequencing of control vs. SMOi-treated murine PDAC identified stromal subpopulations maintaining immunosuppression and revealed specific myeloid subtypes emerging after SMOi. Strikingly, combining SMOi with anti-Gr1 or CCR1 inhibition (CCR1i) markedly increased cytotoxic T cell numbers—up to 20-fold—within the TME. We are now exploring whether these T cells' activity can be further enhanced using additional immunomodulatory agents. Testing therapeutic combinations in the same TME allows us to identify synergistic effects that may inform future PDAC immunotherapy. Our work elucidates complex immunosuppressive mechanisms in PDAC, leveraging a novel tumor explant system and in vivo models. Our ultimate goal is to develop translatable regimens that neutralize local immunosuppression, enabling cytotoxic T cells to infiltrate, proliferate, and effectively attack cancer cells.

#2571 Effects of patient-derived ovarian cancer-associated fibroblasts and tumor progression and metastasis.

Ji-Young Lee, Sung Wan Kang, Min-Seo Lee, Ok-Ju Kang, Yong-Man Kim, Shin-Wha Lee

Obstetrics and Gynecology, Asan Medical Center, University of Ulsan, Seoul, Korea, Republic of

Background: Ovarian cancer (OC) is a highly treatment-resistant malignancy, often diagnosed late due to minimal symptoms and extensive metastasis. Despite platinum-based chemotherapy, recurrence and chemoresistance highlight the need for new therapies. This study focuses on cancer-associated fibroblasts (CAFs), key TME components, to explore their impact on OC progression and identify novel CAF subtypes. Methods: CAFs were isolated from OC patient tumors via enzymatic dissociation and flow cytometry. Their markers, FAP and α-SMA, were analyzed using immunofluorescence and flow cytometry. Conditioned media from CAF cultures were tested on OC cell lines (A2780, A2780-cp, SKOV3, OVCAR3) to assess proliferation and migration via cell viability and scratch assays. Cytokines in CAF-conditioned media were quantified using ELISA.
Results: CAFs were identified within OC tumors, with their expression of FAP validated in high-grade serous ovarian cancer (HGSOC) samples via immunohistochemistry and flow cytometry. CAFs were successfully isolated and cultured from patient-derived OC tumors. Flow cytometry revealed that CAFs represent a minor subset of the total tumor cell population. Morphologically, CAFs exhibited plump spindle or stellate forms. CAFs derived from HGSOC displayed elevated levels of FAP and α-SMA expression compared to those derived from non-HGSOC tumors. Exposure of four OC cell lines (A2780, A2780-cp, SKOV3, OVCAR3) to CAF-conditioned media significantly promoted their growth. Scratch assays revealed enhanced motility in two OC cell lines (SKOV3 and OVCAR3) following treatment with CAF-conditioned media. Notably, the HGSOC-derived CAF subtype, W-OVCAF7, exhibited significantly greater growth- and motility-enhancing effects compared to non-HGSOC-derived CAFs. Scratch assays further confirmed that W-OVCAF7 markedly increased the migratory capacity of both SKOV3 and OVCAR3 cells, suggesting that CAF activity may vary depending on the tumor cell type. These findings underscore the pivotal role of CAFs in modulating the proliferation and migration of OC cells.
Conclusion: Tumor-derived CAFs significantly enhance OC cell proliferation and migration, underscoring their critical role in the TME. This study highlights the importance of patient-derived CAFs for precise analysis of OC dynamics and lays a foundation for therapeutic strategies targeting the CAF-TME interplay.

#2572 Association of cancer-associated fibroblast composition with CD4 T cells in pancreatic ductal adenocarcinoma.

Jessica Hung, Michel DuPage

Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA

Understanding interactions between cancer-associated fibroblasts (CAFs) and immune components in the tumor microenvironment (TME) is crucial for engineering antitumor immunity in pancreatic ductal adenocarcinoma (PDAC). While most studies have focused on CAF-mediated immunomodulation, the impacts of immune cells on CAF heterogeneity and function remain underexplored. In this study, we used flow cytometry and microscopy to profile three major CAF subsets—myofibroblasts (myCAFs), inflammatory CAFs (iCAFs), and antigen-presenting CAFs (apCAFs)—in two orthotopic mouse models of PDAC presenting “immune-hot” (6421c2) and “immune-cold” (FC1245) TMEs. Consistent with subcutaneous models, orthotopic 6421c2 tumors exhibited smaller tumor size, elevated T cell infiltration, and reduced suppressive neutrophils compared to FC1245, highlighting distinct immune microenvironments. Although the overall frequency of $PDPN^+$CAFs in the non-immune compartments was similar, CAF composition differed significantly between the two tumors. FC1245 was predominantly composed of myCAFs (80% of $PDPN^+$CAFs), with low iCAF and apCAF proportions. In contrast, 6421c2 tumors displayed increased iCAFs (30%) and apCAFs (20%) alongside a reduced myCAF frequency (50%). Immunofluorescence and Trichrome staining revealed elevated αSMA^+myCAFs and increased collagen deposition in FC1245, suggesting unique CAF composition and function in immunologically distinct TMEs. To examine whether intratumoral T cells modulate CAF heterogeneity in vivo, we examined CAF compositions in tumor-bearing mice administrated with $CD8^+$ or $CD4^+$ T cell depleting antibodies or in the $Foxp3^{DTR}$ mice given diphtheria toxin (DT) to deplete regulatory T cells (T_{regs}). Unlike CD8 or CD4 depletion, T_{reg} depletion significantly improved primary tumor control in 6421c2, reduced metastasis, and prolonged survival in FC1245. CAF subset analysis also revealed T_{reg}’s unique roles in regulating the myCAF and apCAF subsets in PDAC. Specifically, T_{reg} depletion significantly reduced myCAFs, supporting prior findings that T_{regs} are essential for myCAF development during PDAC precursor lesion formation. Furthermore, T_{reg}-mediated support of myCAF was $CD4^+$ conventional T cell (T_{conv})-dependent, as myCAF level was restored upon CD4 depletion in DT-treated mice. Conversely, apCAFs increased following T_{reg} depletion but were completely abolished with CD4 depletion, suggesting that effector CD4 T cells are required for recruiting or maintaining MHC II-expressing CAFs within the TME. In summary, our study reveals distinct CAF compositions in immunologically diverse TMEs and uncovers the unique roles of T_{regs} and T_{conv} in regulating CAF subsets. These findings provide novel insights into CAF-CD4 T cell interactions in vivo and support further investigation of molecular mechanisms underlying stromal functions in PDAC.

#2573 Single-cell multi-omics analysis identifies a novel GPRC5B-marked endothelial cell subset and its mechanistic role in tumor stemness in colorectal cancer.

Mengying Suo, Hongyu Huang, Tianyou Li, Ziqi Meng, Xueqian Zhang, Shanshan Jiang, Na Li

Nankai University School of Medicine, Tianjin, China

Introduction: Tumor endothelial cells (ECs) support the tumor microenvironment and regulate the status of tumor cells. GPRC5B, enriched in ECs, is linked to poor prognosis in colorectal cancer (CRC). Yet, the influence of GPRC5B on ECs and its regulatory mechanism on CRC stemness are still elusive. Therefore, this study aims to clarify the impact of GPRC5B on ECs states, the specific role of GPRC5B-marked ECs (GPRC5B$^+$ ECs) in tumor/stromal interactions and the mechanisms regulating CRC stemness.

Methods: We analyzed scRNA-seq data from 18 CRC samples to decode the endothelial heterogeneity. We used an original SVM-based algorithm to find EC subsets linked to CRC stemness, ultimately identifying the GPRC5B$^+$ ECs. By deconvoluted bulk RNA-seq data, we evaluated stemness level in GPRC5B$^+$ EC-enriched samples. Cellular interactions and spatial omics analyses were employed to examine the signaling axis between GPRC5B$^+$ECs and CRC stem cells. ChIP-seq and hdWGCNA algorithm pinpointed upstream regulators in the axis.

We also developed a GPRC5B-overexpressing EC line and co-cultured it with CRC cells, whose stemness was then assessed by spheroid formation assays and western blot. Inhibitors targeting this signaling axis were administered, and changes in stemness phenotype and key signaling molecules were measured in co-cultured CRC cells. Finally, we constructed a CT26 subcutaneous tumor mouse model and treated the mice with siRNA targeting GPRC5B (siGPRC5B) to monitor tumor growth. Tumor tissues were collected to detect the expression of stemness markers using IHC and western blot.

Results: GPRC5B$^+$ ECs exhibited high angiogenic potential, with significant ERK pathway activation. ScRNA and bulk analyses showed GPRC5B$^+$ ECs enhance CRC stemness, which was further confirmed by the increased spheroid formation ability and enhanced expression of KLF4 and SOX2 in CRC cells after co-culturing with GPRC5B-high ECs. Mouse models showed that high GPRC5B expression in ECs fosters tumor stemness and progression.

Mechanistically, multi-omics and in vitro analyses revealed that GPRC5B in ECs activated the ERK-SP1 axis to promote the expression of ADAM17, which cleaved and activated JAG1, ultimately enhancing stemness of CRC cells via JAG1/NOTCH1 interaction. Application of ERK pathway or ADAM17 inhibitors to ECs decreased tumor stemness, respectively. Besides, application of siGPRC5B effectively inhibited tumor growth *in vivo*.

Conclusion: Through single-cell multi-omics analyses, we identified a novel GPRC5B-marked EC subset, promoting angiogenesis and CRC progression, and revealed that GPRC5B$^+$ ECs enhance tumor stemness via the ERK/SP1/ADAM17/JAG1-NOTCH1 axis. The study offers insights into CRC endothelial heterogeneity and tumor/stromal interactions, and future targeted therapy will be conducted to elucidate its clinical application potential.

#2574 Cancer associated fibroblasts with high expression of immunoglobulin superfamily containing leucine-rich repeat show tumor inhibitory capabilities in brain metastasis.

Thomas Simon[1], Aspiara Roosmin[1], David N. Buckley[1], Ben Y. Tew[1], Gerald C. Gooden[1], Gabriel Zada[2], Bodour Salhia[1]

[1]Department of Translational Genomics, USC - University of Southern California, Los Angeles, CA,[2]Department of Neurosurgery, USC - University of Southern California - Keck School of Medicine, Los Angeles, CA

Brain metastasis (BM) remains mostly incurable with a dismal mean 2-year survival of less than 2%. The tumor microenvironment (TME) of BM is complex and includes cancer associated fibroblasts (CAFs), which we have shown can either support or inhibit tumor growth, pointing to their heterogeneity. To examine the CAF heterogeneity in the BM TME, we performed a cell multiome sequencing analysis (scMultiome-seq) of BM patient tissues and identified 4 diverse CAF subpopulations in the TME. Among these was a subpopulation of CAFs that expressed high levels of tumor inhibitory CAF marker immunoglobulin superfamily containing leucine-rich repeat (ISLR). High expression of ISLR was also observed in CAFs we have previously reported as tumor inhibitory in an *in vivo* model of BM. We thus hypothesized that ISLR-CAFs could be tumor inhibitory in the BM TME. Further analysis of our scMultiome-seq data to infer cell-cell communication revealed the anti-proliferative IGFBP3-TMEM219 pathway as a potential communication mechanism between ISLR-CAFs and BM tumor cells. In the present study, we thus aimed to investigate the role of ISLR in BM CAFs and to study the significance of the IGFBP3-TMEM219 pathway in the tumor-inhibitory effects of ISLR-CAFs *in vitro.* To do so, patient-derived BM tumor (CM04) and CAF (CM01, CM02, CM03 and CM08) cell lines previously established in our lab were used. BM tumor cells were treated with CAF-derived conditioned media (CM) and cell viability was assessed using the CellTiter-Glo assay. The expression of ISLR, IGFBP3 and TMEM219, along with the activation of caspase-8 signaling linked to the anti-proliferative effects of the IGFBP3-TMEM219 pathway, was analyzed via western blotting. We observed high expression of ISLR in CM03 and CM08 CAFs, as compared to CM01 and CM02 CAFs, which expressed low to no ISLR. CM04 tumor cells showed high expression of TMEM219 compared to CAFs, while CM03 and CM08 CAFs expressed the highest levels of IGFBP3. Both ISLR and IGFBP3 were also detected in CM derived from CM03 and CM08 CAFs. We observed that CM03 and CM08 CAF-derived CM significantly inhibited CM04 tumor cell viability *in vitro*. In addition, activation of the caspase 8 signaling was detected in CM04 tumor cells treated with CM08 CAF-derived CM. Next, ISLR knock-down was performed in CAFs using lentiviral shRNA particles to confirm a role for ISLR in CAF-mediated tumor inhibition. ISLR knock-down in CM08 CAFs decreased expression of IGFBP3, suggesting a role for ISLR in regulating IGFBP3 expression in CAFs. Altogether the present data revealed that CAFs with high ISLR expression could inhibit tumor growth *via* the IGFBP3-TMEM219 pathway in the BM TME. Further in-depth understanding of the roles of ISLR-CAFs in the BM TME could help develop strategies that selectively target tumor supportive CAFs while protecting tumor inhibitory ones.

#2575 Tumor microenvironmental metabolites regulate cancer-associated fibroblast heterogeneity.

Chufan Cai[1], Marta Storl-Desmond[1], Kristof Turan[1], Axel R. Concepcion[2], Jing Chen[1], **Simon Schwoerer**[1]

[1]Medicine, University of Chicago, Chicago, IL,[2]Biochemistry, University of Chicago, Chicago, IL

Pancreatic ductal adenocarcinoma (PDAC) is characterized by a prominent desmoplastic response with a high abundance of cancer-associated fibroblasts (CAFs). CAFs are a functionally and transcriptionally heterogeneous population of cells and include inflammatory CAFs (iCAFs), thought to promote tumor growth via the secretion of cytokines and chemokines, as well as myofibroblasts CAFs (myCAFs), thought to restrain tumor growth via the secretion of extracellular matrix. The iCAF and myCAF states are highly plastic. Preventing the interconversion of CAF populations could have significant clinical benefits, but how this plasticity is regulated is largely unknown. Cytokines like IL1α and growth factors like TGFβ are required but not sufficient to induce changes in the CAF state. Instead, their effect is dependent on the presence of other environmental factors. We recently demonstrated that cancer cell-derived cytokines synergize with hypoxia to induce a transition from a myCAF- towards and an iCAF state, indicating that metabolic factors in the TME can modulate the CAF state in combination with signaling molecules. To identify other extracellular metabolic factors that synergize with cytokines in regulating CAF plasticity, we performed a high-content screen using a human blood chemical library in combination with our recently developed fibroblast reporter (FIRE) system incorporating iCAF (*IL6*-EGFP) and myCAF (*Acta2*-DsRed) transcriptional reporters. Out of hundreds of compounds, we identified and validated one metabolite that synergizes with the cytokines IL1α and TNFα to promote *IL6* expression in a time- and dose-dependent manner. Intriguingly, previous studies have reported that this metabolite accumulates in the PDAC TME to a high micromolar range. Metabolite and cytokine treatment together induce pancreatic stellate cells (PSCs) to upregulate production and secretion of pro-inflammatory cytokines including IL6, CXCL1 and LIF. Pretreatment of PSCs with physiological concentrations of this metabolite is sufficient to alter the fibroblast state and to promote tumor growth when co-injected with PDAC cells *in vivo*. Mechanistically, we find that this metabolite acts in part through the JAK-STAT pathway, a major regulator of the iCAF state. These findings support the idea that TME metabolites can alter PDAC progression by regulating CAF heterogeneity through synergy with cancer cell-derived signaling molecules.

#2576 Crosstalk between cancer-associated fibroblasts and sympathetic nerves promotes pancreatic ductal adenocarcinoma progression.

Ariana Sattler[1], Parham Diba[1], Tetiana Korzun[1], Marigold Kuykendall[1], Kevin MacPherson-Hawthorne[1], Mara Sherman[2], Teresa A. Zimmers[1], Sebnem E. Eksi[1]

[1]Oregon Health & Science University, Portland, OR,[2]Memorial Sloan Kettering Cancer Center, New York, NY

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancers, with a 5-year survival rate of 12.8%. This aggressive malignancy is composed of a heterogenous tumor microenvironment (TME), including cancer-associated fibroblasts (CAFs) and sympathetic neurons. The abundance of CAFs and sympathetic neurons correlates with tumor progression and is often associated with poor prognosis of PDAC patients. However, the precise role of CAF-nerve interactions in cancer progression and metastasis remains unclear. To explore our hypothesis that these bidirectional interactions can be tumor-supportive, we used advanced imaging and sequencing techniques, an *in vivo* tumor denervation murine model, and *in vitro* CAF-sympathetic neuron coculture systems. Compared to human PDAC tumors, we affirmed that sympathetic innervation is recapitulated in murine PDAC tumors, and these intratumor nerves are similarly surrounded by an abundance of CAFs. To focus on the interaction between CAFs and sympathetic nerves, we developed and optimized *in vitro* coculture systems. These cocultures initially revealed that CAFs consistently interact with nerve fibers and significantly increase sympathetic nerve outgrowth. Transcriptional analysis of these co-cultures further identified genes upregulated in CAFs that promote adhesion, ECM deposition, and neuroactive ligand-receptor interactions, while sympathetic nerves upregulated pathways associated with nerve injury response. To further interrogate the impact of sympathetic innervation in the PDAC TME, we specifically and locally ablated sympathetic nerves in murine orthotopic PDAC tumors and spatially evaluated the cellular and molecular changes in response. Collectively, our findings suggest that the crosstalk between sympathetic nerves and CAFs may modulate CAF behavior, enhance tumor innervation, and drive tumor-promoting phenotypes in PDAC progression.

#2577 Antigen-presenting CAFs in PDAC differentially regulate Treg phenotype and function via CXCL9 and CCL22.

S. Y. Maru, J. T. Mitchell, M. Wetzel, N. E. Gross, L. Andaloori, K. Howe, E. Kartalia, G. Mo, J. Leatherman, W. Ho, E. Fertig, L. T. Kagohara, E. J. Pearce, E. M. Jaffee;
Johns Hopkins University School of Medicine, Baltimore, MD

Pancreatic ductal adenocarcinoma (PDAC) is characterized by a complex tumor microenvironment (TME) including stromal cells that influence resistance to therapy. Recent studies have revealed that stromal cancer-associated fibroblasts (CAFs) are heterogeneous in origin, gene expression, and function. One such population, antigen-presenting CAFs (apCAFs), is defined by MHC-II and can induce both $CD4^+$ T cell activation and regulatory T cell (Treg) differentiation. Understanding apCAF biology could uncover mechanisms that can be targeted to promote CAF differentiation and enhance T cell activity.

Methods: Two clones differing in sensitivity to immune checkpoint blockade (ICB), derived from the autochthonous KPC murine PDAC model, were implanted orthotopically into the pancreas of syngeneic C57BL/6 mice. apCAF KO mice were generated by crossing $PDGFR\alpha^{Cre}$ and MHC-II^{flx} mice. All mouse strains were untreated, treated with ICB or anti-mesothelin antibody, or immunized with a KRAS peptide vaccine. Tumors were harvested for TME characterization, functional assays, single-cell RNA sequencing (scRNAseq), and imaging mass cytometry (IMC). apCAFs, DCs, and $CD4^+$ T cells were isolated and peptide-pulsed apCAFs and DCs were cultured with naïve OT-II $CD4^+$ T cells or tumor-infiltrating $CD4^+$ T cells. Cell to cell communication inference using dominoSignal was performed to assess signaling between apCAFs and $CD4^+$ T cells.

Results: Immunosensitive (sKPC) tumors were characterized by higher immune cell and apCAF infiltration than resistant (rKPC) tumors. IMC analysis showed proximity of apCAFs and $CD4^+$ T cells in both sKPC and rKPC tumors implicating interaction within the TME. apCAF-depleted sKPC tumor-bearing mice have diminished sensitivity to ICB. apCAFs activate tumor-infiltrating $CD4^+$ T cells and induce Treg differentiation, but not production of immunosuppressive cytokines in sKPC tumors. scRNAseq revealed greater Th1-like Treg differentiation in CD4s cultured with sKPC apCAFs, and differential chemokine signaling from apCAFs based on TME of origin, such that sKPC apCAFs induce Th1-like Tregs via CXCL9 and rKPC apCAFs induce classical Tregs via CCL22.

Conclusions: apCAFs are more abundant in immunosensitive PDAC tumors, spatially involved with $CD4^+$ T cells, and are functionally relevant for response to ICB as shown by two depletion models. Notably, chemokine signaling from apCAFs varies with tumor of origin and modulates Treg function. Together these data implicate apCAFs as important mediators of the antitumor immune response. Although a small proportion of the TME, modulating apCAF number or function may provide a solution to the currently unmet need of new and more effective treatments for PDAC patients.

#2578 GDF15 reprograms the microenvironment to drive the development of uveal melanoma liver metastases.

Sathya Neelature Sriramareddy[1], Navya Siddarajappa[1], Thanh Nguyen[2], Jeffim Kunetsov[3], Stefan Kurtenbach[3], James Jarad Dollar[3], Jonathan D. Licht[4], Ann Y. Chen[2], William J. Harbour[5], Keiran S. M. Smalley[1]

[1]Department of Tumor Microenvironment & Metastasis, H. Lee Moffitt Cancer Center, Tampa, FL,[2]Department of Biostatistics,, University of Utah, Salt Lake City, UT,[3]Sylvester Cancer Center, University of Miami, Miami, FL,[4]University of Florida Health Sciences Center, Gainesville, FL,[5]Department of Ophthalmology, University of Texas Southwestern, Dallas, TX

Little is known about how interactions between the uveal melanoma (UM) and host cells in the tumor microenvironment (TME) drive the progression of liver metastases. Single cell RNA-seq analysis of UM samples identified marked differences in cell-cell communication between primary and metastatic UM characterized by increased signaling strength and numbers of interactions emanating from the hepatic stellate cells (HSCs) and endothelial cells in the metastatic samples. In co-culture, HSCs modulated the transcriptional heterogeneity of the UM cells, enriching for cell states that expressed genes implicated in cell survival, metabolic reprogramming and the angiogenic response. Co-culture of HSCs and UM cells also led to increased expression of growth factors and receptors implicated in angiogenesis including IL-8, VCAM1, VEGF and ICAM1. One candidate driver of HSC reprogramming was the TGF-beta family member GDF15. Increased expression of GDF15 was associated with a Class 2 metastatic UM phenotype in clinical UM samples and UM cell lines, with silencing of BAP1 in UM cells increasing both GDF15 expression and H3K27ac marks at the GDF15 promoter. Treatment of HSCs with GDF15 led to increased expression of multiple ECM proteins, inflammatory cytokines and angiogenic factors, including IL- 8. Both exogenous GDF15, IL-8 and conditioned media from UM-HSC co-cultures increased endothelial cell network formation in vitro, an effect that could be blocked by anti-GDF15 antibodies. In an eye->liver metastasis model of UM, silencing of GDF15 did not prevent the seeding of UM cells to the liver, but did inhibit the outgrowth of metastatic lesions. Although the GDF15-silenced UM cells were proliferative they did not recruit endothelial cells, leading to a significant reduction in metastatic burden. In sum, we provide the first evidence that UM liver metastasis development is dependent upon GDF15-mediated remodeling of the liver microenvironment leading to an angiogenic response that supports tumor growth.

#2579 Integrated sequencing analysis reveals prognostic thyroid cancer-associated fibroblast subpopulations.

Matthew A. Loberg[1], George J. Xu[1], Claudia C. Wahoski[1], Kailey P. Caroland[1], Megan L. Tigue[1], Sheau-Chiann Chen[2], Hua-Chang Chen[2], Quanhu Sheng[2], Eric C. Huang[3], Fei Ye[2], Ryan H. Belcher[2], Ethan Lee[1], Vivian L. Weiss[2]

[1]Vanderbilt University, Nashville, TN,[2]Vanderbilt University Medical Center, Nashville, TN,[3]University of Washington School of Medicine, Seattle, WA

Thyroid cancers exist on a continuum of differentiation. Our group recently defined dynamic changes in the tumor microenvironment co-occurring with tumor histotypes and de-differentiation. Here, we integrate 423,733 cells from seven distinct single-cell RNA-sequencing cohorts to define unique thyroid cancer- specific fibroblast subtypes occurring across the histologic and mutational spectrum of thyroid cancer. We identify distinct inflammatory, perivascular-like, and myofibroblastic cancer-associated (myCAF) populations. The myCAF population, specifically, is only found in malignant samples, and mapping myCAFs to three thyroid cancer bulk RNA-sequencing cohorts reveals an association with disease progression, with hazard ratio of 3.0 (95% CI, 1.7-5.3). Within this myCAF population, we identify subpopulations with immune-regulating phenotypes that are specifically associated with de-differentiated tumors. Additionally, we identify an invasive tumor cell phenotype in both differentiated and de-differentiated tumors marked by TNC and LAMB3. Ligand-receptor interaction analysis indicates robust interaction of myCAFs and invasive tumor cells through TGF-beta, tenascin, and collagen signaling pathways. Multiplex immunofluorescence, immunohistochemistry, and spatial transcriptomics analysis of 28 tumors (13 differentiated, 8 de-differentiated, 7 with areas of differentiated and de-differentiated histopathology) confirms robust upregulation of myCAFs in de-differentiated tumors. Further, myCAFs spatially localize next to the leading edge of de-differentiated tumors and near invasive cells from differentiated tumors, supporting a tumor-promoting role for myCAFs in aggressive thyroid carcinomas. In summary, our study provides an integrated atlas of thyroid cancer fibroblast subtypes, spatial analysis on patient samples, and an association of fibroblasts with disease progression.

#2580 Direct interaction between cancer cells and fibroblasts drives epithelial-to-mesenchymal plasticity (EMP) and contribute to chemotherapy resistance.

Nilu Dhungel, Ana- Maria Dragoi

Department of Molecular and Cellular Physiology, LSU Health Sciences Center, Shreveport, LA

The tumor microenvironment (TME), composed of immune cells and fibroblasts, blood vessels, signaling molecules and extracellular matrix, provides important cues that govern epithelial-to-mesenchymal transition (EMT) and facilitate the acquisition of aggressive traits such as chemotherapy resistance by cancer cells. Cancer cells exist in multiple hybrid epithelial/mesenchymal (E/M) states responsible for the ever-changing environment during tumor development and metastasis. How are these hybrid states generated and maintained is not fully understood. Using small cell lung carcinoma (SCLC) and normal lung fibroblasts model we show that direct interaction between SCLC and lung fibroblasts induces a hybrid EMT phenotype in cancer cells in which several mesenchymal genes involved in receptor interaction with the extracellular matrix (ECM) and ECM remodeling are upregulated while epithelial genes such as E-cadherin remain unchanged. We also demonstrate that several core EMT-regulating transcription factors (EMT-TFs) are upregulated in cancer cells during direct contact with fibroblasts, including Yes-associated protein (YAP1), a major regulator of the Hippo pathway. Furthermore, we demonstrate that this upregulation of YAP1 in cancer cells which occurs significantly only during the direct interaction with fibroblast makes the cancer cells more resistant to chemotherapeutic agents resulting in more aggressive phenotypes. The cells become sensitive to chemotherapeutic agents when YAP1 is inhibited. Altogether, our results provide evidence that tumor cells' direct contact with the fibroblasts in the TME initiates a signaling cascade responsible for hybrid E/M states of cancer cells. These hybrid states are maintained during the interaction and contribute to therapy resistance via YAP1 pathway, while interference with direct contact will result in slow recovery and a switch to the initial states.

#2581 Establishing a multi-omic spatial ECM proteome and N-glycome of pancreatic ductal adenocarcinoma tissues.

Caroline G. Kittrell[1], Jade Macdonald[1], Blake Sells[2], Lyndsay E. A. Young[1], David DeNardo[2], Peggi M. Angel[1], Richard R. Drake[1]

[1]The Medical University of South Carolina (MUSC), Charleston, SC,[2]Washinton University in St. Louis, St. Louis, MO

Pancreatic Ductal Adenocarcinoma (PDAC), one of the leading causes of cancer related mortality nationwide, is characterized by an extensive desmoplastic stromal reaction and an altered N-glycome. Stromal collagens serve to modulate the immune microenvironment, limit nutrient and oxygen diffusion, influence chemotherapy resistance, and promote tumor progression and metastasis. Dysregulated cancer cell N-glycosylation also promotes immune evasion and neoplastic spread. Despite their important roles in cancer pathogenesis, little is known about the spatial localization and organization of the various stromal collagens, other extracellular matrix proteins, and N-glycans within the PDAC tumor microenvironment. This study utilized Matrix Assisted Laser Desorption Ionization-Mass Spectrometry Imaging (MALDI-MSI) with Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) to characterize the extracellular matrix proteome and glycome of 10 primary PDAC patient tumors in collaboration with the Washington University SPORE in Pancreatic Cancer at the Siteman Cancer Center. Following PNGaseF glycosidase digestion of PDAC tissues, 214 released N-glycans and their spatial distributions were detected by MALDI-MSI. These tissues were then digested with Collagenase III for detection and localization via peptide MALDI-MSI. Tissues were scraped from the slide and further digested with Collagenase III in solution for peptide ID analysis by LC-MS/MS, with 31 identified peptides confirmed and matched to MALDI peaks. Spatially, both ECM peptides and N-glycans uniquely colocalize with various histopathologic features according to multiplex immunohistochemistry. N-glycans with bisecting N-acetylglucosamine structures associated specifically with the invasive tumor front and clusters of immune cell infiltrate, while distinct collagen species encased tumor cell regions and formed stromal nests surrounding immune cells. The degree of proline hydroxylation and other post translational modifications also varied across confirmed ECM peptides and may impact stability and deposition. From this analysis we may begin to link the N-glycome and ECM proteome of PDAC with patient survival, treatment response and other clinical variables.

#2583 A T cell engager targeting cancer associated fibroblasts inhibits tumor growth and modulates the tumor microenvironment.

Joshua R. Tagore[1], **Robert Joseph Norgard**[1], Pratha Budhani[1], Sai Charan Penikalapati[1], Jessica N. Egan[1], Di Feng[1], Leticia Corrales-Pecino[2], Anna Bachmayr-Heyda[2], Claudia Reichel-Voda[2], Abhishek Kashyap[1], Youli Xia[1], Sarah A. O'Brien[1]

[1]Boehringer Ingelheim, Ridgefield, CT,[2]Boehringer Ingelheim, Vienna, Austria

Fibroblast activating protein (FAP) is an attractive target for therapeutic intervention being mainly expressed in malignant tissues. Previous studies have targeted FAP using a variety of methods yet failed in clinics. Here, we utilize FAP-targeted T cell engager (TcE) in KPCY tumor models to recapitulate a pre-clinical environment. The use of FAP- TcE efficiently depleted FAP+ fibroblasts *in vitro* and *in vivo* from tumors. This resulted in bystander killing of tumor cells and tumor growth control *in vivo*. This therapeutic effect was not dependent on priming via lymph node activation as shown in other publications. To understand the complex interactions with FAP expression, we perform comprehensive single cell RNA-sequencing profiling before and after FAP-TcE depletion to understand the pivotal genetic roles of FAP depletion in all cell types. This analysis initially revealed a T cell exhaustion phenotype with upregulated of PD-1 on T cells after FAP-TcE treatment. However, while addition of anti-PD-1 therapy significantly enhanced tumor efficacy, complete control was absent. RNA sequencing of the fibroblasts identified depletion of a myCAF-like fibroblast and an outgrowth of a mixed CAF population with phenotypes of both myCAF and iCAF. This population correlated with tumor growth, and cell cycle gene signatures. Future work will investigate the implications of this fibroblast population's role in subverting immune suppression.

#2584 *In situ* hybridization-based validation of transcriptionally-defined stromal clusters in the prostate cancer microenvironment.

Filippo Pederzoli, Hubert Pakula, Wikum Dinalankara, Jeanne Jiang, Mohamed Omar, Silvia Rodrigues, Pier Vitale Nuzzo, Fabio Socciarelli, Giuseppe Nicolo Fanelli, Luigi Marchionni, Massimo Loda

Pathology and Laboratory Medicine, Weill Cornell Medicine, New York, NY

Introduction: Stromal cells in the prostate tumor microenvironment (TME) affect tumor progression and exhibit both a universal response to tumorigenesis and specific ones determined by the genotype that drives the tumor (*Pakula et al., Nat Commun 2024*). As a result, stromal clusters associated with murine and human prostate cancer identified by single cell RNA sequencing (scRNA-seq) fall into a) common subtypes: myofibroblasts/pericytes and innate immunity-associated; as well as b) two additional ones specific to either AR positive adenocarcinomas or neuroendocrine tumors (NEPC). Here, we defined their spatial localization leveraging spatial transcriptomics and multiplex *in situ* RNA hybridization.

Methods & Results: We computationally identify the most discriminative genes able to distinguish the four stromal groups. Specifically, we first identified highly discriminatory genes representative of each cluster. We then used integer programming-based optimization to obtain small sets of genes ($n \leq 4$) that are differentially expressed in each group compared to the remaining three. For instance, group 0, encompassing myofibroblasts and pericytes, was characterized by the expression of Rgs5 and Myh11, while group 2, whose stromal cells are characterized by the expression of Ar and Wnt signaling genes, was characterized by the expression of Wif1 and Coch. A decision-tree analysis was able to discriminate all the different stromal groups. These were validated using 1) *in situ* RNA hybridization + multiplex immunofluorescence panels and 2) spatial transcriptomics. Myofibroblasts/pericytes were found to be distant to tumor cells, common clusters were associated with all tumors of diverse phenotype (Tmprss2-ERG, cMYC, $Pten^{-/-}$, $Pten^{-/-}$/ $Rb1^{-/-}$ $NMYC^{+/+}$), while periostin high/AR low clusters were immediately adjacent to NEPC foci in mouse models.

Conclusions: Our integrated computational and experimental approach establishes a robust pipeline for validating stromal markers identified through scRNA-seq, enabling spatial mapping of stromal heterogeneity in prostate cancer.

#2585 GABA shunt pathway promotes peri-synaptic brain metastasis by hijacking synaptic signaling.

Shih-Ying Wu[1], Kerui Wu[2], Abhishek Tyagi[1], Ravindra Pramod Deshpande[1], Eleanor Cecile Smith[1], Jee Won Kim[1], Kounosuke Watabe[1]

[1]Wake Forest University School of Medicine, Winston-Salem, NC,[2]University of North Carolina at Greensboro, Greensboro, NC

Metastatic brain tumors are the most common intracranial neoplasms in adults. Among all subtypes of breast cancer, triple-negative breast cancer (TNBC) has a significantly poorer prognosis compared to other subtypes. Pre-menopause and estrogen replacement therapy after menopause have been shown to increase the risk of brain metastases in TNBC patients. Estrogen blockade using tamoxifen demonstrates some efficacy in TNBC, suggesting that estrogen promotes brain metastases by modulating the tumor microenvironment in estrogen receptor (ER)-negative tumors. Neurons, as highly specialized cells responsible for cell-to-cell signal transmission, are among the most critical and abundant cell types in the brain. However, the role of tumor-associated neurons and their contributions to TNBC remain poorly understood. We hypothesize that estrogen promotes brain metastasis in hormone receptor-negative breast cancer by increasing the release of gamma-aminobutyric acid (GABA), which serves as a metabolic substrate to fuel tumor cell growth. We further hypothesize that blocking GABA transporter function will suppress brain metastases in hormone receptor-negative breast cancer. Our findings show that pre-menopausal TNBC patients exhibit higher synaptic formation and increased expression of GABA transporters in brain metastases, suggesting that neurons may contribute to tumor growth. To investigate the effect of estrogen on brain metastasis in ER-negative breast cancer aCnd to understand how estrogen depletion modulates metastatic growth in the brain, we performed bilateral ovariectomy in female mice and intracardially implanted luciferase-labeled human ER-negative brain metastatic breast cancer cells (231BrM). Our results demonstrate that estrogen increases synaptic formation and upregulates the expression of GABA and GABA transporter 1 (GAT1) in brain metastases more prominently than other neurotransmitter receptors. To examine the effect of GABA on tumor progression, 231BrM cells were treated with 0.5 mM GABA, mimicking physiological brain concentrations, or recombinant GABA. We found that GABA treatment upregulated GAT1 expression and enhanced colony formation and migration abilities, strongly suggesting that GABA promotes brain metastasis. Furthermore, treating 231BrM cells with conditioned medium (CM) obtained from GABAergic neurons exposed to estrogen led to increased tumor growth through activation of the GABA shunt pathway in tumor cells. Blocking GAT1 using CRISPR/Cas9 or a small-molecule GAT1 inhibitor effectively suppressed GABAergic neuron-induced tumor progression. Our results suggest that estrogen enhances GABA release from neurons, subsequently promoting activation of the GABA shunt pathway in tumor cells. This study highlights GAT1 inhibitors as promising therapeutic tools for treating TNBC patients with brain metastases.

#2586 PAI-1 promotes tumor angiogenesis and metastasis via crosstalk between NSCLC cells and CAFs.

Kazuhiro Okada, Ken Suzawa, Yin Min Thu, Ryunosuke Fujii, Kousei Ishimura, Ryota Fujiwara, Kazuya Hisamatsu, Ryo Yoshichika, Tomoaki Higashihara, Naohiro Hayashi, Fumiaki Mukohara, Mao Yoshikawa, Yuma Fukumoto, Keiichi Date, Hidejiro Torigoe, Kazuhiko Shien, Shinichi Toyooka

General Thoracic Surgery, Okayama Univ. Graduate School of Med., Okayama, Japan

Plasminogen activator inhibitor -1 (PAI-1) plays a multifaceted role in the tumor microenvironment, with its high expression correlated with poor prognosis in multiple cancers, including non-small cell lung cancer (NSCLC). Our group's previous studies have shown that cancer-associated fibroblasts (CAFs) induced PAI-1 in lung cancer cells and that PAI-1 mediated the MET-targeted therapy resistance in NSCLC. In this study, we discovered that CAFs promoted PAI-1 as a secretory protein and its expression in cancer cells themselves and into the conditioned medium by the stimulation of CAF-derived stimulatory factors of transforming growth factor beta-1 (TGFb-1) and -3. Next, we used several lung cancer cell lines with different mutational profiles to assess the effect of PAI-1 in the tumor microenvironment. We investigated the phenotypic changes of lung cancer cells by recombinant human PAI-1 administration and the PAI-1 overexpressed lung cancer cell lines. PAI-1 increased the migratory ability of cancer cells and promoted epithelial to mesenchymal transition (EMT). PAI-1 contributed to tumor progression via the enrichment of EMT, extracellular matrix signaling, and inflammatory and immune responses according to gene set enrichment analysis (GSEA) revealed by the PAI-1 overexpressed lung cancer cells. Moreover, PAI-1 also promoted angiogenesis via the vascular endothelial growth factor (VEGF) pathway supported by the tube formation assay, western blot pathway analysis, and the GSEA. This impact is further validated through vivo study, in which the inhibition of PAI-1 improves the anti-cancer targeted therapy in vivo, where the tumor growth is inhibited by the combination of target therapy and the PAI-1 inhibitor, tiplaxtinin. The tumors from PAI-1 overexpressed lung cancer cells xenograft models also showed high expression of CD31 compared to control, confirming that angiogenesis is being promoted in these tumors. These findings underscore the clinical importance of PAI-1 as an important target for personalized anti-cancer drug therapy.

#2587 Transcription factor ZEB1 controls lineage specificity of African ancestry-enriched breast stromal multilineage cells.

M. Shukla[1], M. Krishnan[2], M. Niese[2], J. Ji[2], H. Gao[2], Y. Liu[2], M. H. Kaplan[2], H. Nakshatri[2];
[1]IU Simon Comprehensive Cancer Center, Indianapolis, IN, [2]IU School of Medicine, Indianapolis, IN

Although disparity in breast cancer outcomes in women of African Ancestry (AA) as compared to European Ancestry (EA) has been thought to derive from socioeconomic factors, emerging evidence demonstrating genetic ancestry-dependent differences in normal and cancer genome, cancer-associated mutation patterns and immunosuppressed tumor microenvironment suggests the involvement of biological factors. To investigate breast cancer outcome-associated biologic factors, we analyzed breast tissues of healthy donors of distinct genetic ancestry and found that PROCR$^+$/ ZEB1$^+$/ PDGFRα$^+$ stromal cells (hence, called PZP) are more abundant in normal breast tissues of women of AA as compared to EA. PZP cells display the properties of multi-lineage fibroadipogenic mesenchymal stromal cells and can differentiate into adipogenic and osteogenic lineages. Whether and how PZP cells contribute to breast cancer progression is unknown. Using a combination of gene knockout, RNA-sequencing, co-culture and immunophenotyping studies, we investigated the role of PZP cell-enriched Zinc finger E-box-binding homeobox 1 (ZEB1) transcription factor in PZP cell function. ZEB1 knockout in PZP cells resulted in altered lineage specificity. The expression of epithelial markers KRT 7, 8, 18 and adipogenic markers PPARG and PPARGC1B were upregulated upon ZEB1 knockout suggesting that ZEB1 maintains PZP cells in an undifferentiated state. ZEB1 also controlled the expression of several genes that encode extracellular matrix (ECM) associated proteins including Tenascin-C (TNC), an ECM factor linked to aggressive tumors and immunosuppressive microenvironment. Furthermore, the expression of matrix metallopeptidase 1 (MMP1) and a Leucine-rich repeat-containing protein 15 (LRRC15), both of which are markers of myofibroblasts, was downregulated in ZEB1 knockout cells suggesting the role of ZEB1 in trans-differentiation of PZP cells into cancer-promoting myofibroblast lineage. In addition, ZEB1 controlled the expression of prostaglandin-endoperoxide synthase 1 (PTGS1), which synthesizes immunosuppressive prostaglandin E2 (PGE2), in PZP cells. In support of immunosuppressive role of PZP cells, PZP cell secreted factors decreased the expression of Interferon γ and Granzyme B in CD8$^+$ T cells, which are critical for effector function of T cells. Ongoing studies are investigating the potential contributions of ZEB1, PGE2, and TNC in PZP cell-mediated decrease in effector function of CD8$^+$ T cells. Collectively, our results suggest that PZP cells are enriched in breast tissues of AA women and contribute to breast cancer progression through the breast stroma-enriched therapeutic target ZEB1.

#2588 Stromal remodeling promotes immune triad formation in the tumor microenvironment (TME).

Daniel Lagal[1], Duncan Hong[1], Athanasios Papadas[2], Alicia Gibbons[1], Yun Huang[1], Yaling Dou[1], Alexander Cicala[3], Elsa Molina[4], Kristina Matkowskyj[5], Dustin Deming[6], Fotis Asimakopoulos[1]

[1]UC San Diego, La Jolla, CA,[2]Ohio State University, Columbus, OH,[3]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY,[4]Salk Institute for Biological Studies, La Jolla, CA,[5]Mayo Clinic, Rochester, MN,[6]UW-Madison, Madison, WI

Recent evidence has highlighted the critical requirement for “immune triads” of CD8+ T cells, CD4+ T cells and dendritic cells (DC) for tumor elimination in response to checkpoint inhibition (CPI) immunotherapy. However, the mechanistic underpinnings of this cross-talk are poorly understood. Immune triads are often located in peripherally-distributed DC-rich niches within the tumor stroma. We have hypothesized that stromal remodeling in response to tumor expansion generates homeostatic signals that trigger an adaptive immune response that may ultimately fail through effector exhaustion or apoptosis. In particular, stromal proteolysis of the large matrix proteoglycan versican (VCAN) has been associated with T-cell infiltration across multiple tumor types. The objective of this study was to interrogate a mechanistic link between stromal remodeling, immune triad formation and response to CPI. We used HALO image analysis to determine the spatial relationship of CD4+ and CD8+ T cells in a panel of 95 human non-small cell lung cancers. Each tumor was VCAN proteolysis-scored based on a binning system measuring staining intensity for a neoepitope exposed through site-specific VCAN proteolysis. Tumors displaying VCAN proteolysis intensity of zero (0) had an average of 0.122 CD8+ T cells located with 10um of a CD4+ T cell vs. 0.216 in tumors with any VCAN proteolysis (intensity 1,2,3 combined); p=0.04. Similar results were obtained when measuring proximity of CD4+ to CD8+ T cells (0.124 vs 0.223, p=0.02). These data demonstrate that remodeling stroma promotes CD4+:CD8+ crosstalk. Site-specific VCAN proteolysis releases a 441 N-terminal bioactive fragment, versikine. Versikine constitutes a classical “matrikine”, i.e., a bioactive matrix fragment possessing novel functions compared to its parent macromolecule. To determine the mechanisms by which versikine may promote immune triad niches, we generated several transplantable syngeneic tumor cell lines engineered to stably secrete versikine into the TME. One CPI-refractory tumor cell line (Lewis Lung Carcinoma, LLC) and two partially CPI-responsive transplantable tumor lines (CT26 and MC38 colorectal carcinoma) were extensively characterized through flow cytometry, spatial immunofluorescence and scRNAseq. Our results demonstrate that in all contexts, versikine results in expansion of Tfh-like CD4+ T cells expressing Cd40lg and Icos. This expansion is associated with specific patterns of CD8+ T cell exhaustion that will be discussed. Therapeutically, versikine reversed anti-PD-1 pathway refractoriness in the poorly-immunogenic LLC model and demonstrated superior synergy with CPI in the more immunogenic CT26 and MC38 models, leading to tumor eradication with resistance to tumor rechallenge. Our data bolster the mechanistic rationale for exploitation of stromal remodeling signals to promote CD4+T:CD8+T:DC cross-talk for enhanced CPI efficacy.

#2589 Folate receptor α confers colonization advantage of cholangiocarcinoma in peritoneal microenvironment with therapeutic vulnerability.

H. Zhang[1], L. Guo[2], S. Lin[1], X. Li[1], Z. Song[1], L. Xu[1], C. Liu[1];

[1]Sun Yat-Sen Memorial Hospital of Sun Yat-Sen University, Guangzhou, China, [2]Fudan University Shanghai Cancer Center, Shanghai, China

Purpose: Peritoneal metastasis/recurrence results in poor prognosis and failure of radical resection in cholangiocarcinoma (CCA) patients, but the molecular mechanisms and potential therapy strategies remain an enigma.

Methods: Freshly resected primary lesions and peritoneal metastases from CCA patients were collected for single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics to identify the crucial target during peritoneal colonization of CCA cells. Tumor-adipocytes *in vitro* co-culture model and tumor peritoneal implantation mouse model were utilized to investigate the role and therapeutic promises of the identified target in peritoneal metastasis of CCA. Metabolomics, metabolic flux, and flow cytometry assays were employed to delve into the underlying molecular mechanisms.

Results: The scRNA-seq analysis of CCA tumors (N=5 pairs) revealed an up-regulated expression of FOLR1, encoding folate receptor alpha (FRα), in CCA cells after colonization in the peritoneal microenvironment, which was validated by spatial transcriptomics (N=2 pairs). The specifical overexpression of FRα in CCA cells in the peritoneal metastases was validated by immunohistochemistry and multiplex immunofluorescence staining, and it was associated with a poorer prognosis, larger metastases size, and increased ascites volume in CCA patients (N=68). Despite no significant impact on the proliferative and invasive capacities of CCA cells in conventional culturing, overexpression of FRα enhanced their proliferation in the tumor-adipocytes co-culture model and tumor peritoneal implantation mouse model. Mechanistically, utilization of enriched fatty acids from adipocytes for fatty acid oxidation fuels CCA cells outgrowth and colonization in peritoneal metastases, but it also leads to excessive oxidative stress. FRα-dependent folate metabolism and one-carbon metabolism contribute to production of NADPH and GSH, the essential substrates for cellular anti-oxidative stress, thus leading to growth advantage of CCA cells in the peritoneal microenvironment. Herein, the utilization of Mirvetuximab Soravtansine, the antibody-drug conjugate targeting FRα, demonstrated an encouraging therapeutic efficacy in CCA peritoneal implantation mouse models, including reduced tumor size, decreased number of disseminated lesions, lowered ascites volume and ascites CTCs, prolonged mouse survival, and delayed recurrence of peritoneal metastases after peritoneal metastasectomy.

Conclusions: Hence, FRα plays a pivotal role in facilitating the colonization of CCA in the peritoneal microenvironment, rendering it a promising therapeutic target for alleviating peritoneal metastases burden in advanced CCA patients. The potential therapeutic advantages of Mirvetuximab Soravtansine warrant preliminary investigation in our planned clinical trial.

#2590 Characterization of tumor-neuron communication in lung adenocarcinoma brain metastasis.

Sampada Chande[1], Don Nguyen[1], Atsushi Yamasaki[2], Veronica Chiang[1], Declan McGuone[1]

[1]Yale School of Medicine, New Haven, CT,[2]Daiichi Sankyo, New Haven, CT

30-40% of cancer patients with lung cancer develop brain metastasis with poor prognosis. The incidence of brain metastasis has increased in patients that have received standard of care treatment, including targeted therapies. The mechanisms which promote lung cancer cell dissemination and colonization of the central nervous system (CNS) remain understudied and identifying novel drug targets to treat brain metastasis is an unmet clinical need. By using a variety of animal models of lung cancer metastasis, we previously identified shifts in epithelial and neuronal like lineage gene expression programs in malignant cells as they adapt to the brain tumor microenvironment (TME) *in vivo*. Gene set enrichment analysis (GSEA) revealed that most of genes are involved in neuronal guidance and cell adhesion pathways (L2FC > 0.5, padj < 0.05, Log2RPKM > 1 compared to 2D culture). We also examined tumor-neuron interactions in patient derived surgical biopsies and found active areas of interaction between tumors and neurons in human lung adenocarcinoma (LUAD) brain metastasis. Spatial transcriptomics of human brain metastasis also revealed differential profiles for areas of active interaction. *In vitro*, we established co-culture conditions which demonstrate that primary neurons can directly interact with brain metastatic LUAD cells and improve their fitness. Based on these observations, we performed a custom Clustered regularly interspaced palindromic repeats (CRISPR)-Cas9 loss of function screen targeting 470 neuronal and cell adhesion genes that are up-regulated in LUAD cells that seed the brain *in vivo*. Moreover, we performed our screens under different conditions which recapitulate tumor cell-tumor cell clustering in 3-dimensional culture conditions (3D) or tumor-neuron co-clustering in 3D. Results from this differential screening approach suggests that LUAD cells are increasingly, but not exclusively, dependent on several neuronal genes as they are co-cultured with neurons. In conclusion, we have modelled direct tumor-neuronal cell interaction *in vitro*, shown that it is an important component of brain metastasis, and identified putative targets that can be inhibited to disrupt tumor-neuron interactions and potentially limit brain metastatic progression.

#2591 The interaction of pericytes and brain metastasizing cancer cells in the *in vitro* co-culture system.

Shoko Noda-Narita, Atsu Aiba

The University of Tokyo, Tokyo, Japan

Introduction: Brain metastasis significantly reduces the patients' quality of life and survival, but there are only a few choices of treatment for brain metastasis. The research for brain metastasis is difficult because of its complex and unique microenvironment, including blood-brain barrier (BBB). As the role of pericytes in maintaining the BBB function has become clearer, the reconstituted models of the BBB in which endothelial cells (ECs), pericytes, and astrocytes are seeded on the front and back of the insert membrane have gotten our attention. We previously applied a reconstituted BBB model (Ito R, et al. *Mol Pharm* 2019) to develop a co-culture system for the evaluation of brain metastatic cells. This *in vitro* brain metastasis model mimics the brain vascular microenvironment, and successfully reproduces the invasive ability of brain metastatic cell lines. In this study, we figured out the role of pericytes in promoting cancer invasion and elucidated the interaction between pericytes and metastasizing cancer cells.

Methods: Using our *in vitro* co-culture system, we compared the migration ability of MDA231 and Ex3LL in several BBB models: EPA model (ECs, pericytes, and astrocytes), E0A model (ECs and astrocytes), EP0 model (ECs and pericytes), and E00 model (ECs only). To figure out how pericytes promote the migration of Ex3LL, we analyzed the morphology, transcriptome and migration ability of the parental and brain metastatic (BrM) cell lines of Ex3LL with and without pericytes.

Results: Though the migration of MDA231 was inhibited when co-cultured with pericytes or astrocytes, the migration of Ex3LL was promoted by the co-culture with pericytes. BrM cells of Ex3LL showed further increased migration ability when co-cultured with pericytes, compared to the parental cells. The transcriptome analysis of BrM cells of Ex3LL showed upregulated signaling pathway of the cell-cell and cell-matrix interactions, which implied the direct interaction between pericytes and cancer cells. The migration analysis using EP0 model (pericytes were seeded on the back of the insert membrane and had a direct interaction with cancer cells) and E0P model (pericytes were seeded on the bottom surface of the well and only secreted factors affected the cancer cells), also suggested the direct interaction of pericytes promoting the migration of Ex3LL.

Conclusion: Using our BBB reconstituted model, we showed that the role of pericytes in promoting cancer invasion depended on cancer cell types. Our results suggested that the direct interaction between pericytes and cancer cells, and assumedly ECs, was indispensable for the mechanism in which pericytes promote the Ex3LL migration. We need further investigation to elucidate the molecular mechanism underlying the interaction between pericytes and brain metastasizing cancer cells.

#2592 The osteoblastic microenvironment determines the fate of breast cancer cells disseminated in the bone marrow.

Hong-Li Wang, Xiao-Min Yue, Rui Zhang, Yu-Fan Huang, Rong Meng, **Xiao-Qing Li**

Tianjin Medical University Cancer Institute & Hospital, Tianjin, China

Bone is the most common destination for metastatic breast cancer cells. When cancer cells disseminate to the bone, they may either colonize or settle into a quiescent state, depending on their interaction with the bone microenvironment. In this study, we elucidated how the differentiation status of the osteoblastic microenvironment determines the fate of cancer cells disseminated in the bone marrow. We analyzed clinical survival data and demonstrated that luminal-like cancer cells with high Runt-related transcription factor 2 (RUNX2) expression exhibited an increased risk of bone metastases in breast patients. By using RUNX2-overexpressing cancer cells as bone-tropic seeds and establishing various osteoblastic microenvironments with different differentiation statuses in vitro and in vivo, we observed that both luminal-like and basal-like cancer cells remained quiescent as disseminated tumor cells (DTCs) or micrometastases within an inactive osteoblastic microenvironment. In contrast, luminal-like cancer cells colonize the bone within both nonmineralized and mineralized active osteoblastic (osteogenic) microenvironments. Basal-like cancer cells, however, remained quiescent within highly mineralized osteogenic microenvironment but colonized bone in a nonmineralized osteogenic niche rich in nonmineralized bone matrix (osteoid). In the active microenvironments, well-differentiated osteoblasts were observed to be responsible for recruiting cancer cells, whereas the nonmineralized bone matrix supported the proliferation of both luminal-like and basal-like cancer cells, with luminal-like cells gaining additional growth advantages from well-differentiated osteoblasts. Furthermore, we explored the possibility that activation of the osteoblastic microenvironment due to abnormal bone metabolism and fluctuating estrogen levels reactivate quiescent DTCs or micrometastases, leading to increased risk of bone metastasis. Additionally, in a supportive osteogenic microenvironment, both cancer cells and well-differentiated osteoblasts were found to contribute to the activation of osteoclasts, resulting in the development of osteolytic lesions. Thus, this study provides a comprehensive explanation for the divergent outcomes of disseminated cancer cells in the bone, whether they colonize the bone or become dormant, and suggests promising therapeutic strategies to block the reactivation of quiescent tumor cells.

#2593 Caspase-1 dependent pyroptosis converts αSMA^{+} CAFs into collagen-III high iCAFs to fuel chemoresistant cancer stem cells.

Hongbo (Beth) Gao[1], Stephen Q.R. Wong[1], Ethan Subel[1], Mustafa Karabicici[2], Winston Huang[1], Mark Ellie Alonzo[2], Keith Syson Chan[1]

[1]Houston Methodist Research Institute, Houston, TX,[2]Cedars-Sinai Medical Center, Los Angeles, CA

Chemotherapy-induced pyroptotic tumor cells potentially impact on cancer-associated fibroblasts (CAFs), which serve as key cellular components in stroma to construct a favorable tumor microenvironment (TME). Our study identifies three CAFs subtypes: αSMA^{+}IL-6^{-} myofibroblastic CAFs (myCAFs), αSMA^{-}IL-6^{+} inflammatory CAFs (iCAFs), and hybrid i/myCAFs. Caspase-1 activation in tumor cells release inflammatory chemokines/cytokines, promoting iCAFs formation in a CCR6-dependent manner. Interestingly, the reactive iCAFs, instead of myCAFs, contribute to collagen III expression upon chemotherapy-induced cancer cell pyroptosis. Collagen III-enriched microenvironment preferentially harbors CD44^{high} cancer stem cells (CSCs). Consistently, chemoresistant bladder cancers enhance iCAFs markers and collagen III after neoadjuvant chemotherapy. Thus, chemotherapy-caused pyroptotic cancer cells initiates an iCAFs-CSCs feedforward loop to drive the formation of chemoresistant TME.

#2594 Estrogen-regulated cytokine and growth factor secretion from breast cancers of women of diverse ancestry and effects on myelopoiesis.

Marisol Chavez[1], Mario Morales Martinez[1], Eduardo Mauricio Gonzalez[1], Gang Deng[2], Julia Aguade Gorgorio[3], Madhuri Wadehra[4], Nalo Hamilton[5], Michael E. Jung[2], Evelyn Jiagge[6], Sofia Merajver[6], Hannah Mikkola[3], Richard J. Pietras[1], Diana Marquez-Garban[1]

[1]Department of Medicine, UCLA Jonsson Comprehensive Cancer Center, Los Angeles, CA,[2]Department of Chemistry and Biochemistry, UCLA Jonsson Comprehensive Cancer Center, Los Angeles, CA,[3]Molecular Cell and Developmental Biology. Broad and Stem Cell Research Center, UCLA Jonsson Comprehensive Cancer Center, Los Angeles, CA,[4]Department of Pathology and Laboratory Medicine, UCLA Jonsson Comprehensive Cancer Center, Los Angeles, CA,[5]UCLA Jonsson Comprehensive Cancer Center, UCLA School of Nursing, Los Angeles, CA,[6]University of Michigan School of Medicine, Ann Arbor, MI

Breast cancer (BC) is the most common cancer among women in the U.S. It disproportionately affects African American (AA) women who have earlier disease onset, more aggressive subtypes, and lower survival rates compared to European American (EA) women. AA tumors show distinct gene variants linked to poor BC outcomes and immune signatures with differential expression of cytokines/chemokines potentially driving a more immunosuppressive tumor microenvironment (TME). Of note, cancer-related inflammation promotes aberrant myelopoiesis in bone marrow mediated distantly by tumor secretion of specific factors that stimulate accumulation of immunosuppressive myeloid cells. We have previously reported that estrogen signaling induces the expansion of myeloid derived suppressor cells (MDSCs), an action that can be blocked by selective estrogen receptor degraders (SERD), such as fulvestrant and our newly-designed oral SERD, JD128. To explore potential differences in cytokine/chemokine/growth factor secretion patterns in BC cells from EA and AA women, we used a Luminex multiplex assay to detect 38 cytokines/growth factors. Findings indicate different patterns of secretion occur, with AA cells showing significantly higher extracellular levels of CXCL9, MCP1, IL-8, PDGF-AB/BB, VEGF, TNFα and G-CSF under basal conditions as compared to EA cells ($P<0.05$). Further, treatment with estradiol-17β stimulated secretion of TNFα, IL8, IL13, IL18,MCP1, G-CSF and PDGF-AA, an action that was significantly inhibited by use of SERDs fulvestrant or JD128 ($P<0.05$). TNFα and G-CSF are known to promote myelopoiesis and a greater ratio of MDSC/CD8 T-cells, thereby potentially contributing to an immunosuppressive state in the TME. Further, estrogen stimulated proliferation of CD34+ bone marrow stem cells and MDSCs derived from normal female donors, an effect inhibited by fulvestrant and JD128 indicating that hematopoietic stem cells express estrogen receptors and respond to estrogen. Notably, using ELISA and qRT/PCR, SERDs fulvestrant and JD-128 decreased expression of transcript and protein levels of estrogen receptor alpha ($P<0.05$). Our preliminary data provides further evidence of a biologically distinct immunosuppressive profile that may drive more aggressive BC outcomes in AA women. Estrogen-mediated effects on hematopoietic cells may have implications for use of SERDs for estrogen receptor positive or negative BC. Funded by CIRM DISC2-14166, UCLA JCCC Breast Cancer Award, Tower Cancer Research Foundation, NIH/NCI U54 CA143930 CDU-UCLA JCCC Partnership, CBCRP B27IB3869, CDMRP DOD BCRP.

TUMOR BIOLOGY: Developmental Origins and Drivers of Pediatric Cancers
Poster Session

#2598 Fatty origins of a bone tumor? The curious case of Ewing Sarcoma.

Utkarsh Kapoor[1], Christoph Hafemeister[1], Lisa Shaw[2], Karin Muelbacher[1], Julia Moldaschl[3], Caterina Sturtzel[1], Dominik Egger[4], Stefan Toegel[5], Cornelia Kasper[3], Martin Distel[1], Kelly M. Bailey[6], Marc van de Wetering[7], Masanori Hayashi[8], Matthias Farlik[2], Florian Halbritter[1], Heinrich Kovar[1]

[1]St. Anna Children's Cancer Research Institute, Vienna, Austria,[2]Department of Dermatology, Medical University of Vienna, Vienna, Austria,[3]Institute of Cell and Tissue Culture Technologies, Department of Biotechnology, BOKU University, Vienna, Austria,[4]Institute of Cell Biology and Biophysics, Leibniz University Hannover, Hannover, Germany,[5]4. Karl Chiari Lab for Orthopaedic Biology, Department of Orthopedics and Trauma Surgery, Medical University of Vienna, Vienna, Austria,[6]University of Pittsburgh School of Medicine and UPMC Children's, Pittsburgh, PA,[7]Princess Maxima Centre for Pediatric Oncology, Utrecht, Netherlands,[8]Department of Pediatrics, University of Colorado School of Medicine, Aurora, CO

Ewing sarcoma (EwS) is an aggressive pediatric bone tumor with an enigmatic cell-of-origin. The fusion oncogene EWS::FLI1 is pathognomonic for EwS and the primary driver in 85% of EwS patients. Human mesenchymal stem cells (hMSCs) are considered to be the likely cell of origin of EwS for two reasons: their ability to tolerate ectopic EWS::FLI1 expression and the resemblance in the transcriptomes of EWS::FLI1 expressing hMSCs to primary Ewing tumors. However, to date a cellular differentiation-based model of EwS does not exist. To obtain insights into EwS etiology and histogenesis, this study has established the first comprehensive single-cell differentiation atlas of juvenile bone marrow-derived hMSCs with and without EWS::FLI1 expression. A pool of bone-marrow derived hMSCs from nineteen healthy juvenile donors was engineered to conditionally express EWS::FLI1. We used physiology mimicking conditions (hypoxia, spheroid cultures, serum-free growth and differentiation media) and induced EWS::FLI1 oncogene expression for 24h or 72h at daily time points of hMSC differentiation into adipo-, chondro-, osteo- and neuro-genic lineages. This highly resolved atlas of 252 samples representing normal and perturbed differentiation allowed us to map single cell tumor data from EwS patients to multiple lineages and time-points across stages of lineage commitment. Single-cell RNA-seq analysis of ~488,000 transcriptomes revealed loss of lineage identity in the presence of EWS::FLI1 across all four lineages. However, the analysis of genes detected in the EWS::FLI1 high cluster uncovered a specific time and lineage preference towards treated adipogenic cells that shared the transcriptomic signature with tumor cells, indicating the potential cell/stage of tumor-origin. Longer EWS::FLI1 induction led to enhanced loss of lineage identity and higher mapping with patient data. Soft agar colony formation assay confirmed stronger transformation potential of EWS::FLI1 expressing adipogenic cells. Our results offer novel insights into the elusive cellular origins of Ewing sarcoma, thereby establishing a foundation for the development of indispensable preclinical models to inform tailored therapeutic approaches. This study is supported by a generous grant (#20-17258) of the Alex’s Lemonade Stand Foundation (ALSF) Crazy8 Program.

#2599 Biological consequences and mechanisms of neural signatures in fusion-positive rhabdomyosarcoma.

Jack Kucinski, Alexi Tallan, Matthew Kent, Cenny Taslim, Meng Wang, Matthew V. Cannon, Katherine M. Silvius, Benjamin Z. Stanton, Genevieve C. Kendall

Center for Childhood Cancer Research, Nationwide Children's Hospital, Columbus, OH

The chimeric transcription factor PAX3::FOXO1 is a core dependency and monogenic driver of fusion-positive rhabdomyosarcoma, an aggressive pediatric soft-tissue sarcoma. *PAX3::FOXO1* consists *of* the *PAX3* DNA binding domains (paired and homeobox) and the *FOXO1* transactivation domain. Despite the identification of the fusion-oncogene over thirty years ago, there are no targeted therapies for children suffering from this disease. The current therapeutic regimen consists of surgery, radiation, and general chemotherapy, which has been largely unsuccessful in improving patient outcomes. This lack of targeted therapies is partly due to unclear in vivo regulatory mechanisms of PAX3::FOXO1 across cellular contexts and tumorigenic stages. Previously, we found that PAX3::FOXO1 has pioneer-like activity and is transforming in zebrafish models, which generate tumors that recapitulate the human disease. To understand initial PAX3::FOXO1 activities, we developed a zebrafish mRNA injection model that broadly expresses the human fusion oncoprotein during early vertebrate development. We find in vivo evidence to characterize PAX3::FOXO1 as a pioneer transcription factor and demonstrate its ability to bind to inaccessible chromatin. We identified partial homeobox motif recognition as a new mode for PAX3::FOXO1 pioneering activity that diverges from in vitro models. PAX3::FOXO1 binding sites and motif selection preference correlated with DNA methylation. Together, these findings highlight that context, specifically underlying DNA methylation, can impact how pioneer factors interact with chromatin. PAX3::FOXO1 subsequently induced chromatin accessibility and activated enhancers upon binding. This was observed at sites where PAX3::FOXO1 was bound to its full composite motif, showing that motif preferences drive different functional roles. This neomorphic chromatin landscape supports the transcriptional activation of known rhabdomyosarcoma gene signatures and neural-related pathways. Even though rhabdomyosarcoma is characterized by incomplete skeletal muscle differentiation, neural signatures specifically exist within this aggressive subtype and are further enriched upon chemotherapeutic treatment in patient-derived xenografts. One critical target of PAX3::FOXO1 in our zebrafish model is the neural transcription factor Her3, zebrafish ortholog to HES3. PAX3::FOXO1 mRNA injection into *her3/HES3* knockout zebrafish embryos suppresses neural transcriptional programs. Altogether, this work highlights neural pathway activation as an initial activity of PAX3::FOXO1 and that these programs could play a critical role across multiple stages of tumorigenesis. Our long-term goal is to further elucidate and delineate how PAX3::FOXO1 cooperates with neural factors across cellular contexts to identify and assess novel therapeutic vulnerabilities to improve the lives of patients.

#2600 A human stem cell model of neuroblastoma reveals candidate drivers of tumorigenesis on chromosome 17q.

W. Fang[1], B. Cheng[2], S. J. Pastor[3], I. Barbaric[4], A. Tsakiridis[4], T. Porras[2], H.-W. Wu[2], C. Parekh[5], S. Asgharzadeh[1], J. M. Maris[3], M. Huang[1];

[1]University of Southern California, Los Angeles, CA, [2]Children's Hospital Los Angeles, Los Angeles, CA, [3]Children's Hospital of Philadelphia, Philadelphia, PA, [4]University of Sheffield, Sheffield, United Kingdom, [5]City of Hope, Duarte, CA

Background: In neuroblastoma (NB), the most common pediatric extracranial solid tumor, the most frequent genetic abnormality is gain of chromosome 17q (17q+), occurring in >80% of all patient tumors. 17q+, similar to amplification of the oncogene *MYCN*, is one of the hallmarks of high-risk NB, which has a 5-year survival rate of less than 50% and is associated with a high relapse rate. Although *MYCN*-driven mouse models of NB exist, mouse models incorporating human 17q+ cannot be established due to the misalignment of genes on mouse and human chromosomes. Thus, the mechanism by which 17q+ contributes to poor prognosis remains largely unknown.

Methods: Here, we used a human stem cell model of NB to investigate the effects of 17q+. Two human embryonic stem cell lines (H7 and H9), either wild-type (WT) or with spontaneous 17q+, were transduced with doxycycline-inducible *MYCN*, differentiated into sympathoadrenal cells (NB cell of origin), and implanted orthotopically into the renal capsule of immunocompromised mice fed doxycycline chow. RNA sequencing was performed on the resulting *MYCN*/17q+ and *MYCN*-driven NBs. *In vitro* proliferation of the tumor cells was assessed by Incucyte live-cell imaging and EdU incorporation assays.

Results: Although 17q+ alone was insufficient to generate NB tumors, *MYCN*/17q+ tumors compared to *MYCN*-only tumors exhibited higher penetrance (H7: 100% vs 60%; H9: 100% vs 50% at day 104 post-implantation) and shorter latency (median survival H7: 70 vs 90 days, p=0.0186; H9: 69 vs 114 days, p=0.0001, n=10 per arm), suggesting that *MYCN* and 17q+ cooperate in tumorigenesis. *MYCN*/17q+ tumor cells also showed increased proliferation and S-phase occupancy relative to *MYCN*-only tumor cells *in vitro*. Bulk RNA sequencing revealed an enrichment in the activation of ribosome biogenesis, cell cycle transition, and DNA replication pathways in *MYCN*/17q+ tumors. Additionally, 16 genes located on 17q were upregulated in *MYCN*/17q+ tumors compared to *MYCN*-only tumors, as well as in NB patients with 17q+ compared to 17q WT. We confirmed the protein-level overexpression of 6 of these 16 genes in *MYCN*/17q+ tumors: *BRIP1* (helicase), *TK1* (thymidine kinase), *BIRC5* (survivin), and *CDC6* (pre-replication complex subunit) directly regulate the cell cycle, while *IGF2BP1* (mRNA binding protein) stabilizes the *MYCN* transcript, and *CBX2* (polycomb repressive complex subunit) blocks neural differentiation. Indeed, individual knockdown of these 6 genes in *MYCN*/17q+ tumor cells reduced proliferation, and individual overexpression in *MYCN*-only tumor cells enhanced proliferation, implicating these genes in tumorigenesis.

Conclusion: A newly established human stem cell model of NB identified *BRIP1*, *TK1*, *BIRC5*, *CDC6*, *IGF2BP1*, and *CBX2* as candidate genes on chromosome 17q that cooperate in NB tumorigenesis.

#2601 KAT6A and B inhibition increases efficacy of differentiation and GD2 targeting immunotherapy in neuroblastoma.

Nina Weichert-Leahey[1], Alla Berezovskaya[1], Mark W. Zimmerman[1], Francesca Alvarez-Calderon[1], Ulrike Gerdemann[1], Silvi Salhotra[1], Nathaniel Mabe[1], Adam D. Durbin[2], Kimberly Stegmaier[1], Brian J. Abraham[2], A. Thomas Look[1]

[1]Dana-Farber Cancer Institute, Boston, MA,[2]St. Jude Research Hospital, Memphis, TN

Neuroblastoma is a malignancy of the peripheral sympathetic nervous system, accounting for 15% of childhood cancer deaths. Despite multi-modal treatment regimens and post-consolidation therapy, including anti-GD2 antibody treatment combined with retinoic acid, overall survival for patients with high-risk disease is only ~50%, and treatment-associated toxicity is immense. Thus, less toxic and more effective treatment options are desperately needed. Retinoic acid is clinically used to induce growth arrest of neuroblastoma cells and epigenetic rewiring of the enhancer landscape, leading to suppression of oncogenic MYCN expression. However, the epigenetic and growth-suppressing effects of retinoic acid are completely reversible, leading to tumor re-growth. Here, we sought to identify epigenetic modifiers that enhance the antiproliferative effects of retinoids in neuroblastoma. In a drug screen with 452 compounds targeting epigenetic modifiers, we identified PF-9363, an inhibitor of the histone H3K23 acetyltransferases KAT6A and B, as synergistically inhibiting neuroblastoma growth in combination with retinoic acid. Importantly, this growth suppression was durable and persisted beyond retinoid withdrawal in *in vitro* models, in contrast to retinoid treatment alone. Durable growth suppression was due to sustained downregulation of MYCN and the essential transcription factors PHOX2B and GATA3, caused by gene expression silencing through PRC2-dependent deposition of H3K27me3 across their regulatory enhancers. In NSG xenograft models of neuroblastoma, the KAT6A/B inhibitor PF-9363 alone demonstrated significant anti-tumor activity when given at 5 mg/kg daily by oral gavage and tumor volumes were significantly different compared to vehicle treated mice starting at day 4 through the end of treatment on day 28. Moreover, the combination treatment with retinoic acid and PF-9363 exhibited profound and sustained growth suppression compared to vehicle treated mice and exhibited the most sustained growth suppressing effect among all treatment conditions, persisting even after retinoic acid was withdrawn. This outcome was significantly different from retinoid treatment alone, where drug withdrawal resulted in rapid tumor proliferation in NSG mice. Reflecting the epigenetic reprogramming of these tumor cells, we observed that expression of GD2, a cell surface glycosphingolipid that is a target of antibody-based and CAR T cell therapies in neuroblastoma, was induced on $GD2^{low}$ neuroblastoma cells by treatment with retinoic acid plus PF-9363 both *in vitro* and *in vivo*, leading to increased effectiveness of anti-GD2 CAR T cell therapy in neuroblastoma. These studies nominate KAT6A/B inhibition as a novel approach to enhance the effectiveness of differentiation and GD2-immunotherapy in neuroblastoma and may be relevant for other tumors of neuro-ectodermal origin.

#2603 Targeting METTL3 as a novel therapeutic strategy for H3 G34-mutant gliomas.

Maria Eleftheriou[1], Eliza Yankova[1], Rebecca D. Haase[2], Antonella De Cola[1], Michael McNicholas[1], Sian Evans[1], Joao M. L. Dias[1], Natalie Webster[3], Georgia Tsagkogeorga[3], James Russell[1], Manav Pathania[1], Oliver Rausch[3], Mariella G. Filbin[2], Konstantinos Tzelepis[1]

[1]University of Cambridge, Cambridge, United Kingdom,[2]Dana-Farber Boston Children's Cancer and Blood Disorders Centre, Boston, MA,[3]Storm Therapeutics, Cambridge, United Kingdom

Background: Diffuse hemispheric glioma, H3 G34-mutant, is a pediatric-type high-grade glioma with limited treatment options and a poor prognosis. RNA-modifying enzymes, including METTL3, are critical for the initiation and maintenance of various cancers, including brain tumors. We previously presented STM2457, a first-in-class RNA-modifying enzyme inhibitor targeting the catalytic activity of METTL3, preventing m^6A deposition on mRNA. Here, we show small-molecule inhibition of METTL3 as a novel therapeutic strategy for H3 G34-mutant gliomas.

Aims: To study the therapeutic potential of pharmacological inhibition of METTL3 in preclinical models of H3 G34-mutant diffuse hemispheric gliomas (DHG).

Results: Using genome-wide CRISPR/Cas9 screening, we identified METTL3 as a genetic vulnerability in H3.3-G34-mutant primary murine glioma models. We confirmed that METTL3 is essential for the maintenance of glioma through genetic and pharmacological approaches in primary mouse and human glioma models, harboring H3.3-G34 mutations. Notably, METTL3 inhibition did not affect the proliferation of normal murine neuronal stem cells or isogenic primary murine glioma models with H3.3-K27M mutations. m^6A-immunoprecipitation-sequencing revealed downregulation of several stemness-related m^6A substrates, including *Nestin* and *Sox2*, upon pharmacological inhibition of METTL3. In H3.3-G34-mutant human and mouse glioma models, METTL3 inhibition disrupted key glioma pathways, suppressing translation of stem cell-related markers, like Nestin and Sox2, while upregulating glioma differentiation markers, such as Tubb3. In parallel, the transcriptional levels of these markers remained unaltered, highlighting that the effects are primarily on the mRNA translation of glioma-associated m^6A substrates. Notably, pharmacological inhibition in patient-derived samples with H3.3-G34 mutations impaired growth and suppressed the same molecular pathways consistent with our epitranscriptomic and proteomic analyses. Orthotopic transplantation of primary murine H3.3-G34R glioma cells with CRISPR/Cas9-mediated *METTL3* knockout into NSG mice significantly extended survival compared to controls. Importantly, pharmacological inhibition of METTL3 in an H3.3-G34V patient-derived xenograft model recapitulated this effect, significantly prolonging mouse lifespan and validating METTL3 as a therapeutic target in these gliomas.

Conclusions: In summary, here we demonstrate that pharmacological inhibition of METTL3 is a potent therapeutic strategy for H3 G34-mutant gliomas. To our knowledge, this is the first study that reveals that the catalytic function of METTL3 is a critical epitranscriptomic factor in H3 G34-mutant gliomagenesis. Our findings provide strong proof-of-concept that targeting RNA-modifying enzymes offers a promising therapeutic approach for this aggressive glioma subtype.

#2606 In vivo screening reveals therapeutic vulnerabilities in a patient-derived xenograft established from an intraosseous TFCP2-rearranged rhabdomyosarcoma.

Erika Dobrota[1], M. Reza Saadatzadeh[1], Barbara Bailey[1], Keiko Kreklau[1], Kathy Coy[2], Felicia Kennedy[2], Melissa Trowbridge[2], Anthony Sinn[2], Chris Davis[1], Michael Ferguson[3], Steve Angus[1], Pankita Pandya[3], Karen Pollok[3]

[1]Herman B. Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN,[2]Indiana University Simon Comprehensive Cancer Center Preclinical Modeling and Therapeutics Core, Indiana University School of Medicine, Indianapolis, IN,[3]Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Indiana University School of Medicine, Indianapolis, IN

Primary intraosseous rhabdomyosarcoma (IORMS) is a rare, metastatic, spindle cell-like rhabdomyosarcoma (RMS) subset that occurs in craniofacial bones and less commonly in long bones, or pelvis. IORMS patients typically succumb to the disease within 15 months of diagnosis. This disease is distinguished by specific gene fusions, such as FUS-TFCP2 which blocks myogenic differentiation, inhibits DNA repair, and induces transcription of Anaplastic Lymphoma Kinase (ALK) and Telomerase Reverse Transcriptase (TERT). Although case reports of treatment with ALK inhibitors (ALKi) have shown modest results, resistance always emerges and no other treatment options beyond surgery and chemotherapy exist. The ability to understand disease pathogenesis and identify actionable targets for rare RMS subsets is hindered by the lack of appropriate pre-clinical in vivo models. Thus, there is a critical need for developing such models to identify therapeutic options that will block disease progression, enhance quality of life, and ultimately achieve a cure for these patients. In collaboration with the Pediatric Cancer Precision Genomics Program at the Riley Hospital for Children, we developed a set of FUS-TFCP2 fusion-containing IORMS patient-derived xenografts (PDXs). PDX174 was derived prior to ALKi treatment (lorlatinib) and PDX199 from the same patient following disease progression after 16 months of lorlatinib therapy. Consistent with IORMS characterization, PDX174 transcriptome shows significantly increased expression of ALK and TERT, 10.6 and 14.8-fold respectively, compared to normal human skeletal muscle cells. ALK overexpression was confirmed by western blot but was notably absent in the subsequent PDX199 model. To further investigate the PDX174 model, we conducted an in vivo screen of six different small molecule inhibitors (SMIs) based on previously prioritized biomarkers of therapeutic response. Bivalent BET inhibitor (BETi), AZD5153, resulted in an initial block in tumor growth ($p<0.05$) compared to the vehicle and other SMIs. However, resistance emerged after four weeks of AZD5153 treatment. In addition, while CDK4/6 inhibitor, palbociclib, and CHK1 inhibitor, SRA737, induced moderate inhibition of tumor growth, drug resistance quickly followed within 2 weeks. Global kinome analysis indicated a significant increase in the mitogen- and stress-activated kinase 1 (MSK1) in AZD5153-resistant tumors compared to the vehicle group, revealing a potential therapeutic vulnerability in the BETi-resistant tumors. IORMS models such as PDX174/PDX199 are crucial for revealing actionable molecular signatures before and after targeted therapy and will increase our mechanistic understanding of tumor adaptive responses and aid in designing therapies that mitigate the emergence of therapeutic resistance.

#2607 Multi-omic profiling reveals subtype-specific signaling mechanisms in pediatric B-cell acute lymphoblastic leukemias.

Irina Pushel[1], Zachary Clark[2], Michaella J. Rekowski[2], Lisa A. Lansdon[1], Byunggil Yoo[1], Keith J. August[1], Alan S. Gamis[1], Nicole M. Wood[1], Michael P. Washburn[2], Midhat S. Farooqi[1]

[1]Children's Mercy Kansas City, Kansas City, MO,[2]University of Kansas Medical Center, Kansas City, KS

Genetic subtyping has improved the accuracy of diagnosis, and in turn outcomes, for children with B-cell acute lymphoblastic leukemia (B-ALL). Currently, clinical subtyping is based primarily on mutations in, or fusions involving, known driver genes. This approach is restricted in its power, particularly for subtypes such as *BCR::ABL1*-like B-ALL, which is largely characterized by its transcriptional similarity to *BCR::ABL1*+ cases, but has numerous possible driver fusions. Moreover, even transcriptomic profiling does not fully capture the molecular processes driving leukemogenesis, which are carried out by proteins, yet proteomics has been relatively unexplored in pediatric B-ALL to date. To bridge this gap, we compare genomic, transcriptomic, proteomic, and phosphoproteomic profiles between pediatric patients with *BCR::ABL1*-like and *ETV6::RUNX1* B-ALL to characterize subtype-specific molecular mechanisms. For each subtype, we selected at least five blood or bone marrow aspirates collected from patients at diagnosis. We combined genomic findings from clinical assays (karyotyping, FISH, microarray, and whole exome sequencing) with total RNA sequencing (Illumina). Additionally, we generated global proteomic and phosphoproteomic profiles using label-free quantitation. Peptides were enriched for phosphorylation using the SMOAC method, and all samples were run on an Orbitrap Ascend mass spectrometer (Thermo) using data dependent acquisition. Differential gene expression analysis comparing the two subtypes showed elevated expression of *CRLF2* in *BCR::ABL1*-like cases, consistent with clinical data and prior subtype characterization. Pathway enrichment of the 684 differentially expressed genes ($p < 0.05$) revealed upregulation of cell differentiation in *ETV6::RUNX1* samples, but otherwise provided limited mechanistic insights. Differentially expressed proteins ($n = 644$, $p < 0.05$) showed upregulation of translation-related processes in *ETV6::RUNX1* samples, and calcium-dependent signaling in *BCR::ABL1*-like samples. Kinase activity prediction based on phosphoproteomic data showed significantly higher activity of calcium/calmodulin-dependent protein kinases (CAMKs), including CAMK2B and CAMK4, in *BCR::ABL1*-like samples, which is not observed in corresponding gene expression between subtypes. There is a growing body of work suggesting a role for CAMKs in leukemias, though they have not previously been implicated in *BCR::ABL1*-like pediatric B-ALLs. Using a multi-omic profiling approach, we demonstrate that proteomic and phosphoproteomic data reveal previously undescribed subtype-specific activation of signaling cascades in pediatric B-ALL, which are not captured by genomic or transcriptomic assays. These insights add to our understanding of subtype-specific mechanisms, and can facilitate the identification of novel treatment targets.

#2608 The super enhancer-driven long noncoding RNA lncNB promotes neuroblastoma tumorigenesis by interacting with MSI2 protein and is targetable by small molecule compounds.

Sujanna Mondal[1], Pei Y. Liu[1], Janith Seneviratne[1], Antoine De Weck[1], Pooja Venkat[1], Chelsea Mayoh[1], Jing Wu[1], Jesper Maag[2], Jingwei Chen[1], Matthew Wong[1], Nenad Bartonicek[2], Poh Khoo[1], Lei Jin[3], Louise E. Ludlow[4], David S. Ziegler[5], Toby Trahair[1], Pieter Mestdagh[6], Belamy B. Cheung[1], Jinyan Li[7], Marcel E. Dinger[8], Ian Street[1], Xu D. Zhang[3], Glenn M. Marshall[1], Tao Liu[1]

[1]Children's Cancer Institute Australia, Sydney, Australia,[2]Garvan Institute of Medical Research, Sydney, Australia,[3]University of Newcastle, Callaghan, Australia,[4]Murdoch Children's Research Institute, Melbourne, Australia,[5]Kids Cancer Centre, Sydney, Australia,[6]Center for Medical Genetics Ghent, Ghent, Belgium,[7]Shenzhen Institute of Advanced Technology, Shenzhen, China,[8]The University of Sydney, Sydney, Australia

Background: While *MYCN*-amplified neuroblastoma has been the focus of neuroblastoma research in the past three decades, most human neuroblastomas do not harbour *MYCN* oncogene amplification, and their tumorigenic factors are unknown. Long non-coding RNAs (lncRNAs) regulate tumorigenesis by modulating the expression of molecular targets, however, there is limited literature on therapeutic targeting of lncRNAs with small molecule compounds.

Aims: To determine the oncogenic mechanism though which the lncRNA lncNB promotes neuroblastoma cell proliferation, survival and tumour progression; and to identify a small molecule compound that inhibits the interaction between lncNB and its binding protein MSI2 as an effective anticancer strategy.

Methods and results: Kaplan Meier analysis showed that high levels of lncNB expression in neuroblastoma tissues correlated with poor prognosis in 476 patients. We identified lncNB as the lncRNA most over-expressed in *MYCN* non-amplified, compared with *MYCN*-amplified, neuroblastoma cell lines. lncNB expression was controlled by super-enhancers, and lncNB RNA bound to MSI2 protein. RNA immunoprecipitation and sequencing identified BMX mRNA as the transcript most significantly disrupted from binding to MSI2 protein, after lncNB knockdown. lncNB or MSI2 knockdown reduced, while their over-expression enhanced, BMX mRNA stability and expression, ERK protein phosphorylation and *MYCN* non-amplified neuroblastoma cell proliferation. lncNB knockdown significantly suppressed neuroblastoma progression in mice. AlphaScreen of a compound library identified NSC617570 as an efficient inhibitor of lncNB RNA and MSI2 protein interaction, and NSC617570 reduced BMX expression, ERK protein phosphorylation, neuroblastoma cell proliferation *in vitro* and tumor progression in mice.

Conclusions: Our study demonstrates that lncNB RNA interacts with MSI2 protein to induce neuroblastoma tumorigenesis, and that targeting lncNB and MSI2 interaction with small molecule compounds is an effective anticancer strategy.

#2611 Cellular origin of DICER1 tumor predisposition syndrome informed by lineage-traceable genetically engineered mouse model.

Joyce Zhang[1], Felix Kommoss[1], Branden Lynch[1], Shary Chen[1], Janine Senz[1], Yana Moscovitz[1], Lesley Ann Hill[2], Wilder Scott[3], Jonathan Bush[4], William Foulkes[5], Gregg Morin[6], Michael Underhill[2], Yemin Wang[1], David Huntsman[1]

[1]Molecular Oncology, BC Cancer Research Centre, Vancouver, BC, Canada,[2]Cellular and Physiological Sciences, University of British Columbia, Vancouver, BC, Canada,[3]Sunnybrook Research Institute, Toronto, ON, Canada,[4]Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada,[5]Department of Human Genetics, McGill University, Montreal, QC, Canada,[6]Medical Genetics, University of British Columbia, Vancouver, BC, Canada

Introduction: DICER1 tumor predisposition syndrome is a genetic disorder driven by germline *DICER1* pathogenic variants that predisposes pediatric and young adult patients to cancers of various organs. The second, missense mutation in RNase IIIb domain leads to systemic loss of mature 5p-miRNAs. Tumours of various sites are histologically and molecularly similar, suggesting a shared cellular origin and oncogenic mechanisms. To this end, we expanded our first-ever genetically engineered mouse model (GEMM) that recapitulated human Müllerian adenosarcomas, a DICER1 neoplasm, through inducing *Dicer1* mutations in a lineage-traceable, tamoxifen inducible, Hypermethylated in Cancer 1 (Hic1)-creERT2-driven mouse strain. Hic1 marks mesenchymal progenitors. This GEMM histologically resembles DICER1-renal neoplasms such as cystic nephroma and anaplastic sarcoma. We leveraged single-cell whole transcriptomics (scRNA-seq) and Xenium spatial transcriptomics platform (379 genes) to uncover cellular origin and hierarchy of Dicer1 sarcomagenesis.

Methods: We profiled normal kidney mesenchymal stromal cells (n=3 for scRNA-seq, n=3 for Xenium) and mesenchymal tumor components (n=5 for scRNA-seq, n=25 for Xenium). Trajectory analysis deconvoluted hierarchy of sarcomagenesis.

Results: In normal mesenchymal stromal niche, scRNA-seq identified 5 Hic1+ clusters including 2 clusters of universal fibroblasts (UniFibro): *Pi16*^{high} and *Col15a1*^{high}, and a cluster of *Dpt*$^{-}$ *Itga8*$^{+}$ fibroblasts. Spatially,*Pi16*^{high} UniFibro are enriched in renal vascular niche, whereas *Col15a1*^{high} UniFibro reside in subepithelial basement membrane. ScRNA-seq of tumours revealed a *Col15a1*^{high} *Mfap4*^{high} "Progenitor" cluster that is transcriptionally similar to control *Col15a1*^{high} UniFibro, suggesting the latter represent putative progenitors of the tumors. ScRNA-seq also identified a "ground" population that is transcriptionally similar to control *Dpt*$^{-}$*Itga8*$^{+}$ kidney fibroblasts, one cluster of *Pax7*$^{+}$ *satellite cells,* a committed Myog^{+} muscle cluster and a highly proliferative cluster. Pseudotime trajectory analysis showed *Col15a1*^{high} *Mfap4*^{high} progenitors can gives rise to a myogenic lineage that progresses via *Pax7*$^{+}$ transitional state and holds the potential to progress to highly proliferative sarcoma cells via "Ground" state of cells. Spatially, "Progenitor" cells have low-grade morphology, supporting their role in cancer initiation. Lastly, scRNA-seq of tumors revealed the expansion of *Col15a1*^{high}*Mfap4*^{high} UniFibro but not *Pi16*^{high} ones into the "Progenitor" cells.

Conclusion: Single cell profiling of renal tumours from *Dicer1*-GEMM supports *Mfap4*^{high} *Col15a1* ^{high}universal fibroblasts as cell of origin, and delineates progression underlying DICER1 sarcomagenesis, thereby providing opportunities for therapeutic intervention and mechanistic studies.

#2612 Elucidation of PAX3::FOXO1 initiation mechanisms in human induced pluripotent stem cell models.

Bradley T. Stevens, Yang Zhang, Jamaica F. Siwak, Shondra M. Pruett-Miller, Brian J. Abraham, Mark E. Hatley

St. Jude Children's Research Hospital, Memphis, TN

Rhabdomyosarcoma (RMS) is the most common pediatric soft tissue sarcoma. Alveolar RMS (ARMS), an aggressive subtype of RMS, is driven by either t(2;13)(q35;q14) or t(1;13)(p36;q14) resulting in the PAX3-FOXO1 (P3F) or PAX7-FOXO1 (P7F) fusion oncoproteins. Despite rigorous scientific advances and clinical trials, the survival rate for high-risk ARMS has remained at 20% over the last three decades. A deeper understanding of the basic biology driving P3F mediated tumorigenesis is needed to discover novel therapeutic targets. The P3F oncofusion protein is the key oncogenic driver and a potential candidate for therapeutic intervention. Current *in vivo* model systems fail to faithfully recapitulate the human disease in terms of timing and location and are complicated by the formation of other tumor types. A deeper understanding of the cells of origin and mechanisms of P3F tumor initiation are essential to enhancing models and preclinical efforts. Previously, our lab generated an ARMS model system derived from *TP53*-null human induced pluripotent stem cells (iPSCs), in which forced P3F expression during endothelial cell directed differentiation blocked endothelial maturation and instead reprogrammed cells to skeletal muscle-like cells that form ARMS tumors in mice. Generation of iPSC-derived ARMS (iARMS) occurs over 15 days and provides a unique model to determine the mechanism of P3F mediated transformation from non-myogenic origins. To elucidate this mechanism, I utilized CUT&RUN for P3F and H3K27ac to assess P3F occupancy and enhancer landscape, ATAC-seq to assess chromatin accessibility, and RNA-seq to assess gene expression changes at timepoints throughout the transformation event. These data define the fundamental genomic changes required for transformation and uncovers the key determinants of ARMS identity. Removal of P3F utilizing a guide RNA targeting the P3F breakpoint allowed iARMS cells to form endothelial-like tubes *in vitro*, indicating RMS cells retain endothelial potential after oncogene withdrawal. Finally, to explore alternative cells of origin and endogenous regulation of P3F during development, we created t(2;13) iPSCs (tiPSCs). In proof of principle experiments tiPSCs formed teratomas with discrete regions of ARMS in immunocompromised mice and differentiation of tiPSCs into muscle organoids revealed an abrogation in normal developmental trajectory upon P3F induction. Our novel cellular models provide a unique platform to uncover how P3F establishes and maintains ARMS cell state, dissect the specific dependencies required to maintain the ARMS cell state, and uncover how P3F is endogenously regulated during development. Understanding the fundamental mechanism of P3F-driven tumorigenesis from translocation regulation to transformation provides greater resolution into the key disease determinants to generate faithful model systems to focus pre-clinical efforts.

#2613 The role of the RNA methyltransferase RNAMT1 as a tumorigenic driver and therapeutic target in neuroblastoma.

K. Chok[1], K. Ip[2], J. Wu[2], S. Gadde[2], S. Mondal[2], J. Wong[3], B. Cheung[4], T. Liu[5];
[1]Children's Cancer Institute Australia and School of Women's and Children's Health, UNSW, Sydney, Australia, [2]Children's Cancer Institute Australia, Sydney, Australia, [3]Epigenetics and RNA Biology Program Centenary Institute and Sydney Medical School, Sydney, Australia, [4]Children's Cancer Institute Australia and School of Clinical Medicine, UNSW Medicine & Health, UNSW Sydney, Sydney, Australia, [5]Children's Cancer Institute Australia and University of New South Wales Centre for Childhood Cancer Research, Sydney, Australia

Neuroblastoma is the most common extracranial solid tumor in children worldwide. We have found that high expression of the *RNAMT1* gene in tumor tissues is associated with poor prognosis in both *MYCN*-amplified [Overall survival (OS): $p = 0.0409$ and event-free survival (EFS): $p = 0.0061$] and *MYCN* non-amplified neuroblastoma patients (OS: $p < 0.0001$ and EFS: $p < 0.0001$). Multivariable Cox regression analysis for neuroblastoma patient prognosis of RNAMT1 gene expression [OS: hazard ratio (HR) = 1.92, $p = 0.003$ and EFS: HR = 1.72, $p = 0.0001$] is independent from current prognostic markers: MYCN amplification (OS: HR = 3.39, $p = 5.1E^{-8}$ and EFS: HR = 1.77, $p = 0.0001$), age older than 18 months (OS: HR = 2.73, $p = 2.9E^{-5}$ and EFS: HR = 1.59, $p = 0.01$), and International Neuroblastoma Staging System stages 3&4 (OS: HR = 5.68, $p = 5.00E^{-6}$ and EFS: HR = 3.00, $p = 6.83E^{-8}$). Doxycycline (DOX)-inducible RNAMT1 knockdown in *MYCN*-amplified and MYCN non-amplified neuroblastoma cells decreased cell viability and colony formation capability, and induced apoptosis *in vitro*. DOX-inducible RNAMT1 knockdown delayed tumor growth and improved survival in mice bearing SK-N-BE(2)-C shRNA1 (OS: $p = 0.0034$) and SK-N-AS shRNA2 (OS: $p < 0.0001$). RNAMT1 regulates protein translation based on previous literatures, we demonstrated that RNAMT1 did not affect global mRNA translation alteration via puromycin incorporation assay and slightly affected polysome profile. The subset of mRNA transcripts after RNAMT1 knockdown and RNAMT1 inhibitor treatment will be analyzed with translating ribosome affinity purification sequencing (TRAP-Seq). RNAMT1 inhibitor was discovered by AlphaScreen of 2952 small molecule compounds provided by National Cancer Institute (NCI). Unravelling the molecular mechanisms of RNAMT1 in neuroblastoma tumorigenesis and mechanism of actions of RNAMT1 inhibitor, may provide novel insights to develop a more effective treatment for neuroblastoma patients.

#2615 The Open Single-cell Pediatric Cancer Atlas project: Collaborative analysis of pediatric tumor data.

Joshua A. Shapiro, Stephanie J. Spielman, Allegra G. Hawkins, Deepashree Venkatesh Prasad, Jennifer O'Malley, David S. Mejia, Jaclyn N. Taroni

Childhood Cancer Data Lab, Alex's Lemonade Stand Foundation, Wynnewood, PA

The Open Single-cell Pediatric Cancer Atlas (OpenScPCA) project is an open, collaborative project created to analyze publicly available data from the Single-cell Pediatric Cancer Atlas (ScPCA) Portal, with the goal of improving the quality and usability of single-cell pediatric cancer data and driving insights into pediatric cancer biology through deeper analysis of available data sets.

The ScPCA Portal (https://scpca.alexslemonade.org/), developed and maintained by Alex's Lemonade Stand Foundation (ALSF), is an open-source data resource for single-cell and single-nuclei RNA sequencing data of pediatric tumors. The ScPCA Portal currently contains summarized gene expression data for over 680 samples from a diverse set of over 50 types of cancers. All data on the portal is publicly available, uniformly processed with an open-source workflow, and ready for download in formats compatible with popular single-cell data analysis frameworks.

To coordinate further analysis of the ScPCA data, we launched the OpenScPCA project in April 2024. The project aims to engage a broad community of researchers analyzing genomic data from pediatric tumors, building off the previous success of the Open Pediatric Brain Tumor Atlas project (Shapiro et al. 2023). As part of the OpenScPCA project, ALSF provides contributors with support through technical guidance, comprehensive documentation, and access to computational resources.

We focused our initial collaborative efforts on the challenge of labeling cell types in pediatric cancer samples. Automated methods often have limited utility for annotating malignant cells in these samples because many tools and references for single-cell analysis were designed with adult healthy tissues or cancer types in mind. To overcome this limitation, we engage experts in various pediatric cancers who contribute their knowledge and analytical code. All contributions are publicly reviewed on GitHub for scientific content and code quality. In addition, we use automated testing to ensure that the annotation pipelines are robust and reusable. These combined efforts allow us to build a public resource to help improve pediatric cancer cell type annotation in this project and beyond.

As we continue this ongoing work, we invite researchers with expertise in pediatric cancer gene expression and single-cell RNA-seq analysis to join the OpenScPCA Project. We hope those participating will discover new datasets to advance their research, gain experience with cutting-edge technologies, build their research portfolios, and join a supportive community. Get started at https://openscpca.readthedocs.io/en/latest/.

#2616 Epigenetic targeted therapies induce unique MHC-I epitopes identified by immunopeptidome analysis in low mutation-burden sarcomas.

Alice Recho[1], Grace Huang[2], Himavanth R. Gatla[1], Erin E. Resch[1], Stephanie Glavaris[1], Maggie Phillips[1], Isaac Woodhouse[3], Pouya Faridi[3], Brian H. Ladle[1]

[1]Sidney Kimmel Comprehensive Cancer Center, Department of Oncology, Division of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD,[2]Department of Medicine, School of Clinical Sciences, Monash University, Clayton, Australia,[3]Centre for Cancer Research, Hudson Institute of Medical Research and Monash University, Clayton, Australia

Pediatric sarcomas as a group are poorly immunogenic tumors with low mutation burdens and poor responsiveness to immunotherapies such as immune checkpoint inhibitors (ICI). We have been investigating the use of the hypomethylating agent decitabine (DAC) followed by histone deacetylase inhibitor entinostat (Enti) in our murine models of sarcoma to potentially increase the tumor immunogenicity. Using our Kras-driven murine sarcoma KP Sarc, a whole-cell vaccine using DAC and Enti treated tumor cells generates a T-cell dependent, tumor-specific immune response restricted to epigenetically regulated antigens further potentiated with addition of ICI. We showed that epigenetically regulated antigens are shared between unrelated tumors KP Sarc and sarcoma M3-9-M and allow for cross-protection after vaccination. We hypothesized that epigenetically upregulated genes are persistent in vivo and can be used as tumor-specific targets identified by immunopeptidomics. We treated KP Sarc-bearing mice in vivo with DAC or Enti for 5 days, or sequentially with DAC then Enti. Tumors harvested at the end of treatment showed upregulation of the cancer testis antigens (CTA) in DAC- and DAC+Enti-treated mice by expression density analysis and immunohistochemistry compared to no expression in untreated and Enti-treated tumors. Moreover, these changes persisted a week after DAC treatment. We examined the gene expression profile of KP Sarc by RNA sequencing (RNA-Seq). We determined that the sequential in vitro treatment of KP Sarc with DAC then Enti (DAC+Enti) leads to a higher upregulation of CTAs and inflammatory pathways than either treatment alone. We treated human rhabdomyosarcoma (RH30 and RH41) and Ewing sarcoma (CHLA-9) cell lines showing upregulation of 75 shared antigens with DAC+Enti treatment. To investigate changes induced in antigen presentation, we examined the immunopeptidome of KP Sarc under two conditions: Interferon gamma (IFN-γ) treatment alone (n=4) and DAC+Enti+IFN-γ (n=4). Following immunoprecipitation of MHC I, bound peptides were identified via liquid chromatography-mass spectrometry. We combined this analysis with whole transcriptome RNA-seq of those samples. Analysis revealed a total of 4,171 peptides across all conditions and replicates, sourcing from 3,393 proteins, which were matched to the mouse proteome database. On average, 3,417 epitopes were identified in the control group compared to 3,912 epitopes in the treated group. 526 MHC I-bound peptides were unique to the treated group, with no unique peptides observed in the control. We identified epitopes from numerous proteins beyond CTAs indicating broad changes in antigen processing and antigenic peptides in the DAC+Enti treated cells. Further studies will validate the immunogenicity and ways to increase immune responses targeting these shared epigenetically regulated epitopes in sarcomas.

#2617 Uncovering genomic complexity of PAX5 internal tandem duplication using short-read and long-read sequencing.

Yanling Liu[1], Bensheng Ju[1], Li Dong[1], Melanie Loyd[1], Yuan Feng[1], Heather Mulder[1], Pandurang Kolekar[1], Li Fan[1], Hanxia Li[1], Scott Foy[1], Geoff Neale[1], Patrick A. Brown[2], William L. Carroll[3], Mignon L. Loh[4], John Easton[1], Xiaotu Ma[1]

[1]St. Jude Children's Research Hospital, Memphis, TN,[2]Bristol Myers Squibb, Princeton, NJ,[3]NYU Langone Health, New York, NY,[4]Seattle Children's Hospital, Seattle, WA

PAX5 is known to be frequently mutated in childhood B-cell precursor acute lymphoblastic leukemia (B-ALL) via several molecular mechanisms, including chimeric fusion, focal deletion, and point mutations. Among these, patients with PAX5 internal tandem duplication (PAX5-ITD) are reported to have a poor outcome. Unlike other common ITDs (e.g., FLT3), PAX5-ITD often involves gain of more than one copy of involved exons, and the exact nature of these alterations remain elusive. Here we aim to investigate the following questions: 1) what are the typical copy number gains in DNA and RNA among PAX5-ITD cases? 2) are PAX5-ITD always in-frame? 3) are these aberrations a result of multi-step or single-step events? Short-read whole genome sequencing (WGS) and RNA sequencing (RNA-seq) and PacBio ISO-seq, a state-of-the-art long-read sequencing method, are used in this study. By integrative analysis of WGS and RNA-seq data from a relapse cohort (n=584) and a non-relapse cohort (n=1055), we identified PAX5 mutation rate of ~30% in relapsed patients versus that of ~20% in non-relapsed patients. Among the various PAX5 mutation types, the PAX5-ITD mutation shows the greatest degree of enrichment (P=3e-6) in relapsed patients (n=21), compared with those who remain in remission (n=5). Copy number gains in PAX5-ITD cases ranged from 3 to 25 and involved four exon combinations within the gene: E5, E3-E5, E2-E5 and E1-E5 and concordant copy number gains were detected between diagnosis tumor and relapse tumor. We observed a negative correlation between the number of copy gains and the length of duplicated genomic regions. Furthermore, 11 out of the 26 PAX5-ITD cases exhibited multiple structural variants (SVs) and 1 case harbored 2 somatic SNV in ITD region. To investigate the transcriptional consequences of these SV events, we performed ISO-seq on 7 PAX5-ITD cases with available specimen. By mapping SVs and SNVs to full-length RNA transcripts, we observed complex sequences of events driving the formation of ultimate PAX5-ITD in 3 cases. Besides, copy number analysis on full-length RNA transcripts revealed variable copy number gains within tumors due to alternative splicing. Additionally, we identified neo-splice sites in 2 cases, indicating that the splicing process was influenced by the altered DNA context created by SVs. Finally, our data indicated that all PAX5-ITDs can transcribe in-frame mRNAs, suggesting activating nature of these mutations. These findings provide valuable insights into the genomic complexity of PAX5-ITD and highlight the diverse transcriptional and splicing outcomes due to these alterations.

#2618 Methylation-based epigenetic "switches" in group 3/4 medulloblastoma.

Muhammad Awais Khan[1], Arsalan Riaz[2], Faisal F. Khan[2]

[1]Khyber Medical College, Peshawar, Pakistan,[2]Precision Medicine Lab, Peshawar, Pakistan

Medulloblastoma (MB) is the most common primary brain tumor in children, with group 3 and group 4 (G3/4) being the most aggressive. Methylation studies have identified eight distinct subgroups within G3/4 MB, with observed differences in survival patterns. However, the contribution of specific methylation-based epigenetic changes to the ontogeny of the disease remains poorly understood. We analyzed publicly available methylation profiles of 1,370 G3/4 MB samples spanning all subgroups. To identify methylation probes important for classifying G3/4 MB into subgroups, we employed a wrapper-based feature selection machine learning (ML) algorithm on 400 samples. Subsequently, we trained a k-nearest neighbor (kNN)-based ML model to classify the remaining 970 samples, evaluating the sensitivity and specificity of the selected methylation probes. The kNN model achieved an accuracy of 90.41% using a panel of 541 methylation probes. We further refined this panel to 179 probes based on sensitivity and specificity metrics and observed a sequential alteration in beta values, suggesting a common developmental lineage for these subgroups. To identify specific genes driving the differentiation of the cells of origin or their transition to a cancerous state via methylation mechanisms, we selected a cohort of 460 samples that best represented the subgroups. Matched expression profiles for 103 samples were integrated with methylation data to infer candidate genes. To determine the spatiotemporal origins of these subgroups, we performed gene set enrichment analysis (GSEA) using published spatiotemporal gene sets of the developing human cerebellum. Our findings suggest that distinct subgroups originate from the Rhombic Lip Ventricular Zone, Intermediate Zone, Sub-ventricular Zone, and Choroid Plexus Epithelium. Further GSEA traced the cells of origin to Rhombic Lip - Progenitor, Unipolar Brush Cell - Progenitor, Differentiating and Defined, and Excitatory Cerebellar Neuron - Defined. In summary, we identified a prioritized methylation signature capable of accurately classifying G3/4 MB into subgroups and developed an R package, PriorityMeth, for further analysis. We also propose a developmental model outlining the origins of the subgroups of G3/4 MB and provide insights about the probable methylation-based epigenetic “switches” that drive the ontogeny of this group.

#2619 Exploring the genomic landscape of pediatric chronic myeloid leukemia stem cells.

Ryan S. Sathianathen[1], Julietta Picco[2], Hye Na Kim[1], Minyoung Youn[1], Ramesh Nair[3], Nobuko Hijiya[4], Kathleen M. Sakamoto[1]

[1]Pediatrics, Stanford School of Medicine, Stanford, CA,[2]Biology, San Jose State University, San Jose, CA,[3]Genetics, Stanford School of Medicine, Stanford, CA,[4]Pediatrics, Columbia University Irving Medical Center, New York, CA

Pediatric chronic myeloid leukemia (CML) accounts for 2-9% of leukemias in children. CML in children has been reported to present with a more aggressive clinical manifestation than adult CML, including a higher white blood count and larger spleens. The molecular basis for these clinical differences remains unknown. Therefore, we hypothesize that the molecular pathogenesis of pediatric CML differs from that of adult CML. Furthermore, CML relapse and resistance to tyrosine kinase inhibitors are due to the persistence of CML stem cells. Bulk RNA-seq data with mRNA from bone marrow (BM) CD34+ cells previously demonstrated differential gene expression and uniquely enriched pathways in pediatric CML compared to adult CML stem cells. Our data also showed significant downregulation of Rho pathway-related genes such as *ARGHAP27*, *VAV2,* and *DLC1*, suggesting different molecular pathways in pediatric and adult CML stem cells. To study the clonal heterogeneity and diversity of pediatric CML stem cells, we performed single-cell RNA seq on CD34+ cells from pediatric CML (n=7) and healthy pediatric (n=2) BM samples using the Stem Pro $CD34^+$ system (Miltenyi Biotec, Inc.) and Chromium Next GEM Single Cell 3' kit from 10X Genomics. Our preliminary data show 13 clusters from CML and healthy $CD34^+$ cells based on gene expression. Overall, CML clusters show a significant downregulation of immune related genes compared to healthy BM clusters. Pathway enrichment analysis shows differential enrichment of immune-related and inflammatory signaling pathways, such as the immune activation and T cell inhibitory pathways in pediatric CML. Our results suggest that immune dysregulation in pediatric CML stem cells contributes to disease pathogenesis. Current work focuses on characterizing 1) the role of immune-related genes in pediatric CML stem cells and their impact on T cell response and 2) cell surface markers of pediatric CML stem cells to identify potential targets for immunotherapy.

#2620 m^{6}A demethylase ALKBH5 regulates medulloblastoma growth and progression.

Panneerdoss Subbarayalu[1], Daisy Medina[1], Pitta V. Prabhakar[1], Shahad Abdulsahib[1], Saif R. Nirzhor[1], Desiree Denman[1], Santosh Timilsina[1], Deepika Singh[1], Phat Do[1], Krishna Evani[2], Dhiya Billa[3], Esha Reddy[4], Meera Nair[5], Peter Houghton[1], Yogesh Gupta[1], Yidong Chen[1], Suryavathi Viswanadhapalli[6], Ratna K. Vadlamudi[6], Gangadhara R. Sareddy[6], Andrew Brenner[6], Manjeet K. Rao[1]

[1]UT Health San Antonio Greehey Children's Cancer Res. Inst., San Antonio, TX,[2]UT San Antonio, San Antonio, TX,[3]Health Careers High School, San Antonio, TX,[4]Southern Methodist University, Dallas, TX,[5]Brennan High School, San Antonio, TX,[6]UT Health San Antonio, San Antonio, TX

Introduction: Medulloblastoma (MB) is pediatric brain cancer originate from the cerebellum, and accounts for large number of all childhood cancer related deaths. MB grows vigorously, and spreads to central nervous system (CNS) through cerebrospinal fluid, leading to leptomeningeal metastases in 66% brain cancer patients. MB are commonly treated with a combination of surgery, radiation, and chemotherapy drugs like vincristine. Unfortunately, at the young age, radiation exposure and chemotherapy treatment lead to high toxicity which ultimately compromise the quality of life for those kids. Therefore, it is critical to understand the molecular mechanism that regulates MB tumor growth, metastasis and relapse so that safe and novel therapeutics can be developed to treat pediatric MB. In this study, we investigated the role of RNA demethylase ALKBH5, in regulating MB growth and progression.
Methods: Medulloblastoma cell lines including HD-MB03, D556, D425 and DAOY were purchased from the ATCC. MB cells were transfected either with ALKBH5 overexpression (OE) plasmid or siRNA (KD). These OE/KD MB cells were analyzed for cell viability, migration, invasion, colony formation, cell cycle, apoptosis assays, RNA sequencing, RT-qPCR, western blotting, RNA immunoprecipitation, and *in vivo* tumor xenograft study.
Results: Our results showed that depletion of ALKBH5 significantly reduced short term cell viability, migration, invasion and colony forming ability of MB cells. ALKBH5 knockdown MB cells showed increased apoptosis. Further, our results show that ALKBH5 silencing suppressed the self-renewal/proliferation of MB cancer stem cells (MBCSC), including medullosphere formation. In orthotopic xenograft study, ALKBH5 knockdown cells intracranially injected into mouse showed reduced tumor growth compared to the scrambled siRNA transfected MB cells. To understand the molecular mechanism of ALKBH5, we performed transcriptome analysis of ALKBH5 depleted MB cells. Silencing of ALKBH5 showed alterations in several metabolism related pathways. Further, ALKBH5-depletion led to significantly decreased levels of cancer stem cell marker proteins including Nanog, OCT4 and SOX9.
Conclusion: Together, our data suggests that m^{6}A RNA demethylase ALKBH5 may play a critical role in MB growth and tumor progression by regulating MB tumor initiating stem cells and ALKBH5 serves as a potent therapeutic target to treat MB.

#2621 Mitotic activities of Ewing sarcoma proteins.

Evan Schulz, Mizuki Azuma

University of Kansas, Lawrence, KS

Ewing sarcoma (ES) is a pediatric bone tumor in critical need of targeted chemotherapies with fewer side effects. ES's driving hallmark, the t(11:22) chromosomal translocation, generates two aberrations of the EWS protein: the loss of an *EWS* allele and expression of *EWS/FLI1* fusion protein, denoted as EWS aberrations. Identifying drug targetable EWS aberration-induced changes to tumor biology and drug sensitivity is required to optimize current treatment strategies and develop new targeted therapies. To accomplish this, we established a DLD-1 cell line that lacks the t(11:22) chromosomal translocation and EWS aberrations, while enabling conditional *EWS/FLI1 expression* with doxycycline (Tet-On system), single-allele *EWS knockdown* with auxin (auxin-inducible degron system), or a combination of both (*EWS/FLI1-EWS KD*). This allows us to dissect the contribution of each EWS aberration to phenotypic changes in the cell. Since EWS is a known regulator of tubulin acetylation, we investigated whether tubulin acetylation levels changed under EWS aberration conditions. Both immunocytochemistry and western blotting revealed *EWS/FLI1 expressing* and *EWS/FLI1-EWSKD* cells displayed lower acetylated tubulin signal intensity after one and seven days, while *EWS knockdown* cells displayed higher acetylated tubulin signal intensity. As tubulin acetylation prevents microtubule damage, we probed cells with an antibody that visualizes microtubule damage and repair sites. *EWS/FLI1 expressing* and *EWS/FLI1-EWS KD* cells exhibit a higher signal intensity, covering a broader area compared to the control cells. Our data suggests that the combination of *EWS/FLI1-EWS KD* increases microtubule damage sites by reducing microtubule acetylation. When the EWS/FLI1-EWS KD cells were treated with nocodazole, the rate of cell death increased compared to control nocodazole-treated cells, indicating the cells had been sensitized to microtubule disruption. Currently, we are investigating how EWS aberrations change the regulation of microtubule acetylation and how this mechanism affects sensitivity to microtubule disruptors. Understanding this mechanism could identify new drug targets and treatment strategies in ES to improve drug efficacy and reduce secondary side effects for patients.

#2623 Targeted therapy with DFMO and immunotherapy equalizes survival in MYCN-amplified neuroblastoma: insights from genomic and network analyses.

Anderson O. Cox[1], Abhinav Nagulapally[2], Deha Ay[1], Qianqian Song[3], Liang Liu[1], Hong-Gang Wang[2], Abigail Moore[2], Valerie I. Brown[2], Wei Zhang[1], Giselle L. Saulnier Sholler[2]

[1]Atrium Health Wake Forest Baptist Comprehensive Cancer Center, Winston-Salem, NC,[2]Penn State College of Medicine, Hershey, PA,[3]University of Florida College of Medicine, Gainesville, FL

Background: This study evaluates the efficacy of difluromethylornithine (DFMO) combined with immunotherapy for its potential as a targeted therapy to mitigate outcome differences associated with MYCN amplification in neuroblastoma (NB).
Methods: A clinical trial was conducted involving high-risk (HR) NB patients, who completed standard HR induction and consolidation, were then treated with standard of care immunotherapy adding DFMO either during and/or after immunotherapy. Patients were categorized based on MYCN amplification status. Survival analysis, differential gene expression, and pathway enrichment analyses were performed using RNA sequencing data. STRING and Bayesian network analyses were employed to identify biological relationships among differentially expressed genes (DEGs). Centrality analysis was performed to identify the top nodes in the regulatory network. Cox proportional hazards (CPH) models were used to determine the impact of individual genes on survival, and a multivariate Lasso model was developed to predict risk scores based on gene expression profiles.
Results: Gene copy number variation analysis showed that among the 55 cases, 19 cases had MYCN amplification. There are slightly more responders to immunotherapy and DFMO among MYCN-amplified (MNA) patients (not statistically significant). Survival analysis showed that immunotherapy and DFMO treatment achieved similar survival rates for both MNA and non-amplified patients. 112 DEGs were identified between responders and non-responders, with 190 DEGs in the MNA group and 74 in the MYCN non-amplified (MNN) group. Key genes such as PDZRN4, MGDA2, LINC00632, and ENTPD3 were identified as significant for survival in MNA patients. The DFMO-treated cohort with MYCN amplification did not show a significant relationship with poor survival (p-value = 0.8). A multivariate CPH model subjected to Lasso penalization identified five genes that distinguished high-risk and low-risk patients with significant survival disparity (p-value < 0.001). Pathway analysis showed that the Hippo signaling pathway was suppressed in MNN responders. Ribosomal protein gene expression was enriched in responders overall and in MNN responders, but not in MNA responders. STRING analysis revealed highly connected networks in DEGs, with stem cell factor SOX2 being a central node in MNA cases, indicating its role in maintaining stemness and promoting tumor propagation.
Conclusion: DFMO combined with immunotherapy shows promise as an effective targeted therapy for both MNA and MNN NB, equalizing survival rates between MNA and MNN patients. This treatment may particularly target stem cell populations in MNA cases. Further validation is required to confirm these findings and refine the associated risk prediction models.

#2624 Aneuploidy induction mechanism in Ewing sarcoma.

Priyani H. Harsha Hapugaswatta, Mizuki Azuma

University of Kansas, Lawrence, KS

Ewing sarcoma (ES) is the second most prevalent sarcoma that develops in the bones of adolescents and children. A major aberration in Ewing sarcoma is the chromosomal translocation between chromosomes t(11;22), resulting in the loss of one allele of *EWSR1* and acquisition of *EWSR1-FLI1* fusion gene. Trisomy 8 (~50%) is the major aneuploidy in ES tumors and is associated with low survival rate in patients. This study aims to elucidate the molecular mechanism underlying the aneuploidy induction in ES. To study the role of loss of one *EWSR1* allele and gain of the *EWSR1-FLI1* fusion gene inducing aneuploidy, we established a conditional DLD-1 cell line that enables knockdown of EWS derived from one *EWSR1* allele using Auxin-induced Degron system and expression of EWSR1-FLI1 using a Tet-on system. The DLD-1 cell line was employed in this study because it is a near diploid cell line that has been utilized to investigate aneuploidy induction by the gene of interest. Treatment of the cell with Auxin (AUX) degrades EWSR1 protein expressed from one allele of *EWSR1*, while treatment with Doxycycline (DOX) induces the expression of EWS-FLI1. Simultaneous degradation of EWSR1 and EWSR1-FLI1 expression is achieved by treating the cell line with both AUX and DOX. To investigate the effect of loss of one *EWSR1* allele and gain of *EWSR1-FLI1* fusion gene, cells were treated with AUX (EWSR1 knockdown derived from one allele), DOX (EWSR1-FLI1expression), and AUX/DOX (EWSR1 knockdown and EWSR1-FLI1 expression), for 2 to 8 days. The results showed that all three-drug treated samples led to an increase in cell size. Further, Fluorescence-activated Cell Sorting analysis (FACS) revealed that the majority of the small cells were diploid (2N), whereas the majority of large cells were aneuploid (>3N). While Ewing sarcoma cells are described as small round cells, there was a variation observed in Ewing sarcoma cell sizes, A673 and SKES1, but not in the normal MSC cells.Next, we investigated whether the treatment of AUX and DOX induces trisomy 8 using FISH assay. Both AUX and DOX treated cells displayed a higher incidence of trisomy 8 compared to the control group. Furthermore, the AUX/DOX treated cells displayed a significantly higher incidence of trisomy 8 compared to AUX, DOX treated, and control cells, suggesting that the expression of EWSR1-FLI1 fusion protein and EWSR1 knockdown have a cumulative effect on trisomy 8 induction. While trisomy 8 has been reported in various cancers with a poor prognosis, the induction mechanism remains unclear. To the best of our knowledge, this is the first observation describing the collaborative activities between the EWSR1-FLI1 fusion protein and EWSR1 knockdown in inducing trisomy 8. We are currently investigating the molecular function of trisomy 8 induction in this cell line and in Ewing sarcoma cell lines.

#2625 Role of base deaminase and splicing deregulation in pediatric B cell acute lymphoblastic leukemia stem cell persistence.

Mrinal Joshirao[1], Inge Van Der Werf[2], Jessica Pham[2], Antonio W. Ruiz[2], Neha Katragadda[2], Emma E. Klacking[2], Elsa Molina[3], Ludmil Alexandrov[1], Thomas Whisenant[4], Catriona Jamieson[2]

[1]Pediatric Hematology Oncology, University of California San Diego, San Diego, CA,[2]Division of Regenerative Medicine, University of California, San Diego, San Diego, CA,[3]Next Generation Sequencing & Genomics Core, Salk Institute for Biological Studies, La Jolla, CA,[4]Center for Computational Biology and Bioinformatics, University of California San Diego, San Diego, CA

Introduction: B cell acute lymphoblastic leukemia (B ALL) is the most common childhood malignancy with more than ninety percent cure rates and current research efforts are directed towards development of curative therapy in patients who relapse. Leukemia stem cell (LSC) persistence contributes to relapse. Whole genome sequencing and RNA sequencing (RNA seq) have revealed APOBEC3C and ADAR1 base deaminase induced splicing alterations in LSCs in both adult and pediatric acute myeloid leukemia. Through our study we strive to determine the role of base deamination deregulation in LSC persistence in pediatric B ALL.
Methods: We designed a longitudinal early relapse detection study by collecting bone marrow aspirates and/or peripheral blood samples from patients ages 1 to 25 years diagnosed with B ALL at University of California, San Diego according to Institutional Review Board guidelines. Timepoints include diagnosis, end of induction cycle and/or subsequent cycle until they achieve remission or relapse. Cord blood and pediatric non leukemic (pNL) samples were used as controls (*Van Der Werf, ⋯Jamieson. Cell Reports Medicine 2023, March cover*). Mononuclear cells were isolated followed by CD34+ cell selection. Fluorescence activated cell sorting purification of HSCs ($CD34^+CD38^-Lin^-$) and HPCs ($CD34^+CD38^+Lin^-$) was followed by RNA extraction (Qiagen), library preparation, sequencing (Illumina Hi Seq) and RMATS as well as A to I editome analysis.
Results: To date, twelve B ALL patients have been enrolled in the study, with 67 percent belonging to high risk and 33 percent to standard risk NCI subgroups, and 7 patients available for analysis. RNA seq analysis was performed on purified HSCs and HPCs from three patients. While there was a trend toward APOBEC3C overexpression in B ALL versus pNL control HSCs, there was a statistically significant upregulation of ADAR1 in B ALL HSCs and HPCs and ADARp150 splice isoform overexpression in B ALL HPCs. Furthermore, RMATS differential splicing analysis detected alternative splicing deregulation, including exon skipping (ES), intron retention (IR), and alternative 5 prime splice site (A5SS) as well as alternative 3 prime splice site (A3SS) usage. With more than 2000 differential events identified, ES was the most frequent event in our HSC and HPC RNA seq analyses, which has been linked to increased adenosine to inosine (A to I) splice acceptor site editing by ADAR1p150.
Conclusions: Preliminary data suggests RNA splicing deregulation and ADAR1p150 splice isoform mediated A to I RNA editing activity may contribute to LSC persistence following therapy. To validate our findings, we will perform lentiviral ADAR1 nanoluciferase GFP reporter and splicing reporter assays in combination with functional survival and self renewal assays with the goal of developing base deaminase targeted strategies to predict and prevent pediatric B ALL relapse.

#2626 Leveraging FFPE single-nucleus RNA-sequencing for Osteosarcoma.

Danh Truong, Clement Agyemang, Davis Ingram, Alexander Lazar, Valerae O. Lewis, Joseph Ludwig

UT MD Anderson Cancer Center, Houston, TX

Osteosarcoma (OS), a heterogeneous class of bone sarcomas, can be subclassified based on predominant histological features. This molecular heterogeneity cannot fully be resolved with bulk sequencing. However, due to the rarity of several OS subtypes, including low-grade and small-cell, parosteal, periosteal, and high-grade surface, acquiring fresh specimens for single-cell omics is difficult. Logistical challenges exist when acquiring fresh specimens, such as transportation or adequate storage. Here, we leverage archival formalin-fixed paraffin-embedded (FFPE) samples of OS, which are routinely processed and preserved in pathology laboratories. We utilized 10x Flex assay to perform single-nucleus RNA-sequencing (snRNA-seq) from FFPE samples of OS, including osteoblastic OS, chondroblastic OS, low-grade, and periosteal. We compared fixed-frozen to FFPE samples to determine if both methods yielded concordant results. Two 25 µm FFPE scrolls and matched fixed-frozen tissue of approximately 60 mg were dissociated using the gentleMACs Dissociator. After confirming adequate cell count, we performed snRNA-seq on the fixed samples using the 10x Flex assay. Fixed-frozen (5201±3410 cells) had higher cell counts than FFPE (1185±823 cells), which may be attributed to tissue area in the FFPE scrolls. The confidentially mapped reads were similar for fixed-frozen (64%±13%) and FFPE (55%±17%). Higher UMIs and genes were detected in fixed-frozen (8566±110 UMIs and 2342±15 genes detected) than in FFPE (1464±25 UMIs and 835±9 genes detected). Regardless of specimen type, we detected the subtype-specific gene expression. Fixed-frozen and FFPE specimens of osteoblastic OS expressed osteoblastic markers, SPP1, CHD11, and IBSP. Chrondroblastic markers (SOX9 and ACAN) were expressed in both conditions for chondroblastic OS. Significantly, CDK4 and MDM2 were overexpressed in low-grade OS due to the amplification of CDK4 and MDM2 copy numbers, confirmed by CopyKAT and inferCNV. Overall, we found that the 10x Flex-based snRNA-seq adequately preserved each OS subtype's biology. FFPE had lower quality control metrics than fixed-frozen, possibly due to the smaller amount of tissue available from the scrolls. Future work will investigate the common theme of genetic drift from normal osteoblasts and their varied degrees of dedifferentiation observed along mesenchymal lineages among OS subtypes.

#2627 Integrated multi-omics analyses discovers lineage plasticity in desmoplastic small round cell tumors.

Danh Truong[1], Emre Arslan[1], Veena Kochat[1], Roberto Cardenas[1], Jiaqian Fan[1], Clement Agyemang[1], Adewale A. Adebayo[1], Davis Ingram[1], Akshay Basi[1], Hannah C. Beird[1], Jared Burks[1], P Andrew Futreal[1], Alexander Lazar[1], Ravin Ratan[1], Najat C. Daw[1], Andrea Hayes-Dixon[2], Kunal Rai[1], Joseph Ludwig[1]

[1]UT MD Anderson Cancer Center, Houston, TX,[2]Howard University, Washington, DC, DC

Desmoplastic small round cell tumor (DSRCT) is a rare and usually incurable aggressive sarcoma subtype. Less than 20% of patients survive beyond five years with standard of care. All tumor cells harbor a pathognomonic EWS::WT1 fusion protein (FP), but FP-targeted agents are nonexistent. Given the male to female ratio of 6:1, we sought to understand androgen receptor (AR) biology and its role as a therapeutic target in DSRCT. Our preliminary data suggested that a subset of DSRCT cell's epithelial lineage expressed AR, which is the driver of prostate cancer (PC). A separate subset of DSRCT cells exhibited neural (NE) markers without AR expression. We explored gene expression, chromatin accessibility, and protein expression using multi-omic analyses (n=9) to understand the mechanisms driving AR and NE expression in DSRCT. We identified DSRCT, fibroblasts, endothelial cells, immune cells, and smooth muscle cells in the snRNA-seq data. Using AR and NE signatures, we generated three subsets of DSRCT cells. One subset was high in AR expression, and the other was high in NE markers, but not both. The third subset appeared to exhibit both markers - implying a hybrid phenotype or a poorly differentiated phenotype. To validate protein expression, we used the Lunaphore COMET. In AR-positive tumor nests, AR was localized to the nucleus, suggesting downstream activation of this pathway, and correlated with pan-cytokeratin (Ck) expression - an epithelial marker. Tumor nests marked by neural-specific enolase (NSE), an NE marker, demonstrated little to no expression of AR or Ck. However, patient subsets that harbored a hybrid transcriptomic profile had different cell states within the tumor nests, which were AR/Ck positive or NSE positive, but not both. This suggested that tumors may harbor cells of different cell states. The ATAC-seq data found that Epithelial/AR-positive subtypes were enriched in motifs for nuclear receptors (ARE and GRE). The NE subtype lacked the ARE and GRE motifs. EMT-related motifs were associated with the NE and hybrid subtypes, such as ZEB1, SNAI1, and SNAI2 (Slug). Transcription factor binding prediction showed that ZEB1 had higher activity in the NE subtype, while AR, FOXA2, and GRHL2, a co-regulator of AR, had higher activity in the AR subtype. Although the neuroendocrine subtype in PC usually evolves in response to therapy, how the NE phenotype emerges in DSRCT remains unknown. It has yet to be discovered how transcription factors play a role in the Epithelial/AR and NE in DSRCT dimorphic states. ZEB1 and GRHL2 form a double negative feedback loop, where ZEB1 suppresses GRHL2. This suggests that there may be an interaction between the ZEB1 and AR to regulate epithelial and NE phenotype in DSRCT, which may have implications for treating DSRCT with AR inhibitors.

TUMOR BIOLOGY: Invasion and Migration
Poster Session

#2631 Trop-2 is expressed in tumor budding cells and drives colon cancer cell migration and invasion through a β-catenin, CDC42, PKCα, Akt signaling network.

Saverio Alberti[1], Valeria Barresi[2], Rossano Lattanzio[3], Marco Trerotola[3], Emanuela Guerra[3], Martina Ceci[3], Roberta Di Pietro[3], Donato F. Altomare[4], Raffaella Depalo[4], Arcangelo Picciariello[5], Laura Antolini[6], Federica M. Previtera[7]

[1]BIOMORF, University of Messina, Messina, Italy,[2]University of Verona, Verona, Italy,[3]University of Chieti, Chieti, Italy,[4]University of Bari, Bari, Italy,[5]University of Lecce, Lecce, Italy,[6]University of Milano Bicocca, Monza, Italy,[7]University of Messina, Messina, Italy

TROP2 was previously shown to drive metastatic diffusion of colorectal cancer (CRC), upon proteolytic activation by ADAM10. Trop-2 activation was shown to lead to cleavage of E-cadherin, with release from the scaffolding cytoskeleton and disruption of cell-cell junctions. This suggested that Trop-2-driven loss of cell adhesion is pivotal for the acquisition of invasion capacity in CRC. Tumor budding was shown to play a role in CRC invasion at the tumor/stroma interface and to impact on disease outcome. We thus investigated whether Trop-2 is associated with CRC cell budding. Our findings show that essentially all CRC buds express Trop-2. Trop-2-positive tumor cell buds and poorly differentiated clusters associated with metastatic relapse in CRC patients in independent CRC case series. Trop-2 was shown to drive cell migration and matrix invasion upon induction of β-catenin signaling, via expression of CDC42, phosphorylation/activation of PKCα at Ser657 and of Akt at Thr308. The highest impact on CRC patient prognosis was jointly shown by Trop-2, CDC42 and phosphorylated PKCα, supporting a model of Trop-2-driven CRC progression through induction of migration and invasion of tumor budding cells.

#2632 IL10RB is a key molecule that regulates tumor escape through invadopodium formation and immune dysfunction.

C. Kudo-Saito, H. Ozawa, H. Imazeki;
National Cancer Center Research Institute, Tokyo, Japan

Purpose: Many molecular mechanisms underlying tumor escape, in which cancer cells undergo EMT and thereby acquire metastatic potential and cancer stemness, have been elucidated, and these efforts have led to the development of various novel drugs. However, many patients do not necessarily benefit from the treatments. This may be because cancer cells diversify and evolve through intrinsic changes that occur within the cancer cells themselves via EMT, and extrinsic changes that are modified by the host environment, in an attempt to escape the attacks of therapeutics and immune surveillance. Considering this situation, further in-depth research is needed to achieve complete cure in more patients. In this study, we focused on the changes in gene expression that occur in cancer cells after achieving EMT, and attempted to elucidate the evolutionary mechanisms that further promote tumor escape leading to robust treatment resistance.
Results: We comprehensively analyzed gene expression in mouse melanoma B16-F10 cells stably expressing Snail, a key EMT-governing transcription factor, by GeneChip microarray, and found that IL10RB expression was strikingly increased as compared to that of mock control. Transfection with siRNA-snail into the cells abrogated both Snail and IL10RB expression, suggesting that this is regulated by Snail. Transfection with siRNA-il10rb significantly reduced cell invasion, adhesion, and chemoresistance as compared to transfection with control siRNA, suggesting a key functional role of IL10RB in tumor malignancy. Il10rb gene transduction into mouse IL10RB(low) cell lines (NHOS and Colon26) significantly enhanced cell invasion, adhesion, and chemoresistance, accompanied by dramatic morphological changes - formation of numerous tentacles/invadopodia. RNA-sequencing analysis of the IL10RB transfectants revealed that gene expression of actin and related genes, which are required for invadopodia formation and drug resistance, and of enzymes, which are required for invasion and metastasis, was markedly increased than that in mock control. In the in vivo settings, IL10RB+ tumor growth and metastasis were extremely slower after implantation in mice than those of mock control. However, T cells and NK cells were dramatically decreased in the spleens of the IL10RB+ tumor-implanted mice, and their cytotoxic activity was also significantly reduced, suggesting induction of immune suppression and dysfunction. Surprisingly, chemotherapy significantly exacerbated both IL10RB+ tumor growth and metastasis in the mice, while immunotherapy with anti-IL10RB blocking mAb significantly induced anti-tumor effects without such adverse events.
Conclusions: These results suggest that IL10RB is a key molecule that regulates tumor escape and intractability post Snail-induced EMT. Targeting IL10RB may be a promising strategy for improving clinical outcomes in cancer therapy.

#2633 Mechanisms of SEMA4D mediated blood brain barrier transmigration.

A. D. Sunkara, R. Klotz, M. Yu;
University of Maryland, Baltimore, Baltimore, MD

Brain metastasis poses a challenge in cancer treatment due to its prevalence, diagnostic complexities, and the limited efficacy of systemic therapies due to the blood brain barrier (BBB). Previous work in our lab utilized patient-derived Circulating Tumor Cell (CTC) lines established from the peripheral blood of patients with metastatic luminal breast cancers to investigate the molecular features that allow a subset of CTCs to adapt and grow in the brain. This study identified Semaphorin 4D (SEMA4D), a transmembrane receptor that facilitates BBB transmigration, both *in vitro* and *in vivo*, through its interaction with the Plexin B1 (PLXNB1) receptor. Together with oncogene MYC, SEMA4D facilitates the colonization of the brain and promotes brain metastasis of CTCs. In this study, we propose investigating the hypothesis that the reverse signaling via the cytoplasmic region of SEMA4D activates the Yes Associated Protein (YAP) pathway, facilitating BBB transmigration. Our data shows that truncating the cytoplasmic region of SEMA4D (deleting the PDZ binding domain) in the BT474 HER2 positive cell line reduces BBB transmigration. In addition, cells that are overexpressed with SEMA4D showed nuclear localization of YAP via immunofluorescence. Hence, using patient-derived CTC cultures and a model system of breast cancer cell lines we aim to elucidate SEMA4D's link to YAP signaling and the mechanism of BBB transmigration. Understanding the mechanisms underlying metastatic invasion through the BBB is crucial for developing targeted interventions in the future.

#2634 IL34-induced Arg1 positive macrophage cells at the edge of invasion in cerebellum metastasis model of HER2-positive breast cancer.

X. Cheng[1], K. Patel[2], Y. Fu[1], R. Cleary[1], B. Zhou[1], M. Highkin[1], J. Hsia[1], X. Jin[1], J. L. Prior[1], M. Michie[1], R. Sun[1], I. S. Hagemann[1], T. Li[1], V. Jain[2], S. Gregory[2], A. Kim[1], R. Bose[1];

[1]Washington University in St. Louis, St. Louis, MO, [2]Duke University, Durham, NC

Brain metastases occur in about 30% breast cancer patients. The cerebellum is a frequent location for metastases in HER2-positive breast cancer patients, but the mechanisms for this is unknown. We hypothesize that the cerebellar microenvironment plays a crucial role in promoting the growth of metastatic HER2+ breast cancer cells. To study the interaction between cerebellar cells and metastatic breast cancer, we developed a syngeneic, immunocompetent mouse model for breast cancer brain metastasis, by stereotactically injecting mouse breast cancer organoids with HER2V777L; PIK3CAH1047R activating mutations or HER2V777L; TP53fl/fl1 into the cerebellum. Growth of these cerebellar metastasis was monitored both by MRI and also with Herceptin imaging using a Near-Infrared fluorophore conjugated to Herceptin. We observed significant tumor growth in the cerebellum for up to 7 weeks post injection. We further investigated cellular interactions within the pro-tumorigenic microenvironment and HER2+ breast cancer cells using spatial transcriptomics. We identified inflammation-induced ARG1+ macrophage cells at the edge of the cerebellar metastasis. Further, the cytokine IL34 is upregulated in the brain metastasis. IL34, which is secreted by HER2+ breast cancer, induces Arg1 expression in both homeostatic microglial cells and bone marrow derived macrophages. These results suggest IL34 polarizes macrophages facilitating tumor invasion in the brain. These novel mouse models and findings elucidate the molecular mechanisms and the role of the tumor microenvironment that promotes cerebellar metastasis, and suggest a therapeutic approach to inhibit breast cancer invasion into the brain.

#2635 E3 ubiquitin ligase ARIH1 mechanism and regulation in triple-negative breast cancer.

Casey B. Staby[1], Vanessa Zapata[1], Sean Cruz[2], Kelly Anderson[1], Abigail Quesnelle[1], Breege Howley[1], Philip Howe[1]

[1]Medical University of South Carolina, Charleston, SC,[2]College of Charleston, Charleston, SC

We have recently identified a novel role for the ubiquitin E3 ligase ARIH1 in Epithelial-to-Mesenchymal transition (EMT) induction, tumor initiation, and metastatic progression of triple-negative breast cancer (TNBC). In TNBC cells, ARIH1 knockdown (KD) leads to a reduction in cell invasion and mammosphere formation, and loss of tumor initiation and metastasis. In this current study, we aim to define the underlying mechanisms controlling ARIH1 expression and contributing to ARIH1 function in TNBC. Quantitative proteomic analysis of control and ARIH1 silenced TNBC cells revealed an enrichment of mitochondrial proteins consistent with ARIH1's role in mitophagy. Additionally, a striking deregulation of proteins involved in oxidative stress response was observed. This analysis revealed that ARIH1 increases in response to various oxidative stressors, as detected by qPCR and immunoblot. The ARIH1 promotor contains conserved motifs for transcription factors (TFs), including CREB1 and HIF1α sites, linked to oxidative stress. Of note, CREB1 is positively correlated with ARIH1 transcript in breast cancer patient tumors. These findings support the transcriptional upregulation of ARIH1 in response to oxidative stress and may explain why we see an upregulation of this protein during breast cancer progression.As ARIH1 plays a role in both mitochondrial dynamics and EMT, we tested ARIH1 silencing in response to Doxorubicin (Dox), a DNA-damaging agent that induces oxidative stress, and saw improved sensitivity compared to WT MDA-MB-231 cells. Next, we assessed ARIH1 expression in the Dox-resistant cell line of our model, showing ARIH1 is upregulated in these cells at the transcript level, supporting the oxidative stress model. To further understand the crucial players impacting chemoresistance mechanisms in relation to this protein, we will test the contribution of downstream targets in response to chemotherapy. We hypothesize ARIH1's upregulation in response to oxidative stress and DNA-damaging treatment promotes cancer cell survival and enhances chemoresistance. This study expands on our novel discovery of ARIH1's role in EMT and cancer progression. The significance of ARIH1 in TNBC is highlighted in patient data, where high ARIH1 expression is correlated with worse patient survival.

#2636 Targeting the HGF/MET/TWIST1 pathway in lung adenocarcinoma brain metastases.

Xiaxuan Wu[1], Charitha Marni[2], Veronica Zhang[2], Prarthana Mocherla[1], Jacob George[1], Purva Rumde[1], Rohan K. Goyal[3], Lora H. Rigatti[2], Princey Devadassan[4], Autumn Gaither Davis[4], Timothy F. Burns[4], Laura P. Stabile[5]

[1]School of Medicine, University of Pittsburgh, Pittsburgh, PA,[2]UPMC Hillman Cancer Center, Pittsburgh, PA,[3]University of Pittsburgh, Pittsburgh, PA,[4]Department of Medicine, Division of Hematology/Oncology, University of Pittsburgh, Pittsburgh, PA,[5]Department of Pharmacology & Chemical Biology, University of Pittsburgh, Pittsburgh, PA

Lung adenocarcinoma (LUAD) is the most common primary tumor to metastasize to the brain, with ~40% of patients developing brain metastases (BM) which is associated with poor prognosis. No brain specific targeted therapies currently exist, highlighting the need for novel treatments. We identified a significant enrichment of *MET* amplification in LUAD BM compared to primary LUAD and liver metastases. MET, a tyrosine kinase receptor, and its ligand hepatocyte growth factor (HGF), mediate proliferation, epithelial-mesenchymal transition (EMT), angiogenesis, and metastasis. We have found that MET-driven tumorigenesis is dependent on the EMT transcription factor, TWIST1, which is regulated by the HGF/MET pathway and is overexpressed in LUAD BM. These findings led us to hypothesize that the HGF/MET/TWIST1 axis is a potential therapeutic target for LUAD BM. To examine the role of the HGF/MET/TWIST1 pathway in LUAD BM, we utilized an *ex vivo* organotypic brain slice model. Using a patient derived LUAD cell line pair, H2073 (primary tumor, *MET* wildtype; WT) and H1993 (metastasis, *MET* amplified), we found increased brain colonization with the *MET* amplified H1993 cells. In addition, HGF promoted brain colonization of *MET* WT H2073 cells, while the MET inhibitor capmatinib reduced brain colonization of *MET* amplified H1993 cells. Similarly, using our murine syngeneic LUAD lines, FVBW-17 (MET WT) and FVB-HGF (HGF overexpression; OE), we found that HGF OE led to increased brain colonization. Additionally, conditioned medium (CM) from H1993 cells increased astrocyte migration compared to CM from *MET* WT H2073 cells. Capmatinib reduced H1993 CM-mediated astrocyte migration, while HGF induced H2073 CM-mediated astrocyte migration. Further, these HGF/MET driven processes appeared to be TWIST1 dependent as TWIST1 inhibition with harmine decreased brain colonization in both *MET* amplified H1993 and *MET* mutant H1437 cells, and inhibited astrocyte migration induced by CM from H1993. We next examined the role of the HGF/MET/TWIST1 pathway on BM *in vivo*. Using an intracardiac injection metastasis model, we observed a higher frequency of BM in HGF OE mice compared to WT SCID mice in all LUAD lines assessed. In WT SCID mice, time to BM development was significantly shorter in mice injected with *MET* amplified cells compared to *MET* WT cells. Further, BM was accelerated in HGF OE mice compared to WT mice injected with H2073 *MET* WT cells. In syngeneic LUAD models, 40% of mice injected with HGF OE cells developed BM versus 0% of mice injected with WT cells. Moreover, orthotopic intracranial injections showed a two-fold faster growth rate of HGF OE cells compared to WT cells. Finally, capmatinib or harmine treatment in mice with BM led to BM disappearance, with harmine showing a sustained effect lasting over three months after end of treatment. These findings suggest that targeting the HGF/MET/TWIST1 axis may be an effective treatment strategy for *MET* amplified LUAD BM.

#2637 Investigation of cell shape, focal adhesion, and cytoskeletal changes induced by SIAH inhibition in malignant human cancer cell lines.

Daniel J. McWilliams[1], Jonathan M. Baker[1], Amy H. Tang[1], Emanuel Petricoin[2], Julia Wulfkuhle[2], Andrew Howell[3]

[1]Old Dominion University, Norfolk, VA,[2]George Mason University, Fairfax, VA,[3]EVMS, Norfolk, VA

INTRODUCTION: SIAH is an evolutionarily conserved E3 ubiquitin ligase that plays an important gatekeeper role downstream of the tumor-driving EGFR/HER2/RAS pathway. SIAH expression is prognostic in human cancer, including pancreatic ductal adenocarcinoma (PDAC) and triple negative breast cancer (TNBC). Our lab has demonstrated that SIAH is a potent cancer target. Here, we found a novel molecular mechanism of how SIAH inhibition changes cell shape, focal adhesion, mechano-sensing, and cytoskeleton organization in SIAH-deficient cancer cells. In this study, we focus on validating and quantifying the changes of phospho-YAP, phospho-cofilin, and collagen I proteins in MDA-MB-468, MDA-MB-231, and MiaPaCa cancer cell lines.
METHODS: The reverse protein phase array (RPPA) technology in combination with immunofluorescence (IF), Flow, and western blots (WB) were used to determine the SIAH inhibitor-induced changes in phospho-YAP, phospho-cofilin, and collagen I proteins in human PDAC and TNBC cell lines. Triplicated RPPA assays were performed to quantify the altered expression of these phosphorylated proteins utilizing a nitrocellulose coated plate dotted with these pair-matched cell lysates with SIAH proficiency and deficiency in triplicates. We then performed WB/IF/FACS assays to verify the changes of these protein expression in the DOX (+/-) cancer cell lines independently.
RESULTS: We found that phospho-cofilin was upregulated, and phospho-YAP were downregulated in a few selected SIAH-deficient cancer cell lines as compared to their SIAH-proficient cancer cells. These molecular changes were consistent with the altered cellular shape, increased cell detachment, and altered cytoskeleton observed in the SIAH-mutant cells. Further investigations will be conducted to identify the crosstalk of the Hippo/Yap/Cofilin/Collagen signaling pathways with the EGFR/RAS/SIAH signaling pathway in human cancer cells.
CONCLUSION: Our results showed that SIAH deficiency altered the cell shape, adhesion, and cytoskeletal dynamics in malignant cancer cells. Increased phospho-cofilin expression suggested that SIAH inhibition may disrupt actin cytoskeleton organization, alter cell shape, decrease focal adhesion, increase cell detachment, and impede cell motility. The changes in the Hippo/YAP pathway indicate that the mechano-sensing pathway may be altered in SIAH mutant cells. These documented changes in the Hippo/YAP/Cofilin/Collagen Type 1 pathways may provide a novel mechanism to explain tumor suppression phenotype of SIAH-deficient cancer cells in xenograft mouse models.

#2638 Study of the effect of ribosomal protein L23 on the metastasis related protein of human osteosarcoma cell lines.

Chia-Hsuan Chou[1], Shun-Fa Yang[2]

[1]Department of Medical Research, Chung Shan Medical University Hospital, Taichung, Taiwan,[2]Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan

Introduction: Lung metastases are the main cause of death and poor prognosis in patients with osteosarcoma. Its high propensity to metastasize is the leading cause for treatment failure and poor prognosis. Human ribosomal protein L23 (RPL23), a novel RBP, has been reported that involved in various physiological and pathological processes, including cell proliferation, cell apoptosis and cycle arrest. The higher expression of RPL23 is associated with the aggressiveness of the disease. However, the underlying functions and mechanisms of RPL23 in osteosarcoma metastasis has not been explored.
Material and methods: The GSE33382, GSE19276, and GSE 21257 database from Gene Expression Omnibus (GEO) were analyzed. Boyden chamber assay was used to evaluate the effects of cell migration and invasion. The FAM111B mRNA level and transcription activity were assessed by PCR, real-time PCR and luciferase reporter assay in osteosarcoma cells (U-2OS and HOS) after transfected with RPL23 siRNA.
Results: We found that RPL23 was upregulated in osteosarcoma tissues and its high expression was associated with poor prognosis in osteosarcoma patients. Using RNA-seq, microRNA-seq, and bioinformatic analysis, we revealed FAM111B and miR-30b-5p as downstream targets of RPL23. Knockdown of RPL23 significantly reduced the migration, invasion, and metastatic potential of osteosarcoma cell lines in vitro. However, these effects were reversed by the miR-30b-5p inhibitor.
Conclusions: RPL23 may be a novel osteosarcoma treatment strategy and a useful biomarker for predicting osteosarcoma progression.

#2639 Role of annexin A2 in triple-negative breast cancer metastasis.

P. Chaudhary;
University of North Texas Health Science Ctr., Fort Worth, TX

Introduction: Emerging evidence indicates that AnxA2 plays a role in the invasion and metastasis of breast cancer. However, the clinical significance of AnxA2 expression in breast cancer remains unknown.
Methods/Materials: The TNBC cells were purchased from the American Type Culture Collection (Manassas, VA). The expression of AnxA2 in cell lines, tumor tissues, and serum samples of breast cancer patients were analyzed by immunoblotting, immunohistochemistry, and enzyme-linked immunosorbent assay, respectively.
Results: We observed that AnxA2 was significantly elevated in the tumor tissues and serum samples of breast cancer patients compared to normal controls. High levels of serum AnxA2 were significantly correlated with tumor grade and poor survival in breast cancer patients. When we analyzed molecular subtypes, we found that AnxA2 expression was notably higher in the tumor tissues and serum samples of triple-negative breast cancer (TNBC) patients compared to those with other breast cancer subtypes. Our studies on breast cancer cell lines indicated that the secretion of AnxA2 is linked to its phosphorylation at tyrosine 23 (Tyr23). Additionally, phosphorylation of AnxA2 at Tyr23 facilitates its translocation to the cell surface in TNBC cell lines. The increased expression of AnxA2 on the cell surface enhances plasmin generation in these cell lines, and an inhibitory hexapeptide against AnxA2 can neutralize this activity. Furthermore, our analysis of AnxA2 phosphorylation in clinical samples confirmed that the phosphorylation at Tyr23 was significantly higher in the tumor tissues of TNBC patients when compared to matched adjacent non-tumorigenic breast tissues.
Conclusions: These findings suggest that serum AnxA2 concentration could be a potential diagnostic biomarker for TNBC patients.

#2640 Prevention of distant metastasis by disrupting the CTC-platelet interaction.

Lei Zhu[1], Nina Wang[1], Ting Jiang[1], Louisa Channon[1], Lena Lemmer[1], Dominic Helm[2], Sebastian Scholch[1]

[1]Translational Surgical Oncology, German Cancer Research Center, Heidelberg, Germany,[2]Proteomics Core Facility, German Cancer Research Center, Heidelberg, Germany

Circulating tumor cells (CTCs) are the primary mediators of hematogenous metastasis. During their journey in circulation, CTCs actively recruit platelets (PLTs) to form a protective cloak that facilitates immune evasion, enhances survival under shear stress, and promotes vascular adhesion and extravasation. We identified Neuraminidase 1 (NEU1) as a pivotal regulator of CTC-PLT interactions through its control of α2,6-sialylation, a critical post-translational protein modification that mediates PLT recruitment. We observed that diminished NEU1 expression in CTCs is associated with elevated sialylation, increased platelet cloaking, and poor prognosis in gastrointestinal cancer patients. This suggests that CTC sialylation drives PLT-mediated immune evasion and correlates with metastatic efficiency. Glycosylation profiling of CTCs from genetically engineered mouse models revealed a dynamic interplay in which NEU1 regulates α2,6-sialylation on the extracellular domains of glycoproteins. CRISPR-mediated knockout of NEU1 in tumor cells significantly enhanced sialylation, transendothelial migration, and metastatic seeding, whereas NEU1 overexpression suppressed platelet cloaking, inhibited CTC-PLT clustering, and reduced metastasis in vivo. NEU1 depletion increased metastatic efficiency, as evidenced by elevated proliferation of PLT-associated tumor cells in primary and metastatic tissues. Glycoproteomic analyses identified fibronectin 1 (FN1) as a potential NEU1-regulated, α2,6-sialylated target, further linking glycosylation changes to tumor progression. These findings establish a mechanistic connection between NEU1, CTC glycosylation, and PLT interactions, highlighting the functional significance of α2,6-sialylation in driving metastasis. To explore therapeutic potential, we investigate dual strategies targeting NEU1-mediated sialylation and CTC-PLT interactions. Preliminary experiments suggest that combining desialylation treatments with chemotherapy may effectively limit metastatic seeding by reducing CTC sialylation and disrupting PLT cloaking, thereby interfering with key steps of the metastatic cascade. Ongoing studies aim to validate these findings and assess their potential for clinical translation. Our findings underscore the central role of sialic acid metabolism in regulating CTC-PLT dynamics and establish NEU1 as a key regulator of this process. Unraveling how NEU1-mediated sialylation drives platelet cloaking and metastasis, this work opens avenues for novel therapeutic interventions to disrupt CTC-driven dissemination, thus reducing the metastatic burden and improving outcomes in cancer patients.

#2641 Glycosyltransferases at the helm of organ specific metastasis of pancreatic ductal adenocarcinoma.

Venkatesh Varadharaj, Frank Leon, Nivedeta Krishna Kumar, Palanisamy Nallasamy, Wyatt D. Petersen, Zahraa Wajih I. Alsafwani, Annant Bir Kaur, Kirtana Arikath, Sanchita Rauth, Saravanakumar Marimuthu, Surinder K. Batra, Moorthy P. Ponnusamy

Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE

Background: Pancreatic cancer (PC) deaths, incident cases, and disability-adjusted life-years globally have more than doubled from 1990 to 2017 with five-year survival rate of less than 14%, a trend likely to persist with population aging. The most common cause of mortality in pancreatic cancer patients is the metastasis of neoplastic cells to vital organs like the lungs and liver, and patients develop an inoperable condition due to late diagnosis. Malignant transformation of cancer cells is associated mainly with altered tumor-cell glycosylation at the cell surface. The expression of glycosyltransferases (GTs) is instrumental in modulating the cellular phenotype and promoting cancer cell survival at the metastatic niche. Specifically, we found that upregulation of (glucosaminyl (N-acetyl) transferase 3) GCNT3 and (beta-1,3-N-acetylglucosaminyltransferase 3) B3GNT3 expression are key mediators in altering glycosylation patterns in PC cells and enhancing the metastatic proclivity towards the liver and lung, respectively.
Methodology: We utilized two different metastatic models to replicate key aspects necessary to study the lung and liver-specific metastasis of PC. The RT^2 PCR array was used to analyze the global gene expression of GTs between primary PC cells and lung/liver metastatic models. Further, qPCR, ddPCR, and western blot techniques were used to confirm the differential expression of GTs at RNA and protein levels. Immunohistochemistry and Immunofluorescence experiments on the matched human primary and metastatic PC tissues validated the higher expression of specific GTs in the metastatic PC tissues when compared to the primary tumor.
Results: An unbiased transcriptional screen revealed a reciprocal >2-fold increase in GCNT3 and B3GNT3 expression in the liver and lung metastatic cell lines, respectively. Further, qPCR, dd-PCR, and western blot results confirmed the same expression pattern in liver and lung-specific metastatic cells compared to the PC controls. These results were further validated in human tissue from the same patient's primary pancreatic tumor and metastatic lung and liver tissues collected through the UNMC Rapid Autopsy program (UNMC-RAP).
Conclusion: Our data suggest that a clonal population of PC cells that highly express GCNT3 and B3GNT3 plays a pivotal role in reshaping glycosylation profiles, thereby augmenting their tendency for liver and lung-specific metastasis, respectively. This study highlights the critical correlation between glycosylation and organ-specific metastasis in PC.

#2642 High expression of N-acetyl transferase 9 in cholangiocarcinoma and its possible role in tumor progression.

T. Hoteit, H. P. Jones, P. Chaudhary;
The University of North Texas Health Science Center at Fort Worth, Texas Coll+, Fort Worth, TX

Cholangiocarcinoma (CCA) is a rare and aggressive subtype of liver cancer known for its limited number of biomarkers and its resistance to anticancer drugs, leading to difficulty in its early detection. The five-year survival rate for CCA varies between 2% and 24%, depending on factors such as the severity of the cancer, the presence of metastasis, and the timing of diagnosis. Currently, surgery is the most effective treatment option, but only under certain criteria, such as early cancer detection and absence of metastasis. N-terminal acetylation is an epigenetic modification that plays an important role in the regulation of cellular growth and function. This process is modulated by the enzyme N-acetyl transferase (NAT), which is involved in various functions, such as protein stability, membrane targeting, gene silencing, and drug resistance. These functions can regulate cell cycle progression, cell proliferation, and energy metabolism, all of which are key characteristics of cancer. NAT9, a subset of NATs, has abundant transcripts in breast, ovarian, and endometrial cancers; however, its role and expression in CCA have not been studied. In this study, we aimed to assess the expression, role, and function of NAT9 in CCA progression and metastasis using cell lines and patient tissue samples. According to The Cancer Genome Atlas (TCGA) database, NAT9 is overexpressed in patients with CCA, and its expression increases with the increase in the grade of CCA. NAT9 was also overexpressed in CCA cell lines and patient tissue samples compared to their normal and non-cancerous counterparts at both RNA and protein levels. In vitro, NAT9 knockdown experiments showed that NAT9 plays a role in CCA cellular proliferation and migration. These findings suggest that NAT9 plays a critical role in the development and progression of CCA.

#2644 Investigating major vault protein promoting migration and its clinical relevance in oral squamous cell carcinoma.

Sheng-Yen Hsiao[1], Shu-Fang Sun[1], Yuh-Ling Chen[2], Tse-Ming Hong[3]

[1]Hemato-oncology, Chi Mei Medical Center, Liouying, Tainan, Taiwan,[2]Institute of Oral Medicine, College of Medicine, National Cheng Kung University, Tainan, Taiwan,[3]Hemato-oncology, Institute of Clinical Medicine, College of Medicine, National Cheng Kung University, Tainan, Taiwan

Introduction: Head and neck squamous cell carcinoma (HNSCC), particularly oral squamous cell carcinoma (OSCC), has emerged as a significant health concern in Taiwan. It is the sixth leading cause of cancer-related death in the country, posing major challenges to public health and healthcare systems. The limited treatment options for unresectable or metastatic HNSCC highlight the need for new therapeutic targets. Several studies have reported that major vault protein (MVP), the primary component of ribonucleoprotein particles known as vaults, plays a role in regulating drug resistance in cancer therapy. However, the effect of MVP on OSCC progression remains largely unknown. In this study, we investigated the mechanisms through which MVP contributes to OSCC progression and its impact on clinical outcomes.

Materials and Methods: The migration ability of OSCC cells was assessed using wound-healing assays, two-chamber transwell invasion assays, and cell mobility tracking. Cisplatin resistance was evaluated using WST-1 cytotoxicity assays. Western blotting (WB), qRT-PCR, and RNA sequencing (RNA-seq) were conducted to explore potential mechanisms. Immunohistochemical (IHC) staining was used to determine MVP expression in OSCC tissues.

Results: Analysis of the TCGA dataset showed that HNSCC patients with high MVP expression had lower overall survival rates. OSCC cells with MVP knockdown exhibited reduced migration ability, while cells with MVP overexpression demonstrated increased migration capacity compared to control cells in wound-healing, two-chamber transwell invasion assays, and cell mobility tracking. MVP knockdown in OSCC cells led to increased cisplatin sensitivity, whereas MVP overexpression resulted in cisplatin resistance, as demonstrated by WST-1 cytotoxicity assays. RNA sequencing data suggested that MVP may regulate cell differentiation, EGFR signaling, and cisplatin resistance, as indicated by GSEA analysis. Additionally, GO enrichment analysis suggested that MVP may influence the PI3K-AKT signaling pathway, focal adhesion, and ECM-receptor interaction. Western blotting assays confirmed that MVP overexpression in OSCC cells activated the PI3K-AKT-mTOR pathway. Furthermore, HNSCC patients with higher MVP expression levels or IHC H-scores were more likely to experience poorer recurrence-free survival.

Conclusion: MVP may serve as a promising marker influencing clinical outcomes in HNSCC by regulating the PI3K-AKT-mTOR signaling pathway. Further validation of its clinical application is warranted.

#2645 Rnd3 depletion in lung cancer cell lines decreases cell invasion and migration in a ROCK1 independent manner.

Noemi Garcia Garcia[1], **Thanh Ha Vy Nguyen**[1], Dane Richey[1], Cody Ashby[2], Analiz Rodriguez[3], Katie R. Ryan[1]

[1]Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR,[2]Biomedical Informatics, University of Arkansas for Medical Sciences, Little Rock, AR,[3]Neurosurgery, University of Arkansas for Medical Sciences, Little Rock, AR

The purpose of this study is to investigate how altered expression levels of the small Rho GTPase, Rnd3, affects lung cancer cells. Lung cancer is among the most common and deadliest forms of cancer in the US. Most lung cancers metastasize, with lung to brain metastasis being the number one killer of lung cancer patients. Studying mechanisms that drive metastatic potential is key to unlocking new strategies for the treatment of this deadly form of cancer. Our focus for this study is Rnd3, an atypical GTPases, which has roles in regulating proliferation and cell migration in other cancer types. Our analysis of The Cancer Genome Atlas data predicts that lung adenocarcinoma patients with low levels of Rnd3 have significant higher survival rate. We investigated the role of Rnd3 by depleting its expression in the lung adenocarcinoma cell line (A549), and also patient-derived lung to brain metastasis (PDLBM) cell lines (601 and 620) as a proxy for advance disease. We observed no significant difference in cell viability or cell cycle progression between Rnd3-depeled and control groups. We did observe a significant effect on cell invasion and migration, Rnd3 depletion significantly decreases cell invasion and migration across all three tested cell lines. Previous studies on Rnd3 in melanoma cells found that Rnd3 signals via the RhoA-ROCK1 pathway, which impacts cell adhesion and the actin cytoskeleton, ultimately regulating cell invasion. However, when we either chemically inhibited ROCK1 or knocked down ROCK1 expression in our lung adenocarcinoma and PDLBM cells, it did not rescue the invasive phenotype. Our findings indicate that Rnd3 regulates cell invasion and migration, two hallmarks of metastasis, in lung cancer via a distinct pathway, independently from ROCK1. Identifying this novel mechanism will provide crucial insights for developing treatment for lung cancer metastasis.

#2646 Ankrd1 enhances lamellipodia formation and cell motility in clear cell renal cell carcinoma.

Hiromi Ito, Yuki Takai, Sei Naito, Takafumi Narisawa, Norihiko Tsuchiya

Urology, Yamagata University, Yamagata, Japan

Introduction: Clear cell renal cell carcinoma (ccRCC) displays more mesenchymal traits compared to other cancers. These traits include mesenchymal motility, which involves membrane protrusions like lamellipodia formed by actin polymerization. Ankyrin repeat domain 1 (Ankrd1) is regulated by YAP and ERK5, and contains structures that facilitate protein-protein interaction. Ankrd1 has been linked to epithelial-mesenchymal transition (EMT), cancer progression, and drug resistance in various cancers. However, its role in ccRCC remains unexplored. This study investigates Ankrd1 expression, regulation, and function in ccRCC.
Methods: We analyzed Ankrd1 expression using the TCGA database and tissue samples via immunohistochemistry. Regulation of Ankrd1 was examined by observing its response to YAP inhibitor and ERK5 inhibitors. Functional studies included siRNA, MTS assay, cell invasion assay, Western blotting, and immunofluorescence imaging.
Results: High Ankrd1 expression correlated with poor prognosis of ccRCC patients in TCGA cohort. In tissue samples, metastatic tissues showed higher Ankrd1 levels compared to primary tissues in the same patients. YAP regulated Ankrd1 in all ccRCC cell lines, while ERK5 influenced expression in some ccRCC cell lines. Ankrd1 silencing reduced cell viability under low-density conditions, where YAP activity is prominent. Ankrd1 silencing decreased cell motility, whereas overexpression enhanced motility without altering EMT-related protein levels. Ankrd1 co-localized with F-actin in lamellipodia stimulated by TPA. Ankrd1 silencing altered cell shape and reduced lamellipodia formation. Ankrd1 also interacted and co-located in lamellipodia with Talin-1. Ankrd1 depletion repressed activation of integrin pathway by Talin-1.
Conclusion: Ankrd1 is critical for lamellipodia formation and cell motility in ccRCC.

#2647 Essential roles of CRK and CRKL in glioblastoma cell migration and invasion.

Piyanka Hettiarachchi, Neka Large, Taeju Park

Children's Mercy Research Institute, Kansas City, MO

The dysregulation of CT10 regulator of kinase (CRK) and CRK-like (CRKL) expression has been implicated in various human cancers, notably glioblastoma, in which their elevated levels are associated with poor prognosis. Although CRK and CRKL have been proposed as therapeutic targets for invasive cancers, the mechanism underlying the contribution of CRK and CRKL to tumorigenesis was unclear. Previously, we induced transient knockdown of CRK, CRKL, or both in the U-118MG human glioblastoma cell line and demonstrated the requirement of CRK and CRKL for glioblastoma cell migration and invasion. CRKL knockdown induced cell shrinkage and inhibited cell proliferation, adhesion, and invasion, while CRK knockdown exhibited little or modest inhibitory effects. Combined knockdown of both CRK and CRKL knockdown resulted in pronounced morphological alterations and a complete inhibition of cell migration and invasion, highlighting their overlapping roles. To establish this finding as a generally accepted proof of principle for various glioblastoma cells and prepare mutant glioblastoma cell lines for orthotopic xenograft mouse models, we induced transient and stable knockdown of CRK/CRKL in LN-229 and U-87MG glioblastoma cell lines, which are proven to form tumors with high efficacy upon orthotopic transplantation. The gene knockdown of CRK and CRKL using siRNAs demonstrated substantial decreases in CRK and CRKL protein levels in LN-229 cells, as confirmed by Western blot analyses. We utilized the xCELLigence real-time cell analysis system to analyze the effects of CRK/CRKL knockdown on cell migration, adhesion, and invasion. CRKL knockdown caused modest inhibition of cell proliferation and substantial inhibition of cell adhesion, migration, and invasion. CRK/CRKL double knockdown resulted in pronounced morphological alterations, more robust inhibition of proliferation, and complete inhibition of cell adhesion, migration, and invasion. Stable knockdown of CRK and CRKL using lentiviral infection of shRNAs showed similar results. The results suggest similar responses of both U-118MG and LN-229 cells to CRK and CRKL knockdown. The similar and consistent outcomes between the two glioblastoma cell lines underscore the critical requirement of CRK and CRKL in glioblastoma cell migration and invasion. In addition to testing the cellular functions of CRK/CRKL in U-87MG cells, we plan to transplant the glioblastoma cells with stable knockdown of CRK/CRKL to immunodeficient mouse brains and study the requirement of CRK and CRKL for *in vivo* tumor formation and diffuse invasion of glioblastoma into surrounding brain tissues. These *in vitro* and *in vivo* studies will establish CRK and CRKL as therapeutic targets for blocking diffuse invasion of glioblastoma in the brain.

#2648 ZFP36L2 orchestrates stress adaptive plasticity during injury repair and metastasis.

Qingwen Jiang, Manisha Raghavan, Britney Forsyth, Aileen Rodriguez, Cyrus Tam, Sasha Balkaran, Saskia Hartner, Morgan Lallo, Ahmed Mahmoud, Andrew Moorman, Jura Pintar, Dana Pe'er, Richard Koche, Quaid Morris, Joe Chan, Karuna Ganesh

Memorial Sloan Kettering Cancer Center, New York, NY

Phenotypic plasticity is emerging as a key hallmark of cancer progression, yet the molecular mechanisms of cancer cell reprogramming remain poorly understood. Using single cell RNA sequencing of synchronously surgically resected matched trios of normal colon, primary and metastatic tumors from colorectal cancer (CRC) patients, we recently demonstrated that CRC undergoes highly conserved sequential phenotypic transitions during tumor progression: (1) differentiated epithelial cells dedifferentiate into an LGR5+ intestinal stem cell (ISC) state during primary tumor initiation (2) disseminating cancer cells adopt an injury-induced fetal-like state and (3) at the metastatic site again dedifferentiate into LGR5+ ISC states, or alternatively undergo lineage plasticity into non-canonical neuroendocrine and squamous states, associated with aggressive behavior and poor clinical outcomes. Cancer cells thus dedifferentiate into LGR5+ ISC states at two steps during tumor progression. Seeking regulators of dedifferentiation, computational analysis identified the RNA binding protein ZFP36L2 as a stress-associated ISC state marker in normal intestinal crypts and in primary and metastatic tumors. In mouse models, we demonstrate that ZFP36L2 is dispensable for intestinal homeostasis, but is required for epithelial regeneration after DSS induced colitis or ISC ablation. Using human tissue samples, organoids and orthotopic mouse models, we show that loss of ZFP36L2 impairs progenitor regeneration and metastatic seeding—both of which require dedifferentiation into an intestinal stem cell state—while promoting proliferation and lineage plasticity into non-canonical states. Multiplex immunofluorescence staining of clinical samples demonstrates that loss of function mutations in ZFP36L2, found in 5-10% of human CRC, are associated with non-canonical differentiation in metastasis. Mechanistically, using HyperTRIBE and mRNA decay analysis, we show that ZFP36L2 binds to stress-associated mRNAs containing AU-rich 3’UTRs, in turn inducing the formation of dynamic phase-separated biomolecular condensates. Unexpectedly, IP-MS identifies the intestinal differentiation transcription factor CDX2, and the NCOR nuclear corepressor complex as lead ZFP36L2 interactors. Nuclear/cytoplasmic fractionation and super resolution live cell microscopy demonstrate that while ZFP36L2 is largely cytoplasmic, it can shuttle into the nucleus, where it induces transcriptional repression of the intestinal differentiation program through CDX2-NCOR complex formation and chromatin compaction. Together, our work identifies ZFP36L2 as a key orchestrator of stress-induced phenotypic plasticity, driving cellular dedifferentiation and epigenetic reprogramming essential for re-establishing the ISC state during metastatic seeding and wound healing.

#2649 Defining how Rnd3, a novel regulator of breast cancer invasion, affects the metastatic potential of triple-negative breast cancer.

Noemi Garcia Garcia, Thanh Ha Vy Nguyen, Dane Richey, Katie R. Ryan

Department of Biochemistry & Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR

The purpose of this study is to investigate whether the atypical Rho GTPase, Rnd3, affects triple-negative breast cancer (TNBC) cells metastatic potential (invasion and migration rates). One of the most dangerous attributes of cancer is its ability to metastasize and spread. The 5-year survival rate for localized breast cancer is 90%. However, this drops to 29% when breast cancer metastasizes. Identifying key regulatory proteins and signaling pathways involved in cell migration and invasion, two hallmarks of metastasis, will pave the way to potential therapeutic targets for reducing the metastatic potential of breast cancer. Rho GTPases regulate cellular processes that are commonly dysregulated in cancer with many studies focusing on what roles these proteins play in tumorigenesis. The expression level of the atypical Rho GTPase, Rnd3, rather than Rnd3 mutations, has been linked to cancer. High expression of Rnd3 increases cellular migration and invasion in several cancers, while depletion of Rnd3 decreases cellular proliferation and migration. How Rnd3 expression affects breast cancer progression is unknown. Here we assayed a panel of TNBC cell lines and assessed their migration and invasion potential, we observed a correlation of high Rnd3 expression and high cell migration and invasion rates. After knocking down Rnd3 expression in these cell lines we observed a significant reduction in cell invasion in MB-231, HCC1937, and MCF7 cells, and decreased cell migration in MB-231 cells. Rnd3 has been shown to regulate cell migration via the RhoA-ROCK1 pathway in melanoma. We were unable to rescue invasion rates in Rnd3 knockdown MB-231 cells following either chemical inhibition or knockdown of ROCK1, indicating Rnd3 is regulation cell invasion via a novel pathway in TNBC cells. Our findings indicate that Rnd3 regulates cell invasion and migration, two hallmarks of metastasis, in TNBC cells.

#2650 Inhibition of glucose uptake by targeting GLUT3 reduces cell invasion and metastasis of triple-negative breast cancer cells through modulating AMPK-mediated cell mechanics.

Aadil Qadir Bhat[1], Seeun Oh[2], Cristina V. Iancu[3], Jun-yong Choe[3], Dongjoon Lee[4], Donghoon Yoon[4], **Tae-Hyung Kim**[1]

[1]Pathology, University of New Mexico, Albuquerque, NM,[2]Molecular Genetics & Microbiology, University of New Mexico, Albuquerque, NM,[3]Chemistry, University of New Mexico, Albuquerque, NM,[4]Internal Medicine, University of Arkansas for Medical Sciences, Little Rock, AR

Obesity is accompanied by dysregulated metabolic functions including hyperglycemia, hyperinsulinemia, and hypertriglyceridemia. In clinics, obesity has been associated with increased breast cancer metastasis. Since cancer cells are highly dependent on aerobic glycolysis, called the Warburg effect, we hypothesized that the elevated extracellular glucose level that cancer cells experience with hyperglycemia in obesity promotes cancer metastasis.

The capacity of cancer cells to deform and exert contractile force, known as cell mechanics, plays a crucial role in various stages of metastasis. Our previous study demonstrated that elevated extracellular glucose levels boost glycolysis and activate the RhoA signaling pathway in human triple-negative breast cancer (TNBC) cells, resulting in heightened cell stiffness and increased contractility. Such modified cell mechanics are associated with enhanced cell migration and invasion, consistent with other in vitro studies showing that high extracellular glucose levels promote cancer cell motility.

In the current study, we present our new findings indicating that inhibition of glucose transporter 3 (GLUT3) significantly diminishes the motility of TNBC cells, including human (MDA-MB-231) and mouse (EO771) TNBC cells. Mechanistic investigations reveal that inhibition of glucose uptake by targeting GLUT3 pharmacologically using GLUT3-selective inhibitor (G3iA) or genetically using GLUT3 knock-out cells reduces glucose uptake and glycolysis without affecting cell viability. Such reduced glycolysis activates 5' AMP-activated protein kinase (AMPK) and decreases total F-actin levels and non-muscle myosin II activity, which results in decreased cell stiffness. We further elucidated that the decreased F-actin level is mediated by actin regulators including VASP and cofilin. These changes are associated with a reduction in cell migration. To confirm the anti-metastatic effects of GLUT3 inhibition in vivo, we developed two obese-TNBC mouse models using diet-induced obesity (DIO) strategy in NSG mice and C57BL/6J mice with orthotopic implantation of human and mouse TNBC cells, respectively. GLUT3 deficient MDA-MB-231 cells showed significantly reduced lung metastasis compared to wild-type MDA-MB-231 cells.

In summary, our results suggest that inhibiting GLUT3 modifies the mechanics of TNBC cells, leading to a decrease in cell motility and metastasis. Given that GLUT3 is notably elevated in TNBC and is linked to metastatic progression and a poor prognosis in breast cancer patients, our findings underscore the need for further in vivo studies to validate G3iA as a potential anti-metastatic drug in obese TNBC patients.

#2651 Nuclear prothymosin α suppresses TGF-β-induced EMT by stabilizing smad7: implications for lung cancer progression and metastasis.

Bing-Hua Su, Li-Hsin Cheng

Taipei Medical University, Taipei, Taiwan

Elevated expression of prothymosin α (ProTα), a 12.5-kDa acidic protein encoded by the PTMA gene, is frequently observed in cancers, including lung cancer, where it correlates with poor prognosis. Although ProTα is primarily localized to the nucleus, advanced-stage tumors exhibit its translocation to the cytoplasm, indicating a potential involvement in disease progression. In this study, we aim to elucidate the molecular mechanisms by which nuclear ProTα regulates epithelial-to-mesenchymal transition (EMT) and metastasis in lung cancer. Our findings demonstrate that nuclear ProTα suppresses TGF-β-induced EMT by enhancing Smad7 acetylation and stability. This acetylation disrupts the binding of Smad2 to the promoters of EMT transcription factors SNAI1, TWIST1, and ZEB1, thereby reducing their expression. Conversely, the loss of nuclear ProTα resulting from caspase-3-mediated cleavage of its nuclear localization signal (NLS) enables cytoplasmic translocation, associated with increased metastatic potential. Moreover, the use of mutant Smad7 constructs confirmed that the inhibitory effect of ProTα on EMT is dependent on Smad7 acetylation. Our clinical analyses of lung cancer specimens demonstrate that high nuclear ProTα levels are prevalent in early-stage tumors, while metastatic cases exhibit significant nuclear ProTα loss. These observations identify nuclear ProTα as a potential biomarker for tumor progression and a suppressor of lung cancer metastasis through TGF-β signaling interruption. In conclusion, this study underscores the importance of nuclear ProTα in lung cancer progression and highlights its potential as a prognostic indicator and therapeutic target for metastasis prevention. Further research into ProTα-targeted therapies could open new avenues in cancer treatment.

#2652 Glucose-transporter 1 (GLUT1) is highly expressed in many tumor types and often linked to tumor progression.

Seyma Buyucek[1], Katharina Moller[1], Florian Viehweger[1], Ria Schlichter[1], Anne Menz[1], Andreas M Luebke[1], Viktor Reiswich[1], Martina Kluth[1], Claudia Hube-Magg[1], Elena Bady[1], Lisa Hornsteiner[1], Andrea Hinsch[1], Florian Lutz[1], Soren Weidemann[1], Frank Jacobsen[1], **Maximilian Lennartz**[1], David Dum[1], Christian Bernreuther[1], Patrik Lebok[1], Guido Sauter[1], Andreas H Marx[2], Ronald Simon[1], Christoph Fraune[1], Natalia Gorbokon[1], Eike Burandt[1], Sarah Minner[1], Stefan Steurer[1], Till Olchers[3], Sonke von der Weihe[3], Martin Reck[3], Till S Clauditz[1], Till Krech[1], Viktoria Chirico[1]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany,[2]Academic Hospital Fuerth, Fuerth, Germany,[3]German Center for Lung Research, Grosshansdorf, Germany

Glucose transporter 1 (GLUT1) is a key protein for transmembranous glucose uptake of cells which is often overexpressed in cancer. To better comprehend the prevalence and role of GLUT1 expression in different cancer types, a tissue microarray containing 14,966 samples from 134 different tumor entities was analyzed in this study. GLUT1 expression was generally markedly higher in cancer than in corresponding normal tissues. A total of 122 of 134 tumor categories showed GLUT1 expression in at least one case, and 97 tumor categories included at least one case with strong GLUT1 staining. The frequency of GLUT1 positivity was particularly high in squamous cell carcinomas of various sites of origin (92.9%-100%), urothelial neoplasms (91.3%-98.7%), and carcinomas of the ovary and the endometrium (up to 100%). Elevated levels of GLUT1 staining were often associated with parameters of cancer aggressiveness. For example, high GLUT1 staining was associated with invasive growth of urothelial carcinomas ($p<0.0001$), high grade ($p=0.0015$), advanced pT stage ($p=0.0050$), and shorter survival ($p<0.0040$) in clear cell renal cell carcinoma, high grade ($p=0.0500$), advanced pT stage ($p=0.0460$), nodal ($p=0.0460$) and distant metastasis ($p=0.0022$), as well as shorter recurrence free survival ($p=0.0253$) in papillary renal cell carcinoma, high BRE grade in invasive breast cancer of no special type ($p<0.0001$), advanced pT stage ($p<0.0001$), nodal metastasis ($p<0.0001$), V1 status ($p=0.0005$), L1 status ($p<0.0001$), and mismatch repair protein (MMR) deficiency ($p=0.0378$) in colorectal adenocarcinoma, advanced pT stage ($p=0.0153$) and MMR deficiency ($p=0.0006$) in gastric cancer, advanced pT stage in pancreatic adenocarcinoma ($p=0.0068$), nodal metastasis in endometrioid carcinoma of the ovary ($p=0.0136$), advanced pT stage ($p=0.0305$), nodal metastasis and higher grade ($p<0.0001$ each) in lung adenocarcinomas, and with overall survival in non-small cell lung cancer (NSCLC, $p=0.0139$). It is concluded, that GLUT1 is highly expressed in a broad range of tumor entities, that it occurs more often in malignant than in benign tumors, and that it is linked to aggressive behavior in many different entities. These findings identify GLUT1 as a critical prognostic cancer marker, which may be clinically useful in many cancer types.

#2653 Non-muscle myosin II a role in breast cancer proliferation, invasion and metastasis.

Wasim Feroz[1], Sarah Storr[2], Andrew Stiles[3], Marykate Kilroy[1], Olamide Ogunleye[1], Joan T. Garrett[1]

[1]University of Cincinnati, Cincinnati, OH,[2]University of Nottingham, Nottingham, United Kingdom,[3]University of Notre Dame, Indiana, OH

We recently identified non-muscle myosin IIa (NMIIA) as a novel binding partner for HER3 in HER2+ breast cancer cells. This interaction was observed through HER3 immunoprecipitation experiments following inhibition of the HER family, compared to vehicle control treatments. NMIIA, a hexameric protein, is encoded by the Myosin heavy chain 9 (*MYH9*) gene, which provides the heavy chain necessary for its structure and function. NMIIA plays a critical role in cytoskeletal dynamics and cellular mechanotransduction, processes implicated in cancer progression and metastasis. To investigate the clinical significance of NMIIA, we analyzed its protein expression in early-stage invasive breast cancer samples using immunohistochemistry. Tumor samples were stained with an antibody against NMIIA and subsequently stratified into two groups: the lower 50% and the upper 50% based on NMIIA expression levels. This stratification was performed by a trained pathologist. Focusing on patients with lymphovascular invasion (LVI), we found that those with high NMIIA expression levels exhibited significantly worse disease-specific survival compared to patients with low NMIIA levels ($p = 0.010$). Interestingly, no difference in survival was observed among LVI-negative patients, regardless of NMIIA expression levels ($p = 0.948$). These findings underscore a potential interplay between NMIIA expression and tumor vasculature, suggesting NMIIA may contribute to tumor progression in the presence of LVI. Ongoing studies aim to determine whether NMIIA is more highly expressed in matched brain metastases of breast cancer compared to their primary tumors, mirroring a pattern previously reported for HER3. This research will help elucidate NMIIA's role in metastatic progression and its potential as a therapeutic target. Additionally, we are exploring whether NMIIA contributes to breast cancer development in a mechanically sensitive manner using a novel parent NMII inhibitor compound. This compound has already led to the development of a small molecule inhibitor that has entered clinical trials, highlighting the translational potential of targeting NMIIA. Our findings suggest NMIIA as a key player in HER3 signaling and tumor progression, particularly in the context of LVI, and provide a foundation for further research into its mechanistic and therapeutic implications.

#2654 MTAP deficiency is highly homogeneous in advanced, muscle-invasive urothelial carcinoma of the urinary bladder.

Natalia Gorbokon[1], Viktor Reiswich[1], Florian Lutz[1], Sebastian Dwertmann Rico[1], Henning Plage[2], Maximilian Lennartz[1], Margit Fisch[1], Sarah Minner[1], Elena Bady[1], Lisa Hornsteiner[1], Ronald Simon[1], Guido Sauter[1], Henrik Zecha[3], Stefan Steurer[1], Thorsten Schlomm[2], Lukas Bubendorf[4], David Horst[2], Thorsten Ecke[5], Stefan Koch[5], Martina Kluth[1], Florian Viehweger[1], Morton Freytag[1]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany,[2]Charite Berlin, Berlin, Germany,[3]Albertinen Hospital, Hamburg, Germany,[4]University Hospital Basel, Basel, Switzerland,[5]Helios Hospital Bad Saarow, Bad Saarow, Germany

Complete expression loss of S-methyl-5′-thioadenosine phosphorylase (*MTAP*) is caused by homozygous 9p21 deletion and results in a critical vulnerability of cancer cells towards drugs targeting multiple different pathways. MTAP deficiency is common in urothelial cancer, but data on its intratumoral heterogeneity - a potential obstacle for targeted therapies - are lacking. To study the heterogeneity of MTAP expression loss and 9p21 deletions in advanced primary urothelial cancers of the urinary bladder, a tissue microarray (TMA) was constructed from 5 different sites from different tissue blocks of 105 pT2-4 urothelial carcinomas and analyzed by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH). In addition, all 1-15 tumor containing blocks (average 6.1) from 41 consecutive pT2-4 carcinomas were analyzed by IHC. Complete absence of MTAP staining (MTAP deficiency) was seen in 132 of 385 interpretable TMA samples. All 80 samples with a complete MTAP expression loss and FISH data had a homozygous 9p21 deletion while there were no cases with homozygous deletions within 148 samples with retained MTAP expression (100% FISH/IHC concordance). On a patient level, there was a homogeneous MTAP deficiency in 33%, a heterogeneous MTAP deficiency in 1% and a retained MTAP expression in 66% of these 98 tumors with at least 3 interpretable TMA samples. Among the 41 consecutive pT2-4 carcinomas from which whole sections were analyzed, MTAP was homogeneously deficient in 34%, heterogeneously deficient in 4.9% and homogeneously retained in 61%. It is concluded that MTAP deficiency is mostly homogeneous in advanced urothelial carcinoma. MTAP IHC is a near perfect surrogate for the detection of homozygous 9p21 (MTAP) deletions. Drugs targeting MTAP-deficiency could be highly useful in a relevant subset of invasive urothelial bladder cancers.

#2655 Novel stress-induced extracellular regulatory mechanism leads to decreased cellular invasion in prostate cancer.

Katelyn L. O'Neill, Johnny Zigmond, Raymond Bergan

University of Nebraska Medical Center, Omaha, NE

Background: Cancer cells are exposed to multiple stressors in their environment. The ability to overcome stress involves mechanisms that drive aggressive behavior such as cell motility and subsequent metastasis. Expression and secretion of HSP90α has been linked to many forms of cell stress and plays an important role in cancer cell migration and invasion. It's been shown to do so through co-chaperone mediated activation of extracellular proteases such as MMPs, which are responsible for degradation of the extracellular matrix allowing for cell migration/invasion. Yet how MMPs respond to cellular stress and subsequent levels of extracellular HSP90α is not well understood. We conducted a comprehensive evaluation of the above factors focusing on human prostate cancer (PCa).
Methods: Cell stress was induced in PCa cell lines via heat shock (43°C) and serum starvation (SS). Cell lysate and conditioned media (CM) were probed separately to examine intracellular vs. extracellular proteins by Western blot. MMP-2 activation was measured by zymography and gelatin dequenching assays. Cell invasion was measured using scratch wound and Matrigel drop assays. HSP90α knockout (KO) was performed in PC3 cells utilizing CRISPR-Cas9 transient transfection and verified by genomic sequencing and Western blot.
Results: We examined human PCa cells in vitro, demonstrating that under stress conditions (i.e., increased temperature and SS), cytosolic and extracellular HSP90α increased. In contrast to the findings of others, we demonstrate that this increase is associated with a decrease in MMP-2 activity. We went on to corroborate the functional relevance of MMP-2 decrease by demonstrating that CM from stressed cells decreased PCa cell invasion compared to media from non-stressed cells. Investigating further we found that decreased MMP-2 activity is mediated by processes dependent on extracellular protease/phosphatase activity. To examine the primary role of HSP90α in these processes, we created HSP90α KO PCa cells. KO cells exhibit normal proliferation and decreased invasion. We are currently using HSP90α KOs to investigate the direct role of extracellular HSP90α in stress-induced MMP-2 responses.
Conclusion: While others have examined separate components of this complex regulatory process, to our knowledge this is the first comprehensive evaluation integrating cell stress, the composition and function of secreted factors, how they are regulated and the resultant impact on PCa cell motility. Cell stress in all PCa cells tested led to increased expression and secretion of HSP90α. Surprisingly, this is associated with a decrease in MMP-2 activity. Decreased MMP-2 is due to a novel regulatory mechanism involving extracellular protease/phosphatase activity. HSP90α is involved in PCa cell invasion. We are currently testing its role in stress-related changes of MMP-2.

#2656 Sec23b governs the migration of MDA-MB-231 breast cancer cells by regulating collagen I processing and secretion..

E. Emily Joo[1], Audrey Astori[2], Jonathan St-Germain[2], Brian Raught[2], Michael F. Olson[1]

[1]Toronto Metropolitan University, Toronto, ON, Canada,[2]Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada

Cancer metastasis is a complex, multi-step process that begins with the dissociation of primary cancer cells from their original site. These cells then migrate to distant areas, where they establish colonies and undergo uncontrolled proliferation at secondary locations. Thus, dysregulation of cell migration is one of the hallmarks of solid tumour metastasis. Given that the actin cytoskeleton plays crucial roles in normal cell migration, we developed a robust method that combines an amino acid sequence that binds to actin (LifeAct) with biotin-mediated proximal labelling (BioID) to identify actin-binding proteins. Specifically, to identify actin-associated protein complexes during the early stages of cells migrating into scratched areas, we conjugated LifeAct with the newest derivation of BioID (miniTurbo-LifeAct-BioID) and controlled its expression with doxycycline in MDA-MB-231 cells. After inducing cell migration by scratch wounding, we allowed cells to migrate into the scratched areas for 1.5 hours then pulse-labelled with biotin for 30 minutes. As controls, using the same system, we labelled actin-associated proteins in resting cells and in cells that had been treated with cytochalasin D (CD) to depolymerize actin filaments. Compared to the untreated cells, the CD-treated cells increased labelling of the G-actin binding proteins (e.g., profilin and JMY) while decreased labelling of the F-actin binding proteins (e.g., Coronin2A and ABLIM1/ABLIM2/ ABLIM3), validating the ability of miniTurbo-LifeAct-BioID to detect dynamic changes in cytoskeleton-associated protein complexes. By comparing miniTurbo-LifeAct-BioID labelled proteins in migrating versus stationary cells, eighteen proteins were significantly more biotin labelled and eight proteins were less biotin labelled. Unexpectedly, Sec23b, a component of the COPII secretion complex, was labelled to a greater extent in cells migrating into scratched areas. Depletion of Sec23b increased secretion of procollagen I but decreased deposition of collagen I filaments. Interestingly, the reduced migration of cells depleted of Sec23b was rescued by collagen I but not by fibronectin or gelatin. Based on our findings, metastatic breast cancer MDA-MB-231 cells appear to control the secretion and processing of collagen I to govern their extracellular matrix organization for efficient cell migration.

#2657 Impact of STRN4 knockdown on TNBC migration and survival.

Caroline Rinderle, Jordan Wright, Olivia Mayer, Bruce Bunnell

University of North Texas Health Science Ctr., Fort Worth, TX

Introduction: Striatin 4 (STRN4) is a member of the STRIPAK protein complex, including protein phosphatase 2A (PP2A) and other interacting protein kinases. These proteins interact to play a role in a range of signaling cascades, including MAPK and Hippo signaling. STRN4 has been shown to play a role in several cancer types, as it is a negative prognostic marker of renal, ovarian, and liver cancers. It has also been shown to contribute to liver cancer migration, and its knockdown diminishes pancreatic cell migration and proliferation. However, although STRN4 is associated with a trend of decreased overall survival in breast cancer patients, it is relatively understudied in the context of the disease.

Experimental Methods: To better understand the role of STRN4 in breast cancer survival and related processes, an siRNA-mediated knockdown of STRN4 was performed in a patient-derived xenograft-derived triple-negative breast cancer (TNBC) cell line: TU-BcX-4IC (4IC). Using three pre-validated siRNAs (A, B, and C) specific to STRN4 paired with a polymer-based transfection reagent, we knocked down the expression of STRN4. We then monitored the effects of the knockdown on 4IC cell migration and invasion, proliferation, metabolism, and gene expression via Boyden chamber migration assays, colony-forming unit assays, XTT assays, and qRT-PCR, respectively.

Results: STRN4 gene expression was significantly diminished 24 hours post-transfection, which was maintained 72 hours post-transfection. However, there was no significant difference in the migration of 4IC cells through a Boyden chamber, and only minor alterations in the expression of epithelial-to-mesenchymal transition markers E-cadherin, Vimentin, CD24, and Jagged1 24, 48 and 72 hours post-knockdown, despite significantly lower expression of STRN4 at these timepoints. However, there was substantially lower colony-forming unit potential and metabolic output in the C siRNA group despite there being no difference between the A and B siRNA groups.

Conclusions: Overall, STRN4 knockdown has minimal effects on TNBC cell migration and gene expression 24, 48, and 72 hours after the onset of diminished expression. While this may suggest that STRN4 is not as crucial to TNBC-related processes, much research remains. In the future, we plan to add additional cell lines to the knockdown experimental groups and monitor protein expression during STRN4 knockdown.

#2658 GATA2 loss promotes Piezo1 expression and stiffness-dependent invasion in uterine serous carcinoma.

Brittany Baikie, Mayuri Dutta, Pamela K. Kreeger, Daniel R. Matson

Pathology and Laboratory Medicine, University of Wisconsin-Madison, Madison, WI

Background: Piezo1 is a mechanosensitive ion channel that couples extracellular matrix (ECM) properties to intracellular signaling cascades that can modulate cell phenotypes. Piezo1 was recently shown to regulate metastasis in high-grade ovarian serous carcinoma (HGSOC) by promoting tumor budding in an ECM stiffness-dependent manner. Uterine serous carcinoma (USC) is an aggressive gynecologic malignancy that is increasing in incidence and responsible for 40% of uterine cancer deaths. USC rapidly invades and metastasizes via mechanisms that remain incompletely understood. Whether Piezo1 may have a similar function in USC, which shares many molecular and clinical features of HGSOC, is not known. We recently showed that expression of the transcription factor GATA2 is lost in USCs that locally invade into the wall of the uterus versus non-invasive USCs. However, the transcriptional targets of GATA2 that mediate this activity are not known.

Methods: We performed RNAseq in patient-derived USC cells lines after GATA2 depletion to identify differentially expressed genes. We performed anti-GATA2 ChIPseq in patient derived USC cells to identify genome wide sites of GATA2 occupancy. Western blots were performed for GATA2, Piezo1, and Tubulin after GATA2 depletion in patient-derived USC cells modified to express doxycycline-inducible anti-GATA2 shRNAs or shScramble control. *In vitro* models of ECM with different stiffnesses (3.7 and 14 kPa) were generated using an interpenetrating network of collagen I and methacrylated gelatin (GelMA), crosslinked within transwell inserts. USC cells were seeded on top of the gels and monitored over 24 hours for invasion in response to GATA2 depletion.

Results: GATA2 depletion led to an increase in *Piezo1* transcripts in patient-derived USC cells (log2fold change = 0.28, FDR<0.005), while western blot revealed a significant increase in Piezo1 protein expression after GATA2 depletion in two unique patient-derived USC cell lines. Anti-GATA2 ChIPseq identified two significant peaks of GATA2 occupancy within intron 1 of the *Piezo1* gene, each harboring conserved canonical *GATA* motifs. Depletion of GATA2 in patient-derived USC cells led to increased tumor cell invasion, but only on the stiffer substrates.

Conclusions: GATA2 binds two putative GATA enhancers within intron 1 of the *Piezo1* gene in USC. GATA2 depletion leads to increased Piezo1 RNA and protein expression, which is accompanied by increased tumor cell invasion in a stiffness-dependent manner. Our findings support a model where loss of GATA2-mediated Piezo1 suppression facilitates increased Piezo1 expression, and Piezo1-driven ECM stiffness-dependent USC tumor invasion. They justify further studies to evaluate whether a GATA2-Piezo1 axis is a critical driver of USC invasion *in vivo*.

#2659 Erythropoietin stimulation in BRAF mutated metastatic melanoma confers a highly proliferative and invasive phenotype.

Jayesh Menon[1], Raza Ahmed[2], Muneeb Aslam[3], Rakesh Singh[2], Philip Friedlander[4], Omar Aljitawi[2]

[1]University of Rochester School of Medicine, Rochester, NY,[2]Wilmot Cancer Center, Rochester, NY,[3]University of Rochester, Rochester, NY,[4]Icahn School of Medicine at Mount Sinai, New York City, NY

Introduction: Currently, there is a lack of understanding of the mechanisms underlying the progression of primary melanoma to a metastatic phenotype. Interestingly, RNA-sequencing data from the cancer genome atlas has demonstrated that patients with cutaneous melanoma with high expression of EPOR have poor long-term survival outcomes. This finding may be related to why some patients with anemia of malignancy treated with erythropoietin (EPO) have progression of their cancer. There has been significant discussion on this topic and whether membrane-bound EPOR is functional in solid malignancies. Here we report on how modulation of EPOR affects melanoma cell proliferation and invasion in an in-vitro model of metastatic disease.

Methods: A metastatic melanoma cell line, SK-MEL1, with mutant BRAFV600E was treated with varying doses of EPO and a novel EPOR inhibitor, URV3, for 24 hours. Cell proliferation was quantified by determining absolute cell number using a hemocytometer. Further, an invasion assay was utilized to assess the effect of EPOR modulation on cell invasiveness. ~50,000 cells were seeded into 1:5 diluted Matrigel overlaying a porous membrane and allowed to invade for 24 hours. After 24 hours, Matrigel was removed from the membrane and cells were fixed, stained, and imaged in 4 different positions. Invaded cells were quantified by averaging the cell density in each imaged field (cells/micrometer^2) and multiplying by total membrane area. All data were performed in duplicates and reported as mean fold change from control. Non-parametric ANOVA was used for statistical analysis.

Results: Modulation of EPOR with EPO, 8uM URV3, 8uM URV with EPO, 32uM URV3, and 32uM URV3 with EPO yielded a mean fold change in cell number over control of 1.43 (95% CI: 1.09-1.78), 0.89 (95% CI: 0.56-1.24), 1.38 (95% CI: 0.95-1.81), 0.88 (95% CI: 0.46-1.30), and 1.59 (95% CI: 1.15-2.04), respectively (p=0.0027). Cell invasion after 24 hours of treatment with EPO, 8uM URV3, 8uM URV with EPO, 32uM URV3, and 32uM URV3 with EPO yielded a mean fold change in cell invasion over control of 1.96 (95% CI: -0.80-4.71), 0.59 (95% CI: -4.82-5.99), 0.48 (95% CI: 0.03-0.92), 0.48 (95% CI:-1.22-2.19), and 0.60 (95% CI: -1.22-3.96) respectively (p=0.059).

Discussion: We demonstrate that direct EPOR stimulation causes increased cell proliferation and invasion. EPOR blockade with URV3 impedes cell growth; however, it is unable to overcome the proliferative effect of additional EPO stimulation. Interestingly, URV3 demonstrates a strong inhibitory effect on melanoma cell invasion despite EPO stimulation. These results illustrate the functional capabilities of the cell-surface bound EPOR receptor in metastatic melanoma and warrant further investigation of downstream signaling that contributes to this invasive and proliferative phenotype.

TUMOR BIOLOGY: Microenvironment-Driven Cancer Evolution
Poster Session

#2663 Mapping clonal heterogeneity and stromal dynamics in prostate cancer.

Sandy Figiel[1], Mengxiao He[2], Wencheng Yin[1], Emmanouela Perisynaki[2], Renuka Teague[1], Thineskrishna Anbarasan[1], Eleanor O'Roberts[2], Nithesh Ranasinha[1], Sophia M. Abusamra[1], Trishna Desai[3], Dimitrios Doultsinos[1], Andrew Erickson[1], Srinivasa Rao[1], Clare Verrill[1], Richard Colling[1], Pelvender Gill[1], Tuomas Mirtti[4], Ibrahim Kulac[5], Karl Smith-Byrne[3], Ruth Travis[3], Charlotte Stadler[2], Richard J. Bryant[1], Freddie C. Hamdy[1], Dan J. Woodcock[1], Ian G. Mills[1], Joakim Lundeberg[2], Alastair D. Lamb[1]

[1]Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom,[2]Science for Life Laboratory, KTH Royal Institute of Technology, Solna, Sweden,[3]Cancer Epidemiology Unit, Oxford Population Health, University of Oxford, Oxford, United Kingdom,[4]Department of Pathology, University of Helsinki & Helsinki University Hospital, Helsinki, Finland,[5]Department of Pathology, Koc University School of Medicine, Istanbul, Turkey

Introduction: Prostate cancer (PCa) exhibits significant intratumoral heterogeneity, driving a spectrum of phenotypes ranging from indolent disease to aggressive metastasis, challenging risk stratification and treatment. A key feature of this heterogeneity is clonal diversity, encompassing both cancer cells and their interactions with the surrounding stromal microenvironment. Understanding clonal dynamics and stromal influences on tumor progression is crucial for identifying factors that contribute to metastasis and disease lethality, ultimately improving risk stratification and therapeutic strategies.

Methods: We performed organ-wide spatial transcriptomic analyses (Visium^{TM} v2, 10x Genomics) on formalin-fixed paraffin-embedded tissues from ten patients with primary, nodal metastatic PCa and biopsy samples, recruited to a local trial (ISRCTN10046036). Two pathologists meticulously annotated each spot, including benign, Gleason grade, stroma, and immune cells. Fastq data were processed using SpaceRanger^{TM}, and inferred clonal genomic identities were analyzed with SpatialInferCNV (https://github.com/aerickso/SpatialInferCNV). We reconstructed clonal evolution using phylogenetic trees and analyzed stromal gene expression around distinct tumor clones using differential expression analyses. In addition, we performed multiplex imaging on consecutive sections to assess protein expression and correlate it with gene expression data, enabling the identification of key tumor-stroma interactions associated with metastatic progression.

Results: We analyzed 122 spatial transcriptomics capture areas, collectively representing over 1,000,000 spots (55 µm diameter), or approximately 16,000,000 cells. We identified clonal subtypes across biopsy, primary and metastatic tissues, revealing distinct clonal relationships and polyclonal lymph node colonization at different evolutionary stages. Stromal cells around metastatic progenitor clones showed upregulation of antigen presentation and inflammatory pathway genes, contrasting with stromal profiles surrounding lethal clones (as defined by their ability to metastasize to lymph nodes). These findings suggest a dynamic interplay between tumor cells and their stroma, potentially influencing metastatic potential and immune evasion.

Conclusion: This study provides a detailed cellular-level characterization of clonal heterogeneity in PCa and its relationship with stromal dynamics, offering insights into mechanisms driving metastatic progression. These insights could inform strategies for risk stratification and therapeutic intervention, including leveraging dual tumor-stromal profiles as biomarkers to distinguish lethal from indolent disease. Understanding the interplay between tumor clones and their stroma opens new avenues for targeted treatment approaches aimed at preventing metastasis and improving patient outcomes.

#2664 Spatial and single-cell transcriptomics reveal molecular heterogeneity in pulmonary pleomorphic carcinoma.

Atsushi Matsuoka[1], Kazuhiko Shien[2], Shuta Tomida[3], Hirofumi Inoue[3], Kazuya Hisamatsu[1], Ryota Fujiwara[1], Kousei Ishimura[1], Ryunosuke Fujii[1], Ryo Yoshichika[1], Tomoaki Higashihara[1], Naohiro Hayashi[1], Kazuhiro Okada[1], Fumiaki Mukohara[1], Mao Yoshikawa[1], Ken Suzawa[2], Hidetaka Yamamoto[4], Shinichi Toyooka[1]

[1]Thoracic, Breast and Endocrinological Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan,[2]Thoracic, Breast and Endocrinological Surgery, Okayama University Hospital, Okayama, Japan,[3]Center for Comprehensive Genomic Medicine, Okayama University Hospital, Okayama, Japan,[4]Pathology and Oncology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

Background: Pulmonary pleomorphic carcinoma (PPC) is a rare and aggressive subtype of non-small cell lung cancer characterized by the presence of both epithelial and sarcomatoid components. We conducted multi-omics analyses on surgically resected specimens from nine primary PPC cases to explore characteristics of this tumor at molecular level.
Materials and Methods: Whole-exome sequencing and RNA sequencing on bulk tumor specimens to identify mutational profiles. Spatial transcriptomics using the Digital Spatial Profiler was employed to analyze gene expression separately for epithelial and sarcomatoid tumor components. Single-cell RNA sequencing (scRNA-seq) was conducted on three cases to explore intratumoral heterogeneity and clonal evolution. Gene signature based on extracellular matrix (ECM) remodeling was developed and validated in public lung cancer datasets to assess its association with tumor aggressiveness and prognosis.
Results: Mutational analyses revealed *MET* exon 14 skipping in two cases and an *ALK-EML4* fusion in one case, while no *EGFR* mutations were observed. We found various differential expressed genes (DEGs) between components. Further analysis (GSEA and Transcriptional factor scoring) revealed there was a common enrichment trend observed among pathways related to MYC, such as those involved in cell proliferation, division, and the cell cycle. On the other hand, significant differences were observed in pathways related to cancer invasion and cell-cell interactions, such as extracellular matrix (ECM) remodeling and hypoxia. identified an atypical cell population bridging epithelial cells and fibroblasts, which we defined as sarcomatoid cells. The clusters defined as epithelial tumor cells and sarcomatoid cells showed upregulation of DEGs corresponding to the respective component identified through spatial transcriptomics. Trajectory analysis visualized tumor evolution, suggesting a transition from epithelial tumor cells to sarcomatoid cells. A consistent upregulation of seven ECM remodeling-related genes was observed in both tumor components compared to normal lung tissue. When applied to a GEO lung adenocarcinoma cohort, this gene signature was associated with more aggressive histological subtypes ($P < 0.01$). Survival analysis using TCGA demonstrated that high ECM remodeling signature scores correlated with significantly poorer prognosis ($P < 0.01$).
Conclusion: This study provides a comprehensive molecular analysis of PPC, revealing both shared and distinct features between its components. The trajectory analysis supports the hypothesis of a common cellular origin for these components and highlights the transition to a more aggressive sarcomatoid phenotype. These findings provide new insights into the biology of PPC and identify potential therapeutic targets to address its intratumoral heterogeneity and clonal evolution.

#2665 Single-cell analysis and cytokines modulation of intra-tumor subpopulations in colorectal cancer tumoroids.

Sabrina Johanna Fletcher[1], Irene Catalano[2], Elena Grassi[1], Sofia Borgato[1], Barbara Peracino[3], Luca Primo[1], Livio Trusolino[1], Andrea Bertotti[1], Alberto Puliafito[1]

[1]Department of Oncology, University of Turin, Candiolo, Italy,[2]Candiolo Cancer Institute, Candiolo, Italy,[3]Department of Clinical and Biological Sciences, San Luigi Hospital, University of Turin, Orbassano, Italy

Colorectal cancer (CRC) is still one of the most commonly diagnosed cancers worldwide. Treatment strategies include chemotherapy, radiotherapy, targeted therapy, and immunotherapy. Despite the availability of such a diverse set of therapeutic strategies, around a million people still die from this disease each year worldwide. Phenotypic intra-tumor heterogeneity, arising from both genetic and non-genetic origins, has become a significant focus of study due to its impact on treatment response. Among the various subpopulations within a tumor, the stem-like cells have been extensively investigated, as they are recognized as tumor initiators. In contrast, the subpopulations identified by the expression of genes associated with differentiation has received comparatively less attention. An interesting hypothesis is that differentiated populations might plastically give rise to stem cell populations fueling tumor regrowth. Here we examine phenotypic intra-tumor heterogeneity in patient-derived metastatic CRC (mCRC) tumoroids. Our goal was to decipher the unknown role of differentiated cells and to relate it to growth and therapeutic response. In particular, we sought to determine whether manipulating exogenously the proportions of differentiated cells within CRC tumoroids could alter the drug response. We analysed one KRAS wild type (wt) model and one mutant (*kras*) model by scRNAseq. Our data confirmed that tumoroids present two main subpopulations, one associated to stemness (with high expression of LGR5), and the other associated to differentiation genes (with high expression of KRT20), coherently with reported studies. First we identified two novel cell surface markers associated to the KRT20+ subpopulation: GABRA2 and GPRC5C. Using FACS, we confirmed that our models present a double-positive subpopulation at the protein level. RT-qPCR confirmed that the expression of both genes increased over time. We next studied the effect of different cytokines on the modulation of this subpopulation. We found that exogenously adding IL6 and TGF-b1 alters the proportion of our markers, with a stronger effect in the wt sample. For both models, ELISA assays did not indicate any significant secretion into the culture medium of these cytokines. Interestingly, we found a release of IL6 receptor (IL6R) in the medium, particularly from the wt sample, likely as a results of the previously characterized cleavage of its membrane-bound form. Furthermore, RT-qPCR showed that TGF-b1 induced its own expression, a phenomenon known as autoinduction. Our preliminary results using the wt sample indicate that pre-treatment with these cytokines affected the response to Oxaliplatin, making it more sensitive to the chemotherapeutic reagent. Overall, our results indicate that cytokines released in the tumor microenvironment might reshape the intra-tumor phenotypic heterogeneity impacting drug-response.

#2666 Mapping intratumoral heterogeneity in colorectal cancer: Spatially resolved subclones and tumor microenvironment.

YeonJu Lee[1], Woong-Yang Park[2]

[1]Sungkyunkwan Uiversity, Seoul, Korea, Republic of,[2]Samsung Genome Institute, Seoul, Korea, Republic of

Background: Colorectal cancer (CRC) is the third most common malignancy, with over 1.85 million new cases and 850,000 deaths annually. Significant intratumoral heterogeneity in CRC contributes to recurrence, chemotherapy resistance, and poor prognosis. This study aimed to explore the spatial distribution of subtype clones and the diversity of the tumor microenvironment (TME), which are not captured by the traditional gene expression-based CMS classification. Additionally, it sought to investigate cancer trajectories and their contributions to metastatic phenotypes using spatial transcriptomics (ST).
Methods: Spatial analysis was performed using public 10X Genomics Visium data from 4 CRC and 4 CRC-liver metastasis (CRC-LM) patients (GSE226997, GSE217414). Cell-type deconvolution utilized single-cell RNA-seq datasets (GSE178318, GSE132465). Copy number alterations (CNA) were inferred, and subclone development trajectories were modeled using inferCNV, DPT, and PAGA analyses. Pathway and functional enrichment analyses, expression score calculations, and correlation analyses were conducted using ClusterProfiler, AddModuleScore, and statistical methods. A Wilcoxon test with an adjusted p-value < 0.05 was used to determine statistical significance.
Results: Distinct CRC and CRC-LM subclones were identified through CNA inference, revealing spatially segregated CNA clusters. These included malignant subclones and boundary-associated clusters, characterized by varying proportions of differentiation and mesenchymal traits. Immune-related signaling pathways demonstrated differential activation, with increased MAPK, JAK-STAT, and TNFα-NFκB in specific regions and elevated WNT, VEGF signaling, MYC, and E2F4 in others. Certain subclones also showed enrichment of SPP1+ macrophages and myofibroblasts, indicating diverse invasive characteristics. Evolutionary trajectories of subclones, inferred through hierarchical trees, revealed CSC features and a common ancestral clone. Progression toward malignancy was associated with enhanced pathway activity linked to critical biological processes. In CRC-LM, subclones were categorized by TAM subtypes, with one subtype displaying significantly elevated SPP1 expression compared to primary CRC. Immune-related features were reduced in metastatic tissues, reflecting a less immunogenic TME.
Conclusion: Intratumoral heterogeneity in CRC evolves from a single common ancestral clone into distinct progression states, resulting in spatially unique TMEs. Understanding the interactions between clones across different progression states offers valuable insights into disease dynamics and therapeutic opportunities.

#2667 Full spectrum profiling flow cytometry to study cancer-associated fibroblast heterogeneity.

Kevin Munoz Forti, Marta Storl-Desmond, Daniel Martinez, Sayana Issac, Shan Cao, Simon Schworer

Medicine, Section of Hematology and Oncology, The University of Chicago, Chicago, IL

It is now well appreciated that tumor growth depends on the tumor microenvironment (TME) comprising various non-malignant stromal cell types, including cancer-associated fibroblasts (CAFs). Cancer-associated fibroblasts (CAFs) are responsible for secreting the extracellular matrix (ECM) prominent in certain solid tumors such as pancreatic adenocarcinoma (PDAC). Despite our increasing knowledge of CAFs, therapeutic targeting of CAFs is currently not a feasible strategy, as CAFs are highly heterogeneous and extremely plastic, with each CAF population able to adopt both tumor-promoting and -restraining functions. We hypothesize that the plasticity and functional heterogeneity of CAFs can be explained by their flexible metabolism in response to environmental cues and the existence of discrete metabolic states within CAF subpopulations. While metabolic states can be predicted from single-cell transcriptomic data, this does not provide information on metabolic activity and potential, nor does it allow perturbations required for validation. Scalable and tractable methods to assess metabolic activity and CAF heterogeneity in parallel at the single cell level are needed to distinguish numerous existing CAF states do not exist. To address this need, in this work, we developed a strategy to study CAF metabolic activity and phenotypic heterogeneity at the single-cell level. We designed and optimized a 25-plex immunophenotyping panel of CAF and fibroblast markers that can be combined with numerous metabolic probes via Full Spectrum Profiling (FSP) flow cytometry. In addition, we compiled an analysis pipeline to facilitate the analysis of high-dimensional flow cytometry data. Using established 2D and 3D mono- and co-culture assays, we demonstrate the potential of our panel to be multiplexed with chemical probes for cell proliferation, lipid content, lipid uptake, fatty acid oxidation, glucose uptake, cholesterol content, hypoxia, or mitochondrial potential. Deploying our strategy in a KPC organoid-derived murine model of PDAC, we observed subpopulations of CAFs with differing metabolic potentials that are consistent with our predictions from transcriptomic metabolic modeling. With the high throughput and single-cell nature of FSP, our strategy represents a novel and modular tool for studying CAF phenotypic and metabolic heterogeneity.

#2668 Spatial-temporal diversity of extrachromosomal DNA shapes urothelial carcinoma evolution and tumor-immune microenvironment.

Wei Lv[1], Yuchen Zeng[2], Conghui Li[3], Yuan Liang[4], Jens Luebeck[5], Huiying Tao[6], Yanfen Zhu[7], Xiaolong Sui[6], Yue Li[6], Shiqi Jiang[2], Qingqing Gao[2], Elias Rodriguez Fos[8], Gino Prasad[5], Yuanmei Wang[2], Run Zhou[2], Zhe Xu[9], Xiaoguang Pan[3], Linlin Chen[2], Xiang Xi[10], Huajing Teng[11], Chaoyang Sun[12], Tianyu Qin[12], Wei Dong[2], Yongwei Li[6], Xun Lan[13], Xuesong Li[14], Lin Lin[15], Lars Bolund[2], Huanming Yang[2], Roel G. W. Verhaak[16], Bishoy M. Faltas[17], Jacob B. Hansen[15], Sihan Wu[18], Paul S. Mischel[19], Anton G. Henssen[20], Vineet Bafna[5], Birgitte Regenberg[3], Yonglun Luo[2], Chunhua Lin[6], **Peng Han**[10]

[1]Department of Urology & Andrology, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China,[2]Lars Bolund Institute of Regenerative Medicine, HIM-BGI Omics Center, Hangzhou Institute of Medicine (HIM), Chinese Academy of Sciences, Hangzhou, China,[3]Department of Biology, University of Copenhagen, Copenhagen, Denmark,[4]CAS Key Laboratory of Genomic and Precision Medicine, Beijing Institute of Genomics, Chinese Academy of Sciences and China National Center for Bioinformation, Beijing, China,[5]Department of Computer Science and Engineering, University of California at San Diego, La Jolla, CA,[6]Department of Urology, The Affiliated Yantai Yuhuangding Hospital of Qingdao University, Yantai, China,[7]The Fourth Affiliated Hospital of School of Medicine, and International School of Medicine, International Institutes of Medicine, Zhejiang University, Yiwu, China,[8]Department of Pediatric Oncology and Hematology, Charite - Universitatsmedizin Berlin, corporate member of Freie Universitat Berlin, Humboldt-Universitat zu Berlin, Berlin, Germany,[9]Lars Bolund Institute of Regenerative Medicine, Qingdao-Europe Advanced Institute for Life Sciences, BGI-Qingdao, BGI-Hangzhou, BGI-Shenzhen, Qingdao, China,[10]The Seventh Affiliated Hospital, Sun Yat-sen University, Shenzhen, China,[11]Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Department of Radiation Oncology, Peking University Cancer Hospital & Institute, Beijing, China,[12]Department of Gynecological Oncology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China,[13]Department of Basic Medical Sciences, School of Medicine, Tsinghua University, Beijing, China,[14]Department of Urology, Peking University First Hospital, Beijing, China,[15]Department of Biomedicine, Aarhus University, Aarhus, Denmark,[16]Department of Neurosurgery, Yale School of Medicine, New Haven, CT,[17]Department of Medicine, Weill Cornell Medicine, New York, NY,[18]Children's Medical Center Research Institute, University of Texas Southwestern Medical Center, Dellas, TX,[19]Department of Pathology, Stanford University School of Medicine, Stanford, CA,[20]Department of Pediatric Oncology and Hematology, Charite, Universitatsmedizin Berlin, corporate member of Freie Universitat Berlin, Berlin, Germany

Extrachromosomal DNA (ecDNA) presents a promising target for cancer therapy; however, its spatial-temporal diversity and influence on tumor evolution and the immune microenvironment remain largely unclear. Here, we apply computational methods to detect and reconstruct ecDNA from whole-genome sequencing data from 595 patients prospectively recruited into the Chinese Urothelial Carcinoma Genome Atlas (CUGA) study. We demonstrate that ecDNA contributes to clonal evolution through structural rearrangements and copy number variation during malignant transformation and recurrence of urothelial carcinoma (UC). This supports a model wherein tumors evolve via the selective expansion of ecDNA-bearing cells. Through multi-regional sampling of tumors, we demonstrate ubiquitous ecDNA presence across clones in multi-focal cancers, directly linking ecDNA to the evolution of multifocality. Notably, we observe highly variable levels of ecDNA across multiple foci within the same tumor, and ecDNA contributes to increased intratumoral heterogeneity and serves as a substrate for natural selection and evolution. EcDNA is present in 36% of UC tumors and correlates with an unstable cancer genome, an immunosuppressive phenotype, and significantly reduced 5-year survival. Single-cell RNA sequencing analyses reveal that ecDNA$^+$ malignant cells exhibit diminished expression of major histocompatibility complex class I molecules, enabling them to evade T-cell mediated immune responses. Finally, we show that sequencing of urinary sediment-derived DNA has excellent specificity in detecting ecDNA, underscoring its diagnostic potential. This study delineates the evolutionary trajectory of ecDNA during tumor progression and provides further insights into immune evasion mechanisms associated with ecDNA.

#2669 Solid tumor microenvironments cause more mutations than liquid or soft tumor microenvironments -- even before selection.

Dennis E. Discher, Markus Sprenger

University of Pennsylvania, Philadelphia, PA

Sequencing of many types of tumors shows that solid tumors are far more mutated than liquid tumors and soft tumors such as brain tumors, but it is unclear whether the differences are caused by induction of more mutations or subsequent selective pressures. Our pan-cancer analyses of clinical data shows increased chromosomal changes associate with collagen-I and further shows that genetic variance between patients also increases with the mean for a given tumor type. Using a 3D hydrogel-based model with tunable stiffness and of cancer spheroids, we discover that matrix stiffness increases mitotic aberrations including micronuclei and also increases chromosome loss as detected using live cell chromosome reporters (ChReporters). However, stiffness suppresses growth and size variation, which indicates more mistakes per division. Our approach is >10-fold more sensitive than sequencing approaches, and we quantify colonies of ChReporter-negative cells within cancer spheroids. Our results are consistent with Luria-Delbruck's theory of heritable genetic changes which predicts inter-spheroid variance greatly exceeds Poisson statistics. Knockdown of myosin-II, a well-known mechanosensor, increases chromosomal loss and variance without affecting spheroid growth, consistent with a tumor suppressor role. Various drug strategies provide deeper insight into mechanisms, and use of clinically deployed CDK4/6-inhibitor reveals this drug on its own drives micronuclei formation and genetic variation.

#2670 Understanding cell-of-origin influence on cancer-associated fibroblasts in lung adenocarcinoma.

Minxiao Yang[1], Tawfeeq Al-Mansur[2], Chunli Yan[1], Zea Borok[3], Crystal Marconett[2]

[1]Department of Surgery, Keck School of Medicine, USC - University of Southern California, Los Angeles, CA,[2]Department of Integrative Translational Sciences, Beckman Research Institute of City of Hope, Los Angeles, CA,[3]Division of Pulmonary, Critical Care and Sleep Medicine, Department of Medicine, University of California San Diego, San Diego, CA

Lung cancer remains the top cause of cancer mortality worldwide, with lung adenocarcinoma (LUAD) representing the most common and heterogeneous subtype of non-small cell lung cancer (NSCLC). This heterogeneity lies in cell of origin, histological presentation, therapeutic response and clinical outcome, posing challenges for precision in diagnosis, prognosis and effective therapeutics. Our previous research in mouse models determined that GRAM domain containing-2 (Gramd2)-positive alveolar epithelial type 1 (AT1) cells can give rise to LUAD, exhibiting histological, cellular and molecular characteristics that are distinct from LUAD derived from surfactant-secreting type 2 (AT2) cells, which are a widely regarded cell of origin for LUAD. Additionally, we recently leveraged single-nucleus RNA sequencing (snRNA-seq) to dissect the composition of the tumor microenvironment (TME) of the *Gramd2*$^+$ AT1 cell-derived LUAD and *Sftpc*$^+$ AT2 cell-derived LUAD, demonstrating a distinct immune landscape of LUAD based on cell-of-origin. Significantly, immunosuppressive myeloid cells, such as myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs), dominated the TME of *Sftpc*$^+$ AT2 cell-derived LUAD, whereas immunoreactive macrophages and pro-inflammatory monocytes were highly enriched in *Gramd2*$^+$ AT1 cell-derived LUAD. These findings may partly answer why only a subset of LUAD patients respond to immunotherapy. However, the role of mesenchymal cells in the TME and how they influence LUAD progression are unknown. To address this, snRNA-seq data of cancer-associated fibroblasts (CAF/M) cells from both cell of origin models was analyzed. We identified a distinct *Lcn2*$^+$, *Mmp2*$^+$, *Cxcl14*$^+$ CAF/M cell population specifically enriched in *Sftpc*$^+$ AT2-derived LUAD TME. These CAF/M cells exhibited upregulated mitochondrial activity, oxidative phosphorylation, and extracellular matrix remodeling, supporting tumor development and invasion. In contrast, fibroblasts in the TME of *Gramd2*$^+$ AT1-derived LUAD showed elevated RNA processing, mRNA metabolism, and DNA damage repair. To further investigate the aberrations of cancer cells, we employed InferCNV, an R package to infer genomic alterations associated with cancer, to analyze large-scale copy number variations (CNVs) of LUAD cells between two models. The results reveal significantly higher CNV scores in *Sftpc*$^+$ AT2-LUAD compared to *Gramd2*$^+$ AT1-LUAD, suggesting a more pronounced genomic instability and an aggressive tumor phenotype. These significant variations may interact with CAF/M cells and thereby impact survival outcomes. This study demonstrates that it is critical to stratify properties of the LUAD TME and establish how LUAD evolves based on cell-of-origin, yielding knowledge for elucidating LUAD heterogeneity and consequently boost development of precision medicine strategies in the clinic.

#2671 Divergent evolutionary trajectories of the molecular subtypes of breast cancer are driven by differential selection and selective epistasis.

Jessica Liu[1], **Krishna Dasari**[2], Nicholas Fisk[3], Carolyn Kravitz[4], Lajos Pusztai[5], Jeffrey Townsend[2]

[1]Yale University, New Haven, CT,[2]Biostatistics, Yale School of Public Health, New Haven, CT,[3]Computational Biology, The University of Rhode Island, South Kingstown, RI,[4]Pathology, Yale School of Medicine, New Haven, CT,[5]Medical Oncology, Yale School of Medicine, New Haven, CT

The molecular subtypes of breast cancer—defined by estrogen-receptor, progesterone-receptor, and human epidermal growth factor receptor status—have established differences in mutational profiles, prognoses, and response to standard treatments. However, the evolutionary trajectories underlying these differences are not well understood. Knowledge of these differential trajectories would empower predictive modeling of treatment benefit. To characterize these trajectories, we first aggregated a multi-institutional cohort of 23,247 breast cancer patients with whole exome or targeted sequencing data, including from AACR Project GENIE. We then calculated variant-specific background mutation rates, and subsequently quantified the selective benefit of known and potential driver mutations across breast cancers and within each subtype. We then assessed how the selective benefit of such mutations changed throughout cancer progression and due to the epistatic influence of prior mutations. Finally, we confirmed the ordering of mutational acquisition determined by epistasis by performing phylogenetic reconstruction of the evolution of ten breast cancers.

In a subtype-agnostic analysis, we found that PIK3CA H1047R and H1047L, SF3B1 K700E, and multiple TP53 variants experienced the strongest selection. Interestingly, the selective benefit of mutations to oncogenesis varied between subtypes: the PIK3CA H1047R variant experienced maximal selection in the Luminal A subtype, while the TP53 I195T variant experienced maximal selection in TNBC. Additionally, we found that PIK3CA E542K experienced significantly higher selection in the Luminal A and B subtypes than the TNBC subtype. These mutations also experienced differential selection throughout cancer progression: PIK3CA and CDH1 variants were under stronger selection during early carcinogenesis, whereas TP53, ERBB2, and multiple other variants experienced stronger selection in late stage cancer. These differences in subtype- and stage-specific selection are partially explained by asymmetric synergistic and antagonistic epistatic interactions between mutations in PIK3CA, TP53, AKT1, CDH1, and other driver genes, which create preferential mutation orderings and combinations. These analyses enable inference of subtype-specific tumor evolutionary trajectories with quantitative measures of the selective benefit of mutations across molecular contexts and stages of cancer progression. Such measures are useful for predicting response to targeted therapeutics and therefore facilitate the development and testing of targeted therapies—and the optimization of current regimens—for the molecular subtypes of breast cancer.

#2672 Eco-evolutionary profiling of the UV-driven clonal dynamics and tumor initiation.

Stanislav S. Avdieiev[1], Leticia Tordesillas[1], Karol Prieto[1], Omar Chavez Chiang[1], Zhihua Chen[1], Nihir Patel[2], Sofia Cordero[1], Luiza Silva Simoes[1], Y. Ann Chen[1], Noemi Andor[1], Robert A. Gatenby[1], Elsa R. Flores[1], Christopher J. Whelan[1], Joel S. Brown[1], Kenneth Y. Tsai[1]

[1]Moffitt Cancer Center, Tampa, FL,[2]Admera Health, South Plainfield, NJ

Classically, cancer initiation is attributed to a sequential acquisition of discrete genetic events such as driver mutations. However, homeostatically-stable tissues, including skin, harbor numerous somatic mutations spread across the tissue's clones. Yet, most of these clones never give rise to cancer. Using the model of UV-driven cuSCC, we tested our hypothesis that cancer initiation occurs in three phases: 1) tissue disruption and the emergence of unusually large "goliath" clades (ecological driver), 2) clonal selection within a subset of goliaths as evidenced by the presence of cell density hotspots and higher mutational burden (evolutionary driver), and 3) emergence of macroscopic lesions. We tracked these tissue level drivers via *in-vivo* serial imaging and 3-D reconstruction of fluorescently labeled keratinocyte clades, yielding 25,085 clades from 14 mice over 3 months, and 14,525 clades from 4 of these mice over 6 months. While median and mean clade sizes differed little across UV exposure, our ecological survey revealed the emergence of large goliath clades (> cc. 4.2×10^6 μm^3) observed essentially only within UV-exposed skin and occupying the extreme tail of the distribution of clade sizes. Goliath clades arose as serpentine structures, intercalating between adjacent keratinocytes, some with areas of unusually high local densities ("micro-lumps"). Targeted DNA sequencing of goliath revealed *Kmt2a, Kmt2d, Prex2,* and *SCN11A* as the most mutated genes. Giant clades do not typically possess common driver mutations of SCC, e.g. *TP53* and *NOTCH,* and display two extremes of mutational profiles, with some goliaths having dominant clones (VAF >0.75), and others having no discernable clonal structure (VAF <0.025), demonstrating that strong mutational drivers are not a prerequisite for developing goliaths. scRNAseq analysis of both bulk skin and sorted clades revealed upregulation of the fraction of cycling basal-like cells, and downregulation of Alox12e-expressing granular cells, along with increased proportions of plasmacytoid dendritic cells (pDC) and CD4+ regulatory T-cells (Tregs) in UV-exposed samples, reflecting early epidermal de-differentiation and immunosuppression. By month 7, mutational burdens were significantly larger in goliath clades, particularly for those with micro-lumps. Finally, lesions emerged between months 6 and 7, only in UV-exposed skin. Based on our adaptation of the Drake equation, we conclude that the probability of observing macroscopic lesions arising in 2 of the 21 randomly sampled goliaths we followed for 3 months to be less than 10^{-7}. Taken together, our results support the interplay between cell autonomous and non-cell autonomous compartments during cancer initiation, the earliest phase of which *presage* the acquisition of driver mutations. Our data constitute an unprecedented look at clonal dynamics in UV exposed skin defining the transition from normal tissue to tumor.

#2673 Tumor heterogeneity and stemness can be shaped by mechano-structural parameters in osteosarcoma.

Zunaira Shoaib[1], Aleczandria S. Tiffany[2], Brendan Harley[2], Joseph Irudayaraj[3], Timothy M. Fan[4]

[1]University of Illinois at Urbana-Champaign, Urbana, IL,[2]Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign, Champaign, IL,[3]Bioengineering, University of Illinois at Urbana-Champaign, Champaign, IL,[4]Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL

The aim of the study was to identify factors in the physical tumor microenvironment that might participate in Osteosarcoma Cancer Stem-like Cells (OS-CSC) development, which might underlie chemoresistance and recurrence of OS. It was hypothesized that within an OS tumor, spatial localized niches possessing distinct microenvironmental structural and mechanical properties could favor the genesis and survival of different OS cell populations, and certain niches might be more conducive for OS-CSCs formation and maintenance. Mineralized and porous collagen scaffolds mimicking heterogenous bone niches were fabricated by changing crosslinking and pore alignment and their distinct structural and mechanical properties were determined using electron microscopy, Atomic Force Microscopy (AFM) and compression tests. The 3D scaffolds were cultured with highly metastatic human OS cell lines and were assessed for cytoskeletal changes, cell stiffness, epigenetic pathways, chemoresistance and chemoresistance-related pathways, and the differential expression of stemness genes. A consistent trend of OS-CSC phenotype was observed in OS cells grown within softer bone niches (~10 kPa) with aligned pores as compared to niches with non-aligned pores and increased stiffness (~30 kPa). The cell population growing in softer, aligned scaffolds had significantly higher expression of stemness genes (OCT4, NANOG, SOX2, ALDH1A1) and drug efflux gene ABCB1 along with increased chemoresistance to doxorubicin and cisplatin, and gave rise to larger colonies in soft agar. Epigenetic modifications associated with cisplatin resistance in OS including decreased methyltransferase EZH2 and concurrent upregulation of LIF/Notch and PRKCA pathways were observed in softer, aligned niche cultures. Fluorescent imaging of scaffold cultures and AFM measurements showed rounder, softer cells in soft, aligned niches indicative of a CSC phenotype, and stretched, elongated, stiffer cells in the non-aligned, stiffer niche with higher YAP nuclear localization, indicative of a differentiated phenotype. RNA Sequencing showed differential expression of 87 genes between scaffold types, with enrichment in ECM-receptor interaction and PPAR signaling pathways; particularly interesting was the enhancement of stem cell reprogramming mediator RAD50 in aligned, soft cultures. In summary, our investigation demonstrates that both the mechanical and structural properties of bone niches influence the formation of distinct OS cell populations. A softer niche with an aligned structure is more conducive for maintaining OS-CSCs, and these findings could be translated to designing therapeutic strategies that modify the microarchitecture to potentially reduce CSC-favoring niches.

#2674 Cellular plasticity facilitates therapy resistance through enhancing adaptability.

Alicia Bjornberg[1], Xierali Aobuli[1], Matthew Froid[1], Rowan Barker-Clarke[2], Jeff Maltas[2], Jacob Scott[2], Berkley Gryder[3], David Bassanta[1], Alexander Anderson[1], Virginia Turati[4], Andriy Marusyk[1]

[1]H. Lee Moffitt Cancer Center, Tampa, FL,[2]Cleveland Clinic, Cleveland, OH,[3]Case Western Reserve University, Cleveland, OH,[4]Lund University, Lund, Sweden

In oncology, patients treated with targeted therapies often have strong and durable responses early in treatment; however, the development of resistance hinders long-term therapeutic benefit. A subset of tumor cells can develop resistance, often from both genetic and non-genetic mechanisms through gradual, multi-step resistance. Nonetheless, predicting which cells will evolve to become resistant, and strategies to suppress adaptability contributing to resistance remain unknown. While resistance may emerge in cells harboring driver mutations, the adaptation process necessitates substantial modifications to the phenotype facilitated by cellular plasticity. Therefore, plasticity serves as a crucial facilitator of resistance by enhancing adaptability, rather than functioning as a direct cause of resistance. We hypothesize that the direct selection for therapy resistance leads to indirect selection for enhanced plasticity. We propose that as treatment selects for drug-resistant phenotypes, concurrent selection for cells exhibiting greater adaptability occurs. Thus, enhanced plasticity is indirectly favored during selection for drug resistance. We have found strong support for this hypothesis through in silico modeling, with subsequent validation through functional assays. In our functional assays, we utilize the H3122 cell line derived from a patient with ALK+ non-small cell lung cancer (NSCLC) to model targeted therapy resistance. This well-studied model effectively mimics the initial responsiveness and subsequent resistance observed in clinical settings. Utilizing single-cell sorting and the expansion of clonal isolates from the parental H3122 population, we identified heterogeneity in the ability to adapt where subgroups exhibited a range of high adaptation potential (HAP) to low adaptation potential (LAP) based on their response to numerous independent stressors, including targeted ALK inhibitors. We have found that therapeutic stressors lead to selection of subpopulations with higher adaptability potential, enriching for a more lethal subgroup than the original tumor. HAP cells display increased multi-drug resistance and metastatic potential compared to LAP cells. HAP cells are significantly enriched in plasticity-related pathways overlapping with those found in cells that have developed strong resistance to ALK inhibitors. In subcutaneous tumor models, LAP cells show a 1,000-fold reduction in tumor burden, while HAP cells develop resistance. Our findings suggest that disrupting cellular adaptability can prevent resistance, maintaining cells in a therapy-sensitive state. This approach shifts the focus from killing tumor cells to blocking their ability to adapt, opening up a novel angle for overcoming resistance. Addressing adaptability reveals intervention points that conventional strategies might overlook, offering new, proactive strategies in cancer treatment.

#2675 Tumor clones display intrinsic and stable rates of progression and coordination of the tumor microenvironment in association with epithelial mesenchymal plasticity.

Timothy N. Trotter, Jason McBane, Xing Gao, Carina Dagotto, Tao Wang, H. Kim Lyerly, Zachary C. Hartman

Duke University, Durham, NC

Delayed relapse is attributable to residual tumor cells that remain minimally proliferative/quiescent until favorable conditions for growth are met, but what determines the overall duration of remission is unresolved. Recent evidence suggests that epithelial-mesenchymal plasticity (EMP) facilitates phenotypic switching between dormant and proliferative cell states, in addition to enabling chemoresistance, metastasis, and immune evasion. However, which features of EMP are responsible for each or multiple of these phenotypes, and to what extent EMP is restricted to certain tumor populations remains unclear. To address these questions, we initially enriched for features of EMP (CD44hi, CD104low) in the mouse mammary tumor cell line TSAE1. Interestingly, no difference in tumor growth was observed between parental or EMP-enriched mammary fat pad (MFP) tumors in either syngeneic Balb/c or immune-deficient SCID-beige mice. Individual tumors were then cultured ex vivo, revealing a significant EMP-enrichment in parental tumors after one round of selection in vivo. Similar results were observed in the mouse mammary cell line D2.OR, suggesting that implantation alone gives rise to EMP which may mask important tumor cell-intrinsic differences in a heterogeneous tumor. Thus, EMP-enriched TSAE1 cells were further divided by isolating nine clonal lines. Although each expressed less epithelial markers than parental cells (Epcam, E-cadherin), individual TSAE1 clones displayed remarkably divergent growth rates and patterns in 3D culture. In vivo all clones were capable of forming tumors, but some were significantly delayed regardless of being implanted into Balb/c or SCID-beige animals. Furthermore, individual clones recruited distinct immune and stromal populations. To assess if intrinsic phenotypes change over time, 3D culture assays and bulk RNA sequencing was performed on three representative clones before implantation (“Pre”) and on three Balb/c MFP-derived tumors expanded ex-vivo from each clone (“Post”). Surprisingly, for each clone the Post tumors closely resembled the Pre clone and much of the difference in up/downregulated genes between individual clones and parental TSAE1 cells was already found in Pre samples. Finally, MFP tumors from EMP-enriched TSAE1 clones were differently sensitive to Doxorubicin therapy, suggesting that specific aspects of EMP may be important during different stages of BC survival and progression. Together, these data reveal that proliferation itself is intrinsically determined at the clonal level, independent of adaptive immunity, and highlight the potential for individual tumor clones in various EMP states to shape the tumor microenvironment for long-term survival.

#2676 Single cell spatial profiling reveals ERBB2 and EGFR co-expression in primary esophageal adenocarcinoma and brain metastasis tumors.

Nora Lawson[1], Lingqun Ye[2], Chae Yun Cho[1], Bo Zhao[2], Thomas Mitchell[3], Ines Martin[1], Andy Futreal[2], Kadir Akdemir[1]

[1]Neurosurgery-Research, The University of Texas MD Anderson Cancer Center, Houston, TX,[2]Genomic Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX,[3]Genetics, The University of Texas MD Anderson Cancer Center, Houston, TX

Esophageal adenocarcinoma is a prevalent and serious disease, ranking eighth globally, with a low survival rate of 15-25% and a rising incidence in the United States. However, the occurrence of brain metastasis in esophageal adenocarcinoma (EAC) is rare, affecting only approximately 2-6%. Despite its rarity, brain metastasis in EAC is associated with a poor prognosis, compounded by the limited availability of effective treatment options. Due to the infrequent occurrence of brain metastasis in EAC patients, our understanding of the tumor composition in these cases and how they differ from those in primary sites remains limited. To address these gaps, we analyzed the tumor microenvironment of brain metastasis tumors and their matched primary tumors using spatial transcriptomic data generated by the 10x Xenium Analyzer. By employing a brain tumor panel, an immune panel, and a custom panel, we characterized the tumor microenvironment, including neuronal and immune cell populations present in these samples. Overall, primary samples contained a higher proportion of immune cells such as macrophages, T cells, B cells, dendritic cells, plasma cells, and mast cells, compared to brain metastases, which had a greater composition of neuronal cells. We additionally investigated the oncogenic expression patterns at the single-cell level within these matched samples, revealing areas with high *ERBB2* and *EGFR* expression density within malignant cell populations. Notably, brain metastasis samples contained a higher proportion of malignant cells co-expressing *ERBB2* and *EGFR* than primary samples, suggesting clonal evolution during disease progression from primary to metastatic sites. Our findings indicate that *ERBB2* and *EGFR* amplifications may represent potential targets for therapeutic interventions in these patients. These findings enhance our understanding of brain metastasis in EAC, offering insights into the tumor microenvironment that may guide future therapeutic strategies and improve clinical management for this previously understudied metastatic type.

#2677 Multi-omics profiling identifies two epithelioid sarcoma molecular subtypes with distinct signaling and immune characteristics.

Carine Ngo[1], Leo Colmet-Daage[1], Julien Vibert[1], Clemence Henon[1], Riwan Brillet[1], Jia Xiang Jin[2], Daniel Pissaloux[3], **Clemence Astier**[1], Jean-Yves Blay[3], Axel Le Cesne[1], Charles Honore[1], Matthieu Faron[1], Thomas Grunewald[2], Franck Bourdeaut[4], Franck Tirode[3], Sophie Postel-Vinay[1]

[1]Institut Gustave Roussy, Villejuif, France,[2]DKFZ, Heidelberg, Germany,[3]Centre Leon Berard, Lyon, France,[4]Institut Curie, Paris, France

Background: Epithelioid sarcoma (EpS) is an aggressive sarcoma, characterized by the loss of SMARCB1 expression. EpS is traditionally classified as distal or proximal according to clinicopathological features, but its molecular bases remain largely unknown.
Methods: To establish an EpS molecular classification, we combined multiple bulk omics profiling approaches to systematically characterize the genomic, transcriptional and methylome landscapes of EpS. We further investigated inter- and intra-patient heterogeneity and explored molecular subtype-specific transcriptomic signatures, using single-cell RNA sequencing on fresh samples.
Results: We identified two molecular subtypes of EpS: “distal-like” and “proximal-like”. Distal-like tumors include all morphological distal subtypes and a subset of proximal and hybrid EpS. They express a specific single-cell derived epithelial-to-mesenchymal transition signature, which associates with improved patient survival and includes Desmoglein 2 (DSG2), which we identify as a potential routine immunohistochemichal biomarker to improve diagnosis. Distal-like EpS also display higher peri-tumoral CD8+ T cell infiltrates with specific tumor-immune cell interactions. Conversely, proximal-like EpS harbor a higher inter-tumoral molecular heterogeneity, with some cases resembling other SMARCB1-deficient tumors by DNA methylation profiling, and high intra-tumoral pro-tumoral macrophage infiltrates.
Conclusion: Our study introduces a novel molecular classification of EpS with prognostic value, potential diagnostic markers and distinct tumor immune microenvironments, that extends beyond traditional clinicopathological classification. These insights pave the way for more personalized and effective treatment strategies for patients with EpS.

#2679 Tumor heterogeneity in hepatocellular carcinoma demonstrates disruption in gap junctions and calcium signaling pathways.

Katherine M. Barrows[1], Jonathan M. Hernandez[1], Natalie Porat-Shliom[2]

[1]Surgical Oncology Program, NIH-NCI, Bethesda, MD,[2]Thoracic and GI Malignancies Branch, NIH-NCI, Bethesda, MD

Introduction: The liver is an innately heterogeneous organ responsible for diverse metabolic and biosynthetic functions, which are accomplished by regionalization of hepatocytes based on nutrient availability, oxygen tension, biochemical gradients, and intact local cell communication. When exposed to stress, however, hepatocytes forgo tissue heterogeneity and synchronize functions, which has been shown to occur through calcium transfer via gap junctions and the activation of downstream signaling. Not surprisingly, malignant tumors interfere with local homeostasis and disrupt gap junctions. However, the role of calcium dynamics and its regulation have not been examined in primary hepatocellular carcinoma (HCC).

Aim: Identify changes in gap junction and calcium signaling-dependent proteins in a murine model and samples from patients with HCC.

Methods: *Clinical Data Evaluation* Gene query in the TCGA database to determine the clinical correlation of alterations of gap junctions and calcium regulatory proteins in HCC.

Murine HCC Model Male mice aged between 8 and 12 weeks were administered a hydrodynamic injection of Sleeping Beauty plasmids containing mutant Beta-Catenin and Akt. After a time course to allow the development of microtumors, the mouse livers were fixed, cryo-preserved, and sectioned.

Human Tissue Liver tissue was procured from two human subjects undergoing hepatic resection. Both normal adjacent liver parenchyma and histologically confirmed HCC were fixed with 4% PFA, cryopreserved and sectioned.

Immunofluorescence Antibodies and staining for nucleus, actin, gap junctions, IP3R, for periportal regions and pericentral regions were used in immunofluorescence.

Results: On query of 30 genes that encode calcium regulatory proteins in the TCGA database between 30%-59.4% of 1075 cases of HCC had a genetic alteration as a mutation, copy number variance or change in expression.

Images of the gap junction protein immunofluorescence in both normal mouse and human tissues demonstrated an organized periportal signal. Mouse and human HCC tissue demonstrated cellular heterogeneity as well as variable and disorganized fluorescence intensity.

Discussion: Murine HCC models confirm comparable changes that occur to gap junction proteins in human HCC, offering a unique window of interrogation. Comparing changes in the expression of gap junction proteins and calcium signaling-related proteins over a time course in the development of HCC may provide insight into malignant transformation. The role of calcium signaling in the development of malignancies is understudied, may promote tumor heterogeneity and ultimately treatment resistance.

#2680 Volumetric analysis of spatial tumor phylogeny enabled by 3D pathology-guided macrodissection (3DPM).

H.-C. Hsieh[1], Q. Han[1], G. Gao[1], D. Brenes[1], E. Baraznenok[1], R. Serafin[1], K. W. Bishop[2], E. Q. Konnick[1], C. C. Pritchard[1], S. Figiel[3], F. C. Hamdy[3], I. G. Mills[3], L. D. True[1], M. C. Haffner[4], S. R. Rao[3], D. J. Woodcock[3], J. T. Liu[1];
[1]University of Washington, Seattle, WA, [2]Broad Institute, Boston, MA, [3]University of Oxford, Oxford, United Kingdom, [4]Fred Hutchinson Cancer Center, Seattle, WA

Current methods for studying tumor phylogeny primarily rely on manual macrodissection or laser capture microdissection of tumor regions identified in thin 2D histology sections. With the emergence of non-destructive 3D pathology workflows, the study of 3D spatial tumor phylogeny is proposed, whereby distinct tumor regions may be macrodissected based on 3D pathology datasets for downstream DNA sequencing and clonal reconstruction. The tumor phylogenetic tree can then be mapped into 3D space using the spatial locations of different subclones, providing an improved understanding of the spatial spread of tumors along their evolutionary paths. To implement the concept of 3D spatial tumor phylogeny, we are developing a 3D pathology-guided macrodissection (3DPM) system as an alternative to current techniques that are limited to thin 2D tissue sections. In our workflow, tissues stained with H&E-analog fluorescence dyes are first imaged using an open-top light sheet (OTLS) microscope. Based on 3D pathology datasets digitally false colored to mimic the appearance of H&E staining, regions of interest with distinct tumor morphologies are annotated by pathologists. The tissue specimen along with the digital annotations are then transferred to a co-registered 3D macrodissection system, which is based on a portable desktop five-axis CNC milling machine. For each volume of interest, the unwanted background tissue is first milled away from the top, followed by detachment of the volume through angled cuts. The toolpath is optimized to maximize the purity and volume of the dissected tissue. Large FFPE tissue slices from archived prostatectomies (centimeter-scale lateral dimensions and millimeter-scale axial dimensions) are being used for proof-of-concept demonstrations. First, we aim to show that the 3D macrodissection method can achieve high tumor purity comparable to laser capture microdissection, despite the increased challenge of dissecting 3D contours vs. 2D tissue regions. Second, we anticipate that more distinct subclones can be identified in each tumor region due to improved sampling of volumetric tissue regions with distinct morphologies (e.g. different Gleason patterns and sub-variants) in comparison to 2D thin-section dissection methods. Finally, given that prostate cancer is known to be multifocal and exhibits complex clonal expansion within the primary tumor, we aim to elucidate whether these multiclonal expansions are spatially separated, intermixed, or vary between cases using reconstructed 3D spatial phylogenetic trees. In summary, 3DPM aims to deepen our understanding of prostate cancer evolution and to enable many new directions in tumor phylogenetics research.

TUMOR BIOLOGY: Targeting the Tumor Microenvironment: A Brave New World
Poster Session

#2684 The iRGD tumor-penetrating peptide inhibits TGF-β activation mediated by an αvβ5 integrin-rich tumor microenvironment in pancreatic cancer.

Yukihito Kuroda[1], Kodai Suzuki[1], Yuki Kunisada[1], Hotatka Kawai[2], Henri Havia[3], Tero A. H. Jarvinen[3], Moriya Tsuji[4], Kazuki N. Sugahara[1]

[1]Department of Surgery, Columbia University, New York, NY,[2]Department of Oral pathology and Medicine, Okayama University, Okayama, Japan,[3]Faculty of Medicine and Health Technology, Tampere University & Tampere University Hospital, Tampere, Finland,[4]Department of Medicine, Columbia University Irving Medical Center, New York, NY

αvβ5 integrin is highly expressed in pancreatic ductal adenocarcinoma (PDAC) and predicts poor prognosis in PDAC patients. We previously showed that transforming growth factor-β (TGF-β) produced by cancer-associated fibroblasts and cancer epithelial cells induces the expression of αvβ5 integrin in PDAC, contributing to the αvβ5-rich tumor microenvironment (TME). Here, we show that the αvβ5 integrin is a critical activator of TGF-β in PDAC and that the iRGD tumor-penetrating peptide effectively inhibits the activation cascade causing a dynamic modification of the PDAC TME. TGF-β is secreted as a latent complex (LAP-TGF-β) and activated by various stimuli including receptor-dependent mechanisms. αv integrins potently activate TGF-β by binding to the RGD peptide motif within LAP-TGF-β to mechanically release active TGF-β from the complex. Our results show that the αvβ5 integrin is a major activator of TGF-β in PDAC cells because deleting the integrin significantly reduced TGF-β signaling and target gene expression despite the presence of other αv integrins on the cells. The iRGD peptide, which binds to αv integrins (and neuropilin-1) and delivers drugs deep into the tumor tissue, inhibited the integrin-mediated activation of TGF-β in the PDAC cells in an RGD-dependent manner. iRGD effectively inhibited TGF-β signaling *in vivo* evidenced by a dramatic suppression of phosphorylated Smad-2 in orthotopic tumors in syngeneic PDAC mice. Various changes in the TME accompanied the effect, such as reduced regulatory T cells, altered distribution of CD8+ T cells, normalization of blood vessels, and reduced stromal fibers. Similar results were obtained in humanized orthotopic PDAC mice that allow proper characterization of human immune response to tumor antigens. Adding an immune checkpoint blockade (ICB) to the iRGD monotherapy led to enhanced anti-tumor effects in both mouse models. Of note, iRGD, even as monotherapy, caused a striking inhibition of liver metastasis formation in humanized PDAC mice. Our findings suggest that PDAC has assembled a mechanism to maintain high levels of active TGF-β and αvβ5 integrin expression that likely contributes to its desmoplastic and drug resistant nature. iRGD interferes with the mechanism to cause dynamic TME modification and sensitization to ICB. The results justify the concept of the ongoing iLSTA clinical trial that studies the effect of iRGD-based immunotherapy in patients with locally advanced PDAC (ACTRN12623000223639).

#2685 Sensitizing mouse ovarian cancer tumors to immunotherapy.

Elizabeth Anne Allen, Justine Chen, Supreeti Tallapragada, Huyen Pham, Oliver Dorigo

Stanford University School of Medicine, Palo Alto, CA

Introduction: The overall survival of patients with advanced ovarian cancer (OC) is poor and clinical responses to immunotherapy are low, encouraging development of new strategies that sensitize ovarian cancer to immunotherapy. Many studies in OC and other cancers have linked the presence of intra-tumoral B cells, high endothelial venules (HEVs) and lymphoid aggregates that resemble tertiary lymphoid structures (TLSs) with improved prognosis and increased survival. In contrast to the favorable prognosis associated with the presence of TLS, high levels of immunosuppressive T regulatory cells (Tregs) in OC are associated with reduced survival.
Methods: Single, double, and triple combinations of a Treg depleting antibody, an HEV-inducing LTβR agonist antibody, and the anti-PD1 checkpoint inhibitor were tested in immune-competent C57Bl/6 mice inoculated intraperitoneally with homologous recombination defective (HRD) ID8 Trp53-/-Brca2-/- and HR proficient (HRP) ID8 Trp53-/- bioluminescent murine OC tumor models. Mono- and combination therapy was administered in fixed endpoint and survival studies and assessed for tumor burden, Treg depletion, HEV induction, lymphocyte infiltration, other changes to the tumor microenvironment, and overall survival.
Results: High numbers of Tregs were found in control tumors which were significantly reduced in arms which included the Treg depleting antibody along with significantly increased numbers of CD8+ T cells. HEVs were significantly more abundant in arms which included the LTβR agonist along with increased numbers of B cells and plasma cells. Clonal selection of T cells occurred in treated tumors, most notably with triple combination treatment. In fixed endpoint trials, tumor burden was significantly reduced and sometimes completely eradicated in combination arms that included the Treg depleting antibody. Median survival in mice bearing ID8 Trp53-/-Brca2-/- tumors was increased in triple combination tumors vs IgG (130 vs 57 days) with 5/13 animals that completely eradicated tumors and successfully rejected tumor rechallenges. Analogous studies using ID8 Trp53-/- tumor cells produced 7/7 mice without a detectable bioluminescent tumor signal after 75 days (ongoing) while the IgG group had a median survival of 50 days.
Conclusions: This study indicates that OC tumors in immune-competent mice can be sensitized to immunotherapy and potentially eradicated with checkpoint inhibition using a strategy that combines Treg depletion and/or HEV induction. Data generated in this study can provide the rationale for the design of a clinical trial in OC patients, possibly stratified by HR status, as a biomarker driven approach.

#2686 P2X4 purinergic receptor activation on macrophages inhibits phagocytosis and promotes tumor-promoting cytokine secretion.

Vahinipriya Manoharan, Kennedy M. Rains, Jillian K. Landers, Sharvari S. Saraf, Tristan R. Ibarra, Angelo M. De Marzo, David E. Sanin, Janielle P. Maynard

Johns Hopkins University School of Medicine, Baltimore, MD

Prostate cancer (PCa), the most frequent cancer and the second leading cause of cancer-related deaths among men in the U.S., necessitates improved therapeutic strategies. Our previous studies demonstrated elevated P2X4 purinergic receptor expression in PCa tissues, which correlated with poor prognoses. We found that P2X4 receptor knock down or blockade in PCa cells reduces subcutaneous allograft tumor growth *in vivo* and inhibits human PCa cell viability, migration, and invasion *in vitro.* However, the expression profile and functional role of the P2X4 receptor in the PCa tumor microenvironment (TME) remain poorly defined. This study aims to characterize P2X4 receptor expression and functional significance within the prostate TME. Multiplex immunohistochemistry (IHC) analyses revealed P2X4 receptor expression on $CD163^+$ M2 macrophages, but not in $CD8^+$ T cytotoxic cells, $CD4^+$ T helper cells and FAP^+ fibroblast cells in human PCa tissues. IHC performed on FVB mouse allograft primary and metastatic tumors also suggested P2X4 expression on $F4/80^+$ macrophages. Treatment of human THP-1-derived M2 macrophages with CTP (400 μM), a P2X4-specific agonist, significantly reduced phagocytosis *in vitro* ($p = 0.0115$). A human phospho-kinase array revealed that P2X4 activation decreased phosphorylation of AKT (S473) and p70S6 (Thr389), indicating a potential role in suppressing Fcγ receptor-mediated phagocytosis through the PI3K > AKT > mTORC1 > p70S6 pathway. Additionally, decreased phosphorylation of Src and Src family kinases (Hck, Fgr, and Lyn) suggested a Src-mediated mechanism of impaired phagocytosis. Further, a human cytokine array revealed that P2X4 receptor activation with CTP (400 μM) reduced the secretion of tumor-suppressive factors such as Endoglin, IGFBP3, and Serpin E1, while increasing the secretion of tumor-promoting factors including BAFF, RANTES, FGF-19, IL-8, MCP-1, MIP-3α, PDGF-AB/BB, and Relaxin, contributing to PCa growth, metastasis, and recurrence. We generated a macrophage-specific P2X4 receptor knockout mouse (LyzM-$P2X4^{-/-}$), confirmed by flow cytometry and IHC. Supporting our findings in human THP-1 derived M2 macrophages, peritoneal $TIM4^+$ $F4/80^+$ macrophages from LyzM-$P2X4^{-/-}$ mice exhibited enhanced phagocytosis compared to wild-type controls ($p = <0.0001$). However, we observed no apparent difference in TRAMP-C2 tumor growth rates between wild-type and LyzM-$P2X4^{-/-}$ mice. In summary, our findings demonstrate that P2X4 receptors are present on tumor-associated M2 macrophages in PCa tissues. We identified a role for P2X4 purinergic receptors in regulating macrophage cytokine secretion and phagocytic activity *in vitro,* suggesting that P2X4 receptors in macrophages within the prostate TME may contribute to PCa progression. Our ongoing studies are aimed at determining the efficacy of P2X4 purinergic receptors as a potential therapeutic target.

#2687 Exploring the synergistic mechanisms of VEGFR and immune checkpoint inhibitors in clear cell renal cell carcinoma metastases.

Stefan Maksimovic, Nina Costanza Boscolo, Matthew T. Campbell, Eleonora Dondossola

UT MD Anderson Cancer Center, Houston, TX

M.C. and E.D. equally contributed. Clear cell renal cell carcinoma (ccRCC) is the most common malignancy originated from the kidney, with bone and lungs as the main sites of distant spread in vast majority of patients. A key feature of ccRCC is the loss of function of the von Hippel-Lindau (VHL) protein leading to enhanced angiogenesis; in addition, ccRCC exhibits a rich and heterogeneous immune infiltrate. Consequently, different anti-angiogenic tyrosine kinase inhibitors (TKIs) and immune checkpoint inhibitors (ICIs)have emerged as standard of care for these patients, as single agents or in combination. Yet, limited data are available on their efficacy in different metastatic sites, and their impact on the immune infiltrate is unclear. We hypothesized that modulation of the microenvironment with a TKI provides an additive impact on ICI, and the outcome of combinational treatments depends on metastatic sites, treatment doses and tumor size. To this purpose, we implanted a luciferase-GFP-positive RENCA-VHL$^-$ cell line in the tibia of immunocompetent mice, resulting in synchronous bone and lung metastases. Cabozantinib (C), as one of the most potent TKIs, anti-programmed cell death protein 1 (Anti-PD1) and anti-CTLA4 antibodies, were used as single agents or in combination. The outcomes were monitored by macroscopic bioluminescence detection *in vivo,* and 3D multiparametric spatial analysis of bones and lungs, *ex vivo*. Compared to control-treated mice, all agents significantly reduced tumor progression at both tumor sites. Although all these treatments are clinically applied at stage IV of disease development, differences in their efficacy were noted once treatment was withdrawn. Spatial analysis of tumor cells, immune infiltrate and blood vessels revealed significant changes dependent on treatment, tumor site and time, with potential synergistic mechanism by TKIs when used in combination with ICI. We proved the efficacy of standard of care for bone and lung lesions of ccRCC in an immunocompetent mouse model and identified differences between treatments at the cellular level in the tumor microenvironment. We believe our studies may provide important insights and efficacy predictions for therapeutic applications in metastatic ccRCC patients.

#2688 FXIIIa-mediated transferrin sequestration drives cancer stem cell phenotype in colorectal cancer.

Savannah Gregg[1], Rio Boothello[2], Sareh Bayatpour[3], Shoja M. Haneefa[4], Adam Hawkridge[5], Umesh Desai[6], Gretchen Hubbard[7], Andrew Elliott[8], Jose J. Trevino[9], Leopoldo Fernandez[10], Mary Garland-Kledzik[11], Bhaumik Patel[12], Adam Khader[12]

[1]Surgery, Veterans Health Administration, Richmond, VA,[2]VCU School of Medicine, Richmond, VA,[3]U.S. Department of Veterans Affairs, Richmond, VA,[4]VCU School of Medicine, Veterans Health Administration, Richmond, VA,[5]Proteomics Shared Resource, VCU School of Pharmacy, Massey Comprehensive Cancer Center, Richmond, VA,[6]Center for Drug Discovery, VCU School of Pharmacy, Richmond, VA,[7]Caris Life Sciences, Baltimore, MD,[8]Caris Life Sciences, Phoenix, AZ,[9]Surgery, VCU School of Medicine, Massey Comprehensive Cancer Center, Richmond, VA,[10]Surgery, Veterans Health Administration, VCU School of Medicine, Massey Comprehensive Cancer Center, Richmond, VA,[11]Surgery, West Virginia University, Morgantown, WV,[12]Veterans Health Administration, VCU School of Medicine, Massey Comprehensive Cancer Center, Richmond, VA

Background: Colorectal cancer (CRC) is the third most common cancer worldwide. Tumors, often described as wounds that do not heal, exhibit fibrin clot deposition, which may create a niche for cancer stem cells (CSCs). Factor XIII (FXIII), a transglutaminase, cross-links fibrin (XLF) and functionalizes XLF clots by incorporating additional protein into the matrix. Our previous studies suggest that FXIII-XLF promotes the CSC phenotype. This study aims to identify and validate CSC-promoting proteins enriched in the XLF matrix.
Methods: TCGA and CARIS CODEai databases were analyzed for correlations of FXIII expression on CRC survival in primary and CRC peritoneal metastasis (PM). CRC cell lines were grown in an XLF spheroid model with various levels of activated FXIII (FXIIIa). We performed a comparative LC-MS/MS-based label-free proteomics (LFQ) analysis on high-salt washed XLF clots treated with normal plasma (100% FXIIIa activity, N-XLF) and CSC-promoting FXIIIa (150% FXIIIa activity, CP-XLF) matrices. FXIIIa activity was measured using a transglutaminase assay. Differential protein expression in the matrices was further validated by ELISA. The impact of FXIIIa inhibition was tested using the pan-transglutaminase inhibitor T101, and the role of the candidate protein transferrin (Tf) on spheroid formation was assessed with a Tf-neutralizing antibody (Tf ab). We further validated the effects of iron-loaded holo-transferrin versus apo-transferrin on spheroid numbers. Additionally, the effect of FXIIIa on intracellular iron was assessed using an iron-sensitive dye via flow cytometry.
Results: FXIIIA1 RNA expression was 2.7-fold higher in CRC PM compared to primary sites, and higher expression correlated with poor survival in all stages. Further, XLF protein expression in a CRC tumor microarray was present in 39.5% of patients, correlating with notably reduced survival (HR=2.2, $p<0.05$). Proteomics analysis revealed 21 differentially expressed proteins in CP-XLF compared to N-XLF (FDR <0.05). Tf was upregulated (4.5-fold) in CP-XLF. FXIIIa inhibition with T101 disrupted Tf sequestration in the XLF matrix and the FXIIIa-mediated promotion of spheroid formation. Tf neutralizing antibodies significantly reduced spheroid formation in both N-XLF and CP-XLF conditions. FXIIIa preferentially sequestered iron-loaded holo-transferrin over apo-transferrin. FXIIIa was associated with a 2.2-fold increase in cells with high intracellular iron ($p=0.002$).
Conclusions: FXIII RNA expression is associated with worse overall survival in CRC. Transferrin (Tf) and iron saturation are crucial in promoting the CSC phenotype in the CP-XLF matrix. This finding positions Tf as a potential mechanistic lead in the FXIII-XLF-CSC axis and a target for therapeutic intervention.

#2689 Targeting mesenchymal cells with epigenetic therapy in lung cancer and lung disease.

Dave-Preston Esoe[1], Avery R. Childress[1], Xiulong Song[1], Jamie Sturgill[2], Christopher M. Waters[3], Christine F. Brainson[4]

[1]Department of Toxicology and Cancer Biology, University of Kentucky, Lexington, KY,[2]Department of Microbiology, Immunology and Molecular Genetics, Markey Cancer Center, University of Kentucky, Lexington, KY,[3]Department of Physiology, Augusta University, Augusta, GA,[4]Department of Toxicology and Cancer Biology, Markey Cancer Center, University of Kentucky, Lexington, KY

Lung diseases, including fibrosis and cancer, are major health problems that together lead to millions of deaths each year. Non-small-cell lung cancer (NSCLC) has high morbidity and mortality rates, and patient prognosis and survival are very poor. Similarly, pulmonary fibrosis is a life-threatening respiratory disease characterized by scarring and damage caused by increased abundance of collagen-secreting myofibroblasts. An autopsy study showed that as many as one third of non-small cell lung cancer patients have some amount of pulmonary fibrosis[1], suggesting that these two diseases frequently co-exist. Our laboratory has also found that higher abundance of mesenchymal cells in tumors predicts a poor prognosis[2]. Various literature has identified Enhancer of Zeste Homolog 2 (EZH2), which is responsible for mediating histone 3 lysine 27 trimethylation, as a key regulator of lung disease. Several EZH2 inhibitors (tazemetostat and valemetostat) provide a reliable and therapeutically tolerable treatment option for diseased patients. Our central hypothesis is that EZH2 inhibition can prevent or reverse fibrosis via targeting myofibroblasts. To test this hypothesis, we have established pulmonary mesenchymal cell lines from both normal and diseased human lung. We observed that EZH2 inhibition slowed cell proliferation with or without TGF-beta, a cytokine known to drive a myofibroblast fate. In vivo, we have observed that EZH2 inhibition attenuates the fibrosis that occurs after exposure to bleomycin. Next, we are testing the roles of these mesenchymal cells in responses to cancer therapies, including immunotherapy. For our initial experiments, we used murine pulmonary mesenchymal cells grown with tumor cells and immune cells in a 3-dimensional Matrigel co-cultures. This culture system mimics aspects of the tumor immune microenvironment *in vitro* and has allowed us to test therapies including EZH2 inhibition and anti-PD1 immunotherapy in a tractable system. Our preliminary results suggest that EZH2 inhibition can alter cells to be more susceptible to anti-PD1, and we are now examining the contributions of mesenchymal cells to these phenotypes. Together our results suggest that targeting EZH2 may improve aspects of both pulmonary fibrosis and lung cancer, and be a way to target these co-occurring diseases to improve patient outcomes. [1]doi: 10.7196/ajtccm.2020.v26i1.050, [2] doi: 10.1016/j.labinv.2023.100176. Funded by R01 CA237643, R01 HL170193, and P30 CA177558 (Markey Shared Resources).

#2690 Expression profiles of antibody drug conjugate (ADC) targets in breast and lung cancer subtypes and in new HER2 subgroups with their PD-L1 status.

Marie Cumberbatch[1], Woo Ho Kim[2], Milan Bhagat[1], John Cogswell[1]

[1]TriStar Technology Group, LLC, Rockville, MD,[2]Seoul National University Medical College, Seoul, Korea, Republic of

Antibody drug conjugates (ADCs) are transforming the treatment of breast cancer as well as lung, ovarian, and several other cancer indications. ADCs targeting HER2 and TROP2 are currently approved in breast and lung cancer subgroups. Some HER2-targeted therapies are efficacious in breast cancer in newly defined HER2-low and -ultralow subgroups. There has been recent interest and some successes combining ADC's with immune checkpoint inhibitors. Despite these successes, tumors still develop resistance through both target-dependent and independent mechanisms. Previously we investigated which ADC's may be expressed in HER2-positive versus negative breast cancers and adeno versus squamous NSCLC histological subtypes. We have now extended these analyses to explore which ADC's show the highest relative expression levels in these cancers, co-expression with PD-L1 and expression in HER2-low and -ultralow breast cancer subgroups. Optimized IHC assays for B7-H3, B7-H4, Nectin-4, TROP2, PD-L1 and HER2 on Ventana or Dako automated staining platforms were applied to treatment naïve tumor samples from ER+ BC (n=60), TNBC (n=52) and NSCLC (n=134; adeno and squamous) in tissue microarray format (1mm cores). ADC targets were scored by pathologist-assisted digital image analysis to deliver H-Scores and frequencies of positivity at high, moderate, low and negative expression levels. Diagnostic visual scoring methods were employed for analysis of PD-L1 and HER2 (including addition of ultra-low score for HER2). We demonstrate that immune positivity for PD-L1 (CPS >10) was most frequent in HER2-low TNBC compared with HER2-ultralow or negative samples, and that B7-H3 and Nectin-4 H-Scores were higher in TNBC compared with ER+ BC, regardless of PD-L1+ status suggesting potential for immunotherapy combinations in TNBC and especially HER2-low subtype. Furthermore, B7-H4 H-Scores were higher in HER2-low, ultralow and negative ER+ BC compared with HER2 positive samples, while the inverse was observed for B7-H3 revealing potential opportunities for therapies that may overcome resistance to HER2-targeted therapies in this cancer subtype. Squamous NSCLC displayed higher B7-H3 and B7-H4 H-Scores and percent positive cells compared with adeno subtype, and B7-H3 and B7-H4 H-Scores were higher in PD-L1 positive samples (TPS≥1%) supporting ADC therapy for these targets in combination with PD-1/PD-L1 immunotherapies. Taken together, these data may aid the future selection of targets for upcoming therapeutics that can overcome target-independent resistance and provide rationale for immunotherapy combinations.

#2691 Configurable multiplex immunofluorescence panels for emerging ADC targets.

Yvette Cajigas, Cassandra Kysilovsky, Lauren Duro, **Kevin Hwang**, Lorenz Rognoni, Angela Vasaturo

Vizgen, Cambridge, MA

Background: Antibody-drug conjugates (ADCs) are revolutionizing oncology by selectively delivering cytotoxic payloads to cancer cells while sparing healthy tissues. ADC targets are typically cell-surface proteins overexpressed on tumor cells but with limited expression on normal cells, making spatial proteomics assays an ideal complement to this research. However, standard DAB-based immunohistochemistry techniques often lack the specificity and sensitivity needed to confidently quantify the target expression level in tumors with low target abundance, which can result in discordant scoring by pathologists. To support ADC drug development, we present a flexible and scalable approach to the design, development, and validation of mIF panels tailored to ADC targets using the highly sensitive InSituPlex (ISP) technology.
Methods: Antibodies targeting ADC-specific markers were initially optimized using DAB chromogenic staining. Following this, antibodies were conjugated to DNA barcodes, and titrations of both antibodies and fluorophores were performed to achieve optimal signal intensity and staining patterns as evaluated by a pathologist. Singleplex DAB and ISP assays were conducted on serial FFPE sections across multiple tumor types. Validation experiments were designed and performed to test the platform's precision and configurability for ADC-related targets. AI-driven STARVUE software was used for image analysis and assessment of reproducibility of key metrics including cell density and signal intensity.
Results: This approach integrates adaptability to diverse ADC targets, optimized antibody titration, fluorophore selection, and panel construction, achieving high specificity and sensitivity for multiple biomarkers in a single tissue section. The inclusion of STARVUE image analysis enhances the evaluation of spatial relationships between ADC targets and downstream effectors, offering a deeper understanding of drug efficacy and resistance mechanisms. The platform demonstrated high concordance across configurations, with single-target coefficients of variation (CVs) consistently below 20%. Spatial and intensity metrics further confirmed the robustness of the assay in multiple tumor types.
Conclusions: This validated and scalable mIF platform for ADC targets represents a significant advancement in spatial proteomics, providing a robust solution for researchers and clinicians to accelerate ADC development, optimize therapeutic strategies, and improve clinical outcomes.

#2692 3D in vitro models for identifying primary vs. metastatic tumors in targeted breast cancer treatment.

Shrinwanti Ghosh[1], Sangdeuk Ha[1], Anu Gaba[2], Kalpana Katti[3], Jiha Kim[1]

[1]Biological Sciences, North Dakota State Univ., Fargo, ND,[2]Hematology & Oncology, Sanford Roger Maris Cancer Center, Fargo, ND,[3]Civil, Construction and Environmental Engineering, North Dakota State Univ., Fargo, ND

Metastatic disease is responsible for over 90% of cancer-related deaths, with breast cancer patients exhibiting a survival rate of only 5% once bone metastases develop. Traditional 2D in vitro models often fail to accurately replicate the tumor microenvironment (TME) and drug responses observed in vivo. To address these limitations, we utilized advanced 3D in vitro models to better simulate primary and metastatic tumors, enabling the study of cancer-stromal interactions and drug efficacy. Patient-derived cancer cells (PDCs) from three molecular subtypes of breast cancer were used to model both primary tumors (using a hanging drop system) and bone metastases (using a bioengineered bone-mimetic scaffold). Stromal components, including endothelial cells (ECs), macrophages, and cancer-associated fibroblasts (CAFs), were co-cultured with PDCs to mimic TME more closely. As expected, drug sensitivity profiles generated within 48 hours revealed significant differences between 2D and 3D models when treated with paclitaxel (PTX), doxorubicin (DOX), lapatinib (LAPA), and 4-hydroxy tamoxifen (4-OH TAM). PDCs at bone metastatic sites exhibited higher survival rates post-treatment, as indicated by elevated BCL2 and Ki67 expression and lower CAS9 expression compared to primary tumor sites. This suggests site-specific resistance mechanisms, emphasizing that bone metastases may require higher drug doses to achieve comparable efficacy. Stromal interactions significantly influenced angiogenic capacity. While drug treatments substantially altered network formation when ECs and PDCs were cultured together, the presence of CAFs produced opposing effects. These findings underscore the complexity of bone TME and suggest that higher drug doses or combination therapies may be necessary to effectively target metastatic tumors. Additionally, we are incorporating macrophages into the TME in ongoing experiments to further elucidate their contributions to angiogenesis and therapeutic resistance. Moving forward, we will employ single-cell sequencing of 3D models to investigate tumor heterogeneity and refine dose-specific treatments, advancing precision medicine strategies to combat metastasis and improve patient outcomes. This work is currently in progress. Our single-cell sequencing data aims to reveal distinct gene expression differences between primary tumor sites and metastatic sites within the tumor microenvironment. By identifying unique gene expression profiles in cancer cells, stromal cells, and immune cells at each site, we aim to uncover critical pathways driving metastasis. These findings will guide the development of targeted therapies that specifically address metastatic tumors, thereby preventing metastasis and minimizing off-target effects.

#2693 BA3361, a tumor selective, conditionally active biologic (CAB) anti-nectin4-ADC with a novel NextGen linker system enhances therapeutic efficacy in pancreatic cancer.

Jing Wang, Jian Chen, Gerhard Frey, Haizhen Liu, Cathy Chang, William J. Boyle, Jay M. Short

R&D, BioAtla, Inc., San Diego, CA

Antibody drug conjugates (ADCs) are a unique therapeutic combination of the tumor-targeting monoclonal antibody, high-potency cytotoxic drug, and a chemical linker. Together, these components enable the tumor-specific delivery of the highly potent cytotoxin. However, treatment efficacy is often limited by on-target/off-tumor toxicity due to antigen expression in healthy tissue and by unwanted payload release outside the tumor caused by linker cleavage via circulating proteases. In this study, we present BA3361, a NextGen ADC featuring a conditionally active biologics (CAB) anti-Nectin4-ADC, optimized with novel CAB and linker technologies. BA3361 demonstrates pH-selective enhanced target binding in the tumor microenvironment conditions, improved serum stability, increased solubility, and tumor-specific payload release. These advancements effectively address critical clinical challenges by reducing off-tumor, on-target and off-target toxicities. *In vivo* efficacy data showed complete tumor regression in several patient derived cancer models. Notably, compared to an Enfortumab vedotin analogue, superior efficacy was observed in patient-derived pancreatic xenograft models, and the *in vivo* potency was tightly correlated with target expression. Furthermore, we discovered that intracellular glycosidase activity is high in pancreatic tumor tissue, independent of target expression, indicating efficient tumor selective linker cleavage. In conclusion, our NextGen Nectin4-CAB-ADC BA3361 demonstrates comparable or superior activity in lung, breast, and urothelial patient derived cancer models compared to Enfortumab vedotin and outperforms it in pancreatic PDX models. In addition, the conditional binding in the TME should help to reduce side effects of nonCAB ADCs and potentially widen the therapeutic index.

#2694 ID1-4 proteins' contribution to cancer stem-like cell maintenance and ovarian cancer recurrence following chemotherapy.

Megan Anne Keene[1], Mikella Robinson[2], Darren Lighter[2], Carrie House[2]

[1]Cell and Molecular Biology, San Diego State University / University of California San Diego, San Diego, CA,[2]Cell and Molecular Biology, San Diego State University, San Diego, CA

Although most patients initially respond to chemotherapy, ovarian cancer (OC) remains the most lethal gynecological cancer, primarily because over 80% of advanced cases relapse within 24 months. Current research suggests that cancer recurrence can be attributed to a small subpopulation of cancer stem-like cells (CSCs) that possess the ability to resist chemotherapy, self-renew, and asymmetrically divide. ID1-4 proteins, also known as inhibitors of differentiation or inhibitors of DNA binding proteins, regulate cell fate and differentiation in normal stem and progenitor cells. In gliomas, ID1-4 proteins have been identified as master transcriptional regulators of CSC identity, signaling to downstream pathways important for self-renewal, tumor initiation, and survival, but their role in OC CSCs and recurrence is unclear. We have previously found that ID1-3 gene expression increases following chemotherapy treatment in OC clinical samples and cell lines, and the elevated gene expression of ID1 and ID3 is associated with worse clinical outcomes. In subcutaneous xenograft mouse models, we have found that ID2 and ID4 protein expression significantly increases acutely following carboplatin treatment as compared to vehicle group, but this effect is lost over time. In OVCAR5, OVCAR8, and OV90 OC cell lines, we see a 10-40 fold increase in ID gene expression following two cycles of chemotherapy treatment. We hypothesize that ID proteins are required for OC CSC survival, self-renewal, and tumor recurrence following chemotherapy. Using a pan-ID inhibitor, AGX51, we have significantly knocked down ID expression in chemotherapy-naïve (OVCAR5, OV90) and chemotherapy-resistant (OVCAR4, OVCAR8) OC cell lines. Our data suggest pan-ID inhibition in OC cell lines reduces chemotherapy resistance, and reduces chemotherapy-dependent increases in CSC surface marker expression by flow cytometry, stemness and EMT genes by qRT-PCR, and downstream targets, including IL-6. Future studies will use siRNA silencing to further investigate the role of specific ID proteins in chemotherapy resistance, asymmetric division, spheroid formation, CSC maintenance by gene expression and flow cytometry, and signaling to downstream targets. These studies highlight a novel target important in CSC maintenance in response to chemotherapy that contributes to tumor progression and relapse. Understanding these mechanisms will allow us to develop novel therapeutic approaches to overcome chemotherapy-induced relapse, and better clinical outcomes.

#2695 TGF-β accelerates STING signaling termination in the tumor microenvironment by enhancing AP3D1-mediated STING trafficking.

Xingzhe Liu[1], Yuanjia Wen[1], Jiahao Liu[1], Guannan Li[1], Huayi Li[1], Xiaofei Jiao[1], Mengshi Luo[1], Yuewen Zhang[1], Xuejiao Zhao[1], Gorden B. Mills[2], Ding Ma[1], Guangnian Zhao[1], Qinglei Gao[1], **Yong Fang**[1]

[1]Tongji Medical College - Tongji Hospital, Wuhan, China,[2]OHSU Knight Cancer Institute, Portland, OR

Background: Activation of stimulator of interferon genes (STING) has shown great potential to enhance anti-tumor effect and reshape the tumor microenvironment (TME). Instead, the impact of TME on STING signaling remains elusive. Leveraging patient-derived samples at single-cell resolution from our clinical trial (NCT04507841), we find that TGF-β significantly attenuates STING signaling and downstream type I interferons production, which is related to the accelerated STING trafficking and degradation. Mechanistically, we identify AP3D1, a subunit of the AP-3 complex, could response to TGF-β and accelerate STING signaling termination in the TME.
Methods and results: Utilizing unique samples with HRD precision stratification and PARPi treatment information from our clinical trial (NCT04507841), and employing cutting-edge technologies such as single-cell sequencing and multiplex immunohistochemistry to reveal the complex immune microenvironment, we identified TGF-β suppresses the STING signaling pathway and the production of downstream type I IFN. Additionally, through RT-PCR, western blot, and immunofluorescence co-localization, we discovered that TGF-β promotes STING transport from Golgi to lysosome, accelerating STING degradation. To elucidate the specific mechanism by which TGF-β promotes STING transport, we employed techniques including the STING-APEX2 based proximity biotin labeling system, phosphorylation mass spectrometry and IP-MS, our analysis revealed and verified that TGF-β activates MEK to facilitate phosphorylation of adaptor related protein complex 3 (AP-3) subunit delta 1 (AP3D1) at the S9 site, thereby enhancing the interaction between AP3D1 and STING and accelerating the degradation of STING. Furthermore, we conducted validation experiments in multiple mouse models to confirm the effectiveness of peptides that target the inhibition of AP3D1-STING binding at the AP3D1 R26 site and the blockade of AP3D1 phosphorylation at the S9 site. When administered in combination with PARP inhibitors and STING agonists, these peptides demonstrated satisfactory therapeutic outcomes in upregulating STING signaling, reducing tumor burden, and inducing anti-tumor immune responses, all without evident toxicity.
Conclusions: Our work identified TGF-β as an exogenous STING regulator that accelerates AP3D1-mediated STING trafficking and degradation, providing an unprecedented insight into crosstalk between the TME and the STING signaling. Furthermore, our in-depth dissection of mechanisms offered conceptual strategies for new drug discovery and combined pharmacotherapy in cases where STING agonists fail to provide anticipated efficacy currently.

#2696 Targeting SLC7A11 creates a favorable immune microenvironment in colorectal cancer.

Jichang Li[1], **Xiaoxue Pan**[1], Kang Xia[2], Zeruo Yang[3], Xiaojing Yang[4], Pengyuan Wang[1], Shanwen Chen[1]

[1]Peking University First Hospital, Beijing, China,[2]Peking University Chengdu Academy for Advanced Interdisciplinary Biotechnologies, Chengdu, China,[3]Center of Quantitative Biology of Peking University, Beijing, China,[4]Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, Peking Univerisity, Beijing, China

Background: Cysteine is a key immunoregulatory nutrient in colorectal cancer (CRC). Cystine uptake is primarily mediated by the cystine/glutamate transporter SLC7A11, which consists of 12 transmembrane α-helices that forming a channel through the cell membrane. Given SLC7A11's role in both metabolic competition and resistance to ferroptosis, we aim to explore whether SLC7A11 can be effectively targeted with monoclonal antibodies as a therapeutic strategy for colorectal cancer.
Methods: Using a cell-based in vivo immunoassay and standard hybridoma technology, we generated a human SLC7A11-specific murine monoclonal antibody, designated 1A4. Subcutaneous and orthotopic colorectal cancer models in mice, as well as an AOM/DSS-induced colitis-associated colorectal cancer model was established. We evaluated the effects of the SLC7A11 antibody on the immune microenvironment of colorectal cancer by multicolor immunofluorescence and flow cytometry. Furthermore, single-cell sequencing was employed to analyze alterations in immune cell subpopulations and to investigate the impact of the SLC7A11 antibody on immune cell metabolism and intercellular interactions within the tumor microenvironment. Flow cytometry was further utilized to validate the differential effects of the SLC7A11 antibody on CD4+ and CD8+ T cells.
Results: Subcutaneous and orthotopic cancer model of colon cancer showed that treatment with 1A4 significantly reduced tumor volume. Multiplex immunofluorescence analysis showed that both duel immune checkpoint blockade and 1A4 increased the number of infiltrating CD8+ T cells and the CD8+T/Treg ratio in tumor tissues, with a more pronounced increase observed in tumors treated with 1A4. Single-cell sequencing results demonstrated that 1A4 modulated the tumor immune microenvironment by increasing the infiltration of T cells, B cells, and NK cells within the tumor tissue. RNA sequencing on CD4+ T cells exhibited more pronounced alterations in metabolic pathways. The flow cytometry results showed that treatment of CD8+ T cells with 1A4 enhanced stemness and a reduction in the proportion of exhausted CD8+ T cells.
Conclusions: This study developed an antibody targeting the SLC7A11 and validated its anti-tumor effects in colorectal cancer. Targeting SLC7A11 potently creates a favorable immune microenvironment through inhibiting the activation of Tregs and increasing the stemness of CD8+ T cells.

#2697 Targeting tumor microenvironments of triple negative breast cancer with a novel adeno-associated virus-mediated virotherapy.

Md. Manirujjaman[1], Yong Ran[2], Soroor Heidari[1], Jone Garai[1], Samarpan Majumder[1], Luis Del Valle[1], Jovanny Zabaleta[1], Barbara Osborne[3], Lucio Miele[1], Todd Golde[2], **Fokhrul Hossain**[1]

[1]LSU Health New Orleans, New Orleans, LA,[2]Emory University, Atlanta, GA,[3]University of Massachusetts, Amherst, Amherst, MA

Triple-negative breast cancer (TNBC) is a group of aggressive breast cancers with a higher mortality rate due to their resistance to chemotherapy and radiotherapy. Unfortunately, there are no specific targeted therapies for TNBC, making it a critical medical need. In basal-like TNBC, Notch signaling regulates the recruitment of tumor-associated macrophages (TAMs) and the activation of cancer stem-like cells (CSCs) by TAMs through TGF-β pathways. TGFβ-responsive CSCs have enhanced resistance to T cell immunotherapy and are responsible for tumor relapse. Therefore, TGF-β pathway inhibition is a promising therapeutic target in TNBC. However, due to systemic toxicity, the FDA has not approved TGF-β investigational inhibitors. To avoid these toxicities, we use a recombinant adeno-associated virus (r-AAV) vector encoding a soluble TGF-β receptor decoy (r-AAV-TGFβRD) to inhibit TGF-β intratumorally in TNBC. r-AAVs are non-replicating viruses and, therefore, are a safe platform for in vivo gene therapy delivery. We found that rAAV-GFP transduced both human and mouse TNBC cells. Our rAAV constructs contain a C1QTNF3-derived collagen domain (CD) that facilitates the trimerization and stabilization of secreted fusion proteins. We purified these proteins from cellular supernatants of r-AAV vector-transduced cells using His-Tag affinity chromatography and found that they significantly decreased human TNBC cell's mammosphere growth. Intratumoral injection of the r-AAV-TGFβRD virus significantly reduced syngeneic mouse TNBC tumor growth and increased the tumor-infiltrating lymphocytes (TILs), including CD3 and CD8. NanoString analyses (nCounter Immunology) suggest changes in tumor microenvironments after the treatment. We found significant increases in the innate immune system, host-pathogen interactions, and chemokine signaling with increasing influx of macrophages, DC, and CD8 as TILs. Interestingly, we found decreased B cells and Tregs within TME as TILs. Notably, the r-AAV-TGFβRD virus significantly enhances the efficacy of chemotherapy (paclitaxel) in mediating the anti-tumor tumor effect in the syngeneic TNBC mouse model. These findings indicate that r-AAV-TGFβRD virus can be a promising approach for treating TNBC alone or in combination with chemotherapy for translational application in the future.

#2698 Altered collagen morphology in pancreatic cancer treated with the iRGD tumor-penetrating peptide.

Yoon Ho Choi[1], Minji Kim[1], Hotaka Kawai[2], Yukihito Kuroda[3], Norio Miyamura[3], Seock-Jin Chung[1], Kazuki N. Sugahara[3], Tae Hyun Hwang[1]

[1]Mayo Clinic Florida, Jacksonville, FL,[2]Oral pathology and Medicine, Okayama University, Okayama, Japan,[3]Columbia University, New York, NY

Background: Pancreatic ductal adenocarcinoma (PDAC) is characterized by a dense, collagen-rich stroma that impedes drug delivery. We investigated how iRGD-mediated nanoparticle delivery modifies collagen architecture using humanized mouse models. Computational analysis of Picro-Sirius Red-stained whole-slide images (WSIs) using curvelet transform-based fiber extraction method (CT-FIRE) was performed to quantitatively examine changes in collagen features, thereby understanding treatment-induced alterations in the tumor microenvironment (TME).

Methods: Humanized PDAC mice were prepared with either the human PANC-1 cell line or 0779 primary human PDAC cells and treated with or without iRGD monotherapy for two months (n=12). Primary tumors were sectioned and stained with Picro-Sirius Red to visualize collagen structures. WSIs of Picro-Sirius Red-stained tissues were analyzed with CT-FIRE to extract six morphological features of collagen, including average fiber length, waviness, and density to capture structural changes. WSIs were divided into 256×256 pixel tiles with consistent sampling through bootstrapping, followed by permutation testing for statistical validation (1,000 permutations, $p<0.01$).

Results: Computational analysis revealed significant iRGD-induced collagen remodeling with quantifiable changes in key structural and morphological features. Permutation testing showed that, compared to controls, average fiber length decreased by 3.27% ($p<0.01$), waviness reduced by 3.25% ($p<0.01$), and density diminished by 36.3% ($p<0.01$), compared to controls. The structural changes were more notable in 0779-derived tumors compared to PANC-1, with reductions in average fiber length (1.03%, $p<0.01$), waviness (8.78%, $p<0.01$), and collagen density (24.27%, $p<0.01$), demonstrating cell-line dependent stromal remodeling.

Conclusions: Our findings suggest that long-term iRGD therapy alters collagen morphology in PDAC. Further investigation is warranted to understand how these alterations contribute to enhanced penetration of drugs and immune cells observed in iRGD-treated PDAC tumors. The observed cell-line dependent variations in stromal remodeling suggest tumor heterogeneity influences treatment response, highlighting the potential for personalized therapeutic approaches in stromal-rich tumors like PDAC.

#2699 Investigating fibroblast activation protein alpha (FAPα) as a therapeutic target for delivery of pre|CISION® cancer medicines: Expression, spatial localization and functional insights.

Ruairidh Edwards[1], Curtis Rink[1], Giulia Tagliavini[2], Christopher Mills[2], David Jones[1], Michelle Morrow[1]

[1]Avacta Life Sciences, London, United Kingdom,[2]Concept Life Sciences, Edinburgh, United Kingdom

Fibroblast activation protein α (FAP) is a post-proline protease and solid-tumor target which Avacta's pre|CISION® peptide moiety linker selectively uses as a differentiated means to target the tumor microenvironment (TME). The potential of the pre|CISION® technology is highlighted by the promising clinical data from AVA6000, a pre|CISION® enabled Doxorubicin peptide drug conjugate. FAP expression is tightly regulated in normal adult tissues, with low to undetectable levels in most healthy cells. However, it becomes highly upregulated in the TME where it is expressed by cancer-associated fibroblasts (CAFs). This selective expression pattern has been observed in over 90% of epithelial tumors, including breast, colorectal, pancreatic, lung, brain, and ovarian cancer. We implemented a multi-faceted approach to further the understanding of FAP biology and the potential application for pre|CISION® medicines across a broad range of tumors. We conducted a comprehensive analysis of FAP using real-world data from Tempus AI's LENS database encompassing over 140,000 tumor samples across 30+ solid tumor types. We evaluated FAP pan-cancer RNA expression, spatial organization of FAP in the TME, as well as correlations with biomarkers. We further analyzed FAP expression pre- and post-treatment and performed in-depth analysis to evaluate FAP expression in tumor subtypes and primary or metastatic lesions. Single-cell RNA-sequencing (scRNA-seq) analysis provided insights into FAP-expressing cell populations as well as correlations between CAF abundance and FAP. FAP expression was evaluated at the protein level using IHC analysis across multiple tumor indications. Multi-immunofluorescence IHC (multi-IF) was used to evaluate the spatial localization of FAP relative to tumor cells and vasculature. Our findings reveal high FAP expression across a range of tumor indications and demonstrate a correlation with novel biomarkers of interest. IHC analysis confirms a direct relationship between FAP RNA and protein expression, while multi-IF imaging and concentric partitioning analysis for a range of solid tumor indications shows the spatial association of tumor cells, blood vessels and CAFs expressing FAP. ScRNA-seq data confirms high level FAP expression on fibroblasts in stromal-rich tumors. Finally, co-culture models were established with tumor cells and matched fibroblasts which represent tumors with differing levels of FAP enzyme activity. Avacta's preCISION® peptide-drug conjugates elicit bystander cell kill over a range of physiological FAP levels within these co-culture models. Combined, these analyses emphasize the significance of FAP as an important stromal target for focused delivery of targeted oncology therapeutics, and guide further development of the preCISION® pipeline.

#2700 AXL-positive cancer-associated fibroblasts enhance the metastatic potential of cancer cells and suppress tumor immunity in gastric cancer.

Tuyen Thanh Tran, Yulim Lee, In-Hye Ham, Hur Hoon

Surgery, Ajou University School of Medicine, Gyeonggi-do, Korea, Republic of

Purpose: Cancer-associated fibroblasts (CAFs) are key components of the tumor microenvironment (TME). Numerous studies have demonstrated a significant correlation between increased CAFs accumulation and poor outcomes in gastric cancer (GC) patients. However, no targeting agents against CAFs have been clinically applied in cancer treatments. AXL, a receptor tyrosine kinase, has emerged as a promising target to suppress cancer cell metastasis, yet AXL inhibitors have not been approved due to limited efficacy in clinical trials. However, the role of AXL upregulated in CAFs remains unexplored. In this study, we investigated the effect of AXL-positive CAFs on GC progression and explore their potential as therapeutic targets to enhance GC treatment efficacy.
Methods: We analyzed a single cell RNA-seq (scRNA-seq) dataset of human GC tissues to identify the cell types expressing AXL. Human CAFs and mouse gastric fibroblasts (MGF) expressing AXL were treated with AXL inhibitors, such as CCB3233 and 9im. Additionally, AXL knockdown (KD) was performed in CAFs and MGFs, which were subsequently co-cultured with human GC cell lines (SNU668 or MKN-1) or mouse GC cell lines (ACKPY4113) to assess the effects of AXL+ fibroblasts on GC progression. The impact of AXL-inhibited fibroblasts on T-lymphocytes or macrophages was also evaluated. Furthermore, transcriptome analysis was conducted on AXL KD and control fibroblasts.
Results: The analysis using scRNA-seq dataset revealed that AXL expression was predominantly upregulated in CAFs compared to other cell types. High expression and activation of AXL in fibroblasts were confirmed across various cell lines, including GC cell lines, fibroblasts and immune cells. AXL inhibitors, such as CCB3233 and 9im, effectively reduced AXL phosphorylation in human CAFs and MGFs. Co-culture experiments demonstrated that AXL-inhibited or KD fibroblasts significantly reduced the migration or invasion of GC cells compared to control fibroblasts. Moreover, while control CAFs and MGFs suppressed the activation of T- lymphocytes or macrophages, AXL-inactivated or KD fibroblasts were unable to inhibit immune cell activation. Transcriptome analysis of AXL KD or control fibroblasts revealed reduced expression of chemokines such as CXCL9 and CXCL13, which may explain the observed experimental results.
Conclusions: Our findings highlight the crucial role of AXL-positive fibroblasts in promoting the aggressive phenotype of GC cells and suppressing immune cell activity in the GC TME. Targeting AXL in CAFs holds significant promise as a novel therapeutic strategy for GC treatment.

#2701 iRGD peptide therapy transforms immunosuppressive microenvironment to immune-favorable state in pancreatic ductal adenocarcinoma.

Minji Kim[1], Yoon Ho Choi[1], Hotaka Kawai[2], Yukihito Kuroda[3], Norio Miyamura[3], Kazuki N. Sugahara[3], Tae Hyun Hwang[1]

[1]Department of Artificial Intelligence and Informatics Research, Mayo Clinic, Jacksonville, FL,[2]Department of Oral pathology and Medicine, Okayama University, Okayama, Japan,[3]Department of Surgery, Columbia University, New York, NY

Background: Pancreatic ductal adenocarcinoma (PDAC) exhibits a complex, immunosuppressive tumor immune microenvironment (TIME), necessitating molecular-level understanding to identify therapeutic targets. We investigated how iRGD peptide therapy remodels the immunosuppressive TIME to an immune-favorable state using single-cell spatial molecular analysis.
Methods: Single-cell level in situ molecular imaging was performed using Xenium analyzer with 5K gene panel on tumor samples from humanized PDAC mice (n=7) treated with iRGD monotherapy (n=3) or control (n=4), enabling comprehensive spatial molecular characterization of the TIME.
Results: iRGD therapy significantly transformed the immunosuppressive TIME to an immune-favorable state through T-cell composition remodeling. We observed increased populations of CD4+ memory T cells, CD4+ helper T cells, and cytokine-secreting effector T cells, indicating enhanced immune memory and effector functions. Concurrent decreases in regulatory T cells and activated CD4+ T cells reflected reduced immune suppression. Additionally, decreased TGF-β signaling in the iRGD cohort suggested TGF-β antagonism as a mechanism for overcoming immunosuppression.
Conclusions: iRGD therapy effectively converted the immunosuppressive PDAC microenvironment to an immune-favorable state by enhancing beneficial T cell populations and reducing immunosuppression through TGF-β pathway modulation. These findings support previous work by Kuroda et al. and provide mechanistic rationale for the ongoing iRGD-based immunotherapy clinical trial (iLSTA:ACTRN12623000223639).Acknowledgments: We thank DeepL for the English language editing.

#2702 A novel inhibitor targeting DDR complex to suppress pancreatic cancer growth and progression.

Chrid TP Do, Prabhakar P. Venkata, Jack Y. Prochnau, Deepika Singh, Santosh Timilsina, Panneerdoss Subbarayalu, Daisy Medina, Shahad Abdulsahib, Sajeed Khan, Manjeet K. Rao

UT Health Science Center at San Antonio, San Antonio, TX

Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal malignancy, with a 5-year survival rate of 12%. The dense extracellular matrix (ECM) and cancer-associated fibroblast (CAF)-driven microenvironment contribute to tumor progression and resistance to current therapies, necessitating the development of novel treatments. This study introduces CIDD-8633, a selective small-molecule inhibitor targeting the Discoidin Domain Receptor (DDR) complex, which plays a crucial role in ECM-mediated signaling and PDAC progression. High-throughput screening identified CIDD-8633 as a potent inhibitor of DDR kinase activity. Functional assays demonstrated that CIDD-8633 significantly reduced PDAC cell proliferation, migration, and colony formation while inducing apoptosis and G1/S cell cycle arrest. RNA sequencing revealed that CIDD-8633 treatment downregulated pathways associated with DNA replication and repair, while upregulating apoptosis-related signaling pathways. In vivo, CIDD-8633 effectively suppressed tumor growth in PDAC xenograft models, reducing tumor volume and proliferation markers. Histopathological analysis showed decreased phosphorylated DDR and downstream RTKs expression, accompanied by enhanced apoptotic signaling. Furthermore, combination therapy with gemcitabine demonstrated synergistic effects, enhancing anti-tumor efficacy by reducing PDAC cell viability and metastatic potential more effectively than either agent alone. These findings position CIDD-8633 as a promising therapeutic candidate for PDAC, capable of targeting the DDR complex to disrupt tumor progression. This study highlights the potential of DDR inhibition as a novel therapeutic approach for overcoming the challenges of this aggressive cancer.

#2703 Effect of combination of calcipotriol with paclitaxel on pancreatic ductal adenocarcinoma orthotopic mouse model.

Fatima Dagher[1], Mikayla Skillman[1], Guodong Zhang[2], Lu Dai[1], Amir Mohammad Gholizadeh[1], Chun Li[2], Diana S-L Chow[1]

[1]University of Houston, College of Pharmacy, Houston, TX,[2]Cancer Systems Imaging, The University of Texas M.D Anderson Cancer Center, Houston, TX

Pancreatic ductal adenocarcinoma (PDAC) remains one of the leading causes of cancer-related mortality in the United States, with a dismal 5-year overall survival rate of less than 11%. The progression and treatment resistance of PDAC is heavily influenced by its dense stromal environment, acting as a physical and biochemical barrier, limiting the effective delivery of therapeutics to tumor tissues. This stromal barrier significantly contributes to suboptimal drug efficacy and poor therapeutic outcomes. Calcipotriol (Cal), a known modulator of tumor stroma, may disrupt this barrier and enhance drug delivery to tumors. In this study, we investigated the combination of Cal with paclitaxel (PTX), a commonly used chemotherapeutic agent, to evaluate its ability to improve therapeutic efficacy and drug distribution in a PDAC mouse model. Using an orthotopic Kras* C57bl/6 mouse model of PDAC, we assessed the pharmacokinetics, biodistribution, and antitumor effects of Cal, PTX, and the combination (Cal/PTX). Mice were randomized into four treatment groups: Cal/PTX combination therapy (3 doses of 2 mg/kg /20 mg/kg and 3 doses of 1 mg/kg/10 mg/kg) (N=3), Cal alone (2 mg/kg) (N=3), PTX alone (20 mg/kg) (N=3), and vehicle control (N=4). All treatments were administered intraperitoneally with the solvent Ethanol:Cremophore:Saline (1:1:8) as vehicle and a dosing regimen of 3 days a week (day on day off) for 2 weeks. Tumor intensity was monitored over two weeks using bioluminescence imaging with an IVIS system. Drug concentrations in blood and tumor tissues were quantified using a validated LC-MS/MS method with a lower limit of quantification (LLOQ) of 0.5 ng/ml. In the Cal/PTX group, tumor growth was effectively inhibited, with a 100% response rate observed after 6th dose. Conversely, in the groups treated with Cal or PTX alone, tumor growth continued in 2 out of 3 mice, suggesting limited efficacy of the single agents. Similarly, 3 out of 4 mice showed no signs of tumor regression in the vehicle group. Biodistribution studies further supported these findings, with significantly higher accumulation of both Cal and PTX at the tumor site in the combination group compared to single-agent groups, indicating improved tumor-specific delivery. In the tumor, the mean concentrations (ng/g) (±SD) of Cal and PTX in the combination group were 98.08 ± 55.5 and 147.4 ± 45.07, respectively, compared to 3.71±2.33 and 52.46 ± 21.25 in the alone group. These findings highlight the potential of the Cal/PTX combination therapy to overcome stromal barriers and enhance drug delivery and antitumor efficacy in PDAC. Ongoing work focuses on developing a co-formulated polymeric micelle system (M-Cal/PTX) to be tested on Kras* mouse model to enhance drug delivery further, maximize therapeutic outcomes, and overcome drugs' associated toxicity. This novel approach could pave the path for more effective treatment strategies for PDAC.

#2704 Targeting HCC tumor microenvironment interactions using an advanced HCC patient-derived on-chip model.

Orsola Mocellin[1], Abbie Robinson[1], Aleksandra Olczyk[1], Stephane Treillard[1], Thomas Olivier[1], Chee P Ng[1], Jeroen Heijmans[1], Gilles van Tienderen[2], Monique Verstegen[2], Flavio Bonanini[1], Sebastian J. Trietsch[1], Dorota Kurek[1], Henriette Lanz[1], Jos Joore[1], Paul Vulto[1], **Karla S. Queiroz**[1]

[1]MIMETAS, Oegstgeest, Netherlands,[2]Erasmus MC-University Medical Center Rotterdam, Rotterdam, Netherlands

Hepatocellular carcinoma (HCC) is the most common type of liver cancer. Its incidence is increasing and is closely related to advanced liver disease. Interactions in the HCC microenvironment between tumor cells and the associated stroma actively regulates tumor initiation, progression, metastasis, and therapy response. Current in vitro systems often fail to replicate the HCC complex microenvironment, including vascular structures, hindering accurate efficacy evaluation and limiting the translational value of preclinical studies. This study aimed to address these gaps by developing a scalable, vascularized 3D HCC model using the OrganoPlate Graft platform to enable detailed analyses of drug responses, bridging a critical gap in anticancer drug discovery Here we report a phenotypic drug screen on a HCC patient-derived comprehensive Organ-on-a-Chip setup. The vascularized patient-derived HCC models include relevant cellular players of the HCC microenvironment. These co-cultures are highly suitable for studying specific cell types as well as patient-specific responses in medium- to high- throughput fashion. We assessed the effect of 28 treatment conditions, including standard of care (SoC), single, and combination treatments with compounds targeting different molecular programs. Approximately 960 HCC patient derived cultures were grown under perfusion flow in microfluidic chips, exposed to treatments, and subsequently assessed for viability, chemokine and cytokine changes, and vascular bed responses. The screen showed known therapeutics sorafenib and lenvatinib to reduce culture viability and induce significant alterations in vascular bed organization. Compound 1 reduced viability without affecting vascular structures. All three compounds also affected chemokine and cytokine profiles. Additionally, several compounds including Compound 25, Compound 5 and Compound15 lowered IL6 in culture supernatant, whereas Compound 6 elevated IL6 levels. These findings highlight the utility of the organ-on-a-chip model in visualizing drug-induced responses at both molecular and morphological levels, providing detailed insights into drug effects on specific cellular compartments within the HCC TME. This platform holds significant potential for enabling safer and more effective drug development, offering scalability and ease of use for industrial applications. By addressing critical limitations of conventional in vitro models, this approach facilitates a more accurate understanding of HCC biology and supports the translation of novel anticancer therapies to clinical practice.

#2705 Spatial profiling of tumor microenvironment uncovers potential novel therapeutic approach in pediatric neuroblastoma.

C. Tu[1], C. Tan[1], J. Monkman[1], A. Almeida[2], G. Antonio-Carreon[1], N. Omer[1], G. Hull[1], K. Chung[1], A. Mehdi[1], A. Salerno[3], O. Vittorio[3], A. Mayer[4], C. Souza[2], F. Pinhati[2], S. Elifio-Esposito[2], W. Nicholls[5], L. Noronha[2], A. Kulasinghe[1], F. Souza-Fonseca-Guimaraes[1]; [1]Frazer Institute, Faculty of Medicine, University of Queensland, Woolloongabba, QLD, Australia, [2]Laboratorio de Patologia Experimental, PPGCS da PUCPR, Curitiba, Brazil, [3]School of Biomedical Sciences, Faculty of Medicine and Health, University of New South Wales, Sydney, NSW, Australia, [4]Enable Medicine, Menlo Park, CA, [5]Queensland Children's Hospital, Brisbane, QLD, Australia

Background: Neuroblastoma (NB) is a tumor of the sympathetic nervous system and is the most common extracranial solid malignancy in children. Patients with high-risk NB have much poorer outcomes compared with patients with low- and intermediate-risk neuroblastoma. These striking differences in clinical outcomes led us to investigate how the tumor microenvironment (TME) differs between high-risk and low-risk NB patients. We examined the cancerous and non-cancerous cells, along with other components, in the TME to determine their potential to either promote or suppress tumor growth, and to determine their association with a specific tissue spatial organization.

Methods: In this study, we profiled 27 pediatric NB patients (n = 14 high-risk and 13 low-risk samples) using GeoMx DSP Whole Transcriptome Atlas (WTA) (Nanostring Technologies) and Phenocycler Fusion (Akoya Biosciences). We analyzed a total of 67 regions of interest (ROIs) using spatial transcriptomics, guided by fluorescent markers (CD45, Synaptophysin, CD56, and DAPI). ROIs were segmented into tumor regions (CD56+Synaptophysin+CD45-) and TME regions (CD45+CD56-). We conducted spatial proteomics to phenotype cells, map their locations, and assess functional states by profiling 53 markers. This 53-plex protein panel enabled us to identify various cell types within the NB TME, covering relevant lineages (tumor, lymphoid, myeloid, and stromal), subtypes (e.g., CD4 T cells, CD8 T cells, B cells, macrophages and neutrophils), and functional states (e.g., Ki67 and PD-1). Spatially resolved signatures in the NB identified characteristics of cellular neighborhoods that are linked to NB patients' survival outcomes.

Results: Within the tumor regions, we identified an upregulation of the glutathione metabolism pathways that are involved in the suppression of ferroptosis in high-risk NB tumors, which could be targeted with GPX4 inhibitors. We also discovered that NB with macrophage-rich and stroma-secluded immune cell neighborhoods had worse outcomes. Stroma-secluded immune cells were more frequent in high-risk NB, which may contribute to the resistance mechanisms to immunotherapy and overall survival of patients with high-risk NB.

Conclusion: Our study highlights the need to combine the genomic profile of NB TME with spatially defined cellular neighborhoods to better understand tumor aggressiveness. Considering the upregulation of ferroptosis suppressive pathways and the macrophage-rich neighborhood in high-risk NB, our findings suggest that inducing ferroptosis with GPX4 inhibitors might be a promising therapy for high-risk NB. Overall, our study revealed key genes and pathways in high-risk NB and provides a new therapeutic avenue for patients with high-risk NB. This hypothesis is currently being investigated in our lab using animal models.

#2706 Engineered tumor-targeting bacteria expressing cancer-killing protein for breast cancer.

D. Tran[1], K. Christov[1], B. Ganesh[2], J. Wang[2], A. Green[1], T. Yamada[1];
[1]University of Illinois College of Med. at Chicago, Chicago, IL, [2]University of Illinois, Chicago, IL

Despite significant advancements in the treatment of advanced breast cancer, therapies using chemotherapeutic agents still face considerable challenges. One key issue is the hypoxic tumor microenvironment (TME), characterized by insufficient blood flow and oxygen supply within tumors. This hypoxia reduces the sensitivity of breast tumors to treatment, diminishes therapeutic efficacy, and promotes resistance and metastasis. Approximately 25-40% of invasive breast cancers exhibit hypoxic tumors, which are strongly associated with increased risks of metastasis and chemotherapy resistance. Therefore, there is a pressing clinical need for novel strategies to specifically target these hypoxic tumor regions. To address this limitation, our objective is to develop an innovative approach using tumor-targeting bacteria carrying cancer-killing protein, controlled by an engineered hypoxia-inducible expression system. In this study, four *Escherichia coli* strains from the probiotic Symbioflor® 2 were isolated and characterized based on colony morphology and PCR results. These strains were transformed with luciferase gene, and luciferase activity was monitored to determine biodistributions of each *E. coli* after intravenous injection into mice. Our data showed that G5 strain preferentially colonized in xenografted triple-negative breast tumors. To specifically express cancer-killing azurin gene within the hypoxic tumor TME, we optimized the hypoxia-inducible promoter in *E. coli* G5 cells. To test its anti-tumor efficacy, TNBC cells were orthotopically injected into the mammary fat pads of immunodeficient mice. The engineered *E. coli* G5$^{Hyp*\text{-}azurin}$ strain was administered at varying concentrations (1×10^5, 1×10^6, and 1×10^7 CFU/mouse) through weekly tail vein injections over three weeks. Our results demonstrated that *E. coli* G5$^{Hyp*\text{-}azurin}$, at doses of 1×10^7 and 1×10^6 CFU, effectively inhibited tumor progression. In contrast, lower dose (1×10^5 CFU) showed no significant effect. Additionally, no noticeable side effects were observed, as there was no evidence of weight loss. Overall, our results highlight the potential of our newly designed bacterial approach to develop an innovative treatment strategy.

#2707 An *ex vivo* culture platform of invasive lobular breast cancer for the screening of novel drugs targeting the tumor microenvironment.

Konstantina Strepi, Daisy Batenburg, Martine van Miltenburg, Pim Kloosterman, Eline van der Burg, Anne Paulien Drenth, Frank van Diepen, Guido de Roo, Lex de Vrije, Mariska van Geldrop, Serge Zander, Jos Jonkers, **Julia M. Houthuijzen**

Netherlands Cancer Institute, Amsterdam, Netherlands

The study of invasive lobular breast cancer (ILC) and the development of effective ILC-specific therapies is hampered by the lack of suitable model systems that accurately reflect the human disease. Here, we present a set of four genetically engineered mouse models (GEMMs) of ILC, based on the most common genetic alterations found in human ILC. These GEMMs represent the predominant subtypes of ILC and closely mimic human ILC in growth pattern, invasiveness, tumor microenvironment (TME) composition, and subtype-specific molecular profiling. Using these GEMMs, we developed a novel *ex vivo* culture platform that maintains tissue architecture, TME composition and gene expression for up to seven days. Using this platform, we investigated whether targeting cancer-associated fibroblasts (CAFs), which are commonly found in invasive lobular carcinoma (ILC) and known to hinder therapy effectiveness, in combination with tumor cells, could enhance anti-cancer drug efficacy. To identify potential drug combinations, we conducted single-cell transcriptomics on our four ILC mouse models to investigate the complex interactions between CAFs and other cells in the TME. Building on previous research showing that immune-regulatory CAFs (iCAFs) recruit myeloid cells and promote tumor cell invasion, as well as our current findings of extensive CXCL- and complement-mediated crosstalk between iCAFs and macrophages, we hypothesized that inhibiting these iCAF functions could synergize with immunotherapy; a treatment typically ineffective in ILC. Indeed, inhibiting iCAF functions improved immunotherapy efficacy in *ex vivo* cultures, as measured by enhanced T-cell activation, a finding that was subsequently validated in mice. Furthermore, our *ex vivo* platform enabled the culture and drug screening of human ILC specimens. Overall, our ILC GEMMs effectively model a wide range of human ILC molecular subtypes, and our *ex vivo* culture platform facilitates the faster and more accurate identification of new therapeutic vulnerabilities compared to traditional 2D or 3D cultures. Taken together, these models have the potential to significantly advance ILC research.

#2708 Prediction of radiosensitivity in glioblastoma tumors after standard and low dose rates of ionizing radiation.

I. Rivera, R. Yao, K. Selting, C. Best-Popescu, B. Harley, **S. Pedron-Haba**;
University of Illinois at Urbana-Champaign, Urbana, IL

Glioblastomas (GBM) exhibit high infiltration into the brain parenchyma, impairing surgical resection and leading to recurrence. After standard of care, including tumor resection, radiation therapy (RT) and chemotherapy, most tumors recur at the tumor margins. These tumors show an increased aggressiveness and therapeutic resistance. The underlying mechanisms for these cellular transitions are unclear. Research suggests that RT paradoxically alters the GBM microenvironment promoting cancer cell survival upon recurrence. Some of these alterations in the irradiated tumor microenvironment include increased oxidative stress, hypoxia, cellular senescence, vasculogenesis and ECM compositional and structural changes.We use an *ex vivo* platform that combines the hallmarks of GBM tumors: high cellularity, the neural circuitry that surrounds tumors, a hydrogel-based ECM that mimics the biophysical and biochemical properties of GBM and the intermittent hypoxic environment present in native tumors, to characterize and evaluate the therapeutic benefits of RT and the underlying mechanisms of neurotoxicity to more effectively treat the tumor while minimizing the deleterious side effects.Our results show that radiation treatment increases glycolysis in tumor cells and that immediate response to radiation produces a more significant ATP production decrease in hypoxia environments. We have also investigated the long-term energy production and mitochondrial dysfunction in the tumor cells at different radiation rates and under low oxygen environments. Interestingly, the maximal respiration capacity in GBM cells increases with the presence of conditioned media from neuronal cultures in normoxic conditions but has the opposite effect in hypoxic environments.Proliferation ability of cells on stiffer matrices is greater than that on softer matrices. In the presence of an altered biomechanical microenvironment, a stiffer tumor tissue is more prone to immunosuppression and epithelial-mesenchymal transition. After cells are irradiated, the cell migration is different with different matrix stiffness. Moreover, softer matrices are more likely to promote cellular radiosensitivity, while stiffer matrices can inhibit cellular radiosensitivity.The use of preclinical tools that can model key features of the GBM and normal brain tissue can give a better understanding of the spatial-temporal characteristics of tumor microenvironment alterations during progression and response to therapy.

#2709 Disrupting cancer fitness and pro-tumorigenic fibroblast functions with a first-in-class Pin1 degrader.

Melvyn Chow[1], Morgan O'Shea[1], Lilly Ein[1], Julie Arnold[1], Sydney Alnemy[1], Erica Dube[1], Pradeep Kota[1], Guosen Ye[1], Ronan Hanley[1], Andrew Good[2], Kristin Brown[1], Catherine Sabatos-Peyton[1], Emily Corse[1], Sebastian Hayes[1], Krista Goodman[1]

[1]Larkspur Biosciences, Inc., Cambridge, MA,[2]CAnDiD Consulting, Wallingford, CT

Background: Pin1 plays a pivotal role in regulating cellular processes including growth and differentiation by catalyzing the cis-trans isomerization of peptidyl-prolyl bonds in phosphorylated proteins. As a key gene involved in cancer cell fitness, Pin1 promotes tumor progression and maintains the immunosuppressive tumor niche. Research has extensively documented how Pin1 acts as a catalyst in both the initiation and progression of malignancies by modulating the stability and activity of oncogenes and tumor suppressors such as p53, c-Myc and NF-κB. These interactions underline Pin1's significant role in the intrinsic biology of cancer, influencing cell cycle progression, apoptosis, and cellular senescence. Beyond its direct effects on cancer cell intrinsic pathways, Pin1's influence extends to the tumor microenvironment, the supportive niche a tumor creates to support growth and metastasis. Recent studies have identified Pin1 as a driver of cancer-associated fibroblast (CAF)-mediated suppression, particularly in colorectal cancer, breast cancer and pancreatic cancer. In this study, we present the discovery of a reversible, first-in-class Pin1 degrader. Our findings reveal how Pin1 degradation not only disrupts cancer cell fitness but also reprograms fibroblast phenotypes.
Methods: Our research employed a set of in vitro experiments with Pin1-targeting strategies applied to various colorectal cancer and fibroblast cell lines. The impact on the cancer fitness and fibroblast phenotypes was assessed through a detailed cell phenotype and protein analysis using a combination of imaging analysis, ELISA, and Western Blot techniques.
Results: We demonstrate that targeting Pin1 significantly reprogrammed fibroblast phenotypes, steering them away from a pro-tumorigenic state. We disclose a first-in-class selective Pin1 degrader which phenocopies knockout effects on CAF and cancer cell interactions. We further show that Pin1 degradation or KO has a profound impact on multiple drivers of tumor fitness and fibroblast-mediated suppression. Notably, Pin1 degradation disrupts cancer cell communication with their surrounding microenvironment, leading to reduced tumor growth and decreased survival.
Conclusions: These findings highlight Pin1's pivotal role in driving critical cellular and molecular mechanisms in cancer. Our study demonstrates the potential of a bifunctional Pin1 degrader to selectively and efficiently target Pin1 in both CAFs and cancer cells, altering CAF phenotypes and disrupting mechanisms essential for cancer cell fitness and viability.

#2710 Establishment of a co-culture model to investigate the effects of chemotherapy in pancreatic periampullary intestinal adenocarcinoma.

Panagiotis Sarametidis[1], Jojanneke Stoof[1], Laura Ivers[1], Jean Murphy[2], Niall Swan[2], Luca Benigno[2], Fiona Hand[2], Kevin Conlon[2], Paul Mullan[3], Maeve Lowery[4], Naomi Walsh[1]

[1]Dublin City University, Dublin, Ireland,[2]St.Vincent's University Hospital, Dublin, Ireland,[3]Queen's University, Dublin, United Kingdom,[4]Trinity St James Cancer Institute, Dublin, Ireland

Introduction: Periampullary adenocarcinoma (PAC) refers to cancers originating from the head of the pancreas (60%), ampulla of Vater (20%), distal common bile duct (10%), and duodenum (10%). The pancreatic tumor microenvironment (TME) plays a critical role in driving progression and therapeutic resistance in pancreatic cancer, with cancer-associated fibroblasts (CAFs) emerging as key contributors. CAFs, a heterogeneous population within the TME, are central to stromal remodeling through interactions with tumor cells and immune components. Understanding CAF phenotypes is essential to unravel their role in PAC biology and to identify novel therapeutic targets.

Methods: Patient-derived tumor organoids (PDTOs) and CAFs were isolated from primary PAC specimens. Tissue was minced, digested, and dissociated into single cells. Tumor cells were seeded into 3D domes within a basement membrane extract (BME) and cultured in supplemented medium. CAFs were seeded in 2D and allowed to attach. KRAS mutations were analyzed using Sanger sequencing, and CAFs subtypes were characterized through immunofluorescence staining for key markers. Co-cultures of organoids and CAFs were formed at a 1:4 ratio, stained with CellTracker Dyes and their growth was monitored over 2 weeks by microscopy. Flow cytometry was used to characterize T cell subpopulations from PBMCs. Advanced co-cultures were used to measure cytotoxicity and immune efficacy.

Results: PDTOs and CAFs were successfully derived and cultured from PAC specimens, both confirmed as KRAS wild type. All 3 isolated CAF lines expressed fibroblast markers Smooth Muscle Actin alpha (α-SMA) and vimentin. CAFs showed heterogeneous intensity and expression of specific myofibroblastic (myCAFs) markers like Platelet Derived Growth Factor Receptor (PDGFR) and inflammatory CAFs (iCAFs) markers, like Fibroblast Activation Protein (FAP). Co-culture of $PDGFR^{+}/FAP^{-}$ myCAFs with PDTOs at increasing concentrations of cisplatin showed a 4.0 and 6.8-fold increase in the IC_{50} of the co-culture compared to PDTOs and CAFs alone, respectively. Co-culturing a heterogenous myCAF/iCAF population with PDTOs and treatment with cisplatin, showed no induction of resistance (IC_{50} co-culture 6.6 μM) compared to organoids alone (IC_{50} 9.5 μM), and CAFs alone (IC_{50} 9.4 μM). Enrichment of T cells from PBMCs revealed 95% T cell viability, of which 26.9% were cytotoxic, 63.1% were helper T cells, 4.3% were double positive ($CD4^{+}CD8^{+}$) and 5.7% were double negative ($CD4^{-}CD8^{-}$).

Conclusion: PDTOs and CAFs serve as a useful model for studying the cellular heterogeneity of PAC *in vitro*. CAF subtypes based on protein markers exhibit both tumor-promoting effects and increased cisplatin resistance. High viability and enrichment of T cells for co-culture models will provide valuable insights into PAC biology and support testing strategies targeting the TME.

#2711 Deciphering transcriptional activity of the tumor microenvironment for robust stratification of chemotherapy response in triple-negative breast cancer.

Yaoyi Dai[1], Xiaoxi Pan[2], Shaolong Cao[3], Shuai Guo[4], Shuangxi Ji[5], Matthew Montierth[1], Yujie Jiang[6], Leming Shi[7], Gloria V. Echeverria[8], Lucy Yates[9], Johan Staaf[10], Bora Lim[11], Yinyin Yuan[2], Wenyi Wang[4]

[1]Quantitative and Computational Biology, Baylor College of Medicine, Houston, TX,[2]Department of Translational Molecular Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX,[3]Biogen inc., Cambridge, MA,[4]Department of Bioinformatics and Computational Biology, The University of Texas MD Anderson Cancer Center, Houston, TX,[5]Department of Bioinformatics and Computational Biology, Eisai Co., Ltd., Nutley, NJ,[6]AbbVie Inc., North Chicago, IL,[7]School of Life Sciences and Human Phenome Institute, Fudan University, Shanghai, China,[8]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX,[9]Wellcome Sanger Institute, Hinxton, United Kingdom,[10]Laboratory Medicine, Lund University, Lund, Sweden,[11]Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX

Background: Triple-negative breast cancer (TNBC), defined by the absence of estrogen receptor, progesterone receptor, and HER2 expression, represents an aggressive subtype with poor prognosis due to limited therapeutic options beyond chemotherapy. Current molecular subtyping methods fail to predict outcomes despite known tumor-immune-stromal interactions. The tumor microenvironment (TME) influences treatment resistance, yet capturing its complex dynamics remains challenging. TNBC demonstrates distinct features between European and Asian populations, suggesting ethnicity-specific disease mechanisms. Development of biomarkers capable of delineating TME features holds promise for improving TNBC treatment outcomes across ethnic groups.

Methods: We applied our previously proposed pan-cancer biomarker TmS (tumor-specific total mRNA expression, Cao et al. Nature Biotechnology 2022) to 575 chemotherapy-treated TNBC patients from four cohorts (The Cancer Genome Atlas [TCGA], Molecular Taxonomy of Breast Cancer International Consortium [METABRIC], Sweden Cancerome Analysis Network-Breast [SCAN-B], and Fudan University Shanghai Cancer Center [FUSCC]). TmS analysis uses integrative deconvolution of matched RNA/DNA sequencing data to measure per cell per haploid total mRNA ratio for tumor versus non-tumor cells. Using recursive partitioning on survival outcomes, patients were stratified into high/low TmS groups. We performed survival analyses, gene set enrichment analysis, cell type deconvolution, and computational histology on H&E images.

Results: Across all cohorts, low-TmS patients (n=325) presented significantly faster progression of TNBC (log-rank test P value < 0.05, hazard ratio = 0.1-0.53). The high vs. low TmS dichotomization represents a novel independent biomarker distinct from Lehman's TNBCTypes. TmS stratification is independent of clinical markers including BRCA mutation status, tumor mutation burden and Ki67. Mechanistically, high-TmS tumors (n=250) showed distinct TME features with enhanced immune activation in the Western cohorts but not in the Asian cohort, while low-TmS tumors demonstrated immune exclusion with extra-cellular matrix (ECM) enrichment. These molecular distinctions were validated by computational histology showing increased stromal barriers in low-TmS tumors (Kruskal test, adjusted P value < 0.05), suggesting potential ECM-targeted therapeutic strategies.

Conclusions: TmS represents a clinically actionable biomarker capturing TME characteristics in TNBC. Its ability to summarize distinct TME states across diverse populations is ideal for patient stratification. The identification of ethnic-specific TME patterns and ECM-related targets in low-TmS tumors offers new therapeutic opportunities for chemotherapy-resistant TNBC.

#2712 Therapy-induced senescence promotes tumor immunosuppression in cholangiocarcinoma.

Binbin Li, Meina Cai, Jingchun Yang, Sumera Ilyas

Division of Gastroenterology and Hepatology, Mayo Clinic, Binbin Li (Individual), Rochester, MN

Background and Aims: Senescent cells are characterized by high expression of markers including $p16^{INK4A}$, $p21^{cip1}$, and production of a senescence-associated secretory phenotype (SASP). Therapy-induced senescence can be triggered by cytotoxic drugs and can promote resistance to further chemotherapy and immunotherapy. Cholangiocarcinoma (CCA) is a highly lethal tumor with an abundance of immunosuppressive cells such as myeloid-derived suppressor cells (MDSCs). We have demonstrated that cancer cells can leverage noncanonical proapoptotic tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) signaling to foster MDSC accumulation in murine CCA (PMID: 38219900). We hypothesize that senescent cancer cells may be an important source of TRAIL. In this study, we aim to dissect the effects of TIS on CCA tumor growth and the tumor immune microenvironment (TIME).
Methods: Sequential immunofluorescence (IF) was conducted on resected human CCA specimens. Using murine models of CCA (PMID: 29464042; 38458319), murine CCA cells (SB cells) were implanted into livers of WT C57BL/6J male mice. Mice were treated for two weeks with the potent senescence inducers, alisertib, etoposide, or doxorubicin. Mice were sacrificed and tumor and TIME characterization were conducted. *In vitro* functional studies employed murine bone marrow (BM)-derived MDSCs treated with conditioned medium (CM) from proliferating or senescent. murine (SB) cells, then co-cultured with CD8 T cells isolated from murine splenocytes.
Results: Using IF, we demonstrated the majority of $p16^+$ and $p21^+$ senescent cells in human CCA are cancer cells, rather than stromal cells. Both $p16^+$ and $p21^+$ cancer cells were increased in tumors from patients who have undergone cytotoxic therapy with gemcitabine/cisplatin (Gem/Cis). *In vivo* senescence induction led to an increase in MDSC abundance and reduced cytotoxic T lymphocyte infiltration in mice. Incubation of BM-MDSCs with CM from senescent cancer cells enhanced MDSC viability and suppressive function. scRNA seq data demonstrated that *TRAIL*$^+$ human CCA cells have higher expression of a senescence signature compared to *TRAIL*$^-$ cells. Senescence inducing therapy also significantly increased *Trail* expression in murine tumors *in vivo*. Mechanistically, incubation of BM-MDSCs with senescent murine CCA cells with deletion of *Trail* significantly decreased MDSC viability.
Conclusion: we have demonstrated that senescent cancer cells accumulate in tumors from patients treated with standard-of-care therapy with Gem/Cis. Senescent cancer cells enhance the abundance and suppressive function of MDSCs via TRAIL. Thus, senolysis has the potential to rewire the TIME, and augment response to systemic therapy.

#2713 Characterization of GM-CSF and G-CSF expressing cell subtypes in the tumor microenvironment using the Integrated MultiOmyx-RNAscope assay.

Courtney Todorov, Kevin Gallagher, Victoria Huang, Harry Nunns, Marianne Thio, Eric Leones, Flora Sahafi, Erinn A. Parnell, Qingyan Au

NeoGenomics, Aliso Viejo, CA

Granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) are cytokines known to stimulate differentiation and activation of granulocytes, monocytes, macrophages, and dendritic cells (DCs) as well as non-immune cells including endothelial, epithelial and fibroblasts. GM-CSF and G-CSF can be produced by many different cell types, including immune cells, fibroblasts, endothelial cells and have also been shown to be upregulated in multiple human cancers. Studies have further demonstrated the immune-modulatory effects of GM-CSF/G-CSF can promote both anti-tumorigenic or pro-tumorigenic activity through regulation of neutrophils/M1 macrophages/T-effectors or myeloid-derived suppressor cells (MDSCs)/M2 macrophages/Tregs, respectively. Therefore, modulators to promote or reduce the activity of GM-CSF/G-CSF have been explored for tumor therapeutic intervention, often in combination with other therapies such as chemotherapy. However, to further dissect and target anti or pro-tumor GM-CSF/G-CSF modulation requires a better understanding of the distribution and identity of GM-CSF/G-CSF secreting cells within the tumor micro-environment (TME). To characterize GM-CSF/G-CSF expression in the TME, we use the Integrated MultiOmyx-RNAscope platform. MultiOmyx^{TM} (NeoGenomics Laboratoies, Inc) is a proprietary multiplex immunofluorescence (mIF) platform for the visualization and characterization of up to 60 protein biomarkers in a single formalin-fixed paraffin-embedded (FFPE) section and offers high-resolution spatial and quantitative analysis of protein expression in tissue samples. RNAscope^{TM} (Bio-Techne) Multiplex is a highly sensitive fluorescent in-situ hybridization (ISH) assay that can detect up to 3 RNA markers in a single FFPE section. The Integrated MultiOmyx-RNAscope assay allows for simultaneous detection of both protein and RNA markers in a single sample. Herein we report the design and use of a novel panel of commercially-available RNAscope ISH probes and IF antibodies broad enough to characterize GM-CSF and G-CSF subpopulations in the TME of a variety of tumor indications including lung, prostate, melanoma, and colon cancer. Using this panel in combination with proprietary deep-learning based image analysis, GM-CSF and G-CSF expressing cells can be characterized into different immune and TME subtypes. Understanding of the variety and phenotype of GM-CSF and G-CSF expressing cells in the TME will help improve the understanding and development of targeted therapies for cancer treatment.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Epigenetic Mechanisms and Therapeutic Implications in Cancer Poster Session

#2716 Role for KMT2C mutation in endometrial cancer pathogenesis.

Juveria Ali[1], Swornalata Pukhrambam[1], Sophia Agrusa[1], Areebah Qazi[1], Jessica Back[1], Greg Dyson[1], Michele L. Cote[2], Anna Gottschlich[1], Mike R. Wilson[1]

[1]Wayne State University, Detroit, MI,[2]Indiana University, Indianapolis, IN

Background: Endometrial cancer (EC) is the 4th most common cancer to be diagnosed in women. Although EC has reasonable survival rates, there is a disparity gap for African American (AA) women, as they are twice as likely to succumb to metastatic EC. They are also more likely to develop the more severe subtype, uterine serous carcinoma (USC), and high-grade EC. HER2-positive USC can be treated with Trastuzumab, but response varies. The KMT2C gene encodes a lysine methyltransferase involved in histone modification and transcriptional coactivation. Through a meta-analysis of multiple datasets, we identified KMT2C as highly mutated in High Grade Serous Endometrial Cancer (HGSEC) tumors from AA patients when compared to Non-Hispanic White patients. KMT2C was the top gene mutated when comparing differential mutation rates from this data.
Methods: To explore the relationship between KMT2C and EC, we transfected 12Z endometrial epithelial cells with KMT2C siRNA to induce transient knockdown and performed western blots with the transfected protein lysates. Additionally, we transfected cells with plasmids containing green fluorescent protein (GFP), Cas9, and 3 different guide RNAs targeting KMT2C to generate stable knockouts. Flow cytometry isolated the GFP+ cells. Using those isolated 12Z CRISPRCas9 knockouts, we performed a final western blot.
Results: KMT2C knockdown led to decreased HER2 expression. KMT2C knockdown increased Arid1a expression and minimally increased Snail and Slug expression. GFP expression confirmed successful transfection. CRISPR KMT2C guide 1 silenced some isoforms of KMT2C.
Conclusion: Patients who have KMT2C mutations would likely be less responsive to Trastuzumab due to reduced expression of HER2. Arid1a is mutated in about 10% of human cancers and Snail and Slug are markers of epithelial to mesenchymal transition (EMT), so their overexpression in the KMT2C knockdown may have serious implications. More testing should be done related to EC patients with KMT2C mutations and alternate treatment plans need to be explored for this vulnerable subpopulation.

#2717 Dual inhibition of PRC2 complex subunits EZH1/2 enhances DNA hypomethylation therapy in colorectal cancer.

Ashley K. Wiseman, Ali A. Chomiak, Rochelle Tiedemann, Yanqing Liu, Scott Rothbart

Van Andel Institute (VAI), Grand Rapids, MI

Aberrant DNA methylation patterning is an epigenetic hallmark of human cancers. DNA methyltransferase inhibitors (DNMTi) act by reversing these aberrant DNA methylation patterns and are clinically approved to treat hematologic cancers. However, there is limited evidence for clinical efficacy in solid tumors. Our prior work showed that compensatory transcriptional silencing through the Polycomb Repressive Complex 2 (PRC2) emerges following DNMTi to maintain epigenetic silencing of critical tumor suppressor gene networks. This provides mechanistic rationale for combining DNMTi with inhibitors of EZH2 (EZH2i), the most well studied lysine methyltransferase (KMT) subunit of PRC2 that catalyzes all three orders of lysine 27 methylation on histone H3 (H3K27me1/2/3). EZH1 is an interchangeable KMT subunit of the PRC2 complex whose role in cancer is less well studied. A small molecule screen of PRC2 inhibitors revealed that compounds targeting both EZH1 and EZH2 were more effective in combination with DNMTi than those that were selective for EZH2. Integrative proteomics and epigenomics analyses revealed a mechanism in which H3K27me1, supported by EZH1, blocks the activity of the lysine acetyltransferases p300/CBP towards the H3K27 substrate, which is critical for transcriptional activation downstream of DNMTi and EZH1/2i treatment. Collectively, these studies are defining the division of labor between the two KMT subunits of PRC2 in colon cancer cells, uncovering mechanisms of crosstalk between DNA methylation, histone methylation, and histone acetylation, and revealing therapeutic vulnerabilities that we propose are targetable with dual EZH1/2 inhibitors in combination regimens to enhance the clinical efficacy of DNMTi therapy.

#2718 Investigating the interaction of NF1 with the Polycomb Repressive Complex 2.

Asra Noor, Peng Zhang, Pinpin Sui, Feng-Chun Yang

University of Texas Health Science Center at San Antonio, San Antonio, TX

Neurofibromin 1 (NF1) is a tumor suppressor gene frequently mutated in myeloid leukemias. The encoded neurofibromin protein negatively regulates the RAS pathway in the cytosol. Inactivating mutations in NF1 lead to hyperactive RAS signaling, phenocopying activating mutations in RAS itself. This aberrant signaling drives downstream activation of pathways such as MAPK and PI3K/Akt/mTOR. *Despite the prevalence of NF1-mutated leukemia, effective therapies remain elusive, highlighting the need for a deeper understanding of NF1-mediated leukemogenesis*. To identify key dysregulated genes in NF1-driven leukemogenesis, we performed bulk RNA sequencing on hematopoietic stem cells from Nf1 knockout mice exhibiting myelodysplastic syndrome. Gene set enrichment analysis revealed significant dysregulation of pathways involved in histone methylation and RNA modification. Etv4, Id2, and Bcl6 were among the top upregulated genes, although their dysregulation is likely secondary to RAS activation. To uncover novel, non-canonical targets of NF1, we identified Phf19 as a dysregulated gene not directly regulated by RAS. PHD finger protein 19 (PHF19) is a component of the Polycomb Repressive Complex 2 (PRC2), a complex that mediates histone H3 lysine 27 trimethylation (H3K27me3) through the catalytic activity of EZH2. T*his finding is particularly intriguing as previous studies have suggested nuclear localization of neurofibromin, but its function in the nucleus has remained unclear.* To explore the potential nuclear role of neurofibromin, we employed TurboID proximity labeling to identify its interaction partners in both the nucleus and cytoplasm. We found that neurofibromin interacts with the core PRC2 components EZH2 and SUZ12 in the nucleus, a finding confirmed by co-immunoprecipitation. Ongoing studies aim to elucidate the functional consequences of Phf19 upregulation and the impact of the neurofibromin-PRC2 interaction on apoptosis, cell cycle progression, and proliferation in Nf1 knockout mouse model.

#2719 Decoding role of KMT5C-H4K20me3 in EGFRi resistance in non-small cell lung cancer.

Jihye Son[1], Alexandra Glaws[1], Christina Davidson[2], Ching-Hua Shih[2], Paula Vertino[2], Andrea Kasinski[1]

[1]Purdue University, West Lafayette, IN,[2]Wilmot Cancer Center, Rochester, NY

Epigenetic dysregulation has recently been recognized as a new hallmark of cancer, with dysregulation of chromatin remodelers estimated to affect 10-20% of cancer. Thus, understanding how epigenetic-based mechanisms contribute to cancer initiation and progression is critical. KMT5C is a histone methyltransferase that catalyzes histone H4 lysine 20 trimethylation (H4K20me3), which has historically been associated with formation and maintenance of constitutive heterochromatin regions. Perturbations in KMT5C-H4K20me3 in cancer has been extensively characterized, including our own work in which we discovered that loss of KMT5C promotes development of resistance to EGFR inhibitor (EGFRi) in EGFR-mutant non-small cell lung cancer (NSCLC) cells. Patient data further supports that the KMT5C transcript level is downregulated following resistance to osimertinib, a third-generation EGFRi. Here, we present data to support a non-canonical role for H4K20me3 where it regulates genes outside of heterochromatin. Notably, deposition of H4K20me3 in these regions occurs without the need for H3K9me3, which was thought to be a prerequisite for H4K20me3 deposition. This uniquely deposited H4K20me3 is associated with pathways such as DNA replication and cell cycle transition. H4K20me3 also co-localizes with activating histone modifications including H3K4me3, requiring additional studies to evaluate how dysregulation of H4K20me3 is involved in transcriptional programming in NSCLC to promote EGFRi resistance. Finally, we identifed the KMT5C interactome, which reveals novel interacting partners of KMT5C, potentially uncovering mediators of KMT5C dysregulation. Taken together, our findings unveil novel roles of KMT5C-H4K20me3 in regulating chromatin architecture and suggest etiological mechanism of KMT5C-driven EGFRi resistance in NSCLC.

#2720 Investigating the functional role of SUV420H1 in HPV-negative head and neck squamous cell carcinoma.

Arfa Moshiri[1], Sohyoung Kim[2], Marie Luff[1], Vassiliki Saloura[1]

[1]Thoracic and GI Malignancies Branch, National Cancer Institute, Bethesda, MD,[2]Laboratory of Receptor Biology and Gene Expression, National Cancer Institute, Bethesda, MD

Human Papilloma Virus (HPV)-negative head and neck squamous cell carcinoma (HNSCC) remains a significant clinical challenge due to limited treatment options and poor prognosis. *SUV420H1*, encoding a protein lysine methyltransferase (PMT) responsible for H4K20 trimethylation (H4K20me3), is recurrently amplified in approximately 5% of HPV-negative HNSCC tumors, indicating a potential oncogenic role. Our previous studies demonstrated that while SUV420H1 depletion using siRNAs reduced the proliferation and colony-forming capacity in HPV-negative HNSCC cell lines, enzymatic inhibition with the SUV420H1 inhibitor A-196 induced only a modest effect in these phenotypes. In this study, we generated enzymatically inactive SUV420H1 mutants with a confirmed decrease in global H4K20me3 levels and conducted proliferation and colony-forming assays which concordantly showed only a modest effect in these phenotypes. GSEA analysis of RNA-seq obtained from HPV-negative HNSCC cells treated with SUV420H1 siRNAs or the SUV420H1 inhibitor A-196 showed commonly enriched pathways, including EMT, cell cycle and proliferation, and interferon alpha response pathways. In vivo experiments using Suv420h1 knockout (KO) MOC1 mouse cell lines in a syngeneic HPV-negative HNSCC flank mouse model demonstrated a marked decrease in tumor size. Furthermore, tumor flow cytometry revealed increased CD8+ T-cell, B-cell and macrophage infiltration. These findings provide strong evidence for the role of SUV420H1 in modulating immune responses. Ongoing studies aim to evaluate the combinatorial impact of SUV420H1 depletion and anti-PD-1 therapy in vivo to determine whether SUV420H1 loss sensitizes tumors to immune checkpoint blockade. Collectively, our data supports SUV420H1 as a potential therapeutic target in HPV-negative HNSCC.

#2721 Mechanism Studies of HDACi combined with SP/KLF inhibitors in the treatment of H3K27M-mutant diffuse intrinsic pontine gliomas.

B. Wang[1], Y. Kong[2], X. Lv[2], H. Zhuang[3], X. Wu[2];
[1]UCSF, San Francisco, CA, [2]Tianjin Medical University, Tianjin, China, [3]UC San Diego, San Diego, CA

Diffuse intrinsic pontine gliomas (DIPGs) pose significant challenges in pediatric oncology with limited treatment options. Frequently harboring H3K27M mutations, these tumors are insensitive to histone deacetylase inhibitors (HDACi) in clinical trials for yet unclear reasons. Here we find that HDACi induce histone hyperacetylation including H3K27 acetylation, and increased accessibility at genomic regions linked to tumor microenvironment adaptation and invasiveness. And these regions gain occupancy of the SP/KLF family of transcription factors. Encouragingly, targeted intervention with EC-8042 effectively reverses this activated regulatory network by HDACi, thereby mitigating tumor adaptation and invasiveness. Furthermore, the combination of EC-8042 with HDACi enhances the repression of transcription in cell cycle-associated genes, leading to the inhibition of H3K27M-DIPG cell proliferation and impeding tumor progression in orthotopic xenograft models. Transcriptomic analysis further supports that the combination treatment drives transcriptional programs correlating with favorable prognosis in DIPG patients. Therefore, employing DIPG models, this study provides critical mechanistic understanding of the limited responsiveness to HDACi in solid tumors, thereby laying the groundwork for the innovation of novel combination strategies.

#2722 An added twist: drug-mediated pan-inhibition of KDM catalysis causes selective reduction of mRNAs that encode a small subset of KDM enzymes.

Hartmut Martin Hanauske-Abel[1], Mainul Hoque[1], Seema Husain[1], Sukhwinder Singh[1], Axel R. Hanauske[2], Patricia Soteropoulos[1]

[1]Rutgers New Jersey Medical School, Newark, NJ,[2]Technical University, Munich, Germany

Background: The class of 2-oxoglutarate-requiring mono-iron dioxygenases (MIDOs) that demethylate specific lysine, histidine, or arginine residues of histones is receiving attention for its role in human diseases, in particular cancer (10.1016/j.ejmech.2022.114143). The steric events during MIDO catalysis were resolved at subatomic scale in 1982 by two students at Philipps University, Marburg/Germany (HAG mechanism [10.1016/0022-5193(82)90320-4, 10.1016/0022-5193(82)90320-4]). They presented a classification of all *conceivable* inhibitors and rapidly reduced their seminal approach to practice, establishing pyridine dicarboxylates and oxalylglycines as MIDO inhibitors, now in global use. In 1995, Cornell researchers employed HAG metrics to identify, among FDA-approved drugs, deferiprone (DFP) as MIDO inhibitor, enabling control of key protein families *in vitro* and *in vivo* (e.g. US-6080766-A). In 2019, DEF was identified as pan-selective inhibitor of histone lysine demethylating MIDOs (KDMs [10.1038/s41598-019-39214-1]). KDMs have, beyond their enzymatic activity, critical non-enzymatic functions in the physical assembly of the transcriptional machinery (10.3390/ijms25136900). We noted that DFP not only inhibits key MIDOs at clinically relevant serum concentrations, but also decreases selectively their encoding mRNAs, a precondition for reducing their biosynthesis.

Hypothesis: DEF not only inhibits KDM catalysis pan-selectively, but also reduces the biosynthesis of particular KDMs via knockdown of their mRNAs.

Methods: Cell culture of ARK1; repeated, timed (12/24/48 hrs) RNA-Seq using clinical concentrations of DFP (and deferoxamine [DFOX] to test for iron depletion); flow cytometry for MIDO-dependent proteins (collagens, collectins, fibrillins) to confirm biologically effective intracellular/intracompartmental concentrations of DFP.

Results: mRNAs encoding 15 histone lysine demethylating MIDOs were detected. 11 mRNAs for KDMs were expressed irrespective of time point monitored, duration of DFOX (15 µM) or DFP (100 and 150 µM) exposure, or DFP concentration. These non-reactive mRNAs were: KDM2A, 3B, 4A, 4C, 4D, 5A-C, 6A, 7A, 7C. The expression of KBM2B, KBM3A, KBM4B, and KBM6B increased at least 1.8 fold between 12 and 48 hrs. (FDR p <0.00000) in controls, with marginal effect of DFOX. By contrast, DFP uniformly abolished this rise of expression, causing 2-fold reductions of the four mRNAs relative to controls at 48 hrs. The slopes of the dose-response curve changed markedly, e.g. from +3 in control to below -0.5 for KBM2B, KBM3A, and KBM4B on DFP. Remarkably, at 12 hrs. DFP *increased* their mRNAs distinctly above controls, e.g. doubled KDM3A expression. The disruptive DFP effects were fully evident at 100 µM.

Conclusions: Drug inhibition of KDM catalysis can reduce KDM expression, thus also affecting non-enzymatic KDM functions.

#2723 Unveiling epigenetic mechanisms driving RasGRP3 upregulation via oncogenic Gαq signaling in uveal melanoma.

Zaizhou Liu, Boris Bastian, Xu Chen

UCSF - University of California San Francisco, San Francisco, CA

Uveal melanoma (UM) is a rare but aggressive cancer originating from melanocytes within the eye. It is distinct from cutaneous melanoma (CM) and genetically defined by somatic mutations activating the Gαq signaling pathway, most commonly affecting the Gαq subunits GNAQ or GNA11. UM remains a highly lethal melanoma subtype that cannot be effectively treated by current treatment regimens, including immune checkpoint blockade and targeted therapies. RasGRP3 (RAS Guanyl Releasing Protein 3), a guanine nucleotide exchange factor (GEF) for Ras, is markedly (>100-fold mRNA levels compared to CM) and selectively upregulated in UM and serves as a critical signaling hub connecting Gαq signaling to the MAPK pathway. Elucidating the mechanisms underlying the RasGRP3 upregulation unique for UM promises to unveil alternative therapeutic targets. We compared the epigenetic landscape of the RasGRP3 locus in UM and CM cells using ChIP-seq for H3K27ac (marking active enhancer/promoters), H3K4me1 (active enhancers), and H3K4me3 (active promoter). In UM but not CM cells, the RasGRP3 locus was highly enriched for H3K27ac and H3K4me1 modifications, and two of its three transcription start sites were marked with elevated H3K4me3 levels, indicating the expression of two major isoforms in UM. Treatment of UM cells with the Gαq inhibitor YM-254890 (YM) or the PKC inhibitor LXS-196 (LXS) for 24 hours significantly reduced all three histone modifications (H3K4me3, H3K27ac, H3K4me1) at the RasGRP3 locus. No changes were observed in CM cells. Our findings indicate an active super-enhancer that regulates RasGRP3 expression in UM, which is under control of the Gαq pathway through epigenetic modifications at its promoter and enhancer regions. These findings explain the selective upregulation RasGRP3 characteristics of UM. Ongoing investigations aim to delineate the precise mechanisms by which Gαq signaling drives these epigenetic changes. Our results highlight the potential of targeting specific histone modifiers as a novel therapeutic strategy for UM treatment.

#2724 TGF-β signaling activity induces co-localization of both H4K20me3 and H3K27Ac at active enhancers during breast cancer epithelial-to-mesenchymal transition.

Bachelard Dieujuste, Christina Davidson, Ching-Hua Shih, Laura McLane, Paula Vertino

Biomedical, Genetics and Genomics, University of Rochester School of Medicine & Dentistry, Rochester, NY

In 2022, 2.3 million women worldwide were diagnosed with breast cancer, and more than670,000 breast cancer-related deaths were reported. A leading contributor to breast cancer mortality is metastatic progression driven in part by the epithelial-to-mesenchymal transition (EMT). EMT is a reversible plastic state during which cancer cells spread beyond their primary environment. This plasticity in cell states can be mediated by epigenetic factors that influence gene expression through the regulation of chromatin accessibility. Our lab and others found that inhibition of Suv420H2/KMT5C, the methyltransferase that tri-methylates lysine 20 of histone H4 (H4K20), is typically associated with gene repression that promotes EMT in breast epithelial cells, suggesting that Suv420H2-mediating H4K20me3 plays a role in regulating epithelial vs. mesenchymal identity. To further dissect the role of H4K20me3 in EMT plasticity, MCF10Aimmortalized breast epithelial cells were treated with transforming growth factor-β1 (TGF-β1); a growth factor that acts as a tumor suppressor by limiting cell proliferation but can also promote tumor progression by driving EMT. Using a TGF-β treatment, recovery, and rechallenge approach followed by RT-qPCR analysis, I confirmed that TGF-β triggers EMTs by upregulating mesenchymal marker genes, such as EMT-transcription factors (ZEB1 and SNAI1/2), and downregulating epithelial marker genes (E-cad and EpCAM). In addition, cells gain a fibroblast-like morphology. TGF-β-induced EMT was reversible in response to TGF-β withdrawal during which cells regain their baseline morphology and re-express epithelial marker genes, showing a plastic state behavior. Additionally, we performed both a Cleavage Under Targets and Tagmentation and a Precision Run-On Sequencing on MCF10A cells to identify H4K20me3 distribution and nascent transcription activity. Collected data have revealed TGF-β-induced regions that gain H4K20me3, and these regions also acquire histone H3 lysine 27 acetylation (H3K27Ac), an epigenetic mark associated with active promoter and enhancer regions in response to TGF-β. TGF-β-induced H4K20me3 and H3K27Ac co-localization sites are transcriptionally active and distal to transcriptional start sites, suggesting that they may be active enhancers. The significant accumulation and co-localization of both H4K20me3 and H3K27Ac at active enhancers in response to TGF-β suggests that H4K20me3 may have additional functions, beyond that of its known role as a repressor. A better understanding of the role of TGF-β-induced H4K20me3 sites at active enhancers in the context of EMT will provide insight to better target epigenetic modifiers to prevent breast cancer progression.

#2725 Noncanonical regulation of genes by KMT5C-H4K20me3 in EGFR inhibitor resistant non-small cell lung cancer.

Alexandra Glaws, Jihye Son, Andrea Kasinski

Purdue University Institute for Cancer Research, West Lafayette, IN

Lung cancer is the leading cause of all cancer-related deaths, with non-small cell lung cancer (NSCLC) making up ~85% of lung cancer cases. Activating mutations in the epidermal growth factor receptors (EGFR) are associated with NSCLC development, comprising ~32% of patients. Standard treatment for NSCLC patients involves targeting EGFR mutations with EGFR inhibitors (EGFRi), yet most patients develop resistance to EGFRi over time. Approximately 55% of the mechanisms involved in EGFRi resistance involve EGFR T790M secondary mutations; however, nearly 15% of these mechanisms are unknown. We have previously shown that the loss of KMT5C drives resistance to EGFRi in EGFR-mutant NSCLC cells. Patient data further supports that the KMT5C transcript level is downregulated following resistance. KMT5C is a histone methyltransferase that catalyzes the trimethylation of H4K20 (H4K20me3), a repressive modification historically known for the involvement of heterochromatin formation and maintenance. KMT5C recruitment through H4K20me3 to heterochromatic regions is a well-known mechanism occurring in H3K9me3-dependent manner. Our preliminary data also suggests KMT5C-H4K20me3 is associated with the regulation of gene-rich euchromatin regions; yet the mechanism of recruitment remains unknown. We hypothesize that recruitment of KMT5C to euchromatin is distinct from the mechanism involved in KMT5C recruitment to heterochromatin. Here, we study the interactome of KMT5C using an unbiased proximity labeling technique, BioID, and aim to identify factors that form a novel complex with KMT5C to enable the recruitment of KMT5C to its target genes in euchromatic regions that drive resistance to EGFRi.

#2726 A circadian regulator drives tumor lineage plasticity through chromatin bivalency resolution.

Y. Yang[1], X. zhang[1], h. zou[1], E. Corey[2], R. Evans[3], A. Zoubeidi[4], H. Chen[1];
[1]UC Davis, Sacramento, CA, [2]University of Washington, Seattle, WA, [3]Salk Institute for Biological Sciences, La Jolla, CA, [4]University of British Columbia, Vancouver, BC, Canada

Chromatin bivalency plays an important role in cell lineage specification during development. Cancer therapy resistance involves an extensive reprogramming of gene expression resulting in tumor lineage plasticity (LP). However, the underlying mechanisms are poorly understood. Rev-erb-alpha, a member of the nuclear receptor transcription factor family, plays a key role in regulation of circadian rhythm and metabolism primarily through repression of its target genes. We performed sequential ChIP-seq epigenome profiling of tumor tissues from the clinic and PDX models of prostate cancer and integrated analysis of tumor epigenome and transcriptome. We also treated the PDX models with small-molecule inhibitors of the receptor. We found that most of the tumor LP genes, including those in programs of neurodevelopment, neural signaling, stemness and EMT, are controlled by bivalent promoters with concurrent marking of H3K27me3 and H3K4me3. Our further studies revealed that the bivalent chromatin state resolves during the development of cancer therapy resistance. Interestingly, Rev-erb-alpha plays a critical role in promoting the bivalency resolution and activation of the LP programs. Its pharmacological targeting effectively reverses the bivalency resolution and diminishes the LP in anti-AR therapy-resistant tumors. Therefore, our study establishes that resolution of chromatin bivalency is a major epigenetic mechanism of tumor LP and demonstrates that therapeutic targeting of Rev-erb-alpha represents a novel treatment of advanced prostate cancer.
Keywords: Lineage plasticity; Bivalent chromatin; Rev-erb-alpha; Prostate cancer.
This work was supported by NIH and UC Davis comprehensive cancer center and U.S. Department of Defense.

#2727 Histone deacetylase (HDAC1) and IKAROS are critical regulators of histone methylation and epigenomic landscape in T-cell acute lymphoblastic leukemia.

Jiaxing Tan[1], Yali Ding[1], Bing He[1], Daniel Bogush[1], Joseph Schramm[1], Chingakham Singh[1], Diwakar Tukaramrao[1], Charyguly Annageldiyev[2], Avinash Kudva[1], Dhimant Desai[3], Arati Sharma[3], Sinisa Dovat[1]

[1]Department of Pediatrics, Penn State College of Medicine, Hershey, PA,[2]Department of Medicine, Penn State College of Medicine, Hershey, PA,[3]Department of Pharmacology, Penn State College of Medicine, Hershey, PA

Objective: To dissect the role of IKAROS-HDAC1 complexes in global regulation of gene expression and chromatin landscape in T-ALL.

Methods: We studied IKAROS and HDAC1 role in epigenomic regulation of gene expression in primary human T-ALL, *IKZF1*-null human T-ALL, *Ikzf1*-null mouse T-ALL cells and following IKZF1-re-introduction into IKZF1-null human and mouse T-ALL cells. We used ATAC-seq and ChIP-seq of IKAROS, HDAC1, Enhancer of zeste homolog 2 (EZH2), along with CUT&Tag of histone modification markers, coupled with RNA-seq to study dynamic effect of IKAROS in *IKZF1*- null T-ALL and at several time points following *IKZF1* re-introduction.

Results: Deletion of IKZF1 results in severely reduced HDAC1 DNA occupancy and reduced H3K27me3 enrichment. Re-introduction of *IKZF1* results in the increased HDAC1 recruitment to promoters, EZH2 re-distribution, and de novo formation of H3K27me3 facultative heterochromatin. This was associated with cessation of cellular proliferation and induction of T- cell differentiation. The newly-formed heterochromatin was often located at promoters of known oncogenes and the genes that promote cell cycle progression. In contrast, IKAROS binding to DNA, without concomitant HDAC1 recruitment results in increased H3K27 histone acetylation with de novo formation of active enhancers, characterized with H3K4me1+/H3K27ac+ and super- enhancers, with a broad enrichment of histone H3K27 acetylation across the large genomic areas. Pathway enrichment analysis showed that newly-formed active enhancers positively regulate genes that are involved in cell cycle suppression and T cell differentiation.

Conclusion: Our results demonstrated that IKAROS and HDAC1 are the key regulators of histone acetylation and epigenomic landscape in T-ALL. HDAC1 recruitment by IKAROS is critical for formation of facultative heterochromatin, while IKAROS DNA binding without HDAC1 promotes of histone H3K27 acetylation. Results provide novel insight into mechanisms of regulation of histone acetylation, gene expression and tumor suppression in T-ALL.

#2728 Lysine acetyltransferase inhibitor MG149 reduces tumor recurrence in triple-negative breast cancer.

Christiana O. Appiah[1], Manjulata Singh[1], Christiane Morecock[1], Aashka Shah[1], Abigail Andrews[1], Nasser Alabdulaly[1], Akash Jagdeesh[1], Sidney Umana-Guardado[1], Jonathan Yun[1], Laura Graham[2], Carson Walker[3], Madelyn Lorenz[4], Shengzhe Shang[3], Anthony Faber[5], Andrew Souers[6], Dipankar Bandyopadhyay[7], Steve Grant[8], Gordon Ginder[3], Jennifer Kobilinski[4], Harry D. Bear[2], Joshua C. Harrell[3], Joseph W. Landry[1]

[1]Department of Human and Molecular Genetics, Virginia Commonwealth University - VCU, Richmond, VA,[2]Departments of Surgery and Microbiology & Immunology, Virginia Commonwealth University - VCU, Richmond, VA,[3]Department of Pathology, Virginia Commonwealth University - VCU, Richmond, VA,[4]VCU OVPRI, Virginia Commonwealth University - VCU, Richmond, VA,[5]Department of Oral and Craniofacial Molecular Biology, Philips Institute for Oral Health Research, Virginia Commonwealth University - VCU, Richmond, VA,[6]AbbVie Inc, Chicago, IL,[7]Department of Biostatistics, School of Population Health, VCU Massey Comprehensive Cancer Center, Richmond, VA,[8]Department of Internal Medicine, Virginia Commonwealth University, Massey Cancer Center, Richmond, VA

Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer characterized by the absence of estrogen receptor, progesterone receptor, and HER2 expression. This lack of targetable receptors makes TNBC particularly challenging to treat. Identifying therapeutic targets which can be targeted to prevent TNBC recurrence is crucial for developing better treatments. By studying mechanisms driving recurrence we hypothesized that post-therapy changes in cancer cell epigenetics contribute to recurrence and that using epigenetic drugs to inhibit these changes could reduce the likelihood of relapse and improve patient outcomes. Thus, the aim of this study was to identify novel epigenetic regulators of tumor recurrence in TNBC. Using TNBC cell line MDA-MB-231, we generated a model of recurrence using Doxorubicin (Doxo). We performed a drug screen using commercially available epigenetic inhibitors *in vitro* and *in vivo* to identify novel epigenetic regulators of tumor recurrence. Cell count (*in vitro*) and tumor volumes (*in vivo*) were used to determine the anti-tumor effects. Weight loss was used as an index of toxicity in mice. Supporting our hypothesis, we observed notable differences in the levels of various epigenetic regulators before and after Doxo treatment and during recurrence. Additionally, we discovered that certain epigenetic drugs could prevent recurrence in culture, but only when administered sequentially following Doxo exposure, and not when used in prepriming or in combination. Sequential administration of the KAT inhibitor MG149 effectively controlled tumor growth in NSG mice with MDA-MB-231 and PDX1 tumors. MG149 was also found to enhance the effectiveness of other standard chemotherapy drugs, including paclitaxel and cyclophosphamide. *In vitro* and *in vivo* studies showed that sequential use of MG149 with Doxo increases DNA damage (gamma H2AX) and apoptosis (annexinV/7AAD) and significantly reduces the expression of cell cycle inhibitors p21, p27, and p53. These findings suggest a model where suppression of cell cycle inhibitors and persistent DNA damage post-therapy exposure with MG149 leads to increased apoptosis, reduced recurrence from therapy, and enhanced tumor growth control. Our findings indicate that new epigenetic states are formed following chemotherapy exposure, which can be therapeutically targeted to prevent recurrence in TNBC.

#2729 Investigating the interplay between RNA methyltransferase PCIF1 and DNA hypomethylating drug decitabine in *TP53*-mutated acute myeloid leukemia.

L.-Y. Shiau[1], P.-S. Ko[2], K.-J. Chang[2], S.-H. Chiou[2];

[1]National Yang Ming Chiao Tung University, Taipei City, Taiwan, [2]Taipei Veterans General Hospital, Taipei City, Taiwan

PCIF1 is an RNA methyltransferase that interacts with RNA polymerase during transcription and catalyzes N^6,2'-O-dimethyladenosine (m6Am) modification on nascent mRNA, Decitabine (DAC), a DNA hypomethylating drug treating acute myeloid leukemia (AML), would activate hypermethylated genes in a way of non-specific action, which might inadvertently promote the expression of anti-apoptosis gene, leading to drug resistance. In this study, we unveil the role of the RNA modification m6Am and its writer protein PCIF1 in modulating AML's sensitivity to DAC. Utilizing KG-1a, a *TP53*-mutated AML cell line, we confirmed that the overexpression of catalytically active PCIF1 sensitizes leukemic cells to DAC treatment. This is evidenced by a higher apoptosis rate, increased S phase arrest observed through propidium iodide (PI) staining, and significant DNA demethylation on given genes detected via methylated DNA immunoprecipitation (MeDIP). Additionally, in terms of the second role of PCIF1 in this study, we noted elevated mRNA and protein levels of p21 in PCIF1 overexpressing cells, with p21 expression becoming even more pronounced following DAC treatment. RNA immunoprecipitation (RIP) confirmed increased direct binding of overexpressed PCIF1 to p21 mRNA. Correspondingly, there was no significant upregulation of well-established upstream regulators of p21, such as the p-Akt signaling pathway, DNA damage response cascade, or Sp1 protein. To elucidate the role of elevated p21 levels in modulating DAC's cytotoxic effects, we used Annexin-V-conjugated magnetic beads to separate dead and alive cells post-DAC treatment and found a skewed distribution of p21 in live cells. Moreover, treating PCIF1 overexpressing cells with a combination of DAC and UC2288, a p21 transcription inhibitor, proved more effective at eliminating leukemic cells than DAC alone. In short, we conclude that the second role of PCIF1 is promoting p21 expression through its catalytic function and further neutralizing the cytotoxicity effect of DAC. Nevertheless, the DAC-sensitizing pathway mediated by PCIF1 ultimately prevails, resulting in increased apoptosis in the PCIF1 overexpression model. Comparing the two axes, from a prognostic perspective for AML, high PCIF1 and low p21 expression levels appear to be more favorable. Clinical data from the TCGA LAML dataset supports this, showing significantly better survival rates in patients with high *PCIF1* and low *CDKN1A* (the gene name of p21 protein) expression compared to those with low *PCIF1* and high *CDKN1A* expression. These findings pave the way for clinical research to explore whether PCIF1 and p21 protein levels influence the response rate or prognosis of AML patients receiving DAC treatment.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Epigenetic Mechanisms of Cancer Resistance and Progression Poster Session

#2733 Super-enhancers in oncogene upregulation in high-grade ovarian serous carcinoma proliferation and platinum-resistance.

Zhongmou Jin, Kristin Adams, Kendra Wendt, Josie Wood, Sydney Olson, Jessica D. Lang

Pathology, University of Wisconsin-Madison, Madison, WI

The most common subtype of ovarian cancer, High-Grade Serous Ovarian Carcinoma (HGSC), bears an exceptionally high level of chromosomal instability including chromosomal recombination and copy number variation. Many oncogenes are amplified in HGSC, of which some of the most frequently examined ones include MYC, PAX8 and CCNE1. Although downstream pathways of those genes have been repeatedly studied, epigenetic features of many of them are relatively unknown, as they could involve mechanisms that additively and continuously promote oncogene expression that may contribute to the aggressiveness of HGSCs. In our study, we specifically focus on super-enhancers (SEs), large transcriptional hubs known to drive oncogene expression. We aim to assess whether SEs are hijacked coordinately with copy number alteration in HGSCs. We were able to apply a multi-omic approach that includes bulk RNA sequencing, histone post-translational modification CUT&Tag, and Hi-C analysis to not only identify SE regions in HGSC cell lines but link them to the genes they regulate. With integration of these layers of data, we show that there is a positive correlation between HGSC SEs and gene expression changes in HGSCs, in the context of both cancer growth as well as drug resistance acquisition. We observed that SEs are almost always statistically associated with higher gene expression of their target genes compared to enhancers. We also explored changes in SEs in the context of platinum resistance acquisition in our HGSC cell lines, with the observation that gain of new SEs are associated with increase in gene expression and vice versa. Together, these findings support the possibility that SEs could be critical for essential oncogene expression and changes that lead to drug resistance. We then explored if the copy number amplification (CNA) known associated with cancer genome also plays a role in SE regulation of oncogene expression. We found in almost all HGSC cell lines tested that compared to non-oncogene-related SEs, oncogene-related SEs are significantly more likely to be in copy number amplified regions of the genome ($p < 0.001$), rendering it a potential mechanism contributing to SE associated oncogene upregulation. Based on this result, we are launching screens for specific SE-oncogene interaction in locating functionally important epigenetic marks with CRISPRi, as well as to leverage our existing Hi-C contact data in combination with long-read sequencing to understand the chromatin architecture in the context of CNV.In summary, in this study we observed promising trends supporting SE hijacking in oncogene upregulation and CNA as a potential factor that contributes to SE regulation. We hope that understanding the mechanisms and context behind epigenetic regulation may lead to rational use of epigenetic inhibitors for cancers driven by copy number alterations to otherwise untargetable oncogenes.

#2734 Exploring epigenetic control of immunogenicity in small cell lung cancer through multi-omics analyses.

Sana Parveen[1], Ramesh Adhinaveni[1], Meijun Du[1], Neshatul Haque[2], Tianxiang Liu[3], Kun Fang[3], Raul Urrutia[2], Victor X. Jin[3], Hui-Zi Chen[1]

[1]Medicine, Medical College of Wisconsin, Milwaukee, WI,[2]Linda T. and John A. Mellowes Center for Genomic Sciences and Precision Medicine, Medical College of Wisconsin, Milwaukee, WI,[3]Data Science Institute, Medical College of Wisconsin, Milwaukee, WI

Background: Small Cell Lung Cancer (SCLC) is an aggressive neuroendocrine malignancy characterized by epigenetic dysregulation. EZH2, a key component of the Polycomb Repressive Complex 2 (PRC2), is overexpressed in SCLC and correlates with poor prognosis. Recent studies show that EZH2 inhibition enhances SCLC cell immunogenicity, though the underlying mechanisms remain unclear.

Aim: This study investigates how EZH2 function alters the epigenetic landscape in SCLC using an integrated multi-omics approach. In SCLC cell lines treated with EZH1/2 dual inhibitor valemetostat, we examine changes in chromatin accessibility (ATAC-seq), three-dimensional chromatin organization (micro-C profile), and gene expression (RNA-seq).

Key Findings: Gene set enrichment analysis of RNA-seq data revealed upregulation of genes involved in antigen processing, presentation, and inflammatory response pathways following EZH1/2 dual inhibition in SCLC cell line H146. ATAC-seq analysis showed increased chromatin accessibility of genes with increased expression, primarily in distal regulatory regions. Crucially, we addressed the limitation of ATAC-seq in providing only one-dimensional accessibility information by performing Micro-C profiling. This approach confirmed that EZH1/2 dual inhibition led to the formation of neo-loops between accessible promoters and their enhancers. Specifically, we observed gained loops leading to increased HLA-B and HLA-C expression with valemetostat treatment. Validating these findings at the transcriptional and protein levels, qRT-PCR and flow cytometry analyses confirmed increased surface expression of pan-HLA molecules following valemetostat treatment. Functional inhibition of EZH1/2 was confirmed by Western blot analyses showing decreased H3K27me3 levels. These results collectively demonstrate that EZH1/2 controls gene expression in SCLC by establishing long-range interactions between promoter and enhancer elements through modulation of chromatin accessibility, ultimately impacting the expression of key immunogenic factors.

Conclusion: Our integrative multi-omics approach reveals a comprehensive model of EZH1/2 contribution to SCLC pathogenesis, highlighting its role in chromatin reorganization and gene expression regulation. Future work will focus on identifying additional therapeutically relevant genes within the EZH1/2-regulated network for potential intervention strategies.

#2735 The role of ca2 in regulating 3d chromatin structure in tamoxifen resistant breast cancer.

Lavanya Choppavarapu, Kun Fang, Tianxiang Liu, Aigbe G. Ohihoin, Victor X. Jin

Medical College of Wisconsin, Wauwatosa, WI

Background: Estrogen receptor-positive breast cancer frequently develops resistance to tamoxifen in one third of patients, driven by various mechanisms. Our work has linked 3D chromatin alterations to this resistance. However, most of the work in 3D chromatin regulation was mostly conducted in in vitro cell culture system. It is becoming very clear that to expand the characterization of 3D genome organization in patient samples is an emerging task.
Hypothesis: We hypothesize that 3D chromatin alterations, including compartment switching, TAD reorganization, and dysregulated chromatin looping, drive tamoxifen resistance (TR) in breast cancer. Looping-mediated genes may play critical roles in resistance and serve as potential therapeutic targets.
Methods and Results: To investigate the role of 3D chromatin changes in tamoxifen resistance, we performed Hi-C profiling on normal tissues (NTs), primary tumors (PTs), and tamoxifen-treated recurrent tumors (RTs), uncovering significant chromatin alterations. These included 46.8% A-to-B and 32.8% B-to-A compartment switching from NTs to PTs, and 58.3% B-to-A flipping during PT-to-RT progression. TAD analysis identified RT-specific Neo and Fuse alterations as well as tumor-specific chromatin loops. CA2 was identified as a enhancer-promoter looping (EPL)-mediated gene with its higher expression linked to poor relapse-free survival in endocrine-treated patients. Functional studies using the CA2 inhibitor brinzolamide demonstrated its ability to reduce cell viability, loop intensity, and CA2 expression in MCF7TR and T47DTR cells. In vivo, brinzolamide significantly reduced tumor growth in tamoxifen-resistant cell-derived xenograft (CDX) models. Additionally, CRISPR deletion of CA2 EPL enhancer region in MCF7TR cells resulted in a marked reduction in cellular viability, gene expression and protein expression.
Conclusion: Our findings suggest CA2 as a key looping-mediated gene play an oncogenic role in driving tamoxifen resistance in breast cancer. Our study yields significant mechanistic insights into the role and clinical relevance of 3D chromatin architecture in tamoxifen resistant breast cancer.

#2736 Mutation of histone H2A at glutamate 121 is frequently observed in cancer patient samples and promotes cancer-associated cellular properties.

Richard L. Bennett, Jixiu Shan, Anthony J. Lamberto, Rishika Cherukuru, Kimberly Espinoza Pereira, Pierre Priam, Jonathan D. Licht

University of Florida Health Cancer Center and Department of Medicine, University of Florida, Gainesville, FL

Background: Histones are essential proteins that facilitate DNA compaction into nucleosomes and chromatin fibers. Their dynamics are crucial for various cellular processes, including regulation of gene expression, cellular differentiation, maintenance of cell identity, DNA replication and DNA repair. By surveying the cBioPortal of Cancer genomics we previously found that approximately 11% of cancer patients had alterations of histone H2A genes, primarily missense mutations of unknown significance. The most commonly mutated H2A residue was a glutamate at amino acid position 121. Prior work by others has demonstrated that mutation of H2A at E121 caused decreased ACF-dependent nucleosome sliding *in vitro* and impaired yeast growth.
Objective: To determine the effect of the histone H2A glutamate to lysine mutation at amino acid 121 (H2A-E121K) on gene expression and cell growth properties relevant to cancer.
Methods: We expressed HA-tagged wild type or mutant histone H2A-E121K at near physiological levels in the lung epithelial cell line BEAS-2B using a "minigene" construct which drove expression from a native H2A promoter and 3' UTR regulatory region. Using live cell imaging we determined the effect of H2A-E121K on cell proliferation, apoptosis and migration in normal serum free media or growth factor deprived conditions. We measured the rate of serum-induced EMT signatures by immunoblotting for proteins such as claudin and cadherins. We investigated how expression of H2A-E121K may alter chromatin accessibility and gene expression by ATAC-seq and RNA-seq.
Results: Expression of H2A-E121K promoted both proliferation and survival of lung epithelial cell line BEAS-2B when cultured in normal media or under growth factor deprived conditions. The H2A-E121K mutant promoted colony formation. Cells expressing H2A-E121K had altered chromatin accessibility and gene expression compared to cells expressing WT H2A.
Conclusions: Expression of the histone H2A-E121K mutation promotes cancer-associated cellular properties in lung epithelial cells.

#2737 Enhancing the efficacy of DNA methyltransferase inhibitors in colon cancer through dual targeting of EZH1 and EZH2.
Rochelle Tiedemann, Alison Chomiak, Ashley Wiseman, Yanqing Liu, Scott Rothbart

Van Andel Institute (VAI), Grand Rapids, MI

DNA methyltransferase inhibitors (DNMTis) have shown limited success in solid tumors, despite the prevalence of aberrant DNA methylation and associated tumor suppressor gene silencing. Colon cancer cells exposed to DNMTi accumulate lysine 27 tri-methylation on histone H3 (H3K27me3), and we and others have proposed this EZH2-dependent repressive histone modification limits DNMTi therapeutic potential. Recently we demonstrated that low-dose DNMTi treatment, which mimics solid tumor exposure in patients, sensitizes colon cancer cells to EZH2 inhibitors (EZH2is) and synergistically upregulates tumor suppressor genes and transposable elements (TEs). Importantly, the observed synergy varies among EZH2 inhibitors. Molecules targeting both EZH2 and its interchangeable catalytic counterpart, EZH1, demonstrate superior efficacy in tumor suppressor gene activation and cancer cell growth inhibition. In this study, we profiled the epigenomic landscapes that result from combination therapy with DNMTi and EZH1/2i to understand how these compounds alter the transcriptional states of solid tumors. We show that dual EZH1/2i reduces all states of H3K27 methylation (me1/me2/me3), providing additional substrates for p300/CBP deposition of H3K27 acetylation (H3K27ac) and enhancing transcriptional outcomes. Furthermore, H3K27ac is required for tumor suppressor gene activation observed with combination DNMTi and EZH1/2i treatment. These results underscore the therapeutic promise of combining DNMTi with dual EZH1/2i to reprogram the epigenetic landscape, restore tumor suppressor gene activity, and enhance the efficacy of epigenetic therapies in solid tumor treatment.

#2740 Mechanisms of phase-separation driven chromatin looping in acute myeloid leukemia.

Tooba Rashid, Zachary Drum, Doris Cruz Alonso, Gelila Petros, Yoseli Quiroga, Douglas Phanstiel

UNC Chapel Hill, Chapel Hill, NC

New therapies are desperately needed for NUP98-fusion-driven Acute Myeloid Leukemia (AML) patients as they exhibit a poor prognosis on existing therapies, with a 5-year overall survival rate of ~30%. We and others have shown that one fusion, NUP98-HOXA9 drives leukemogenesis through the formation of liquid-liquid phase separated (LLPS) condensates. Specifically, NUP98-HOXA9 binds DNA and pulls bound regions into the nuclear condensates, forming 3D chromatin loops that activate oncogenes' transcription. Mutation of the phenylalanine-glycine (FG) repeats responsible for NUP98-HOXA9 phase separation abolishes the formation of these condensates and inhibits malignant transformation. These findings suggest a new class of chromatin loops, that form via a mechanism distinct from loop extrusion and drive oncogene expression. We set out to characterize the necessary components for the formation and maintenance of these aberrant loops. Using an Auxin Inducible Degron (AID) system in HCT116 cells, we rapidly degraded loop extrusion proteins, CTCF and RAD21, followed by in situ Hi-C to observe impact on loop formation. We observe approximately 8,000 canonical loops, which are bound by CTCF and lost upon auxin treatment. Additionally, we identify approximately 60 phase-separation driven loops, bound by NUP98-HOXA9 which do not require CTCF. We additionally explore the role of ATP, BRD4, and p300 as requirements for the formation of these loops. Future work includes further characterization of loop maintenance and screening for compounds to disrupt condensate formation with the goal of using a deeper understanding of phase-separation driven loops to create new promising therapeutic avenues for NUP98-fusion-driven AMLs.

#2741 Novel murine models of epigenetic dysregulation in SWI/SNF-mutated cholangiocarcinoma.

Jayla Millender[1], Jennifer Tomlinson[2], Ryan Watkins[2], Danielle Carlson[1], Jack Sample[2], Nathan Werneburg[1], Rory Smoot[2], Keith Robertson[3], Gregory Gores[1], Caitlin Conboy[4]

[1]Department of Gastroenterology & Hepatology, Mayo Clinic, Rochester, MN,[2]Department of Surgery, Mayo Clinic, Rochester, MN,[3]Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN,[4]Division of Medical Oncology, Mayo Clinic, Rochester, MN

SWI/SNF chromatin remodeling complexes are frequently mutated across human cancers, including cholangiocarcinoma (CCA). Dysregulation of this complex due to loss of function mutations in AT-rich interaction domain-1A (*ARID1A)* and polybromo-1 (*PBRM1)* occur in up to 18% and 10% of cases respectively. SWI/SNF subunit mutations frequently co-occur alongside PI3K/AKT pathway activation. Preexisting literature suggests altered gene expression and protein synthesis contribute to CCA oncogenesis, but our understanding of these mechanisms remains immature. Genetically accurate preclinical models are needed to define the roles of ARID1A and PBRM1 in CCA and identify therapeutic targets. We developed novel murine models of CCA with either *ARID1A*, *PBRM1* or dual *ARID1A/PBRM1* (APAC) knockout in the context of AKT activation, utilizing a surgical biliary transfection method for somatic gene editing with CRISPR/Cas9. Within 8 months, mice formed numerous liver tumors, predominantly CCA and mixed CCA/HCC. Knockout of *ARID1A* and *PBRM1* was confirmed through genomic PCR, qRT-PCR, and immunoblot assays. Histologically, murine APAC tumors displayed phenotypic similarities to human CCA, including dense cancer-associated fibroblast infiltration and variable CD45+ immune cell infiltration. A novel, syngeneic CCA cell line was isolated from a dual *ARID1A/PBRM1* knockout tumor (APAC-2). RNA-sequencing of primary tumors and the APAC-2 cell line versus a non-invasive cholangiocyte cell line with AKT activation only (AC), was performed to identify differentially expressed genes (DEGs) of interest in ARID1A and PBRM1-deficient CCA. Periostin (*Postn)* was among the top upregulated genes in the APAC-2 cell line. POSTN is a secreted glycoprotein that is upregulated in human CCA and may be therapeutically targetable. Knockdown of periostin inhibited the *in vitro* growth of APAC-2 cells and a human CCA cell line with *ARID1A* mutation and periostin overexpression (HuH-28). These studies serve to characterize and validate a genetically relevant murine model of epigenetic dysregulation in ARID1A and PBRM1-deficient CCA. This model can be used for identification and validation of potential drug targets. Ongoing work focuses on studying multiple dysregulated pathways and testing dependence on genes of interest, including periostin, as potential treatment strategies for CCA.

#2743 The H2B-E76K oncohistone alters chromatin accessibility, composition, and gene expression to induce EMT.

Pierre Priam[1], Kimberly Espinoza Pereira[1], Jixiu Shan[1], Anthony Lamberto[1], Jay Sarthy[2], Wuyue Zhou[3], Ciaran Seath[3], Richard L. Bennett[1], Jonathan D. Licht[1]

[1]Division of Hematology/Oncology, University of Florida, Health Cancer Center, Gainesville, FL,[2]Seattle Childerns's Hospital, Seattle, WA,[3]Herbert Wertheim UF Health Scripps Institute for Biomedical Research, Jupiter, FL

Background: Histones are essential nucleosome components that regulate DNA accessibility and transcription. Mutations in histones, such as H2B-E76K, are prevalent in various cancers, including lung and genitourinary cancers. The H2B-E76K mutation destabilizes nucleosomes by disrupting H2A/H2B dimer binding to H3/H4 tetramers, altering chromatin structure and function.
Objectives: Investigate the effects of the H2B-E76K mutation on gene expression, chromatin interactions, signaling pathways, and cellular phenotypes, focusing on the induction of epithelial-mesenchymal transition (EMT) in lung cancer models.
Methods: CRISPR-Cas9 created BEAS-2B bronchial epithelial cell lines with heterozygous and homozygous H2B-E76K mutations in the H2BC4 gene. Phenotypic assays assessed cell growth, migration, invasion, and EMT marker expression. Transcriptomic changes were analyzed using scRNA-seq, bulk RNA-seq, and PRO-seq to evaluate pathway deregulation, transcriptional instability, and RNA polymerase activity. The µMap technique mapped protein-chromatin interactions disrupted by the mutation. Chromatin profiling with CUT&RUN and ATAC-seq examined histone incorporation and chromatin accessibility changes.
Results: H2B-E76K mutant cells showed enhanced migration and invasion, increased mesenchymal markers (N-cadherin, vimentin), and decreased epithelial markers (E-cadherin), consistent with EMT. These cells also resisted apoptosis upon growth factor withdrawal. Transcriptomic analyses revealed transcriptional instability, with increased cell-to-cell variability in gene expression, altered patterns linked to oncogenic signaling, transcript switching, and disrupted RNA polymerase activity. The µMap technique identified disrupted interactions between chromatin and RNA-processing enzyme RAVER1, correlating with altered splicing, exon skipping, and gene expression variability. ATAC-seq showed enhanced binding of EMT-related transcription factors (e.g., AP1, CEBPB, ZNF384, FOXC2) and increased chromatin accessibility at EMT-related promoters. CUT&RUN revealed diffuse genome-wide deposition of H2B-E76K histones, consistent with replication-coupled incorporation. These chromatin changes promoted transcriptional programs associated with EMT and cancer progression.
Conclusions: H2B-E76K histones are broadly distributed throughout the genome, causing transcriptomic instability, altered splicing, and transcription factor binding changes. These alterations drive EMT phenotypes, including enhanced invasion, migration, and resistance to apoptosis, contributing to aggressive cancer behavior. H2B-E76K acts as a key epigenetic driver of oncogenesis by modifying chromatin accessibility, gene regulation, and cellular plasticity.

#2744 Interrogating the functional roles of differential CTCF-binding sites in IDH-mutant gliomas using a novel CRISPR library.

Ebru Yilmaz[1], Alisan Kayabolen[2], Dogancan Ozturan[3], Tahsin E. Tekoglu[2], Ester Calvo Fernandez[1], Sedef Sarac Yalcinkaya[2], Nathan A. Lack[2], Mario L. Suva[1], Tugba Bagci Onder[2]

[1]Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA,[2]School of Medicine, Koc University, Istanbul, Turkey,[3]School of Biomedical Engineering, University of British Columbia, Vancouver, BC, Canada

Isocitrate dehydrogenase (IDH) mutations are frequently observed in gliomas, and their mutation status is accepted as a CNS tumor classification marker. IDHs are enzymes that convert isocitrate into alpha-ketoglutarate (α-KG). Mutations in IDHs cause neomorphic enzymatic activity, by which D-2-hydroxyglutarate (2- HG), the oncometabolite, is produced from α-KG. This leads to the inhibition of α-KG-dependent enzymes, such as DNA and histone demethylases, thus altering chromatin structure and gene expression profile. CTCF is a methylation-sensitive structural protein that recognizes specific DNA motifs. It dimerizes, together with cohesin, forms chromatin loops, and determines the boundaries of topologically associated domains. Previous work demonstrated that IDH-mutant gliomas have hypermethylation at CTCF-binding sites, leading to reduced CTCF binding and permitting a constitutive enhancer to interact aberrantly with a glioma oncogene. Despite the unique epigenetic features of IDH-mutant gliomas and the ever-increasing importance of IDH mutation in patient prognosis, there has been no comprehensive study on the functional roles of CTCF binding sites in these tumors.We reanalyzed publicly available CTCF ChIP-seq data of three IDH-wild type (wt) and three IDH-mutant glioma cell lines, determined differential CTCF ChIP-seq regions, and aimed to explore their functional roles in glioma survival. To investigate the effect of methylation and demethylation of a specific CTCF binding site in both IDH-wt and -mutant gliomas, we developed a novel paired gRNA library targeting the left and right sides of these differential regions, which consists of 899 paired gRNAs. The CRISPR droplet screens are being conducted using dCas9-fused DNMT3A&3 and TET1 effector proteins, causing DNA methylation and demethylation, respectively. Beyond the alterations of gRNAs in the population, the single-cell transcriptomic readout will be obtained at the end of the screen.Our library design offers advantages over available libraries as it can be utilized with different CRISPR approaches. This high-throughput epigenome editing approach will allow for identifying regulatory interactions leading to tumor cell survival in IDH-wild type and -mutant gliomas.

#2745 Identification of a selective and orally bioavailable BPTF PROTAC with anti-tumor activity in SWI/SNF mutant lung cancer.

W. Yan[1], J. Chung[1], D. E. Reyna[2], D. Sherman[2], S. Li[1], S. Linker[1], J. Lapek[1], S. Thomas[2], J. Pizzano[2], E. Bortolon[2], S. Brun[1], S. Kaiser[1], S. Sakata[1], I. Taylor[2], J. Cantley[2], A. Gilbert[1], T. Paul[1];
[1]Pfizer, Inc., San Diego, CA, [2]Arvinas, Inc., New Haven, CT

BPTF is a bromodomain and PHD finger domain-containing protein that serves as a scaffolding subunit of the ISWI family member NURF chromatin remodeling complex. Together with interchangeable catalytic subunits SMARCA1 and SMARCA5 and additional subunits RBBP4, RBBP7, the NURF complex catalyzes ATP to slide nucleosomes on DNA, exposing or occluding transcription factor binding sites, leading to transcriptional activation or repression. Dysregulation of BPTF-containing NURF complexes disrupts gene expression and contributes to oncogenesis. BPTF overexpression is observed in various cancers and correlates with poor survival. Here we report the discovery of potent and selective BPTF heterobifunctional protein degraders A123497 and A126329 utilizing BPTF bromodomain ligand NVS-BPTF-1. While BPTF bromodomain ligands are inactive across a panel of lung cancer cell lines, BPTF degraders demonstrate potent anti-tumor activity in a subset of SCLC and NSCLC models, recapitulating the genetic dependency observed in CRISPR inhibition studies. Anti-tumor activity of BPTF degraders exists in a subset of lung cancer cell line models enriched in mutations in the SMARCA2/4 catalytic subunit of SWI/SNF chromatin remodeling complex. BPTF degradation leads to loss of protein stability and chromatin binding to additional NURF complex members including catalytic subunits SMARCA1 and SMARCA5 and chromatin accessibility changes at specific genes. Unique chromatin accessibility changes observed with BPTF degraders in cell lines harboring mutant SWI/SNF suggest interplay between chromatin remodeling functions of NURF and SWI/SNF complexes. We present optimization of BPTF degraders for in vivo dosing further demonstrating the therapeutic potential for targeting BPTF-containing NURF complex in cancer.

#2746 3D genome structural alterations in biological pathway genes drive the progression of cells from normal epithelium to metastatic cancer.

Huy Nguyen[1], Shyamtanu Chattoraj[1], Jude Dunne[2]

[1]Bruker Spatial Genomics, Inc, Newton, MA,[2]Bruker Spatial Genomics, Inc, San Jose, CA

Large scale structural variation in the genome is known to be linked to cancer pathways. Breast cancer progression from normal epithelium to localized, and metastatic cancer involves genomic perturbations such as mutations, deletions, and duplications. Emerging research has shown that the structure of the 3D genome, specifically how chromosomes and genes are folded and organized in the nucleus, directly impacts gene expression and thus, may contribute to disease progression. In fact, local folding around critical genes, especially those involved in important biological pathways, may have direct impact on cancer initiation and progression. Limits in technology has restricted a more complete understanding linking 3D genome to disease. Biochemical methods require many cells and are unable to preserve single cell and sub-population level information. Previous imaging methods are limited by the number of analyzable targets, reducing the generalizability of results across other genomic loci and limited a comprehensive genome-wide understanding.

Here, we use jebFISH^{TM} and the PaintScape^{TM} platform to resolve 3D structural features of genomic loci at high fidelity. With our Biological Pathways Panel, we focus on genes that are known to play critical roles in DNA repair, cell cycle, apoptosis, chromatin modifications, and transcriptional regulation. Using breast cancer progression as the model, we apply jebFISH^{TM} to cell lines from normal epithelium, localized cancer, and metastatic cancer. We observe dramatic changes in 3D genome at both single cell and population levels. At each stage of breast cancer progression, we identify specific alterations to 3D genome features including changes in structure and switching of A/B chromatin compartments. Structural alterations are also present at whole chromosome level. We see disruption and repositioning of chromosome territories as well as interchromosomal interactions. jebFISH^{TM} single-cell, single-homolog resolution enables identification of cellular sub-populations at each stage of breast cancer progression. We observe changes in the frequency of sub-populations with disease progression. Our results suggest that genome organization, at the local gene as well as chromosome level is modulating the expression of genes important in biological pathways. The presence of sub-populations suggests that the heterogeneous nature of 3D genome organization within the population is an important feature, impacting our understanding and treatment of diseases. While this study focuses on breast cancer, we envisage that these results are generalizable and further the understanding of genome organization and gene expression.

#2748 Integrative analysis of 3D chromatin organization revealed regulation mechanisms in angioimmunoblastic T-cell lymphoma.

Makoto Iwasaki[1], Caroline MacVicar[1], Anna Baird Rider[1], Aliyah Sohani[1], Valentina Nardi[1], Josie Ford[1], Kusha Chopra[1], Eric Jacobsen[2], Jeffrey Barnes[1], Salvia Jain[1]

[1]Massachusetts General Hospital, Boston, MA,[2]Division of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA

Molecular studies have highlighted recurrent alterations in epigenetic modifier genes, including *TET2*, *DNMT3A*, and *IDH2,* in angioimmunoblastic T-cell lymphoma (AITL). However, the direct impact of gene regulatory elements on gene expression changes leading to T-cell lymphomagenesis remains poorly understood. We focus on 3D chromatin organization. Hi-C and RNA sequencing was performed on diagnostic FFPE tumor samples from seventeen patients with AITL. Two CD4+ T cells from individual donors and one follicular helper T cells (TFH) from four donors were used as controls. To annotate AITL-specific loops in association with enhancer status, we identified H3K27ac histone marks in TFH cells using CUT&Tag. Promoter-super enhancer (P-SE) loops or stripes were defined as those with non-promoter anchors containing SE predicted by ROSE. In comparison to normal CD4+ T-cell controls, recurrent AITL-specific alterations in A/B (active/inactive) compartments, topologically associating domains (TADs), and chromatin loops were identified. Principal-component analysis (PCA) of genome-wide compartment scores indicated AITL samples and normal CD4+ T cells clearly separated by the first two components. On average, there was 3.5% (range: 2.0-5.3) A-to-B compartment switch and 1.9% (range: 0.8-4.8) B-to-A switch when comparing AITL samples with normal CD4+ T cell controls ($p=5.17 \times 10^{-5}$, two-sided paired t-test). Alterations in TADs were recurrently observed in 434 of 743 cancer census genes in COSMIC, including *MYC, TCL1A,* and *RHOA*. On average, 60.1% of loops include promoters in at least one anchor. Genes within AITL-specific loops exhibited significantly higher expression than those outside of loop regions ($p=7.39 \times 10^{-88}$, Kruskal-Wallis test). Genes within P-SE loops/stripes showed significantly higher expression than those within promoter-inactive enhancer (P-IE) loops/stripes, which were defined by ENCODE as enhancers without TFH SE (loops: $p = 5.17 \times 10^{-5}$, stripes: $p=1.55e^{-15}$, Kruskal-Wallis test). Based on the differential loop analysis using diffpeakachu, significant chromatin loops were observed in the *MYC* region and genes associated with pathways related to *MYC*, such as the Wnt signaling pathway. AITL-specific loops in the *MYC* region were observed in eight of seventeen patients. Recurrent MYC promoter-TFH SE loop (chr8:127730000-127740000,chr8:127960000-127970000) was located between ME1 and ME2 of MYC enhancer in AML (Pulikkan et al., 2018). Our findings indicate that AITL exhibits unique chromatin conformations, suggesting the formation of a lymphoma-specific gene regulatory landscape. Recurrent changes in chromatin conformation involve *MYC*. Distinct gene regulatory elements associated with enhancer regions in AITL is implicated. Correlation of the regulatory candidates with their mutational signature is underway and will be reported at the meeting.

#2749 Single-cell 3D genome structure analysis reveals clonal evolution and heterogeneity in acute myeloid leukemia.

Yu Luan[1], Ye Hou[2], Yutong Lei[3], Qiushi Jin[1], Jie Xu[2], Bei Jia[4], Siwei Xu[3], Ping Wang[2], Alok Swaroop[2], Yihao Fu[2], Juan Wang[2], Qixuan Wang[2], Hong Zheng[4], Jing Zhang[3], Feng Yue[2]

[1]UT Health Science Center at San Antonio, San Antonio, TX,[2]Department of Biochemistry and Molecular Genetics, Northwestern University, Chicago, IL,[3]Department of Computer Science, University of California, Irvine, Irvine, CA,[4]Department of Medicine, Division of Hematology and Oncology, Penn State University, Hershey, TX

Alterations in the 3D genome organization have been reported in different types of cancer and have been linked with gene dysregulation and cancer progression. However, majority of the studies were performed using bulk chromatin interaction assays and therefore, cannot address the heterogeneity and clone evolution in primary human tumors. In this study, we generated single cell Hi-C and single cell ATAC-Seq data in a cohort of AML patients, comprising samples at different disease stages, including diagnosis, remission, and relapse. To our knowledge, this is first large collection of single cell Hi-C data in primary tumor. In total, we profiled 153,517 cells across 13 samples, revealing distinct contact patterns and significant heterogeneity in genome-wide contact features between different samples, as well as within the same sample. We demonstrated the impact of 3D genome alterations on AML-specific gene expression programs, highlighting the role of compartmentalization in driving AML pathogenesis. Moreover, we identified hundreds of AML-specific chromatin domains with decreased heterogeneity during the transition from initial diagnosis to post-treatment relapse, suggesting the emergence and expansion of therapy-resistant subclones. By integrating a clonal evolution model, we delineated the dynamic relationship between somatic mutation, structural variation, and 3D genome architecture alterations. Finally, we discovered a chromatin loop uniquely present in a subclone of the relapse sample, which hijacked a distal enhancer to the known chemo-resistance-related oncogene and led to its upregulation. To summarize, our findings demonstrated the heterogeneity of 3D genome structure in AML and provide insights on the disease progression and relapse mechanisms.

#2750 Alternative enhancer usage of *PER2* locus regulating 3D genome architecture in epithelial-mesenchymal states.

Yi-Chia Chiu[1], Qing You Pang[2], Tuan Zea Tan[3], Wendy W. Hwang-Verslues[4], **Ruby Y. J. Huang**[1]

[1]National Taiwan University College of Medicine, Taipei, Taiwan,[2]National Neuroscience Institute, Singapore, Singapore,[3]National University of Singapore, Singapore, Singapore,[4]Academia Sinica, Taipei, Taiwan

The regulation of epithelial (E) and mesenchymal (M) states requires orchestrated adaptation at the epigenomic level. The 3D genome hierarchical orders, together with chromatin accessibility, histone modifications, and local DNA looping, establish the architectural backbone of the genome in response to functional ques for EM plasticity during cancer progression. Here, we delved into alternative enhancer usage and the accompanied changes in 3D genome architecture which could govern the interconversion between these two states. Using ovarian cancer (OC) cells as a model, distinct enhancer usage at the circadian gene Period 2 (*PER2*) loci was identified in OC cells of epithelial-like Epi-A (PEO1 and OVCA429) and mesenchymal-like Mes (SKOVE3 and HeyA8) subtypes. Intron 6-14 of *PER2* consisted of multiple enhancers in PEO1 and OVCA429 suggesting an open chromatin state compared to a relatively closed chromatin in SKOV3 and HeyA8 marked by latent/inactive regions. OC tumors of epithelial-like Epi-A subtype with high *PER2* expression were also correlated with better overall survival, suggesting the clinical relevance of this region. CRISPR/Cas9-mediated deletion of 3 enhancers, namely A, B, and C regions, was performed to generate clones in OVCA429. Deletion of specific enhancer B and C regions of *PER2* resulted in a spindly mesenchymal morphology, compared to the cobblestone epithelial morphology of parental OVCA429 and clones with region A deletion. These changes corresponded with the altered expression of known EMT markers. RNA-seq analysis showed a significant shift in the EMT scores of OVCA429_*PER2*_B towards a Mes-like profile. Hi-C analysis was done in OVCA429_*PER2*_B to decipher the 3D genome architecture. Changes at compartments, topologically associated domains (TADs), and long-range DNA interaction loops were explored. Our findings highlighted that alternative enhancer usage is crucial in EM plasticity and subtype conversion, offering genome level insights into EMT regulatory mechanisms in cancers.

#2751 Role of transcriptional regulation in chromatin architecture and immunotherapy resistance of hepatocellular carcinoma.

Siyun CHEN[1], Yingnan LIN[1], Chengpeng Zhong[2], Yalin TU[1], Haoran WU[1], Yiling ZHANG[1], Jia WANG[3], Alfred Sze Lok CHENG[1]

[1]School of Biomedical Sciences, Chinese University of Hong Kong (CUHK), Hong Kong, Hong Kong,[2]Department of Liver Surgery, Renji Hospital Affiliated to Shanghai Jiao Tong University, Shanghai, China,[3]GMU-GIBH Joint School of Life Sciences, Guangzhou Medical University, Guangzhou, China

Introduction: Chromatin architecture is frequently altered in cancers, including one of the most prevalent cancers, hepatocellular carcinoma (HCC). Emerging evidence suggests that liquid-liquid phase separation plays a crucial role in shaping chromatin architecture and regulating gene transcription. Immune checkpoint blockade (ICB) therapy has become a new standard of care for HCC. However, the majority of HCC patients exhibit either primary or adaptive resistance to this immunotherapy, highlighting the urgent need for effective combination therapies. This study aims to uncover the differences in chromatin architecture between ICB-sensitive and resistant HCC tumors and dissect the underlying regulatory mechanisms.
Methods: Hi-C, ATAC-seq, CUT&Tag, and RNA-seq were performed on RIL175 cells from a well-established adaptive ICB-resistant mouse model to generate the multi-omics dataset. To explore the functional role of F1 in ICB resistance of HCC, RIL175-PD1-R cells with F1 knockdown were constructed using the lenti-virus infection. To explore the phase separation ability of F1, F1 protein tagged with mCherry was expressed in E. coli and purified. Droplet formation assay and fluorescence recovery after photobleaching (FRAP) assay were applied.
Results: Three-dimensional genomic analysis by Hi-C reveals comparable chromatin interactions between ICB-sensitive and resistant cells at both compartment and topologically associated domain (TAD) levels but an increase in loops within ICB-resistant cells. In accord with this, ATAC-seq results indicate upregulated chromatin accessibility in a subset of chromatin regions in ICB-resistant cells compared to ICB-sensitive cells. Motif analysis identifies F1 as the top transcription factor enriched for the open chromatin regions. CUT&Tag shows that F1 binds to those accessible regions and alters chromatin looping in ICB-resistant cells. Modulating F1 expression in HCC tumor cells significantly increases the therapeutic efficacy of ICB treatment. We also found that F1 undergoes phase separation, which may mediate the changes in chromatin architecture between ICB-sensitive and resistant cells.
Discussion and Conclusion: F1 undergoes phase separation in HCC, particularly in ICB resistant cells. This characteristic of F1 may shape specific three-dimensional chromatin architecture in HCC cells, contributing to their resistance to ICB treatment. Targeting F1 condensates may provide a novel therapeutic strategy to reprogram the genome architecture to improve ICB efficacy for patients with HCC.

Keywords: chromatin architecture, phase separation, hepatocellular carcinoma, immune checkpoint blockade (ICB) therapies
Acknowledgement: This work is supported by NSFC/RGC (460795048) & Li Ka Shing Foundation.

#2752 Analysis of epigenetic regulation by mutant p53 gain-of-function in anaplastic thyroid cancer.

Wataru Kawase[1], Yukihiko Hiroshima[1], Takashi Oshima[2], Katsuhiko Masudo[2], Yohei Miyagi[1]

[1]Kanagawa Cancer Center Research Institute, Yokohama, Japan,[2]Kanagawa Cancer Center, Yokohama, Japan

Thyroid cancer is one of the most common endocrine malignancies. It is primarily classified into four types : papillary thyroid cancer, follicular thyroid cancer, medullary thyroid cancer and anaplastic thyroid cancer (ATC). Among them, ATC is particularly rare, has a very poor prognosis and is insensitive to conventional treatments. Consequently, the development of effective therapeutic strategies for ATC is urgently needed. Mutations in *TP53* have been reported to play a role in the anaplastic transformation of thyroid cancer. *TP53* is the most frequently mutated gene across all human cancers. Although common *TP53* missense mutations cause loss of its tumor-suppressive function, 'gain-of-function' (GOF) mutations of *TP53* have recently been reported to promote several types of cancer. Mutant p53 (mut-p53) GOF can manipulate epigenetic regulators and alter the chromatin landscape. However, the impact of mut-p53 GOF on epigenetic regulation in ATC remains unclear. In this study, we aim to elucidate the mechanisms of epigenetic regulation underlying tumor progression driven by mut-p53 GOF in ATC. Hotspot gene mutation analysis revealed the presence of known mut-p53 GOF mutations in three out of seven ATC cell lines. Distribution analysis of ATAC-seq peaks in enhancer regions revealed that certain promising transcription factor binding motifs were enriched in ATC cell lines with mut-p53 GOF. Moreover, we demonstrated that these candidate transcription factors are involved in cell proliferation and migration of ATC cell lines with mut-p53 GOF. These results suggest that the exploration of gene expression regulatory mechanisms by mut-p53 GOF may provide a clue for a novel therapeutic strategy for ATC.

#2753 Potential novel interventions of oncohistones, epigenetic modifications, and RNA processing machinery in betel-nuts related HNSCC in Taiwan.

Jo-Pai Chen[1], Jui-Ying Chang[1], Ruey-Long Hong[2]

[1]National Taiwan University Hospital, Yun-lin Branch, Taipei, Taiwan,[2]National Taiwan University Hospital, Taipei, Taiwan

Background: Betel nut chewing leads to strong invasion & recurrence. We found betel-nuts exposed HNSCC cell line, TW2.6, caused treatment refractoriness in Taiwan with high PDL1, defective p53 mutation, p16 loss, & BCL2 overexpression. PI3K/AKT/mTOR interventions, ALK/IGF1R inhibitor, CDK4/6 inhibitor, BCl2 inhibitor, WEE1 inhibitor, ATR inhibitor, DNA-PK inhibitor, AT2AR inhibitor, Mcl-1 inhibitor, MEK1/2 inhibitor, JAK2 inhibitor, CXCR4 inhibitor, FAK inhibitor, p53 reactivator, MDM2 inhibitor, SHP2 inhibitor, PARP7 inhibitor, IAP inhibitor, GLS1 inhibitor, eribulin, & VEGFR2/ PDGFR/FGFR or VEGFR2/c-MET/Axl triple blockage might be effective on TW2.6 and reverse treatment refractoriness, through the inhibition of mesenchymal transformation, pRB, & PI3K/AKT /mTOR signaling and modulation of stemness & PD1/PDL1 pathway. Besides, PLK inhibitor & GLS1 inhibitor seemed good radiosensitizers for TW2.6(AACR2024). The genomic signature of TW2.6 was figured out(*AACR2020*) with PIK3CA H1047R mutation, high TMB/MSS, VEGF-A amplification, p53/MYC/HRAS/DDR2/ PDGFRbeta/EPHB1/ATM mutations, FAT1 loss, amplification of TERT/ FGF10/CCND3/SOX9/IL-7R/SDHA/ RICTOR/FLCN, CDK12/PTPRD loss of function, deletions of STK11/ARID1B/MITF/ TNFAIP3, & SETD2(a histone lysine methyltransferase)/KDM5A(a lysine demethylase)/CDK8 mutations. H3K36 mutation and NSD1 defects altering H3K36 methylation will block cellular differentiation and promote oncogenesis. Disrupting NSD1 in HNSCC cell lines led to CpG hypomethylation & enhanced cisplatin sensitivity.

Methods: TW2.6 used to test (1)*in vitro* drug sensitivity to (a)Chemical Modulators of Splicing; (b)PRMT5 inhibitor; (c)CDK9 inhibitor, EZH2i, DNMT3i, BRD/BET4i, and HDACi; (d)NSD1/SETD2 inhibitor; (e)METTL3 inhibitor; (2)synergistic effects with other therapies by MTT assay, colony formation, flow cytometry, & western blotting.

Results: Epigenetic modifications were tested over TW2.6. AZD4573(CDK9 inhibitor), tazemetostat(EZH2 inhibitor), azacitidine(DNMT3 inhibitor), AZD5153(BRD4/BET inhibitor), and romidepsin(HDAC inhibitor) were effective over TW2.6 and enhanced ICIs efficacy. AZD4573 can synergize with tazemetostat, AZD5153, AZD6094(c-MET inhibitor), AZD5991(Mcl-1 inhibitor), & AZD2014(TOC1/2 inhibitor); but no synergistic effects with DDR interventions, such as AZD6738(ATR inhibitor) & olaparib except AZD1775(WEE1 inhibitor). AZD4573 will cause the drop of EMT signals, pRB, & p70S6K1 and prominent elevation of p-PARP. TW2.6 was also effective towards STM2457(METTL3 inhibitor) and JNJ64619178(PRMT5 inhibitor).

Conclusions: TW2.6 was sensitive to epigenetic modifications due to CDK8/SETD2/KDM5A mutations & ARID1B loss. Novel RNA processing interventions and epigenetic modifications for radiosensitization & immuno-modulation may be future trends.

#2754 Long-read direct RNA sequencing identifies m^3C modification sites in poly(A) RNAs from pancreatic cancer cells.

Kenzui Taniue[1], Shuhei Mitsutomi[1], Anzu Sugawara[1], Kenji Takahashi[2], Yusuke Mizukami[2], Nobuyoshi Akimitsu[1]

[1]University of Tokyo, Tokyo, Japan,[2]Asahikawa Medical University, Hokkaido, Japan

Covalent modifications provide a precise and effective mechanism to regulate the biogenesis, stability, and function of biological molecules, including DNA, RNA, proteins, and lipids. More than 300 distinct chemical modifications have been identified in both coding and non-coding RNAs, forming a diverse RNA modification landscape known as the epitranscriptome. RNA modifications are mediated by three classes of proteins: writers, which add modifications; erasers, which remove them; and readers, which recognize and interpret modified RNAs. RNA modifications play critical roles in cell homeostasis and cell developmental processes by regulating gene expression, RNA metabolism, and translation into proteins. Dysregulation of RNA modifications has been linked to the pathogenesis of various human diseases, including cancer. Recent studies has shown that 3-methylcytidine (m^3C) is primarily located at position 32 on the anticodon loop of certain transfer RNAs (tRNAs). This modification is catalyzed by m^3C writers, such as METTL2A, METTL2B, METTL6, and METTL8. These writers and their cofactors have been associated with human diseases. m^3C has also been detected in polyadenylated RNA (poly(A) RNA) at levels approximately 1000-fold lower than in tRNA through LC-MS/MS analysis. However, its transcriptome-wide distribution and functions remain unknown due to its low abundance and the absence of commercially available anti- m^3C antibodies suitable for immunoprecipitation. Here, we demonstrated that METTL2A expression is upregulated in pancreatic tumor tissues and that high METTL2A levels are associated with poor prognosis in pancreatic cancer patients. To further investigate the role of METTL2A, we performed comparative direct RNA sequencing on the pancreatic cancer cell line AsPC-1 to identify potential METTL2A-mediated m^3C sites in poly(A) RNA. These m^3C sites were enriched in the CC motif and predominantly located in the coding sequences of mRNA, mitochondrial mRNA, and mitochondrial ribosomal RNA. Moreover, METTL2A downregulated m^3C RNAs and altered the balance between specific isoforms containing METTL2A-mediated m^3C sites by promoting exon inclusion and shifting isoform expression, underscoring a potential role of m^3C in poly(A) RNA in regulating gene expression. In conclusion, our approach may contribute to uncovering the functions and characteristics of previously unknown RNA modifications regulated by various factors.

#2755 The m^{6}A epitranscriptomic landscape of bladder cancer is characterized by sequential 3'UTR hypermethylation and global methylation dilution.

Jonas Koch[1], Felix Bormann[2], Vitor Coutinho Carneiro[1], Manuel Neuberger[3], Katja Nitschke[3], Malin Nientiedt[3], Philipp Erben[3], Maurice S. Michel[3], Frank Lyko[1]

[1]German Cancer Research Center, Heidelberg, Germany,[2]Bioinformatics.Expert UG, Berlin, Germany,[3]Department of Urology and Urosurgery, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

N^6-Methyladenosine (m^{6}A) is the most prevalent internal modification of eukaryotic mRNAs and affects the regulation of its target transcripts in all aspects of the mRNA life cycle. Cancer-associated changes in the m^{6}A epitranscriptomic landscape as well as the deregulation of m^{6}A regulators have been linked to cancer development. However, most studies have focused on *in vitro* or *in vivo* models and antibody-dependent mapping techniques, which have limited resolution and lack quantitative methylation information. Here, we provide the first transcriptome-wide, base-resolution map of m^{6}A in cancer and determine methylation levels with absolute stoichiometric information by comparative sequencing of nine urothelial carcinoma of the bladder (UCB) and nine non-malignant uroepithelial control samples. Our results define key signatures of the cancer-associated m^{6}A epitranscriptomic landscape of UCB, which was characterized by differential methylation of cancer-related mRNAs. m^{6}A hypermethylation primarily occurred near stop codons and was associated with upregulation of the VIRMA subunit of the m^{6}A methyltransferase complex, and alternative polyadenylation. Furthermore, the integration of methylation data and expression data revealed a global dilution of methylation marks in UCB, which was caused by the decreased expression of highly methylated transcripts, combined with the increased expression of unmethylated transcripts. Notably, both changes appeared sequentially during the progression to muscle-invasive bladder cancer. Our study thus characterizes the systematic differences in the m^{6}A methylome of UCB and establishes the potential of m^{6}A profiling for cancer biomarker development.

#2756 Epigenetic drivers of enzalutamide resistance in prostate cancer: targeting of DNMTs and EZH2 as therapeutic strategies to overcome enzalutamide resistance in prostate cancer.

Santosh Lamichhane, Brandon E. Burow, Sweaty Koul, **Hari K. Koul**

LSU Health New Orleans, New Orleans, LA

Background: Prostate cancer results in over 35,000 deaths in USA, annually. Enzalutamide (ENZ) treatment enhances overall patient survival. The development of ENZ-R is marked by adaptive cellular mechanisms, including cellular plasticity and the emergence of CRPC-NEPC and CRPC-DNPC phenotypes. The role of epigenetic mechanisms in CRPC remains less understood. Recent studies, including those from our laboratory, have pointed to increased expression and activity of DNA methyltransferases (DNMTs) and the PRC2 component EZH2 during prostate cancer progression. However, the direct role of DNMTs in ENZ-R has not been thoroughly evaluated. In this study, we explored the potential of DNMT inhibitors and EZH2 inhibitors to sensitize prostate cancer cells to enzalutamide.
Methods: Enzalutamide-resistant (LNCaP-ENZ-R) cells were developed by maintaining the cells in a medium containing 5 μM ENZ for a minimum of six months, while Short-term treatment with ENZ was conducted for 72h. Where indicated DNMTi (5-AZA-dC) and EZH2i (GSK-126) and Enz were used at 5uM concentrations. Proliferation was assessed using Incucyte. Colony formation and cell migration were assessed. Protein and mRNA level were determined by western blot and RT-PCR techniques.
Results: We observed that treatment of PCa cells with ENZ increased levels of DNMTs in both short-term (treatment of the LNCaP cells for 72 h) and established ENZ resistant (LNCaP-ENZ-R) cells. We also observed a gradual transformation from epithelial morphology to neuroendocrine phenotype in ENZ-R cells, characterized by spindle-shaped and elongated structures in LNCaP-ENZ-R cells. Moreover, in ENZ-R cells AR levels did not change but AR target (PDEF, PSA, and KRT18) were significantly downregulated, while markers associated with EMT (Snail) and NEPC (CHGA and enolase) were upregulated. Treatment of the cells with a DNMT inhibitor (5-AZA-dC) resulted in a decrease in DNMT levels in both ENZ-sensitive and -resistant cells. EZH2 expression showed no notable changes in either ENZ-sensitive or -resistant cells, however, H3K27me3 levels were increased with ENZ treatment. Additionally, LNCaP cells were sensitive to DNMTs and EZH2 inhibitors, and inhibiting both DNMTs and EZH2 substantially inhibited the growth and proliferation ENZ-sensitive as well as of ENZ-R-PCa cells. We also observed that treatment with inhibitors of DNMT and EZH2 decreased the scratch wound closure and clonogenic activity of PCa cells.
Conclusion: Overall, our findings reveal that the DNA and histone methylation pathways play an important role in prostate cancer and development of ENZ-Resistance, and that targeting these epigenetic regulators by small molecule inhibitors, may help overcome therapy resistance in PCa.
Supported in part by: Funds from VA Merit Award: I01BX005351, NIH/NCI RO1: CA242839, and LSUHSC, NOLA.

#2757 Hypermethylation of FAT4 linked to tumor progression in colorectal adenocarcinoma.

Dalma Muller, **Balazs Gyorffy**

Semmelweis University, Budapest, Hungary

Introduction: The gene *FAT4* encodes a cell adhesion molecule of the protocadherin family. *FAT4* can be linked to cancer progression at multiple levels, including metastasis, autophagy, epithelial-to-mesenchymal-transition, and as a tumor suppressor. Aberrant methylation, a common hallmark of colorectal adenocarcinoma, disrupts normal gene regulation.
Purpose: We explored the methylation changes of FAT4 during colorectal cancer progression, using a large patient cohort with methylation microarray data.
Methods: Raw data files of a collection of 2,356 colorectal tumor, adenoma, and normal samples from two microarray platforms (Illumina HM450K and EPIC) were processed in R using the *minfi* and the *watermelon* libraries. Based on a matrix of β-values, methylated regions and CpG loci were identified using Kruskal-Wallis-test complemented with ROC analysis. For data visualization we employed www.epigenplot.com, a web platform established based on our database. False discovery rate was set at 10%.
Results: Based on UCSC RefGene data, 18 of the HM450K and 27 of the EPIC array probes were assigned to the *FAT4* gene. All the probes hypermethylated in adenocarcinoma tissues compared to normal ones belonged to the CpG island chr4:126235895-126238930 of the proximal promoter region. When comparing to normal tissues, the TSS200 region had the most prominent hypermethylation (HM450K $\Delta\beta$ =0.28, AUC 0.9; EPIC $\Delta\beta$ = 0.22, AUC 0.85, $p < 0.0001$), while the probes of the open sea CpGs in the protein coding region have shown a slight hypomethylation (HM450K $\Delta\beta$ = 0.1; EPIC $\Delta\beta$ = 0.14, $p < 0.0001$). These changes were also present in adenoma tissues compared to normal ones. Based on KEGG classification, three other genes associated to the mTOR signaling pathway were similarly hypermethylated in the TSS200 region ($\Delta\beta >= 0.2$), including *WNT2, FZD10*, and *WNT7A*.
Conclusions: *FAT4,* a tumor suppressor gene with previously reported downregulation in CRC, shows significant hypermethylation in colon cancer, including the precursor lesions. Our results suggest that detection of these methylation changes in the promoter region of *FAT4* might be utilized as a prognostic biomarker and in the early detection of CRC.

#2758 Methylome plasticity as a biomarker of treatment response in small cell lung cancer.

Danielle Benedict Sacdalan[1], Sami Ul Haq[2], Luna Jia Zhan[1], Janice J. N. Li[1], Vivek Philip[1], Scott V. Bratman[1], Geoffrey Liu[1], Benjamin H. Lok[1]

[1]UHN Princess Margaret Cancer Centre, Toronto, ON, Canada,[2]Schulich School of Medicine & Dentistry, Western University, London, ON, Canada

Background: Small cell lung cancer (SCLC) is an aggressive disease with poor treatment outcomes, in part due to epigenetic mechanisms driving tumor growth and resistance. Cell-free DNA methylome profiles in untreated SCLC can identify prognostic sub-groups, making it a useful biomarker. In this study, we hypothesize that changes in the cell free methylome of SCLC influence disease response to therapy and drive treatment resistance.

Methods: Cell free methylated DNA immunoprecipitation followed by sequencing (cfMeDIP-seq) was performed on 34 baseline-relapse paired samples belonging to a cohort of SCLC patients treated at the Princess Margaret Cancer Centre (Toronto, Canada). Matched leukocyte DNA methylation profiles were incorporated to exclude the contribution of non-cancer methylation to the cfMeDIP signal and allow focused profiling of changes in the SCLC methylome through first-line treatment. The plasticity of the SCLC methylome was determined by subtracting the reads per kilobase of transcript per million mapped reads (RPKM) at the time of progression compared to the pre-treatment timepoint. A methylome demonstrated plasticity if it showed an increase or decrease in reads compared to the median RPKM across all samples in the cohort. Associations between methylation groups and relevant clinical data were identified. Kaplan-Meier and Cox regression analysis were performed to determine if methylome changes were associated with overall survival and progression-free survival, anchored from the time of SCLC diagnosis. KEGG pathways corresponding to the top differentially methylated windows between baseline and relapse pairs were identified and characterized.

Results: Plasticity of the SCLC methylome were seen in 67% of patients (n=23/34). Methylome plasticity was found to be associated with a shorter time to progression from the end of first-line treatment (mean difference = 70 days; 95% CI 25-115, p = 0.0031). Accordingly, patients with greater methylome plasticity were more likely to be platinum resistant (57% vs. 17%; χ-squared p = 0.033). Cox regression showed that methylome plasticity was significantly associated with worse progression free survival (PFS) (adjusted hazard ratio [aHR] = 5.7, p = 0.0010) and overall survival (OS) (aHR = 1.9, p = 0.11), after adjusting for VA stage at diagnosis. Pathway analysis of differentially methylated windows mapped to genes related to neuronal polarity and axonal guidance, as well as Wnt signaling.

Conclusion: Changes in cell-free DNA methylomes serve as a biomarker for treatment response in SCLC. Increased plasticity of the methylome is associated with shorter PFS. The underlying biology of this relationship may involve changes in pathways that govern neuronal change and established cancer-associated pathways.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Functional Genomics / Large-Scale Approaches to Cancer Gene Discovery
Poster Session

#2763 Molecular QTL analyses identify functional variants at inherited risk loci in chronic lymphocytic leukemia.

Huihuang Yan, Zhiquan Wang, Mingma Hoel, Cristine Allmer, Chantal McCabe, Daniel O'Brien, Dennis Robinson, James Cerhan, Sameer Parikh, Esteban Braggio, Susan Slager

Mayo Clinic, Rochester, MN

Most disease-associated single-nucleotide polymorphisms (SNPs) identified by genome-wide association studies (GWASs) reside in noncoding regions. There is increasing evidence that they play regulatory roles through epigenetic mechanisms by altering local chromatin state, 3D chromatin structure, and looping interactions. We and others have identified 42 CLL risk loci from GWASs. At 12 risk loci located outside of gene promoters, we previously demonstrated that functional SNPs are highly enriched in enhancers and super-enhancers that mostly target *BCL2* family genes and transcriptional regulators. However, the study was limited by using only public epigenomic data generated in lymphoblastoid cell lines (LCL) and normal immune related cell types. To gain a better understanding of how the functional SNPs alter local chromatin state and modulate target gene expression in CLL, we generated RNA-seq data (n=588) and transposase-accessible chromatin with sequencing (ATAC-seq) data (n=69) in CLL tumor B cells. We performed molecular quantitative trait loci (QTL) analysis with index and correlated SNPs (R^2 0.5) at 41 risk loci (excluding the HLA locus). The analyses identified cis expression QTLs (eQTLs), within 1Mb of genes' transcription start site, at 25 risk loci and chromatin accessibility QTLs (caQTLs, within peak) at 11 risk loci, with both eQTLs and caQTLs at 8 risk loci. We identified a total of 54 eGenes, for which the expression is affected by nearby SNPs within 1Mb, including 40 novel eGenes at 16 CLL risk loci not previously reported. Notably, based on publicly available chromosome interaction data from CLL, LCL, and normal B cells, for over 70% of the novel eGenes, their promoters showed looping interactions with the regions carrying the eQTLs. Further, 13 SNPs were identified as both caQTL and eQTL, with the associated novel eGenes including *CD81* at 11p15.5, *IPCEF1* at 6q25.2, as well as *CXXC1* and *MBD1* at 18q21.1. The result suggests that these SNPs play a role in altering chromatin accessibility contributed to gene expression variation. At 11p15.5 risk locus, SNP rs2651823 (A/G) represents a novel caQTL and eQTL. The alternative G allele is associated with increased chromatin accessibility and *CD81* expression. Hi-C data from GM12878 and CD20+ B cells supported interaction between the open chromatin region carrying rs2651823 and *CD81* promoter. CD81 facilitates the trafficking of CD19 to the surface of activated B cells, and CD81-null mice showed impaired immune response. In a mouse model, CD81 contributes to tumor progression and metastasis in Lewis lung carcinoma. In summary, our molecular QTL analysis revealed that functional SNPs, located in CLL risk loci, modulate chromatin accessibility and expression of downstream target genes that could impact CLL risk.

#2764 Cas9 cytosine base editors for precision genome editing with high specificity and activity.

Erin Brettmann[1], Stephen Beishir[1], Xiao Chen[1], Xiaokang Zhang[2], Rajagopalan Lakshmi Narasimhan[3], Ajaya Kumar Kotekar Manjunath[3], Daniel Taglicht[4], Jiajian Liu[1], Fuqiang Chen[1]

[1]MilliporeSigma, Saint Louis, MO,[2]MilliporeSigma, Shanghai, China,[3]MilliporeSigma, Bangalore, India,[4]MilliporeSigma, Jerusalem, Israel

Base editing technologies allow for the precise and efficient installation of defined nucleotide substitutions into a target genome without double strand breaks. These editing methods can be used to generate cell lines and animal models for the study of a diverse range of topics, from molecular mechanisms of pathogenesis to drug resistance and therapeutics. However, overexpression of base editors can lead to off-target editing, both genome-wide in a guide-independent fashion and enriched at sequences similar to the intended target. To counter this challenge, we have developed a suite of recombinant protein-based cytosine base editor (CBE) tools. This suite includes two separate CBE proteins with complementary editing windows, a protein format uracil glycosylase inhibitor (UGI), and a guide design tool to facilitate experimental planning and computationally predict editing outcomes. The first CBE, “Flexible,” has a wide editing window, enabling editing over a large fraction of the target sequence and facilitating flexible placement of the guide RNA relative to the target cytosine residue. The second CBE, “Precision,” has a narrowed editing window, enabling discrimination between the target cytosine and nearby, untargeted cytosines. We show that co-transfection with the UGI protein increases the proportion of substitutions that are C-to-T and the ratio of C-to-T editing to indel formation, thus reducing undesired editing outcomes. We further show that co-transfection with a transfection enhancer results in an increase in overall editing rates, and that the transfection enhancer and UGI can be combined to maximize the rate of C-to-T editing while minimizing undesired outcomes. We use *in vitro* editing experiments to characterize the editing profiles of our CBEs and identify a preference for cytosine residues preceded by thymine (TC dinucleotides) and unmethylated cytosine residues. Finally, we show that RNP delivery of Flexible and Precision CBEs results in lower rates of mismatch editing and genome-wide nonspecific editing than plasmid delivery of the same editors, demonstrating their improved specificity profiles. To facilitate use of these editors, we have developed a guide design tool that will identify candidate guide RNAs in a user-provided sequence, identify potential off-target sites, and design primers to amplify the on- and off-target sites. The tool further uses a machine learning model trained on experimental data to predict which cytosine residues are most likely to be edited, and a joint probability model to predict the potential bystander outcomes, enabling the user to predict their editing outcomes before performing the experiment. Together, these tools will facilitate precision genome editing experiments.

#2765 Extracellular vesicle (EV) RNA transcriptome analysis: An optimized RNA-Seq workflow reveals distinctive transcript structures.

Srikanth Perike, Angelica Olcott, Cissy Jiang, Linda Lingelbach, Elizabeth Dreskin

Bio-Rad Laboratories, Hercules, CA

Introduction: Extracellular vesicles (EVs) are crucial membrane-bound particles containing DNA, RNA, proteins, metabolites, and bioactive molecules, which serve as conduits for essential cell-to-cell communication. EV RNA studies often focus on miRNAs due to their prevalence, potentially overlooking non-miRNA transcripts. A wide array of RNA species are found in EVs, including long non-coding RNAs (lncRNAs), messenger RNAs (mRNAs), and small non-coding RNAs (sncRNAs); many linked to disease. To gain molecular insights into the EV transcriptome, we devised a novel protocol for isolating EV RNA, enabling comprehensive RNA-Sequencing (RNA-Seq) profiling.
Methods: We used six cell lines (A549, Jurkat, MCF-7, HeLa, HEK293, and NTERA-2) to demonstrate our workflow and results. Our protocol involves EV precipitation from a minimal volume (1.4mL) of conditioned cell culture media prepared with exosome-depleted FBS, followed by EV lysis using SingleShot Cell Lysis reagent which preserves RNAs of all sizes. In each experiment, cellular RNA from the source cells was prepared as a control. Whole transcriptome libraries were constructed using the SEQuoia Complete Stranded RNA Library Prep Kit with ten microliters of EV RNA lysate. Libraries were sequenced on the Illumina NextSeq 1000. After secondary sequence analysis, we visualized transcript differences in EV and cellular transcripts on the UCSC genome browser. Novel qPCR assays were developed as an orthogonal method to confirm the transcript structure observations.
Results: Our EV RNA-Seq workflow identified diverse RNA biotypes, including miRNAs and numerous sncRNAs in both cellular and EV transcriptomes. Notably, within EVs, our study revealed distinct structural configurations in many sncRNA transcripts that were not observed in the originating cell lines. These novel structural findings were confirmed using 1-step quantitative reverse transcription polymerase chain reaction (RT-qPCR) with custom assays designed for several transcripts. These findings are consistent across tested cell lines and other methods of EV and EV RNA isolation.
Discussion and Conclusion: Many sncRNAs which we observed to have unique transcript structures are known components of ribonucleoprotein (RNP) complexes. Given their proposed role in cell-to-cell communication, we hypothesize that the pivotal element of EVs in intercellular communication may be the incorporation of these unique RNA transcripts as components of the RNP complex. Our efficient EV RNA-Seq workflow reduces sample handling, preserves RNA samples and allows for detection of RNAs of varying sizes. This will allow researchers to uncover the complex landscape of the EV transcriptome.

#2766 Metabolic and tumor immune cell landscapes are significantly different amongst *KRAS* mutational variants in non-small cell lung cancer.

Frank D. Weinberg[1], Jen Godden[2], Denise Shieh[2], Stamatina Fragkogianni[2], Jacob Mercer[2], Melissa Stoppler[2], Daniel Principe[3], Ryan Nguyen[1], Kamya Sankar[4], Koosha Paydary[5], Mary Jo Fidler[5]

[1]University of Illinois Cancer Center, Chicago, IL,[2]TEMPUS, Chicago, IL,[3]University of Wisconsin, Madison, WI,[4]Cedars-Sinai Medical Center, Los Angeles, CA,[5]Rush University, Chicago, IL

Approximately 30% of patients (pts) with non-small cell lung cancer (NSCLC) have alterations in the *KRAS* oncogene. In NSCLC *KRAS* mutational variants are diverse and therapeutically relevant. However, it is unclear how each variant is associated with the tumor biology, including lipid metabolism and the immune microenvironment. Since perturbed tumor immune infiltration and lipid metabolism are linked to NSCLC outcomes, we evaluated and characterized *KRAS* variants and association with lipid metabolism and immune infiltration. Using the Tempus Database, 5,925 de-identified records from pts with NSCLC and *KRAS* alterations (G12A (n=464), G12C (n=2,510), C12D (n=937), G12R (n=61), G12V (n=1,251), G13C (n=244), G13D (n=185), Q61H (n=273)) were retrospectively analyzed. Samples were sequenced with the Tempus xT and xR RNA assays. Tumor microenvironment cell proportions were estimated using QuanTIseq. Single-sample gene set enrichment analysis based on 775 lipid metabolic genes was used to compute enrichment scores (ES) for each pt. We analyzed neoantigen tumor burden (NTB) and tumor mutational burden (TMB) as mutations per megabase (mut/Mb). P-values were calculated using Pearson's Chi-squared and Kruskal-Wallis rank sum test. Pairwise comparisons of median ES were performed using the Wilcoxon test and the FDR method was used to correct for multiple comparisons. Among the overall *KRAS* cohort, *KRAS* G12C and G12V represented over 60% of cases. Pts with *KRAS G12D* alterations had the highest proportion (23%) of never-smokers ($p<0.001$). We identified small but significant differences in the median lipid ES in pts with *KRAS* G12C variants compared to pts with G12V and G12D, with G12C having lower median lipid ES (0.89 versus 0.91 and 0.91 respectively, both $p<0.001$). Differences were also identified in pts with KRAS G13C compared to pts with G12D (0.9 versus 0.91, $p=0.03$). Median TMB and NTB differed amongst pts with *KRAS* variants with a higher TMB and NTB found in G12C, G13C and G13D cohorts while the lowest TMB and NTB was observed in the G12D cohort ($p<0.001$). *KRAS* G12C variant tumors had the highest median percentage of CD8 T cells while G12D and Q61H variants had the lowest median percentage of CD8 T cells ($p=0.016$). Interestingly, G12D variant tumors had the highest median percentage of infiltrating B cells ($p=0.006$). Our data show *KRAS* variants are associated with changes in the lipid and tumor immune landscape in NSCLC. There were significant differences in the proportion of tumor immune cells amongst tumors with different *KRAS* variants. Notably, pts with *KRAS* G12D variants had a less immunogenic immune microenvironment indicated by low proportion of TMB, NTB and CD8 T cells compared to G12C variants, which could affect immunotherapy efficacy. Further analysis is needed to determine if immunotherapy efficacy is altered by the distinct molecular phenotypes in pts with *KRAS* variants.

#2767 Aneuploidy-induced VHL haploinsufficiency in lung squamous cell carcinoma and clear cell renal cell carcinoma.

Joanna Kopko, Joan J. Castellano-Perez, Gayatri Bulsara, Alison Taylor

Columbia University Irving Medical Center, New York, NY

Aneuploidy is present in 90% of solid tumors, making it a hallmark of cancer. Despite its prevalence, aneuploidy has been difficult to model in human cells due to the technical challenges of generating targeted events. One high-frequency aneuploidy alteration is chromosome 3p (chr3p) deletion, which is found in 80% of lung squamous cell carcinomas (LUSC) and 90% of clear cell renal cell carcinomas (ccRCC). Cells with chr3p deletion are missing one copy of the Von Hippel-Lindau (VHL) gene, a master regulator that targets Hypoxia Inducible Factor Subunit Alpha (HIF-α) for degradation. When HIF-α is not degraded, it promotes the transcription of genes involved in cell metabolism, cell survival, and proliferation. The role of VHL has already been extensively studied in ccRCC, but only using models with homozygous VHL loss, therefore the effect of heterozygous VHL loss is not known. I hypothesize that partial VHL loss is enough to stabilize HIF and activate transcriptional targets involved in tumor progression in LUSC. To study the effects of chr3p deletion, we developed a CRISPR-based approach to generate isogenic cell lines with and without the aneuploidy event in lung airway epithelial cells. Seven of eleven chr3p deleted clones had HIF-α protein stabilization in normoxic conditions. We also generated cells with smaller regions of chr3p deleted instead of whole arm loss. If the deletion region included VHL, we observed HIF-α accumulation. To test whether VHL haploinsufficiency was sufficient for this phenotype, we used CRISPRi to reduce VHL to 50% levels and found stabilization of HIF-α. In hypoxic conditions, VHL knockdown cells had increased HIF-α protein accumulation and gene expression of HIF-α targets GLUT1 and VEGF. These preliminary data suggest that VHL haploinsufficiency is enough to cause HIF-α stabilization. Additionally, we looked at VHL knockdown in renal epithelial cells and saw no HIF stabilization. This indicates that VHL haploinsufficiency and HIF stabilization may differ in lung vs. kidney cells. To our knowledge, our research is among the first to study VHL in lung cancer, and our results could have significant implications for drug development in LUSC. HIF-α inhibitors have recently been approved by the FDA, so determining the dose dependency of VHL on HIF-α activity will increase knowledge on treatment efficacy for patients with heterozygous deletion of VHL.

#2768 Highly parallel integrated functional and sequencing analysis of single cells using the Beacon® optofluidics platform.

Leqian Liu, David Coit, Or Gadish, Julie Perreau, Andrei Rajkovic, Brandon Bruhn, Minha Park, Edward Han, **Alireza Salmanzadeh**, Vikram Devgan

Bruker Cellular Analysis, Emeryville, CA

Single-cell RNA sequencing has emerged as a powerful tool for uncovering cellular heterogeneity and its connections to disease progression and treatment efficacy, including immunotherapy. However, many critical phenotypes cannot be deduced solely from gene expression changes. To gain deeper insights, functional readouts of these cells are essential, yet current methods are limited by low throughput and restricted assay capacity. To tackle this challenge, we developed a high-throughput workflow that integrates functional and sequencing analyses at the single-cell level. This workflow combines versatile phenotypic assays with highly parallel single-cell RNA sequencing, leveraging the advanced capabilities of the Beacon® optofluidics platform from Bruker Cellular Analysis. Using this platform, we demonstrated the ability to link cell surface marker staining with gene expression profiling using single cells derived from PBMC samples. We then utilized this platform to analyze the functional and transcriptomic heterogeneity within adoptive cell therapy products. Specifically, we quantified multiple secreted cytokines (IFNγ, TNFα, and IL-2) alongside gene expression in thousands of *in vitro*-expanded T cells expressing antigen-specific T cell receptors. These non-growth-based, single live-cell functional assays were performed with minimal crosstalk, followed by cDNA capture and synthesis using barcoded beads in a highly parallel manner on the Beacon platform. Single-cell RNA sequencing data were subsequently integrated with functional assay results to uncover the molecular mechanisms underlying the functional heterogeneity of cell therapy products. We discovered that polyfunctional cells—those secreting all three cytokines—exhibit upregulated expression of genes associated with metabolic reprogramming, activation regulation, and DNA replication, hallmarks of T cell activation. Additionally, increased expression of chemokine genes in polyfunctional cells suggests ongoing immune cell recruitment beyond cytokine secretion. Validating these findings could pave the way for strategies to develop enhanced cell therapy products. Beyond uncovering the molecular drivers of functional heterogeneity in single cells, we envision this approach being further applied to advance single-cell functional genomics through integration with high-content CRISPR screening. The Beacon optofluidics platform enables direct functional profiling of gene function and regulation at single-cell resolution, making it uniquely suited to tackle previously intractable questions in cancer research and therapeutic development.

#2771 CRISPR/Cas9 essentiality screens identify stand-alone and IRF4-linked genetic vulnerabilities in melanoma cells.

Ulduz Sobhiafshar, **N. C. Tolga Emre**

Department of Molecular Biology and Genetics, Bogazici University, Istanbul, Turkey

Melanomas have poor prognosis at the metastatic stage despite impressive progress in targeted- and immuno-therapies. Therefore, identification of novel critical genes involved in melanoma biology with targeting potential is desirable.
We and others have previously demonstrated the transcription factor interferon regulatory factor 4 (IRF4) as a dependency factor in various lymphocyte-derived cancers. These findings align with the therapeutic efficacy attained by immunomodulatory drugs which indirectly target IRF4 expression in these cancers.
IRF4 also takes part in pigmentation in melanocytes, and its overexpression is prevalent in melanomas. Recently, we showed that high expression of IRF4 is linked to dependency in melanoma cells and mortality in patients. Mechanistically, we demonstrated that IRF4 regulates epigenetic silencing factors involved in DNA and histone H3K27 methylation, and consequently, controls the expression of melanoma-critical tumor suppressor genes and key downstream oncogenic pathways.
In order to identify novel genes that melanoma cells rely on for survival or proliferation, we performed CRISPR/Cas9 drop-out screens. One set of screens was aimed at identifying single individual genes necessary for cell fitness. The other set of screens was aimed at uncovering genes, when knocked-out, compromised cell fitness synthetically with IRF4 deficiency. For each set, we screened in replicate two publicly available sub-genomic guide RNA libraries, one targeting genes involved in gene expression and the other targeting genes involved in apoptosis and cancer processes. In order to detect drop-outs between the start and the end of the experiments, we analyzed samples quantitatively via next generation sequencing of guides and the MAGeCK algorithm. Depletion of guides targeting core essential genes verified the screens' ability to detect dependency genes. We then defined all significant dependency genes in their respective screens. We observed that, in addition to core essential genes, several genes that are deemed druggable show up as essential for melanoma cell fitness, some of which not previously reported. Several additional genes show up only in IRF4-knock out state, suggesting synthetic dependency. We followed up with pathway enrichment analyses to identify the prominent cellular pathways with dependency genes. Here, we observed gene transcription and translation, and cell cycle/mitosis as pathways enriched for melanoma dependency genes, among others. In addition, RNA processing and protein degradation are among the pathways enriched for dependency genes in the IRF4-knockout setting.
The dependency genes uncovered in these CRISPR/Cas9 drop-out screens are expected to pave the way for novel targeting approaches in melanoma by themselves or in combination with IRF4.

#2772 Comparison of three single-cell CRISPR-Seq protocols on the 10X Genomics platform.

Donato Tedesco, Tianbing Liu, Mikhail Makhanov, Nadya Isachenko, Dongfang Hu, Paul Diehl, **Alex Chenchik**

Cellecta, Inc., Mountain View, CA

Different methodologies exist for single-cell CRISPR (scCRISPR) screens, each with its own requirements concerning sgRNA scaffold, sgRNA library vector design, instrumentation, and reagents. This study compares the performance of three distinct scCRISPR protocols: 10X Genomics 3' scCRISPR, CROP-Seq (using a modified 10X 3' scCRISPR protocol), and 10X Genomics 5' scCRISPR. 10X 3' scCRISPR relies on the CS1 capture sequence inserted in the sgRNA tracr to detect expressed sgRNAs. CROP-Seq protocol requires the sgRNA sequences to be embedded in an expressed mRNA for detection. 10X Genomics 5' scCRISPR detects sgRNAs without the need for scaffold modifications or inclusion in a mRNA transcript. A modification of the 10X 5' scCRISPR protocol also allowed us to incorporate sc-targeted RNA-seq, decreasing NGS costs and potentially leading to better transcriptional profiling data. The findings of the study showed that while the CROP-Seq and 10X-CS1 approaches are equally effective for single-cell sgRNA detection and gene expression profiling, the CROP-seq approach was superior in the sgRNA's ability to induce the expected phenotype. The 10X 5' scCRISPR protocol proved to be superior to both CROP-Seq and 10X-CS1 in sgRNA detection, and comparable if not better to CROP-seq in gene expression profiling and sgRNA activity. Also, the 5' scCRISPR targeted RNAseq approach proved to be technically successful, although it did not provide the expected transcriptional profiling improvement. Given the ease of use, the quality of the results, and the lack of need for specialized sgRNA scaffold or library design, 10X 5' scCRISPR should be the protocol of choice for scCRISPR screens utilizing the 10X Genomics platform.

#2773 The Gundersen Precision Oncology Cohort: a biospecimen resource for mutation characterization and target discovery.

Peter Feiszt, **Paraic A. Kenny**

Gundersen Medical Foundation, La Crosse, WI

Background: The generation and widespread sharing of large somatic mutation datasets has revolutionized our understanding of genetic mechanisms driving cancer initiation and progression. For well-understood mutations, this has created the opportunity for individualized therapeutic intervention in a wide range of malignancies. Beyond these well-characterized mutations, there exists a wide range of alterations that remain poorly understood and which require some kind of functional genomics analysis to determine whether such patients are eligible for the targeted therapy used for more typical mutations in that gene, whether these alterations are passenger mutations that don't affect throughput on the associated signaling pathway, or reflect another more nuanced phenomenon. Whether using ectopic overexpression in cell lines or generation of genetically modified mice, addressing this problem at scale using functional genomics approaches is challenging. A key limitation is the absence of the original tissue context in which that variant co-evolved with other genetic alterations as well as data on the clinical response of the patient to successive lines of therapy. This can be addressed, in part, by the banking of tissue from patient cohorts who have had comprehensive genomic profiling as part of their routine clinical care. For this purpose, we have established the Gundersen Precision Oncology Cohort.
Methods: The Gundersen Precision Oncology Cohort is a retrospective collection of tissue specimens, NGS reports and associated clinical data from deceased patients who received cancer care at the Gundersen Health System. The cohort is updated twice per year and banked tissue specimens are available for collaborative research studies. High resolution digital pathology whole slide images are available for artificial intelligence and machine learning studies.
Results: At November 26, 2024 there were data from 908 patients (913 tumors) in the cohort, with specimens tested between 2015 and 2024. 55 tumor types were represented, of which lung (n = 225), colorectal (n = 101), pancreas (n = 69), esophageal (n = 62) and breast (n = 56) were most common. The most common diagnostic companies were STRATA Oncology (n = 533) and Tempus (n = 332). Most patients had testing at a single timepoint, but longitudinal testing is available for 40 patients. We present data from some of the studied subgroups, including atypical point mutations in BRAF as well as fusions affecting BRAF and ROS1.
Conclusions: This cohort, available for collaborative studies via the Gundersen Cancer Biobank, illustrates the value of banking well-annotated biospecimens to address outstanding questions in oncology.

#2774 Pan-cancer chromatin state profiling suggests enhancer-heterogeneity and precision medicine approach.

Mayinuer Maitituoheti, Emre Arslan, Ayush T. Raman, Christopher Terranova, **Kunal Rai**

Genomic Medicine and MDACC Epigenomics Therapy Initiative, The University of Texas MD Anderson Cancer, Houston, TX

Epigenetic aberrations are a hallmark of cancer; however, systematic chromatin state maps of cancer cells are unavailable. We generated and analyzed 803 histone mark profiles in 142 cancer cell lines and 114 human tumors belonging to 9 solid tumor types. Irrespective of their cell-of-origin, cancer cells segregate from normal tissues based on their enhancer patterns, suggesting enhancer deregulation is a fundamental epigenetic feature in cancer. Enhancer based clustering defined 5 distinct subgroups of cancer cells (EpiC1-5) with unique developmental trajectories, molecular features and dependencies. Importantly, we define a set of core TFs that are critical for EpiC-specific enhancer patterns and survival. Notably, EpiC4 represented a predominantly epigenetic, pan-cancer subtype that displays poor survival, activation and dependence on a FN1-CAV1-SRC-PI3K-AKT signaling network. Together, these data uncover enhancer heterogeneity in pan-cancer systems with identification of a novel enhancer-based subtype and identify potential new therapeutic targets associated with unique epigenetic features.

#2775 High-resolution mapping of oncogenic structural changes in osteosarcoma.

Andrew Scott Clugston

Pediatric Oncology, UCSF - University of California San Francisco, San Francisco, CA

We have leveraged long-read and spatial sequencing modalities to identify cancer-specific oncogenic alterations for treatment of osteosarcomas.

Osteosarcoma is archetypal of cancers driven by aneuploidy and structural rearrangement rather than point mutations. The functional consequences of many SVs stem from their effect on multidimensional genome organization. SVs can enable enhancer hijacking, alter boundaries of topologically associated domains (TADs), and move gene loci to different regulatory compartments to affect gene expression. SVs can also lead to the formation of extrachromosomal DNA amplicons (ecDNA), which can be megabases in size and can incorporate both genes and regulatory elements to result in substantial increases in gene transcription and intratumor heterogeneity. Complex SVs may have profound implications for prognosis and treatment in OS and other cancers yet they remain poorly understood. By incorporating long-read optical genome mapping (OGM) and chromatin conformation capture sequencing (HiC), we studied SVs in osteosarcoma with the goal of elucidating their contribution to cancer development.

We studied a unique panel of 11 OS patient-derived xenograft (PDX) cell lines which have been previously characterized regarding response to targeted therapies as well as genomically via whole genome (WGS), RNA (RNA-seq), and chromatin accessibility (ATAC-seq) sequencing. To understand the complex genomic reorganization that occurs in osteosarcoma, we integrated OGM and WGS data for all 11 cell lines to describe detailed genome-wide SVs including ecDNA formation. PDX lines assayed using OGM exhibited between 106 and 704 translocations each (versus zero detected in germline controls) and we identified dozens of potential ecDNA amplicons, including one that may explain exceptionally high expression of YAP1 and BIRC3 transcripts. Further, we use HiC to determine which ecDNA amplicons appear to be free-floating based on their genome-wide interaction scores, noting significantly greater interaction in between 33% and 67% of ecDNA detected using OGM and break point graph-based methods. We also used HiC to describe the topological environment resulting from genome-wide SVs and observed potential enhancer hijacking events affecting dozens of known oncogenes including MYC and CDK4. HiC interaction maps among six cell lines reveal genomic compartments and TAD boundaries in detail, with 4,167 unique TAD boundaries identified among six cell lines using 50kb genomic bins. Changing TAD boundaries are associated with numerous SVs and/or copy number discrepancies, and are associated with genomic regions that contain genes with dysregulated RNA transcript levels. Overall, OGM and HiC modalities add considerably to our ability to detect oncogenic SVs and the genes they affect.

#2776 Leveraging HiChIP to assess chromatin interactions of lung cells upon smoking-related BaP exposure and identify target genes of lung cancer risk-associated variants.

Elelta Sisay, Thong Luong, Chia-Han Lee, Mai Xu, Jinhu Yin, Jiyeon Choi

NIH-NCI, Rockville, MD

Lung cancer is the leading cause of cancer-related deaths in the United States, with lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) as the most frequent subtypes. These subtypes have distinct biological origins and risk loci, as identified through genome-wide association studies (GWAS), which are influenced by environmental exposures such as smoking. GWAS variants, largely found in non-coding regions, are hypothesized to function as cis-regulatory elements (CREs) that influence gene expression. However, CREs do not always regulate the nearest gene and can act over megabase distances. Chromatin conformation capture (Hi-C) assays can link GWAS variants to their target genes over long ranges, but these datasets are scarce and often derived from lung cancer cell lines, which may not recapitulate early tumor development. To address this gap, we performed HiChIP, which combines Hi-C and chromatin immunoprecipitation (ChIP) to profile chromatin interactions at active enhancer regions (marked by H3K27ac) in two non-tumorigenic lung cell lines: HPL1-D and BEAS-2B. These cell lines model the alveolar and bronchial tissues where LUAD and LUSC originate, respectively. To determine molecular effects of smoking on chromatin interactions, we exposed cells to the tobacco smoke carcinogen benzo[a]pyrene (BaP). We tested multiple time points to establish acute (48-hour) and chronic (10-day) BaP protocols, evaluating transcriptional responses, DNA damage, and viability. Acute exposure induced DNA damage marked by γ-H2AX and dose-dependent *CYP1A1/CYP1B1* responses by 48 hours, while chronic exposure elicited stronger nuclear metabolic and inflammatory responses measured by *RRM2* and *IL1R1* expression, respectively. The IC_{20} dose of BaP, determined as 0.10 µM after 10- and 20-day treatments, guided the selection of 0.10 µM and 0.25 µM BaP for chronic exposure and 0.25 µM and 0.50 µM for acute exposure, with DMSO as a control. Notably, BEAS-2B cells showed significant responses to BaP, while HPL1-D did not. Critical optimization steps in HiChIP to ensure ChIP efficiency, sensitivity, and specificity include complete cell lysis and efficient DNA shearing by sonication. Incorporating a FACS filter successfully reduced clumped cells and improved cell lysis. Optimizing lysis time and sonication power achieved the desired chromatin fragment distribution range. With an optimized workflow, we use the Arima-adapted MAPS and FitHiChIP pipelines to perform quality control, identify interacting regions, and analyze differential loops between conditions. By integrating GWAS data with chromatin interaction profiles, we aim to pinpoint functional variants and their regulatory genes influenced by cell type and BaP exposure. This work will further our understanding of lung cancer etiology while providing a unique dataset for the public.

#2777 Smoking, clonal evolution, and lung cancer risk.

Edward J. Evans[1], Fabio Marongiu[2], Emilia Lim[3], Faiz Jabbar[4], Ferriol Calvet[5], Shi Biao Chia[1], Amy Briggs[1], Nuria Lopez-Bigas[5], Moumita Ghosh[1], Mariam Jamal-Hanjani[6], York Miller[1], Charles Swanton[4], James V. DeGregori[1]

[1]University of Colorado Anschutz Medical Campus, Aurora, CO,[2]University of Cagliari, Cagliari, Italy,[3]University of British Columbia, Vancouver, BC, Canada,[4]The Francis Crick Institute, London, United Kingdom,[5]Institute for Research in Biomedicine Barcelona, Barcelona, Spain,[6]University College London, London, United Kingdom

Lung cancer is the leading cause of cancer mortality in the U.S. and in the world. Although tobacco use has steadily declined with a corresponding decrease in lung cancer incidence, the impact of smoking on the progression to lung cancer and its mechanisms are largely unresolved. This is further highlighted by the fact that the presence of oncogenic mutations persists in histologically normal tissue for years, even decades. Therefore, we sought to understand the mechanisms by which smoking exposure alters the somatic mutational landscape to result in lung oncogenesis. To observe somatic mutations, we used a rare-mutation detection technique, DuplexSeq, to analyze somatic variants across a 50kb panel of 31 genes associated with lung cancer from 400+ lung samples. We acquired DNA from lung cells from bronchoscopy brushings (BDRE cohort) and surgical samples of histologically normal tissue (PEACE and TRACERx cohorts) from people who currently, formerly, or never smoked cigarettes. We characterized mutations using bioinformatic pipelines and various databases. For each cohort, we analyzed the mutational landscape at the panel, gene, and nucleotide level. Across the panel, we observed higher summations of the variant allele frequencies (VAFs) from those with a smoking history than those who abstained from smoking, indicating that smoking selected for larger clones in cancer-associated genes. Despite the different nature of the samples across cohorts, numerous genes showed increased VAFs and altered dN/dS measures of selection independent of smoking status when compared to the control *TIAM2* gene, suggesting that selection is already at play in histologically normal tissue. When looking at the nucleotide level, the most deleterious and cancer-associated mutations and drivers (based on the Cancer Genome Atlas and COSMIC) are most prevalent in the people who smoked. We also conducted Principal Component Analysis on the mutations from multiple lung regions of the same individual. Here, we observe higher intra-lung similarity of mutational landscape than similarity based on smoking status, highlighting the uniqueness of mutational landscapes to each individual. Using CRISPR technology, we recapitulated mutations observed in the human tissue samples in air-liquid interface and mouse models of the lung, where we observed disruptions in cell proliferation and differentiation. With the duality of characterizing the mutational landscape in the lung and modeling the observed mutations, we can begin to elucidate the mechanisms by which smoking can induce oncogenesis and understand the intermediate phenotypes in the progression of lung cancer. Altogether, these results show that while lung tissue exhibits mutation-driven clonal expansions independent of smoking history, that smoking enhances selection for particular cancer-associated mutations consistent with the increased risk of lung cancers, emphasizing the value of precision diagnoses and prevention.

#2778 Large-scale multi-omic analysis identifies noncoding somatic driver mutations and nominates *ZFP36L2* as a driver gene for pancreatic ductal adenocarcinoma.

Jun Zhong[1], Aidan O'Brien[1], Minal Patel[1], Daina Eiser[1], Michael Mobaraki[1], Irene Collins[1], Li Wang[2], Konnie Guo[2], ThucNhi TruongVo[2], Ashley Jermusyk[1], Maura O'Neill[3], Courtney D. Dill[1], Andrew D. Wells[4], Michelle E. Leonard[4], James A. Pippin[4], Struan F. A. Grant[5], Tongwu Zhang[1], Thorkell Andresson[3], Katelyn E. Connelly[1], Jianxin Shi[1], H. Efsun Arda[2], Jason W. Hoskins[1], Laufey T. Amundadottir[1]

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, MD,[2]Center for Cancer Research, National Cancer Institute, National Institutes of Health, Rockville, MD,[3]Frederick National Laboratory for Cancer Research, Leidos Biomedical Research Inc, Rockville, MD,[4]Center for Spatial and Functional Genomics, Children's Hospital of Philadelphia, Rockville, MD,[5]Division of Human Genetics and Center for Applied Genomics, Children's Hospital of Philadelphia, Rockville, MD

Pancreatic ductal adenocarcinoma (PDAC) is predicted to become the second leading cause of cancer-related mortality in the U.S. by the end of this decade. Due mainly to a late stage at diagnosis and lack of effective treatment, PDAC is a highly lethal malignancy with an overall 5-year survival rate of 13%. This highlights an urgent need for further research into the etiology of PDAC to improve outcomes for this cancer of unmet need. Cancer develops due to somatic and germline genetic factors exerting their influence within the cellular context of environmental factors. Most somatic mutations are in noncoding regions of the genome, but identification of noncoding somatic driver mutations remains challenging. To comprehensively characterize noncoding driver mutations for PDAC, we first created genome-scale maps (2,929,610 elements) of accessible chromatin regions (ACRs) (using ATAC-Seq) and histone modification marks (HMMs) (using ChIP-Seq for H3K27Ac, H3K27me3, H3K4me1 and H3K4me3 marks) in pancreatic cell lines and purified pancreatic acinar and duct cells. Integration with whole-genome mutation calls from 506 PDACs revealed 314 ACRs/HMMs significantly enriched with 3,614 noncoding somatic mutations (NCSMs). Although a majority of these were only observed in a single patient, using enrichment analysis, we identified 988 NCSMs located to ACRs/HMMs where at least two PDAC patients had a mutation within a 31bp window (selected as the longest ENCODE transcription factor (TF) motif). On one hand, to identify the potential target genes for the 988 NCSMs highlighted by our enrichment analysis, chromatin interactions between promoter and enhancer regions by high-resolution promoter-focused capture C (in-house datasets) and HiChIP revealed 416 target genes putatively implicated by these NCSMs, and differential expression analysis revealed six significant NCSMs/target gene pairs by RNA-seq. One the other hand, to functionally assess if the 988 NCSMs alter gene regulatory activity, the functional assessment using massively parallel reporter assays (MPRA) (in-house datasets) identified 178 NCSMs impacting reporter activity (19.45% of those tested) in one or more cell lines. Focused luciferase validation confirmed negative effects on gene regulatory activity for NCSMs near *CDKN2A* and *ZFP36L2*. For the latter, CRISPR interference (CRISPRi) further identified *ZFP36L2* as a target gene (16 - 24% reduced expression, $P = 0.0230\text{-}0.0047$) with disrupted KLF9 binding likely mediating the effect. Our integrative analysis provides a catalog of potentially functional noncoding driver mutations, nominates *ZFP36L2* as a PDAC driver gene and highlights potential therapeutic targets. Moreover, our approach provides a paradigm for employing a multi-omics approach to decipher complex noncoding mutations related to tumorigenesis.

#2779 Whole transcriptome spatial exploration of cultured tumor cells at subcellular resolution.

Yi Cui, **Shanshan He**, Margaret Hoang, Rachel Liu, Tushar Rane, Rustem Khafizov, Haiyan Zhai, Christine Kang, Michael Rhodes, Joseph Beechem

Bruker Spatial Biology, Seattle, WA

Cancer research has long sought to understand the complex tumor microenvironment and the molecular underpinnings of tumor progression. Traditional methods often fail to capture the spatial organization and cell-cell interactions within tumors. The CosMx® Spatial Molecular Imager (SMI) with its CosMx Whole Transcriptome (WTX) panel offers a transformative solution, providing spatially resolved, single-cell transcriptomics with nanometer-scale precision. This platform enables the mapping of RNA molecules within their native tissue context, which is essential for uncovering critical biological insights related to cancer cell behavior, tumor microenvironment interactions, and therapeutic responses.

We developed and optimized a tailored protocol for in vitro cell-based assays using the CosMx WTX platform. Key steps, including permeabilization and enzymatic digestion, were fine-tuned to preserve RNA integrity and cellular morphology, ensuring high-quality transcript detection. Cancer cell lines, U2OS (osteosarcoma) and SH-SY5Y (neuroblastoma), were cultured and analyzed using CosMx WTX to map the spatial distribution of RNA transcripts. The data were compared to bulk RNA-seq results to validate reproducibility and accuracy. The assay's spatial resolution allows for precise mapping of individual RNA molecules within their spatial context, facilitating phenotype-associated spatial transcriptomics.

In our analysis, the CosMx WTX platform successfully quantified approximately 7,300 transcripts per cell in U2OS cells and 1,900 transcripts per cell in SH-SY5Y cells, showing high reproducibility and strong agreement with bulk RNA-seq data. This high-throughput capability allows for robust transcriptomic profiling of diverse cancer cell types. By correlating gene expression data with morphological and functional phenotypes, we were able to visualize the spatial organization of transcripts within cells. The platform's nanometer-scale resolution also supports mapping of cancer organoids, offering insights into intratumoral heterogeneity, treatment resistance, and metastatic processes.

The CosMx WTX cell-based assay represents a significant advancement in spatially resolved transcriptomics. By enabling high-resolution analyses, this platform provides valuable insights into cancer biology and offers a powerful tool for drug screening and companion diagnostic development. The integration of spatial transcriptomics with protein-level data holds the potential to transform our approach to cancer diagnostics and therapy, advancing precision medicine and improving patient outcomes.

#2780 Topoisomerase IIb binding underlies frequently mutated elements in cancer genomes.

Juri Reimand[1], Christian A. Lee[1], Robin H. Oh[2], Zoe P. Klein[1], Nina Adler[1], Sana Akhtar Alvi[3], Ellen Langille[2], Elisa Pasini[4], Kevin C. L. Cheng[1], Diala Abd-Rabbo[1], Huayun Hou[3], Ricky Tsai[2], Mamatha Bhat[1], Daniel Schramek[2], Michael D. Wilson[3], Liis Uuskula-Reimand[3]

[1]Ontario Institute for Cancer Research, Toronto, ON, Canada,[2]Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada,[3]Hospital for Sick Children, Toronto, ON, Canada,[4]University Health Network, Toronto, ON, Canada

Type-II topoisomerases resolve topological stress in DNA through controlled double-strand breaks. While TOP2A is a chemotherapy target in proliferating cells, the ubiquitously expressed TOP2B is a potential off-target. Here we explore roles of TOP2B in mutagenesis by generating DNA-binding maps of TOP2B, CTCF, and RAD21 in human cancer samples and analyzing these maps for driver mutations and mutational processes in 6500 whole cancer genomes. TOP2B-CTCF-RAD21 and TOP2B-RAD21 sites are enriched in somatic mutations and structural variants (SVs), especially at evolutionary conserved sites displaying high transcription and long-range chromatin interactions. TOP2B binding underlies SVs and hotspot mutations in cancer-driving genes such as TP53, MYC, FOXA1, and VHL, and many cis-regulatory elements. We show that the TOP2B-bound mutational hotspot at RMRP drives tumor initiation and growth in vivo. These data highlight TOP2B as a protector of the genome from topological challenges whose aberrant activity promotes driver and passenger mutations in cancer genomes.

#2781 Enabling low cell input and high throughput processing of cell fixation for single cell RNA profiling using combinatorial barcoding.

Olivia Kaplan, Sarah Schroder, Vuong Tran, Simone Marrujo, Alec Salvino, Ajay Sapre, Ian Dowsett, Zeynep Sayar, Alex Koriath, Alex Sova, Gary Geiss, Charles Roco, Alex Rosenberg

Parse Biosciences, Seattle, WA

Single cell analysis is a critical approach to tease out nuanced information in any biological system. Combinatorial barcoding is an extremely efficient method for scRNA-seq at scale, achieving both sensitivity in gene detection and flexibility in sample input. This approach, however, is challenging when starting with sample inputs of less than 100,000 cells. To address this, we have developed a modified fixation and cell capture method for low sample inputs that is compatible with the combinatorial barcoding approach. This allows researchers working with low sample inputs to fix, store and barcode their cells efficiently without the need for costly or specialized instruments. Low Input Fixation can be used to fix and profile up to 384 samples in parallel, results in a shorter workflow, and enables consistent handling (which minimizes both experimental error and burden on scaling). This method maintains the stability of fixed samples at -80℃ and capture efficiency remains consistently high for inputs as low as 10,000 cells. The workflow maximizes sample recovery while providing accurate and comprehensive results of transcriptional changes from experimental perturbations. As proof-of-concept we screened a cancer drug panel with cell input amounts between 10,000 - 20,000 cells per treatment. Robust cell capture minimizes loss during drug perturbations resulting in consistent gene detection. Whole transcriptome analysis using Evercode WTK identifies changes in gene expression associated with each drug class and cell type. Results of the screen validate putative drug targets in the panel. Additionally, panel-based perturbation allows for detection of off-target impacts at scale. This work demonstrates the combination of low input fixation with combinatorial barcoding to enable high throughput cancer drug and compound screening.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: microRNAs and Other Noncoding RNAs 2
Poster Session

#2785 Effects of melatonin and 17β-estradiol on the miR-222-3p's expression and activity in IDCs cells.

Bruna Cristine De Almeida, Kelly Pedrozo Ferreira, Jose Maria Soares-Junior, Juciara Costa Silva, Katia Candido Carvalho, Edmund Chada Baracat

Departamento de Obstetricia e Ginecologia, Faculdade de Medicina da Universidade de Sao Paulo, Sao Paulo, Brazil

Background: The *DICER* gene encodes a protein that is required for RNA interference and microRNA (miRNA) synthesis. It is known that miRNAs have a substantial impact on cancer origin and progression, and they are deeply involved in the posttranscriptional regulation of genes expression. A growing body of evidence indicates that the oncomiR *miR-222-3p* overexpression promotes proliferation, migration, invasion, and metastasis of breast cancer (BC) cells as well as chemoresistance, by the regulation of its target genes. However, in this circuit, the *miR-222* negatively regulates the expression of *DICER*, which can be involved with tumor malignant behavior. In an attempt to modulate the *miR-222* expression, we treated invasive ductal carcinoma (IDC) cells with melatonin (Mel), which exerts anti-tumor actions by regulating miRNA expression and may protect against BC. In addition, we stimulated the IDC cells with 17β-estradiol (E2) to mimic the hormonal conditions. Hence, our goal was to evaluate the *miR-222* expression under the effects of Mel and E2 treatments in IDC cells and to assess the expression of its target gene *DICER*.
Methods: MCF7 and MDA-MB231 cell lines treatments were performed with 3000 µM Mel and 100 nM E2, and the two drugs were combined (M+E2) and were replaced daily. After the treatment, the RNA was extracted and the reverse transcription was performed. Expression of *miR-222* and *DICER* were assessed by RT-PCR. Data analysis was performed with ExpressionSuite Software v1.3.
Results: *miR-222* showed a significant decrease with Mel (fold: 0.701) and M+E2 (fold: 0.829) treatments in MCF7 cells, compared to control. However, the E2 treatment did not significantly affect the *miR-222* expression. Inversely, *DICER* expression was increased with suppression of *miR-222* after the treatments, both Mel (fold: 1.436) and M+E2 (fold: 1.368). After the E2 treatment, *DICER* presented low expression levels (fold: 0.720), compared to control. In MDA cells, the expression levels of *miR-222* were increased after Mel (fold: 1.095) and M+E2 (fold: 1.109) treatments, compared to control. *miRNA-222* showed a slight reduction in expression with E2 treatment (fold: 0.856). Nonetheless, *DICER* did not present differences of expression. Although Mel and M+E2 led to a greater expression of *miR-222*, these treatments also increased the expression of *DICER* (Mel fold: 2.011 and M+E2 fold: 1.979).
Conclusion: These data revealed that *miR-222* contributes to the tumorigenicity of IDC through the down-regulation of *DICER* in MCF7 cells. In addition, the anticancer effect of Mel modulates the critical expression of *miR-222-3p* and its target *DICER* in IDC cells.
Supported by: FAPESP 2022/04174-2 and 2018/24224-9

#2786 Investigation on the role of tumor suppressive microRNAs in angiosarcoma.

Bozhi Liu, Jillian Stachon, Annaleigh Powell-Benton, Sagar Utturkar, Nimod Janson, Anthony Murphy, Nadia Lanman, Jason Hanna

Purdue University, West Lafayette, IN

Angiosarcoma (AS) is an aggressive tumor resulting in a very poor prognosis for patients. MicroRNAs (miRNAs) regulate gene expression and play important roles in a variety of diseases including cancer. Our previous studies demonstrate miRNA loss leads to AS in mice. To further understand the role of miRNAs that function as critical tumor suppressors in AS, we performed a miRNA-focused CRIPSR-Cas9 screen. The gRNA library was transduced into a human AS cell line expressing doxycycline (dox)-inducible Cas9. After passaging the cells for 28 days, sgRNA amplicon sequencing was performed to determine the change in the frequency of gRNAs. We anticipated that the loss of function of tumor suppressing miRNAs would lead to gRNA enrichment in the cells. Indeed, three miRNAs were identified as hits with significant enrichment of multiple gRNAs, including miR-200b, miR-181b, and miR-410. We hypothesize these miRNAs act as tumor suppressors and reduce proliferation and cell viability in AS. Based on our RNA-seq data, all three miRNAs are expressed in normal endothelial cells. Additionally, miR-410, miR-181b and a miR-200 family member are significantly downregulated in a human AS cell line and miR-410 is frequently deleted in AS patient samples. We are currently conducting functional validation studies by overexpressing these miRNAs in a panel of AS cell lines by lentiviral based expressing the pre-miRNAs. In preliminary results, the overexpression of miR-410 consistently inhibits cell proliferation and colony formation ability in both human and mouse AS cell lines. Additionally, to gain mechanistic insights for these miRNAs, a novel technique called AgoTRIBE was used to identify important mRNA targets regulated by miRNAs. In AgoTRIBE, the miRNA effector Ago2 is fused to ADAR's RNA editing domain, directing ADAR activity to miRNA natural targets. The ADAR editing events on miRNA targets can be detected by RNA seq. We used a dox-inducible lentiviral vector expressing human ADAR (hADAR) or hADAR-Ago in a mouse AS cell line. In preliminary data, we validated the protein expression of ADAR and ADAR-Ago upon dox treatment and the cellular localization of these complexes in cytoplasm. We are working on validating the function of AgoTRIBE by RNA seq and utilize this tool to identify targets regulated by our screen hits. This project represents the first global screen on miRNAs in AS. Functional validation of the hits from the screen in combination with the mechanistic insights from AgoTRIBE can provide fundamental evidence for the role of tumor suppressive miRNAs in AS.

#2787 Effect of miR-494 modulation in colorectal cancer (CRC): Clinical outcome, target expression and cell characteristics.

P. Mittal[1], J. Millstein[1], S. Soni[1], F. Battaglin[1], K. Ashouri[1], S. Algaze[1], P. Jayachandran[1], Y. Yang[1], L. T. Gonzalez[1], U. H. Shah[1], W. Zhang[1], F.-S. Ou[2], A. P. Venook[3], I. K. Singh[4], H. J. Lenz[1];

[1]USC - University of Southern California, Los Angeles, CA, [2]Mayo Clinic Rochester, Rochester, MN, [3]University of California, San Francisco, CA, [4]University of Delhi, New Delhi, India

Background. MicroRNAs are small, single-stranded non-coding RNA molecules that play major role in gene regulation. miRNAs have been reported as both tumor suppressive and oncogenic and are promising candidates for cancer prognosis and treatment. miR-494 is known to promote cell migration and invasion in CRC. Our study aimed to further explore the role of miR-494 modulation (inhibition and overexpression) in CRC.

Methods. We evaluated the impact of tumor gene expression of *MIR494* on outcomes (overall survival: OS; progression-free survival: PFS) in data for 433 metastatic CRC (mCRC) patients (pts) (bevacizumab [bev] (N = 226) vs cetuximab [cet] (N = 207)) enrolled in randomized phase III CALGB/SWOG 80405 trial obtained from Alliance for Clinical Trials in Oncology. RNA extracted from FFPE tumor samples was sequenced using the HiSeq 2500 (Illumina). Gene expression was divided into high, medium and low. Associations were assessed by log-rank test and multivariable Cox proportional hazards regression model. Treatment-gene interactions were analyzed using the likelihood ratio test. CRC cell lines HCT116 and HT29 were transfected with miR-494 inhibitors and mimics for 48 hours and total RNA was collected, followed by RT-qPCR (miR-494 and target genes *BMAL1*, *PAK1*, *CDH1*, *CDH2*, *SNAI1*, *TWIST*) and clonogenic assays.

Results. High miR-494 was significantly associated with worse PFS in overall (median survival: high vs low: 9.3 vs 11.3 months, $P<0.025$) and cet-treated (median survival high vs low: 9.2 vs 9.6 months, $P<0.025$) mCRC cohorts, while no significance was observed in bev-treated mCRC. Multivariable Cox model showed significant association between high miR-494 and worse PFS and OS in the cet-treated cohort; however, treatment-gene interaction was not statistically significant. Expression analysis revealed miR-494 was significantly reduced and increased following inhibitor and mimic transfection, respectively. Differential gene expression of the target genes analyzed was observed between the miR-494 inhibitor and mimic-treated HCT116 and HT29 samples. In HCT116 cells, inhibition of miR-494 significantly ($P < 0.01$) reduced expression of *PAK1*, while increased the expression of *CDH1*. The number of colonies formed relatively increased after transfection with miR-494 inhibitor compared to miR-494 mimic.

Conclusion. Our findings showed that high miR-494 expression correlated with worse PFS overall and in cet-treated mCRC pts. It would be interesting to check for potential associations with EGFR signaling (direct or indirect). Further studies are warranted for efficient targeting and to gain better understanding of the associated signaling involved in miR-494 modulation in CRC. Support: U10CA180821, U10CA180882; U10CA180888 (SWOG): Genentech. https://acknowledgments.alliancefound.org; P30CA014089, UG1CA180830 [HJL]. Clinicaltrials.gov Identifier: NCT00265850

#2788 Targeting miR-92b reduces neurosphere formation in glioblastoma cancer stem cells.

Annelis O. Sanchez-Alvarez[1], Diego Garcia-Ortiz[2], Misael Rivera-Garcia[3], Alanis M. Labeaga-Sainz[3], Pablo E. Vivas-Mejia[2]

[1]Clinical & Translational Research, University of Puerto Rico Comprehensive Cancer Center, San Juan, Puerto Rico,[2]Biochemistry, University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico,[3]Interdisciplinary Studies of Natural Sciences, University of Puerto Rico Rio Piedras Campus, San Juan, Puerto Rico

Glioblastoma (GBM) is a grade IV brain tumor with a dismal prognosis, driven by aggressive brain tumor-initiating cells (BTICs). This study focuses on targeting microRNA-92b-3p (miR-92b-3p), a microRNA aberrantly overexpressed in GBM that correlates with poor patient outcomes. We utilized primary BTIC neurospheres M1123 and GBM1A, derived from GBM patients, to investigate miR-92b-3p's role in tumor progression and therapeutic resistance. Our findings revealed a striking 40-fold higher basal expression of miR-92b-3p in GBM1A compared to M1123. Functional studies showed that miR-92b-3p inhibition significantly disrupted neurosphere formation in both cell lines up to 96 hours post-transfection, highlighting its essential role in maintaining GBM stemness. Additionally, the IC50 values for temozolomide, the standard GBM chemotherapeutic agent, were 27 µM for M1123 and 30 µM for GBM1A, suggesting similar baseline resistance. Ongoing experiments are exploring whether miR-92b-3p inhibition sensitizes BTICs to temozolomide, potentially enhancing its efficacy. These results underscore miR-92b-3p’s critical role in neurosphere maintenance and chemoresistance in GBM cancer stem cells. Its overexpression may serve as a biomarker for aggressive tumor behavior, and its inhibition represents a promising therapeutic strategy to target BTICs, disrupt GBM progression, and improve patient outcomes.

#2789 miR-6794-3p inhibits human pancreatic cancer metastasis by regulating the RBBP4/GRHL2 signaling pathway.

H. Kim, E.-T. Oh, Y. Cho, H. Park;
Inha University College of Medicine, Incheon, Korea, Republic of

Metastasis is a major cause of treatment failure in patients with pancreatic cancer, highlighting the urgent need for effective therapeutic strategies. Here, we focused on identifying novel miRNAs with key roles in metastasis of pancreatic cancer. Microarray analysis of miRNA expression in metastatic and non-metastatic pancreatic cancer samples revealed significantly lower expression of miR-6794-3p in the metastatic tumor group. Gain- and loss-of-function approaches using the pancreatic cancer cell lines MIA-PaCa-2 and HPAF-II expressing low and high levels of miR-6794-3p, respectively, indicated a role of miR-6794-3p in suppression of cell invasion, migration, and EMT signaling. Importantly, our results showed that miR-6794-3p exerts its effects by inhibiting expression of the chromatin remodeling factor, RBBP4. The resulting suppression of RBBP4 induced an increase in the levels of GRHL2 involved in regulating invasion, migration, and EMT signaling in metastatic pancreatic cancer cells. Consistent with these findings, low miR-6794-3p expression levels correlate with poor pancreatic cancer patient survival. Additional preclinical experiments on nude mice clearly demonstrated inhibitory effects of miR-6794-3p on pancreatic cancer cell metastasis. The collective results highlight the functional significance of miR-6794-3p as a suppressor of metastasis and support its predictive utility as a prognostic biomarker and therapeutic target in pancreatic cancer.

#2790 Identification of tumor suppressor miRNA to regulate FXYD3 in pancreatic cancer cells.

Ke Xin Yee, Kuen-Haur Lee

PhD program of Cancer Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University-Shuang Ho Campus, New Taipei, Taiwan

Pancreatic cancer (PC) continues to be one of the deadliest cancers globally, ranking among the top causes of cancer-related mortality. Despite significant progress in medical research, effective methods for early diagnosis and treatment remain challenging, contributing to poor survival rates. MicroRNAs (miRNAs), small non-coding RNA molecules that consist of 18-25 nucleotides, have emerged as critical regulators of gene expression as therapeutic agents and diagnostic biomarkers. Recently, the oncogene FXYD3 has gained attention as a candidate for early detection, offering a promising result for research. In this study, we applied bioinformatic analyses to identify potential interactions between tumor-suppressing miRNAs and the oncogene FXYD3. Our findings revealed that several miRNAs directly target FXYD3, highlighting their role in regulating its expression. To validate these predictions, we performed immunohistochemistry (IHC) analysis. The results confirmed that these miRNAs were significantly overexpressed in normal pancreatic tissues compared to PC tissues. These observations suggested that the loss of specific miRNAs could contribute to the upregulation of FXYD3, promoting tumor progression. Interestingly, our study also found differential expression patterns of these miRNAs across various pancreatic cancer cell lines, indicating cell-type-specific regulatory mechanisms. The findings of the functional assays using miRNA mimics or inhibitors provide a foundation for developing miRNA-based therapeutic strategies targeting FXYD3. In summary, this study emphasizes the importance of miRNAs that target FXYD3 in pancreatic cancer. Our research supports their role as tumor suppressors and underscores their therapeutic potential in combating one of the most aggressive and challenging cancers.

#2792 PiRNA as a novel therapeutic in hepatocellular carcinoma: insights from population screening.

Rui Han[1], Qin Qin[1], Ran Liu[1], Tianshu Wu[1], Yaya Guo[1], Peyton Cook[2], Kinsey Garofalo[2], Yujuan Guo[2], Bolni Nagalo[2], **Yong Zhu**[2]

[1]Yale University School of Public Health, New Haven, CT,[2]University of Arkansas for Medical Sciences, Little Rock, AR

Hepatocellular carcinoma (HCC) ranks as the third leading cause of cancer-related death globally and is the fastest-growing cause of cancer-related deaths in the United States. Current therapies, such as chemotherapy and radiotherapy, are largely ineffective in advanced HCC due to tumor-specific characteristics, resistance to drugs and radiation, and substantial adverse effects. Despite breakthroughs in immunotherapy, approximately 80-85% of American patients with advanced HCC fail to respond to treatments. These challenges underscore the urgent need for more effective therapeutic strategies to treat HCC. One emerging therapeutic strategy involves the use of small non-coding RNAs (ncRNAs), which have shown promising efficacy in both preclinical and clinical studies for various cancer types. Piwi-interacting RNAs (piRNAs) are an abundant of small non-coding RNAs (ncRNAs) involved in maintaining genomic stability and represent a promising but largely unexplored avenue for cancer treatment. The current study identified a piRNA candidate and examined its therapeutic implication for the treatment of HCC. First, we performed piRNA arrays for small RNAs isolated from 12 pairs of HCC tissues and normal liver tissues and detected significantly downregulated piRNAs in HCC tissues compared to normal liver tissues. Downregulation of piR-37213 was confirmed using the TCGA small RNA-seq data of HCC tissues (N=331) and normal liver tissues (N=48). Lipid nanoparticles (LNP) were used as a delivery vehicle to capsulate piR-37213 in functional tests. Significant tumor-suppressing effects of piR-37213 were observed in a series of *in vitro* assays ($P<0.01$), including cell proliferation assays, colony formation assays, 3D formation and invasion assays. Systemic delivery of LNP-piR-37213 was administered in animal experiments. Mice (N=44) were treated twice a week for four consecutive weeks via tail vein injection. Tumor signals were significantly reduced by 90% $P<0.001$) after 2 weeks of treatment. Survival was significantly improved ($P<0.05$). Both *in vitro* and *in vivo* experiments suggest that the identified piRNA candidates, which are downregulated in HCC tissues, play a tumor-suppressing role. A genome-wide expression profiling analysis was further performed and found that genes involved in cell cycle proliferation, replication, and DNA repair were significantly downregulated, which further provided molecular mechanisms accounting for the anti-tumor effect of under-expressed piR-37213. This preclinical study represents the crucial initial step in developing a novel, piRNA-based therapeutic strategy for liver cancer and provides proof of principle for the use of piRNAs in future clinical treatment. Future studies are needed to explore the transformative potential of this early-stage, highly innovative, piRNA-based technology in cancer treatment.

#2793 LncRNA CRNDE facilitates head and neck cancer progression by modulating the tsmiRs-oncogenes network regulating multiple oncogenic signaling pathways.

T. Lin, A. Cheng, Y. Chen, H. Huang;
Chang Gung University, Taoyuan, Taiwan

Recent studies have emphasized the pivotal role of long non-coding RNAs (lncRNAs) in tumorigenesis, with CRNDE identified as dysregulated in several cancers. This study explores the role of CRNDE in head and neck cancer (HNC), a globally prevalent malignancy. We demonstrate that CRNDE drives key malignant phenotypes in HNC, including enhanced cell migration, invasion, proliferation, and chemoresistance. RNA sequencing identified numerous downstream effectors, with 195 oncogenes significantly enriched in cancer-related pathways, such as focal adhesion, actin cytoskeleton organization, and PI3K-AKT, Ras, and Rap1 signaling. To elucidate the regulatory mechanisms of CRNDE, we observed that it predominantly localizes in the cytosol of HNC cells, suggesting its primary role in miRNA modulation. Bioinformatic analysis identified 14 miRNAs with strong regulatory potential. Among these, three tumor-suppressive miRNAs (TsmiRs)—miR-181c-5p, miR-29a-3p, and miR-29b-3p—were prioritized based on their clinical relevance from the TCGA-HNSC dataset. Further analysis revealed that these miRNAs potentially regulate 391, 417, and 137 target genes. Integrative analysis demonstrated that these hub miRNAs co-regulate 69 oncogenes, significantly influencing five cancer-related pathways. We constructed a comprehensive CRNDE-TsmiRs-Oncogenes regulatory network, uncovering a complex cross-regulatory mechanism. Moreover, we validated the CRNDE-miR-181c-PI3K/AKT3 axis as a critical pathway in HNC progression. Overexpression of miR-181c-5p markedly suppressed CRNDE-driven cancer progression by downregulating PI3K/AKT expression and mitigating malignant phenotypes, including migration, invasion, and chemoresistance. This study presents a novel CRNDE-TsmiRs-Oncogenes molecular network and highlights the CRNDE/miR-181c-5p/AKT axis as a promising biomarker and therapeutic target for improved HNC management.

#2797 N-linked and O-linked glycosylated RNA enrichment in human prostate cell lines with low/no malignancy.

Esther Jones, Samantha McGuire, Spencer Moen, **Aurora Esquela Kerscher**

Biomedical & Translational Sciences, Eastern Virginia Medical School at ODU, Norfolk, VA

Our lab is the first to characterize glycosylated RNA in the context of human prostate cancer (PCa). PCa is the 2nd leading cancer-related cause of male deaths in the United States. As high-grade forms of PCa often lead to poor prognoses, it is imperative to improve patient stratification screening tools and treatment options to increase patient survivorship. Noncoding RNAs are widely misexpressed in clinical PCa tissues and many act as key tumor suppressors, oncogenes, and metastatic factors in the prostate. Noncoding RNA harbor a range of post-transcriptional modifications (m6A, pseudouridine, uridine tailing) important for RNA folding, stability, transport, and localization. Dysregulation of these RNA modifications is often associated with malignancy, disease aggressiveness, and drug resistance. Noncoding RNA carrying a newly discovered class of glycosylation modifications was recently discovered in cultured human and mouse cells metabolically labeled using bioorthogonal chemistry methods normally employed for glycosylated protein and lipid enrichment. Nothing is known about the existence/significance of prostate glycoRNA or its role in disease. We hypothesized that RNA requires carbohydrate modifications to maintain prostate homeostasis and predicted that the glycosylated state of RNA would correlate with disease progression. We first confirmed that prostate glycoRNA exists in the human prostate. Our studies employed a stringent RNA isolation workflow and were tested using two independent approaches; metabolic labeling with azide click chemistry for biotin-tag detection & lectin northern blot analysis. Using metabolic azidosugar reagents that preferentially labeled N-linked (Ac4ManNAz) or O-linked (Ac4GalNAz) modifications, we detected small (<200 nt) noncoding RNA (but not large RNA) carrying N-linked and O-linked sugars that showed sensitivity to endoglycosidases (PNGase F, O-glycosidase) and glycosylation inhibitors. GlycoRNA expression correlated with PCa progression with preferential enrichment in human prostate cell lines and syngeneic panels with low/no malignancy compared to metastatic lines. Lectin northern blot studies (WGA, PNA, ConA) verified that prostate RNAs carry N-linked and O-lined sugars. Subcellular fractionation studies indicated glycoRNA localization predominantly at the plasma membrane of prostate cells. Results from our RNAseq and glycomics studies are ongoing and will also be discussed. Furthermore, we verified that *C. elegans* grown on Ac_4GalNAz plates expressed glycoRNA, indicating conservation across species. This work will provide novel insight into how RNA modifications impact PCa progression and identify first-in-class clinical tools to improve patient outcomes.

#2798 Long non-coding RNA DUXAP10 regulates mediators of endoplasmic reticulum stress-induced epithelial-to-mesenchymal transition in anaplastic thyroid cancer.

Nicole R. DeSouza[1], Kate Nielsen[1], Danielle Quaranto[1], Michelle Carnazza[1], Tara Jarboe[1], Kaci Kopec[1], Robert Suriano[1], David Garber[2], Steven Hemmerdinger[2], Augustine Moscatello[2], Humayun K. Islam[1], Raj K. Tiwari[1], Jan Geliebter[1]

[1]New York Medical College, Valhalla, NY,[2]Westchester Medical Center, Valhalla, NY

Anaplastic Thyroid Cancer (ATC) is a fast-growing, metastatic and lethal carcinoma with mortality rates reaching ~100%. Understanding the molecular landscape that separates ATC from other slow-growing, highly survivable thyroid carcinoma subtypes is key to biomarker detection, therapeutic successes, and increased patient survival. Long non-coding RNAs (lncRNA) use their extensive DNA, RNA, and protein-binding domains to orchestrate cell function. These multi-ranged regulatory capabilities make their study in ATC salient. LncRNA DUXAP10 is overexpressed in NCBI GEO datasets by 40-fold and 20-fold (GSE33630, GSE85457) in ATC patient tissue and primary ATC cell lines compared to normal thyroid tissue. DUXAP10 expression is higher in samples taken from primary ATC sites (cBioPortal) and its overexpression is correlated with distant metastasis and lower patient survival (cBioPortal, GEPIA).

Our data supports DUXAP10 overexpression in ATC cell line T238, and the transcriptional reduction of its expression was achieved using CRISPRi technology. Supporting experiments show a significant role for DUXAP10 at the interface of metastatic phenotypes driven by the convergence of the Unfolded Protein Response (UPR) and Wnt/β-catenin-induced epithelial-to-mesenchymal transition (EMT). Hypoxia is a central issue at ATC tumor sites, resulting in an unremitting survival adaptation that by-passes ER-stress induced apoptotic branches of the UPR. Our data showed that ER-stress induced upregulation of EMT phenotypes was ameliorated by CRISPRi-reduced DUXAP10 expression- functioning via β-catenin-driven rapid growth induction of PERK-mediated EMT.

DUXAP10 was determined to have strong nuclear localization in T238. Current work includes investigation of its protein-scaffolding role in the nucleus, and how this specific function is impacting mediators of the UPR and Wnt/β-catenin regulation. Other supporting data confirms a role for DUXAP10 in T238 proliferation, clonogenicity, apoptosis, cell-cycle regulation, invasion, migration and rate of RNA synthesis. These data reveal the novelty of DUXAP10, and that its over-expression may increase the negative consequences of ATC metastasis.

#2799 SNORD80-guided 2'-O-methylation stabilizes the lncRNA GAS5 to regulate cellular stress responses.

L. Xu, X. Zhao, Y. Zhang, L. Jin, X. Zhang;
University of Newcastle, Australia, Newcastle, Australia

The introns of the gene encoding the long noncoding RNA (lncRNA) GAS5 host up to 10 C/D box small nucleolar RNAs (snoRNAs). However, whether there is a regulatory and functional relationship between these snoRNAs and GAS5 is unknown. Here, we show that the expression of SNORD80, but not the other snoRNAs, parallels GAS5 expression and is regulated alongside GAS5 in response to cellular stress. The 2′-O-methylation at the A496 site, located within a segment of GAS5 complementing the conserved RNA binding region on SNORD80, promotes GAS5 stability and consequent upregulation. This methylation requires SNORD80, as it is diminished by knockdown of SNORD80 and increased by SNORD80 overexpression, similar to the effects of manipulating the expression of fibrillarin, the methyltransferase of the box C/D small nucleolar ribonucleoprotein particle (snoRNP). The upregulation of SNORD80 in response to cellular stress is due to an enhancement in its stability, which is associated with an increase in its interaction with fibrillarin. Collectively, these results identify a novel role for SNORD80 in guiding 2′-O-methylation to stabilize GAS5. This uncovers a feedforward regulatory loop at the GAS5 gene locus in response to cellular stress and sheds new light on posttranscriptional mechanisms governing lncRNA expression.

#2800 Unraveling long non-coding RNA dynamics in uveal melanoma: Insights into pathogenesis, biomarkers, and targeted therapies.

Vasileios Alevizos[1], Nikitas Gerolimos[2], Sabrina Edralin[3], Clark Xu[4], Akebu Simasiku[5], Georgios Priniotakis[6], George A. Papakostas[7], Zongliang Yue[8]

[1]Karolinska Institutet, Solna, Sweden,[2]University of Aegean, Syros, Greece,[3]University of Illinois Urbana-Champaign, Champaign, IL,[4]Artificial Intelligence & Discovery, Mayo Clinic, Rochester, MN,[5]Zambia University, Lusaka, Zambia,[6]University of West Attica, Attica, Greece,[7]Department of Informatics, Democritus University of Thrace, Kavala, Greece,[8]Auburn University, Auburn, AL

Uveal melanoma (UM), a rare yet aggressive intraocular cancer with an estimated 5 cases per million people annually, presents a high metastatic risk and poor prognosis, highlighting an immeasurable need for novel diagnostic and therapeutic strategies. Not only is UM characterized by complex genetic and epigenetic aberrations, but it is also prone to deregulation of non-coding RNAs that could be used as therapeutic targets of diagnostic markers. In this study, we analyzed whole exome sequencing data from tumor and control samples of patients diagnosed with both uveal and cutaneous melanoma, focusing on a patient with a germline mutation in MBD4. Our framework, made up to work with long non-coding RNAs (lncRNAs), aims to flesh out these molecules' novel roles in disease etiology and progression. We identified several lncRNAs significantly associated with UM progression and computationally validated them in conjunction with microenvironmental factors in existing literature. LncRNAs are instructive in maintaining cellular homeostasis and, when deregulated, contribute to oncogenesis. Our results implied that the interaction between lncRNAs and other non-coding RNAs might be one of the most relevant mechanisms influencing UM pathogenesis. More interestingly, microenvironmental factors and lncRNA interplay are crucial in disease progression. The clinical relevance of our findings was tested using radiation treatment to evaluate potential therapeutic implications. Over and above that, we assessed the potential of lncRNAs as biomarkers and therapeutic targets in personalized medicine for UM patients to address ineffective treatments. The new lncRNA panels could serve as a new avenue for targeted therapy. With an in-depth understanding of the descent of molecular alterations and how lncRNAs interact within the tumor microenvironment, we can better size up potential strategies to cast off the challenges UM poses. Our work provides instructive insights into lncRNAs underpinnings of UM, emphasizing the significance of lncRNAs in tumor biology and their interactions within the microenvironment. We believe our findings will lay the groundwork for future therapeutic strategies and underscore the necessity for continued research to unravel the complex mechanisms in UM pathogenesis.

#2801 Functional characterization of long non-coding RNA *PAINT* and mechanistic insights into its crosstalk with splicing factor hnRNPM in cancer progression.

Subhoja Chakraborty[1], Kavya Ganapathy[1], Angela Rodio[1], Manasi Joseph[1], Mitchell Campbell[1], Julia Soulakova[1], Domenico Coppola[2], Ratna Chakrabarti[1]

[1]University of Central Florida, Orlando, FL,[2]Florida Digestive Health Specialist, Lakewood Ranch, FL

Long non-coding RNAs (lncRNAs) modulate crucial cellular processes in nexus with miRNAs, transcription factors and RNA-binding proteins (RBPs) whose dysregulation is often linked with cancer progression and drug resistance. In aggressive, recurrent and often fatal invasive cancers, there is an unmet need to identify newer targets and their mechanistic roles in cancer progression for improved therapies. Previously, we reported an association of lncRNA *PAINT* with aggressive prostate cancer (PCa) and its involvement in dysregulated expression of several cancer hallmark genes including genes in WNT and AKT signaling pathways. In this study, we tested our hypothesis on the general involvement of *PAINT* in cancer. Our study showed an oncogenic role of lncRNA *PAINT* in metastatic breast cancer (BCa) cells. GSE database-based expression analysis showed *PAINT* upregulation in tumor samples compared to normal patient samples. *In-situ* hybridization (ISH) of tissue microarrays revealed differential upregulation of *PAINT* in aggressive BCa subtypes. *PAINT* inhibition decreased proliferation and migration of triple negative BCa (TNBC) cell lines MDA-MB-231 ($BRCA^+$) and MDA-MB-436 ($BRCA^-$), and improved the sensitivity of MDA-MB-231 cells to Doxorubicin, Docetaxel and Carboplatin. *PAINT* over-expression in an inducible MDA-MB-231-*PAINT* stable subline augmented cell proliferation and migration and increased S-phase cell population. Mass spectrometry of *PAINT*-bound proteins using *in-vitro* transcribed biotinylated *PAINT* and PC-3 cell extracts revealed hnRNPM as an interactor of *PAINT*, which was validated by RNA immunoprecipitation in both BCa and PCa cell lines. Ectopic expression of *PAINT* in an inducible PC-3-*PAINT* PCa subline showed an increased hnRNPM concentration upon induction, promoting CD44 exon-skipping function of hnRNPM in PC-3-*PAINT* and C4-2B-*PAINT* PCa stable sublines. *In-silico* analysis showed prospective hnRNPM-interacting sites on *PAINT*, overlapping with its stem-loop structure forming regions. hnRNPM knockdown in induced MDA-MB-231-*PAINT* cells diminished the effect of *PAINT* over-expression on cell proliferation and migration. Our study demonstrates that the oncogenic role of *PAINT* is partly mediated by the stabilization of its interactor hnRNPM, in turn exerting its tumorigenic and epithelial-mesenchymal transition effects on cancer progression. This study establishes the benefit of *PAINT*-silencing on enhancing the antitumor effects of hnRNPM knockdown, that can be utilized in combination with chemotherapeutics for treating aggressive forms of BCa and PCa.

#2802 Circular RNA variants of the*NUMB* gene modulate the oncogenicity of oral squamous cell carcinoma.

C.-H. Chou, P.-Y. Chen, S.-C. Lin, **K.-W. Chang**;
National Yang Ming Chiao Tung University, Taipei, Taiwan

NUMB adaptor protein confers pleiotropic biological and pathological functions by binding with ubiquitin ligases to break down targeted molecules and other activities. Our previous studies have identified that NUMB protein suppresses oral squamous cell carcinoma (OSCC) oncogenicity, and it binds monocarboxylate transporters to elicit the degradation and the associated metabolic disruption. Besides, several oncogenic miRNAs co-target *NUMB* to abrogate its activity. However, in the TCGA head and neck squamous cell carcinoma (HNSCC) dataset and our OSCC cohort, *NUMB* expression is slightly unregulated. Despite several isoforms of *NUMB* owing to alternative slicing, our studies have disclosed the resemblance in oncogenic potential from different *NUMB* isoforms. Circular RNAs deriving from exons' back-splicing are new RNA entities mediating versatile biological functions. This study identifies several uncharacterized circular RNA variants of the *NUMB* gene (*circNUMB*s) in OSCC cell lines and tissues. Following the induction of *NUMB* promoter activity using the Crispr SAM activation system, the endogenous linear and circular *NUMB*s are upregulated similarly. The *NUMB*-null cell subclones, devoid of linear and circular *NUMB* transcripts, exhibit decreased oncogenicity. However, *circNUMB*s expression increases the oncogenicity of parental OSCC cells, and it profoundly reverses oncogenic phenotypes of cell subclones as being repressed by *NUMB* deletion. Analysis using transcriptomic algorithms reveals that *circNUMB*s modulate cancer, cell development, cell injury, and cell movement genes. The MYC and WNT/β-catenin are the leading signals affected by *circNUMB*s. This study concludes that *circNUMB*s mediate oncogenic functions opposite to linear *NUMB* transcript. Illuminating the interplay of linear *NUMB*, *circNUMB*, and their effectors would enable mechanistic insight into HNSCC/OSCC pathogenesis associated with *NUMB* expression.

#2803 Genome-wide CRISPR-Cas9 functional screens map long noncoding RNA dependencies in triple-negative breast cancer.

N. M. Alajez;
Qatar Biomedical Research Institute (QBRI), Doha, Qatar

Long noncoding RNAs (lncRNAs) are increasingly recognized as crucial regulators of gene expression, significantly influencing complex biological processes and disease progression, including cancer. Their transformative potential was underscored by the awarding of the 2024 Nobel Prize in noncoding RNA research, spotlighting lncRNAs as powerful modulators of biological regulation and therapeutic innovation. However, the precise functions of most lncRNAs remain largely unexplored. While GENCODE has cataloged over 20,000 lncRNA genes, the true diversity of lncRNAs is likely much greater. To address this, we constructed an expanded lncRNA reference by integrating GENCODE, BIGTranscriptome, and MiTranscriptome databases. This enhanced reference enabled a comprehensive mapping of lncRNA dependencies in aggressive triple-negative breast cancer (TNBC) using a genome-wide CRISPR-Cas9 screen. Our screen specifically targeted the transcription start sites of over 1,000 TNBC-associated lncRNAs, encompassing both annotated and novel transcripts. By employing a dual-excision CRISPR strategy in two TNBC cell models, we identified lncRNAs that are essential for TNBC cell growth and chemoresistance, validating several candidates using antisense oligonucleotides (ASOs). In total, 102 lncRNA hits were identified across both MDA-MB-231 and BT-549 TNBC models. Of these, three novel lncRNAs were found to be indispensable for TNBC survival in both two-dimensional and three-dimensional cultures. Analysis of a large TNBC cohort (n=360) further associated the expression of these lncRNAs with more aggressive TNBC subtypes. Loss-of-function studies with ASO Gapmers underscored the roles of these novel lncRNAs in regulating key cellular processes, including extracellular matrix organization, lipid metabolism, and GTPase signaling. Our study provides the first comprehensive map of lncRNA vulnerabilities in TNBC, highlighting their potential as therapeutic targets. This approach, while focused on TNBC, is adaptable for exploring lncRNA functions in other biological settings, paving the way for novel RNA-based therapies and clinical applications.

#2804 Long non-coding RNA LINC01614 regulates ferroptosis-mediated carcinogenic phenotypes in papillary thyroid cancer.

Danielle Quaranto[1], Michelle Carnazza[1], Nicole R. DeSouza[1], Sina Dadafarin[2], Augustine Moscatello[3], Humayun K. Islam[1], Codrin Iacob[4], Raj K. Tiwari[1], Jan Geliebter[1]

[1]New York Medical College, Valhalla, NY,[2]University of Washington, Seattle, WA,[3]Westchester Medical Center, Valhalla, NY,[4]New York Eye and Ear Infirmary of Mount Sinai, New York, NY

Papillary thyroid cancer (PTC) is the most common endocrine malignancy and is increasing annually in incidence by about 3%. PTC has a significantly higher prevalence in females of childbearing age and aggressiveness increases among males. Despite a high survival rate, disease recurrence and metastasis remain prominent issues-even decades after diagnosis. Therefore, the identification of prognostic biomarkers and actionable therapeutic targets remains a critical need. A newer class of epigenetic and regulatory molecules, known as long non-coding RNAs (lncRNAs), have demonstrated differential expression patterns in a variety of cancer types. lncRNAs modulate gene expression and drive carcinogenic behavior- making them attractive biomarkers and/or therapeutic targets for investigation. Bioinformatic analysis of our PTC vs. normal thyroid genomic repository identified the lncRNA LINC01614 transcript as significantly 12-fold upregulated in PTC. Thus, we are studying LINC01614 as a potential PTC biomarker. LINC01614 was found to be upregulated in thyroid cancer cell lines. Two PTC cell lines, TPC1 (~5 fold upregulated; RET/PTC rearrangement; female) and K1 (~2 fold upregulated; BRAFV600E; male), were selected for in vitro study. For phenotypic evaluation, CRISPRi technique was employed to achieve transcriptional repression of LINC01614 in TPC1and K1. LINC01614 knockdown in TPC1 and K1 resulted in decreased migration (~20%), clonogenicity (~50% and ~34%, respectively), and proliferation (~55% and ~45%, respectively). RNA sequencing analysis of CRISPRi knockdown cell lines vs. CRISPRi controls demonstrated reduced expression of key ferroptosis-related genes-SLC7A11 and SLC3A2. Both genes encode for the xCT system (cystine/glutamate reverse transporter) that regulates the uptake of cystine - a key precursor for glutathione (GSH) synthesis. GSH is a critical antioxidant defense against lipid peroxidation, further preventing ferroptosis. Total cell iron levels were quantified and found to be significantly higher in our LINC01614 knockdowns, signifying increased ferroptosis induction. Studies are currently underway to identify the impact of LINC01614 on lipid peroxidation levels, reactive oxygen species production, as well as identification of ferroptosis-related protein expression in our PTC in vitro model. Elucidation of LINC01614's carcinogenic mechanism of action through ferroptosis regulation is a promising and novel avenue of exploration for PTC prognosis and therapy.

#2805 A lncRNA-specific CRISPRi screen identifies an essential role of *PDCD4-AS1* in colorectal cancer growth and survival.

Vida Kufrin[1], Silvia Schafer[1], Silke Brilloff[1], Maria Gabriela Pereira dos Santos[1], Elahe Rahimian[1], Sandra Kuchler[1], Denis M. Schewe[2], Claudia R. Ball[1], Hanno Glimm[1], Marius Bill[2], Alexander A. Wurm[1]

[1]National Center for Tumor Diseases Dresden, Dresden, Germany,[2]University Hospital Carl Gustav Carus, Dresden, Germany

Colorectal cancer (CRC) ranks as the third most commonly diagnosed cancer worldwide and is the second leading cause of cancer-related mortality. Recent studies have highlighted the critical role of long non-coding RNAs (lncRNAs) in CRC development, influencing processes such as proliferation, invasion, and therapy resistance. However, the functional roles of most annotated lncRNAs in CRC remain poorly understood, underscoring the need to uncover novel lncRNAs that may impact CRC initiation, progression, and treatment response.

To identify lncRNAs involved in CRC cell growth, we conducted a comprehensive lncRNA-specific CRISPR interference (CRISPRi) dropout screen targeting 675 lncRNAs highly expressed across various cancer entities. We utilized four CRC models with diverse molecular backgrounds, including two patient-derived 3D models. As a control, we included HEK293T cells to filter out common essential lncRNAs. This approach revealed 11 lncRNAs significantly depleted across all CRC models, indicating a shared regulatory role in CRC. To validate these findings, we inhibited expression of selected top-hit lncRNA candidates using CRISPRi and antisense oligonucleotide-mediated knockdown. In both approaches, we observed reduced cell growth compared to control conditions, closely aligning with the dropout screen findings. Among the candidates, *PDCD4-AS1* was prioritized for further characterization, as it is not located near an oncogenic or common essential protein-coding gene, minimizing the likelihood of off-target effects. By generating loss-of-function patient-derived spheroid models, we showed that *PDCD4-AS1* silencing significantly reduced cell growth, induced G2/M cell cycle arrest, and promoted apoptosis. To evaluate the therapeutic potential of targeting *PDCD4-AS1*, we performed knockdown experiments in a panel of patient-derived organoids (PDOs) both *in vitro* and *in vivo*. In a PDO xenograft mouse model, *PDCD4-AS1* knockdown significantly suppressed tumor growth and prolonged overall survival. To explore the molecular mechanisms underlying the *PDCD4-AS1* knockdown phenotype, we performed transcriptomic analysis by RNA sequencing. Here, we identified significant alterations in amino acid metabolism, suggesting a *trans*-regulatory role of *PDCD4-AS1* in CRC. However, further analyses are required to identify the interaction partners of *PDCD4-AS1* and gain deeper insights into its mechanism of action.

Collectively, our findings highlight the potential of CRISPRi screens in uncovering novel molecular vulnerabilities in patient-derived CRC models. We present the previously uncharacterized lncRNA *PDCD4-AS1* as a mediator of CRC progression and provide strong evidence for lncRNAs as promising therapeutic targets and/or biomarkers for personalized oncology.

#2806 The putative role of the lncRNA (*SOCS3 DT*) as a potential biomarker for ovarian cancer diagnosis and progression.

Yahya Tamimi[1], Safa Al-Battashi[1], Shika Hanif Malgundkar[2], Sidika Sevde Yenigun[1], Hamad Al Riyami[1], Mohammed Al Toubi[1], Moza AL Kalbani[1], Aikou Okamoto[1], Ikram Burney[1]

[1]Sultan Qaboos University, Muscat, Oman,[2]Biochemistry, Sultan Qaboos University, Muscat, Oman

Ovarian cancer (OC) is a major global health challenge due to its high rates of cancer-related morbidity and mortality. The majority of OC cases are diagnosed at advanced stages, leading to poor survival outcomes. Thus, the identification of effective screening methods for early detection is crucial. The emergence of long non-coding RNAs (lncRNAs) as key regulators of gene expression has opened new avenues for investigating cancer biology. Previous *in-silico* analyses have indicated elevated expression levels of the lncRNA *SOCS3-DT* in OC, suggesting its potential as a biomarker. However, the exact functional role of *SOCS3-DT* in OC remains elusive. In this study, we conducted bioinformatic analysis using R software to identify differentially expressed lncRNAs in recurrent OC samples compared to primary ovarian tumor samples. Our analysis revealed increased expression of *SOCS3-DT* in recurrent OC samples compared to primary samples. To validate these findings, we utilized qRT-PCR to monitor *SOCS3-DT* expression in OC cell lines and human tissue samples. The qRT-PCR analysis revealed the overexpression of *SOCS3-DT* in the high-grade OC cell line OVSAHO, with a gradual increase in expression across early and late stages of OC tissue samples compared to healthy individuals. This suggests its potential as a promising marker for OC detection.Moreover, we suppressed *SOCS3-DT* expression in OVSAHO cells and assessed its impact on proliferation, colony formation, and wound healing. Additionally, to assess the effect of *SOCS3-DT* knockdown on crucial markers associated with cancer pathways, we conducted qRT-PCR and western blotting. Compared to the negative control, silencing *SOCS3-DT* in OVSAHO cells resulted in reduced cell proliferation and colony formation abilities, decreased expression of apoptotic markers (CASPASE-3, BCL-2), downregulation of autophagy markers (ATG5 and BECLIN), and mesenchymal markers (VIMENTIN and CDH2). In conclusion, this study provides new insights into identifying an effective early detection biomarker (*SOCS3-DT*) implicated in the early stages of the disease. This biomarker shows promise as a novel therapeutic target, presenting new approaches for the effective treatment of OC.

#2807 Determining a role for a long noncoding RNA involved in the DNA damage response of lung adenocarcinoma.

Michele Ramos Correa[1], Jonathan Castillo[2], Patrick Pirrotte[3], Crystal N. Marconett[4]

[1]Surgery, University of Southern California, Los Angeles, CA,[2]Integrative Translational Sciences, Beckman Research Institute of The City of Hope, Glendale, CA,[3]Department of Immuno-Oncology, Beckman Research Institute, Duarte, CA,[4]Integrative Translational Sciences, Beckman Research Institute, Duarte, CA

Lung adenocarcinoma (LUAD) is the most common histological subtype of the deadliest cancer in the United States. This cancer accounts for 40% of cases that can arise from a high rate of mutations that is exaggerated by dysregulation in the DNA damage and repair signaling pathways that cause genomic instability. Historically, the role of protein coding genes in regulation of these pathways has been extensively studied but developments in new technology has identified noncoding RNAs as critical regulators involved in physiological and pathological processes related to DNA stability. Specifically, long noncoding RNAs (lncRNAs) have been implicated as critical regulators of gene expression in the DNA damage response; controlling the repair mechanism response's magnitude, duration, and resolution, but very few have been characterized and ascribed a function. We have previously identified the lncRNA, *LINC00261*, that acts as a tumor suppressor in LUAD by regulating the activation of the DNA damage repair system. However, its exact mechanisms of action in the signaling pathway remain unknown. We found that reintroduction of *LINC00261* in LUAD is associated with G2/M cell cycle arrest and phosphorylation-mediated activation of key DNA damage response pathway components. We hypothesize that *LINC00261* maintains DNA fidelity through direct interaction with repair components. To gain critical insights into the essential functions that *LINC00261* plays in the DNA damage response, a mediation analysis including *LINC00261* and tumor mutational burden was conducted on the TCGA LUAD dataset. Results of this mediation analysis suggested a role for *LINC00261* in nucleotide excision repair (NER) pathway in lung adenocarcinoma. To mechanistically characterize the function of *LINC00261* in NER, we have conducted ChIRP-MS in the A549 LUAD cell line. Our preliminary results indicate LINC00261 direct interaction with mitochondrial translation (p=1.34E-13), rRNA processing in the nucleolus and cytosol (p=3.76E-10), and branched-chain amino acid catabolism, (p=5.68E-09) pathways. Further validation of the relationship between *LINC00261* and NER was performed on RNA isolated from A549 cells upon UVC DNA damage. *LINC00261* was subcellularly localized to both cytoplasm and nucleus, inferring that *LINC00261* can function in cis and trans. We are currently investigating *LINC00261* direct binding partners that mediate DNA damage and repair responses. Our findings provide evidence for the role of *LINC00261* in the DNA damage response and will lead to a further understanding of how lncRNA-mediated dysregulation of DNA repair leads to genomic instability and oncogenic activation.

#2808 LUCAT1 facilitates areca nut-induced cell invasion and therapeutic resistance in head and neck cancer through STAT1 transcriptional modulation.

H.-H. Huang, A.-J. Cheng;
Chang Gung University, Taoyuan, Taiwan

Areca nut is a significant carcinogen associated with head and neck cancer (HNC) in Southeast Asia, but the mechanisms, particularly those involving long non-coding RNAs (lncRNAs), remain unclear. We previously identified the lncRNA LUCAT1 as induced by arecoline, a key areca nut component, and this study explores its crucial role in HNC progression. Silencing LUCAT1 reduced several arecoline-induced malignant features. These include cell invasion through epithelial-mesenchymal transition (EMT) modulation and chemo-radiotherapy resistance via reactive oxygen species (ROS) scavenging and autophagy regulation. RNA sequencing (RNA-seq) of LUCAT1-silenced cells revealed 2,326 downstream effectors enriched in oncogenic pathways, including EGFR, VEGF, HIF-1, and extracellular matrix-receptor interactions. This underscored LUCAT1's pivotal role in cell motility and survival. LUCAT1 predominantly localizes in the nucleus, suggesting transcriptional regulation. Bioinformatic analysis of its promoter revealed 491 transcription factors binding to a DNase hypersensitivity site (DHS). Integrative proteomic analysis of areca nut-treated HNC cells narrowed this to three key transcription factors (IRF6, CUX1, and STAT1). Among these, STAT1 was confirmed as the primary factor induced by arecoline to activate LUCAT1 transcription. Silencing STAT1 or inhibiting its phosphorylation using fludarabine suppressed LUCAT1 expression, reduced ROS-scavenging, and reversed arecoline-induced malignancy. Thus, this study demonstrates that areca nut upregulates LUCAT1 via STAT1 activation, contributing to cell invasion and therapeutic resistance in HNC. Additionally, fludarabine shows potential as a therapeutic agent to counteract areca nut-induced malignancy, offering a promising avenue to improve survival outcomes for HNC patients with areca nut-chewing.

#2809 Targeting the LINC00355-MENIN interaction with CDK4/6 inhibitors to reduce proliferation in late relapse breast cancer.

Richa Mishra[1], Brianna Duhart[1], Dhanusha Duraiyan[1], Prasanth Thunuguntla[1], Kyla Gelev[1], Debbie C. Crans[2], Cynthia X. Ma[3], Jessica Silva-Fisher[3]

[1]Division of Oncology, Department of Internal Medicine, Washington University School of Medicine in St. Louis, St. Louis, MO,[2]Department of Chemistry, Colorado State University, Fort Collins, CO,[3]Division of Oncology, Department of Internal Medicine, Washington University School of Medicine in St. Louis and Siteman Cancer Center, St. Louis, MO

Breast cancer is the most common cancer diagnosed among women in the United States, with approximately 310,720 new cases estimated in 2024. Late relapse refers to disease recurrence after being disease-free for at least five years following the initial curative treatment. Late relapse is particularly relevant to ER+/HER2- breast cancer as it accounts for over 50% of recurrences for patients with this subtype of breast cancer. Cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors in combination with endocrine therapy are standard first-line therapy for metastatic relapse. While these therapies initially show promise, resistance to endocrine therapy and CDK4/6 inhibitors inevitably develops, presenting a major clinical challenge and unmet medical need for late relapse. Long non-coding RNAs (lncRNAs) are key regulators of cancer-related processes, including cell cycle progression. Our lab has identified deregulated lncRNAs in metastatic late relapse breast cancer patient tumor samples, but their role in CDK4/6 inhibitor resistance remains unclear. To investigate this, we treated the ER+ primary breast cancer cell line MCF7 (wild-type) and the long-term estrogen-deprived (LTED) cell line - mimicking late relapse with CDK4/6 inhibitors, including Abemaciclib, Palbociclib, and Ribociclib. Wild-type cells were more sensitive to these inhibitors than LTED cells. Further, the analysis identified that *LINC00355*, the topmost upregulated lncRNA in late relapse breast cancer binds to the MENIN protein. This was demonstrated using RNA pulldown assays and multiplexed RNA in situ hybridization in LTED cells and patient samples. The *LINC00355*-MENIN interaction decreases CDKN1B expression, promoting cellular proliferation. Notably, MENIN has been shown to regulate the CDK4/6 pathway and inhibit cell proliferation. To explore the therapeutic potential, we reduced *LINC00355* expression using GapmeRs, locked nucleic acid-based antisense oligonucleotides (LNA ASOs), which significantly decreased cell proliferation. In contrast, treatment of LTED cells with Palbociclib or Ribociclib alone for 72 hours showed no significant changes in proliferation. However, combination treatment with *LINC00355* LNA ASOs and Palbociclib or Ribociclib resulted in a notable reduction in cell proliferation. These findings highlight the critical role of lncRNA-protein interactions, such as *LINC00355*-MENIN, in promoting cellular proliferation and provide proof-of-concept for targeting lncRNAs that can cause resistance to CDK4/6 inhibitors for late relapse breast cancer.

#2810 Investigating the oncogenic function of the long noncoding RNA KCNQ1OT1 in rhabdomyosarcoma.

Ara Jo[1], Alex Kovach[1], Christian Stackhouse[1], Samantha Weitzel[1], Brian Masters[1], Justina McEvoy[2], Wenyue Sun[3], Frederic G. Barr[3], Mauro Calabrese[4], Xiang Chen[5], Corinne M. Linardic[1]

[1]Pediatrics, Duke University, Durham, NC,[2]St. Jude Children's Research Hospital, Memphis, TN,[3]National Cancer Institute, Bethesda, MD,[4]Pharmacology, University of North Carolina, Chapel Hill, NC,[5]Computational Biology, St. Jude Children's Research Hospital, Memphis, TN

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children and adolescents, with survival for high-risk cases stagnant at less than 30%. Contemporary molecular classification of RMS is into fusion-positive (FP) and fusion-negative (FN) groups, based on expression of fusion oncogenes. Although the majority of FN-RMS tumors exhibit mutations in protein coding genes, especially in the RAS pathway, about 25% instead show only copy number alterations and LOH at chromosome 11p15.5, which harbors tumor suppressor genes (TSGs) *CDKN1C*, *PHLDA2*, and *SLC22A18*. The 90kb long noncoding RNA KCNQ1OT1 (OT1) is a known *cis* silencer of this locus. We hypothesize that OT1 is upregulated in RMS, contributes to RMS tumorigenesis by repressing critical TSGs, and that targeting OT1 could provide a novel therapeutic approach. Using qPCR and epigenomic interrogation of available RMS tissue, we find OT1 highly expressed in FN-RMS cell lines and banked tumor samples. Complementary genomic methylome data of RMS tumors shows silencing and activation of the *CDKN1C* and *OT1* promoters, respectively. Suppression of *OT1* using shRNA against various sites along the transcript, or a dual excision CRISPR knockout (DECKO) of the promoter, inhibits RMS cell proliferation, induces apoptosis, and recovers TSG expression. In a candidate approach, using both gain and loss of function approaches, the Hippo pathway YAP1 transcriptional co-activator was found to modulate OT1 expression. Unbiased genomic screens are planned. Finally, using *in silico* binding prediction models, we designed OT1-directed anti-sense oligonucleotides (ASOs), demonstrating they can be delivered to RMS cell lines. These ASOs, designed to degrade or inhibit OT1 function, will be tested in RMS cell-based and xenograft models to determine their efficacy and specificity. To generate additional information about regions of OT1 that might be therapeutically tractable, antisense DNA capture probes will be applied to OT1 in RMS cells to generate higher nucleotide resolution maps of OT1 structure and protein binding. This study not only deepens our understanding of OT1's role in RMS but also advances the development of innovative RNA-based treatments, addressing a critical need for effective therapies in this aggressive childhood cancer.

#2811 Mapping the eRNAs regulatory networks associated with clinical traits in gastric cancer.

Yanxin Yao, Xiangnan Zhang, Lijie Ji, Mengyue Qiu, Huahuan Liu, **Kexin Chen**, Ben Liu

Tianjin Medical University Cancer Institute and Hospital, Tianjin, China

Gastric cancer (GC) is one of the top five common malignancies worldwide and the fourth leading cause of cancer-related death. Enhancer RNA (eRNA) is a type of non-coding RNA transcript originating from enhancers. In different tumors, eRNAs exhibit significant tumor specificity. In different tumors, eRNAs showed significant tumor specificity. However, the expression characteristics of eRNAs and their potential transcriptional regulatory functions still need to be further explored. More than two hundred local gastric adenocarcinoma samples were included in this study. We define two classes of eRNAs, the shorter bi-directionally transcribed 2D eRNAs and the longer 1D eRNAs. We identified about 30,000 eRNAs expressed in gastric cancer or normal gastric cancer tissues, and most of these eRNAs are located in intergenic and antisense strands of genes. Some of the eRNAs showed strong tissue specificity, aberrant activation or silencing of eRNAs leads to tumorigenesis in gastric cancer. Previous reports have demonstrated that eRNAs play a complex scaffolds role in maintaining chromatin ring stability and regulating the expression of target genes. HiC and HiChIP data were collected as evidence of spatial proximity of enhancers and promoters. Anchors annotation was performed using previously quantified eRNAs to obtain eRNA-targe gene pairs. Co-expression analysis of eRNAs and their target genes, combined with the above 3D genomic results, total of about 30,000 eRNA-target gene pairs were identified. Using edge perturbation in the gene interaction network, the edge-perturbation matrix of eRNA-target gene interaction pairs was obtained, and a value was assigned to each interaction pair. About 30,000 of these action pairs were associated with clinical characteristics. It is noteworthy that a large proportion of the action pairs were associated with the degree of differentiation of gastric cancer. To further explore the role that eRNAs play in tumor proliferation, we selected representative eRNAs and constructed CRISPRa and CRISPRi screening libraries to explore the potential role of eRNAs in gastric cancer proliferation. Bulk screening results showed that gastric cancer cells proliferated faster after activating or knocking down eRNAs. In addition, CROP-seq was used to verify the changes in target genes after activation/knockdown of eRNAs expression. Taken together, these results demonstrate that eRNAs can regulate the progression of gastric cancer by modulating the expression of their target genes, and that eRNAs can be used as potential targets for gastric cancer therapy. In summary, we have provided an in-depth characterization of the eRNAs expression landscape and its regulatory functional network in gastric cancer, and validated the eRNA-target gene action, demonstrating the possibility of eRNAs as a potential target for gastric cancer therapy.

#2812 Prognostic implications of gastric cancer-specific tsRNA subtypes and functional validation.

Yuan Liu, Xin Hu, Ye Tian, Wei Wang, **Kexin Chen**, Hongji Dai, Ben Liu, Fengju Song

Tianjin Medical University Cancer Institute and Hospital, Tianjin, China

Background: Gastric cancer is a global malignant disease of the digestive tract. The marked heterogeneity of gastric cancer leads to prognostic and therapeutic challenges. tRNA-derived fragments (tsRNAs), a novel class of small non-coding RNAs closely associated with tumor progression, have an imperfect role in prognostic assessment and treatment of gastric cancer. We aimed to identify specific tsRNA isoforms associated with the tumor microenvironment of gastric cancer, establish a tsRNA-based prognostic model for gastric cancer, and screen-specific factors that may inhibit gastric cancer to guide prognosis and treatment.
Methods: We performed matrix score analysis and consensus clustering analysis using tsRNA expression profiles from the tsRFun database and gastric cancer sample information from the TCGA database. Ten machine learning (Lasso, Enet, plsRcox, CoxBoost, StepCox, GBM, Ridge, RSF, survival-SVM, and SuperPC) were used to construct tsRNA-related prognostic models (TRS). In-house development of an R package called 'GCtsRNAscore' for calculating tsRNA score (RS) in gastric cancer patients. TMB and IMvigor210 cohorts were evaluated for immunotherapy efficacy. Pandora sequencing screened for gastric cancer-specific tsRNAs for functional validation in cellular, organoid, and animal models for functional validation.
Results: We classified 80 gastric cancer-specific tsRNAs into three immunological subtypes, Stromal_L, Stromal_M, and Stromal_H, based on stromal cell infiltration analysis, with Stromal_L having the best prognosis. A multi-omics approach revealed significant differences in biological characteristics, CNVs, and mutation patterns among the subtypes ($P < 0.05$). The TCGA and GEO gastric cancer cohorts validated that TRS was the best prognostic model for tsRNA-associated tsRNAs in gastric cancer ($P < 0.0001$, AUC = 0.967). Gastric cancer patients were categorized into high-risk and low-risk based on their respective RS. High-risk patients responded well to axitinib, bexarotene, and dasatinib, while low-risk patients responded well to immunotherapy. In addition, we identified six subtype-specific tsRNAs, which were combined with Pandora sequencing to screen for the gastric cancer-specific suppressor Gly-tsRNA-4. Overexpression of Gly-tsRNA-4 was found to inhibit tumor growth in cellular, organoid, and animal experiments.
Conclusion: In this study, tsRNA-related indicators accurately predicted the prognosis of gastric cancer and provided insight into the response of GC patients to therapeutic drugs. Gly-tsRNA-4 may act as a tumor suppressor to inhibit gastric cancer progression. The above study can help to explore new targets for gastric cancer treatment, but further comprehensive validation is needed.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Non-Apoptotic Cell Death
Poster Session

#2816 Lipid composition differentiates ferroptosis sensitivity between in vitro and in vivo systems.

Vivian S. Park[1], Lauren E. Pope[2], Justin Ingram[1], Grace A. Alchemy[2], Julie Purkal[1], Eli Y. Andino-Frydman[2], Sha Jin[1], Sanjana Singh[1], Anlu Chen[3], Priya Narayanan[3], Sarah Kongpachith[3], Darren C. Phillips[1], Scott J. Dixon[2], Relja Popovic[1]

[1]AbbVie Inc., North Chicago, IL,[2]Stanford University, Stanford, CA,[3]AbbVie Bay Area, South San Francisco, CA

Ferroptosis is a regulated non-apoptotic cell death process characterized by iron-dependent lipid peroxidation. This process has recently emerged as a promising approach for cancer therapy. Peroxidation of polyunsaturated fatty acid-containing phospholipids (PUFA-PLs) is necessary for the execution of ferroptosis. Ferroptosis is normally suppressed by glutathione peroxidase 4 (GPX4), which reduces lipid hydroperoxides to lipid alcohols. Some evidence indicates that GPX4 may be a useful target for drug development, yet factors that govern GPX4 inhibitor sensitivity *in vivo* are poorly understood. We find that pharmacological and genetic loss of *GPX4* function was sufficient to induce ferroptosis in multiple adherent ("2D") cancer cell cultures. However, reducing GPX4 protein levels did not affect tumor xenograft growth when these cells were implanted in mice. Furthermore, sensitivity to GPX4 inhibition was markedly reduced when cells were cultured as spheroids ("3D"). Mechanistically, growth in 3D versus 2D conditions reduced the abundance of PUFA-PLs. 3D culture conditions upregulated the monounsaturated fatty acid (MUFA) biosynthetic gene stearoyl-CoA desaturase (SCD). SCD-derived MUFAs appear to protect against ferroptosis in 3D conditions by displacing PUFAs from phospholipids. Various structurally related long chain MUFAs can inhibit ferroptosis through this PUFA-displacement mechanism. These findings suggest that growth-condition-dependent lipidome remodeling is an important mechanism governing GPX4 inhibitor effects. This resistance mechanism may specifically limit GPX4 inhibitor effectiveness *in vivo*.

Disclosure Statement: V.S.P., J.P., S.J., S.S., A.C., P.N., S.K., D.C.P. and R.P. are employees of AbbVie and may own stock. J.I. was an employee of AbbVie at the time of the study. S.J.D., L.E.P., G.A.A. and E.Y.A are affiliated with Stanford University. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

#2817 ATR-mediated phosphorylation of RIPK1 inhibits DNA damage-induced necoptosis and chemosensitivity in ovarian cancer.

Q. Xu[1], S.-n. Shi[1], X. Jiao[1], X. Zhao[1], G. B. Mills[2], Q. Gao[1], Y. Fang[1];

[1]Tongji Hospital, Tongji Medical College, Wuhan, China, [2]Department of Cell, Development and Cancer Biology, Oregon Health and Sciences University, Portland, OH

Background: Cell death under DNA damage induced by chemotherapy is considered a highly significant and effective treatment for epithelial ovarian cancer. Nevertheless, ovarian cancer to date persists as the leading cause of death among all gynecological malignancies, as the majority of patients eventually obtain chemotherapy resistance. chemotherapy-resistant patients, while the underlying mechanism remains unclarified. When tumor cells are undergoing persistent DNA damage, ATR is activated, yet the cell fate mediated by ATR remains unknown. RIPK1 is the crucial regulatory protein for cell death, which is activated by upstream signals and mediates RIPK1-dependent cell apoptosis and necroptosis. However, the mechanism through which RIPK1 regulates cell death in DNA damage still requires further exploration.

Methods and results: Western blot and flow cytometry were employed to detect the cell death induced by etoposide-induced DNA damage, which revealed this mode of cell death was dependent on RIPK1 activation-induced necroptosis. Through Co-immunoprecipitation, it was determined that RIPK1 and ATR had an obvious interaction. Subsequently, by using ATR inhibitors or si-RNA to knockdown ATR, the activation of RIPK1 increased, the necroptosis induced by DNA damage was enhanced, and the downstream Necrosome formed within a shorter period. Furthermore, a kinase-specific phosphorylation site-prediction program, GPS 6.0, and a mass spectrometry analysis were employed to determine that RIPK1 is phosphorylated at Ser335 by ATR. Additionally, the WT and S335A mutant plasmids were complemented in RIPK1 knockout tumor cells, which revealed that in the case of the S335A mutation, the repression of RIPK1 was released, and downstream necroptosis was also augmented. Finally, RIPK1 was knocked out with the complementation of WT and S335A in ovarian cancer cell lines. The results demonstrated that ATR phosphorylation of RIPK1 at S335 led to chemoresistance, while the acquisition of the S335A mutation significantly enhanced chemosensitivity, as indicated by the intense activation of the necroptosis pathway, decreased cell viability, and increased apoptotic cells.

Conclusions: Our study reveals a pathway that governs ATR-mediated RIPK1 regulation and RIPK1-dependent cell death under DNA damage, suggesting for the first time that the ATR-RIPK1 axis plays an unrecognized role in integrating DNA damage checkpoints and cell death. Parallel to its other substrates, ATR directly inhibits RIPK1 and downstream necrosome formation by phosphorylation at Ser335, thereby suppressing RIPK1-dependent cell death triggered by DNA damage. This work broadens our comprehension of the interplay between DNA damage and cell death regulation, which could facilitate the development of therapeutic drugs aimed at enhancing DNA damage-induced tumor cell death and promoting chemosensitivity.

#2818 Targeting PIKfyve sensitizes gastroenteropancreatic neuroendocrine tumor to mTOR inhibition by disrupting ferritinophagy and lipid homeostasis.

Yizhi Cao, Yitong Yin, Caleb Cheng, Shannon VanAkon, Sarah Nicole Yee, Jacinda Jiali Liu, Yang Zheng, Andrej Coleski, Radha Paturu, Somnath Mahapatra, Fan Yang, Rupam Bhattacharyya, Rahul Mannan, Yuanyuan Qiao, Arul Chinnaiyan

University of Michigan, Ann Arbor, MI

Gastroenteropancreatic neuroendocrine tumors (GEP-NETs) have shown a significant global increase over the past decade. The mTOR signaling pathway is a positive regulator of cell cycle progression, with its activation playing a key role in the pathogenesis of NETs. Since 2011, the FDA-approved mTOR inhibitor everolimus has been used as an anti-tumor agent for treating progressive NETs, and its clinical efficacy is limited due to rapid disease relapse and progression. In this study, we investigated the role of autophagy, specifically ferritinophagy, in mediating resistance to mTOR inhibition in GEP-NETs. Enhanced ferritinophagy followed by mTOR inhibition increases intracellular iron availability, supporting tumor cell energy production and survival. Genetic or pharmacological inhibition of PIKfyve, a lipid kinase integral to lysosomal functioning, disrupted ferritinophagy, reducing the intracellular iron pool elevated by mTOR inhibition. Interestingly, mTOR inhibition also restored cholesterol and fatty acid biosynthesis resulting from PIKfyve inhibition. Notably, dual inhibition of mTOR and PIKfyve exhibited a strong synergistic antitumor effect in vitro and significantly prolonged survival in an orthotopic GEP-NET preclinical model. Together, our findings highlight the therapeutic potential of combining mTOR and PIKfyve inhibitors in the treatment of GEP-NETs.

#2819 Metabolic determinants of ferroptosis in B-cell malignancies.

Etienne Leveille[1], Eden Bramson[1], Mark Robinson[1], Thierry Bertomeu[2], Andrew Chatr-Aryamontri[2], Shalin Kothari[1], Markus Muschen[1]

[1]Center of Molecular and Cellular Oncology, Yale University, New Haven, CT,[2]The ChemoGenix pooled CRISPR screening platform of the Institute for Research in Immunology and Cancer, Universite de Montreal, Montreal, QC, Canada

Although the development of targeted approaches has been effective for the treatment of B-cell malignancies, outcomes remain poor in the relapsed and refractory settings. To expand the portfolio of B-cell-selective drugs, we developed an interactive computational tool (lymphoblasts.org) and identified ferroptosis, a form of cell death driven by iron-dependent membrane lipid peroxidation, as a previously unrecognized selective vulnerability in B-cell malignancies. Although ferroptosis has shown potential in therapy-resistant tumors, no potent ferroptosis inducers are available clinically, and a better understanding of the factors regulating ferroptosis is required to allow its therapeutic targeting and the development of clinical grade ferroptosis inducers. Our comparative analyses of CRISPR dependency (DepMap) and drug (CTD, GDSC) screens revealed that B-cell malignancies are particularly sensitive to ferroptosis and depend on several anti-ferroptotic molecules that notably include components of the glutathione synthesis machinery (GCLC, GCLM, GSS) and selenoprotein synthesis (SEPHS2, LRP8). Strikingly, this analysis also identified pro-ferroptotic genes involved in iron (*TFRC*) and polyunsaturated fatty acid (PUFA; *ACSL4*) metabolism as selective B-cell dependencies, suggesting that addiction to these pro-ferroptotic processes makes B-cell malignancies intrinsically vulnerable to ferroptosis. Accordingly, iron chelation treatment showed a much higher toxicity in B-cell malignancies compared to solid tumors, and Cre-mediated ablation of *Tfrc* in murine models of B-cell acute lymphoblastic leukemia (B-ALL) and B-cell lymphoma induced rapid cell death. Cre-mediated loss of ACSL4, while making cells almost completely resistant to ferroptosis, significantly compromised murine B-ALL cell viability, suggesting a key role of ACSL4 and PUFA in B-cell malignancy survival. An important role of ACSL4 and PUFA metabolism in B-cell malignancies was also suggested by analysis of clinical data from the diffuse large B-cell lymphoma MMMLNP trial, which revealed that greater than median expression of *ACSL4* is associated with significantly worse survival ($p = 0.0007$).To uncover new ferroptotic regulators in B-cell malignancies, we performed whole-genome CRISPR knockout screens under the selective pressure of the ferroptosis inducers RSL3, Erastin, and $FINO_2$, which highlighted several known ferroptosis regulators and uncovered new ferroptosis-related genes and pathways including sphingolipid metabolism (*KDSR*), phosphatidylcholine synthesis (*FLVCR1*, *PCYT1A*), inositol metabolism (*ITPK1, IPMK*), and lipid membrane remodelling (*ATP8B2*). Taken together, our findings uncover iron and PUFA metabolism as selective B-cell dependencies that likely contribute to their exquisite sensitivity to ferroptosis and identifies novel regulators of ferroptosis in B-cell malignancies.

#2820 Intracellular calcium dynamics affect ferroptosis sensitivity.

Veronica T. Brooks[1], Ronan Talty[2], Simon F. Roy[2], Marcus W. Bosenberg[3]

[1]Department of Dermatology, Yale University, New Haven, CT,[2]Department of Dermatology, Pathology, Yale University, New Haven, CT,[3]Department of Dermatology, Pathology, Immunobiology, Yale University, New Haven, CT

Background: Melanoma is the deadliest form of skin cancer, and its incidence continues to rise. While advances in immunotherapy, in the form of anti-PD-1, anti-CTLA-4, and anti-LAG-3 immune checkpoint inhibitors, have led to extended cancer-free outcomes for many patients with advanced disease, most patients do not have durable responses. Therefore, improvements in long-term treatment of melanoma will depend on the identification of methods to enhance existing immunotherapy regimens. Recent studies highlight ferroptosis, an iron-dependent form of regulated cell death, as a necessary component of immunotherapy-induced tumor clearance. Cytosolic calcium influx is a key aspect of ferroptosis, preceding cell bursting and linking increased plasma membrane permeability with the activation of membrane repair mechanisms.
Design: This study investigated the ability of calcium modulators to alter sensitivity to ferroptosis in cancer cells. SKF96365, an inhibitor of calcium release-activated channel (CRAC) activity and store-operated calcium entry (SOCE) channels, was selected for its effects on cellular calcium homeostasis and its limited exploration as an anti-melanoma agent and ferroptosis regulator. The ability of SKF96365 to sensitize melanoma cells to ferroptosis induction was assessed by evaluating key ferroptotic hallmarks, including cell death, lipid peroxidation and reduced glutathione levels. To further elucidate the underlying mechanism, three additional calcium modulators—GSK7975A, 2-Aminoethoxydiphenyl borate (2-APB), and Ruthenium Red (RR)—were tested.
Results: SKF96365 enhanced sensitivity to ferroptotic cell death, but not apoptosis or autophagy. Melanoma and colon adenocarcinoma cells treated with SKF96365 had increased levels of lipid peroxides and decreased reduced glutathione levels. GSK7975A similarly sensitized cells, supporting the role of store-operated calcium entry (SOCE) channels in ferroptosis. In contrast, 2-APB, which shows a dose-dependent bimodal effect on SOCE, increased resistance to ferroptosis despite elevating lipid peroxides and reducing glutathione. RR, a mitochondrial calcium uniporter inhibitor, also sensitized cells to ferroptosis.
Conclusion: SKF96365, GSK7975A, and RR enhance ferroptosis sensitivity, whereas 2-APB confers resistance. These results provide a foundation for further exploration of calcium signaling pathways in ferroptotic cell death.

#2821 Regulation of autophagy by the DDI2-CCN1 axis.

Nayyerehalsadat Hosseini, Ahmed M. Elshazly, Holly A. Byers, Janakiram R. Vangala, Senthil K. Radhakrishnan

School of Medicine, Virginia Commonwealth University, Richmond, VA

Background: The two primary mechanisms of protein degradation in cells are the ubiquitin-proteasome system (UPS) and the autophagy-lysosome pathway (ALP). In cases of UPS dysfunction, compensatory autophagy is activated to alleviate proteotoxic stress. This phenomenon can influence the efficacy of cancer treatments. Despite significant progress in understanding these systems, the specific molecular pathways linking proteasome dysfunction to the activation of compensatory autophagy remain poorly understood. DDI2 (DNA Damage Inducible 1 Homolog 2) functions as both a protease and a ubiquitin shuttle factor. Its loss sensitizes cancer cells to proteasome inhibition through the accumulation of polyubiquitinated proteins. However, these cells may activate autophagy to clear ubiquitinated protein aggregates. This study investigate how DDI2 depletion promotes autophagy in cancer cells to improve the efficacy of cancer treatments.

Methods: Autophagy activity was assessed in various cell lines, including non-cancerous (NIH-3T3 murine fibroblasts, MRC5 human fetal lung fibroblasts) and cancerous (EW16 and ES1 human Ewing sarcoma, MIA PaCa-2 human pancreatic cancer) cell lines, either with or without DDI2 depletion. Proteomic analysis revealed an upregulation of cellular communication network factor 1 (CCN1) in DDI2-deficient cells, suggesting its potential role in autophagy activation. Then, siRNA and viral constructs were used to examine CCN1's role in autophagy induction. Moreover, Co-immunoprecipitation (Co-IP) and confocal microscopy were applied to investigate the possible interactions between DDI2 and CCN1.

Results: Autophagy flux analysis showed increased LC3B-II levels, a key marker of autophagy, in DDI2-deficient cells compared to controls. Furthermore, western blotting confirmed elevated levels of CCN1 protein in DDI2-deficient cells. Downregulation of CCN1 by siRNA resulted in reduced autophagy activity in DDI2-deficient cells, demonstrating that CCN1 is essential for the autophagic induction. In contrast, overexpression of CCN1 in wild-type cells led to an increase in LC3B-II levels, confirming that CCN1 alone can trigger autophagy activity. Co-IP analysis revealed a direct interaction between DDI2 and CCN1, and confocal microscopy further confirmed their colocalization.

Conclusion: Autophagy plays a critical role in effectiveness of cancer treatments. Our findings show that the loss of DDI2 leads to the accumulation of CCN1, which consequently activates autophagy. This research highlights the interaction between protein degradation pathways, UPS and autophagy, suggests that targeting both DDI2 and autophagy could provide a promising strategy to improve cancer therapy outcomes.

#2822 AdipoRon suppresses breast cancer growth through autophagy-mediated ferroptosis.

Yong-Han Su, Yi-Zhen Wu, Hsin-Yi Yang, Tzu-Yi Chia, Jou-Fang Hsu, Ching-Ying Kuo

National Taiwan University College of Medicine, Taipei City, Taiwan

Excess adipose tissue and the release of large amounts of adipokines are recognized as contributing factors in the development of various cancer types. Adiponectin, distinct from other adipokines, is negatively correlated with cancer progression. Low serum levels of adiponectin have been correlated with dysregulated cellular metabolism, including lipid and glucose metabolism, as well as increased cancer cell aggressiveness. Additionally, elevated adiponectin has been characterized as an anti-tumor factor with potential as a therapeutic target. However, due to its large size and protein instability, the development of adiponectin as a drug has been challenging. Recently, an oral adiponectin receptor agonist, AdipoRon, has been identified and is currently under investigation as a potential cancer treatment. Given that breast cancer is known to be highly influenced by surrounding adipose tissue, we utilized the breast cancer cell models to further evaluate the effects of AdipoRon on cancer progression. Cell viability of various breast cancer cell lines across subtypes was reduced by AdipoRon supplementation, and autophagy was induced in a dose-dependent manner, with significant LC3 lipidation and autophagosome formation. In addition, AdipoRon led to the accumulation of free radicals in mitochondria, accompanied by morphological changes and functional disruption, which in turn caused mitophagy. Lipid metabolism of cancer cells was also impaired by AdipoRon, with a decline in fatty acid storage capacity. The increased free fatty acids were further oxidized, resulting in lipid peroxide accumulation, a hallmark of ferroptosis. Other characteristics of ferroptosis, including ferritinophagy, iron accumulation and a decrease in glutathione peroxidase 4 level, were also observed in AdipoRon-treated cells. Moreover, AdipoRon-induced cell death could be rescued by a ferroptosis inhibitor, ferrostatin, suggesting that ferroptosis was induced by AdipoRon in breast cancer cells. Mechanistically, we identified BECN1, the key regulator of autophagy, as a critical factor in AdipoRon-associated ferroptosis. Knockdown of *BECN1* significantly rescued the ferroptotic cell death and related characteristics in breast cancer cells. Collectively, our data demonstrate that AdipoRon induced cell death in breast cancer cells by disrupting cellular metabolic capacity and triggering ferroptosis, a mechanism that has not been previously reported. These findings suggested the therapeutic potential of AdipoRon in inhibiting breast cancer progression.

#2823 CHAC1 regulates paraptosis by promoting glutathione degradation in cancer cells.

Ling-Chu Chang[1], Shih-Kai Chiang[2], Shuen-Ei Chen[2]

[1]China Medical University Hospital, Taiwan, Taichung, Taiwan,[2]Department of Animal Science, National Chung Hsing University, Taichung, Taiwan, Taichung, Taiwan

Paraptosis is a type of programmed cell death characterized by cytoplasmic vacuolation, merging as a potential therapeutic strategy for cancer treatment. Paraptosis has been associated with disruption of proteostasis and redox homeostasis, however, the detailed mechanisms remain to be explored. CPYPP, a known paraptosis inducer, was employed to investigate the underlying mechanisms in breast cancer and melanoma cells. CPYPP significantly increased the production of reactive oxygen species (ROS). Treatment with *N*-acetyl-$_L$-cysteine (NAC) and glutathione (GSH), but not oxidized glutathione (GSSG), mitigated CPYPP-induced cytoplasmic vacuolation and cell viability inhibition. CPYPP significantly decreased the intracellular GSH/GSSG ratio. Next-generation sequencing analysis indicated that CPYPP upregulates the expression of genes related to the cystine metabolism pathway, including SLC3A2, SLC7A11, cystathionine-γ-lyase (CTH), glutamate-cysteine ligase (GCLC, GCLM), and ChaC glutathione specific γ-glutamylcyclotransferase 1 (CHAC1). CPYPP increased the uptake of cystine. Cystine deprivation attenuated CPYPP-induced protein ubiquitination, cytoplasmic vacuolation, and cell viability inhibition. Furthermore, CHAC1 knockdown ameliorated CPYPP-induced endoplasmic reticulum stress, unfolded protein response, protein ubiquitination, cell viability inhibition, and ROS production. CHAC1 is transcriptionally upregulated by CPYPP via the PI3K-mTOR-CDK7/CDK9 axis. Taken together, these results suggest that the upregulation of CHAC1 mediates paraptosis. CHAC1 enhances the degradation of GSH, resulting in a decline in the GSH/GSSG ratio, a more oxidized intracellular state, and increased ROS production. This leads to the disruption of proteostasis, ultimately inducing paraptosis.

#2824 Physiologic regulation of lipid oxidation and ferroptosis by vitamin E, high-density lipoprotein and scavenger receptor class B type 1.

Jonathan S. Rink[1], Andrea E. Calvert[1], Adam Y. Lin[1], SonBihn T. Nguyen[2], Leo I. Gordon[1], C Shad Thaxton[1]

[1]Northwestern Univ. Feinberg School of Medicine, Chicago, IL,[2]Chemistry, Northwestern Univ. Feinberg School of Medicine, Evanston, IL

Introduction: Ferroptosis is a form of cell death caused by oxidative (-OOH) damage to phospholipids (PL) in the cell membrane. Understanding mechanisms of ferroptosis is important because of the role it plays in cancer. Previous work has focused on the intracellular antioxidant enzyme glutathione peroxidase 4 (GPx4), which detoxifies PL-peroxides (PL-OOH) and prevents ferroptosis. Studies show that alpha-tocopherol (α-toc), the active form of Vitamin E, is an exogenous lipophilic antioxidant that inhibits ferroptosis in cancer cells in vitro. The mechanisms by which α-toc engages cells to manipulate PL redox balance and ferroptosis are unknown. Lipoproteins, like high- and low-density lipoproteins (HDL and LDL), carry α-toc in the circulation. Using diffuse large B cell lymphoma (DLBCL) and clear cell renal cell carcinoma (ccRCC) models, two malignancies sensitive to ferroptosis, we show that lipoproteins, particularly HDL, deliver α-toc by binding the cell membrane receptor scavenger receptor class B type 1 (SR-B1) to prevent ferroptosis.
Methods: Ramos (lymphoma), SUDHL4 (lymphoma) and 786-O (ccRCC) cell lines were used in these experiments. Ferroptosis was induced using ML210 and erastin. α-toc loading of native HDL and LDL was increased by incubation with free α-toc and spin filter purification. To isolate α-toc as the component responsible for modulating ferroptosis, we developed synthetic α-toc-loaded HDLs (sHDL). siRNAs were used to reduce SR-B1 and LDL Receptor (LDLR) expression. Blocker of lipid transport-1 (BLT-1) was used to inhibit uptake of lipoprotein cargo via SR-B1. Cell viability was measured by MTS assay and lipid peroxidation was measured by C11-BODIPY.
Results: The α-toc level was higher in LDLs compared to HDLs (6.0 ± 0.1 α-toc/ apoB-100 vs. 1.0 ± 0.1 α-toc/ apoA-I) and increased in both HDL and LDL following incubation with free α-toc (α-toc LDL, 10.1 ± 0.1; α-toc HDL, 1.6 ± 0.3). HDL, α-toc HDL and α-toc LDL, but not LDL, rescued DLBCL and ccRCC cells from ferroptosis induced by the addition of ML210 and erastin, with a corresponding decrease in PL-OOH accumulation. Similarly, α-toc sHDLs, but not empty sHDLs, prevented ferroptosis in DLBCL and ccRCC. Knockdown of SR-B1 resulted in down-regulation of GPx4 expression and ferroptosis in ccRCC cells, requiring the use of BLT-1 to investigate SR-B1's role in α-toc delivery. Addition of BLT-1 prevented α-toc lipoprotein rescue from ferroptosis. Knockdown of LDLR expression did not alter the ability of HDL and α-toc HDL to prevent ferroptosis and enhanced the ability of α-toc LDL to prevent ferroptosis.
Conclusion: We reveal a tunable cellular redox axis whereby HDLs containing α-toc target SR-B1 to reduce PL-OOH and prevent ferroptosis. Accordingly, this work reveals that the α-toc / HDL/ SR-B1 axis of ferroptosis prevention must be accounted for when targeting ferroptosis in vivo.

#2825 Oncosis-inudcing hydrazones complexes with selective anticancer activity.

Alzbeta Srovnalova[1], Robert Kakplanek[2], Zdenek Kejlik[2], Jan Hajduch[2], Pavel Martasek[2], Josef Srovnal[1], Sona Gurska[1], Katerina Jecmenova[1], Petr Dzubak[1], Marian Hajduch[1], Milan Jakubek[2]

[1]Palacky Univ. Faculty of Medicine, Olomouc, Czech Republic,[2]Charles University, Prague, Czech Republic

INTRODUCTION: The molecular hybridization of three pharmacophores—benzothiazole, cyclobut-2-ene-1,2-dione, and hydrazone—has led to the development of novel compounds with diverse biological activities, including anticancer, anti-inflammatory, and antiviral properties. Among these, certain derivatives demonstrated potent and selective anticancer activity, particularly in CCRF-CEM and HCT116 cell lines. Some derivatives also emitted green fluorescence, enabling organelle-specific imaging by fluorescence microscopy. In U2OS cells, these fluorescent derivatives induced cytoplasmic vacuolization within 24 hours, suggesting a non-apoptotic cell death mechanism consistent with oncosis, characterized by energy depletion, increased membrane permeability, organelle swelling, and protein denaturation.

METHODS: Various microscopy techniques were employed to explore the fluorescent features of target organelles in U2OS cells. Compound 6, with its stable green fluorescence, was selected for detailed analysis. Mitochondria were visualized using MitoTracker dye, with colocalization assessed via Pearson correlation. Mitochondrial membrane potential was evaluated using TMRM dye, which accumulates only in cells with intact membrane potential. Endoplasmic reticulum colocalization with cytoplasmic vacuoles was analyzed using mCherry-tagged calreticulin in a recombinant U2OS cell line. Lysosomal integrity and vacuole colocalization were examined with LysoTracker dye. To determine if cytoplasmic vacuolization is depended on caspase activation, cells were treated with compound 6 in the presence or absence of the pancaspase inhibitor Z-VAD-FMK, and caspase-3/PARP-1 cleavage was assessed by Western blot. The FUCCI reporter system was used for cell cycle analysis.

RESULTS: Compound 6 strongly colocalized with mitochondria and induced mitochondrial dysfunction, ER vacuolization, and oncosis-like non-apoptotic cell death. This suggests that compound 6 exerts anticancer effects by targeting multiple cellular compartments, involving ER stress and mitochondrial disruption. The oncosis-like pathway is significant as it may bypass apoptosis resistance, a key hurdle in cancer therapy. Additionally, compound 6 reduced LysoTracker staining, indicating lysosomal dysfunction. Cell cycle analysis revealed a concentration-dependent G2/M arrest, further supporting its anticancer efficacy.

ACKNOWLDEDGEMENT: This work was supported by the Technology agency of the Czech Republic project PERMED: T2BA (TN02000109) and the National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102). Infrastructure was supported by projects CZ-OPENSCREEN (LM2023052) and EATRIS-CZ (LM2023053). We also acknowledge support from Charles University projects [SVV260637; SVV260521; UNCE 204064; Cooperation ONCO] and IGA_LF_2024_038.

#2826 Celastrol-Cu nanoparticles induce self-amplified cuproptosis augmented cancer immunotherapy.

Huawei Pan, Liuxian Meng, Yifan Li, **Tj (Tiejun) Bing**

ICE Bioscience, Inc., Beijing, China

Cuproptosis, a recently identified copper-dependent programmed cell death pathway, has emerged as a promising strategy in cancer therapy. Its effectiveness is closely linked to intracellular copper accumulation but is mitigated by elevated levels of glutathione (GSH) in tumor cells. A significant barrier to its clinical application lies in the challenge of facilitating the cellular uptake of copper ions. Additionally, intracellular copper ions rapidly bind with GSH, further complicating the induction of tumor cell death. Notably, no existing ionophore drug simultaneously acts as both a copper ionophore and a GSH scavenger to effectively induce cuproptosis.
In summary, we have developed a celastrol-based nanomedicine that, upon complexation with copper ions, effectively promotes cuproptosis in a self-amplifying manner, as validated in both 4T1 subcutaneous and metastatic breast cancer models. This Cel-Cu NP platform provides a novel option with significant translational potential for the integrated programmed therapy of triple-negative breast cancer (TNBC) in immunologically “cold” tumors.

#2827 Heat shock factor 1 regulates the cellular toxicity-induced autophagy in pancreatic ductal adenocarcinoma.

Rejina Shrestha, Shruti Ghai, Ahmad Hegazi, Kuo-Hui Su

Department of Cell and Cancer Biology, The University of Toledo, Toledo, OH

Background: Heat shock factor 1 (HSF1) is a master transcriptional regulator of the proteotoxic stress response and has been known to be hyperactivated in pancreatic ductal adenocarcinoma (PDAC). Recent research has shown that ion-induced cellular toxicity (ferroptosis or cuproptosis) triggers autophagy—a critical mechanism that maintains cellular homeostasis. HSF1 has been established to mediate autophagy in a context-dependent manner. It has been reported that HSF1 prevents copper-induced toxicity in PDAC; however, it remains unclear whether HSF1 mediates autophagy as a mechanism of stress adaptation in response to aforementioned cellular toxicity.
Objective: The objective of this study is to investigate the molecular mechanism of the role of HSF1 in cellular ion toxicity-induced autophagy.
Methods: Human PDAC cell line MIA PaCa-2 was used for this study. A CellTiter Blue Cell Viability Assay was used to determine the IC50 of Erastin, a well-known ferroptosis inducer, and Elesclomol/copper, a copper ionophore, in human PDAC cells. An HSF1 overexpressed (HSF1_WT) MIA PaCa-2 cell was used to test ion-induced toxicity in comparison to the control (LacZ) MIA PaCa-2 cells. Western blotting was used to detect the protein HSF1, autophagy markers, and internal loading control.
Results: In human MIA PaCa-2 cells, Elesclemol/copper and Erastin induced cell death. Elesclomol/copper and Erastin induced increased autophagy marker microtubule-associated protein 1A/1B-light chain 3β (LC3β) lipidation and decreased the expression of total HSF1 protein in a dose-dependent manner. The overexpression of HSF1 prevented the Erastin-induced LC3β lipidation.
Conclusion: HSF1 plays a role in the induction of autophagy, which functions as a stress-adaptive mechanism in the tumor cell in response to the toxicities induced by ferroptosis and cuproptosis inducers.

#2828 Anthocyanin-induced autophagosome accumulation and mitochondrial dysfunction drive apoptosis in liver cancer cells.

Anyssa Rodriguez[1], Samantha Tamayo[1], **Aarya Tripathi**[1], Sumeet Chauhan[1], Subash Chauhan[2], Nirakar Sahoo[1]

[1]School of Integrative Biological and Chemical Sciences, The University of Texas Rio Grande Valley, Edinburg, TX,[2]Medicine and Oncology, School of Medicine, The University of Texas Rio Grande Valley, Edinburg, TX

Introduction: Liver cancer remains one of the most lethal malignancies worldwide. Hepatocellular carcinoma (HCC) accounts for most cases. Existing therapies provide limited efficacy, especially in advanced stages, highlighting the urgent need for alternative treatments. Recent studies have demonstrated that anthocyanins, a natural plant-derived compound, show anticancer potential. Dracorhodin Perchlorate (DP), a synthetic analog of anthocyanin found in *Daemonorops draco* fruit, exhibits cytotoxic effects in cancer cells. This study investigates the mechanisms underlying DP's cytotoxicity in HCC.
Methods: We examined the effect of DP on human liver cancer cells (SK-HEP1). We employed an integrative experimental approach combining cell viability assay, death pathway analysis, autophagy monitoring, lysosomal function evaluation, oxidative stress assessment, and mitochondrial integrity. Western blot, immunocytochemistry, and live cell imaging were employed.
Results: Our result shows that DP significantly reduced SK-HEP1cell viability in a time- and dose-dependent manner. The IC50 values decreased from 60 µM at 24 hours, 26 µM at 48 hours, and 18 µM at 72 hours, reaching 12 µM at 96 hours. We observed decreased pro-caspase-3 levels in DP-treated cells, confirming apoptosis. We found no change in phosphorylated MLKL levels, ruling out necroptosis. Interestingly, we observed a significant increase in LC3II/I ratios and LC3 puncta, indicating autophagosome accumulation. However, we noted no increase in lysosomal activity-based DQ-BSA and Lysotracker assays, suggesting autophagic flux inhibition. Furthermore, we detected a significant rise in reactive oxygen species (ROS) levels following DP treatment, accompanied by elevated HO-1 expression. We found evidence of mitochondrial dysfunction, as Mitotracker assays revealed fragmented mitochondria and reduced cytochrome c release. We observed that Bafilomycin A1 amplified DP-induced apoptosis, supporting the role of autophagic flux inhibition in driving cell death.
Conclusions: Taken together, these findings reveal that DP induces apoptosis in liver cancer cells by driving autophagosome accumulation, oxidative stress, and mitochondrial damage. Our work suggests that DP holds potential as an anthocyanin-based therapy for liver cancer.

#2829 Multifaceted characterization of ferroptosis using live-cell imaging and AI-driven analysis.

J. E. Trigg, G. Lovell, N. Bevan;
Sartorius, Royston, United Kingdom

Ferroptosis is a form of regulated cell death which is distinct from apoptosis and therefore represents a new mechanism by which cancer cells can be targeted and killed. Live-cell imaging was used to capture the dynamic and non-discrete morphology changes associated with ferroptosis in a range of cell types, and to characterize this mechanism of cell death and its contrast with apoptosis. Incucyte® AI Cell Health Analysis uses a trained neural network (NN) to segment cells in phase contrast images. The NNs were trained on a wide range of cell types, including healthy and non-viable, and therefore adapts robustly to unseen cells, yielding highly accurate delineation across a variety of morphologies as well as live/dead classification of ferroptotic cells. Using Incucyte® Advanced Label-free Classification software to perform machine learning-based classification of cells based on morphology, the characteristic ferroptotic transition state was selectively identified using images of cells treated with FIN56. This method was used for label-free quantification of compound efficacy in HeLa cells, comparing apoptosis inducer CMP to ferroptosis inducers Erastin (ERA), FIN56 and RSL3. Pharmacological validation of the mechanism of action showed ferroptosis inhibitor Ferrostatin-1 (FERR) inhibited ERA-induced cell death but not CMP-induced cell death; apoptosis inhibitor Z-VAD-FMK displayed converse selectivity. In addition, we demonstrated that ferroptosis inducer ERA, but not apoptosis inducer staurosporine (STP), was sensitive to the density of cells in the assay. ERA EC_{50} in SK-OV-3 cells decreased from 2.2×10^{-6} to 8.2×10^{-6} as the cell density increased from 500 to 3,000 cells per well while STP EC_{50} remained constant at 1.3×10^{-7} at all cell densities. Fluorescence labeling of live and dead cells was explored to evaluate ferroptosis in 2D monolayer as well as 3D spheroid culture. Fibroblasts were treated with FIN56 in the presence of Incucyte® Cytotox Dye which enters non-viable cells via permeable cell membranes. Rapid ferroptosis was observed with the area of green fluorescence experiencing a rapid increase over 12h post treatment, and cell death was concentration-dependent. Ramos B cell line and adherent A549 cells were labeled with nuclear restricted orange and Near IR fluorophores, respectively. Upon treatment with ferroptosis inducers, both Ramos Nuclight Orange cells and A549 Nuclight NIR spheroids displayed a decrease in fluorescence intensity, consistent with cell death. Ferroptotic cell death in the spheroids was also characterized by an increase in brightfield area as the spheroids lost compactness, in contrast to apoptotic cell death which reduces the total spheroid area. Live-cell analysis provided a valuable platform for characterizing the dynamic process of ferroptotic cell death, yielding insight into the cellular mechanisms, and enabling quantification across multiple cell types.

#2830 Aurora kinase B phosphorylates ZBP1 to drive PANoptosis following treatment with a combination of PARP and ATR inhibitors.

Wenjian Gong[1], Zhiqi Liao[1], Shennan Shi[1], Fan Xiong[1], Mengshi Luo[1], Yuewen Zhang[1], Qiuyang Xu[1], Yijie Wu[1], Linghui Wang[1], Li Zhu[2], Dong Kuang[3], Marilyne Labrie[4], Xuejiao Zhao[1], Gordon B Mills[5], Ding Ma[1], Guangnian Zhao[1], Qinglei Gao[1], Yong Fang[1]

[1]Department of Gynecological Oncology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China,[2]Department of Hematology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China,[3]Department of Pathology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China,[4]Department of Immunology and Cell Biology, Faculty of Medicine and Health Sciences, Universite de Sherbrooke, Sherbrooke, OH, Canada,[5]Division of Oncological Sciences, Oregon Heath and Sciences University, Portland, OR, USA; Knight Cancer Institute, Portland, OR

Background: The combination of DNA damage response inhibitors (DDRis) to induce unrepaired DNA damage entry into mitosis prematurely has emerged as a promising strategy for anticancer therapy. However, the efficacy is often limited by their tolerability due to associated toxicities.
Method & Result: Here we demonstrate that combining poly (ADP-ribose) polymerase (PARP) and ATR inhibitors induces PANoptosis in both cancer and non-cancer cells as a result of abnormal mitotic progression. This is highly dependent on CDK1 activity. Furthermore, ZBP1-dependent PANoptosome (ZBP1-RIPK1-CASP8-CASP6) mediated PARP and ATR inhibitors combination therapy induced PANoptosis. Further exploration revealed that the kinase activity of aurora kinase B (AURKB) was essential for ZBP1-mediated PANoptosis following the treatment. ZBP1 could be phosphorylated by AURKB during mitosis, and phosphorylated ZBP1 subsequently facilitates the assembly and activation of PANoptosome. Finally, our findings were validated in an ovarian cancer patient-derived xenograft (PDX) model in which PARPi and ATRi combination induced PANoptosis in both tumor and normal tissue Importantly, the toxicity to normal tissues was mitigated in ZBP1-knockout mice.
Conclusion: Overall, these results suggest that phosphorylation of ZBP1 by AURKB triggers the activation of ZBP1-dependent PANoptosome after treatment with PARPi and ATRi combination, leading to cell-intrinsic antineoplastic activity and toxicity. Our study elucidates the specific mechanisms underlying cell fate determination during aberrant mitosis progression resulting from PARPi and ATRi combination therapy. These findings provide a theoretical foundation for understanding their anticancer effects and toxicity, crucial for optimizing the use of DDRis combination approaches in the clinic.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Origins and Mechanisms of Genomic Instability
Poster Session

#2834 CyclinE1 amplification promotes chromosomal instability and distinct vulnerabilities in gastroesophageal adenocarcinoma.

Zhong Wu[1], Ankur Nagaraja[2], Shangwei Sun[1], Ling Lan[1], **Jin Zhou**[1], Adam Bass[2]

[1]West China Hospital of Sichuan University, Chengdu City, China,[2]Dana-Farber Cancer Institute, Boston, MA

Background: Gastroesophageal adenocarcinomas (GEA) are characterized by chromosomal instability (CIN) predominantly consisting of high amplitude focal amplifications of oncogenes, including Cyclin E1. An initial pan-cancer analysis revealed that *CCNE1* is one of the top genes whose expression correlates with focal CIN.
Objective: We proposed a causal role for excess CCNE1 as a driver of CIN and gastroesophageal tumorigenesis, and investigated the therapeutic vulnerabilities of *CCNE1* amplification/overexpression.
Design: We engineered a novel *CCNE1*-overexpressing mouse gastric organoid model with *Trp53* deletion and studied the functional effects on tumor initiation and progression. Whole genome sequencing of tumors was performed to characterize the effects of *CCNE1* overexpression on genomic instability. To identify dependencies of *CCNE1*-amplified GEA, we executed a CRISPRi functional genomic screen. Top genetic dependencies were translated into potential pharmacologic vulnerabilities and tested in monotherapy and combination.
Results: *CCNE1* overexpression accelerated tumor initiation and progression of mouse gastric organoids with *Trp53* deletion. These tumors demonstrated increased chromosomal complexity, mainly focal amplifications. Our functional genomic screen identified specific genetic dependencies of *CCNE1*-amplified GEA, including *CCNE1*/*CDK2* as the strongest hits, and *SKP2* as a novel dependency likely through its function as a p27 degrader. Pharmacologic inhibition of CDK2 was insufficient and antagonistic with concurrent inhibition of the DNA damage response via targeting the ATR/CHEK1 axis. However, staggered inhibition of these distinct vulnerabilities demonstrated efficacy.
Conclusions: Our study identifies CCNE1 as a driver of CIN in GEA and builds a foundation for further exploration of therapies for *CCNE1*-amplified GEA characterized by genomic instability.

#2835 Lamin AC deficiency promotes genomic instability through increased R-loop accumulation.

Christopher William Schultz[1], Sourav Saha[1], Anjali Dhall[1], Yang Zhang[1], Parth Desai[2], Lorinc Pongor[3], David A. Scheiblin[4], Valentin Magidson[4], Yilun Sun[5], Christophe Redon[1], Suresh Kumar[1], Manan Krishnamurthy[1], Henrique B. Dias[6], Vasilisa Askenova[7], Elizabeth Giordanio[7], Nobuyuki Takahashi[8], Michael Nirula[1], Yasuhiro Arakawa[9], Ukhyun Jo[10], Beverly A. Teacher[11], Mirit I. Aladjem[1], Stephen Lockett[4], Marry Dasso[7], Yves Pommier[1], Ajit K. Sharma[1], Anish Thomas[1]

[1]National Cancer Institute, Bethesda, MD,[2]Fox Chase Cancer Center, Philadelphia, PA,[3]Cancer Genomics and Epigenetics Core Group, Hungarian Center of Excellence for Molecular Medicine, Szeged, Hungary,[4]Optical Microscopy and Analysis Laboratory, Cancer Research Technology Program, Frederick National Laboratory for Cancer Research, Frederick, MD,[5]Department of Pharmacology, Physiology, and Drug Development, University of Maryland School of Medicine, Baltimore, MD,[6]Cancer Innovation Laboratory, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD,[7]Division of Molecular and Cellular Biology, National Institute of Child Health and Human Development, Bethesda, MD,[8]Department of Medical Oncology, National Cancer Center Hospital East, Kashiwa, Japan,[9]Department of Clinical Pharmacology and Therapeutics, Jikei University School of Medicine, Tokyo, Japan,[10]Developmental Therapeutics Branch and Pediatric Oncology Branch, Center for Cancer Research, Bethesda, MD,[11]Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health, Bethesda, MD

Lamin A/C (*LMNA*), a key component of the nuclear envelope, plays an essential role in maintaining nuclear integrity and regulating genome organization. While *LMNA* dysregulation has been linked to genomic instability in both cancer and aging, the specific mechanisms remain poorly understood. We investigated the role of *LMNA* in small cell lung cancer (SCLC), a highly lethal form of lung cancer characterized by neuroendocrine (NE) differentiation, low *LMNA* expression, and extreme genomic instability. We show that *LMNA* depletion leads to increased R-loop formation, transcription-replication conflicts, replication stress, DNA breaks, the formation of micronuclei and increases sensitivity to TOP1 and ATR inhibition. Mechanistically, *LMNA* loss compromises RNA export efficiency by disrupting the nuclear pore complex distribution causing clumping and reducing incorporation of phenylalanine-glycine-nucleoporins (FG-nucleoporins). We found that *LMNA* expression is dynamically regulated during SCLC differentiation from NE to a non-NE state by EZH2, a component of the polycomb repressive complex that epigenetically silences *LMNA*. Inhibition of EZH2 restored LMNA expression in SCLC cells, and functionally led to a reduction in R-loops. Low *LMNA* levels were associated with significantly reduced survival in SCLC patients implicating R-loops as potential detrimental factor in SCLC patients. To confirm this, we utilized multiplexed immunofluorescence and confirmed LMNA protein was inversely correlated with R-loops both within the same SCLC tumor and across SCLC tumors using an SCLC rapid autopsy sample alongside an SCLC tumor microarray. Interestingly, low expression of canonical R-loop suppressing genes (*RNASEH1, BRD4, SETX and DHX9*) also predicted poorer prognosis in SCLC patients, supporting R-loops as a detrimental factor for SCLC prognosis. Critically, we were able to replicate key findings linking low LMNA to decreased FG-nucleoporin incorporation and increased R-loops using patient derived young and old fibroblasts lines demonstrating generalizability of this finding across disease models. Our results reveal a novel mechanism by which *LMNA* safeguards genome integrity by repressing R-loops, with important implications for both cancer and aging.

#2837 Mechanisms promoting chromosomal instability following the loss of p53 and Rb.

Risha Banerjee[1], David G. Kirsch[2]

[1]Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada,[2]Radiation Medicine Program, Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada

p53 and Rb co-mutations are common in several human cancers and are associated with the onset of aggressive tumors with complex karyotypes. Canonically, these tumor suppressors respond to DNA damage and cellular stress by inducing cell cycle arrest, activating pro-apoptotic and anti-proliferative signalling, and promoting cellular senescence. While these pathways are sufficient to partially explain the tumor suppressive abilities of p53 and Rb, recent studies in genetically engineered mouse models suggest additional, non-canonical means of tumor suppression, including modulation of cellular differentiation and DNA topology, and interestingly, influencing cellular response to chromosomal instability. Chromosomal instability (CIN) is a hallmark of aggressive, therapy-resistant cancers and is associated with mitotic dysfunction, faulty DNA damage responses, immune evasion, and cellular stress response pathways. While it is known that p53 and Rb maintain the integrity of the genomic landscape, the mechanisms by which they prevent CIN remain understudied. Here, we use mouse embryonic fibroblasts (MEFs) to show that p53 + Rb double knock-out (DKO) causes a dramatic increase in ploidy at very early passages, which stabilizes at tetraploid levels. These cells also harbour high levels of CIN, indicated by the presence of micronuclei, DNA double-stranded breaks, and lagging chromosomes. Additionally, we observed CIN in $p53^{25,26}$ transactivation domain (TAD) mutant MEFs that are unable to activate most canonical p53 targets yet retain the ability to suppress tumorigenesis in vivo, raising the possibility that the maintenance of chromosomal stability is dispensable to the tumor suppressive functions of p53. Using a combination of bulk and scRNA sequencing of aneuploid and diploid DKO MEFs, in future experiments we will identify p53- and Rb-dependent changes in gene expression associated with the onset and progression of CIN. Overall, the results of this study are expected to provide critical insights into the molecular mechanisms by which tumor suppressors p53 and Rb work together to repress CIN.

#2838 Modeling putative oncogenic variants of human topoisomerase II by genome editing of a near haploid cell line.

Karin C. Nitiss, Brigid Conroy, Maureen N. McCoy, **John L. Nitiss**

Retzky College of Pharmacy, University of Illinois Chicago, Rockford, IL

DNA topoisomerases (Topos) carry out changes in DNA structure by introducing transient breaks in DNA. These breaks are needed to change DNA conformation, and the enzyme mechanisms minimize possible genome instability from a failure to resolve the cleaved DNA intermediates. Nonetheless, small molecules can interfere with the Topo reaction, leading to elevated DNA cleavage, genome instability and cell death, and this property is the basis of the action of important anti-cancer drugs. Recent work has led to the identification of mutations in eukaryotic Top2 that also result in high levels of Top2 cleavage; including Lys743Asn in the Top2 alpha isoform which was observed in multiple human cancers. Hyper cleavage Top2 mutants are genome destabilizing in yeast and have been shown to induce 2-5 nucleotide *de novo* duplications, as well as other genetic changes. To generate mammalian cell lines expressing hyper cleavage Top2 mutants, we used Piggybac mediated scarless editing in the haploid mammalian cell line eHap. We accomplished successful editing of both Top2 alpha and Top2 beta in this cell line. Successful editing was assessed by sequencing Top2 cDNAs as well as sequencing of the edited genomic loci. Two independent successfully edited cell lines resulted in changes of Lys600Thr in Top2 beta and Lys579Thr in Top2 alpha; allowing us to compare the effects of mutations at orthologous positions in the two mammalian Top2 isoforms. Both edited cell lines show strong constitutive induction of DNA damage responses with elevated levels of DNA-PKcs and histone H2AX phosphorylation. Interestingly, elevated levels of ubiquitylated H2AX were also seen, suggesting that H2AX ubiquitylation may be indicative of constitutive topoisomerase induced damage. These cell lines are being used to measure genetic changes induced by topoisomerase induced damage and will be a unique tool to identify pathways that repair Topo-induced DNA damage. These cells will also provide insights into topoisomerase mediated mechanisms of oncogenesis.

#2839 The functional role of tumor suppressor WW domain-containing oxidoreductase in maintaining genome integrity.
Li-Jin Hsu

National Cheng Kung Univ. Medical College, Tainan, Taiwan

The induction of DNA damage response (DDR) to ensure accurate duplication of genetic information during DNA replication is crucial for maintaining genome integrity. Cellular senescence is a DDR mechanism that prevents the proliferation of cells with deleterious DNA damage to avoid mitotic anomalies and inheritance of the damage over cell generations. A decreased ability of the cells to undergo senescence has been suggested to be associated with cancer development. Using *Wwox* knockout models, we determined that *Wwox* knockout mouse embryonic fibroblasts (MEFs) failed to undergo replication stress-induced cellular senescence after multiple passages in culture. Importantly, accelerated cell cycle progression and increased apoptosis were detected in *Wwox* knockout MEFs at late passages. Increased γH2AX-positive cells and microsatellite instability were examined in late-passage *Wwox* knockout MEFs, indicating massive accumulation of DNA damages in these cells. We detected significantly reduced expression of p53 and CDK inhibitors $p16^{INK4A}$ and $p21^{cip1/waf1}$ in late-passage *Wwox* knockout MEFs. Inhibition of oxidative stress during *en vitro* passage culture reversed $p16^{INK4A}$ and $p21^{cip1/waf1}$ downregulation and induced senescence in *Wwox* knockout MEFs. Importantly, we demonstrated that WWOX protein expression was upregulated in cells during DNA replication. Significantly enhanced phosphorylation of Chk-1 protein was detected in proliferating WWOX-knockdown HEK-293T cells and *Wwox* knockout MEFs, suggesting that WWOX plays an important role in regulating DDR signaling for maintaining genome stability upon replication stress. Our findings delineate the regulatory role of WWOX in DDR and have great impact on our understanding of molecular pathogenesis of cancer and senescence-related diseases.

#2842 Dual-specificity phosphatase 12 as a key modulator of DNA damage response and nucleolar stress in hepatocellular carcinoma.

Viktor Kalbermatter Boell, **Fabio Luis Forti**

Department of Biochemistry, Institute of Chemistry, University of Sao Paulo, Sao Paulo, Brazil

The dual-specificity phosphatase 12 (DUSP12) has been implicated in liver pathologies, such as hepatocellular carcinoma (HCC), through molecular mechanisms poorly understood. Our group identified protein partners of DUSP12 in tumor cells under genotoxic stress, including the nucleolar proteins NAT10 and TCOF1, well-recognized for their roles in ribosome biogenesis, but which involvement in DNA damage response (DDR) mechanisms has gained attention. Given these proteins are associated with HCC progression and poor prognosis, this study aimed to explore the role of DUSP12 in the hepatic cell lines HepaRG and HuH-7 under treatments with cisplatin (CP) or doxorubicin (DX). Datamining the TCGA database revealed frequent amplification of DUSP12 in HCC patients, resulting in elevated mRNA and protein expression levels, which were strongly correlated with NAT10 and TCOF1 expression. Gene ontology analyses of DUSP12-correlated genes in HCC patients highlighted novel functional relationships between DUSP12 and processes related to DNA repair and DDR. In vitro, DX treatment produced marked cytotoxic and antiproliferative effects in both monolayer cultures of HepaRG and HuH-7 cells. However, noteworthy resistance was observed in 3D spheroid cultures of these cell lines. At the molecular level, DX treatment elicited an early and robust activation of DDR pathways compared to CP treatment, as evidenced by increased levels of p-Chk2 (Thr68), p-p53 (Ser15), and γH2AX (Ser139) in both cell lines. Intriguingly, DX treatment also induced distinct nucleolar stress features, nuclear accumulation of DUSP12, and the redistribution of NAT10 and TCOF1 to the nucleoplasm, although their expression levels remained unchanged. CRISPR-Cas9-mediated knockdown (KD) of DUSP12 in HuH-7 cells sensitized these cells to DX treatment in both monolayer and spheroid cultures in a manner proportional to DUSP12 expression levels. This sensitization to DX occurs independently of reactive oxygen species (ROS) modulation and was accompanied by increased levels of p-p53 (Ser15) and γH2AX, as well as the modulation of double-strand break levels as assessed by comet assay, suggesting that DUSP12 KD affects DDR signaling and DNA repair in these cells. Similarly, transfection with DUSP12 siRNA enhanced γH2AX focus formation in HepaRG cells treated with DX. Notably, DUSP12 KD in HuH-7 cells induced nucleolar stress characteristics, as evidenced by the mislocalization of NAT10 and TCOF1, as well as hyperploidy, increased micronuclei frequency, and a higher mitotic cell population, indicated by elevated p-H3 (Ser10) levels. Altogether, our findings reveal that DUSP12 plays a pivotal role in hepatocellular carcinoma by affecting genomic stability and modulating DNA damage response and nucleolar stress pathways under genotoxic conditions, underscoring its potential as a therapeutic target in HCC.

#2843 Neoantigen evaluation in viably cryopreserved dissociated tissue via large scale proteomic profiling.

Shawn P. Fahl, Casey Smith, Kerri Colwell, Jessica Moore, Jaison Arivalagan, Danielle Gutierrez

Discovery Life Sciences, Huntsville, AL

Genomic instability is a hallmark of cancer where the acquisition of numerous genetic alterations leads to transformation and carcinogenesis. These alterations result in the generation of potentially immunogenic neoantigens, and the clinical success of checkpoint inhibitor therapies has highlighted the importance of neoantigens in cancer immunotherapy. Evaluation of the next generation of neoantigen therapies is complicated by the difficulty in sourcing primary patient material. During tumor resection and pathology review, the tissue is fixed, which prevents the ability to test in vitro model systems for therapy efficacy. While fresh tissue is amendable to in vitro culture, it is not possible to perform large scale genomic and proteomic screens in a timeframe for the culture to occur. By leveraging viably cryopreserved dissociated tissue, the tumor can be analyzed for genetic alterations and then, when novel neoantigens are determined, cultured at a later date. To test this workflow, we profiled dissociated tissues from cancer indications with a high degree of genomic instability for microsatellite instability (MSI). Consistent with previous studies, 17-55% of endometrial, colorectal, and gastric cancer samples were MSI-high. MSI-high and microsatellite stable (MSS) samples were further analyzed for peptide identification using LC-MS/MS (Orbitrap Astral) and over 10,000 protein groups and 80,000 peptides were identified. Samples were also amendable to T cell receptor (TCR) repertoire sequencing to uncover tumor-reactive T cell clones. These deep molecular analyses provided the foundation for the development of short-term spheroid and long-term tumoroid culture systems to understand immune responses using autologous tumor-infiltrating and peripheral lymphocytes. Collectively, this workflow provides a scalable system to identify neoantigens and evaluate the immune response to these antigens in a robust and reproducible pipeline.

#2844 A cytosolic DNA/Ca^{2+}-dependent signaling pathway for genome protection during replication stress.

Z. You;
Washington University School of Medicine, St. Louis, MO

Protecting the replication fork structure, especially under replication stress, is essential for maintaining genome integrity and stability. Defects in fork protection, often linked to mutations in tumor suppressor genes like BRCA1, BRCA2 and FANCD2, can lead to genomic instability and consequently, cancer progression. Such defects may also influence cancer progression by altering the tumor microenvironment through the generation of cytosolic DNA fragments that activate the innate immune response. Intriguingly, our recent findings indicate that cytosolic DNA, produced after replication stress, unexpectedly also supports replication fork protection. This protective effect is mediated through a complex signaling pathway that involves the activation of the cGAS/cGAMP/STING axis, Ca2+ release from the endoplasmic reticulum via the TRPV2 ion channel, activation of CaMKK2 and AMPK protein kinases, and the inhibition of the EXO1 nuclease. Disruption of this pathway results in excessive fork processing, chromosomal instability and heightend sensitivity to replication stress inducers. The newly uncovered links between innate immune factors, intracellular Ca2+ and genome maintenance in this novel signaling pathway may open new avenues for cancer treatment. (Li *et al.*, *Mol Cell* 2019, 74:1123-1137; Li, Kong *et al., Mol Cell* 2023, 85:556-573; Kong, Cheng *et al., Nat Commun* 2024, 15:4609)

#2845 Midasin overexpression drives intrinsic endocrine resistance by stabilizing DNA damage.

Manasa Kotina[1], Afia Ohemeng[1], Mounika Pamukuntla[1], Bipika Banjara[1], Alicia Hudson[1], A. Micheal Davidson[1], Jillian L. Pope[2], Selina Darling-Reed[1], Syreeta L. Tilghman[1]

[1]Basic Pharmaceutical Sciences, Florida A&M University, Tallahassee, FL,[2]Biological Sciences, Florida A&M University, Tallahassee, FL

Midasin (MDN1), a AAA-ATPase protein critical to ribosome biogenesis, has emerged as a key player in the progression of triple-negative breast cancer (TNBC), a subtype characterized by its heterogeneity, absence of therapeutic targets, and poor prognosis. Previous work from our lab and others, demonstrated that estrogen receptor negative (ER-) breast cancer patients with high MDN1 expression are positively correlated with a worse relapse-free survival compared to their ER+ counterparts and breast tumors with MDN1 mutations are associated with high tumor mutation burden and unfavorable prognosis. Additionally, MDN1 alterations are associated with increased expression of a signature of proteins involved in cell cycle dysregulation and genomic instability. Thus, we hypothesize that MDN1 overexpression drives intrinsic endocrine resistance by stabilizing DNA damage which promotes tumor cell survival and progression. To gain mechanistic insight into the role of MDN1 in ER- breast cancer, we employed, Ribozinoindole-2 (i.e., Rbin-2), a potent MDN1 pharmacologic inhibitor to study two TNBC cell lines, which express high (MDA-MB-468) and low (MDA-MB-231) levels of MDN1. Both cell lines were treated with 1-12 μM Rbin-2, and after 72 hour, 12 μM Rbin-2 treatment induced a 50% reduction in cell viability. Western blot analyses were conducted, and MDA-MB-468 cells expressed a 1.5-fold increase in MDN1 expression compared to the MDA-MB-231 cells. Rbin-2 treatment reduced MDN1 expression by 30-50% in both cell lines. To gain an in depth understanding of the impact on MDN1 alterations on the fate of the cell, we conducted a series of *in silico* studies and observed that breast cancer patients with alterations in MDN1 are associated with various forms of DNA damage including higher mutation counts, microsatellite instability, and fractions of the genome that are altered. To confirm this finding, a focused DNA damage antibody array study was performed which revealed that 12 μM Rbin-2 treatment decreased mismatch repair proteins and DNA damage checkpoint markers. We validated this finding by immunoblots and Rbin-2 treatment reduced MSH2, MSH6, and phospho-p53, by 13.8-15.6% in MDA-MB-231 cells and 20-25% in MDA-MB-468 cells further supporting the *in silico* and protein array data. Immunofluorescence and immunoblotting of γH2AX, a marker for DNA double-strand breaks, confirmed that MDN1 inhibition caused a 55-75% increase in DNA damage in both cell lines, suggesting that MDN1 plays a key role in maintaining genomic stability and regulating DNA damage responses in TNBC. Given these findings elevated MDN1 expression may drive DNA repair dysregulation, thereby providing a survival advantage for *de novo* endocrine resistant breast cancer. These preliminary studies suggest that MDN1 may represents a potential therapeutic strategy to exploit TNBC's inherent genomic instability and improve treatment outcomes.

#2846 Association of cyclin E1 expression with genomic instability in ovarian cancer.

Ajay Obla[1], Michelle Kinder[1], Chifei Sun[1], Heather Bullins[1], Cynthia Timmers[1], Ming Poi[2], Sharon Wu[2], Edoardo Missiglia[3], Bettina Bisig[3], Krisztian Homicsko[4]

[1]Incyte Research Institute, Wilmington, DE,[2]Caris Life Sciences, Phoenix, AZ,[3]Institute of Pathology, Department of Laboratory Medicine and Pathology, Lausanne University Hospital (CHUV) and Lausanne University, Lausanne, Switzerland,[4]Department of Oncology, Lausanne University Hospital (CHUV); Ludwig Institute for Cancer Research, Lausanne Branch; Swiss Cancer Center Leman, Lausanne, Switzerland

Background: Cyclin E1 (CCNE1) expression has been associated with replicative stress and defects in double-strand break repair in multiple tumor types; however, this has yet to be demonstrated in ovarian cancer. Herein, we describe *CCNE1* DNA amplification (AMP), messenger RNA (mRNA) expression, and cyclin E1 protein expression in 2 large cohorts of high-grade serous ovarian cancer (HGSOC) (The Cancer Genome Atlas [TCGA] and Caris Life Sciences, Phoenix, AZ, USA).
Methods: 8127 HGSOC samples were analyzed by next-generation sequencing (NextSeq, 592 genes or NovaSeq, whole exome sequencing) and RNA (NovaSeq, whole transcriptome sequencing) (Caris Life Sciences). For genomic loss of heterozygosity (LOH), 22 autosomal chromosomes were split into 552 segments and the LOH of single nucleotide polymorphisms within each segment was calculated (LOH-H ≥16%). Homologous repair deficient (HRD+/−) status was determined by high genomic scar score (GSS) or *BRCA1/2* mutant (MT) [HRD+], or low GSS and *BRCA1/2* wild-type (WT) [HRD−], respectively. GSS was calculated from large-scale state transitions (LSTs) and LOH (GSS-H ≥46). 1177 samples from TCGA were also included in this analysis.
Results: In the Caris data set, HGSOC *CCNE1* AMP was significantly more frequent in *BRCA1/2* WT HGSOC tumors (*CCNE1* AMP: 4.88% *BRCA1/2* MT vs 21% *BRCA1/2* WT; $P<0.0001$), as reported in other studies. Additionally, median expression of *CCNE1* mRNA was higher in *BRCA1/2* WT samples when compared with *BRCA1/2* MT samples (1.11-fc; $P<0.0001$). Median *CCNE1* mRNA was higher in HRD− HGSOC compared with HRD+ HGSOC (Caris: 1.11-fc, $P<0.0001$; TCGA: $P<0.0001$). In both data sets, *CCNE1* AMP was associated with lower global LOH prevalence (CCNE1-AMP %: 22.6% in LOH-L vs 13.9% LOH-H in Caris data, $P<0.0001$; TCGA, $P<0.0001$). However, there was a significant increase in *CCNE1* mRNA expression in LOH-H compared with LOH-L HGSOC Caris samples (1.27-fc, $P<0.0001$). There were no associations between *CCNE1* AMP or RNA/protein expression and LST in either data set, or with telomeric allelic imbalance score in TCGA. *CCNE1* AMP was associated with aneuploidy in TCGA ($P<0.001$). We also examined an independent set of commercial ovarian cancer samples for CCNE1 protein and pH2AX expression by immunohistochemistry to evaluate for an association with double-strand breaks, which will be included in the presentation.
Conclusion: Together, these data demonstrated that *CCNE1* AMP and RNA/protein expression were associated with various types of replicative stress in HGSOC. These data warrant further investigation on the impact of *CCNE1* AMP and CCNE1 expression on clinical outcomes.

#2847 Patterns of APOBEC3-mediated chromosomal rearrangements in solid cancers.

Taek-Chin Cheong[1], Qi Wang[1], Ahram Jang[1], Elif Karaca-Atabay[1], Jake June-Koo Lee[2], Roberto Chiarle[1]

[1]Department of Pathology, Boston Children's Hospital and Harvard Medical School, Boston, MA,[2]Department of Biomedical Informatics, Harvard Medical School, Boston, MA

Chromosomal translocations are complex biological processes that require the formation of DNA double-strand breaks (DSBs) at two different loci, followed by the joining of the broken ends by DNA repair mechanisms. In B-cell lymphoma, chromosomal translocations are directed by the on- or off-target activity of activation-induced cytidine deaminase (AID) that converts cytosine to uracil in single-stranded DNA (ssDNA), facilitating DSBs when two unpaired nicks are close together. However, no such enzymes have been discovered in solid tumors. While AID, a B-cell-specific APOBEC enzyme, is responsible for somatic hypermutations and chromosomal translocations in B-cell malignancies, apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3 (APOBEC3; A3) enzymes have been primarily studied for their mutagenic potential in solid cancers. In this study, we present detailed characterizations of A3-induced chromosomal translocations in multiple cell models. While shared features of AID- and A3-driven translocations are expected because they deaminate cytosine at similar consensus sequences which should result in similar patterns of DNA DSB hotspots, their translocation patterns are partially distinct. As expected, AID generated focal DSB clusters (FDCs) near transcription start site (TSS) regions, but AID also induced new types of FDCs in the gene body and in both TSS and gene body, as well as dispersed DSB cluster (DDC)-type translocations. Interestingly, A3s enhanced FDCs and DDCs in fibroblasts with the similar level of AID, but A3s were only able to induce FDCs in the gene body, not in the TSS region. Both AID- and A3-mediated chromosomal translocations were enriched in genomic regions associated with early-replicating constant timing regions (CTRs), A compartments, and topologically associated domain (TAD) boundaries. Moreover, we revealed an enrichment of A3-attributed mutational signatures at the vicinity of A3-induced chromosomal rearrangements and the oncogene amplification boundaries in patient samples. Finally, we found that A3 expression significantly increased the number of oncogenic tyrosine kinase (TK) fusions, such as ALK, RET, ROS1, and NTRK1 fusions that are recurrently found in non-small cell lung cancer (NSCLC). Furthermore, deletion of APOBEC3 genes significantly decreased the number of oncogenic TK fusions. This study highlights that A3 enzymes induce chromosomal translocations and the formation oncogenic TK fusions in human cancers, with their translocation patterns distinct from but partially shared with those induced by AID, suggesting A3s as promising therapeutic targets for reducing genomic instability in cancer patients.

#2849 Comparative analysis of mutational signatures in TumorGraft PDX bank and TCGA dataset to improve patient tumor modeling.

Stefano Cairo, Mara Gilardi, Marianna Zipeto, Michael Ritchie, Gilad Silberberg

Champions Oncology, Inc., Hackensack, NJ

Mutational signatures are unique patterns of DNA alterations that arise from specific mutagenic processes, such as exposure to genotoxic agents or DNA repair deficiencies. Tumors with common mutational signatures can harbor defects in specific DNA repair pathways that may underlie common genetic vulnerabilities. Furthermore, exposure patterns of mutational signatures can vary among clinical types of cancer. Champions' TumorGraft platform, a collection of 1500 patient-derived xenografts generated from more than 50 different types of cancer, is one of the most comprehensive preclinical oncology platforms worldwide and aims to recapitulate the variety of the patient population and tumor biology complexity. We performed the mutational signatures by profiling our models through whole exome sequencing. The results obtained were crossed with the patient's clinical history and showed a good correlation between the mutational signatures identified and tumor etiology and DNA damage repair alterations known to be associated with some indications. To assess the clinical relevance of our findings, we applied the molecular signature profiling method used with our PDXs to the TCGA dataset. This analysis revealed that the mutational profiles enabled the co-clustering of patients' tumors and PDXs from the same indications. As expected, we also observed co-clustering of tumors from indications that share exposure to a specific environmental or therapeutic genotoxic agent (e.g., tobacco smoke, chemotherapy, or radiation), or common pro-oncogenic genetic events associated with DNA damage repair alterations, such as homologous recombination repair, that can be detected in tumor samples across different indications. In addition to this, we found other signatures, associated with known, inferred or unknown etiology, detected across indications and affecting a limited number of tumors per indication. By combining the insights from these mutational signatures with the clinical data and the comprehensive pharmacological and molecular characterization of our models, including whole transcriptome, proteomics, and phospho-proteomics data, we evaluated the association of the tumors that harbor these signatures with tumor biology and clinical outcome. This approach led to the identification of mechanisms of action and biomarkers that might allow the identification of personalized therapies for patients with these tumors that are driven by similar mutational profiles, regardless of tumor indication.

#2851 YAP1-TFE3 fusion protein promotes EndMT in epithelioid hemangioendothelioma.

Ant Murphy, Sam Hartzler, Paula Carranza, Madison Yates, Nimod Janson, Shyaman Jayasundara, Majid Kazemian, **Jason A. Hanna**

Purdue University, West Lafayette, IN

Epithelioid Hemangioendothelioma (EHE) is an ultra-rare vascular cancer driven by chromosomal translocations resulting in the TAZ-CAMTA1 or YAP1-TFE3 fusion transcription factors. Despite some knowledge of the disease defining fusion proteins, the mechanisms driving tumorigenesis remain largely unclear. To study the effects of EHE fusion protein expression on the biology of endothelial cells, we generated EHE cell models using the murine endothelial cell line MS-1 and the human endothelial cell line HUVEC. Expression of TC and YT led to increased Hippo target gene expression, as expected. However, we observed a striking phenotype in YT-expressing endothelial cells associated with morphologic changes, enhanced migratory potential, and anchorage independence growth. These phenotypic changes are consistent with a process known as endothelial-to-mesenchymal transition (EndMT). EndMT is a physiological process where endothelial cells lose their endothelial identity and become more mesenchymal-like. Indeed, we observe transcriptional changes consistent with EndMT such as the reduction in expression of multiple endothelial-specific markers and a significant increase in transcription of mesenchymal marker, *Acta2.* We utilize several gain- and loss-of-function approaches to further investigate the mechanisms and impact of EndMT in tumorigenesis.

#2852 Mapping single cell spatial 3D genome landscape of localized and metastatic breast cancer sub-types.

Shyamtanu Chattoraj, Huy Nguyen, **Jude Dunne**

Bruker Spatial Genomics, San Jose, CA

Metastatic breast cancer is the most aggressive form of breast cancer leading to poor disease-free patient survival and accounting for 90% of all breast cancer-related deaths. The specific mechanisms that promote metastasis are not fully understood owing to the complex interplay between cancer cells and their surrounding microenvironment. This microenvironment involves clonal and sub-clonal heterogeneity in genomic, transcriptomic and protein signaling network across different cancer cell sub-populations. Understanding the molecular mechanism behind cancer metastasis demands state of the art single cell and spatial technologies that can provide deeper insight on disease progression.

Since, cancer is heavily influenced by genetic changes, the study of the 3D- genome organization in single cells helps to improve understanding of disease progression and prognosis. The genome is organized and packaged in a highly structured manner inside the nucleus of mammalian cells and exhibits dynamic reorganization throughout disease progression. Understanding this 3D organization at the single-cell level with high spatial resolution is crucial for disease research.

In this abstract, we present a novel jebFISH^{TM} protocol on the PaintScape^{TM} platform that can be used to understand differences in global 3D genome organization among several localized and metastatic breast cancer cell lines at single cell, sub-population and population level. Using a highly multiplexed genome wide panel of chromosomal targets, we will show disruption in chromosome territory, radius of gyration, chromosomal instability between localized and metastatic breast cancer cells at single cell and sub-population level. We will also show the signature of specific 3D genome structural alterations and interchromosomal interactions related to breast cancer metastasis compared to normal breast cells. In addition, we investigated differences in global 3D genome architecture of different breast cancer sub-types such as HER2 enriched vs triple negative breast cancer cells. We will show how different breast cancer sub-types carry specific chromosomal instability and differential interchromosomal interaction patterns at single cell, sub-chromosomal and sub-population level. We envision this study will improve our understanding on breast cancer disease progression and provide deeper insight into the underlying genomic heterogeneity of single cancer cells and sub-populations which might help to design better treatment options in the future. We propose that this study serves as proof-of-principle and is generalizable to other types of cancers where changes in 3D genome organization may occur at different cancer stages.

#2853 Engineered extrachromosomal oncogene amplifications promote tumorigenesis.

M. A. Yao[1], D. Pradella[1], M. Zhang[1], R. Gao[1], K. M. Gluchowska[1], Y. Cendon Florez[1], T. Mishra[1], G. La Rocca[1], M. Weigl[1], Z. Jiao[1], H. H. M. Nguyen[1], M. Lisi[1], M. M. Ozimek[1], C. Mastroleo[1], K. Chen[1], F. Grimm[1], J. Luebeck[2], S. Zhang[3], A. Zolli[1], E. G. Sun[1], B. Dameracharla[2], Z. Zhao[4], Y. Pritykin[4], C. Siegel[1], H. Y. Chang[3], P. S. Mischel[3], V. Bafna[2], C. R. Antonescu[1], A. Ventura[1];
[1]Memorial Sloan Kettering Cancer Center, New York, NY, [2]UC San Diego, La Jolla, CA, [3]Stanford University, Stanford, CA, [4]Princeton University, Princeton, NJ

Focal gene amplifications are among the most common cancer-associated mutations but have proven challenging to engineer in primary cells and model organisms. Here we describe a general strategy to engineer large (more than 1 Mbp) focal amplifications mediated by extrachromosomal DNAs (ecDNAs) in a spatiotemporally controlled manner in cells and in mice. By coupling ecDNA formation with expression of selectable markers, we track the dynamics of ecDNA-containing cells under physiological conditions and in the presence of specific selective pressures. We also apply this approach to generate mice harbouring Cre-inducible Myc- and Mdm2-containing ecDNAs analogous to those occurring in human cancers. We show that the engineered ecDNAs spontaneously accumulate in primary cells derived from these animals, promoting their proliferation, immortalization and transformation. Finally, we demonstrate the ability of Mdm2-containing ecDNAs to promote tumour formation in an autochthonous mouse model of hepatocellular carcinoma.These findings offer insights into the role of ecDNA-mediated gene amplifications in tumorigenesis. We anticipate that this approach will be valuable for investigating further unresolved aspects of ecDNA biology and for developing new preclinical immunocompetent mouse models of human cancers harbouring specific focal gene amplifications.

#2854 The role of NONO in genome stability: Implications for RNA-DNA hybrid accumulation, genomic instability, and inflammatory signaling.

Vikas Gaur, Sara Knowles, Weihang Chai

Center for Genetic Diseases, and Department of Microbiology and Immunology, Rosalind Franklin Univ. of Med. & Science, North Chicago, IL

Genomic instability, often resulting from replication stress and defects in DNA damage repair pathways, is a hallmark of cancer. The NONO protein, a multifunctional RNA-binding factor, plays a key role in DNA damage repair, transcriptional regulation and genome stability. Emerging evidence has linked NONO to cancer progression, though its mechanistic roles in maintaining genomic stability remain unclear. In our study, we demonstrate that NONO localizes at replication forks, as revealed by iPOND and SIRF assays, and interacts with replication polymerases. Loss of NONO in knockout (KO) cells leads to increased replication speed, significant RNA-DNA hybrid (R-loop) accumulation, and heightened genomic instability. These cells exhibit elevated micronuclei formation, abnormal chromosome segregation during metaphase, and increased 53BP1 foci, a marker of DNA double-strand breaks. NONO KO cells also show higher RPA loading under replication stress, highlighting their vulnerability to DNA damage. Additionally, the loss of NONO activates inflammatory response genes, including phospho-NFκB, p-IKKα, cGAS, and IRF3, suggesting that DNA-RNA hybrid accumulation and DNA damage may drive inflammatory signaling pathways. These findings position NONO as a critical regulator of genome stability, replication dynamics, and inflammation suppression. Our study highlights the essential role of NONO in maintaining genome integrity, interacting with replication machinery, and resolving R-loops. We will present ongoing work to further elucidate the mechanisms by which NONO contributes to genome stability and inflammatory responses in cancer cells.

#2855 CDK12 inhibition remodels DNA replication to drive selective gene amplification.

Shivani Yalala[1], Julia Kutz[2], Helmut Pospiech[2], Harri M. Itkonen[1]

[1]University of Helsinki, Helsinki, Finland,[2]Leibniz Institute on Aging - Fritz Lipmann Institute, Jena, Germany

Background and Objective: Transcription is controlled by phosphorylation of the C-terminal domain (CTD) of RNA Polymerase II (RNA Pol II). Cyclin-dependent transcriptional kinase 12 (CDK12) phosphorylates the CTD to sustain productive transcription elongation of long genes. Unexpectedly, because cancer cells typically exhibit high levels of transcription, CDK12 inactivation defines a subset of aggressive prostate cancers, which have a high number of gene amplifications in the absence of simpler mutations. Defects in transcription of specific genes and collisions between transcription and DNA replication machineries have been proposed as the source of this mutational signature. We hypothesize that DNA replication machinery and program are directly impacted by CDK12 inactivation, and some of the genes selectively amplified in the mutant tumors are required to sustain proliferation of these cells.
Methods: To assess the effects of CDK12 inactivation on DNA synthesis, we have measured nucleotide incorporation and relied on DNA combing assays. ChIP-seq experiments against the DNA replication machinery were used to probe the DNA replication program. Finally, proteomic-profiling of DNA replication machinery in the presence and absence of CDK12 inhibitor was used to evaluate the effects on its composition.
Results: CDK12 inhibition leads to defects in DNA replication as measured by nucleotide incorporation, which are explained, at least in part, due to replication fork stalling as determined using DNA combing assays. Integration of ChIP-seq data against the DNA replication machinery with data from CDK12 mutant prostate cancer patients revealed accumulation of the machinery on a specific sub-set of genes amplified in the patient samples. We termed this gene set as replication-defective and CDK12 mutant amplified (ReDeAmp). Mass spectrometry-based profiling of DNA replication machinery revealed significant remodeling in response to CDK12 inhibition. Currently, we are probing if the genes of the ReDeAmp-signature or the proteins accumulating to DNA replication machinery are essential for the proliferation of the CDK12-mutant cells.
Conclusions: Our data show that decrease in CDK12 activity disrupts DNA replication, which we propose explains the unique mutational signature observed clinically when CDK12 is inactivated. These findings further pose that selective gene amplifications are required in the CDK12-deficient cells. If this was the case, CDK12 inactivation would serve as a biomarker of sensitivity for the acquired synthetic lethality - this is currently under intense investigation in our laboratory.

#2856 Pks+ E. coli trigger intestinal stem cell plasticity and early onset colorectal cancer.

Stefanie Gerstberger[1], Melissa Lumish[2], Saskia Hartner[3], Farheen Shah[1], Seongmin Choi[1], Krystal Lum[4], Christopher Cowley[1], Anisha Luthra[1], Qingwen Jiang[1], Hyung Jun Woo[1], Ahmed Mahmoud[1], Henry Walch[1], Asha Saxena[1], Tavis J. Reed[4], Andrea Cercek[1], Rona Yaeger[1], Ileana Cristea[4], Andrew McPherson[1], Francisco Sanchez-Vega[1], Karuna Ganesh[1]

[1]Memorial Sloan Kettering Cancer Center, New York, NY,[2]University Hospitals Cleveland Medical Center, Cleveland, OH,[3]McGill University, Montreal, QC, Canada,[4]Princeton University, Princeton, NJ

Escherichia coli (E.coli) strains carrying the polyketide synthase (pks) island produce the genotoxin colibactin associated with pathogenesis of colorectal cancer (CRC). The colibactin polyketide induces DNA damage through direct alkylation of DNA, which leads to characteristic mutational A-T rich signatures in the genome (SBS28 and SBS88) that can be identified by whole genome sequencing (WGS). Since WGS is not routinely used clinically, we developed an approach to specifically identify SBS-pks using a clinical targeted exon capture assay, MSK-IMPACT. In an institutional pan-cancer clinical cohort of 78,905 tumors we identified 15,967 samples with >20 mutations detected by MSK-IMPACT. We identified 149/1845 samples with microsatellite stable CRC with >10% mutations attributable to SBS-pks. Examining clinicopathologic and genomic features of SBS-pks+ compared to SBS-pks- CRC tumors, we find that SBS-pks+ signatures are significantly enriched in younger age of onset compared to SBS-pks- CRC patients (median age 52 vs 57, p=0.00059). While global genomic features and oncogenic pathways are similar, we find that the APC:c835-8A>G splice site mutation is overrepresented in SBS-pks+ tumors with 21.7% enrichment compared to 4.99% in SBS-pks- tumors, even further enriched in young onset SBS-pks+ CRC (27% <50 y, 31% <45 y, p<0.0001). To mechanistically investigate colibactin-associated damage we developed a novel long-term human colon organoid-microbe co-culture model system that takes advantage of reversal of polarity in suspension culture. WGS of human CRC organoids co-cultured for three months with pks+ and delta-pks E.coli NC101 confirmed induction of SBS-pks signatures, validating the model. 48h acute exposure to pks+ E.coli upregulates DNA replication and DNA damage repair (DDR) pathways coupled with cell cycle stalling in S phase with activation of ATM/ATR, indicative of replication stress. Strikingly, pks+ E.coli exposure drives a phenotypic shift of tumor and normal epithelial cells into an intestinal stem cell (ISC) state, with pks+ exposed cells demonstrating increased regeneration and proliferation, while thermal proximity coaggregation-MS analysis of protein complexes reveals global chromatin remodeling changes. Thus, our findings suggest that colibactin SBS signature is associated with young onset CRC and that the colibactin genotoxin phenotypically promotes cells to enter a proliferative intestinal stem cell states as precursors to CRC carcinogenesis. Correlating our clinicogenomic findings of an increased pks+ signature presence in young onset patients, we propose a model of colibactin injury in which younger, DNA replication and repair proficient cells are able to overcome colibactin-associated DDR stress, while aged colonic cells exit DDR stress through cell cycle arrest and apoptosis, resulting in the overall observed enriched genomic colibactin signature in young onset CRC.

#2857 Genomic Analysis of UV-induced Mutations in Normal Skin Cells of Dark and Light-skinned Individuals.

Rojina Nekoonam, Aravind K. Bandari, Delahny Deivendran, Bishal Tandukar, Harsh Sharma, Alan H. Shain

Dermatology, University of California San Francisco, San Francisco, CA

Objectives/Goals: Despite the documented link between UV exposure and skin cancer, the role of pigmentation in preventing skin carcinogenesis is not fully understood. This project aims to investigate how pigmentation influences the earliest stages of skin cancer development by examining the genomic landscape in skin cells across individuals of diverse skin tones, which will uncover potential differences in susceptibility and mutation patterns.

Methods/Study Population: We collected skin biopsies from various body sites exposed to different levels of habitual sun exposure. Next, we cultured individual skin cells, including keratinocytes, melanocytes, and dermal fibroblasts, ex vivo to form small colonies of daughter cells. We extracted DNA and RNA from these colonies and amplified the nucleic acids in vitro. Subsequently, we sequenced the expanded clones at exome-level resolution. For our study, we collected samples from seven dark skin donors ranging in age from 35 to 83 years. For comparison, we also analyzed mutations in skin cells from 33 donors with light skin tones, who served as the control group.

Results: Our preliminary analysis of the existing data suggests expected trends. The average mutation burden in individuals with light skin tones was 4.967 Mut/Mb, whereas in those with dark skin tones, it was only 1.609 Mut/Mb. These findings align with the prevailing hypothesis that melanin acts as a protective factor against skin cancer, as it appears to reduce the mutagenic effects of UV radiation. There is evidence to suggest that individuals with darker skin may have fewer cumulative mutations, possibly decreasing their risk of skin cancer. One limitation of our study is that we are underpowered to draw definitive conclusions, as we had to group specific anatomical sites and lacked data from all skin cell lineages across all sites. Despite this, our results revealed variations in mutational burdens between different anatomical locations, with higher burdens observed in areas subjected to greater UV exposure. These findings suggest a potential link between UV exposure and mutational load. To build on our current findings, we plan to increase the sample size and refine our control over site and cell lineage factors to enhance the strength of our results.

Conclusion/Implications: Understanding how skin tone affects the risk of skin cancer is imperative for gaining a deeper insight into the mechanisms driving cancer development across diverse populations. While individuals with darker skin tones are less commonly diagnosed with skin cancer, they often experience more advanced stages at diagnosis due to delayed recognition of symptoms. By understanding the differences in mutational patterns and the role of UV exposure in darker skin, we can improve early detection and treatment strategies for this underserved group and refine screening and prevention methods for lighter-skinned individuals who are at higher risk.

#2858 Pleiotropy of experimental signatures across species and model systems.

Maria Zhivagui[1], Shams Al-azzam[2], Mark Barnes[2], Ludmil B. Alexandrov[2]

[1]School of Medicine, University of Nevada Las Vegas, Las Vegas, NV,[2]Moores Cancer Center, University of California San Diego, San Diego, CA

Mutational signatures in cancer genomes provide crucial insights into the etiology and progression of malignancies. While numerous signatures have been identified, many lack known causes, hindering our understanding of cancer development. This study presents a comprehensive analysis of mutational patterns induced by diverse environmental agents across multiple model systems. We compiled data from over 4,000 genome-wide sequencing experiments, exposing various biological models—including human cell lines, rodent tumors, avian cells, and C. elegans—to more than 120 different agents under controlled conditions. By comparing mutational profiles across 30+ model systems, we identified significant inter-model disparities, underscoring the importance of diverse experimental approaches in confidently characterizing mutational signatures observed in human cancers. Our findings enhance the interpretation of clinical and epidemiological data related to specific carcinogen exposures and provide a robust framework for elucidating the role of environmental factors in cancer etiology. This research contributes to a more nuanced understanding of the complex interplay between environmental exposures, genetic susceptibility, and cancer development, potentially informing novel prevention strategies and therapeutic interventions.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Antibody-Based Cancer Therapeutics 2
Poster Session

#2862 Anti MSLN ADC RC88 engages unique binding to target positive tumor cells with less interference from the soluble MSLN protein.

Lili Wang[1], Yidan Xu[1], Mingyang Li[1], Kailin Wang[1], Xiao Wang[1], Xiaoping Zhang[1], Zhanjiao Yu[1], Yinghao Xin[1], Chenglin He[1], Xiangyi He[1], Xiaoshan Min[2], Hang Chen[2], Min Li[1], Zhulun Wang[2], **Dong Li**[3], Yuanhao Li[1]

[1]RemeGen Co., Ltd., Yantai, China,[2]RemeGen Biosciences,Inc., South San Francisco, CA,[3]RemeGen (Shanghai) Co.,Ltd, Shanghai, China

Background: Mesothelin (MSLN) is a cell surface protein in various cancers, including ovarian, pancreatic and malignant mesothelioma. Soluble mesothelin (sMSLN) can be detected in the circulation of patients and may reduce the efficacy of antibody drugs targeting surface MSLN. Acting as a decoy receptor, sMSLN competes for binding or neutralizes the drug, affecting antibody properties. This study examined the anti MSLN antibody-drug conjugate (ADC) RC88, focusing on its ability to target cancer cells and the interference caused by sMSLN, thereby highlighting its competitive advantage over other drugs in clinical studies.
Methods: RC88, a novel anti MSLN ADC developed by RemeGen, is in phase I/II trials for advanced solid tumors, either as monotherapy (NCT04175847, NCT06173037) or in combination therapy (NCT05804526). We compared RC88 with other anti MSLN antibodies from relevant trials by antigen and cell binding assays. To show the unique binding of RC88, we treated cellular MSLNs in various conditions to mimic *in vivo* pathological environments. Additionally, we expressed a truncated MSLN fragment in 293 cells to focus on the C-terminal region near the cell membrane for binding comparisons.
Results: Our analysis revealed the distinctive properties of RC88 that may provide clinical advantages. RC88 had a strong primary binding site in the N-terminal domain and a weaker secondary site in the C-terminal region. The primary binding site at amino acids 317-352 was characterized by cross-linking mass spectrometry and mutagenesis. This epitope also facilitates CA125 binding, and RC88 effectively blocks CA125 from binding to MSLN. The secondary binding site located in the C-terminal stem region (amino acids 577-606) was less prominent and appeared to enhance tumor cell binding of RC88. Although further studies are required to refine the location of this secondary site, RC88 demonstrated its ability to bind and induce ADC-mediated cell death in cells expressing the C-terminal fragment of MSLN, a response not seen with other anti MSLN antibodies. When comparing cell binding in the presence of sMSLN, RC88 showed stronger cancer cell binding than others.
Conclusions: Our findings show RC88 has unique binding kinetics to different MSLN regions, with a dominant N-terminal binding site by rigorous methods. RC88 displayed superior binding to MSLN-positive cancer cells than other anti MSLN antibodies and maintained effectiveness despite sMSLN interference. Its stronger binding to the C-terminal MSLN fragment compared with other antibodies further underscores its targeting potential in tumor cells. Given its distinctive binding properties, RC88 may bring enhanced clinical benefits for treating MSLN-positive cancers, even with sMSLN and various truncated forms of MSLN in tumor cells. Therefore, RC88 is likely to have greater clinical benefits than other MSLN ADCs in clinical studies.

#2863 TAVO307, a novel ADC targeting CDH17, for the therapy of gastrointestinal cancers.

Guangmao Mu[1], Fulai Zhou[1], Zhengxia Zha[1], Sheng Huang[1], Ying jin[1], Yuanting Chen[2], Mark Chiu[2], **Di Zhang**[2]

[1]Tavotek Biotherapeutics, Suzhou, China,[2]Tavotek Biotherapeutics, Lower Gwynedd, PA

Background: Gastrointestinal (GI) cancers account for about 28% of all cancers globally, however, the treatment options remain limited partly due to the lack of appropriate cancer targets. Cadherin-17 (CDH17) is a cell adhesion membrane protein predominantly expressed at the tight junctions of intestinal epithelial cells of healthy individuals. However, the aberrant expression of CDH17 in 50-90% of GI tract tumors (including stomach, pancreas, liver/bile duct and colon cancers) is associated with poor prognosis and shorter survival of GI cancer patients. In these tumors, CDH17 interacts with α2β1 integrin to drive the proliferation, adhesion, and metastasis of tumor cells. Such overexpression of CDH17 makes it an attractive target for the developing novel treatments of GI tract cancers.
Methods and Results: We discovered an anti-CDH17 antibody with high binding affinity to CDH17 with an EC50 at sub nM range. We prepared an ADC molecule loaded with the anti-mitotic toxin MMAE/MMAF via a lysosomal cleavable dipeptide linker. CDH17-ADC showed potent cytotoxicity (IC50 at sub nM level) to multiple GI and pancreatic cancer cell lines in *in vitro* cell cytotoxicity assays. In addition, CDH17-MMAE (DAR=4) treatment led to potent tumor growth inhibition (70% to 95% TGI) in multiple CDX and PDX pancreatic, colon, and gastric cancer xenograft models. The mice in the CDH17-MMAE treatment group maintained normal body weight throughout the treatment course without observed toxicity.
Conclusions: Targeting CDH17 in GI tract cancers, our lead CDH17-MMAE molecule (TAVO307) showed potent tumor cell cytotoxicity in multiple tumor cell lines in cytotoxicity assays as well as in CDX and PDX tumor models. Further preclinical safety assessment, pharmacokinetics and IND-enabling studies are on-going with the goal to develop TAVO307 as the “best-in-class” ADC molecule for the treatment of colon, gastric, pancreatic and oesophagus cancers with significant unmet medical needs. This antibody can also be applied to alternative targeting modalities.

#2864 DXA023-G017, a bispecific PD-1/VEGF conjugate elicits potent anti-tumor efficacy in preclinical studies.

Xiaobo Cai, Wei Zheng, Junxiang Jia, Shishan Hu, Hui Xia, Qingliang Yang, Zhixiang Guo, Wei Liu, Lu Bai, Jun Zheng, Wenjun Li, Yuanyuan Huang, Zhongliang Fan, Binbin Chen, Meng Dai, Robert ZHAO

Hangzhou DAC Biotechnology Co., Ltd., Hangzhou, China

Background: Programmed cell death protein 1 (PD-1) and vascular endothelial growth factor (VEGF) are often co-expressed in the tumor microenvironment. VEGF not only promotes tumor vessel normalization but also effectively alleviates immunosuppression, thereby enhancing the infiltration and activation of immune effector cells. The synergistic effect of combining immunotherapy with anti-angiogenic agents (anti-PD-1 in combined with anti-VEGF) has been validated in the clinical treatment of advanced non-small cell lung cancer (NSCLC). Although Ivonescimab, a PD-1/VEGF bispecific antibody, has exhibited a notable prolongation of progression-free survival (PFS) versus Keytruda in individuals with PD-L1-positive NSCLC, the impact on overall survival (OS) necessitates further validation. Furthermore, the combination of Ivonescimab with chemotherapy has demonstrated more extensive antitumor efficacy compared to Ivonescimab monotherapy. Consequently, a chemotherapeutic drug conjugates to the anti-PD-1/VEGF bispecific antibody with functional linker may represent a novel and promising strategy for enhancing clinical outcomes. DXA023, the bispecific antibody, possesses a tetravalent structure similar to Ivonescimab, demonstrated both efficacy and safety against both PD-L1 and VEGF targets. DXA023 was conjugated to a small molecule/functional linker complex, G017 to form a PD-1/VEGF conjugate (DXA023-G017), which holds promise for further enhancing antitumor activity.
Methods: The antigen binding activities of DXA023 to PD-1 and VEGF were evaluated using ELISA and ForteBio assays. The protein binding affinities of both DXA023 and DXA023-G017 to PD-1 and VEGF were assessed by ELISA. The blockade of the PD-1/PD-L1 signaling pathway was determined using a luciferase reporter cell assay. Cell proliferation inhibitory assay was used to test in-vitro pharmacology of DXA023 and DXA023-G017. In vivo anti-tumor activities of DXA023 and DXA023-G017 were studied in an HCC827 tumor mouse model reconstituted with human peripheral blood mononuclear cells (PBMCs).
Results: DXA023 exhibits specific and high-affinity binding to human PD-1 and VEGF. The AAS mutations introduced significantly reduces FcγR binding, thereby effectively diminishing the antibody's ADCC effect, while still maintaining FcRn affinity. Both DXA023 and DXA023-G017 effectively block the PD-1/PD-L1 signaling pathway and demonstrate notable in-vitro antitumor activity. Furthermore, DXA023-G017 exhibits superior antitumor efficacy compared to DXA023 in the HCC827-PBMC mouse model.
Conclusions: DXA023-G017, a dual-blocking anti-PD-1/VEGF bispecific conjugate, demonstrates impressive antitumor efficacy in a mouse tumor model, providing robust support for its clinical development as a treatment for various advanced solid tumors.

#2866 An innovative T cell engager platform with optimized CD3 affinity and formats for targeting hematologic and solid tumors.

Cuicui Guo, Yu Pang, Zhen Han, Fenglin Yun, Ao Li, Hai Wu, Xun Gui

Mabwell (Shanghai) Bioscience Co., Ltd., Shanghai, China

Background: T-cell engagers (TCEs) are a promising class of immunotherapies that bridge T cells to tumor cells, triggering T-cell activation and subsequent tumor cell cytotoxicity. While TCEs demonstrated substantial efficacy in hematologic malignancies, their clinical benefit in solid tumors remains less established. The development of effective TCEs is hindered by challenges related to the complex interplay between CD3 affinity, TCE format, tumor-associated antigen (TAA) density, and specificity. To address these challenges, we have developed an innovative TCE platform designed to optimize CD3 affinity and TCE format to better match TAA expression profile and improve therapeutic efficacy.
Methods: Humanized CD3 and TAA-targeting antibodies were generated by Mabwell's integrated novel antibody discovery system. CD3 activity was assessed through T-cell binding and activation assays, while TAA binding potency was measured using tumor cell binding assays. Various bispecific and trispecific TCE formats were evaluated and optimized for developability, stability, and functional activity. Tumor-directed cytotoxicity (TDCC) assays were used to assess the efficacy of TCEs *in vitro*, while cytokine release syndrome (CRS) potential was also evaluated.
Results: CD3 parental antibodies with varying T-cell binding and activation potencies, including cross-reactivity with monkey CD3, were generated. TAAs were identified and prioritized using a bioinformatics analysis system, leading to the selection of tumor-specific targets such as LILRB4, CLL-1, CLDN6, MUC16, MSLN, CDH17, GUCY2C, SEZ6, DLL3, and ENPP3. Several optimized 1+1, 1+2, and 2+2 TCE formats were developed, demonstrating excellent developability and potent *in vitro* tumor cell killing. Importantly, these TCEs exhibited minimal cytokine release syndrome (CRS) potential. Our platform also integrates comprehensive indication analysis, target validation, and TCE format optimization, facilitating the identification of multiple TAAs across a broad range of cancer types.
Conclusion: Our innovative TCE platform offers a flexible and powerful approach for designing TCEs with enhanced efficacy and minimal CRS. This platform holds significant promise for developing novel T-cell engagers with robust therapeutic activity and favorable safety profiles for both hematologic and solid tumor indications.

#2868 Preclinical evaluation of LM-350: An innovative anti-CDH17 antibody drug conjugate for gastrointestinal cancer therapy.

Wentao Huang, Jin Li, Xia Qin, Da Fei, **Runsheng Li**

LaNova Medicines Limited, Shanghai, China

Background: Cadherin 17 (CDH17) is a membrane cell adhesion protein selectively expressed in gastrointestinal cancers, including colorectal (CRC), gastric (GC), and pancreatic cancers (PC). Its expression in normal intestinal epithelial cells is limited. LM-350, a CDH17-targeted antibody-drug conjugate (ADC), is composed of a humanized monoclonal antibody (LM-150) conjugated to the next-generation topoisomerase I inhibitor LDX2 via a cleavable linker, with a drug-antibody ratio of 8.

Methods: Target binding activity, specificity, and cross-species reactivity of LM-350 was evaluated by flow cytometry. Internalization was evaluated using an acidic pH-sensitive dye, with fluorescence signal detection via flow cytometry. Cytotoxicity and bystander effects were evaluated using the CellTiter-Glo luminescent cell viability assay. *In vivo* anti-tumor activity of LM-350 was evaluated in CDH17-positive CRC and GC cell line-derived xenografts (CDX) and patient-derived xenografts (PDX). Immunohistochemistry was used to assess CDH17 expression in tumor tissues.

Results: LM-350 demonstrated concentration-dependent binding to CDH17-expressing human CRC and GC cells, with EC_{50} values ranging from 0.13 to 0.94 nM, which was comparable to the un-conjugated monoclonal antibody LM-150. LM-150 exhibited high specificity and binding affinity to monkey CDH17 but not to rodent CDH17. In addition, LM-350 induced concentration and time dependent cellular internalization and potent cytotoxic effects in CDH17-positive cells. *In vivo*, LM-350 treatment at doses of 3 or 6 mg/kg significantly inhibited or regressed tumor growth in multiple CRC and GC xenograft models, with tumor growth inhibition rates ranging from 70% to 129%.

Conclusion: The novel ADC LM-350 targeting CDH17 exhibited potent anti-tumor activity *in vitro* and in CRC and GC xenograft models. LM-350 demonstrated high therapeutic potential in CDH17-positive gastrointestinal cancers, particularly in CRC and GC.

#2869 DXC014, a novel bispecific ADC targeting B7-H3 and PSMA, demonstrates favorable antitumor efficacy and safety profiles in preclinical evaluations.

Huihui Guo, Zhicang Ye, Yuanyuan Huang, Weibin Li, Qingliang Yang, Hangbo Ye, Junxiang Jia, Jun Zheng, Yongxiang Chen, Xiafen Wu, Yunxia Zheng, Binbin Chen, Meng Dai, Naiyan Yang, Gaituo Chen, Li Chen Chen, Xiaolei Liu, Junpeng Wang, Yi Luo, Xiaoqiang Xie, Xia Zhou, Lu Bai, Miaomiao Chen, Wenjun Li, Xiang Cai, Wei Liu, Robert Y. Zhao

Hangzhou DAC Biotechnology Co., Ltd, Hangzhou, China

B7-H3 (also known as CD276), a member of the B7 ligand family, is overexpressed on differentiated malignant cells and cancer-initiating cells, with limited heterogeneity, and high frequency (60% of 25,000 tumor samples), such as the cells of prostate cancer, non-small cell lung cancer (NSCLC), melanoma, bladder cancer, breast cancer, clear cell renal carcinoma, and head and neck squamous cancer cell (HNSCC), but scarcely detected in normal tissues. PSMA is highly expressed in prostate cancer cells and the endothelial neovasculature of several solid human malignancies. High PSMA expression is an independent biomarker of poor prognosis throughout the course of prostate cancer and across anatomical sites. Normal human tissues, including prostate epithelium, small intestine, renal tubules, and salivary glands, demonstrate considerably lower levels of PSMA expression than prostate cancer. DXC014 is an innovative bispecific antibody-drug conjugate (ADC) targeting both B7H3 and PSMA, utilizing a topoisomerase I inhibitor as its payload. This dual-target design aims to enhance therapeutic efficacy across a wide range of solid tumors, in particular, for prostate cancers. In vitro studies demonstrated that DXC014 binds to B7H3 and PSMA with high specificity and affinity, enabling precise targeted cytotoxicity. DXC014 induces DNA damage in cancer cells while exhibiting a significant bystander effect, and thus kills both target-positive cells and surrounding target-negative cells. Furthermore, DXC014 exhibited broad cytotoxic activity across various cancer cell lines, including prostate cancer and small-cell lung cancer cells with different levels of target expression. Overlapping pharmacokinetic profiles of both ADC and total antibody indicated excellent linker stability. DXC014 was well-tolerated at doses up to 300 mg/kg in mice and 40 mg/kg in repeat-dose studies in cynomolgus monkeys. In xenograft models, including C4-2B, 22RV1, and SHP77 models, DXC014 showed superior or comparable antitumor efficacy to Dxd conjugate at equivalent doses. These findings underscore the therapeutic potential of DXC014 as a dual-targeting ADC for the treatment of various solid tumors. Its innovative design and excellent preclinical performance position DXC014 as a promising candidate for further clinical development.

#2870 Enhancing Topo1i ADC efficacy: development of homogeneous dual-payload ADCs combining Topo1i with microtubule inhibitors or PARP inhibitors.

Gang Yin, Daniel Calarese, Alice Yam, Werner Rubas, Krishna Bajjuri, Xiaofan Li, Helena Kiefei, Miao Wen, Guifen Xu, Abigail Yu, Hans-Peter Gerber

Sutro Biopharma, Inc., South San Francisco, CA

There is a critical need to safely enhance the potency of antibody-drug conjugates (ADCs) for targeting low-expression and heterogeneous tumors, as well as to overcome drug resistance and relapse. Cancer cells often develop resistance to single-agent therapies, making it essential to explore combination approaches. One promising strategy presented in this work is combining two payloads with different mechanisms of action (MOA) into a single ADC, creating dual-payload ADCs. Clinical data have shown that if resistance develops to a topoisomerase I inhibitor (Topo1i) ADC, it will also result in resistance to a Topo1i ADC administered sequentially, regardless of the target antigen. This implies that anti-tumor activity of Topo1 ADCs is limited by formation of resistance towards the payload (cross-payload resistance). In contrast, switching to a payload with different MOAs, such as transitioning from Topo1i to microtubule inhibitor or vice versa, can help to restore the effectiveness of ADCs. The combination study of sacituzumab govitecan and enfortumab vedotin has clinically validated the concept of concomitant administration of a Topo1i payload with a microtubule inhibitor (MTi) payload to increase response rates without significantly increasing toxicity. Dual-payload ADCs that combine Topo1i and MTi agents are specifically designed to maximize their anti-tumor activity by overcoming resistance and broadening the therapeutic potential. Another promising dual-payload strategy involves combining PARP inhibitors (PARPi) with Topo1i, as PARP plays a critical role in repairing DNA damage induced by Topo1i. Such a Topo1i/PARPi dual-payload ADC has the potential to improve anti-tumor activity while avoiding toxicities commonly associated with conventional PARPi chemotherapy, due to the lack of targeted delivery. By leveraging Sutro's cell-free expression platform and its precise dual conjugation technology, we fine-tuned the drug-to-antibody ratio (DAR) and dual payload ratios to achieve optimal synergy between the two MOAs. The homogeneous, dual-payload ADCs demonstrate desirable physicochemical properties, enabled by the careful selection of conjugation sites and a best-in-class hydrophilic β-glucuronide linker. These design features also resulted in favorable pharmacokinetics and stability of dual-payload ADCs. Using this approach, we identified superior anti-tumor activities against low-antigen and Topo1 ADC-resistant tumor models treated with dual-payload ADCs compared to mono-payload ADCs. Furthermore, the dual payload approach streamlines preclinical, clinical development and the manufacturing process compared to the parallel development of two mono-payload ADCs.

#2871 Comparative anti-tumoral efficacy of endoglin-targeting ADCs harbouring different payloads for the treatment of gastric cancer.

Isabel Egana[1], **Patricia Gonzalez-Callejo**[1], Alvaro Gonzalez[1], Cristina Ferrer[1], Roland Kontermann[2], Oliver Seifer[2], Klaus Pfizenmaier[2], Myriam Fabre[1]

[1]Oncomatryx Biopharma, S.L., Derio, Spain,[2]Institute of Cell Biology and Immunology, Stuttgart University, Stuttgart, Germany

Endoglin (CD105), a TGFb-RII co-factor, overexpressed in tumor microvasculature and endothelial-derived CAFs, has been extensively validated as a therapeutic target for cancer treatment. Endoglin-targeting ADCs have been developed in this work, using a human IgG1 antibody, OMTX003, conjugated to different microtubule inhibitors such as eribulin (OMTX603-3) or MMAE (OMTX103), with DAR values between 4 and 8. Target binding was maintained within subnanomolar values for each ADC, similar to the unconjugated antibody. Internalization was efficient, with 75% intracellular localization within 60 minutes, regardless of the conjugated payload. *In vitro* activity in Endoglin-expressing HT1080 cells was target- and concentration-dependent, with IC50 values of 6 and 95.5pM, respectively. ADCs were tested for their *in vivo* anti-tumor efficacy in a gastric cancer PDX murine model. Complete tumor regression was observed. No re-growth occurred up to 40 days after treatment end, showing the lack of resistance to treatment. ADCs were well tolerated with no weight loss. These encouraging results are postulating these anti-ENG ADCs for further development.

#2872 DXC018: A novel HER2 dual-payload bispecific ADC with promising potential for gastrointestinal cancers and other solid tumors.

Huihui Guo, Yong Du, Yuanyuan Huang, Juan Wang, Qingliang Yang, Junxiang Jia, Jun Zheng, Hangbo Ye, Fang Du, Meng Dai, Hui Xia, Xiaoxiao Chen, Xingyan Jiang, Ziyu Zhao, Wei Liu, **Robert Yongxin Zhao**

HANGZHOU DAC BIOTECHNOLOGY CO., LTD, Hangzhou, China

HER2 (human epidermal growth factor receptor 2) is a transmembrane tyrosine kinase receptor critical for cell growth and differentiation. As a pivotal therapeutic target, HER2 has been extensively investigated in ADC drug development, resulting in numerous innovative designs along with approval in recent years. Among these, dual-payload ADCs, particularly those utilizing small molecules with distinct mechanisms of action, represent a promising yet still evolving area of research. DXC018 is a novel HER2-targeting bispecific antibody-drug conjugate (ADC) featuring dual epitope binding and dual payload delivery. The bispecific feature of DXC018 to bind HER2 domains II and IV with high affinity, enables its target specificity and therapeutic efficacy. Its payload, comprising an antimetabolite inhibitor and a topoisomerase I inhibitor, effectively induces DNA damage and cytotoxic effects. Pharmacokinetic studies in mice revealed favorable properties, with overlapping pharmacokinetic profiles for the ADC and total antibody, indicating excellent linker stability. Toxicological evaluations confirmed its tolerability at doses up to 300 mg/kg. In xenograft models of human cancer cells, including NCI-N87, JIMT-1, and CFPAC-1 cells, DXC018 exhibited superior or comparable antitumor activity to Dxd conjugate at equivalent doses. These findings suggest that DXC018, by leveraging dual HER2 binding and the complementary mechanisms of the antimetabolite and the topoisomerase I inhibition, offers significant therapeutic potential for the treatment of gastrointestinal malignancies and the other solid tumors. This innovative dual-action design establishes DXC018 as a promising next-generation ADC to address unmet needs in gastrointestinal cancers.

#2873 Preclinical development of SMP-656: An eribulin-SuperHydra^{TM} linker based ADC.

Jinkun Huang, Chenglong Wu, Chaoyang Feng, Xiaojie Heng, Tingting Yang, Rang Gao, Tianyi Ke

Xiling Lab Co., Ltd., Chengdu Sichuan, China

Background: To date, 15 ADCs have been globally approved, 9 of which employ Michael addition reactions between maleimide and the free thiol groups of antibodies. This reaction is characterized by high selectivity, mild conditions, and rapid kinetics. However, the retro-Michael addition reaction in vivo can reverse this process, leading to payload detachment, which compromises the drug's efficacy and safety. The SuperHydra^{TM} linker promotes maleimide ring-opening via ortho effects, preventing retro-Michael deconjugation and enhancing ADC stability. Consequently, ADCs with the SuperHydra^{TM} linker demonstrate remarkable efficacy, safety, and better stability as represented by our clinical stage candidate, SMP-656, a HER2-eribulin ADC.

Methods: SuperHydra™ linkers were designed with varying ortho groups, distances, and pro-hydrolysis group optimizations. A medium hydrolysis-rate linker was chosen for SMP-656 to balance stability during CMC stages with controlled hydrolysis in circulation, ensuring ADC homogeneity. Comparative studies of SMP-656 vs. DS-8201a were performed in vitro and in vivo. Additionally, we evaluated SMP-656's cytotoxicity against DS-8201a-induced resistant cells and cells overexpressing the DXd efflux pump. Bystander effects, critical for DS-8201a efficacy, were assessed for SMP-656. Repeat-dose PK and toxicology studies in non-human primates provided PK data and HNSTD.

Results: SMP-656 demonstrated significantly higher efficacy than DS-8201a in various cancer cell lines, including DS-8201a-resistant gastric cancer and HER2-low breast cancer cells. It retained potency in resistant models where DS-8201a was ineffective, highlighting its potential for treating resistant and HER2-low patients.In vivo studies showed that SMP-656 achieved higher tumor growth inhibition rates than DS-8201a, with only half the DAR and one-third the dosing amount. This translates to superior tumor inhibition with one-sixth the toxin dosage, highlighting its enhanced safety profile. In NCI-N87 xenografts model, SMP-656 extended tumor inhibition to 62 days at an equivalent payload dose 0.08 mpk (single dose), compared to DS-8201a's 23 days. SMP-656 also demonstrated significant bystander effects, addressing tumor heterogeneity.Repeat-dose PK studies showed high concordance between ADC and total antibody concentration curves, confirming SuperHydra^{TM} linker stability. Additionally, SMP-656 achieved an HNSTD of 10 mpk, representing the highest safety profile among eribulin-based ADCs known to date.

Conclusions: The SuperHydra^{TM} linker enabled SMP-656 to combine superior stability, efficacy, and safety with the ability to overcome DS-8201a resistance. SMP-656 represents the safest and most effective eribulin-based ADC and is currently in Phase I clinical trials, showing promising tolerability and efficacy.

#2874 ZW327, a novel Ly6E-targeting antibody-drug conjugate bearing a topoisomerase 1 inhibitor payload.

Dunja Urosev, Saki Konomura, Michael Lee, Germanna Righetto, Elizabeth Porter, Katina Mak, Ambroise Wu, Amos Chua, Linglan Fu, Diego Alonzo, Spencer Boisjoli, Edward Lau, Janice Tsui, Vidhi Khanna, Alex Wu, Devika Sim, Mark E. Petersen, Jamie R. Rich, Stuart D. Barnscher

Zymeworks Inc., Vancouver, BC, Canada

Ly6E (lymphocyte antigen 6 family member E) is a GPI anchored cell surface protein frequently expressed in a number of cancers such as TNBC, NSCLC, HNSCC, ESCC, OVCA, and PDAC, with minimal presence in normal tissues. ZW327 is an antibody-drug conjugate (ADC) targeting human Ly6E, composed of a humanized IgG1 antibody bearing Fc-silencing mutations, conjugated to a novel camptothecin-based topoisomerase 1 inhibitor, ZD06519, via a maleimide anchor and a glycyl glycyl phenylalanyl glycine (GGFG)-aminomethyl (AM) cleavable linker with DAR8. The antibody was discovered from animals immunized with Ly6E HEK293 transfected cells, utilizing proprietary B-cell culture-based technology followed by humanization. Antibody binding to human and cynomolgus monkey Ly6E was evaluated by KinExA^{TM} and flow cytometry. To understand therapeutic potential of ZW327, antibody internalization was assessed by flow cytometry in several Ly6E-expressing tumor cell lines and *in vitro* cytotoxicity of ADC was assessed in tumor spheroids using a panel of breast, lung and other tumor cell lines. Tumor spheroid penetration of ZW327 was also investigated, as well as tumor cell co-culture assays performed to assess bystander-mediated cell killing by ZW327. Developability was assessed utilizing methods such as, NS-ELISA, AC-SINS, cIEF, LC/MS and UPLC-SEC, under normal and stressed conditions. Anti-tumor activity of ZW327 and PK were investigated in a number of xenograft mouse models representing a range of Ly6E-expression. ZW327 antibody was successfully humanized and demonstrated low nanomolar binding affinity to both human and cynomolgus monkey Ly6E, as well as strong binding to target-expressing tumor cell lines. Robust internalization of ZW327 antibody was observed in Ly6E-expressing tumor cell lines, as well as spheroid penetration and ADC exhibited potent and target-specific cytotoxicity in a panel of breast, lung and other tumor cell spheroids. The antibody binding, internalization and spheroid penetration were all superior to the hu9B12.v12 clinical benchmark antibody and ZW327 exhibited pronounced cytotoxicity when compared to DLYE5953A clinical benchmark ADC. ZW327 showed good bystander-mediated killing of Ly6E-negative cells when co-cultured with target positive tumor cells. A single administration of ZW327 resulted in tumor regression across xenograft models and demonstrated a favorable PK profile. Overall, these results support the potential of ZW327 as a novel therapeutic agent against Ly6E-bearing cancers.

#2875 IL1RAP-targeting antibody-drug conjugate: a novel therapeutic targeting both tumor cells and the tumor microenvironment.

Elin Jaensson Gyllenback[1], Camilla Rydberg Millrud[1], Olga Ab[2], David Liberg[1]

[1]Cantargia AB, Lund, Sweden,[2]AbbVie, Waltham, MA

Background: Overexpression of Interleukin-1 receptor accessory protein (IL1RAP) has been linked to various malignancies, including acute myeloid leukemia, renal, urothelial and ovarian cancer as well as pancreatic ductal adenocarcinoma and non-small cell lung cancer, making it a promising therapeutic target. Expression on tumor cells, as well as throughout the tumor microenvironment, including expression on cancer associated fibroblasts, cancer endothelium and suppressive infiltrating immune cells sets it apart from most existing antibody-drug conjugate (ADC) targets. Here, an IL1RAP-targeting ADC was developed, consisting of a monoclonal antibody conjugated via a linker to a potent cytotoxic payload, designed to selectively deliver the cytotoxic agent to IL1RAP-expressing cells. The ADC was tested *in vitro* and *in vivo* to evaluate its therapeutic potential.

Methods: A library of IL1RAP-binding clones was screened for affinity, cell binding, cynomolgus cross-reactivity and internalization. The selected antibody was conjugated to a maytansinoid using a cleavable linker. *In vitro* cell killing was assessed using SK-MEL5 WT and SK-MEL5 IL1RAP KO cells. The anti-IL1RAP ADC was tested *in vivo* using the human subcutaneous SK-MEL5 melanoma or the human BxPC3-CAF model (BxPC3 pancreatic cancer cells co-inoculated with pancreatic cancer-associated fibroblasts). Mice were treated with single (5mg/kg, SK-MEL5 and BxPC3-CAF) or multiple (3mg/kg or 10mg/kg in SK-MEL5; 5mg/kg or 10mg/kg in BxPC3-CAF) doses of the IL1RAP-targeting ADC to evaluate anti-tumor activity. A surrogate anti-mouse IL1RAP ADC was developed and used to assess toxicity in mice (C57Bl/6 1, 3, 5 or 10mg/kg antibody, Q1W x 2 doses; Balb/c 20mg/kg antibody, Q1W x 2 doses).

Results and Conclusions: The selected clone showed low nanomolar affinity to human (KD 4.9nM) and cynomolgus (KD 5.4nM) IL1RAP, strong binding to IL1RAP-expressing cells (IC50 6.9nM) and robust internalization in IL1RAP-expressing cells. Conjugation to the payload did not compromise binding of the antibody to IL1RAP-expressing cells. *In vitro*, the IL1RAP-targeting ADC showed IL1RAP-dependent cell killing. Furthermore, the IL1RAP-targeting ADC demonstrated significant dose-dependent anti-tumor activity, with tumor regression observed in both *in vivo* models used. Both the anti-human IL1RAP ADC and the anti-mouse IL1RAP ADC were well tolerated in mice as indicated by kidney and liver histology, hematology assessment and body weight. These results suggest that ADCs targeting IL1RAP may have promising therapeutic potential and support clinical development for IL1RAP-targeting ADCs across a broad spectrum of cancers.

#2876 BR116: An antibody drug conjugate targeting CDH17 for gastrointestinal cancer therapy.

Xiaobei Zhao[1], Jie Zhu[1], Zhenhua Wu[2], Yiqun Li[1], Cindy Wu[1], Lei Nie[2], Haibin Wang[2], Gang Chen[1]

[1]BioRay Pharmaceutical Co., San Diego, CA,[2]BioRay Pharmaceutical Ltd., Hangzhou, Zhejiang, China

Cadherins are Ca^{2+}-dependent cell adhesion molecules that mediates cell-to-cell adhesion and plays a key role in tissue development. Classical cadherins, such as E-cadherin (CDH1), N-cadherin (CDH2), P-cadherin (CDH3), and VE-cadherin (CDH5) are implicated in cancer progression, invasion, and metastasis when their expression or function changes.

CDH17 (Cadeherin-17, LI-cadherin) is a structurally unique member of the cadherin superfamily, with seven extracellular EC repeats, a transmembrane domain, and a shorter cytoplasmic domain. Originally discovered in rat liver and intestine, CDH17 is also called liver and intestine cadherin. In humans, the expression of CDH17 is mainly limited to the digestive tract, including the duodenum, jejunum, ileum, colon, pancreas, and is rarely detected in other tissues. In the intestinal epithelium, CDH17 is mainly localized to lateral and basolateral cell membranes.

CDH17 overexpression is observed in various gastrointestinal malignancies, such as gastric cancer, colorectal cancer, bile duct cancer, pancreatic cancer and hepatocellular carcinoma, with almost no expression in normal tissues and organs. High expression of CDH17 is also associated with poor prognosis. The tumorigenic role of CDH17 involves interaction with α2β1 integrin to regulate cell adhesion, proliferation and migration, particularly in colon cancer liver metastasis, making CDH17 a promising therapeutic target in cancer research.

BR116 is a novel anti-CDH17 ADC that consists of a humanized, highly specific monoclonal antibody, stably conjugated to an innovative topoisomerase I inhibitor with hydrophilic linker. Notably, our unique conjugation platform CysX™ offers site-specific, stable and irreversible conjugation. CysX™ not only prevents payload detachment during circulation and reducing the off-target toxicity, improved therapeutic window, but also enables a uniformed drug-to-antibody ratio (DAR) of 4. The toxin was carefully selected based on the target characterization and cancer cell sensitivity.

BR116 has demonstrated remarkable antitumor activity in both *in vitro* and *in vivo* studies. Preclinical evaluations have shown strong safety profile in cynomolgus monkeys, indicating its potential as a therapeutic treatment for gastrointestinal cancers. With its enhanced safety profile and promising efficacy, BR116 holds significant potential for further clinical development. BR116 is aiming to file for an IND in 2025.

#2877 MRG007, a cadherin 17-targeted, glycan-linked, exatecan-based antibody-drug conjugate, demonstrated potent anti-tumor activity and a good safety profile in preclinical studies.

Peng Zeng[1], Qi Wu[1], Yongjun An[1], Yili Feng[1], Yanchun Wang[2], Shoujia Liu[2], Deliang Li[3], **Feifei Cui**[1], Lei Fang[1]

[1]Concept to Medicine Biotech Co., Ltd., Shanghai, China,[2]Shanghai Miracogen Inc., Shanghai, China,[3]Lepu Biopharma Co., Ltd., Shanghai, China

Background: Colorectal cancer (CRC) is the one of the most challenging cancers with unmet medical needs. Cadherin 17 (CDH17) is a membranous cell adhesion molecule. It is frequently overexpressed in CRC and several other gastrointestinal (GI) cancers, with limited expression in normal intestinal tissue and pancreatic duct. The differential expression profile in tumor versus normal tissue makes it an attractive target for antibody-drug conjugate (ADC) in GI cancers, particularly CRC. MRG007 is a CDH17-targeting ADC consisting of a novel humanized IgG1 antibody site-specifically conjugated with the cytotoxin Exatecan using Synaffix's GlycoConnect^{TM} technology with a well-balanced DAR value. This differentiated drug design will potentially improve anti-tumor efficacy and broaden the therapeutic window.

Materials and Methods: The biological activity and safety profile of MRG007 were investigated in a series of preclinical studies, including (1) binding ability to CDH17 expressed on GI cancer cells as well as rhesus CDH17 evaluated by flow cytometry; (2) internalization rate in GI cancer cells evaluated by pHAb Reactive Dye labeling; (3) in vitro cytotoxicity against a panel of GI cancer cell lines; (4) anti-tumor activity evaluated in several GI cancer cell line-derived xenograft (CDX) and CRC patient-derived xenograft (PDX) mouse models with different levels of CDH17 expression; (5) plasma stability evaluated in human, rhesus, rat and mouse plasma; (6) an exploratory pharmacokinetic (PK) and toxicology study in rhesus monkey.

Results: MRG007, the CDH17-targeting ADC, and its antibody moiety showed strong binding activity to CDH17 expressed on a panel of GI cancer cell lines. Similar binding potency to both human and rhesus monkey CDH17 makes rhesus monkey suitable for toxicological evaluation. Rapid internalization of MRG007 was observed in a panel of CDH17-expressing GI cancer cells, and MRG007 maintained comparable internalization efficiency to the naked antibody. MRG007 demonstrated efficient and CDH17-dependent cytotoxic activity in a panel of GI cancer cells expressing varying levels of CDH17. MRG007 exhibited a robust and dose-dependent tumor growth inhibition in multiple GI cancer CDX and CRC PDX mouse models with varying levels of CDH17 expression, which was significantly better than GGFG-DXd DAR8 ADC conjugated with the same naked antibody. Remarkable stability of MRG007 was shown in the plasma of various species tested with minimal payload release and stable DAR value. MRG007 demonstrated a favorable PK and safety profile with good tolerability in the exploratory toxicology study.

Conclusion: Overall, the preclinical study results suggest that MRG007 is a differentiated and potent ADC drug candidate for the treatment of CDH17-expressing cancers in clinical trials.

#2878 Preclinical characterization of GLR1061, a B7H3-targeted ADC with a novel DNA topoisomerase I inhibitor, demonstrated superior bystander effect to overcome tumor heterogeneity.

Jiao Jiao, Yingjie Sun, Jiaxing Wang, Menghan Cui, Siyao Li, Wenjie Shao, Hairui Yuan, Haitong Sheng

Gan & Lee Pharmaceuticals, Beijing, China

B7H3 (CD276) belongs to the B7 superfamily of immune checkpoint molecules. It is present at low levels in most normal tissues but is overexpressed in a variety of cancers, such as lung cancer, prostate cancer, breast cancer, head and neck squamous cell carcinoma, and esophageal squamous cell carcinoma. Notably, B7H3 overexpression has been found to be associated with a poor prognosis in a broad spectrum of malignant tumors, and it has become an attractive target for cancer therapy in recent years. B7H3-targeted antibody-drug conjugates (ADCs), such as DS7300, have shown positive clinical efficacy in small cell lung cancer, squamous non-small cell lung cancer, metastatic castration-resistant prostate cancer. GLR1061 is a B7H3 targeting ADC comprised of a humanized IgG1 monoclonal antibody, a cleavable linker and a novel DNA topoisomerase I inhibitor. Our independently developed topoisomerase I inhibitor had super strong cancer cell inhibition activity, which was 5 to 10 times stronger than DXd, and enhanced key advantages such as ADC bystander effect. Conjugated with B7-H3 antibody, which had superior binding and internalization, GLR1061 demonstrated more significant anti-tumor efficacy across various cancer types than DS7300 both in vitro and in vivo. In addition, in the CDX model of co-culture of antigen-positive and antigen-negative cells, GLR1061 exhibited much stronger bystander activity than DS7300, which was expected to overcome tumor heterogeneity and effectively eliminate cancer cells with different antigens expressions. In plasma stability studies, GLR1061 showed significantly lower free-payload release than DS7300 in different species (PBS, rat, monkey, and human). In a preclinical repeated-dose toxicology study in mice, GLR1061 showed good tolerability and reversible toxicity. Overall, GLR1061 demonstrated excellent specificity, efficacy, and safety as a next-generation therapy for the treatment of B7H3-positive tumors.

#2879 A novel therapeutic approach to treat patients with Type I interferon autoantibodies.

E. Garcia-Reino[1], N. Gao[1], T. Udumulla[1], E. Sosa[1], N. Ayoluwa[1], P. Mashati[2], A. Patera[2], J.-L. Casanova[3], **P. Premsrirut**[1];
[1]Mirimus, Inc., Brooklyn, NY, [2]SUNY Downstate Medical Center, New York, NY, [3]Rockefeller University, New York, NY

Type I interferons (IFNs) are a group of nonredundant pleiotropic cytokines that play an important role in eliciting antiviral responses and have also been shown to have antineoplastic effects. Deficits in this signaling pathway have been shown to increase susceptibility to viral infection and decrease immune surveillance to block tumorigenesis. Prior studies demonstrate that inhibition of the type I IFN pathway markedly compromises the anti-neoplastic effects of chemotherapies, such as anthracycline-based drugs like doxorubicin, and dramatically decreases the number of tumor-free mice in experimental settings. Recent large-scale clinical studies revealed that circulating anti-type I interferon autoantibodies capable of neutralizing type I IFN signaling contributed to disease severity in patients infected by SARS-CoV-2 and may be responsible for nearly 20% of COVID-19 deaths. These IFN autoantibodies were directly capable of hindering innate antiviral responses, thus allowing viral replication to occur more robustly and spread to distal tissues. Additional studies revealed that these type I IFN autoantibodies are quite prevalent in the aging population, found at high levels in nearly 1 in 15 over the age of 65 and confer a 100-fold risk of severe life-threatening viral illness. Despite their high prevalence and ability to block IFN signaling, no study to date has examined the role of type I IFN autoantibodies in cancer progression and tumor response. Here, we investigate whether neutralizing type I IFN autoantibodies can block critical innate immune signaling pathways that result in a higher conversion of pre-malignant cells to malignant tumors. We hypothesize that carriers of type I IFN autoantibodies may respond poorly to primary anticancer therapies, including standard chemotherapy regimens and immunotherapies. Furthermore, using a first-in-class therapeutic decoy, we test whether blockade of type I IFN autoantibodies can provide a synergistic benefit when combined with standard anticancer agents.

#2880 TM4SF1 as a novel target for different modalities (mAb, bispecific ADC, targeted siRNA) for the treatment of different solid tumor cancers.

Mingquan Xie[1], Peter Li[1], Han Lu[1], ShuTing Dong[1], Nicole Wilski[2], Poonam Pandey[2], Nishida Motohiko[2], Peter Haytko[2], **Mark Chiu**[2], Maria P. MacWilliams[2]

[1]Tavotek Lab Inc., Suzhou, China,[2]Tavotek Lab Inc., Lower Gwynedd, PA

Background: Transmembrane-4-L-six-family member-1 (TM4SF1) is a tetraspanin superfamily member that is overexpressed in cancers such as breast, colon, lung, liver, and prostate. A contributor to tumor development and progression, TM4SF1 participates in microdomains that regulate cancer cell migration and invasion via induction of the epithelial-mesenchymal transition, and angiogenesis. The overexpression of TM4SF1 makes it an attractive target for the developing novel treatments of GI tract cancers.
Methods and Results: We have engineered an anti-TM4SF1 antibody that binds with nM affinity to TM4SF1-expressing cells. The mAb has anti-tumor growth inhibition in gastric, ovarian cancer cell lines. This mAb can serve as an efficacious targeting carrier for toxins in an ADC and for siRNA in an ARC. To broaden the scope of cancer cell engagement, we prepared constructed a TM4SF1-cMET bispecific ADC which showed potent cytotoxicity in multiple GI, lung, ovarian, breast, TNBC, and pancreatic cancer cell lines in in vitro cell cytotoxicity assays. The bispecific TM4SF1-cMET MMAE (DAR=4) treatment led to potent tumor growth inhibition (70% to 95% TGI) in CDX pancreatic and gastric cancer xenograft models. The mice in the TM4SF1-cMET MMAE treatment group maintained normal body weight throughout the treatment course without observed toxicity. The TM4SF1 - MYC siRNA conjugates were stable. Treatment of MYC-dependent cancer lines led to MYC protein knockdown and cell growth inhibition.
Conclusions: A bispecific TM4SF1 ADC had activity in multiple cancer cell lines. Notably, the TM4SF1-MMAE molecule showed potent in vivo tumor growth inhibition in ovarian, pancreatic, and gastric cell lines. The TM4SF1 - siRNA ARC had tumor cell proliferation inhibition in lung, pancreatic, and gastric cancer cell lines. Preclinical safety assessment, pharmacokinetics and IND-enabling studies are on-going with the goal of developing TM4SF1 as the “best-in-class” ADC molecule for the treatment of lung, gastric, breast, and pancreatic cancers with significant unmet medical needs. This antibody can be applied to alternative targeting modalities.

#2881 QLS5133-a novel CDH6-targeting antibody-drug-conjugate for solid tumors.

Ying Huang[1], Fanrui Meng[1], Liuqing Yang[1], Shuyong Zhao[2], Xinmei Wang[2], **Ling Zhang**[1], Yang Yang[1], Hua Ying[1], Weikang Tao[1]

[1]Shanghai Qilu Pharmaceutical Research and Development Center LTD., Shanghai, China,[2]Qilu Pharmaceutical Co., Ltd., Jinan, China

Background: Cell-cell adhesion molecule cadherin-6 (CDH6), a member of the CDH family, is expressed in the basolateral membrane of epithelial cells and mediates calcium-dependent cell-cell adhesion. Although the function of CDH6 has not been fully elucidated, it is believed to be associated with epithelial-mesenchymal transition (EMT) and metastasis during tumor development. CDH6 is upregulated in various cancers, including ovarian cancer, renal cell carcinoma, and thyroid carcinoma, while its expression in normal tissues is limited. The therapeutic potential of CDH6 as an ADC target was recently unraveled by DS-6000a, a Dxd-based ADC from Daiichi/MSD. In this study, we present the development of a novel CDH6 ADC that is in the IND enabling stage. The new ADC demonstrates a superior safety profile compared to DS-6000a and shows robust anti-tumor activities in preclinical studies.
Method: QLS5133 is built on the QILU's proprietary novel topoisomerase-1(TOPO 1) inhibitor QLS6917 platform, with site-specific conjugation to yield a uniform DAR 8 product. The payload QLS6916 displayed nanomolar activity across various cancer cell lines and much faster systematic clearance than Dxd in vitro.
Result: QLS5133 exhibited a higher affinity and internalization compared to DS-6000a. Treatment with QLS5133 induced significant tumor regressions in multiple CDH6-expressing human cancer cell line xenograft studies encompassing ovarian cancer and renal cell carcinoma. Notably, in one CDX model with ultralow and highly heterogeneous CDH6 expression, QLS5133 still demonstrated strong efficacy. Additionally, QLS5133 was able to achieve a durable response even after resistance to Mirvetuximab soravtansine (FRα ADC/ImmunoGen) in preclinical models. This suggests that QLS5133 could potentially serve as a late-line treatment following treatment failure with Mirvetuximab soravtansine. More importantly, QLS5133 showed much less severe hematotoxicity than DS-6000a particularly regarding changes in platelets, white blood cells, neutrophils, as well as having a lesser impact on body weight reduction when dosed up to 80 mg/kg (single dose administration) in cyno monkeys. Meanwhile, QLS5133 demonstrated a better PK profile than DS-6000a in cyno monkeys, with longer $T_{1/2}$ and slower systematic clearance as intact ADC, and sustained DAR retention for a period of 21 days.
Conclusion: The promising efficacy, superior tolerability, and favorable PK profile support the potential of QLS5133 as a novel therapeutic agent to address the high unmet needs in patients with variable CDH6-expressing cancers. QLS5133 will enter clinical trials.

#2882 Development and preclinical characterization of HER2×Trop-2 bispecific antibody-drug conjugates (bsADCs) with auristatin microtubule inhibitors.

Daniel He, Greg Liang, Haixia Chen, Mingqi Shao, Songyan Huang, Qiong Wang, Yinfei Yin, **Qikuan Chen**

Shanghai ChemPartner Co., Ltd., Shanghai, China

Background: The emerging field of biparatopic (bpADC) and bispecific antibody-drug conjugates (bsADC) provides an innovative therapeutic category combining the merits of ADCs and bispecific/biparatopic antibodies. Promise in the field stems from the ability of bsADCs to overcome the existing clinical challenges associated with ADCs, such as poor internalization, drug resistance, and off-target toxicity. To date, more than 10 bsADCs are undergoing clinical trials, including HER2×Trop2 BsADCs. HER2 is a well-established target for ADCs such as trastuzumab emtansine and trastuzumab deruxtecan, demonstrating significant clinical benefits in HER2-positive breast cancers. The human cell-surface trophoblast protein 2 (Trop-2) is overexpressed in a wide range of solid tumors, including HER2-low expressing tumors, with minimal expression in normal tissues. Trop-2-directed ADCs such as sacituzumab govitecan and datopotamab deruxtecan have demonstrated potent antitumor efficacy in a broad range of solid tumors including patients with advanced triple-negative breast cancer, lung cancers and urothelial cancers. The dual-targeting approach offered by bsADCs has the potential to increase tumor cell binding affinity and internalization, leading to enhanced payload delivery and better tumor efficacy.
Methods and Results: We developed two different HER2×Trop-2 bispecific antibodies (Saci/Tras-hIgG1 and Dato/Tras-hIgG1) leveraging a monovalent bispecific IgG1 format, which is monovalent for each antigen based on the Fab fragments of trastuzumab, sacituzumab or datopotamab. The fully human anti-HER2/Trop-2 bispecific antibodies were conjugated with monomethyl auristatin E (MMAE) via a lysosomally cleavable linker to generate uniform HER2×Trop-2 bsADCs (Saci/Tras-vc-MMAE and Dato/Tras-vc-MMAE) with an average DAR of 4.2. The binding affinity and specificity of anti-HER2/Trop-2 bsADC was evaluated by ELISA and FACS, respectively. The bsAb-vc-MMAE conjugates exhibited greater tumor killing efficiency and specificity with an IC50 in sub nM level in cell lines with high Trop-2 expression and medium/low HER2 expression. Furthermore, in vivo studies demonstrated antitumor activity with improved efficacy compared to monotherapy ADCs targeting either HER2 or Trop-2 alone, with favorable safety and pharmacokinetic profiles.
Conclusion: HER2×Trop2 bsADCs represent a novel and exciting class of therapeutic agents with the potential to transform the treatment of multiple cancer types. Preclinical studies of HER2×Trop2 bsADCs have demonstrated that bsADCs can effectively inhibit tumor growth and prolong survival in xenograft animal models, with a favorable safety profile and low incidence of severe adverse events.

#2883 Cetuximab increases LGR5 expression and enhances efficacy of LGR5 antibody-drug conjugates in patient-derived models of colorectal cancer.

Peyton High, Zhengdong Liang, Cara Guernsey-Biddle, Adela Aldana, Yukimatsu Toh, Qingyun Liu, Kendra S. Carmon

UT Health Houston, Houston, TX

Colorectal cancer (CRC) is the second-leading cause of cancer-associated deaths in the United States with a need for improved therapies. Antibody-drug conjugates (ADCs) are a rapidly expanding class of anticancer drugs that utilize monoclonal antibody (mAb) specificity to hone cytotoxic payloads to cancer cells. We generated ADCs targeting leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5), a cancer stem cell biomarker that is highly overexpressed in CRC. LGR5 ADCs demonstrated high specificity and efficacy in CRC cells and xenograft models without notable toxicity, although tumors eventually relapsed following treatment cessation. Interestingly, FDA-approved therapies targeting epidermal growth factor receptor (EGFR) have been shown to increase LGR5 expression in patient-derived models of CRC. This study therefore aims to determine the mechanisms of EGFR-mediated regulation of LGR5 expression and evaluate the therapeutic efficacy of EGFR-targeted therapies in combination with LGR5 ADCs for the improved treatment of CRC. We have demonstrated that cetuximab (CTX), an EGFR-targeting mAb approved for *KRAS*^{WT} metastatic CRC (mCRC), increases LGR5 protein levels independent of *KRAS*/*PIK3CA* mutational status in CRC cell lines, tumor organoids, and cell line-derived xenografts. Additionally, we identified a novel EGFR-LGR5 interaction that is enhanced by CTX treatment. Furthermore, we have generated a camptothecin-based LGR5 ADC (8E11-CPT2) utilizing site-specific conjugation technology that demonstrates target- and dose-dependent cytotoxicity in CRC cells and was well-tolerated. Importantly, combination treatment of 8E11-CPT2 with CTX in *RAS*^{mut} CRC patient-derived xenograft models significantly reduced tumor burden with notable regression in some mice and extended survival compared to LGR5 ADC and CTX monotherapies. These results demonstrate that combination treatments of LGR5 ADCs with CTX may prove more effective than ADC monotherapies in treating CRC and implicate expanded clinical utility for CTX to treat mCRCs with *KRAS* and/or *PIK3CA* mutations.

#2884 Development of antibody drug conjugates targeting MUC16 in ovarian cancer subtypes.

Mengyao Xu[1], Bo Rueda[2], David Spriggs[1], Yeku Oladapo[1]

[1]Massachusetts General Hospital Cancer Center, Boston, MA,[2]Massachusetts General Hospital Vincent Center for Reproductive Biology, Boston, MA

Background: Ovarian cancer (OC) remains a formidable challenge due to limited treatment options and inevitable development of multidrug resistance. Antibody-drug conjugates (ADCs) have emerged as a promising therapeutic modality, particularly for cancers with high unmet needs such as ovarian cancer (OC). MUC16, also known as CA125, is a high-molecular-weight glycoprotein overexpressed in ovarian cancer, serving as a biomarker for diagnosis and monitoring while contributing to tumor progression and immune evasion. Our laboratory previously identified 4H11, an antibody that preferentially binds to the retained (ectodomain) portion of MUC16. To exploit this target, we have systematically evaluated a panel of ADC payloads and linkers, including Auristatins (MMAE, MMAF), Camptothecin (SN-38), Maytansinoids, Calicheamicin, Nemorubicin, and Belotecan, conjugated to our proprietary antibody 4H11. Given the heterogeneity of OC, we investigated the efficacy of these ADCs across various subtypes, including high-grade serous ovarian carcinoma (HGSOC) and patient derived organoids.

Methods: ADCs were synthesized by conjugating 4H11 to different payloads via established linker chemistries. The resulting ADCs were characterized for drug-to-antibody ratio (DAR) and stability. In vitro cytotoxicity was assessed using a panel of OC cell lines and patient-derived organoids representing multiple subtypes. Dose-response curves were generated to evaluate IC50 values, and selectivity for antigen-positive cells was confirmed. Additionally, we analyzed differential sensitivity among subtypes to identify the most effective payload and ADC formulation.

Results: All ADCs demonstrated high stability and target specificity in vitro. Notably, ADCs with maytansinoids exhibited superior potency across HGSOC and other subtypes, achieving sub-nanomolar IC50 values in MUC16-positive cell lines. However, nemorubicin-conjugated ADCs showed enhanced efficacy in certain non-HGSOC subtypes, suggesting subtype-specific vulnerability. In organoid models, ADC efficacy closely mirrored cell line data, highlighting the translational potential of these findings. Importantly, payload and linker selection significantly impacted ADC performance, with maytansinoids yielding the best overall therapeutic index across of broad spectrum of OC histologic subtypes.

Conclusion: The retained portion of MUC16 is a viable target for ADC development. The maytansinoid conjugates emerged as leading candidates for further preclinical evaluation and potential clinical development based on their broad spectrum of activity against multiple histological subtypes. Our findings further underscore preferential susceptibility of different OC histologic subtypes to different ADC payloads.

#2885 IKS04, an antibody drug conjugate with a highly potent DNA crosslinker payload for the treatment of gastrointestinal cancers.

Jutta Deckert, Justyna Mysliwy, Adam Lodge, James Stephenson, Sarah Robinson, Robert J. Lutz

Iksuda Therapeutics Ltd, Newcastle-upon-Tyne, United Kingdom

IKS04 is an antibody drug conjugate (ADC) targeting human CA242, also known as CanAg, a tumor-associated carbohydrate antigen. This glycotope is highly expressed in most colorectal, pancreatic, gallbladder, and gastric cancers, with limited expression in normal tissues. While prior experience demonstrated that CA242 can be safely targeted with an ADC, the limited clinical efficacy observed with microtubule inhibitor payloads in gastrointestinal indications suggests that alternate payloads such as DNA crosslinkers could provide an improved therapeutic to target CA242+ tumors more effectively.

The ADC was generated by site-specific bioconjugation of a pyrrolobenzodiazepine (PBD) prodrug payload to the antibody light chain at a drug-to-antibody ratio of 2. The linker-prodrug incorporates two glucuronide-trigger moieties that require lysosomal cleavage by beta-glucuronidase to both release and activate the PBD dimer payload. In vitro antiproliferative and bystander activity was evaluated by CellTiterGlo assay. In vivo efficacy studies for ADC alone and antibody co-administration were performed with subcutaneous human xenografts in CB17-SCID mice. CA242 expression was determined in formalin fixed tumor samples by immunohistochemistry and on cell lines by quantitative flow cytometry.

In vitro, IKS04 results in potent and specific cell killing of panel of CA242+ target cells and potency correlated with antigen expression determined by flow cytometry. The PBD payload confers strong bystander activity in co-culture studies in vitro, an attribute that may allow treating tumors with heterogeneous antigen expression. The ADC was active against gastric and colorectal cancer xenograft models at single doses of 0.5 to 1 mg/kg, while the maytansinoid-based ADC benchmark cantuzumab ravtansine was inactive even at 2 mg/kg. Contrary to in vitro results, we observed a trend towards greater in vivo activity in models with lower antigen expression. This is suggestive of an antigen barrier effect where high target expression results in ADC accumulation at the tumor surface, precluding deeper penetration into tumor tissue. As the maximum tolerated ADC dose can be limited by target-independent payload toxicities, we increased the overall in vivo dose by addition of antibody to improve delivery. Consistent with this concept, co-administration of the unconjugated antibody with the ADC at a 5 to 50-fold excess improved the minimally effective dose in the CA242-high SNU16 gastric and HT29 colon cancer models compared to ADC alone while the antibody itself had no anti-tumor activity.

IKS04 is an ADC with a DNA crosslinker payload that shows potent in vitro and in vivo efficacy against gastric and colorectal cancer tumor models. Co-administration of the unconjugated antibody can improve ADC delivery which drives increased efficacy in preclinical models without increasing toxicity.

#2886 A *de novo* designed miniprotein enables modular and specific CAR-T targeting.

M. E. Llases[1], C. McKay[2], S. Syed[2], V. Andrusyshyn[1], E. Carcamo Noriega[1], J. W. Bowman III[1], T. Howard[1], N. Rodriguez[1], F. Teets[1], I. Truebridge[1], **X. Michelet**[1], Z. Caruolo[2], L. Webb[2], J. Newcomb[2], F. Mermelstein[2], C. Novina[2], C. Bahl[1];
[1]AI Proteins, Boston, MA, [2]Dynamic Cell Therapies, Watertown, MA

A definitive cure for acute myeloid leukemia (AML) remains elusive. Inter and Intra-patient heterogeneity in antigen expression profiles is a major challenge for AML treatment. Adapter CAR-T cell therapies utilize a chimeric antigen receptor (CAR) which binds to a non-natural small molecule, which in turn can be attached to a soluble affinity domain that binds the tumor antigen. Decoupling cancer cell binding and CAR engagement provides flexibility and control in directing the CAR-T therapy through dosing of the adapter, which can improve safety and decrease T cell exhaustion. A critical challenge for the success of adapter CAR-T is finding an ideal adapter molecule.

De novo designed miniproteins are a novel therapeutic modality. They are approximately 45 amino acids in length and adopt a highly stable tertiary structure, are easily produced recombinantly or synthetically, and present many additional advantages over traditional biologics. The structure of the miniprotein is created from scratch using generative artificial intelligence and is therefore controllable, permitting specific design of the conjugation site, as well as precise tuning the affinity. The small size, high solubility, and compact structure confers better biodistribution and lower internalization in the tumor cell surface. Here, we show that miniproteins outperform antibodies as the targeting moiety of an adapter CAR-T therapy, and we demonstrate this using CD123 directed treatment of AML.

We engineered AIP26GQ, a 5.6 kDa miniprotein that binds selectively to CD123 with KD = 0.5 pM. AIP26GQ is thermostable, and can be conjugated to a fluorescein molecule (FITC) via an engineered lysine residue. We demonstrated that AIP26GQ can direct anti-FITC CAR-T cells to kill AML model cell lines (MV-4-11) in vitro. We evaluated multiple positions for selective attachment of FITC, and identified the optimal geometric orientation for maximizing CAR-T activation. In vivo efficacy studies using MV-4-11 in NCG mice showed that AIP26GQ-directed CAR-T treatment reduces tumor burden to background levels and the remission is stable at least 15 days after the last dose. In the same study, a talacotuzumab antibody based adapter molecule and a direct anti-CD123 CAR-T were used for comparison. The miniprotein-directed CAR exhibited multiple advantages over both the antibody and the direct CAR-T, including increased efficacy with lower dose required of both the adapter and the CAR-T cells, improved tumor occupation, and less CAR-T exhaustion.

Our system combining a FITC-conjugated miniprotein with anti-FITC CAR-T cells yields complete remission in an MV-4-11 xenograft mice model, outperforming both an antibody adapter and direct CAR-T approaches. We demonstrated that the use of controllable CAR-T cells combined with miniprotein adapters exhibits an unprecedented potential for the design of dosable, redirectable and multiplexable cancer treatments.

#2887 Next generation antibody drug conjugates targeting HER2 and TROP2: Multi-Payload Conjugates™ targeting orthogonal mechanisms of cell killing.

Richard L. Kendall[1], Devin Trinter[1], Maxwell Nguyen[1], Daniel Gutierrez[1], Samantha Brady[1], Chanez Symister[1], Andrew Lau[1], Derek Garcia-Almedina[1], Saurabh Johri[1], Matthew Francis[2], Marco Lobba[1]

[1]CatenaBio Inc., Berkeley, CA,[2]Chemistry, University of California at Berkeley, Berkeley, CA

Introduction: Antibody-Drug Conjugates (ADCs) have had tremendous impact on patient outcomes in breast and other cancers. T-DXd, for example, is second-line therapy for stage IV, HER2 positive metastatic breast cancer as well as HER2 low expressing tumors. Sacituzumab govitecan, a TROP2 targeted ADC, has been approved as a 3rd line therapy in locally advanced or metastatic HER2 negative breast cancer. However, many patients fail to respond or relapse after treatment with ADC therapies due to tumor heterogeneity and eventual resistance to the ADC payload. We are developing next generation Multi-Payload Conjugates™ (MPCs™) that deliver targeted combination chemotherapies within a single molecule to address shortcomings in current ADCs.
Method: CatenaBio has developed novel, highly stable, single-molecule targeted combination therapies, MPCs™, with tunable payload ratios. Our selective conjugation platform allows the attachment of distinct payloads targeting different mechanisms of action at three unique sites on antibody scaffolds.
Results: Catena's MPCs show superior inhibition and excellent tolerability in mouse models of tumor growth in HER2 high and HER2 low/TROP2 expressing xenograft models including models of TNBC (triple negative breast cancer). In head-to-head comparisons MPCs out-perform current standards of care T-DXd and sacituzumab govitecan, demonstrating tumor elimination at low mg/kg doses. To produce the MPCs, combinations of different payloads targeting orthogonal mechanisms of cell division attached to trastuzumab as well as sacituzumab were screened at different DARs and payload ratios to optimize tumor cell killing. These targeted combination ADCs demonstrated robust killing in multiple cell lines containing high and low expression of HER2 and TROP2, as well as a T-DXd resistant cell line.
Conclusion: While advances have been made in the design of ADCs to expand to previously unaddressed populations, high patient relapse and the failure of recent mono-payload ADCs in late-stage trials indicate a need for novel conjugate modalities. Multi-payload Conjugates™ offer the next step in ADC design and allow for the targeted combination of multiple mechanisms of action with a single MPC™ that are highly efficacious at eliminating tumors across multiple cell line derived xenograft models with a range of target expression. Successfully constructed with a variety of targeting antibodies, these molecules offer the potential to circumvent tumor resistance pathways to deliver deeper and more durable patient responses.

#2888 DB-1418, a bispecific antibody-drug conjugate (ADC) targeting EGFR and HER3, demonstrates superior and broad antitumor efficacy and favorable safety in preclinical models.

Yunhua Zhou, Jun Yao, Rong Shi, Yang Qiu, **Haiqing Hua**

Duality Biologics, Shanghai, China

Background: EGFR and HER3 are co-overexpressed in a spectrum of tumors, including lung, breast, gastrointestinal, renal cell, ovarian, bladder, and prostate cancers. Importantly, HER3 expression is further enhanced upon inhibition of EGFR, suggesting that simultaneous targeting of both EGFR and HER3 could enhance tumor selectivity and improve therapeutic efficacy in EGFR tyrosine kinase inhibitors resistant tumors. This hypothesis has been supported by the clinical data of BL-B01D1, a bispecific ADC currently in clinical development. Here, we developed a differentiated bispecific ADC with stronger antitumor activity in preclinical studies.
Methods: We generated DB-1418, an asymmetric IgG-like bispecific ADC, featuring a Fab arm targeting EGFR with medium affinity and a scFv arm targeting HER3 with high affinity. The antibody is conjugated to a linker-payload L101P1021 at a drug-to-antibody ratio (DAR) of 6.
Results: The bispecific antibody showed low affinity on EGFR-only or HER3-only cell lines but demonstrated high affinity on tumor cells co-expressing both EGFR and HER3 compared to the parent monospecific antibodies. It also showed higher internalization capacity than the parent monospecific antibodies. DB-1418 exhibited broad anti-tumor activity across different expression levels of EGFR and HER3. Superior tumor inhibition was observed compared to a BL-B01D1 analog, based on both equal molar and mass concentrations. In the EGFR-dominant CAL-27 model, DB-1418 demonstrated a significant tumor growth inhibition (TGI) of 83% at a dose of 1.9 mg/kg Q3W, whereas the BL-B01D1 analog achieved only a 73% TGI at a dose of 3 mg/kg Q3W. In the HER3-dominant SW-620 model, DB-1418 treatment at a dose of 10 mg/kg Q3W resulted in complete tumor regression in 4 out of 5 mice and persisted up to 63 days after administration, whereas the BL-B01D1 analog at the same dose level and frequency failed to inhibit tumor growth. Notably, in an osimertinib-resistant NSCLC xenograft model with the C797S mutation, DB-1418 induced tumor regression with a TGI of 98% at a dose of 6 mg/kg Q3W, significantly higher than the BL-B01D1 analog at 6 mg/kg Q3W (TGI of 68%, $p<0.01$). DB-1418 demonstrated in vitro plasma stability in human, monkey, and rat models. In cynomolgus monkeys, the dose-range finding study showed favorable safety, with the highest non-severely toxic dose (HNSTD) reaching up to 45 mg/kg.
Conclusion: DB-1418 has shown promising pharmacological and pharmacokinetic properties, differentiated from the current clinical trial bispecific ADC targeting EGFR and HER3, with a broader therapeutic window. DB-1418 offers additional treatment options for patients who have developed resistance to EGFR tyrosine kinase inhibitors (TKIs) and addresses other unmet medical needs.

#2889 Multabodies: A next-generation approach for cancer immunotherapy and 4-1BB agonist therapy.

Joanne Hulme, Peter Bayliss, Abilasha Rao, Shrayasee Saha, Xinwen He, Debbie Jin, Yvonne Tsao, Amy Berkley

Radiant Biotherapeutics Inc., Toronto, ON, Canada

4-1BB agonism represents a promising strategy in cancer immunotherapy, targeting the 4-1BB receptor (CD137, TNFRSF9) expressed on activated T cells and NK cells. This co-stimulatory receptor, when activated by receptor multimerization, enhances immune cell proliferation, survival and effector functions. Agonistic antibodies against 4-1BB, optimized for FcγR cross-linking, have demonstrated potent anti-tumor activity in preclinical models. However clinical development has been hindered by severe liver toxicity (i.e., urelumab), or insufficient single agent activity, as seen with utomilumab. Here we describe a novel MULTi-specific, multi-Affinity antiBODY (Multabody™, MB) platform that exploits multi-valency to deliver an innovative 4-1BB Multabody™ with broad therapeutic potential. To understand the impact of epitope on 4-1BB agonism, we generated a panel of 4-1BB Multabodies to unlock comprehensive comparisons of potency and maximal activity of these molecules in a suite of assays including a 4-1BB Jurkat reporter assay vs urelumab and utomilumab. We identified 4-1BB MBs that demonstrated a range of Fc-independent agonistic profiles, including those mimicking the natural ligand, 4-1BBL. Identified lead 4-1BB MB candidate, RBT101, was further evaluated in vivo using the syngeneic MC38 murine tumor model. RBT101 demonstrated robust in vivo efficacy resulting in durable complete tumor regressions. Unlike urelumab which demonstrated splenomegaly and liver inflammation, RBT101 showed no signs of spleen or liver toxicity. Furthermore, RBT101 also provided long-term protection in a tumor rechallenge study, suggesting generation of a durable immunological memory response driven by RBT101. These data suggest the Multabody™ platform provides a novel approach to delivering a 4-1BB therapeutic with a differentiated profile to address an unmet clinical need in cancer immunotherapy for patients with solid malignancies.

#2890 Anti-tumor effect and mechanism of action of a nuclear transportable antibody drug conjugate Y-TR-1: Anti CD26 antibody conjugated with RNA polymerase II inhibitor triptolide.

M. Hayashi[1], H. Madokoro[1], K. Yamada[2], R. Hatano[3], C. Morimoto[3], T. Yamada[1];

[1]Saitama Medical University, Saitama, Japan, [2]The Jikei University, Tokyo, Japan, [3]Graduate School of Medicine, Juntendo University, Tokyo, Japan

Introduction: CD26 is a multifunctional transmembrane glycoprotein that is overexpressed in many cancers. We established the humanized anti-CD26 monoclonal antibody YS110, which has a direct anti-tumor effect through nuclear translocation of CD26 and transcriptional suppression of the POLR2A gene, a subunit of RNA polymerase II (Pol II). Subsequently, we developed an antibody-drug conjugate Y-TR-1 with YS110 and the Pol II inhibitor triptolide and evaluated its in vitro and in vivo antitumor efficacy and toxicity. We then elucidated the mechanism of action of Y-TR-1.

Experimental procedures: 5 triptolide molecules on average were crosslinked to YS110 by the heterobifunctional linker SMCC (designated Y-TR-1). The in vitro cytotoxicity of Y-TR-1 was analyzed in CD26-positive or -negative cancer cells and normal cells. The in vivo antitumor effect of Y-TR-1 was assessed using a xenograft assay. Pharmacokinetics/pharmacodynamics studies and toxicity tests were conducted using cynomolgus monkeys. Western blotting and immunofluorescence imaging were performed to confirm the nuclear translocation of Y-TR-1. The inhibitory effect of Y-TR-1 against Pol II was analyzed using quantitative polymerase chain reaction and a live cell imaging assay. The molecular mechanism of Y-TR-1 nuclear translocation was elucidated by immunofluorescence imaging.

Results: A conjugation method of Y-TR-1 was established, and the average drug-to-antibody ratio (DAR) was 4.76 by LC/MS. Y-TR-1 showed in vitro cytotoxicity on CD26-positive cancer cells, whereas non-cancer cells were less susceptible to Y-TR-1. The in vivo antitumor effect of Y-TR-1 was confirmed in a xenograft study using three CD26-positive cancer cell lines. Toxicity studies of cynomolgus monkeys with cross-reactivity to Y-TR-1 revealed no severe toxicity. The animals that received 30 mg/kg body weight of Y-TR-1 showed some decline in the reticulocyte count and slight increase in the thrombocyte count, whereas no histological damage was observed in the liver, kidney, heart, or lungs. Nuclear translocated Y-TR-1 was confirmed in the nuclear fraction of CD26-positive cancer cells by western blotting or immunofluorescence imaging. Quantitative polymerase chain reaction and live cell imaging assays revealed Pol II inhibition by Y-TR-1. Y-TR-1 was internalized via caveolae-dependent endocytosis and subsequently colocalized with endosomes before translocation to the nucleus.

Conclusions: Y-TR-1 is an ADC with a novel mechanism of action, i.e., nuclear translocation and subsequent Pol II inhibition. Transcriptional repression of POLR2A expression by nuclear-localized CD26 and Pol II inhibition by internalized triptolide are expected to have additive effects. Y-TR1 exhibited potent antitumor activity against CD26-positive cancer cells in vitro and in vivo.

#2891 In vitro comparison of Kadcyla and Enhertu in breast cancer with varying HER2 expression: proliferation, internalization, bystander effects and toxicity.

Veronica Bergo, Philipp Metzger, Valerie Oberhardt, Jan Eric Ehlert, **Holger Weber**

Reaction Biology Europe GmbH, Freiburg, Germany

HER2-positive breast cancer is characterized by overexpression of the HER2 receptor, driving aggressive tumor growth. Antibody-drug conjugates (ADCs), like trastuzumab emtansine (Kadcyla, T-DM1) and trastuzumab deruxtecan (Enhertu, T-DXd), are effective in targeting HER2-positive tumors, though their efficacy across varying HER2 levels remains under study. This study compares Kadcyla and Enhertu regarding cell proliferation, ADC internalization, and bystander cytotoxicity in cancer cell lines with high (SK-BR-3/SKOV-3), moderate (JIMT-1), and low (MDA-MB-231/MDA-MB-435) HER2 expression. It also examines off-site ADC toxicity in primary non-tumor cells caused by off-target payload delivery.
The xCELLigence system, which works via label-free impedance measurements on living cells, was employed to monitor real-time cell proliferation and viability. Both, Kadcyla and Enhertu, eliminate tumor cells, effectively at nanomolar concentrations, correlating with HER2 expression levels. Interestingly, Kadcyla's effect appears at least 24 hours earlier than Enhertu, whereas the unconjugated antibody Trastuzumab itself has little to no effect on tumor cell growth. Low HER2-expressing cells were killed at 1 µM, the highest dose tested. ADC internalization was analyzed using pH-sensitive, fluorescently labeled Trastuzumab in flow cytometry and microscopy, assessing uptake by mean fluorescence intensity (MFI) in viable cells. This analysis allowed the comparison of the rate and extent of Trastuzumab uptake across the tested cell lines. A co-culture system evaluated bystander effects, with high HER2-expressing SKOV-3/SK-BR-3 cells co-cultured with luciferase-labeled HER2-negative LN-229 cells. After treatment with Kadcyla or Enhertu, luciferase assays were conducted to assess whether the cytotoxic payload released by the ADCs in HER2-positive cells could affect the viability of neighboring HER2-negative cells. Enhertu demonstrated a bystander effect, whereas Kadcyla, on the other hand, showed a more limited activity, reflecting the non-cleavable linker and the membrane-impermeable nature of its payload DM1.
Toxicity analyses of Kadcyla and Enhertu on non-tumor cells, and especially on bone marrow, complement on-target investigations by revealing critical results about their safety profiles. While these ADCs are designed to selectively target tumor cells, off-site toxicity can affect healthy tissues and impair immune system function. Studies on hematopoietic stem cells are essential to assess potential adverse effects on hematopoietic regeneration and differentiation, aiming to reduce unintended immunosuppression. These findings support the optimization of ADC design, to enhance both selectivity and therapeutic windows.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: DNA Damage Response and Modulation of DNA Repair 1
Poster Session

#2894 NTX-452: a non-covalent, potent, selective and highly efficacious WRN inhibitor with best-in-class potential for the treatment of MSI-H tumors.

Rob Svensson, Scott Boiko, Beth Browning, Sam Carreiro, Derun Li, Angela Toms, Justin Caravella, Silvana Leit, Suzanne Jacques-O'Hagan, Hyesun Oh, Emily Florine, Zhenhong Li, Avinash Phadke, Yuet Mei Khong, Anita Scheuber, Peter Tummino

Nimbus Therapeutics, LLC, Boston, MA

Microsatellite instability (MSI-H) is a phenotypic consequence of defective mismatch repair (dMMR) in cancer and is observed in over 20 solid tumor types, with the highest prevalence in colorectal, gastric and endometrial cancers. While the treatment landscape of MSI-H is improving with the use of immune checkpoint inhibitors, substantial unmet medical need remains for patients with MSI-H cancers that fail to respond or relapse on standard-of-care therapies. Werner Syndrome Helicase (WRN) has been validated as a promising synthetic lethal drug target for MSI-H tumors, thus inhibitors of WRN may offer a novel therapeutic option for patients with MSI-H tumors. Our efforts to develop inhibitors of WRN led to the generation of two series of novel small molecules that had either a covalent or non-covalent mechanism of action. Treatment of MSI-H cell lines with exemplars from each series led to dose-dependent and rapid degradation of WRN protein, followed by an accelerated WRN protein re-synthesis rate that was independent of inhibitor mechanism. Washout studies using covalent inhibitors revealed that substantial target engagement was lost in MSI-H cells within 24- hours, suggesting rapid WRN resynthesis may limit sustained target inhibition. Prioritization of our non-covalent inhibitor series led to the generation of NTX-452, our lead candidate with best-in-class potential for the treatment of MSI-H cancers. The preclinical profile of non-covalent NTX-452 was characterized and compared to clinical-stage WRN inhibitors, including those from Novartis (HRO761, non-covalent) and Roche/Vividion (RO7589831, covalent). NTX-452 displayed excellent metabolic stability- with low in vivo clearance, good oral bioavailability, and high oral exposures in rodents and non-rodents. In vivo, low dose NTX-452 treatment induced robust pharmacodynamic and tumor regression across multiple MSI-H Cell line Derived (CDX) and Patient Derived (PDX) xenograft models. Notably, NTX-452 outperformed existing clinical stage WRN inhibitors at lower doses across multiple MSI-H tumor models. NTX-452 has broad efficacy across MSI-H CDX and PDX models from colorectal, gastric and endometrial lineages and is highly efficacious in models with diverse genomic and clinical characteristics. Moreover, NTX-452 promoted durable tumor regression and complete responses in MSI-H PDX models that were refractory to immunotherapy (anti-PD1) or chemotherapy. Lastly, resistance to clinical stage WRN inhibitors was explored and the potential for NTX-452 efficacy in WRN inhibitor resistant cell lines and tumors was evaluated.Taken together, our results highlight the broad, best-in-class potential of NTX-452 in MSI-H tumors and support its advancement to clinical evaluation.

#2895 Efficacy of DNA-PK inhibitor as maintenance strategy after cisplatin induction in SCLC.

Luisa Amato[1], Caterina De Rosa[1], Concetta Tuccillo[1], Francesca Iommelli[2], Viviana De Rosa[2], Virginia Tirino[3], Federica Papaccio[4], Kavya Ramkumar[5], Qi Wang[6], Jing Wang[6], Alberto Servetto[7], Floriana Morgillo[1], Lauren Averett Byers[5], Fortunato Ciardiello[1], Carminia Maria Della Corte[1]

[1]Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Napoli, Italy,[2]Institute of Biostructures and Bioimaging, National Research Council, Napoli, Italy,[3]Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Napoli, Italy,[4]Department of Medicine, Surgery and Dentistry, "Scuola Medica Salernitana", University of Salerno, Napoli, Italy,[5]Departments of Thoracic/Head & Neck Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX,[6]Bioinformatics & Computational Biology, The University of Texas MD Anderson Cancer Center, Houston, TX,[7]Department of clinical medicine and surgery, University of Naples Federico II, Napoli, Italy

Given the aggressive nature of small cell lung cancer (SCLC) and the frequent relapse after initial chemotherapy, this work aims to identify novel approaches to enhance the immune response. DNA-dependent protein kinase (DNA-PK) is a targetable enzyme involved in non-homologous end joining (NHEJ) and antiviral responses. DNA-PK plays a role during cGAS/STING dependent and independent viral DNA sensing pathway. Thus, we investigated the role of DNA-PK inhibition (DNA-PKi) as a maintenance strategy following cisplatin treatment in SCLC patients, focusing on its ability to enhance antitumor immune responses. We included different models of SCLC, in particular SCLC patient-derived peripheral blood mononuclear cells (PBMCs), SCLC cell lines and cocultures of PBMCs with SCLC cells and SCLC tumor spheroids. SCLC cells were treated in vitro with low-dose cisplatin (0.5 μM), and blood was collected from SCLC patients on day 3 of cisplatin treatment. SCLC cells and PBMCs were subjected to transcriptomic sequencing and gene expression profiling to assess the effect of DNA-PKi on PBMC subsets (CD8+ T cells, NK cells and monocytes). Gene set enrichment analysis (GSEA) of RNA-seq data revealed that cisplatin treatment enriched several DDR pathways and upregulated immune-related pathways in both SCLC cell lines and patient-derived PBMCs. Sequential treatment with DNA-PKi after cisplatin significantly activated CD8+ T-cell and NK-cell pathways, as well as enhancing interferon-related pathways and gene signatures associated with viral infections. In vitro treatment with DNA-PKi resulted in increased expression of pro-inflammatory markers in PBMCs, promoting M1 polarization of macrophages (TNF-α, IL-6 and CXCL10) while inhibiting M2 polarization (IL-10, IL4 and CCL22). This shift towards an anti-tumor phenotype was confirmed by enhanced NK cell-mediated cytotoxicity against SCLC cell lines. In addition, combination therapy with DNA-PKi and anti-PD-L1 resulted in increased immune cell infiltration into tumor spheroids, demonstrating a synergistic effect on anti-tumor immunity. In conclusion, our data suggests that DNA-PKi may improve immune responsiveness and potentially prolong antitumor effects in SCLC patients. Future investigations are warranted to further elucidate these mechanisms and to evaluate the clinical applicability of DNA-PKi in combination with existing therapies for SCLC.

#2896 Transforming treatment paradigms: A cutting-edge polθ Inhibitor (DWP223) targeting PARP resistance in BRCA-mutant cancers.

Juno Kim[1], Jie Luo[2], Bin Cao[2], Ling Zhao[2], Xiaojing Zhang[2], Xiaohua Lin[2], Xiaojing Lin[2]

[1]Daewoong Pharmaceutical, Seoul, Korea, Republic of,[2]XtalPi, Shanghai, China

A significant portion of tumors with BRCA1 or BRCA2 mutations exhibit only weak responses to PARP inhibitor (PARPi) treatment, and often develop resistance to PARPi over time. Both Polθ and PARP1 play critical roles in MMEJ for DNA double-strand break repair, and Polθ inhibition holds the potential to overcome PARPi resistance driven by BRCA reversion mutations. To address these challenges, we have developed DWP223, a novel selective and potent Polθ inhibitor. This compound can be used as a single agent or in combination with PARPi to treat tumors harboring BRCA mutations.DWP223 has shown potent and selective inhibition of BRCA-deficient cell proliferation in vitro, achieving an IC50 value below 20 nM, while demonstrating minimal impact on parental cell lines. In vivo, DWP223 monotherapy is effective at doses as low as 30 mg/kg, and when used in combination with PARP inhibitors, it induces over 50% tumor regression at a lower dose. Notably, even at high doses, DWP223 is well-tolerated, with no observable weight loss or hematological toxicity in animal models.Furthermore, DWP223 extends therapeutic potential by exhibiting synthetic lethality in tumors deficient in non-homologous recombination (HR), broadening its application to patients without homologous recombination repair (HRR) mutations. The compound’s favorable pharmacokinetic profile and robust safety data across multiple species underscore its suitability for clinical development, highlighting the opportunity to reduce hematological toxicity and target PARP inhibitor-resistant cancers.Collectively, DWP223 represents a promising therapeutic candidate, either as a standalone treatment or in combination with PARPi, for targeting HR-deficient cancers and potentially overcoming PARPi resistance. DWP223 is advancing toward IND filing in 2025.

#2898 SS induces BRCA1 reduction and sensitizes breast cancer cells to PARP inhibitor therapy.

J. Chen, B. Yi, Y. Xi;
University of Georgia, Athens, GA

Background: PARP inhibitors (PARPi) have demonstrated substantial clinical efficacy in treating BRCA-mutant breast cancers through synthetic lethality, where the inhibition of DNA single-strand break repair in BRCA-mutant cells leads to the accumulation of DNA damage and cell death. However, in the majority of breast cancer patients with BRCA wild-type (BRCAwt) tumors, PARPi therapy has limited effectiveness. Several approaches, such as CRISPR/Cas9-mediated BRCA1 gene editing, have been explored to induce BRCA deficiency in BRCAwt cancers, but these strategies face challenges for clinical application. Our recent work has focused on novel methods to sensitize BRCAwt breast cancer cells to PARPi. Sulindac sulfide (SS), the active metabolite of the FDA-approved NSAID sulindac, has emerged as a potential agent to enhance cancer cell death when combined with DNA-damaging agents, potentially by impairing DNA repair mechanisms.
Methods: This study investigates the mechanisms by which SS inhibits DNA repair, potentially through modulating BRCA1 expression. We assessed the effects of SS on BRCA1 protein levels using western blot analysis and examined BRCA1 mRNA levels using RT-PCR. DNA damage and repair were evaluated by γH2AX foci formation, RAD51 recruitment assays, and slot blot assays. To assess the therapeutic potential of combining sulindac sulfide with PARPi, we conducted both *in vitro* and *in vivo* studies.
Results: Sulindac sulfide treatment resulted in a dose-dependent reduction of BRCA1 protein levels via ubiquitin-proteasomal degradation. This reduction led to impaired homologous recombination repair capacity and increased DNA damage accumulation. *In vitro*, the combination of sulindac sulfide and olaparib exhibited synergistic cytotoxic effects in BRCA1wt triple-negative breast cancer (TNBC) cells. *In vivo*, the combination therapy significantly inhibited tumor growth compared to either agent alone. These findings suggest that sulindac sulfide can sensitize BRCA1wt TNBC cells to PARPi therapy by inducing homologous recombination deficiency.
Conclusion: This study provides a mechanistic basis for the combination of sulindac sulfide and PARPi as a potential therapeutic strategy for BRCA1wt TNBC. By downregulating BRCA1 and impairing DNA repair, sulindac sulfide induces a synthetic lethality effect, expanding the therapeutic reach of PARPi to BRCAwt cancers.
Keywords: Sulindac Sulfide, Olaparib, TNBC, BRCA1, and homologous recombination repair

#2899 PARG inhibition provokes a DNA damage-dependent innate immune reaction that enhances ICI-driven anti-tumor immunity.

Reeja Maskey, Megan Conway, Pei Xin Lim, Amber C. Donahue, Emily Fitz, Vidhya Nagarajan, Arjun A. Rao, Zabrisky Roland, Natalie Bresnahan, David Trinh, Ivan Shabalin, Kelly Trego, Jonathon Ryan, Rebeca M. Choy, Peter Teriete, Christian Frey, Yuchen Bai, Paul A. Barsanti, Claire Neilan, Mike White, **Diana M. Munoz**

IDEAYA Biosciences, South San Francisco, CA, CA

Poly (ADP-Ribose) glycohydrolase (PARG) is an emerging oncology drug target with a unique role in the resolution of DNA damage repair and DNA replication fork restart through hydrolysis of Poly (ADP-ribose) (PAR) chains. IDE161 is a potent, orally bioavailable, small molecule that directly binds the active site of PARG to impede PAR chain hydrolysis and exhibits robust anti-tumor activity in preclinical models associated with replication stress. Molecular profiling of baseline cell states across 257 tumor cell lines indicated innate immune signaling was strongly associated with responsiveness to IDE161. Moreover, induction of interferon regulatory factor (IRF) activity was the dominant gene regulation program detected in tumors in response to IDE161 exposure in vivo. Given the body of evidence indicating replication stress can lead to a type 1 interferon response via nucleic acid sensing, we speculated that IDE161-induced innate immune signaling is a consequence of cytosolic exposure to DNA repair intermediates including RNA: DNA heteroduplexes produced by failed resolution of stalled DNA replication forks. Consistent with this, IDE161-dependent cancer cell death was preceded by transcription-replication conflicts, replication catastrophe, innate immune pathway activation, and IFN-β production and secretion. To evaluate the potential for innate immune priming of an adaptive immune reaction, IDE161/immune checkpoint inhibitor (ICI) combination activity was assessed in a syngeneic mouse tumor model reported to be partially responsive to PARG inhibition. The combination of IDE161 with RPM1-14, an antibody directed against the mouse-programmed cell death protein 1 (PD-1) receptor, showed greater anti-tumor activity than either agent alone and altered the frequency of tumor-infiltrating lymphocytes towards an immune-activated phenotype. Moreover, T-cell receptor sequencing of clinical PBMC specimens from IDE161 monotherapy patients revealed clonal expansion in the T-cell receptor repertoires on treatment as compared to baseline. These cumulative observations suggest that IDE161 can induce an innate-immune-pathway-mediated priming of an antitumor adaptive immune response, and thus may deliver both tumor autonomous and nonautonomous therapeutic benefits. This relationship is currently under evaluation with IDE161/pembrolizumab combination therapy In patients with advanced endometrial cancer who have previously progressed on anti-PD-1 therapy (NCT05787587)

#2900 Musashi-1 regulated oncogenic switch enhances therapy resistance to DNA damage in glioblastoma.

X. Liu, T. Kong, M. Wong, L. Lin;
Hong Kong Polytechnic University, Hong Kong, Hong Kong

Glioblastoma (GBM) is notoriously resistant to treatment, largely due to its inherent enhancement of DNA repair mechanisms. Standard therapies, which include ionizing radiation and alkylating agents, primarily exert their effects by damaging DNA. Therefore, targeting the DNA damage response (DDR) has emerged as a promising strategy to sensitize tumor cells to these treatments. The RNA-binding protein Musashi-1 (MSI1) is known to contribute to the aggressive nature of GBM, yet its specific role in DNA damage-mediated therapy resistance remains largely unexplored. This study employed three GBM cell lines, an orthotopic GBM model in immunodeficient mice, and tissue samples from 35 GBM patients. We developed a specialized antibody to detect phosphorylation at the MSI1-S347 site in both cells and tissues. Site-directed mutagenesis and transcriptome sequencing were used to investigate the effects of MSI1 phosphorylation on GBM. The comet and colony formation assays were conducted to assess therapy resistance in GBM cells following treatment with carmustine (BCNU) or X-ray irradiation. Flow cytometry, immunofluorescence, and immunohistochemistry were utilized to identify cancer stem cell markers in GBM. A luminescence reporter system was created to pinpoint the binding site of non-phosphorylated MSI1 and to establish a visual drug screening platform targeting MSI1-S347. We identified a new phosphorylation site at serine-347 (S347) on the C-terminal of MSI1, which transcriptome analysis linked to the processing of DNA double-strand breaks in GBM. Patient tissue samples showed significantly higher levels of phosphorylated MSI1-S347 compared to normal brain tissues, regardless of tumor location or GBM subtype. Phosphorylation at MSI1-S347 enhanced BCNU resistance by promoting DNA repair mechanisms, and it also increased resistance to X-ray irradiation by preserving colony-forming ability. Additionally, higher levels of S347 phosphorylation were associated with reduced survival rates in GBM animal models. Mechanistically, we observed a positive correlation between phosphorylated S347 and the cancer stem cell marker CD133 expression. Further analysis revealed changes in binding interactions between MSI1 and p21 mRNA depending on the S347 phosphorylation state. To address this, we developed a luminescence reporter system to visually screen drugs that can modulate MSI1-S347 phosphorylation. This platform measures S347 phosphorylation levels in vivo and serves as a drug screening tool targeting GBM therapy resistance. This study uncovers a novel mechanism by which MSI1 enhances DNA damage repair, contributing to therapy resistance in GBM. Understanding the DNA damage response can guide clinical strategies to inhibit the MSI1 binding switch and enhance treatment efficacy. Targeting the MSI1 binding switch could offer a more effective approach to overcoming GBM therapy resistance.

#2901 Targeting *miR-19b*/PPP2R5E regulatory axis to enhance alkylating agent efficacy: a new avenue to counteract temozolomide resistance in recurrent glioblastomas.

Erik Vassella, Elham Kashani

University of Bern, Bern, Switzerland

Glioblastoma (GBM), the most aggressive primary brain tumor, notorious for its inevitable recurrence despite standard of care, presents a pressing need for novel treatment paradigms. Temozolomide (TMZ), a frontline DNA alkylating agent, induces extensive DNA damage. However, TMZ response is counteracted by intricate or acquired survival mechanisms. Through functional TMZ-resistance screens using microRNAs, we uncovered a novel mechanism involving the *miR-19b* target, *PPP2R5E*, a subunit of PP2A serine-threonine phosphatase, in TMZ response. Specifically, the induced expression of *PPP2R5E*, resulting from *miR-19b* attenuation, triggers genotoxic stress, priming GBM cell lines and primary GBM cells for heightened TMZ cytotoxicity. Elevated DNA damage in *PPP2R5E*-overexpressing cells is attributed to increased nuclear and mitochondrial ROS production, which is accompanied by enhanced ROS-mediated senescence and ferroptosis. Of note, all these phenotypes are reversed in GBM cells with concomitant *miR-19b* and *PPP2R5E* attenuation, indicating that *miR-19b* exerts its role in TMZ resistance by targeting *PPP2R5E*. In line with this finding, our orthotopic human-derived GBM stem cell xenograft model confirmed that *PPP2R5E* attenuation not only decreases TMZ cytotoxicity in the brain, but also at the spinal metastatic location. Consistently, treating cells with the PP2A-activating drug FTY720 or knocking down endogenous PP2A-inhibiting proteins mirrors the effects of *miR-19b* attenuation in enhancing TMZ cytotoxicity. This intricate interplay between *miR-19b* and *PPP2R5E* underscores a novel strategy to modulate the TMZ response, highlighting promising results in our pre-clinical models. Intriguingly, PP2A inhibiting proteins CIP2A and ANP32A (PHAP1) are upregulated in TMZ-resistant GBM cells and recurrent GBM tumors following TMZ treatment, suggesting a role of the PPP2R5E axis in TMZ resistance. In conclusion, our findings advocate for the therapeutic targeting of the *miR-19b*/PPP2R5E axis as a promising strategy to overcome TMZ resistance in GBM, indicating a potential novel strategy in the pursuit of effective therapeutic interventions for the formidable recurrent GBM tumors.

#2902 Systematic mapping of chemo-genomic interactions between DNA damaging agents and DNA damage repair genes with targeted CRISPR knockout screening.

Collin D. Heer[1], Spenser S. Johnson[1], James L. Elia[1], Sam Friedman[1], Xin Pei[2], Sofia R. Arbelaez[1], Ranjini Sundaram[1], Vijay Menon[1], Joseph Edmonds[1], Karlie Lucas[1], Nadeem Riaz[2], Susan E. Gueble[1], Ranjit S. Bindra[1]

[1]Therapeutic Radiology, Yale University, New Haven, CT,[2]Radiation Oncology, Memorial Sloan Kettering, New York, NY

Genetic instability is a hallmark of cancer, often arising from mutations in DNA damage repair and response (DDR) proteins. It is our goal to map the repair dependencies associated with different DNA-damaging agents (DDA) and DDR inhibitors (DDRi). To this end, we developed a DDR-targeted, CRISPR knockout screening approach to assess relationships among 355 DNA repair genes and 30 DDA and DDRi in LN229 glioma cells.

Systems-level analysis of over 11,000 interactions revealed that DDA cluster into distinct classes. In contrast, DDRi including ATR, Pol-θ, DNA-PK, WRN, Wee1, USP1, ATM and PKMYT1 inhibitors exhibited a variety of unique dependency profiles. Pathway analysis revealed clear associations between the knockout of Fanconi anemia pathway genes and sensitivity to bifunctional alkylating agents (e.g. chlorambucil), nucleotide excision repair and monofunctional alkylating agents (e.g. illudin S), homologous recombination and PARP inhibitors (e.g. talazoparib), and non-homologous end-joining and radiomimetics (e.g. bleomycin).

Furthermore, this platform identified differences in repair dependencies of compounds with subtle variations in chemical structure, highlighted by the imidazotetrazines temozolomide (TMZ) and KL50. In contrast to the methyl adducts deposited by TMZ, DNA modifications deposited by KL50 slowly evolve into DNA interstrand crosslinks (ICLs), circumventing mismatch repair (MMR)-driven resistance observed with TMZ. Indeed, screening revealed that resistance to TMZ, but not KL50, is driven by loss of MMR genes. Furthermore, we observed that BRIP1, a helicase known to play a key role in repair of ICLs, emerged as a unique sensitizing hit for KL50, but not TMZ, providing biological support for the formation of ICLs by KL50.

Finally, we sought to identify the most clinically actionable chemo-genomic interactions from our screens. Bi-allelic inactivating mutations (BI) of DNA repair genes are often necessary for manifestation of a DDR deficiency phenotype. Thus, we subjected our gene list to a custom bioinformatic pipeline to quantify rates of BI in samples from The Cancer Genome Atlas (TCGA). Excluding genes listed as Level 1 biomarkers on OncoKB, we found that 4.73% of TCGA samples harbored putative BI of at least one of the 138 genes that emerged as a hit (FDR < 0.01) in our screens.

Overall, our screens validated established chemo-genomic interactions while also identifying genetic dependencies associated with DDA of previously unknown mechanisms of action. Furthermore, we elucidated subtle differences in repair dependencies between structurally similar compounds. Finally, we identified novel genetic biomarkers with frequent BI in human tumor samples that sensitized to specific therapeutic agents upon loss, nominating chemo-genomic interactions with potential clinical relevance.

#2903 Evaluation of SMP-3124, a novel liposome-encapsulated CHK1 inhibitor, in patient-derived preclinical models of treatment-resistant ovarian cancer.

Yuka Kumagai[1], Junzo Hamanishi[2], Ryusuke Murakami[2], Kana Shimizu[3], Yosuke Ota[3], Yusuke Sawayama[4], Naoaki Shimada[3], Kento Hayashi[3], Yoshiko Fukuoka[3], Steven L. Warner[1], Jason M. Foulks[1], Masaki Mandai[2], Makoto Matsuoka[3], Seiji Kamioka[3], Hitoshi Ban[3], Hiroki Umehara[3]

[1]Translational Research and Medicine, Sumitomo Pharma America, Inc., Marlborough, MA,[2]Department of Gynecology and Obstetrics, Kyoto University Graduate School of Medicine, Kyoto, Japan,[3]Cancer Drug Discovery Research Unit, Sumitomo Pharma Co Ltd, Osaka, Japan,[4]Sumitomo Pharma Co Ltd, Osaka, Japan

Purpose: Checkpoint kinase 1 (CHK1) plays a critical role in DNA damage repair by responding to and managing DNA replication stress. CHK1 activation results in cell cycle arrest and the induction of DNA damage repair pathways. Targeting CHK1 is a promising therapeutic strategy due to high levels of replication stress in cancer cells. CHK1 inhibition causes replication catastrophe and inhibits cell growth in a wide range of cancers (Toledo L, 2017). In the past decade, multiple drugs targeting this pathway have been tested in clinical trials (de Costa AABA, 2023). Although some of the CHK1 inhibitors showed promising clinical responses as monotherapies or in combination therapies in patients with gynecological cancers, efficacies were limited due to toxicities. To maximize the potential for efficacy and limited toxicities, we utilized a CHK1 inhibitor formulated as a liposomal nanomedicine since liposomal drugs are reported to alter drug distribution to achieve longer drug retention in plasma and higher accumulation in tumors in preclinical and clinical settings (J Vaage, 1997, Barenholz Y, 2012, Northfelt DW, 1996).
Methods: SMP-3124 is an investigational selective CHK1 inhibitor encapsulated within a liposome. SMP-3124 showed a durable pharmacodynamic response due to the liposomal accumulation in the tumor in an ES-2 ovarian cancer xenograft model.
Results: We confirmed potent anti-tumor activities of SMP-3124 in multiple preclinical subcutaneous xenograft models without weight loss. To further evaluate the potential of SMP-3124, especially in recurrent ovarian cancer, we established a novel preclinical model from the ascites of a patient with multiple treatment-resistant ovarian cancers. The preclinical study using this model revealed resistance to the standard treatment drugs in ovarian cancer, while showing sensitivity to SMP-3124. Furthermore, we investigated the therapeutic potential of SMP-3124 in patient-derived xenograft models of ovarian cancer, including platinum-resistant models generated at Kyoto University. SMP-3124 showed more than 40% tumor regression relative to baseline in seven out of fourteen of the preclinical patient-derived xenograft models and achieved complete remission in three cases. These results support the investigation of SMP-3124 in ovarian cancer patients and the ongoing phase 1/2 study of SMP-3124 in adults with advanced solid tumors overall.

#2904 Synthetic lethal relationship between pharmacologic PARP inhibition and loss of LIG1 in chordoma.

Kwang Seok Lee[1], Tatjana Walther[1], Stefanie Reinhart[2], Lena Wickenhauser[2], Christina Schniederjohann[2], Maria Frolova[1], Dominic Helm[3], Schneider Martin[3], Mikolaj Slabicki[4], Claudia Scholl[2], Stefan Frohling[1]

[1]Translational Medical Oncology, German Cancer Research Center, Heidelberg, Germany,[2]Applied Functional Genomics, German Cancer Research Center, Heidelberg, Germany,[3]Proteomics Core Facility, German Cancer Research Center, Heidelberg, Germany,[4]Krantz Family Center for Cancer Research, Massachusetts General Hospital Cancer Center, Charlestown, MA

Impaired homologous recombination (HR) repair of DNA double-strand breaks (DSB) renders cancer cells sensitive to poly(ADP-ribose) polymerase inhibitors (PARPi). This synthetic lethal relationship is used to treat several epithelial cancers. However, the efficacy of PARPi is often limited by primary or acquired resistance, which might be overcome by rational combination therapies. We previously observed that most patients with advanced chordoma, a spinal neoplasm with no standard treatments, show genomic imprints of HR deficiency without mutations in HR-related genes such as *BRCA1/2 or PALB2*. Notably, a patient with HR-deficient, refractory chordoma responded to the PARPi olaparib for 10 months before developing resistance through a secondary PARP1 mutation (PMID: 30967556). To identify genes whose depletion increases the sensitivity of chordoma cells to olaparib, we conducted a genome-wide CRISPR/Cas9 screen in a patient-derived chordoma cell line (UM-Chor1). We identified several strong synthetic lethal interactors whose depletion conferred higher sensitivity to olaparib than knockout of *BRCA1*. These included *LIG1*, encoding an ATP-dependent DNA ligase involved in Okazaki fragment maturation and base excision repair, and *PRDX1*, encoding an enzyme that mitigates oxidative stress by neutralizing reactive oxygen species (ROS). Functional validation showed that *LIG1* knockout alone had only a minor effect on chordoma cell lines. In contrast, its combination with olaparib induced significant toxicity, associated with increased DSB, as determined by γH2AX staining, G2/M cell cycle arrest, and apoptosis. Furthermore, the screening results could be recapitulated with the second-generation PARPi talazoparib. Proteome analysis of *LIG1*-depleted, olaparib-treated chordoma cells revealed significant downregulation of proteins involved in DNA repair and oxidative phosphorylation. Concomitant phosphoproteome profiling identified increased kinase activity in pathways responsive to ROS, DNA damage, and apoptosis. Proteomic analysis of nuclear fractions revealed that olaparib treatment of *LIG1*-depleted cells upregulated the E3 ligase TRIM4, which collaborates with PRDX1 to mediate cell death caused by oxidative stress. Consistent with this, we observed significant ROS induction in *LIG1*-depleted, olaparib-treated cells. In summary, our integrated genomic and proteomic analysis revealed that *LIG1* loss enhances the effect of PARPi in chordoma cells, suggesting a potential strategy for combination therapies. Further investigation is required to determine if LIG1 is a viable therapeutic target and to unravel the mechanisms underlying olaparib-induced cell death in *LIG1*-deficient chordoma cells. This process may be influenced by the collaborative roles of TRIM4 and PRDX1, offering avenues for future research.

#2905 Combination of the ATR inhibitor, ART0380, with irinotecan for treating ATM-negative tumors.

Helen M. R. Robinson, Desiree Piscitello, Elias Elinati, Lucy Armstrong, Emily Graham, Silvia Peripolli, Giorgia Lillo, Joana F. B. P. Neves, Jayesh Majithiya, Eeson Rajendra, Marina Roy-Luzarraga, Lerin Geo, Vera Grinkevich, Ian C. Smith, Alessandro Galbiati, Arya Ashok Nair, Graeme C. M. Smith

Artios Pharma, Cambridge, United Kingdom

Ataxia telangiectasia mutated (ATM) and Ataxia telangiectasia and Rad3-related (ATR) proteins function in concert to regulate the cellular response to both intrinsic and extrinsic factors that induce replication stress such as oncogene activation and DNA damaging agents. Loss or inhibition of ATR has been well documented to be synthetic lethal with ATM deficiency in cancers and this concept is being explored in clinical trials, however, the efficacy of monotherapy with ATR inhibitors has been limited with objective response rates of ~10%. Here, using a novel ATR kinase inhibitor, ART0380, we provide mechanistic preclinical evidence that exacerbating replication stress with a low dose of the TOPO1 inhibitor, irinotecan, further sensitizes ATM negative tumors to ATR inhibition. This enhanced cancer cell killing by the combination of ART0380 and irinotecan in ATM negative cells is linked to loss of cell cycle control and entry of ATM negative cancer cells into mitosis with DNA damage, leading to mitotic catastrophe. In a range of CDX and PDX mouse models we show that the combination of ART0380 with irinotecan leads to robust tumor growth inhibition and, in some instances, regressions across diverse ATM negative tumor types and is well tolerated. These findings highlight ART0380 in combination with low-dose irinotecan as a promising therapeutic strategy for ATM negative cancers, which supports the ongoing clinical investigation NCT04657068.

#2906 Functional and therapeutic significance of DNA ligase I (LIG1) depletion in triple-negative breast cancer (TNBC).

Anh M. Tran Huynh[1], Sumimasa Arimura[2], Lacey E. Dobrolecki[1], Mariah J. Berner[1], Xuxu Gou[3], Junkai Wang[1], Michael T. Lewis[1], Jason Mills[2], Jun Xu[4], Gloria V. Echeverria[1], Susan M. Rosenberg[5], Christopher J. Lord[6], Simon Powell[7], Jonathan T. Lei[1], Matthew J. Ellis[1], Meenakshi Anurag[1]

[1]Breast Center, Baylor College of Medicine, Houston, TX,[2]Medicine-Gastroenterology, Baylor College of Medicine, Houston, TX,[3]University of California, San Francisco, Houston, CA,[4]Biochemistry, Baylor College of Medicine, Houston, TX,[5]Molecular and Human Genetics, Baylor College of Medicine, Houston, TX,[6]Cancer Genomics, The Institute of Cancer Research, London, United Kingdom,[7]Breast Center, Memorial Sloan Kettering Cancer Center, New York, NY

Background: We recently reported a proteogenomic study of TNBCs that demonstrated a correlation between chemoresistance and heterozygous LIG1 loss, a critical ligase serving DNA replication and repair (PMID: 36001024). Thus, this translational study aims to functionally characterize and investigate therapeutic opportunities driven by LIG1 depletion.

Methods: Heterozygous LIG1 knockouts ($LIG1^{het\text{-}KO}$) were engineered into the non-tumorigenic breast epithelial cell line, MCF-12A. Since TP53 was also mutated in almost all TNBC cases with LIG1 het deletion, $LIG1^{het\text{-}KO}$ was attempted in both $TP53^{wt}$ and $TP53^{null}$ MCF-12A. In contrast to $LIG1^{het\text{-}KO}$ in $TP53^{null}$ model, $LIG1^{het\text{-}KO}$ in $TP53^{wt}$ cells were not viable. Roles of LIG1 in cellular transformation, cell cycle dynamics, and the effect of carboplatin and PARP inhibition (PARPi) on these cells were investigated. RNA-seq was also employed to determine the impact of $LIG1^{het\text{-}KO}$ loss at a molecular level.

Results: $LIG1^{het\text{-}KO}$ in MCF-12A $TP53^{null}$ cell lines enhanced cell migration, decreased epithelial and increased mesenchymal gene expression, suggesting LIG1 depletion induced epithelial-to-mesenchymal transition (EMT). Recapitulating patient data, $LIG1^{het\text{-}KO}$ cells were less responsive to carboplatin than LIG1 intact cells. RNA-seq and western blotting demonstrated upregulated homologous recombination (HR) genes in MCF-12A $TP53^{null,}$ $LIG1^{het\text{-}KO}$ versus MCF-12A $TP53^{null}$ $LIG1^{wt}$ cells. Consistent with enhanced DNA repair, lower DNA repair foci (yH2AX) were observed in $TP53^{null}$ $LIG1^{het\text{-}KO}$ and the proportion of S-phase cells remained unchanged. This suggests that progression through cell cycle was not delayed despite $LIG1^{het\text{-}KO}$, due to compensatory upregulation of HR. To further assess therapeutic vulnerabilities in $LIG1^{het\text{-}KO}$ cells, a published CRISPR KO screen of PARPi was reanalyzed to identify LIG1 loss as a robust determinant of PARPi sensitivity (PMID: 29973717). This was confirmed in genome-wide CRISPR screenings with different PARPi(s) in the MCF-10A $TP53^{null}$ cells. Experimentally, MCF-12A $TP53^{null}$ $LIG1^{het\text{-}KO}$ cells displayed a significant increase in PARPi sensitivity, which was reversed upon over-expressing LIG1. Knocking down LIG1 in the LIG1 intact, HR-proficient TNBC line (MDA-MB-468) induced PARPi sensitivity, while overexpressing LIG1 in the LIG1-low, HR-deficient TNBC cell line (MDA-MB-436) reduced PARPi sensitivity. PARPi and carboplatin in combination was more effective than either drug alone in MCF-12A $TP53^{null}$ $LIG1^{het\text{-}KO}$ cells.

Conclusion: LIG1 depletion drives chemotherapy resistance through EMT and HR pathways activation. LIG1 depleted TNBC tumors could benefit from combination of PARPi and carboplatin, potentially driving future studies to investigate biomarker-guided TNBC treatment strategies.

#2908 WEE1 as a therapeutic target in *TP53* and *ARID1A* concurrent mutant colorectal cancers.

C. Zhang, K. Peng, Q. Liu, Y. Zhu, L. Ai, J. Wang, Q. Huang, T. Liu;
Zhongshan Hospital Fudan University, Shanghai, China

The development of next-generation sequencing (NGS) technology has provided us a comprehensive mutation profile of colorectal cancer (CRC). However, large number of genetic alterations, especially tumor suppressers, remain undruggable. *ARID1A*, as a member of tumor suppresser complex SWI/SNF, is frequently mutated (~9%) in CRC and its mutations contribute to the tumorigenesis of CRC. Recent evidence indicates targeting DNA damage response pathways exhibits promising efficacy in *ARID1A*-mutant cancers. WEE1 is a key regulator of G2/M cell cycle checkpoint, and WEE1 inhibition results in the accumulation of DNA damages and ultimately leads to cell death. Our present study demonstrates that WEE1 inhibitor (WEE1i) exhibits the highest sensitivity [ln (IC50) median difference = -1.85, $P < 0.0001$] among a panel of 199 compounds in *ARID1A*-mutant CRC cells based on GDSC2 dataset screening. As *ARID1A* mutation is considered as loss of function (LoF), *ARID1A* knockout was performed to simulate LoF in multiple CRC cell lines. Notably, *ARID1A* knockout sensitizes *TP53*-mutant, but not *TP53*-wildtype CRC cell lines, to WEE1 inhibitor MK-1775 or ZN-c3. In vivo study also indicated preferable efficacy to MK-1775 in COLO205 *ARID1A* knockout xenografts, compared with their wildtype counterparts. Mechanistically, *TP53*-wildtype cells exhibited robust rebound of P53 and P21 pathway under WEE1i treatment which significantly limited the efficacy. Besides, ARID1A deficiency enhanced replication stress (EdU incorporating assay) and DNA damage (γH2AX) through accumulation of pathological R-loops, which were detected by S9.6 nuclear intensity. Immunofluorescence colocalizing analysis also indicated that after WEE1i treatment, γH2AX signal tended to culminate in S9.6 positive loci, suggesting an R-loop associated DNA damage. These studies paralleled a phase 1b/2 clinical trial with SC0191 (a novel WEE1 inhibitor) based combination therapy in a cohort of late-line metastatic CRC patients (NCT06363552). In this cohort, one patient who had previously undergone 6 treatment regimens and harbored concurrent mutations in *TP53* and *ARID1A*, achieved stable disease for at least five months, accompanied by tumor regression in the liver lesion. In conclusion, our research showed that targeting WEE1 is effective in *TP53* and *ARID1A* mutant colorectal cancers. This therapeutic strategy is being tested in a phase 2 study for mCRC patients.

#2909 Targeting Polθ suppresses CCNE1-amplified cancers via transcription-replication conflicts mediated DNA damage and RAD51 toxicity.

Chuancong Zhu[1], Yu Xu[1], Xuejiao Zhao[2], Gordon B. Mills[2], Ding Ma[1], Qinglei Gao[1], Yong Fang[1]

[1]Department of Gynecological Oncology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China,[2]Department of Cell, Development and Cancer Biology, Oregon Health and Sciences University;Knight Cancer Institute, Portland, OR

Background: CCNE1 amplification is prevalent in various cancers, including ovarian, endometrial, and breast cancers, and is associated with elevated cyclin E levels, chemotherapy resistance, and poor prognosis. Despite its clinical relevance, no approved therapeutic strategies currently target CCNE1-amplified tumors. DNA polymerase θ (Polθ) has emerged as a promising therapeutic target in DNA repair-deficient cancers, such as HRD tumors. However, its function in oncogene-amplified cancers characterized by high replication stress and endogenous DNA damage remains underexplored.

Methods & Results: Public dataset analyses revealed a positive correlation between Polθ and CCNE1 expression across various tumor types in both clinical samples and cancer cell lines. Elevated Polθ expression was linked to worse survival outcomes exclusively in CCNE1-high tumors. Using a doxycycline (Dox)-inducible CCNE1 overexpression HeLa cell model and a panel of CCNE1-amplified, CCNE1-gain, and CCNE1-low cell lines, we demonstrated that CCNE1 overexpression upregulated Polθ expression, enhanced MMEJ repair activity, and increased sensitivity to Polθ inhibitors. Polθ inhibition, achieved through chemical inhibitors or shRNA knockdown, significantly impaired cell viability and exacerbated DNA damage, replication stress, and genomic instability in CCNE1-overexpressing cells. RNA-seq analysis revealed that CCNE1 overexpression promoted replication and transcription initiation events, while immunofluorescence and proximity ligation assays confirmed transcription-replication conflicts (TRCs) as a key outcome of CCNE1 overexpression. Polθ inhibition exacerbated TRC-associated DNA damage, both at the end of early S phase and after a round of cell cycle. In CCNE1-high cells, Polθ inhibition resulted in the accumulation of large RAD51 foci. Unlike normal CCNE1-high cells, where RAD51 foci resolved during cell cycle progression, Polθ inhibition caused persistent foci, indicating failed homologous recombination (HR) repair. Colocalization studies implicated genome G4 sites in this process, suggesting that Polθ compensates for stalled RAD51-mediated repair at G4-prone regions.

Conclusions: Polθ inhibition suppresses CCNE1-amplified tumors by amplifying replication stress, DNA damage, and genomic instability. We propose that Polθ supports tumor cell survival in CCNE1-amplified contexts by resolving TRC-associated DNA damage and toxic HR intermediates. Additionally, CCNE1-induced TRCs primarily occur at G4-prone regions, where Polθ mitigates RAD51 repair failure, underscoring its critical role in maintaining genomic stability under oncogene-induced replication stress.

#2910 Pamiparib-induced replication stress augments the efficacy of temozolomide in GBM PDX models.

Shiv K. Gupta, Ann C. Mladek, Sonia Jain, Katrina K. Bakken, Zeng HU, Brett L. Carlson, Danielle M. Burgenske, Jann N. Sarkaria

Department of Radiation Oncology, Mayo Clinic, Rochester, MN

Temozolomide (TMZ) is the main chemotherapy for GBM, but resistance develops in most patients, highlighting the need for sensitizing strategies. PARP inhibitors (PARPi), which disrupt DNA repair, are being investigated as TMZ-sensitizing agents. Here we evaluated the efficacy and explored molecular mechanisms of pamiparib-mediated TMZ sensitization. In vitro, low concentrations of pamiparib (0.1-1.0 μM) synergized with TMZ and enhanced DNA damage, particularly during the S- and G2/M phases. In vivo, pamiparib monotherapy (0.75 or 3 mg/kg, twice daily) was ineffective, however, TMZ afforded survival benefit in orthotopic patient-derived xenograft (PDX) models (GBM12, GBM22, and GBM84) of *MGMT*-methylated primary GBM. Pamiparib combined with TMZ (25 or 50 mg/kg daily) prolonged survival in GBM12 [median survival 272 vs. 114 days with TMZ (P=0.02), 30 days with placebo (P<0.001)] and GBM22 (median survival 88 vs. 60 days with TMZ (P=0.05), 26 days with placebo, (P<0.001)] but not in GBM84 [median survival 355 vs. 404 days with TMZ (P=0.44), 37 days with placebo (P<0.001)]. These findings align with prior studies indicating that PARPi can potentiate TMZ efficacy in a subset of primary GBM models sensitive to TMZ. TMZ induces cytotoxic DNA adducts, N_7MeG, N_3MeA, and O_6MeG. While N_7MeG and N_3MeA are repaired by base-excision repair (BER), MGMT removes O_6MeG. Suppressed *MGMT* expression, due to promoter methylation, drives TMZ sensitivity in ~40% of primary GBM cases. In *MGMT*-methylated tumors, O_6MeG-thymine mispairs trigger futile mismatch repair, causing replication stress. Although BER is PARP-dependent, BER inhibition did not replicate the TMZ-sensitizing effects of pamiparib. Beyond DNA repair, PARP regulates replication stress by recruiting translocases like SMARCAL1 and controlling endonucleases such as MRE11 and EXO1, protecting stressed forks and nascent DNA. We targeted these components using siRNA and small-molecule inhibitors to elucidate their roles in TMZ sensitization. Unexpectedly, silencing SMARCAL1 or MRE11, or inhibiting MRE11 with mirin, did not affect pamiparib-mediated sensitization in U251 cells. While EXO1 silencing attenuated DNA damage signaling, it had minimal impact on TMZ sensitivity or pamiparib-mediated sensitization. These results suggest that fork protection mechanisms may be epistatic to PARP but redundant for TMZ sensitivity. Alternatively, replication stress may be mitigated by lesion bypass via translesion synthesis (TLS), where replicative polymerases are replaced with low-fidelity polymerases. Consistent with this, silencing RAD18, involved in TLS, enhanced sensitivity to both TMZ and pamiparib, with the combination being more effective than either drug alone. In conclusion, pamiparib enhances TMZ efficacy in a subset of primary GBM. While further *in vivo* validation is needed, TLS deficiency may be a key determinant of pamiparib-mediated sensitization.

#2911 Unravelling the role of AP-1 transcription factor in DNA damage signaling and response (DDR), platinum and PARP inhibitor resistance in ovarian cancers.

Shatha Alqahtani, Rinad Mahmoud, Mashael Algethami, Asmaa Ibrahim, Ahmed Shoqafi, Jennie N. Jeyapalan, Sophie Kellaway, Nigel P. Mongan, Emad A. Rakha, Alan McIntyre, Srinivasan Madhusudan

University of Nottingham, Nottingham, United Kingdom

Background: Hypoxia is a feature of high grade serous ovarian cancer (HGSOC) microenvironment and contributes to platinum and PARP inhibitor resistance. Hypoxia induces activator protein-1 (AP-1) transcription factor (TF) activity leading to sustained proliferation, invasion, metastasis and angiogenesis. The role of AP-1 in DNA damage signaling and repair (DDR) and platinum/PARP resistance is unclear in HGSOC.
Methods: PARP sensitive and resistant HGSOC cells (PEO1, PEO1R) were monitored for the activity of 48 different TFs using a luciferase-based reporter assay. AP-1 subunits including c-JUN, JUND, JUNB, cFOS and FOSL2 were profiled for protein expression under normoxia and hypoxia (1% O_2). PEO1 and PEO1R tumor xenografts were immunohistochemically evaluated for JUNB, FOSL2, MRE11, CA-9 and CD-31 expression. CRISPR knock outs (KO) of JUNB and FOSL2 were generated and investigated for DNA repair gene expression (by DNA repair profiler PCR arrays), whole genome RNA sequencing, proliferation, invasion, cisplatin sensitivity (clonogenic and 3D-spheroids). Functional assays included DNA double strand break (DSB) accumulation, cell cycle progression, apoptosis, immunofluorescence, protein stability assay, co-immunoprecipitation and chromatin immunoprecipitation. The clinicopathological significance of FOSL2, JUNB and MRE11 expression was investigated in 331 clinical epithelial ovarian cancers.
Results: In platinum/PARP resistant PEO1R cells, upregulation of AP-1 transcription activity was evident compared to PEO1. Overexpression of JUNB and FOSL2 proteins was observed in normoxia and hypoxia (1% O_2). PEO1 and PEO1R tumor xenografts showed high levels of JUNB and FOSL2 in tumor hypoxic areas. Compared to controls, JUNB and FOLS2_KO cells were less proliferative and have increased sensitivity to cisplatin/PARPi (olaparib), which was associated with increased DSBs, G2/M cell cycle arrest and increased apoptosis. DNA repair profiling revealed down regulation of several DNA repair genes in KO cells including MRE11, a key DDR factor. JUNB/FOSL2 proteins physically interacted with MRE11 and promoted its stability. RNA sequencing revealed enrichment of pathways such as platinum response, oxidative phosphorylation, translation and others in KO cells compared to control. In clinical cohorts, high FOSL2, high JUNB and high MRE11 expression was significantly associated with shorter progression-free survival (PFS) and worse overall survival (OS).
Conclusion: Our data provides evidence that JUNB and FOSL2 may operate at the hypoxia-DDR interface in HGSOC. JUNB and FOSL2 not only have predictive and prognostic significance but could also be attractive anti-cancer targets including in platinum/PARP resistant HGSOC.

#2913 Preclinical characterization of GSK4418959 (IDE275): A potent, selective, and highly efficacious WRN inhibitor for MSI-H tumors across multiple cancer types.

Yang Lee[1], Phil Landis[2], Nanhua Deng[1], Diana Munoz[3], Jay Prakash Jain[3], Brian T. Jones[3], Edward J. Brnardic[2], Joshua Cottom[2], Jianxia Shi[3], Youyou Zhang[1], Robert Silvers[1], Xin Linghu[3], Ian Young[3], Yang Peng[2], Dawin Khiev[1], Gabriele Picco[4], Mathew Garnett[4], Joshua Taygerly[3], Mike P. DeMartino[2], Claire Neilan[3], Paul Barsanti[3], Michael White[3], Gul Y. Serbest[2], Yanhua Rao[2], Benjamin Schwartz[1], Geeta Sharma[1]

[1]GlaxoSmithKline, Cambridge, MA,[2]GlaxoSmithKline, Upper Providence, MA,[3]IDEAYA Biosciences, San Francisco, CA,[4]Wellcome Sanger Institute, Cambridge, United Kingdom

Large-scale genome-wide CRISPR screens have identified WRN helicase, RecQ family, as a promising synthetic lethal target for microsatellite instability-high (MSI-H) cancers across tumor types. GSK4418959 (IDE275) was discovered as a novel WRN helicase inhibitor to treat MSI-H cancer. Here, we present the cellular and in vivo evidence to demonstrate the selectivity and efficacy of GSK4418959 (IDE275) in multiple MSI-H models across various solid tumor types, including colorectal (CRC), endometrial, and gastric cancers. GSK4418959 (IDE275) demonstrates potent anti-proliferative effects in MSI-H cancer cell lines and patient-derived organoids across multiple tumor types, with no measurable impact on microsatellite-stable (MSS) models. In vivo, GSK4418959 (IDE275) induces tumor regression and activates DNA damage response (DDR) markers in several MSI-H CDX and PDX models across diverse tumor types, including CRC, endometrial, and gastric cancers with varying oncogenic drivers and tumor suppressor mutations, while sparing MSS models. One notable model is an MSI-H CRC PDX derived from a patient who had failed three prior lines of therapy, including the immune checkpoint inhibitor Nivolumab. With its unique binding mode, GSK4418959 (IDE275) showed tumor regression in an MSI-H CRC CDX model resistant to other existing WRN inhibitors. Additionally, GSK4418959 (IDE275) showed efficacy in chemotherapy-refractory MSI-H, HER2-negative gastric cancer patient-derived models. Furthermore, GSK4418959 (IDE275) also demonstrated combinatorial effects with existing standard-of-care therapies to benefit MSI-H cancer patients. These findings underscore GSK4418959 (IDE275)'s potency and highly selective activity against MSI-H cancer models in preclinic, highlighting its potential as a promising treatment option for MSI-H cancer patients in clinical setting, both as in monotherapy for those unresponsive to current cancer treatments and in combination with existing therapies for enhanced clinical benefit.

All studies were conducted according to GSK's Policy on the Care, Welfare and Treatment of Animals and reviewed by the Institutional Animal Care and Use Committee at GSK or by the ethical review process at the institution where the work was performed.

#2914 The WEE1 inhibitor Debio 0123 is synergistic with the PKMYT1 inhibitor lunresertib in preclinical models of ovarian and breast cancer.

Luke Piggott[1], Diana Bianca Rocha Gomes[1], Paula Martinez Sanz[2], Violeta Serra[2]

[1]Debiopharm International S.A., Lausanne, Switzerland,[2]Vall D'Hebron Institute of Oncology, Barcelona, Spain

Background: Debio 0123 is a highly selective and brain penetrant WEE1 inhibitor that has previously shown significant preclinical efficacy in combination with DNA damaging agents and is currently being investigated in several phase 1 clinical studies either as a monotherapy or in combination. WEE1 plays an important role in regulating the cell cycle during the S-phase and at the G2/M transition. Lunresertib (RP6306) is a selective and potent PKMYT1 inhibitor currently in phase 1 clinical studies as a monotherapy or in combination. Like WEE1, PKMYT1 regulates the G2/M transition, arresting cells with DNA damage to permit repair before progressing into mitosis. Herein, we present pre-clinical data that demonstrates the synergistic activity of the lunresertib and Debio 0123 combination in breast and ovarian tumor models.

Methods: Knockdown studies were performed utilizing siRNA against PKMYT1 and WEE1 to explore potential synergistic relationship of these targets in different cancer cell types. *In vitro,* combination efficacy of the selective WEE1i (Debio 0123) and PKMYT1i (lunresertib) was assessed by cytotoxicity in cell lines across different cancer types. Additionally, combination efficacy of lunresertib and Debio 0123 was further assessed *in vivo* in subcutaneous breast and ovarian cancer cell line-derived and patient-derived xenograft models.

Results: Knockdown of either PKMYT1 or WEE1 alone resulted in minor decrease in cell viability, however, concomitant knockdown of both targets resulted in no surviving cell populations. *In vitro*, combination of lunresertib and Debio 0123 resulted in significant synergy (Bliss) at low nM doses of both compounds. *In vivo*, in models of ovarian or breast cancer, monotherapy treatments with either Debio 0123 or lunresertib only resulted in minor tumor growth inhibition. Strikingly, the combination of Debio 0123 and lunresertib at all dose levels and schedules tested across all 5 models resulted in complete and sustained regression of all tumors.

Conclusions: These results demonstrate, in preclinical models of ovarian and breast cancer, the WEE1 inhibitor, Debio 0123, is synergistic with the PKMYT1 inhibitor, lunresertib, and consistently leads to deep and sustained regressions *in vivo*.

#2915 WRN depletion sensitizes the ARID1A-mutated cancers by inducing a distinct DNA damage response.

Jiwon Kim, Sung-Yup Cho

Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Korea, Republic of

SWI/SNF complex, which remodels chromatin structure to regulated transcription, is frequently mutated in various cancer types. ARID1A, a critical subunit of the SWI/SNF complex, is mutated in approximately 20% of colorectal cancers. Several synthetic lethal targets have been identified to address ARID1A-mutated cancers in various studies; ARID1B, EZH2, ATR, and AURKA-CDC25C. Notably, around 15% of ARID1A mutations are associated with microsatellite instability (MSI). In this study, we propose that WRN, already established as a synthetic lethal target of MSI cancers, is a promising therapeutic target in ARID1A-mutated cancers. In ARID1A-mutated cancer cells, WRN depletion reduced proliferation and increased apoptosis, underscoring its potential as a selective vulnerability. Interestingly, we observed distinct DNA damage response pathways triggered by WRN depletion depending on the presence of ARID1A mutations. In ARID1A wild type cells, WRN knockdown predominantly activated Chk1, whereas in ARID1A-mutated cells, Chk2 activation was more prominent. Further investigation revealed that ATR and Chk1 expression were downregulated in ARID1A-mutated cells. These findings suggest that Chk1 defects drive Chk2 activation in ARID1A-mutated cancers, presenting a distinct therapeutic opportunity to exploit this differential DNA damage response mechanism in ARID1A-mutated cancers.

#2916 DNA polymerase theta inhibitor, ART6043, potentiates the efficacy of 177Lu- and 225Ac-based radioligand therapies in vitro and in vivo.

Marco Ranzani, Alessandro Cicconi, Joseph Barlow, Paula Costales, Elias Elias, Alessandro Galbiati, Jayesh B. Majithiya, Silvia Peripolli, Eeson Rajendra, Marina Roy-Luzarraga, Nan Shao, Martin L. Stockley, Asmita Thapa, Robert A. Heald, Graeme C. M. Smith, Helen M. R. Robinson

Artios Pharma, Cambridge, United Kingdom

DNA polymerase theta (Polθ) is a DNA repair enzyme critical for microhomology mediated end joining (MMEJ). Loss or inhibition of Polθ has been shown to potentiate external beam radiation in a tumour-specific manner[1,2]. However, the ability of Polθ inhibitors to potentiate open source radiation has not been explored. This potential is pertinent to cancer therapy, as radioligand therapy (RLT), where a radionuclide is targeted to tumour cells by linking to peptides binding tumour-specific receptors, has had recent clinical success, in particular with ^{177}Lu-PSMA-617 for prostate cancer. Hence, we investigated the ability of the clinical Polθ inhibitor, ART6043[3], to potentiate different forms of RLT. Here, we show that ART6043 treatment significantly potentiate the cell killing activity of ^{177}Lu-RLT and ^{225}Ac-RLT in different prostate cancer cell models. Mechanistic studies show that the combination of ART6043 with ^{177}Lu-RLT caused a significant activation of DNA damage response pathways, accumulation of DNA damage and an increase in apoptosis compared to RLT or ART6043 alone. Key findings were confirmed for the combination of ART6043 with ^{225}Ac-RLT.Furthermore, *in vivo* studies in mouse prostate cancer xenografts showed that the combination of ART6043 with ^{177}Lu-RLT induced significant tumour growth inhibition and a significant improvement in median survival with regimens that were well tolerated. This work supports the clinical development of the combination of ART6043 with RLT to improve the clinical efficacy of RLT.
References: 1 Rodriguez-Berriguete G, Ranzani M, et al., Clin Cancer Res. 2023. 2 Higgins GS, et al., Cancer Res. 2010. 3 NCT05898399.

#2917 Utilizing artificial intelligence for the discovery of a novel CNS-penetrating ATR inhibitor.

Sarah Truong[1], Beibei Zhai[1], Louise Ramos[1], Mona Marzban[1], Fariba Ghaidi[1], Peter Guzzo[2], Mehran Khodabandeh[2], Ahmad Issa[2], Seyed A. Saberali[2], Jason Rolfe[2], Sarah Omar[2], Marshall Drew-Brook[2], John Langlands[3], Dennis Brown[3], Jeffrey Bacha[3], Mads Daugaard[4]

[1]Vancouver Prostate Centre, Vancouver, BC, Canada,[2]Variational AI, Vancouver, BC, Canada,[3]Rakovina Therapeutics, Vancouver, BC, Canada,[4]University of British Columbia, Vancouver, BC, Canada

Introduction: Ataxia telangiectasia and Rad3-related protein serine/threonine kinase (ATR) plays a crucial role in regulating DNA damage repair. ATR inhibitors have shown promise against solid tumors, not only as monotherapies, but also in combination with chemotherapy, radiotherapy, and immunotherapy with several clinical trials ongoing. The ATR inhibitors currently in clinical development have low CNS-penetration and are therefore sub-optimal for the treatment of brain tumors and brain metastases. It has been proposed that ATR inhibitors could be used as a monotherapy or could be used to potentiate the cytotoxicity of radiotherapy, one of the mainstay treatments for brain tumors. Development of novel, potent, CNS-penetrating ATR inhibitors could provide a new treatment alternative for patients with few options. Traditional drug discovery methods are time-consuming and costly, necessitating innovative approaches. Here, we describe the application of the Enki™ platform, an artificial intelligence approach, in the discovery of a novel ATR inhibitor for use against brain tumors.

Methods: The Enki™ platform utilizes generative AI and deep machine learning to search a broad chemical space to identify hits. It uses variational autoencoders to optimize many properties simultaneously within the search space, including maximal potency against the primary target, selectivity, ADMET, and physicochemical properties. This platform was used to generate de novo molecules with optimized properties to penetrate the blood-brain barrier and specifically target ATR.

Results: We will present preliminary results from the de novo molecule generation by the Enki™ platform. Validation of in vitro data, including ATR inhibition and selectivity, BBB permeability and metabolic stability, will be described.

Conclusion: The Enki™ platform is being leveraged for the development of a CNS-penetrant ATR inhibitor, significantly accelerating the drug discovery and development process. Use of this system enables a broader exploration of the vast chemical space and refinement of drug properties, both shortening the drug development process and increasing specificity and efficacy of potential therapeutics.

#2918 Wild-type IDH1 inhibition induces BRCAness to sensitize pancreatic cancer to PARP inhibitors.

Mehrdad Zarei[1], Omid Hajihassani[1], Hallie J. Graor[1], Soubhi Tahhan[1], Alexander W. Loftus[2], Faith Nakazzi[1], Semmer A. Ali[1], Vinayak Uppin[1], Luke D. Rothermel[2], Jonothan R. Brody[3], Jordan M. Winter[2]

[1]Case Western Reserve University School of Medicine, Cleveland, OH,[2]University Hospitals, Cleveland, OH,[3]Oregon Health & Science University, Portland, OR

Introduction: Pancreatic ductal adenocarcinoma (PDAC) remains one of the most fatal cancers in the United States. Homologous recombination (HR) deficiency has facilitated the development of targeted therapies like Poly (ADP-ribose) polymerase (PARP) inhibitors. However, only 10-20% of PDAC cases exhibit HR deficiency, leaving a majority of patients without targeted therapeutic options. This study identifies wild-type isocitrate dehydrogenase 1 (wtIDH1), a cytosolic metabolic enzyme, as a critical factor for HR repair in HR-proficient tumors. Inhibiting wtIDH1 disrupts genomic stability, sensitizing HR-proficient PDAC to PARP inhibitors.
Methods: We evaluated wtIDH1 inhibition on HR and non-homologous end joining (NHEJ) activity using DNA repair protein quantification and reporter assays. We also examined its effect on histone methylation. Synergistic anti-tumor effects of IDH1 and PARP inhibitors were assessed through drug combination assays, DNA damage response analysis, and apoptosis quantification. In vivo efficacy was studied in xenograft and orthotopic murine PDAC models.
Results: WtIDH1 inhibition impaired HR repair pathways, induced a BRCAness phenotype by reducing α-ketoglutarate levels, and promoted histone hypermethylation. Combination therapy with IDH1 and PARP inhibitors significantly reduced cell viability in vitro and amplified DNA damage and apoptosis markers. In murine models, co-administration of ivosidenib and olaparib synergistically reduced tumor growth and improved survival compared to monotherapies.
Conclusion: This study uncovers a novel strategy to target HR-proficient PDAC using combined IDH1 and PARP inhibition, demonstrating significant preclinical efficacy. Future studies will focus on optimizing and translating this approach into clinical practice.

#2919 Homologous recombination deficiency in non-small cell lung cancer: genetic and epigenetic causes beyond HRR.

Katerina Tsilingiri[1], Anna Chalari[1], Georgia Christopoulou[2], Alexandra Voutsina[3], Pantelis Constantoulakis[4], Konstantinos Potaris[5], Ioannis Vamvakaris[6], Dora Hatzidaki[7], Georgina Zachou[7], Giannis Vatsellas[8], Vassilis Georgoulias[7], Athanasios Kotsakis[9], **Apostolos Klinakis**[10]

[1]Department of Basic Research, BRFAA, Athens, Greece,[2]Genotypos SA, Athens, Greece,[3]National Hellenic Research Foundation, Athens, Greece,[4]Genotypos S.A., Athens, Greece,[5]Department of Thoracic Surgery, General Hospital of Thoracic Diseases Sotiria, Athens, Greece,[6]Department of Pathology, General Hospital of Thoracic Diseases Sotiria, Athens, Greece,[7]Hellenic Oncology Research Group, Athens, Greece,[8]Greek Genome Center, BRFAA, Athens, Greece,[9]Department of medical oncology, University General Hospital, Larissa, Greece,[10]Department of Rasic Research, BRFAA, Athens, Greece

Introduction: The mechanisms of homologous recombination deficiency (HRD) in non-small cell lung cancer (NSCLC) remain understudied, mainly due to the low prevalence of germline or somatic mutations in relevant genes such as BRAC1/2 in this cancer type. However, recent reports suggest that an important subset of patients present high genomic scarring score (GSS), indicating HRD, although the causes are not always clear. In this work, we examined a cohort of 147 patients and sought to correlate GSS, mutational profile and relevant gene expression with response to PARP inhibition in preclinical functional experiments.
Experimental Procedures: We used next generation sequencing to evaluate the GSS of each primary tumor with a commercially available sequencing kit which also provides information on the genetic status of relevant HRR and other genes. We performed rt-PCR analyses to assess the expression of certain genes, and we established patient-derived xenografts to examine the response of high and low GSS (h-GSS and l-GSS respectively) tumors to PARP inhibition.
Results: 42 out of 147 tumors (28.6%) presented a GSS>50 (h-GSS). The prevalence of germline or somatic mutations in HRR genes was not significantly different between h-GSS and l-GSS tumors (18/42 vs 37/105, p=0.388). On the contrary, TP53 mutation frequency ($p<0.001$) as well as variant allele frequency ($p<0.0001$) correlated significantly with GSS. Expression of HRR genes was marginally higher in h-GSS tumors, whereas expression of the epigenetic factor KMT2C was significantly lower in that subset and presented an inverse correlation with both GSS (p=0.0059) and TP53 variant allele frequency (p=0.02). The growth of h-GSS PDXs was significantly hampered upon PARP inhibition.
Conclusions: Our data show that for early-stage NSCLC, the GSS might be a valuable biomarker to stratify patients who might benefit from PARP inhibition. As this is not dependent on HRR genetic status in this cohort, further experimentation is needed to elucidate the complex relationship between TP53 status, KMT2C expression and HRD.

#2920 ZBP1 antagonizes MRE11-mediated DNA end resection and confers synthetic lethality to PARP inhibition in Ovarian cancer.

Shi Shennan[1], Qiuyang Xu[1], Xuejiao Zhao[1], Gordon B. Mills[2], Qinglei Gao[1], Yong Fang[1]

[1]Tongji Hospital, Tongji Medical College, Wuhan, China,[2]Department of Cell, Development and Cancer Biology, Oregon Health and Sciences University, Portland, OR

Background: DNA double strand breaks (DSB) results in the accumulation of abnormal nucleic fragments in the cytosol, which activates nucleic acid sensors such as cGAS, DHX9, and AIM2, leading to innate immune responses. Intriguingly, these sensors can influence DNA damage repair (DDR) within the nucleus conversely, which might indicate an unexpected feedback loop between these sensors and their functions in both the cytosol and nucleus. ZBP1, is also a classic nucleic acid sensor, which leads to programmed cell death and innate immune responses. Previous studies have demonstrated that ZBP1 plays a significant role in the downstream of DNA damage. However, it remains largely unclear whether ZBP1 exerts upstream feedback effects on DDR.
Methods and Results: ZBP1 was overexpressed in two human tumor cell lines, and its expression was induced in mouse L929 cell lines by IFN-γ. Subsequently, γ-H2AX foci were detected through immunofluorescence staining after DNA damage induced by etoposide, indicating that DNA damage repair was decelerated in cells expressing ZBP1. Immunohistochemical staining on ovarian cancer (OV) tissues demonstrated that the expression level of ZBP1 was positively correlated with that of γ-H2AX. Additionally, Co-IP assay revealed that ZBP1 bound with γ-H2AX, indicating ZBP1 localized to the DSB site. Then the DR-GFP and EJ5/GFP reporter system showed that ZBP1 inhibited Homologous Recombination (HR), but had no significant effect on Non-homologous End Joining (NHEJ). Subsequently, immunofluorescence and chromatin fractions extraction proved that ZBP1 affected DNA end resection. Co-IP assays verified that ZBP1 affected HR by binding to MRE11 and inhibiting MRE11 recruitment to chromatin. Furthermore, a kinase-specific phosphorylation site-prediction program, GPS 6.0, and western blot assay demonstrated that ZBP1 was phosphorylated at Ser106 by ATM, functioning as the initial signal for ZBP1 to inhibit downstream HR. Truncated mutants of ZBP1 for the binding site with MRE11, as well as a dephosphorylation mutation at Ser106, both attenuated the effect of ZBP1 in HR. In Addition, the results of flow cytometry and CCK8 assay on multiple ovarian cancer discovered ZBP1 overexpression cells displayed an enhanced sensitivity to PARP inhibitors. Moreover, the data from NANT study also indicated OV patients with high expression level of ZBP1 have better outcomes.
Conclusions: This study reveals that ZBP1 is binding on the DSB site upon the exogenous DNA damage, where it undergoes phosphorylation at ser106 by ATM and subsequently binds to MRE11 to prevent downstream end resection and inhibit HR, ultimately enhancing the synthetic lethality effect of PARPi. These findings indicates a cellular protective mechanism that prevents excessive repair mediated by ZBP1 and also suggests that PARPi could be an effective treatment strategy for OV with elevated ZBP1 expression.

#2921 SNV5686 a differentiated inhibitor of Werner helicase with potent activity against both WT WRN and WRN-C727S mutant proteins for treatment of MSI-H tumors.

Phillip C.C. Liu, Jeffrey Yang, Daniele Ferrante, Lin You, Mingming Gao, Yu Bai, Qipeng Fang, Yu Li, Zhentian Li, Chunhong He, Liyang Wang, Fei Zhou, Robert Landman, Hewen Zheng, Ken Mukai, Sabiha Chowdhury, Colin Zhang, Chao Qi, Liangxing Wu, Wenqing Yao

Synnovation Therapeutics, Inc., Wilmington, DE

Tumors with defects in mismatch repair genes can present with microsatellite instability (MSI) characterized by expansions of short, repeated DNA sequences. Functional genomic screens revealed that MSI-high (MSI-H) cell lines were uniquely vulnerable to depletion of Werner (WRN) which encodes an ATP-dependent helicase. Importantly inhibitors of WRN phenocopy genetic depletion leading to selective inhibition of MSI-H tumor growth. SNV5686 is a novel, reversible inhibitor of WRN with potent inhibitory activity in biochemical and cellular assays. SNV5686 shows exquisite selectivity against related RECQ helicases. The viability of MSI-H cells is selectively inhibited by SNV5686 through a mechanism involving activation of the DNA Damage response, loss of WRN protein and time- and dose-dependent increases in DNA breaks. In vivo, oral administration of low daily doses of SNV5686 potently depletes WRN and induces p21 in tumors and leads to deep tumor regression in an MSI-H colorectal xenograft model.
Irreversible WRN inhibitors and the non-covalent inhibitor HRO761 leverage binding to CYS727 in the active site to attain potency and selectivity, and published data show that mutation of CYS727 severely abrogates their activity. SNV5686 in contrast retains potent inhibition of WRN-C727S in both biochemical and cellular assays thereby exhibiting a differentiated profile. Acquired resistance to covalent inhibitors against other cancer targets such as EGFR and BTK though mutation of the covalent-bonded CYS (EGFR-C797, BTK-C481) is a well-documented clinical phenomenon. By maintaining potency SNV5686 has the potential to be a best-in-class inhibitor of WRN through its activity against both WT and mutant WRN as well as favorable physical-chemical properties and excellent preclinical PK profile. Furthermore, toxicology studies with SNV5686 have not revealed adverse findings in hematology or clinical chemistry supporting the potential to combine with standard of care agents including chemotherapy. These data support the clinical investigation of SNV5686 for tumors characterized by MSI-H.

#2922 RAD51AP1 deficiency enhances the synthetic lethality of the PARP inhibitors in lung cancer.

Jabunnesa Khanom[1], Nanditi N. Thangaraju[1], Sonia Batan[1], Kunal Kumar[1], Snigdha Ganjikunta[1], Subash Sundaram[1], Nikhil Patel[2], Santhakumar Manicassamy[3], Puttur D. Prasad[1], Muthusamy Thnagaraju[1]

[1]Biochemistry and Molecular Biology, Augusta University, Augusta, GA,[2]Pathology, Augusta University, Augusta, GA,[3]Medicine, Augusta University, Augusta, GA

Lung cancer (LC) is the most common cause of cancer-related death in men and women. Currently, the 5-year survival rate for LC is ~15%. Non-small cell lung cancer (NSCLC) accounts for more than 80% of all LC cases. About two-thirds of all patients with newly diagnosed NSCLC have advanced disease (stage III or IV), and they can be managed using palliative chemotherapy if they cannot benefit from the targeted therapy. The standard primary treatment for these patients is a combination of platinum-based chemotherapeutic agents. However, the critical barrier to using platinum-based chemotherapy is that it has high toxicity, especially nephrotoxicity and myelosuppression, and acquired drug resistance. Therefore, the development of a new and effective therapeutic strategy is a major unmet medical need and should have a high clinical impact on the treatment of NSCLC. Recently, poly (ADP-ribose) polymerase (PARP) inhibitors, Olaparib (AZD2281) and Talazoparib (BMN 673) offer an attractive new therapeutic avenue for the treatment of NSCLC as they are largely less toxic than platinum-based drugs. Although PARP inhibitor treatment alone has a significant impact on the overall survival of NSCLC patients, PARP inhibitors should have more clinical benefit in patients with a deficiency in homologous recombination (HR). RAD51AP1, HR protein involved in DNA repair and chemotherapy resistance. Therefore, the RAD51AP1 deficiency should potentiate the anticancer potential of PARP inhibitors and have a high clinical impact on the treatment of NSCLC, including *KRAS*-mutant NSCLC patients. We found that *RAD51AP1* knockdown significantly enhances PARP inhibitor response in both *KRAS*-wildtype NSCLC cell lines (H1299 and HCC827) and *KRAS*-mutant NSCLC cell lines (H1734 and H358). However, *RAD51AP1* knockdown did not affect the normal lung epithelial cells (HEL299 and HSAEC). *Rad51ap1* knockout mice are resilient in the *KRAS* mutation-induced spontaneous mouse lung cancer (*Rad51ap1*$^{-/-}$*-KrasLA2*) and significantly enhanced PARP inhibitors, Olaparib and Talazoparib, response. However, PARP inhibitor treatment does not have a significant response in *Rad51ap1*$^{-/-}$*-KrasLA2*. *RNA*-seq analysis provides evidence that *Rad51ap1* deficiency enhances chemotherapy response by limiting signaling pathways that are associated with drug resistance and efflux. Overall, our results provide a mechanism-based novel therapeutic strategy for the treatment of NSCLC and the identification of RAD51AP1 inhibitor should have a significant clinical impact on the prevention and treatment of NSCLC

#2923 Targeting DNA repair mechanisms to overcome CIC::DUX4 undifferentiated sarcomas.

Zeinab Kosibaty, Cuyler Luck, Ross A. Okimoto

UCSF - University of California San Francisco, San Francisco, CA

Transcription factor (TF) fusion oncoproteins represent cancer specific alterations that arise from chromosomal rearrangements. Through target gene recognition, TF fusions can disseminate transcriptional responses that collectively work to drive tumorigenesis. Thus, identifying the molecular targets that operate as a disease driving network can potentially uncover key actionable dependencies. We have taken this strategy to dissect the underlying biological mechanism by which CIC::DUX4, a fusion oncoprotein associated with dismal outcomes, drives sarcomagenesis. We and others have defined a CIC::DUX4 fusion mediated network that dysregulates cell-cycle and DNA replication checkpoints. Specifically, CIC::DUX4-mediated *CCNE1* upregulation compromises the G1/S transition leading to high DNA replication stress and conferring a dependence on the G2/M checkpoint kinase, WEE1. WEE1 provides a molecular brake to enable effective DNA repair prior to mitotic entry. Importantly, the mechanism by which CIC::DUX4 regulates DNA repair remains unknown. Here we show that the catalytic subunit of DNA polymerase epsilon (POLE) is essential for DNA integrity and cellular division in CIC::DUX4 sarcoma. Mechanistically, POLE loss increases DNA damage and induces p21-mediated cellular senescence to limit CIC:DUX4 tumor growth *in vitro* and tumor formation *in vivo*. Collectively, we credential POLE as a CIC::DUX4 target and further characterize a functional network through which CIC::DUX4 operates to drive tumor progression and survival.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Growth Factor Receptors and Other Surface Antigens as Targets for Therapy 1
Poster Session

#2927 CS2011: A novel bispecific antibody targeting EGFR and HER3 that demonstrates promising antitumor activity in preclinical evaluation.

Chuan Wang, Ning Zhang, Xinling Zhang, Yongwang Li, Yongli Yang, Mengyao Zhu, Yuxin Qian, Jingyu Sun, Xuelian Liu, Fei Ma, Yi Sun, Jianxin Yang

CSTONE Pharmaceuticals, Suzhou, China

Background: EGFR (also known as HER1) is an epidermal growth factor receptor with tyrosine kinase activity implicated in both tumorigenesis and tumor progression, and its overexpression in approximately 70% of colorectal cancer (CRC), 60% of lung cancers, and over 90% of head and neck squamous cell carcinoma (HNSCC) have been shown associated with advanced diseases. Approved anti-EGFR therapies, including cetuximab, pantitumumab, and necitumumab, are widely used in treating the aforementioned malignancies. Amivantamab, a bispecific antibody targeting EGFR and c-MET, has been approved for treating advanced NSCLC with EGFR mutations. HER3, another member of the HER family, is overexpressed in cancers including CRC and lung cancer. The upregulation of HER3 has been reported in tumors when treated with MAPK/PI3K inhibitors, EGFR TKIs, or hormone therapies. Daiichi's HER3-ADC, U3-1402, has shown promising clinical results in advanced EGFR-mutant NSCLC, confirming HER3 as a valid therapeutic target. CS2011 is a bispecific antibody composed of a potent anti-EGFR arm and a fully functional anti-HER3 arm. Unlike anti-EGFR, anti-HER2 or anti-HER3 mono-targeting antibodies, CS2011 inhibits almost all functional membrane dimers formed with HER members, and therefore acts as a broad epithelial growth factor signaling blocker to address tumor heterogeneity effectively.
Methods & Results: The lead molecule of CS2011, J17, was constructed with monovalent arms targeting EGFR and HER3. J17 showed high binding affinities to EGFR or HER3 respectively and dual binding to EGFR & HER3 concurrently, as assessed by BLI. Our data further demonstrated that J17 possessed single-digit nanomolar affinities to EGFR-positive and/or HER3-positive tumor cells, while demonstrating no binding activity to EGFR/HER3-negative cells. J17 induced faster and deeper internalization compared to anti-EGFR and anti-HER3 mono-targeting antibodies. J17 also effectively inhibited EGFR downstream signaling to levels similar to anti-EGFR antibodies. Furthermore, J17 inhibited NRG1 (HER3 ligand)-induced proliferation of A431 cells. *In-vivo* studies further demonstrated potent tumor growth inhibition by J17. J17 also demonstrated favorable stability and developability, achieving yields comparable to those of monoclonal antibodies. Pilot pharmacokinetic (PK) and toxicity studies in cynomolgus monkeys suggested a monoclonal antibody-like PK profile and favorable tolerability at 30 mg/kg.
Conclusion: CS2011 is a promising bispecific antibody for the treatment of various advanced solid tumors. Current preclinical data support further IND-enabling development and clinical research on CS2011.

#2928 Combination surface targeting strategies in relapsed small cell lung cancer (SCLC) to overcome intratumoral heterogeneity associated with treatment resistance.

C. Allison Stewart, Kavya Ramkumar, Runsheng Wang, Yan Yang, Bingnan Zhang, Yuanxin Xi, Lixia Diao, Qi Wang, Alberto Duarte, Ping Li, Azusa Tanimoto, Alejandra G. Serrano, Jody Vykoukal, Mukulika Bose, Loukia G. Karacosta, Luisa Solis Soto, Samir Hanash, Jing Wang, John V. Heymach, Lauren Averett Byers, Carl M. Gay

UT MD Anderson Cancer Center, Houston, TX

Small cell lung cancer (SCLC) is an aggressive neuroendocrine malignancy characterized by rapid onset of chemoresistance and poor clinical outcomes. Following decades of, at best, modest clinical advances, the recent FDA approval of tarlatamab, a DLL3 targeting bispecific T-cell engager (BiTE), alongside unprecedented response rates observed with multiple antibody-drug conjugates (ADCs), have ushered in a paradigm shift towards surface targeting strategies in relapsed SCLC patients. These same agents are demonstrating similar efficacy in more rare high-grade neuroendocrine carcinomas, both pulmonary and extrapulmonary; however, they are being largely tested in unselected populations. While providing much-needed optimism for SCLC patients, resistance, both *de novo* and acquired, is common and must be better characterized to maximize the potential of these new therapeutic classes. We hypothesize that combinatorial targeting of multiple surface proteins using distinct strategies (i.e., BiTEs, ADCs, etc.) represents a novel way to overcome intratumoral heterogeneity common to relapsed SCLC and enhance antitumor immunity engendered by ADC payloads (i.e., topoisomerase 1 [TOP1] inhibitor). To better define the surfaceome of relapsed SCLC, we performed surfaceome mass spectrometry analysis of SCLC cell lines, naïve and relapsed patient derived xenografts (PDXs), and PDXs treated with frontline chemotherapy until relapse occurred, and identified a number of novel and known surface proteins (i.e., TROP2, HER2, B7H3). Surface targeting strategies against HER2, TROP2, and DLL3 are effective in preclinical models (i.e., cell lines and xenograft models) resistant to other common SCLC therapies (i.e., platinum chemotherapy). In particular, ADCs with TOP1 inhibitor payloads were more effective in models with high SLFN11 and target levels, suggesting that sensitivity requires both surface target expression and SLFN11 positivity for greatest response. Notably, single-cell transcriptional profiling of relapsed patient biopsies revealed mutually exclusive expression of surface genes in distinct cell populations, including senescent, drug tolerant persister cells (DTPCs), representing an unique opportunity to target heterogeneous populations. We show that combination targeting against different surface proteins (e.g., DLL3, TROP2, HER2) using both immune (i.e., chimeric antigen receptor T-cells, BiTEs) and payload-based modalities (i.e., ADCs) was more effective than single-agent targeting in resistant, neuroendocrine-low models. Therefore, intratumoral heterogeneity associated with relapsed SCLC, which limits efficacy of single-agent surface targeting strategies, may be exploited with combinatorial therapies to target resistant cell populations, including DTPCs, using payload and immune-based methods.

#2929 LX-101, a novel, clinical stage, payload-bearing targeted therapy directed to the insulin-like growth factor receptor (IGF-1R), demonstrates potent anti-tumor activity in Ewing sarcoma animal models and desmoplastic small round cell tumor.

Matt Hoberman[1], Danh Dinh Truong[2], Roberto Cardenas Zuniga[2], **Joseph A. Ludwig**[2]

[1]Lirum Therapeutics, Inc., New York, NY,[2]MD Anderson Cancer Center, Houston, TX

Objective: Multiple aggressive cancers of unmet need have alterations involving the insulin-like growth factor 1 receptor (IGF-1R) pathway, including gene fusions and/or high IGF-1R expression, that correlate with poor outcomes. In Ewing sarcoma (ES) and desmoplastic small round cell tumor (DSRCT), the gene fusion-produced oncogenic proteins upregulate the IGF signaling pathway. Although prior attempts at inhibiting IGF-1R with non-payload bearing naked monoclonal antibodies or small molecules produced some tumor responses, none of these agents were ultimately approved, potentially due to suboptimal potency and escape mechanisms via redundant signaling. LX-101 is a next-generation, targeted therapy directed to the IGF-1R, consisting of a proprietary IGF-1 variant coupled to a cytotoxic methotrexate payload. LX-101 was previously evaluated in Phase 1 trials of adult patients with advanced, pretreated cancers, where it was well-tolerated and demonstrated single agent activity. Neither a dose limiting toxicity nor a maximum tolerated dose was reached, leaving room for additional dose and schedule optimization. Herein, we investigated the in vitro and in vivo anti-tumor activity of the payload-bearing agent LX-101 in ES and DSRCT.

Methods: ES and DSRCT cell lines were incubated with LX-101 (1 - 10,000 nM) for 72 h. Cell viability was assessed by CellTiter-Glo; IC_{50}s were calculated using GraphPad PRISM software. Pretreatment IGF-1R expression levels were analyzed by RNA-seq & Western blot. NOD-SCID mice bearing subcutaneous A-673 tumors were treated i.v. with vehicle or LX-101 (16 uEq/kg, twice a week, for 3 weeks). Tumor volume and body weight were measured 3 times a week.

Results: LX-101 displayed anti-tumor activity against ES and DSRCT cell lines, including EWSR1::FLI1+ cell lines A-673 (IC_{50} = 693 nM), ES-8 (IC_{50} = 354 nM), and TC-71 (IC_{50} = 199 nM), as well as the EWSR1::WT1+ cell line BOD-DSRCT (IC_{50} = 453 nM). IGF-1R mRNA levels correlated with LX-101 anti-tumor activity. In mice bearing A-673 tumors, LX-101 significantly inhibited tumor volume, in vivo, following twice a week treatment for 3 weeks (55% tumor growth inhibition relative to vehicle group on Day 21, $p < 0.01$). No adverse effects were observed, demonstrating that LX-101 was well-tolerated.

Conclusion: These results demonstrate that LX-101 has potent anti-tumor activity against ES, in vivo and in vitro, and DSRCT, in vitro, providing a differentiated approach from previous IGF-1R targeting agents. These data support further clinical development of LX-101 in ES, DSRCT, and other IGF-1R-involved cancers. Phase 2 clinical trials are expected to begin in 2025.

#2930 Intrinsic payload resistance governs responses to datopotamab deruxtecan, a TROP2 antibody drug conjugate, in pancreatic cancer model systems.

Panot Sainamthip, Kazuki Takahashi, Jiajia Chen, Junning Wang, Julien Dilly, Seema Chugh, David Lui, Andrew J. Aguirre, Harshabad Singh

Dana-Farber Cancer Institute, Cambridge, MA

Introduction-TROP2, a transmembrane glycoprotein encoded by the TACSTD2 gene, is overexpressed in pancreatic cancer (PC) and contributes to cancer progression. Datopotamab deruxtecan (Dato-DXd) is an antibody-drug conjugate targeting TROP2, utilizing a novel linker technology that is only cleaved following internalization. It has demonstrated clinical activity in breast and lung cancers and is under investigation in PC. Determinants of response and resistance to ADCs are not well studied. Here, we investigate predictors of Dato-DXd efficacy in PDAC using in vitro models. *Method*-Twenty six PC cell lines with varying TROP2 mRNA levels were tested using Dato-DXd, isotype control-DXd, and naked toxin DXd in 96-well plates using CellTiter-Glo®. Drug sensitivity was correlated with mechanism based predictors using Pearson's correlation. Cell surface TROP2 was quantified using QIFIKIT; antibody internalization was analyzed using iQue® Human Antibody Internalization Reagent and quantified with Quantum™ MESF microsphere kit. Cathepsin B activity was measured with the Magic Red® Fluorescent Cathepsin B Assay Kit.*Result*-To identify factors influencing response and resistance, we used the ratio of AUC (Area Under the Curve) of Dato-DXd to AUC of Isotype-DXd (AUC Dato/Isotype) as a standardized measure of efficacy. AUC Dato/Isotype approaching 1 indicate no activity of Dato-DXd over isotype control, and lower values signify selective cytotoxicity mediated by Dato-DXd. We noted no activity of Dato-DXd in cell lines lacking surface expression of TROP2 (n = 4), whereas responses varied among TROP2+ lines. To explore predictors of Dato-DXd response in TROP2+ cell lines (n = 22), we examined the relationship of TROP2 mRNA levels, TROP2 surface expression, Dato-DXd internalization, and Cathepsin B activity with Dato-DXd sensitivity, using Pearson correlation. AUC of the DXd (payload) is highly correlated with the Dato-DXd sensitivity ($r = 0.63$, $p = 0.002$). Further review of sensitivity of to DXd showed natural separation of tested models into DXd sensitive and resistant models at 1nM DXd concentration. Lines resistant to DXd at 1 nM showed significantly higher resistance to Dato-DXd (two-tailed t-test, $t = 7.34$, $p < 0.0001$). *Conclusion*-PC in vitro models reveal a range of sensitivities to Dato-DXd. We found that TROP2 expression is necessary but not sufficient for sensitivity to Dato-DXd and intrinsic resistance to payload is highly correlated with resistance to Dato-DXd. Ongoing analyses to identify additional predictors of response to Dato-DXd using whole transcriptome profiles of tested models will be presented.

#2931 AXL-directed surface targeting approaches in recalcitrant pulmonary high-grade neuroendocrine carcinomas.

Kavya Ramkumar, C. Allison Stewart, Yan Yang, Bingnan Zhang, Qi Wang, Yuanxin Xi, Runsheng Wang, Alejandra G. Serrano, Luisa M. Solis Soto, Jing Wang, John V. Heymach, Carl M. Gay, Lauren A. Byers

UT MD Anderson Cancer Center, Houston, TX

High-grade neuroendocrine carcinomas of the lung (hgNECs), which include small cell lung cancer (SCLC) and pulmonary large-cell neuroendocrine carcinoma (LCNEC), are characterized by poor prognoses, limited treatment options and rapid emergence of resistance. While recent surfaceome-directed approaches targeting DLL3, SEZ6, and TROP2 using chimeric antigen receptor T cells (CAR-Ts), bispecific T cell engagers (BiTEs) and antibody drug conjugates (ADCs) show considerable promise in hgNECs, novel strategies to target the heterogenous resistant disease are needed. AXL, a TAM family receptor tyrosine kinase, is known to mediate resistance to chemotherapy, radiation and targeted therapies in SCLC and other cancers, through its roles in epithelial to mesenchymal transition (EMT), DNA damage repair and replication stress tolerance. We hypothesize that targeting cell surface AXL would provide an effective therapeutic strategy in hgNECs, especially for relapsed disease. We analyzed the transcriptomic (bulk and single-cell RNA sequencing) and proteomic (immunohistochemistry) expression profiles of AXL in treatment-naïve and relapsed SCLC, LCNEC and other extrapulmonary hgNECs patient tumors. High levels of AXL expression were seen in distinct tumor subsets. Notably, AXL-high tumors exhibited molecular signatures associated with resistance (higher EMT scores, lower replication stress and cisplatin response). To evaluate AXL as a viable surface target in hgNECs, we generated a preclinical CAR T cell against AXL. Anti-AXL CAR T cells were effective at inducing cytotoxicity in AXL-expressing SCLC and LCNEC cell lines. Similarly, an anti-AXL ADC also showed selective and potent cytotoxicity in AXL-positive models. These findings establish AXL as both a valuable biomarker and a promising target for the drug-resistant hgNECs, supporting the development of AXL-directed therapeutics such as anti-AXL CAR T cells and anti-AXL ADCs.

#2932 GNX1021, a novel ADC targeting glycans with branched Lewis B/Y, demonstrated preclinical tumor control activity in gastric cancer models and favorable safety.

Y.-Y. Chen, C.-C. Chang, W.-H. Lee, C.-K. Chen, Y.-R. Shen, Y.-T. Lin, C.-W. He, Y.-J. Wang, L.-Y. Liu, M.-C. Yang, T.-H. Chang; GlycoNex Inc., New Taipei City, Taiwan

Background: Branched Lewis B/Y (bLeB/Y) is a unique tumor-associated glycan antigen overexpressed in a wide panel of solid tumors including gastric cancer. GNX1021 is a novel bLeB/Y-targeting ADC, linked to the potent antimitotic agent Monomethyl auristatin E (MMAE) via an enzyme-cleavable linker. The mAb backbone of GNX1021 has established safety profile in Ph1 oncology study. Disease control was maintained over 4 months as stable disease in 4/46 advanced solid tumor patients. This study presents *in vitro* pharmacodynamics and *in vivo* anti-tumor activity of GNX1021 in multiple gastric cancer models as well as its safety profile in rats.
Methods: Target engagement was verified via ELISA, with detailed characterization of cell-binding, internalization, and cytotoxicity. Prevalence of bLeB/Y expression and other therapeutic targets are assessed through IHC over TMAs. Next, the *in vivo* efficacy was assessed in cell line-derived xenograft (CDX) models. MMAE-associated PD markers including Ki67 for cell proliferation and cleaved caspase-3 for apoptosis were characterized with IHC in the tumors isolated from CDX models. Nonclinical safety was evaluated in a pilot repeat-dose study of Sprague-Dawley (SD) rats.
Results: The mAb of GNX1021 showed specific binding to bLeB/Y glycans without cross-reactivity to other types of glycans in ELISA. Among the positive-stained solid tumors, bLeB/Y showed high prevalence in 50% to 60% of overall gastric tumors tested, whereas HER2 had a limited expression in less than 10% of the same cohorts. *In vitro* cytotoxicity and internalization of GNX1021 was positively correlated with target expression in gastric cancer cells. Moreover, single administration of GNX1021 at low doses exhibited significant *in vivo* tumor burden reduction activity in high bLeB/Y-expressing gastric models, with sustained efficacy to the study end. The intratumoral activation of cleaved caspase-3 and down-regulation of Ki67 was observed along with tumor control as expected. Furthermore, hematotoxicity of GNX1021 was not detected with weekly administration at a preclinically efficacious low dose over 4 weeks. A mild reduction in neutrophils and reticulocytes was noted as the weekly dose increased to more than the double, while no detectable lesion in bone marrow was correlated with this observation. No obvious sign of hepatic and renal toxicity was identified. In general, GNX1021 was well tolerated in SD rats.
Conclusions: GNX1021, a novel glycan-targeting ADC to branched Lewis B/Y, showed promising preclinical efficacy in gastric cancer models and favorable safety profile in SD rats. Through targeting such a unique glycan, GNX1021 demonstrated strong potential to address gastric cancer patients' unmet medical needs for novel targets.

#2933 IDP-001 is a potent bispecific ADC with in vivo activity against mouse xenograft tumor models expressing EGFR and CDCP1.

Hayley Ma[1], Sophia Romero[1], Anna de Regt[1], Clare Malone[2], Ben Setter[1], Brian Woodruff[1], Hengyu Xu[1], Rob Oslund[2], Niyi Fadeyi[2], Heath Klock[2], Ertan Eryilmaz[2], Pamela Holland[1], Scott Lesley[1]

[1]InduPro, Seattle, WA,[2]InduPro, Cambridge, MA

EGFR is a clinically-validated target that is overexpressed and activated in many human cancers and associated with poor prognosis. However, ADCs targeting EGFR exhibit toxicity due to expression on healthy tissue (1), and EGFR can show heterogeneous tumor expression (2). We utilized InduPro's proprietary MIntTM platform to identify proteins inherently proximal to EGFR on tumor cells. CUB domain-containing protein 1 (CDCP1) was identified as a TAPA (tumor associated proximity antigen) of interest. Like EGFR, CDCP1 is over-expressed in multiple cancers and plays key roles in oncogenic signaling and malignant transformation (3).

IDP-001 is a novel MMAE-conjugated EGFR-CDCP1 bispecific antibody with low DAR. Here we report the preclinical in vivo assessment of IDP-001 in established tumor cell line-derived xenograft (CDX) models representing a range of target expression (measured by flow cytometry and immunohistochemistry) across multiple solid tumor indications. We also generated isogenic pairs of tumor cell lines with CDCP1 knocked out. IDP-001, along with versions of varying EGFR affinity, or monospecific derivatives thereof, were administered I.V. at a single dose in tumor-bearing mice.

Unlike other EGFR ADCs, IDP-001 does not engage immune effector cells and has a serum half-life of ~16 hours in mice. It is well tolerated at all doses evaluated, with no signs of body weight loss. Robust, dose-dependent anti-tumor activity was observed in tumor models with dual-target expression, regardless of expression level. Activity is reduced but not absent in CDCP1 knockout tumors and mice treated with EGFR or CDCP1 monospecific ADC controls, demonstrating more selective anti-tumor activity through co-binding. The activity of IDP-001 was also evaluated in patient-derived xenograft (PDX) models of squamous lung carcinoma, where a high degree of target co-expression is observed in clinical samples. IDP-001 is a first-in-class bispecific ADC with the potential for broad therapeutic activity and best-in-class efficacy. These encouraging in vivo results support advancement of IDP-001 to non-human primate studies.

References

1. Dan-yun Ruan et al., JCO 42 (2024) 6013-6013.

2. Passaro A et al ESMO open vol. 5,5 (2020) e000919.

3. Khan T et al., Cancer Res 81 (2021) 2259-2978.

#2934 Novel hydrophilic CDH17-targeting antibody-drug conjugate exhibits anti-tumor efficacy in preclinical xenograft models.

Yang Ye, Yurong Qin, Xingxing Fang, Xiaoya Liu, Wanting Wang, Tingting Li, Chengze Ni, Chao Chu, Mi Ye, Guojin Wu, Jianming Sun, Yue Zhao, Xiao Huang, Jordan Zhu, Shoupeng Lai, Xiaoqiang Kang, **Hong Ling**

Nanjing Leadsbiolabs Co., Ltd., Nanjing, China

Background: CDH17 is a member of the cadherin superfamily, a group of calcium-dependent cell adhesion molecules critical for organ development, tissue integrity, and cancer progression. In normal tissues, CDH17 is highly restricted to the lateral membrane and hidden within intestinal tight junctions which is inaccessible. In contrast, it is overexpressed and redistributed in 50% to 90% of gastrointestinal cancers, leading to its exposure on the cancer cell surface, therefore becoming more accessible. This unique feature makes CDH17 a promising target for antibody-based therapies. Here, we describe the preclinical development of LBL-054, a novel CDH17-targeting antibody drug conjugate (ADC) that is developed from a humanized CDH17 IgG1 monoclonal antibody conjugated with our proprietary linker-payload platform.

Methods: LBL-054 targeting CDH17 monoclonal antibody was generated using mouse hybridoma technology. The binding affinity of mAb to CDH17 was determined using Fortebio and flow cytometry. The mAb internalization was assessed with anti-Fc-MMAE method. The internalization of both the mAb itself and LBL-054 was assessed by FACS method. Binding of LBL-054 to CDH17 was confirmed in human cancer cell lines and cells engineered to overexpress CDH17. Furthermore, plasma stability of LBL-054 was assessed with LC-MS methods. The killing activity of cancer cells was evaluated on CDH17 positive, negative and the mixed cancer cells. Finally, the anti-tumor effects of LBL-054 were evaluated in xenograft mouse tumor models with pharmacokinetics studies.

Results: LBL-054 exhibited proper affinity, and rapid internalization. In the killing assays with CDH17 positive cancer cells LBL-054 was more potent than LBL-054-Dxd conjugate while LBL-054 was less prone than LBL-054-Dxd conjugate to kill CDH17 negative cells. And also, LBL-054 exhibited stronger bystander effect than LBL-054-Dxd conjugate. LBL-054 was highly stable in plasma stability test. A single dose of LBL-054, which induced tumor regression, was more potent than LBL-054-Dxd conjugate in the xenograft models, and better PK than LBL-054-Dxd conjugate as well.

Conclusions: By conjugating with high potent/bystander/non-pgp substrate TOP1i payload exatecan with a cleavable/hydrophilic linker, LBL-054 targeting CDH17 demonstrates high stability in plasma, robust anti-tumor activity and good tolerability in the xenograft models, supporting clinical development of LBL-054 for patients with CDH17-expressing tumors.

#2935 Development of selective HER2 covalent inhibitors with brain penetration for effective treatment of CNS metastases.

Hyunjin Park, Yu-Yon Kim, Jiyoung Jeon, Sun Young Jang, Young Gil Ahn, Haemin Chon

Hanmi Pharmaceutical Co., Ltd., Hwaseong-si, Korea, Republic of

HER2 alterations, including amplification, overexpression, and mutations, are implicated in the development and progression of brain metastases (BM) and leptomeningeal metastases (LM) across various cancer types. In breast cancer, BM occurs in 15-25% of patients, with up to 50% of HER2-positive cases developing BM, underscoring the importance of HER2 amplification in metastatic progression. Similarly, HER2 alterations are positively associated with a higher risk of LM, affecting up to 25% of HER2-positive metastatic breast cancer patients. Although HER2 alterations are less common in lung cancer and melanoma, the high incidence of BM (20-40% in lung cancer and up to 50% in melanoma) and LM (3-5% in lung cancer and up to 23% in melanoma) highlights the importance of targeting HER2-driven pathways to manage these aggressive metastatic complications. The role of HER2 mutation and amplification in promoting metastatic progression establishes its significance as both a biomarker and target for tailored therapies. BM and LM are associated with poor prognosis and significantly reduced overall survival. Therefore, effective management of BM and LM requires addressing HER2-driven mechanisms, highlighting the unmet need for selective HER2 inhibitors with brain-penetrating properties capable of targeting both HER2 mutants and HER2WT amplification in HER2-altered BM and LM. Herein, we report the development of potent and selective covalent HER2 inhibitors, the Hanmi compounds, which exhibit strong anti-tumor activity in BM and LM models with high selectivity over EGFRWT. The growth-inhibitory effects of these compounds were evaluated in vitro using cell lines harboring HER2G776insVG/C mutations and HER2WT amplifications. Additionally, their anti-tumor efficacy was assessed in BM and LM mouse models. Hanmi compounds significantly reduced tumor burden and markedly improved survival in mice intracranially implanted with HER2WT-amplified N87 cells. Moreover, in the H1781 LM model harboring HER2G776insVG/C mutations, these compounds demonstrated superior efficacy by significantly increasing median overall survival (mOS) and inhibiting tumor growth compared to the approved HER2 targeting agent. These findings highlight the potential of Hanmi compounds as effective treatments for central nervous system (CNS) metastases, including BM and LM, addressing a critical unmet need in the management of HER2-altered metastatic diseases.

#2936 Preclinical characterization of XB371, a novel anti-tissue factor antibody-drug conjugate.

Kathleen Gogas[1], Brian A. Mendelsohn[1], Marlene Hennessy[1], Hui Zhao[1], Jeffrey Higaki[1], Maxine Bauzon[2], Fangjiu Zhang[2], Stepan Chuprakov[2], Robyn Barfield[2], Penelope Drake[2], Seema Kantak[1]

[1]Exelixis, Alameda, CA,[2]Catalent Pharma Solutions, Emeryville, CA

Background: Tissue factor (TF) is a transmembrane glycoprotein that plays an important role in the extrinsic coagulation cascade. TF is aberrantly expressed in various cancers, and its expression is generally associated with poor disease outcomes. XB371 is an anti-TF antibody-drug conjugate, developed using the SMARTag® platform, and is designed to deliver a cytotoxic payload to TF-expressing tumors while minimizing adverse events related to the disruption of TF function, notably bleeding. XB371 is composed of a tandem-cleavage topoisomerase inhibitor-based linker-payload conjugated to a monoclonal antibody that binds to TF with high affinity and does not interfere with the clotting cascade.1 Here, we describe the preclinical characterization of XB371, including its in vitro cytotoxicity, bystander, and immunogenic cell death (ICD) activity, and in vivo efficacy in cell line-derived xenograft (CDX) and patient-derived xenograft (PDX) models.

Methods: In vitro cytotoxicity of XB371 was evaluated in HPAF-II, BxPC3, and A431 cells using a luminescence assay. To evaluate bystander effect, the potency and maximal effect of XB371 on the viability of non-TF-expressing Jurkat cells were compared for a mono-culture of Jurkat cells and co-cultures with TF-expressing MDA-MB-231 cells. ICD activity was assessed by measuring immunostimulatory molecule release (HMGB1) in A431 cells, and monocyte activation and cytokine release in tumor cell/PBMC co-cultures following XB371 treatment. Tumor suppression was evaluated in HPAF-II, BxPC3, and A431 CDX models using a single dose level or a range of doses of XB371. Tumor growth inhibition was assessed in colorectal cancer (CRC) and non-small cell lung cancer (NSCLC) PDX models using a single dose of XB371 (10 mg/kg intravenously).

Results: XB371 displayed potent in vitro cytotoxic activity at subnanomolar concentrations. XB371 demonstrated in vitro bystander cell-killing effect and induced ICD in all assays evaluated. In vivo anti-tumor activity was observed following a single intravenous dose in the HPAF-II, BxPC3, and A431 xenograft models. In the HPAF-II CDX model, XB371 demonstrated dose-dependent anti-tumor activity, with significant increases in median survival and tumor volume quadrupling time at higher doses. A single dose of XB371 inhibited tumor growth in both CRC and NSCLC PDX models. Tumor regression and complete responses were observed in the NSCLC PDX model.

Conclusions: XB371 demonstrated in vitro cytotoxicity, bystander, and ICD activity, and in vivo efficacy across multiple xenograft cell lines and PDX models. Taken together, these preclinical results support further development of XB371; IND-enabling studies are ongoing.

Reference: 1. Theunissen JW, et al, Mol Cancer Ther. 2018;17:2412-2426.

#2937 Animal models for evaluating the efficacy of anti-human HER2 ADC alone or in combination with IO therapy.

Jean Huang, Chong Li, Ruili Lv, Qinjun Wang, Qingqing Xu, Heling Shen, Xiaofei Zhou

Biocytogen, Waltham, MA

Antibody-drug conjugates (ADCs) combine the targeting ability of antibodies with the killing power of cytotoxins, allowing for the release of highly toxic payloads within tumor cells, thereby enhancing therapeutic efficacy while reducing systemic toxicity. ADCs have demonstrated significant efficacy as monotherapy in the treatment of various tumors. However, like many cytotoxic agents, the issue of drug resistance limits the sustained efficacy they can achieve as standalone treatments. To overcome this challenge, the combined application of ADCs with other therapies is becoming a new focus of research. Among these, the combination of immuno-oncology inhibitors (IO) with ADCs has shown strong therapeutic potential in clinical settings. An appropriate preclinical evaluation model can effectively assess the therapeutic potential of ADCs.

Biocytogen developed a HER2-expressing MC38 cell line called B-hHER2 MC38 plus. The HER2 expression level of B-hHER2 MC38 plus is similar to that of the human cell line NCI-N87, and it can form tumors normally in B-hHER2 mice. In a syngeneic transplantation model of B-hHER2 mice with MC38 plus cells, the anti-HER2 antibody-drug conjugate (ADC) analogous to trastuzumab, MMAE, exhibited a dose-dependent tumor suppression effect. When combined with the anti-mPD-1 antibody, it demonstrated a higher tumor suppression rate. In another syngeneic transplantation model where B-hHER2 MC38 plus cells were inoculated into B-h4-1BB/hHER2 mice, the commercial product DS8201 showed significant tumor growth inhibition. In the xenograft models using a diverse array of human tumor tissues - including breast cancer tumor tissues (BP0818 and BP1056), colorectal cancer tumor tissues (BP0170 and BP0847), lung cancer tumor tissues (BP0508 and BP0638), as well as breast cancer cell lines (BT-474 and MDA-MB-231), and the gastric cancer cell line (NCI-N87) - the commercial product DS8201 demonstrated significant tumor growth inhibition.

#2938 Avaren-Fc: a novel lectin-Fc fusion protein for treatment of ovarian cancer.

Katarina Mayer[1], Noel Verjan-Garcia[2], Nobuyuki Matoba[3]

[1]Pharmacology and Toxicology, University of Louisville, Louisville, KY,[2]Center for Preventative Medicine, University of Louisville, Louisville, KY,[3]Pharmacology and Toxicology, Center for Preventative Medicine, and Brown Cancer Center, University of Louisville, Louisville, KY

Ovarian cancer (OVCA) is the deadliest gynecological malignancy and the fifth leading cause of cancer death in females in the United States. Despite advancements in the treatment options, current therapies lack specificity for tumor cells and fail to generate a robust anti-tumor immune response. In this study, we use a novel lectin-Fc fusion protein, named Avaren-Fc (AvFc), to target the abnormal, increased density of *N*-linked high-mannose-type glycans on the OVCA cell surface. Upon binding to clusters of these glycans on malignant cells, AvFc is postulated to induce antibody-dependent cell-mediated cytotoxicity (ADCC) by engaging Fc-bearing effector immune cells, such as natural killer cells. This study explores AvFc's therapeutic potential against OVCA utilizing *in vitro* experiments and *in vivo* evaluations using the syngeneic ID8 OVCA mouse model. AvFc's effects were evaluated utilizing flow cytometry and an *in vitro* luciferase-based ADCC reporter assay using a FcγRIIIa-expressing cell line, where AvFc exhibited dose-dependent binding and demonstrated a variable, yet significant degree of ADCC to four human (A2780, CAOV3, SKOV3, and SW626) and two murine (ID8 and ID8-VEGF-Def2b) OVCA cell lines. The antitumor effects of AvFc were evaluated in the ID8 OVCA model, where mice were intraperitoneally (i.p.) challenged with 2 million ID8 cells and 7 days later AvFc treatment was administered at 25 mg/kg i.p. every other day, for a total of 15 doses. The survival analysis revealed that, as of 28 days post-treatment, 75% of AvFc-treated animals remain alive, in contrast to 40% in the mutant AvFc control group and 0% in the vehicle control group. To assess the impact of this 15-dose regimen of AvFc on the tumor immune microenvironment in the ID8 OVCA model, immune cell profiling was performed at 36 days post-tumor challenge using cytometry by time-of-flight (CyTOF) on both the peritoneal fluid and spleen. CyTOF analysis revealed that AvFc treatment significantly increased total leukocytes ($p<0.05$; one way ANOVA with Tukey's multiple comparisons) including $TCR\gamma\delta^+$, $TCR\beta^+$, and $TCR\beta^+CD4^+$ T cells in the spleen compared to all other treatment groups. Additionally, AvFc treatment significantly increased $CD8^+$ T cells expressing the degranulation marker CD107a in the peritoneal fluid ($p<0.05$) relative to vehicle and healthy controls, suggesting that AvFc treatment induced a significant adaptive immune response against the murine ID8 OVCA challenge. These findings highlight AvFc's promising efficacy and immunostimulatory activity, supporting its potential as a novel targeted immunotherapeutic candidate for the treatment of OVCA.

#2939 The effect of cSNX1.3 on MMTV-PyMT tumor growth and metastasis.

Delina J. Denogean, Barbara Sands, Joyce Schroeder

Molecular and Cellular Biology, University of Arizona, Tuscon, AZ

The Epidermal Growth Factor Receptor (EGFR) belongs to a group of Receptor Tyrosine Kinases (RTKs) that are a part of the HER family. EGFR mediates many processes in the cell including proliferation, migration, and survival however its overexpression is correlated with poor prognosis in several epithelial cancers including Triple Negative Breast Cancer (TNBC). In healthy cells, EGF binds to EGFR dimerizes and undergoes transphosphorylation, and can then be intracellularly imported where it is taken to the early endosome and awaits ubiquitination. This signals readiness to be taken to the lysosome where it undergoes degradation or in some cases, gets recycled. In cancerous cells, however, ubiquitination gets inhibited due to their loss of polarity. This results in failure to signify readiness to be taken to the lysosome which allows EGFR to completely evade degradation and undergo nuclear localization. This process is initiated by the binding of Sorting Nexin 1 (SNX1), a protein responsible for the regulation of trafficking, to EGFR. Considering the interaction between Sorting Nexins and RTKs causes oncogenic activity, it was hypothesized in this lab that a therapeutic that can modify this interaction can alter tumor-specific retrotranslocation. In order to target these interactions, the therapeutic peptide, cSNX1.3, was developed. cSNX1.3 is a cell-penetrating peptide with a Protein Transduction Domain that allows for the delivery of peptides across the cell membrane and into the intracellular domain. cSNX1.3 interacts with EGFR when it's in the long-lived endosome and binds in competition with SNX1. When cSNX1.3 is bound to EGFR, it is able to inhibit the nuclear localization of the RTK and as a result, inhibit its oncogenic activity. In order to fully evaluate the tumor-regressing abilities of cSNX1.3, we used an immunocompetent model of transgenic Whey Acidic Protein - Transforming Growth Factor alpha (WAP-TGFα) mice, whose mammary gland tumors are EGFR dependent. These mice were treated with cSNX1.3 or the control drug, cPTD4, over the course of 4 weeks. After completion of treatment, it was determined that the tumors of the cPTD4-treated mice continued to grow whereas the tumors of the cSNX1.3-treated mice began to regress. We then decided to construct an additional study using another immunocompetent mouse model that would also demonstrate how cancer metastasis reacted to cSNX1.3 treatment, given that metastasis is typically what makes breast cancer fatal in humans. For these reasons, we decided to use a model of transgenic Mouse Mammary Tumor Virus - Polyoma Middle T (MMTV-PyMT) mice. Conducting an evaluation of cSNX1.3 using this model that closely resembles the function of metastatic breast cancer in humans, will allow us to better understand the future of cSNX1.3 as a breast cancer therapeutic for human applications.

#2940 Preclinical development of a next generation antibody drug conjugate (ADC) targeting CDH17 for treatment of solid tumors.

Wei Lian, Qing Zong, Chun Deng, Peng Zou, Liang Xiao, Tongtong Xue, Jiaqiang Cai

MediLink Therapeutics (Suzhou) Co., Ltd., Suzhou, China

Cadherin-17 (CDH17) is a membranous cell adhesion protein predominantly expressed in intestinal epithelial cells. High CDH17 expression is associated with metastatic progression in different cancers that mainly originate from gastrointestinal tracts. YL217 is a novel CDH17 -targeting antibody-drug conjugate (ADC) and built on MediLink's TMALIN® platform, which can release the payload both in the tumor cell and in the tumor microenvironment. This study evaluated efficacy, safety and pharmacokinetic profiles of YL217 in non-clinical studies. YL217 could specifically bind to CDH17 on tumor cell surfaces, followed with payload release both in the lysosome through internalization and in the tumor microenvironment. These translate *in vivo* where YL217 showed superior efficacy in causing tumor regressions and good tolerability in both cell lines and patient-derived xenograft (CDX and PDX) mouse models with low to high CDH17 expression levels. The *in vivo* pharmacokinetics in monkeys showed that YL217 is highly stable with less than 0.1% (molar ratio) payload released in circulation. These non-clinical data suggest the high stability of the linker in circulation as well as efficient payload release in tumors. Moreover, acceptable safety profiles were also noted in monkey GLP toxicity studies. Taken together, preclinical data suggest that YL217 could be further developed in the treatment of CDH17-positive cancer patients.

#2941 Physiologic epidermal growth factor modeling improves sensitivity of KRAS^{G12D}inhibitor MRTX1133.

Lauryn Elise Flannagan[1], Michela Cadarso[1], Eleanor Riedl[2], Md Shahadat Hossan[1], Ethan S. Lin[2], Jeremy D. Kratz[1]

[1]School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI,[2]University of Wisconsin-Madison, Madison, WI

Background: Pancreatic Ductal Adenocarcinoma (PDAC) remains a formidable cancer type with a median 5-year survival rate of 13%, largely due to ineffective systemic therapy. MRTX1133 is an emerging small molecule inhibitor of KRAS^{G12D} which has been shown to be effective across multiple treatment settings. Epidermal growth factor (EGF) is often integrated at supraphysiologic levels in models of luminal cancer types. Here, we evaluate KRAS^{G12D} targeting as a function of EGF supplementation to understand both sensitivity and selectivity across EGFR signaling.

Methods: Organoids were seeded in low volume 10uL 96 well plate (Ibidi, Inc) with a density of 500 cells per microliter across experimental groups. Organoids were treated with MRTX1133 in serial dilutions with either standard EGF concentrations (50 ug/mL) or physiological (0.5 ug/mL). Brightfield imaging was captured using time course data from 0h to 144h via Cytation 5. Viability was assessed 144 hours post treatment via Cell-Titer Glo assay, to calculate IC50 of MRTX1133 (n= 8). Response to MRTX1133 was tracked via effect size Glass's Delta, which is defined by difference between the mean of control and treatment group normalized to the standard deviation of the control. Protein analysis was performed by western blot for MAP kinase effector signaling.

Results: Removal of EGF in media lead to growth arrest over the course of 5 weeks (+17.8% v. + 120% in control; GΔ = 0.64). Indicating that its addition is required for proper organoid propagation. When established across 5 weeks, physiologic levels of EGF in cultures did not impact the growth of KRAS^{G12D} pancreatic organoids (+101.3% v. 102% in control; GΔ = 0.005). However, the use of physiologic EGF had improved sensitivity to MRTX1133 (IC_{50} 0.46± 11.03 nM) when compared to standard media (IC_{50}=33.6±3.40 nM), which shows a 60-fold decrease in sensitivity. At lower concentrations of MRTX1133 (0.5nM), resistance was observed in standard EGF conditions (+133.8% v. +106.4% in control; GΔ = 0.437), in comparison to physiological EGF media which reduced proliferation (+55.8% v. +85.7% in control; GΔ= -0.47). Western blot analysis revealed few changes between standard media conditions and physiologic when probing for effectors in Ras signaling, such as AKT and EGFR. Treatment of MRTX1133 (10nM) over 72 hours, also did not yield improved inhibition at either media conditions.

Conclusion: Physiologic EGF stimulation yielded improved sensitivity to MRTX1133 in a manner independent of organoid growth rate. While resistance to MRTX1133 can be observed in organoid models, understanding this as a function of accurate EGF stimulation remains a critical need particularly at informing relevant resistance mechanisms. Future work includes evaluating this across a diversity of KRAS^{G12D} cancer models and comparing physiologic EGF across therapeutic screening applications in combination.

#2942 Efficacy of a claudin 18.2/TGFβ bi-specific CAR-T in preclinical mouse models of gastric cancer.

Ralph F. Fleuranvil[1], Naryan Rustgi[1], Michael James[1], Zachary Hammer[1], Thomas J. Ryan[1], Jessica Reyes[2], Melanie L. Munguiam[2], Orit Foord[2], Jiajia Cui[2], Sandra Ryeom[1]

[1]Columbia University Irving Medical Center, New York, NY,[2]ImmPACT Bio, West Hills, CA

Gastric cancer (GC) is one of the 4th leading causes of cancer-related deaths worldwide. For decades, chemotherapy and surgery has been the primary treatment modality, however, the overall five-year survival rate for GC remains at 36% and new therapies are needed. Claudin 18.2, a tight junction protein that is expressed on the GC cell surface, has been validated as a bona fide target in GC with recent FDA approval of zolbetuximab, a Claudin 18.2 directed antibody. Chimeric antigen receptor (CAR)-T cell therapies are human T cells modified to express synthetic engineered receptors designed to target and eliminate cells expressing specific antigens. However, many barriers limit the therapeutic potential of CAR T cells in solid tumors. Our study examined the anti-tumor function of a bispecific Claudin18.2/TGFβ-targeting CAR T-cell candidate in preclinical orthotopic mouse models of GC in an immune competent background, as well as in GC patient-derived organoids (PDO) in an immunodeficient orthotopic mouse model. Our data indicate prolonged tumor suppression in our PDO GC model as well as in our murine gastric tumor-bearing mice treated with Claudin 18.2/TGFβ CAR T cells relative to mice treated with untransduced control T cells. We also demonstrate remodeling of the tumor immune microenvironment in our GC model in an immune competent background with an increase in CD19+ B cells in mice treated with our bispecific Claudin18.2/TGFβ CAR-T versus mice treated with untransduced T cells. Our data suggest that a bi-specific claudin18.2/TGFβ CAR T cell candidate may be an effective therapeutic approach for the treatment of gastric tumors that express Claudin 18.2.

#2943 SWY2321, a novel topoisomerase I inhibitor linked bispecific ADC targeting EGFR/cMet, exhibits potent anti-tumor efficacy.

J. Yang[1], J. Qiao[2], M. Dan[1], **X. Hui**[2], B. Yao[2], C. Yuan[2], M. Wei[1], X. Li[1], X. Lv[1];

[1]CSPC Pharmaceutical Group Ltd., Beijing, China, [2]CSPC Megalith Biopharmaceutical Co., Ltd., Shijiazhuang, China

EGFR and cMet are two important clinically validated targets in cancer therapy. They exhibit overexpression across various cancer types with a poor prognosis. The signaling pathways of EGFR and cMet demonstrate intricate interconnections, with evidence suggesting that activation of one pathway can trigger the other. These interactions contribute to augmented cell proliferation, survival, invasion, ultimately tumor growth as well as therapeutic resistance. Consequently, targeting both EGFR and cMet has the potential to improve treatment in patients with solid tumors characterized by EGFR and/or cMet dysregulated pathways. SWY2321 is comprised of a bispecific antibody against EGFR/cMet, a cathepsin B cleavable linker, and a novel topoisomerase I inhibitor agent. The ADC was constructed on the backbone of the clinically validated monovalent bispecific IgG platform and was engineered with fine-tuned affinity on EGFR and cMet, with the aim of enhancing tumor inhibition capacity while reducing EGFR and/or cMet-driven toxicity in normal tissues. SWY2321 demonstrated in vitro binding to all the tested cancer cell lines with various expression of EGFR and cMet, and showed higher internalization and cytotoxicity on the tumor cells co-expressing EGFR and cMet than single targeted ADC or first in class ADC. Furthermore, SWY2321 had potent efficacies on the tumor xenograft models of different malignant types including non-small cell lung cancer (NSCLC), gastric cancer (GC), cutaneous squamous cell cancer (CSCC), and with various expression levels of these two targets, including $EGFR^{High}cMet^{Low}$, $EGFR^{Low}cMet^{High}$, $EGFR^{moderate}cMet^{moderate}$ and $EGFR^{Low}cMet^{Low}$. The tumor inhibition efficacies of SWY2321 on these models were superior to first in class ADC, and superior to or equivalent to single-targeted ADCs given at the same dose with SWY2321. Especially in EGFR/cMet co-expressed NSCLC NCI-H1975 tumor model, SWY2321 showed excellent efficacy at 1.5 mg/kg single administration. In summary, the promising efficacy of SWY2321 supports that SWY2321 can be an effective treatment targeting multiple EGFR- or cMet- expressing solid tumors.

#2944 Synergistic lethality of combination treatment with Trop2-directed antibody-drug conjugate (IMMU-132) and Apo2L/TRAIL in triple negative breast cancer (TNBC).

Y. A. Addissie, M. Kundu, Y. E. Greer, S. Lipkowitz;
National Cancer Institute, Bethesda, MD

Background: Sacituzumab govitecan (IMMU-132) is an antibody drug conjugate targeting trophoblastic cell surface antigen 2 (Trop2) that's approved for treatment of patients with metastatic TNBC who have received two or more prior systemic therapies. The cytotoxic payload of IMMU-132 is a topoisomerase I inhibitor (SN-38) that kills cancer cells by causing DNA damage. Efforts to enhance the efficacy of IMMU-132 treatment include combination therapy with other agents. Apo2L/TRAIL interacts with death receptors on the cell surface to induce apoptosis in cancer cells, sparing normal cells. We previously have shown that TNBC is most sensitive to Apo2L/TRAIL. We investigated whether combination treatment with IMMU-132 and Apo2L/TRAIL synergistically inhibit TNBC cell growth.

Methods: Human TNBC cell lines treated *in vitro* with IMMU-132 and Apo2L were assessed for cell viability via a propidium iodide-based cell death assay and ATP cell viability assay. The mode of cell death elicited by combination treatment was also investigated by using inhibitors of apoptosis, necroptosis and ferroptosis. Effects of treatment on cell cycle arrest were explored using flow cytometry. *In vivo,* NCr athymic nude (nu/nu) female mice with HCC1806 TNBC xenografts were treated with IMMU-132 and Apo2L after which tumor growth and survival were monitored.

Results: Combination treatment with IMMU-132 and Apo2L synergistically induced cell death in triple negative breast cancer cell lines with high Trop2 expression (e.g. HCC1806, MDA-MB-468) but did not in the Trop2 low expressing MDA-MB-231 cell line. In some Trop2 low TNBC cell lines (e.g. BT-549, SUM-159), combination treatment also showed synergistic induction of cell death, suggesting extracellular deconjugation of the SN-38 cytotoxic payload and diffusion into cells. The synergy seen in Trop2 low TNBC cell lines was abrogated by limiting drug incubation periods - which minimizes the extracellular deconjugation. In contrast, the synergy seen in Trop2 high cells was maintained in these short incubation assays. Caspase activation assays and viability assays with caspase inhibitors showed that the primary mechanism of cell death for Apo2L and combination treatment with IMMU-132 is through apoptosis. Furthermore, flow cytometry-based cell cycle assays showed that IMMU-132 and combination treatment with Apo2L induce cell cycle arrest at the G2/M stage. A xenograft model investigating the effect combination treatment *in vivo* is ongoing.

Conclusions: The data suggest a synergistic effect of combination treatment with Trop2 antibody drug conjugate IMMU-132 and Apo2L/TRAIL in killing TNBC cells.

#2945 PLB-002 is a novel Claudin 6 antibody-drug conjugate for ovarian cancer and testicular germ cell cancer.

Haitao Pan*[1], Qing Zhang*[1], Ganyuan Xiao*[2], Guangchao Zhang[2], Jiaxin Li[3], Ling Xin[2], **Kia J. Puan**[1], Ling Xu[3], Yingdong Lu[2], Mao Yin[4]

[1]Biology, PrimeLink BioTherapeutics, Suzhou, China,[2]Chemistry, PrimeLink BioTherapeutics, Suzhou, China,[3]Analytical, PrimeLink BioTherapeutics, Suzhou, China,[4]R&D, PrimeLink BioTherapeutics, Suzhou, China

Background: Claudin 6 (CLDN6) is a clinically validated target for many solid tumors notably ovarian, endometrial, and testicular cancer. Its expression is restricted to tumor cells and fetal tissues with little or no detectable in normal tissues. As such, CLDN6 represents an attractive therapeutic target for the development of an antibody drug conjugate (ADC). Here, we describe the development and preclinical characterization of a novel ADC called PLB-002, which consists of a highly selective CLDN6 targeting antibody, PLB-002-ab7, conjugated to a FDA-approved microtubule inhibitor, eribulin, via a Primelink Biotherapeutics proprietary enzyme-cleavable linker with an optimized average drug-to-antibody ratio (DAR) of 4.0. PLB-002-ab7 is a novel humanized anti-CLDN6 single-domain antibody (Mw. 78.8 kDa) with highly binding affinity and selectivity to CLDN6 with barely binding to related CLDN family members (CLDN9, CLDN3, and CLDN4) in protein and cell based binding assays.
Methods: The in vitro binding affinity of the PLB-002 was determined by flow cytometry on OVCAR3, PA-1, OVCA429, OV90, and NEC-8 cells. Cell internalization effect was evaluated by an in-direct flow cytometry assay on OVCAR3, PA-1, and OV90 cells. In vitro cytotoxicity was measured using Cell-Titer Glo assay on OVCAR3, OV90, and NEC-8 cells. In vivo anti-tumor efficacy was investigated using several cancer cell derived xenograft (CDX) models of OVCAR3, OV90, and NEC-8 cells, as well as patient derived xenograft (PDX) models of ovarian cancer with CLDN6 high, moderate, low and negative expression. A dose-range finding (DRF) safety study of PLB-002 was performed in cynomolgus monkeys. Pharmacokinetics (PK) and pharmacodynamics (PD) data of PLB-002 in CDX mice were determined by LC-MS/MS method.
Results: PLB-002 exhibited strong binding, rapid internalization effect, and potent antitumor activity in vitro on OV90 (CLDN6 low expression) and OVCAR3 (CLDN6 high expression) cells with nano-molar range of IC50. When evaluated in OVCAR3 and OV90 CDXs of ovarian cancer, NEC-8 CDX of testicular germ cell tumor, and OV PDXs, PLB-002 showed strong tumor regression in a dose-dependent manner, which is consistent with the PK analyses showing high exposure, slow clearance and long half-life for both total antibody and conjugated drug. In a non-human primate study using cynomolgus monkey, PLB-002 showed favorable safety profile with no clinical symptoms of toxicities up to the highest tested dose.
Conclusions: These results show that PLB-002 has remarkable antitumor activity and support the clinical development as a therapeutic ADC for the treatment of ovarian cancer and other CLDN6 expressing solid cancer.
*, Equal contributions.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Growth Factor Receptors and Other Surface Antigens as Targets for Therapy 2
Poster Session

#2949 LX-101, a novel, clinical stage, payload-bearing targeted therapy directed to the insulin-like growth factor receptor (IGF-1R), demonstrates potent preclinical anti-tumor activity against multiple cancer types with elevated IGF-1R expression.

Matt Hoberman

Lirum Therapeutics, Inc., New York, NY

Objective: LX-101 is a next-generation, targeted therapy directed to IGF-1R. LX-101 consists of a proprietary IGF-1 variant coupled to a cytotoxic methotrexate payload. LX-101 was previously evaluated in Phase 1 trials of adult patients with advanced, pretreated cancers, where it was well-tolerated and demonstrated single agent activity. Neither a dose limiting toxicity, nor a maximum tolerated dose was reached, leaving potential room for additional dose and schedule optimization. Multiple aggressive cancers of unmet need harbor alterations affecting the IGF-1R pathway, including gene fusions and/or high IGF-1R expression, that correlate with poor outcomes, including certain subsets of cancers of the breast, head and neck, esophagus, stomach, and lung, as well as Ewing's sarcoma, osteosarcoma, and others. Prior attempts at targeting IGF-1R with non-payload bearing naked monoclonal antibodies or small molecules produced a range of clinical outcomes including some partial and complete responses. None of these agents, however, were ultimately approved, potentially due to redundant signaling, escape pathways, and/or the lack of enrichment for select tumor types with strong ties to the IGF-1/IGF-1R pathway. Herein, we investigated the anti-tumor activity of the payload-bearing agent LX-101 against IGF-1R-expressing cancer cell lines to overcome these past shortcomings.
Methods: Cell lines known to highly express IGF-1R were incubated with LX-101 (~1.6 - 2500nM). Cell viability was assessed by CellTiter-Glo after 4 days. IC_{50}s were calculated using GraphPad PRISM software.
Results: LX-101 displayed high levels of potency against lung cancer (A549, IC_{50} = 29 nM; NCI-H2122, IC_{50} = 10 nM; NCI-H526, IC_{50} = 23 nM), esophageal cancer (TE-1, IC_{50} = 16 nM; KYSE-70, IC_{50} = 7 nM), and stomach cancer (MKN74, IC_{50} = 19 nM; NUGC-4, IC_{50} = 30 nM).
Conclusion: These results demonstrate that LX-101 has potent preclinical anti-tumor activity against multiple cancer cell lines expressing IGF-1R. These data support further clinical development of LX-101 in IGF-1R-involved cancers. New clinical trials are planned.

#2950 Identification of sensitivity predictors and differentiators for HER2-directed ADCs.

Daphne J. F. Kluitmans, Jeffrey J. Kooijman, Janneke J. T. M. Melis, Sabine Wetemans, Jeroen A. D. M. de Roos, Mandy W. E. Drossaert-Smeets, Guido J. R. Zaman

Oncolines B.V., Oss, Netherlands

Antibody drug conjugates (ADCs) is a promising class of anti-cancer drugs that combines a monoclonal antibody with a small therapeutic agent for cell-specific delivery. With many ADCs in clinical trials and over 10 approvals by the U.S. Food and Drug Administration (FDA) since the beginning of this century, it is a rapidly expanding field. However, many clinical studies have been discontinued or terminated because of challenges such as low efficacy or off-target toxicity.

Trastuzumab emtansine (T-DM1, Kadcyla®) and trastuzumab deruxtecan (T-DXd, Enhertu®) are two ADCs approved for HER2-positive (HER2+) breast cancers that share the same anti-HER2 monoclonal antibody, but differ in their payload and linker chemistry. T-DM1 consists of trastuzumab and tubulin inhibitor emtansine (mertansine) connected by a non-cleavable linker, while T-DXd combines trastuzumab and topoisomerase I inhibitor deruxtecan with a cleavable linker. T-DXd is also approved for non-HER2+ breast cancers, which reflects that the ADCs have different effects on tumors even though they share the same antibody. Further insights into the cellular targeting of the two ADCs and their respective free payloads may help in better understanding important differences.

T-DXd and T-DM1 were profiled on 108 cancer cell lines in cell viability assays. Additionally, the free payloads, trastuzumab and the small molecule HER2 inhibitor tucatinib were profiled as single agents on the same cell lines. The cell line panel represents a broad range of tumor types including seven HER2+ cell lines. All cell lines were incubated with a 9-point dose range of each drug, followed by intracellular ATP readout to determine the cell viability. Half-maximum inhibitory concentrations (IC_{50}) were used for bioinformatic analyses to identify response predictors or differentiators based on genomic, transcriptomic and proteomic status of the cell lines.

Comparison of cell line responses between the ADCs and their free payloads showed a higher selectivity of the ADCs. Linking trastuzumab to deruxtecan or mertansine thus improves selectivity *in vitro*. Nonetheless, the ADCs targeted more cell lines than trastuzumab alone, showing that the ADCs also have non-antibody directed effects. Additionally, the HER2+ cell line SK-OV-3 showed striking resistance to T-DXd (IC_{50} > 10,000 ng/mL), while T-DM1 effectively targeted this cell line (IC_{50} = 393 ng/mL). Although a significant Pearson correlation between *ERBB2* (HER2) upregulation and sensitivity to T-DXd (r = -0.39) and T-DM1 (r = -0.63) was found, the results above show that HER2 status alone is not sufficient for response prediction of HER2-directed ADCs. Other sensitivity predictors were determined by bioinformatic analyses on resistant HER2+ and sensitive non-HER2+ cells. This study provides insights into sensitivity predictors and differentiators of the two HER2-directed ADCs and the small molecule HER2 inhibitor tucatinib.

#2951 Development of TNFR2, TNFR1 and LTBR humanized mouse model for evaluating anti-human LTBR antibodies.

Qiurong Wang, Chong Li, Zhixia Li, Zan Zhang, Jiansu Zhang

Biocytogen, Waltham, MA

The Lymphotoxin B receptor (LTBR) is primarily expressed on epithelial, stromal, and myeloid cells. LTBR signaling has diverse roles, including regulation of lymphoid organ development, induction of tertiary lymphoid structures (TLS), and promotion of liver regeneration. Recently, the therapeutic strategy of activating LTBR to induce and mature TLS has gained momentum, with LTBR-targeting antibodies now advancing into clinical trials. However, traditional preclinical models generally lack expression of human LTBR, which limits the progress of anti-human LTBR antibody development.

To address this, Biocytogen has developed a humanized mouse model expressing TNFR1, TNFR2, and LTBR through Biocytogen proprietary gene editing technology, known as B-hTNFR2/hTNFR1/hLTBR mice. In these mice, humanized TNFR1, TNFR2, and LTBR expression is detected in granulocytes, regulatory T cells, and macrophages, respectively. Testing of anti-human LTBR antibodies in this model has shown effective tumor growth control, underscoring the B-hTNFR2/hTNFR1/hLTBR mouse as a valuable preclinical platform for in vivo evaluation of anti-human LTBR antibodies.

#2952 The oncomucin MUC4 EGF domains: A new paradigm to target ErbB/HER and RTK receptors in pancreatic adenocarcinoma using inhibitory small molecules.

Tristan Girgin, Nicolas Stoup, Elsa Faillon, Maxime Liberelle, Nicolas Lebegue, Isabelle Van Seuningen

Inserm, Lille, France

Pancreatic adenocarcinoma is one of the deadliest cancers with an increasing incidence. It is mainly due to absence of diagnosis and no effective treatments, and a strong resistance to therapies. It is thus crucial for this devastating cancer to find new therapeutic alternatives. In this aim, we work on the interaction of the mucin MUC4 with receptor tyrosine kinases (RTK), and the targeting of MUC4 using small inhibitory molecules. MUC4, a large glycoprotein overexpressed at the surface of pancreatic cancer cells, is associated with poor prognosis of the tumor, increase of proliferation and migration as well as apoptosis and resistance to therapies. Moreover, we have shown that MUC4EGF domains interact with and upregulates the activity of ErbB2/HER2, this complex being a strong promoter of tumorigenesis. Use of small inhibitory molecules would circumvent the steric hindrance played by MUC4 glycosidic chains. We have now, using molecular dynamics and peptide library screening, identified a specific MUC4EGF domain inhibitory peptide (i) capable of inhibiting MUC4-ErbB2 complex formation and activity, (ii) having anti-proliferative activity and (ii) resensitizing pancreatic cancer cells to Trastuzumab and Pertuzumab ErbB2-targeted therapies.During these studies, we also observed that MUC4 interaction with ErbB2 led to the activation of the other members of the ErbB/HER family as well as other RTKs showing structural similarities with the ErbB/HER family. Our hypothesis is thus that MUC4 may play the role of an oncogenic interaction platform at the surface of pancreatic cancer cells. The interactions between MUC4 and the ErbB/HER family, Met, Axl, EpCAM, and IGF-1R were studied and characterized using Proximity Ligation Assay, MicroScale Thermophoresis, co-immunoprecipitation, GST pull-down. Our results show that MUC4 interacts directly via its EGF domains with and activate ErbB1,3,4, Met, Axl, IGF-1R and EpCAM. These interactions (i) are independent of ErbB2 expression and (ii) activate downstream oncogenic signaling pathways to maintain proliferation and migration capacities of pancreatic cancer cells. At this time, other drugs (tyrosine kinase inhibitors, mAbs) are currently being screened in association with our MUC4 inhibitory peptide to find the best therapeutic combination possible. Taken together, these results provide new paradigms on the inhibitory small molecule-based therapeutics targeting of ErbB/HER and RTK receptors as partners of the oncomucin MUC4 in pancreatic cancer but also in all epithelial cancers overexpressing these complexes.

#2953 CS5006: A novel integrin β4-targeted antibody-drug conjugate (ADC) with robust antitumor activity in preclinical studies.

Chuan Wang, Yamin Wang, Xinling Zhang, Ning Zhang, Yilin Huo, Yongwang Li, Xuelian Liu, Mengyao Zhu, Jingyu Sun, Yongli Yang, Yuxin Qian, Xueqin Dai, Fei Ma, Yi Sun, Jianxin Yang

CSTONE Pharmaceuticals, Suzhou, China

Background:The integrins are a family of cell surface receptors existing as heterodimers formed with one α subunit and one β subunit, and there are 24 heterodimers identified so far. These receptors play diverse roles in cellular adhesion, motility, and cytokinesis. Integrin β4 (ITGB4), also known as CD104, binds to integrin α6 (ITGA6) to form α6β4 integrin heterodimer that is recognized as a ligand of laminin and plays a critical role in strengthening and stabilizing the skin. The cytoplasmic tail of ITGB4 contains approximately 1000 amino acids which is longer than any other integrin β subunit. ITGB4 interacts with and plays important roles in multiple tumor-promoting signaling pathways including ErbB2, PI3K, FAK/AKT, and c-MET, and has been frequently reported as a potential prognostic and predictive marker in various cancers.
Methods & Results: Utilizing our proprietary AI-based target identification platform, ITGB4 was identified as a promising therapeutic target for multiple malignancies. Immunohistochemistry (IHC) staining confirmed high ITGB4 expression in tumor tissues from patients with colorectal carcinoma (CRC), non-small cell lung cancer (NSCLC), squamous carcinoma of head and neck (SCCHN) and esophageal squamous cell carcinoma (ESCC). CS5006, an ITGB4-targeted ADC, comprises a humanized anti-ITGB4 antibody, a proprietary CSL linker, and the well-validated topoisomerase 1 inhibitor exatecan (EXd). The plasma-stable, hydrophilic, beta-glucuronide CSL linker enables effectively tumor-selective toxin release. Both CS5006 and the humanized ITGB4-targeted antibody H86.2 exhibited selective binding to ITGB4 with high affinity, and triggered rapid, high-level internalization. *In vitro* cytotoxicity assays demonstrated CS5006's potent, target-dependent killing of ITGB4-expressing tumor cell lines. CS5006 also showed robust in vivo antitumor activity in CDX models originated from multiple cancer types, with minimal effective dose (MED) of 5 mg/kg. Pilot toxicity studies in non-human primate demonstrated a favorable safety and pharmacokinetics (PK) profile of CS5006.
Conclusion: CS5006 is a novel ITGB4-targeted ADC with robust preclinical efficacy and a favorable safety and PK profile. These findings support further IND-enabling and clinical evaluation of CS5006 as a promising ADC therapy for various advanced solid tumors.

#2954 CS5007: A novel EGFR and HER3 dual-targeted antibody-drug conjugate (ADC) with potent antitumor activity in preclinical studies.

Chuan Wang, Yamin Wang, Ning Zhang, Xinling Zhang, Yongwang Li, Yuxin Qian, Yongli Yang, Mengyao Zhu, Xuelian Liu, Yaxin Chen, Fei Ma, Yi Sun, Jianxin Yang

CSTONE Pharmaceuticals, Suzhou, China

Background: EGFR (also known as HER1) is an epidermal growth factor receptor with tyrosine kinase activity implicated in both tumorigenesis and tumor progression, and its overexpression in approximately 70% of colorectal cancer (CRC), 60% of lung cancers, and over 90% of head and neck squamous cell carcinoma (HNSCC) has been shown associated with advanced diseases. EGFR has been clinically validated as a therapeutic target for ADC. For instance, MRG003, an EGFR-targeted ADC, has shown encouraging clinical outcomes in NPC and HNSCC. HER3, another HER family member, is overexpressed in CRC and lung cancer. The upregulation of HER3 has been reported in tumors when treated with MAPK/PI3K inhibitors, EGFR TKIs, or hormone therapies. Daiichi's HER3-ADC, U3-1402, has shown promising clinical results in advanced EGFR-mutant NSCLC, confirming HER3 as a valid therapeutic target for ADC. Additionally, BL-B01D1, an EGFR/HER3 bispecific ADC, has shown encouraging phase 1 data in advanced cancers. CS5007 is designed to be a potential best-in-class, dual-targeted ADC with 1) fully functional EGFR/HER3 targeted arms to tackle tumor heterogeneity; 2) a proprietary, hydrophilic and stable beta-glucuronide linker to ensure mAb-like PK profile and tumor selective cleavage; and 3) a clinically validated, potent and well-tolerated topoisomerase 1 inhibitor exatecan (EXd).
Methods & results: CS5007 demonstrated high-affinity binding (single digit nM) to EGFR single-positive (A431), HER3 single-positive (SW620), and EGFR/HER3 double-positive (BxPC3, FaDu) tumor cells, comparable to its naked bispecific antibody. CS5007 also triggered high-rate internalization on these cells, resulting in efficient cell killing, thus posing the potential to address tumor heterogeneity by targeting tumor cells expressing either or both of EGFR and HER3. In the *in vivo* studies using CDX models, CS5007 demonstrated robust, antigen-dependent tumor growth inhibition across a range of EGFR and HER3 expression levels. Its efficacy was comparable or superior to the control ADCs composed of the same bispecific antibody with GGFG-DXd and to the monospecific (targeting EGFR or HER3) ADCs bearing GGFG-DXd. CS5007 was well tolerated in rodent with maximum tolerated dose (MTD) at 200 mg/kg and demonstrated a mAb-like PK profile. Its stability in circulation was confirmed in the human/cynomolgus monkey serum incubation assays. In pilot cynomolgus monkey studies, CS5007 was well tolerated at 30 mg/kg administered three times with two weeks interval, with toxicokinetic data confirming its excellent circulating stability.
Conclusion: CS5007 is a promising dual-targeted ADC that demonstrates potent antitumor efficacy with a favorable safety and PK profile. These preclinical findings support its further IND-enabling studies and clinical investigations for the treatment of various advanced solid tumors.

#2955 Preclinical development of TJ101, a potent bispecific ADC targeting EGFR and B7-H3 for the treatment of solid cancers.

Li Qi, **Shugaku Takeda**, Yangxin Zhang, Mao Huang, Dongdong Zhou, Yanli Mao, Zhaoyuan Chen, Siyi Hu

Phrontline Biopharma Co., Ltd, Shanghai, China

Antibody-drug conjugates (ADCs) have emerged as a promising class of targeted therapeutic agents; however, off-tumor toxicity and drug resistance remain as major challenges. Bispecific ADCs are designed to target two distinct antigens or different epitopes on the same antigen. Compared to monoclonal antibody ADCs, bispecific ADCs can enhance targeting to the tumors that co-express both antigens and reduce the likelihood of off-tumor toxicity. EGFR is a validated cancer therapeutic target that is upregulated in various solid cancers such as lung, head and neck, breast, and kidney cancers. Activation of EGFR through mutation, overexpression, or amplification drives proliferation, survival, and metastasis of tumor cells. The B7 family protein B7-H3, also known as CD276, is widely overexpressed in a range of solid cancers including non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), head and neck squamous cell carcinoma (HNSCC), prostate cancer, and breast cancer. Notably, B7-H3 expression is limited in normal tissues, making it an attractive target for ADC approaches. Interestingly, EGFR is co-expressed with B7-H3 in the majority of lung cancer and HNSCC cases (TCGA dataset) and in many solid cancer cell lines (DepMap Portal). TJ101 is a bispecific ADC directed against human EGFR and B7-H3. Its backbone is a natural IgG1 format bispecific antibody heterodimerized using knobs-into-holes (KIHs) technology. The affinity of the parental humanized anti-EGFR antibody is reduced to minimize on-target toxicity, while the anti-B7-H3 antibody retains high affinity. The warhead of TJ-101 is PY-4car2, a novel payload derived from camptothecin, conjugated with a cleavable linker. TJ101 exhibited synergistic internalization and cytotoxicity in multiple cancer cell lines expressing EGFR and B7-H3. Inhibition of antigen binding through pre-treatment with parental unconjugated antibody prevented the cytotoxicity of TJ101. TJ101 treatment in NSCLC, HNSCC, and colon cancer CDX models demonstrated potent, dose-dependent anti-tumor activity. Importantly, TJ101 also achieved significantly better anti-tumor activity than monoclonal anti-EGFR or anti-B7-H3 ADCs in EGFR/B7-H3 co-expressing models. Furthermore, single administration of TJ101 produced durable tumor regressions in PDX models derived from NSCLC, SCLC, HNSCC, breast cancer, and esophageal cancer. TJ101 was well-tolerated in a non-GLP toxicity study in cynomolgus monkeys with the highest nonseverely toxic dose (HNSTD) exceeding 30 mg/kg. In summary, TJ101 is a promising bispecific ADC with highly potent anti-tumor efficacy across multiple solid cancer types and preferable toxicity profiles, enabling an increased therapeutic index. Additionally, the dual-targeting approach of TJ101 offers a significant advantage in overcoming potential tumor resistance that may arise during future clinical applications.

#2956 Anti-tumor activity of camptothecin analog conjugate of a RSPO4-based peptibody targeting LGR4/5/6 in preclinical models of colorectal cancer.

Yukimatsu Toh[1], Ling Wu[1], Jianghua Tu[1], Zhengdong Liang[1], Adela M. Aldana[1], Li Li[1], Jake J. Wen[1], Sheng Pan[1], Julie H. Rowe[2], Martha E. Hensel[3], Carolyn L. Hodo[3], Rick A. Finch[3], Kendra S. Carmon[1], Qingyun J. Liu[1]

[1]Center for Translational Cancer Research, Brown Foundation Institute of Molecular Medicine, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, TX,[2]Department of Internal Medicine, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, TX,[3]Department of Comparative Medicine, Michale E. Keeling Center for Comparative Medicine and Research, University of Texas MD Anderson Cancer Center, Bastrop, TX

Antibody-drug conjugates (ADCs) have emerged as a major modality of targeted cancer therapy, yet no ADC has been approved for colorectal cancer (CRC). LGR4/5/6 (leucine-rich repeat containing, G protein-coupled receptor 4, 5, 6) are three related receptors that are expressed at high levels together or alternately in nearly all cases of CRC. ADCs targeting LGR5 have been shown to have robust anti-tumor potency, but not all CRC cells express LGR5 and LGR5-positive tumor cells may lose LGR5 expression due to cancer cell plasticity. R-spondin 4 (RSPO4) is a natural protein ligand of LGR4/5/6 with high affinity for all three receptors. We fused a mutant form of RSPO4 that retains high affinity binding to LGR4/5/6 to IgG1 Fc to create a peptibody designated R462. Conjugation of R462 with a camptothecin analog (CPT2) at eight drugs per peptibody led to the synthesis of R462-CPT2 that showed highly potent cytotoxic activity in vitro in CRC cell lines expressing any of LG4/5/6. In cell line xenograft and PDX models of CRC, R462-CPT2 demonstrated robust anti-tumor effect. Importantly, R462-CPT2 showed no major adverse effect at therapeutically effective dose levels. These results strongly support the use of RSPO ligand drug-conjugates that target LGR4/5/6 simultaneously for the treatment of CRC.

#2957 Establishing preclinical activity of topoisomerase I inhibitor (TOPO1i) antibody-drug conjugate AZD9592 in patient-derived xenograft (PDX) models of metastatic colorectal cancer (mCRC) in context of irinotecan pretreatment and resistance.

Mel Ehudin[1], Lara McGrath[2], Simon Christ[3], Italia Grenga[2], Fernanda Arnaldez[4], Frank Comer[4], Edward Rosfjord[4]

[1]Oncology R&D, AstraZeneca, Gaithersburg, MD,[2]Early Oncology R&D, AstraZeneca, Waltham, MA,[3]Computational Pathology, AstraZeneca, Munich, Germany,[4]Early Oncology R&D, AstraZeneca, Gaithersburg, MD

Background: Each year an estimated 1.9 million people are diagnosed with colorectal cancer worldwide and approximately 930,000 people die due to mCRC. The TOPO1i irinotecan is used frequently in combination chemotherapy regimens to treat mCRC that cannot be managed with surgery. Colorectal tumor cells often overexpress the cell membrane proteins epidermal growth factor receptor (EGFR) and mesenchymal-epithelial transition tyrosine kinase receptor (cMET). Overexpression of either EGFR or cMET is associated with cancer progression and poor clinical prognosis in mCRC. AZD9592 is a bispecific antibody-drug conjugate that binds EGFR and cMET and delivers a TOPO1i payload.

Methods: We evaluated a set of 9 colorectal PDX models with a broad range of EGFR and cMET protein expression. Eight of these models were mCRC obtained from tumors biopsied from the liver (n=7) or lung (n=1). We evaluated these models for a response to AZD9592 (administered in a single dose at 2, 4, or 8 mg/kg), irinotecan, or irinotecan plus oxaliplatin. We also examined whether AZD9592 induced responses in PDX models with tumors that progressed after irinotecan treatment. A response was defined as ≥30% tumor volume reduction from baseline.

Results: A single dose of AZD9592 resulted in responses in 100% (9/9) of tumors treated with 8 mg/kg, 67% (6/9) of tumors treated with 4 mg/kg, and 33% (3/9) of tumors treated with 2 mg/kg. A single dose of irinotecan resulted in a response in 33% (3/9) of tumors. Irinotecan plus oxaliplatin resulted in a response in 55% (5/9) of tumors. Subsequent dosing with AZD9592 was feasible in 4 of the PDX models that did not respond to irinotecan; responses to a single dose of AZD9592 were observed in 50% (2/4) and 75% (3/4) of these models treated with 4 or 8 mg/kg, respectively. A similar experiment was conducted in 2 of the 4 models that did not respond to irinotecan plus oxaliplatin; a response was observed in 1 of these models with subsequent AZD9592 dosing at both 4 and 8 mg/kg. We also evaluated the effect of multiple doses of AZD9592 on the magnitude and duration of response. In 22% of mCRC PDX models (2/9), multiple doses of AZD9592 increased the magnitude of response at 4 mg/kg compared to a single dose. In 78% of models (7/9), multiple doses of AZD9592 4 mg/kg increased the duration of response. Dosing AZD9592 every 3 weeks improved the magnitude of response in some models and increased the time until tumor relapse.

Conclusions: AZD9592 was effective in mCRC PDX models, even at the lowest doses tested. AZD9592 was effective in mCRC PDX models that did not respond to irinotecan-based treatment. Ongoing research is evaluating whether multiple doses of AZD9592 can induce resistance in PDX models, and potential resistance mechanisms are being assessed by molecular characterization.

#2958 SAR408701: An anti-CEACAM5-maytansinoid antibody-drug conjugate to treat CEACAM5-positive neuroendocrine prostate cancer (NEPC).

Loic Poiraudeau[1], Bigot Ludovic[1], Cedric Pobel[1], Naoual Menssouri[1], Marine Aglave[1], Catline Nobre[1], Alice Bernard-Tessier[1], Natacha Naoun[1], Anna Patrikidou[1], Claudio Nicotra[1], Maud Ngocamus[1], Benjamin Besse[1], Karim Fizazi[1], Jean-Yves Scoazec[1], Anne-Marie Lefebvre[2], Celine Nicolazzi[2], Nathalie Mace[2], Anne-Laure Bauchet[2], Yohann Loriot[1]

[1]Gustave Roussy, Villejuif, France,[2]Sanofi, Vitry sur Seine, France

Background: Neuroendocrine prostate cancer (NEPC) is a very aggressive subtype of metastatic castration-resistant prostate cancer (mCRPC), for which therapeutic strategies are currently lacking. NEPC is rare *de-novo* (<1% of the cases) but can represent 15 to 20% of cases after failure of the treatment targeting the androgen receptor (AR) axis (such as enzalutamide, abiraterone) in. Carcinoembryogenic antigen-related cell adhesion molecule 5 (CEACAM5) has been previously identified as an attractive NEPC cell surface antigen. Here, we investigate its expression in NEPC patient specimens and its therapeutic potential as a target for Antibody-Drug Conjugate (ADC) therapy.

Methods: Tumor biopsy specimens were obtained from the prospective trial MATCH-R (NCT02517892). Patient-derived xenografts (PDX) were established and both patient biopsies (n=59) and PDX (n=16) were characterized by RNAseq and immunohistochemistry (IHC). 5 PDX from our 16 models were selected based on their CEACAM5 expression for our pharmacological assay: 2 NEPC models with a high expression of CEACAM5, 1 NEPC model with a mild (weak) expression of CEACAM5, 1 NEPC model with no expression of CEACAM5 and 1 mCRPC with a luminal phenotype and no expression of CEACAM5. SAR408701, an anti-CEACAM5 ADC or an irrelevant ADC with no specific target was administrated to immunodeficient mice. Tumor growth and body weight were monitored twice a week for 21 days post-injection.

Results: 54 patient biopsies were collected for IHC and 59 for RNAseq analysis. 33 cases out of the 54 presented evidence of neuroendocrine differentiation (in IHC analysis)tusamitamab and 21 expressed synaptophysin in more than 50% of the tumor cells. CEACAM5 expression in tumor cells was detected in 12 cases which all expressed synaptophysin (>50%), suggesting a strong correlation between CEACAM5 expression and neuroendocrine differentiation. An unsupervised hierarchical clustering based on RNAseq analysis of patient samples showed an enriched neuroendocrine cluster, in which CEACAM5 was significantly overexpressed. In the 5 PDX models selected to test the activity of Tusamitamab Ravtansine based on CEACAM5 expression, a strong correlation between CEACAM5 expression in the PDX models and
response to Tusamitamab Ravtansine was observed, with Tusamitamab Ravtansine showing a strong antitumoral activity on CEACAM5 expressing PDX models and no efficacy in models lacking CEACAM5 expression.

Conclusion: Overall, these results demonstrate the efficacy and specificity of SAR408701 against NEPC tumors harboring CEACAM5.This study provides preclinical evidence of the antitumor activity of SAR408701 in CEACAM5-positive NEPC.

Funding: Gustave Roussy, Cancer Campus, Grand Paris

#2959 PF-08046031: A promising first-in-class antibody-drug conjugate for targeting novel tumor antigen in melanoma.

Raj Giraddi, Steven Jin, Devra J. Olson, Iliyana Mikell, Eliana Moskovitz, **Rebecca Mazahreh**, Sharsti Sandall

Pfizer, Inc., Bothell, WA

MFI2, also known as CD228, melanotransferrin, p97, MTf, or MELTF, is a GPI-anchored membrane protein that belongs to the iron-transporting transferrin family. CD228 expression is elevated in multiple solid tumors such as melanoma and non-small cell lung cancer (NSCLC) but has limited expression in normal tissues. Here, we have expanded our expression analysis and found that other solid tumors, including head and neck cancer (HNSCC) and esophageal cancer (ESCC), also show positivity rates of 86% and 94%, respectively. PF-08046031 is an antibody-drug conjugate (ADC) that consists of the anti-CD228 human IgG1 antibody conjugated with an average of 4 molecules of monomethyl auristatin E (MMAE) by a cleavable valine-citrine (vc) linker. The proposed mechanism of action (MoA) of PF-08046031 is to bind to CD228-expressing cells where it is internalized and transported to lysosomes to release MMAE, causing microtubule disruption and cytotoxic cell death. This drug-linker and conjugation methodology have been clinically validated and approved. In this study, pharmacological evaluations of antibody binding, internalization, cytotoxic cell death and in vivo anti-tumor activity were conducted using various in vitro and in vivo models. PF-08046031 specifically binds to human CD228 with high affinity in both high and low CD228-expressing tumor cells. In vitro studies showed that the ADC had strong cytotoxic effects on CD228-positive cell lines but not on CD228 negative or genetically modified CD228KO cells. The anti-tumor activity of PF-08046031 was tested in vivo using two cutaneous melanoma models derived from cell lines (A2058 and Colo853). After a single intravenous dose of PF-08046031 at 3 and 6 mg/kg, significant tumor growth inhibition (TGI) was observed in a dose-dependent manner compared to the untreated group. At the highest dose of 6.0 mg/kg, TGI reached 87% for A2058-derived tumors and 90% for COLO853-derived tumors. These findings support the clinical development of PF-08046031 for targeting multiple solid tumors.

#2960 MYTX-011: A pH-dependent anti-cMET ADC exhibits potent activity in multiple epithelial tumor models with clinically actionable mutations and varying levels of cMET expression.

Deepak Kanojia, Federico Colombo, William J. Israelsen, Vijay Ramesh, Nimish Gera, Thomas Chittenden, Brian P. Fiske

Mythic Therapeutics, Waltham, MA

Small-molecule inhibitors (SMI) of acquired genetic alterations in cancer-driver genes have advanced patient care, especially in non-small cell lung cancer (NSCLC). Tyrosine kinase inhibitors (TKI) targeting the cMET receptor currently benefit a subset of patients whose tumors are dependent on cMET signaling. However, tumors frequently become refractory to SMI therapy. Antibody-drug conjugates (ADCs) targeting cMET have shown promising efficacy in a subset of NSCLC patients whose tumors express high levels of cMET. MYTX-011, an investigational, pH-sensitive, vcMMAE-based ADC targeting cMET, is designed to benefit a broader set of patients whose tumors express low to moderate levels of cMET and are not dependent on cMET signaling, in addition to patients that express high levels of cMET. We previously demonstrated that MYTX-011, which is engineered to rapidly dissociate from cMET at the acidic pH of endolysosomes, drives increased net internalization and cytotoxicity in tumor cells expressing low or moderate levels of cMET compared to a matched non-engineered ADC. Moreover, MYTX-011 was efficacious in NSCLC, gastric, esophageal, and HNSCC xenograft models with various cMET adenocarcinoma/squamous histology. Here, we demonstrate by live cell imaging that the pH-dependent binding of the antibody component of MYTX-011 leads to its rapid dissociation from cMET in the early endosomal lumen, whereas the non-engineered antibody remains localized to the endosomal membrane. These data support a model where MYTX-011 avoids non-productive recycling to the cell surface thereby enhancing delivery to lysosomes, payload release and cytotoxicity. To further study the mechanism of MYTX-011 across multiple tumor mutational backgrounds, we showed potent efficacy of MYTX-011 in tumor xenografts harboring clinically relevant mutations such as MET exon 14 skipping (METex14), KRAS, and EGFR, demonstrating that pH-dependent binding of MYTX-011 translated to potent efficacy regardless of actionable mutation status. MYTX-011 was active in low or moderate cMET-expressing NSCLC KRAS mutant (G12C) xenografts as well as in TKI-resistant xenograft models bearing EGFR mutation (T790M), whereas a benchmark ADC was either inactive or had greatly reduced efficacy. We also show that MYTX-011 was highly active in a METex14 Hs746T cell line derived xenograft (CDX) and NSCLC patient-derived xenografts (PDX) including a model derived from a patient with acquired resistance to MET TKI. Further, there were no antagonistic effects of combining MET TKI and MYTX-011 in METex14 PDX models, indicating the potential of MYTX-011 in combination with approved MET TKIs. Together, these findings highlight the potential of MYTX-011 as a therapeutic candidate for treating a broad range of cMET-expressing cancers with clinically actionable mutations.

#2961 AZD9592, an EGFR/cMET bispecific antibody-drug conjugate (ADC), demonstrates target-dependent efficacy in colorectal cancer (CRC) patient-derived xenograft (PDX) models.

Ying Zheng[1], Edward Rosfjord[1], Simon Christ[2], Stephanie Zalesak-Kravec[3], Italia Grenga[4], Fernanda Arnaldez[1], Frank Comer[1], Lara McGrath[4]

[1]Early Oncology R&D, AstraZeneca, Gaithersburg, MD,[2]Computational Pathology, AstraZeneca, Munich, Germany,[3]Translational Medicine - CBD, Early Oncology, AstraZeneca, Gaithersburg, MD,[4]Early Oncology R&D, AstraZeneca, Waltham, MA

Background: AZD9592 is a bispecific ADC designed to target both epidermal growth factor receptor (EGFR) and mesenchymal-epithelial transition tyrosine kinase receptor (cMET), delivering a topoisomerase 1 inhibitor payload to tumor cells. Despite recent advances in ADC development for CRC, such as the use of trastuzumab deruxtecan to treat HER2-positive CRC, a significant unmet need remains for novel therapeutic approaches, particularly for patients with limited targeted treatment options.

Methods: We evaluated the antitumor efficacy of AZD9592 and explored biomarker-response relationships in CRC PDX models across a range of very low to high EGFR expression (optical density of membrane [OD] 6.7-162.6) and cMET expression (OD 18.5-142.6) measured by immunohistochemistry-quantitative continuous score (IHC-QCS), as well as other molecular features like oncogene drivers. Responses were defined as ≥30% tumor volume reductions from baseline.

Results: AZD9592 demonstrated dose-dependent efficacy, with responses observed in 34.5% of models at 8 mg/kg and 14.8% at 4 mg/kg. Biomarker analyses of tumors revealed a significant association between cMET expression levels by IHC-QCS and responses at 8 mg/kg. cMET levels were significantly higher in AZD9592-responsive models than in antibody-responsive and isotype control-responsive models ($p<0.02$ for both). An optimized cMET expression cutoff had a predictive performance with an area under the curve of 0.73. Compared to wildtype tumors, response rates were not significantly different in tumors harboring mutations in oncogenes that share signaling pathways with EGFR and cMET and where clinical activity of EGFR antibody therapies is limited, including KRAS and BRAF. Moreover, response rates were not significantly different across tumors derived from patients with or without prior irinotecan treatment, a chemotherapy that also inhibits topoisomerase I. Proteomics identified additional molecular features associated with response.

Conclusions: AZD9592 demonstrated dose-dependent antitumor activity in CRC PDX models, including KRAS-mutant and KRAS-wildtype tumors. These responses suggest opportunities for clinical development of AZD9592 in CRC and the potential for biomarker-guided patient selection.

#2962 Enhanced HER2 internalization by clathrin-dependent endocytosis in non-small cell lung cancer positive for *HER2* mutations.

A. Shimauchi[1], E. Iwama[1], R. Ibusuki[1], H. Tsutsumi[1], D. Shibahara[1], K. Otsubo[1], Y. Yoneshima[1], K. Tanaka[2], H. Inoue[3], I. Okamoto[1]; [1]Kyushu University, Fukuoka, Japan, [2]Kagoshima University, Kagoshima, Japan, [3]Fukuoka University Hospital, Fukuoka, Japan

Introduction: Mutations in the gene for human epidermal growth factor 2 (HER2) result in constitutive phosphorylation of the protein and are driver alterations in cancer. HER2 mutations are present at a frequency of 1% to 4% in non-small cell lung cancer (NSCLC), with exon-20 insertion mutations such as A775_G776insYVMA and G776delinsVC being among the most common of these latter mutations. Although HER2-targeted ADCs have shown marked efficacy for HER2 mutation-positive NSCLC, the detailed molecular mechanisms underlying such efficacy are not well understood.

Methods: The intracellular trafficking and dimerization patterns of HER2 were examined in cells expressing wild-type (WT) or mutant forms of the protein with the use of live cell imaging and an in situ proximity ligation assay, respectively. Proteins that preferentially associate with mutant HER2 were identified by liquid chromatography and tandem mass spectrometry to shed light on the pathway of HER2 trafficking in HER2-mutated cells.

Results: HER2 internalization was enhanced in NSCLC cells expressing mutant forms of HER2 compared with those expressing HER2(WT). Homodimers of HER2(WT) were localized mainly at the cell surface, whereas those of mutant HER2 were present mostly in the cytoplasm. Although siRNA interference of EGFR or HER3 effectively suppressed the HER2 internalization in cells stably expressing WT forms of HER2, such internalization was not affected in the cell lines expressing mutant forms of HER2. The enhanced internalization of mutant HER2 was mediated by clathrin-dependent endocytosis, as was reflected by increased binding of the ubiquitin ligase c-Cbl to mutant HER2 and its consequent ubiquitination. Zongertinib, a HER2-specific tyrosine kinase inhibitor, attenuated HER2 phosphorylation, the binding of c-Cbl to HER2, and HER2 internalization in HER2-mutated cells.

Conclusions: Upregulation of HER2 phosphorylation promotes internalization of mutant forms of HER2 mediated by clathrin-dependent endocytosis, which may contribute to the efficacy of HER2-targeted ADCs in NSCLC positive for HER2 mutations.

#2964 SCR-A002, a novel FGFR2b-targeting ADC for solid tumors.

Yayuan Fu, Yuanjie Ge, Xiaohui Shao, Qi Deng, Chunlei Xia, Renhong Tang

Simcere Zaiming Pharmaceutical Co., Ltd., Shanghai, China

Background: FGFR2b (fibroblast growth factor receptor 2 IIIb), an isoform of transmembrane tyrosine kinase FGFR family, is overexpressed in various cancers, including gastric, esophageal, breast, hepatocellular, pancreatic, ovary, uterine, cervical, endometrial, lung, colorectal cancers. Bemarituzumab (FPA144) is an afucosylated humanized IgG1 monoclonal antibody targeting FGFR2b with enhanced ADCC activity, which had promising clinical efficacy in phase 2 study of FGFR2b-selected gastric or gastroesophageal junction adenocarcinoma. Simcere Zaiming has developed a potential first-in-class FGFR2b-targeting ADC (SCR-A002) at IND-enabling status, displaying significantly superior anti-tumor potency compared to Bemarituzumab.
Methods: Using hybridoma technique, anti-FGFR2b antibody was generated from immunization of FGFR2b protein in mice. SCR-A002 was comprised of a humanized anti-FGFR2b IgG1 monoclonal antibody conjugated through a cleavable linker to proprietary TOPO1 inhibitor payload (DAR 8). Binding activity and cytotoxicity were assessed in FGFR2b+ tumor cell line. And anti-tumor efficacy was evaluated in several CDX and PDX models.
Results: SCR-A002 specifically bound to FGFR2b on tumor cells and could be internalized, and the TOPO1 inhibitor could be cleaved intracellularly and then exhibited potent cytotoxicity to tumor cells. And tumor regression was achieved after single-dose administration of SCR-A002 in different FGFR2b expression level CDX and PDX models, even with big-size tumor or Bemarituzumab resistance.
Conclusion: SCR-A002 showed remarkable anti-tumor efficacy in preclinical data, suggesting that SCR-A002 is expected to provide a new treatment option as single agent or combo with SoC for patients with FGFR2b-overexpressing solid tumor.

#2965 SCR-A006, an innovative and potential best-in-class EGFR/cMet bispecific antibody-drug conjugate.

Yayuan Fu, Chaochao Cen, Lei Jiang, Xiaohua Yan, Chuanchuan Lu, Qian Wang, Ye Hong, Renhong Tang

Simcere Zaiming Pharmaceutical Co., Ltd., State Key Laboratory of Neurology and Oncology Drug Development, Simcere Pharmaceutical Group, Shanghai, China

Background: EGFR and cMet are widely co-expressed in solid tumors, and there is crosstalk between their signaling pathways, which is the general escape mechanism for each other in clinic. Although various drugs targeting EGFR and/or cMet have been approved or are in clinical development, primary and acquired resistance limits their prolonged efficacy. There still remains a large unmet need in solid tumor therapy. SCR-A006 is an antibody-drug conjugate (ADC) comprising a bispecific mAb targeting EGFR&cMET conjugated to a topoisomerase 1 inhibitor (TOP1i) via a cleavable and hydrophilic linker, which potentially benefits a broad population of solid tumor patients.

Methods: Physiochemical and biological properties of SCR-A006 were evaluated. The binding affinity and cytotoxicity of SCR-A006 were investigated *in vitro* using cancer cell lines containing different target expression levels, tumor types (lung, head and neck, breast, gastric, colorectal and pancreas), as well as genomic landscapes. *In vivo* pharmacodynamic studies were conducted in several CDX and PDX models with different EGFR expression levels or mutations. Preclinical PK studies and tissue distributions were performed by intravenous injection of SCR-A006 at 5mg/kg single in WT mice and tumor cell xenograft mice.

Results: SCR-A006 exhibited strong cytotoxicity against a broad range of tumor cell lines with higher potency than benchmark ADC, but did not enhance toxicity to normal cells, suggesting a larger therapeutic window. *In vivo*, SCR-A006 demonstrated dose-dependent tumor inhibition and was more effective than benchmark ADC in xenograft models regardless of tumor types and genetic profiles. SCR-A006 also showed good efficacy and was superior to benchmark ADC in amivantamab resistant xenograft models. Excitingly, we found complete response and prolonged efficacy in NSCLC PDX model. To figure out the mechanism of better efficacy compared to benchmark ADC, PK and cleavage efficiency in tumor or normal tissue from CDX model of SCR-A006 were carried out, which showed better tumor penetration and higher cleavable efficiency in tumor homogenate than benchmark ADC but very limited cleavage in normal tissue. SCR-A006 also displayed higher hydrophilicity and more excellent plasma stability than Benchmark ADC. All of above performance potentially resulted the better efficacy and good safety of SCR-A006.

Conclusions: Taken together, these findings highlight the potential of SCR-A006 as a therapeutic candidate for broad treatment of solid tumors and with a larger therapeutic window than other EGFR/cMET BsADCs. IND enabling studies of SCR-A006 are currently underway, and planned to initiate clinical study in 2025.

#2966 The anti-tumor activity of a novel anti-Her2 and anti-c-Met bispecific antibody-drug conjugate.

Hongyan Zhong, Daniel Li, Daniel Guo, Phuong Nguyen, **Ming Yang**

Bright Biologics LLC, Sunnyvale, CA

Background: Her2 and c-Met, two tumor-associated antigens (TAAs), are well-established cancer targets, as evidenced by the approval of several anti-Her2 antibody-drug conjugates (ADCs), a bispecific anti-c-Met/EGFR antibody, and promising Phase 2 efficacy of an anti-c-Met ADC. Her2 and c-Met are expressed separately or co-expressed in various cancer types, including lung, pancreatic and kidney cancers, reflecting the heterogeneity of tumor TAA expression. Dual TAA-targeting may reduce the probability of cancer escape, commonly seen in the treatment of single TAA-targeting ADCs, due to the heterogeneity of TAA expression.
Methods: We developed a novel bispecific anti-Her2/anti-c-Met ADC, BB-205. BB-205 was engineered using humanized anti-Her2 and anti-c-Met VHH fragments, fused with a human IgG1 Fc, and conjugated with vc-MMAE. Whole cell binding assays were conducted to determine the binding affinity of BB-205 to TAAs. *In vitro* cytotoxicity assays were performed to evaluate the cytotoxic effects of BB-205 on various cancer cell lines. The *in vivo* efficacy of BB-205 was tested in HPAC and NCI-H1975 xenograft mouse models.
Results: BB-205 maintained the high binding affinities of its parental VHH-Fc antibodies to Her2 and c-Met. It exhibited remarkable cytotoxic effects on cancer cells that express either Her2 or c-Met, or both. Its cytotoxic potency was comparable to Trastuzumab-MMAE (T-MMAE) in Her2-high expressing cancer cells and to Telisotuzumab vedotin (Teliso-V) in c-Met-high expressing cancer cells. BB-205 demonstrated superior cytotoxicity than either reference ADC or combination of both reference ADCs in dual TAA-expressing cancer cells with low to medium level of expression. Additionally, BB-205 showed higher internalization compared to either reference ADC in cancer cells. In the *in vivo* HPAC xenograft model, where HPAC cells express medium levels of c-Met and low levels of Her2, both BB-205 and Teliso-V significantly inhibited tumor growth, achieving 100% and 91% tumor growth inhibition (TGI), respectively. In contrast, treatments with T-MMAE had a low efficacy in suppression of tumor growth. At the end of study, 50% of mice in the BB-205 group were tumor-free, whereas no mice in other groups were tumor-free. BB-205 was also tested in an NCI-H1975 xenograft model, where NCI-H1975 cells express medium levels of Her2 and c-Met. Treatment with either BB-205 or T-MMAE significantly reduced tumor size. Four weeks after a single dose, 50% mice in BB-205 group and 25% mice in T-MMAE group were tumor-free. While Teliso-V initially shrank tumors, the tumors gradually regrew in the fifth week post-treatment. No obvious side effects were observed in both *in vivo* studies.
Conclusions: The novel anti-Her2/anti-c-Met bispecific ADC BB-205 demonstrates superior anti-tumor activity in preclinical studies. BB-205 shows promising therapeutic potential as a first-in-class ADC drug candidate.

#2967 Bone voyage: FGFR1, stemness, and the Wnt trail to prostate cancer metastasis.

Agustina Sabater[1], Pablo Sanchis[1], Nicolas Anselmino[2], Juan Bizzotto[3], Jun Yang[2], Jiabin Dong[2], Peter D. A. Shepherd[2], Paul G. Corn[2], Elba Vazquez[4], Christopher J. Logothetis[2], Nora Navone[2], Geraldine Gueron[4], Estefania Labanca[2]

[1]Universidad de Buenos Aires (UBA) - IQUIBICEN - CONICET - Universidad Argentina de la Empresa (UADE), CABA, Argentina. MD Anderson Cancer Center, Houston, TX,[2]MD Anderson Cancer Center, Houston, TX,[3]Universidad de Buenos Aires (UBA) - IQUIBICEN - CONICET - Universidad Argentina de la Empresa (UADE), CABA, Argentina,[4]Universidad de Buenos Aires (UBA) - IQUIBICEN - CONICET, CABA, Argentina

Prostate cancer (PCa) lethality largely stems from the progression to castration-resistant PCa (CRPC) bone metastasis. We previously reported that fibroblast growth factor receptor 1 (FGFR1) is overexpressed in a subset of human CRPC bone metastases, and that FGFR1 overexpression of both α and β isoforms drives bone metastases and reduces survival in experimental metastatic murine models with intracardiac PCa cells injection, posing its blockade as a promising therapeutic strategy for advanced PCa. An on-going clinical study at our department at MD Anderson is currently evaluating erdafitinib, an FGFR inhibitor, for treating men with CRPC bone metastases (ClinicalTrials.gov Identifier: NCT04754425). However, the dynamics behind FGFR-driven PCa progression remain elusive. As FGFR signaling has previously been linked to the development and proliferation of PCa stem-cells, we hypothesized that FGFR promotes PCa bone metastasis by modulating cell plasticity. *In vitro* assays confirmed that FGFR1α- and FGFRβ-expressing PCa cells exhibited increased colony formation and confluency, and a more stem-like phenotype, evidenced by altered morphology, smaller cell size, elevated aldehyde dehydrogenase (ALDH) levels, and enhanced tumorsphere formation ($p<0.0001$). Consistently, RNA-seq analysis of PCa cells and MDA PCa patient-derived xenografts (PDX) showed a significantly positive correlation between FGFR1 levels and stem-cell markers ($p<0.05$). Furthermore, PCa patients from TCGA-PRAD (497 primary PCa) and SU2C (81 metastatic CRPC) datasets with enrichment of FGFR-correlated stem-cell categories exhibited increased risk of progression (log-rank $p<0.05$). Among the stem-categories that correlate with FGFR expression, genes from Wnt signaling, a pathway involved in stem-cell traits regulation and implicated in PCa, emerged prominently (*FERMT2*, *FZD7*, NES, *CORO1C*, *BMP4*, *MEF2C*, *GJA1*, *SFRP1*, *WNT2B*, *CDH2*). Bioinformatics analysis also revealed significant correlation between TCF/LEF factors and FGFR1 in TCGA-PRAD patients. FGFR1-expressing PCa cells showed increased *TCF* reporter activity, indicating Wnt activation, and nuclear β-catenin immunostaining in FGFR1-expressing PDXs and human CRPC bone metastases further confirmed this activation. Overall, our findings suggest that FGFR1 drives PCa progression through Wnt signaling-induced stemness, conferring cancer cells the plasticity to adapt to the bone microenvironment. Future studies aim to further characterize the FGFR1-induced stemness and dedifferentiation mechanisms underlying PCa bone dissemination and evaluate FGFR-targeted therapies.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Identification of Molecular Targets 1
Poster Session

#2971 Characterization of targeted ADC and small molecule therapy efficacy toward HER2-mutated XPDX models.

Andre Villegas[1], Johnnie R. Flores[1], Alyssa Simonson[1], Anna Stackpole[1], Natalia Banos[2], Amy Fredrickson[3], Jim Lund[1], Rose Garcia[1], Ashley Jamison[1], Jennifer Garcia[1], Alexis Nunez[1], Karen Hernandez[1], Kyriakos P. Papadoupolos[4], Victor Moreno[5], Manish Sharma[6], Drew Rasco[4], Andrew Cunningham[1], Michael J. Wick[1]

[1]XenoSTART, San Antonio, TX,[2]XenoSTART, Madrid, Spain,[3]XenoSTART, Grand Rapids, MI,[4]START San Antonio, San Antonio, TX,[5]START Madrid, Madrid, Spain,[6]START Midwest, Grand Rapids, MI

Background: Somatic mutations in the human epidermal growth factor receptor 2 (HER2) gene occur in several cancer types, including bladder, colorectal, lung and cervical. Multiple antibody-drug conjugate (ADC) molecules and small molecule treatments targeting HER2 have been approved; however, comparative in vivo sensitivity of these therapies toward known and novel HER2 mutations is unclear. To better understand the effects of these targeted therapies on specific HER2 variants, we established and characterized a panel of XPDX models harboring various HER2 mutations and benchmarked in vivo sensitivity to targeting ADCs and small molecule therapies.
Methods: 18 XPDX models were included in this study. Models representing the S310F mutation in the extracellular domain included ST174 (bile duct), ST524 (colorectal), ST1243 (lung) and ST5025B (bladder) and one cervical model (STM602) with a reported S310Y variant. Models harboring an R678Q mutation in the juxtamembrane domain included ST022 (ovary), ST026 (colorectal) and STF001 (pancreas). Models with mutations in the kinase domain included ST5960 breast (Y735C), STF117 lung (L755F), ST1616B breast (D769Y), ST2594 colorectal (D769Y), ST427 colorectal (V777L), ST4295 ovary (S792F), and ST1456 breast (V842I). Two models ST3107 (lung) and STF059 (colorectal), harboring a YVMA insertion, and the ST3013 (lung) with a 776-777 G/LC indel were also evaluated. Models were characterized for HER2 expression by immunohistochemistry and profiled using WES and RNA_{seq}. For in vivo studies, T-DXd, T-DM1, neratinib, tucatinib, and afatinib were evaluated at standard treatment regimens. Study endpoints included tumor volume and time from treatment initiation with %T/C values and tumor regression reported at study completion; a %T/C of ≤ 20 versus control was considered sensitive. Tumor regression (%T/C<0%) versus Day 0 tumor volume was also reported.
Results: The majority of models stained for HER2 at 2+ or 3+ and RNA based expression correlated with IHC results. In vivo, T-DXd or T-DM1 treatment resulted in regressions in ST1243 and ST3013 but were ineffective toward ST5025B. Neratinib reported activity toward ST5025B (%T/C=-80%), ST3107 (%T/C=1%), and ST3013 (%T/C=2%) while tucatinib was found efficacious toward ST5025B (%T/C=12%) and STF117 (%T/C=13%). Afatinib treatment resulted in regressions toward ST427 (%T/C=-25%) and was active in ST3013 (%T/C=8%).
Conclusion: We have established and characterized a panel of XPDX models harboring various HER2 mutations and benchmarked in vivo sensitivity toward targeting ADCs and small molecule therapies. This data and model panel can be utilized as a valuable tool in better understanding the utility of HER2-targeting therapies in HER2-mutated cancers.

#2972 Trabectedin exerts its potent and selective antitumor effects by irreversibly covalently bonding to duplex stem loops in G-quadruplexes.

Alena Safarova[1], Marta Martinez Diez[2], Raquel M. Altares[2], Pablo M. Aviles[2], Marcelo Lima Ribeiro[2], Carmen Cuevas[2], **Laurence H. Hurley**[1]

[1]University of Arizona, Tucson, AZ,[2]Pharma Mar, S.A., Madrid, Spain

Trabectedin, a marine-derived anti-tumor agent from the ecteinascidin family, shows effectiveness when treating cancers such as Ewing Sarcoma, small cell lung cancer (SCLC) and ovarian cancer. It features a unique carbinolamine group that forms reversible bonds with the N2 position of guanine, distinguishing it from typical DNA alkylating agents. Structurally, trabectedin also includes a C-subunit that widens the DNA minor groove, inducing transcriptional replication stress and genome instability. Given the sensitivity of Ewing Sarcoma to trabectedin and the role of G-quadruplexes (G4s) in binding EWSR1—especially those with longer stem loops—this study aims to redefine trabectedin's molecular target by exploring its potential interaction with G4 structures. Building on these insights, we designed experiments using trabectedin in combination with wild-type (WT) and mutant MYCN G4s to assess whether trabectedin could irreversibly bond covalently to a consensus sequence within a duplex stem loop associated with a G4. Circular dichroism (CD) analysis confirmed that trabectedin stabilized a G4 containing a consensus covalent bonding site within the associated hairpin loop. Mass spectrometry (MS) demonstrated that a guanine in the trabectedin consensus binding site is essential for this irreversible covalent binding. Additionally, CD and MS showed that when trabectedin was bound covalently to the hairpin stem loop, it displaced GSA0932, a molecule that binds non-covalently to both the core structure and stem loop. Finally, we observed that trabectedin treatment significantly increased G4 frequency within cells. On a final note, trabectedin irreversibly binds to the N2 of guanine within a duplex stem loop of a G4, fully accounting for its downstream effects on transcription-dependent replication stress and genomic instability. While this data specifically pertains to trabectedin, other members of the ecteinascidins, which possess structurally distinct C-subunits, appear to exhibit varied downstream effects, such as on transcription. These differences may arise from variations in sequence selectivity at the stem-loop level, leading to the targeting of distinct G-quadruplex structures.

#2973 Discovery of novel synthetic lethal targets for effective and safe colorectal cancer therapies.

Andrzej Mazan[1], Marcin Duleba[1], Eliza Zimolag[1], Joanna Szuszkiewicz[1], Marcin Serocki[1], Joanna Krawczyk[1], Oleksii Bryzghalov[1], Agata Stachowicz-Walaszek[1], Izabella Wieckowska[1], Marcelina Chmiel[1], Alexander Kurzejamski[1], Barbara Lipert[1], Katarzyna Sarad[1], Michal Mikula[2], Rafal Dziadziuszko[3], Tomasz Rzymski[1], Andrew Thomason[1], Krzysztof Brzozka[1]

[1]Ryvu Therapeutics, Krakow, Poland,[2]Maria Skłodowska-Curie Institute of Oncology, Warsaw, Poland,[3]Medical University of Gdansk, Gdansk, Poland

Colorectal cancer (CRC) poses a significant global health burden, highlighting the urgent need for innovative and effective treatment options. This study focuses on the discovery and validation of novel therapeutic targets, particularly synthetic lethal (SL) interactions, to exploit specific genetic vulnerabilities in CRC cells and enable the development of safer and more effective therapies. We employed a comprehensive strategy integrating healthy cells, genetically engineered isogenic cancer models derived from human intestinal stem cells (hISCs) with patient-derived xenografts (PDXs) and primary cell cultures. Using CRISPR/Cas9 technology, we introduced key CRC-associated mutations into the hISCs to replicate disease-specific contexts. Transcriptome profiling, coupled with machine learning, validated these models as robust, clinically relevant representations of tumor biology by aligning them with clinical datasets. Genome-wide CRISPR/Cas9 screenings conducted across the isogenic and patient-derived models revealed actionable vulnerabilities, including the identification of genes that are synthetically lethal with APC and KRAS mutations. Discovered SL targets were rigorously validated *in vitro* and *in vivo* using patient-derived cells with matching mutational profiles. This integrative approach bridges preclinical and clinical research, ensuring translational relevance. By identifying and validating SL targets tailored to CRC mutational landscapes, we provide a robust foundation for the development of novel treatment options for CRC patients.

#2974 Bortezomib resistant multiple myeloma shows a specific dependency on Ecm29 in vitro and in vivo.

Andrej Besse[1], Lenka Besse[2], Sara C. Stolze[3], Marianne Kraus[4], Ondrej Slaby[2], Hirofumi Nakagami[3], Christoph Driessen[4]

[1]Dept. of Biology, Masaryk University, Brno, Czech Republic,[2]Masaryk University, Brno, Czech Republic,[3]Max Planck Institute for Plant Breeding Research, Cologne, Germany,[4]Cantonal Hospital St. Gallen, St. Gallen, Switzerland

Background: Resistance to proteasome inhibitors (PI) bortezomib (BTZ) is a major obstacle to the successful treatment of multiple myeloma (MM). Thus, the identification of a specific target in PI-resistant MM is an unmet medical need. The highly specialized and adapted nature of PI-resistant MM may offer novel areas of vulnerability. Using genome-wide CRISPR/Cas9-based functional screening in BTZ-adapted cell lines *in vitro* we identified the proteasome adaptor and scaffold protein Ecm29 as an essential survival factor for BTZ-adapted cells under BTZ treatment. We aimed to characterize its function in myeloma *in vitro* and *in vivo*.
Methods: Genome-wide CRISPR/Cas9 screening was used in BTZ-resistant cells to identify overlapping BTZ sensitivity candidates, which were further validated in a set of four BTZ-resistant cells. Viability was determined by CCK8 viability assay. Cell cycle, apoptosis induction, and aggresome formation after BTZ treatment were determined by flow cytometry. NGS, LC-MS/MS, and SILAC were used to determine major changes in Ecm29 knock-down (KD) or knock-out (KO) cells. RT-PCR and westernblot were used to determine unfolded protein response. Two *in vivo* orthotopic mouse models were used to determine the effect of Ecm29 on BTZ resistance.
Results: Ecm29 was identified as a consistent candidate for BTZ sensitivity in two independent screens with BTZ-adapted MM cell lines exposed to BTZ. Validation with specific Ecm29 KD or KO sensitized BTZ-adapted cells to BTZ, in contrast to BTZ-naïve parental cells. Pull-down of proteins bound to Ecm29 in BTZ-adapted cells, in contrast to BTZ-naïve cells, showed significant changes, related to Ecm29 and proteasome localization towards the cytoskeleton and vacuolar compartment. Following, Ecm29 silencing induced changes in the cytoskeleton and vesicular trafficking, decreased protein synthesis and diminished the accumulation of poly-ubiquitinated protein. BTZ treatment caused accumulation of poly-ubiquitinated proteasome substrate proteins, induction of the unfolded protein response, induction of chaperones and aggresome formation, G2-phase cell cycle arrest, and apoptosis in Ecm29-silenced cells, in contrast to BTZ-adapted control cells. Ecm29 depletion significantly reduced the growth of two independent BTZ-adapted cells *in vivo* in murine bone marrow, following BTZ treatment.
Conclusion: The proteasome adaptor and scaffold protein Ecm29 controls proteasome trafficking and localization. It is of central importance in BTZ-adapted MM cells undergoing BTZ treatment due to dependance on different proteasome localization. Ecm29 may therefore represent a novel therapeutic target to specifically re-sensitize PI-resistant MM cells to PI treatment.
Support: Supported by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union - Next Generation EU.

#2975 Characterization of the RAC1/CDC42 inhibitor MBQ-167 to assess its utility as a tool for interrogating Rho GTPase biology.

Szilvia Barsi, Arpad Varga, Csaba Szantai-Kis, Maria Victoria Ruiz-Perez, Ivan Fekete, Imre Gaspar, Csilla Polner-Szundi, Boldizsar Elek, Magdolna Djurec, Nora Ordasi, Istvan Taisz, Amrik Basran, **Daniel Veres**

Turbine Simulated Cell Technologies Ltd., Budapest, Hungary

The Simulated Cell platform highlighted RAC1 as a target with great antitumor potential. RAC1 is a small Rho GTPase with roles in cell polarity and migration, and contributes to cancer metastasis. To identify good chemical tools for RAC1, we evaluated multiple RAC1 inhibitors, and selected MBQ-167 as a potent RAC/CDC42 inhibitor with publicly available efficacy data.

We analyzed the *in silico* effects of individual and dual RAC1/CDC42 inhibitors using the Simulated Cell. *In vitro* we studied MBQ-167 using viability, apoptosis and scratch assays. Viability of RAC1 KO cells treated with MBQ-167 was also analyzed. Post-treatment RNA-Seq and bioinformatics analysis of pathway activity signatures revealed MBQ-167 downstream effects. The SignalChem kinase assay was used to validate off-target identification.

Our simulations revealed that, beyond their role in cytoskeleton, RAC1 and CDC42 exert effects through apoptosis signaling, with a stronger contribution of RAC1. *In vitro*, we confirmed the activity of MBQ-167 against RAC1 and CDC42. We observed a negative impact of MBQ-167 on cell migration and an induction of apoptosis. However, RAC1 KO did not affect sensitivity to MBQ-167, suggesting that effects of MBQ-167 on cell viability are not dependent on RAC1.

Post-treatment RNA-Seq analysis of MBQ-167 treated cells and calculation of the similarity scores with LINCS consensus signatures, revealed that the MBQ-167 signature is highly similar to those of various kinase KOs and inhibitors targeting PLKs, AURKA, MET, CDK1, MAPK14, FLTs, RET and PDGFRs. RAC KOs were not involved. A kinase off-target panel validated that MBQ-167 inhibits the aforementioned kinases. It inhibited PLK2 and AURKA with an IC50 of around 200 nM, similar to its primary described targets. Other kinases were also inhibited in the nanomolar range, such as CDK1, MET and PLK1.

Targeting RAC proteins and CDC42 holds great anti-tumoral potential. We investigated MBQ-167 *in vitro* and showed that it does not exert its effects on cancer cell viability through RAC1. Besides RAC and CDC42, this compound can inhibit cancer-relevant kinases such as AURKA and PLK2. Simulations helped understanding that MBQ-167 is not a specific inhibitor and therefore not adequate as a tool for interrogating the biology of interest. Further studies are needed to find additional inhibitors against RAC/CDC42 and to understand the effects of MBQ-167 on cancer cells and as a clinical asset tested in Phase I clinical trials as a single agent against advanced breast cancer.

#2976 XPR1 and SLC34A2 (NaPi2B)- A tale of targeting the phosphate homeostasis in cancer cells.

Mansi Babbar[1], Dion Daniels[1], Katie Vowell[1], Rajesh Sundaresan[1], Shuzhen Wu[2], Feng Wang[1], James Lin[1], Jenny Laraio[1], Justin Munro[1], Joseph Kozole[1], John Kreeger[1], Geeta Sharma[2], Bryan Joosse[1], Florence Patel[1], Joseph Tomlinson[1], Katherine Welbeck[3], Richard Priest[3], Jennifer Percival-Alwyn[3], Trevor Wattam[3], Gurjinder Heer[1], Iain Moal[3], Sreenivas Nannapaneni[1], Benjamin Schwartz[2], Biju Mangatt[1], Anthony Mazurek[1]

[1]GlaxoSmithKline, Collegeville, PA,[2]GlaxoSmithKline, Cambridge, MA,[3]GlaxoSmithKline, Stevenage, United Kingdom

SLC34A2 is overexpressed in 61% of ovarian cancer and 18% of uterine cancer tumors. SLC34A2 overexpression is believed to be driven by the aberrant activity of the essential PAX8 transcription factor in these tumors. Overexpression of SLC34A2, a phosphate importer, sensitizes the cells to the genetic depletion of XPR1, the sole phosphate exporter. Importantly, pharmacologic inhibition of XPR1 by XRBD, a peptide molecule that binds the extracellular domain 3 (ECL3) of XPR1 to antagonize its function, phenocopies the lethal impact of XPR1 knockdown on SLC34A2 overexpressing cancer cells. This observation supported the hypothesis that an antibody that binds to XPR1 will antagonize its function and selectively kill cancer cells that overexpress SLC34A2. There are no XPR1 therapeutics reported in clinical development indicating a first-in-class opportunity for an XPR1 therapeutic antibody. The goal of this program was to identify an antagonist antibody that inhibits XPR1 phosphate export function as a novel therapy for ovarian and uterine cancers patients with tumors that overexpress SLC34A2 (SLC34A2-high). We utilized XRBD to understand XPR1 biology in SLC34A2-high and low expressing cancer cell line models. Mechanistically, in SLC34A2-high expressing cell lines, genetic or pharmacologic inhibition of XPR1-dependent phosphate efflux led to the toxic accumulation of intracellular phosphate. XPR1 inhibition in these cell lines also resulted in increased cellular nucleotide triphosphate levels. Interestingly, we found altered mitochondrial gene expression and metabolism upon XPR1 inhibition and/or loss. Further, XPR1 inhibition induced gamma H2AX suggesting increased DNA damage. This finding was also supported by synergistic growth inhibition and cytotoxicity when XPR1 inhibitor was combined with cisplatin. On our path to therapeutic XPR1 antibody discovery, we identified antibodies that bound to overexpressed and endogenous XPR1, however they did not induce expected pharmacodynamic responses or efficacy against SLC34A2 -high expressing cancer cell lines. These results indicate that the binding of these antibodies to XPR1 does not recapitulate the antagonistic activity of XRBD against the viability of SLC34A2 overexpressing cancer cells.

#2978 PSMD6 is a promising therapeutic target in multiple myeloma.

Minxing Wang, Arghya Ray, Teng Fang, Xueping Wan, Ting Du, Sindhu C. Pillai, Md Abu Musa, Yan Song, Kenneth C. Anderson

Dept of Medical Oncology, LeBow Institute for Myeloma Therapeutics and Jerome Lipper Myeloma Center, Dana-Farber Cancer Institute, Boston, MA

Background: The 26S proteasome is essential for maintaining protein homeostasis by degrading misfolded or unneeded proteins regulating critical cellular processes such as the cell cycle, apoptosis, and immune response. It consists of 20S catalytic core particle and 19S regulatory particle, with the PSMD family playing a key role in proteasome assembly. Multiple myeloma (MM), a plasma cell malignancy characterized by abnormal immunoglobulin production, heavily depends on proteasome function. While proteasome inhibitors (PIs) are commonly used in MM treatment, resistance to inhibitors targeting the 20S subunit frequently develops. Targetting the PSMD family of ubiquitin receptors upstream of the proteasome in the ubiquitin proteasome cascade represents a promising therapeutic target to overcome PI resistance. This study aimed to identify key PSMD subunits as therapeutic targets in MM, with a focus on validating PSMD6.

Methods: Gene expression and cell line dependencies were analyzed using the Gene Expression Omnibus datasets and DepMap portal. Kaplan-Meier plotter was used to assess the correlation between PSMD expression and MM patient survival. PSMD6 was knocked down via siRNA or CRISPR/Cas9 in MM cell lines, followed by immunoblotting to evaluate K48 polyubiquitinated protein accumulation, apoptosis, endoplasmic reticulum (ER) stress, and cell cycle progression.

Results: 1) DepMap identified that PSMD1-4, PSMD6-8, and PSMD11-14 as essential for MM cell line survival. 2) PSMD3, PSMD5-13, and PAAF1, are highly expressed in MM patients (GSE6691, GSE6471, GSE153380). 3) Elevated expression of PSMD1-3, PSMD6, PSMD8, PSMD9, PSMD11, and PSMD13 was correlated with poor MM patient survival. 4) Our real-time PCR and immunoblot analysis confirmed higher PSMD6 expression in MM patient samples and cell lines compared to normal controls. 5) Functional studies revealed that PSMD6 KD significantly inhibited proliferation of MM cell lines (AMO1, KMS11, H929, and RPMI-8226), independent of p53 status. 6) To assess whether PSMD6 KD disrupt proteasome function, a ubiquitin-GFP reporter cell line was used, where GFP is constitutively degraded by the proteasome. Immunoblot analysis revealed significant accumulation of GFP in PSMD6 KD cells. This was further confirmed by live-cell imaging using the IncuCyte system. 7) Mechanistic studies showed that PSMD6 KD led to increased apoptosis (early and late apoptotic phases), G0-G1 cell cycle arrest, accumulation of K48-linked polyubiquitinated proteins, caspases activation, and induction of the ER stress pathway.

Conclusion: Our study highlights the potential of PSMD subunits, particularly PSMD6, as therapeutic targets in MM. Our data validate targeting PSMD6 to enhance cytotoxicity and overcome PI resistance in MM and provide the preclinical rationale for developing PSMD6 inhibitors to improve patient outcomes in MM.

Keywords: PSMD6, proteasome, multiple myeloma, therapeutic target

#2979 Deletion of p53-related protein kinase (TP53RK) suppresses solar UV-induced photocarcinogenesis by inhibiting PDL1 expression and enhancing CD8+ T cell infiltration.

Qiushi Wang[1], Eunmiri Roh[2], Asad Khan[1], Sally Dickinson[3], Georg T. Wondrak[3], Ann Bode[1], Clara Curiel-Lewandrowski[3], Tianshun Zhang[1]

[1]The Hormel Institute, University of Minnesota, Austin, MN,[2]Department of Cosmetic Science, Kwangju Women's University, Gwangju, Korea, Republic of,[3]The University of Arizona Cancer Center, The University of Arizona, Tucson, AZ

Nonmelanoma skin cancers (NMSCs), including cutaneous squamous cell carcinoma (cSCC), are primarily caused by solar ultraviolet (UV) exposure and represent the most common cancers in the United States. TP53RK (p53-related protein kinase, also known as PRPK) is a protein kinase involved in multiple cancer progression such as colon cancer, myeloma and skin cancer. In this study, we generated epidermal-specific TP53RK knockout mice using CRISPR/Cas9 technology in SKH1 hairless mice with loxP-flanked TP53RK alleles, crossed with keratin 14-Cre (K14.Cre) mice. Our findings reveal that epidermal-specific deletion of TP53RK significantly suppresses tumor growth in solar-simulated light (SSL)-induced NMSC. Mechanistically, TP53RK deletion inhibits proliferating cell nuclear antigen (PCNA) and programmed death-ligand 1 (PDL1) expression, as well as the transcription factors c-Myc, c-Jun, and NF-κB, which mediate PDL1 expression. Using a 3D culture system, we further demonstrate that TP53RK deletion suppresses cSCC cell growth. Flow cytometry analysis indicates that TP53RK deletion enhances CD8+ T cell infiltration into the tumor microenvironment. This is accompanied by significant reductions in IL-6, MIP-2, and VEGF levels, reprogramming the tumor microenvironment to support CD8+ T cell infiltration. In summary, our study demonstrates that TP53RK deletion suppresses solar UV induced photocarcinogenesis by inhibiting PDL1 expression and enhancing CD8+ T cell infiltration, highlighting its potential as a therapeutic target for NMSC.

#2980 Therapeutic potential of combined targeting of casein kinase 1 alpha (CK1 alpha) and CDK7/9 with the inhibitor BTX-A51 in human liposarcomas.

Renyan Liu[1], Nicole L. Solimini[1], Caoibhne McSweeney[1], Patrick Bhola[1], Daryl Griffin[1], Tim B. Branigan[1], Michael J. Wagner[1], Audrey Turchick[1], Ricardo de Matos Simoes[1], Joshua M. Dempster[2], Jie Hao[1], Xin Wang[1], Roshen Alharthi[1], Michael Yorsz[1], Shaili Soni[1], Cing-siang Hu[3], Irit Snir-Alkalay[4], Francisca Vazquez[2], Prafulla C. Gokhale[3], Constantine Mitsiades[1], Anthony Letai[1], Yinon Ben-Neriah[4], George D. Demetri[1], Geoffrey I. Shapiro[1]

[1]Dana-Farber Cancer Institute, Boston, MA,[2]Broad Institute of MIT and Harvard, Boston, MA,[3]Experimental Therapeutics Core, Dana-Farber Cancer Institute, Boston, MA,[4]The Lautenberg Center for Immunology and Cancer Research, Institute of Medical Research Israel-Cana, Hebrew University-Hadassah Medical School, Jerusalem, Israel

Introduction: Liposarcomas (LPS) are mesenchymal cell malignancies that are diagnosed in more than 3500 patients in the US each year. LPS are characterized by DNA amplifications of *MDM2* (100%) and *CDK4* (90+%) genes on neochromosomes, generally with wild-type *TP53*. Management of metastatic or surgically unresectable LPS remains purely palliative. Recent clinical trials targeting MDM2 or CDK4/6 with small-molecule inhibitors have shown modest activity but have generally failed in phase 3 trials. The development of new therapeutics is greatly needed to improve outcomes for patients with LPS.
Results: To identify unique liposarcoma-specific vulnerabilities, we utilized the Dependency Map (DepMap) database to analyze gene-specific dependencies of 9 LPS cell lines as compared to non-LPS cancers and identified *CSNK1A1* as one of the top essential genes preferentially for LPS. siRNA-mediated depletion of *CSNK1A1* confirmed that CK1α negatively regulates p53 and is critical for the proliferation and survival of LPS cells. DepMap analyses identified *CDK7* and CDK9 as two additional top LPS-essential genes. Three CDK9 inhibitors suppressed LPS cell growth and induced apoptosis by decreasing MDM2 levels while inducing expression of p53 and PUMA. The cytotoxic effects of CDK9 inhibitors were enhanced upon CK1α depletion. Furthermore, we quantitatively determined synergism between CDK7 and CDK9 inhibitors in LPS cells. These data led us to examine combined targeting of CK1α and CDK7/9 in LPS with the novel small molecule inhibitor BTX-A51, which has previously also been shown to inhibit CK1α, CDK7, and CDK9 with nanomolar potency in acute myeloid leukemia (AML) models. BTX-A51 treatment significantly reduced expression of MDM2 with marked induction of p53, resulting in increased PUMA expression and profound apoptosis of LPS cells. Through CRISPR/Cas9-mediated *TP53* knockout, we established that BTX-A51-treatment-induced apoptosis was in part mediated in a p53-dependent manner, which was further validated transcriptionally using RNA-Seq based pathway analysis. BTX-A51 also reduced expression of the apoptosis inhibitor MCL1 and primed LPS cell lines and PDX-derived cells for BIM/PUMA-induced apoptosis. Importantly, *in vivo* experiments in
two LPS PDX models demonstrated that BTX-A51 is well-tolerated with anti-tumor efficacy.
Conclusions: These data have confirmed CK1α, CDK9, and CDK7 as essential for LPS cells and indicate that BTX-A51 has potent preclinical efficacy in LPS, through combined inhibition of CK1α, CDK9, and CDK7. Our data form the scientific foundation for our ongoing pilot clinical trial evaluating BTX-A51 in patients with advanced WDLPS or DDLPS (NCT06414434).

#2981 Glioblastoma reduced sensitivity to tumor treating fields (TTFields) is associated with changes in electrical membrane properties of tumor cells.

Michele Mazzanti[1], Francesca Cianci[1], Guido Rey[1], Sara Torabi[1], Elisa Meraviglia[1], Davide Ceresa[2], Paolo Malatesta[2], Kerem Wainer-Katsir[3], Tullio Florio[2], Dietmar Krex[4]

[1]University of Milan, Milano, Italy,[2]University of Genova, Genova, Italy,[3]Novocure, Haifa, Israel,[4]6Klinik und Poliklinik fur Neurochirurgie, Universitatsklinikum Carl Gustav Carus der Technischen Universitat Dresden, Dresden, Germany

Glioblastoma (GBM) is recognized as the most aggressive type of brain tumor, and current treatments generally fail to prevent recurrence. Tumor-treating fields (TTFields) is an innovative therapy that improves patients' life expectancy. Although the initial application of TTFields slows tumor progression, it typically does not prevent tumor relapse in most patients. To model tumor recurrence after surgical intervention, we exposed patient-derived GBM primary cultures to continuous electric field stimulation. Remarkably, three days of uninterrupted TTFields stimulation completely halted the proliferation of tumor cells. However, the surviving cells eventually become less sensitive to TTFields, and rescue proliferation at their maximum rate within 8 to 10 days. In this study, we analyzed transcripts of several patients' tumor tissues derived from primary and secondary surgical resection. Eight of these patients received TTFields treatment between the two surgical interventions. Their transcriptomic profile was compared with those who received the standard therapy but not TTFields between the surgeries. The tumor samples collected during initial surgeries and untreated GBM cells displayed significant inter-heterogeneity in their transcript profiles. These differences were less pronounced following TTFields application, which unveiled several commonly altered cytosolic pathways including inhibition of proliferation. Many of the upregulated pathways are not viable therapeutic targets, as they involve positive or negative modulations of physiological cellular functions. In addition, RNA extracts from human glioblastoma primary cultures were matched with those of patients treated with TTFields. After the TTFields application, several stemness pathways were significantly upregulated in isolated cells and human biopsies. Previous studies demonstrate that glioblastoma stem cells are enriched in transmembrane Chloride Intracellular Channel 1 (tmCLIC1) and its inhibition slows down tumor cell proliferation. CLIC1 transcript does not appear to be modulated in patients by electric field stimulation. However, following prolonged TTFields exposure in primary cell lines we observed a significant increase in the membrane protein activity. This indicates that tmCLIC1 could serve as a valuable target for a combined antitumoral therapy alongside TTFields to impair glioblastoma development.

#2982 Accelerating antigen-targeting therapy discovery with a scalable pan-cancer bioinformatics platform.

Eleonore Fox, Lea Meunier, Guillaume Appe, Abdelkader Behdenna, Lucas Hensen, Akpeli Nordor, Solene Weil, Camille Marijon

Epigene Labs, Paris, France

Cancer drug discovery is lengthy and nearly 90% of clinical trials fail due to efficacy or safety concerns. Identifying safe and effective drug targets using data-driven approaches in the earlier stages of research is critical to improve success rates. Over 40 antigen-targeting therapies have been FDA-approved in the past three decades but have yet to reach their full potential, especially in solid tumors where selecting the right antigen targets is a major challenge. To address this, we developed a comprehensive and scalable translational bioinformatics platform leveraging underutilized public omic data to discover, and prioritize novel antigen targets across various cancers.We integrated 168 microarray datasets using our AI-powered, human-supervised clinical data curation and transcriptomic data normalization pipeline. These were aggregated into 18 indication-specific cohorts encompassing 7,885 tumor and 1,569 healthy samples, and profiling 20,347 genes. We leveraged our proprietary target discovery pipeline, starting with a differential gene expression analysis, followed by proteomic filters selecting cell surface proteins with transmembrane or glycosylphosphatidylinositol -anchored domains. We further prioritized targets using a proprietary framework incorporating bulk and single cell transcriptomic data, proteomic, biological and pharmacological data metrics into a weighted scoring system designed to anticipate clinical relevance. Metrics were classified into 3 categories (efficacy, safety and novelty) and weights were optimized using FDA-approved targets.We identified an average of 216 potential antigen targets per indication, ranging from 41 to 556. Among these, we successfully rediscovered FDA-approved targets across several indications, including CD19 and CD22 in acute lymphoblastic leukemia, ERBB2 and TACSTD2 in breast invasive carcinoma, PDL1 in skin melanoma, PSMA in prostate cancer and FOLR1 in ovarian serous carcinoma, alongside targets currently in clinical trials for antigen-based therapies. Our prioritization framework was then applied to our candidate targets and revealed novel antigen targets exhibiting characteristics similar to those of FDA-approved targets. For example in breast invasive carcinoma, we identified 16 highly promising targets with efficacy scores matching or exceeding FDA approval benchmarks, including 9 with better predicted safety and 2 with high novelty scores in this indication. Additionally, 14 of these promising targets were identified in two or more indications, highlighting their tissue-agnostic potential. Our platform integrates unbiased data-driven tools with cancer biology insights to streamline antigen target discovery, from data integration to target prioritization. Scalable to any cancer type or antigen-targeting modality, it offers a robust framework for accelerating oncology drug discovery.

#2983 BUB1B is an inactive kinase required for survival of chromosomally unstable cells.

Nazario Bosco[1], Rafael S. Depetris[1], Jasmina Prabhakara[1], Akanksha Verma[1], Jared L. Johnson[2], Lewis C. Cantley[2], Scott Drutman[1], Shinsan M. Su[1], Rumin Zhang[1], Derek A. Cogan[1], Christina H. Eng[1]

[1]Volastra Therapeutics, New York, NY,[2]Department of Cell Biology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA

Chromosome instability (CIN) is a prevalent hallmark of aggressive cancers that is largely absent in normal cells. Targeting tumors with CIN is a therapeutic strategy that is rapidly advancing. Volastra Therapeutics has undertaken multiple orthogonal approaches to target CIN as a cancer vulnerability. One such approach that relies on the Cancer Dependency Map (DepMap) identified BUB1B (BUB1 Mitotic Checkpoint Serine/Threonine Kinase B) as a top scoring gene required for survival of cell lines with increased genomic instability. BUB1B, a core component of the spindle assembly checkpoint that functions in cell cycle progression and chromosome segregation, was also highlighted in published screens as a major cancer vulnerability in cells with aneuploidy and whole-genome duplication.

To corroborate the dependency of chromosomally unstable cancer cells on BUB1B, we used three independent cell models: 1) an isogenic CIN system based on modulating kinetochore-microtubule dynamics, 2) a Cas9-engineered system to induce dicentric chromosomes through telomere deprotection and 3) a non-isogenic cell line model based on differential levels of aneuploidy and micronuclei. Data in these three orthogonal systems confirmed that chromosomally unstable cells rely heavily on BUB1B for survival.

We next explored the development of BUB1B small molecule inhibitors. The C-terminus of BUB1B is responsible for chromosome congression and contains the putative kinase domain, suggesting that catalytic activity may be responsible for CIN suppression. Whether BUB1B possesses enzymatic activity has been controversial, with some groups reporting low but inhibitable BUB1B kinase activity, while others indicate that BUB1B lacks key kinase residues and is catalytically inactive.

To probe the function of BUB1B, we generated full-length and kinase domain recombinant proteins. Both constructs showed very weak ATPase activity comparable to published and commercially available proteins. Efforts to boost the kinase activity through improved substrate specificity or enhanced phosphorylation were not successful, and additional purification resulted in further loss of kinase activity. Furthermore, modeling of the BUB1B kinase domain suggests that ATP binding to the ATP cleft is physically hindered by bulky residues. Finally, substrate sequence mapping did not match sequences predicted for BUB1B. Together the data suggest that BUB1B is not enzymatically active and that the measured low ATPase activity may be attributable to a co-purifying kinase.

Our work confirms chromosomally unstable cells have greater dependency on BUB1B. However, the protein lacks catalytic activity and thus cannot be targeted with a kinase inhibitor strategy. BUB1B's function as a protein interaction hub with specific partners could permit alternative therapeutic strategies that may result in effective treatments for patients with chromosomally unstable cancers.

#2984 High throughput drug screening of patient-derived organoids reveals the ATR-mediated DNA damage response pathway as an esophageal cancer vulnerability.

Wataru Hirose[1], Samuel Flashner[1], Yasuto Tomita[1], Kento Kawasaki[1], Masaki Morimoto[1], Izabella Pacheco[1], Nour Kandil[1], Christian Lee[1], Cecilia Martin[1], Charles Karan[2], Joel T. Gabre[1], Anil K. Rustgi[1], Hiroshi Nakagawa[1]

[1]Columbia University Herbert Irving Comprehensive Cancer Center, New York, NY,[2]Department of Systems Biology, JP Sulzberger Columbia Genome Center, New York, NY

Introduction: Esophageal squamous cell carcinoma (ESCC) is common worldwide and the deadliest of all squamous cell carcinomas (SCC). Standard-of-care chemotherapy and immune checkpoint inhibitors offer limited survival benefits, highlighting the need for novel therapeutic strategies to combat ESCC. Patient-derived organoids (PDO) recapitulate therapy response and are powerful tools to meet this need; however, current PDO drug screening protocols are limited to fewer than 100 drugs, restraining their use in high-throughput screening (HTS) campaigns.

Methods: We developed a novel protocol enabling HTS of therapy-naïve ESCC (n=7) and normal esophageal mucosa (n=4) PDOs with >10,000 bioactive compounds. We validated our results by preforming a dose response assay to determine the IC50 of select drugs using three representative ESCC PDO lines. We further validated our results in an orthogonal model of murine-derived organoids (MDO) generated from mice bearing normal mucosa, preneoplasia and ESCC, the latter two induced by esophageal carcinogen 4-nitroqunoline-1-oxide. We analyzed DNA damage response (DDR) genes in the Cancer Genome Atlas, ESCC human tissue microarrays, and MDO. We evaluated DNA damage in tissues and organoids by alkaline comet assay and γH2AX staining. We depleted ATR by CRISPR interference (CRISPRi) in MDO.

Results: We identified 222 drugs (many FDA-approved) that killed (viability <80%) the majority of ESCC PDO (≥4 of 7) at 10 μM. We next selected drugs based on their pharmacodynamic properties. First, we selected drugs with IC50 values significantly lower than their Cmax values, the maximum plasma drug concentration documented in patients. We then selected 19 drugs that killed ESCC PDO significantly more (≥three-fold, $P<0.05$) than normal PDO, highlighting a potential therapeutic window for these strategies. Four of such ESCC-selective killers target the DDR regulators ATR, CHK1, and WEE1. We found that in DDR genes were significantly upregulated in ESCC patient tissue (n=88) relative to normal adjacent mucosa (n=11). Moreover, we determined that γH2Ax was significantly elevated in ESCC (n=215) relative to normal mucosa (n=93). We confirmed our results in MDO: γH2Ax labeling index, comet tail moments, and expression of DDR genes were significantly increased during ESCC pathogenesis. CRISPRi-mediated ATR depletion killed ESCC but not normal MDO.

Conclusions: Through an innovative HTS campaign in PDOs, we determined that ESCC are addicted to DDR genes to combat endogenous DNA damage that would be lethal to cancer cells if left unrepaired. ATR and other DDR checkpoint kinases are therefore promising therapeutic targets for selectively killing ESCC cells, and possibly other SCC, underscoring the translational utility of PDO in precision cancer medicine.

Grants: P01-CA098101, Torrey Coast Foundation, Mark Foundation

#2985 UHRF1 drives small cell lung cancer progression and immune evasion via epigenetic regulation.

Yijun Gu, Claudia Benavente

University of California, Irvine, Irvine, CA

Small cell lung cancer (SCLC) is a highly aggressive subtype of lung cancer, with a five-year survival rate of less than 8%. This poor prognosis stems primarily from rapid tumor growth, early metastasis, and an immunologically “cold” tumor microenvironment. A hallmark of SCLC is the near-universal inactivation of the RB transcriptional corepressor 1 (RB1), yet therapeutic advancements have been hindered by the lack of targetable oncogenic mutations. Our research has identified ubiquitin-like containing PHD and RING Finger domains 1 (UHRF1) as a direct downstream target of the RB pathway and a key protein overexpressed in RB-deficient cancers, including SCLC. Elevated UHRF1 expression correlates with poor survival outcomes in SCLC patients, but its specific role in disease progression remains poorly understood. We hypothesized that UHRF1 overexpression is critical for SCLC tumor progression. To test this hypothesis, we employed CRISPR-Cas9 and shRNA to disrupt UHRF1 expression in human SCLC cell lines. We assessed changes in tumor cell proliferation, clonogenicity, migration, and invasion in vitro, alongside primary tumor growth, metastasis, and survival in vivo. Additionally, we developed genetically engineered mouse models of SCLC to explore Uhrf1’s role of in tumor development, particularly its interaction with the immune microenvironment. Our findings reveal that UHRF1 overexpression drives a more aggressive tumor phenotype by promoting SCLC proliferation, angiogenesis, and maintaining an immune “cold” environment. Loss of UHRF1 in both human and mouse models led to significantly improved survival outcomes. These results underscore UHRF1’s critical role in SCLC progression and highlight its potential as therapeutic target. To elucidate the molecular mechanisms behind UHRF1’s oncogenic roles, we performed transcriptomic analysis using bulk RNA-seq, further validated by RT-qPCR. Upon UHRF1 KO, we observed significant gene expression changes, including downregulation of ST6GALNAC5 and upregulation of MAGEA4, RERG, and TGFBI. Pathway analysis suggests that UHRF1 modulates these downstream targets epigenetically, potentially through DNA methylation. In conclusion, our study identifies UHRF1 as an oncogenic driver in SCLC, facilitating tumor growth, metastasis, and immune evasion via epigenetic regulation. These findings provide a foundation for developing targeted therapies aimed at UHRF1 or its key downstream effectors to improve outcomes for SCLC patients.

#2986 Genomic amplification of MCL1 as a therapeutic target for osteosarcoma.

Satoshi Takagi, Tianyi Huang, Ryohei Katayama

Japanese Foundation for Cancer Research, Tokyo, Japan

Osteosarcoma (OS) is the most common primary malignant bone tumor that predominantly occurs in children, adolescents, and young adults. The treatment for OS that combines surgery with chemotherapy, which consists of a four-drug combination of doxorubicin (DOX), cisplatin (CDDP), high-dose methotrexate (MTX), and ifosfamide, was established in 1970s, and it is still used as a standard therapy. The lack of progress in drug development for OS is partly due to unidentified actionable oncogenic drivers common to OS. In this study, we demonstrate that copy number gains of MCL1 frequently occur in OS, leading to increased vulnerability to therapies based on Mcl-1 inhibitors. Fluorescence in situ hybridization analysis of formalin-fixed paraffin-embedded specimens revealed MCL1 amplification in 46.3% of patients with OS at the JFCR hospital (N = 41). Furthermore, analysis of the TARGET OS dataset, which comprises pediatric patients with OS (N = 81), found that the frequency of MCL1 amplification was notably higher, at 70.4%. Genetic inhibition of MCL1 induced significant apoptosis in MCL1-amplified OS cells. Pharmacological inhibition of MCL1 demonstrated a negative correlation between MCL1 copy numbers and sensitivity to Mcl-1 inhibitors, including MIK665 and AZD5991, with a Pearson's r value of -0.65 ($p = 0.03$) across OS cell lines. The chromosome 1q21.2-3 region, where MCL1 is located, contains multiple genes related to the IGF-1R/PI3K pathway, including PIP5K1A, TARS2, OUTD7B, and ENSA, which also showed increased copy numbers in MCL1-amplified OS cells. Additionally, the combination of MIK665 with IGF-1R inhibitors, including OSI906, AEW541, and AZD3463, induced synergistic cell death by overcoming drug tolerance conferred by the activation of IGF signaling in OS cells. In a NOS-10 (which has 16 copies of MCL1) xenograft model, MIK665 significantly suppressed tumor growth, and its antitumor effect was remarkably enhanced by combining with OSI906. This trend was also observed in a NOS-1 (with 5 copies of MCL1) xenograft model, but the enhancement was more modest than in the NOS-10 model. Moreover, the combination therapy of AZD5991 with OSI906 also reduced tumor growth in the NOS-10 xenograft model. These results suggest that genomic amplification of MCL1 in the 1q21.2-3 region, observed in nearly half of OS patients, may act as a predictive biomarker for combination therapy with an Mcl-1 inhibitor and an IGF1-R inhibitor.

#2987 Role of EIF4G1 network in non-small cell lung cancers (NSCLC) pathogenesis and development of targeted therapy.

Z. Qin[1], S. R. Post[1], H.-y. Li[2], L. Dai[1];

[1]University of Arkansas for Medical Sciences, Little Rock, AR, [2]University of Texas Health San Antonio, San Antonio, TX

Although the Eukaryotic Translation Initiation Factor 4 Gamma 1 (EIF4G1) has been found overexpressed in a variety of cancers, its role in non-small cell lung cancers (NSCLC) pathogenesis, clinical relevance and therapeutic potential remain largely unknown. By using cancer patients tissue assays, our results indicate that EIF4G1 expressional levels are much higher in NSCLC tissues than in adjacent or normal lung tissues, which are also associated with NSCLC patient survival. By using an RNA-Sequencing based pipeline, the data show that EIF4G1 has a significant association with immune checkpoint molecules such as PD-1/PD-L1 in NSCLC. Protein array results identify the signature of proteins controlled by EIF4G1 in NSCLC cells, in which new candidates such as MUC1 and NRG1 are required for NSCLC survival and tumorigenesis with clinical relevance. EIF4G1 small-molecule inhibitors effectively repress NSCLC growth in cell culture and xenograft animal models. To further improve the specificity of EIF4G1 targeted therapies, we recently have designed and synthesized O'PROTAC-based EIF4G1 specific degraders which showing effective anti-NSCLC activities. Taken together, these results demonstrate the immunoregulatory functions, clinical relevance and therapeutic potential of the EIF4G1 network in NSCLC, which may represent a promising and novel target to improve lung cancer treatment.

#2988 Patient-survival linked *TTLL12* isoform switching event sensitizes EAC cells to standard-of-care chemotherapy agents via chaperone-mediated autophagy.

Y. Zhang[1], R. Israel[1], B. Vandenburg[1], S. Barnett[1], J.-J. Riethoven[2], J. Clarke[2], M. P. Lee[1], D. A. Ntsiful[1], K. H. Lagisetty[1], J. Lin[1], R. M. Reddy[1], C. Ekeke[1], A. C. Chang[1], D. Odell[1], L. A. Kresty[1];

[1]University of Michigan, Ann Arbor, MI, [2]University of Nebraska - Lincoln, Lincoln, NE

Esophageal adenocarcinoma (EAC) represents a growing health problem characterized by rising incidence and poor prognosis, despite current multimodal therapy. Although EAC has a high mutational burden, it lacks highly prevalent oncogenic drivers that can be therapeutically targeted, as *TP53* mutation accounts for over 80% of mutational events in EAC. Recently, our group characterized isoform switching events, defined as a change of dominant gene isoforms between conditions, associated with EAC progression and identified 75 isoform switching events between Barrett's low-grade dysplasia (BE.LGD) and Barrett's high-grade dysplasia (BE.HGD) patient samples. Herein, we further investigated whether isoform-switching events may offer prognostic or therapeutic value in EAC. Isoform switching analysis was performed using RNA-seq data derived from 57 patient samples comparing BE.LGD to BE.HGD combined with EAC (BE.HGD+EAC) alone or in combination with *TP53* mutations. Patients were further stratified into tertiles based on isoform expression levels, followed by survival analysis. Forty-two isoforms were significantly ($P<0.05$) linked with all-cause mortality, comparing BE.LGD with BE.HGD+EAC, whereas 39 isoforms were identified when *TP53* mutation status was included. Among those identified to be significantly associated with all-cause mortality comparing BE.LGD with BE.HGD+EAC, isoforms of tubulin tyrosine ligase like 12 (*TTLL12*), a gene linked with aberrant cell proliferation, oxidative stress and cancer progression in other targets, were among the top isoforms switched. Isoform-specific genetic knockdown was performed via siRNA to target the protein-coding isoform of *TTLL12* in two EAC cell lines (OE19 and OE33). Knockdown of the *TTLL12* protein-coding isoform significantly inhibited cell viability and migration in both cell lines. In addition, synergistic effects on cell viability were observed for *TTLL12* knockdown of EAC cells treated with standard-of-care chemotherapeutic agents (Paclitaxel and Carboplatin), suggesting a role for specific isoform-switching events in therapeutic sensitization. Protein-interaction prediction using STRING and immunoblot analysis further supported that TTLL12 is linked with the regulation of chaperone-mediated autophagy (CMA), as evidenced by the induction of LAMP2A and HSC70 in EAC cells. Because CMA is linked with the regulated degradation of CHK1 and mutant TP53 in other cancers, we are currently assessing the role of CMA in regulation of CHK1 and degradation of TP53 in EAC. Future directions include using FDA-approved agents to target CMA in EAC. In conclusion, isoform switching may provide fresh insight for the identification of prognostic markers and inform new potential therapeutic targets for EAC treatment or prevention.

#2989 PIM1 contributes to tumor progression, immune evasion and metastasis of triple-negative breast cancer.

Chandrajeet Singh[1], Patrick Faughnan[1], Wasif A. Mohi[1], Bao Le[1], Jason M. Foulks[2], Golam Mohi[1]

[1]University of Virginia, Charlottesville, VA,[2]Sumitomo Pharma America, Inc, Marlborough, MA

Triple-negative breast cancer (TNBC), which is characterized by the absence of expression of estrogen, progesterone and HER2/Neu receptors, is the most aggressive subtype of breast cancer and associated with poor prognosis. Due to the absence of definitive molecular targets, chemotherapies are commonly used to treat TNBC, but these patients eventually develop resistance to chemotherapies. Therefore, there is a critical need to develop novel targeted therapies for TNBC. Analysis of the TCGA gene expression datasets and CCLE breast cancer cell line database revealed that PIM1 expression is significantly elevated in TNBC patients and TNBC cell lines. We also observed increased levels of PIM1 expression in metastatic breast cancer patients. Furthermore, high expression of PIM1 is associated with poor survival in TNBC patients. PIM1 belongs to the PIM family of serine/threonine kinase that controls cell growth and survival. Depletion of PIM1 by shRNA-mediated knockdown or CRISPR/Cas9-mediated genome editing significantly reduced the proliferation, migration, invasion and 3D tumor sphere formation of TNBC cells. To study the *in vivo* roles of PIM1 in breast cancer progression and metastasis, we crossed the MMTV-PyMT metastatic breast cancer mouse model with Pim1 knockout mice. Genetic ablation of Pim1 significantly inhibited breast tumor growth and reduced lung metastasis in these mice. Treatment of TP-3654, a novel second generation PIM1 kinase inhibitor, also significantly reduced proliferation, migration, invasion and tumor sphere formation in TNBC cells, and reduced breast tumor growth and lung metastasis in MMTV-PyMT mice. Genetic deletion or pharmacologic inhibition of Pim1 also resulted in a significant reduction of tumor-associated macrophages (TAMs) and cancer stem cells (CSC) while increased the infiltration of cytotoxic and helper T cells in the mammary tumors of MMTV-PyMT mice, suggesting a role of Pim1 in the maintenance of tumor microenvironment and immune evasion. Mechanistically, we show that deletion or inhibition of PIM1 significantly inhibited the activation of mTORC1 signaling pathway and reduced the expression of Slug, Vimentin and c-Met in TNBC cells. Additionally, we performed RNA-sequencing analysis on MDA-231 TNBC cells without or with PIM1 deletion or TP-3654 treatment to identify which pathways were affected by PIM1 deletion or inhibition. Gene Set Enrichment Analysis (GSEA) revealed significant downregulation of genes related to cell proliferation, translation, EMT and metastasis in PIM1-deleted or TP-3654-treated TNBC cells compared to control TNBC cells. Collectively, our results suggest that PIM1 plays an important role in tumor progression, immune evasion and metastasis of TNBC, and inhibition of PIM1 with TP-3654 might be potentially useful for treatment of TNBC.

#2990 Single-slide FFPE proteomic profiling enables therapeutic target quantification and molecular subtyping in non-Hodgkin's lymphoma and bone marrow tumor biopsies.

Matthew Loya[1], Tine Casneuf[2], Helene Bon[2], Manling Ma-Edmonds[1], Angelo Harris[1], Daniel Martin[1], Anantharaman Muthuswamy[1], Dominique Kamber[3], **Martin Mehnert**[3], Amaury Lachaud[3], Yuehan Feng[3], Mark Fereshteh[1], Omar Jabado[1]

[1]Genmab, Plainsboro, NJ,[2]Genmab, Utrecht, Netherlands,[3]Biognosys AG, Schlieren, Switzerland

Molecular profiling of formalin-fixed paraffin-embedded (FFPE) tumors in clinical research is performed with low-plex immunohistochemistry or genome-wide transcriptomics. The application of unbiased, highly multiplexed proteomics to FFPE is limited by sample input requirements, assay throughput and complex bioinformatics. Here we report a proteomic method amenable to low-input FFPE profiling which complements transcriptomics.

We applied Biognosys' mass-spectrometry platform (TrueDiscovery, DIA-MS) to samples encountered in routine histopathology laboratories (e.g. B-cell malignancies [n = 35], and breast, pancreatic and colorectal tumors [n=8]). FFPE samples were deparafinzed, de-crosslinked followed by trypsin digestion, and resulted peptides were analyzed by directDIA. Impact of tissue staining by Hematoxylin and Eosin (H&E) and acid decalcification was also assessed. We compared a deep-proteome workflow on a Thermo Scientific Orbitrap Exploris 480 to a novel high throughput workflow on a Bruker timsTOF HT.

The deep-proteome workflow with single-slide FFPE tissues (median area 35mm^2) generated comprehensive proteome coverage: ~12,000 proteins detected overall and ~7,000 detected in the majority of samples. The high throughput Bruker workflow delivered similar data with 80% less input in 20% of the time. Proteomes from H&E stained, cover-slipped slides were nearly identical to adjacent unstained tissue. Interestingly, proteomics profiling was successful in acid decalcified leukemia bone marrow biopsies whereas RNA was not recoverable. To assess the detection of key tissue biomarkers for therapeutic decision-making, we compiled a list of FDA approved antibody based therapeutic targets and found 20 of 21 were detectable in our samples (e.g. PDL1, PD1, LAG3, CD19, CD20 and CD38). Similarly, >80% of 55 immuno-oncology targets undergoing clinical development were detected. A key effector function of antibody therapeutics is complement-dependent cytotoxicity. The presence of complement components was confirmed by proteomics, however, some were undetectable by transcriptomics. Diffuse Large B-cell Lymphoma samples in this study were molecularly subtyped for a key prognostic, Cell of Origin (COO), using a CLIA validated RNA assay (Lymph2Cx). Well studied proteins (e.g. MUM1 and CD10) showed expected distribution based on patient COO, demonstrating proteomics can recapitulate established subtyping methods.

We demonstrate that proteomics profiling can be conducted on single FFPE slides even with pre-processed tissue. This high-throughput workflow enables real-time analysis of Phase I trials or large tissue banks. Furthermore, proteome profiling provides an opportunity to consolidate testing for multiple therapeutic targets instead of serial IHC assays, maximizing the use of precious tissue biopsies.

#2991 Systematic discovery of regulators of adoptive T-cell therapeutic resistance using *in vivo* CRISPR screening.

Chenlin Zhao[1], Sharif Ahmed[2], Xiyue Hu[1], Ranjit S. Atwal[1], Zongjie Wang[3], Sophie Xiao[1], Shana O. Kelley[1]

[1]Biochemistry and Molecular Genetics, Feinberg School of Medicine, Northwestern University, Chicago, IL,[2]Department of Chemistry, Northwestern University, Chicago, IL,[3]Department of Biomedical Engineering, McCormick School of Engineering, Northwestern University, Evanston, IL

Background: Adoptive T-cell therapy has emerged as a promising approach for the treatment of several malignancies, including melanoma. Despite promising outcomes, therapeutic resistance remains a significant challenge, with limited or transient responses observed in many patients. The molecular mechanisms driving resistance pathways have not been thoroughly studied *in vivo*. Systematic characterization of the genetic regulators of adoptive T-cell therapeutic resistance is thus critical for designing next-generation of synergistic combination therapies and treatment regimens to enhance efficacy and durability.

Methods: We developed an *in vivo* CRISPR knockout screening system to identify genetic factors mediating resistance to adoptive T-cell therapy in a melanoma mouse model. To explore potential synergistic therapeutic combinations, we opted to target the easily druggable subset of the human genome, encompassing all known kinases. CRISPR-edited B16-GFP-OVA cells transduced with a mouse CRISPR KO library (Brie: comprising 2,852 sgRNAs targeting 713 mouse kinase genes) were engrafted into C57BL/6J mice. The mice were divided into two groups: a PBS control group (n=6) and a T-cell treatment group (n=14). Tumors were harvested either at an early (Day X) or later time points (Day Y) for genomic DNA extraction and next-generation sequencing (NGS)-based guide RNA enrichment analysis. Deep sequencing data were analyzed using the MAGeCK pipeline. Functional validation of our top candidates was performed by generating single clones of single-gene CRISPR knockouts and conducting functional profiling using orthogonal assays, including flow cytometry, LDH cytotoxicity assay, and pharmacological inhibition studies to assess target engagement and efficacy.

Results: The CRISPR screen identified two key regulators of adoptive T-cell resistance, termed NU-ATCTr-101, NU-ATCTr-102. Single-gene knockouts of these targets significantly enhanced T-cell-mediated killing *in vitro*. Pharmacological inhibition of NU-ATCTr-101 demonstrated a dose-dependent increase in cytotoxicity and enhanced T-cell response. Mechanistic studies revealed alterations in antigen presentation, PD-L1 expression, and T-cell activation pathways. Bulk RNA sequencing and additional pathway analyses are ongoing to further elucidate precise molecular mechanisms underpinning these enhanced therapeutic outcomes.

Conclusion: Our findings identify NU-ATCTr-101 and NU-ATCTr-102 as potential therapeutic targets to overcome resistance to adoptive T-cell therapy. Synergistic pharmacological interventions targeting these kinases may serve as promising adjuvants towards the development of effective immunotherapeutic strategies. Future studies will focus on validating these findings across additional human cancer model systems to evaluate and expedite their translational potential.

#2992 Uncovering metabolic dysregulation and therapeutic targets in aneuploid models of squamous cell carcinoma.

Nadja Zhakula[1], Sejal Jain[2], Laura Byron[3], Joan J. Castellano[4], Matthew L. Meyerson[5], Alison M. Taylor[4]

[1]Genetics and Development, Columbia University Irving Medical Center, New York, NY,[2]Medical Oncology, Dana-Farber Cancer Institute, Boston, MA,[3]Cell and Developmental Biology, Rutgers University, New York, NY,[4]Pathology and Cell Biology, Columbia University Irving Medical Center, New York, NY,[5]Genetics and Medicine, Harvard Medical School, Boston, MA

Squamous cell carcinomas (SCCs) arise from epithelial tissues in the lung, head and neck, esophagus, bladder, and cervix. SCCs lack canonical oncogenic mutations and urgently need targeted therapies. Aneuploidy—chromosomal losses or gains, is rare and poorly tolerated in normal cells but occurs in 90% of tumors in tissue-specific patterns. SCCs are characterized by near-universal early chromosome 3p deletion and 3q gain, which correlate with poor prognosis and metastasis. To study effects of 3p deletion in human cells, we used CRISPR-Cas9 to delete a copy of 3p in human immortalized lung epithelial cells, representing the basal cell (lung SCC cell-of-origin). In a subset of clones with 3p deletion, we observed whole chromosome duplication of the unaltered chromosome 3 copy. As a result, these cells transitioned to a state of 3q gain, providing us models for 3p loss, 3q gain, and 3 disomy.

To uncover vulnerabilities associated with chromosome 3 arm aneuploidies, we performed drug-repurposing and CRISPRi screens in our engineered cells. In 3q gain cells, the top genetic hit was sterol regulatory element-binding factor 1 (SREBF1), a key regulator of cholesterol and fatty acid biosynthesis. Compared to cells without 3q gain, 3q gain cells were more sensitive to knockdown of SREBF1, co-factor SCAP, and downstream target HMG-CoA reductase (HMGCR), the rate-limiting enzyme in cholesterol synthesis. HMGCR inhibitors (several statins, including mevastatin) were top chemical hits, inducing higher cell death in 3q gain cells. Chemical inhibition of SREBF1 using fatostatin recapitulated effects seen with genetic knockdown. Rescue with mevalonate or geranylgeranyl pyrophosphate (GGPP, a product of the mevalonate pathway involved in protein prenylation) demonstrated the toxicity of pathway inhibition are on-target. Transcriptomic and lipidomic profiling suggest that increased statin sensitivity of 3q gain cells is due to their reduced activation of SREBF1 and HMGCR, causing increased cell death upon inhibition of the mevalonate pathway. Ongoing mechanistic studies will investigate how aneuploidy alters lipid metabolism and contributes to cancer.

Statins have been shown to induce tumor-specific apoptosis and reduce cancer risk, though these effects can be highly variable and context-dependent. Preliminary studies in organoid cultures and SCC cancer cell lines suggest that 3q gain is a biomarker of statin response and sensitivity in SCCs. Mevastatin and fatostatin co-treatment, inhibition at two points in the mevalonate pathway, showed synergy, suggesting statin potentiation that could be beneficial in cancer therapy. Additionally, 3q gain cells showed increased toxicity in response to other cholesterol-lowering compounds. Understanding aneuploidy-induced vulnerabilities will reveal how aneuploidies promote tumorigenesis and help identify precision medicine targets for SCCs with 3q gain.

#2993 Loss of p53 sensitizes cancer cells to oxidative phosphorylation agonism.

Jianhuang Lin[1], Dimitra Georgopoulou[2], Shuzhen Wu[3], Svaty Sachania[2], Rebecca Saunders[2], Biju Mangatt[1], Geeta Sharma[3], Euan Stronach[2], **Anthony Mazurek**[1], Fiona M. Behan[2], Benjamin Schwartz[3], Brendan A. Veeneman[3]

[1]GSK, Collegeville, PA,[2]GSK, Stevenage, United Kingdom,[3]GSK, Boston, MA

Large scale efforts using CRISPR based gene loss-of-function screens have produced comprehensive catalogs of gene essentiality in hundreds of well annotated cancer models. The equivalent catalogs have not been generated for gene gain-of-function. While loss-of-function (LOF) mutations in *TP53* are frequent in cancer, targeting these mutations pharmaceutically remains challenging. To explore synthetic lethalities with p53 LOF we performed genome-wide CRISPR activation screens in wildtype and *TP53* knockout isogenic cancer cell lines. We discovered a cluster of genes associated with oxidative phosphorylation (OxPhos) whose overexpression were synthetic lethal with p53 loss. This is consistent with the literature describing a metabolic switch from OxPhos to glycolysis after p53 mutation/loss. Our screen results suggest that this metabolic switch could be a protective action for cancer cells with mutant p53 if OxPhos activation is synthetic lethal with functional p53 deficiency. Indeed we found that overexpression of two of the top OxPhos gene hits from the screen, namely ADCK3 and SCO2, were synthetic lethal with p53 loss *in vitro*. Moreover, overexpression of ADCK3 resulted in significant growth inhibition of tumors with p53 loss *in vivo*. Mechanistically, overexpression of either ADCK3 or SCO2 significantly induced cellular ROS production and mitochondrial DNA damage in cancer cells with p53 loss. Our results suggest a model that wildtype p53 is important for maintaining the homeostasis between OxPhos and glycolysis. In the absence of p53 cells cannot tolerate the ROS generated by OxPhos and they switch to glycolytic metabolism. Our results are consistent with the model that hyperactivation of ADCK3, a critical component for electron transport chain, results in an increase in OxPhos and subsequently ROS which in the context of p53 deficient cells is lethal. Similar to p53 deficient cells, cancer cells with hotspot *TP53* mutations have been reported to undergo a similar OxPhos to glycolysis metabolic shift and are deficient in the repair of mitochondrial DNA damage caused by OxPhos generated ROS. Our results support that an ADCK3 agonist therapeutic could be an effective strategy to target mutant p53 cancers.

#2994 Co-targeting the HER family and mutant KRAS is efficacious in colorectal cancer.

Mary Kate Kilroy[1], Cecilia Wischmeier[2], Briley SoYoung Park[3], Rosalin Mishra[4], Wasim Feroz[1], Joan T. Garrett[1]

[1]Pharmaceutical Sciences, University of Cincinnati, Cincinnati, OH,[2]University of Cincinnati, Cincinnati, OH,[3]Dana Farber Cancer Institute, Boston, MA,[4]MedPace, Cincinnati, OH

We are investigating the role of naturally co-occurring HER3 and KRAS mutations in colorectal cancer, as about 6% of all colorectal cancers contain a HER3 mutation and 41% contain a KRAS mutation. After investigating the frequency of co-occurring mutations in publicly available data sets, we observed that there is a statistically significant co-occurrence of HER3 in KRAS mutations while there is mutual exclusivity with mutations in the other members of the HER family (EGFR, HER2, HER4) and mutant KRAS. We have found that there is a striking increase in total HER3 levels in SNU-407, LS180, LS513 cells and 572918-348-R and 172845-121-B patient derived organoids (PDOs) when treated with the $KRAS^{G12D}$ inhibitorsMRTX1133 and RMC9805, creating an adaptive response that could limit the efficacy of a KRAS inhibitor as a single agent. We next aimed to determine if this was biologically meaningful by genetically knocked down HER3, KRAS or the combination in SNU-407 and LS-513 cells. We have found that genetic knockdown of both KRAS and HER3 with siRNA targeting KRAS and HER3 in the CRC cell lines SNU-407 ($HER3^{V104M}$,$KRAS^{G12D}$) and LS513 ($HER3^{WT}$, $KRAS^{G12D}$) results in a statistical reduction in cell proliferation in comparison to genetic knockdown with only KRAS or HER3. Next, we examined HER3 binding partners in a panel of CRC and observed HER3 bound to EGFR in SNU-407 cells and HER3 bound to MET in LS513 cells. Co-targeting EGFR and mutant KRAS resulted in a slight synergistic effect in $KRAS^{G12D}$ mutant cell lines usingMRTX1133 in combination with sapitinib, afatinib or peltinib. Currently, we are assessing if there is a synergistic effect directly targeting HER3 with a HER3 antibody-drug-conjugate in combination with a KRAS inhibitor in CRC cells. Our findings may present a new paradigm for targeted combination therapies in colorectal cancer with the eventual goal of increased overall patient survival.

#2995 Inhibition of pyrimidine synthesis and the cell cycle in mesothelioma.

George Scaria, Marian Kratzke, Stephen Porter, Betsy Kren, **Mark A. Klein**

Minneapolis VA Health Care System, Minneapolis, MN

Introduction: Novel approaches to the treatment of advanced mesothelioma are needed, as mesothelioma is considered incurable in the vast majority of cases. Targeting 1) pyrimidine biosynthesis via inhibition of dihydroorotate dehydrogenase (DHODH) and 2) DHODH in combination with inhibition of select cyclin-dependent kinases (CDKs) are potential approaches for treating mesothelioma, as the cell cycle is altered most mesothelioma cells.

Methods: We conducted cell proliferation assays in mesothelioma cell lines to determine the IC_{50} for the DHODH inhibitor BAY2402234 as compared with the older generation DHODH inhibitor brequinar. BAY2402334, brequinar, or control were incubated with mesothelioma cells for 72 hours and cell viability was determined using a Cell Counting Kit-8 spectrophotometric assay. Experiments were conducted with and without uridine at 100 mM to determine the effect of “uridine rescue” on the IC_{50} values for each DHODH inhibitor. We also determined mRNA expression via RNA-sequencing (RNA-Seq) and protein expression via immunoblotting of key mRNA transcripts and corresponding proteins. We then conducted cell proliferation assays to determine if the combination of BAY2402234 plus dinaciclib (inhibitor of CDKs 1, 2, 5, and 9) could be additive or synergistic.

Results: BAY2402234 exhibited a significant degree of potency against 4 mesothelioma cell lines with IC_{50} values ranging from 55 picomolar (pM) to 4.63 nanomolar (nM). Uridine rescue resulted in a significant shift in the fitted curves (suggesting decreased efficacy of BAY2402234 due to uridine supplementation), which supports the notion that DHODH is a major mechanism of BAY2402234 as anticipated. Overall, BAY2402234 was between 59 to 1,811 times more potent than brequinar against mesothelioma cells. After exposure to BAY2402334 or control for 48 hours, H2373 cells were evaluated via RNA-Seq and immunoblotting. Of note, PD-L1 mRNA increased approximately 7-fold, and PD-L1 protein expression increased 1.5-2-fold. In addition, mRNA expression of CDKN1A (p21 protein), Cyclin D1, Cyclin A1, and CDK1 increased. Cytokine pathway interactions also increased. For several concentration combinations, the combination index was < 1, suggesting a synergistic effect of that combination of drugs on decreasing cell proliferation.

Conclusions: BAY2402234 is an extremely potent inhibitor of cell growth in multiple mesothelioma cell lines and 59-1,881 times more potent than brequinar; 2) BAY2402234 and dinaciclib act synergistically, suggesting an interaction between pyrimidine biosynthesis and one or more of the following CDKs: CDK1, CDK2, CDK5, and/or CDK9; and 3) BAY2402234 results in increased PD-L1 expression and increased cytokine pathway mRNA expression, suggesting the potential for enhancement of immune checkpoint blockade.

#2996 TNG462, an MTA-cooperative PRMT5 inhibitor, demonstrates strong efficacy in combination with clinically relevant targeted therapies in MTAP-null preclinical models.

Minjie Zhang, Alice Tsai, Kevin M. Cottrell, Brian B. Haines, Alan Huang, Jannik N. Andersen, Adam S. Crystal, **Kimberly J. Briggs**

Tango Therapeutics, Boston, MA

TNG462 is a clinical-stage, MTA-cooperative PRMT5 inhibitor currently being evaluated in an ongoing phase I/II clinical trial for solid tumors with MTAP loss. Approximately 10-15% of all human cancers exhibit MTAP deletions, including 10-15% of lung cancer and more than 20% of pancreatic adenocarcinomas. MTAP deletion frequently co-occurs with other genetic alterations: ~30% of MTAP-deleted lung adenocarcinomas and ~92% of MTAP-deleted pancreatic ductal adenocarcinomas (PDAC) also harbor KRAS mutations, while ~20% of MTAP-deleted lung adenocarcinomas are also EGFR mutant. Although TNG462 monotherapy drives deep and durable tumor regressions in MTAP-null preclinical models, we investigated its potential in combination with therapies targeting actionable genetic alterations. In relevant models, the combination of TNG462 with either KRAS or EGFR inhibitors led to significant tumor regressions, surpassing the effects of either agent alone. This supports our clinical development plans for TNG462, which include targeted combinations with two RAS(ON) inhibitors, RMC-6236 and RMC-9805 from Revolution Medicines, as well as the EGFR inhibitor osimertinib from AstraZeneca. Additionally, nearly all MTAP-deleted cancers are also CDKN2A-deleted due to the proximity of the two genes on chromosome 9p, resulting in de-repression of the cyclin D-dependent CDK4/6 complexes, which creates a susceptibility to CDK4/6 inhibition. Significant efficacy was observed in preclinical models with the combination of TNG462 and CDK4/6 inhibitors, supporting a potential development path in GBM for our next generation CNS penetrant PRMT5 inhibitor, TNG456, with abemaciclib. Lastly, MTAP loss also sensitizes cells to MAT2A inhibition, providing strong rationale for combining PRMT5 inhibitors with MAT2A inhibitors. Preclinical data demonstrated significant efficacy when subtherapeutic doses of both agents are combined. However, TNG462 monotherapy at a clinically relevant dose achieved comparable benefits to the combination. Collectively, these findings strongly support evaluating TNG462 in combination with other targeted therapies in clinical trials for patients with cancers that exhibit MTAP loss.

#2997 Cell-based assay platforms for NLRP3 inflammasome drug discovery.

Jianghong Wu, Li Liang, Peter Gallagher, Yong Wan, Haiching Ma

Reaction Biology Corporation, Malvern, PA

Inflammasomes have recently emerged as the exciting and promising drug targets for inflammatory disease therapy. The best characterized is the NLRP3 inflammasome. The NLRP3 inflammasome is associated with onset and progression of various diseases, including metabolic disorders, multiple sclerosis, inflammatory bowel disease, cryopyrin-associated periodic fever syndrome, as well as other auto-immune and auto-inflammatory diseases. The NLRP3 inflammasome is a cytosolic multiprotein complex composed of the innate immune receptor protein NLRP3, adapter protein ASC, and inflammatory protease caspase-1 that responds to microbial infection, endogenous danger signals, and environmental stimuli. The assembled NLRP3 inflammasome can activate the protease caspase-1 to induce gasdermin D-dependent pyroptosis and facilitate the release of IL-1β and IL-18, which contribute to innate immune defense and homeostatic maintenance. We have established cell-based Caspase-Glo 1 Inflammasome Assay and NanoBRET target engagement intracellular NLRP3 assay for high throughput screening of inflammasome inhibitors. MCC950 is a cell-permeable, bioavailable, non-toxic sulfonylurea-derived compound. Here, we demonstrate that MCC950 inhibits LPS/Nigericin-induced caspase-1 activation and pyroptotic cell death in dose response mode in PMA primed THP-1 cells in Caspase-Glo 1 inflammasome and CellTiter-Glo viability assays. Our NanoBRET target engagement NLRP3 assay results show that MCC950 binds to cellular NLRP3 within 2 hours of incubation. Interestingly, NanoBRET NLRP3 residence time analysis in living cells indicates that a slow dissociation kinetics (long residence time) is observed for MCC950. Furthermore, Western blot analysis confirms that MCC950 blocks LPS/Nigericin-induced caspase-1 cleavage and activation in PMA primed THP-1 cells. Taken together, our results indicate Caspase-Glo 1 inflammasome and NanoBRET target engagement NLRP3 assays serve as great cell-based assay platforms to facilitate inflammasome drug discovery against inflammatory diseases.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Kinase and Phosphatase Inhibitors 2
Poster Session

#3000 Targeting ERK and PI3K signaling in pancreatic ductal adenocarcinoma.

Gauthami Nair, Katie Reindl, Mohiuddin Quadir

North Dakota State Univ., Fargo, ND

Pancreatic ductal adenocarcinoma (PDAC) remains one of the deadliest malignancies, with a dismal five-year survival rate of only 13%. The high mortality is largely due to late-stage diagnosis, limited surgical options (available in just 10-20% of cases), and the failure of current chemotherapy regimens, which are often plagued by toxicity and drug resistance. Therefore, innovative therapeutic strategies targeting key signaling pathways driving tumor progression are urgently needed. Mutations in the *KRAS* gene, present in approximately 95% of PDAC cases, lead to hyperactivation of the KRAS protein and downstream pathways, including extracellular signal-regulated kinase (ERK) and phosphatidylinositol 3-kinase (PI3K). In this study, we hypothesized that simultaneous inhibition of these critical pathways could effectively suppress PDAC cell proliferation. Using SCH772984 (an ERK 1/2 inhibitor) and pictilisib (a class I PI3K inhibitor), we evaluated their combined effects on human PDAC cell lines (MIA PaCa-2 and PANC-1) and a patient-derived xenograft (PDX) cell line. Our results demonstrate significant synergy between these two inhibitors, as evidenced by reduced cell viability and colony formation in clonogenic assays compared to single-agent treatments. Western blot analysis confirmed that SCH772984 was able to inhibit its target ERK 1/2 and pictilisib was able to inhibit AKT, that is downstream of PI3K. These findings suggest that dual targeting of ERK and PI3K pathways effectively disrupts PDAC cell growth. Future studies will focus on formulating these inhibitors into nanoparticle-based drug delivery systems to enhance solubility, stability, and tumor-specific delivery. Additionally, preclinical testing in animal models will provide critical insights into the therapeutic potential of this combination. This approach represents a promising avenue for improving PDAC treatment outcomes and addressing unmet needs in this aggressive disease.

#3001 Highly selective and oral bioavailable PI3Kγ inhibitor for cancer immunotherapy by targeting myeloid-derived suppressor cells in tumor.

Hanning Wen, Shuai Mao, Mahamadou Djibo, Chengyi Li, Jinsong Tao, Hongyi Zhao, Qiyan Wang, Weijia Zheng, Bo Wen, Wei Gao, Duxin Sun

University of Michigan, Ann Arbor, MI

Introduction: Phosphoinositide 3-kinases (PI3Ks) are crucial for cell growth, survival, and metabolism, with dysregulated signaling common in cancer. The PI3K family includes four isoforms—PI3Kα, PI3Kβ, PI3Kδ, and PI3Kγ—each with distinct roles, where pan-PI3K inhibition often results in toxicity due to broad isoform expression. Existing various PI3K isoform inhibitors, despite *in vitro* specificity, often act as pan-inhibitors at high *in vivo* clinical effective concentrations (1μM to 5μM). We developed a highly selective PI3Kγ inhibitor that retains its specificity even at therapeutic *in vivo* levels, effectively targeting tumor immunosuppressive environment to inhibit tumor growth, while reducing toxicity associated with pan-PI3K inhibitions.

Methods: Newly synthesized compounds were screened for PI3K isoform selectivity by kinase binding assays. After oral administration (p.o.), tissue distribution studies compared lead compounds with IPI-549 across various tissues, including tumor, spleen, tumor-draining lymph nodes (TDLNs), plasma and liver. The lead PI3Kγ inhibitors were further evaluated *in vivo* using MC38 colon cancer model and MMTV-PyMT breast cancer model, in both monotherapy (p.o.) and combination therapy with paclitaxel (PTX) or anti-PD-1 antibody (aPD-1). Immune cell profiling in tumor microenvironment was analyzed by flow cytometry post-treatment.

Results: The lead compounds, SH-315 (γ IC_{50}110.4nM, δ IC_{50}>10000nM, β IC_{50}>10000nM, α IC_{50}3212nM) and SH-327(γ IC_{50}297.4nM, δ IC_{50}>10000nM, β IC_{50} >10000nM, α IC_{50}2438nM), demonstrated significantly higher selectivity for PI3Kγ compared to IPI-549 (γ IC_{50} 5.5nM, δ IC_{50} 968.8nM, β IC_{50} 1566nM, α IC_{50} 1248nM). Concentrations of SH-315 and SH-327 were higher in target tissues (tumors, spleen, TDLNs), while plasma levels were lower than IPI-549, potentially reducing PI3K inhibitor-related side effects. In MMTV-PyMT model, SH-327 and SH-315 at 20 mg/kg, combined with PTX and aPD-1, led to significantly smaller tumors compared to IPI-549. In MC38 model, SH-315 at 10mg/kg combined with PTX and aPD-1 achieved Complete Response (CR) in 60% mice, where SH-327 at 20mg/kg achieved CR in 75% mice. SH-315 at 10mg/kg decreased CD11b+Ly6C+ myeloid-derived suppressor cells (MDSCs) in MC38 tumor by 24% compared to IPI-549 and increased CD8+ T cells in tumor by 41% than IPI-549, suggesting enhanced immune activation and reduced immunosuppression.

Conclusion: The PI3Kγ inhibitors SH-315 and SH-327 demonstrated superior anti-tumor efficacy compared to IPI-549. Treatment with SH-315 and SH-327 reduced immunosuppressive MDSCs and increased cytotoxic CD8+ T cell activity, highlighting their potential to drive anti-tumor responses through targeted immune modulation and supporting their further development for cancer immunotherapy.

#3002 GSC002639, a brain penetrating and mutant-selective PI3Kα inhibitor, exhibits potent mono- and combination therapy against PI3CA mutant tumors.

Xing Fan, Sheng Zhao, Hang Xu, **Biao Lu**, Yuanfeng Xia, Fanglong Yang

Changchun GeneScience Pharmaceutical Co., Ltd., Shanghai, China

Background: PIK3CA gene mutation is recognized as a significant driver of oncogenesis in various human cancers. Approximately 30% of breast cancers, 25% of endometrial cancers, 15% of colorectal cancers, 10% of ovarian cancers, and 5% of lung cancers exhibit mutations in the PIK3CA gene. The approvals of Alpelisib and Inavolisib have demonstrated the therapeutic benefits of PI3Kα inhibition for patients with PIK3CA mutations. However, their inhibition of wild-type PI3Kα leads to side effects such as hyperglycemia with insulin resistance, rash, and diarrhea, which limit their efficacy.
Material and methods: The suppressive effects on cell proliferation and phosphorylated AKT were analyzed across cellular models harboring various PIK3CA mutations. The capacity of GSC002639 to inhibit the isoforms PI3Kβ, PI3Kγ, and PI3Kδ, along with additional kinases, was determined through in vitro kinase activity tests. CDX model were used for in vivo efficacy evaluation. Furthermore, the pharmacodynamic properties of GSC002639 and its impact on glucose imbalance were investigated following established procedures. Preclinical toxicology studies were conducted with GSC002639 over 28 days of dosing in rats and dogs.
Results: GSC002639 exhibited nanomolar potency against PI3Kα hotspot mutations while maintaining approximately 30-fold selectivity over the wild-type PI3Kα. Furthermore, GSC002639 displayed excellent selectivity over other PI3K isoforms, including PI3Kβ, δ, and γ. GSC002639 showed favorable pharmacokinetic properties and strong antitumor activity against cancer cells that express the PI3Kα H1047X mutations, without causing elevated plasma insulin levels, even at high dosages. Our study also revealed a notable observation: In an HR-positive and HER2-negative PI3Kα H1047X CDX model, the combination of GSC002639 with Fulvestrant exhibited greater efficacy than the combinations of either Alpelisib or Inavolisib with Fulvestrant. In addition, GSC002639 showed good brain penetrating capability with a brain/plasma ratio of 0.26. In preclinical toxicology studies, GSC002639 demonstrated excellent therapeutic safety window in F/M rats with 14.6/12.7 folds and in F/M dogs with15.3/28.1 folds, respectively.
Conclusions: In this report, we present the identification and characterization of GSC002639, a selective inhibitor targeting the PI3Kα H1047X mutation. GSC002639 demonstrates high specificity, the ability to cross the blood-brain barrier, and robust in vivo efficacy, while avoiding the insulin elevation. The promising pharmacokinetic and toxicological profiles of GSC002639 suggest its potential as a safe and effective cancer therapy. GSC002639 is undergoing IND-enabling studies, with plans for its clinical evaluation as both a single-agent therapy and in combination regimens for patients with PI3Kα mutant cancers by early 2025.

#3003 Targeting mechanisms of adaptive resistance to the PI3Kαmutant selective inhibitor RLY-2608 in HR+/PIK3CA mutant breast cancer.

Fabiana Napolitano[1], Chang-Ching Lin[1], Khushi Ahuja[2], Dan Ye[1], Maria Rosario Chica-Parrado[1], Yasuaki Uemoto[1], Pamela Luna[1], Yuki Matsunaga[1], Saurabh Mendiratta[1], Nisha Unni[1], Jeon Lee[2], Ariella B. Hanker[1], Carlos L. Arteaga[1]

[1]Harold C. Simmons Comprehensive Cancer Center, Dallas, TX,[2]Lyda Hill Department of Bioinformatics, University of Texas Southwestern Medical Center, Dallas, TX

The *PIK3CA* gene is mutated in ~40% of HR+/HER2- breast cancers. Alpelisib and inavolisib are currently FDA approved for treatment of *PIK3CA*-mutant breast cancers, although neither is selective for mutant *PIK3CA*. Inhibition of wild-type PI3K results in multiple adverse events, including hyperglycemia, which limit the dose and duration of treatment. Further, inhibition of PI3K/AKT relieves feedback suppression of receptor tyrosine kinase (RTK) expression and activity, thus limiting complete inhibition of PI3K/AKT signaling in cancer cells and resulting in adaptive drug resistance. Whether PI3K mutant-selective inhibitors can more potently block this compensatory adaptive resistance is unclear. In this study, we interrogated early adaptive resistance mechanisms to the PI3Kα mutant-selective inhibitor RLY-2608 in HR+ breast cancer cells with *PIK3CA* mutations. Phospho-RTK arrays of MCF7 and T47D cells treated with the IC_{75} of RLY-2608 for 0-48h revealed feedback activation of the ERBB family of RTKs (EGFR, HER2, HER3, HER4) at 24 h. These results were confirmed by immunoblot analysis with phospho-specific antibodies. After an initial inhibition of MAPK and PI3K/AKT signaling at 6 h, we found rebound activation of pERK1/2, pAKT1, and pS6 at 24 h, which was maintained for 48 h, suggesting a potential mechanism of adaptive resistance. To identify the ERBB RTKs causing this adaptation, we tested the TKIs erlotinib, neratinib, and tucatinib, and the HER3 antibodies patritumab and zenocutuzumab, each in combination with RLY-2608. RLY-2608 combined with the pan-HER TKI neratinib and the HER2-selective TKI tucatinib most effectively reduced pERK1/2, pAKT1 S473, and pS6 at 48 h, suggesting inhibition of HER2 could block this adaptive mechanism. In short and long-term proliferation assays, neratinib and tucatinib also synergized better with RLY-2608 compared to the other inhibitors. GSEA of RNA-seq data from MCF7 and T47D cells treated with RLY-2608 for 48 h revealed upregulation of pathways related to tyrosine kinase signaling, extracellular matrix, and redox signaling. FoxO signaling was one of the top upregulated KEGG and Reactome pathways, suggesting that activation of FoxO transcriptional activity may compensate for PI3K/AKT inhibition. The Hallmark Estrogen signaling pathway was also upregulated following RLY-2608 treatment; this was validated by immunoblot analysis, showing that ERα levels increased in T47D and MCF7 cells treated with RLY-2608 over a time course. Other upregulated Hallmark pathways in both cell lines included Myogenesis, Hypoxia, and KRAS signaling down. These findings suggest that, in addition to RTK activation, adaptive resistance to RLY-2608 may involve metabolic reprogramming, increased oxidative stress, and hypoxia signaling, highlighting the need for combination strategies to overcome resistance and enhance treatment efficacy.

#3004 Preclinical characterization of CGT6297, a novel PI3Kα H1047R mutant-selective inhibitor.

Aaron C. Smith, Ben Arwood-Levine, Abiezer Blandon, Alexandra Born, Richard Brizendine, Payal Chatterjee, Mark J. Chicarelli, Michael L. Conner, Brad Fell, Jennifer Fulton, Anna Guarnieri, Hannah Hubert, Ravi Jalluri, Hailey Knox, Keith Koch, Daniel Krischlunas, Vijay Kumar, Sara Kuzbiel, Matt McDonald, Colin McHugh, Brent Mclean, Kelsey W. Nassar, Paul Larsen, Brad Newhouse, Scott Niman, Rob Rieger, John Robinson, Mareli Rodriguez, Leah Salituro, Vinny Scarato, Lee Stunkard, Francis Sullivan, Patrick Sutter, Roy Turton, Rob Van Gulick, Brooklynn Venteicher, Logan Vine, Shannon Winski, Shannon Winski, Hannah Work, Yeyun Zhou

Cogent Biosciences, Inc., Boulder, CO

PIK3CA encodes the p110α catalytic subunit of PI3-kinase alpha (PI3Kα) and is the most frequently mutated kinase in human cancer with common mutations occurring in the kinase domain (H1047R) and helical domain (E542K/E545K). The approved PI3Kα inhibitor, alpelisib, shows promise for this targeted class of agents with improvements in progression-free survival in ER+/Her2- breast cancer patients in combination with fulvestrant. However, toxicities attributed to the inhibition of wild-type PI3Kα, such as hyperglycemia, gastrointestinal issues, and skin reactions, lead to sub-optimal target engagement due to requisite dosing modifications. A PI3K mutant inhibitor that spares WT PI3K is predicted to be better tolerated, require fewer dosing modifications, and therefore, have the potential to provide improved clinical benefit. Herein, we present the preclinical characterization of CGT6297, a wild type sparing PI3Kα H1047R inhibitor.

#3005 Discovery of a potential therapeutic mechanism for selected tumors via targeting PI3K and DDR pathways simultaneously with a novel orally available small molecule.

W. Chen, W. Xu, Y. Wang, Z. Zhou;
LittDD Medicines Ltd, Hangzhou, China

The genomic instability and hyperactivation of PI3K pathway have been observed in many types of tumors. Targeting some of the key proteins among these pathways such as ATR or mTOR individually has been studied in many clinical trials with various small molecular drug candidates, but so far these efforts have failed to deliver therapeutic benefits to patients. One of the key challenges for targeting these targets is the lack of therapeutic window due to their significant toxicities in clinical trials. It has been evidenced that both PI3K and DDR pathways can be activated simultaneously in many tumor types. We hypothesize that targeting both mTOR and ATR simultaneously could generate synergistic effect to lower the toxicities while maintaining the efficacy in selected tumors. Through rational design, we discovered a novel orally available small molecule LIT-00814 which solely inhibits both mTOR and ATR with great selectivity against other kinases. In preclinical studies, LIT-00814 demonstrates much larger therapeutic window while maintaining good efficacy in selected tumor models with higher DDR mutations and PI3K pathway activities. A specific Akt1-S473 phosphorylation antibody LIT-A0001 has also been developed to precisely stratify patients in clinical trials which is critical to eliminate Akt2 and Akt3 signals and identify right patients for LIT-00814. The clinical phase I trials for LIT-00814 were initiated and the preliminary results hold promise to deliver beneficial therapy to selected cancer patients. In summary, we have discovered a novel orally available small molecule LIT-00814 targeting both mTOR and ATR with novel therapeutic mechanisms of action which could deliver beneficial therapy to selected cancer patients.

#3006 Efficacy of novel CDK9/CDK4/6/PI3K triple inhibitors, LCI133 and LCI139, in Ewing sarcoma.

Kaitlyn H. Smith[1], Cody C. McHale[2], Kenzie Wells[1], Krishnaiah Maddeboina[2], Kimberly Q. McKinney[1], Poornima Gourabathini[1], Jeffrey S. Huo[1], Donald L. Durden[2], Javier Oesterheld[1]

[1]Pediatric Oncology Research Laboratory, Atrium Health, Charlotte, NC,[2]Molecular Targeted Therapeutics Laboratory, Atrium Health, Charlotte, NC

Introduction: The survival rate of patients with Ewing sarcoma (ES) has seen slow improvement over the past several decades and remains dismal for those with recurrent or metastatic disease. Initial clinical trials in relapsed/refractory ES with single agent targeted therapies, or combinations of single agents, have not yet improved patient outcomes. To overcome this barrier, the Molecular Targeted Therapeutics Laboratory has developed by *in silico* design “first in class” small molecule multikinase inhibitors (MKIs) which simultaneously target multiple tumor-promoting pathways with a single drug at nM potency. LCI133 (CDK4/6/CDK9) and LCI139 (PI3K/CDK4/6/CDK9) potently inhibit key kinases that control mitogenic signaling, proliferation, and transcription. The purpose of this study is to evaluate the potency, efficacy, and toxicity of LCI133 and LCI139 in ES model systems.
Methods: ES cell lines and normal human skeletal muscle cells (SkMC) were treated with MKIs in logarithmic fashion and IC_{50}s were determined by CellTiter-Glo 2.0 viability assays. Incucyte® Annexin V Dye was used to evaluate apoptosis over time by Incucyte® S3 following MKI treatment. Cell cycle analysis was performed over time using Incucyte® Cell Cycle Lentivirus Reagent and analyzed using an Incucyte® S3. Western blot and qPCR analysis was used to assess protein and RNA expression changes, respectively, of targeted pathways to confirm on-target activity of these MKIs following 3-24 hr treatment.
Results: LCI133 and LCI139 were nanomolar potent in ES cell lines TC71 (392.5nM/312nM) and A673 (471.5nM/407.4nM), while only LCI139 was nanomolar potent in the RDES (262.2nM) cell line. Critically, LCI133 and LCI139 were less toxic against SkMC compared to single agent CDK9 inhibitors. In TC71 and A673 cells, both compounds induced statistically significant dose-dependent apoptosis, as well as G1 phase cell cycle arrest. Three hr LCI133 and LCI139 treatment decreased expression of CDK9-RNA polymerase II response genes *MCL1* ($P \leq 0.001$) and *MYC* ($P \leq 0.001$). 24 hr treatment had differential effects on pRb and pAKT levels. pRpb1 and MCL-1 were reduced in all cell lines by LCI139 and/or LCI133. Increased cleaved PARP (cPARP) was observed in all cell lines following 24 hr treatment with LCI133 and LCI139 confirming the induction of apoptosis.
Conclusion: Novel small molecule MKIs allow for the simultaneous targeting of multiple pathways while mitigating the toxicity of single agent combination therapy. This is a promising potential treatment strategy for ES patients for whom new treatment approaches are desperately needed. These studies provide preclinical evidence of the potential efficacy of small molecule MKIs in ES and support the future efforts of *in vivo* models evaluating efficacy and safety of these agents.

#3007 The epigenetic regulators KDM5C and KAT6A influence AKT inhibitor response in ER positive breast cancer.

Valentina Cutano[1], Shanade Dunn[1], Lorna Hopcroft[1], Eleanor M. Wigmore[1], Lambert Montava Garriga[1], Sungmi Park-Chouinard[2], Wu Qing[2], Lee Hyung Joo[2], Cath Eberlein[1], Michele Chirichella[1], Daniel Barrell[1], Toby Gurran[1], Omid Tavana[2], Neeraj Aryal[2], Jerome T. Mettetal[2], Huayang Liu[2], Ho Man Chan[2], Susan E. Critchlow[1], Ultan McDermott[1], Simon T. Barry[1]

[1]Astrazeneca, Cambridge, United Kingdom,[2]Astrazeneca, Boston, MA

Breast cancer is one of the most common cancers worldwide, and around 80% of breast cancers are oestrogen receptor positive (ER+ BC). For patients with ER+ advanced BC with one or more *PIK3CA/AKT1/PTEN* tumor alterations, capivasertib (AKT inhibitor) in combination with fulvestrant (selective oestrogen receptor (ER) degrader) is a recommended treatment. An innate PI3K/AKT pathway inhibitor resistance can occur in patients with ER+ BC, limiting their efficacy. Therefore, novel therapeutic strategies are needed to increase sensitivity to treatments in patients with ER+ BC. With this aim, from CRISPR-based sensitization screens in ER+ BC cells, we have found that the effectiveness of the AKT inhibitor capivasertib is improved by targeting the epigenetic regulators, KDM5C (lysine demethylase 5C) or KAT6A (lysine acetyltransferase 6A). Knockout (KO) of KDM5C or KAT6A strongly enhances the response to capivasertib monotherapy and its treatment in combination with fulvestrant in *PIK3CA*-mut and *PTEN*-null ER+ BC cells, as well as re-sensitizes capivasertib-resistant ER+ BC cells. Importantly, the effects of the genetic KO were recapitulated with a pan-KDM5 inhibitor and a selective KAT6A/B inhibitor in combination with capivasertib in ER+BC cells, suggesting that combination therapies, consisting of capivasertib with KDM5 or KAT6A inhibitors, could be beneficial in patients. Moreover, transcriptomic analysis and chromatin profiling techniques show that both KDM5C and KAT6A KO downregulate expression and alter chromatic accessibility of a subset of ER-target genes and this phenotype is enhanced in combination with capivasertib, suggesting that targeting KDM5C or KAT6A sensitizes cells to AKTi through a novel epigenetic regulation of ER-signaling. Also, this study reports for the first time a landscape of epigenetic differences upon capivasertib monotherapy treatment and in combination with fulvestrant in ER+BC cells, revealing changes in chromatin accessibility as a consequence of treatment with AKTi and the ER degrader. This suggests that epigenetic remodelling can lead to resistance to capivasertib and fulvestrant therapy in cancer cells. In summary, this findings identified and validated KDM5C and KAT6A as novel epigenetic targets, the inhibition of which enhances efficacy of AKTi in PI3K pathway mutated ER+ breast cancer. Inhibitors of KDM5C and KAT6A may be potential combination partners for capivasertib in ER+ breast cancer.

#3008 The interplay between FOXO3 and FOXM1 influences sensitivity to AKT inhibition in PIK3CA and PIK3CA/PTEN altered estrogen receptor positive breast cancer.

Valentina Cutano[1], Ming Li Chia[2], Eleanor M. Wigmore[1], Stuart C. Williamson[1], Amanda L. Christie[3], Brandon Willis[3], James Kerr[1], Sophie D'Arcy[1], Lauren Bradshaw[1], Cath Eberlein[1], Lambert Montava Garriga[1], Elza de Bruin[1], Susan E. Critchlow[1], Kevin M. Brindle[2], Simon T Barry[1], Susana Ros[1]

[1]Astrazeneca, Cambridge, United Kingdom,[2]Cancer Research UK, Cambridge, United Kingdom,[3]Astrazeneca, Boston, MA

Combining fulvestrant (a selective estrogen receptor (ER) degrader) with the PI3K-AKT signalling inhibitors such as alpelisib (PI3Kα inhibitor) or capivasertib (AKT inhibitor) gives benefit to patients with ER+ breast cancer (ER+ BC) harboring *PIK3CA* mutations (alpelisib) and *PIK3CA*, *PTEN* and *AKT-1* altered tumors (capivasertib). While *PIK3CA* mutations are common, PI3K-AKT signalling activation can also occur due to other alterations, including in *PTEN* or *AKT-1*, which alter pathway function and dependency. Deletion of *PTEN,* leading to ablation of PTEN protein, attenuates the effectiveness of PI3Kα inhibition in *PIK3CA* altered tumors. Our study demonstrates that in ER+ BC tumors with *PIK3CA* activating mutations, co-occurring *PTEN* homozygous or hemizygous loss is common. This study evaluates the impact of reduced PTEN protein expression on the efficacy of AKT inhibition with capivasertib in the presence of *PIK3CA* mutations. In *PIK3CA* altered and intact PTEN protein, *in vivo* tumor and *in vitro* cell line models, PI3Kα and AKT inhibitors are equally effective. However, in *in vivo* and *in vitro PIK3CA* altered models, reduction in PTEN protein expression reduces the effectiveness of PI3Kα inhibition alone and in combination with fulvestrant, while AKT inhibition remains effective. The anti-tumor activity of both monotherapy and combination treatment with PI3Kα or AKT inhibitors is associated with a decrease in FOXM1 protein and FOXM1-mediated gene expression. The regulation of FOXM1 expression is FOXO3-dependent and is directly related to the ability of the PI3Kαi or AKTi to reduce AKT dependent downstream signalling. When FOXO3a is deleted from PTEN deleted - T47D and MCF7 cells, treatment with capivasertib did not reduce FOXM1 expression. Indeed, FOXO3a loss leads to an increase in FOXM1 expression, resulting in no impact on cell viability despite the presence of capivasertib. These findings indicate that inhibiting AKT signalling is a more effective strategy than targeting PI3Kα to address this crucial effector node across a heterogeneous array of PI3K-AKT tumor drivers. Furthermore, the study highlights the pivotal role of the AKT-FOXO3-FOXM1 axis in mediating responses to AKT inhibitors in ER+ breast cancer with *PIK3CA* mutations regardless of *PTEN* expression. In addition, loss of FOXO3a expression can mediate resistance, and FOXM1 downregulation serves as a crucial biomarker of response to the combination treatment.

#3009 STX-478, a *PIK3CA* mutant-selective inhibitor, alone and in combination with romidepsin for *PIK3CA* mutant CRC.

Addison T. Zick, Alexa E. Schmitz, Alec B. Cornelio, Cheri A. Pasch, Dustin A. Deming

University of Wisconsin-Madison, Madison, WI

Background: Hyperactivation of PI3K has been linked to enhanced tumor cell growth and survival, highlighting its importance as a therapeutic target in colorectal cancer (CRC). In this study, we aim to explore the effects of STX-478, a mutant-selective, allosteric *PIK3CA* inhibitor, to assess its impact on *PIK3CA* mutant CRC. Additionally, this study aims to investigate a potential therapy in combination with STX-478 that may amplify its efficacy. A histone deacetylase inhibitor, romidepsin, was chosen as a therapy of interest due to previous work that has shown enhanced activity in CRC lines in combination with PI3K inhibitors.

Methods: CRC cell lines SW48 and SW48PK (Horizon Discovery, SW48 with a $PIK3CA^{H1047R}$ mutation) were utilized in a WST assay to determine the impact of STX-478 treatment. Immunoblotting was performed on SW48 and SW48PK with STX-478 single agent or in combination with romidepsin. ImageJ was used to quantify these blots to evaluate PI3K protein expression. Patient derived cancer organoids (PDCOs) were utilized to determine how growth was affected by STX-478 treatment. PDCO lines were derived from biopsies and surgical procedures, after approved Institutional Review Board protocols and with patient consent. Their response was determined by measuring the longest diameter of any given PDCO at Day 0 versus Day 2. Glass's Delta was determined to quantify effect size and assess any significant growth reduction. Romidepsin was also tested as a secondary agent in both western blots and diameter analysis.

Results: SW48 did not have a significant change in cell viability at high concentrations of STX-478 compared to controls. In contrast, SW48PK showed a significant response to STX-478 treatment at even 500 nM ($p<0.001$). Immunoblotting showed little difference in phosphorylation of AKT in both SW48 and SW48PK, however, in SW48PK, at low concentrations of STX-478, a decrease of phosphorylated ribosomal S6 was seen. In a $PIK3CA^{H1047R}$ mutant PDCO line, a significant response was seen at 750 nM, (Glass's delta = 1.16) 1,000 nM (1.29), 1500 nM (1.51), and 2000 nM (1.62). When treated with the combination of STX-478 and romidepsin, PDCOs had a significant response compared to control and single agents (median percent relative change 12%; control: 39.1% $p<0.001$, STX-478: 27.8%, $p<0.001$, Romidepsin: 19.4%, $p = 0.013$).

Conclusion: This study demonstrates that STX-478, a mutant-selective, allosteric *PIK3CA* inhibitor, exhibits varying effects on CRC cell lines in both 2D and 3D culture models. The combination of STX-478 and romidepsin is promising and deserves further *validation in vivo.*

#3011 Novel CDK12 inhibitors with preclinical anti-tumor activity and best-in-class profile on HER2 low breast cancer patient samples.

C. Seth Nanda, M. New, A. Velentza-Almpani, J. Martinez-Torres, N. K. Bah, N. A. Leonard, F. A. B. Sangkolah, K. K. Nan, E. Peerani, D. Ranatunga;
Pear Bio, London, United Kingdom

CDK12 is an oncogenic ATP-dependent kinase that participates in transcriptional regulation by directly phosphorylating RNApol-II when in complex with cyclin K. Overexpression or amplification of CDK12 is associated with hyper-transcription including upregulation of DNA damage repair enzymes (e.g., RAD51) which then interact with BRCA1, BRCA2 and modulate genomic instability (CDK12 directly phosphorylates cycE1 in the pre-replication complex) and induce uncontrolled cellular proliferation. Immunohistochemistry (IHC) experiments have shown high expression of CDK12 in multiple solid tumors and is associated with poor prognosis. Genomic or chemical inhibition of CDK12 reduces cancer cell proliferation in vitro and in vivo.

A series of novel CDK12 inhibitors were designed, synthesized and screened in a panel of efficacy, selectivity, safety/toxicity, target engagement and functional assays. PB301, PB316, PB325 and PB338 were identified as lead reversible inhibitors exhibiting high on-target potency and low off-target inhibition (non-transcriptional CDKs e.g., CDK2, CDK4, CDK6). For instance, PB325 and PB301 were, respectively, ~800x and 16x more potent than SY-143 (a selective CDK12/13 inhibitor) in 2D cell proliferation assays on a panel of cancer cell lines (e.g., IC50 of 0.1nM for PB325, 5nM for PB301 and 81nM for SY-143 on CAL51 cell line). Moreover, in efficacy assays, the median IC50 for PB316 was 42.99nM (n=7 cell lines), 66.4nM (n=10 patient-derived organoids) and 51.25nM (N=10 primary breast microtumors). Based on this, 50nM was chosen as the CDK12i dose to be tested as an exploratory drug (monotherapy and combo therapy) using fresh ex vivo tissue samples from patients enrolled in the observational clinical trial PEAR-TNBC (NCT05435352) to identify potential combination drug partners and compare drug efficacy to standard-of-care neoadjuvant therapies. Using in vivo models, good bioavailability, plasma and tissue distribution was observed in PK/PD studies upon oral dosing for all the lead compounds in Balb/c mice and key biomarkers like RNApol-II pSer2 and RAD51 showed significant decrease upon successful inhibition of CDK12 in vitro and in vivo. Moreover, our novel compounds demonstrated efficacy in reducing tumor burden in xenografted Balb/c mice (SUM149PT cell line) with once/twice-daily oral dosing.

In summary, we identified a novel series of CDK12 inhibitors which have drug-likeness and high therapeutic potential in solid tumors. Our lead compounds have been assessed and carefully selected using our precision medicine platform to highlight drug molecules that can treat fresh tumor samples as a monotherapy and in combination with standard of care therapeutics. These molecules will be investigated further to nominate a development candidate and commence IND-enabling studies.

#3012 A highly potent selective CDK9 inhibitor OBP-004 reduces metastatic tumor burden in bone, brain, lung and lymph nodes and decreases growth of triple-negative breast cancer cells in bone.

Tiina E. Kahkonen[1], Gergana Galabova[2], Ru Yang[3], Jie Wen[3], MIchael Thormann[2], Jussi M. Halleen[1]

[1]OncoBone Therapeutics Ltd, London, United Kingdom,[2]Origenis GmbH, Planegg, Germany,[3]PharmaLegacy LLC, Shanghai, China

CDK9 is a promising target in many cancers. Development of first generation CDK9 inhibitors has been stopped due to severe adverse effects, but next generation selective CDK9 inhibitors are considered safer and more effective. OBP-004 is a highly potent selective small-molecule inhibitor of CDK9 with favorable physicochemical properties, 17-20 h in vivo half-life and high tissue biodistribution in brain, lung, spleen and kidneys. Triple-negative breast cancer (TNBC) patients have limited treatment options together with high incidence of metastatic relapse. TNBC typically forms multi-organ metastases and has a high incidence of bone metastases. In this study, we demonstrate preclinical efficacy of OBP-004 on metastatic tumor burden, and more specifically on bone metastatic TNBC.
For studying efficacy on metastatic disease, NMRI nude mice were inoculated intravenously with luciferase-labelled MV4-11 human acute monocytic leukemia cells. OBP-004 was given orally at a dose of 1.0 mg/kg three times a week (3-day on, 4-day off). The mice were randomized to groups based on body weight and bioluminescence imaging (BLI) signal at day 15, and tumor growth was monitored by BLI during the study. Treatment was started after the randomization at day 15 and continued until day 32. For studying efficacy on tumor growth in bone metastatic microenvironment in more detail, BALB/c mice were inoculated intratibially with aggressive luciferase-labelled 4T1 mouse TNBC cell line. OBP-004 was given orally at a dose of 0.8 mg/kg three times a week (Mon/Wed/Fri). The mice were randomized to groups based on body weight and BLI signal at day 4. Treatment was started after the randomization at day 4 and continued until day 21. During the study, tumor growth was monitored by BLI, cancer-induced bone changes by X-ray radiography, and bone pain by mechanical allodynia using Von Frey filaments.
The 1.0 mg/kg dosing (3-day on, 4-day off) of OBP-004 in the MV4-11 study resulted in slight decrease of body weight without any clinical signs. The optimized dosing with 0.8 mg/kg (Mon/Wed/Fri) in the 4T1 study was well tolerated. In the MV4-11 model, OBP-004 showed strong decrease of bone, brain, lung and lymph node metastases. In the intratibial 4T1 model, OBP-004 decreased tumor growth in bone from day 10 forward, bone pain at day 14, and cancer-induced bone loss at day 21.
We conclude that the highly potent selective CDK9 inhibitor OBP-004 reduces metastatic tumor growth in bone, brain, lung and lymph nodes and decreases metastatic growth of TNBC cells in bone microenvironment, bone pain and cancer-induced bone loss in an aggressive preclinical TNBC model.

#3013 GSC000829, a novel selective FGFR2/3 inhibitor, displays superior antitumor activity in preclinical FGFR-driven neoplasms models.

Tian Han, Donglin Fu, Tao Cheng, **Yuanfeng Xia**, Zhilong Hu, Fanglong Yang

Changchun GeneScience Pharmaceutical Co., Ltd., Shanghai, China

Fibroblast growth factors (FGF) and their receptors (FGFRs) are pivotal in a myriad of cellular functions. Tumorigenesis onset due to FGFR genomic alterations is commonly observed across diverse solid tumors. Although several FGFR inhibitors targeting FGFR1-3 or FGFR1-4 have gained approval for treating various solid tumors, their widespread application is hindered by the development of resistant gatekeeper mutations in FGFR proteins and toxicities linked to FGFR1 and/or FGFR4, necessitating precise dosing or even drug withdrawal. Here, we report the discovery of a novel pre-clinical candidate compound GSC000829 with a strong inhibition towards FGFR2/3 and a significantly improved selectivity over FGFR1 and FGFR4. Furthermore, GSC000829 shows excellent efficacy in various biochemical and cellular assays corresponding to different FGFR fusions or mutations. It also demonstrates enhanced antitumor activity at lower dosage in FGFR3-driven CDX models compared to the pan-FGFR inhibitor Erdafitinib, as well as other FGFR3-selective inhibitors such as TYRA300 and LOXO-435. GSC000829 exhibits favorable physicochemical and pharmacokinetic properties along with an excellent preclinical toxicity profile with no obvious phosphate increase at quite high exposure level in rats. Taken together, these findings bolster the rationale for advancing GSC000829 into clinical trials as a potential best-in-class FGFR isoform-selective inhibitors, offering an approach for treating advanced solid tumors harboring FGFR2/3 aberrations.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Antibody-Based Cancer Therapeutics 2
Poster Session

#3014 The mechanism of action of ADCs are dependent on Rab GTPases.

Astrid Medhus[1], Kay Oliver Schink[2], Ane Sager Longva[1], Olav Engebraaten[1], Kristian Berg[1], Anette Weyergang[1]

[1]Oslo University Hospital, Institute for Cancer Research, Oslo, Norway,[2]University of Oslo, Institute of Basic Medical Science, Oslo, Norway

Antibody-drug conjugates (ADCs) are therapeutic compounds designed to exert targeted toxicity as compared to conventional chemotherapy. The conjugation of a cytotoxic payload to an antibody trough a chemical linker serves to lower the systemic toxicity and thereby increase the therapeutic window. However, an enhanced understanding of the mechanism of action of ADCs is needed, as little is known about how these drugs are trafficked and processed within the cell.
The tumor-targeted activity of ADCs depend on the presence of target molecules on the tumor cell surfaces, and the overall response of ADCs is generally dependent on the expression level of these surface receptors. However, ADCs are dependent on cellular uptake and intracellular trafficking for the cytotoxic payload to be released and reach its target within the cell. This process of endocytosis and subsequent intracellular trafficking is regulated by a group of small molecular switches called Rab GTPases. These proteins acts as key regulators of the endo/lysosomal machinery, and we hypothesized them to impact ADC efficacy.
We used cell lines from breast and lung cancer origin to study the mechanism of action of different ADCs dependent on RAB5 and RAB4 expression. We have found that the makeup of the ADC - comprising the antibody moiety, linker design, and choice of payload - dictates how it is taken up and processed, and thereby determines which Rab GTPases are critical for its therapeutic effect. Our results demonstrate how ADC design influences intracellular trafficking and processing, and points towards linker design as a major regulator of the intracellular fate of an ADC.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Kinase and Phosphatase Inhibitors 2
Poster Session

#3014 ASN-7350, a highly selective FGFR2/3 dual inhibitor, for FGFR2/FGFR3 driven solid tumors.

Qiaoling Sun[1], Jingmin Guan[1], Haifeng Song[1], Jing Lv[1], Peng Zeng[1], Mengyuan Wang[1], Yonghui Yu[1], Shuai Luo[1], Jinna Yu[1], Liang Wu[1], Tielin Wang[1], Alice Chen[2]

[1]Asieris Pharmaceuticals (Shanghai) Co,Ltd, Shanghai, China,[2]Asieris Pharmaceuticals, Palo Alto, CA

Alterations in fibroblast growth factor receptors (FGFRs) are recognized as oncogenic drivers in numerous human cancers. Several FGFR inhibitors, with similar potencies against FGFR1-3 or FGFR1-4, have been approved and demonstrated modest clinical benefits in FGFR-altered urothelial carcinoma and bile duct cancer. The clinical benefit was limited partly due to FGFR1- and FGFR4-mediated side effects. In addition, FGFR2/3 acquired resistance mutations after initial response to pan-FGFR inhibitor limit the long-term clinical efficacy as well. 60% of patients develop these secondary FGFR2 kinase domain mutations in FGFR2-altered cholangiocarcinoma, and 13-25% of patients develop FGFR3 resistance mutations in urothelial carcinoma. ASN-8639 and ASN-7350 were designed to address these challenges in treating multiple solid tumors harboring FGFR2/3 alterations. ASN-7350 is a novel, highly selective, and potent non-covalent FGFR2/3 inhibitor (IC50 < 5 nM in kinase assays). It showed greater than 200-fold selectivity for FGFR3 over FGFR1/4 in cellular assays. The prodrug ASN-8639 is in preclinical developed to improve the pharmacokinetic profile of ASN-7350. ASN-8639 was efficiently converted to ASN-7350 and showed dose-proportional increases in exposure and clinically acceptable oral bioavailability across four different species. ASN-7350 was more potent in inhibiting FGFR3 wt/mut signaling than competitor compounds. Its cell killing potency was 10× stronger compared to a reference compound currently in clinical development in cell lines harboring FGFR3-TACC3 fusion or erdafitinib-resistant FGFR3-V555M mutation, and meanwhile with a better the cellular selectivity of FGFR3 over FGFR1 and FGFR4. ASN-8639 demonstrated significant anti-tumor efficacy in cancer model of RT112 with FGFR3-TACC3 fusion and erdafitinib-resistant mutations of FGFR3-V555M. ASN-7350 also exhibited high potency against FGFR2. It produced comparable activity to a reference FGFR2 inhibitor in clinical development in multiple FGFR2-altered cell lines. Notably, ASN-8639 was more efficacious than reference FGFR2 inhibitor in the endometrial tumor model with erdafitinib-resistant FGFR2-N549K mutation at the same dose. These findings support the further clinical development of ASN-8639 as a potential best-in-class FGFR2/3 dual inhibitor for the treatment of bladder, endometrial, and other solid tumors with FGFR2/3 aberrations.

#3015 EGFR x MET tsAb: Differentiated MET biparatopic design with optimal MET inhibitory activity to pursue best-in-class opportunity.

Yanzhou Zhang[1], Ming Jiang[2], Hongyu Chen[2], Mengran Qian[1], Renke Li[1], Huang Yan[1], Huan Li[1], Xiaona Sun[2], Sujuan Huang[2], Xuechun Zhang[2], Ming Fang[2], Yue Wu[2], Fan Wang[2], Mingchao Kang[2], Xiaomin Song[2], Xi Wu[2]

[1]BeiGene (Shanghai) Biotechnology Co., Ltd., Shanghai, China,[2]BeiGene (Beijing) Co., Ltd., Beijing, China

Acquired resistant mutation after small molecule inhibitor treatment in non-small cell lung cancer (NSCLC) develops invariably through mutations in EGFR or through activation of compensatory pathways such as MET. BG-T187 is a tri-specific antibody (TsAb) targeting EGFR and MET with a differentiated MET biparatopic designation designed to treat tumors driven by activated EGFR and/or MET signaling. Stronger antiproliferative effects and MET signaling inhibition by BG-T187 compared to traditional EGFR/MET bi-specific antibody were observed in vitro. Additionally, potent in vivo anti-tumor activity was observed upon BG-T187 treatment of human tumor xenograft models driven by EGFR activation mutation and/or MET amplification. Interestingly, stronger anti-tumor activity of BG-T187 than that of traditional EGFR/MET bi-specific antibody was observed in xenograft models with MET amplification. Through a comprehensive assessment of EGFR on-target toxicity risk, we demonstrated that BG-T187 show better HEKn selectivity than EGFR/MET bi-specific antibody, indicating potential lower on-target toxicity risk of BG-T187. Collectively, our findings represent a novel EGFR/MET tri-specific antibody with differentiated MET biparatopic and pursue best-in-class potential.

#3016 Therapeutic efficacy of a novel combination of ck2 inhibitor cx-4945 with cytotoxic therapy in neuroblastoma preclinical models.

Marudhu Pandiyan Shanmugam, Muhammed Danial, Rajesh Rajaiah, Koby Duke, Katie Mercer, Yasin Uzun, Sinisa Dovat, Giselle Saulnier Sholler, Chandrika Gowda Behura

Pediatrics, Penn State University College of Medicine, Hershey, PA

Introduction: Neuroblastoma is a pediatric cancer of the sympathetic nervous system, which is often diagnosed at a clinically advanced stage with primary or acquired resistance to conventional chemotherapy. Protein Kinase CSNK2A1 (CK2) is an intrinsically active, ubiquitously present serine-threonine kinase overactive in various solid cancers, including neuroblastoma (NBL). Recurrent genetic alteration in CK2 is not detected in NBL. CX-4945 (Silmitasertib) is an ATP-competitive CK2 selective small-molecule irreversible inhibitor of CK2 with inhibition constant (Ki) values in the low nanomolar range and high selectivity (49 of 250 kinases) versus other kinases. CX-4945 has shown *in vivo* anti-tumor activity in solid tumors and leukemia has favorable pharmacokinetic properties and is well-tolerated in human subject studies. Here, we report the therapeutic efficacy of CX-4945 in combination with temozolomide and irinotecan NBL preclinical models.

Methods: The susceptibility of NBL cell lines with and without MYCN amplification was tested against CX-4945. We measured CK2 mRNA expression, protein level, and CK2 kinase activity in NBL cell lines and NBL PDX samples (n=18). CK2 knockdown was achieved in SMS-KCNR and SK-N-BE2 cell line using shRNA against CK2α and phenotype was assessed using cell viability assay, apoptosis, spheroid formation, migration, invasion assay. Cells were also injected into the flank of athymic mice to assess engraftment potential. We performed gene expression analysis using RNA sequencing of CX-4945 treated and gene knockdown NBL cells. MYCN-NBL cell line-derived subcutaneous model was developed in athymic mice. Mice were treated with a combination of Irinotecan (IRN) and temozolomide (TMZ) with and without CX-4945 for up to 28 days. Cohorts were followed for tumor growth and survival.

Results: CK2α shRNA silencing in NBL cell lines resulted in significantly reduced proliferation, increased apoptosis, reduced spheroid formation, and reduced migratory potential. The IC_{50} of CX-4945 (3-6uM) in NBL cell lines also showed synergy with the other standard chemo drugs, Temozolomide and Irinotecan. Gene expression analysis showed a decrease in the expression of several known MYC target proteins. The cohort receiving IRN + TMZ with CX-4945 showed significant tumor inhibition (50-60%) and prolonged survival time compared to the TMZ+IRN group.

Conclusion: Protein kinase CK2 is intrinsically active and negatively impacts survival in HR-NBL patients. In MYCN-NBL xenograft models, CX-4945 is well tolerated and shows superior therapeutic efficacy when combined with cytotoxic drugs Irinotecan and temozolomide. The ongoing Phase 1/II study is evaluating the safety, tolerability, and efficacy of combining CX-4945 with TMZ+IRN in relapsed refractory NBL patients ((NCT06541262).

#3017 Profiling patient response to TTK/PLK1 dual antagonist BAL0891 using 3D patient-derived organoid model in a microphysiological system.

Junfeng Wang[1], MinKyung Kang[2], Delaney Donnelly[1], Jihye Baek[1], Bushra Rajput[1], Byungjun Lee[1], Hyeon-geun Park[1], Yunha Shin[1], Tsung-Li Liu[1], Aneesh Sathe[1], Yangji Kim[1], Kyusuk Baek[1], Sanghee Yoo[1], Salman Ahmed[3], Jae Jung Lee[2], Seunghyun Ma[3]

[1]Qureator, Inc., San Diego, CA,[2]SillaJen, Inc., Seoul, Korea, Republic of,[3]Medical Affair, SillaJen Bio, San Francisco, CA

Introduction: Dysregulation of cell cycle checkpoints in cancer cells drives uncontrolled proliferation and tumor progression. Threonine Tyrosine Kinase (TTK) and Polo-like Kinase 1 (PLK1) are key regulators of the spindle assembly checkpoint (SAC) during mitotic division. Their overexpression is linked to tumor aggressiveness and poor prognosis in multiple malignancies. BAL0891, a first-in-class dual TTK/PLK1 inhibitor disrupts SAC integrity, increases cytoplasmic DNA, activates the cGAS/STING pathway, induces senescence-associated secretory phenotypes (SASP), and elevates PD-L1 expression, suggesting potential for synergy with immune checkpoint inhibitors. To explore patient-specific responses and identify predictive biomarkers of sensitivity to BAL0891, we utilized 3D patient-derived organoid (PDO) models within a microphysiological system (MPS), offering physiologically relevant insights beyond traditional models.

Methods: PDOs from renal cell carcinoma, triple-negative breast cancer, gastric cancer, and colorectal cancer were established in collaboration with Qureator. These PDOs were cultured in Qureator's CurioChips, which incorporate hydrogel-based extracellular matrix (ECM) to mimic the tumor tissue. Following organoid stabilization, BAL0891 was administered and cytotoxicity was assessed through live/dead dye staining and organoid volume measurements. PD-L1 expression was analyzed via flow cytometry to evaluate the potential for combination therapy with anti-PD-L1/PD-1 inhibitors. Cytokine profiling was conducted using multiplex secretome analysis to assess changes in the co-cultures. Additionally, RNA sequencing was performed to identify gene expression signatures associated with sensitivity and resistance to BAL0891.

Results: BAL0891 exhibited variable cytotoxicity across PDOs, with a subset demonstrating resistance. Responding PDOs showed SASP, confirmed by β-Galactosidase staining, increased PD-L1 expression, and distinct pro-inflammatory cytokine profiles compared to non-responders. Preliminary cytokine analyses indicates that BAL0891 reshapes the immune landscape in sensitive PDOs. Ongoing RNA sequencing analysis aims to identify gene clusters associated with sensitivity to BAL0891, offering potential biomarkers or therapeutic targets.

Conclusion: This study highlights the value of PDO-based 3D MPS in evaluating patient-specific responses to BAL0891 and identifying predictive biomarkers. These findings establish a foundation for patient stratification and personalized treatment strategies in clinical trials combining BAL0891 with immune checkpoint inhibitors. Further analysis of gene signatures may provide insights into potential therapeutic targets and improve our understanding on the mechanism driving TTK/PLK1 inhibition and its combination with immunotherapy.

#3018 Discovery of SIGX1094R, a novel FAK inhibitor simultaneously blocking FAK and SRC signaling.

Haisheng Zhang, Shu Zhuo, Lei Zhang, Lin Li, Wanfen Peng, Fengtao Jiang, Yufang Chen, Lin Lin

Signet Therapeutics, Shenzhen, China

Diffuse gastric cancer (DGC) is a highly lethal malignancy with only minimally effective systemic therapies. Our previous studies identified the Focal Adhesion Kinase (FAK) as a novel and promising target for DGC. To enhance the efficacy of FAK inhibition in cancer therapy, we developed a potent small-molecule inhibitor, SIGX1094R. This inhibitor targets both FAK and the related kinase SRC, which complexes with FAK, whereas current FAK inhibitors in clinical trials only inhibit p-FAK.In DGC cell line models and mouse organoid models, SIGX1094R significantly inhibited cell growth and demonstrated much greater potency than current FAK inhibitors in clinical trials, with similar results observed in DGC patient-derived organoids. To further evaluate efficacy, we found that SIGX1094R significantly blocked tumor growth in several DGC patient-derived xenografts, whereas current first-line drugs demonstrated limited efficacy. This aligns with clinical results, highlighting SIGX1094R's strong potential to replace the existing standard of care. Additionally, SIGX1094R demonstrated significantly greater efficacy in ascites models (both in vitro and in vivo) compared to the current standard treatments. In addition to treating DGC, SIGX1094R shows promise in preclinical studies for treating ovarian, triple-negative breast, and pancreatic cancers. It also has high potential for use in combination therapies with targeted treatments for KRAS and EGFR mutations. GLP tox studies demonstrated that most of the lesions in high dose of SIGX1094R animals at the end of the recovery period were fully recovered.The drug received investigational New Drug (IND) clearance both from the U.S. Food and Drug Administration (FDA) and the National Medical Products Administration (NMPA) in China. SIGX1094R, poised to be the world’s first targeted therapy for DGC, is the inaugural project in a pipeline discovered by AI and validated using organoid disease models.

#3019 Targeting transcriptional regulation using CDK7 and CDK9 inhibition as a novel combination therapy in fibrolamellar carcinoma.

Austin Stram, Manabu Nukaya, Patrick Carney, Eleanor Riedl, Jeremy D. Kratz, Sean Ronnekleiv-Kelly

University of Wisconsin-Madison, Madison, WI

Background: Fibrolamellar carcinoma (FLC) is a rare liver cancer with a 5-year survival rate between 35-40%. FLC typically affects a younger patient population, the majority receiving a diagnosis between the ages of 10-35. There are currently no curative systemic therapies and an unmet need for novel and effective therapeutics to improve survival. A unique molecular hallmark of FLC is a fusion event between heat shock protein 40 (*DNAJB1*) and the catalytic subunit alpha of protein kinase A (*PRKACA*). This event creates the FLC specific oncoprotein (DNAJ-PKAc) which has been shown to promote FLC tumor formation. Downstream signaling of DNAJ-PKAc remains unknown, however, this fusion protein has been shown to cause enhancer remodeling to upregulate FLC specific drivers of proliferation.

Methods: Western blot analysis was performed on patient tumors, normal tissue, and patient derived organoids (PDOs) for active RPB1, including phosphorylation of serine 2, serine 5, and serine 7 (pRBP1-s2, s5, s7). Single agent activity of SY5609 was assessed at 144 hours in PDOs. Synergy was assessed at 144 hours using a dose titration grid. SynergyFinder 3.0 was used to produce Highest Single Agent (HSA) synergy scores. Viability was determined using CellTiterGlo (CTG, 50% v/v). RNA was extracted from PDOs for qPCR to measure relative FLC specific driver gene expression, including *SLC16A14* and *LINC00473*. SY5609 was treated continuously while VIP152 was removed after 24 hours to mimic *in vivo* pharmacokinetics.

Results: Relative to normal tissue, FLC tumors had an increase in pRPB1-s5 (6.38, $p<0.05$) and pRPB1-s7 (5.82, $p<0.05$) compared to cholangiocarcinoma (CCA) (2.42, n.s.; 1.10, n.s.) and hepatocellular carcinoma (HCC) (2.61, n.s.; 0.95, n.s.). CDK7 inhibition with SY5609 in FLC PDOs had a 9x greater selectivity than normal liver PDOs and 216x greater than CCA PDOs with IC_{50}=0.39nM, 3.14nM, and 84.22nM, respectively. FLC PDOs treated with physiologic concentrations of SY5609 showed downregulation of FLC specific enhancer driven gene *SLC16A14* but not *LINC00473* with fold changes of 0.51 and 0.84. SY5609 did not regulate *SLC16A14* in CCA PDOs (fold change=2.78) and *LINC00473* was undetectable in all samples. The addition of CDK9 inhibition with VIP152 was synergistic in FLC PDOs (HSA=8.79) which was not seen in CCA PDOs (HSA=-1.82). Combination treatment synergistically downregulated FLC specific driver gene *LINC00473* (fold change=0.41), and decreased pRBP1-s2, 5, and 7 shown via western blot.

Conclusion: Here, we show FLC has an increase in phosphorylated RPB1 specifically at s5 and s7, and a unique sensitivity to CDK7 inhibition with SY5609. The addition of CDK9 inhibition using VIP152 is synergistic at downregulating FLC specific driver genes and decreasing activated RPB1 and viability. Ongoing work is validating this combination in animal models to assess toxicity and *in vivo* efficacy.

#3021 Discovery of a novel and selective CDK2 inhibitor for targeted cancer therapy.

Douglas D. Fang, Yueqin Liu, Yang Liu, Wei Sha, Charles Z. Ding

Shenzhen Yangli Pharma. Tech. Co., Ltd., Shanghai, China

Targeted inhibition of CDK2 presents a promising therapeutic strategy for breast cancer patients who experience disease progression following CDK4/6-based therapies. In addition, CDK2 hyperactivity or CCNE1 amplification drives the progression of many cancer types, making it an attractive target. However, developing CDK inhibitors is often complicated by toxicities resulting from off-target inhibition of other CDK family members, particularly CDK1 and CDK9, which are essential for normal cell proliferation and survival. Achieving selectivity for CDK2 is therefore crucial to maximize therapeutic efficacy while minimizing adverse effects, enabling a broader therapeutic window in clinical applications. Here, we present VB18214 as a novel and selective CDK2 inhibitor. In biochemical assays, VB18214 demonstrated potent inhibition of the CDK2/cyclin E1 complex (IC_{50} = 0.6 nM) and exhibited 200-fold selectivity over CDK1/cyclin B1. Intracellular NanoBRET assays confirmed its high affinity for CDK2/cyclin E1 (IC_{50} = 3.8 nM), along with exceptional selectivity—124-fold over CDK1/cyclin B1, 12-fold over CDK4/cyclin D1, 94-fold over CDK6/cyclin D3, 334-fold over CDK7/cyclin H, and 1348-fold over CDK9/cyclin T1. In CCNE1-amplified OVCAR3 ovarian cancer cells, VB18214 effectively inhibited cell proliferation (IC_{50} = 18.5 nM). Compared to the clinically advanced CDK2 inhibitor PF-07104091, VB18214 demonstrated 5-fold greater potency in inhibiting CDK2 kinase activity, 16-fold higher affinity for CDK2 in NanoBRET assays, 2-fold greater selectivity over CDK1 in both biochemical and intracellular assays, and 9-fold higher antiproliferative activity in OVCAR3 cells.These findings position VB18214 as a potent and selective CDK2 inhibitor with significant potential for clinical development in cancers driven by CDK2/cyclin E dysregulation, as well as CDK4/6 treatment progressed mBC. More characterization data for VB18214 will be updated at the meeting.

#3022 ODY-CDK, a brain-penetrant, selective CDK2 inhibitor that demonstrates antitumor activity in an intracranial *CCNE1*-amplified breast tumor model.

Joe Gozgit[1], Gerald Lelais[1], Ellen Vieux[1], Sam Zelman[1], Laura Stransky[2], Hyeseok Shim[1], Kim Aardalen[1], Casey Landry[1], Arun Thottumkara[1], Jin Choi[1], Chip Lesburg[1], Sebastian Schneider[2], Chaya Stern[1], Elizabeth McMillan[1], Hanbin Lu[1], Caroline Ho[1], Nigel Waters[1], John Shen[1], Natalie Dales[1], Shifeng Pan[2], Bob Abraham[2], Gary D. Glick[1], Elaine M. Pinheiro[1]

[1]Odyssey Therapeutics, Inc., Boston, MA,[2]Odyssey Therapeutics, Inc. (former employee), Boston, MA

Cyclin-dependent kinase 2 (CDK2) is a member of the serine/threonine family of protein kinases that acts in complex with cyclins E1/E2 and A1/A2 to drive entry into and progression through S/G2-phases of the cell cycle. Amplification of *CCNE1* or overexpression of cyclin E1 protein leads to persistent CDK2-cyclin E1 activation and phosphorylation of the retinoblastoma protein (pRb), thereby promoting uncontrolled cell cycle progression. In addition, cancer cells with loss-of-function (LoF) mutations in the retinoblastoma gene (*RB1*) exhibit variable levels of dependence on CDK2-cyclin A2 activity for progression through S/G2 phases of the cell cycle and have shown vulnerability to CDK2 inhibition. Brain metastases are frequently observed in cancers that harbor *CCNE1* or *RB1* alterations, such as small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC), and triple-negative breast cancer (TNBC). Clinical-stage CDK2 inhibitors are not brain penetrant, and a brain-penetrant CDK2 inhibitor could provide a targeted therapeutic option for these high need patients. Here, we report on a brain-penetrant, selective CDK2 inhibitor, ODY-CDK, for the treatment of cancers with *CCNE1* or *RB1* genetic alterations with brain metastases or at high risk of developing them. ODY-CDK potently inhibits CDK2 (Ki = 3.1 nM) and demonstrates 40 to 180-fold selectivity over CDK1/5/6/7/9 as measured by cellular NanoBRET assays. ODY-CDK inhibits pRb in *CCNE1*-amplified ovarian and breast cancer cells and demonstrates antiproliferative activity in *CCNE1*-amplified and *RB1*-null ovarian, lung and breast cancer cells. In a clinically relevant human bone marrow assay, ODY-CDK is predicted to have a low risk of neutropenia that enables safe combinations with other agents. In vivo, once daily oral administration of ODY-CDK to mice with subcutaneous *CCNE1*-amplified HCC1569 breast or *RB1* LoF NCI-H526 SCLC xenografts was well tolerated and demonstrated antitumor activity comparable to the clinical-stage CDK2 inhibitor PF-07104091. ODY-CDK treatment provoked sustained pRb inhibition in HCC1569 tumors. Pharmacokinetic evaluation of ODY-CDK demonstrated brain penetration across species with equivalent free exposure observed between cerebrospinal fluid (CSF) and plasma in dog (CSF Kp_{uu} 0.9). To evaluate the intracranial efficacy, *CCNE1*-amplified HCC1569 cells were implanted into the cranium of NOG mice and tumor growth was measured by MRI. Daily oral dosing of ODY-CDK resulted in tumor regression in 8 out of 11 mice, while PF-07104091 showed modest activity with regression in only a single mouse. These data demonstrate that ODY-CDK is a selective brain-penetrant CDK2 inhibitor with differentiating intracranial antitumor activity to existing clinical-stage CDK2 inhibitors and support the evaluation of ODY-CDK in patients with brain metastases that have a high unmet medical need.

#3023 Preclinical study of TR-412, a novel and highly selective CLK inhibitor for acute myeloid leukemia.

Wentao Zhang, **He Tian**, Mengyang Yan, Qian Shao

Tarapeutics Pharmaceutical Science Inc, Bengbu, China

Alternative splicing (AS) is a major mechanism for diversifying mRNA transcripts and regulating protein expression. Most tumors exhibit widespread splicing abnormalities relative to peritumoral healthy tissues, including frequent retention of normally excised introns, inappropriate expression of isoforms normally restricted to other cell types or developmental stages, and splicing errors that disable tumor suppressors or promote oncogene expression. The CDC2-like kinases (CLKs) and dual-specificity tyrosine-regulated kinases (DYRKs) are thought to govern the efficiency and specificity of AS by directly phosphorylating serine/arginine-rich splicing factors (SRSFs) and thereby influencing pre-mRNA splice junction selection. TR-412 is a novel inhibitor of CLK2/3 and DYRK1A/B, which was evaluated its anti-leukemic activities both in vitro and in vivo. TR-412 showed strong inhibitory effects in an exon-skipping luciferase reporter assay, achieving an IC50 of 120 nM. Furthermore, it significantly suppressed the proliferation of hematological malignancies, with a median IC50 of 350 nM. To understand the mechanism of anti-cancer cell growth, the effect of TR-412 on AS and protein expression of key tumor related biomarkers were examined. The experimental results showed that TR-412 effectively inhibited pSRSF (IC50 <100 nM in AML cells). In addition, RNA-seq analysis revealed that TR-412 induced global splicing changes, which typically resulted in exon exclusion (exon skipping) of cassette exon. To further investigate the effect of TR-412 on tumor growth in vivo, we established multiple AML-derived subcutaneous xenograft models (CDX), which were treated with varying doses of TR-412. In particular, complete tumor regression was achieved in the MV4-11 tumor model. Overall, the preclinical findings strongly support that this novel CLK inhibitor TR-412 is worthy of further exploration in hematologic malignancies.

#3024 The novel selective CDK9 Inhibitor ORI-15412 reduces PD-L1 expression and drives the efficacy in combination therapies for melanoma and triple-negative breast cancer.

Gergana Galabova, Michael Almstetter, Nicole Brusis, Christiane Gilch, Roland Koestler, Julie Kondrotas, Jan Kubitzki, Henrik Kuhn, Hendrik Liebers, Federico Medda, Patricia Perez-Galan, Jean Tassel, Nadine Traube, Andreas Treml, David Wifling, Nasser Yehia, Peter Zegar, Michael Thormann

Origenis GmbH, Planegg, Germany

Aggressive cancers like melanoma and triple-negative breast cancer (TNBC) are challenging to treat due to limited therapeutic options and high relapse rates. Advances in combination therapies offer promise by targeting multiple cancer progression and resistance pathways. This study explores ORI-15412, Origenis' novel, oral CDK9 inhibitor, as monotherapy and in combination therapies in syngeneic melanoma (B16F10) and TNBC (4T1) murine models. ORI-15412, developed with Origenis' proprietary MOREsystem®, MolMind®, BRAINstorm™, and Cippix® technologies, shows unhindered brain penetration, high selectivity, and potency. Its CDK9 inhibition effectively targets the pTEFb complex and downstream pathways, including phospho-RNA Pol II, MCL-1, and c-MYC, inducing apoptosis and inhibiting osteoclast differentiation in cancer cell models (L363, MV4-11, and huPBMCs). ORI-15412 also dose-dependently reduces PD-L1 expression, highlighting its potential to modulate immune responses. In vivo, ORI-15412 monotherapy showed significant tumor growth inhibition (TGI) in B16F10 melanoma and 4T1 TNBC models. Combined with anti-PD-1 antibody, Vemurafenib, or Trametinib, it enhanced anti-tumor activity, achieving 72-84% TGI in the B16F10 model. In the 4T1 TNBC model, combining ORI-15412 with anti-PD-1 antibody achieved a 63% TGI, outperforming monotherapies or controls. ORI-15412 was non-toxic at effective doses in mono and combination therapies. These findings highlight ORI-15412 as a pivotal component in combination therapy for hard-to-treat cancers. Its effects on tumor growth inhibition and immune checkpoint modulation support its selection as a clinical candidate for melanoma, TNBC, and other malignancies.

#3025 Optimization of therapeutic index of SGR-3515, a first-in-class Wee1/Myt1 inhibitor through intermittent dosing for monotherapy and combination with chemotherapy in xenograft tumor models.

Shaoxian Sun, Felicia Gray, Sarah Silvergleid, Robert Pelletier, Lin Tang, Andrew Placzek, Jiashi Wang, Pieter Bos, Jennifer Knight, Anthony Clark, Zhijian Liu, Naveen Thakral, Aleksey Gerasyuto

Schrodinger, New York, NY

Background: Cancer cells often have deregulated G1-S phase in cell cycle and rely on the G2-M checkpoint to delay mitosis allowing for completed DNA damage repair. Wee1 and Myt1 kinases regulate G2 to M phase transition by inhibitory phosphorylation of cyclin dependent kinase (CDK1) to prevent premature entry to mitosis. Small molecule inhibitors targeting either Wee1 or Myt1 have shown promising clinical benefit in solid tumors as a monotherapy and/or combination therapy. Emerging evidence suggests that Wee1 and Myt1 have a synthetic lethal relationship in tumor cells, and Myt1 upregulation contributes to acquired resistance to Wee1 inhibitors. Here we describe the superior anti-tumor activity of SGR-3515, a first-in-class Wee1/Myt1 inhibitor, compared to single inhibition of Wee1 or Myt1 as a monotherapy, synergistic combination with chemotherapy and optimization of dosing schedule to achieve improved therapeutic index in xenograft tumor models.

Methods: SGR-3515 was identified internally using Schrödinger's computational platform. Its binding affinity to Wee1 or Myt1 and inhibition potency of kinase activity was measured by surface plasmon resonance (SPR) assay and ADP-Glo kinase activity assay, respectively. SGR-3515 effects on DNA damage and cell cycles were investigated in tumor cell lines using gH2Ax and DNA staining high content imaging assays, respectively. The cellular target engagement was measured by pCDK1 Y15 for Wee1 inhibition and pCDK1 T14 for Myt1 inhibition in tumor cell lines. The in vivo anti-tumor activity was evaluated in cell line derived xenograft models.

Results: SGR-3515 is a novel and potent small molecule inhibitor of Wee1/Myt1. The co-inhibition of Wee1 and Myt1 induces rapid DNA damage and causes significant tumor cell apoptosis. SGR-3515 demonstrates superior anti-tumor activity in vivo in xenograft models compared to single inhibition of Wee1 or Myt1 alone, consistent with the Wee1 and Myt1 synthetic lethal relationship. Furthermore it suppresses the acquired resistance associated with a Wee1 inhibitor (ZNc3). Optimization of intermittent dosing schedule of SGR-3515 allows sufficient recovery of Wee1 inhibition mediated myelosuppression while maintaining similar anti-tumor activity as the continuous dosing in xenograft tumor models. Intermittent dosing of SGR-3515 in combination with carboplatin achieved synergistic anti-tumor activity without significantly worsening hematological effects.

Conclusions: The robust anti-tumor activity of SGR-3515, a first-in-class Wee1/Myt1 inhibitor confirms the synergy of Wee1 and Myt1 co-inhibition. SGR-3515 dosing schedule optimization improves therapeutic index as a monotherapy and in combination with Carboplatin. The preclinical data supported the progression of SGR-3515 to clinical study (NCT06463340).

#3026 ARTS-023: a potent and selective CDK4 Inhibitor demonstrating anti-tumor activity in preclinical ER+ breast cancer models.

yali guo[1], jinhong chen[1], nathan schomer[2], jiaqi liang[1], tingru zhou[1], xipan liu[1], wenheng liang[1], wei pang[1], jia fu[1], xiaobin zhang[2], dai cheng[1], john campbell[2], tao ji[2], fang li[2], yaoyu chen[2], **qing sheng**[2]

[1]Allorion Therapeutics, Guangzhou, China,[2]Allorion Therapeutics, Natick, MA

Cell cycle deregulation is a hallmark of cancer, and cyclin-dependent kinases (CDKs) play a pivotal role in driving tumor proliferation by regulating cell cycle progression. Among these, CDK4 and CDK6 are critical in promoting cell G1 to S transition. While three CDK4/6 inhibitors have been approved for treating estrogen receptor-positive (ER+), human epidermal growth factor receptor 2-negative (HER2-) breast cancers, eventual relapse and severe hematological toxicities, particularly neutropenia, remain major clinical challenges. Preclinical data suggest that CDK6 dependence is predominantly associated with hematopoietic and lymphoid cell lines, whereas CDK4 dependence is enriched in breast cancer cell lines. This distinction highlights the potential of developing a next-generation CDK4-selective inhibitor to minimize hematological toxicity, allow for higher CDK4 inhibition, combination with other CDK inhibitors and improve therapeutic efficacy in ER+ breast cancer. Here we report the development and preclinical characterization of ARTS-023, an orally bioavailable CDK4 inhibitor with high selectivity over CDK6. In enzymatic assay, ARTS-023 potently inhibits the activity of CDK4/CyclinD1 complex with 50% inhibitory concentration (IC50) in the sub-nanomolar range, displaying near 60-fold selectivity against CDK6 and over 130-fold selectivity against other CDKs assessed. A very low hit rate (S (10) = 0.017, at 1uM) is observed when ARTS-023 is assessed in whole Kinome profiling, further revealing the selectivity of this compound. ARTS-023 also demonstrates potent CDK4 inhibition and selectivity against CDK6 in NanoBRET and other cellular assays. ARTS-023, at low nanomolar concentrations, inhibits Rb phosphorylation and blocks the G1/S transition, resulting in growth arrest in ER+ breast cancer cell lines. Compared to palbociclib, ARTS-023 is 32-fold more selective in inhibiting the proliferation of ER+ breast cancer cell lines over human hematopoietic stem cells, suggesting that a CDK4-selective inhibitor may have reduced cytotoxicity on hematopoietic lineages. In vivo, ARTS-023 exhibited strong anti-tumor activity as a single agent across various ER+ breast cancer xenograft models. Additionally, it demonstrated robust synergistic effects when combined with the CDK2 inhibitor AVZO-021 in both palbociclib-sensitive cell line-derived xenograft models and palbociclib-resistant patient-derived xenograft models. Taken together, these findings establish ARTS-023 as a promising next-generation CDK4-selective inhibitor, offering improved efficacy and reduced toxicity for patients with ER+ breast cancer. Furthermore, the favorable toxicity profile of ARTS-023 supports the potential combination with CDK2 inhibitors to enhance therapeutic outcomes.

#3027 Killing two birds with one stone: veratridine functions as a dual inhibitor of both hyperactive mTORC2 and mitochondria in colon cancer cells.

Morgan Eikanger, Khosrow Rezvani

University of South Dakota, Vermillion, SD

Despite considerable advances to improve colorectal cancer (CRC) survival over the last decade, therapeutic challenges remain due to the rapid metastatic dissemination of primary tumors. There has been an increase in early-onset CRC (patients under age 50) incidences in all ethnicities. Early-onset CRC introduces another major health challenge as these patients are commonly diagnosed with advanced, metastatic forms of CRC, resulting in higher mortality rates, showing the clear need to develop safer and more effective therapies. There has been significant progress in understanding the tumorigenic pathways responsible for the invasion and metastasis of human CRC. Current evidence indicates that overactive mitochondria and hyperactivation of the Rictor-mTORC2-AKT signaling pathway drive key metastatic players and support the formation and progression of metastatic cells in diverse malignant tumors, including early-onset CRC. Small molecule inhibitors that selectively interfere with these key metastatic pathways have become a primary focus of targeted therapy as they control proteins involved in tumor dissemination without affecting normal cells. Selective mTORC2 inhibitors are becoming a potential optimal treatment strategy for patients with CRC because they eliminate therapeutic limitations and drug resistance reported for mTORC1 and mTORC1/mTORC2 inhibitors. Veratridine (VTD), a lipid-soluble alkaloid extracted from Liliaceae plants, can transcriptionally increase UBXN2A, a ubiquitin-like protein. UBXN2A's E3 ubiquitin ligase partners enable UBXN2A to target dually and selectively induce 26S proteasomal degradation of the following substrates: mitochondrial HSP70 (mortalin) proteins and Rictor in the mTORC2 signaling pathway. This study explains the dual-action molecular mechanisms of the VTD-UBXN2A axis in patient-derived xenograft (PDX) cell lines. Longitudinal analysis of cells after completing real-time cell analysis using xCELLigence technology and generating a 3D spheroid CRC model using PDX cells indicate that VTD can effectively restrict cell migration. Additionally, Seahorse bioenergetic assays showed that VTD reduces mitochondria respiration and glycolysis. These results indicate the dual-targeting efficiency of VTD on metastatic cancer cells. This study opened a new platform for repurposing VTD, a supplemental anti-hypertension molecule, into an effective targeted therapy in CRC tumors.

#3028 Tumor suppressor activation: unlocking a novel path to overcome CDK4/6 inhibitor resistance in HR-positive breast cancer.

Hee-Sung Park, Kyu-Kwang Cho, Martin Pettersson, Chakrapani Subramanyam

Promedigen, Daejeon, Korea, Republic of

Clinically approved CDK4/6 inhibitor drugs, though initially effective, often generate acquired resistance in HR-positive breast cancer, leading to 25-35% of patients becoming nonresponsive. A number of drivers for this resistance have been identified, including cyclin D1 upregulation. Herein, we present an innovative strategy to tackle this pressing challenge. We have developed a novel small molecule, PMG-A7, that activates a key tumor suppressor critical to maintaining cellular homeostasis and regulating the cell cycle. PMG-A7 induces a robust, dose-dependent reduction in resistance drivers, demonstrating efficacy at single-digit nanomolar concentrations. Further, PMG-A7 upregulates cell cycle inhibitors such as p27, effectively countering CDK4/6 inhibitor resistance. When used in combination with a CDK4/6 inhibitor, PMG-A7 significantly suppresses resistance mechanisms. These findings highlight tumor suppressor activation as a promising combination therapeutic strategy to help overcome drug resistance to CDK 4/6 inhibitors in the clinic.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Novel Antitumor Agents 2
Poster Session

#3032 Selective pharmacological activation of the mitochondrial protease OMA1 inhibits tumor growth and induces regression in tumors expressing low levels of FAM210B.

Matthew Kostura, Michael Luther, Michael Stocum, Todd Hembrough, Zahid Bashir

Bantam Pharmaceutical, Durham, NC

1-Thiazol-2-yl-N—3-methyl-1H-pyrozole-5-carboxylic acids have been described as anti-tumor agents. Two members of this family, BTM-3528 and BTM-3566, exhibit robust, single agent *in vivo* activity in models of Diffuse Large B-Cell Lymphoma (DLBCL). Drug activity is correlated with activation of the OMA1-ATF4 ISR axis. *In vitro*, response to drug is associated with G1/S growth arrest and , in DLBCL, apoptosis.

We evaluated the tumor response activity profile of BTM-3528 and BTM-3566 *in vitro* in panels of solid and hematopoietic tumor lines. Cell growth inhibition was determined using Cell Titre Glo (Crown Bio OmniScreen). Both compounds demonstrated growth inhibitory activity in a broad range of solid and hematopoietic tumor lines. Biomarker analysis did not reveal correlations between drug activity and mutation or copy number changes associated with tumor oncogenes. Analysis of RNAseq data identified a panel of 9 genes whose expression predicted response to drug. Amongst these is a mitochondrial protein, FAM210B, where response to drug is inversely correlated to RNA expression. We demonstrate that ectopic expression of FAM210B specifically regulates the activity of BTM-3566 but no other compounds that also activate OMA1.

BTM-3566 was evaluated *in vivo* using 16 PDX models of diverse origin including uterine, esophageal and biliary tract (Crown Bio HuBase) . Tumors were prospectively selected based on FAM210B RNA expression (quintile binning). Seven tumors were in the first, lowest quintile, whereas the remaining nine were distributed in the 2nd through 5th quintiles. Response to BTM-3566 occurred exclusively in 5 tumors (uterine, esophageal and biliary tract) within the first quintile of FAM210B expression. Two of the tumors exhibited signs of regression.

Selective activation of OMA1 leads to changes in mitochondria including alterations in cristae structure, morphology and expression of BH3 proteins such as MCL1. The outcome is cell growth inhibition and in DLBCL, Bax-dependent apoptosis. Such changes in mitochondrial structure and function may prime tumor cells to apoptosis in the presence of a BH3 mimetic. Combinations of BTM-3566 with the BH3 mimetic navitoclax results in synergistic cell killing activity in select solid tumor lines *in vitro*. BTM-3566 increased the potency of navitoclax by approximately 7-fold, increasing both cell killing and caspase activation.

Conclusions: BTM-3566 targets an pathway important in mitochondrial quality control leading to tumor cell growth and regression. In vivo, drug activity correlated with low expression of RNA for the mitochondrial protein FAM210B. BTM-3566 demonstrated robust activity in vivo in solid tumors of diverse origin with low FAM210B RNA expression. Rational combinations with BH3 mimetics will extend the therapeutic utility of BTM-3566 in otherwise difficult to treat tumors.

#3033 Increased mitochondrial protein import necessitates greater reliance on the mitochondrial unfolded protein response (UPR^{mt}) and the protease LONP1 to maintain mitochondrial protein solubility and cell viability in acute myeloid leukemia (AML).

Matthew Tcheng, Veronique Voisin, Marcela Gronda, Rose Hurren, Lan Xin Zhang, Yue Feng, Zaynab Mamai, Brady Stock, Yulia Jitkova, Andrea Arruda, Steven M. Kornblau, Mark D. Minden, Aaron D. Schimmer

University Health Network Princess Margaret Cancer Centre, Toronto, ON, Canada

Almost all mitochondrial proteins are encoded by nuclear DNA, translated in the cytosol, and imported into the mitochondria. Once inside the mitochondria, these unfolded, aggregation prone precursors are processed and folded into mature forms. Failure to properly process and fold these newly imported proteins results in the formation of toxic aggregates. To counter the mitochondrial stress from newly imported proteins, cells developed the mitochondrial unfolded protein response (UPR^{mt}), a conserved pathway which activates the transcription of mitochondrial proteases and chaperones to fold newly imported precursors and degrade protein aggregates. There are no UPR^{mt} gene sets in the KEGG, Gene Ontology or Reactome databases, so we compiled a 39-gene signature of the known components of the human UPR^{mt}. In primary AML cells, expression of this UPR^{mt} signature was increased compared to normal hematopoietic cells. UPR^{mt} expression was strongly correlated with mitochondrial protein import expression, highlighting the protective role of the UPR^{mt} in countering the stress of increased protein import. Leading edge analysis identified the protease LONP1 as a top driver of the UPR^{mt} signature and analysis of AML cells' dependency on UPR^{mt} genes (Depmap) also identified LONP1 as a top essential gene. We focused additional studies on LONP1 and its role in protecting AML cells from mitochondrial stress. LONP1 mRNA expression was increased in AML compared to normal hematopoietic cells. LONP1 protein was increased >2-fold in 26/39 primary AML samples, compared to bulk (n=14) and CD34+ (n=3) hematopoietic cells. In AML, LONP1 expression positively correlated with expression of protein import and UPR^{mt} genes as well as decreased overall survival. LONP1 folds newly imported precursors and degrades aggregated mitochondrial proteins. To assess the role of LONP1 in mitochondrial proteostasis, we developed a novel assay to measure mitochondrial protein aggregation. LONP1 genetic depletion and chemical inhibition with Omaveloxolone and bardoxolone methyl increased mitochondrial protein aggregation and cell death in AML lines and primary AML with high LONP1 but not hematopoietic cells or primary AML with low LONP1. In primary AML, sensitivity to LONP1 chemical inhibition or genetic depletion positively correlated with LONP1 expression (Omaveloxolone (n=16, R^2=0.64); Bardoxolone methyl (n=30, R^2=0.65); LONP1 shRNA (n=9, R^2=71)). In summary, AML cells have increased mitochondrial protein import, necessitating a heightened dependence on the UPR^{mt} to maintain mitochondrial proteostasis. Targeting the UPR^{mt} at the level of LONP1 selectively induces mitochondrial protein aggregation and eliminates AML cells while sparing normal hematopoietic cells.

#3034 SY-12696, a novel and potent MALT1 protease inhibitor, for the treatment of B-cell lymphomas.

Xintao Cao[1], Jijun Li[2], Yongliang Sun[1], Chang Lu[1], Yuan Ji[1], Miao Zhang[1], Zhifeng Chen[2], Shenyu Zhao[3], Yan Zhu[2], Hong Luo[3], **Yinghui Sun**[1]

[1]Department of Discovery Biology, Shouyao Holdings (Beijing) Co., Ltd, Beijing, China,[2]Department of Medicinal Chemistry, Shouyao Holdings (Beijing) Co., Ltd, Beijing, China,[3]Department of DMPK, Shouyao Holdings (Beijing) Co., Ltd, Beijing, China

Background: MALT1 (Mucosa-associated lymphoid tissue lymphoma translocation protein 1) is a key component of the CBM complex (CARD9/10/11/14-BCL10-MALT1) downstream of Bruton's tyrosine kinase (BTK) in the B-cell receptor signaling pathway. Through its unique scaffolding and proteolytic functions, MALT1 precisely modulates the NF-κB pathway and regulates cell proliferation, differentiation and immunity. MALT1 is major driver of a subset of B-cell lymphomas, making it an attractive target for therapeutic intervention. Inhibition of MALT1 is considered a promising therapeutic approach for the treatment of several B-cell lymphomas, including tumors with acquired BTK inhibitor (BTKi) resistance.

Methods: Based on structure-activity relationship analysis, series of MALT1 inhibitors were synthesized and screened. The potency and downstream effects of compounds were evaluated using biochemical enzyme assay, cellular phenotype and functional studies and *in vivo* xenograft models. Furthermore, the potential for enhanced efficacy through combination with BTKi was investigated in TMD8 cells overexpressing the BTK C481S mutation.

Results: SY-12696 was identified as a novel, potent and small molecular MALT1 inhibitor, it showed excellent potency on MALT1 protease activity both in biochemical and in cellular assays with nanomolar IC_{50}s. SY-12696 suppressed cleavage of MALT1's substrate (e.g. Rel B) in the activated B-cell subtype of diffuse large B cell lymphoma (ABC-DLBCL) cell lines, highlighting its capability to disrupt the NF-κB pathway. In cellular proliferation assays, SY-12696 effectively inhibited the growth of ABC-DLBCL cell lines, including the BTKi-resistant cell line OCI-Ly3 (carrying CARD11 L224P, MYD88 L265P mutations) and the BTKi-sensitive cell line OCI-Ly10 (carrying CD79A Δ191-208, MYD88 L265P mutations). In the OCI-Ly3 xenograft model, oral administration of SY-12696 at 10 mg/kg twice daily (bid) resulted in >90% tumor growth inhibition (TGI). Additionally, a synergistic effect of SY-12696 with the BTKi was observed on cell viability in TMD8-BTK ^{C481S} cells. The compound also exhibited desirable drug-like properties, including favorable pharmacokinetic properties and safety profile.

Conclusion: Based on current studies, SY-12696 has demonstrated significant inhibitory potency on ABC-DLBCL cancer cells *in vitro* and *in vivo*, which makes SY-12696 a promising candidate for further development as a monotherapy or in combination with BTKi to treat relapsed/refractory B-cell lymphoma, particularly those resistance to BTKi.

#3035 Effects of novel isoform selective HSP90 beta inhibitors alone and in combinations against experimental esophageal adenocarcinoma.

Md Sazzad Hassan[1], Erin Garzella[1], Annie Ritter[2], Elizabeth Heffernan[2], Aktar Ali[2], Brian Blagg[2], Urs von Holzen[1]

[1]Indiana University School of Medicine (South Bend, IN), South Bend, IN,[2]University of Notre Dame, South Bend, IN

Background: Inhibition of the heat shock protein 90 (HSP90) chaperone activity with small molecules is an attractive therapeutic approach for cancer treatment because more than 300 client proteins are involved in aggressive cancer growth and metastasis. To date, ~20 inhibitors have undergone clinical trials, however, all have failed due to pan-inhibitory activities and insufficient isoform selectivity. In this study, we have investigated isoform selective HSP90β inhibitors, NDNB21, NDNB25 and NDNB11.82 on esophageal adenocarcinoma (EAC), which is one of the most aggressive human cancers with increasing incidence in the United States. A major target for EAC therapies is the human epidermal growth factor receptor 2 (HER2), which is a well-known client protein of HSP90. In this research, we tested the effect of NDNB21 and NDNB25 on HER2 overexpressing EAC cell lines, including one cell line that acquired lapatinib resistance either alone or in combination with HER2/MET targeting by lapatinib/capmatinib.

Methods: We first evaluated HER2/pHER2 and MET/pMET expression status by Western blot analysis in a panel of EAC cell lines (Flo-1, ESO26, OE19, OE33, SK-GT-2, ESO51, OACM5.1C, and LPR-OE19). Lapatinib-resistant OE19 (LPR-OE19) cell line was established from OE19 cells by intermittent exposure to increasing concentrations of lapatinib for a period over six months. Lapatinib is a potent EGFR/HER2 inhibitor and capmatinib is a potent c-MET inhibitor. Antiproliferative activities were measured by WST-1 assay and Western blot analyses were performed to evaluate apoptosis.

Results: NDNB21 and NDNB25 dose-dependently inhibited *in-vitro* cell proliferation of HER2 overexpressing EAC cell lines with NDNB11.82 maintaining much lower potency. All three HER2 overexpressing EAC cell lines showed significant sensitivity to NDNB21 and NDNB25 mediated inhibition of cell proliferation with IC_{50} values between 16 nM to 2 μM and degree of sensitivities are OE19>LPR-OE19>OE33. NDNB21 and NDNB25 dose-dependently induced apoptosis as measured by the expression of cleavage of poly ADP-ribose polymerase (cleaved PARP). Treatment with increasing doses of NDNB21/NDNB25 resulted in a dose-dependent decrease in HER2, p-HER2, AKT, and p-AKT. Coadministration of NDNB21 or NDNB25 with lapatinib effectively inhibited HER2 phosphorylation and significantly enhanced inhibition of cell proliferation in OE19/LPR-OE19 but not in OE33. The OE33 cells with the strongest expression of phosphorylated MET showed reduced sensitivity to HER2 targeted NDNB21/NDNB25/lapatinib induced cell death compared to that of OE19/LPR-OE19. OE33 showed enhanced cell death when HER2 targeted NDNB25 were combined with MET inhibitor capmatinib.

Conclusions: In conclusion, our data shows that NDNB21/NDNB25 exhibited potent apoptotic activity against HER2-positive EAC cells either alone or in combination.

#3036 Direct co-targeting of Bcl-xL and Mcl-1 exhibits synergistic anti-cancer effects in AR-V7-expressing mCRPC preclinical models.

Benjamin C. Brim[1], Andres F. Leon[1], Erica L. Beatson[1], Jessica D. Kindrick[1], Kinjal Bhadresha[1], Xiaohu Zhang[2], Giulia C. Napoli[1], Emily N. Risdon[1], Keith T. Schmidt[1], Erin Beck[2], Carleen Klumpp-Thomas[2], Michele Cerebelli[2], Douglas K. Price[1], Cindy H. Chau[1], Craig J. Thomas[2], William D. Figg[1]

[1]Genitourinary Malignancies Branch, National Cancer Institute, Bethesda, MD,[2]Division of Preclinical Innovation, National Center for Advancing Translational Sciences, Rockville, MD

There is an unmet need to develop novel treatment options for patients with metastatic castration-resistant prostate cancer (mCRPC). Patients often develop resistance to hormonal therapies that target the AR-axis (e.g., abiraterone, enzalutamide). A splice variant of AR, AR-V7, is associated with resistance to these inhibitors as well as mCRPC progression and poor prognoses. We embarked upon a high-throughput screen to identify synergistic combinations of targeted therapies using two CRPC cell lines, LNCaP95 and VCaP-CR. The goal of the drug screen is to identify rational targeted combination therapies for mCRPC, where efficacy can be achieved at lower, equally effective doses in order to minimize toxicity. Extensive bioinformatic analysis identified several synergistic combinations, including dual targeting of Bcl-2 family proteins using BH_3 mimetics. Combinations targeting Bcl-xL (A-1331852 and Navitoclax) and Mcl-1 (S63845) synergistically decreased cell viability and induced apoptotic activity via cleavage of PARP, caspase 3, and caspase 7 across AR-V7 expressing CRPC cell lines (LNCaP95, VCaP-CR, 22Rv1) as early as two hours post-treatment. The effect of the treatment combination on downstream targets was assessed via western blot analysis. We demonstrated synergistic anti-tumor effects with Mcl-1/Bcl-xL co-targeting in three-dimensional (3D) spheroid CRPC models and in the patient-derived LuCaP-167CR organoid model, which represents a more physiologically relevant model for evaluating drug response and efficacy. We also explored the use of a Bcl-xL-specific PROTAC to minimize platelet toxicity associated with Bcl-xL inhibitors in the clinic. We showed similar synergistic efficacy with the S63845/Bcl-xL targeting PROTAC combination in the 3D spheroid models compared to the small-molecule Bcl-xL inhibitors while also inducing apoptosis. Our findings provide unique insight into the dependence on Bcl-2 family proteins in mCRPC. Our findings also support further preclinical development of Bcl-xL and Mcl-1 inhibitors for mCRPC.

#3037 Targeting pro-survival MYC oncogene expression in small cell lung cancer and large cell neuroendocrine carcinoma of the lung with a novel small molecule PIM2 inhibitor.

Katherine G. Minton, Anthony H. Cuadros, Olivia C. Terry, Phu D. Pham, Mateusz Opyrchal, Kelvin P. Lee, Misty D. Shields

Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, IN

Introduction: Small cell lung cancer (SCLC) and large cell neuroendocrine carcinoma of the lung (LCNEC) are aggressive neuroendocrine carcinomas (NECs) that exhibit frequent chemoresistance and lack effective treatments for relapsed disease. *MYC* paralogs are frequently amplified on extrachromosomal DNA in lung NECs. Recent interrogation into the evolution of SCLC has identified *c-MYC* amplification as a major driver of tumor plasticity across the neuroendocrine spectrum. As such, targeting molecular underpinnings related to *MYC* amplification provides a therapeutic vulnerability for lung NECs. JP11646, a potent non-ATP competitive inhibitor of PIM family members that function to phosphorylate Ser329 on c-MYC, has preclinical efficacy in a number of cancers including triple negative breast cancer and multiple myeloma. Here, we investigate the efficacy and functional effects of JP11646 in lung NECs.

Methods: JP11646 sensitivity was evaluated in triplicate with eight serial dilutions for 72 h (hours). The transient effects of JP11646 after 24 h were evaluated in H1155, H524, H209, and H1417, which represent the spectrum of MYC paralog amplification in lung NECs, through RNAseq. Protein expression was evaluated via western blot analysis (PIM2 1:1000). *PIM2* RNA interference (50 nM) was performed to investigate effects on JP11646 sensitivity and interactions between *PIM-* and *MYC-* paralogs after 72 h.

Results: A panel of MYC paralog amplified cell lines differentially express PIM2 and exhibit nanomolar potency for JP11646 (IC_{50}: mean 87 nM, range 7 - 117 nM). Transient knockdown of PIM2 results in reductions in *PIM3* and *l-MYC* in H209. Following 24 h JP11646 treatment, we find that cells exhibit reduction of PIM2 and MYC paralogs and extensive downregulation of oncogenes including amplified *MYC* paralogs and *AURKA*. Interestingly, a pattern of upregulation of reciprocal *MYC* paralogs is observed in H1155, H209, and H1417. Insignificant changes are observed in *PIM* family expression with 24 h JP11646 treatment. After 72 h, we find that *PIM2* knockdown further enhances JP11646 sensitivity in H1155, reducing IC_{50} two-fold.

Conclusion: Given the observed reduction of PIM2 protein levels following treatment, we hypothesize that JP11646 functions through a feedback mechanism, disrupting PIM2:MYC interactions to reduce MYC paralog RNA/protein expression in lung NECs and exert its anticancer effects. Future studies are underway to further examine the mechanism and preclinical effects of JP11646, including xenograft studies, proteasome inhibitor studies, and CRISPR knockout of *PIM2*.

#3038 Preclinical evaluation of combination therapy of HJ-037 with anti-PD-1 antibody treating KRAS^{G12C} mutated tumors.

Na Guo, Xiangyu Yang, Longjun Yang, Fengtian Du, Huiqiao Ling, Ke Xue, Lina Zhang, **Jianxin Ji**, Zhigang Wu

Chengdu Huajian Science Co., Ltd., Chengdu, China

Background: The development and clinical application of KRAS^{G12C} inhibitors have made significant progress in recent years and some groundbreaking clinical results have been reported. However, overcoming resistance of KRAS^{G12C}-OFF covalent blockade remains a key focus in the fight against KRAS^{G12C} mutated cancers. Combination therapy may be one of the best treatment strategies to overcome the resistance.

Methods and Results: HJ-037 is an orally available, potent, and highly selective covalent KRAS^{G12C} inhibitor, which showed significantly superior inhibitory activities against NCI-H358 and Mia PaCa-2 cells to current marketed KRAS^{G12C} inhibitors, and favorable safety profile was observed in pre-clinical study, providing a good safety foundation for its combination with PD-1 inhibitor. In a Colon Cancer CT26-mKRAS^{G12C} cell line subcutaneous syngeneic model, animals treated with HJ-037 plus anti-PD-1 antibody showed complete tumor regression without recurrence. A proof-of-concept clinical trial combining HJ-037 with PD-1 inhibitor is currently ongoing, and preliminary data shows a positive trend in efficacy and safety profile.

Conclusions: Preclinical study results indicate that the combination therapy of HJ-037 with PD-1 inhibitor could enhance the intensity and duration of antitumor activity compared to monotherapy, with satisfactory safety, therefore possessing a better potential for the treatment of cancer patients harboring KRAS^{G12C} mutation.

#3039 Elucidating hsa_circ_0001038 and circRNA_400029 circular RNAs mechanisms in response to splicing factor 3 inhibitor in cervical cancer cells.

Amahle Nyalambisa, Zodwa Dlamini, **Rahaba Makgotso Marima**

University of Pretoria, Pretoria, South Africa

Cervical cancer (CCa) is the fourth leading cause of cancer-related death among women worldwide. Around 90% of CCa cases are reported in low- and middle-income countries (LMICs), with Sub-Saharan Africa (SSA) bearing over 80% of the global burden. CircRNAs hsa_circ_0001038 and circRNA_400029 have been implicated in cervical cancer. Splicing factor 3 key a key factor in circRNA generation. However, mechanisms of these ceRNAs in CCa is poorly understood. This study aimed at elucidating hsa_circ_0001038 and circRNA_400029 mechanisms in response to splicing factor 3 inhibitor, theophylline in CCa. Using bioinformatics, CircAtlas, the study identified miRNA binding sites for hsa_circ_0001038, selecting miR-205-5p for further analysis due to its tumor-suppressive role. Similarly, miRTarBase predicted miR-16-5p as a key interacting miRNA for circRNA_400029. The downstream gene targets of these miRNAs were explored, focusing on those associated with CCa. Cell cycle FACS analysis and apoptosis assay were used to study cell biological response of theophylline to CCa cells SiHa and C33A. Furthermore, gene specific primers of hsa_circ_0001038, circRNA_400029 and SRSF3 were used to study gene expression in drug treated and control groups. Interestingly, ceRNA network was constructed, showing that one gene can be targeted by more than one miRNA. Additionally, pathway analysis using KEGG, STRING, and Reactome highlighted key signaling pathways, such as the HPV E5-EGFR-RAS-ERK and HPV E6-Notch pathways, which are implicated in cell proliferation, survival, and oncogenesis in CCa. Cell cycle analysis revealed G2/M arrest in response to theophylline treatment, while there was no significant cytotoxic effects of the drug, as revealed by apoptosis FACS analysis. hsa_circ_0001038, circRNA_400029 and SRSF3 were downregulated by theophylline treatment. Understanding circular RNAs and their interaction with splicing factors holds potential translational and clinical applications.

#3040 Memantine, (a dual α7-nAChR/NMDAR antagonist) displays anti-tumor activity in human squamous cell lung cancer.

J. C. Christian[1], K. L. Denning[1], K. J. Modi[1], R. S. Light[1], S. L. Miles[1], Y. Chen[2], P. Dasgupta[1];
[1]Marshall University School of Medicine, Huntington, WV, [2]Bluefield State University, Bluefield, WV

Purpose of the study: Squamous cell lung carcinoma (LUSC), also called epidermoid carcinoma, is a type of non-small cell lung cancer (NSCLC) that typically develops in one of the air passages, or bronchi, of the lungs. It is the second most common type of NSCLC and accounts for roughly 30 percent of all lung cancer cases. The development of LUSC bears an 85% correlation with smoking habits. Nicotine, the addictive component of cigarettes, promotes the growth and progression of lung cancers via the α7-nicotinic acetylcholine receptor (α7-nAChR) subunit in human lung cancer cells. Published reports show that the α7-nAChR is over expressed on LUSCs relative to normal lung epithelial cells. Therefore, agents which block the function of α7-nAChR should inhibit the growth of human LUSC cells. The drug memantine is a dual α7-nAChR/NMDAR antagonist which is used clinically to combat dementia associated with mild -to-moderate Alzheimer's disease in patients. Receptor binding assays have shown that the affinity of memantine for α7-nAChR receptors is greater than that of NMDARs. The primary objective of our study was to determine the anti-cancer activity of memantine in human LUSC.

Experimental procedures. The expression of α7-nAChR on human LUSC cells was analyzed by ELISA. The effect of memantine of nicotine-induced proliferation of LUSC cells was measured by Proliferating Cell Nuclear Antigen (PCNA) ELISA assays. The growth-inhibitory activity of memantine was confirmed in chicken chorioallantoic membrane (CAM) model systems of LUSC. Next, the anti-tumor activity of memantine was measured in two independent mice models, namely the cell derived xenograft (CDX) mouse model and the patient derived xenograft (PDX) mouse model. Finally, siRNA methodology was used to delineate the signaling pathways underlying the anti-proliferative activity of memantine.

Results: Memantine displayed robust anti-proliferative activity in a panel of human LUSC cell lines. The anti-proliferative activity of memantine was also observed in chicken CAM models of LUSC. The treatment of LUSC cells with memantine induced cell cycle arrest at G1/S phase. Finally, the administration of memantine via dietary methods decreased nicotine-induced growth of LUSC tumors in both CDX and PDX tumors in immunodeficient mice models.

Conclusions: The drug memantine could be repurposed for the treatment of LUSC in patients.

Support or Funding Information:
Funding for our study was supported by an NIH R15-AREA Grant (2R15CA161491-02), Edwards Cancer Center Pilot grant, Cabell Huntington Hospital, Marshall University. This work was supported in part by the West Virginia IDeA Network of Biomedical Research Excellence (WV-INBRE) grant GM103434 (PI: Dr. G. Rankin).

#3041 Arsenoplatin-5: A small molecule that delivers arsenous acid.

D. Miodragovic[1], W. Qiang[2], B. Hong[3], T. O'Halloran[3];
[1]Northeastern Illinois University, Chicago, IL, [2]Northwestern University, Evanston, IL, [3]Michigan State University, East Lansing, MI

Arsenic trioxide (ATO), an FDA-approved drug for treating Acute Promyelocytic Leukemia (APL), increased the survival of newly diagnosed and relapsed APL patients. Despite its success in treating blood cancer, this success did not translate in clinical trials treating solid tumors due to low bioavailability, rapid renal clearance, and cardiotoxicity when higher doses are applied. The search for agents that deliver ATO to solid tumors has been ongoing for almost two decades. While a number of nanoformulations have been evaluated, none have been approved for clinical use. Our solution to this problem is developing small molecules that release arsenous acid, the aqueous and active form of ATO. Inspired by the synergy between cisplatin and ATO, we designed arsenoplatins, dual pharmacophore agents in which an aqueous form of arsenic(III) is covalently bound to the platinum(II) center. Given superior arsenoplatin-5 (AP-5) activity against TNBC (triple-negative breast cancer) cell lines relative to cisplatin or ATO used as single drugs, its potency was evaluated in a five-dose NCI-60 screen using the Sulforhodamine B (SRB) assay by Developmental Therapeutics Program at the National Cancer Institute (DTP-NCI). The COMPARE algorithm was used to compare GI_{50}, TGI, and LC_{50} values, thereby allowing evaluation of the inhibitory and cytotoxic activity of AP-5 to all other agents in the NCI database. A very high Pearson correlation coefficient (PCC) of 0.84 is obtained when AP-5's GI_{50} values were compared to GI_{50} values of ATO, indicating a similar mechanism of action. The uptake of AP-5 was evaluated in TNBC MDA-MB-231 cells using ICP-MS (inductively coupled plasma mass spectrometry). Significantly more arsenic atoms than platinum atoms in cells were taken up upon incubation of MDA-MB-231 cells with 10 μM AP-5. These results are consistent with a model in which the Pt-As bond in AP-5 breaks inside the cell and releases arsenous acid moiety, with arsenic being more retained in cells than platinum, in correlation with high PCC revealed. Interestingly, when comparing LC_{50} values of AP-5 with all other agents in the NCI database using a COMPARE algorithm, the highest PCC was obtained for alkylating agents, pipobroman and cisplatin (0.47 and 0.46, respectively). These results support a dual mechanism for arsenoplatins wherein the inhibitory activity of AP-5 is due to arsenic pharmacophore and cytotoxic activity due to platinum pharmacophore. Remarkably, although bearing both platinum and arsenic pharmacophores, AP-5 is significantly less toxic than cisplatin, based on single maximum tolerated dose (MTD) studies in vivo in female C57BL/6 mice. These results demonstrate that AP-5 is a potent, well-tolerated dual pharmacophore agent in vivo and represents the first small molecule capable of delivering an aqueous form of arsenic(III).

#3042 Non-covalent thioredoxin reductase inhibitors for TNBC.

B. Flowers[1], A. Rullo[1], P. Petukhov[2], I. Kastrati[1];
[1]Loyola University Chicago, Maywood, IL, [2]University of Illinois Chicago, Chicago, IL

Triple negative breast cancer (TNBC) has the lowest 5-year mortality and lacks targeted therapeutics. Therefore, there is a need to explore more therapeutic targets and strategies. Previous research has indicated that TXNRD1 may be a relevant target in other cancers and is elevated in TNBC and high levels of TXNRD1 and TXNRD2 correlate to lower recurrence free survival and lower overall survival (TXNRD1) and has emerged as a possible therapeutic target.The thioredoxin system includes thioredoxin reductase (TXNRD) and thioredoxin (Trx). TXNRD takes electrons from NADPH to reduce the disulfide bond in thioredoxin. Reduced thioredoxin then goes on to reduce reactive oxygen species (ROS), other oxidized species, and proteins within the cell. This system is critical in maintaining intracellular redox homeostasis, especially in breast cancer, where tumor cells heavily rely on these detoxifying systems to balance ROS produced by the dysregulated metabolism in cancer.Previous research has shown that targeting the thioredoxin reductase system using FDA approved auranofin has anticancer activity. However, auranofin is a covalent inhibitor of thioredoxin reductases, inhibits isoforms indiscriminately, and has significant side effects.Here, we show that new non-covalent inhibitors developed to target thioredoxin glutathione reductase in *Schistosoma mansoni*, also inhibit human TXNRD as demonstrated through fluorescent biochemical assays and qPCR of TXNRD related genes. Additionally, these non-covalent TXNRD inhibitors (TXNRD(i)s) show anti-cancer activity in both *in vitro* and *in vivo* TNBC models. Treatment with these TXNRD(i)s reduces viability and collagen invasion in TNBC cell lines. Similarly, knocking down TXNRD1 reduces TNBC cell invasion through collagen. Indicating TXNRD1 may be the primary target. In a TNBC xenograft, these TXNRD(i)s inhibit primary tumor growth, alter gene expression levels of multiple epithelial-mesenchymal transition (EMT) associated genes within the tumor and inflammatory markers within the host animal. These data also indicate there is a potential use of these inhibitors in a metastatic model that remains to be explored.Overall, we have shown that these TXNRD(i)s inhibit human TXNRD, have anti-cancer effects in TNBC *in vitro* and *in vivo*, inhibit collagen invasion, and alter EMT and inflammatory gene expression levels. Further research on the downstream mechanisms and optimization of these TXNRD(i)s is needed.

#3043 Preclinical efficacy assessment of brincidofovir against glioblastoma.

K. Seo[1], T. Ozawa[1], D. R. Raleigh[1], S. Nagatani[2], **M. Hazama**[2];

[1]UCSF Brain Tumor Center, San Francisco, CA, [2]SymBio Pharmaceuticals Limited, Minato-Ku, Tokyo, Japan

Introduction: Clinical use of the cytidine-analogue antiviral, cidofovir (CDV), is limited by nephrotoxicity. The lipid-conjugated CDV, brincidofovir (BCV), is characterized by a lack of nephrotoxicity, as well as superior activity compared to CDV. The objectives of this study were to examine the preclinical efficacy of BCV against glioblastoma (GBM), a malignant tumor with an extremely poor prognosis, and to identify biomarkers predicting the efficacy of BCV against GBM.

Methods: BCV cytotoxicity was tested *in vitro* in 11 human GBM cell lines, using the CellTiter-Glo® (CTG) assay. Those cell lines were then analyzed by whole-transcriptome sequencing, and differential gene expression analysis between BCV-sensitive and non-BCV sensitive cell lines was performed using DESeq2 software. To examine the influence of O^6-methylguanine-DNA methyltransferase (MGMT) expression on BCV sensitivity, monoclonal MGMT-overexpressing cell lines were established using lentiviral vector-based gene transfer in U87MG cells. MGMT gene and protein expression were confirmed using qPCR and western blot, respectively, and the BCV IC_{50} values were compared among these cell lines using the CTG assay. Finally, the *in vivo* anti-tumor efficacy of BCV was evaluated in nude mice that were orthotopically implanted with firefly luciferase-transfected U87MG cells. Mice were i.p. administered vehicle or BCV (10, 20, or 40 mg/kg) twice weekly, and bioluminescence imaging (BLI) was performed twice weekly after i.p. D-Luciferin injection to evaluate intracerebral tumor growth. Survival was evaluated using the Kaplan-Meyer method.

Results: The CTG assay revealed a median BCV IC_{50} value of 2.2 μM. Sub-micromolar BCV IC_{50} values were obtained in KALS-1, T98G, and LN-18 cells. In these highly sensitive cell lines, differential gene expression analysis identified glypican 6 (GPC6) as the gene showing the most significantly decreased expression, and neuronal tyrosine-phosphorylated phosphoinositide-3-kinase adaptor 2 (NYAP2) as the gene with the most significantly increased expression. Compared to the parent MGMT-negative U87MG cells, two MGMT-overexpressing cell lines exhibited BCV IC_{50} values that were lower by 16.3-fold and 2.5-fold. *In vivo* experiments demonstrated that BCV significantly decreased intracranial tumor growth, determined as BLI, in a dose-dependent manner during the treatment period compared with the vehicle group. With the exception of weight loss in the BCV 40 mg/kg group, no apparent systemic toxicity was observed in the other BCV dose groups. Compared with the vehicle group, the BCV 20 mg/kg group exhibited significantly increased survival.

Conclusion: BCV exerted antitumor effects on GBM *in vitro* and *in vivo*, regardless of MGMT status, and improved survival *in vivo*. Low GPC6 expression and high NYAP2 expression could serve as clinical pharmacologically significant biomarkers that predict high sensitivity to BCV.

#3044 Butyrate modulates key oncogenic pathways in triple-negative breast cancer: insights from transcriptomic profiling.

Gabriel Borges Velez[1], Diego N. Bracero-Sanchez[2], Liah M. Roman-Calderon[1], Ariana S. Garcia-Lopez[1], Angel J. Amaral-Maisonet[3], Jeannette L. Salgado-Montilla[1], Esther A. Peterson-Peguero[4], Josue Perez-Santiago[1]

[1]Division of Clinical & Translational Cancer Research, UPR - Comprehensive Cancer Center, San Juan, Puerto Rico,[2]Division of Clinical & Translational Cancer Research, University of Maryland, College Park, MD,[3]Science-Interdisciplinary Studies, UPR - Rio Piedras, San Juan, Puerto Rico,[4]Department of Biology, UPR - Rio Piedras, San Juan, Puerto Rico

Introduction: Triple-negative breast cancer (TNBC) is a highly aggressive subtype with a poor prognosis, affecting approximately 5% of breast cancer patients and lacking targeted therapies. Recent studies highlight the potential of short-chain fatty acids (SCFAs), metabolic byproducts of the microbiome, as anticancer agents. Among these, butyrate exhibits anti-inflammatory, immunomodulatory, and antineoplastic effects in several cancer types. Our preliminary data shows that butyrate exerts cytotoxic, anti-migratory, and antiproliferative effects on TNBC cells. However, to understand molecular mechanisms related to butyrate treatment and investigate its therapeutic potential in TNBC, this study measured transcriptomic changes after butyrate treatment of TNBC. **Methods:** MDA-MB-231 cells (TNCB cell model) were treated with 5 mM butyrate for 24 hours. RNA was extracted, quantified, and assessed for integrity before sequencing on the Illumina NextSeq 550. Bioinformatics workflows included quality control (FastQC), trimming (Trimmomatic), and alignment to the GRCh38 human genome using STAR. Read quantification was performed with RSEM, and differential gene expression was analyzed using R statistical software (tximport and DESeq2 packages). Functional annotation, pathway enrichment, and protein interaction analyses of significantly differentially expressed genes were conducted using Metascape and STRING (adj. p-value<0.05). **Results:** Butyrate treatment significantly altered the expression of 4,352 transcripts in MDA-MB-231 cells. The most upregulated transcript, DHRS2, a cell cycle regulator with tumor suppressor properties, exhibited an 8.8-fold increase. Conversely, ZNF709, a zinc finger protein frequently overexpressed in cancers, showed a 7.8-fold decrease. Pathway analysis revealed that affected transcripts are involved in critical oncogenic processes, including cell adhesion, cytokine signaling, wound healing, regulation of cell migration, and MAPK signaling. STRING analysis identified clusters of proteins significantly associated with protein phosphorylation regulation, along with binding-related molecular functions and phosphoprotein. **Conclusions:** Our results demonstrate that butyrate modulates the transcriptome of MDA-MB-231 cells, targeting pathways involved in oncogenic processes such as cell adhesion, migration, and signaling, which is consistent with our cellular results. Key transcripts, such as DHRS2 and ZNF709, are novel prognostic markers for prostate and gastric cancer respectively, highlighting butyrate's capacity to regulate tumor suppressor and oncogenic pathways. These findings support butyrate's potential as a microbiome-derived therapeutic for TNBC.

#3045 Development of a first-in-class PDIA6/IRE1 modulator for selectively sensitizing cancer cells to ER stress and regulated cell death.

Wei Jia[1], Xiaobing Zhang[1], Bo Kang[2], Zhao Xu[1], Chencong Bo[1], Mingxing Yin[1], Shujian Ren[1], Zhengshu Chen[1], Amar Al-Ansi[1], Hongban Zhong[1], **Ying-Jie Wang**[1]

[1]Nain Biotech Co., Ltd., Hangzhou, China,[2]Zhejiang University, Hangzhou, China

Background: A high proliferation rate of cancer cells requires increased protein synthesis leading to ER stress, a condition characterized by accumulation of unfolded or misfolded proteins in the ER lumen. Three interconnected Unfolded Protein Response (UPR) pathways, IRE1, PERK, and ATF6 pathways, are activated to relieve ER stress or to initiate apoptosis if prolonged strong ER stress remains unsolved. Thus, the chronically elevated ER stress levels in cancer cells constitute an Achilles' heel and provide a window of opportunity for development of therapeutic regimens. Despite multiple ongoing preclinical and clinical studies using various agents and regimens to overactivate ER stress and UPR, agents that can specifically induce UPR in cancer cells but not in normal cells are still lacking.

Methods: Metabolic activity-based phenotypic assay was employed to identify NAI003 as the most potent anticancer compound during lead optimization where over three hundred small-molecule NCEs were derived from the hit compounds. HuProt™ Human Proteome Microarray and chemoproteomics approaches were used to identify target proteins to which NAI003 can directly bind. The top ranked target proteins were each validated by specific siRNAs, followed by Biacore analysis to quantify target binding affinity. Subsequently, multiple approaches were used to further understand the MoA of NAI003 and to assess its developability.

Results: Our preclinical candidate compound NAI003 directly binds with high affinity (Kd=1.8 nM) and selectivity to PDIA6 protein that is known to catalyze protein folding and thiol-disulfide interchange reactions in the ER. NAI003 blocks the PDIA6/IRE1 protein-protein interaction in ER-stressed cancer cells and thereby inducing constitutively activated IRE1 signaling that ultimately leads to regulated cell death (RCD). NAI003 series compounds have subnanomolar to low micromolar IC50s against selected cervical, lung, ovarian, liver, pancreatic, skin and brain cancer cells *in vitro*, and potently inhibit their *in vivo* propagation in multiple CDX and PDX models. Remarkably, they completely spare normal cells that usually have much lower levels of ER stress. Furthermore, in rodent models, NAI003 compounds exhibit low acute toxicity, desirable pharmacokinetics profile and reasonable oral bioavailability. Importantly, NAI003 compounds exhibit synergism with PARP inhibitors (e.g. Talazoparib), proteasome inhibitors (e.g. Ixazomib) and metabolic stressors (e.g. glucose deficiency) that are all known to induce ER stress in cancer cells.

Conclusion: NAI003 is a potential first-in-class small-molecule PDIA6/IRE1 modulator that sensitizes cancer cells to ER stress and RCD by selectively overactivating IRE1 signaling. It holds great promise in treating selected cancers either as a single agent or as a combination therapy with marketed anticancer drugs.

#3046 Combination therapy targeting genomic and signal transduction vulnerabilities in epithelioid hemangioendothelioma.

Gillian DeWane, Nicholas Scalora, Yuliia Drebot, Ali Khan, Souradip Sinha, Krishnendu Ghosh, Nicole Merritt, Colleen Fullenkamp, Munir Tanas

Pathology, University of Iowa, Iowa City, IA

Background: Epithelioid hemangioendothelioma (EHE) is a vascular sarcoma that frequently presents with early onset metastasis and lacks an effective medical therapy. EHE sarcomas are defined by the presence of gene fusions, where 85-90% of all EHE tumors contain TAZ-CAMTA1 and 10-15% contain YAP-TFE3. These fusion proteins bypass regulation by the Hippo pathway and act as important transcriptional coactivators by activating a TEAD-based transcriptional program, which is responsible for activating genes in several downstream pathways, including the PI3K pathway. Topoisomerase II, a chromatin modifier, was found to act as a key cofactor for the TAZ-CAMTA1 and YAP-TFE3 transcriptional programs. Herein, we propose utilizing inhibitors as a combination therapy targeting the PI3K-Akt axis, topoisomerase II, and TEAD binding of the TAZ-CAMTA1 and YAP-TFE3 transcription factors.
Methods: Experiments were completed utilizing NIH 3T3 cells or a human liposarcoma cell line, SW872, that have been engineered to express the TAZ-CAMTA1 or YAP-TFE3 fusion proteins, or human EHE extended primary cell cultures. Several *in vitro* hallmarks of cancer assays were performed including 3D soft agar or poly-HEMA assays to measure anchorage independent growth and 2D clonogenic assays to measure cell survival. In each assay, cells were treated with various concentrations of vehicle control, everolimus, an mTORC1 inhibitor, IK930/VT-107, TEAD inhibitors, teniposide, a topoisomerase II inhibitor, or a combination of two or all three.
Results: In the cells mentioned above, treatment with everolimus, IK930, teniposide and the combination of everolimus and IK930 and teniposide and IK930 showed a dose dependent decrease in anchorage independent growth when compared to the vehicle control. In soft agar and poly-HEMA, synergy was found with the combination of everolimus and IK930 and teniposide and IK930. To examine the cytotoxicity of the drugs, a clonogenic assay was performed on cells treated with everolimus and IK930 and observed no apparent cytotoxicity in cells treated with these inhibitors, however we did see cytotoxicity when EHE cells were treated with VT-107.
Discussion/Conclusion: Because most EHE cells contain either the TAZ-CAMTA1 or YAP-TFE3 fusion proteins that activate a transcriptome that promotes several hallmarks of cancer, targeting the main way the fusions interact with DNA could mitigate these effects. Previous studies investigating the use of monotherapies to target cells expressing the fusion proteins show limited effectiveness. However, targeting both the interaction of the fusion proteins' ability to interact with DNA as well as an important pathway for EHE cells, such as the PI3K pathway, could lead to a greater effectiveness in decreasing EHE tumor progression. These studies here show everolimus, teniposide and IK930/VT-107 as a potential combination therapy for EHE.

#3047 Glycan-targeted therapy for effective treatment of pancreatic cancer.

Erica J. Peterson[1], Vignesh Vudatha[2], Vashti Bandy[2], Praveen Bhoopathi[2], Arunima Punjala[2], Thomas M. Clausen[3], Jennifer E. Koblinski[4], Chunqing Guo[5], Larisa Litovchick[1], Jose G. Trevino[2], Nicholas P. Farrell[6]

[1]Internal Medicine, Virginia Commonwealth University, Richmond, VA,[2]Surgery, Virginia Commonwealth University, Richmond, VA,[3]University of Hawaii at Manoa, Oahu, HI,[4]Pathology, Virginia Commonwealth University, Richmond, VA,[5]Immunology, Virginia Commonwealth University, Richmond, VA,[6]Chemistry, Virginia Commonwealth University, Richmond, VA

Patients with the most common pancreatic malignancy, pancreatic ductal adenocarcinomas (PDAC), typically present with advanced disease and their cancer progresses 6-12 months after treatment with the front-line regimen, FOLFIRINOX (5-fluorouracil, oxaliplatin, irinotecan and leucovorin). Therefore, there is a critical need for new and effective treatment regimens for PDAC patients. The novel polynuclear platinum agent, Triplatin (BBR3464), showed objective responses in a subset of patients, including a pancreatic cancer patient with liver metastasis in clinical trials. Although Triplatin did not advance beyond Phase II, our new understanding of its mechanism of action supports the rationale for its re-examination against tumors expressing high levels of glycosaminoglycans (GAGs), specifically chondroitin-4-sulfate (C4S). C4S is a tumor-specific GAG attached to proteoglycans such as CD44, glypicans and syndecans, expressed on the cell surface and in tumor stroma. The positive charge of Triplatin increases its binding affinity to negatively charged GAGs, which facilitates drug accumulation into the tumor cells and increases the level of platinum-DNA adducts. Recent studies suggest that overexpression of GAGs, particularly CS, contributes to the dense fibrotic stroma associated with PDAC, and resistance to chemotherapeutics and new immunotherapeutic agents. Our preliminary data indicates that alterations in the expression of GAG levels in PDAC cell lines affect the cytotoxicity and cellular uptake of Triplatin. First, we show Triplatin is generally more active than oxaliplatin against human and mouse PDAC cell lines. Secondly, a GAG-deficient CHX1990 cell line (CRISPR KO of β-1,4-galactosyltransferase 7 (B4GalT7), verified by reduction in binding of CS-binding peptide, rVAR2-V5, shows a correlative loss in cytotoxicity. Interestingly, the reduction in Triplatin Pt-DNA adduct formation observed in the CHX1990 B4GalT7 KO (HS and CS deficient), CSGalNAcT1/2 KO (CS deficient) and CHST 11/12 KO (chondroitin-4-sulfate (C4S) deficient) is at similar levels, suggesting DNA platination by Triplatin is dependent specifically on C4S. Third, we show significant activity of Triplatin against high GAG-expressing PDAC PDX and mouse CHX1990 models *in vivo*. Finally, we show high GAG expression in PDAC patient samples vs normal tissues, highlighting the potential of GAG-targeted platinum therapy. Together, our results show that GAGs may provide a viable biomarker for Triplatin in PDAC patients, producing a significant clinical advantage over oxaliplatin in this setting. A more active platinum would not only increase response rates and durations of response, but also avoid the toxicity of relatively inactive therapy.

#3048 *In vitro* and i*n vivo* anti-cancer properties of novel synthetic inhibitor targeting human NAD(P) dependent steroid dehydrogenase-like.

H. Kim[1], S.-H. Yoon[2], S. Lee[2], S. Ryu[2], H.-B. Lee[3], B.-J. Lee[4], W. Han[3];

[1]Seoul National University Hospital, Seoul, Korea, Republic of, [2]Seoul National University Graduate School, Seoul, Korea, Republic of, [3]Seoul National University College of Medicine, Seoul, Korea, Republic of, [4]Ajou University College of Pharmacy, Suwon-si, Korea, Republic of

Background: NAD(P)-Dependent Steroid Dehydrogenase-Like (NSDHL), which is involved in cholesterol synthesis, has been found to play a role in breast cancer stemness, growth, and metastasis. NSDHL is emerging as a potential new therapeutic target for breast cancer. This aim is to evaluate molecular mechanisms underlying the anti-cancer properties of a novel NSDHL inhibitor in breast cancer models *in vitro* and *in vivo*.

Methods: A structure-based inhibitor of NSDHL (Compound 9) was developed by Dr. Lee Bong-Jin. Compound 9 (1-250 μM) was treated in diverse human breast cancer cell lines (MCF-7, ZR75-1, BT474, BT-20, MDA-MB-231) for 2-7 days and administered by oral gavage (2.5 mg/kg/day) for 30 days in an orthotropic breast tumor model of BALB/c Nude mouse. Anti-cancer activities and molecular mechanisms of Compound 9 were evaluated by cell cycle analysis using DNA staining with PI, apoptosis analysis using annexin V/PI-labeling, cell proliferation analysis using crystal violet assay, cell migration analysis using transwell migration assay, western blot, immunohistochemistry, and TEM image.

Result: Rhodamine B-conjugated Compound-9 was easily internalized into breast cancer cells within an hour. Compound 9 exerted anti-proliferative and anti-migratory activity, G0/G1 phase arrest, and apoptotic and autophagic death along with the downregulation of XIAP and BCL2, the upregulation of P62 and LC3II, and modulation of the Akt/mTOR/MAPK signaling pathway. A combination of Compound 9 and paclitaxel enhanced the anticancer activity of paclitaxel in cultured breast cancer cells. Compound-9, when given orally to mice, inhibited the breast tumor growth without affecting their body weight.

Conclusions: We suggest that Compound 9 can be employed as a potential therapeutic drug to target NSDHL in cancer as well as cholesterol-related diseases.

[#]Corresponding Authors: Wonshik Han (hanw@snu.ac.kr) and Hoe Suk Kim (hoeskim@snu.ac.kr)

#3049 Synergistic growth-inhibitory efficacy of LSD1 and BCL-XL inhibitors in JAK2+ AML/MPN cells.

Mark A. Forsberg[1], Zhe Wang[2], Sovira Chaudhry[1], Anna Skwarska[1], Raajit Rampal[3], Alexandra Schurer[1], Kira Gritsman[1], Guangrong Zheng[4], Daohong Zhou[5], Marina Konopleva[1]

[1]Montefiore Einstein Comprehensive Cancer Center, Bronx, NY,[2]Division of Immunobiology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH,[3]Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY,[4]Department of Medicinal Chemistry, UF College of Pharmacy, Gainesville, FL,[5]Department of Biochemistry and Structural Biology and Center for Innovative Drug Discovery,, The University of Texas Health Science Center at San Antonio, San Antonio, TX

This study explores a potential synergy between Lysine-specific demethylase 1 inhibitors (LSD1i) and BCL-XL inhibitors for acute myeloid leukemias (AML) arising from myeloproliferative neoplasms (MPN). LSD1i prolongs the survival of post-MPN AML murine models (Rampal et al. Blood 2020) and induce apoptosis in AML cell lines via upregulating BAX (Wang et al. Blood, 2019). Our analysis of the RNA-seq and data from the Beat 1.0 AML cohort, CRISPR screen data from Cancer Cell Line Encyclopedia, Public Avana 21Q2 dataset, and multi-omics from post-MPN sAML patients (Rodriguez-Meira et al., Nat Genet. 2023) showed that post-MPN AML exhibited high expression of *BCL2L1*/BCL-XL (p=0.0026), compared to de novo AML (Wang, ASH 2024, #2749). We explored the synergy between the LSD1i Iadademstat (Selleckchem), and BCL-2/BCL-XL inhibitor, Navitoclax, or the platelet-sparing BCL-XL degrader, DT2216 (Dialectic Therapeutics). JAK2 mutated cell lines, Hel and Set2, and Ruxolitinib-resistant (RR) lines derived from them were utilized. The activities of LSD1i, navitoclax, and DT2216, alone and in combination, were tested using Cell Titer Glo assays of 20,000 cells at 72 hours. LSD1i as a single agent (0.6-50 nM) reduced viability plateauing at 50-68% in all tested cell lines. Navitoclax and DT2216 effectively reduced cell viability; when combined with 10 nM of LSD1i, IC_{50} values were significantly lower (Table 1). These findings were next verified using the Annexin 5 flow cytometry at 24 hours co-treatment. All combinations showed a significant increase in apoptosis over either compound alone (Table 1). Synergy was confirmed with SynergyFinder+ software (mean: 18.86-72.47 zip model). In summary, $JAK2^{+}$-mutant post-MPN AML cell lines and isogenic Ruxolitinib-resistant lines showed sensitivity to BCL-XL inhibitors and a synergistic increased sensitivity to these compounds when combined with low dose LSD1 inhibitor, Iadademstat. Mechanisms behind the synergy are being studied and will be reported.

Summary of Glo Titer Assays and Annexin 5 Flow Cytometry Data with mean Synergy Scores

			Glo Titer Assay			
		Navitoclax	DT2216	LSD1i	LSD1i+Navitoclax	LSD1i+DT2216
Hel Parental	IC_{50}	1.3±0.2μM	2±0.8μM	Not Reached	6.5±3 nM	0.25±0.08 μM
Hel RR	IC_{50}	0.5±0.3μM	1.8±0.7μM	Not Reached	6±3nM	0.3±0.15 μM
Set 2 Parental	IC_{50}	0.4±0.4μM	2.5±1 μM	Not Reached	6.5±02.5nM	2±1.3 μM
Set 2 RR	IC_{50}	1±0.2μM	0.6±0.3μM	Not Reached	20±9 nM	0.525±0.5μM
			SynergyFinder+ Glo Titer			
		Hel Parental	Hel RR	Set 2 Parental	Set 2 RR	
Zip Model Mean Synergy:	LSD1i + Navitoclax	68.92	55.32	48.72	69.88	
Zip Model Mean Synergy:	LSD1i + DT2216	31.42	45.22	19.25	18.86	
			Annexin-5 Flow Cytometry			
	Navitoclax 10 nM	DT2216 1μM	LSD1i 5 nM	LSD1i + Navitoclax	LSD1i + DT2216	Control
Hel Parental (Annexin %+)	13.76%	25.4%	12.24%	22.51%	52.9%	9.92%
Hel RR (Annexin %+)	32.3%	11.34%	26.37%	26.37%	52.7%	9.04%
Set 2 Parental (Annexin %+)	21.36%	29.18%	7.15%	57.9%	46.11%	7.48%
Set 2 RR (Annexin %+)	33.20%	45.3%	14.86%	87%	47.8%	12.33%
			SynergyFinder+ for Annexin-5 Dilutional Series			
		Hel Parental	Hel RR	Set 2 Parental	Set 2 RR	
Zip Model Mean Synergy:	LSD1i + Navitoclax	34.23	32.99	68.97	72.47	

Zip Model Mean Synergy:	LSD1i + DT2216	58.93	23.44	44.24	20.22	

#3050 Targeting SREBP-driven transcriptional programs in metastatic prostate cancer.

Prashanth Parupathi, Lakshmi Sirisha Devarakonda, Enkiel Francois, Bhaskar C Das, Avinash Kumar

Long Island University, Brooklyn, NY

Prostate cancer (PCa) is the most commonly diagnosed cancer and the second leading cause of cancer-related deaths among men in the United States. Metastasis is the major cause of mortality associated with prostate cancer, and despite the availability of targeted therapeutic agents, metastatic prostate cancer remains a lethal disease. Therefore, there is a significant unmet need to identify new drug targets and develop novel targeted therapeutic agents. Sterol regulatory element binding proteins (SREBPs) are a family of transcription factors, comprising SREBP-1 and SREBP-2, that govern lipid homeostasis by regulating the expression of numerous enzymes involved in fatty acid and cholesterol synthesis (lipogenesis). The role of SREBPs in regulating non-lipogenic transcriptional programs is beginning to be elucidated. Published findings associate perturbation of SREBP-dependent transcriptional programs in the development, progression, and metastasis of various cancers, including PCa. Our analysis of publicly available patient datasets revealed increased gene expression for SREBPs in prostate tumors compared to normal prostate. Further, we discovered significantly higher gene expression for SREBPs in metastatic samples compared to primary prostate tumors. Moreover, we found significantly elevated levels of SREBPs in more invasive PC3M and C4-2B compared to the less invasive PC3 and LNCaP prostate cancer cell lines. Hence, we hypothesized that pharmacological inhibition of SREBPs might be an effective therapeutic strategy against metastatic prostate cancer. We have developed a novel boron-containing compound, BF175, which inhibits the expression and transcriptional activity of SREBPs, but the information on the anticancer action of BF175 in various cancers, including prostate cancer, is very limited. In this study, we investigated the SREBP-mediated anticancer activity of BF175 in prostate cancer. We found that BF175 inhibited the viability, proliferation, and metastatic potential as well as induced apoptosis in all prostate cancer cell lines tested. We also confirmed that BF175 inhibited the expression of SREBPs in the tested prostate cancer cell lines. Of note, BF175 exhibited significantly higher potency as an anticancer agent in more invasive prostate cancer cell lines compared to their less invasive counterparts. Taken together, our results demonstrate that BF175 shows promise as a novel anticancer agent that may improve outcomes in lethal metastatic prostate cancer.

#3051 DiaXCIP3M: A new small drug molecules conjugate displaying a strong antiproliferative in vitro and in vivo activity against human small cell lung cancer.

Gregoire Pierre Prevost[1], Gwenael Forcher[2], Anne-Caroline Chany[2], Laurent Fontaine[2]

[1]CIPREVO, Antony, France,[2]Institut des Molecules et Materiaux du Mans - UMR 6283 CNRS -, Le Mans, France

The high medical need for new, original therapeutic molecules remains clear for all those fighting small cell lung cancer (SCLC). DiaXCIP3M based on patented technology belongs to a new class of small drug molecules conjugates (SDMCs). Traditional chemotherapy mostly relies on the use of a cocktail of several anticancer drugs. However, two different drugs display two different pharmacokinetics and two different tissue biodistributions meaning that it is very challenging to anticipate the ratio of these two drugs in the tumor or normal cells. Small molecule drug conjugates (SMDCs) are a promising strategy to reduce these limits by coupling two distinct cytotoxic molecules in one new drug therefore inducing only one pharmacokinetic and one biodistribution. The inter-variability of the drug exposure between patients is thus expected to be reduced compared to the cocktail approach. Reduction of the variability allows a better definition of the recommended dose to actively and safely treat the cancer patients. Preclinical studies performed today indicate that DiaXCIP3M has a strong product profile for further development with 1/ a patented chemical structure, 2/ an original mechanism of action that involves simultaneously inhibition of two validated anticancer targets, resulting in activity in a large range of cancer subtypes, 3/ a powerful in vitro antitumor activity at low nM against human SCLCs, 4/ a suitable in vivo plasma stability assessed by iv route in mouse superior to 2 hrs, and 5/ an induction of in vivo tumor reduction at safe dose in human SCLC xenografted model in mice.DiaXCIP3M has the potential to become an integral part of the therapeutic landscape for ES-SCLC, particularly as a second-line alternative to the currently validated chemotherapy, topotecan, or as a third-line treatment after topotecan failure.

#3052 Butyrate reduces NLRP3 RNA expression in oral squamous cell carcinoma.

Oscar Antonio Loperena Gonzalez, Angel J. Amaral Maisonet, Jurelis Torres Reyes, Jeannette L. Salgado Montilla, Gabriel Borges Velez, Josue Perez Santiago

University of Puerto Rico Comprehensive Cancer Center, San Juan, PR

Background: Oral squamous cell carcinoma (OSCC) is a type of oropharyngeal cancer associated with elevated levels of IL-1β, which can be secreted with the activation of the inflammasome NLRP3 and caspase 1 signaling pathway. High levels of IL-1β in OSCC leads to cancer progression, invasiveness, autocrine signaling which promotes metastasis, and prolonged inflammation that contributes to tumorigenesis. Inflammasomes are proteins essential for rapid responses in the innate immunity and they work with caspases to promote inflammation signaling, but persistent signaling can induce malignant transformation of cells. Additionally, OSCC is exposed to microbial products, short chain fatty acids (SCFA) which can influence transcription of inflammasomes and caspases; yet it has not been evaluated in OSCC. We have observed that butyrate treatment reduces cell viability, migration, and proliferation in OSCC cell model (OECM-1). Therefore, we aimed to investigate the SCFAs effect in regulating genes *NLRP3*, *NLRP6*, *Caspase 1* and *Caspase 4* in OECM-1.

Methods: We extracted RNA from OECM-1 untreated and treated either with butyrate, acetate, or propionate at 5mM for 24 hours. We quantified RNA expression NLRP3, NLRP6, caspase 1, and caspase 4 by probe-based qRT-PCR using the gene RPP30 as the endogenous control. We calculated the fold change using comparative Ct method (ΔΔCt) and t-test to determine significance difference in the levels of inflammasome and caspase after SCFA treatment vs untreated cells.

Results: Levels of NLRP3 significantly decreased (fold change: -2.43, $p=0.022$) and levels of caspase 1 significantly increased (fold change: 1.51, $p<0.001$) after 24 hours exposure of butyrate. There were no significant changes in gene expression levels of NLRP6 or caspase 4. Treatment with propionate resulted in the increase of NLRP3 (fold change: 1.94, $p=0.086$), NLRP6 (fold change: 2.22, $p=0.058$), caspase 1 (fold change: 2.84, $p=0.026$), caspase 4 (fold change: 1.87, $p=0.057$). No significant changes in levels of the inflammasomes or caspases were observed with acetate treatment.

Conclusion: This study demonstrates that butyrate reduces NLRP3 inflammasome expression on OSCC, promotes caspase 1 expression and does not alter NLRP6 and caspase 4 expression. Conversely, our data shows that propionate increases NLRP3 and caspase 1 transcription, which increases IL-1β levels; and increments NLRP6 and caspase 4 transcription, both associated with inflammation. This proposes butyrate's ability to reduce IL-1β levels in OSCC and potentially be promoting alternate pathways with caspase 1. Modifying the microbiome to induce greater butyrate secretion or direct inhibition of NLRP3 inflammasome through medications, can represent a novel treatment strategy for OSCC.

#3053 SHP2 inhibition with RMC4630 demonstrates antitumor efficacy and potentiates nanoparticle-paclitaxel response in pancreatic cancer.

Nicola Grimaldi, Lily Darman, Mitchel Ramos, Margaret Schwarz, Niranjan Awasthi

Indiana University School of Medicine, South Bend, IN

Objective: Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal malignancy with a grim prognosis. The combination of nab-paclitaxel and gemcitabine (NPT-GEM) remains the standard treatment for advanced and metastatic PDAC, achieving a median survival of approximately 8.5 months. An activating mutation in the KRAS gene is central to PDAC pathogenesis, driving cancer cell proliferation, survival, and tumor progression through downstream pathways, including PI3K/AKT/mTOR and RAS-RAF-MEK-ERK. Despite being a critical target, KRAS has long been considered "undruggable" due to its small size and the lack of accessible binding sites. The SHP2 inhibitor RMC4630, targeting a key coactivator of KRAS signaling, represents a promising therapeutic approach for PDAC.

Methods: In vitro cell proliferation assays were performed using the colorimetric WST-1 method. Protein expression levels were assessed by immunoblotting. In vivo tumor growth studies were conducted in 4-6-week-old female NOD/SCID mice bearing AsPC-1 subcutaneous xenografts.

Results: In vitro assays demonstrated dose-dependent inhibition of proliferation in PDAC-associated cell lines treated with RMC4630 and NPT-GEM. A synergistic effect was observed with the combination therapy. In $KRAS^{G12D}$AsPC-1 cells, NPT-GEM, RMC4630, and their combination inhibited proliferation by 33%, 13%, and 74%, respectively. Similarly, in $KRAS^{G12C}$ MIA PaCa-2 cells, inhibition rates were 46%, 20%, and 78%, while in $KRAS^{wt}$ BxPC-3 cells, inhibition was 63%, 26%, and 73%. PDAC Stromal cell proliferation was inhibited by 48%, 25%, and 60% with NPT-GEM, RMC4630, and their combination, respectively. Immunoblot analysis of KRAS ^{G12D}-mutant cells revealed that RMC4630 treatment decreased phosphorylation of PI3K/AKT and RAS-RAF-MEK-ERK pathway proteins, while increasing apoptosis marker proteins cleaved-PARP-1 and cleaved-caspase-3. In vivo, RMC4630 and NPT-GEM significantly delayed tumor growth, with the combination demonstrating synergistic effects. Tumor weights were reduced from 0.22g in controls to 0.09g with NPT-GEM, 0.077g with RMC4630, and 0.06g with the combination. No significant body weight changes were observed, indicating minimal toxicity.

Conclusion: These findings underscore the therapeutic potential of RMC4630, especially in combination with standard chemotherapy, as a promising approach for managing KRAS-mutant PDAC.

#3054 A potent triterpene combination treatment for eliminating castration resistant prostate cancers.

Kunj Bihari Gupta[1], Kanza Mamouni[1], Georgios Kallifatidis[2], Truett Taylor[1], Bal. L. Lokeshwar[1]

[1]Georgia Cancer Center, Augusta University, Augusta, GA,[2]Biology, Augusta University, Augusta, GA

Patients with advanced prostate cancer (PCa) with castration resistance and neuroendocrine phenotype (CRPC) respond poorly to most existing therapies (median survival, <2y). The studies of antitumor activities of the bioactive pentacyclic triterpenes (PTTs), such as Oleanolic Acid (OA) and Ursolic Acid (UA), have been reported in multiple tumor models. Here, we tested the efficacy of OA and UA, individually and in combination, against PCa cells in both in-vitro and in-vivo systems. We found ~ 50% ($p<0.05$) reduction in tumor growth and terminal tumor weight in the CRPC line 22Rv1 tumors that were implanted subcutaneously in athymic nude mice following oral gavage at 50 mg/kg with OA and UA alone and in 1:1 combination three times a week. There was no detectable systemic toxicity to the host mice. Further, *in-vitro* mechanistic investigation of the effect of OA and UA unveiled that at higher concentrations, a 1:1 combination ratio of OA and UA shows synergistic activity against PCa cells (e.g. 22Rv1, PC3, LNCaP, and C4-2), in contrast, this combination showed insignificant toxicity on a non-tumorigenic human prostate epithelial cell line (NHPrE1). To investigate the molecular mechanism of tumor cell-specific cell death, we explored the effect of these PTTs on ROS production, glucose metabolism, extracellular oxygen consumption rate, and ATP production in both 22Rv1 and PC3 cells. The combination treatment of OA and UA significantly increased the ROS level up to 80% ($p<0.05$) and considerably decreased the mitochondrial Oxygen Consumption Rate (OCR). The levels of intermediates of glycolysis were significantly increased, but tricarboxylic acid (Krebs cycle) metabolites decreased significantly. Immunocytochemical and Immunoblotting analyses of tumor tissues from xenografts showed excessive cytotoxic autophagy as the leading cause of cell death. An increased autophagy-associated membrane-bound LC3B corroborated and higher expression of other markers of autophagy (e.g., Beclin-1, P62/SQSTM1, ATGs, and pULK-1) were seen in cells treated with OA, UA, and their combination. In contrast to increased autophagy markers, markers associated with apoptosis (e.g., cleaved PARP-1, Caspase-3, etc.) were either undetectable or insignificant. Overall, ongoing studies showed that OA and UA in a 1:1 combination induce cancer-cell-specific toxicity mainly via autophagy. This study demonstrated that the triterpenes (OA, UA, and OA+UA) have compelling potential to inhibit CRPC in patients, and because they exhibit little systemic toxicity, yet show significant antitumor activity, they could be used as a neo- or adjuvant therapeutics with current FDA-approved cytotoxic chemotherapy drugs.

#3055 Developing the cyclic peptide AD05 as a novel Hsp90 inhibitor for triple-negative breast cancer therapy.

Ilham Zarguan[1], Sumin Lu[2], vamsi Krishna Kommalapati[2], Alisha Hussain[2], Dhruvi Paladiya[2], Wayne Fang[2], Lamiae Belayachi[1], Abdelaziz Benjouad[1], Abdessamad Debbab[3], Ahmed Chadli[2]

[1]CReSS, Health Sciences Research Center, International Faculty of Medicine, International University of Rabat, Rabat, Morocco,[2]Georgia Cancer Center, Augusta University, Augusta, GA,[3]Institut fur Pharmazeutische Biologie und Biotechnologie, Dusseldorf, Germany

The Heat-shock protein 90 (Hsp90), emerged as an exciting molecular target in the late 90s after being recognized as the driving force behind the Ansamycin antibiotic geldanamycin's antitumoral activity. Since then, extensive research has been conducted to develop Hsp90 inhibitors for clinical applications in cancer therapy. Specifically in triple-negative breast cancer (TNBC), studies have shown that using Hsp90 inhibitors as single agents, or in combination with immunotherapy can offer new leads to better therapeutic efficacy. However, traditional inhibitors targeting the N-terminal domain of Hsp90 often trigger a heat shock response (HSR) or are pan-inhibitors which results in detrimental side effects in clinical trials. Considering this, this study aims to identify and characterize small molecules that can inhibit Hsp90 and degrade its oncogenic client proteins, without inducing the HSR. Using a high-throughput progesterone reconstitution screening assay, a cyclic peptide, AD05, was identified from medicinal plant extracts and their symbiotic microorganisms. This compound is intriguing because it inhibits Hsp90 in a distinct manner from the classical N-terminal Hsp90 inhibitor tested in Phase III clinical trials; 17-N-allylamino-demethoxygeldanamycin (17-AAG) and does not induce the HSR. The binding of AD05 to Hsp90 was further characterized in purified and cell-based systems using IP linked to LC-MS, molecular docking analysis, cellular thermal shift assay (CETSA), and Western blot that showed an efficient degradation of oncogenic client proteins. AD05 exhibited significant cytotoxicity against cancer cells evaluated in MTT assays compared to normal cells, immunohistochemistry (IHC), immunofluorescence, electron microscopy, and Seahorse metabolic analyses. Our findings suggest that bioactive natural products are effective and promising inhibitors against the Hsp90 machine for cancer research and therapy.

#3056 Structural optimization of lapatinib analogs for overcoming CNS metastases in breast cancer.

Mostafa Mohammed Ahmed Aref, Eneye D. Ajayi, Md A. Emran Hossain, Rokaia S. Abdullah, Hamed I. Ali

Pharmaceutical Sciences, Texas A&M University, College Station, TX

Due to its role in aggressive disease progression and poor clinical outcomes, HER-2 kinase was considered a vital target in breast cancer (BC). Central nervous system (CNS) metastases represent a significant challenge in HER2+ BC, as they are often refractory to existing therapies such as lapatinib. Our previous work focused on the structural optimization of lapatinib analogs to enhance potency, selectivity, and pharmacokinetics. The HER2-selective derivatives were synthesized and characterized, with nanomolar IC_{50} values range of [5.4-12 nM], more the reference drug lapatinib (IC_{50}: 95.5 nM). Compound 17d demonstrated outstanding HER2 selectivity and minimal off-target kinase activation. Broad-spectrum activity was observed (GI_{50}: 1.43-2.09 μM), with a strong preference for HER2+ BC cells over HER2– cells, achieving up to 20-fold selectivity in antiproliferative assays. Compound 17d emerges as a promising candidate with the potential to overcome the limitations of current HER2 therapies. Its potent HER2-selective inhibition, robust *in vivo* efficacy, and favorable pharmacokinetic profile highlight its therapeutic potential for HER2+ breast cancer. Our current study mainly focuses on the structural optimization of our lead compound to be more BBB penetrable to overcome the HER-2 CNS Metastatic cancers by applying *in-silico, in-vitro*, and *in-vivo* models.

#3057 Telomerase and G-quadruplex dual-targeting chimera inhibits melanoma via telomere disruption.

Yutong Sui[1], qi xu[1], mingsheng liu[1], jinyu liu[1], geng qin[2]

[1]Jilin University, Changchun City, China,[2]Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun City, China

Interference with telomerase and telomere maintenance have historically been viewed as a highly attractive target for anticancer therapies by restraining proliferative capacity via telomere length. A range of screens have identified many small molecule inhibitors of telomerase, but these inhibitors have all encountered obstacles in the early stages of preclinical studies primarily due to their limited potency and delayed onset of cytotoxic effects. Ligand-induced stabilization of telomeric G-quadruplexes (G4s) formation have been thought to be negative regulators of telomerase activity. However, given that G4-forming sequences are pervasive in human genome, G4 ligands display quite limited intra-G4 selectivity, resulting in a risk of side effects in therapeutic use. Here, we introduce an innovative telomere targeting strategy leveraging telomerase and G4 dual-targeting chimera. We design and synthesize such chimera by connecting telomerase inhibitor BIBR1532 to G4 ligands pyridostatin via a linker. On one hand, pyridostatin can enhance the anticancer potency of BIBR1532 by targeting telomere G4 structures. On the other hand, BIBR1532 can recruit pyridostatin to the telomere region by directly interacting with telomerase, thereby mitigating its off-target effects. As expected, the chimeric compound is capable of telomerase inhibition and suppress melanoma progression in vitro and in patient-derived xenograft (PDX) model with no observable toxicity. Notably, the anti-melanoma effects of the chimeric compound outperform that of BIBR1532 or pyridostatin alone. Our study provides a more effective strategy for telomerase inhibition, and the telomerase and G4 dual-targeting chimera is a promising candidate for anticancer therapy.

#3058 AR-dependent anticancer action of BF175 in hormone sensitive prostate cancer.

Lakshmi Sirisha Devarakonda, Prashanth Parupathi, Ekniel Francois, Bhasker C. Das, Avinash Kumar

Long Island University, Brooklyn, NY

Androgen receptor (AR) is a well established key biological contributor to prostate cancer progression. Despite significant efforts which have resulted in the therapeutic agents that target the AR signaling being the therapy of choice, prostate cancer remains one of the topmost causes of cancer-related deaths among men in the United States. Hence, development of novel targeted therapeutics that augment or supplant AR-based therapy remains a top priority. Sterol regulatory element binding proteins (SREBPs)are a family of transcription factors, consisting of SREBP-1 and SREBP-2, that control lipid synthesis. Available literature provides evidence for the cooperation between SREBPs and AR signaling in promoting prostate cancer progression. We observed that the SREBP expression is higher in LNCaP, C4,and C4-2 AR-positive/androgen-dependent compared to DU145, and PC3 AR-negative/androgen-independent prostate cancer cell lines. Therefore, we hypothesized that pharmacological targeting of SREBPs might be a promising therapeutic approach in hormone sensitive prostate cancer. We developed a novel boron-containing small molecule inhibitor of SREBPs, BF175, whose anticancer activity against prostate cancer has not been elucidated. In our study, we investigated the AR-dependent anticancer activity of BF175 in prostate cancer. We observed inhibition of viability, proliferation, and metastatic potential as well as induction of apoptosis in all prostate cancer cell lines treated with BF175. We also observed reduction in the levels of SREBPs in all prostate cancer cell lines treated with BF175. Notably, the anticancer action of BF175 was dependent on the AR status and BF175 demonstrated significantly more potent anticancer action in AR-positive/androgen-dependent compared to AR-negative/androgen-independent prostate cancer cell lines. Altogether, our data shows that BF175 may be a potential novel therapeutic agent for hormone sensitive prostate cancer.

#3059 Targeting tumor progression: The impact of SMR peptide and miRNA on exosome-inflammasome-EMT axis in breast cancer.

M. Huang[1], E. L. Johnson[1], L. Yang[2], J. Stiles[1], A. Uren[3], F. Yan[1], V. C. Bond[1];

[1]Morehouse School of Medicine, Atlanta, GA, [2]Emory University School of Medicine, Atlanta, GA, [3]Georgetown University, Washington, DC, WA

Background: In breast cancer, the interplay between exosomes, the inflammasome, and epithelial-mesenchymal transition (EMT) significantly influences tumor progression and metastasis. Exosomes carry molecules that affect cellular processes like inflammation and EMT. The inflammasome produces pro-inflammatory cytokines, promoting tumor-associated inflammation and stimulating EMT, which enhances cancer cell invasiveness. Exosomes also deliver miRNAs that modulate gene expression and promote EMT.
Objective: This study investigates the role of the secretion modification region (SMR) peptide in regulating interactions between exosomes, the inflammasome, and EMT in breast cancer, focusing on miRNA influence on gene expression.
Methods: MDA-MB-231 breast cancer cells were treated with tumor exosomes or exSMRwt. Exosomes were isolated and characterized using nanoparticle tracking analysis. Inflammasome activation was assessed by measuring caspase-1 and pro-inflammatory cytokines (IL-1β, IL-18). miRNA expression was evaluated using the nCounter Human miRNA assay.
Results: The SMR peptide suppressed MDA-MB-231 cell activity and inhibited exosome release, crucial for cancer progression. exSMRwt-treated cells showed reduced inflammasome activity and altered miRNA expression, with significant upregulation and downregulation of specific miRNAs. Nine miRNAs were identified as closely related to breast cancer progression, inhibiting cell proliferation, EMT, migration, invasion, and metastasis.
Conclusion: The SMR peptide is a promising therapeutic agent in breast cancer, targeting multiple pathways involved in tumor progression. It inhibits inflammasome activation, reduces pro-inflammatory cytokine production, and modulates exosome-mediated signaling. The peptide also influences miRNA regulation, suppressing inflammation and inhibiting tumor growth and metastasis. This study highlights the therapeutic potential of the SMR peptide and provides direction for further miRNA research in breast cancer.

#3060 The cyclopeptide enniatin a inhibits the chaperone hsp90 and induces mitophagy in breast cancer cells.

Vamsi Krishna Kommalapati[1], Asif Elahi[2], Nada M. Eisa[3], Michael A. Serwetnyk[4], Abdessamad Debbab[5], siva panda[6], Daniela Marasco[7], Brain Blagg[4], Ahmed Chadli[1]

[1]Biochemistry and Molecular Biology, Augusta University, Augusta, GA,[2]University of Alabama at Birmingham, Birmingham, AL,[3]Department of Biochemistry, Mansoura University, Mansoura, Egypt,[4]Department of Chemistry and Biochemistry, The University of Notre Dame, Notre Dame, IN,[5]Institute of Pharmaceutical Biology and Biotechnology, Heinrich-Heine-University Dusseldorf, Dusseldorf, Germany,[6]Chemistry and Biochemistry, Augusta University, Augusta, GA,[7]Department of Pharmacy, University of Naples Federico II, Naples, Italy

Heat shock protein 90 (Hsp90) is a molecular chaperone that plays a key role in protecting the proteome. In cancer cells, this Hsp90 function is hijacked to promote cancer cell proliferation. Except for the Pimitespib (TAS-116), approved for clinical use in Japan in 2022, no Hsp90 inhibitors have yet been approved by the FDA. One of the main side effects of the current Hsp90 inhibitors tested in the clinic is their induction of the heat shock response (HSR). Therefore, identifying new inhibitors that destabilize the oncoproteins clients of Hsp90 without inducing HSR is an attractive strategy to harness the clinical potential. We previously reported that the natural product Enniatin A (EnnA) is a novel Hsp90 inhibitor satisfying this criterion. Here, we report that EnnA potently induces mitophagy in breast cancer cells. The cytoplasmic form of Hsp90 has been shown to be more abundant in the mitochondria of tumor cells compared to mitochondria of normal tissues. We show that EnnA can enter multiple compartments of cancer cells, including the mitochondria, and inhibits Hsp90. Furthermore, we show that EnnA induces mitochondrial dynamics impairment as reflected by loss of mitochondrial membrane potential, reduced oxygen consumption rate, and loss of ATP production. We also show that targeting Hsp90 by EnnA reduced the level of cox4, a component of the electron transport chain, leading to parkin-mediated mitophagy. Our data indicate that EnnA represents a member of a new class of Hsp90 inhibitors that impair mitochondrial function in cancer cells and is a promising compound to treat breast cancer.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Novel Drug Resistance Mechanisms
Poster Session

#3064 The interaction of calcium-sensing receptor with KIF11 enhances cisplatin resistance in lung adenocarcinoma.

Fuhao Wang, Ming Chang, Xiao Zhang, Xiaoyi Li, Zhiying Chen, Wei Zhang, **Jian Zhang**

Southern University of Science and Technology (SUSTech Shenzhen), Shenzhen, China

Lung cancer remains the deadliest form of cancer globally, and non-small cell lung cancer (NSCLC) accounts for 80%-85%. Chemotherapy remains the foundational treatment for NSCLC, with platinum-based regimens serving as the primary first-line therapy for advanced stages of the disease. Cisplatin is a widely used platinum drug with strong broad-spectrum anticancer activity. However, the development of chemotherapy resistance frequently results in treatment failure. Consequently, further research into the mechanisms underlying tumor chemoresistance is crucial for enhancing our understanding of tumor cell biology. This research will aid in identifying new resistance-related genes and molecules, facilitate the development of novel chemotherapeutic agents, and improve treatment regimens. In this study, we first established two cisplatin-resistant lung adenocarcinoma cell lines, A549-DDP and H1299-DDP, by exposing them to escalating concentrations of cisplatin. Through transcriptome analysis and examination of the correlation between gene expression and the prognosis of lung adenocarcinoma patients treated with chemotherapy, we discovered that CaSR was significantly upregulated in cisplatin-resistant LUAD cells. Additionally, high expressions of CaSR were closely associated with poor prognosis in LUAD patients receiving chemotherapy. As a member of the Gprotein-coupled receptor (GPCR) family, the role of CaSR in tumor chemoresistance has been infrequently reported. To further investigate this, we generated LUAD cell lines with stable overexpression of CaSR and confirmed its essential role in cisplatin resistance. Meanwhile, we observed that CaSR knockdown significantly enhanced the sensitivity of cisplatin-resistant LUAD cells. Importantly, a combination of cisplatin with the CaSR inhibitor NPS-2143 significantly inhibited the growth of cisplatin-resistant LUAD cells *in vitro* and *in vivo*. Furthermore, CaSR was found to be involved in the cell cycle of cisplatin-resistant LUAD cells and affected the protein levels of BRCA1 and cyclin B1. Moreover, we have observed an interaction between CaSR and KIF11. In LUADcells, KIF11 was identified as a crucial hub within the CaSR signaling pathway, regulating the expression of cyclin B1 and BRCA1 and promoting cisplatin resistance. Our study highlighted the therapeutic potential of targeting CaSR to mitigate the chemotherapy resistance of LUAD. Furthermore, it explores a novel mechanism underlying chemoresistance in LUAD and provides essential experimental evidence, presenting the case as a promising therapeutic target for overcoming cisplatin resistance in clinical settings. This work was supported by the NSFC Project (82173336, 81972766,81972420), The Science and Technology Project of Shenzhen(JCYJ20210324104214040), the Medical Research Innovation Project (G030410001).

#3065 Tribbles 2 drives lineage maintenance and survival in neuroendocrine prostate cancer by regulating N-Myc stability.

Jitender Monga[1], Rohith Guddeti[1], Craig Rogers[1], Shirish Gadgeel[2], Dhananjay Chitale[3], Jagadananda Ghosh[1]

[1]Urology, Henry Ford Health, Detroit, MI,[2]Hematology & Oncology, Henry Ford Health, Detroit, MI,[3]Pathology, Henry Ford Health, Detroit, MI

Neuroendocrine prostate cancer (NEPC) is a highly aggressive and treatment-resistant subtype of prostate cancer, characterized by an increased propensity for visceral metastasis. As prostate cancer develops resistance to second-generation androgen receptor inhibitors, such as enzalutamide, many patients exhibit tumors with highly proliferative, metastatic cells that adopt neuroendocrine features. This form of treatment-emergent NEPC (t-NEPC) occurs in over 20% of cases, contrasting with the much rarer de novo NEPC, where neuroendocrine (NE) cells (~1%) within the prostate transform into malignant entities. The clinical management of NEPC is challenging due to a limited understanding of molecular targets that can selectively combat these rapidly growing and invasive cells. One intriguing target is the pseudokinase Tribbles 2 (TRIB2), which has been found to be overexpressed in both t-NEPC and de novo NEPC. Elevated TRIB2 expression is implicated in promoting neuroendocrine differentiation in prostate adenocarcinoma cells, facilitating their escape from androgen-dependence and contributing to resistance against AR inhibitors. Functionally, TRIB2 enhances tumor cell survival and proliferation by fostering the adoption of neuroendocrine traits, thereby accelerating disease progression. Inhibition of TRIB2, however, reverses neuroendocrine traits and restores sensitivity to anti-androgen therapies, indicating that TRIB2 plays a critical role in therapeutic resistance by driving cellular trans-differentiation. Despite this, the precise mechanisms by which TRIB2 contributes to the onset and maintenance of neuroendocrine features in prostate cancer remain unclear. Our studies using well-characterized NEPC tumors, and established NEPC cell lines have consistently shown that TRIB2 is closely associated with neuroendocrine phenotypes both *in vitro* and *in vivo*. Notably, TRIB2 appears to regulate these features by promoting the upregulation and stabilization of N-Myc, a key oncogene in neuroendocrine differentiation, through inhibition of its ubiquitination mediated proteasomal degradation. CRISPR-Cas9 mediated modulation of TRIB2 expression levels in NEPC cells and tumors results in corresponding changes in their neuroendocrine characteristics, reinforcing the notion that TRIB2 is a critical driver of NE trans-differentiation. These findings highlight TRIB2 as a novel regulator in the progression and maintenance of NEPC, with its effects likely mediated through downstream modulation of N-Myc activity.

#3066 Fumarylacetoacetate hydrolase (FAH) stimulates resistance to CDK4/6 inhibitors in hormone receptor-positive breast cancer via a novel non-canonical function.

Jenny Hogstrom[1], Boryana Petrova[2], Hanna Doh[1], Camila Perea[1], Su Min Hong[1], Nina Kozlova[1], Kayla Cruz[1], Conor L. Evans[3], Jonah Lee[1], John Clohessy[1], Gerburg Wulf[1], Laura Collins[1], Naama Kanarek[2], Taru Muranen[1]

[1]Beth Israel Deaconess Medical Center, Boston, MA,[2]Boston Children's Hospital, Boston, MA,[3]Massachusetts General Hospital, Boston, MA

Breast cancer is the most prevalent cancer among women worldwide with hormone receptor-positive (HR+) cancer constituting 70% of all breast cancers. HR+ breast cancer patients are usually treated with targeted therapies, which include hormone therapy, as well as CDK4/6- and PI3K-inhibitors. Unfortunately, the tumors usually become resistant over time. In addition to genetic drivers of resistance, metabolic perturbations and enhanced activity of metabolic enzymes can also contribute to tumor growth, resistance and poor survival. We have discovered that Fumarylacetoacetate hydrolase (FAH), the last enzyme in tyrosine catabolism pathway, is enriched in HR+ breast tumors and correlates with poor survival. By using HR+ patient-derived organoid cultures (PDOs), we show that FAH stimulates resistance to multiple standard-of-care therapies. Despite its canonical function in tyrosine catabolism, FAH overexpressing cells do not preferentially utilize the tyrosine catabolism pathway. Instead, we surprisingly observe increased nuclear localization of FAH in patients treated with endocrine therapy and/or CDK4/6-inhibitors. Similarly, ER degraders and CDK4/6-inhibitors increase the ratio of nuclear FAH in patient-derived organoids, suggesting that the nuclear localization could drive resistance. To investigate this, we cloned a nuclear localization signal (NLS) to FAH and observed that FAH-NLS expressing PDOs were more resistant to CDK4/6-inhibitors. In contrast, blocking the nuclear translocation reversed the FAH-mediated resistance, showing that the nuclear localization is essential for the resistance phenotype. Collectively this suggests a yet uncharacterized moonlighting function of FAH in the nucleus and links this metabolic enzyme to treatment resistance in HR+ breast cancer.

#3067 The long non-coding RNA CDH3-AS1 facilitates gemcitabine resistance and lung metastasis via TGFβ activation in pancreatic ductal adenocarcinoma.

M. Yang[1], R. Wang[1], H. Sun[1], M. Dong[2], **Q. Chen**[1];

[1]University of Macau, Taipa, Macao, [2]Guangzhou Laboratory, Guangzhou, China

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies, with chemotherapy serving as the primary treatment for PDAC when surgery is not feasible. Gemcitabine is commonly used as the first-line chemotherapeutic agent in PDAC treatment, yet resistance to gemcitabine frequently emerges, necessitating the development of novel strategies to improve its efficacy. In our study, we established a gemcitabine resistant PDAC cell model through an evolutionary process involving gradually increasing gemcitabine concentrations. RNA-Seq analysis revealed significant upregulation of extracellular matrix (ECM) signaling in resistant cells. ECM dysregulation impeded gemcitabine uptake, leading to resistance. In addition, resistant cells showed reduced proliferation compared to parental cells, further exacerbating resistance. Mechanically, we found enhanced activation of the transforming growth factor β (TGFβ) pathway in resistant cells, resulting in increased expression of ECM-related genes and cyclin kinase Inhibitor p57. Moreover, we found that the long non-coding RNA (LncRNA) CDH3-AS1, the antisense of CDH3, was significantly upregulated in resistant cells and exhibited a positive correlation with ECM signaling in SMAD4 mutant PDAC, suggesting its role as a regulator of TGFβ signaling. Disrupting CDH3-AS1 impaired TGFβ activation in resistant cells by inhibiting TGFBR1 and SMAD4, thereby reversing gemcitabine resistance. Interestingly, CDH3-AS1 also regulated the expression of CDH3 in resistant cells, promoting lung metastasis through mediating epithelial-to-mesenchymal transition (EMT). The study reveals CDH3-AS1 as a novel regulator of TGFβ activation that contributes to PDAC progression, providing a new potential therapeutic target for the treatment of this aggressive cancer.

#3068 The clonal nature of FGFR3-TACC3 in glioma: Mechanism discovery and preclinical study.

Y. Li[1], T. Li[1], X. Ren[1], J. Liang[2], J. Fan[1], X. Wang[2], S. Yu[1], W. Zhang[3], X. Yang[2];

[1]Tianjin Medical University General Hospital, Tianjin, China, [2]Beijing Tsinghua Changgung Hospital, Beijing, China, [3]Wake Forest School of Medicine, Winston-Salem, NC

During the course of treatment, tumor cells can undergo a Darwinian process of clonal evolution, where resistant cell clones emerge, proliferate and replace more vulnerable ones, ultimately leading to increased treatment resistance. Numerous oncogenic fusion genes have recently been identified in gliomas, promoting malignant transformation and serving as potential therapeutic targets. Among these, the FGFR3-TACC3 (F3-T3) fusion gene was initially detected in glioblastoma multiforme (GBM), with its fusion protein comprising primarily FGFR3 structural domains and a smaller portion of TACC3. F3-T3 fusions are consistently expressed in both untreated and recurrent tumors, confirming the clonal persistence of these fusion events. Our previous study implicated F3-T3 in chemotherapy resistance in GBM, though the underlying mechanism remained unclear. In this study, we further investigate the clonal nature and functional role of F3-T3 in GBM cells. Through bioinformatics data mining, we discovered that F3-T3 functionally enriches DNA repair pathways, as shown by Gene Set Enrichment Analysis (GSEA). Experimental results demonstrated that GBM cells harboring F3-T3 maintain their proliferation, invasion and migration capabilities under temozolomide (TMZ) treatment. Subsequent RNA sequencing revealed activation of the PI3K-AKT signaling pathway in F3-T3 GBM cells under TMZ conditions. Further analysis identified PKM2 as a downstream substrate of the F3-T3 protein, linking it to the DNA damage response. Experimental evidence highlights the critical role of the F3-T3/PI3K-AKT/PKM2 axis in mediating chemoresistance in F3-T3 GBM. Importantly, our preclinical models demonstrated synergistic efficacy when combining TMZ with either FGFR or PI3K inhibitors in F3-T3 GBM. In summary, our findings underscore the significance of the F3-T3/PI3K-AKT/PKM2 axis in driving chemotherapy resistance in F3-T3 GBM and present a potential therapeutic strategy for gliomas with F3-T3 fusions, providing empirical support for future fusion gene-targeted investigations.

#3069 Inhibition of PGE2 signaling by an EP2/4 dual antagonist OCT-598 prevents acquisition of therapy-resistance and tumor relapse.

Y. Lee[1], H. Choi[1], J.-W. Lim[1], S. Lee[2], Y. Kim[2], C. Choi[2], T. Yoon[1];

[1]OSCOTEC Inc., Seongnam, Korea, Republic of, [2]Samsung Medical Center, Seoul, Korea, Republic of

Conventional antitumor drug discovery focuses primarily on selective killing of cancer cells. However, more often than not, cancer cells become resistant to the therapy and tumors eventually relapse in a more malignant form, potentially even accelerating disease progression afterwards. Therefore, it is of paramount importance to curtail the process by which cancer cells adapt to the therapeutic pressure to re-invent themselves and regenerate. Prostaglandin E2 (PGE2) is widely recognized as an important mediator of wound healing and tissue regeneration after injury by orchestrating reparative immune responses and mobilizing tissue stem cells. Likewise, in cancer, the PGE2 pathway has been shown to promote tumor growth, immune evasion, angiogenesis, and cancer stem cell (CSC) survival. Thus, upon cytotoxic insults, the apoptotic signal in dying cells induces PGE2 production, which enables, in a paracrine manner, the surviving cells to persist and eventually repopulate. Therefore, blockade of the PGE2 signals concurrently with cell-killing is anticipated to prevent tumors from acquiring therapy resistance. Of the known PGE2 receptors EP1-4, EP2 and EP4 are best known to convey its pro-tumor and immune-evasive signals. OCT-598 is a potent and selective dual antagonist of EP2 and EP4 with Ki values of 23 nM and 0.2 nM vs EP2 and EP4, respectively. OCT-598 has been shown previously to elicit complete regression in a mouse syngeneic TC-1 tumor model when used in conjunction with the standard of care chemo-immunotherapy regimen (Abstract 3234, AACR 2023). In this study, we present “anti-resistance” activities of OCT-598. In vitro, chemotherapy-induced cell deaths are followed by a dormant period, where the ‘un-dead’ cells show enlarged morphology and hyperploidy, and subsequently by repopulation of smaller, proliferative para-diploid cells. Addition of OCT-598 along with the chemotherapeutic agent decreased the number of the transient, enlarged cells and inhibited eventual repopulation in a dose-dependent manner. In vivo, OCT-598 abrogated tumor relapse after irradiation in CT26 mouse syngeneic tumor model, leading to complete remissions. Based on these observations, a clinical trial is set to be initiated to test the “anti-resistance” activity of OCT-598 as an adjuvant to the standard-of-care treatment in patients with advanced solid tumors.

#3070 Cytomegalovirus infection enhances spheroid formation and drives aggressive phenotype in glioblastoma via upregulation of SOX2 pRB pAKT and STAT3/IL-6 signaling.

Vida Tajiknia[1], Noe Mercado[1], Jacquelin Real[1], Eain Murphy[2], Sean Lawler[1]

[1]Legorreta Cancer Center at Brown University, Providence, RI,[2]Upstate Medical University, Syracuse, NY

Background: Cytomegalovirus (CMV) infection has been implicated in glioblastoma (GBM) progression, with effects on stemness, immune evasion, and angiogenesis. Our work investigates the impact of CMV infection on spheroid formation, oncogenic pathways including SOX2, retinoblastoma protein (pRB), phospho-AKT (pAKT), and STAT3/IL-6 signaling, as well as cytokine modulation. Additionally, we evaluate the therapeutic potential of ganciclovir in reversing CMV-induced effects.
Methods: GBM cell lines LN229, U251, U87, U138, and T98G were infected with the GFP-expressing CMV TB40 strain at a multiplicity of infection (MOI) of 0.5. Infection was confirmed via fluorescence microscopy and Western blotting for CMV pp65 protein. The stable cell line with infection was confirmed after multiple passage and presence of CMV protein. Spheroid formation assays assessed morphological changes and aggressiveness in vitro. Western blotting analyzed the expression of SOX2, pRB, pAKT, and phospho-STAT3 (pSTAT3), while cytokine profiles, including PD-L1, VEGF, angiopoietin-2, and IL-6, were measured in non-infected, infected, and ganciclovir-treated cells using Luminex panel.
Results: CMV infection was confirmed in all GBM cell lines, with infection rates ranging from 60% to 90%. CMV-infected cells exhibited accelerated and more aggressive spheroid formation compared to non-infected cells. Infection led to upregulation of SOX2, pRB, pAKT, and pSTAT3, accompanied by elevated IL-6, indicating enhanced stemness, proliferation, and pro-inflammatory signaling. Cytokine profiling revealed significant increases in PD-L1and angiopoietin-2 with decrease in soluble VEGF levels following infection and treatment with Gancylovir, reflecting immune suppression and angiogenesis. Ganciclovir treatment modulate pro-tumorigenic cytokines, creating a more favorable tumor microenvironment.
Conclusions: CMV infection accelerates spheroid formation and enhances the aggressive phenotype in GBM via upregulation of SOX2, pRB, pAKT, and STAT3/IL-6 signaling, alongside immune evasion and angiogenic cytokine shifts. Our data suggest the therapeutic potential of targeting CMV and its downstream pathways in GBM. These findings highlight the critical role of CMV in GBM pathogenesis and its promise as a therapeutic target.

#3071 Improving the docetaxel-based chemotherapy in therapy resistance prostate cancer.

Md Rakibul Alam, Amos Olalekan Akinyemi, Jianlin Wang, Mithu Howlader, Lixiang Gu, Zhiguo Li

Toxicology and Cancer Biology, University of Kentucky, Lexington, KY

Prostate cancer is known to have a relatively good prognosis, but long-term hormone therapy can lead to castration-resistant prostate cancer (CRPC). Docetaxel, a third-generation chemotherapy drug based on inhibiting depolymerization of microtubules, provide excellent initial response in patients for the CRPC treatment. Despite the improved survival duration and reduction of tumor size observed in some patients, many have no response to the drugs or develop resistance over time. In this study, to elucidate the molecular basis of docetaxel resistance, we established docetaxel resistance prostate cancer PC3-TR, C4-2-TR and DU-145-TR cell lines, which exhibited higher LD_{50} and lower drug uptake against docetaxel than parental PC3, C4-2 and DU-145 cell lines. We employed quantitative high throughput screen with a collection of 2500 FDA approved drugs to identify drugs that can enhance the docetaxel sensitivity of docetaxel resistance cell lines. ML385 has been identified as a potent NF-E2 related factor-2 (Nrf2) synthetic inhibitor, and its treatment of docetaxel resistance cells significantly suppressed the Nrf2 signaling by decreasing the cytosolic and nuclear Nrf2 levels. In addition, docetaxel resistance cell lines showed marked increases in nuclear accumulation of Nrf2 compared to parental cell lines, suggesting that Nrf2 signaling is homeostatically activated in docetaxel resistance cell lines. Here we identified that silencing of Nrf2, docetaxel sensitivity of resistance cell lines was increased, and that of parental cell lines were reduced by activation of Nrf2. Combination treatment of ML385 with docetaxel induced cell death in docetaxel resistance cell lines. Together, these results provide new insight in the understanding of docetaxel resistance and direct a therapeutic approach to overcome docetaxel resistance in CRPC treatment.

#3072 Novel approach to target trastuzumab resistant, HER2+ breast cancer: Activation of PKCdelta.

Maria Mejia[1], Debra Wyatt[2], Mitchell F. Denning[2], Kelvin Billingsley[3], **Clodia Osipo**[2]

[1]Biochemistry and Molecular Program, Loyola University Chicago, Maywood, IL,[2]Cancer Biology, Cardinal Bernardin Cancer Center, Loyola University Chicago, Maywood, IL,[3]Chemistry and Biochemistry, Loyola University Chicago, Maywood, IL

The ERBB2/HER2 oncogene is amplified in 15% of breast cancers leading to overexpression of the HER2 protein. Trastuzumab based therapy has improved survival outcomes for patients with localized and regional HER2+ tumors. However, the prognosis for patients with HER2+ metastatic breast cancer remains poor, with survival rates as low as 30%. Resistance to anti-HER2 targeted therapy is a key factor contributing to disease progression. Previously, we showed that Notch1 is a driver of trastuzumab resistance and PKCα attenuates Notch signaling to prevent resistance. There are many isoforms of PKC in breast cancer cells. This study investigated the contributions of at least 6 PKC isoforms in the context of trastuzumab sensitive and resistant breast cancer cell lines (isogenic BT474 sensitive and resistant and de novo resistant HCC1954). PKCδ mRNA and protein were expressed higher than any other PKC isoform and further increased in resistant compared to sensitive cells. The activity of PKCs overall was less in resistant compared to sensitive cells. Exogeneous addition of TPA activated PKC and potently inhibited proliferation, cell cycle progression, and mammosphere formation only in resistant cells and not in isogenic sensitive cells. Knockdown of PKCδ rescued these growth phenotypes, suggesting that TPA-mediated activation of PKCδ was sufficient to inhibit growth of trastuzumab resistant cells lines. Based on these encouraging findings, we tested other PKC modulators to identify potentially safer alternatives to TPA. Among the seven compounds (TPPB, Prostratin, iPr-ILV, Ac-ILV, 1,2-dioctanoyl-sn-glycerol, 1-oleoyl-2-acetyl-sn-glycerol and Bryostatin) screened for growth phenotypes of HCC1954 and BT474 resistant cell lines, TPPB was as potent as TPA (IC50=14nM TPPB vs. 0.45nM TPA). TPPB-mediated growth inhibition was dependent on PKCδ as knocked down of PKCδ using an RNAi approach almost completely rescued the inhibition of growth. An RNA sequencing approach was used to determine the mechanism by which TPA-mediated activation of PKCδ inhibits proliferation and cancer stem cell survival of trastuzumab resistant breast cancer cell lines. The most significant pathway activated by TPA was the TNFα- NFKβ pathway, suggesting that the mechanism by which activated PKCδ inhibits growth of trastuzumab resistant breast cancer could be through activation of a pro-inflammatory signaling cascade. In particular, the S100 family of cytokines were significantly upregulated by TPA, and their expression was reversed by PKCδ knockdown. These results suggest that even though TPA has been shown to be an effective agent to activate PKCδ, TPPB could possibly be a safer alternative. Future studies will determine mechanisms by which activating PKCδ selectively inhibits growth of trastuzumab resistant breast cancer *in vivo* and if PKCδ is a novel vulnerability for trastuzumab resistant disease.

#3073 Metabolic dependency on de novo pyrimidine synthesis in platinum resistant ovarian cancer.

H. Cardenas, G. Zhao, A. M. Isac, **D. E. Matei**;
Northwestern University - Chicago, Chicago, IL

Background: High grade serous ovarian cancer (OC) is lethal due to near universal development of resistance to platinum-based chemotherapy.
Materials and Methods: We used cisplatin resistant (Cis-R) cells generated by repeated cisplatin treatment of sensitive (Cis-S) OC cell lines, transcriptomic and metabolomic analyses to identify key metabolic pathways associated with platinum resistance. In vivo experiments used intraperitoneal (ip) OC xenografts and patient derived xenografts (PDX).
Results: Pathway analysis of differentially abundant metabolites in Cis-R vs. Cis-S OVCAR5 and COV362 cells identified enrichment of pyrimidine metabolism (FDR<0.05) related to increased de novo pyrimidine synthesis. 15N-glutamine flux analysis confirmed increased de novo pyrimidine synthesis in Cis-R cells. Inhibition of this pathway using the DHODH inhibitor brequinar (BRQ) decreased cell viability and induced expression changes in genes of the mitochondrial electron transport in Cis-R compared with Cis-S cells. Oxygen consumption rate (OCR) measured with a Seahorse assay indicated lower (P<0.05) OCR (baseline, ATP-related, and maximal) and spare respiratory capacity (SRC) in Cis-R vs. Cis-S OVCAR5 cells. BRQ induced dose-related inhibition of OCR in both Cis-R and Cis-S cells. SRC was inhibited by BRQ only in Cis-R cells. In vivo, BRQ attenuated growth of ip Cis-R OC xenografts but did not alter Cis-S tumors. Tumor growth was also inhibited (p<0.01) by BRQ in a Cis-R PDX model. BRQ plus carboplatin enhanced (p<0.05) the inhibitory effects of carboplatin alone in ip Cis-R OC xenografts.
Conclusions: Our results support that Cis-R cells acquire dependency on the de novo pyrimidine synthesis and maintain relatively low levels of mitochondrial oxidation, rendering Cis-R cells and tumors vulnerable to inhibitors of this pathway.

#3074 Sorafenib resistance and cholesterol metabolism in hepatocellular carcinoma.

Veerababu Nagati, Dennis Kwabiah, Yamile Abuchard Anaya, Manish K. Tripathi

Medicine and Oncology ISU, University of Texas Rio Grande Valley, McAllen, TX

Hepatocellular carcinoma (HCC) is among the most prevalent cancers and a leading cause of cancer-related deaths worldwide. Sorafenib, a multikinase inhibitor, serves as a key first-line treatment for HCC when surgical intervention is not an option. However, primary and acquired resistance to sorafenib has significantly limited its effectiveness, reducing the disease control rate. Dysregulation of numerous genes that influence cancer cell proliferation, survival, and drug resistance contributes to this challenge. Identifying the molecular drivers of sorafenib resistance remains critical to improving treatment outcomes. Several factors, including activation of oncogenic pathways (e.g., Akt/mTOR), hypoxia, cholesterol metabolism, multidrug resistance, and autophagy, play pivotal roles in driving resistance. RNA/DNA binding proteins, such as Y-box binding protein 1 (YBX1), are central to cancer progression, metastasis, and drug resistance. Elevated YBX1 expression correlates with poor prognosis in HCC. Our preliminary data indicate that YBX1 overexpression enhances oncogenic phenotypes such as proliferation, migration, and colony formation. Additionally, it upregulates oncogenic markers like CD44, PDL1, SNAIL, and TWIST, further promoting HCC progression. Conversely, YBX1 inhibition increases sensitivity to sorafenib, as evidenced by the downregulation of drug resistance markers in HCC cell lines. Kinases also play a critical role in drug resistance, and activation of Akt/mTOR-associated genes was observed in YBX1 overexpression models. Moreover, cholesterol metabolism pathways appear to be significantly impacted by YBX1. Specifically, sterol regulatory element-binding transcription factors (SREBF1 and SREBF2) and downstream lipid metabolism genes, such as COL1A1, are positively correlated with YBX1 expression and are activated in HepG2 and Huh7 sorafenib-resistant cells. Notably, YBX1 knockdown reversed the effects of SREBF2-associated lipid metabolism genes, reducing their role in drug resistance. Collectively, these findings suggest that YBX1 contributes to sorafenib resistance in HCC through cholesterol metabolism and oncogenic signaling. This research highlights YBX1 as a potential therapeutic target, with the promise of sensitizing sorafenib-resistant HCC cells and improving treatment outcomes.

#3075 Targeting endoplasmic reticulum stress pathways to combat enzalutamide resistance in prostate cancer.

A. O. Akinyemi, M. Alam, M. Howlader, J. Wang, L. Gu, X. Liu, Z. Li;
University of Kentucky, Lexington, KY

Prostate cancer (PCa) is a significant public health issue, ranking as the second leading cause of cancer-related death in men in the United States, with projections for 2024 estimating 299,101 new cases and 35,250 deaths. A critical driver of PCa progression, the androgen receptor (AR) signaling pathway remains a major therapeutic target, particularly in castration-resistant prostate cancer (CRPC). Although AR-targeted therapies such as abiraterone and enzalutamide were developed to delay the need for chemotherapy, they provide only modest survival benefits of approximately five and two months, respectively, emphasizing the need for more effective strategies. This research investigates a resistance mechanism in CRPC mediated by a kinase-driven phosphorylation of GRP78, a key chaperone protein that aids in cellular survival independent of conventional AR signaling pathways. In this study, we examine how this phosphorylation of GRP78 triggers post-translational modifications that support CRPC cell survival under AR-targeted therapy. Using both in vitro and in vivo approaches, we characterize this adaptive resistance pathway. In vitro assays using CRPC cell lines include RNA sequencing (RNA-Seq) for transcriptomic profiling, cell viability assays, proteasome activity measurements, and Western blot analysis to evaluate protein expression changes. Confocal microscopy and flow cytometry are employed to assess cellular responses, while kinase assays and mass spectrometry elucidate specific GRP78 modifications. Chromatin immunoprecipitation sequencing (ChIP-Seq) further investigates the impact of GRP78 modification on AR's genomic binding. In vivo, CRPC xenograft models are treated with enzalutamide, allowing us to assess tumor progression and validate cellular findings via immunohistochemistry (IHC) and RNA-Seq analysis. Preliminary data indicate that phosphorylation-induced modifications of GRP78 enhance CRPC cell resilience under AR inhibition, supporting a proteostasis mechanism that counters therapeutic pressure. Targeting this adaptive response pathway may offer a novel strategy to disrupt resistance and improve the effectiveness of AR-directed therapies in advanced CRPC. By advancing insights into the cellular reprogramming associated with AR resistance, this work contributes to identifying new therapeutic targets for enhanced PCa management.

#3076 A niche driven mechanism determines response to ATRA and a mutation-independent therapeutic approach forMDS and AML.

Ioanna Mosialou[1], Abdullah M. Ali[1], Alvaro Cuesta-Dominguez[1], Brian Chernak[1], Marta Galan-Diez[1], Mael Heiblig[2], Ellin Berman[3], Joseph G. Jurcic[1], Steven Kornblau[4], Guiliermo-Garcia Manero[4], Azra Raza[1], Stavroula Kousteni[1]

[1]Columbia University Irving Medical Center, New York, NY,[2]Centre Hospitalier Lyon Sud, Lyon, France,[3]Memorial Sloan-Kettering Cancer Center, New York, NY,[4]MD Anderson Cancer Center, Houston, TX

Myeloid cancers remain resistant to standard of care (SOC) and targeted therapies. An oncogenic signal from the niche, constitutive activation of β-catenin/JAG1 signaling in osteoblastic cells (osb-β-catenin), leads to myelodysplasia (MDS) and acute myeloid leukemia (AML) and occurs in ~40% of MDS and AML patients and patients with del(5q)-associated myeloid malignancies. We show that its levels increase with disease severity and correlate with MDS to AML transformation and a worse overall survival. Searching for regimens that block osb-β-catenin activity we identified all-*trans*-retinoic acid (ATRA), a regimen with disparate results in MDS and AML. By analyzing publicly available RNA-seq data and bone marrow biopsies collected from 14 ATRA-treated patients from four different study sites we found a strong association between inhibition of osb-β-catenin activity and/or Notch signaling in AML cells with ATRA, leading to AML improvement. An overall response rate of 100% was reached in patients with active baseline osb-β-catenin, as compared to 22% among patients without this pathway active. Responsiveness was observed across patients with diverse cytogenetic and mutational profiles belonging even to adverse risk groups. Improvement occurred despite a diverse ATRA dosing schedule in combination with other therapeutic agents (AZA, VEN, VPA). Indeed, whereas there was no association between response and the degree of global DNA hypomethylation or histone acetylation among patients treated in combination with AZA/VPA, we observed high association of treatment response with baseline osb-β-catenin activity (p=0.021). Moreover, first-line AZA/VEN treatment of an AML patient resulted in relapse and CR was reached only upon ATRA addition to AZA/VEN backbone. Supporting this conclusion, treatment with ATRA monotherapy of an MDS patient with high levels of active osb-β-catenin improved disease status and was associated with complete inhibition of osb-β-catenin activity and a parallel decrease in Notch signaling in the patient's MDS cells. The patient remained transfusion independent for at least 9 years. Mechanistically, ATRA-mediated inhibition of osb-β-catenin activity suppressed cell growth and survival and promoted differentiation of MDS/AML cells solely from patients with active osb-β-catenin without toxicity in healthy cells. Confirming findings in patient samples, ATRA administration in leukemic mice suppressed osb-β-catenin signaling thus improving disease outcome and prolonging survival by at least six-fold with no evidence of relapse and a superior safety profile to SOC. These results suggest that responsiveness to ATRA may be predicted by measuring activation of β-catenin in osteoblasts. Thus, repurposing ATRA use for the treatment of this specific group of patients may significantly improve disease outcome.

#3077 Pharmacological inhibition of NUAK1 and NUAK2 synergizes with chemotherapy in pancreatic cancer models by abrogating drug resistance and suppressing fibrosis.

Minji Seo, Jihye Yoon, Song-Eun Park, Jong-Won Lim, Sungho Park, Yuntae Kim, **Taeyoung Yoon**

OSCOTEC Inc., Seongnam-si, Korea, Republic of

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive and treatment-resistance cancers with a 5-year survival rates of around 10%. Since most PDAC cases are diagnosed at an advanced stage, current treatment options are essentially limited to chemotherapy, which is unfortunately barely effective. Therefore, the development of new, innovative therapies is desperately needed. PDAC is characterized by its desmoplastic nature and exceptionally high recurrence rates, both of which are also known to be furthered by chemotherapy. It is hypothesized that therapy-induced cell death exacerbates fibrosis and paradoxically facilitates resistance development. Therefore, concurrent inhibition of these pathways alongside cytotoxic therapy may enhance treatment outcomes in PDAC patients. NUAK family kinase1 and 2 are members of AMP-related kinase family, known to be implicated in cancer progression, cell adhesion, cell proliferation and metastasis. Elevated NUAK1/2 expression correlates with poor prognosis and patient survival rate in many cancer types including pancreatic cancer. Recently, it was also shown that NUAK1 inhibition by genetic knockdown or a chemical inhibitor reduced organ fibrosis via Hippo pathway modulation in mouse models. In this study, we demonstrate anti-chemoresistance and anti-fibrotic activities of P4899, a potent and selective NUAK1/2 dual inhibitor, in pancreatic cancer. Treatment of pancreatic cell lines with chemotherapeutic agents such as gemcitabine and cisplatin initially causes massive cell death, but the surviving cells undergo hyperploidy that eventually leads to repopulation of para-diploid cells, mimicking chemoresistance and tumor recurrence. In the presence of P4899, however, the number of the transient hyperploid cells as well as ensuing repopulation was dramatically reduced. Furthermore, administration of the orally bioavailable NUAK1/2 inhibitor in combination with gemcitabine in a mouse orthotopic PDAC model resulted in significant inhibition of tumor growth as well as reduction of collagen deposition and expression levels of fibrotic genes in tumor tissue. These data suggest that NUAK1/2 inhibition can effectively ameliorate desmoplasia and impede the development of chemo-resistance in pancreatic tumors, which warrants further investigation in the clinical setting.

#3078 Targeting a ROS-induced oxidation and O-GlcNAcylation cascade to overcome drug resistance across multiple cancer types.

Juanjuan Li[1], Huadong Pei[2], Feng Yao[1], Hemeng Zhang[3], Junfeng Ma[2]

[1]Renmin Hospital of Wuhan University, Wuhan, China,[2]Georgetown University Medical Center, Washington, DC,[3]The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China

Reactive oxygen species (ROS) are critical in lipid peroxidation and the initiation of ferroptosis, which significantly impacts chemotherapeutic drug resistance. However, the mechanisms by which ROS function and are sensed remain poorly understood. In this study, we identified that *O*-GlcNAc transferase (OGT), a key enzyme for protein *O*-GlcNAcylation, acts as a sensor for ROS induced by ferroptosis. The ROS-induced oxidation of OGT at C845 in its catalytic domain activates OGT. Once activated, OGT *O*-GlcNAcylates FOXK2, which enhances its interaction with importin α, promoting its nuclear translocation and subsequent transcription of SLC7A11, thereby inhibiting ferroptosis. Moreover, ferroptosis-induced activation of the ATM-CHK2 pathway and subsequent phosphorylation of FOXK2 further boost FOXK2 *O*-GlcNAcylation and SLC7A11 transcription. Clinically, the elevated OGT-FOXK2-SLC7A11 axis drives tumorigenesis and promotes resistance to chemoradiotherapy across various cancer types, including hepatocellular carcinoma (HCC). Our study uncovers a ROS-induced oxidation-O-GlcNAcylation cascade specific to ferroptosis, which integrates ROS signaling, O-GlcNAcylation, FOXK2-mediated SLC7A11 transcription, and chemoradiotherapy resistance. Targeting this cascade represents a promising approach to overcoming drug resistance in multiple cancer types.

#3079 Uncovering novel molecular mechanisms of PI resistance in multiple myeloma through multi-omics analysis.

Seungbin Han[1], Chien-Yun Lee[2], Lenka Besse[3], Umair Munawar[1], Shilpa Kurian[1], Andrej Besse[3], Ann-Sohpie Hainold[1], Silvia Nerreter[1], Cornelia Vogt[1], Johanna Thurner[2], Nina Rein[1], Max Koppel[1], Xiang Zhou[1], Larissa Haertle[1], Christoph Driessen[4], Hermann Einsele[1], Leo Rasche[1], Ondrej Slaby[3], Johannes Waldschmidt[1], Bernhard Kuster[2], Klaus Martin Kortum[1]

[1]Internal Medicine II, University Hospital Wurzburg, Wurzburg, Germany,[2]Technical University of Munich, Munich, Germany,[3]Masaryk University, Brno, Czech Republic,[4]Cantonal Hospital St. Gallen, St. Gallen, Switzerland

Background: Even with considerable advancements in multiple myeloma (MM) therapy, proteasome inhibitors (PIs) continue to be an indispensable treatment pillar in MM. However, the development of PI resistance remains a significant clinical challenge. Previous studies proposed different causes contributing to PI-resistance, though, these mechanisms explain only a subset of PI resistance cases. Hence, we employed a multi-omics approach to identify further molecular pathways underlying PI resistance in MM.
Methods: We applied proteomic analysis on PI-naïve parental cell line models and their Bortezomib (BTZ) and Carfilzomib (CFZ) resistant derivatives. RNA-sequencing data and the clinical outcome of MM patients were obtained from CoMMpass to derive the impact of differentially expressed proteins. Identified candidate protein was then overexpressed in MM cell line models and the drug sensitivity changes was measured through Alamarblue.
Results: Proteomic screening identified 14 significantly upregulated proteins in CFZ-resistant cells and 9 overexpressed proteins in BTZ-resistant cells. NQO1 was notably overexpressed in both CFZres and BTZres models, confirmed by Western blot and qPCR. To investigate the functional impact of NQO1 on PI sensitivity, we generated NQO1-overexpressing cell lines and assessed their response to BTZ and CFZ. These engineered cell lines exhibited increased IC50 values for both BTZ and CFZ compared to their parental wild-type counterparts, indicating that NQO1 overexpression confers resistance to proteasome inhibition. To validate the clinical relevance of NQO1 expression, we analyzed its impact on progression-free survival (PFS) in MM patients. Specifically, we compared PFS in patients with high NQO1 levels vs. normal/low level, in a subcohort of the CoMMpass dataset (n=409) receiving PI-based first-line therapy. Remarkably, Patients with high NQO1 expression demonstrated significantly shorter PFS (median: 582 days, $p < 0.0001$) compared to those with normal/low NQO1 levels (median: 1032 days). This adverse prognostic impact was comparable to or even worse than that observed in high-risk myeloma patients with del(17p) (median: 630 days). Importantly, this effect appeared specific to PI-based therapy, as no significant PFS difference was observed between high and normal/low NQO1 expressers in a separate cohort (n=79) receiving PI-free first-line treatment (median PFS: 844 days vs. 1096 days, $p = 0.6756$).
Conclusions: In summary, we identified NQO1 overexpression as a mechanism of proteasome inhibitor resistance in multiple myeloma. Overexpression of NQO1 *in vitro* confirmed its impact on PI sensitivity, increasing resistance to both BTZ and CFZ. Clinically, high NQO1 expression correlated with significantly shorter PFS in patients receiving PI-based therapy, proposing NQO1 as a functional mediator of PI resistance in MM.

#3080 PKM2 inhibition overcomes lapatinib resistance in HER2 overexpressing esophageal adenocarcinoma.

Annie Ritter[1], Md Sazzad Hassan[2], Elizabeth Heffernan[1], Niranjan Awasthi[2], Urs von Holzen[2]

[1]University of Notre Dame, South Bend, IN,[2]Indiana University School of Medicine (South Bend, IN), South Bend, IN

Background: The human epidermal growth factor receptor 2 (HER2) is overexpressed in esophageal adenocarcinoma (EAC) and is a major target for EAC therapies. Lapatinib blocking HER1 and HER2 pathways, fails to improve patient survival. The molecular mechanisms of this lapatinib resistance are still unclear. We therefore explored the role of the glycolytic enzyme pyruvate kinase M2 (PKM2), a key regulator of the Warburg effect, in the lapatinib resistance mechanism of EAC cells.
Methods: HER2/pHER2 expression status was evaluated by Western blot analysis in a panel of EAC cell lines (Flo-1, ESO26, OE19, OE33, SK-GT-2, ESO51, OACM5.1C, and LPR-OE19). The lapatinib-resistant OE19 (LPR-OE19) cell line was established from the OE19 cell line by intermittent exposure to increasing concentrations of lapatinib for a period over six months. We investigated the cell growth/migration inhibition and apoptotic signaling effects of treating HER2-overexpressing OE19, LPR-OE19, and OE33 cell lines with lapatinib, PKM2 inhibitor shikonin, and a chemotherapeutic agent nanoparticle albumin-bound paclitaxel (nab-paclitaxel) either alone or in combinations. Lactate production and cellular localization of PKM2/HSP40 were also detected. In addition, we explored the in-vivo antitumor efficacy following lapatinib, shikonin, and nap-paclitaxel mono and combination therapies in murine subcutaneous cell lines and patient-derived xenograft models of human EAC.
Results: LPR-OE19 cells showed significantly reduced sensitivity to lapatinib-induced cell growth/migration inhibition and apoptosis compared to parent OE19 cells. Augmented cell growth/migration inhibition and apoptosis were observed in LPR-OE19 cells compared to parent OE19 cells when lapatinib was combined with shikonin. Knockdown of PKM2 in LPR-OE19 cells abolished the reduced sensitivity of lapatinib-induced cell growth inhibition and abolished the augmented cell growth inhibition response when lapatinib and shikonin were combined. Knockdown of HSP40 in OE19 cells enhanced PKM2 expression. LPR-OE19 cells showed enhanced lactate production compared to parent OE19 cells. PKM2 and HSP40 co-localize with each other in the cell nucleus, suggesting that PKM2 bound to molecular chaperone HSP40. In subcutaneous xenografts, shikonin displayed a remarkable tumor regression compared to that of control as monotherapy, and shikonin in combination with either lapatinib or nab-paclitaxel showed a significant enhancement effect of tumor regression.
Conclusions: These data suggest that combination therapy of the PKM2 inhibitor shikonin with lapatinib or nab-paclitaxel should be tested as a potent option for lapatinib-resistant HER2-positive EAC patients and could be a novel treatment strategy for specific EAC. In addition, we suggest targeting HSP40-PKM2 interaction as an innovative therapeutic approach to overcome lapatinib resistance in EAC.

#3081 The multiciliated transcription factor FOXJ1 modulates microtubule dynamics and mediates taxane resistance in prostate cancer.

Ada Gjyrezi[1], Fang Xie[2], Daniel E. Fein[2], Maryam Labaf[2], Larysa Poluben[2], Olga Voznesensky[2], Aniket Gad[2], Eva Corey[3], Rupal S. Bhatt[2], Steven Balk[2], Paraskevi Giannakakou[1]

[1]Medicine, Weill Cornell Medicine, New York, NY,[2]Medicine, Beth Israel Deaconess Medical Center, Boston, MA,[3]Urology, University of Washington School of Medicine, Seattle, WA

Docetaxel is the first-line chemotherapy for metastatic castration-resistant prostate cancer, but clinically meaningful mechanisms of resistance remain to be established. We generated an in vivo model of docetaxel resistance using castration-resistant patient-derived xenografts and found increased expression of genes that drive development of multiciliated cells, including FOXJ1 and its effector genes, many of which regulate ciliary microtubules. Mechanistically, FOXJ1 overexpression conferred docetaxel resistance in vitro and in vivo, which was associated with decreased docetaxel-mediated microtubule bundling. Overexpression of a microtubule-associated FOXJ1-regulated gene (TPPP3) had similar effects. Conversely, FOXJ1 knockdown impaired basal microtubule function, enhanced taxane binding to microtubules, and increased docetaxel sensitivity. These results establish mechanistic causality between the FOXJ1 signaling axis, microtubule biology, and taxane resistance. Clinically, FOXJ1 gene amplification was increased in taxane-treated prostate cancer patients. Moreover, in the CHAARTED clinical trial of docetaxel combined with androgen deprivation for metastatic prostate cancer, higher baseline FOXJ1 was predictive of decreased survival in prostate cancer patients treated with docetaxel, further supporting clinical relevance. Together these findings identify a previously unrecognized clinically impactful mechanism of taxane resistance whose exploitation could stratify patients that will not benefit from taxane treatment.

#3082 Activation mevalonate pathway via HMGCS2 introduced resistance to molecular targeted therapy for cancer.

Y. Kato;
Yasuhiro Kato (Individual), Tokyo, Japan

Introduction: Recently, some oncogenic driver mutations found in any kinds of cancer. NTRK fusion is one of the oncogenic driver mutations express in any kinds of malignancy tumors and neurotrophic tropomyosin receptor kinase (NTRK) tyrosine kinase inhibitors (TKIs) is specific treatment for cancer harboring *NTRK* fusion. We investigated resistance mechanism of cancer harboring NTRK fusion to NTRK-TKI.

Method: We established resistant cells to NTRK-TKIs (larotrectinib, entrectinib and selitrectinib) using KM12 harboring *NTRK* fusion. We investigated resistant cells for finding resistant mechanism to NTRK-TKI.

Results: Whole exome sequence and microarray analysis conducted to resistant cells (KM12-LR, KM12-ER, and KM12-SR). Only KM12-LR have G595R as resistant solvent front mutation previously reported. We confirmed the overexpression of 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2) in resistant cell lines from result of microarray, western blotting and quantitative RT-PCR. SREBP2 and PPARα which associated mevalonate pathway showed lower expression correlated HMGCS2 expression in resistant cells. After knocking down of HMGCS2, sensitivity to NTRK-TKI recovered in KM12-ER and KM12-SR. Mevalonic acid reintroduced NTRK-TKI resistance in KM12-ER and KM12-SR. after knocking down HMGCS2. Simvastatin and silibinin showed synergic effect with NTRK-TKIs to resistant cells, and delayed emergence of resistance in KM12 cells. In vivo study, resistant tumor to NTRK-TKI created in mice showed HMGCS2 over expression. NTRK-TKI and simvastatin reduced resistant tumor growth compared with entrectinib alone in vivo.

Conclusion: Activation mevalonate pathway via HMGCS2 overexpression introduced resistance to NTRK-TKI. It found novel resistance mechanism to molecular targeted therapy. Statin showed possibly useful for overcoming resistance mechanism introduced by HMGCS2.

#3083 NT219 overcomes immune evasion-resistant mechanisms in head and neck squamous cell carcinoma (HNSCC).

Hadas Reuveni, Hava Ben David, Katy Sigalov, Daphna Miron

Purple Biotech Ltd., Rehovot, Israel

Background: NT219 is a small molecule that promotes insulin receptor substrate 1/2 (IRS) degradation and suppresses STAT3 phosphorylation, two major complementary signaling pathways that play a key role in cancer and drug resistance. We present NT219 suppressive effect on HNSCC tumor and tumor microenvironment (TME) and on cancer stem cells (CSC) which contribute to drug resistance and disease recurrence

Method: Tumor microarrays (TMA) of 54/57 HNSCC and 9 normal tissue cases were analyzed for pSTAT3(Y705) and IRS2 by IHC NT219 and cetuximab effect on CSC was assessed using 3D spheroid assay of HPV^- HNSCC lines, evaluating size (microscopic measurements), cell viability (CellTiterGlo) and stemness marker expression (FACS analysis). Ex vivo patient-derived tumor explant (PDE), treated with NT219 and/or nivolumab was analyzed for efficacy and cytokine secretion. In vivo efficacy was tested in a stem cell-enriched (NutriStem®) patient-derived xenograft (PDX).

Results: NT219 with cetuximab suppressed established spheroids of SCC-9 HNSCC, in terms of size (100% disintegration) and CSC viability (94% reduction), while cetuximab had no effect ($P<0.001$). NT219 alone showed a significant effect on CSC viability (82% reduction, $P<0.001$). HNSCC FaDu spheroids showed the same trend. Spheroids showed enrichment in OCT3/4 or NANOG positive CSC (4-fold vs 2D growth), completely reversed by NT219 and cetuximab. Inhibitory effect was also demonstrated in established FaDu spheroids including major stemness markers; OCT3/4, NANOG, SOX2, ALDH, CD166, CD44. In a stem cell-enriched PDX HNSCC model, NT219 synergized with cetuximab to suppress tumor growth. Accordingly, delayed tumor recurrence demonstrated. A significant upregulation of STAT3(Y705) phosphorylation and IRS1/2 were detected in tumor and/or TME fibroblasts and TILs vs normal tissue. PDX models demonstrated that blockage of both STAT3 and IGF1R/IRS pathways is required to overcome resistance to EGFR therapy in terms of survival and tumor size. In a HNSCC PDE resistant to nivolumab, NT219 and nivolumab combination induced synergistic tumor cell death (66%) accompanied by immuno-cytotoxic activation (10 and 1.5-fold increase of IL2 and granzyme B). Nivolumab-induced immunosuppressive effects (8-fold increase of IL-10, 2-fold decrease of TNFα) were completely blocked by the combination. In a phase 1 study of HNSCC patients treated with NT219 50-100 mg/kg and cetuximab standard dose weekly, significant differences in patient's biopsy pIGF1R and pSTAT3 were observed in 2 responders (PR) compared to the 4 progressed disease pts.

Conclusions: NT219 mitigates several immune evasion mechanisms including CSC-mediated resistance and tumor recurrence, and sensitizes resistant HNSCC tumors to PD1 and EGFR therapies. Upregulation of pIGF1R and pSTAT3 were correlated with patient response and suggested as potential biomarkers for NT219 treatment.

#3084 Claudin-11 promotes cisplatin resistance by inducing autophagy through activation of the PHF23-ERK signaling pathway in ovarian cancer cells.

Ha Yeong Chae[1], So Young Lee[1], Hyojin Jeong[1], Hye Ri You[1], Jinil Han[2], Young Kee Shin[3], Mi Jeong Kwon[1]

[1]Kyungpook National University, Daegu, Korea, Republic of,[2]Gencurix, Seoul, Korea, Republic of,[3]Seoul National University, Seoul, Korea, Republic of

Claudin-11, a member of claudins consisting of tight junction has been shown to be associated with prognosis and to play dual roles in cancer. However, its role in the response to anti-cancer agents is unknown. In this study, we investigated the role and mechanism of claudin-11 in cisplatin resistance in ovarian cancer. Kaplan-Meier survival analysis using The Cancer Genome Atlas data revealed that high *CLDN11* expression is significantly associated with poor prognosis in ovarian cancer. Claudin-11 expression was significantly upregulated in cisplatin-resistant ovarian cancer cells. Consistent with this, claudin-11 overexpression significantly decreased the sensitivity to cisplatin in ovarian cancer cells. Cisplatin-induced apoptosis was reduced in claudin-11-overexpressing ovarian cancer cells, which was associated with decreased DNA damage. Moreover, claudin-11 overexpression inhibited cisplatin-induced apoptosis by promoting autophagy through increased ERK phosphorylation in ovarian cancer cells. The cisplatin sensitivity of claudin-11-overexpressing cells was restored by co-treatment with chloroquine, an autophagy inhibitor, and cisplatin, indicating that claudin-11 promotes cisplatin resistance by inducing autophagy. RNA sequencing data analysis further showed that autophagy-related PHD finger protein 23 (*PHF23*) is significantly upregulated in claudin-11-overexpressing cells compared with vector-transfected cells. PHF23 knockdown reversed cisplatin resistance by inhibiting ERK phosphorylation and then cisplatin-induced autophagy and apoptosis in claudin-11-overexpression cell. Taken together, these results indicate that claudin-11 promotes cisplatin resistance by activating autophagy through the PHF23-ERK signaling pathway and thereby reducing apoptosis in ovarian cancer cell, suggesting that inhibition of claudin-11 may overcome cisplatin resistance in ovarian cancer.

#3085 Upregulation of TAGLN2-PI3K-AKT signaling promotes therapeutic resistance in glioblastoma through a positive feedback mechanism.

Ashok Kumar[1], Sasha Beyer[1], Bryan Perez-Soto[2], Anca-Ligia Grosu[3], Steffen Lindert[2], Heather R. Manring[1], Saikh Jaharul Haque[1], Arnab Chakravarti[1]

[1]Radiation Oncology, The Ohio State University Wexner Medical Center, Columbus, OH,[2]Department of Chemistry & Biochemistry, The Ohio State University, Columbus, OH,[3]Department of Radiation Oncology, University of Freiburg, Freiburg, Germany, Freiburg, Germany

Objective: Despite aggressive treatment with surgery, radiation and temozolomide chemotherapy glioblastoma (GBM) remains the most lethal tumor of the human brain, which is largely due to inevitable recurrence of treatment refractory tumor. Studies have shown that aberrant activation of AKT significantly contributes to the development of treatment resistance in GBM. Molecular profiling and in vitro studies from our group identified TAGLN2, an actin binding cytoskeletal protein, as a top upregulated gene in GBM. In other cancer types, TAGLN2 has been shown to activate the PI3K-AKT pathway resulting in treatment resistance. This study aims to explore how TAGLN2 drives treatment resistance in GBM.
Methods: PDX primary human GBM cell lines (08-387 and GBM 3359) were used. Co-immunoprecipitation (Co-IP) assays were performed to study the interaction of PTEN with TAGLN2 in vitro. Biacore/Surface plasmon resonance (SPR) analyses were performed to study binding affinity and kinetics of their interaction. Protein-protein interactions were validated by in silico (AlphaFold2) analysis. Protein expression was quantified by WB analysis. Cell viability was assessed by MTS assays.
Results: We have previously shown that RNAi-mediated knockdown (KD) of TAGLN2 significantly reduced cell proliferation, survival, clonal expansion and the invasive potential of GBM PDX cell lines in vitro and reduced tumor growth using preclinical in vivo models. We found that KD of TAGLN2 reduced the levels of activated/phosphorylated AKT (pAKT) in GBM cells resulting in downregulation of this pathway. Subsequently, we found that TAGLN2 physically associated with PTEN as evidenced by Co-IP, SPR and in silico analysis. Binding of TAGLN2 to PTEN may attenuate PTEN function resulting in the activation of downstream oncogenic pathways. mTORC2 and DNA-PK are known to phosphorylate and activate AKT resulting in the upregulation of downstream signaling. Here, we show that inhibition of these kinases either individually or in combination reduced TAGLN2 protein levels in GBM cells in vitro. Importantly, direct inhibition of Akt phosphorylation also resulted in the reduction of TAGLN2 protein levels indicating that activated Akt stabilized TAGLN2 (likely by phosphorylation), which in turn promoted oncogenic pathways via a positive feedback mechanism. Also, KD of TAGLN2 increased the sensitivity of GBM cells to both TMZ and RT as assessed by multiple in vitro models.
Conclusion: Taken together, our data suggest that Transgelin-2 promotes chemo-radiation treatment resistance in GBM through a novel positive feedback mechanism that involves the TAGLN2-PTEN-PI3K-AKT axis. Therefore, TAGLN2 may serve as potential therapeutic target in GBM and to this end, development of small molecule inhibitors of TAGLN2 is actively underway in our laboratory.

#3086 TFAP2C promotes mitochondrial fitness required for sustained kinase reprogramming in endocrine therapy resistance in breast cancer.

Attila Szenasi, Austin Whitman, Santiago Haase, Philip Spanheimer

University of North Carolina, Chapel Hill, NC

Endocrine therapy resistance (ETR) remains a formidable challenge in the treatment of estrogen receptor-positive (ER+) luminal breast cancer, frequently leading to therapeutic failure. Our previous research identified the transcription factor TFAP2C as a key regulator of the antioxidant enzyme glutathione peroxidase 1 (GPX1), as well as c-ErbB2/HER2, RET, and EGFR, which lead to ERK/MAPK activation and promote ETR. Here, we aimed to elucidate the role of TFAP2C in the redox regulation of kinase reprogramming in acquired ETR. Our current study reveals that TFAP2C acts as a metabolic switch, triggering cellular reprogramming and redox modulation of kinase signaling through post-translational cysteine modifications. TFAP2C knockdown in tamoxifen-resistant cell lines and patient-derived organoids induces mitophagy, leading to reduced mitochondrial content, metabolic reprogramming, and altered Reactive oxygen species (ROS) profiles via GPX1 and SOD1 signaling. This disruption enhances phosphatase activity in the PI3K and MAPK pathways, resulting in cell cycle arrest and attenuated HER2- and EGFR-mediated signaling, both of which are pivotal for sustaining ETR. This study not only underscores the role of TFAP2C as a central player in maintaining the metabolic and signaling adaptations required for kinase reprogramming associated with ER+ targeting therapies, but also highlights the broader implications of mitochondrial redox signaling in the etiology of ETR, providing a novel avenue for therapeutic intervention.

#3087 Cancer-associated fibroblasts promote resistance to chemotherapy through the p53/ERK1/2/DKK1 pathway.

Zhijie Yin, Yuning Zhou, Xudong Zhu, Chi Wang, Heidi L. Weiss,B. Mark Evers, Qingding Wang

Markey Cancer Center, The University of Kentucky, Lexington, KY

Introduction: Chemotherapy constitutes the cornerstone of adjuvant therapy for colorectal cancer (CRC); however, acquired resistance issues can lead to a decrease in the efficiency of chemotherapy. Dickkopf-1 (DKK1) promotes MDSC infiltration, immunosuppression, and cancer relapse. Cancer-associated fibroblasts (CAFs) play a pivotal role in the tumor microenvironment. The purpose of this study was to examine whether CAFs play a critical role in acquired chemotherapy resistance.
Methods: CAFs were treated with either 5-FU, oxaliplatin or sn38 in a time- and dose-dependent manner. Expression of DKK1, p53, fibroblast activation protein (FAP), and phosphorylated ERK1/2, JNK, p38 were determined by either RNA-seq, western blot, real-time (RT)-PCR, ELISA, or immunofluorescence (IF) staining. To determine whether targeting DKK1 would enhance the efficacy of 5-FU treatment, $APC^{f/f}$; CDX2-Cre/ERT2 mice were injected with tamoxifen to induce adenoma formation followed by treatment with 5-FU in combination with administration of either normal IgG or anti-DKK1 antibody. Tumor tissues were harvested and flow cytometric analyses performed.
Results: Treatment of CAFs with either 5-FU, oxaliplatin, or sn38 (an active metabolite of irinotecan) increased DKK1 expression and secretion, suggesting a general induction of DKK1 by chemotherapeutic agents. Treatment with these agents resulted in the activation of MAPK as noted by the increased phosphorylation of ERK1/2, JNK, and p38. Inhibition or knockdown of MEK/ERK1/2, but not JNK and p38, blocked the induction of DKK1 expression, suggesting that chemotherapy increased DKK1 expression through activation of ERK1/2 signaling pathway in CAFs. Consistently, elevated levels of phospho-ERK were found in CAFs in surgically resected samples from patients treated with neo-adjuvant therapy prior to surgery compared to non-chemotherapy controls as determined by double IF staining for phospho-ERK1/2 and FAP. Chemotherapeutic agents induce DNA damage-related upregulation of p53 in CAFs. Knockdown of p53 blocked increased ERK1/2 phosphorylation and DKK1 expression after treatment with the chemotherapeutic agents. These results suggested that chemotherapeutic agents increased DKK1 expression through p53-dependent activation of ERK1/2. Treatment with 5-FU increased levels of DKK1 in plasma, and coincidently, increased myeloid-derived suppressor cell (MDSC) tumor infiltration as determined by FACS analysis; these increases were blocked by treatment with anti-DKK1 neutralizing antibody. Moreover, anti-DKK1 antibody enhanced the antitumor efficacy of 5-FU as noted by the significantly decreased tumor size and numbers.
Conclusions: Our current study identified a novel mechanism contributing to chemotherapy resistance. Importantly, our findings provide evidence for targeting ERK1/2/DKK1 to counteract chemotherapy resistance for CRC treatment.

#3090 miRNA-221-5p-mediated epigenetic regulation promotes chemoresistance and offers therapeutic potential in ovarian cancer.

Tasmin Rahman Omy, Naresh Sah, Mark Reedy, Ganesh Acharya, Komaraiah Palle

Texas Tech University Health Sciences Center, Lubbock, TX

Epithelial ovarian cancer (EOC) is a highly aggressive malignancy and the leading cause of gynecological cancer-related deaths. The majority of patients are diagnosed at advanced stages (III-IV), where treatment involves surgical debulking followed by platinum-based chemotherapy. While initial responses to therapy are often favorable, over 70% of advanced-stage EOC patients experience recurrence, typically accompanied by resistance to standard of care treatments. This chemoresistance significantly limits therapeutic success and worsens patient prognosis. Understanding the molecular mechanisms driving resistance is critical for developing effective therapeutic strategies. Emerging evidence highlights the role of miRNAs in regulating gene expression networks associated with DNA damage repair and chemoresistance. miRNA-221-5p has been implicated in modulating tumor progression and therapeutic responses in various cancers. Here, we investigate its role in ovarian cancer, focusing on regulating RAD18 and RAD51—key mediators of DNA damage tolerance and homologous recombination repair (HRR). Our analysis of miRNA expression in a panel of human ovarian cancer cell lines revealed low to undetectable levels of miRNA-221-5p, coupled with elevated expression of DNA damage response and repair genes such as **RAD18** and **RAD51**. Our experimental data demonstrate that miRNA-221-5p regulates RAD18 and RAD51 transcripts through 3'-UTR, leading to their post-transcriptional repression and subsequent restoration of ovarian cancer cell sensitivity to platinum drugs. Mechanistically, our results confirm that miRNA-221-5p epigenetically regulates RAD18- and RAD51-mediated DNA damage tolerance and homologous recombination repair. Importantly, restoring miRNA-221-5p downregulates RAD18 and RAD51 and sensitizes chemoresistant ovarian cancer cells to platinum drugs, offering a promising strategy to enhance therapeutic outcomes. Overall, our data demonstrate that miRNA-221-5p is a critical regulator of chemotherapy-induced epigenetic and transcriptional alterations, making it a prospective therapeutic target for overcoming chemoresistance and avoiding EOC recurrence.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: The Microenvironment in Therapeutic Response
Poster Session

#3093 Molecular profiling of tisotumab vedotin-treated patients identified immune pathways associated with clinical activity, supporting the multimodal mechanism of action.

Sriram Sridhar[1], Guy Roukens[1], Christina Yu[1], Han Si[1], Steven Xu[1], Lauren Brady[1], Merzu Belete[1], Mark Bieda[2], Khyati N. Shah[3], Regina Aquino[1], Denise Gallagher[1], Brandon Higgs[1], Mark Fereshteh[1], Maria Jure-Kunkel[1], Jeffrey R. Harris[1]

[1]Genmab US, Inc., Plainsboro, NJ,[2]Pfizer Inc., Bothell, WA,[3]Pfizer Inc., South San Francisco, CA

Introduction: Tisotumab vedotin (TV) is an antibody drug conjugate comprised of a human tissue factor (TF)-specific IgG1κ monoclonal antibody chemically conjugated via a protease-cleavable valine-citrulline linker to the microtubule-disrupting agent monomethyl auristatin E. TV is approved in the US based on the pivotal single arm innovaTV 204 study and the randomized phase 3 innovaTV 301 study for patients with recurrent/metastatic cervical cancer who have progressed on/after chemotherapy. The purpose of this study was to explore the tumor microenvironment of patients with cervical cancer for characteristics associated with immune-mediated mechanisms of action (MoA) of TV.
Methods: RNA sequencing was performed on available pre-treatment biopsies from 41 patients enrolled in the innovaTV 204 study. Differentially expressed genes and gene signatures were identified by comparing responders (per RECIST v1.1, complete or partial response) and non-responders (stable or progressive disease) using a linear model adjusting for clinical factors, where mean fold differences and nominal p-values between groups were determined. Associations with overall survival (OS) were determined using a Cox proportional hazards model. To further explore signatures involved in TV MoA, Fc-mediated effector functions of TV were analyzed using tumor cell lines with varying TF expression levels co-cultured with variations of TV/tisotumab antibodies and immune cells.
Results: Mean levels of a natural killer (NK) cell signature were higher in TV responders vs. non-responders. The NK cell signature, as well as an M1 macrophage and monocyte signatures were also associated with better OS among patients from innovaTV 204. Levels of these signatures in untreated patients with cervical cancer from TCGA were not associated with better OS, suggesting that their link with this outcome was specific to TV treatment. Immune cell effector mechanisms were confirmed in vitro by assessing viability of tumor cell with various TF expression levels co-cultured with immune cells. Tumor cell viability analysis suggested TV may contribute to immune cell-mediated clearance of target tumor cells via Fc-mediated effector mechanisms, which was reduced with an Fc-inert variant of TV.
Conclusions: Immune gene signatures associated with the Fc-mediated effector mechanisms of antibody-dependent cellular cytotoxicity (NK cells) and antibody-dependent cellular phagocytosis (macrophages/monocytes) were associated with improved OS and overall response rate in innovaTV204 patients. Preclinical results demonstrate Fc-dependent immune cell engagement as a MoA of TV. Together, these results provide additional evidence of TV's multimodal MoA in patients with advanced cervical cancer.

#3094 Aurora-A inhibition sensitizes HPV-driven cancers to anti-CTLA-4 immunotherapy by modulating the tumor immune microenvironment.

Soma Ghosh[1], Madison P. O'Hara[1], Tuhina Mazumdar[1], Lacin Yapindi[1], Li Shen[1], Jing Wang[1], Mitchell J. Frederick[2], Jagannadha K. Sastry[1], Faye M. Johnson[1]

[1]UT MD Anderson Cancer Center, Houston, TX,[2]Department of Otolaryngology, Baylor College of Medicine, Houston, TX

About 5% of cancers are caused by human papillomavirus (HPV), most of which are resistant to immune checkpoint inhibitors (ICIs). Alisertib (Ali), an Aurora kinase A inhibitor, may sensitize these cancers to ICIs by inducing apoptosis and DNA damage. This study investigates combining Ali with ICIs to enhance antitumor immunity and overcome resistance. We assessed Ali's effects on apoptosis, DNA damage, and immunogenic cell death in HPV-positive cancer cell lines. Ali was tested alone or with ICIs in the mEER model, a surrogate for HPV+ oropharyngeal cancers. Changes in the tumor immune microenvironment (TIME) were analyzed by flow cytometry. RNA-seq evaluated gene expression across four groups: control, Ali, anti-CTLA-4, and combination. Gene set enrichment and Gene Ontology (GO) analyses identified key biological processes.Combination therapy with Ali and anti-CTLA-4 significantly reduced tumor size and improved survival, with 75% of treated mice surviving at 60 days compared to 0% of controls. Ali induced increased cleaved caspase-3 and PARP expression in tumor tissues, indicating enhanced cancer cell apoptosis. Flow cytometry showed enhanced T-cell polyfunctionality, with increased IFN-γ and granzyme production in tumor-infiltrating lymphocytes, along with reduced T-cell exhaustion (e.g., LAG3). A significant reduction in the Gr-1low Ly6G+ granulocytic subset of myeloid-derived suppressor cells (MDSCs) suggested an improved immune microenvironment. RNA-seq identified 22,059 protein-coding genes with ≥1.5-fold increase compared to controls. Of these, 400 genes were upregulated by Ali, 3 by anti-CTLA-4, and 640 by the combination, with 191 genes upregulated in both Ali and combination groups. GO analysis revealed that shared genes activated immune-related processes like cell surface receptor signaling, macrophage activation, and T-cell cytokine regulation. In the Ali group, immune activation was linked to cytokine production and immune effector responses, while the combination showed enrichment for metabolic processes, suggesting metabolic reprogramming as a mechanism for enhanced immune responses and efficacy. The combination therapy increased CD8+ central memory T-cells, CD4+ memory T-cells, and elevated naive CD4+ T-cells, indicating enhanced T-cell activation and recruitment to the tumor, correlating with increased immune cell infiltration and improved survival. The combination of Ali and anti-CTLA-4 enhances outcomes in an HPV-driven murine model by boosting immune activation and reshaping the TIME. RNA-seq analysis shows that Ali drives immune responses, further amplified by anti-CTLA-4, promoting both immune and metabolic reprogramming. These findings suggest that combining Ali with ICI could overcome resistance and improve therapeutic outcomes in HPV-positive cancers.

#3095 Comparative analysis of WRN inhibitors HRO761 and VVD-133214 in a cancer cell panel: insights into MSI status-dependent drug responses.

Lili Chai, Tingting Yang, Huawei Pan, Wei Liu, Zhengtai Li, Xiaojian Wang, **Tj (Tiejun) Bing**

ICE Bioscience, Inc., Beijing, China

High-throughput screening of a cancer cell panel represents a pivotal instrument in the armamentarium of drug discovery and development. The sensitivity profiles of these cell lines serve as a cornerstone for the refinement of in vivo model systems and for the stratification of patient cohorts in clinical trials. Werner Syndrome RecQ Helicase (WRN), a member of the RecQ helicase family, is instrumental in the preservation of genomic integrity. Its multifaceted role encompasses DNA repair, replication, recombination, and telomere maintenance. WRN has garnered significant attention as a potential therapeutic nexus in oncology, particularly within the context of tumors exhibiting microsatellite instability (MSI). The synthetic lethality between WRN and MSI has been the subject of intensive research, demonstrating that WRN inhibition is selectively toxic to cancer cells harboring a high microsatellite instability (MSI-H) phenotype, which is a characteristic observed across a spectrum of malignancies, including colorectal, endometrial, and gastric cancers.

The present investigation assesses the therapeutic potential of two advanced WRN inhibitors, HRO761 and VVD-133214, within a meticulously curated panel of cancer cells that display a continuum of MSI and microsatellite-stable (MSS) statuses, as well as HRO761 resistant cell lines. Employing cell viability assays, we conducted a comparative analysis of the growth-inhibitory effects of these compounds on MSI and MSS cell lines. Our data reveal a pronounced anti-neoplastic effect of both HRO761 and VVD-133214 in some MSI-H cell lines, juxtaposed with a diminished impact on MSS cell lines. Further, transcriptomic profiling elucidated discrete transcriptional landscapes modulated by each inhibitor, shedding light on their divergent pharmacological mechanisms. This study not only showcases our proficiency in high-throughput screening and bioinformatics but also contributes a wealth of knowledge regarding the MSI-dependent therapeutic responses to WRN-targeted agents, potentially informing future clinical strategies in cancer treatment.

#3096 The RXR agonist MSU-42011 and the MEK inhibitor selumetinib reduce tumor burden by decreasing pERK levels and modulating immune cell populations within the NF1 tumor microenvironment.

Pei-Yu Peggy Hung[1], Jessica A. Moerland[1], Karen T. Liby[2]

[1]Michigan State University, East Lansing, MI,[2]IU School of Medicine, Indianapolis, IN

Neurofibromatosis type 1 (NF1) is a common genetic disease that predisposes approximately 50% of affected individuals to develop plexiform neurofibromas (PNFs), which can progress to highly aggressive malignant peripheral nerve sheath tumors (MPNSTs) in approximately 10% of patients. NF1 is caused by mutations in the tumor suppressor gene *NF1*, which encodes for neurofibromin, a negative regulator of RAS activity. Selumetinib, a specific inhibitor of MEK1/2, is the only FDA-approved drug for NF1-associated PNFs. However, the anti-tumor effects of selumetinib are limited in MPNSTs, and the drug has dose-limiting side effects. Deficiency of the *NF1* gene not only promotes tumorigenesis but also has broad effects on the immune cells and cytokine signaling driven by hyperactive RAS signaling. Because macrophages account for almost half of cells in NF1 lesions and their infiltration correlates with disease progression, we hypothesized that targeting tumor-promoting immune cells is an alternative approach for treating NF1. The novel retinoid X receptor (RXR) agonist MSU-42011 reduces tumor growth in experimental Kras-driven cancers by decreasing pERK expression, reducing tumor-promoting immune cells like CD206+ macrophages and regulatory T cells, and increasing activated cytotoxic T cells. Here, we treated NF1-deficient cells and macrophages with MSU-42011 and selumetinib, either alone or in combination, using monoculture and conditioned media (CM) conditions. In human PNF and mouse MPNST cells, treatment with 200 nM MSU-40211 or 50 nM selumetinib for 3 hours reduced pERK protein levels compared to untreated controls, and the combination treatment enhanced this reduction in pERK protein levels. Additionally, there was a trend toward reduction in cell viability with increasing drug concentrations after 72 hours of the combination treatment. Moreover, CM from human and mouse PNF cells increased the mRNA expression of monocyte chemoattractant *CCL2* and the secretion of IL-6 and TNFα in human THP1 monocytes/macrophages and bone marrow derived macrophages (BMDM). Notably, MSU-42011 and selumetinib alone inhibited *CCL2* mRNA expression in THP1 macrophages and BMDM stimulated with CM from human and mouse PNF cells, respectively, and the inhibition of *CCL2* mRNA expression was greatest with the combination treatment. The combination of MSU42011 and selumetinib also significantly reduced tumor growth and reduced pERK levels and tumor-promoting CD206+ macrophages in both a LL2 model of lung cancer driven by an activating *Kras* mutation and a syngeneic mouse model of MPNST. Based on the similarities in RAS activation and immune cell infiltration in NF1 and lung cancer, our next step is to confirm the immunomodulatory and anti-tumor effects of MSU-42011 and selumetinib in a genetically engineered model of PNF and MPNST.

#3097 MNK1/2 kinase expression in immune cells dictates tumoral response to MNK inhibitors.

Thao D. Pham, Apurba Majumder, Alejandra M. Marinelarena, Jeffrey H. Becker, Hidayatullah G. Munshi

Medicine, Northwestern University, Chicago, IL

Background: MAPK-interacting serine/threonine-protein kinase 1 and 2 (MNK1 and MNK2) are downstream effectors of the MEK/ERK and p38 MAPK pathways. Increased expression and activity of MNK kinases are linked to tumor growth and therapeutic resistance. As a result, several classes of MNK inhibitors (MNKi) have been developed and evaluated preclinically. Previously, we reported that treatment of murine pancreatic tumors with MNKi resulted in increased tumoral infiltration of PD-1^{hi}CD8+ T cells. We further found that MNKi-induced increase in immunosuppressive tumor-associated macrophages (TAMs) accounted for the infiltrating T cell exhaustion. Finally, co-treatment with MNKi and anti-PD-1 rescued T cell activity and sufficiently controlled tumor growth. However, the extent to which MNKi efficacy relies on the immune cells was not clearly defined. In this study, we set out to better understand the roles of MNK kinases in T cells and macrophages, and how they contribute to MNKi anti-tumor efficacy.

Methods: We evaluated the proliferation and effector functions of bone marrow-derived macrophages (BMDMs) and splenocyte-derived T cells collected from either mice with wild-type background or mice with body-wide knockout (KO) of MNK1, MNK2, or both MNK1 and MNK2 expression. In addition, we assessed murine pancreatic tumor growth in wild-type, MNK1-, MNK2-, or MNK1/2-KO and their response to anti-PD-1 therapy, either alone or in combination with MNKi.

Results: We first found that BMDMs isolated from MNK1-, MNK2-, or MNK1/2-KO (collectively referred to as MNK^{KO} hereon) mice are more potent at suppressing T cell proliferation in vitro. Interestingly, T cells isolated from MNK^{KO} spleen demonstrated higher proliferative capacity and activation following CD3/CD28 ligation. Compared to the wild-type, MNK^{KO} T cells are also capable of better proliferation when co-cultured with MNK^{KO} BMDMs. Similarly, T cells isolated from the tumor-bearing MNK^{KO} mice showed enhanced effector functions compared to those isolated from the wild-type mice. Significantly, tumor-bearing MNK^{KO} mice showed an exceptional response to an anti-PD-1 antibody, whereas the wild-type mice did not respond to the treatment. Finally, the addition of a MNKi was found to potentiate anti-PD-1 therapy in the wild-type, but not the MNK^{KO}, mice.

Conclusion: Together, these new findings demonstrate previously unknown roles of MNK kinases in T cells, macrophages, and their crosstalk within the tumor microenvironment. These data also suggest that while MNKi have been designed to target cancer cells, their reported efficacy relies primarily on the immune cells, specifically T cells. Thus, efforts to enhance MNKi efficacy should focus on combining these drugs with immune-modulatory agents, particularly those that can boost T cell activity or suppress immunosuppressive macrophages.

#3098 Linker cleavable enzymes determine anti-tumor activity of trastuzumab deruxtecan in HER2-positive lung cancer and HER2-low breast cancer.

Akari Shiraishi[1], Asumi Iesato[2], Yuki Takahashi[3], Ken Uchibori[4], Tomo Osako[5], Reo Maruyama[6], Makoto Nishio[4], Ryohei Katayama[1]

[1]Div of Experimental Chemotherapy, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo, Japan,[2]NEXT-Ganken program, Japanese Foundation for Cancer Research, Tokyo, Japan,[3]Technical Research Institute, TOPPAN Holdings Inc., Tokyo, Japan,[4]Department of Thoracic Medical Oncology, The Cancer Institute Hospital, Japanese Foundation for Cancer Research, Tokyo, Japan,[5]Division of Pathology, Cancer Institute, Japanese Foundation for Cancer Research, Tokyo, Japan,[6]Department of Cancer Epigenomics, Cancer Institute, Japanese Foundation for Cancer Research, Tokyo, Japan

HER2 genetic aberrations are found across various cancer types. Trastuzumab deruxtecan (T-DXd), an anti-HER2 antibody-drug conjugate (ADC), has been approved for the treatment of HER2-amplified breast and gastric cancers, HER2-mutant lung cancer, and HER2-overexpressing solid tumors. The approval of T-DXd has recently been expanded to include patients with HER2-low breast cancer. In addition, a clinical trial in lung cancer has demonstrated that the efficacy of T-DXd is not correlated with HER2 expression or specific genetic mutations. These findings suggest the presence of factors beyond HER2 expression that affect T-DXd sensitivity. To identify novel determinants of T-DXd efficacy in HER2-positive cancers, we first evaluated its anti-tumor activity in *in vitro* and *in vivo* using patient-derived HER2-positive lung cancer cell lines, JFCR-007 and JFCR-381. Interestingly, these cells showed significant shrinkage only by a single dose of T-DXd when implanted in BALB/c-nu mice, they were resistant to T-DXd in a regular 2D culture condition. Therefore, we hypothesized that tumor microenvironment affected T-DXd sensitivity. Cancer cells in patient tumor tissue are surrounded by non-cancerous cells, so we used a three-dimensional (3D) layered co-culture model with fibroblasts and extracellular matrix (ECM). We developed a 3D-in model, in which cancer cells are mixed with fibroblasts and ECM. We also made 3D-on, in which cancer cells were layered on the top of approximately 30 layers of fibroblasts, and 3D-out, in which cancer cells were seeded outside the chamber insert filled with 30 layers fibroblasts. Strikingly, the two lung cancer cell lines became sensitive to T-DXd in the 3D-in and the 3D-on state compared to the 3D-out model. Next, this co-culture model was applied to the HER2-low breast cancer, for which T-DXd has been shown to significantly prolong progression-free survival (PFS) in more than half of cases. We therefore established HER2-knockout and -low expressing breast cancer cell lines (HCC1954 and MDA-MB-453) using the CRISPR/Cas9 system followed by sorting with cell sorter. These HER2-low and -null cells were resistant to T-DXd in 2D culture state compared to parental cells. However, they became sensitive to T-DXd when cultured in 3D-in and 3D-on state. To identify the mechanism by which cancer cells become T-DXd-sensitive in 3D co-culture systems, we focused on cathepsins, lysosomal peptidases that cleave the T-DXd linker. The cathepsin subtypes capable of cleaving the amino acid sequence in the T-DXd linker were knocked down and examined the T-DXd sensitivity. Notably, cancer cells exhibited resistance to T-DXd when some cathepsin subtypes were individually knocked down. Collectively, our results suggest that certain types of cathepsins in the tumor microenvironment may play a critical role in the anti-tumor effects of T-DXd.

#3099 Predicting response to trastuzumab deruxtecan in breast cancer using flow cytometry.

Daniel S. Peiffer[1], Muriel Laine[2], Long Nguyen[1], Natalia Povarova[2], Frederick M. Howard[3], Nan Chen[3], Kimberly Cole[4], Olufunmilayo I. Olopade[3], Rita Nanda[3], Geoffrey L. Greene[2]

[1]The University of Chicago Medicine Comprehensive Cancer Center, Chicago, IL,[2]Ben May Department of Cancer Research, The University of Chicago Medicine Comprehensive Cancer Center, Chicago, IL,[3]Medicine, The University of Chicago Medical Center, Chicago, IL,[4]Pathology, The University of Chicago Medical Center, Chicago, IL

Background: Historically, characterization of HER2 status has focused on stratifying tumors that over express HER2, known as HER2-positive (HER2+) breast cancer (BC), and those that do not, known as HER2-negative (HER2-) BC. Approximately half of all BCs, considered HER2-, express low levels of HER2 by immunohistochemistry (IHC). Although HER2-low BCs are resistant to traditional HER2-targeted therapies, recent trials have demonstrated they are sensitive to the antibody drug conjugate (ADC) trastuzumab deruxtecan (T-DXd). Tumor expression of HER2 was hypothesized to be required for response to T-DXd, however studies have demonstrated its efficacy in patients with tumors that stained <1+ on IHC, considered HER2-zero (HER2-0) disease. We hypothesize that the HER2 heterogeneity in HER2-low and HER2-0 tumors makes IHC a suboptimal prediction tool. Thus, there is a clinical need for other quantification methods to determine if HER2 represents a valid biomarker to predict response to T-DXd.
Methods: We measured HER2 levels by flow cytometry (FC) in IHC-determined HER2-low and HER2-0 BC cell lines and patient-derived organoids (PDOs) and correlated the results to T-DXd sensitivity in vitro. To determine if HER2 is required for response to T-DXd, ERBB2 knocked down by siRNA and CRISPR-CAS9 HER2-knock out (HER2-KO) variants from the HER2-low and HER2-0 parental cell lines and PDOs were generated. To model intratumor HER2 heterogeneity, mixtures of HER2 depleted cells with control cells were made to assess T-DXd efficacy in tumors with decreasing amounts of HER2-expressing cells. To assess ADC uptake, cell lines and PDOs were treated with fluorescently tagged T-DXd and intracellular ADC levels were measured by immunofluorescence. Relative amounts of deruxtecan deposition in the TME were quantified by mass spectrometry. In vivo studies using mixtures of CAS9-control and HER2-KO HER2-low cells implanted into NSG mice are ongoing.
Results: FC quantification of HER2 levels across BC cell lines and PDOs were reproducible and agreed with IHC results. Levels of HER2 expression directly correlated with sensitivity to T-DXd, and HER2 expression was required for response. Cell mixtures of HER2 knockdown + SCBi-control cells or CAS9-control and HER2-KO cells were more representative of PDO HER2 heterogeneity as quantified by FC. Growth assays of cell mixtures showed sensitivity to T-DXd was the same regardless of if there were 100%, 50% or <25% HER2-expressing cells.
Conclusions: FC represents a novel method for predicting tumor cell response to T-DXd as it can reproducibly quantify HER2 heterogeneity within the TME. Our results suggest that small amounts of HER2-expressing cells are sufficient to elicit response to T-DXd, which may be missed on IHC. Thus, use of techniques like FC to quantify HER2 levels may identify T-DXd responders in the IHC-defined HER2-0 population, thus broadening the eligible patient population that benefits.

#3100 Spatially resolved effects of oligonucleotide therapeutics on cellular phenotypes in cancer tissues.

Corinne Ramos, Sandra Boute, Amandine Gerstenberg, Richard Ruez

Aliri, Lille, France

Introduction: Oligonucleotide therapeutics, such as antisense oligonucleotides (ASOs) and small interfering RNAs (siRNAs), are promising tools for targeting oncogenic pathways in cancer. However, their impact on tumor microenvironment (TME) architecture and cellular phenotypes within cancer tissues remains insufficiently understood. This study integrates oligonucleotide-based treatments with advanced spatial phenotyping to explore their effects on cancer tissues at a high-definition, single-cell scale.
Methods: Cancer tissues treated with oligonucleotide therapeutics were analyzed using multiplexed immunohistochemistry (mIHC) and spatial transcriptomics. Cellular phenotypes, including immune and stromal cell populations, were characterized in situ to assess changes in the TME. High-resolution imaging and transcriptomic profiling were used to localize therapeutic effects, while computational analysis integrated phenotypic and molecular data to uncover spatially resolved mechanisms.
Results: Oligonucleotide treatment led to distinct spatial alterations in the TME, including reprogramming of tumor associated macrophages and increased infiltration of cytotoxic T cells in peritumoral regions. Spatial transcriptomics revealed suppression of target oncogenes, alongside upregulation of immune activation pathways in responsive regions of the tissue. Notably, these effects were localized to specific niches, suggesting cell-type-specific and spatially restricted therapeutic responses.
Conclusion: This study highlights the power of integrating oligonucleotide therapeutics with spatial phenotyping to uncover their impact on the TME in cancer tissues. The findings provide valuable insights into the spatial dynamics of oligonucleotide therapy, paving the way for more effective design and deployment of these treatments in oncology.

#3101 Multitargeting of SHMT2 and purine biosynthesis is an effective antitumor treatment under hypoxia.

Mathew Joseph Schneider[1], Carrie OConnor[1], Xun Bao[1], Md. Junayed Nayeen[2], Zhanjun Hou[1], Jing Li[1], Aleem Gangjee[2], Larry Matherly[1]

[1]Oncology, Wayne State University School of Medicine, Detroit, MI,[2]Division of Medicinal Chemistry, Duquesne University, Pittsburgh, PA

These studies investigate the antitumor effects of targeting serine hydroxymethyltransferase (SHMT) 2 under hypoxia, the downstream metabolic consequences, and how to exploit unique metabolic vulnerabilities for cancer therapy. Mitochondrial one-carbon (C1) flux through SHMT2 and 5,10-methylene tetrahydrofolate (THF) dehydrogenase 2 (MTHFD2) is the major source of C1 units in cancer cells. In mitochondria, THF serves as a cofactor for the catabolism of serine by SHMT2 to produce glycine and 5,10-methylene-THF which is subsequently metabolized by MTHFD2 and MTHFD1L. The major products of this mitochondrial C1 flux include formate and NADH, along with glycine for protein, purine and glutathione (GSH) synthesis. Thus, mitochondrial C1 metabolism is important for maintaining redox homeostasis and scavenging reactive oxygen species (ROS). Under hypoxic conditions, the flux through SHMT2 and MTHFD2 is the major source of NADH; therefore, inhibition of SHMT2 under hypoxia may increase oxidative stress resulting in cell death. However, inhibition of SHMT2 under hypoxia may facilitate cell growth as a result of aspartate accumulation due to depletion of NADH and suppression of the TCA cycle. Aspartate accumulation preserves de novo purine biosynthesis under these conditions via donation of an amine group to IMP. We discovered novel 5-substituted pyrrolo[3,2-*d*]pyrimidine multitargeted antifolates (e.g., AGF347) which inhibit SHMT2 in the mitochondria in addition to cytosolic targets (SHMT1 and folate-dependent enzymes in purine biosynthesis). We hypothesized that these agents may overcome inherent challenges of targeting SHMT2 under hypoxia, reflecting their inhibition of purine biosynthetic targets to preserve antitumor activity. Further, since all current SHMT2 inhibitors also inhibit SHMT1, to uncover the respective impacts of inhibiting these homologous enzymes, we utilized tetracycline-inducible SHMT1 or SHMT2 HCT116 tumor cell models to simulate selective inhibition of these targets by removal of doxycycline. We demonstrate that the pyrrolo[3,2-*d*]pyrimidine compounds retain potent antitumor efficacy under hypoxia, as reflected in inhibition of cell proliferation and colony formation. We assessed the metabolic consequences of antifolate treatment under hypoxia and normoxia by measuring malate, aspartate, and purine levels by LC-MS/MS in addition to effects on ROS and GSH levels. We also demonstrated that loss of SHMT2 activity alone is sufficient for anticancer effects under both normoxia and hypoxia, whereas loss of SHMT1 alone had no effect. These studies demonstrate the considerable promise of multitargeted pyrrolo[3,2-*d*]pyrimidine antifolates for cancer therapy under conditions under which standard-of-care agents may be less effective.

#3102 Spliceosome perturbation enhances immunotherapy in SCLC by triggering ZBP1-driven necroptosis in cancer-associated fibroblasts.

Xinpei Jiang[1], Xueying Ma[2], Yunyun Zhou[3], Siddharth Balachandran[4], Israel Canadas[2]

[1]Center for Immunology, Cancer Epigenetics Institute, Nuclear Dynamics and Cancer Program, Lewis Katz School of Medicine at Temple University, Fox Chase Cancer Center, Philadelphia, PA,[2]Center for Immunology, Cancer Epigenetics Institute, Nuclear Dynamics and Cancer Program, Fox Chase Cancer Center, Philadelphia, PA,[3]Fox Chase Cancer Center, Philadelphia, PA,[4]Center for Immunology, Fox Chase Cancer Center, Philadelphia, PA

Small cell lung cancer (SCLC) is the most lethal form of lung cancer with a 5-year overall survival of 5%. Despite advancements in immunotherapy, SCLC is characterized by an immunologically ‘cold’ tumor microenvironment (TME) and the majority of SCLC patients remain unresponsive to these treatment strategies. This combination of poor survival outcomes and limited therapeutic options underscores the critical urgency of identifying innovative strategies to treat SCLC. A potential approach to activate an innate immune response within tumors is by promoting the accumulation of endogenous "virus-like" nucleic acids in cancer cells, thereby initiating an antiviral response, concept known as ‘viral mimicry’. Here, we demonstrated that targeting the spliceosome component SF3B1 is an effective strategy to induce viral mimicry not only in cancer cells but also in cells from the TME, including cancer associated fibroblasts (CAFs). SF3B1 inhibition induced the formation of Z-form RNAs (Z-RNAs), which are unique, left-handed dsRNA species and necroptosis-activating ligands for Z-DNA binding protein 1 (ZBP1). Notably, we found that SF3B1 inhibition triggered ZBP1-dependent necroptosis, a highly immunogenic cell-death, through the RIPK3- ZBP1-MLKL axis in necroptosis-competent cancer cells and patient-derived CAFs. In vivo, SF3B1 inhibition promoted a decreased tumorigenicity and increased immunogenicity, as well as ZBP1-driven necroptosis in CAFs, enhancing response to immune checkpoint blockade (ICB). These findings have significant implications for inducing viral mimicry in immunologically "cold" tumors. Since SF3B1 inhibition can trigger necroptosis by targeting any necroptosis-competent cell, including CAFs within the TME, our results suggest that this approach could create a more immunogenic TME in all SCLC patients, regardless of whether their tumor cells have impaired necroptosis signaling. Thus, SF3B1 inhibitors and potentially other spliceosome-targeted therapies represent a promising strategy with exceptional translational potential for inducing necroptosis and enhancing ICB responsiveness in aggressive “cold” tumors, such as SCLC.

#3103 Perspectives of novel tissue engineering technologies for next generation cancer therapeutics.

Preeya D. Katti

American University of the Caribbean, Cupecoy, Sint Maartin

Cancer is the second leading cause of death in the United States, behind cardiovascular disease. For the first time, new cancer cases in the US will exceed 2 million in 2024 while 611,720 cancer deaths are projected for 2024. Further, the global economic burden of cancer is estimated to reach $25.2 trillion by 2050. Cancer metastases are responsible for 90% of the deaths due to cancer. While the discovery of new biomarkers for early detection, also enhanced by recent efforts in machine learning for image diagnostics, of various cancers, such as breast cancer, has improved mortality rates; therapeutics in the event of metastasis are limited. Further, *in vivo,* experiments for metastasis are primarily limited by the need for intracardiac injections to initiate metastasis and the use of immunocompromised animals. Tissue engineering, the holy grail of regenerative medicine, presents an innovative technology in the cancer metastasis arena. Besides providing a novel means for reconstruction surgeries, tissue engineering technologies enable the development of robust and reliable *in vitro* models of metastasis that present new tools to evaluate cancer pathophysiology to assess specific cancer phenomena such as adhesion to metastatic site and migration of cells and cell clusters. We demonstrate here current emerging technologies that are expected to greatly impact the mortality of patients with metastasis. These include two specific developments in the tissue-engineered *in vitro* models. These include the development of an accurate tumor microenvironment and the development of fluid-flow-enabled systems that exert physiologically relevant shear stresses on the cancer cells and tumors. While 5,541 articles (as reported by the National Library of Medicine) illustrate various in vitro models of cancer metastases developed using tissue engineering, only 58 report the concurrent use of flow characteristics in conjunction with the development of accurate tumor microenvironment, and 45 of those just in the past decade. The role of fluid flow-derived shear stresses during extravasation and tissue invasion are key factors that initiate and propagate the metastasis cascade. We present a state-of-the-art analysis of current tissue engineering technologies that are at the forefront in the development of tumor models of breast, prostate, colon, and pancreatic cancer metastases as a tool for screening therapeutic agents. Micro-engineered tumor environments enabled using microfluidics cancer-on-a-chip also offer advantages of a concurrent tumor microenvironment mimicry in combination with fluid-based cancer transport, which resulted in 213 publications with 185 in the last decade. The concurrent gene and protein analysis enabled via fluid flow-based bioreactors with tissue-engineered scaffolds makes this a promising technology, and it is expected to test new therapies that will help reduce the large cancer burden.

#3105 Unveiling the potential of humanized bispecific antibody: a novel approach to tumor suppression in oncology.

Anna Jablonska, Damian Kolakowski, Tomasz Kornatowski, Aleksandra Sowinska, Sylwia Wieczorek, Krzysztof Lacek, Filip Mitula, Bartosz Wiernicki, Olga Abramczyk, Maciej Wieczorek, Delfina Popiel

Celon Pharma SA, Kazun Nowy, Poland

Background: AXL is a critical regulator in tumor progression, contributing to proliferation, invasiveness, epithelial-mesenchymal transition (EMT), angiogenesis, and immune modulation. High AXL levels are associated with poor prognosis in aggressive cancers. Concurrently, PD-L1 can suppress the antitumor immunity by binding to PD-1 on immune cells, a pathway already targeted by several immunotherapies. However, recent studies indicated that PD-L1's role extends beyond PD-1 binding. It seems to be involved in regulation of tumor growth, stemness, immune modulation, and DNA damage responses, many independently of PD-1. Our humanized bispecific antibody (BsAb), CPL976H, offers a unique dual-target approach by inducing internalization and lysosomal degradation of both PD-L1 and AXL, effectively diminishing their cell surface expression.
Methods: The BsAb CPL976H was engineered in a VHH-Fc-VHH format, incorporating dual VHH domains against PD-L1 and AXL. The antibody's activity, specificity, internalization, and target degradation were assessed using Western blot and flow cytometry across multiple cancer cell lines with varying target expression profiles (e.g., MDA-MB231, MCF7, A549, H1299), and after target induction with INFγ or its silencing with siRNA. RT-PCR and Western blot analyses were performed to assess impact on EMT and immune-modulating pathways.
Results: CPL976H demonstrated specific, high-affinity binding to PD-L1 and AXL on diverse cancer cell lines, inducing rapid internalization and lysosomal degradation of these targets. What is important, we were able to achieved efficient targets degradation in sub-nanomolar concertation's. This degradation led to downstream modulation of critical pathways, particularly those governing EMT and immune evasion. Notably, CPL976H impacted PD-L1 and AXL signaling disrupting cellular processes essential for tumor survival, regardless of PD-1 interaction, indicating its potential efficacy in PD-L1/PD-1 blockade-resistant cancers.
Conclusions: CPL976H represents a groundbreaking advancement in oncology by targeting PD-L1 and AXL through a novel mechanism that extends beyond traditional PD-L1/PD-1 inhibition. This bispecific antibody not only mitigates immune escape but also disrupts crucial survival pathways within the tumor, offering a potent therapeutic option for patients with resistance to conventional PD-L1/PD-1 therapies. Its pronounced internalization capabilities make CPL976H a promising candidate for antibody-drug conjugate (ADC) development, which could further amplify its antitumor effects. Notably, in vivo studies confirmed the high efficacy of CPL976H, both as a standalone antibody and as an ADC, demonstrating significant tumor-suppressive effects. This *in vivo* validation underscores CPL976H's potential to reshape the therapeutic landscape for challenging oncologic cases.

#3106 The first-in-class PRX3 inhibitor RSO-021 modulates the protein composition of malignant pleural effusions by increasing immunomodulating phenotypes and decreasing epithelial to mesenchymal protein expression.

Terri Messier[1], Bradley Cech[2], Joanna Dzialo[3], Charlotte Poile[3], Apostolos Nakas[4], Kudzayi Kutywayo[3], Aleksandra Bzura[3], Jan Rogel[3], Victoria Gibson[1], Ying Wai Lam[5], George Naumov[6], Dean Fennell[3], Christopher C. Landry[2], **Brian Cunniff**[1]

[1]Larner College of Medicine at The University of Vermont, Burlington, VT,[2]Department of Chemistry, The University of Vermont, Burlington, VT,[3]University of Leicester, Leicester, United Kingdom,[4]University Hospitals of Leicester, Leicester, United Kingdom,[5]Department of Biochemistry, The University of Vermont, Burlington, VT,[6]RS Oncology, Cambridge, MA

Malignant pleural effusion (MPE) is the buildup of fluid, cellular material, cytokines, and excreted proteins in the pleural space due to tumor burden. Approximately 90% of pleural mesothelioma (PM) patients and 20% of all advanced cancer patients will present with an MPE. The composition of MPE has been shown to be predicative of tumor burden and response to therapy. In the ongoing MITOPE phase 1/2 clinical trial, RSO-021 is being tested for efficacy in patients with MPE arising from mesothelioma or other metastatic tumors to the lung (NCT05278975). Results from the Phase 1 trial of RSO-021 showed promise that this approach may extend survival of PM patients (J Clin Oncol. Vol 42, 16). RSO-021 is delivered via an intrapleural catheter, limiting systemic exposure. RSO-021 is an inhibitor of the mitochondrial antioxidant enzyme peroxiredoxin 3 (PRX3) which protects tumor cells from oxidative stress induced apoptosis. Covalent inhibition of PRX3 with RSO-021 has shown cytotoxic activity in cell, mouse models, and human clinical trials, giving promise to a new therapy for aggressive cancers. To evaluate patient responses to RSO-021, we conducted mass spectrometry (MS) of MPE from patients pre and post RSO-021 treatment and identified a composition of proteins with differential abundance related to RSO-021 treatment. These data correlated with cytokine ELISA assays conducted with the same fluid. In addition, RNA sequencing of tumor explants identified common immunologic responses, stress response pathways and downregulation of pro-metastatic markers. Using Metascape Gene Annotation software we determined enrichment pathways for differential levels of abundant peptides within the PE fluid collected pre and post treatment. Pathways increased by RSO-021 include neutrophil degranulation, cell killing, hydrogen peroxide metabolic processes, aerobic glycolysis, and apoptosis. These findings support our current data for the proposed MOA of RSO-021. Increased levels of the cytokine resistin were identified in treated patient MPE by proteomics and ELISA assays. Resistin has been shown to activate tumor associated macrophages to increase anti-tumor activity in mesothelioma. Lastly, across cell lines, tumor explants and humans treated with RSO-021 we find significant downregulation of proteins related to the epithelial to mesenchymal transition (EMT) including TGFBI and periostin. Studies are underway correlating patient responses to MPE composition to identify biomarkers of sensitivity and resistance to RSO-021. Together, these data show the positive effects of RSO-021 on anti-tumor protein signatures related to tumor immunophenotypes and EMT and support the continued investigation of RSO-021 in MPE associated solid tumors.

#3107 Nuciferine inhibits proliferation, invasion, and migration of colorectal cancer cells via the RBM15-JAG2 axis.

KaiHe Zhu[1], JiaPeng RU[1], Yichen Zhang[2], Fangyue Guo[3], Mengying Liu[1], Shize Xiao[1], Xufeng Wang[4], Jing Wu[3], Peizhi Ma[3], Pei Li[1], **Shanfeng Zhang**[1]

[1]Zhengzhou University of Basic Medical College, Zheng Zhou City, China,[2]Henan Institute of Medical Sciences, Zheng Zhou City, China,[3]Department of the First Clinical Medicine, Zhengzhou University, Zheng Zhou City, China,[4]Practice Base of Basic Medical Innovation and Entrepreneurship Education in Henan Province, Zheng Zhou City, China

Background: Colon cancer is the third most common cancer globally, with high incidence and mortality rates. Dietary supplementation therapy, due to its safety, painlessness, high patient acceptance, and practical efficiency, often becomes an adjunctive treatment for colon cancer. Therefore, developing new health foods and exploring new treatment mechanisms are of great significance for the adjuvant treatment of colon cancer. Methods: This study investigated the effects of nuciferine treatment and RBM15 inhibition on the m6A modification of JAG2 using MeRIP-qPCR and actinomycin D experiments. Additionally, the impact on the biological behavior of colorectal cancer cells was assessed through plate colony formation assays, scratch assays, Transwell assays, and Western blot analyses.
Results: In this study, through transcriptome sequencing, RBM15 was discovered as a potential target of Nuciferine. The RBM15/JAG2 regulatory axis was found through bioinformatics analysis. Through total m6A detection, MeRip-qPCR, and actinomycin D experiments, it was confirmed that Nuciferine inhibits colon cancer by suppressing the m6A modification of JAG2 by methyltransferase RBM15. Additionally, plate colony experiments, scratch experiments, Transwell experiments, and Western blot experiments demonstrated that Nuciferine can inhibit the proliferation, invasion, and metastasis of colon cancer. In vivo experiments showed that Nuciferine has the effect of inhibiting the proliferation and metastasis of colon cancer.
Conclusion: The results of this study indicate that nuciferine inhibits the proliferation, invasion, and migration of colorectal cancer cells by regulating RBM15 expression to mediate JAG2 m6A methylation. Therefore, Nuciferine is an ideal health food for adjuvant treatment of colon cancer.

#3108 Imipridones ONC201, ONC206, and ONC212 promote immune-mediated cell death and anti-tumor activity in biliary tract cancer models *in vitro*.

Archana Saxena[1], Alexis J. Lannigan[1], Grace Sun[1], Jasper Chan[1], Jane Trainor[1], Lanlan Zhou[1], Varun V. Prabhu[2], Wafik S. El-Deiry[1], Alexander G. Raufi[1]

[1]Legorreta Cancer Center at Brown University, Providence, RI,[2]Chimerix Inc., Durham, NC

Background: Biliary tract cancers (BTCs) are an aggressive group of malignancies with 5-year survival rates of less than 2% in those diagnosed with metastatic disease. Chemoimmunotherapeutic approaches have limited efficacy highlighting an urgent need for novel approaches. Imipridones (Dordaviprone/ONC201, ONC206, and ONC212) have demonstrated promising antitumor activity in multiple preclinical cancer subtype models but remain underexplored in BTCs. ClpP agonist and DRD2/3 antagonist ONC201 exerts antitumor effects by upregulating TRAIL, activating the Integrated Stress Response (ISR), inhibiting AKT/ERK signaling, disrupting oxidative phosphorylation, and altering cancer cell metabolism. Second-generation imipridones (ONC206, ONC212) show enhanced potency relative to ONC201. To further investigate their therapeutic potential, we employed co-culture systems with natural killer (NK) cells to evaluate the immunomodulatory effects of these compounds. Additionally, the roles of ferroptosis, necroptosis, and autophagy modulation in sensitizing BTC cells to apoptosis and immune-mediated destruction were explored.

Methods: Two BTC cell lines, RBE and HuCCT1, were tested for drug sensitivity and synergy using the CellTiter-Glo® luminescent cell viability assay. for imipridones (ONC201, ONC206, ONC212) and trametinib. Co-culture experiments involved RBE cells (blue CMAC dye) and NK-92MI cells (green CMFDA dye), treated with ONC201 (2.53 μM), ONC206 (917 nM), ONC212 (46.99 nM), and trametinib (5.94 μM). Live/dead fluorescent imaging was conducted at 0, 24, 48, and 72 hours using DAPI, FITC, and cherry red channels on the ImageXpress® system. Cell death pathways were analyzed in RBE and HuCCT1 cells treated with ONC201, ONC206, or ONC212 (100 μM) alone or combined with inhibitors (Ferrostatin-1, Z-VAD-FMK, Bafilomycin A1, Necrostatin-1s).Cell viability was measured after 72 hours with the CellTiter-Glo® Assay.

Results: Imipridones and MEK inhibitors exhibit potent antineoplastic effects in BTC cell lines. In RBE cells, half maximal inhibitory concentrations (IC50) were 2.53 μM (ONC201), 917 nM (ONC206), 46.99 nM (ONC212), and 5.94 μM (trametinib), while in HuCCT1 cells, they were 1.97 μM (ONC201), 1.06 μM (ONC206), 38.95 nM (ONC212), and 3.71 μM (trametinib). Imipridones (ONC201 and ONC212) with trametinib showed synergy in HuCCT1 cells. ONC201 primarily results in cell death through apoptosis, and further analysis of alternative cell death pathways including ferroptosis, necroptosis, and autophagy is underway. RNA-seq studies are also ongoing. Imipridones enhanced NKcell-mediated tumor death. These findings underscore the therapeutic potential of imipridones in BTC, driven by immune-mediated signaling and offering promising prospects for future treatment strategies.

#3109 Pre-clinical efficacy of tumor treating fields and imipridones in glioblastoma and colorectal cancer cell lines.

Viva Voong, Tyler Roady, Connor Purcell, Vida Tajiknia, Lanlan Zhou, Shengliang Zhang, Wafik El-Deiry

Legorreta Cancer Center at Brown University, Providence, RI

Tumor treating fields (TTFields) utilize alternating electric fields of intermediate frequency and low intensity to disrupt cell division, demonstrating strong promise for tumor treatment. Currently, TTFields are approved for glioblastoma, unresectable mesothelioma and metastatic non-small cell lung cancer following progression on or after platinum based chemotherapy. This project aims to explore the synergistic effects and molecular mechanism of TTFields and imipridones in vitro using colorectal and glioblastoma cell lines. Imipridones are a class of anti-cancer drugs that target ClpP and GPCRs and lead to p53 independent apoptosis through chronic activation of the integrated stress response pathway. Both imipridones and TTFields have been shown to inhibit AKT and ERK phosphorylation. Thus, we theorized that co-treatment will have a synergistic effect.

We performed ATP-based (Cell Titer Glo) viability assays to determine the IC50 dosage for imipridones ONC201, ONC206 and ONC212 in HT29 and HCT116 colorectal cancer (CRC) cell lines and U251 and T98G glioblastoma (GBM) cell lines. We then treated cells with imipridones at the identified IC50 doses either alone or together with 200kHz TTFields and collected cell lysate after 72 hours.

We found differences in AKT phosphorylation across our treatment conditions (control, ONC201, ONC206, ONC212, TTF, TTF + ONC201, TTF + ONC206, and TTF + ONC212). These findings are significant in guiding future in vivo experiments and offering insight on whether TTField and imipridone treatment can be further explored in clinical trials.

#3110 A clinically actionable lipid metabolic approach in potentiating the response to anti-androgen therapy in a castration resistant prostate cancer model.

Cynthia Sprenger[1], Silvia Rodrigues[2], Ali Talebi[3], Kathryn Soriano Epilepsia[1], Shihua Sun[1], Caroline Fidalgo Ribeiro[4], Fabio Socciarelli[2], Jonas Dehairs[3], Lisa Butler[5], Johannes V. Swinnen[3], Massimo Loda[2], Stephen Plymate[1]

[1]University of Washington, Seattle, WA,[2]Weill Cornell Medicine, New York, NY,[3]KU Leuven, Leuven, Belgium,[4]Bristol Myers Squibb, San Diego, CA,[5]University of Adelaide, Adelaide, Australia

Purpose: Despite better management of prostate cancer (PCa) and good response rates to androgen deprivation therapies (ADT), patients that progress towards metastatic disease remain refractory to therapy with poor prognosis. There is a significant unmet need in sensitizing castrate-resistant PCa (CRPC) to agents such as enzalutamide (ENZ). A significant body of literature has shown saturated fatty acids (SFA) and mono-unsaturated fatty acids (MUFA) protect cancer cells from the deleterious effects of reactive oxygen species (ROS), and conversely, membrane poly-unsaturated fatty acids (PUFA) promote it. Considering that PCa is driven by androgen signaling, and that AR is upstream of lipogenesis and therefore SFA and MUFA production, we aimed to develop a pharmacological approach to sensitize CRPC to treatment, using a combination of the fatty acid synthase (FASN) inhibitor TVB-3664, enzalutamide (ENZ), and the ROS inducer arsenic trioxide (ATO).

Experimental Design: We treated castration sensitive (CS) and CRPC cell lines with combinations of TVB, ENZ and ATO and recorded live and dead cell counts. We then tested these treatments in vivo on a therapy resistant patient derived xenograft, LuCaP35 CR. We used a phospholipidomics approach to evaluate the effect of these treatments individually and in combination on membrane lipid profiles of the LuCaP35CR tumors.

Results: CS cell lines were sensitive to single agent treatments with ENZ ($p<0.0001$), ATO ($p<0.0001$) and TVB ($p<0.01$ to $p<0.0001$); combination therapies also reduced cell proliferation significantly (all $p<0.0001$). CR lines were resistant to ENZ but responded to TVB ($p<0.0001$) and ATO treatments ($p<0.0001$); combined treatments also reduced the number of live cells (all $p<0.0001$). In the pre-clinical LuCaP35CR model, TVB exposure led to a sensitization to ENZ, which was augmented by further exposure to ATO. The triple combination kept tumors at the baseline volume throughout treatment ($p<0.0001$). Phospholipidomics demonstrated that TVB directed FASN inhibition, alone and in combination with ENZ and ATO, led to a significant decrease in membrane MUFA ($p<0.0001$) and a significant increase in membrane PUFA ($p<0.0001$). In addition, there was a significant decrease in AR expression in the ENZ+TVB treated tumors compared to control ($p<0.0001$), ENZ alone ($p<0.001$), or TVB alone ($p<0.0001$).

Conclusions: We adopted a therapeutic strategy in rendering CRPC ROS sensitive. Specifically, we used the FASN inhibitor TVB-3664 to elevate membrane PUFA, together with ENZ and the ROS elevating drug ATO. Critically, this clinically actionable combination therapy dramatically decreased the growth of pre-clinical cell lines and PDX models. Our results demonstrate the applicability and potential of a ROS sensitizing strategy in the treatment of CRPC.

#3111 PI3K-dependent GAB1/Erk phosphorylation renders head and neck squamous cell carcinoma sensitive to PI3Kα inhibitors.

Xu Zhang[1], Jiao Xu[1], Xuan Wang[1], Lan Xu[1], Xi Zhang[1], Yi Wang[1], Shujuan Jiang[2], Yixiang Zhang[2], Jian Ding[1], Chen Qing[3], Linghua Meng[1]

[1]Shanghai Institute of Materia Medica, Shanghai, China,[2]Haihe Biopharma, Shanghai, China,[3]Kunming Medical University, Yunnan, China

Head and neck cancer is the seventh most common cancer in the world, of which about 90% are head and neck squamous cell carcinoma (HNSCC). Frequent alterations of *PIK3CA* in HNSCC suggest that PI3Kα is a promising target for HNSCC treatment. CYH33 is a novel PI3Kα-selective inhibitor discovered by our group, which is currently undergoing a phase I clinical trial (NCT03544905) for the treatment of advanced solid tumors, including HNSCC. However, there is an urgent need to elucidate its mechanism of action and improve its efficacy against HNSCC. In this study, we found CYH33 displayed promising but variable therapeutic activity against HNSCC. Inhibition of PI3K/Akt pathway by CYH33 was not sufficient for its activity against HNSCC. Tandem-Mass-Tag (TMT) proteomics and phosphoproteomics were performed to reveal comprehensive regulation of kinome by CYH33. Among them, phosphorylation of MAPK1/Erk at activation sites (T185/Y187) were significantly attenuated by CYH33 in sensitive HNSCC cells. CYH33 significantly suppressed phosphorylation of Erk in sensitive but not resistant cells. Further inhibition of Erk phosphorylation enhanced the activity of CYH33 against HNSCC. Signaling network of differentially phosphorylated proteins illustrated that phosphorylation at multiple sites (Y659, Y406, S651, S648) of the adaptor protein GAB1, which integrates the signaling from PI3K and receptor tyrosine kinases, were significantly downregulated upon CYH33 treatment. CYH33 dose-dependently suppressed the phosphorylation of GAB1 at Y659 in sensitive HNSCC cells, whereas it had little effect in resistant cells. We found that CYH33 attenuated the membrane localization and phosphorylation of GAB1, resulting in reduced Erk phosphorylation and ultimately inhibiting the proliferation of sensitive cells. Meanwhile, activation of EGFR induced GAB1 phosphorylation independent of PI3K in HNSCC. Concurrent inhibition of EGFR synergistically potentiated the activity of CYH33 against HNSCC.In conclusion, we found that PI3Kα-selective inhibitor CYH33 displayed potent activity against HNSCC, which was associated with inhibition of Akt signaling as well as attenuation of PI3K-dependent GAB1/Erk phosphorylation. Simultaneous inhibition of GAB1 phosphorylation independent of PI3K potentiated the anti-HNSCC activity of CYH33. These findings revealed the insight mechanism of CYH33 against HNSCC and provided rational combination regimen for HNSCC.

#3112 Pralsetinib induces opportunistic infection in RET fusion-positive NSCLC patients via inhibition of IL-2 production by blocking Jak3/Stat5 activation.

Shinkyo Yoon[1], **Hyun-Min Ryu**[1], Eunjin Lee[1], Yunkyung Sung[1], Ho-Su Lee[2], Ji Eun Park[3], Chang Hoon Lee[4], Seyoung Seo[1], Sang-We Kim[1], Kang-Seo Park[1], Dae Ho Lee[1]

[1]Oncology, Asan Medical Center (AMC), Seoul, Korea, Republic of,[2]Biochemistry and Molecular Biology, University of Ulsan College of Medicine, Seoul, Korea, Republic of,[3]Radiology and Research Institute of Radiology, Asan Medical Center (AMC), Seoul, Korea, Republic of,[4]Oncology, SCBio, Daejeon, Korea, Republic of

Background: Oncogenic alteration in RET, representing 1-2% of NSCLC, is an important target. Pralsetinib is a selective RET inhibitor targeting RET fusions. We present a case series on opportunistic infections and the mechanism of immunosuppression.
Methods: From October 2021 to March 2022, we administered pralsetinib to a total of 15 patients with NSCLC harboring RET fusion. We retrospectively analyzed the clinical efficacy and adverse events related to pralsetinib. To investigate pralsetinib's impact on T-cells, we examined cytokine release using ELISA from Jurkat T (JT) cells after pralsetinib treatment. To test if Stat5 binds to the IL-2 promoter, we conducted Chromatin Immunoprecipitation (ChIP).
Results: Out of 18 patients with measurable disease, 14 (93%) achieved a partial response. With a median follow-up duration of 8.7 months, four cases of opportunistic infections occurred. Notably, three patients (16.6%) experienced invasive pulmonary aspergillosis, and one patient (5.5%) experienced cytomegalovirus pneumonia. To investigate mechanisms behind these observations, we aimed to replicate conditions similar to those of activated T cells. Thus, JT cells were treated with PMA and ionomycin to induce IL-2 release through NFAT, AP-1, and NF-κB binding to the IL-2 promoter. After ~2 weeks of pralsetinib treatment, Jak3 signaling was inhibited, leading to decreased IL-2 release by downregulating transcription factors associated with IL-2 production, such as JunB/c-Jun and Stat5, which are downstream components of Jak3 signaling. Additionally, to investigate the direct correlation between Jak3 and IL-2 release, JT cells were treated with ritlecitinib, a Jak3-selective inhibitor, for ~2 weeks. Like pralsetinib, a reduction in IL-2 release was observed. Furthermore, based on the evidence that Stat5 inhibition leads to reduced IL-2 release, we showed that Stat5, a direct downstream component of Jak3, binds to the IL-2 promoter during activation of Jurkat T cells.
Conclusion: Pralsetinib, not Selpercatinib, inhibits additional IL-2 production by blocking Jak3/Stat5 activation triggered by IL-2 released during early T cell activation. Consequently, Jak3 inhibition by pralsetinib suppresses IL-2 release by inhibiting the activation of transcription factors for IL-2, such as JunB/c-Jun and Stat5, thereby inducing opportunistic infections, including invasive pulmonary aspergillosis (IPA), cytomegalovirus (CMV) pneumonia, CMV viremia, and pneumocystis pneumonia.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Therapeutic Approaches to Attack the Tumor Microenvironment Poster Session

#3118 IBI3026, a first-in-class anti-PD-1/IL-12 fusion protein, demonstrates the potential to be a new immuno-oncology therapy by releasing the break in immune response and strongly activating T and NK cells in the tumor microenvironment.

Xinran Liu, Fenggen Fu, Shuaixiang Zhou, Weiwei Wu, Haoyu Wen, Yang Liu, Min Wu, Meng Ni, Yan Liu, Enhong Zhong, Yayao Yan, Ying Zhang, Zhihai Wu, Yuting Wei, Kaijie He

Innovent Biologics (Suzhou) Co. Ltd, Jiangsu, China

Interleukin 12 (IL-12) is a cytokine that is able to activate both innate (NK cells) and adaptive (T cells) immunities, making it an ideal candidate for immuno-oncology therapy. Administration of IL-12 drugs locally has shown promising clinical results in treating tumors. However, when applying IL-12 systemically, severe dose-limiting toxicities are induced, greatly impeding the drug development process and its potential in cancer treatment. Herein, we designed IBI3026, an immune agonist that targets both IL-12 receptor and PD-1. By weakening the potency of IL-12, the safety profile of IBI3026 is extensively improved, guaranteeing the feasibility of systemic drug administration. By incorporating a high affinity, bivalent anti-PD-1 antibody, in addition to blocking PD-1/PD-L1 signaling pathway, IBI3026 could be efficiently enriched to PD-1+ T cells for maximal IL-12 activation in the tumor microenvironment. This “cis-activation” mode largely widened the therapeutic window of IL-12. *In vitro*, IBI3026 potently activates CD3/CD28-pretreated human PBMCs (hPBMCs) by showing strong STAT4 phosphorylation and high IFN-gamma secretion, while leaving untreated hPBMCs intact. In EMT6 (triple-negative breast cancer) and CT26 (colorectal cancer) cell line-derived xenograft (CDX) models, IBI3026 mouse surrogate strongly inhibited tumor growth with complete response in nearly all mice. In humanized A375 (melanoma) and BxPC-3 (pancreatic cancer) CDX models, IBI3026 showed much stronger tumor inhibitory effects than IBI308 (anti-PD-1 monoclonal antibody) and showed good safety profiles. In pilot toxicity studies, cynomolgus monkeys were administered 3 weekly doses of 10, 30, 80, and 150 mg/kg IBI3026 intravenously. No mortality or serious toxicities were observed in any group. The highest non-severely toxic dose (HNSTD) of IBI3026 is 150 mg/kg and the calculated therapeutic index is 63. These results demonstrate the good safety profile of IBI3026. In conclusion, IBI3026 is a first-in-class anti-PD-1/IL-12 immune agonist with high tumor inhibition potential and excellent safety profiles.

#3119 Next-generation multi-specific and conditionally activated CD3 Switch-DARPins with CD2 co-stimulation to tackle the current limitations of T cell engagers in solid tumors.

M. Bianchi[1], M. Franchini[1], T. Lekishvili[1], E. Tselempi[1], J. Robinson[1], Y. Kaufmann[1], G. Ems[1], C. Friang[1], C. Tissier[2], M. R. Muller[1], A. Link[1], C. Borg[2], A. Croset[1], V. Calabro[1], A. Goubier[1], **M. Guzman Ayala**[1];

[1]Molecular Partners AG, Schlieren, Switzerland, [2]UMR1098 INSERM, University of Bourgogne Franche-Comte, Besancon, France

Introduction: T-cell engagers (TCE) are powerful and clinically validated cancer therapeutics but are limited by high toxicity, lack of tumor-specific targets, and presence of exhausted/dysfunctional T cells particularly in solid tumors. Our CD3 Switch-DARPin platform provides a logic-gated “on/off” function (“Switch”) to increase tumor specificity and enable strong CD3 engagement and activation without compromising safety. Additionally, a DARPin with a co-stimulatory function can be added to enhance T-cell responses, especially in the context of an adverse tumor microenvironment. Using ovarian cancer (OC) as a proof-of-concept indication, we designed a multi-specific CD3 Switch-DARPin with CD2 co-engagement (CD2 x CD3 Switch-DARPin) which targets two tumor-associated antigens (TAAs); mesothelin and EpCAM. The logic-gated function of our Switch-DARPin ensures that the CD3 moiety is able to bind and activate T cells only when the TAAs are expressed on target cells. Herein we present a Switch-DARPin TCE that safely sustains anti-tumor efficacy, providing new TAA options and expanding TCE use in solid tumors.

Methods: RNAseq phenotypic analyses of OC patient samples were validated on resected tumors and ascites using flow cytometry and immunohistochemistry. The specific activity and safety of our CD2 x CD3 Switch-DARPin were assessed *in vitro* and *ex vivo* using reporter cells, primary human T cells from healthy donors and OC patient ascites, whole blood cytokine release assays, and *in vivo* in PBMC-humanized NXG mice engrafted subcutaneously with mesothelin- and EpCAM-expressing cells.

Results: Our analysis confirmed that mesothelin and EpCAM are highly co-expressed in OC tumors. Furthermore, reduced expression of CD28 compared to CD2 on tumor-infiltrating lymphocytes, combined with CD58 downregulation on primary tumors and ascites, support the use of CD2 as a co-stimulatory receptor for T cell engagement in OC. Functionally, the CD2 x CD3 Switch-DARPin abrogated TAA-independent T-cell activation and reduced potential on-target off-tumor toxicity, while CD2 co-engagement sustained T-cell response and prevented T-cell dysfunction. Notably, *in vivo,* the CD2 x CD3 Switch-DARPin induced significant tumor regression without causing systemic release of cytokines in the blood. In contrast, a control molecule without the Switch led to systemic cytokine release and showed no antitumor efficacy, likely due to fratricide-mediated depletion of T cells.

Conclusions: Our CD3 Switch-DARPin platform provides a novel approach for sustained tumor-specific T-cell engagement, by combining a logic-gated on/off Switch mechanism with CD2 co-stimulation. This allows immune activation to be directed to tumors, increasing efficacy and safety and paving the way for new opportunities for cancer treatment.

#3120 Evaluation of PYX-201, an EDB+FN-targeting ADC, in a comprehensive PDX mini-trial study enables identification of gene signatures associated with anti-tumor activity.

Amanda Facklam, Justin Trickett, Sara Lewandowski, Chuan Shen, Frank Wang, Marsha Crochiere, Nicolas Severe, Jan Pinkas

Pyxis Oncology, Boston, MA

PYX-201, a first-in-concept non-cellular targeting antibody-drug conjugate (ADC), is currently being tested in a Phase 1 clinical trial in a variety of solid tumors (NCT05720117). PYX-201 is designed to bind specifically to the extra-domain B splice variant of fibronectin (EDB+FN), a component of the extracellular matrix found in various solid tumors with absent or low expression in normal adult tissue (Hooper, 2022). Extracellular proteases can cleave the valine-citrulline peptide linker to release the optimized toxic payload Auristatin0101 into the tumor microenvironment which diffuses into tumor cells and induces cell killing. A comprehensive mini-trial study has been conducted to measure PYX-201 anti-tumor efficacy in multiple patient-derived xenograft (PDX) mouse models selected from ten solid tumor indications. Building on our previous analysis (Severe, 2024), full mini-trial results demonstrate that PYX-201 (3mg/kg, Q4Dx4) was well tolerated and resulted in broad anti-tumor activity as calculated by the Tumor Growth Inhibition percentage (%TGI) at study end for each individual PDX model. Our results show that 25% were non-responders (TGI<25%), 30% moderate (25% <TGI<70%), 20% strong (70%<TGI<90%) and 25% very strong responders (TGI>90%). Remarkably, PDX models with very strong PYX-201 responses (TGI>90%) were found across 9/10 solid tumor indications. Additionally, mice with complete responses to PYX-201 (tumor volume reached 0mm^3 for at least two consecutive measurements) were found across several tumor indications. To investigate gene expression changes relevant to PYX-201 mechanism of action, a satellite mouse was included on study for tumor collection for each PDX model prior to treatment. To determine which gene signatures may be associated with PYX-201 response, analysis was performed on RNA extracted from satellite tumor samples. The Nanostring ADC Development panel was selected for expression analysis across 750 human genes. A comparison between PDX models with very strong PYX-201 responses (TGI>90%) to PYX-201 non-responders (TGI<25%) with all tumor indications combined identified several genes which were differentially expressed. In addition, gene set analysis was performed to identify gene categories which were associated with PYX-201 response. The gene sets with the greatest number of differentially expressed genes in our analysis were tumor stroma and enzymes, indicating these categories are important for PYX-201 response in pre-clinical PDX models. Overall, PYX-201 showed broad anti-tumor activity in PDX pre-clinical models, suggesting it is a promising ADC for further testing across solid tumor indications. Furthermore, tumor stroma and protease genes were associated with PYX-201 activity in PDX models, providing further insight into PYX-201 mechanism of action.

#3121 DWP217, a novel Arginase inhibitor enhancing tumor immunity through tumor microenvironment reprogramming.

Seung Jae Jeong[1], Wolyoung Kim[1], Ji-Hae Kim[1], Sujin Ha[1], Youngwoo Choi[1], Jiyoon Seok[1], Ye Gi Han[1], Jong Cheol Shon[1], Hyoungrok Bak[1], Eunmi Hong[2], Jonghyeon Son[2], Kyung Seok Oh[1], Ji-Duck Kim[1], Joon Seok Park[1]

[1]Daewoong Pharmaceutical Company Ltd., Yongin-si, Korea, Republic of,[2]New Drug Development Center, Daegu-Gyeongbuk Medical Innovation Foundation, Daegu, Korea, Republic of

Checkpoint inhibitors have transformed cancer immunotherapy, yet their efficacy is often undermined by the immunosuppressive tumor microenvironment (TME). Arginase, a key enzyme secreted by myeloid-derived suppressor cells and tumor-associated macrophages, contributes to this suppression by depleting arginine, a critical nutrient for T-cell and NK cell activation. Elevated Arginase activity is associated with immune resistance and diminished response to immunotherapy, highlighting it as a promising target to reprogram the TME and restore antitumor immunity. Preclinical evaluation of DWP217 revealed its potent ability to inhibit Arginase activity, enabling T-cell proliferation and reversing immune cell dysfunction. Structural analyses uncovered a unique binding mechanism, supporting the rational design of an optimized compound series with enhanced potency. In combination with anti-PD-1 antibodies, DWP217 achieved significantly better tumor control and survival outcomes in syngeneic cancer models compared to anti-PD-1 monotherapy. Notably, DWP217 outperformed competing Arginase inhibitors that had advanced to clinical trials, underscoring its superior efficacy and potential as an immune-oncology therapeutic candidate. Immune profiling revealed that DWP217 enhances tumor-infiltrating lymphocyte activation and reprograms the TME toward a pro-inflammatory state. These findings indicate that DWP217 not only counteracts Arginase-mediated immune suppression but also reshapes the TME to support durable antitumor immunity. With its strong preclinical efficacy and favorable immune modulation profile, DWP217 is a promising therapeutic candidate for overcoming immuno-oncology resistance. Its further development holds the potential to significantly improve therapeutic outcomes in refractory solid tumors.

#3122 A novel solid tumor-tissue-penetrating platform technology: Overcoming tumor microenvironment barriers for an effective targeted delivery of therapeutic agents into solid tumors.

Serry Koh, Ji-young Lee, Young Pil Jung, Won-seok Choi, Min Gyu Soung

Cellicon Lab Inc., Daejeon, Korea, Republic of

The tumor microenvironment (TME)—comprising tumor vasculature, stromal cells, immune cells, and extracellular matrix (ECM)—presents significant challenges for effective anticancer therapy delivery. To address these challenges, we developed a novel solid tumor-tissue penetrating (STTP) platform featuring DST-516, a tumor-penetrating peptide designed to enhance tumor-specific delivery and deep penetration of therapeutic agents into solid tumors. Preclinical studies demonstrated that DST-516 outperformed the iRGD control in terms of tumor penetration. Confocal laser scanning microscopy (CLSM) revealed that co-administration of DST-516 with fluorescently labeled doxorubicin (10 mg/kg) and 70 kDa albumin (10 mg/kg) resulted in profound and extensive tissue penetration. Enhanced distribution was observed over a period of 1 to 24 hours in AsPC-1 xenograft mouse models. The efficacy of fluorescently labeled doxorubicin penetration was further validated in both 3D PANC-1 and 3D AsPC-1 spheroids. Co-administration of DST-516 with gemcitabine and paclitaxel resulted in a significantly greater reduction in pancreatic tumor volume compared to either drugs alone or in combination with iRGD, demonstrating enhanced therapeutic efficacy *in vivo*. The efficient delivery of 70 kDa albumin to pancreatic tumors via co-administration with DST-516 highlights its potential for transporting large molecules, laying a strong foundation for future applications in antibody-drug conjugates (ADCs) and other advanced cancer therapies through the STTP platform. Mechanistic studies demonstrated that DST-516 preferentially binds to neuropilin-1 (NRP-1) and integrins αvβ3/αvβ5 in AsPC-1 cells, with minimal binding in siNRP-1-transfected AsPC-1 cells, emphasizing DST-516's tumor specificity. This specificity suggests that DST-516 could minimize off-target toxicity while maximizing therapeutic efficacy. Comprehensive safety evaluations, including nine preclinical toxicology studies, confirmed DST-516’s tolerability, with no significant adverse effects observed in single- or repeated-dose intravenous toxicity or genetic toxicity assessments. These results support its translational feasibility and clinical development potential. In conclusion, our findings establish DST-516 and the STTP platform as a promising strategy to overcome TME-mediated resistance, enabling safe and efficient delivery of therapeutic agents to solid tumors. Future studies will expand its applications across various solid tumor types and further refine its mechanism of action. This platform represents a pivotal step toward advancing precision oncology and improving outcomes for patients with hard-to-treat cancers.

#3123 Fracturing the tumor-bone alliance in metastatic prostate cancer with Ra-223.

Pablo Sanchis[1], Agustina Sabater[2], Juan Bizzotto[3], Gaston Pascual[4], Nicolas Anselmino[5], Jiabin Dong[5], Jun Yang[5], Ganiraju Manyam[6], Bradley Broom[6], Zhongcheng Shi[7], Shixia Huang[8], Rocio Seniuk[4], Eleonora Dondossola[5], Teocharis Panaretakis[5], John Araujo[5], Peter Shepherd[5], Patricia Troncoso[9], Nora Navone[10], Christopher J. Logothetis[5], Daniel E. Frigo[5], Elba Vazquez[4], Paul Corn[5], Geraldine Gueron[4], Estefania Labanca[5]

[1]1-IQUIBICEN (UBA-CONICET), Argentina; 2-Universidad Argentina de la Empresa (UADE), Instituto de Tecnologia (INTEC), Buenos Aires, Argentina; 3-Department of Genitourinary Medical Oncology, The University of Texas MD Anderson Cancer Center, Caba, Argentina,[2]1-IQUIBICEN (UBA-CONICET), Argentina; 2-Universidad Argentina de la Empresa (UADE), Instituto de Tecnologia (INTEC), Buenos Aires, Argentina., Caba, Argentina,[3]1-IQUIBICEN (UBA-CONICET), Argentina; 2-Universidad Argentina de la Empresa (UADE), Instituto de Tecnologia (INTEC), Buenos Aires, Argentina, Caba, Argentina,[4]IQUIBICEN (UBA-CONICET), Argentina, Caba, Argentina,[5]Department of Genitourinary Medical Oncology and The David H. Koch Center for Applied Research of Genitourinary Cancers, The University of Texas MD Anderson Cancer Center, Houston, TX,[6]Advanced Technology Cores, Baylor College of Medicine, Houston, Texas, Houston, TX,[7]Department of Molecular and Cellular Biology, Huffington Department of Education, Innovation & Technology, Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, TX,[8]Advanced Technology Cores, Baylor College of Medicine, Houston, Texas; Department of Molecular and Cellular Biology, Huffington Department of Education, Innovation & Technology, Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, Texas, Houston, TX,[9]Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX,[10]Department of Genitourinary Medical Oncology and The David H. Koch Center for Applied Research of Genitourinary Cancers, The University of Texas MD Anderson Cancer Center, -Deceased, Houston, TX

Radium-223 dichloride (Ra-223) is a calcium-mimetic alpha-emitting radiopharmaceutical homing to bone, approved for the treatment of bone metastases in castration-resistant prostate cancer (CRPC), significantly prolonging overall survival in patients with advanced disease. This study integrates preclinical and clinical studies to investigate the effects of Ra-223 on tumor volume, bone structure, transcriptomics alterations, and to identify biomarkers of therapeutic response to Ra-223. Using patient-derived xenografts (MDA PCa PDX), which mimic the hallmark characteristics of clinical bone-forming metastases, mice with tumor-bearing and non-tumor-bearing bones were treated with Ra-223 or vehicle. Tumor burden was monitored via magnetic resonance imaging (MRI), bone parameters were assessed through microCT and bone histomorphometry, and transcriptomics changes were evaluated using RNA sequencing in both, tumor and stroma. Ra-223 treatment significantly reduced tumor volume and altered bone architecture, promoting bone remodeling and increasing bone density. Histomorphometric analysis revealed suppressed osteoclast activity in both tumor-bearing and non-tumor-bearing bones treated with Ra-223, with a more pronounced effect in non-tumor-bearing sites. Transcriptomic profiling identified differential expression of immune, metabolic, and cell-cycle pathways, with TP53 and MYC as central regulators. Additionally, we analyzed human serum samples from patients with CRPC bone metastases. We profiled novel candidate protein markers of Ra-223 response on serial serum samples collected at baseline (prior to initiation of Ra-223 treatment), on-treatment (OT), and at end of treatment (EoT) from 25 patients enrolled in a single-center, open-label clinical study (NCT02135484). Subjects received six intravenous doses of Ra-223 (50 kBq/kg) every four weeks, unless they experienced disease progression or toxicity, or until withdrawal. Our analysis highlighted IL18, SPARC, MET, TFF3 and TNFSF11 as survival-associated proteins significantly deregulated in patients receiving Ra-223 therapy. In particular, multivariable analyses showcased that elevated levels of TFF3 were independently linked to lower overall survival and possible resistance to Ra-223, suggesting its potential as a predictive biomarker of treatment efficacy. In summary, our findings suggest that Ra-223 has a more extensive impact on the tumor-bone microenvironment than previously understood. The increases in bone density and associated transcriptomics alterations pave the way for future investigations into combination therapies that enhance Ra-223's benefits while addressing potential risks. Considering the clinical importance of TFF3, further validation in larger patient cohorts is essential to refine treatment strategies to improve outcomes for patients with CRPC bone metastases.

#3126 Tumor microenvironment composition is shaped by tumor cell line-derived xenograft subtype and tumor burden in BRGSF-HIS mice.

Florent Creusat, Siham Hedir, Alexis Gonon, Amelie Marguier, Yacine Cherifi, Fabiane Sonego, Gaelle Martin, **Kader Thiam**

genOway, Lyon, France

Emergence of immunotherapies for treating cancer has been an important step towards a more efficient, selective and safe approach for patients. However, only a fraction of patients can respond to treatments currently available, requiring the identification of additional therapeutic targets. The relevance of preclinical models in the identification of new therapeutic targets and assessment of immunotherapies has vastly improved with mice bearing a human immune system. BRGSF (Balb/C $Rag2^{-/-}$, $IL2R\gamma^{-/-}$, SIRPαNOD and $Flt3^{-/-}$) is a highly immunodeficient mouse featuring reduced murine myeloid cells. BRGSF mice reconstituted with human cord blood $CD34^+$ cells (BRGSF-HIS) develop functional lymphoid and myeloid compartments. This engraftment is stable for over a year (Labarthe *et al.*, 2019) and mice do not develop GvHD. Human myeloid compartment can be transiently boosted with exogenous human Flt3L injections. In contrary to other models, which overexpress human cytokines to develop human myeloid cells, Flt3L-treated BRGSF-HIS mice do not show side effects. BRGSF-HIS mice are permissive to mouse and human cancer cell lines engraftment and represent a valuable preclinical model to study cancer development and evaluate novel therapeutics. Here, we show that composition of tumor microenvironment (TME) is tumor burden-dependent. Indeed, BRGSF-HIS mice engrafted with triple negative cancer cell line MDA-MB-231 have a diverse TME, enriched in myeloid cells. The major cell type present in tumors of approximately 200mm^3 is $CD206^+$/$CD163^+$ M2-like macrophages, which express high levels of PD-L1 and other immunomodulatory proteins. At this tumor volume, TME is also composed of a small fraction of T cells. However, the frequency of conventional $CD4^+$T cells increase from <5% to 15% of human infiltrate when tumors reach approximately 500 mm^3. Interestingly, NK cells are detected at very low levels in tumors of 200 mm^3, but increase to 10% in the TME of tumors of 500 mm^3. The infiltration of human immune cells in the TME is also tumor type-dependent, as in a colon-derived adenocarcinoma SW480 tumor model, conventional $CD4^+$ T cells are the major cell type present in the TME (40%) of tumors of 200 mm^3. Interestingly, these tumors show an infiltrate of γδ T cells as well, ranging from 6% to 8%of human immune cells in tumors of 200 mm^3 and 800 mm^3, respectively. BRGSF-HIS mice hence represent a valuable tool to investigate immune cell infiltration in the TME, enabling a translatable assessment of mechanism of action of immunotherapies.

#3127 An mRNA-encoded masked IL-12 improves systemic tolerability while maintaining anti-tumor efficacy in preclinical studies.

Kanchana Ravichandran[1], Erwan LeScolan[2], Na Cai[2], Bruce Howng[2], Robert Lau[2], Lisa Clark[2], Joshua Millet[2], Pankaj Kumar[3], Sushma Gurumurthy[1], Raghava Sriramaneni[2], Sean McAllister[2], Vangipuran Rangan[2], Olga Vasiljeva[2], Madan Paidhungat[2], Dylan Daniel[2], Marcia Belvin[2], Lin Guey[1]

[1]Moderna, Cambridge, MA,[2]CytomX Therapeutics, San Francisco, CA,[3]Demeetra, Lexington, KY

IL-12 is a potent pro-inflammatory cytokine that has shown promising anti-tumor activity through its ability to activate both innate and adaptive immune cells. However, systemically administered IL-12 has limited clinical utility due to striking inflammatory toxicity and short half-life leading to a narrow therapeutic margin. Alternative strategies to reduce systemic exposure or restrict IL-12 activity to the tumor microenvironment and limit activity in circulation and non-tumor tissues have been explored. Using CytomX proprietary PROBODY® Therapeutics masking technology and Moderna mRNA technology, we describe a novel product concept of an mRNA encoded masked IL-12 molecule that is designed to have reduced activity systemically. Masked IL-12 is activated by elevated protease activity associated with the tumor microenvironment (TME), leading to preferential IL-12 activity in the tumor microenvironment. mRNA-encoded masked IL-12 protein showed 200X lower cell-based potency relative to an unmasked IL-12 counterpart and regained full activity upon protease-mediated cleavage of the mask *in vitro*. In an *in vivo* MC-38 tumor-bearing mouse model, systemically administered LNP encapsulated mRNA encoding masked IL-12 showed significantly improved tolerability (>30X) compared to unmasked IL-12. Consistently, masked IL-12 mRNA led to significantly lower serum IFNγ levels relative to unmasked IL-12 mRNA dosed at 10X lower dose. Most importantly, masked IL-12 mRNA dosed systemically resulted in complete remission of tumors in all dose levels and demonstrated protease-dependent intra-tumoral activation and efficacy. Taken together, these data show a novel and attractive engineering approach to develop an mRNA-encoded IL-12 therapeutic that significantly improves tolerability while maintaining potent anti-tumor activity.

#3128 Nonclinical efficacy and safety evaluation of IL-12 mRNA delivered by a novel lipid nanoparticle.

Qing He[1], Chunyan Dong[1], Dejiang Tan[1], Huimin Sun[1], Yiyang Zheng[1], Zhuang Li[1], Linyu Zhang[1], Sihan Liu[1], Yu Zhang[1], Jijun Yuan[2], Peng Gao[2], Liang Du[2], Huayuan Yan[2], Bo Ying[2]

[1]State Key Laboratory of Drug Regulatory Sciences, National Institutes for Food and Drug Control, Beijing, China,[2]Suzhou Abogen Biosciences, Suzhou, China

Interleukin-12 (IL-12) is considered to be a promising cytokine for enhancing anti-tumor immune response. However, systemic recombinant IL-12 had significant toxicity and limited efficacy in early clinical trials. Recently, many strategies to express IL-12 in the tumor tissue are being pursued preclinically and in clinical trials. Here, we report on ABO2011 (LNP-IL12 mRNA), developed by Abogen Bioscience, an innovative mRNA therapeutic cancer vaccine with novel lipid nanoparticles encoding IL-12.The LNP-IL12 mRNA significantly activates a robust T-cell-mediated adaptive immune response and exhibits strong anti-tumor potential. In the anti-tumor efficacy evaluation of B16F10, MC38 and EMT6 tumor-bearing mice, LNP-IL12 mRNA successfully eradicated tumors with a single administration. In the preliminary evaluation of safety, LNP-IL12 mRNA treatment did not significantly change body weight, HE staining of major organs did not significantly change, and serum cytokine levels were extremely low, indicating a good safety. Flow cytometry detected that LNP-IL12 mRNA induced large infiltration of total lymphocytes in B16F10, MC38 and EMT6 tumor-bearing mice, especially the proportion of cytotoxic $CD8^+$ T cells and NK cells increased significantly, while the proportion of immunosuppressive Treg cells decreased. In addition, the levels of pro-inflammatory factors such as IL-1β, IL-6, IFN-γ and TNF-α in tumors induced by LNP-IL12 mRNA were significantly increased by CBA method, but IL-10 was not detected. It was shown that LNP-IL12 mRNA mediated immunotherapy can safely and effectively induce a strong immune response. In conclusion, we conducted relevant evaluation studies with LNP-mIL12 mRNA as an example, trying to establish non-clinical efficacy evaluation criteria for mRNA therapeutics.

#3129 Fibroblast activation protein directed CAR T cells engineered *in situ* using targeted lipid nanoparticles inhibit progression of pancreatic ductal adenocarcinoma.

K. Bajbouj[1], Z. Xiao[1], L. A. Hopper[1], L. Huang[1], T. E. Papp[1], J. Ramani[1], A. Bot[2], Y. Bao[2], M. Butcher[2], H. Aghajanian[2], C. H. June[1], D. Weissman[1], H. Parhiz[1], S. M. Albelda[1], E. Pure[1];

[1]University of Pennsylvania, Philadelphia, PA, [2]Capstan Therapeutics, San Diego, CA

The growth of solid tumors is greatly influenced by stromal components including cancer-associated fibroblasts (CAFs) and desmoplastic matrix through both immune-dependent and immune-independent mechanisms. Fibroblast activation protein (FAP) is a stromal cell target that is selectively expressed at high levels on the cell surface of CAFs. We previously established an *ex vivo* retroviral-transduced CAR T cell approach to target FAP^+ CAFs that resulted in depletion of FAP^+ stromal cells, disruption of desmoplastic matrix, enhanced immune infiltration, and inhibition of tumor growth. In this study, we generated FAP-CAR T cells *in situ* using a novel platform to deliver modified FAP-CAR mRNA encased in anti-CD5 conjugated targeted lipid nanoparticles (tLNPs) and assessed the efficacy of this approach in a preclinical model of pancreatic ductal adenocarcinoma utilizing a cell line derived from a spontaneous pancreatic tumor isolated from a genetically engineered KPC mouse. We found that a single intravenous dose of anti-CD5 tLNP loaded with 30 µg of FAP-CAR mRNA resulted in transient FAP-CAR expression on >45%, >69% and >35% of CD3 T cells in spleen, blood and tumor tissues, respectively. Although CAR expression was more transient, the percentage of $FAP\text{-}CAR^+$ T cells generated using this approach was significantly greater than the percentage of $FAP\text{-}CAR^+$ cells detected following administration of $1X10^7$ *ex vivo* retrovirally transduced FAP-CAR T cells. Notably, this *in situ* mRNA CAR T-cell engineering resulted in significantly increased inhibition of tumor growth, as compared to adoptive transfer of *ex vivo* retroviral-engineered T cells (74% vs 48%) respectively. Given that this approach circumvents the need for autologous T cell isolation, *ex vivo* viral transduction and expansion (thereby reducing the potential risk of insertional mutagenesis, while also eliminating the need for pre-conditioning by lymphodepletion), our data indicate a promising therapeutic delivery platform for treating desmoplastic solid tumors using a more accessible and cost-efficient technology.

#3130 Differential gene expression regulation by the RXR agonist V-125 in MMTV-Neu mammary tumors.

A. Chowdhury[1], L. A. Reich[2], C. J. Occhiuto[2], K. T. Liby[1];
[1]Indiana University School of Medicine, Indianapolis, IN, [2]Michigan State University, East Lansing, MI

Retinoid X receptors (RXRs) are nuclear receptors that play a pivotal role in regulating gene expression by forming homodimers and heterodimers with other nuclear receptors. When activated, RXRs influence various cellular processes, including cell differentiation, proliferation, and apoptosis. This regulatory activity makes RXR agonists promising candidates in cancer research. However, the specific gene expression changes induced by RXR agonists can vary depending on the compound, reflecting their unique interactions within cellular environments and signaling networks. In this study, RNA sequencing was performed to compare the molecular pathways activated by the novel compound V-125 and FDA-approved drug bexarotene in mammary tumors of a mouse model of HER2+ breast cancer (MMTV-Neu mice). By analyzing the transcriptomic profiles, we identified that each treatment differentially regulated cancer-relevant gene categories, including focal adhesion, regulation of actin cytoskeleton, and extracellular matrix-receptor interaction. The most significantly altered genes by V-125, including *Lrrc26, Mgarp, Aldh1a3*, and *Colca2* are positively correlated with improved survival in breast cancer patients. While V-125 and bexarotene act on many common pathways, they induce distinct gene expression profiles. V-125 primarily targets focal adhesion and extracellular matrix-receptor interaction pathways, whereas bexarotene affects the regulation of actin cytoskeleton and NF-kappa B signaling pathways. We confirmed the significant differential expression ($p<0.05$) of key genes (*Nrg1, Nfasc, Chi3l1, Slit2*) at both the mRNA and protein levels using quantitative PCR (qPCR) and immunohistochemistry (IHC). Beyond cancer cells, RXR modulation influences the tumor microenvironment, including immune cells. Our transcriptomic analysis revealed that RXR agonist treatment affects pathways associated with macrophage activation and phagocytosis. This suggests the role of RXR in the polarization of macrophages, an important immune population related to breast cancer. To investigate this further, we utilized bone marrow-derived macrophages (BMDMs) to evaluate the impact of V-125 and bexarotene treatment on macrophage polarization toward a tumor-suppressive phenotype. Upon treatment, the polarization status of macrophages was assessed through gene expression analysis by qPCR. We observed that BMDMs treated with 300nM of either RXR agonist significantly ($p <0.05$) increased mRNA expression of IRF7 and TLR9, associated with a pro-inflammatory, anti-tumor phenotype. This comprehensive approach allowed us to determine the relative effectiveness of V-125 and bexarotene in modulating gene expression and polarizing macrophages toward a tumor-suppressive phenotype, providing valuable insights into their therapeutic potential and the underlying molecular mechanisms.

#3131 In vitro analysis of collagen deposition in lung cancer-associated fibroblasts using clinically validated biomarkers.

Annika Hettich, Morten Karsdal, Nicholas Willumsen

Nordic Bioscience, Herlev, Denmark

Background: Lung cancer remains the leading cause of cancer-related mortality worldwide, affecting both men and women. This high mortality rate is largely due to the frequent ineffectiveness of interventions and the late-stage diagnosis of the disease, which typically limits available treatment options. Within the tumor stroma of lung cancers, cancer-associated fibroblasts (CAFs) play a crucial role in tumor progression and metastasis by depositing extracellular matrix (ECM) components, such as collagens. To further understand lung cancer biology and support drug discovery, novel tools that replicate the tumor microenvironment are essential. In this study, we developed a pseudo-3D in vitro model incorporating healthy normal lung fibroblasts (lNFs) or lung cancer-associated fibroblasts (lCAFs), combined with clinically validated collagen-based biomarkers, as a translational platform for anti-fibrotic drug screening.
Methods: Primary human CAFs from lung adenocarcinoma (lCAFs) and normal lung fibroblasts (lNFs) were cultured for 12 days in ficoll-based media supplemented with ascorbic acid under unstimulated conditions and with TGF-β stimulation. Additionally, cells were treated with the ALK5/TGF-β1 receptor kinase inhibitor (ALK5i) and Fresolimumab, a human monoclonal antibody targeting all TGF-β isoforms. Collagen type I (PRO-C1), III (PRO-C3), and VI (PRO-C6) levels were measured in the cell supernatant on days 3, 6, 9, and 12 using competitive enzyme-linked immunosorbent assay.
Results: TGF-β1 stimulation increased type I, III, and VI collagen deposition in both lCAFs and lNFs. Notably, lCAFs showed higher type I collagen formation than lNFs, even after treatment. While untreated lCAFs and lNFs exhibited no type III collagen formation, TGF-β1 treatment significantly induced PRO-C3, with higher levels in lNFs than in lCAFs. PRO-C6 levels were initially comparable in untreated lCAFs and lNFs, yet TGF-β1 stimulation led to a significant increase in type VI collagen in lCAFs compared to a smaller increase in lNFs. Treatment with ALK5i and Fresolimumab reduced levels of PRO-C1, PRO-C3, and PRO-C6 in both lCAFs and lNFs.
Conclusion: The pseudo-3D in vitro model using lCAFs and lNFs successfully replicates key aspects of the lung tumor microenvironment, particularly the differential collagen deposition patterns. The model demonstrated that lCAFs exhibit distinct collagen formation responses to TGF-β1, especially in type I and VI collagens, compared to lNFs. Additionally, treatment with ALK5i and Fresolimumab effectively reduced collagen formation, underscoring the model's potential for preclinical anti-fibrotic drug screening. These findings support the model's application in studying CAF-driven ECM remodeling and assessing therapeutic responses in lung cancer.

#3132 Innovative development of IMB-201, an anti-HLA-G antibody-drug conjugate (ADC), for enhanced tumor immunotherapy.

Hyeonju Kang, Inyoung Lee, Joseph Kim, Yoojin Kim, Sungmuk Kang, Yongjun Kang, Hongjai Lee, Chungmin Lee, Chihye Park, Gyongsik Ha

IMBiologics Corp., Suwon-si, Korea, Republic of

Human leukocyte antigen-G (HLA-G), a non-classical MHC class I molecule, is expressed only in the placenta, where it functions as an immune suppressor to protect the fetus from maternal immune recognition. Recent studies have demonstrated that HLA-G is enriched in various malignant cells, providing evidence that HLA-G is strongly associated with tumor immune escape. HLA-G, a tumor-specific antigen, mediates immune suppression in tumor-infiltrating immune cells, including T cells, natural killer (NK) cells, and macrophages, by engaging inhibitory receptors such as Ig-like transcript 2 (ILT2) and 4 (ILT4). Therefore, the development of an HLA-G-targeting ADC can be a novel therapeutic strategy not only to induce direct tumor cell killing through payload delivery upon binding to HLA-G, but also to take advantage of the fact that HLA-G is minimally expressed on normal cells and predominantly restricted to tumor cells. This selective expression pattern makes HLA-G a promising target compared to other cancer antigens, in which off-target effects on normal tissues are expected to be minimal. Moreover, by disrupting the interaction between HLA-G and inhibitory receptors such as ILT2, this approach enhances anti-tumor immunity, followed by boosting immune activation and improving therapeutic outcomes. We developed an antibody-drug conjugate (ADC) targeting HLA-G (IMB-201) to selectively deliver cytotoxic drugs to HLA-G-expressing tumor cells, thereby overcoming immune suppression and promoting tumor regression. We generated a monoclonal antibody specific to HLA-G and conjugated it with a potent cytotoxic drug via a stable linker. The IMB-201 demonstrated high binding affinity and selectivity for HLA-G-positive tumor cells. *In vitro* serum and preliminary stability assays confirmed that the stability of IMB-201 is comparable to that of other developed ADCs. *In vitro* studies revealed significant dose-dependent cytotoxicity when treated to HLA-G-expressing cell lines, with minimal off-target effects on HLA-G-negative cells. *In vivo* studies employing mouse models demonstrated significant tumor regression and prolonged survival. Collectively, the IMB-201 could be a promising therapeutic approach for the treatment of HLA-G-positive solid tumors, such as gastric and pancreatic cancers. As HLA-G is a tumor-specific antigen, it has the potential to widen the therapeutic index (TI) compared to other conventional therapies. This strategy selectively targets tumor cells, delivering cytotoxic agents and enhancing immune response, thereby overcoming immune evasion mechanisms.

#3133 PF-08046032: a CD25-directed vedotin ADC leveraging a well characterized safety and pharmacokinetic profile for enhanced regulatory T cell depletion in the tumor microenvironment.

Christopher Carosino, Sherif Abdelhamed, Priyanka Gupta, Xinqun Zhang, Bryan Grogan, Weiping Zeng, Lauren Bou, Devra Olson, Paromita Raha, Andrea R. Lim, Brian P. O'Connor, Ryan A. Heiser, Matthew R. Levengood

Pfizer, Inc., Bothell, WA

PF-08046032 is a novel vedotin antibody-drug conjugate (ADC) engineered to target and deplete immunosuppressive regulatory T cells (Tregs) within the tumor microenvironment (TME) through direct cytotoxicity. PF-08046032 directs delivery of the cytotoxic MMAE (monomethyl auristatin E) vedotin payload to cells expressing CD25, the alpha chain of the IL-2 receptor, which is highly expressed on intratumoral Tregs and lymphoma cells. The MMAE-based vedotin drug linker platform, utilized in four approved ADCs for hematologic and solid tumors, has a well-characterized mechanism of action (MoA), exposure, and target-independent toxicity profile. PF-08046032 leverages this platform, exerting anti-tumor activity through the multifaceted MMAE-driven MoA, including direct cytotoxicity, bystander killing, and immunogenic cell death. Additionally, PF-08046032 is differentiated from other vedotin ADCs and anti-CD25 depleting agents through affinity-tuned binding, which enables selective targeting of $CD25^{hi}$ intratumoral Tregs while sparing peripheral Tregs. Here, we present preclinical data demonstrating safety, pharmacokinetics, and anti-tumor activity of PF-08046032 which is highly consistent with the vedotin ADC platform, while additionally harnessing a Treg-directed anti-tumor approach. PF-08046032 selectively kills Tregs compared to CD8 T cells, with preferential activity on Tregs with the highest CD25 expression and shows dose-dependent antitumor activity in CD25-expressing lymphoma xenograft models. In human CD25-expressing mice bearing MC38 colorectal carcinoma tumors, PF-08046032 significantly suppressed intratumoral Treg levels with minimal activity on peripheral Tregs and no effect on intratumoral CD8+ T cells. A mouse surrogate of PF-08046032 demonstrated that targeted depletion of immunosuppressive tumoral Tregs resulted in robust antitumor activity as both a single agent and in combination with anti-PD1 checkpoint inhibitor blockade. Pharmacokinetic and toxicity studies in non-human primates demonstrated safety and PK profiles consistent with the vedotin platform despite targeting CD25. Leveraging the cumulative knowledge of the vedotin platform, PF-08046032 enables a novel additional mechanism of action for vedotin ADCs at potentially efficacious and safe doses. These data support further investigation of PF-08046032 in clinical trials for advanced malignancies.

#3134 Preclinical anti-glioblastoma pharmacology of HX301, a novel CSF-1R inhibitor.

T. Yang[1], H. Ke[1], J. Wang[2], S. Xiong[2], L. Zhang[1], F. Zhang[1], **H. Q. Li**[1];
[1]Hanx Bio, Hangzhou, China, [2]Crown Bioscience, Taicang, China

Background. GBM is the most malignant and frequently occurring glioma (>60% of all brain tumors in adults) with poor prognosis (12-18 months survival time after diagnosis and 5-year survival of ~5%) and an unmet medical need. Glioma-associated macrophages/microglia (GAMs) are the prominent and suppressive immune cells in the tumor microenvironment (TME), promoting the growth, migration, invasion and stemness of tumor cell . GAMs also secret VEGF to promote angiogenesis and TGF-β to suppress immunity. Notably, the numbers of surrounding GAM inversely correlate with the prognosis. CSF1R, together with its two ligands, CSF1 (M-CSF) and IL-34, governs the proliferation, polarization, differentiation and functions of the myeloid lineage cells, including macrophage/microglia in GBM. Blocking CSF1/CSF1R signaling inhibits microglia/macrophage proliferation and delayed tumor growth by depleting GAMs and diminishing their tumor-promoting activities. HX301 (Narazaciclib or ON123300) is a clinical-stage tyrosine kinase inhibitor (TKI, NCT#05731934) with great potency against CSF1R (IC_{50} 0.285nM) and has demonstrated significant inhibition on macrophage proliferation *in vitro/in vivo* as well as capability of penetrating brain-blood-barrier (BBB). We hypothesized it has anti-GBM activity.
Methods. A single dose of HX301 (30 mg/kg) was orally administrated in NOD/Scid mice, followed by plasma and brain tissues collection at designated timepoints and the drug concentration determination by LC-MS. Anti-GBM pharmacology of the drug was assessed in the following preclinical models: 1) subcutaneous U87-MG xenograft; 2) orthotopic GL261-Red-Fluc syngeneic mouse GBM model; and 3) orthotopic BN2289-Luc PDX model, respectively. HX301, Temozolomide (TMZ), or their combination were oral-administrated daily. tumor-focused-irradiation was also performed using a X-RAD320 (Precision X-Ray, 15-Gy two days after randomization).
Results. The mouse PK study showed that HX301 efficiently penetrate BBB resulting in adequate exposure in brain for activity. In the three GBM pharmacology modeling, HX301 alone significantly inhibited the tumor growth, of which the anti-tumor activity seen in the orthotopic GL261-Red-Fluc and BN2289-Luc models also confirmed HX301 penetration BBB to exert its anti-tumor effects, possibly acting on the macrophages or microglia in the TME. Moreover, although HX301 and radiation combo failed to show any synergistic effects in orthotopic GL261-Red-Fluc model, HX301 in combination with TMZ exhibited significantly stronger anti-tumor activities over either HX301 alone or TMZ alone in the subcutaneous U87-MG model, suggesting a synergistic effect between the two.
Conclusions. HX301 demonstrated great ability to penetrate BBB and robust anti-tumor efficacy, as well as enhanced activity when combined with TMZ, in GBM models, which warrants a further clinical investigation.

#3135 First-in-class NR2F6 antagonist TES-ONC-07 enhances anti-cancer host immunity reducing tumor growth in a “cold” cancer model.

Francesco A. Greco[1], Daniela Passeri[1], Gianmario Ciaccioli[1], Cipolloni Marco[1], Carolina Colliva[1], Cristina Geroni[1], Paride Liscio[1], Emiliano Rosatelli[1], Giorgia Manni[2], Francesca Fallarino[2], Giuseppe Damiano[3], Vincenzo Russo[3], Roberto Pellicciari[1]

[1]Tes Pharma Srl, Corciano, Italy,[2]University of Perugia, Perugia, Italy,[3]Ospedale San Raffaele, Milano, Italy

Anti-cancer immunotherapy has been a groundbreaking approach in the oncology field. However, currently approved immune checkpoint inhibitors (ICI) suffer from several drawbacks, such as low patient response rate and acquired resistance. The reasons why certain patients do not respond to ICI have not been fully elucidated. Recently, the high expression of the orphan Nuclear Receptor Subfamily-2 Group-F Member-6 (NR2F6) has been suggested as a biomarker for ICI sensitivity in melanoma patients (Kim H. et al. Science, 2023). NR2F6 expression correlates with low IFNγ signature and response to anti-PD-1. In T cells, NR2F6 acts as a transcriptional repressor of specific cytokines (IL-2, IFNγ), pivotal for anti-tumor response. Thus, NR2F6 is a pivotal factor that limits immune infiltration and activation in the tumor microenvironment. So far, the evaluation of NR2F6 as a potential drug target has been limited by the lack of small molecule ligands. A Tes Pharma drug discovery project has led to the identification of TES-ONC-07, an orally available first-in-class NR2F6 antagonist. TES.ONC-07 binds NR2F6 recombinant protein in the nanomolar range. TES-ONC-07 binding to NR2F6 leads to the detachment of the nuclear receptor from the promoter of target genes such as IL-2. Ex vivo, TES-ONC-07 stimulation increases the production of specific cytokines, including IL-2 and IFNγ, when added to CD4+ and CD8+ T cells. In a melanoma B16-F1 mouse model, TES-ONC-07 increases infiltration of several myeloid and lymphoid cell subsets, resulting in a significantly reduced cancer growth. Finally, we demonstrated that orally administered TES-ONC-07 has antitumor activity. In conclusion, we have identified the first small molecule NR2F6 antagonist endowed with characteristics that allowed in vivo proof of concept. TES-ONC-07 administration leads to increased immune infiltration in a notably “cold” cancer model and this effect remarkably slows down cancer growth. Overall, our data suggests that NR2F6 is a valuable novel drug target. Potentially, treatment with TES-ONC-07 may increase sensitivity to ICI, also in those patients with limited response. Further studies are ongoing to test combination therapies as well as complete the preclinical development of TES-ONC-07.

#3136 EOS-215, a first-in-class TREM2 antagonist, designed to reprogram the tumor microenvironment and overcome resistance.

Thibaut Janss, Romain Pirson, Virginie Rabolli, Iain Welsby, Clotilde Hoyos, Quentin Dubray, Laura Saerens, Jean-Philippe Deglasse, David Carbonez, Lennart Raman, Francesco Strozzi, Julie Chesne, Joao Matos Marchante, Reece Marillier, Yvonne McGrath, **Catherine Hoofd**

iTeos Therapeutics, Charleroi, Belgium

TREM2 (triggering receptor expressed on myeloid cells 2) is a central regulator of macrophage functions involved in tissue damage and resolution of inflammation in multiple pathologies. TREM2 macrophages have recently emerged to play a critical role in oncology, shaping a tolerogenic tumor microenvironment, favoring resistance to multiple therapeutic approaches (including anti-PD-1) and promoting metastasis, across solid tumor indications. EOS-215 a first-in-class human TREM2 antagonist, has been specifically designed to reprogram $TREM2^{+}$ macrophages and block their pleiotropic pro-tumoral functions. This antibody demonstrates a sub-nanomolar affinity to human, cynomolgus and mouse TREM2. *In Silico* modeling has predicted that EOS-215 could compete with multiple TREM2 ligands such as phosphatidylserine (PS) and low density lipoprotein (LDL), and thereby prevent the TREM2 multimerization required for downstream signaling. Those predictions were confirmed in *in vitro* competition and signaling assays. Importantly EOS-215 was specifically selected among a panel of antibodies to potently inhibit efferocytosis, a key mechanism driving macrophage pro-tumoral functions, LAM (lipid associated macrophage) formation and involved in the metastatic niche formation. *In vitro*, EOS-215 profoundly impacted the transcriptomic program of various models of In monocyte derived macrophages. Hallmark gene set enrichment analysis (GSEA) revealed a significant enrichment in genes related to metabolism and regulation of the immune response, supporting the ability of EOS-215 to reprogram macrophages. This was further confirmed by increased secretion of pro-inflammatory markers from macrophages in response to EOS-215. In vivo efficacy of EOS-215 was first tested in the orthotopic 4T1 triple negative breast cancer model. This model shows limited response to anti-PD-1 and spontaneously leads to the development of lung metastasis. In this model, EOS215 decreased metastasis burden in monotherapy and this effect is reinforced when combined with anti-PD-1 treatment. In another anti-PD-1 resistant model, CT26, EOS-215 combined with anti-PD-1 significantly delayed tumor growth. In conclusion, EOS-215 is a potent TREM2 antagonist with a sub-nM potency proven preclinically to block pro-tumoral function in macrophages, consequently leading to in vivo efficacy. EOS-215 is now ready to enter clinical trials in 2025.

#3137 The combination of anti-PD1 and a mouse analog of PYX-201, an antibody-drug conjugate targeting the extra-domain B splice variant of fibronectin (EDB+FN), shows greater anti-tumor efficacy than either treatment alone.

Anthony Burt Rodriguez, Amanda Facklam, Matthew Iovino, Chuan Shen, Justin Trickett, Sara Lewandowski, Frank Wang, Marsha Crochiere, Nicolas Severe, Jan Pinkas

Pyxis Oncology, Boston, MA

Antibody-drug conjugates (ADC) are transforming cancer therapy, with some showing potential for enhanced efficacy with immunotherapy (Hoimes et al., 2023; Powles et al., 2024). PYX-201 is a first-in-concept, non-cellular ADC targeting EDB+FN, an extracellular matrix protein that is highly expressed in the stroma of various solid tumors but minimally present in normal adult tissues, making it an ideal drug delivery target. Currently, PYX-201 is being evaluated as a monotherapy in a phase 1 clinical trial for various solid tumors (NCT05720117). Preclinical studies have shown that PYX-201 exhibits broad anti-tumor activity across an array of patient-derived xenograft mouse models (Severe et al., 2024). PYX-201 operates through multiple mechanisms, including modulating the tumor immune compartment and increasing sensitivity to immunotherapy (Hooper et al., 2022). Combining PYX-201 with immunotherapy could be a potent strategy for tackling difficult-to-treat cancers. This study aims to demonstrate that combining a mouse analog of PYX-201 with anti-PD1 is more effective than either treatment alone in a syngeneic mouse breast tumor model. The mouse analog of PYX-201 was generated using the variable regions of the heavy and light chains of an anti-human EDB+FN monoclonal antibody (L19), fused to mouse IgG2a constant regions, and conjugated to an optimized Auristatin0101 payload via an mcValCitPABC linker, with an average drug-to-antibody ratio of 4 (Hooper et al., 2022). For efficacy studies, immunocompetent mice bearing subcutaneous EMT6 breast tumors were treated with the mouse analog of PYX-201 (3 mg/kg) and anti-PD1 (10 mg/kg), either alone or in combination. Tumor volume and body weight were measured bi-weekly, with tumors collected for pharmacodynamic analysis and immune profiling by flow cytometry and imaging. Treatment with either anti-PD1 or the mouse analog of PYX-201 resulted in significant tumor growth inhibition (TGI) and some complete responders (CR) compared to the vehicle control group (anti-PD1: 53.76% TGI, 3/15 CR; mouse analog of PYX-201: 65.87% TGI, 3/15 CR). Notably, the combination of the mouse analog of PYX-201 and anti-PD1 resulted in significantly greater tumor regression and clearance than either treatment alone (93.21% TGI, 8/15 CR). Flow cytometry and image analysis revealed an increased number of infiltrating lymphocytes post-treatment with the mouse analog of PYX-201. Further studies are underway to characterize changes in the activation status and tumor localization of these lymphocytes after treatment. Overall, these results provide compelling evidence that combining PYX-201 with anti-PD1 is a highly effective and promising strategy for improving outcomes in patients with difficult-to-treat cancers.

#3138 EGR1 is a master regulator of tumor growth and macrophage polarization in inflammatory breast cancer.

Verena Martinez-Rodriguez[1], Van Ngu Trinh[2], Suguru Ogata[1], Manuel Van Gijsel-Bonnello[1], Andrew C. Montgomery[1], Naoto T. Ueno[1], Xiaoping Wang[1]

[1]Cancer Biology, University of Hawaii Cancer Center, Honolulu, HI,[2]The University of Texas MD Anderson Cancer Center, Houston, TX

Background: Inflammatory breast cancer (IBC) is one of the most aggressive forms of breast cancer, accounting for 2-4% of cases but 8-10% of breast cancer-related deaths in the United States. Patients with IBC face poor prognoses and limited treatment options, highlighting the urgent need to understand the mechanisms driving this disease. The transcription factor early growth response 1 (EGR1) is highly dysregulated in various cancers, including IBC, where its expression is significantly elevated compared to stage-matched non-IBC samples. Lower EGR1 expression correlates with improved survival outcomes. Our previous research has identified EGR1 as a key mediator of the EGFR-regulated immunosuppressive tumor microenvironment (TME), a critical driver of IBC's aggressiveness. The molecular mechanisms through which EGR1 modulates the IBC TME remain poorly understood. This study aims to fill this significant knowledge gap by exploring EGR1's role in IBC and testing the hypothesis that targeting EGR1 is a novel therapeutic strategy for IBC.

Methods: We depleted EGR1 expression in IBC cells using CRISPR/Cas9 knockout (KO) or shRNA knockdown (KD) and examined the effect on cell proliferation, migration, and invasion *in vitro* and tumor growth *in vivo*. We depleted EGR1 in THP-1 monocytes to test the impact on M2 polarization and subsequent effect on IBC cell migration. Coculture experiments were performed to investigate the crosstalk between IBC cells and M2 macrophages. RNA-sequencing (RNA-seq), real-time PCR, and Western blot analyses were performed to determine the molecular mechanisms by which EGR1 regulates the IBC TME.

Results: EGR1 KO significantly reduced proliferation, colony formation, and migration of IBC cells *in vitro. In vivo* tumor xenograft models confirmed that EGR1 KD reduced tumor burden, decreased M2 macrophages, and increased M1 macrophages, indicating EGR1's role in modulating the TME. Depleting EGR1 in macrophages reduced M2 polarization and their migratory and phagocytic abilities, suggesting that EGR1 impacts both IBC cells and macrophages. *In vitro* coculture experiments showed that EGR1 KO in IBC cells reduced M2 macrophage recruitment, while EGR1 KO in macrophages attenuated IBC cell migration. RNA-seq analysis of IBC Ctrl and EGR1 KO cells showed gene downregulation in cell signaling, cancer progression, and metabolism. In EGR1 Ctrl and KO M2 macrophages, RNA-seq indicated downregulation of immunosuppressive macrophage signature genes.

Conclusion: Our findings establish EGR1's dual role in promoting cancer cell proliferation and shaping an immunosuppressive TME in IBC. Targeting EGR1 impairs M2 macrophage polarization and fosters an anti-inflammatory TME, positioning EGR1-targeted therapy as a novel IBC treatment strategy. These results warrant further investigation to elucidate the underlying molecular mechanisms and validate its therapeutic potential.

#3139 The novel peptide drug conjugate AVA6103 is a FAP-enabled pre|CISION® medicine which targets exatecan, a topoisomerase I inhibitor, to the tumor microenvironment following FAP cleavage.

C. Rink, T. Clough, E. Watts, F. Orafidiya, M. Houee, C. Tong, V. Juskaite, M. Morrow, D. Jones, F. Wilson;
Avacta Life Sciences, London, United Kingdom

AVA6103 is a novel peptide drug conjugate (PDC) based on proprietary pre|CISION® technology which incorporates a dipeptide that is specifically cleaved by Fibroblast Activation Protein α (FAP). FAP is a post-proline protease that is overexpressed on the surface of cancer associated fibroblasts (CAFs) in many solid tumors, and facilitates delivery of pre|CISION® medicines specifically to the tumor microenvironment (TME). AVA6103 consists of exatecan, a potent Topoisomerase I warhead, covalently linked to a dipeptide containing a cleaving sequence (D-Ala-L-Pro), which is susceptible to hydrolysis by FAP but is resistant to hydrolysis by other mammalian proteases. Exatecan has been investigated both as a single agent and as a warhead in ADC therapeutics in the clinic. However, clinical utility of this family of warheads is limited by dose-limiting toxicities, including severe neutropenia. The exquisite selectivity of the pre|CISION® substrate to FAP results in release of exatecan warhead in the TME, greatly increasing the therapeutic window and hence reducing systemic exposure and associated toxicities. AVA6103 is a pre|CISION-enabled exatecan programme, with a candidate in IND-enabling studies. A series of compounds have been engineered with modifications in the capping-group and linker portions. Using structure-based drug design, clear structure-activity relationships have been established for affinity to, and susceptibility of linker cleavage by FAP and subsequent warhead release. Concentric partitioning algorithms show differential FAP expression in stromal regions closest to cancer cells vs. those more distal to cancer cells, in multiple indications. Multiplex-immunofluorescence (mIF) data also shows the spatial organization of blood vessels, FAP-positive CAFs, and adjacent FAP-negative cancer cells provide an ideal architecture for the delivery, cleavage, and warhead uptake of a FAP-enabled PDC. In a cancer cell line/fibroblast cell co-culture, AVA6103 demonstrates FAP-dependent release of exatecan and bystander cancer cell kill. *In vivo* studies demonstrate targeting and accumulation of warhead in the TME, resulting in tumor growth inhibition and complete tumor regressions. Tolerability profiles of AVA6103, allied with tumor growth inhibition efficacy, shows a greatly increased therapeutic window compared to conventional exatecan, with pharmacodynamic biomarkers demonstrating on-target activity of the warhead. The enhanced therapeutic index and efficacy data shown here supports the rationale to progress AVA6103 towards clinical development and supports wider utility of the pre|CISION® platform to target warheads to the tumor while reducing systemic dose-limiting toxicities.

#3140 Preclinical development of YL242, a non-internalized antibody-drug conjugate (ADC) targeting soluble VEGF for treatment of solid tumors.

Tongtong Xue, **Wei Lian**, Qing Zong, Chun Deng, Qigang Liu, Peng Zou, Jiaqiang Cai

MediLink Therapeutics (Suzhou) Co., Ltd., Suzhou, China

Traditional ADC technologies require overexpressed targets, high internalization and efficient intracellular processing. To this end, a non-internalized antibody-drug conjugate (ADC) targeting soluble VEGF was developed using MediLink's TMALIN® platform. VEGF is overexpressed in virtually all human tumors and contributes to key aspects of tumorigenesis and angiogenesis. YL242 incorporates an anti-VEGF antibody, protease-cleavable tripeptide linker and novel DNA topoisomerase I inhibitor. It introduces a novel cleavage mechanism that occurs in the tumor microenvironment while stable during systemic circulation. YL242 is designed to leverage anticancer activity through both anti-angiogenesis by the VEGF antibody and the cytotoxicity of the extracellularly released payload. The linker of YL242 can be enzymatically cleaved by enzymes in TME (tumor microenvironment) to release payload C24, subsequently inducing cytotoxic effects to kill cancer cells. Significant dose-dependent antitumor effects, including complete tumor regressions were observed in tumor xenograft mouse models. The stability of YL242 has been demonstrated by incubation with various plasmas under physiological conditions. This high stability was further evidenced by the pharmacokinetic profiles in non-human primate (NHP) studies, where both the ADC and TAb curves are overlapped. YL242 demonstrated promising safety profiles in pivotal non-human primate (NHP) toxicity studies with no drug-related adverse effects observed. In summary, VEGF-targeted ADC YL242 synergistically combines both antitumor activity of cytotoxic payload and anti-angiogenesis effect of antibody and demonstrated both antitumor efficacy and safety. Consequently, YL242 holds promise for further development in the treatment of cancer patients.

#3141 Development and comprehensive preclinical evaluation of dual-drug polymeric micelles ONP-001 for PDAC and HCC.

Qiu-Xu Teng[1], Guodong Zhang[1], Lu Dai[2], Defeng Deng[1], Ping Pan[1], Diana S-L Chow[2], Guorong Ma[3], Peiying Yang[1], **Chun Li**[1]

[1]UT MD Anderson Cancer Center, Houston, TX,[2]University of Houston, Houston, TX,[3]OmniNano Pharmaceuticals, Houston, TX

Introduction: Pancreatic ductal adenocarcinoma (PDAC) and hepatocellular carcinoma (HCC) remain the leading causes of cancer-related mortality, with poor outcomes. This study comprehensively evaluated the mechanisms of polymeric micelles co-encapsulating cyclopamine (CPA) and paclitaxel (PTX) (M-CPA/PTX, ONP-001) and the effectiveness against HCC and PDAC. The dual-drug delivery system targets embryonic signaling pathways, such as Hedgehog (Hh), which drives tumor progression and resistance.
Methods: CPA and PTX were encapsulated in micelles (~55 nm in size) to improve bioavailability and tumor concentrations. Preclinical experiments included *in vitro* assays for cytotoxicity, tumorsphere, and colony formation in both cancer cell lines. *In vivo* models included orthotopic Kras* cell implanted PDAC and spontaneous HCC in transgenic LAP-tTA/TRE-MYC mice. The antitumor efficacy was examined by intravenously injection of ONP-001 at 2.5, 5.0, and 7.5 mg/drug equivalent/kg thrice weekly for 4 weeks, as well as combination regimens with irreversible electroporation (IRE) and an immune checkpoint inhibitor. Survival, tumor burden, immune modulation, and cytokine profiles were analyzed using Kaplan-Meier survival curves, bioluminescence imaging, and multiplex cytokine assays.
Results: In the PDAC model, ONP-001 significantly extended median survival at 2.5 mg equivalent drug/kg ($p<0.01$), 5.0 mg/kg ($p<0.0001$), and 7.5 mg/kg ($p<0.05$) compared untreated control. ONP-001 administered at 7.5 mg/kg displayed shorter median survival than 5.0 mg/kg suggesting that the optimal regimen tested was 5 mg/kg. ONP-001 reprogrammed tumor stroma, and reduced cancer stem cell (CSC)-associated tumorsphere formation. Combination of ONP-001 with IRE and anti-PD-1 further enhanced survival and immune infiltration, overcoming PDAC's immunosuppressive microenvironment. In HCC, ONP-001 significantly inhibited tumor growth and colony formation. Tumor cytokine profiling revealed significant reductions in markers associated with CSC maintenance (e.g., IL-15, IL-33) and tumor progression, highlighting an anti-inflammatory effect on the tumor microenvironment. Studies in the transgenic HCC model also confirmed significantly greater antitumor efficacy for ONP-001, with no observed hepatotoxicity, compared to sorafenib treatment.
Conclusion: ONP-001 was an effective therapeutic strategy for PDAC and HCC, targeting CSCs, modulating the stroma, and enhancing immunotherapy, at 5.0 mg/kg, TIW, with enhanced benefits when in combination regimens. Ongoing studies will further characterize the pharmacokinetics, tumor uptake and biodistribution of both drugs. The data supports further development of ONP-001 for potential future clinical development.

#3142 Patient-derived models of breast cancer for drug screening and precision medicine approaches.

Rui Li[1], Xiao Hu[2], Ning Ge[3], Michael Kerin[1], Laura Barkley[1]

[1]Lambe Institute, University of Galway, Galway, Ireland,[2]State Key Lab of New Drug and Pharmaceutical Process, Shanghai Institute of Pharmaceutical Industry, China State Institute of Pharmaceutical Industry, Shanghai, China,[3]Pharmacology, Northwestern University Feinberg School of Medicine, Chicago, IL

Introduction: The breast tumor microenvironment (TME) consists of a complex and heterogeneous network of cancer cells, cancer-associated fibroblasts (CAFs), immune cells, and an acellular extracellular matrix (ECM). These components critically impact treatment responses, emphasizing the utilization of patient-derived models that closely mimic the TME. Such models provide valuable insights into chemotherapy responses and facilitate the development of personalized treatment strategies. Natural products represent a vast and diverse source for anti-tumor drug discovery due to their structural diversity and wide range of biological activities. This study aims to utilize patient-derived models to screen a library of natural products to identify lead compounds with potential for personalized breast cancer therapy.

Methods: A primary screen was performed using a patient-derived co-culture system comprising breast cancer cells and matched CAFs. A library of 61 natural products and 6 standard chemotherapy drugs was evaluated for their impact on cell viability, with IC_{50} values calculated to assess efficacy. Positive hits were further investigated in a secondary screen using 3D patient-derived organoid (PDO) models derived from four breast cancer subtypes. These PDOs included patient-derived cancer cells, matched CAFs, and autologous tumor-infiltrating lymphocytes (TILs), representing the tumor immune microenvironment. The anti-tumor effects of these compounds on PDO viability, growth, TIL recruitment, TIL activity and induction of apoptosis were assessed.

Results: The patient-derived co-culture and PDO models retained characteristics of their respective primary breast cancers and showed varied responses to different chemotherapeutics. Among standard drugs, doxorubicin and camptothecin showed uniform IC_{50} values across all PDOs, while paclitaxel primarily inhibited proliferation rather than inducing significant cell death. Conversely, 5-fluorouracil and etoposide displayed limited efficacy. In the natural product screen, Emodin (an anthraquinone) and Platycodin D (PD, a triterpenoid steroid) demonstrated potent anti-tumor activity. Both compounds caused a dose-dependent reduction in PDO size and a significant increase in cell death. Apoptosis assays revealed enhanced tumor cell and CAF apoptosis following treatment. Additionally, Emodin and PD increased TIL cytotoxicity, evidenced by elevated Granzyme B expression in $CD4^+$ and $CD8^+$ T cells.

Conclusion: This study highlights the utility of patient-derived models in precision medicine and drug screening, providing a valuable platform for identifying effective therapeutic agents for breast cancer. Emodin and PD emerge as promising candidates with broad efficacy across multiple breast cancer subtypes, highlighting their potential for future development in breast cancer therapies.

#3143 Leveraging chemo-immunotherapy to remodel the tumor microenvironment and enhance treatment efficacy in osteosarcoma lung metastases.

Raneem R. Aldaqqa[1], Fatemah S. Sunbul[2], Victoria A. Garcia[1], Asma M. Al-terawi[1], Joshua J. Reineke[3], Sandro R. P. da Rocha[1]

[1]Pharmaceutics, Virginia Commonwealth University - VCU, Richmond, VA,[2]Department of Respiratory Therapy,, Mohammed Al-Mana College for Medical Sciences, Dammam, Saudi Arabia,[3]Pharmaceutical Sciences, South Dakota State University, Brookings, SD

Osteosarcoma (OS), the most common primary bone cancer across all ages, predominantly metastasizing to lungs. At diagnosis, 20% of OS patients present gross OS lung metastases (OSLM), while most others harbor undetectable micrometastases. Despite current treatments, including systemic chemotherapy and experimental therapeutics like immune checkpoint inhibitors, the 5-year survival rate for OSLM continues to be low (ca. 20-30%), making it the leading cause of death in OS patients. Thus, there is an urgent need for innovative therapies to shift OSLM tumor microenvironment (TME) towards a more immunogenic state to overcome tumor tolerance. Gemcitabine (GMT), has shown limited efficacy in relapsed OSLM when administered systemically. Pulmonary administration (PA) of GMT demonstrated promise in reducing tumor burden but does not eradicate it. To address this gap, we propose a novel strategy combining inhaled GMT with colony-stimulating factor-1 receptor inhibitors (CSF-1Ris); pexidartinib (PLX-3397), that target tumor-associated macrophages (TAMs), both delivered locally to lungs via PA. GMT's pulmonary efficacy is partially linked to its ability to upregulate Fas, a death ligand on tumor cells, promoting Fas-mediated apoptosis, and CSF-1Ris are expected to shift TAMs towards a more inflammatory, M1-like phenotype. Our studies began with evaluating GMT's immunomodulatory effects on Fas expression *in vitro* using OSLM cells. Moving to 3D multicellular tumor spheroid (MCT) model composed of OSLM cells, macrophages, and fibroblasts, we confirmed GMT's ability to increase Fas expression, simulating the results we obtained in OSLM *in vivo* model. Concurrently, we tested the ability of PLX to shift macrophages from tumor-promoting M2-like phenotype to tumor-suppressing M1-like phenotype. RAW 264.7 macrophages polarized to M2-like state with IL-4 were treated with PLX, resulting in a significant reduction of M2-like macrophages. Similar effects were observed in the 3D MCT model, which recapitulates again our *in vivo* studies. *In vivo*, Balb/c mice with OSLM were treated via PA with GMT (5 mg/kg weekly for 2 weeks) and PLX (2 mg/kg every other day for 3 weeks). Tumor burden, tracked with bioluminescence imaging (BLI), has significantly reduced for combination therapy. GMT increased Fas expression in tumor cells, while PLX shifted TAM toward an anti-tumorigenic state, reducing M2 TAMs and enhancing M1/M2 ratio. The treatment was well-tolerated without affecting body weight or overall health.

In conclusion, we showed that chemo-immunotherapy combination via PA can effectively remodel the TME in an immunocompetent OSLM mouse model. We also developed a 3D spheroid model with the relevant immune-cells that mimic key aspects for the *in vivo* model. These studies help us identify clinically translatable combination therapies for OSLM as both GMT and PLX are approved clinically.

#3144 Selective HDAC10 inhibitor enhances innate antitumor immunity.

Nithya Gajendran[1], David Quiceno-Torress[1], Xintang Li[1], Bryan Weselman[1], Manasa Suresh[1], Sonia Sebaoui[1], Fereshteh Mahmoudi[2], Marie Durr[1], Satish Kumar Reddy Noonepalle[1], Wolfgang Sippl[2], Alejandro Villagra[1]

[1]Oncology, Georgetown Lombardi Comprehensive Cancer Ctr., Washington, DC,[2]Medicinal chemistry, Institute of Pharmacy, Martin-Luther- Universitaet Halle, Wittenberg, Germany

Most anticancer therapies aim to target specific mechanisms that regulate or potentially inhibit the proliferation and survival of cancer cells. Macrophages play a crucial role in the tumor microenvironment (TME), exhibiting both pro and antitumoral functions depending on the polarization state. Our lab focuses on transforming the immune suppressive TME into an antitumoral environment, explicitly enhancing the proinflammatory M1 macrophage phenotype using HDAC inhibitors. Many research studies have shown the aberrant expression of HDAC10 in cancer and various pathologies. However, the deacetylation targets and biological functions of HDAC10 remain poorly characterized due to a lack of specific and potent HDAC10 inhibitors with cellular activity. HDAC10 has a unique structure and substrate preference. Understanding its mechanisms of action is crucial for developing effective therapies. In our study to identify the potential HDAC10-specific inhibitor, we screened 13 HDAC10 inhibitors for their ability to suppress the M2 macrophage phenotype. We also identified six potent inhibitors that suppress the M2 phenotype and tested for cytotoxicity and HDAC inhibition. Interestingly, FM60 with 0.9 nM IC50 against HDAC10 in cells showed negligible cytotoxicity and more than 60 % HDAC inhibition. Inhibition of HDAC10 in primary mouse bone marrow-derived macrophages results in upregulating proinflammatory M1 markers-iNOS and TNFa while concurrently downregulating the expression of anti-inflammatory M2 markers Arginase1 and Fizz1. Flow cytometry analysis demonstrated reduced CD206 + and increased CD80 + macrophages in BMDMs treated with FM60, indicating potential antitumor effects. M1-like macrophages are antitumoral due to their intrinsic phagocytosis and antigen presentation capability. To investigate the phagocytic potential of FM60, BMDMs from GFP mice were left unpolarized (M0) or polarized to M1, pretreated with HDAC10i FM60, and co-cultured with cell trace far red stained SM1 murine melanoma cells. Images captured at every 20-minute interval demonstrated a significant increase in phagocytosis by M1 macrophages treated with FM60. Principal Component Analysis (PCA) showed the closest association between BMDMs from HDAC10 KO mice and FM60-treated in M1 macrophage phenotype, additionally, RNA seq analysis indicated that treatment with FM60 significantly regulates polyamine biosynthesis, crucial for tumorigenesis. To evaluate the antitumor immunity in syngeneic murine melanoma, FM60 was given intraperitoneally at varying doses. 50 mg/kg body weight of FM60 significantly reduced the tumor growth, enhancing its antitumor effects. These findings provide the rationale for using HDAC10is as an immunotherapeutic anticancer agent.

CHEMISTRY: High-Throughput Screening Assays and Libraries
Poster Session

#3149 Desorption electrospray ionization mass spectrometry (DESI-MS) high-throughput screening of small-molecule inhibitors for human sulfotransferase 2B1b & biophysical and crystallographic characterization of target-hit interaction.

Beinan Yang[1], Nicolas M. Morato[2], Samadhi Kulathunga[2], Timothy L. Ratliff[3], Graham Cooks[2], Andrew D. Mesecar[1]

[1]Biochemistry, Purdue University, West Lafayette, IN,[2]Chemistry, Purdue University, West Lafayette, IN,[3]Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN

Upon malignancy and metastasis to distant organs such as brain, lung and bone marrow, the 5-year survival rate of castration-resistance prostate cancer (CRPC) is only around 30%. Therefore, there is an urgent need for identifying new CRPC drug targets and developing new CRPC treatments. A potential new drug target for CRPC is human sulfotransferase isozyme SULT2B1b. It catalyzes the transfer of the sulfate group from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to cholesterol to produce cholesterol sulfate (CS). Increased expression of SULT2B1b has been shown to correlate to prostate cancer cell proliferation with decreased level of TNF-mediated apoptosis. Knockdown of SULT2B1b leads to elevation of TNF expression and NF-κB activation. SULT2B1b knockdown also enhances TNF-mediated apoptosis in both TNF-sensitive (LNCaP)and TNF-resistant (C4-2) cells. We demonstrate herein that catalytic knockout mutants, H125A and K70A, of SULT2B1b abolish CS production in cells mimicking genetic knockdown studies. This result suggests that small molecule inhibitors of SULT2B1b activity could serve as potential CRPC therapeutics. Towards this goal, we established a novel high-throughput (50nL, <1s per sample) label-free DESI-MS platform for the screening of small-molecule compounds with excellence Z' scores ranging from0.73 to 0.86. Using DESI-MS, we first determined Kcat and KM values for catalysis of cholesterol by SULT2B1b to be 0.05 ± 0.002 min^{-1} and 19.8 ± 2.2 μM respectively. We then screened 4500compounds from three chemical libraries (ChemBridge Kinase Inhibitor, LOPAC 1280 &AnalytiCon MEGx Natural Products) and identified 3 bona fide hit compounds (#099, #170 and#954) that had IC50 values ranging from 1.2 to 1.6 μM. We also showed via DESI-MS that these three compounds, each containing multiple hydroxyl groups, could not be sulfated. NanoDSF was used to show that the hit compounds stabilize the SULT2B1b melting temperature by 1.7°C to3.0°C. Binding interactions of the hit molecules were also examined using microscale thermophoresis (MST) and dissociation constants (KD) of 13.5 μM (#099), 6.9 μM (#170) and 7.2μM (#954) were determined. X-ray structures of SULT2B1b in complex with #099 and #170 were determined to 2.8 Å and 2.9 Å resolution respectively. Both compounds are found bound within the cholesterol substrate binding site. One important structural observation is the N-terminus helix (residues 1 - 28) is displaced due to the binding of the inhibitors. In summary, we used DESI-MS to identify three compounds that potently inhibit SULT2B1b through interactions at the cholesterol binding site. These results should now allow for the structure-based design and development of more potent lead compounds of SULT2B1b which ultimately may be useful forCRPC treatment.

#3150 Utilizing the HiBiT system to identify CARM1 degraders for targeted cancer therapy.

Megan Bacabac[1], Fabao Liu[1], Eui-Jun Kim[1], Isaac Hayes[1], Yidan Wang[1], Weiping Tang[2], Tim S. Bugni[2], Wei Xu[1]

[1]Oncology, University of Wisconsin-Madison, Madison, WI,[2]Pharmaceutical Sciences, University of Wisconsin-Madison, Madison, WI

Coactivator-associated arginine methyltransferase 1 (CARM1) is a member of the protein arginine methyltransferase (PRMT) family that asymmetrically dimethylates proteins on arginine residues. Methylation of BAF155 by CARM1 in triple-negative breast cancer (TNBC) drives cancer metastasis. Amplification and overexpression of CARM1 has been observed in a variety of cancers, including breast cancer, and its overexpression correlates with poor prognosis. Potent small molecule inhibitors for CARM1 have been developed but the effects of inhibiting CARM1 differ from the effects of knocking out (KO) CARM1 in cancer cells. CARM1 KO, but not CARM1 inhibition, decreases cancer cell proliferation. This implies that CARM1 also has non-enzymatic roles in driving cancer progression which necessitates the development of small molecule degraders of CARM1 to inhibit both structural roles and enzymatic activity. We have developed CARM1-specific proteolysis targeting chimeras (PROTACs), which contain a CARM1 ligand (TP-064), a rigid chemical linker, and an E3 ligase ligand (von-Hippel Lindau) to target CARM1 for proteasomal degradation. We characterized the degradation activity of the best compound, 3b, which has a DC_{50} of 8.1 nM and a D_{max} of 97%. While PROTAC 3b is potent and selective, many challenges remain in using PROTACs in clinical settings. There are still no PROTACs that have received FDA-approval. A possible way to overcome the potential toxicity and side effects of chemically synthesized drugs is to utilize naturally occurring products. We generated a breast cancer cell line containing HiBiT-tagged CARM1 to perform high-throughput unbiased screening of 1408 natural products. Two natural product fractions, named 5_9 and 10A, decreased luciferase signal and CARM1 protein levels by Western blot. Treatment with either natural product did not impact the protein levels of another PRMT family member, PRMT6. Ongoing studies will determine the biological consequences of 5_9 and 10A on breast cancer cell lines and organoids. CARM1 PROTACs and natural products may be developed as therapeutic agents for targeting CARM1-driven cancers more effectively than currently available inhibitors.

#3151 Designing and utilizing a c-Rel specific bioassay in drug repurposing screen for HNSCC.

Kristin A. Altwegg[1], Kathryn E. King[2], Natalia J. Martinez[1], Ty C. Voss[1], Dvir Blivis[1], Wendy C. Weinberg[2], Mark J. Henderson[1]

[1]NIH-NCATS (National Center for Advancing Translational Sciences), Rockville, MD,[2]Food and Drug Administration, White Oak, MD

Head and neck squamous cell carcinoma (HNSCC) is the 7th most common cancer worldwide, with incidence anticipated to increase by 30% by 2030. HNSCC occurs most often in men in their 50-60s, although the incidence among women and younger individuals is rising. The current standard-of-care practices are surgical resection and chemoradiotherapy. HNSCC tumors exhibit frequent mutation of TP53 (72% of tumors). Two additional members of the TP53 gene family, TP63 and TP73, are frequently altered in HNSCC. TP63 encodes two major isoforms, ΔNp63 and TAp63 (containing an alternate or p53-like transactivation domain, respectively). Specifically, ΔNp63α is overexpressed in ~80% of HNSCC tumors. Our lab has reported that ΔNp63α interacts with activated c-Rel (a nuclear factor-κB family member) in HNSCC, and this interaction is associated with uncontrolled proliferation, inflammation, and metastasis. Consistent with a role in growth regulation, ΔNp63α:c-Rel complexes bind a promoter motif and repress the cyclin-dependent kinase inhibitor p21WAF1 in both human HNSCCs and normal keratinocytes overexpressing ΔNp63α. A robust nuclear co-localization in the proliferating compartment of primary HNSCC reflects the relationship between ΔNp63α and activated c-Rel. Stimulation of HNSCC cells with TNFα results in the activation and nuclear localization of c-Rel, which binds to ΔNp63α. This binding displaces and inactivates the tumor suppressor TAp73 from ΔNp63:TAp73 complexes. By western blot, we confirmed that TNFα stimulation enhances nuclear c-Rel localization in human HNSCC cell lines. We then designed and optimized a high-throughput screening bioassay to evaluate compounds inhibiting c-Rel nuclear localization under TNFα stimulation. A 1536-well immunofluorescence assay was developed using high-content image analysis to quantify nuclear translocation. The specificity of the c-Rel antibody was validated using CRISPR/Cas9-generated c-Rel knockout cell lines. The optimized bioassay was used to screen the NCATS pharmaceutical collection of approved drugs. Of the 2,807 compounds screened, we identified 168 inhibitors (6% hit rate). Interestingly, 17 compounds promoted/activated c-Rel nuclear translocation. These “hits” were then validated in dose-response to confirm activity. Other NCATS compound libraries will be screened to discover new candidate inhibitor compounds. Top candidates will undergo further investigation for drug synergy with current standard-of-care therapeutics in vitro, ex vivo, and in vivo to explore the possibility of repurposing drugs for HNSCC therapeutics.

#3152 Identification and characterization of inhibitors SHOC2-MRAS-PP1C complex assembly.

Alexei Brooun[1], Patrick Fagan[1], Simon Bergqvist[2], Oleksii Hrabovsky[3], Fang Fang[4], Yecenia Peraza[1], Yin Wu[4], Dongming Quan[4], Meiying Cui[5], Liang Huang[5], Yujie Chen[5], Huihui Zhu[5], Ping Chen[1], Adriana Irimia[1], Peter Chua[1], Mykola Protopopov[3], Yurii Moroz[6], Olga Tarkhanova[3], Jean-Michel Vernier[1], Robert Shoemaker[1]

[1]ERASCA, Inc., San Diego, CA,[2]Biofizik, Inc., San Diego, CA,[3]Chemspace LLC, Kiev, Ukraine,[4]Viva Biotech Ltd., Shanghai, China,[5]Suzhou AlphaMa Biotechnology Co., Ltd, Suzhou, China,[6]Enamine LTD, Kyiv, Ukraine

Inhibition of SHOC2 function by either genetic ablation or protein degradation potently sensitizes RAS-driven cells to MEK inhibition and impairs RTK-mediated adaptive reactivation of MAPK signaling induced by a MEK inhibitor (MEKi). The SHOC2-MRAS-PP1C (SMP) complex is responsible for full activation of RAF via dephosphorylation of the phosphoserine in conserved region 2 (CR2-pS). Based on the SMP complex's critical role in RAF activation and SHOC2 synthetic lethal interaction with MEKi, the SMP complex represents an attractive target for the development of novel therapeutic agents that could prevent adaptive resistance to MEKi. We have conducted a systematic DNA encoding library (DEL) screening campaign against the purified intact SMP complex and all three individual components using the HitGen OpenDEL kit. A series of SHOC2 selective hits were identified and profiled using direct binding and biochemical assays. The lead compounds bind to full-length SHOC2 protein with K_Ds in the low nM range, inhibit SMP complex formation, and block dephosphorylation of BRAF CR2-pS peptide. Hydrogen/deuterium exchange mass spectroscopy (HDX-MS) analysis of SHOC2 in complex with a lead compound identified a putative binding site, consistent with biochemical studies and supporting the MRAS competitive nature of the lead compounds. To our knowledge, these compounds represent first-in-class examples of direct modulators of the SMP complex, via SHOC2 engagement, that have the potential for optimization as either PPI modulators or selective degraders.

#3153 High-throughput identification of radiosensitizers and synergistic drug combinations with potent therapeutic efficacy against diffuse midline glioma.

Syed M. Faisal[1], Monika Yadav[1], Garrett R. Gibson[1], Adora T. Klinestiver[1], Ryan Sorenson[1], Anuradha Roy[2], Carl Koschmann[3], Scott J. Weir[4], Stefan H. Bossmann[4], Viveka N. Yadav[5]

[1]Children's Mercy Research Institute, Kansas City, MO,[2]High-throughput Screening Laboratory, University of Kansas, Lawrence, KS,[3]University of MIchigan Medical School, Ann Arbor, MI,[4]University of Kansas Medical Center, Kansas City, KS,[5]Department of Pediatrics, Children's Mercy Research Institute, Kansas City, MO

Diffuse midline gliomas (DMGs), including diffuse intrinsic pontine glioma (DIPG), are the most aggressive pediatric brain tumors with a median survival of less than one year. Over 85% of DMGs harbor the H3K27M mutation in histone genes H3F3A (H3.3) and H3C2 (H3.1), which inhibits EZH2, the catalytic subunit of the Polycomb Repressive Complex 2 (PRC2), leading to global loss of H3K27 trimethylation (H3K27me3). This epigenetic disruption drives oncogenesis by reprogramming gene expression and activating oncogenic pathways. Current treatments, including chemotherapy and radiotherapy (IR), offer marginal benefits, with 95% of patients dying within 1.5 years of diagnosis. ONC201 has shown clinical benefits in H3K27M-mutant DMGs, extending median survival to 21.7 months by disrupting metabolic and epigenetic pathways and reversing H3K27me3 loss. Novel therapeutic strategies targeting H3K27M oncohistone are urgently needed. We performed high-throughput screening (HTS) of 1,520 FDA-approved compounds to identify drugs that selectively target H3K27M-mutant primary DMG cell lines, but not CRISPR-corrected H3K27M-KO lines, derived from distinct neuroanatomical location: the thalamus (BT-245) and the pons (DIPGXIIIP). From this screening, we identified 86 promising drugs, further narrowed down to five lead compounds: floxuridine, gemcitabine, cytarabine, pyrimethamine, and cladribine (HTS-identified lead candidates, HTS-ILCs). These HTS-ILCs exhibited potent cytotoxicity against other patient-derived H3K27M-mutant DMG lines (DIPG007, SF8628) and murine PPK cells (DNp53/PDGFRA/H3.3K27M) from genetically engineered mouse models (GEMMs). HTS-ILCs were further tested for radiosensitizing potential, with floxuridine, gemcitabine, cytarabine, and pyrimethamine showing enhanced efficacy when combined with IR. Western blot analysis confirmed H3K27me3 restoration, indicating epigenetic modulation and reduced DMG growth. Combining IR, HTS-ILCs, and ONCs (201/206/212) showed synergistic effects and reversed epigenetic dysregulation. Gemcitabine exhibited strong anti-invasive properties, followed by floxuridine against H3K27M mutant DIPGXIIIP and SF8628 cells. Conditioned medium from HTS-ILCs-treated PPK cells showed enhanced HMGB1 secretion, suggesting activation of an immune-stimulatory response. Ongoing *in vivo* testing includes orthotopic implantation of PPK cells in immune-competent murine models and IUE-generated GEMMs of H3K27M DMGs. Spatial transcriptomics and phosphoproteomic microarray analysis are being used to elucidate the molecular mechanisms. Our findings suggest that combining HTS-ILCs with IR, alone or with ONC therapies, offers a promising strategy for treating H3K27M-mutant gliomas, potentially leading to more effective, personalized therapies for children with DMGs.

#3154 Targeting of the Elongator tRNA-modifier complex through high-throughput screening revealed series of compounds with novel mechanism of action.

Mariantonietta Rubino[1], Kerstin-Maike Schmitz[1], Andrea Lauri[1], Debora Zian[1], Nazareno Menegatti[1], Samuel Bradberry[1], Francesca Rapino[2], Pierre Close[2], **Daniele Carettoni**[1], Cinzia Nucci[1], Angela Molteni[1], Jean-Francois Rolland[1]

[1]AXXAM SpA, Bresso, Italy,[2]THERAtRAME SA, Liege, Belgium

Numerous human diseases, such as cancers and neurodegenerative disorders, have been associated with the dysregulation of tRNA-modifying enzymes' activity. These post-transcriptional modifications can affect tRNA structure stability, codon-anticodon base pairing during ribosomal decoding, translation efficiency, and proper protein folding during protein synthesis, impacting both cell cycle and metabolism. It is therefore not surprising that several tRNA-modifying enzymes have recently emerged as attractive therapeutic targets in drug discovery. Nevertheless, these enzymes often form large complexes with multiple subunits, and assessing their activity *in vitro* using reconstituted protein complexes and tRNA substrates is particularly challenging.The aim of the present study was to identify inhibitors of a cancer-implicated tRNA-modifying enzyme. For this purpose, we have developed a TR-FRET-based protein-RNA interaction assay suitable for high-throughput screening (HTS) in 384 MTP format. The protein-RNA interaction was assessed using a tagged recombinant catalytic subunit and a biotinylated RNA oligo derived from a specific tRNA. The optimization through buffer component screening and the validation with tool inhibitors yielded a thorough biochemical characterization of the *in vitro* protein-RNA binding. A HTS campaign with a proprietary library of ~100,000 compounds was performed in search of protein-RNA interaction inhibitors. A secondary assay was used to identify those hit compounds interfering with the primary assay's readout system. The primary screening hit rate was 1 % (cut-off: 49% inhibition) and 84% of those hits were confirmed as putative protein-RNA interaction inhibitors with IC_{50} values in the nM-µM range and no interference effects.A few of these primary hits have progressed to the hit-to-lead stage, and the preliminary results obtained thus far are highly promising.This study illustrates a hit discovery program targeting the interaction between the catalytic subunit of a tRNA-modifying enzyme and a tRNA-derived RNA, aimed at identifying specific inhibitors. Targeting tRNA modification systems represents an attractive strategy for treating human cancers.

#3155 High-throughput drug repositioning identifies dinaciclib as a potent osteosarcoma inhibitor.

Mithun Sarangdhar[1], **Jonah Snyder**[2], Eric O'Brien[1], Brett VanCauwenbergh[1], Mitali Basu[1], Joseph Pressey[1], John Perentesis[1]

[1]Department of Pediatrics, CBDI, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH,[2]CBDI, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Background: Osteosarcoma (OS) is the most common primary malignant bone tumor, predominantly affecting adolescents and young adults. Despite multimodal treatment combining chemotherapy and surgical resection, survival rates for metastatic OS remain below 20%, underscoring the urgent need for novel therapies. Recent studies implicate chromothripsis, a phenomenon of chromosomal shattering and reassembly, in OS pathogenesis, contributing to its aggressive behavior and treatment resistance. However, therapeutic options for OS have remained largely stagnant over the past two decades. Advancing our understanding of OS biology and identifying new therapeutic approaches are essential to improving outcomes for patients with metastatic and relapsed disease.
Methods and Findings: We conducted a high-throughput screen of 3,800 compounds across eight OS cell lines. Two-thirds of the drug library consists of FDA approved drugs and one-third consists of drugs in early phase clinical trials. Each compound was tested in five concentrations (1 nM to 10,000 nM), generating over 200,000 data points to identify active single agents. Dinaciclib, a CDK1/2/5/9 inhibitor, emerged as one of the top-performing agents, ranking in the top 0.5% based on cytotoxicity across all cell lines. Notably, clinically relevant agents Gemcitabine and Docetaxel, components of active retrieval regimens for relapsed OS, also ranked in the top 0.5%. To investigate drug synergy, combinations of Gemcitabine and Docetaxel were tested at varying molar ratios (1:10, 10:1, 1:5, 5:1, and 1:1) across the eight cell lines. The 1:10 molar ratio demonstrated the highest cytotoxicity, consistent with the clinically established 1:9 ratio. Subsequently, an iterative screen assessed the synergy of third agents with the Gemcitabine+Docetaxel regimen in the MG-63 OS cell line. Dinaciclib exhibited additive effects when layered onto this regimen, further supporting its therapeutic potential.
Conclusions: This study identifies Dinaciclib as a potent inhibitor of osteosarcoma, with additive cytotoxicity when combined with the clinically relevant Gemcitabine+Docetaxel regimen. These findings, coupled with prior evidence of Dinaciclib's efficacy in reducing metastatic burden in OS patient-derived xenograft models, highlight its potential as part of a triple combination therapy. This research underscores the value of high-throughput drug repurposing in identifying innovative treatment strategies for osteosarcoma and supports further preclinical and clinical evaluation of Dinaciclib to improve outcomes for patients with metastatic or treatment-resistant disease.

#3156 A surface plasmon resonance-based screening platform to identify potential PD-1/PD-L1 inhibitors from a FDA-approved drugs library for cancer immunotherapy.

H. Li, A. Cai, N. P. Seeram, **C. Liu**;
University of Rhode Island, South Kingstown, RI

The PD-1/PD-L1 axis plays a pivotal role in immune checkpoint regulation in cancer, making its inhibition a key strategy in cancer immunotherapy. However, the development of small molecule inhibitors targeting PD-1/PD-L1 interaction has been hindered by the lack of robust high-throughput screening platforms. In this study, we developed a high-throughput screening platform based on surface plasmon resonance (SPR) technology, utilizing a 384-well plate format. This platform integrates a dual-step coupling method, employing both amine and bio-streptavidin coupling to optimize PD-1 orientation on the sensor chip.We screened a library of 2,764 FDA-approved drugs using this SPR platform with a 1:1 ratio of drug to PD-L1, alongside two positive control PD-L1 inhibitors. Three compounds, RB, MB, and VP, were identified as hits, showing PD-1/PD-L1 blockade rates of 13.1%, 9.6%, and 6.7%, respectively, compared to the positive controls BMS-1166 (33%) and BMS-202 (8.4%). Structural validation through saturation transfer difference nuclear magnetic resonance (STD-NMR) spectroscopy confirmed the binding of RB to PD-L1, with a unique STD peak at 7.6 ppm, further supported by a WaterLOGSY control experiment indicating a negative nuclear overhauser effect to water. The dissociation constants KD of RB were determined using SPR, revealing moderate binding affinities to PD-L1.Cytotoxicity assays conducted on Jurkat T cells and A375 melanoma cells demonstrated that RB exhibited no toxicity across a concentration range from 100 μM to 0.01 μM. Functional assays using a co-culture model of T cells and cancer cells, designed to replicate the tumor microenvironment, showed that RB treatment modulated IL-2 secretion and induced apoptosis in A375 cancer cells. Proteomic analysis of sorted Jurkat T cells from the co-culture model via bottom-up proteomics revealed key proteins and signaling pathways regulated by RB.Overall, these findings suggest that RB exerts significant PD-1/PD-L1 inhibitory activity and may hold promise as a repurposed drug candidate for cancer immunotherapy.

Keywords: PD-1/PD-L1, FDA-approved drugs, surface plasmon resonance, STD-NMR, co-culture model, proteomics

#3157 Novel synthetic lethality-based cellular assays for cancer drug discovery.

G. Maresca[1], F. Scagnoli[1], F. Sassone[1], A. Poli[1], P. Bonetti[1], L. Curti[1], S. Goldoni[2], H. Batoulis[3], E. Nevedomskaya[4], J.-F. Rolland[1], E. Pook[3], L. Redaelli[1], **T. Grammatopoulos**[1];

[1]AXXAM SpA, Bresso, Italy, [2]Bayer AG R&D Pharmaceuticals, Cambridge, MA, [3]Bayer AG R&D Pharmaceuticals, Wuppertal, Germany, [4]Bayer AG R&D Pharmaceuticals, Berlin, Germany

In the present study we illustrate how, using the concept of synthetic lethality (SL), we developed cellular assays suitable for the screening of new drugs targeting cancer-specific weaknesses. SL is a promising strategy in cancer therapy that exploits the genetic vulnerabilities of cancer cells. This approach targets pairs of genes where the mutation or changes of one gene does not affect cell viability, but the simultaneous occurrence of these events in both leads to cell death. This concept is particularly valuable in cancer treatment, as it allows for the selective targeting of cancer cells while sparing normal cells. In this study we coupled powerful molecular approaches such as CRISPR/Cas9 or RNA interference (siRNA and shRNA) with cell viability tests (CellTiter-Glo®), to generate cellular assays suitable for assessing SL of two different genes of interest (GOIs) in proof-of-concept studies and perform drug screening to identify novel inhibitors able to mimic loss or mutations of the GOIs, inducing specific cell death of tumor cells. First, we induced SL by silencing VPS4A in cells already exhibiting loss or reduced function of VPS4B, ultimately triggering selective tumor cell death while sparing normal cells. Indeed, the homozygous loss of VPS4B is seen in up to 4% of colorectal, ovarian, and pancreatic cancers. The SL was then assessed by using siRNA to silence VPS4A and VPS4B in various cancer cell lines expressing different levels of the 2 GOIs and evaluating cell viability. Conversely, to generate stable cellular assays, CRISPR/Cas9 technology was applied to develop both VPS4B knockout (KO) and heterozygous (HET) cell lines, validated through Sanger sequencing and protein expression analysis. These genetically modified lines were critical for understanding the molecular mechanisms driving SL, as they were further engineered to obtain an inducible VPS4A silencing (shRNA technology), allowing for controlled VPS4A depletion. These experiments demonstrated that VPS4A silencing in VPS4B KO cells significantly reduced cell viability, confirming the synthetic lethal relationship between the two GOIs. In conclusion, our survival assay approach could be applied to validate the SL between 2 GOIs, identify a suitable genetic context for applying customized therapeutic options, and new drugs to treat cancer. Proof of principle studies could be conducted in transient using siRNA to silence GOIs in various cancer cell lines expressing different levels of GOIs; while stable modulation of the expression of GOI might be obtained using *ad hoc* engineered cell lines, thus allowing long-term studies and screening campaigns. Cellular assays able to elucidate SL mechanisms may have a significant impact on the cancer drug discovery identifying therapies that specifically target cancer cells with known genetic alterations, while minimizing the impact on normal cells and therefore reducing potential side effects.

#3158 CARP: a high-throughput platform for rapid VHH discovery of potent CAR-T therapy.

Weihong Wang[1], Huiyuan Tang[1], John Lee[1], Xinhao Wang[1], Leo Ng[1], Yong Wang[1], Toya N. Baral[1], Jia Yu[2], Jiaguo Li[2], Wenfeng Xu[1]

[1]Chantibody Therapeutics Inc., Menlo Park, CA,[2]Shanghai Cell Therapy Group Co., Shanghai, China

Background: The conversion of suitable antibodies into functional CAR-T therapy presents challenges. Traditional methods for antibody discovery and screening face significant bottlenecks, including the difficulty of high-throughput identification of high-quality antibodies with diverse epitope and affinity coverage, also carry a high risk of missing functionally relevant candidates or falsely identify unsuitable candidates.

Methods: Here, we introduce **CARP** (**C**AR-T **A**I-powered **R**apid high-throughput screening **P**latform), a novel solution designed to rapidly identify VHHs with clinical potential in CAR structures. Our screening platform was applied for the development of CAR T-cell therapy targeting Cadherin 17 (CDH17). Following immunization of alpacas and llamas, hundreds of CDH17 VHH hits were analyzed using an AI-powered platform for lineage determination (epitope coverage), humanization, and immunogenicity profiling. A library of >400 CDH17 VHHs was screened in serial proliferation tumor cell line co-culture assays simulating long-term tumor-CAR-T interactions to evaluate their target-specific expansion capacity in CAR-T format.

Results: After screening and validation of individual VHH candidates, CDH17 CAR hits consistently outperformed VHHs via traditional methods in functional assays, demonstrating superior activity and robustness. Moreover, CARP successfully identified multiple VHHs with identical target binding domain that demonstrated superior target-dependent expansion compared to clinical CDH17 CAR benchmarks and traditionally selected VHHs.

Conclusion: Our CAR VHH platform not only accelerates the discovery process by enabling high-throughput screening to identify highly target-dependent and proliferative VHH leads, but also eliminates the need for additional rounds of immunization and immunogenicity assessments once lead VHHs are identified. CARP offers a powerful, efficient approach for uncovering VHHs that enhance CAR-T cell functionality, providing significant promise for advancing CAR-T therapy development.

#3159 The 5-base genome, a simultaneous detection of genomic and methylation signatures using a concise, integrated multiomic library prep.

Evgenii Chekalin, William Jordan, Oscar Murillo, Danielle Goldberg, James Baye, Joshua Beytebiere, Eric Brustad, Kayla Busby, Bridget Dorsey, Sophie Fertel, Stephen Gross, Rebekah Karadeema, Huy Lam, Valerie Montel, June Pais, **Suzanne Rohrback**, Angelica Schalembier, David Tse, Kat Tzeng, Ali Crawford, Lisa Watson, Allison Yunghans, Fiona Kaper

Illumina, Inc., San Diego, CA

Multiomic (multiple omic) approaches provide an integrated tool set to power biological discovery due to their ability to generate comprehensive information about the molecular underpinnings of distinct phenotypes. Such integrated analyses have shown broad utility across a range of applications, such as cancer classification and rare disease prognosis. For example, a recent population-scale study on breast cancer by Staaf et al showed that the combination of WGS and DNA methylation can be useful to detect BRCA1/BRCA2-deficient tumors with greater sensitivity than DNA sequencing alone. However, multiomic studies that employ single-ome technologies are often time-consuming, costly, and require considerable sample input. A streamlined workflow which provides both genome and methylome annotations stands to greatly accelerate discovery and translational research. Here we present a novel methyl-conversion technology, capable of simultaneous genome variant detection and DNA methylation profiling. This methodology introduces a simplified workflow, requires less than seven hours turnaround time, as little as 50ng of genomic DNA input, and comes with comprehensive analysis pipelines, the combination of which deliver accurate dual-omic annotations for discovery. Here were present a comparative analysis of our 5-base genome technology against existing methodologies, including Whole Genome Sequencing, Enzymatic Sequencing (EM-Seq) and Illumina Methylation EPIC arrays.

#3160 Silicon membrane technology enables novel live-cell assays for screening impermeable compounds prior to medchem optimization.

Zhihui Song, Sophia Hirsch, Andrew Laroque, Jacquelyn Hanson, Alec Barclay, Armon Sharei, Darby Kreienberg, Eleni Rogers, Monique Richards, Crystal Tsui

Portal Biotechnologies, Inc., Watertown, MA

Cell permeability poses a significant challenge to understanding target biology and exploring structure/function interactions of potential drug candidates. As a result, many experiments are performed in biochemical or lysate-based systems, which often fail to replicate the conditions of a live cell, leading to expensive and time-consuming downstream failures. Portal Biotechnologies has created an innovative intracellular delivery platform that leverages silicon membrane-based mechanoporation technology to directly deliver otherwise non-permeable cargos, such as small molecules, peptides, PROTACs, DELs, macrocycles, and probes, into the cytosol. Our research shows that mechanoporation causes minimal disruption to cellular function compared to other techniques like electroporation, which tend to impose stricter cargo limitations and inflict more cellular damage. This platform has also shown early success in delivering probe molecules for innovative intracellular assays, including LgBiT/HiBiT systems for protein degradation studies. Furthermore, we have demonstrated efficient co-delivery of diverse cargos like oligonucleotides, antibodies, and nucleic acids, achieving delivery rates exceeding 80% while maintaining cell viability above 80%.The platform has been validated across a variety of cell types, including widely used cancer cell lines and primary cells such as human immune cells and stem cells. The straightforward mechanical delivery approach enables scalability and seamless integration with liquid-handling systems for high-throughput applications. This capability allows for the automated screening of molecules previously unsuitable for live-cell assays, thereby enhancing the efficiency of drug discovery at multiple stages. By enabling live-cell assays, the Portal platform aims to offer novel biological insights, reduce target-related risks, and lower the likelihood of failures during lead optimization.

#3161 High throughput drug screening to uncover molecular vulnerabilities in pancreatic ductal adenocarcinoma.

Nikta Feizi[1], Eugenia Flores-Figueroa[2], Karen Ng[2], Zhen-Mei Liu[2], Farnoosh Abbas Aghababazadeh[2], Gun Ho Jang[3], Daniela Bevacqua[2], Stephanie Ramotar[2], Shawn Hutchinson[2], Anna Dodd[2], Julie Wilson[3], Robert Grant[1], Ronan Mclaughlin[2], Erica Tsang[1], Jennifer Knox[1], Steven Gallinger[1], Benjamin Haibe-Kains[1], Faiyaz Notta[1]

[1]University of Toronto, Toronto, ON, Canada,[2]University Health Network, Toronto, ON, Canada,[3]Ontario Institute for Cancer Research, Toronto, ON, Canada

Introduction: High-throughput drug screening enables rapid testing of numerous drugs on tumor samples and has achieved breakthroughs in treatment selection for fatal diseases such as acute myeloid leukemia. This study leverages high-throughput screening to identify molecular vulnerabilities in pancreatic ductal adenocarcinoma (PDAC), a highly lethal cancer with a 5-year survival rate of just 13%.

Methods: Using PDAC patient-derived organoids (PDOs), we developed a platform to screen approximately 3,000 compounds at a single dose and 600 compounds across six doses on 120 PDOs. Additionally, we designed a tailored focused screening of 22 drugs at 20 doses, prioritizing Health Canada-approved drugs identified as promising in earlier screenings. To ensure robust data analysis, we developed an optimized bioinformatics pipeline incorporating a three-tier evaluation: Area Under the Curve (AUC), Drug Sensitivity Score (DSS), and maximum efficacy (Emax). Results from each PDO were systematically compared against the entire cohort, ensuring that sensitivity profiles reflect reliable outcomes. Additionally, we integrated drug response data with genomics and transcriptomics obtained through whole-genome and whole-exome sequencing of PDOs.

Results: We identified highly potent compounds capable of suppressing tumor growth in over 55% of PDOs, prioritizing clinically approved drugs to facilitate rapid translation to patient care. Drug sensitivity patterns from single-dose screenings were corroborated through six-dose validation and a focused screening platform. By integrating drug response data with multi-omics analyses, we uncovered patient-specific drug response profiles linked to molecular vulnerabilities. Notably, we validated a gene-drug association involving anagrelide, a selective agent that induces cytotoxicity in cancer cells with elevated phosphodiesterase PDE3A levels, highlighting the robustness of our approach. Preliminary findings demonstrate a strong concordance between PDO-derived and patient drug responses, establishing a foundation for actionable clinical insights in the ADOPT trial to guide personalized treatment strategies.

Conclusion: In conclusion, our high-throughput drug screening platform, integrated with multi-omics analysis and robust bioinformatics, enables the identification of patient-specific molecular vulnerabilities in PDAC. This approach advances the potential for precision oncology, providing a foundation for tailored therapeutic strategies in this highly lethal cancer.

#3162 New hit compounds for p53 identified with a CETSA based high throughput screen.

Tomas Friman, Merve Kacal, Laurence Arnold, Stina Lundgren, Daniel Martinez Molina

Pelago Bioscience AB, Solna, Sweden

The p53 tumor suppressor protein is of central importance in tumor biology because of its role as guardian against neoplastic growth. The importance of p53 is highlighted by the frequency of mutations that have been identified in cancers. Most of these mutations are inactivating the original functions of the protein but some mutations also provide p53 with new functions that act pro-tumorigenic. Therefore, therapeutic strategies and initiatives that either restore the original function of p53 or limit gained functions have been applied. Numerous compounds have been reported in the literature to modulate p53 function. Still, to date, no project has proven successful in the clinical setting, which highlights the difficulty of finding compounds to manipulate p53. Reasons for the undruggable nature of p53 comprise the absence of accessible deep pockets and its lack of enzymatic activity. To understand the nature of the interaction between a set of reference compounds and p53, we subjected these molecules to a high throughput (HT) assay based on the Cellular Thermal Shift Assay (CETSA). Three different breast cancer cell lines with different p53 mutational status were used for this study: SK-BR-3 (p53R175H), BT-474 (p53E285K), and MCF-7 (p53WT). We generated ten-point concentration - response curves for all compounds in each of the three cell lines. In addition, knowing the complex nature of both p53 and its central role in the cell, we ran the assay both in lysate and intact cells. Target engagement could be established for some of the compounds in intact cells but was lacking in lysate. There was also a cell type specific variation in effects of the compounds. As an example, Butein exhibited single digit micromolar potency in intact SK-BR-3 cells, whereas there was very little effect in MCF-7 cells, which could be attributed to the difference in p53 mutational status between these cells. To learn more about the reference compounds and to investigate if their effects could be attributed to other cellular protein interactions, we subjected them to a proteome wide CETSA profiling, using mass spectrometry as a read out. The target engagement profile of the tested compounds was as diverse as their chemical structures, with very little overlap. Next, we again turned to the CETSA HT platform to screen a library of low molecular weight compounds aiming to identify new hit compounds for mutant p53. The screen was successful in identifying compounds that induced a thermal shift of mutant p53 in intact SK-BR-3 cells. These hits were then confirmed by QPCR based efficacy assays which showed effects on p53 regulated transcription. Here we highlight the applicability of CETSA in investigating the relevance of annotated tool compounds and how the properties of CETSA make it well-suited for screening for new hit matter, also when aiming for notoriously hard to drug targets.

#3163 Discovery of highly selective ligands for the Bcl-2 family of proteins using DNA-encoded chemical libraries.

Ying Zhang, Sana Bazzaz, Corey Bienstock, Paolo A. Centrella, Marie-Aude Guie, John P. Guilinger, Anthony D. Keefe, Matthew A. Clark

X-Chem Pharmaceuticals, Waltham, MA

DNA-encoded chemical library (DEL) technology enables the efficient screening of large collections of encoded compounds and has emerged as a powerful tool for hit identification. De Novo design and synthesis of non-traditional encoded libraries such as a reversible covalent compound library and beyond Lipinski's rule of five (bRo5) macrocyclic (MC) libraries have significantly expanded the chemical space available for DEL screening, and further enhances the feasibility of discovering highly selective ligands for challenging oncology targets. We will present examples of affinity-based ligand discovery targeting protein-protein interactions within the Bcl-2 family of proteins, specifically focusing on MCL1 and BFL1. We highlight the multiplexed DEL selection strategy employed and elucidate the molecular details around the uniqueness of the ligand interactions.

#3164 Micro-Tag^{TM} cell target engagement platform - From discovery to optimization.

Ivan Babic, **Elmar Nurmemmedov**

CellarisBio, San Diego, CA

Smartly designed drug discovery programs can amplify the success rate and accelerate the progress from hit discovery to optimization. Drug discovery for challenging or “undruggable” drug targets such as transcription factors necessitates the proteomic complexities of the cellular milieu for proper folding and function. Therefore, conventional drug discovery methods are not sufficient for most of these proteins. The incorporation of recent cell target engagement methodologies has helped improve drug discovery efforts. However, currently available high throughput cell target engagement methods are limited to measurement of end-point signal, poor utility across multiple challenging target families and insufficient resolution for kinetic and mechanistic readouts. Here we present MICRO-TAG^{TM} cell target engagement platform for challenging drug targets. We provide data demonstrating utility of MICRO-TAG platform for rapid discovery of novel interacting drug molecules to several challenging drug targets. The platform enables cross experimentation with the same cell reporter system for comprehensive hit identification, validation, lead identification and optimization employing several experimental strategies.

#3165 Leveraging chemoinformatics and high-throughput screening for the identification of novel ubiquitin specific protease 7 (USP7) inhibitors.

R. Oliveira[1], C. Franco[2], M. Mano[3], A. Leal[4], K. Liby[4], J. Salvador[1];

[1]Faculty of Pharmacy - University of Coimbra, Centre for Innovative Biomedicine and Biotechnology & Center for Neuroscience and Cell Biology - University of Coimbra, Coimbra, Portugal, [2]Center for Neuroscience and Cell Biology - University of Coimbra, Coimbra, Portugal, [3]Centre for Innovative Biomedicine and Biotechnology & Center for Neuroscience and Cell Biology - University of Coimbra, Coimbra, Portugal, Coimbra, Portugal, [4]IU School of Medicine, Indianapolis, IN

Ubiquitin-Specific Protease 7 (USP7) is a member of one of the most largely studied families of deubiquitylating enzymes, proteases that play a key role in controlling the dynamics of ubiquitination within the cellular ubiquitome. USP7 plays a pivotal role in modulating the levels of multiple proteins, including tumor suppressors, transcription factors, epigenetic modulators, DNA repair proteins, and regulators of the immune response. This suggests USP7 as a promising druggable target that offers interesting new avenues for cancer therapy. Wherefore, the main goal of this study was the identification of promising small molecules that inhibit USP7 enzymatic activity. The work was conducted according to an integrated workflow combining Computer-Aided Drug Discovery (CADD) and High-Throughput Screening (HTS) of chemical libraries to discover and characterize selective USP7 inhibitors with new chemotypes and adequate pharmacological properties as potential drug candidates to be used for anticancer therapy. Such protocol screened a large set of databases, disclosing 9 novel USP7 hit compounds with *in vitro* USP7 inhibitory activities against both USP7 full-length and USP7 catalytic domain, displaying IC_{50} values between 4.5 μM to 33 μM. The dose-response curves of these compounds tested against cancer cell lines showed promising selective cytotoxicity in breast and lung cancer cells, with IC_{50} values ranging from low nanomolar to micromolar. These results highlight the utility of using CADD and HTS in the early steps of drug discovery and pave the way for the identification of novel USP7 inhibitors that might represent a steppingstone for cancer treatment.

#3166 High-throughput screening and triage assays for the identification of PPI disruptors in cancer cells.

A. Dos Santos, R. De Gioia, A. Segnali, D. Zian, C. Nucci, M. Catalani, A. Molteni;
Axxam, Bresso, Italy

Epigenetic and transcriptional dysregulations play pivotal roles in cancer development, progression aggressiveness, and recurrence of malignancies. In collaboration with a pioneering stealth company sprouted from the academic world, we leveraged an innovative drug discovery platform designed to target the disruption of the target protein-protein interaction (PPI). Using a luminescence complementation assay, we identified small molecules capable of selectively disrupting the target PPI within cells (native conformation, *i.e.* target assay) as well as disrupting the interacted proteins in cellular extracts. Over 4.10^5 compounds were screened using the target assay, successfully adapted to a 384-well plate format, in the search of a specific compound disrupting the native conformation of the PPI. An overall statistical hit rate of 0.4% (cut-off ranging between 46%-57% inhibition, depending on the collection tested) was obtained at the completion of the compounds' libraries screening tested at one concentration (20 µM). Up to 1% of screened compounds were selected for further validation via dose response assessment on both the target and the counter-screening assays (i.e. unrelated protein-protein interaction related to cancer progression). Among the 5,000 compounds promoted, 184 compounds were identified as putative selective disruptors of the target, while 107 were selected for further assessments due to a > 0.7 log shift in IC_{50}. Similarity search using Tanimoto coefficient from confirmed hits was performed to select additional analogs compounds from the original pool. A total of 1,279 confirmed hits and their analogs were promoted to potency evaluations on the target and three counter-screening assays. These assays included dynamic interactions in cellular assays and protein disturbance in cell lysates, confirmed the activity on the target assay of more than 60% of the compounds. Notably, 27 hits were confirmed as specific in the native conformation assays, while 88 compounds demonstrated specificity in lysate-based assays. Among these, seven compounds were validated as selective and specific disruptors, and one compound showing a >1 log shift in IC_{50} between target PPI and unrelated cancer PPI. A subset of compounds has undergone further validation through co-immunoprecipitation and target-specific in vitro growth arrest models, showing encouraging results. Following a well-defined screening cascade that integrates bioactivity in cancer-specific cell lines with cytotoxicity assays in non-transformed cells, the compounds will be further triaged to select the best qualified hits. Targeting PPI directly or indirectly has been a challenging and long-standing goal in cancer research. By integrating diverse expertise and resources, from academia and industry, this drug development program has not only been accelerated but it was enhanced by the robustness of the findings.

#3167 Longer and brighter live cell bioluminescence prosubstrates.

C. Gao[1], B. Binkowski[2], H. Wang[1], W. Zhou[1];
[1]Promega Corporation, San Luis Obispo, CA, [2]Promega Corporation, Madison, WI

Bioluminescence prosubstrates are chemically protected bioluminescence substrates which, upon introduction to cells, can gradually and slowly release free substrate for generating luminescent signals in cellular assays. Prosubstrates can generate steady signals that last for several hours or days, enabling live-cell, kineticassays. Noticeable examples of bioluminescence prosubstrates include Nano-Glo Endurazine and Vivazine Live Cell Substrates. Although these prosubstrates have been serving the scientific community for years, they still have drawbacks, including reduced brightness, sensitivity, and signal duration. Herein, we describe a new generation of live cell bioluminescence prosubstrates that can overcome the drawbacks of current prosubstrates.

#3168 High-throughput hybridoma monoclonality characterization using the Celigo™ Image Cytometer.

M.-S. Lin[1], **C. Franco Nitta**[2], M. Pierce[2], B. Lin[2], L. L. Chan[2], J. Ni[1];
[1]BioLegend, San Diego, CA, [2]Revvity, Lawrence, MA

Hybridomas are hybrid cells produced by fusing an antibody producing B cell with a myeloma cell to produce a specific monoclonal antibody. In the process of generating stable clones, developers are often required to perform several rounds of subcloning by plating cultures with a density of a single cell per well. The wells with a single colony are then selected to produce the target antibodies. Traditionally, this lengthy process was heavily relied on researchers with extensive training and experience; however, even experienced researchers may suffer from human error of selecting clones that are not truly monoclonal, thus potentially delaying antibody development and increasing costs. In this work, we demonstrate a high-throughput solution that has successfully established monoclonality of our hybridoma by employing the Celigo™ Image Cytometer (Revvity Health Sciences, Inc., Lawrence, MA) with the robotic arm accessory to screen potential candidates for downstream antibody production under a GMP environment. The Celigo™ is a plate-based, whole well, high-throughput image cytometry system with brightfield and fluorescence imaging capabilities. In combination with the robotic arm, automation can be implemented to further streamline the monoclonality workflow, and subsequently reduce subcloning personnel time by 25-50%, while also minimizing human handling errors by at least 10-fold. Following imaging acquisition, an in-house monoclonality analytical software was utilized to provide concrete documentation for every clone selected for production. Lastly, we demonstrated that time for hybridoma development in clone confirmation screening as well as subcloning could be further improved by incorporating ClonePix technology.

#3169 Dependency-agnostic drug discovery: A dual-screening strategy to uncover novel cancer vulnerabilities.

Olasunkanmi Olaoye[1], Colleen Harrington[2], Kenneth N. Ross[1], Allen T. Basanthakumar[1], Jamie O. Brett[3], Shakchhi Joshi[1], Jonathan H. Shrimp[4], Min Shen[4], Lucy Merickel[1], Audrey Taillon[1], Silvi Salhotra[1], Bo Kyung A. Seong[1], Ji Seul Han[3], Yara R. Zabala[1], Ashleigh Meyer[1], Qi Liu[1], Luc de Waal[2], Jun Qi[1], Matthew D. Hall[4], Marcia C. Haigis[3], Jennifer Roth[2], Kimberly Stegmaier[1]

[1]DFCI/Harvard Medical School, Boston, MA,[2]Broad Institute, Boston, MA,[3]Department of Cell Biology, Harvard Medical School, Boston, MA,[4]National Center for Advancing Translational Sciences, Rockville, MD

The current landscape of small-molecule drug discovery prioritizes genetic dependencies considered as viable protein targets. While this approach has driven significant therapeutic advancements, it has also constrained the exploration of non-dependency targets harboring tractable vulnerabilities. Moreover, many genetic dependencies remain undruggable, precluding their therapeutic exploitation and leaving critical gaps in addressing cancers with unmet clinical needs. To overcome these limitations, we developed a tandem screening approach that integrates large-scale chemical and genetic screens to identify novel vulnerabilities independent of traditional dependency paradigms. Using PRISM, a multiplexed drug-screening platform, we identify compounds with selective toxicity against specific cancer lineages, revealing novel biology readily amenable to small-molecule drug discovery. Subsequent genome-wide CRISPR screens elucidate the mechanistic underpinnings of these compounds, offering actionable insights into new therapeutic opportunities. We applied this approach to Ewing sarcoma, an aggressive bone tumor characterized by the fusion oncoprotein EWS-FLI1, a known dependency that drives epigenetic reprogramming and disease progression. Despite its critical role in disease initiation and maintenance, therapeutic targeting of EWS-FLI1 has remained elusive, underscoring the urgent need for alternative strategies. From our PRISM screen of 4,518 compounds across 935 cancer cell lines, including 17 Ewing sarcoma lines, we identified several compounds with selective toxicity against Ewing sarcoma. Chemical clustering and mechanism-of-action analysis revealed diverse chemical motifs and biological targets, none of which were directly linked to Ewing sarcoma dependencies. A CRISPR rescue screen with three top compounds identified a shared vulnerability: UGT3A2, a UDP-glucose transferase specifically expressed in Ewing sarcoma under the transcriptional control of EWS-FLI1.Our mechanistic studies revealed that UGT3A2-mediated glycosylation of these compounds depletes intracellular UDP-glucose, disrupting glycolysis and triggering ER stress via the PERK-EIF2A pathway, culminating in cell death. This unique vulnerability of UGT3A2, while not a dependency itself, represents a tractable target for therapeutic exploitation in Ewing sarcoma. Building on these findings, we have developed a library of lead compounds exploiting the vulnerability imposed by UGT3A2 in Ewing sarcoma and are currently advancing them in later-stage preclinical optimization. This study underscores the potential of our tandem screening approach to uncover vulnerabilities in cancers with undruggable targets and unmet clinical needs. Beyond Ewing sarcoma, this platform offers a promising strategy to expand the therapeutic landscape for difficult-to-treat malignancies.

#3170 DMSO 2.0 - An advanced DMSO product for 2D and 3D cell culture of PROTACs and hydrophobic drugs.

Ketan Patel

St. John's University, Queens, NY

Dimethyl sulfoxide (DMSO) is the most commonly used aprotic solvent for solubilizing poorly water-soluble molecules in cell culture assays. It is an inert, biocompatible solvent with high solubilization capacity. In the last decade, there has been a big parade shift in cancer drug discovery with the development of next-generation cancer therapeutics, especially oncogenic protein-specific small molecules and targeted protein degraders, also known as Proteolysis Targeting Chimera (PROTACs). Such novel drugs are efficiently degrading/inhibiting the key oncoproteins that remained “undruggable” for many years before 2010 e.g., KRAS, PARP, BRAF, etc. Though very efficient, the majority of them fall in beyond Lipinski rule 5 space (bRo5) due to extreme hydrophobicity. Due to poor solubility and rapid precipitation tendency, such molecules pose great challenges during *in vitro* cell culture and preclinical testing. In our lab, we have observed that stock solution of anticancer molecules (BRD4 PROTAC, EGFR PROTAC, BRD4/MDM2 PROTAC, Sorafenib, Adagrasib, Trametinib, Vemurafenib, Lenvatinib) prepared in DMSO showed immediate to sustained precipitation of drug in DMEM/RPMI media with 5-10% serum. It resulted in significant variation and inaccuracy in cytotoxicity assays. Precipitation of the molecule within the tip (while mixing it from stock solution to media) led to inaccuracy in concentration. Thus, designing and developing an advanced DMSO formulation for testing such novel anticancer molecules is imperative. After screening over 15 hydrophilic adjuvants and their combinations, DMSO 2.0 was developed and tested using the above anticancer molecules. 50 mM stock solution of molecules was prepared in DMSO and DMSO 2.0, followed by addition into cell culture media. Precipitation was monitored by microscopy and high-performance liquid chromatography (HPLC) method. DMSO 2.0 led to the easy mixing of stock solutions with the media by preventing the precipitation of molecules within the tip. Most importantly, DMSO 2.0 led to marked inhibition/slowing down the precipitation of molecules in media compared to DMSO. Precipitation was inhibited at as high as 50 µM and all the subsequent dilutions. DMSO 2.0 showed no apparent cytotoxicity at a concentration below 0.5% v/v in lung cancer cells. Overall, DMSO 2.0 could be a promising solvent for cancer drug discovery, and its drug-specific composition will provide crucial insight into early formulation development strategy for preclinical testing.

CLINICAL RESEARCH: Adoptive Cell Therapy 1
Poster Session

#3173 JL-lightning CAR-T, a next generation non-viral CAR-T without *in-vitro* culturing procedure, shows high clinical efficacy, good safety with low treatment dosage.

Shuya Wang[1], Yan Sun[1], Jinxing Lou[2], Hongfan Zhu[2], Zhicai Lin[1], Dan Sun[1], Jiaguo Li[1], Lijie Rong[1], Jun Guo[1], Jinjing Zhang[1], Yi Liu[1], Wenfeng Xu[3], Qijun Qian[1]

[1]Shanghai Cell Therapy Group Co., Ltd, Shanghai, China,[2]Shanghai Mengchao Cancer Hospital, Shanghai, China,[3]Chantibody Therapeutics Inc., Menlo Park, CA

Background: CAR-T with high in vivo proliferation and long persistency could benefit hematologic malignancy patients with low relapse and long survival. Previous studies reveal that CAR-T stemness (T-stem) and exhaustion level are major determinants of CAR-T quality that contribute to clinical outcome. However, conventional manufacturing process with 9-14 days *in vitro* culture sacrifices T-stem and prolong the vein-to-vein process. Researchers have tried to shorten this process, but many failed due to T-cell activation insufficiency, transfection toxicity, and poor quality control, especially in non-viral transfection.

Methods: Two versions of non-viral JL-Lightning-CAR-T fast manufacturing process have been developed to shorten *in vitro* culture process, V-30h and V-6h. Of note, there is no culture after transfection to enhance CAR-T-stem and *in vivo* expansion. JL-transposon has been developed and proved with high transfection efficiency and low toxicity on both activated and resting T cells. We generated a tri-targeting CAR-T (BZE2204) using VHHs against CD19, CD22 and BCMA, then evaluated its safety and efficacy with both V-30h and V-6h *in vitro* and *in vivo* using Raji orthotropic model. One investigator-initiated trial (IIT) is designed to assess the safety and preliminary efficacy of BZE2204 CAR-T in relapsed/refractory Non-Hopkins Lymphoma (r/r NHL) patients.

Results and Conclusion: *In vitro* experiments showed both versions of BZE2204 exerted strong cytotoxicity on CD19-, CD22- and BCMA-positive tumor cells with high CAR-T cell expansion rates, superior stemness and low exhaustion phenotype compared with conventional CAR-T. V-6h, which has the shortest process, displayed the highest T-stem and proliferation among all groups. *In vivo* models showed V-30h BZE2204 inhibited tumor growth and significantly prolonged mice lifespan under super low dosage (1E5 CAR-T/mouse). Three r/r NHL patients with high tumor burden were enrolled and infused with the dosage of 40-fold lower than conventional CAR-T. The data showed that CAR-T BZE2204 had promising clinical response with manageable safety profile, two achieved complete remission (CR) within 1 month, and the third patient with a single tumor over 7000mm^2 in-size, achieved partial remission (PR) with >80% of tumor shrinkage. Pharmacokinetics data showed high CAR-T proliferation with Cmax of 5066 CAR-T cells/ul and AUC_{0-28} of 46,486 cells*d. Our results show JL-Lightning CAR-T achieves high anti-tumor efficacy and good safety in large tumor-burden r/r NHL patients with super low dosage, likely due to short non-viral manufacturing process, high stemness and proliferation, and VHH-based multi-targeting design in CAR-T product. It offers a more effective, affordable and short vein-to-vein process option for hematologic malignancies CAR-T treatment.

#3174 BiTE® molecule vs CAR-T targeting FLT3 in AML: How positive co-stimulation tips the scale.

Lisa Rohrbacher[1], Daniel Nixdorf[1], Helena Stadler[1], Bettina Brauchle[1], Florian Markl[1], Adrian Gottschlich[1], Gordon Hoffmann[1], Nora Philipp[1], Gerulf Hanel[1], Martin Kirmaier[1], Anetta Marcinek[1], Maryam Kazerani[1], Alica-Joana Emhardt[1], Giulia Magno[1], Rebecca L. Goldstein[2], Sebastian Theurich[1], Tobias Straub[3], Sebastian Kobold[1], Tara Arvedson[4], Veit L. Bucklein[1], Marion Subklewe[1]

[1]LMU University Hospital, LMU Munich, Munich, Germany,[2]AMGEN Inc., South San Francisco, CA,[3]Core Facility Bioinformatics, Biomedical Center, LMU Munich, Munich, Germany,[4]AMGEN Inc, South San Francisco, CA

T cell-based immunotherapy has revolutionized the treatment of B-cell malignancies, yet applying it to acute myeloid leukemia (AML) is challenging due to difficulties in identifying suitable target antigens without on-target off-leukemia toxicity. Prior studies identified FLT3 as a promising target antigen with restricted expression in the healthy hematopoietic compartment[1]. Here, we evaluate a FLT3-directed BiTE® molecule and 2nd generation FLT3-specific CAR T cells in a preclinical AML model. We hypothesize that positive costimulatory molecules on AML cells enhance BiTE® molecule effectiveness, while CAR T cells may be less dependent due to their built-in costimulatory domain. Cytotoxicity was assessed in cocultures over time using MPFC. The impact of positive costimulation was tested using our established Ba/F3 model system lacking any human costimulatory molecules. On-target off-leukemia toxicity was evaluated in cytotoxicity and CFU assays of pAML cells and healthy bone marrow (hBM). T-cell exhaustion was examined in a longterm culture system with continuous antigen exposure. Lastly, xenograft AML models and RNA sequencing explored differences between the two platforms *in vivo*. BiTE® molecule and CAR effectively mediated cytotoxicity against various AML cell lines and pAML cells. Overexpression of the costimulatory CD86 on the target cells significantly increased BiTE® molecule-mediated cytotoxicity cells, while CAR-mediated lysis was unaltered. In both scenarios, low E:T-dependent toxicity was observed against hBM. Mixing experiments showed efficient pAML cell lysis with minimal impact on hBM. CFU assays indicated no effect on hematopoiesis of healthy $CD34^+$ stem cells. 28-day cocultures led to T-cell exhaustion in both settings, marked by reduced proliferation, cytokine secretion, metabolic fitness, and cytotoxicity. CAR T cells demonstrated superior antileukemic activity, with enhanced proliferation and splenic homing in an AML xenograft model. RNA sequencing revealed more exhaustion-related markers in BiTE® molecule-redirected T cells, while CAR T cells showed upregulated glycolysis and fatty acid metabolism. Repeating our *in vivo* xenograft experiment with CD86-overexpressing AML cells supported our hypothesis that costimulation might be a key factor providing a head start to CAR T cells by increasing T-cell proliferation and persistence *in vivo*. Our *in vitro* data show similar cytotoxicity and specificity, while xenograft mouse models suggest CAR T cells offer a significant survival advantage over BiTE® molecules. Our data support the hypothesis that positive costimulation integrated within the CAR constructs boosts T-cell activation, homing, and efficacy, thereby delaying T-cell exhaustion *in vivo*. Future studies are needed to further dissect differences between BiTE® molecule vs CAR T cell-based immunotherapy and identify suitable patient groups. [1]Brauchle et al 2020

#3175 Exploring the potential of CEA-CAR-NK cells in colorectal cancer.

Alice D'Andrea[1], Marco Cortese[2], Enzo Medico[1]

[1]Candiolo Cancer Institute, FPO IRCCS, University of Torino, Torino, Italy,[2]Oncology, Candiolo Cancer Institute IRCCS, Candiolo, Italy

Chimeric Antigen Receptor (CAR) therapy targeting carcinoembryonic antigen (CEA/CEACAM5) is a promising strategy for colorectal cancer (CRC). CAR-T cells, however, carry significant side effects, and the fact that CEA is also expressed at low levels in normal tissues makes CAR-NK cells a safer alternative, offering reduced toxicity while maintaining effective anti-tumor activity. Despite this potential, CAR-NK systems targeting CEA remain underexplored. In this study, we generated and evaluated clones of the NK-92 cell line expressing two CAR constructs with distinct affinity for CEA: BW431/26 (moderate affinity) and hMN-14 (high affinity). The BW431/26-based CAR, with its lower affinity, may offer a safer profile by reducing potential on-target, off-tumor effects. On the other hand, the hMN-14-based CAR demonstrated superior cytotoxicity in *in vitro* studies due to its higher affinity. Sorted populations and clones of CAR-NK cells transduced with these constructs were evaluated for killing activity both *in vitro* and *in vivo*. *In vitro*, the high affinity hMN-14 CAR exhibited enhanced killing capacity compared to the BW431/26 CAR. However, *in vivo* experiments on CRC xenografts in NOD/Scid mice showed comparable tumor growth inhibition between the two constructs. These findings suggest that higher scFv affinity does not necessarily translate to improved *in vivo* efficacy of CEA-CAR-NK cells, emphasizing the multiplicity of factors influencing their activity—such as persistence and activation—and highlighting the need for further modifications to optimize their functionality.

#3176 CD19/CD22 bispecific CAR-T cells are effective in CD19 loss B-cell tumor models.

Jiao Jiao

Gan & Lee Pharmaceuticals, Beijing, China

Background: Chimeric antigen receptor T (CAR-T) cell therapy targeting CD19 can significantly improve the long-term survival rate of patients with relapsed or refractory leukemia. However, CD19 absence or loss leads to resistance of the B-lymphoblastic leukemia cells to CD19 CAR-T cells and the patients will relapse. To solve the problem, we developed bispecific CAR-T cells that target CD19 and CD22 and evaluated their antitumor effects.
Methods: The CD19/CD22 bispecific CAR-T cells were generated through the transduction of lentiviruses that simultaneously carry the genes for both CD19 CAR and CD22 CAR, including an anti-CD19 scFv, an anti-CD22 scFv, a CD8 hinge region, a CD8 transmembrane region, a 41BB intracellular region and a CD3ζ intracellular region. In vitro tumor killing assay on NALM6 and Ramos cells and in vivo efficacy studies on Ramos cell line-derived xenografts (CDX) were performed.
Results: In in vitro studies, the CD19/CD22 CAR-T cells killed wild-type (WT) NALM6 and WT Ramos cells effectively like CD19 CAR-T; the CD19/CD22 CAR-T cells but not CD19 CAR-T cells killed NALM6 cells with loss of CD19 protein but expression of CD22 protein (CD19-CD22+) and CD19-CD22+ Ramos cells effectively. In in vivo studies, the CD19/CD22 CAR-T cells significantly prolonged the survival of mice bearing CD19-CD22+ Romas cells than CD19 CAR-T.
Conclusion: The CD19/CD22 bispecific CAR-T cells effectively kill B-cell tumors with CD19 loss both in vitro and in vivo, which will have a superior therapeutic effect on patients with relapse due to loss of CD19 protein.

#3177 Advancing CAR T-cell therapy in AML: Enhancing specificity and bone marrow homing via chemokine receptor engineering.

Lis Winter[1], Katharina Konnerth[1], Anne-Sophie Neumann[1], Luisa Schmid[1], Stefanos Michaelides[2], Yingshuai Wang[1], Louisa von Baumgarten[3], Sebastian Kobold[2], Veit L. Bucklein[1], Marion Subklewe[1]

[1]Department of Medicine III, University Hospital, LMU Munich, Munich, Germany,[2]Division of Clinical Pharmacology, Department of Medicine IV, University Hospital, LMU Munich, Munich, Germany,[3]Department of Neurosurgery, University Hospital, LMU Munich, Munich, Germany

To date, the success of CD19-directed CAR T cells in B-cell malignancies has not been replicated in other hematological or solid malignancies. Key hurdles include the absence of suitable target antigens and limited tumor site homing. CAR T-cell efficacy can be enhanced by engineering T cells to express receptors specific to chemokines released by cancer or cancer-associated cells[1]. Thus, we aimed to identify receptors that promote directed migration to the bone marrow niche, thereby enhancing CAR T-cell efficacy in AML. Köhnke et al.[2] identified upregulated expression of CCR2 and CXCR2 on the surface of AML cells via mass spectrometry, which we confirmed on primary AML cells using MPFC. Next, we used a retroviral-based transduction to engineer αCD33-28ζ-CAR T cells to co-express CCR2 or CXCR2. We analyzed $CCR2^{high}$- or $CXCR2^{high}$-CD33 CAR T-cell mediated cytotoxicity against the AML cell lines MV4-11 and MOLM-13 by MPFC. Next, we evaluated the specific migration towards the ligands CCL2 and CXCL8, conditioned AML cell line medium as well as towards bone marrow serum from primary AML samples using transwell assays and 3D migration chambers. The engineered $CCR2^{high}$- and $CXCR2^{high}$- CD33 CAR T cells showed no significant phenotypic differences compared to CD33 CAR T cells, as shown by activation or exhaustion marker expression or T-cell subset formation. To our surprise, we observed a reduced target cell killing together with reduced inflammatory cytokine secretion at high E:T ratios (1:20 & 1:30) with the $CCR2^{high}$- or $CXCR2^{high}$- CD33 CAR T compared to CD33 CAR T cells. These effects were more prominent in MOLM-13 than MV4-11 cells and we are currently investigating the underlying mechanism. Next, we tested for the migratory capacity towards the specific ligands CCL2 and CXCL8. Enhanced migration, cell velocity and speed of $CCR2^{high}$- and $CXCR2^{high}$-CD33 CAR T cells were observed compared to CD33 CAR T cells. As expected by the surfacome data of primary AML cells, we identified CCL2 and CXCL8 to be highly abundant in the bone marrow serum of AML patients. Accordingly, transwell assays using bone marrow serum of primary AML patients demonstrated a higher targeted migration of $CCR2^{high}$- and $CXCR2^{high}$ CD33 CAR T cells compared to CD33 CAR T-cell controls. Taken together, we demonstrated that $CCR2^{high}$- and $CXCR2^{high}$- CD33 CAR T cells show enhanced specific migration towards their ligands but also towards AML bone marrow serum. Surprisingly, we detected a reduction in specific lysis after transduction of the chemokine receptors into CD33 CAR T cells. Further studies are needed to understand the influence of the chemokine receptors on killing capacity. Next, we aim to validate these findings in a model system permitting *in vivo* live-cell imaging.

1. Biondi M et al.; Blood Journal 2023:blood. 20220183302. 2. Köhnke T et al.; Blood 2017;130:3968

#3178 Discovery and characterization of small molecules to enhance NK cell cytotoxicity against solid tumors.

Indrani Das, Olubukola Abiona, Riufu Liu, David N. Wald

Case Western Reserve University School of Medicine, Cleveland, OH

Adoptive cell therapies (ACT), such as CAR-T and NK therapy, have transformed hematologic cancer treatment but show limited efficacy against solid tumors. NK cell therapies in particular have an excellent safety profile due to their specific, innate recognition of cancer cells, but fail to elicit adequate and sustained tumor clearance *in vivo*. To address this hurdle, we developed a high-throughput phenotypic small molecule screen to identify novel compounds that can enhance NK cell cytotoxicity against tumor cells.
We screened 300,000 compounds for their ability to enhance NK cytotoxicity against ovarian cancer cells (OVCAR-3). Our primary screen and subsequent hit validation identified 11 lead compounds that increase NK cell killing of OVCAR-3 cells (>10% increase over vehicle-treated control). We performed secondary screening using a flow cytometry-based assay on 5 of these 11 compounds to date, confirming that 3 significantly enhance NK cytotoxicity against OVCAR-3. After completing the secondary screening of the remaining 6 compounds, we will identify the most promising lead compound(s) through a series of secondary and orthogonal assays to characterize the biological activity and toxicity profile of validated hits. We will then pursue medicinal chemistry efforts to increase potency of our best lead compound, coupled with mechanistic studies for how the compound promotes NK cytotoxic activity. Ultimately, we hope to translate our findings into NK cell therapy clinical trials to achieve greater efficacy against solid cancers.

#3179 A comprehensive serial killing workflow for assessment of CAR T-cells.

Xiaoyu Zhang, Jyothi Thyagabhavan Mony, Natalia Romero

DGG, Agilent Technologies, Inc., La Jolla, CA

Adoptive cell therapy with chimeric antigen receptor T cells (CAR-T cells) has been highly successful in individuals with hematological malignancies. However, its efficacy against most solid tumors remains elusive due to short-term persistence and inadequate expansion of CAR T-cells within the tumor microenvironment. Traditional in vitro cytolysis assays often fail to reflect the true antitumor potential of the CAR T cells, as they typically involve brief co-culture periods and assess CAR T-cell performance only from a single perspective. To address these limitations, we developed a long-term multiplexed workflow that can robustly and comprehensively assess CAR T cell products. In our study, the EpCAM CAR T-cells were repeatedly challenged with an EpCAM expressing breast cancer cell line, T47D cells, over seven consecutive rounds. During each challenge, the EpCAM T-cells were directly added to the T47 D cells cultured on an E-Plate VIEW 96 (E-Plate) for 24 hours, at the effector to target ratio (E:T) of 0.5 :1. The CAR T-cell cytotoxicity against T47D cells was continuously measured over three days using an xCELLigence RTCA eSight system (eSight). After each co-culture round, the CAR T-cell metabolism, immunophenotype and percentage were evaluated using Agilent XF Pro analyzer and NovoCyte flow cytometer respectively. Following assessment, the challenged CAR T-cells were transferred to a new tumor cell-containing E-Plate to repeat the assay sequence. Our results indicate that: 1) although the percentage of CAR T-cells progressively increased from 25% to 80% by the fifth challenge round, a significant decline was observed in the sixth round; 2) CAR T-cells exhibited a gradual reduction in proliferation and cytotoxicity against tumor cells, along with an increase in the PD1+Tim3+ population across repeated challenges; and 3) CAR T-cells demonstrated a distinct metabolic profile, characterized by progressively decreased maximal respiration, spare respiratory capacity (SRC), and ATP production rate throughout the successive challenges. In conclusion, this multiplexed workflow enables comprehensive evaluation of CAR T-cell proliferation, killing potency, exhaustion phenotype, and metabolic profile over repeated tumor cell challenges, providing a more accurate prediction of their in vivo therapeutic efficacy.

#3181 Single cell characterization and anti-tumor efficacy of single & multi-armored CAR Ts against CLDN18.2.

Megha Sah, Liang He, Gundula Povysil, Cassandra Kontur, Ashley Chui, Stephen Pu, Annie Dyatel, Malavika Sabu, Alice HK Kwon, Xinchen Wang, **David Phizicky**

Waypoint Bio, Inc., New York, NY

Adoptive cell therapies such as CAR T have struggled to translate to solid tumors due to challenges posed by the solid tumor microenvironment (TME) and the low-throughput of *in vivo* testing. Pooled CAR T screens are typically restricted to single readouts (e.g. proliferation) and cannot measure the cell-cell and cell-TME interactions that are critical for CAR T success in solid tumors. We performed *in vivo* spatial pooled screening to profile >250 different CAR T designs to identify efficacious constructs based on spatial-dependent CAR T properties and CAR T-TME interactions. Mouse xenograft models of pancreatic cancer were each injected with hundreds of unique CAR T constructs, including variations in CAR designs and armors. For each library, we prepared 30-40 FFPE slides, harvested from different mice across time points and treated with CAR T cells from different donors. Each slide was stained for 20-30 protein markers, and we also performed *in situ* genotyping to determine CAR T construct identities in the same microscope images. We obtained image-based phenotypes of ~20M total single cells and used custom computer vision pipelines to define single cell spatial phenotypes for each CAR T design, including cytotoxicity, exhaustion, local-neighborhood phenotypes, tumor infiltration, on-target/off-tumor toxicity, proliferation, and activity in immunosuppressive tumor regions. We evaluated selected screen hits in single-construct studies, and trained a random forest model with cross-validation to identify the combination of single cell spatial phenotypes for CAR T designs that were most predictive of tumor shrinkage in the efficacy studies. Overall, we identified multiple single- and double-armored constructs that had superior spatial single cell-level phenotypes (e.g. improved infiltration, increased persistence, decreased PD1/LAG3, etc.) compared to both unarmored 2nd gen CLDN18.2 CAR Ts and TGFβRIIDN-armored CLDN18.2 CARs. We then confirmed superior anti-tumor efficacy of these CAR modifications and armors in multiple xenograft models, at multiple doses ranging from $0.5x10^6$ to $2x10^6$ cells. Combinations of these top armors will be further tested in WP-001, a CAR T construct for solid tumors expressing CLDN18.2.

#3182 Profiling of B7-H3 CAR T cells generated from patients with pancreatic ductal adenocarcinoma.

Giulia Cattaneo[1], Liti Zhang[1], Marco Ventin[1], Shahrzad Arya[1], Enrica Quattrocchi[1], Arsen Osipov[2], Andrew Hendifar[2], Russel W. Jenkins[3], Xinhui Wang[4], Cristina R. Ferrone[1]

[1]Department of Surgery, Cedars-Sinai Medical Center, Los Angeles, CA,[2]Department of Medicine (Medical Oncology), Cedars-Sinai Medical Center, Los Angeles, CA,[3]Department of Medicine, Mass General Cancer Center, Massachusetts General Hospital, Boston, MA,[4]Department of Surgery, Massachusetts General Hospital, Boston, MA

Background: Chimeric antigen receptor (CAR) T cells targeting B7-H3 represent a promising treatment option for pancreatic ductal adenocarcinoma (PDAC), but their application in patients with advanced-stage disease is challenging. CAR T cell fitness is key for therapeutic success, and it correlates with the quality of T cells obtained via leukapheresis. T cell quality can be affected by several factors, including prior therapies and global T cell suppression driven by high tumor burden. In this study we aim to identify immune determinants of CAR T cell fitness in PDAC.

Methods: Peripheral blood mononuclear cells (PBMC) were isolated from pre-operative blood of 9 PDAC patients and analyzed by flow cytometry. CAR T were generated from patients' PBMC by retroviral transduction with a second generation B7-H3-CAR. Results are described as median values and interquartile range.

Results: Of the 9 PDAC patients, 5 were treatment naïve (TN) and 4 had previously received neoadjuvant (NA) chemotherapy. Pathologic tumor size and CA 19-9 level were higher in NA patients than in TN patients (4.7cm [6.4-3.2] vs. 3.3cm [4.5-2.3] and 464 [700-110] vs. 54 [186-13], respectively). TN patients' PBMC presented with a higher T cell count, as compared to the NA group (CD4: 1251 cells/ul [2356-274] vs. 675 cells/ul [1175-512]; CD8: 434 cells/ul [3124-255] vs. 225 cells/ul [1090-140]). The number of circulating Treg was similar between the two groups, while the level of circulating myeloid derived suppressor cells was higher in NA patients as compared to TN patients (875 cells/ul [1317-452] vs. 285 [1147-59]). T cells isolated from TN patients demonstrated a superior proliferation ability upon either acute or chronic *in vitro* activation with anti-CD3 and anti-CD28 antibodies, and were characterized by a higher presence of early memory T cell subsets, as compared to NA patients (percentage of CD45RA+CD45RO-: 17% [31-10] vs. 14% [17-12] for CD4 , and 44% [72-36] vs. 31 [33-28] for CD8). B7-H3 CAR T generated from either TN or NA patients demonstrated a good transduction efficiency (80% [84-73] vs. 73% [82-70]). However, NA-B7-H3 CAR T had a reduced *in vitro* expansion rate than TN-B7-H3 CAR T (18-fold [30-10] vs. 15-fold [21-8]). When cocultured with the human PDAC2 cell line, TN-B7-H3 CAR T demonstrated a significantly higher antitumor activity as compared to NA-B7-H3 CAR T (percentage of PDAC cell killing: 70% [82-65] vs. 50% [63-42], p=0.028). At the end of the coculture, NA-B7-H3 CAR T displayed a more differentiated phenotype, indicated by a higher presence of effector memory CD8 T.

Conclusions: Our findings support the hypothesis that PDAC patients with a greater tumor burden present with a higher degree of T cell dysfunction and that this might hamper the fitness of the related CAR T product. This study provides the basis to further investigate mediators of CAR T cell biology to advance the therapeutic potential of CAR T cell therapy with PDAC.

#3183 GPRC5D and BCMA bi-specific CAR-T: Optimized CAR design to mitigate antigen escape and elicit deep and durable response in heterogeneous multiple myeloma.

Jincai Zhou, Xinran Luo, Kai Wu, Feifei Shi, Bixia Lei, Yanhong Xu, Joy Zhou, **Xiaowen He**

Oricell Therapeutics Co., Ltd., Shanghai, China

Introduction: BCMA CAR-T cell therapies have demonstrated superior efficacy, including in patients with extramedullary disease and high-risk cytogenetics, compared to BCMA-directed TCE or ADC. The longitudinal single cell analysis revealed that pre-existing T cell exhaustion and presence of highly proliferative plasma cells with Ag downregulation predisposed to early relapse post BCMA CAR-T. Moreover, shedding BCMA and suppressive TME also pose significant challenges for sustained clinical response. To tackle these obstacles, BCMA and GPRC5D bi-specific CAR-Ts were designed. GPRC5D is a promising therapeutic target for multiple myeloma (MM), as it - expresses exclusively on MM cells but not in normal tissues (except for hair follicles) and is independent of BCMA expression. Clinical studies confirmed the co-existence of single or double Ag positive MM subsets within the same patient, along with a rare occurrence of double-negative multiple myeloma plasma cells.
Methods: Aiming to broadly and deeply eradicate MM subsets and achieve long-term remission, we've developed bi-specific CAR-Ts exclusively targeting BCMA and GPRC5D. Our design incorporates novel VHH binders with optimized CAR design to enhance sensitivity and efficacy toward heterogenous MM cells in the presence of soluble BCMA, mitigate Ag escape, restore T cell functionality and improve expansion. We've conducted the pre-clinical investigations to validate the activity, functionality and druggability of the bi-specific CAR-Ts.
Results: Binding to GPRC5D and BCMA resulted in bi-specific CAR-T exhibiting improved immune cell activation, superior anti-tumor activity, and functionality over mono-CAR-T or benchmark, and enhanced target-dependent expansion under re-stress. Moreover, the bi-specific CAR-T retained its functionality against BCMA.KO or GPRC5D.KO cells, whereas the mono-CAR-T could not. Importantly, the optimized bi-specific CAR designs incorporating the novel BCMA VHH binder(s) showed robust cytotoxicity against MM cells with low Ag expression in the presence of high concentrations of soluble BCMA, suggesting their potential to effectively counteract the inhibitory effects of soluble BCMA in the serum of MM patients. Compared to mono-specific CAR-Ts, the bi-specific CAR-T showed robust efficacy in tumor eradication at low dose(s) in NSG mice xenografted with heterogeneous MM cell lines.
Conclusions: The pre-clinical data of GPRC5D and BCMA bi-specific CAR-T exhibited superior anti-tumor efficacy and persistence, mitigated Ag escape and preserved potency with shedding BCMA. Our findings provide support for the clinical investigation of the bi-specific CAR-T as a therapeutic approach to broadly and deeply deplete heterogenetic MM cell clones and subsets, potentially leading to durable responses in both newly diagnosed and R/R MM patients.

#3184 Development of novel anti-mesothelin CAR-T cells for the treatment of solid tumors.

Sayyed Hamid Zarkesh Esfahani[1], Kun Luo[1], Michael Wagner[2], Ngoc Tran[1], Brittany Steimle[1], Rimas J. Orentas[1], Peirong Hu[1], Dina Schneider[1]

[1]Lentigen Technology Inc., a Miltenyi Biotec Company, Gaithersburg, MD,[2]Miltenyi Biotec B.V. & Co. KG, Bergisch Gladbach, Germany

Introduction: Mesothelin (MSLN) is overexpressed in many human solid tumors and absent from most healthy tissues. Despite multiple clinical trials, little success has been achieved by MSLN CARs. Shed MSLN accumulates in patient sera and tumor draining fluids, potentially blocking MSLN CAR function (1,2). Alpaca-derived VHHs are an attractive option for CAR design that offer exquisite epitope targeting and the potential to circumvent the challenges of cleaved and shed MSLN.

Methods: Eight alpaca-derived VHHs targeting the MSLN membrane-proximal region were incorporated into a second-generation CD28-CD3ζ CAR framework. A previously developed scFv-based MSLN CAR with either CD28 or 4-1BB co-stimulation was used as comparison. MSLN CAR-T cells from healthy donors were tested *in vitro* for CAR surface expression, overnight killing and real-time consecutive tumor re-challenge. Cytokine production by CAR-T cells was measured after overnight co-culture with $MSLN^+$ tumor cells. *In vivo* CAR-T cell function was evaluated in an AsPC1 pancreatic tumor NSG mouse xenograft model. Persistence of CAR-T cells *in vivo* was investigated by measuring T cells in peripheral blood (PB) and at tumor site. Additionally, shed MSLN in plasma samples was quantified by ELISA. We also tested the effect of soluble MSLN on the potency of CAR-T cells as analyzed by spiking MSLN into *in vitro* killing assays, modeling *in vivo* conditions.

Results: VHH CAR D0492 and scFv CAR D0405 were selected for their high surface expression, *in vitro* cytotoxicity and expansion. *In vitro*, scFv-based CARs with a CD28 co-stimulatory domain demonstrated higher anti-tumor activity, in comparison to 4-1BB. CAR-T cell production of TNFα, IL2 and IFNγ in response to $MSLN^+$ A431 tumor cells was consistently stronger in CD28 CARs. The VHH CAR showed moderate cytokine release without target stimulation, assumed to be via self-association on the T cell surface. *In vivo,* tumor-bearing mice treated with MSLN CAR-T cells each delayed tumor progression without adverse effects on body weight. In contrast to *in vitro* studies, the scFv-based 4-1BB CAR D0181 showed the highest expansion and persistence in PB. IHC staining of tumor samples demonstrated the presence of T cells within tumors in mice treated with anti-MSLN CARs. Shed MSLN plasma concentration *in vivo* at the end of study take down (TD) correlated with tumor size. Importantly, spiking with equivalent or greater MSLN concentrations did not impede CAR-T cell activity in an *in vitro* cytotoxicity assay.

Conclusions: A series of novel MSLN CARs demonstrated potent *in vitro* and *in vivo* anti-tumor activity, were well-tolerated and insensitive to inhibition by soluble MSLN. Second generation scFv-based CARs with 4-1BB co-stimulation demonstrated greater CAR expansion *in vivo*. VHH CARs demonstrated equivalent *in vivo* tumor control to scFv, highlighting the importance in both formats to targeting MSLN membrane-proximal domains.

#3185 Optimizing Stim-R^{TM}, a synthetic stimulatory agent, for feeder-free tumor-infiltrating lymphocyte manufacturing.

Riley P. Allen, Jason Duecker, Mikayla Min, Samantha Chan, Christina Ta, Benjamin Harris, Emily Fu-Sum, Stefan Siebert, Purnima Sundar, Yogin Patel, Shobha Potluri, Suman Vodnala, Gary Lee, Alexander Cheung, Aileen Li

Lyell Immunopharma, South San Francisco, CA

The choice of stimulation agent (e.g. irradiated feeder cells) utilized to expand cells for adoptive cell therapy may limit both scalability and potency of the product. Synthetic cell stimulating agents are tunable, consistent, scalable, and can be adapted to a range of cell therapies. Previously, we reported the development of Stim-R^{TM} for use in melanoma tumor infiltrating lymphocyte (TIL) manufacturing. To broaden the potential application of the technology, we describe optimization of Stim-R in the TIL manufacturing process in additional cancer types. To achieve robust TIL expansion from multiple types of cancer, screens for additional feeder activation cues were conducted by assessing expression of T-cell stimulating ligands on the feeder cell surface, assessing the secretome of feeder cells, and assessing expression of stimulable surface receptors on the initial TIL populations. These data were utilized to develop a new, optimized Stim-R process, which incorporated both new surface cues on the Stim-R platform and soluble signals in the TIL culture media. The optimized Stim-R process was utilized to replace feeder cells in the second expansion step of the TIL manufacturing process. TIL products manufactured with the optimized Stim-R process or feeder cells were evaluated for total cell yield, the expression of stemness markers using flow cytometry, and retention of putative tumor-reactive clones, assessed as the highest frequency clones from the starting tumor material using T-cell receptor sequencing. We developed a new formulation of Stim-R, Stim-R$_{V2}$, which engages CD2, CD3, CD28, CD137 (41BB), and ICOS, and a set of feeder-secreted proteins (FSPs), which are supplemented in the TIL culture media. An optimized Stim-R process that incorporates both Stim-R$_{V2}$ and the FSPs resulted in increased TIL expansion from all melanoma and NSCLC tumors evaluated compared to products generated using feeder cells. TIL products generated with the optimized Stim-R process demonstrated similar CD8 T-cell stemlike phenotypes to feeder-produced TIL and retained a similar proportion of the tumor reactive cells compared to a feeder-based production process. Stim-R can serve as a synthetic replacement for feeder cells in TIL manufacturing. We have optimized soluble and tethered signals incorporated into the Stim-R process to increase TIL expansion in multiple types of tumors. TIL produced with the optimized Stim-R process were phenotypically comparable to TIL produced with a feeder-based process and retained a similar proportion of the putative tumor reactive clones. The Stim-R technology has potential to overcome scalability limitations in TIL manufacturing and may make TIL therapy accessible for more patients. Furthermore, we demonstrate the versatility of the Stim-R platform as a stimulating agent that can be tuned with activation signals based on cell population and desired phenotype.

#3186 Functional analysis of chimeric ILT receptor natural killer (CIR™NK) cells that target tumor cells expressing HLA-G *in vitro* and *in vivo*.

MyLinh Duong, Jihyun Park, Joseph Senesac, Raphael Ognar, Simon Wain-Hobson, **Henri Bayle**

NKILT Therapeutics, Houston, TX

Background: Human leukocyte antigen (HLA)-G exerts immunosuppression by binding to ILT2 and ILT4 on myeloid and lymphoid cells. While HLA-G expression is repressed in normal tissues except the placenta and cornea, more than 50% of cancers express HLA-G to evade immune surveillance. We developed Chimeric ILT Receptors (CIRs) that link the extracellular domains of ILT2 or ILT4 receptor with novel intracellular costimulatory domains (4-1BB.DAP10 ± TLR2) to drive HLA-G specific targeting and activation of Natural Killer (NK) cells.

Methods: $CD56^+$ NK cells were activated and transduced with γ-retroviruses directing multi-cistronic expression of CIR, soluble IL-15, and a ΔCD19 marker. Coculture assays were performed with GFPFluc-expressing tumor cell lines and NK cells transduced with RFP and monitored using the IncuCyte. In vivo, Molm13 tumor-bearing NSG mice were injected i.v. with a single dose of 7.5E6 mock or CIR-NK cells and tumor and NK cell bioluminescence were tracked via GFPFluc and ONLRluc, respectively. Cytokines from cocultures and mouse plasma were analyzed with a 26-panel multiplex kit.

Results: CIR-NK cells demonstrated target-specific elimination of HLA-G^+ multiple myeloma and AML cancer cells while sparing HLA-G^- cancer cells. ILT4.4-1BB.DAP10.TLR2 CIR-NK cells eliminated 2 fold more HLA-G^+MM1S tumor cells than mock-transduced NK cells (GFP intensity: mock = 4.21E5 ± 5.14E4 vs. ILT4.4-1BB.DAP10.TLR2 = 2.15E5 ± 2.26E4) but no killing was observed in coculture against HLA-G^- Molm14 AML cells. In repeated target coculture where tumor cells were added up to 4 times every 2 days, ILT4.4-1BB.DAP10-2A.TLR2 CIR-NK cells eliminated more than 60% of Molm13 AML cells (GFP intensity: 4-1BB.ζ = 3.96E7 ± 5.37E6 vs. 4-1BB.DAP10.2A-TLR2 = 1.45E7 ± 3.34E6) and expanded more than 2 fold relative to CIR-NK cells containing 4-1BB.ζ (RFP intensity: 4-1BB.ζ = 1.49E5 ± 5.72E3 vs. 4-1BB.DAP10.2A-TLR2 = 3.26E5 ± 5.1E4). Comparison of chemokine and cytokine production with and without Molm13 target revealed that that IL-33 (42 fold), CD40L (45 fold), IP-10 (26 fold), and IL-12 p70 (73 fold) were among the many analytes highly upregulated by ILT4.4-1BB.DAP10 CIR-NK cells that arm NK cells against the cancer. In vivo, a single dose of ILT4.4-1BB.DAP10 CIR-NK cells induced 60% greater clearance of Molm13 tumor burden in NSG mice in 10 days relative to ILT4.4-1BB.ζ counterparts and 3 fold greater in NK expansion on day 14. Proinflammatory cytokine and chemokines secreted into mouse plasma were remarkably higher as early as 7 days post CIR-NK injection (IFN-γ = 4134 ± 127pg/ml; GM-CSF = 594 ± 56pg/ml; granzyme B = 1529 ± 233pg/ml).

Conclusions: We demonstrated that engineered CIR-NK cells, coupled with potent intracellular costimulatory domains, can convert HLA-G expression in cancer cells from an inhibitor of immune surveillance into a practical target for anti-cancer therapy.

#3187 Assessing large-scale CAR-T cell cytotoxicity ex vivo using 2-D and 3-D multimodal imaging techniques for industrial standards.

Catherine McManus, John Foulke, Meghan Sikes, Luping Chen, Hyeyoun Chang, **Fang Tian**, Zhizhan Gu

ATCC, Gaithersburg, MD

CAR-T cell therapy is a revolutionary and highly effective treatment for hematologic malignancies, with rapid advancements particularly in applications to solid tumors. The development and refinement of this innovative therapy rely on efficient ex vivo assays to evaluate CAR-T cell cytotoxicity. Furthermore, advancement in the treatment of solid tumors using CAR-T cell therapy requires the development of ex vivo assays specifically using solid tumor models. Here, we report the development of luciferase reporter cancer cell lines that endogenously express high levels of key CAR-T target antigens such as CD19, CD20, BCMA, or HER2. Using targeted and mock-engineered CAR-T cells, we evaluated cytotoxicity against these reporter cells via a bioluminescence assay and phase contrast/fluorescence live imaging assays. Our multimodal imaging approach demonstrated significantly higher cancer cell killing by targeted CAR-T cells compared to mock CAR-T cells. To enhance our live imaging capabilities and enable assays for CAR-T cell infiltration into 3-D tumor models, we engineered GFP-Luc2 dual reporter cancer cell lines. Using 3-D confocal live imaging, we observed reduced spheroid size and decreased luciferase signal in Raji-GFP-Luc2 spheroids co-cultured with CD19 CAR-T cells versus mock CAR-T controls, indicating superior cancer cell killing when using targeted CAR-T cells. Upon embedding Raji-GFP-Luc2 spheroids in 3-D matrices to mimic a more physiologically relevant tumor microenvironment, we captured CAR-T cell infiltration into spheroids by time-lapse 3-D imaging. Our results highlight the utility of combining luciferase bioluminescence and live fluorescence imaging to assay CAR-T cell cytotoxicity and infiltration in 2-D and 3-D co-culture models. The luciferase assay provides sensitive, quantitative measurements, while live fluorescence imaging reveals the spatial and temporal dynamics of CAR-T cell-cancer cell interactions. These scalable assays hold significant potential for large-scale industrial applications in CAR-T cell therapy development.

#3188 Functional interrogation of human T cells using the accessible Beacon Discovery™ optofluidic system.

Patrick N. Ingram[1], Qiong Gao[1], Leqian Liu[2], Julie Perreau[2], Ritika Kukreja[2], Or Gadish[1], Brandon R. Bruhn[1], Minha Park[2], Edward Han[3], Alireza Salmanzadeh[3]

[1]Applications R&D, Bruker Cellular Analysis, Emeryville, CA,[2]Biology R&D, Bruker Cellular Analysis, Emeryville, CA,[3]Marketing & Business Development, Bruker Cellular Analysis, Emeryville, CA

Adoptive cell therapies (ACTs), such as chimeric antigen receptor therapy (CAR-T) and T-cell receptor therapy (TCR-T), involve the extraction, *ex vivo* study, genetic engineering, and reimplantation of live T cells. These therapies have proved invaluable in our ongoing battle against cancer, with *over 1,500* active clinical trials. Despite their promise, cell-based therapies are uniquely challenging to discover, validate, and translate, requiring deeper characterization compared to small molecules or other therapies. Researchers' success at each stage depends on accessible tools that enable the measure of viability, variability, persistence, and functionality of thousands of single cells. To this end, we have implemented Bruker's Opto® T Cell Profiling workflow on the new Beacon Discovery system and investigated on-instrument the resulting cytotoxicity, cytokine secretion, and surface marker expression for single human MART-1 T cells interacting with T cell activating beads (TABs) and antigen-presenting live cells (APCs).

The Beacon™ optofluidic single-cell analysis system provides multi-dimensional characterization and recovery of live cells using Opto-Electronic Positioning (OEP) to sort live cells into NanoPens. With applications in immune cell profiling (such as T cell profiling), antibody discovery, and cell line development, these platforms are utilized by top pharma companies and backed by 30+ published studies. These same capabilities have been enabled in the more accessible Beacon Discovery system. The Beacon Discovery has integrated fluidic handling, single cell sorting, culture and cell incubation, fluorescent microscopy, and immunoassay capabilities, all in a benchtop automated, user-friendly platform. In parallel experiments on the Beacon Discovery, single human T cells were paired with TABs or APCs, where they were cultured and assayed over 2 days. When paired with APCs, target cell cytotoxicity measurements were performed via continuous perfusion of a caspase-3 substrate and time-lapse fluorescent imaging, enabling differentiation of fast-killing and slow-killing T cells. We also assessed cytokine secretion upon activation by co-loading 3 customizable cytokine capture beads in each NanoPen. Comparing T cells paired with TABs or APCs, we observed differing levels of expression for TNF-α, IL-2, and IFN-γ. We also utilized the Beacon Discovery to detect the presence of surface markers (CD4/CD8) on the T cells during co-culture with TABs or APCs. These functional measures, combined with cell recovery for sequencing, are ideal for accelerating ACT development. In summary, the Beacon Discovery optofluidic system is an approachable, easy-to-use instrument that offers an intuitive user interface and powerful data analysis and visualization software, empowering labs of all sizes to revolutionize their single-cell functional analysis.

#3190 Enhancing chimeric antigen receptor (CAR)-T cell therapy for solid tumors through photobiomodulation.

Jude S. Franklin

Biomedical Engineering, CWRU, Cleveland, OH

Chimeric Antigen Receptor (CAR)-T cell therapy has demonstrated remarkable success in treating hematological malignancies, yet its efficacy in solid tumors remains limited, with response rates as low as ~9% and limited by high relapse rates (~50% within one year). Furthermore, CAR-T therapies have demonstrated challenges for reduced efficacy, largely attributed to poor penetration and poor infiltration of CAR-T cells into the tumor microenvironment (TME), combined with immune suppressive factors such as T cell exhaustion, metabolic fatigue, and anti-inflammatory cytokines. To address these barriers, we investigate the potential of photobiomodulation (PBM), a non-invasive light-based treatment to enhance CAR-T cell function in both solid tumors and hematological malignancies. PBM, which stimulates cellular activity, could improve CAR-T cell survival, metabolic fitness, and persistence, and thus, therapeutic efficacy. Using optimized wavelengths, we evaluated CAR-T cell function in vitro, focusing on cellular metabolism and expansion. Metabolic analysis using the Seahorse XF platform revealed a marked upregulation in oxygen consumption rates (OCR) indicative of enhanced glycolytic activity ($p<0.001$) in CAR-T cells exposed to PBM. Furthermore, we observed a significant increase in T cell counts over a 14-day period, validated by CFSE labeling and flow cytometry with AO/PI and hemacytometer counting, in addition to evaluation of cytotoxicity and differentiation of PBM-treated CAR-T cells compared to control CAR-T cells. These findings suggest PBM preconditioning significantly enhances CAR-T cell energy production, proliferation, metabolic activity, and persistence, potentially overcoming challenges related to TME penetration, immune suppression, and exhaustion in solid tumors. Additionally, the PBM treatment could help address issues of persistence and proliferation of CAR-T cells in both solid tumors and hematological malignancies, improving their overall therapeutic potential and optimizing treatment protocols for clinical application. This combinational approach of CAR-T cell therapy and PBM represents a promising strategy to refine and optimize CAR-T functional longevity in both hematological and solid malignancies for clinical translation.
As my lab experience focuses on immunology and the development of CAR-T therapies for pre-clinical use, this project will be in collaboration with the Jenkins/Moffitt Lab for their expertise in PBM.
An invention disclosure was made to CWRU for the use of PBM for adoptive cell therapy. It is expected that a provisional application will be filed in the future prior to public disclosure. CWRU is the full owner of this intellectual property.

#3192 GT316: a next-generation TIL therapy identified through genome-wide CRISPR screening with promising efficacy in advanced solid tumors.

Jingwei Sun[1], Yao Sheng[1], Jingman Wang[1], Jiahui Jin[1], Zhao Xu[1], JIng Yu[1], Jing Guo[2], Zhongping Cheng[2], **Pin Wang**[3], Yarong Liu[1]

[1]Grit Biotechnology, Shanghai, China,[2]Shanghai Tenth People's Hospital, Shanghai, China,[3]USC - University of Southern California, Los Angeles, CA

Adoptive cell therapy using tumor-infiltrating lymphocytes (TILs) has demonstrated therapeutic efficacy in late-stage melanoma patients. However, expanding its applicability to other solid tumors and improving clinical outcomes remain significant challenges. To address these limitations, we developed *ImmuTFinder*, a genome-wide CRISPR/Cas9 screening platform designed to identify key immunoregulatory targets that enhance TIL functionality and broaden their therapeutic potential. Functional validation of over 120 top candidate targets was performed in TCR-T cells using a high-throughput 96-well array. Promising targets were further tested for combinatorial effects in TILs through in vitro assays and in vivo models. A dual knockout of GT304 and GT312, termed GT316, emerged as the most effective combination, significantly improving TIL proliferation, cytokine production, tumor eradication in CDX and PDX mouse models, and long-term persistence with reduced dependency on IL-2. Specific and highly efficient sgRNAs for these targets were selected, and a scalable manufacturing process for GT316 TILs, named *KOReTIL*, was developed. A first-in-human trial of GT316 demonstrated good tolerability and encouraging preliminary antitumor activity in patients with advanced solid tumors. Notably, a patient with treatment-refractory cervical cancer achieved a confirmed complete response (CR) four weeks post-infusion, with the response persisting for 50 weeks to date (RECIST 1.1). Another patient with ovarian cancer, previously treated with nine lines of systemic therapy, achieved a confirmed partial response (PR) at six weeks post-infusion, with a response lasting over 50 weeks (RECIST 1.1). Data collection on additional responses is ongoing. In summary, these findings highlight the utility of the *ImmuTFinder* platform for discovering novel immunoregulatory targets and demonstrate the potential of GT316 as a next-generation TIL therapy for advanced solid tumors.

#3193 Overcoming glioblastoma heterogeneity and plasticity through the development of novel chimeric antigen receptor (CAR)-T cells targeting PTPRZ1.

Aditya A. Mohan, Barbara Lipes, Charla Gentry, Rushil Yerrabelli, Daniel Wilkinson, Kisha Patel, Emily Blandford, Andrew Hardigan, Peter Fecci, Michael Gunn, Anoop Patel

Duke University Medical Center, Durham, NC

Glioblastoma (GBM) is an aggressive and heterogeneous brain tumor characterized by extensive plasticity, which contributes to resistance against conventional therapies. To address this challenge, we generated a unique single nuclei RNA sequencing atlas, integrating GBM, normal brain tissue, and developmental brain datasets. Utilizing single-cell RNA sequencing (scRNA-seq) analysis methods, we comprehensively characterized GBM heterogeneity and plasticity, focusing on identifying homogeneous cell surface antigens suitable for chimeric antigen receptor (CAR) T-cell targeting. scRNA-seq analysis revealed PTPRZ1 as a promising candidate antigen, uniformly expressed across tumor cells, offering a compelling therapeutic target for CAR-T cell therapy. Following antigen identification, we initiated mouse immunization campaigns to stimulate antibody production, coupled with phage display technology to generate a diverse library of single-chain variable fragments (scFvs). A rigorous cell-based positive and negative selection protocol was employed to isolate scFvs that specifically bind to PTPRZ1 while avoiding binding to closely related proteins from the same family, thereby minimizing off-tumor effects. Protein language models were used and experimental site-directed mutagenesis was performed to affinity mature and humanize selected scFv clones. Once PTPRZ1-specific scFvs were identified, they were incorporated into novel CAR constructs designed to overcome the low surface antigen density challenge commonly observed with GBM antigens. These CARs incorporated several signaling domains and structural modifications to enhance sensitivity and efficacy in low antigen-density settings. Our approach integrates cutting-edge single-cell sequencing, phage display technologies, and CAR-T cell design principles to address the heterogeneity of GBM and develop precision-engineered CAR-T cells targeting PTPRZ1. This study presents a step forward in overcoming GBM's plasticity, offering a potential therapeutic strategy with translational potential.

#3194 Opposing roles of product CD27 and TIGIT expression in outcome to CAR-T cell therapy in R/R LBCL.

Justin Budka[1], Subing Cao[1], Chad Williams[1], Gabriela Balderrama-Gutierrez[1], Soumya Poddar[1], Mike Mattie[1], Jenny J. Kim[1], Saran Vardhanabhuti[1], Yohei Arihara[2], Yusuke Kamihara[2], Shogo Miura[2], Jerome Ritz[2], Davide Bedognetti[1], **Simone Filosto**[1]

[1]Kite Pharma, Inc., Santa Monica, CA,[2]Dana-Farber Cancer Institute, Boston, MA

Background: CAR-T cell therapy provides transformative outcomes for patients with relapsed/refractory (R/R) large B cell lymphoma (LBCL). However, a large fraction of patients remains at risk for relapse, underlying the need to uncover mechanisms of resistance and predictive biomarkers. We leveraged ZUMA-1, -6 and -7 studies of R/R LBCL to discover product features associated with outcome to CAR-T cell therapy [Axicabtagene Ciloleucel (axi-cel)]. We explored ligand-receptor interactions between product cells and the tumor microenvironment, identifying CD27-CD70 and TIGIT-PVR axes as putative mediators of durable response and disease progression, respectively.

Methods: Cytometry by time of flight (CyTOF) with 34 markers pertinent to T cell phenotyping and activation was performed on CAR-T cell products from ZUMA-1 (3rd line(L) LBCL; n = 57), ZUMA-6 (CAR-T cells + Atezolizumab; 3rd L LBCL; n = 14), and ZUMA-7 (2nd L LBCL; n = 45). Individual marker intensity and unsupervised clustering (by flowSOM algorithm) analysis were performed to identify features associated with outcome and a P-value < 0.05 was considered significant by descriptive statistics. The CyTOF analysis was combined with transcriptomic Nanostring IO360™ from ZUMA-7 pre-infusion tumors (N=35) for ligand-receptor interaction studies. scRNAseq analysis was performed (CellRanger v7.0 and Scanpy) on a set of ZUMA-1 products (n=36).

Results: Cross-study analysis of individual CyTOF markers presented increased expression of activation and exhaustion proteins CD29, OX40 and TIGIT in patients who experienced disease progression (lack of response or relapse after response), while co-stimulatory CD27 and NKG2D associated with ongoing response (P < 0.05). Unsupervised clustering analysis demonstrated that product CD8 stem central memory (T-SCM) cells, expressing CCR7, CD45RA, CD95, NKG2D, and CD27 associated with ongoing response (P < 0.01), while activated memory or senescent CD4+ cells associated with disease progression (P < 0.05). All patients with > 25% CD8 T_{SCM} (% of T cells) responded to axi-cel (100% ORR), with TIGIT expression representing the only marker significantly associated with Relapse Vs Ongoing response status in these patients (P < 0.05). The analysis of receptor expression on product cells Vs ligand expression in the tumor uncovered associations of the CD27-CD70 and TIGIT-PVR axes with durable response and disease progression, respectively. scRNAseq of ZUMA-1 products corroborated the above observations, with elevated TIGIT expression in two cell clusters enriched in non-responding patients.

Conclusions: Using orthogonal methods on three clinical studies, we identified product features consistently associated with CAR T-cell efficacy in r/r LBCL, informing design of next-generation products and combinatorial approaches to improve outcome, including product enrichment of CD27+ CD8 T_{SCM} and TIGIT inhibition.

#3195 Advancing CAR T clinical development with high-parameter CyTOF technology.

Ling Wang, Shakir Hasan, Stephen Li, Michael Cohen, Deeqa Mahamed, Christina Loh

Standard BioTools, Markham, ON, Canada

Adoptive immunotherapy using chimeric antigen receptor (CAR) T cells is considered a recent revolutionary treatment in cancer therapy. CAR T therapy has achieved great success in hematological B cell malignancies. However, it has faced significant challenges in solid tumors due to various factors, such as complex tumor microenvironments, restricted trafficking, impersistent antitumor activity and toxicities. A better understanding of CAR T biology will accelerate development of CAR T therapies with improved antitumor efficacy, durability and decreased toxicities. High-parameter flow cytometry has been a powerful tool to functionally characterize CAR T cells at multiple stages of clinical development, from product characterization during manufacturing to longitudinal evaluation of the infused product in patients. However, fluorescence-based cytometry faces significant challenges with signal overlap and autofluorescence, limiting sensitivity and the number of targets that can be detected in CAR T cells. Consequently, rare cell populations are poorly resolved and functional readouts of CAR T cells are unreliable. CyTOF technology overcomes these limitations with low signal overlap and no autofluorescence. Further, CyTOF technology enables a streamlined and flexible workflow in clinical research using freezing antibody cocktails and stained cell samples. Here, we present a 40-plus-marker CyTOF panel to simultaneously analyze phenotypic and functional protein expression in CAR T cells from *in vitro* co-culture with tumor cells. CD19 CAR T cells were expanded *in vitro* and co-cultured with Nalm6 cells at different E:T ratios for 2-5 days. A high-parameter CyTOF panel including over 40 surface, cytoplasmic and nuclear markers was used to stain CAR T cells. Samples co-cultured at different time points were stained, barcoded, frozen and simultaneously acquired on a CyTOF XT system at a later date. The cytotoxicity, activation, proliferation, differentiation and exhaustion of CAR T cells were evaluated. Comprehensive profiling revealed that CAR T cells became activated, proliferated and produced cytokines in *in vitro* co-culture with tumor cells and showed reduced cytotoxicity and exhaustive phenotype after an extended period of expansion. Overall, we demonstrate that the high-parameter CyTOF panel enables deep functional characterization of CAR T cells by simultaneous detection of surface, cytoplasmic and nuclear markers, supporting the continuous clinical development of CAR T products.

#3196 CRTAM defines effector functionality and robust anti-tumor response of cytotoxic CD8+T cells.

Zheng Liu[1], An Nguyen[1], Amy Olex[2], Mikail Bala[2], Jose Trevino[3], Bhaumik Patel[4], Jolene Windle[1], Xiang-Yang Wang[1], **Syed Ammer Shah**[5]

[1]Human and Molecular Genetics, Virginia Commonwealth University - VCU, Richmond, VA,[2]Bioinformatics Core, Virginia Commonwealth University - VCU, Richmond, VA,[3]Surgery, Massey Cancer Center, Virginia Commonwealth University - VCU, Richmond, VA,[4]Internal Medicine Massey Cancer Center, Virginia Commonwealth University - VCU, Richmond, VA,[5]Surgery, Massey Cancer Center, Central Virginia VA Healthcare System, Virginia Commonwealth University - VCU, Richmond, VA

The effectiveness of current cancer immunotherapies, such as immune checkpoint blockade (ICB), adoptive T cell therapy (ACT), and chimeric antigen receptor (CAR) T-cell therapy, is limited in solid tumors due to the scarcity of tumor-specific immunogenic targets, impaired tumor antigen processing, and immune cell dysfunction. Cytotoxic $CD8^+$ T lymphocytes (CTLs) are the primary effectors in antitumor immunity, yet their dysfunctional or exhausted states present significant challenges to successful immunotherapy. Recent study of pre-infusion CAR T cells in non-Hodgkin lymphoma (NHL) have identified polyfunctional T cell subsets that exhibit engagement of diverse immune programs, characterized by the production of various cytokines and chemokines (IFN-γ, GMZB, and MIP1a) which corroborated with enhanced patient survival. In our previous clinical study involving ACT in melanoma patients (NCT00665470), we observed engraftment of $CD8^+$ T cell clones without objective antitumor responses (non-responders), while spontaneous complete responses were noted in two patients prior to ACT (responders). A comparative analysis of $CD8^+$ T cell clones derived from responders versus non-responders identified Cytotoxic and Regulatory T cell Molecule (CRTAM) as a differentially expressed gene in activated $CD8^+$ T cell clones from responders. We propose CRTAM as a predictive marker for a potent and durable $CD8^+$ T cell-mediated antitumor response and to explore its potential role in enhancing the antitumor efficacy of ACT and CAR T cell therapy in solid tumors. We have thus far established the role of CRTAM in defining enhanced effector functions of murine cytotoxic $CD8^+$ T cells by examining antigen specific P - Mel $CRTAM^{Hi}$ Vs $CRTAM^{Lo}$ $CD8^+$ T cell subsets with $CRTAM^{Hi}$ T cells showing enhanced cytotoxic profile (perforin/ GMZB), cytokine production (IFN- γ), antitumor cytotoxicity, immunophenotype and gene profile using both in vitro and in vivo models. RNA sequencing analysis reveals that $CRTAM^{Hi}$ P-Mel T cells exhibit a unique gene expression profile associated with cytokine activity, signal transduction, cell migration, and cytotoxicity. Furthermore, increased overall survival in patients with solid tumors exhibiting T cell infiltration and increased CRTAM expression was demonstrated by query of publicly available databases, such as The Cancer Genome Atlas (TCGA). Therefore, CRTAM identifies a unique subset of CTL with enhanced effector functions, with deployment of diverse immune programs leading to improved antitumor activity. T cells engineering to knockout and express CRTAM is currently underway to evaluate the feasibility of enhancing antitumor responses in, in vivo murine models of ACT. Engineering $CD8^+$ T cells for CRTAM may generate more effective and durable T cell products for ACT and CAR T cell therapy in solid tumors.

#3197 Macrophage-ferritin-drug conjugates: a novel approach to overcome glioblastoma drug resistance and induce long-term tumor immunity.

Maciej Białasek[1], Miaomiao Sun[2], Bartłomiej Taciak[1], Tomasz Rygiel[3], Michael Weller[2], Tobias Weiss[2], **Magdalena Krol**[1]

[1]Center of Cellular Immunotherapies, Cellis AG | Warsaw University of Life Sciences, Zurich, Switzerland,[2]University Hospital Zurich and University of Zurich, Zurich, Switzerland,[3]Cellis AG, Zurich, Switzerland

Glioblastoma (GBM) remains a major clinical challenge due to its resistance to conventional therapies and immunosuppressive tumor microenvironment. To address these challenges, we developed Macrophage-Ferritin-Drug Conjugates (MDCs), leveraging the unique ability of macrophages to infiltrate gliomas and transfer therapeutic ferritin-drug complexes directly to cancer cells. In orthotopic glioma models (GL-261 and CT-2A), MDC-735, consisting of macrophages loaded with ferritin conjugated to inhibitor of tubulin polymerization, demonstrated significant tumor reduction and improved survival compared to controls, including unloaded macrophages or cell-free ferritin-drug conjugates. MDC-735 also outperformed current standard treatments such as temozolomide, radiation, and second-line agents like lomustine, which fail to achieve long-term survival. Remarkably, rechallenge studies showed that mice treated with MDC-735 developed immunological resistance to tumor regrowth, indicating a role for adaptive immunity. In vitro co-culture experiments revealed that MDC-735-loaded macrophages induced T-cell activation and pro-inflammatory cytokine production. In vivo, MDC-735 enhanced tumor-infiltrating lymphocyte populations, reduced exhaustion marker expression, and decreased regulatory T-cell frequencies, further supporting its immune-modulating effects. Functional validation in immunodeficient models confirmed the critical involvement of lymphocytes in MDC-735's therapeutic efficacy. To explore clinical potential, we produced cryopreserved human MDC-735 using macrophages differentiated and loaded via automated platforms. MDC-735 administration in human glioblastoma-bearing mice was well-tolerated and led to tumor regression and survival benefits. Patient-derived glioblastoma samples co-cultured with MDC-735 exhibited preferential ferritin transfer to glioma cells and enhanced phagocytosis, underscoring its selective anti-tumor activity. These findings highlight MDC-735 as a promising immunotherapeutic strategy for GBM, capable of targeting drug-resistant cancer cells, modulating the tumor microenvironment, and inducing durable anti-tumor immunity. Importantly, MDC-735 is designed as an allogeneic, off-the-shelf cell-based therapy, enabling scalable and timely delivery for clinical applications, addressing a significant unmet medical need in glioblastoma treatment.

#3198 TK-6302, a supercharged PRAME TCR-T cell therapy containing a high affinity TCR, an activating CD8 coreceptor (costimCD8 CoR) and a Fas-based switch receptor, drives sustained anti-tumor responses.

Paul Najm, Kathrin Skibbe, Lia Walcher, Alvaro Haroun-Izquierdo, Laure Twyffels, Mikhail Steklov, Lorenz Knackstedt, Lucia Poncette, **Marleen M. van Loenen**, Elisa Kieback, Panagiota A. Sotiropoulou

T-knife Therapeutics Inc., San Francisco, CA

Background: T cell receptor (TCR) T cell therapy targeting PRAME mediates durable responses in melanoma and sarcoma but has shown limited efficacy in other solid tumors. Suboptimal T cell engraftment and fitness and immune suppression by the tumor microenvironment (TME) are main causes of the limited efficacy. We have developed TK-6302, a PRAME targeted TCR-T cell therapy with best-in-class potential, manufactured with a non-viral gene editing process, and incorporating a high-affinity TCR, a chimeric CD8 co-receptor that engages CD4 T cells and provides co-stimulation upon TCR engagement (costim CD8 CoR), and a Fas switch receptor (SwR) that boosts fitness in the periphery and prevents apoptosis in the tumor.

Methods: High-affinity PRAME TCRs were identified using T-knife's MyT^{TM} platform. Transcriptomic analysis of 4,622 stage III/IV tumors using Tempus^{TM} database identified consistent expression of FasL in cancer types with high PRAME prevalence. Supercharged PRAME TCR-T cells were generated using a non-viral gene editing process inserting all elements into the TRAC locus, and then compared to benchmark products based on dextramer binding, memory and activation/exhaustion phenotype by flow cytometry, cytokine secretion by LEGENDplex^{TM}, and cytotoxicity upon chronic or repeated antigen stimulation using Incucyte.

Results: Supercharged PRAME TCR-T cells incorporate three functional elements: (1) high-affinity TCR with EC50 $4.73x10^{-10}$ M, (2) costimCD8 CoR, a single-chain CD8 CoR with TRAF-binding costimulatory motifs, and (3) Fas-CD40/OX40 (Fas-TNFR) SwR selected based on inhibitory ligand screening of PRAME expressing indications and designed to provide higher resistance to FasL-induced apoptosis compared to the commonly used Fas-4-1BB SwR. Supercharged PRAME TCR-T demonstrated higher *in vitro* anti-tumor activity compared to known PRAME TCR-T approaches, including clinical candidates, with a minimum of two additional rounds of serial killing and higher levels of pro-inflammatory cytokines against cancer cells with low PRAME expression. Supercharged PRAME TCR-T maintained early memory phenotype upon serial killing compared to benchmark approaches. When PRAME TCR-T products were tested in a multi-cellular model with TME resembling that of PRAME-expressing indications, Supercharged PRAME TCR-T cells were protected from inhibitory signals, as shown by elevated cell numbers and higher cytotoxicity and cytokine production, while benchmark PRAME TCR-T cells failed to control long term growth of cancer cells.

Conclusions: Supercharged PRAME TCR-T therapy demonstrates best-in-class preclinical anti-tumor efficacy and T cell fitness compared to current clinical stage PRAME TCR-T approaches, providing the potential for deep and durable responses in hard-to-treat indications.

#3199 Radiomic features associated with anti-tumor activity in Non-Hodgkin lymphoma patients treated with CD19 directed allogenic CAR T therapy.

Andrew R. Dunn[1], Shashwat Deepali Nagar[1], Donald J. Kirsch[1], Leah M. Williams[1], Annie Y. Weaver[1], Philipp M. Altrock[2], William S. Denney[3], Chan Y. Cheah[4], P. Joy Ho[5], Joseph P. McGuirk[6], Paul Shaughnessy[7], Constantine S. Tam[8], Armin Ghobadi[9]

[1]CRISPR Therapeutics, South Boston, MA,[2]Modeling & Simulation, Altrock Scientific Consulting, Kronshagen, Germany,[3]Human Predictions, LLC, Boston, MA,[4]Sir Charles Gairdner Hospital, Perth, Australia,[5]Institute of Hematology, Royal Prince Alfred Hospital, New South Wales, Australia,[6]Division of Hematologic Malignancies & Cellular Therapeutics, University of Kansas Medical Center, Kansas City, KS,[7]Sarah Cannon Transplant and Cellular Therapy Program, Methodist Hospital, San Antonio, TX,[8]Alfred Hospital and Monash University, Melbourne, Australia,[9]Division of Oncology, Washington University School of Medicine, St. Louis, MO

Introduction: Radiomic features extracted from ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography ([^{18}F] FDG PET/CT) provide valuable insight into the anti-tumor activity of CD19-directed CAR T therapy in patients with non-Hodgkin lymphoma (NHL). This study examined an integrative approach combining radiomic features and patient-specific attributes to determine if these parameters are associated with anti-tumor response across two investigational allogeneic CD19-directed CAR T programs, CTX110 and CTX112. CTX112 contains two novel CRISPR-mediated gene edits to Regnase-1 and Transforming Growth Factor Beta Receptor 2 (TGFBR2), which are intended to increase CAR T cell expansion, functional persistence and potency.
Methods: Patients with R/R NHL of a variety of histologies were given LD chemo followed by either CTX110 or CTX112. Radiomic features were extracted from [^{18}F] FDG PET/CT scans at baseline (screening) and the first assessment at day 28. 48 patients treated with CTX110 and CTX112 at four dose levels ($3x10^7$ [DL1], $1x10^8$ [DL2], $3x10^8$ [DL3], and $6x10^8$ [DL4] CAR+ T cells) were analyzed. Potential features associated with anti-tumor activity were identified at three different levels: (1) subject-level, (2) total tumor burden-level, and (3) lesion-level. To understand the interplay between CAR T cells and tumor lesions, multiple feature selection techniques were employed to delineate different sets of features that correlate with anti-tumor activity.
Results:Median values for the total tumor burden (TB) as measured by the total metabolic tumor volume (MTV) for patients treated with CTX110 were less than CTX112 (CTX110 = 96 [38 to 291] mL and CTX112 = 146 [91 to 187], median [IQR]) indicating that the patients treated with CTX112 may have been more difficult to treat. CTX110 and CTX112 both showed dose-dependent activity, producing larger reductions in MTV with higher CAR T cell doses. However, CTX112 was observed to have similar % MTV reduction at $3x10^7$ cells (DL1) as CTX110 does at $3x10^8$ cells (DL3), suggesting CTX112 possess an estimated 10-fold higher anti-tumor activity than its predecessor. By estimating the effector to target (E:T) ratio in patients, whereby the CAR+ cells dose is normalized to the baseline MTV, patients with high E:T ratios were shown to have increased anti-tumor activity compared to low ratios for both CTX110 and CTX112.
Conclusions: Multivariate analysis of [^{18}F] FDG PET/CT scans shows that CTX110 and CTX112 exhibit consistent dose-dependent anti-tumor activity, as measured by decrease in MTV, across patients with diverse radiomic features. Across multiple methods estimating MTV-reduction efficacy, CTX112 is approximately 10-fold more efficacious than CTX110, despite the CTX112-treated cohort containing patients with baseline radiomic features associated with poor response.

CLINICAL RESEARCH: Immune Checkpoints
Poster Session

#3205 Artificial intelligence (AI) -powered H&E whole-slide image (WSI) analysis of tertiary lymphoid structure(TLS) to predict response to immunotherapy in non-small cell lung cancer (NSCLC).

Young Kwang Chae[1], Leeseul Kim[2], Sangwon Shin[3], Sooick Cho[3], Sanghoon Song[4], Borislav Alexiev[1], Behtash Nezami[1]

[1]Northwestern University Feinberg School of Medicine, Chicago, IL,[2]Department of Medicine, The University of Chicago, Chicago, IL,[3]Lunit Inc., Seoul, Korea, Republic of,[4]Lunit, Seoul, Korea, Republic of

Background: The efficacy of ICIs in treating NSCLC is limited to a subset of patients, necessitating reliable predictive biomarkers. TLSs within the tumor microenvironment have emerged as potential indicators. This study aims to investigate the association between an AI-powered TLS analysis and treatment response of NSCLC patients treated with ICIs.
Method: We collected H&E-stained WSI of primary and metastatic tumors and clinical data in a retrospective study of advanced-stage NSCLC patients treated with ICI (with or without chemotherapy) at at Northwestern University from May 2015 to November 2023. Following the assessment of the presence of TLS by a pathologist, the identical WSIs were evaluated by the AI-powered H&E WSI analyzer. We used Lunit SCOPE IO, an AI-powered H&E WSI analyzer developed using 21,096 H&E stained WSIs, which can segment various classes of tissue area including TLS within the TME. Survival analysis was performed using Kaplan-Meier curves, and differences in survival outcomes among TLS groups were evaluated using the log-rank.
Result: A total of 102 patients with advanced-stage NSCLC were included in the study. 30.3% (31/102) and 22.5% (23/102) of cases were found to contain TLS as determined by the AI analyzer and a pathologist respectively. The AI model achieved an accuracy of 88.2% (95% confidence interval [CI], 80.4%-93.8%) in identifying the presence of TLS compared to the pathologist's interpretation. The concordance between the pathologist's assessment and the AI model, as evaluated by Cohen's kappa, was 0.70. The median follow-up duration was 40.0 months. Based on TLS presence as assessed by a pathologist, the TLS (+) group demonstrated significantly improved PFS (HR: 0.29, 95% CI: 0.12-0.68, $p < 0.01$) and OS (HR: 0.43, 95% CI: 0.23-0.78, $p < 0.01$) compared to the TLS (-) group. When TLS presence was assessed by an AI analyzer, the TLS (+) group had a median PFS (mPFS) that was not reached (95% CI: 31 months-not reached), whereas the TLS (-) group had a mPFS of 11 months (95% CI: 6-28 months), with an HR of 0.39 (95% CI: 0.20-0.79, $p < 0.01$). The median OS (mOS) for the TLS (+) group was 26.2 months (95% CI: 17 months-not reached) compared to 13 months (95% CI: 9-21 months) in the TLS (-) group, with an HR of 0.55 (95% CI: 0.33-0.92, $p = 0.02$). There was no significant different in overall response rate between the two groups (17.7% in TLS (+) and 17.4% in TLS (-))
Conclusions: AI-powered TLS analysis can be potentially utilized as a predictive biomarker of survival outcomes of NSCLC patients treated with ICI.

#3206 Cadonilimabplus chemotherapy as first-line treatment in PD-L1 negative advanced non-small cell lung cancer (LungCadX): a phase II study.

Li Wang[1], Haoran Tang[2], Hang Dong[2], Chunxia Su[1]

[1]Department of Medical Oncology, Shanghai Pulmonary Hospital & Thoracic Cancer Institute, Tongji University School of Medicine, Shanghai, China,[2]Huidu (Shanghai) Medical Sciences, Ltd., Shanghai, China

Introduction: Immunotherapy against non-small-cell lung cancer (NSCLC) with PD-L1-negative remain an unmet need. Cadonilimab is a bispecific antibody that simultaneously targets programmed cell death receptor-1 and cytotoxic T lymphocyte-associated antigen-4. In this study, we aimed to assess the efficacy and safety of cadonilimab combined with chemotherapy for the first-line treatment of PD-L1-negative advanced NSCLC.

Methods: LungCadX (NCT06424821) is a multi-center, open-label, single-arm, phase II study. Patients received 4 cycles cadonilimab 10 mg/kg every three weeks plus chemotherapy, followed by cadonilimab maintenance therapy. The primary endpoint was the 12-month progression-free survival (PFS) rate by investigator assessment per RECIST 1.1. Secondary endpoints included overall survival (OS), PFS, objective response rate (ORR), duration of response (DOR), disease control rate (DCR) and the safety. Blood samples were collected at C1D1 and C3D1 for biomarker analysis. The study utilized PredicineALERT, a liquid biopsy methylation assay, to profile DNA methylation profiles in circulating tumor DNA (ctDNA) and monitor disease response.

Result: As of data cutoff on November 8, 2024, 56 patients were enrolled with a median age of 64 years old, and 47 (83.9%) of them had stage IV NSCLC. 46 patients had at least 1 post-baseline tumor assessment. At a median follow-up of 6.7 months (range, 3.3 -11.5 months), the investigator-assessed ORR was 60.9% (28/46), with a 80.9% ORR for squamous (17/21) and 40.9% for non-squamous (11/25), and disease control rate (DCR) was 100.0% (46/46). Of the 50 patients who received at least two cycles of treatment, treatment-related adverse events were reported in 45 (80.3%), with grade ≥3 TRAEs occurring in 27 (48.2%), serious TRAEs reported in 12 (21.4%). The most common adverse events included leukopenia, neutropenia, thrombocytopenia, and anemia. No treatment-related deaths were reported. We completed ctDNA methylation analysis on samples collected at C1D1 and C3D1 from 18 patients. Changes in methylation scores from baseline to C3D1 were correlated with the RECIST 1.1. Specifically, all nine patients with a partial response exhibited a decrease in the differentially methylated fragment (DMF) score. In contrast, two patients with stable disease showed a greater than 20% increase in the DMF score, indicative of methylation-based progression and suggesting potential treatment resistance.

Conclusions: Our preliminary result showed that cadonilimab plus chemotherapy showed promising anti-tumor activity and manageable safety as first-line treatment in patients with PD-L1 negative advanced NSCLC and may potentially be a promising treatment option. The ctDNA analysis underscores the potential of monitoring treatment efficacy by utilizing cfDNA methylation dynamic profiles.

#3207 TIM-3 expression in human cancer: A tissue microarray study on 17,172 patients.

Seyma Buyucek[1], Sam Etebar Farimani[1], Katharina Moller[1], Florian Viehweger[1], Ria Schlichter[1], Anne Menz[1], Andreas M Luebke[1], Ahmed Abdulwahab Bawahab[2], Viktor Reiswich[1], **Elena Bady**[1], Lisa Hornsteiner[1], Claudia Hube-Magg[1], Andrea Hinsch[1], Florian Lutz[1], Soren Weidemann[1], Frank Jacobsen[1], Maximilian Lennartz[1], David Dum[1], Christian Bernreuther[1], Patrick Lebok[1], Guido Sauter[1], Andreas H Marx[3], Ronald Simon[1], Christoph Fraune[1], Natalia Gorbokon[1], Eike Burandt[1], Sarah Minner[1], Stefan Steurer[1], Till S Clauditz[1], Till Krech[1]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany,[2]University of Jeddah, Jeddah, Saudi Arabia,[3]Academic Hospital Fuerth, Fuerth, Germany

TIM-3 (T-cell immunoglobulin and mucin-domain containing-3) is an inhibitory immune checkpoint molecule which mainly occurs in subsets of inflammatory cells. On the surface membrane of cancer cells TIM-3 may represent a druggable target. To assess the prevalence of TIM-3 expression in cancer, a tissue microarray containing 17,172 samples from 134 different tumor types and subtypes was analyzed by immunohistochemistry (IHC). TIM-3 expression of tumor cells and macrophages were semiquantitatively recorded. In cancer cells, a membranous TIM-3 positivity was predominantly seen in clear cell (61.9%) and papillary (62.5%) renal cell carcinomas (RCCs) but - less commonly - also in Hodgkin's (25.0%) and non-Hodgkin's lymphomas (up to 9.1%), epithelial thymomas (6.9%), ovarian carcinomas (0.7%-1.4%), seminomas (0.4%), and several mesenchymal neoplasms (up to 16.0%). High TIM-3 expression of tumor cells was linked to low grade (p=0.0079), low UICC stage (p=0.0085), low pT (p<0.0001), and pN0 (p=0.0067) in clear cell RCC (ccRCC), and to pM0 (p=0.0177) in papillary RCC (pRCC). TIM-3 expression of macrophages occurred in all tumor entities but its relationship with parameters of aggressive disease varied between entities. A high quantity of TIM-3 positive macrophages was linked to unfavorable tumor parameters such as high grade (p<0.0001), advanced pT stage (p=0.0126), and distant metastasis (p=0.0069) in ccRCC, as well as high grade (p<0.0001), HER2 positivity (p=0.0015), and absence of estrogen (p<0.0001) and progesterone receptor (p=0.0187) expression in invasive breast carcinomas of no special type. A high number of TIM-3 positive macrophages was linked to favorable tumor parameters such as L0 (p=0.0333) and V0 (p=0.0161) in mismatch repair protein proficient colorectal carcinoma, low pT (p=0.0128), L0 (p=0.0333), and V0 (p=0.0161), as well as a lack of rete testis (p=0.0053) and spermatic cord infiltration (p=0.0365) in testicular seminoma. It is concluded that tumor cell TIM-3 expression among epithelial or mesenchymal tumors is largely restricted to RCCs where high expression is associated with parameters of non-aggressive disease. TIM-3 expression in tumor associated macrophages is abundant and its clinical significance varies between tumor entities.

#3208 Immunomodulatory potential of mormodica balsamina crude extract as an immune checkpoint inhibitor on human cancer cells using chicken egg chorioallantoic membrane model.

Portia Pheladi Raphela-Choma, Mpho Suzan Choene, Lesetja Raymond Motadi

biochemistry, University of Johannesburg, Johannesburg, South Africa

The chorioallantoic membrane (CAM) is widely used as an *in vivo* model for the evaluation of tumorigenesis. Immune checkpoint molecules play a critical role in the immune system, respond to various infections, and protect against tissue damage. The purpose of this study was to investigate the immunomodulatory potential of *Mormodica balsamina (MMB)* crude extract in colorectal cancer cells using the CAM model. Human colon cancer cells were transplanted onto the CAM membrane of fertilized eggs, resulting in tumor development. The crude extract was injected intravenously into the chicken egg at different dosages. Comparative studies of the effect of MMB crude extract on colon cancer xenografts were conducted. The crude extract exhibited efficacy in inhibiting tumor progression and metastasis. The proteins (PDL1 and LAG3) were lowly expressed to inhibit cancer proliferation and promote apoptosis. In conclusion, the chicken egg tumor model has shown to be valuable in testing medicinal plants as therapies for human colon cancers. The crude extract may have the potential to be used as a drug target in future studies.

#3209 IND enabling study of NRT-YHD_001, a macrophage immune checkpoint inhibitor in liver cancer.

S. Nam[1], J. Ha[1], S. Kim[1], M. Na[1], S. Jeon[1], B. Yoo[2], I. Yoon[2], H. Lee[2], C. Park[2];
[1]Catholic University of Korea, College of Medicine, Seoul, Korea, Republic of, [2]NEORNAT Inc, Seoul, Korea, Republic of

NRT-YHD_001, a modified antisense miRNA targeting let-7i-5p for liver cancer therapy, acts as a key regulator of macrophage activity within the tumor microenvironment. Through *in vitro* pharmacology studies, we identified anticancer effects not only on cell growth, proliferation, viability, migration, and invasion but also through the enhancement of macrophage phagocytosis. To evaluate the *in vivo* efficacy of NRT-YHD_001, we used a *Ras*-transgenic mouse liver cancer model which develops spontaneous liver cancer mass after 15 ~ 20 weeks of birth. Based on dose-dependent and injection interval studies, the optimal regimen was determined to be 1 mg/kg administered weekly. We confirmed that weekly intravenous injections of 1mg/kg NRT-YHD_001 showed significantly greater therapeutic efficacy than the sorafenib-treated group. Additionally, xenograft assay using human liver cancer cells in athymic-nude mice showed that weekly injection of NRT-YHD_001 led to improved survival and tumor growth inhibition compared to the untreated group. We performed Absorption, Metabolism, Distribution, and Excretion studies for NRT-YHD_001, stability studies in serum and liver homogenate, and metabolite identification and profiling analysis were completed in mice and cynomolgus monkeys. In maximum tolerated dose (MTD) studies conducted in mice and cynomolgus monkeys, no toxicity of NRT-YHD_001 was observed at doses up to 500 mg/kg administered intravenously. Furthermore, comprehensive evaluations across 4-week repeated dose toxicity and toxicokinetic in mice and monkey, safety pharmacology, and genetic toxicity studies revealed no adverse findings with NRT-YHD_001. For NRT-YHD_001, Drug Substance (DS) and Drug Product (DP) were produced by a global CDMO with experience in U.S. FDA approval of oligonucleotide drugs, and the analysis method was established.These results enabled us to complete the IND-enabling study package for NRT-YHD_001, a liver cancer therapy candidate with FTO analysis completed and intellectual property (IP) fully secured. Regulatory approval submissions to the Korea MFDS and the U.S. FDA are planned for 2025.

#3210 Neoadjuvant-adjuvant pembrolizumab (PEMBRO) in advanced basal cell carcinoma (BCC) induces memory-like T cell expansion with therapeutic promise.

Yee Peng Phoon[1], Beatriz Coutinho de Oliveira[1], Grace Jones[1], James M. Isaacs[1], Jennifer Ko[1], Lucy B. Kennedy[1], Pauline Funchain[2], Thach-Giao Truong[1], Brian Gastman[1], Jan J. Melenhorst[1]

[1]Cleveland Clinic, Cleveland, OH,[2]Stanford Cancer Institute, Stanford, CA

Background: BCC is a prevalent form of skin cancer, and immunotherapy has been explored as a treatment option. Advancements in checkpoint inhibitors (CPI) and other systemic therapies have improved outcomes for unresectable BCC. However, alternatives for high-risk resectable disease remain scarce, with no approved neoadjuvant or adjuvant therapies. Our team conducted a phase IB trial evaluating PEMBRO in patients with resectable locally advanced BCC of the head and neck, with clinical outcomes previously reported. Here, we present our findings on immune cell function and the spatial tumor microenvironment dynamics in the enrolled patients.

Method: 13 patients were enrolled from July 2020 to May 2023, with analysis focusing on 11 who received surgery after at least one dose of PEMBRO. Immune modulation was assessed using 32-color flow cytometry on peripheral blood pre- and post-CPI, T cell analysis in FlowJo, and 33-plex spatial multiplexed immunofluorescence (mIF) analysis in QuPath for tumor biopsies. Cytokine profiling was conducted with IsoPlexis, and data were analyzed in GraphPad Prism.

Results: In this trial, 3 patients (27%) achieved a pathologic complete response at surgery and were classified as responders. With a median follow-up of 30 months, none of the responders experienced a relapse.

Flow cytometry analysis showed variation in CD4 levels across the groups. In some patients, CD4 levels increased by up to 2-fold post-CPI, accompanied by higher CD8 levels. Cluster analysis revealed an increase in CD4 central memory (T_{CM}) and CD8 effector memory (T_{EM}) T cells post-CPI, while naïve and exhausted T cells were more prominent pre-CPI. These clusters, particularly in responders, underscore the importance of a sustained anti-tumor immune response for better therapeutic outcomes.

Spatial mIF of tumor biopsies identified 204,601 cells across 45 sections, averaging 4,547 cells per section. Responders showed a significantly higher level of T cell infiltration than non-responders, with increased CD4 and CD8 T cell counts. Notably, responders had elevated CD45RO-expressing CD4 and CD8 T cell counts compared to non-responders. Patients showed a marked increase in CD8 T cell percentage post-CPI.

In responders, post-CPI plasma levels of IL-6, IL-17, and IFNγ were significantly elevated, indicating increased immune activation that likely enhanced treatment effectiveness.

Conclusion: Our study demonstrates that neoadjuvant-adjuvant PEMBRO stimulates T cell expansion and selectively enhances memory T cells in locally advanced BCC. The rise in memory T cells after CPI therapy indicates a more sustained immune response, which is associated with better recurrence-free survival in our trial cohort. These T cell modifications offer valuable insights into the potential effectiveness of CPI in other immune-sensitive tumor types.

#3211 A multiple blockade of PD-L1/TIGIT/TIM3 to enhance the therapeutic efficacy on B-cell lymphomas.

Jihyun Park[1], Seojeong Kim[1], Youngil Koh[1], Byung-Su Kim[2], Sung-Soo Yoon[1]

[1]Seoul National University Hospital, Seoul, Korea, Republic of,[2]Eunpyeong St. Mary Hospital, Seoul, Korea, Republic of

Hodgkin lymphoma exhibits high PD-L1 expression and responds well to PD-1 blocking antibodies. In contrast, most non-Hodgkin lymphomas express multiple checkpoints, reducing their sensitivity to PD-1/PD-L1 blockade. These findings highlight the complexity of immune evasion mechanisms and the potential of combinatorial therapeutic approaches in lymphoma. We established cell lines overexpressing murine CD155 and luciferase using lentivirus to measure tumor growth in mouse models of B cell lymphoma. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Seoul National University Hospital (protocol 22-0333-S1A0). Female Balb/c mice (7-10 weeks old) were used, sourced from Koatech. For A20 tumor models, 5×106 cells were injected intraperitoneally. Mice received isotype control or specific blocking antibodies (anti-PD-L1, TIGIT, TIM3) three times per week. Tumor growth was monitored weekly via luminescence. After four weeks, spleen and lymph nodes were collected for lymphocyte analysis. Data acquisition was performed using a BD Symphony A3 flow cytometer, and analysis was conducted with FlowJo V10 software. We compared intraperitoneal versus intravenous injection of several different cell doses of hCD20-mCD155-A20 cells to optimize the lymphoma murine model. Intraperitoneal injection of $5x10^6$ cells was determined as the optimal dose, showing stable tumor growth for experimental analyses. Anti-PD-L1 and anti-TIGIT antibodies significantly inhibited tumor progression, whereas anti-TIM3 antibody promoted tumor growth. Mechanistic studies revealed that anti-PD-L1 antibody restored $CD4^+FoxP3^-$ and $CD8^+$ T cell populations and reduced Treg cells, modulating PD-1 expression differentially across immune subsets. Anti-TIGIT antibody increased the CD226/TIGIT ratio in splenic cells. Interestingly, treatment with anti-PD-L1 antibodies significantly increased the expression of both PD-1 and TIGIT on Tregs. Furthermore, the application of an anti-TIGIT blocking antibody decreased PD-1 expression on CD4+FoxP3- T cells, while concurrently enhancing PD-1 expression on CD8+ T cells. Immune checkpoint molecules on Tregs appear to be less affected compared to those on other immune cell populations. Conversely, anti-TIM3 antibody decreased effector T cells while increasing Tregs and PMN-MDSCs, fostering an immunosuppressive microenvironment. Our findings showed the distinct immunological effects of these checkpoint inhibitors, offering insights into their therapeutic potential and limitations in B cell lymphoma models. This research was a collaborative effort made possible through support from F. Hoffmann-La Roche for the imCORE Network.

#3212 IL2-mediated modulation of sEV secretion and PD-L1 expression: A novel perspective for neutralizing immune suppression within cancer cells.

Soojeong Noh[1], Suyeon Ryu[2], Dokyung Jung[2], Sanghee Shin[1], Inseong Jung[1], Sung-Min Kang[2], Christine Seulki Kim[1], Sung-Jin Choi[1], Hanchae Cho[2], Melanie Schwammle[3], Youngtae Jeong[1], Felicitas Bucher[4], Il-Kyu Choi[1], Shin Yup Lee[2], Sin-Hyeog Im[5], Kyungmoo Yea[1], Moon-Chang Baek[2]

[1]Daegu Gyeongbuk Institute of Science and Technology (DGIST), Daegu, Korea, Republic of,[2]Kyungpook National University, Daegu, Korea, Republic of,[3]University of Freiburg, freiburg, Germany,[4]University of Freiburg, Freiburg, Germany,[5]Pohang University of Science and Technology, Pohang, Korea, Republic of

IL2 is known to induce anticancer effects by activating immune cells expressing the IL2 receptor complex (IL2R). However, the potential impact of IL2 on cancer cells expressing IL2R, such as melanoma cells, has been understudied. Here, we demonstrate that IL2 significantly reduces the secretion of small extracellular vesicles (sEVs) and expression of programmed death ligand 1 (PD-L1) in melanoma cells, while increasing their sensitivity to $CD8^+$ T cell-mediated cytotoxicity. These effects are mediated through the activation of IL2R-MEK/ERK signaling in melanoma cells. To evaluate the direct effects of IL2 on melanoma cells *in vivo*, we developed a mouse model in which melanoma cells were engineered to express IL2 on their surface for self-stimulation. In this model, IL2 stimulation inhibited tumor growth and reduced PD-L1 expression in tumor tissues and plasma. Similar patterns were observed in lung cancer clinical samples. Our findings suggest that IL2 can directly elicit an anticancer immune response in IL2R-bearing cancers, highlighting its potential application in immunotherapy as an immune checkpoint modulator.

#3213 IA-05592: A breakthrough oral PD-L1 inhibitor with exceptional anti-tumor efficacy and immune activation potential.

Yeo Wool Kang[1], Ye Eun Kim[1], Garam Choi[1], Sol Lee[1], Deokmo Yang[1], Jongmin Sung[1], Kye Jung Shin[2], Tae-Hwe Heo[2], Taegwon Oh[1], Myung Jin Kim[1]

[1]ILAb Co.,Ltd., Bundang-gu, Seongnam-si, Gyeonggi-do, Korea, Republic of,[2]Integrated Research Institute of Pharmaceutical Sciences, College of Pharmacy, The Catholic University of Korea, Wonmi-gu, Bucheon, Gyeonggi-do, Korea, Republic of

Programmed death-ligand 1 (PD-L1) is a key immune checkpoint protein that interacts with PD-1 on T cells to suppress anti-tumor immunity. While PD-1/PD-L1 antibodies have become a cornerstone of cancer therapy, their limitations, including poor tumor penetration, immunogenicity, immune-related risks due to long half-life, and high production costs, have driven interest in small-molecule alternatives. IA-05592, a novel orally bioavailable small-molecule PD-L1 inhibitor, was developed using structure-based drug design to disrupt PD-1/PD-L1 interactions. This compound offers advantages such as oral bioavailability, reduced costs, and a novel mechanism of action involving PD-L1 internalization and degradation. Biochemical assays using fluorescence resonance energy transfer (FRET) demonstrated IA-05592's potency in disrupting PD-1/PD-L1 interactions, while cellular activity assays confirmed its ability to activate T cells, with an EC_{50} of 14 nM in NFAT reporter assays. IA-05592 reduced PD-L1 expression on tumor cells by inducing internalization and degradation, a mechanism confirmed in MC38 cells engineered to express human PD-L1 (MC38-hPD-L1). *In vivo*, daily oral administration of IA-05592 in a human PD-L1 knock-in MC38 syngeneic mouse model at doses of 10, 30, and 90 mg/kg resulted in dose-dependent tumor reduction. Combination studies with Fingolimod confirmed that IA-05592's anti-tumor effects are immune-mediated. Pharmacokinetic and ADME studies in rodents revealed high oral bioavailability and systemic exposure, further supporting its therapeutic potential. These findings position IA-05592 as a promising candidate for next-generation cancer immunotherapy, offering a unique mechanism of action and robust preclinical anti-tumor efficacy comparable to anti-PD-L1 antibodies. Ongoing studies aim to advance IA-05592 as a key component of all-oral cancer immunotherapy regimens.

#3214 The duration of immune checkpoint inhibitors (ICI) in oncology: Longer is not better.

Farah Mazahreh, Samuel L. Schach, Liyan Mazahreh, A. Mazin Safar

University of Arkansas for Medical Sciences, Little Rock, AR

ICI addition to the oncology armamentarium provided significant therapeutic benefits. Standard schedule of ICI calls for 2-year regimen without rational justification. Doses selected clearly do not adhere to classic maximal tolerated dose since ICIs were doubled at longer dose intervals. Theoretically however, multiple concerns can be proposed to question the 2-year duration. In human neoadjuvant melanoma studies examining ICI effect on effector T-cells comprehensively documented the expected clonal expansion in target cells but this was noted only for few weeks and did not continue upon repeated dosing, a major challenge to the current standard dosing schedule. Therapeutic T-cell transfers administer a single dose of 10^9 , a bulk that is expected to require 2-3 weeks of lymphocyte proliferation. Neutralizing antibodies have been reported in multiple studies, but notably without detriment to the therapeutic impact. Lastly, published and unpublished cases confirm lasting benefits (in years) after single ICI dosing. We hypothesized that benefits of ICI depend mostly, if not entirely, on the first, or few initial doses; that doses later in time can be delivered but add little if anything to the therapeutic impact.

Methods/Results: we reviewed published ICI monotherapy or chemo-ICI which provided individual swimmers' plot in stage 4 melanoma, renal cell and NSCLC. We extracted the median survival (MS) of those who received variable ICI durations excluding progression as the cause for halting therapy. Patients with progressive disease beyond 6 months, were included because they received considerable durations of ICI and might enjoy lasting benefits. We report the MS in the times depicted in the table. Our data reveal comparable results in all subgroups supporting our hypothesis.

Conclusions: Despite standard recommendations, there is considerable rationale in addition to our current data to challenge the common duration of ICI and substantiate a far more limited exposure to ICI.

Table

ICI Duration	Median Survival (Months)	Number of Cases
1-3 months	27	29
3-6 months	31.5	24
6-12 months	32	63
12-18 months	35	30
18-24 months	37	47
24+ months	48	32

#3215 Cystatin F changes in melanoma patients during therapy with immune checkpoint inhibitors.

Milica M. Perisic Nanut[1], Emanuela Senjor[1], Tanja Mesti[2], Simona Miceska[3], Janja Ocvirk[2], Janko Kos[4]

[1]Jozef Stefan Institute, Ljubljana, Slovenia,[2]Department of Medical Oncology, Institute of Oncology Ljubljana, Ljubljana, Slovenia,[3]Department of Medical Oncology, Institute of Oncology Ljubljana,Department of Medical Oncology, Institute of Oncology Ljubljana,, Ljubljana, Slovenia,[4]Department of Pharmaceutical Biology, Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia

Although immune checkpoint inhibitors (ICI) have achieved undeniable success in the treatment of metastatic melanoma, primary and secondary resistance to ICI therapy continues to pose a significant challenge for melanoma patients. Around 40-60% of patients respond to immunotherapy, achieving long-term benefits, but over two-thirds experience immune-related adverse events (irAEs) with ICIs. About 10% show pseudoprogression, marked by transient inflammation, while up to 30% experience hyperprogression with rapid cancer advancement. Despite irAEs, treatment interruption often leads to better outcomes and prolonged survival. The lack of clinically validated predictive biomarkers remains a major factor contributing to the unpredictable effects of immunotherapy. Implementing biological markers in clinical practice is essential to enhance personalized treatment and predict responses. We have identified a protease inhibitor cystatin F as a potent down regulator of cytotoxic efficacy of cytotoxic T lymphocytes (CTLs). Its expression was shown to be increased in hypo responsive and exhausted CTLs but also in the cancer tissue in several types of cancer. In order to elucidate its role in CTLs of patients with melanoma and correlation its expression with response to treatment we have examined cystatin F expression, along with the CTL PD-1 expression and IFNγ in peripheral blood CTLs form patients with advanced melanoma (TNM classification, stage IIID and IV) receiving first-line therapy with ICIs. Study-specific data were collected at three different time points: up to 4 weeks before the start of treatment, at the 12th (+/−2 weeks) and 28th week (+/−2 weeks) and the expression of markers on whole blood preparations or isolated CTLs was analysed using flow cytometry. We characterized the cellular sources of cystatin F in tumor tissue using multicolor immunofluorescence staining and confocal analysis on formalin-fixed melanoma tissue samples. Our findings reveal cystatin F expression in predominantly in non-lymphoid cells within the tumor microenvironment. Analysis of cystatin F in peripheral blood CTLs shows higher expression in non-responders and patients with irAEs, suggesting its potential as a marker for enhancing personalized treatment and predicting treatment responses.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Identification of Molecular Targets 1
Poster Session

#3216 A dual-acting chimeric peptide inhibits tumor growth by inhibiting PD-L1 and inducing immunogenic cell death.
S.-Y. Park, S. Gurung, B. Lee;
Kyungpook National University, Jung-gu, Korea, Republic of

Blockade of the immune checkpoint proteins such as PD-L1 enables to overcome the resistance of cancer cells against the immune surveillance. Nevertheless, the blockade therapy itself lacks cytotoxic killing ability to cancer cells. In this study, we exploited PD-L1-targeted cytotoxic peptides that block PD-L1 on the surface of tumor cells and induce the tumor cell death as well. As targeting peptides, we used PD-L1Pep-1 and PD-L1Pep-2 that bind to and block PD-L1. As a cytotoxic peptide, the KLAKLAKKLAKLAK peptide (named K) was fused to the targeting peptide. PD-L1Pep-1-K and PD-L1Pep-2-K decreased cell survival in PD-L1-expressing tumor cells. Both peptides were internalized into tumor cells through the receptor-mediated endocytosis and localized at lysosomes and also at mitochondria. The peptide-induced cell death was accompanied by an increase in the exposure of CRT on the cell surface and extracellular release of ATP and HMGB1, which are markers of immunogenic cell death (ICD). PD-L1Pep-2-K rekindled the T-cell activity including proliferation and cytokine secretion when co-cultured with tumor cells. Systemic administration of PD-L1Pep-2-K inhibited tumor growth more efficiently than PD-L1Pep2 and anti-PD-L1 antibody. These results suggest that the PD-L1-targeted cytotoxic peptide is a novel dual-acting therapeutic agent capable of PD-L1 blocking and ICD induction for cancer immunotherapy.

CLINICAL RESEARCH: Immune Checkpoints
Poster Session

#3218 The Secretome: a multigene biomarker for the prognosis and selection of solid tumor patients evaluated for treatment with immune checkpoint inhibitors..

Alessandro Porrello[1], Marjory Charlot[2], Michael M. Cunningham[3], Shruti Saxena-Beem[4], Leon G. Coleman[5], Rumey G. Ishizawar[6], Benjamin G. Vincent[7], Chad V. Pecot[1]

[1]Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC,[2]Department of Medicine, Division of Oncology, University of North Carolina at Chapel Hill, Chapel Hill, NC,[3]Department of Medicine, Division of Rheumatology, Allergy and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC,[4]Thurston Arthritis Research Center, University of North Carolina at Chapel Hill, Chapel Hill, NC,[5]Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill, Chapel Hill, NC,[6]Department of Medicine, Division of Rheumatology, Allergy & Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC,[7]Department of Medicine, Division of Hematology, University of North Carolina at Chapel Hill, Chapel Hill, NC

Introduction: The advent of immune checkpoint inhibitors (ICIs) has been revolutionary for cancer treatment. However, only a minority of solid tumor patients significantly benefit from them as a monotherapy. Approximately 3 out of 4 patients receiving ICIs experience a broad range of immune-related (ir) adverse events (AEs), including many AEs of grades 3 and 4 as well as fatalities (0.3 - 1.3%). Accurate criteria to select the best candidates, sparing other patients from unnecessary physical and financial burden, are urgently needed.
Methods: We developed a Secretome signature, based on 50 genes, utilizing The Cancer Genome Atlas (TCGA) lung squamous cancer (LUSC) RNA-Seq data, to identify patients whose tumor is characterized by abundant immune cell infiltration. This signature was generated through gene selection and generalized linear models. An algorithm was created to make this signature portable across multiple solid tumor types and sequencing platforms. We used this algorithm to assign a Secretory-like (SL) status to a cohort of 78 patients of the University of North Carolina (UNC) - Chapel Hill, mostly diagnosed with lung and head and neck cancer and having received ICIs. A retrospective chart review collected their socioeconomic and racial/ethnicity demographics, smoking and alcohol use, and characterized cancer outcome and irAEs. We used categorical data analysis to investigate salient features of the SL group. After dichotomizing patients according to their SL status, we performed Gene Set Enrichment Analysis (GSEA) of their RNA-Seq data. Using Kaplan-Meier Plotter - Immunotherapy, we assessed the role played by Secretome genes in overall survival (OS).
Results: GSEA shows that main features found in TCGA LUSC cohort (e.g. enrichment of the ‘Hallmark TNFA signaling via NFKB’ gene set) are also present in the UNC SL cohort. These results reveal that our model, which strongly constraints the number of genes, can still retrieve patients with key biological features displayed by the group of TCGA LUSC Secretory. SL lung cancers are more prevalent in Black and Hispanic/Latinx (BHL) than in White ($P = 0.02$), which generally points towards a worse prognosis for BHL patients. SL patients have > 3 times post-ICIs dermatologic AEs than non-SL ($P = 0.01$). Remarkably, the hazard ratios of high vs. low gene expression levels show that > 80% of testable Secretome genes are individually predictive of OS in patients receiving anti-PD1 ICIs, supporting the combined use of Secretome genes for optimal performance.
Conclusions: The Secretome can be used to identify patients, who are most likely to be associated with prolonged survival after ICI therapy. The Secretome can also characterize patients (i) expected to have a worse prognosis and (ii) who should be more closely monitored when using ICIs, since they have an increased risk of developing dermatologic AEs.

#3219 Assessing strategies to activate DNAM1 signaling and inhibit the TIGIT/PVRIG axis using mathematical modeling.

Oleg Demin Jr, Galina Kolesova, Dmitry Shchelokov

InSysBio CY, Paphos, Cyprus

PVR (CD155) and CD112 are ligands from the Nectin/Nectin-like family, frequently expressed on various tumor cells. PVR binds to the inhibitory receptors TIGIT and CD96, triggering a direct inhibitory signal in T and NK cells, and binds to the co-stimulatory receptor DNAM1 (CD226), leading to its downregulation. CD112 binds to the inhibitory receptor PVRIG and to DNAM1, stimulating T and NK cells. Late-stage clinical trials of anti-TIGIT monoclonal antibodies (mAbs) have shown mixed results regarding efficacy (Cho et al. ***Lancet Oncol***. 2022 Jun;23(6):781-792; Rudin et al. ***J Clin Oncol***. 2024 Jan 20;42(3):324-335). Numerous anti-PVR, anti-CD96, and anti-PVRIG mAbs, along with their combinations with anti-TIGIT mAbs and other therapies, are currently in early clinical development. This study aimed to compare how the complete inhibition of TIGIT, PVRIG, PVR, or their combinations affects the engagement of TIGIT, CD96, PVRIG, and DNAM1.

We developed a mechanistic model describing the surface expression of PVR and CD112 on cancer cells, and TIGIT, CD96, DNAM1, and PVRIG on CD8 T-cells. This model captures the interactions of these ligands and receptors within the immunological synapse (IS) between CD8 T-cells and cancer cells in the tumor. In vitro data and expression data of these ligands and receptors were used to identify parameters. The model was validated against data on the downmodulation of DNAM1 and TIGIT on CD8 T-cells in the tumor compared to the blood. We simulated total DNAM1 on the surface of CD8 T-cells in the tumor and the engagement of TIGIT, CD96, PVRIG, and DNAM1 in the IS (i.e., the number of PVR:TIGIT, PVR:CD96, CD112:PVRIG, PVR:DNAM1, and CD112:DNAM1 complexes) under 100% inhibition of TIGIT, PVR, PVRIG, TIGIT and PVRIG, or PVR and PVRIG.

TIGIT blockade did not increase DNAM1 engagement. Moreover, 100% inhibition of TIGIT led to the release of additional free PVR, resulting in the downmodulation of total DNAM1 and an increase in CD96 engagement. Inhibition of PVRIG resulted in an approximately 1.3-fold increase in bound DNAM1, but not in total DNAM1. The combination of TIGIT and PVRIG blockade did not increase engaged DNAM1 beyond the levels observed with anti-PVRIG monotherapy. Inhibition of PVR led to a significant increase in total DNAM1 and a moderate increase in bound DNAM1 (approximately 1.3-fold), along with the disruption of TIGIT and CD96 engagements. The combination of PVR and PVRIG blockade resulted in the strongest increase in engaged DNAM1 (approximately 2-fold), in addition to disrupting TIGIT, CD96, and PVRIG engagements.

The inability to increase DNAM1 engagement during TIGIT inhibition may be one of the reasons for the lack of efficacy observed in the phase 3 trial of tiragolumab (anti-TIGIT mAb) used in combination with atezolizumab. Targeting PVR alone or in combination with PVRIG appears to be a better strategy than targeting TIGIT or TIGIT in combination with PVRIG.

#3220 PD-1 antibody-bound progenitor-exhausted CD8$^+$T cells in lymph nodes enhance PD-1 blockade anti-tumor immunity in gastrointestinal cancer.

Takuro Saito[1], Yohei Nose[1], Yoshiaki Yasumizu[2], Naganari Ohkura[2], Hisashi Wada[3], Hidetoshi Eguchi[1], Yuichiro Doki[1]

[1]Department of Gastroenterological Surgery, Osaka University, Suita-shi, Osaka, Japan,[2]Experimental Immunology, WPI Immunology Frontier Research Center, Suita-shi, Osaka, Japan,[3]Department of Clinical Research in Tumor Immunology, Osaka University, Suita-shi, Osaka, Japan

Introduction: Progenitor-exhausted T (Tpex) cells, marked by TCF1 and PD-1 expression, are essential for the efficacy of immune checkpoint inhibitors (ICIs) like anti-PD-1 antibodies. These cells serve as precursors to intratumoral exhausted T (Tex) cells and may play a pivotal role in maintaining immune responses during cancer therapy. While Tpex cells are recognized for their importance, the detailed mechanisms by which ICIs influence their activation, proliferation, and migration remain unclear. Specifically, the dynamics of ICI-bound Tpex cells across lymph nodes (LNs) and tumors have not been fully characterized. To address this point, we investigated the spatial and functional behavior of Tpex cells in gastrointestinal cancer patients, focusing on how ICIs reprogram these cells to exert anti-tumor immunity.

Methods: We collected tumor, lymph node (LN), and blood samples from gastrointestinal cancer patients treated with or without anti-PD-1 antibodies. Purified lymphocytes were analyzed with flowcytometry and sequencing analysis. To identify ICI-binding T cells, we developed a method using IgG4 detection, which allowed us to detect cells directly bound by therapeutic anti-PD-1 antibodies. We further used combined sequencing technique including single-cell RNA sequencing, T cell receptor sequencing and CITE-sequencing for IgG4 detection (scRNA/TCR/CITE-seq) to profile 29,388 T cells across tissue compartments, including LNs, tumors, and blood.

Results: Our flowcytometry analysis revealed that CD8-Tpex cells were enriched in proximal LNs and exhibited significant proliferation following ICI treatment. Using IgG4 detection and sequencing, we confirmed that PD-1$^+$ Tpex cells in LNs directly bound therapeutic anti-PD-1 antibodies and underwent clonal expansion. Notably, these ICI-bound Tpex cells shared TCR clonotypes with intratumoral Tex cells, suggesting that Tpex cells migrate from LNs to tumors after treatment.

Conclusion: ICIs primarily target PD-1$^+$ Tpex cells in proximal LNs, driving their proliferation and migration to tumors, where they differentiate into Tex cells and contribute to sustained tumor destruction. These results not only deepen our understanding of the mechanisms underlying ICI therapy but also provide a robust methodological framework for tracking ICI-binding T cells across tissues.

#3221 Identification and characterization of FAK kinase as a novel regulator of PD-L1 stability and activity in cancer immune evasion.

A.-I. Boyd, P. Khanal, Y. Hao, X. Yang;
Queen's University, Kingston, ON, Canada

The PD-1/PD-L1 axis is an essential immune checkpoint that modulates T cell-mediated immune responses. Cancer cells exploit this checkpoint to inhibit anti-tumour immune activity, enabling immune evasion. Thus, blocking PD-1/PD-L1 using antibodies has become a cornerstone of cancer immunotherapies. However, despite promising results, these therapies show limited response rates in many cancer patients and often lead to adverse effects, impacting its overall efficacy. Therefore, understanding of the molecular mechanism underlying immunotherapy resistance is critical for successful cancer immunotherapy. Emerging research indicates that phosphorylation of PD-L1 by kinases significantly influence PD-L1 stability and functionality, thereby contributing to cancer cell immune evasion and influencing immunotherapy outcomes. However, although 560 kinases have been identified in the human kinome, there is no systematic screening of kinases regulating PD-L1 stability and its function in immune evasion. By using a PD-L1 NanoLuc luciferase biosensor, in this study, we performed a systematic kinome-wide screening and identified focal adhesion kinase (FAK) as a novel regulator of PD-L1 stability in cancer. Further investigation of FAK-mediated PD-L1 signalling and its role in immune evasion may provide a new therapeutic strategy for cancer treatment.

#3223 A highly selective and novel TCR-like antibody, IMB-202, targeting NK and T cell immune checkpoint for enhanced cancer immunotherapy.

Inyoung Lee, Hyeonju Kang, Chaerin Yoo, Hong Jai Lee, Sungmuk Kang, Sunghyun Byun, Yongjun Kang, Chungmin Lee, Gyongsik Ha

IMBiologics Corp., Suwon, Korea, Republic of

Human natural killer (NK) and T cells are essential cytotoxic effectors in tumor surveillance and elimination, with an increasingly important role in immune checkpoint inhibitor (ICI) therapies. While current ICIs have focused on inhibitory receptors on T cells, creating a paradigm shift in cancer treatment, challenges remain due to non-responders and treatment-associated adverse events. There is a growing need for alternatives that address these limitations and for strategies that enhance efficacy through combination with existing ICIs.

In this study, we developed IMB-202, a therapeutic anti-cancer antibody designed to target the ligands (TOI, Target of Interest), which are overexpressed on tumor cells, of an immune checkpoint receptor on NK and T cells. This TOI plays a crucial role in tumor escape mechanisms by transmitting inhibitory signals to NK and T cells. While this target holds significant promise for ICIs, its development as a therapeutic antibody has been particularly challenging due to the high sequence homology among related family proteins, which complicates achieving the specificity.

Through advanced phage display screening and affinity maturation, we successfully developed IMB-202 as a highly selective TCR-like antibody, capable of recognizing even a single amino acid difference in peptide sequences. IMB-202 binds with high affinity and specificity to TOI in various cancer cell lines and efficiently kills cancer cells by disrupting the immune evasion mechanisms, followed by activating NK and T cells, as well as through antibody-dependent cellular cytotoxicity (ADCC) *in vitro*. Additionally, IMB-202 demonstrated significant tumor growth inhibition in an *in vivo* xenograft mouse model. When applied to ePENDY technology, IMBiologics' proprietary multivalent antibody platform, IMB-202-ePENDY demonstrated enhanced avidity and increased NK and T cell cytotoxicity against cancer cells. Furthermore, its rapid internalization of IMB-202 supports its potential as an antibody-drug conjugate (ADC) candidate, with robust internalization also observed in the ePENDY platform format.

These findings highlight IMB-202 as a promising alternative to existing ICIs and as a first-in-class therapy, targeting a novel immune checkpoint ligand with exceptional selectivity. Additionally, IMB-202 demonstrates significant potential for superior anti-tumor efficacy, with the versatility to be applied across various therapeutic modalities.

#3224 An indirect Meta-analysis of efficacy and safety of PD-1 inhibitors versus PD-L1 inhibitors as preoperative therapy in patients with solid tumors.

Peng Wu, Chaoqi Zhang, Dongyu Li, Xuanyu Gu, Junhan Zhou, Chuqi Lin, Nan Sun, Jie He

Department of Thoracic Surgery, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing, China

Background: Programmed cell death 1 (PD-1) and ligand (PD-L1) immune checkpoint inhibitors (ICIs) in perioperative settings have improved clinical outcomes. However, it remains controversial which agent should be selected. We aim to compare the difference between anti-PD-1 and anti-PD-L1 efficacy and safety in neoadjuvant and/or adjuvant settings across randomized controlled trials.
Methods: We systematically searched PubMed, Embase, Cochrane CENTRAL, and major oncology conferences up to May 20th, 2024. Clinical trials comparing anti-PD1 or anti-PD-L1 with standard care in perioperative settings were collected. These studies were further paired based on comparable characteristics and integrated as mirror groups. The Preferred Reporting Items for Systematic Reviews and Meta-Analysis guideline was followed for data extraction. Specifically, trial names, author, publication year, study design, blinding status, phase, pathologic characteristics, number of patients, settings of treatment, drugs, biomarker status, incidence of adverse events (AEs), pathological complete response (pCR), and major pathological response (MPR), and hazard ratios (HRs) with 95% confidence interval (CI) for event-free survival (EFS), disease-free survival (DFS), and overall survival (OS) were extracted. A random or fixed model was used based on intertrial heterogeneity. Primary outcomes included pCR, MPR, EFS, DFS, OS, and AEs. Indirect comparisons between anti-PD-1 and anti-PD-L1 were conducted using Frequentist and Bayesian approaches.
Result: Totally, 31 studies involving 14974 patients were included. Overall, anti-PD-1 demonstrated superior pCR (risk ratio (RR): 1·65, 95% CI: 1·18-2·29, P = 0·003) and MPR (RR: 1·43; 95% CI: 1·04-1·96, P = 0·026) when compared with anti-PD-L1, and was associated with improved DFS (HR = 0·82, 95% CI: 0·71-0·96, P = 0·0106). For PD-L1 expression, no differential benefits were observed between anti-PD-1 and anti-PD-L1. No significant discrepancies in overall safety profiles were noted, however, anti-PD-1 was linked to a higher incidence of 3-5 grade immune-related adverse event (irAE). Frequentist and Bayesian data were largely consistent.
Conclusions: Anti-PD-1 therapy showed better clinical outcomes and higher incidence of serious irAEs to anti-PD-L1 in perioperative settings. These results may guide clinical decisions and trial designs, and further tumor-specific evaluations and mature trial data are needed.

#3225 A meta-analysis of single agent versus combination immune checkpoint inhibitors in metastatic triple negative breast cancer.

Phaedon D. Zavras[1], Hugo R. Lara-Martinez[2], Qi Nie[3], Julius Y. Kattoula[3], Nicole Strossman[3], Zehui He[3], Ming Li[3], Romualdo Barroso-Sousa[4], Isaac Chan[5], Sara M. Tolaney[6], Vered Stearns[7], Cesar A. Santa-Maria[1], Evanthia T. Roussos Torres[3]

[1]Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins, Baltimore, MD,[2]University of Texas, MD Anderson Cancer Center, Houston, TX,[3]University of Southern California, Keck School of Medicine, Norris Comprehensive Cancer Center, Los Angeles, CA,[4]Dasa Institute for Education and Research (IEPD), Brazilia, Brazil,[5]Department of Internal Medicine, Division of Hematology and Oncology, University of Texas Southwestern Medical Center, Dallas, TX,[6]Dana Farber Cancer Institute, Boston, MA,[7]Weill Cornell Medicine, Meyer Cancer Center, New York, NY

Background: Immune checkpoint inhibitors (ICIs) are active against triple-negative breast cancer (TNBC), and approved in combination with chemotherapy, or as a single agent in patients with high tumor mutational burden and/or microsatellite instability/mismatch repair deficiency. A number of studies have been conducted with single-agent inhibitors of programmed cell death 1 or its ligand (PD1/PD-L1i), however, responses are typically low, especially in the later line setting. Few studies have evaluated the efficacy of PD1/PD-L1i combined with inhibitors of cytotoxic T-lymphocyte associated protein 4 (CTLA-4i) in metastatic TNBC, and none compared single agent vs combination ICI approaches. The purpose of this meta-analysis is to evaluate the efficacy of PD1/PD-L1i with or without CTLA-4i in TNBC.

Methods: A comprehensive literature search across PubMed, Embase, and Cochrane Library identified 870 unique clinical trials from 2010 - 2024. Studies eligible for inclusion were those of patients with: (1) metastatic TNBC, (2) >1 line of prior therapy, (3) received PD1/PD-L1i with or without CTLA-4i. Studies of combination of ICI with chemotherapy or targeted treatments were excluded with the exception of histone-deacetylase inhibitor (HDACi) combinations. Data were selected and extracted by two reviewers based on the PRISMA-A guidelines. The primary objective of the meta-analysis was objective response rate (ORR). Progression-free (PFS) and overall survival (OS) were also determined when available.

Results: A total of 10 clinical trials were eligible and included in the meta-analysis, six for single ICI and four for combination ICI. The heterogeneity test indicated no significant heterogeneity (I^2) in either of the groups, thus, a fixed-effect model was used. A total of 611 patients with metastatic TNBC (>1L) treated with single ICI showed a pooled ORR of 8.3% (95% CI, 6.1-10.7%), median PFS of 1.7 months (95% CI 1.40-2.06) and OS of 9.13 months (95% CI, 8.09-10.17). Among 42 patients treated with combination ICI, a pooled ORR was 27.7% (95% CI, 14.1%-43.3%). A sensitivity analysis excluding patients that received an HDACi showed an ORR of 7.2% (95% CI, 4.4%-10.6%) vs. 24% (95% CI, 9.5%-41.6%) for the single and combination ICI groups, respectively.

Conclusion: The use of combination ICI therapy led to an overall response rate of 27% compared to 8% in the single ICI studies. Our results provide rationale for further studies evaluating combination ICI approaches in patients with metastatic TNBC.

#3226 Intratumoral manipulation of melanoma using novel hyperthermic treatment to enhance anti-PD-L1 therapy.

Nicole DeSouza[1], Danielle Quaranto[1], Kaci Kopec[1], Jan Geliebter[1], Raj K. Tiwari[1], Mark D. Hurwitz[2]

[1]New York Medical College, Valhalla, NY,[2]Westchester Medical Center, Valhalla, NY

Despite tremendous advancement in the understanding and manipulation of immune checkpoint molecules in immunotherapeutic design, there are still vast limitations in treatment efficacy. Immunotherapy manipulates the immune checkpoint blockade (ICB), blocking negative regulators of the immune system-preventing tumor immune evasion. Combination therapy enhances the mechanistic actions of each individual component, enhancing tumoricidal behavior. Thermal ablation to induce cancer cell death as a therapeutic adjuvant is an understudied area.

B16-F10 melanoma cells were grown at 37°C or 41°C. B16-F10 cells grown at 41°C decreased cell migration by 70% in 24 hours. B16-F10 cell proliferation was decreased at 41°C by 62% at 48 hours and 94% at 72 hours.

Protein expression profiles were evaluated under hyperthermic conditions in vitro. Expression of pERK and ERK decreased by 86% and 50% and Caspase-3 increased by 31% at 41°C. Cell stress can induce death pathways and the heat shock protein response simultaneously, therefore we investigated inducible Hsp70 expression. Hsp70 expression was undetectable at 37°C and 188% at 41°C. Cytokine analysis of conditioned media showed that at 41°C, TNF-α expression was increased and IL-4 expression was decreased, suggesting a proinflammatory shift in cytokine profiles at higher temperatures. In support of our data, hyperthermia-induced TNF-α apoptotic responses have been reported.

Programmed cell death ligand (PD-L1) is expressed by tumors to suppress immune cells and induce tolerogenic phenotypes within the tumor microenvironment. We observed that hyperthermic potentiation decreased the expression of this checkpoint molecule in B16-F10 by 35%. Therefore, we hypothesize that hyperthermia modulates the tumor microenvironment to enhance their responsiveness to checkpoint immunotherapies. Based on these promising in vitro results, we plan to further explore the clinical aspect of our findings through a translation in vivo. B16-F10 cells will be implanted onto C57BL/6 mice and treated with infrared radiation in combination with either anti-PD-L1, anti-PD-1, or IL-15.

#3227 Components of interleukin-4 signaling are enriched in melanoma patients who respond to anti-PD-1 or anti-CTLA-4 therapy.

Szonja Anna Kovacs, Janos Tibor Fekete, **Balazs Gyorffy**

Semmelweis University, Budapest, Hungary

Background: Immune checkpoint inhibitors represent an advanced class of immunotherapy drugs used to treat various cancers, including melanoma, particularly in advanced stages. These therapies work by targeting immune checkpoints — proteins such as PD-1, PD-L1, and CTLA-4 — that act as brakes on the immune system. Prognostic biomarkers are essential for predicting disease outcomes, thereby enabling improved patient management.
Methods: We obtained transcriptomic and clinical data from melanoma patients treated with immune checkpoint inhibitors through the NCBI Gene Expression Omnibus and CRI iAtlas. After reviewing 246 datasets, we excluded those involving cell lines, unrelated diseases, or treatments. The expression data were quantile-normalized, and statistical analyses were conducted using the R environment. Survival data were excluded for patients without an event if follow-up ended before 12 months. Cox regression was applied to overall survival (OS) data.
Results: Altogether, 423 melanoma samples had sufficient data for survival analysis. Elevated expression of IL4R (interleukin-4 receptor, HR=0.33, FDR=2%, log-rank P=5.7E-04), JAK1 (Janus kinase 1, HR=0.3, FDR=1%, log-rank P=2.8E-04), GFI1 (growth factor independence 1, HR=0.35, FDR=5%, log-rank P=2.2E-03), and PTPN6 (protein tyrosine phosphatase non-receptor type 6, also known as SHP-1, HR=0.31, FDR=5%, log-rank P=1.9E-03) correlated with improved OS after anti-PD-1 therapy. Similar findings were observed after anti-CTLA-4 therapy for IL4R (HR=0.13, FDR=1%, log-rank P=3.2E-04), JAK1 (HR=0.14, FDR=1%, log-rank P=9.3E-04), and PTPN6 (HR=0.09, FDR=1%, log-rank P=2E-04). Signals mediated by interleukin-4 and interleukin-13 through these genes promote improved immune responses, cell survival, and differentiation. Finally, the Kaplan-Meier Plotter (www.kmplot.com) web platform was updated with our newly compiled immunotherapy cohort.
Conclusions: In melanoma patients, elevated expression of IL4R, JAK1, GFI1, and PTPN6 following anti-PD-1 and anti-CTLA-4 immunotherapies may enhance immune cell recognition, increase treatment efficacy, and extend survival. Our analytical platform supports a deeper understanding of patient responses and facilitates biomarker validation in a large cohort of melanoma patients.

#3228 Characterization of multi-organ system involvement of immune-related adverse events after anti-PD-(L)1 monotherapies: A pooled analysis of clinical trial data submitted to FDA.

Abhilasha Nair[1], **Matthew J. Hadfield**[2], Yue Huang[3], Flora Mulkey[4], Ilynn Bulatao[3], Marc R. Theoret[3], Kerry L. Reynolds[5]

[1]Office of Oncologic Diseases, Center for Drug Evaluation and Research, US Food and Drug Administration, Silver Spring, MD,[2]Division of Hematology/Oncology, Dept of Medicine, Warren Alpert Medical School, Brown University, Legorreta Cancer Center, Providence, RI,[3]Oncology Center of Excellence, US Food and Drug Administration, Silver Spring, MD,[4]Office of Biostatistics, Center for Drug Evaluation and Research, US Food and Drug Administration, Silver Spring, MD,[5]Department of Medicine, Division of Hematology-Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, MA

Background: Treatment with PD-(L)1 blocking antibodies has dramatically improved outcomes across multiple cancer types. Such benefits must be carefully weighed against the risks of serious immune-related adverse events (irAEs) involving various organs, the occurrence patterns of which have not yet been well characterized. This large novel analysis of pooled clinical trial data submitted to FDA aims at uncovering and gaining deeper insights into the multiorgan patterns of irAEs associated with these agents.
Methods: This retrospective pooled analysis is based on patient-level, monotherapy, PD-(L)1 blocking antibody data from 18 clinical trials completed between 2019 and 2023, supporting FDA approvals across 7 cancer types. Only FDA authors had access to and analyzed patient level data. Patients with irAEs were identified using each PD-(L)1 development program's pre-defined definitions based on MedDRA preferred terms. Preferred terms were grouped into 30 irAEs based on the involved organs. IrAEs starting before or within 7 days of the end date of the preceding irAE, were considered co-occurring.
Results: Among the 5863 patients, 64% (n=3771) had at least one irAE; the most common irAEs (>10%) were skin reaction (53.5%), colitis/diarrhea (33.2%), hepatitis (23%), hypothyroidism (19.3%), and nephritis/renal dysfunction (11.7%). 50% (n= 1890) had more than one irAE type, with 59.7% experiencing 2, 24.8% experiencing 3, 9.7% experiencing 4, and 5.8% experiencing 5 to a maximum of 8 distinct irAE types, regardless of timing during their treatment course. Median time-to-onset of irAE in patients with a single type of irAE was 45 days while median time-to-onset of first irAE in subjects with more than one type of irAE was 30.5 days. Among the 1743 patients evaluable for co-occurrence analysis, 1285 (73.7%) experienced co-occurring irAEs. The 5 most frequently co-occurring irAEs were: skin reaction and colitis/diarrhea (15.8%); skin reaction and hepatitis (8.2%); skin reaction and hypothyroidism (7.5%); skin reaction and nephritis/renal dysfunction (4.8%); and colitis/diarrhea and hepatitis (4.2%).
Conclusions: This is the largest study of patient level clinical trial data evaluating the incidence and occurrence patterns of multi-system irAEs. The study demonstrated that the median time-to-onset of the first irAE was shorter for patients who experienced multi-organ irAEs than in those with single organ irAEs. Notably, half of the patients with an irAE experienced more than one distinct irAE during treatment, the majority of whom demonstrated co-occurrence of irAEs. These findings underscore the importance of vigilant monitoring and comprehensive management strategies to address the interconnected nature of irAEs, aiding providers in understanding, predicting, and managing patients experiencing irAEs.

#3229 Investigating the transcriptomic signature of HHLA2-mediated suppression of NK cell activity.

Nahuel Perrot[1], Nikolaos Kalavros[1], Deepthi Chowbene[1], Shoushuo Wang[1], Yered Pita-Juarez[1], Antonella Arruda de amaral[1], David McDermott[1], Gordon Freeman[2], Ioannis Vlachos[1], **Kathleen Mahoney**[1]

[1]Harvard Medical School/Beth Israel Deaconess Medical Center, Boston, MA,[2]Harvard Medical School/Dana-Farber Cancer Institue, Boston, MA

Determining how immune checkpoint inhibitors (ICI) alter functional characteristics of lymphocytes can improve our understanding of ICI. HERV-H LTR-associating 2 (HHLA2, B7-H7) is an immune checkpoint expressed by many tumors. Multiple HHLA2:KIR3DL3 blocking antibodies are currently in Phase I clinical trials and enrolling cancer patients (NCT05958199, NCT06240728), but little is known about how different subsets of NK cells are affected by HHLA2 expression. Our group previously found that in addition to an activating receptor, TMIGD2, HHLA2 has an inhibitory receptor, KIR3DL3. The KIR family of receptors is expressed in NK cells as well as T cells. Through co-culture experiments of CD56+ NK cells in the presence of K562 cells, we sought to investigate the mechanisms of NK cell engagement. Additionally, we generated transgenic K562 cell lines, over-expressing HHLA2, validated through flow cytometry, to maximize our ability to glean insight into HHLA2-KIR3DL3 mediated activation of NK cells. To this end, we generated a high quality scRNA-seq dataset of more than 100,000 cells, comprising of more than 90,000 NK cells, with approximately 30,000 cells either cultured alone, co-cultured with K562 or K562 over-expressing HHLA2 tumor cells, allowing unprecedented granularity. In conjunction with published NK cell states and newly identified ones through this study, we recapitulate important known cytokines, growth factors and other regulators underlying NK cell maturation and substates that are differentially affected by treatment, in abundance, expression, and regulation. Furthermore, we shed some light on the complex interplay of positive and negative regulators, including CD48 - 2B4 and CD56 related interactions in CCL4 and XCL2 high NK cells, as well as TGFb related pathways. Uncovering NK cell activity modulators, such as amphiregulin, NKG7, FAM49B, and TXNIP, we then focused on NK cells in the context of KIR3DL or TMIGD2 expression. We add to previous efforts to understand immune checkpoint inhibition by the HHLA2-KIR3DL3 axis and in a concerted effort with blocking antibodies, provide evidence of enhanced NK cell efficacy. Better understanding of how HHLA2 pathway affects the function of different subsets of lymphocytes could determine which patients may best benefit from targeting this pathway.

#3230 Preclinical double humanized CD47/SIRPA mouse model and MC38 cell line expressing human CD47 for efficacy and safety assessment of anti-CD47/SIRPA-targeting therapies.

Philippe De La Rochere, Angela Pappalardo, Gaelle Martin, Yacine Cherifi, Patricia Isnard-Petit, **Fabiane Sonego**, Kader Thiam

genOway, Lyon, France

CD47 is overexpressed on many human tumor cells and interacts with its ligand, the signal-regulating protein alpha (SIRPα), a protein expressed on macrophages and dendritic cells, to induce a “don’t eat me” signal. CD47 and its interaction with phagocytes is therefore a promising therapeutic target to treat cancer (S.B. Willingham *et al.*, Proc Natl Acad Sci U S A. 2012). Herein, we describe a double humanized CD47/SIRPα model for efficacy and safety assessment of human CD47/SIRPα-targeting therapies in immunocompetent mice. The double humanized CD47 and SIRPα mouse model (hCD47/hSIRPα) was generated by intercrossing hCD47 and hSIRPα mice. Both hCD47 and hSIRPα are humanized and their model design enables expression of all human isoforms. hCD47 and hSIRPα are expressed at physiological levels, which enabled the biodistribution assessment of anti-SIRPα nanobodies, used as tracers in non-invasive in vivo imaging of tumors (T.R. Wagner et al., Front.Immunol. 2023). Activity of anti-CD47-targeting compounds were investigated in the model and a MC38 tumor cell line expressing hCD47 was developed to reproduce as much as possible the interactions between hCD47 and hSIRPα upon tumor engraftment in the mouse model. Tumor growth inhibition studies in the hCD47/hSIRPα model with the corresponding cell line have shown significant reduction of tumor growth in mice treated with Magrolimab (Hu5F9-G4). Furthermore, safety assessment of Magrolimab in MC38-hCD47-bearing hCD47/hSIRPα mice in tumor context recapitulates the clinical findings (C.K. Brierley *et al.*, Transfusion. 2019), with a decline in hemoglobin and red blood cells post treatment. Altogether, these data suggest that the hCD47/hSIRPα model enables assessment of efficacy and safety of CD47 and/or SIRPα-targeting agents. The model is currently being upgraded to express humanized FcγR to enable assessment of Fc-mediated function of human antibodies targeting human CD47 and/or SIRPα.

#3231 Inhibition of focal adhesion kinase enhances antitumor response of immune checkpoint inhibitor therapy in laryngeal cancer.

Atish Ranjan Mohanty[1], Dana Do[1], Sharad Singhal[1], Haiqing Li[1], Sravani Ramisetty[1], Raju Pillai[1], Sultatna Shoukath[2], Prakash Kulkarni[1], Michelle Afkhami[1], Sue Chang[1], Ellie Maghami[1], Ravi Salgia[1], Erminia Massarelli[1]

[1]City of Hope National Medical Center, Monrovia, CA,[2]University of Texas at Tyler School of Medicine, Tyler, TX

Introduction: Laryngeal squamous cell carcinoma (LSCC), a subtype of head and neck squamous cell carcinoma (HNSCC), is projected to account for 12,650 new cases and 3,880 deaths in the United States in 2024. Key risk factors include smoking, alcohol abuse, and exposure to carcinogens like agent orange and asbestos. Active-duty service members and veterans face a heightened risk of developing LSCC. Curative standard treatment involves surgery, radiation, and platinum-based chemoradiation for laryngeal preservation. However, 25-50% of patients develop recurrence, often requiring salvage laryngectomy, which severely impacts their quality of life. We investigated the efficacy of Focal adhesion kinase inhibitors (FAKi), alone and in combination with immune checkpoint inhibitors (ICI), as potential therapeutic options for platinum-resistant LSCC.

Methods: We conducted an IRB-approved retrospective pilot study of 12 formalin-fixed paraffin-embedded (FFPE) LSCC tumor samples from smokers treated at the City of Hope. RNA was extracted from tumors and normal tissues and analyzed by NanoString Cancer and Immune Profiling Panels. FAKi screening was conducted using the CCK-8 cell viability assay. RNA-seq was performed to assess global changes in gene expression profiles. Syngeneic mouse models using MOC2 cell lines were utilized to evaluate the therapeutic efficacy of the FAKi in combination with ICI.

Results: Comparative analysis between normal and tumor tissues of 12 FFPE LSCC revealed upregulation of genes associated with cell-to-cell and cell-to-matrix adhesion, contributing to PI3K/AKT signaling. Tumor tissues also showed increased expression of genes involved in cell cycle regulation and DNA repair. Immune profiling of the same LSCC samples revealed CD4+ regulatory cells as the primary tumor infiltrating lymphocytes, underscoring the predominant immunosuppressive environment of LSCC. To investigate the significance of adhesion-dependent signaling, we used two human laryngeal cancer cell lines resistant to cisplatin: SCC17a and SQ20B. Among ten FAKi, TAE226 showed the most powerful inhibitory effect on cell growth in both cell lines. RNA-seq analysis revealed that TAE226 inhibits FAK phosphorylation, leading to the inhibition of genes that contribute to PI3K signaling. Additionally, it suppresses the expression of genes involved in the cell cycle and DNA repair. In the syngeneic mouse model, TAE226 showed a powerful synergistic effect with the ICI on the suppression of tumor growth, significantly prolonging the survival of the mice compared to ICI or the FAKi monotherapy groups.

Conclusion: Our study is the first to show synergism of FAKi and ICI in platinum-resistant LSCC in vitro and in a syngeneic HPV-negative HNSCC mouse model *in vivo*. This represents an important rationale for the translation of this therapeutic combination in platinum-resistant LSCC.

#3232 Developing PRMT5 inhibition-based Immuno-oncology combination therapies in MTAP-loss tumors.

Si Chen

Biology and Biochemistry, University of Houston, Houston, TX

Hyperactivated protein arginine methyltransferases (PRMTs) are implicated in human cancers. Inhibiting tumor-intrinsic PRMT5 has been shown to enhance antitumor immune responses, suggesting the potential of combining PRMT5 inhibitors (PRMT5i) with immunotherapy. However, global PRMT5 inhibition can impair immune cells functions. This study aims to identify strategies for tumor-specific PRMT5 inhibition and PRMT5i-based immuno-oncology (IO) combinations, especially for methylthioadenosine phosphorylase (MTAP)-loss cancers. Isogeneic tumor lines with and without MTAP loss were generated using CRISPR/Cas9 knockout. MTAP inactivation was confirmed by methylthioadenosine (MTA) accumulation and reduced symmetric dimethylarginine levels in MTAP-KO cell lines. Two PRMT5 inhibitors, GSK3326595 (substrate-competitive) and MRTX1719 (MTA-cooperative), were evaluated on tumor and immune cells. As shown in the Table 1, GSK3326595 suppressed PRMT5 activity and cell proliferation in both tumors and immune cells, regardless of MTAP status. Conversely, MRTX1719 selectively inhibited PRMT5 in MTAP-loss tumors, while treated T cells retained cytokine production and cytotoxicity. Transcriptomic and proteomic analyses revealed that MRTX1719 reduced PI3K pathway activation, a well-characterized immune resistant mechanism. In MTAP-loss syngeneic murine models, MRTX1719 combined with anti-PD-1 slowed tumor growth and extended survival (e.g., MC38/gp100 MTAP-KO tumor day 21: Ctrl 95.82±8.87 mm^2, PRMT5i 99.99±6.34 mm^2, anti-PD-1 94.47±6.76 mm^2, combination 72.81±4.14 mm^2; B16 MTAP-KO tumor day 18: Ctrl 108.24±9.13 mm^2, PRMT5i 106.45±22.54 mm^2, anti-PD-1 92.72±28.07 mm^2, combination 47.97±22.23 mm^2). The combination also increased $CD8^+$ T cell proliferation in MTAP-KO tumor tissues. Collectively, our results provide a strong rationale for the clinical development of MRTX1719-based IO combinations in MTAP-loss tumors.

Characterization of tumors and T cells in response to PRMT5i treatment

Cell line	MTA level (ng/ml) Mean±SEM	IC50 of GSK3326595 (uM)	IC50 of MRTX1719 (uM)
MC38 WT	2.93±0.96	3.118	2.747
MC38 MTAP-KO	362.50±0.71	3.315	0.356
B16 WT	1.89±0.08	0.834	3.853
B16 MTAP-KO	117.50±4.95	0.346	0.068
D4M WT	1.70±0.04	6.848	7.756
D4M MTAP-KO	266.00±11.31	0.488	0.152

CLINICAL RESEARCH: Liquid Biopsy: Circulating Nucleic Acids 1
Poster Session

#3236 Analytical validation of a tumor-agnostic DNA methylation-based assay for minimal residual disease detection in lung cancer.

Bo Yang[1], Yuan Sun[1], Lei Wang[1], Peiru Liu[1], Simei Qiu[1], Lei Chen[1], Fujun Qiu[1], Yalan Jin[1], Shuailai Wu[1], Yu Xu[1], Lei Zhang[1], Zhihong Zhang[1], Kezhong Chen[2]

[1]Burning Rock Dx, Guangzhou, China,[2]Department of Thoracic Surgery, Peking University People's Hospital, Beijing, China

Background: Minimal residual disease (MRD) detection methods are pivotal for non-invasive tumor fraction assessment in lung cancer, enabling disease monitoring and prognosis. Mutation-based assays, such as Signatera and PROPHET [1], have shown favorable performance in detecting MRD. Since DNA methylation can also reflect cancer features, we hereby developed the STELLA, a tumor-agnostic assay leveraging targeted DNA methylation sequencing of cell-free DNA (cfDNA) from blood, to explore the efficacy of DNA methylation in tracking MRD in lung cancer.
Methods: To demonstrate proof-of-concept, we designed and validated a STELLA pilot panel, which includes 341 lung-cancer-specific hypermethylated regions with low background noise level (≤1e-5) derived from 2,485 healthy samples. The ctDNA fraction in plasma samples was quantified using a maximum likelihood method based on the cumulative probability p-values across the selected regions.
Results: Analytical validation revealed that our assay can detect tumor fractions as low as 0.02% with a DNA input of 30 ng. In a cohort of 126 PROPHET-positive and 436 PROPHET-negative lung cancer samples, STELLA achieved a PPA of 73.8% and NPA of 97.9% using PROPHET as an orthogonal reference, with tumor fraction measurements strongly correlating with PROPHET ($R = 0.73$, $P < 0.001$). Probit regression analysis established a limit of detection (LOD) of 0.019%. In an independent cohort of 87 PROPHET-positive and 338 PROPHET-negative samples from 148 surgically treated lung cancer patients in the MEDAL cohort [1], STELLA demonstrated a PPA of 41.4% and NPA of 98%, using PROPHET as an orthogonal reference relative to PROPHET. In preoperative plasma samples, STELLA detected circulating tumor DNA (ctDNA) with a sensitivity of 32.6%, outperforming a mutation-based tumor-naïve assay (21.7%) but underperforming PROPHET (58.7%). Critically, postoperative STELLA-positive results correlated with significantly shorter disease-free survival (hazard ratio [HR]: 3.98; 95% CI: 1.74-9.13; $P < 0.001$).
Conclusion: These findings underscore the potential of DNA methylation-based, tumor-agnostic cfDNA methods like STELLA to detect MRD in lung cancer with a tumor fraction as low as 0.02% and an NPA of 98%. STELLA offers a promising approach for tumor burden assessment, providing timely insights for treatment decisions and enhancing patient care.
Reference: 1. Chen K, Yang F, Shen H, et al. Individualized tumor-informed circulating tumor DNA analysis for postoperative monitoring of non-small cell lung cancer. Cancer Cell. 2023;41(10):1749-1762.

#3237 Advancing non-invasive multiomics for early breast cancer detection: Transitioning from cfMeDIP-seq to single-molecule sequencing of cell-free DNA in the RENOVATE study.

Martina Dameri[1], Francesco Ravera[2], Pamela Becherini[2], Mario Stabile[2], Alberto Tagliafico[3], Marilena Iorio[4], Gianmaria Frige[5], Luca Mazzarella[5], Giovanni Scala[6], Michele Ceccarelli[7], Massimo Calabrese[8], Michele Piana[9], Alberto Ballestrero[2], Lorenzo Ferrando[1], Gabriele Zoppoli[2]

[1]IRCCS Ospedale Policlinico San Martino, Genoa, Italy,[2]Department of Internal Medicine and Medical Specialties, University of Genoa, Genoa, Italy,[3]Department of Health Sciences (DISSAL), University of Genoa, Genova, Italy,[4]Department of Experimental Oncology, Fondazione IRCCS Istituto Nazionale Dei Tumori, Milan, Italy,[5]Department of Experimental Oncology, IEO European Institute of Oncology IRCCS, Milan, Italy,[6]Department of Biology, University of Naples Federico II, Naples, Italy,[7]Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL,[8]Department of Radiology, IRCCS Ospedale Policlinico San Martino, Genoa, Italy,[9]Department of Mathematics, University of Genoa, Genoa, Italy

Introduction: Tissue biopsy for suspicious breast lesions (BI-RADS 3-5) is invasive, costly, anxiety-inducing, and often yields false positives, with benign lesions (BLs) like fibroadenomas identified in approximately one-third of cases. Liquid biopsy, which analyzes the genomic and epigenomic features of cell-free DNA (cfDNA), offers a promising alternative. Techniques such as cell-free methylated DNA immunoprecipitation and high-throughput sequencing (cfMeDIP-seq) and cfDNA profiling using single-molecule Nanopore sequencing (NP-seq) could leverage the potential of cfDNA multiomics in early cancer detection.
Methods: In the RENOVATE trial (NCT04781062) conducted at IRCCS Ospedale Policlinico San Martino (Genoa, IT), we collected PAXgene plasma samples from 366 women. Over a recruitment period of 18 months, we included in the final analysis, per protocol criteria, 64 patients with pT1N1/pT2N0 breast cancer (BC) and 115 women with BLs. After extracting cfDNA from these samples, we prepared cfMeDIP-seq and low-pass whole genome sequencing (WGS) libraries following the protocol described by Shen et al. (Nat Prot, 2019) with minor modifications. Libraries were sequenced on a NovaSeq6000 (Illumina). Differentially methylated regions were analyzed using the MEDIPS package. Additionally, a subset of RENOVATE cfDNA samples was used to implement NP-seq. Libraries were prepared according to Ligation Sequencing V14 Human cfDNA multiplex protocol and sequenced on the Oxford Nanopore Technologies P2 Solo device.
Results: CfMeDIP-seq libraries achieved an average of 45 MegaReads per sample (3-5x coverage), while low-pass WGS libraries were sequenced to obtain 1-2x coverage. CfMeDIP-seq analysis identified 2,133 hypomethylated regions and 1,299 hypermethylated regions, allowing us to determine methylomic variables that distinguish BC from BLs. For representative RENOVATE samples, we obtained NP-seq data showing that an average of 30 million basecalled reads and 11 GB of output data can be achieved for each sample. Preliminary data indicate that NP-seq can replicate genomic and epigenomic features while also enabling complex analyses due to the richness of information generated.
Conclusions: The assessment of cfDNA multiomics to differentiate BLs from BC holds great potential in early BC management. Advanced sequencing technologies like cfMeDIP-seq and NP-seq extract key cfDNA features, including copy number variations (CNVs), fragment length, and 5mC for both methods, but unprecedented capability to simultaneously capture native fragment features, as well as previously poorly characterized epigenetic modifications for NP-seq, such as 5hmC and 8-oxodG. This approach could have clinically relevant implications, reducing unnecessary biopsies and benefiting both patients and healthcare systems globally.

#3238 Prognostic significance of ctDNA tumor fraction quantification in advanced HR-positive breast cancer.

Hao Liao[1], Jianxin Zhong[1], Xiaoran Liu[1], Bin Shao[1], **Haoran Tang**[2], Cancan Jia[2], Feng Xie[2], Yue Zhang[2], Huiping Li[1]

[1]Peking University Cancer Hospital, Haidian District, Beijing, China,[2]Huidu Shanghai Medical Sciences, Ltd., Fengxian District, Shanghai, China

Introduction: Hormone receptor (HR)-positive breast cancer is the most common breast cancer subtype worldwide. While treatments with CDK4/6 inhibitors and endocrine therapies like aromatase inhibitors (AIs) have improved patient outcomes, the risk of disease progression persists. This study employs circulating tumor DNA (ctDNA) Next-Generation Sequencing (NGS) assays to profile gene variations and quantify tumor fraction in patients with advanced HR-positive breast cancer, aiming to identify relevant gene biomarkers and enhance patient management.

Methods: In this retrospective study, plasma samples were collected from 136 patients with advanced HR-positive breast cancer before initiating first-line treatments, including CDK4/6 inhibitors, endocrine monotherapies, and chemotherapies. The study employed PredicineCARE™, a targeted NGS liquid biopsy assay with 20,000X coverage, to detect somatic alterations and calculate tumor fractions in ctDNA. Additionally, plasma samples were collected from 58 patients after first-line AI therapy and before initiating second-line treatment with fulvestrant. To detect potential residual or progressive disease in these patients, the study utilized PredicineCARE™ Ultra, an ultra-sensitive NGS liquid biopsy assay with coverage exceeding 100,000X.

Results: In baseline samples collected before first-line treatments from 136 patients, the ctDNA assay identified positive tumor fractions in 92 patients, detecting a total of 247 somatic mutations and 311 gene copy number variations. Patients with positive tumor fractions had significantly poorer progression-free survival (PFS) (median PFS: 9.4 months vs. 15.7 months; p=0.006) and overall survival (OS) (median OS: 82 months vs. 108 months; p=0.030). Among patients with positive tumor fractions, the median tumor fraction was 9.4%, ranging from 0.5% to 88.2%. When applying a cutoff value of 1%, patients with higher tumor fractions experienced significantly poorer PFS compared to those with lower tumor fractions (median PFS: 9.4 months vs. 15.7 months; p=0.006). In the post-AI-treatment samples from 58 patients, the ultra-sensitive ctDNA assay identified positive tumor fractions in 43 patients, detecting 93 somatic mutations. When applying the same tumor fraction cutoff of 1%, patients with high tumor fractions exhibited significantly poorer OS compared to those with low tumor fractions (median OS: 19 months vs. 46 months; p=0.015).

Conclusion: Quantification of tumor fractions using ctDNA NGS assays demonstrated significant prognostic value in advanced HR-positive breast cancer, not only at baseline but also in patients undergoing treatment with potential residual or progressive disease. These findings emphasize the critical role of tumor fraction as a prognostic indicator and underscore the potential of integrating ctDNA tumor fraction assessment into clinical practice.

#3239 Advancing cancer genomics with Agilent Avida DNA onco panels: a breakthrough in targeted sequencing.

Akanksha Khare, Nishita M. Balaguruvappa, Katherine Wilkins, Bonita Lam, Semen Leyn, Carole Durand, Alessandro Agostino, Kyeong S. Jeong, Michael Ruvolo

Agilent Technologies, Inc., Santa Clara, CA

Introduction: The Agilent Avida DNA Onco panels, developed to address the requirements of the European OncNGS Consortium, represent a significant leap forward in next-generation sequencing (NGS) solutions for cancer research. Optimized for low-input circulating cell-free DNA (cfDNA) from liquid biopsies & compatible with fragmented genomic DNA (gDNA) from diverse sample types, including FFPE & fresh/frozen tissues, the Avida technology delivers ultra-sensitive & rapid analysis of cancer-related genomic alterations. Designed for modularity & cost-effectiveness, these panels enable genomic profiling across solid tumor cancers & lymphoma samples. Its innovative synergistic-probe system hybridization chemistry ensures unparalleled recovery & efficiency, achieving deep DNA analysis within hours. With a streamlined workflow & ready to use design, these panels set a new standard for high-performance, reliable genomic insights in both liquid biopsy research applications & translational cancer research.
Methods: The Avida DNA Onco LB Panels focus on Tier 1 & 2 targets in ESCAT & OncoKB Levels 1 & 2, ensuring relevance for standard research applications. For broader research applications, the Avida DNA Onco LB Plus Panel combines Onco LB panel with additional content, spanning 1.75Mb with 437 genes, enabling expanded capabilities. The Avida DNA Lymphoma Panel encompasses 85 genes across 378kb to include key markers relevant to hematologic malignancies for research applications. The performance of these panels was evaluated using a comprehensive set of reference materials, including Seraseq ctDNA Mutation Mix v4, Seraseq® ctDNA Lymphoma Mix (Lymphoma Panel) reference samples with known biomarkers, human cfDNA control (Biochain Z2121001), & Coriell male HapMap gDNA (NA24385). Libraries were prepared using the Avida targeted sequencing workflow & sequenced to assess capture specificity & target region coverage.
Results: Using a set of well-characterized reference standards, the Avida DNA Onco LB Panels & Avida DNA Lymphoma Panels both demonstrated robust performance, achieving >90% sensitivity for SNPs, indels, & translocations at a limit of detection (LOD) of 0.5% allele frequency (AF) across all input amounts. For copy number variant (CNV) detection, the LOD was determined to be 1.3-fold amplification, demonstrating high accuracy. The panel achieved >90% sensitivity & >97% specificity for most samples with variants at or above the LOD, irrespective of input amount, confirming its reliability & consistency in detecting genomic alterations. Translocation detection sensitivity was >90% for AF ≥0.5% & DNA input ≥25ng.
Conclusions: The newly developed Agilent Avida DNA Onco panels offer a modular approach designed to address diverse genomic profiling needs across cancer research & applications. PR7001-3583For Research Use Only. Not for use in diagnostic procedures.

#3240 Analytical validation of CancerDetect™ RUO assay: a minimal residual disease detection test that integrates tumor-informed and tumor-agnostic approaches with large-scale mutation monitoring.

Sunghoon Heo[1], Seon-Kyu Ham[1], Hayoon Lee[1], Hwang-Phill Kim[1], Duhee Bang[2], Sang-Hyun Song[1], Tae-You Kim[1]

[1]IMBdx, Inc, Seoul, Korea, Republic of,[2]Department of Chemistry, Yonsei University, Seoul, Korea, Republic of

Background: Detection of circulating tumor DNA (ctDNA) has demonstrated clinical utility as a prognostic marker for recurrence by indicating minimal residual disease (MRD). Because the abundance of ctDNA is below the detection limit (LoD) of current tumor genotyping methods (<0.1%), novel technology is required. Currently developed methods adopted personalized tumor genotype informed (tumor-informed) strategy with suboptimal LoD (~0.01%). Increasing MRD positivity by lowering LoD is required to screen high-risk recurrence patients. Additionally, most of the currently developed MRD test lack clinically actionable information.

Methods: A clinically validated personalized large-scale mutation profiling strategy MRD platform, CancerDetect™, was improved by adding actionable mutation targets across 15 genes recurrently mutated in solid cancers. For analytical validation of personalized mutation regions, well-characterized mixtures of two germline gDNA (NA12891, NA12892) mixture were used, varying mixing ration from 0%, 0.0001%, 0.001%, 0.005%, 0.01%, 0.05% and 0.1%. For analytical validation of the clinically actionable regions, commercially available reference standard (Seraseq® ctDNA MRD Panel Mix) was used with dilution ranged from 0.01% to 0.5%. The panel was designed to target more than 100 different genotype loci (Twist Biosciences).

Results: The new iteration of CancerDetect™ integrates personalized tumor-informed large-scale mutation profiling strategy and drug target mutations (tumor-agnostic region) targeting strategy. Results showed that the limit of detection (LoD_{95}) was reached down to 0.001% for tumor-informed region with 99.9% specificity and 0.1% for drug target mutations with 95% specificity. The precision analysis showed 100% and interference test showed that the test performance was not affected by interfering materials (Bilirubin. Wash Buffer) near LoD fraction. Increased sensitivity achieved MRD positivity if 27.2% in a prospective cohort of 551 patients with stage II/III CRC after one month of curative surgery and 45.2% in stage II/III gastric cancer after 3-10 weeks of surgery.

Conclusion: The new iteration of CancerDetect™ showed the limit of detection of 0.001% with 99.9% specificity for tumor-informed region and 0.1% with 95% specificity for the tumor-agnostic region. The test result was not affected by the interfering materials and precision analysis showed 100% result. This indicates the new iteration of CancerDetect™ is a robust, sensitive MRD test.

#3241 Cell-free DNA fragmentomes for treatment response monitoring in patients with metastatic colorectal cancer: the DOLPHIN study.

Denise E. van Steijn[1], Jamie Medina[2], Lorenzo Rinaldi[2], Adria Closa[1], Lana Meiqari[1], Erica Peters[2], Alissa Konicki[2], Frederieke H. van der Baan[3], Mariska Bierkens[1], Haoyue Wang[4], Marjolein J. E. Greuter[4], Birgit I. Lissenberg-Witte[4], Veerle M. H. Coupe[4], Marie V. Coignet[2], Victor E. Velculescu[2], Daan van den Broek[1], Gerrit A. Meijer[1], Max J. Lahaye[1], Manon N.G. J.A Braat[3], Jeanine M. L. Roodhart[3], Nicholas C. Dracopoli[2], Geraldine R. Vink[3], Niels F. M. Kok[1], Remond J. A. Fijneman[1]

[1]Netherlands Cancer Institute, Amsterdam, Netherlands,[2]Delfi Diagnostics, Baltimore, MD,[3]University Medical Center Utrecht, Utrecht, Netherlands,[4]Amsterdam University Medical Centers, Amsterdam, Netherlands

Accurate monitoring of treatment response in patients with metastatic colorectal cancer (mCRC) is essential for optimizing therapeutic strategies. Current response evaluation relies on imaging-based assessment of tumor size changes. However, this approach is limited by suboptimal sensitivity for detecting lymph node and peritoneal metastases, as well as inter-reader variability. Circulating tumor DNA (ctDNA) is indicative of the number of neoplastic cells and could have clinical value to CT imaging for assessment of treatment response. A mutation- and tumor-independent ctDNA assay was recently developed, demonstrating its potential for longitudinal assessment of treatment response: the DELFI-tumor fraction (DELFI-TF) score. The DOLPHIN study aims to investigate the added clinical value of the DELFI-TF score compared to CT imaging for treatment response monitoring in patients with mCRC. DOLPHIN is a prospective, observational, multi-center substudy of the Prospective Dutch ColoRectal Cancer cohort (PLCRC). Clinical data, images, and blood samples from 400 patients receiving systemic therapy in 11 hospitals in the Netherlands are being collected. Blood samples are drawn longitudinally in conjunction with routine CT imaging. The plasma cfDNA fragmentomes will be assessed using the DELFI-TF. DELFI-TF is a locked and validated machine learning model that quantifies tumor burden uses using cell-free DNA (cfDNA) fragmentomes data derived from low-coverage whole genome sequencing. Droplet digital PCR (ddPCR) ctDNA testing will be used as a reference for patients with confirmed RAS/BRAF mutations. The primary objective is to evaluate the association between ctDNA changes and clinical response. Secondary objectives include analyzing the association between ctDNA changes and RECIST criteria (I), correlation with serum carcinoembryonic antigen (CEA) levels at specified points during systemic therapy (II), lead time of ctDNA-testing versus CT imaging for detecting disease progression (III), the prognostic value of longitudinal ctDNA-testing (IV). Until November 2024, 385 patients have been enrolled, with the anticipated inclusion target of 400 patients expected to be reached by December 2024. A comprehensive overview of the DOLPHIN study population will be presented at the conference. Application of DELFI-TF and ddPCR ctDNA testing on the initial cohort of collected samples is scheduled to commence in early 2025. The DOLPHIN study will assess the clinical validity of the DELFI-TF in monitoring treatment response and investigate whether longitudinal ctDNA-testing can complement or partially replace imaging-based treatment response monitoring. These findings could pave the way for ctDNA-guided decision-making, supporting and enhancing accurate assessment of therapeutic effectiveness and therapeutic decision-making for patients with mCRC.

#3242 DNA methylation profiling of Epstein-Barr virus (EBV) in lymphoproliferative disease reveals diagnostic and therapeutic applications.

Christoph Weigel[1], Haley L. Klimaszewski[2], Cara Noel[2], Ada C. Sher[1], Kurtis M. Host[1], Yue-Zhong Wu[1], Sarah Y. Schlotter[1], Eric Brooks[1], Charles T. Gregory[1], Esko Kautto[1], Elshafa H. Ahmed[1], Rosemary Rochford[3], James S. Blachly[1], Mark Lustberg[4], Timothy Voorhees[1], Christopher C. Oakes[1], Robert A. Baiocchi[1]

[1]Division of Hematology, Department of Internal Medicine, The Ohio State University, Columbus, OH,[2]College of Medicine, The Ohio State University, Columbus, OH,[3]Department of Immunology and Microbiology, School of Medicine, University of Colorado, Aurora, CO,[4]Division of Infectious Diseases, Yale School of Medicine, New Haven, CT

Introduction: Epstein-Barr Virus (EBV) is an oncogenic herpesvirus linked to hematologic malignancies. EBV enters latency within host cells and can undergo lytic activation, resulting in release of infectious virions. Latent EBV mediates malignant transformation, while lytic EBV is a frequent complication in immune suppression settings like post-transplant lymphoproliferative disease (PTLD). Current clinical assays detect only the presence of EBV via qPCR, limiting their diagnostic use. DNA methylation on EBV genomes is essential for interconversion of lytic/latent states, making it a promising virology biomarker.
Methods: We have developed a high-throughput multiplex PCR profiling assay (iPLEX) to assess quantitative EBV DNA methylation at n=35 CpG dinucleotide sites in liquid biopsies (plasma/serum). We applied this assay to a cohort of n=246 plasma samples collected at the OSU Wexner Medical Center via the Leukemia Tissue Bank Shared Resource. Our cohort consisted of viremic patients with EBV-associated lymphoma or non-malignant EBV activation, including n=118 organ transplant recipients. We complemented our analysis with single molecule readout of EBV plasma methylomes (n=27) using Nanopore sequencing of native DNA methylation.
Results: Low EBV methylation distinguished lytic from latent EBV (sensitivity: 93.3%, specificity: 97.2%), outperforming clinical EBV qPCR. We revealed distinct EBV methylation in lymphoma subtypes, ranging from intermediate (Hodgkin lymphoma, polymorphic PTLD) to high (Burkitt-, NK/T-cell lymphoma, monomorphic PTLD). Assessment of EBV methylation in treatment follow-up (n=55) highlighted malleable EBV methylation was associated with improved survival under treatment (p=0.0005). We further identified unique EBV methylation dynamics under epigenetic therapies, including DNA methyltransferase (DNMT) inhibitor decitabine. DNA methylation in the EBV protein kinase gene *BGLF4* was further associated with response to antiviral ganciclovir (GCV) use. Lastly, assessment of overall methylation patterns revealed four EBV methylation groups. Single molecule methylome analysis via Nanopore sequencing validated our data and identified distinct states of molecular epigenetic heterogeneity in EBV lymphoma and PTLDs. Methylation groups showed highly significant association with overall survival in multiple disease entities, including Hodgkin- and diffuse large B-cell lymphoma (DLBCL).
Conclusion: Our data reveal the epigenetic EBV landscape in lymphoma and non-malignant viremic conditions. EBV methylation can identify lymphoma through a minimally invasive plasma test for earlier and more reliable diagnosis of EBV-driven malignancy. EBV methylation could guide informed treatment decisions, especially with regard to epigenetic- and antiviral drug use, and aid in personalized care in the future.

#3243 Fragmentomic features of plasma cell-free DNA in patients with osteosarcoma.

Anastasia E. Mackeracher[1], Joanna Przybyl[2]

[1]Cancer Sciences, McGill University, Montreal, QC, Canada,[2]McGill University, Montreal, QC, Canada

The rapid advancement of liquid biopsy technologies represents a major breakthrough in cancer management, allowing for sensitive detection of tumor-derived molecules and providing real-time insights into disease progression. Osteosarcoma (OS), the most common primary bone malignancy, currently lacks robust biomarkers for therapy response. We hypothesize that a multi-omic and multi-modal approach to identifying OS-specific markers in circulating tumor DNA (ctDNA) will facilitate precise patient stratification and improve treatment outcomes. It has been demonstrated by others that plasma ctDNA in OS patients carry specific genomic markers, such as point mutations and copy number alterations. However, the fragmentomic features of cell-free DNA (e.g., fragment length and nucleotide composition) remain poorly characterized in OS patients.

To provide the rationale for studying fragmentomic features of plasma DNA in OS, we sought to demonstrate the feasibility of detecting distinct fragmentomic patterns in the publicly available whole genome sequencing data from cfDNA of 3 OS patients (NCBI SRA accession number PRJNA917431) together with our previously published shallow whole genome sequencing data from cell-free DNA of 7 patients with leiomyosarcoma, 12 patients with leiomyoma, 3 patients with dedifferentiated liposarcoma and 3 patients with well-differentiated liposarcoma (PMIDs: 29463554, 32232185, 34986184). We determined the first 4 nucleotide sequence (i.e., a 4-mer motif) on each 5′ fragment end of plasma DNA molecules. We calculated the frequency of each of the 256 possible motifs and normalized it to the total number of reads in each specimen. We applied multiclass differential analysis of the frequency of these motifs in 5 different types of tumors, and identified 64 motifs significantly enriched in OS (mean frequency difference between groups > 2.5-fold, false discovery rate < 0.05). In OS cell-free DNA, we observed decrease of motifs associated with DNASE1L3 nuclease (e.g., CCCA, CCAG, CCTG) and increase of motifs such as TAAA, AAAA, and TTTT. In the next steps, we will examine the expression of different DNA nucleases in patient tissue specimens by immunohistochemistry and will analyze plasma DNA from a new cohort of 50 OS patients to validate our preliminary findings. We will investigate whether these fragmentomic patterns of cell-free DNA have the predictive and prognostic value in OS patients. Our preliminary results warrant further studies of the frequency and diversity of the 4-mer motifs and expression of different DNA nucleases in OS.

#3244 *cfTrack-methyl*: A personalized approach using cfDNA methylomes for ultra-sensitive MRD detection.

Li Shuo[1], Weihua Zeng[1], Xiaohui Ni[2], Chun-Chi Liu[2], Yonggang Zhou[1], Mary L. Stackpole[2], Wenyuan Li[1], Arjan Gower[3], Kostyantyn Krysan[3], Andrew Melehy[4], Angela H. Yeh[1], Megumi Yokomizo[5], Preeti Ahuja[5], Lopa Mishra[6], Kirti Shetty[7], Yazhen Zhu[1], Hsian-Rong Tseng[8], Clara Lajonchere[9], Daniel H. Geschwind[9], David S. Lu[5], Steven S. Raman[5], William Hsu[5], Clara E. Magyar[1], Samuel W. French[1], Denise R. Aberle[5], Steven-Huy B. Han[3], Edward B. Garon[3], Vatche G. Agopian[4], Wing H. Wong[10], Steven M. Dubinett[3], Xianghong Jasmine Zhou[1]

[1]Pathology and Laboratory Medicine, UCLA - University of California Los Angeles, Los Angeles, CA,[2]EarlyDiagnostics Inc, Los Angeles, CA,[3]Medicine, UCLA - University of California Los Angeles, Los Angeles, CA,[4]Surgery, UCLA - University of California Los Angeles, Los Angeles, CA,[5]Radiological Sciences, UCLA - University of California Los Angeles, Los Angeles, CA,[6]Surgery, George Washington University, Washington, DC,[7]Medicine, University of Maryland, Baltimore, MD,[8]Molecular and Medical Pharmacology, UCLA - University of California Los Angeles, Los Angeles, CA,[9]Institute for Precision Health, UCLA - University of California Los Angeles, Los Angeles, CA,[10]Statistics, Stanford University, Stanford, CA

Methylation alterations are pervasive in cancer, making cfDNA methylomes a promising tool for cancer detection and minimal residual disease (MRD) monitoring. However, cfDNA methylation-based MRD detection faces unique challenges. Unlike genomic mutations, methylation signals are prone to noise from non-tumor-related factors, such as heterogeneous cell types and environmental influences. This noise introduces confounding signals and dilutes tumor-specific methylation markers. While patient-specific tumor methylation profiles offer personalized insights, they often contain inherent noise that compromises their performance. Existing approaches failed to address this challenge, as they either (1) disregard personal tumor methylation profiles, yielding suboptimal performance, or (2) fail to account for noise, treating all personal markers as equally informative. These limitations underscore a crucial need for a novel method that integrates personal markers while mitigating noise.

To address this challenge, we developed *cfTrack-methyl*, a personalized approach that integrates personal and population cancer methylome data (i.e., data from other patients). This method identifies methylome-wide personal tumor markers from the patient's tumor tissue or pre-treatment plasma and evaluates their prevalence across population cancer methylome data. Population prevalence quantifies the cancer relevance of noisy personal markers, filtering out non-tumor-related signals and prioritizing true tumor markers. With these purified personal markers, *cfTrack-methyl* achieves sensitive and specific MRD detection, even at ultra-low tumor fractions. In the titration series between fragmented liver tumor and WBC gDNA, the detection limit of *cfTrack-methyl* was calculated as < 0.01% with 100% sensitivity and 100% specificity. In simulated data with plasma spike-ins from various cancer types, *cfTrack-methyl* achieved a median AUC of 99.6% at a tumor fraction of 0.01% when tumor tissue was informed and maintained robust performance with a median AUC = 100.0% at a tumor fraction of 0.01% when tumor tissue was unavailable and pre-treatment plasma was used. Further, in liver cancer patients with complex comorbidities (cirrhosis or HBV), *cfTrack-methyl* achieved comparable detection limits, with a median AUC = 99.9% at a tumor fraction of 0.01%. In all scenarios, *cfTrack-methyl* outperformed the MRD detection using general cancer markers.

In conclusion, *cfTrack-methyl* delivers unprecedented sensitivity for MRD detection in cfDNA at low tumor fractions across diverse clinical scenarios. By integrating personal and population data, this approach maximizes the potential of cfDNA methylomes for ultra-sensitive MRD detection. Ongoing studies using serial samples from patients with surgically resected tumors aim to further validate this personalized strategy.

#3245 Analytical validation of a sensitive, tissue-free blood test for tumor burden monitoring.

Qiancheng You, Jing Su, Jiayue Xu, Xingyu Yang, Sa Zhang, Zeliang Deng, Guangyou Li, Xinyue Kang, Xiaoling Li, Si Zhang, Meifang Wu, **Bingsi Li**

Innovation Center, Burning Rock Biotech, Shanghai, China

Background: Liquid biopsy stands as a valuable tool in the field of precision medicine, with its utility extending across cancer detection, recurrence monitoring, and treatment selection. However, achieving the desired level of sensitivity often requires access to tumor tissue, which can be a limiting factor. Recent studies have shown that analyzing methylation patterns in circulating tumor DNA (ctDNA) enables a sensitive and accurate measurement of tumor burden in a tissue-independent manner, thus opening up new applications in relapse monitoring and therapy response assessment. Here, we report the initial validation of OverC Monitor™, a multi-cancer blood test that interrogates methylation information from over 2,000 genes at single-base resolution across various solid tumors.

Methods: Analytical performance of OverC Monitor™ was assessed using 672 contrived cell line samples and cell-free DNA (cfDNA) samples with 10-30ng input amounts. Analytical sensitivity was evaluated using a dilution series of cell line and cfDNA samples across different tumor allele fraction (TAF) levels, ranging from 0.001% to 0.5%. The limit of detection (LoD_{95}) was determined as the lowest tumor methylated fraction (TMeF) that achieved ≥95% detection probability through Probit regression for each cancer type. In addition, both ultra-deep sequencing (HS-UMI) and droplet digital PCR (ddPCR) were employed to verify the accuracy of TMeF in these dilution mixtures. The limit of blank (LoB) was evaluated by per-sample positive rate in 72 age-matched cancer-free donors. The combined precision and accuracy study was carried out by testing clinical samples from patients with lung cancer (LC), colorectal cancer (CRC), liver cancer (HCC) and non-cancer donors for a total of 216 replicates across different conditions.

Results: For sample-level methylation, the limits of detection (LoDs) were reached as low as 0.004% for cell line dilutions, and 0.02% for circulating-free DNA (cfDNA) titrations. The per-sample false positive rate was 0% (95%CI, 0-5.0%) among age-matched cancer-free donors. The positive predictive agreement (PPA) and negative predictive agreement (NPA) across different instruments, operators, and reagent lots were 100% (95%CI, 97.1-99.8%) and 100% (95%CI, 91.7-100%), respectively. Additional validation is ongoing, and data will be presented as it becomes available.

Conclusions: OverC Monitor™ is a patient-care-ready liquid biopsy capable of detecting common solid tumors even at ultra-low tumor burden. It requires no prior knowledge for downstream analysis, offering a new opportunity to improved sensitivity in a cost-effective manner. In addition, it is designed to be compatible with conventional tumor genotyping methods, adding a new dimension in the continuum of cancer management.

#3247 Liquid based methylation profiling for quantification of breast cancer subtypes.

Caroline Weipert, Denis Tolkunov, Sara Wienke, Derek Dustin, Michael Weindel, Catalin Barbacioru, Justin Odegaard, Darya Chudova

Guardant Health, Palo Alto, CA

Background: Hormone receptor (HR) status, typically determined through immunohistochemistry (IHC), and human epidermal growth factor receptor 2 (HER2) status, assessed by IHC and FISH, are critical factors in guiding breast cancer treatment. However, in metastatic breast cancer, resistance to endocrine or anti-HER2 therapies often develops, and receptor status may change over time due to tumor evolution under selective treatment pressures. Both IHC and FISH require tissue biopsies, which can become increasingly difficult to obtain in later treatment stages. Moreover, HR and HER2 status may evolve as the tumor adapts to therapy, highlighting the need for ongoing monitoring. A non-invasive, blood-based method could provide a practical alternative, enabling real-time assessment of receptor status. DNA methylation offers a potential avenue for identifying subtype changes in breast cancer. This could serve as an early indicator of resistance to treatment. In this study, we investigated whether methylation-based signatures in cell-free DNA (cfDNA) could act as a surrogate to predict breast cancer subtypes.

Methods: A novel cfDNA methylation-based deconvolution model was developed to quantify the proportional contributions of each breast cancer subtype in patient blood samples. The model leverages methylation signals across 4,500 cancer-associated genomic regions to estimate the contribution of each subtype while simultaneously predicting patient-specific subtype methylation signatures. A novel probabilistic model was developed to calculate the posterior probability of methylation for each region, based on hypermethylated molecule counts. To improve reliability, regions with high noise were filtered out, and penalized logistic regression models were created for each subtype using these posterior probabilities. Using a training set of 1,078 patients with breast cancer subtype derived from real-world data (HER2+ N=114, HR+ N=277, and TNBC N=71, with tumor fraction > 0.5%), logistic regression models were trained and optimized to predict HR, TNBC, and HER2 statuses.

Results: Model performance was evaluated by comparing the predicted histological subtypes to the known histological subtype annotations of 366 breast cancer samples. The overall accuracy on the test set was 80.1%, with the highest accuracy for TNBC (90.5%), followed by HR+/HER2+ (88%), HR+/HER2- (80%) and HR-/HER2+ (80%). Importantly, the model demonstrated the ability to detect sub-type in samples with tumor fraction as low as 0.5%.

Conclusions: Our methylation-based subtype deconvolution model provides a highly accurate method for predicting breast cancer subtypes from patient blood samples. This approach may enable the early non-invasive detection of growing treatment-resistance amongst breast cancer subtypes, with the potential to allow for more informed clinical decision-making and personalized treatment selection.

#3248 Circulating tumor DNA analysis of patients with BRAF-mutated advanced unresectable solid tumors treated with plixorafenib (FORE8394/PLX8394) in phase 1/2a study.

Rona Yaeger[1], Eric J. Sherman[1], Macarena de la Fuente[2], Frank Q. Tsai[3], Nicholas Butowski[4], Carl E. Allen[5], Natraj R. Ammakkanavar[6], Philip Lammers[7], **Jessica C. Jang**[8], Michael Paz[8], Sam Wang[8], Stacie Peacock Shepherd[8], Ping Jiang[8], Jordi Rodon[9]

[1]Memorial Sloan Kettering Cancer Center, New York, NY,[2]University of Miami, Miami, FL,[3]HonorHealth Cancer Care, Scottsdale, AZ,[4]University of California, San Francisco, San Francisco, CA,[5]Texas Children's Hospital, Houston, TX,[6]Community Health Network, Anderson, IN,[7]Baptist Cancer Center, Bartlett, TN,[8]Fore Biotherapeutics Inc., Philadelphia, PA,[9]The University of Texas MD Anderson Cancer Center, Houston, TX

Plixorafenib is a selective, potent BRAFi that inhibits class 1, 2, and fusion BRAF mutations. Plixorafenib evades paradoxical MAPK activation by disrupting BRAF dimerization and reduces toxicities and resistance that were observed in early generation BRAFi. Circulating tumor DNA (ctDNA) has proven its predictive or prognostic value for patients (pts) on treatment. We present here the analysis of longitudinal ctDNA from plixorafenib-treated pts, an exploratory endpoint from this Phase 1/2a study (NCT02428712).

A total of 211 plasma samples from 71 pts, 65 with BRAF alterations ($BRAF^{mut}$ not required in dose escalation study) were tested using PredicineCARE, which detects SNVs, INDELs, CNVs and fusions in ctDNA down to 0.05% allele frequency. Samples were obtained prior to treatment, at subsequent cycles, and at disease progression.

The ctDNA concordance with tissue-based NGS data at baseline (BL) was 83.3% for V600E, 72.7% for class 2 SNVs, and 33.3% for BRAF fusions. Most discordance in V600E was found in papillary thyroid carcinoma pts. V600E variant allele frequency (VAF) decreased from C1D1 to C2D1 in 17/20 pts. Decreases were greater in responders. BL V600E VAF was higher in non-responders. In 6 pts with available paired tumor biopsies, decreases in pERK validated ctDNA results after treatment. Class 2 and fusion VAFs also declined after treatment, supporting differentiated activity from early generation BRAFi. In longitudinal samples, changes in ctDNA correspond to tumor size across tumor types, suggesting that ctDNA may be a surrogate marker for monitoring disease. Genomic profiles at disease progression were compared to BL to assess new mutations and pathways between subjects in response group (CR+PR+SD) and non-response (PD) group (non-CRC due to intrinsic resistance). No new mutations in MAPK pathway genes were observed, supporting the dimer breaker property of plixorafenib. In ovarian cancer pts, BRAF VAF dominated over other mutations and matched tumor volume, suggesting that BRAF VAFs drove disease outcome. In non-responders, heterogeneity of genomic profile, co-occurrent RAS, MAPK-associated or NF1 mutations were seen, including melanoma pts who all received prior MAPKi therapies. In CRC, multiple driver mutations were found at BL associated with receptor tyrosine kinase activation, confirming resistance pathways and supporting clinical utility of combination. Despite genomic heterogeneity in V600+ melanoma and CRC, early decreases in BRAF VAF were observed.

ctDNA accurately detects BRAF mutations identified in tumor biopsy and longitudinal ctDNA levels is a potential surrogate marker for monitoring disease. Response to plixorafenib was observed through declines in ctDNA BRAF VAF and pERK. Furthermore, the “paradox breaking” property of plixorafenib was confirmed, as no new MAPK mutations emerged after treatment.

#3249 The development of a tissue-agnostic genome-wide methylome enrichment assay for lung cancer.

H. I. Pass[1], C. A. Melton[2], S. Shen[2], B. Brown[2], J. M. Burgener[2], J. Zhang[2], Y. Wang[2], J. Min[2], O. Hall[2], K. Budhraja[2], A. Licon[2], A. Williams[2], S. V. Bratman[3], B. A. Allen[2], J. Zhang[2], D. D. De Carvalho[3], A.-R. Hartman[2];

[1]New York University, New York, NY, [2]Adela, Inc., Foster City, CA, [3]Princess Margaret Cancer Centre, University Health Network, University of Toronto, Toronto, ON, Canada

Background: Lung cancer remains the leading cause of cancer-related death in the United States. Precision oncology is revolutionizing early-stage non-small cell lung cancer (NSCLC) treatment, showing promising results in improving outcomes. Utilization of molecular residual disease (MRD) testing has the potential to improve outcomes by detecting relapse ahead of clinical presentation. Currently, up to 38% of patients are ineligible for tumor-informed MRD tests due to insufficient tissue, often resulting from risks associated with invasive procedures, small biopsy sizes (e.g., FNAs), low tumor purity, competing demands for tissue (e.g., PD-L1 staining), or complete response to neoadjuvant treatment.[1,2] Herein, we demonstrate the feasibility of a tissue-agnostic genome-wide methylome enrichment platform utilizing cell-free methylated DNA immunoprecipitation followed by high-throughput sequencing (cfMeDIP-seq) for cancer detection, cancer signal quantification, and prognostication in lung cancer. [3,4]

Methods: In this feasibility study, a total of 24 patients (136 samples) with early-stage lung cancers (87.5% Stage I [IA (50%), IB (37.5%), IIA (4.2%) and IIB (8.3%)]); 19 were analyzed based on inclusion/exclusion criteria and assessed for their correlation with recurrence-free survival (RFS). Blood collections for each patient occurred prior to surgery, after surgery and at irregular intervals before recurrence or last clinical follow-up. 5-10 ng of cfDNA isolated from each plasma sample was used for cfMeDIP-seq. The analysis considers multiple MRD tests over a surveillance period following surgery. RFS is compared between groups using a log-rank test. The Hazard Ratio (HR) is estimated using Cox proportional hazards model.

Results: With a median follow up duration of 85.6 months, 10 patients experienced recurrence, and 9 patients remained recurrence-free. Patients with a positive MRD test showed significantly worse RFS than those who tested negative (HR 3.58; 95% CI, 1.00,12.89). The lead time between MRD positivity and clinical recurrence was up to 35.91 months, with a median of 13.08 months.

Conclusions:The analysis demonstrates that MRD detection with a tissue-agnostic, genome-wide methylome enrichment platform in patients with lung cancer after curative intent treatment correlates strongly with RFS. References: 1. Khan S, et al. JCO 2024. 2. Aggarwal C, et al. JAMA Oncology 2019.3. Shen SY, et al. Nature 2018 4. Liu G, et al. Annals Oncol 2024

#3250 Tumor-independent monitoring of minimal residual disease in multiple myeloma using cfDNA fragmentomics.

Dor D. Abelman[1], Jenna Eagles[2], Aimee Wong[3], Saumil Shah[3], Stephanie Pedersen[2], Stephenie Prokopec[2], David S. Scott[2], Sarah Bridges[4], Darrell White[5], Irwindeep Sandhu[6], Kevin Song[7], Esteban Braggio[8], Alli Murugesan[9], Anthony Reiman[10], Suzanne Trudel[1], Trevor J. Pugh[11]

[1]Princess Margaret Cancer Centre and Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada,[2]Princess Margaret Cancer Centre, Toronto, ON, Canada,[3]Department of Biological Sciences, University of New Brunswick, Saint John, NB, Canada,[4]Maritime SPOR SUPPORT Unit, Saint John Regional Hospital, Saint John, NB, Canada,[5]Dalhousie University and QEII Health Sciences Centre, Halifax, NS, Canada,[6]Cross Cancer Institute, Edmonton, AB, Canada,[7]BC Cancer Agency, Vancouver General Hospital, and Department of Medicine, Division of Hematology, University of British Columbia, Leukemia Bone Marrow Transplant Program of British Columbia and Vancouver General Hospital, Vancouver, BC, Canada,[8]Mayo Clinic Arizona, Phoenix, AZ,[9]Faculty of Medicine, Dalhousie University, and Department of Nursing and Health Sciences, Faculty of Science, Applied Science and Engineering, University of New Brunswick, Saint John, NB, Canada,[10]Department of Biological Sciences, University of New Brunswick and Faculty of Medicine, Dalhousie University, and Department of Oncology, Saint John Regional Hospital, Saint John, NB, Canada,[11]Princess Margaret Cancer Centre; Department of Medical Biophysics, University of Toronto, and Ontario Institute for Cancer Research, Toronto, ON, Canada

Introduction: Monitoring minimal residual disease (MRD) is critical in multiple myeloma (MM) to predict outcomes and guide therapy. Traditional bone marrow (BM) aspirates for MRD detection are invasive and limited by sample quality. We therefore explored cell-free DNA (cfDNA) fragmentation as a less invasive alternative for MRD detection.

Methods: We performed 30-40X whole-genome sequencing (WGS) on peripheral blood cfDNA from 45 MM patients, collecting baseline (n = 45) and follow-up samples (n = 98) from eight Canadian sites (M4 and IMMAGINE studies) and one U.S. site (SPORE study), plus 25 healthy controls. Samples were collected post-induction therapy (n = 13), 100 days post-autologous stem cell transplantation (ACST, n = 36), and during maintenance therapy (n = 49). Multiparameter flow cytometry (MFC) MRD testing was conducted on 77 samples. We evaluated insert size metrics and MM-specific chromatin accessibility (from Ordoñez et al., 2020) using Griffin (described in Doebley et al., 2022).

Results: Baseline samples had a higher proportion of short fragments (20-150 bp) than follow-up samples ($p = 0.0037$) and healthy controls ($p = 0.019$). A fragment score (FS) based on weighted fragment distribution (per Vessies et al., 2022) was highest at baseline (mean = -0.384), followed by MRD-positive (mean = -0.495) and MRD-negative (mean = -0.550) samples. MRD-negative FS was lower than baseline samples ($p < 0.001$) but higher than healthy controls (mean = -0.639; $p = 0.016$).

Baseline samples had lower coverage at MM-specific chromatin regions than MRD timepoints ($p < 0.05$; baseline mean = 0.982; MRD-positive = 0.989; MRD-negative = 0.994; healthy = 0.993), suggesting higher transcriptional activity pre-treatment. MRD-negative samples had higher coverage than MRD-positive cases ($p < 0.01$).

Using logistic regression, we assessed the predictive performance of FS and coverage at MM-specific sites. FS alone achieved an AUC of 0.632 (sensitivity [SN] 39.4%, specificity [SP] 92.1%, accuracy [AC] 67.6%). Coverage yielded an AUC of 0.681 (SN 75.8%, SP 60.5%, AC 67.6%). Combining FS and coverage at MM sites improved performance (AUC = 0.734; SN 48.5%, SP 92.1%, AC 71.8%). We next evaluated whether adjusting the BM tumor cell percentage cutoff by MFC could improve performance. At 0.017% (1 in 5,789 cells) for MRD positivity, the combined model's accuracy reached 83% with an AUC of 0.757 (SN 17%, SP 97%).

Conclusions: cfDNA fragmentomic analysis achieved high specificity (92-97%) in detecting MRD, providing a less invasive alternative to facilitate serial monitoring. While sensitivity remains an area for improvement, the high specificity of cfDNA makes it valuable for confirming MRD negativity and reducing invasive procedures. Future efforts aim to enhance sensitivity by integrating additional fragmentomic features.

#3251 Immediate postoperative minimal residual disease detection with MAESTRO predicts recurrence and survival in head and neck cancer patients.

Edward S. Sim[1], Justin Rhoades[2], Kan Xiong[2], Laurel Walsh[2], Andjela Crnjac[2], Timothy Blewett[2], Yana Al-Inaya[1], Julia Mendel[1], Daniel Ruiz[1], Vasileios Efthymiou[1], Gjystina Lumaj[1], William J. Benjamin[1], G. Mike Makrigiorgos[3], Shervin Tabrizi[2], Viktor A. Adalsteinsson[2], Daniel L. Faden[1]

[1]Department of Otolaryngology-Head and Neck Surgery, Massachusetts Eye and Ear, Boston, MA,[2]Gerstner Center for Cancer Diagnostics, Broad Institute of MIT and Harvard, Cambridge, MA,[3]Department of Radiation Oncology, Dana-Farber Cancer Institute, Boston, MA

Introduction: Most head and neck squamous cell carcinoma (HNSCC) patients are diagnosed with locoregionally advanced disease and face high recurrence rates despite standard-of-care treatments. Using circulating tumor DNA (ctDNA) to detect minimal residual disease (MRD) has emerged as a promising biomarker for assessing recurrence risk and treatment efficacy across various cancers. However, the sensitivity of commercially available assays for detecting MRD remains limited, often failing to capture subtle dynamic changes. As a result, the dynamics of ctDNA immediately following treatment and its potential as a reliable biomarker for guiding early post-operative adjuvant treatment decision making remain poorly understood.

Methods: Our group has developed MAESTRO (minor-allele-enriched sequencing through recognition oligonucleotides) that uses patient-specific probes to selectively enrich ctDNA carrying thousands of genome-wide tumor mutations, enabling highly sensitive MRD detection with minimal sequencing. We utilized MAESTRO to prospectively monitor MRD in HNSCC patients after surgery during the immediate post-operative window—serial blood samples taken while patients were in the hospital recovering from surgery—and the surveillance period, aiming to evaluate its potential to predict disease progression. Kaplan Meier survival analysis and Cox proportional hazards regression were applied to assess the association of MRD detection during the immediate post-operative window with event-free survival (EFS).

Results: 24 HNSCC patients were enrolled. Patients were pre-dominantly HPV-negative [23/24 (95.8%)] and had advanced stage III/IV disease [22/24 (91.7%)]. Serial sampling in the days following surgery revealed rapid ctDNA clearance on the first post-operative blood draw (1-3 days postop) of patients without MRD. MAESTRO detected ctDNA in 13 of the 15 (86.7%) patients who experienced an event (recurrence or death). In 8 of the 13 (61.5%) patients with an event and detectable MRD, tumor fractions were below the limit of detection of leading commercial assays. MRD detection was associated with worse EFS (HR = 27.4; 95% CI 3.5-214.5; $P < 0.0001$) and remained an independent predictor of EFS when controlling for high-risk pathologic features (23.8; 3.0-192.1; $P = 0.003$).

Conclusions: Tumor fractions of HNSCC patients treated effectively with surgery were undetectable as early as the first postoperative day. Most tumor fraction levels detected by MAESTRO were outside the sensitivity range of leading commercially available assays. MRD detection of patients in the immediate post-operative period was predictive of recurrence and worse survival. MAESTRO-based MRD detection may enable highly-sensitive identification of high-risk HNSCC patients and guide therapy including whether treatment escalation may be necessary.

#3252 Circulating tumor DNA (ctDNA) enables enhanced measurable residual disease (MRD) detection and non-invasive clonal profiling in acute myeloid leukemia (AML).

Ruwan Gunaratne[1], Crystal Zhou[1], Sanjeeth Rajaram[1], Jesse W. Tai[1], Sky Kim[1], Kailee Tanaka[1], Charu Tiwari[1], Grace Gao[1], Mia Carleton[2], Brian J. Sworder[3], Gabriel N. Mannis[1], Michael S. Khodadoust[2], Ravindra Majeti[1], David Kurtz[2], Tian Yi Zhang[1]

[1]Division of Hematology, Stanford University School of Medicine, Stanford, CA,[2]Division of Oncology, Stanford University School of Medicine, Stanford, CA,[3]Division of Hematology and Oncology, University of California Irvine, Irvine, CA

Introduction: Current MRD detection methods for AML (multiparameter flow cytometry and single-gene assays) are limited by suboptimal sensitivity, narrow coverage for molecular monitoring and clonal resolution, and/or reliance on invasive bone marrow biopsies. To address these issues, we developed AML-CAPP-Seq, a ctDNA-based assay leveraging a customized Cancer Personalized Profiling by Deep Sequencing (CAPP-Seq) approach to detect MRD and track clonal evolution.
Methods: AML-CAPP-Seq integrates patient-specific hybrid capture panels (based on whole exome sequencing, WES) with a shared panel of 58 recurrently mutated 'canonical' AML genes to generate personalized MRD assays. We analyzed > 1000 biological samples from 56 AML patients (median follow-up: 19.5 months) and 21 healthy controls, comparing MRD detection in bone marrow mononuclear cells (BMMCs), peripheral blood mononuclear cells (PBMCs), and cell-free DNA from plasma (PB-ctDNA) and bone marrow (BM-ctDNA) against standard-of-care (SOC) MRD assays.
Results: WES identified a median of 32.5 tumor single nucleotide variants (SNVs) per patient, allowing MRD detection in 100% of patients with a detection limit of <0.01% tumor fraction. Use of only canonical AML genes reduced coverage to 84%, while SOC single gene molecular MRD methods were applicable to just 32% of patients. Tumor burden in PB-ctDNA was highly correlated ($r > 0.9$) with other compartments and showed significantly higher tumor DNA levels at low disease burden states (median 3.8- and 2.9-fold enrichment compared to PBMCs and BMMCs respectively, $p < 0.02$), particularly in cases of extramedullary involvement. PB-ctDNA detected residual disease in 98.5% of samples with +MRD by SOC marrow-based methods and identified MRD in 47.4% of SOC-negative samples. Of these, 46.8% were from patients who later relapsed, while 50% ultimately reached MRD-negative status after further therapy. In CR1 patients (n = 48), PB-ctDNA identified MRD in 89.3% of those who relapsed, compared to 46.4% by SOC MRD and 35.7% by flow alone. PB-ctDNA MRD robustly predicted relapse-free survival (RFS, HR = 11.9, p = 7e-8) and overall survival (HR = 19.7, p = 5e-9), outperforming SOC methods (HR = 3.4, p = 5e-4 and HR = 5.2, p = 4e-6 respectively). For patients undergoing allogeneic SCT (n = 27), PB-ctDNA MRD clearance before and after SCT predicted RFS (p = 4e-4). Additionally, by monitoring dozens of tumor variants including indels and copy number alternations in a time-resolved fashion, AML-CAPP-Seq allowed detailed tracking of clonal dynamics over time.
Conclusions: AML-CAPP-Seq offers ultrasensitive, noninvasive MRD detection in ctDNA superior to SOC methods without need for invasive BM evaluation. Personalized variant tracking ensures universal applicability across AML patients, enabling both MRD monitoring and clonal evolution profiling.

#3254 Cell-free DNA molecular response predicts clinical efficacy in HGSOC patients treated with azenosertib.

Jinkil Jeong[1], Jianhui Ma[1], Mona Abed[1], Heekyung Chung[1], Doris Kim[1], Nandini Molden[1], Divya Rajendran[1], Chang Shim[1], Danielle D. Jandial[1], Mark R. Lackner[1], Fiona Simpkins[2], Funda Meric-Bernstam[3], Leslie M. Randall[4], Olivier Harismendy[1]

[1]Zentalis Pharmaceuticals, San Diego, CA,[2]University of Pennsylvania, Philadelphia, PA,[3]University of Texas MD Anderson Cancer Center, Houston, TX,[4]Inova Health, Fairfax, VA

Background: Azenosertib is a novel selective WEE1 inhibitor showing promising activity in the treatment of gynecologic tumors, including high-grade serous ovarian cancer (HGSOC). The clinical development of azenosertib in HGSOC is conducted via several efficacy studies using Objective Response Rate (ORR) and progression free survival (PFS) as primary and secondary endpoints. Molecular response (MR), as measured by longitudinal changes of tumor DNA fraction in cell free DNA (cfDNA), can represent a cost-effective, and early efficacy endpoint, which needs further evaluation in HGSOC clinical studies.
Method: Patients (N=139) enrolled in single-agent azenosertib studies (NCT04158336, NCT05128825, NCT05198804) were included in the analysis when 1) assigned to receive at least 300mg QD of azenosertib and 2) plasma cfDNA successfully profiled (Tempus xF+ Liquid Biopsy Assay) at baseline (C1D1) and at least one on-treatment timepoint (C2D1 or C3D1). Efficacy was extracted from an unlocked clinical database. MR positivity was defined as a decrease of TP53 variant allelic fraction greater than 50% between baseline and the earliest evaluable sample.
Results: Most patients (123/139 88.5%) had detectable baseline TP53 mutations and were evaluable for MR of which 70/123 (57%) were MR+ including 28 with complete MR (mCR: 100% decrease). MR+ patients had a higher ORR (20/70 29% vs 4/53 8%, p=5.1e-3, Odds ratio=0.20). Stages of molecular response correlated with best tumor size change (Kruskal-Wallis p<2e-4) including 31% median tumor shrinkage in mCR patients. Consistently, MR predicted time to progression (TTP median 5.12 mo vs 2.69 mo, p=2.8e-4, HR=0.46) and a longer TTP was observed in mCR patients (median 5.68 mo). Remarkably, MR helped further stratify patients with stable disease at first assessment (SD1A patients), with MR+ patients showing a longer TTP (6.08 mo vs 3.42 mo, p=0.012, HR=0.45). In particular, 5/6 SD1A patients who eventually responded had positive MR identified at least 12 weeks (median 14w) prior to confirmation, while 24/39 SD1A patients who progressed had negative MR identified at least 2 weeks (median 7w) prior to progression. Of the 65 patients profiled at both C2D1 and C3D1, 38/65 (58%) had confirmed MR at C3D1. These confirmed MR were more likely to respond to the therapy (16/38 42% vs 4/27 15%, Odds ratio=0.24) and had a longer TTP (5.58 mo vs 3.25 mo, p=0.044, HR=0.52). Interestingly, confirmed MR patients were not more likely to have mCR at C2D1 (16/38 in confirmed vs 3/7 in unconfirmed MR) suggesting that the depth and duration of MR provide independent prognostic values.
Conclusion: Molecular response by cfDNA is an early and reliable surrogate endpoint to measure clinical efficacy in HGSOC studies. Molecular response, duration, and depth were predictive of response or stable disease from azenosertib treatment, supporting its further clinical development.

#3255 Analytical validation of cancer gene promoter methylation detection in cfDNA liquid biopsy assay.

Janice A. Patterson, Martina Lefterova, Brooke Overstreet, Colby Jenkins, Katie Quinn, Darya Chudova

Guardant Health, Palo Alto, CA

Introduction: Promoter methylation (PM) is a critical epigenetic mechanism that is involved in gene silencing and is associated with key pathways in cancer progression. Traditionally, PM detection has relied on single-gene testing of tissue biopsies, which are invasive, less clinically accessible, and impractical for tracking tumor evolution. Here, we evaluate PM within clinical multi-modal assessments using Guardant Infinity smart liquid biopsy platform, providing an innovative approach for monitoring epigenetic alterations.
Methods: Data across >17k samples, including >5k cancer-free samples, and cancer-positive samples from >100 cancer types were used to define promoter regions associated with hypermethylation in cancer-related genes. PM regions were refined to ensure (a) the methylation signal is present exclusively in cancer and low in cancer-free samples, and (b) a negative correlation between gene expression and methylation in The Cancer Genome Atlas Program (TCGA) cancer samples. PM regions are quantified relative to constitutively hypermethylated control regions, measuring gene PM relative to total cfDNA. Analytical accuracy was demonstrated using an orthogonal Enzymatic Methyl Sequencing (EM-seq) assay on a cohort of clinical cfDNA and contrived samples. Limit of detection, precision, and limit of blank were established based on cancer cfDNA samples, cell line dilutions, and healthy donor samples. Gene PM prevalence was assessed in a test cohort of >7k samples across multiple cancer types to compare the relative prevalence to published tissue data sets.
Results: Guardant Infinity PM limit of detection (>95% variant level) was established at PM mutant allele frequency (MAF) 1.6% across a panel of promoter genes. PM above the limit of detection is highly concordant with orthogonal EM-Seq, with 98.2% positive and 98% negative concordance. MLH1 PM was identified in 54% of patients with colorectal cancer (CRC) and 100% of patients with endometrial cancer (EC) who also had MSI-H detected. MLH1 PM in our cohort of CRC showed significant enrichment for BRAF V600E (36%, $p<0.001$). High prevalence of BRCA1 PM was observed in breast (BC) and ovarian cancer (OC), consistent with what is observed in literature. Enrichment of BRCA1 PM in BC was observed with loss of heterozygosity (LOH) (6%, $p=0.02$). The relative PM prevalence for individual genes across tumor types is largely consistent with what has been reported in tissue datasets.
Conclusion: PM in liquid biopsy is consistent with tissue-based findings and correlates with underlying tumor characteristics, such as MSI-H and LOH events, thus demonstrating Guardant Infinity's ability to identify potentially actionable PM patterns in a non-invasive manner. This analysis represents a fraction of the PM landscape observed, highlighting the broader potential of methylation as a novel biomarker for cancer detection and characterization.

#3256 Circulating tumor DNA as a prognostic biomarker in locally advanced esophageal cancer.

Marisa Sewell[1], Matthew Bott[1], Katherine Gray[1], Steve Maron[2], Yelena Janjigian[2], Hans Gerdes[3], Pari Shah[3], Abraham Wu[4], Bernard J. Park[1], David R. Jones[1], Daniela Molena[1]

[1]Thoracic Service, Department of Surgery, Memorial Sloan Kettering Cancer Center, New York CIty, NY,[2]Gastrointestinal Oncology Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York CIty, NY,[3]Gastroenterology, Hepatology, and Nutrition Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York CIty, NY,[4]Radiology, Memorial Sloan Kettering Cancer Center, New York CIty, NY

Introduction: The role of plasma circulating tumor DNA (ctDNA) as a prognostic biomarker in locally advanced esophageal cancer has not been well defined. We aim to evaluate the prognostic value of pre- and on-treatment ctDNA detection.
Methods: In this retrospective single institution study, patients with esophageal cancer undergoing definitive local therapy via endoscopic resection (ER), esophagectomy, or definitive chemoradiation (CRT) underwent baseline and post-treatment ctDNA analysis utilizing a plasma-based tumor informed assay. ctDNA detection and quantification was annotated to clinicopathologic characteristics.
Results: Between 2022 and 2024, 37 treatment-naïve esophageal cancer patients with stage I-IVA disease were analyzed. Overall, 28 underwent esophagectomy, 7 underwent definitive CRT, and 2 underwent ER. At baseline, ctDNA was detectable in 62% (23/37), with a mean 2.74 mean tumor molecules per milliliter of plasma (MTM/ml) (SD 6.37). Among those with detectable baseline ctDNA, 87% (13/15) achieved ctDNA clearance within a median of 45 days postoperatively. The rate of achievement of ctDNA clearance did not differ between the surgical or CRT groups (92% v. 83%, p>0.99).
Of those patients who underwent surgery, 79% (22/28) had neoadjuvant therapy. Baseline ctDNA was detectable in 73% (16/22) of those patients, with a mean ctDNA level of 2.34 MTM/ml (SD 6.10). ctDNA remained detectable in 18% (3/17) after neoadjuvant therapy. Following surgery, 93% (14/15) achieved ctDNA clearance. When examining pathologic response among patients that had neoadjuvant therapy, 27% (6/22) had no residual disease at the time of resection. Patients with no residual disease had significantly lower ctDNA at baseline than those who did have residual disease (0.37 MTM/ml v 1.51 MTM/ml, p=0.005). Of the 14 patients that were detectable at baseline who achieved ctDNA clearance preoperatively, only 3 of those had no residual disease. Among the 28 esophagectomy patients, 11 had nodal metastases. While ctDNA detection rates at baseline were similar between pN0 and pN+ patients (58% v. 64%, p=0.70), patients with nodal metastases had significantly higher pre-treatment ctDNA levels (3.0 MTM/ml vs. 1.2 MTM/ml, p<0.001).
Conclusion: Local therapy for esophageal cancer effectively clears ctDNA. Additionally, quantitative analysis of baseline ctDNA may be a useful prognostic indicator for patients likely to have nodal metastases and poor treatment response at the time of surgery.

#3258 Predicting genitourinary cancer tissue-of-origin using urine cell-free DNA fragmentomics and cell-free RNA.

Pradeep Singh Chauhan[1], Irfan Alahi[1], Jessica Linford[1], Arpit Panda[2], Nathan Colon[3], Ryan Mueller[3], Faridi Qaium[1], Peter K. Harris[3], John Sheng[3], Eric H. Kim[3], Melissa A. Reimers[3], Zachary L. Smith[4], Woodson W. Smelser[3], Aadel A. Chaudhuri[1]

[1]Mayo Clinic, Rochester, MN,[2]University of Chicago, Chicago, IL,[3]Washington University School of Medicine in St. Louis, St. Louis, MO,[4]Advent Health, Orlando, FL

Introduction: We previously developed a urine liquid biopsy assay to measure urine tumor DNA (utDNA) levels in bladder cancer (BC) patients. Here, we extended this approach significantly by layering in cell-free DNA fragmentomic and cell-free RNA analysis, and extending the method to the full spectrum of genitourinary (GU) cancers, to enable both GU cancer detection and tumor tissue of origin prediction.
Methods: A total of 155 GU cancer patients and 34 healthy adults were enrolled in this prospective study. Preoperative urine was collected from 94 bladder cancer (BC) patients, including 65 with muscle-invasive disease, all undergoing cystectomy. Additionally, urine samples were collected from 18 renal cell carcinoma (RCC) patients undergoing nephrectomy and 42 metastatic prostate cancer (mPC) patients. Urine cfDNA and cfRNA were isolated and sequenced, with cfRNA sequenced to 100 million reads per sample on an Illumina NovaSeq X and cfDNA sequenced at 5x genome-wide coverage on a Novoseq S4 flow cell. To identify malignant tissue of origin, we analyzed somatic copy number alterations (CNAs), genome-wide fragment length ratios, and 4-mer end motifs. Tumor fraction (TFx) was estimated using ichorCNA, while DELFI was employed for fragment length ratios in 5 megabase bins across the genome and chromosome arm-level z-scores. Individual logistic regression models were developed based on each fragmentomic feature, and validated using a training and held-out testing approach. Outputs from these models were combined into a single comprehensive model, also trained and held-out tested using the same approach. Urine cfRNA transcript isoform abundance was quantified using Salmon and normalized to Transcripts Per Million (TPM) to account for sequencing depth variations.
Results: Bladder cancer (BC) showed the highest mean tumor fraction (TFx, 10.5%), followed by RCC (3.8%) and mPC (3.4%). Among 94 BC patients, 42% achieved a pCR, while 58% had residual disease and did not. Patients without pCR had significantly higher urine TFx compared to those with pCR (14.7% vs. 4.7%, p=0.0008). A machine learning model based on genome-wide fragment length ratios achieved an ROC AUC of 0.74, while models using genome-wide CNAs and end motifs achieved AUCs of 0.81 and 0.88, respectively. Combining all features into a single model yielded an AUC of 0.92, with classification accuracies of 89% for bladder cancer, 80% for RCC, and 60% for prostate cancer. Additionally, urine cfRNA analysis using the top 1,000 coding transcripts achieved an AUC of 0.95, with classification accuracies of 100% for mPC, 87% for BC, and 78% for RCC.
Conclusion: Fragmentomic features from urine cfDNA and transcriptomic features from urine cfRNA demonstrate strong potential for predicting tumor tissue-of-origin in GU cancers, underscoring the promise of non-invasive diagnosis across the spectrum of GU malignancies through urine liquid biopsy.

#3259 Beyond tumor size: Biological and clinical predictors of ctDNA shedding in colon cancer.

Nerma Crnovsanin*[1], **Carmen Rubio Alarcon***[2], Vincent Pappalardo[2], Ingrid Franken[3], Steven L. C. Ketelaars[2], Renske de Wit[4], Soufyan Lakbir[4], Marcos da Silva[2], Geraldine R. Vink[5], Gerrit A. Meijer[2], Mark Sausen[6], Renee Menezes[2], Jeanine M. L. Roodhart[2], Sanne Abeln[7], Remond J. A. Fijneman[2]

[1]Netherlands Cancer Institute / University Hospital Heidelberg, Amsterdam / Heidelberg, Netherlands,[2]Netherlands Cancer Institute, Amsterdam, Netherlands,[3]University Medical Center Utrecht / Utrecht University, Utrecht, Netherlands,[4]Netherlands Cancer Institute / Vrije Universiteit Amsterdam, Amsterdam, Netherlands,[5]University Medical Center Utrecht / Utrecht University / Netherlands Comprehensive Cancer Organisation, Utrecht, Netherlands,[6]Labcorp, Baltimore, MD,[7]Vrije Universiteit Amsterdam / Utrecht University, Amsterdam / Utrecht, Netherlands

Introduction: Circulating tumor DNA (ctDNA) detection in liquid biopsies has multiple applications in oncology and is becoming part of routine diagnostics. However, the inter-patient variability regarding ctDNA presence and levels in blood hampers the interpretation of ctDNA testing results. A better understanding of the underlying biology of ctDNA shedding could facilitate ctDNA testing implementation, by identifying the patients for whom the test might be informative to guide clinical decisions. This study aims to explore the clinicopathological and molecular characteristics influencing ctDNA shedding in patients with colon cancer.
Methods: Pre-surgery ctDNA levels were measured in 150 stage III colon cancer patients (PLCRC-PROVENC3 cohort) using Labcorp Plasma Detect, a highly sensitive tumor-informed, whole genome sequencing (WGS)-based ctDNA test. Chromosomal instability was assessed via WGS and SNVs using a 505-gene panel (PGDx elio tissue complete). RNA exome sequencing evaluated gene expression and immune cell composition. Features were analyzed independently and integrated using Lasso regression.
Results: High ctDNA levels were associated with large tumor diameter (FDR<0.001), necrosis (FDR = 0.003), angioinvasion (FDR = 0.008), MSI status (FDR = 0.032) and more advanced substages (FDR = 0.004). In the MSS subgroup, genomic analyses showed correlation with more indels (FDR=0.001) and loss of chr10p/chr10q (FDR = 0.018). Transcriptomic analyses revealed association with epithelial-mesenchymal-transition (FDR < 0.001), TNF-alpha signaling (FDR < 0.001), angiogenesis (FDR < 0.001) and higher fraction of cancer-associated fibroblasts (FDR = 0.008) in high shedders, while low shedders showed enrichment in oxidative phosphorylation (FDR < 0.001). Importantly, factors influencing ctDNA shedding independent of tumor diameter included advanced lymph node involvement (FDR = 0.049), necrosis (FDR = 0.066), enrichment in the E2F-Targets (FDR = 0.006), G2M-checkpoint (FDR = 0.005), and Wnt-β- catenin (FDR = 0.022) pathways. Low ctDNA levels were associated with upregulation of KRAS signaling (FDR=0.049) and high expression of CPS1 (FDR < 0.001) and MUC4 (FDR=0.015). Lasso regression identified large tumor diameter and necrosis as the strongest overall predictors of ctDNA levels.
Conclusion: Tumor diameter is the most important feature determining ctDNA levels in blood. High-shedding tumors are larger, show advanced lymph node involvement, necrosis, and activation of proliferation and invasion pathways driving ctDNA release. In contrast, low-shedding tumors are smaller, less aggressive, and metabolically distinct, relying on mitochondrial processes like oxidative phosphorylation. Our findings improve the understanding of the biology behind ctDNA shedding in non-metastatic colon cancer and have the potential to improve patient selection for ctDNA-based clinical decisions.

#3260 Perioperative tumor-informed ctDNA monitoring for minimal residual disease in triple-negative and HER2-positive breast cancer patients.

Yongjun Cha[1], Junghoon Shin[2], Jongseong Ahn[1], Sunghoon Heo[1], Hwang-Phill Kim[1], Duhee Bang[3], Sang-Hyun Song[1], Young-Hyuck Im[2], Ji-Yeon Kim[2], Tae-You Kim[1]

[1]IMBdx, Inc, Seoul, Korea, Republic of,[2]Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of,[3]Department of Chemistry, Yonsei University, Seoul, Korea, Republic of

Introduction: Circulating tumor DNA (ctDNA) enables non-invasive monitoring in solid tumors. This study evaluates the prognostic and predictive value of minimal residual disease (MRD) detection using a personalized ctDNA assay during neoadjuvant chemotherapy (NAC) and post-surgical follow-up in triple-negative breast cancer (TNBC) or HER2-positive breast cancer (BC) patients.
Methods: Patients with stage IA-IIIC TNBC or HER2-positive BC scheduled for NAC followed by curative surgery were prospectively enrolled. Peripheral blood samples (20 mL) were collected at three time points: before NAC (P0), perioperatively (pre- or one month post-operatively, P1), and one year post-operatively (P2). The CancerDetect™ assay (IMBdx, Inc.), a tumor-informed MRD test, identified patient-specific mutations (up to 100) through whole exome sequencing (WES) of tumor tissue and peripheral blood mononuclear cells. CtDNA positivity was defined as the presence of more than two tumor-derived mutations in plasma.
Results: Of 145 patients analyzed (68 TNBC, 77 HER2-positive, median age 49 years), stages included stage I (2.1%), stage II (64.1%), and stage III (33.8%). The median number of mutations identified through WES was 59 (range 9-234), higher in TNBC (71 vs. 50, P = 0.002) but not by stage (P = 0.668). Baseline ctDNA positivity (P0) was 86.9% (median 20 mutations; range 0-97), significantly higher in TNBC (100% vs. 75.3%, P < 0.001) and stage III (95.9% vs. 82.3%, P = 0.021).After NAC, ctDNA clearance occurred in 86.5% of P1-positive patients, with overall ctDNA positivity at P1 dropping to 11.7%, slightly higher in TNBC (14.7% vs. 9.1%, P = 0.314). Pathologic complete response (pCR) rates post-NAC were 55.6%, similar across subtypes and stages. P1 positivity tended to be higher in non-pCR patients (15.6% vs. 7.5%, P = 0.202). CtDNA clearance was associated with higher pCR (60.6%) compared to persistent positive (37.5%, P = 0.106) or persistent negative (42.1%, P = 0.207). For TNBC, P1 positivity was associated with non-pCR (22.6% vs. 5.6%, P = 0.070), unlike HER2-positive BC (9.1% for both).At P2, 90.4% were ctDNA-negative, with positivity in 8.2% (6/73; 4 persistent positives, 2 conversions). At 23 months' median follow-up, 5 patients developed distant metastases. Persistent positivity at P0-P1 (23.5% vs. 0.8%, P < 0.001) or P1-P2 (75.0% vs. 0%, P < 0.001) was linked to higher recurrence rates.
Conclusion: The personalized ctDNA assay detected BC effectively and correlated with subtypes and stages. Post-NAC ctDNA monitoring predicted treatment response and recurrence risk. We are planning longer-term follow-up in the enrolled cohort to better validate its power in recurrence prediction.

#3261 Plasma whole-genome sequencing to monitor pancreatic cancer.

Yuanchang Fang[1], Michelle Chan-Seng-Yue[2], Karen Ng[3], Amy Zhang[2], Nguyen Hoang[3], Gun Ho Jang[2], Sabiq Chaudhary[2], Eugenia Flores-Figueroa[3], Daniela Bevacqua[3], Stephanie Ramotar[3], Ayelet Borgida[3], Shawn Hutchinson[3], Anna Dodd[3], Julie Wilson[2], Barbara Grunwald[4], Robert Grant[3], Erica Tsang[3], George Zogopoulos[5], Jennifer Knox[3], Masoom Haider[1], Steven Gallinger[3], Faiyaz Notta[1]

[1]University of Toronto, Toronto, ON, Canada,[2]Ontario Institute for Cancer Research, Toronto, ON, Canada,[3]University Health Network, Toronto, ON, Canada,[4]University Hospital Essen, Essen, Germany,[5]McGill University, Montreal, QC, Canada

Pancreatic ductal adenocarcinoma (PDA) is a highly aggressive disease with a 5-year survival rate of 12%. Patients are typically diagnosed at the metastatic stage due to lack of symptoms and current treatment options do not effectively control the disease. There is an urgent need to develop sensitive strategies to detect PDA at an early stage before it spreads. Characterizing circulating tumor DNA (ctDNA) in plasma is an effective approach to detect and monitor cancer. Typically, cancer genomes contain thousands of mutations, but targeted sequencing approaches only focus on pre-determined diver genes, which largely limits its performance in detecting ctDNA. Whole-genome sequencing (WGS) however, tracks all the tumor mutations simultaneously, and has a higher sensitivity than targeted approaches, especially in low tumor burden samples. In this study, we aim to use plasma WGS to build a liquid biopsy platform for PDA detection and characterization. To date, we have established the largest plasma WGS (20-60x) cohort for PDA which consists of over 200 baseline and longitudinal plasma samples from patients, with additional 45 plasma samples from age-matched healthy individuals. Paired tumor tissue WGS and RNAseq are also available. We have developed and validated a bioinformatics tool to detect cancer mutations in plasma and estimate plasma tumor fraction (TFx) for tumor burden. In the PDA cohort, the median of plasma TFx was 1% with a wide range between statistically undetectable and 70%. Plasma TFx increased with clinical stages, from a median of 0.5% in resectable PDA to 5% in metastatic cases. Plasma TFx was positively correlated with liver metastasis size, but not with the primary pancreatic tumor size. These results suggest that in PDA, metastases are the major site to shed ctDNA, but not the primary tumors. A positive correlation between serum CA19-9 and plasma TFx was found in metastatic but not in resectable PDA. Higher baseline plasma TFx was associated with worse prognosis, which was more evident in the metastatic PDA. Using the paired tumor WGS and RNAseq, we found that higher ctDNA shedding was associated with higher cell cycle, lower immunity and polyploid genomes in the tumors. No difference was found in plasma TFx between Basal-like and Classical PDA subtypes. In summary, the current study provides insights of the interplay between ctDNA dynamics and clinical features in PDA. We aspire to deliver a freely accessible computational platform for cfDNA genomic profiling, PDA early detection and progression monitoring using plasma.

#3262 Whole genome sequencing of ctDNA enables disease monitoring and residual disease detection in head and neck cancer patients.

Shetal A. Patel[1], Melanie Lou[2], Eddie Ho[2], Michelle Harwood[2], Seng Saelee[2], Dinesh Kumar[2], Steven Johnson[1], Siddarth Sheth[1], Jeffrey Blumberg[1], Catherine Lumley[1], Wendell Yarbrough[1], Michael K. Gibson[3], Jessica Bauman[4], Trevor Hackman[1], Jared Weiss[1], Sowmi Utiramerur[2]

[1]University of North Carolina at Chapel Hill, Chapel Hill, NC,[2]Roche Diagnostics Solutions, Pleasanton, CA,[3]Vanderbilt-Ingram Cancer Center, Nashville, TN,[4]Fox Chase Cancer Center, Philadelphia, PA

Detection of circulating tumor DNA (ctDNA) has clinical value as a non-invasive method for therapeutic response monitoring and detection of minimal residual disease (MRD). In this study, we employed a tumor-informed approach for detecting ctDNA from plasma in a cohort of head and neck cancer (HNSCC) patients with matched sets of FFPE tissue (FFPET), peripheral blood, and plasma samples. Patients with locally advanced HNSCC were treated with induction chemoimmunotherapy, followed by surgical resection, and risk-adapted adjuvant therapy. Plasma was collected pre-treatment, post-induction (on-treatment), and after completion of all treatment (post-treatment). By leveraging whole genome sequencing (WGS), we can overcome the sparsity of ctDNA fragments in low tumor fraction (TF) settings *via* tracking of hundreds to thousands of single nucleotide variants (SNVs). Our approach identifies a patient's unique compendium of tumor-related SNVs from their FFPET sample (after germline subtraction) and tracks this compendium of variants in their plasma samples to detect the presence of ctDNA. Three patients failed quality control criteria and were excluded from further analysis due to the presence of high proportions of C>T:G>A and C>A:G>T variants, known to be FFPE-related and oxidative damage-related artifacts, respectively. Three plasma samples from the post-induction timepoint were also excluded due to insufficient material for sequencing. Post-induction ctDNA was detected in 13 of 22 evaluable samples. Among the ctDNA positive (ctDNA+) post-induction patients, all of them (13/13) had measurable residual tumors on pathologic review, while (2/9) in the ctDNA negative (ctDNA-) group had measurable tumors. For patients with measurable tumors, mean tumor size in the ctDNA+ group (n=13) was 4 cm, while the mean in the ctDNA- group (n=2) was 2.6 cm. Post-treatment ctDNA was detected in 2 of 18 evaluable samples. Among the ctDNA+ post-treatment patients, 100% (2/2) had disease recurrence within 6 months, while disease recurrence was observed in 12.5% (2/16) of the ctDNA- group (occurring 7- and 32-months post-treatment completion). Lastly, we estimated plasma tumor fraction (TF) following Zivran et al (2020). ctDNA+ post-induction samples had a mean TF of 2.4×10^{-4} (n=13; ranging from 2.3×10^{-5} to 1.1×10^{-3}) and ctDNA+ post-treatment samples had an order of magnitude lower mean TF of 5.8×10^{-5} (n=2; ranging from 5.7×10^{-5} to 5.8×10^{-5}) indicating partial molecular response to treatment even in the presence of detectable residual ctDNA. In this albeit small clinical study, our tumor-informed ctDNA assay achieved clinical sensitivity of 87% and 50% at post-induction and post-treatment, respectively, and clinical specificity of 100% at both time points. Our findings underscore the utility of ctDNA as a promising biomarker for disease monitoring in head and neck cancer patients.

#3263 Assessing molecular residual disease via early postoperative plasma circulating tumor DNA for recurrence risk evaluation in non-small cell lung cancer following radical resection.

J. Tang[1], Y. Tian[1], S. Wang[2], Y. Liu[2], M. Chen[1], X. Yang[1], X. Tong[1], M. Wang[1], Y. Zhao[1], J. Pang[2], Q. Ou[2], **H. Tang**[2], X. Chen[1]; [1]The First Affiliated Hospital of Kunming Medical University, Kunming, China, [2]Nanjing Geneseeq Technology Inc., Nanjing, China

Background: The significant risk of tumor relapse in resectable non-small cell lung cancer (NSCLC) highlights the need for biomarkers to identify patients at increased risk of recurrence and inform personalized treatment. Investigating the potential of plasma-derived circulating tumor DNA (ctDNA) for molecular residual disease (MRD) detection and prognosis is essential, particularly in the context of lung cancer risk variations. Xuanwei, a city in Yunnan province, China, provides a valuable model for exploring molecular features related to lung cancer susceptibility.

Methods: This study included 226 stage I-III NSCLC patients who received radical tumor resection. Paired surgical tumor and postoperative plasma samples within 3-7 days (median: 4.5 days, interquartile range: 4.0-6.0 days) were analyzed to evaluate post-surgical ctDNA detection and its association with disease-free survival (DFS) and overall survival (OS) using a tumor-informed method.

Results: Among the 226 patients with surgically resectable NSCLC, 31 were from the Xuanwei region, exhibiting a higher incidence of multifocal lesions (P<0.01) and elevated tumor mutation burden (P<0.001). Subgroup analysis revealed that Xuanwei patients had a higher prevalence of uncommon *EGFR* mutations (P<0.001 and P<0.01 for p.G719X and p.S768I, respectively), as well as mutations in oncogenes and tumor suppressor genes, including *KRAS* (P<0.001), *LRP1B* (P<0.01), *MED12* (P<0.01), and *PIK3CA* (P=0.03). Detectable ctDNA demonstrated high specificity for predicting tumor relapse (0.96; 95% confidence interval [CI]: 0.92-0.98), with specificity increased to 0.97 (95% CI: 0.94-0.99) in stage I patients and 0.73 (95% CI: 0.39-0.94) in stage II-III patients. While sensitivity was relatively lower, it still reached 0.67 (95% CI: 0.49-0.81) in the overall population, with 0.38 (95% CI: 0.14-0.68) for stage I and 0.83 (95% CI: 0.61-0.95) for stage II-III patients. Multivariate Cox regression analysis, incorporating clinical and mutational features with a mutation frequency of ≥ 5%, revealed that post-surgical MRD status was significantly associated with DFS independent of clinical stage and *RB1* variants, and served as the most significant predictor among all these clinicopathological risk factors (hazard ratio [HR]: 9.25; 95% CI: 3.57-24.00; P<0.001). Detectable ctDNA also effectively stratified OS in subgroups, including patients above 60 years of age and those with stage II-III disease.

Conclusions: Our findings reinforce the clinical significance of evaluating ctDNA status within one-week post-surgery for detecting MRD, thereby informing precise individualized treatment and surveillance strategies for NSCLC patients.

#3264 Digital profiling of tumor-derived extracellular vesicle RNA: a novel approach for early diagnosis and monitoring of pancreatic cancer patients.

Taewon Son[1], Hyojin Um[2], Yeochan Kim[2], Elizabeth Maria Clarissa[1], Hanna Kim[3], Jihye Ahn[2], Juhee Park[1], Beomhee Ahn Ahn[2], Kyusang Lee[3], Song Cheol Kim[4], **Beomseok Lee**[3], Yoon-Kyoung Cho[1]

[1]Biomedical Engineering, UNIST, Ulsan, Korea, Republic of,[2]LS Research Institue, LabSpinner, Inc, Ulsan, Korea, Republic of,[3]ExoDiscovery Research Institue, ExoDiscovery LLC, Cedar Park, TX,[4]Department of Hepatobiliary and Pancreatic Surgery, Asan Medical Center, Seoul, Korea, Republic of

Introduction: Pancreatic cancer remains a leading cause of cancer-related deaths due to challenges in early diagnosis and the lack of effective biomarkers. Current diagnostic methods are insufficient, necessitating novel approaches. This study explores the use of extracellular vesicle RNA (EV-RNA) from plasma as a diagnostic tool, leveraging a new assay that facilitates membrane fusion between EVs and liposomes.
Methods: The study introduces a digital EV-RNA profiling assay (LiquiDyne) that uses charge-mediated fusion between extracellular vesicles and charged liposomes (CLIPs) carrying molecular beacons. This process occurs on a droplet microfluidic chip, where the surface charge of CLIPs is adjusted to optimize fusion efficiency. The assay was validated analytically using EVs from pancreatic cancer cell lines (MIA-PaCa-2, PANC-1, PSN-1, Capan-2) with known KRAS mutations (G12C, G12D, G12R, G12V). Clinical validation involved testing retrospective patient samples across all stages of pancreatic cancer.
Results: The assay requires only 20 µL of plasma, removing the need for prior EV isolation or RNA preparation and thus minimizing sample loss. It successfully detected KRAS mutations in both cell lines and patient samples with high accuracy, demonstrating its potential as a reliable diagnostic tool.
Conclusions: This innovative assay offers a promising method for early pancreatic cancer diagnosis by enabling precise quantification of rare EV subpopulations. It holds potential not only for diagnosis but also for monitoring minimal residual disease, thus addressing critical gaps in current pancreatic cancer diagnostics.

#3265 5-methylcytosine and 5-hydroxymethylcytosine are synergistic biomarkers for early detection of colorectal cancer.

Fabio Puddu[1], Annelie Johansson[1], Aurelie Modat[1], Jamie Scotcher[1], Riccha Sethi[1], Nicholas J. Harding[1], Mark S. Hill[1], Ermira Lleshi[1], Casper K. Lumby[1], Jean Teyssandier[1], Michael J. Wilson[2], Robert Crawford[1], Tom Charlesworth[1], Robert J. Osborne[1], Shankar Balasubramanian[3], **Paidi Creed**[1]

[1]biomodal, Cambridge, United Kingdom,[2]Princeton University, Princeton, NJ,[3]Yusuf Hamied Department of Chemistry, University of Cambridge, Cambridge, United Kingdom

Early cancer detection has the potential to significantly improve treatment outcomes and survival rates. Epigenetic biomarkers in cell-free DNA, including DNA methylation, have been shown to differentiate between cancer and non-cancer and are already being integrated into liquid biopsy development programs. Traditional DNA methylation sequencing provides a conflated modified Cytosine (modC) readout, measuring CpGs that are 5-methylcytosine (5mC) or 5-hydroxymethylcytosine (5hmC) but not discriminating between the two states. Dynamic DNA demethylation occurs through TET enzyme activity, with conversion of 5mC to 5hmC preceding eventual loss of methylation. Hence, we hypothesized that obtaining separate measurements of 5mC and 5hmC would improve the ability to detect the development of colorectal cancer at the earliest stage.

We have therefore applied a technology which provides the 6-base genome (the complete genetic sequence whilst simultaneously distinguishing 5mC and 5hmC) from low nanogram input quantities of cfDNA. We generated whole genome 6-base data from cfDNA extracted from plasma of 32 healthy volunteers and 37 patients with colorectal cancer (CRC) at stages I and IV. We used machine learning approaches to build classifiers with features based on modC, 5mC alone, 5hmC alone, and both 5mC and 5hmC, to differentiate between cfDNA from patients with cancer and individuals without cancer, as well as between stage I CRC and Stage IV CRC.

Our findings indicate that using features based separate measurements of 5mC and 5hmC significantly enhances diagnostic accuracy for the detection of stage I CRC (AUC = 0.95) compared to traditional approaches that conflate these markers (modified C, AUC = 0.66). Notably, most regions with an increase in 5hmC in stage I CRC cfDNA also decreased in 5mC in stage IV CRC, suggesting that 5hmC can effectively track regions undergoing demethylation during tumor development. These results support the hypothesis that distinguishing between 5mC and 5hmC can improve the sensitivity of liquid biopsy tests for early cancer detection.

We feel there is merit in applying 6-base sequencing to larger clinical cohorts, across different indications, to more broadly evaluate the potential of 6-base data to improve the earlier detection, diagnosis, and treatment of disease.

CLINICAL RESEARCH: Modifiers of the Tumor Microenvironment
Poster Session

#3269 LRRC15/COL11A1$^+$cancer-associated fibroblasts drive immunosuppression and chemotherapy resistance in pancreatic ductal adenocarcinoma.

Juanfei Peng[1], Kristina Althoff[1], Sven-Thorsten Liffers[1], Luay Farah[1], Marija Trajkovic-Arsic[1], Rui Fang[1], Savvatakis Konstantinos[1], Jan Mengeler[1], Stefan Selbert[2], Doron Shmerling[2], Phyllis Fung-Yi Cheung[1], Jens T. Siveke[1]

[1]Bridge Institute of Experimental Tumor Therapy, West German Cancer Center, Essen University Hospital, Essen, Germany,[2]PolyGene AG, Rumlang, Switzerland

Background and Objectives: Pancreatic ductal adenocarcinoma (PDAC) is characterized by a highly immunosuppressive tumor microenvironment (TME), which contributes to immune evasion, chemotherapy resistance, and poor prognosis. Cancer-associated fibroblasts (CAFs) are key components of the TME and are closely associated with tumor progression. This study investigated the role of LRRC15/COL11A1$^+$ CAFs in shaping the TME, mediating immunosuppression, and driving chemotherapy resistance in PDAC.
Methods and Results: Using single-cell transcriptomic analysis, we identified a CAF subset with co-expression of LRRC15 and COL11A1, which was significantly associated with reduced CD8$^+$ T cell infiltration and increased infiltration of MARCO$^+$ macrophages. These LRRC15/COL11A1$^+$ CAFs were enriched in chemotherapy non-responsive groups and exhibited higher activity in modulating the immune microenvironment by secreting soluble factors such as TGF-β, GAS6, GRN, EGF, VEGF, and CXCL12, which interact with myeloid cells and T cells. To study the temporal dynamics of LRRC15/COL11A1$^+$ CAFs during chemotherapy, we established ultrasound-guided orthotopic PDAC models in mice and administered FIRINOX-based chemotherapy. Serial tumor biopsies were collected from the same tumor before treatment and one and two weeks post-treatment, and at treatment endpoint. Transcriptomic analysis of sequential biopsy samples using ClusterGVis revealed downregulation of various immune response-related pathways (e.g., T-cell receptor signaling, natural killer cell-mediated cytotoxicity, and allograft rejection) after chemotherapy. In contrast, immunosuppressive cytokines including CCL12, CCL2, CCL9, CXCL12, CXCR1, CXCR4, CCR2, CCR5, and IL10, were all significantly upregulated. Additionally, marker genes of LRRC15/COL11A1$^+$ CAFs were also upregulated, recapitulating findings in chemotherapy-treated PDAC patients. We found high correlation of COL11A1 with LRRC15. Multiplex immunofluorescence of the biopsy tissues showed an increase in COL11A1$^+$ CAFs after chemotherapy, which was accompanied by reductions in CD8$^+$ and CD4$^+$ T cell infiltration, and increase in MARCO$^+$ macrophage infiltration. Using a new genetic mouse model we observed an increase in CD8$^+$ T cell infiltration upon diphteria toxin-mediated targeted genetic ablation of COL11A1$^+$ CAFs.
Conclusions: LRRC15/COL11A1$^+$ CAFs reshape the TME by immunosuppression and contribute to chemotherapy resistance in PDAC. Targeting LRRC15/COL11A1$^+$ CAFs may represent a potential strategy to enhance immune responses and improve the efficacy of chemotherapy of PDAC.

#3270 Modulating the tumor microenvironment and boosting T cell immunity with smart enzyme-loaded virus-like particles.
Orikeda Trashi, Jeremiah Gassensmith

UT Dallas, Richardson, TX

Virus-like particles (VLPs) are biomaterials engineered to structurally and functionally mimic viruses without carrying viral genetic material, making them non-infectious. These icosahedral structures, composed of coat proteins, offer a versatile platform for chemical and genetic modifications. In this project, we modify VLPs with receptors targeting the overexpressed Platelet-Derived Growth Factor Receptor Beta (PDGFRβ) in cancers such as lung, liver, and colorectal cancers, enabling precise tumor targeting. Additionally, we address the metabolic challenges posed by the Warburg effect, which leads to lactate accumulation, acidifying the tumor microenvironment and impairing T cell function. To counteract this, we encapsulate lactate oxidase (LOX) and catalase (CAT) within VLPs. LOX reduces lactate levels by converting it to pyruvate and hydrogen peroxide (H_2O_2), while CAT mitigates excess H_2O_2, alleviating oxidative stress and hypoxia. Together, these enzymes reprogram the tumor microenvironment, enhance anti-tumor immunity, and suppress tumor progression. This innovative strategy combines targeted tumor delivery and immune activation, positioning enzyme-loaded VLPs as a promising platform for next-generation cancer therapies.

#3271 Targeted cancer therapy using a novel recombinant il-24s/il-15 chimeric fusion cytokine.

Amit Kumar, Padmanabhan Mannangatti, Swadesh K. Das, Paul B. Fisher

Virginia Commonwealth University, Richmond, VA

Melanoma Differentiation Associated Gene-7/Interleukin-24 (IL-24) and IL-15 provide therapeutic benefit in treating specific cancers. IL-24 displays cancer-specific apoptosis/toxic autophagy-inducing properties in a broad spectrum of human tumors without harming normal cells. IL-15 induces both differentiation and proliferation of T lymphocyte (T) and natural killer (NK) cells, enhances the cytolytic activity of CD8+ T cells, and induces the maturation of dendritic cells (DCs). *In vivo* administration of IL-15 has antitumor effects in several mouse tumor models; however, administration of IL-15 alone is not optimal. Various combination strategies have been explored to increase the efficacy of IL-15 immunotherapy, including coadministration of other cytokines or inhibitory antibodies against immune-suppression molecules. These approaches enhance antitumor responses as compared with IL-15 alone. Using an engineered form of IL-24 (IL-24S; a Superkine) that is secreted more robustly and is more stable, we created a recombinant IL-24S/IL-15 fusion construct in which IL-24S and IL-15 are linked by a flexible linker. This fusion construct was introduced into an adenovirus type 5 for effective delivery and expression. In the present study, we used focused ultrasound (FUS) paired with microbubbles (MB) to deliver the adenoviruses equipped with the IL-24S/IL-15 fusion construct into the mouse brain containing glioblastoma (GBM) and compared the antitumor effect of IL-24S/IL-15 with the individual factors IL-24S and/or IL-15. IL-24S/IL-15 displayed higher antitumor activity and survival benefits than the combination of the individual cytokines in GBM cancer models. There was a significant induction of apoptosis of cancer cells as well as an increase in tumor infiltration of T cells, DCs, Macrophages and NK cells. Antibody neutralization indicates that a primary immune mediator of anticancer activity of FSK is through recruitment and activation of NK cells. However, unlike immunocompetent mice, there was no increase in the survival rate by IL-24S/IL-15 when compared to the IL-24S or IL-15 treated group in SCID mice. Therefore, the increase in the survival rate by IL-24S/IL-15 in immunocompetent mice was achieved through the regulation of the anti-tumor immune response. Global cytokine analyses indicated no changes in inflammatory cytokines during therapy, suggesting that this strategy will be safe. In addition, IL-24S/IL-15 treatment resulted in enhanced GBM cell death vs. the individual cytokines. The antitumor inducing activity of IL-24S/IL-5 is likely due to induction of cancer-specific apoptosis (a function of IL-24S) as well as diverse effects on immune cells (both IL-15 and IL-24S). In summary, IL-24S/IL-15 may provide a new therapeutic reagent to enhance antitumor immunity and treat cancer.

#3273 BTK inhibitors reshape tumor transcriptome and lymph node microenvironment in CLL.

V. M. Rogness, R. Herur, B. Dileep Kumar, C. Gruessner, C. Sun, C. Underbayev, A. Wiestner;
National Institutes of Health, Bethesda, MD

Bruton tyrosine kinase inhibitors (BTKis) have transformed the treatment landscape for patients with chronic lymphocytic leukemia (CLL). BTKis target pathways critical for CLL cell survival and proliferation. We evaluated the effects of BTKis on CLL cell transcriptome and the composition of the tumor microenvironment (TME) in CLL.

Lymph node (LN) biopsies from 65 patients treated with ibrutinib (n=35) collected pre-treatment and at 24 and 72 hours from first dose or acalabrutinib (n=30) collected pre-treatment and after 3 days of treatment at 4, 12, 24, and 36 hours from the last dose of drug were submitted for RNA sequencing. We used gene set enrichment analysis (GSEA) to interrogate activation of signaling pathways and cellular response and quantified the degree of CLL activation using a previously published gene signature derived from single-cell RNA sequencing. Immune cell composition was assessed with CIBERSORT. GSEA highlighted significant downregulation of key CLL-related pathways by BTKis, including MYC, KRAS, HIF-1, NOTCH, NF-κB, and BCR, all with adjusted $p = 0.007$. Consistently, the CLL activation gene signature showed a significant decrease on BTKis ($p = 0.015$). To better understand the timing of decreased activation, we analyzed ibrutinib timepoints separately. Activation score decreased by 23% after 24 hours ($p = 0.24$) and by 87% after 72 hours of ibrutinib ($p = 0.014$). We then investigated the acalabrutinib timepoints: the activation score decreased by 73.7% at 4 hours ($p = 0.013$), 61.7% at 12 hours ($p = 0.016$), 101.6% at 24 hours ($p = 0.056$).

How BTKis affect the LN TME is not well known. The LN TME in CLL differs from normal LNs in several respects, including increased presence of T follicular helper (Tfh) cells, and decreased presence of CD4+ naïve T cells and M0 macrophages. In ibrutinib treated LN biopsies, CIBERSORT identified a significant decrease of Tfh after 72 hours ($p=0.001$) but not after 24 hours, while the proportion of resting CD4 memory T cells increased ($p = 0.003$). LN biopsies taken >72 hours after the start of acalabrutinib revealed significant decreases in Tfh cells ($p = 0.00003$), CD4 memory activated T cells ($p = 0.03$), M0 macrophages ($p = 0.028$), and increases in CD4 naïve T cells ($p = 0.018$), CD4 memory resting T cells ($p = 0.012$), M2 macrophages ($p = 0.03$), and resting mast cells ($p = 0.0097$).

In conclusion, BTKis not only rapidly reduced activating signaling in CLL cells but also significantly altered the composition of the LN TME. The virtually immediate impact on tumor transcriptomes was paralleled by equally rapid if slightly delayed reorganization of the TME. These data suggest that the effects of BTKis spread beyond the immediate drug target and disrupt the tumor-supportive TME.

#3274 Stage-specific deregulation of the Wnt signaling pathway in colorectal cancer among Puerto Rican Hispanics.

Elba V. Caraballo, **Camille Zenon-Melendez**, Josue Perez-Santiago, Sheila Lopez-Acevedo, Marcia R. Cruz-Correa

University of Puerto Rico Comprehensive Cancer Center, San Juan, Puerto Rico

Background: Colorectal cancer (CRC) is a significant global public health concern. It remains one of the leading causes of cancer-related deaths among Puerto Rican Hispanics (PRH), with 60-70% of cases diagnosed at advanced stages (III/IV). This highlights the urgent need for novel diagnostic biomarkers for early-stage CRC detection while monitoring those in advanced stages, which is crucial for understanding progression and guiding therapy. The *Wnt* signaling pathway plays a vital role in cell proliferation, differentiation, apoptosis, and cancer progression. This study aims to identify stage-specific biomarkers linked to the *Wnt* signaling pathway and the molecular mechanisms associated with CRC disease progression.

Methods: A retrospective case-case study was conducted on 30 tissue samples from PRH recruited through the Puerto Rico Familial Colorectal Cancer Registry (PURIFICAR). Transcriptomic analysis was performed in tissue samples from early (n=7) and advanced CRC stages (n=8) compared to their respective adjacent mucosal tissue as controls (adjusted p-value < 0.05). We used R statistical software (package DESeq2) for gene expression analysis between groups stratified by CRC tumor stage. Protein-protein interaction networks were generated using the STRING database to explore functional associations. Specific *Wnt* signaling markers, target genes, and molecular processes associated with each CRC stage will be validated using Quantinova PCR.

Results: Functional interaction networks analysis (≥ 5 genes) revealed 10 *Wnt* signaling-associated genes uniquely deregulated in early-stage CRC and 10 in advanced-stage CRC. Differentially expressed genes in early stages included WNT1, DKK1, and DKK2, while WNT3 was exclusive to advanced stages. Additional *Wnt*-related genes, such as WNT2, WNT7B, and WNT5, were differentially expressed in both early and advanced stages. These findings highlight distinct patterns of *Wnt* signaling pathway involvement across CRC stages.

Conclusion: Differential gene expression analysis of early and advanced CRC stages revealed significant deregulation of *Wnt* signaling pathway genes, emphasizing their potential as stage-specific diagnostic biomarkers and therapeutic targets. Future studies will focus on the modulation of these genes to study the expression and molecular mechanisms that can contribute to CRC disease progression. Understanding these molecular mechanisms is of utmost importance, as it will facilitate the development of more effective, targeted strategies for CRC diagnosis and treatment, improving outcomes for at-risk populations particularly PRH.

#3275 Reprogramming of MDSCs by ST316, a clinical peptide antagonist of b-Catenin/BCL9, enhances anti-tumor immuno- and chemotherapy.

Claudio Scuoppo, Julia Diehl, Ricardo Ramirez, Barry J. Kappel, Abi Vainstein-Haras, Jim A. Rotolo

Sapience Therapeutics, Inc., Tarrytown, NY

Myeloid-derived suppressor cells (MDSCs) are a diverse group of immune cells that play critical roles in mediating immune-suppression. They are often associated with poor responses in various cancer types and represent an attractive target for immunotherapy. Challenges in defining the genetic dependencies of MDSCs have hindered the development of effective therapeutic strategies aimed at targeting them. The Wnt/β-catenin signaling pathway is a key oncogenic driver that has been implicated in immune-exclusion, although additional roles in immune-suppression remain unclear. ST316 is a clinical-stage peptide antagonist of the interaction between β-catenin and its co-activators, BCL9 and BCL9L. Currently, ST316 is being evaluated in a Phase 2 study (NCT05848739) in patients with advanced colorectal cancer (CRC) in combination with standard-of-care agents. We demonstrated in a Phase 1 dose escalation study that ST316 suppresses MDSC expansion in the peripheral blood of treated patients and corroborated these findings in non-clinical CRC murine models. Here, we characterize the role of the Wnt/β-catenin/BCL9 pathway in MDSC programming and investigate the mechanism by which ST316 reduces MDSC expression and immuno-suppressive activity. Transcriptional profiling of MDSCs from tumor-bearing mice indicates that β-catenin and other Wnt pathway components are highly expressed in these cells. Our findings indicate that exposure to ST316 reduces the expression of receptor systems linked to MDSC-mediated T-cell suppression, thereby activating T-cell responses in vivo. In combination studies, ST316 significantly enhances the anti-tumor efficacy of anti-PD-1 immunotherapy and standard-of-care chemotherapies for advanced CRC such as FOLFIRI. Moreover, while anti-PD-1 and FOLFIRI alone each resulted in expansion of immunosuppressive MDSCs, combination with ST316 prevented this expansion. Our results reveal a novel role for Wnt/β-catenin signaling in MDSC biology and demonstrate that ST316 can suppress the increase of therapy-driven MDSCs in vivo. These data suggest that therapeutically targeting the BCL9/β-catenin interaction may be an effective strategy to enhance both chemotherapy and immunotherapy in Wnt-driven tumors that respond poorly to monotherapy.

#3276 Clinical PK, PD and safety analysis of a phase I clinical trial of DT-9081, an EP4R-antagonist, for RP2D determination in patients with advanced solid tumours.

Christophe Le Tourneau[1], Zahra Castel-Ajgal[1], Jean-Pascal Machiels[2], Rachel Galot[2], Nuria Kotecki[3], Christiane Jungels[3], Jean-Pierre Delord[4], Iphigenie Korakis[4], Anne-Laure Blayo[5], Lola Lecru[5], Thibaut Brugat[5], **Stephan Schann**[5], Nathalie Lenne[5], Samira El-Farouk[5], Claire Jouffroy-Zeller[5], Anne Quesnel[5], Carolina Duarte[5], Karim Taamma[5]

[1]Institut Curie, Paris, France,[2]UC Louvain, Woluwe-Saint-Lambert, Belgium,[3]Institut Jules Bordet, Bruxelles, Belgium,[4]IUCT Oncopole, Toulouse, France,[5]Domain Therapeutics, Illkirch, France

Prostaglandin E2 (PGE2) is a lipid known to promote tumor growth and immune evasion through the EP4 receptor (EP4R). DT-9081, a novel EP4R antagonist, is under investigation for its potential to inhibit tumor growth and enhance immune response. Here are presented the preliminary pharmacokinetic (PK), pharmacodynamic (PD) and safety assessments from the dose escalation phase 1 clinical trial (NCT05582850) allowing the determination of the recommended phase 2 dose (RP2D).

This dose-escalation study employed a modified 3+3 design. DT-9081 was administered orally once daily in doses ranging from 25 to 600 mg. Plasma concentrations of DT-9081 were quantified using LC-MS/MS for PK analysis. PD endpoints included changes in immune activation markers as well as blocking of EP4R agonist (TCS2510)-induced cytokine modulation in blood. Safety assessments were based on NCI-CTCAE v5.0 criteria, with adverse events (AEs) recorded by occurrence and severity.Currently, a total of 29 patients treated with DT-9081 over 6 dose levels (25 mg to 600 mg) are available for analysis. Primary cancer sites included head and neck, ovary, kidney, oesophagus, cervix, gall bladder, lung, pancreas, stomach, prostate, thyroid and rectum carcinoma. Overall, DT-9081 was well tolerated at all tested dose levels. MTD was not reached. Treatment-related AEs (TRAEs) were mostly grade 1 or 2. The most frequent TRAEs (n≥5) were diarrhoea, decreased appetite, nausea and abdominal pain across all cohorts. Grade ≥3 TEAEs included diarrhoea and abdominal pain in one participant from the 50 mg cohort. One dose-limiting toxicity (hepatic enzyme increased grade 3) was observed for another participant at the 50 mg cohort.PK data showed linear increases of C_{max} and AUC in a dose-dependent fashion, with suitable exposure to cover the target IC_{50} for 24h with once-daily dosing. Blood PD data demonstrated that DT-9081 was able to prevent the immunosuppressive activity of TCS2510 by restoring IFN-γ and TNF-α secretion at all dose levels, with a maximum reversion observed around 2h post dosing, in line with PK profile (T_{max}). A long-lasting effect until 10h post-dosing was also observed after 15 days of treatment. Analysis of paired biopsies and PBMCs is ongoing to evaluate the impact of DT-9081 on intra-tumoral and peripheral immune response.

DT-9081 was well tolerated and exhibited a linear PK profile and an exposure supportive of once-daily dosing scheme. PD findings demonstrated the EP4R engagement and inhibition induced by DT-9081 across all dose levels tested and were consistent with the PK data. The collective evaluation of the available PK, PD, safety and tolerability data appoints the 600 mg dose as the potential RP2D, upon finalization of the last ongoing cohort at this dose. Additional PD assessments in peripheral blood and paired tumor biopsies will be available in the poster presentation.

#3277 Investigating the link between metabolic reprogramming and immune modulation in tumors: exploring the therapeutic potential of BPM31510.

Maria-D. Nastke[1], Sylwia Stopka[1], Abbas Khojestah[2], Juan J. Aristizabal-Henao[1], Archna Ravi[1], Kaila M. Bennett[1], Niven R. Narain[1], Lawrence Recht[2], Michael A. Kiebish[1], Stephane Gesta[1]

[1]BPGbio Inc., Framingham, MA,[2]Stanford University, Palo Alto, CA

BPM31510, a CoQ10-lipid nanoparticle, has been shown in previous studies to deliver supraphysiological levels of oxidized CoQ10 to tumors, leading to the induction of mitochondrial Reactive Oxygen Species (ROS) and subsequent apoptotic cell death. Since mitochondrial metabolism is crucial for immune cell function, we investigated whether BPM31510 could influence the tumor microenvironment (TME) to influence immune-mediated tumor elimination. In tumor models such as MC38 and PAN02, we have demonstrated that BPM31510 induces cytotoxic T cell infiltration while concurrently reducing tumor-associated macrophages (TAMs). Additionally, in a rat glioblastoma (GBM) model, BPM31510 was found to metabolically reprogram tumors, further suggesting a potential link between immune modulation and metabolic changes within the TME. These findings support the hypothesis that BPM31510's ability to reprogram tumor metabolism and induce T cell infiltration may be interconnected. This dual mechanism positions BPM31510 as a promising therapeutic for enhancing immune responses in 'immunologically cold' tumors, such as GBM and pancreatic cancer. To explore this connection further, we propose focused studies in syngeneic murine models to examine how BPM31510's immune-modulatory and metabolic effects interact within the TME. These studies will involve detailed analysis of both tumor and lymphoid tissues using advanced flow cytometry and TIMSTOF FLEX spatial omics technology. The goal is to investigate how different dosing and scheduling regimens of BPM31510 impact the relationship between tumor metabolism, immune cell phenotype, and immune activity. Ultimately, this study aims to provide new insights into optimizing BPM31510 treatment strategies, potentially enhancing the effectiveness of immune-based therapies for these difficult-to-treat cancers. By understanding how BPM31510 influences both the immune landscape and metabolic reprogramming of tumors, we hope to develop more effective therapeutic approaches for 'cold' tumors like GBM and pancreatic cancer.

#3278 Neoadjuvant EGFR-TKI therapy reshapes tumor immune microenvironment in resectable EGFR-positive non-small cell lung cancer.

Fang Wu[1], Dan Fan[1], Yingzhe Zhang[1], Wenliang Liu[2], Fenglei Yu[2], Wen Hu[2], Yue Zeng[1], Yurong Peng[1], Chunhong Hu[1], Xianling Liu[1], Fang Ma[1], Chao Deng[1], Zhenhua Qiu[1], Shijiao Yao[1], Shuxing Wang[1], Xiaoqiao Lin[1], Aoxi Liang[1], Xinzhu Lin[1], Qian Guan[1], Han Yang[1], Yiyang Li[1]

[1]Department of Oncology, The Second Xiangya Hospital of Central South University, Changsha, China,[2]Department of Thoracic Surgery, The Second Xiangya Hospital of Central South University, Changsha, China

Background: Patients with EGFR-positive non-small cell lung cancer (NSCLC) do not respond as well to immunotherapy as patients without an EGFR mutation. Nevertheless, recent studies indicate that subsequent immunotherapy following EGFR-TKI treatment may be effective in EGFR-positive NSCLC. Given the critical role of the tumor immune microenvironment (TIME) in determining immunotherapy efficacy, understanding the influence of EGFR-TKI on the TIME in NSCLC remains an important yet unresolved area of research.

Methods: Single-cell RNA sequencing (scRNA-seq) was performed to investigate the TIME of NSCLC patients. Post-treatment samples were analyzed from three EGFR-positive NSCLC patients treated with neoadjuvant EGFR-TKI (Neo-TKI) and two EGFR-negative NSCLC patients treated with neoadjuvant immunotherapy (Neo-IO). The scRNA-seq datasets of seven EGFR-positive NSCLC patients undergoing surgery only (TN-TKI) from the National Genomics Data Center (CRA001477, CRA001963) and six EGFR-negative NSCLC patients received surgery only (TN-IO) from the Gene Expression Omnibus (GSE131907) were analyzed to assess the TIME in treatment-naïve conditions.

Results: A total of ~173,000 single cells were sequenced, revealing 6 subpopulations. Compared to TN-TKI samples, Neo-TKI samples showed a higher proportion of T and B cells, whereas the proportion of myeloid cells significantly decreased. Further analysis of T cell composition in Neo-TKI samples revealed a trend towards a higher prevalence of GZMK+CD8+T cells and enrichment of positive regulation of T cell activation pathway. The expression of TLS-associated genes in CD4+ T cells was significantly elevated, along with the upregulation of immune-related pathways, such as B cell activation signaling and IL-17-related signaling, underscoring the immunomodulatory role of EGFR-TKI. Regarding B cell composition, Neo-TKI samples showed a higher proportion of germinal center B cells with an enrichment in positive regulation of B cell signaling pathway compared to TN-TKI samples. Notably, a shift in tumor-associated macrophages from an M2 phenotype to a neutral phenotype was observed in Neo-TKI samples, supporting the notion that EGFR-TKI impacts the immune landscape. Further, we conducted an exploratory analysis comparing the effects of immunotherapy and EGFR-TKI treatment on the TIME. Distinct differences were observed in cellular subpopulation distribution, enriched genes, and activated pathways. Overall, EGFR-TKI therapy appears to have a more pronounced effect on the TIME of patients with NSCLC.

Conclusion: EGFR-TKI therapy alleviated the immunosuppressive TIME of EGFR-positive NSCLC, providing valuable insights for optimizing subsequent immunotherapy strategies in EGFR-positive NSCLC.

#3279 Smoking burden is associated with immunotherapy resistance in extensive stage small cell lung cancer.

Kyle Concannon[1], Benjamin Morris[1], Lixia Diao[1], Moushumi Sahu[2], Whitney Lewis[1], Xiaowen Sun[1], Simon Heeke[1], Bingnan Zhang[1], Hai Tran[1], Lingzhi Hong[1], Waree Rinsurongkawong[1], Minh Nguyen[1], Kang Qin[1], Hui Li[1], Jainjun Zhang[1], Jack Lee[1], Jing Wang[1], Robert Cardnell[1], Carl Gay[1], John Heymach[1], Lauren Byers[1]

[1]The University of Texas MD Anderson Cancer Center, Houston, TX,[2]Joe R. & Teresa Lozano Long School of Medicine, The University of Texas Health Science Center at San Antonio, San Antonio, TX

Small cell lung cancer (SCLC) remains the deadliest histologic lung cancer subtype with a median survival of only ~12 months among patients with extensive stage (ES) disease. SCLC is strongly associated with heavy tobacco use, yet the effect of tobacco use on therapeutic outcomes remains understudied. Recent data from our group showed that the subtype of SCLC with inflammatory gene signatures (SCLC-I) responds better to immunotherapy (IO) than the other subtypes. Given the association between smoking and inflammation, we hypothesized that heavy smoking may correlate with better IO responses and enrichment in the SCLC-I subtype. To investigate this, we evaluate the outcomes of ES-SCLC patients treated at MD Anderson between 2017-2024 (N=521) and compared those who received chemotherapy plus IO (N=263) to those who received chemo alone (N=258). Transcriptional changes were evaluated across different tobacco pack-year cohorts using the GEMINI bulk RNAseq database of MD Anderson SCLC tumors (N=81).

The median overall survival (mOS) for all ES-SCLC patients was 13.3 months. Immunotherapy provided an added 1-month benefit in mOS over chemotherapy alone, extending mOS from 12.1 to 13.1-months (HR=0.88; p=0.21); consistent with historic cohorts. Patients were then stratified by the cohort pack-year median of 40. For patients with ≤ 40 pack-year smoking histories, the addition of IO improved mOS by 3.7-months from 11.5 to 14.3-months (HR=0.74; p=0.03). However, those with >40 pack-year smoking histories demonstrated no benefit from added IO (13.0 to 12.2-months (HR=1.06; p=0.70). Further analysis by pack-year tertiles (0-24, 25-49, 50+) showed mOS benefits of 5.4 months (HR: 0.67, P=0.05), 1.6m (HR:0.91, P=0.62), and 0.7m (HR=1.01, P=0.679) respectively, for chemo plus IO vs chemo alone.

Despite better IO outcomes among patients with 0-40 pack-years, the SCLC-I subtype was underrepresented in the 0-40 pack-year group compared to expectation using the GEMINI RNAseq database (chi-squared 8.2; p=0.04). Tobacco exposure positively correlated with absolute immune cell infiltration by CIBERSORTx (R=0.24; p=0.03), although driven by higher immunosuppressive T cell signatures. Tumors from the 50+ pack-year (upper smoking tertile) group also demonstrated elevated chronic inflammatory GSEA signatures and genes associated with microenvironment immune suppression compared to the 0-24 (lower smoking tertile) pack-year group.

The discovery that smoking is linked to immunotherapy resistance in SCLC represents a novel contribution to our understanding of the disease and its treatment. By identifying immunosuppressive cellular infiltrates and immunosuppressive gene signatures among patients with heavy vs light smoking histories, we have identified potentially targetable mechanisms of immune evasion. This represents a new lens through which to study immunotherapy resistance in SCLC.

#3280 Type I IFN receptor signaling in myeloid and T cells drives antitumor immunity elicited by intratumoral delivery of immunogenic recombinant modified vaccinia virus Ankara.

Yueqi Wang[1], Shanza Baseer Tariq[1], Ning Yang[1], Shuaitong Liu[1], Bharath Salla[1], Zeda Zhang[1], Juan Yan[1], Weiyi Wang[1], Yi Wang[1], Gregory Mazo[1], Juan Angelo-Lozano[2], Jiahu Wang[3], Tuo Zhang[4], Jenny Zhaoying Xiang[4], Juan C. Osorio[2], Jeffrey V. Ravetch[2], Ming O. Li[1], Ping Chi[1], Scott W. Lowe[1], Charles M. Rice[2], **Liang Deng**[1]

[1]Memorial Sloan Kettering Cancer Center, New York, NY,[2]The Rockefeller University, New York, NY,[3]Genvira Biosciences, Ottawa, ON, Canada,[4]Weill Cornell Medical College, New York, NY

Type I interferons (IFNs) are critical in mediating antitumoral and antiviral responses. However, the roles of IFN and IFNAR signaling within the tumor microenvironment remain poorly understood. To address this, we utilized a Cre-loxP system to generate IFNAR1 conditional knockout mice, enabling us to dissect the role of IFNAR signaling in innate and adaptive immune cells during antitumor responses elicited by immune-stimulating vaccinia viruses. Our first-generation virus, MQ710, was developed by deleting *E5R*, which encodes a cGAS inhibitor, from the non-replicative modified vaccinia virus Ankara (MVA) and incorporating transgenes for membrane-bound Flt3L and OX40L. MQ710 has shown promising early results in a Phase I clinical trial for solid tumors. Building on this platform, the second-generation virus, MQ833, was engineered by deleting two additional viral immunosuppressive genes (*E3L* and *WR199*) and introducing extracellular matrix-anchored murine interleukin-12 (IL-12) as the third transgene. MQ833 was found to be more effective than its oncolytic vaccinia counterpart, OV-IL12, expressing the same transgenes in various tumor models, including murine ovarian cancer. The third-generation virus, MQ954, incorporated further modifications to MQ833, including deletion of *C12L* and insertion of human granulocyte colony-stimulating factor, G-CSF, also anchored to the extracellular matrix. Intratumoral (IT) administration of MQ954 elicited more potent antitumor effects than MQ833 in preclinical models of malignant peripheral nerve sheath tumor (MPNST) and bladder cancer. Using $Irf3^{-/-}Irf7^{-/-}$ and $Stat2^{-/-}$ mice, we found that type I IFN production and signaling are critical for the antitumor effects induced by these viruses. We evaluated the efficacy of MQ833 and MQ954 in $Lyz^{cre}Ifnar1^{fl/fl}$, $Fcgr^{cre}Ifnar1^{fl/fl}$, $MRP8^{cre}Ifnar1^{fl/fl}$, $Foxp3^{cre}Ifnar1^{fl/fl}$, and $CD4^{cre}Ifnar1^{fl/fl}$ mice, in which Ifnar1 gene is depleted exclusively in myeloid cells, monocytes/macrophages, neutrophils, and regulatory T cells, respectively, and compared with $Ifnar1^{fl/fl}$ controls. Our findings revealed that IFNAR signaling in myeloid cells, regulatory T cells (Tregs), and CD4/CD8 T cells is essential for the observed antitumor effects. Single-cell RNA sequencing of CD45+ cells isolated from MB49 tumors from conditional knock-out mice and controls treated with MQ954 twice three days apart further highlighted the pivotal role of type I IFN signaling in monocyte recruitment, macrophage and neutrophil activation and the differentiation of stem-like T cells into effector T cells. This study underscores the multifaceted role of type I IFN signaling across diverse immune cell types in the context of virus-based cancer immunotherapy and provides valuable insights into the intricate immune mechanisms driving antitumor responses.

#3281 CRISPR activation of endogenous STING as an innovative strategy for targeted immunotherapy.

Emil L. Lauridsen, Stine H. Godsk, Anders Etzerodt, Martin R. Jakobsen

Biomedicine, Aarhus University, Aarhus, Denmark

Background: Stimulator of Interferon Genes (STING) has long been of interest within the field of cancer immunology, as the cyclic GMP-AMP synthase (cGAS)-STING pathway can drive inflammation in the tumor microenvironment through the sensing of cytosolic DNA and production of 2'3'-cGAMP. Importantly, in cancer cells with high levels of chromosomal instability (CIN), we and others have observed selective suppression of STING expression, which we hypothesize benefit cancer cells to avoid activating an immune response and to promote immune escape.

Method: The murine triple-negative breast cancer model 4T1, which presents with CIN was explored. We conducted both *in vitro* and *in vivo* experiments to characterize cGAS-STING pathway signaling in response to exo- as well as endogenous DNA stimulation. Utilizing a CRISPR-dCas9 activation (CRISPRa) approach we selectively regulated STING expression in cancer cells and evaluated immune responsiveness *in vitro*. The anti-tumoral effect of activating the cGAS-STING signaling cascade was tested *in vivo* using aEpCAM-labelled lipid-nanoparticles (LNPs) for delivering mRNA to support selective uptake in 4T1 cells.

Results: Initially, 4T1 cellswere found to have low STING expression, in conjunction with high levels of micronuclei and cGAS activity, supporting endogenous accumulation of 2'3'-cGAMP. Using CRISRPa of STING, we observed a rapid cGAS dependent type-I interferon and inflammatory cytokine response correlating with STING expression in a time-dependent manner. Using EpCAM-targeted LNPs we enabled specific mRNA delivery to 4T1 cells *in vitro* and *in vivo*. Our current efforts focus on exploring the *in vivo* anti-tumoral effects of this STING-targeting immunotherapy which will be presented at AACR.

Conclusion: Our data reveal that reestablishing endogenous STING expression in cancer cells that exhibit elevated CIN, may result in an anti-tumoral inflammatory response. We believe that the altered immune profile determined by STING reactivation presents a therapeutic potential to promote tumor regression. Using this specifically targeted LNP-mRNA system, this type of epigenetic regulation may become a cornerstone of future personalized cancer therapies in addition to traditional immunotherapy.

#3282 Versican abundance/proteolysis as a biomarker for immunotherapy efficacy in intestinal cancers.

Elizabeth L. Field[1], Sean Kraus[1], Madeleine Buratti[1], Heidi Koehnke[1], Cheri A. Pasch[1], Fotis Asimakopoulos[2], Dustin A. Deming[1]

[1]University of Wisconsin-Madison, Madison, WI,[2]University of California San Diego, San Diego, CA

Background: The composition of the tumor microenvironment (TME) can regulate T cell infiltration and potentially immunotherapy efficacy. Versican (VCAN), an extracellular matrix proteoglycan, regulates the presence of tumor infiltrating lymphocytes (TILs) and their activation, while its proteolytic byproduct, versikine (Vkine), can stimulate an immune response. Here we aim to validate our prior findings that VCAN status correlates with CD8+ T cell infiltration in intestinal cancers and evaluate the predictive role of VCAN/Vkine for patients undergoing immunotherapy for microsatellite instability high (MSI-H) cancers.
Methods: A total of 28 consented subjects were identified with intestinal cancers that were MSI-H, high tumor mutation burden, or with alterations in mismatch repair genes as part of an IRB-approved protocol. Baseline characteristics, immunotherapy response, and survival data were collected. Cancer samples were obtained and stained for VCAN, Vkine, and CD8+ TILs. An intensity binning system was used to score VCAN and Vkine, ranging from 0-3. TILs were averaged across high power fields (HPF). Cancers with high VCAN (2-3) and any Vkine were categorized as VCAN proteolytic weak (VPW), cancers with low VCAN (0-1) and high Vkine (2-3) as VCAN proteolytic predominant (VPP), and cancers with both low VCAN (0-1) and low Vkine (0-1) as VCAN/Vkine low (VVL).
Results: Clinical characteristics of the 28 subjects studied: median age 62.5 years (32-90); 17 females; 19 colon cancers; six rectal cancers; one rectosigmoid cancer and two small intestinal cancers. Further, nineteen were stage 3, nine were stage 4 and 71% were MSI-H. Six were VVL, one was VPP and 21 were VPW. The VVL and VPP cancers had a median of 27 CD8+ TILs/HPF, while the VPW cohort had a median of 15.7 (p=0015). CD8+ TILs in the MSI-H VPW subset trended higher than the microsatellite stable VPW cancers (21.7 versus 8.5; p=0.35). Thirteen subjects with MSI-H cancers received immunotherapy (10 pembrolizumab (PEMBRO), three ipilimumab (IPI) and nivolumab (NIVO)). Of these cancers, six were VPW and completed PEMBRO. None of the VPW cancers achieved a complete response to PEMBRO alone with most developing progressive disease. An additional VPW subject is still receiving therapy with a partial response to PEMBRO to date. Two patients with VPW cancers received IPI and NIVO and had complete responses. The four subjects with VVL or VPP cancers have all achieved a complete response and are alive without cancer recurrence. Three were treated with PEMBRO and one with IPI and NIVO.
Conclusion: The VCAN status in the TME of intestinal cancers shows promise as a biomarker for T cell infiltration and response to immune checkpoint blockade. The ability of VCAN status to identify patients who are more likely to benefit from dual immune checkpoint blockade deserves further investigation.

#3283 RNAi mediated silencing of *STAT3 or PD-L1* in tumor-associated immune cells induces robust anti-tumor effects in immunotherapy resistant tumors.

Shanthi Ganesh[1], Minju KIm[1], Jenny Lee[1], Xudong Feng[1], Harini Krishnan[1], Amber Beaudry[2], Jennifer A. Lockridge[2], John Hanrahan[2], Sam Moskowitz[2], Vinita Uttamsingh[2]

[1]Pharmacology, Novo Nordisk, Lexington, MA,[2]Early Development, Novo Nordisk, Lexington, MA

Most refractory malignant solid tumors create an immunosuppressive tumor microenvironment (TME), rendering them resistant to standard-of-care immune checkpoint inhibitors (CPIs). We investigated the feasibility of applying systemically delivered RNAi agent to silence the mRNA of a historically undruggable target, that involved in mediating immune suppression in TME, directly in tumor-associated immune cells. In preclinical rodent tumor models, GalXC-Plus^{TM} conjugates selectively silenced the *Stat3* mRNA in multiple relevant cell types, reduced the protein levels, and increased cytotoxic T-cell infiltration. In immunotherapy resistant tumors, RNAi-mediated silencing resulted in tumor growth inhibition, which was further enhanced in combination with CPIs. We also demonstrated that silencing *Cd274* (PD-L1) in antigen-presenting cells using another GalXC-Plus^{TM} conjugate was effective in treating tumors that are resistant to PD-L1 antibody therapy. Currently, both human active STAT3 and PDL1 conjugates are in Phase 1 clinical trials for treating immunotherapy-refractory cancer types (NCT06098651, NCT06504368).

#3284 Dual blockade of EGFR and TGF-B with ficerafusp alfa has the potential to overcome resistance mechanisms in 1L R/M HNSCC in combination with pembrolizumab.

Brenda C. O'Connell[1], Shiv R. Krishn[2], Pradip Nair[2], John Kaczmar[3], Christine H. Chung[4], Dan Zandberg[5], Debora J. Wong[6], David Raben[1], Rachel Salazar[1]

[1]Bicara Therapeutics Inc., Boston, MA,[2]Syngene International Limited, Bengaluru, India,[3]Medical University of South Carolina, Hollings Cancer Center, Charleston, SC,[4]Moffitt Cancer Center, Tampa, FL,[5]UPMC Hillman Cancer Center, Pittsburgh, PA,[6]UCLA Medical Center, Los Angeles, CA

Objective: Resistance mechanisms, such as epithelial-mesenchymal transition (EMT) and hypoxia diminish the efficacy of standard treatments in head and neck squamous cell carcinoma (HNSCC), including anti-EGFR and anti-PD-1 therapies. TGF-β is a cytokine involved in these resistance pathways, promoting a mesenchymal tumor phenotype through EMT. TGF-β-expressing cancer-associated fibroblasts (CAFs) are associated with T cell exclusion and resistance to anti-PD-1/PD-L1, while EMT is linked to anti-EGFR resistance. Ficerafusp alfa, a bifunctional antibody targeting EGFR and TGF-β in the tumor microenvironment (TME), showed clinical activity in a Phase 1b study for 1L R/M HNSCC (NCT04429542), particularly among HPV-negative (neg) patients (IJROBP 2024). Analysis of paired biopsies from HPV-neg tumors highlight the effects of ficerafusp alfa in combination with pembrolizumab (P).
Methods: Paired tumor biopsies collected from the Ph1b 1L R/M HNSCC cohort (NCT04429542) were analyzed by immunohistochemistry for pSMAD2 and CD8 and for gene expression within regions of the TME by whole genome spatial transcriptomics. Cytokine analysis was performed on pre- and post-treatment plasma samples.
Results: Data from paired tumor biopsies demonstrated inhibition of TGF-β signaling within tumor cells and an increase in CD8+ T-cell infiltration (n=7), including in HPV-negative tumors with low PD-L1 (CPS 1-19). Spatial transcriptomics revealed down-regulation of genes and pathways associated with drug resistance including hypoxia and EMT in both tumor and stromal regions (n=8). Up-regulation of immune activation pathways such as interferon-gamma and interferon-alpha was also observed in both tumor and stromal regions. Plasma analyses revealed complete neutralization of TGF-β1 (0.5% of pre-dose at day 21; p<.0001) and increased pro-inflammatory cytokines CXCL9, CXCL10 and IFNG (p<.01) with the ficerafusp alfa combination (n=24).
Conclusion: Down-regulation of hypoxia and EMT pathways in both tumor and stromal regions with ficerafusp alfa and P treatment supports the potential role of TGF-β inhibition in preventing the mesenchymal phenotype associated with drug resistance. These findings provide proof of concept that ficerafusp alfa effectively blocks TGF-β signaling in both tumor cells and tumor supporting stromal cells. Association of translational findings with clinical data will be presented. This supports the rationale for combining ficerafusp alfa with P in HPV-negative R/M HNSCC and potentially in other settings where anti-EGFR and anti-PD-1 drug resistance is a factor.

#3285 Targeting interactions between tumor-associated macrophages and tumor cells to enhance chemotherapy response in a resection model of triple-negative breast cancer.

M. E. Fernandez[1], Y. Alshehry[1], R. R. Aldaqqa[1], H. D. Bear[2], D. H. Sweet[1], S. R. P. da Rocha[1];

[1]Virginia Commonwealth University - VCU, Richmond, VA, [2]Massey Comprehensive Cancer Center, Virginia Commonwealth University, Richmond, VA

Triple-negative breast cancer (TNBC) is the most lethal breast cancer subtype and lacks targeted treatment options. While immune checkpoint blockade leads to significant benefits when combined with chemotherapy, recurrence, metastasis, and chemoresistance remain major challenges. The purpose of this study was to assess the ability of a colony-stimulating factor 1 receptor inhibitor (CSF-1Ri) and an anti-CD47 (aCD47) monoclonal antibody to remodel the tumor microenvironment (TME) in a murine resection model of TNBC, and the benefit of such priming when administered with chemotherapy. We focused on targeting tumor-associated macrophages (TAMs) as they are the most abundant immune cell in the TME and are associated with worse metastasis free survival (MFS) and overall survival (OS). Targeting TAMs, specifically M2-like TAMs, has been shown to delay recurrence and prolong MFS and OS. We also sought to block the CD47-SIRPα interaction between TNBC cells and TAMs, because TNBC cells have higher levels of CD47 expression compared to other breast cancer subtypes, and high rates of CD47 expression are associated with worse MFS and OS and higher cancer stemness. We selected pexidartinib (PLX) as CSF-1Ri given its translational potential (FDA approved) and gemcitabine (GEM) as chemotherapy, given its immunomodulatory effects. To recapitulate the clinical setting, we utilized a survival resection model, allowing us to administer neoadjuvant and adjuvant therapy, and track the development of metastases. BALB/c mice were injected with 100,000 4T1 mammary carcinoma cells in the flank, and tumors established over the course of six days. Mice were then exposed to daily administrations of PLX (8 mg/kg), every other day administration of aCD47 (8 mg/kg) and weekly administrations of GEM (60 mg/kg). The primary tumor was resected on day thirteen and mice were then exposed to adjuvant therapy using the dosing regimen outlined previously. The TME of the resected tumors was characterized by flow cytometry, and MFS and OS were recorded over a ninety-day period. Both PLX+GEM and aCD47+GEM significantly reduced total TAMs (39% and 40% reduction, respectively). However, Only PLX+GEM significantly shifted the M1:M2 ratio towards the pro-inflammatory M1-like phenotype. Both combinations led to a significant prolongation of MFS and OS compared to GEM therapy alone. Triple therapy (PLX+GEM+aCD47) led to the most significant depletion of TAMs (62% reduction compared to vehicle), and a favorable M1:M2 shift, while reducing cancer stem cells and increasing dendritic cells. Triple therapy also had the greatest prolongation of MFS and OS (prolonged by 30% and 31% compared to GEM, respectively). Thus, the therapeutics investigated led to a remodeling of the TNBC TME towards a favorable phenotype, leading to enhanced chemotherapy efficacy and prolonged survival.

#3286 Targeting ALKBH5 to enhance the anti-tumor immunity of natural killer cells.

Yuan Che, Tingting Tang, Zunsong Hu, Chao Shen, Zhenhua Chen, Wei Li, Kitty Wang, Lillian Sau, Ying Qing, Dong Wu, Keith Leung, Zhaoxu Xu, Xiaolan Deng, Jianjun Chen

Department of Systems Biology and Center for RAN Biology and Therapeutics, Beckman Research Institute of City of Hope, Duarte, CA

Natural killer (NK) cell-based immunotherapy is emerging to be a promising strategy to treat cancers due to its high safety profile. Compared with traditional chimeric antigen receptor (CAR) T therapy, NK cell-based therapy has much lower side effects such as cytokine release syndrome (CRS) or neurotoxicity. However, the limited persistence and lower levels of infiltration to the tumor microenvironment has impeded the outcomes for NK cell-based clinical trials. How to genetically engineer NK cells to a better priming activation state has yet to be explored. Here, we identify ALKBH5, a well-established RNA N6-methyladenosine (m^6A) demethylase, as a key regulator that restricts NK cells cytotoxicity. Xenograft model studies using B16F10 and MC38 tumor cells demonstrated that tumor progression was dramatically inhibited upon Alkbh5 knockout ($Alkbh5^{-/-}$) in the recipient mice. Single-cell RNA sequencing (scRNA-seq) revealed the enriched abundance of NK cells among tumor-infiltrating lymphocytes in $Alkbh5^{-/-}$ mice. Also, NK cells extracted from $Alkbh5^{-/-}$ spleen are hyperactivated, exhibiting enhanced cytotoxic function and proliferation rate, facilitating robust tumor suppression. Using MeRIP-seq, RIP-seq and lipid metabolomics, we demonstrated that Alkbh5 deficiency in NK cells reprogramed lipid metabolism pathway, enhancing the conjugation rate between NK cells and tumor cells. This increased synapse formation leaded to elevated release of perforin and granzyme, further improving NK cell killing capacity. Based on these findings, we genetically reprogrammed NK cells by conjugating anti-NK1.1 antibodies to lipid nanoparticles (LNPs) embedding Alkbh5-targeting oligonucleotides. Using the in vivo LNPs target delivery system, we want to explore whether this NK-based immunotherapy could inhibit tumor growth/progression in mice. Altogether, our findings uncover a previously unrecognized role for ALKBH5 in regulating NK cell cytotoxicity and proliferation, providing a new therapeutic strategy by targeting ALKBH5 in NK cell-based immunotherapies for solid tumors.

#3287 TOX modulates methionine availability and enhances CD8$^+$ T cell anti-tumor function in colorectal cancer.

Yujie Chang, Zhao Hui

Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China

Colorectal cancers (CRC) with high TOX expression exhibit greater infiltration of CD8$^+$ T cells, which display stronger tumor-killing capacity. This study investigates the metabolic mechanisms underlying the role of TOX in shaping the tumor microenvironment (TME) and its impact on CD8$^+$ T cell function. Using LC-MS/MS metabolomics, we found that methionine and its metabolites are enriched in the TME of TOX-high CRC tumors. Further analysis revealed that TOX-high tumor cells express lower levels of the methionine transporter SLC43A2, reducing their methionine uptake. This methionine pool supports CD8$^+$ T cell cytotoxicity and decreases apoptosis. In TOX-low tumors, we tested intratumoral methionine supplementation to assess its effect on tumor growth and CD8$^+$ T cell activity. Supplementing methionine in TOX-low CRC tumors led to a significant reduction in tumor size and an increase in the proportion and activity of infiltrating CD8$^+$ T cells. These results indicate that TOX modulates methionine metabolism by downregulating SLC43A2 in tumor cells, reducing competition with CD8$^+$ T cells and enhancing their anti-tumor function. Our findings highlight TOX as a critical regulator of tumor metabolism and immune cell function, with potential as a therapeutic target for both immunotherapy and metabolic intervention strategies in CRC.

#3288 Wildtype IDH1 blockade as a strategy to modulate the tumor microenvironment and boost immune responses in pancreatic cancer.

Priyashree Sunita[1], Soubhi Tahhan[1], Mehrdad Zarei[1], Christina S. Boutros[1], Omid hajjihassani[1], Hallie J Graor[1], Faith Nakazzi[1], Parnian Naji[1], Claudia Marcela Diaz[2], Jordan M Winter[1]

[1]Case Comprehensive Cancer Centre, Department of Surgery, Case Western Reserve University, Cleveland, OH,[2]Department of immunology, Cleveland Clinic, Cleveland, OH

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive malignancy characterized by a dense tumor stroma and a five-year survival rate of less than 13%. Its poor prognosis arises from rapid progression, early metastasis, and resistance to therapy. Despite, advancements in chemotherapy and immunotherapy, outcomes remain dismal, largely due to the immunosuppressive tumor microenvironment (TME). Previous work in our lab demonstrated the efficacy of ivosidenib, an FDA-approved inhibitor of mutant isocitrate dehydrogenase 1 (IDH1), also inhibits wild-type IDH1(wtIDH1) under conditions mimicking the PDAC TME. Here we show that targeting wtIDH1 suppresses cancer cell proliferation by inducing apoptosis, increasing reactive oxygen species (ROS) levels, and enhancing antitumor immune responses by reducing immunosuppressive immune cell populations. In vitro studies were conducted using Miapaca-2, Panc-1 and KPC cell lines. Cells were cultured in low glucose (2.5mM) and low magnesium (0.08mM) media to mimic the TME. Cell viability was assessed using Titer-Glow, Pico Green and colony formation assays. Cellular and mitochondrial ROS levels were determined via DCFDA and Mito-SOX assays using colorimetric and flow cytometry analysis. The therapeutic potential of ivosidenib, was evaluated through cell proliferation (Cell Trace assay), cell cycle arrest (BrdU) assay via flow cytometry and immunofluorescence, and apoptosis detection (Annexin-V/PI staining). In vivo studies employed orthotopic and autochthonous murine models of PDAC to assess drug efficacy and its impact on immune cell infiltration in the TME. Our data revealed that targeting wtIDH1 suppress cell proliferation and DNA synthesis and elevated cellular and mitochondrial ROS levels leading to increased apoptosis. In orthotropic pancreatic murine models of pancreatic cancer, ivosidenib treatment significantly increased the infiltration of immune cells ($CD45^+$), and $CD4^+$ T cells in particular, including naïve T (CD45RA) and central memory T cells (CD45RO). Additionally CD127 expression was enhanced, promoting the survival and proliferation of naïve and memory T cells, potentially boosting anti-tumor immunity. Notably, treatment led to reduction of anti-inflammatory M2-like monocytes and adaptive immunity associated $CD8^+$T cells, suggesting reshaped immune landscape favoring antitumor responses. Wild-type IDH1 plays a critical role in tumorigenesis and chemoresistance in pancreatic cancer. Our data demonstrates that IDH1 inhibition not only reduces cancer cell proliferation in vitro but also
remodels the TME in in vivo, enhancing immune responsiveness. Modulation of the TME via IDH1 inhibition offers a promising avenue to improve efficacy of existing therapies, including chemotherapy and immune checkpoint inhibitors as a part of combination treatment strategies.

#3289 Radiation supports the polarization of M1 macrophages against triple-negative breast cancer cells via the IKZF1 - CCL5 axis.

Kuo Han-Hsi, Yu-Chan Chang

National Yang Ming Chiao Tung University, Taipei, Taiwan

Triple-negative breast cancer (TNBC), known for its therapeutic challenges and high metastatic potential, is commonly treated with ionizing radiation (IR). In the tumor microenvironment (TME) of TNBC, macrophages occupy a place and are reprogrammed into two main types, M1 and M2, in response to different stimuli such as IR or various cytokines. However, the detailed molecular mechanism remains to be elucidated. Using our cell model, we applied IR dose-dependently (0.5/8 Gy) to observe macrophage polarization and found that high-dose IR could increase the proportion of M1 macrophages. These trends are consistent with polarization by LPS (M1) and IL-4/IL-13 (M2), respectively. By integrating transcriptomics and cytokine array profiling, we demonstrated that IKAROS family zinc finger 1 (IKZF1), a transcription factor involved in DNA repair, downregulates secretion of the downstream target, CCL5, in macrophages under high-dose IR exposure. In the collected conditioned medium, the high-dose IR group could significantly inhibit the migration and proliferation of TNBC cells. Even, recombinant CCL5 protein was recruited to modulate the TME of TNBC, and the migration ability of TNBC cells was reversed. In the direct co-culture system, phagocytic event was enhanced by high-dose IR. In conclusion, under high-dose irradiation conditions, IKZF1 is inhibited and CCL5 secretion is reduced. The TME environment is conducive to polarization of the M1 subtype and enhances its ability to phagocytose TNBC cells, thereby inhibiting TNBC progression. This finding suggests that the benefits of radiotherapy in TNBC may also be linked to a new chapter in immunotherapy.

#3290 Targeting CREB reprograms the tumor immune microenvironment and enhances immunotherapy efficacy in smoking-associated pancreatic cancer.

Varunkumar Krishnamoorthy, Siddharth Mehra, Sudhakar Jinka, Haleh Amirian, Edmond Box, Chen Zheng, Jashodeep Datta, Nipun Merchant, Nagaraj Nagathihalli

Department of Surgery, University of Miami Miller School of Medicine, Sylvester Comprehensive Cancer Center, Miami, FL

Background: Smoking, a major risk factor for pancreatic cancer, significantly attenuates the survival benefits of standard therapies, contributing to poor clinical outcomes in this malignancy. Previously, we have identified that hyperactivation of cyclic AMP response element-binding protein (CREB) as driver of immunosuppressive tumor immune microenvironment (TME), which limits the therapeutic potential of immune checkpoint blockade (ICB) therapies. The present study explores the therapeutic strategy of targeting CREB to overcome TME associated immunosuppression and to enhance the efficacy ICB in smoking associated preclinical models of pancreatic cancer.

Methods: An *in vivo* smoking model was developed using syngeneic orthotopic tumor implantation in C57BL/6 mice and genetically engineered mouse model (GEMM) of pancreatic cancer, including *Creb* deleted ($Creb^{fl/fl}$, CREBcKO) $LSL\text{-}Kras^{G12D/+}$; $Trp53^{R172H/+}$; $Pdx1^{Cre/+}$ (KPC) mice. CRISPR/Cas9 technology was used to generate *Creb* knockout ($Creb^{KO}$) in KPC tumor cells, which were orthotopically implanted into the pancreata of C57BL/6 mice. Mice with established tumors were randomized and treated with CREB inhibitor (CREBi, 666-15) alone or in combination with anti-PD-1 monoclonal antibody. This combination therapy was further evaluated in $Ptf1a^{Cre/+}$; $LSL\text{-}Kras^{G12D/+}$;$Tgfbr2^{flox/flox}$ (PKT) GEMM. Multiplex immunophenotyping was performed on harvested tumors to assess immune subsets changes, along with histological analyses including H&E, immunohistochemistry (IHC) and Sirius red staining.

Results: Smoking-exposed KPC GEMM mice showed accelerated PDAC progression, characterized by increased high-grade PanIN lesions and an enhanced fibroinflammatory TME. CREB deletion (CREBcKO) significantly attenuated these effects, highlighting its role in smoking-induced tumorigenesis. Genetic ablation or pharmacological inhibition of CREB using 666-15 attenuated the immunosuppressive TME, promoted effector $CD4^+$ and $CD8^+$ T cell infiltration with elevated PD-1 expression on T cells. In combination with anti-PD-1 therapy, CREB inhibition enhanced the anti-tumor benefits in both PKT GEMMs and in KPC orthotopic models. Similarly, $Creb^{KO}$ tumor cells implanted in smoke-exposed C57BL/6 mice showed significant tumor suppression and modulates TME, with or without anti-PD-1 therapy.

Conclusions: This study demonstrates the therapeutic advantage of targeting CREB to reprogram the stromal immune microenvironment and improve the efficacy of immunotherapy in preclinical models of smoking-associated pancreatic cancer. These findings provide a strong rationale of integrating CREB inhibition into immunotherapeutic strategies for pancreatic cancer, particularly in high-risk smoking populations.

#3291 Development of anti-B7H3 CAR T cells for treatment of osteosarcoma in a canine model.

Theresa Higgins, Payal Agarwal

Auburn University, Auburn, AL

Characterized as a highly aggressive and metastatic solid bone tumor, osteosarcoma (OS) remains a difficult to treat cancer. Even with the current treatment options of chemotherapy, radiation, and tumor removal surgery there is poor patient prognosis and low survival rates, highlighting the need for novel treatments. Over the last decade, chimeric antigen receptor (CAR) T cells have shown promising results in the treatment of certain leukemias and lymphomas and are further being explored in solid tumor types. We aim to develop CAR T cells against the immune checkpoint cell surface receptor B7H3 (anti-B7H3 CAR T cells), which is over expressed in several cancers, including OS. We hypothesize that anti-B7H3 CAR T cells will be able to successfully lyse osteosarcoma in a canine model. We have verified that B7H3 is expressed and demonstrates significant antibody binding in four canine OS cell lines (D17, CF11, MCKOS, and D22). We have successfully isolated and activated T cells by incubating in CD3 and CD28 coated plates. We will transduce active T cells with anti-B7H3 encoding lentivirus. The cytotoxic effects of anti-B7H3 CAR T cells will be explored in both two-dimensional (2D) and three-dimensional (3D) cell cultures. Additionally, IFNγ, TNFα, and IL-2 cytokine levels will be measured and analyzed. We expect cell lysis and increased cytokine levels post-CAR T therapy in our canine OS cell lines.

#3292 Unraveling molecular mechanisms underlying the awakening of dormant melanomas.

Ianca R. Dias[1], Debora K. Alves-Fernandes[1], Lih Hongtao[2], Frank J. Slack[3], Gangning Liang[2], **Miriam G. Jasiulionis**[1]

[1]Pharmacology, Federal University of Sao Paulo (UNIFESP), Sao Paulo, Brazil,[2]Urology, University of Southern California, Los Angeles, CA,[3]Pathology, Harvard Medical School, Boston, MA

Tumor recurrence, which can occur many years after therapeutic intervention, represents the main challenge in clinical oncology. Clinically, this period between the "cure" of the primary tumor and its local or distal metastatic relapse is known as the "dormancy state." Tumor dormancy has been linked to therapeutic resistance and a high metastatic potential of malignant neoplasms. During tumor immunoediting, the immune response shapes the tumor to adapt and thrive within an immunocompetent host. Epigenetic modulation is an important way by which cells can quickly regulate gene expression and respond to environmental pressures. Another way of rapid modulation can be through regulatory RNAs, such as lncRNAs which can interact at the level of transcription and translation of other molecules, such as protein-coding genes, and regulate gene expression. To better understand the molecular basis of this tumor dormancy and awakening, we established a murine immune-mediated model of awakening of dormant melanoma cells. In this model, a large number of apoptotic cells are used to generate an acute inflammation process in C57BL/6 mice that promotes a permissive environment for a sub-tumorigenic dose of viable metastatic melanoma cells grown into a fully formed tumor, that would not be formed otherwise. Our study shows that the sub-tumorigenic dose of viable cells only generates tumors in immunosuppressed (NSG) mice (100% of animals), indicating that in immunocompetent animals, the primary defense system - T and B lymphocytes, and NK cells - appears to contain it. Considering the role of epigenetic modulation in rapid cell response to the pressure of the environment, we also show that treatment with the global demethylating agent 5-aza-2'-deoxycytidine causes delay and reduction of tumor mainly through immune activation. This suggests the need for future investigations regarding the epigenetic machinery in our model. Thereby, genome-wide DNA methylome analysis was conducted (EPICarray), and differential gene expression (RNA-seq) analysis of tumors from animals treated (or not) with the demethylating agent. Differentially expressed genes are being selected for further *in vitro* and *in vivo* studies. To elucidate this model, we are carrying on assays to understand the role of acute inflammation in the awakening of melanoma dormancy by analyzing the inflammatory profile of the defense system using flow cytometry meanwhile we are conducting other *in vivo* experiments to better characterize our model. Now, we investigated lncRNAs that are differentially expressed in tumors of animals treated with that drug aiming for new potential biomarkers, or regulators of important immune-response protein-coding genes. All *in vivo* data were statistically validated using the Student's t-test. We aim to discover potential therapeutic targets with a functional role in the immune-mediated progression of melanoma. Supported by CNPq, CAPES, FAPESP

#3293 Exploiting PEGylation of CCN5 for tumor immunity and therapy of triple-negative breast cancer: A new and effective approach for TNBC therapy.

Sunil P. Upadhyay[1], Axel H. Breier[2], Inamul Haque[2], Archana De[2], Snigdha Banerjee[3], Mohiuddin Quadir[4], Sushanta K. Banerjee[5]

[1]Cancer Research Unit, Kansas City VA Medical Center, Kansas City, MO,[2]Kansas City VA Medical Center, Kansas City, MO,[3]Pathology and cancer Research unit, University of Kansas Medical Center and Kansas City VA Medical Center, Kansas City, MO,[4]Coatings and Polymeric Materials, North Dakota State University, Fargo, ND,[5]University of Kansas Medical Center, Kansas City, MO

Triple-negative breast cancer (TNBC) is defined by a lack of expressions of three receptors. These include estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor rector 2 (HER2). TNBC accounts for ~15-17% of all breast cancers (BC) and is the most aggressive subtype of metastatic BC. TNBC incidence is high among BRCA1 mutation carriers, women with the age of 50 or below, and African American descent. The therapeutic opportunities for TNBC are limited to chemotherapy as targeted options are deficient, resulting in elevated risk of recurrence and mortality risk. Over the last five years, new strategies for treating TNBC have transformed significantly by introducing PD-1/PD-L1-immune checkpoint inhibitors (ICIs) and others. However, only eighteen-20% of PD-L1 positive TNBC patients with metastasis respond to ICIs with or without chemotherapy and enhance the antitumor response of therapeutic CD8+ T cells. The low overall response rate of ICIs in TNBC patients is due to insufficient PD-L1/PD-1 interaction blockade. Consequently, an unmet need exists to revolutionize treatment regimens by replacing or modifying them with ones that are more effective and less toxic. Previous studies demonstrate that CCN5 (also known as WISP-2), which is family of cellular communication Network (CCN) factors, is considered a tumor suppressor and anti-invasive protein as it promotes the growth arrest and progression of TNBC cells. Our previous studies showed that recombinant CCN5 (hrCCN5) therapy is weak for systemic delivery due to poor bioavailability. Thus, only intratumoral injection of CCN5 was considered and found effective with no toxicity in animal studies, but this is not an ideal therapeutic strategy for progressive BC therapy. To overcome this limitation, we implemented the protein PEGylation/PEG conjugation technique that increased the bioavailability and activities of CCN5 and reverses tumor growth and metastasis and increases prolonged overall survival in an MDA-MB-231tumor xenograft model. Further, we found that ^{PEG}CCN5 significantly increased CD8+ T cells decreased myeloid infiltration and PD-L1 expression in tumor cells. Thus, we consider ^{PEG}CCN5 can emerge as a new, beneficial immunotherapeutic drug for TNBC treatment alone or in combination with αPD-1, chemotherapy, or both.

#3294 Subtype-specific genetic drivers of immune evasion in breast cancer.

Otilia Menyhart, **Balazs Gyorffy**

Semmelweis University, Budapest, Hungary

Introduction: Immune evasion is a hallmark of cancer and a significant driver of therapeutic resistance. While immunotherapy has shown promise in highly immunogenic cancers, its efficacy in breast cancer (BC) remains limited, particularly in less immunogenic subtypes. We aimed to investigate the prognostic relevance of two immune-related gene signatures—106 genes associated with evasion of immune destruction (EID) and 182 genes linked to evasion of killing by cytotoxic T lymphocytes (ECTL)—across distinct BC subtypes.

Methods: Transcriptomic and clinical data from three integrated datasets (GEO, n=1,965; TCGA, n=1,069; and GSE96058, n=2,976) were analyzed. Expression of immune evasion-related genes was stratified by BC subtype using PAM50 classification. Survival analysis was conducted using Kaplan-Meier curves and Cox regression models, stratifying gene expression into high- and low-expression groups. A False Discovery Rate (FDR) correction was applied to ensure statistical robustness. Subtype-specific survival associations were further evaluated with individual gene-level analyses.

Results: High mean expression of the ECTL signature was significantly associated with improved overall survival (OS) in triple-negative BC (TNBC) patients (HR = 0.25, 95% CI = 0.16-0.4, p = 8.6e−10, FDR < 1%). Similar trends were observed in HER2-enriched BC but were less pronounced after FDR correction. For the 106 EID genes, high mean expression correlated with favorable OS in TNBC (HR = 0.3, 95% CI = 0.18-0.49, p = 3.4e−7). Gene-level analysis identified essential immune evasion genes, including *CXCL10*, *CXCL9*, *CXCR4*, and *JAK3*, as robust predictors of survival in TNBC, validated across independent datasets. The new, combined four-gene signature showed significant predictive power for response to immune checkpoint inhibitors (ICI), with an AUC of 0.722 (p = 1.3e−7).

Conclusions: Immune evasion-related gene signatures exhibit subtype-specific prognostic value, particularly in TNBC and HER2-enriched BC. A signature based on genes within these signatures represent potential biomarkers for predicting survival outcomes and guiding immunotherapy.

CLINICAL RESEARCH: Predictive Biomarkers 4
Poster Session

#3298 LRP12 promotes triple-negative breast cancer metastasis via recruiting the activation of β-catenin and PI3K/AKT signaling pathways.

Kung-Ju Chen, Yuan-Feng Lin

Graduate Institute of Clinical Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan

Background: Triple-negative breast cancer (TNBC) metastasis is the major cause of failure in TNBC therapy. The challenges in treating TNBC may stem from their heightened cellular plasticity, and characterized by significant heterogeneity at the molecular level. As a result, understanding the molecular mechanism for TNBC metastasis is urgently needed for the precise management of TNBC clinically. Low-density lipoprotein receptor-related proteins (LRPs) are the multifunctional transmembrane proteins involved in receptor endocytosis and signal transduction and associated with tumor progression. The aim of this study was made to delineate the role of LRP12 in the metastatic progression of TNBC.
Methods: The transcriptional profile of normal tissues and primary tumors derived from TNBC patients obtained from The Cancer Genome Atlas (TCGA) database was used to examine the expression of LRPs. Kaplan-Meier analysis was performed to estimate the prognostic significance of LPRs. Trans-well migration and lung colony-forming assays were established to evaluate the metastatic potentials of TNBC cell variants. RNA-sequencing was employed to generate differentially expressed genes (DEGs) followed by the prediction of possibly activated pathways by pathway enrichment analysis after LRP12 knockdown in the tested TNBC cells. Cycloheximide-chased assay was used to measure the degradation of β-catenin in response to LRP12 knockdown in TNBC cells.
Results: Transcriptional profiling and Kaplan-Meier analysis demonstrated that LRP12 upregulation is commonly found in primary tumors compared the normal adjacent tissues and highly associates with the poor prognosis in TNBC patients. LRP12 knockdown predominantly suppressed the cellular migration and lung colony-forming abilities. Pathway enrichment analysis against DEG result from RNA-sequencing indicated that LRP12 knockdown dramatically suppresses the activity of β-catenin and PI3K/AKT signaling pathways in Hs578T cells. Western
blot analysis conformed that LRP12 knockdown predominantly reduced the phosphorylated/active β-catenin and PI3K/AKT protein in Hs578T cells. Cycloheximide-chased assay further showed that LRP12 knockdown significantly accelerates the protein degradation of β-catenin in Hs578T cells.
Conclusion: These findings highlight the potential role of LRP12 as a prognostic biomarker but also a therapeutic target in metastatic TNBC.

#3299 Anti-FRα antibody-drug conjugate luveltamab tazevibulin for the treatment of FRα-expressing non-small cell lung cancer.

Robert Yuan, Andrew McGeehan, Sihong Zhou, Mark Armanini, Jennifer Smith, Millicent Embry, Rhoneil Pena, Danielle K. Lewis, Krishna Bajjuri, Cuong Tran, Gang Yin, Xiaofan Li, Hanspeter Gerber, Alice Yam, Helena Kiefel, **Genevive Hernandez**

Sutro Biopharma, Inc., South San Francisco, CA

Luveltamab tazevibulin (luvelta; STRO-002) is a novel antibody-drug conjugate (ADC) designed to target folate receptor alpha (FRα)-positive cancers across a broad range of expression levels. It is composed of an anti-FRα antibody conjugated to a tubulin-directed hemiasterlin payload via a protease-cleavable linker. The linker-payload is site-specifically conjugated via non-natural amino acids incorporated into the antibody sequence using Sutro's Xpress CF+® system, yielding a homogenous ADC with a drug-antibody ratio of four (DAR4). Preliminary results from Phase 1 and 2/3 studies in endometrial and ovarian cancer, respectively, show robust clinical activity and enhanced tolerability of luvelta as a single agent and in combination with bevacizumab. Here, we report preclinical data to support therapeutic targeting of advanced non-small cell lung cancer (NSCLC), for which there remains an unmet medical need, with luvelta.

IHC staining of NSCLC patient tumor microarrays shows a prevalence of 48% and 14% FRα-positive tumors in non-squamous and squamous NSCLC tumors, respectively, as defined by ≥1% tumor cells with FRα membrane staining at any intensity. In NSCLC patient-derived xenograft (PDX) models, luvelta treatment resulted in robust tumor growth inhibition, with best responses strongly correlating with FRα levels. The majority of PDX models evaluated were EGFR wild type, but responses were also observed in some EGFR mutant models.

An existing standard-of-care option for NSCLC is immunotherapy. ADCs and chemotherapeutic drugs have been shown to promote an inflamed tumor environment by triggering immunogenic cell death (ICD), which can synergize with immune checkpoint blockade. We provide evidence that luvelta is a potent inducer of ICD. In *in vitro* assays, luvelta induced all three hallmarks of ICD: HMGB1 release, ATP release, and surface expression of calreticulin. Furthermore, in *in vivo* vaccination studies, injection of luvelta-treated tumor cells established protective immunity against subsequent tumor challenge. Consistent with ICD induction, luvelta treatment in combination with anti-PD-1 or anti-PD-L1 immune checkpoint inhibitors resulted in enhanced *in vivo* efficacy in syngeneic mouse tumor models. Luvelta treatment in tumor-bearing mice also altered tumor immune cell infiltration and activation, demonstrating its ability to modulate the tumor immune microenvironment.

In conclusion, the work presented here provides support for luvelta as a promising targeted therapy for NSCLC, either as monotherapy or in combination with immune checkpoint blockade. Luvelta is now being evaluated in a Phase 2 study in patients with advanced or metastatic NSCLC (NCT06555263).

#3300 A longitudinal, circulating tumor molecular response biomarker as a predictor of clinical outcomes in a real-world cohort of patients with advanced solid tumors treated with tyrosine kinase inhibitors.

John Guittar[1], Akash Mitra[1], Rossin Erbe[1], Shannon Mulmat[1], Michelle Stein[1], Xifeng Wang[1], Seung Won Hyun[1], Chithra Sangli[1], Halla Nimeiri[1], Rotem Ben-Shachar[1], Wade T. Iams[2], Charu Aggarwal[3]

[1]Tempus AI, Inc., Chicago, IL,[2]Tennessee Oncology, Nashville, TN,[3]University of Pennsylvania, Philadelpha, PA

Studies have shown that early changes in quantitative measures of circulating tumor DNA (ctDNA) in response to immunotherapy can predict clinical outcomes and may be useful for identifying molecular responders (MRs) and molecular non-responders (nMRs) who may benefit from a change in therapy. Yet few studies have evaluated treatment with tyrosine kinase inhibitors (TKis).

The cohort consisted of deidentified patients from the Tempus clinicogenomic database with stage IV solid tumors who underwent ctDNA NGS. Patients who had a pre-treatment baseline liquid biopsy (T0) and a longitudinal sample 21-180 days after initiating TKi therapy (T1) were included. TKis were classified by the Tempus Medical Ontology, which was validated using the NCI Metathesaurus TKi concept (NCI Code C1967). xM for treatment response monitoring estimates tumor fraction (TF) via an ensemble algorithm that incorporates pathogenic variant allele frequencies, copy number information, and germline information. Evaluable patients had at least one blood sample with a TF ≥ limit of blank of 0.09%. MR was quantified as a ≥ 50% decrease in TF from T0 to T1. Patients with consistently low TF < 1% at both T0 and T1 were also classified as MRs given previous data demonstrating that baseline low TF is correlated with prolonged clinical outcomes. All other patients were classified as nMRs.

Real-world overall survival (rwOS) was defined from T1 to death or last known clinical record in event-free patients. Stratified Cox proportional hazards models were used to estimate the hazard ratio (HR) for MR status (MR vs. nMR), stratified by line of therapy (LOT, first line, 1L vs. ≥ second-line, 2L+). A 1-sided Wald test was conducted using a 5% significance level. Predicted rwOS estimates were obtained from the Cox models.

The evaluable cohort consisted of 109 patients (1L=62, 57%, 2L+=47, 43%). Cancer types included non-small cell lung cancer (NSCLC, n=52, 48%), breast cancer (BC, n=35, 32%), and 10 other cancer types each comprising < 5% of the patient cohort. The most common therapies were osimertinib in NSCLC patients (n=34) and alpelisib + fulvestrant in BC patients (n=11). Median time from the start of TKi therapy to T1 was 93 days and median follow-up was 10.3 months. Sixty patients had MR and 49 patients were classified as nMRs.

MR was significantly associated with longer rwOS than nMRs (HR = 0.45; P = 0.006). Median predicted rwOS among MRs receiving TKis in 1L (n=43) was 28.4 months vs. 16.5 months for nMRs (n = 19). The HR for death between MR vs nMR was consistent across different cancer subgroups and LOT: HR=0.38 for NSCLC patients, HR=0.57 for BC patients, HR=0.46 for 1L patients and HR=0.44 for 2L+ patients.

These data demonstrate that longitudinal molecular treatment response can stratify rw-outcomes to TKi therapy in a heterogeneous, real-world pan cancer cohort.

#3301 A sequential treatment strategy for ex vivo profiling of live tumor fragments that mitigates tumor heterogeneity and tissue scarcity from core needle biopsies to characterize response to immunotherapies.

Christina Scribano[1], Pichet Adstamongkonkul[1], **Sean Caenepeel**[2], Nicholas Dana[1], Jackie Derrick[1], Evan Flietner[1], Hilary Hernan[1], Christin Johnson[1], Nathan Marhefke[1], Payton McDonnell[1], Betsy Mulligan[1], Amreen Nasreen[1], Josh Porter[1], Jordyn Richardson[1], Sidney Schneider[1], Mikaela Schultz[1], Mike Smith[1], Chetan Sood[1], Aishwarya Sunil[1], Lindsey Vedder[1], Ellen Wargowski[1], Hinco Gierman[1], Andreas Friedl[3], Laura Hrycyniak[1], Michael Korrer[1], T.S. Ramasubramanian[1]

[1]Elephas, Madison, WI,[2]Elephas, Thousand Oaks, CA,[3]University of Wisconsin, Madison, WI

Immunotherapies are highly effective treatment options for cancer; yet they do not work in all patients, and current companion diagnostic (CDx) biomarkers do not always align with response. Ex vivo profiling of live tumor samples provides the ability to measure T cell activity in response to treatment and is a potential means of improving response prediction. The limited amount of tumor tissue available from core needle biopsies (CNBs) presents a challenge when split into control and treatment groups for ex vivo profiling, as tumor heterogeneity causes increased variability and sampling errors. Elephas has developed a treatment strategy where control (IgG) and immune checkpoint inhibitor (ICI) treatments are performed sequentially on the same tissue within a single well. Changes in the rate of cytokine secretion are then compared between ICI and IgG treatment to characterize response to therapy. For these experiments, human CNBs (n>150) were collected from diverse tumor types during standard of care diagnostic procedures and sliced using a proprietary cutting instrument. Each biopsy was profiled for response to ICI using a multiplexed cytokine immunoassay. Sequential aliquots of conditioned media were collected from cultured biopsy slices at three time points: ~4 hrs post-encapsulation with a proprietary hydrogel, following ~16 hrs of IgG treatment and following ~28 hrs of treatment with ICI. Cytokine results were assessed for data quality to inform inclusion/exclusion of data points for analysis/algorithm development. CDx biomarker status (PD-L1, MMR/MSI and TMB) for individual biopsies was obtained from medical records. Our results show live tumor fragments, obtained from resections where tumor tissue amount was sufficient to address inter-well heterogeneity, exhibit comparable cytokine response profiles when stimulated with CD3/CD28 at experiment start vs. following 20 hrs of IgG treatment. An algorithm to define ex vivo response to ICI treatment in CNBs was then developed using tissue viability measurements and treatment-induced changes in cytokines. Response on the Elephas platform was subsequently compared to CDx biomarker status and clinical response from patient medical records. A sequential treatment strategy for the assessment of response to immunotherapy is an effective approach to capture treatment-induced changes in cytokine response. Sequential treatment of CNBs circumvents issues of tumor heterogeneity inherent to cross-well comparison in settings of limited tissue availability. Implementation of this strategy enables the Elephas platform to differentiate potential responder and non-responder CNB specimens to immunotherapy ex vivo.

#3302 Comprehensive analysis of B7-H7/HHLA2 expression in pan-solid tumors and its potential significance in anti-tumor immunity.

Yasmin L. Hashambhoy-Ramsay, Karishma Vekaria, Emilien Loeuillard, Melissa A. W. Hayes, Emma Ya-Ting Wang, Matthew Rausch, Tatiana Novobrantseva, Katalin Kis-Toth, Leena Gandhi

NextPoint Therapeutics, Inc., Cambridge, MA

BACKGROUND: B7-H7/HHLA2 is a B7 family member expressed on select normal epithelium and upregulated in many tumors. B7-H7 suppresses T and NK cell function through an inhibitory receptor, KIR3DL3, and stimulates lymphocyte activation through a distinct receptor, TMIGD2. High levels of B7-H7 expression in tumor cells are frequently associated with poor prognosis and it may represent a novel target for therapeutic interventions. We have previously demonstrated that higher levels of B7-H7 are associated with increased tumor cell killing by a B7-H7-targeting T cell engager. Here, we systematically characterized B7-H7 expression across solid tumor histologies to assess the potential of B7-H7 as a biomarker for use in therapeutic targeting of B7-H7.
METHOD: B7-H7 protein expression was assessed by immunohistochemistry (IHC) using a monoclonal antibody that has been widely used in the literature (566.1). Hundreds of tumors across many indications and select normal tissues were characterized for B7-H7 expression. To develop a B7-H7 IHC assay that could be used for patient selection for B7-H7 targeting therapeutics including NPX887, a monoclonal antibody currently in clinical trials, the performance of 566.1 and a commercially available monoclonal antibody (E1U6X) were compared. Quantitative flow cytometry (qFC) was used to measure the number of molecules per cell across cell lines displaying different levels of B7-H7, and concordance of qFC and IHC measurements by E1U6X was established. To investigate the effects of B7-H7 levels on immunomodulation and cell killing, *in vitro* tumor and immune cell co-culture systems were used.
RESULTS: Tumor cells from colorectal, non-small cell lung, pancreatic, renal and gastric cancer displayed the highest protein levels of B7-H7 and defined patient populations of interest for NPX887. Normal B7-H7 expression was restricted to substructures of intestine and lung and showed only faint cytoplasmic staining in the kidney. There was no detectable B7-H7 expression on any circulating immune cells. During assay development, similar patterns of B7-H7 expression were detected using the two monoclonal antibodies, however the optimized assay using E1U6X displayed superior performance. The levels of B7-H7 expression on tumor samples were comparable to tumor cell lines effectively killed by immune cells in the presence of anti-B7-H7 therapeutic antibodies *in vitro*.
CONCLUSION: We demonstrated that normal tissue expression of B7-H7 is restricted to organ substructures within the intestine, lung, and kidney. We observed significant expression of B7-H7 across tumor cells of varying histologies, highlighting patient populations for B7-H7 targeting therapies. The IHC assay using E1U6X was selected for investigational use in ongoing (NPX887-001; NCT06240728) and potential future clinical studies of therapeutic molecules targeting the B7-H7 axis.

#3303 Standardizing peripheral blood mononuclear cells processing for *ex vivo* evaluations to predict response to immunotherapy.

Katelyn Jansen[1], Farah Sagin[2], Claire Girten[2], Joanna Khoury[2], Tyler Hendrickson[2], Kelsey Dillehay McKillip[2], Dalia El-Gamal[1], Trisha Wise-Draper[1]

[1]Hematology and Oncology, University of Cincinnati College of Medicine, Cincinnati, OH,[2]University of Cincinnati College of Medicine, Cincinnati, OH

In many cancers, including head and neck squamous cell carcinoma (HNSCC), expression of programmed death ligand-1 (PD-L1) is upregulated, which inhibits the anti-tumorigenic immune response of cytotoxic T-cells. Immune checkpoint inhibitors (ICIs) targeting the PD-1/PD-L1 axis has greatly enhanced survival rates in these patients, but many progress and/or relapse due to other immunosuppressive factors. Therefore, predicting patient response to current ICIs and identifying new biomarkers is imperative to advance tailored therapeutics. The current "gold standard" for diagnosing and assessing treatment response and tumor progression is tumor biopsy, however, it is not always feasible, safe, or sufficient for additional molecular testing. While research into alternative methods such as circulating tumor cells (CTCs) are ongoing, these methods are expensive and lack standardization. As a result, our laboratory is investigating a liquid biopsy approach utilizing peripheral blood mononuclear cells (PBMCs) as a surrogate for tumor biopsy, as longitudinal peripheral blood sampling of patients is safe and more readily available in patients before, during, and following treatment. Previous work has demonstrated that preanalytical variables affect PBMC viability and suitability for functional assays. The purpose of our work so far has been to elucidate the optimal collection tube condition and timing of blood processing to standardize the collection and mitigate pre-analytical bias of PBMC processing for downstream critical functional analyses. Peripheral blood samples from eligible HNC patients were collected in EDTA or sodium heparin (SH) tubes and either processed for isolation of PBMCs immediately or at delayed timepoints (8- and 24-hours post-collection) at room temperature (22-30°C) to account for same-day and overnight processing delays. Additionally, the delayed (8 and 24 hour) timepoint samples were diluted 1:1 with sterile PBS immediately following collection and compared to matched undiluted specimens to assess the effects of granulocyte contamination on PBMC viability and functionality following cryopreservation. Functional tests included cytotoxicity assays with patient-derived CD8+ T-cells or NK cells that were co-cultured with HN5 target tumor cells at a 1:5 target to effector cell ratio and stained with 7AAD Viability Solution for flow cytometry analysis. NK or T cell-killed HNC cells are calculated by dividing the percent dead (7AAD-positive) in co-culture and the basal cell death. Preliminary findings suggest that dilution enhances cell recovery but may slightly reduce NK cell cytotoxicity, while the blood collection tube type has no significant influence on recovery or cytotoxicity. Although this research is focused on one solid tumor class, our rigorous approach and findings could be readily expanded to other solid tumors.

#3304 HCAR2 activation limits tumor growth by the activation of p53 signaling in breast cancer.

SONIA BATAN, Nanditi Thangaraju, Jabuneesa Khanom, Kunal Kumar, Snigdha Ganjikunta, Subash Sundaram, Nikhil Patel, Santhakumar Manicassamy, Puttur D. Prasad, Muthusamy Thangaraju

Augusta University, Augusta, GA

Breast cancer (BC) is the most common cause of cancer-related death in women. BC affects ~2.3 million women worldwide, with ~685,000 deaths every year. Therefore, a clear understanding of the molecular mechanism(s) associated with BC formation and the development of more effective therapeutic strategies for the prevention and treatment of this disease are sorely needed. The p53 tumor suppressor protein, encoded by the TP53 gene, prevents multicellular organisms from developing cancer. However, p53 mutations occur in almost all tumors, emphasizing its role as a formidable barrier that must be breached to allow oncogenic transformation. *p53* is the most frequently mutated gene in BC, especially in triple-negative breast cancer (TNBC). Although *p53* mutation has been seen in 18-25% of primary BC, about 80% of TNBC is associated with *p53* mutation or inactivation. p53 is a multi-functional protein that controls the cell cycle, cell proliferation, DNA repair, senescence, and apoptosis, thereby blocking the proliferation of damaged cells and preventing tumor formation. Therefore, the development of therapeutic strategies that will rejuvenate wild-type p53 (*WTp53*) function or target mutant *p53* (*p53α*) should have a high clinical impact on the prevention and treatment of BC. We found that HCAR2 (Hydroxycarboxylic acid receptor 2) or NIACR1 (Niacin receptor 1) or HM74A or GPR109A, a G-protein coupled receptor for the vitamin B3 Niacin (NA), the bacterial fermentation product butyrate (BTR), and the ketone body β-hydroxybutyrate (BHB), activates WTp53 and also destabilizes *p53*α in BC cells. p53 is an NAD^+-dependent molecule, and deficiency of NA, the precursor of NAD^+ and HCAR2 agonist impairs p53 function. NA- and BTR-induced HCAR2 activation can enhance p53 function by decreasing cAMP, thereby attenuating the binding of MDM2 to p53 and the resulting proteasomal degradation and inhibiting SIRT1, a p53 deacetylase. Similarly, BHB, an HCAR2 agonist and the primary ketone body in mammals, potentiates p53 function by increasing p53 acetylation. Further, HCAR2 activation induces cytotoxicity in *p53*α BC cells via inhibition of HSP90-HDAC chaperone, which stabilizes the transcription of p53α in the mammary epithelium**.** Therefore, HCAR2 activation should have a high clinical translational potential for the prevention and treatment of BC by rejuvenating *WTp53* and destabilizing p53α in BC cells. Overall, our study provides preclinical evidence to show the functional implication of ketogenic signaling in the regulation of p53 and its relevance to BC prevention and treatment.

#3305 Notch mutations in subjects with recurrent or metastatic adenoid cystic carcinoma treated with rivoceranib from study RM-202.

Ari Rosenberg[1], James Bugni[2], Lissa Nazal[2], Chris Galloway[2], Kristin Ryan[2]

[1]Department of Medicine, Hematology and Oncology, University of Chicago, Chicago, IL,[2]Elevar Therapeutics, Fort Lee, NJ

Background: Activating *NOTCH* mutations are present in approximately 20% of metastatic adenoid cystic carcinomas (ACC) leading to an aggressive tumor phenotype and poor clinical prognosis, yet less common pathogenic *NOTCH* mutations are poorly characterized. RM-202 was an international, single-arm, open-label phase 2 trial of oral VEGFR-2 tyrosine kinase inhibitor, rivoceranib, in patients with histologically confirmed recurrent or metastatic (R/M) ACC (Hanna *et al.* 2023 Clin Cancer Res 29(22): 4555). Herein, we report the mutational analysis *NOTCH* genes 1-4 *(NOTCH1-4)* in a subset of ACC samples from RM-202.
Methods: Patients (n=72) enrolled in RM-202 received oral rivoceranib 700 mg once daily for a 28-day cycle. The co-primary endpoints were objective response rate (ORR) assessed by both investigator and a blinded independent review committee (BIRC). RNA seq was performed on ACC tumor samples from 27 patients. Complete coding sequences were analyzed with *NOTCH1-4* variants identified and cross-referenced with Tempus and public databases to categorize mutations according to pathogenicity.
Results: The investigator and BIRC assessed objective response rates were 15.3% (95%CI, 7.9-25.7) and 9.7% (95% CI, 4.0-19.0), respectively. The median duration of response was 14.9 months (95% CI, 4.9-17.3) and median progression free survival was 9.0 months (95% CI, 7.3-11.5). Grade 3 treatment related adverse events occurred in 70% of patients, with hypertension (42.5%) and stomatitis (7.5%) being the most common. A total of 477 variants were identified in *NOTCH1-4* from 27 RM-202 ACC tumor samples, of which 463 (97%) mutants had been previously reported by Tempus DNA sequencing of germline and somatic variants across multiple tumor types. Three pathogenic *NOTCH1* mutations (two nonsense mutations and 1 in-frame deletion) were identified across 2 tumor samples. Two of these mutations had not been previously reported in ACC. Forty-five additional non-synonymous variants were identified in *NOTCH1-4*. Of those, *NOTCH4* L16 mutations occurred in 17/27 (63%) of tumors, with 12/27 (44%) tumors harboring L16 deletions, 4/27 (15%) of tumors with L16 duplications, and 1/27 (4%) that had both an L16 deletion and an L16 duplication. The frequency of L16 mutant sequences in tumors ranged from 8%-67% suggesting subclonal variation and potential pathogenicity.
Conclusions: Previously unreported pathogenic *NOTCH1-4* mutations were identified in ACC tumor samples from the RM-202 phase II trial. High rates of *NOTCH4* L16 mutations suggest a novel mechanism of Notch pathway activation in ACC and represents a potential therapeutic target in this rare disease.

#3306 Comprehensive plasma profiling in NSCLC patients undergoing IO- and chemotherapy identifies potential proteomic signatures for progression-free survival.

A. Rigutto[1], S. Schaer[2], P. Shichkova[2], L. Raess[2], A. Viode[2], C. Below[2], R. Bruderer[2], M. Joerger[3], J. Musilova[3], S. Hayoz[3], V. Somasundaram[3], N. Nunez[4], N. Fournier[5], J. Lourenco[5], R. Gottardo[5], B. Becher[4], A. Addeo[6], A. Gupta[1], **Y. Feng**[2], A. Curioni-Fontecedro[7];

[1]University of Fribourg, Fribourg, Switzerland, [2]Biognosys AG, Schlieren, Switzerland, [3]Swiss Group for Clinical Cancer Research (SAKK), Bern, Switzerland, [4]University of Zurich, Zurich, Switzerland, [5]Agora Cancer Research Center, Swiss Institute of Bioinformatics, Lausanne, Switzerland, [6]University Hospital Geneva, Geneva, Switzerland, [7]HFR Hospital Fribourg, Fribourg, Switzerland

In NSCLC, the identification of circulating predictive biomarkers is critical to improve patient selection. Circulating biomarkers have the advantage of accessibility of samples but challenges arise in detecting disease-relevant biomarkers in plasma, where low-abundance circulating analytes are often obscured by highly abundant proteins. This underlines the need for highly sensitive, reproducible assays to detect subtle changes. Therefore, developing a sensitive, reproducible method for biomarker discovery in plasma could significantly enhance clinical outcomes for patients with NSCLC.

To address this need, we developed a novel workflow combining particle enrichment (P2) and quantitative mass spectrometry (DIA-MS). We deployed this method on plasma samples from a multicentric phase II clinical trial (SAKK17/18)[1] in patients with NSCLC. Longitudinal plasma samples were collected at baseline (n=33) and at two post-dose time points (C1D8, n=31 and C2D1, n=31). The samples were subjected to the developed P2 workflow and unbiased mass spectrometry analysis, leading to the quantification of 9283 proteins across all 95 samples. With this depth at plasma proteome profiling, we could cover up to 12 orders of magnitude, detecting low-abundance tissue/cell proteins from leakage events as well as proteins from extracellular vesicles[2].

We conducted weighted gene correlation network analysis (WGCNA) incorporating plasma protein expression and clinical outcome measures. This analysis identified a cluster of key proteins significantly associated with progression-free survival (PFS), including DERL1 and SEPRB, both known to be upregulated in NSCLC, as well as MOSPD1, which has been previously reported to promote epithelial-mesenchymal transition (EMT). To enhance our understanding of the biological context surrounding these plasma proteomic findings, we also incorporated data from two orthogonal methods: 1) the characterization of circulating immune cells from blood samples taken from patients at the same time points, performed using high-dimensional spectral flow cytometry; and 2) the integration of information on cell type markers from published single-cell datasets to delineate potential sources of plasma proteomic signatures.

In conclusion, this integrated proteomics approach provides a powerful platform for identifying predictive biomarkers, enabling more precise patient stratification and potentially improving clinical outcomes in NSCLC treatment.

1 Rigutto et al. WCLC 2024 abstract MA09.05 2 Lattmann et al. Cell Mol Life Sci. 2024

#3307 Utilizing endocytosis as a predictive biomarker for ADCs.

Anette Weyergang[1], Astrid Medhus[1], Kay Oliver Schink[2], Kristian Berg[1], Olav Engebraaten[1]

[1]Oslo University Hospital, Institute for Cancer Research, Oslo, Norway,[2]University of Oslo, Institute of Basic Medical Sciences, Oslo, Norway

Antibody drug conjugates (ADCs) consist of an antibody conjugated to a chemotherapeutic drug. These therapeutic compounds are designed to exert improved targeted toxicity as compared to conventional chemotherapy. However, variable therapeutic outcome has been experienced and predictive markers for response are warranted. ADCs are dependent on endocytosis to exert their effect, and we have hypothesized that proteins regulating the endocytic machinery can be utilized as predictive biomarkers. We have shown, for the first time, that the expression level of Rab5A, a protein regulating endocytosis correlates to the efficacy of Trastuzumab Emtansine, a HER2 targeted ADC. Furthermore, we have documented Rab5A as a predictive biomarker for Trastuzumab emtansine in three independent clinical cohorts. We are currently investigating the interplay between different ADCs and the endo/lysosomal machinery, and aim to develop this concept to a diagnostic tool for patients to be treated with ADCs. We use Trastuzumab emtansine, Trastuzumab deruxtecan and Sacituzumab Govitecan as model ADCs and evaluate the impact of different Rab GTPases on their mechanism of actions in cell lines from breast- and lung-cancer.

#3308 Use of biosimulation to predict immune checkpoint inhibitor resistance in patients with high microsatellite instability.

Adity Ghosh, Mamatha Patil, Anuj Tyagi, Ansu Kumar, Chandan Kumar, Muthiyah MR, Rahul KR, Swati Khandelwal, Jyoti Chauhan, Michael Castro, Shweta Kapoor, **James Wingrove**

Cellworks Group, Inc, South San Francisco, CA

Background: Immune checkpoint inhibitors (ICI) have become a standard treatment option in patients with high microsatellite instability (MSI-H). Although immune checkpoint inhibitors are largely effective in these patients, MSI is an imperfect biomarker and other therapies may be warranted. A mechanistic Computational Biology Model (CBM) was developed by Cellworks that can be personalized based on a patient's tumor-based genomic profile, revealing signaling pathway dysregulation and patient-specific drug response. Model output was used to identify MSI-H patients who may have a poorer response to ICIs.
Design: Computational biosimulation was performed using real-world retrospective cohorts of 423 STAD patients and 534 CRC patients (TCGA). MSI measurements were provided by TCGA. Efficacy scores (ES) based on biosimulated composite cell growth in response to disease and therapy were generated on all patients for pembrolizumab. Molecular rationales for ICI resistance were identified for MSI-H patients with low pembrolizumab ES (ES-L).
Results: Model efficacy scores for pembrolizumab were significantly higher in MSI-H patients for both STAD (average ES 20.5 vs 3.2 respectively, $p\text{-value} < 0.001$) as well as CRC (average ES 13.4 vs 2.4 respectively, $p\text{-value} < 0.001$). Of the MSI-H patients, 59% and 81% were ES-L (STAD and CRC respectively) MSI-H/ES-L STAD patients showed a significantly higher percentage of NOTCH2, EGFR, and EZH2 amplifications as well as a higher percentage of TP53-SOF mutations, whereas MSI-H/ES-L CRC patients showed a significantly higher percentage of MYC amplifications ($p\text{-value} < 0.05$)
Conclusions: In this study, a therapy efficacy score produced through biosimulation was significantly associated with microsatellite instability levels, and markers of ICI resistance were identified in MSI-H/ES-L patients. Future studies will be performed to confirm the hypothesis that biosimulation has utility in deciding which MSI-H patients might benefit from therapies other than ICI.

#3309 Characterization of long non-coding RNAs in ependymomas: Uncovering potential biomarkers and therapeutic targets.

R. Malik, D. Kumar, D. Suri, D. C. Sharma;
All India Institute of Medical Sciences (AIIMS) New Delhi, New Delhi, India

Introduction: Ependymomas (EPNs), accounting for 5-6% of glial tumors, arise from radial glial cells and exhibit significant heterogeneity, leading to variable prognosis. Despite their clinical significance, the oncogenic drivers and potential therapeutic targets for EPNs remain poorly understood. Long non-coding RNAs (lncRNAs) are emerging as crucial regulators in many cancers, influencing tumorigenesis and therapeutic resistance. However, their role in EPN pathobiology has not been explored. This study aims to unravel the lncRNA landscape in EPNs to identify novel diagnostic biomarkers and therapeutic targets, improving patient stratification and treatment outcomes.

Methods: 38 EPN tumor samples were classified into 21 supratentorial (ST), 13 posterior fossa (PF), and 4 spinal (SP) subtypes based on WHO 2021 criteria and subjected to RNA sequencing to map their RNA landscape, using 6 normal brain tissue samples as control. Raw data was normalized to identify significantly deregulated genes (padj < 0.05, -1 ≤ log2 fold change ≥ 1). qPCR validated the top deregulated lncRNAs (n = 60 extended cohort, $p < 0.05$). Pan-cancer analysis, functional annotation, target prediction, co-expression network construction, and prognostic relevance were assessed using ECORI, LncExplore, LncHub2, RNAInter, and Prognoscan respectively.

Results: RNA sequencing identified 2,271 upregulated and 1,728 downregulated lncRNAs in EPN sub-groups, with 108 commonly upregulated and 187 downregulated across subtypes. qPCR confirmed LINC01971 overexpression in ST-EPNs ($p < 0.001$), FAM74A7 upregulation in PF and SP subtypes ($p < 0.0001$, $p < 0.001$), and LINC00320 downregulation in all subgroups ($p < 0.001$). Pan-cancer analysis revealed high LINC01971 in uterine endometrial and lung carcinoma, slightly increased FAM74A7 in kidney clear cell carcinoma, and low LINC00320 across all cancers. LINC01971, co-expressed with RELA, is involved in NF-kB-driven tumorigenesis and immune modulation. Pathway analysis associated with transcription factors (ESR1, SOX2, TP53, FOXA1) and chromatin modifiers (H3K27me3, H3K4me3), indicating a role in epigenetic reprogramming. FAM74A7, correlated with transcription factors (EOMES, SOX2, NANOG, FOXA1), is linked to tumor progression and epigenetic regulation, with disease associations (papillary, cellular, and myxopapillary EPNs) supporting its potential as a diagnostic and therapeutic target. LINC00320, reduced in malignant gliomas, regulates FOXA1 and H3K4me3, affecting metabolism and protein lipidation. Co-expression patterns in EPNs and gliomas highlight their role in tumor progression and neurodegenerative diseases Prognoscan links RELA, FOXA1, and SOX2 overexpression to poor prognosis in various cancers. This comprehensive transcriptomic analysis of EPNs unveils novel lncRNAs, as potential prognostic biomarkers and therapeutic targets.

#3311 Uncovering methylation signatures and pathways associated with resistance to anti-EGFR therapy in colorectal cancer.

A. de Abreu[1], Y. Heylen[1], J. Ibrahim[1], M. Peeters[2], G. Van Camp[1], K. Op de Beeck[1];
[1]University of Antwerp, Edegem, Belgium, [2]Antwerp University Hospital, Edegem, Belgium

Background: Although anti-epidermal growth factor receptor (EGFR) therapy has established efficacy in *RAS*/*BRAF* wild type (WT) metastatic colorectal cancer (mCRC), a significant proportion (30-50%) of WT patients exhibit primary resistance to this treatment. Existing biomarker discovery has predominantly focused on tumor-specific DNA mutations, which suffer from variable mutation frequencies among patients. In contrast, DNA methylation represents a common and early event in carcinogenesis. For this reason, methylation markers hold great promise for detecting resistance mechanisms in primary tumors. Several studies have shown an association between DNA methylation patterns and resistance to anti-EGFR therapy in CRC. To validate and build upon these findings, we investigated the methylomes of primary tumors with resistant and responsive phenotypes.
Material and Methods: We isolated DNA from FFPE tissue of 40 primary CRC tumors. DNA was subjected to bisulfite conversion and Infinium MethylationEPIC v2.0 BeadChip (Illumina). Subsequently, ChAMP was used to determine the methylation values (β values) and perform differential methylation analysis. The CpG sites were ranked based on the largest Δβ values and the significance of p-values across the patient groups. To ensure the reliability of our findings, we performed a false discovery rate (FDR) evaluation using the Benjamini-Hochberg method, which led to the selection of a subset of differentially methylated CpG sites (DMCs) with the highest likelihood of being genuine associations. Additionally, a gene set enrichment analysis (GSEA) was performed with methylGSA.
Results: 48 DMCs were identified with adjusted p-values < 0.05. The affected genes are involved in cancer progression and invasiveness, suggesting a role in a yet-to-be discovered resistance mechanism. The GSEA revealed significant enrichment in 53 KEGG and 33 Reactome pathways, including MAPK-related and Akt signaling pathways. There were 470 significant gene ontology (GO) pathways: 347 in biological processes, 71 in cellular components and 52 in molecular functions.
Conclusion and future work: We have found methylation sites associated with resistance to anti-EGFR therapy in CRC patients. In addition to identifying critical DMCs, the pathways uncovered hold potential as a rich source of predictive biomarkers. Next, we will include 24 additional patients. When increasing the sample size, we expect a higher statistical power and an enrichment of CpGs with significant p-values. Afterwards, we will independently validate the most significant CpG sites in a follow-up study using a highly sensitive multiplexed droplet digital PCR assay on additional tissue biopsies.

#3313 GenialisTM Supermodel: A large molecular model enabling monotherapy and combination therapy selection in KRAS-mutated cancers.

Josh Wheeler, Luka Ausec, Miha Stajdohar, Rafael Rosengarten, Mark Uhlik

Genialis, Inc., Boston, MA

KRAS mutations drive approximately 25% of human cancers, resulting in 3.9 million new cases annually. Recently approved KRAS inhibition (KRASi) monotherapies demonstrate limited efficacy and durability. Diverse combination therapies are under investigation. This necessitates biomarker strategies for identifying patients most likely to respond to KRASi monotherapy or combination therapy.The Genialis™ Supermodel is a foundation model of cancer biology that can be used to derive novel biomarkers for diagnosis, clinical development, and treatment decision-making in oncology. Trained on nearly 1 million harmonized transcriptomic samples, this large molecular model (LMM) maps individual patient RNA-seq samples into biologically meaningful spaces defined by distinct combinations of biological signatures. Each biological subspace reflects mechanisms relevant to a specific therapeutic class. These mappings enable the selection of core biological features, which are then used in downstream machine-learning models to predict therapeutic response and stratify patients.The Supermodel captures biology linked to responses to KRASi, EGFR inhibitors, ICI (immune checkpoint inhibitors), and standard-of-care chemotherapy, among other therapeutic mechanisms, generating classifiers that predict monotherapy responses in diverse preclinical and clinical cohorts. We further demonstrate that the intersection of these individual classifiers identifies clinical contexts where monotherapy is sufficient and where combination therapies may provide additional benefit. These results refine patient stratification and guide clinical decision-making by aligning treatment selection with the underlying tumor biology and therapeutic context.The Genialis™ Supermodel establishes a novel and robust framework for precision oncology, enhancing patient stratification, optimizing clinical decision-making, and expanding opportunities for effective KRAS-driven cancer treatments.

#3314 AML patients characterized by BH3 profiling and the response to venetoclax-based regimen.

Qi Tatarata[1], Zhe Wang[2], Lina Han[2], Tapan Kadia[2], Courtney DiNardo[2], Marina Konopleva[3]

[1]Internal Medicine, SUNY Downstate Health Sciences University, Brooklyn, NY,[2]Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX,[3]Medical Oncology, Albert Einstein College of Medicine, Bronx, NY

Venetoclax (VEN), a selective BCL-2 inhibitor, has dramatically changed acute myelogenous leukemia (AML) treatment. The AML patient respond heterogenous to VEN-based therapy and frequently require additional conjugated treatment.

BCL-2 family, crucial in apoptosis regulation, comprises three categories: anti-apoptotic protein, pro-apoptotic protein, and BH3-only sensitizers which bind to specific anti-apoptotic proteins at resting state. By exposing cells to various synthesized BH3-only peptides and measuring cytochrome c release, we are able to detect the cells dominate anti-apoptotic proteins, namely BCL-2 family dependency. This method, known as BH3 profiling, has been validated by multiple studies as a way to correlate the cells response to different BH3 inhibitors in cell lines and primary samples (*Bhatt et al. Cancer Cell 2020*). In this study, we performed BH3 profiling on samples collected from 52 patients on VEN-based treatments, including 40 patients achieved response (28 CR, 7 CRi, 2 CRp, 1 CR with MRD, 2 CR then relapse), 12 non-response (NR). We exposed the primary cells to peptides of hBIM and PUMA (general priming, all anti-apoptotic proteins), HRK-Y (priming BCL-XL), mBAD (priming of BCL-W in addition to BCL2- and BCL-XL), MS-1 and mNoxaA (priming MCL-1), FS-1 (priming of BFL-1), and BCL-2 inhibitor Veneotoclax (priming BCL-2) for 1 hour, and then measure the cytochrome C by flow cytometry. The priming was defined as CytC loss by median fluoresce intensity relative to Alamethicin and DMSO controls. AML dependency on anti-apoptotic proteins is heterogenous, as reflected by varying responses to BH3-only peptides. 69.23% (36 out of 52) of the samples showed intact apoptotic capacity with more than 50% priming to hBIM. 43.15% (24/52) of samples depended on BCL-2, as indicated by more than 40% priming to VEN, while 34.62% (18/52) depended on BCL-XL, as indicated by more than 40% priming to HRK-Y. In addition, 51.92% (27/52) had a dependency on MCL-1 (more than 40% priming to MS-1), and 25.00% (13/52) were dependent on BFL-1 (more than 40% priming to FS-1). Overlapping co-dependencies were also observed. Among the 24 samples with BCL-2 dependency, 16 also depended on MCL-1, 10 on BCL-XL, 9 on BFL-1, and 6 were co-dependent on BCL-2, MCL-1, BCL-XL and BFL1.

Comparing the samples from patients who achieved response with who did not, response samples had higher priming capacity, as shown by greater median CytC release in response to hBIM (p=0.0378). Non-CR samples exhibited higher dependency on non-BCL-2 proteins, with greater priming to peptides mNoxaA (p=0.0893), MS-1 (p=0.0153), and FS-1 (p=0.0147).

In summary, AML shows heterogenous and co-dependent relationships withc multiple anti-apoptotic proteins. Patients who responded to VEN-based therapy typically retain the apoptotic capacity and show lower co-dependency on MCL-1 and BFL-1.

#3315 Unveiling a protective role of B-cells in classical Hodgkin lymphoma progression through gene expression signatures.

Stefano Pozzi[1], Francesca Reggiani[2], Tanja Lazic[2], Nizzoli Maria Elena[2], Alberto Bavieri[2], Benedetta Donati[2], Durmo Rexhep[2], Riccardo Valli[2], Attilio Gennaro[2], Cristian Ascione[2], Alessia Ruffini[2], Annibale Versari[2], Francesco Merli[2], Massimo Dominici[3], Alessia Ciarrocchi[2], Stefano Luminari[2]

[1]University of Modena and Reggio Emilia, Clinical and Experimental Medicine PhD Program, Modena, Italy,[2]AUSL-IRCCS Reggio Emilia, Reggio Emilia, Italy,[3]University of Modena and Reggio Emilia, Modena, Italy

Background: Classical Hodgkin Lymphoma (cHL) is considered highly curable, but identifying patients at risk of relapse or progression after initial treatment remains a challenge. Disease progression may involve biological factors not accounted for by current prognostic models, which could potentially be revealed by molecular analysis. In this study, we performed comprehensive gene expression profiling to identify molecular markers that could help to develop new prognostic tools in cHL patients.
Patients and Methods: We conducted a retrospective review of local clinical records to identify patients with confirmed cHL diagnosed between 2004 and 2019, aged 18-65, at any disease stage, and treated with systemic chemotherapy. Gene expression analysis using nCounter Nanostring Technology with the PanCancer Immune Profiling Panel was performed on baseline diagnostic biopsies. Genomic data were correlated with clinical, laboratory, and radiomic information. Progression-Free Survival (PFS) was the primary endpoint. Immunohistochemistry was used for validation.
Results: We identified 185 patients with classical Hodgkin lymphoma (cHL), of whom 155 had available formalin-fixed paraffin-embedded (FFPE) tissue samples. Of these, 32% were aged over 45 years, 46% had stage III-IV disease, and 10% presented with bulky disease. After a median follow-up of 72 months (range, 6-171 months), 31 PFS events occurred, with a 4-year PFS rate of 80.4% (95% CI 74.1-87.3). Through Cox Proportional Hazards modeling, we identified 66 genes that were significantly associated with PFS ($p<0.05$). Of these, 41 genes were positively associated with better PFS, suggesting a protective effect, while 25 genes were linked to a reduced probability of survival.
Through correlation analysis and gene ontology, we were able to identify a 7-gene signature associated with B-cell signaling pathways. An unsupervised analysis on the entire cohort based on the expression of the identified B cell signature has allowed us to define two main patient clusters: low- B cell and high- B cell. The former cluster displayed a significant increase in the incidence of clinical events ($p=0.03$) and worse PFS compared to the high B-cell cluster ($p=0.007$). Additionally, elevated expression of PAX5, a key regulator of B-cell differentiation, was significantly associated with better PFS, with a 4-year PFS rate of 90% (95% CI 82.7-97.3) compared to 71% (95% CI 61.3-82.4) in patients with lower expression levels. Immunohistochemical evaluation of PAX5 in the tumor microenvironment further supported its potential as a prognostic marker.
Conclusion: These results suggest that gene expression analysis may assist in the early detection of disease relapse and highlight the immune-modulatory role of B-cells in cHL progression.

#3316 Comparative analysis of Ki67 and BCI for risk of recurrence and prediction of extended endocrine therapy benefit in the BCI Registry study.

Sami Diab[1], Natalia Siuliukina[2], Brandon O'Neal[2], Amanda K. L. Anderson[2], Yi Zhang[2], Kai Treuner[2]

[1]Intermountain Health, Denver, CO,[2]Biotheranostics, a Hologic Company, San Diego, CA

Background: The BCI Registry study is an ongoing trial with over 3000 enrolled patients diagnosed with HR+ early-stage breast cancer to assess long-term outcomes as well as the overall impact of BCI in treatment decisions. The BCI (H/I) ratio has been validated to predict benefit from extended endocrine therapy (EET) in the MA.17, Trans-aTTom, IDEAL and B-42 trials. The BCI score is prognostic for the risk of distant recurrence. Ki67 is a well-known prognostic marker for invasive breast cancer disease. A high expression of Ki67 relates to a higher proportion of actively dividing cells within the tumor, which is associated with more aggressive behavior and poorer prognosis. Conversely, low Ki67 expression indicates a lower proliferation rate and may be associated with a less aggressive tumor phenotype and better prognosis. This analysis investigates the relationship between BCI (H/I) and BCI score against Ki67 score in patients enrolled in the BCI Registry study.

Methods: BCI Registry patients with available BCI and Ki67 results were incorporated in this study (n=1872), consisting of 1483 node negative and 389 node-positive patients. BCI (H/I)-Low and BCI (H/I)-High groups were derived from a pre-specified cut-point. Ki67 score was stratified into the following risk groups: Low risk (Ki67 < 10%), Intermediate risk (Ki67: 10 to 25%), and High risk (Ki67 > 25%). Kendall correlation (R) was used to examine the correlation between BCI (H/I), BCI score and Ki67 score as continuous variables. Descriptive statistics were used to summarize the distribution of BCI (H/I) categories and BCI prognostic groups across all Ki67 risk categories for all patients and subsets based on nodal status.

Results: In the overall cohort, 37.2%, 27.2% and 35.6% patients were classified by Ki67 as Low, Intermediate and High Risk, respectively. In addition, 61.5% of patients were classified as BCI (H/I)-Low and 38.5% as BCI (H/I)-High. Continuous analysis of BCI (H/I) versus Ki67 revealed a weak correlation (R= 0.14), but a positive correlation for BCI score versus Ki67 (R= 0.46). Within Ki67 Low Risk patients, 27.4% were classified as BCI (H/I)-High, and 48.1% of Ki67 High Risk patients were classified as BCI (H/I)-Low. The prognostic analysis identified 28.6% of Ki67 Low Risk patients classified as BCI high risk and 17.3% of Ki67 High Risk patients classified as BCI low risk. In addition, 51.8% and 48.2% of Ki67 Intermediate Risk patients were classified as BCI low risk and high risk, respectively.

Conclusions: These results confirm only a weak correlation between BCI (H/I) and Ki67 but a moderate correlation between the BCI score and Ki67. These findings further highlight the important distinction between prognostic and predictive biomarkers, where prognostic risk assessment by Ki67 does not predict the likelihood of benefit from extended endocrine therapy.

#3317 IFITM3: A predictive biomarker for therapeutic outcomes in advanced hepatocellular carcinoma.

Yinchao Zhang[1], Linze Xu[1], Jin Zhang[1], Dandan Wu[1], Huikai Li[1], Yueguo Li[2], Yang Liu[1]

[1]Tianjin Medical University Cancer Institute & Hospital, National Clinical Research Center for Cancer, Key Laboratory of Cancer Prevention and Therapy, Tianjin's Clinical Research Center for Cancer, Tianjin, China,[2]Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer, Tianjin Key Laboratory of Digestive Cancer, Key Laboratory of Cancer Prevention and Therapy, Tianjin's Clinical Research Center for Cancer, Tianjin, China

Background: Hepatocellular carcinoma (HCC) is a major global health challenge, with China bearing a significant burden due to its high incidence and mortality rates. Vascular invasion (VI), particularly microvascular invasion (MVI), is a critical prognostic factor in HCC , profoundly influencing treatment decisions. Despite its clinical importance, reliable and non-invasive biomarkers for stratified prediction and therapeutic response remain scarce. Extracellular vesicles (EVs) are emerging as promising tools in cancer progression and therapy assessment.

Methods: Sixty-five patients with China Liver Cancer (CNLC) stage III HCC treated with hepatic artery infusion chemotherapy (HAIC) plus sintilimab and a bevacizumab biosimilar (IBI305) were stratified based on RECIST criteria and surgical status for survival analysis and Kaplan-Meier (KM) curve construction. Univariate and multivariate regression analyses were employed to identify independent predictors of surgical eligibility. Cellular and molecular investigations followed to uncover potential biomarkers. scRNA-seq data (GEO: GSE242889), bulk RNA-seq data (GEO: GSE19977, TCGA), and hdWGCNA analysis were utilized to identify hub genes linked to MVI. TCGA data were divided into training and validation sets at a 7:3 ratio. Machine learning algorithms (LASSO regression, SVM-RFE, random forest) identified high-risk MVI genes. A prognostic nomogram model was constructed and significant predictors were explored with its performance assessed via ROC curves. Immunohistochemical staining and exosomal IFITM3 analysis were applied to evaluate gene expression across treatment stages.

Results: Data from 65 patients (April 2021 to July 2023) with a median age of 57 years, predominantly male (83.1%), were analyzed. Patients who underwent surgery (30.8%) or achieved partial response (PR) per RECIST (38.5%) demonstrated significantly improved overall survival. Multivariate regression indicated that patients with VI, distant metastasis, ascites, and Child-Pugh grade B as factors reducing surgical eligibility, with VI as an independent predictor. Six MVI high-risk genes (VTN, DYNC1I2, HABP2, IFT43, IFITM3, NDUFS4) were identified, among which IFITM3 emerged as a key prognostic marker. High IFITM3 expression was associated with shorter survival ($P < 0.001$). The nomogram model showed strong predictive accuracy for survival (AUC1=0.641, AUC2=0.613, AUC2.5=0.579). Immunohistochemical analysis and exosomal analysis further validated IFITM3's role in influencing therapeutic outcomes.

Conclusions: In summary, IFITM3 levels are closely linked to therapeutic outcomes in HCC patients. Non-invasive blood-based detection of IFITM3 holds promise for enhancing therapeutic stratification. Further research may refine prediction models and deepen understanding of IFITM3's role in poor therapeutic outcomes in cancer.

#3318 Serum Insulin-Like Growth Factor-1 levels predict outcomes in patients with advanced hepatocellular carcinoma receiving immunotherapy.

Tsung-Che Wu[1], Ching-Tso Chen[1], Chien-Huai Chuang[2], Ann-Lii Cheng[2], Chih-Hung Hsu[3], Yu-Yun Shao[3]

[1]Department of Oncology, National Taiwan University Hospital, Hsinchu Branch, Hsinchu, Taiwan,[2]Department of Medical Oncology, National Taiwan University Cancer Center, Taipei, Taiwan,[3]Department of Oncology, National Taiwan University Hospital, Taipei, Taiwan

Background: The combination of immune checkpoint inhibitors (ICIs) with antiangiogenesis agents or another ICI has become the standard of care for advanced hepatocellular carcinoma (HCC). Elevated serum levels of insulin-like growth factor-1(IGF-1) levels have been previously associated with better outcomes of patients with advanced HCC treated with sorafenib or atezolizumab plus bevacizumab. However, the prognostic value of serum IGF-1 level for other immunotherapy regimens remains unexplored.
Methods: Patients with advanced HCC who received anti-PD-1/anti-PD-L1 monoclonal antibody monotherapy or combinations were enrolled. Serum samples were collected prior to initiating immunotherapy, and serum IGF-1 levels were quantified using enzyme-linked immunosorbent assay (ELISA). Based on prior findings from our group, a cutoff value of 63.6 ng/mL was used to stratify patients into high and low serum IGF-1 groups. Patient demographics, tumor characteristics, treatment responses, and survival outcomes were analyzed.
Results: From August 2015 to July 2024, 111 patients were enrolled. Baseline characteristics were as follows: 90% male, 71% HBsAg-positive, 18% anti-HCV-positive, Child-Pugh scores: 72% (5 points)/23% (6 points)/5% (7 points), 43% with macrovascular invasion (MVI), 72% with extrahepatic involvement (EHI), and 46% treated with antiangiogenic-containing regimens. Patients with higher serum IGF-1 levels demonstrated fewer adverse clinical features, including lower Child-Pugh scores, ALBI grades, and MVI rates. Patients who achieved an objective response to therapy had significantly higher IGF-1 levels compared to those who did not (57.2 vs. 46.2 ng/mL, $p = 0.037$). Additionally, patients with high IGF-1 levels exhibited improved overall survival (OS) compared to those with low IGF-1 levels ($p = 0.024$). When stratified by regimen types, the prognostic value of high IGF-1 levels was maintained in patients treated with antiangiogenic-containing regimens ($p = 0.001$) but was not observed in patients treated without antiangiogenic agents ($p = 0.967$).
Conclusions: High serum IGF-1 levels are associated with improved OS in patients with advanced HCC, but this prognostic value is confined to regimens containing antiangiogenic agents. These findings underscore the potential of IGF-1 as a biomarker for optimizing immunotherapy strategies in advanced HCC.

#3319 Evaluating and targeting DNA repair defects in pediatric high grade glioma.

Evan Savage[1], Ke Xu[2], Miaomiao Li[3], Jason Myers[2], Evadnie Rampersaud[2], Rebekah DeVries[1], Kamran Mohammad Zahid[3], Krista Millican[1], Yawei Hui[2], Ti-Cheng Chang[2], Gang Wu[2], Anang Shelat[1], Christopher Tinkle[3]

[1]Chemical Biology and Therapeutics, St. Jude Children's Research Hospital, Memphis, TN,[2]Center for Applied Bioinformatics, St. Jude Children's Research Hospital, Memphis, TN,[3]Radiation Oncology, St. Jude Children's Research Hospital, Memphis, TN

Homologous recombination deficiency (HRD) has been extensively studied in breast cancer and ovarian cancer given its correlation with sensitivity to Poly (ADP-ribose) polymerases (PARP) inhibitors. In these cancers, HRD is most frequently caused by biallelic inactivation of BRCA1/2 and these tumors often exhibit ‘genomic scars’ (Nik-Zainal, 2016; Telli, 2016; Davies, 2017). Our group profiled a cohort of 255 pediatric high-grade glioma (pHGG) patient samples for DNA damage response (DDR) gene variants and genomic scars. We found no evidence of BRCA1/2 loss in any sample, but we did detect evidence for HRD-associated genomic scars in 30% of our samples. Interestingly, we also detected biallelic loss of ATM, which is reported to be synthetically lethal with ATR inhibition (Marwan, 2016). Given these findings, our group set out to answer the following questions: 1) Does the presence of BRCA deficiency or HRD-related genomic scars predict sensitivity to PARP inhibition in pHGG tumors? 2) Do the ATM mutations we found impair ATM pathway activation and confer radiosensitivity, and do they induce synthetic lethality with ATR inhibition? Overall, this work underscores the importance of looking beyond simple mutation status to identify robust biomarkers of drug response in pHGG tumors.

#3320 A comprehensive pan-cancer analysis of BCOR as a promising biomarker for immune checkpoint inhibitor therapy.

C. Li[1], S. Sun[2], K. Dong[2], Y. Zhu[1], X. Zhong[3];

[1]Dali Bai Autonomous Prefecture People's Hospital (The Third Affiliated Hospital of Dali University), Dali, Yunnan, China, [2]The first Affiliated Hospital of Dali University, Dali, Yunnan, China, [3]3D Medicines Inc., Shanghai, China

Background: *BCOR* is a gene that encodes an epigenetic regulator, which is indispensable for guiding cell differentiation and body structure development. While immune checkpoint inhibitors (ICIs) have emerged as a significant therapeutic option for tumor patients, the correction between *BCOR* mutations and the efficacy of ICIs in solid tumors remains uncertain.

Methods: Next-generation sequencing (NGS) and clinical data of pan-cancer patients were sourced from two MSK-IMPACT cohorts: MSK-2017, comprising 10336 patients, and MSK-2019, consisting of 1661 patients. The MSK-2019 cohort treated with ICIs underwent analysis to investigate the correlation between *BCOR* mutations and the therapeutic efficacy of ICIs. mRNA expression data of 10228 pan-cancer patients were retrieved from the TCGA database, aiming to explore the relationship between BCOR expression levels and immune cell infiltration.

Results: Totally, 2.9% (304/10336) of pan-cancer patients in the MSK-2017 cohort and 3.9% (64/1661) in the MSK-2019 cohort harbored *BCOR* mutations. In the MSK-2017 cohort, the top three tumor types with the highest frequency of *BCOR* mutations were endometrial cancer (28/218, 12.8%), adrenocortical carcinoma (3/25, 12.0%), and salivary gland cancer (9/114, 7.9%), respectively. Differently, the MSK-2019 cohort showed the highest frequency of *BCOR* mutations in colorectal cancer (11/110, 10.0%), whereas in the MSK-2017 cohort, *BCOR* mutations were only observed in 3.5% (35/1007) of colorectal cancer patients. The most common types of *BCOR* mutations in both cohorts were missense and truncating mutations. In the MSK-2019 cohort, the *BCOR*-mutated group exhibited a significantly higher tumor mutational burden (TMB) compared to the *BCOR*-wild group (median TMB: mutated-type vs. wild-type = 36.7 vs. 5.9 Muts/Mb, $P<0.0001$). Additionally, the overall survival (OS) of the BCOR-mutated group was significantly longer than that of the wild-type group (median OS: 36.0 vs. 15.0 months; hazard ratio [HR]=0.5, 95% confidence interval [CI]: 0.6-0.7, $P=0.003$). However, in MSK-2017 cohort treated without immunotherapy, there was no difference in OS between *BCOR*-mutated group and *BCOR*-wild group (median OS, 30.0 vs. 26.0 months; HR=0.9, 95%CI: 0.7-1.1, $P=0.3$). The immuno-correlation analysis showed that across most tumor types, the infiltration levels of CD4+ memory resting T cell, plasma B cell, and naive B cell exhibited a positive correlation with BCOR expression. Conversely, the infiltration levels of regulatory T cells, gamma delta T cells, and follicular helper T cells exhibited a negative correlation with BCOR expression.

Conclusion: The results indicated that the *BCOR* mutation frequency varied across different tumor types and cohorts. Notably, in patients treated with ICIs, *BCOR* mutations were associated with higher TMB and improved OS, suggesting its potential as a biomarker in immunotherapy response.

#3321 Real-world clinical outcome analyses in head and neck squamous cell carcinoma (HNSCC) to uncover TIGIT biology in patients treated with first-line immune checkpoint inhibitors.

Meghna Das Thakur, Xiaoyong Fu, Shahed Iqbal, Joon Rhee

Gilead Sciences, Inc., Foster City, CA

Introduction: ICIs are approved for HNSCC. T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibition motif domain (TIGIT) is a potential ICI anti-tumor target. Understanding how effector T cell (Teff) and *TIGIT* expression levels in tumors correlate with real-world outcomes of 1L treatment can highlight the role of TIGIT as a potential target.

Methods: Deidentified real-world pt data, including whole-transcriptome RNA sequencing (RNA-seq) and progression-free survival (PFS), were acquired from Tempus AI, Inc. Data from pts with metastatic HNSCC (mHNSCC) who received 1L therapy with ICI, ICI + chemotherapy (chemo), or chemo were analyzed. Gene expression correlations, including between *TIGIT* and a Teff gene set (geometric mean of: *CD8A, GZMA, GZMB, IFNG, EOMES, and PRF1*) were assessed by Pearson correlation coefficients. Human papilloma virus (HPV) status was extrapolated by an HPV gene signature (*GGA2*, *SPIB*, *CD200*, *ADAM28, BCL2*, *VCAM1*, *ICOSLG*, *STAG3*). Real-word PFS (rwPFS) was assessed using the Kaplan-Meier method and compared by expression level (high vs low) of *TIGIT*, Teff gene set, and *TIGIT* normalized by Teff gene set.

Results: The cohort included pts with 1L ICI (n = 27), ICI + chemo (n = 62), and chemo (n = 159). At baseline, *TIGIT* expression (N = 276 biopsies with RNA-seq) was highly correlated with Teff gene set ($R = 0.852$, $P < 0.001$) and *FOXP3* expression ($R = 0.853$, $P < 0.001$). Correlations were consistent when stratified by HPV status (HPV+ or HPV−) or programmed death-ligand 1 (PD-L1) status (combined positive score: < 1, 1-19, and ≥ 20). Higher immune marker expression and numerically improved rwPFS for ICI + chemo were observed for HPV+ vs HPV− status. High vs low *TIGIT* expression was associated with numerically improved rwPFS for ICI (n = 13 vs 14; HR [95% CI], 0.86 [0.23-3.22]) or ICI + chemo (n =31 each; HR [95% CI], 0.4 [0.18-0.9]; $P = 0.023$). Similar results were observed for high vs low Teff gene set expression (ICI: HR [95% CI], 0.54 [0.13-2.16]; ICI + chemo: HR [95% CI], 0.66 [0.3-1.42]). When *TIGIT* expression was normalized to Teff gene set expression, rwPFS was numerically shorter for high vs low expression in ICI (HR [95% CI], 1.25 [0.33-4.8]) or ICI + chemo (HR [95% CI], 1.37 [0.64-2.95]).

Conclusions: *TIGIT* expression was highly correlated with Teff gene set expression and *FOXP3* in HNSCC tumors. Improved rwPFS with HPV+ status was likely due to a more immunogenic tumor microenvironment. High Teff or *TIGIT* expression in tumors was associated with improved rwPFS with 1L ICI-containing regimens. However, after normalization of *TIGIT* expression by Teff infiltration, the presence of TIGIT-high tumors was associated with worse rwPFS with ICI-based treatment. Thus, combining anti-TIGIT and anti-PD-1 treatments could bring more benefit in a 1L mHNSCC setting to pts with TIGIT-high tumors.

#3322 Comprehensive genomic profiling (CGP)-informed circulating tumor DNA analysis for postoperative monitoring of non-small cell lung cancer: BEACON study.

Guanggui Ding[1], Jinquan Yuan[2], Peikun Ding[1], Xiaoxiang Huang[1], Junjun Li[3], Hongsheng Xie[1], Yuxin Wen[1], Jiaochun Shi[3], Shihua Dou[1], Wohua Han[2], Xuning Fan[3], Yuan Sun[3], Zhihong Zhang[3], Bing Li[3], Zhou Zhang[3], Jianping Zhou[2], **Lin Yang**[1]

[1]Department of Thoracic Surgery, Shenzhen People's Hospital（The Second Clinical Medical College，Jinan University；The First Affiliated Hospital, Southern University of Science and Technology）, Shenzhen, China,[2]Department of Thoracic Surgery, Dongguan People's Hospital, Dongguan, China,[3]Burning Rock Biotech, Guangzhou, China

Background: Detection of circulating tumor DNA (ctDNA) after surgery in early-stage non-small cell lung cancer (NSCLC) is associated with poorer outcomes. The previous MEDAL study (PMID: 37683638) demonstrated that PROPHET, a whole-exome sequencing (WES)-based, individualized ctDNA detection assay, outperformed fixed-panel assays in a direct comparison for NSCLC. However, WES requires high-quality tissue samples and is associated with high costs. Here, we further aim to validate CGP sequencing-based individualized ctDNA assay for minimal residual disease (MRD) detection in NSCLC.

Methods: The BEACON study is a multicenter, prospective cohort study that collected plasma samples at pre-surgery, 30 days post-surgery, and at longitudinal time points from patients with early-stage NSCLC between February 2022 and September 2024. A 520-gene panel sequencing-based personalized tumor-informed technology known as PROPHET_lite was used, which employs deep sequencing up to 25 patient-specific variants. PROPHET_lite was developed to detect MRD with a limit of detection (LoD) of 0.01% and a sample-level specificity exceeding 99%, as established during analytical validation.

Results: In our cohort, excluding two patients with no detectable mutations, 130 patients successfully underwent ctDNA assays, with a median of five tumor-specific tracking variants. Of these, 77.7% had stage I NSCLC, and 86.2% had adenocarcinoma. ctDNA was detected pre-surgery in 129 patients, with a 31% positivity rate. Among patients with stage I NSCLC, this rate was comparable to the PROPHET assay in the MEDAL study. After a median follow-up of 445 days (95% CI: 376-536), 18 patients experienced distant recurrence. Landmark MRD status was significantly associated with DFS (HR 3.36, 95% CI: 1.3-8.71, $p = 0.008$); the negative predictive value (NPV) and positive predictive value (PPV) for recurrence were 88.3% and 53.8%, respectively. Longitudinal MRD status was also significantly associated with DFS (HR 15.1, 95% CI: 4.33-52.61, $p < 0.001$); the NPV and PPV for recurrence were 95.8% and 66.7%, respectively. Among the 18 patients with recurrence, MRD was positive in 14 cases at or before recurrence, with a median lead time of 295 days before CT detection. The remaining four MRD-negative cases were associated with brain metastases or prolonged intervals without ctDNA detection. Multivariate Cox analysis further revealed that longitudinal MRD status (HR 52.28, 95% CI: 7.37-370.8, $p < 0.001$) was an independent predictor of recurrence.

Conclusion: Our study demonstrated that the MRD approach, with its high clinical accessibility, broad applicability, and good sensitivity, enables prognostic predictions and supports disease monitoring during surveillance in NSCLC patients.

#3323 nELISA high-throughput protein profiling applied to the RADIOHEAD cohort: Insights from the largest plasma proteomics study of patients receiving checkpoint inhibitor therapy.

Nathaniel Robichaud[1], Samantha I. Liang[2], Grant Ongo[1], Enjun Yang[2], Kiran Edwardson[1], JiaMin Huang[1], Andy Lee[1], John E. Connolly[2], Milad Dagher[1]

[1]Nomic Bio Inc, Montreal, QC, Canada,[2]Parker Institute for Cancer Immunotherapy, San Francisco, CA

Proteomics holds great promise for cancer immunotherapy, with intensive efforts being exerted for early disease identification, patients selection, and adverse event prediction. Despite this potential, the high cost and low throughput of existing tools to profile circulating proteins render such studies prohibitively slow and costly, limiting their wide-spread application. As a result, proteomics studies in the field have been constrained to sample sizes in the 10s and 100s , restricting the power to discover key biomarkers. Here, we leverage a novel proteomics tool, the nELISA, to quantify ~600 circulating proteins across ~3000 samples from the RADIOHEAD (Resistance Drivers for Immuno-Oncology Patients Interrogated by Harmonized Molecular Datasets) cohort, a prospective study of 1070 immunotherapy naive pan-tumor patients on standard of care immune checkpoint inhibitor (ICI) therapy regimens from community oncology clinics.

The nELISA is a highly multiplexed immunoassay platform that enables the profiling of hundreds of proteins across 1536 samples per instrument daily, at significantly reduced costs. The method miniaturizes sandwich immunoassays by pre-packaging antibody pairs on the surface of color-coded microparticles, which can then be analyzed via high-throughput flow cytometry. Using this technology, our preliminary data identified several markers of response to treatment; for example, PD-1 inhibitors resulted in increased circulating levels of soluble PD-1, and ICIs increased levels of several chemokines including CXCL9 and CXCL10, as seen in several other small-scale studies. We also identified potentially novel markers associated with response to treatment and irAEs, requiring a much larger dataset for validation.

The scalable nature of the nELISA platform now allows us to validate these findings in a large longitudinal cohort, providing the power needed for such a broad biomarker discovery effort. In this presentation, we share the results of applying nELISA to the RADIOHEAD serum samples from pretreatment, early on-treatment, 6-month, and 12-month timepoints. For participants who experienced immune-related adverse events, additional samples were collected upon presentation and in follow-up visits - these samples were also analyzed in this study. Pairing nELISA protein profiling of these longitudinal samples with associated demographic metadata and clinical outcomes provides an opportunity to identify clinically actionable mechanisms for ICI resistance and adverse events, discover targets for combination therapies and post-ICI treatment, and inform system biology approaches to elucidate disease pathways. Such identified biomarkers and protein signatures are expected to further accelerate research and improve clinical decision making in the field of cancer immunotherapy.

#3324 Co-occurrence of gene fusions with SNV/Indels and with CNVs on solid tumors in a cohort of 795 patients from the community setting.

Chaugiang Duong, Nathan Montgomery, Derek Lyle, Fernando Lopez-Diaz

NeoGenomics, Aliso Viejo, CA

Background: Co-occurrence of gene alterations often plays a relevant role in the selection of potential targeted therapies for a cancer patient. It has been reported that patients with fusions respond to fusion-targeted therapy regardless of the tissue type. Less is known about the co-occurrence of SNVs and Indels with gene fusions and copy number variation.
Methods: Data from 795 patients tested with a CGP assay that interrogated SNVs/Indels on 517 genes, fusions of 59 genes, and CNV on 55 genes were included in the study. Variant clinical significance was determined as described by the FDA-recognized OncoKB database or the National Comprehensive Cancer Network, NCCN. The study was performed with an IRB approved research protocol.
Results: We examined the co-occurrence of copy number variation, RNAseq fusion detection, and actionable SNV and Indel mutations in community care settings to understand their combined potential clinical implications. We analyzed the co-occurrence of fusion events with targetable mutations, the overlap of fusions with copy number variation detection, and simultaneous copy number variation and actionable SNV/Indels. Roughly 5.3% (38/ 713) of tumors with pathogenic/likely pathogenic OncoKB gene mutation also had a fusion event. Conversely, 80.9% (38/47) of patients harboring a gene fusion had pathogenic OncoKB mutation(s). When analyzed alone, amongst the 40 fusions, the most frequent were SDCCAG8::AKT3 (n=5), TMPRSS2::ERG (n=4), ESR1::CCDC170, and ROS1 fusions (n=3). Fifteen (15) of these fusions have a potentially actionable fusion as defined by NCCN and/or OncoKB database. ROS1 fusions most commonly had TP53 mutations (66.66%), with equal detection of other pathogenic oncoKB co-mutations in BRCA2, EGFR, and BRAF. Meanwhile, 6 of the patients had PIK3CA oncogenic mutations with an available therapeutic selection, and 2 had a fusion that offers additional clinical implications. Additionally, ESR1 was the most frequent fusion partner with PIK3CA co-mutation with OncoKB described actionability. The most common SNV/Indel mutation observed was TP53, with 31 unique tumors bearing co-occurring TP53 mutation with a fusion. In addition, 36.2% (17/47) of fusion positive tumors had concurrent copy number variation, whereas 6.9% (17/245) of tumors with copy number alteration detection (n=245) concurrently had a fusion. Moreover, 32.0% (228/713) of samples with pathogenic gene mutation also had copy number variation(s). Conversely, 93.1% (228/245) of tumors with copy number variation had pathogenic OncoKB mutation.
Conclusion: Genomic rearrangements, albeit relatively infrequent, can often be concomitantly expressed in the context of other actionable DNA mutations or CNVs, which can lead to better understanding of tumorigenesis and improve clinical care as tumors are tested with comprehensive genomic profiling assays.

#3325 PELO is a synthetic lethal target in FOCAD deleted cancers.

Michelle Patricia Cicchini[1], Yang Peng[2], Yanhua Rao[1], Biju Mangatt[1], Benjamin Schwartz[1]

[1]Intracellular Targeted Cancer Therapeutics, GlaxoSmithKline, Collegeville, PA,[2]Synthetic Lethality, GlaxoSmithKline, Collegeville, PA

During protein translation, ribosomes may stall for a number of reasons including the presence of mRNA defects, cell stress, nonoptimal codon clusters, ribosome collisions, etc. One mechanism of resolving these stalled ribosomes is to extract the mRNA using the SKI complex. Focadhesin (FOCAD) provides an essential function to the SKI complex by stabilizing and protecting SKI proteins from degradation, but it is also a common collateral deletion with CDKN2A- and MTAP-loss in many cancers. In the absence of a functional SKI complex, stalled ribosomes are resolved via the No-go decay (NGD) pathway which leverages PELO (protein pelota homolog), HBS1L (HBS1-like protein), and ABCE1 (ATP-binding cassette sub-family E member 1) . Together PELO and HBS1L recognize stalled ribosomes and recruit ABCE1 to split and recycle ribosomal subunits. Consistent with the NGD pathway compensating for the loss of a functional SKI complex in normal cells, unbiased whole-genome knockout screens identify FOCAD-deleted cell lines to be dependent on PELO for their survival. In this work, PELO-dependency is validated in FOCAD-deleted cancer cell lines and it is demonstrated that FOCAD-deletion drives PELO-dependency. Furthermore, it is shown that PELO-knockout induces ribosomal readthrough, GCN2 activation, and EIF2α induction in FOCAD-deleted cell lines prior to the robust anti-proliferative and apoptotic readouts observed at longer timepoints. Together, these data support that loss of PELO in FOCAD-deleted cancer cells is synthetic lethal due to the persistence of stalled ribosomes that initially activate cell survival pathways but ultimately lead to cell death.

#3326 Putative value of HLA-neoantigen-TCR interactions as a response biomarker to neoadjuvant chemoradiotherapy in locally advanced rectal cancer.

R. Torreglosa do Carmo[1], V. Lira[2], F. Hinterholz Knebel[1], F. Bettoni[1], G. Pagin Sao Juliao[3], N. M. Felicio[3], L. Corbi[3], A. Habr-Gama[4], A. Aranha Camargo[1], C. Masotti[1], R. Oliva Perez[2];

[1]Hospital Sirio-Libanes, Sao Paulo, Brazil, [2]Hospital Beneficencia Portuguesa de Sao Paulo, Sao Paulo, Brazil, [3]Hospital Alemao Oswaldo Cruz, Sao Paulo, Brazil, [4]Instituto Angelita e Joaquim Gama, Sao Paulo, Brazil

The standard initial treatment for locally advanced rectal cancer (LARC) is neoadjuvant chemoradiotherapy (nCRT), which aims to reduce tumor size before total mesorectal excision. A notable proportion of patients (10-30%) achieve a complete pathological response (pCR) post-nCRT, allowing organ-preserving approaches, such as the Watch and Wait protocol. However, predicting pCR and differentiating between responders (nCRT-R) and non-responders (nCRT-NR) remains challenging, as current clinical, radiological, and molecular tools lack sufficient accuracy. Identifying predictive biomarkers for nCRT response could thus transform LARC clinical management. Research consistently highlights that tumor-infiltrating lymphocytes (TILs), especially those enriched with cytotoxic CD8+ T-cells, correlate with improved outcomes across various cancers and treatments. Recent findings indicate that some LARC tumors exhibit “hot” immune microenvironments, which may be associated with enhanced nCRT responses. Furthermore, an immunohistochemistry-based scoring system that evaluates CD3+ and CD8+ T-cell enrichment in LARC TILs is being explored as a potential preoperative predictor of response. This study assesses whether elements of the anti-tumor immune response, such as the antigen-presentation pathway, might serve as viable molecular biomarkers for response. We conducted exome and T-cell receptor (TCR) sequencing on 69 LARC tumors (15 nCRT-R and 37 nCRT-NR) to investigate possible associations between HLA, neoantigens, and TCR genetic diversity and nCRT response. Results showed that the neoantigen load, a measure of tumor immunogenicity, was significantly higher in nCRT-NR patients (Wilcoxon test, p=0.03). HLA somatic mutations, however, had no significant impact on antigen presentation or drug resistance, with only 8 mutations identified in 8 patients (6 nCRT-NR and 2 nCRT-R; Pearson’s Chi-squared, p=0.76). HLA polymorphism did not reveal significant differences between response groups, showing no enrichment for specific HLA alleles or loss of heterozygosity (Pearson’s Chi-squared, p=0.64). TCR diversity also showed no association with response (Wilcoxon test, p=0.25), and no differences were detected in TCR gene composition between nCRT-NR and nCRT-R. Notably, we could not quantify the specific repertoire of anti-tumor CD8+ T-cells. Finally, we estimated the probability of HLA-neoantigen-TCR interactions through a machine-learning algorithm and observed that they are more frequent in nCRT-R patients (Wilcoxon test, p&lt0.001). This is the first time that the anti-tumor adaptative immune response was associated with nCRT response based on tumor and host immunogenetic data (tumor-specific neoantigen, HLA, and TCR sequences from TILs), corroborating the growing evidence of the predictive value of the basal immune repertoire.

#3327 Obesity-mediated extracellular vesicle secretion: A potential target for preventing endometrial hyperplasia and cancer initiation.

Lakshmi Narasimhan Chakrapani[1], Kalpana Deepa Priya Doraiyappan[1], Ganesh Yadagiri[1], Jessica Velasquez[1], Shyam Sundaram[1], Takahiko Sakaue[2], Casey Cosgrove[1], George Larry Maxwell[3], David M. O'Malley[1], David E. Cohn[1], Selvendiran Karuppaiyah[1]

[1]Obstetrics and Gynecology, The Ohio State University, Columbus, OH,[2]Gastroenterology, Kurume University, Kurume, Japan,[3]Gynecology, Inova Fairfax Hospital, Falls Church, VA

Introduction: Endometrial cancer (EC) is the most common gynecologic malignancy in the U.S., with obesity contributing to 57% of cases. This study investigates the molecular mechanisms linking obesity to EC, focusing on extracellular vesicle (EV) secretion and oncogenic protein regulation in adipose and uterine tissues. Understanding these pathways could lead to innovative preventive and therapeutic strategies for obesity-related EC.

Methods: Endometrial hyperplasia and cancer were induced in immunocompetent mice using high-fat diets (HFD; 45% or 60% kcal from fat) for 25 weeks. Molecular profiling was performed using LC-MS/MS, while EV quantification and size analysis were conducted via nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM), respectively. The expression of TMEM205, STAT5, FAS, and PIAS3 was assessed using immunohistochemistry (IHC), ELISA, and RT-PCR in patient and HFD-treated mouse adipose and uterine tissues.

Results: EV secretion and the regulation of oncogenic proteins were significantly elevated in adipose and uterine tissues from obese EC patients compared to non-obese controls. In mice fed a 45% or 60% kcal HFD for 25 weeks, we observed increased body weight, abdominal adipose tissue, uterine horn enlargement, and heightened inflammation, correlating with endometrial hyperplasia and cancer initiation. This was associated with increased EV secretion, upregulated TMEM205, FAS, and STAT5 expression, and downregulation of the tumor suppressor PIAS3. Furthermore, HFD-induced EV secretion carrying oncogenic proteins were linked to elevated serum glucose, lipid and Free fatty acid levels. A small-molecule inhibitor, DAP-5, selectively targeting EV secretion, significantly reduced body weight and adipose tissue accumulation in HFD-treated mice. DAP-5 treatment also restored normal uterine morphology and reduced the expression of EV-related oncogenic proteins.

Conclusion: This study highlights obesity-mediated EV secretion as a key contributor to the pathogenesis of EC and identifies it as a potential target for prevention. Preclinical findings support further investigation into EV-targeted therapies, including initiating first-in-human trials to prevent obesity-driven EC.

CLINICAL RESEARCH: Prognostic Biomarkers 2
Poster Session

#3331 Genomic landscape and prognostic indicators in Taiwanese high-grade glioma.

Chun-Yu Liu, Ji-Lin Chen, Chun-Fu Lin, Jui-To Wang, Wei-Hsin Wang, Cheng-chia Lee, Hsin-Hung Chen, Yi-Wei Chen, Shih-Chieh Lin, Chih-Chun Wu, Chi-Cheng Huang, Sanford P. C. Hsu

Taipei Veterans General Hospital, Taipei, Taiwan

Purpose: High-grade gliomas, including glioblastoma (GBM), are characterized by aggressive phenotypes and a high recurrence rate. Molecular subtyping within high-grade gliomas has advanced diagnostic precision and opened pathways for personalized medicine. IDH mutation status, a critical determinant of high-grade glioma subtypes, reflects distinct patterns in genetic profiles and treatment responses. Given the poor prognosis of high-grade gliomas, there is an urgent need for developing diagnostic tools and therapeutic strategies.
Experimental Design: In this VGHTPE cohort, we recruited 192 patients diagnosed with high-grade glioma/GBM between 2009 and 2024. Tumor samples were collected for genomic DNA extraction and analyzed using the FoundationOne CDx test. We assessed the association between genomic alterations and clinical outcomes.
Results: Tumor samples from 192 patients (ages 4-82) included 134 IDH-wildtype glioblastomas (69.8%) and 58 IDH-mutant high-grade gliomas (30.2%) based on IDH1/2 status. In the VGHTPE cohort, 21 genes displayed genomic alterations in at least 5% of cases. Over 50% of IDH-wildtype glioblastoma patients exhibited TERT promoter or CDKN2A/B mutations, while over 50% of IDH-mutant glioma patients had TP53 and ATRX mutations. Notably, genes predominantly altered in recurrent tissues were linked to Rap1 signaling. Our analysis showed that IDH1 mutations co-occurred with ATRX and CIC alterations but were mutually exclusive with CDKN2A/B, EGFR, MTAP, and PTEN alterations. Alterations in CDKN2A/B, MTAP, EGFR, PTEN, PIK3CA, and KIT were associated with unfavorable outcomes, whereas patients with IDH1, ATRX, and CIC alterations demonstrated better progression-free survival.
Conclusion: This study provides a comprehensive genomic profile of Taiwanese patients with high-grade glioma or GBM, supporting the identification of potential prognostic biomarkers.

#3332 Cancer organoid growth rate as a phenotypic marker to predict adjuvant chemotherapy outcomes in ovarian cancer patients.

Dong Woo Lee[1], Sang-Yun Lee[2], Sung Hun Ju[2], Bosung Ku[3], Yongmun Choi[3], Jeong Woo Lee[4]

[1]Gachon Univ. of Medicine and Science, Suwon-si, Korea, Republic of,[2]Gachon Univ. of Medicine and Science, Seongnam-si, Korea, Republic of,[3]Medical & Bio Decision (MBD) Co., Ltd, Suwon-si, Korea, Republic of,[4]Sungkyunkwan University School of Medicine, Suwon-si, Korea, Republic of

Background: Patient-derived organoid (PDO)-based drug sensitivity tests have been utilized to predict patient outcomes, such as recurrence after adjuvant chemotherapy, by assessing the drug response of patient-derived organoids. This study introduces a multi-parameter assay platform called Organoid Growth-based Oncological Sensitivity Test (OncoSensi) for predicting recurrence of adjuvant therapy in patients with Epithelial Ovarian Cancer (EOC). Patient-derived organoid (PDO)-based drug sensitivity tests have been utilized to predict patient recurrence after adjuvant therapy by assessing the drug response of PDOs. However, we found that PDO growth rates vary among individuals, and it is a very important factor in predicting patient recurrence.
Methods: Among 135 patients, 95 ovarian cancer patients were followed up for recurrence over a 2-year period after receiving adjuvant chemotherapy (Paclitaxel/Carboplatin). PDOs derived from these 95 patients were utilized to measure growth rates and drug responses without subculturing. Recurrence-free survival rates were compared between responders and non-responders identified by OncoSensi.
Results: In this study, we propose an organoid growth-based Oncological Sensitivity Test (OncoSensi) that utilizes the PDO growth rate-based approach to specifically improve the prediction of adjuvant therapy outcomes (recurrence) in patients with EOC. Comparing conventional drug sensitivity tests that considered only single parameters such as the area under the curve (AUC), the proposed OncoSensi algorithm showed improved 6-month, 1-year, 1.5-year recurrence prediction performance in patients with EOC. The recurrence rate within one year for responder groups in OncoSensi is 4.76 %, which is three times lower than the 14.29 % recurrence rate of the previous sensitivity test. For non-responder groups, the recurrence rate within one year for OncoSensi is 41.51 %, which is higher than the 33.96% of the previous sensitivity test. In addition, the responder and non-responder groups were statistically well distinguished (P-value < 0.0001) under OncoSensi index-based analysis conditions. Thus, the good recurrence prediction performance was confirmed using the OncoSensi algorithm. The recurrence rate within one year for responder groups in OncoSensi is 4.76 %, which is three times lower than the 14.29 % recurrence rate of the previous sensitivity test. For non-responder groups, the recurrence rate within one year for OncoSensi is 41.51 %, which is higher than the 33.96% of the previous sensitivity test.

#3333 Ultra-sensitive detection of KRAS mutations in circulating tumor DNA predicts survival in pancreatic adenocarcinoma.

Madison Cox[1], Amy Wells[1], Dominic Vitello[1], Larissa Masnyk[1], Chengwei Peng[2], John Abad[1], Qiang Zhang[2], Pan Du[3], Shidong Jia[3], Akhil Chawla[1]

[1]Department of Surgery, Division of Surgical Oncology, Northwestern University Feinberg School of Medicine, Chicago, IL,[2]Department of Medicine, Division of Medical Oncology, Northwestern University Feinberg School of Medicine, Chicago, IL,[3]Predicine, Inc., Hayward, CA

Introduction: The Kirsten rat sarcoma viral oncogene homolog (*KRAS*) gene is mutated in over 90% of pancreatic adenocarcinoma (PDAC). These mutations are predominantly single-base missense variants, 98% of which are found at codon 12 (*G12*), codon 13 (*G13*), or codon 61 (*Q61*). Numerous studies have demonstrated the clinical utility of these *KRAS* mutations in PDAC, more recently employing liquid biopsy technology as a preferred method to analyze mutations non-invasively. This study aimed to investigate the prognostic potential of pathogenic *KRAS G12*, *G13*, or *Q61* mutations harbored by circulating tumor DNA (ctDNA) in resectable PDAC prior to treatment initiation using an ultra-sensitive liquid biopsy panel.
Methods: Plasma samples for patients at our institution with resectable disease at diagnosis were collected prior to chemotherapy initiation. This study cohort included 45 patients (median age 73; 42% male and 58% female; 86.7% Non-Hispanic White, 2.2% Non-Hispanic Black, 8.9% Hispanic White, 2.2% American Indian). At diagnosis, 68.8% had resectable disease (Stage IA-IIA), 15.6% had borderline resectable disease (Stage IIB), and 15.6% had locally advanced disease (Stage III). 38 patients received 3-12 cycles of neoadjuvant chemotherapy with modified FOLFIRINOX and/or Gemcitabine/Abraxane, 27 patients received 1-8 cycles of adjuvant chemotherapy, and 29 patients underwent upfront or interval surgery with Whipple procedure or distal pancreatectomy. Baseline ctDNA was analyzed for *KRAS G12*/*G13*/*Q61* mutations using the PredicineCARE liquid biopsy assay (20,000x sequencing depth) and the PredicineCARE Ultra assay (>100,000x sequencing depth), providing full *KRAS* gene coverage.
Results: The PredicineCARE assay identified pathogenic *KRAS* mutations (*G12*/*G13*/*Q61)* in 11 patients. Of these, 8 had co-occurring *G12* and *Q61* mutations. Presence of any *KRAS* mutation determined by CARE assay at baseline was not significantly associated with overall survival (OS) (HR 2.96, 95% CI 0.85-10.29), however, detection of a *G12* mutation was significantly predictive of worse OS (HR 4.93, 95% CI 1.39-17.44). Co-occurrence of *KRAS G12* and *Q61* mutations in baseline ctDNA was also predictive of worse OS (HR 3.89, 95% CI 1.12-13.54). The PredicineCARE Ultra assay identified KRAS mutations in an additional 7 patients who were mutation-negative by the standard CARE assay. Baseline *KRAS G12/G13* mutations detected with the Ultra assay were predictive of OS (HR 3.71, 95% CI 1.04-13.28), in addition to demonstrating 100% concordance with CARE assay results for overlapping mutations.
Conclusion: Ultra-sensitive liquid biopsy assays such as the PredicineCARE Ultra panel demonstrate robust detection of pertinent KRAS mutations that significantly improves prognostic stratification for PDAC patients, demonstrating the potential of advanced liquid biopsy technology in improving outcomes in PDAC.

#3334 Expression analyses of SLC3A2 and its associated genes in glioma progression.

Birnur Sinem Karaoglan[1], Ozan Topcu[1], Ecren Ecem Uzlu[1], Osman Aliskan[1], Thomas Mohr[2], Nazli Cakici[3], Ahmet Tulgar Basak[3], Asli Cakir[4], Nihal Karakas[5]

[1]Cancer Research Center, Research Institute for Health Sciences and Technologies (SABITA), Istanbul Medipol University, Istanbul, Turkey,[2]Center of Cancer Research, Department of Medicine, Medical University of Vienna and Comprehensive Cancer Center, Vienna, Austria,[3]Department of Neurosurgery, Istanbul Medipol University School of Medicine, Istanbul, Turkey,[4]Department of Pathology, Istanbul Medipol University School of Medicine, Istanbul, Turkey,[5]Department of Medical Biology, Istanbul Medipol University International School of Medicine, Istanbul, Turkey

High grade gliomas (grade III and IV) including glioblastomas (GBM, grade IV, IDH wild type), are highly aggressive brain tumors with limited treatment options and poor prognosis. Tumor recurrence is inevitable with standard methods including surgical debulking, radio- and chemotherapies. For this reason, a broad range of studies focus on the discovery of diagnostic and therapeutic markers to cure the malign disease. SLC3A2, a polyamine transporter, has been implicated in chemoresistance of tumors and identified as a prognostic marker in renal cell carcinoma. Accordingly, in this study, we examined i) the prognostic impact of SLC3A2 expression in gliomas, ii) its association with differentially expressed gene networks, and iii) its molecular characteristics to elucidate its role in glioma biology. Immunohistochemical and western blot analyses revealed significant overexpression of SLC3A2 in high grade glioma tissues compared to controls. Robust SLC3A2 expression was also observed in a pannel of GBM cell lines. Cohort analysis of the TCGA French glioma patient dataset (n=264) stratified patients by SLC3A2 expression, showing a significant survival advantage for low expressors across glioma grades (p=.0021). This survival benefit was particularly pronounced in high-grade gliomas, underscoring the link between SLC3A2 expression and tumor aggressiveness. Differentially expressed gene analysis identified several druggable genes co-regulated with SLC3A2. Differential expression of VEGFA and ADM in SLC3A2 high-expressors suggests a potential role of Slc3a2 in tumor angiogenesis, while links to CHI3L1 and CXCL14 imply roles in enhancing tumor invasiveness and immune modulation. In contrast, TOX3 and SNAP91 were enriched in SLC3A2 low-expressors, pointing to suppression of neuronal differentiation and maintenance of glioma stem-like properties. Weighted gene co-expression analysis linked SLC3A2 to genes involved in cytoskeletal remodeling (DMPK), and chromatin regulation (RNF40), reinforcing its role in tumor plasticity. Negative correlations with SIRT1 and KAT6B suggest a metabolic shift favoring tumor-specific survival and growth. These findings lay the groundwork for exploring SLC3A2 as a therapeutic target and its potential in personalized therapies. Further studies will clarify its mechanistic role in gliomagenesis and its utility in clinical applications. This study was supported by The Scientific Research Commision of İstanbul Medipol University with project number 2023-66.

#3335 Non-tumor-bearing tissue: A prognostic biomarker for cancer.

Ji Hye Choi[1], Yeong Hak Bang[2], YeonJu Lee[1], Woong-Yang Park[2]

[1]Samsung Advanced Institute of Health Sciences and Technology, Sungkyunkwan University, Seoul, Korea, Republic of,[2]Samsung Genome Institute, Samsung Medical Center, Seoul, Korea, Republic of

Background: We hypothesized that distinct microenvironments either support or inhibit the growth of cancer cells, and that the non-tumor bearing tissue (NBT) reflects these microenvironments. Additionally, we assumed that the more closely the characteristics of the NBT resemble those of the tumor, the greater the likelihood of supporting cancer cell progression and promoting disease recurrence.
Method: We investigated TCGA dataset with more than 20 samples of NBT and paired tumors. We employed a scoring system using the top 10-50 DEGs with high fold-change values in tumors. We scored the expression of the tumor-supportive signature genes in the NBT using single-sample gene set enrichment analysis (ssGSEA). We evaluated the mean score derived from the top 10-50 in the NBT to categorize the patients into two groups that most effectively facilitated prognostic differentiation. The groups were defined as follows: patients showing relatively high tumor supportive signatures in NBT (patients with tumor-supportive microenvironment in NBT; TSM), and patients showing relatively low supportive signatures in NBT (patients with healthy microenvironment in NBT; HM).
Results: Among the 12 cancers, the significantly poorer overall survival (OS) in TSM were identified in head and neck cancer squamous cell carcinoma (HNSC; hazard ratio [HR]: 2.06 [1.03 - 4.10]), lung squamous cell carcinoma (LUSC; hazard ratio [HR]: 3.87 [1.69 - 8.90]), kidney renal cell carcinoma (KIRC; hazard ratio [HR]: 2.85 [1.31 - 6.21]). In other cancer types, there are no prognostic differentiations. The 3-year overall survival in the TSM group compared to the HM group were markedly lower: HNSC (0.569 vs. 0.813), LUSC (0.242 vs. 0.492), and KIRC (0.250 vs. 0.689). Pathway analysis suggested that leukocyte-mediated immunity and inflammatory pathways were upregulated, whereas the maintenance of epithelial barriers was decreased in the NBT of the TSM group in HNSC, LUSC and KIRC. Immune cell deconvolution analysis revealed that the TSM group had a significantly higher proportion of neutrophils compared to that of the HM group in HNSC and LUSC. In KIRC, significantly higher proportion of CD8+ T cells and memory CD4+ T cells were observed in the NBT of the TSM group.
Conclusions: Our findings suggest that NBT serves as a prognostic biomarker that effectively predicts cancer invasiveness and tumor-promoting inflammation, particularly in HNSC, LUSC, and KIRC. This also highlights the importance of maintaining a healthy microenvironment.

#3336 Early detection of tumor relapse, racial disparity, and treatment resistance in triple negative breast cancer.

Amy H. Tang[1], Richard A. Hoefer[2], Mary L. Guye[3], Billur Samli[4], Janet S. Winston[4], Jennifer Koblinski[5], Valentina Robila[6], Michael O. Idowu[7], Rick J. Jansen[8], Harry D. Bear[9]

[1]Biomedical and Translational Sciences, Macon & Joan Brock Virginia Health Sciences at Old Dominion University, Norfolk, VA,[2]Sentara Surgery Specialists, Sentara CarePlex Hospital, Sentara Cancer Network, Sentara Healthcare, Norfolk, VA,[3]Sentara Cancer Network, Sentara Healthcare, Sentara Surgery Specialists, Sentara CarePlex Hospital, Newport News, VA,[4]Pathology Sciences Medical Group, Sentara Norfolk General Hospital, Norfolk, VA,[5]Massey Cancer Center, Virginia Commonwealth University, Richmond, VA,[6]Department of Pathology, Division of Anatomic Pathology, Virginia Commonwealth University, Richmond, VA,[7]Pathology, Virginia Commonwealth University, Richmond, VA,[8]Masonic Cancer Center, University of Minnesota, Minneapolis, MN,[9]Departments of Surgery and Microbiology & Immunology, Massey Comprehensive Cancer Center, Virginia Commonwealth University/VCU Health, Richmond, VA

Introduction: Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype that disproportionately affects BRCA1 mutation carriers and young Black/white women. Black/AA patients have the highest mortality and the shortest survival of any racial/ethnic group in the US. Pembrolizumab and neoadjuvant chemotherapy (NACT) has become the standard of care (SOC) for high-risk early-stage TNBC. Pathologic complete response (pCR) predicts good outcome, whereas pathologic incomplete response (pIR) with high residual cancer burden (RCB) is correlated with early tumor relapse, treatment resistance, and poor survival. Many similarly treated patients with identical TNM and RCB classifications experience treatment disparity, distinct relapse rates, and disparate survival. Current methods fall short in predicting early tumor relapse/chemo-resistance/patient survival with high accuracy in the clinic.

Methods: We have conducted IHC staining of EGFR, Ki67, SIAH and PD-L1 expression in a large cohort of TNBC patients (> 1,000 patients), of which 48% patients are Black/AA and 48% patients are white patients, from the Sentara Cancer Network and VCU Massey Comprehensive Cancer Center, both of which serve socio-economically-disadvantaged, medically underserved, and underinsured racial/ethnic minorities in low-income communities in Virginia.

Results: We found that high SIAH expression in residual tumors reflects ineffective therapy and persistent EGFR/K-RAS/SIAH pathway activation (ON) and predicts early relapse, chemo-resistance, and poor survival. Conversely, low SIAH expression in residual tumors reflects effective therapy and EGFR/K-RAS/SIAH pathway inactivation (OFF) and predicts tumor remission and good survival. We detected a major racial disparity in the large TNBC cohort. Black/AA breast cancer patients suffer a higher MBC mortality and reduced survival compared to their white counterparts in our clinical studies similar to the SEER/CDC databases.

Conclusions: We found that persistent EGFR/RAS/SIAH pathway activation is a major driving force in TNBC malignancy. As an evolutionarily conserved RING-domain E3 ligase, SIAH is a new prognostic biomarker for patient risk stratification, tumor relapse prediction, and racial disparity detection in TNBC. We aim to validate the prognostic power of SIAH and develop a SIAH-centered biomarker panel for patient risk stratification and relapse/resistance/survival prediction in TNBC.

#3337 Unveiling m6A epitranscriptome-related genomic variations in bladder cancer: predictive biomarkers for disease progression and treatment outcome towards tailored therapeutics.

A. Scorilas[1], K.-M. Pilala[1], P. Tsiakanikas[1], K. Panoutsopoulou[1], M.-A. Papadimitriou[1], K. Soureas[1], G.-C. Giagkos[1], P. Levis[1], G. Kotronopoulos[1], Z. Kanaki[2], I. Prassas[3], L. Dimitrakopoulos[3], G. Yousef[3], K. Stravodimos[1], S. Koroneou[1], M. Avgeris[1];
[1]National and Kapodistrian University of Athens, Athens, Greece, [2]Biomedical Research Foundation Academy of Athens, Athens, Greece, [3]University of Toronto, Toronto, ON, Canada

Due to its highly heterogenous molecular landscape, bladder cancer (BlCa) is still characterized by non-personalized prognosis and treatment decisions. N6-methyladenosine (m6A) has emerged as the most common and conserved internal mRNA modification, regulating RNA metabolism and translation. Herein, we have profiled mutations and copy number variations (CNVs) within m6A RNA machinery genes and assessed their clinical relevance in BlCa patients' prognosis and treatment outcome. DNA-seq libraries were prepared from 96 bladder specimens (tumors: n=87; normal urothelium: n=9) using a custom-designed panel of enrichment probes for m6A writers (*METTL3, METTL14, METTL16, VIRMA, WTAP, RBM15, RBM15B*, *ZC3H13*), erasers (*FTO, ALKBH5*) and readers (*YTHDF1, YTHDF2, YTHDF3, YTHDC1, YTHDC2, IGF2BP1, IGF2BP2, IGF2BP3*). *METTL3* methyltransferase expression was quantified by RT-qPCR. Mutational and CNV analyses of DNA-seq data were performed by SNPNexus and CNVkit, respectively. Kaplan-Meier curves and Cox regression analysis were implemented for patient's survival analysis. Internal validation was performed by bootstrap analysis. DNA-seq on DNBSEQ-G400 revealed that ~30% of bladder tumors (25/87) harbored deleterious mutations in m6A writers, while m6A erasers (3,2%) and readers (5,3%) were less mutated. CNVs were found in ~60% of tumors (52/87), with *VIRMA* frequently amplified (22%) in superficial (TaT1) tumors. Reduced *METLL3* expression was detected in muscle-invasive tumors (p=0.009) and associated with increased risk for NMIBC relapse (p=0.002) and progression (p=0.028) to MIBC. Moreover, MIBC patients with deleterious mutations in m6A writer's complex presented significantly higher progression risk (p=0.036) and worse survival (p=0.010), while *VIRMA* copy-number gain/amplification was associated with significantly increased risk of short-term relapse (p=0.019) in NMIBC. Finally, multivariate Cox regression confirmed m6A writer complex mutations as independent marker for patients' progression (HR=3.242, p=0.021) and death (HR=5.388, p=0.006) in MIBC, and of *VIRMA* gain/amplification as an independent indicator of NMIBC relapse risk (HR=3.564, p=0.010).Genomic variations in m6A machinery emerge as modern molecular markers to address BlCa clinical heterogeneity and guide personalized prognosis and treatment/monitoring decisions. *Acknowledgements:* The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "2nd Call for H.F.R.I. Research Projects to support Faculty Members & Researchers" (Project Number: HFRI-FM20-3765)

#3338 A CpG methylation based epigenetic signature for recurrence prediction in stage II/III colorectal cancer: *The CENSURE Study*.

Caiming Xu[1], Silei Xu[1], Joan Maurel[2], Man Li[3], Ajay Goel[1]

[1]Beckman Research Institute of The City of Hope, Monrovia, CA,[2]Department of Medical Oncology, Hospital Clinic of Barcelona, Barcelona, Spain,[3]Department of Oncology, The Second Affiliated Hospital of Dalian Medical University, Dalian, China

Introduction: Adjuvant chemotherapy (ACT) is the standard of care for patients with stage III or high-risk stage II colorectal cancer (CRC) following curative-intent resection. However, over 30% of these patients experience recurrence despite treatment, highlighting the urgent need for developing predictive biomarkers of response to ACT. This study sought to identify epigenetic biomarkers for predicting recurrence in stage II/III CRC.

Patients and Methods: This study included three phases: discovery, training, independent testing. In the discovery phase, we performed genomewide methylation profiling analysis between recurrent (recurrence-free survival, RFS < 5 years) and non-recurrent patients with CRC. A LASSO-based machine learning algorithm was employed to refine and optimize the candidate CpG sites. Subsequently, we developed and tested a methylation biomarker panel by quantitative pyrosequencing assays, followed by eXtreme Gradient Boosting (XGB) machine-learning algorithm in both the training (n = 236) and testing (n = 175) cohorts. Finally, we established the CENSURE (***C****pG m****E****thylatio****N****-based epigenetic ****S****ignat****URE***) model, which combined the CpG methylation panel and carcinoembryonic antigen (CEA) levels, to predict recurrence in stage II/III CRC.

Results: A panel of 8 CpG candidates identified in the discovery phase was refined to 6 using XGB in the training cohort. The 6-CpG panel demonstrated robust ability to predict recurrence, with AUC values of 91.2% and 79.3% in the training and testing cohorts, respectively. Multivariate Cox regression analysis revealed that CEA ≥ 5 ng/mL (HR 2.92; 95% CI: 1.81-4.70) and the CpG panel (HR 19.5; 95% CI: 8.78-43.1) were independent predictors of recurrence. Building on this, we developed the CENSURE model, which integrates the CpG panel with CEA. The CENSURE model achieved AUC values of 95.2% and 86.1%, with sensitivities of 83.9% and 83.3% in the training and testing cohorts, respectively. CENSURE high-risk patients exhibited significantly higher 5-year (71.5% vs 8.0%) and 2-year recurrent rates (48.0% vs 5.4%). Notably, the CENSURE model outperformed CEA risk stratification alone, correctly predicting an additional 46.5% (40 out of 86) of recurrence cases in the entire study cohort.

Conclusions: In conclusion, the CENSURE assay represents a novel potential advance with a significant clinical impact for postoperative recurrence prediction in stage II/III CRC.

#3339 Evaluation of GATA6 and KRT17 immunostaining for subtype classification in pancreatic ductal adenocarcinoma.

Ruomeng Fang[1], Joan Miguel Romero[1], Yifan Wang[2], Alicia Gomez Mendez[3], Adeline Cuggia[3], George Zogopoulos[3]

[1]Clinical and Translational Research, McGill University, Montreal, QC, Canada,[2]Surgical Oncology, MD Anderson Cancer Center, Houston, TX,[3]Research Institute of the McGill University Health Centre, Montreal, QC, Canada

Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal malignancy with poor survival outcomes. Transcriptomic profiling has identified distinct PDAC subtypes associated with survival, highlighting their clinical relevance. However, reliance on RNA sequencing for subtype classification is not feasible in routine clinical practice, underscoring the need for surrogate biomarkers that are both effective and practical for predicting patient outcomes. This study evaluated GATA6 and KRT17 as multiplex immunohistochemistry (IHC) biomarkers to develop a prognostic model for PDAC subtype classification. Using a tissue microarray (TMA) comprising 130 patient-derived xenografts (PDXs), we quantified cell IHC positivity and stratified samples into subtypes based on GATA6 and KRT17 expression. Whole-slide staining of representative patient tumors validated the consistency of subtype classifications between PDXs and corresponding patient tumors. Patient TMAs (224 cores from 82 tumors) were subsequently analyzed to refine the classification system, incorporating GATA6 and KRT17 positive cell percentages, staining intensity (H-scores), and clinical variables into a comprehensive prognostic model. Initial classification based on PDX models demonstrated non-significant distinct survival trends ($p=0.14$), likely due to sample size limitations. However, a refined model applied to patient TMAs that incorporated additional parameters effectively stratified patients into prognostic groups with significantly different overall survival ($p=0.042$). These findings highlight the utility of GATA6 and KRT17 as surrogate classifiers for PDAC subtype classification and prognostication, offering a practical alternative to transcriptomic sequencing in HR-proficient PDAC.

#3340 A miRNA based signature for the individual assessment of the prognosis of patients with seminoma.

Farzaneh Zohari[1], Lea Eckhart[2], Hiresh Ayoubian[1], Hans-Peter Lenhof[2], Rainer Bohle[3], Felix Bremmer[4], Fabian A. Gayer[5], Yue Che[6], Marieke Vermeulen-Spohn[6], Tim Nestler[7], Michael Stockle[8], Julia Heinzelbecker[8], Kerstin Junker[8]

[1]Department of Urology and Pediatric Urology, Saarland University, Homburg/Saar, Germany,[2]Saarland Informatics Campus, Saarland University, Center for Bioinformatics, Saarbrucken, Germany,[3]Institute of Pathology, Saarland University Medical Center and Saarland University, Homburg/Saar, Germany,[4]Institute of Pathology, University Medical Center Gottingen, Gottingen, Germany,[5]Department of Urology, University Medical Center Gottingen, Gottingen, Germany,[6]Department of Urology, Heinrich-Heine University, Medical Faculty, Dusseldorf, Germany,[7]Department of Urology, Bundeswehr Central Hospital Koblenz, Koblenz, Germany,[8]Department of Urology and Pediatric Urology, Saarland University Medical Center and Saarland University, Homburg/Saar, Germany

Introduction: Due to the risk of long-term toxicities in young seminoma patients, surveillance regimens nowadays are the standard of care in patients without metastases. Nevertheless, up to 20% will relapse with the necessity of definitive treatment exposing them to an even higher amount of therapy compared to in the adjuvant setting. This study seeks to establish a prognostic miRNA profile on primary tumors utilizing bioinformatics tools to differentiate between metastatic (m) and non-metastatic (nm) seminomas.
Materials and methods: MiRNA was extracted from formalin-fixed paraffin-embedded sections. Two cohorts of patients with seminoma (each 24 patients, each 12 nm and 12 m) were investigated by microarray analysis. Validation was performed in 103 patients (47 nm and 56 m) through quantitative PCR (qPCR) using the TaqMan® assay, with RNU48, miR-191-5p, and miR-361-5p serving as references. Statistical and bioinformatics analyses were performed by using the Mann-Whitney U test and machine learning algorithms.
Results: The bioinformatics analysis of microarrays identified 16 miRNAs exhibiting an accuracy >70% in differentiating m tumors from nm ones. qPCR analysis of seven selected miRNAs demonstrated significant expression differences between m and nm tumors for miRNA-371a-5p, miR-512-3p and miR-517a-3p ($p<0.05$), contingent upon the reference used. Analyzing metachronous and synchronous m tumours separately, all 3 miRNAs showed significantly different expression only in synchronous m, although a trend was observed in metachronous m.
Conclusions: By employing machine-learning on microarray data, we established a miRNA signature that differentiates metastatic from non-metastatic seminomas with high accuracy. The findings have been validated for 3 miRNAs in independent cohorts by qPCR, reinforcing the potential of miRNAs as prognostic markers in seminomas. Ongoing validation efforts are currently being conducted in independent cohorts to define a robust miRNA signature.

#3341 The co-expression and prognostic significance of the HER-family members, EGFRvIII, CD44, CD109 and Claudin 18.2 in patients with stomach adenocarcinoma.

Tina Al-Janaby[1], Izhar Bagwan[2], Said Khelwatty[1], Helmout Modjtahedi[3]

[1]Kingston Univ. London School of Life Sci., Kingston, United Kingdom,[2]Royal Surrey Hospital, Guildford, United Kingdom,[3]Kingston Univ. London School of Life Sci., Kingston upon Thames, United Kingdom

Stomach cancer remains a major cause of death worldwide. While targeted therapies with anti-HER2 monoclonal antibodies (mAbs) such as trastuzumab, antibody-drug-conjugate trastuzumab deruxtecan, and more recently zolbetuximab, an anti-claudin 18.2 (CLDN 18.2) mAb, have been approved for the treatment of stomach cancer, many patients may not respond or have a response of short duration to such treatments. Therefore, there is an urgent need for identification of novel biomarkers for the response to therapy, and the development of more effective therapeutic interventions. In the past few decades, the crosstalk between the HER-family and other biomarkers such as cancer stem cells have been proven to promote tumorgenicity, metastasis and resistance to anti-HER2 therapy. Therefore, in this study, for the first time, we examined the relative expression levels, and prognostic significance of all members of the HER-family, the type-III EGFR mutant (EGFRvIII), CD44, CD109, and CLDN 18.2 in patients with stomach cancer by immunohistochemistry. The staining was scored at different cut off values (i.e. >5%, 10%, 20% and 50% of tumours with positive staining), the cellular location, and the intensity of staining. In addition, the prognostic significance of the expression and co-expression of these biomarkers were also investigated. We found the expression of wild-type EGFR (12.8%), HER2 (82.1%), HER3 (9.0%), HER4 (33.3%), EGFRvIII (33.3%), CD44 (41.0%), CD109 (60.3%) and CLDN 18.2 (39.7%) in tumour specimens from 78 patients. Furthermore, the expression of HER2 was accompanied with the co-expression of EGFR (9%), HER3 (8%), HER4 (27%), EGFRvIII (28%), CD44 (33%), CD109 (49%) and CLDN18.2 (32%) in the cases examined respectively. [SK1] [SK2] Interestingly, at a cut-off value of >5% of tumour cells with positive staining, the co-expression of HER2/EGFRvIII (7.26 vs 4.43 years, P=0.028), EGFRvIII/CD44 (7.02 vs. 2.17 years, P=0.003) HER2/EGFRvIII/CD44 (6.93 vs. 2.33 years, P=0.011), and CD109/CLDN 18.2 (7.01 vs 3.99 years, P= 0.021) were associated with a poorer overall survival. Moreover, the membranous expression of CD109 (7.06 vs. 3.93 years, P=0.028), and its co-expression with EGFRvIII/CD44 were also associated with a poorer overall survival (7.01 vs 1.65 years, P=0.0009) and a higher risk of poor overall survival [SK3] (HR=4.21, P=0.0009) as well as CLDN 18.2 immunostaining of intensity of 3+ (6.83 vs. 3.50 years, P=0.032). All these remained as independent prognostic factors for overall survival in multivariate analysis. Taken together, our results suggest co-expression of HER-2 with other members of the HER family, EGFRvIII, CD44, CD109 and CLDN 18.2 occurs in patients with stomach cancer and warrants further investigation on the predictive value of such biomarkers for the response to therapy with inhibitors to HER family members and CLDN 18.2.

#3342 Congruence of tumor-specific ctDNA and radiographic recurrence in high-risk sarcoma.

Chahat Rana, Daniel Y. Wang, Amanda M. Dann, Nissy A. Alex, **Sanjay Chandrasekaran**

Harold C. Simmons Comprehensive Cancer Center, Dallas, TX

Background: Surveillance imaging remains the best method for detecting recurrence of high-risk sarcoma in patients off therapy. Whether tumor-specific ctDNA (circulating tumor DNA, Signatera) adds prognostic value to radiography remains unknown. We hypothesize that ctDNA conversion from negative to positive (mean tumor molecules per milliliter [MTM/ml]) will correlate with radiographic recurrence.

Methods: We retrospectively assessed congruence of tumor-specific ctDNA and radiographs at serial timepoints in high-risk sarcoma patients 1) off therapy and on surveillance, with 2) scans showing no evidence of disease (NED) and a negative ctDNA +/- 28 days of those scans, and 3) ≥ 1 follow-up data point. Congruence was achieved if ctDNA and scans remained negative or if ctDNA conversion to positivity occurred prior to or ≤ 21 days post imaging recurrence.

Results: 16 patients (9 male/7 female; median age 57yrs, Range 39-77) treated from 9/2021-9/2024 with bone or soft tissue sarcoma (STS) met study criteria. Histologies included 3 ewing sarcoma, 3 leiomyosarcoma (LMS), 3 liposarcoma (LPS), 1 each for osteosarcoma, sclerosing epithelioid fibrosarcoma (SEF), malignant pecoma, clear cell, undifferentiated pleomorphic, and other (2). 3 patients with metastatic disease (LMS, ewing, osteosarcoma) met study criteria. Prior therapies included surgery (14/16), radiation (7/16), and chemotherapy (4/16, 3/3 metastatic). Median follow-up time was 330.5 days (range 73-810) with 6 median assessments per patient (2-11). The median interval between the initial negative ctDNA and NED scan was 10 days (1-28) and 16 days (1-63) across all assessments. 10/16 patients (62.5%) remained NED on scans and 6 (37.5%) recurred, with ctDNA congruence in 9/10 (90%) and 5/6 (83.3%), respectively. 1/10 (10%) NED patients with myxoid LPS had a single positive ctDNA event (0.18 MTM/mL) with 2 subsequent negative events. All 3 metastatic patients recurred along with 1 SEF, 1 LMS, and 1 pleomorphic LPS. 5/6 positive ctDNA levels were detected 66 (pleomorphic LPS), 49, and 14 (both LMS) days before scans and 8 days post scans (ewing & SEF). An osteosarcoma patient with progression in pulmonary metastasis had 2 negative ctDNA levels before conversion (13.94 MTM/mL, 158 days) and required 2 lung biopsies prior to pathologic confirmation. Altogether, ctDNA was congruent in 14/16 (87.5%) cases, with sensitivity of 83.3% (35.9-99.6%) and specificity of 90% (55.5-99.8%). PPV and NPV were 83.3% (42.9-97.1%) and 90% (59.8-98.2%).

Conclusions: Our findings show that tumor-specific ctDNA may be a useful prognostic sarcoma surveillance tool in high-risk bone and STS patients with local or metastatic disease. ctDNA positivity in 2 cases >6 weeks prior to scans suggests potential to detect early DNA shedding and the need to further examine temporal associations between ctDNA and scans in surveillance patients.

#3343 Centrosomal and mitotic gene expression profiles across cancer types: Implications for tumor progression and survival outcomes.

Christopher L. Farrell, Amy Turner, Alex Feltus, Victoria Cipollino

Clemson University, Clemson, SC

Introduction: Genes involved in centrosome function, microtubule dynamics, and mitotic regulation are critical for normal cell division. In cancer, dysregulation of these processes can lead to chromosomal instability and tumor progression. The genes CEP72, HAUS4, TUBGCP4, HAUS2, PLK1, and OFD1 play essential roles in cell cycle regulation, mitosis, and microtubule dynamics, particularly in spindle assembly and centrosome function. These genes are involved in interconnected cellular processes related to mitotic progression, and their altered expression may be linked to cancer progression and survival outcomes.
Objective: This study analyzes the expression profiles for these six genes across thirteen cancer types, aiming to identify associations between dysregulation of these genes and cancer progression, as well as their impact on survival outcomes.
Methods: We analyzed the expression of these six genes and the tumor suppressor genes APC and PTEN using RNA sequencing data from TCGA cancerous tissues and normal control tissues from GTEx for the thirteen cancer types. Differential gene expression was assessed using the DESeq2 R package, which models RNA sequencing data using a negative binomial distribution. Results:
Our analysis identified significant changes in gene expression across cancers, including bladder (BLCA), colon (COAD, READ), esophagus (ESCA), kidney (KICH, KIRC, KIRP), liver (LIHC), prostate (PRAD), stomach (STAD), and thyroid (THCA). Elevated CEP72 expression was associated with lower progression-free survival (PFS). We observed consistent downregulation of CEP72, HAUS2, and PLK1 across several cancers, with significantly adjusted p-values (padj < 1E-5). OFD1, HAUS4, and PTEN showed tissue-specific expression patterns, with upregulation in cancers such as prostate (padj = 1.1E-11) and thyroid (padj = 8.5E-5). We further identified cancer-specific expression profiles for these six genes involved in cell division. These findings suggest complex regulatory mechanisms influencing tumor progression and metastasis. Further analysis of these mitotic and centrosomal proteins revealed significant cancer-specific correlations between gene expression levels of CEP72, HAUS4, TUBGCP4, HAUS2, PLK1, OFD1 and patient outcomes, including PFS, disease-specific survival, and overall survival.
Conclusion: This study underscores the interconnectedness of centrosomal and mitotic genes, suggesting that downregulation of key proteins like CEP72, HAUS2, and PLK1 disrupts essential cell migration, proliferation, and adhesion pathways. Conversely, upregulation of HAUS4 may enhance tumor suppressor functions and immune modulation. Analyzing these genes within their broader biological networks provides valuable insights into their roles in cancer progression, offering potential as cancer-specific biomarkers or therapeutic targets.

#3344 The 31-gene expression profile identifies patients at risk of developing early distant metastases and can guide risk-appropriate surveillance strategies.

Merve Hasanov[1], Elshad Hasanov[1], David Pariser[2], Sonia Morgan-Linnell[3], Samuel Dierks[3], Brian Martin[3], Abel Jarell[4]

[1]Comprehensive Cancer Center, The Ohio State Univeristy, Columbus, OH,[2]Pariser Dermatology, Norfolk, VA,[3]Research and Development, Castle Biosciences, Inc., Friendswood, TX,[4]Northeast Dermatology Associates, Portsmouth, NH

Introduction: Up to 20% of patients with stage I-II cutaneous melanoma (CM) will develop distant metastases (DMs), with the most frequent DM sites being the liver, lungs, bone, and brain. Although patients who develop DM have poor prognosis, trials of new immunotherapies in patients with DM have reported 5-year survival rates as high as 50%. Critically, early detection of DM, when tumor burden is lower, tends to lead to better treatment responses. Thus, it is important to identify patients at the highest risk of developing DM so clinicians can recommend risk-appropriate surveillance and treatment plans. We previously assessed the ability of the 31-gene expression profile (31-GEP) to predict CNS metastases, and here we expand our analysis to assess 31-GEP risk prediction for DMs to the lung, liver, and bone.

Methods: We conducted a retrospective analysis of patients with stage I-II CM clinically tested with the 31-GEP from 2013-2017 (n=1,661). Differences in DM rates between groups were assessed with Chi-squared test. Survival estimates (DM-free survival, DMFS) were estimated using Kaplan-Meier and compared using the log-rank test. (*, $p<0.05$; **, $p<0.001$).

Results: Among patients with stage I-II CM, a total of 90 DMs developed in 67 (4.0%) patients to the liver (n=48), lung (n=22), and/or bone (n=20). A higher percentage of patients with Class 2B than Class 1A developed DM to the liver (7.4% vs. 1.2%, **), lung (4.5% vs. 0.6%, **), and bone (3.0% vs. 0.8%, *). A 31-GEP Class 2B result was associated with significantly lower 5-year DMFS than Class 1B/2A or Class 1A for liver (91.7%, 92.2%, vs. 98.9%, **), lung (95.0%, 97.6%, vs. 99.4%, **), and bone (96.4%, 98.0%, vs. 99.2%, *). Of those with DM, the median time to develop DM (Class 1A, 1B/2A, vs. 2B) was 2.43, 2.22, vs. 2.27 years for liver; 1.74, 3.20, vs. 1.10 years for lung; and 3.2, 2.05, vs. 1.30 years for bone.

Conclusion: Among patients with stage I-II CM, a greater percentage of those with a Class 2B 31-GEP developed DM at all studied sites, and 5-year DMFS was significantly lower for those with a Class 2B result. Thus, including 31-GEP testing with AJCC staging can allow clinicians to identify patients at higher risk of DM and recommend risk-appropriate surveillance modalities and frequency for early detection and treatment of DM.

#3345 Application of cGAS-immunopositive micronuclei as an indicator of tumor aggressiveness in liposarcoma.

Kiyota Kato[1], Toru Motoi[1], Yu Naito[2], Masumi Ogawa[1], Chihiro Onda[1], Hideo Arai[1], Shin-ichiro Horiguchi[1], Toshihide Hirai[3], Tsunekazu Hishima[1]

[1]Pathology, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan,[2]Nagoya University Graduate School of Medicine, Nagoya, Japan,[3]Musculoskeletal Oncology, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan

Background: Recent molecular biological studies have revealed that chromosomal instability (CIN) promotes the malignant phenotype of tumors. CIN causes chromosomal segregation errors, leading to micronuclei (MN) formation. Ruptured MNs activate the cGAS-STING pathway, an intracellular DNA sensor, and induce metastasis and invasion. Histological confirmation of MNs is thought to be uncertain and not reproducible. On the other hand, cGAS is localized in a subset of MN by immunohistochemistry, so we attempted to apply cGAS-positive MN as an indicator of CIN and tumor aggressiveness in liposarcoma, which is reported as one of the tumors with the highest frequency of CIN among various malignant tumors.
Study Design: Formalin-fixed paraffin-embedded biopsy and surgical specimens of liposarcoma of four histological types, namely well-differentiated (WLPS) (n=18), dedifferentiated (DLPS) (n=32), myxoid (MLPS) (n=6), and pleomorphic liposarcoma (PLPS) (n=7) were examined. In addition, benign lipomatous tumors (n=17) were also tested. Ten cases of non-lipomatous high-grade sarcoma were used as controls. Because DLPS results from the dedifferentiation of WLPS, it comprises differentiated (W) and dedifferentiated (D) components. Immunohistochemical staining for cGAS was performed, and the number of cGAS-positive micronuclei in 500 tumor cells of each tumor was counted. For DLPS, dedifferentiated cells (D cells) in the D component and lipogenic cells (L cells) and non-lipogenic cells (N cells) in the W component were evaluated separately.
Results: cGAS immunostain highlighted a subset of micronuclei, as well as mitotic figures, and some small punctate structures within the main nuclei. The average number of cGAS-positive MNs was 1.2 for benign lipomatous tumor (n=17), 1.0 for myxoid liposarcoma (MLPS, n=6), 34.1 for pleomorphic liposarcoma (PLPS, n=7), 23.2 for D cells in (DLPS, n=32), 3.1 for L cells and 3.6 for N cells in WLPS / W-component (n=43), 24.2 for referenced sarcomas, respectively. The number of cGAS-positive micronuclei was significantly higher in aggressive sarcomas (PLPS and D components of DLPS) than in translocation-associated sarcoma (MLPS), WLPS / W-component, and benign tumors. Although the D-component and W-component / WLPS share a similar complex karyotype, the number of c-GAS positive MNs was significantly higher in D cells rather than L cells (t-test, p=0.0001) and N cells (t-test, p=0.0002).
Conclusion: MNs can be visualized by cGAS immunostain. Significant increases in cGAS-positive MNs were observed in aggressive liposarcomas with complex karyotypes. Furthermore, increased cGAS-positive MNs are associated with malignant progression in DLPS. cGAS-positive MNs may be applicable as an objective indicator of tumor aggressiveness and CIN.

#3346 CD44 is an independent predictor of lymph node metastasis in differentiated thyroid cancer.

Syed Zeeshan Qadri, Abdul K. Siraj, Sandeep K. Parvathareddy, Nabil Siraj, Padmanaban Annaiyappanaidu, Khawla S. Al-Kuraya

King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia

Background: Differentiated Thyroid Carcinoma (DTC), a common endocrine malignancy, has witnessed a significant rise in global incidence over the past few decades. Despite its indolent nature and favorable prognosis, the clinical course of DTC varies extensively, with lymph node metastasis being an important risk factor in prognosis. CD44 has been shown to be associated with prognosis in various cancers. However, its role in DTC is not well explored.
Methods: A total of 1703 DTC cases of Middle Eastern ethnicity were included in the study. CD44 expression was evaluated by immunohistochemistry using tissue microarray to analyze the clinico-pathological associations. Univariate and multivariate logistic regression analysis was used to determine the predictors of lymph node metastasis.
Results: CD44 over-expression was noted in 69.7% (1187/1703) of DTC cases and was significantly associated with younger age (< 55 years; $p < 0.0001$), multifocality ($p < 0.0001$), lymphovascular invasion ($p = 0.0007$) and lymph node metastasis ($p < 0.0001$). Interestingly, on multivariate logistic regression analysis, CD44 was found to be an independent predictor of lymph node metastasis (Hazard ratio = 1.79; 95% confidence interval = 1.40 - 2.30; $p < 0.0001$).
Conclusion: Our study showed that CD44 expression was an independent predictive biomarker of lymph node metastasis in Middle Eastern patients with DTC. Detection of CD44 expression could help identify patients at risk for developing lymph node metastasis and help in individualization of clinical management for DTC patients.

#3347 Developing a prognostic biomarker of EGFR/RAS/SIAH pathway activation/inactivation following neoadjuvant chemotherapy to risk stratify patients, detect racial disparity, and predict relapse and survival in triple-negative breast cancer in Hampton Roads, Virginia.

Z. Bouker[1], A. H. Tang[1], C. B. Piatak[1], T. N. Drake[1], C. D. Lee[1], E. L. Breeding[1], J. S. Winston[2], B. Samli[2], R. J. Jansen[1], M. Danso[3], R. A. Hoefer[2];

[1]Eastern Virginia Medical School, Norfolk, VA, [2]Sentara Health, Norfolk, VA, [3]Virginia Oncology Associates, Norfolk, VA

Background: Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype that disproportionately affects BRCA1 mutation carriers and young Black/white women. Hampton Roads, Virginia is a major cosmopolitan area with 1.8 million population, in which 30% are Black/African Americans. TNBC is highly prevalent in our area where high mortality and racial disparity are reported by CDC/SEERS databases. Supported by strong evidence in developmental, evolutionary, and cancer biology, we hypothesize that persistent EGFR/K-RAS/SIAH pathway activation is a major driving force of TNBC malignancy, and a new prognostic biomarker of racial disparity, early tumor relapse, and high mortality in the clinic.

Methods: Chart review was conducted using Sentara MD Office/EPIC and VOA iKnowMedicine portals to update tumor relapse and survival of 535 TNBC patients. In a sub-cohort study, we focused on 183 high-risk TNBC patients who received neoadjuvant chemotherapy (NACT). We will determine if 5-year TNBC survival is associated with $SIAH^{High/Low}$ expression pre- and post-NACT, race, and/or other clinicopathological parameters.

Results: We report that a higher SIAH expression in residual tumors post-NACT was associated with lower 5-year survival rates. In a cohort of 48 incomplete responders (pIR) post-NACT, the 5-year survival rate was 2.25-fold higher in patients whose residual tumors had a lower SIAH expression (< 50%) than those with a higher SIAH expression (> 50%) (61.5% vs 27.3%; p=0.02). Similarly, the 5-year survival rate was 3-fold higher in 18 pIR patients with stage 2 residual tumors that had a lower SIAH expression (<50%) than those with a higher SIAH expression (> 50%) post-NACT (100% vs 37.5%, p=0.01). A significant racial disparity in 5-year survival was detected between Black/white TNBC patients with stage 2 diseases post-NACT (95 vs 74%; p=0.005).

Conclusion: A persistent high SIAH expression post-NACT was associated with reduced survival in high-risk TNBC patients. Encouraged by the preliminary data, we aim to develop a SIAH-centered biomarker panel, $SIAH^{High\ (>\ 50\%)}/SIAH^{Low\ (<50\%)}$ by measuring the EGFR/K-RAS/SIAH pathway activation (ON)/inactivation (OFF) to risk stratify TNBC pIR patients, detect cancer racial disparities, forecast early tumor relapse, and predict survival at 1st-line neoadjuvant settings. We aim to delineate the molecular underpinning of the Black/white racial disparity in TNBC so that we can provide better, more equitable, and effective therapies to more high-risk TNBC patients with residual diseases in our local communities.

#3348 Clinical relevance and tumor microenvironment characteristics of SMARCD3 expression in gastric cancer.

K. Yuza[1], M. Oshi[2], Y. Takeshita[1], T. Takahashi[1], S. Sato[2], T. Kosaka[2], T. Kumamoto[1], T. Sato[1], K. Takabe[3], I. Endo[2];
[1]Yokohama City University Medical Center, Yokohama City, Japan, [2]Yokohama City University School of Medicine, Yokohama City, Japan, [3]Roswell Park Comprehensive Cancer Center, Buffalo, NY

Background: SMARCD3, a member of the SWI/SNF family, plays a role in chromatin remodeling and gene transcription. Although its involvement in mesenchymal-epithelial transition in breast cancer cells has been reported, its clinical significance in gastric cancer (GC) remains unclear. This study aims to investigate the clinical relevance of SMARCD3 expression in GC and its impact on the tumor microenvironment, survival outcomes, and tumor biology.
Methods: We analyzed clinical and gene expression data from 807 GC patients in The Cancer Genome Atlas (TCGA) and GSE57495 cohorts. SMARCD3 high expression was defined as the top tertile in each cohort. Gene set enrichment analysis (GSEA) was performed to explore biological pathways, while the xCell algorithm was used to evaluate immune and stromal cell infiltration in the tumor microenvironment.
Results: High SMARCD3 expression was significantly associated with worse overall survival in both cohorts ($p = 0.044$, and $p = 0.005$, respectively), underscoring its potential as a prognostic marker. Tumors with elevated SMARCD3 expression exhibited deeper invasion and more aggressive pathological features (all $p < 0.001$). SMARCD3-high tumors showed lower tumor mutation burden but higher intratumoral heterogeneity, indicating a complex genomic landscape. Immune profiling of SMARCD3-high GC revealed reduced infiltration of both anti- and pro-tumor immune cells, including CD4+ memory T cells, Th1, Th2, and NK cells (all $p < 0.001$). Conversely, these tumors demonstrated increased infiltration of stromal cells, including fibroblasts, adipocytes, microvascular endothelial cells, lymphatic endothelial cells, and pericytes (all $p < 0.001$), reflecting a distinct tumor microenvironment. GSEA revealed significant enrichment of pro-cancerous pathways, including the epithelial-mesenchymal transition, the Hedgehog signaling, the Apical Junction, and Myogenesis, while proliferation-related pathways were inversely correlated with SMARCD3 expression.
Conclusions: SMARCD3-high GC is associated with poor prognosis, characterized by aggressive tumor behavior, distinct immune and stromal profiles, and activation of pro-cancerous pathways compared to SMARCD3-low GC. These findings suggest that SMARCD3 is a potential prognostic biomarker, aiding in the stratification of GC patients and informing personalized treatment strategies. Further validation in clinical settings may establish its utility in guiding therapeutic decisions.

#3349 Loss of GATA2 promotes invasion and predicts cancer recurrence and survival in FIGO stage I uterine serous carcinoma.

Usha S. Polaki[1], Trey E. Gilpin[1], Apoorva T. Patil[1], Emily Chiu[2], Paula M. Manan Mejias[2], Pei Hui[3], Maria Virumbrales-Munoz[2], Lisa Barroilhet[2], Stephanie M. McGregor[1], Emery H. Bresnick[4], Daniel R. Matson[1]

[1]Pathology and Laboratory Medicine, University of Wisconsin - Madison, Madison, WI,[2]Obstetrics and Gynecology, University of Wisconsin - Madison, Madison, WI,[3]Pathology, Yale University School of Medicine, New Haven, CT,[4]Cell and Regenerative Biology, University of Wisconsin - Madison, Madison, WI

Background: Uterine cancer is the 4th most common cancer in women. Its incidence is increasing and its mortality rate also is rising. Uterine serous carcinoma (USC) is an aggressive uterine cancer subtype that is responsible for 40% of uterine cancer deaths. Although 50% of USC patients present with cancer localized to the uterine body (FIGO stage I) and should be cured by staging hysterectomy, 30% of these patients later recur in the absence of adjuvant combination paclitaxel-carboplatin chemotherapy. There is no reliable method to identify which USC patients are at risk of USC recurrence, so most patients receive risk-reducing adjuvant chemotherapy associated with significant morbidity. A priori knowledge of recurrence risk would enable a risk-stratified approach to adjuvant therapy to reduce morbidity and optimize survival. GATA Binding Protein 2 (GATA2) is a transcription factor that supports progesterone receptor signaling in the uterus. We recently generated anti-GATA2 monoclonal antibodies that can routinely detect GATA2 by standard immunohistochemistry (IHC).

Methods: We assembled a retrospective multi-institutional cohort of 81 patients with primary FIGO stage I USCs. Associations between GATA2 levels and clinicopathologic metrics were evaluated using Student's t-test, Fisher's exact test, Kaplan-Meier method, and Cox proportional hazards ratio. Gene targets including GATA2 were depleted from Ark1 and Ark2 patient-derived USC cell lines using siRNA or shRNA, and cell invasion evaluated using Matrigel-coated membranes and organoid-based natural hydrogel. RNA-seq, anti-GATA2 ChIP-seq, and western blotting enabled identification of GATA2 gene targets.

Results: GATA2 expression in USC tumors ranged from 0-100% GATA2+ tumor nuclei. USCs with >15% GATA2+ nuclei were defined as GATA2-high based on an initial inflection point in the data that optimally delineated patients by outcome. The 39% of patients with GATA2-high USCs had 100% recurrence-free, 100% disease-specific, and 87% overall survival. These outcomes were significantly better than patients with GATA2-low USCs. Depletion of GATA2 in USC cell lines increased invasion *in vitro*. In patients who received no adjuvant chemotherapy (n=18), those with GATA2-high USCs had 100% recurrence-free survival compared to 50% recurrence-free survival in GATA2-low USC patients. A multi-omic approach identified SIN3 Transcription Regulator Family Member B as a GATA2 target gene that suppressed USC invasion *in vitro*.

Conclusions: GATA2 IHC identifies FIGO stage I USC patients with a greatly reduced risk of USC recurrence. A GATA2 guided personalized medicine approach using standard IHC methods could be rapidly implemented in most hospital settings, would reduce treatment-related morbidity, and may optimize survival for USC patients.

#3350 Prognostic importance of physical activity in the chemotherapy of esophagogastric cancer; associations with skeletal muscle, systemic inflammation, and lifestyle.

Koichi Hayano, Natsiko Arimatsu, Yasunori Matsumoto, Yoshihiro Kurata, Ryota Otsuka, Akira Nakano, Tadashi Shiraishi, Nobufumi Sekino, Takeshi Toyozumi, Masaya Uesato, Gaku Ohira, Hisahiro Matsubara

Department of Frontier Surgery, Chiba Univ. School of Medicine, Chiba, Japan

Background: Physical activity (PA) must be an important intervention in the treatment of cancer. However, it is unknown what kinds of PAs can improve patients' outcome in the chemotherapy of esophagogastric cancer (EGC).
Methods: Unresectable or recurrent EGC patients treated with chemotherapy were prospectively investigated. PA was measured using an accelerometer for 1 week before and after the chemotherapy administration. PA was classified as sedentary behavior (< 1.5METs, SB), light-intensity PA (1.6-2.9 METs, LPA), and moderate-to-vigorous PA (≥ 6.0 METs, MVPA). PA parameters were compared with disease specific survival (DSS), inflammatory and nutritional markers, subsequent skeletal muscle (SM) volume change, and lifestyle.
Results: Thirty-nine patients were evaluated. In Cox regression analyses, better DSS was significantly correlated with longer times of LPA (P=0.02), but not with MVPA (P=0.2). LPA showed significant negative correlations with WBC, CRP, and modified GPS score (P=0.03, 0.03, 0.004, respectively), and positive correlations with serum total protein (TP) and albumin (Alb) levels and SM change (P=0.01, 0.003, 0.01, respectively). The longer times of LPA was significantly associated with better socialization with neighbors (P=0.001).
Conclusions: LPA would be recommended during the chemotherapy of advanced EGC patients, keeping their better inflammatory and nutritional status and preventing SM loss. Socialization might play a key role in increasing LPA.

#3351 From bench to bedside: generalizable AI model for ADC biomarker evaluation in NSCLC.

Philipp Anders[1], Philipp Erwin Seegerer[2], Katja Lingelbach[2], Suhas Pandhe[2], Sandip Ghosh[2], Cornelius Bohm[2], Stephan Tietz[2], Rosemarie Krupar[2], Lars Tharun[1], Marie-Lisa Eich[1], Julika Ribbat-Idel[2], Verena Aumiller[2], Sabine Merkelbach-Bruse[3], Alexander Quaas[3], Nikolaj Frost[4], Georg Schlachtenberger[5], Matthias Heldwein[5], Ulrich Keilholz[6], Khosro Hekmat[5], Jens-Carsten Ruckert[7], Reinhard Buttner[3], David Horst[1], Maximilian Alber[2], Lukas Ruff[2], Frederick Klauschen[8], Gabriel Dernbach[2], Simon Schallenberg[1]

[1]Institute of Pathology, Charite - Universitatsmedizin Berlin, corporate member of Freie Universitat Berlin, Humboldt-Universitat zu Berlin and Berlin Institute of Health, Berlin, Germany,[2]Aignostics GmbH, Berlin, Germany,[3]Institute of Pathology, University Hospital Cologne, Cologne, Germany,[4]Department of Infectious Diseases and Respiratory Medicine, Charite - Universitatsmedizin Berlin, corporate member of Freie Universitat Berlin, Humboldt-Universitat zu Berlin and Berlin Institute of Health, Berlin, Germany,[5]Department of General, Visceral, Oncological and Thoracic Surgery, University Hospital Cologne, Cologne, Germany,[6]Charite Comprehensive Cancer Center, Charite - Universitatsmedizin Berlin, corporate member of Freie Universitat Berlin, Humboldt-Universitat zu Berlin and Berlin Institute of Health, Berlin, Germany,[7]Department of General, Visceral, Vascular and Thoracic Surgery, Charite - Universitatsmedizin Berlin, corporate member of Freie Universitat Berlin, Humboldt-Universitat zu Berlin and Berlin Institute of Health, Berlin, Germany,[8]Institute of Pathology, Ludwig-Maximilians-University Munich, Munich, Germany

Background:Antibody-drug conjugates (ADCs) targeting trophoblast cell-surface antigen 2 (TROP-2) and hepatocyte growth factor receptor (cMET) are promising therapies for non-small cell lung cancer (NSCLC). However, their clinical application requires robust and rapid biomarker evaluation that addresses expression heterogeneity and avoid interobserver variability. Current approaches based on pathologist assessments are limited by subjectivity and scalability. This study aimed to develop a generalizable AI model for ADC biomarker evaluation, trained on TROP-2 and inferred on cMET, to validate its adaptability across markers. Additionally, the model's performance was compared with expert pathologists to assess its clinical utility. Finally, biomarker prevalence in the two main NSCLC subtypes, namely adenocarcinoma (LUAD) and squamous cell carcinoma (LUSC), was evaluated.
Methods: We collected a bicentric real-world sample group of 1142 patients with resected NSCLC from the Charité, Berlin and the University Hospital Cologne. For tissue microarray construction, two 1.5-mm tissue cores were punched from formalin-fixed and paraffin-embedded tumor blocks. Immunohistochemical staining for TROP-2 and cMET was performed and sections were scanned for AI-based analysis. The AI pipeline comprised three models: a cell detection model for the identification of cells, a cell classification model for the differentiation of tumor and other cells, and an expression scoring model for membranous biomarker quantification. The model was trained on TROP-2, and subsequently inferred on cMET, thus enabling an evaluation of cross-marker generalization. Five pathologists with varying levels of expertise manually evaluated a representative subset, using H-scoring. Finally, the results were compared with those yielded by the AI model.
Results: The expression scoring model achieved a macro-averaged F1 score of 94% for TROP-2 and 91% for cMET. Moreover, the model demonstrated excellent concordance with expert pathologists (TROP-2: 93%; cMET: 95% average pair-wise Pearson correlation). TROP-2 overexpression was significantly higher in LUSC (mean H-score: 154.67) than LUAD (mean H-score: 86.57), while cMET showed the opposite trend (mean H-score LUAD: 59.52; LUSC: 25.68).
Conclusion:This study highlights the potential of AI models to address key challenges in ADC biomarker evaluation, including expression heterogeneity, interobserver variability, and reduction in time expenditure. By successfully generalizing between TROP-2 and cMET, the model demonstrates adaptability and scalability for broader clinical applications. These findings pave the way for integrating AI into clinical workflows, improving patient stratification, and optimizing ADC therapy selection. Future efforts will focus on expanding this approach to additional biomarkers and validating its utility in prospective clinical trials.

#3352 Profiling impact of genomic alterations on the BCL2-family protein interactions in acute myeloid leukemia using the SPID platform.

Saem Hong[1], Yunseo Lee[1], Byungsan Choi[1], Hee-Je Kim[2], Byung-Sik Cho[2], **Hongwon Lee**[1]

[1]Proteina Co., Seoul, Korea, Republic of,[2]Department of Hematology, Catholic Hematology Hospital, Seoul, Korea, Republic of

Introduction: Acute myeloid leukemia (AML) is a genetically heterogeneous malignancy, leading to varied therapeutic outcomes with targeted therapies. Venetoclax, a BCL2 inhibitor, has shown efficacy in treating AML patients by disrupting BCL2 functions. Genomic mutations like TP53, IDH1/2, PTPN11, and NRAS are associated with response rates to venetoclax. However, profiling the association between these mutations and BCL2-family protein interactions remains limited in clinical specimens. This study utilized the single-molecule protein interaction detection (SPID) platform to map BCL2-family interactions and examine their links to TP53, IDH1/2, PTPN11, FLT3, and NRAS mutations.

Methods: Bone marrow mononuclear cells (BMMCs) from AML patients were retrospectively analyzed using the SPID platform to measure BCL2-family protein expression and complex levels. 10 assays evaluated Active BAX, Active BAK, BCL2, BIM levels, and complexes like BCL2-BAX and BCLxl-BAK. A BIM-BH3 mimicking probe quantified unoccupied anti-apoptotic protein levels. Mutational status was determined using next-generation sequencing, and Mann-Whitney U tests compared protein profiles between wildtype and mutant groups.

Results: Among 80 patients (median age: 65 years), 43 (48.75%) had TP53, IDH1/2, PTPN11, FLT3, or NRAS mutations. IDH1/2 mutations were linked to higher BCL2-BAX complexes and unoccupied BCL2 levels ($p<0.05$). TP53 mutations were associated with reduced Active BAX and BCL2-BAX complex levels ($p=0.015$ and $p=0.003$). NRAS mutations led to reduce BCLxl-BAK ($p=0.0019$) and MCL1-BAK ($p=0.0504$) complexes, while PTPN11 mutations lowered BIM-el and MCL1-BAK levels ($p<0.05$). No significant biomarkers were linked to FLT3 mutations.

Conclusion: This study reveals how genomic alterations in AML impact BCL2-family signaling, influencing venetoclax response. IDH1/2 mutations enhance BCL2-BAX complexes, potentially associating with favorable venetoclax sensitivity. Conversely, TP53 mutations reduce pro-apoptotic signaling, conferring resistance to venetoclax. NRAS and PTPN11 mutations affect anti-apoptotic complexes, further influencing therapeutic outcomes. Taken together, these discoveries provide how genomic alterations can impact on the BCL2-family signaling.

#3353 Predicting the prognosis of ductal carcinoma in situ through chemical analysis of breast microcalcifications and soft tissue using infrared and Raman spectroscopy.

Jayakrupakar Nallala[1], Doriana Calabrese[1], Sarah Gosling[2], Esther Lips[3], Ihssane Bouybayoune[4], Rachel Factor[5], Sarah Pinder[4], Lorraine King[5], Jeffrey Marks[5], Thomas Lynch[5], Donna Pinto[6], Alastair Thompson[7], Elinor Sawyer[4], Jelle Wesseling[3], Shelley Hwang[5], Keith Rogers[2], Nick Stone[1], Grand Challenge PRECISION consortium

[1]Physics and Astronomy, University of Exeter, Exeter, United Kingdom,[2]Cranfield Forensic Institute, Cranfield University, Cranfield, United Kingdom,[3]Department of Pathology & Division of Molecular Pathology, The Netherlands Cancer Institute, Netherlands, Netherlands,[4]Comprehensive Cancer Centre at Guy's Hospital, School of Cancer & Pharmaceutical Sciences, Kings College London, London, United Kingdom,[5]Department of Surgery, Duke University School of Medicine, Durham, NC,[6]www.DCIS411.com, San Diego, CA,[7]Division of Surgical Oncology, Baylor College of Medicine, Houston, TX

Introduction: Ductal carcinoma in situ (DCIS) is a potential precursor to invasive breast cancer (IBC). The trajectory of an individual's DCIS, if it will progress to IBC or remain as DCIS is difficult to predict. Currently >80% of DCIS is detected through mammographic screening of breast calcifications. Despite the close association of calcifications with DCIS, their role in the development of DCIS and/or its progression to IBC remains largely unexplored. In this study, we present a spectroscopy based analytical approach to probe chemical compositional changes of both DCIS associated breast calcifications and surrounding soft tissue aiming to identify a cohort at increased risk for invasive progression.

Experimental Procedures: Tissue samples from 316 DCIS patients without invasive cancer were obtained from multiple centres as part of the PRECISION consortium (The Netherlands, UK and USA). Three consecutive tissue sections were obtained each of the tissue biopsies. Mid-infrared (mid-IR) and Raman hyperspectral imaging was performed independently on two sections that were left unstained. The third H&E-stained section was used to annotate calcifications and histopathological features. All DCIS samples had known outcome (i) 'pure DCIS as controls' (DCIS without progression to invasion) (n=193), (ii) 'DCIS with progression to invasion as cases' (DCIS from patients who subsequently developed invasive disease after initial treatment) (n=123). Spectral features of DCIS calcifications and surrounding soft tissue were used as inputs for analysis. Data was divided into a discovery and a validation set. Cluster analysis followed by Principal component analysis fed linear discriminant analysis was carried out on the discovery set to develop DCIS prediction models.

Results: For the Raman data, a mean area under the receiver operating characteristic curve (AUROC) value of 0.85 was obtained using calcification spectral features, and 0.75 using soft tissue spectral features in distinguishing controls from cases (N=118 vs 52). Similar analysis on the IR data showed a mean AUROC value of 0.68 for calcification, 0.78 for epithelial and 0.80 for stromal components (N=97 vs 61). Preliminary analysis shows changes in phosphate to carbonate ratio and variations in magnesium whitlockite content in calcifications, and protein secondary structural changes in soft tissue, between the two groups. The models will be tested independently on the validation set and the outcomes will be presented at the AACR conference.

Conclusion: Spectroscopic chemical analysis of breast calcifications and soft tissue show promise in predicting the likely progression of DCIS to IBC. Pending further independent validation, these techniques appear to be novel image-based risk assessment tools that can potentially be utilised to inform DCIS prognosis and treatment options.

#3354 Hsp70 is an independent predictor of recurrence-free survival in differentiated thyroid cancer.

Sandeep K. Parvathareddy, Zeeshan Qadri, Abdul K. Siraj, Padmanaban Annaiyappanaidu, Nabil Siraj, Khawla S. Al-Kuraya

King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia

Background: Differentiated Thyroid Cancer (DTC) has an indolent nature and generally has favorable prognosis. However, tumor recurrence is a major concern and can be noted several years after initial treatment. Identifying markers that can predict recurrence could lead to better management of DTC. Heat shock protein 70 (Hsp70), a molecular chaperone, has been shown to be associated with tumor recurrence and poor prognosis in other tumors. However, its role in predicting outcome in DTC is not well explored.
Methods: A total of 1710 DTC cases were included in the study. Hsp70 expression was evaluated by immunohistochemistry using tissue microarray to analyze the clinico-pathological associations. Kaplan-Meier curve analysis was used to compare recurrence-free survival (RFS). Cox proportional hazards model was used for multivariable analysis.
Results: Hsp70 over-expression was noted in 71.6% (1224/1710) of DTC cases and was significantly associated with adverse clinico-pathological characteristics such as extrathyroidal extension ($p = 0.0039$), T4 tumors ($p = 0.0002$), lymph node metastasis ($p = 0.0118$), distant metastasis ($p = 0.0130$), advanced tumor stage ($p = 0.0312$) and tumor recurrence ($p < 0.0001$). Interestingly, HSP70 over-expression was found to be associated with shorter RFS ($p < 0.0001$). On multivariable analysis, HSP70 was an independent predictor of RFS (Hazard ratio = 1.82; 95% confidence interval = 1.33 - 2.55; $p = 0.0001$).
Conclusion: Our data suggests that Hsp70 is associated with poor prognosis in DTC and is a useful biomarker for predicting tumor recurrence. DTC patients whose tumor shows over-expression of Hsp70 should be subjected to more intense follow-up to identify potential tumor recurrence.

#3355 FTO in smoking-associated lung cancer and lung cancer tumor progression.

Rachel Campoli, Rong Qiu, Robert Hillwig, Neelu Puri

University of Illinois at Chicago, Chicago, IL

In 2024, an estimated 234,580 patients will be diagnosed with lung cancer and 125,070 are expected to die, making lung cancer the leading cause of cancer-related deaths. Early detection of lung cancer is critical for improving prognosis, hence the need for early detection biomarkers. Fat mass and obesity-associated gene (FTO) had earlier been linked with obesity, however current studies indicate it may have a role in tumor development and could be a potential biomarker. In this study, we aimed to identify a possible link between FTO and non-small cell lung cancer (NSCLC), which accounts for 85% of lung cancer diagnosis. We hypothesize FTO is involved in increasing tumorigenicity and mortality among lung cancer patients. The role of FTO was studied in tumor progression, patient survival analysis, and smoking induced lung cancer, which is not established. Using immunohistochemistry, 115 tumor sections of NSCLC patients were studied; of these patients, 64 were early-stage NSCLC and 51 were late-stage. Patient tissues were obtained from local hospital pathology archives. Staining was completed using FTO primary antibody and a biotinylated secondary antibody. FTO brightness scores were generated using the BZX-800 analyzer software. FTO expression was also calculated using an H-score with the help of a pathologist. Both imaging studies and pathology grading indicated that late-stage lung cancer tissues had significantly ($p<0.05$) higher FTO expression compared to early-stage cases. When subdivided into low and high expression levels, 69% of early-stage tissue samples exhibited low expression levels and 55% of late-stage tumor tissues had high FTO expression. To assess the role of FTO expression in lung cancer survival, a Kaplan-Meier analysis was done with 47 patients. Results showed that NSCLC patients with higher expression had a shorter median survival time (14 months) compared to NSCLC patients with low expression (27 months) ($p=0.03$). These results suggest that patients with higher FTO expression may have poorer prognosis. Since 80-90% of lung cancer patients had a history of smoking, we studied the connection between FTO expression and smoking associated lung cancer. 50 NSCLC tumor sections were stained, 28 being smokers and 22 being non-smokers. The average FTO expression level was 24% higher in smokers compared to non-smokers ($p<0.02$). Using H-scores, smokers were divided into low and high expression levels. 93% of smokers had a high FTO expression compared to 7% in non-smokers indicating that smokers had higher expression of FTO compared to non-smokers ($P=0.001$) In conclusion, FTO may have a role in tumor progression as it is highly expressed in late-stage lung tumor tissues. High expression of FTO is also a potential indicator of patient survival, as patients who had an upregulation of FTO had shorter survival time. Finally, we found a possible link between high FTO expression and smoking-induced lung cancer.

#3356 Construction of a 23-gene risk scoring system to predict disease recurrence and chemotherapy response in mucinous colorectal cancer.

J.-W. Seo[1], J.-Y. Kim[1], Y.-J. Ha[2], S.-W. Baek[1], J.-L. Park[1], S.-W. Lee[1], J.-L. Lee[2], S.-Y. Kim[1], S.-K. Kim[1];

[1]Korea Research Institute of Bioscience & Biotechnology (KRIBB), Daejeon, Korea, Republic of, [2]Asan Medical Center, Seoul, Korea, Republic of

Backgrounds: Mucinous adenocarcinoma (MuC), a unique subtype of colorectal cancer (CRC), exhibits distinct molecular features and is associated with poorer outcomes compared to non-mucinous adenocarcinoma (NMuC). Standard CRC treatments often overlook the molecular diversity within CRC, especially in stage II, where the benefit of adjuvant chemotherapy remains debated. Biomarkers are needed to improve risk assessment and guide tailored treatments.

Methods: We conducted molecular profiling of 259 CRC samples, including MuC and NMuC subtypes, to identify MuC-specific gene signatures. A 23-gene risk score (MuC-RS) was developed and validated in four independent cohorts (n = 1,157). Prognostic significance of MuC-RS was assessed using various statistical methods, including Kaplan-Meier survival analysis, log-rank tests, and Cox regression.

Results: In the discovery cohort of fresh-frozen tissues, unsupervised clustering revealed distinct gene expression patterns differentiating MuC from NMuC. MuC showed notable enrichment in fibroblast-associated genes, EMT pathways, and mucin glycosylation processes. The MuC-RS effectively stratified patients into high-risk (MuC-H) and low-risk (MuC-L) categories. Multivariate analysis confirmed MuC-RS as an independent prognostic factor. Importantly, stage II MuC-L patients showed limited response to standard chemotherapy but increased responsiveness to immune checkpoint inhibitors (ICIs), associated with higher tumor mutation burden and immune activation. MuC-RS's prognostic utility was further validated through comparisons with consensus molecular subtypes.

Conclusion: MuC-RS offers a promising tool for predicting recurrence and informing personalized treatment strategies in CRC, potentially enhancing risk stratification and supporting novel therapies, such as ICI, particularly for stage II MuC patients.

Keywords: Mucinous colorectal cancer, biomarker, risk score, chemotherapy, immunotherapy

Grant Support: This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MSIT) (2022R1A2C2008480 to S.K. Kim, 2018R1C1B6008571 and 2022R1F1A1074317 to J.L. Lee). This study was also supported by grants from the Korea Research Institute of Bioscience and Biotechnology (KRIBB) Research Initiative Program (KGM5192423).

#3357 Identification of outcome-associated tumor perivascular features in renal cell carcinoma.

Alfonso Martin-Bernabe[1], Victor Sanchez-Minarro[1], Philipp Schmucker[1], Magnus Lindskog[1], Ulrika Harmenberg[1], Martin Johansson[2], Lars Egevad[1], Arne Ostman[1]

[1]Karolinska Institutet, Stockholm, Sweden,[2]Goteborgs Universitet, Gothenburg, Sweden

Background: One third of patients with clear cell renal cell carcinoma (CCRCC) eventually develop metastasis. The introduction of anti-angiogenic therapy and immunotherapy in metastatic CCRCC (mCCRCC) has significantly improved outcomes for many patients. Among vascular endothelial growth factor tyrosine kinase inhibitors (TKIs), sunitinib is the most commonly used first-line drug. However, treatment is administered without specific patient selection criteria and relapse occurs in approximately 40% of cases. Given the lack of clinical stratification of these patients, predictive and prognostic biomarkers are needed to improve their clinical outcome.
Methods: This work explored the clinical impact of perivascular (PV) characteristics in RCC by performing multiplex-based tissue profiling in a population-based cohort (N = 70) and a sunitinib-treated mCCRCC cohort (N = 61). Vessel features and tumor PV cell subsets were characterized based on multimarker (CD34, PDGFRα, PDGFRβ, αSMA and pSMAD2) expression combinations. In both cohorts, patients were categorized using a tertile-based dichotomization of the variables, with the upper tertile compared against the combined lower and middle tertiles to explore associations.
Results: Two PV cell subsets, PER4 (PDGFRB+/ASMA+/PDGFRA-/pSMASD2-) and PER5 (PDGFRB+/ASMA-/PDGFRA-/pSMASD2-), showed statistically significant survival associations using multiple endpoints in both cohorts. In the sunitinib-treated cohort (OS from treatment), PER4 (log-rank $p < 0.001$) and PER5 (log-rank $p = 0.002$) were associated with poor survival. Similarly, in the population-based cohort (OS), PER4 (log-rank $p = 0.043$) and PER5 (log-rank $p = 0.001$) were also linked to worse survival. Univariate and multivariate analyses confirmed robust survival associations for the identified PV cell subsets PER4 and PER5.
Conclusions: Collectively, this study identified candidate prognostic or TKI predictive response markers in RCC. These data provided in-depth characterization of PV cell subsets associated with clinical outcome in RCC. These data could be further explored to find clinically useful patient selection to improve the accuracy of anti-angiogenic therapy administration in RCC.

#3358 Tertiary lymphoid structures (TLS) estimated by AI tools from digital H&E slides significantly enhance survival prediction in high-risk AJCC stages III/IV melanoma.

Ahmad A. Tarhini[1], Tingyi Li[1], Ranran Tao[1], Sandra J. Lee[2], Islam Eljilany[1], F. Stephen Hodi[3], Howard Streicher[4], Arivarasan Karunamurthy[5], John M. Kirkwood[5], Xuefeng Wang[1]

[1]H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL,[2]Dana Farber Cancer Institute, Harvard Medical School, Boston, MA,[3]Dana-Farber Cancer Institute, Boston, MA,[4]National Cancer Institute, Investigational Drug Branch, Cancer Therapy Evaluation Program, Bethesda, MD,[5]UPMC Hillman Cancer Center, Pittsburgh, PA

Tertiary lymphoid structures (TLS) in tumors have emerged as novel disease prognostic and predictive biomarkers with immunotherapy. Recent advances in whole-slide image (WSI) digitization of pathology slides and AI tools are making in situ prediction of TLS more feasible.

Methods: We applied HookNet-TLS, a deep learning-based automated TLS quantification tool, to identify and quantify TLS and germinal centers (GC) in digitized H&E slides from 376 patients (pts) with resected cutaneous melanoma enrolled in the E1609 phase 3 trial that tested adjuvant ipilimumab (IPI) vs interferon-α (IFN). We tested the prognostic value of TLS by correlating the presence of TLS and the normalized number of TLS (number of TLS divided by total area of segmented tissue) in E1609 pts with AJCC7 stages IIIB (n=180), IIIC (n=151), M1a (n=30), M1b (n=15), by stage group, sex, age, treatment and tumor ulceration, utilizing Kaplan-Meier analysis and log-rank tests.

Results: TLS were present in the tumors of 207 pts (55%) and predicted better overall survival (OS) ($P = 0.09$), especially in those with >1 TLS ($P = 0.0096$). TLS density (TLS per unit area) was even more prognostic ($P = 0.0036$ for median cutoff; $P = 0.0012$ for optimal cutoff). Presence of both TLS and GC within TLS (n = 114) demonstrated significantly better OS compared to those without GC ($P = 0.022$). Higher TLS scores were associated with significantly better OS in IIIB ($P = 0.021$) and IIIC ($P = 0.019$) with a similar trend towards improved recurrence free survival (RFS). Associations in M1a and M1b were limited by sample size, although improvement in RFS was seen in M1a with higher TLS ($P = 0.07$) and presence of GC ($P = 0.007$). By treatment, higher TLS predicted better OS ($P = 0.033$) and RFS ($P = 0.018$) among IPI treated patients (n=246). A similar trend in OS was seen among IFN treated patients (n=130) that did not reach significance. By sex, higher TLS & presence of GC were associated with better OS ($P = 0.0062$ & $P = 0.072$) and RFS ($P = 0.01$ & $P = 0.04$), respectively in males (n=226). By age, higher TLS was associated with better OS among pts <65 (n = 310) ($P = 0.0002$). Similar trends in females ($n = 150$) and age ≥65 (n = 66) that were not significant, likely limited by sample size. Higher TLS predicted better OS and RFS in pts with non-ulcerated melanoma (n=138) ($P = 0.001$ & $P = 0.022$) but not those with ulceration (n=165).

Conclusions: Automated TLS quantification using a deep learning tool (HookNet) effectively identified and characterized TLS in H&E slides where the application of TLS significantly enhanced survival prediction by AJCC stage group, sex, age, treatment and tumor ulceration. These findings highlight the potential of AI-driven approaches to standardize TLS assessment using low-cost H&E staining, with the potential to improve prognostic stratification within AJCC, that warrants further investigation and validation.

#3359 Prevalence of heterozygous and homozygous 9p21 deletions in human cancer: a tissue microarray study on 4999 tumors from 125 different tumor types.

Natalia Gorbokon, Katharina Teljuk, Katharina Moller, Seyma Buyucek, Maximilian Lennartz, Sebastian Dwertmann Rico, Simon Kind, Viktor Reiswich, Florian Viehweger, Elena Bady, Lisa Hornsteiner, Guido Sauter, Ronald Simon, Stefan Steurer, David Dum, Sarah Minner, Frank Jacobsen, Till S Clauditz, Martina Kluth

University Medical Center Hamburg-Eppendorf, Hamburg, Germany

9p21 deletions including CDKN2A belong to the most common chromosomal aberrations in cancer. Most of the topical interest in 9p21 comes S-methyl-5′-thioadenosine phosphorylase (MTAP) which is also included in most 9p21 deletions. Homozygous 9p21 deletions are the major cause for MTAP deficiency which results in a critical vulnerability of cancer cells towards drugs targeting several different pathways. Fluorescence in situ hybridization (FISH) is the gold standard method for deletion detection because single cells are always analyzed independent of any admixture of non-neoplastic cells. To assess the prevalence of heterozygous and homozygous deletions in cancer, a tissue microarray containing 7,172 samples from 125 different tumor entities was analyzed by FISH. Consecutive sections were immunostained for MTAP and p16. Among 4,999 evaluable cases, 10.7% had a heterozygous and 7.4% a homozygous deletion. Heterozygous and homozygous did not predominate in the same tumor categories. Homozygous deletions were most frequent in mesotheliomas (up to 47.8%), pancreatic ductal adenocarcinoma (44,7%), and in urothelial carcinoma (up to 36.4%) while the heterozygous deletions were most common in squamous cell carcinomas (up to 34.3%), leiomyosarcoma (31.4%), adenocarcinomas of the esophagus (30.1%), malignant melanoma (29.4%), and cholangiocarcinoma (21.2%). Accordingly, the proportion of homozygous deletions within the 9p21 deleted cases varied markedly. It was high in mesothelioma (up to 91.7%), urothelial carcinoma (up to 80%), pancreatic ductal adenocarcinoma (76.4%), and mucinous carcinoma of the ovary (66.7%). It was intermediate in squamous cell carcinomas of different organs (10-50%) and adenocarcinoma of the lung (46.3%), and it was particularly low in endometrioid (9.1%) and in high-grade serous (4%) carcinoma of the ovary. Both MTAP and p16 were not detectable in cancers with 9p21 deletion, but otherwise the results differed markedly between these proteins. The expression of MTAP was markedly lower in heterozygously deleted cancers as in wild type cancers ($p<0.0001$) while p16 expression did not change significantly from normal to heterozygously deleted cases. In summary, these data provide a unique FISH based catalogue of 9p21 deletions in cancer and demonstrate that the proportions of heterozygous and homozygous MTAP deletions vary markedly between tumor entities. The near perfect concordance between homozygous MTAP deletions and absence of MTAP immunostaining further supports the use of IHC for detecting MTAP deficiency.

#3360 Pre- and post-operative analysis of circulating tumor DNA in patients with breast cancer treated with neoadjuvant therapy using a tissue-free, methylation-based approach.

Arielle J. Medford[1], Justine Knape[2], Derek Dustin[3], Haiyang Zhang[3], Caroline Weipert[3], Shivahamy Maheswaran[4], Irene Kuter[1], Jeffrey Peppercorn[1], Seth Wander[1], Steven J. Isakoff[1], Beverly Moy[1], Dejan Juric[1], Laura M. Spring[1], Aditya Bardia[5]

[1]Hematology/Oncology, Mass General Cancer Center/Harvard Medical School, Boston, MA,[2]Hematology/Oncology, Mass General Cancer Center, Boston, MA,[3]Guardant Health, Redwood City, CA,[4]UMass Chan Medical School, Boston, MA,[5]Hematology/Oncology, UCLA Health Jonsson Comprehensive Cancer Center, Los Angeles, CA

Background: Detection of circulating tumor DNA (ctDNA) in early breast cancer (BC) is a prognostic biomarker for disease recurrence. This pilot study evaluates the performance of a tissue-free epigenomic ctDNA assay in pre-neoadjuvant therapy (NAT), on-NAT and post-op plasma samples from patients (pts) with BC.

Methods: Pre-NAT, on-NAT and post-op plasma samples from 13 pts that received NAT for BC were retrospectively analyzed for ctDNA using Guardant Reveal powered by Infinity, a tissue-free assay that analyzes differentially methylated regions of DNA using an applied bioinformatics filter optimized to detect BC. It results in binary ctDNA detection calls and a quantitative estimate of tumor fraction (TF) in ctDNA+ samples.

Results: A total of 27 samples from 14 pts were evaluated, with 96% (26/27) samples passing quality control and included in the analysis (10 pre-NAT, 3 on-NAT, and 13 post-op). ctDNA was detected in 42% (11/26) of samples: 70% (7/10) pre-NAT, 33% (1/3) on-NAT and 23% (3/13) post-op (Table). For post-op samples collected within one year of recurrence, sensitivity was 100% (3/3). 2/5 pts who experienced recurrence, whose post-op samples were collected during adjuvant therapy and were ctDNA-, had a recurrence >15 months after the sample collection. No pts (0%, 0/8) without recurrence were ctDNA+ post-op. A pt with a brain-only recurrence had the lowest TF fraction amongst ctDNA+ post-op samples at 0.9%, with a median post-op TF of 2.5% (range, 0.9-23%) among the 3 ctDNA+ samples.

Conclusion: ctDNA was detectable in 70% of pts pre-NAT, 33% on-NAT and 23% post-op, with persistent ctDNA associated with recurrence. In the subsequent full study, ~ 600 samples will be evaluated at these timepoints. The dataset will provide a rich clinical landscape to understand sensitivity and specificity of methylation-based ctDNA testing, optimal timing and key areas to study therapeutic interception.

	Pre-op samples(n=10)	**Pre-op ctDNA+(n=7)**	**On-NAT samples (N=3)**	**On-NAT ctDNA+(N=1)**	**Post-op samples (n= 13)**	**Post-op ctDNA+ (n=3)**
Receptor subtype						
HR+/HER2-	0 (0%)	N/A	1 (33.3%)	1 (100%)	1 (7.7%)	1 (100%)
HER2+	4 (40.0%)	3 (75.0%)	1 (33.3%)	0 (0%)	5 (38.5%)	0 (0%)
TNBC	6 (60.0%)	4 (66.7%)	1 (33.3%)	0 (0%)	7 (53.8%)	2 (28.6%)
Lymph nodes at surgery						
Negative	4 (40.0%)	3 (75.0%)	2 (66.7%)	0 (0%)	6 (46.2%)	1 (16.7%)
Positive	6 (60.0%)	4 (66.7%)	1 (33.3%)	1 (100%)	7 (53.8%)	2 (28.6%)
Grade						
2	2 (20.0%)	1 (50.0%)	0 (0%)	N/A	2 (15.4%)	0 (0%)
3	8 (80.0%)	6 (75.0%)	3 (100%)	1 (33.3%)	11 (84.6%)	3 (27.2%)
Recurrence						
Yes	4 (40.0%)	4 (100%)	1 (33.3%)	1 (100%)	5 (38.5%)	3 (60.0%)
No	6 (60.0%)	3 (50.0%)	2 (66.7%)	0 (0%)	8 (61.5%)	0 (0%)

CLINICAL RESEARCH: Real-World Data and Real-World Evidence: Clinical Informatics and Data Science 1
Poster Session

#3365 Preview of early adverse event landscape and association with clinical outcomes in advanced non-small cell lung cancer.

Dung-Tsa Chen, Jhanelle Gray, Andreas Saltos, Timothy Shaw

Moffitt Cancer Center, Tampa, FL

Background: AE incidence and the association with clinical outcomes are still not well defined in advanced NSCLC. Most trial studies published AE results limited in treatment-related (Tr) AE using worst grade approach in descriptive manner. As a result, it fails to delineate AE landscape and explore AE potential role as a prognostic factor.
Methods: All AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA) and classified by MedDRA system organ class and preferred term. The severity of AEs was assessed according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE). AE data were collected from 7 advanced NSCLC trial cohorts, including 3 immunotherapy cohorts, 2 chemotherapy cohorts, and 2 targeted therapy cohorts. Early AE was defined as AE occurrence since first treatment until day 30, equivalent to period time of dose limiting toxicity. AE incidence was calculated by percentage of patients with AE experiences. Cox proportional hazards model and log-rank test were used for survival analysis.
Results: Analysis of early AE landscape showed (a) low-grade AEs were more common than high-grade AEs regardless of treatment types. It makes sense otherwise the trials would be closed in early stage due to safety concern, (b) non-Tr AEs were not always more common than Tr AEs. It depends on degree of toxicity of treatments. There were one immunotherapy cohort, two chemotherapy cohorts, and one targeted therapy cohort had a higher incidence of Tr AEs over non-Tr AEs. Likely more toxic drugs were used in these cohorts, and (c) patients without non-Tr AEs may not tend to experience more low-grade Tr AEs than patients with non-Tr AEs. Two immunotherapy cohorts and one chemotherapy cohort had a higher incidence of low-grade Tr AEs in patients without versus with non-Tr AEs. In contrast, one immunotherapy cohort, one chemotherapy cohort (all patients experienced Tr AEs), and two targeted therapy cohorts had an opposite trend with higher or comparable incidence level of low-grade Tr AEs in patients with non-Tr AEs. Regarding survival association in early AEs, we found some early AE biomarkers were associated with survival outcomes in 5 cohorts. Specifically, one immunotherapy cohort and one chemotherapy cohort showed patients experienced treatment related grade 1 (Tr-G1) early overall AE had improved PFS or OS. Other significant associations were non-Tr AEs either low or high-grade level associated with poorer PFS or OS in three immunotherapy cohorts and one targeted therapy cohort. Another common pattern observed was hazard ratio (HR)>1 in high-grade AEs and HR<1 in some low-grade AEs.
Conclusion: This is the first study to portray early AE outlook in advanced NSCLC. Further analysis of survival association in early AEs identified low-grade treatment related early AEs correlated with better PFS or OS.

#3366 Determinants of survival in hemophagocytic lymphohistiocytosis: A real-world database analysis.

P. A. Haddad, S. Chennapragada, D. Kantamani, S. Gaddam;
LSUHSC-S Feist-Weiller Cancer Ctr./Overton Brooks VAMC, Shreveport, LA

Background: Hemophagocytic lymphohistiocytosis (HLH) is a severe, life-threatening syndrome characterized by the uncontrolled activation of lymphocytes and macrophages, resulting in hyperinflammation and tissue damage. HLH manifests in two forms: primary (familial), driven by genetic mutations affecting cytotoxic function, and secondary (acquired), triggered by infections, malignancies, autoimmune disorders, or other conditions. This study aims to identify key prognostic factors, clinicopathologic characteristics, and therapeutic strategies that impact survival outcomes in HLH patients through a pooled database analysis.
Methods: We analyzed a real-world database of confirmed HLH cases, evaluating demographic features, molecular and immunohistochemical profiles, potential prognostic indicators, treatment interventions, and survival outcomes. Kaplan-Meier survival curves were generated, and Cox proportional hazards models and Log-rank tests were applied to assess the effect of various clinicopathologic factors on overall survival (OS).
Results: The study included 255 confirmed HLH patients, with a median age of 32 and a male-to-female ratio of 1.5. Primary HLH comprised 8% of cases, with PRF1 mutations present in 33%. Triggering etiologies included infections (49%), malignancies (19%), autoimmune conditions (8%), and unknown causes (24%). Tissue hemophagocytosis was observed in 56% of cases, while liver (58%), pulmonary (25%), renal (23%), neurological (15%), and cardiac (9%) dysfunction were also noted. Median OS for the entire cohort was 24 months. Factors significantly associated with worse OS included age $\geq$50 (p=0.003), splenomegaly (p=0.02), malignancy (p=0.0004), LDH $\geq$1000 U/L (p=0.003), sIL2R $\geq$5000 U/ml (p=0.03). Patients with pulmonary or renal dysfunction also had worse OS. Etoposide-based regimens and non-chemotherapy immunosuppression improved median OS compared to supportive care (24 vs. NR vs. 3 months, p=0.038). Of note, etoposide-based regimens arm had a greater percentage of high-risk patients. Complete responders had superior OS compared to those with partial and no response, respectively (NR vs. 18 vs. 0.75; p<0.0001). Moreover, patients with relapse <2months had worse survival (6 vs. NR months, p=0.01).
Conclusion: This analysis, from the largest real-world HLH cohort, highlights key prognostic factors such as age, splenomegaly, malignant etiology, LDH, sIL2R, organ dysfunction, treatment approach, and quality of response as critical determinants of overall survival in HLH patients.

#3367 Determinants of survival in acute megakaryocytic leukemia: A real-world database analysis.

P. A. Haddad[1], S. Vutukuri[2], A. Gupta[2];

[1]LSUHSC-S Feist-Weiller Cancer Ctr./Overton Brooks VAMC, Shreveport, LA, [2]Overton Brooks VAMC, Shreveport, LA

Background: Acute megakaryocytic leukemia (AMKL) is a rare and aggressive subtype of acute myeloid leukemia (AML), characterized by the malignant proliferation of megakaryoblasts. It accounts for less than 1% of AML cases in adults but is more prevalent in pediatric patients, particularly those with Down syndrome. AMKL poses unique diagnostic and therapeutic challenges due to its distinct clinical and biological features, and overall prognosis remains poor. The prognostic factors influencing survival in AMKL are not well-defined, prompting this pooled real-world database analysis to identify clinicopathological characteristics and treatment strategies associated with survival outcomes.

Methods: A pooled database of confirmed AMKL cases was analyzed to assess demographic characteristics, molecular and immunohistochemical profiles, therapeutic interventions, and survival outcomes. Kaplan-Meier survival curves were used to estimate overall survival (OS), while Cox proportional hazards models and Log-rank tests assessed the impact of clinicopathologic factors on OS.

Results: A total of 220 patients with confirmed AMKL were included, with a median age of 3 years. The M:F was 1.3 overall, differing between pediatric (M:F of 1) and adult (M:F of 2) subgroups. The median duration of symptoms before diagnosis was 1 month. Constitutional symptoms, lymphadenopathy, splenomegaly, hepatomegaly, and bone involvement were present in 40%, 19%, 34%, 26%, and 13% of cases, respectively. The cohort median OS was 13 months, with pediatric patients showing significantly better OS compared to adults (44 vs. 5.9 months, $p<0.0001$). Patients with constitutional symptoms ($p=0.05$), lymphadenopathy ($p=0.06$), or elevated LDH (>500 U/L, $p=0.07$) had worse OS, with borderline statistical significance. However, patients with Down syndrome had significantly improved OS (NR vs. 12 months, $p=0.01$). Positive staining for CD4 (<0.0001), CD7 ($p=0.04$), or CD13 ($p=0.03$) was associated with better OS. Sex, WBC count, hemoglobin levels, platelet count, t(1;22), inv(16), and splenomegaly did not significantly impact OS. AMKL arising in the context of malignancy or myeloproliferative disorders was associated with worse OS ($p<0.0001$). Chemotherapy and stem cell transplantation provided superior OS compared to no treatment (10 vs. 23 vs. 1.5 months, $p<0.0001$). Low-intensity regimens offered no significant benefit over no treatment. Patients who achieved complete remission (CR) had significantly improved OS compared to those who did not (44 vs. 4 months, $p<0.0001$).

Conclusion: The analysis of this large pooled cohort of AMKL patients identified key factors influencing survival, including age, Down syndrome status, association with malignancy or myeloid disorders, treatment intensity, and quality of response. These findings provide valuable insights for improving prognostication and guiding treatment strategies in AMKL.

#3368 Hemophagocytic lymphohistiocytosis provisional prognostic score.

P. A. Haddad, S. Chennapragada, D. Kantamani, S. Gaddam;
LSUHSC-S Feist-Weiller Cancer Ctr./Overton Brooks VAMC, Shreveport, LA

Background: Hemophagocytic Lymphohistiocytosis (HLH) is a life-threatening syndrome characterized by the uncontrolled activation of lymphocytes and macrophages, leading to excessive cytokine production, hyperinflammation, and tissue damage. HLH presents in two forms: primary (familial) HLH, associated with autosomal recessive genetic mutations affecting cytotoxic function, and secondary (acquired) HLH, triggered by infections, malignancies, autoimmune diseases, or other underlying conditions. Given the variability in HLH outcomes, this study aimed to develop a provisional prognostic score (HLHPS) to stratify patient risk.
Methods: We analyzed data from a real-world HLH database, comprising 255 cases. Data included demographic information (sex, age, race), blood counts, comorbidities, immunohistochemical and molecular disease phenotypes, treatment types, and survival outcomes. Of these, 219 cases with complete survival data were selected for analysis. A Cox proportional-hazards model and Log-rank tests were applied to identify clinicopathologic factors significantly affecting overall survival (OS), which were then incorporated into a provisional prognostic score based on the impact of hazard ratios.
Results: The median OS for the cohort was 24 months. The key prognostic factors identified were: age $\geq$50 (9 vs. 60 months; p=0.003), underlying malignancy (2 vs. 60 months; p=0.0004), splenomegaly (22 vs. NR months; p=0.02), LDH $\geq$1000 U/L (24 vs. NR months; p=0.003), and sIL2R $\geq$5000 U/ml (6 vs. NR months; p=0.03). A prognostic model was created using these variables, with each variable assigned 1 point when present, for a total score ranging from 0 to 5. Risk categories were defined as low (0-1 points), intermediate (2 points), and high (3-5 points). This prognostic score system led to our cohort's most optimal risk discriminatory model, with low, intermediate, and high risk groups having a median OS of NR, 22, and 1.8 months, respectively (p<0.0001).
Conclusion: The HLHPS is a promising tool for risk stratification in HLH patients, offering a new approach to predict outcomes. However, further prospective validation is necessary to confirm its utility.

#3369 Identifying biomarkers of resistance for immunotherapy-treated patients with non-small-cell lung cancer using real-world evidence and machine learning.

Chao Fang[1], **Natasha Markuzon**[1], Sajan Khosla[2]

[1]AstraZeneca Oncology, Waltham, MA,[2]AstraZeneca Oncology, Cambridge, United Kingdom

Immunotherapy (IO) has revolutionized the management of non-small-cell lung cancer (NSCLC), leading to groundbreaking improvements in patient survival. However, resistance to IO in some patients remains a challenge. Existing IO resistance biomarkers are based on either clinical or genomic factors. Combining genomic factors with patient health characteristics is pivotal in developing new resistance markers and improving the accuracy of predictions of treatment resistance.

The objective of this study is to develop a data-driven model with no a priori assumptions using real-world evidence (RWE) data to identify predictors of treatment resistance in NSCLC patients treated with first-line IO. By integrating clinical and genomic features, we aim to assess the differences in the importance of genomic mutations under various clinical conditions and identify new biomarkers associated with IO resistance.

Using clinical and genomic features from real-world evidence (RWE) Flatiron dataset for an NSCLC cohort treated with first-line IO, we developed a machine learning (ML) model to identify predictors of treatment resistance. Using progression-free survival (PFS) as an endpoint, the model identified key contributing factors, a combination of clinical and genomic features, where the importance of genomic mutations varies under different clinical conditions. Specifically, in patients with ECOG scores of 0 or 1 and no metastasis, the CDKN2A mutation was associated with reduced PFS; for those with ECOG scores of 0 or 1 and with metastasis, CDKN2A was associated with decreased PFS and KRAS with improved PFS; and for patients with ECOG scores of 2-4, KRAS was associated with improved PFS.

Our results underscore the value of RWE in identifying resistance biomarkers, demonstrate the efficacy of a data-driven methodology able to extract combinations of important features without prior assumptions, and highlighted the importance of integrating clinical and molecular data to understand patient-level risk factors of treatment resistance. Using our methodology, we pinpointed statistically significant biomarkers linked to an increased risk of treatment resistance in NSCLC patients undergoing first-line IO treatment.

#3370 MCC-MELD: A diverse lung cancer registry with detailed molecular and environmental annotation.

Luchang Cui[1], Juhong Lee[2], Sajjad Abedian[2], Minmeng Tang[3], James Solomon[4], Robert Crupi[5], Juliet Miller[6], H. Oliver Gao[3], Evan Sholle[2], Christine Garcia[6], Eunji Choi[1], Yiwey Shieh[1]

[1]Department of Population Health Sciences, Weill Cornell Medicine, New York, NY,[2]Information Technologies & Services Department, Weill Cornell Medicine, New York, NY,[3]School of Civil and Environmental Engineering, Cornell University, Ithaca, NY,[4]Department of Pathology and Laboratory Medicine, Weill Cornell Medicine, New York, NY,[5]Ambulatory Care and Population Health, NewYork-Presbyterian Queens, New York, NY,[6]Department of Medicine, Weill Cornell Medicine and New York Presbyterian Hospital, New York, NY

Background: Research on EGFR-mutated lung cancers has been constrained by the scarcity of large, diverse datasets integrating molecular, clinical, and environmental data. To address this gap, we built the Meyer Cancer Center Molecularly Enhanced Lung Cancer Database (MCC-MELD), which combines molecular testing results, detailed clinical information, and fine-resolution environmental data while leveraging the diversity of our catchment area.

Methods: MCC serves as the cancer center for Weill Cornell Medicine, comprising three clinical sites in New York City: Manhattan, Queens, and Brooklyn. We extracted electronic health record (EHR) data for patients diagnosed with lung cancer from all sites using Epic, and from Queens (AllScripts) and Brooklyn (Cerner Millennium) sites prior to Epic implementation in 2020. We obtained tumor characteristics and treatment history from the ONCOLog internal tumor registry (2010-2020) and a manually curated lung cancer database maintained by our thoracic oncology clinic (2010-2024). We retrieved molecular testing results from an in-house pathology database (cBioPortal) and from the EHR. We used natural language processing (NLP) powered by generative artificial intelligence (AI) using Gemini-1.0-pro to abstract additional factors, including quantitative smoking data. Area-level factors - including air pollution, neighborhood socioeconomic status, and built environment - were linked to geocoded patient addresses at diagnosis. To address the challenges of measuring air pollution in densely populated urban areas, we will use a hyperlocal AI model that estimates longitudinal air pollutant levels on a 1000-by-1000-meter grid scale.

Results: We identified a maximally inclusive cohort of 12,285 patients, with 8,754 comprising the analytic cohort (2010-2024) of confirmed lung cancer patients. The cohort included 53% female patients (compared to 44% nationally), with racial and ethnic representation of 20% Asian, 13% Black, 8% Hispanic, and 46% White patients. Lung cancer cases among nonsmokers increased from 9.9% to 20.0% over the study period. Of the cases that received in-house comprehensive genomic profiling, EGFR alterations were found in 28.7% (766 of 2,671). Our preliminary NLP achieved high accuracy in extracting smoking status (87.1%) and smoking intensity (94.4%, measured as packs smoked per day).

Discussion: MCC-MELD shows the feasibility of integrating AI-powered annotation and prediction into clinical research, providing a robust platform to study risk factors, prognostic indicators, and care patterns for EGFR-mutated lung cancers. Our planned analysis using our hyperlocal air pollution model will allow us to assess the relationship between air pollutants and EGFR-mutated lung cancer at a uniquely fine scale. We expect MCC-MELD will be a valuable resource for advancing research on lung cancer risk and outcomes in a diverse urban population.

#3371 Gene deletions in estrogen receptor positive HER2-negative breast cancer are associated with worse outcome than predicted by diagnostic-grade gene expression assays.

Jonathan T. Lei[1], Kim Chu[2], Brian Li[3], Eugene Schuster[4], Zachary L. Skidmore[3], Shyam M. Kavuri[1], Vikrant Mehta[1], Nicholas C. Spies[3], Jason Kunisaki[3], Catrina Fronick[3], Robert S. Fulton[3], Malachi Griffith[3], Richard Buus[4], Jack Cuzick[2], Mitch Dowsett[4], Meenakshi Anurag[1], Obi L. Griffith[3], Matthew J. Ellis[5]

[1]Baylor College of Medicine, Houston, TX,[2]Queen Mary University of London, London, United Kingdom,[3]Washington University School of Medicine, St Louis, MO,[4]Royal Marsden Hospital, London, United Kingdom,[5]Guardant Health, Palo Alto, CA

Introduction: Currently, risk of recurrence in ER+ HER2- breast cancer is determined by pathological stage and histological grade, in combination with a Gene Expression Assay (GEA) including Oncotype, Prosigna, Breast Cancer Index and EPClin. Higher risk disease justifies systemic therapy beyond endocrine therapy alone. GEAs need improving as these assays can misclassify risk, i.e., recurrences still occur in patients assigned low risk. Thus, approaches to identify high-risk cases more accurately would reduce under-treatment. Herein we explore whether somatic mutation and copy number data from an established 83 gene targeted sequencing panel can improve risk stratification based on clinical factors and GEA alone.

Methods: Targeted gene-sequencing (TGS) was undertaken in the Anastrozole (A) or Tamoxifen (T) alone or Combined (C) (ATAC) adjuvant trial for estrogen receptor-positive (ER+) breast cancer to identify mutations and copy number variants that are prognostic variables independent of clinical factors and GEA-based risk assessments. Patients in the combination arm were excluded for this study. Forward Stepwise Cox Regression was applied to identify candidates, whose prognostic effects were validated in independent datasets.

Results: Investigation of prognostic interactions of gene alterations with four GEA mentioned above identified three adverse gene deletions (*del*) including: 1) MAGI3*del* at chr1p.13.2 (HR=2.43-2.86, p-value< 0.005), a newly assigned tumor suppressor, that associated with other adverse deletion events on chr1p13.2-36.22 including ARID1A/JAK1/mTOR; 2) MET*del* on chr7q.31.2 (HR=1.86-2.15; p<0.05), adjacent to the tumor suppressor CAV1; and 3) RB1CC1*del* on chr8q.11.23 (HR=3.41-4.30; p<0.05), also a putative tumor suppressor. Potential clinical impact included high-relapse risk reassignment for Oncotype/Prosigna/Breast Cancer Index (BCI)-defined low and/or intermediate-risk cases if MAGI3*del* was present, and high-risk reassignments of BCI intermediate-risk cases if any of MAGI3/MET/RB1CC1 deletions were present, with lower-risk reassignment if absent.

Conclusions: Copy number variation (CNV) analysis identified three high-risk deletion events (MAGI3, MET, RB1CC1) in ER+ HER2- breast cancer that predicted worse clinical course than predicted by diagnostic-grade gene expression assays. Thus, the incorporation of CNV-detection into prognostic algorithms has the potential to reduce under-treatment due to low/intermediate-risk mis-assignment. These data are proof-of-concept that prognostication in ER+ HER2- disease can be improved by TGS, while also identifying under-studied gene deletions underlying the highly variable relapse trajectories that typifies ER+ breast cancer.

#3372 Building precision oncology via real-world data standardization, privacy preserving infrastructures, and collaboration among IT and clinical experts: The DIGICORE network experience.

Alberto Traverso[1], Giovanni Tonon[2], Piers Mahon[3], Gennaro Ciliberto[4], Andre Dekker[5], Xose Fernandez[6], **Adriana Albini**[7]

[1]Department of Artificial Intelligence, Faculty of Medicine, San Raffaele Hospital, Milano, Italy,[2]School of Medicine, Libera Universita Vita-Salute San Raffaele, Milano, Italy,[3]DIGICORE, Bruxelles, Belgium,[4]IRCCS National Cancer Center Regina Elena, Roma, Italy,[5]Department of Radiation Oncology (Maastro, GROW School for Oncology and Reproduction, Maastricht University Medical Centre, Maastricht, Netherlands,[6]DigiONE, IQVIA Cancer Research, Zaventem, Belgium,[7]IRCCS IEO - European Institute of Oncology, Milano, Italy

Precision oncology necessitates personalized treatments maximizing efficacy while minimizing toxicity. Real-World Data (RWD) presents a powerful resource to achieve this, offering insights to clinical trials and facilitating cross-national comparisons of treatment effectiveness. However, significant obstacles persist: data sparsity, unstructured formats, and limited digitization; complex and internationally diverse data privacy regulations; heterogeneous IT infrastructures and expertise across institutions. The DIGICORE (Digital Institute for Cancer Outcome Research) consortium—a network of European hospitals and companies—directly addresses these challenges. Its initiatives, among the DIGIONE research plan, include: (1) the definition of a set of minimum data elements related to electronic health record systems required to setup RWD studies (MEDOC) (2) a standardized survey assessing the digitalization of these key cancer-related data elements; (3) AI-based tools such as natural language processing to support data digitization; (4) a federated learning infrastructure for privacy-preserving clinical research; (4) the development of standards for comprehensive cancer center infrastructure, (5) the establishment of disease-focused working groups to foster collaboration, and (6) dedicated training programs for clinicians to enhance their IT knowledge. To date, three projects have been completed, with additional 10 projects underway. DIGICORE's multi-faceted approach promises to unlock the transformative potential of RWD in advancing personalized cancer care. (https://digicore-cancer.eu/) 1. Mahon P, et al. A federated learning system for precision oncology in Europe: DigiONE. Nat Med. 2024 doi: 10.1038/s41591-023-02715-8. 2. Mahon P, et al. Harnessing oncology real- world data with AI. Nat Cancer. 2023 doi: 10.1038/s43018-023-00689-7.

Study Name	Initiating countries	Disease	Clinical question(s)	Sample size
Analysis of the number of new primary cancers diagnosed and 12-month survival changes during COVID-19 lockdowns	Belgium Germany Italy Norway UK The Netherlands	Pan cancer	To Investigate the impact of COVID19 lockdowns on new cancer diagnoses. To estimate 12-month survivals.	124.682 patients
A disease natural history and outcomes study with care quality assessment in metastatic non-small cell lung cancer	Germany Italy Norway UK The Netherlands	Lung cancer	To investigate the survival of patients according to the location of the metastases. To describe treatment patterns by line of therapy prescribed to patients: 1. All the cohort 2. Subgroups by locations of the metastases To benchmark care quality between centers based on ESMO recommendation.	1294 patients
A disease natural history and outcomes study with care quality assessment in HR+/HER2- metastatic breast cancer	Belgium Italy UK	Breast cancer	To Describe the demographic, clinical, molecular phenotypes, and next-generation sequencing (NGS) results for patients with HR+/HER2– metastatic breast cancer (mBC): Cohort A: HR+/HER2– de novo mBC, Cohort B: HR+/HER2– recurrence after locoregional disease. To Compare HER2-low versus HER2-zero within both cohorts. .	5k-10k patients (estimate)

#3373 Event-free survival among polycythemia vera patients treated with novel JAK inhibitors: a comparative analysis using real-world genomic data and electronic health records.

Stacy Charlerie[1], Nathan Lee[1], Jaime Smith[1], **Angela X. Qu**[2]

[1]Real World Research, PAREXEL International, Research Triangle Park, NC,[2]Translational Medicine, PAREXEL International, Research Triangle Park, NC

Known as a chronic myeloproliferative neoplasm, Polycythemia Vera (PV) is a rare blood cancer characterized by an abnormal increase in the number of red blood cells. PV is associated with increased risk of thrombotic events and mortality as well as other complications, including splenomegaly, macrovascular and microvascular events (e.g., transient ischemic attacks, erythromelalgia, ocular migraines), and aquagenic pruritus. The exact cause is unknown, but genetic mutations associated with a protein called Janus kinase 2 (JAK2) are often involved, and as such genomic testing is often part of the diagnostic journey and treatment options. Real-world data (RWD) and real-world evidence (RWE) have emerged as powerful tools for unlocking the potential of healthcare data generated in routine clinical practice. In oncology, RWD has been instrumental in multiple areas including 1) Discovering novel biomarkers by uncovering associations of genetic, molecular, and clinical outcomes that predict treatment response and disease progression; 2) Identifying comorbidities by recognizing coexisting conditions that may impact treatment outcomes and patient management 3) Optimizing treatment pathways by identifying effective treatment sequences and combinations for specific patient populations; 4) Enriching clinical trials by selecting the most appropriate patient populations for clinical trials and accelerating drug development. In this study, we aim to leverage electronic health records (EHR) data to conduct a comparative analysis of event-free survival (EFS) in PV patients on novel JAK inhibitor therapy versus other standard of care first-line treatments. By utilizing EHR data, we can identify key clinical factors associated with EFS and potentially develop predictive models to inform personalized treatment strategies. Additional exploration of real-world genetic/genomic data for insights to predict thrombotic events or mortality is forthcoming. This research has the potential to improve the management of PV patients and reduce the burden of disease. We will present our findings with some preliminary data, describe the data sources used, outline the technologies leveraged, and conclude with lessons learned.

#3375 Demographic characteristics and incidence of cardiovascular events, infections and cytokine response syndrome (CRS) in patients treated with chimeric antigen receptor (CAR) T cell therapy: a nationwide analysis.

Himil Mahadevia[1], Karnav Modi[1], Akhil Jain[2], Furha Cossor[3], Deepthi Vodnala[3], Taiyeb Khumri[3]

[1]University of Missouri - Kansas City School of Medicine, Kansas City, MO,[2]University of Iowa, Iowa City, IA,[3]Saint Luke's Hospital of Kansas City, Kansas City, MO

Introduction: CAR-T cell therapy represents a significant advancement in the management of relapsed/refractory hematological malignancies. Adverse events include cytokine release syndrome (CRS), infections, and neurological and cardiovascular events. Though real-world data from referral centers regarding the uptake of CAR-T cell therapy in different populations and the incidence of various adverse events are available, we used the National Inpatient Sample (NIS) with weighted analysis to provide contemporary data representing national estimates for patients and complications profile with CAR-T therapy.
Methods: A retrospective study was conducted by obtaining patients who underwent CAR-T cell therapy using ICD-10-PCS procedure codes (XW033C3, XW043C3, XW23346, XW24346, XW23376 and XW24376) from NIS 2021 dataset. Demographic characteristics, as well as the incidence of infectious complications and adverse cardiovascular events, were obtained using ICD-10-CM diagnostic codes. Different grades of CRS were identified using specific ICD-10 codes (Grade 1-5: D89.831-D89.835).
Results: Of 2,235 patients who underwent CAR-T cell therapy between January 2021 and December 2021, 64.2% were male, 73.5% identified as Caucasian, 12.3% as Asian and 5.5% as African American. 50.6% had private insurance, 36.5% had Medicare, and 6.7% had Medicaid. Most patients had lymphomas (64.9%), followed by myelomas (17.9%) and leukemias (13%). Acute myocardial infarction and stroke were documented in 0.4% of patients each. Notably, 21.7% of patients experienced cardiac arrhythmias, and major cardiovascular events were recorded in 4.3% of the cohort. Additionally, 4.9% of patients were diagnosed with pneumonia, while sepsis occurred in 7.6% of patients, and 3.4% experienced septic shock. Moreover, 1.1% of patients developed HLH. CRS was observed in 59.3% of the patients. Among those with CRS, grade 1 and grade 2 reactions were reported in 32% and 21% of patients, respectively, while grade 3 and grade 4 reactions were noted in 4.7% and 2% of patients, with no cases of grade 5 identified. The mean total cost of hospitalization was 324,972.8 USD. The overall in-hospital mortality rate was found to be 3.4%.
Conclusion: In our study, most patients were males, and the majority belonged to the Caucasian ethnicity. Over half of the patients had private insurance. CRS was common but mostly low-grade intensity. Infections present a considerable challenge with CAR-T therapy, making early identification and appropriate antimicrobial treatment critical. Adverse cardiovascular events were frequently reported among patients receiving CAR-T cell therapy, underscoring the necessity for close cardiac monitoring to detect such events and to implement comprehensive care strategies effectively.

#3376 Real-world cancer treatment patterns and their effects in older adults with lung cancer: a SEER-MHOS analysis.

Shuyan Qiu[1], Yueyi Xu[1], Laura Pinheiro[2], Jialin Mao[2], Yushu Shi[2], Yiwey Shieh[2], Christine Garcia[2], Eunji Choi[2]

[1]Mailman School of Public Health, New York, NY,[2]Weill Cornell Medicine, New York, NY

Introduction: Lung cancer primarily affects older adults, with a median diagnosis age of 71. Due to high competing death in older adults, the feasibility and efficacy of treatment may differ from clinical trials. This study examined treatment patterns and mortality outcomes in older adults with lung cancer using real-world analytic methods.
Methods: We used SEER-Medicare Health Outcomes Survey (MHOS) to identify adults aged ≥65 years with non-small cell lung cancer (2007-2019). The study had 2 primary outcomes: (1) uptake for first-course treatments, and (2) lung cancer survival. Treatment uptake was calculated by age, frailty at diagnosis (assessed with a cancer-specific frailty index from MHOS), and neighborhood socioeconomic status (nSES) across disease stages. We used inverse probability of treatment weighting (IPTW) and clone-censoring methods in multivariable Cox models to mitigate selection and immortal time biases.
Results: In 11,443 patients (mean age, 77.3 years; 72% White; 47% distant-stage), treatment uptake varied by age, frailty, and nSES. Surgery rates for localized-stage patients declined with age (67% vs. 16% for ages 65-69 vs. ≥85, $P<10^{-3}$), as did chemotherapy rates for distant-stage patients (58% vs. 21% for ages 65-69 vs. ≥85, $P<10^{-3}$). Patients who received surgery or chemotherapy were less frail at diagnosis ($P<10^{-3}$) compared to those who did not, while frailty score was comparable by radiotherapy. Survival benefits for all three treatments were not observed in patients aged ≥85 using both IPTW and clone-censoring models, though IPTW-only models suggested survival benefits, indicating residual bias. Conclusion: Age at diagnosis, frailty, and nSES influence treatment uptake in older adults with lung cancer. Rigorous real-world analytic methods indicate limited survival benefits from intensive treatments for patients≥85 years, underscoring the need for tailored treatment decision-making in this vulnerable population.

Table. Association between treatment and lung cancer survival

Real-world Analytics	Age at LC	Surgery (vs. no surgery)		Chemotherapy (vs. no chemotherapy)		Radiation (vs. no radiotherapy)	
		aHR	(95% CI)	aHR	(95% CI)	aHR	(95% CI)
IPTW	65-74	0.34	(0.31-0.37)*	0.74	(0.69-0.80)*	0.86	(0.81-0.92)*
	75-84	0.40	(0.38-0.43)*	0.88	(0.84-0.93)*	0.86	(0.82-0.90)*
	85+	0.62	(0.56-0.68)*	0.75	(0.67-0.83)*	0.73	(0.67-0.80)*
IPTW + Clone-censoring	65-74	0.56	(0.52-0.59)*	1.08	(1.02-1.14)*	0.84	(0.80-0.89)*
	75-84	0.61	(0.59-0.63)*	1.12	(1.07-1.16)*	0.88	(0.85-0.92)*
	85+	0.97	(0.90-1.04)	1.08	(1.00-1.16)*	1.00	(0.93-1.08)

Abbreviations: IPTW, inverse probability of treatment weighting; aHR, adjusted hazard ratio; CI (confidence interval); LC (lung cancer); * denotes *P*<0.05; Hazard ratios were adjusted for key prognostic factors.

#3377 The impact of gender and age on real-world progression-free survival (rwPFS) in patients without a smoking history with NSCLC treated with checkpoint inhibitors.

Hye Sung Kim[1], Li Zhang[2], Margie L. Clapper[2], Jill Hasler[2], Tia Nicolas[2], Hossein Borghaei[2], Martin J. Edelman[2], Joseph Treat[2], J. Nicholas Bodor[2]

[1]Temple University Hospital, Philadelphia, PA,[2]Fox Chase Cancer Center, Philadelphia, PA

Introduction: While the emergence of checkpoint inhibitors has changed the treatment landscape of non-small cell lung cancer (NSCLC), most never-smokers derive little benefit. This lack of benefit is due to many never-smokers possessing oncogene-driven cancers (e.g. EGFR, ALK, ROS1, etc.) that have “cold” tumor microenvironments (TME) that poorly respond to anti-PD-1(-L1). However, being female and young are also associated with inferior responses to immunotherapy in several cancer types, possibly due to these patients having more endogenous hormones that may be immunosuppressive. To our knowledge, the impact of gender and age on the efficacy of anti-PD-1(-L1) has not been studied in NSCLCs that occur in never-smokers. Such an investigation is particularly relevant given the frequency of this diagnosis in young women.

Methods: A nationwide de-identified EHR-derived database (Flatiron Health) was used to evaluate patients without a smoking history with advanced NSCLC treated with single-agent anti-PD-1(-L1). De-identified data originated from approximately 280 US cancer clinics (~800 sites of care). Kaplan-Meier curves estimated rwPFS (based on clinician documentation of progression) in months (m) with gender and age comparisons evaluated using the log-rank test and multivariable Cox regression.

Results: In our cohort of 1535 patients, 1029 (67%) were female, 836 (55%) had a tumor oncogene, 144 (9%) were <55 years of age (yoa), 782 (51%) were 55-75 yoa, and 609 (40%) were >75 yoa. Real-world PFS did not differ between females (2.9 m, 95% CI 2.8-3.1) and males (2.8 m, 95% CI 2.7-3.2) (p=0.52). However, among female patients, those < 55 yoa had shorter rwPFS (2.1 m, 95% CI 1.9-2.7) than females 55-75 yoa (2.9 m, 95% CI 2.7-3.2) and >75 yoa (3.2 m, 95% CI 2.9-3.6) (p=0.001). In a Cox regression model that adjusted for performance status, race, therapy line, and presence of a tumor oncogene, female patients >75 yoa (HR 0.74, 95% CI 0.58-0.95) and 55-75 yoa (HR 0.82, 95% CI 0.65-1.03) were at lower risk for disease progression as compared to females < 55 yoa (reference group). Differences by age in rwPFS were not observed in males.

Conclusion: Our analysis revealed that among women without a smoking history, young patients < 55 yoa with NSCLC experience especially poor survival with anti-PD-1(-L1). Research is needed to elucidate the biological mechanisms underlying this disparity. While data on menopausal status were not available for this analysis, most women < 55 yoa are pre- or perimenopausal. The contribution of greater levels of endogenous estrogens to immunosuppression in the lung TME warrants further study. (Supported by a Flatiron-FCCC Academic Partnership Research Grant Award)

CLINICAL RESEARCH: Real-World Data and Real-World Evidence: Clinico-Genomics
Poster Session

#3381 Patient characteristics and recommendations from a multi-institution Precision Oncology Tumor Board.

Aseem Aseem, Noor Naffakh, Michael Weinfeld, Shikha Jain, V K Gadi, Kathleen Kennedy, Nan Sethakorn, Sarah Khan, Natalie Reizine, Frank Weinberg, Ryan Nguyen

University of Illinois at Chicago, Chicago, IL

Background: The Precision Oncology Tumor Board (POTB) at the University of Illinois Chicago (UIC) advances cancer treatment through molecular profiling and personalized recommendations. Studies link molecular tumor boards to improved outcomes. POTB integrates multidisciplinary expertise across institutions to provide therapeutic and trial recommendations based on clinical and genomic data. The PROMOTE (PRecision Oncology: Measuring Outcomes and Treatment Effectiveness) study is a retrospective analysis of cases presented at the UIC POTB and here we report the patient demographics, clinical characteristics, and recommendations from the first two years of this program.
Methods**:** We retrospectively analyzed attendee specialties, patient demographics, tumor characteristics, and treatment recommendations from cases reviewed (September 2022-October 2024). Descriptive statistics captured data on age, sex, race, ethnicity, cancer type, stage, insurance, diagnosis timing, biomarker class, FDA approval status, recommendation type, and trial options.
Results: A total of 120 healthcare professionals participated across 22 POTB sessions, averaging 29 attendees per session, including medical oncologists and hematology/oncology fellows (53%), pharmacists (33%), pathologists, genetic counselors, industry partners, and trainees. Of the 81 cases presented, the mean patient age was 59 years, with 33% aged >64 years and 58% aged 40-64 years. Of the patients, 49% were female, 35% were Black, 40% White, and 19% Hispanic. The most common cancer types included lung (33%), genitourinary (19%), and gastrointestinal (14%), with 79% of cases involving stage IV disease. Treatment recommendations included clinical trial recommendations (68% of cases) and FDA-approved therapies outside their labeled indications (56% of cases). Targeted therapies were recommended in 50% of cases, followed by additional testing (16%) and immunotherapy (12%). Germline testing was completed or recommended in 62% of cases.
Conclusions: This retrospective analysis highlights one of the most racially and ethnically diverse molecular tumor board patient cohorts reported in the literature. Recommendations from UIC POTB demonstrate the feasibility of integrating precision oncology into practice, with implications for expanding access to targeted therapies and clinical trials for underrepresented populations. Future efforts will focus on linking recommendations to outcomes, refining treatment strategies, and enhancing equity in precision oncology.

#3382 The predictive effect of gBRCAs mutations on rates of pathological complete response (pCR) after neoadjuvant chemotherapy (NAC) in Chinese breast cancer (BC) patients: a single-center study in Xijing Hospital, Northwest China.

Jing Fan[1], Ting Wang[1], Jiajun Ding[1], Yidi Wang[1], Mengxuan Li[1], Huimin Meng[1], Jing Kong[1], Haoyi Zi[1], Qingxia Li[1], Shuai Wang[1], Fei Yao[2], Changbing Zhu[2]

[1]Department of Thyroid, Breast and Vascular Surgery, Xijing Hospital, Air Force Medical University, Xi'an, China,[2]Department of Medicine, Amoy Diagnostics Co., Ltd., Xiamen, China

Background and Objective: The link between germline *BRCA* (gBRCA) mutations and the effectiveness of neoadjuvant therapy (NAC) in early-stage breast cancer (BC) is debated. It prompted us to conduct this retrospective study to determine NAC efficacies according to gBRCA variant status overall or further by BC molecular subtypes.

Methods: A cohort of 452 female patients receiving standard NAC and following surgery with gBRCAs mutational signatures were enrolled in this study. Pearson Chi-square test, continuous correction chi-square test and fisher's exact test were used to explored the association between gBRCA1/2 mutation and the response to NAC.

Results: Overall, 131 patients with triple negative breast cancer (TNBC), 215 patients with Luminal B ($HER2^-$ or $HER2^+$) BC as well as 57 $HER2^+$ patients were included. All enrolled patients classified as stageII-III. 60.6% (20/33) of gBRCA1 mutation ($gBRCA1^{mut}$) patients, 28.6% (8/28) of gBRCA2 mutation ($gBRCA2^{mut}$) patients and 39.6% (155/391) of noncarriers patients achieved pathological complete response (pCR) (p=0.049). For achieving RCB 0/I, the rates were 69.7% (23/33), 39.3% (11/28) and 50.4% (197/391) respectively (p=0.045). Among $HER2^-$ patients, $gBRCA1^{mut}$ patients were more likely to be pCR compared to non-$gBRCA1^{mut}$ carriers (53.8% vs. 25.2%, p=0.005), so as in $gBRCA1/2^{mut}$ patients compared to non-$gBRCA1/2^{mut}$ carriers (42.9% vs. 25.7%, p=0.023). In Luminal B ($HER2^-$) patients, only $gBRCA1^{mut}$ was associated with better pCR rate (57.1% in $gBRCA1^{mut}$ patients compared to 13.9% in non-$gBRCA1^{mut}$ patients, p=0.013). While in TNBC patients, pCR was more frequent in $gBRCA1/2^{mut}$ patients compared to noncarriers (60.7% vs. 37.9%, p=0.034). Additionally, in clinically node-positive patients, in spite of the difference was not statistically significant, $gBRCA2^{mut}$ patients tended to be worse response to NAC compared to non-$gBRCA2^{mut}$ patients (0.0% vs. 29.3%, p=0.077).

Conclusion: Though the outcome of a single-center study with small sample size was restricted, it could be found that $BRCA1^{mut}$ patients had better response to NAC, while $BRCA2^{mut}$ patients achieved less from NAC.

#3383 Clinical and genomic utility of smoking pack years in a real world cohort.

Tejiri E. Agbamu[1], Kelly Pekala[2], Xinran Bi[3], Xuechun Bai[1], David Ma[1], Nicholas Pickersgill[1], Michele Waters[1], Jian Carrot-Zhang[1]

[1]Memorial Sloan Kettering Cancer Center, New York, NY,[2]Multicare Urology, Puyallup, WA,[3]Princeton University, Princeton, NJ

Introduction: In this work, we employ pack years (PYs) to characterize the relationship between smoking exposure and clinical and genomic features in a diverse clinical genomic cohort of lung (NSCLC) and bladder cancer (BLCA) patients.
Methods: Clinical annotations including sex, age and stage at diagnosis were extracted from electronic health records (EHR) for 6,441 NSCLC and BLCA patients who underwent clinical genomic sequencing at Memorial Sloan Kettering Cancer Center after 2014. PYs were extracted from EHR using regular expressions and manually curated. To account for the non-parameteric distribution of PYs we split PYs into four groups: 0(0), 1(0-20), 2(20-40), and 3 (>40). Age at diagnosis was split into 4 groups: Under 45, 46-65, 66-80, and Over 81. Categorical variables such as age, sex, and stage were evaluated by wilcoxon rank-sum test. While continuous variables such as tumor mutational burden (TMB) and fraction genome altered (FGA) were tested by linear regression controlling for age, sex, and stage. Overall survival (OS) was measured from time of sequencing to last follow up and evaluated with a Cox proportional hazards model (CoxPH). Genetic ancestry was determined from MSK-IMPACT sequencing data using reference populations from the 1000 Genomes project, including European (EUR), African (AFR), East Asian (EAS). Ancestry was assigned based on proportion greater than 80%
Results: Our cohort consisted of 4,953 NSCLC and 1,488 BLCA patients including 3,975 former/current smokers and 2,466 never smokers. There was a significant difference among age and PYs in groups 46-65 and 66-80 in NSCLC (Mdn=1.5 v 20;p <0.0001) and BLCA (Mdn=0 vs 6;p <0.0001). Male patients also tended to have a higher PYs in comparison to female patients in NSCLC (Mdn=9 vs 23;p<0.0001) and BLCA (Mdn=0 vs 6; p<0.05). We did not observe a significant association between FGA and PYs in NSCLC or BLCA patients. However, we did notice a positive relationship between PYs and TMB in NSCLC patients (LR:1.77; CI[1.62-1.92] ; p<0.0001). We also evaluated the relationship between ancestry and PYs where patients with EUR ancestry had a higher PYs than patients with AFR in NSCLC (Mdn=20 vs 3; p<0.0001). Interestingly, we identified a novel genomic association between PYs and STAG2 in BLCA (OR:1.21, CI[1.08-1.36],p=0.001). STAG2 which could be a potential therapeutic target. Lastly, we performed a CoxPH to investigate the relationship between smoking exposure and OS. PYs were associated with an incremental decrease in OS among NSCLC patients where those in PYs group 3 had the highest risk (HR:1.50; CI:[1.34-1.66,];p<0.0001) and outperform TMB (HR:1.17; CI:[1.06-1.28]; p=0.0012). PYs also predicted OS in BLCA (HR:1.90; CI:[1.49-2.42]; p<0.0001).
Conclusion: By utilizing PYs we were able to identify clinical and novel genomic associations such as STAG2 in BLCA and lower OS in NSCLC. PYs can serve as an alternate clinical variable to smoking history alone.

#3384 Comprehensive profiling of g*BRCA1* and g*BRCA2* variants in Hormone receptor-positive breast cancer of Chinese patients.

Jiajun Ding[1], Ting Wang[1], Jing Fan[1], Huimin Meng[1], Mengxuan Li[1], Haoyi Zi[1], Shuai Wang[1], Qingxia Li[1], Yidi Wang[1], Jing Kong[1], Fei Yao[2], Changbing Zhu[2]

[1]Department of Thyroid, Breast and Vascular Surgery, Xijing Hospital, Air Force Medical University, Xi'an, China,[2]Department of Medicine, Amoy Diagnostics Co., Ltd., Xiameng, China

Background and Objective: The identification as well as the interpretation of germline *BRCA1 (*gBRCA1*)* and germline *BRCA2* (gBRCA2) variants are becoming increasingly critical in the treatment of breast cancer (BC), particularly in cases of hormone receptor-positive BC (HR^+ BC). However, a thorough analysis of these variants within the HR^+ BC population is still insufficient.

Methods: In this single-center retrospective study, a total of 1033 female invasive BC patients getting gBRCAs mutation testing with signatures were enrolled, and 652 of them were HR^+ BC patients. Pearson chi-square test and continuous correction chi-square test were used to explored the association between gBRCAs mutation and the characteristics of BC. Then clinical and pathological characteristics related to gBRCAs mutation enrollment on univariate logistic regression were selected for multivariate logistic regression.

Results: Overall, 108 Luminal A (LA) BC patients, 411 Luminal B represented HER2 negative ($LB\text{-}HER2^-$) BC patients, 133 triple-positive BC (TPBC) patients, 109 HER2 positive ($HER2^+$) patients as well as 272 triple-negative BC (TNBC) patients were enrolled in the study. There were 5.6% (58/1033) patients, 4.7% (49/1033) patients and 10.4% (107/1033) patients carrying $BRCA1^{mut}$, $BRCA2^{mut}$ and $BRCA1/2^{mut}$ individually. As for HR^+ patients, the rates were 2.6% (17/652), 5.2% (34/652) and 7.8% (51/652) respectively. It was the most frequent to found $gBRCA1^{mut}$ in TNBC (14.7% in TNBC compared to 2.36% in non-TNBC, $p<0.001$) while for $gBRCA2^{mut}$, it was $LB\text{-}HER2^-$ BC (6.8% in $LB\text{-}HER2^-$ BC compared to 3.4% in others). Even if in HR^+ BC patients, the rate of $gBRCA1^{mut}$ was higher in ER-low patients compared that in ER-high ones (10.5% vs. 1.8%, $p<0.001$), while this phenomenon could not be found for gBRCA2 mutation. According to the multivariate logistic regression in overall patients, those with bilateral BC ($p=0.007$) and PR negative BC ($p=0.026$) were more likely to be $BRCA1^{mut}$. Though the difference without statistically significant, it seemed that patients younger than 40 (≤40) ($p=0.075$) and grade III ($p=0.056$) tended to be $gBRCA^{mut}$. While patients ≤40 ($p=0.008$), in T3 or T4 stage ($p=0.034$) with higher Ki-67 ($p=0.015$) were more frequent to be $BRCA2^{mut}$. Among $LB\text{-}HER2^-$ patients ≤40 in T3 or T4 stage, the rate of $gBRCA2^{mut}$ increased to 26.9% (7/26), no less than the rate of $gBRCA1^{mut}$ in young TNBC patients (24.7%, 20/81), who were recommended the gBRCAs testing.

Conclusion: The clinical and pathological characteristics differed in $gBRCA1^{mut}$ and $gBRCA2^{mut}$ among HR-positive BC patients**.** The screening for gBRCA2 mutation could be meaningful in young $LB\text{-}HER2^-$ patients with tumor in T3 or T4 stage.

#3385 Real-world data analysis of somatic mutations using the GenMineTOP, a dual-targeted DNA and RNA comprehensive genomic profiling panel.

Kousuke Watanabe[1], Miho Ogawa[1], Eri Habano[2], Aya Shinozaki-Ushiku[2], Masakazu Akahori[3], Toshimitsu Ichijo[3], Shuichi Tsutsumi[4], Kenji Tatsuno[4], Hiroyuki Aburatani[4], Hidenori Kage[5], Katsutoshi Oda[2]

[1]Next-Generation Precision Medicine Development Laboratory, Graduate School of Medicine, University of Tokyo, Tokyo, Japan,[2]Division of Integrative Genomics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan,[3]Konica Minolta, Co. Ltd., Tokyo, Japan,[4]RCAST, University of Tokyo, Tokyo, Japan,[5]Department of Respiratory Medicine, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

Introduction: In Japan, comprehensive genomic profiling (CGP) tests have been covered by insurance since 2019, and the dual-targeted DNA and RNA CGP panel, GenMineTOP, has been covered since 2023. Of the CGP tests conducted under insurance, 99.6% of cases are registered in the nationwide database: Center for Cancer Genomics and Advanced Therapeutics (C-CAT). This study aims to elucidate somatic genomic alterations detected by GenMineTOP using real-world data from C-CAT.
Method: We analyzed 1,356 cases of GenMineTOP registered in the C-CAT database by June 2024. Clinical significance was determined based on the evidence level defined by C-CAT. We examined DNA mutations, copy number alterations, fusion/exon skipping, and TMB-high cases. Additionally, we performed multivariate analysis to assess the impact of DNA mutations, copy number amplifications, fusion genes, and primary organ on RNA expression levels.
Results: The top five cancer types were bowel (15.4%), soft tissue (11.6%), CNS/brain (11.1%), pancreas (8.3%), and head and neck (5.6%). Oncogenic DNA mutations, copy number amplifications, and fusion/exon skipping were found in 1,140, 342, and 117 cases, respectively. Fusion/exon skipping was more common in cases without DNA mutations (68/1140 vs. 49/216). Frequent DNA mutations included *TP53*, *KRAS*, *APC*, *PIK3CA*, and *TERT*. Copy number amplifications were most common in *MDM2*, *CDK4*, *MYC*, *EGFR*, and *CCND1*. TMB-high was observed in 72 cases (5.3%). The RNA panel detected 105 fusion cases and 13 exon skipping cases; 17 fusions were evidence level A (Japan- or FDA-approved drug for the cancer type) and 39 were diagnostically significant. NTRK fusions were detected in 11 cases (0.8%). Exon skipping was observed in *EGFR* (exon 2-7, n=5), *CTNNB1* (exon 3, n=7), and *ERBB2* (exon 16, n=1). Gene expression was influenced by the primary organ and the presence of gene amplification, fusion genes, and/or mutations. All 27 analyzed genes showed differences in expression depending on the primary organ, with *ALK*, *NTRK2*, and *NTRK3* exhibiting particularly significant variations. These genes had the highest expression levels in the CNS/brain. The effect of gene amplification on expression levels varied by gene, with the most significant impact observed in *CDK4* and *MDM2*. Mutations in *KIT* and *TERT* were linked to increased expression. Statistically significant overexpression was observed in cases positive for ALK, FGFR3, NTRK1, NTRK3, and RET fusions.
Conclusion: GenMineTOP detected clinically significant fusion/exon skipping. Factors affecting RNA expression were quantitatively elucidated.

#3386 Clinicogenomic comparison of patients with liver-avid/lung-adverse and lung-avid/liver-adverse patterns of metastases highlights commonalities across cancer types.

John Nguyen[1], Walid K. Chatila[1], Paolo Manca[1], Henry Walch[1], Subhiksha Nandakumar[1], Pedram Razavi[2], Eileen M. O'Reilly[2], Yelena Y. Janjigian[2], Rona Yaeger[2], Karuna Ganesh[3], Michael F. Berger[4], Nikolaus Schultz[1], Francisco Sanchez-Vega[1]

[1]Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY,[2]Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY,[3]Cancer Biology and Genetics Program, Memorial Sloan Kettering Cancer Center, New York, NY,[4]Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY

Background: The analysis of metastatic trajectories and their association with genomic features can provide valuable insights to improve patient stratification and prognostication. We analyzed real-world data from a large multi-cancer cohort of patients who developed metastasis to either the liver but not the lung or the lung but not the liver, and we compared their clinical and molecular profiles.

Methods: We queried a pan-cancer database of more than 85,000 patients treated at Memorial Sloan Kettering Cancer Center. All patients were profiled using MSK-IMPACT, a targeted sequencing panel that identifies somatic alterations in 341-505 genes using matched tumor and blood. Liver-avid/lung-adverse (“LiverA”) patients were defined as having developed liver metastasis but not lung metastasis, while lung-avid/liver-adverse (“LungA”) patients were defined conversely. We identified 4 cancer subtypes with at least 100 patients in each metastatic cohort: pancreatic adenocarcinoma (PAAD, n=1247, 968 LiverA/ 279 LungA), microsatellite stable colon adenocarcinoma (MSS COAD, n=905, 653/252), esophageal adenocarcinoma (ESCA, n=344,193/151), and HR+/HER2− ductal breast adenocarcinoma (HR+/HER2− BRCA, n=298,156/142). Overall survival (OS) was measured from the time of diagnosis.

Results: LiverA patients had shorter OS: MSS COAD (median 53 vs. 106 months, p<0.0001), ESCA (median 21 vs. 41 months, p<0.0001), HR+/HER2− BRCA (percentage surviving after 9 years, 22% for LiverA vs. 69% for LungA patients, p<0.0001), and PAAD (median 16 vs. 33 months, p<0.0001). LiverA patients were diagnosed at a significantly younger age in 4 cancer types: HR+/HER2− BRCA (median 54 vs. 61 years, p<0.0001), ESCA (median 63 vs. 68 years, p<0.0001), PAAD(median 67 vs. 71 years, p<0.0001), and MSS COAD (median 59 vs. 61.5 years, p=0.017). Genomic analyses of primary tumors identified significantly higher chromosomal instability in the LiverA patients quantified as the fraction of genome altered (FGA) by copy number changes in 3 cancer types: ESCA (median 0.69 vs. 0.59, p=.036), HR+/HER2− BRCA (median 0.64 vs. 0.43, p=0.001), and PAAD (median 0.28 vs. 0.18, p=1.1e-5). Among HR+/HER2− BRCA patients, LiverA patients had higher rates of 11q13 (36% vs. 11%, q=0.002), MYC (14% vs. 2%, q=0.02) and FGFR1 (21% vs. 9%, q=0.06) amplifications. TP53 alterations were more frequent in LiverA patients within PAAD cohorts (84% vs. 59%, q<0.0001). MSS COAD patients in the LiverA group had higher rates of SMAD4 (19% vs. 9%, q=0.02) and lower rates of KRAS alterations (44% vs. 56%, q=0.05).

Conclusions: Our data demonstrate distinct outcomes and clinicogenomic features associated with LiverA and LungA patients consistent across several cancer types, suggesting potential commonalities in underlying tumor biology that could be exploited to improve patient stratification.

#3388 Proteomic analysis of Latin American patients and machine learning strategies for developing a prognostic signature for prostate cancer.

Juan Bizzotto[1], Agustina Sabater[1], Pablo Sanchis[1], Pia Valacco[2], Elba Vazquez[2], Javier Cotignola[2], Geraldine Gueron[2]

[1]Departamento de Quimica Biologica, IQUIBICEN - CONICET, Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales; Universidad Argentina de la Empresa (UADE), Instituto de Tecnologia (INTEC), Buenos Aires, Argentina,[2]Departamento de Quimica Biologica, IQUIBICEN - CONICET, Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Buenos Aires, Argentina

Prostate cancer (PCa) is the leading cause of cancer-related deaths among males in Latin America (LATAM). While some PCa patients are classified as intermediate-risk based on histopathological characteristics, their outcomes can vary widely. There is an unmet need for biomarkers that are cost-effective, easy to implement in low-resource clinical settings, and capable of identifying patients at risk for aggressive disease to tailor treatments. These biomarkers must be developed specifically for the LATAM population to ensure their relevance and accuracy within this demographic. This study aimed to identify molecular biomarkers capable of stratifying the risk of disease progression in LATAM PCa patients, independent of histological grading and other clinicopathological variables. To this end, we performed protein extraction from formalin-fixed paraffin-embedded (FFPE) PCa and benign prostatic hyperplasia (BPH) tissue samples from patients at Buenos Aires, Argentina (n=20). Tandem mass spectrometry (LC ESI-MS/MS) analysis identified 109 proteins enriched in PCa. These proteins were subsequently analyzed through a custom made bioinformatic pipeline using publicly available transcriptomic datasets (16 datasets, n = 2,954). We designed a sequential workflow that included survival analyses to evaluate the association of each gene with disease progression, as well as machine learning algorithms to identify key predictors of PCa progression. Random forest was used for feature selection, identifying the most important genes, and LASSO regression was applied to construct a predictive model based on these genes. Our analysis yielded a 10-gene expression signature capable of identifying patients at higher risk of disease progression, independent of clinicopathological parameters, specifically within the intermediate-risk subgroup ($HR > 1$, $p < 0.05$ across datasets). We validated these findings in 4 independent PCa datasets not used in the training phase, reinforcing the robustness and generalizability of our methodology. This study leveraged experimental and transcriptomic data to develop a prognostic gene-expression signature based on genes with confirmed expression and detectability in the LATAM population, ensuring its relevance and applicability within this demographic. The signature has the potential to enable risk assessment among intermediate-risk patients, facilitating personalized treatment strategies, improving clinical outcomes, and reducing unnecessary interventions. Additionally, the small size of the gene panel makes it cost-effective and compatible with standard RT-qPCR platforms, enabling widespread implementation in decentralized settings. Our results lay the foundation for future clinical trials integrating this signature into personalized therapeutic approaches.

#3389 Genetic and clinical landscape of NUTM1 structural variants.

Sara R. Selitsky[1], **Brandon A. Price**[1], Michael Rollins[1], Adam J. Hockenberry[1], Ezra Cohen[1], Brian Piening[2], Alexander Rubinsteyn[3], Jennifer H. Choe[4], Kim A. Ely[4], Henry Kaplan[5]

[1]Tempus, Chicago, IL,[2]Earle A. Chiles Research Institute, Portland, OR,[3]University of North Carolina at Chapel Hill, Chapel Hill, NC,[4]Vanderbilt University Medical Center, Nashville, TN,[5]Swedish Cancer Institute, Seattle, WA

Introduction: NUT carcinoma is an aggressive cancer, originally described in midline structures of adolescents and young adults but now known to occur in a variety of anatomic sites with growing numbers of variant histologies being reported. As such, NUT immunoreactivity and/or demonstration of the NUTM1 fusion is required for definitive diagnosis.
Methods: We identified patients within the Tempus multimodal real-world database who had either a diagnosis of NUT carcinoma based on clinically abstracted diagnosis fields or via the presence of a NUTM1 fusion, as detected either by Tempus xT (targeted DNA panel) or Tempus xR (whole-exome capture RNA-seq).
Results: There were 59 patients in the Tempus database with NUT carcinoma annotated as their primary diagnosis. Tempus NGS testing confirmed the presence of a NUTM1 fusion for 48 (81%). Of the remaining 11 patients, none underwent RNA-seq testing at Tempus and only 2 had solid-tissue DNA-seq performed at Tempus. Additionally, there were 106 patients with a NUTM1 fusion based on NGS evidence that did not have an initial NUT diagnosis, including: non-small cell lung cancer (n=33), head and neck (n=10), cancer of unknown primary (n=10), thyroid cancer (n=11), soft tissue sarcoma (n=11), and several other cancer types. Eight out of 31 sweat gland adenocarcinomas/porocarcinomas in the cohort had a NUTM1 rearrangement. Several other NUTM1 fusion rearrangements with partners not previously associated with NUT carcinoma were identified, the clinical significance of which is unclear.Among the 154 patients where we detected a NUTM1 rearrangement, the most common fusion events were BRD4-NUTM1 (n = 55), NSD3-NUTM1 (n = 31), BRD3-NUTM1 (n = 17), SLC12A6-NUTM1 (n=11), YAP1-NUTM1 (n = 11), MXD4-NUTM1 (n=3), and CIC-NUTM1 (n=3)other fusion partners occurred in only one or two patients. The overall potential underdiagnosis rate (patients with the detected fusion but not an annotated formal NUT carcinoma diagnosis) was 69% (106/154); including BRD4-NUTM1 51% (28/55), BRD3-NUTM1 47% (8/17), NSD3-NUTM1 81% (25/31), and 91% for YAP1-NUTM1 (10/11).Some cancer types were enriched for specific fusion gene partners. Eleven of 12 patients (92%) that were diagnosed with thyroid cancer had a NSD3-NUTM1 fusion. Of the 8 sweat gland adenocarcinomas/porocarcinomas, 6 had a YAP1-NUTM1 fusion.The median overall survival was 5.29 months (n = 72) and the median age was 51 (n = 114) at diagnosis. The median age at diagnosis for patients annotated with NUT carcinoma was 41 years compared to 58.5 years for patients with a NUTM1 fusion but lacking the diagnosis.
Conclusions: NUT carcinomas may be underdiagnosed the majority of the time. Certain cancer types with a high enrichment of NUTM1 fusions, such as sweat gland adenocarcinomas/porocarcinomas, thyroid cancers, and sarcomas, may benefit from universal NGS sequencing to ensure correct diagnosis.

#3390 Impact of genomic alterations in *HMGB1*, *ROS1*, *IL6*, *FGFR1*, *FGFR2*, and *TLR4* on long-term survival outcomes in esophageal squamous cell carcinoma (ESCC).

V. C. Kok;
Kuang Tien General Hospital, Taichung, Taiwan

Background. To test the hypothesis that the genomic alterations of *HMGB1, ROS1, IL6, FGFR1, FGFR2*, and *TLR4* would be associated with long-term outcomes in patients with ESCC through disrupting normal immune surveillance and promote mechanisms that allow tumor cells to evade immune detection and destruction. These genes were also variously involved in the RTK-Ras pathway (*ROS1, FGFR1, FGFR2*), PI3K-Akt signaling (*FGFR1, FGFR2, IL6* and *TLR4*), TLR signaling (*TLR4* and *IL6*) and the SHP2 signaling (*FGFR2* and *IL6*). Methods. This study merged five datasets and sorted out pure squamous cell carcinoma for analysis. GSE53624 was used for gene expression correlating to survival analysis on the genes of interest. Disease-free survival (DFS) and overall survival (OS) were calculated in patients whose survival times were available for Kaplan-Meier analysis. The log-rank test was used for comparison. Only patients with available survival data were put into the survival modes. Hazard ratios (HR) with 95% confidence interval (CI) were derived from the log-rank test. The research was conducted via the KEGG.jp, cBioPortal (accessed November 2024), and ESCCdb/Sichuan. Results. A total of 437 patients with 437 samples were selected. Male accounted for 78.5%. The altered and unaltered group comprises 66 (15%) and 371 (85%) patients. The top 10 mutated genes in this cohort are *TP53, TTN, KMT2D, MUC16, CSMD3, PKHD1L1, ZNF804B, SYNE1, NOTCH1* and *PCLO.* Patients were assigned to either altered group (any alteration in the six genes of interest) or unaltered group. Compared with the unaltered group, the altered group has a higher nonsynonymous tumor mutational burden (TMB) [(median (IQR), 3.37 (2.07-5.23) vs 5.33 (4.32-6.3), p = 5.22e-8 by Wilcoxon test)] and higher mutation count [(median (IQR), 56 (13-117) vs 158.5 (125-187), p < 10e-10)]. DFS can be calculated in 76 patients. The altered group has a nonsignificant worse DFS with an HR of 1.171 (95% CI, 0.512-2.681). With more than 85 months of follow-up in 288 patients, compared with the altered group, the altered group shows significantly worse overall survival: median and 95% CI, 40.93 mo. (28.42 mo. - not reached) vs. 18.63 mo. (18.17 mo. - 28.13 mo.), HR = 2.16 (1.33 - 3.52)]. Gene expression versus survival analyses revealed that higher HMGB1 expression harbors a significantly worse prognosis (p < 0.008). Conclusions. These in silico and dataset experiments leveraging the genomic data from ESCC demonstrate that despite having a higher mutation count and TMB, which might elicit more tumor neoantigens for immune recognition and eradication, the altered group with genomic alterations in any of the six genes might still have a significantly worse long-term survival prognosis. The novelty of the study findings needs further investigation.

#3391 Clonality and allelic imbalance of driver genes in a large retrospective cohort of colorectal cancer patients.

Henry Walch, Paolo Manca, Jierui Xu, Christopher Fong, Justin Jee, Karl Pichotta, Adam Price, Allison L. Richards, Mark Donoghue, Jinru Shia, Efsevia Vakiani, Neil H. Segal, Andrea Cercek, Nikolaus Schultz, Francisco Sanchez-Vega, Walid K. Chatila, Rona Yaeger

Memorial Sloan Kettering Cancer Center, New York, NY

Intro: The genomic alteration landscape of colorectal cancers (CRCs) is heterogeneous, which can have implications for treatments and outcomes. In this analysis, we investigated tumor sequencing data from a large cohort of CRC patients to unpack the relevance of clonality and allelic imbalance heterogeneity.

Methods: We analyzed a cohort of 6789 CRC patients treated at Memorial Sloan Kettering Cancer Center from 2014 to 2024. Every patient had a tumor/normal pair sequenced with MSK-IMPACT, a targeted sequencing assay that profiles genomic alterations across 341-505 cancer genes. The FACETS copy number algorithm was used to determine whole-genome duplication (WGD) status, as well as assess allelic imbalance and the clonality of mutations. Of the 6789 tumors, 5520 passed FACETS QC criteria. Tumors were classified as microsatellite stable (MSS), microsatellite instable (MSI), and POLE-mutant (POLE). Overall survival was calculated from time of diagnosis to last follow up.

Results: The median age at diagnosis of our cohort was 56 years. Left-sided primary tumors were the most common (38.0%) and the majority of patients were stage IV (50.3%). The most frequent molecular subtype was MSS (87.6%), followed by MSI (11.4%) and POLE (1.0%).

Within the MSS cohort, we investigated the clonality and allelic imbalance of mutations in canonical CRC genes, such as TP53, APC, KRAS, BRAF, and PIK3CA. Across all genes, clonal mutations demonstrated significantly higher rates of allelic imbalance favoring the mutant allele relative to subclonal mutations. Consistent with the other genes, tumors with a single clonal APC mutation had higher rates of allelic imbalance favoring the mutant allele when compared to those with a single subclonal mutation (81.5% vs 63.1%, $p<0.001$). However, this difference was not seen in tumors with more than 1 APC mutation.

Tumors with a clonal TP53 mutation had higher median FGA estimates relative to subclonal TP53 mutant tumors in both WGD- (42% vs 28%, $p<0.001$) and WGD+ (77% vs 69%, $p=0.011$) tumors. TP53 clonal mutant tumors were enriched for loss across several chromosomal arms, whereas TP53 wild-type tumors were enriched for gains of chromosome 12, which contains both MDM2 and KRAS (32.4% vs 10.8%, $p<0.001$). Among patients with left-sided primary tumors and KRAS mutations, presence of KRAS gain was associated with a higher risk of death (HR=1.58, 95%CI:1.17-2.12, $p=0.003$)

Conclusions: The presence of allelic imbalance in CRC might contribute to the clonal expansion of mutations important for early tumorigenesis, particularly in the absence of multiple mutations leading to loss of the wild-type allele, as is the case with APC. Further, stratification by TP53 mutation clonality demonstrated its role in regulating genomic instability. Copy number alterations might shape CRCs fate toward a higher dependency on the TP53 or RAS pathway, with possible prognostic implications.

#3393 A pan-cancer analysis of cancer-predisposition germline pathogenic variants (gPVs) utilizing the cancer genome atlas (TCGA) reveals distinct survival trends associated with immune response pathways.

Alan Shen[1], Lin Li[2], Joshua Arbesman[3], Ying Ni[2], Pauline Funchain[4]

[1]Cleveland Clinic Lerner College of Medicine, Cleveland, OH,[2]Cleveland Clinic Center for Immunotherapy and Precision Immuno-Oncology, Cleveland, OH,[3]Department of Dermatology, Medical Specialty Institute, Cleveland Clinic, Cleveland, OH,[4]Department of Medicine, Division of Oncology, Stanford University School of Medicine, Palo Alto, CA

A family history of cancer has previously been shown to positively impact survival outcomes in various cancers. Additionally, specific cancer-predisposition germline pathogenic variants (gPVs) have also been shown to have a beneficial effect on survival across multiple cancers including colorectal cancer, ovarian cancer and melanoma. However, the impact on survival of commonly accepted cancer-predisposition gPVs across cancers has not been widely studied. Here, we examine the effect of 85 common cancer-predisposition gPVs on survival outcomes across all cancer types in the Cancer Genome Atlas (TCGA). When examining survival trends, we observed that cancers with a greater proportion of gPVs generally had improved survival ($p = 0.0088$). When examining each cancer indication separately, those with significantly improved survival amongst gPV positive patients included bladder [$HR = 0.35$, $p = 0.01$] and ovarian cancer [$HR = 0.55$, $p = 0.0003$], whereas head and neck squamous cell carcinoma had significantly worse survival [$HR = 1.43$, $p = 0.04$]. Interestingly, bladder and ovarian cancer gPV positive patients had significant upregulations in immune-stimulatory transcriptomic pathways with the majority of gPVs in the homologous recombination deficient (HRD) variant category, whereas the opposite was observed in head and neck squamous cell carcinoma. Thus, by examining a large array of cancer-predisposition gPVs in all cancer subtypes included in TCGA, we not only demonstrate the prevalence of a wider variety of gPVs across cancer indications but that specific categories of gPVs can influence survival across cancers in a beneficial manner, potentially by creating a pro-inflammatory immune response.

#3394 Impact of gBRCAs mutation on the surgery decisions of Chinese breast cancer (BC) patients: a single-center real world study in Xijing Hospital, Northwest China.

Ting Wang[1], Jing Fan[1], Jiajun Ding[1], Jing Kong[1], Mengxuan Li[1], Yidi Wang[1], Huimin Meng[1], Haoyi Zi[1], Qingxia Li[1], Shuai Wang[1], Fei Yao[2], Changbing Zhu[2]

[1]Department of Thyroid, Breast and Vascular Surgery, Xijing Hospital, Air Force Medical University, Xi'an, China,[2]Department of Medicine, Amoy Diagnostics Co., Ltd, Xiamen, China

Background and Objective: Germline *BRCA* (gBRCA) mutations complicate clinical surgery management due to a higher risk of initial breast cancer, as well as increased chances of new primary cancers or local recurrence. In this study, we examined the surgical decisions for individuals carrying gBRCA mutations.

Methods: In this real world study, 1050 female patients with unilateral operable invasive breast cancer getting gBRCA mutation test were enrolled. Pearson chi-square test, continuous correction chi-square test and Fisher's exact test were used to explored the association between gBRCA mutation and surgery choice appropriately.

Results: Germline *BRCA1* mutations (gBRCA1^{mut}), Germline *BRCA2* mutations (gBRCA2^{mut}) and gBRCA1/2^{mut} were found in 5.1% (54/1050), 4.9% (51/1050), 10.0% (105/1050) BC patients separately. Among all patients, those with gBRCAs^{mut} preferred contralateral prophylactic mastectomy than those without mutation (9.3% gBRCA1^{mut} patients compared with 2.0% gBRCA1^{wt} patients, 7.8% gBRCA2^{mut} patients compared with 2.1% gBRCA2^{wt} patients, 8.6% gBRCA1/2^{mut} patients compared with 1.7% gBRCA1/2^{wt} patients, all $p<0.05$). This tendency could also be found in early-stage BC patients (n=699). While in young (≤40) BC patients (n=346), there were no significant differences in choice between gBRCAs^{mut} cohort and gBRCAs^{wt} cohort. Though in all patients, the selection of specific surgery [breast-conserving surgery (BCS), mastectomy or reconstruction surgery] was not affected by gBRCAs mutations. In young BC patients, patients with gBRCA2^{mut} were more likely to choose mastectomy or reconstruction surgery rather than BCS (10.3% gBRCA2^{mut} patients selected BCS compared with 34.4% gBRCA2^{wt} patients, $p=0.011$). The same tendency could also be found in early-stage BC patients though the difference was not statistically significant ($p=0.074$). The gBRCA1 mutation had no influence on the specific option.

Conclusion: Northwest Chinese BC patients made their surgery choice under the influence of gBRCA1/2 mutation. gBRCA2^{mut} played an more crucial role on it.

#3395 Investigating clinical features and molecular mechanisms of long-term response and acquired resistance to immunotherapy in non-small cell lung cancer by applying real-world-data.

Si Wu, **Weilong Zhao**, Baoyi Zhang, Christy Choi, Saeed Salehin Akhand, Qu Zhang, Jack Chen, Peter Ansell, Rong Chen, Xi Zhao, Josue Samayoa

Abbvie South San Francisco, South San Francisco, CA

The introduction of immunotherapy (IO) has transformed cancer treatment, particularly for non-small cell lung cancer (NSCLC), yet molecular mechanisms of IO resistance remain elusive due to incomplete clinical and omic data. Notably, while some patients, “long-term responders” (LTR), experience lasting benefits, ~60% of initial responders develop “acquired resistance” (AR), differing from those with primary resistance (PR) who exhibit no initial response.

Our study leverages real-world data (RWD) for its comprehensive clinical data, capturing therapy lines and responses with extensive follow-up. We examined RWD from ConcertAI PT360® EHRs linked to Caris Life Science molecular data across 2,176 NSCLC patients, focusing on a filtered cohort (N = 407) to explore IO response and resistance mechanisms. Within this cohort, 43% received IO monotherapy and 57% combination therapy with chemotherapy, predominantly using pembrolizumab. Most patients initiated IO in the first line (90%) or second line (8.2%).

Responders to IO noted improved overall survival (p = 0.0037, median OS 27.6 vs. 12.6 months for non-responders) and longer treatment durations (p = 4.04E-04, median 210 vs. 99 days), compared to non-responders. Biomarker analysis highlighted significantly higher tumor mutational burden (TMB) and immunologic constant of rejection (ICR), along with more PD-L1 positive patients (p = 0.03) among responders, indicating increased immune infiltration. The RNA-seq data revealed 604 significantly differential genes; responders showed higher levels of immune biomarkers (e.g., CD274, CXCL-9, GZMK, TNFRSF9), whereas non-responders exhibited increased immunosuppressive markers such as NNMT and some antigen targets. Gene Set Enrichment Analysis (GSEA) revealed enriched pathways in lymphocyte activation and extracellular structure organization, TGF-b signaling, etc.

Integrative analyses of LTR, AR, and PR groups identified mutations, notably ARID1A, PREX2, and PRKN, enriched in the LTR group, potentially rendering tumor sensitivity to IO and potential new drug targets. Additionally, six distinct molecular patterns were identified, highlighting differences between LTR, AR, and PR groups. Notably, Immunoglobulin G, B cells, and plasma cell activity scores varied significantly between AR and PR, with AR mirroring LTR, implicating these immune cells in initial IO response, potentially linked to immature tertiary lymphoid structures (TLS) before transitioning to AR, manifested with TLS scores differing significantly across groups (p = 1.7E-04).

In summary, our study uses multi-modal RWD to establish an analytical workflow, revealing key clinical features and biomarkers while offer insights into molecular underpinnings of IO long-term response and resistance through multi-omic RWD integration.

#3396 Genomic landscape and ancestry-associated variations of gynecological cancers.

Vasily N. Aushev[1], Carly B. Scalise[1], Rebecca C. Arend[2], Avinash Ramu[1], J. Bryce Ortiz[1], Alyssa Antonopoulos[1], Faraz Salmasi[1], Alexey Aleshin[1], Adam C. ElNaggar[1], Laura Chambers[3]

[1]Natera, Inc., Austin, TX,[2]School of Medicine, The University of Birmingham, Birmingham, AL,[3]Wexner Medical Center and James Cancer Hospital, The Ohio State University, Columbus, OH

Background: Gynecological cancers are a diverse set of malignancies, each with distinct genomic alterations that influence tumorigenesis and disease progression. Here, we analyzed genomic data from endometrial, ovarian, and cervical cancer patients, stratified by genetic ancestry, to assess differences in genomic mutational landscapes.
Methods: Whole-exome sequencing (WES) data from >8,000 gynecological cancer cases in Natera's proprietary Real-World Database were utilized. WES was performed on tumor tissues for designing personalized Signatera™ circulating tumor DNA tests ordered from June 2019 - November 2024. After exclusions, 6,485 patients remained: 2,956 (46%) endometrial, 2,709 (42%) ovarian, and 820 (12%) cervical cancer. Variant calling used Mutect2 and VarScan2; only non-synonymous mutations were included in prevalence analysis. Genetic ancestry was determined using EthSeq algorithm for four groups: Africa (AFR), East Asia (EAS), Europe (EUR), South Asia (SAS).
Results: In endometrial cancer, top mutated genes were PTEN (43.2%), ARID1A (37.9%), and PIK3CA (34.5%). The most common variants being SETD1B H8X (7.6%), RNF43 G659X (6.8%), PTEN R130G (5.8%) and PIK3CA H1047R (5.8%). EUR and AFR ancestry comprised 85% and 14% of cases, respectively. The top 4 most frequently mutated genes were the same in EUR and AFR; but EUR ancestry had significantly higher ARID1A (40.7% vs 23.6%, $p<0.01$), PIK3CA (36.1% vs 23.8%, $p<0.05$), and PTEN (46.8% vs 22.5% $p<0.001$) mutations, while AFR ancestry had higher prevalence of TP53 (51.2% vs 30.5%, $p<0.01$). In ovarian cancer, TP53 (53.3%), ARID1A (10.4%), and PIK3CA (9.7%) were the top mutated genes. Common variants were KRAS G12D and G12V (each 2.9%), and PIK3CA H1047R (2.5%). EUR and AFR groups comprised 79% and 8% of cases, respectively. No significant ancestry-based differences were found. In cervical cancer, PIK3CA (26.7%), KMT2C (14.0%), and KMT2D (11.5%) were most commonly mutated. The most common variants were PIK3CA E545K (13.0%), E542K (7.1%), and E726K (2.7%). EUR and AFR comprised 75% and 13% of cases, respectively, and harbored the same top two mutated genes: PIK3CA and KMT2C. The AFR group had a lower prevalence of PIK3CA E545K mutation (7.6% vs 14.4%) but a higher E542K prevalence (10.5% vs 6.8%).
Conclusions: This extensive real-world genomic analyses of gynecological cancers revealed distinct mutational landscapes that distinguish ovarian, endometrial, and cervical cancers. The data highlight the genomic diversity of these malignancies and frequent alterations in genes and variants across ancestries, offering potential research targets. Future studies will investigate links between genomic alterations and clinical outcomes to inform disease management.

#3397 Survival stratification for *TP53*-mutated endometrial cancers under platinum-based chemotherapy.

Xiaotian Zhao, **Haimeng Tang**, Xue Wu, Qiuxiang Ou

Geneseeq Research Institute, Nanjing Geneseeq Technology Inc., Nanjing, China

Background: Endometrial cancers (EC) with mutated *TP53* or abnormal p53 show unfavorable clinical outcomes, with high recurrence rates, and additional chemotherapy has been considered within these patients. Genomic biomarkers may be critical to increasing the precision in developing treatment strategy for *TP53*-mutated EC.

Methods: We applied an in-house developed targeted sequencing homologous recombination deficiency (HRD) assessment bioinformatic algorithm (GeneseeqPrime® HRD) on 529 the Cancer Genome Atlas (TCGA) EC samples, covering all exons and translocation-related introns of 437 cancer-relevant genes. The HRD score threshold to determine HRD status as well as to stratify survival outcomes under platinum-based chemotherapy, and associations of HRD status with signaling pathways and protein expression were investigated.

Results: Of 529 EC samples, 18.0% was determined as HRD-positive (HRD score threshold: 38), including 85 patients carrying mutated *TP53*, which was associated with increased HRD scores (p<0.001). However, pathogenic *BRCA1/2* mutations appeared to be less common in *TP53*-mutated EC (p=0.08). HRD-negative EC had higher tumor mutation burden (p=0.03), with more prevalent homologous recombination-related *ATM*, and *RAD50* mutations, whereas positive HRD status was associated with higher aneuploidy score (p<0.001) and fraction of genome altered (p<0.001). Serous and endometrioid carcinomas were enriched in HRD-positive (p<0.001) and HRD-negative EC (p<0.001), respectively. HRD-positive EC exhibited higher patient age (p<0.001), disease stage (p=0.03), and histologic grade (p=0.04) than HRD-negative EC. Under platinum-based chemotherapy, HRD-positive *TP53*-mutated EC had improved progression-free survival [adjusted hazard ratio (HR), 0.56; 95% confidence interval (CI), 0.29-1.10; p=0.09] and superior overall survival (adjusted HR, 0.49; 95% confidence interval (CI), 0.24-1.00; p=0.05) compared to HRD-negative patients, when adjusting for clinical characteristics and molecular subtypes. Suppressed p53, Hedgehog, and hippo signaling pathways were observed in HRD-positive EC with *TP53* mutations (p.adjust <0.05). Additionally, MAPK14 (p<0.001) and PIK3CA (p<0.001) expression were significantly associated with higher HRD score.

Conclusions: HRD status may identify patients with *TP53*-mutated EC who could get survival benefits from platinum-based chemotherapy. Further research is required to determine the utilization of HRD assessment in EC receiving PARP inhibitors.

#3398 Gene-level and global genomic insights into colorectal cancer ovarian metastasis: Molecular mechanisms and determinants.

Y. Fang[1], Y. Sun[1], X. Zhao[2], X. Wu[2], J. Pang[2], **H. Tang**[2], Q. Ou[2], C. Chen[3], Z. Xu[4];

[1]Jiangsu Cancer Hospital, Jiangsu Institute of Cancer Research, The Affiliated Cancer Hospital of Nanjing Medical University, Nanjing, China, [2]Nanjing Geneseeq Technology Inc., Nanjing, China, [3]Jiangsu Province Hospital of Chinese Medicine, Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing, China, [4]The First Affiliated Hospital of Nanjing Medical University, Nanjing, China

Background: Approximately 5% of female patients with colorectal cancer (CRC) develop ovarian metastasis (OM), predominantly in young women and leading to poorer survival. As the molecular mechanisms underlying OM remain unclear, this study aimed to identify the genetic determinants of OM and to investigate the mechanisms of synchronous and metachronous metastasis.

Methods: We retrospectively enrolled 81 patients with CRC who had developed OM (28 with matched primary and metastatic lesion samples, 30 with primary lesion, 23 with metastatic samples). Treatment-naïve primary and metastatic lesion tissue samples underwent next-generation sequencing targeting 437 genes to identify gene-level and global genomic determinants of OM. Two external CRC cohorts (MSKCC cohort, N=643; TCGA cohort, N=448) were analyzed for further mechanism exploration.

Results: Among 81 included patients, copy number variations (CNVs) of *SMAD4* ($p<0.01$) and *SMAD2* ($p<0.01$) were more frequently detected in metastatic lesions than primary lesions, with similar findings in 28 pairs of matched samples (*SMAD4*, $p=0.05$; *SMAD4*, $p=0.02$). Associations of *SMAD4* ($p<0.001$) and *SMAD2* ($p=0.09$) CNV loss with unfavorable overall survival were observed in the MSKCC cohort. RNA sequencing data from the TCGA cohort demonstrated up-regulated angiogenesis pathway related to *SMAD4* ($q=0.03$) and *SMAD2* ($q<0.001$) CNV loss. Higher chromosome instability was observed in metastatic lesions than primary lesions in the study cohort ($p<0.01$; matched samples, $p=0.08$), consistent with the findings in the MSKCC cohort ($p<0.001$). The APOBEC mutational signature was related to OM synchronous metastasis, with heavier burdens in both the primary ($p=0.04$) and metastatic ($p=0.01$) lesions of synchronous metastasis than those of metachronous metastasis; however, primary lesions of metachronous metastasis exhibited higher whole-genome doubling fraction ($p=0.04$) and ploidy ($p=0.02$). Moreover, in metastatic lesions, *FLT1* (17.6%, $p=0.05$) and *ZNF217* (17.6%, $p=0.05$) CNV gains were exclusively detected of synchronous metastasis when compared to metastatic lesions of metachronous metastasis.

Conclusions: *SMAD4*/*2* CNV loss and chromosome instability may drive OM in CRC. Synchronous metastasis displays distinct gene-level and global genome features different from metachronous metastasis.

#3399 Real-world experience of the clinical correlation between ARID gene alterations /RNA expression levels and treatment benefit of immunotherapy in malignant solid tumors.

Yuliang Sun, Tobias Meissner, Rachel Elsey, Bing Xu, Crystal Hattum, Casey Williams

Avera Cancer Institute, Sioux Falls, SD

Background: Cancer immunotherapies provide durable benefits for many, but not all patients with various types of solid tumor. Accumulating research suggests that patients with ARID gene alterations of the human switch/sucrose nonfermentable (SWI/SNF) chromatin-remodeling complex are more likely to respond to anti-PD-1/ PD-L1 and anti-CTLA-4 immunotherapy. To investigate the correlation between ARID gene alterations/RNA expression levels and clinical outcomes after immunotherapies, we reviewed patients with solid malignancies seen at our Institute.

Methods: A review of patients (from 2014 to 2024) with solid malignancies from our clinical database at the Avera Cancer Institute, whose tissue DNA and RNA were analyzed by next generation sequencing (NGS) by Tempus and Foundation Medicine, Inc. Patients who have alterations in the ARID gene (ARID1A, ARID1B, ARID2 and ARID5B) and received therapeutic regimens with immune checkpoint inhibitors (ICIs, including PD-1/PD-L1 and CTLA-4 inhibitors) alone or in combination with other treatments were assembled as Cohort 1. Patients with wild-type ARID gene and received ICIs were used as Cohort 2. The outcome differences of the patients between Cohort 1 and 2 were then assessed. Furthermore, patients in Cohort 1 were stratified to subgroups by RNA levels, microsatellite instability (MSI), as well as tumor mutational burden (TMB) status, and the outcomes were analyzed.

Results: **1.** Of the 3620 patients in our database, we found 591 (16.3%) patients harboring ARID gene alterations with one of 15 types of cancer. This includes 160 (4.4% of 3620 and 27.1% of 591 patients) patients (Cohort 1) that received ICIs (excluding 10 patients unevaluable due to treatment termination per drug toxicity or lack of follow-up information), and 470 (13.0%) patients (Cohort 2) with wild-type ARID gene received ICIs. **2.** No statistical difference of RNA expression level between Cohort 1 (*N*= 50) and Cohort 2 (*N*= 135). **3.** Patients in Cohort 1 (*N*= 160), the median progression-free survival (PFS) and overall survival (OS) measured from date of the initiation of ICIs were 8.3 and 20.3 months. **4.** PFS and OS of patients in Cohort 2 appear less than in Cohort 1. Assessment for patients of Cohort 2 and subgroups is ongoing. Updated results will be presented at the meeting.

Conclusion: Improved response rates to ICIs will require the efforts of researchers and clinicians to advance our understanding of tumor immunology, biology as well as omics and to integrate them with other important components of precision oncology. Our single-center observations of real-world data provide a reference for the clinical correlation between ARID gene alterations /RNA expression levels of the SWI/SNF complex and treatment outcomes after immunotherapy in malignant solid tumors, these findings might be beneficial for cancer patients' treatment in the future.

#3400 Co-mutations in FGFR3 and CDKN2A in bladder cancer: a descriptive analysis.

Muhammad A. Shahid, Radhika Gutta, Sunita Ghosh, Nallasivam Palanisamy

Henry Ford Health System, Detroit, MI

Introduction: Bladder cancer is the 9th most common cancer worldwide. Two key genetic alterations, in Fibroblast Growth Factor Receptor (FGFR3) and Cyclin-Dependent Kinase Inhibitor 2A (CDKN2A), are known to contribute to its development. Interactions between these genes have been suggested in bladder cancer progression. However, limited data exists on patients' demographics with co-mutations in these genes.
Methods: This retrospective study evaluates the patient demographics and survival analysis in patients with co-mutated CDKN2A and FGFR3 in bladder cancer. We utilized the cBioPortal MSK Cell Reports 2022 Database for Bladder cancer for our samples. We quantified our samples based on an FGFR3 mutation, CDKN2A mutation, co-mutation in FGFR3/CDKN2A, or wild-type mutation. Further associations of these mutations were tested with race, gender, smoking status, and cancer stage. Kaplan-Meier estimates for overall survival (OS) were compared for the mutational status and log-rank tests were used to compare survival curves. Statistical analysis was performed using Chi-square tests for categorical variables, and time to event analysis was performed using log-rank test. A p-value <0.05 was used for statistical significance.
Results: We evaluated 1462 samples in our study. 139 patients had a co-mutated CDKN2A/FGFR3 (9.5%), 243 with FGFR3 alteration (16.7%), 184 with CDKN2A alteration (12.5), and 896 (61.2 %) with no alteration. There was a statistically significant difference in smoking status when stratified by mutational status: current or former smokers compiled 67.9% of the co-mutated CDKN2A/FGFR3 cohort, 59.6% in the FGFR3 group, 65.9% in the CDKN2A group, and 64.4% in the wild type group ($p=0.010$). 76% of patients were male, 26% were female, and 92% were of Caucasian race; no statistical significance was noted when stratified based on gender or race. When stratifying based on cancer stage, co-mutated CDKN2A/FGFR3 was most commonly seen in Stage I and IV bladder cancer. The 5-year survival probability for patients with co-mutated FGFR3/CDKN2A was 57.9%, compared to 60.9% in FGFR3 samples, 56.8% in CDKN2A samples, and 54.7% in the wild-type samples($p=0.261$). Median OS was only reached for the wild-type mutation.
Conclusions: Our retrospective study outlines the different population groups with aberrations in either FGFR3 or CDKN2A. Patients who were current/former smokers were more likely to have a co-mutated FGFR3/CDKN2A mutation. Patients with wild-type CDKN2A/FGFR3 fared worse when looking at median overall survival (77.4 months), and patients with co-mutated CDKN2A/FGFR3 had the best response (median OS not reached). One of the considerable limitations of this database is the homogeneity of the population, with the vast majority of patients being of Caucasian descent. We hope to evaluate further our bladder cohort of patients, where we have a diverse group of patients that may give us better insight into these alterations.

#3401 Pharmacogenomic landscape of UGT1A1 in cancer patients from the Avera Sequencing and Analytics Protocol (ASAP) registry.

Kobina A. Amoah[1], Crystal Hattum[2], Tobias Meissner[2], Cheryl P. Jones[2], Stephanie B. Hastings[1], Michelle F. Green[1], Heidi C. Ko[1], Bing Xu[2], Zachary D. Wallen[1], Neil Matthiesen[3], Richard Conklin[2], Benjamin Solomon[2], Brian J. Caveney[1], Eric A. Severson[1], Shakti Ramkissoon[1], Casey Williams[2], Rachel Elsey[2]

[1]Labcorp, Durham, NC,[2]Avera Cancer Institute, Sioux Falls, SD,[3]Avera Genetics, Sioux Falls, SD

Purpose: Pharmacogenomic (PGx) testing enables clinicians to identify patients at increased risk of adverse events after chemotherapy. Specific genomic alterations that change drug metabolism can lead to increased toxicity or decreased efficacy. Irinotecan is a chemotherapy drug used to treat solid tumors like colorectal and pancreatic cancer. The UGT1A1 gene metabolizes irinotecan, and genomic alterations can lead to significant dose-limiting toxicities like neutropenia and diarrhea. We investigated the link between UGT1A1 genotype with irinotecan treatment-related adverse events (TRAEs).

Methods: We performed a retrospective analysis of 564 patients with cancer from a prospective observational registry, termed ASAP, who had PGx testing. The ASAP registry is a multicenter precision oncology initiative in a large community-based health system. Using the ASAP registry database, we characterized the demographics, genotype-phenotype correlation, cancer diagnosis, and TRAEs after irinotecan-based chemotherapy.

Results: UGT1A1 phenotypes were classified as normal (*1/*1), intermediate (*1/*80), or poor (*80/*80) metabolizers. Within the study population, 47% (264) were normal metabolizers (57% female, 43% male); 42% (236) were intermediate metabolizers (60% female, 40% male); and 11% (64) were poor metabolizers (56% female, 44% male). Of the 564 patients analyzed, 19 underwent irinotecan-based chemotherapy. Most of the UGT1A1 results were received after the patients had been treated with irinotecan and dose adjustments were made based on other patient-specific factors. Normal metabolizers accounted 37% (7) of patients, with 43% (3) requiring dose reductions at the start of treatment, 57% (4) receiving dose reductions during therapy, and 29% (2) experiencing grade 3 TRAEs. Intermediate metabolizers accounted for 32% (6) of patients, with 33% (2) requiring initial dose reductions, 67% (4) needing dose reductions during treatment, and 33% (2) experiencing grade 3 TRAEs. One patient in this group discontinued treatment. Poor metabolizers represented 32% (6) of the patients, with 50% (3) requiring initial dose reductions, 33% (2) needing dose reductions during treatment, and 17% (1) experiencing grade 3 TRAEs. Overall, 68% (13) of patients experienced grade 1-3 adverse events, which most commonly were neutropenia, diarrhea, and fatigue with 69% (9) occurring in intermediate or poor metabolizers.

Conclusion: This study analyzes UGT1A1 pharmacogenomic profiles in patients with cancer, treated with irinotecan-based chemotherapy. Individuals with intermediate or poor metabolizer phenotypes were more likely to experience grade 1-3 TRAEs resulting in dose adjustments. These results highlight the importance of UGT1A1 testing for minimizing irinotecan toxicity. Future research is underway to explore the clinical impact of UGT1A1 polymorphisms on survival.

#3402 Number of variants of unknown significance (VUS) by next generation sequencing (NGS) and response to systemic therapies in advanced urothelial carcinoma (aUC).

Thomas L. Enright[1], Jacob B. Leary[1], Dimitra R. Bakaloudi[1], Rafee Talukder[2], Michael T. Schweizer[1], Jessica E. Hawley[1], Andrew C. Hsieh[1], Todd Yezesfski[1], Bruce Montgomery[1], Evan Y. Yu[1], Petros Grivas[1], Ruben Raychaudhuri[1], Ali R. Khaki[3]

[1]Division of Hematology Oncology, Department of Medicine, University of Washington, Seattle, WA,[2]Section of Hematology Oncology, Baylor College of Medicine, Houston, TX,[3]Division of Oncology, Deptartment of Medicine, Stanford University, Palo Alto, CA

Background: NGS is routinely used in aUC to identify clinically actionable alterations. NGS assays tier variants by the likelihood of clinical significance, reporting both pathogenic variants and VUS. The number of VUS may serve as a rough estimate for neoantigen load, e.g. tumor mutational burden (TMB). We hypothesized that the number of VUS may represent an easy-to-use biomarker associated with response and overall survival (OS) with certain therapies e.g. immune checkpoint inhibitors (ICI).
Methods: We conducted a retrospective study from patients (pts) from a single academic center with aUC and available NGS data derived from FoundationCDx using tumor tissue who had received ICI, enfortumab-vedotin (EV), and/or sacituzumab govitecan (SG) in the first line (1L) or as salvage (2L+). Patients at or above the median were defined as VUS high (VUS-H); those below were VUS low (VUS-L). We analyzed observed response rate (ORR) and OS measured from each therapy start between groups. Cox proportional hazards test was used to compare OS; Fisher's exact test was used to compare ORR. Kaplan-Meier method was used to calculate survival curves.
Results: 90 pts had available NGS data and received ICI, EV, and/or SG [VUS-H (≥9 VUS) N = 51; VUS-L (0-8 VUS) N = 39]. VUS was correlated with TMB (Pearson coefficient = 0.78). Baseline characteristics including age, sex, ECOG PS, prior extirpative surgery rate, and primary tumor site were similar between groups, except for presence of liver metastases at diagnosis (VUS-H = 10%, VUS-L = 36%; p= 0.003). Results are described in the table.
Conclusions: Higher number of VUS detected by tissue-based NGS was associated with higher ORR and OS with 1st line ICI. Limitations include retrospective nature, lack of randomization, moderate sample size and power, selection and confounding biases. Results are hypothesis-generating and need external validation.
Table: ORR and OS analyses by group

		N	ORR %	OR (95%CI)	p	mOS, months(95%CI)	HR(95%CI)	p
1L ICI	VUS-H	23	60.9%(14/23)	7.78(1.74 – 34.71)	0.009	39(24-NR)	0.30(0.14, 0.66)	0.003
	VUS-L	18	14.3%(3/18)			8.0(5.3-29)		
2+L ICI	VUS-H	16	18.8%(3/16)	1.15(0.16 – 8.27)	1.0	4.3(3.0-24)	1.74(0.73 – 4.12)	0.2
	VUS-L	12	18.2%(2/12)			11(6.7-NR)		
2+L EV	VUS-H	20	45.0%(9/20)	2.86(0.47 – 17.35)	0.41	26(12-NR)	0.63(0.24, 1.62)	0.3
	VUS-L	9	22.2%(2/9)			10(8.8-NR)		
2L + SG	VUS-H	13	7.7%(1/13)	undefined	1.0	10(6.1-NR)	0.35(0.11 – 1.13)	0.08
	VUS-L	9	0.0%(0/9)			7.1(2.5-NR)		

#3403 The landscape of TP53 Y220C mutations in cancer.

Alison M. Schram, Fatema Nagib, Mara Rao, Rogelio Velasco, Michael Gormally, Alexandra Waksmundzki, Henry Walch, Michael Berger, Nikolaus Schultz, Mark Donoghue, Walid Chatila

Memorial Sloan Kettering Cancer Center, New York, NY

Background: *TP53* encodes the critical tumor suppressor p53 and is the most commonly mutated gene in cancer. The *TP53* Y220C hotspot mutation occurs in approximately 1% of solid tumors, reduces thermal stability of *TP53*, and impairs its DNA binding. *TP53* Y220C creates a unique conformational change that is amenable to small molecules that can restore *TP53* function. Several *TP53* Y220C reactivators are in development. Despite emerging evidence that *TP53* Y220C is a therapeutic target, the clinicopathologic features of patients with these tumors remain poorly understood. We describe the landscape of *TP53* Y220C solid tumors at Memorial Sloan Kettering Cancer Center (MSK).

Methods: This retrospective study included patients with TP53 Y220C solid tumors at MSK from July 2014 to March 2024. Patients were identified using paired tumor-normal next-generation sequencing (NGS) with MSK-IMPACT, a hybridization capture-based NGS assay that detects mutations, copy number alterations, and select structural rearrangements. Clinicopathologic data including sex, age and stage at diagnosis, race, and cancer type were automatically extracted from the medical record. Allele-specific copy number analysis was performed using FACETS, which infers purity and ploidy corrected integer DNA copy number calls from sequencing data. Genomic characteristics of *TP53* Y220C solid tumors were compared to those with other *TP53* missense mutations, truncating mutations, and complex alterations involving *TP53*, in addition to *TP53* wild-type tumors.

Results: 498 patients were identified with *TP53* Y220C-mutated solid tumors. The majority were female (67%), White (71%), never smokers (52%), with a median age of 63 years-old (range 16-90, n=479). Approximately half (56%) had stage I-III disease at diagnosis, 33% stage IV, 11% unknown. The most common tumor types were ovarian (22%), pancreatic (11%), breast (11%), and non-small cell lung cancer (10%). *TP53* Y220C prevalence was highest in high-grade serous ovarian cancer (4.5%). *TP53* Y220C rarely co-occurred with other *TP53* mutations (0.07%). The co-mutational profile, tumor mutational burden, copy number profile, and fraction of genome altered of *TP53* Y220C-mutated tumors was similar to tumors with p53 non-Y220C mutations. Whole genome doubling (WGD) occurred in approximately half of *TP53* altered cases, with similar frequencies for *TP53* Y220C and *TP53* non-Y220C mutated cancer. The frequency of WGD was higher in the altered groups compared to the *TP53* wild-type group. Mutations were predominantly clonal, with subclonal alterations found in only 0.7% of cases.

Conclusion: *TP53* Y220C is a newly described clinically actionable target found across solid tumors at low frequency. The genomic features of *TP53* Y220C-mutated cancers are similar to *TP53* non-Y220C mutated cancers. Research is ongoing to determine the natural history of *TP53* Y220C mutated cancers and whether this biomarker has unique predictive or prognostic implications.

IMMUNOLOGY: Enhanced Antibodies, TCR Constructs, Cytokines and Chimeric Proteins
Poster Session

#3406 GPRC5D multispecific antibodies with potent anti-tumor activity against multiple myeloma.

Hayley Roth, Daniel Rogers, **Ileine Sanchez**, Breanna Tyrell, Amelia Snyder, Kyle Doolan, Benjamin Doranz, Ross Chambers, Joseph Rucker

Integral Molecular, Philadelphia, PA

Background: GPRC5D is a G protein-coupled receptor that is expressed on multiple myeloma cells but absent from most healthy tissues. It is an attractive target for anti-tumor modalities including T cell-engaging bispecific antibodies. Recent clinical data demonstrate that combination therapy using T cell-engaging molecules, which individually target GPRC5D and BCMA on tumor cells, offers unprecedented therapeutic benefits for patients with relapsed/refractory multiple myeloma. A trispecific antibody that simultaneously engages these molecules while activating T cells is expected to provide comparable and additional benefits as a monotherapy.
Methods: Multipass membrane proteins are valuable therapeutic targets but are largely inaccessible for antibody discovery due to structural complexity and high conservation. We developed an antibody discovery platform (MPS) tailored for membrane proteins that utilizes advanced immunization techniques including DNA, mRNA, and Lipoparticles (virus-like particles) and also utilizes evolutionarily divergent host species. From the parental antibodies isolated, we engineered panels of GPRC5DxCD3 bispecific and GPRC5DxBCMAxCD3 trispecific antibodies using multiple formats and CD3 arms that encompass different geometries and binding stoichiometries. GPRC5D multispecific antibodies were tested for target specificity using Integral Molecular's Membrane Proteome Array (MPA). Both bispecific and
trispecific antibodies were tested for *in vitro* potency and cytokine release using MM.1R and RPMI 8226 multiple myeloma cell lines. *In vivo* efficacy for a subset of antibodies was determined using xenograft models in humanized mice.
Results: We immunized chickens with GPRC5D to obtain high-titer immune responses and configured resulting antibodies as T cell-engaging bispecific molecules with a CD3 binding arm. A subset of GPRC5DxCD3 bispecific antibodies displayed potent T cell-mediated cytotoxicity with picomolar potency on multiple myeloma cell lines. Antibody specificity profiling showed high specificity binding to its intended targets, and not to other proteins across the membrane proteome. Bispecific molecules were then re-engineered as trispecific antibodies with additional binding to BCMA, a well-validated multiple myeloma target. GPRC5DxBCMAxCD3 trispecific antibodies showed improved cytotoxic activity against multiple myeloma cells. Both bispecific and trispecific antibodies demonstrated a large window between cell killing and cytokine release. GPRC5D multispecific antibodies also showed robust tumor control against multiple myeloma xenografts in a humanized mouse model.
Conclusions: GPRC5D multispecific antibodies hold promise as potent and safe therapeutics for multiple myeloma.

#3407 Development of bispecific antibodies targeting claudin-6 for precision cancer therapy.

Siret Tahk[1], Paule Hermet[1], Anu Ustav[1], Robin Pau[1], Ana Shahpazir[1], Mario Plaas[2], Gaily Kivi[1], Andres Mannik[1], Joan Teyra[1], Tanel Mahlakõiv[1], **Mart Ustav**[1]

[1]Icosagen Cell Factory OU, Kambja, Estonia,[2]Tartu University, Tartu, Estonia

Background: Claudin 6 (CLDN6), a tight junction protein, is an oncofetal antigen with limited expression in adult tissues but aberrantly overexpressed in several cancers, including ovarian, testicular, and gastric tumors. Its tumor-specific expression and critical role in maintaining cell adhesion make CLDN6 a compelling target for cancer therapy. Recent studies have linked CLDN6 to tumor progression, metastasis, and chemoresistance, highlighting its potential as both a biomarker and a therapeutic target in precision oncology. This study presents the development of bispecific antibodies for the treatment of CLDN6-positive tumors.
Methods: Monoclonal antibodies specific to CLDN6 were generated using virus-like particles (VLPs) displaying CLDN6 as immunogens. Chickens were selected for immunization due to their ability to produce antibodies against highly conserved, multi-transmembrane proteins. Antibody discovery was performed using HybriFree B cell cloning technology and phage display platforms.
Results: Bispecific antibodies that specifically recognize CLDN6 and CD3 were developed and evaluated for their effectiveness in killing CLDN6-positive cells while sparing CLDN6-negative cells. Various bispecific antibody formats were assessed, showing differences in non-specific T-cell activation. These findings underscore the importance of selecting appropriate bispecific antibody modalities to achieve maximum therapeutic potency while ensuring a favorable safety profile. The functionality and potency of these novel bispecific antibodies were further demonstrated in vivo using ovarian cancer xenograft models.
Conclusions: This study highlights the development of novel bispecific antibodies targeting CLDN6 and CD3, emphasizing their superior safety profile. The use of specifically engineered antibody modalities and highly selective CLDN6 binders ensures minimal cross-reactivity with other homologous family members. These findings support the potential of these bispecific antibodies as a promising therapeutic approach for CLDN6-positive cancers.

#3408 Cancer-specific anti-HER2 monoclonal antibodies demonstrate antitumor activities in human breast cancer xenograft models.

Yukinari Kato[1], Tomokazu Ohishi[2], Tomohiro Tanaka[1], Hiroyuki Suzuki[1], Mika K. Kaneko[1]

[1]Tohoku University Graduate School of Medicine, Sendai, Japan,[2]Institute of Microbial Chemistry (BIKAKEN), Numazu, Numazu, Japan

Monoclonal antibody (mAb) therapy represents cutting-edge strategies for cancer treatment. In contrast, safety concerns persist due to the potential targeting of normal cells that express reactive antigens. Developing cancer-specific mAbs (CasMabs) that bind to cancer-specific antigens and exhibit antitumor activity in vivo is critical for reducing the risk of adverse effects. Herein, we selected mAbs targeting human epidermal growth factor receptor 2 (HER2) and successfully established an anti-HER2 CasMab (clone H2CasMab-2; mouse IgG1, kappa). We investigated both the in vitro and in vivo antitumor efficacy using three types of H2CasMab-2: the mouse IgG2a type of H2CasMab-2 (H2CasMab-2-mG2a), the mouse-human chimeric H2CasMab-2 (H2CasMab-2-hG1), and the humanized H2CasMab-2 (humH2CasMab-2). H2CasMab-2 showed lower reactivity to HER2-overexpressed Chinese hamster ovary-K1 (CHO/HER2) and breast cancer cell lines (BT-474 and SK-BR-3) than trastuzumab in flow cytometry. Every format of H2CasMab-2 also showed lower antibody-dependent cellular cytotoxicity (ADCC) against CHO/HER2 and the breast cancer cell lines than trastuzumab. In contrast, every format of H2CasMab-2 exhibited significantly higher complement-dependent cellular cytotoxicity (CDC) in CHO/HER2 and breast cancer cells than trastuzumab. Furthermore, every format of H2CasMab-2 demonstrated compatible in vivo antitumor effects against breast cancer xenografts with trastuzumab. These findings highlight the distinct roles of ADCC and CDC in the antitumor effects of H2CasMab-2 and trastuzumab and suggest a potential direction for the clinical development of H2CasMab-2 for HER2-positive tumors.

#3409 T cell receptors and antibodies targeting shared indel-derived neoantigens.

Kazuma Kiyotani, Peng Zhao, Xiaojing Wu, Yusuke Nakamura

Center for Intractable Diseases and ImmunoGenomics, National Institutes of Biomedical Innovation, Health, and Nutrition, Osaka, Japan

T cells play a pivotal role in cancer immunotherapy by recognizing neoantigens presented on HLA molecules, which are specifically expressed by cancer cells. While neoantigens are generally unique to individual cancers, shared antigens that are common in a subset of cancer patients, represent promising immunotherapeutic targets. Since frameshift insertions or deletions (indels) are reported to generate highly immunogenic neoantigens through novel open reading frames. However, few studies have explored shared neoantigens from frameshift indels, which are shred among multiple cancer patients. In this study, we analyzed frameshift neoantigens from frameshift indels in TCGA exome sequencing data, identifying 760 recurrent frameshift mutation clusters (FSCs) that shared frameshifted open reading frames and premature stop codons. Among these, we focused on two FSCs in the APC gene, which generate potential neoantigens (AS3 and AS10) presented the HLA-A24 molecule. We successfully identified the T cell receptor (TCR) sequences for APC frameshift neoantigens from healthy donor's blood. Genetically engineered T cells expressing these TCRs demonstrated recognition and cytotoxic activity against target cells presenting these APC frameshift neoantigens. Additionally, we explored the development of an antibody targeting a complex formed by the APC shared neoantigen AS10 and the HLA-A24 molecule. Using phage display library screening, we isolated single-chain variable fragments (scFvs) that specifically recognized the AS10-HLA-A24 complex. A bispecific antibody (BsAb) was then designed to connect T cells via an anti-CD3 scFv to the AS10 neoantigen on HLA-A24. We found that the AS10 BsAb specifically recognized both the AS10-HLA-A24 complex and CD3 protein, and showed significant IFN-γ release and cytotoxicity against target cells expressing frameshift neoantigens. By focusing on recurrent frameshift mutations and shared neoantigens, this study provides compelling evidence for the development of more effective and broadly applicable immunotherapeutic strategies.

#3410 Fc engineering of CD9 antibodies mitigates platelet toxicity and retains potency against acute lymphoblastic leukemia.

Wing Hei Ng[1], Shengyu Fu[2], Kathy Yuen Yee Chan[1], Kin Yi Chung[1], Yuen Ching Tiffany Hui[1], Qi Zhao[2], Kam Tong Leung[1]

[1]Department of Paediatrics, The Chinese University of Hong Kong, Hong Kong, Hong Kong,[2]Institute of Translational Medicine, Faculty of Health Sciences, University of Macau, Macau, Macao

Background: Albeit significant progress in CD19/CD22-based immunotherapies, the outcomes of patients with relapse/refractory acute lymphoblastic leukemia (ALL) remains suboptimal. Tetraspanin CD9 endows proven prognostic impact and therapeutic relevance in ALL (Leung et al, 2020/2024). Yet, lethal platelet toxicities incurred by CD9 antibodies have hampered their clinical translation. This study aims to develop a safe and effective CD9 biologics through antibody engineering.
Methods: Murine CD9 antibodies were generated by standard immunization/hybridoma technologies and humanized via complementarity-determining region (CDR) grafting. Antibody affinity and specificity were assessed against soluble CD9 antigen and CD9-expressing/depleted ALL cell lines, while efficacy was confirmed with cell line-derived and patient-derived xenograft (PDX) models. Platelet toxicity was evaluated by CD62P expression, optical aggregometry and in FcγRIIA transgenic mice. Mechanisms were explored by cell proliferation, apoptosis, imaging and antibody-dependent cellular cytotoxicity (ADCC) assays.
Results: Two isolated murine monoclonal antibodies (mAbs) displayed distinct binding affinity and specificity towards the CD9 antigen, with biophysical properties preserved following humanization (hAbs). Administration of either mAbs or hAbs in 697-transplanted NOD/SCID mice markedly suppressed leukemia progression, alongside with substantial extension of animal survival. Consistent with previous reports, platelets were fully activated within 30 minutes after exposure to hAb IgGs but not their Fab or scFv fragments, indicating that platelet activation is a Fc-dependent event. By introducing a N297A mutation to the antibody Fc domain, platelet activation and aggregation was completely extinguished compared with the non-engineered version as reflected by absence of phenotypic CD62P expression (5.1±0.9% *vs.* 89.6±4.8%, $P<0.0001$) and physical aggregation (AUC: 18.9-29.5 *vs.* 382-395, $P=0.0016$). Additionally, no thrombocytopenia was observed in FcγRIIA transgenic mice with platelet counts in the hAb arm similar to those in the human IgG control arm (929±84 *vs.* 968±118, $P=0.7935$). Notably, N297A-modified hAbs profoundly reduced leukemia burden by >98% in PDXs developed from ALL patients who relapsed from upfront immunotherapies displaying CD19 antigen loss. Underlying its potency, Fc-modified hAbs triggered direct cell death and proliferation inhibition in CD9+ ALL cell lines following homotypic aggregation, a feature associated with type II antibodies. Cytotoxicity was mediated via an ADCC-independent mechanism.
Conclusion: Fc engineering mitigates the platelet toxicity of CD9 antibodies but retains potency against refractory ALL, potentially breaking the decade-long hurdle for their clinical translation. Large animal studies are ongoing.

#3411 Transcriptional modulations of tumor microenvironment by BH3120, a PD-L1x4-1BB bispecific antibody, in patient-derived organotypic tumor spheroids (PDOTS) model.

Jun Wang, **Jiangcheng Xu**, Jing Wang, Jiawang Liu, Kyoungwoo Lee

Beijing Hanmi Pharm. Co. Ltd., Beijing, China

BH3120 is an heterodimeric IgG like bispecific antibody in clinical stage targeting PD-L1 (CD274) and 4-1BB (CD137) simultaneously. It binds 4-1BB with moderate affinity while binds PD-L1 with relatively higher affinity, with the aim to focuss PD-L1 binding dependent 4-1BB agonism in tumor microenvironment. De-coupling of immune modulation between tumor and normal tissues were confirmed in multiple non-clinical studies, and the results were consistent with favorable safety profiles. In the meantime, BH3120 shows synergistic anti-tumor efficacy with PD-1 inhibitors without systemic safety concerns in vivo.

To understand how BH3120 and the combination with a PD-1 inhibitor modulate the tumor microenvironment, ex vivo studies with patient-derived organotypic tumor spheroids (PDOTS) were generated from freshly obtained tumor tissues of non-small cell lung cancer (NSCLC) patients, and cultured in microfluidic devices containing extracellular matrix. After incubation of the test articles in the ex vivo model, anti-tumor activities and immune modulations were observed by RNA analysis as the response to BH3120, a PD-1 inhibitor, and the combination.

In responding tumor spheroids, multiple genes were significantly upregulated or down-regulated. In particular, RNAs representing $CD4^+$ T cell, $CD8^+$ T cell, NK cell, and B cell signals, and certain co-stimulators, proinflammatory cytokines, and chemokines were notably increased. Combination of BH3120 and PD-1 inhibitor resulted in the relevant genetic modulations with high magnitudes implicating potential synergism in RNA level.

#3412 Cis demasking cytokine technology improves therapeutic index of immunocytokine.

Caroline Mary, Margaux Seite, Virginie Thepenier, Geraldine teppaz, Justine Durand, Kevin Biteau, Cecile Batty, Ariane Desselle, Julien Taurelle, Virginie Aillerie, Nicolas Poirier, **Aurore Morello**

OSE Immunotherapeutics, Nantes, France

Immunocytokines are promising therapeutic approaches to enhance T cell response by delivering interleukins directly to the tumor site. The fusion of wild-type or attenuated interleukins with anti-PD-1 antibodies has shown some efficacy in preferentially cis-activating PD1+ T-cells in tumor and lymph nodes. However, for many immunocytokines (*e.g.* IL-2/IL-15/IL-21/IL-12), off-target activation of PD1-negative cells remains a significant challenge due to the high affinity of the cytokine for their receptors, leading to unwanted systemic effects, toxicity, high clearance and low tumor biodistribution, which ultimately limits their clinical potentials. To address this low therapeutic index issue, strategies have emerged to achieve localized cytokine activation using conditional masking strategies (e.g. Enzymatic cleavable linkers) but their clinical translation remains challenging due to off-tumor activation. To overcome these limitations, we developed OSE-Cytomask® Platform, a universal and non-cleavable linker technology allowing cytokine CIS-demasking upon binding of the fused antibody to its target. This platform enables precise cytokine activation on the right T cell population to minimize toxicity and improve therapeutic index of immunocytokine. A series of linkers were screened for optimal cis activation of the cytokine on PD1+ vs PD1neg T cells fused to a high-affinity anti-PD1 antibody. OSE-Cytomask® linker technology strongly decreases IL-2 or IL-15 cytokine activity on PD1neg cells while maintaining strong activation of PD1+ T cells even in a co-culture system. We confirmed OSE-Cytomask® activity on PD1+ primary T cells while very low activation has been observed in naïve T cells. OSE-Cytomask® linker technology does not induce TRANS-activation on PD1neg T-cells illustrating a strict CIS-demasking activity. Importantly, Anti-PD1/IL-15 and anti-PD1/IL-2 OSE-Cytomask® technology strongly reduced toxicity of the immunocytokine while maintaining in vivo anti-tumor in vivo efficacy. At least 10-fold higher therapeutic index for OSE-Cytomask® Anti PD1/IL2v was obtained compared to the conventional Anti PD1/IL2v. In conclusion, OSE-Cytomask® linker technology illustrates specific intrinsic property to mask cytokine on naïve peripheral immune cells not expressing the target of the antibody while allowing selective CIS-demasking and activation of the cytokine on PD1+ cells. Unlike protease-cleavable linker technologies, OSE-Cytomask® does not rely on enzymatic activity, making it a more stable and adaptable solution across different TME conditions. It has been validated with multiple cytokines (6 tested) and can be fused with various antibodies. By reducing off-tumor cytokine activity and selectively activating immune cells, OSE-Cytomask® platform offers a promising strategy for the clinical development of more effective and safer immunocytokines for cancer therapies.

#3413 Discovery of GRC 65327, a best-in-class, selective and potent Cbl-b inhibitor for the treatment of advanced solid cancers.

Venkatesha Udupa[1], Atul Akarte[1], Jiju Mani[1], Nilanjana Biswas[1], Vikas Karande[1], Murugan Chinnapattu[1], Megha Marathe[1], Akshata Virdikar[1], Sneha Pusadkar[1], Shruti Talekar[1], Anuj Singh[1], Pandurang Lambade[1], Gopal Rajput[1], Akshaya Wagh[1], Pramod Pawar[1], Ajit Jagadale[1], Dayanidhi Behera[1], Balasaheb Gavhane[1], Manoj Pawar[1], Prashant Ingle[1], Pankaj Jain[1], Amol Walunjkar[1], Pavan Payghan[1], Sachin Chaudhari[1], Vidya Kattige[1], Sravan Mandadi[1], Madhavi Mulay[1], Ramesh Senaiar[1], **Mario Perro**[2], Pratima Deshpande[1], Nagaraj Gowda[1]

[1]Ichnos Glenmark Innovation (IGI), Mumbai, India,[2]IGI Biotherapeutics SA, Epalinges, Switzerland

Casitas B-lineage lymphoma-b (Cbl-b), a key member of RING finger E3 ligases, regulates both innate and adaptive immunity by mediating the degradation of signaling transducers. Cbl-b is a master regulator downstream of TAM receptors and CD28, promoting an immunosuppressive tumor microenvironment (TME) that limits the anti-tumor functions of T and NK cells. The absence of CD28 co-stimulation has been associated with T cell exhaustion and resistance to anti-PD-1 therapy. Inhibition of Cbl-b activates T cells even without CD28 co-stimulation, offering a potential therapeutic strategy to overcome immune suppression in the TME. GRC 65327 is a potential best in class, highly active and selective Cbl-b inhibitor. It significantly potentiates IL-2 and IFN-γ production in assays across human and preclinical species, with sub-nanomolar potency. Importantly, GRC 65327 restores function in anti-PD1 resistant exhausted T cells, mimicking the immunosuppressive conditions of the TME. The compound also engages its target in vivo, modulating biomarkers such as Notch1, enhancing its expression on T cells. Additionally, GRC 65327 demonstrated potent tumor cell killing in vitro, further highlighting its therapeutic potential. Efficacy studies in CT26 and MC38 colon tumor models demonstrated significant inhibition at tested doses. When combined with anti-PD1 therapy, GRC 65327 enhanced tumor growth inhibition and immune responses. Complete tumor regression was achieved in combination treatment in approximately 50-65% of animals, suggesting a synergistic effect between GRC 65327 and anti-PD1. Importantly, no treatment-related adverse effects, including changes in body weight, clinical signs, or mortality, were observed in these efficacy models. In vitro safety receptor profiling revealed minimal off-target activity, and hERG inhibition at an IC_{50} > 10 µM. In a one-month repeat-dose study in mice, no adverse effects were observed at the maximum tested dose of 300 mpk/day. However, liver toxicity was noticed in dose-range finding studies in dogs and monkeys. Despite this, a robust pharmacological response in the tissues was observed around $AUC_{0\text{-}24}$ of 1500 ng·h/mL and an increased number of circulating immune cells were seen at the highest dose tested compared to controls and baseline levels, with no adverse findings up to $AUC_{0\text{-}24}$ of ~13,400 ng·h/mL in a one-month repeat-dose study in monkeys. Importantly, no cytokine storm and myeloproliferative effects attributed to c-cbl in the literature were observed in the same study. Additionally, unlike a previous less-selective lead which exhibited RTK-mediated toxicities in various organs during exploratory tox study in rodents, GRC 65327 did not show adverse effects, highlighting its improved selectivity and safety profile. Based on these findings, a first-in-human clinical trial of GRC 65327 is scheduled to begin in advanced solid cancers.

#3414 PD-1 targeting increases the tolerance of IL-12 while maintaining potent anti-tumor immunity.

Stephen Searles, Kritika Mohan, Wenjing Yang, Elena Galitovskaya, Lynn Wang, Haikuo Zhang, Lidia Mosyak, Changhua Ji, Javier Chaparro-Riggers, Tzu-Hsuan Huang, Gabriel Starbeck-Miller

Pfizer Oncology, San Diego, CA

Preclinical studies have shown that IL-12 is a potent immunostimulatory cytokine that is capable of directly boosting CD8 T cell and IFNγ-dependent anti-tumor immunity. However, fully potent IL-12 therapies have limited clinical safety profiles. We have designed PF-07921585 to overcome this challenge by targeting IL-12 pharmacology on PD-1^{+} immune cells, a subset of CD8 T cells often enriched in the tumor microenvironment. PF-07921585 is a fusion protein consisting of an effector null bivalent anti-human PD-1 full length IgG1 and a single, potency reduced, human IL-12 mutein. PF-07921585 was specifically designed to permit simultaneous binding of PD-1 antagonists and enable combination with PD-1/L1 agents (PDx). Combination potential was confirmed in a mixed-lymphocyte reaction assay where we observed that PF-07921585 promoted robust IFNγ-responses as a single agent and boosted PDx-dependent IFNγ responses when given in combination. These data also suggested that targeted IL-12 receptor agonism and PDx checkpoint blockade have potential to non-redundantly amplify anti-tumor immune responses. In further support of this hypothesis, we found that combining a PF-07921585 mouse surrogate with PDx therapy can elicit greater efficacy in both PDx-sensitive and resistant mouse syngeneic tumor models than when either the surrogate or PDx therapy were administered as single agents. Administration of mouse surrogate as a single-agent also elicited potent anti-tumor CD8/IFNγ-dependent efficacy in PD-sensitive/resistant models and less peripheral toxicity compared to an untargeted, fully potent IL-12. Mechanistically, single-agent surrogate treatment enhanced the effector states (including increased IFNγ production) of several PD-1^{+} exhausted CD8 T cell subsets including the most dysfunctional subset, terminally exhausted cells. This latter effect was most striking as many published studies suggest PDx therapies only elicit efficacy by affecting the least dysfunctional exhausted T cell subsets, stem-like/progenitor cells. In summary, these data suggest that PF-07921585 may be better tolerated than other IL-12 therapies and has potential to deliver efficacy in PDx-refractory patients by heightening the effector function of CD8 T cell subsets not previously achieved by existing PDx therapies.

#3415 Preclinical characterization of LG anti-LILRB1, a potent antagonist targeting LILRB1.

Seok-Joo Kim, Younghoon Kim, Han Byoul Kim, Junhaeng Cho, Minsoon Kim, JungA Kim, Suji Hong, Heehang Kim, Shin-Young Kang, Hyungjin Cho, Jeeyeon Roh

LG Chem, Seoul, Korea, Republic of

LILRB1 is an inhibitory receptor found on various immune cells, including T cells, B cells, NK cells, and monocytes. It interacts with multiple ligands, including HLA class I molecules, with the highest affinity for HLA-G, and induces inhibitory signals that allow cancer cells to escape cancer immunosurveillance, impeding clearance from immune cells such as NK cells, T cells, and macrophages. This inhibitory effect in the tumor microenvironment may lead to disease progression, tumor metastasis, and poor clinical outcomes. For this reason, the blockade of LILRB1/HLA-G interaction could be a novel therapeutic target for the treatment of cancer. Additionally, recent studies indicate that LILRB1 may play an independent role in T cell functions beyond those regulated by PD1, suggesting that anti-LILRB1 therapy could potentially help in overcoming resistance mechanisms to anti-PD1 treatments. Therefore, LILRB1 is a promising target for cancer immunotherapy in human cancers due to its potential to mediate immune suppression across a wide range of tumor-associated immune cells. The protein-based and cell-based binding affinity of anti-LILRB1 antibody was measured using SPR and FACS analysis. A competitive ligand blocking assay was used to evaluate the ability of anti-LILRB1 antibody to block the interaction of LILRB1 with HLA-G. NK cell-mediated cellular cytotoxicity was evaluated by flow cytometry against HLA-G expressing tumors. The activities of NK cells and T cells were evaluated following the blockade of LILRB1. The in vivo anti-tumor efficacy of anti-LILRB1 antibody was evaluated in the human LILRB1 transgenic mouse and MC38-HLA-G syngeneic model. The binding affinity of the anti-LILRB1 antibody against LILRB1, quantified as 60 kD, was measured using a protein-based assay, and the EC50 was determined to be 20 nM using a cell-based assay. The binding of anti-LILRB1 antibody blocked interaction with HLA-G. In studies using human NK cell line and primary NK cells demonstrated that the anti-LILRB1 antibody enhanced their killing activity against HLA-G expressing tumors. In both systems, anti-LILRB1 antibody increased expression of CD69 and CD137, which are activation markers of NK cells, and IFN-Γ secretion. In LIR-overexpressing Jurkat cell line, anti-LILRB1 antibody enhanced IL-2 secretion. Importantly, the anti-LILRB1 antibody showed tumor growth inhibition in syngeneic model of human LILRB1 transgenic mice with MC38-HLA-G tumor. Based on these preclinical data, phase 1a study is currently ongoing (NCT06332755). Blockade of LILRB1/HLA-G interaction by antibody targeting LILRB1 enhances the immuno-modulatory effect of target immune cells. Furthermore, LILRB1 blockade enhanced anti-tumor efficacy in tumor bearing LILRB1-Tg mice model. These findings suggest that LILRB1 blockade antibodies have the potential to be developed as a therapeutic agent for the cancer immunotherapy for further investigation.

#3416 Advancing cancer immunotherapy: PD-L1-targeted IFNa2a fusion protein RO7694744.

Moritz Rapp[1], Harald Duerr[1], Gabriel Schnetzler[2], Davide Bertoldo[1], Jack Bates[1], Laura Kruempelmann[1], Alexandra Baumgartner[1], Marion Lichtenauer[1], Sandra Bunte[1], Kerstin Paetzold[1], Martina Antoniolli[1], Malika Ait-Goughoulte[2], Yvonnne Kienast[1], Johannes Sam[3], Steffen Dettling[1], Martin Lechmann[1], Robert Mader[2], Daniela Willend[2], Barbara Leutgeb[2], Anja Irmisch[3], Claudia Mueller[1], Metodi Donev[4], Andreas Roller[2], Nino Gochitashvili[5], Kerstin Meier[6], Yan Li[2], **Meher Majety**[1], Marina Bacac[3], Christian Klein[3], Pablo Umana[3]

[1]Roche Innovation Center Munich, Roche Pharma Research and Early Development, Penzberg, Germany,[2]Roche Innovation Center Basel, Roche Pharma Research and Early Development, Basel, Switzerland,[3]Roche Innovation Center Zurich, Roche Pharma Research and Early Development, Zurich, Switzerland,[4]Regulatory Affairs, F. Hoffmann-La Roche AG, Basel, Switzerland,[5]Clinical Development, Roche Products Limited, Welwyn, United Kingdom,[6]Legal, Roche Diagnostics GmbH, Penzberg, Germany

Interferon alpha (IFNa) is recognized for its potential in cancer immunotherapy by activating immune responses against tumor cells. However, untargeted IFNa's application is limited by systemic toxicity and non-specific activity, affecting both tumor and healthy tissues and resulting in undesirable side effects. To overcome these challenges, we developed the novel trivalent bispecific antibody-cytokine fusion protein RO7694744 designed to provide a specific activation of IFNa on programmed cell death ligand 1 (PD-L1) positive cells, such as tumor cells and immune cells. One antibody arm binds to human PD-L1 with high affinity, whereas the other arm can bind mutually exclusive either to human PD-L1 (with lower affinity) or to IFNa2a, which is fused to the antibody via a peptide linker. Both PD-L1 binders are non-blocking. This antibody design, called DutaFlip, protects the IFNa2a moiety from interacting with IFNa receptors (IFNAR1/2) until it is activated by binding to PD-L1 on the target cells.

Our research explored the PD-L1 selective activity of RO7694744 in multiple preclinical models. Using reporter cell assays, human whole blood cell assays, and various tumor cell lines with different PD-L1 expression levels, we demonstrated that IFNa activity depends on PD-L1 expression. Cells with elevated PD-L1 expression showed significantly higher activation compared to those with low PD-L1 levels. In vivo studies further validated these findings. In MDA-MB-231 tumor-bearing mice, RO7694744 treatment resulted in significant tumor growth inhibition (TGI), accompanied by intratumoral immune cell activation, and demonstrated a synergistic effect when combined with checkpoint inhibitor (CPI) treatment. To ensure translational relevance, we confirmed these in vivo observations in patient-derived tumor explants that mimic the human tumor environment. The explant data supported our previous findings, demonstrating PD-L1 specificity and dependency of RO7694744.

Our preclinical findings illustrate that RO7694744 selectively targets and activates PD-L1 expressing tumor and immune cells. Clinical experience is needed to confirm whether this targeted strategy can enhance therapeutic efficacy while minimizing adverse effects, thus addressing the key challenge of untargeted IFNa in cancer immunotherapy.

#3417 Preclinical studies of BR-2302: A HER3xEGFR bi-specific Exd-containing ADC.

Y. Wang, B. Xia, L. Cao, J. Yang, C. Feng, F. Jiang, C. Li, L. Gu, Y. Yang, J. Tian, J. Geng, Q. Zhang, Z. Wei, Y. Zhou;
Bliss Biopharmaceutical (Hangzhou) Co., Ltd., Hangzhou, China

HER3 has emerged as a critical player in the resistance mechanisms against therapies targeting the epidermal growth factor receptor (EGFR). Its upregulation can activate alternative signaling pathways, enabling cancer cells to bypass the effects of EGFR inhibitors, which often results in treatment failure. To combat this issue, novel HER3-targeting therapies are under investigation, including antibody-drug conjugates (ADCs) such as Patritumab Deruxtecan (U3-1402), which have shown encouraging results in clinical trials, underscoring the potential of HER3 targeting to overcome resistance. The development of bispecific ADCs that target both HER3 and EGFR represents a pioneering approach that may enhance therapeutic efficacy through dual targeting. Izalonatamab brengitecan (BL-B01D1), an ADC designed for simultaneous targeting of EGFR and HER3, has demonstrated substantial clinical benefits in heavily pretreated patients with EGFR-mutant non-small cell lung cancer (NSCLC). Building on this strategy, we are advancing a novel bispecific ADC, BR-2302, which employs a human IgG1 format bi-specific antibody with high affinity for HER3 and moderate affinity for EGFR. This innovative design aims to maximize therapeutic effectiveness while minimizing on-target/off-tumor toxicity associated with the widespread expression of EGFR in normal tissues. Our comprehensive studies on BR-2302 have focused on its *in vitro* cytotoxicity and *in vivo* tumor inhibitory effects across various cancer cell lines and xenograft models expressing the target antigens. BR-2302 exhibited significant anti-tumor activity *in vitro*, achieving subnanomolar IC_{50} values against HER3-expressing cell lines regardless of their EGFR expression levels. *in vivo* experiments demonstrated remarkable tumor growth inhibition and sustained regression in human lung and colon cancer xenograft models, surpassing the tumor inhibitory effects of established benchmark ADCs. Collectively, our findings indicate that BR-2302 possesses promising anti-tumor properties. This innovative therapeutic approach has the potential to improve treatment outcomes for patients with solid tumors expressing both HER3 and EGFR, warranting further clinical exploration.

#3418 IL6/1 fusion cytokine as a novel adjuvant for manufacturing of T cells for the treatment of ovarian cancer.

Sejal Tarun Sharma[1], Rahul Das[2], Lisa Barroilhet[1], Manish Patankar[1], Jacques Galipeau[2]

[1]Obstetrics & Gynecology, University of Wisconsin-Madison, Madison, WI,[2]Department of Medicine, University of Wisconsin-Madison, Madison, WI

Background: Autologous adoptive T cell therapy (ACT) is an emerging approach for the treatment of ovarian cancer. ACT is more effective if T cells manufactured ex vivo exhibit memory phenotype rather than exhaustive. IL-6 cytokine is known to effect T cell activation, proliferation, survival, and polarization whereas IL-1β is known to augment *in vivo* expansion of antigen-primed CD8 and CD4 T cells, $CD8^+$ T cell compartmentalization, and activation of T cells by antigen presenting dendritic cells. Due to known effects of these cytokines, we developed a novel biological product, IL6/1, a chimera of IL-6 and IL-1β, for effective manufacturing of T cells for ACT against ovarian cancer. We demonstrate that manufacturing of modified T cells with IL6/1 fusion cytokine improves T cell biological properties and hope to improve success of ACT in ovarian cancer.
Methods: Media from the HEK293 cells lentivirally transduced with IL6/1 was used to purify and characterize the fusokine. T cells were isolated from healthy donor PBMCs by negative selection and activated with IL2, anti CD3 - CD28 beads in the presence IL6, IL1β, IL6 + IL1β, IL6/1, conditional media, or no treatment control. Flow cytometry was used to monitor (a) T cell proliferation, (b) expression of CD45Ra, CD45Ro, CD3, CD4 and CD8 to define T cell phenotype and (c) assess cell death by labeling with annexin V and DAPI. Western blotting was performed to study the mechanism of action of IL6/1. Two-way ANOVA was used for statistical analysis.
Results: IL6/1 enhanced human T cell survival and increased the CD4 and CD8 memory (CD45Ro+) T cells as compared to vehicle controls or T cells exposed to IL6 or IL1β. T cells exposed to IL6/1 also showed a 10-15% increase in protection from cell death and apoptosis as compared to vehicle controls and T cells exposed to IL-6 or IL1β. Western blotting showed an increase in phosphorylation of STAT3, suggesting the involvement of this key signaling molecule in IL6/1-mediated T cell activation.
Conclusion: These findings suggest that IL6/1 fusion cytokine holds promise as a novel adjuvant for increasing the efficacy of T cell manufacturing for therapy in ovarian cancer. Overall, the IL6/1 fusion cytokine represents a novel approach to promote T cell survival, and memory T cell proliferation. Ongoing studies are focusing on evaluating the efficacy of IL6/1-based adjuvants in combination with ACT for the treatment of ovarian cancer in relevant mouse models.

#3419 EPS 226, a novel HER2 targeting IgE therapeutic, delivers immune dependent anti-tumor activity in preclinical models of breast cancer.

Elizabeth Hardaker[1], Lais C. GF Palhares[2], Melanie Grandits[2], Katie Stoker[2], Jitesh Chauhan[2], Tim Wilson[1], Sophia N. Karagiannis[2], Heather J. Bax[2], Kevin FitzGerald[1]

[1]Epsilogen Ltd, London, United Kingdom,[2]Kings College London, London, United Kingdom

HER2 is expressed at low levels in approximately 30-40% of cancers, including in breast cancer patients. Therapeutic IgE antibodies have been shown to deliver robust immune-dependent preclinical anti-tumor activity across a wide range of antigen levels. To explore the potential utility of a HER2-targeting IgE for the treatment of low HER2 breast cancer, EPS 226 was profiled in two in vivo models.EPS 226 was tested in the HER2-low, trastuzumab-resistant, human breast cancer model, JIMT1, in PBMC engrafted mice and the rat metastatic breast cancer model, MTLn3, in a fully immune competent rat. Briefly, female NXG mice were implanted with JIMT1 cells subcutaneously and engrafted with PBMC in the presence of FLT3 ligand once the tumors reached 50mm3. Female Fischer rats were implanted with MTLn3 subcutaneously and once tumors reached 50mm3, they were randomized into treatment groups. Animals were dosed twice weekly with EPS 226 or appropriate controls by intravenous administration. Tumor volumes were assessed, and the tumors were formalin fixed, or snap frozen, for further analysis at the end of study.EPS 226 induced an antigen specific tumor cell killing of JIMT1 and MTLn3 cells via antibody dependent cellular cytotoxicity (ADCC) in vitro, in the presence of human or rat PBMCs respectively. In the JIMT1 model, EPS 226 resulted in a dose dependent tumor growth inhibition. At 20mg/kg, EPS 226 resulted in a 62±10% tumor growth inhibition in the presence of PBMCs. No anti-tumor activity was observed in the absence of PBMCs demonstrating this efficacy was immune dependent. To fully explore the impact of EPS 226 on the immune system a rat syngeneic model, MTLn3, was established and shown to express low levels of HER2. Across multiple experiments EPS 226 resulted in an average of 50% tumor growth inhibition. In contrast, in this model, anti-PDL1 did not statistically significantly impact tumor growth. The efficacy of EPS 226 was associated with a 1.9-fold increase in tumor resident macrophages compared to the PBS control. Histology analysis also demonstrated that EPS 226 resulted in a 2.7-fold increase in T cells, comprising of predominantly CD8+ T cells. RNA Seq analysis of the bulk tumor showed a statistically significant increase ($p\ adj < 0.05$) in the activated CD8 T cell signature, compared to PBS controls.These studies are the first to demonstrate immune dependent anti-tumor efficacy of a novel HER2-targeting IgE antibody in models that have low HER2 expression. Treatment with EPS 226 resulted in an upregulation of immune cells, including an increased level of activated T cells, within the tumor microenvironment. These findings suggest that a HER2 targeting IgE has the potential to deliver clinical efficacy, in the context of low HER2 expressing patients, via a unique mechanism of action.

#3420 Coordinated stimulation of APC and T cell functions by first-in-class, tri-specific AN8025 for next-generation cancer immunotherapy.

Youping Wang, Wenjing Yang, Wanwan Chen, Kun Zhu, Zhihong Liu, Guolong Xu, Nanhai He

Adlai Nortye Ltd., Hangzhou. Zhejiang Province, China

Background: Immune checkpoint inhibitors (ICI), the cornerstone of current immunotherapies, primarily target T cells to reinvigorate the immune system against tumors. Recently, combinatory approaches, particularly in the form of bi-specific molecules such as Akeso's PD-1/VEGF and Innovent's PD-1/IL2, have garnered significant interest for their potential to deliver superior clinical efficacy beyond the ICI era. Building on this paradigm, AN8025 emerges as a tri-specific antibody fusion protein derived from a clinically validated and approved anti-PD-L1 antibody, fused with functionally optimized extracellular domain of CD86 and LAG3 on its C- and N-termini, respectively. Here we report that AN8025 leverages the LAG3 component to stimulate the differentiation and maturation of antigen-presenting cells (APCs), while the CD86 and anti-PD-L1 components synergistically amplify tumor-specific T cell responses. Together, these actions orchestrate a multifaceted enhancement of immune function.

Method: The *in-vitro* binding activities of AN8025 to corresponding targets and targets expressed on cell lines or primary cells were assessd by BLI, ELISA, or flow cytometry. Its *in-vitro* functional activities were measured using both reporter and primary immune cells. *In-vivo* efficacy was evaluated in MC38-hPD-L1 syngeneic model and hPBMC-engrafted NOG mouse bearing the MDA-MB-231 model. The developability of AN8025 was assessed under various stress conditions, and the preliminary pharmacokinetic (PK) profile was characterized in SD rats and Cynomolgus monkeys.

Results: AN8025 exhibited low single-digit nanomolar binding affinity to PD-L1 and CTLA4, while demonstrating over ten nanomolar binding to CD28. It effectively blocked the PD-1/PD-L1 interaction and the interaction between CTLA4 and CD80 proteins. Functionally, AN8025 demonstrated superior activation of T cells compared to the PD-L1 antibody alone and the combination of individual components of AN8025 (PD-L1 antibody, CD86-Fc, and LAG3-Fc). Significantly, the heightened T cell activation, primarily driven by the interaction of CD86 and CD28, is strickly dependent on the presence of PD-L1. In addition, AN8025 promoted the differentiation and maturation of antigen-presenting cells (APCs), as indicated by morphological changes, increased secretion of cytokines and chemokines, and induced expression of APC activation/maturation markers such as CD80/CD83. *In-vivo*, AN8025 alone showed superior efficacy compared to either ICI or the combination of individual components of AN8025. Furthermore, AN8025 exhibited favorable *in-vitro* stability and *in-vivo* pharmacokinetic profiles, with a long serum half-life in rats and in monkeys.

Conclusion: AN8025 demonstrates superior *in-vitro* and *in-vivo* activities compared to ICIs, supporting its further preclinical and clinical development.

#3421 XON7, a new emerging multitarget immunotherapy against metastatic soft tissue sarcomas.

Carine Ciron, Gwenaelle Evanno, Pierre-Joseph Royer, Odile Duvaux, **Firas Bassissi**

Xenothera, Nantes, France

Context: Soft tissue sarcomas (STS) are rare and heterogeneous malignancies originating from mesenchymal tissues, comprising over 50 subtypes. While surgery and radiotherapy are effective for localized disease, advanced and metastatic STS have poor outcomes due to limited efficacy of standard treatments like doxorubicin and frequent therapeutic resistance. Advances in molecular profiling have enabled targeted therapies, such as PARP inhibitors, but their effectiveness remains limited by the diversity of STS and resistance mechanisms. In this study, we evaluate the safety and efficacy of XON7, a first-in-class glyco-humanized polyclonal antibody (GH-pAb), as a novel therapeutic approach to address these challenges.
Methods: XON7's antitumor efficacy was tested in a panel of primary patient-derived sarcoma tumors and established sarcoma cell lines using CellTiter-Glo assay. Mechanisms of action assessed included complement-dependent cytotoxicity (CDC), apoptosis, antibody-dependent cell cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP). Apoptotic pathways were explored via caspase 8/9 activation, reactive oxygen species (ROS) production, and mitochondrial membrane potential. In vitro 3D cultures, XON7's impact on tumor growth, spheroid formation, and metastasis was evaluated. In vivo XON7 efficacy was evaluated in patient-derived xenograft (PDX) models of STS, with tri-weekly treatments initiated at tumor volumes of 150-200 mm^3.
Results: XON7 acts via plural MOA (CDC, apoptosis and ADCP), it demonstrated a potent anti-tumor activity in a panel of soft tissue sarcoma cells, with cytotoxicity ranging from 60% to 100% at a concentration of 300 µg/ml. Apoptotic induction involved both intrinsic and extrinsic pathways as evidenced by caspase 8/9 activation, ROS generation and loss of mitochondrial membrane potential. In 3D culture, repeated exposure to XON7 at a concentration of 300µg/ml significantly reduced the surface and viability of spheroids (liposarcoma SW872 cell line). XON7 also demonstrated its ability to reduce tumorogenicity and metastasis in a transwell invasion assay. In a PDX mice model of dedifferentiated liposarcoma, XON7 inhibited tumor growth by 50% over 48 days.
Conclusion: XON7 characterized by its plurality of action and potent antitumor efficacy, currently under clinical evaluation, XON7 emerges as a promising immunotherapy for soft tissue sarcomas.

#3422 Targeted optimized interferon alpha demonstrates improved safety and efficacy in tumors with low PD-L1 expression.

Kristopher Steward[1], Wuyi Wang[1], Yanmei Sun[2], Chengwei Shang[2], Chun Yang[2], Tom Boone[1], Hai Yan[1]

[1]REMD Biotherapeutics, Camarillo, CA,[2]Sichuan Luzhou Buchang Biopharmaceutical Co., Luzhou, China

Introduction: Type 1 interferons (IFN), including IFN alpha, have potent antitumor and immune stimulating effects. Though approved for treatment of multiple cancers, clinical use of IFN alpha is hindered by its short serum half-life and poor tolerability due to the broad expression of the Type 1 IFN receptor (IFNAR). A systematic approach was taken to improve the therapeutic window of IFN alpha. First, site specific mutations were designed to attenuate binding to IFNAR resulting in modified interferons with optimized activity. Then, the optimized interferons were fused to tumor antigen targeting antibodies (Abs) to generate immunocytokine molecules called Tumor Activated Interferon (TAI). Behaving as agonists, TAIs exhibit improved on-target activity once bound to antigen-positive cells. Moreover, TAIs also display significantly reduced systemic toxicity due to their attenuated non-targeted IFN activity. These characteristics contribute to the improved therapeutic window of TAIs. REMD-456 is our lead candidate consisting of optimized IFN alpha fused to PD-L1 Ab.

Methods: TAIs were screened for their IFN activity by reporter assay using cells with or without PD-L1 expression. Tumor cell growth inhibition activity of TAIs was assessed by MTS and CellTiter-Glo proliferation assays. Equimolar doses of pegylated IFN alpha-2a (PEGASYS) and TAI were evaluated in human tumor xenograft models using cell lines with various levels of PD-L1 expression. A single dose non-GLP tox study was conducted in Syrian golden hamsters with both PEGASYS and TAIs to assess PK and safety. Furthermore, PK, toxicity and biomarkers of TAIs were assessed in cynomolgus monkeys.

Results: Various TAIs containing different peptide linkers and optimized IFNs fused to PD-L1 Ab were screened for their PD-L1 targeted and non-targeted IFN activity. Compared to IFN alpha, the TAIs showed up to 9000-fold reduced non-targeted activity and up to 14-fold improved targeted activity. Tumor cell growth inhibition assays showed a similar activity profile. TAIs, at doses as low as 1 ug/kg, demonstrated superior in vivo anti-tumor activity compared to PEGASYS against NCI-N87 (PD-L1 low) and NCI-H820 (PD-L1 high) human tumor xenografts. In Syrian golden hamsters, high dose TAI did not affect body weight while PEGASYS at equimolar dose caused significant body weight loss. In cynomolgus monkeys REMD-456 had an acceptable safety profile at all doses tested.

Conclusions: REMD-456 was selected for its efficacy and safety profile in vitro and in vivo and manufacturability. It showed enhanced IFN activity only on PD-L1^{+} cells, resulting in an improved therapeutic window. In vivo, REMD-456 demonstrated superior anti-tumor efficacy in human tumor xenografts. Moreover, REMD-456 displayed an improved safety profile in hamster and primate single dose toxicity studies. IND enabling studies are in progress with anticipated filing in late 2025.

#3423 A novel triple action of SLC-3010 strengthens anti-tumor immunity by *in vivo* free TCB2/endogenous IL-2 complexing.

Junhyeok Heo[1], Daeun Kim[1], Geona Kim[1], Kyungmi Han[1], Junyoung Lee[1], Sun Young Rha[2]

[1]Selecxine Inc., Pohang-si, Korea, Republic of,[2]Song-dang Institute for Cancer Research, Yonsei University College of Medicine, Seoul, Korea, Republic of

Background: CD122-biased signaling has arisen as a promising approach to enhance anti-tumor T cell responses. However, the limited efficacy of CD122-biased IL-2 in clinical trials suggests that a more delicate approach beyond CD8 activation is needed. The secretion of IL-2 by activated T cells following CD122-biased IL-2 eventually leads to the expansion of Tregs, which can dampen the strength of the anti-tumor immune responses. Based on prior studies that anti-mouse IL-2 antibody (S4B6) can suppress Treg survival by blocking mouse IL-2 in vivo but boost CD8 T cells when it is complex with mIL-2, we devised a strategy leveraging this mechanism. To this end, we developed SLC-3010, which is hIL-2 and an anti-hIL-2 antibody (TCB2) conjugate via antigen-antibody interaction. Since TCB2 binds with IL-2 through CD25-binding epitope on hIL-2, the SLC-3010 provides CD122-biased IL-2 signaling. Although the primary MoA, anti-tumor efficacy and crystal structure of SLC-3010 have been previously reported, the fate and therapeutic implications of the non-covalent conjugated drug are not fully understood.
Methods: PK properties of SLC-3010 and TCB2 were assessed by ELISA after SLC-3010 administration. The role of free TCB2 in augmenting anti-tumor immune responses was investigated using flow cytometry. The therapeutic impact of free TCB2 was assessed by comparing the anti-tumor efficacy of SLC-3010 in tumor-bearing hIL-2 tg and wt mice. The anti-tumor efficacy of SLC-3010 and IL-2-CD25 fusion protein, which is unable to regulate endogenous IL-2 beyond primary CD122-biased signaling, was evaluated in hIL-2 tg mice.
Results: Free TCB2 dissociated from SLC-3010 remains detectable in the bloodstream for an extended duration than SLC-3010. Circulating free TCB2 can bind with lately encountered hIL-2 and provide a CD122-biased signaling again. Tregs expansion and/or survival were significantly reduced in hIL-2 tg mice, leading to a 3.65-fold higher tumor infiltrating MP CD8/Treg ratio than in wt mice. The anti-tumor efficacy of SLC-3010 was more pronounced in hIL-2 tg mice compared to wt mice. SLC-3010 exhibited superior anti-tumor efficacy compared to IL-2-CD25 fusion protein in hIL-2 tg mice.
Conclusions: SLC-3010 exhibits a "triple action" mechanism: SLC-3010 selectively stimulates anti-tumor immunity, disrupts Treg homeostasis, and re-boosts the immune system through in vivo complexing with endogenous IL-2. Currently, SLC-3010 is undergoing evaluation in an open-label, multi-center phase I/II clinical trial with dose escalation and expansion. SLC-3010 has been well-tolerated and has showed promising clinical activity, including confirmed partial response and sustained stable diseases with tumor shrinkage in patients with advanced solid tumors. (NCT05525247)

#3424 DuoBody®-FAPαxDR4 induces tumor cell death through FAPα-dependent, DR4 transactivation-mediated apoptosis.

I. Jongerius[1], J. Laoukili[2], B. de Goeij[1], E. Souteyrand[1], G. Andringa[1], M. Paauwe[1], M. Luijten[1], D. Satijn[1], E. Breij[1], O. Kranenburg[2], M. Overdijk[1];

[1]Genmab B.V., Utrecht, Netherlands, [2]University Medical Center Utrecht, Utrecht, Netherlands

Apoptosis can be induced through activation of death receptors (DRs), which, upon binding to their natural ligands, hypercluster to initiate caspase-dependent cell death. This process can be mimicked by agonistic antibodies, making it a promising approach for anticancer therapy. However, DR-targeting cancer therapies evaluated in the clinic have shown limited success, due to insufficient clinical activity and/or safety concerns primarily related to hepatotoxicity. We hypothesize that conditional activation of DR4 in the tumor microenvironment (TME) can improve the therapeutic window of DR agonism.

Fibroblast activation protein (FAP)α-expressing cancer-associated fibroblasts (CAFs) are abundant in the stroma of many solid cancers, making FAPα an attractive target to direct DR4 agonism to the TME. Here we present the novel Fc-silenced bispecific antibody DuoBody-FAPαxDR4 (GEN1057) designed to simultaneously bind to DR4 expressed on tumor cells and FAPα expressed on CAFs, to conditionally activate DR4 on the tumor cells. Functional activity of DuoBody-FAPαxDR4 was assessed in tumor cell lines and colorectal carcinoma (CRC) patient-derived organoids (PDOs) using viability and bioluminescent reporter assays and potential hepatotoxicity was evaluated using human primary liver spheroids *in vitro*. *In vivo*, antitumor activity of DuoBody-FAPαxDR4 was evaluated in patient-derived xenograft (PDX) models from different tumor types and in a model for spontaneous multiorgan metastasis of CRC.

DuoBody-FAPαxDR4 induced dose-dependent caspase-8 activation and cytotoxicity in DR4-expressing tumor cell lines and CRC PDOs, when cocultured with FAPα-expressing cells *in vitro*. Cytotoxicity was strictly dependent on the presence of FAPα-expressing cells, with no direct effect on their viability. Furthermore, no cytotoxicity was observed in cocultures treated with monospecific control antibodies containing either the FAPα- or DR4-specific Fab arm of DuoBody-FAPαxDR4, confirming that binding to both DR4 and FAPα is required for DR4 transactivation. DuoBody-FAPαxDR4 induced caspase-8 activation and cytotoxicity in the cocultures to levels in the same range as an antibody that induced unconditional DR4 hyperclustering. Importantly, DuoBody-FAPαxDR4 did not induce cytotoxicity in human primary liver spheroids. *In vivo*, DuoBody-FAPαxDR4 showed potent antitumor activity in PDX models and against multiorgan metastases in a CRC PDO cecum transplantation mouse model.

In conclusion, DuoBody-FAPαxDR4 is a novel bispecific antibody that activates DR4 on cancer cells exclusively in the presence of FAPα-expressing cells, leading to targeted caspase activation and tumor cell death. DuoBody-FAPαxDR4 represents a promising therapeutic agent for the treatment of cancer and is currently under evaluation in a first-in-human trial in patients with solid tumors (NCT06573294).

#3425 A novel anti-EGFR × IL-10M (FP010) fusion protein for solid tumor therapy.

J. Guo, J. Ding, C. Gu, L. Ye, D. Zhu, Y. Ou, Z. Zhang, B. Ren, C. Zhou, B. Shen, X. Wu, K. Chen, D. Lu, Y. Huo;
Guangdong Fapon Biopharma Inc, Dongguan, China

$CD8^+$ T cell exhaustion has been recognized as an important factor contributing to the resistance to PD-1/PD-L1 inhibitors. Recently, IL-10 was found to reinvigorate tumor-infiltrating terminally exhausted $CD8^+$ T cells via metabolic reprogramming, and enhance their proliferation, cytotoxicity and cytokine secretion. Notably, combining IL-10 with anti-PD-1 or CAR-T therapies markedly enhanced their antitumor efficacy. In a Phase 1b clinical trial, PEGylated IL-10 showed promising therapeutic potential in NSCLC and RCC. However, severe hematologic toxicities limited its clinical applications.

To overcome this challenge, we engineered the dimeric IL-10 into a monomeric form (IL-10M) to reduce its downstream activity, thus minimizing its toxicity. We then developed FP010, an anti-EGFR × IL-10M fusion protein of which IL-10M was fused to the C-terminus of the heavy chain of an engineered anti-EGFR antibody (panitumumab Fab combined with IgG1 Fc). This design allowed FP010 to preferentially accumulate in EGFR-expressing tumors, thereby enhancing its therapeutic effect. In this study, we evaluated FP010's antitumor activity in vitro and in vivo, as well as its safety profile in cynomolgus monkeys.

FP010 showed binding and blocking activity similar to that of panitumumab on A431 cells, and could significantly inhibit their proliferation. Additionally, FP010 exhibited strong ADCC activity. FP010 exhibited much lower downstream signal activity than wild type IL-10 in a STAT3 reporter assay. However, FP010 still kept its ability in promoting IFN-γ secretion and inhibiting apoptosis in exhausted $CD8^+$ T cells, indicating its ability to reinvigorate these cells. In vivo studies demonstrated potent antitumor activity of FP010 in both MC38-cEGFR and B16-cEGFR models, better than IL-10M or anti-EGFR antibody alone. We further assessed the hematologic toxicities of FP010 in cynomolgus monkeys. No evident hematologic toxicity was observed with FP010 at a dose of 10 mg/kg IV QW. In contrast, Panitumumab × wt IL-10 caused a remarkable decrease of erythrocytes at the same dose, consistent with the adverse effects of PEGylated IL-10 observed in clinical trials.

FP010 has demonstrated significant antitumor activity in vitro and in vivo, with acceptable adverse effects observed in cynomolgus monkeys. These data indicate that FP010 has great potential for cancer immunotherapy, especially for tumors with resistance to PD-1/PD-L1 inhibitors due to T cell exhaustion.

#3426 Preclinical development of CD-001, a novel fusion protein combining PD-1 blockade with IL-21 for solid tumors.

Y. Li[1], X. Luan[2], S. Zhou[2], X. Cheng[2], J. Zhao[1], G. Sang[2], H. Shen[2], N. Yang[2], J. Yang[2], J. Xu[2], S. Wang[1];
[1]Institute of Biophysics, Chinese Academy of Scienses, Beijing, China, [2]CD (Suzhou) Biopharma Co., Ltd., Suzhou, China

Background: Immune checkpoint blockade (ICB) has revolutionized cancer immunotherapy, offering significant clinical benefits. However, these successes are unfortunately limited to a minority of patients. Meanwhile, cytokine-based therapies have shown promise in cancer treatment, but are accompanied by potential severe systemic toxicity. CD-001 is a novel fusion protein integrating anti-PD-1 antibody with mutated IL-21. This dual-targeting approach enables simultaneous blockade of the PD-1/PD-L1 axis and targeted delivery of IL-21 to PD-1$^+$ T cells, aiming to enhance tumor-specific immune responses while minimizing systemic adverse effects.

Methods: Comprehensive nonclinical studies, including pharmacology, pharmacokinetics (PK), and toxicology assessments, were conducted to evaluate the safety and efficacy of CD-001. Pharmacology studies were performed to assess CD-001's binding affinity to PD-1 and IL-21R as well as its functional targeting of PD-1$^+$ T cells. In vivo efficacy was explored in various mouse tumor models including melanoma, lung cancer, and lymphoma. Toxicology and PK were also evaluated in cynomolgus monkeys.

Results: CD-001 exhibited high affinity to both PD-1 and IL-21R, and effectively targeted PD-1$^+$ T cells to exert its biological function. In vivo, CD-001 demonstrated superior antitumor activity compared to anti-PD-1 monotherapy or its combination with IL-21. Enhanced tumor-reactive CD8$^+$ T cell responses were observed. PK studies showed dose-proportional systemic exposure of CD-001 with no significant accumulation after repeated dosing in cynomolgus monkeys, and receptor occupancy results illustrated more than 30% of target engagement within 7 days. GLP toxicity studies in cynomolgus monkeys demonstrated a favorable toxicity profile, with no significant cardiovascular or neurological dysfunctions observed. Immunotoxicity observed in the GLP study tended to resolve or was fully resolved by the end of the recovery period.

Conclusion: CD-001 exhibits a well-characterized safety profile and potent antitumor activity in preclinical models, supporting its future development as a therapeutic candidate for advanced solid tumors. The dual mechanism of CD-001, combining PD-1 inhibition with targeted IL-21 delivery, represents a novel therapeutic strategy to enhance T cell-mediated immunity in cancer patients.

#3427 HY07121, a new generation of immune oncology therapeutics overcoming tumor immune resistance.

X. Zheng, Y. Chen, G. Dong, Y. Zhang, M. Zhang, B. An, C. Li, J. Zhan, X. Chai, J. Dai, P. Yang, D. Sun, A. Fu, T. Wei;
Sichuan Huiyu Seacross Pharmaceutical Technology Co., Ltd., Chengdu, China

Objective: The majority of cancer patients develop resistance to immune checkpoint inhibitors (primary and acquired drug resistance), which may be related to immune cell exhaustion mediated by additional immune checkpoints and the immunosuppressive tumor microenvironment. Developing next-generation immunotherapy drugs to address tumor immune resistance is a pressing clinical need.
Methods: We engineered and optimized a bispecific fusion protein, HY07121, by integrating PD-1 and TIGIT-targeted antibodies with IL-15/IL15Ra fusion cytokine. The molecule utilizes steric hindrance and tumor-specific targeting of PD-1 and TIGIT to minimize IL-15 exposure to peripheral tissues and balance toxicity with efficacy. Additionally, HY07121 synergistically enhances the intratumoral targeting of stem-like CD8+ T cells through PD-1 and IL-15 co-stimulation, inducing their expansion and differentiation. This replenishes tumor-killing effector T cells, thus overcoming immune exhaustion-induced resistance. The pharmacodynamic (PD), mechanistic, pharmacokinetic (PK), and toxicological profiles of HY07121 were evaluated through in vitro, in vivo, and cynomolgus monkey studies.
Results: In vitro pharmacodynamic studies demonstrated that HY07121 significantly stimulated IFN-γ secretion from human PBMCs at levels superior to the combination treatment group, demonstrating a strong synergistic potential that went beyond additive effects. In vivo, HY07121 exhibited dose-dependent tumor inhibition in both the human bladder cancer cell derived xenograft model and the immune-resistant human melanoma cell (A375) derived xenograft model, significantly outperforming the combination of Pembrolizumab and Tiragolumab. Mechanistically, HY07121 markedly upregulated the transcription of stem-like CD8+ T cell and NKT-related gene sets. Cynomolgus monkey pharmacokinetic analysis revealed dose-dependent systemic exposure (Cmax and AUClast) in the 0.2 mg/kg, 0.6 mg/kg, and 1.8 mg/kg groups, indicating nonlinear pharmacokinetics. The half-life (T1/2) across different doses ranged from 6.25 to 13.7 hours, with an accumulation ratio of less than 1.17 after four consecutive doses. During the entire GLP toxicity study, all monkeys were in good condition, with no abnormal clinical findings, and all animals survived until the planned necropsy. The maximum tolerated dose (MTD) was determined to be 2.4 mg/kg, and the highest non-severe toxic dose (HNSTD) was 1.8 mg/kg, indicating a therapeutic safety window of approximately 10-fold.
Conclusion: The in vitro and in vivo findings suggest that HY07121 is a novel, mechanism-driven therapeutic with the potential to enhance the efficacy of immunotherapy and address both primary and acquired resistance. The pharmacological and toxicological profiles strongly support the continued clinical development of HY07121.

#3428 Mechanistic insights into IFx-Hu2.0 responses in the first human trial after prior anti-PD-1 therapy failure.

Joseph Markowitz, Vishanna Balkaran, Andrew S. Brohl, Amod S. Sarnaik, Zeynep Eroglu, Nikhil I. Khushalani, Deanryan B. De Aquino, Pei-Ling Chen, Vernon K. Sondak, Ahmad A. Tarhini, Youngchul Kim, Shari Pilon-Thomas

Moffitt Cancer Center, Tampa, FL

Introduction: IFx-Hu2.0 is a plasmid encoding Streptococcal Emm55 protein for transfection into mammalian cells. The first-in-human clinical trial of IFx-Hu2.0 for unresectable melanoma showed that intra-tumoral IFx-Hu2.0 is safe. Melanoma patients responded to anti-PD1-based treatments after IFx-Hu2.0 treatment despite being previously refractory to treatment. Antibodies to Emm55 protein were seen in patient-derived plasma (Markowitz et al., Mol Cancer Ther., 2024). We now report on TCRseq and BCRseq from patients who received IFx-Hu2.0 on study. We also report on murine studies investigating how IFx-Hu2.0 produces a systemic immune response.
Methods: Samples were available pre/post therapy from 6/6 patients who completed the dose-limiting toxicity period on trial. TCRseq from peripheral blood and BCRSeq from peripheral blood and tumors were measured on purified RNA using Adaptive Biotechnologies™ company. The B16 murine model was utilized to study IFx-Hu2.0 systemic effects. Thirteen mice had tumors implanted in both right and left flanks. The mice received weekly injections of IFx-Hu2.0 or empty plasmid vehicle control into the right flank, and tumor growth curves were constructed. Tumor growth curves were compared using the CGGC permutation test. To determine whether antibodies are developed against Emm55, we transfected an unrelated cell line (EL4) with the Emm55 plasmid (EL4-Emm55) and exposed it to murine serum obtained from mice bearing B16 tumor, treated with IFx-Hu2.0 treatment using an empty plasmid as a control. Following this, we utilized a fluorescently tagged secondary goat anti-mouse IgG to assess whether antibodies in the serum bound to the cells, with measurements taken via flow cytometry.
Results: TCRSeq and BCRSeq demonstrated unique T and B-Cell clones in melanoma patients treated with IFx-Hu2.0. Mice injected with IFx-Hu2.0 demonstrated a statistically significant reduction in tumor growth compared to control mice (mean tumor volume at Day 17; 104 ± 22 mm^3 vs 702 ± 100 mm^3, $p < 0.01$). Within mice treated with IFx-Hu2.0, uninjected tumors grew more slowly than tumors in uninjected mice ($p = 0.03$), supporting an abscopal effect in mice. Anti-emm55 IgG antibodies were measured via flow cytometry on EL4-emm55 cells pulsed with murine sera from mice treated with IFx-Hu2.0 and IgG did not bind to EL4 cells transfected with Emm55 but not pulsed with serum.
Conclusions: The expression of Emm55 in a B16 murine melanoma model induces a direct anti-tumor and abscopal effect, with antibodies to Emm55 detected in murine sera. Further research is needed to assess the role of these antibodies in anti-tumor immunity. Unique clones from TCRSeq and BCRSeq suggest that IFx-Hu2.0 may be a personalized vaccine. Ongoing studies aim to understand how patients become re-sensitized to anti-PD-1 therapy, with subsequent clinical trials in development.

#3429 Improving druggability of a promising tumor target by the novel glycosylation-dependent mAb GT-008 for treatment of solid tumors.

Johanna Gellert, **Andreas Franz**, Manon Weis, Evelyn Hartung, Stephanie Gurka, Antje Danielczyk, Lisa Wei?, Patrik Kehler

Pentixapharm AG, Berlin, Germany

Aberrant O-glycosylation is a hallmark of cancer and can be used to improve druggability of targets for cancer immunotherapy. Tumors express cell surface proteins decorated with truncated O-glycans like Thomsen-Friedenreich (TF), Thomsen-nouveau (Tn) or sialylated Tn (sTn) antigens, which are virtually absent from normal tissues. Development of therapeutic mAbs against these hapten glycotopes is often hampered by weak affinities and low specificity, whereas peptide epitope targeting mAbs usually cannot distinguish between normal and tumor tissue. Both drawbacks can be circumvented by addressing combined protein/carbohydrate epitopes with mAbs that enable high affinity binding and high tumor specificity at the same time. We generated mAb GT-008 recognizing the glycoprotein CD24 with fundamental role in tumorigenesis and tumor progression, which is expressed on solid tumors, but also on healthy immune cells and tissues. GT-008 binds CD24 only in presence of a tumor-associated glycosylation thereby leading to reduced binding of the protein expressed on healthy cells.GT-008 was generated by immunization and single B cell cloning using different on- and off-target glycoforms for selection and screening. Binding and tumor selectivity were analyzed by ELISA, flow cytometry and immunohistochemistry on healthy and tumor tissues. Functionality was investigated by *in vitro* internalization and cytotoxicity assays with tumor and healthy cells. Compared to a conventional glycosylation-independent control antibody, GT-008 binds the protein target only in presence of TF O-glycosylation, but not the non-glycosylated protein, other glycoproteins carrying TF nor a synthetic TF O-glycoconjugate. GT-008 differentiates against the sialylated glycoform present on normal tissues. This leads to reduced binding to healthy human immune cells and tissues compared to the control mAb, whereas binding to cancer tissues from different indications (e.g. endometrium, breast, ovarian, prostate, a.o.) was retained. Additionally, its glycosylation dependent binding translates into tumor-selective internalization of GT-008 *in vitro* while sparing out CD24-positive immune cells. GT-008 has the potential to overcome limitations of conventional protein-targeting mAbs currently in clinical development by circumventing immune cell depletion and on-target/off-tumor toxicities associated with target expression on healthy cells. With its improved therapeutic window due to the strong differentiation between healthy and tumor tissue, GT-008 is exceptionally suitable for different therapeutic modalities, such as drug delivery (ADC, RIT) or cellular therapies (CARs). An *in vitro* and *in vivo* proof-of-concept study as therapeutic radioimmunoconjugate has been initiated.

#3430 FP008, a novel anti-PD-1×IL-10M fusion protein for cancer therapy.

Ce Gu, Jiaojiao Ding, Baiguang Ren, Mengying Liang, Linhui Ye, Yingye Ou, Chang Zhou, Bingquan Shen, Jian Guo, Xiaodong Wu, Ke Chen, Yongting Huo, Di Lu

Guangdong Fapon Biopharma Inc., Dongguan, China

Immune checkpoint inhibitors (ICI) such as anti-PD-1/PD-L1 antibodies have achieved remarkable clinical successes in cancer therapy. However, over 70 % of patients receiving ICI treatments are not responsive or experience disease progression after an initial period of response. Terminal exhaustion of $CD8^+$ T cell was supposed to contribute to this low responsiveness. On the other hand, IL-10 could enhance the anti-tumor activity of ICI by reinvigorating and expanding terminally exhausted $CD8^+$ T cells. Encouraging efficacies have been observed in phase 1 clinical trials of IL-10 therapy, but severe hematologic toxicity and low dosage greatly hindered its efficacy in further clinical trials. We therefore developed FP008, a novel anti-PD-1 × IL-10M (IL-10 monomer) fusion protein, and demonstrated its promising potential for cancer therapy.

FP008 exhibited strong anti-PD-1 blocking performance and activity comparable to its parental anti-PD-1 antibody. More importantly, FP008 significantly decreased $CD8^+$ T cells apoptosis and promoted IFN-γ secretion when co-cultured with exhausted $CD8^+$ T cells induced in vitro. This function was mainly caused by the IL-10M moiety, which was modified into a monomer to reduce downstream signaling and the resulting hematologic toxicity. Compared with wild-type (wt) IL-10, IL-10M significantly decreased the phagocytosis of erythrocytes by macrophages in vitro. The result was consistent with the observation in the toxicity study in monkeys. FP008 induced less STAT3 phosphorylation without PD-1 crosslinking in activated $CD8^+$ T cells or reporter cells than wt IL-10. Furthermore, the STAT3 signaling was greatly enhanced after PD-1 crosslinking, indicating FP008 may have strong activity on TILs where PD-1 is highly expressed.

In both CT26 and MC38 tumor models in hPD-1 knock-in mice, FP008 dose dependently inhibited tumor growth, with stronger anti-tumor efficacy and longer survival than Keytruda. In a CT26 rechallenge model after FP008 treatment and complete tumor regression, mice were re-challenged with CT26 tumor cells but remained tumor-free without further treatment, indicating that FP008 treatment could induce immunological memory. TILs analysis showed that the number of tumor-infiltrating $CD8^+$ T cells increased, while Treg cells decreased after FP008 treatment. In a GLP toxicology study in cynomolgus monkeys, FP008 was well tolerated at the dose of 10 mg/kg, and produced minimal decrease of erythrocyte and no tissue damage.

In conclusion, FP008 showed novel characteristics such as the synergistic function of anti-PD-1 and IL-10M, cis-activation of IL-10M by PD-1 enrichment and improved safety profiles. It demonstrated promising potential for clinical therapy for the anti-PD-1 naïve or resistant patients.

#3434 Evaluation of TCR-engineered T cells specific for KRAS G12V mutant peptide presented by HLA-A*02:01.

Joshua Ferrell, Xingyue He, Hongjing Qu, Allison Drain, Monique Peyreau, Nicole Danek, Ping Zhu, Hubert Lam, Dongmei Xiao, Joshua Francis, Gary Shapiro, Loic Vincent

Affini-T Therapeutics, Inc., Watertown, MA

Adoptive cellular therapies (ACT) have had limited success in the treatment of solid tumors. Recent approvals of both Tumor Infiltrating Lymphocyte (TIL) and T cell receptor-engineered T cell (TCR-T) therapies herald the promise of autologous T cell strategies. Unfortunately, existing therapeutics face significant challenges including an existing T cell response to immunogenic tumors (TILs) or relying on targets lacking tumor dependence (TCR-T). Engineered T cell therapies utilizing TCRs specific for public oncogenic driver mutations would overcome these hurdles with the intent of driving potent durable responses. Single residue alterations in KRAS are the most frequent oncogenic driver mutations in solid tumors, with G12V arising in indications with high unmet medical need such as PDAC, CRC, and NSCLC. HLA-A*02:01 is the most frequently occurring HLA class I allele in North American and Western European populations, and thus targeting the mutant KRAS G12V peptide presented in the context of HLA-A*02:01 offers the opportunity to treat a large patient population. However, therapeutics designed to provoke immunogenic responses to the KRAS G12V peptide presented by HLA-A*02:01 have proven challenging thus far, and physical evidence of the presentation of the G12V peptide by HLA-A*02:01 has been limited. Here, we employed a non-viral knock in system to screen TCRs via delivery of a transgene cassette comprised of 1) a transgenic TCR, 2) a chimeric cytokine receptor, and 3) the CD8αβ coreceptor to the *TRAC* locus of human primary T cells. We show specific TCR-T activation by KRAS G12V exogenous peptides loaded into HLA-A*02:01. We also demonstrate the potent induction of functional responses by engineered T cells to endogenous HLA-A*02:01 cancer cell lines both 1) engineered to express tandem minigenes (TMGs) encoding the KRAS G12V peptide and 2) transiently transfected with mRNA to express full length KRAS G12V proteins. These data provide evidence for the processing and presentation of KRAS G12V peptides by endogenous HLA-A*02:01 and illustrate its immunogenicity. This finding is further supported by the recognition of HLA-A*02:01 cell lines endogenously harboring KRAS G12V mutations and the specificity of the engineered T cells as assessed by X-Scan analysis. Our work provides evidence for the processing and presentation of the KRAS G12V mutant peptide in the context of HLA-A*02:01 and demonstrates the feasibility of targeting this public tumor-specific neoantigen widely expressed in indications with high unmet medical need.

#3435 B cell TuLibs: Immortalized tumor-derived B cell libraries for the interrogation and unbiased discovery of novel therapeutic targets and antibodies from patients.

Casper Marsman, Jurgen Heinen, Kelly Maijoor, Arjen Bakker, Rob Roovers, Michael Koslowski, Stefano Gulla, **Alessandra Micaela Villa**

Kling Biotherapeutics BV, Amsterdam, Netherlands

The use of B cell repertoires for antigen and antibody discovery is on the rise due to the emergence of new techniques to tap into the human antigen-experienced B cell repertoire. Recently, many research groups have shown that the presence of tumor-infiltrating B cells or Tertiary Lymphoid Structures (TLS) at tumor sites correlate with improved survival and response to Immune Checkpoint Inhibitors. Here, we present Kling Biotherapeutic's platform that enables the discovery of novel therapeutic targets and antibody-pairs. We overcome the limited proliferative lifespan of primary human and animal B cells by transduction with a proprietary vector to express Bcl6 and Bcl-xL, which prevents both terminal differentiation and apoptosis. Through the highly efficient isolation and immortalization of tumor- or TLS-derived B cells, our approach captures the B cell diversity and all Ig-isotypes from all tissues. As opposed to the labour intensive and costly approach of ex vivo BCR sequencing and subsequent recombinant antibody production and validation, our B-cell Tumor Libraries (TuLibs) secrete antibodies into the culture supernatant while retaining indefinite expansion capabilities, allowing for immediate functional screening of antibodies and rapid identification of unique tumor-specific B cell clones. Moreover, our immortalized B cells retain the ability to undergo somatic-hypermutation, allowing for in vitro affinity maturation of immortalized B cell clones against targets of interest. Altogether, Kling Biotherapeutic's proprietary platform allows the comprehensive functional screening and affinity maturation of human and animal B cell repertoires from multiple tissues sources. TuLibs represents a significant advancement in our ability to identify new immuno-functional targets and antibodies for biopharmaceutical development of tumor targeted therapies.

IMMUNOLOGY: Inflammation and Cancer: Tumor Initiation and Progression / Metastasis
Poster Session

#3438 The CD24-SEC14L2 drives the neoplastic progression of oral squamous cell carcinoma.
S.-R. Chang, C.-H. Chou, K.-W. Chang, **S.-C. Lin**;
National Yang Ming Chiao Tung University, Taipei, Taiwan

Immune stimulation or escape are critical factors determining the survival of malignancies, including oral squamous cell carcinoma (OSCC) and head and neck SCC (HNSCC). CD24 is a glycoprotein anchoring on cell membranes, modulating macrophages' immune responses and tumor stemness. However, its roles in the neoplastic progression are still controversial. As the inhibition of multiple oncogenic miRNAs downregulates CD24 expression, but not other immune checkpoint molecules, in murine OSCC cell lines, the functional roles of CD24 are investigated using a knockdown approach and the stable *CD24* tet-off expression cell subclones. CD24 expression modulates the increase of migration and invasion and the upregulation of phosphatidylcholine/phosphatidylinositol transfer protein SEC14 Like 2 (*SEC14L2*) expression. CD24 expression enriches the orthotopic growth of syngeneic tumor grafts. This is accompanied by the slight change in the myeloid lineage of immune cell infiltration within tumors and the mild disruption of cell profile in recipient mice's neck lymph node and spleen. The shutdown of CD24 with doxycycline treatment drastically suppresses the growth of CD24 tet-off tumors. Concordance in CD24 and SEC14L2 expression is noted in murine tumors. *SEC14L2* is upregulated in TCGA-HNSCC tumors and our OSCC tumors. High *SEC14L2* expression defines the decreased B cells and myeloid cell population. The integrative analysis of combined TCGA-HNSCC and our OSCC cohort using random effect mode illustrates *SEC14L2* as an unfavorable survival predictor of tumors. The oncogenic potential of the CD24-SEC14L2 axis being elucidated in this study may signify the therapeutic efficacy of CD24 targeting for oral cancer.

#3439 Study on the mechanism of sesamin inhibiting the malignant metastasis of prostate cancer.

Y. Chen[1], A.-C. Chang[2], C.-H. Tang[1], T.-S. Hwang[2];
[1]Medicine, China Medical University, Taichung, Taiwan, [2]Shin-Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan

Purpose: Prostate cancer (PCa), a malignant tumor of the urinary tract, is one of the most common cancers in men. PCa tends to metastasize to bones and lymph nodes, resulting in increased mortality. Sasamin is a plant compound extracted from sesame seeds. Some studies suggest that sesame lignans may have the potential for antioxidative, anti-inflammatory, and anti-tumor effects. Here, we aim to investigate the inhibitory effects of sesamin on the invasiveness of PCa cells *in vitro* and tumor progression *in vivo*.
Materials and Methods: Human PCa cell lines DU145 and PC-3 were obtained from the Bioresource Collection and Research Center (BCRC). Cell survival was detected by Colony formation assay. Wound healing assay, Anoikis assay and Transwell migration/invasion assay were performed to analyze cell aggressiveness. The subcutaneous tumor model was performed to study the inhibition of tumor growth by sesamin treatment. The levels of ADAM9, PD-L1 and Ki67 protein expression in tumor tissues were detected by immunohistochemistry (IHC) staining.
Results: Firstly, PCa cells were treated with different concentrations of sesamin, revealing a dose-dependent inhibition of both cell survival rate and proliferative ability. The co-administration of sesamin with a chemotherapeutic drug (docetaxel & cabazitaxel) enhances chemosensitivity in PCa cells. Next, we found that sesamin inhibits cell motility, invasion, and anoikis resistance via ADAM9 down-regulation through the JNK and c-Jun signaling pathway in PCa cells. Interestingly, sesamin can reduce ADAM9-mediated cleavage of membrane-bound PD-L1 (mPD-L1) into its soluble form, thereby inhibiting immune escape in PCa. Moreover, subcutaneous injection of PCa cells in mice, followed by twice-weekly intraperitoneal injection of sesamin, resulted in tumor growth inhibition. IHC staining of mice tumor tissues revealed a decrease in the levels of ADAM9 and Ki67 expression, and an increase in mPD-L1 expression in the sesamin-treated group compared to the non-treated group.
Conclusion: These results suggest that sesamin exhibits inhibitory effects on cell proliferation, migration, invasion, anoikis resistance, and immune escape *in vitro*, as well as tumor growth *in vivo*. The strategy of co-administration of sesamin can benefit clinical chemotherapy treatment, highlighting the potential of sesamin as an adjunctive treatment in PCa.

#3440 Local regulatory T cell dysfunction in gut mucosa post-infection prevents colorectal cancer establishment.

L. M. Goncalves[1], L. B. da Conceicao[1], J. B. de Assis[1], G. W. da Silva[1], G. M. B. Rodrigues[1], B. d. Oliveira[1], M. V. de Araujo[1], G. d. Lima[1], A. M. Abreu[1], C. B. Alves[1], F. Moreira[2], M. S. Coelho[1], L. C. R. da Silva[1], E. A. d. Nascimento[1], R. G. Ludwig[3], M. R. Vinolo[3], S. M. Muxel[1], E. E. de Oliveira[4], A. Lepique[1], D. M. da Fonseca[1];

[1]Institute of Biomedical Sciences (ICB), University of Sao Paulo, Sao Paulo, Brazil, [2]Johannes Gutenberg University Mainz, Mainz, Germany, [3]Institute of Biosciences (IB), University of Campinas, Campinas, Brazil, [4]University of Rio de Janeiro State - UERJ, Rio de Janeiro, Brazil

Colorectal cancer (CRC) is among the most lethal intestinal diseases, claiming approximately 1 million lives globally each year, with an incidence rate of around 2 million cases annually, presenting a significant public health challenge. Investigations into the etiology of CRC and strategies to enhance patient survival have highlighted the role of infectious episodes in disrupting intestinal immune homeostasis. Our research indicates that the resolution of certain acute gastrointestinal infections, typically considered benign, leads to permanent remodeling of the immune and lymphatic systems in the gastrointestinal tract and adjacent organs, such as the mesentery and mesenteric lymph nodes. This remodeling results in chronic local inflammation, impairing specific regulatory responses in the intestinal mucosa. We have demonstrated that this chronic inflammatory environment increases susceptibility to inflammatory bowel diseases, prompting the hypothesis that a similar process could occur with CRC. To test this, we infected C57BL/6 mice with *Yersinia pseudotuberculosis* (YP) and, after bacterial clearance, we induced CRC by azoxymethane and dextran sodium sulfate treatment. On the contrary to our initial hypothesis, Mice previously infected with YP exhibited fewer countable tumors compared to naïve mice, suggesting that the prior infection might protect against cancer development. Concurrently, we observed that Treg lymphocytes in the colonic lamina propria of YP-infected mice are dysfunctional, as they fail to control inflammation in both the T cell transfer colitis model and in vitro suppression assays. We believe that Treg dysfunction post-YP infection may favor immune surveillance and support the reduction in tumor development, given that the tumor microenvironment typically recruits Tregs as an immune escape mechanism, and post-infection, these cells are unable to function correctly.

#3442 Multi-omics comparison of early vs late-onset colorectal cancer reveals distinct tumor phenotype of early-onset patients.

David Cohen[1], Aleksandar Obradovic[2], Luka Jovanovic[2], Soobeom Lee[3], Dechokyab De[4], Casidhe-Nicole Bethancourt[1], Caitlin Rogers[4], Yosuke Mitani[4], Tianxia Li[4], Viktoria Jakubikova[5], Yoanna Pumpalova[2], Beatrice Dionigi[1], Sanja Vickovic[6], Joel Gabre[2]

[1]Department of Surgery, Columbia University Irving Medical Center, New York, NY,[2]Department of Medicine, Columbia University Irving Medical Center, New York, NY,[3]New York Genome Center, New York, NY,[4]Columbia University Irving Medical Center, New York, NY,[5]Human Immune Monitoring Core, Columbia University Irving Medical Center, New York, NY,[6]Department of Biomedical Engineering and Herbert Irving Institute for Cancer Dynamics, New York Genome Center, New York, NY

Though incidence and mortality of colorectal cancer (CRC) in individuals older than 50 (late-onset, LO-CRC) have been declining over the last 30 years, they have been increasing among adults less than 50 (early onset, EO-CRC), constituting a significant public health concern. Detailed molecular comparison of EO-CRC to LO-CRC tumors has thus far been limited to whole-exome sequencing and confounded by the complex tumor immune microenvironment (TME), with significant presence of non-tumor cells. Here, we present integrated comparison of EO- and LO-CRC by single-cell RNA-Sequencing (scRNA-Seq) and multi-plex immuno-fluorescence (mIF). We combine public data with in-house clinical samples to comprise a rich resource of scRNA-Seq on 43,000 cells from 11 EO-CRC tumors and 19 LO-CRC tumors and perform mIF on a tissue microarray of 50 EO- and 50 LO-CRC samples, focusing on major immune cell subsets. Our ScRNA-Seq analysis utilizes an algorithm that leverages gene regulatory networks for Virtual Inference of Protein Activity by Enriched Regulon (VIPER) to cluster cell populations in the TME, identify cell types over-represented in EO-CRC (by Fisher's Exact Test), and characterize regulatory proteins aberrantly activated in those cell types. By this approach, we have found small, but statistically significant elevation in fibroblasts (p=0.042), endothelial cells (p=0.025), and B-cells (p=0.042) in EO-CRC compared with LO-CRC, though no difference in T-cells. Further, scRNA-Seq VIPER analysis of tumor cells demonstrates a sub-phenotype conserved across patients and significantly over-represented in EO-CRC (p=0.028), marked by up-regulation of TLR4, C5AR2, LILRB4, PTPRE, and CCR5 (proteins known to play a role in immune signaling and inflammation) – and with specific activity of druggable proteins EPHB4, FGFR4, and IKBKB. Overall, we find that EO-CRC is transcriptionally similar to LO-CRC and is composed of a similar cellular milieu. mIF validates no differences in T-cell subsets but is unable to confirm increase in endothelial cells in EO-CRC found in our scRNA-Seq data set. Our finding of similar T-cell density is of particular interest and helps dispel the notion that EO-CRC is more immune "cold" than sporadic CRC in older cohorts. Most strikingly, we find that EO-CRC is marked by a particular cluster of TLR4-high tumor cells. It has previously been found that high fat diets can lead to increased CRC growth in a TLR4-dependent manner in murine models – thus suggesting a link between a known environmental risk factor and EO-CRC. Work is ongoing to validate TLR4-high tumor cells in human tissues and patient-derived organoids (PDOs). Further studies will utilize PDO as an *in vitro* model for drug screens targeting aberrantly active druggable protein targets in EO-CRC tumors and to further explore the mechanisms that contribute to development of EO-CRC.

#3443 Bipotential role of retinoblastoma binding protein 9 in sporadic colorectal cancer and colitis-associated cancer.

Yuki Nakanishi, Kensuke Hamadaa, Yu Muta, Mayuki Omatsu, Kosuke Iwane, Munehiro Ikeda, Jiayu Chen, Yoko Masui, Naoki Aoyama, Hiroki Kitamoto, Nobuyuki Agatsuma, Go Yamakawa, Takahiro Utsumi, Makoto Okabe, Shuji Yamamoto, Akihisa Fukuda, Hiroshi Seno

Kyoto Univ. Graduate School of Medicine, Kyoto, Japan

Retinoblastoma-binding protein 9 (RBBP9) is expressed in various human cancers and has been identified as a serine hydrolase promoting cancer progression by antagonizing TGFβ signaling. While its roles in pancreatic cancer have been explored, little is known about its function in intestinal tumorigenesis. Using human samples and mouse models, we investigated the bipotential role of RBBP9 in sporadic colorectal cancer (CRC) and colitis-associated cancer (CAC).Human surgical samples revealed that RBBP9 expression is significantly lower in CAC compared to sporadic CRC. Sporadic CRC is driven by Wnt/β-catenin activation, whereas CAC involves inflammation-associated pathways such as mTORC1 and IL6/JAK/STAT3 signaling. Consistent with these findings, *Rbbp9*$^{-/-}$ mice exhibited increased colitis severity and enhanced CAC tumorigenesis in the azoxymethane/dextran sulfate sodium (AOM/DSS) model. Transcriptomic analysis of AOM/DSS-treated tumors revealed upregulation of inflammatory and proliferation-associated pathways, such as mTORC1 and IL6/JAK/STAT3, while Wnt/β-catenin and mitotic signatures were downregulated, indicating inflammation-driven tumorigenesis independent of Wnt signaling in CAC.In the *Apc*$^{Min/+}$ model of spontaneous intestinal tumorigenesis, RBBP9 loss led to fewer and smaller tumors in the small intestine, accompanied by reduced proliferative activity and downregulation of cell cycle-related pathways (e.g., Myc targets and G2M checkpoint). Interestingly, tumors from *Apc*$^{Min/+}$;*Rbbp9*$^{-/-}$ mice exhibited enrichment of inflammatory and interferon-related signatures, including “oxidative phosphorylation” and “interferon alpha/gamma response,” though these changes did not lead to increased tumor proliferation without inflammatory stimuli. However, when *Apc*$^{Min/+}$;*Rbbp9*$^{-/-}$ mice were treated with DSS, a significant increase in inflammation-mediated tumorigenesis was observed in the colon, accompanied by elevated Ki67 and pS6 staining, markers of proliferative activity.Collectively, these findings highlight the bipotential role of RBBP9 in intestinal tumorigenesis. In human CAC, reduced RBBP9 expression correlates with inflammation-driven pathways, while in sporadic CRC, higher RBBP9 expression supports tumor progression via Wnt/β-catenin and cell cycle pathways. These results demonstrate that RBBP9 functions as a context-dependent regulator, suppressing inflammation-mediated tumorigenesis while supporting spontaneous tumorigenesis through proliferative pathways. Targeting RBBP9 may offer novel therapeutic strategies tailored to distinct CRC subtypes, depending on their inflammatory or non-inflammatory origins.

#3444 Characterization of immune environment in SV40C3-tag mouse model of triple-negative breast progression.

Janvi Sandhu[1], Anjana Bhardwaj[1], Alexander Koh[1], Raniv D. Rojo[2], Rim Ouni[3], Matthew Embury[1], Roza Nurieva[3], Isabelle Bedrosian[1]

[1]UT MD Anderson Cancer Center, Houston, TX,[2]College of Medicine, University of the Philippines, Manila, Philippines,[3]Department of Immunology, UT MD Anderson Cancer Center, Houston, TX

Background: Triple-Negative Breast Cancer (TNBC) is aggressive subtype and studies have reported impact of immune system on prognosis and treatment response. The role of the immune system during TNBC tumorigenesis, in the transition from precancer to cancer in unknown. We hypothesize that progression towards tumor is associated with both quantitative and functional changes to the immune microenvironment.

Methodology. SV40C3-TAg female mice, a TNBC progression model that develops tumors resembling human Ductal Carcinoma In-situ (DCIS) and Invasive Ductal Carcinoma (IDC) was used. To characterize immune population, mammary glands were harvested at 6-weeks (precancer) and 22-weeks (tumor) and immune cell population was analyzed by Fluorescence Assisted Cell Sorting (FACS). Animals were also sacrificed at multiple timepoints prior to tumor development to investigate immune cell populations and cytokine mileu during tumorigenic progression. Histopathological grades were assigned to mammary tissues: 0 for simple epithelium, 1-3 for mid to atypical hyperplasia (non-malignant low grade) and 4-5 for in-situ to invasive carcinoma (high grade lesions). Average number of $B220^+$ B cells and $CD8^+$ T cell per high-power-field (HPF) across the mammary tissue samples was determined. To understand functional characteristics of immune population mammary tissue samples were analyzed for levels of cytokines IL-4, IL-10, INF- ɣ using ELISA and Luminex Discovery Assay.

Results: The average number of $B220^+$ B cells increased from 0.73 cells/HPF in low grade (G0-3) to 1.61 cells/HPF in high grade (G4-5) mammary lesions ($p=0.0329$). Similarly in flow cytometry analysis, a 2-fold increase ($p=0.0051$) of B cell population was seen when comparing mammary tumors in 22-week animals to mammary glands from 6-week tumor free animals. Similarly, a significant infiltration of $CD8^+$ T cells from low (0.6 cells/HPF) to high (3.62 cells/HPF) grade lesions was seen by IHC ($p<0.0015$), mostly localized in peri-tumoral region, which is substantiated with 10-fold increase in $CD8^+$ T cells ($p=0.0012$) in FACS analysis of mammary tumors vs mammary tissue from 6 week animals. Furthermore, when we investigated the subpopulation of CD8+ T cells, there is an 2-3-fold increase in $CD8^+$ T FoxP3+ and $CD8^+$T $CTLA^+$ ($p=0.0004$) cells when comparing 22 week tumor bearing glands to mammary glands at the 6 week timepoint and 2-3-fold elevation in $CD8^+$ T IL-10 ($p=0.0291$). In line with FACS data, we observed increasing trend of IL-10 and IL-4 along with reduction in INF- ɣ when comparing cytokine levels in high grade mammary tissues vs low grade mammary tissues.

Conclusions: The influx of immune B cells and immune regulatory cells T cells indicate active response to tumor. The patterns of change in quantitative cell counts and immune mileu suggest changes from immune-stimulatory (Th1) to suppressive environment (Th2) with tumor progression.

#3445 Germline BRCA2 heterozygosity drives gastric lesion formation via myeloid-biased differentiation in bone marrow under chronic IL-1β overexpression and *Helicobacter felis* infection.

Feijing Wu, Quin Waterbury, Puran Zhang, Hiroki Kobayashi, Biyun Zheng, Hualong Zheng, Yi Zeng, Ruhong Tu, Jin Qian, Xiaofei Zhi, Yosuke Ochiai, Mashahiro Hata, Leah B. Zamechek, Timothy C. Wang

Columbia University Irving Medical Center, New York, NY

Background: BRCA2 plays a crucial role in DNA homologous recombination repair, and mutations in this gene significantly increase cancer risk. Previous studies have shown that heterozygous mutations in BRCA2 enhance pancreatic tumorigenesis driven by $KRAS^{G12D}$, but their impact on gastric tissues is less understood. This study investigates how knockout of a single BRCA2 allele affects inflammation-induced gastric damage under chronic interleukin-1β (IL-1β) overexpression and Helicobacter felis infection.
Methods: Mice heterozygous for BRCA2 were crossed with ATP4b-IL1β transgenic mice, which overexpress IL-1β in parietal cells to mimic chronic gastric inflammation. These $BRCA2^{+/-}$; ATP4b-IL1β mice were then infected with H. felis for three months and compared to ATP4b-IL1β control mice. Stomach histology and immune response dynamics were assessed, including a complete blood count (CBC) and flow cytometry of bone marrow to analyze hematopoietic stem cells (HSCs), myeloid-biased multipotent progenitors (MPP3), and neutrophils.
Results: We observed no significant atrophy or metaplasia histological changes in the stomachs of $BRCA2^{+/-}$; ATP4b-IL1β or ATP4b-IL1β mice at three months. However, H. felis infection significantly intensified gastric inflammation, metaplasia, dysplasia, and atrophy in $BRCA2^{+/-}$; ATP4b-IL1β mice compared to the ATP4b-IL1β mice. CBC results showed a 52.3% increase in neutrophil counts in the blood of infected of infected $BRCA2^{+/-}$; ATP4b-IL1β mice. Flow cytometry of bone marrow hematopoietic cells indicated a 72.3% reduction in HSCs and a 21.0% increase in MPP3 populations in $BRCA2^{+/-}$ mice compared to $BRCA2^{+/+}$ mice overexpressing IL-1β, suggesting a shift towards myeloid differentiation. Analysis of gastric tissue showed a 77.9% increase in neutrophil infiltration in $BRCA2^{+/-}$ mice, which correlated with the increased severity of lesions.
Conclusions: Our results indicate that heterozygosity for BRCA2 mutations can worsen gastric injuries in the context of chronic inflammation and H. felis infection, potentially due to hematopoietic imbalances and a shift towards neutrophil differentiation that exacerbates gastric pathology. This underscores the critical role of bone marrow dysfunction in driving inflammation-induced gastric damage in individuals with BRCA2 mutations. Future studies are needed to clarify the effects of BRCA2 mutations on hematopoietic progenitors and potential strategies to mitigate these effects.

#3446 Acinar to ductal metaplasia is an evolutionary dead end and does not actively contribute to pancreas regeneration after damage.

Rutvi Shah[1], I-Lin Ho[1], Er-Yen Yen[1], Charles Dyke[1], Hyuk Jean Kwon[1], Matthew Wong[1], Francesca Citron[1], Zhaoliang Liu[1], Sergio Attanasio[1], Ruparoshni Jayabalan[2], Ricardo Gonzalez Delgado[2], Luca Cecchetto[1], Elisabetta Granato[1], Ashish Damania[1], Shan Jiang[3], Haoqiang Ying[4], Giulio Draetta[1], Mauro Di Pilato[2], Nadim Ajami[1], Andrea Viale[1]

[1]Genomic Medicine, UT MD Anderson Cancer Center, Houston, TX,[2]Immunology, UT MD Anderson Cancer Center, Houston, TX,[3]Traction, UT MD Anderson Cancer Center, Houston, TX,[4]Molecular and Cellular Oncology, UT MD Anderson Cancer Center, Houston, TX

One of the major risk factors strongly associated with PDAC is pancreatitis and meta-analysis studies have revealed that inflammation of the pancreas increases the relative risk of developing pancreatic cancer by up to 13-fold. During pancreatitis, acinar cells transiently undergo metaplasia known as acinar to ductal metaplasia (ADM). It is well known that in the presence of activated oncogenes, for example mutated *Kras*, this metaplastic state can progress into PDAC with ADM being the first step towards tumorigenesis. To understand the pathophysiological role of ADM during pancreatitis and tumorigenesis, we utilized different mouse models to study the function and fate of ADM cells. We found that although ADM can effectively mitigate pancreatitis-induced tissue damage, it is not a regenerative process. Instead, cells that underwent ADM were eventually exhausted. We confirmed these findings by performing lineage tracing experiments to understand the fate of the cells and found that they were indeed not responsible for regenerating the pancreas. To better capture the dynamic process distinct sub-populations of epithelial cells undergo, during ADM induction and resolution, we are leveraging spatial transcriptomics of the entire pancreatic tissue at different timepoints after damage induction. We are also actively studying how oncogenic *Kras* activation reverts this process and contributes to tumorigenesis. This study will shed light on initiation of pancreatic cancer and how it can be prevented in the context of evolution from pancreatitis.

#3447 Neutrophil extracellular traps drive the tumorigenic potential in pancreatic ductal adenocarcinoma.

Sakshi Bansal[1], Anjali Aggarwal[1], Vinit Sharma[1], Rajesh Gupta[2], Harjeet Singh[2], Naresh Sachdeva[3], Alka Bhatia[4]

[1]Anatomy, Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India,[2]GI Surgery, HPB and Liver Transplantation, Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India,[3]Endocrinology, Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India,[4]Experimental Medicine & Biotechnology, Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India

Background: Tumor-infiltrating neutrophils (TINs) have been observed to be linked with poor prognosis in patients with pancreatic ductal adenocarcinoma (PDAC). In the inflammation and oxidative stress-rich tumor microenvironment, neutrophils are likely to undergo neutrophil extracellular traps (NETs) formation which have unique capability of shielding the tumor cells from anti-tumor immune response and facilitate tumor progression. However, the extent of NET infiltration in PDAC and the specific mechanisms by which NETs contribute to tumorigenesis remain unclear. This study aims to investigate NETs infiltration in PDAC and their impact on PDAC progression.

Methodology: Tumor-infiltrating neutrophils (CD15) and NETs specific markers (citrullinated histone H3, Myeloperoxidase) in the tumor tissue and blood of 15 PDAC patients and 15 healthy volunteers were determined by flow cytometry, gene expression and immunofluorescence. NETs were cocultured with Panc1 cells to assess their effect on tumor growth and behavior through various tumorigenic assays (MTT, colony formation assay, scratch assay). The gene expression analysis was done for epithelial-mesenchymal transition (EMT), extracellular matrix (ECM) modulation, stemness and dormant cell markers through qRT-PCR and further confirmation at protein level by western blot.

Results: In both blood and tissue samples of PDAC patients, relative percentage of NETs were markedly increased and the levels of citrullinated histone is positively correlated with higher tumor stage & grade. Moreover, circulating NETs shows a significant linear positive correlation with local pancreatic tumor NETs, indicates an association between NETs infiltration and disease severity. *In vitro*, NETs increased tumor migration and induced a more aggressive mesenchymal phenotype. They promoted tumor progression and invasion by downregulating epithelial markers (E-cadherin, ZO-1) and upregulating mesenchymal markers (N-cadherin, Vimentin, Snail, Twist, Zeb1, IL8, TGFβ) as well ECM-modulating markers (αSMA, Desmin, MMP, TIMP). Additionally, NETs contributed to tumor relapse by awakening dormant cancer cells (Dec2, p21, p27, p38) and increasing the expression of proliferative markers (Ki67, ERK, Bcl2) along with stemness markers (Sox2, Oct4, Nanog).

Conclusion: NETs contribute to a pro-metastatic cancer phenotype by activating EMT and reactivating dormant cancer cells. Therefore, NETs may serve as independent diagnostic biomarker and a potential therapeutic target in PDAC.

#3448 Profiling the urinary bladder cancer composition and inter-cellular proximity patterns using single-cell RNA-sequencing and spatial transcriptomics.

Alexandros Kostakis, Andrea Behanova, Iliana Kerzeli, Ramy Elgendy, Aikaterini Chourlia, Sven Nelander, Per-Uno Malmstrom, Ulrika Segersten, Milena Doroszko, Anca Dragomir, Carolina Wahlby, Martin Lord, **Sara Mangsbo**

Uppsala University, Uppsala, Sweden

Urinary bladder cancer (UBC) has shown to be difficult to treat, attributed to tumor heterogeneity and differences in response to treatment based on molecular subtypes. It is essential that we increase our knowledge the interplay between the tumor, stroma and immune system to improve our knowledge of the TME in UBC and thereby therapeutic options. Herein we employed single-cell RNA sequencing and *in situ* spatial transcriptomics on bladder samples from two UBC mouse models (model development in PMID 34232958), including in-depth immune clustering to characterize the TME during tumor progression from non-muscle invasive bladder cancer (NMIBC) to the muscle-invasive bladder cancer (MIBC) stage in a semi-spontaneous UBC model. Granulocytes and lymphoid cells were enriched in the carcinogen-induced tumors, while monocytes/macrophages were more prevalent in the reference MB49 cell line-transplantable orthotopic tumor model. Immune activation was generally increased in tumor-bearing mice overall. Lymphoid cell analysis identified NK cells and various T and B cell types, with effector CD8 T cells in carcinogen-induced tumors and scarce naïve CD4 and CD8 cells in MB49 mice. Differentially expressed genes between NMIBC and MIBC stages were predominantly linked to cell proliferation and signaling pathways. Interestingly, inferred cell interaction analysis by CellChat predicted increased *Cd6-Alcam* signaling within the lymphoid populations between T cells and basal or myeloid cells. Myeloid cell analysis displayed distinct profiles between the two models with infiltration of Ms4a7+ macrophages in the carcinogen-induced model and Iftim1+ macrophages in the MB49 model. Also, stage played a role for myeloid infiltration, with primarily Ccl8+ macrophages enriched in NMIBC, while those in MIBC were predominant of Spp1+ type. Granulocyte analysis identified eight clusters with diverse distributions in carcinogen-induced tumors, including four with strong neutrophil-like phenotypes (Isg, Retnlg, Hif-1, and SigF). Spatial transcriptomics indicated increased monocyte/macrophage tumor influx and interestingly, a significant interaction with bladder mesenchymal cells upon tumor progression. This technique also showed noticeable granulocyte tumor infiltration of penetrated tumor tissue at both the NMIBC and MIBC stages, the latter at higher levels, possibly suggesting spiraling pro-inflammatory TME. This data provide an example of that we need to further improve our knowledge of the myeloid/granulocyte role in UBC and consider how to selectively target subtypes of these cells with pharmacological interventions to improve therapeutic outcome in this severe disease, a disease that often recurs at the NMIBC stage and that presents with a high mortality in the MIBC stage.

#3449 The role of N-terminal cleavage of Trop2 in tumor progression and therapeutic resistance in triple negative breast cancer.

Andrea E. Wilson[1], Junping Wei[2], Gangjun Lei[2], Tao Wang[2], Xiao-Yi Yang[2], Melissa Gajda[2], Cong-Xiao Liu[2], Zachary C. Hartman[2]

[1]Pathology, Duke University School of Medicine, Durham, NC,[2]Surgery, Duke University School of Medicine, Durham, NC

Background: Triple negative breast cancer (TNBC) is the most aggressive subtype of breast cancer (BC), with risk factors including greater frequency of recurrence and metastasis and lower overall survival rate compared to other BC subtypes. While the drivers of TNBC are unclear, Trop2 is a transmembrane protein that has been found to be highly expressed in TNBC, whose role in tumor progression is not well understood. High expression of Trop2 has led to the development of antibody-drug conjugates (ADCs) targeting wild-type Trop2, including Sacituzumab govitecan (SG) and Datopotamab deruxtecan (Dato-dxd). However, Trop2 is an intronless gene but can be posttranslationally cleaved by ADAM10 and other proteases in malignant tissues. Cleavage of Trop2 and its effect on tumor progression and drug resistance have not been previously explored, which is becoming increasingly crucial to understand as more Trop2-targeting therapies enter clinical practice.

Experimental Methods and Results: To determine the oncogenic capacity of cleaved Trop2, our lab has generated a construct of N-terminally cleaved Trop2 ΔQ31-R87 called Trop2-ΔN and have stably transduced mouse TNBC MM3MG cells, a cell line that is not well transformed and grows poorly in immune competent mice. SCID-beige mice who lack T and B cells and have non-functional NK cells and immune competent BALB/c were injected with wild-type Trop2, an uncleavable form of Trop2 called Trop2-87/88, Trop2-ΔN, and control to find that Trop2-ΔN conferred a proliferative phenotype, which was comparable to the known oncogene HER2Δ16. Additionally, MM3MG cells were stained for SG, Dato-dxd, and their parental monoclonal antibodies and analyzed for binding via flow cytometry. We found that SG and Dato-dxd had lowered binding capacity towards Trop2-ΔN expressing cells. To determine if the killing capacity of these ADCs were altered, we transduced the human breast epithelial cell line MCF10AT with our constructs of uncleavable Trop2-87/88 and Trop2-ΔN to find that both SG and Dato-dxd to have lowered killing against Trop2-ΔN expressing cells.

Conclusions: Our studies found that N-terminally cleaved Trop2 induces a proliferative phenotype in vivo, reduce binding capacity of Trop2 ADCs, and are more resistance to ADC-induced killing in vitro. These data provide insight into the potential importance of N-terminally cleaved Trop2 in tumor progression and drug resistance that have not been explored. Future experiments will elucidate the mechanisms that underlie cleavage of Trop2 and refining Trop2-targeting therapies.

#3450 The role of NRF2-HO-1 expressing macrophages in lung cancer progression.

Ana Sofia Leal, Karen T. Liby

Indiana University School of Medicine, Indianapolis, IN

Macrophages are a highly plastic cell, in the lung they have important physiological roles, such as removing reactive oxygen species (ROS). One of the main pathways that is used by macrophages to remove ROS is the NRF2->HO-1, a detoxifying mechanism. KRAS mutated lung cancer, one of the most common diagnosed lung cancers, can deregulate the NRF2->HO-1 pathway on macrophages within the lung microenvironment, promoting cancer progression. Bone marrow derived macrophages (BMDM) from both A/J WT and full body NRF2 KO mice were used. Monocytes were differentiated into macrophages using M-CSF for 5 days. On day 5, BMDM are further stimulated with conditioned media (CM) from murine lung cancer cells (VC1 and LL2). HO-1 and NRF2 ($p<0.05$) mRNA levels are higher in BMDM of WT mice compared with NRF2 KO mice. Assuming HO-1 is a “first” response mechanism, stimulation with CM from VC-1 and LL2 was extended to 48 hours. At 48 hours BMDM from WT mice compared with NRF2 KO mice have increased mRNA HO-1 expression in both VC-1 and LL2 stimulated ($p<0.05$). Moreover, other markers, such as CCL2 and IL6 are differently expressed between WT and NFF2 KO BMDM. CCL2 appears to be predominantly expressed in NRF2 KO BMDM. To determine the evolution of HO-1 expression in macrophages in the lung after tumorigenesis initiation with vinyl carbamate (VC), lung of A/J mice were collected at 1, 2, 4, and 8 weeks after VC injection. VC is a carcinogenic compound that induces KrasQ61L mutations and consequently lung adenocarcinomas 12 weeks after administration. CD11b cells were isolated from the lungs of mice after VC administration. CD11b cells show an increase in HO-1 mRNA at week 2 post VC, and in NRF2 one week after VC. IL6 and CD206 mRNA values do not significantly change over time. CCL2 mRNA levels increase 2-fold ($p<0.001$) at 4 weeks in CD11b positive cells when compared with mice receiving saline. Zinc (II) propoporphyrin IX (ZN-IX) is an HO-1 inhibitor, that has shown anti-tumor and immune modulatory effects. A/J WT mice 6 weeks after stimulation with VC, were treated with ZN-IX for 2 weeks, every 2 days at 50mg/Kg ip. Flow cytometry of lungs of mice treated with ZN-IX showed an increase in CD4 T cells ($p=0.002$) and increase in the activation of CD8 T cells (CD107, $p=0.007$). The number of macrophages infiltrating the lungs did not change between mice treated with control-DMSO or ZN-IX, however, significant reductions ($p<0.05$) in the macrophage tumor promoting marker CD206 were observed. Moreover, the same macrophage populations showed and increased expression of IA-IE (MHC-II, $p<0.05$), an anti-tumor marker in macrophages. Immune cell-cancer cell interactions and regulation are still not fully understood. Here we show that KRAS mutated lung cancer cells can hijack the natural detoxifying process in macrophages to promote cancer. Interrupting this process can unlock new therapeutic vulnerabilities for lung cancer prevention in high-risk populations.

#3451 Exploring the impact of neoantigen expression on lung tumor development.

Jennifer Loza, Ishan Bansal, Brian Hunt, Kelli Connolly, Srividhya Venkatesan, Advait Jeevanandam, Nikhil Joshi

Yale School of Medicine, New Haven, CT

Lung adenocarcinoma (LUAD) stands as a formidable challenge in oncology, despite the emergence of immunotherapy as a promising treatment avenue. The dynamic interplay between the immune system and cancer plays a pivotal role in shaping cancer development and progression. Central to this process are neoantigens, tumor-specific antigens recognized by cytotoxic T cells, which trigger the elimination of tumor cells. It has been implicated that the presence of T cells also decreases tumor grade. However, the precise impact of neoantigen expression on LUAD development and progression remains poorly understood.

Our lab previously developed a genetically engineered mouse model with the ability to induce expression of a fluorescent neoantigen in oncogenic KRAS, P53-null, tdTomato-expressing lung adenocarcinoma cells. Using this system, we track tumor initiation, progression, and advanced LUAD development across 20 weeks in both immune-deficient and -sufficient settings. Further, tumors initiated in this system give rise to heterogeneous (neoantigen+ and -) tumors, enabling the study of T cell-driven tumor selection. Through multiplexed immunofluorescence, we identified that neoantigen+ tumors experience increased T cell infiltration and reduced volume, ultimately leading to prolonged animal survival. Surprisingly, advanced lung tumors maintain neoantigen+ cells, indicating that endogenous lung T cells are ineffective at clearing neoantigen-expressing tumor cells. Yet, we detected that infiltrated tumors exhibit reduced p-ERK expression, suggesting that T cells may slow tumor progression through non-cytotoxic mechanisms. Transferring stimulated neoantigen-specific T cells to tumor-bearing mice largely eliminated neoantigen+ tumor cells. All these data together suggest complex mechanisms for the role of endogenous T cells in controlling lung tumors.

Through molecular characterization of tumor progression and T cell-mediated tumor suppression, this study reveals specific pathways that LUAD cells undergo during the process of development and progression, providing valuable insights into the immune response against neoantigen-expressing tumors. Ultimately, by unraveling the intricate interplay between neoantigen expression, immune surveillance, and tumor progression, this project aims to contribute significantly to our understanding of how neoantigens shape the dynamics of tumor biology.

#3452 Investigating the relationship between obesity-related dyslipidemia, inflammatory myeloid cells, and cancer.

Maria D. Sanchez-Pino[1], Ramesh Thylur Puttalingaiah[2], Dorota Wyczechowska[2], Timothy Kayes[2], Julia Panov[3], Jone Garai[2], Timothy Foster[4], Jovanny Zabaleta[1]

[1]Interdisciplinary Oncology, LSU Health Science Center, New Orleans, LA,[2]LSU Health Science Center, New Orleans, LA,[3]Tauber Bioinformatics Research Center, Haifa, Israel,[4]Microbiology, LSU Health Science Center, New Orleans, LA

Background: Since people with obesity generally have altered immune responses, a high level of cholesterol, and a higher risk for cancer development and progression, it is important to investigate the relationship between cholesterol, immune cells, and cancer. The current study aims to assess the contribution of obesity-related excess lipids in priming subset of myeloid cells.
Methods: The carcinogen diethylnitrosamine (DEN) was used to induce hepatocellular carcinoma (HCC) in mice (Wild-type and liver-specific Alb-CPT1-Knockout) and diet-induced obesity (DIO) was induced to evaluate the impact of obesity compared to lean mice. Tumor burden and transcriptomic profiling were performed. *In vitro* exposure to immature myeloid cells (IMCs) from mice bone marrow with LDL-cholesterol (LDLc) was performed for molecular (protein, RNAseq, ATACseq) assessments and functionality.
Results: Obesity induced by diet increased the tumor burden on mice associated with steatosis and accumulation of myeloid-derived suppressor cells in the liver identified by flow cytometry. CPT1-KO mice showed increased gene expression of markers for immunosuppressive neutrophilic subtype and macrophages. Among different pathways, differentially expressed genes were involved in the MET and TGFb Signaling- ECM Remodeling & Metastasis, Inflammation and Epigenetic & Transcriptional Regulation. LDLc *in vitro* stimulation of IMCs increased the expression of immunosuppressive markers. Integration of RNAseq-ATACseq suggests that lipid-exposed IMCs have an epigenetically regulated activation program.
Conclusion: Excess lipids facilitate liver cancer where inflammatory neutrophilic cells and macrophages may play a major contributing role.
Funding: Pennington Research Center NORC U54-GM104940 and LSU LCMC Health Cancer Center.

#3453 TNF augments ovarian cancer metastasis in an omentum-independent manner.

R. L. Mintz[1], E. E. Erlich[2], H. S. Ruiz[1], Q. Alayo[1], M. Wohltmann[1], G. J. Randolph[1];
[1]Washington University School of Medicine in St. Louis, St. Louis, MO, [2]Memorial Sloan Kettering Cancer Center, New York, NY

The mechanistic role of the inflammatory cytokine tumor necrosis factor (TNF) in ovarian cancer pathogenesis has been widely debated. The Randolph lab has developed a new mouse model, TNF^{D69Au}, in which CRISPR/Cas9 was used to target the 3' AU rich-response element of TNF mRNA transcripts in immunocompetent C57BL/6J mice, resulting in increased stability of TNF RNA and thus elevated inflammation. When TNF^{D69Au} mice and their wild-type (WT) littermate controls were intraperitoneally injected with the aggressive ID8p53$^{-/-}$Brca2$^{-/-}$ murine ovarian cancer cell line that recapitulates high-grade serous ovarian cancer, the TNF^{D69Au} mice had significantly increased tumor burden compared to their littermates as early as one-week post-injection. This discrepancy continued consistently until the endpoint when ascites accumulated. The greater omentum, a visceral fat pad along the stomach, is a well-established early and predominant metastatic niche in ovarian cancer. Yet, the TNF^{D69Au} mice demonstrate broad tumor dissemination across peritoneal organs with minimized omental preference. When TNF^{D69Au} mice were omentectomized, their tumor burden was equivalent to that of TNF^{D69Au} mice that received sham surgery, while WT mice expectedly exhibited decreased tumor burden when omentectomized. Regardless of omentectomy, the tumor burden in these TNF^{D69Au} mice was higher than in WT mice, highlighting how increased TNF production in the peritoneal cavity leads to increased tumor burden independent of the omentum. To determine if the source of TNF driving the elevated tumor burden was hemopoietic-derived, bone marrow from TNF^{D69Au} mice or WT littermates was transplanted into WT or TNF^{D69Au} mice recipients. TNF^{D69Au} mice that received bone marrow from WT or TNF^{D69Au} mice had increased tumor burden compared to WT mice that received WT bone marrow. These results suggest that the elevated TNF responsible for increased tumor burden can be stromal-derived, which is surprising given the canonical role of hematopoietic cells in TNF-signaling. Altogether, our data suggest that TNF increases peritoneal ovarian cancer spread and minimizes omental dominance, potentially through stromal signaling.

#3454 Charting single-cell metabolism co-evolution during metastasis.
Y. C. Wu;
Fudan University, Shanghai, China

Metastasis, the leading cause of cancer death, exhibits a highly heterogeneous and suppressive cancer microenvironment. Here, we sequenced 97 matched samples by using single-cell RNA sequencing and spatial transcriptomics of colorectal cancer liver metastasis. Strikingly, the metastatic microenvironment underwent remarkable spatial reprogramming of immunosuppressive cells such as MRC1+ CCL18+ M2-like macrophages. We further developed scMetabolism, a computational pipeline for quantifying single-cell metabolism, and observed that those macrophages harbored enhanced amino acid metabolic activity. Interestingly, neoadjuvant chemotherapy could block this status and restore the antitumor immune balance in responsive patients, whereas the nonresponsive patients deteriorated into a more suppressive one. In parallel, metastatic cancer cells displayed the metabolic switch from OXPHOS to glycolysis, which is dependent on the metabolic master regulator LY6E. Our work described the metabolism co-evolution of both immune and cancer cells during metastasis and uncovered the black box of how tumors respond to neoadjuvant chemotherapy.

#3455 Mesenchymal tumors direct macrophage-mediated vascular editing and premetastatic remodeling to promote PDAC metastasis.

Nicole Elizabeth Gross[1], Soren Charmsaz[1], Tiffany Chu[2], Jae W. Lee[1], Emma Kartalia[1], Yiyang Gao[3], Alexei G. Hernandez[1], Sarah M. Shin[1], Erin M. Coyne[1], Courtney Cannon[1], Xuan Yuan[1], Cheng-Chieh Huang[1], James Leatherman[1], Pei-Hsun Wu[2], Laura D. Wood[1], Elizabeth M. Jaffee[1], Won Jin Ho[1]

[1]Johns Hopkins University School of Medicine, Baltimore, MD,[2]Department of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD,[3]Johns Hopkins University, Baltimore, MD

Background: Patients with pancreatic ductal adenocarcinoma (PDAC) often have metastatic disease at diagnosis and respond poorly to therapies. High myeloid-to-T cell ratios correlate with worse prognoses, but a more detailed, integrated understanding of mechanisms by which myeloid cells regulate metastasis is critically needed. Using high-parameter spatial profiling, we recently observed that a highly metastatic tumor cell line utilizes mesenchymal invasion and harbors a tumor microenvironment with increased endothelial cell abundance, lower M1-like to M2-like tumor associated macrophage (TAM) ratios, and Arginase-1+ TAMs situated closer to tumor and endothelial cells, compared to a less metastatic model. These models were further interrogated to study the myeloid drivers of metastasis in the primary and metastatic sites.

Methods: We used two murine cell lines driven by *Kras*^{G12D} and *Trp53*^{R172H} under *Pdx1*-Cre, KPCY 2838c3 (“c3”) and KPCY 6419c5 (“c5”), which exhibit low-metastatic and high-metastatic propensities, respectively. Imaging mass cytometry was used to determine the changes in the primary tumor and pre-metastatic microenvironments. TAMs and endothelial cells from the primary site were evaluated using RNAseq. *In vitro* tube formation assays were performed to evaluate the potential factors driving vascularization.

Results: In the pancreatic site, the highly metastatic c5 model was significantly enriched for metastasis- and angiogenesis-promoting pathways, including the RANK/RANKL axis. *In vitro* tube formation assays revealed that c5-derived TAMs induce greater tube formation over those from the c3 model and that RANKL significantly promotes tube formation. Moreover, in the hepatic premetastatic site, the c5 tumors expanded Kupffer, stellate, and endothelial cells to a greater extent than c3 tumors. Finally, the c5 tumors recruit fewer lymphocytes and more granulocytes to the liver, suggesting poorer immune protection against metastasis.

Conclusions: Our study highlights multiple tumor-intrinsic and extrinsic factors responsible for metastasis, and that TAMs serve as a critical integration point. Interestingly, we found the RANK/RANKL axis, for which there is already an inhibitory agent that is clinically used, to be a potential mechanism underlying the TAM-endothelial crosstalk. Together, these data prompt further exploration of the observed TAM-endothelial relationship and the premetastatic remodeling in human PDAC, as well as the targetability of the RANK/RANKL axis for disrupting PDAC metastasis.

#3456 Differential impact of oncostatin M (OSM) on EMT and biomechanical properties of HER2+ and HER2- breast cancer.

Maria Pou[1], Kali Woods[2], Laura Bond[2], Cheryl Jorcyk[1]

[1]Boise State University, Boise, ID,[2]Biomedical Research Institute, Boise, ID

In the United States, breast cancer (BC) is projected to cause over 43,000 deaths in 2025, despite significant advances in diagnostic and therapeutic strategies. Mortality is primarily attributed to late-stage diagnosis with distant metastasis. One key mechanism that enhances the metastatic potential of breast carcinoma cells is the epithelial-to-mesenchymal transition (EMT), a process in which cells lose their apical-basal polarity and acquire a mesenchymal-like phenotype; thereby increasing their motility and invasiveness. The treatment choice for each BC patient is very complex since it depends on the presence or absence of hormonal receptors and epidermal growth factor receptor 2 (HER2) expressed by the tumor cells. This research distinguishes between patients whose cancer cells overexpress HER2 (HER2-positive; HER2+) and those who do not (HER2-negative; HER2-), representing around 30% and 70% of all BC diagnoses, respectively. Oncostatin M (OSM) is an interleukin 6 (IL-6)-family proinflammatory cytokine produced in the BC tumor microenvironment in a paracrine manner by macrophages and neutrophils, as well as in an autocrine fashion by the tumor cells. Clinical data indicates a negative correlation between elevated OSM tumor levels and patient survival. OSM plays a critical role in metastasis through multiple mechanisms, including promoting the secretion of pro-metastatic molecules such as VEGF, LOXL2, and IL-6, increasing circulating tumor cell (CTC) numbers, and facilitating metastasis to distant organs such as bone and lung as observed *in vivo*. Our laboratory has demonstrated that OSM induces EMT in HER2- BC cell lines, but little is known about its role in HER2+ BC. Thus, we aimed to investigate the differential effects of OSM on metastatic potential, focusing on EMT and cell motility in HER2+ versus HER2- BC models. Using qRT-PCR, western blot analysis, and immunofluorescent staining on HER2+ and HER2- BC cell lines, we show that OSM treatment downregulates tight junction proteins, essential for maintaining epithelial apical-basal polarity. Additionally, we detected upregulation of key EMT transcription factors, including SNAIL, and a marked rearrangement of actin filaments, which collectively promote a mesenchymal-like phenotype in these cells. By employing nanoindentation assays with atomic force microscopy (AFM) to measure biomechanical properties, we establish for the first time, a link between OSM and increased membrane elasticity, a hallmark of motile cells in breast cancer. This research aims to understand the unique role OSM plays in HER2+ and HER2- breast cancer in promoting metastasis and will provide a mechanistic understanding of how this occurs and offer a molecular framework for identifying patients who may benefit from anti-OSM therapies that are in development.

#3457 CYLD regulates cGAS-STING pathway and metastasis in HNSCC.

Shiyang Deng[1], Satoru Shinriki[1], Yasuhito Tanaka[1], Hideki Nakayama[2]

[1]Molecular Laboratory Medicine, Kumamoto University, Kumamoto University, Kumamoto, Japan,[2]Oral & Maxillofacial Surgery, Kumamoto University, Kumamoto University, Kumamoto, Japan

Chromosomal instability, a hallmark of cancer, is related to cancer progression including metastasis and known to promote the activation of the cGAS-STING pathway. Cylindromatosis (CYLD), a well-known negative regulator of NF-kappaB, is down-regulated in aggressive head and neck squamous cell carcinoma (HNSCC), correlating with reduced apoptosis, enhanced invasiveness, and poor prognosis of patients with HNSCC. However, its role in metastasis, immune responses and interaction with the cGAS-STING pathway remain unclear. We found that CYLD protein was rapidly up-regulated and phosphorylated under Poly(dA:dT) or cGAMP stimulation. In addition, by using various cancer cell lines expressing shRNA targeting CYLD, we found that CYLD was essential for sustained phosphorylation of TBK1, a main STING downstream mediator. These results suggest that modulation of the CIN-related activation of cGAS-STING pathway and hence immune responses by down-regulation of CYLD regulates HNSCC progression. Further studies focusing on possible relationship between the NF-kappaB and cGAS-STING pathways would contribute to better understanding of the molecular mechanisms underlying CIN-related metastasis. Furthermore, our preliminary data revealed that CYLD knockdown significantly enhanced metastasis of murine HNSCC cells in immunocompetent mice.

#3458 Antagonism between tumors and lymphocytes drives coevolutionary processes throughout metastasis.

Cort B. Breuer, Marcos Labrado, Nathan E. Reticker-Flynn

Otolaryngology - Head & Neck Surgery, Stanford University School of Medicine, Stanford, CA

The development of metastatic disease is a conserved feature across most solid organ malignancies and accounts for the majority of cancer-related deaths. While adaptations within the local immune microenvironment of primary tumors are well-appreciated hallmarks of tumor progression, there remains much unknown about the specific impact of metastasis on both local and systemic immune responses. Here, we present an analysis of changes in the adaptive immune response to tumors during metastasis and clonal selection. Our group previously developed a syngeneic melanoma mouse model of spontaneous lymph node metastasis, performing nine generations of in vivo serial passaging to select for metastatic phenotypes. As previous work suggests, we find that tumors undergo clonal selection during metastasis using lentivirus integration mapping to measure clonality. Even amidst reduced genomic diversity, we find that metastases exhibit comparable or increased transcriptomic diversity when compared to primary tumors. In light of this discordance between genomic and transcriptomic diversity, we ask whether the immune response, especially T cells, exhibit similar dynamics. Performing both bulk and single cell T cell receptor (TCR) sequencing, we find that T cell clonality mirrors the reduced clonal diversity of metastatic tumors both in the total T cell compartment and specifically in regulatory T cells. Through paired investigation of evolution of tumors and the responding immune cells, we develop a model of tumor-immune coevolution in which reduced tumor clonality selects for narrower T cell repertoires; yet, diverse tumor transcriptional profiles support the differentiation of a variety of T cell subsets that are advantageous for tumors including regulatory T cells and exhausted CD8+ T cells. We further perturb this dynamic by evolving new metastatic cell lines in the absence of T cell pressure, finding unique evolution of metastatic pathways when the immune system is altered, impairing metastasis when reimplanted into immunocompetent mice. This model of tumor-immune coevolution establishes the importance of ongoing, dynamic communication between the host and tumors throughout metastatic progression.

IMMUNOLOGY: Local Treatments, Novel Tools, and Delivery Systems to Manipulate Tumor Immunity
Poster Session

#3462 Novel delivery platform for temporally-controlled release of immunocytokines and immune checkpoint inhibitors to enhance cancer therapy.

Pilar O'Neal[1], Julia Lu[2], Yicheng Zhang[2], Marek Kovar[3], Hai-Quan Mao[2], Jamie Spangler[1]

[1]Biomedical Engineering, Johns Hopkins University, Baltimore, MD,[2]Department of Material Science and Engineering, Johns Hopkins University, Baltimore, MD,[3]Laboratory of Tumor Immunology, Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic

Interleukin-2 (IL-2) is a multifunctional cytokine that plays a critical role in the differentiation, proliferation, activation, and survival of immune cells and has been used therapeutically for over 30 years as a cancer treatment. Unfortunately, the cytokine's narrow therapeutic window and its short circulation half-life have severely limited its clinical application. Strategies designed to bias the activities of IL-2 represent emergent and promising avenues in the treatment of cancer, as they afford the opportunity to selectively target and stimulate certain immune cell subtypes. Recently, our lab has developed one such therapy, termed an immunocytokine (IC), which fuses IL-2 to an anti-IL-2 antibody that biases the cytokine towards immunostimulatory activities while also extending its persistence in the bloodstream. Additionally, we further established that the IC was able to synergize with immune checkpoint inhibitors (ICIs) to effectively clear tumors through expansion of CD8+ T cells and stimulation of their effector functions. Interestingly, it was observed that the therapeutic effects of this combination treatment were highly sensitive to dosing regimen; specifically, robust antitumor activity was observed when IC was delivered after ICI, but not when the order was reversed. Building on this observation of time-dependent anti-cancer activity, here we expand upon this work through the development of a biomaterial system that will enable further exploration of the kinetics surrounding the combinatorial delivery landscape using IC and ICIs. Our biomaterial system involves formulation of nanoparticles that encapsulate IC and ICIs using a flash nano complexation and flash nanoprecipitation system. These particles are further enclosed within a hydrogel system for protection and sustained release within the tumor environment, controlling both the temporal and spatial distribution of the encapsulated immunotherapies. We have successfully designed and characterized the delivery platform and established time-dependent release of the therapeutic cargo. In vivo immune stimulation and tumor clearance studies are ongoing to further evaluate our approach. Overall, our work introduces an innovative and highly versatile bioengineering approach that can be tuned to optimize immunotherapy strategies in a wide range of cancer types.

#3463 A novel nanobody based IL-18 surrogate cytokine agonist, used alone or combined with immune checkpoint inhibitors, demonstrated synergistic tumor suppression.

Yakun Wan[1], Min Zhu[1], Junwei Gai[1], Yanyang Nan[2], Yuping Huang[1], Xuyao Zhang[2]

[1]Novamab Biopharmaceuticals Co,. Ltd, Shanghai, China,[2]School of Pharmacy, Fudan University, Shanghai, China

IL-18 is a cytokine belonging to IL-1 family regulating both innate and acquired immune responses, acting on innate lymphocytes and antigen-experienced T cells to produce interferon gamma (IFNγ). However, clinical efficacy of IL-18 monotherapy in cancer patients has been proved to be slight, which is mainly caused by the upregulation of IL-18 binding protein (IL-18BP), a decoy protein that binds IL-18 with extremely high affinity, produced in the tumor microenvironment. In this study, we have developed an innovative approach to activate IL-18 receptors without the need for the cytokine itself. We achieved this by employing human IL-18Ra and human IL-18Rb-targeting nanobodies, which mimic the natural IL-18 cytokine. This strategy leads to the formation of functional receptor dimers and initiates the subsequent signaling cascade. IL-18Ra and IL-18Rb nanobodies with high affinity were selected out from the immune libraries constructed from PBMCs isolated from camels immunized with IL-18Ra and IL-18Rb respectively. And then IL-18Ra/IL-18Rb bispecific nanobodies were constructed and evaluated for their induction of IFNγ release in primary peripheral blood mononuclear cells (PBMCs). Modality with best IFNγ stimulation was chosen for further development. And the humanized most potent IL-18Ra/IL-18Rb nanobody was named LQ058. Compared to wild type IL-18, LQ058 demonstrated superior IFNγ stimulation from PBMCs. And $CD8^+$ T cells RNA-sequencing evaluation demonstrated that LQ058 is IL-18BP resistant. In human PK59 and H23 tumor xenograft models, LQ058 illustrated better tumor growth suppression compared with wild type IL-18. Meanwhile, LQ058 showed synergistic tumor inhibition when administrated together with anti-PD-1 or anti-CTLA4 therapies. Besides, LQ058 is designed to have extended half-life which would improve medication convenience. Overall, these positive characteristics of LQ058 make it a promising therapy possible to overcome the limitations of current IL-18 therapies for malignant tumor treatment.

#3464 Advancing antibody therapeutics: a 3D *in vitro* model for penetration assessment of immunoglobulins in human tumor spheroids.

Sue Fontaine Grepper, Maja Nedic, Charlotte Veser, Michal Rudnik, Matthias Zbinden, Irina Agarkova, Madhu Nag, Laure-Anne Ligeon

InSphero AG, Schlieren, Switzerland

The advancements in biotechnology and the successful application of monoclonal antibody therapeutics have incited the development of innovative therapeutic antibody modalities, including bi-or tri-specific antibodies, antibody fragments (e.g. Fab), and other formats (e.g. DARPin). These have transformed cancer treatments; however, monoclonal antibodies often demonstrate limited efficacy in cancer therapy due to poor tumor penetration, leaving regions of untargeted and untreated tumor areas that can escape therapy. Addressing this limitation requires the development of advanced *in vitro* culture models that accurately replicate tumor-tumor microenvironment (TME) interactions. To bridge the gap between traditional *2D in vitro* cultures and animal models, 3D human tumor spheroids offer a promising solution. In this context, InSphero has developed an *in vitro* 3D Antibody Penetration Assay. We demonstrate that 3D InSight™ Human Tumor Microtissues closely mimic the complexity and heterogeneity of the TME, while maintaining uniform composition and size across spheroids, providing reliable and reproducible models to enhance drug discovery. Using 3D InSight™ human tumor spheroids, we evaluated the efficacy, distribution, and penetration depth of a therapeutic antibody and an antibody-drug conjugate (ADC). Results showed that both modalities successfully penetrated the 3D InSight™ lung cancer tumor (A549) model. After 30 minutes of treatment, antibodies were detected at the periphery of the spheroids, while longer treatment windows resulted in progressively deeper penetration. This assay offers a robust and reliable platform for assessing the distribution and penetration of antibody therapeutics, biologics, ADCs, and other agents in solid tumor models, leveraging advanced *in vitro* 3D human tumor models to improve drug discovery and therapeutic development.

#3465 T cells on a crossroad between cytosolic and mitochondrial BCAA transamination in the lymphoma microenvironment.

Tanner Wetzel[1], Christie Adam[1], Sheila Erfan[1], Lucas Figueroa[1], Lindsey Croll[1], Anthony Diehl[1], Taha Khan[1], Alexander Martin[2], Maxwell Swain[1], Michael Boyer[1], **Elitsa Ananieva**[1]

[1]Des Moines University, West Des Moines, IA,[2]Flushing Hospital Medical Center, Flushing, NY

Neoplastic cells of the lymphoma microenvironment demonstrate substantial plasticity allowing their escape from immune surveillance and subsequent rapid growth. A distinct feature of this plasticity is the release of immunosuppressive signals that lead to T cell exhaustion. Metabolic enzymes, including the cytosolic (BCATc) and mitochondrial (BCATm) branched chain aminotransferases may contribute to the immunosuppressive repertoire of lymphoma cells as these enzymes break down and thus limit the availability of the branched chain amino acids (BCAAs) for helper and cytotoxic T cells. Because of the significant impact BCATc and BCATm have on the biosynthetic and energy demands of T cells, we hypothesized that T cell differentiation and effector function depend on a switch between cytosolic and mitochondrial BCAA transamination. Mice with a single or a combined deletion of BCATc and BCATm in T cells were subcutaneously injected with $2.5x10^5$ murine EL4-OVA lymphoma cells [n=8-9/group] for 15 days and tumor development and growth were monitored. In addition, splenic and lymphatic T cells were isolated, activated with anti-CD3/CD28, and differentiated into $CD4^+$ T cell subsets or $CD8^+$ T cells, followed by RT-PCR, Flow Cytometry and ELISA to measure lineage-specific transcription factors and effector cytokine secretion. Results showed that T cells deficient in BCATm afforded mice with a local (as seen during an in vitro cell killing assay) but not with a systemic resistance to EL4-OVA lymphoma. In contrast, T cells with a single (BCATc), or a combined (BCATc-BCATm) deletion, led to ~ 50 % reduction in tumor growth over time. This difference in response was further observed at the level of T cell subset differentiation, where a loss of BCATc caused an increased secretion of IFNγ (53 %) and upregulation of Tbet expression by 31 % in Th1 cells. In contrast, BCATm had no impact on Th1, however, in Th2 cells, a loss of BCATm expression resulted in a 50 % decrease in the secretion of IL13. A loss of both isoenzymes from Treg cells, however, favored a switch to Th1-like phenotype where Treg cells demonstrated 2.3- and 1.7- fold increases in the expression of Tbet in the absence of BCATc or BCATm, respectively. Lastly, deletion of BCATc positively affected $CD8^+$ T cell activation, while deletion of BCATm stimulated $CD8^+$ T cell expansion. The observed effects were directly bound to the transaminase activity of the BCAT enzymes as seen by the reverse effects of the leucine structural antagonist, n-acetyl-leucine amide [NALA], on T cell differentiation and function. These findings suggest that T cells switch between cytosolic and mitochondrial transamination of BCAAs during their activation and lineage commitment, a behavior that may likely impact the interactions of T cells with cancer cells in the lymphoma microenvironment.

#3466 Bubble therapy: Histotripsy ablation of pancreatic tumors in an *in vivo* murine model affects the tumor microenvironment and increases systemic anti-tumor immunity.

Tamalika Paul, Victor Lopez, Cora Youngs, Carley Elliott, K.M Imran, Tyler Moore, Manali Powar, Benjamin Tintera, Eli Vlaisavljevich, Irving Coy Allen

Virginia Tech, Blacksburg, VA

Pancreatic cancer is one of the leading causes of cancer-related deaths, highlighting the urgent need for innovative treatment strategies. Histotripsy is a non-invasive, non-ionizing, non-thermal, image-guided focused ultrasound ablation treatment method that uses high-pressure pulses to create acoustic cavitation, a “bubble cloud,” at the target. The rapid expansion and collapse of this bubble cloud ablates the tumor into an acellular homogenate. In this study, we evaluated the hypothesis that histotripsy ablation of pancreatic tumors reduces tumor burden, activates systemic immune responses, and induces an abscopal-like effect. Our *in-vivo* study is a contralateral study conducted in C57BL/6J mice, employing a specialized histotripsy setup designed for precision. This setup featured a customized 1 MHz, 8-element histotripsy transducer engineered with a tailored focal point geometry. We targeted partial ablation around 50-60% to activate the immune responses and convert the “cold” pancreatic tumor microenvironment to “hot” tumor microenvironment. Treatment parameters included a peak negative pressure (PNP) of 250 Hz, a pulse repetition frequency (PRF) of 250 Hz, and 250 pulses per focal point. Mice were harvested in 5 groups/timepoints (2 hours, 24 hours, 7 days, 14 days and 21 days), with biological assessments generally focused on ablation zone, structural effects of histotripsy, chemokines and cytokines released and immune cell populations. The study’s significance lies in understanding how histotripsy influences immune responses, particularly T cell infiltration and proliferation, from the treated tumor site to the untreated contralateral tumor site. Our data demonstrated that histotripsy effectively reduced tumor volume, with significant reductions in tumor diameter observed in both treated and contralateral untreated tumors compared to control groups. Flow cytometry analysis revealed significant changes in immune cell populations across treated and control groups. Transcriptomic analyses identified differentially expressed genes and pathways associated with systemic anti-tumor immune responses, while mass spectrometry highlighted antigens presented by antigen-presenting cells. Overall, the results indicated that histotripsy promotes a localized immune response, leading to immune system activation and substantial alterations in the tumor microenvironment. These systemic immunological changes present potential targets for adjuvant therapies, underscoring the promise of histotripsy as a therapeutic modality in pancreatic cancer treatment.

#3467 Delivery of CpG and GM-CSF in a novel silica-based scaffold leads to differentiation of AML blasts and T cell-dependent immunity in syngeneic AML tumor models.

Fernanda Langellotto, Jessica McDonough, Benjamin Seiler, Brena Barra, Edward Doherty, Robert Pierce

Attivare Therapeutics Inc., Natick, MA

Background: Acute Myeloid Leukemia (AML) has poor prognosis, however, differentiation therapy results in a high cure rate for a subset of patients with acute promyelocytic leukemia. Prior preclinical studies using a cryogel-based “antigen free” system demonstrated complete remission in AML models and a vaccine-like response. We set out to recapitulate these effects using a silica-based, biodegradable scaffold (mesoporous silica rod, MSRs) in order to capitalize on their ease of manufacturing and injectability. Building on this prior work, here we show that MSRs loaded with GM-CSF and CpG (ATT-01) can similarly induce differentiation of AML blasts *in vitro*, a significant vaccine-like T cell response, and durable remission in murine models of AML.

Methods: Syngeneic mouse models [C1498, C1498-eGFP, and WEHI-3] were conducted by injecting tumor cells into the mouse tail vein. Tumors were treated with chemotherapy (ara-C, doxorubicin) on days 7-12 and then mice were treated with a subcutaneous injection of ATT-01 on 2 flanks on day 14. For takedown studies, flow cytometry was conducted on mouse organs on day 7 and 14 post-ATT-01 treatment. In a cellular depletion experiment, mice were treated with antibodies to decrease CD8+T-cells, CD4+T-cells and NK cells prior to running the C1498 therapeutic model. *In vitro* experiments were conducted by co-culturing MSRs with THP-1 cells and tumor cells, and APC functionality of THP1 cells (no PMA) were assayed using flow cytometric phenotyping.

Results: Chemotherapy plus ATT-01 treatment resulted in complete remissions [CRs] in C1498 and WEHI-3 models, compared to chemotherapy or GM-CSF and CpG injection alone, which resulted in no CRs. The cellular depletion study demonstrated that CD8+ T cells were required for the therapeutic effect. The takedown study demonstrated that ATT-01 therapy resulted in increased CD8+ T cells in the bone marrow at day 7, compared to controls. *In vitro*, MSR and ATT-01 caused NETosis in THP-1 cells. *In vitro* studies demonstrated that Murine AML cell lines cultured with ATT-01 and MSR resulted in tumor cell death for a portion of cells. Surprisingly, a subset of murine AML cell lines expressed immune cell markers after culture with ATT-01 or MSR. In addition, THP-1 cells (human leukemic cell line, no PMA) cultured with MSR and ATT-01 differentiated into immune phenotypes.

Conclusions: Treatment of murine AML with chemotherapy and ATT-01 resulted in durable remissions. We propose that generating peripheral inflammation in AML can lead to differentiation of AML blasts into APCs and presentation of AML antigens, leading to immune-mediated tumor control. Future experiments will further elucidate the differentiation effect of ATT-01 *in vivo*.

#3468 Intratumoral interleukin-7 delivery via human iPS cell-derived neural stem cells for glioblastoma immunotherapy.

Yi Fu, **Irfaan Karim**, Hernando Lopez-Bertoni, Mingyao Ying

Neuro-Oncology, Kennedy Krieger Institute, Baltimore, MD

Glioblastoma (GBM) is the most aggressive primary brain tumor characterized by severe immunosuppression and a poor response to conventional treatments. The immunologically "cold" tumor microenvironment of GBM makes immunotherapy still challenging. Here, we studied the immunostimulatory cytokine interleukin-7 (IL7) for GBM immunotherapy. We first showed that IL7 delivery to GBM tumors effectively induced an immune-active tumor microenvironment and anti-tumor immunity. Next, we developed a therapeutic strategy based on intratumoral IL7 delivery via human iPS cell-derived neural stem cells (iNSCs). This iNSC-IL7 treatment in two mouse syngeneic GBM models (CT2A and GL261) led to robust tumor regression with a complete response rate (CR) of 40%. It also significantly extended the survival of animals with partial responses. This treatment induced an immune-active microenvironment, as evidenced by increasing cytotoxic CD8+ T cell infiltration and activity and established anti-tumor immunity. Following RNA-sequencing analysis, we defined transcriptomic changes of tumors in response to iNSC-IL7 treatment, including various tumor microenvironment modulators and the upregulation of immune checkpoint pathways (e.g., PD-1 and CTLA4). This discovery guided the combination regimens using iNSC-IL7 and immune checkpoint inhibitors (anti-PD1 or anti-CTLA4 monoclonal antibody). These two combination regimens led to CR of 80% (iNSC-IL7 + anti-PD1) and 60% (iNSC-IL7 + anti-CTLA4), more effective than monotherapies (iNSC-IL7: 40%; anti-PD1: 40%; anti-CTLA4: 30%). Our results demonstrate that IL7 is a potent immunostimulatory cytokine for GBM immunotherapy. They support iNSC-IL7 treatment for future clinical translation as an autologous cellular immunotherapy for GBM and likely other solid tumors based on human iPS cell-derived cell progenies. The transcriptomic analysis dataset will facilitate the future development of an optimal iNSC-IL7 therapy through combination strategies with other immunotherapy regimens, such as immune checkpoint inhibitors.

#3470 Intratumoral cytokine delivery combined with checkpoint blockade improves immunotherapy responses in immune-infiltrated oral cancers.

A. H. Molina[1], G. M. Sunga[1], N. Dharmaraj[1], N. I. Hussein[1], A. G. Sikora[2], S. Young[1];

[1]The University of Texas Health Science Center at Houston, Houston, TX, [2]The University of Texas M.D. Anderson Cancer Center, Houston, TX

Cytokine therapies improve antigen priming, increase effector immune cells in the tumor immune microenvironment (TIME), and enhance cytolytic activity. There is strong interest in the interleukin (IL) family to activate effector immune cells in the TIME. Interleukin-12 (IL-12) is known for its pro-inflammatory effects, T-cell differentiation properties, regulation of natural killer (NK) cell responses, and production of interferon-gamma (IFNγ). The TIME in head and neck squamous cell carcinoma (HNSCC) impairs tumor-infiltrating lymphocyte function, decreases the production of effector cytokines, and contains suppressive immunocytes. Immune checkpoint inhibitors (ICIs) have shown promise in several solid cancers with similar mutation burden and immune infiltration as HNSCC. However, most HNSCC patients do not respond to treatment with single-agent immunotherapy, in large part due to the immunosuppressive TIME. Therefore developing strategies to mitigate the suppressive tumor immune microenvironment and elicit a robust anti-tumor immune response through combinatorial treatments is key. Herein, we combined anti-programmed cell death protein-1 (α-PD-1) with IL-12 to reverse exhausted phenotypes and activate effector immune cells in the HNSCC TIME. We hypothesize that the combination of IL-12 and α-PD-1 therapy will stimulate anti-tumor effector immunocytes to target immune-infiltrated and immune-excluded TIME in the preclinical oral cancer models, MOC1 and MOC2. Continuous treatments of intratumoral IL-12 and systemic α-PD-1 either as monotherapies or in combination were administered to MOC1 tumor-burdened mice. We observed tumor regression in the MOC1 tumor-burdened mice with IL-12 + α-PD-1 combinatorial treatments, IL-12, and α-PD-1 monotherapies. Mice that rejected the primary tumor challenge were rechallenged with MOC1 cells. We observed complete responses in IL-12 + α-PD-1 treatment groups and tumor regression in IL-12 monotherapy groups. Additionally, in the more aggressive HNSCC preclinical model, MOC2, IL-12 treatments led to prolonged survival. Overall, our results demonstrate the effectiveness of IL-12 + α-PD-1 combinatorial treatments in an immune-infiltrated HNSCC preclinical model. Future studies will include the addition of small molecules, such as Stimulator of Interferon Genes (STING) agonists, to enhance antigen presentation and activate anti-tumor effector cells to combat an aggressive TIME.

#3471 CD47 blockade-based local immunotherapy for endometrial cancer.

Kerem Yucebas[1], Sooah Ko[1], Omair Khan[2], Irving Weissman[2], **Shiying Jin**[1]

[1]University of Missouri - Columbia, Columbia, MO,[2]Stanford University, Stanford, CA

Endometrial cancer (EC) incidence and mortality have been persistently increasing, indicating the limitations of current therapeutic measures. We have developed an immunotherapy for EC targeting the immune checkpoint molecule CD47. CD47 is a transmembrane protein that serves as a ligand for receptor signal regulatory protein-α (SIRPα) expressed on macrophages. When CD47 on tumor cells and SIRPα on macrophages interact, phagocytosis of malignant cells is inhibited. We found that macrophage phagocytosis of human EC cells is enhanced by an anti-CD47 monoclonal antibody (mAb). Our preclinical studies revealed the anti-CD47 mAb effectively reduces tumor burden without displaying overt signs of toxicity when administered through transvaginal local treatment in *PR-cre*$^{+/-}$*;Pten*$^{flox/flox}$ female mice with endometrial carcinoma. Thus, our findings provide rationale for the clinical application of anti-CD47 local immunotherapy to benefit women at risk for endometrial cancer development.

#3472 STX-003: cancer immunotherapy with systemic delivery of mRNA utilizing programmable genetic circuits for precise regulation of IL-12 expression and reduced toxicity.

Gautam N. Shenoy, Wen Allen Tseng, Emily A. Clinton, Ryan A. Patenaude, Wuhbet Abraham, Zhijun Su, Madhuri Dey, Justin H. Letendre, Maeve M. Pattie, John T. Sexton, Emily Tjon, Nandita S. Menon, Isma Zarin, Breanna Burgett, Aidan Coia, Akshay Mavani, Weiyu Zhao, Kevin Shi, Alexander C. Lemaire, Julian Quinones, Ryan T. Sowell, Jaspreet S. Khurana, Joseph Barberio, Prashant R. Nambiar, Jacob R. Becraft, Tasuku Kitada

Strand Therapeutics, Boston, MA

Introduction: Immunotherapies have revolutionized the treatment of solid tumors, which represent 90% of adult human cancers. Therapeutic delivery of IL-12, a potent immunostimulatory cytokine, is robustly effective in preclinical models. However, systemically delivered IL-12 is poorly tolerated potentially due to its off-target activity. To overcome this challenge, we developed an mRNA platform that utilizes programmable genetic circuits to regulate the expression of an encoded protein in response to microRNAs (miRNAs), enabling precise control of payload expression in the target tissues. Using this platform we developed STX-003, a systemically delivered self-replicating mRNA encoding IL-12 which bears programmable genetic circuitry to limit the expression of payload in off-target tissues while preserving expression in the tumor. Here, we tested the activity of the genetic circuitry as well as its effects on the efficacy and tolerability of systemically delivered IL-12 using preclinical models.

Methods: To reduce payload expression in (i.e., detarget) healthy tissues, we first identified unique miRNA signatures using in silico modeling and high-throughput in vitro screening. We then selected candidate circuits, characterized their effectiveness and formulated them in LNPs. Payload expression was tested in vitro using the human cancer cell lines and patient-derived breast cancer organoids. Proof-of-concept studies were conducted in non-human primates and naive mice (with a murine surrogate) to demonstrate successful detargeting of the off-target tissues and its impact on tolerability following systemic delivery. Efficacy was tested in the B16.F10 syngeneic mouse model of melanoma. Tolerability was assessed by monitoring body weights and clinical signs, while efficacy was tracked by measuring tumor volumes over time.

Results: STX-003 as well as the uncircuited control expressed the IL-12 payload when transfected into patient-derived tumor organoids and cancer cell lines. Intravenous injection of a murine surrogate of STX-003 improved tolerability and induced anti-tumor responses in a syngeneic mouse model. While STX-003 yielded similar levels of mIL-12 in tumors compared to an uncircuited control, markedly lower levels of mIL-12 were observed in off-target tissues. In healthy non-human primates, systemic administration of STX-003 demonstrated a substantial reduction in circulating IL-12 levels compared to a control without genetic circuitry.

Conclusion: These data demonstrate the potential of STX-003, our programmable RNA circuit platform, for expanding the therapeutic index of potent drugs that are limited by systemic toxicities associated with off-target expression.

#3473 iPS-derived MSC secreting IL7 and IL15 exert immune mediated anti-tumor activities.

Sandeep Singh[1], Andrea Bedoy[1], Dipmoy Nath[2], Kyung Hee Chang[1], Lauren B. Ostermann[1], Ivo Veletic[3], Christopher D. Pacheco[3], Po Yee Mak[1], Edward Ayoub[1], Muharrem Muftuoglu[1], Mahesh Basyal[1], Taeyun Kim[4], Raven Dance Hinkel[4], Kyle M. Garland[4], Matthew Angel[4], Sanjeev Luther[2], Christopher B. Rohde[4], **Michael Andreeff**[1]

[1]Section of Molecular Hematology & Therapy, Department of Leukemia, UT MD Anderson Cancer Center, Houston, TX,[2]Eterna Therapeutics, Inc., Cambridge, MA,[3]Leukemia, UT MD Anderson Cancer Center, Houston, TX,[4]Factor Bioscience, Inc., Cambridge, MA

We previously discovered that BM-derived Mesenchymal stromal cells (BM-MSCs) are migrating to the stroma of numerous cancers and their metastasis, forming TAFs and secrete proteins intra-tumorally. MSCs have not been used in cancer therapy because of their immune suppressive properties, limited replicative capacity, source dependent quality and controversial roles in cancer biology. Here, we report the characterization of novel MSCs (iMSCs), which were generated from adult skin fibroblast-derived induced pluripotent stem cells (iPSC). These iPSCs were generated via a unique, transient mRNA transfection technique and subsequently differentiated into iMSCs. Notably, iMSCs displayed contact inhibition and significantly enhanced proliferative capacity under both normoxic and hypoxic conditions, outperforming bone marrow-derived MSCs (BM-MSCs) while fully retaining trilineage differentiation potential. Comprehensive molecular profiling, including RNA sequencing, single-cell mass cytometry (CyTOF), and Luminex assays, revealed strong phenotypic and functional resemblance between iMSCs and BM-MSCs. Crucially, no sarcoma formation was detected in NSGS mice following intraperitoneal, subcutaneous, or intravenous administration of iMSCs, underscoring their safety profile. We further engineered a DNA cassette into these cells to enable IL-7 and IL-15 cytokine production, expressed as either individual molecules (P2A) or a single fused molecule (FUS). Both P2A and FUS cells demonstrated the capacity to drive T cell proliferation autonomously in co-culture. Cytokine secretion from these modified iMSCs enhanced tumor cell death in a triple co-culture system comprising iMSCs, the ovarian cancer cell line ID8, and human PBMCs. In a syngeneic mouse model of ovarian cancer (ID8 in C57BL/6 mice), administration of P2A or FUS MSCs resulted in reduced tumor growth and extended survival. Immunohistochemical and flow cytometric analyses revealed massive infiltration of T cells, macrophages, NK cells, and other immune cells into the tumor microenvironment (TME) in both FUS or P2A groups, but not in control, unmodified MSC, or PBS injected animals. Moreover, the TME in P2A- and FUS-treated mice showed enrichment in M1-type macrophages, with no detection of any of the T cell exhaustion markers tested, in contrast to the control groups (PBS or GFP-iMSC). In conclusion, our findings indicate that IL7-IL15-secreting iMSCs migrate into solid tumors, induce massive immune cell infiltration into the TME and enhance antitumor immunity in a syngeneic mouse model of cancer. These cytokine-producing iMSCs represent a promising therapeutic strategy for modulating the immune response against tumors and advancing anticancer immunotherapy by converting “cold” into “hot” tumor microenvironments.

#3474 Breast cancer control and increased TIL with intratumoral TLR2 agonist and IFNγ.

Ileana Soto Mauldin, Elaheh Najafianashrafi, Kelly T. Smith, Marya E. Dunlap-Brown, Chengli Shen

Surgery, University of Virginia, Charlottesville, VA

Background: High densities of tumor-infiltrating lymphocytes (TILs) in triple-negative breast cancer (TNBC) are associated with better outcomes and response to anti-PD-1 checkpoint blockade therapy. CXCL10 is a critical chemoattractant for activated T-cells and is lowly expressed in breast cancers, thus, approaches to increase CXCL10 may boost TIL, and enhance TNBC control. We have previously found that a TLR2 agonist (FSL-1) plus IFN-gamma enhances the production of CXCL10 by human melanoma cells. We hypothesized that intratumoral therapy with these agents would enhance CXCL10 production, TIL, and tumor control in TNBC.
Methods: Chemokine production and TNBC cell viability were evaluated in vitro after treatment of TNBC cell lines with PBS, FSL-1, IFNγ, or FSL-1+IFNγ. In vivo, changes in TIL, tumor outgrowth, and murine survival were evaluated after intratumoral therapy of E0771 tumors grown in the mammary fat pad of C57BL/6 mice.
Results: CXCL10 production by 12 human and 3 murine TNBC cell lines was evaluated by flow cytometry and ELISA assay. FSL-1+IFNγ stimulation synergistically enhanced CXCL10 production by human and murine TNBC cell lines when compared to IFNγ, FSL-1, or PBS alone ($p \leq 0.03$). Luminex assay revealed that CXCL10 was the most abundant chemokine produced by murine TNBC cells in response to FSL-1+IFNγ (1185 pg/ml), while human TNBC cells dominantly produced CXCL10 (36498 pg/ml) and CXCL9 (31480 pg/ml) and produced low levels of CXCL11, (341 pg/ml) CXCL12 (8 pg/ml), and CCL5 (391 pg/ml). Intratumoral therapy with FSL-1+IFNγ treatment increased overall survival when compared to those receiving PBS, FSL-1, or IFNγ alone (58 vs 34 days $P<0.0001$). FSL-1+IFNγ induced visible tumor necrosis and induced sustained full tumor regression in 2 of 11 mice evaluated for long-term survival. Gene signatures associated with T-cell infiltrates and T-cell activation were elevated in FSL-1+IFNγ treated tumors evaluated by Nanostring analysis. $CD8^+$ T-cell infiltrates, evaluated by multiparameter immunofluorescence histology (mIFH), were increased in FSL-1+IFNγ treated tumors (117 cells/mm^2) compared to those treated with PBS, FSL-1, IFNγ (52, 29, 33 cells/mm^2 respectively, $p \leq 0.02$). Additionally, in flow cytometric assays, FSL-1+IFNγ treated tumors had increased percentages of $CD8^+$ T-cells (27% $p \leq 0.001$) relative to control groups treated with PBS 12%, FSL-1 8%, and IFNγ 5%.
Conclusions: FSL-1+IFNγ treatment increases TIL, murine survival, and tumor growth control, and promotes the complete regression of a subset of tumors. Thus, further studies are warranted to identify the mechanisms that modulate response to this treatment to improve treatment efficacy and to evaluate whether FSL-1+IFNγ can drive immunogenic cell death of TNBC and impart a systemic benefit for tumor control.

#3475 KGX103, a conditionally active prodrug of PD-1-targeting IL-15 induces antitumor activity by preferentially activating PD-1+ intratumoral T cells in cis.

Jiangfeng Fan, Zhiqiang Bai, Jing Wang, Shumin Yang, Yucheng Luo, Jie Huang, Hui Chen, Haixiang Wu, Yi Wu, **Weidong Jiang**

Shanghai KangaBio Co., Ltd., Shanghai, China

Interleukin-2 (IL-2) and IL-15 have both similar and contrasting functional roles in the proliferation and regulation of lymphocytes. They share the β/γ heterodimeric receptors while having distinct α-receptors. IL-15 is thought to be superior to IL-2 for the treatment of cancer in the aspects of lower vascular endothelium associated toxicity, much weaker Treg-stimulating activity and stronger expanding NK and CD8+ T cells abilities. However, the application of IL-15 in tumor immunotherapy remains challenging due to short half-life and severe adverse events caused by excessive and systemic immune activation.

To address these challenges, we have developed KGX103, a PD-1-targeting non-α attenuated IL-15 prodrug fused with anti-HSA antibody. The IL-15 mutein is concealed by an inactivation domain linked with tumor-specific protease cleavable peptide, to prevent systemic immune activation. Once mask is cleaved in tumor microenvironment (TME), KGX103 could effectively stimulate intratumoral antigen-experienced CD8+ T cells with elevated PD-1 expression. With PD-1 targeting and tumor-specific activation, KGX103 could greatly potentiate tumor-specific T cell proliferation and function while ameliorating systemic immune activation. The pSTAT5 activation of either fresh or pre-activated peripheral blood mononuclear cells (PBMCs) were tested with KGX103 and unmasked KGX103. KGX103 showed almost no pSTAT5 activation signal in fresh PBMCs with low PD-1 expression and weak activation in pre-activated PBMCs with elevated PD-1 expression. Meanwhile, KGX103 showed significantly reduced potency of >3000-fold than the unmasked KGX103, which could be recovered after tumor-specific protease cleavage. Then we evaluated KGX103's *in vivo* antitumor efficacy in xenograft models using B-NDG β2m KO mice plus. Mice were subcutaneously implanted with a mixture of A375 tumor cells and hPBMCs. After grouping, mice were treated with vehicle or KGX103. Compared to the vehicle group, KGX103 demonstrated its antitumor efficacy and safety as evidenced by significant tumor growth inhibition while no obvious body weight change during the dosing period. The pilot toxic study to evaluate the safety and toxicokinetics of KGX103 is ongoing in *Cynomolgus* monkeys.

#3476 A single intratumoral injection of IL-12 bound to mesoporous silica rods generates effective anti-tumor immune responses and tumor growth control in multiple syngeneic tumor models.

Fernanda Langellotto, **Jessica McDonough**, Everton Mandley, Benjamin Seiler, Brena Barra, Edward Doherty, Robert Pierce

Attivare Therapeutics Inc., Natick, MA

Background: Interleukin-12 (IL-12) is a potent inflammatory cytokine, which demonstrated anti-tumor activity in both experimental model systems and in a number of clinical trials. Unfortunately, the development of IL-12 as a therapeutic is hampered by severe toxicity in the event of significant systemic exposure. Therefore, intralesional IL-12 therapy has been actively pursued for intratumoral therapy. When delivered into the TME, IL-12 increases intratumoral interferon-γ expression leading to conversion of myeloid cells from an immunosuppressive state into an immunogenic one, recruits $CXCR3^+$ NK and effector T cells, upregulates antigen presentation/processing machinery, and generates anti-tumor immunity. Mesoporous silica rods [MSRs] are currently in development as a modular localized therapeutic platform, due to their large payload capacity, slow-release kinetics, high biocompatibility and intrinsic proinflammatory nature. In this study, we evaluated the anti-tumor effect of MSR plus IL-12 [ATT-02] as an “in situ” (“antigen-less”) vaccine and characterized the immune effects of MSR alone and in in combo with IL-12.

Methods: Syngeneic mouse models [B16F10, B16-OVA, CT26, and EMT-6] were conducted by challenging the mice with tumor cells on one flank. Tumors were treated one time with ATT-02 i.t. [Murine IL-12, MSR] when tumors reached the target size. Abscopal models were conducted by injecting tumor cells [CT26, B16F10, and B16-OVA] on both flanks at day zero. The tumor on one flank was treated when it reached the target size. Lymph nodes and spleens were collected at day 7 and analyzed by flow cytometry. *In vitro* work was conducted by co-culturing MSRs with THP-1 cells that had been initially polarized to an “M2” like phenotype. Repolarization (“M2 to M1”) and APC functionality of THP-1 cells were assayed using flow cytometric phenotyping and proliferation of co-cultured antigen-specific T- cells.

Results: ATT-02 i.t. generated potent anti-tumor immunity compared to PBS and IL-12 alone in 3 single tumor syngeneic models and 3 abscopal syngeneic models. Complete responses [CRs] were observed in the CT26 and EMT-6 model after a single dose of ATT-02. Upon rechallenge no tumor growth was observed. In the B16-OVA group, SIINFEKL+ T cells and CD86+ macrophages were observed at a higher level than controls in the draining lymph node of ATT-02 treated mice. Interestingly, MSR i.t. alone resulted in delayed tumor progression the B16F10 model. *In vitro*, we demonstrated that MSR alone results in a repolarization of macrophages to an immunogenic phenotype capable of driving T cell activation. Addition of IL-12 enhances this effect.

Conclusions: I.t. injection of ATT-02 (MSR_IL-12) leads to significant anti-tumor immune responses, antigen-specific responses, and tumor growth inhibition in multiple syngeneic tumor models.

#3477 Direct in vivo activation and expansion of tumor infiltrating lymphocytes using multifunctional immunotherapy nanoengager.

X. Wen[1], L. Ruan[1], M. Wang[1], K. Au[1], L. Zhang[2], T. Zhang[1], A. Wang[1];

[1]The University of Texas Southwestern Medical Center, Dallas, TX, [2]Xuzhou Medical University, Xuzhou, China

INTRODUCTION: TILs (Tumor infiltrating lymphocytes) therapy has been shown to be highly effective in melanoma and holds great potential for improving cancer immunotherapy in other cancers. The key limitation for TIL therapy is the complex process to activate and expand TILs. Thus, there is a strong need for refining strategies to enable TIL immunotherapy. To address this challenge, we hypothesized that we can directly activate and expand TILs *in vivo*, thereby eliminating the complex production process. We took advantage of a multifunctional nanoparticle (NP) platform to engineer NPs with multiple co-stimulatory molecules. We aimed to show that our in *vivo* approach can effectively stimulate TILs.

METHODS: Co-stimulatory molecules functionalized NPs (TIL-NPs) were synthesized through reacting Azide-functionalized NP cores with DBCO-functionalized molecules. TIL-NPs were injected intratumorally to promote the expansion and activation of TILs *in vivo*. The MC38 colorectal tumor model was used for evaluating the effects of TIL-NPs on TILs activation. For the *in vitro* experiment, TILs from tumors of MC38-bearing mice were incubated with MC38 cells following various stimulation strategies. For the *in vivo* experiment, a bilateral tumor model was established, with only the right flank receiving TIL-NP injection.

RESULTS: We successfully engineered NPs containing anti-CD3, anti-41BB and IL-2. These NPs were 126.2 nm in size. Per miligram NP conjugated with approximately 50μg immune activating molecules. TILs from MC38 tumors were incubated with TIL-NPs. Free antibodies were used as control. We found TILs stimulated by TIL-NPs demonstrated greater cytotoxicity against MC38 cells compared to the groups treated with free antibodies or unstimulated TILs (Figure 1A). IFN-γ levels were elevated in all treatment groups, with the most significant increase observed in the TIL-NPs group (Figure 1B).To determine if TIL-NPs increased T-cell activation *in vivo*, we evaluate the antitumor effects of TIL-NPs in a bilateral tumor model. One tumor received TIL-NPs while the other tumor was used to assess systemic treatment responses. Intratumorally injected TIL-NPs were highly effective in eradicating the injected tumor when compared to that of control and free antibodies groups (Figure 2A-B). All of the tumors on the right flank/injected completely regressed, achieving a 100% complete response (CR) rate. Importantly, the TIL-NPs also provided a robust systemic response, providing 60% CRs in the contralateral flank tumors. Overall, TIL-NPs significantly prolonged survival of mice (Figure 2C). Functionalizing these molecules on NPs also induced clustering of their respective receptors and improved signaling.

CONCLUSION: TIL-NPs engineered with multiple co-stimulatory molecules can directly stimulate TILs *in vivo*, providing a systemic immunotherapy response against cancer.

#3478 Pre-clinical characterization of AZD0240, a CRISPR-engineered autologous TCR-T cell product targeting KRAS-G12D/HLA-A*11:01 that is multi-armored to increase epitope sensitivity.

Lianne Kok[1], Sander Eshuis[1], Xiangjun Kong[1], Jennifer Ma[2], Michelle Mojadidi[2], Paula Kroon[1], Vanessa Tubb[1], Ton Schumacher[3], Arianne Perez[1], Carsten Linnemann[1], Mark Cobbold[4], Jeroen van Heijst[1], Gavin Bendle[1]

[1]Neogene Therapeutics / AstraZeneca, Amsterdam, Netherlands,[2]Neogene Therapeutics / AstraZeneca, Santa Monica, CA,[3]Netherlands Cancer Institute, Amsterdam, Netherlands,[4]AstraZeneca, Boston, MA

KRAS is one of the most commonly mutated genes in human cancer, with the G12D mutation having the highest frequency, occurring in 33% and 28% of colorectal cancer (CRC) and pancreatic cancer (PDAC), respectively. KRAS G12D-containing peptides are presented by the common HLA allele HLA-A*11:01, making this cancer neoantigen a highly attractive target for the development of adoptive TCR-T cell therapy. However, the levels of cancer neoantigen presentation are typically low and combined with the immunosuppressive tumor microenvironment this compromises the activation and reactivity of TCR-engineered T cells, posing a major hurdle for these cells to eradicate tumor lesions. To establish a TCR-T cell therapy clinically intended for the treatment of solid cancers, including CRC and PDAC, we developed AZD0240, a CRISPR-engineered autologous TCR-T cell product targeting KRAS-G12D/HLA-A*11:01. To improve the reactivity of AZD0240 to the KRAS-G12D neoantigen, we developed a novel multi-armoring strategy, involving the combined disruption of a key negative regulator of T cell function and the introduction of a positive enhancement of TCR signaling. Employing this multi-armoring strategy, we observed enhanced cytokine production in responds to low levels of exogenously loaded KRAS-G12D peptide, as well as HLA-A*11:01$^+$ tumor cells that express the KRAS-G12D mutation. Moreover, assessment of the *in vitro* and *in vivo* proliferative and cytotoxic capacity revealed that the AZD0240 armoring strategy can profoundly increase both the proliferative capacity and the anti-tumor efficacy of TCR edited T cells. Notably, the armored AZD0240 T cells were unable to respond to the KRAS G12 wild-type epitope, and no signs of increased TCR cross- or allo-reactivity, or malignant transformation were detected, derisking the clinical use of this armoring strategy. Taken together, AZD0240 is a KRAS-G12D/HLA-A*11:01 targeted TCR-T cell therapy that employs a novel multi-armoring strategy and is currently in preparation for clinical testing, with encouraging signs of pre-clinical efficacy and safety.

#3479 iPSC-derived γδT with novel combinatorial KO demonstrated significant anti-tumor activity and extended longevity without cytokine support.

Jhang-Sian Yu, Ming-Chin Ko, Chia-Hung Lin, Yi-Han Dai, Ke-Hsun Hsu, I-Ting Chen, Ting-Yi Wu, Jin-Yi Lu, Ying-Tsen Tung, Edward Po-Fan Chu, Yuan-Yu Hsia, Yo-Chuen Lin, Chieh-Teng Cheng, Tzu-Chien Kuo, Yi-Ting Lai, Wei-Ting Chen, Darien Zhing Herr Chan, Lee-Yieng Lim, Chih-Lung Chen, Chun-Yu Lin, Lin-Yu Liao, Wan-Chen Tsai, Lien-Szu Wu, Ching-Ying Huang, De-Kuan Chang, Alex Shih-Min Huang

BeiGene, Ltd., Grand Cayman, Cayman Islands, United Kingdom

Induced pluripotent stem cell (iPSC)-derived cell treatment offers several competitive benefits, including consistency, homogeneity and unlimited dosage, in contrast to autologous and allogeneic donor-derived cell therapies. These attributes improve affordability and accessibility of medicines worldwide. Noteworthy, the effectiveness of T cell therapy is constrained by factors such as cell potency and persistence. Our research on iPSC-derived gamma-delta T (γδT) cells has identified a novel combinatorial KO to overcome these challenges. IL-2 administration has been approved by FDA for durable, complete, and apparently curative regressions in cancer patients. Previous studies demonstrated that IL-2 receptor beta (IL-2Rβ) chain-mediated signaling pathway is critical for killing potency and cell persistence. The suppressor of cytokine signaling (SOCS) family helps control cytokine signaling by blocking the JAK/STAT pathway. For example, cytokine-inducible SH2-containing protein (CISH) is a member of the SOCS protein which is essential for managing cytokine signaling, cell growth, differentiation, and immune system activities. Our findings indicated that iγδT cells deficient in both CISH and another SOCS family protein exhibited increased persistence and enhanced cytotoxic capabilities compared to cells with only one of the knockouts. Notably, we demonstrated the proliferative advantage of double SOCS protein KO by using a humanized mouse model. Cytokine withdrawal- and activation-induced cell death were key factors that diminish effector cell persistence post infusion. For prolonging cell survival, we identified a mediator of mitochondrial apoptotic pathway and found that this proapoptotic protein is necessary for extending the survival of iγδT cell post IL-15 withdrawal. In addition, FAS-FASL signaling was recently identified as auto-regulatory circuit for CAR-engineered lymphocyte persistence. We presented biochemical data and performed gain-of-function experiments to elucidate the mechanisms by which this proapoptotic protein contributes to the extended survival of effector cells. Furthermore, we demonstrated FAS deficiency protects functional enhanced iγδT cells from FASL-induced apoptosis and then enhances its durable killing ability against HepG2 cells. Altogether, we identified a novel combination of gene knockouts that extends longevity and profound anti-tumor efficacy without cytokine support. We achieved the goldilocks signaling balance could strengthen the efficacy and longevity of iγδT cell, while avoid the possible dysfunction, such as exhaustion, caused by introducing constitutively active cytokine receptors as many others have attempted. Building on this engineering platform, we could incorporate tumor-targeting receptors for treating different indications in the near future.

#3480 Gene electrotransfer in cancer: different immunomodulatory profiles and cell death pathways depend on different electric pulse protocols.

Mariangela De Robertis[1], Tim Bozic[2], Iva Santek[2], Flaviana Marzano[3], Bostjan Markelc[2], Domenico Alessandro Silvestris[1], Apollonia Tullo[3], Graziano Pesole[1], Maja Cemazar[2], Emanuela Signori[4]

[1]Department of Biosciences, Biotechnology and Environment, University of Bari Aldo Moro, Bari, Italy,[2]Department of Experimental Oncology, Institute of Oncology Ljubljana, Ljubljana, Slovenia,[3]Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies, Consiglio Nazionale delle Ricerche, Bari, Italy,[4]Laboratory of Molecular Pathology and Experimental Oncology, Institute of Translational Pharmacology, CNR, Roma, Italy

Gene electrotransfer (GET) is an excellent strategy for delivering therapeutic genes and vaccines into the target cell using electric pulses. In view of the insights gained during the last COVID-19 pandemic, it is widely acknowledged that genetic vaccines represent a safe and affordable option with considerable promise for addressing a range of diseases, including infectious diseases and cancer. Growing evidence from preclinical studies and clinical trials, which include both intramuscular and intratumoral immunotherapy, suggests that GET-based methods could be adapted for application in veterinary and clinical treatment protocols. At present, a key focus of research is on enhancing plasmid DNA (pDNA) electrotransfer techniques to reduce tissue damage and improve gene transfection efficiency. However, the molecular effects of electroporation (EP)-assisted DNA delivery in vivo are still not well understood. We assessed two GET protocols for the intratumoral administration of pDNA: Short and High Voltage (SHV) electric pulses or an adapted High Voltage - Low Voltage (HV-LV) pulses protocol, both of which are used for reversible electroporation. We investigated the ability of these two electric pulse conditions to trigger immune and tissue responses in murine CT26 colorectal tumors treated with intratumoral non-coding pDNA EP, instead of using an antigen or immunostimulatory agent. Immune cell infiltration in the tumors was assessed after both treatments, and a transcriptomic analysis through RNA sequencing was conducted to examine the gene expression changes induced by GET, with a particular focus on immune and cellular signaling pathways. We observed no significant differences between the two GET protocols in counteracting tumor development in the absence of immunomodulatory molecules. However, important variations in intratumoral macrophage, CD4+ T cells and even higher CD8+ T cells recruitment were reported between the two treatment methods. Transcriptomic analysis revealed that apoptosis is a predominant mode of cell death after GET by SHV pulses, whereas, GET by HV-LV pulses is associated with immunogenic necrotic pathways as well as a prominent activation of both innate and adaptive immune response. We showed that varying pulse parameters can trigger different immunomodulatory profiles within the tumor microenvironment, emphasizing the significance of choosing the right pulse settings in EP-based therapies. This analysis could provide valuable insights for selecting the most effective EP method or for developing new EP-based strategies to enhance the success of GET protocols used for vaccination or gene therapy.

#3481 Transarterial embolization for HCC: how embolic agents shape tumor immunity and systemic responses.

Andrea C. Cortes[1], Simone Anfossi[2], Kiyoyuki Minamiguchi[1], Rony Avritscher[1]

[1]Interventional Radiology, UT MD Anderson Cancer Center, Houston, TX,[2]Translational Molecular Pathology, UT MD Anderson Cancer Center, Houston, TX

Transarterial embolization (TAE) is an effective therapy for hepatocellular carcinoma (HCC). Effects of the treatment on tumor immune microenvironment are poorly understood. Different TAE agents induce varying degrees of tumor and peritumoral hypoxia and nutrient-depletion. The purpose of this study is to investigate the immunological effects of the most commonly used embolic agents. Using a preclinical rat HCC model, tumors treated with oil or microspheres were compared to untreated controls. Tumor and serum samples collected at 7 and 14 days post-treatment were analyzed by flow cytometry, cytokine assays, and gene expression profiling using nanoString CodeSet. Ethiodized oil significantly altered the TME, reducing exhausted CD8+Tcells and PD-1+regulatory T cells while increasing CD45+CD3+immune cell infiltration ($P \leq 0.05$). Microsphere-treated tumors showed less pronounced effects. Serum cytokine analysis revealed a shift from Th2- to Th1-dominated immunity in the Ethiodized oil group, with elevated Th1 cytokines (IL-12,IL-18,IFNγ) and reduced Th2 cytokines (IL-4,IL-5,IL-10) at 14 days ($P \leq 0.05$). Both embolic agents reduced pro-inflammatory cytokines (IL-1α,IL-1β, TNFα,G-CSF), indicating a dampened systemic inflammatory response. Gene expression analysis showed significant downregulation of Th2-associated pathways, in tumors treated with Ethiodized oil. This treatment promoted a Th1-dominant immune response, contrasting with the Th2-skewed signaling seen in microsphere-treated tumors. Pathway analyses further demonstrated a reduced activity of Th2-associated chemokine receptors and cytokine pathways in Ethiodized oil-treated tumors compared to microspheres, highlighting distinct immunological modulations driven by embolic agent choice. The findings demonstrate how embolic agents impact the tumor microenvironment and systemic immunity. Ethiodized oil exhibits superior immunomodulatory effects, promoting a Th1 phenotype and reducing pro-tumoral factors. This information is critical for devising new combinatorial immunotherapy strategies in HCC that consider the distinct effects of different embolic agents.

#3482 Enhanced targeting of HPV positive cancers using gamma delta 1 T cells armored with an HPV E7-specific TCR: a novel approach to overcome HLA downregulation.

Andres Mosquera[1], Diego F. Chamorro[1], Paul Shafer[2], Lauren K. Somes[1], Emily Madaras[1], Valentina Hoyos[1]

[1]Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX,[2]Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, TX

T-cell receptor (TCR) engineered T cells have emerged as a promising cancer therapeutic. While the clinical trial using ab T cells targeting the HPV E7 protein showed promise, it also highlighted a concerning issue: tumor escape due to the acquisition of defects in peptide-HLA presentation. As an alternative cellular platform, Gamma Delta One T cells (DOT) offer several advantages over alpha/beta T cells. These include their tissue-homing abilities, their use as allogeneic cells without GVHD risk, and their potential to target cells through both TCR and NK-like receptors (which can kill HLA Class-I deficient cells). *The overall purpose of this study is to establish DOTs as an effective platform for TCR-T therapy targeting solid tumors, demonstrating enhanced therapeutic efficacy in overcoming tumor escape through mutations in the antigen-processing machinery (APM).* We developed a novel protocol to isolate and expand DOTs from peripheral blood using a Delta 1 TCR-specific antibody and EasySep positive selection. By day 4, DOTs reached 37.3% (SD ± 10.3%) enrichment, increasing to 91.85% (SD ± 2.15%) by the end of culture. From transduction day, cells expanded over 100-fold by day 15, demonstrating scalability for therapeutic use. Efficiently transduced with an E7-specific TCR engineered to minimize mispairing (murine constant alpha and beta chains), we achieved transduction levels comparable to alpha/beta T cells. DOTs expressed high levels of NK-like receptors, including NKG2D (94.85% SD ± 0.55%), DNAM-1 (99.1% SD ± 0.3%), and NKp30 (76% SD ± 11%), crucial for targeting HLA-negative tumors. Using HPV+ E7+ SCC90 and CaSki cell lines with varying NKG2D ligand expression, we performed CRISPR/Cas9-mediated B2M knockout to generate HLA class I-deficient variants. Cytotoxicity was evaluated using in vitro chromium release (short-term) and IncuCyte (long-term) assays, where DOTs consistently outperformed alpha/beta T cells in killing HLA-heterogeneous tumors. DOT TCR-T cells exhibited comparable cytotoxicity to alpha/beta TCR-T cells against HLA-A*02:01+ E7+ targets while excelling against HLA-negative variants. These findings establish DOTs as a robust platform for TCR-engineered therapies, capable of overcoming tumor immune evasion across diverse HLA settings. We are currently validating these results in vivo using murine models. In summary, DOTs offer a promising platform for TCR-engineered therapies, excelling in targeting HLA-heterogeneous tumor populations through their dual recognition mechanisms. This work highlights their potential for developing off-the-shelf TCR-T therapies to overcome HLA downregulation and tumor heterogeneity in solid tumors.

#3483 MyT^{TM}platform-identified PRAME TCRs for T cell therapy demonstrate superior efficacy and best-in-class potential compared to clinical benchmarks.

Lia Walcher, Lena Immisch, Mira Heichenko, Alexei Leliavski, Robert Hoeltzel, Sharesta Khoenkhoen, Niklas Fellmer, Elisa Kieback, Lorenz Knackstedt, **Lucia Poncette**

T-Knife GmbH, Berlin, Germany

Introduction: PRAME is a clinically validated target for T cell receptor-modified T cell (TCR-T) therapy with high prevalence in multiple solid tumor indications. T-knife's MyT^{TM} platform generates best-in-class, high-affinity and high-specificity PRAME-directed TCRs for use in next-generation TCR-T therapy.

Methods: We identified PRAME-directed TCRs using the MyT platform, a TCR discovery engine based on transgenic mice expressing the human TCR repertoire with the ability to overcome central tolerance. Reactive TCR candidates were identified by rapid throughput screening using an indicator cell line. Selected TCR candidates were subsequently expressed retrovirally in primary T cells in combination with a CD8αβ coreceptor and characterized for peptide dose-response, cytokine release and cytotoxicity. We performed in-depth TCR safety profiling by rapid alloreactivity screening of single-HLA-transfected cells and by deep mutational cross-reactivity scans (X-scans). Finally, we supercharged TCR-modified T cells by co-expression with an activating CD8 coreceptor with built-in co-stimulation and a FAS-CD40/OX40 switch receptor using CRISPR-Cas9 gene editing combined with knockout of the endogenous TCR.

Results: PRAME was highly immunogenic leading to a 100% response rate in mice after immunization. We identified 85 TCRs reactive to the immunodominant HLA-A*02:01 PRAME epitope SLLQHLIGL. Peptide dose-response assays as well as co-cultures with a panel of PRAME-expressing cancer cell lines from a broad range of tumor indications demonstrated high avidity, high cytokine secretion and high cytotoxicity of T cells expressing MyT TCRs. Three lead TCR candidates were selected, which showed no alloreactivity against 149 tested HLA-subtypes covering 98% of class-I HLA alleles. Furthermore, testing over 300 putative off-target peptides predicted *in silico* or experimentally in X-scans, revealed that MyT TCRs show no off-target recognition. Importantly, benchmarking showed that the selected lead TCR candidate displays higher *in-vitro* efficacy compared to publicly disclosed clinical-stage TCRs, which was particularly evident at low E:T ratios and retained during multiple rounds of tumor cell restimulation. Finally, as part of the development of a Supercharged PRAME TCR-T product, TCR expression and functionality was further enhanced, by dual T cell armoring and using gene editing combined with endogenous TCR knockout, without impacting safety.

Conclusions: Our data demonstrate that the MyT platform delivers best-in-class, high-affinity/high-specificity PRAME TCRs. Administration of Supercharged PRAME-TCR-T cells incorporating these MyT TCRs, a single-chain co-stimulatory CD8 coreceptor and a switch receptor in a non-viral process, provides potential to induce deep and durable responses in hard-to-treat solid tumor indications.

#3484 Lipid nanoparticle-mediated *in vitro* transfection of microbubble-activated T cells.

Seeta Nyayapathy[1], Paige Gronevelt[1], Erin Holcomb[2], Julie M. Rumble[1]

[1]Cayman Chemical Company, Ann Arbor, MI,[2]Medical School, University of Michigan, Ann Arbor, MI

Immunotherapy of cancer has revolutionized approaches to controlling and curing cancer, with adoptive transfer of chimeric antigen receptor T cells (CAR-T cells) representing a potentially curative treatment for previously unresponsive tumors. CAR-T cell therapy consists of ex vivo expansion of autologous T cells, transfection with the chimeric antigen receptor (CAR) and transfer into the patient. The CAR allows those T cells to identify and kill any cells expressing the antigen of interest, typically a surface protein expressed abundantly and primarily by the tumor. While these methods are extremely effective in a subset of patients, there is opportunity for improvement that may broaden the efficacy of this type of therapy. First, the application of selection, activation and expansion microbubbles has been shown to result in a less “exhausted” phenotype of the T cells at the end of the expansion phase, resulting in better overall T cell responses. In addition, alternative methods of transfection such as lipid nanoparticles (LNPs) may improve both transfection efficiency and viability. LNP lipid components can be tuned to optimally encapsulate different cargo types and transfect different cell types. Therefore, we formulated a variety of different LNPs to encapsulate either mRNA or pDNA encoding a reporter gene and assessed the relative transfection efficiency in microbubble-activated T cells. Additionally, we evaluated the final activation/exhaustion profile of the transfected cells to determine how uptake of different LNPs may affect the killing effectiveness. These results may ultimately suggest process improvements that have the potential to expand the number of CAR-T recipients who have improved responses to therapy.

#3485 T cell receptor (TCR)-Based cellular therapy for Fibrolamellar HCC.

Kayla J. Bendinelli[1], Allison M. Kirk[2], Paul G. Thomas[2], Mark Yarchoan[1]

[1]Johns Hopkins University, Baltimore, MD,[2]St. Jude Children's Research Hospital, Memphis, TN

Background: Fibrolamellar carcinoma (FLC) is a rare and often lethal liver cancer that primarily affects children and young adults without cirrhosis. There is no standard systemic treatment for FLC and the median survival for patients with advanced, unresectable FLC is approximately 12 months. The FLC transcriptome is characterized by an in-frame fusion of exon 1 of DNAJB1 with exons 2-10 of PRKACA, the catalytic subunit of protein kinase A (PKA), leading to expression of a chimeric protein, DNAJ-PKAc that has thus far been “undruggable” due to unacceptable on-target toxicity. Our overall goal is to target this “undruggable” oncogenic driver using T Cell Receptor (TCR)-Based cellular therapy.
Methods: We are performing a clinical trial of a peptide-based therapeutic vaccine targeting DNAJ-PKAc, in combination with the immune checkpoint inhibitors nivolumab and ipilimumab. We analyzed PBMCs from a subset of patients who demonstrated a clinical response to this immune-based combination therapy to identify TCRs with specificity for DNAJ-PKAc.
Results: To identify fusion specific T cell receptors (TCRs) generated in response to the vaccine patient peripheral lymphocytes were expanded and 10x single cell transcriptomic with paired TCR sequencing was performed. Candidate TCRs were reconstructed and expressed in T cells prior to co-culturing with HLA matched antigen presenting cells that were pulsed with DNAJ-PKAc peptides to verify fusion specificity via flow cytometry for T cell activation. We reconstructed and verified fusion specificity for multiple TCRs from several patients, and characterized them functionally by HLA specificity, affinity, and killing capacity in vitro. We identified TCRs recognizing DNAJ-PKAc-derived peptides in the context of class II HLAs, including HLA-DRB1*13:01 and HLA-DRB3*01. The TCRs that were identified recognize distinct DNAJ-PKAc-derived epitopes, with variable HLA specificity, TCR affinity, and cytokine response profiles after DNAJ-PKAc antigen stimulation. In general, TCRs with specificity for DNAJ-PKAc presented in the context of HLA-DRB1*13:01 were higher affinity than those recognizing HLA-DRB3*01, even when obtained from the same original patient.
Conclusion: This work identifies and characterizes multiple TCRs with specificity for DNAJ-PKAc. Our data provides evidence that the therapeutic efficacy of a peptide-based therapeutic vaccine may be mediated by a CD4 T cell response, and provides potential candidates for off-the-shelf TCR therapy for FLC.

#3486 Click-to-release for controlled immune cell activation: tumor-targeted unmasking of an IL-12 prodrug *in vivo*.

Martijn H. den Brok[1], Kim E. de Roode[1], Luc M. J. Zijlmans[1], Laurens H. J. Kleijn[1], Marleen H. M. van Stevendaal[1], Ron M. Versteegen[2], Lieke W. M. Wouters[1], Raffaella Rossin[1], Marc S. Robillard[1]

[1]Tagworks Pharmaceuticals B.V., Nijmegen, Netherlands,[2]SyMO-Chem, Eindhoven, Netherlands

Immunotherapy utilizing immune stimulating cytokines such as IL-12 holds great promise for the treatment of cancer. However, clinical use of IL-12 is hampered by severe toxicity following systemic administration resulting in a narrow therapeutic window. Tagworks is pioneering the use of *in vivo* click chemistry (Click-to-Release platform) to enable effective on-tumor activation of prodrugs, with the aim to increase the therapeutic index and application scope of potent anticancer drugs. We here present a novel treatment strategy in which IL-12 is inactivated by lysine conjugation to *trans*-cyclooctene(TCO)-linked PEG masks that can be released through the Click-to-Release reaction between the TCO linker and a tetrazine molecule (the trigger), restoring the native cytokine. Selective activation in the tumor microenvironment is achieved by pre-targeting the trigger to the tumor by i.v. injection of a tetrazine-protein conjugate, triggering local activation of the masked IL-12 when it is given in a subsequent i.v. injection. Evaluation of several Click-to-Release designs *in vitro* resulted in the selection of an IL-12-TCO-PEG construct with 9 conjugated lysines, exhibiting superior masking (>100 fold attenuated) combined with instantaneous and complete re-activation upon reaction with tetrazine. Next, we developed a tumor-targeted tetrazine based on a diabody against TAG72, a pan-carcinoma marker in the tumor microenvironment, modified with 4 tetrazine moieties. The diabody-tetrazine showed good tumor uptake in mice bearing colorectal (LS174T) xenografts combined with rapid blood clearance (< 0.5 %ID/g 24h p.i.). Also IL-12-TCO-PEG showed nearly complete clearance in 24 hours (< 0.5 %ID/g 24h p.i.). Administration of diabody-tetrazine to tumor-bearing mice followed by IL-12-TCO-PEG 24 hours later afforded selective IL12 unmasking in tumors and not in blood, demonstrating the feasibility of locally activating systemically administered immune stimulating cytokines with the versatile Click-to-Release platform.

#3487 Mechanistic role of CD70 in UV exposed keratinocytes and cutaneous squamous cell carcinoma (cSCC).

Asad Khan[1], Qiushi Wang[1], Chengcheng Hu[2], Emannuel Petricoin[3], Rebecca Morris[1], Georg T. Wondrak[2], Ann M. Bode[1], Clara Curiel-Lewandrowski[2], **Tianshun Zhang**[1]

[1]The Hormel Institute, University of Minnesota, Austin, MN,[2]The University of Arizona Cancer Center, The University of Arizona, Tucson, AZ,[3]Center for Applied Proteomics and Molecular Medicine, College of Medicine, George Mason University, Fairfax, VA

The incidence of cutaneous squamous cell carcinoma (cSCC) is rising due to limited prevention strategies. Significant gaps persist in understanding the mechanisms of UV-induced DNA damage and immune modulation in skin cancer development. By analyzing expression data from sun-exposed and sun-protected skin in the Genotype-Tissue Expression (GTEx) database and the Gene Expression Omnibus (GEO), we identified CD70 as a key gene linking solar UV exposure, immune responses, and photocarcinogenesis. Reverse Phase Protein Microarray Analysis (RPPA) revealed a significant upregulation of CD70 in sun-damaged skin compared to sun-protected skin. CD70 expression was also markedly higher in actinic keratosis (AK) and cSCC compared to normal tissue. In this study, we investigated the mechanistic role of CD70 in solar simulated light (SSL,149 kJ/m^2 UVA & 7.2 kJ/m^2 UVB)-exposed keratinocytes and cSCC. CD70 expression was found to increase in keratinocytes following SSL irradiation in a dose- and time-dependent manner. Deletion of CD70 effectively suppressed cSCC cell growth in both 2D and 3D culture models. RNA sequencing (RNA-seq) analysis (shCon vs. shCD70) revealed that CD70 regulates gene expression changes induced by solar UV exposure through multiple oncogenic pathways. CD70-associated genes are strongly linked to AK and cSCC and involve critical signaling pathways, including MAPK, Rap1, and others. Additionally, CD70 deletion inhibits NFkappa B nuclear translocation, as demonstrated by luciferase assays and immunofluorescence analysis. In summary, these findings underscore the pivotal role of CD70 in SSL-induced skin damage and photocarcinogenesis. The CD70 inhibition may offer a novel targeted strategy for preventing and treating cSCC.

#3488 MERTK inhibition regulates tumor-associated myeloid cell phenotypes and potentiates host-versus-lung cancer immunity.

Dan Yan[1], Xiaodong Wang[2], Stephen V. Frye[2], H. Shelton Earp[3], Deborah DeRyckere[1], Douglas K. Graham[1]

[1]Pediatrics, Emory University, Atlanta, GA,[2]Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC,[3]Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC

The TAM (TYRO3, AXL, MERTK) family receptor tyrosine kinases are important for clearing apoptotic cells and maintaining tissue homeostasis. They promote an immunosuppressive environment during cell clearance, which can be hijacked by tumor cells to promote immune evasion. This study aimed to determine how MERTK inhibition affects immune cells within the tumor microenvironment (TME) in lung cancer using a subcutaneous *Kras*-mutant murine lung cancer tumor model in wild-type (WT), *Axl* knock-out (KO), or *Mertk* KO mice. Tumor growth was inhibited in *Mertk* KO mice compared to WT mice, while *Axl* KO mice had no significant change in tumor growth. The anti-tumor effect observed in *Mertk* KO mice was lost in Mertk KO *scid* mice, which lack functional T and B cells, suggesting MERTK as an immunotherapeutic target in *Kras*-mutant lung cancer. MRX-2843 is a novel first-in-class MERTK-selective inhibitor that is currently being tested in phase 1/1b clinical trials. The murine cancer cell line used in this study did not express detectable MERTK and was unresponsive to MRX-2843 *in vitro.* However, *in vivo* treatment with MRX-2843 led to dose-dependent inhibition of tumor growth. Conditional knockout of *Mertk* in LysM expressing (i.e. myeloid) cells resulted in reduced tumor growth compared to tumors in WT mice. These data suggest that MERTK functions in myeloid cells to inhibit anti-tumor immunity, although contributions of homozygous LysM KO could not be eliminated in this study. Additionally, MERTK was only expressed in WT tumor myeloid cells ; myeloid cell MERTK was not detected in the TME in conditional *Mertk* KO mice. Furthermore, tumors from *Mertk* KO mice had evidence of increased antigen-presenting capacity with significantly increased numbers of tumor-associated macrophages expressing high levels of MHC-II and increased incidence of dendritic cells. Together these findings suggest that MERTK inhibits anti-tumor immunity by regulating myeloid cell functions and implicate MERTK as an immunotherapeutic target in *Kras*-mutant non-small cell lung cancer.

#3489 Functional enhancement of hypoxia-activated chimeric antigen receptor T-cells by a chimeric switch receptor recognizing TGF-β.

Zhihong Huang, Deping Han, Jean-Paul Thiery, **Xi Zhang**

Biosyngen Pte Ltd, Singapore, Singapore

Background: We have previously demonstrated that hypoxia-activated chimeric antigen receptor (CAR) T-cells could execute robust anti-tumor efficacy with controllable on-target, off-tumor (OTOT) toxicities in the clinical setting. However, some normal tissues or organs, such as bone marrow, rectum, stomach, gallbladder, and epidermis, also exhibit hypoxic microenvironment, which might lead to potential side effects or constraints for the clinical use of hypoxia-responsive CAR T-cells. TGF-β is a significant immunosuppressive factor that impedes immune response in the tumor microenvironment, while its expression in the hypoxic normal tissues is limited. We optimized the construct by introducing an AND-gated circuit complementing the hypoxia-driven conditional activation to reduce OTOT toxicities further.

Methods: The AND-gated CAR, BTRPTB001L, consisted of a hypoxia-activated CAR and a constitutively expressing TGF-β chimeric switch receptor (CSR). T-cells isolated from human peripheral blood mononuclear cells were stimulated with anti-CD3/CD28 beads, lentivirally transduced, and expanded *ex vivo*. CAR/CSR expression was assessed by flow cytometry. IncuCyte was used to analyze the *in-vitro* cytotoxicity in the presence of TGF-β. The TGF-β sponging capability was assessed by ELISA. Anti-tumor efficacy was evaluated in subcutaneously inoculated xenograft mouse models. Safety evaluation was performed using the AND-gated CAR T-cells targeting the murine orthologous antigen (BTRPTB001L') in immunocompromised mice.

Results: In BTRPTB001L, the fold change of CAR expression level in 1% O_2 compared to 21% O_2 was 6.33 ($P < 0.05$), while the CSR expression remained unaffected under either condition. BTRPTB001L showed significantly reduced cytotoxicity under normoxic conditions compared to second-generation (2nd-gen) hypoxia-activated CAR T-cells by co-culturing with target cells. Interestingly, in the presence of TGF-β and under hypoxic conditions, BTRPTB001L showed enhanced cytotoxicity, indicating that the CSR could reverse the inhibitory signal and be specifically activated by the soluble TGF-β in the tumor microenvironment. Notably, the CSR could also sponge TGF-β in the culture supernatant. The improved anti-tumor efficacy of BTRPTB001L was also demonstrated in a xenograft mouse model of pancreatic cancer. Moreover, while the constitutively expressing 2nd-gen CAR recognizing the murine orthologous TAA caused a lethal weight loss phenotype in mice, the BTRPTB001L'-treated group showed no prominent adverse events.

Conclusion: BTRPTB001L demonstrated robust anti-tumor potential and improved safety profile compared to hypoxia-responsive 2nd-gen CAR T-cell, supporting its further clinical development.

IMMUNOLOGY: T Cell Engagers
Poster Session

#3493 A novel T cell engager antibody for the treatment of HLA-A*01/KK-LC-1-positive tumors.

Athanasia Dasargyri, **Melissa Vrohlings**, Stephanie Jungmichel, Blaz Pavlovic, Martina Priola, Zoi Barou, Hannes Merten, Philip Knobel, Luca Scheu, Fabian Scheifele, Gonzalo Acuna, Matteo Morotti, Christian Leisner, Swethajit Biswas, Leonardo Borras

CDR-Life Inc., Horgen, Switzerland

Kita-Kyushu lung cancer antigen-1 (KK-LC-1) is a cancer-testis antigen prevalently expressed in multiple tumors including lung and gastric cancers but not in normal tissues. Here, we describe CDR505, a novel antibody-based T cell engager (TCE) for the treatment of KK-LC-1-expressing solid tumors. Since most HLA-restricted TCEs currently in clinical development are targeted against peptides presented on HLA-A*02, there is a high unmet need to develop TCEs for other HLA-A patient subpopulations. CDR505 is the only TCE to date, which is being developed against an intracellular target presented on HLA-A*01$^+$ tumors. CDR505 is a humanized, affinity-matured, and stability-engineered TCE that binds with one Fab arm to CD3 on T cells and with two scFv arms to the HLA-A*01-restricted KK-LC-1 peptide NTDNNLAVY on cancer cells. With a molecular weight of 100 kDa, this format is predicted to achieve a favorable PK likely allowing Q2W or Q3W dosing. CDR505 shows very efficient cancer cell killing as demonstrated with KK-LC-1$^+$/HLA-A*01$^+$ cancer cell lines NCI-H1703 and EKVX in co-cultures with human PBMCs. Upon CDR505-mediated cancer cell killing, increased expression of activation (CD69, CD25) and proliferation (Ki67) markers were observed on CD3$^+$ cells. T cell activation was further confirmed by the presence of cytokines detected in culture supernatants, including IFNγ, IL2, TNFα, IL6, and granzyme B. KK-LC-1 is a highly challenging target due to high levels of similar off-target peptides presented in healthy tissues. Extensive assessments for binding specificity and cross-reactivity in normal human cells were performed with CDR505. The binding profile of CDR505 was determined using surface plasmon resonance (SPR). This binding profile was used to identify potential off-target peptides in mass-spectrometry databases containing peptides presented in healthy tissues. None of the peptides identified caused relevant T cell activation in presence of CDR505 when presented on TAP-deficient, (empty) HLA-A*01-expressing T2 cells. Furthermore, CDR505 did not induce relevant T cell reactivity towards a panel of HLA-A*01 positive normal human cells from different organs and tissues. These results support a low risk for CDR505 off-target reactivity and a favorable safety profile. Collectively, CDR505 potently activates T cells and induces efficient cytotoxicity against KK-LC-1$^+$/HLA-A*01$^+$ cancer cells. It shows high specificity for KK-LC-1/HLA-A*01 and shows an overall safe profile in the presence of normal human cells. Future studies will include in vivo efficacy testing and a broader range of cellular assays using tissues and cultured cells as part of the preclinical studies that will be included in the IND application.

#3494 Durable and potent in vitro T cell activity with repeated exposure to CDR404, a potential best-in-class T cell engager targeting MAGE-A4.

Alessio Vantellini, Nora Wettstein, **Melissa Vrohlings**, Stephanie Jungmichel, Andre Luis Fonseca da Silva, Alice Langer, Anna Howald, Zoi Barou, Daniel Lenherr-Frey, Matteo Morotti, Christian Leisner, Swethajit Biswas, Leonardo Borras

CDR-Life Inc., Horgen, Switzerland

T cell engagers (TCEs) targeting the melanoma antigen gene A4 (MAGE-A4) have entered first-in-human trials for HLA-A*02:01+ patients with MAGE-A4 expressing solid tumors. CDR404 is a bispecific antibody-based TCE that binds bivalently to the MAGE-$A4_{230-239}$ peptide on HLA-A*02:01 and monovalently to CD3. The safety, tolerability and preliminary therapeutic efficacy of CDR404 are currently being evaluated in a dose-finding Phase 1 trial in multiple solid cancers including NSCLC (NCT06402201). The only other MAGE-A4 targeting TCE currently in clinical trial is a TCR-based TCE with a low affinity T cell recruiter, bearing an Fc region (TCR-TCE-Fc). Therefore, there are fundamental differences in format, functionality and pharmacology between these two TCE molecules. This preclinical study provides novel mechanistic insights into the persistence of cancer cell killing with repeated dosing of CDR404, comparing it to the TCR-TCE-Fc molecule. Since upregulation of PD-1 is an obligate immunosuppressive brake to T cell activation, we additionally investigated the in vitro killing activity of a CDR404 and Pembrolizumab combination compared to CDR404 alone. In co-culture assays of MAGE-A4+/HLA-A*02:01+ NSCLC cells (NCI-H1755) and human PBMCs, CDR404 induced higher levels of T cell activation, reflected by activation (CD69, CD25) and proliferation (Ki67) markers, and more potent tumor cell killing compared to the TCR-TCE-Fc. Higher potency of CDR404 was confirmed on different MAGE-A4+ cell lines from various cancer types. CDR404 also showed more efficient cancer cell killing than the TCR-TCE-Fc at low E:T ratios (down to 1:1), indicating that it has the potential to achieve higher efficacy in tumors with low T cell infiltration (“cold tumors”). Strikingly, potency of CDR404 was efficiently maintained over multiple rounds of serial killing of NSCLC cells (NCI-H1755), in contrast to the TCR-TCE-Fc molecule. In this experiment, CDR404 induced significantly less upregulation of T cell exhaustion markers than the TCR-TCE-Fc. These results highlight the potential of CDR404 to achieve more durable responses in solid tumors. Since T cell engagers treatment is accompanied by upregulation of PD-1/PD-L1 axis, we evaluated CDR404 in combination with the anti-PD-1 therapy pembrolizumab, showing enhanced potency of in vitro exhausted T cells on a PD-L1 overexpressing NSCLC cell line compared to CDR404 alone. In conclusion, these results demonstrate the potential of CDR404 to achieve improved efficacy and more durable responses for HLA-A*02:01+ patients with MAGE-A4+ tumors. The combination with an anti-PD-1 therapy may have additive anti-tumor activity in patients, and could potentially allow a transition into earlier, anti-PD-1/PD-L1 containing lines of treatment for multiple solid cancers including NSCLC, thus benefiting a larger patient population.

#3495 QL535, A novel CD2-costimulating T cell engager targeting PSMA-positive prostate cancer matching CD28 trispecific antibody in cytotoxicity, minimizing cytokine release and T-cell exhaustion.

Xiao Liu[1], Shenda Gu[1], Ke Liu[2], Joshua Morrissette[1], Jiantao Kong[3], Guangjie Guo[1], Yuxia Wang[2], Xin Zhang[2], Jiaming Cao[3], Moyan Hu[2], Min Sun[3], Ruixue Ma[2], Jane Chen[1], Nathan Ngo[1], Hejie Zhu[3], Liwei Wang[3], Yan Fang[3], Hao Liu[3], Shuyong Zhao[2], Shihao Chen[1]

[1]QLSF Biotherapeutics, South San Francisco, CA,[2]QILU Pharmaceutical Co., Ltd., Jinan, China,[3]QILU Pharmaceutical Co., Ltd., Shanghai, China

Prostate cancer is a leading cause of cancer-related mortality in men, highlighting the resistance to current treatments and need for new therapeutic options. PSMA (Prostate-Specific Membrane Antigen) is overexpressed in prostate cancer tissues compared to normal prostatic tissue, rendering it an ideal target for prostate cancer therapies, enabling precise delivery of therapeutic agents to cancer cells. We evaluated PSMA expression in 77 Chinese prostate cancer patients using immunohistochemistry (IHC), confirming its high expression in Chinese malignant prostate tissues. Due to the lack of clinical activity from T cell targeted therapies in solid tumors, recent developments in the field have focused on providing a co-stimulatory signal via CD28 or more recently, CD2. Analysis of single-cell RNA sequencing data from 2,170 cells across 14 mCRPC patients revealed that CD2 expression on tumor-infiltrating lymphocytes (TILs), particularly $CD8^+$ T cells, exceeds that of CD28, underscoring the potential for CD2 targeting in enhancing antitumor immunity. We developed QL535, a novel trispecific antibody in a 2+1+1 format designed to bridge tumor cells via PSMA and activate T cells through CD3 (signal 1) and CD2 (signal 2) via its ligand CD58. This design aims to stabilize the immune synapse and enhance T-cell cytotoxicity. Additionally, this combination overcomes T-cell exhaustion, with fine-tuned CD3 affinity to improve safety and reduce the risk of cytokine release syndrome (CRS). Comparative analysis was conducted with three trispecific antibody formats targeting CD28, and three formats targeting CD2, resulting in a lead CD2 trispecific antibody, QL535, alongside a CD28 trispecific antibody as a control. QL535 demonstrated superior tumor-specific killing both *in vitro* and *in vivo* compared to bispecific CD2-deficient antibodies, and exhibited a favorable safety profile, characterized by low IL-6, TNFα, IFN-γ and IL-8 levels compared to the CD28 trispecific control, which achieved comparable cytotoxicity. Further evaluation using repeated stimulation assays of prostate cancer patient-derived PBMCs showed that QL535 outperformed the CD28 trispecific control, an analog of the clinical benchmark PSMA targeted T cell engager, and our bispecific antibody control in overcoming T-cell exhaustion. Whereas other agents showed diminished cytotoxicity, potentially due to T-cell exhaustion, QL535 maintained durable tumor-specific lysis. Favorable manufacturing (CMC) studies further support QL535's developability for clinical applications. In conclusion, QL535 is a novel trispecific T cell engager with improved anti-tumor efficacy and safety profile. These findings warrant further clinical investigation to evaluate its safety and efficacy in patients.

#3498 Generation of an NG2 targeting bispecific antibody for induction of T cell immunity against melanoma.

Nisha Prakash, Sebastian Horner, Ilona Hagelstein, Gundram Jung, Helmut R. Salih

Clinical Collaboration Unit Translational Immunology, German Cancer Consortium (DKTK), University Hospital Tubingen, Tubingen, Germany

Analysis of mRNA levels using The Cancer Genome Atlas (TCGA) identifies high expression of neuron-glial antigen 2 (NG2) in several tumor tissues, particularly in melanoma, where it is selectively overexpressed in 90% of cases. NG2, also known as Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP) was originally identified on the surface of cutaneous melanoma. Due to its tumor-restricted expression, NG2 is an attractive target for immunotherapeutic approaches. Strategies that aim at the direct and specific lysis of malignant cells are needed in melanoma treatment despite the advent of immune checkpoint inhibiting antibodies. The latter lack tumor-specific activity and the capacity to directly recruit T cells to the tumor site. As a consequence, a considerable proportion of patients do not respond to immune checkpoint inhibitors, or for a limited time only.
Here we report on the generation and characterization of two NG2 bispecific antibodies (bsAbs) with differing T cell binding properties. NG2xUCHT-1 contains a CD3 binder with high affinity, whereas NG2xM18 was generated using a modified UCHT-1 with approximately 100-fold lower CD3 affinity. The purity and integrity of the generated bsAbs were biochemically confirmed using SDS-PAGE and size exclusion chromatography. Dose titration of the two bsAbs on NG2 expressing cell lines documented dose-dependent binding with similar EC_{50} values of ~0.5nM. Functional analysis in-vitro revealed that both bsAbs potently induce T cell activation and proliferation, resulting in strictly target-dependent tumor cell lysis. Of note, despite the significantly reduced binding affinity to T cells, NG2xM18 mediated comparable antitumor efficacy as NG2xUCHT-1, but induced substantially lower secretion of cytokines like IL-2, IFN-γ, and TNF, pointing to an optimized ratio of efficacy and potentially dose limiting side effects caused by cytokine release syndrome (CRS).
Our findings indicate that NG2xM18 is a promising therapeutic compound that may allow for effective clinical dosing with reduced side effects.

#3499 Engineering and construction of anti-B7-H3 antibody-based T cell engagers (TCEs) and antibody-drug conjugates (ADCs) for cancer therapy.

Chuan Chen, Yue Wu, Liang Tian, Chenpeng Su, Xiaoou Xu, Xiaoqian Chen, Dandan Liu, Yang He, Jiyuan Tian, Jian Peng, Zhenping Zhu

Helixon US Inc., New York, NY

B7-H3 (CD276), an immunomodulatory protein of the B7 superfamily, is overexpressed in a wide range of solid tumors. Various antibody-based therapeutic modalities targeting B7-H3 have shown antitumor response in preclinical and clinical studies. Ifinatamab deruxtecan (I-DXd), a B7-H3-directed DXd antibody drug conjugate (ADC) has demonstrated promising efficacy in patients with pretreated ES-SCLC in phase II clinical study. Further, several bispecific T-cell engagers (TCE) targeting B7-H3 and CD3 have also entered early phase clinical trials. In this study, leveraging our streamlined AI-guided antibody engineering platform, we generated several biparatopic TCEs targeting B7-H3 and CD3 as well as a number of biparatopic anti-B7-H3 ADCs constructed with various linkers and cytotoxic payloads. Our biparatopic TCEs were designed to simultaneously target two distinct epitopes on B7-H3 and with monovalent binding to CD3 on T cells with moderate to low affinity, in an attempt to enhance T cell-mediated specific cytotoxicity while mitigating the risk of cytokine release syndrome (CRS). The lead biparatopic TCE molecule demonstrated a robust B7-H3-specific T cell-dependent cellular cytotoxicity towards multiple B7-H3-positive tumor cells with various B7-H3 expression levels. The biparatopic ADCs demonstrated high avidity-driven binding activity to B7-H3 positive cancer cells and were capable of mediating strong internalization activity with higher tumor cell killing efficiency compared to their counterpart ADCs targeting to a single binding epitope. Further in vitro assays and in vivo efficacy studies for the lead biparatopic TCE and ADC molecules in xenografted tumor animal models are being investigated. Taken together, our studies provide support for the further development of the biparatopic B7-H3-targeting ADCs and TCEs for the treatment of B7-H3-expressing solid cancers.

#3500 LBL-054-CD3: An anti-CDH17 and CD3 bispecific antibody for the treatment of CDH17-positive gastrointestinal cancer.

Xingxing Fang, Yurong Qin, Xiaoya Liu, Chengze Ni, Tingtng Li, Peng Zhang, Chao Chu, Mi Ye, Guojin Wu, Jianming Sun, Yue Zhao, Xiao Huang, Jordan Zhu, Shoupeng Lai, Xiaoqiang Kang, **Hong Ling**

Nanjing Leadsbiolabs Co., Ltd., Nanjing, China

Background: CDH17 is a member of the cadherin superfamily, a group of calcium-dependent cell adhesion molecules critical for organ development, tissue integrity, and cancer progression. In normal tissues, CDH17 is highly restricted to the lateral membrane and concealed within intestinal tight junctions, making it inaccessible and blocking immune cell infiltration. In contrast, it is overexpressed and redistributed in 50% to 90% of gastrointestinal cancers, leading to its exposure on the cancer cell surface, therefore becoming more accessible to target. This unique feature makes CDH17 a promising target for T cell engager based immunotherapy for GI cancer. Here, we have developed a novel bispecific antibody, LBL-054-CD3, targeting CDH17 and CD3 to selectively kill CDH17-positive tumor cells by engaging T cells.
Methods: LBL-054-CD3 was developed using our proprietary LeadsBody™ T-cell engager platform, comprising high-affinity arms targeting CDH17 and one finely tuned arm targeting CD3, engineered to balance efficacy and safety characteristics. The binding affinity of LBL-054-CD3 to CDH17 and CD3 was assessed using Fortebio, while its biological activity was evaluated through several cell-based assays, including reporter gene assay, TDCC to various levels of CDH17 expressing tumor cells, and T cell activation. The anti-tumor activity of LBL-054-CD3 antibody was evaluated in PBMC-humanized mice bearing GI tumor cell line xenograft models.
Results: LBL-054-CD3 was shown potent binding affinity for CDH17 with no cross-reactivity to other family proteins. In vitro cytotoxicity assays revealed that LBL-054-CD3 mediated tumor cell killing was dependent on CDH17 expression level, inducing moderate cytokine release and cell proliferation while exhibiting no cytotoxic effects on CDH17-negative cells. In a PBMC-humanized mouse CDX model, LBL-054-CD3 demonstrated significant antitumor activity.
Conclusions: The study demonstrates that LBL-054-CD3 has potent binding affinity to the membrane-proximal region of CDH17 and mediates T-cell cytotoxicity against various levels of CDH17 expressing tumor cells. Further, the bispecific antibody is shown excellent antitumor activity in both in vitro and in vivo experiments.

#3501 A novel CD2-targeted costimulatory bispecific antibody platform for enhancing CD3 T cell engager-based cancer immunotherapy.

Gerhard J. Niederfellner[1], Welbeck Danquah[2], Melanie Pichery[3], Thomas Eden[4], Aurelien Boyance[3], Lise Pasquet[3], Virginie Roure[1], Alice Marchand[3], Marion Mars[3], Ellen Gumz[4], Mylene Gador[3], Lou Valente[3], Marion Contini[3], Lukas Czernecki[4], Sanjit Shanmuganathan[4], Markus Dangl[1]

[1]Evotec International GmbH, Gottingen, Germany,[2]Evotec International GmbH, Hamburg, Germany,[3]Evotec France SAS, Toulouse, France,[4]Evotec SE, Hamburg, Germany

CD3-based T cell engagers (TCEs) are a clinically successful class of bispecific antibodies that has delivered 11 approved agents to date, albeit primarily for treatment of blood cancers. These agents provide a strong signal 1 for redirecting polyclonal T cells, regardless of their native specificity, to kill cancer cells. However, when only receiving signal 1 for prolonged periods of time without a costimulatory signal, T cells become anergic and exhausted. In part due to the lack of costimulatory signals in the tumor microenvironment, TCEs have had limited therapeutic success in solid tumor indications so far. Interestingly, single cell RNA sequencing data from multiple solid tumor types revealed that a high percentage of tumor infiltrating T cells express high levels of CD2 but not CD28 or other costimulatory receptors. Based on a proprietary antibody that binds a unique epitope on the costimulatory T cell receptor CD2, we have developed a novel bispecific antibody platform (BiTco) that provides costimulation to T cells via CD2 in the context of a tumor-associated antigen. Using primary human T cells as effectors *in vitro*, we found that CD2 and CD28 costimulation potentiated T cell activation and T cell-mediated tumor cell killing to a comparable extent. However, the CD2 BiTco format triggered less pronounced cytokine release making it a potentially safer clinical option. Moreover, we demonstrated that, unlike CD28, our CD2 BiTco format also provides robust costimulation to CD28-negative CD8 T cells that possess high cytotoxic capacity and constitute the majority of CD8 T cells found in elder patients. Further, by using a fixed sub-efficacious dose of CD3 TCE we simulated *in vitro* a clinical setting where reduced expression of tumor antigen can result in loss of CD3 TCE monotherapy efficacy. When adding increasing doses of CD2 BiTco in this setting we could fully restore optimal anti-tumor cytotoxicity and T cell activation. In addition, we knocked out CD58 on tumor target cells to mimic loss of expression of this primary ligand of CD2, which is another known mechanism of immune escape by tumors. We showed that BiTco treatment allows to recover and even enhance TCE-mediated T cell cytotoxicity in the absence of tumor-expressed CD58. Finally, we established a BT474 clone 5 xenograft model using immunodeficient mice, in which a human T cell compartment was reconstituted by injection of human PBMCs. In this in vivo model we showed that combination treatment of CD2 BiTco with a sub-efficacious dose of a CD3 TCE resulted in highly effective control of tumor growth by human T cells. In summary, combination therapy with our differentiated CD2 BiTco platform can improve CD3 TCE response and limit acquired resistance mediated by loss of tumor antigen and/or CD58.

#3502 Preclinical development of an mRNA-encoded T cell engager targeting Glypican-3 with tissue- and tumor-specific enrichment in hepatocellular carcinoma.

Yan Huang, Shaoli Liu, Xiaoju Zhang, Bingxu Zhang, Hongya Han, Andong Liu, Xiaoyun Ma, **Wei Xu**

METiS Pharmaceuticals, Hangzhou and Beijing, China

Background: T cell engagers (TCEs) have been clinically validated but are mostly limited to hematological cancers. Developing BiTEs for solid tumors faces hurdles such as T cell penetration into tissue, suppressive nature of the tumor microenvironment and systemic toxicity, etc. This study aims to tackle these challenges by delivering an mRNA-encoded TCE targeting the hepatocellular carcinoma antigen Glypican-3 (GPC3) directly to liver tissue, thus enhancing the local TCE concentration to promote anti-tumor activity and exhibiting a favorable pharmacokinetics (PK) profile.
Methods: The BiTE was engineered by fusing single-chain variable fragments (scFvs) targeting GPC3 and CD3, separated by a flexible peptide linker. The mRNA encoding the BiTE was engineered using a combinatorial approach to optimize codon usage and enhance translational efficiency and stability, balancing the Codon Adaptation Index (CAI) and Minimal Free Energy (MFE). The mRNA was encapsulated in a novel lipid nanoparticle (LNP) for efficient liver-tropic delivery, forming the complex named MTS105. BiTE's cell-killing activity was tested using human GPC3-expressing cell lines and human PBMC. Anti-tumor efficacy was evaluated in liver orthotopic tumor-bearing mouse models. GLP Tox studies including PK and biodistribution were assessed in rodents and cynomolgus monkeys.
Results: Mechanistic studies demonstrated that MTS105-translated TCE mediated specific killing of GPC3-positive cells, T cell activation and proliferation *in vitro* and *ex vivo*. In the two liver orthotopic tumor-bearing mouse models, MTS105 achieved complete tumor regression in a dose-dependent manner, accompanied with intra-tumoral T cell activation. In mice, rats and cynomolgus monkeys, MTS105-translated TCE consistently exhibited higher exposure levels in the liver than the plasma. The sustained release of MTS105-translated TCE resulted in higher functional protein exposure in liver/tumor, while showing lower peripheral TCE Cmax (maximum concentration) and AUC (area under the curve) than the Fc-domain containing antibody-based TCE. In the cynomolgus monkeys, MTS105 displayed favorable and linear plasma PK profiles including mRNA, lipid and translated TCE in the up to 100 μg/kg following a single and 4-time repeat dosing weekly. The time to peak (Tmax) was 6 to 12 h in both periphery and targeting tissues, which correlated with the observation of fast Intra-tumoral T cell activity in mice. All monkeys tolerated well to a dose up to 100 μg/kg, with no sever adverse effects and gross pathology observed.
Conclusion: The mRNA-encoded TCE demonstrated optimal tissue/tumor-specific PK, safety and potent anti-tumor activity in preclinical models. These preclinical studies enabled a first-in-human study to assess its safety and preliminary efficacy which is currently ongoing.

#3503 ZW171, a differentiated 2+1 T cell-engaging bispecific antibody with antitumor activity in a range of mesothelin-expressing cancers.

Nicole J. Afacan, Patricia Zwierzchowski, Wingkie Wong, Maya Poffenberger, Chayne Piscitelli, Thomas Spreter von Kreudenstein, Nina E. Weisser

Zymeworks Inc., Vancouver, BC, Canada

Mesothelin (MSLN) is a tumor associated antigen overexpressed in many cancer indications and is an attractive target for immunotherapies including bispecific T cell engagers (TCE) and chimeric antigen receptor T (CART) cells. While MSLN-targeting immunotherapies have shown signs of clinical activity, their success has been hindered by dose-limiting toxicities associated with on-target off-tumor effects and cytokine release syndrome (CRS). To overcome these issues, we engineered ZW171, a MSLN-targeting TCE, with enhanced safety and antitumor activity. ZW171 is a 2+1 IgG1-like antibody, built with Azymetric^{TM} and EFECT^{TM} technologies, consisting of two MSLN binding domains and one low affinity CD3ε binding domain. We previously showed that the unique geometry and 2+1 design of ZW171 facilitates tumor selective binding and potent preferential killing of MSLN-mid and -high target cells, while sparing MSLN-low expressing target cells, and enhanced antitumor activity compared to other 2+1 TCE formats and the clinical benchmark HPN536 in MSLN-expressing PBMC-engrafted CDX models. To address the evolving clinical landscape and assess antitumor activity in additional indications and more translationally relevant models, we assessed ZW171 activity in advanced patient-derived organoid and xenograft models and benchmarked to other MSLN-targeting TCE including AMG 305, JNJ-79032421 and CT95. Additionally, activity in expanded MSLN-positive indications including pancreatic, endometrial, and gastric cancer, and the in the presence of soluble MSLN (sMSLN), which is observed in the serum of patients and can impede MSLN-targeted antibody-based therapies, was assessed in vitro. *Ex vivo*, ZW171 mediated potent tumor cell killing and T cell activation in patient-derived ovarian cancer organoid models. ZW171 induced complete tumor regressions in established patient-derived *in vivo* MSLN-positive non-small cell lung cancer and pancreatic cancer models. Comparison of ZW171 to AMG 305, JNJ-79032421 and CT95 showed reduced binding to T cells and equivalent or greater antitumor activity against MSLN-overexpressing cells. *In vitro*, ZW171 demonstrated potent MSLN-dependent killing in MSLN-positive indications including pancreatic, endometrial, and gastric cancer, and maintained activity in the presence of clinically relevant sMSLN concentrations observed in patient serum. Overall, ZW171 demonstrates differentiated and potent antitumor activity in a range of MSLN-expressing cancers. ZW171 is being evaluated in a Phase 1 clinical trial in MSLN-expressing solid tumors (NCT06523803).

#3504 Evaluation of T cell-engaging bispecific antibody using perfusable three-dimensional model.

R. Tsumura[1], R. Singh[2], N. Mori[3], Y. Kida[3], R. Asano[4], H. Takashima[1], H. Fuchigami[1], N. Sakamoto[1], M. Yasunaga[1], Y. Koga[1]; [1]National Cancer Center, Kashiwa, Japan, [2]University of Tsukuba, Tsukuba, Japan, [3]National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan, [4]Tokyo University of Agriculture and Technology, Tokyo, Japan

Introduction The development of T cell-engaging bispecific antibody (T-BsAb) has been an attractive field in cancer research due to its clinical success, especially in the treatment of hematologic cancer. In recent years, several T-BsAbs have been investigated for treating solid cancers. To improve T-BsAbs in treating solid cancers, it would be important to develop a robust *in vitro* evaluation system that mimics *in vivo* tumors. A conventional two-dimensional (2D) model is often used to evaluate *in vitro* efficacy of T-BsAb. However, T cells can directly contact cancer cells without any obstacles in this assay, resulting in a limited correlation with their *in vivo* efficacy. Although a three-dimensional (3D) model using the extracellular matrix such as matrigel can also be applied for drug evaluation, the lack of a perfusable vascular network may be a limitation for evaluating some therapeutic modalities, including T-BsAb. Here, we established the novel perfusable 3D *in vitro* model and compared the efficacy of T-BsAb in several *in vitro* and *in vivo* models, in order to determine which *in vitro* model best mimics *in vivo* in the evaluation of T-BsAb.

Material and Method We used three colorectal cancer cell lines, DiFi (EGFR+++), HT29 (EGFR++) and SW620 (EGFR-), and T-BsAb (named hEx3) targeting EGFR and CD3. As the conventional models, 2D and 3D *in vitro* models were adopted to evaluate the efficacy of hEx3. In the 3D model, T cells were applied on the matrigel with cancer cells. The perfusable 3D tissue is formed from a collagen gel composed of cancer cells and human umbilical vein endothelial cells (HUVECs). The tissue has a main blood vessel in the center through which the culture medium flows. The HUVECs in the gel have the self-organizing activity of a capillary-like structure from the main vascular vessel. To investigate *in vivo* efficacy, the cell line-derived xenograft (CDX) models were used.

Results In the conventional 2D model, hEx3 exerted high cytotoxicity against DiFi and HT29 cells but not SW620 cells. On the other hand, in the conventional 3D model, the cytotoxicity of hEx3 was limited even for EGFR-positive cells. In addition, hEx3 treatment showed anti-tumor effects in only DiFi CDX model but not in HT29 and SW620 CDX models. These results suggested that the efficacy of T-BsAb in the CDX model is not reflected by the conventional 2D and 3D *in vitro* models. We attempted to perform T cell perfusion in the perfusable 3D *in vitro* model. T cells were distributed near the capillary-like blood vessels, indicating that T cells infiltrated into the tissue through the blood flow. We are currently using this model to evaluate the efficacy of hEx3 *in vitro.*

Conclusion The conventional *in vitro* models would not be suitable to evaluate the efficacy of T-BsAb because their results were inconsistent with those in CDX models. Therefore, it would be necessary to develop an *in vitro* evaluation system that mimics *in vivo* blood vessel structures.

#3505 AM105, first-in-class T cell engaging bispecific antibody for metastatic colon cancer, demonstrates strong anti-tumor activity by both neutralizing EGFR activity and engaging T cells.

Hyun-Jong Lee, Dong-Wook Kim, Seong-Yeol Kim, Min Yoon, Young-ha Lee, In-Sik Hwang, Yoon Lee, Jong-Hoon Kim, Jong-Seo Lee

AbClon, Inc., Seoul, Korea, Republic of

Globally, colorectal cancer is a significant health concern. In 2020, there were over 1.9 million new cases and more than 930,000 deaths attributed to this disease and 5 year survival rate for distant colorectal cancer is 14.7 percent. Cetuximab, an approved anti-EGFR antibody, is effective in treating metastatic colorectal cancer with wild-type KRAS, improving survival. However, about 80% of patients develop resistance, leading to disease recurrence. To address this high recurrence rate, ongoing research focuses on developing bispecific antibodies that can overcome this resistance, aiming to improve long-term outcomes in metastatic colorectal cancer patients.AM105, a novel T cell-engaging bispecific antibody comprising a newly developed monoclonal antibody (15E3) and an affibody targeting EGFR and 4-1BB respectively. Characterized by its 2+4 architecture, it enhances selective binding to cancer cells by creating a difference in affinity between TAA and 4-1BB. It includes four Affibodies targeting 4-1BB, thereby increasing the likelihood of binding with T cells. Additionally, by enhancing the “clustering of CD137” on T cells, the T cell response is significantly amplified. In addition, AM105 was found to efficiently induce tumor cell death in CRC cell lines, even at very low effector-to-target cell ratio, in a target expression dependent manner. In vivo experiment using humanized 4-1BB mice have demonstrated excellent tumor cell elimination effects, and this therapeutic effect was still observed after newly introduction of tumor cells even in the absence of AM105 in the blood, suggesting the induction of T-cell memory function. Taken together, these results strongly suggested that AM105, a novel 4-1BB based bispecific antibody, exhibits potent anti-tumor T cell activity and could be promising therapeutic agent.

#3506 Clinic enabling development of a TCR-Ab half life extended T cell engager against a novel solid tumor target.

Sarah Leonard[1], Hemza Ghadbane[1], Dominic Hine[1], Samantha Drennan[1], Peter Cain[1], Louise Holland[1], Eleanor Bagg[1], Jana Rundle[1], Helena Horvatic[1], Christos Gavriel[1], Sophie Wells[1], Samhita Rao[1], Mathew Veal[1], Michela Marongiu[1], Juan Bolivar[1], Xiaoyan Pan[1], Maria Busz[1], David Cook[1], Wilawan Bunjobpol[1], Alexander Stephens[1], Sophie Johnson[1], Oleksandr Dudchenko[1], Jong Fu Wong[1], Sophie Richard[1], Samuel Ward[1], Simon Wright[1], On Kan[1], Amalia Martinez[1], Dzmitry Batrakou[1], Mitchell P. Levesque[2], Rebecca Ashfield[1], Mark Lees[1], Graham Ogg[3], Nathaniel Davies[1], Thomas L. Andresen[1]

[1]T-Cypher Bio, Oxford, United Kingdom,[2]Department of Dermatology, University of Zurich, Zurich, Switzerland,[3]University of Oxford, MRC Translational Immune Discovery Unit, Oxford, United Kingdom

Introduction: T cell engagers (TCEs) are a promising class of targeted therapies with recent clinical successes in solid tumors. A unique advantage of HLA-presented targets is that they can be derived from any part of the human genome providing an opportunity to build next generation TCEs against novel targets. We here present our discovery and development of a half-life extended TCE against a novel tumor-target towards clinical candidate selection. Our proprietary TCE drug format is based on a solubilized TCR, engineered as an Fc-based bispecific, showing high potency and safety in pre-clinical evaluation.
Experimental Procedures: We have performed T cell repertoire analysis on billions of T cells to identify the most potent TCRs from CD8 T cells against 100s of targets. This has resulted in isolation of specific TCRs against multiple novel targets. We have then taken the most potent TCRs (low micromolar affinity) through affinity engineering using a proprietary approach that combines machine learning-guided mutagenesis with high-throughput functional screening. A library of proprietary TCE formats with engineered TCRs and an anti-CD3 engager arm were optimized for yield, stability and function. The novel target in focus was selected based on high copy number on cancer tissue determined using epitope staining approaches. Finally, lead TCEs were assessed for efficacy, safety, longevity & biodistribution in ex vivo primary human cell assays and humanized xenograft models.
Results: We have developed a half-life extended TCE in a unique format with specific picomolar binding affinity against a novel tumor target. Our machine learning guided affinity engineering process progressed a set of uM wild-type TCR molecules to pM affinity, enhancing TCR:target binding >100,000 fold. High yielding TCRs were formatted into TCR-Ab TCE engagers and tested on primary tumor cells taken from patients. The TCEs showed activation of primary T cells and subsequent killing of these novel target positive cancer patient cells. We observed no T cell activation or killing of primary normal cell panels. Selected TCEs tested in humanized mouse models showed long circulating properties and efficacy. We have identified a novel TCE lead candidate which is potent, specific and safe in ex vivo human samples with a large therapeutic window.
Conclusions: We have developed a half-life extended T cell engager against a novel solid tumor target utilizing machine learning-guided TCR engineering that allows for the formation of an Fc-based anti-CD3 TCE. Currently, we are finalizing the preclinical evaluation and will enter IND enabling development mid 2025.

#3507 Discovery and preclinical characterization of novel TCR-mimetic T-cell engagers targeting TERT peptide-HLA complex for the treatment of solid and hematologic malignancies.

Yu Huang, Bhupal Ban, Jessica Jimenez, Preethi Sankaran, Christine A. Devlin, Melissa Bikowitz, Qunyan Yu, Ricard Masia, Delainey O'Connor, Emily McNally, Sarah Jaffe, Tanzila Rahman, Jennifer Helble, Alona Kulesha, Andrew Wolpert, Brendan Whalen, Shawn O'Malley, Yue Li, Michael Jennings, Galina Gabriely, Amanda Mak, Tao Wang, Benjamin Lee, Michi Schebesta, Michael Princiotta, Geraldine L. C. Paulus, Dmitri Wiederschain

Crossbow Therapeutics, Inc., Cambridge, MA

TERT (telomerase reverse transcriptase) is highly expressed in 85-95% of all tumors, making it a compelling target for cancer therapy. Although several TERT-targeting approaches are being investigated in clinical trials, only one therapeutic (RYTELO®, imetelstat) has received FDA approval for the treatment of low-risk myelodysplastic syndrome. Targeting HLA-restricted peptides (pHLA) derived from intracellular cancer antigens allows for broader exploration of the cancer proteome. The HLA-A*02:01-restricted $TERT_{540}$ peptide is presented by human tumors and can be a target for T cell-mediated tumor lysis. TCR mimicking (TCRm) antibodies recognize pHLA complexes and are capable of re-directing T cells once incorporated into a T Cell Engager (TCE) format. Here we report the preclinical characterization of potent and specific TCRm-based TCEs targeting $TERT_{540}$ pHLA. $TERT_{540}$-targeted TCEs consist of a single humanized CD3 binding domain and either one or two fully human TCRm domains, thus recognizing $TERT_{540}$/HLA-A*02:01 in either monovalent or bivalent forms. Monomeric affinity for $TERT_{540}$ pHLA was found to be in sub-nM range, and target binding was greatly enhanced through avidity effects in the presence of two TCRm binding domains. Alanine/glycine scan identified multiple amino acid residues in the $TERT_{540}$ peptide sequence that are required for TCE binding. Extensive specificity profiling revealed no meaningful activity of $TERT_{540}$ TCEs against cells pulsed with supra-physiological levels of computationally predicted HLA-A*02:01 cross-reactive peptides or multiple unrelated HLAs (alloreactivity). Furthermore, both $TERT_{540}$ TCEs showed no interactions with a large panel of normal primary cells from multiple vital tissues and organs *in vitro*. A modest IFNγ response was noted against bone marrow (BM) CD34+ hematopoietic stem cells at high concentrations of $TERT_{540}$ TCEs. In contrast, both compounds induced potent, target-dependent T cell activation and T cell-mediated killing of multiple TERT- and HLA-A*02:01- positive leukemia, lung, and ovarian cancer cell lines, achieving sub-nM EC50 values *in vitro*. Consistent with the specificity profile of $TERT_{540}$ TCEs, no activity was observed against TERT-negative or HLA-A*02:01-negative cells. *In vivo* efficacy studies with $TERT_{540}$-targeted TCEs are currently in progress. Comprehensive analytical and biophysical assessments revealed favorable developability profiles for both TCEs. In summary, these data provide strong preclinical evidence of potency, specificity, safety, and developability of novel, TCRm-based TCEs for TERT-targeted cancer therapy.

#3509 ATG-110, a novel “2+1” LY6G6D-targeted T-cell engager (TCE) for the treatment of MSS colorectal cancer.

Gang Bian[1], Jiahui Hua[1], Ge Lin[1], Xueli Chen[1], Huiling Liu[2], Peng Chen[1], Jay Mei[3], **Bing Hou**[3]

[1]Shanghai Antengene Corporation Limited, Shanghai, China,[2]Antengene (Hangzhou) Biologics, Hangzhou, China,[3]Antengene Corporation, Shaoxing, China

Background: Colorectal cancer (CRC) is one of the most common cancers worldwide and requires more effective and safer therapies to improve its poor survival outcome. LY6G6D is a GPI-anchored cell surface protein with expression that is particularly specific to colorectal cancer (CRC). LY6G6D exhibits significantly elevated expression levels in colorectal cancer (CRC) tissue comparing to normal tissue, particularly in pMMR/MSS CRC, which demonstrates primary resistance to immune checkpoint inhibitor (ICI) therapy. T cell engagers have shown encouraging efficacy in treating hematological malignancies, while exhibiting suboptimal performance in solid tumors. The potential of cytokine release syndrome (CRS) remains significant issues. We present a novel 2+1 LY6G6DxCD3 T cell engager, ATG-110, which demonstrated LY6G6D-dependent CD3 binding and significant anti-tumor activity with reduced cytokine release.

Methods: ATG-110 was constructed by introducing a novel conformational epitope-targeted anti-CD3 single chain fragment variable (scFv) to the hinge region of one of the heavy chains of a LY6G6D monoclonal antibody. It was evaluated in a series of preclinical *in vitro* studies for binding affinity, T cell activation and cytokine release, T cell dependent cytotoxicity (TDCC), and drug developability. The *in vivo* anti-tumor efficacy was evaluated in PBMC-humanized immunodeficient NDG mice engrafted with LY6G6D^{medium}-expression HT55 or LY6G6D$^{very\ low}$-expression SW480 MSS CRC cells.

Results: ATG-110 bound to LY6G6D-positive cell lines, LY6G6D-overexpression 293T, HT55, LS1034, with the nanomolar grade EC50. The CD3 binding site of ATG-110 is concealed by the LY6G6D Fab arm before binding to LY6G6D, due to the steric hindrance. Therefore, ATG-110 demonstrated limited binding capability to CD3+ cells before LY6G6D crosslinking. It activated T cells and induces cytokine release only in the presence of LY6G6D+ cells. *In vitro*, ATG-110 resulted in potent T cell dependent cytotoxicity with single-digit pM IC50 values on HT55 and SW480 cells. ATG-110 also showed potent anti-tumor activity in PBMC-humanized SW480 xenograft model and exhibited complete response (CR) in PBMC-humanized HT55 xenograft model. ATG-110 demonstrated good drug developability.

Conclusion: ATG-110 demonstrated LY6G6D-dependent CD3 binding and activation with low risk of CRS. It showed powerful *in vitro* and *in vivo* anti-tumor efficacy against colorectal cancer, which warrants further clinical evaluation.

#3510 A 2+1 format MUC16 targeting T cell engager induces MUC16-dependent T cell activity and superior anti-tumor efficacy.

Jinyang Li, Yang Li, Nan Gao, Yi Wang, Mengqiang Di, Min Wu, Yiyi Li, Ran Xue, Shiyu Hao, Weiwei Wu, **Xiguang Zhang**, Li Li, Shuaixiang Zhou, Huizhong Xiong

Innovent Biologics (Suzhou) Co. Ltd, Jiangsu, China

T cell engager (TCE) therapies targeting specific tumor associated antigens (TAAs) have shown clinical efficacy in multiple types of solid tumors. Indeed, TCE has been approved to treat melanoma and small cell lung cancer. Nevertheless, it remains necessary and challenging to improve the efficacy and safety profile of TCE to deliver benefits to more cancer patients. Recurrent and chemotherapy resistant ovarian cancer remains an urgent global unmet medical need. These is limited therapeutic options for this highly lethal and refractory disease. Recently, a MUC16 TCE demonstrated promising and durable clinical efficacy against heavily pretreated MUC16-high ovarian cancer, with acceptable safety profile. This underlines the potential to develop a MUC16 TCE with favorable safety profile and enhanced anti-tumor efficacy to possibly expand the benefits to MUC16-medium and MUC16-low ovarian cancer patients. MUC16 is a glycoprotein overexpressed in several types of solid tumors, especially in the majority of ovarian cancer. Here, we developed a 2+1 format MUC16 TCE, with improved tumor cell binding affinity, enhanced tumor cell killing potency, and MUC16-dependent T cell binding and activation. This MUC16 TCE targets membrane-proximal domains of MUC16 and achieves avidity-driven tumor cell binding in the presence of soluble CA125. Moreover, this MUC16 TCE elicited limited T cell binding and activation in the absence of MUC16, compared to benchmark molecule, to minimize off-target activity. This 2+1 format MUC16 TCE also showed favorable pharmacokinetics profile in mice. Functionally, systemic administration of this MUC16 TCE showed superior anti-tumor efficacy, with undetectable toxicity, in xenograft tumor models. Importantly, this MUC16 TCE demonstrated potent and synergistic anti-tumor efficacy in combination with clinically validated therapeutic strategies for ovarian cancer in pre-clinical tumor models. This provides rationale to develop combination therapies in future clinical trials. Indeed, combination of TCE with other therapies, e.g. ADC, chemotherapy, PD1/PDL1 mAb, are being tested clinically. Together, our pre-clinical data demonstrated that a 2+1 MUC16 TCE elicits potent anti-tumor efficacy and minimized off-target T cell activation, with potential to deliver benefits to ovarian cancer patients and patient with other MUC16-positive cancer.

#3511 A T cell engager (TCE), HB134, targeting LILRB4 and CD3, exhibits potent anti-tumor activity with a favorable safety profile for monocytic AML.

Juan Liu, Fangfang Ren, Chunmei Liu, Jing Shao, Fang Yang, Jianhe Chen, **Bin Ye**, Jianhua Sui

Huahui Health, Ltd., Beijing, China

Background: Acute myeloid leukemia (AML), is a heterogeneous and highly aggressive malignancy, characterized by poor prognosis and limited treatment options. Monocytic AML, including M4 and M5 subtypes, presents a significant clinical challenge due to its dismal prognosis and high relapse rates post-transplant. The leukocyte immunoglobulin-like receptor B4 (LILRB4) is overexpressed in monocytic AML but is absent in normal hematopoietic stem cells, highlighting its potential as a therapeutic target. Here, we report the development of a novel TCE targeting LILRB4 and CD3, HB134, for the treatment of monocytic AML.
Methods: The HB134 TCE was generated by fusing an anti-LILRB4 nanobody and an anti-CD3 Fab to a human IgG1 with a silent Fc region. Surface plasmon resonance, enzyme-linked immunosorbent assay, and flow cytometry were used to measure HB134's binding activities to LILRB4 and CD3. Luciferase-based cellular reporter assays and a primary T cell-dependent cellular cytotoxicity (TDCC) assay using peripheral blood mononuclear cells (PBMCs) were employed to assess HB134's T cell activation activity. Additionally, *in vivo* anti-tumor efficacy and pharmacokinetic (PK) profile were evaluated in mouse models.
Results: HB134 exhibited sub nanomolar high binding affinity for human LILRB4 and low binding affinity to human CD3. In the cellular luciferase reporter assays, in the presence of LILRB4-positive monocytic AML cells (THP-1 or MOLM-13 cell line), HB134 induced robust T cell activation; in contrast, no T cell activation was observed in the presence of LILRB4-negative cells, indicting HB134 can specifically induce T cell activation in a LILRB4-engagement-dependent manner. In the TDCC assays using PBMCs, HB134 induced potent cytotoxicity against LILRB4-positive THP-1 and MOLM-13 cells with limited induction of cytokine release syndrome-related proinflammatory cytokines, including IL-6 and TNF-α. In an immunodeficient mouse model engrafted with human PBMCs and LILRB4-positive THP-1-luciferase cells, HB134 showed potent anti-tumor efficacy. Additionally, HB134 demonstrated a favorable PK profile in BALB/c mice.
Conclusions: HB134, a TCE designed to engage T cells via CD3 and specifically target LILRB4, exhibits potent anti-tumor activity and favorable PK and safety profiles, positioning HB134 as a promising therapeutic candidate for treating LILRB4-positive monocytic AML.

#3512 Development of a novel T cell engager for solid tumors presenting KRAS G12V mutant peptide in HLA-A*11:01.

Lauren Pitt, Rasika Premaratne, Rebecca Wood, Ruban Kannan, Shereen Jabar, Dylan James, Brendan Eames, Kal Gunarathne, Nicole Church, Zac Rosenes, Fiona Gracey, Idris Bello, Eleanor Leung, Brad McColl, Ben Kiefel, Matt Beasley

Myrio Therapeutics Pty Ltd, Blackburn North, Australia

Background: KRAS neoantigens present on the surface of tumors as peptide-HLA complexes are promising targets for immunotherapy. Here, we describe preclinical development of a half-life extended T cell engager, MA022-2037, designed to redirect T cells to kill solid tumors driven by KRAS G12V mutation in HLA-A*11:01-positive patients.
Methods: The antibody discovery platform, Retained Display (ReD), was used to isolate fully human single chain variable fragments (scFv) selectively recognizing a 10-mer peptide derived from KRAS G12V in the context of HLA-A*11:01, while avoiding the corresponding wildtype KRAS peptide differing by a single amino acid. Target selectivity was assessed in bead-based binding assays involving arrays of irrelevant peptide-HLA-A*11:01 complexes and HLA-A*11:01-restricted peptides with sequence homology to the target peptide. Scanning mutagenesis was used to map specific binding motifs for prediction of cross-reactive peptides. Following conversion to T cell engager format, activity was assessed in co-cultures of *in vitro* expanded T cells isolated from donor PBMC with HLA-A*11:01-positive tumor cell lines with or without KRAS G12V mutation. The pharmacokinetics of half-life extended MA022-2037 was assessed in animals prior to evaluation of efficacy in tumor-bearing mice engrafted with donor PBMC.
Results: MA022-2037 binding was restricted to KRAS G12V/HLA-A*11:01, with no binding observed to any other HLA-A*11:01-restricted peptide examined, including those with similarity to the target peptide. No cross-reactive peptides from the known human proteome were predicted to bind MA022-2037 based on analysis of its specific binding motif. MA022-2037 mediated robust killing of HLA-A*11:01-positive, KRAS G12V mutant tumor models in the presence of donor T cells *in vitro*, while KRAS wildtype or KRAS mutation-mismatched tumors were spared. As a half-life extended T cell engager, MA022-2037 displayed a favorable pharmacokinetic profile with a half-life of ~20 days, enabling *in vivo* evaluation of efficacy.
Conclusions: Our preclinical data support continued development of MA022-2037 for HLA-A*11:01-positive patients with KRAS G12V mutant tumors and IND-enabling studies are in progress. We anticipate delivery of a new precision immunotherapy for patients whose high unmet need is not currently addressed by molecularly targeted inhibitors.

#3513 CC312, a trispecific CD19-targeting co-stimulatory T cell engager, for the treatment of B cell malignancies and autoimmune diseases.

Yingfeng Huang, Ruixia Zhang, Xiaofang Zhang, Zhen Jing, Caizhi Zhao, Fangfang Pan, Beibei Zheng, Ruixue Dai, **Li Zeng**

CytoCares (Shanghai) Inc., Shanghai, China

Background: B cell depletion therapy (BCDT), such as monoclonal antibodies, bispecific T cell engagers(TCE) and CAR-T,has an extensive history of effectively treating B cell malignancies. Certain BCDT monoclonal antibodies,e.g. rituximab,are also approved to treat autoimmune diseases(ADs), however demonstrate poor B cells depletion in deep tissue which may limit the long-term disease control or remission in patients. Recently, anti-CD19 CAR-T, through thorough depletion and “resetting” of B cell populations, have achieved multi-year drug-free remission in systemic lupus erythematosus (SLE),idiopathic inflammatory myopathy(IIM)and systemic sclerosis(SSc). Here, we developed a trispecific TCE CC312, targeting CD3 and CD28 on T cells and CD19 on B cells. Consistent with CAR-T, CC312 showed potent T cell activation and B cell depletion in a prior study in B cell malignancies. In the current study, the potential of CC312 in treating ADs was explored in translational models and in the clinical setting.

Methods: B cell killing of CC312 was assessed in *ex vivo* cytotoxicity assays using PBMC isolated from AD patients. T cell activation, proliferation and differentiation were also evaluated with patient PBMC. The *in vivo* efficacy for SLE was investigated in mice engrafted with SLE patient PBMC. In addition, clinical safety, tolerability, and efficacy of CC312 in refractory SLE, IIM, SSc, and other B cell-related ADs was evaluated in an ongoing investigator-initiated clinical trial.

Results: *In vitro*,CC312 induced a concentration-dependent potent depletion of B cells in PBMCs derived from donors with ADs, with EC_{50} in pM range across donors and disease types. Compared with anti-CD3/CD19 bispecific benchmark, CC312 significantly enhanced T cell activation, proliferation, and memory T cell differentiation due to CD28 co-stimulation. *In vivo*, CC312 thoroughly depleted peripheral B and plasmablast cells,as well as eliminated IgG and anti-dsDNA in serum. At the end of this study, neither IgG deposits nor CD20+B cell infiltrates were observed within kidney tissues. In the ongoing clinical study of ADs, CC312 is well-tolerated, with no Grade 3+ CRS or ICANS. Even at the lowest dose level(5 μg/dose), peripheral B cells and B cell subgroups can be comprehensively depleted, accompanied with improvement or remission of disease.

Conclusion: CC312’s capacity to potently and thoroughly eliminate B cells has been demonstrated in *in vitro* and *in vivo studies* as well as in the clinical setting, indicating the potential benefit in treating B cell-related ADs. As the first CD28 co-stimulatory TCE to be explored in ADs, additional clinical studies will be conducted to further evaluate the efficacy and safety in autoimmune patients.

#3514 AZD6621: Improving the therapeutic index for prostate cancer T cell engagers with a next-generation CD8-guided format.

Suzanne Sitnikova[1], Natalie Burrows[1], Nikolaos Ioannou[1], Even Walseng[2], Chunning Yang[2], Nadia Luheshi[1], Yariv Mazor[2], Mark Cobbold[2], Saso Cemerski[2], Simon Dovedi[1]

[1]AstraZeneca, Cambridge, United Kingdom,[2]AstraZeneca, Gaithersburg, MD

Prostate cancer is the second most common malignancy in males with a 5-year survival rate for metastatic castration-resistant prostate cancer of only 37%. Six-transmembrane epithelial antigen of the prostate 2 (STEAP2) is a metalloreductase with plasma membrane expression that is restricted to the prostate but is highly overexpressed across all stages of prostate adenocarcinoma. This expression profile makes STEAP2 an ideal target for a T cell engager (TCE). Despite promising efficacy, the toxicity profile of current TCEs remains challenging with patients often experiencing cytokine release syndrome and on-target, off-tumor adverse effects. This dose-limiting toxicity profile restricts their therapeutic window and with it the potential for combination therapy. We sought to improve this therapeutic index in 3 ways: 1) targeting the tumor-restricted STEAP2 antigen, 2) affinity-optimizing the CD3 binding domain and 3) integrating a CD8 binding domain to preferentially engage $CD8^+$ T cells over $CD4^+$ T cells, as the latter have been shown to contribute to the risk of cytokine release syndrome. Here we provide preclinical *in vitro* and *in vivo* evidence demonstrating that this design can potentially increase the therapeutic index when compared to a conventional TCE. We demonstrate that AZD6621 mediates potent, STEAP2-dependent cytotoxicity *in vitro* with cytotoxicity EC50 values below 10 pM. AZD6621 also induces preferential activation of $CD8^+$ T cells over $CD4^+$ T cells, with an approximately 5-fold increase in maximal activation of $CD8^+$ T cells, as well as significantly reduced cytokine release compared to a conventional TCE. AZD6621 mediates complete anti-tumor regressions as a monotherapy in the STEAP2-expressing 22Rv1 subcutaneous xenograft model in humanized mice. Anti-tumor activity of AZD6621 *in vivo* was also observed in a C4-2 intratibial xenograft model. Furthermore, when matched for anti-tumor activity in an intravenous 22Rv1 xenograft model, systemic cytokine levels were significantly reduced in AZD6621-treated mice compared to a conventional TCE. These data provide reason to believe that AZD6621 can improve the therapeutic index of conventional TCEs via CD8-guided, affinity-optimized CD3 engagement of STEAP2 on prostate cancer cells and support a first-in-human clinical trial planned to start in 2025.

#3516 TriNKETs®: trispecific immune cell engager therapies exhibit potent preclinical in vivo anti-tumor activity and broad combinability in diverse cancer models.

Colin LaCroix, Adam Stockmann, **Mitchell Bigelow**, Cornelis van der Poel, Jolanta Dubauskaite, Jeremy Beech, Samia Khan, Eva Gutierrez, Nathan Felix, Stuart W. Hicks, Nicolai Wagtmann, Ann F. Cheung

Dragonfly Therapeutics, Inc., Waltham, MA

The trispecific immune cell engager TriNKET® platform is designed to direct the activity of cytotoxic immune cells against cancer cells through simultaneous engagement of a tumor-associated antigen and the activating receptors CD16a and NKG2D (natural killer group 2 member D), a receptor expressed on natural killer (NK) cells, γδ T cells, and CD8+ T cells. To evaluate the preclinical efficacy of the TriNKET platform, a TriNKET targeting the murine Tyrosinase-related protein 1 (TYRP1), a melanocyte protein expressed on melanomas and normal melanocytes, was developed and evaluated using TYRP1-expressing immunocompetent syngeneic models. The TYRP1-TriNKET model system bridges the gap between in vitro studies and potential clinical activity, providing a robust system to validate the immune-based activities of TriNKETs, and their ability to be combined with standard-of-care therapies, such as checkpoint blockade or chemotherapy.

The anti-tumor activity of the TYRP1-targeting TriNKET was evaluated in the B16F10 melanoma model, which endogenously expresses TYRP1, as well as in MC38 and CT26 colon tumor models that were engineered to express TYPR1. Immunological changes within the tumor microenvironment were characterized using flow cytometry. In addition, the potential for enhanced activity was explored by testing the TYRP1-TriNKET in combination with anti-PD1, Abraxane, and various chemotherapy regimens.

The TYRP1-TriNKET demonstrated strong binding to TYRP1+ cell lines and robustly activated NK cells in vitro when co-cultured with tumor cells. TYRP1-TriNKET monotherapy showed superior in vivo anti-tumor efficacy compared to a TYRP1-targeting monoclonal antibody in all tumor models tested. TYRP1-TriNKET increased infiltration of NK cells and CD8+ T cells, promoted immune cell activation, and increased levels of pro-inflammatory cytokines and chemokine within the tumor microenvironment without inducing peripheral immune activation or cytokine release. Depletion of immune cells prior to treatment confirmed that the observed anti-tumor responses are mediated through both NK cells and CD8+ T cells. Furthermore, TriNKET efficacy was enhanced when combined with standard-of-care therapies, including anti-PD-1, Abraxane, cyclophosphamide, gemcitabine, and 5-FU, with no evidence of antagonism in combination treatments.

TriNKETs display potent preclinical in vivo anti-tumor responses driven by NK cell and CD8+ T cell effector functions. These findings highlight the potential of TriNKETs as promising immunotherapeutic agents, demonstrating their potential both as standalone treatments and as components of combination strategies with standard-of-care therapies.

#3517 A bispecific pan-γδT cell engager with IL-15 fusion that activates both δ1 and δ2 types of γδT cells for the treatment of acute myeloid leukemia.

Mingcan Yu[1], Peng Chen[2], Ying Jin[2], Fulai Zhou[2], Honglei Bi[2], Mark Chiu[1], **Di Zhang**[1]

[1]Tavotek Biotherapeutics, Lower Gwynedd, PA,[2]Tavotek Biotherapeutics, Suzhou, China

Background: Bispecific T cell engagers redirecting pan $CD3^+$ T cells for tumor immunotherapy have demonstrated clinical promise in treating mainly hematologic cancers but are often accompanied with severe toxicity due to cytokine storms. An alternative approach involves engaging innate-like γδ T cells instead which possess inherent antitumor activity but are less prone to toxicity. Unfortunately, most γδ T cell-based therapeutics reported so far exhibit suboptimal clinical efficacy due to subtype-specific engagement, limited quantity, and poor durability of γδ T cells in cancer therapy.

Methods and Results: We characterized a pan γδ T cell receptor antibody capable of binding and activating γδ T cell receptors from both δ1 γδ T cells (mainly resident in mucosal tissues and tumors) and δ2 γδ T cells (predominantly found in blood circulation). When paired with an antibody targeting the acute myeloid leukemia (AML) associated target CD123, this bispecific pan γδ T cell engager, TAVO317A, mediated tumor cell dependent activation and degranulation of both δ1 and δ2 γδ T cells and redirected them to potently kill antigen-specific hematologic cancer cells. To enhance the durability of bispecific pan γδ T cell engagers in tumor therapy, we engineered an attenuated IL-15 along with the IL-15 receptor α sushi domain as a fusion component on the CD123 bispecific pan γδ T cell engager. The addition of the IL-15 domain further boosted the ability of the engager to promote the activation and proliferation of γδ T cells. In humanized AML cell xenograft models, the CD123 bispecific pan γδ T cell engager effectively recruited γδ T cells to control AML tumor growth. Moreover, the CD123 engager with IL-15 fusion exhibited even better efficacies in tumor cell elimination and sustained γδ T cell activity without requiring IL-2 supplementation.

Conclusions: By recruiting both δ1 and δ2 γδ T cells, our bispecific pan γδ T cell engager can mobilize more γδ T cells that may otherwise be of limited quantity for tumor therapy. In addition, the engineered IL-15 domain for immune modulation can facilitate enhanced durability of γδ T cells in tumor cytotoxicity. Our novel proprietary bispecific pan γδ T cell engager platform has the potential to serve as a potent and efficacious alternative to CD3-based therapy by offering enhanced liquid and solid tumor immunotherapy with a better safety profile.

#3519 EVOLVE104, a differentiated T cell engager targeting the novel antigen ULBP2/5/6, exhibits potent preclinical efficacy for the treatment of squamous tumors.

Oksana A. Sergeeva, Jennifer Zeiger, Colleen Brown, William DeMaria, Donal Ryan, Antonio Ward, Guixian Jin, Martin Preyer, Yanhuai Ding, Bruce Andrien, Mark Aimone, Agnes Meade, Jay S. Fine, Jeremy S. Myers, Stella Martomo

EvolveImmune Therapeutics, Inc, Branford, CT

EVOLVE104 is the first CD3 T cell engager (TCE) being developed for solid tumors which utilizes integrated CD2 T cell co-stimulation. EVOLVE104 is a trispecific molecule which targets ULBP2/5/6 on the tumor cell and uses affinity-tuned CD3 engagement together with a CD2-selective fusion protein to deliver coordinated costimulatory signal integration to the T cell to optimize its effector function. ULBP2/5/6 are closely related proteins of the UL16 binding protein family which are recognized by the NKG2D receptor and induced on tumor cells while maintaining restricted expression on normal human tissues.

In patient tumor samples, at both the RNA and protein level, ULBP2/5/6 expression is higher in carcinomas of squamous pathology as compared to adenocarcinomas. For example, squamous non-small cell lung cancer (NSCLC), squamous head and neck, and squamous urothelial cancer demonstrate > 70% ULBP2/5/6 positivity. In addition, transitional urothelial cancer, which encompasses most bladder cancers, also shows 70% ULBP2/5/6 positivity. We have verified that matched primary and metastatic urothelial, head and neck, and NSCLC tumors retain ULBP2/5/6 expression, and that standard of care treatments as late as post-third line do not decrease ULBP2/5/6 expression levels. These observations support the positioning of EVOLVE104 in squamous tumors and transitional urothelial cancers in initial clinical trials.

Using a variety of indication-specific tumor cell lines, we show an in vitro T-cell directed cell cytotoxicity EC50 of ~100 pM for EVOLVE104 with concomitant T cell activation, proliferation, and cytokine release. EVOLVE104 demonstrates a minimal efficacious dose of 0.25 mg/kg in indication-specific human cell line derived in vivo xenograft models in NSG mice engrafted with human PBMCs. In non-human primates, EVOLVE104 was well tolerated at doses up to 24 mg/kg, with no adverse findings or organ-specific toxicities at supra-pharmacologic levels compared to predicted human efficacious exposures. We show binding of EVOLVE104 in vivo on more than 95% of peripheral T cells for at least 168 hours post dose and no evidence for cytokine excursions throughout the duration of the study. Thus, preclinical data demonstrate safety and efficacy of EVOLVE104 and support the upcoming Phase I clinical study.

EVOLVE104, a trispecific TCE with integrated affinity-tuned CD3 binding and CD2 co-stimulation, optimizes effector T cells for anti-tumor activity. The enriched expression of its tumor antigen target, ULBP2/5/6, in primary and metastatic squamous tumors and transitional bladder cancers, together with its compelling in vitro and in vivo pharmacology and nonclinical safety profile, position EVOLVE104 as a first-in-category immunotherapy to address underserved patient populations with high unmet needs.

#3520 EM1031, a novel KLK2 x CD3 bispecific T-cell engager with highly effective efficacy in preclinical models.

Z. Ma, B. Shi, Z. Wei, W. Guo, L. Hu, Y. Li, X. Wu, S. Gong, D. Wu, C. Wu;
EpimAb Biotherapeutics Inc, Shanghai, China

Therapeutic options for metastatic castration-resistant prostate cancer (mCRPC) are limited within hormonal therapies, taxane-based chemotherapies and palliative radiotherapy. Currently available targeted therapies or immunotherapies have limited efficacy and can only benefit a small population of mCRPC patients. Human kallikrein 2 (KLK2) is a membrane-bound trypsin-like serine protease that shares 78% homology with prostate specific antigen (PSA). Directly regulated by androgen receptor (AR), KLK2 is specifically expressed in epithelial cells of prostate and prostate cancer cells and was considered as a promising tumor associated target. In this case, we have developed EM1031, a novel KLK2 x CD3 bispecific T-cell engager (TCE) based on EpimAb's proprietary CD3 antibody and bispecific antibody platform, adopting optimized CD3 affinity and binding arm valency to achieve potent redirected tumor cell lysis while maintaining minimal cytokine release. *In vitro* T-cell activation and cytokine release assay showed that EM1031 activates T cells only in the presence of KLK2 positive target cells. A clinical stage reference KLK2 TCE was also generated with published sequences for comparison. In preclinical studies, EM1031 showed less cytokine release against KLK2 positive prostate cancer cell lines than the reference KLK2 TCE, while it maintained comparable *in vitro* tumor cell killing activity. In xenograft models derived from KLK2 positive prostate cancer cell lines, EM1031 completely eradicated tumors with lower cytokine release compared to the reference TCE. Taken together, these data suggest that EM1031 has the potential in the treatment of prostate cancer.

#3521 A novel T cell engager targeting HLA-A02:01 TP53-R175H for cancer immunotherapy.

Mark Ng, Emily Zygiel, Alexander McQuown, Jinsheng Liang, Luhua Shen, Joshua Francis, Xingyue He, Hubert Lam, Gary Shapiro, Loic Vincent

Affini-T Therapeutics, Inc., Watertown, MA

Background: The tumor suppressor protein p53 (TP53) plays a pivotal role in preventing tumor formation by inducing cell cycle arrest and apoptosis in response to DNA damage. However, TP53 mutations are prevalent across various cancer types, and these mutations not only result in loss of tumor suppressive functions but also confer gain-of-function properties that drive oncogenesis. Among these mutations, HLA-A0201 TP53-R175H represents the largest TP53 population with >34,000 cases per year in the US and EU across all solid tumor indications, with no therapies explicitly targeting this mutation in the clinic. Leveraging Affini-T's TETHER™ platform, we present the development of a novel TCR-based T cell engager designed to redirect the cytotoxic potential of T cells against mutant TP53-expressing cancer cells.

Methods: A bispecific T cell engager was engineered to simultaneously engage T cells via CD3 and target TP53-R175H-expressing cancer cells by an affinity-matured specific TCR. Affinity maturation of the TCR was performed to increase its specificity and binding affinity towards the mutant peptide. Tumor co-culture assays were conducted to evaluate T cell-mediated cytotoxicity. T cell activation was assessed by measuring cytokine production. The tolerability profile of the engagers was established by X-scan, evaluating T cell activation by normal tissue.

Results: The data demonstrate successful affinity maturation of the TCR, resulting in enhanced recognition of the HLA-A0201 TP53-R175H peptide with high specificity. Tumor co-culture assays revealed potent T cell-mediated killing of mutant TP53-R175H-expressing cancer cells by the engager, accompanied by robust T cell activation characterized by cytokine production. Importantly, the engager exhibited favorable tolerability profiles, demonstrated by minimal off-target activation via X-scan and with minimal T cell activation towards normal tissue.

Conclusions: These findings highlight the promising activity and tolerability profile of a novel T cell engager targeting HLA-A02 TP53-R175H for cancer immunotherapy. Harnessing the cytotoxic potential of T cells against mutant TP53-expressing tumors presents a promising approach for the development of innovative cancer treatments.

IMMUNOLOGY: Vaccines, In Situ Vaccines, and Vaccine Combinations
Poster Session

#3525 Neo20 mRNA vaccine induces neoantigen-specific T cell immunity in humanized patient-derived xenograft mouse models of triple-negative breast cancer.

Maria F. Chervo[1], Chiara Mancino[2], Raghav Shroff[3], Wei Qian[1], Jenying Deng[1], Karina A. Ortega-Martinez[1], Jianying Zhou[1], Fotis Nikolos[4], Liliana Guzman-Rojas[1], Francesca Taraballi[2], Keith S. Chan[4], John P. Cooke[5], Jimmy D. Gollihar[3], Jenny C. Chang[1]

[1]Breast Oncology Department, Dr. Mary and Ron Neal Cancer Center, Houston Methodist Research Institute, Houston, TX,[2]Center for Musculoskeletal Regeneration, Houston Methodist Research Institute, Houston, TX,[3]Department of Pathology and Genomic Medicine, Houston Methodist Research Institute, Houston, TX,[4]Department of Urology, Dr. Mary and Ron Neal Cancer Center, Houston Methodist Research Institute, Houston, TX,[5]Department of Cardiovascular Sciences, Center for Cardiovascular Regeneration, Houston Methodist Research Institute, Houston, TX

Triple-negative breast cancer (TNBC) is the most aggressive breast cancer subtype, carrying a higher risk of recurrence and metastasis within 3 years, with poor prognosis. Neoadjuvant chemotherapy and immune checkpoint blockade are the standard of care. However, many patients still do not benefit from these treatments. Here we developed an mRNA lipid nanoparticle (LNP) vaccine (Neo20) as a potential therapeutic strategy for TNBC. We aimed to test the preclinical efficacy of Neo20 vaccine in humanized patient-derived xenograft (hu-PDX) mouse models of TNBC expressing HLA class I. Tumor neoantigens were identified in the TNBC PDX model MC1 using a proprietary algorithm that prioritizes vaccine candidates on their likelihood to elicit neoantigen-specific T cells and associated antitumor responses. mRNA encoding 20 HLA-A2-restricted neoantigens was synthesized and encapsulated into LNPs for in vivo administration. Human immunity was reconstituted in female HLA-A2 transgenic NSG mice through I.V. transplantation of CD34+ hematopoietic stem cells (HSCs) from HLA-A2+ cord blood donors. Mice showing efficient human CD45+ (hCD45+) cell engraftment were implanted with MC1 tumors into the mammary fat pad. Once the tumors were established, hu-PDX mice received Neo20 vaccine (10 μg mRNA/mouse) or vehicle/empty LNPs as control by intramuscular (I.M.) injection. Animals were treated every three days for one week, and splenocytes were harvested 7 days after the last dose for restimulation with vaccine peptides followed by IFN-γ ELISpot assay. Flow cytometry analysis of hCD45+ cells showed human reconstitution with T cells, B cells, myeloid cells, and NK cells in blood, spleen, and bone marrow (BM) collected from humanized mice at 12 weeks post-HSCs injection. Within the human T cell fraction, we identified naive, central memory and effector memory CD8+ subsets in the recipient spleen. We also confirmed the ability of T cells to produce IFN-γ, TNF-α, and granzymes A and B, demonstrating their potential to drive effective antitumor responses. Human dendritic cells differentiated into plasmacytoid and conventional subsets in the recipient BM, further supporting this model for the study of acquired immunity post-vaccination. When treated with Neo20 vaccine, hu-PDX mice mounted HLA-A2-restricted immune responses against multiple vaccine peptides as measured by ex vivo IFN-γ production. Luminex analysis of mouse serum demonstrated an increased secretion of proinflammatory cytokines and T cell activation markers in the Neo20-treated group. Overall, our findings provide evidence associated with mRNA vaccines against TNBC and support the use of hu-PDX mice as a model to preclinically validate vaccine candidates. Ongoing studies are directed to evaluate the antitumor response to Neo20 vaccine in hu-PDX mice upon repeated I.M. administration.

#3526 A novel strategy to treat advanced, late-stage tumors with real-time tumor vaccination “Online Vaccine”.

Piotr Jachimczak[1], Paul Jachimczak[2]

[1]RealTVac, Piotr Jachimczak (Individual), Wuerzburg, Germany,[2]MD Program, Gustaw Herling-Grudzinski University of Cracow, Cracow, Poland

Malignant cells acquired immunoevasive properties by numerous mechanisms allowing them to form locally invasive and metastatic tumors resistant to current therapeutic strategies. TGF-beta overexpression in tumor cells correlates with malignant progression by induction of “immune-escape phenomenon” in cancer bearing patients. Real-Time approach to generate a Tumor Vaccine *in vivo* (“Online Vaccine”) aims at avoiding this ,,immune-escape phenomenon“ by intratumoral inhibition of TGF-beta function while simultaneously and synergistically inducing an efficient anti-tumor immune response. The synergistic effects of the proposed combination of a TGF-beta inhibitor with immunostimulating cytokines IL-2 and GM-CSF (RealTVac®) upon human immune cell activation has been demonstrated *in vitro* and *in vivo*. This, four-dimensional (4D)-approach to treat advanced, late-stage, malignant tumor by use of repetitive spacio (intratumoral) - temporal (real-time) combination therapy allow the host immune system to scan in real-time all Tumor Associated Antigens (TAAs) being currently expressed by those tumor cells that are exposed to sufficient TGF-beta inhibition. The repetitive administration of the RealTVac® approach is furthermore expected to provide a continuous update of the immune response, reflecting the changing pattern of TAAs related to the dynamic intratumoral heterogeneity in malignant tumors. Thus, intratumoral immunization as applied for RealTVac® may create a new paradigm for cancer therapy in the future.

#3527 Low-intensity pulsed focused ultrasound, with microbubbles and immune adjuvants, promotes CNS epitope spreading, improving CAR T cell therapy for heterogeneous brain tumors.

Marco Gallus[1], Akane Yamamichi[1], Victor Andres. Arrieta[2], Atsuro Saijo[1], Lan Phung[1], Pavlina Chuntova[1], Su Phyu[1], Jeffrey Haegelin[1], Aishi Zhao[1], Haoyu Long[1], Senthilnath Lakshmanachetty[1], Takahide Nejo[1], Kaori Okada[1], Li Chen[2], Karl Habashy[2], Andrew Gould[2], Christina Amidei[2], Catalina Lee Chang[2], Roger Stupp[2], Michael Canney[3], Adam M. Sonabend[2], Hideho Okada[1]

[1]UCSF - University of California San Francisco, San Francisco, CA,[2]Northwestern University, Chicago, IL,[3]Carthera, Lyon, France

While chimeric antigen receptor (CAR) T-cell therapies present novel therapeutic opportunities, their clinical success has been limited when targeting malignant brain tumors, such as malignant gliomas. Marked antigenic heterogeneity of those tumors underlies their treatment resistance. Ultimately, it is crucial to promote endogenous T cell responses against tumor cells not targeted by the CAR ("epitope-spreading"). However, natural anatomical barriers and an immunosuppressive environment allow neoplasms within the brain to evade detection by the endogenous immune system. We identified that low-intensity pulsed focused ultrasound combined with microbubbles (LIPU/MB)-mediated transient blood-brain barrier (BBB) opening enables the trafficking of antigen-presenting cells from the central nervous system (CNS) into the periphery utilizing intravital two-photon microscopy, multiparametric spectral flow cytometry, bone marrow chimera mice and blood samples from glioblastoma patients treated with LIPU/MB. Because mouse glioma models do not allow us to study the immune response with the intact BBB, we created a novel transgenic mouse model in which MHC class I- or II-presented T-cell epitopes are restrictedly expressed in glial cells. A combination of LIPU/MB with immune adjuvants poly-ICLC and interleukin-2 promoted priming, activation and homing of CNS-antigen-specific naïve T cells into the brain. Genetic depletion of β2 microglobulin in systemic hematopoietic cells by bone marrow chimera showed that this phenomenon was independent of antigen presentation by peripheral bone marrow-derived cells. Furthermore, T cell activity in the brain also induced MHC-I and MHC-II epitope spreading, shown as significantly increased endogenous epitope-specific T cells in the deep cervical lymph nodes and spleen. Ultimately, the combination of LIPU/MB with poly-ICLC and IL-2 significantly enhanced the efficacy of EGFRvIII CAR T cell therapy in mice harboring heterogenous (CAR+ and CAR-) SB28 tumors. Therapeutic efficacy was associated with the presence of endogenous immune cells targeting other tumor antigens within the CNS. Collectively, our findings suggest that LIPU/MB, in combination with poly-ICLC and Interleukin-2, can overcome the inherent limitations of CAR T cell therapy by promoting CNS antigen presentation to the peripheral immune system inducing endogenous tumor-tumor-specific immune responses that enhance the efficacy of CAR T cell therapy.

#3528 Combination therapy with HIF1α/c-MET peptide vaccine and bone-modifying agents is a promising strategy for suppressing bone metastases though a synergistic effect in mouse model of metastatic triple-negative breast cancer.

JinHwa Hong, Soon Young Lim, Hye Yeon Rho, Dong Ge Ra Mi Moon, Ju Won Kim, Jiwon Lee, Kyong Hwa Park

Korea University College of Medicine, Seoul, Korea, Republic of

Metastatic triple-negative breast cancer (mTNBC) remains incurable due to its aggressiveness and high metastatic potential, particularly to bones. Bone metastases activate osteoclasts, resulting in severe pain and bone destruction. While bone-modifying agents such as denosumab (anti-RANKL) and bisphosphonate (zoledronic acid; ZA) have been commonly used to inhibit the activity of osteoclasts, they have limitations such as high cost and jaw osteonecrosis. Due to the high immunogenicity and hypoxic tumor microenvironment (TME) of TNBC, immunotherapies such as immune checkpoint inhibitors (ICIs) and cancer vaccines are widely explored as treatment options. In our previous study, we demonstrated that HIF1α/c-MET peptide vaccine combined with anti-PD-1 effectively inhibited bone metastases. However, in clinical practice, many patients exhibit low response rates due to the heterogeneity of TNBC and its diverse immune evasion mechanism, highlighting the need for new strategies to reverse the TME. The synergistic effects of bone-modifying agents, such as denosumab or ZA have not yet been explored. This study aimed to evaluate whether the combination therapy of HIF1α/c-MET peptide vaccine with bone-modifying agents will provide a synergistic effect for suppressing bone metastases. We established the bone metastatic model by intracardiac inoculation of M6-bone cells in C3(1)Tag mouse. Mice were vaccinated with HIF1α/c-MET peptide vaccine weekly for three times and treated anti-PD-1 or anti-RNAKL or ZA. Control group received PBS vaccination and treated with IgG. Weekly In vivo imaging showed that combination therapy with HIF1α/c-MET peptide vaccine and bone-modifying agents reduced metastases. Through micro-CT and bone volume-to-total volume analysis, bone metastasis and destruction were reduced in the combination therapy of peptide vaccine with bone-modifying agents compared to the anti-PD-1 combination group. To understand the TME changes more comprehensively, immunofluorescence staining of HIF1α and c-MET is ongoing to confirm the inhibitory effects of the peptide vaccine on targeted oncogenic protein. Osteoclast activity is also being quantitatively assessed by analyzing the ratio of osteoclast surface to bone surface using tartrate-resistant acid phosphatase (TRAP) staining. To determine whether combination therapy can modulate the TME, tumor infiltrating lymphocytes (TILs) are being evaluated by assessing the expression of CD4 and CD8. Although further analysis of TME is necessary, we propose that the combination therapy of HIF1α/c-MET peptide vaccine with bone-modifying agents may provide a promising strategy for suppressing bone metastases in mTNBC.

#3530 Utilizing a humanized HLA-A2.1 mouse model for non-clinical studies of mRNA vaccines.

Leila Kokabee, Zhenlan Niu, Jing Guo, Shuaiqiang Zhang, Zhiyuan Shen, Hui Xu

Biocytogen, Waltham, MA

Substantial genetic discrepancies exist between the major histocompatibility complex (MHC) systems of animals and the human leukocyte antigen (HLA) system. The utilization of HLA humanized mouse model systems constitutes a superior methodology for examining fundamental mechanisms associated with HLA-A-related vaccine development and HLA-A-restricted immune responses against diseases such as infections and cancers in humans.

To study both *in vitro* and *in vivo* the requirements for shaping an effective T cell response against clinically relevant cancer targets such as NY-ESO-1, WT1 and other newly discovered targets, Biocytogen generated a C57BL/6 background HLA-A2.1 humanized mouse model that is restricted to the human HLA-A2. This humanized mouse model was utilized to evaluate immune responses to a variety of cancer peptide and mRNA vaccines, including analyses of immune cell populations, cytokine production, and *in vivo* anti-tumor efficacy.

Immunoprofiling analysis of this novel humanized HLA-A2.1 mouse strain revealed that the introduction of the HLA-A2.1 gene significantly influences the immune cell composition in these humanized mice. Following vaccination with various cancer-specific epitopes, HLA-A2.1-restricted, epitope-specific cytotoxic T lymphocytes (CTLs) were induced in the B-HLA-A2.1 mouse model. Furthermore, we established an mRNA vaccination treatment model in B-HLA-A2.1 mice, demonstrating a synergistic antitumor effect when combined with the mRNA vaccine. These findings indicate that the B-HLA-A2.1 mouse model serves as a powerful research tool, not only for investigating HLA-A2.1-restricted CTL responses but also for the preclinical evaluation of vaccines.

We have established a promising preclinical research model using HLA-A2.1 humanized mice. This model could accelerate the identification of novel HLA-A2.1-restricted epitopes and facilitate vaccine development, while also supporting the study of HLA-A2.1-restricted responses against cancers in humans.

#3531 Development of a mRNA-LNP vaccine for Lynch syndrome.

Yuri Koboziev[1], Ligia A. Pinto[2], Robert H. Shoemaker[3], **Jason D. Marshall**[1]

[1]NCI-Frederick/Leidos Biomed. Research, Inc., Frederick, MD,[2]Vaccine, Immunity and Cancer Directorate, NCI-Frederick/Leidos Biomed. Research, Inc., Frederick, MD,[3]Division of Cancer Prevention, NCI-Frederick, Frederick, MD

Lynch syndrome (LS) is a genetic disorder in which colorectal cancer is caused by mutations in DNA mismatch repair enzymes. Prophylactic mRNA vaccines have become the subjects of increasing interest as platforms for the potential prevention of such disorders. Several neoantigen sequences caused by defective mismatch repair were identified as expressed in a GEMM model of Lynch Syndrome, VCMsh2, and were also confirmed to be immunogenic for T and B cell responses in the background strain C57BL/6, as measured by IFN-g ELISPOT and ELISA in peptide/adjuvant-vaccinated mice. Vaccine mRNA constructs coding for 3 neoantigen peptides were designed with the HA signal peptide sequence and BioNTech 3' and 5' UTR regions and synthesized on the BioXp 3250 platform using pseudouridines. Monovalent mRNA vaccines were generated by encapsulating LS neoantigen mRNA into lipid nanoparticles (LNPs) on the NanoAssemblr Spark platform. High mRNA encapsulation efficiency was verified, and LNP size, polydispersity index, concentration, and zeta potential passed vaccine specifications. In vitro translation of vaccine mRNAs in transfected HEK-293 and RAW 264.7 cells were confirmed by FLAG Western Blot and mass spectrometry. The mRNA vaccines were administered 2X monthly via IM route to C57BL/6 mice. Substantial splenic CD4 T cell responses to the LS neoantigen peptides Nacad, Maz, and Senp6 were detected although lower than T cell responses to the corresponding peptide/adjuvant vaccine. In an attempt to adjuvant the mRNA vaccine, the TLR9 agonist CpG-2395 was encapsulated into LNPs via methods identical to mRNA packaging. In vitro testing on cell lines revealed that the CpG-LNPs enhanced Luciferase mRNA expression in RAW264.7 macrophages, triggered TLR9 signaling in mTLR9-HEK Blue reporter cells, and promoted IL-12 production by transfected RAW264.7. When LS neoantigen mRNA-LNPs were co-vaccinated with CpG-LNPs in C57BL/6 mice, T cell responses were augmented to be comparable to peptide-vaccinated T cell responses. Our current efforts involve optimizing the LNP lipid composition for uptake efficiency, optimizing the in vivo trafficking of the LNPs, and determining a suitable method for adjuvanting mRNA vaccines to boost T and B cell responses before evaluation in the VCMsh2 Lynch Syndrome GEMM model.

#3532 Development of mouse models for preclinical evaluation of cancer mRNA vaccines.

Hongyan Sun[1], Jialu Fan[1], Yunlong Jiang[1], Jianming Xu[1], Xiang Gao[1], Jun Wang[2]

[1]GemPharmatech Co., Ltd, Nanjing, China,[2]GemPharmatech Co., Ltd, San Diego, CA

Since the successful development of vaccines against COVID, mRNA vaccine strategy showed superior efficacy and lower manufacturing costs compared to conventional vaccine approaches. The broad usage of mRNA vaccines was initially hampered by the early degradation before reaching to the target tissues. With the development of improved delivery systems to overcome the aforementioned problems *in vivo,* mRNA vaccine has become a promising strategy against various diseases including cancer. However, wild type mouse models are not suitable for the evaluation of cancer mRNA vaccines due to the fact that effective MHC-restricted antigen presentation is critical for mRNA vaccines to work *in vivo* in order to trigger a robust cellular and humoral immune response.

GemPharmatech has developed a series of MHC-humanized mouse models to better evaluate the *in vivo* immunogenicity, efficacy and safety of mRNA vaccines, eliminating the inconsistencies in antigen peptide recognition and immune response due to the MHC structural discrepancies between humans and mice.

Human immune cells can be reconstituted in immunodeficient mice (NCG) engrafted with human hematopoietic stem cells (HSC). However, since the species differences that murine cytokines provide limited support to human immune cells, HSC-NCG mice lack sufficient numbers of functional DC cells and the T cells are functionally defective because they are educated by murine MHC molecules. To address these two issues, we have introduced human HLA-A2 molecules into HSC-NCG-M mice, which is the ideal model for human myeloid cell reconstitution. HSC-NCG-M-hHLA-A2 mice have been developed to better reconstitute DCs and functional T cells educated by human MHC I molecules. An improved quantity of DC cells can be detected in HSC-NCG-M-hHLA-A2 mice. In addition, HLA-A2-restricted immune responses can be observed in HSC-NCG-M-hHLA-A2 mice. The HSC-NCG-M-hHLA-A2 mouse model is an ideal mouse model for the evaluation of *in vivo* immunogenicity and anti-tumor efficacy of mRNA cancer vaccines.

In addition to the humanized models, the adoptive cell transfer mouse model, in which splenocytes from immunocompetent MHC-humanized mice are injected into tumor-bearing (HLA matched) immunodeficient mice, is also an excellent choice, utilizing the complete immune system of immunocompetent mice and the susceptibility of immunodeficient mice to human tumor cells. This is the ideal model for the evaluation of TAA targeting mRNA vaccines, which is time and cost efficient without the need for cell line modification.

In conclusion, we have developed a series of MHC-humanized mouse models to provide more options for preclinical evaluation of tumor vaccines.

#3533 Claudin 6 vaccines effectively inhibit tumor growth in a syngeneic mouse colon cancer model.

N. Wang, M. Toh, H. Wang;
CK Life Sciences Development Limited, Hong Kong, China

Human Claudin 6 (CLDN6) is a member of the Claudin family of proteins, which play a crucial role in the structure and function of tight junctions. The expression of CLDN6 is restricted to stem cells and embryonic tissues. In human adults, CLDN6 mRNA is only detected at very low levels in testis, pancreas, and placenta. However, several types of cancers have been found to express CLDN6 at high levels, suggesting that CLDN6 is an ideal tumor-associated antigen and target for immunotherapy, albeit its precise role in cancer remains to be defined. Here, we designed and produced vaccine proteins consisting of multiple human B- and/or T-cell epitopes of CLDN6, which were selected from computer-predicted sequences, fused with an immune enhancer/carrier, and investigated their immunogenicity and anti-cancer activities in mice. First, C57BL/6 mice were vaccinated subcutaneously once a week for 5 times with the different vaccine proteins. CpG oligodeoxynucleotides were used as the vaccine adjuvant. Using the IFNγ ELISpot assay, we found that splenocytes collected from mice vaccinated with vaccine proteins containing T-cell epitopes displayed potent IFNγ release activity after stimulation with T-cell epitope peptides, suggesting that these CLDN6 vaccine proteins may inhibit the growth of CLDN6-expressing cancer by activating specific cytotoxic T-cells to destroy cancer cells. To explore this potential, we investigated the anti-cancer activity of the vaccine proteins by monitoring the tumor growth of CLDN6-humanized MC38 mouse colon cancer cells inoculated subcutaneously in vaccinated C57BL/6 mice for 4 weeks. We found that the growth of tumors was inhibited 98-100% (compared to the adjuvant-only control group) in two groups of mice (N=8 in each group) vaccinated with proteins containing multiple T-cell epitopes. In the first group, all 8 tumors disappeared within 4 weeks. In the second group, 7 of 8 tumors disappeared within 3 weeks and the last tumor stopped growing after the second week. In mice treated with adjuvant only, all tumors (N=8) displayed the same growth rate and reached an average of ~1,000 mm^3 in volume, similar to mice treated with PBS. Collectively, our results demonstrate that CLDN6 vaccines effectively inhibited the growth of CLDN6-expressing cancer cells in mice. These findings suggest that CLDN6 protein vaccines hold promise for treating CLDN6-expressing cancers in humans, and this potential will be further explored in future studies.

#3534 TPIPE, a pipeline for the identification and functional assessment of therapeutic cancer vaccine candidates.

C. Schifflers[1], M. Vlaming[1], E. Sidot[1], J. Lefevre[1], E. Pepermans[2], E. Boelen[1], S. Pattyn[1];
[1]IQVIA, Gosselies, Belgium, [2]Immunespec, Niel, Belgium

Therapeutic vaccination is a promising strategy for personalized cancer therapy. Using this approach, anadaptive T cell mediated immune response can be mounted against peptides presented by cancer cells,allowing for a systemic cancer cell eradication and immune memory formation.A key challenge for this approach is the identification of target peptides that are ubiquitously presented bycancer cells and capable of eliciting a cytotoxic T cell response. In this context, IQVIA Laboratories, In Vitro Immunology (formerly known as ImmunXperts) developed a pipeline for the Target Peptide Identification and the (functional) Peptide Evaluation (*TPIPE*) using *in vitro* generated peptide-specific T cells. This approach can be customized to be applicable to various therapeutic vaccine platforms. In brief, *in silico* prediction tools are used for the identification and pre-selection of candidate peptides.Peptide presentation is assessed by immunopeptidomics/MHC-associated peptide proteomics (MAPPs) in collaboration with *ImmuneSpec*. Upon identification of relevant targets, peptide-specific T cells aregenerated *in vitro* by co-culturing T cells with peptide-loaded/transfected dendritic cells. Peptide-reactivityis monitored over time by assessing the ability of the antigen-specific T cells binding to multimers via flowcytometry and in peptide re-stimulation FluoroSpot assays. The cytotoxic functionality of *in vitro* enriched peptide-specific T cells is subsequently evaluated in live-cell imaging, flow cytometry or colorimetry-based killing assays. Taken together, the *TPIPE* pipeline provides a customizable approach of the identification and functionalevaluation of therapeutic vaccines. TPIPE relies on high-quality pre-screened PBMCs, as provided by IQVIA Laboratories, In Vitro Immunology's own biobank, covering a wide range of 4-digit HLA-typed healthy donor preparations.

#3535 Targeting cervical cancer: VC-2 as a vaccine and treatment for HPV infections.

Mohammed T. Hussain, Robert Ellis, Kiran Fida, Mariano Carossino, Brent A. Stanfield

Department of Pathobiological Sciences, Louisiana State University, Baton Rouge, LA

Cervical cancer is a major cause of mortality worldwide and has been attributed to long-lasting (persistent) infections with the human papillomavirus (HPV). Although more than 50 types of HPVs are known the infect the genital tract, fewer than a dozen of them are regularly linked to cervical cancers leading to these types being designated as “high risk” HPVs. The infectious nature of HPVs procures the need for preventative vaccines against cervical cancer, and current vaccines such as Gardasil-9 and Ceravix achieve high efficacy in protection. However, once infections are established, preventative vaccines are inefficient in treating the malignancies. In HPV-associated tumors, the E7 oncoprotein is an ideal target for immunotherapeutic strategies and has been shown to be a likely target to treat cervical cancer. HSV, particularly HSV-2, often coexists with HPV due to similar transmission routes. Studies suggest that HSV infections may enhance the oncogenic effects of HPV by increasing inflammation, immune system modulation, and promoting a more favorable environment for HPV persistence and progression to cancer. Engineered Herpes Simplex Virus Type-1 (HSV-1) has been a promising candidate in treating established cancers and has been shown to be efficacious in improving anti-tumors responses and controlling tumor metastasis. Therefore, in this study, we aim to utilize the engineered HSV-1 strain (VC-2) to treat established cervical cancers and evaluate its efficacy as a prophylactic vaccine. The mice inoculated with the Tal-3 cervical cancer cell line show metastasis in the lungs and formation of large tumors. Moving forward, we would like to conduct therapeutic and prophylactic studies evaluating the reengineered VC-2 towards treating cervical cancer.

#3536 Synergistic effects of combined PD-L1 and B7-H3 fusion protein vaccination in a mouse model of pancreatic cancer.

Kenneth Nansheng Lin, Melvin Toh, Hong Wang

CK Life Sciences Limited, Hong Kong, Hong Kong

Background: Expressing immune checkpoint ligands is a mechanism for cancer cells to escape immune surveillance. Inhibiting these checkpoints effectively disrupts their escape mechanisms, improving cancer treatment outcomes. Various strategies have been developed to induce immune checkpoint inhibition. In this study, we developed cancer vaccines targeting checkpoint regulators, PD-L1 and B7-H3, and studied the anti-cancer activity in a syngeneic mouse cancer model using Panc02 cancer cells, known for their expression of both PD-L1 and B7-H3.
Methods: Vaccines targeting PD-L1 and B7-H3 were recombinant proteins consisting of immune enhancers fused with mouse PD-L1 and B7-H3 extracellular domains, respectively, and produced in E.coli. C57BL/6 mice were vaccinated multiple times with vaccine protein(s) adjuvanted with Poly(I:C) and Freund's Adjuvant. Vaccine-induced humoral responses were measured by ELISA using serum samples collected from the mice. Anti-cancer activity was investigated using mice vaccinated four times followed by subcutaneous implantation of Panc02 cells and additional four vaccinations. Tumor growth was recorded. Tumor-infiltrated immune cells were characterized by immunofluorescence staining.
Results: PD-L1 and B7-H3 vaccines induced specific antibodies against PD-L1 and B7-H3. Serum samples collected from PD-L1-vaccinated mice displayed neutralizing activity, blocking the binding of PD-L1 to PD-1. In mice inoculated with Panc02 cells, vaccination with PD-L1, B7-H3, and PD-L1/B7-H3 mixture inhibited tumor growth by 10%, 30%, and 66%, respectively. In the tumors, vaccination with the PD-L1/B7-H3 mixture increased CD4+ T cells significantly, indicating that vaccine-induced immune cell infiltration plays a critical role in tumor inhibition.
Conclusions: Our results demonstrate that vaccination is an effective strategy for inducing neutralizing antibodies that inhibit immune checkpoints in cancer treatment. The most significant tumor growth inhibition observed with the combination of PD-L1 and B7-H3 vaccination underscores the necessity of simultaneously targeting multiple checkpoint regulators in cancers that express these proteins.

#3537 Development and validation of humanized HLA-A11.1 mouse model for preclinical evaluation of novel peptide vaccines.

Dong Han, Leila Kokabee, James Jin, Zhenlan Niu, Ying Zhao, Shuaiqiang Zhang, Zhi Zhang, Hui Xu

Biocytogen, Waltham, MA

The Major Histocompatibility Complex (MHC) is responsible for presenting diverse antigenic peptides and initiating T cell-mediated immune responses. Given the significant genetic differences between animal MHC systems and the human leukocyte antigen (HLA) system, HLA humanized mouse models offer an efficient *in vivo* system for HLA-A-related vaccine development and for studying HLA-A-restricted response mechanisms in humans.

In this study, we present a novel B-HLA-A11.1 mouse model and have thoroughly investigated its phenotypic and immunological attributes. Our findings indicate that the blood routine and biochemistry of B-HLA-A11.1 mice are comparable to those of wild-type mice, suggesting that the humanization of HLA-A11.1 does not adversely alter blood cell composition or morphology, nor does it affect the health status of organs such as the heart, liver, or kidneys. Collectively, these data demonstrate that B-HLA-A11.1 mice develope normally and do not exhibit any evidence of spontaneous pathology due to the humanization of HLA-A11.1. To gain a deeper understanding of the role of MHC molecules, we extended our research using a humanized mouse model to assess immune responses against various cancer target peptides.

In summary, our data indicate that humanized HLA-A11.1 mice provide a powerful preclinical model for *in vivo*/*ex vivo* evaluation of novel peptide and mRNA vaccines.

#3538 The development of neoantigen-derived peptide vaccine driven by AI epitope design and computer assisted epitope enhancement.

Chi Han Samson Li[1], Hong Wang[1], Kin Tak Chan[1], Genwei Zhang[2], Jiewen Du[2], Lipeng Lai[2], Melvin Toh[1]

[1]CK Life Sciences Development Limited, Hong Kong, Hong Kong,[2]Xtalpi, Beijing, China

Background and objective: Neoantigens are the ideal targets for developing cancer vaccine for cancer treatment. However, the development of cancer vaccines is often hindered by poor antigen presentation of the vaccine peptide by major histocompatibility complex (MHC) molecules. Our AI epitope prediction model showed prediction accuracy superior to several state-of-the-art algorithms for MHC-I antigen presentation. As studies showed that amino acid substitution in key positions of MHC-I epitope provides strong activating signals to cytotoxic T cells, we aimed to develop MHC-I restricted neoantigen vaccines by coupling our AI model with computer-assisted modification of neoantigen-derived epitopes to improve their HLA presentation probability and immunogenicity.
Methods: The AI model was used to analyze some common TP53 neoantigen sequences and predict epitopes for HLA-A*02:01. The epitopes underwent computer-assisted saturation mutagenesis that simulated single amino acid substitutions to obtain the associated presentation score changes. Peptide sequences with improved presentation scores were synthesized, and their binding affinities with HLA-A*02:01 were analyzed by surface plasmon resonance (SPR). The peptides were immunized into the HLA-A*02:01-humanized C57BL/6 mice, and their immunogenicity was determined by mouse interferon γ (mIFNγ) ELISpot assay and intracellular cytokine staining (ICS) using splenocytes of the mice.
Results: Our AI model predicted epitopes from common TP53 neoantigens that showed high HLA-A*02:01 presentation probability. Through saturation mutagenesis, dominant mutations with at least 5% increase of presentation probability were screened out. Seven novel modified peptides were synthesized and showed high binding affinity with HLA-A*02:01/ß2-microglobulin heterodimer. After these peptides were pooled to immunize HLA-A*02:01-humanized mice, mIFNγ ELISpot assay showed that 6 out of the 7 modified peptides reactivated splenocytes from the peptide-immunized mice, while mIFNγ ICS showed that $CD8^+$ cytotoxic T-cells were activated. The splenocytes were then restimulated with the native neoantigen peptides corresponding to the modified peptides. One native neoantigen peptide reactivated T-cells from the immunized mice, suggesting that immunization of the modified peptides could induce cytotoxic T-cell response against the native neoantigens.
Conclusion: Our study demonstrates a highly efficient approach by coupling MHC-I presentation prediction AI model and computer assisted saturation mutagenesis to design single amino acid substituted MHC-I peptides derived from neoantigens. Immunization of the peptides induced cytotoxic T-cell responses which may inhibit the growth of cancer cells carrying such a neoantigen. This novel approach to developing cancer vaccines will be further tested in future studies.

#3540 Proteolysis-targeting vaccines for robust combination immunotherapy of melanoma.

Qiyan Wang[1], Ting Su[2], Furong Chen[3], Shurong Zhou[1], Xiang Liu[1], You Xu[1], Ri Tang[1], Shimiao Liao[1], Jordan Dailey[1], Guolan Xiao[1], Guizhi Zhu[1]

[1]University of Michigan, Ann Arbor, MI,[2]State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, College of Biologica, Donghua University, Shanghai, China,[3]School of Life Sciences, Shanghai University, Shanghai, China

Introduction: Protein/peptide vaccines show great promise for combination tumor immunotherapy with immune checkpoint blockade (ICB). However, conventional protein/peptide vaccines often elicit insufficient antitumor T cell responses, resulting in suboptimal therapeutic outcomes. Here, we introduced proteolysis-targeting vaccines (PROTAVs), which integrate melanoma protein/peptide antigens, E3 ligase-binding ligands, and linkers. This design enhances antigen proteolytic processing and cross-presentation, resulting in robust activation of T cell responses. By co-delivering PROTAVs with a bi-adjuvant system through lipid nanoparticles (LNPs), we aim to significantly improve the quantity and quality of specific $CD8^+$ T cells, thereby enhancing the efficacy of ICB-based combination therapies.

Method: Ovalbumin (OVA) was employed as a model antigen, with pomalidomide serving as the E3 ligase ligand, conjugated via a series of linkers. Linker optimization was conducted using an in vivo T-cell response assay. Protein degradation and antigen presentation efficiency were assessed using Western blotting, co-immunoprecipitation, and flow cytometry. Leveraging these findings, PROTAV-TgT (Trp2-gp100-Trp1), incorporating melanoma-specific antigens, was strategically developed to enable a comprehensive evaluation of its immunological mechanisms and therapeutic potential.

Result: A series of PROTAV-OVA constructs incorporating linkers of varying water solubility and lengths were synthesized, eliciting robust $SIINFKEL^+$ T cell responses in mice, with the EG_4 linker achieving superior immunogenicity, inducing up to 18% $SIINFKEL^+$ T cells. Mechanistic studies demonstrated that PROTAV-OVA facilitated enhanced antigen degradation, as evidenced by increased DQ-OVA fluorescence and OVA-ubiquitin colocalization in dendritic cells. Additionally, it significantly improved antigen presentation, as shown by elevated H_2K^b-SIINFEKL staining both in vitro and in vivo. Based on these, the PROTAV-TgT was designed, incorporating melanoma-specific antigens and co-delivery with CpG using SM-102 LNPs. Immunization with PROTAV-TgT induced 4-fold increasing gp100-$specific^+$ T cell responses, compared with TgT alone. In the B16F10 model, PROTAV-TgT combined with ICB resulted in complete tumor regression (CR) in 50% of mice treated. Remarkably, in the aggressive SM1 $Braf^{V600E}$ melanoma model, this combination therapy achieved 100% CR by day 24, an outcome not observed in other group at the time point.

Conclusion: PROTAVs effectively elicit robust $CD8^+$ T cell responses and alleviate tumor immunosuppression, enhancing the efficacy of ICB combination immunotherapy for melanoma. These findings highlight the potential of PROTAVs as a versatile platform for developing cancer therapeutic vaccines, offering broad applicability for robust immunotherapy across various cancer types.

#3541 A novel chimeric RNA as a source of neoantigens in CRC.

Sarah A. Lynch[1], Amanda M. Lulu[2], Samir Lalani[3], Justin Elfman[3], Craig L. Slingluff[4], Hui Li[1]

[1]Pathology, University of Virginia, Charlottesville, VA,[2]Pediatrics, University of Virginia, Charlottesville, VA,[3]Biochemistry and Molecular Genetics, University of Virginia, Charlottesville, VA,[4]Surgery, University of Virginia, Charlottesville, VA

Colorectal cancer (CRC) is the second-leading cause of cancer-related death worldwide, affecting over 100,000 men and women in the United States every year. There is an unmet need for early biomarker discovery and the identification of novel therapeutic targets in CRC. Chimeric RNAs and their protein products are well-established as ideal biomarkers and drug targets for malignant cancers. Additionally, cancer-specific fusion proteins present attractive targets for neoantigen vaccines. Using RNA-seq from The Cancer Genome Atlas (TCGA) database, we have identified a chimeric RNA prevalent in approximately 50% of CRC samples, but absent in non-cancer colon tissue. This transcript correlates with worse overall survival, is expressed as early as stage I CRC, and is present in both microsatellite stable and instable disease. This chimeric RNA arises from readthrough transcription and cis-splicing between adjacent genes, producing a previously unidentified chimeric protein. Importantly, this protein includes a splice variant of the 5' gene, leading to a frameshift in the 3' gene and resulting in a novel 101 amino acid peptide sequence. This sequence is not detected by mass spectrometry in any healthy tissue but is present in multiple CRC cases within the Clinical Proteome Tumor Analysis Consortium (CPTAC) data portal. Using an antibody generated against the frameshift sequence, we have confirmed this protein is expressed in patient CRC samples, regardless of disease stage, sex, age, race, or microsatellite instability status. Finally, we have demonstrated that T cells from healthy donors and patients with CRC exhibit activation following co-culture with autologous dendritic cells pulsed with peptides derived from this protein. Additionally, these T cells display cytotoxicity against HLA-matched CRC cell lines. These findings highlight the potential of chimeric RNAs and their protein products as both biomarkers and therapeutic targets, presenting a previously unexplored class of tumor antigens as an innovative strategy for improving patient outcomes.

#3542 Nanofilament immunotherapy as an antigen-agnostic cancer vaccine.

Kevin Neil[1], Samuel Genier[1], Marie-Eve Poisson[1], Hugo Giguere[2], Lauren Daniel[2], Julie Douchin[1], Lee-Hwa Tai[2], Sebastien Rodrigue[3], Jean-Francois Millau[1]

[1]TATUM Bioscience, Sherbrooke, QC, Canada,[2]IRCUS, Universite de Sherbrooke, Sherbrooke, QC, Canada,[3]Biology, Universite de Sherbrooke, Sherbrooke, QC, Canada

Checkpoint inhibitors revolutionized cancer treatment by potentiating antitumor immune responses. However, many patients do not respond to these therapies, often due to a lack of pre-existing immune response against cancer cells. Personalized cancer vaccines promote cancer cell antigen recognition and mount antitumor immune responses, thus helping to improve response rates. However, personalized cancer vaccine interventions imply complex and costly procedures that make the technology difficult to scale. As an alternative to personalized cancer vaccines, we established multimodal nanofilament immunotherapy as an antigen-agnostic *in situ* cancer vaccine modality. Through genetic engineering of the M13 bacteriophage, nanofilaments displaying combinations of therapeutic agents were generated to steer the immune system against cancer cells. TAT003, a multimodal nanofilament combining the natural adjuvant properties of M13 with the display of anti-PD-L1 and IL-2 molecules, was developed to bind the surface of cancer cells and transform them into immunological targets. After validation of TAT003 biological activities *in vitro* and assessment of biodistribution, the potency of the candidate was evaluated upon intratumoral administration in syngeneic mouse models both as a single agent as well as in combination with PD-1 blockade therapy. The mode of action of TAT003 was also characterized using cytokine paneling, immune profiling, and T-cells activation assays. TAT003 nanofilaments simultaneously displayed biologically active anti-PD-L1 and IL-2 molecules. Upon intratumoral injection, TAT003 anchored durably in the tumor, which limited systemic exposure to the drug. TAT003 profoundly remodeled the tumor microenvironment of injected lesions, where it sparked a strong myeloid cell infiltration, and promoted the infiltration of T-cells in non-injected contralateral lesions, promoting T-cells infiltration. This translated into potent tumor regression of both lesions in multiple cancer models, and potentiated PD-1 blockade therapy. TAT003 treatment induced the expansion of cancer-cell specific effector T cells systemically, providing a long-lasting antitumor vaccine response. Multimodal nanofilament immunotherapy is a novel approach to mount systemic antitumor immune responses *in situ* by physically coupling large immunostimulatory molecules to cancer cells. TAT003 induced marked tumor regressions by leveraging synergies between the therapeutic agents displayed on its surface while offering a favorable tolerability profile. These results establish multimodal nanofilaments as an innovative and versatile immunotherapy platform for developing *in situ* cancer vaccines.

#3543 Combinatorial mRNA-LNP vaccines for triple-negative breast cancer.

Sara L. Woessner[1], Xiaolei Cai[2], Caroline Kellogg[3], Jun Huang[2]

[1]Committee on Cancer Biology, University of Chicago, Chicago, IL,[2]Pritzker School of Molecular Engineering, University of Chicago, Chicago, IL,[3]University of Chicago, Chicago, IL

Breast cancer is the most commonly-diagnosed tumor in women, and there remains a need for effective treatment options for metastatic disease. mRNA-lipid nanoparticle (LNP) vaccines targeting tumor neoantigens are a potential avenue for treating triple-negative and basal-like breast cancers. Using established whole-exome and RNA-sequencing data, we identified a combination of neoantigens that induce CD4 and CD8 T cell responses. Administration of mRNA-LNPs encoding 4 neoantigens significantly delayed growth of 4T1 murine syngeneic tumors. When the vaccine is combined with IV injection of engineered IL7 and IL12, the median overall survival is increased by 14 days. Taken together, these improvements to current breast cancer vaccination approaches may represent a way to induce tumor regression and clearance in treatment-refractory disease.

#3544 Synergistic efficacy of a PSMA DNA vaccine and PD-1 inhibition in anti-tumor immunity against prostate cancer.

Perry Ayn Mayson Maza, Leslie Flores, Dana Rae Cyril-Ramirez, Dafei Chai, Delana Bui, Dan Zhao, Junhao Wang, Yifei Wang, Haiwei Ni, Xiaohui Xie, Xinfang Yu, Yong Li

Baylor College of Medicine, Houston, TX

Prostate cancer (PCa) remains one of the leading causes of cancer-related morbidity and mortality in men. A promising target for immunotherapy is prostate-specific membrane antigen (PSMA), a transmembrane protein predominantly overexpressed in PCa, with expression levels correlating with tumor aggressiveness and invasiveness. In this study, we evaluated the prophylactic and therapeutic potential of a plasmid DNA vaccine targeting PSMA for the treatment and prevention of prostate cancer using the RM-1 mouse PCa cell line engineered to overexpress human PSMA. A DNA plasmid which encoded the human PSMA antigen, was administered prophylactically, prior to tumor challenge, or therapeutically, following tumor establishment using electroporation. In the prophylactic approach, vaccination resulted in significant tumor volume reduction and marked tumor growth inhibition. Moreover, the immune response was robust, characterized by increased infiltration of tumor-infiltrating lymphocytes (TILs), including CD8+ cytotoxic T cells, CD4+ helper T cells, B cells, NK cells, neutrophils, macrophages, and dendritic cells within the tumor microenvironment. Immune cell activation was also observed in the spleens, indicating systemic immune priming. Peptide-MHC tetramer staining revealed a significant increase in PSMA-specific CD8+ T cells in the spleen of vaccinated mice, confirming the induction of a targeted immune response against PSMA-expressing tumor cells. Additionally, PSMA-specific antibodies were significantly increased in the serum of vaccinated mice, suggesting humoral immunity in response to the vaccine. In the therapeutic approach, vaccination with the PSMA DNA vaccine alone slowed tumor growth. Like the prophylactic approach, therapeutic vaccination resulted in increased TILs and immune activation in the spleen. Upon the addition of PD-1 antibody, tumor growth was significantly inhibited, resulting in a greater reduction in tumor volume. The combination of PSMA vaccination and PD-1 blockade led to a more potent anti-tumor immune response, characterized by further activation and expansion of PSMA-specific CD8+ cytotoxic T cells, increased production of PSMA-specific antibodies, and enhanced infiltration of immune cells. These findings underscore the potential of PSMA-targeted DNA vaccines in combination with immune checkpoint blockade (e.g., anti-PD-1) as a promising preventive and therapeutic strategy for PCa, highlighting their ability to enhance immune surveillance and induce more effective anti-tumor immunity

#3545 Enhancing anticancer immunity with SARS-CoV-2 B cell epitope-guided neoantigen nanovaccines.

Chengyi Li, **Fang Ke**, Shuai Mao, Zera Montemayor, Mohamed Dit Mady Traore, Alejandra Duran Balsa, Mahamadou Djibo, Neha Karekar, Hanning Wen, Wei Gao, Duxin Sun

University of Michigan, Ann Arbor, MI

Background: Current neoantigen cancer vaccines primarily rely on dendritic cell- and macrophage-mediated antigen presentation to activate T cell immunity. However, the potential of incorporating B cell-mediated antigen presentation into these vaccines to enhance CD4 and CD8 T cell responses has not been fully explored.

Methods: We developed $B_{SARS}T_{NeoAg}$Vax, a SARS-CoV-2 B cell epitope-guided neoantigen peptide or mRNA cancer nanovaccine, designed to leverage B cell-mediated antigen presentation. $B_{SARS}T_{NeoAg}$Vax was tested in three mouse cancer models (melanoma, pancreatic cancer, and breast cancer) using two types of T cell tumor neoantigens (peptide or mRNA) and two types of nano delivery systems (I or lipid nanoparticles, LNP). SARS-CoV-2 B cell epitopes were conjugated on the surface of I or LNP to facilitate crosslinking with the B cell receptor (BCR) on SARS-CoV-2-specific B. Immune responses and therapeutic efficacy of $B_{SARS}T_{NeoAg}$Vax were compared with existing neoantigen vaccines.

Results: $B_{SARS}T_{NeoAg}$Vax enabled SARS-CoV-2 B cell-mediated antigen presentation to tumor-specific CD4 T cells, significantly enhancing the activation of tumor-specific follicular and non-follicular CD4 T cells. This interaction also promoted B cell-dependent tumor-specific CD8 T cell immunity, resulting in robust antitumor responses. Importantly, the efficacy of $B_{SARS}T_{NeoAg}$Vax was independent of antibody production, as the SARS-CoV-2 B cell epitopes on the vaccine generated anti-SARS-CoV-2 antibodies that lacked anticancer efficacy. In the mouse cancer models, $B_{SARS}T_{NeoAg}$Vax demonstrated superior efficacy, showing improved tumor control and enhanced immune activation compared to current neoantigen vaccines.

Conclusion: Our findings establish a universal platform for cancer immunotherapy, utilizing SARS-CoV-2 B epitope-guided neoantigen nanovaccines to enhance CD4 and CD8 T cell antitumor immunity through viral-specific B cell-mediated antigen presentation. This approach holds significant promise for improving the efficacy of neoantigen-based cancer vaccines across diverse tumor types.

#3547 Therapeutic efficacy of genetically modified CD34-derived allogeneic dendritic cell cancer vaccine platform in a pancreatic tumor model.

Jose Dominic Gonzalez[1], Sara Huerta-Yepez[2], Neha Sheik[1], Senay Beraki[1], Elango Kathirvel[1], Ariel Rodriguez Frandsen[1], **Saleemulla Mahammad**[1], Lu Chen[1], Francois Binette[1], David Weinstein[1], Anahid Jewett[2]

[1]Renovaro Biosciences, Los Angeles, CA,[2]Division of Oral Biology and Medicine, School of Dentistry, University of California, Los Angeles, CA

Background: Pancreatic cancer is the fourth leading cause of all cancer-related deaths. Dendritic cells are the primary antigen presenting cells that have the capacity to activate both innate and adaptive immune systems. Although peripheral blood derived mature dendritic cell (DC)-based vaccines have shown the potential to activate multiple anti-tumor immune responses *in-vitro* and in animal studies, they have yielded disappointing results in clinical trials. Here we report on a novel allogeneic dendritic cell cancer vaccine (DCCV) platform derived from CD34+ Hematopoietic Stem Cells (HSCs) that are transduced with lentiviral vector encoding CD93, CD40L, and CXCL13. We are reporting therapeutic efficacy of such allogeneic dendritic cell cancer vaccine therapy platform in a pancreatic tumor bearing humanized mouse model.

Methods: We have developed a robust manufacturing method to generate large numbers of engineered allogenic DCs overexpressing CD93, CD40L, and CXCL13. A tri-cistronic lentiviral vector was constructed to co-express these genes. The engineered DCs were evaluated for the expression of transgenes and functionality via flow cytometry, PCR, and ELISA. Antigen education of engineered DCs was performed via coculturing them with whole tumor lysate. *In vivo* therapeutic efficacy was tested in Bone-Liver-Thymus (BLT) and PBMC humanized NOD-SCID gamma (NSG) mouse models with Mia Paca-2 (MP2) orthotopic pancreatic tumors. Chromium release assay, E-sight and IHC methods were employed to quantify the cytotoxicity and cell proliferation of T cells and infiltration of immune cells into the tumors upon treatment with the engineered DC.

Results: We have investigated the therapeutic efficacy of our engineered DCCV in curtailing pancreatic tumor growth. Tumor-bearing Humanized mouse models treated with engineered DCCV demonstrated significant tumor regression compared to untreated controls. This is accredited to DCCV's ability to activate the host immune system, proliferation of CD4+, CD8+, and NK cells in the peripheral blood and subsequent infiltration of these immune cells into the tumor tissue.

Conclusion: These results demonstrate the potency of our allogeneic DCCV expressing CD93, CD40L, and CXCL13, in controlling pancreatic tumor growth by stimulating the immune system and therefore could provide an effective therapeutic opportunity for the treatment of various solid tumors. We report on a DCCV platform to treat multiple solid tumors such as triple negative breast cancer (TNBC), hepatocellular carcinoma, pancreatic, head and neck, oral and prostate cancers.

#3548 Systemic antitumor immunity stimulated by intratumoral CD40 agonist in combination with PD1 therapy in immune-cold melanoma.

Barbara Pazdrak, Heather M. Sonnemann, Barbara M. Nassif, Salah E. Bentebibel, Greg Lizee, Adi Diab

UT MD Anderson Cancer Center, Houston, TX

Agonistic CD40 antibodies in combination with checkpoint inhibitors have shown beneficial outcomes in clinical trials for the treatment of malignancies. We demonstrated that intratumoral injection of CD40 agonist in combination with systemic anti-PD1 therapy activates innate immune responses and promotes selective expansion of effector CD8 T cells while decreasing immunosuppressive Treg and M2 populations in melanoma tumors. However, the mechanisms of systemic antitumor immune responses driven by this combination therapy in patients are not well defined. Therefore, the aim of this study was to evaluate the impact of this combination therapy on systemic antitumor immunity using a bilateral, immunologically “cold” tumor model. Mice bearing bilateral B16-F10 melanoma tumors were injected with anti-CD40 Ab into right flank tumors, either alone or in combination with anti-PD1 Ab on day 8 post-tumor implantation. Mice were received treatment every 3 days for a total of 5 doses. Our results demonstrated that intratumoral CD40 Ab alone or in combination with anti-PD1 therapy inhibited the growth of both treated and distant (non-treated) tumors. The combination therapy showed superior efficacy compared with single Ab treatments. CD40 Ab-injected tumor weights measured at day 17, were ~9-fold reduced in mice treated with the combination therapy as compared to PD1 Ab alone (0.09±0.02g vs 0.83±0.17g). Importantly, similar antitumor efficacy of the combination therapy was observed in non-treated tumors (0.09±0.02g vs 0.74±0.23g) on the left flanks of these mice, demonstrating an abscopal treatment effect that was associated with enlargement of spleen and TdLNs. Flow cytometry analysis showed that CD40 agonist stimulated the proliferation of CD4 and CD8 T cells, and B cells in TdLNs and spleen. Furthermore, CD40 Ab treatment increased the frequency of blood circulating effector (40% vs 7% CTR) and central memory (25% vs 15% CTR) CD8 T cells as determined at day 21. Our data indicated that intratumoral CD40 agonist in combination with anti-PD1 therapy stimulated systemic antitumor immunity resulting in inhibition of distant tumor growth in a “cold” melanoma model, which was accompanied by increased proliferation of T cells and B cells in lymphoid organs and frequency of effector and memory CD8 T cells in the circulation. The results provide new insights about the mechanisms of systemic antitumor responses induced by intratumoral CD40 agonist.

#3549 Engineering an artificial antigen presenting system: A novel immunotherapeutic strategy for lung cancer.

Sindhu Yalavarthi[1], Li Wang[1], Yusif-Abdul Rashid[1], Kiki Parker[2], I'yanah Gunter[1], Ethan Li[3], Emily Cao[3], Jianjun Wei[1], Zhenquan Jia[4], Kounosuke Watabe[5], Kerui Wu[1]

[1]Nanoscience, Joint School of Nanoscience and Nanoengineering, University of North Carolina Greensboro, Greensboro, NC,[2]Nanoenigneering, Joint School of Nanoscience and Nanoengineering, North Carolina Agricultural and Technical State University, Greensboro, NC,[3]Nanoscience, Joint School of Nanoscience and Nanoengineering, Greensboro, NC,[4]Department of Biology, University of North Carolina Greensboro, Greensboro, NC,[5]Department of Cancer Biology, Wake Forest University School of Medicine, Winston Salem, NC

Lung cancer continues to be the major cause of cancer-related deaths worldwide, indicating the urgent need for innovative and effective treatment strategies. Immune checkpoint inhibitors (ICIs) have demonstrated great promise as a new therapy, offering new hope for patients with advanced lung cancer. However, resistance to ICIs is frequently observed in cancer patients, with many failing to achieve durable responses. One of the very important factors contributing to ICI resistance is the dysfunction of antigen-presenting cells (APCs), especially dendritic cells (DCs), which are key initiators of the anti-tumor immunity. In lung cancer patients, DCs are compromised with a low capability of generating cytotoxic T cells, which are indispensable for the effectiveness of ICIs. To overcome this challenge, we have designed an artificial antigen-presenting system (aAPS), a new bioengineered platform that enables the direct stimulation of anti-tumor T cell responses independent of host APCs. The platform comprises specifically engineered nanostructures presenting membrane-bound IL-2, co-stimulatory molecules (4-1BBL and CD40L), and major histocompatibility complex (MHC)-bound antigens. aAPS thus efficiently performs direct antigen presentation to lymphocytes, leading to a robust and specific adaptive immune response. By mimicking these three necessary signals—cytokine stimulation, co-stimulatory activation, and MHC-antigen presentation—aAPS can elicit and amplify T cell activation independently of dysfunctional host APCs. The membrane-bound IL-2 on aAPS is extensively oriented toward highly targeted T-cell engagement, improving immune cell activation and lowering the systemic toxicity usually linked with the administration of free cytokines. Our preliminary studies have shown that aAPS potently induces an antigen-specific T cell response, which is superior to the effects seen in conventional dendritic cell-based approach. The efficacy profile of aAPS has been quite promising in preclinical murine lung cancer models, showing a huge reduction in tumor growth, enhancement of tumor-infiltrating lymphocytes, and augmentation of the efficacy of ICIs. Such data indicate that aAPS may overcome one of the major immune suppressive barriers to ICI therapy in lung cancer, paving the way for potentially potent new tools to enhance anti-tumor immunity and improve patient outcomes. Further studies will confirm these results and move aAPS toward clinical application in advanced lung cancer by transforming the bioengineering strategies to reduce technical barriers, enabling high-yield and high-purity production of this innovative therapy.

#3550 Dendritic cells pulsed with cancer stem cell antigen showed profound anti-tumoral efficacy in ovarian cancer.

Haixi Liang, Tong Xiang, Lin Zhou, Zhiwen Xie, Mao Ye, Chunyan Lan

Sun Yat-sen University Cancer Center (SYSUCC), Guangzhou, China

Background: Ovarian cancer serves as the first leading lethally gynecologic cancer, of which around 70% of patients would relapse soon even have undergone standard treatment. Several mechanisms work together attributing to the early relapse. Among these, cancer stem cells (CSCs) act as the root of metastasis and relapse in the early reports. However, limited methods directly targeting these rare populations have been developed so far. Here we develop DC vaccine directly targeting CSCs by loading DCs with enriched cancer stem cell antigen (CSC-Ag) in vitro.

Methods: Freshly dissected ovarian tumors are digested into single cell suspension with digestive enzymes. Subsequently, fluorescence-activated cell sorting (FACS) is used to isolate CD44CD133 double-positive cells, previously reported surface markers of CSCs. Basic medium supplied with several cytokines is used to maintain and enrich CSCs. Suspension of enriched stem cell spheres is repeated five freeze-thaw cycles in liquid nitrogen and 37-degree Celsius water bath, and the supernatant after centrifugation is regarded as CSC-Ag. CD14+ cells of patients' PBMCs are isolated using CD14 microbeads according to manufacturer's instruction. IL-4 and GM-CSF are added to induce DC. Immature DC are loaded with previously prepared CSC-Ag. Meanwhile, CD14- cells are cultured with the supplement of anti-CD3, anti-CD28 antibodies, and rhIL-2. T cells are soon co-cultured with mature DC for further antigen-presenting. In vitro cytotoxicity against OC cell lines are tested. Additionally, we developed a peritoneal metastatic ovarian cancer cell model by injecting OVCAR8 transfected with luciferase into NSG mice. IVIS system is used later to monitor tumor growth twice a week.

Result: Both CD44 and CD133 were highly expressed in ovarian cancers compared with normal tissue in matched TCGA and GTEx datasets. CD44CD133 double-positive cells ranged from 0.00% to 59.10% in our OC cohort (n=21). CSCs were successfully isolated and enriched, confirmed by the expression of stem cell markers, including SOX2, OCT4, and NANOG. CSC-Ag also expressed those markers comparable to freshly cultured CSC. DC-CSC+T showed remarkable efficacy both in vitro and in vivo against ovarian cancer, with more cancer cell apoptosis.

Conclusion: Dendritic cells pulsed with cancer-stem cell antigen (DC-CSC+T) could significantly lower the growth rate of OC, and may serve as a potential adjuvant therapy for improving OC disease-free interval.

#3551 Signal 1 boosters that address the antigen-access problem for solid tumor cell therapy.

Diane Manry, Kelly Radecki, Alexander Partin, Charles Kirsh, Anagha Krishnagiri, Navneet Kumar, Aaron D. Flynn, Yuta Ando, Jon Torres, Breanna DiAndreth, Timothy P. Riley, Alexander Kamb

A2 Biotherapeutics, Inc, Agoura Hills, CA

Background: Access to tumor antigens in solid tumors is one of the major impediments to progress in oncology, exemplified by the stark difference between the success of cell therapies for blood cancers vs. solid tumors. To overcome this obstacle, cell therapy research and development has explored a wide range of methods to enhance potency without worsening toxicity. Special attention has been paid to onboard potency boosters expressed by engineered immune cells, because they more readily avoid systemic toxicity. Investigation has focused primarily on signals 2 (co-stimulatory factors) and 3 (cytokines), but other mechanisms have also been considered, including expression of transcription factors, anti-apoptotic proteins, and TGFβ pathway inhibitors. Despite the magnitude and range of these efforts to boost potency, little attention has been directed at enhancers of signal 1, beyond designs intended to improve acute sensitivity of chimeric antigen receptors (CARs) and t-cell receptors (TCRs).
Methods: Three different small-molecule-regulated systems were explored as inducible modules to regulate tonic signaling by CARs and TCRs. Acute and long-term behavior was examined, with an emphasis on inducer-dependent activation of immune function. Fusions of known members of the TCR signaling pathway were compared to identify modules with different functional properties; for example, background signaling level and ligand-induced induction ratio.
Results: A variety of constructs, including fusions to LAT, SLP76, CD3ε, and CD3ζ were shown to affect tonic signaling in a fashion that could be controlled by small-molecule inducers. Acute effects measured in Jurkat cells were also observed in primary T cells. Three clinically-tested classes of small molecule were able to act with their cognate ligand-binding fusion proteins as signal 1 boosters, demonstrating flexible small molecule control of the booster. This tunable-booster approach was extended to Tmod^{TM}, a dual-receptor NOT gate based on the LIR-1 inhibitory receptor (Hamburger AE, et al. *Mol Immunol*. 2020).
Conclusions: We have developed an approach to mitigate a major challenge for solid tumor cell therapy—access to tumor antigens in the blood. The system utilizes signal 1 agonists and is tunable, flexible, and modular. These signal 1 booster constructs can be added to CARs, TCRs, and other receptor systems to mimic an antigen stimulus with a small molecule, thereby triggering tonic (antigen-independent) signaling that reversibly activates the cells in the absence of antigen.

#3552 An mRNA-encoded adjuvant-enzyme activates STING to enhance the immunogenicity of vaccines targeting human papillomavirus positive cancers.

Jonathan Chow, Kelsey Finn, Anastasia Nikiforov, Dahlia Alkekhia, Stephan Matissek, Saisha Nalawade, Angela O'Neil, Carolyn MacFarlane, Philip Coblentz, Milap Chokshi, Colin Kelly, Brenna Sherbanee, Nicolina Lamberti, Evan Kennedy, Andrew Cornforth, Sivan Elloul, Jonathan C. Kagan

Corner Therapeutics, Watertown, MA

Despite the clinical success of prophylactic vaccines against human papillomavirus (HPV), vaccines administered post-infection are unable to prevent HPV-associated cancers. Therapeutic HPV vaccines that can treat pre-malignant and established oropharyngeal and anogenital tumors therefore represent an unmet clinical need. Using a novel mRNA-encoded catalytic vaccine adjuvant, Corner Therapeutics is developing a therapeutic vaccine against HPV.The cyclic GMP-AMP synthase/stimulator of interferon genes (cGAS/STING) signaling pathway is a robust inducer of inflammation and protective immunity. No effective means to activate this pathway in the context of mRNA-based vaccines is known. Towards this end, we engineered an mRNA construct encoding a self-DNA reactive form of the enzyme cGAS (hereafter cGASΔN) to use as a catalytic vaccine adjuvant. Herein we show that cGASΔN mRNA was successfully formulated into lipid nanoparticles (LNPs) of a size and consistency matching LNPs used clinically, and that the cGASΔN mRNA is translated into a functional protein allowing for downstream STING signaling in primary human dendritic cells. When treated with cGASΔN mRNA-LNPs, human dendritic cells produced cytokines, chemokines and interferons, and expressed cell surface activation markers associated with T cell co-stimulation. The immunostimulatory activities of cGASΔN-LNPs resulted in robust ability of DCs to activate HPV-specific human T cells in *in vitro* cocultures. In contrast, DCs stimulated with LNPs encoding non-stimulatory proteins (akin to those used clinically), were ineffective inducers of T cell activation. In murine vaccination studies, intramuscular injection of LNPs encoding cGASΔN and HPV16 E6 and E7 antigens enhanced antigen-specific T cell responses to both viral proteins. These results establish cGASΔN mRNA-LNPs as a potent adjuvant to enhance the immunogenicity of mRNA-based HPV antigens, and that this promising approach could be utilized to develop therapeutic cancer vaccines.

#3553 Bria-OTS+: A versatile therapeutic platform for inducing anti-cancer immunity.

Miguel A. Lopez-Lago[1], Vikas Bhardwaj[2], Pravin Kesarwani[2], Xiaoyi Zheng[2], sagarika Pachhal[2], Patience Cournoo[2], Renee Cortez[1], George Woodfield[1], Charles Wiseman[2], William Williams[3]

[1]BriaCell, Philadelphia, PA,[2]BriaCell Therapeutics Corp., Philadelphia, PA,[3]BriaCell Therapeutic Corp., Philadelphia, PA

Background: Despite advances, achieving curative cancer therapies remains challenging due to the complex, multi-stage nature of the disease. Recently, therapeutic cancer vaccines have shown promising efficacy, driven by advances in immune profiling, antigen selection, immunotherapy combinations, mRNA technology, and delivery platforms. However, challenges persist, including identifying highly immunogenic tumor antigens, overcoming T-cell immune escape, and achieving scalable, cost-effective manufacturing. To address these, BriaCell is developing a gene-modified allogeneic tumor cell platform to stimulate both innate and adaptive immunity. This platform provides a broad range of tumor antigens and includes immune-stimulatory factors to induce a comprehensive immune response. Initial results with the SV-BR-1-GM (Bria-IMT) breast cancer cell line, expressing GM-CSF, have shown promise. Bria-OTS+ further enhances immune activation by expressing additional cytokines, co-stimulatory factors, and HLA alleles across tumor cells, offering a personalized, adaptable immunotherapy platform.
Methods: Bria-OTS+ cell lines are derived from various cancer lines, including breast, prostate, lung, and melanoma. The cells are modified to express co-stimulatory molecules, cytokines and a range of HLA alleles through lentiviral transduction. To assess Bria-OTS+'s therapeutic potential, we developed an in vitro vaccination assay. Briefly, peripheral blood mononuclear cells (PBMCs) from healthy donors were co-cultured with Bria-OTS+ cells in a four-phase assay—priming, resting, boosting and effector phases—to evaluate immune activation, memory formation, exhaustion, and cytotoxicity.
Results: Bria-OTS+ robustly activated innate and adaptive immune responses in vitro. Co-culture with PBMCs led to T and NK cell activation, marked by increased activation markers and cytokine production. The platform induced immune memory, with enhanced cytokine release, cytotoxicity and memory marker expression upon re-stimulation. Cytotoxicity assays confirmed effective tumor cell targeting, and blocking antibody studies identified key activation pathways.
Conclusions: Bria-OTS+ is an adaptable cancer immunotherapy platform with strong clinical potential. It expresses a broad range of cancer antigens, including Tumor-Associated Antigens and Post-Translational Modifications, to stimulate robust immune responses. The platform activates both adaptive and innate immunity, overcoming immune escape by engaging NK cells in cases of HLA deletion. Bria-OTS+ is flexible, easily updated with new innovations, and suited for both personalized and ready-to-use applications. Its simplified administration and anticipated favorable side effect profile support broad clinical potential and patient tolerance.

SCIENCE AND HEALTH POLICY: Cancer Disparities and Costs of Care / Grant Peer Review
Poster Session

#3559 Racial and ethnic disparities in medication adherence between Black and Caucasian cancer survivors using oral anticancer medications: A Blinder-Oaxaca decomposition analysis.

Meng-Jung Wen[1], Olayinka O. Shiyanbola[2]

[1]Health Services Research in Pharmacy, School of Pharmacy, University of Wisconsin-Madison, Madison, WI,[2]Department of Clinical Pharmacy, College of Pharmacy, University of Michigan, Ann Arbor, MI

Background: About half of cancer survivors do not adhere to prescribed oral anticancer medications (OAMs), leading to higher relapse rates, hospitalizations, and healthcare costs. Nonadherence disproportionately impacts Black cancer survivors, often due to barriers such as limited healthcare access, healthcare mistrust and health literacy challenges. However, limited research has examined adherence among Black cancer survivors using OAMs, highlighting the need to explore factors contributing to nonadherence for future interventions. This study aimed to (1) assess disparities in medication adherence among Black and Caucasian cancer survivors on OAMs, and (2) examine the extent to which these disparities may be attributed to patient demographics and psychosocial factors.
Methods: A total of 335 cancer survivors currently taking OAMs were recruited through two online panels. Participants completed a survey on medication adherence, psychosocial factors, and demographics. Medication adherence was measured with the 12-item Adherence to Refills and Medications Scale, designed to assess the extent of adherence to OAMs. Independent t-tests and multiple linear regression analyses examined differences and predictors of nonadherence between Black and White patients. A two-fold Blinder-Oaxaca decomposition analysis was used to further decompose the disparities in medication adherence identified within the regression model.
Results: Compared to Caucasian patients, Black patients had significantly lower adherence (-2.06 points, $p = .04$) to OAMs. The model attributed a total of 2.77 points (SE = .64) of adherence disparities to socio-demographic and psychosocial factors. Specifically, age and metropolitan residence explained 1.18 points (51.5%) and 0.18 points (7.9%) of the variance, respectively. Health literacy emerged as the most significant psychosocial factor, accounting for 1.24 points (54.2%) of the observed disparities in medication adherence.
Conclusion: Blinder-Oaxaca decomposition analysis offered a nuanced way to assess disparities in medication adherence and their contributing behavioral factors. The results indicate that younger Black cancer survivors and those who live in metropolitan areas showed lower engagement in medication use. Future interventions should adopt a multi-faceted approach to address modifiable factors, such as enhancing health literacy and providing support for OAM adherence among Black cancer survivors.

#3560 Understanding health-seeking delays for post-menopausal bleeding among subpopulations of Black women.

Maurice J. Chery, LaShea D. Rolle, Wilmar Belizaire, Alejandra Casas, Sara M. St. George, Sophia HL George, Matthew Schlumbrecht

University of Miami Miller School of Medicine, Miami, FL

Background: Black women face disproportionate risks of endometrial cancer (EC). They more often present with advanced stage and high-grade histologies, and have lower survival rates compared to White women. Subpopulations of the Black community have been shown to have variable risks of disease and poor outcomes; the underlying causes for this observation are unknown. Without specific screening tests for EC, timely diagnosis relies on recognizing postmenopausal bleeding (PMB), the most common symptom. Our objective was to explore perceptions of PMB and factors influencing care-seeking behavior when PMB occurs among subpopulations of healthy Black women to determine if these factors influence observed intra-racial disparities.

Methods: We utilized a community based participatory research approach guided by the Safer and Anderson model of Total Patient Delay. We conducted focus groups with three subpopulations of healthy Black women (US-born [USB], Caribbean-born English speaking [CBB], and Haitian-Creole speaking [HC]) who were ≥50 years old. We collected demographic information and queried participants about their perceptions of PMB if they were to personally experience it, including its meaning, disclosure of the symptoms to others, and strategies to self-appraise and engage the healthcare system. We transcribed, thematically coded, and analyzed each session using Dedoose software.

Results: Fifty-four women (median age 60 [54-65]) participated (USB 40%; HC 40%, CBB 20%). Over 60% were non-USB, and 90% had at least a high school education. Cultural and religious beliefs significantly shaped health management across all groups. HC prioritized prayer and divine guidance over immediate medical care, preferring to use natural remedies aligned with traditional health practices. USB integrated spiritual beliefs with conventional medical approaches but expressed concerns over stigma, leading to selective sharing of health concerns within families. CBB highlighted hereditary factors, linking PMB symptoms to family history and genetics. Across all groups, family dynamics shaped health-seeking behaviors, with non-USB preferring to rely more heavily on familial networks for guidance. Information-seeking behaviors varied, from strong reliance on digital resources (USB) to direct validation from healthcare professionals (CBB) or community networks (HC). Despite differences, all groups highlighted delays in seeking care, often attributed to symptom misconceptions and normalization of PMB.

Conclusion: In the hypothetical setting of PMB, cultural beliefs, familial dynamics, and information-seeking behaviors shape health perceptions and care-seeking actions among Black women. Culturally tailored interventions to address knowledge gaps, promote timely recognition of PMB, and encourage early engagement with healthcare providers are needed to reduce EC disparities.

#3562 Associations between premature aging and cancer among middle-class Black and White women in the US: A case-control NHANES analysis.

Kayla S. Ingram[1], Brittany Jenkins-Lord[1], Kassandra L. Alcaraz[2], Avonne E. Connor[1]

[1]Johns Hopkins Bloomberg Sch. of Public Health, Baltimore, MD,[2]Johns Hopkins School of Medicine, Baltimore, MD

Although overall cancer mortality rates have decreased, Black women in the U.S. continue to have higher mortality rates from cancer than their White counterparts. Recent studies reveal that both genetic and environmental factors combine to contribute to these racial disparities in cancer, and may be modulated by differences in tumor biology. With cancer being an aging-related disease, it has previously been hypothesized that racial disparities in cancer outcomes are exacerbated by prolonged exposures to social and economic stressors that lead to premature aging. Our study aims to explore these effects on cancer status among U.S. Black and White women with annual household incomes of > $75K/year. Using data from the National Health and Nutrition Examination Survey (NHANES) from 1999 to 2021, we quantified social and economic stressors using three factors: (1) allostatic load (AL) score to predict confounding stress factors on an individual's health, (2) telomere length to predict premature aging, and (3) validated epigenetic aging markers from NHANES methylation data. We calculated the total AL score by summing the individual components for a score ranging from 0 to 9. Participants with high AL have a score ≥3. Mean telomere length was measured continuously. All aging metrics represent different aspects of accelerated epigenetic aging (Horvath Age and Hannum Age), phenotypic age (PhenoAge), pacing of aging (DunedinPoAm), and predicted age of mortality (GrimAge2Mort). Adjusted regression models were used to identify associations between these metrics, any cancer status, and race. We restricted our study sample to women with annual household incomes >$75K/year, resulting in a final NHANES cohort of 3,690 women (cancer cases, n=302; controls, n=3,388). In this cross-sectional study of Black (n=850) and White (n=2,840) women, increasing allostatic load was associated with decreased odds of cancer case status overall (odds ratio (OR)=0.89, 95%CI=0.81,0.98, p=0.02) and specifically for White women (OR=0.89, 95%CI=0.80,0.99, p=0.03). Increasing Horvath Age was associated with a 20% increased odds of cancer case status overall (OR=1.20, 95%CI=1.01,1.43, p=0.04) and significantly for White women (OR=1.36, 95%CI=1.11,1.166, p=0.003), but not Black women. We also found that AL and telomere length were both associated with several accelerated aging metrics in cases and controls. Our results suggest that increasing allostatic load had a protective effect in this cohort of high-earning women, specifically among White women. Increasing epigenetic aging metrics correlated to odds of a cancer diagnosis overall and with race specific implications. Some differences by race may be attributed to the low sampling of Black women in this cohort with corresponding telomere and methylation data, emphasizing the need for future epigenetic studies to include more Black populations.

#3563 Racial and ethnic disparities in mental health care and psychotropic medication utilization and expenditure among cancer survivors with serious psychological distress.

Hyunjung Lee[1], So-Yeon Kang[2], Alexandre Chan[3], Jason Zheng[1], Ahmedin Jemal[1], Farhad Islami[1]

[1]American Cancer Society, Atlanta, GA,[2]Department of Health Management and Policy, Georgetown University School of Health, Washington, DC,[3]School of Pharmacy & Pharmaceutical Sciences, University of California, Irvine, CA

Background: Although National Comprehensive Cancer Network provides the standardized guideline for mental health screening and treatment during cancer care, mental health care has been still underutilized among cancer survivors. Cancer survivors may experience stress and mental health issues for many reasons, including the unexpected diagnosis; cancer treatment; and fear of reoccurrence and premature death. Access to mental health care is critical to improve their quality of life and avoid unnecessary costs due to mental illness. Although previous research has shown racial and ethnic inequities in mental health services and medication utilization in the general population, few studies have examined these disparities among cancer survivors with serious psychological distress (SPD).
Method: Nationally-representative pooled 2012-2021 Medical Expenditure Panel Survey was used. The key outcomes included 1) the total number of each type of office-based visits (psychiatrist, psychologist, and psychotherapy/mental health counseling), 2) expenditures related to these visits, 3) whether medication was prescribed during these visits, and 4) psychotropic medication utilization (purchased). Zero-inflated negative binomial model for count outcomes, two-part regression models for cost outcomes, and logistic regression model for a binary outcome, adjusting for multiple individual socioeconomic factors and health status.
Results: Approximately 5% of cancer survivors in 2012-2020 MEPS data had SPD (N=1,016). Compared with non-Hispanic White (NHW) survivors with SPD, non-Hispanic Black (NHB) with SPD were less likely to receive counseling (−1.74 visit, 95%CI=−2.70, −0.79) and non-Hispanic other (NHO) were less likely to visit psychiatrist (−1.74 visit, 95%CI=−2.70,−0.79). Among cancer survivors who visited office-based mental health professionals, psychotropic medications were more likely to be prescribed to NHB survivors than NHW survivors by 22.2 percentage points (95%CI=9.22,35.21). The expenditure on mental health visits was lower among NHB survivors than NHW survivors (total cost: $−226, 95%CI=−384,−68; out-of-pocket cost: $-75, 95%CI=−110, −40). Compared with NHW survivors, antidepressants utilization was lower among NHB (−15.0%, 95%CI=−24.8,−5.2) and Hispanic (−9.6%, 95%CI=−18.8,−0.5) and anxiolytics utilization was lower among NHB (−12.5%, 95%CI=−19.8,−5.2) and NHO (−14.9%, 95%CI=−24.7,−5.1) survivors.
Conclusions: We found that Black-White disparities existed in number of counseling visits, total and out-of-pocket expenditures, and antidepressant and anxiolytic utilization, and Hispanic or NHO were less likely to utilize antidepressant or anxiolytics, calling for improvements in adherence of mental health treatment and access to psychotropic medication among racial and ethnic minorities.

#3564 Analyzing state policies that impact cancer care access for individuals with intellectual and/or developmental disabilities.

Esha Mathur[1], Carly Camplain[2], Freya Abraham[1], Erin Vinoski[3], Julie Armin[1]

[1]University of Arizona, Tucson, AZ,[2]Indiana University, Bloomington, IN,[3]Georgia State University, Atlanta, GA

Background: Almost 2 million people in the US are diagnosed with some form of cancer, with a large proportion due to environmental, occupational, or lifestyle factors. People with intellectual and/or developmental disabilities (IDD) have the same risks for cancer as the general population, and are diagnosed with cancer at similar rates to the general population; however, they may experience worse cancer-related outcomes. Studies show the life expectancy of people with IDD has improved, largely due to better health management. As this population ages , it is important to address diseases of age, like cancer. There has been little exploration into how health-promoting federal and state policies influence the cancer outcomes of people with IDD. To understand how policies may affect cancer-related experiences of those with IDD, our team is conducting in-depth analysis of state programs and Medicaid waivers of two states: Kentucky, a state that expanded Medicaid eligibility with the Affordable Care Act; and Georgia, a state that chose not to expand.
Methods: We compiled every Kentucky and Georgia Medicaid waiver (vehicles states use to deliver and pay for health care services in Medicaid) and health-focused program (17 for Georgia, and 14 for Kentucky). Our team also conducted in-depth interviews with policy advocates in each state (N=4), where we gained insight into the effectiveness of these policies in practice. We thematically coded each waiver with 3 broad categories-overall health promoting, disability-specific, and cancer-specific initiatives-and with 6 social determinants of health (SDoH) categories- economic stability, education, social context, living conditions and physical environment, health care system, and food.
Results: For both Georgia and Kentucky, we found that every waiver/program (100%) addressed health care access. In Georgia, waivers and programs were coded as follows: food (4, 23.5%), economic stability (1, 5.8%), living conditions/physical environment (4, 23.5%), education (0), social context (6, 35.3%). In Kentucky, waivers and programs were coded: food (3, 21.4%), economic stability (2, 14.3%), living conditions/physical environment (7, 50%), education (1, 7.1%), social context (5, 35.7%). In interviews, advocates mentioned transportation, health literacy, and provider education as major barriers to health care.
Conclusions: While most Medicaid waivers address access to health care, there is a need for waivers to address the other SDoHs that create significant accessibility barriers and may affect cancer outcomes.

#3565 Personalized Drug Discovery Process (PDDP): An AI-driven, sustainable, and equitable solution for cancer drug discovery.

Pietro Veronesi[1], Adekunle Odunsi[2], Marciano Siniscalchi[3], **Marina Chiara Garassino**[2]

[1]Booth Business School, University of Chicago, Chicago, IL,[2]University of Chicago, Chicago, IL,[3]Economics, Northwestern University, Chicago, IL

Background: Personalized medicine has improved survival rates for lung cancer patients with common targetable alterations. However, developing drugs for rare or ultra-rare molecular alterations is likely to become economically unsustainable, potentially creating unequal access to personalized therapies for patients with these mutations.
Methods: We formulate a new mathematical model and use Monte Carlo simulations to calculate pharma companies' projected profits from targets that affect an increasingly smaller fraction of the world population, considering the costs of R&D and Randomized Clinical Trials (RCTs) and the probability of drug approval. We then use similar methods to calculate projected profits for an alternative, feasible, AI-driven approach called the Personalized Drug Discovery Process (PDDP), which has four steps: target identification, protein synthesis, drug matching or repurposing, and bioassay synthesis.
Findings: At the current annual price for targeted therapies of around $200,000/patient and RCT costs of $100,000 per subject, the financial incentives for further drug discovery under the current approval paradigm evaporate for targets affecting less than 0.5% of the lung cancer population, even if Phase 0 and I R&D costs are as low as $100 million/target. To ensure proper incentives for Pharma companies to innovate even for targets affecting less than 0.5% of lung cancer patients, targeted therapies would require margins of over $828,000/year per patient over manufacturing costs, implying global societal costs of over $1.45 trillion/year. PDDP is instead projected to be sustainable, even accounting for the required investments in infrastructure. At peak, we calculate PDDP will cost $1,250 per lung cancer patient, net of drug manufacturing costs. Pharma companies will invest in PDDP and hence foster further research if successful personalized drugs yield margins between $25,000 - $90,000/year per patient.
Conclusions: PDDP is already a feasible and sustainable drug discovery process. This can create a paradigm shift in drug discovery by testing the process instead of specific drugs. By contrast, under current regulatory agency requirements, the drug discovery and development process is unsustainable for targets with a frequency of less than 0.5%. This approach promotes innovation and equity in access for all patients with any identifiable target mutation

#3566 OncoPSM as a tool for cost-effectiveness analysis in oncology trial using Kaplan Meier curve data.

Baohui Han[1], Feilong Zhao[2], Ding Zhang[2]

[1]Shanghai Chest Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China,[2]3D Medicine, Shanghai, China

Introduction: Cost-effectiveness analysis (CEA) is a key tool for evaluating the economic viability of new treatments compared to current standards of care. However, many CEA methodologies are not specifically designed to address the intricate cost structures in oncology trials.

Methods: We employed a public image recognition tool to extract data from Kaplan-Meier (KM) curves published in clinical trials. Using an iterative algorithm based on the iKM method, we reconstructed individual patient data (IPD). A partitioned survival models (PSM) were then fitted to the IPD data. Under this model, we estimated the probability of each survival state for each model-cycle and combine these probabilities with utility values to calculate both the effect per model-cycle and the incremental effect for the experimental group. For cost analysis, we performed a treatment-cycle-specific cost evaluation, accounting for cost uncertainty through a gamma distribution. By setting model-cycle equals to the treatment-cycle, we could calculate the state-weighted cost, applied a discount rate, and determined the incremental cost for the experimental group. The Incremental Cost-Effectiveness Ratio (ICER) was then calculated based on the incremental effect and incremental cost.

Results: The OncoPSM application, available at (https://zhaofeilong.shinyapps.io/app_oncopsa/), was validated with real-world data from the CHOICE-01 trial. OncoSPM accurately reconstructed IPD from KM curve data, with root mean square error (RMSE) values below 0.004 across all curves. Log-rank p-values for the experimental and control groups (PFS: <0.0001; OS: 0.0104) closely aligned with those reported in the original publication (PFS: <0.0001; OS: 0.0108). Hazard ratios (HR) from the reconstructed IPD data (PFS: 0.504, 95%CI: 0.4-0.63, OS: 0.731, 95%CI: 0.57-0.93) were consistent with the original study (PFS: 0.49, 95%CI: 0.39-0.61, OS: 0.73, 95%CI: 0.57-0.93). The log-logistic model provided the best fit for both PFS and OS curves, based on the Akaike Information Criterion (AIC). Extrapolating the survival curve to a 10-year horizon, we created the PSM, deriving the average state probabilities per treatment-cycle to calculate state-weighted costs. The incremental cost for the experimental group was ¥42,291, with an incremental quality-adjusted life year (QALY) of 0.35, yielding an ICER of 122,319 ¥/QALY, which is well below the willingness-to-pay (WTP) threshold of 268,200 ¥/QALY. Uncertainty analysis showed a 99.6% probability that the experimental group was cost-effective at the WTP threshold.

Conclusions: OncoPSM offers an accurate and user-friendly treatment-cycle-based cost analysis tool that addresses the complexities of costs in oncology research. By integrating and visualizing the entire CEA process, OncoPSM supports decision-makers in making well-informed choices based on both statistical and intuitive insights.

#3567 Immunotherapy combined with chemotherapy versus biomarker-guided immunotherapy: A cost-effectiveness analysis.

Fubing Wu[1], Feilong Zhao[2], Ding Zhang[2], Wen Huang[3]

[1]Sir Run Run Hospital, Nanjing Medical University, Nanjing, China,[2]3D Medicine, Shanghai, China,[3]The Fourth Affiliated Hospital of Nanjing Medical University, Nanjing, China

Background. PD-L1 and TMB are established biomarkers for predicting immunotherapy efficacy. However, with the substantial reduction in the cost of domestic PD-L1 testing, the value of expensive genetic testing compared to its limited benefits has become a pertinent question.

Methods. Data from 309 NSCLC receiving immunotherapy and 156 NSCLC undergoing chemotherapy in CHOICE-01 clinical trial were analyzed. Using a decision tree model, patients were categorized into All-in group receiving immunotherapy combined with chemotherapy, and two control groups guided by TMB and PD-L1, respectively. Cost-effectiveness was assessed using Incremental Cost-Effectiveness Ratio (ICER).

Results. The decision tree comprised three chance nodes: All-in combinational therapy, TMB-guided, and PD-L1-guided strategies (Table 1). The TMB-H group exhibited the highest response rate, followed by the PD-L1 positive group, both exceeding an overall response rate of 65.7%. Utility values for partial response (PR), stable disease (SD), and progressive disease (PD) were retrieved from previous studies, then adjusted by weighted average of SD and PD distribution and adverse reaction-related disutility. After model evaluation, the All-in, TMB-guided, and PD-L1 guided strategies yielded utility of 0.653, 0.638, and 0.628, respectively. Corresponding costs were ¥3,202, ¥3,833, and ¥11,395. Given the decreasing utility and increasing cost across the strategies, calculating the ICER was deemed unnecessary.

Conclusion. The All-in strategy demonstrated the highest utility and lowest cost, indicating it as the most cost-effective option for patients considering immunotherapy combined with chemotherapy. However, as this study focused on ORR, it only reflects short-term cost-effectiveness. Further investigation into long-term cost-effectiveness regarding survival outcomes is warranted.

Table 1. Structure and parameters of the decision tree.

Decision	Probability, %	Regimen	ORR mean, %	ORR Distribution	Adjusted mean utility
All-in	100	Comb	65.70	Beta(203,106)	R: 0.698 NR: 0.567
TMB	TMB-H: 30.96	Comb	72.73	Beta(56,21)	R: 0.698 NR: 0.567
	TMB-L: 69.04	Chemo	45.88	Beta(39,46)	R: 0.690 NR: 0.547
PD-L1	Pos: 65.16	Comb	68.00	Beta(136,64)	R: 0.698 NR: 0.567
	Neg: 34.84	Chemo	39.62	Beta(21,32)	R: 0.690 NR: 0.547

#3568 Evaluating recent drug approvals for hematologic malignancies utilizing the New European Society of Medical Oncology Magnitude of Clinical Benefit Scale for Hematology (ESMO-MCBS:H).

Ryan Hirsh[1], Justin Moyers[2], David Benjamin[3], Aysche Stern[4]

[1]University of Kansas Medical Center, Kansas City, KS,[2]Cedars-Sinai The Angeles Clinic and Research Institute - West Los Angeles, Los Angeles, CA,[3]Hoag, Newport Beach, CA,[4]Department of Internal Medicine - Temecula Valley Hospital, Temecula, CA

Introduction: Over the last decade, the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have steadily leaned towards granting regulatory approval based on earlier phase trials and surrogate endpoints. Despite obtaining regulatory approval, many countries hold additional standards to assess the value of a new drug approval. The ESMO-MCBS is a tool created to evaluate the values and reduce bias or hype of drug approvals using various metrics: efficacy, quality of life, and duration of response. In 2023, the ESMO-MCBS:H was created for hematological malignancies. We aimed to evaluate recent drug approvals using the new ESMO-MCBS:H criteria for hematologic malignancies.

Methods: We included all drugs approved for a hematologic malignancy between 2019 and 2024. Drugs were assessed using ESMO-MCBS:H forms 2a, 2b, 2c, or 3 depending on the trial type and primary endpoints leading to drug approval. Approvals were independently scored by two reviewers with a third reviewer to adjudicate scoring differences.

Results: 66 drugs were approved by either FDA or EMA during this time. 88% (n=58) were approved by both EMA and FDA, 12% (n=8) by FDA alone. No drugs were approved by the EMA alone without preceding or subsequent FDA approval. 4 drug approvals were for pediatric indications. 36 approvals (n=35/66, 53 %) were based on early phase 1 and/or 2 trial data. Approvals were for leukemias (n=16; 24%), lymphomas (n=25, 38%), myeloma (n=18, 27%), or CLL/SLL (n=6; 9%). 13% of approvals (n=9/66) were for drugs with a biomarker matched indication. Substantial benefit criteria were met by 15 of 66 approvals (23%). Substantial benefit criteria were met by 0% (N=0/8) of drug indication by approved by FDA alone versus 26% of drugs approved by both the FDA and EMA (n=15/58).Approvals based on early phase (phase 1 and/or 2) trials met criteria in 17% (n=6/35) of trials and 29% of later phase (phase 3) trials met criteria (n=9/31) without statistical significance (p=0.25).

No significant difference in approvals meeting substantial benefit were seen for biomarker matched therapies (33%; n=3/9) versus unmatched therapies (21%; n=12/57) (p=0.41). No therapies approved for a pediatric indication met criteria for substantial benefit (n=0/4).

Conclusion: Few drugs approved by both agencies met the rigorous ESMO-MCBS:H criteria for substantial benefit. Recent approvals meeting substantial benefit criteria were not statistically correlated with approval agency, phase of trial utilized for approval, or biomarker selection of therapies. Drug approvals must balance proven benefit to access to innovative and new therapies.

#3569 Patient characteristics, treatment complications, and factors associated with inpatient outcomes and treatment burdens among CAR-T recipients: Insights from the 2021 National Inpatient Sample.

Simo Du[1], Yuqing Wang[2], Jiahao Peng[2], Junmin Song[1], Roha Memon[1], Hasiya Yusuf[1], Ruirong Yuan[3], Abhishek Kumar[1]

[1]Internal Medicine, Jacobi Medical Center/Albert Einstein College of Medicine, Bronx, NY,[2]Internal Medicine, Montefiore Medical Center, Bronx, NY,[3]Medical Oncology, East Orange VA Medical Center, Bronx, NY

Background: Chimeric antigen receptor T-cell (CAR-T) therapy has transformed cancer treatment since FDA approval, with increasing utilization. Despite its potential, however, inpatient CAR-T therapy remains costly and is often complicated by cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), leading to longer hospital stays and higher costs. Identifying factors influencing outcomes is critical for optimizing care and targeting therapy to appropriate populations.
Methods: We conducted a cross-sectional study using the 2021 National Inpatient Sample (NIS). CAR-T recipients and associated complications including CRS, ICANS, and hemophagocytic lymphohistiocytosis (HLH) were identified using ICD-10 codes. The Elixhauser comorbidity index was derived from 31 comorbidities. Weighted analyses were used to provide national estimates. Generalized linear models were used to assess factors influencing length of stay (LOS) and costs, and logistic regression was employed to evaluate mortality predictors.
Results: Among 6,666,752 weighted discharges, 525 CAR-T cases were identified, with 468 adult admissions analyzed, representing 2,340 national encounters. The mean age was 56.1 years; most patients were White (72.4%), male (64.1%), and admitted electively (76.1%). Non-Hodgkin lymphoma (NHL) was the most common diagnosis (65.6%), followed by Multiple Myeloma (19.7%). Hospitals were predominantly large (76.9%), private (78.6%) and teaching institutions (100%). Complications included CRS (61.3%), ICANS (8.3%), and HLH (1.1%). Median LOS was 14 days (IQR: 9-19) and median charges were $980,000 (range: $180,000-$1,700,000).Higher Elixhauser comorbidity scores were associated with increased mortality (OR 1.21 per point, $p < 0.01$), longer LOS (+1.6 days/10-point increase, $p < 0.01$), and higher costs (+$91,192/10-point increase, $p < 0.01$). CRS added 2.1 days to LOS ($p < 0.01$) and $255,634 to costs ($p = 0.003$). ICANS added 2.6 days to LOS ($p = 0.05$) but did not significantly affect mortality. Non-Hodgkin lymphoma increased LOS by 3.5 days ($p < 0.01$) and costs by $1,368,424 ($p < 0.01$). Medicaid patients experienced 4.1 more days of LOS ($p < 0.01$). Medium-sized hospitals increased LOS by 4.2 days ($p < 0.01$), and large hospitals by 2.0 days ($p = 0.02$).
Conclusions: Inpatient CAR-T therapy is associated with substantial complications, prolonged LOS, and high costs. Comorbidities, diagnosis and treatment-related complications, particularly CRS and ICANS, are primary drivers of resource utilization. Additionally, hospital size and insurance type also significantly influence outcomes. These findings highlight the need to address both clinical and non-clinical factors to optimize care delivery and patient outcomes for CAR-T therapy.

#3570 Impact of private equity ownership on quality of cancer care in U.S. hospitals.

Megan Worthington[1], Ruth Carlos[2]

[1]Touro College of Osteopathic Medicine, Middletown, NY,[2]Radiology, Columbia University, New York, NY

Introduction: This study examined the potential impact of private equity (PE) acquisitions on patient satisfaction in U.S. hospitals with cancer care centers. Given the growing trend of PE involvement in healthcare, we hypothesized that ownership changes could correlate with measurable declines in perceived quality of care.

Methods: From a national dataset of 5,395 Medicare-participating hospitals, we filtered to 2,405 facilities that met criteria for dedicated oncology services and comprehensive cancer care, including defined treatment facilities and national program affiliations. Using Hospital Consumer Assessment of Healthcare Providers and Systems (HCAHPS) survey data (2016-2023), which provides patient satisfaction scores on a 1-to-5-star scale, we analyzed satisfaction ratings for 118 hospitals acquired by PE to identify changes pre- and post-acquisition. Pitchbook provided insights into deal type, deal frequency, and financing status. Julius AI was utilized for analysis, with particular attention to deal structures and rating trends.

Results: Over half of the hospitals studied (51.7%) experienced declines in satisfaction ratings post-acquisition, with an average decrease of 0.061 stars. Bankruptcy and debt-related deals corresponded with more substantial declines (-0.318 and -0.095 stars, respectively), suggesting that financial pressures may directly or indirectly impact patient experiences. Comparatively, single-deal hospitals showed a mean decrease of 0.061 stars, with 56.7% reporting declines, versus 44.9% for multiple-deal hospitals, which showed a more stable rating profile. Although a weak negative correlation (-0.101) existed between multiple deals and rating stability, hospitals with three or more deals showed minimal fluctuation, possibly reflecting stronger organizational adaptation over time.

Conclusion: This analysis reveals a consistent trend: patient satisfaction ratings tend to decrease in a considerable portion of PE-owned hospitals, with variations based on deal type and structure. The findings underscore the need for ongoing evaluation of PE ownership models in healthcare, particularly as financial motivations intersect with patient-centered care priorities. These preliminary trends merit deeper investigation into the mechanisms through which PE acquisitions may influence hospital service quality, especially in oncology-focused settings where patient care and continuity are critical. This abstract was prepared with the assistance of generative AI (Julius AI) for data analysis.

#3571 Drivers of cancer genomics research productivity in high income countries (HICs) versus lower middle income countries (LMICs): Which interventions can help close the gap?.

R. Chown[1], G. Curtis[2], C. Quigley[2], N. Jansler[2], R. Jacome[2], R. G. Bristow[2], G. F. Njoroge[3], E. Njoki Mwangi Maina[4], D. C. Wedge[2]; [1]The Christie NHS Foundation Trust and The University of Manchester, Manchester, United Kingdom, [2]The University of Manchester, Manchester, United Kingdom, [3]Kenyatta University Teaching Research and Referral Hospital, Nairobi, Kenya, [4]The University of Nairobi, Nairobi, Kenya

Purpose: Productivity disparity exists in cancer research systems between High Income Countries (HICs) versus Lower-Middle-Income-Countries (LMICs). Capacity and productivity for cancer genomics research is an exemplar metric for overall cancer research capacity. Kenyan cancer genomics research capacity was used as a case study for an LMIC benchmarked against the HIC countries of the UK and USA. A conceptual framework was used to analyse drivers of productivity of cancer genomics research within institutions based on research environment resources. Preliminary recommendations which can help close the research productivity gap were then made.
Methodology: This research study was carried out by conducting sixteen semi-structured interviews with researchers based across the United Kingdom, United States, Australia, South Africa, and Kenya, and analysis of this qualitative data was then conducted via NVivo. Further primary research data was sought via surveying of genomics researchers and specific institutions based in case study countries. Secondary research was conducted through online and database searches, and in-depth literature reviews on cancer genomics research and global disparities in cancer research. We applied and considered 'The Matthew Effect', that relates to cumulative advantage, and theories of productivity.
Results: This study found that drivers of the productivity gap between national cancer research systems across HICs and LMICs can be attributed to interrelated factors within three key areas: Data and Technology, Funding and Partnerships, and Knowledge and Capacity Build. These are critical to the productivity and eventual impact of cancer research in a particular environment. This study identified that external context factors are as important as the drivers of research productivity, and that research needs to be context relevant and designed to be relative to the local environment.
Conclusions: This study concludes that The Matthew Effect is clearly observable within cancer research systems. Whilst this has amplified global disparity to date, the correct application of stated recommendations can change this trajectory and help close the gap to ultimately improve global progress and outcomes for comparative cancer research.

POPULATION SCIENCES: Cancer Disparities: Environmental, Microbiome, and Tumor Biology
Poster Session

#3576 Socio-environmental exposure leads to disparities in colorectal cancer mortality and tumor biology in metropolitan Detroit.

Natalie Gail Snider[1], R. Blake Buchalter[2], Theresa Hastert[1], Gregory Dyson[1], Carina Gronlund[3], Elena Stoffel[4], Laura Rozek[5], Ann Schwartz[1], Kristen Purrington[1]

[1]Oncology, Wayne State University School of Medicine, Detroit, MI,[2]Quantitative Health Sciences, Cleveland Clinic, Cleveland, OH,[3]University of Michigan, Ann Arbor, MI,[4]Internal Medicine, University of Michigan Health System, Ann Arbor, MI,[5]Oncology, Georgetown University School of Medicine, Washington, DC

Non-Hispanic Black (NHB) patients face the highest colorectal cancer (CRC) mortality rates in the US, heavily influenced by social determinants of health (SDOH). Neighborhood quality, a key SDOH, impacts both socioeconomic factors and exposure to cancer-related environmental contaminants which has direct implications in tumor biology. Thus, in studying the role of environmental justice on health outcomes it is crucial to account for the separate and combinatory effects of environmental and socioeconomic burden. We expect socio-environmental exposures to increase the risk of CRC mortality among NHB patients, as well as alter tumor biology among those with the highest exposure. We utilized the Centers for Disease Control and Prevention's (CDC) comprehensive socio-environmental variable available at the census tract level called the Environmental Justice Index (EJI) to first compare social vulnerability (SVM), environmental burden (EBM), and their combined Social Environmental Score (SER) to the risk of CRC mortality by race. Patient data including census tract at diagnosis, demographics, and clinical features were obtained from the Metropolitan Detroit Cancer Surveillance System population-based cancer registry for 9727 Non-Hispanic White (NHW) and 3778 NHB invasive CRC cases. EJI variables were converted to quartile-level variables, and mortality risk was evaluated for quartile 4 (highest burden) compared to quartile 1 (lowest burden). Associations were tested via Cox Proportional Hazards regression. In nearly all models, EJI variables increased the risk of mortality. SVM showed stronger effects overall compared to EBM, and most models showed similar effects among race and age groups in both overall and cancer-specific mortality. A notable exception to this was that NHB early onset (EO) patients showed an elevated risk of cancer-specific mortality due to EBM (HR=1.98, 95% Confidence Interval (CI)=1.20-3.26, p=0.0071) compared to SVM (HR = 1.10, 95% CI = 0.61-2.00, p=0.75), and also compared to NHW EO EBM (HR=1.40, 95% CI=0.88-2.25, p=0.16) and NHW EO SVM (HR=2.12, 95% CI = 0.97-4.61, p=0.059). Additionally, we have noted that NHB patients are more likely to have KRAS mutations than BRAF, and comparative analyses are underway to compare molecular subtypes to exposure level. Our results suggest that environmental burden may play a role in racial disparities by age, and further analyses investigating the molecular implications of these exposures are needed to better understand the molecular mechanisms driving tumor progression and mortality disparities.

#3579 Racial disparity in microbial risk score for predicting subsequent risk of head and neck squamous cell cancer.

Soyoung Kwak[1], Chan Wang[1], Mykhaylo Usyk[2], Feng Wu[1], Steve C. Moore[3], Wen-Yi Huang[3], Caroline Y. Um[4], Martha J. Shrubsole[5], Qiuyin Cai[5], Huilin Li[1], Jiyoung Ahn[1], Richard B. Hayes[1]

[1]NYU Grossman School of Medicine, New York, NY,[2]Albert Einstein College of Medicine, Bronx, NY,[3]National Cancer Institute, Bethesda, MD,[4]American Cancer Society, Atlanta, GA,[5]Vanderbilt University Medical Center, Nashville, TN

Background: The association between oral bacteria and head and neck squamous cell cancer (HNSCC) risk has been investigated in recent years. Traditional approaches often explore individual microbes, overlooking the interaction and compositional profiles within microbial communities. We propose the use of a Microbial Risk Score (MRS) to examine the collective effects of bacterial communities in predicting HNSCC risk, with a focus on racial disparities.
Method: We conducted a prospective nested case-control study within 3 large US cohorts: the ACS-Cancer Prevention Study-II Nutrition Cohort, the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, and the Southern Community Cohort Study. Among 159,840 cohort participants who provided oral wash samples, we selected HNSCC cases and their matched controls (2:1 frequency matching on cohort, age, sex, race/ethnicity, and time since oral wash sample collection) during a mean of 5.1 years of follow-up. Our study population included 440 White participants (149 HNSCC cases and 291 controls) and 222 Black participants (76 HNSCC cases and 146 controls). We characterized the oral bacterial microbiome using whole-genome shotgun sequencing. We explored the probability of MRS to predict HNSCC risk across different racial groups and at different p-value(p) thresholds for bacterial community selection. The MRS was calculated as a weighted sum of relative abundance of the selected bacterial species, with weights assigned according to their effect size determined via Analysis of Compositions of Microbiomes with Bias Correction. The association between MRS and HNSCC risk was estimated using logistic regression after adjusting for age, sex, cohort, smoking, drinking, and oral human papillomavirus 16 status.
Results: The MRS derived from Whites at $p<0.01$ yielded an OR in Whites of 1.38 (95% CI: 1.07-1.80) per 1 SD increase in the MRS, while this MRS yielded a weaker OR of 1.23 (0.86-1.75) in Blacks. The MRS derived from Blacks at $p<0.01$ yielded an OR of 1.54 (1.01-2.33) in Blacks, while it yielded a weaker OR of 1.19 (0.92-1.53) in Whites. Decreasing the stringency of bacterial community selection (e.g., to $p<0.20$) tended to yield overall stronger associations which were also more closely comparable for the counterpart racial groups; MRS derived from Whites at $p<0.20$ showed an OR of 1.74 (1.20-2.52) in Blacks, and the MRS derived from Blacks showed an OR of 1.39 (1.07-1.79) in Whites. These associations between MRS and HNSCC tended to hold for oral cavity, pharynx, and larynx, and were unrelated to tobacco and alcohol use.
Conclusion: Our findings suggest that while the MRS tends to perform better within racial groups, race-specific MRS do associate with disease risk in another racial group, particularly under less stringent selection of bacteria that comprise the score. The MRS offers a promising approach for HNSCC risk prediction across diverse racial groups.

#3580 Contemporary neighborhood redlining and racial mortgage lending bias and disparities in prostate cancer survival.

Wayne R. Lawrence[1], Neal D. Freedman[1], Jennifer K. McGee-Avila[1], Hyokyoung G. Hong[1], Scarlett L. Gomez[2], Andrew P. Loehrer[3], Kirsten M. M. Beyer[4], Yuhong Zhou[4], Jared W. Magnani[5], Harold W. Neighbors[6], Jarrett A. Johnson[7], Ziqiang Lin[8], Amy J. Davidoff[1], Adana A. M. Llanos[9], Meredith S. Shiels[1]

[1]National Cancer Institute, Bethesda, MD,[2]University of California San Francisco, San Francisco, CA,[3]Dartmouth-Hitchcock Medical Center, Lebanon, NH,[4]Institute for Health & Equity, Medical College of Wisconsin, Milwaukee, WI,[5]Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA,[6]School of Public Health, University of Michigan, Ann Arbor, MI,[7]Division of Community Health and Population Science, National Institute on Minority Health and Health Disparities, Bethesda, MD,[8]School of Medicine, Jinan University, Guangzhou, China,[9]Department of Epidemiology, Columbia University, New York, NY

Introduction: Mortgage lending bias (based on property location or applicant's race) is a critical driver of residential segregation and may contribute to inequities in cancer survival. We investigated the association between contemporary redlining and racial mortgage lending bias and prostate cancer survival among Medicare-insured men.
Methods: This cohort study used the Surveillance, Epidemiology, and End Results (SEER)-Medicare database that included 34,163 non-Hispanic Black (13%) and White (87%) men diagnosed with prostate cancer between 2010-2013 and followed-up through 2019. Home Mortgage Disclosure Act data (2010-2017) were used to calculate census-tract (1) redlining index (odds of a mortgage application denial for a property in a local area, compared with all properties within the same metropolitan statistical area) and (2) racial lending bias index (odds of a mortgage application denial for a Black applicant, compared to a White applicant for a property in the local area). Both indices were assessed continuously and categorically (low (<1), moderate (1-<2), or high (≥2)). Multivariable-adjusted Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (95%CIs) for prostate cancer-specific and all-cause mortality.
Results: 44.6% of Black and 7.4% of White men lived in high redlined neighborhoods; 60.3% of Black and 63.7% of White men lived in neighborhoods with high racial lending bias. In the overall cohort, as the redlining index increased, men experienced poorer prostate cancer survival. Compared to those residing in low redlined neighborhoods, those in high redlined neighborhoods had an increased risk of prostate cancer-specific (HR=1.21; 95%CI 1.03-1.42) and all-cause (HR=1.25; 95%CI 1.17-1.34) mortality. Associations between redlining and all-cause mortality were observed among both Black (HR=1.14; 95%CI 1.03-1.26) and White (HR=1.30; 95%CI 1.19-1.43) men. Among White men, compared with those residing in low racial lending bias neighborhoods, those in high racial lending bias neighborhoods had an increased risk of all-cause mortality (HR=1.11; 95%CI 1.03-1.21).
Conclusions: Neighborhood redlining was associated with poorer prostate cancer survival in the overall population. However, the association between neighborhood racial lending bias and elevated mortality was only observed among White men. Findings suggest mortgage lending discrimination may contribute to inequities in prostate cancer survival through shaping neighborhood health conditions.

#3581 Psychosocial factors associated with lung cancer incidence and survival among individuals with low income in the Southeastern U.S..

Anna Junkins[1], Jennifer Richmond[2], Douglas DeMoulin[1], Xijing Han[1], Heather Munro[1], Martha J. Shrubsole[1], Wei Zheng[1], Xiao Ou Shu[1], Melinda C. Aldrich[1]

[1]Vanderbilt University Medical Center, Nashville, TN,[2]Wake Forest University School of Medicine, Winston-Salem, NC

Background: Lung cancer is the leading cause of cancer-related mortality in the U.S. and significant racial inequities exist. Psychosocial factors, including stressful life experiences, have been linked to increased incidence and poorer outcomes. The purpose of this study was to identify psychosocial factors associated with lung cancer incidence and survival among a low-income, racially diverse population in the U.S. South.

Methods: We utilized data from the Southern Community Cohort Study (SCCS), a large, prospective study across 12 Southeastern states, enrolling participants from 2002-2009, to examine psychosocial factors and lung cancer incidence and survival. Incident cancers and deaths were identified by linkage with state cancer registries and the National Death Index, respectively. Individual-level social, behavioral, and medical history information was ascertained at baseline via questionnaire. We examined depression, spirituality, attendance at faith-based services, social support, and use of anti-depressant/anxiety medication. We also analyzed data from a subset of participants who responded to psychosocial questions collected on a follow-up survey. We used Cox regression analyses to identify factors associated with lung cancer incidence and survival overall and by race.

Results: Among 74,295 SCCS participants, approximately 69.8% self-reported as Black persons, 58.4% female, and 56.3% had an annual household income below $15,000. There were 1,569 incident lung cancer cases identified, of whom, almost half (49.2%) were diagnosed at distant stage. Overall, lower spirituality was associated with a reduced risk of lung cancer. Among Black participants, identifying as “fairly” vs. “very” spiritual was associated with a 15% lower risk (HR: 0.85; 95% CI: 0.74-0.97); a similar pattern was observed among White participants but was not statistically significant. Similar patterns were observed with mortality. In subset analyses among 367 participants with lung cancer, depression and less frequent attendance at faith-based services were associated with greater mortality. Among Black participants, those with depression had 2.75-fold greater mortality (95% CI:1.11-6.84) compared to those without depression, and those who attended faith-based services at least once per week had 50% lower mortality compared to those who attended less than once per week (HR: 0.50; 95% CI: 0.30-0.83). Among White participants, identifying as “slightly/not at all” vs. “very” spiritual was associated with 4.31-fold greater mortality (95% CI: 1.47-12.66).

Conclusions: Psychosocial factors and spirituality may play a role in inequities observed in lung cancer outcomes. Future research should investigate how psychosocial factors may impact lung cancer outcomes.

#3582 Lung cancer screening in breast cancer survivors: a comparison of the USPSTF guidelines and risk-based criteria.

Baitong Wang[1], Marissa Michael[1], Justin Lee[2], Yiwey Shieh[1], Lauren Groner[1], Leah M. Backus[2], Allison W. Kurian[2], Rulla M. Tamimi[1], Summer S. Han[2], Eunji Choi[1]

[1]Weill Cornell Medicine, Cornell University, New York, NY,[2]Stanford School of Medicine, Stanford University, Palo Alto, CA

Introduction: Improved survival rates among breast cancer survivors highlight the need to address long-term health risks, including second primary cancers. Second primary lung cancer (SPLC) is the second most common second primary cancer among breast cancer survivors and is associated with high mortality. However, the optimal approach for identifying high-risk breast cancer survivors to be surveilled for SPLC remains uncertain. This study seeks to fill this gap by comparing the US Preventive Services Task Force (USPSTF) criteria for lung cancer screening vs. a risk-based criteria leveraging a SPLC risk prediction model tailored for breast cancer survivors.

Methods: We analyzed 5,563 patients diagnosed with initial primary breast cancer (IPBC) in 1993-2014 from the PLCO Cancer Screening Trial. SPLC was defined as a primary lung cancer occurring at least six months after the IPBC. We utilized cause-specific Cox regression to build a prediction model for 5-year SPLC risk in breast cancer survivors, informed by existing literature and the Bayesian information criterion for model parsimony. Predictive accuracy was assessed through discrimination using the area under the curve (AUC) and calibration via plots derived from 200 bootstrapped samples. We compared the risk-stratification ability of the USPSTF and risk-based criteria for SPLC screening using the Aalen-Johanssen estimator.

Results: In a cohort of women with breast cancer (mean age at diagnosis: 71 years; stages: in situ [20.7%], localized [58.8%], regional/distant [18.5%]), 91 individuals (1.6%) developed SPLC over 108,783 person-years of follow-up. The SPLC risk model exhibited strong discrimination (AUC: 0.86 [95% CI, 0.79-0.92]) and good calibration in internal validation. Key model features included risk-decreasing factors (hysterectomy, menopausal hormone therapy, BMI, ibuprofen use) and risk-increasing factors (prior cancer, liver comorbidities, smoking-related factors). The stratification by a predicted risk threshold of ≥2% (90th percentile) showed that the observed incidence of SPLC was higher in high vs. low-risk groups (8.7% vs. 0.8%), surpassing the stratification performance of the USPSTF criteria (6.6% vs. 0.7%) when comparing eligible and ineligible subgroups.

Conclusions: We developed an SPLC risk model tailored for breast cancer survivors. Although the USPSTF criteria showed reasonable risk stratification in SPLC risk, the risk-based approach provided superior performance. This method provides a promising strategy for identifying high-risk breast cancer survivors for SPLC who may benefit from low dose computed tomography screening. Given the potential value of integrating breast cancer screening with effective lung cancer screening strategies, breast cancer survivors could serve as a model to advance early detection of lung cancer during routine surveillance.

#3583 The Breast Cancer Risk Prediction Project: a resource of 1.8 million women with diverse backgrounds from 20 prospective cohorts to improve breast cancer risk prediction and advance breast cancer epidemiology.

Peter Kraft[1], Laura Beane-Freeman[1], Julie Palmer[2], Ellen O'Meara[3], Jeanine Genkinger[4], James Lacey[5], Thomas Rohan[6], Lauren Teras[7], Marian L. Neuhouser[8], Kala Visvanathan[9], Celine Vachon[10], Roger Milne[11], Christopher Haiman[12], Yu Chen[13], Heather Eliassen[14], Cari Kitahara[1], Katie O'Brien[15], Emily White[8], Garnet Anderson[8], I-Min Lee[14], Archie Campbell[16], Renee Fortner[17], Ylva Lagerros[18], Sven Sandin[18], Mia Gaudet[1], Montserrat Garcia-Closas[19], Nilanjan Chatterjee[9]

[1]National Cancer Institute, Rockville, MD,[2]Boston University, Boston, MA,[3]Kaiser Permanente Washington Health Research Institute, Seattle, WA,[4]Columbia University, New York, NY,[5]City of Hope, Duarte, CA,[6]Albert Einstein College of Medicine, Bronx, NY,[7]American Cancer Society, Atlanta, GA,[8]Fred Hutchinson Cancer Center, Seattle, WA,[9]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD,[10]Mayo Clinic, Rochester, MN,[11]Cancer Council Victoria, Melbourne, Australia,[12]Keck School of Medicine of the University of Southern California, Los Angeles, CA,[13]NYU Grossman School of Medicine, New York, NY,[14]Harvard T.H. Chan School of Public Health, Boston, MA,[15]National Institute of Environmental Health Sciences, Research Triangle, NC,[16]University of Edinburgh, Edinburgh, United Kingdom,[17]Cancer Research of Norway, Oslo, Norway,[18]Karolinska Institutet, Stockholm, Sweden,[19]Institute of Cancer Research, Sutton, United Kingdom

Breast cancer risk assessment tools are widely used in clinical practice to guide decisions regarding screening timing and modality, lifestyle interventions, genetic testing, preventive therapy, and risk-reducing surgery. Although a number of tools designed for the general population are used in practice, they face a series of challenges including: (i) modest discriminatory ability due to lack of a unified model that flexibly incorporates a comprehensive set of risk factors; (ii) inability to produce subtype-specific risk estimates, relevant for tailoring screening and/or preventive strategies (e.g. prophylactic endocrine therapy that is effective only for hormone receptor positive tumors); (iii) lack of data from diverse populations to build models that work well for all women; and, (iv) scarcity of opportunities for comparative validation of different models. The Breast Cancer Risk Prediction Project (BCRPP), a collaboration currently including 20 prospective cohorts from the National Cancer Institute's Cohort Consortium, is addressing these challenges by harmonizing, sharing, and analyzing data on over 1.8 million women, over 115,000 of whom have developed breast cancer. The BCRPP is developing a comprehensive tool that will predict breast cancer risk, overall and by subtypes, across major racial and ethnic groups in the United States. This tool incorporates known and emerging risk factors (e.g. reproductive history, anthropometry, smoking, alcohol intake, medication use, mammography, family history, and measured genotypes). In parallel to model building and validation efforts, BCRPP investigators are developing privacy-preserving web and mobile interfaces to deliver the risk tools to patients and clinicians. Here we present descriptive data on participating cohorts (e.g. dates of enrollment, ages at enrollment, length of follow up, numbers of cases by subtype), summarize missing data patterns and our approach to model development and validation that accounts for heterogeneity in missingness patterns across cohorts, and describe data governance procedures and data sharing infrastructure (including pooled and federated analyses, to account for policy limitations on sharing individual data). Descriptive information and procedures for requesting and accessing data are available via the BCRPP data platform (https://epidataplatforms.cancer.gov/bcrpp/). Although originally assembled for risk model development, these data on a large and diverse sample of women with extensive harmonized risk factors and covariates can be a resource to address other outstanding questions in breast cancer epidemiology across time, the lifecourse, and diverse populations.

#3584 Artificial intelligence-derived body composition measurements reveal sex- and metformin-specific predictors of overall survival in non-small cell lung cancer.

Xinan Wang[1], Akinori Hata[2], Noriaki Wada[3], Yi Li[4], Mark Schiebler[5], Hiroto Hatabu[3], David Christiani[6]

[1]Harvard T.H. Chan School of Public Health, Boston, MA,[2]Radiology, Osaka University, Osaka, Japan,[3]Radiology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA,[4]Biostatistics, University of Michigan, Ann Arbor, MI,[5]Radiology, University of Wisconsin, Madison, WI,[6]Pulmonary & Critical Care Medicine and Cancer Center, Massachusetts General Hospital, Boston, MA

Background: Body composition (BC) is emerging as a prognostic factor in cancer, with metformin showing potential anti-tumor effects in overweight/obese patients with non-small cell lung cancer (NSCLC). However, the sex-specific impact of BC on overall survival (OS), its underlying mechanisms, and relationship to metformin's beneficial effects remain unclear.
Methods: A total of 1,275 patients with NSCLC were included. Six AI-derived BC measurements were extracted from chest CT scans: muscle density and volume, visceral (VAT) and subcutaneous adipose tissue (SAT), VAT/SAT ratio, and intra-muscular adipose tissue (IMAT). Clinical data included age, sex, histology, stage, smoking status, diabetes, metformin use, and BMI. Multivariable Cox proportional hazard model was used to assess the associations with OS. Correlations between BC and eight circulating inflammatory cytokines were evaluated in a subset of 93 patients and BMI-related mutation profiles were compared in an independent cohort of 5,363 patients with NSCLC.
Results: Sex-specific distributions showed significantly higher muscle volume, density, VAT, and VAT/SAT ratio in males, while females had higher SAT and IMAT. Higher muscle volume is associated with prolonged OS (HR = 0.71, T3 vs. T1 and HR = 0.78, T2 vs. T1, $P < 0.01$). In females, higher muscle density was associated with prolonged OS (HR = 0.76, T3 vs. T1, $P = 0.04$), while higher IMAT correlated with shortened OS (HR = 1.39, T3 vs. T1, $P = 0.01$). In males, increased VAT and SAT tended to associate with prolonged OS. Higher SAT (HR = 0.48, 0.42 for T3 and T2 vs. T1, $P = 0.02$ and 0.01) and IMAT (HR = 0.52, 0.42 for T3 and T2 vs. T1, $P = 0.04$ and 0.01) predicted prolonged OS specifically in metformin users, but not in diabetic non-metformin users. Cytokine analysis showed positive correlations between VAT, SAT, IMAT and leptin in both sexes, while IMAT positively correlated with IL-8 and MCP-1, and muscle density negatively correlated with leptin and IL-8 in females. *EGFR* mutation was significantly less frequent and *KRAS* mutations more frequent in patients with BMI > 25, particularly in females. Mutation analysis revealed lower EGFR and higher KRAS mutation frequencies in patients with BMI > 25, particularly in females. Mutations in metabolism and immune response genes (STK11, NF1, ATM, and SMARCA4) showed reversed sex-specific trends related to BMI, suggesting obesity may drive distinct mutation selection and immune responses between sexes.
Conclusions: AI-derived BC measurements revealed sex-specific associations with OS in NSCLC, supported by distinct patterns in circulating cytokines and somatic mutations. Metformin's beneficial effects were specifically associated with adipose tissue measurements, suggesting its anti-tumor activity may be mediated through metabolic and immunomodulatory pathways.

#3585 Persistent poverty and late-stage breast cancer diagnosis in the United States: Impacts of rurality, race, and time within Surveillance, Epidemiology, and End Results (SEER) registries, 2004 to 2021.

H. R. Sherr[1], A. Soliman[2], K. A. Bertrand[3], K. A. Hirko[4];

[1]Boston University School of Public Health, Boston, MA, [2]City University of New York School of Medicine, New York, NY, [3]Boston University Chobanian & Avedisian School of Medicine, Boston, MA, [4]Michigan State University College of Human Medicine, Traverse City, MI

Background: County-level poverty is associated with late-stage breast cancer (BC) diagnosis, though the effects of persistent poverty (>20% residents in poverty for 30+ years) are unclear. Persistent poverty's role may also vary by rurality, race, and over time.

Methods: We analyzed county-level data from the Surveillance, Epidemiology, and End Results (SEER) Program for females (ages 20-74 years) diagnosed with late-stage (regional and distant) BC from 2017-2021 (excluding 2020). We estimated mean rate differences (RDs) in late-stage BC between persistently poor (n = 156) and non-persistently poor (n = 923) counties using multivariable linear regression. Models were adjusted for age, current poverty, race, lack of insurance, unemployment, and educational attainment using data from the American Community Survey (ACS) between 2017 and 2021. We stratified results by rurality (rural vs. urban based on Rural-Urban Continuum Codes) and race (≥ 5% vs. < 5% Non-Hispanic Black). Joinpoint regression was used to estimate annual percent change (APC) in BC stage at diagnosis rates from 2004-2019 according to persistent poverty status and rurality.

Results: Age-adjusted late-stage BC rates were higher in persistent poverty compared to non-persistent poverty counties (RD = 5.78 per 100,000 person-years, 95% CI = 2.46, 9.10). Differences in late-stage BC rates by persistent poverty status were similar in rural and urban counties but elevated in counties with larger non-Hispanic Black populations. Associations were attenuated in adjusted models (overall RD = 3.22, 95% CI = -1.13, 7.56). However, fully adjusted late-stage BC diagnosis rates were higher in persistently poor counties with small non-Hispanic Black populations (RD = 8.32, 95% CI = 1.39, 15.26).

Overall rates of breast cancer diagnosed at the distant stage increased from 2004 to 2010 (APC = 2.01, 95% CI = 1.09, 4.87) and have remained constant since (APC = 0.04, 95% CI = -1.65, 0.52). The rise in distant BC was driven by counties characterized by persistent poverty (APC = 1.79, 95% CI = 0.95, 2.62) and rurality (APC = 1.78, 95% CI = 0.68, 2.87). Regional breast cancer rates declined from 2004-2017 (APC = -1.05, 95% CI = -2.04, -0.74), but have been rising since (APC = 1.57, 95% CI = -0.93, 3.02).

Conclusions and Implications: Late-stage BC diagnosis rates were elevated in persistent poverty counties, but the difference was reduced after adjusting for current poverty. Rates of distant BC have risen over time, particularly in persistent poverty and rural counties. Efforts to alleviate the burden of poverty and address barriers to screening are needed to promote early BC detection, particularly in medically underserved areas.

#3586 Fusobacterium nucleatum presence and colorectal cancer-specific mortality in a racially and ethnically diverse patient population.

Nicole C. Lorona[1], Scott D. LaBrie[2], Claire E. Thomas[2], Hang Yin[2], Jeroen Huyghe[2], Conghui Qu[2], Sushma Thomas[2], Sosun Nayemi[2], Hamza Ammar[2], Orsalem Kahsai[2], Li Hsu[2], Diana Redwood[3], Li Li[4], Christopher I. Li[2], Ulrike Peters[2], Timothy K. Thomas[3], Amanda I. Phipps[2], Jane C. Figueiredo[1], Meredith Hullar[2]

[1]Department of Medicine, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA,[2]Fred Hutchinson Cancer Center, Seattle, WA,[3]Alaska Native Tribal Health Consortium, Anchorage, AK,[4]Ochsner Clinic Foundation, New Orleans, LA

Purpose: There are persistent racial and ethnic disparities in colorectal cancer (CRC) mortality. We examine the association between prevalence of *Fusobacterium nucleatum* (Fn) in tumor tissue and CRC-specific mortality in a diverse population.
Methods: The present study includes 569 African American, Alaska Native, Latinx, and non-Hispanic White adults with stage I-III CRC who were part of the Translational Research Program in Colorectal Cancer Disparities (TRPCD). 188 patients with CRC who died of CRC were matched to 381 patients with CRC who did not die of CRC. Genomic DNA was extracted from colorectal tumor tissue samples. Droplet digital polymerase chain reaction (ddPCR) was used to analyze 1) the nusG gene from Fn (Fn(nusG)), and 2) the SLCO2A1 gene as a housekeeping (HK) gene reference from the human genome. Fn(nusG) positive droplet counts were normalized by HK gene positive droplet counts for each duplicated assay, and then averaged. Fn(nusG) prevalence was analyzed as a dichotomous variable in primary analyses (not present, present) and as a categorical variable (not present, low prevalence, high prevalence). The median normalized average Fn(nusG) count among those with Fn(nusG) present was the cut-point between low and high prevalence. We fit logistic regression models for the association between Fn(nusG) presence and CRC-specific mortality, adjusted for matching factors (age at diagnosis, year of diagnosis, sex, stage, tumor site, race and ethnicity). Effect modification by age, sex, stage, tumor site, and racial and ethnic group was assessed using a likelihood ratio test.
Results: Fn(nusG) was present in tumors for 24% of patients with CRC who died of CRC (N=46) and 16% of patients with who did not die of CRC (N=61). Presence was lower in patients who were Alaska Native (14%), with stage I CRC (12%), and with rectal cancer (16%), and higher in those diagnosed in 2011-2017 (22%). Presence of Fn(nusG) was associated with a higher odds of CRC-specific mortality (OR: 1.76; 95% CI: 1.12-2.74), relative to no presence of Fn(nusG), after adjustment for matching factors. Estimates for the association between low prevalence (OR: 1.77; 95% CI: 0.97-3.21) and high prevalence (OR: 1.74; 95% CI: 0.95-3.14), relative to no presence of Fn(nusG), and CRC-specific mortality were similar. Further analysis is underway.
Conclusions: These findings provide evidence of an association between presence of fusobacterium in CRC tumor tissue and CRC-specific mortality in a diverse population. As the association did not differ by low or high prevalence of fusobacterium, any amount of fusobacterium in CRC tumor tissue may confer poorer prognosis. Fusobacterium may contribute less to CRC-specific mortality in Alaska Native patients with CRC. Identifying microbiota associated with CRC mortality can inform targeted interventions to address racial and ethnic disparities in this disease.

#3587 Identifying clinical and lifestyle factors which mediate the association between socioeconomic status and colorectal cancer mortality.

Shaneda Warren Andersen[1], Thomas Lawler[1], Oluwatoyosi Ogunmuyiwa[1], Rida A. Khatri[1], Mark Steinwandel[2], Martha Shrubsole[2]

[1]University of Wisconsin-Madison, Madison, WI,[2]Vanderbilt University Medical Center, Nashville, TN

Background: Higher socioeconomic status (SES) is associated with lower mortality risk for people with colorectal cancer (CRC) and contributes to geographic and racial disparities for CRC. However, the factors that mediate the association between SES and CRC mortality have not been comprehensively investigated. We performed a mediation analysis to identify clinical and lifestyle- factors that may mediate the association between household income as a proxy for SES with overall, and CRC-specific mortality in a longitudinal cohort where most participants are non-Hispanic Black with lower SES.

Methods: Data arise from 1,038 participants of the Southern Community Cohort Study (SCCS) who developed incident CRC after enrollment. Participants were aged 40-79 years at enrollment between 2002-2009; 73% self-identify as having Black racial identity. Annual household income was assessed via questionnaire at enrollment and 59% of the cohort had household income < $15,000/year. Overall and CRC-specific mortality were determined via linkages to the National Death Index through December 31, 2020. Proportional hazards models were used to estimate hazard ratios (HRs) with 95% confidence intervals (CIs) for mortality outcomes by income level, adjusted for age, sex, and marital status. Mediation analysis was used to estimate the indirect effect of stage, healthcare-related factors (CRC screening, insurance, and surgical resection) and lifestyle-related factors (smoking status, pack years smoked, alcohol consumption, diabetes duration, diet, and physical activity) on mortality, expressed as a percentage of the association between income and mortality attributable to the mediator.

Results: Income ≥ $15,000/year was associated with lower risk for mortality, however associations were more apparent in White participants (all-cause: mortality HR 0.44, CI: 0.29-0.67; CRC-specific HR: 0.43, CI: 0.24-0.76) than Black participants (all-cause: HR 0.76, CI: 0.62-0.94; CRC-specific: HR: 0.81, CI: 0.62-1.05). Stage at diagnosis and completing surgical resection were the largest mediation factors accounting for 35% of the association between income and CRC-specific mortality among white participants and 20% of the association among Black participants. Results were similar for overall mortality. For participants identifying as Black, history of and pack-years of smoking mediated an additional 28% of the association. For White participants, smoking and the other lifestyle-related factors did not mediate the association between income and mortality outcomes.

Conclusions: Higher income is associated with lower risk for mortality after CRC diagnosis. However, the factors that mediate the association may differ by self-reported race. Interventions to promote early detection of CRC and equitable access to curative surgery may help to mitigate CRC disparities related to SES.

#3588 Modifiable factors associated with survival among a racially diverse and low-income population with prostate cancer.

Sobia T. Siddiqui[1], Martha J. Shrubsole[1], Heather Munro[1], Xijing Han[1], Francesca Kolitsopoulos[2], Julia S. Perkins[3], Leo J. Russo[4], Loren Lipworth[1], Wei Zheng[1]

[1]Epidemiology, Vanderbilt University Medical Center, Nashville, TN,[2]Pharmacoepidemiology and Safety, Gilead Sciences, New York, NY,[3]Oncology and Hematology, Pfizer, New York, NY,[4]Epidemiology, Pfizer, New York, NY

Background: Prostate cancer is the most diagnosed cancer among men in the United States and is the second leading cause of cancer-related deaths. Black men have the highest incidence and mortality rates compared to all other racial or ethnic groups and these disparities are not fully understood.
Objective: In this study, we aim to investigate various sociodemographic, lifestyle, health and dietary factors that might be associated with survival in men diagnosed with prostate cancer within a study designed to evaluate cancer health disparities.
Methods: The Southern Community Cohort Study is a prospective cohort study of 34,313 adult men recruited between 2002 and 2009, with a focus on Black individuals and those with low socioeconomic status. At recruitment, a baseline questionnaire was administered to assess sociodemographic and lifestyle factors. Participants were followed over time by linkages to state cancer registries for cancer diagnosis and the National Death Index for death. Chi-squared and ANOVA tests examined associations between baseline characteristics and racial group. Cox proportional hazards regression models were used to assess relationships between covariates and survival, calculating hazard ratios for overall survival with 95% confidence intervals.
Results: Our study included 1,367 men with prostate cancer of whom 350 died, including 291 Black and 59 White men. Only current cigarette smoking was associated with increased risk of mortality among both Black (HR= 2.22, 95% CI=1.57-3.14) and White (HR= 4.09, 95% CI=1.56-10.75) men in comparison to never-smokers and only higher intake of added sugars was associated with reduced risk among both Black (HR=0.66, 95% CI=0.46-0.96) and White men (HR=0.31, 95% CI: 0.13-0.89). Among Black men only, diabetes history (HR=1.58, 95% CI=1.17-2.14) was associated with an increased risk of mortality and hypercholesterolemia (HR=0.74, 95% CI=0.55-0.99) was associated with decreased risk. Among White men only, living in an area of highest deprivation (HR=3.12, 95% CI=1.18-8.27) and consuming higher quantities of red meat (HR=3.66, 95% CI=1.37-9.73) were associated with increased risk of death and higher intake of whole grains (HR=0.34, 95% CI=0.15-0.76) was associated with a decreased risk.
Conclusion: Our study identified an increased risk of mortality in men with prostate cancer associated with several modifiable lifestyle, health and dietary factors. Although not all these factors were associated with increased risk among both Black and White men, differing lifestyle behaviors based on race highlight the need for studies to better understand mortality risk. However, cigarette smoking was strongly associated with risk for all groups, thus, tobacco cessation should be considered for men with prostate cancer. These findings highlight the need for targeted interventions to address disparities in outcomes in men with prostate cancer.

#3589 Serum organochlorine insecticide concentrations and risk of aggressive prostate cancer.

D. A. Tadesse[1], L. M. Hurwitz[1], R. B. Biritwum[2], Y. Tettey[2], A. Adjei[2], E. Tay[2], A. Sjodin[3], R. Jones[3], M. Davis[3], F. Menegaux[4], M. Abubakar[1], E. A. Akpakli[5], K. Klufio[5], J. E. Mensah[2], S. Berndt[1], S. Koutros[1];
[1]National Cancer Institute, Rockville, MD, [2]University of Ghana Medical School, Accra, Ghana, [3]Centers for Disease Control and Prevention, Atlanta, GA, [4]Universite Paris-Saclay, Villejuif, France, [5]Korle Bu Teaching Hospital, Accra, Ghana

Introduction: Prostate cancer is one of the leading causes of cancer morbidity and mortality among men worldwide, with heightened rates in Western, Central and Southern Africa, and the Caribbean. Although few risk factors have been identified, studies have shown positive associations with exposure to persistent organochlorine pesticides (OCPs) and aggressive prostate cancer. Few studies have yet been conducted in Africa despite the greater burden of prostate cancer and continued OCP use for vector control. Here we described predictors of serum levels of OCPs in 300 prostate cancer cases and 300 population-based controls from Ghana.
Methods: Serum concentrations of three DDT (dichlorodiphenyltrichloroethane) metabolites were measured: p,p'-dichlorodiphenyldichloro-ethylene(pp'-DDE), p,p'-dichlorodiphenyltrichloroethane(pp'-DDT), and o,p'-dichlorodiphenyltrichloroethane(op'-DDT). Chi-Square tests were used to examine the relationship between quartiles of DDT and demographic/lifestyle factors in controls. Levels were reported in cancer cases by clinical factors, including high-grade (Gleason≥8) and advanced stage (T3 or T4) disease.
Results: Among 300 controls, the geometric means (and percent detected) of pp'-DDE, pp'-DDT, and op'-DDT were at 737.6 ng/g lipid (99.7% detect), 48.4 ng/g lipid (95.6% detect), and 16.7 ng/g lipid (45.3% detect), respectively. Increasing BMI was associated with higher serum levels of pp'-DDE and pp'-DDT (chi-square p-value=0.064 and 0.002, respectively). Among prostate cancer cases, higher mean levels of pp'-DDE were observed in men with high-grade compared to low-grade disease (1109.3 ng/g lipid vs. 760.4 ng/g lipid, p-value=0.038) and in men with advanced disease (1139.2 ng/g lipid vs. 838.7 ng/g lipid, p=value=0.028).
Conclusion: OCPs are a class of insecticides that are banned in most countries due to their slow degradation and ability to bioaccumulate. Measurable levels of DDT were abundant in serum samples in men from Ghana (pp'-DDE =737.6 ng/g lipid vs. 262 ng/g lipid and nondetectable op'-DDT in Black men in the U.S. as reported by the 2003-2004 National Health and Nutrition Examination Survey), suggesting ongoing exposure. Consistent with reports that DDT is highly fat soluble, higher BMI was associated with higher serum concentrations. Serum levels of pp'-DDE were higher for those with aggressive prostate cancer, offering evidence of a plausible link between exposure and aggressive prostate cancer risk in Africa. Results from the ongoing case-control analysis will be presented.

#3590 Survival among individuals diagnosed with cancer during the COVID-19 pandemic in the United States.

Chenxi Jiang, Xuesong Han, Ahmedin Jemal, Hyuna Sung

American Cancer Society, Atlanta, GA

Background: The COVID-19 pandemic has impacted cancer outcomes, but research on its impact on survival outcomes, especially cause-specific death across different cancer types and socioeconomic factors, is limited.
Methods: Individuals diagnosed in 2019 and 2020 with one of 14 types of cancer at ages 20-89 in 17 Surveillance, Epidemiology, and End Results registries were included. We used Cox proportional hazards model to compare the risk of death from all causes, cancer, and COVID-19 within the first year of diagnosis between cases from 2020 vs 2019, adjusting for demographic factors and socioeconomic status. Additionally, for 2020 cases, Cox models were used to identify risk factors for COVID-19, cancer, and all causes death.
Results: Compared to 2019 cases (N=274,605), 2020 cases (N=248,840) had a higher risk of death from all causes and COVID-19 for each of the 14 types of cancer, while increased risks of cancer-specific death were limited to cases with 8 of 14 cancer types. Highest hazard ratios (HRs) were estimated among those with cancers of the thyroid (HR=1.57, 95%CI: 1.42-1.72) and prostate (HR=1.51, 95% CI:1.44-1.58) for all-cause death; among those with leukemia (HR=11.42, 95%CI: 6.31-20.64) and lung (HR=6.58, 95%CI:5.02-8.62) cancers for COVID-19 death; and among those with colorectal (HR=1.15, 95%CI:1.10-1.20) and breast (HR=1.12, 95%CI:1.04-1.21) cancers for cancer-specific death. Among 2020 cases, cancer-specific death and Covid-19 death showed distinct patterns in racial/ethnic and urban/rural disparities (Table 1). Specifically, individuals living in large metropolitan areas and those identifying as Hispanic were found to be at higher risk of death from COVID-19, but not from cancer.
Conclusion: The Covid-19 pandemic's impact on survival of individuals with cancer varied by cause of death, cancer type, and socioeconomic factors, highlighting the need for targeted healthcare interventions to ensure equitable care and reduced mortality.

Table 1: Association of patients characteristics with the risk of death from all-cause, cancer-speci

Risk factors (reference group)	All-cause Adjusted HR (95% CI)	cancer-specific Adjusted HR (95% CI)	covid-related Adjusted HR (95% CI)
Age (ref: 20-39 y)			
40-49 y	1.35 (1.25-1.45)	1.71 (1.50-1.96)	1.51 (0.74-3.09)
50-59 y	1.42 (1.33-1.52)	2.36 (2.10-2.66)	2.65 (1.42-4.97)
60-69 y	1.60 (1.50-1.71)	3.07 (2.73-3.45)	4.79 (2.61-8.79)
70+ y	2.23 (2.10-2.38)	4.49 (4.00-5.04)	8.26 (4.52-15.11)
Race (ref: Non-Hispanic White)			
Hispanic	1.42 (1.38-1.46)	1.00 (0.96-1.05)	1.91 (1.62-2.25)
Non-Hispanic American Indian/Alaska Native	1.13 (1.00-1.27)	1.03 (0.88-1.20)	1.36 (0.64-2.86)
Non-Hispanic Asian or Pacific Islander	1.20 (1.16-1.25)	1.02 (0.97-1.07)	1.16 (0.90-1.49)
Non-Hispanic Black	1.12 (1.08-1.16)	1.08 (1.04-1.13)	1.73 (1.44-2.07)
Urban/Rural (ref: Nonmetropolitan)			
Counties in metropolitan areas with >1 million population	1.02 (0.98-1.06)	0.89 (0.85-0.94)	1.30 (1.02-1.66)
Counties in metropolitan areas with less than 1 million population	0.98 (0.95-1.02)	0.94 (0.90-0.98)	1.21 (0.96-1.52)
Sex (ref: Female)			
Male	1.04 (1.02-1.06)	1.04 (1.01-1.07)	1.16 (1.00-1.34)
Income (ref: Upper income level)			
Low	1.28 (1.23-1.33)	1.27 (1.21-1.34)	1.80 (1.40-2.31)
Middle	1.13 (1.10-1.15)	1.13 (1.09-1.16)	1.57 (1.34-1.84)
Stage (Ref: Stage 1)			
Stage 2	1.41 (1.35-1.46)	2.13 (1.98-2.28)	1.44 (1.15-1.81)
Stage 3	2.22 (2.13-2.31)	4.22 (3.96-4.50)	1.95 (1.55-2.46)
Stage 4	3.22 (3.10-3.35)	6.84 (6.45-7.26)	1.82 (1.44-2.31)

#3591 Global burden and trends in cancer incidence and mortality among young adults.

Kieran Patrick Kelly[1], Yejun Son[2], Nikita Sandeep Wagle[1], Dong Keon Yon[2], Ahmedin Jemal[1], Hyuna Sung[1]

[1]Surveillance & Health Equity Science, American Cancer Society, Atlanta, GA,[2]Kyung Hee University College of Medicine, Seoul, Korea, Republic of

Background: Increasing cancer incidence among young adults has been reported for several types of cancers, primarily in certain high-income countries. However, there is a lack of comprehensive examination of the global burden and trends in cancer incidence and mortality among young adults.

Methods: We presented estimated numbers of cancer cases, cancer deaths, age-standardized incidence, and mortality rates per 100,000 people among young adults (YAs, ages 25-49 years) for all cancers combined and 35 cancer sites in 185 countries and 20 UN regions using GLOBOCAN 2022. We further quantified trends in age-standardized incidence (ASIR; 52 countries) and age-standardized mortality (ASMR; 62 countries) for the top five incident cancers (female breast, cervix uteri, thyroid, colorectum, and trachea, bronchus and lung cancers) among YAs over the most recent decade by estimating average annual percent change (AAPC), using the Cancer Incidence in Five Continents (CI5; 2008-2017) and WHO mortality data (2011-2020; thyroid mortality data was not available), respectively.

Results: In 2022, an estimated 2.9 million cancer cases and 962,000 cancer-related deaths occurred in YAs worldwide, corresponding to 108.1 cases per 100,000 people per year and 35.9 deaths per 100,000 people per year. Globally, ASIR and ASMR were higher in women than men (143.7 vs. 73.5 for incidence; 40.0 vs. 32.0 for mortality). By UN region, ASIR per 100,000 people was highest in Australia-New Zealand (185.7) and lowest in South Central Asia (72.5), while ASMR per 100,000 was highest in Eastern Africa (55.9) and lowest in Northern Europe (21.9). For the most recent 10 years, increases in ASIRs were observed for thyroid cancer (AAPC, 2.5-19.6%) in 27 countries, for colorectal cancer (AAPC, 1.2-11.5%) in 20 countries, for female breast cancer (AAPC, 0.7-5.9%) in 17 countries, and for cervix uteri cancer (AAPC, 1.8-15.7%) in 4 countries. Increases in ASMR were observed for colorectum cancer (AAPC, 0.6-8.0%) in 13 countries, for female breast cancer (AAPC, 0.9-4.8%) in 11 countries, and for cervix uteri cancer (AAPC, 2.1-4.7%) in 6 countries. Incidence and mortality both increased in YAs for female breast cancer in Czechia, ovarian cancer in the Republic of Korea, cervix uterine cancer in the Netherlands, and colorectum cancer in Australia, Canada, Chile, and the USA. Trachea, bronchus and lung cancers ASIR and ASMR decreased in 24 (AAPC, -1.5 to -11.3%) and 47 (AAPC, -2.0 to -11.4%) countries among YAs, respectively.

Conclusions and Relevance: The burden and trends in cancer among young adults vary significantly by sex and region. Rising incidence and mortality rates for multiple cancer types across the world underscore the need for renewed attention to cancer prevention efforts and improvements to cancer surveillance systems targeting this often overlooked age group.

#3592 Hospital-treated infectious diseases and pancreatic cancer risk: Findings from a large population-based cohort.

K. R. Murison[1], R. J. Hung[2];
[1]University of Toronto, Toronto, ON, Canada, [2]Sinai Health, Toronto, ON, Canada

Rationale: Inflammation has been established as a mechanism for pancreatic cancer development. The longitudinal relationship between infection, and the resulting inflammation, and pancreatic cancer risk has yet to be elucidated. A large population-based cohort, such as the United Kingdom (UK) Biobank, offers a unique opportunity to assess these relationships with long follow-up time and a large number of infections.
Objectives: Estimate the effect of hospital-treated infection on pancreatic cancer risk by infection type and infection burden.
Methods: UK Biobank participants with no record of pancreatic cancer at cohort entry were included. Follow-up was to February 29, 2020 and January 31, 2021 for participants from England and Wales, and Scotland, respectively. Hospital-treated infection status was obtained through linkage to hospital inpatient data and allowed for characterization of exposure to over 900 infectious diseases using the International Classification of Diseases and Related Health Problems 10th Revision. Incident pancreatic cancer diagnosis was obtained through linkage to national cancer registries. Relative risk (RR) and associated 95% confidence intervals (CI) were estimated for infectious disease groups and infectious disease burden categories using negative binomial regression models.
Results: From cohort entry to the end of follow up, 502,219 people were included in the cohort. 93,123 participants had at least one recorded exposure to hospital-treated infection preceding pancreatic cancer diagnosis and 1,255 were diagnosed with pancreatic cancer throughout the follow up period. Exposure to any hospital-treated infection was associated with increased risk of pancreatic cancer (aRR 2.56 [95% CI 2.27, 2.88]). When considering infection burden, a dose-response relationship was seen ($p_{trend} < 0.001$) where a greater number of infections was associated with a greater risk of pancreatic cancer. When compared to those with no infection, participants had increasing risk with 1 infection (aRR 1.99 [95% CI 1.69, 2.35]), 2 infections (aRR 2.84 [95% CI 2.34, 3.43]), and $\geq$3 infections (aRR 3.21 [95% CI 2.72, 3.78]).
Conclusions: Infections which require treatment in hospital are associated with risk of pancreatic cancer. This association was strongest when considering bacterial infection and its impact on longitudinal risk of pancreatic cancer. Further, the dose-response relationship observed when investigating infection burden may suggest a cumulative risk of pancreatic cancer conferred with severe infections.

#3593 Effect of regular antidepressants use on cancer morbidity and mortality: A propensity score matched cohort study.

Jing He[1], **Yue Ma**[1], chenyang Li[1], Fubin Liu[1], Yaogang Wang[2], Fengju Song[1]

[1]Department of Epidemiology and Biostatistics, Key Laboratory of Molecular Cancer Epidemiology, Key Laboratory of Prevention and Control of Major Diseases in the Population, Ministry of Education, National Clinical Research Center for Cancer, Tianjin's Clinical Research Center for Cancer,Tianjin Medical University Cancer Institute & Hospital, Tianjin, China,[2]School of Public Health, Tianjin Medical University, Tianjin, China

Background—Antidepressant use is rising globally and has shown potential anticancer properties in vitro, but findings from observational studies have been inconsistent. We aimed to investigate the association between regular antidepressant use and cancer risk in a large prospective cohort with long-term follow-up.
Methods—A total of 421,529 participants without cancer or cardiovascular disease at baseline were obtained from the UK Biobank, recruited between 2006 and 2010 from 22 assessment centres across the UK. Regular antidepressant users were matched to nonusers using propensity score matching (PSM), adjusting for 21 key covariates, including age, sex, race, socioeconomic status, lifestyle factors, and medical history. The primary outcomes were overall cancer morbidity and mortality, with site-specific cancer morbidity as a secondary outcome. The association between different antidepressant subtypes and cancer risk was also examined.
Findings—The median follow-up was 13.6 years. Of the participants, 26,796 were regular antidepressant users and 394,733 were nonusers. After 1:1 PSM, 26,372 matched pairs were identified. Regular antidepressant use was associated with a reduced risk of overall cancer morbidity (HR 0.83, 95% CI 0.79-0.88), particularly for colorectal cancer (HR 0.75, 95% CI 0.65-0.86), and lower cancer-related mortality (HR 0.91, 95% CI 0.84-0.99) compared with nonusers. Among antidepressant subtypes, selective serotonin reuptake inhibitors (SSRIs) were associated with a 17% reduced risk of overall cancer morbidity (HR 0.83, 95% CI 0.79-0.88), especially for fluoxetine, citalopram, and sertraline. SSRIs were also associated with a 23% reduced risk of cancer-related mortality (HR 0.77, 95% CI 0.70-0.85), particularly with fluoxetine and citalopram. However, tricyclic antidepressants (TCAs) were linked to an increased risk of cancer-related mortality (HR 1.19, 95% CI 1.07-1.32), especially for amitriptyline.
Interpretation—These findings provide important evidence for the potential repurposing of antidepressants in cancer prevention and treatment. However, TCAs, such as amitriptyline, were associated with an increased risk, warranting caution. Further research is needed to confirm these findings.

#3594 Performance of common general-population breast cancer risk prediction models in 14 cohorts.

Kristen D. Brantley[1], **Thomas U. Ahearn**[2], Emily Norton[3], Julie Palmer[4], Gary Zirpoli[4], Matt Barnett[5], Marian L. Neuhouser[5], Lauren Teras[6], James Hodge[6], Thomas E. Rohan[7], Roger Milne[8], A. Heather Eliassen[9], Hongyan Huang[9], Yu Chen[10], Katie M. O'Brien[11], Cari Kitahara[12], Garnet Anderson[5], I-Min Lee[13], Nilanjan Chatterjee[3], Montserrat Garcia-Closas[14], Peter Kraft[2], Breast Cancer Risk Prediction Project

[1]Dana-Farber Cancer Institute, Boston, MA,[2]National Cancer Institute, Bethesda, MD,[3]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD,[4]Slone Epidemiology Center at Boston University, Boston, MA,[5]Fred Hutchinson Cancer Center, Seattle, WA,[6]American Cancer Society, Atlanta, GA,[7]Albert Einstein College of Medicine, Bronx, NY,[8]University of Melbourne, Melbourne, Australia,[9]Harvard TH Chan School of Public Health, Boston, MA,[10]New York University Langone Medical Center, New York, NY,[11]National Institute of Environmental Health Sciences, Durham, NC,[12]Radiation Epidemiology Branch, National Cancer Institute, Bethesda, MD,[13]Harvard Medical School, Boston, MA,[14]Institute of Cancer Research, London, United Kingdom

Background: Several breast cancer (BC) risk prediction models are used in clinical practice to identify women eligible for enhanced screening or prevention strategies. While these models have been independently validated in specific and often separate contexts, their performance has not been systematically evaluated and compared across a wide range of populations or age ranges.
Methods: We collected individual-level baseline questionnaire data and incident cancer diagnoses from 14 cohorts participating in the Breast Cancer Risk Prediction Project (BCRPP), representing the United States (N=12), Canada (N=1) and Australia (N=1). After harmonizing data across cohorts, five-year absolute risk estimates for invasive breast cancer were derived using four models: BCRAT, iCARE-Lit, Tyrer-Cuzick (all estimating risk in women aged ≥20-75 years) and the Black Women's Health Study (BWHS) calculator (estimating risk in Black women aged ≥30-70 years). Using the iCARE-calibrate function in R we estimated the area under the curve (AUC) and 95% confidence intervals (CI) within each cohort, using absolute risk designations to incorporate age. Expected to observed (E/O) absolute risks were estimated on average and within expected absolute risk deciles for each cohort. To align with its intended use in clinical practice, calibration of the BWHS calculator (and comparison to other models) was performed within the subset of Black women pooled from all cohorts.
Results: A total of 1,041,708 women, enrolled in studies between 1976-2015, were included. Within five years of baseline cohort entry, 116,113 invasive breast cancer cases were diagnosed. Mean age at baseline ranged from 34 to 62 years. Within cohorts, discrimination was similar across models but varied substantially across cohorts. Age-incorporated AUCs ranged from 0.55-0.77, with most cohort-specific AUCs under 0.65 and higher AUCs observed among younger cohorts. Calibration, measured by E/O ratios, differed substantially by both cohort and risk prediction model. For the majority of cohort-model combinations, risk was overestimated in the upper risk deciles. In general, E/O ratios were more similar for BCRAT and Tyrer-Cuzick models compared to iCARE-Lit, which tended to overestimate risk more on average. In the subset of Black women aged ≥30-70 years (N=84,594, N=824 invasive cases in 5 years), age-incorporated AUCs ranged from 0.61 to 0.64. While risk was overestimated in the upper risk deciles for all models, overestimation was 13-118% lower for the BWHS calculator.
Conclusion: The discrimination and calibration of existing risk prediction models varied across studies. Future model development including additional risk factors (e.g., genetic and mammographic information) should leverage diverse training data and flexible models to ensure risk estimates perform well across different regions, countries, and ethnicities.

#3595 Estimating excess mortality due to cancer during the COVID-19 outbreak in South Korea.

Linh Thi My Bui[1], Jinwoo Cho[2], Yun-Chul Hong[3], Yoon-Jung Choi[1]

[1]Cancer Control and Population Health, National Cancer Center Graduate School of Cancer Science and Policy, Goyang, Korea, Republic of,[2]Department of Statistics, University of Pittsburg, Pittsburgh, PA,[3]Department of Preventive Medicine, Seoul National University College of Medicine, Seoul, Korea, Republic of

Background: Excess mortality serves as a valuable metric for evaluating the comprehensive health impact of healthcare systems worldwide during the COVID-19 pandemic, particularly affecting vulnerable populations, including cancer patients. This study aims to estimate excess mortality attributable to all causes and cause-specific mortality, including cancer, during the COVID-19 pandemic in South Korea. ***Methods:*** Monthly mortality data from January 2000 to December 2022 was obtained from Statistics Korea. Using the World Health Organization International Classification of Diseases and Related Health Problems (ICD-10), we identified deaths due to neoplasia (C00-D48), infectious diseases (A00-B99, U07.1, U07.2, U10), cardiovascular diseases (I00-I99), and respiratory diseases (J00-J98, U04). Excess mortality during 2020-2022 was calculated by subtracting the predicted monthly number of deaths from the observed deaths from January 2020 to December 2022. The predicted number of deaths in 2020-2022 was estimated by using exponential smoothing, specifically, Error-Trend-Seasonal (ETS) models based on the death in 2000-2019. AIC, BIC and Log likelihood criteria were used to evaluate the different models of ETS, which showed that (additive, additive, additive) model was the most well-fitting. ***Results:*** All-cause excess mortality was highest in March 2022, with a total of 44,616 observed deaths (predicted deaths: 26,874, 95% confidence interval (CI): 22,271 - 31,476 deaths, statistically significant). There was significant excess mortality due to infectious and parasitic diseases in every month from 2021 to 2022, peaking at March 2022 (1367.75%), which corresponded to 11,893 observed deaths (predicted deaths: 810, 95% CI 676 - 945). The number of deaths attributable to cardiovascular or respiratory diseases was not statistical different from predicted values. Notably, excess mortality attributable to cancer was significantly associated with the COVID-19 pandemic, peaking in February 2022 at a rate exceeding 4.45%, equating to 6,740 observed deaths (predicted deaths: 6453, 95% CI 6212 - 6704). Among those deaths, males experienced a higher excess mortality rate of 3.91%, compared to 1.74% among females. ***Conclusion:*** We observed a significant overall increase in all-cause excess mortality during 2022 in South Korea related to the COVID-19 outbreak. Although cancer deaths remained relatively stable and closely aligned with predicted values during the study period, there was 4.45% increase of cancer death in 2022 February which exceeded the upper limit of 95% CI. Our study provides valuable insights into the resilience of cancer-related mortality patterns in South Korea amidst the challenges posed by the pandemic in accessing healthcare services and medical resources.

#3596 Risk of gastrointestinal cancer by sex and age in U.S. veterans with new-onset iron deficiency anemia.

Andrew J. Read[1], Sarah L. Krein[1], Tony Van[2], Kennedy Dubose[2], Jennifer Burns[2], Akbar K. Waljee[3], Sameer D. Saini[1]

[1]Internal Medicine, University of Michigan, Ann Arbor, MI,[2]VA HSR&D Center for Clinical Management Research, Ann Arbor, MI,[3]Learning Health Sciences, University of Michigan, Ann Arbor, MI

Background: New onset iron deficiency anemia (IDA) may indicate undiagnosed gastrointestinal (GI) cancer. Current guidelines strongly recommend colonoscopy and upper endoscopy (bidirectional endoscopy) for new onset IDA in men and postmenopausal women. In younger women, guidelines conditionally recommend bidirectional endoscopy, as IDA can occur from benign menstrual blood loss. Given rising rates of GI cancers in younger individuals, disregarding IDA in premenopausal women could inadvertently lead to delayed diagnoses of GI cancer. We assessed the likelihood of GI cancer in a national cohort of Veterans with new onset IDA by age and sex, using common clinical cutoffs.

Methods: We conducted a retrospective cohort study in the Veterans Health Administration (VA). We identified regular users of VA primary care (≥1 primary care visit every 2 years over a 5-year period) who developed a new anemia (hemoglobin <12 g/dL for women, <13 g/dL for men), and who had a ferritin within 1 year. We defined IDA as ferritin <45 ng/ml and varied this definition in sensitivity analysis. We identified luminal GI cancers (esophageal, gastric, small intestine, colon, rectum) diagnosed within 1 year of incident anemia by the VA cancer registry. We calculated the odds ratio (OR) of IDA and a GI cancer diagnosis in the overall cohort, stratified by age and sex.

Results: 308,250 individuals met inclusion criteria, with 18,672 (6.1%) women and 289,578 (93.9%) men. 94,713 (30.7%) had IDA, and 33,711 (10.9%) were <50 years old. 3,035 GI cancers were diagnosed within 1 year of incident anemia (0.98%), the majority of which were colon cancers (n=1835, 60.5%). The odds of cancer were significantly higher in those with IDA versus non-IDA (OR 4.47, 95% CI: 4.14-4.82). For women with IDA age ≥ 50 compared to those < age 50, the OR of GI cancer in 1 year was 6.62 (95% CI: 3.54-12.36). In those < age 50 with IDA, men had an OR of GI cancer in 1 year of 9.74 compared to women (95% CI: 5.45-17.40). In those age ≥ 50 with IDA, men had an OR of GI cancer of 2.24 compared to women (95% CI: 1.64-3.05). In sensitivity analysis, lowering the ferritin cutoff to 10 ng/ml did not change the directionality of these relationships.

Conclusions: Women age < 50 with new onset IDA were substantially less likely to develop GI cancer than comparable age men and older women. These findings support current guidelines, which strongly recommend bidirectional endoscopy in men and postmenopausal women and conditionally recommend endoscopy in premenopausal women. At the same time, clinicians should carefully assess for the presence / absence of symptoms that point to GI tract blood loss in premenopausal women. Additionally, future work should explore the role of computational methods and the electronic health record to leverage data from longitudinal complete blood counts (CBCs), moving beyond simple ferritin cutoffs.

#3597 Tumor bacteria, tumor-infiltrating T cells, and colorectal cancer survival outcomes.

Tomotaka Ugai[1], Jennifer Borowsky[2], Yuxue Zhong[1], Satoko Ugai[3], Mai Chan Lau[4], Kota Arima[5], Koichiro Haruki[6], Marios Giannakis[7], Jonathan Nowak[7], Shuji Ogino[1]

[1]Brigham and Women's Hospital, Boston, MA,[2]The University of Queensland, Brisbane, Australia,[3]Harvard T.H. Chan School of Public Health, Boston, MA,[4]A*STAR's Bioinformatics Institute, Singapore, Singapore,[5]Kumamoto University, Kumamoto, Japan,[6]The Jikei University School of Medicine, Tokyo, Japan,[7]Dana-Farber Cancer Institute, Boston, MA

Background: Colorectal cancer (CRC) is a heterogeneous tumor with complex interactions between tumor cells, immune cells, and microbiota in the tumor microenvironment. We hypothesized that T cell infiltrates might differ by colorectal tumor tissue bacteria. We also hypothesized that the presence of T-cell-associated tumor bacteria might be associated with worse clinical outcomes.
Methods: We used a prospective cohort tumor biobank database of 831 incidence CRC cases with available tissue data in the Nurses' Health Study and the Health Professionals Follow-up Study. To identify the tumor microbiota, we developed 16S rRNA gene sequencing and validated the data using quantitative PCR assays. We assessed tumor-infiltrating T-cell subsets using multiplexed immunofluorescence assays with digital image analyses and machine-learning algorithms. Spearman correlation test was performed to examine the correlation between the abundance of tumor microbiota and $CD3^+CD4^+$ or $CD3^+CD8^+$ T-cell densities. Multivariable Cox proportional hazards regression model was used to calculate hazard ratios (HRs) and 95% CIs for CRC-specific mortality. P values <0.005 were considered statistically significant.
Results: Strong negative correlations were observed between $CD3^+CD4^+$ T-cell density and the abundance of *Bacteroides fragilis* (rho=-0.12, P<0.001), *Fusobacterium canifelinum* (rho=-0.11, P=0.001), *Fusobacterium nucleatum* (rho=-0.10, P=0.003), and *Moryella indoligenes* (rho=-0.08, P=0.007). We observed similar strong negative correlations between $CD3^+CD8^+$ T-cell density and *Bordetella trematum* (rho=-0.10, P=0.004), and *Prevotella salivae* (rho=-0.075, P=0.023). We further evaluated whether T-cell-associated bacteria were associated with poor prognosis. Among identified $CD3^+CD4^+$ T cell-associated bacteria, the presence of *Moryella indoligenes* [HR (95% CI), 2.82 (1.40-5.66); P=0.004]*, Campylobacter gracilis* [HR (95% CI), 2.10 (1.22-3.61); P=0.008], and *Fusobacterium nucleatum* [HR (95% CI), 1.56 (1.17-2.30); P=0.004] were associated with higher CRC-specific mortality. We did not observe significant associations of $CD3^+CD8^+$T-cell-associated bacteria with poor prognosis.
Conclusions: We identified immune-suppressive bacteria that were correlated with lower $CD3^+CD4^+$ and $CD3^+CD8^+$T cell densities in the tumor microenvironment. Among these immune-suppressive bacteria, *Fusobacterium nucleatum*, *Moryella indoligenes,* and *Campylobacter gracilis* were associated with both lower $CD3^+CD4^+$T cell density and worse survival. Our findings indicate that certain tumor microbiota, such as *Fusobacterium nucleatum,* suppresses anti-tumor T cell response, leading to the development and progression of CRC. By contrast, other tumor bacteria, such as *Bacteroides fragilis,* may suppress anti-tumor T cell response and have a key role in tumor initiation, but may not strongly affect clinical outcomes.

#3598 Environmental circadian misalignment and cancer prevalence in the United States.

Seigi Karasaki[1], Matthew D. Weaver[2], Amanda I. Phipps[3], Trang VoPham[1]

[1]Epidemiology, Fred Hutchinson Cancer Center, Seattle, WA,[2]Medicine and Neurology, Brigham and Women's Hospital, Boston, MA,[3]Epidemiology, University of Washington, Seattle, WA

PURPOSE: Circadian disruption (e.g. from shift work) is associated with increased risk for certain cancers. Emerging evidence suggests that sources of environmental circadian misalignment, such as solar jetlag (due to differential light exposure based on time zone position) and polycyclic aromatic hydrocarbons (PAHs; from combustion of compounds such as gasoline, damaging the transcription translation feedback loop that drives circadian rhythms at the cellular level) may impact cancer development. We examined the associations between solar jetlag, PAHs, and cancer prevalence.

METHODS: We examined cancer prevalence (proportion of adults in 2022 ≥18 years diagnosed with cancer, excluding non-melanoma skin cancer) in the United States at the census tract level using Population Level Analysis and Community EStimates (PLACES). We estimated solar jetlag exposure (unitless) using a high-resolution geospatial model incorporating data on time zone position, sunrise and sunset times, and elevation. We estimated exposure to PAHs using data on smoking prevalence from PLACES; benzo[a]pyrene (BaP) and residential wood combustion from chemical transport and atmospheric dispersions models; black carbon (BC), carbon monoxide (CO), nitrogen dioxide (NO_2), particulate matter <10 microns in diameter (PM_{10}), and $PM_{2.5}$ from land-use regression; and traffic volume from averaged traffic counts. We used geographic information systems to aggregate and join these data to census tracts with areal weighting. We used Poisson regression with robust variance estimation to calculate prevalence ratios (PRs) and 95% confidence intervals (CIs) for associations between solar jetlag, PAHs, and cancer prevalence adjusting for census tract-level sociodemographics, health conditions, behaviors, socioeconomic status, and light at night.

RESULTS: This analysis included 77,494 census tracts with an average cancer prevalence of 8.0%. There were no independent associations of solar jetlag or PAHs with cancer prevalence. However, there were statistically significant interactions between solar jetlag and BC, NO_2, PM_{10}, $PM_{2.5}$, and traffic volume in associations with cancer prevalence. The strongest associations were observed within $PM_{2.5}$ exposure strata, in which higher solar jetlag exposure was associated with increased cancer prevalence in areas with relatively higher $PM_{2.5}$ levels (adjusted PR per interquartile range [45.33] increase in highest quintile of $PM_{2.5}$ [8.62-35.40 μg/m^3]: 1.05, 95% CI 1.04-1.07 vs. adjusted PR in lowest quintile of $PM_{2.5}$ [2.05-5.89 μg/m^3]:1.01, 95% CI 0.99-1.03; p interaction <0.01).

CONCLUSIONS: We observed interactions between solar jetlag and PAHs on cancer prevalence, suggesting that these sources of environmental circadian disruption may jointly act to impact cancer. Future research should utilize geocoded addresses, examine other sources of PAHs such as from diet, and data on cancer incidence.

#3599 Proteomic risk factors for prostate cancer: A case-cohort study in EPIC.

Zhe Huang[1], Mahboubeh Parsaeian[1], Vivian Viallon[2], Ziqiao Wang[3], Keren Papier[1], Trishna Desai[1], Stephanie Chan[1], Antonio Agudo[4], Carlotta Sacerdote[5], David C. Muller[6], Domenico Pali[7], Giovanna Masala[7], Nicholas Wareham[8], Raul Zamora-Ros[9], Roel C. H. Vermeulen[10], Rosario Tumino[11], Ian G. Mills[12], Nilanjan Chatterjee[3], Elizabeth A. Platz[13], Pietro Ferrari[2], Marc Gunter[6], Elio Riboli[6], Tim J. Key[1], Joshua R. Atkins[1], Karl Smith-Byrne[1], Ruth C. Travis[1], The EPIC SomaLogic Working Group

[1]Nuffield Department of Population Health, University of Oxford, Oxford, United Kingdom,[2]International Agency for Research on Cancer, Lyon, France,[3]Department of Biostatistics, Johns Hopkins University, Baltimore, MD,[4]Institut Catala d'Oncologia, Barcelona, Spain,[5]University of Turin, Turin, Italy,[6]School of Public Health, Imperial College London, London, United Kingdom,[7]Institute for Cancer Research, Prevention and Clinical network, Florence, Italy,[8]University of Cambridge, Cambridge, United Kingdom,[9]Unit of Nutrition and Cancer, Cancer Epidemiology Research Program, Catalan Institute of Oncology (ICO), Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain,[10]Division of Environmental Epidemiology and Veterinary Public Health, Institute for Risk Assessment S, Utrecht University, Utrecht, Netherlands,[11]Iblea Association, Epidemiological Research (AIRE-ONLUS), Ragusa, Italy,[12]Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom,[13]Department of Epidemiology, Johns Hopkins University, Baltimore, MD

Background: Prostate cancer (PCa) is the most prevalent cancer among men in Europe, yet its aetiology is poorly understood. Proteins contribute to the development of carcinogenesis and are also the target of most pharmacological interventions. The examination of associations between plasma proteins and PCa may enhance understanding of the aetiology of the PCa.
Aim: Identify proteins associated with PCa risk within a large European prospective cohort, with emphasis on associations with aggressive subtypes of the disease.
Methods: We conducted a case-cohort study in the European Prospective Investigation into Cancer and Nutrition (EPIC) study, with data for a sub-cohort of 1,573 individuals and for 982 incident PCa cases. The SomaLogic® 7K panel was used to measure 7,363 aptamers (representing 6,412 unique proteins) in plasma samples drawn at recruitment. PCa diagnosis was ascertained by linkage to cancer and death registry data, and was further stratified into aggressive subtypes based on histological grade, tumour stage and mortality information. The Prentice-weight Cox regression model was applied to estimate associations between individual proteins and PCa risk, with adjustment for body mass index, smoking, alcohol intake and education level. Subgroup analyses examined associations with high-grade, advanced-stage, aggressive and extended lag-time (diagnosis more than 15 years after blood draw) PCa risk. We sought external replication for our significant findings in an independent multi-ancestry cohort, the Atherosclerosis Risk in Communities (ARIC) study (SomaLogic® 5K).
Results: Following a median follow-up period of 16.2 years, the sub-cohort developed 79 cases of PCa. ACP3, FLT4, and KLK3 [HRs (95% CI): 1.20 (1.10, 1.31), 1.26 (1.13, 1.40) and 2.28 (1.96, 2.65), respectively] were associated with overall PCa risk in EPIC at FDR (false discovery rate) <0.05. In the subgroup analyses, 12 proteins were associated with high-grade, 9 with advanced stage, and 7 with aggressive PCa risk. Among these, ANKRD1 was associated with approximately a 25% higher risk of all three of these clinically relevant subtypes per SD increase. Twelve proteins were associated with PCa risk diagnosed more than 15 years after blood draw, including ANXA2, OSCAR, and SAT2. Two well-established biomarkers of PCa (ACP3 and KLK3) were replicated in ARIC, together with two novel proteins (ANXA2 and APOC2).
Conclusion: Multiple novel circulating proteins were associated with PCa risk, particularly with risk for aggressive subtypes. While some proteins may serve as early biomarkers of PCa, those with evidence from long-lag time highlight underlying aetiological mechanisms in the development of PCa.

#3600 Methylation-based biological age and risk of colorectal cancer recurrence: Findings from the ColoCare Study.

A. Richards[1], E. Jean-Baptiste[1], T. Barrow[2], S. Hardikar[3], B. Gigic[4], J. Figueiredo[5], C. Li[6], D. Shibata[7], A. T. Toriola[8], D. A. Byrd[1], C. M. Ulrich[3], P. Stewart[1], E. M. Siegel[1], **J. K. Kresovich**[1];

[1]H. Lee Moffitt Cancer Center, Tampa, FL, [2]University of Essex, Essex, United Kingdom, [3]Huntsman Cancer Institute, Salt Lake City, UT, [4]Heidelberg University Hospital, Heidelberg, Germany, [5]Cedars-Sinai Medical Center, Los Angeles, CA, [6]Fred Hutchinson Cancer Center, Seattle, WA, [7]University of Tennessee Health Science Center, Memphis, TN, [8]Washington University School of Medicine, St. Louis, MO

Introduction: Approximately 20% of colorectal cancer (CRC) patients experience disease recurrence, contributing significantly to CRC-specific mortality in those with non-metastatic disease. Recurrence risk is typically assessed after treatment, potentially delaying interventions for high-risk patients. Assessing recurrence risk earlier could enable personalized therapy strategies that balance remission potential with treatment side-effects. Although tumor characteristics provide some predictive value, they are insufficient for accurately identifying patients at high risk of recurrence. Biological age metrics derived from blood DNA methylation (DNAm) data, called “epigenetic clocks,” integrate information from known recurrence risk factors and are attractive targets for improving risk assessment. However, the relationship between epigenetic clocks and CRC recurrence risk has not been examined in detail.

Methods: The ColoCare Study is an international, multi-center, prospective cohort of newly diagnosed CRC patients. For this study, we selected a nested case-control sample of 137 patients enrolled at Moffitt Cancer Center, with recurrence cases matched 1:3 to recurrence-free controls by age, sex, body mass index, stage, and follow-up time. Pre-treatment whole blood DNAm profiles were assayed and used to calculate seven epigenetic clocks (Hannum, Horvath13, Horvath18, PhenoAge, GrimAge, DunedinPACE, DNAm Frailty). Logistic regression models, adjusted for matching factors and tumor location, estimated odds ratios (ORs) for associations between each epigenetic clock (per 1-standard deviation [SD] increase) and risks of CRC recurrence and all-cause mortality.

Results: Patients had a mean age of 62.7 years at diagnosis (SD= 13), with a majority being male (57%), overweight (78%), and White (90%). During follow-up, 35 (25%) patients experienced CRC recurrence, and 47 (34%) died. Higher GrimAgeAccel, DunedinPACE, and DNAm Frailty were associated with higher recurrence risk (GrimAgeAccel, OR: 1.60, 95% CI: 1.02, 2.50, P= 0.04; DunedinPACE, OR: 1.47, 95% CI: 0.91, 2.36, P= 0.12; DNAm Frailty, OR: 2.13, 95% CI: 1.17, 3.85, P= 0.01). PhenoAgeAccel, GrimAgeAccel, DunedinPACE, and DNAm Frailty were also positively associated with all-cause mortality (PhenoAgeAccel, OR: 1.52, 95% CI: 0.99, 2.35, P= 0.06; GrimAgeAccel, OR: 1.78, 95% CI: 1.15, 2.76, P= 0.01; DunedinPACE, OR: 1.43, 95% CI: 0.91, 2.23, P= 0.12; DNAm Frailty, OR: 1.69, 95% CI: 0.97, 2.95, P= 0.06).

Conclusions: Several epigenetic clocks measured in pre-treatment blood samples are associated with CRC recurrence risk and mortality. These findings suggest epigenetic clocks are novel risk factors for CRC recurrence that may be useful for enhancing risk assessment before treatment initiation.

#3601 Elevated short-chain fatty acid level and increased risk of hepatocellular carcinoma in a prospective cohort study.

Sindhu Karnam[1], Wei Jia[2], Renwei Wang[3], Jennifer Adams-Haduch[3], Woon-Puay Koh[4], Jian-Min Yuan[1]

[1]UPMC Hillman Cancer Center, University of Pittsburgh School of Public Health, Pittsburgh, PA,[2]The University of Hong Kong, Hong Kong, China,[3]UPMC Hillman Cancer Center, University of Pittsburgh Medical Center, Pittsburgh, PA,[4]Yong Loo Lin School of Medicine, National University of Singapore, Kent Ridge, Singapore

Background: Hepatocellular carcinoma (HCC) makes up 80-90% of primary liver cancers and is typically detected at an advanced stage because of its silent nature of progression and a lack of effective non-invasive early detection biomarkers. Gut-derived metabolites such as short-chain fatty acids (SCFAs) play a key role in liver health and may impact the progression of early non-malignant stages of liver disease to malignant transformation to HCC. Although most of studies have shown that SCFAs including acetate, butyrate, and propionate have been associated with lower risk of intestinal disease, some experimental studies have shown dysregulated metabolism of SCFAs may induce proinflammatory milieu for liver disease progress. The goal of this study is to investigate whether metabolomic profiling of SCFAs in baseline serum is associated with risk of HCC development.
Methods: We performed a nested case-control study with 100 incident HCC cases and 100 individually matched healthy controls within the Singapore Chinese Health Study, a cohort of 63,257 men and women aged 45-74 years at enrollment in 1993-1998. The incidence of HCC among cohort participants was annually surveilled using the nationwide Singapore Cancer Registry. Serum acetic, butyric, propionic, and valeric acids were quantified using ultra-performance liquid chromatography triple-quadrupole mass spectrometry assays. The conditional logistic regression was used to evaluate the association between SCFAs and HCC risk using odds ratios (ORs) and their 95% confidence intervals (CI).
Results: Cases and controls were comparable in distributions by age, body mass index (BMI), alcohol consumption and smoking status. Individuals with HCC were more likely to be HBsAg positive and had a history of diabetes than controls. Among controls, the summed all SCFAs and total butyric acid and its metabolites were positively associated with BMI (P trend < 0.05). The summed and most of individual SCFAs measured were significantly elevated in HCC compared with controls. Compared to the lowest tertile, the OR (95% CI) of HCC for the highest tertile of summed all SCFAs and total butyric acids was 3.24 (1.45-7.24) and 2.78 (1.27-6.12) after adjusting for age, sex, BMI, alcohol consumption, smoking status, type 2 diabetes, and HBsAg status (both P's for trend < 0.01).
Conclusion: The present analysis demonstrated a statistically significant association between elevated SCFA levels and increased risk of HCC in a prospective cohort of Singaporean Chinese. These results align with increased risk of HCC within this cohort with elevations in other metabolites such as bile acids. Mapping metabolic profile changes in liver disease progression to cancer will provide clinicians with a novel biomarker for risk stratification and early detection.

#3602 The association between longitudinal lipid trajectories and all-cause mortality in breast cancer survivors: A population-based study.

Shana Jean Kim[1], Vanessa De Rubeis[2], Brendan T. Smith[3], Husam Abdel-Qadir[4], Jennifer D. Brooks[1]

[1]University of Toronto, Toronto, ON, Canada,[2]McMaster University, Hamilton, ON, Canada,[3]Public Health Ontario, Toronto, ON, Canada,[4]Women's College Hospital, Toronto, ON, Canada

Background: Given breast cancer is the second leading cause of cancer-related death in women, it is important to identify breast cancer patients at high risk of mortality. Lipid levels before and after diagnosis may be key indicators for overall health and prognostic outcomes for breast cancer patients. This study aimed to identify distinct lipid trajectories in those diagnosed with breast cancer and examined their association with all-cause mortality.

Methods: This population-based retrospective cohort study included females diagnosed with incident breast cancer who survived at least five years after diagnosis (i.e., breast cancer survivors). Total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides (mmol/L) were used to develop trajectory patterns for each lipid through group-based trajectory modelling. Cox proportional hazards models estimated hazard ratios (HR) and 95% confidence intervals (CI) of all-cause mortality by lipid trajectories.

Results: The study included 32,537 female breast cancer survivors. Approximately 30% of the study population lipid levels within recommended ranges before and after a breast cancer diagnosis (moderate-stable trajectory). Nearly 10% of the population had very low lipids before and after a breast cancer diagnosis (very low-stable trajectory); this pattern was associated with an increased risk of all-cause mortality for total cholesterol (HR 1.38, 95%CI 1.16, 1.66), LDL (HR 1.24, 95%CI 1.04, 1.49), and HDL (HR 1.20, 95%CI 1.02, 1.40), compared to the moderate-stable trajectory. Approximately 5-6% had very high total cholesterol or LDL that rapidly declined after a breast cancer diagnosis (very high-declining trajectory); this pattern was associated with an increased risk of all-cause mortality compared to a moderate-stable trajectory (HR 1.59, 95%CI 1.23, 2.05; HR 1.40, 95%CI 1.09, 1.81, respectively). The association between lipid trajectories and all-cause mortality did not significantly differ by lipid-lowering agent use, suggesting pharmacologic management did not impact the findings. However, those not on a lipid-lowering agent had a mortality risk of higher magnitude than those on pharmacologic intervention.

Conclusions: Breast cancer patients with very low lipids before and after diagnosis, and patients with very high lipids that rapidly decline to low levels after diagnosis were identified as subgroups at high risk of mortality. These findings highlight the importance of considering longitudinal, repeat lipid measures to improve long-term outcomes in this population.

#3603 Racial and ethnic disparities in colorectal cancer-specific mortality: The Multiethnic Cohort.

Wei (Melody) Xiong[1], Adelynn Paik[1], Iona Cheng[2], Anna H. Wu[1], Ugonna Ihenacho[1], Lynne R. Wilkens[3], Loic Le Marchand[3], David V. Conti[1], Christopher A. Haiman[1], Brian Z. Huang[1]

[1]Center for Genetic Epidemiology, Department of Population and Public Health Sciences, USC - University of Southern California, Los Angeles, CA,[2]School of Medicine, University of California, San Francisco, San Francisco, CA,[3]Epidemiology Program, University of Hawaii Cancer Center, Honolulu, HI

Background: Colorectal cancer (CRC) is a leading cause of cancer-related mortality in the United States. However, few studies have investigated the racial and ethnic disparities of CRC-specific mortality in multiethnic populations of adults with CRC while accounting for detailed individual- and neighborhood-level data.
Methods: We evaluated racial and ethnic disparities in CRC-specific mortality among 5,738 (2,911 men, 2,827 women) invasive colorectal adenocarcinoma cases (1993-2019) from the Multiethnic Cohort Study (MEC). Cases included African American (n=1,166), Latino (n=1,178), Native Hawaiian (N=358), Japanese American (n=1,960), and non-Hispanic White (NHW) (n=1,178) adults. Using Cox regression with age since diagnosis as the time metric, we estimated hazard ratios (HRs) for CRC-specific mortality, adjusting for sex, CRC grade/stage, treatment (surgery, chemotherapy, radiotherapy), family history of CRC, history of polyps, education, neighborhood SES (accounting for census block group), BMI, smoking, alcohol, physical activity, diabetes, NSAID and multivitamin use, daily calories, intake of red/processed meats, folate, fiber, and number of comorbidities (0, 1, ≥2). Stratified analyses were performed by sex, tumor stage, and site (colon, left colon, right colon, rectum).
Results: Over a mean follow-up period of 7.5 years, 1,695 (29.5%) CRC cases (874 men and 821 women) died of colorectal adenocarcinoma. Compared to NHW CRC cases, Japanese American (HR=0.74, 95% CI: 0.61, 0.89) and Latino (HR=0.65, 95% CI: 0.51, 0.82) cases had significantly lower CRC-specific mortality. These associations were significant in men (Japanese American: HR=0.74, p=0.02; Latino: HR=0.62, p<0.001), but not in women (HRs = 0.72-0.77, p<0.10). Across stage, Japanese American cases had 36% lower CRC-specific mortality for localized tumors (HR=0.64, 95% CI: 0.43-0.97), while Latino cases had >50% reduced mortality for distant tumors (HR=0.46, 95% CI: 0.31-0.69). For colon cancer specifically, both groups showed significant lower CRC-specific mortality (Japanese American: HR=0.67, 95% CI: 0.41-0.77; Latino: HR=0.65, 95% CI: 0.50-0.85). Results were similar by colon tumor site but were stronger for Japanese American cases for left-sided colon cancer (HR =0.57, 95% CI:0.41-0.77), and for Latino cases for right-sided colon cancer (HR =0.57, 95% CI: 0.41-0.80). There were no significant differences in CRC-specific mortality among African American or Native Hawaiian cases compared to NHWs overall and by subgroups.
Conclusions: Japanese American and Latino CRC cases had significantly lower CRC-specific mortality compared to NHWs in the MEC adjusting for prognostic factors. These findings were consistent across CRC stage, tumor site, and among males. Further research is needed to explore the structural, social and biological factors underlying these disparities in CRC-specific mortality.

#3604 Diabetes and prostate cancer-specific mortality in the Multiethnic Cohort Study: Looking deeper into diagnostic timing and disease duration.

Wei (Melody) Xiong[1], David Bogumil[1], Peggy Wan[1], Gertraud Maskarinec[2], David V. Conti[1], Lynne R. Wilkens[2], Loic Le Marchand[2], Christopher A. Haiman[1], Fei Chen[1]

[1]Center for Genetic Epidemiology, Department of Population and Public Health Sciences, USC - University of Southern California, Los Angeles, CA,[2]Epidemiology Program, University of Hawaii Cancer Center, Honolulu, HI

Background:Diabetes is found to be associated with higher cancer-specific mortality for several cancers, but the findings have been conflicting for prostate cancer (PCa). The goal of this study was to evaluate the association between diabetes and PCa-specific mortality (PCSM) in a racial/ethnically diverse population of PCa patients.
Methods:The association between diabetes and PCSM was assessed in 10,464 African American (20.9%), Latino (23.6%), Native Hawaiian (5.4%), Japanese American (28.2%), and non-Hispanic White men (21.9%) diagnosed with incident PCa in the Multiethnic Cohort (MEC). Based on questionnaires and administrative data, diabetes status was defined as non-diabetic, pre-diagnostic diabetic, and post-diagnostic diabetic (relative to PCa diagnosis). In a subsequent analysis, diabetes status was further categorized given the timing of diabetes diagnosis (0-5 or > 5 years pre- or post-diagnosis of PCa). Sub-hazard ratios (SHRs) and 95% confidence intervals (CIs) were estimated using the Fine and Grey subdistribution hazards models, with time since PCa diagnosis as the time metric and adjustments on potential confounders. All analyses were performed in all PCa patients and stratified by tumor stage (localized, advanced) and Gleason score (high-grade, low-grade).
Results: During an average follow-up of 8.5 years, 1,311 (12.5%) men with PCa died of the disease. Compared to men without diabetes, men with pre-diagnostic diabetes had a significantly lower PCSM (SHR=0.73, 95% CI: 0.62-0.86) and the reduction in PCSM appeared to be greater for men with post-diagnostic diabetes (SHR=0.58, 95% CI: 0.48-0.70). Similar trends of the associations for pre- and post-diagnostic diabetes were observed among patients with high-grade (SHR 0.72 vs. 0.51) or advanced (SHR 0.72 vs. 0.56) disease. Among patients with low-grade or localized disease, the inverse associations with PCSM were similar for men with pre- (SHR 0.70-0.76) or post-diagnostic diabetes (SHR 0.65-0.75). Further categorization given the timing of diabetes diagnosis did not lead to differences in trends of the associations for pre- and post-diagnostic diabetes on PCSM among all, high-grade and advanced PCa cases. Among low-grade or localized cases, the inverse association was more evident among those with 5+ years of diabetes before cancer diagnosis and those who became diabetic within 5 years of cancer diagnosis (SHR 0.48-0.69 vs. 0.81-0.91). Statistical significance was defined as $P < 0.05$.
Conclusions:We found that both pre- and post-diagnostic diabetes were significantly associated with a lower PCSM among PCa patients, and the association appeared to be stronger for post-diagnostic diabetes. Further studies are needed to confirm these findings in other multiethnic populations and to understand the mechanisms driving these associations.

POPULATION SCIENCES: Environmental, Immunological, and Tumor Microenvironment Drivers of Cancer Disparities Poster Session

#3608 Non-smoking lung cancer: The impact of cooking-related household air pollution.

B. J. McAllister, K. Brown, S. Cai, S. Khan;
UNIVERSITY OF LEICESTER, Leicester, United Kingdom

Lung cancer is increasingly prevalent in the non-smoking population, with Household Air Pollution (HAP) recognised as a major contributor, and some evidence that women of South-Asian backgrounds are at greater risk than other demographics. A systematic review of available literature was performed to explore the risks of lung cancer posed by HAP for never-smokers in high-income countries. We identified three papers fulfilling our criteria, two conducted in Taiwan, and one in Hong Kong, with no identified studies fitting the criteria in Western countries. The review found that cooking-related HAP increases the risk of LCINS in Chinese women. There is a clear research gap regarding the impact of HAP on LCINS in high-income countries outside Taiwan and Hong Kong. Building on this evidence, the aim of this project is to determine whether South Asian households are exposed to higher levels of cooking-related HAP compared to households of White British ethnicity, leaving them at a greater risk of non-smoking lung cancer.
Methods: Twenty households have been recruited, with White British, and South-Asian families equally represented among participants. Kitchen air quality is monitored for pollutants including PM_1, $PM_{2.5}$, PM_{10}, gases including NO_x, CO, and O_3, VOCs and mould, using active and passive methods. Air sampling is conducted over a week, once in winter and once in summer to account for seasonal variation. Participants complete a daily diary noting activities related to cooking, cleaning and ventilation. A home survey is completed noting structural features e.g., age and style of house, layout of living spaces, floor coverings and renovation work.This study is ongoing, with the first round of data collection complete in January 2025. This data will include accurate, real time air quality measurements allowing for direct comparison with the daily activity diaries to assess the impact of cooking on kitchen air quality, as well as initial analysis of the impact of ethnicity and cultural practices on the quality of household air in non-smoking households.To date, data from eight houses has been collected, with 12 more scheduled. Initial Findings have shown that the use of gas cookers and hobs increases PM_1, $PM_{2.5}$, PM_{10}, while also raising concentrations of NO_x and O_3. Homes using gas appliances frequently breached recommended NO_x exposure levels. While air fryers and electric hobs also increase levels of PM_1, $PM_{2.5}$, PM_{10} during use, there is no corresponding increase in NO_x or O_3.To the best of our knowledge, this is the first UK study to explore how cooking practices affect the presence and concentrations of PM, VOCs and mould in household environments, with a focus on ethnicity and cultural practices. It will provide valuable data on how household air quality affects the risks of non-smoking lung cancer in a high-income country.

#3610 Healthcare segregation experienced by colorectal cancer patients residing in high-poverty areas and its impact on health outcomes.

X. Zheng[1], L. C. Pinheiro[1], P. Tehranifar[2], E. Phillips[1], R. M. Tamimi[1], S. Y. Chao[1], M. Pisu[3], C. Gao[1], A. G. Rundle[2], **J. Mao**[1]; [1]Weill Cornell Medicine, New York, NY, [2]Columbia University Mailman School of Public Health, New York, NY, [3]The University of Alabama at Birmingham School of Medicine, Birmingham, AL

Background: Existing evidence indicates that colorectal cancer (CRC) patients from high-poverty areas (HPA), defined as areas with 20% or more residents living below federal poverty level, are more likely to receive surgery at low-volume, non-accredited hospitals than those from low-poverty areas (LPA). The systematic difference in surgical care location between patients from HPAs and LPAs, healthcare segregation, could represent a structural equity through which disparities in health outcomes arise. We sought to determine the existence of healthcare segregation and assess its impact on patient health outcomes.

Methods: We identified patients undergoing surgery for non-metastatic CRC diagnosed in 2009-2019 from SEER-Medicare. Patients living in a county which had been an HPA for at least 30 years were classified as living in a persistent-poverty area (PPA). Patients living in a county which was an HPA during the year of cancer diagnosis but not a PPA were classified as living in a current-poverty area (CPA). Otherwise, they were classified as living in an LPA. Experiencing healthcare segregation was defined as being treated at a poverty-area-serving (PAS) hospital where ≥50% of the patients were from HPAs. We examined the association between area-level poverty, treatment at PAS hospitals, and health outcomes (postoperative adverse events, 30-day readmission, long-term all-cause mortality (ACM), and cancer-specific mortality (CSM)) using logistic and Cox regression. We performed a subgroup analysis for metropolitan, urban, and rural areas.

Results: We included 81,767 patients (7.5% from PPAs, 8.4% from CPAs) with a median age of 78 (IQR: 73-84) years. 53.9% of patients were females, 14% were non-White, and 1.5% lived in rural areas. 81% of the hospitals (180/991) were PAS hospitals, which treated 64% of patients from PPAs and CPAs vs. 3% from LPAs. Compared with patients from LPAs treated at non-PAS hospitals, those treated at PAS hospitals who were from PPAs (ACM HR=1.17(1.08-1.26), CSM HR=1.23(1.11-1.37)) and CPAs (ACM HR=1.15(1.08-1.22), CSM HR=1.23(1.13-1.35)) had worse short and long-term outcomes. Patients from CPAs treated at non-PAS hospitals also had worse outcomes, to a lesser degree (ACM HR=1.12(1.05-1.19), CSM HR=1.16(1.06-1.28)). Among rural residents, being treated at PAS-serving hospitals but not area-level poverty was independently associated with worse outcomes.

Conclusions: Patients living in HPAs experience healthcare segregation in CRC treatment, contributing to their worse health outcomes. Healthcare segregation reflects systemic inequities and structural barriers limiting access to resources that patients from HPAs may deploy to mitigate consequences of cancer. Understanding healthcare segregation and factors shaping it can help identify areas for intervention to reduce associated health disparities.

#3611 Association of ambient air pollution with hypertension among cancer survivors.

Da-eun Lee[1], Juyeon Hwang[2], Seohyun Ahn[2], Kyounghyeon Kim[1], Kyeezu Kim[1], Hyun-Jin Kim[2]

[1]Sungkyunkwan University, Seoul, Korea, Republic of,[2]National Cancer Control Institute, National Cancer Center, Goyang, Korea, Republic of

Introduction: Hypertension is one of the leading risk factors for cardiovascular disease and premature death worldwide. Ambient air pollutants such as particulate matter (PM) and toxic gases have been linked to an increased risk of hypertension. Cancer survivors are more susceptible to the adverse effects of air pollution and tend to have a higher prevalence of hypertension compared to cancer-free individuals, potentially due to shared intrinsic and extrinsic factors that contribute to both oncogenesis and elevated blood pressure. This study aimed to investigate the association between long-term exposure to ambient air pollution and blood pressure in cancer survivors.

Methods: This study utilized data of 2,492 cancer survivors obtained from the Korea National Health and Nutrition Examination Survey between 2007 and 2021. Five air pollutants, $PM_{2.5}$ (μg/m3), PM_{10} (μg/m3), SO_2 (ppb), NO_2 (ppb), and CO (ppb), were included, and moving average concentrations over 1 to 3 years prior to the survey date were calculated using air quality models and satellite data. We adopted multivariable regression models adjusting for socio-demographic and lifestyle factors to assess the association of air pollution with hypertension status and high blood pressure. We further conducted age- and sex-stratified analyses to examine the potential differences in hypertension.

Results: Greater exposure to air pollution was associated with high blood pressure in cancer survivors, particularly among females and individuals older than 65 years. In male cancer survivors, higher 1-year exposure to SO_2 was associated with greater odds of hypertension (OR= 1.08, P= 0.05). In female cancer survivors, higher exposure to $PM_{2.5}$, PM_{10}, NO_2, and CO over 1- to 3-year periods was associated with elevated diastolic blood pressure (β ranged 0.47 1.09, all $P < 0.05$). Cancer survivors aged 65 or older had greater odds of hypertension ($PM_{2.5}$: OR= 1.27, P= 0.01; PM_{10}: OR= 1.14, P= 0.04) and elevated systolic/diastolic blood pressure (β ranged 0.68 1.56, all $P< 0.05$) per 1-unit increase in PM exposure over 3-years. In cancer survivors aged under 65, 3-year exposures to PM_{10}, NO_2, and CO were associated with higher diastolic blood pressure (β ranged 0.64 0.98, all $P< 0.05$).

Conclusions: Our findings suggest that long-term exposure to air pollution may impact high blood pressure among cancer survivors, across diverse air pollutants. These results underscore the potential health risk posed by air pollution particularly for female cancer survivors and those aged 65 or older.

#3612 Three aging biomarkers differentially mediate cardiotoxicity after irradiation to the heart or exposure of anthracyclines in survivors of childhood cancer.

Xiaoxi Meng, Tiffany Eulalio, Noel-Marie Plonski, John Easton, Heather Mulder, Emily Walker, Geoffrey Neale, Kyla Shelton, Deo Kumar Srivastava, Stephanie B. Dixon, Jinghui Zhang, Melissa M. Hudson, Kirsten K. Ness, Gregory T. Armstrong, Zhaoming Wang

St. Jude Children's Research Hospital, Memphis, TN

Background: Childhood cancer survivors experience accelerated aging, largely due to treatment toxicity. We investigated how aging biomarkers, which quantify accelerated aging and capture multifaceted mechanisms, differentially mediate treatment-related cardiotoxicity.

Methods: Peripheral blood samples from survivors in the St Jude Lifetime Cohort were profiled with EPIC BeadChip to generate DNA methylation (DNAm) data, from which three established aging biomarkers were subsequently derived: epigenetic age (EA, reflecting epigenetic alterations), leukocyte telomere length (LTL, reflecting cellular senescence), and C-reactive protein (CRP, reflecting chronic inflammation). EA (Levine's PhenoAge) and LTL were calculated with the methylCIPHER R package, while CRP was computed as a weighted (regression coefficients from a prior study) sum of serum CRP-associated CpGs. Cardiomyopathy (CMP) with a CTCAE (Common Terminology Criteria for Adverse Events) grade ≥2 and myocardial infarction (MI) with a CTCAE-grade ≥3 were analyzed. Multivariable logistic regression assessed associations of CMP or MI with each biomarker (standardized sample age adjusted residuals), adjusting for sex, attained age, age at primary cancer diagnosis, and cardiotoxic exposures including mean heart irradiation (heart-RT) dose and cumulative anthracycline dose. Linear regression evaluated associations between each biomarker and cardiotoxic exposures, adjusting for sex, age at blood draw and other treatments.

Results: Among 3,059 survivors (all of European ancestry; 46.85% female; mean [SD] = 29.35 [0.22] years of age at blood draw), the incidence of CMP and MI was 8.0% and 1.6%, respectively. Biomarker correlations ranged from moderate to high (r^2 = 0.32 to 0.67). CRP was associated with increased risk of CMP (Odds ratio [95% CI] = 1.28 [1.11-1.47], P = 8.30×10^{-4}) and MI (1.43 [1.09-1.88], P = 1.08×10^{-2}). EA was associated with MI risk only (1.76 [1.30-2.38], P = 2.45×10^{-4}), and LTL was associated with risk of CMP (1.23 [1.05-1.42], P = 7.86×10^{-3}) and MI (1.71 [1.26-2.31], P = 1.19×10^{-3}). Furthermore, CRP specifically mediated the cardiotoxicity of heart-RT (13.87%, P < 2.00×10^{-16}) and anthracyclines (3.07%, P = 1.20×10^{-2}) on CMP, whereas EA specifically mediated the cardiotoxicity of heart-RT on MI (24.36%, P = 2.00×10^{-3}). In contrast, LTL mediated the cardiotoxicity of heart-RT on both CMP (15.06%, P = 8.0×10^{-3}) and MI (20.77%, P = 4.00×10^{-3}), albeit less significantly.

Conclusion: These findings suggest specific aging biomarkers are more informative for certain outcomes, potentially driven by various underlying aging mechanisms. There is no one-fits-all biomarker to identify survivors with greater accelerated aging and hence higher risk of particular adverse outcomes, emphasizing the need for integrative approaches in future clinical research.

#3613 Association between cumulative lifetime exposure of reproductive hormones and TILs among women with breast cancer.

Yidan Zhang[1], Rand Akasheh[1], Bo Qin[2], Angela Omilian[3], Nur Zeinomar[2], Song Yao[3], Elisa V. Bandera[2], Christine Ambrosone[3], Thaer Khoury[4], Ting-Yuan David Cheng[1]

[1]Department of Internal Medicine, The Ohio State University Wexner Medical Center, Columbus, OH,[2]Cancer Epidemiology and Health Outcomes, Rutgers Cancer Institute, New Brunswick, NJ,[3]Department of Cancer Prevention and Control, Roswell Park Comprehensive Cancer Center, Buffalo, NY,[4]Department of Pathology & Laboratory Medicine, Roswell Park Comprehensive Cancer Center, Buffalo, NY

Background: Tumor-infiltrating lymphocytes (TILs) within the tumor and its surrounding microenvironment reflect an active immune response and are regarded as a prognostic marker for breast cancer. However, host factors that affect TILs remain less explored. The study investigated the association between lifetime hormone exposure and TILs.
Methods: The study included newly diagnosed invasive breast cancer cases in the Women's Circle of Health Study. Lifetime endogenous hormone exposure (LHEendo) was defined as the sum of reproductive years and cumulative pregnancy duration, minus cumulative breastfeeding duration. The reproductive years were defined as the duration between age at menarche and age at diagnosis for premenopausal women, and as the duration between age at menarche and age at menopause for postmenopausal women. Lifetime exogenous hormone exposure (LHEexo) was estimated by summing the years of oral contraceptive (OC) use and hormone replacement therapy (HRT) use. TILs were scored as 0% to 100% of stromal tissue based on established guidelines. Generalized linear models with Gamma distribution and log link function were used to assess the associations between LHEendo or LHEexo and TILs. Directed acyclic graphs (DAGs) were used to select covariates. When assessing the association between LHEendo and TILs, we adjusted for race, family history of breast cancer, and duration of OC use. For the association between LHEexo and TILs, adjustments included race, BMI at enrollment, age at diagnosis, and menopausal status.
Results: A total of 1315 patients (920 Black and 395 White women) were included in the study. Overall, we did not observe a significant association between either LHEendo or LHEexo and TILs. However, when analyses were stratified by race, shorter LHEendo or LHEexo was associated with higher TILs among White women (exponentiated (exp) beta=0.98, 95% CI=0.96 to 0.99 for LHEendo, and (exp) beta= 0.98, 95% CI=0.97 to 1.00, respectively; P-interaction=0.042 for LHEexo and 0.15 for LHEendo), but not among Black women. When stratifying the analyses by breast cancer molecular subtype, a shorter LHEendo was associated with higher TILs in hormone receptor (HR)+/HER2- patients ((exp) beta=0.99, 95% CI=0.97 to 1.00), while a shorter LHEexo was associated with higher TILs in HR+/HER2+ patients ((exp) beta=0.97, 95% CI=0.95 to 1.00). The interactions between LHEendo or LHEexo and molecular subtype were not significant (P>0.05).
Conclusions: The associations of lifetime endogenous and exogenous hormone exposure with TILs may vary by race and breast cancer molecular subtype. These findings contribute to our understanding of the roles of hormonal factors in the breast tumor microenvironment.

#3614 Examining social networks within public housing to better direct cancer screening interventions for Pacific Islanders.

Mark L. Willingham[1], Kevin Cassel[1], Ian Pagano[1], Grazyna Badowski[2]

[1]University of Hawai'i Cancer Center, Honolulu, HI,[2]College of Natural & Applied Sciences, University of Guam, Mangilao, Guam

Background: Social networks and social interactions play an important role in an individual's health and cancer screening behaviors. We examined the relationship between types of social interactions to better understand where to direct efforts for cancer screening interventions for Pacific Islanders (PIs) in Hawai'i (Native Hawaiians, Chuukese, and Marshallese adults) in public housing communities where PIs are often overrepresented.

Methods: We utilized a mixed methods research design, examining an existing survey dataset of 1,010 PIs and 16 semi-structured interviews from the same sites where we surveyed individuals, to determine the role of social networks on health outcomes within PIs living in public housing. As a measure of social networks, we formed a social network index from three survey items, including, the number of individuals a person knows personally, interacts with, and discusses personal matters with utilizing a range of 0-9 for each item. Findings from the 16 semi-structured interviews regarding social networks used by individuals for information sharing and with whom participants discussed personal health matters provided qualitative data used for examining the social network index. We applied means testing between the groups (515 Native Hawaiians, 305 Chuukese, 180 and Marshallese) to determine if there were differences in the groups' social networks, and grounded theory to extract the major themes from the narratives.

Results: Interviewees discussed the role of small social circles consisting of their families and households for information and health resource sharing. Within the dataset, Chuukese had significantly smaller personal networks ($\bar{x}$= 4.05, p=.003); however, they had significantly more individuals ($\bar{x}$= 4.90, p=.04) with whom they would discuss personal matters, more than Native Hawaiian and Marshallese adults. Marshallese indicated significantly more individuals ($\bar{x}$= 5.10, *p*=.04) that they have interacted with over the past two weeks.

Conclusions: Chuukese adults have small personal networks; however, their small social circles used to discuss personal and important matters is larger than the other groups, Marshallese and Native Hawaiian. This thus indicates more meaningful social networks for this group. Marshallese adults may interact with more people; however, they did not indicate more close relationships than Chuukese to discuss personal matters. These findings indicate social networks and the type and frequency of social interactions have meaningful roles in health and cancer information sharing for PIs. Future interventions must recognize the frequency and types of meaningful social networks within PIs to potentially change cancer screening behaviors by increasing resource sharing within the most valuable networks.

#3615 Clonal hematopoiesis in ovarian cancer survivors: Correlation with carboplatin and PARP inhibitor exposure.

Marc R. Radke, Nithisha Khasnavis, Carly Mondoy, Mayumi Rubin-Saika, Enna Manhardt, Jennifer Ochoa, Melanie R. Dillon, Roseanne J. Gamboa, David Wu, Sioban Keel, Sergei Doulatov, **Elizabeth M. Swisher**

University of Washington, Seattle, WA

Background: Ovarian cancer (OC) survivors have high rates of therapy-related myeloid neoplasia. We characterized clonal hematopoiesis (CH) in OC survivors and its relationship to therapeutic exposures.
Methods: We sequenced peripheral blood mononuclear cell (PBMC) DNA from 121 OC survivors treated with carboplatin +/- PARP inhibitors and 71 controls: 37 cancer-free females and 34 with OC prior to treatment. Our BROCA-MY sequencing panel identifies all classes of mutations in 72 susceptibility genes for gynecologic cancer, bone marrow failure, and leukemia and genes frequently altered in CH or myeloid malignancies. To accurately detect CH pathogenic variants (CHV) at ≥1% variant allele frequency (VAF), unique molecular identifiers (UMIs) were used to construct duplex consensus reads. CHVs were included if present in at least three consensus reads, had a VAF ≥1%, had a QUAL score (VarDict) ≥100, and were predicted to be pathogenic.
Results: Of the 121 PBMCs collected post treatment from OC survivors, 57 (47.1%) had no CHVs, 36 (29.8%) had 1 CHV, and 28 (23.1%) had ≥2 CHVs, not significantly different than controls: 37 (52.1%) with no CHVs, 26 (36.6%) with 1 CHV, and 8 (11.3%) with 2 or more (P=0.14). However, there was a trend (P=.055) for more cases with ≥2 CHVs in cases vs controls. Within treated OC cases, PARP inhibitor exposure was significantly associated with CHV detection: 14 (34.1%) with 0 CHV, 11 (26.8%) with 1 CHV and 16 (39.0%) with ≥2 CHVs vs cases without PARP inhibitor exposure: 43 (54.1%) with 0 CHV, 24 (30.4%) with 1 CHV, and 12 (15.2%) with ≥2 CHVs (P=0.0045). CHVs were significantly more common in cases than controls for *CHEK2* (11.8% vs 1.4%, P=0.006) and *PPM1D* (19% versus 0, P< 0.0001) while CHVs in *DNMT3A* (17.4% vs 11.6%) and *TET2* (9.1% vs 5.6%) were similarly frequent in cases and controls. *TP53* CHVs were more common in case (8.3%) versus controls (1.4%) but this did not reach statistical significance (P=0.057). In 2 patients with paired PBMC samples collected 6 months apart, CHVs detected in the second sample were not detectable in the baseline (*ATM* and *TET2* in one case and *TP53* in the other). A third case had a baseline blood sample with 5 CHVs (2 in *TP53*, 2 in DNMT3A and 1 in *SMC3)* that were detectable 6 years prior to TMN diagnosis. At the time of TMN diagnosis, the same 5 CHVs were identified with 2 additional CHVs in *PPM1D* and *CHEK2.*
Conclusions: The gene distribution and number of CHVs differed in PBMCs from ovarian cancer survivors treated with platinum chemotherapy compared to controls, although the overall frequency of CHVs was similar. CHVs in *PPM1D* and *CHEK2* were significantly more common in cases, while *DNMT3a* and *TET2* CHVs were present at similar frequencies. Within OC survivors, PARP inhibitor exposure was significantly associated with the presence of a CHV. We are following OC survivors prospectively and will present additional sequential data on CHVs in patients undergoing varying treatments.

#3616 Cultural racism as a driver of health disparities.

O. L. Osazuwa-Peters[1], T. Brown[2], N. Krieger[3], T. Akinyemiju[1];
[1]Duke University School of Medicine, Durham, NC, [2]Duke University, Durham, NC, [3]Harvard T.H. Chan School of Public Health, Boston, MA

Background: Cultural racism (CR), which refers to ideologies, values, belief systems, and tangible manifestations that enable racial oppression, is a distinct component of systemic racism with hypothesized links to health disparities. However, relative to other more widely recognized components of systemic racism— interpersonal and structural racism— CR is poorly studied with sparse epidemiological evidence on its direct relationship with health outcomes and contributions to disparities.
Methods: To bridge this gap, we developed the cultural racism factor (CRF), a latent measure derived using confirmatory factor analysis to model interrelationships among seven indicators that are hypothesized to reflect CR: i) anti-immigrant sentiments, ii) racial resentment, iii) online racial animus, iv) number of public symbols of the confederacy/100,000, v) aggregate implicit racial bias for White residents, vi) lack of White support for Medicaid expansion, and vii) lack of community-oriented policing. These indicators were quantified at the state level, given that data was consistently available at this level, and states are influential political actors that shape population health and allocate resources. Associations between the CRF and a variety of health outcomes (four mortality outcomes, life expectancy at birth, and two mental health outcomes) were evaluated using demographics-adjusted regression models.
Results: In analysis of US mortality data between 2018 and 2021 obtained from CDC Wonder, each unit increase in the CRF was associated with ~136 (95% confidence interval (CI): 90-182) all-cause deaths per 100,000, ~40 (21-59) cardiovascular-specific deaths per 100,000, ~6 per 100,000 (3-9) diabetes-specific deaths per 100,000, and ~11 per 100,000 (5-17) cancer-specific deaths per 100,000. Also, the CRF was associated with a one-year decline in life expectancy at birth (~ -1 (-2 - -1); National Center for Health Statistic data). Overall, these associations were consistent for both Black and White adults. Also, CRF's association with all-cause mortality appeared to be independent of structural racism. Further, CRF showed significant associations with mental health outcomes including a higher risk for depression history ranging from 3% overall to 25% among Native Hawaiian/PI and higher risk for poor mental health days ranging from 3% overall to 14% among Hispanics, in CDC's Behavioral Risk Factor Surveillance System data (2018-2021).
Conclusion: In sum, CRF was adversely associated with numerous health outcomes, overall and by racialized groups. These findings highlight cultural racism's role as a contemporary, consistent, and universally harmful social exposure for US adults that critically needs to be addressed. Further, the CRF makes it possible to advance the science of racism and health and provides an empirical basis for efforts to address US health inequities that result from cultural racism.

#3617 Distinct immune signature and regulated genes in prostate cancer disparity.

Shiv Shankar Verma[1], Pingfu Fu[1], Gregory T MacLennan[2], Lee E Ponsky[3], Chen-Han Wilfred Wu[4], Jonathan E Shoag[1], Sanjay Gupta[1]

[1]Urology, Case Western Reserve University School of Medicine, Cleveland, OH,[2]Department of Pathology,, Case Western Reserve University School of Medicine, Cleveland, OH,[3]Department of Urology, Case Western Reserve University School of Medicine, Cleveland, OH,[4]Department of Genetics and Genome Sciences,, Case Western Reserve University School of Medicine, Cleveland, OH

Prostate cancer is a biologically and clinically diverse disease, with incidence and mortality rates varying significantly among different racial and ethnic groups. In African American (AA) men, the incidence of prostate cancer is approximately 60% higher, and the mortality rate is two to three times greater compared to Caucasian American (CA) men. These disparities are complex and encompass various biological and social determinants, including genetic predisposition, comorbid conditions, socioeconomic status, and environmental influences. The interplay of these elements has a clear association with disease outcomes. Recent research highlights the role of the tumor immune landscape and its associated genes in regulating prostate cancer biology at every stage of progression, ultimately impacting patient outcomes. We hypothesize that variations in immune cell gene signatures within tumors across different racial groups may influence prostate cancer outcomes. To investigate this, we analyzed transcriptomic data from 35 radical prostatectomy specimens obtained from treatment-naive patients, comprising 20 AA men and 15 CA men with prostate cancer. This analysis focused on the gene expression profiles of primary prostate tumors and adjacent normal tissues. We specifically selected immune-related genes to assess the immune-oncological differences between AA and CA prostate tumors, utilizing bioinformatics platforms such as EPIC, xCell, and IPA. To evaluate race-specific differential gene expression, we employed a rank test, and the effect of each gene's expression on overall survival was further analyzed using a univariate Cox regression model. Prostate tumors in AA patients exhibit significant enrichment in the tumor microenvironment and a distinct gene immune profile compared to those in CA men. In AA patients we observed elevated z-scores in pathways such as CREB signaling, Th1 and Th2 pathways, dendritic cell maturation, IL-17 signaling, TREM1 signaling, and cytokine production in macrophage and T Helper cells mediated by IL-17A and IL-17F. We also noted increased cytokine levels in epithelial cells influenced by IL-17A and IL-17F. Conversely, pathways like PD-1, PD-L1, ILK, IL-3, chemokine signaling, adrenomedullin, erythropoietin signaling, IGF-1, VEGF, and B-cell receptor signaling exhibited low z-scores in AA prostate tumors. Additionally, our analysis identified 13 major immune cell types, and 27 genes significantly associated with immune cell infiltration in AA prostate cancer. The expression levels of ARSL, BAALC, CCL23, EPHA2, IGFL2, KRT5, NTM, and NDP ($p < 0.05$) were linked to overall survival in AA prostate cancer patients. Taken together, prostate cancer in AA patients demonstrates a unique immune repertoire and gene profile, characterized by significant enrichment in pro-inflammatory immune pathways that correlate with poorer outcomes.

#3618 Biosocial determinants of health disparities among racial/ethnic minorities with chronic liver disease and liver cancer.

Tagari Samanta, Jun Hyoung Park, Panagiotis Katsonis, Benny Abraham Kaipparettu

Baylor College of Medicine, Houston, TX

Background: Metabolic disorders and chronic liver disease (CLD) play crucial roles in the development and progression of liver cancer (LC) like hepatocellular carcinoma (HCC). Previous studies suggest that the ethnic minority population increasingly suffers from CLD and LC. Thus, the impact of biosocial factors contributing to CLD and LC among minority populations requires further investigation. Using the All of Us database, this study aimed to understand the biosocial factors contributing to CLD and LC among racial subgroups.
Methods: Using All of Us Researcher Workbench, we analyzed in a cohort of 33767 CLD [non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease (ALD), and Hepatitis B and C] patients among different ethnic groups. We investigated the impact of various social determinants of health (SDOH) factors and genetic variations in key liver disease genes (PNPLA3, TM6SF2, MBOAT7, GCKR, and HSD17B13). Additionally, we explored metabolic pathways contributing to LC progression were analyzed using MACdb, GENT2, and TCGA databases and publicly available RNASeq data.
Results: Several confounding factors associated with CLD in Hispanic (HA) and African American (AA) populations compared to the non-Hispanic White (NHW) populations were identified in this study. HA and AA CLD patients suffer more from high socioeconomic status (SES) barriers. While genetic variations in the aforementioned genes were associated with CLD risk, their impact varied across ethnic groups. Metabolomic data of urinary metabolites from LC patients and healthy volunteers suggest hypoxanthine is upregulated in LC patients. TCGA analysis of hypoxanthine-related genes in the LC tumors confirmed that xanthine dehydrogenase (XDH), which converts hypoxanthine to uric acid, is down-regulated in the tumors of Asian patients. Survival analysis confirmed poor overall survival among the Asian and AA LC patients with lower XDH expression in their tumors.
Conclusions: Our study highlights the complex interplay of social, genetic, and metabolic factors in CLD and LC disparities. Further research is needed in a larger cohort to eliminate the root causes of these SES and cancer signaling disparities to obtain a more equitable survival outcome from CLD and LC in minority populations.

#3619 Urban-rural variations in racial/ethnic differences in time to treat among individuals newly diagnosed with cancer in the United States 2015-2021.

Hui Cheng, Oxana Palesh, Susan Hong

Virginia Commonwealth University - VCU, Richmond, VA

Timely treatment is crucial for effective cancer care. Studies have shown longer durations from diagnosis to treat among non-white racial/ethnic groups in the United States. In this study, we assessed whether racial/ethnic differences in the time to treat vary across urban/rural residents. We conducted a retrospective cohort study of over 3.5 million individuals newly diagnosed with cancer from 2015 to 2021, using data from the Surveillance, Epidemiology, and End Results (SEER) program. We estimated the median number of days from diagnosis to treatment by five racial/ethnic groups (non-Hispanic [NH] white, NH black, NH American Indian/Alaskan Native, NH Asian and Pacific Islander, and Hispanics) and five strata of urban/rural classifications of residence (large, mid-sized, small, adjacent, and non- metropolitan areas). NH black individuals living in large metropolitan areas experienced the longest median duration from diagnosis to treatment initiation (33 days), which was 8 days (95% CI=7.8, 8.2) longer than NH white individuals in large metropolitan cities. Longer durations among NH black were also present among those who live in mid-sized (6 days difference, 95% CI=5.6, 6.4), small (6 days, 95% CI=5.4, 6.6), adjacent (4 days, 95% CI=3.4, 4.6), and non-metropolitan areas (5 days, 95% CI=3.9, 6, .1), albeit smaller in size. Among Hispanic individuals newly diagnosed with cancer, those in large to small metropolitan cities also experienced longer duration from diagnosis to treatment when compared to NH white individuals in those areas (coef.=3 days [95% CI=2.8. 3.2], 2 days [95% CI=1.7, 2.3], and 3 days [95% CI=2.4, 3.6] for large, mid-sized, and small metropolitans); those in adjacent and non-metropolitan areas had slightly shorter duration from diagnosis to treatment (coef.=-1 day [95%=-1.7, -0.3] and -1 day [95% CI=-1.9, -0.1] respectively). NH American Indian/Alaskan Native also experienced longer duration from diagnosis to treatment compared to NH white individuals, and there were no variations across urban/rural areas. Similarly, NH Asian and Pacific Islander individuals had an estimated 3 days longer duration from diagnosis to treatment regardless of their residential areas, except that those who lived in non-metropolitan areas experienced even longer delays (coef.= 7 days [95% CI=5.7, 8.3]) compared to NH white individuals. This study highlights significant racial/ethnic disparities in time to cancer treatment, particularly for NH black individuals in large metropolitan areas. Addressing these disparities is essential for improving cancer care equity.

#3620 A weighted multi-modal transfer learning model for alleviating racial disparities in breast cancer.

Min-jeong Baek[1], Lusheng Li[1], Jieqiong Wang[2], Vimla Band[1], Shibiao Wan[1]

[1]Department of Genetics, Cell Biology and Anatomy, University of Nebraska Medical Center, Omaha, NE,[2]Department of Neurological Sciences, University of Nebraska Medical Center, Omaha, NE

Breast cancer (BC) is the second leading cause of cancer-related death among women in the United States. Previous studies have shown that African American (AA) women have disproportionately high mortality rate in BC compared to European American (EA) women in the United States. Existing studies have suggested that transfer learning could reduce health disparities in BC by transferring information from a majority group (e.g., EA women) to a minority group (e.g., AA women). However, their performance may be decreased due to limited training patient samples and using single-omics data only. We recently presented a transfer learning method by integrating two multi-omics data for reducing cancer disparities. However, the integration model was not optimized, and its performance in reducing disparities was not robust. To address these concerns, we propose a novel weighted multi-modal transfer learning model that can optimize the integration of multi-omics data to alleviate racial disparities in BC. Specifically, we first calculated the patient-patient similarity by using the Pearson Correlation Coefficient (PCC), based on which we performed weighted integration of multi-omics data. Then, we performed a nested grid search method to find the optimal weights for each type of omics data, which were subsequently fed into a transfer learning model. To demonstrate the effectiveness of our model, we performed the tasks of predicting Disease-Specific Survival (DSS) prognosis for the 1098 female BC patient samples from The Cancer Genome Atlas (TCGA) database. Results suggested that our model with optimized weighted integration of three multi-omics (including mRNA, miRNA and methylation) data significantly outperformed previous multi-omics transfer learning models in terms of AUC. In addition, our multi-modal transfer learning model performed remarkably better than single-omics and two-omics-integration transfer learning models, suggesting that the inclusion of more diverse omics data contributes to a better reduction of racial disparities in BC. Furthermore, the performance of our transfer learning model was also superior to that of conventional mixed models and independent models. In summary, our proposed weighted multi-modal transfer learning model achieves far better performance than existing state-of-the-art methods for reducing health disparities in BC. We expect that our proposed model will provide a new way to reduce health disparities in BC diagnosis, prognosis, and treatment, and it can be extensible to alleviate health disparities in other types of cancer.

#3621 Geographic and racial disparities in bladder cancer care in the U.S..

Atreyee Majumder[1], Daniel Erim[1], Arveen Kaur[2], Lisa Dwyer Orr[1]

[1]Johnson & Johnson, Titusville,, NJ,[2]Johnson & Johnson, Horsham, PA

Introduction: This study aims to elucidate variations in bladder cancer health outcomes and social determinants of health across diverse geographic regions in the US. It specifically investigates racial disparities in care delays among patients with bladder cancer, identifying critical intervention areas to enhance health equity.
Methods: A retrospective database analysis was conducted utilizing Medicare fee-for-service data for patients with bladder cancer diagnosed between January 1, 2021, and December 31, 2022, to measure and compare rates of diagnosis, interventions (surgical or pharmacological), specialist visit, time-to-specialist visit, and diagnosis-to-treatment initiation across US racial and ethnic groups. The reference group for rates was White patients. For example, the diagnosis rate ratio for Black patients was the quotient of diagnosis rates among Black residents and diagnosis rates among White residents diagnosed with bladder cancer during the study period. Diagnosis claims with ICD-10 code C67 and ICD-9 code 188 were used. Medicare patients with at least one year of enrollment were included. Patients were excluded if their first bladder cancer diagnosis was outside the study period.
Results: Diagnosis rate ratios were 0.5 for Black patients and 0.4 for Hispanic patients. Intervention rate ratios were 0.9 for Black patients and 0.8 for Hispanic patients. Specialist visit rate ratios were 0.9 for both Black patients and Hispanic patients. Median time-to-specialist visit was 21 days for all patients, while mean (standard deviation) times were 89 (162) days for Black patients, 77 (128) days for Hispanic patients, and 85 (148) days for White patients. Mean (standard deviation) diagnosis-to-treatment initiation were 29 (91) days for Black patients, 33 (94) days for Hispanic patients, and 27 (90) days for White patients; the corresponding 95% confidence interval was (29,36) and (26,28) for Hispanic and White patients, respectively.
Conclusion: At the national level, diagnosis-to-treatment initiation for bladder cancer was significantly delayed for Hispanic patients and disparities in intervention were identified for Black and Hispanic patients in hotspots of economic inequity. Stakeholders, including population health decision makers, healthcare providers, payers, advocacy groups and policymakers, can use the study findings to design and implement strategies to reduce geographic and racial disparities, foster collaboration among key stakeholders, and improve overall healthcare quality.

#3622 Racial disparities in second primary cancers in stomach cancer survivors: a comparative study of US and Korea cancer registries.

Yuntong Wang[1], Dong-Woo Choi[2], Minjin Kim[2], Jialin Mao[1], Art Sedrakyan[1], Kui Son Choi[2], Heejung Chae[2], Eunji Choi[1]

[1]Weill Cornell Medicine, New York, NY,[2]National Cancer Center, Goyang-si, Korea, Republic of

Background: Stomach cancer shows racial disparities in incidence and mortality, influenced by biological, lifestyle, and healthcare factors globally. With improved survival rates, stomach cancer survivors face an elevated risk of second primary cancer (SPC). However, racial differences in SPC risk and post-SPC survival remain unclear. We examined these disparities, leveraging disaggregated racial data from the U.S. and Korea SEER Registries.

Methods: Data from 47,627 U.S. stomach cancer survivors (stage I-III, diagnosed 2000-2021, survival ≥1 year) were analyzed. SPCs were defined as primary cancers diagnosed >1 year after the initial stomach cancer. The cumulative SPC incidence (stomach vs. other SPCs) were estimated using the Aalen-Johansen estimator, accounting for competing death. Cox regression evaluated the impact of SPC development on overall survival by treating SPC as a time-varying factor. Comparative analysis included 200,456 stomach cancer survivors in Korea (2012-2020).

Results: Among 47,627 U.S. stomach cancer survivors (49% localized-stage), 4,918 developed SPC (996 stomach SPC and 3,911 non-stomach SPC) over 238,135 person-years. The cumulative incidence of stomach SPC increased over time, with Hispanic survivors having the highest 15-year incidence (3.6%, CI: 3.1-3.6%). While grouped Asian data suggested a moderate stomach SPC incidence (3.0% CI: 2.4-3.5), disaggregated analysis revealed heterogeneity (1.4%-3.3%), highest in Korean and lowest in Filipino survivors. The cumulative incidence of non-stomach SPC, however, showed less variability among Asian subgroups. Survival analysis found that any SPC development (vs. those without SPC) was associated with 2.2 (CI: 2.07-2.29) times higher mortality risk. In race-specific subgroup analyses, the mortality impact of SPC development was more pronounced in Asians (adjusted hazard ratio, HR=3.2) vs. in Whites (HR=1.9; Interaction *P*<.1). Among Asian subgroups, the mortality impact of SPC was highest in Koreans and Chinese (HR=3.5 vs. 1.8 in Filipinos). Data from the Korean SEER Registry, including 200,456 survivors (75.1% localized stage [vs. 50.7% in Korean Americans]), showed that SPC incidence was half of that observed in Korean American patients. The post-SPC mortality risk was lower in Korea (HR: 2.0) than in Korean Americans in the US (HR: 3.49).

Conclusions: SPC incidence and post-SPC survival in stomach cancer survivors vary by race, with marked heterogeneity among disaggregated Asian subgroups. Patient groups with a better prognosis from initial stomach cancer, such as Asians and Koreans, experience a higher incidence of SPC and greater post-SPC mortality, possibly due to the longer survival time allowing for SPC development. Comparative studies linking US and Korean SEER with claims data could advance understanding of SPC patterns and post-treatment surveillance outcomes.

#3623 Racial disparities in front-line use of novel agents and stem cell transplants among patients with multiple myeloma.

Phyu Thin Naing[1], Anthony Corsi[2], Kelvin Zheng[2], Divya Chukkalore[1], Richa Thakur[1], Juliet Meir[1], Jessica Caro[1]

[1]Hematology and Oncology, Northwell Health Cancer Institute, New Hyde Park, NY,[2]Internal Medicine, Northwell Health, New Hyde Park, NY

Background: Racial disparities in incidence, mortality, and access to care persist in multiple myeloma (MM). Black/African Americans remain underrepresented in pivotal clinical trials, such as MAIA and PERSEUS, that led to the approval of novel agents like daratumumab (Dara). Dara received FDA approval for frontline use for transplant-ineligible (2018) and transplant-eligible (2019) patients. This study aims to analyze the disparities in the utilization of novel agents and autologous stem cell transplants (ASCT) in MM patients.

Methods: We conducted a single-center retrospective study of MM patients diagnosed between January 2022 and December 2023 at Northwell Health, the largest healthcare system in New York. Patients without confirmed MM or complete diagnostic data were excluded. Demographics, MM risk (cytogenetics and Revised International Staging System), time to treatment, time to ASCT, and treatments received were collected and analyzed.

Results: A total of 115 patients were included, of which 40 (34.8%) were Black/African American (AA), 46 (40%) White, 9 (7.8%) Asian, and 17 (14.8%) other races; 2.6% had missing race data. The median age at diagnosis was 68.8 years, with no significant difference across racial groups (p=0.21). High-risk cytogenetics, marked by t(4;14), t(14;16), t(14;20), 17p deletion, or 1q amplification, were identified in 22 patients. Stage 2 or 3 disease was observed in 76 patients. At a median follow-up of 13.6 months, 18 patients had died. Only 33.9% of patients received Dara as initial therapy. White patients had the highest proportion of recipients (17/46, 37%), whereas AA patients had the lowest (10/40, 25%). The median time to first treatment was 15 days, with no significant differences by race (p=0.08) or gender (p=0.6). The median time to ASCT was 259 days, with only 23 patients (20%) undergoing ASCT. White patients had the highest transplant rate (30.4%), followed by AA (15%), other races (11.8%), and Asians (11.1%). Non-White patients experienced a longer time to ASCT.

Discussions: Despite its demonstrated efficacy and widespread FDA approval, Dara use in frontline treatment remains low, particularly among Black/African Americans, who continue to have disproportionately limited access to novel agents. Similarly, access to ASCT remains inequitable, with lower utilization and longer times to transplant among non-White patients. Barriers such as delayed clinician adoption, drug accessibility, referral patterns, and systemic inequities should be addressed through education, streamlined access pathways, and targeted interventions to ensure equitable care for all MM patients.

#3625 Urban-rural and racial disparities in the utilization of immune checkpoint inhibitor (ICI) treatments for non-small cell lung cancer (NSCLC).

Mary T. Davis[1], Nikita Nikita[1], Krupa Gandhi[2], Scott W. Keith[2], Sarah Gordon[1], Rebecca Hartman[2], Swapnil Sharma[1], Amy Shaver[1], Grace Lu-Yao[1]

[1]Medical Oncology, Sidney Kimmel Comprehensive Cancer Center at Jefferson, Philadelphia, PA,[2]Biostatistics, Sidney Kimmel Comprehensive Cancer Center at Jefferson, Philadelphia, PA

Background: While ICIs have revolutionized NSCLC management, studies regarding real-world ICI uptake are limited. Before ICIs became the standard of care, NSCLC research uncovered inequities in treatment outcomes by race and other social determinants, like rurality. It is unclear if similar disparities exist for ICI treatment. This population-based study aims to investigate differences in ICI treatment uptake by race and rurality among late-stage NSCLC patients.

Methods: Patients ≥ 66 years diagnosed with stage III or IV NSCLC, with continuous Medicare coverage ≥ 12 months prior to diagnosis, were identified using the SEER-Medicare database. ICI treatment uptake was analyzed between January 1, 2014 and December 31, 2019. A multivariable logistic regression model was used to assess the likelihood of receiving ICI treatment, adjusting for age, year of diagnosis, sex, race, rurality, Charleson comorbidity index, cancer histology, dual eligibility for Medicaid, and state buy-in to Medicaid. Race was self-reported. Rurality was identified using SEER's Rural-Urban Continuum Codes 04-09.

Results: Of the 18,083 patients identified with NSCLC, 1,316 (7.38%) were Black and 15,972 (88.34%) were White; 3,230 (17.86%) lived in rural areas and 14,853 (82.14%) in urban areas. ICI treatment uptake increased from 8.70% of patients diagnosed in 2014 to 31.35% in 2017. ICI treatment rates were 15% lower in Black patients compared to White patients, and 25% lower for rural residents compared to urban residents (Table 1). No significant difference was found comparing patients of other races to White patients. (Table 1).

Conclusion: This population-based study documented increasing ICI usage over time, with significant rural-urban and racial disparities in treatment. These findings highlight the need to implement initiatives addressing rural and racial differences in both accessing ICI treatment for NSCLC and improving overall treatment equity.

Adjusted Odds Ratios (aOR) and 95% Confidence Intervals (CI) of Receiving Immunotherapy Treatment

Effect	aOR (95%CI)	P-value
***Metropolitan Status*: Rural vs Urban**	0.75 (0.68–0.83)	<0.001
***Race:* Black vs White**	0.85 (0.73–0.99)	0.040
***Race:* Other vs White**	1.03 (0.86–1.23)	0.245

#3626 Deoxycytidine kinase (dCK) gene expression patterns and racial disparities: Findings from the Multiethnic Cohort study.

Saunjoo Yoon[1], Sherise Rogers[2], David Bogumil[3], Edward Agyare[4], Renee Reams[4], Mariana Stern[3], Seth Ablordeppey[4], Diana J. Wilke[1], Bo Han[3]

[1]University of Florida, Gainesville, FL,[2]Yale Universtiy, New Haven, CT,[3]University of Southern California, Los Angeles, CA,[4]Florida Agricultural and Mechanical University, Tallahassee, FL

Background: Significant disparities exist in pancreatic cancer outcomes across different racial and ethnic groups, with African Americans experiencing disproportionately higher incidence and mortality rates than other ethnic groups. The genetic underpinnings of these disparities, particularly concerning drug metabolism and treatment response, remain poorly understood. Deoxycytidine Kinase (dCK) plays a crucial role in activating nucleoside analogs for pancreatic cancer treatment. Our study aim was to characterize dCK expression patterns across different racial/ethnic groups and evaluate the association between genetic variations in dCK and survival outcomes.

Methods: Germline genetic variation was analyzed on a subset of 756 cases in the Multiethnic Cohort (MEC) study. Incident pancreatic cancer cases were identified using SEER state cancer registries. Samples were genotyped across various arrays and imputed to the 1000 genomes reference panel using the Minimac4 algorithm. Imputed data was filtered to SNPs and short indels within the dCK gene coding region. Genetically determined dCK gene expression was estimated using imputed data. Associations between genetic variation and mortality among cases were estimated using Cox proportional hazards regression.

Results: Sample characteristics are shown in Table 1. Nine variants from the dCK gene with an imputation quality score of $R^2 \geq 0.8$ across all samples were highly correlated within each self-reported ethnic group—the highest correlation was among African Americans. There was no association between any variants and pancreatic cancer survival nor interactions between dCK variants dosage and chemotherapy treatment on mortality rate.

Conclusions: To further evaluate the potential influence of the variants on survival and mortality, we are testing the protein levels using Immunohistochemistry (IHC) staining and Western blot.

Table 1. Sample Characteristics: (N =756)

Race/Ethnicity	African-American	Japanese-American	Latino	Native Hawaiian	White
Cases (n)	129	266	152	76	133
Females (%)	60.5%	59.4%	45.4%	55.3%	47.4%
Deaths	122	238	136	67	127
Pancreatic Cancer Deaths	101	207	112	59	111
Person-Months Total	1253	3778	1921	1201	2488
Mean Age at Dx (years)	79.4	79.0	78.4	75.8	77.0
Mean Survival (months)	9.7	14.2	12.6	15.8	18.7
Median Survival (months)	4.1	4.9	5.2	5.5	5.0

#3627 Looking beyond the aggregate: uncovering ethnic-specific differences in breast cancer among young Asian American women.

Alice W. Lee[1], Diana Aguilar-Cruz[1], Cindy Do[1], Peyton Fisher[2], Aleah La Flair[2], Mojgan Sami[1], Nicole Wells[2]

[1]California State University, Fullerton, Fullerton, CA,[2]The Young Breast Cancer Project, Chula Vista, CA

Background: Emerging data suggest that breast cancer incidence rates in Asian American women are on the rise, and this has also been observed specifically among young women (i.e., under age 40). However, most breast cancer studies often aggregate data across all Asian Americans, overlooking the distinct sociodemographic features and health disparities that may characterize each Asian ethnic subgroup. Therefore, it is unclear if the observations noted in young Asian American breast cancer patients as a single group translate to each Asian subgroup as well. This is especially important when it comes to young women since they do not have established breast cancer screening guidelines and are more likely to be diagnosed with advanced stage tumors.
Methods: We analyzed the Surveillance, Epidemiology, and End Results Program's (SEER's) specialized Asian groups database, which includes cancer incidence data from 13 population-based registries. Overall, 33,960 female breast cancer cases who were diagnosed under age 40 and between the years of 1990 and 2014 were identified; the 1990-2014 time period for our analysis reflects the available data in this database. We calculated incidence rate ratios (IRRs) and 95% confidence intervals (CIs) comparing young breast cancer incidence by stage for seven Asian subgroups relative to Non-Hispanic Whites (NHWs). Joinpoint regression models were also run to determine annual percent change (APC) using 5-year grouping intervals for the racial/ethnic groups.
Results: Most Asian ethnic subgroups showed lower incidence rates of regional/distant breast cancer when compared to NHWs with the only exception being Hawaiian/Pacific Islanders (IRR=1.14, 95% CI 0.98-1.31). Regional/distant breast cancer incidence had also been trending upwards for most racial/ethnic groups; it increased 2.72% for Asian Indian/Pakistanis (95% CI -0.52 to 7.61) and 1.97% for Koreans (95% CI 0.79 to 3.18). Japanese constituted the only Asian subgroup to be significantly more likely to receive a localized breast cancer diagnosis relative to NHWs (IRR=1.21, 95% CI 1.09-1.35). In addition, significant increasing trends of localized tumors were observed for Asian Indian/Pakistanis (APC=3.35, 95% CI 1.20 to 6.71) and Chinese (APC=1.18, 95% CI 0.23 to 2.23).
Conclusions: Breast cancer in young women is understudied, particularly in Asian Americans who have historically been thought to be at lower risk of the disease. With the growing literature highlighting the increasing incidence of breast cancer in Asian Americans, including young women, our findings highlight important disparities in which specific ethnic subgroups appear to be at higher burden of advanced stage breast cancer. This type of work is essential for developing targeted preventive strategies to address the needs of young Asian females.

#3628 Associations between per and polyfluoroalkyl substances (PFAS) and inflammatory markers in a racially diverse cohort of breast cancer patients.

Alicia Arredondo Eve[1], Ashlie Santaliz Casiano[2], Haihley Connors[1], Oana Danciu[3], Garth Rauscher[4], Natalie Pulliam[5], Elona Liko-Hazizi[5], Sarah Friedewald[6], Seema Khan[6], Julie Kim[6], William Gradishar[6], Kent Hoskins[3], Zeynep Madak-Erdogan[7]

[1]Food Science and Human Nutrition, University of Illinois Urbana-Champaign, Urbana, IL,[2]Division of Nutritional Sciences, University of Illinois Urbana-Champaign, Urbana, IL,[3]Division of Hematology/Oncology, University of Illinois at Chicago, Chicago, IL,[4]School of Public Health, University of Illinois at Chicago, Chicago, IL,[5]Northwestern Memorial Hospital, Chicago, IL,[6]Northwestern University Feinberg School of Medicine, Chicago, IL,[7]Cancer Center at Illinois, University of Illinois Urbana-Champaign, Urbana, IL

Background: Per- and polyfluoroalkyl substances (PFAS) are persistent environmental pollutants known for their resistance to degradation and their potential to accumulate in biological systems. These compounds have been associated with various adverse health effects, including promoting cancer progression.
Aim: This study aimed to examine the associations between plasma PFAS levels and inflammation-related proteins identified through targeted proteomics in a racially diverse cohort of menopausal women and breast cancer patients.
Methods: Three PFAS compounds—perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and perfluorobutane sulfonic acid (PFBS)—were quantified in plasma samples from both cancer-free individuals and breast cancer patients using targeted liquid chromatography-mass spectrometry (LC-MS).
Results: We found higher levels of PFBS in African American patients with breast cancer compared to Non-Hispanic White patients. Analysis of variance (ANOVA) revealed marginal differences in PFAS levels between racial groups for PFBS ($p=0.056p$) with no significant differences for PFOA or PFOS ($p>0.05$). Logistic regression models showed no significant associations between normalized PFAS levels (PFOA, PFOS, and PFBS) and health outcomes (tumor grade or status) in the overall cohort or within racial subgroups. Lastly, Among African American patients, PFBS levels were strongly associated with inflammatory and cancer-related markers including CASP8, CCL3, MMP10 and TRANCE, which are implicated in immune regulation tissue remodeling and cancer progression. These findings highlight the potential role of PFAS in contributing to inflammation and cancer progression, particularly among African American breast cancer patients, emphasizing the need for further research on PFAS exposure and its impact on cancer disparities.

#3630 First-line therapies and subjective cognitive function trajectories among international metastatic hormone-sensitive prostate cancer survivors.

Michelle O. Sodipo[1], Alicia K. Morgans[2], Erica T. Warner[3], Emily M. Rencsok[1], Lauren E. Howard[4], Colleen B. McGrath[1], Anna Siefkas[1], Konrad H. Stopsack[3], Christopher Sauer[5], Robert Drecier[6], Emilio Esteban[7], Hassan M. Dogo[8], Ademola Popoola[9], Charles Waihenya[10], Anders Bjartell[11], Kim Chi[12], Sebastien Hotte[13], Richard Cathomas[14], Deborah Enting[15], Scott Tagawa[16], Michael Ong[17], David Lorente[18], Elisabeth Heath[19], Rana McKay[20], Stefanie Fischer[21], Heather H. Cheng[22], Young E. Whang[23], Frederic Pouliot[24], Simon Crabb[25], Monica Chatwal[26], Miguel A. Climent[27], Raymond McDermott[28], Ian D. Davis[29], Camille Ragin[30], Folakemi Odedina[31], Natalie Greaves[32], Simone Badal[33], Simon Anderson[34], Sharon Harrison[30], Karen Autio[35], Laurel Cannon[36], Marie Grant[36], Alyssa Chan-Cuzydlo[36], Travis Gerke[36], Hannah D. McManus[4], Philip W. Kantoff[37], Daniel George[4], Sebastien Haneuse[38], Lorelei A. Mucci[1], on behalf of the IRONMAN Registry

[1]Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA,[2]Dana-Farber Cancer Institute, Boston, MA,[3]Clinical and Translational Epidemiology Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA,[4]Duke Cancer Institute, Durham, NC,[5]West German Cancer Center, University Hospital Essen, Essen, Germany,[6]University of Virginia, Charlottesville, VA,[7]Department of Medical Oncology, Hospital Universitario Central of Asturias, Oviedo, Spain,[8]University of Maiduguri Teaching Hospital, Maiduguri, Nigeria,[9]University of Ilorin Teaching Hospital, Ilorin, Nigeria,[10]Department of Surgery, Faculty of Health Sciences, University of Nairobi, Nairobi, Kenya,[11]Department of Urology, Skane University Hospital, Malmo, Sweden,[12]British Columbia Cancer Agency, Vancouver, BC, Canada,[13]Juravinski Cancer Centre, Hamilton, ON, Canada,[14]Kantonsspital Grabunden, Chur, Switzerland,[15]Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom,[16]Weill Cornell Medical Center, New York, NY,[17]Ottawa Hospital Cancer Centre, Ottawa, ON, Canada,[18]Hospital Provincial de Castellon, Castellon, Spain,[19]Wayne St. University Karmanos Cancer Institute, Detroit, MI,[20]University of California San Diego, San Diego, CA,[21]Kantonsspital St. Gallen, St. Gallen, Switzerland,[22]University of Washington Medical Center and Fred Hutchinson Cancer Center, Seattle, WA,[23]University of North Carolina, Chapel Hill, NC,[24]CHU de Quebec-Universite Laval, Quebec City, QC, Canada,[25]University Hospital Southampton NHS Foundation Trust, Southampton, United Kingdom,[26]Moffitt Cancer Center, Tampa, FL,[27]Instituto Valenciano de Oncologia, Valencia, Spain,[28]Tallaght University Hospital, Dublin, Ireland,[29]Monash University Eastern Health Clinical School, Melbourne, Australia,[30]Fox Chase Cancer Center, Philadelphia, PA,[31]Mayo Clinic, Jacksonville, FL,[32]Faculty of Medical Sciences, The University of the West Indies, Cave Hill, Barbados,[33]University of the West Indies, Mona, Jamaica,[34]George Alleyne Chronic Disease Research Centre, The University of the West Indies, Cave Hill, Barbados,[35]Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY,[36]Prostate Cancer Clinical Trials Consortium, New York, NY,[37]Convergent Therapeutics,, Cambridge, MA,[38]Department of Biostatistics, Harvard T. H. Chan School of Public Health, Boston, MA

Introduction: The standard of care for metastatic hormone-sensitive prostate cancer (mHSPC) has evolved with the expanded approval of androgen receptor pathway inhibitors (ARPIs) alongside androgen deprivation therapy (ADT). ADT alone has been shown to affect cognitive function. However, the cognitive impact of distinct treatment combinations remains unclear. We investigated the association between first-line systemic treatments and the trajectory of subjective cognitive function among mHSPC survivors in the International Registry for Men with Advanced Prostate Cancer (IRONMAN).

Methods: This analysis included 2435 newly diagnosed mHSPC patients enrolled across 15 countries between July 2017 and August 2024. Treatments were reported by site personnel and physicians. For this analysis, we focused on first-line systemic treatment and classified treatment groups at enrollment as ADT monotherapy (referent), ADT + ARPIs, or ADT + chemotherapy. Subjective cognitive function was assessed via the EORTC QLQ-C30 Cognitive Function subscale, with patient-reported scores (range 0-100) collected at enrollment and every 3 months for up to 5 years; higher scores reflect better cognitive function. Associations were estimated using linear mixed effects models with clustering by study site and country to examine differences at enrollment and across trajectories of subjective cognitive function by first-line treatment, adjusting for demographic and clinical factors.

Results: Median age at enrollment was 70 years, with a median follow-up of 1.7 years. 724 (30%) participants were treated with ADT monotherapy, 1317 (54%) with ADT + ARPIs, and 394 (16%) with ADT + chemotherapy. Mean cognitive function scores at enrollment were 85.4 (Standard Deviation [SD] 19.5) for participants treated with ADT monotherapy, 86.7 (SD 17.3) for ADT + ARPIs, and 86.7 (SD 18.0) for ADT + chemotherapy. Cognitive function scores decreased by -0.60 points (95% Confidence Interval [CI] -0.72, -0.48) per year among those treated with ADT monotherapy, with similar rates observed for those treated with ADT + chemotherapy. Participants treated with ADT + ARPIs had a slower decline in cognitive function, with scores decreasing by -0.36 points (95% CI -0.60, -0.08) per year.

Conclusion: In this international cohort of mHSPC survivors, subjective cognitive function scores were generally high at enrollment but declined over time. There was no evidence of worse cognitive function in those treated with ADT + ARPIs nor ADT + chemotherapy over time compared to ADT monotherapy. Cognitive functioning is a key component of survivorship, and defining cognitive trajectories associated with first-line treatment can inform supportive interventions, patient treatment decision-making, and clinical guidelines.

#3631 The role of distress-related metabolic dysfunction in ovarian cancer development.

Nan Lin[1], Raji Balasubramanian[2], Heather A. Eliassen[1], Britton Trabert[3], Mary K. Townsend[4], Shelley S. Tworoger[4], Oana A. Zeleznik[1]

[1]Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA,[2]Department of Biostatistics and Epidemiology, University of Massachusetts, Amherst, MA,[3]Department of Obstetrics and Gynecology, University of Utah, and Huntsman Cancer Institute at the University of Utah, Salt Lake City, UT,[4]Division of Oncological Sciences, Oregon Health & Science University, Portland, OR

Background: Ovarian cancer has few known risk factors and most of them are not easily modifiable on a population level. Thus, there is an urgent need to identify novel and modifiable risk factors for ovarian cancer. Growing evidence suggests chronic stress and subsequent distress influence ovarian cancer etiology. Metabolites represent the end-products of metabolism, reflect genetic and environmental influences, and are associated with chronic distress. Here, we evaluated the association of a 20-metabolite distress score (MDS) and its constituting metabolites with ovarian cancer risk.
Methods: Our study includes two 1:1 matched case-control studies, one (N=594) nested within the Nurses' Health Studies (NHS and NHSII) and another (N=348) nested within the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO). Metabolites were measured 3-27 years before diagnosis using liquid-chromatography tandem mass spectrometry in two batches. We calibrated and pooled metabolite values across two studies. Of the 20 MDS metabolites, we aligned 19 across batches, which were used in calculating the MDS. We estimated odds ratios (OR) and 95% confidence intervals (CI) for ovarian cancer corresponding to a one standard deviation (SD) increase in MDS and the individual metabolites using unconditional logistic regression, adjusting for matching factors and menopausal status, race, tubal ligation, oral contraceptive use, and family history of breast/ovarian cancer.
Results: A 1-SD increase in MDS was not associated with ovarian cancer risk, however, point estimates were consistent across studies and in the expected directions (Overall: OR 1.07; CI 0.97-1.19, p=0.17; NHS: OR 1.07; CI 0.94-1.21, p=0.31; PLCO: OR 1.09; CI 0.90-1.31, p=0.39). We observed heterogeneity in the MDS-ovarian cancer association by menopausal status, where a 1-SD increase in MDS was associated with a 19% (OR 1.19; CI 0.98-1.14, p=0.08) increased ovarian cancer risk in pre-menopausal women and 3% (OR 1.03; CI 0.91-1.17, p=0.62) increased risk in post-menopausal women.
Conclusion: Pooling two independent studies, higher circulating MDS levels were associated with a modest increase in ovarian cancer risk, consistent with our hypothesis. Further, the MDS-ovarian cancer association was more pronounced among pre-menopausal women, consistent with the previously reported association between depression and ovarian cancer risk. Additional analyses investigating heterogeneity in the MDS-ovarian cancer association by lifestyle and behavior factors is ongoing. Our findings suggest that metabolic dysregulation associated with chronic distress may represent a novel risk factor for ovarian cancer.

#3632 Analysis of supportive care medication use in metastatic colorectal cancer across racial and ethnic groups using SEER-Medicare.

Olga Trejos Kweyete[1], Yi Guo[2], Sherise Rogers[2], Lisa Scarton[2], David Deremer[2], Diana Wilkie[2], John Allen[3]

[1]University of Florida, Orlando, FL,[2]University of Florida, Gainesville, FL,[3]Purdue University, Indianapolis, IN

Background: This study investigates disparities in the use of adjunctive supportive care medications (SCM) among metastatic colorectal cancer (CRC) patients across different racial and ethnic groups. SCMs are central to symptom management and improving health-related quality of life in cancer treatment. Our goal was to identify potential disparities in medication use for neurological symptoms that may impact quality of life and symptom management in end-of-life care for metastatic colorectal cancer patients.

Methods: Data were acquired from Surveillance, Epidemiology, and End Results (SEER)-Medicare-linked database covering 2005-2017. We included patients diagnosed with metastatic CRC who died between 2005-2017 with more than one year of Medicare enrollment. Logistic regression models evaluated the relationship between SCM use and race while adjusting for various covariates, including sex, race, age, marital status, education, income, residence, and Charlson Comorbidity Index. Non-Hispanic White (NHW) CRC patients served as the reference group for all comparisons. Results are reported as the adjusted Odds Ratio and 95% Confidence Interval.

Results: After applying the inclusion/exclusion criteria, 28,212 patients were included in the final analysis (NHW:72.81%, Black 12.67%, Hispanic 9.51%, Asian/Pacific Islander 4.65%). Racial and ethnic disparities in the use of multiple SCM drug classes were identified. The results are summarized in Table 1.

Conclusion: Black CRC patients had significantly lower use of multiple SCM drug classes than NHW patients. Hispanic and Asian CRC patients' results were more heterogeneous, demonstrating both increased and decreased use for various SCM drug classes than NHW patients. These findings suggest varying patterns in medication use across racial and ethnic groups and require further investigation to understand further the causes and potential consequences of these differences in use

	Black/African American (n=3575)	**Hispanic (n=2683)**	**Asian (n=1312)**
Medications for neurological symptoms	aOR (95 % CI)	aOR (95 % CI)	aOR (95 % CI)
Anti-emetics	**0.78 (0.72, 0.84)**	1.03 (0.94, 1.12)	1.04 (0.93, 1.17)
Anti-epileptics	**1.21 (1.02, 1.43)**	1.10 (0.90, 1.33)	**0.68 (0.49, 0.94)**
Headache aids	**0.77 (0.68, 0.87)**	0.96 (0.84, 1.1)	**1.22 (1.03, 1.45)**
Steroids	**0.72 (0.66, 0.79)**	**0.89 (0.80, 0.97)**	1.03 (0.91, 1.18)
Cognitive aids	**1.20 (1.01, 1.42)**	**1.31 (1.09, 1.57)**	1.04 (0.79, 1.34)
Other			
Sleep aids	**0.67 (0.63, 0.77)**	0.97 (0.88, 1.07)	0.98 (0.85, 1.12)
Appetite stimulants	1.00 (0.92, 1.08)	**1.09 (1.00, 1.19)**	**1.41 (1.26, 1.58)**

#3633 Exploring racial and ethnic disparities in supportive medication use for nonmetastatic colorectal cancer: a SEER-Medicare database analysis.

Olga Trejos Kweyete[1], Yi Guo[2], Sherise Rogers[2], Lisa Scarton[2], David Deremer[2], Diana Wilkie[2], John Allen[3]

[1]University of Florida, Orlando, FL,[2]University of Florida, Gainesville, FL,[3]Purdue University, Indianapolis, IN

Background: Colorectal cancer (CRC) is a devastating disease with CRC patients experiencing physical disease and treatment-related symptoms that seriously impact the quality of life and psychological health. These symptoms often require supportive care medications (SCM) to manage and improve health-related quality of life throughout the illness trajectory. This study explores racial and ethnic differences in using SCMs in nonmetastatic CRC, aiming to uncover barriers and promote equitable care.
Methods: Data from the SEER-Medicare-linked database (2005-2017) were analyzed for metastatic CRC patients who died during this period and had over a year of Medicare enrollment. Logistic regression assessed the relationship between SCM use and race, controlling for variables like sex, age, marital status, education, income, residence, and Charlson Comorbidity Index, with non-Hispanic White patients as the reference group. Results are presented as the adjusted Odds Ratio and 95% Confidence Interval.
Results: After applying the inclusion/exclusion criteria, 81784 patients were included in the final analysis (White:72.6%, Black: 10.0%, Hispanic: 7.9%, Asian/Pacific Islander: 4.2%). Racial and ethnic disparities in the use of multiple SCM drug classes were identified. The results are summarized in Table 1.
Conclusion: Black patients tended to have lower odds of supportive care medication use across several SCM classes, particularly anti-emetics, headache aids, steroids, and sleep aids, while showing higher use of anti-epileptics and cognitive aids than White patients. Hispanic patients showed higher odds than White patients of using anti-emetics, cognitive aids, and appetite stimulants but had lower odds for muscle relaxants and steroids. Asian patients displayed higher odds for using anti-emetics and appetite stimulants but lower odds for using anti-epileptics, muscle relaxants, and sleep aids.

	Black/African American n=8214	**Hispanic n=6491**	**Asian n=3392**
Medications for neurological symptoms	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)
Anti-emetics	**0.88 (0.83,0.93)**	**1.13 (1.07, 1.2)**	**1.05 (1.05, 1.23)**
Anti-epileptics	**1.22 (1.11, 1.33)**	0.97 (0.87, 1.08)	**0.78 (0.67, 0.91)**
Headache aids	**0.8 (0.73, 0.89)**	0.97 (0.88, 1.07)	1.04 (0.90, 1.19)
Steroids	**0.73 (0.69, 0.77)**	**0.92 (0.86, 0.97)**	1.00 (0.92, 1.09)
Cognitive aids	**1.18 (1.1, 1.28)**	**1.13 (1.04, 1.23)**	0.97 (0.86, 1.09)
Other			
Muscle relaxants	0.97 (0.87, 1.08)	**0.78 (0.67, 0.89)**	**0.70 (0.57, 0.86)**
Sleep aids	**0.74 (0.67, 0.79)**	0.95 (0.89, 1.01)	**0.88 (0.81, 0.96)**
Appetite stimulants	0.99 (0.94, 1.04)	**1.07 (1.01, 1.13)**	**1.30 (1.21, 1.40)**

#3634 Short-term outcomes of breast cancer surgery by race-ethnicity in the Military Health System.

Yvonne Eaglehouse[1], **Sarah Darmon**[1], Matthew Nealeigh[1], Tracey Koehlmoos[2], Craig D. Shriver[1], Kangmin Zhu[1]

[1]Surgery, Uniformed Services University of the Health Sciences, Bethesda, MD,[2]Preventive Medicine and Biostatistics, Uniformed Services University of the Health Sciences, Bethesda, MD

Background: Racial-ethnic differences in receiving breast cancer treatment in the U.S. are well documented and have been partly attributed to access to care. However, little is known about possible disparities in short-term outcomes following breast cancer surgery. This study aimed to compare racial-ethnic groups in experience of post-operative outcomes following breast cancer surgery in the equal access U.S Military Health System.

Methods: We identified a cohort of women aged 18 and older diagnosed with stage I-III breast cancer between 2001 and 2014 who received mastectomy without reconstruction for their cancer in the MilCanEpi database. Information on 30-day complications, reoperation, and inpatient admission were captured. Multivariable logistic regression estimated the adjusted odds ratios (AORs) with 95% confidence intervals (CIs) in association with race-ethnicity for the outcomes.

Results: Among 886 Asian or Pacific Islander (API), 1,246 non-Hispanic Black (NHB), 497 Hispanic, and 4,898 non-Hispanic White (NHW) women receiving mastectomy for stage I-III breast cancer, the odds of 30-day complications were lower for API (AOR=0.63, 95% CI=0.47, 0.86) and similar for NHB (AOR=0.79, 95% CI=0.62, 1.02) and Hispanic (AOR=0.97, 95% CI=0.70, 1.34) compared to NHW women. The likelihood of reoperation was similar between the racial-ethnic groups. Hispanic women had higher odds of 30-day inpatient admission (AOR=1.55, 95% CI=1.10, 2.17) and the likelihood was statistically similar for API and NHB compared to NHW women.

Conclusions: In the equal access Military Health System, API women were less likely and NHB and Hispanic women as likely as NHW women to experience 30-day complications following primary surgical treatment without reconstruction for non-metastatic breast cancer. This highlights the importance of access to care in providing quality surgical care regardless of a patients' racial or ethnic background. The reason for higher likelihood of 30-day inpatient admission despite similar likelihood of complications among Hispanic women warrants further research.

#3635 Racial-ethnic comparisons in surgical treatment and outcomes of non-metastatic renal cell cancer in an equal access system.

Yvonne L. Eaglehouse, **Sarah Darmon**, Craig D. Shriver, Kangmin Zhu

Surgery, Uniformed Services University of the Health Sciences, Bethesda, MD

Background: Access to care has been implicated in racial-ethnic disparities in surgical treatment and survival for patients with renal cell carcinoma (RCC) in the United States. We assessed whether there were racial-ethnic disparities in surgery receipt and clinical outcomes of RCC in the U.S. Military Health System (MHS), which provides equal access to care for beneficiaries.
Materials and Methods: We used the MilCanEpi database to study a cohort of patients aged 18 and older who were diagnosed with stage I-III RCC between 1998 and 2014. Treatment with nephrectomy was evaluated in logistic regression models expressed as adjusted odds ratios (AORs) with 95% confidence intervals (CIs). Risk of recurrence and all-cause death were estimated using multivariable Cox regression models.
Results: The study included 1,371 non-Hispanic White, 362 non-Hispanic Black, and 177 Hispanic patients. Black patients were less likely to receive nephrectomy as non-Hispanic White patients overall (AOR=0.69, 95% CI=0.47, 1.00) and in strata for stage I (AOR=0.62, 95% CI=0.41, 0.95) or clear cell subtype (AOR=0.46, 95% CI=0.22, 0.96). Hispanic patients were as likely to receive nephrectomy as non-Hispanic White patients overall (AOR=1.13, 95% CI=0.49, 2.62) and in strata by stage or histologic subtype. There were no statistically significant differences in risk of recurrence or all-cause death for Black or Hispanic patients compared to non-Hispanic White patients in multivariable models.
Conclusion: In the equal access MHS, nephrectomy receipt was similar for Hispanic patients compared to non-Hispanic White patients while Black patients, especially with stage I or clear cell RCC, were less likely to receive nephrectomy. Nevertheless, there were no differences in recurrence or survival for non-Hispanic Black or Hispanic patients compared to non-Hispanic White patients with non-metastatic RCC. Further research on factors other than access to care that may explain the lower likelihood of receiving nephrectomy for Black patients is needed.

BIOINFORMATICS / COMPUTATIONAL BIOLOGY / SYSTEMS BIOLOGY / CONVERGENT SCIENCE: Artificial Intelligence and Machine Learning for Therapeutic Election and Discovery
Poster Session

#3639 A generative AI architecture integrates molecular, clinical and functional data to develop explainable predictive models and therapeutic strategies for multiple myeloma.

Praneeth Reddy Sudalagunta, Rafael Renatino Canevarolo, Daniel DeAvila, Rachel Howard, Maria Coelho Siqueira Silva, Alexandra Achille, Angel Perez, Robert Gatenby, Mark B. Meads, Kenneth H. Shain, Ariosto Siqueira Silva

H. Lee Moffitt Cancer Center, Tampa, FL

We present a novel Generative AI pipeline designed to integrate molecular, clinical, and functional data, specifically tailored to develop explainable predictive models and therapeutic strategies for Multiple Myeloma (MM). MM is an incurable, biologically complex, and a heterogeneous disease characterized by multifactorial progression and therapy-resistant phases, driven by genetic, cytogenetic, epigenetic, and transcriptomic alterations. These alterations exhibit patient-specific variability but converge on conserved biological pathways. The lack of consensus in treating pre-malignant, high-risk, or therapy-refractory MM patients underscores the need for innovative approaches like the one described here.
Our AI architecture leverages the Mistral 7 Large Language Model (Mistral AI), fine-tuned using Low-Rank Adaptation (LoRA), and incorporates a Retrieval-Augmented Generation (RAG) layer to enhance contextual understanding and decision-making. The pipeline was trained on a unique cohort of 2419 MM patient samples (PMRC DB), spanning the disease spectrum from pre-malignant to late-refractory stages, using integrated data from molecular (RNAseq - 1175, whole exome sequencing - 1232, cytogenetics - 2008), clinical (treatment history and response - 1413), and ex vivo functional assays (986), and validated in CoMMpass cohort (MMRF).
The RAG framework integrates a literature database curated from PubMed abstracts relevant to MM biology and clinical trials through the R library easy PubMed. The documents were embedded into dense vector representations using the sentence-transformers/all-MiniLM-L6-v2 model. These embeddings were indexed using FAISS's IndexFlatL2, for fast similarity-based retrieval of relevant documents for a query run on structured PMRC DB. The pipeline retrieves the top-k relevant documents based on proximity to the query's embedding, providing essential context. Retrieved documents and the user query are combined into a structured prompt, which is passed to the model for contextually aware text generation. The model uniquely combines an MM patient's features and biological/clinical context from published literature to generate text output that provides patient-specific insight from published literature.
The AI system provided explainable insights into MM treatment strategies and can potentially aid in therapeutic decision-making. Its accuracy as a predictive biomarker and capacity to elucidate underlying biology require further validation, especially for clinical cohorts with sparse or missing data. This work represents a proof-of-concept for the integration of generative AI with domain-specific biological and clinical datasets, setting the foundation for more precise and explainable AI-driven therapeutic strategies in MM.

#3640 Mapping drug-gene interactions in cancer with large language models.

Ying-Ju Lai[1], Li-Ju Wang[2], Yufei Huang[2], Yu-Chiao Chiu[2]

[1]Department of Biostatistics and Health Data Science, University of Pittsburgh, Pittsburgh, PA,[2]UPMC Hillman Cancer Center, Pittsburgh, PA

Understanding drug-gene relationships is critical for advancing targeted cancer therapies, identifying opportunities for drug repurposing, and improving patient outcomes. However, the vast and growing volume of biomedical literature presents significant challenges for researchers attempting to efficiently extract relevant insights. To address this, we developed an automated pipeline that integrates state-of-the-art large language models (LLMs) with up-to-date literature to reliably infer drug-gene interactions. By seamlessly interfacing PubMed with LLMs, the pipeline generates efficient, evidence-based inferences, effectively overcoming the limitations of static LLMs, including outdated knowledge and the potential for inaccurate conclusions. We systematically validated the pipeline using curated collections of drug-gene pairs from established knowledgebases. Comparative performance evaluations of various LLMs, including GPT-4o (OpenAI), Gemini 1.5 Pro (Google), and several open-source models, revealed that GPT-4o slightly outperformed Gemini 1.5 Pro, with both achieving an area under the ROC curve (AUROC) exceeding 90%. These proprietary models demonstrated substantial performance advantages over open-source alternatives. Using this pipeline, we analyzed hundreds of key oncogenes and FDA-approved drugs to construct a comprehensive pan-cancer drug-gene interaction network. This network identifies therapeutic targets and drug repurposing opportunities. The highest density of drug-gene pairs was observed in breast, colorectal, and lung cancers, reflecting significant therapeutic development and research interest in these prevalent cancer types. Additionally, our analysis of liver cancer, the third leading cause of cancer-related deaths worldwide, uncovered intricate multi-target effects of approved drugs such as sorafenib and lenvatinib. Beyond cancer-type-specific findings, the analysis also revealed numerous drug-gene associations spanning multiple cancer types. In summary, our pipeline integrates real-time literature retrieval with advanced LLMs and offers a robust and accessible resource for anti-cancer drug development. Designed with user accessibility in mind, the pipeline has the potential to enable cancer researchers to analyze drug-gene relationships without requiring programming expertise or extensive computational resources. This work provides a foundation for advancing therapeutic exploration and supporting innovation in oncology.

#3641 Predicting treatment outcomes using cross-modality correlations in multimodal oncology data.

Aakash Gireesh Tripathi[1], Asim Waqas[1], Yasin Yilmaz[2], Matthew B. Schabath[1], Ghulam Rasool[1]

[1]H. Lee Moffitt Cancer Center, Tampa, FL,[2]University of South Florida, Tampa, FL

Accurate prediction of treatment outcomes in oncology requires modeling the intricate relationships across diverse data modalities. This study investigates cross-modality correlations by leveraging imaging and clinical data curated through the Multimodal Integration of Oncology Data System (MINDS) and HoneyBee frameworks to uncover actionable patterns for personalized treatment strategies. Using data from over 10,000 cancer patients, we developed a machine learning pipeline that employs advanced embedding techniques to capture associations between radiological imaging phenotypes and histopathological features, aiming to predict survival outcomes and treatment efficacy.

The HoneyBee framework played a key role in preprocessing and feature extraction, using foundation models for imaging and text data. Radiological imaging, such as computed tomography (CT) and magnetic resonance imaging (MRI), was transformed into high-dimensional embeddings with vision transformers and convolutional neural networks. Clinical data, including electronic health records and pathology reports, was structured into embeddings using biomedical language models. These embeddings ensured standardization and cross-modal alignment while capturing modality-specific features. HoneyBee also applied data augmentation and preprocessing, such as stain normalization and tokenization, to maintain data quality. MINDS provided a scalable, metadata-driven architecture for integrating datasets like patient demographics and treatment histories, supporting seamless ingestion, harmonization, and storage. Together, these frameworks created a unified, multimodal dataset optimized for predictive modeling and resilient to missing data.

In training, embeddings were combined through late-stage fusion techniques to generate unified patient-level representations in latent space. These representations were used to train machine learning models, including gradient-boosted trees and neural networks, for survival analysis and treatment response prediction. Testing was conducted using stratified cross-validation to ensure generalizability across cancer subtypes and demographic groups. Results demonstrated significant predictive correlations, such as imaging-derived phenotypes linked to histopathological indicators of treatment response. The machine learning pipeline achieved a 12% improvement in concordance indices over unimodal approaches, underscoring the enhanced accuracy of cross-modality learning.

#3642 TLSL: Transfer learning for synthetic lethality (SL) experiment design and SL prediction.

Birkan Gokbağ, Lijun Cheng, Lang Li

Department of Biomedical Informatics, The Ohio State University College of Medicine, Columbus, OH

Introduction: In a synthetic lethality (SL) gene pair, the cell dies when the function of both genes is disrupted but remains viable when the function of only one of the genes is compromised. While carrying strong therapeutic potential in the treatment of cancer, SL interactions are context specific and sparse. CRISPR-Cas9 based gene combination double knockout (CDKO) experiments are purposely designed to discover novel SL gene pairs. However, none of the published CDKO studies have considered how to use existing SL data to choose gene pair targets and cell lines for new experiments. In this project, for the first time, we present a transfer learning framework for SL prediction, namely TLSL. We anticipate TLSL can illustrate whether and how existing SL data can assist in gene and cell line selection for new CDKO experiments.
Methods: Signature-iSL is the foundation model for TLSL. It is designed to be interpretable, perform cell line specific SL prediction, and incorporate pathway-based co-membership of gene pairs and multi-omics signatures. In transfer learning, Signature-iSL shall transfer information from source data (i.e. existing CDKO experiment data) to target data (i.e. data from new CDKO experiment). Our transfer learning covers four different CDKO experiment design scenarios: (1) experiment keeps the same genes, but on a new cell line; (2) experiment keeps the same cell line, but selects different genes; (3) experiment targets a similar mechanism, but selects different cells and genes; and (4) big data (i.e. all other existing SL CDKO experiments in Synthetic Lethality Knowledge Base (SLKB)) to small data (i.e. targeted genes in a cell). TLSL was applied to all datasets under four scenarios in SLKB. It covers 11 studies, 22 cell lines, 6,127 genes, 16,059 SL, and 264,424 non-SL gene pairs. Each computational experiment was repeated five times and evaluated in out-of-sample gene pair prediction; 20% of genes and their gene partners were held out from training.
Results: As a standalone model, Signature-iSL outperformed two previous state-of-the-art methods, MVGCN-iSL and MLEC-iSL. While MVGCN-iSL achieved 0.62 and MLEC-iSL achieved 0.65 AUC-ROC, Signature-iSL reached 0.75. On average, across all four scenarios, TLSL improved accuracy, AUC-ROC, and F-1 metrics by 15%, 9%, and 10% respectively. The most successful scenario was big data to small data transfer learning, where accuracy, AUC-ROC, and F-1 metrics improved by 23%, 18%, and 15% respectively.
Conclusion: The proposed transfer learning approach, TLSL, is shown to be highly informative and beneficial for selecting genes and cells in new CDKO experiments by borrowing SL data from existing CDKO experiments. Under all four transfer learning scenarios, TLSL has significantly improved SL prediction model performance.

#3643 AI driven discovery of novel synthetic lethal gene pairs for targeted cancer therapy.

Amalia Soenens[1], Daniel Miller*[2], Oliver Vipond[2], Florencia Skuras[2], Alfie Brennan[2], Anna Hercot[2]

[1]EditCo Bio, Redwood City, CA,[2]Evariste Ltd, London, United Kingdom

Synthetic lethality (SL) offers a promising avenue for cancer therapy by exploiting vulnerabilities unique to tumor cells and minimizing off-target effects on normal tissue. However, the application of SL-based therapies remains constrained by a limited repertoire of validated targets and biomarkers. Using data from the Cancer Dependency Map (DepMap) and advanced in silico modeling, Evariste's Frobenius Target platform identified 50 potential SL gene pairs with druggable nodes and rational mechanisms linking targets and biomarkers. From this set, 24 gene pairs were prioritized for validation based on translational relevance and impact on the patient population.

Validation was achieved through a collaborative high-throughput workflow with EditCo Bio, leveraging their CRISPR-based cell line engineering platform to generate 72 knockout pools in under three weeks. Validation studies in multiple cell lines demonstrated >10 high-confidence SL pairs, including a novel SL relationship involving focal adhesion kinase (FAK). To further substantiate this relationship, novel inhibitors were developed with best-in-class selectivity over the SL partner gene. Functional assays confirmed these inhibitors' effectiveness in biomarker-low cell lines, with promising implications for clinical translation.

This study highlights the potential of AI-driven approaches to uncover the next generation of SL targets, expanding the therapeutic landscape in cancer research. By focusing on biomarker-low gene expression as a predictor of SL, our work establishes a robust framework for the discovery and validation of clinically actionable SL relationships.

#3644 Mechanistically explainable AI model for predicting synergistic cancer therapy combinations.

H. Si[1], S. Kumar[1], S. Lata[1], A. Ahmad[1], S. Ghosh[1], K. Stephansen[1], D. Nagarkar[1], E. Zhou[2], B. W. Higgs[1];
[1]Genmab US, Inc., Plainsboro, NJ, [2]Lamini, Menlo Park, CA

Background: Resistance to single-agent cancer therapies often arises through complex mechanisms such as genetic mutations, compensatory signaling, and tumor microenvironment changes. These adaptive strategies make multi-drug combinations essential to effectively target canonical pathways simultaneously. However, common *in vitro* and *in vivo* models often fail to capture these complexities, resulting in limited translational success to human clinical settings. This highlights the need for predictive frameworks that not only identify synergistic drug combinations from human clinical trials, but also provide mechanistic insights to inform clinical application. This study presents a novel Large Language Model (LLM)-based framework that integrates extensive drug combination data with a knowledge graph to predict drug combinations with biological explainability.
Methods: A retrieval-augmented generation (RAG) approach was employed to enhance predictive specificity and reduce hallucinations. *In vitro* data on drugs, targets, cell lines, tumor types, and synergy scores were compiled from over 50,000 cell line results from the DrugComboDb database. Using NCBI's APIs, BeautifulSoup, and GPT-4, biomedical information was extracted from 1,092 phase I-III clinical trials covering 700+ drug combinations across 22 tumor types. This RAG corpus, combined with targeted prompts, fed into the LLM Mistral v0.2 for prediction generation, while a knowledge graph - PrimeKG provided mechanistic insights. Model validation included unseen cases (N=42) across various drug modality combinations (N=8), including 34 immuno-oncology therapies (IO) combined with an IO, chemo, or targeted therapy, 1 targeted therapy combination and 7 antibody drug conjugate (ADC) combinations.
Results: The model achieved an F1 score of 0.80 on the entire validation set, with accuracy at 0.74, precision at 0.71, and recall at 0.92. Among drug modalities, combinations of ADC agents with IO/targeted/chemo/other ADC agents performed best (F1 score: 100%), while IO-IO combinations scored the lowest (F1 score: 62%). Each prediction included detailed mechanistic explanations, highlighting whether the drug combination was supported or unsupported with biological rationale.
Conclusions: This study presents a novel framework integrating LLMs with biological knowledge graphs to predict the efficacy when combining 2-3 oncology drugs across a range of indications. By linking generative AI with explainability tools, we address two critical challenges in AI-driven drug discovery: predictive accuracy and mechanistic transparency. Such predictive models can accelerate the discovery of synergistic therapies, thereby reducing costly experiments, and improve overall patient outcomes. Specific test cases with explainable results will be presented.

#3645 Pathway-informed deep learning enhances cancer dependency prediction using multi-source CRISPR screening data.
Chien-Hung Shih, Yu-Chiao Chiu

UPMC Hillman Cancer Center, Pittsburgh, PA

Precision cancer therapy relies on identifying tumor-specific genetic dependencies, which are genes critical for cancer cell survival and proliferation that can be leveraged as therapeutic vulnerabilities. The Cancer Dependency Map (DepMap) datasets from the Broad and Sanger Institutes provide a comprehensive resource for systematically uncovering these dependencies through genome-wide loss-of-function CRISPR screens. These datasets have enabled the development of predictive models for cancer cell lines or tumors that cannot be directly screened. Recent machine and deep learning approaches have utilized gene expression data, with or without the integration of other omics profiles, to predict such dependencies. However, existing methods often fall short in capturing pathway-level interactions among genes and in effectively integrating insights from both Broad and Sanger DepMap datasets due to limitations in model design and training strategies. To address these challenges, we present a novel deep learning model that integrates pathway-level information and both DepMap datasets to predict the dependency of individual genes in cancer cell lines. In our approach, each cancer cell line is represented by activity scores across thousands of biological pathways and functions, while each gene is modeled based on its involvement in these pathways. This design enables the model to comprehensively capture functional interactions and dependencies within the cellular context, rather than treating genes in isolation. To further enhance the representation of genes targeted for knockout, we implemented a contrastive learning framework. This framework was pretrained on the Sanger CRISPR screens to classify whether two genes exhibit similar dependency profiles and was subsequently fine-tuned on the Broad CRISPR screens to predict the dependency score of each gene in a specific cell line. The final deep learning model demonstrated strong predictive performance, achieving a median correlation coefficient of 0.86 between actual and predicted dependency scores across genes in individual cell lines. The model significantly outperformed baseline configurations that excluded pathway integration or contrastive learning. Notably, cell lines from lung, head and neck, esophageal, and gastric cancers exhibited particularly high prediction accuracy, potentially due to shared biological characteristics within these tumor types. Further investigation is warranted to identify factors contributing to this performance. In summary, our novel deep learning model improves cancer dependency prediction by leveraging pathway-level insights and integrating multiple DepMap datasets. Ongoing efforts include further model refinement, expanded validation experiments, and systematic model interpretation to uncover the underlying mechanisms captured by the model.

#3646 DeepSynBa: A deep learning method to improve drug combination predictions using full dose-response matrices and contextual features of cell lines and cancer indications.

Marta Milo[1], Haoting Zhang[2], Halil ibrahim Kuru[3], A. Ercument Cicek[4], Oznur Tastan[5], Carl Henrik Ek[6], Magnus Rattray[7]

[1]Oncology Data Science, AstraZeneca, Cambridge UK, United Kingdom,[2]The Computer Laboratory, University of Cambridge, Cambridge UK, United Kingdom,[3]Computer Engineering, Bilkent University, Ankara, Turkey,[4]Computational Bilogy, Carnegie Mellon University, Pittsburg, PA,[5]Faculty of Engineering and Natural Sciences, Sabanci University, Istanbul, Turkey,[6]The Computer laboratory, University of Cambridge, Cambridge UK, United Kingdom,[7]Division of Informatics, Imaging and Data Sciences, University of Manchester, Manchester, United Kingdom

Introduction: Many cancer monotherapies have limited activity in clinic, making combinations a relevant treatment strategy. The number of possible combinations is vast, and the responses can be context-specific, making it challenging to predict combination effects. Existing computational models typically predict a single aggregated synergy score, i.e. Bliss or Loewe, for a given drug combination. However, these approaches exhibit high prediction uncertainty and limited actionability because they fail to differentiate between potency and efficacy by oversimplifying the drug-response surface using a single synergy score.

To address these limitations, we introduce DeepSynBa, which models the full dose-response matrix of drug pairs rather than an aggregated synergy score. DeepSynBa formulates this task as a regression problem and uses cell line-specific features and drug embeddings to predict the entire drug-response matrix within a deep learning framework. Following SynBa's approach of modelling the dose-response surface [1], DeepSynBa includes an intermediate layer that estimates pharmacological parameters, which are then used to predict dose-response values across dosages. This design also enables post-hoc calculation of traditional synergy scores like Loewe and Bliss, maintaining compatibility with existing synergy predictors.

Results: We evaluated DeepSynBa on the NCI-ALMANAC dataset, which contains 104 FDA-approved cancer drugs, 60 cell lines, and nearly 300,000 drug pair-cell line combinations. DeepSynBa outperforms comboFM [2] and comboLTR [3], two state-of-the-art methods for direct dose-response matrix prediction across multiple evaluation scenarios, such as novel drug combinations, cell lines, and drugs. DeepSynBa achieves RMSE values of 7.72 and 7.71 in the new drug combination and new cell line settings, respectively, which is an improvement of approximately 65% compared to comboFM, the next-best model.

DeepSynBa also consistently attained lower RMSE and higher Pearson and Spearman correlations across nine tissue types including breast, colon, and leukemia.

Conclusions: DeepSynBa provides a more accurate framework for predicting the full dose-response matrix than existing methods. Additionally, DeepSynBa supports experimental design by providing detailed predictions of drug combination responses across dosages. For untested combinations, by utilizing the parameter layer which separates efficacy from potency, biologists can select dosage ranges that optimize efficacy while limiting off-target toxicity, hence increasing the likelihood of identifying optimal therapeutic dosages.

References: 1.Zhang H. et al. *Bioinformatics, 39*,1:i121-i130 (2023).2.Julkunen H. et al. *Nature Communications, 11*, 1:1-11 (2020).3.Wang T. et al. *Bioinformatics, 37*,1:i93-i101(2021).

#3647 Validation of a computational reasoning model for precision oncology with large-scale patient-derived xenograft data.

Robert Doczi[1], Akos Takacs[1], Anna Dirner[1], Dora Lakatos[1], Maria Kocsis-Steinbach[1], Barbara Vodicska[1], Dora Tihanyi[1], Eniko Kispeter[1], Reka Szalkai-Denes[1], William T. Beck[2], **Petak Istvan**[1]

[1]Genomate Health Inc, Cambridge, MA,[2]University of Illinois Chicago, Chicago, IL

Comprehensive tumor molecular testing is key in precision oncology. However, interpretation of complex molecular profiles in clinical practice can be challenging and subjective. The Digital Drug Assignment (DDA) system is a computational reasoning model that scores treatment options based on full tumor genomic data. This approach was predictive of relative benefit of the agents as used in the SHIVA01 trial (DOI: 10.1038/s41698-021-00191-2). Here, we analyzed patient-derived xenograft (PDX) data from Gao et al. (DOI: 10.1038/nm.3954). Molecular profiles of 179 solid tumors were processed by DDA, the resulting personalized drug scores were evaluated with treatment outcome data in PDX models for 1151 molecularly targeted agent (MTA) monotherapies, using 23 MTAs, targeting key oncogenic signaling pathways. In the PDX avatar system, multiple treatments can be tested for the same tumor, and outcomes can be measured in clinical metrics, enabling direct evaluation of drug ranking. To this end, cohorts were formed based on DDA ranking positions for each MTA-tumor pair. DDA ranking position was predictive of relative treatment benefit (Table, upper panel). Because the difference between neighboring ranks is probably minor, and to increase group sample sizes, larger cohorts were formed by combining neighboring rank groups. Here, a clear pattern of gradual decrease of treatment benefit with ranking distance from the top cohort was demonstrated (Table, lower panel). In this study, utilizing the unique advantages of the PDX system, we could demonstrate the predictive power of DDA treatment ranking. Digital drug assignment can serve as a practical and economic in silico avatar system for treatment planning and can potentially address challenges with complex molecular profiles in routine clinical settings.

Rank	**N**	**mPFS (days)**	***P* value vs 1st**	**HR (95% CI)**
1	178	38.0	n/a	n/a
2	178	29.6	1.84e-01	1.15 (0.87 - 1.70)
3	172	26.4	1.11e-01	1.15 (0.87 - 1.71)
4	168	19.1	1.72e-08	1.67 (1.50 - 2.93)
5	160	21.4	9.62e-07	1.57 (1.37 - 2.69)
6	133	19.3	1.16e-07	1.65 (1.46 - 2.91)
7 - 10	161	17.1	5.43e-11	1.88 (1.78 - 3.50)
Rank	**N**	**mPFS (days)**	***P* value vs top**	**HR (95% CI)**
1 - 2	356	34.4	n/a	n/a
3 - 4	340	28.0	8.09e-04	1.27 (1.02 - 1.97)
5 - 6	293	23.2	4.40e-09	1.51 (1.30 - 2.51)
7 - 10	161	17.1	4.03e-11	1.76 (1.61 - 3.19)

#3648 AI-enabled 3D multimodal integration platform reveals subcellular architecture of tumor immune microenvironment for therapeutic target and biomarker discovery and patient selection.

Inyeop Jang[1], Yoonho Choi[1], Minji Kim[1], Jeonghwan Park[2], Jiyeon Sung[3], Byungsoo Ann[4], Clemenceau R. Jean[1], TaeHyun Hwang[1]

[1]Mayo Clinic Florida, Jacksonville, FL,[2]Seoul National University College of Medicine, Seoul, Korea, Republic of,[3]KyungHee University, Seoul, Korea, Republic of,[4]Severance Hospital Yonsei University College of Medicine, Seoul, Korea, Republic of

Background: Tumor immune microenvironment (TIME) organization fundamentally shapes cancer progression and immunotherapy outcomes. Traditional 2D spatial molecular approaches inadequately capture the complex three-dimensional architecture and cellular interactions within TIME. We developed an AI-driven platform integrating multiple spatial molecular datasets, enabling comprehensive 3D visualization for therapeutic discovery and patient selection.
Methods: Our platform combines holotomography imaging, providing 3D refractive index maps, with diverse spatial molecular data: in situ molecular imaging, spatial proteomics, multiplex immunohistochemistry, and spatial transcriptomics. AI algorithms align datasets within a unified 3D coordinate system, while Poisson implicit surface reconstruction generates subcellular-resolution models. Platform validation used clinical samples from immunotherapy-treated patients.
Results: The platform integrated multimodal spatial data into high-resolution 3D TIME representations, revealing novel relationships between protein expression, transcriptional patterns, and cellular architecture. 3D surface protein analysis with subcellular morphology identified patients with target protein expression outside tight junctions, indicating higher risk for adverse effects from antibody-drug conjugates and cellular therapies. This insight, unavailable through 2D analysis, enabled precise patient selection.
Conclusions: This platform provides detailed subcellular-resolution TIME architecture analysis, offering actionable insights for therapeutic targeting, biomarker discovery, and patient selection . AI-driven 3D spatial multimodal TIME analysis advances precision medicine across multiple therapeutic approaches, including immunotherapy, targeted therapy, and drug delivery optimization.
AI Disclosure: Original abstract content was written by the authors. Claude was used for editorial assistance and formatting without altering the scientific content or context of the work.

#3649 Artificial intelligence accelerates shared neoantigen peptide cancer vaccine development.

Genwei Zhang[1], Jiewen Du[1], Xiangrui Gao[1], Zhenghui Wang[1], Qingxia Zhang[1], Tongren Liu[1], Dong Chen[1], Chi Han Samson Li[2], Kin Tak Chan[2], Hong Wang[2], Melvin Toh[2], **Lipeng Lai**[1]

[1]XtalPi, Beijing, China,[2]CK Life Sciences Development Limited, Hong Kong, China

Background: Many cancers remain intractable despite the recent breakthroughs in immune checkpoint inhibitors and gene and cell therapies, making the pursuit of novel, efficacious, and safe treatment options critical. Recently, there is a renewed interest in cancer vaccines as a promising cancer immunotherapy approach. Nevertheless, precisely predicting the immune response elicited by cancer vaccines continues to be a significantly hurdle.

Methods: From published literature and available datasets, we collected over 17 million peptide entries ranging from 8 to 11 amino acids, and approximately 4 million peptide entries ranging from 13 to 21 amino acids to train models predicts the probability of a given peptide to be presented by MHC-I or MHC-II, respectively. Those peptides were restricted to 150 unique MHC-I alleles and 19 unique MHC-II alleles. Overall, both MHC-I and MHC-II presentation models were constructed with about 30 million parameters. In addition, we developed algorithms to specifically introduce single point mutations to further enhance binding to MHCs, and in the meanwhile, not influence cognate recognition. To validate the model prediction accuracy, automated peptide synthesis and surface plasmon resonance (SPR) technologies were applied. We then examined the presentation of designed peptides using MHC allele-engineered cell line, and further validated the peptide immunogenicity in mice by assessing T cell activation and tumor cell killing capabilities.

Results: Our advanced AI models has outperformed the state-of-the-art prediction algorithms, including NetMHCpan and MixMHCpred, in predicting antigen presentation for both MHC-I and MHC-II. To validate our model accuracy, 28 novel peptides based on mutated TP53 protein were predicted and their binding to corresponding HLA alleles were measured using SPR. >80% of the peptides displayed stronger binding affinities than the positive controls, demonstrating a significantly improved algorithm accuracy for peptide neoepitope design. In the cellular presentation study, one out of six designed neoepitope peptides were validated by mass spectrometry to be presented by HLA-A*02:01 expressing cell line. Importantly, to further evaluate the immunogenicity, we immunized mice with several mouse-derived neoepitope peptides . *Ex vivo* ELISpot assay showed that >20% mouse-derived neoepitope peptides could elicit strong immune responses.Moreover, the activated T cells demonstrated potent (>50%) tumor cell killing capability in the *ex vivo* cellular experiment.

Conclusions: We crafted sophisticated AI algorithms capable of rapidly designing shared neoantigen T cell epitopes that exhibit strong immunogenicity. We believe that our AI technology has substantial promise for propelling the development of ready-made cancer vaccines, and will eventually improve patient outcomes when successfully applied in clinical settings.

#3650 AI-based MTB assistant in cancer personalized treatment: Analysis from a real-world, multi-center retrospective study.

Minghui Wang[1], Yanhong Shang[2], Zhengang Yuan[3], Xin Huang[4], Qing Hao[4], Shaohua Yuan[4], Guobang Shi[4], Hui Chen[4], Wenjin Liu[4], Lili Wang[5], Shiwang Wen[6], Huilai Lv[6], Zhizheng Wang[4], Dong You[4], Yang Liu[4], Fei Pang[4], Wei Rao[7], Haitao Wang[5], Leilei Lu[4], **Kai Wang**[4]

[1]Sun Yat-sen University, Guangzhou, China,[2]Affiliated Hospital of Hebei University, Baoding, China,[3]Eastern Hepatobiliary Surgery Hospital, Shanghai, China,[4]OrigiMed Co., Ltd, Shanghai, China,[5]The Second Hospital of Tianjin Medical University, Tianjin, China,[6]Fourth Hospital of Hebei Medical University East, Shijiazhuang, China,[7]The Affiliated Hospital of Qingdao University, Qingdao, China

Background: Developing personalized treatment plans in cancer care is a complex and time-intensive process. The multidisciplinary Molecular Tumor Board (MTB) approach offers a promising solution for tailoring precision treatments but is often limited by the manual analysis of vast molecular data, which is laborious and error-prone. To address these challenges, we developed SmartMTB, an artificial intelligence (AI)-based assistant, designed to support clinicians in formulating cost-effective and efficient personalized treatment plans.
Methods: SmartMTB leverages a medical-field-customized large-language model and a database tailored to the Chinese population. It integrates patient-specific tumor types and gene variation data to suggest personalized treatment strategies. In a real-world, multi-center retrospective study on the precision medicine platform, we included clinical data of 1032 Chinese cancer patients and asked SmartMTB for treatment plan suggestions.
Results: The study cohort included 1,032 patients spanning 13 major tumor types, with a median age of 58 years (range: 7-87 years). Clinical stages ranged from I to IV (stage I: 11.4%, stage II: 10.3%, stage III: 21.9%, stage IV: 51.6%, unknown: 4.8%). Among them, 92% of the patients got treatment plan suggestions from SmartMTB. Eventually, 39.4% of the patients received SmartMTB-recommended treatment plans, while 52.5% did not. Patients who received SmartMTB-recommended treatment plans exhibited higher partial response rate (PR, 31.4% vs. 11.3%), objective response rate (ORR, 34.1% vs. 12.3%) and longer median progression-free survival (mPFS, 163 vs. 92 days, $P < 0.0001$) than those who did not. Similar benefits were observed across cancer types, including lung cancer (PR: 31.9% vs. 10.2%; ORR: 35.2% vs. 11.9%; mPFS: 185 vs. 92 days, $P < 0.0001$) and hepatobiliary tumors (PR: 28.6% vs. 13.3%; ORR: 31.4% vs. 15%; mPFS: 121 vs. 87 days, $P = 0.024$). Excluding lung cancer patients treated with EGFR-TKI, SmartMTB's recommendations maintained efficacy for the remaining lung cancer patients (PR: 31.7% vs. 8.9%; ORR: 35% vs. 10.7%; mPFS: 153 vs. 90 days, $P < 0.0001$). Furthermore, SmartMTB took about 2.5 h to analyze data from 1032 patients and provide treatment suggestions, which was much more efficient than the traditional manual MTB.
Conclusion: In this study, patients using treatment strategy SmartMTB recommended had much better clinical treatment results than those who didn't. This study highlights the clinical utility of SmartMTB in enhancing treatment outcomes for cancer patients. By providing timely, accurate, and personalized treatment recommendations, SmartMTB addresses critical gaps in traditional approaches, reducing time and cost burdens. Its potential for widespread clinical adoption could transform precision oncology practices and improve patient care globally.

#3651 AI-derived immune cell knowledge graphs to advance immunotherapy.

Shan (Chloe) He, Yukun Tan, Vakul Mohanty, Hind Rafei, Katy Rezvani, Ken Chen

UT MD Anderson Cancer Center, Houston, TX

Immune cells are critical units in human immunity, yet our current understanding of their roles remains limited. A critical barrier is a lack of human natural language in describing the complex molecular roles of immune cells in diverse contexts. Current descriptors of molecular features such as genes and pathways are limited by biological terms available in the functional annotation databases, which were not created specifically to describe immunity, lacking granularity and completeness. Although richer language exists in biological literature, they require labor-intensive curation. We hypothesize that by applying cutting-edge AI techniques such as large language models (LLMs), we can curate a knowledgebase specific for describing human immunity, consisting of rich molecular-cellular relationships and functional descriptors not available in current databases. Querying this knowledgebase can substantially enrich our language in describing and understanding the complex roles of immune cells, and generate novel, verifiable hypotheses for discovery and development of immunotherapy. To address these challenges, we have constructed immune cell type-specific knowledge graphs (KG) for T cells, NK cells, B cells, and macrophages by parsing 9,995, 3,665, 7,503, and 3,615 relevant published abstracts since 2020, respectively. We have fine-tuned BioBERT models to perform named entity recognition (NER) on genes, diseases, and cell types, formulated rule-based algorithms to extract pathways and biomedical concepts. We then employed the LLaMA3-70B model to infer relationships between the extracted entities based on abstract using carefully designed prompts. We present the first immune cell type-specific KGs. Using LLMs, we overcome limitations in immune vocabularies, extracting concise biomedical phrases to expand vocabularies and better characterize immune cell biology beyond existing knowledge bases. These enriched vocabularies can annotate gene sets, providing annotations that reflect recent literature. The KGs, represented as directed networks of biomedical entities, support hypothesis generation and experimental design. By querying relationships among genes and concepts, researchers can identify genes linked to specific phenotypes and predict phenotypic changes following interventions such as gene silencing or cytokine stimulation. These KGs enable dynamic queries to explore immune cell complexity, identify therapeutic targets, and design experiments to investigate signaling pathways. To enhance accessibility, we provide a web application for exploring genes and phenotypes in a cell type-specific manner: https://kchen-lab.github.io/immune-knowledgegraph.github.io/.

#3652 OCTO-virtual cell: a Foundation Model of Cell and Tissue Spatial Biology with Application to Patient Stratification and Target Discovery.

Yubin Xie, Eshed Margalit, Tyler Van Hensbergen, Dexter Antonio, Jake Schmidt, Yu Phoebe Guo, Jeremie Decalf, Maxime Dhainaut, Lucas Cavalcante, Hargita Kaplan, Rodney Collins, Francis Fernandez, Rob Schiemann, Eric Siefkas, Michela Meister, Joy Tea, Carl Ebeling, Anastasia Mavropoulos, Nicole Snell, Shafique Virani, Ron Alfa, Lacey Padron, Jacob Rinaldi, Daniel Bear

Noetik Inc, South San Francisco, CA

Background: Understanding cellular behavior within the context of the tumor-immune microenvironment is essential to developing next-generation cancer therapies and advancing precision medicine. The inherent challenges of this problem reflect the complexity of human biology - patient and tissue heterogeneity, a multitude of interacting signaling pathways, dynamic short- and long-range interactions between the tumor and the immune system, and the intrinsic limitations of measurement techniques. Machine Learning foundation models trained on multimodal patient data present an opportunity to grapple with this complexity and push the field forward leveraging recent advances in spatial biology.

Method: A custom multimodal transformer was trained on 1399 primary resections from lung cancer patients profiled via H&E, CosMx spatial transcriptomics, whole-exome sequencing, and a custom multiplex immunofluorescence panel. To our knowledge this is the largest extant spatial transcriptomics dataset, comprising more than 40 million cells. The transformer was trained via self-supervised learning to predict expression of each CosMx panel gene for a single cell, conditioned on both spatially proximal and patient-level data from all modalities. This training task induces the model to learn fundamental rules that govern cell state and cell-cell interactions within the context of disease. The resulting model, Celleporter, can generate gene expression in a “virtual” cell at a particular location within real or simulated patient tissue. Counterfactual simulations with modified patient data can be used to predict the effects of genetic alterations, gene expression changes, or external interventions on the tumor-immune microenvironment.

Result: Celleporter accurately predicted spatial gene expression patterns from sparsely sampled data, resolving the limitations of traditional experimental approaches. Virtual cell simulations reproduced distinct biological states, such as cytotoxic and naïve transitions of CD8+ T cells within and outside tumor regions and reproduced foundational immunology, including the relationship between MHC-I and T cell activation. Comparative analyses across patient cohorts identified immune-suppressive mechanisms in STK11-mutant tumors resistant to immunotherapy. Perturbation simulations highlighted therapeutic targets predicted to restore cytotoxic activity in STK11-mutant tumor microenvironments.

Conclusion: This study demonstrates that a self-supervised foundation model trained on large-scale multimodal patient data can learn fundamental aspects of cancer immunology, and accurately reproduce the impact of the tumor-immune microenvironment on cell state in a patient-specific manner. This flexible system for interrogating cell and tissue biology has direct application to patient stratification and target discovery.

#3653 Comparison between rule-based, AI-powered and manual clinical trial matching using OncoSolver, a newly-developed clinical decision support tool.

Edoardo Crimini[1], Matteo Repetto[2], Tommaso Fougier[3], Giuseppe Curigliano[1]

[1]Division of New Drugs and Early Drug Development, IEO - European Institute of Oncology, Milan, Italy,[2]Memorial Sloan Kettering Cancer Center, New York, NY,[3]Independent researcher, Milan, Italy

This study compared OncoSolver (OS), a novel clinical decision tool for matching oncologic patients to clinical trials (CTs), with the manual matching (MM) process at European Institute of Oncology (IEO) Phase I Unit. Twenty volunteer investigators screened for suitable CTs 100 advanced cancer patient profiles (PP) comprehensive of treatment history, pathological, genomic and clinical characteristics, modeled on publicly available precision oncology cohorts. OS used rule-based (RB) and AI modalities to match PP concurrently. AI matching used GPT4 as large language model. Results were compared for accuracy, precision, and recall, with discordant matches reviewed for eligibility assessment. Time spent and correlation between experience and performance were analyzed for MM. Analyses were performed with R version 4.3.3 and RStudio. Neuroendocrine, breast and biliary tract were the most represented cancer types (n=19, 11 and 11, respectively). Seventy-one PP had molecular alterations of interest for at least one of the 25 CTs active during the study period. *PIK3CA* and *KRAS* mutations were the most common alterations of interest (n=21 and 17). The concordance among the 3 methods was 2350/2500 (94.0%), between MM and RB 2395/2500 (95.8%), between MM and AI 2388/2500 (95.5%), and between RB and AI 2418/2500 (96.7%). The review of the discordances across the 3 methods led to the definition of 100 correct CT-PP matches, with 57 PP eligible for at least one CT. The number of false positive was 17, 26 and 46 for MM, RB and AI, while false negatives were 68, 2 and 33, respectively. Accuracy was 96.6, 98.8 and 96.7, precision 65.3, 78.2 and 57.5 and recall 32.3, 98.0 and 65.7 for MM, RB and AI. RB had the highest AUC (0.98), followed by AI (0.82) and MM (0.66). Median time for OS matching was 2 minutes per PP (range 1-4), significantly shorter than MM (4 minutes, range 1-17, $p < 0.001$). Recall showed a significant positive correlation with the increase of experience in MM (range 1-5 years, $p=0.04$). MM process is inefficient and carries the risk of missed therapeutic opportunities for cancer patients, which could negatively affect their outcomes. As a proof of concept, OS has the potential to improve patient enrolment in CTs and to reduce physician workload. Both RB and AI modalities outperformed MM in terms of recall, the most important metric in this context, and time efficiency. RB matching relies on rules derived from actual CT characteristics, requiring costly updates and maintenance to sustain performance. In contrast, AI has the advantage of continuous learning, potentially enhancing its effectiveness over time and offering more scalability. Clinical validation of OS is necessary before its integration into routine practice. To the best of our knowledge, this study is the first to compare CT matching performance of human, RB and AI approaches on the same cohort of PP.

#3654 Comparative efficacy of FOLFIRINOX versus gemcitabine plus nab-paclitaxel in metastatic pancreatic cancer: computing IPTW estimator while limiting patient data exposure with federated learning across 3 institutions.

Quentin Klopfenstein[1], Jean Ogier du Terrail[1], Honghao Li[1], Imke Mayer[1], Nicolas Loiseau[1], Mohammad Hallal[1], Michael Debouver[1], Thibault Camalon[1], Thibault Fouqueray[1], Jorge Arellano Castro[1], Zahia Yanes[1], Laetitia Dahan[2], Julien Taieb[3], Pierre Laurent-Puig[4], Jean-Baptiste Bachet[5], Shulin Zhao[4], Remy Nicolle[6], Jerome Cros[7], Daniel Gonzalez[8], Robert Carreras-Torres[9], Adelaida Garcia Velasco[10], Kawther Abdilleh[11], Sudheer Doss[11], Felix Balazard[1], Mathieu Andreux[1]

[1]Owkin, Paris, France,[2]Department of Digestive Oncology, Hopital la Timone, Marseille, France,[3]GI oncology department Georges Pompidou European Hospital, Universite Paris Cite, CARPEM CCC, APHP, Paris, France,[4]Centre de Recherche des Cordeliers, Sorbonne Universite, Inserm, Universite Paris Cite, Paris, France,[5]Hepatogastroenterology and digestive oncology department, Sorbonne University, Pitie Salpetriere hospital, APHP, Paris, France,[6]Universite Paris Cite, Centre de Recherche sur l'Inflammation (CRI), INSERM, U1149, CNRS, ERL 8252, Paris, France,[7]Department of Pathology, Universite Paris Cite - FHU MOSAIC, Beaujon Hospital, Clichy, France,[8]Federation Francophone de Cancerologie Digestive, Dijon, France,[9]Institut d'Investigacio Biomedica de Girona (IDIBGI), Girona, Spain,[10]Department of Medical Oncology, Catalan Institute of Oncology, Doctor Josep Trueta, University Hospital, Girona, Spain,[11]Pancreatic Cancer Action Network, Manhattan Beach, CA

Background: Two first-line treatments are commonly used for metastatic pancreatic cancer: FOLFIRINOX and gemcitabine plus nab-paclitaxel. However, a direct head-to-head comparison in a randomized clinical trial has not yet been conducted. Collecting enough data for this comparison requires collaboration among multiple partner institutions. Herein we use a privacy enhancing technology called federated learning combined with a novel algorithmic methodology: FedECA. This method aims to facilitate collaboration by enabling institutions to retain ownership of their data, sharing only aggregated information across a network.
Methods: We deployed a federated network across three cancer centers: the Fédération Francophone de Cancérologie Digestive (FFCD) hosting data from two past clinical trials (PRODIGE 35 - NCT02352337, PRODIGE 37 - NCT02827201), the Institut d'Investigació Biomèdica de Girona (IDIBGI) and the Pancreatic Cancer Action Network (PanCAN) that hold data from clinical practice. Patients with metastatic pancreatic adenocarcinoma, a performance status eastern cooperative oncology group (ECOG) no larger than 2, and receiving one of the two treatments as first-line were included. To compare the efficacy of the two treatments, we applied FedECA, the federated version of Inverse Probability of Treatment Weighting (IPTW). The following covariates were used as confounders to estimate the propensity score: age, biological gender, liver metastasis and ECOG score. Missing covariates were imputed using the MissForest algorithms independently on each center. The hazard ratio computed with a propensity score-weighted Cox Model is the estimand of the treatment effect on overall survival. The bootstrap variance estimator is used as advised when using IPTW. The entire analysis was performed without data leaving the servers of the corresponding institutions.
Results: 514 patients were included for the treatment effect estimation (215 FOLFIRINOX and 299 gemcitabine plus nab-paclitaxel). The estimated hazard ratio output by our method, FedECA, is 0.75 (95% CI=0.63-0.92, p=0.005) in favor of FOLFIRINOX over gemcitabine plus nab-placlitaxel. These results show the survival benefit of using FOLFIRINOX over gemcitabine plus nab-paclitaxel. The same analysis conducted in each center independently failed to demonstrate a significant effect likely due to the statistical tests being underpowered.
Conclusion: In this observational study leveraging historical trial data and real-world data, we find that FOLFIRINOX is superior to gemcitabine plus nab-paclitaxel. FedECA is the first method to allow performing IPTW in a federated setting i.e. without pooling data on a central server, facilitating collaboration across institutions and countries while ensuring data privacy.

#3655 Leveraging alternative AIML technologies to identify predictive biomarkers for chemotherapy selection: An analysis of the COMPASS trial evaluating chemotherapy response in advanced PDAC.

Joseph Geraci[1], **Bessi Qorri**[1], Mike J. Tsay[1], Christian Cumbaa[1], Paul Leonchyk[1], Larry Alphs[2], Luca Pani[3]

[1]Netramark Corp., Toronto, ON, Canada,[2]Larry Alphs, Consulting, Princeton, NJ,[3]Miller School of Medicine, University of Miami, Miami, FL

Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal malignancy with limited effective therapeutic options. Gemcitabine plus nab-Paclitaxel (GnP) and FOLFIRINOX (FFX) are commonly used first-line chemotherapies for advanced PDAC. However, disease heterogeneity and late-stage diagnosis remain to result in a poor prognosis. There is a need for predictive biomarkers for selecting patients likely to respond to either regimen. Here, we analyzed data from the COMPASS trial to identify predictive mutational and transcriptional features that can guide treatment selection between GnP and FFX in advanced PDAC patients.

We utilized NetraAI, an alternative artificial intelligence/machine learning (AI/ML) technology to analyze 87 PDAC patients. NetraAI identifies explainable patient personas characterized by a set of 2-4 variables that favor a specific treatment outcome and is capable of learning from smaller data. By focusing on uncovering causal high effect size subpopulations, this approach avoids overfitting and the reinforcement of biases about PDAC. The patient population was categorized into two groups: GnP responders or FFX non-responders (GnPR/FFXNR; n=45) and GnP non-responders or FFX responders (GnPNR/FFXR; n=42). Tumor responses were assessed according to standard criteria (stable disease, partial response, and complete response).

NetraAI identified specific variables that characterize preferential response to either GnP or FFX. A key FFX preferential responder persona (n=26; 16 FFXR/GnPNR, 10 GnPR/FFXNR) was characterized by low expression levels of C3AR1 (<3), MIEF1 (<8), and HOXB6 (<20), demonstrating a significant effect size (Cohen's D=1.086, p=0.00799). A key GnP preferential responder persona (n=28; 17 FFXR/GnPNR, 11 GnPR/FFXNR), was characterized by TRIM25 expression between 22-25, low expression levels of RAB40B (<10), and NUTF2 (<35), with a significant effect size (Cohen's D=1.2, p=0.00294). Additionally, lower levels of Major Vault Protein (MVP) were found to be associated with response to chemotherapy in both regimens, particularly favoring GnP response when combined with other variables.

Our findings suggest that specific transcriptional and mutational biomarkers can predict preferential response to GnP or FFX in advanced PDAC patients. The use of NetraAI enables the identification of patient subpopulations characterized by a small set of variables, paving the way towards personalized treatment selection. These results highlight the potential of AI/ML modeling approaches in enhancing precision medicine for PDAC and could inform clinical decision-making to improve patient outcomes.

#3657 Optimal prescriptive treatments for ovarian cancer with genetic data.

A. Mertzios[1], M. Gjika[1], X. Xu[1], S. Guha[2], N. M. Bardhan[1], S. Kumar[3], A. Belcher[1], G. Perakis[1];
[1]Massachusetts Institute of Technology (MIT), Cambridge, MA, [2]Florida International University, Miami, FL, [3]Temple University, Philadelphia, PA

Among the cancers affecting the female population, Ovarian Cancer (OC), while being relatively rare, is the leading cause of gynecological cancer-related deaths, with overall 5-year survival rates of approximately 50% for all stages combined. This is because of the challenges associated with the diagnosis, resulting in detection at advanced stages of OC, coupled with the slow progress in effective treatment options since the approval of platinum-based chemotherapy in the late 1970s. There has been a relative lack of sophisticated methods based on Machine Learning (ML) models that use genetic data for better prediction of Ovarian Cancer outcomes and result in more effective treatment recommendations. Therefore, there is an unmet clinical need to create models that allow physicians to make informed decisions based on all available data, including patient demographic, social, health, and genomic data. Hence, we develop new techniques for leveraging genetic information in prescribing optimal treatments for patients with OC, using a publicly available dataset from the Prostate, Lung, Colorectal and Ovarian Cancer (PLCO) trial. Our approach is able to transform genotype sequencing information into a simple tabular form that can then be used as the input to any ML model. Coupled with the recorded treatment regimen and clinical parameters of matched patients from the genetic dataset, we estimate the treatment effect in terms of mortality prediction and use it to prescribe the optimal treatment for any given patient. By including the genetic features engineered through our proposed method, our models have a higher accuracy than the models without genetic information embedded. The increase in predictive accuracy demonstrates the improved efficacy of our method in the predictive setting. Furthermore, in the prescriptive setting, the models including genetic features output different treatment choices for patients, showing the impact of their inclusion. This is further highlighted by the feature importance of the genetic features such as mutations in the FAT3, BRCA1, BRCA2, and NF1 genes, where they rank highly with a tighter aggregation of the top features, relative to the sharp drop-off in feature importance after the top feature in the models without genetic data. Taken together, in summary, our models will allow oncologists to make more informed and accurate decisions, incorporating a patient's genetic data with all other available clinical information, which has the potential for improved prognosis and better long-term survival outcomes for Ovarian Cancer patients.

#3658 LINE1 ORF1P is a druggable target for early onset colorectal cancer and cancer prevention.

Dan Zhao[1], Yifei Wang[1], Praveen Neeli[2], Yong Li[1]

[1]Baylor College of Medicine, Houston, TX,[2]University of Texas MD Anderson Cancer Center, Houston, TX

Colorectal cancer is the second most common cause of cancer deaths in the United States. In recent years, there has been a surprising increase in early-onset colorectal cancer, making it the leading cause of cancer-related death in young men under 50 years old. With aging as the primary risk factor for human cancers, we argue that anti-aging agents are a viable option for cancer prevention. One common scientific approach in anti-aging is partial cell reprogramming using Yamanaka factors. However, there is concern that activation of stem cell-specific transcription factors may promote tumor formation. Transposable element LINE1 ORF1p expression has been reported to be elevated in both aging and cancer across multiple species and cancer types, making it a promising target to address both aging and cancer simultaneously. Interestingly, we discovered that LINE-1 expression is higher in the serum of early-onset colorectal cancer patients and hypothesize that ORF1p activation accelerates aging and promotes cancer progression. Using artificial intelligence-based small molecule drug screenings from 10 million compounds, we identified two LINE1 ORF1p targeting drugs, B7 and C1, that significantly kill cancer cells but not normal cells in vitro. A surface plasmon resonance (SPR) assay confirmed high binding affinity of B7 and C1 to the ORF1p protein. Consistently, B7 and C1 inhibited retrotransposition using a LINE1-specific retrotransposition reporter assay. When we transplanted ORF1p high-expressing human cancer cells into NSG mice, treatment with B7 or C1 significantly inhibited tumor growth by 57% (C1) and 75% (B7) and improved survival of tumor-bearing mice compared to vehicle control. Additionally, we designed an antibiotic-free nanoplasmid DNA vaccine targeting ORF1p. We confirmed its high expression level in vitro and then performed electroporation of the DNA vaccine using Ichor's TriGrid™ intramuscular electroporation technology. In a prophylactic model, we vaccinated the mice three times before inoculating them with syngeneic mouse colon tumor cells. The vaccination significantly delayed tumor onset and inhibited tumor growth by more than 50%. In conclusion, our data suggest ORF1p as a druggable target and a promising DNA vaccine target for both cancer prevention and treatment in human early-onset colorectal cancer.

#3659 Biology-driven, machine learning-based development of a biomarker to predict response to WEE1 inhibitor Debio 0123.

Jeannette Fuchs[1], Luke Piggott[1], Kristian Urh[2], Matjaz Zganec[2], Eva Lavrenčič[2], Mark Uhlik[2], Carolina Haefliger[1]

[1]Debiopharm International S.A., Lausanne, Switzerland,[2]Genialis Inc., Boston, MA

Introduction: The WEE1 kinase is a central regulator of G2/M and S phase cell cycle checkpoints which is potently activated in response to DNA damage. To date, a number of biomarker candidates have been proposed for WEE1 monotherapy sensitivity, including TP53 alterations, however, there is currently limited clinical evidence that biomarker-positive patient populations are significantly enriched in response to WEE1 inhibitors. Debio 0123 is a WEE1 inhibitor that has previously shown significant preclinical efficacy as a single agent and is currently being investigated in phase 1 clinical studies either as a monotherapy or in combination with different therapies. Herein we present a first-generation digital biomarker utilizing the ResponderID™ framework and the Genialis™ Supermodel to configure a model capable of predicting responders from non-responders to the Debio 0123 WEE1 inhibitor.

Methods: The initial model is based on an extra-trees regression algorithm spanning five biological pathways related to DNA damage response as features. It was primarily trained on public and proprietary datasets to build a foundation DDR biology model, prior to further training on Debio 0123 patient-derived organoid RNA-seq datasets. The model was then tested on Debio 0123 datasets to measure model performance utilizing leave-one-out cross-validation.

Results: The first-generation DDR biomarker showed potential to predict Debio 0123 IC50 values for viability (prediction accuracy of 0.7). Further refinement of optimal activity thresholds, tuning of features by tissue transferability, and establishment of a classifier-based model yielded an improved prediction accuracy (0.8 vs. null model accuracy of 0.5). Furthermore, this optimized model accurately predicted independent PDX model responses. Specifically, the model accurately predicted a non-responder (tumor growth inhibition <5%) in vivo and strikingly, an in vivo responder that was considered a non-responder by ex vivo organoid IC50.

Conclusion: This biology-driven, machine learning-based classifier predicts response to Debio 0123 in patient derived organoid and xenograft models at an accuracy significantly greater than that of a dummy model and serves as a basis for further optimization and retrospective analyses of Debio 0123 clinical studies.

#3660 Machine learning-based combination prediction for Wee1 inhibitor.

Tianduanyi Wang[1], Juho Rousu[2], **Lin Tang**[3]

[1]Aalto University & University of Helsinki, Espoo, Finland,[2]Aalto University, Espoo, Finland,[3]Schrodinger Inc., San Diego, CA

Motivation: Wee1 is the gatekeeper gene that allows proper DNA damage repair (DDR) during the G2/M cell cycle transition. Inhibiting the Wee1 gene will abrogate the DDR mechanism and lead cancer cells with high replication stress to premature catastrophic mitosis and eventually apoptosis. Several Wee1 inhibitors (Wee1i) are under active development in both preclinical and clinical studies. They have shown efficacy in multiple cancer types as monotherapy or as a combination with chemotherapies or targeted therapies. These studies suggest that a broader combination potential for Wee1i should be explored. Machine learning-based methods are promising and efficient approaches to model drug combination in a broader combination space. Here we have integrated two large combination studies and applied a tensor reconstruction based polynomial regression (comboLTR) method to predict the combination effects for Wee1i in expanded combinations. This work supports a machine learning approach in generating novel combination hypotheses in an expanded space from existing experimental data.

Method: Two large published screening studies containing the Wee1i were normalized and integrated. This resulted in a combined screening study of 131 drugs and 53 cell line models. ComboLTR was applied to train drug combination response prediction models using collected known drug combination responses, drug molecular fingerprints, and model genomic features. The model performance was evaluated under different prediction scenarios, including the prediction of missing entries in a dose-response matrix and the prediction of entire dose-response matrices in new cell lines or with unknown drugs. The hyper-parameters were tuned separately for different prediction scenarios to reach the best performance. Three different synergy scores (HSA, Bliss, Loewe) were used to summarize the synergy effects of the predicted drug combinations. The average of the synergy scores was used to evaluate the combination partners for Wee1i.

Results: We integrated drug combination response data from two publications and trained LTR models based on the data. In 5-fold cross-validations for the different prediction scenarios, new entry, new matrix, new drug combo, the model achieved a Pearson correlation of above 0.8 between predicted and measured response data. The final model was trained to predict responses of drug combinations between Wee1i and 130 other drugs in 53 cell lines. Based on these predictions, we calculated synergy scores for each drug combination and cell line triplet and ranked the synergy effect within each cancer type. Several compounds reported to be synergistic with Wee1i in selected cancer types were reproduced in the prediction, including dasatinib in ovarian cancer, SN38 in breast cancer, and CHK1i in ovarian cancer. The model also predicted these compounds as having synergistic effects with Wee1i in potential new cancer types which may be of future interest.

#3661 TeloPred: A machine learning classification webserver for prediction of small molecules as telomerase inhibitors for anti-cancer drug development.

Divpreet Kaur[1], Daman Saluja[2], Madhu Chopra[3]

[1]Medical Biotechnology Lab and Laboratory of Molecular Modeling and Anti-Cancer Drug Development, Dr. B.R. Ambedkar Ctr. for Biomedical Res., Delhi, India,[2]Medical Biotechnology Lab, Dr. B.R. Ambedkar Ctr. for Biomedical Res., Delhi, India,[3]Laboratory of Molecular Modeling and Anti-Cancer Drug Development, Dr. B.R. Ambedkar Ctr. for Biomedical Res., Delhi, India

Introduction: Telomerase activity is upregulated in 85-90% of cancers, making it a critical therapeutic target. However, the development of small-molecule inhibitors targeting telomerase is hindered by structural complexity and limited resources for three decades since its discovery. To bridge the gap, we present TeloPred—a pioneering structure-activity machine learning classification model for predicting small-molecules as telomerase inhibitors, advancing anti-cancer drug discovery.
Methodology: TeloPred was developed using a curated dataset of telomerase inhibitors with IC_{50} values from ChEMBL. After preprocessing, key molecular properties (e.g., molecular weight, TPSA) were calculated using RDKit, and compounds were classified as active/inactive based on a pIC_{50} cutoff of 5.2 nM. Informative features were selected using variance thresholding and Recursive Feature Elimination (RFE) from each of the 12 molecular fingerprints generated with PaDEL software. The dataset was split (80% training, 20% testing), and six machine learning algorithms (e.g., Random Forest, SVC, XGBoost, AdaBoost) were trained, fine-tuned, and evaluated using metrics like accuracy, F1 score and AUC-ROC. The best model underwent external validation with a decoy set, SHAP analysis for interpretability, and screening of a natural compound library. TeloPred will be soon available on a public webserver for global use.
Results: Data preprocessing reduced 388 compounds to 281. Exploratory analysis showed distinct clustering of active/inactive compounds based on properties like molecular weight, aromaticity, TPSA and H-Bond donor and acceptors. Among tested models, Support vector classifier performed best, achieving 87.2% accuracy on the test set and 89% on training, with low false positives/negatives. External validation yielded an enrichment factor of 21, indicating strong predictive strength of model. SHAP analysis revealed aromatic groups, amide linkage, and carbonyl groups as critical for telomerase inhibition. Screening a natural compound library narrowed the search space by 83%, identifying 10 leads with high predicted probabilities and QED scores which are under experimental validation.
Conclusion: TeloPred is the first ML classification model designed to effectively distinguish telomerase inhibitors, enabling efficient virtual screening of large libraries. It minimizes attrition rates compared to traditional high-throughput screening (HTS) methods, offering a powerful tool to accelerate telomerase-targeted drug discovery for cancer treatment. Its availability as a publicly accessible resource further amplifies its impact on global research efforts to drive precision medicine.

#3662 DNA-encoded chemical library screening and machine-learning for hit identification for WD40 repeat targets including oncology targets such as DCAF1, WDR5 and WDR12.

Anthony D. Keefe, Arrash J. Baghaie, Paolo A. Centrella, Matthew A. Clark, Marie-Aude Guie, John P. Guilinger, Jenny Liu, Ryan T. Walsh, Ying Zhang

X-Chem Pharmaceuticals, Waltham, MA

Training machine-learned models using DNA-Encoded Chemical Library screening data “DEL-ML” and using these models to rank compounds in virtual catalogs has been successful for hit identification for a range of individual oncology targets including ER alpha and c-KIT [McCloskey et al 2020]. Here we describe the importance of library quality and diversity, protein quality, screen design, appropriate profile choice, training set preparation and evaluation, promiscuity labeling, chemical representations, modeling algorithms and their tuning in the context of a systematic target class-based evaluation for a range of oncology targets including DCAF1, WDR5 and WDR12. This approach yielded first-in-class, drug-like ligands for multiple targets that were characterized using a range of biophysical approaches including x-ray crystallography.

#3663 A digital health solution for monitoring skin toxicities in cancer treatment.

Ramya sita Palacholla[1], Ali Soltani[1], Josep A. Pous[1], Monika Dutkiewicz-piasecka[2], Debra Carter[3], Elisabeth Piault-Louis[4]

[1]Digital Science, Evinova (AstraZeneca), Durham, NC,[2]Clinical Development, Astrazeneca, Warsaw, Poland,[3]Astrazeneca, Gaithersburg, DC,[4]Digital Science, Evinova (AstraZeneca), San Fransisco, CA

Introduction: Skin toxicities such as maculo-papular rash, xerosis, or pruritis, can hinder the optimal dosing of immunotherapy, chemotherapy, and targeted therapies1-4. Their management is primarily palliative and prompted by patients' report of skin symptoms or impact on their activities of daily living (ADL). A digital product for the collection, transfer and visualization of data relevant to skin toxicities could contribute to early identification and palliative management and ultimately decreased morbidity for patients between clinic visits

Methods: A review of the published literature and clinical expert interviews (n=8) informed the content of a digital product. Behavioral psychology, measurement and data science drove the design of the interfaces and functionalities with a focus on user-engagement and on supporting diversity. Patients (n=20) receiving anticancer therapies and clinicians (oncologists and nurses, n=8) participated in qualitative user testing of the instructions, question flow, response options, educational material and disclaimers to document comprehension and usage.

Results: Key determinants in identifying and reporting skin symptoms include the actual hue of the skin and the body location and surface affected by the skin manifestation(s). The app supports selection of the closest match to the user skin tone; then the patients access tone-matched pictures from an AI-generated library of skin lesions as relevant references to report and rate symptoms severity. A 3D interactive body avatar allows participants to zoom, record and annotate the symptom(s) location and extent. Patient-captured pictures of the skin lesions are automatically anonymized by an AI- photo processing to ensure patient privacy. The HCP portal provides an easy visualization of patient-reported symptoms and pictures within context of ADL impact and patient's skin history. The digital solution includes a symptom-worsening alert to nudge a discussion between the patient and clinician. In addition, patients receive non-medical tips to self-manage non-severe symptoms to prevent higher morbidity. Most patients appreciated personalizing the app based on their skin tone. Content was easy to understand yet adapted on rewording suggestions. Tracking skin symptoms was seen as enabler of more precise discussions with clinicians and the main intrinsic usage driver. Clinicians were positive of the potential for useful insights into patients' skin status. HCPs reflected on a positive tradeoff between lower risk of significant morbidity and additional workload.

Conclusion: The digital solution features several innovative components for reliable and sustained data capture of the onset and evolution of skin symptoms providing relevant insight for the remote monitoring of patient at risk of skin toxicities; Further research will inform its clinical validity and utility.

#3664 SafetyNet: a knowledge graph framework for toxicity management and safety profile prediction.

Miguel Goncalves[1], Mark O'Donoghue[1], Abigail Morrison[1], Heidi Lloyd-Williams[1], Sanjana Singarayer[1], Neki Patel[1], Samad Jahandideh[2], Ken Twomey[1], Krishna C. Bulusu[1]

[1]AstraZeneca UK Limited, Cambridge, United Kingdom,[2]AstraZeneca US, Gaithersburg, MD

Background: Anti-cancer treatments can range from systemic therapy, like chemotherapy, to targeted approaches, such as antibody-drug conjugates. In any case, such therapies cause adverse events (AEs) in cancer patients. In this study, we have developed a dedicated knowledge graph (KG) for AE risk factor prediction - SafetyNet. Specifically, this study aims to deliver class-specific risk factors, inform toxicity management guidance, and elicit a strategy for risk mitigation and monitoring. Ultimately, we aim to devise a standardized framework to monitor AEs in clinical trials.

Methods: SafetyNet was built from synthetic data of 21 oncology trials, which was created from and follows the same distribution as AstraZeneca trial data and is therefore a suitable interim substitute. Real ADC trial data will replace the synthetic data in the near future. In addition, SafetyNet includes biomedical entities and relationships from AstraZeneca's internal Biological Insights Knowledge Graph, with prior knowledge from literature as well as safety intelligence data from OFF-X. We find trends in the data by querying SafetyNet via the personalized PageRank (PPR) method. Briefly, PPR works by simulating multiple random walks in the graph, from which we count the number of visits to each node. Nodes that are visited more often have higher PPR, meaning they are more important to the starting node.

Results: There are different strategies that can be employed to obtain insights. We can, for example, focus on a given AE: for patients that had nausea as an AE of the primary treatment, we found hypertension to be the most frequent comorbidity, with 367 patients affected. To complement this observation, we found that there are 543 concomitant medications that have both nausea and hypertension as a known AE. We can also analyze trends between serious and non-serious cases of a given AE: serious vomiting was more tightly linked to the use of tinzaparin sodium, an antithrombotic drug. Accordingly, pulmonary embolism and deep vein thrombosis were more frequent in patients experiencing serious vomiting vs non-serious. We can equally analyze patients that had a particular concomitant medication: diarrhea, nausea and vomiting were all higher ranked in patients that were taking cisplatin concomitantly with the primary treatment.

Conclusions: By utilizing SafetyNet, we can identify the risk factors associated with AEs for a given ADC as well as their relationship with concomitant medications. By doing these analyses across ADC trials, we gain generalized insights that can help our understanding of AEs in the ADC space. In turn, these insights can be used to create standardized guidelines for ADC toxicity guidance and AE monitoring. Furthermore, SafetyNet brings considerable time saving and human error reduction relative to manually analyzing all the clinical, literature and OFF-X data.

#3665 Insilco evaluation of multiple ubiquitin-specific protease 1 (USP1) inhibitors and selectivity profiling over other USPs using Alphafold.

Prashant Kashinath Bhavar[1], Uday Kumar Surampudi[1], **Appaji Baburao Mandhare**[2], Rupesh Chikhale[3]

[1]VRise Therapeutics Inc., Cambridge, MA,[2]VeGen Therapeutics, Hyderabad, India,[3]Department of Pharmaceutical and Biological Chemistry, School of Pharmacy, University College London, London, United Kingdom

DNA damage triggers cell signaling cascades that mediate repair. This signaling is frequently dysregulated in cancers. The proteins that mediate this signaling are potential targets for therapeutic intervention. Ubiquitin-specific protease 1 (USP1) is one such target, with small-molecule inhibitors already in clinical trials. Here, we used USP1 crystal structures (PDB: 7ZH4 and 9FCJ) to study the comparison of multiple novel USP1 inhibitors alongside the clinical USP1 inhibitor KSQ-4279 and a well-established tool compound, ML323. Our findings indicate that each of these novel USP1 inhibitors binds to the same cryptic site on USP1 as ML323/KSQ4279, yet they induce subtle variations in the protein structure. Notably, one of the novel compounds exhibit additional interactions with D751 and D752 which we hypothesize may contribute to the unique structural disruptions associated with this novel inhibitor. This binding would likely enhance the thermal stability of USP1 significantly, potentially due to the inhibitors occupying a hydrophobic tunnel-like pocket within the USP1 structure. Furthermore, we have successfully employed AlphaFold structures of various USP proteins to demonstrate the selective binding of these novel compounds to USP1, setting them apart from all other known USP's. Our results advance the in silico understanding of the binding modes of these novel ligands and underscore their selective potential. We aim to utilize this data for the design of new USP1 inhibitors.

#3666 Proteomic-based drug sensitivity prediction algorithms: generation, optimization, and assessment.

Robert F. Gruener

University of Minnesota, Minneapolis, MN

Personalized medicine uses genomic information to inform therapeutic decisions. In the past decade, much effort has been made to develop machine learning models to link drug response with large “-omics” expression patterns in pursuit of personalizing medicine. However, until recently, large-scale proteomic datasets have not been widely available and therefore have not often been incorporated into these machine learning methods. Proteomics has the advantage of directly assaying the functional molecular machinery of cells, leading to better understanding of pathway dynamics and quantifying the targets of small molecule inhibitors. Thus, we hypothesize that proteomic data in drug response modeling could offer distinct advantages and better performing drug response models.
In this study, we sought to determine if drug response models built on proteomics data can outperform other transcriptomic or multi-omic based models. To this end, we developed and tested a novel framework to predict drug response using only proteomic data. We leveraged several recent large-scale cell line proteomics datasets from DepMap (CTRP) and Sanger (GDSC) to build machine learning models trained on baseline protein expression and drug sensitivity data. We used robust testing to determine optimal parameters, drug sensitivity processing, while comparing performance across multiple machine-learning methods. Our optimized framework demonstrated strong predictive power across multiple drug classes and cancer types. Moreover, our approach successfully predicted clinical outcomes in independent, publicly available datasets, illustrating its translational potential. It was worth noting that across most therapies, our models performed similarly to gene expression-based approaches in identifying responders to both cytotoxic and targeted therapies. The work to improve and optimize these models are on-going with the intent that these tools will be made available to all proteomic researchers looking to directly develop drug response hypotheses from their proteomic data.

#3667 AI-based models integrating serum proteomics and clinical features to predict immune-related adverse events in NSCLC patients undergoing immunotherapy.

Junho Song[1], Leeseul Kim[2], Donghoon Shin[3], Jongwoo Kim[1], Taegyu Um[1], Allen Cho[4], Jieun Park[1], **Liam IL-YOUNG Chung**[1], Jeeyeon Lee[5], Young Kwang Chae[1]

[1]Northwestern University, Chicago, IL,[2]University of Chicago, Chicago, IL,[3]Metrowest Medical Center, Framingham, MA,[4]Weiss Memorial Hospital, Chicago, IL,[5]Kyungpook National University, Daegu, Korea, Republic of

Background: Immune checkpoint inhibitors (ICIs) improved outcomes in NSCLC treatment, but causing irAEs. Serum proteomic biomarkers combined with clinical data may offer a better approach for toxicity risk prediction.
Methods: To predict five irAE types (pneumonitis, rash, arthritis, thyroiditis, and other adverse events) at multiple time points, pre-treatment serum samples and clinical data (age, gender, smoking history, cancer stage, performance status, and treatment regimen) were analyzed from consecutive 173 NSCLC patients treated with ICI monotherapy or ICI-chemotherapy combination. Serum proteomics was performed via mass spectrometry, and three machine learning models (XGBoost, HistGradientBoosting, and logistic regression) were evaluated using 5-fold cross-validation. Primary outcomes were five toxicity types evaluated at 1, 3, 6 months, and 1 year. Model performance was assessed using the area under the receiver operating characteristic curve (AUC).
Results: The median age was 70.7 years, with 50.9% female and 66.5% adenocarcinoma. 16.2% had never smoked, 68.2% former smokers, and 15.0% current smokers. Performance status (PS) was 0 in 17.9%, 1 in 55.5%, 2 in 20.2%, and 3 in 5.2%. The median tumor mutational burden (TMB) was 5.4 mut/Mb, with PD-L1 negative in 30.1%, positive in 58.4%. Treatment regimens included mono-ICI (35.3%), dual ICI (12.7%), and ICI + chemotherapy (52.0%). 91.3% received palliative treatment. Median overall survival (OS) was 20.9 months, progression-free survival (PFS) was 7.1 months, and toxicity-free survival was 35.2 months. The multimodal analysis demonstrated distinct predictive patterns across toxicity types and timepoints. At 30 days, combined modality approaches performed best: Logistic Regression for pneumonitis (AUC=0.60), HistGradientBoosting for rash (AUC=0.60), and XGBoost for arthritis (AUC=0.60). Feature importance analysis identified KRAS mutation status and smoking history as primary predictors for pneumonitis, ECOG status and histology type for rash, disease stage and treatment regimen for arthritis, and PD-L1 expression for other adverse events. Proteomics-based models performed best at 3 months for pneumonitis (AUC=0.60) and thyroiditis (AUC=0.57). Notably, the HistGradientBoosting model achieved superior performance (AUC=0.75) for overall irAE prediction at 9 months using both proteomics and clinical features, demonstrating the value of integrated multimodal analysis.
Conclusions: The models showed moderate discrimination, with AUCs ranging from 0.556 to 0.75, particularly excelling in early toxicity prediction. These findings support multimodal machine learning for personalized toxicity risk assessment and proactive management.

#3668 AI-based models integrating serum proteomics and clinicopathologic features to predict survival outcomes in NSCLC patients undergoing immunotherapy.

Junho Song[1], Leeseul Kim[2], Donghoon Shin[3], Jongwoo Kim[1], Taegyu Um[1], Allen Cho[4], Jaeyoung Bae[1], **Liam IL-YOUNG Chung**[1], Jeeyeon Lee[5], Young Kwang Chae[1]

[1]Northwestern University, Chicago, IL,[2]University of Chicago, Chicago, IL,[3]Metrowest Medical Center, Framingham, MA,[4]Weiss Memorial Hospital, Chicago, IL,[5]Kyungpook National University, Daegu, Korea, Republic of

Background: Responses to immune checkpoint inhibitors (ICIs) vary among patients with NSCLC. Combining serum proteomics with clinical data offers a novel approach to individualized outcome prediction.
Methods: This study analyzed 148 consecutive NSCLC patients treated with ICI monotherapy or ICI-chemotherapy combinations. Serum proteomics quantified via mass spectrometry were integrated with clinicopathological data (age, gender, smoking history, cancer stage, performance status, tumor mutational burden (TMB), PD-L1 IHC, and treatment regimen). Machine learning models(XGBoost, logistic regression, and multilayer perceptron) were used with single and multimodal approaches and were evaluated using 5-fold cross-validation. Primary outcomes were progression-free survival (PFS) and overall survival (OS) at 90, 180, 270, and 360 days. Performance was assessed using the area under the receiver operating characteristic curve (AUC).
Results: The cohort's median age was 71 years, with 52.0% being female. 14.2% never smoked, 71.0% former smokers, and 14.2% current smokers. Histologically, 68.2% had adenocarcinoma, 27.7% squamous cell carcinoma, and 4.1% other types. Performance status was 0 in 20.3%, 1 in 54.7%, 2 in 17.6%, and 3 in 6.1%. Most patients had stage IV disease (82.1%). Median TMB was 5.4 mut/Mb; PD-L1 >1% in 56.8%, <1% in 29.7%. Treatment regimens included mono-ICI (35.8%), dual ICI (14.2%), and ICI + chemotherapy (50.0%). Median OS and PFS were 19.5 and 7.1 months, respectively. Proteomics-based models outperformed other approaches for PFS prediction, with XGBoost achieving a peak AUC of 0.67 at 3 months and maintaining strong performance through 6 months (LogReg, AUC=0.65) and 9 months (XGBoost, AUC=0.64). For OS prediction, model performance improved over time, reaching the highest AUC of 0.61 with XGBoost using proteomics at 360 days. Risk groups were stratified at 9 months OS using XGBoost regression, leveraging 4,000 highly variable proteomic features identified by mass spectrometry (m/z). Significant differences in PFS between high- and low-risk groups were confirmed by a log-rank test ($p < 0.01$) and Cox proportional hazards analysis (HR = 1.72, 95% CI: 1.18-2.51, $p < 0.01$), with median PFS of 166 days for the high-risk group and 297 days for the low-risk group.
Conclusions: The AI-based models demonstrated moderate to strong discrimination, with AUCs ranging from 0.70 to 0.77 for the best-performing models. These results highlight the potential of multimodal machine learning approaches for personalized prediction of outcomes with immunotherapy in NSCLC.

BIOINFORMATICS / COMPUTATIONAL BIOLOGY / SYSTEMS BIOLOGY / CONVERGENT SCIENCE: Convergence Science Diagnostic and Predictive Approaches
Poster Session

#3672 High-sensitivity CRISPR-Cas12a detection of prostate-specific antigen based on computationally optimized aptamers.

Peizhi Ma[1], Jing Wu[2], Yichen Zhang[3], Fangyue Guo[1], Mengying Liu[1], Shuying Sun[1], Shize Xiao[1], Shanfeng Zhang[4], **Pei Li**[1]

[1]Zhengzhou University School of Basic Medical Sciences, Zhengzhou, China,[2]Department of the First Clinical Medicine, Zhengzhou University, Zhengzhou, China,[3]Henan Institute of Medical Sciences, Zhengzhou, China,[4]Practice Base of Basic Medical Innovation and Entrepreneurship Education in Henan Province, Zhengzhou, China

Background: Prostate cancer (PCa) is one of the most common malignant tumors worldwide. Early detection of PCa via Prostate-Specific Antigen (PSA) blood testing is essential for efficient and accurate screening. However, current diagnostic approaches are costly, labor-intensive, require large sample volumes and have relatively low interference capability. Additionally, the limitations of Systematic Evolution of Ligands by Exponential Enrichment (SELEX) technology often result in PSA aptamers with insufficient affinity, compromising the sensitivity of some aptamer-based detection methods.
Methods: In this study, we developed a PSA detection strategy based on the trans-cleavage mechanism of the CRISPR-Cas12a-aptamer system, integrating computationally optimized PSA aptamers with CRISPR-Cas12a-based technology. A series of experiments were conducted, including the optimization of PSA aptamers, the design of crRNA, the optimization of aptamer concentration, the establishment of the CRISPR-Cas12a-aptamer detection system, and the validation of clinical samples.
Results: Through sequence alignment and structural analysis of existing PSA aptamers, we identified conserved and potential binding sequences, enabling the construction of optimized aptamers. Molecular docking revealed aptamers with lower ΔG values and higher ZDOCK scores, which were selected as candidates. Optimal aptamer concentrations were identified experimentally, and the system's sensitivity and specificity were assessed using PSA standards and non-specific controls. Comparative studies demonstrated enhanced detection sensitivity with optimized aptamers, exhibiting a linear PSA detection range of 1.44-23 ng/ml with steeper slopes and higher R^2 values compared to original aptamers. In the validation of clinical samples, the detection values of this method showed good consistency with the data provided by hospitals.
Conclusions: This study demonstrates the ultrasensitivity and rapidity of the CRISPR-Cas12a-aptamer detection system and presents a robust strategy for improving aptamer affinity through sequence analysis and molecular docking, offering significant potential for diagnostic applications.

#3673 Identifying druggable target in medulloblastoma.

Dongxue Mao[1], Li Zhao[1], Ying Zhu[1], Chunjie Jiang[1], Kyle Smith[2], Paul Northcott[2], Bissan Al-Lazikani[1]

[1]Genomic Medicine, Institute for Data Science in Oncology, The University of Texas MD Anderson, Houston, TX, [2]St. Jude Children's Research Hospital, Memphis, TN

Background: Approximately 15,000 children in the United States are diagnosed with cancer annually. While pediatric cancers boast a 10-year survival rate of approximately 80%, they present unique challenges in drug discovery due to the low frequency of recurrent mutations and limited therapeutic targets. Brain cancers in children, including medulloblastoma, remain the leading cause of cancer-related deaths in this age group. Certain subtypes, such as group 3 medulloblastoma, are still associated with poor outcomes despite advances in therapy. Current treatment strategies, while effective at increasing survival, frequently lead to severe long-term toxicities, emphasizing the critical need to identify drug targets that can reduce toxicity and enhance outcomes.

Method: Our lab has previously demonstrated that AI-based models can successfully facilitate drug discovery in common adult cancers by identifying and prioritizing key druggable molecular vulnerabilities (Zhu Y et al, *Probabilistic graph-based model uncovers previously unseen druggable vulnerabilities in major solid cancers*, bioRxiv 2024.06.04). Building on this success, we applied this approach to medulloblastoma by analyzing 491 whole-genome sequencing and 167 RNA sequencing to identify potential druggable targets, and systemically evaluated the predictions.

Results: A total of 120 genes were identified as significantly recurrently altered in the cohort, including *TERT*, *PTCH1*, *DDX3X*, *KMT2D*, *CTNNB1*, and *KMT2C*. These genes were used to seed our AI-based model to generate patient network and predict molecular vulnerabilities. Predictions were validated using DepMap medulloblastoma cell lines, confirming 15 of the top 50 ranked genes as dependencies, despite no cell line data being used as input or for model training. Unsupervised clustering of the patient data revealed four distinct molecular clusters consistent with the established medulloblastoma subtypes. Differential expression analysis and elastic net regression were employed to identify representative subtype-specific genes. By combining DNA and RNA data, we constructed subtype-specific molecular networks and identified dependencies unique to each subtype.

Conclusions: This study is the first to apply an AI-based model to identify druggable targets in medulloblastoma. By amplifying weak signals within cancer molecular networks, the model successfully prioritized key vulnerabilities and revealed subtype-specific therapeutic opportunities. These findings provide a framework for advancing precision oncology in pediatric brain cancers, with the potential to reduce treatment-related toxicity and improve outcomes for medulloblastoma patients.

#3674 Predicting chromatin organization from cell-free DNA fragmentation patterns.

Ravi Bandaru[1], Zhihan Zhou[2], Hailu Fu[1], Weimin Wu[2], Haizi Zheng[3], Rashmi S. Hegde[4], Li Wang[1], Han Liu[2], Yaping Liu[1]

[1]Department of Biochemistry and Molecular Biology, Northwestern University Feinberg School of Medicine, Chicago, IL,[2]Department of Computer Science, Northwestern University, Chicago, IL,[3]Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH,[4]Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Epigenetic regulation, including the three-dimensional (3D) architecture of the genome, plays a critical role in gene expression and cellular function. Aberrations in chromatin organization are implicated in various diseases, particularly cancer. While Hi-C is the gold standard for mapping chromosomal conformations, it has significant limitations: it requires fixed cells, limiting its capacity for longitudinal monitoring of dynamic chromatin changes. Cell-free DNA (cfDNA), which consists of short DNA fragments released into circulation through apoptosis and necrosis, offers a promising alternative. CfDNA fragmentation patterns are non-random and influenced by underlying epigenetic features, providing a window into chromatin organization *in vivo*.

Here, we hypothesize that the correlation of cfDNA fragmentation patterns between two genomic regions can potentially reflect the 3D chromatin architecture. Since cfDNA fragments *in vivo* are released from multiple cell types, we generated *in situ* Hi-C and matched cfDNA whole-genome sequencing data from a xenograft mice model as the training dataset. We trained a contrastive deep learning model to infer Hi-C contact maps directly from cfDNA fragmentation data. The model demonstrated high accuracy in predicting chromatin compartments and topologically associating domains (TADs) from cfDNA fragmentation patterns, outperforming conventional machine learning approaches. These findings suggest that cfDNA fragmentation can enable the non-invasive and longitudinal monitoring of 3D genome aberrations *in vivo*.

#3675 An integrated approach for simultaneous detection of genomic and epigenetic alterations in blood-based ctDNA analysis.

Qin Tang, Becky Wong, Varahenage Namali Perera, Yu Sun, Nafisa Bulsara, Brian Giacopelli, James Yu, Jason Close, Peter George, Andy Eang, Ashley Linares, Andrew S. Lee, Neil Peterman, Alex Robertson, Nicole Lambert, Yexun Wang

Foundation Medicine, Inc., San Diego, CA

Cancer harbors abnormalities in the genome and the epigenome, and both can serve as biomarkers to facilitate its detection. We developed an integrated multi-omic approach to identify genomic and methylation alterations simultaneously from blood-based circulating tumor DNA (ctDNA) analysis. In contrast to traditional methods of separated lab workflows for each signal modality, our lab workflow generates both genomic and methylation sequencing libraries from the same input sample. During the workflow, a cytosine conversion-resistant analog of the original cell-free DNA (cfDNA) molecule is synthesized such that its genomic sequence is preserved. Following cytosine conversion of the original cfDNA and its analog in a single reaction, methylation and genomic libraries are respectively derived. A new signal modality identification design is used for separation of genomic and methylation libraries during data processing. Our strategy has the benefit of reducing operational complexity while maximizing ctDNA capture efficiency from small quantities of analyte. Our results show that the multi-omic library is efficiently synthesized (52.8% of genomic reads and 47.2% methylation reads after parsing signal modality identifier), and that the genomic library is effectively protected from cytosine conversion (99.98% protection efficiency). Filtered conversion efficiency exceeds 99.9% in CH (where H corresponds to A, T or C) context from the methylation library, supporting high signal integrity in the methylation workflow. Genomic variant calling detected known cancer variants in FFPE tissue and plasma samples (>96% variants in FFPE tissue samples from stage II and III cancers). In a small clinical cohort study, the methylation signal modality detected 40% of stage I, 95% of stage II and 87% of stage III lung cancer plasma samples at 100% specificity without requiring matching tissue. Taken together, our integrated multi-omic approach provides a versatile tool for combined sensitivity and actionability in blood-based ctDNA detection.

#3676 Ultrasensitive protein detection using proximity initiated nucleic acid target amplification with digital biosensing.
Skye Shepherd, Weinan Liu, Brian T. Cunningham

University of Illinois at Urbana-Champaign, Urbana, IL

Proteins are vital biomarkers and provide a real-time dynamic look into cancer progression and disease states. Point-of-care simple diagnostics for rapid, ultrasensitive testing is necessary to study longitudinal changes of protein biomarkers for treatment monitoring, prognosis, or early detection. Proximity assays are a solution-based protein assay using antibody pairs that can achieve high sensitivity, specificity, and be used for high-throughput or multiplexing applications. However, standard proximity assays require complicated design, enzymatic amplification such as polymerase chain reaction (PCR), and thermocycling with expensive laboratory equipment and specialized laboratories. This limits the use for longitudinal monitoring or liquid biopsies. We demonstrate here the Proximity Initiated Nucleic Acid Target Amplification (PINATA) assay, a novel proximity immunoassay for protein detection that can be performed completely at room temperature in a single step using a small, portable, point-of-care benchtop detection instrument. The PINATA assay implements linear amplification using toehold-mediated strand displacement reactions of antibody-oligonucleotide conjugates along with digital detection of individual gold nanoparticle tags for ultrasensitive, rapid protein quantification in the femtomolar range, allowing detection in less than two hours for sub picogram per milliliter concentrations of human interleukin 6, a cytokine biomarker of inflammation that has been linked to certain cancers. These results showcase the promise of entropy-driven strand displacement proximity assays for fast and ultrasensitive point-of-care applications and disease diagnostics.

#3677 Early detection of pancreatic cancer using methylated circulating cell-free DNA of *ZFP30* and *ZNF781*genes.

Ruo-Kai Lin[1], Yao Yu Hsieh[1], Hsieh-Tsung Shen[2]

[1]Taipei Medical University, Taipei, Taiwan,[2]EG BioMed Co. Ltd., Taipei, Taiwan

Background:
Pancreatic cancer (PC) is often diagnosed at advanced stages, leading to limited treatment options and poor survival rates. Current biomarkers, such as CA19-9, have high false-positive rates, with only 4 out of 1,000 abnormal cases confirmed as cancer. Aberrant DNA methylation, a stable and early epigenetic event in tumorigenesis, has emerged as a promising biomarker for early detection and disease monitoring.
Objective:
This study presents a novel blood test analyzing methylated cell-free DNA (mcfDNA) of *ZFP30* and *ZNF781*genes for the early detection of pancreatic cancer. Functional assays were also performed to explore the tumor-suppressive roles of *ZFP30* and *ZNF781* in pancreatic cancer cell lines.
Methods:
ZFP30 and *ZNF781* were identified through analyses of plasma samples from Taiwanese pancreatic cancer patients combined with data from The Cancer Genome Atlas (TCGA). A novel test was developed to assess methylation levels in cfDNA extracted from plasma. Clinical validation was conducted on PC patients and healthy controls, while functional studies assessed the effects of exogenous *ZFP30* and *ZNF781* expression on cancer cell proliferation and migration.
Results:
The methylation levels of *ZFP30* and *ZNF781* were significantly elevated in 87% of pancreatic cancer (PC) tumor tissues compared to adjacent normal tissues in PC patients. Furthermore, these methylation markers were detected in circulating cell-free DNA (cfDNA) in 99% of plasma samples from PC patients, demonstrating an area under the curve (AUC) of 0.96, with a sensitivity of 99% and a specificity of 93.9%. Methylation signals were nearly undetectable in plasma from healthy subjects, and the test accurately identified PC patients across all stages. Comparative analysis demonstrated superior performance over traditional biomarkers such as CA19-9 and CEA. Functional studies showed that exogenous expression of *ZFP30* and *ZNF781*significantly inhibited pancreatic cancer cell proliferation and migration, supporting their roles as tumor suppressor-like genes.
Conclusion:
The methylated cfDNA of *ZFP30* and *ZNF781* offers a highly sensitive and specific biomarker for the early detection of pancreatic cancer. Integrating this blood test into clinical practice could greatly enhance diagnostic accuracy. Additionally, the tumor suppressor-like functions of *ZFP30* and *ZNF781* highlight their potential as therapeutic targets, providing further avenues for pancreatic cancer management and treatment.

#3678 Liquid biopsy fragmentomics approach for the diagnosis of uterine tumors.

Meagan S. Cobb[1], Philippe Jolivet[2], Kristen N. Ganjoo[3], Deirdre A. Lum[3], Matt van de Rijn[3], Joanna Przybyl[4]

[1]Department of Pathology, McGill University, Montreal, QC, Canada,[2]Research Institute of the McGill University Health Centre, Montreal, QC, Canada,[3]Stanford University, Stanford, CA,[4]Department of Surgery, McGill University, Montreal, QC, Canada

Background: Leiomyomas (LM), also known as fibroids, are common benign tumors of the smooth muscle of the uterus that can cause pain, infertility, and abnormal menstrual bleeding. Diagnosing LM is challenging using clinical indications alone, as they share symptoms with leiomyosarcoma (LMS), a rare, aggressive uterine malignancy with a ~50% disease-specific survival. Pre-operative distinction between LM and LMS is difficult because these tumors are rarely biopsied before surgery. We hypothesize that a non-invasive circulating tumor DNA (ctDNA) test based on LMS- and LM-specific molecular markers will provide accurate pre-operative diagnosis and guide appropriate surgical treatment.

Methods: We previously analyzed point mutations and copy number alterations (CNAs) in ctDNA using deep targeted sequencing and shallow whole-genome sequencing (WGS) from plasma specimens of 7 LMS and 12 LM patients (PMIDs: 29463554, 32232185). To build on this, we performed a new analysis of the first 4 nucleotide sequences (4-mer motifs) on the 5' end of the cell-free DNA fragments using shallow WGS data. The frequency of 256 possible 4-mer motifs was calculated using R programming and normalized to total reads in each specimen. Two-class differential analysis identified motifs enriched in LM and LMS (false discovery rate < 0.05).

Results: Our previous studies demonstrated the feasibility of ctDNA detection in LMS and LM patients. In LMS patients, ctDNA was detected in 6 of 7, with >98% specificity; in LM patients, ctDNA was detected in 6 of 12, with 76% specificity. To increase ctDNA detection sensitivity, we incorporated fragmentomics as a new ctDNA marker. Our new analysis identified 66 significantly enriched and 21 significantly decreased motifs in cell-free DNA from LMS patients compared to LM patients. Motifs associated with DNASE1L3 nuclease activity (e.g., CCCA) were significantly decreased in LM compared to LMS patients.

Conclusion: We identified new tumor-specific fragmentomic markers in cell-free DNA from LMS and LM patients. Combining detection of point mutations, CNAs, and fragmentomic patterns could enable a highly sensitive ctDNA assay for accurate pre-operative distinction between LM and LMS. Next, we will validate DNASE1L3 and other nucleases in tissue microarrays of 100+ LM and 200+ LMS specimens by immunohistochemistry, and validate distinct fragmentomic patterns using expanded plasma collections. This research addresses an unmet clinical need for accurate pre-operative diagnosis of uterine tumors.

#3679 Plasma-based multimodal profiling of urothelial carcinoma to characterize tissue-based subtypes.

A. Cauwels[1], E. Levy[1], A. D. Tang[1], V. Cheung[1], R. Gupte[1], A. Tseng[1], T. Qing[1], K. Walter[1], Z. Assaf[2], S. Mariathasan[2], R. Banchereau[2], E. Tabari[1], J. C. Lin[1];

[1]Freenome, Inc., Brisbane, CA, [2]Genentech, South San Francisco, CA

Background

Urothelial carcinoma (UC) is a molecularly and clinically heterogeneous malignancy with notable diversity in patient outcomes and response to treatment such as immune checkpoint blockade. There have been various efforts to identify and enrich for patient populations which benefit most from these immunotherapies. Cell-free DNA (cfDNA) can be utilized as a non-invasive method to detect the extent of residual tumor, and also provide molecular characterization through indirect measures of gene expression. Here, we use a multimodal plasma-based platform on samples from the IMvigor130 trial to explore biomarkers associated with tissue-based UC subtypes, which have been previously shown to have differing responses to checkpoint blockade.

Methods

Plasma samples from baseline metastatic and locally advanced UC patients with known tissue-defined subtypes (n=120) were evaluated with a targeted sequencing approach that characterizes chromatin accessibility and cfDNA methylation. Fragment length and positioning around each transcription start site were used to indirectly infer gene expression. We also identified UC subtype-associated CpG hypermethylation patterns. We trained binary and multinomial regression models utilizing the individual plasma analytes as well as the combined multimodal features to predict the tissue-based subtypes.

Results

The luminal marker KRT20 and basal marker CXCL6 were previously observed to be subtype markers in tissue data; here we show similar associations with subtype using cfDNA inferred gene expression. An analysis of differentially methylated promoters revealed many other subtype-specific genes that show potential in plasma cfDNA for UC classification. We observed concordance in predicted gene activity from both inferred gene expression and methylation for the majority of genes (>75%). We compared these signals to subtypes previously identified by tissue RNA expression, which featured luminal, stromal, immune, or basal characteristics. The classes with the strongest classification performance were luminal, with an AUC of 0.66, and basal, with an AUC of 0.72. These groups are characterized by tumor-intrinsic features, as opposed to the stromal and immune-driven subtypes. Notably, the basal subtype is known to be enriched for responders to PD-L1 inhibition.

Conclusions

Here, we show a proof-of-concept for how a non-invasive multimodal cfDNA assay can be used to interrogate and recapitulate subtypes of UC. Our findings are aligned to the known biology of tissue transcriptomics-defined subtypes and reveal additional insight into potential plasma biomarkers. Finally, the capacity to subtype UC from a blood-based multiomics approach can be leveraged for the longitudinal monitoring of subtype and disease characteristics over the course of treatment.

#3680 Plasma DNA methylome profiling predicts response to olaparib and durvalumab in recurrent IDH-mutant gliomas: Results from a phase II trial.

Yosef Ellenbogen, Xin Wang, Vikas Patil, Alexander Landry, Justin Wang, Jeffrey Zuccato, Mathew Voisin, Andrew Ajisebutu, Leeor Yefet, Farshad Nassiri, Eric Chen, Gelareh Zadeh

University of Toronto, Toronto, ON, Canada

Background: Combing PARP and Immune Checkpoint inhibition has been proposed as a potentially synergistic strategy in IDH mutant glioma, targeting dysregulated homologous recombination repair pathways. We analyzed the cell-free DNA (cfDNA) methylome of patients in a phase 2 trial using the PARP inhibitor olaparib and the PD-1 inhibitor durvalumab. The primary objective was to evaluate cfDNA methylome as a non-invasive biomarker of response.
Methods: Patients with recurrent IDH-mutant gliomas were enrolled in a phase II open-label study (NCT03991832). Blood samples were collected at baseline and monthly while on treatment. Cell-free methylated DNA immunoprecipitation and high-throughput sequencing (cfMeDIP-seq) was performed. Samples were separated into 50 random discovery and validation sets with an 80:20 split. Binomial regularized regression models were developed for each response class versus others using the discovery set and model performance was assessed using the validation set. Area under receiver operating characteristic (ROC) curve (AUROC) values were calculated for each model in the validation set.
Results: 29 patients (median age 40.5; 41% female) enrolled between January 2020-February 2023. The initial tumor grade was 2 (n=9), 3 (n=8), and 4 (n=12). Patients received olaparib 300 mg twice daily and durvalumab 1500 mg IV every 4 weeks. The overall response rate was 10% (95% CI 2.2-27%) via RANO criteria. 144 plasma samples (from 29 patients) were profiled with cfMeDIP-seq along with 30 healthy controls. The circulating tumour DNA methylome was enriched by normalizing to differentially methylated regions not found within the normal controls. The enriched circulating tumour DNA methylome during response periods exhibited a highly specific signature, accurately discriminating response versus failure (AUC 0.98 ± 0.02). Additionally, on-treatment samples were able to be discriminated from samples off therapy (AUC 0.74 $\pm$ 0.11). Lastly, the specific differentially methylated gene pathways between groups were comprehensively examined in both plasma samples and baseline tumor tissue.
Conclusions: Plasma cfDNA methylome exhibits highly specific signatures that enable accurate prediction of response to olaparib and durvalumab in recurrent IDH-mutant glioma.

#3681 A tumor-naive method for estimating tumor fraction using 5-hydroxymethylation cytosine in cell-free DNA.

Ceyda Coruh[1], Onur Sakarya[2], Yuhong Ning[2], Yuan Xue[2], Michael Cipriano[2], Kyle Hazen[2], Alexander W. Wyatt[3], David Quigley[4], Felix Feng[4], Martin Sjostrom[4], Gulfem Guler[2], Wayne Volkmuth[2], Samuel Levy[1]

[1]ClearNote Health, San Diego, CA,[2]ClearNote Health, San Mateo, CA,[3]Department of Urologic Sciences, Vancouver Prostate Centre, University of British Columbia, Vancouver, BC, Canada,[4]Helen Diller Family Comprehensive Cancer Center, University of California at San Francisco, San Francisco, CA

Accurate measurement of the tumor fraction (TF) in circulating cell-free DNA (cfDNA) allows estimation of tumor burden non-invasively, potentially useful in guiding treatment decisions, monitoring treatment response, and detecting minimal residual disease. 5-hydroxymethylation of cytosine (5hmC) marks activation of cancer drivers and downstream gene targets, and has been shown to positively correlate with gene expression and gene regulation. Here we trained a machine learning (ML) model to predict TF in a tumor-naive way from 5hmC-enriched sequencing data together with low-pass whole genome sequencing data. In cross-validation the ML model showed excellent performance, showing high correlation ($R > 0.8$) and low composite absolute error (CAE < 5%) compared to copy-number variation (CNV) based estimates. The high correlation was confirmed in an independent test set which also showed $R > 0.8$, with only a modest increase in CAE (<10%) using targeted point mutation-based TF estimates as an external reference comparator. Using in silico cancer mixtures that created contrived cfDNA samples targeting concentrations below 10%, our 5hmC-based TF prediction method achieved detection sensitivity of approximately 90% with a specificity of 97%. Finally, we observed that 5hmC-based predicted TF was significantly associated with overall survival ($HR = 1.04$, $p < 0.001$) in an independent prostate patient cfDNA cohort when controlling for other covariates. In summary, we find that the 5hmC-based TF estimate is a reliable metric of tumor burden irrespective of tumor mutational status. The 5hmC-based TF estimate may be used either alone or in conjunction with mutation and CNV-based estimates to provide more accurate measures of survival risk and therapy response during treatment.

#3682 Body mass index impacts blood-based markers signatures in advanced colorectal cancer.

Gabriela Felix[1], Jun Gong[2], Francesca Aguirre[1], Lisa Zhou[1], Aaron Denmark[1], Rocio Ramirez[1], Hayan Lee[3], Megan Hitchins[1]

[1]Department of Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA,[2]Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA,[3]Department of Medical Genetics and Molecular Biochemistry, Fox Chase Cancer Center, Philadelphia, PA

Background: In the USA it is estimated that more than half-million cancer cases are associated with obesity. Colorectal Cancer (CRC) is one of the most common types of cancer, and rising death rates underscores the need for a feasible and tailored algorithm to aid in disease management such as a blood-based test, i.e., liquid biopsy. Here, we investigate a multimodal assessment of blood-based markers, cell-free DNA (cfDNA) methylation signatures, levels of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and Carcinoembryonic Antigen (CEA), and the impact of Body Mass Index (BMI) in CRC cases at presentation.
Method: A multimodal assessment was conducted on sixteen treatment-naive CRC cases. The evaluation consisted of a review of clinical records to collect (i) blood levels of CEA, AST, and ALT, and (ii) BMI data. Additionally, cfDNA methylation analysis was performed by Whole Genome Bisulfite Sequencing (WGBS), and methylated *SEPTIN9* (mSEPT9) by Real-Time PCR. Statistical analysis was conducted on GraphPad and SPSS.
Results: All CRC cases were advanced stages (III and IV), and most had a familial history of cancer. The mean age of diagnosis was 51.50 years (SE ± 3.42). Healthy BMI was observed in most cases (56.3%), followed by overweight/obese (31.3%) and underweight (12.5%). ALT, AST, and CEA levels were abnormal in 25%, 31.3%, and 56.3% of the cases, respectively. Overall cfDNA analysis showed aberrant methylation patterns in all cases, and 75% were mSEPT9 positive. Principal component analysis (PCA) showed a significant correlation among AST (0.93), CEA (0.89), ALT (0.83), mSEPT9 positivity (0.80), and cfDNA overall methylation (0.50) (Kaiser-Meyer-Olkin score 0.60, Bartlett's test $p<0.05$) but negative correlation with BMI (-0.33) (Kaiser-Meyer-Olkin score 0.50, Bartlett's test $p<0.05$).
Conclusion: Blood-based multimodal assessment could be a valuable method for managing CRC clinically. However, there is an inverse correlation BMI and blood markers. This highlights the importance of considering BMI when evaluating liver function and tumor markers in advanced CRC cases.

#3683 AI assisted MRI volumetrics improve recurrent glioblastoma patient stratification following immunotherapy treatment.

Alexander L. Ling[1], Joshua D. Bernstock[1], Erickson Torio[2], Naoyuki Shono[3], Junfeng Liu[4], Ana Montalvo Landivar[5], Nafisa Masud[1], Ethan Chen[1], E. Antonio Chiocca[1]

[1]Neurosurgery, Brigham and Women's Hospital, Brookline, MA,[2]Neurosurgery, Medical College of Wisconsin, Milwaukee, WI,[3]The University of Tokyo, Tokyo, Japan,[4]Neurosurgery, Tongji Hospital, Wuhan, China,[5]Stanford University, Stanford, CA

Background: MRI-based monitoring of tumor progression after treatment plays a critical role in patient care decisions and clinical trial monitoring for glioblastoma patients. However, traditional MRI assessments of glioblastoma tumor burden (i.e. RANO-based criteria) can be significantly confounded by inflammatory pseudo-progression—especially in the context of immunotherapy treatment.
Methods: In an effort to improve MRI assessments of tumor burden in glioblastoma patients, we performed volumetric tumor segmentation of >1000 MRI timepoints from 41 recurrent high-grade glioma patients who underwent oncolytic herpesvirus treatment. Volumetric assessments were correlated with patient outcomes and clinical covariates to assess their utility in stratifying patient outcomes relative to RANO analysis. Furthermore, to reduce the cost and time required for tumor segmentation, we leveraged our dataset and the Brain Tumor Segmentation (BraTS) 2023 challenge dataset to train a convolutional neural network to automate glioblastoma tumor segmentation. Automated segmentation volumes were validated against manually segmented tumor volumes and optimized for high-throughput processing.
Results: While routine RANO assessment of tumor progression in the months following therapy failed to separate short- and long-surviving patients, volumetric assessment enabled clear stratification of patients into short, medium, and long-surviving groups using only a few months of post-treatment imaging follow up. Furthermore, automated segmentation volumes showed a high-concordance with manual segmentation volumes in most patients, with deviations between the two being largely restricted to misclassification of resection cavities. In most cases, such errors could be easily detected and corrected, enabling rapid and accurate segmentation.
Conclusions: Volumetric tumor segmentation significantly improves stratification of recurrent glioblastoma patients following immunotherapy treatment versus traditional RANO classification. While such segmentation is time and skill intensive when performed manually, AI assisted segmentation can result in accurate segmentation volumes with greatly reduced time and skill. Surgical artefacts remain challenging for automated segmentation approaches; however, we expect these limitations to be overcome as we improve our training datasets. We expect this to enable rapid and cheap volumetric analyses capable of improving glioblastoma patient monitoring and, ultimately, providing clinicians with more accurate information when making patient care decisions.

#3684 Integrating multiparametric MRI with spatial transcriptomics to identify "Radio-Spatial Genomic" features of prostate cancer using artificial intelligence..

Thineskrishna Anbarasan[1], Sandy Figiel[1], Sophia M. Abusamra[1], Wencheng Yin[1], Nithesh Ranasinha[1], James T. Grist[2], Dan J. Woodcock[1], Richard J. Bryant[1], Ruth McPherson[1], Freddie C. Hamdy[1], Bartlomiej Papiez[3], Ian G. Mills[1], Alastair D. Lamb[4]

[1]Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom,[2]Department of Academic Radiology, University of Oxford, Oxford, United Kingdom,[3]Big Data Institute, University of Oxford, Oxford, United Kingdom,[4]Barts Cancer Institute, Queen Mary University of London, London, United Kingdom

Introduction: Risk stratification remains a key challenge in prostate cancer (PCa) management involves risk stratification, and identification of the subgroup of patients at highest risk of progressing from localised to metastatic disease is critical. Multiparametric MRI (mpMRI) is key in the PCa diagnostic pathway. By integrating clinical parameters, mpMRI radiomics and spatial transcriptomics (ST), this novel "Radio-Spatial Genomics" platform offers an exciting opportunity to identify mpMRI radiomic features associated with important biological aspects of PCa linked to an aggressive disease phenotype.
Methods: Multi-regional spatial transcriptomics (Visium 10x Genomics) was performed on archived formalin-fixed paraffin-embedded prostatectomy sections from patients recruited to a local trial (ISRCTN10046036). Axial sections were sequenced using ST (8 per patient) from 2 patients with Gleason 4+4 PCa and preoperative mpMRI available was used for this study. Anatomical landmarks on mpMRI were segmented by a radiologist. Using a proportional size algorithm and a convolutional neural network (ProsRegNet), T2-axial MRI slices were aligned and registered to histopathology sections. An application, SpatialStitcher, was developed on Python 3.7.0 to digitally stitch separate ST sections for image registration.
Results: Using the prostatic capsule and urethra as landmarks, histopathology sections from 2 patients were co-registered to corresponding T2-axial MRI slices. In total, a median of 114670 whole transcriptome sequenced barcoded ST spots were co-registered to 30424 pixels on MRI per patient. A median DICE correlation score of 0.942, 0.738 and 0.756 was achieved for capsule, tumour and BPH nodules respectively. AMACR (marker for PCa) expression inversely correlated with T2 MRI intensity-based radiomic features ($r = -0.763$), consistent with the tumour being hypointense. Differential gene expression analysis between hyperintense peri-tumoural and hypointense tumour regions revealed enrichment for genes involved in mucosal immune response.
Conclusion: In this study, we report preliminary results of mapping MRI with ST using machine learning to identify genotypic changes based on radiomics. This novel "Radio-Spatial Genomics" model may allow the detection of clinically relevant genotypic features from diagnostic prostate mpMRI imaging.

#3685 Virtual Gleason score: Towards personalized predictive identification of prostate cancer risk via integration of clinical and imaging data into spatiotemporal, biomechanistically-informed classifiers.

Guillermo Lorenzo Gomez[1], Chengyue Wu[2], Joshua P. Yung[2], John F. Ward[2], Hector Gomez[3], Thomas E. Yankeelov[4], Aradhana M. Venkatesan[2], Thomas J. R. Hughes[4]

[1]University of A Coruna, A Coruna, Spain,[2]The University of Texas MD Anderson, Houston, TX,[3]Purdue University, West Lafayette, IN,[4]The University of Texas at Austin, Austin, TX

The Gleason score (GS) is a fundamental predictor of prostate cancer (PCa) behavior and cancer-specific survival. The GS is determined by analyzing the tumor cell architecture in tissue samples. As PCa exhibits significant heterogeneity within any single tumor, whole-prostate tissue specimens enable a comprehensive assessment of the tumor's GS. However, a biopsy, upon which treatment decisions are made, is inherently prone to sampling bias. Thus, a more accurate method to predict tumor biology and clinical disease progression is needed. To address this unmet critical demand, we propose the concept of virtual Gleason Score (vGS).
The vGS consists of a personalized prediction of the local map of GS over the 3D tumor morphology extracted from MRI and informed by biomechanistic markers that characterize spatiotemporal tumor dynamics. The vGS is obtained through a three-step pipeline hybridizing physics-driven and data-driven models. First, we personalize a biomechanistic model of PCa growth using MRI and serum PSA data collected as part of routine management of the disease. Second, we calculate a panel of biomechanistic biomarker maps probing quantities and mechanisms included in the model that have shown strong correlation with GS. Finally, a machine learning algorithm leverages the local values of these biomechanistic markers to render a spatiotemporally-resolved map of vGS over the tumor geometry.
Here, we present preliminary results in a cohort with 16 PCa cases that were longitudinally monitored with PSA and MRI in the context of active surveillance. The underlying biomechanistic model was the Fisher-Kolmogorov equation, and we used logistic classifiers to build the vGS. Our study shows that local proliferation activity and tumor cell density weighted by the ratio of tumor to prostate volume achieve an AUC of 0.96 as well as optimal sensitivity and specificity of 86.4% s and 90.7% for predicting GS. Despite the coarse nature of the vGS predictions obtained from the biomechanistic model used herein, these forecasts accurately anticipated the emergence of higher GS more than one year earlier than standard PCa monitoring. Future work will seek to advance the biomechanistic and data-driven models to enhance local vGS forecasts, as well as leveraging them to optimize disease management on a patient-specific basis.

#3686 Multi-tensor AI/ML uniquely able to identify mechanistically interpretable and actionable predictors from small-cohort and noisy high-dimensional multi-omic clinical data.

Orly Alter[1], Marissa Coppola[2], Tessa O. House[2], Jessica W. Tsai[2]

[1]University of Utah and Prism AI Therapeutics, Inc., Salt Lake City, UT,[2]Children's Hospital of Los Angeles and University of Southern California, Los Angeles, CA

Two decades after the human genome, prediction in cancer remains limited. The entire multi-ome affects the disease. And, unlike typical artificial intelligence and machine learning (AI/ML), e.g., neural networks and deep learning, our multi-tensor AI/ML is uniquely able to identify predictors from multi-omic clinical data. We have been developing the algorithms, i.e., the “multi-tensor comparative spectral decompositions,” to extend the mathematics that underlies quantum mechanics to overcome the limitations of typical AI/ML. We demonstrated the algorithms in the discovery and validation of novel predictors of survival and response to treatment in, e.g., astrocytoma, including glioblastoma (GBM), brain, lung, nerve, ovarian, and uterine cancers.

The algorithms, first, blindly, i.e., without any assumptions or a-priori knowledge, removed batch effects and separated demographic variations from the data. This enabled identifying, e.g., a predictor in ovarian cancer, which is encompassed within the normally varying X chromosome. Second, the algorithms identified the predictors repeatedly, in federated and imbalanced public datasets from as few as 50-100 patients each, showing that the algorithms are batch- and demographics-agnostic, and the predictors are actionable in the general population. Third, the predictors were interpreted in terms of known and new disease mechanisms and drug targets and combinations of targets to sensitize the tumors to the treatment. The predictors are independent from all standard-of-care indicators, additionally showing that this information that they contain was missing from the understanding and management of cancer. Fourth, the GBM predictor, which is the first biomarker to encompass the whole genome, was prospectively and retrospectively experimentally validated to be the most accurate and precise. All other attempts to associate a GBM tumor’s DNA copy numbers with the patient’s outcome failed. For 70 years, the best indicator has remained age at diagnosis, establishing that the algorithms find what all others miss, and the predictors outperform all others where they exist.

Here, we present recent experiments demonstrating that, fifth, the GBM predictor correctly identified previously unrecognized drug targets, i.e., genes that are required for the tumor’s cell proliferation and viability, further validating the mechanistic interpretability of the multi-tensor AI/ML. Guide RNAs were designed and a lentiviral CRISPR-Cas9 all-in-one vector was harnessed to knock out the candidate genes. Knockout validation at the protein level was performed using Western blot. Knockout in patient-derived GBM cell lines resulted in significantly attenuated cell viability and proliferation.

We conclude that our multi-tensor AI/ML is uniquely suited for personalized medicine.

#3687 Multi-tensor AI/ML discovery and validation of whole-transcriptome predictors of survival in response to immunotherapy from multi-omic clinical data.

Orly Alter[1], David B. Oberman[2], Asaf Zviran[2]

[1]University of Utah and Prism AI Therapeutics, Inc., Salt Lake City, UT,[2]Prism AI Therapeutics, Inc., Salt Lake City, UT

Prediction in cancer remains limited, and drugs continue to fail clinical trials and post-market validation. The entire multi-ome affects the disease, but typical artificial intelligence and machine learning (AI/ML) cannot generate predictors from multi-omic clinical data. Because, first, the data are “skinny,” with orders of magnitude more features than samples. Neural networks and deep learning, e.g., would require ≥3B-patient sets to generate models from the 3B-nucleotide genome alone. Second, batch and demographic variations mask the disease-specific patterns. Third, unavoidable imbalances and unavailable labels obscure the distinctions among subpopulations. Fourth, accuracy and interpretability are usually traded off, but (pre)clinical models need to accommodate both. Fifth, the data are of different types, numbers, and dimensions.

Our multi-tensor AI/ML is uniquely able to identify accurate, precise, actionable, and mechanistically interpretable predictors from multi-omic clinical data. We have been developing the algorithms, i.e., the “multi-tensor comparative spectral decompositions,” to extend the mathematics that underlies quantum mechanics to overcome the limitations of typical AI/ML. We demonstrated the algorithms in the discovery and validation of predictors in, e.g., astrocytoma, including glioblastoma (GBM), brain, lung, nerve, ovarian, and uterine cancers. The algorithms identified the predictors repeatedly, in federated and imbalanced public datasets from as few as 50-100 patients each. The GBM predictor, the first to encompass the whole genome and the first to associate a GBM tumor’s DNA copy-number alterations with the patient’s outcome, was additionally prospectively and retrospectively experimentally validated to be the most accurate and precise predictor of survival and response to treatment. Recent experiments also show that the GBM predictor correctly identified previously unrecognized drug targets, i.e., genes that are required for the tumor’s cell proliferation and viability.

Here, we demonstrate the multi-tensor AI/ML in the discovery and validation of whole-transcriptome predictors of survival in response to atezolizumab PD-L1 inhibitor immunotherapy. The predictors were discovered in pre-treatment metastatic disease profiles from an open-source clinical trial, and validated in primary disease profiles from the Cancer Genome Atlas (TCGA). One predictor is additionally correlated with the response to the treatment, and the other - with the tissue of metastasis. Kaplan-Meier survival analyses and Cox proportional hazards models show that the two predictors outperform the best indicator of response to atezolizumab to date, i.e., tumor mutation burden (TMB).

We conclude that our multi-tensor AI/ML is uniquely suited for personalized medicine.

#3688 Correlation between natural killer cell sensitivity and epithelial mesenchymal transition morphology: A cell morphology based screening for NK cell susceptible tumor cells.

Yin Zou[1], Harrison Ball[1], Yuru Chen[2], Nithya Ramnath[3], Venkateshwar Keshamouni[4], Sunitha Nagrath[1]

[1]Chemical Engineering, University of Michigan, Ann Arbor, MI,[2]Biomedical Engineering, University of Michigan, Ann Arbor, MI,[3]Veteran Affairs, Ann Arbor Healthcare system, Ann Arbor, MI,[4]Internal Medicine, University of Michigan, Ann Arbor, MI

Natural killer (NK) cell-based immunotherapy has demonstrated promising anti-tumor efficacy and safety in multiple early clinical trials against hematological and solid tumors. However, intrinsic NK resistance in tumors has resulted in a variable response to adoptively transferred NK cells. To enhance the treatment efficacy, a personalized approach must be adopted. Current methods for screening NK cell sensitive tumors using transcriptomic or proteomic techniques can be costly and often biased by the limited markers detected. Studies showed that cancer cells undergoing the process of epithelial mesenchymal transition (EMT) increase their vulnerability to NK mediated cytotoxicity. We hypothesize that by utilizing image-based analysis to quantify EM morphology change we can correlate EMT morphology with NK cell sensitivity. To analyze EM morphology, we trained a machine learning model based on cell morphological features using the A549 cell line treated with transforming growth factor-β (TGF- β), which depicts various cell morphology across the EMT spectrum. We optimized an organelle specific and live cell compatible staining panel that targets the nucleus, nucleic acid, cell membrane, and F-actin, which does not rely on antigen presentation. The images were processed via a Cell Painting cell segmentation and feature extraction pipeline. In parallel, we conducted a NK cell cytotoxicity assay on cells undergoing the same treatments and confirmed the EMT status via transcriptomic analysis. Our machine learning model successfully identified the EM status of A549 cells treated with TGF- β for different durations (0, 24, 48, and 72 hours) by assigning each cancer cell a quantitative EM score (zero be most epithelial and one be most mesenchymal). We identified a correlative trend between higher EM scores and increased NK cell sensitivity. The same trend was observed when we evaluated the workflow on various non-small lung cancer (NSCLC) cell lines, including two in-house patient derived CTC cell lines. In conclusion, we have established a quantitative measurement for EM status in NSCLC cancer cell lines and observed a correlation between EM score and NK cell sensitivity. We will extend this work by transferring our EM score model to process clinic patient tumor samples, paving the way for developing a rapid screening method for personalized NK cell therapy.

#3689 Hydrogel-based specimen transfer method enhances compatibility and integration of spatial omics.

Amos Chungwon Lee[1], Sumin Lee[1], **Changhee Lee**[2], Sunghoon Kwon[2]

[1]Meteor Biotech, Seoul, Korea, Republic of,[2]Seoul National University, Seoul, Korea, Republic of

Spatial omics technologies have revolutionized our understanding of biological systems by enabling the molecular mapping of RNA, DNA, and proteins within their tissue context. Despite these advancements, a major limitation of spatial omics tools is their lack of compatibility with traditional methods and with each other. Traditional gold-standard methods such as Hematoxylin and Eosin (H&E) staining on formalin-fixed paraffin-embedded (FFPE) tissue sections and Wright-Giemsa (W&G) staining on hematological smears mounted on standard glass slides are fundamental to routine medical practice, and their widespread use highlights the significant challenge posed by the incompatibility of advanced spatial omics tools with these established protocols. To overcome these challenges, there is a growing practical need for technology that can transfer specimens from one substrate to another while preserving both spatial and molecular information. For tools where extremely fine spatial information, context, and morphology are crucial markers, existing sample transfer techniques such as floating techniques are largely primitive, leading to inadequate preservation of critical data and hampering the effectiveness of spatial omics analyses. Here, we present a novel hydrogel-based technique for transferring samples from standard slides to spatially omics-compatible slides. By comparing the spatial information of samples before and after transfer, as well as consecutive samples, we show that the technique preserves spatial information extremely finely. In addition, we compared proteomics, genomics, and transcriptomics analyses according to the number of transfers, showing that the integrity of molecular information is maintained even after multiple transfers. Furthermore, we demonstrated that it is possible to transfer multiple samples from diverse slides onto a single spatially omics compatible slide. To showcase its applicability, we applied SLACS (Spatially resolved laser-activated cell sorting) to an archival slide containing a rare co-occurring multiple myeloma and acute myeloid leukemia (AML) sample. After transferring the sample to a Cosmoslide, cells were identified using W&G and fluorescent staining, isolated, and sequenced. Whole-genome sequencing linked cytogenetic profiles to clonal variations, uncovering critical changes leading to myeloma and AML cell formation. This hydrogel-based specimen transfer technology bridges the gap between traditional methods and spatial omics tools, enabling seamless integration into clinical and research workflows. It offers a transformative solution for studying rare samples and complex diseases, unlocking new opportunities for spatial omics applications in biomedical research.

#3690 Biomarker Navigator - empowering the augmented scientist in biomarker discovery.

Ioannis Kagiampakis[1], Mishka Gidwani[2], Iaroslav Korkodinov[2], Georgiy Ponomarev[2], Sun Gerald[3], Etai Jacob[3], Damian Bikiel[4]

[1]Oncology Data Science, AstraZeneca US, South San Francisco, CA,[2]Oncology Data Science, AstraZeneca US, Gaithersburg, MD,[3]Oncology Data Science, AstraZeneca US, Waltham, MA,[4]Oncology Data Science, AstraZeneca US, New York, NY

The advent of large language models (LLMs) is poised to revolutionize every domain of the pharmaceutical industry, from early-stage drug development to clinical trial design. By harnessing both public datasets and scientific literature, LLMs offer unparalleled access to critical knowledge for researchers, while proprietary datasets provide a unique competitive edge, enabling organizations to derive exclusive insights and drive innovation. To excel in biomarker development within large organizations, two critical goals must be prioritized: maximizing the extraction of insights from proprietary datasets and ensuring the seamless dissemination of knowledge across the organization. Large Language Models (LLMs) facilitate these goals by connecting expertise across diverse scientific domains and delivering knowledge in natural language. This enables the creation of an “augmented scientist” empowered to make data-driven decisions and generate hypotheses through the integration of cross-disciplinary knowledge. The Biomarker Navigator program exemplifies AstraZeneca’s commitment to this augmented scientist vision through three core components: 1) an automated analysis framework for biomarker identification. 2) a comprehensive repository of standardized, detailed biomarkers insights. 3) an intuitive interface for fast, seamless knowledge access.

AstraZeneca is expanding the Biomarker Navigator by integrating public datasets to make it accessible to the broader scientific community, with an initial focus on renal cell carcinoma (RCC) and melanoma. This dataset expansion includes whole exome sequencing (WES), copy number alterations (CNA), RNA sequencing (RNA-seq), and clinical metadata. The resulting system empowers researchers to seamlessly transition from a high-level overview of the biomarker landscape—within or across datasets—to detailed insights about a single biomarker, ensuring no information is lost. By combining an intuitive user interface with LLM-driven insights, this platform showcases the transformative potential of AI in biomarker discovery, paving the way for a robust, open-source framework for the scientific community in the near future.

#3691 Integrated multiomics unlocks holistic phenotypic insights allowing for deep molecular exploration.

Andrea J. O'Hara[1], Tom Cohen[2], Ethan Stancliffe[2], Adam Richardson[2], David Corney[1], Laure Turner[1], Haythem Latif[1], Ginger Zhou[1]

[1]GENEWIZ from Azenta Life Sciences, South Plainfield, NJ,[2]Panome Bio, St Louis, MO

The omics era has greatly expanded the repertoire of approaches available for researchers and clinicians to unravel the complexity underpinning human health: Next Generation Sequencing (NGS) approaches can characterize genomes, epigenomes, transcriptomes, and proteomes. The analyses are critical to assess in individuals both pre- and post-treatment during therapeutic development and early-stage clinical trials. Peripheral blood mononuclear cells (PBMCs) offer a non-invasive approach that, when combined with omics tools, can provide a near holistic view of immune processes across patient cohorts. For fresh blood draws, this starts with automated sample handling and processing to ensure high viability and yield of PBMCs, along with simultaneous plasma separation and collection, which is then aliquoted for downstream analysis. These PBMC and plasma aliquots were then processed such that whole exome sequencing, whole genome methylation sequencing, single cell and bulk RNA sequencing, Olink Explore HT and Seer proteomic analysis, and metabolomic analysis can all be collected from patient blood draw. Integrated analysis across multiple data modalities allows for holistic views of pathways and processes that are highly impacted, with increased statistical significance than any one modality alone. Inclusion of dual proteomics assays provided a greater breadth of downstream functional results, revealing complementarity between assays. While genomics, transcriptomics, and proteomics provide information about genetic and functional potential, inclusion of metabolomics grants deeper phenotypic insights within and across individuals within a patient cohort. Ultimately, this approach reduces the need for repeated patient collections and lowers bio-storage requirements, making it a more patient-friendly and cost-effective solution, while rapidly producing a diverse set of multiomics results and allows for deep exploration of the molecular underpinnings of cancerous tissue.

#3692 Computational modeling of human microbiome evolution in the context of esophageal adenocarcinoma progression.

C. E. Guccione[1], I. Sfiligoi[1], A. Gonzalez[1], J. Shaffer[2], M. Kazachkova[1], Y. Weng[1], D. McDonald[1], S. Shah[1], S. S. Minot[3], T. Paulson[3], L. Alexandrov[1], R. Knight[1], K. Curtius[1];

[1]University of California San Diego - UCSD, San Diego, CA, [2]Fresno State, Fresno, CA, [3]Fred Hutchinson Cancer Center, Seattle, WA

Elucidating the role of microbes in precancer evolution could transform clinical risk prediction and management strategies. In the case of esophageal adenocarcinoma (EAC) progression, microbial communities have been detected across precancerous and neoplastic stages. However, unlike prior studies that statistically compare microbial abundances at single time-points, we employed mathematical modeling of microbial population dynamics within human hosts to determine likely modes of evolution that drive changes in microbial composition over time.

We first aggregated whole genome sequencing (WGS) datasets from esophageal tissue samples taken across stages of EAC progression, which included healthy esophagus, gastroesophageal reflux disease (GERD), Barrett's esophagus (BE), and EAC. In particular, we analyzed baseline WGS data from a case-control study of patients with BE who later progressed to EAC ("progressors") versus those patients with BE who did not progress to cancer within 5 years ("non-progressors"). We employed a robust bioinformatics host filtration pipeline to remove human DNA reads, mapped remaining reads to a human-scrubbed Web of Life microbial database, and filtered out taxa with low genomic coverage. After extracting occurrence and abundance data for taxa in each disease stage, we applied mathematical models of both neutral and non-neutral microbial evolution. We found evidence of neutral dynamics across stages of normal to pre-cancer (goodness-of-fit to the neutral expectation $R^2 > 0.85$ for normal esophagus and GERD, $R^2 > 0.65$ for BE non-progressors and BE progressors) but not for EAC ($R^2 = 0.14$ in EACs from the International Cancer Genome Consortium, and $R^2 = 0.34$ in a validation set of 19 EACs who also had surrounding BE).

We also found that *Helicobacter pylori* (*H. pylori*), a bacterium inversely associated with EAC risk, deviated significantly from the expected neutral model in BE non-progressor patients but not in BE progressor patients. To test hypotheses that accounted for potential selection effects, we performed simulations for both neutral and non-neutral processes. Using simulated data for the EAC cohorts, we found that assuming widely varying growth rates best reflected the data, implying that selection pressures likely influence largely niche-based population dynamics in the tumor microenvironment. For the BE non-progressor cohort, we found that assuming a lower death rate for *H. pylori* compared to other taxa recapitulated the data. This implies that *H. pylori* may have a selective advantage in non-progressing BE (as it does when present in the stomach), and further studies are needed to understand the impact on EAC progression. Overall, considering metagenomic data as the result of a dynamic process within a human host will enhance our understanding of the human microbiome's role in precancer and cancer evolution.

#3693 Modeling VEGF dynamics in cancer: An evolutionary game theoretic approach.

Ranjini Bhattacharya[1], Anuraag Bukkuri[2], Joel S. Brown[3]

[1]Department of Integrated Mathematical Oncology, Moffitt Cancer Center, Tampa, FL,[2]Department of Computational & Systems Biology, University of Pittsburgh, Pittsburgh, PA,[3]Cancer Biology and Evolution Program, Moffitt Cancer Center, Tampa, FL

Cancer cells in rapidly growing tumors often experience nutrient deprivation and hypoxia. They adapt by upregulating pro-angiogenic factors such as VEGF, which promote blood vessel formation within the tumor. Targeting angiogenesis, therefore, aims to starve cancer cells and inhibit tumor growth. This was the driving principle behind the development of anti-angiogenics like bevacizumab and sunitinib. While anti-angiogenics have shown modest improvements in progression-free survival, patients often relapse within the first year of treatment. This underscores the need to understand “why” and “how” VEGF expression evolves in cancer to better inform therapy.

Empirical, conceptual, and mathematical models of angiogenesis often assume that VEGF expression by cancer cells represents cooperative behavior towards a shared public good. To evaluate this assumption, we develop an evolutionary game theoretic model to explore how nutrient sharing among cancer cells influences VEGF expression. We find that habitats with high nutrient sharing—where nutrients redirected from blood vessels are evenly distributed among cells—support larger cell population sizes. In contrast, in habitats with low nutrient sharing—where nutrients are distributed in proportion to each cell’s investment in VEGF—cells over-invest in VEGF expression in an attempt to outcompete their neighbors. Individual cancer cells are incentivized to increase VEGF expression not to enhance overall blood supply but to pre-empt the blood supply that otherwise would have gone to others. This over-expression of VEGF comes at the cost of lower cell population sizes. This phenomenon, where individual cells prioritize their own fitness over collective welfare, is known as the tragedy of the commons (TOC).

Using the model we evaluate the efficacy of different dosing regimens of anti-angiogenics, glucose transporter (GLUT) inhibitors, and chemotherapy. We find that the TOC diminishes the effectiveness of therapy in habitats with proportional nutrient sharing. Moreover, anti-angiogenic drugs that neutralize VEGF are ineffective at controlling the tumor size. Instead, combining anti-angiogenic drugs with GLUT inhibitors shows greater promise. We are using patient-derived histology samples to observe these trends *ex vivo* and validate the relevance of our findings in a clinical setting. Our modeling efforts highlight: 1) how mathematical models can provide fresh insights into well-studied biological phenomena, 2) how testing alternative hypotheses about the interactions in the tumor microenvironment is important, and 3) how understanding cancer cell traits as adaptations to selective pressures in the tumor microenvironment aids in developing effective therapeutic strategies.

#3694 Clinical validation of a quantitative systems pharmacology (QSP) model of ISB 2001 used for deriving first in human (FIH) dose and efficient phase 1 dose escalation design in relapsed refractory multiple myeloma (RRMM) patients.

Vinu Menon[1], Beata Holkova[1], Lida Pacaud[1], Sunitha GN[2], Andrew Garton[1], Maria Pihlgren[3], Tomomi Matsuura[4], Piet H. van der Graaf[4], Mario Perro[3], Cyril Konto[1]

[1]Clinical Development, Ichnos Sciences Inc, New York, NY,[2]DMPK, Glenmark Pharmaceuticals Ltd, Mumbai, India,[3]Research, Ichnos Sciences Biotherapeutics SA, Lausanne, Switzerland,[4]Applied BioSimulation, Certara UK Limited, Canterbury, United Kingdom

Background: ISB 2001 is the first trispecific antibody that simultaneously targets BCMA and CD38 on MM cells, and CD3 to engage T-cells, forming tumor/immune cell synapses (TICS) that cause tumor cytotoxicity. Since ISB 2001 lacks cross-reactivity to common preclinical toxicology species including monkeys, no preclinical toxicology studies were conducted to inform the FIH dose calculation. The conventional minimal anticipated biological effect level (MABEL) approach for FIH dose derivation for T-cell engagers (TCE) often results in exposing patients to non-therapeutic doses. Instead, we developed a QSP model to predict an optimal FIH dose and efficacious dose range. This informed the design of the ongoing phase 1 dose escalation study of ISB 2001 in RRMM patients (NCT05862012), starting at 5 µg/kg subcutaneously with the initial three single-patient cohorts followed by standard 3+3 cohorts beginning at 150 µg/kg.
Method: A QSP model utilizing binding affinities, target expressions, cell counts, and preclinical pharmacokinetic (PK) data was developed to characterize TICS profiles in the tumor compartment. Normalized TICS formation was linked to tumor cytotoxicity, and the model was calibrated with ISB 2001 *in vivo* efficacy data to enable efficacy predictions. This QSP model was benchmarked with teclistamab's clinical data to derive the minimal pharmacologically active dose (MPAD) of ISB 2001[1]. The MPAD was further adjusted using *in vitro* cytokine secretion data to establish the FIH dose, which was preceded by fractionated step-up doses to mitigate potential cytokine release syndrome concerns. Serum concentration of ISB 2001 across doses up to 1200 µg/kg were quantified using a validated electrochemiluminescence assay and compared with the model predictions.
Results: No CRS was observed in the FIH cohort, and only one grade 1 case was reported at doses up to 15 µg/kg in third cohort, supporting the QSP model-based FIH dose selection while acknowledging the limited sample size. As predicted, objective tumor responses were observed at dose levels as low as 50 µg/kg, just 10-fold higher than the FIH dose. Measured ISB 2001 serum concentration time profiles across multiple dose levels up to 1200 µg/kg (n=20) aligned well with model simulations.
Conclusion: Despite the absence of monkey PK and toxicology data, this QSP-guided approach successfully predicted an optimal FIH dose, clinical PK, safety, and efficacy, while minimizing patient exposure to non-therapeutic doses. With this method, TCEs can be optimized solely for activity against human tumors without requiring cross-reactivity to animal species. Overall, the QSP based FIH calculation approach offers an effective pathway for designing phase 1 studies for future TCEs. References: [1] Lancet. 2021;398(10301):665-674.

#3695 A mechanistic model of ctDNA shedding and its relevance for clinical interpretation.

Thomas Rachman[1], Patrick Wagner[2], William LaFramboise[2], David Bartlett[2], Russell Schwartz[1], Oana Carja[1]

[1]Ray and Stephanie Lane Dept. of Computational Biology, Carnegie Mellon University, Pittsburgh, PA,[2]Allegheny Health Network, Pittsburgh, PA

Circulating tumor DNA (ctDNA) in plasma is a key biomarker of tumor dynamics, yet the quantitative relationship between ctDNA levels and clinical variables—such as tumor volume, vascularization, and cell death—remains unclear. The release of ctDNA is thought to depend on cell death mechanisms and the transport of extracellular tumor DNA (excDNA) to blood vessels. We hypothesized that ctDNA concentrations are modulated by the interplay between proximity to blood vessels and excDNA transport kinetics. To test this hypothesis, we develop a spatial mechanistic model to first predict hypoxia-mediated cell death based on blood vessel distribution and then assess the amount of excDNA reaching blood vessels after release, incorporating diffusion and degradation parameters within empirically plausible ranges. In our model, we find that ctDNA concentration in plasma depends on both vascularization and transport dynamics. For example, when diffusion dominates, ctDNA yield is high, and increasing hypoxic cell death from 0 to 80% leads to a tenfold increase in ctDNA. When degradation dominates, ctDNA yield is much lower and the same change in hypoxia results in a tenfold decrease. When both terms are relevant, ctDNA depends nonlinearly on vascularization. These findings suggest that the relationship between blood vessel proximity and ctDNA is highly sensitive to excDNA transport dynamics, underscoring the importance of experimental validation of key parameters, which may vary significantly according to site and release mechanism.

#3696 Evolutionary immunotherapy in NSCLC: identifying optimal dosing strategies in TIL therapy using agent-based modeling.

Sandhya Prabhakaran[1], Mark Robertson-Tessi[1], Kimberly Luddy[2], Rafael R. Bravo[1], Taylor M. Bursell[2], Julian Pineiro[2], Jeffrey West[1], Megan Johnson[1], Jhanelle A. Gray[3], Amer A. Beg[3], Scott J. Antonia[4], Robert A. Gatenby[5], Alexander R. A. Anderson[1]

[1]Integrated Mathematical Oncology, Moffitt Cancer Center, Tampa, FL,[2]Clinical Science lab, Moffitt Cancer Center, Tampa, FL,[3]Departments of Immunology and Thoracic Oncology, Moffitt Cancer Center, Tampa, FL,[4]School of Medicine, Duke University, Raleigh, NC,[5]Department of Radiation Oncology, Moffitt Cancer Center, Tampa, FL

Purpose: Our study examines tumor growth dynamics and immunosuppression under the presence of immune attack to identify optimal immune pulsing treatment strategies.

Motivation: While immunotherapy has shown remarkable success in treating many cancers like non-small cell lung cancer (NSCLC), not all patients respond well to immunotherapy. This mixed response can be attributed to the complexity of the tumor-immune system dynamics. TIL therapy is an emerging immunotherapy where activated T cells are injected into the patient. This therapy can fail due to tumor-induced immunosuppression, for example via the PDL1/PD1 axis. PDL1 expression has been studied and can increase under IFN-γ, released by activated T cells, but PD-L1 relaxation is not understood. We hypothesize that a) the regimen of TIL therapy administration should impact the dynamics of PDL1, and that b) there may be better strategies of therapy delivery that maximize tumor kill while minimizing immune suppression. We investigate these complex PD-L1 driven dynamics through a unique combination of *in vitro* studies and mathematical modeling.

Methods: We have collected *in vitro* and temporal confocal microscopy images of PD-L1 expression on NSCLC cells, which were either untreated or treated for 48 hours with high dose IFN-γ followed by chronic low dose IFN-γ for 21 days. To reinforce our *in vitro* experiments and findings, we developed a hybrid agent-based model (ABM) to study PD-L1 dynamics. The agents in the ABM are tumor cells having variable PD-L1 expression, and immune cells that secrete IFN-γ and can kill tumor cells. Tumor cells can be randomly- or cluster-seeded. IFN-γ is modeled as either a binary well-mixed pulse (to simulate the *in vitro* experimental setup), or as a diffusible via a reaction-diffusion process (to simulate the tumor-immune cell interactions in an *in vivo* spatial manner). We model the TIL therapy as a pulse of immune cells, given at regular or irregular intervals and with different quantities of cells.

Results: Our ABM is calibrated to capture *in vitro* data dynamics. Under certain combinations of spatial configurations of the tumor and intermittent immune dosing schedules, we observe the presence of ‘sweet spots’ where tumor extinction is possible and immune exhaustion is avoided. This indicates that an appropriate pulsing of TIL therapy may lead to better overall immune efficacy than a bolus injection or continuous immunotherapy, by preventing sustained suppression that increases immune cell exhaustion, despite the potential for tumor regrowth between pulses.

Conclusion: We have built an ABM to study tumor-immune evolution under various pulsing strategies of TIL therapy. Our novel findings can potentially benefit clinical cancer research by giving multiple insights related to the tumor extinction, equilibrium and escape phenomena, and lead to improved schedules in patients receiving immunotherapy.

#3697 Cell-free DNA fragmentome dynamics as a biomarker for immune-checkpoint inhibition in advanced carcinoma.

Linh Nguyen Phuong[1], Frederic Fina[2], Romain Zakrajsek[3], Laurent Greillier[4], Pascale Tomasini[4], Jean-Laurent Deville[5], Audrey Boutonnet[6], Frederic Ginot[6], Jean-Charles Garcia[6], Sebastien Salas[4], Sebastien Benzekry[3]

[1]Aix-Marseille University, Marseille, France,[2]ID-Solutions, Marseille, France,[3]INRIA, Marseille, France,[4]Assistance Publique - Hopitaux de Marseille - Aix-Marseille University, Marseille, France,[5]Assistance Publique - Hopitaux de Marseille, Marseille, France,[6]Adelis Technologies, Labege, France

Background: Immune-checkpoint inhibitors (ICIs) have revolutionized cancer treatment, but a large proportion of patients is resistant to the treatment. Genome-wide cfDNA fragmentation profiles (fragmentome) represent a promising non-invasive approach able to monitor and predict response.
Methods: The SChISM (Size cfDNA Immunotherapies Signature Monitoring) enrolled 128 advanced pan-cancer ICI-treated patients. Using Adelis technologies (France), based on the patented BIABooster system, pre- and on-treatment cfDNA size profiles were generated from the plasma, without prior DNA extraction. First, associations of pre-treatment fragmentome-derived metrics (e.g., concentration within multiple size ranges, position of peaks in the distribution) with early progression (EP, defined as progression at the first imaging evaluation) and progression-free survival (PFS) were assessed using logistic/Cox proportional hazard regression models. To assess predictive performances, data were split into training (n=90) and test (n=38) sets. Then, a mechanistic model for the joint dynamics of cfDNA and tumor lesions at the systemic level was developed. It relies on the following biological hypotheses: 1), wild-type cfDNA is primarily released through the apoptosis of hematopoietic cells, 2) cancer cells can be either resistant or sensitive to treatment, 3) cancer cells release a higher amount of DNA fragments due to the chaotic apoptosis-proliferation cycle characteristics of tumor cells, 4) cfDNA fragments are continuously cleared by liver and kidneys, with half-life ranging from 15min to 2h, 5) immunotherapy induces apoptosis in cancer cells, leading to the release of short cfDNA fragments, and 6) in the tumor microenvironment, cells undergo necrosis as the tumor grows, resulting in the release of longer fragments.
Results; The ratio of long fragments (≥ 1650 base pairs (bp), LF) - normalized by total concentration - was significantly associated with non-EP (odds ratio = 0.3 (95%: 0.16-0.55), $p < 0.001$) and longer PFS ($p<0.001$, hazard ratio 0.44 (95%: 0.31 - 0.64)). It also showed the best predictive performances of EP, on the test set: AUC = 0.74 (95% (CI): 0.65-0.82)), accuracy = 0.71 (95% CI: 0.62-0.77), positive predictive value 0.58 (0.46-0.69). The mechanistic model was able to accurately describe cfDNA-cancer dynamics under ICI, including steady decrease/increase of cfDNA concentration in either the long- or short-size ranges, but also non-trivial patterns, such as initial cfDNA concentration bumps.
Conclusions: High-molecular-weight fragments are associated with early progression in ICI-treated patients. Mechanistic modeling offers biological insights for the interplay between cfDNA and tumor kinetics.

#3698 A longitudinal investigation of temporal/intratumoral heterogeneity by plasma NGS profiling of mutation clones in metastatic colorectal cancer.

Petra Kroupova, Barbora Minarikova, Andrea Chmelarova, **Marek Minarik**

Elphogene, Inc., Prague, Czech Republic

Intratumoral tumor heterogeneity characterized by the presence of genetically and phenotypically distinct cell populations (clones) within the same lesion, represents a significant obstacle to accurate diagnosis, prognosis, and therapeutic targeting based on molecular investigation, when biopsy may yield incorrect results due to insufficient representation of all tumor clones in the sample. Liquid biopsy, defined as the detection and quantification of somatic aberrations of circulating tumor DNA (ctDNA) in blood plasma, has long been considered an approach that can be effectively applied to obtain full spectrum of mutations without any bias. This assumption based on condition that the release of ctDNA fragments occurs quantitatively from all present cell populations.
Our study aims to verify this hypothesis based on continuous liquid biopsies in patients undergoing treatment for advanced solid tumors. In the field of liquid biopsy, the use of ctDNA detection for continuous monitoring of ongoing therapy has recently come to the forefront. The so-called "tumor informed" approach, in which somatic mutations found in tumor tissue are subsequently detected in ctDNA. The procedure is characterized by relatively low costs, which allows repeated examinations based on frequent sampling for continuous monitoring of the disease and treatment. In ctDNA-positive patients, we subsequently perform broad-spectrum longitudinal profiling of mutational clones in ctDNA using an NGS panel based on the principle of hybrid capture of a panel covering a total of 127 genes. This method is used to monitor mutation profiles in ctDNA from plasma of patients with metastatic colorectal cancer undergoing multimodal treatment including surgical resection of metastatic tissue and chemotherapy.
The results indicate that the response to treatment, which is manifested by a decrease or increase in ctDNA represented by different mutations, may not occur homogeneously in all present mutational clones. In fact, some clonal populations appear to indicate earlier response than others, characterized by shifts in mutation fraction dynamics.

BIOINFORMATICS / COMPUTATIONAL BIOLOGY / SYSTEMS BIOLOGY / CONVERGENT SCIENCE: Molecular and Cellular Modeling and Engineering
Poster Session

#3702 Unraveling the mechanism of action for research of cancer therapies using AVITI24.

Vivian Dien, Connor Thompson, Daniel Honigfort, Tristin Rammel, Grace Yeo, Marielle Krivit, Ramreddy Tippana, Jane Yao, Sinan Arslan, Matthew Kellinger, Amirali Kia, Michael Previte

Element Biosciences, Inc., San Diego, CA

For decades, the mechanism of action towards research of cancer therapies have been deciphered by the isolated analyses of transcriptomics, proteomics, or cell morphology to resolve heterogeneous responses in a series of assays requiring multiple biological samples which limit the ability to capture the full picture of the biological response. Integrating these readouts through multiplexed analysis enables a comprehensive understanding of how gene expression relates to functional outcomes and phenotypic effects, offering a holistic view for research of cellular processes impacted by treatment. Tyrosine kinase inhibitors (TKIs) have significantly advanced the treatment of non-small cell lung cancer (NSCLC) yet achieving consistent and durable patient responses remains a challenge. In this research study, we utilized AVITI24 Teton CytoProfiling, a platform that enables spatial co-detection of RNA, protein, phospho-proteins, and cell morphology at subcellular resolution within a single biological sample. This approach allowed us to resolve complex systemic responses to TKIs in three NSCLC cell lines. Specifically, three NSCLC cell lines were treated with Gefitinib or Osimertinib to investigate therapy-induced molecular and morphological changes in both TKI-sensitive and TKI-resistant cells. Single-cell analysis uncovered significant heterogeneity in RNA and protein expression, with distinct patterns emerging between drug-sensitive and drug-resistant cells. For example, in Gefitinib-treated A549 cells, we observed notable pathway inhibition, including reductions in phosY1068-EGFR (Log2FC -0.108) and phosTY-ERK12 (Log2FC -0.237). Conversely, the resistant cell line NCI-H1975 showed minimal changes alongside other distinct responses within the MAPK pathway. Drug-specific morphological changes further highlighted how these therapies influence cellular architecture in ways that correlate with functional outcomes. And by resolving cellular heterogeneity at the single-cell level, these insights—often obscured by bulk analyses—shed light on the intricate mechanisms of both therapeutic effects and resistance development. Our findings demonstrate the potential of multi-omics platforms like AVITI24 Teton CytoProfiling to address critical challenges in cancer research. By consolidating transcriptomic, proteomic, and morphological data in a single assay with subcellular spatial resolution, this platform facilitates the resolution of multidimensional mechanisms underlying tumor responsiveness and drug resistance. By bridging molecular and morphology insights, our study provides a powerful framework for studying the intricate dynamics of targeted therapies in NSCLC, paving the way for innovative strategies to enhance cancer treatment and personalize patient care.

#3703 Cell identity revealed by precise cell cycle state mapping links data modalities.

Saeed S. Alahmari[1], Andrew Schultz[2], Jordan Albrecht[2], Vural Tagal[2], Zaid Siddiqui[3], Sandhya Prabhakaran[2], Issam El Naqa[2], Alexander Anderson[2], Laura Heiser[4], Noemi Andor[2]

[1]Department of Computer Science, Najran University, Najran, Saudi Arabia,[2]Moffitt Cancer Center, Tampa, FL,[3]Baylor College of Medicine, Houston, TX,[4]Oregon Health and Science University, Portland, OR

Purpose: Despite advances in single-cell technologies, integrating multimodal data to capture both the genetic and phenotypic dynamics of tumor cells in their microenvironment remains a challenge.
Approach: We leverage variations in cell cycle state as a natural barcode to link data across different modalities. We have demonstrated the feasibility of this method in a gastric cancer cell line (NCI-N87)using two widely different technologies: single-cell RNA sequencing and live-cell imaging. Our approach begins with the transfection ofFUCCI probes into cells, followed by acquisition of 3D images using a Leica microscope. These images are then utilized to train a label-freeU-Net-based prediction model (FNet)to infer subcellular organization, specifically for the nucleus, mitochondria, and cytoplasm. FNet achieved correlation coefficients of 0.76, 0.70, and 0.78 for these compartments, respectively. Segmentation masks generated by CellPose enabled cell statistics calculations. We further applied deep learning methods, including a customized convolutional neural network (CNN)and a truncated ResNet50, to distinguish between cells in G1, S and G2M phases of the cell cycle. The CNN achieved an accuracy of 90.67% when using the nucleus alone and 91.63% when using all three compartments (nucleus, mitochondria, and cytoplasm).
Deep features were extracted by removing the classification layer and flattening the convolutional layer feature maps. These features were then employed for cell cycle pseudotime inference. When projecting the features onto pseudotime, we observed a strong correlation between the inferred pseudotime and FUCCI-derived cell cycle states (Pearson r =-0.899, p-value < 1E-6). This finding demonstrates the ability to assign continuous cell cycle states directly from imaging data. Comparing imaging- and sequencing-derived cell cycle pseudotime obtained for the same cell line, we assigned 449 imaged cells to their corresponding 693 sequenced cells, achieving an average resolution of 3.4 cells per sequencing profile and 2.4 cells per imaging profile.
Conclusion: This integration has opened new possibilities for linking genotype (from sequencing)and phenotype (from imaging), providing a forward-looking approach to genotype-phenotype mapping at single-cell resolution. Assigning the transcriptome of a sequenced cell to its closest living relative (still actively growing and expanding), will enable tracing the cellular trajectory in real-time, enabling a deeper understanding of how tumor cells adapt to their microenvironment and evolve over time.

#3704 A semiconductor-based biosensor for sensitive T cell detection and common neoantigen identification in cancer immunotherapy.

Po-Yuan Chen[1], I-Fen Ko[1], Kuan-Hsing Chen[1], Wai-Shan Chung[2], Che-Ming Lin[1], Yuh-Shyong Yang[1], **Tai-Ming Ko**[1]

[1]National Yang Ming Chiao Tung University, Hsinchu, Taiwan,[2]Chang Gung Memorial Hospital, Taipei, Taiwan

Background: Identifying common neoantigens and detecting T cell responses to these antigens are critical for providing therapeutic targets and monitoring treatment efficacy. However, current cancer targeted sequencing panels miss up to 95% of genes corresponding to immunogenic neoantigens, limiting treatment options. Detecting specific T cells responsive to these neoantigens is also challenging due to their low abundance and limitations of existing detection methods.
Objective: We developed an innovative extended-gate field-effect transistor (EG-FET) biosensor for sensitive detection of T cells within heterogeneous immune populations and a comprehensive common neoantigen database to identify widely shared tumor antigens, addressing limitations in molecular profiling and aiding generalized treatment design.
Methods: The EG-FET biosensors leverage semiconductor technology for label-free, real-time detection with high sensitivity. Antibodies targeting T cell surface markers were immobilized on the chip, enabling antigen-antibody interactions to differentiate T cells from other immune cells. Its capability to detect multiple samples simultaneously was assessed, maximizing throughput. We developed a common immunogenic neoantigen database using a pipeline that predicts neoantigens from TCGA data and quantifies their prevalence.
Results: The biosensor detected and differentiated T cells with high sensitivity and specificity. The platform detected as few as 1,000 T cells, significantly reducing sample requirements compared to traditional methods needing 100,000 cells. Detection was completed in under 30 minutes, decreasing processing time and costs. The ability to detect multiple samples simultaneously enhanced efficiency. The common neoantigen database aids in identifying widely shared tumor antigens, improving generalized treatment strategies and expanding coverage of immunogenic targets missed by current sequencing panels.
Conclusion: Combining EG-FET biosensors with a common neoantigen database offers a transformative approach in cancer immunotherapy. By enabling sensitive detection of T cells within heterogeneous immune populations and identifying common neoantigens, the platform addresses tumor heterogeneity and limited treatment options. These advancements pave the way for scalable, efficient, and more effective cancer immunotherapy, offering promising strategies to overcome drug resistance and improve patient outcomes.

#3705 *In silico* modeling of ctDNA shedding using a novel computational pipeline which models tumorigenesis, progression, and metastasis.

Jasmine R. Alvarez[1], Ajay Subramanian[2], Everett J. Moding[2], Erik S. Blomain[3]

[1]Department of Pharmacology, Physiology, and Cancer Biology, Thomas Jefferson University, Philadelphia, PA,[2]Department of Radiation Oncology, Stanford University, Stanford, CA,[3]Department of Radiation Oncology, Thomas Jefferson University, Philadelphia, PA

Introduction: Liquid biopsies are shifting the paradigm for diagnosis and treatment of many types of cancer. Circulating tumor DNA (ctDNA), defined as small fragments of tumor DNA shed into circulation from tumor cells, represents a major analyte for liquid biopsy approaches. ctDNA has potentially wide translational applications, including early detection of cancer, monitoring disease response, detection of minimal residual disease (MRD), and biological-sampling of tumor biology noninvasively. Although there is significant interest and a growing body of work in applying ctDNA to the clinic, existing studies are generally limited by the access to patient plasma samples. Importantly, computational models are often useful to simulate data in contexts where actual experiments require such precious resources; however, there are currently no existing computational models of tumor ctDNA shedding. In that context, the current study overcomes this limitation and describes a novel bioinformatic pipeline which is able to model ctDNA shedding during tumor initiation, growth, progression, and metastasis.

Methods: We developed a novel method for generating simulated ctDNA next-gen sequencing (NGS) data in a variety of tumor scenarios. To accomplish this, we enhanced a previously-characterized spatial model of tumor growth which generates simulated whole exome sequencing data *in silico* to also generate ctDNA data. Outputs from this spatial model include a list of variants called from the simulated exome data. To generate the simulated ctDNA data, the distribution of these variant allele frequencies (VAF) were split into quantile bins with an associated scale factor. All variants had their mean tumor VAFs divided by their respective scale factors to determine corresponding ctDNA VAFs utilizing a validated “downsampling” technique.

Results: First we utilized the novel pipeline to generate simulated ctDNA in the setting of both a non-metastatic primary tumor and a metastatic tumor. We next validated the fidelity of simulated ctDNA data to real-world biologic data using published datasets of ctDNA from both metastatic and nonmetastatic sarcomas. In that validation analysis, we demonstrated a high degree of similarity between the simulated and published ctDNA data.

Conclusions: Here we demonstrate a novel computational approach to model the paradigm-shifting technology of liquid biopsy completely *in silico*. Our ability to generate simulated data with validated biologic fidelity has potential to augment discovery-based ctDNA studies. Furthermore, the potential of this technology is underscored by the emerging potential of AI-based approaches, digital twin technologies, and simulated clinical trial data as viable synergistic approaches to conventional clinical studies.

#3706 Identifying first-in-class targets for anti-cancer therapy in KRAS mutant NSCLC using the systems biology-based drug discovery platform.

Namhee Kim, Jae Il Joo, Sea Rom Choi, Kwang-Hyun Cho

biorevert Inc., Daejeon, Korea, Republic of

Background: Activating mutations in KRAS are the most common oncogenic drivers, occurring in approximately 30% of non-small cell lung cancer (NSCLC). The G12C mutation, a glycine-to-cysteine mutation at residue 12, is particularly prevalent in NSCLC patients. Developing KRAS inhibitors has long been challenging, but recent FDA approvals of sotorasib and adagrasib for treating KRAS G12C-mutant NSCLC patients represent significant breakthroughs, establishing a new treatment paradigm. However, they are still limited in their clinical applications by low efficacy, side effects, and resistance. Moreover, it is currently unclear how to predict responsive patient subsets and suggest effective therapeutic strategies for clinical application.
Methods: For this, we used our drug discovery platform, MObyDiCK (Mechanistic target Optimization by Discovering and Controlling networK), which incorporates systems biology and AI-based computational analysis to identify first-in-class targets and their regulatory mechanisms. MObyDiCK includes (a) analysis of single-cell omics data, (b) construction of simulation-capable network models, (c) identification of first-in-class targets, and (d) elucidation of their mechanisms based on network control technologies. In addition, we predicted the efficacy and side effects of the identified targets using different public data, and experimentally validated that regulating the identified targets can effectively overcome KRAS inhibitor resistance.
Results: In this study, we utilized public patient single-cell datasets of NSCLC with KRAS mutations to construct corresponding network models using our MObyDiCK platform. We explored specific pseudo-time trajectories to understand the dynamics of KRAS inhibitor resistance, tailoring the analysis to the distinct cancer subpopulations exhibiting resistance potential in each patient. Next, we binarized single-cell transcriptome data by examining the distribution of gene expression values for each individual. We then inferred gene regulatory networks and constructed their mathematical models. Finally, by analyzing dynamic changes within the mathematical models of NSCLC, we identified the combinatorial targets and their mechanisms to overcome KRAS inhibitor resistance in NSCLC. We further predicted the effectiveness of the targets in NSCLC with public data analysis and validated their efficacy through in vitro experiments.
Conclusions: By analyzing single-cell datasets of NSCLC patients with the MObyDiCK platform, we gained a comprehensive understanding of the dynamics of regulatory interactions in KRAS inhibitor-resistant cells and ultimately identified the prominent target genes. With these findings, we will further expand this study on how these targets are related to the normal developmental processes or immune system in NSCLC and other cancer types.

#3708 Targeting the ATG101 And ULK1 complex in pancreatic ductal adenocarcinoma: computational approach.

Raasil R. Basha[1], Vaishnavi Kuppala[2], Ganji Purnachandra Nagaraju[2]

[1]Missouri Southern State University, Joplin, MO,[2]The University of Alabama at Birmingham, Birmingham, AL

Introduction: Pancreatic Ductal Adenocarcinoma (PDAC) is an aggressive malignancy with poor prognosis (five-year survival rate around 13%). PDAC progression mainly depends on autophagy signaling for cell homeostasis. The current study holds significant importance in this context as it aims to identify the interaction between ULK2 and ATG101 and evaluate hydroxychloroquine's effect on the ULK2 and ATG101 complex.

Methods: The proteins ULK2 (Q9QY01, MOUSE) and ATG101 (Q9D8Z6, MOUSE) were retrieved from the UniProtKB database. The domain region of the protein structures was carefully trimmed using BIOVIA Discovery Studio 4.5 Visualizer, and the interacting residues were identified with precision using HawkDock. The ULK2-ATG101 complex was then imported into DimPlot to recognize and display the presence of intermolecular hydrogen bonds. To validate the complex structure, we employed the Desmond Program, Molecular Dynamic (MD) Simulations, Principal Component Analysis (PCA), Root Mean Square Deviation (RMSD), and Root Mean Square Fluctuation (RMSF). To confirm the functional activity of the interaction, we conducted an *in vitro* pulldown assay. All samples were lysed with RIPA buffer, and the lysate (500 µg) was added to 30 µL of agarose beads and anti-ULK2. The samples were resolved on the SDS page and transferred to the PVDF membrane. The membrane was probed with ATG101, and the intensity of protein bands was quantified using the Image J program. Unpaired t-tests were conducted to acknowledge the difference between treated and untreated samples.

Results: The formation of hydrogen bonds between these complexes are Arg19 vs. Glu146 and Glu150; Gln67 vs. Ser160; Arg4 vs. Thr63. Among the top 10 scoring ATG101-ULK2 PPI complex models, the highest binding energy was found at -48.13 kcal/mol. The RSMD graph displayed a stable trajectory, following 80 ns of simulation time using RMSD values ~3.1A to ~3.7A, depicting stabilization during 100 ns for the PPI complex. The pulldown assay results indicated hydroxychloroquine reduced the interaction between the ATG101 and ULK2, suggesting a potential impact on PDAC treatment.

Discussion/Conclusion: Results suggested that the interaction of ATG101-ULK1 was strong, and hydroxychloroquine reduced this interaction. Site-directed mutagenesis is essential to understanding the molecular interaction (ATG101-ULK2) and the role of residues in PDAC.

#3709 Ezekiel: a simulated digital twin for virtual diagnostic biomarker screening.

Aaron Chevalier, Maciej Pacula, Gabrielle N. Barcaskey, MacKenzie S. King, Lauren Cuoco, Brittany Grimes, Lukasz Kornilowicz, Katherine S. Yang, Daniel Salem, Anthony Couvillon, Toumy Guettouche, Troy B. Hawkins

Mercy Bioanalytics, Waltham, MA

Background: Extracellular vesicles (EVs) are released from all stages of tumors and are highly abundant in blood, making them an attractive analyte for cancer screening diagnostics. However, background noise from non-tumor cells presents a significant challenge to cancer-specific signal detection. We have developed a novel assay platform for measuring combinations of surface biomarkers co-localized on EVs that are highly specific for tumor-associated EVs and a computational platform to simulate individual patient EV profiles for the purpose of nominating candidate biomarkers for use in this assay.

Discovery Pipeline: Because the EV-based assay is a multi-marker test, combinatorial expansion of biomarkers presents a significant challenge to experimental validation. To discover biomarker candidates, we simulate performance using a virtual assay of simulated patients using a novel digital twin platform called Ezekiel. In these simulated patients, tumor gene expression profiles from multi-center cancer genomic consortia (e.g. TCGA) are combined with background gene expression from healthy tissue using tumor size and EV abundance estimates to generate a virtual analyte pool. Initial candidate biomarkers are selected based on a carefully curated set of quantitative and qualitative features, and pairs and triplets of biomarkers are ranked by signal-to-noise ratio in a simulated assay to refine a library of targets for screening.

Validation: Biomarkers and biomarker combinations identified by this computational approach are validated experimentally. Individual biomarkers and immunoaffinity capture reagents are first validated using Western blotting and an abbreviated version of the EV-based assay in pooled plasma. Pairs or triplets of biomarkers are then evaluated in the full assay to select top candidates for development. We have confirmed co-localization of these biomarkers using Single Molecule Localization Microscopy (SMLM) and tumor gene co-expression in matched patient tissue and plasma.

Results: We present a novel computationally driven discovery workflow for a single representative cancer indication. Starting from a pool of 4,782 biomarkers, we virtually screened 11,431,372 combinations resulting in 435 candidates, down selected to 145 biomarkers following manual review. 24 biomarkers were selected based on availability of high-quality antibodies, from which 13 biomarkers were identified that show activity with our assay platform. Representative orthogonal validation data is presented demonstrating the computationally-nominated biomarker’s success in enabling discrimination of cancer samples from healthy controls.

#3711 An RNA-binding phosphoprotein twist with BRCT domains in DNA damage repair: computational modeling of nucleolin.

Nitu Farhin, Andriele S. Eichner, Anjana Saxena, Shaneen Singh

Brooklyn College, Brooklyn, NY

Nucleolin (NCL), an RNA binding protein (RBP), and BRCA1, a tumor suppressor protein linked to breast and ovarian cancer susceptibility, colocalize at the sites of DNA damage and collaborate in homologous DNA repair pathways. Even so, the underlying mechanism where NCL and BRCA1 function together during DNA damage repair (DDR) remains unexplored. We utilized in silico approaches to unveil and characterize the shared interactome of BRCA1 and NCL. Our analysis revealed 49 common proteins interacting with BRCA1 and NCL, potentially influencing their functions. These proteins were systematically categorized into five distinct Gene Ontology (GO) annotation classes: DNA repair, RNA-binding, chromatin remodeling, DNA damage, and cell cycle regulation. Here, we predict interaction scenarios between full-length NCL and five BRCT-containing proteins crucial for DNA repair: BRCA1, MDC1, NBN, PARP1, and TP53BP1, to provide molecular insights into the interface. We strongly predict that the N-terminal acidic region of NCL which is also a site of several CK2 and CDK1 phosphorylation sites is involved in interaction with these five BRCT-containing proteins. Our computational models predict that RNA binding domains (RBDs) of NCL remain free to bind RNA or DNA, potentially facilitating its role in DDR. We further present the mutational landscape of the BRCT regions of these five proteins using the COSMIC database and identify the sites that might affect their interactions with NCL. Identifying the common interactome that associates with NCL and BRCA1 duo will facilitate our understanding of the intricate molecular interplay during the DDR. These protein-protein interactions potentially provide targetable interfaces, especially to combat aggressive triple-negative breast cancer where the DNA-repair pathway is predominantly deregulated.

#3712 A computational method for quantifying circulating tumor DNA (ctDNA) in plasma using methylation profiling data.

Ping Lan[1], Xiaowen He[1], Ying Mei[2], Yangjunyi Li[3], Nina Guanyi Xie[3], Liyuan Zhao[3], Zhihui Xu[3], Chen Zhu[3], Yujie Chen[3], Min Li[3], Baoliang Zhu[3], Xiaohui Wu[3], Xueguang Sun[3]

[1]The Sixth Affiliated Hospital, Sun Yat-sen University, Guangzhou, China,[2]Health Medical Center, The Second Affiliated Hospital, Chongqing Medical University, Chongqing, China,[3]Shanghai Weihe Medical Laboratory Co., Ltd, Shanghai, China

Background: Abnormal DNA methylation is a hallmark of tumorigenesis and cancer development, where patients exhibit distinct methylation patterns compared to healthy individuals, referred to as tumor-specific methylation haplotypes. These haplotype markers are widely used for cancer screening and diagnosis. Accurate quantification of ctDNA in cell-free DNA (cfDNA) is important in evaluating the analytical performance of clinical assays. Conventional methods generally employ 120-nt DNA probes to capture both the Watson and Crick strands, assuming that CpGs are either fully methylated or fully unmethylated, and therefore are unbiased. With sufficient sequencing depth, ctDNA quantification can be performed by calculating the proportion of tumor-related methylation haplotype reads. However, in enrichment-capture methods, where DNA probes are designed to specifically target tumor-related signals, quantification of ctDNA can be challenging due to varying enrichment efficiencies across numerous probes. Here, we present a novel computational method for precise quantification of ctDNA in plasma based on methylation profiling data, where tumor-related methylation signals were enriched experimentally.
Methods: We constructed an Enrichment Factor Model (EFM) based on the thermodynamic characteristics of probes that target methylation haplotypes from a panel. The enrichment factor is determined by comparing the true values and observed values generated from multiple reference samples with different haplotype frequencies captured by that panel. This enrichment factor remains stable when applied to unknown samples captured by the same panel, and therefore the model can be used to predict the ctDNA amount in any unknown clinical samples.
Results: 16 reference samples with a variety of haplotype frequencies were generated by mixing the 0% and 100% HCT116 at different ratios. Using 12 reference samples, we were able to calculate the enrichment factor. The remaining 4 samples were used to validate the EFM, accurately predicting their values with an R^2 of 0.99 and a mean squared error (MSE) of 0.0011. We further applied the model to 59 colorectal cancer (CRC) cases and 103 healthy control clinical plasma samples. The estimated ctDNA fraction closely aligned with clinical tumor stages, with higher fractions consistently observed in late-stage patients. Additionally, a clear distinction was observed between cases and controls with a p-val of $4.73\ E^{-6}$.
Conclusion: We developed a computational method to accurately quantify ctDNA fractions using methylation profiling data, specifically optimized for experimental methods that enrich tumor-related signals. Our method offers a reliable tool for ctDNA quantification in cancer detection and monitoring applications.

#3713 Immunoengineering by design: generative AI and high throughput cell screening to develop therapeutic TCR-guided bispecifics against multiple targets.

Alex S. Powlesland, Aaron Sim, Dubravka Pezic, **Valerie Coppard**, Victoria Koullourou, Joshua Meyers, Lewis Cornwall, James Day, Neil Dalchau, Lilly Wollman

Synteny Biotechnology, London, United Kingdom

T cell receptors (TCRs) are a unique class of targeting molecule. Through highly specific interactions with peptide:HLA complexes present on the cell-surface, they offer a means of therapeutically targeting any protein, with this specificity highlighted by their ability to selectively recognize cancer-associated single amino acid mutations. However, identifying TCRs that have the desired specificity toward a target peptide has traditionally been challenging; often screening large numbers of peripheral T cells to find only a handful of potential molecules. Even when a target peptide-specific TCR has been identified, its selectivity becomes crucial in developing a successful therapeutic, with reactivity toward off-target peptides having potentially serious safety implications. To increase the speed and success of TCR discovery, we have developed a in silico TCR design process leveraging recent advances in machine learning for protein sequences combined with high-throughput, rapid cell-based screening, where both specificity and selectivity are considered at the point of design.

Targets for TCR generation are assessed for potential off-target reactivity through a combination of immunopeptidomic data, sequence similarity, and machine learning predicted binding similarity. Large protein language models are fine-tuned on experimental data which captures binding specificities between known TCRs and their peptide ligands, learning meaningful latent representations of both sequences. These trained models are then used to generate TCRs in silico that are optimized for selective target binding, leveraging an energy-based diffusion process that has been specially adapted to handle multiple conditioning. In this way, we can guide the model towards TCR sequences that bind to multiple target peptide-HLA sequence(s), enabling reactivity to heterogeneous neoantigens, cross-HLA recognition and off-target (self-peptide) avoidance. Designed sequences are then synthesized and expressed in a cell-based high throughput screen, providing binding data on up to a million TCR sequences per cycle, enabling further model refinement based on desired binding properties. Our pipeline has been applied to design novel TCRs against public neoantigens derived from the common KRAS mutations at the position 12. We show how a generative model produces TCRs optimized for multiple-antigen specificity, binding to one or more of the target peptide(s) but not self-peptides including the unmutated wild type KRAS protein. The TCRs are optimized for high-affinity interactions to drive development of TCR-guided bispecific therapies.

Combining state of the art generative AI with high throughput screening empowers a TCR design process that considers both selectivity and specificity, opening up the next generation of rapid development of TCR-based therapeutics.

#3714 Microscopic mayhem: Serial killing and other immune cell dynamics captured in situ.

W. Gregory Sawyer[1], Diego Pedro[1], Alfonso Pepe[1], Reginald Atkins[2], Chandler Gatenbee[2], Luis Felipe[2], Carlos Garcia Fernandez[2], Soraya Zorro Manrique[2], Said Cifuentes Maury[2], Athena Tsingelis[2], Duy Nguyen[2], Alexander Anderson[1], Erin George[2], Gilmer Valdes[2], Fredrick Locke[2], Patrick Hwu[2]

[1]Moffitt Cancer Center, Gainesville, FL,[2]Moffitt Cancer Center, Tampa, FL

Critical to advancing immunotherapy and cell therapy in cancer is developing a deeper understanding pf the dynamics of immune cell-mediated cytotoxicity. The results from the multidisciplinary effort reported here include numerous movies of immune cell-mediated cytotoxicity with striking examples of serial killing, foraging, path-tracking, cytokine gradients at tumor margins, and killing dynamics, in some cases revealing peak apoptotic signatures just minutes after T Cell engagement.

In vitro studies of immune cell killing are traditionally performed using time-lapse imaging and biochemical assays, but these methods are often limited by spatial and temporal resolution, throughput, and the ability to extract the dynamics of cellular interactions. This study integrates high-resolution and high-speed laser scanning confocal microscopy with artificial intelligence (AI), and machine learning (ML) approaches to provide a high-resolution data-driven analysis of immune cell killing dynamics in vitro.

We have engineered a perfusion-enabled 3D culture system integrated microscopy to assess cellular dynamics for extended periods of time. Perfusion culture maintains the interstitial flow of liquid culture media, clearing the microenvironment of toxic metabolites and reactive oxygen species. This platform uses a Liquid-Like Solids (LLS) to mimic the transport dynamics of a capillary bed. Integrated microscopy allows in situ quantification of spatiotemporal cytokine concentrations, immune cell tracking, immune cell killing dynamics, and invasion dynamics.

In one example, we used Human CAR T cells with an anti-cd19.28z CAR that included a fluorescent mCherry protein fused to the carboxy terminus of the CAR vs. target cancer cells (Burkitt lymphoma) that express GFP. The media contained a 0.1% concentration of Propidium Iodide as a terminal maker of cell death. Such multi-channel data highlights the critical stages of immune-mediated cytotoxicity; however, the complexity and volume of confocal imaging data necessitates advanced computational tools for analysis.

The ML algorithm used in this study comes from computer vision using object detection, which is a cornerstone technique in medical imaging. We utilized the YOLOv8X model, which is distinguished by its high accuracy and efficiency in object detection tasks. The YOLO (You Only Look Once) architecture is designed to predict both bounding boxes and class probabilities in a single forward pass, making it exceptionally fast and suitable for real-time applications.

Finally, by integrating robotics, imaging, and AI/ML detection we are able to capture immune cells of interest in situ. This integrated analysis of complex imaging data from confocal microscopy has been significantly accelerated, enabling the identification of subtle patterns and dynamic interactions between immune cells and their targets, and the actual collection of cells of interest.

#3715 Disrupting the effects of KRAS in colorectal cancer: Investigation of protein pathways linking KRAS and PRMT5.

Isaac Silverman[1], Aaron Shaykevich[2], Netanel Louloueian[1], Danbee Chae[1], Sanjay Goel[3], Radhashree Maitra[1]

[1]Biology, Yeshiva University, New York, NY,[2]Public Health and Preventive Medicine, SUNY Upstate Medical University, Syracuse, NY,[3]Medical Oncology, RWJBarnabas Health Rutgers Cancer Institute, New Brunswick, NJ

Introduction: Numerous therapeutic methods have been explored to inhibit the oncogenic effects of mutant KRAS. Previous in-vitro and in-silico research in our lab indicated that PRMT5 (Protein arginine methyltransferase), a well studied transcription regulator, is a possible surrogate target through which the negative effects of mutant KRAS can be mediated. Our previous preliminary in-silico data developed potential protein pathways linking KRAS and PRMT5 utilizing digital protein models and molecular dynamics simulations. Our current study expands on the preliminary molecular dynamic simulation results, enhancing the understanding of structural and kinetic components of the proteins' interactions and the strength of the theorized pathways.
Methodology: In-silico: The STRING database was used to determine two complementary pathways connecting KRAS and PRMT5. AlphaFold protein structure files downloaded from UniProt were docked with the ClusPro server. Using PyMOL, proteins were separated by chain identities. The Molecular Dynamics Simulation software GROMACS was used to simulate interactions between proteins in a computational topology regulated by Newton's Laws. GROMACS, gmx_MMPBSA, and XmGrace softwares were used to analyze kinetic and structural components.
In-vitro: 4 cell lines, HCT116 and SW620 (KRAS mutant), and Hke3 and LIM420 (KRAS wild-type), were used in this study. Cells were treated with the PRMT5 inhibitor, GSK, for either 6, 9, 24, or 48 hours. Subsequently, quantitative polymerase chain reaction (qPCR) was performed to determine mRNA expression of (HRAS, SNRPE, CLNS1A, WDR77). The difference in fold change between untreated and treated was calculated at each time point.
Results: The proteins HRAS, SNRPE, CLNS1A, and WDR77 were found to be significant interactors between KRAS and PRMT5, forming two possible pathways abbreviated: K-H-S-C-P and K-H-S-W-P. RMSD data confirmed complex stability. RMSF data of each protein per complex displayed regions of significant interaction. qPCR data indicated altered expression of pathway mRNA in mutant cells as compared to in wild-type.
Conclusion: Targeting the crosstalk between KRAS and PRMT5 may be a suitable method of inhibiting mutant KRAS' oncogenic effects. Inhibition of PRMT5 has confirmed distinct retroactive effects on the pathway proteins' gene expression in mutant vs. wild-type cell lines. Therapeutic advances can be made against KRAS using this surrogate target approach with detailed information of pathway interactions.

#3716 Discovery of macrocyclic peptides TTA-121 as insulin receptor modulators with anticancer potential: An integrated *in-silico* and *in-vitro* investigation.

Veera Chandra Sekhar Reddy Chittepu[1], Poonam Kalhotra[2], Tzayhri Osorio-Gallardo[3], Tzayhri Gallardo-Velazquez[4], Guillermo Osorio-Revilla[5]

[1]Medicine, Harvard Medical School/Brigham and Women's Hospital, Boston, MA,[2]Medical Oncology, Jerome Lipper Multiple Myeloma Disease Center, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA,[3]Departamento de Microbiologia e Immunologia, Facultad de Medicina Veterinaria Y Zootecnia, Universidad Nacional Autonoma de Mexico, Mexico City, Mexico,[4]Departamento de Biofisica, Escuela Nacional de Ciencias Biologicas, Instituto Politecnico Nacional, Mexico City, Mexico,[5]Departamento de Ingenieria Bioquimica, Escuela Nacional de Ciencias Biologicas, Instituto Politecnico Nacional, Mexico City, Mexico

Insulin receptor (INSR), and Insulin-like growth factor (IGF-1R) are significantly similar to each other, and upon activation by ligands increases cell proliferation, differentiation and metastasis. In addition, for the cancer cells to acquire resistance to existing FDA approved one or downstream of it signaling pathways. So, identification of peptides to participate in the INSR/IGF-1R signaling pathway and possess anticancer is one of the upcoming strategies to treat cancer, and diabetes. In this study, we applied bioinformatics tools to analyze structure-activity relationships (SAR) of chemicals or peptides as insulin receptor (INSR) modulators to identify and gain the molecular insights into structural features responsible for INSR modulators. Later, commercially available cyclic peptide libraries are screened to possess the INSR modulator features, followed by molecular docking simulations/molecular dynamics revealed the binding mode, flexibility, and interacting residues of cyclic peptides which were similar to the binding of insulin-to-insulin receptor. To further validate our *in-silico* predictions, we conducted an intrinsic fluorescence assay, that demonstrated peptides like TTA-121 changed emission levels like insulin, mimicking insulin function or participating in insulin signaling pathway. Further, we carried out cancer cell proliferation assays in different cancer models like breast, lung and prostate cancer models to validate the potential of chosen peptides as anticancer agents. Our findings establish TTA-121 as a promising anticancer agent. On going studies are aimed to understand further TTA-121, its derivatives and in combination with standard drugs in clinical care are synergistic or additive and elucidating the mechanism of action. Overall, this study highlights the potential of cyclic peptides and its derivatives as INSR modulators and supports their use as standalone treatments or in combination with existing anticancer drugs would ultimately help in development of new cancer therapeutics to benefit cancer care.

#3718 Characterization of chromosomal instability-related dependencies through cell-specific metric for gene dosage compensation potential.

Peter Szikora, Ivan Fekete, Maria Victoria Ruiz-Perez, Dora Kallai, Csilla Hegedűs, Bence Szalai, **Daniel Veres**

Turbine Simulated Cell Technologies Ltd., Budapest, Hungary

Numerical chromosomal instability (n-CIN) is a defining characteristic of most human cancers with serious implications of large-scale alterations of the genome - such as chromosome arm level copy number changes. These kinds of somatic alterations are frequently targeting oncogenes and tumor suppressors, enriched in cancer driver events in order to shape the transcriptome in favor of tumor progression and proliferation. While oncogenes are mostly associated with copy number gains and tumor suppressors are more frequent in chromosome arm losses, neighboring genes are also affected in both cases. This collateral damage may be able to create synthetic lethal relationships and context-specific dependencies. Cancer cells might be able to avoid partial loss and gain-related lethal effects by triggering different dose compensation mechanisms, occurring at the genomic, epigenetic and post-translational levels, which leads to deviations across copy number and expression profiles.

To propose a metric for dose compensation potential, we analyzed copy number and gene expression profiles of the Cancer Cell Line Encyclopedia in order to characterize cancer cell lines with the ability to compensate dependencies arising from chromosomal losses and gains. Our metric consists of the sum of gene expression residuals from linear model fits, describing how much a cell line tends to under or overexpress genes compared to what we expect from copy number counts. By comparing other publicly available datasets with our results, such as perturbation transcriptomics data from the LINCS-L1000 project and the DepMap genome-wide gene essentiality measurements, we aim to identify CIN-related therapeutic vulnerabilities associated with chromosome arm losses and gains. Besides finding potential target candidates, we show that our method is capable of finding well-known pathway-specific compensation mechanisms. As an example, we show a significant correlation between the activity of the Nonsense mediated decay (NMD) pathway and our proposed compensation potential score (CPS) of cancer cell lines.

We propose our method to be used to detect context-specific dependencies associated with gene dosage compensation, and also able to provide pathway-level evidence - both novel and previously known from the literature.

#3719 Docking analyses of miR-21 with RNA-binding proteins that interact with BRCA1..

Yelyzaveta Khayman, Shaneen Singh, Anjana Saxena

Brooklyn College, Brooklyn, NY

RNA binding proteins (RBPs) are key post-transcriptional regulators that predominantly control gene expression through RNA metabolism. Dysregulation of RBPs is implicated in therapy resistance of breast cancer and targeting key RBPs can be a promising therapeutic approach to reverse this resistance. However, the RBP-related mechanisms in breast cancer are complex, influencing the critical proteins involved in cellular DNA damage response (DDR) at one end and modulating the processing of primary miRNA (pri-miRNA) structures to precursor forms on the other end. Studies strongly demonstrated that a stress-responsive RBP, namely nucleolin (NCL) co-localizes with the tumor suppressor breast and ovarian cancer susceptibility gene (BRCA1) during DDR and regulates the expression of a specific subset of miRNAs (miR-21, miR-221, miR-222, and miR-103) that are causally involved in breast cancer initiation, progression, and drug resistance. Earlier we generated docking scenarios with this subset of miRNAs to demonstrate a role of NCL-RNA-binding domain (RBD) 3 and 4 playing a significant role in such interactions. We also identified a group of RBPs as common interactors of BRCA1 and NCL, namely heterogeneous nuclear ribonucleoprotein A1 **(**HNRNPA1), heterogeneous nuclear ribonucleoprotein D (HNRNPD), polyadenylate binding protein cytoplasmic 1(PABPC1), and TAR DNA-binding protein 43 (TDP-43). Interestingly, miR-21 is revealed as a known predicted target of this subset of RBPs. In this study, we therefore focus on modeling docking scenarios of miR-21 with these four RBPs and assessed NCL-binding to miR-21. In this study, we utilized complete RBD regions of these RBPs to predict models for RBP-miR-21 interactions, to highlight the conserved motifs participating in such an interaction. Next, we propose models of competitive versus synergistic binding of miR-21 with the different RBPs. The computational models generated in this study will provide key insights about the RBP- miR-21 interacting interfaces. Additionally, RBP-BRCA1 collaborations further provides insights about how miR-21 regulation by RBP play a role in defective DNA repair mechanism in breast cancer that could serve as potential for therapeutic intervention.

#3720 Histone dynamics and minor groove width: structural drivers of mutations in *KRAS* and *NRAS*.

Yisrael Wiener[1], Isaac Silverman[1], Sanjay Goel[2], Radhashree Maitra[1]

[1]Biology, Yeshiva University, New York, NY,[2]Medical Oncology, RWJ Barnabas Health Rutgers Cancer Institute of New Jersey, New Brunswick, NJ

Introduction: Cancer development is driven by genetic mutations, particularly in genomic hotspots, which are sequences prone to mutations. This study seeks to understand the structural properties of DNA in these regions, focusing on the minor groove width (MGW), helical parameters (ProT, HelT, roll, EP), and DNA-histone interactions.

Methods and Materials: An expanded hotspot database was developed by including additional information about mutation-associated sequences and structural properties. A control group of random exon sequences was established to serve as a baseline for analysis. Structural differences were assessed using statistical tests to quantify clustering of hotspot sequences with higher mutation counts around specific MGW values. The *KRAS* and *NRAS* genes were selected for molecular dynamics simulations due to their role in oncogenesis. Several simulations were conducted: one each for both the normal *KRAS* and *NRAS* sequences and others representing the most common mutations. The simulations, lasting 100 ns each and using the AMBER99SB-ILDN force field.

Results and Discussion: The average minor groove width (MGW) values observed in the hotspots revealed structural differences that may impact stability. The normal *KRAS* and *NRAS* sequences exhibited a narrower MGW of 5.4 Å, while sequences with common mutations displayed wider MGW values. This variation in MGW may influence stability. Molecular dynamics simulations indicated that the normal *KRAS* and *NRAS* sequences exhibited greater fluctuations compared to its mutated counterparts. The root mean square fluctuation (RMSF) showed that the normal sequence had higher fluctuations around the guanine-containing regions in *KRAS* or the adenine-containing region in *NRAS*. This instability may be linked to the enhanced mutation rates observed in *KRAS* and *NRAS* hotspot regions. Figures illustrating RMSF plots of *KRAS* demonstrated that the normal sequence's interaction with histone proteins was more unstable than that of sequences with common mutations. The presence of guanine appears to introduce significant strain in the DNA-histone interaction, further correlating with the narrower MGW values. This strain may lead to increased mutational frequency, contributing to the oncogenic potential of *KRAS* mutations. For *NRAS*, the introduction of the adenine nucleotide contributed to the instability of the DNA-histone interaction, particularly when the DNA curved closer to the histone.

Conclusion: The simulations provide valuable insights into the dynamics of the *KRAS* and *NRAS* gene, revealing how specific mutations affect DNA stability and interaction with histones. By focusing on the structural factors that predispose certain regions to mutations, this research contributes to a greater understanding of cancer biology.

#3721 The transcriptional signature of KSHV infection in the guinea pig host.

Kiran Fida, Mohammed Tanvir Hussain, Robert Ellis, Brent A. Stanfield

Pathobiological Sciences, Louisiana State University, Baton Rouge, LA

Introduction: Kaposi's sarcoma-associated herpesvirus (KSHV) is a human oncogenic virus implicated in various malignancies, including Kaposi's sarcoma, primary effusion lymphoma, and Multicentric Castleman disease. The study investigates the similarities in gene expression profiles during KSHV infection of human and guinea pig lung cell lines, aiming to uncover conserved host responses and viral mechanisms across species.

Methods: The human lung cell line MRC5 and guinea pig lung cell line JH4 are infected with KSHV and analyzed against non-infected cell lines as controls. RNA sequencing will be done using Illumina NovaSeq6000 platform. Raw data will be applied to the bioinformatic pipeline, which includes quality control with FastQC and MultiQC, adapter and quality trimming with Cutadapt, filtering to the genome of KSHV with STAR, transcript quantification with Salmon tool. Differential expressed genes will be identified using R package DESeq2 with FDR-adjusted p-value < 0.001 and absolute value of log2(FC) >1. Gene Ontology (GO) for functional annotation of genes and KEGG pathway analysis to determine difference in responses of intracellular pathways will be done.

Expected Results: The RNA sequencing and transcriptome comparison between MRC5 and JH4 is expected to produce significant similarities in immune response and viral manipulation of cellular pathways. RNA sequencing should reveal an upregulation of interferon-stimulated genes (ISGs), and antiviral cytokines. Comparative transcriptomic analysis is expected to show a common pattern of dysregulation in genes related to cell cycle arrest, and apoptosis. Species-specific differences in the transcriptional regulation of metabolic pathways, as well as differences in cell-type-specific transcription factors are anticipated. This may influence the overall magnitude and nature of response to KSHV. Overall, this comparative study will enhance our understanding of KSHV-host interactions and could suggests the use of guinea pig as a viable model for KSHV research offering insights into conserved and species-specific antiviral defense mechanisms.

Conclusion: Both human and guinea pig lung cell lines expected to exhibit similar activation of innate immune response genes, particularly interferon-stimulated genes and pro-inflammatory cytokines, highlighting a conserved antiviral defense response across species during KSHV infection. The similarity in gene expression profiles between human and guinea pig cell lines would be able to suggest that JH4 cells may serve as a viable model for studying KSHV infection, with minor species-specific differences providing additional insights into host-virus interactions.

PREVENTION / EARLY DETECTION / INTERCEPTION: The Role of Physical Activity, Diet, Microbiome, Obesity, and Energy Balance in the Cancer Continuum
Poster Session

#3725 Long-term leisure-time physical activity and digestive system cancer risk and mortality.

Yiwen Zhang, Edward L. Giovannucci

Harvard School of Public Health, Boston, MA

Introduction: Accumulating evidence indicates physical activity (PA) protects against digestive system cancer (DSC) risk. However, less is clear about its optimal amount, intensity, joint association with sedentary behavior, or its role in DSC mortality.
Methods: A total of 231,067 adults from 3 prospective US cohorts (Health Professionals Follow-up Study, 1988-2020; Nurses' Health Study [NHS], 1988-2020; NHS II, 1991-2021) were analyzed. Leisure-time PA was assessed biennially via validated questionnaires and expressed in MET-h/week. Time spent sitting watching TV, a widely used surrogate of sedentary behavior, was assessed biennially. DSC encompassed the digestive tract (mouth, throat, esophagus, stomach, small intestine, colon and rectum) and accessory organs (pancreas, gallbladder, and liver).
Results: Over up to 32 years of follow-up, we documented 6537 incident DSC and 3789 DSC deaths. Higher PA levels were associated with lower DSC risk (≥45 vs. <3 MET-h/week: HR [95% CI], 0.82 [0.74-0.92]; P-trend<0.001) and mortality (0.72 [0.62-0.84], P-trend<0.001). The inverse association was evident for both digestive tract and digestive accessory organ cancers. The maximum benefit appears to be reached at approximately 50 MET-hours/week for DSC risk (P non-linearity=0.01) and mortality (P non-linearity<0.001). Moderate and vigorous PA was independently associated with lower DSC risk and mortality. Consistently meeting the PA guidelines was further associated with lower risk of DSC. Participants who were highly active and less sedentary had a 22% lower DSC risk (95% CI, 0.69-0.87) than those who were highly sedentary and less active.
Conclusion: Higher PA, whether moderate or vigorous, was associated with lower DSC risk and mortality. The optimal benefit was observed at approximately 50 MET-hours/week of total PA. Being consistently physically active may further reduce DSC risk. Increasing PA and reducing sedentariness may be strategies for preventing DSC risk and mortality.

Table 1. Association between total physical activity and digestive system cancer risk and mortality.

	Total physical activity, MET-hour/week						
	<3	3 to <7.5	7.5 to <15	15 to <30	30 to >45	≥45	P-linear
Total digestive system cancer risk							
N.case	693	1074	1512	1764	792	702	
MV model	Ref.	0.92 (0.84, 1.02)	0.93 (0.85, 1.02)	0.88 (0.81, 0.97)	0.84 (0.75, 0.93)	0.82 (0.74, 0.92)	<0.001
Digestive tract cancer risk							
N. case	527	801	1154	1327	596	527	
MV model	Ref.	0.92 (0.82, 1.03)	0.96 (0.86, 1.06)	0.90 (0.81, 1.00)	0.86 (0.76, 0.97)	0.84 (0.74, 0.96)	0.008
Digestive accessory organs cancer risk							
N. case	171	276	360	442	200	177	
MV model	Ref.	0.93 (0.76, 1.13)	0.84 (0.69, 1.01)	0.82 (0.68, 0.98)	0.76 (0.61, 0.95)	0.73 (0.58, 0.92)	0.007
Total digestive system cancer mortality							
N. case	389	597	859	1027	486	431	
MV model	Ref.	0.85 (0.74, 0.97)	0.84 (0.75, 0.95)	0.77 (0.68, 0.88)	0.73 (0.63, 0.84)	0.72 (0.62, 0.84)	<0.001
Digestive tract cancer mortality							
N. case	227	333	495	570	281	247	
MV model	Ref.	0.81 (0.69, 0.97)	0.85 (0.73, 1.00)	0.76 (0.65, 0.90)	0.76 (0.63, 0.92)	0.76 (0.62, 0.92)	0.03
Digestive accessory organs cancer risk							
N. case	162	264	364	457	205	184	
MV model	Ref.	0.90 (0.74, 1.09)	0.83 (0.69, 1.00)	0.79 (0.65, 0.95)	0.69 (0.56, 0.86)	0.67 (0.54, 0.85)	<0.001

#3726 Physical activity levels are associated with transcriptomic pathways in colon tumors and tumor-adjacent visceral adipose tissue: Results from the ColoCare Study.

V. M. Bandera[1], C. Himbert[1], E. M. Glenny[2], T. Lin[1], S. Hardikar[1], A. Tan[1], J. Ose[3], V. Damerell[4], P. Erickson[1], R. Viskochil[5], C. Warby[1], O. Aksonova[1], C. Stubben[6], D. Nix[6], K. Boucher[1], P. Schirmacher[4], I. Strehli[1], A. Sanchez[1], J. Jedrzkiewicz[1], L. C. Huang[1], V. Florou[1], J. N. Cohan[1], A. Brobeil[4], H.-U. Kauczor[7], C. Kahlert[4], M. Karchi[8], E. H. Wood[8], D. A. Byrd[9], E. M. Siegel[9], A. T. Toriola[10], D. Shibata[8], C. I. Li[11], J. C. Figueiredo[12], B. Gigic[4], J. Roper[13], S. D. Hursting[2], C. M. Ulrich[1];

[1]University of Utah Huntsman Cancer Institute, Salt Lake City, UT, [2]University of North Carolina at Chapel Hill, Chapel Hill, NC, [3]University of Applied Sciences and Arts, Hannover, Germany, [4]Heidelberg University Hospital, Heidelberg, Germany, [5]University of Massachusetts Boston, Boston, MA, [6]Huntsman Cancer Institute, Salt Lake City, UT, [7]University Hospital Heidelberg, Heidelberg, Germany, [8]University of Tennessee Health Science Center, Memphis, TN, [9]Moffitt Cancer Center, Tampa, FL, [10]Washington University School of Medicine, St. Louis, MO, [11]Fred Hutchinson Cancer Center, Seattle, WA, [12]Cedars-Sinai Medical Center, Los Angeles, CA, [13]Duke University, Durham, NC

Physical activity has consistently been associated with improved colon cancer survival, yet the biological mechanisms for this association are not yet fully understood. We investigated the transcriptome of colon tumors and tumor-adjacent visceral adipose tissue (VAT) in relation to colon cancer patients' physical activity levels.

Methods: Fresh-frozen tumor and VAT samples were collected from 134 stage I-III colon cancer patients recruited at Huntsman Cancer Institute, Utah, and Heidelberg University Hospital, Germany, and profiled with bulk RNA sequencing. Habitual physical activity levels were self-reported by the International Physical Activity Questionnaire and patients were classified as either active (≥7.5 metabolic equivalents per week (MET hrs/week), or 150 minutes/week of moderate to vigorous activity) or as inactive (≤ 2.5 MET hrs/week or ≤1 hour of light activity). To evaluate robustness and consistency of findings, patients were also categorized as ≥7.5 MET hrs/week vs. ≤ 3.5 MET hrs/week. Normalized differentially expressed genes were identified (DESeq2 R package) and analyses were adjusted for age, sex, body mass index (BMI), study site and tumor stage. Gene set enrichment analysis identified enriched pathways within the 2024 Hallmark gene sets. Significance was assessed using false discovery rate *padj* <0.05.

Results: Active patients (n=57) were on average younger (61±14 years vs. 64±14 years), with less disease severity (84% Stage II/III, 95% Stage II/III), lower BMI (26.8±5 kg/m^2 vs. 32.1±7 kg/m^2) compared to inactive patients (n=55). There were 21 and 27 significant gene sets (*padj* <0.05) in colon tumor tissue and VAT, respectively, when comparing active to inactive patients. Colon tumors of active patients displayed downregulation of oncogenic pathways relative to the inactive patients, including epithelial mesenchymal transition and angiogenesis (|Normalized Enrichment Score (NES)|≥1.5, *padj* < 0.05). Tumors from active patients also exhibited downregulation of oxidative phosphorylation (|NES|≥1.5, *padj* < 0.05) relative to inactive patients. VAT of active patients had significantly upregulated oxidative phosphorylation, fatty acid metabolism and glycolysis relative to inactive patients (|NES|≥1.4, *padj* ≤ 0.05). These tumor and adipose gene set results were consistent across the comparison of ≥7.5 MET hrs/week vs. ≤ 3.5 MET hrs/week.

Conclusions: Colon tumors from patients meeting or exceeding physical activity guidelines displayed significant downregulation of oncogenic pathways relative to tumors from inactive patients. Similarly, VAT of active patients had transcriptional profiles promoting metabolic pathways. These findings suggest that physical activity levels meeting or exceeding the current guidelines are associated with favorable gene expression both in tumors and tumor-adjacent VAT.

#3727 Physical activity patters according to demographic, social, and clinical correlates among breast cancer survivors.

M. Kebede[1], C. E. Matthews[2], M. R. Dunn[1], N. R. Burse[1], A. G. Howard[1], K. R. Evenson[1], M. A. Troester[1];
[1]University of North Carolina at Chapel Hill, Chapel Hill, NC, [2]National Cancer Institute, Rockville, MD

INTRODUCTION: Moderate to Vigorous Physical Activity (MVPA) after breast cancer diagnosis is associated with improved survivorship, suggesting the need for interventions to increase MVPA. However, differences in MVPA by race and age among breast cancer survivors are not well described in population-based studies.
METHODS: We analyzed data from Carolina Breast Cancer Study Phase 3 (CBCS3, n=2994, 50% Black) participants to evaluate the trajectory of MVPA up to 18-month post-diagnosis. Participants were classified as having Any MVPA (and Sufficient or Insufficient according to national guidelines) or No MVPA. Associations between MVPA and demographic, social, and clinical variables were estimated using multivariable logistic regression.
RESULTS: At baseline, 84.0% of participants reported Any MVPA pre-diagnosis, which dropped to 55.4% at 6-month post-diagnosis, then rebounded to 85.1% by 18-month post-diagnosis. Among those who had No MVPA pre-diagnosis, 32.5% and 71.0% became active at 6- and 18-month post-diagnosis, respectively. Race was not significantly associated with Any MVPA [OR=1.12, 95%CI (0.90,1.39) Non-black vs. Black], but higher income [OR=1.33, 95%CI (1.02, 1.74) >$30K vs. <15K], lower Body Mass Index (BMI) [OR= 1.30, 95%CI (1.00, 1.73) <25 vs. >30], low area deprivation [OR= 1.35, 95%CI(1.08, 1.67) vs. high], high area assets [OR=1.54, 95%CI (1.23, 1.93) vs. low], and stage I [OR= 1.72, 95%CI(1.22, 2.43) vs. 3 or 4], were associated with Any MVPA at 18-month post-diagnosis.
CONCLUSION: Individual and community-level socio-economic status (SES) are strong predictors of Any MVPA, and together with established clinical correlates (such as late disease), these factors may contribute to breast cancer disparities.

#3728 Extracellular vesicle dynamics in alternate-day fasting combined with low-carbohydrate weight loss intervention.

Faiza Kalam[1], Kalpana Deepa Priya Dorayappan[1], Rand T. Akasheh[1], Zhipeng Tao[2], Ting-Yuan David Cheng[1], Krista Varady[3], Selvendiran Karuppaiyah[1]

[1]The Ohio State University, Columbus, OH,[2]Texas Woman's University, Denton, TX,[3]University of Illinois at Chicago, Chiago, IL

Background: Obesity is associated with systemic metabolic dysregulation and chronic inflammation, which are reflected in alterations in extracellular vesicles (EVs) that mediate intercellular communication. EVs, marked by CD9 and CD63, may also play a critical role in cancer risk by mediating pro-inflammatory and metabolic signaling. This study investigates how an alternate-day fasting and low-carbohydrate (ADF-LC) intervention impact EV secretion and subpopulation dynamics, exploring their potential as candidates for further protein-level investigations to delineate functional roles in inflammation and metabolic dysfunction.
Methods: Serum samples were analyzed from 10 participants with obesity enrolled in a 24-week ADF-LC intervention, comprising of a 12-week weight loss phase and a 12-week weight maintenance phase. EVs were isolated from serum collected at baseline (Week 0), after weight loss (Week 12), and after weight maintenance (Week 24). EV quantification and characterization were performed using ImageStream flow cytometry, focusing on EV surface markers CD9 and CD63. Fluorescent antibody labeling was used to detect these tetraspanins, allowing precise measurement of EV subpopulations. Total EV concentrations and subpopulation dynamics were assessed, while protein cargo consistency was evaluated to identify functional shifts.
Results: Body weight significantly decreased during the weight loss period (-5.5 ± 0.5%; $p < 0.001$) and stabilized during the weight maintenance phase ($p = 0.57$). Net weight loss by Week 24 was -6.3 ± 1.0%, accompanied by a significant reduction in fat mass ($p < 0.01$). Total EV concentrations significantly declined from baseline to Week 24 ($p < 0.05$), with notable reductions in CD9+ EV populations at Weeks 12 and 24 ($p < 0.05$) and CD63+ EV populations at Week 24 ($p < 0.05$). Despite these changes, EV protein cargo remained consistent, suggesting shifts in specific EV populations and EV-mediated signaling rather than alterations in total protein load. These findings support the need for further investigation into protein changes within EVs to better understand their functional roles in inflammation and metabolic dysfunction.
Conclusions: This study highlights significant changes in EV dynamics and subpopulations during ADF-LC, providing novel insights into metabolic adaptations associated with obesity and weight loss interventions. EVs, particularly CD9+ and CD63+ subpopulations, emerge as potential candidates for further exploration of protein-level changes to better delineate their functional roles in mediating inflammation and metabolic dysfunction, and potential involvement in the obesity-cancer connection.

#3729 The BE FIT study: feasibility of an exercise intervention on the microbiome and immune function in a high-risk cohort for lung cancer.

Marisa A. Bittoni[1], Angela Lontoc[2], Matt Soileau[3], Jennifer Moon[2], Liliana Marin[2], Victoria Hockenhull[2], Amoni Lewis-Hughes[2], Daniel Spakowicz[1], David A. Carbone[1], Zachary Chaplow[3]

[1]Division of Medical Oncology, The Ohio State University, Columbus, OH,[2]Comprehensive Cancer Center, The Ohio State University, Columbus, OH,[3]Department of Human Sciences, The Ohio State University, Columbus, OH

Background/Objective: Modifiable lifestyle risk factors, including diet and exercise, have shown to be associated with reduced lung cancer risk. We previously implemented the BEWELL diet study, which assessed the effects of a black raspberry beverage on bodily inflammation and the microbiome in high-risk smokers. We recently launched the BEFIT exercise study with similar outcomes and objectives which are to: a) confirm the feasibility of an exercise intervention in high-risk smokers, and b) examine the intervention impact on the microbiome and inflammatory biomarkers. This report will describe trial progress, including participant characteristics, recruitment challenges and adherence in this ongoing study.

Methods: Consenting enrollees from The Ohio State University Lung Cancer Screening Clinic (OSULCSC) were randomized to one of two arms: (a) Exercise/Behavioral Support Intervention - an ACSM-approved 12-week virtual resistance training plus aerobic program, or (b) Standard of Care - an information-only, control condition (e.g. light walking). Eligibility criteria include: ages 40-80 years, exercise<150 minutes/week, 20 pack-year smoking history and computer/internet access. Blood draws, stool samples, physical activity measures and behavior change predictors will be evaluated at baseline and intervention completion, with 1-year follow-up to assess adherence.

Results: We have consented 47 out of 115 eligible participants, with 23 randomized to the intervention arm and 24 to the control arm (5 and 6 withdrew from each group, respectively). Individuals have primarily been recruited through the OSULCSC and social media ads. Our goal is to recruit 40 participants who complete the 12-week study (20 per arm). Common reasons for withdrawal include too busy, changed mind and no longer interested. Participant characteristics include mean age 64, 75% female, 89% white, 45% current smokers and mean BMI of 35 (obese). Adherence to the intervention has been high with 90% of sessions attended and no adverse events reported. Biospecimen collection and social cognitive intermediates are currently underway.

Discussion: We have successfully launched the BEFIT exercise intervention study for smokers at high risk for lung cancer with high adherence and no adverse events reported. Due to initial recruiting challenges and dropout, with many being ineligible initially, we adjusted the eligibility criteria (lower age and exercise levels) and increased the study compensation. Our BEWELL study showed statistically significant improvements in the gut microbiome with a few non-significant changes in inflammation. We will assess similar outcomes in the BEFIT study and look forward to reporting these when completed. These lifestyle studies provide unique strategies for lung cancer prevention, which are greatly needed.

#3730 Oral microbiome and breast cancer risk in the NIH-AARP Diet and Health Study.

Giovanny A. Herrera Ossa[1], Xing Hua[2], Linda M. Liao[1], Yunhu Wan[1], Casey L. Dagnall[2], Kristine Jones[2], Belynda D. Hicks[2], Amy Hutchinson[2], Mitchell H. Gail[1], Jianxin Shi[1], Gretchen Gierach[1], Rashmi Sinha[1], Emily Vogtmann[1]

[1]NCI Div. of Cancer Epidemiology & Genetics, Bethesda, MD,[2]Frederick National Laboratory for Cancer Research/Leidos Biomedical Research Laboratory, Inc., Frederick, MD

Introduction: Breast cancer is the most commonly diagnosed cancer and leading cause of cancer death among women worldwide, with approximately 310,720 new cases and 42,250 deaths in 2024 in the US. The oral microbiome has been observed to be associated with breast cancer in case-control studies, but few studies have evaluated whether the oral microbiome may influence the risk of breast cancer. It has been hypothesized that the oral microbiome may be related to breast cancer risk through multiple mechanisms, including periodontal disease-related systemic inflammation or bacterial translocation. We evaluated the prospective association between the oral microbiome and breast cancer risk in the NIH-AARP Diet and Health Study cohort.

Methods: The NIH-AARP cohort recruited 566,398 participants aged 50 to 71 from 1995 to 1996. In 2005, 34,262 oral wash specimens were collected by mail from a subset of living participants who had never been diagnosed with cancer and participants who had previously developed colorectal, breast, or prostate cancer. Within the women with an oral wash specimen without a prior cancer diagnosis, 348 women who later developed breast cancer were matched to a control using incidence density sampling by age at sample collection, race, and smoking history. DNA was extracted from the oral wash using the PowerSoil Pro, and shotgun metagenomic sequencing was conducted on the Illumina NovaSeq. After host filtering, the taxonomy of the unmapped reads was assigned using MetaPhlAn3, and functional profiling was performed using HUMAnN3. Relative abundances of the taxa from phylum to species level and the functional data of genes and pathways were calculated. Conditional logistic regression was conducted for alpha diversity, beta diversity, species-, and genes-level metrics to calculate odds ratios (OR) and 95% confidence intervals (CI) with adjustment for potential confounders including demographic and lifestyle characteristics, and reproductive factors.

Results: The average age of participants was 61.5 (SD 5.2). The cases developed breast cancer an average of 3.4 years after the oral wash sample collection. There were no significant differences between cases and controls by education level, age at menarche, or menopausal status. Results from the first two batches of samples (44 cases and 44 controls) suggested that alpha diversity was inversely, but not statistically significantly, associated with breast cancer risk (e.g., OR observed species=0.79; 95% CI=0.45-1.40). Similarly, the presence of some periodontal pathogens was inversely, but not significantly, associated with breast cancer risk (e.g., OR *Porphyromonas gingivalis*=0.62; 95% CI=0.21-1.80).

Conclusions: There are some indications that the oral microbiome may be prospectively associated with breast cancer risk. However, results from the complete dataset will provide better statistical power to adequately investigate potential associations.

#3731 The role of dietary patterns and microbiome composition in colorectal cancer survival: The ColoCare Study.

Biljana Gigic[1], Tian Jin[1], Mmadili N. Ilozumba[2], Victoria Damerell[1], Doratha A. Byrd[3], Tengda Lin[2], Maria Gomez[3], Van Manh H. Le[1], Alireza Shamsian[1], Farzaneh Malekian[1], Jennifer Ose[2], Sheetal Hardikar[2], Jane C. Figueiredo[4], Christopher I. Li[5], David Shibata[6], Adetunji T. Toriola[7], Gianni Panagiotou[8], Ute Nothlings[9], W. Zac Stephens[2], June L. Round[2], Cornelia M. Ulrich[2], Caroline Himbert[2], Christoph Kahlert[1]

[1]Department of General, Visceral and Transplantation Surgery, Heidelberg University Hospital (UKHD), Heidelberg, Germany,[2]Huntsman Cancer Institute, Salt Lake City, UT,[3]Department of Cancer Epidemiology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL,[4]Department of Medicine, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA,[5]Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA,[6]Department of Surgery, University of Tennessee Health Science Center, Memphis, TN,[7]Department of Surgery, Washington University St. Louis, St. Louis, MO,[8]Microbiome Dynamics, Leibniz Institute for Natural Product Research and Infection Biology- Hans Knoll Institute, Jena, Germany,[9]Institute for Nutrition and Food Sciences, University of Bonn, Bonn, Germany

Introduction: Diet plays a crucial role in shaping the gut microbiome, which in turn can influence cancer progression and patient outcomes. Specific dietary patterns - such as those rich in fiber, protein, and healthy fats - can promote a balanced microbiome, support immune function, reduce inflammation, and improve treatment response. In contrast, diets high in processed foods and unhealthy fats may disrupt the microbiome, potentially leading to poorer outcomes. Understanding the impact of dietary patterns on the microbiome and cancer progression could provide valuable insights into personalized nutrition strategies aimed at improving colorectal cancer (CRC) survival. This study explores the relationship between nutrient-based dietary patterns, microbiome composition, and overall survival (OS) among CRC patients.

Methods: Pre-surgery fecal samples were collected from 129 stage I to IV CRC patients in the ColoCare Study, a prospective cohort of newly diagnosed CRC patients, in Utah, US and Heidelberg, Germany. To characterize the gut microbiome, we performed 16S rRNA sequencing. We calculated estimates of alpha and beta diversity, relative abundance, and *a priori* selected bacterial presence or absence. DESeq2 was used to quantify differential abundance. Principal component analysis was applied to identify nutrient-based dietary patterns, including 23 *a priori* selected macro- and micronutrients from Food Frequency Questionnaires. Nutrients were log-transformed and adjusted for total energy intake. OS was assessed in 117 stage I to III CRC patients, using multivariate Cox proportional hazards regression.

Results: We identified two major dietary patterns: a “Western diet” characterized by e.g. high intake of protein, fats, and carbohydrates, and a “vitamins & minerals diet” with high intake of vitamins and minerals, especially vitamin B_6, potassium, and calcium. Alpha diversity was significantly higher in stage I patients compared to stage IV (Shannon index, $p=0.04$) and significantly different between study centers (Shannon index, $p<0.01$). Beta diversity was significantly different comparing patients adhering to a low *vs.* high “Western diet” (Bray Curtis distance, $p=0.02$). We did not observe statistically significant differences in relative abundance among patients following a “Western diet” or a “vitamins & minerals diet”. Additionally, no significant association was found between microbiome composition and OS.

Conclusion: While dietary patterns were linked to gut microbiome diversity, no significant association was observed between microbiome composition and OS in CRC patients. To further substantiate these findings, examining dietary patterns at the food group level may provide additional insights. Additionally, metagenomic sequencing is essential to investigate species, gene, and functional profiles in larger patient populations.

#3732 Associations between gut microbiome changes and quality of life in colorectal cancer patients: a pre- and post-surgery comparison.

Hyeree Park[1], Donghyeok Seol[1], Ji Won Park[2], Min Jung Kim[2], Seung-Yong Jeong[2], Aesun Shin[3], Eun Sung Jung[4], Sunyoung Lee[4]

[1]Seoul National University Bundang Hospital, Seongnam, Korea, Republic of,[2]Seoul National University Hospital, Seoul, Korea, Republic of,[3]Seoul National University College of Medicine, Seoul, Korea, Republic of,[4]HEM Pharma Inc., Suwon, Korea, Republic of

With the increasing number of colorectal cancer (CRC) survivors, understanding how treatment impacts health-related quality of life (QoL) and microbiome composition is critical. This study investigated the relationship between QoL metrics and microbiome changes before and after surgery in CRC patients, utilizing the EORTC QLQ-C30 and QLQ-CR29 questionnaires alongside clinical metadata. Additionally, the study explored how persistent symptoms, weight changes, and physical activity levels post-surgery influence QoL and microbiome diversity.
Survey data were collected from 38 patients who completed pre- and post-surgery QoL assessments and provided matched fecal samples. Part I of the survey assessed health and lifestyle habits, including exercise levels quantified by MET-minutes, while Part II evaluated QoL using functional and symptom scales. Patients were classified as high-risk based on functional (≤33.32) or symptom scale scores (>66.66). Matched fecal samples were analyzed by sequencing the V3-V4 regions of the 16S rRNA gene, with taxonomy assigned using a besttax approach and processed via the QIIME pipeline. Microbiome analyses included differential abundance testing (using ALDEx2 and DESeq2) and diversity metrics (alpha and beta diversity). Significant changes were reported in functional scales, including body image and weight concern, and in symptom scales, such as blood and mucus in stool and stool frequency, between baseline and follow-up surveys.
Microbiome analysis identified significant differences in taxa such as *Peptostreptococcus, Parvimonas,* and *Prevotella* pre- and post-surgery. While trends toward higher microbial diversity were observed in patients with increased physical activity or weight gain, these were not statistically significant. Symptom severity was associated with distinct microbial profiles: patients with frequent symptoms like blood and mucus in stool exhibited higher levels of *Collinsella* and *Senegalimassilia,* alongside lower abundances of beneficial genera like *Ruminococcus* and *Dialister.*
By integrating QoL survey data with microbiome profiles, this study highlights the potential for personalized symptom management based on distinct microbial patterns linked to specific symptoms. Such insights enable tailored interventions to address both colorectal cancer-related symptoms and broader QoL concerns in survivors. A nuanced understanding of these relationships can guide targeted strategies to improve symptom control, enhance quality of life, and support comprehensive recovery through optimized microbial diversity.

#3733 Increased risk of EOCRC after multiple exposures to antibiotics in A/J mice.

Niti Jani, Maredith Richardson, Kristen Hogan, Maria M. Pena

University of South Carolina, Columbia, SC

Colorectal cancer (CRC) is the fourth most common cancer and the second leading cause of cancer-related deaths in the United States and globally. When diagnosed in individuals under 55 years of age, it is referred to as early onset colorectal cancer (EOCRC). EOCRC has been increasing in incidence and mortality since the 1990's with an 11% to 20% increase from 1995 to 2019 and projected to rise by 140% by 2030. Although EOCRC has been associated with known risk factors for CRC, the exact causes and mechanisms remain unclear. Based on current knowledge, we propose that EOCRC may be driven by exposure to internal and external factors (collectively known as exposomes) that have been on the rise over the past four decades, potentially affecting the microbiome and leading to dysbiosis and inflammation in the distal colon and rectum. One significant contributing factor may be the use of antibiotics (Abx), especially in infants and young children. In this study, we hypothesized that early life exposure to Abx could lead to dysbiosis and inflammation in the distal colon and rectum, which in turn may cause increased mutations and epigenetic changes that promote development of EOCRC. To test this hypothesis, we investigated the impact of commonly prescribed pediatric Abx on the gut microbiota and inflammatory environment in the colon, and their potential role in tumor development when exposed to a carcinogen. A/J mice were treated with several rounds of Abx and then exposed to azoxymethane (AOM), a known carcinogen, to assess their effect on tumor formation. The results revealed that Abx exposure induced dysbiosis and inflammation in the colon, along with systemic inflammation in the treated mice. When AOM was introduced in the presence of Abx-induced dysbiosis, this led to increased tumor burden in the distal colon. These findings suggest that Abx may play a role in elevating the risk of EOCRC. Future studies are aimed at examining age-dependent effects of Abx on EOCRC risk and in combination with other common exposomes.

#3734 Associations between microbiota-derived indoles and inflammation markers among colorectal cancer patients.

Victoria Damerell[1], Doratha A. Byrd[2], Sheetal Hardikar[3], Mmadili N. Ilozumba[3], Christoph Kahlert[1], Jane C. Figueiredo[4], Christopher I. Li[5], David Shibata[6], Adetunji T. Toriola[7], Cornelia M. Ulrich[3], Gianni Panagiotou[8], Biljana Gigic[1]

[1]Department of General, Visceral and Transplantation Surgery, Heidelberg University Hospital (UKHD), Heidelberg, Germany,[2]Department of Cancer Epidemiology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL,[3]Huntsman Cancer Institute, Salt Lake City, UT,[4]Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA,[5]Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA,[6]Department of Surgery, University of Tennessee Health Science Center, Memphis, TN,[7]Department of Surgery, Washington University St. Louis, St. Louis, MO,[8]Leibniz Institute for Natural Product Research and Infection Biology, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knoll Institute, Jena, Germany

Introduction: Gut microbiota-derived indole metabolites, produced from dietary tryptophan, play significant roles in modulating inflammation and intestinal immune responses. However, despite emerging interest, still little is known about the roles of indoles in the context of colorectal cancer (CRC). Given the microbiome's potential impact on CRC progression, understanding the associations between indoles and inflammatory markers may reveal novel therapeutic insights for CRC management.
Methods: Pre-surgery serum samples from 77 stage I-IV CRC patients participating in the ColoCare Study, Heidelberg, have been contributed to the German PerMiCCion Consortium to measure circulating levels of indoles and cytokines using untargeted metabolomics analyses and LEGENDPLEX Cytokine Array, respectively. Multivariable linear regression models were conducted to quantify associations between Indole-3-Acetic Acid (IAA), Methyl-IAA (mIAA), 5-Hydroxyindoleacetic Acid (5-HIAA), Indole-3-Propionic Acid (IPA), Indole-3-Acetamide (IAM), Indole-3-Lactic Acid (ILA), Indole-3-Aldehyde, Indole-3-Carboxylic Acid (ICA) and 44 inflammation markers. Models were adjusted for age, sex, tumor stage, tumor site, and circulating creatinine levels. Sensitivity analyses with false discovery rate (FDR) correction were conducted to adjust for multiple testing.
Results: We found mixed associations for indoles with inflammation markers. For example, IAM was positively associated with IL-6 (β=0.23, p=0.04), CD25 (β=0.68, p=0.01), Tim-3 (β=0.29, p=0.03) and Angiopoietin-2 (β=0.31, p=0.04). In contrast, IPA and ICA showed inverse associations with IL-6 (β=−0.37, p=0.01; β=−0.07, p=0.02, respectively). Strong associations between IPA and CD68 (β=1.01, p=0.02) and PDGF-AA (β=0.62, p=0.03) were observed. Additionally, IAA, 5H-IAA, and ILA were positively correlated with chemokines CXCL9, CXCL11, and CCL4 (β=0.30, p=0.02; β=0.19, p=0.01; β=0.17, p=0.03, respectively). Weak associations of ILA and mIAA with PD-L1 were found (β=0.14, p<0.01; β=0.14, p<0.01, respectively). In sensitivity analyses, associations did not remain statistically significant after FDR adjustment.
Conclusion: This study highlights the varied roles of indole metabolites in CRC. Metabolites like IAM appear to foster a pro-tumor immune landscape via inflammation, immune exhaustion, and angiogenesis. While IPA and ICA may counteract these processes through anti-inflammatory effects, IPA may also have pro-angiogenic properties. IAA, 5H-IAA, and ILA seem to be involved in immune cell recruitment to the tumor site and ILA and mIAA may play a role in suppression of anti-tumor immune responses. Larger cohort studies are needed to gain a deeper understanding of the interplay between indoles and inflammatory processes during colorectal carcinogenesis which may ultimately help identify therapeutic targets for CRC.

#3735 A prospective study of excess weight and weight change in adulthood and risk of colorectal cancer incidence and mortality.

Wen-Yi Huang[1], Steven C. Moore[1], Kathryn Hughes Barry[2], Sonja I. Berndt[1]

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD,[2]Department of Epidemiology and Public Health, University of Maryland School of Medicine, Baltimore, MD

Obesity is a rising public health problem in the U.S. and worldwide and excess body mass index (BMI, kg/m^2) is a recognized risk factor for colorectal cancer (CRC). However, the role of weight change over the adult life course and timing of the excess BMI in relation to the incidence and mortality of CRC is not well understood. With >15 years of follow-up, we prospectively evaluated the risk of CRC incidence (n=3,092) and mortality (n=931) in association with adult weight change among men and women (n=131,814) recruited in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial at ages 55 to 74 between 1993 and 2001. At baseline, participants filled out a questionnaire reporting their weight and height at ages 20 years, 50 years, and baseline, allowing us to assess weight change from early to middle, middle to late, and early to late adulthood. Hazard ratios (HRs) and 95% confidence intervals (CI) were computed using multivariable-adjusted proportional hazard regression models. Higher BMI at all ages was associated with an increased risk of CRC incidence (*p*-trend=0.05, 0.0003 & 0.0001 for ages 20, 50 and baseline, respectively). Compared with individuals whose BMI never exceeded 25 kg/m^2 over their lifetime, those who first exceeded a BMI of 25 kg/m^2 in early adulthood had a slightly higher risk of CRC (HR=1.3, CI:1.1-1.4) than those whose BMI did not exceed 25 kg/m^2 until middle age (HR=1.2, CI:1.1-1.3) or later (HR=1.2, CI: 1.0-1.3). Moreover, compared with stable weight, weight gain >4 kg per 5 years from early adulthood to middle or late adulthood was associated with an increased risk of CRC (HR=1.2, CI: 1.1-1.4 for both periods). The increased risks for weight gain >4 kg/5 years between early to middle adulthood appeared more pronounced for those with normal BMI at age 20 (HR=1.3, CI: 1.1-1.5). For CRC mortality, higher BMI at age 50 and baseline, but not age 20, was associated with increased risk (*p*-trend = 0.0002, 0.0002, and 0.3, respectively). Compared to those whose BMI never exceeded 25 kg/m^2, those who first exceeded a BMI of 25 kg/m^2 in early adulthood had an increased risk of mortality (HR=1.2, CI: 1.0-1.5), but no association was found for those whose BMI did not exceed 25 kg/m^2 until middle adulthood or later. A higher risk of CRC mortality was observed for weight gain >4 kg/5 years from early adulthood to middle or late adulthood (HR=1.5, CI: 1.1-1.9 and HR=1.7, CI:1.4-2.1, respectively), especially among those with normal BMI at age 20 (HR=1.5, CI: 1.1-2.0 and HR=1.7, CI:1.3-2.2, respectively). Exceeding normal weight in early adulthood and weight gain from early to middle or late adulthood were associated with increased risks of both CRC incidence and mortality. These findings highlight the harms of excess BMI in early adulthood, the importance of maintaining healthy weight throughout the adulthood, and the potential benefit of weight control programs for CRC prevention.

#3736 Persistent threat: The role of modifiable risk factors in US cancer mortality.

Kyle Edwards[1], Chinmay Jani[2], Aysswarya Manoharan[1], furkan bahar[3], Samuel Kareff[4], Gilberto Lopes[1]

[1]University of Miami Miller School of Medicine, Miami, FL,[2]University of Miami Miller School of Medicine, Doral, FL,[3]Mount Auburn Hospital, Cambridge, MA,[4]Baptist Health, Boca Raton, FL

Objectives: Cancer is the second leading cause of death globally, with many cases linked to modifiable risk factors. It is crucial to improve our preventive strategies and screening guidelines to reduce preventable cancer. Our study analyzes trends in modifiable risk factors linked to cancer-related mortality in the US over past 30 years..

Methods: The Global Burden of Disease (GBD) database was utilized to extract the overall and risk factor-associated Age-Standardized Mortality Rate (ASMR) for all cancers, as well as for 23 specific cancer in the US, from 1990 - 2021. Our analysis involves the stratification and sub-stratification of modifiable risk factors, encompassing behavioral, environmental/occupational, and metabolic categories, to define risk factor-outcome pairs. Joinpoint Trends were analyzed.

Results: In 2021, 44.1% of cancer-related deaths (315,572) in the US were due to risk factors, with the top five proportional risk factors being smoking (21.4%), high BMI (6.6%), high plasma glucose (6%), occupational carcinogens (5.1%), and high alcohol use (3.4%). From 1990-2019, metabolic risk factors saw the highest associated increase in cancer mortality (+66.4%), especially plasma-glucose (+87.2%) and high BMI (+At 51.5%), contributing to 9 of top 10 increases in ASMR for risk factor-outcome pairs. The greatest reduction was from household air pollution (-81.9%). The highest ASMR among risk factor-outcome pairs was for smoking- trachea-bronchi-lung cancer (TBLC) overall (17.2), in males (21.6), and in females (13.8). Other common pairs included dietary risks (low whole grain, high red meat) - colorectal cancer (CRC), asbestos-TBLC, high fasting glucose-pancreatic cancer, and high BMI-CRC. For males, the top five remained the same except for smoking-esophageal cancer, while for females, the top five also included unsafe sex-cervical cancer and dietary risks-breast cancer replacing asbestos-TLBC and high BMI-CRC. The highest proportional ASMR for risk factor-outcomes pairs included unsafe sex-cervical cancer (99%), occupational carcinogens-mesothelioma (m:96.1%; f:89%), smoking-larynx (m: 72%; f:67%), smoking-TBLC (m;62%; f:56%).

Conclusions: A substantial proportion of cancer-related mortality in the US is linked to modifiable risk factors, particularly smoking, high BMI, and high plasma glucose. Notably, over 50% of the mortality in cancers like cervical, mesothelioma, larynx, TBLC, and liver is associated with modifiable risk factors. Despite a significant decline, smoking remains the leading risk factor for cancer mortality. While mortality associated with environmental and occupational risk factors has improved, that linked to metabolic risk factors has increased both proportionally and absolutely for certain cancers. These findings underscore the urgent need for targeted prevention and screening strategies to address these risk factors and reduce preventable cancer mortality.

#3737 Effect of a potential cancer preventive nutraceutical derived from olive oil processing on hematological, and metabolic parameters.

Adriana Albini[1], Luana Calabrone[2], Anna Aiello[3], Paola Corradino[1], Sara Nofri[4], Simone Cristoni[2], Giulia Accardi[3], Giuseppina Candore[3], Angelo Zinellu[5], Calogero Caruso[3], **Douglas M. Noonan**[6]

[1]IRCCS IEO - European Institute of Oncology, Milano, Italy,[2]ISB-Ion Source & Biotecnologie Srl, Rho, Milan, Italy,[3]Department of Biomedicine, Neuroscience and Advanced Diagnostics, University of Palermo, Palermo, Italy,[4]University of Florence, Florence, Italy,[5]Department of Biomedical Sciences, University of Sassari, Sassari, Italy,[6]Unit of Molecular Pathology, Biochemistry and Immunology, IRCCS MultiMedica, Milano, Italy

Introduction: Extra virgin olive oil is a basic component of the Mediterranean diet; it contains several molecules with antioxidant, anti-inflammatory, anti-angiogenic anti ageing, and pro-apoptotic properties. Olive mill waste water (OMWW), a liquid residue produced in vast amounts during olive oil extraction, represents an environmental issue but on the other side it is a rich source of phytochemicals particularly, phenolic compounds, that show promise in preventing the development of several disease disorders, including cancer. We performed in vitro and in vivo experiments and in a pilot study we measured hematochemical, anthropometric, and metabolomic parameters in volunteers after dietary supplementation of an OMWW purified nutraceutical preparation.
Experimental procedures: OMWWs ability to affect cell proliferation and survival on different cancer cell lines was analyzed by MTT, and by gene expression analysis. The supplementation of OMWW-OL (Oliphenolia) 25 mL twice daily for 30 days was tested, upon ethical committee approval, on a pilot cohort of volunteers with characteristics close to metabolic syndrome. Hematochemical, anthropometric, serum biomarkers and serum metabolomic parameters were analyzed before the intervention, at 30 days, and 30 days after.
Results: OMWW extract shown a potent inhibitory effect inhibiting cells proliferation of cancer cell lines and HUVECs. We analyzed sera from volunteers with pre-metabolic syndrome, and we assessed several biological parameters before and after nutraceutical intervention with Oliphenolia. Body mass index, slightly decreased, particularly in the women. Also, hydration increased, especially in women, while blood pressure, glycemia, and insulin decreased. Cholesterol, high-density lipoproteins, and triglycerides were stable, and LDL levels decreased, while vitamin D levels, alongside calcium, perceptibly increased. Albumin also increased. All the values were in support of a trend towards a beneficial effects. By mass spectrometry analysis, we also found favorable changes in the vitamin D.
Conclusions: OMWW extract has shown stronger anti-angiogenic and anti-tumor properties in vitro and in vivo, with potential cancer preventive potential. A small pilot study, with limited potency, suggested that Oliphenolia extract represents a promising dietary supplement, that can could be potentially valuable for people at risk of metabolic syndrome and relevant in supporting healthy ageing and in cancer prevention

#3738 Triangulating the metabolic crossroads: understanding the complex interplay between metabolic dysregulation and obesity cancer risk.

Marinella Temprosa[1], Bing Li[2], Liza Makowski[3], James Hebert[4], Cornelia Ulrich[5]

[1]The George Washington University, Rockville, MD,[2]University of Iowa, Iowa City, IA,[3]University of Tennessee, Memphis, TN,[4]University of South Carolina, Columbia, SC,[5]University of Utah, Salt Lake City, UT

Obesity is on the verge of surpassing tobacco use as the leading modifiable cause of cancer in the U.S., contributing to over 40,000 new cancer cases annually. With obesity rates rising, especially in younger populations, its association with increased cancer risk is becoming more evident. There is an urgent need to understand the mechanisms underlying the relationship between obesity related metabolic dysregulation and cancer risk, including factors that may mitigate health disparities. Recent advances in molecular oncology and metabolic research provide opportunities in understanding the intricate connections between metabolic dysregulation, obesity, and cancer. This session will describe a novel triangulation approach, synthesizing evidence from randomized controlled trials, mechanistic animal studies, and large-scale secondary data analyses to build a comprehensive understanding of these relationships from the NCI-sponsored Metabolic Dysregulation and Obesity Cancer Risk Consortium (MeDOC). MeDOC is guided by team science and transdisciplinary approach to advance our understanding of the underlying mechanisms that connect obesity, metabolic dysregulation, and cancer risk to identify markers that will enhance cancer risk prediction and identify targets for intervention. Both obesity and metabolic dysregulation contribute to a cascade of derangements in adipocyte function, growth factors, inflammation, gut microbiome, immune function, sex hormones, lipid and glucose metabolism which, in turn, can disrupt several downstream signaling pathways related to cancer initiation and progression. These derangements occur systemically and within the tumor microenvironment itself. Through 5 individual projects and 6 collaborative projects in human and animal studies, we are currently evaluating the role of A-FABP (adipocyte fatty acid binding protein), ceramides, inflammation, gut mediated metabolites, gut microbiome dysbiosis in cancers of the colon, breast, and liver, facilitated by the coordinating center. Methodological triangulation will be emphasized throughout, demonstrating how randomized trials provide causal inference, animal studies reveal mechanistic insights, and secondary data analyses establish population-level patterns and clinical relevance. Experts from the MeDOC Consortium will review current understanding, describe the ongoing human and animal studies and big data analytic methods to establish robust causal frameworks. Open discussion will include our early career members to engage the community to identify emerging areas.

Chairperson: Marinella Temprosa
Hallmarks of metabolic dysregulation and their role in obesity cancer risk: Bing Li
Harmonization of Animal Studies: Liza Makowski
Design of Randomized and Observational Studies: James Hebert
Transdisciplinary Collaboration Discussion: Neli Ulrich

IMMUNOLOGY: Enhanced Antibodies, TCR Constructs, Cytokines and Chimeric Proteins
Poster Session

#3767 Enhanced anti-tumor efficacy through prolonged plasma membrane retention of a novel anti-CCR8/IL2 mutein fusion antibody.

Andres Zucchetti[1], Benjamin Pelletier[1], Fabien Delisle[1], Bere Diallo[1], Anupama Karnam[1], Marco Pretti[1], Erica Russo[1], Caroline Janot-Sardet[1], Yann Courbebaisse[1], Elsa Lheriteau[1], Charlotte Muller[1], Marine Dubois[1], Reno Winter[1], Mercedes Tkach[1], Alvaro Lladser[2], Jonathan Back[1], Bernard Vanhove[1], Sebastien Lemoine[1], Monica Gostissa[1], Eliane Piaggio[3], Ilgar Mamedov[1], **Fiorella Kotsias**[1]

[1]EGLE THERAPEUTICS, Suresnes, France,[2]Centro Cientifico y Tecnologico de Excelencia Ciencia & Vida, Fundacion Ciencia & Vida, Santiago, Chile,[3]Institut Curie, INSERM U932, PSL Research University, Institute Curie Research Center, Paris, France

Background: Infiltrating regulatory T cells (Tregs) are part of the tolerogenic microenvironment of tumors and key players in resistance to immune checkpoint inhibitor (ICI) therapy. Selective depletion of Tregs within the tumor microenvironment has the potential to overcome this resistance and to increase the proportion of patients responding to immunotherapy. The CCR8 chemokine receptor, which is preferentially upregulated on tumor-resident Tregs, represents an attractive and specific target for antibody-dependent cell cytotoxicity (ADCC)-mediated Treg depletion. Several ADCC-enhanced anti-CCR8 antibodies are currently being evaluated in clinical trials to assess their therapeutic potential. Our findings suggest that rapid internalization of anti-human CCR8 antibodies upon receptor binding significantly hampers their ADCC efficacy. To address this limitation, we engineered EGL-002, a novel anti-CCR8 antibody fused to an IL2 mutein designed to preferentially block the CD25 receptor and to prevent CCR8 internalization.
Methods: Retention of EGL-002 on the plasma membrane was assessed through microscopy and flow cytometry using in vitro-expanded and tumor-infiltrating human Tregs. ADCC and antibody-dependent cellular phagocytosis (ADCP) potency were evaluated in reporter assays and confirmed using donor-derived natural killer (NK) cells and macrophages, respectively. Pharmacodynamics, biodistribution, and anti-tumor activity were evaluated in tumor-bearing mice genetically engineered to express the human target receptors. Treg-depleting activity and specificity were further confirmed in human tumor samples.
Results: EGL-002 demonstrated prolonged retention on the plasma membrane of Tregs across in vitro, ex vivo, and in vivo models, compared to the parental antibody. This resulted in enhanced ADCC and ADCP potency, leading to complete Treg depletion in tumors while sparing other tumor-infiltrating effector T cell populations as well as Tregs in the periphery. In mouse models, EGL-002 effectively depleted tumor Tregs, achieving robust anti-tumor efficacy as single-agent and in combination with anti-PD-1 therapy.
Conclusion: Preclinical data highlight EGL-002 as a highly effective tumor Treg-depleting agent with potent anti-tumor activity. Its unique design positions it as a best-in-class therapeutic candidate for solid tumors, either as a standalone treatment or in combination with ICIs.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: DNA Damage Response and Modulation of DNA Repair 1
Poster Session

#4198 Unraveling the molecular interactions between DDR and autophagy in response to oxidative stress.

Kavya Ajit Pandya, Neeru Singh

Institute of Advanced Research, Gandhinagar, India

The DNA damage response (DDR) is essential for maintaining genomic integrity by detecting and repairing DNA damage. In conjunction with autophagy, DDR functions as a critical protective mechanism under oxidative stress, mitigating the risk of cancer development. Our study investigates the link between oxidative stress and DDR, particularly focusing on ATM kinase's role in reactive oxygen species (ROS)-mediated genotoxic stress for double-strand break (DSB) repair. Cancer cells' heightened reliance on DDR, due to high proliferation and genomic instability, underscores their susceptibility to DDR inhibitors. However, the effectiveness of DDR inhibitors is often limited by the evolution of resistance in tumors. Resistance mechanisms can include the restoration of DNA repair pathways or activation of compensatory pathways. This adaptability limits the long-term efficacy of DDR-targeted therapies, emphasizing the need to understand the molecular drivers underlying resistance to enable the development of more effective strategies. Our study focuses on the potential interaction between Mediator of DNA damage checkpoint 1 (MDC1), a key DDR protein, and Beclin-1, a vital autophagy inducer. We have employed a comprehensive approach using the HeLa cell line, including wild type, MDC1 knockdown (KD), and MDC1 overexpressing (MDC1-Ov) variants. Oxidative stress was induced using hydrogen peroxide (H_2O_2), and we conducted co-immunoprecipitation, immunofluorescence, and immunoblotting to investigate the interactions between MDC1 and Beclin-1 under these conditions. Our results demonstrate that MDC1 interacts with Beclin-1, with the interaction enhanced over time, indicating its essential role in cellular responses to oxidative stress. We also show that oxidative stress prompts the relocation of Beclin-1 from the cytoplasm to the nucleus, suggesting that Beclin-1 may be involved in regulating DDR in response to oxidative stress-induced DNA damage. Given the central role of MDC1 in recruiting various effector proteins at the site of DNA repair, we hypothesized that MDC1 may facilitate the nuclear translocation of Beclin-1. Consistent with this hypothesis, we found that nuclear localization of Beclin-1 was significantly reduced in MDC1 knockdown cells compared to wild type when treated with H_2O_2. These findings underscore the critical role of MDC1 in modulating the nuclear localization of Beclin-1 in response to oxidative stress. The significance of our study lies in its potential implications for chemotherapy. Given the prevalence of oxidative stress in various cancers and the therapeutic reliance on chemotherapy-induced oxidative stress, understanding this novel interplay between DDR and autophagy pathways may provide insights for developing constructive therapeutic strategies.

CHEMISTRY: High-Throughput Screening Assays and Libraries
Poster Session

#615 The effect of novel natural compounds on papillary thyroid cancer cell viability.
Michelle Carnazza[1], Nan Yang[1], Robert Werner[1], Raj K. Tiwari[2], Jan Geliebter[2], Xiu-Min Li[2]

[1]General Nutraceutical Technology, LLC, Elmsford, NY,[2]New York Medical College, Valhalla, NY

Thyroid cancer (TC) is the most common endocrine malignancy and has been increasing in incidence in the United States over the last few decades. Incidence and mortality rates are largely owed to papillary TC (PTC), as they constitute approximately 80% of TC cases. PTC is usually contained within the thyroid gland and has an excellent prognosis, with a 5-year survival rate of more than 90% following surgery. Many survivors still need ongoing surveillance, continued treatment, and lifelong thyroid hormone supplementation, while also undergoing the consequences of current therapeutics including hormone fluctuations, surgical complications, and radioactive iodine side effects. Therefore, novel treatments, including herbal medicine, have been suggested. General Nutraceutical Technology, LLC. has a library of over 300 compounds and we screened twenty from different herbs including *Glycyrrhiza glabra* and *Phellodendri chinensis*, to name a few. Literature has supported their immunomodulatory effects and therefore we determined their dose-dependent effects at 40 ug/mL, 20ug/mL, 10ug/mL, 5ug/mL, and 2.5ug/mL on PTC cells, K1 and TPC1. Cell viability was assessed with an XTT assay over three days. Those with the lowest IC_{50} were further screened for synergy through the calculation of an interaction index. Initial screening demonstrated dose-dependent reductions in cell viability over three days in several of the single-blind coded compounds, plateauing at 48 hours. Compounds C35L, C7Q, and C3I were among the lowest IC_{50} for K1 cells at 4.4ug/mL, 9.5ug/mL, and 23 ug/mL respectively. These compounds also had the most pronounced effects on TPC1 cells with determined IC_{50} of 8ug/mL, 9.8ug/mL, and 23 ug/mL, respectively. Synergistic interactions were evaluated and concluded that in K1 a combination of all three compounds reduced the IC_{50} to 4.565ug/mL after 72 hours of treatment (II=0.57). This data warrants further investigation, including the mechanism of cell viability reduction upon treatment using Trypan Blue Exclusion, Western Blot and Flow Cytometry. Apoptosis can be assessed through the protein expression of apoptosis markers by Western Blot and Annexin V/Propidium Iodide staining by Flow Cytometry. Flow Cytometry can also determine effects on the cell cycle. Natural compounds have demonstrated long-lasting effectiveness that is safe and able to regulate multiple targets at once with advantages to Western medicine being more readily available, more convenient, and affordable. Therefore, these novel natural compounds, especially in combination, may provide safe and inexpensive relief to PTC survivors.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Therapeutic Approaches to Attack the Tumor Microenvironment
Poster Session

#6391 OMTX705: A dual-mechanism ADC targeting FAP-positive CAFs, to overcome chemo and immunotherapy resistance in solid tumors.

Patricia Gonzalez[1], Isabel Egana[1], Cristina Ferrer[1], Roland Kontermann[2], Oliver Seifert[2], Klaus Pfizenmaier[2], Wolfgang Richter[1], Laureano Simon[1], **Myriam Fabre**[1]

[1]Oncomatryx Biopharma, S.L., Derio, Spain,[2]Institute of Cell Biology and Immunology, Stuttgart University, Stuttgart, Germany

ADCs targeting the tumor stroma offer a promising strategy to address tumor heterogeneity. Traditional tumor-targeting ADCs face limitations, as intratumor and interpatient variability in target protein expression can result in incomplete targeting. In contrast, the tumor stroma is more genetically stable and consistent across patients. CAFs, the predominant stromal cell type within the tumor microenvironment, play a critical role in cancer progression and immune evasion in multiple cancers. OMTX705, a clinical-stage ADC, targets FAP-positive CAFs in the tumor microenvironment. OMTX705 presents an optimized and highly stable linker and it is quickly internalized and transported to late endosomal compartments in FAP-expressing cells. Its novel cytolysin-based payload demonstrates strong cell killing activity upon specific ADC internalization in FAP positive cells. Indeed, enhanced cytotoxicity was observed when compared to MMAE, particularly in cells with high PgP expression, offering a potential solution for tumor drug resistance. OMTX705 has demonstrated preclinical robust efficacy, achieving 100% tumor growth inhibition and even regression, in PDX murine models for pancreatic, gastric, ovarian, triple negative breast and lung cancers, with or without humanized immune systems, and showing no significant toxicity. Higher anti-tumoral effect was evidenced over both classical chemotherapy and modern immunotherapy drugs, as a single agent or in combination therapies with Gemcitabine/Abraxane or immune checkpoint inhibitors. OMTX705 even displays superiority compared to other FAP-targeted ADCs bearing different payloads such as deruxtecan in pancreas cancer models. Moreover, it exhibits high plasma stability and excellent safety profile, with no adverse effects observed in non-human primates at doses up to 100 mg/kg. OMTX705 operates through a dual mechanism of action. First, upon ADC internalization, the bystander effect of the payload released from FAP-positive CAFs leads to efficient killing of neighboring tumor cells. This effect has been validated through different experimental techniques *in vitro* and in preclinical tumor samples. The second mechanism involves immune cell infiltration within the tumor microenvironment, further strengthening its therapeutic potential. This dual effect, combined with a favorable safety profile, positions OMTX705 as a promising candidate among cancer therapeutics, targeting the stroma in solid tumors and enhancing antitumor immune responses.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Novel Antitumor Agents 2
Poster Session

#861 N17350 outperforms cytotoxic agents in killing tumors of treatment-naïve and chemotherapy-treated ovarian carcinoma patients.

Chang Cui[1], Ravindra Gujar[1], Maria Fumagalli[1], Nicole Martinez[1], Peter Haberz[1], Afshin Bahador[2], Alain Algazi[3], Lev Becker[1]

[1]Onchilles Pharma, Inc., San Diego, CA,[2]South Coast Gynecologic Oncology, Inc, San Diego, CA,[3]UCSF Head and Neck Medical Oncology, San Francisco, CA

Background: Tumor heterogeneity leads to suboptimal responses to chemotherapy as well as primary and acquired resistance. Advanced ovarian cancer (OvCA) patients with chemotherapy resistant disease have significantly poorer prognosis and limited treatment options and thus there is an urgent need for new therapies. N17350 is a novel therapeutic modality that kills cancer cells independent of anatomical locations, tumor genetics, and tumor immune status while preserving normal cells. N17350's broad efficacy and specificity are mediated by elevated levels of histone H1 isoforms in cancer cells. A single intra-tumoral injection of N17350 induces tumor regression and triggers anti-tumor immunity in a wide range of pre-clinical models [1 - 4]. Here, we compared tumor killing by N17350 to standard-of-care chemotherapies *in vitro* and *in vivo* in OvCa patients that had not received chemotherapy or received neoadjuvant chemotherapy (NACT) prior to cytoreductive surgery.
Methods: Cytoreductive surgery tissue specimens were collected from 27 consecutive patients treated by author AB. Cancer cells and $CD45^+$ immune cells were isolated, treated with N17350 24h post-isolation, and cell viability was quantified by calcein-AM and immunogenic cell death markers. *In vitro* tumor killing by N17350 was compared to carboplatin, paclitaxel, doxorubicin, or oxaliplatin in chemotherapy naïve and NACT patients. OvCa cells from 3 patients were injected into nude mice to create cell-derived xenograft (CDX) models. Mice were treated with N17350 (400µg/100mm^3, intratumoral) or carboplatin (100mg/kg, intraperitoneal) and tumor growth was assessed.
Results: Of 27 patients, 18 had OvCa, 9 had peritoneal carcinoma, 1 had fallopian carcinoma, 1 had high-grade invasive squamous cell carcinoma and 1 had sarcoma. 15 were chemotherapy naïve prior to cytoreduction, 11 received 3-7 cycles of NACT, and 1 had recurrent disease. N17350 induced immunogenic cell death in cancer cells and preserved immune cells in all tested patients, whereas chemotherapies were toxic to both cell types equally. N17350 was more effective at killing cancer cells than chemotherapy in patients with NACT. Additionally, N17350 showed robust tumor killing in primary, metastatic, and recurrent tumors whereas chemotherapy was inferior in killing metastatic and recurrent tumors. N17350 rapidly regressed tumors in all OvCa CDX models and exhibited markedly improved efficacy over carboplatin.
Conclusions: Our data demonstrate that, compared to standard chemotherapy, N17350 yields superior and more selective tumor killing, particularly following exposure to NACT. Tumors that are resistant to chemotherapy retain sensitivity to N17350, and N17350 could improve clinical outcomes in OvCa, including patients with chemotherapy resistant disease.

: Advocates Poster Session 1
Poster Session

#ADV01 Hereditary cancer, beyond BRCA mutations..
Debbie Denardi

FORCE (Facing our Risk of Cancer Empowered), Tampa, FL

Hereditary cancers or inherited cancers are caused by a genetic mutation that can be passed from one generation to the next; mothers and fathers can pass these mutations to sons and daughters. A person who inherits a genetic mutation from their parent will have a higher lifetime risk for certain types of cancer. A family with hereditary cancer may have many family members who have been diagnosed. Many different genes have been identified that can increase the risk for different types of cancer. Genetic testing on blood or saliva can tell if a person has inherited a gene mutation that increases cancer risk. Inherited mutations have been linked to many different types of mutations. Some types of cancer are more likely to be hereditary. Below is a list of common cancers, and the most common genes that have been linked to increased risk for each. Breast Cancer in women: gene mutations ATM, BARD1, CHEK2, CDH1, PALB2, PTEN, RAD51C, RAD51D, TP53 (Besides BRCA1, BRCA2). Breast Cancer in men: gene mutations CHEK2, PALB2 (Besides BRCA1, BRCA2). More research is needed to help patients manage their care based on their predisposition to many cancer types.

#ADV02 Results of a Patient Survivorship and Supportive Services Needs Assessment.

Alique Topalian

University of Cincinnati Cancer Center, Cincinnati, OH

A cancer patient needs assessment focuses on patients' needs, how well they are being met, and how to best support them. The purpose of this project is to understand the knowledge cancer patients have of support services, their satisfaction with those services, and what additional services patients desire.

We hypothesize that patients: (1) have a lack of knowledge regarding supportive services offered to them, (2) would like more services than the current offerings, and (3) and face a variety of barriers to accessing services.

We collected data from over 500 patients seen at urban midwestern academic cancer center and satellite hospital. We found that 56.57% of patients were not receiving any supportive services, 27.68% of patients were receiving services from the health system, 4.85% were receiving services outside of the health system, and 10.91% were receiving services from a combination of the health system and outside agencies. Of patients who are receiving supportive services the most common services accessed are individual counseling (9.69%), Cancer Survivorship (9.44%) Nutrition (8.96%), and the Exercise program (7.99%). Of patients surveyed 39.42% said they received the right amount of information on supportive programs, 26.97% were unaware of any supportive programs for cancer patients, and 24.91% were aware of some programs but would have wanted more information. Symptoms most patients would like supportive services for are fatigue (20.39%), fear of recurrence (18.42%), sleep (17.11%), anxiety/distress (17.32%), nutrition (15.57%) and weight (15.35%). Patients said the supportive services they are the most interested in are the exercise program (19.42%), individual counseling (17.86%), cancer survivorship (16.07%), nutrition (14.73%), cancer wellness clinic (11.83%), and group support for cancer type (11.61%). Most patients were interested in alternative services such as massage (33.98%), relaxation/meditation (24.24%), yoga (17.10%), acupuncture (17.10%), and culinary classes (16.45%). The greatest barriers to accessing these services are cost (29.77%), programs not happening during availability (29.53%), fatigue (24.88%), lack of knowledge (22.56%), distance from home (20.70%), and lack of time (20.23%).

Patients need more information about services that are available to them. Majority of patients said that they would like to learn about supportive services from their My Chart Portal. Patients would also like their health care professionals to know more about services available to them to provide referrals. Supportive services are a crucial part of healing and wellness during the cancer journey, and we need to equip our patients with the best programs possible.

#ADV03 Sarcoma Patient Community Data Collaboration to Advance Research.

Brandi Felser

Sarcoma Foundation of America, Damascus, MD

The sarcoma community data project is a collaboration among sarcoma patient advocacy organizations to combine data collected across organizations through registries and other mechanisms. Ethical data sharing, especially in a rare cancer like sarcoma with many different subtypes is beneficial to patients as it allows for the opportunity for more research across the sarcoma landscape. Only by pooling data from many patients, across different institutions, and from studies can there be enough statistical power to derive meaningful conclusions and insights in sarcoma, and importantly rare and ultra-rare subtypes of sarcoma where very few patients exist. Currently, sarcoma organizations and individual patients are housing data in some data platforms not tied to sarcoma, and most of which do not collaborate or work together. Even if the sarcoma community wanted to look across subtypes for specific research questions, it would be difficult because sarcoma data is so fractured. A central repository allows the sarcoma advocacy community to monitor, be collaborators in sharing and providing access to data, and become integrated in outcomes research utilizing patient donated data. This is an opportunity for sarcoma patient advocacy organizations to work collaboratively to combine health records, patient experience data, and genomics and other information in one location across subtypes. Essentially creating a leading registry for sarcoma to advance research.

#ADV04 Youthful Patients and the Importance of Early Detection .

Colt J. Blunt

Debbie's Dream Foundation, Fort Lauderdale, FL

This poster will highlight recent trends of younger people being diagnosed with stomach cancer. It will discuss the need for better education regarding younger stomach cancer patients and highlight the importance of early detection.

#ADV05 Empowering Underserved Communities: Bridging the Gap in Clinical Research.

Valarie Traynham

HealthTree, Lehi, UT

Black, Hispanic, and Rural communities face significant health disparities, often rooted in a lack of awareness, historical mistrust, and systemic inequities in clinical research. This abstract highlights a targeted educational initiative designed to empower those communities with accurate, culturally relevant information about clinical research.

The poster focuses on addressing barriers to participation, such as mistrust stemming from historical injustices, limited access to research opportunities, and misconceptions about clinical trials. Through community workshops, faith-based partnerships, and tailored educational materials, the initiative fosters trust, transparency, and engagement.

Key outcomes include increased awareness of the importance of clinical research in improving health outcomes, growth in participant trust, and higher levels of interest in trial enrollment. By amplifying the voices of advocates within the underrepresented communities and providing resources that resonate culturally, the program lays the foundation for equitable representation in clinical research.

This abstract emphasizes the critical role of education in dismantling systemic barriers and achieving health equity for underserved populations.

#ADV06 Influence of peer support on patient decision-making.

Ben Nathanson

AnCan Foundation, Tumacacori, AZ

We're currently undertaking a survey of those who participate in AnCan -- we have more than 370 responses already and plan to get 500. One goal is to make AnCan better, but another is precisely to demonstrate to medical professionals the importance of organizations like ours.

#ADV07 Comparing base LLMs vs. RAG-enhanced systems in Rare Cancers Such as Chromophobe RCC.

Katie Coleman

Independent Advocate, Buda, TX

This poster outlines the development and goals of the Chromophobe Renal Cell Carcinoma (chRCC) Patient Registry, an initiative led by my nonprofit, the Chromophobe and Oncocytic Tumor Alliance (COA). As one of the most underrepresented and rare subtypes of kidney cancer, chRCC requires dedicated efforts and champions to bridge gaps in research and patient care. The registry aims to collect patient-reported outcomes, medical records, and treatment data in a secure, anonymized format to empower research and improve understanding of this rare cancer.

With a flexible survey design and strong focus on data privacy, the registry enables patients to share their experiences while ensuring their personal information remains protected. By fostering collaboration among patients, researchers, and clinicians, this initiative aims to uncover actionable insights, identify trends in treatment efficacy, and build a foundation for future studies. This poster will also highlight the technical infrastructure and the critical role of patient advocacy in shaping a registry that serves the community's unique needs.

#ADV08 The Influence of Mentorship and Social Media on the Journey of Cholangiocarcinoma (CCA) patients.

Lisa Craine

Cholangiocarcinoma Foundation, Akron, OH

My research will be surveying cholangiocarcinoma patients and how social media, and mentoring impacted their cancer journey, both medically and emotionally. The survey link will be posted on social media sites, emailed, and texted to CCA patients. My goal is to evaluate the role of mentoring and social media and the importance of community, mentoring and social media.

#ADV09 Bridging the Gap: Collaborative Strategies to Advance Molecular Profiling in Brain Tumor Care - Insights from the 3rd Annual Leadership Fly-In.

Georgia Pinner

The Sontag Foundation, Ponte Vedra Beach, FL

This poster will provide an overview of the 3rd Annual Brain Tumor Organization's Leadership Fly-In and its outcomes. This event was dedicated to advancing the understanding and implementation of molecular profiling in the treatment of brain tumors. This event brought together leaders in the field - clinicians, researchers, advocates, and nonprofit leaders to foster collaboration and accelerate the adoption of molecular profiling as a standard practice at diagnosis.

#ADV10 Moving Abroad After Cancer: Is It a Good Idea?.

Carmen Montero

Youth Cancer Europe, Cluj-Napoca, Romania

For many young people, traveling the world and experiencing new cultures is a natural desire. But after a cancer diagnosis, is this still an option? Adolescent and young adult (AYA) cancer survivors face unique challenges beyond medical treatment, including mental health struggles, stigma, and long-term effects on employment and relationships. For migrants, there are extra challenges such as healthcare disparities, cultural barriers, and systemic inequities. Drawing from my lived experience as a migrant cancer survivor across seven countries, this poster explores cancer care and migration, highlighting both the barriers and opportunities encountered along the way. My journey has shaped my role as an advocate and researcher, leading efforts such as the Equitable, Diverse, and Inclusive (EDI) Cancer Carerecommendation for the European Parliament, the Right to Be Forgotten policy proposal in Costa Rica, the Can-Can book series to reduce childhood cancer stigma, and MANO Beyond Cancer, a global platform sharing survivor stories. My experience meeting survivors worldwide underscores the urgent need for culturally sensitive, equitable, and psychologically supportive survivorship care. By fostering collaboration among healthcare professionals, researchers, and patient advocates, we can build a more inclusive cancer care system that supports all survivors, regardless of where they come from.

#ADV11 Patient-centered education in genitourinary malignancies.

Darrell Nakagawa

SWOG Cancer Research Network, Portland, OR

This is a program designed to increase effectiveness in patient facing material to achieve multiple aims: (1) clarify trial eligibility, identify barriers to enrollment, and effective methods of distribution, especially in the community setting; (2) increase understanding of documents related to the trial while ensuring adherence to established regulatory requirements; and (3) quantify the impact of the material in trial accrual.

Patients have expressed great frustration in understanding different clinical trials, and the underlying science beneath them. This project is intended to increase that understanding to stimulate enhanced accrual for our trials. Our intent is to develop new templates for patient communication that are easy to understand, assimilate, and facilitate informed choice.

#ADV12 Engaging Patients to Transform Experience: Building ACCESS.

Adrienne Co-Dyre

ACCESS: Advancing Childhood Cancer Experience, Science & Survivorship, Toronto, ON, Canada

In Canada, roughly 1,500 children and adolescents are diagnosed with cancer each year. Advocacy for enhanced childhood cancer research and care in Canada by patients, families and the academic community led to a $23M investment through the Canadian Institutes of Health Research to establish a national pediatric cancer consortium (termed ‘ACCESS’). ACCESS’ mission is to ensure every child with cancer in Canada has access to the latest scientific advances, diagnostic tools, therapies, and supportive care leading to better outcomes and quality of life. ACCESS is a unique collective of scientists, healthcare providers, industry leaders, partner organisations, advocates, patients, and their families who are conducting research, changing policy, and developing new tools to ensure better lives for children who experience cancer in Canada and for their loved ones. ACCESS is driven and led by people with lived experience of pediatric cancer and framed by the patient journey. To date, ACCESS has unified a national community of more than 750 people and stimulated a culture of open communication in which diverse viewpoints, including those of people with lived experience (PWLE) of cancer, are sought and valued. The inclusion of the diverse perspectives and insights of people who have been directly affected by pediatric cancer drives meaningful improvements through research, policies, practices, processes and programs at all levels of ACCESS. Over 70 persons with lived experience guide and contribute to ACCESS’ 50+ projects. They hold leadership positions in all governance bodies of ACCESS, including as: PWLE Co-Chair of ACCESS; representatives on the ACCESS Senior Leadership Committee; co-leads of major projects and thematic areas; members the PWLE Advisory Committee; engage together in the ACCESS PWLE Community group; and participate in the national PWLE network. Together we will improve outcomes and quality of life for all children who experience pediatric cancer.

#ADV13 Survivor Turned Advocate: Importance of Advocacy and How to Prepare.

Lauren Bendesky

St Baldrick's Foundation, Monrovia, CA

After a diagnosis with stage IV neuroblastoma, Lauren turned to advocacy, using her voice to support others. Through her efforts, she identified gaps in training for advocates, with little integration of medical knowledge. Motivated by this, she worked to help establish the Creating Advocates through Research Education (CARE) Training Program, empowering others to make a meaningful impact.

#ADV14 Bridging the gap in pancreatic cancer: A perspective from a stage IV survivor on the importance of words, testing, and collaboration.

Elise Roth Tedeschi

Independent Advocate, Smyrna, GA

I'm a stage IV pancreatic cancer survivor who was given just a 1% chance of surviving five years. I beat those odds—and that experience shaped my mission: to ensure that patients are not only heard, but meaningfully involved in shaping the future of cancer care and research. In this poster, I emphasize the crucial need for enhanced collaboration among survivors, researchers, and oncologists. Far too often, patients are left confused, overwhelmed, and underinformed due to communication gaps within the healthcare system. In pancreatic cancer specifically, the rates of genetic and molecular tumor testing are still too low, especially in community practices compared to academic centers. Many patients are not receiving care that aligns with NCCN guidelines, which highlights a deeper issue of inconsistency and missed opportunities in treatment.

Survivors offer something that clinical data and textbooks can't: lived experience. When we're included in research design, clinical trials, and medical education, we help shape more relevant studies, build better support systems, and foster more compassionate care. Our perspectives bring humanity to the science, and that leads to better outcomes for everyone.

We are not just stories. We are not just statistics. We are experts in what the system often overlooks. By bringing survivors to the table as true partners, we can close critical gaps and create a future where care is not only scientifically sound but also deeply personal.

#ADV15 Long-term side effects of platinum-based chemotherapy drugs among people treated for colorectal cancer.

Michael Holtz

Fight Colorectal Cancer and Man Up to Cancer, St Louis, MO

Drugs like Oxaliplatin are commonly used in the treatment protocol for colorectal cancers. While the short-term side effects of these drugs are explained to patients, including cold sensitivity, fatigue and neuropathy, the long-term side effects are only now being understood. Long-term side effects can include permanent neuropathy, hearing loss, dental issues, cognitive issues, mental distress, heart problems. fatigue and more. My poster would examine the current research into long-term side effects and the potential gaps in research.

#ADV16 Addressing Colorectal and Anal Cancer Disparities in the LGBTQ+ Community.

David Russo

Cheeky Charity Inc., Palm Springs, CA

This poster will highlight Cheeky Charity's efforts to reduce health disparities in colorectal and anal cancer prevention, screening, and survivorship care for the LGBTQ+ community. It will showcase key initiatives, including the LGBTQ+ Community Cancer Needs Survey, which identifies barriers and motivators to cancer screening, and the PrideCare program, which provides culturally competent support services for patients, survivors, and caregivers.

The poster will also describe how data collected through the survey is used to inform education campaigns, foster partnerships with Federally Qualified Health Centers (FQHCs) and LGBTQ+ clinics, and advocate for health equity. By combining data-driven strategies with community engagement, Cheeky Charity is addressing stigma, mistrust of healthcare providers, and other systemic barriers to improve early detection and outcomes for LGBTQ+ individuals affected by colorectal and anal cancers.

#ADV17 Prevention Is the Cure: Many Paths to Wellness.
Judith A. Fitzgerald

Judith Fitzgerald (Individual)

My poster will represent the issues I am most passionate about in my advocacy work. It will reflect my passion for diet and lifestyle strategies to help with healing, whole body wellness, and prevent recurrence. I developed my “Wheel of Wellness” when I was invited to present in an online webinar hosted by NIH/NCI. The message is that there are many paths to wellness and it’s acceptable to take small steps to avoid feeling overwhelmed. I also advocate for less toxic treatments and better quality of life for patients. By participating in the FDA’s “Project Optimus” I endorsed the paradigm of “individualized oncology” where one size does not fit all. My hope for the future is that we achieve the primary prevention of breast cancer, so the disease never has a chance to start. My role as an advocate on the Phase I clinical trial of the alpha-lactalbumin breast cancer vaccine invented by Dr. Vincent Tuohy at Cleveland Clinic has been one of the greatest honors of my life. The greatest gift I can give my three granddaughters is a world without breast cancer.

: Advocates Poster Session 2
Poster Session

#ADV18 The AYA Cancer Experience: A Qualitative Exploration of Quality of Care.
Rebecca Munoz

Independent Advocate, Austin, TX

Adolescents and Young Adults (AYAs) with cancer face unique challenges in navigating their cancer journey. This qualitative multiple case study aimed to explore the quality of care experienced by AYA patients across pediatric and adult oncology settings. Through semi-structured interviews, I investigated the AYA experience across three key domains of the Donabedian model: Structure, Process, and Outcome. Findings revealed a complex landscape of care, highlighting the importance of patient advocacy, effective communication, and comprehensive support services. This research underscores the need for tailored interventions and policies to improve the quality of care for AYA cancer patients.

#ADV19 Hope For People Living With Metastatic Breast Cancer..

Aditya Manna

MAS Clinic and Hospital, Purba Medinipur, India

As in any developing countries state of West Bengal in India has a huge burden of metastatic breast cancer patients in advanced stage coming from rural area where awareness regarding the usefulness of palliative care in rather poor. Our goal is to give a pain free good quality of life in these advanced stage breast cancer patients.

#ADV20 Survivor Advocacy: Using Social Media to Break Stigma, Raise Awareness, and Empower Change in Cancer Care.

Aisha McClellan

Independent Advocate, Snow, NC

In this poster, I will explore how social media platforms, particularly TikTok, can be used to drive awareness, reduce stigma, and advocate for better cancer care. By sharing my personal journey as a cancer survivor, I've created a space to foster open conversations around the importance of early screening, treatment options, and support for those affected by cancer.

#ADV21 From Guidelines to Action: A Personalized Screening Intervention to Address Breast Cancer Disparities.

Tara Latta

My Density Matters, Hawthorn Woods, IL

The stark reality is that Black women have a 38% higher breast cancer mortality rate than White women, despite a 5% lower diagnosis rate (ACS, 2024-2025). This disparity is, in part, driven by later-stage diagnoses, which primarily result from inequities in breast cancer screening access and quality (ACS, 2024-2025).

To address the need for earlier surveillance, the American College of Radiology® (ACR®) updated its guidelines in 2023, recommending universal breast cancer risk assessments by age 25, with a particular focus on Black women, and early surveillance for high-risk individuals. However, there is no clear evidence of the implementation of these crucial guidelines.

To bridge this gap, I developed the Breast Cancer Screening Guide (BSCG) in 2023, guided by the 2023 ACR® guidelines. Designed to empower women of color in medically underserved areas of Chicago's South Side, the BSCG provides personalized recommendations, facilitates informed screening discussions with physicians, and includes a follow-up questionnaire to evaluate effectiveness and identify barriers.

To ensure the BSCG's ongoing optimization, we will employ a data-driven approach, analyzing pilot data to derive actionable insights for the next iteration of the BSCG. Future development will prioritize refining the questionnaire, enhancing the infographic, and actively engaging community members for feedback on cultural relevance and usability. These efforts will create a powerful tool to promote early detection and reduce breast cancer disparities.

#ADV23 Patients Make a Difference!.

Angus Pratt

Independent Advocate, Surrey, BC, Canada

Lung cancer remains the leading cause of cancer-related deaths in Canada, underscoring the urgent need for a unified approach to improve outcomes. The Canadian Action Plan for Lung Cancer represents a groundbreaking effort to incorporate patient perspectives into national cancer strategy development. This initiative, co-designed by patient advocates, researchers, clinicians, and policymakers, emphasizes the value of lived experience in shaping evidence-based solutions.

The development process began with a nationwide survey using open-ended questions. Patients, caregivers, clinicians, and policymakers shared their priorities, challenges, and unmet needs. Key themes emerged: reducing stigma, enhancing early detection, improving access to treatment, and investing in research for innovative therapies. These insights informed a second survey, in which a select group of constituents were asked to prioritize these themes.

The plan culminated in a one-day summit, during which Patient advocates worked alongside scientific and medical experts to cocreate actionable goals. The day included a mix of panels and workshops presenting specific issues and viewpoints.

To ensure inclusivity, special focus was given to engaging underrepresented groups, such as Indigenous communities and rural populations, whose experiences with lung cancer often differ. The resulting Action Plan integrates patient-centered recommendations with measurable objectives, such as increasing lung cancer screening participation rates, funding research on targeted therapies, and implementing supportive care programs.

This initiative exemplifies the transformative potential of patient-researcher partnerships in addressing complex health challenges. The Canadian Action Plan for Lung Cancer sets a precedent for future cancer strategies globally by amplifying patient voices and prioritizing equitable access.

#ADV24 Implementation and evaluation of a 365-day global cancer podcast for patients and survivors delivering research-backed strategies and comedy content daily in 131 countries..

Saranne Rothberg

The Comedy Cures Foundation, Tenafly, NJ

Cancer patients and survivors (CPAS) commonly report elevated levels of depression, stress, anxiety, loneliness, overwhelm, confusion plus unique stressors compared to other adults. This study tested the feasibility and efficacy of a free, global, on-demand, daily supportive podcast with comedy content.

The Beating Cancer Daily Podcast is streamed on the web and on major podcasting sites, delivering a free audio-guided intervention for those 18+ years old. Before launching, The ComedyCures Foundation sent out a press release, posted graphics on social media, and mentioned the podcast in several emailed newsletters. No listener compensation was provided. The program is designed to provide daily support, humor, and evidence-based strategies to individuals navigating a cancer diagnosis and survivorship. Hosted by stage IV cancer survivor, researcher, and humorist Saranne Rothberg, the podcast aims to empower listeners worldwide through the fusion of expert insights and clean comedy. Recognizing the emotional and informational gaps faced by cancer patients, survivors, and caregivers, the podcast provides 365 original daily episodes, creating a trusted, accessible, and uplifting global resource.

Beating Cancer Daily episodes have achieved remarkable organic global reach without paid advertising or marketing. The podcast has grown monthly by word-of-mouth, eventually helping listeners in 131 countries on 7 continents: North America, Europe, South America, Asia, Africa, Oceania, and recently in Antarctica - including listeners in regions experiencing major conflict, e.g., Ukraine, Russia, Middle East, Syria, Taiwan, Democratic Republic of Congo, Sub-Saharan Africa. Over 80% of listeners are on mobile devices with an average daily listen-through rate of 90% to 94%. Presently, over 8500+ websites are organically linking to podcast's main website. Enthusiastic public and private testimonials are received from listeners, including tennis star and recent survivor Gaby Dabrowski, highlighting the impact on improved mood, reduced isolation, and actionable takeaways. Other recognitions include: Rated 'Top 5 Cancer Podcast for Patients' 2024 & 2025 and '#1 Cancer Survivor Podcast' 2024.

Beating Cancer Daily demonstrates that accessible audio content, combining humor with research-backed strategies, can be a cost-effective and meaningful way to support CPAS globally every day. This podcast's rapid expansion by word-of-mouth, positive ratings and listener feedback validate its unique approach and the effectiveness of the host and survivor, Saranne Rothberg. Extensive audience surveys and examination of advanced listener demographics will bring enhanced understanding of the needs of CPAS.

#ADV25 Survivorship Science: A Call to Expand Pediatric Cancer Research Beyond Treatment.

Sarah Rostock

Independent Advocate, Norfolk, VA

Discuss the need for research into long-term survivor care, including addressing late effects, mental health, and quality of life. Advocate for a holistic approach to childhood cancer research that goes beyond just curing the disease.

#ADV26 Fostering positive and productive interactions between patients and researchers.

Terlisa Sheppard

Independent Advocate

To bring together a diverse audience to create a collaborative research environment with a long-term goal of developing curative approaches for metastatic breast cancer.

#ADV27 Why I left Cancer Advocacy, and Why I Came Back.

Sharon Batt

Independent Advocate, Halifax, NS, Canada

My poster uses my 35-year journey through cancer advocacy to explore how values and advocacy are intertwined. Following a breast cancer diagnosis in 1988, I spent a decade of intense involvement in the breast cancer movement in Canada and internationally. Disturbed by the commercialization of cancer advocacy, I disengaged to study, teach and write about health policy and the rise of “patient power.” My central concern is that corporate partners can shape patient perspectives on health policy to the detriment of patients. With a clearer understanding of what’s at stake, I have reengaged in health advocacy with like-minded patients, citizens, physicians and policy researchers. I co-founded the non-profit organization Independent Voices for Safe and Effective Drugs, became a research fellow at PharmedOut, a project to advance evidence-based prescribing based at Georgetown University's Medical Center, and began working with Common Sense Oncology, a cancer research and advocacy organization based in Kingston Ontario.

#ADV28 Empowering Underserved Communities: Bridging the Gap in Cancer Awareness and Support from Africa to the Diaspora.
Edith Meto'o

Independent Advocate, Longueuil, QC, Canada

Educational Outreach in Africa: Plans and initiatives to bring cancer awareness programs to African communities, focusing on early detection, prevention, and treatment options tailored to local cultural and healthcare realities.
Building Global Bridges: How collaboration between African and diaspora communities can improve access to care, knowledge sharing, and resource mobilization.
Survivorship Support Across Borders: Initiatives like wig donations, mental health resources, and wellness kits designed to support women undergoing treatment in Africa and the diaspora.
Advocacy for Equity in Healthcare: Using training from the Parole-Onco program at CHUM, the organization advocates for patient-centered care while addressing the challenges of stigma, limited resources, and access disparities in Africa.

#ADV29 Chemo at the Edge of the World: Caring for my Mom through Pancreatic Cancer on Haida Gwaii.

Dana Bellis

Canadian Institutes of Health Research, Ottawa, ON, Canada

This poster will feature the lived experience of providing cancer care through chemotherapy on Haida Gwaii, a remote northern archipelago off the coast of BC. By sharing my firsthand experience of caring for my mom through her pancreatic cancer, I will highlight the challenges and opportunities faced by a northern, rural, remote, and largely Haida community. The poster will address key issues related to accessing care, services, and supplies in a community setting, especially through the changing seasons and weather conditions. Key focus areas include barriers to travel, particularly the differences between winter and summer, and the personal costs involved in accessing care and supplies. The poster will overview our current chemo program on island, how chemo is flown in on a 1950's float planes each week, and how our two-seat chemo room provides service to our island of about 4,500 people. It will explore how mail is redefined as an essential service for basic needs, how continuity of care is supported during hospital diversions, and how a telepharmacy programs work to support people going through chemo. Finally, the poster will highlight the importance of leaning into community support and resilience to provide the best care possible.

The presentation of the poster will be done through using photos for visual understanding and impact, written text, and brief charts/graphs as needed to quantify relevant information.

This poster will share my lived experiences with the cancer research community, offering insight into what accessing chemotherapy and cancer care is like in Haida Gwaii. This goal is to help inform future research and foster reciprocal relationships, where researchers feel comfortable reaching out to ask questions and learn more about life on the edge of the world.

#ADV30 Integrating the Patient Voice in Training the Next Generation of Scientists.

Megan Sewell

Canadian Institutes of Health Research, Dollard-des-Ormeaux, ON, Canada

Inform and inspire junior cancer researchers on the advantages of patient partners and how the bi-lateral relationship will shape impactful research.

- Both researchers and patient partners learn from each other
- Patient partners feel empowered and valued
- Early engagement lays the groundwork for long-term collaborations

#ADV31 Community-led efforts examining the quality of life over time in young adults diagnosed with cancer..

Carolyn Van

Canadian Institutes of Health Research, Ottawa, ON, Canada

The Recover Study is a mixed-method and longitudinal study that seeks to engage young adults with lived/living experience with cancer to understand the holistic quality of life (e.g. physical, mental, social, and financial) over time and to identify recommendations (e.g. policy, practice, research, and programming) to support their recovery. The Recover Study is a Pan-Canadian 5-year cohort study that is informed by participatory action research (PAR) and patient-oriented research (POR).

Candidly is a podcast that sheds light on everyday scenarios and circumstances of those living life with and after cancer. From changes in work and finances, to friendships and dating, to fatigue and brain function, Candidly is a space for listeners to ask questions, gain diverse perspectives and food for thought from those with shared experiences and professional practitioners, and listen in on authentic human conversations to relate and connect with.

The Recover Study is led with Young Adult Cancer Canada (YACC), a national, non-profit organization dedicated to helping Young Adults (YAs) live better with, through and beyond cancer through various online and in-person services, including online groups, social events, intensive retreats, and survivor conferences designed to address the unique needs of this population.

Candidly is hosted by non-hodgkin lymphoma cancer survivor and health equity advocate, Carolyn Van and is available across all major podcast platforms. It is committed to shedding light on how various aspects of life are impacted by cancer while prioritizing the voices and perspectives of those living through it. Candidly addresses topics that are not always discussed amongst friends, family members, and in the public discord and calls those with various cultural, ethnic, sexual, educational and financial backgrounds into the conversation.

Carolyn Van hopes that through The Recover Study and Candidly, cancer futures will represent and function with a broadened, more nuanced and holistic understanding of recovery.

#ADV32 Smart Decisions about Breast Cancer.

Martina Wood

Canadian Institutes of Health Research, Ottawa, ON, Canada

My “Smart Decisions about Breast Cancer - choices, risks, living well, preventing recurrence” book helps patients navigate their BC journey proactively. Written in an easy to read format, with decision charts (if this, then⋯ - real steps for real people), practical tips, pathology report understanding, and research citations, this book is essential reading for anyone who wants to understand and participate in decision making, cope better, and achieve a sense of control over what happens to them.

I started my research when I was asked to make a treatment decision without any oncology knowledge, because it was so upsetting. This uncovered so much relevant information that I wanted to share it with other women and resulted in a 200+ page book. My advocacy started with supporting other women as I sat in waiting rooms, developed further when I was writing my book, and continued when I became an admin for a Facebook survivor group (12,000+ strong), and joined Canadian Cancer Trials Group (CCTG) as a patient representative. I recently also became a patient partner at Trillium Health Group (THP) in Mississauga for both the hospital group generally as well as the oncology group.

Both at CCTG and THP I’ve been involved in specific patient facing communications initiatives, and helped train other patient reps in how to review such documents when developed for new trials. I reviewed and scored cancer study proposals for funding approval on a one-year term of four sessions as a full member of the Clinical Trials Committee at CCTG. I have written letters of support for a young investigator and research proposals and supported numerous letters of support written by other patient reps at CCTG.

In 2024 I participated in workshops to identify the research priorities most important to patients, first with the CCTG Patient Rep Committee, which resulted in the August 2024 report “CCTG Patient Representative Priorities in Cancer Research”, and the second with the Canadian Cancer Society in November, which will be compiled in 2025 into a report that will contain recommendations of voices heard on the challenges associated with clinical trials in Canada. I have also had a video made by the College of American Pathologists, about my story, and CCTG about my role as a patient rep and the importance of clinical trials.

#ADV33 EmPOWERing Research that leverages scientist-survivor collaboration.

Marsha Biven

Powell-Drescher Ovarian Cancer Research Foundation, Seattle, WA

The goal of the Powell-Drescher Ovarian Cancer Research Foundation is to foster breakthrough ovarian cancer research that will meaningfully improve ovarian cancer outcomes. We believe that this breakthrough research requires close collaboration between individuals who experience the disease and scientists who are trying to address the problem. A major obstacle is that advocates - ovarian cancer survivors, patients, family members, caregivers, previvors, or other persons touched by ovarian cancer - who bring the experience of living with ovarian cancer do not typically interact with the scientists who are working on its prevention, detection, treatment and/or cure. We intend to address this obstacle through a range of innovative programs and projects as by working together, the physician and the patient, the scientist and the survivor, have the power to change the statistics.

#ADV34 Patient Advocate Advisors for ISPY 2.

Jan Tomlinson

University of California, San Francisco Carol Franc Buck Breast Care Center, San Francisco, CA

Dedicated Patient Advocate Advisors for the I-SPY 2 breast cancer clinical trial, bring the patient voice to the forefront of research design and implementation. Their work focuses on enhancing patient-centered communication, informed consent processes, and trial accessibility. Through close collaboration with researchers and trial participants, patient advocates help ensure that the lived experiences and priorities of patients shape the future of breast cancer treatment.

Tuesday, April 29, 2025

IMMUNOLOGY: CAR-T Cells
Poster Session

#1219 CAR-T migration and cytotoxicity in a vessel-cancer co-culture.
Luuk de Haan, Johnny Suijker, Todd Burton, Michel Ziegler, **Nick Saites**, Pleun Hombrink, Job komen, Karla Queiroz

Mimetas B.V, Oegstgeest, Netherlands

Several CAR-T therapies have been approved for hematologic malignancies. However, despite starting hundreds of clinical trials in solid tumors, no CAR-T therapy has been successful against solid tumors.[1] Efficacy in solid tumors is hampered by various factors such as antigen variation, lack of infiltration of T-cells in tumors from blood vessels, and an immune suppressive tumor micro-environment (TME)[2]. These factors lack in current *in vitro* and preclinical animal models. Therefore CAR-T cells are not evaluated on their efficacy with these critical parameters in place, which makes it impossible to select promising candidates for the clinic. This study aims to reduce the *in vitro-in vivo* gap by introducing these parameters in our vascularized tumor models. In earlier work we evaluated T-cell infiltration from a vessel towards chemotactic gradients and tumor cells[3]. In our organ-chip platform we now have created a model which contains a blood vessel, through which CAR-T cells can be perfused, and an adjacent co-culture of cancer tissue. The adjacent co-culture can consist of cancer cell lines, cancer cells and stroma, or primary tissue containing cancer cells and stroma to recapitulate the TME. With an EPCAM targeting second generation CAR-T, extravasation and migration into the tumor compartment could be quantified. Furthermore, after 72h the CAR-T cells reduced tumor volume in the EPCAM-positive HT29 colorectal cancer cell line, while volume of the EPCAM-negative A375 melanoma cell line grew. There was a clear dose response. The vasculature was shown to be intact at most concentrations of CAR-T cells, while being destroyed at highest concentrations. Ultimately there was a ‘therapeutic window’ in which the CAR-T cells reduced tumor mass but left a viable vessel. With the model the effects of modifications to the CAR-T cells or stimulatory cytokines could be evaluated. Changing the co-stimulatory domain from CD28 to 4-1BB reduced the potency of CAR-T, whereas increasing IL-2 concentrations in the expansion or treatment phase enhanced the potency of CAR-T. Hence, we created a co-culture of a vessel and cancer tissue for evaluating CAR-T functionality. In the future the system will be used for testing novel cell therapy variants in panels of patient cancers to support drug discovery and translational medicine by evaluating the efficacy and therapeutic index of different CAR-T variants and identifying responders.
[1] Saez et al. The changing landscape of cancer cell therapies: clinical trials and real-world data. Nature Reviews Drug Discovery (2024). [2] Hou et al. Navigating CAR-T cells through the solid tumor micro-environment. Nature Reviews Drug Discovery (2021). [3] De Haan et al. A Microfluidic 3D Endothelium-on-a-chip model to study transendothelial migration of T cells in health and disease. Int. J. Mol. Sciences (2021)

IMMUNOLOGY: Epigenetic Regulation of Tumor Immunity
Poster Session

#2226 Combined inhibition of MEK and HDACs improves the cytotoxicity of CD4 and CD8 T cells in NRAS;ASXL1-driven acute myeloid leukemia mice.

M. Bolisetti[1], J. Li[1], B. Jia[2], E. Keewan[1], E. A. Ranheim[3], K. V. Nadiminti[4], H. Zheng[2], X. You[1], J. Zhang[5];
[1]University of Wisconsin-Madison, Madison, WI, [2]Penn State Cancer Institute, Hershey, PA, [3]University of Wisconsin School of Medicine and Public Health, Madison, WI, [4]University of Wisconsin - Madison, Madison, WI, [5]University of Wisconsin Carbone Cancer Center, Madison, WI

Acute myeloid leukemia (AML) is a heterogeneous malignant blood cancer that occurs mainly in the elderly. Its treatment outcome is greatly influenced by leukemia-driving mutations. Oncogenic *NRAS* mutations are associated with both AML progression and multi-drug resistance and treatment failure in AML. We have previously developed a NRAS; ASXL1-driven secondary AML (NA-AML) mouse model, which is characterized by a suppressive immune microenvironment resulting in reduced leukemia killing activities of T cells. Targeting hyperactive RAS/MEK signaling via trametinib (T, a MEK inhibitor) attenuates the exhaustion of CD8 T cells and prolongs the survival of NA-AML mice. To further boost the efficacy of T, we performed a re-purpose screen of ~2,500 drugs, either approved by FDA for treating various human diseases or currently under clinical evaluation, in the absence or presence of T. We identified that quisinostat (Q), a 2nd generation of HDAC inhibitor, potently inhibited the growth of both mouse and human NA leukemia cells and significantly synergized with T in vitro. In addition, combined T and Q (TQ) downregulated AP-1 transcription factors and expression of immune checkpoint ligands (PD-L1/L2, CD80, and CD86) while upregulated expression of MHC I/II in mouse NA-AML cells. We further validated our results in NA-AML mice. Vehicle (Veh) - treated mice rapidly succumbed to AML and died within 4-5 weeks post-treatment. T alone and Q alone slowed down AML progression and prolonged the survival of leukemia mice, while all TQ-treated mice remained alive ~22 weeks post-treatment. Surprisingly, TQ only provided moderate survival benefits in immunodeficient NSG mice engrafted with NA-AML cells, suggesting an important role of cytotoxic T cells in TQ-mediated AML alleviation. We then analyzed CD4 and CD8 T cells from moribund vehicle-treated mice and age-matched drug-treated mice. TQ decreased the exhaustion of CD4 and CD8 T cells and expanded the activated effector/memory T (Tem) cells. Single-cell RNA-Seq analysis showed increased expression of gene signatures associated with cytotoxicity of Tem cells, including CXCR3 and CXCR5. Consistent with these findings, leukemia: T cell co-culture experiments demonstrated significantly enhanced cytotoxicity of TQ-treated CD4 and CD8 T cells, which appeared to be mediated by increased expression of MHC I and II in AML cells. We are currently pursuing the underlying molecular mechanisms. Our data provide a strong rationale to develop immunotherapies via epigenetic modulation of both leukemia and leukemia-associated T cells.

TUMOR BIOLOGY: Angiogenesis
Poster Session

#3836 Discovery of a highly potent fully human VEGF VHH antibody from RenNano transgenic mice that exhibits a superior blocking activity on VEGF function.

Yi Yang, Weiqiu Lan, Baihong Liu, Yang Chen

Biocytogen, Waltham, MA

Background: VEGFA (or VEGF hereafter) is the prototypic member of a family that also includes placental growth factor (PLGF, also known as PGF),VEGFB,VEGFC,VEGFD . **VEGFR2** is the major mediator of the physiological and pathological effects of VEGF. VEGFR2 mediates EC proliferation, migration and arterial fate specification [1]. VEGFA monoclonal antibodies have significant therapeutic effects in the treatment of tumors and macular degeneration [2]. In this study, we evaluated a fully human anti-VEGFA mAb generated from our fully human antibody RenNano^{TM} mice.
Methods: Blocking activity of the anti-VEGFA mAb against VEGF/VEGFR2 interaction was assessed using ELISA. The affinity to human VEGFA165 was measured by surface plasmon resonance (SPR). At the same time, we conducted relevant physicochemical property tests on the obtained nanobodies, including purity, Tm value, Tagg, and hydrophobicity assessment. To evaluate the in vitro inhibitory effect of anti-VEGFA on endothelial cell proliferation, we used human umbilical vein endothelial cells (HUVEC) for the experiment.
Results: We identified an anti-VEGFA nano-antibody with high affinity to human VEGFA165,and it was able to block the interaction of VEGFA and VEGFR2 in vitro. Our antibody molecule exhibits desirable physicochemical properties, as we found that the purity is above 95% after one step of affinity chromatography. Furthermore, our anti-VEGFA nano-antibody exhibited a strong inhibitory activity on the proliferation of HUVEC cells compared to Bevacizumab analog.

#3837 Defining the mechanism of semaphorin4D tumorigenesis in prostate cancer models.

Kavin Velur Ganesan, Maolake Aerken, Renyuan Zhang, Kent L. Nastiuk

Cancer Genetics and Genomics, Roswell Park Comprehensive Cancer Center, Buffalo, NY

Background: Advanced and recurrent prostate cancer exhibits a “cold” immune phenotype characterized by an influx of immunosuppressive cells and fewer tumor-infiltrating lymphocytes (TILs), limiting the efficacy of checkpoint inhibitors. Semaphorin4D (SEMA4D/CD100), via the PlexinB receptors, regulates cell-cell interactions and migration in the nervous system, also responsible for cancer metastasis. Further, SEMA4D affects angiogenesis and immune regulation in other cancers. We hypothesize that SEMA4D promotes the recruitment of immunosuppressive cells via tumor blood vessels, further impairing TIL activity and anti-tumor immunity. Loss of SEMA4D function could disrupt vascularization, creating hypoxia that induces tumor cell apoptosis.
Methods: Two mouse models—subcutaneous Myc-CaP allografts in immunocompetent mice and Hi-Myc prostate cancer GEMM were treated with a SEMA4D blocking mAb (pepinemab) to inhibit SEMA4D signaling. Tumor volume changes, vascularity, and oxygen saturation were assessed during treatment using high-frequency ultrasound, Power doppler, and photoacoustic imaging followed by FACS and IHC analyses on tumor tissues to evaluate immune cell phenotypes, hypoxia, apoptosis, and microvascular damage. To further elucidate genotypic changes in the tumor immune microenvironment, RNA sequencing gene expression changes associated with SEMA4D inhibition, including signaling pathways and Immune cell constituents.
Results: Blocking SEMA4D significantly arrested tumor growth and reduced vasculature in Hi-Myc mice, with no prominent changes in immune phenotype by FACS after 4 weeks pepinemab treatment. IHC will test for changes in hypoxia, apoptosis, and microvascular damage. Allografts of prostate tumor cells similarly demonstrated tumor growth arrest. Effects on vasculature and microvascular integrity, leading to hypoxia and apoptosis, in this more tractable imaging environment, are anticipated. Transcriptomically, gene ontology and enrichment analyses may reveal pathways related to vessel formation, immune regulation, and metastasis.
Conclusion: Our findings suggest that SEMA4D inhibition effectively arrests tumor growth by disrupting angiogenesis and potentially regulating immune activity, alongside inducing hypoxia and apoptosis. Ongoing transcriptomic analyses will further explore molecular mechanisms, including pathways involved in angiogenesis and immune regulation.

#3838 KRAS dependency and thrombospondin-1 levels are inversely associated with TIMP1.

Ilamathi M. Thirusenthilarasan[1], Bilal Siddiqui[2], Pankaj Ahluwalia[3], Ravindra Kolhe[4], Amyn M. Rojiani[5], Mumtaz V. Rojiani[6]

[1]Pathology, Penn State Health Milton S. Hershey Med. Ctr./Penn State Cancer Institute, Hershey, PA,[2]Pathology, Penn State Health Milton S. Hershey Med. Ctr./Penn State College of Medicine, Hershey, PA,[3]Pathology, Medical College of Georgia, Augusta University, Augusta, GA,[4]Pathology, Medical College of Georgia/Augusta University, Augusta, GA,[5]Pathology, Penn State Health Milton S. Hershey Med. Ctr., Hershey, PA,[6]Pharmacology and Pathology, Penn State College of Medicine/Penn State Cancer Institute, Hershey, PA

Beyond its role as an inhibitor of matrix metalloproteinases, tissue inhibitor of metalloproteinase 1 (TIMP1) has been associated with poor prognosis in many cancer types including non-small cell lung cancer (NSCLC), and substantial evidence suggests it plays a role in tumor progression. The most frequent mutation identified in NSCLC is KRAS. Recently, we described an inverse relationship between expression of KRAS and TIMP1 in NSCLC cell lines. These cells fall into two distinct categories, KRAS dependent and KRAS independent, depending upon their need for sustained expression of KRAS or if the cells have become independent of KRAS expression. We have shown that KRAS-dependent cells with high levels of KRAS, exhibit lower levels of TIMP1. Inversely, KRAS-independent cells with low levels of KRAS show high levels of TIMP1. We have demonstrated that modulation of TIMP1 using knockdown clones or TIMP1 overexpressing cells, alters KRAS dependency features. In the present study, based on preliminary observations on TIMP1 and angiogenesis, we postulate a similar inverse relationship between KRAS-dependent cells expressing high levels of Thrombospondin-1 (TSP1), whereas the independent cells expressing low levels of TSP1. Overexpression of TIMP1 led to a decrease in thrombospondin (TSP1) levels, while TIMP1 knockdown resulted in an upregulation of TSP1. Furthermore, serum-free conditioned media (SFCM) from TIMP1 overexpressing (OE) cells significantly enhanced capillary networking tube formation of HUVEC cells in Matrigel, indicating increased angiogenic potential. By contrast, SFCM from TIMP1 knockdown (KD) clones reduced capillary networking and tube formation compared to parental cells. Additionally, CDH5 (VE-cadherin), a marker of endothelial cells, was upregulated in TIMP1-OE cells and downregulated in the TIMP1-KD clone relative to parental cells. These findings suggest that TIMP1 modulates endothelial cell behavior, likely through regulation of TSP1 and CDH5. In KRAS dependent cells, we also observed the upregulation of VEGFα secreted in the SFCM of TIMP1-OE cells. Furthermore, we investigated the clinical significance of TSP1 and TIMP1 in the TCGA LUAD dataset. Immune deconvolution analysis revealed that TIMP1 expression is associated with a higher infiltration of immune cells, along with a significant enrichment of exhausted immune phenotypes compared to TSP1. These findings highlight the dichotomy between TIMP1 and TSP1, demonstrating both quantitative and qualitative impacts on immune infiltration, with crucial therapeutic implications. Future studies will be directed at investigating the underlying mechanisms linking KRAS dependency and TIMP1 in angiogenesis along with the complex interplay of TSP1, which could provide novel insights into therapeutic strategies targeting TIMP1 to impair angiogenesis and tumor progression in NSCLC.

#3839 MIG-6 plays as a tumor suppressor to inhibit angiogenesis by downregulating HDAC1 in PTEN-deficient endometrial cancer.

Shamsun Nahar[1], Jiyoung Yu[2], Haeam Lee[1], Rong Li[1], Jin-Seok Jung[3], Tae Hoon Kim[1], Kyunggon Kim[2], Jung-Yoon Yoo[3], Jae-Wook Jeong[1]

[1]Univ. of Missouri School of Medicine, Columbia, MO,[2]Asan Medical Center, Seoul, Korea, Republic of,[3]Yonsei University Mirae Campus, Wonju, Korea, Republic of

Endometrioid endometrial cancer (EEC) is the most prevalent gynecological malignancy. Metastatic and recurrent endometrial cancer is incurable with currently available standard therapies. While *Mig-6* mutation develops distant metastasis and recurrence of EEC with *Pten* deficiency, *Mig-6* overexpression ($Pten^{d/d}Mig\text{-}6^{over}$ mice) suppresses tumor development. Therefore, there is an urgent need to explore the mechanisms of tumor metastasis and recurrence to further elucidate the progression of EEC. We performed RNA-seq analysis to identify targets of *Mig-6* in $Pten^{d/d}$ and $Pten^{d/d}Mig\text{-}6^{over}$ mice. The transcriptomic analysis identified 104 and 518 genes significantly increased or decreased transcripts in $Pten^{d/d}Mig\text{-}6^{over}$ mice compared to $Pten^{d/d}$ mice. The pathway analysis of these dysregulated genes showed significant inhibition of immune, inflammatory, and angiogenesis pathways in $Pten^{d/d}Mig\text{-}6^{over}$ compared to the $Pten^{d/d}$ mice group, where the key regulator was hypoxia-inducible factor-1 (HIF1A). Immunohistochemical analysis of HIF1A and PECAM1 validated a significant reduction of angiogenesis in $Pten^{d/d}Mig\text{-}6^{over}$ mice. Moreover, combined interactome and immunoprecipitation analyses revealed that MIG-6 directly binds with histone deacetylase 1 (HDAC1) in the EEC of $Pten^{d/d}Mig\text{-}6^{over}$ mice. Our double immunofluorescence results confirmed colocalization of two proteins in EEC. Finally, we evaluated the therapeutic effect of Panobinostat, a HDAC inhibitor, in EEC of $Pten^{d/d}$ mice. Our results demonstrate the tumor suppressor role of MIG-6 as an HDAC1 interacting protein in angiogenesis of EEC. Our findings highlight potential molecular targets of EEC that could significantly enhance therapeutic strategies for endometrial cancer.

#3841 Unique and redundant roles of Notch1 and Notch4 in retinal angiogenesis.

Nivedha G. Krishnan, Christie Kang, Taliha Nadeem, Braulio Aguilar, Krishna Thakkar, L.A. Naiche, Jan K. Kitajewski

Physiology and Biophysics, University of Illinois College of Med. at Chicago, Chicago, IL

Angiogenesis plays a vital role in the progression of cancer: the proliferation and metastasis of tumor cells relies on extending the vascular network that supplies oxygen and nutrients necessary for growth. Understanding the modulators and regulators of angiogenesis thus allows us insight into the mechanisms of a developing cancer. Notch signaling plays a critical role in the mediation of angiogenesis. Of the four Notch genes, the mechanisms of the Notch1 pathway are most well-understood, whereas the impact of Notch4 regulation on the vasculature is poorly studied and has shown contradictory phenotypes. In recent work, we have shown that an anti-Notch4 antibody, E7011, reduces tumor growth in multiple tumor models that express Notch4 in tumor endothelium and changes the number and phenotype of tumor associated macrophages. To better understand the role of Notch4 in angiogenesis, we have examined endothelial-specific loss of Notch4 during developmental retinal angiogenesis and determined that loss of Notch4 has substantially different effects than loss of Notch1. Loss of Notch4 is anti-angiogenic, characterized by reduced radial outgrowth of vessels and decreased vessel density, in contrast to the loss of Notch1, which results in hypersprouting and increased density. However, the structural similarity and common transcriptional targets of Notch1 and Notch4 suggest they have overlapping functions that are not evident until both genes are removed. To investigate the distinct and overlapping roles of Notch1 and Notch4, we generated mice with endothelial-specific knockouts of Notch1 (Notch1ECKO), Notch4 (Notch4ECKO), or both genes (Notch1Notch4ECKO). We harvested retina from mice at postnatal day (P)5, P10, and P12 timepoints to analyze vascular growth in the superficial and deeper plexus of the retina. Harvested retina were stained for Isolectin B4 to identify vasculature and Iba1 to identify microglia and analyzed for vascular growth measurements, including density, outgrowth, and tip cell counts. Quantitative analysis of the postnatal day 5 timepoint showed a highly abnormal double-mutant Notch1Notch4ECKO phenotype characterized by a significant reduction in radial outgrowth and a significant increase in tip cell density at the vascular front. Qualitative analysis suggested diminished vascular density near the center of the retina. Ongoing analysis is defining changes unique to Notch4ECKO, Notch1ECKO, and Notch1Notch4ECKO to clarify each gene’s individual function in angiogenesis.

#3842 ZNF217 promotes breast tumor progression by facilitating angiogenesis.

Ann Dharshika Shangaradas, Parinda Tennakoon, Margaret A. Schwarz, Laurie E. Littlepage

Department of Chemistry and Biochemistry, University of Notre Dame, South Bend, IN

The transcription factor ZNF217 is amplified in 20-30% of breast cancers and is identified as a putative oncogene. Its overexpression accelerates tumor progression, metastasis, and chemoresistance *in vivo* and correlates strongly with poor prognosis in patients. To identify growth factors that are released from mammary epithelial cells that overexpress ZNF217, we analyzed the conditioned media from MCF7 cells with or without knockdown of ZNF217 using a 71-Plex Discovery assay. Conditioned media from MCF7 cells deficient for ZNF217 showed a significant downregulation in the secretion of VEGF-A, PDGF-AA, and PDGF-AB/BB and was validated by ELISA in conditioned media from both MCF7 cells and in MDA-MB-231 cells that overexpress ZNF217. VEGF-A, PDGF-AA, and PDGF-AB/BB are key components in promoting angiogenesis, a key hallmark of cancer. RNA sequencing data and qRT-PCR revealed no significant difference in the transcriptional expression of VEGF-A and PDGF-AA/AB/BB after ZNF217 knockdown. To evaluate if the secretion of the identified angiogenic factors promotes endothelial cell sprouting, we co-cultured endothelial progenitor cells with the conditioned media from MCF7 cells ± siZNF217. Endothelial cells treated with media from MCF7 ± siZNF217 induced a sprouting phenotype in endothelial cells grown in conditioned media from ZNF217 expressing cells compared to knockdown cells. Together, these data suggests that ZNF217 is a promotor of VEGF-A secretion and promotion of angiogenic phenotypes in endothelial cells. We also evaluated the role of ZNF217 in the expression of the VEGFR-2 signaling. Knockdown of ZNF217 also decreased the expression of KDR (VEGFR-2 receptor for VEGF-A) in MCF7 cells, suggesting a potential autocrine signaling role for VEGF-A in the cancer cells. Single cell RNA sequencing data of mammary tumors derived from orthotopic injection of PyMT±Zfp217 revealed an increased population of endothelial cells in FVB mice injected with Zfp217 overexpressing cells compared to vector expressing control cells. These findings highlight a novel role for ZNF217 as a promoter of angiogenesis during breast cancer progression. Thus, unravelling the mechanisms of ZNF217-mediated angiogenesis will be an instrumental step in determining if anti-angiogenic therapies are an effective and novel strategy used to overcome metastatic growth in breast cancer patients with tumors that overexpress ZNF217.

#3844 Tumor endothelial cell lines and their use for the identification of new tumor vascular targets during dysbiosis.

Samir V. Jenkins, Mohammad Alimohammadi, Kieng B. Vang, Robert J. Griffin, **Ruud P. Dings**

UAMS Winthrop P. Rockefeller Cancer Institute, Little Rock, AR

The overall use of antibiotics has increased by more than 30% in recent years according to the centers of disease control and prevention (CDC). Moreover, many cancer patients are prescribed antibiotics, as a prophylactic or to treat infection, a frequent complication during cancer progression and treatment. Yet, tumor endothelial cell lines are scarce at best and tumor endothelial cell lines derived under dysbiotic pressure non-existent. In order to address this we generated ‘normal’ or orthobiotic and dysbiotic tumor endothelial cell lines. After validating their endothelial cell origin on a genomic, proteomic and functional level, we found that tumor endothelial cells derived from dysbiotic mice (TEC-Dys) were more sensitive to hypofractionated radiation doses (i.e. 6 Gy) as compared to tumor endothelial cells derived from orthobiotic mice, TEC-Ortho. In order to identify relevant tumor vasculature drug targets during dysbiosis, we used tandem mass tag mass spectroscopy and focused on the extracellular proteins significantly overexpressed in TEC-Dys, as compared to TEC-Ortho. C-Met fulfilled these criteria and we validated this histologically by comparing tumors with or without dysbiosis. Moreover, c-Met inhibitors Foretinib, Crizotinib, and Cabozantinib were significantly more effective against TEC-Dys than TEC-Ortho. Foretinib inhibited tumor growth to a greater extend during dysbiosis as compared to orthobiotic conditions. Thus, we surmise that tumor response in dysbiotic patients may be greatly improved by targeting dysbiosis-induced pathways, such as c-Met, as compared to the targets suppressed due to dysbiosis. These matched tumor endothelial cell lines generated under orthobiotic and dysbiotic conditions can now be used in in vitro and in vivo cell-based models, and provide an improved and specialized platform for current and next-generation anti-cancer drug discovery and development.

#3845 A matching panel of tumor organoids and patient derived orthotopic xenograft mouse models (PDOX) of high-grade gliomas in EGFP-based SCID mice.

Xin (Alice) Zhai, Milagros Suarez Palacios, Zilu Huang, Jinnan Chen, Tongchao Jiang, Nitin Wadhwani, Michael DeCuypere, Yuchen Du, Xiaonan Li

Northwestern University - Chicago, Chicago, IL

Background: High grade glioma, also known as glioblastoma multiform (GBM) a highly aggressive brain tumor, requires advanced systems to study tumor-stroma interactions. Paired in vitro and in vivo model systems such as 3D organoids and PDOX models derived from EGFP-SCID mice offered a unique opportunity to precisely and rapidly differentiation of mouse (EGFP positive) from human tumor cells, thereby facilitating the visualization and analysis of tumor/normal cell interactions in vitro and in vivo.
Methods: EGFP-SCID mice received intra-cranial implantation of pediatric (n=6) and adult (n=6) GBM tumors to form PDOX. Tumor-bearing mouse brains were dissected, and tumor cells were cultured to generate organoids by seeding at cell densities of 1250, 2500, 5000 cells/well and monitored for size and count over 1-21 or 28 days. Immunohistochemistry (IHC) tracked the distribution of EGFP-positive mouse cells and GBM markers, including GFAP, Nestin, Ki67, GFP, $CD276^{+}$ and so on. Additional staining for lysosomes and nuclei (Hoechst/DIPA) confirmed cellular localization and interactions.
Results: Hybrid organoids were successfully generated, integrating mouse and human GBM cells in 12 PDOX models. Organoid size and count varied by cell density and culture duration, with similar growth patterns between pediatric and adult samples. IHC confirmed expression of cellular proliferation (Ki67), glial cells (GFAP), differentiation (SYN), stem cell (Nestin) as well as normal mouse cells (GFP) that were in and surrounding tumor cells. In organoids, EGFP fluorescence revealed dynamic tumor-stroma interactions, with changing proportions of mouse cells and GBM cells over time, reflecting the organoid's developmental complexity.
Conclusion: This EGFP-based organoid and PDOX model system offers a robust platform for studying tumor-stroma interactions and characterizing cellular dynamics in GBM. The system's integration of IHC provides a detailed view of tumor and stromal components, making it a valuable tool for biological and translational GBM studies.

#3846 BNIP3-dependent muscle atrophy in cancer cachexia promotes PDAC progression.

Alexandra Sophia Ware[1], Elizabeth C. S. Foster[2], Kay F. Macleod[2]

[1]University of Chicago, Chicago, IL,[2]Ben May Department for Cancer Research, University of Chicago, Chicago, IL

Cancer cachexia is a debilitating effect of several human cancers, including pancreatic ductal adenocarcinoma (PDAC), that involves involuntary body weight loss due to wasting of skeletal muscle (with or without loss of adipose tissue). Cancer cachexia negatively affects the long-term survival of cancer patients, partly due to reduced tolerance of and response to therapy, and increased mortality due to cardiac and respiratory failure. Here, we present new data demonstrating a role for the mitochondrial cargo receptor BNIP3 in driving muscle atrophy during cachexia caused by PDAC in genetically engineered mouse models. Muscle-specific deletion of BNIP3 in CB mice (Mck-Cre;Bnip3$^{fl/fl}$) inhibited muscle atrophy in the KPF (FSF-KRas^{G12D};p53 $^{Frt/+}$;p48-FlpO) mouse model of PDAC such that skeletal muscle in age-matched compound KPFCB mice (FSF-KRas^{G12D};p53 $^{Frt/+}$;p48-FlpO; Mck-Cre;Bnip3$^{fl/fl}$) exhibited increased weight, increased myofiber cross-sectional area, and decreased expression of muscle atrogenes MuRF1 and Atrogin1, compared to KPF mice. Orthotopic injection of KPF and KPC PDAC lines and sub-cutaneous injection of LLC lung tumor lines similarly revealed key differences in muscle atrophy between WT and CB mice. The sustained health of myofibers in KPFCB mice compared to KPF mice was associated with increased mitochondrial mass, increased mTOR activity, and decreased autophagy markers. In addition to limiting muscle atrophy, loss of BNIP3 in muscle attenuated PDAC tumorigenesis with tumors exhibiting delayed progression from pre-malignant pancreatic intra-epithelial neoplasia (PanINs) to PDAC, and muscle-specific loss of BNIP3 extended lifespan of KPF mice. These findings indicate that degradation of muscle driven by BNIP3-dependent mitophagy is “feeding” the PDAC tumor via factors circulating in plasma. Steady state metabolomics revealed that purines were amongst the most down-regulated metabolites in muscle and plasma of cachectic mice consistent with a model in which BNIP3-dependent mitophagy in muscle is limiting 1C metabolism due to decreased mitochondrial mass. Together, our findings identify BNIP3 as a molecular target in muscle for therapeutic intervention to limit both cancer cachexia and tumor progression.

#3847 APE1 mediates angiogenesis by stabilizing SMAD3 and blocking its ubiquitin induced degradation in esophageal adenocarcinoma.

Farah Ballout, Heng Lu, Nadeem Bhat, Lei Chen, Dunfa Peng, Zheng Chen, Alexander Zaika, Oliver McDonald, Wael El Rifai

University of Miami Miller School of Medicine, Miami, FL

Background: The incidence of esophageal adenocarcinoma (EAC) is rapidly rising in the United States and Western countries. Unfortunately, EAC has one of the lowest survival rates due to limited treatment options. Identifying disease drivers through functional studies is crucial for improving treatment options. This study aimed to explore the role of APE1 in regulating SMAD3 and promoting EAC progression.

Methods and Results: Analysis of publicly available datasets revealed that APE1 and SMAD3 are upregulated and positively correlated in EAC compared to normal samples. Immunofluorescence staining of esophageal cancer progression tissue arrays confirmed significantly higher levels of APE1 and SMAD3 in EAC than in normal tissues. Western blot analysis showed that silencing APE1 reduced SMAD3 nuclear expression, which was also confirmed by immunofluorescence staining showing a loss of nuclear SMAD3 accumulation following APE1 knockdown. Immunoprecipitation and proximity ligation assays indicated a direct interaction between APE1 and SMAD3 in the nucleus. Further analysis revealed that APE1 binds to the C-terminal MH2 domain of SMAD3, protecting it from ubiquitin-mediated proteasomal degradation by preventing its interaction with the RING finger protein, ROC1. Notably, inhibiting APE1's redox or endonuclease activity with pharmacological inhibitors or mutants disrupted APE1-SMAD3 binding, suggesting a conformational regulation. Interestingly, knocking down APE1 reduced angiogenesis in tube formation assays and 3D co-cultures; however, this effect was reversed by overexpressing SMAD3, suggesting that APE1 regulates angiogenesis through SMAD3.

Conclusion: The findings establish the role of APE1 in regulating SMAD3 in EAC and suggest a potential therapeutic approach for treating EAC patients.

#3848 Smoking promotes angiogenesis and epithelial to mesenchymal transition in breast cancer.

V. Katkoori[1], S. R. Kandukuri[1], M. V. Burra[1], J. E. Trosko[1], D. M. Tamkus[2], C. Leece[1], L. R. Kareti[1], G. S. Abela[1], H. L. Bumpers[1];
[1]Michigan State University, East Lansing, MI, [2]John H. Stroger, Jr. Hospital of Cook County, Chicago, IL

Introduction: Nicotine, the addictive component of cigarettes, induces tumor progression via the α7-nicotine acetylcholine receptor (α7-nAChR) subtype in lung cancer. However, the molecular mechanisms by which nicotine impacts tumor progression of breast cancer (BC) remain unclear. In this study, we evaluated the effects of nicotine/smoking on tumor angiogenesis, and epithelial-mesenchymal transition (EMT) signaling mechanisms that are involved in BC progression.
Experimental Design: BC cell spheroids from the MCF7, a non-invasive cell line was treated with nicotine (0.3 to 3μM) for 14 days. Sections of tumor tissue from the human BC (smoking vs. never smoking) were assessed for phenotypic expressions of α7-nAChR, and vimentin by immunostaining (IHC). CD31 staining was used to evaluate tumor angiogenesis, as measured by microvessel density (MVD). Western blot (WB) analyses were also performed on lysates of both cell lines and tumors to assess the effect of nicotine on the signaling pathways that promote BC progression.
Results: The present study revealed that nicotine treated spheroids have increased expression of Ki67, Mdr1, Oct4 signatures that are indicative of BC progression. IHC analysis indicated that tumor from patients that smoked displayed increased expression of α7-nAChR. Furthermore, the expression of Oct4 (marker for cancer stem-cell), and vimentin (marker for EMT) that correlates with advanced disease and metastasis were found to be higher in smokers. However, tumors from non-smokers had low expression of Oct4 and vimentin. It was also found that tumors from patients who smoked had well established vascularity, while tumors from non-smokers had very little vascularization. Indeed, the mean MVD was lower in tumors from non-smokers compared to tumors from smokers.
Conclusions: These findings offer significant novel insights into the mechanism by which nicotine contributes to the increased aggressiveness of BC. This work was supported in part by NIH/NCI Workforce Diversity Grant R21-CA171251.

#3849 Pygo2 deletion stimulates tertiary lymphoid structure formation and forms the cornerstone for a quadruple combination immunotherapy of prostate cancer.

Yan Liu, Ziyu Zeng, Yini Zhu, Xin Lu

Department of Biological Sciences, University of Notre Dame, South Bend, IN

Introduction: Advanced prostate cancer (PCa) shows overwhelming *de novo* resistance to immune checkpoint blockade (ICB). We previously identified Pygopus 2 (Pygo2) as a critical epigenetic regulator in PCa to orchestrate the Pygo2/p53/Sp1/Kit/Ido1 pathway to foster a microenvironment hostile to cytotoxic T lymphocytes (CTLs), and inhibiting Pygo2 genetically or pharmacologically sensitized PCa mouse models to ICB and adoptive CTL transfer therapy (Sci Immunol. 2023; PMID: 36897957). Here, we continued this line of research and examined the effect of Pygo2 targeting on castration (androgen deprivation therapy), anti-MDSC therapy and ICB in genetically engineered mouse models.

Methods: We crossed Pygo2 conditional null allele to the PB-Cre/Pten/Smad4 (DKO) mice and generated PB-Cre/Pten/Smad4/Pygo2 (TKO) PCa mouse model. Using this model, we performed a series of combination treatments including ICB, surgical castration, and anti-MDSC therapy SX682 (Cxcr1/2 inhibitor). Various immune-phenotyping techniques (single-cell RNA-seq, bulk RNA-seq, flow cytometry and immunohistochemical staining) were performed on treated tumors.

Results: The best tumor protection phenotype came from the combination of Pygo2 deletion, castration, ICB and SX682 treatment. This quadruple combination regimen led to nearly curative outcome, rendering the mouse tumors extensively necrotic. scRNA-seq and IHC revealed that Pygo2 deletion promoted the formation of tertiary lymphoid structures (TLS) within the tumor microenvironment. The quadruple combination demonstrated the highest level of infiltration of B cells and CTLs. Emerging data suggest that Pygo2 loss instigates cancer-cell-intrinsic signaling distinct from the previously identified Kit/Ido1-dependent pathway and connects an antiviral mimicry state of the cancer cells to adaptive tumor immunity that culminates in TLS formation and maturation.

Conclusions: Our study further consolidates Pygo2 as a valid and promising therapeutic target in combination immunotherapy of prostate cancer. The quadruple combination immunotherapy composed of Pygo2 inhibition, androgen deprivation therapy, SX682 and ICB demonstrates superior preclinical efficacy and should be further investigated for its clinical implications.

#3850 Aberrant tumor pericytes in pancreatic ductal adenocarcinoma-a barrier and opportunity for therapeutic advances.

Vikneshwari Natarajan, Sangdeuk Ha, Jiha Kim

North Dakota State Univ., Fargo, ND

There is a lack of effective treatment for PDAC, often due to abnormal and leaky blood vessels and a lack of proper pericyte coverage, contributing to the inability of the drugs to reach the tumor. We and others showed that pericyte (PC) coverage significantly correlates with vascular integrity, function, and intratumoral hypoxia. However, in PDAC, the PC-endothelial (EC) cell communication is decreased or non-existent, thereby affecting drug delivery and immune function. We propose to re-program tumor-associated pericytes to normalize the tumor blood vessels and revert them to an optimal functional state. We previously showed that tumor-associated pericytes in PDAC exhibit ectopic αSMA expression up to ten times higher than normal pericytes, correlating strongly with vascular instability and hypoxia. Due to significant involvement of αSMA in cellular morphology and structure, tumor-associated pericytes presented biomechanical abnormalities, which diminished intimate association with endothelial cells during tube formation assay. We employed scRNA sequencing to define the pathological signature of tumor-associated pericytes and identify therapeutic targets for vascular normalization. Our analysis revealed considerable heterogeneity in lung pericytes compared to PDAC pericyte clusters, indicating a disrupted vessel hierarchy. Additionally, we identified a distinct pericyte subpopulation characterized by elevated expression of αSMA, MCT1, and gap junction proteins. Alterations in basement membrane gene expression and smooth muscle markers further emphasized the disrupted vascular architecture and impaired pericyte-endothelial cell adhesion within the tumor microenvironment. Our data reveals consistently upregulated monocarboxylate transporters in vascular cells. Therefore, we investigated lactate transporters as a potential target for improving PC-EC adhesion. We have shown that in PDAC, MCT1 lactate exporters are upregulated in EC and PCs. The high amount of lactate produced by PCs decreases the pH in the basement membrane. So, we knocked down MCT1 transporters in PCs to increase basement pH, leading to improved PC-EC attachment and pericyte homeostasis. In conclusion, our study indicates that tumor-associated pericytes undergo phenotype switching and disturbed metabolic communication under the influence of pancreatic cancer cells. These aberrant pericytes might contribute to non-optimal vascular integrity and function. Single-cell RNA sequencing helped understand the molecular signature of the PDAC pericyte, providing a powerful tool to develop novel targeting strategies for vascular normalization. Future work includes exploring the vascular normalization approach by suppressing pericyte phenotype switching to enhance vascular function and chemo- and immunotherapeutic efficacy.

#3851 Vascular regulation of immune exclusion in melanoma.

Ha-Ram Park[1], Nika Kozolv[1], Shawn Jaeha Kim[2], Eugene Son[1], Sungsoo Kim[1], Hee Won Yang[1], Minah Kim[1]

[1]Columbia University Irving Medical Center, New York, NY,[2]Columbia University, New York, NY

The infiltration of immune cells into the tumor is as crucial as their activity in determining the responses to immune checkpoint inhibitors (ICIs). Immune cell exclusion from tumors has been linked to poor responses to ICIs across various cancers, including melanoma; however, the underlying biological mechanisms remain poorly understood. Abnormal tumor vasculature, characterized by leakage and instability, represents a key barrier to immune cell infiltration, but the mechanisms underpinning these vascular changes and their impact on immune exclusion development remain poorly understood.To address this, we utilized a syngeneic mouse melanoma model to investigate the role of vascular changes in regulating immune cell positioning and exclusion during tumor progression. Spatial distribution of various immune cell populations and vascular integrity were assessed through multiplexed immunofluorescence staining across whole tumor cross-sections. While early-stage tumors exhibit well-infiltrated $CD8^+$ T cells and neutrophils throughout the tumors, these immune cells became confined to the tumor periphery as tumors grew, leaving the core devoid of infiltration. This immune exclusion development correlated with vascular instability and leakage, particularly observed at the tumor periphery. Transcriptomic analysis of endothelial cells, sorted from the tumor periphery and core revealed increased gene expression of chemotaxis and immune migration in endothelial cells at the tumor periphery. Through multi-omics approaches including single-cell and spatial transcriptomic analysis, we aim to unravel the molecular mechanisms by which vascular abnormalities shape immune cell exclusion. These findings will guide us in building strategies to restore vascular integrity, enhance immune cell infiltration, and improve the efficacy of ICIs, addressing critical unmet needs for melanoma patients resistant to current immunotherapies.

#3852 The role of endothelial cell remodeling in driving immunotherapy resistance of hepatocellular carcinoma.

Baoyi Yin[1], Shufen Chen[1], Zhewen Xiong[1], Patrick Wong[1], Zhuo Yu[2], Stephan Chan[1], Alfred Sze-Lok Cheng[1]

[1]The Chinese University of Hong Kong, Hong Kong, Hong Kong,[2]Shanghai University of Traditional Chinese Medicine, Shanghai, China

Introduction: Hepatocellular carcinoma (HCC) is the most common liver cancer and a leading cause of cancer-related deaths globally. Immune checkpoint blockade (ICB) therapy has shown promise but has yielded limited responses in HCC patients. Combining anti-vascular endothelial growth factor (VEGF) with ICB enhances therapeutic efficacy, however, a deeper understanding of endothelial cell (EC) remodeling is crucial for further improving ICB effectiveness. Our integrated analyses identified a transcription factor-4 (TCF4)-driven transcriptional program, coupled with elevated BRD4 expression, associated with EC remodeling in ICB-resistant HCC. Emerging studies have shown that TCF4 functions depend on BRD4 through recruitment to a specific enhancer region, and BRD4 inhibitors can reduce TCF4 expression. We aim to delineate the microenvironmental cue and transcriptional network that direct EC remodeling for tumor immunosuppression and their role in promoting ICB resistance.

Methods: Single-cell transcriptomic profiling was performed on samples from a Phase II trial of pembrolizumab (anti-PD-1) in hepatitis B-related HCC patients (NCT03419481). An ICB-resistant HCC mouse model generated via iterative selection was used for validation. The BRD4 inhibitor AZD5153, administered in oral and nanoparticle formulations, was used to suppress BRD4 and TCF4 expression in systemic and endothelial cells. Quantitative immunofluorescence assessed ECs and detected TCF4 and BRD4 levels.

Results: scRNA-seq analysis revealed that tumor vessels from ICB non-responders were enriched with disorganized macrovascular-like endothelial cell (MaVEC), which further increased upon ICB. RNA velocity analysis predicted that MaVEC in non-responders likely originate from liver sinusoidal endothelial cell (LEC), indicating LEC-to-MaVEC trans-differentiation during ICB resistance acquisition. TCF4 was identified as the top regulon in MaVEC, with increased activity along the LEC-to-MaVEC trajectory and association with BRD4. Using AZD5153, we observed that TCF4 can be suppressed in ECs, which caused a significant intra-tumoral reversal from MaVEC to LEC phenotypes, resulting in tumor cell apoptosis and regression. Additionally, nanoparticle-delivered EC-targeted AZD5153 achieved similar tumor-suppressive effects at a lower dosage.

Conclusions: Our study reveals that the dynamic trans-differentiation from LEC to MaVEC is associated with ICB resistance in HCC, which can be reversed by BRD4 inhibition and possibly mediated by TCF4 transcriptional regulation, targeting their expression in ECs showed improved therapeutic outcomes.

Keywords: Hepatocellular carcinoma, Immune-checkpoint blockade, Endothelial cells, Transcriptional network.

Acknowledgement: Funded by General Research Fund (14118424) & AstraZeneca Pre-clinical Oncology Research Programme & Li Ka Shing Foundation.

#3853 Plasma activin A levels are significantly elevated for a month after minimally invasive colorectal resection (MICR) for cancer and may support early growth of residual tumor.

Chandana S. K. Herath Mudiyanselage[1], Yi-Ru Chen[1], Neil Mitra[1], Hiromichi Miyagaki[2], Elizabeth Nilsson Sjolander[1], Xiaohong Yan[1], Vesna Cekic[1], Richard L. Whelan[1]

[1]Department of Surgery, Colorectal Division, Lenox Hill Hospital, Northwell Health, New York, NY,[2]Department of Surgery, Otemae Hospital, Osaka, Japan

Introduction: Activin A (ActA) is a cytokine from the TGF-β family. ActA has a wide range of effects in different tissues and plays a role in inflammation, fibrosis, and wound healing. Its actions are mediated mainly via SMAD signaling. A variety of tumors express ActA which has been associated with increased MMP levels and tumor invasion. ActA is also found in the tumor microenvironment (fibroblasts, EC's, and immune cells). ActA mediates the development of cancer-associated fibroblasts that facilitate tumor invasion. Higher tumor ActA levels are associated with worse outcomes. Surgery's impact on ActA levels is not understood. This study's purpose was to determine plasma ActA levels before and after MICR for colorectal cancer (CRC).

Method: CRC patients (pts.) undergoing MICR enrolled in an IRB approved data/tissue bank for whom periop plasma samples were available were eligible. Clinical and pathological data was collected prospectively. Blood samples were obtained preoperatively (PreOp) and on various postoperative days (POD) including POD 1, 3, and at least one late postoperative day (POD 7 to 41). Late samples were grouped into 7-day bundles and used as single time points. Plasma ActA levels were measured in duplicate via ELISA and results expressed as mean ±SD. The Wilcoxon paired t-test was used (sig. $p<0.05$). Correlation between plasma ActA levels and incision length, surgical length, etc was determined using Spearman's rank correlation (rs.). Plasma Act A levels between Laparoscopic and hand-assisted groups were compared by Mann-Whitney U test.

Results: A total of 83 CRC MICR patients met inclusion criteria (48 males: 35 females mean age 65.31± 14.09; rectal, 32%/colon, 68%). The mean incision length was 8.21± 3.63 cm and mean length of stay was 7.9± 6.9 days. The cancer staging (STG.) breakdown was; STG I, 40%, STG II, 29%, STG III, 28% and STG IV, 3%. The mean PreOp ActA level (pg/ml) was 235.8±119.8 (n=83). Elevated mean plasma levels were noted on POD 1(300.4±150.1, n=82, $p< 0.001$), POD3 (325.0±217.4, n=72, $p< 0.001$), POD7-13 (314.9±245.4,n=57,p=0.03), POD14-20 (316.0±144.8,n=29, p=0.003),POD 21-27 (279.3±115.7,n=15, p=0.03) and on POD 28-41(246.4±86.86,n=18, p=0.02) when compared to PreOp levels. Postop ActA levels correlated with the duration of surgery on POD1 ($rs = 0.3$, $p= 0.002$). The mean ActA levels in the hand group were elevated at all Postop time points (9-26%) with (sig. only POD1;P=0.02).

Conclusion: Plasma ActA levels remained significantly elevated from preop levels for 6 weeks after CRC MICR. The increases were 37-39% over Preop levels. The source of the increase may be linked to inflammation associated with wound healing and angiogenesis. Elevated plasma ActA concentrations could potentially facilitate metastasis or the proliferation of remaining cancer cells during the initial month post-surgery.

#3854 Lymphatic endothelial progenitors promote tumor lymphatic formation through cell-cell fusion.

S. Athaiya[1], L. Volk-Draper[1], E. Cox[1], N. Zinkevich[2], S. Ran[1];
[1]Southern Illinois University School of Medicine, Springfield, IL, [2]University of Illinois, Springfield, IL

Introduction: Bone marrow (BM) derived myeloid-lymphatic endothelial cell progenitors (M-LECP) promote formation of tumor lymphatics and metastasis to lymph nodes. Regenerative capacity of BM progenitors to many other lineages is mediated through cell-cell fusion, a process that efficiently inserts a pro-mitotic message directly into damaged or division-restricted cells. We aimed to determine whether M-LECP use a similar mechanism to induce division of tumor lymphatic endothelial cells (LEC) which is an essential step towards the formation of new sprouts.

Methods: Fusogenic markers were determined in M-LECP differentiated by Th2 and TLR4 ligands as well as in RAW264.7 macrophages by qPCR, flow cytometry, and immunohistochemistry (IHC) applied to mouse and clinical breast cancers. Fusion of myeloid and lymphatic cells stably expressing GFP and mCherry tags, respectively, was quantified using flow cytometry and microscopy by determining the percent of yellow cells resulting from merging of green- and red-colored cell partners. Fusion of lymphatic progenitors *in vivo* was determined in female chimera mice bearing MDA-MB-231 or EMT6 breast tumors that have been grafted with male BM prior to tumor implantation. Staining for LEC-specific markers in combination with probes for Y-chromosome quantified tumor lymphatic vessels fused with BM progenitors.

Results: 10% to 25% of myeloid cells fused with co-cultured LEC quantified by two independent methods. M-LECP and RAW264.7 macrophages expressed a variety of fusogenic markers such as SIRP-a, CD36, DAP12, Stabilin-1, and DC-Stamp. These proteins were also expressed in M-LECP residing in human and mouse breast tumors. *In vivo* models showed up to 48% of tumor lymphatic vessels containing nuclear Y-chromosome indicating fusion. Supporting the hypothesis, Y-chromosome in LEC nuclei correlated with mitotic markers, nuclei doubling, lymphatic formation, and metastasis to nodes. Both *in vitro* and *in vivo* assays showed dependency of fusion on Th2 and TLR4 pathways.

Conclusion: We show for the first-time using *in vivo* and *in vitro* assays that bone marrow derived myeloid-lymphatic progenitors induced by Th2 or TLR4 ligands are able to fuse with LEC. This event correlated with LEC proliferation and ultimately, metastatic behavior. These findings suggest a fundamentally novel mechanism of generating new lymphatic sprouts triggered by fusion with lymphatic endothelial cell precursors.

TUMOR BIOLOGY: Approaches for Metastasis Inhibition
Poster Session

#3858 Therapeutic targeting of cofilin-1 phosphorylation by peptidomimetics inhibits lung cancer metastasis.

M.-Y. Lin[1], J.-D. Leu[2], C.-Y. Wu[1], **Y.-J. Lee**[1];
[1]National Yang Ming Chiao Tung University, Taipei, Taiwan, [2]Division of Radiation Oncology, Taipei City Hospital RenAi Branch, Taipei, Taiwan

Background: The main challenges in the clinical management of lung cancer are distant metastasis and recurrence. Targeting the destabilization of actin dynamics has become a critical approach to slowing the rapid proliferation of tumor cells. However, a specific class of anti-metastatic drugs is still being uncovered.
Methods: The analysis of gene expression of ADF/cofilin family by public database in clinically relevant. Combination of the endogenous protein level of CFL-1, its phosphorylation status, and cellular invasion activity in various lung cancer cell lines. We used synthetic peptide to interfere LIMK/CFL-1 regulatory pathway to produced anti-metastatic and survival benefits in a xenograft lung cancer model. Furthermore, the biological characteristics of peptide labeled with metal radionuclide both *in vitro* and *in vivo* were also be evaluated.
Results: Over-expression of cofilin-1 (CFL-1) gene was associated with the incidence and survival rate of lung adenocarcinoma using the in-silico analysis. CFL-1 proteins were also differentially elevated in various NSCLC cell lines. Notably, we found that the phosphorylation level of CFL-1 at serine 3 (Ser3), rather than the total CFL-1, was better correlated with the invasion potential of NSCLC cells. Here, we developed synthetic CFL-1 mimetic peptides (CMP) to inhibit endogenous CFL-1 phosphorylation and evaluated their effects using highly metastatic NSCLC cells. The SPECT/CT imaging demonstrated that CMP labeled with ^{99m}Tc can be detected in an orthotopic lung tumor. Furthermore, CMP can act as a competitive decoy of cofilin kinase, demonstrating anti-metastatic effects and prolonged survival in a xenograft model, with no significant toxicity observed.
Conclusion: This study demonstrates that the phosphorylation level of CFL-1 at serine 3 is strongly correlated with the metastatic potential of lung cancer, and interfering with its phosphorylation could be a promising strategy for lung cancer treatment.

#3859 Ephrin inhibition disrupts stromal-cancer crosstalk and reduces metastasis in pancreatic cancer.

C. L. Bolton[1], R. O'Sullivan[1], S. Menezes[1], A. J. M. Cameron[1], G. Marin[1], J. F. Marshall[1], E. P. Carter[2], M. Henkemeyer[3], R. P. Grose[1]; [1]Queen Mary University of London, London, United Kingdom, [2]University of Bath, Bath, United Kingdom, [3]UT Southwestern Medical Centre, Dallas, TX

Pancreatic Ductal Adenocarcinoma (PDAC) is the 10th most common cancer in the UK but has devastating mortality rates world-wide. Reasons underpinning this include delays in diagnosis and treatment resistance, where the desmoplastic nature of the disease hampers treatment efficacy. One of the most influential cell types within the PDAC stroma is the cancer associated fibroblast (CAF). CAFs are known to have both pro- and anti-tumoral roles, making them an attractive cell type to target for re-programming. To investigate CAF-cancer cell crosstalk we developed a 3D *in vitro* model of CAF-led invasion using heterocellular spheroids of cancer cells and CAFs. RNA sequencing of chimeric mouse/human versions of these spheres identified complementary expression of ephrin family receptors (Eph-B2) and ligands (EFNB2) between cancer and CAFs respectively, implicating their involvement in invasion. Part of the receptor tyrosine kinase superfamily, the ephrin family are unique in that both the ligand and receptor can signal, with implications of this having been well described in vasculogenesis and axon guidance. Utilising CAFs expressing either EFNB2 knockout or a gain-of-function mutation in EFNB2, we have found that both forward and reverse signalling is required for invasion, suggesting that disruption of both elements could be beneficial therapeutically. Using first-in-class inhibitors of this pathway, we observed a significant decrease in PDAC invasion in our 3D spheroid invasion model. Extending this *in vivo*, we have utilised a KPC-derived PDAC orthotopic mouse model that recapitulates human biology through the formation of both primary and metastatic disease. We found that tumour growth and size were not affected by ephrin blockade. However, eph-ephrin signalling inhibition did cause a significant decrease in metastatic tumour presence across multiple organ sites. This highlights a potential for this treatment to halt disease progression and reverse disease severity. We hope to extend these results further to test the potential clinical applications of this compound as a treatment option for both early and late-stage PDAC patients.

#3860 Metastasis score: A powerful tool to determine the metastatic potential and the appropriate adjuvant therapy of melanoma.
K. Shen, C. Wei, J. Gu;
Fudan University, Shanghai, China

Metastasis is the onerous challenge in the prognosis of melanoma patients. Accurately predicting the metastatic potential of non-metastatic melanoma (NMM) and determining effective postoperative adjuvant therapy for inhibiting metastasis remain uncertain. In this study, we conducted comprehensive analyses of melanoma metastases using bulk and single-cell RNA sequencing data, enabling the construction of a metastasis score (MET score) through multiple machine-learning algorithms. The reliability and robustness of the MET score were validated by various in vitro and in vivo assays. Our findings revealed a distinct molecular landscape in metastatic melanoma summarized by the enrichment of metastasis-related pathways, complex cell-cell communication, and heightened infiltration of pro-angiogenic tumor-associated macrophages compared to NMM. Importantly, patients of the high MET score group exhibited poorer prognoses and immunosuppressive microenvironment, featuring increased infiltration of regulatory T cells and decreased infiltration of CD8+ T cells, compared to the low MET score patient group. Expression of PD-1 was markedly higher in patients of low MET scores. Anti-PD-1 (aPD-1) therapy profoundly affected antitumor immunity activation and metastasis inhibition in these patients. In summary, our study demonstrates the effectiveness of the MET score in predicting melanoma metastatic potential. For patients with low MET scores, aPD-1 monotherapy may be a potential treatment strategy to inhibit metastasis. Patients with high MET scores may benefit from combination therapies.

#3862 OC201 and OC202e, potential inhibitors of cancer metastasis, suppress EMT in pancreatic cancer.

Youngjin Kim, Dong Sub Jung, Sewon Kim, So Jung Sung, Yenny Kim, Yi Rang Kim, **Jihoon Kang**

Oncocross Co., Ltd, Seoul, Korea, Republic of

Objective: Pancreatic cancer is among the most aggressive malignancies, associated with a very poor prognosis due to its rapid progression and resistance to conventional therapies, underscoring the urgent need for novel treatment approaches. Epithelial-mesenchymal transition (EMT) is a key process that enhances the migration and invasion capabilities of cancer cells and is recognized as a pivotal mechanism driving malignancy in pancreatic cancer. Inhibiting EMT is therefore a critical therapeutic strategy. This study aimed to evaluate the anti-metastasis efficacy and define the mode of action of OC201 and OC202e in EMT-induced pancreatic cancer cells.
Method: EMT was induced in Panc-1 and MIA PaCa-2 cells through treatment with TGF-β1 (5 ng/ml) and TNF-α (10 ng/ml). Cell migration and invasion were assessed through functional assays, while EMT-related markers (E-cadherin, ZEB1, Snail, Vimentin, and MMP-2) were analyzed via qPCR and Western blot. The cells were subsequently treated with OC201 and/or OC202e, and changes in expression of EMT-related signaling proteins were evaluated. An *in vivo* hepatic metastasis model was also established using MIA PaCa-2 cells to evaluate the effects of OC201 and/or OC202e treatment on metastasis following four weeks of therapy.
Results: Treatment with TGF-β1 and TNF-α significantly increased migration and invasion in both Panc-1 and MIA PaCa-2 cells, accompanied by decreased E-cadherin expression and increased expression of ZEB1, Snail, and Vimentin. Treatment with either OC201 or OC202e reduced cell migration and invasion, suppressed the expression of EMT markers (ZEB1, Snail, Vimentin, and MMP-2), and increased E-cadherin expression. Both compounds inhibited the activation of key EMT signaling proteins, AKT and IκBα, with combination treatment resulting in a stronger inhibition of AKT and IκBα activation and an enhanced E-cadherin expression compared to monotherapy. OC202e alone inhibited autophagy, while OC201 showed no significant effect. In the hepatic metastasis model, both OC201 and OC202e monotherapy significantly reduced liver metastatic nodules in mice compared to control, with combination therapy yielding a more substantial reduction, confirming the efficacy in reducing metastasis to the liver.
Conclusion: This study demonstrated that OC201 and OC202e effectively inhibited TGF-β1- and TNF-α-induced EMT via the AKT and NF-κB signaling pathways, highlighting their potential to target critical mechanisms underlying pancreatic cancer metastasis. Both compounds effectively suppressed EMT while maintaining cell viability, with combination therapy exhibiting superior efficacy. These findings suggest that the combined use of OC201 and OC202e may serve as a promising therapeutic strategy for pancreatic cancer.

#3863 Targeting TACC3 inhibits metastasis in breast cancer with centrosome amplification.

Mustafa Emre Gedik[1], Ozge Saatci[1], **Kubra Calisir**[1], Metin Cetin[1], Burge Ulukan Altun[1], Ozlem Sener Sahin[1], Rony Hull[2], Lauren Ball[2], Elizabeth Hill[3], Chaemin Lim[4], Sridhar Vempati[4], Burcu Caliskan[5], Erden Banoglu[5], Ioulia Chatzistamou[6], Ozgur Sahin[1]

[1]Department of Biochemistry and Molecular Biology, MUSC Hollings Cancer Center, Charleston, SC,[2]Department of Cell and Molecular Pharmacology and Experimental Therapeutics, MUSC, Charleston, SC,[3]Department of Public Health Sciences, MUSC, Charleston, SC,[4]A2A Pharmaceuticals, Inc., New York, NY,[5]Department of Pharmaceutical Chemistry, Gazi University, Faculty of Pharmacy, Ankara, Turkey,[6]Department of Pathology, Microbiology & Immunology, University of South Carolina, Columbia, SC

Centrosome amplification (CA) is highly prevalent in cancer. It is strongly associated with tumor progression and worse prognosis in a variety of different cancers. Preclinical studies have demonstrated that CA can trigger tumor initiation as well as cancer cell invasion. Thus, CA is known to be a promising therapeutic target. We have previously identified Transforming Acidic Coiled-Coil Containing Protein 3 (TACC3) as being overexpressed in tumors with CA. TACC3 forms distinct functional interactomes regulating spindle stability, clustering extra centrosomes and transcription of key tumor suppressors in mitosis and interphase to ensure proliferation and survival of cancer cells with CA. Despite the known mechanisms in tumor growth inhibition, the molecular mechanisms by which TACC3 drives tumor invasion and metastasis in cancers with CA remain underexplored. Here, we aim to test TACC3 targeting as a novel strategy to block invasion and metastasis and to elucidate the underlying mechanisms. We found that higher TACC3 levels are strongly associated with worse metastasis-free survival in breast cancer patients with CA. Using a combination of *in vitro* assays, transcriptomics, proximity labeling (APEX2-TACC3), and mass spectrometry, we demonstrate that TACC3 promotes cancer cell migration and invasion. In addition, we provide evidence that targeting TACC3 via a small-molecule inhibitor or CRISPR-Cas9-mediated knockout significantly reduces cell migration and invasion. Furthermore, TACC3 inhibition reduces the number of circulating tumor cells and spontaneous metastasis to the lungs in the MMTV-PyMT transgenic mouse model and lung-tropic breast cancer xenografts without any toxicity. Overall, our findings demonstrate the potential for TACC3 as a therapeutic target to inhibit metastasis in breast cancer with CA. This work offers new insights into the molecular underpinnings of TACC3's role in metastatic breast cancer, particularly in CA-driven tumors and underscores its therapeutic relevance.

#3864 AOH1996: A multi-faceted inhibitor of metastasis via PCNA and tumor microenvironment targeting.

Robert G. Lingeman[1], Robert Hickey[1], Linda Malkas[1], Mustafa Raoof[1], Jacob Cawley[2]

[1]Beckman Research Institute of The City of Hope, Duarte, CA,[2]Case Western Reserve University, Cleveland, OH

Metastasis remains the leading cause of cancer-related mortality, emphasizing the urgent need for therapies to prevent and treat this process. AOH1996, a selective inhibitor of proliferating cell nuclear antigen (PCNA), has emerged as a promising agent with anti-metastatic properties. AOH1996 disrupts critical pathways such as angiogenesis, cancer cell migration, and tumor microenvironment modulation. Through bulk RNA-seq analysis of cancer cells, AOH1996 was found to downregulate genes involved in angiogenesis and migration, processes essential for metastasis. Functional assays using highly metastatic osteosarcoma cell lines revealed that AOH1996 inhibited cell proliferation and migration in both 2D monolayer and 3D spheroid models. In 3D cultures, which closely mimic the tumor microenvironment, AOH1996 significantly suppressed cell invasion and growth, demonstrating its efficacy in a more physiologically relevant context. The anti-metastatic potential of AOH1996 was further validated using an ex vivo Pulmonary Metastasis Assay (PuMA), where osteosarcoma cells implanted into mouse lungs were effectively killed by AOH1996. This highlights its ability to directly target metastatic cells in the lung. Complementary cell invasion assays using Matrigel showed that AOH1996 altered the morphology of invadopodia—structures critical for tissue invasion—rendering them non-functional and significantly reducing invasive capacity. Preliminary data from a Phase I clinical trial (NCT05227326) in patients with advanced, chemo-refractory solid tumors demonstrated AOH1996's clinical potential. In one patient with pancreatic cancer, AOH1996 reduced the size of two liver metastases by 50%, underscoring its efficacy in a clinical setting. Tumor-associated macrophages (TAMs) facilitate immune evasion, angiogenesis, and metastasis. Oncogenic MYC influences the tumor microenvironment (TME) by recruiting TAMs to the TME. We have found that AOH1996 inhibits MYC expression in cancer cells, but not in non-malignant cells. We hypothesize that this suppression of MYC by AOH1996 disrupts the formation of a supportive metastatic niche through reduced TAM recruitment. Thus, AOH1996 may enhance immune responses and impede angiogenesis and metastasis. Together, these findings establish AOH1996 as a multi-faceted therapeutic with significant anti-metastatic potential. By targeting both PCNA-dependent processes and the tumor microenvironment, AOH1996 could provide a unique approach for combating metastatic cancers.

#3865 Targeting MYC high osteosarcoma through EYA2 modulation.

Ryota Shirai, Sydney Hakim, Avery Bodlak, Masanori Hayashi

University of Colorado Anschutz Medical Campus, Aurora, CO

Osteosarcoma is the most common bone cancer in children, adolescent and young adults. Recent large scale genomic studies have started to reveal that Osteosarcoma (OS) patients who have tumors that highly express the transcription factor MYC, are significantly at high risk of treatment failure. However, MYC has long been known to be extremely difficult to target with drugs. The EYA protein is an evolutionarily conserved transcriptional co-activator of the SIX1 family of homeobox proteins, which also has phosphatase activity. Multiple studies have shown that EYA is involved in the initiation and progression of various cancers. EYA Tyrosine Phosphatase has been shown to regulate MYC expression and protein stability in Brain tumors. In OS cells, EYA2 is highly expressed and has been indicated to be involved in tumor progression and invasion, but the interaction between EYA2 and MYC is unknown. The major goal of our study is to clarify the effect of EYA2 on the expression of MYC in OS cells, and evaluate it as a therapeutic target.
First, EYA2 and MYC expression in various OS cell lines were confirmed using western blotting. Next, we established EYA2 shRNA knock-down in 143B cells and CHCO-OS1 cells, which highly express both EYA2 and MYC. Interestingly, in these cells, EYA2 knock-down led to decreased MYC expression, decreased clonogenicity and proliferation. Furthermore, EYA2 knock-down led to decreased cell migration in 143B cells when measured by a scratch assay using the Incucyte live cell imaging. Next, we also tested 9987, an allosteric EYA2 phosphatase inhibitor. Here, 9987 was shown to inhibit the clonogenicity, proliferation and migration compared to DMSO control.
These results indicate that in MYC-high OS cells, EYA2 knockdown leads to decreased MYC expression, decreased proliferation, clonogenicity, and migration, and EYA2 phosphatase inhibition is a promising therapeutic strategy for this tumor. We are currently confirming whether the EYA2 inhibitor 9987 can reduce metastatic rate and extend overall survival in our orthotopic-implantation-amputation mouse model, and further identifying the mechanisms of how EYA2 regulates MYC activation in OS.

#3866 A new approach to suppress triple-negative breast cancer metastasis by blocking apurinic/apyrimidinic endonuclease 1 interaction with G-quadruplex.

Yingling Chen[1], Suravi Pramanik[1], Mason Tarpley[1], Achyuth Kalluchi[1], Sutapa Ray[2], Bhopal Mohapatra[1], Kyle Hewitt[1], Jordan Rowley[1], Vimla Band[1], Kishor K. Bhakat[1]

[1]Department of Genetics, Cell Biology and Anatomy, University of Nebraska Medical Center, Omaha, NE,[2]Department of Pediatrics, University of Nebraska Medical Center, Omaha, NE

Triple-negative breast cancer (TNBC) has a relatively high metastasis rate as compared to other breast cancer subtypes. Metastasis and tumor recurrence remain major challenges for TNBC patients, resulting in poor prognosis. Therefore, it is imperative to identify and elucidate the molecular mechanisms that drive metastasis to allow the development of new therapeutic agents to inhibit TNBC metastasis and improve patients' outcomes. Herein, we uncovered a new mechanistic connection between a multifunctional DNA repair protein APE1 and G-quadruplex (G4), a secondary DNA structure present in many metastasis-related signature genes promoters in TNBC. We found that APE1 is highly enriched at the G4 regions on the promoters of metastasis related genes and APE1 knockout or knockdown impairs the tumorigenic and pro-metastatic traits of TNBC cell lines in vitro. Intriguingly, we found that G4 is required for APE1's enrichment at these promoters and APE1 promotes transcription factors (TFs) binding to these promoters, thereby promoting the expression of these metastasis-related genes. Deletion of APE1 impeded TFs' binding to promoters and downregulated expression of these genes. In addition, we found that G4 deletion in some target gene promoters exerted similar inhibitory effects as APE1 knockout on cell migration, cell invasion and tumor sphere formation. We discovered that a small molecule which disrupts APE1: G4 interaction significantly disrupted APE1's enrichment to G4 on promoters, thereby blocking TFs binding, gene expression, cell migration, invasion and tumor sphere formation in vitro. We generated orthotopic TNBC metastasis mouse model using an aggressive TNBC cell line, MDA-MB-231 and found that disrupting G4-APE1 axis either by APE1 knockout or G4 deletion or small molecules significantly inhibited the primary tumor growth and impeded TNBC distant metastasis to lungs. Overall, our study uncovered a novel functional axis of G4-APE1 in regulating TNBC growth and metastasis and identified that this regulatory axis has the potential to serve as a new therapeutic target to prevent metastasis in TNBC patients.

#3867 Small molecule inhibitors targeting TG2 in ovarian cancer and identification of drug escape pathways.

Monica Tudor[1], Cristian Munteanu[1], Gabriela Chiritoiu[1], Stefana Orobeti[1], Alexandra Bran[2], Florin Jipa[2], Felix Sima[2], Gary E. Schiltz[3], Daniela Matei[3], **Livia Elena Sima**[1]

[1]Molecular Cell Biology, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania,[2]Cetal, National Institute of Laser Plasma and Radiation Physics, Magurele, Romania,[3]Northwestern University, Chicago, IL

Ovarian cancer (OC) is the 8th most common cancer in women. Most patients with high-grade serous OC present with intraperitoneal disseminated disease when diagnosed, due to mild symptoms in earlier disease stages. They respond efficiently to cisplatin or taxol-based compounds in the front-line treatment; however, resistance emerges in the majority of cases, which allows tumors to progress. There is an urgent need for improved treatment to increase patients' survival. Tissue transglutaminase (TG2) is a protein overexpressed in many solid tumors, including ovarian; it interacts with fibronectin (FN) in the extracellular matrix and promotes OC intraperitoneal dissemination. This makes TG2 a promising target for treatment. We previously identified MT4 as a promising small molecule inhibitor (SMI) able to disrupt TG2-FN protein-protein interaction, prevent cancer cell adhesion and spheroid formation, and sensitize OC cells to chemotherapeutic drugs, such as paclitaxel (*Sima LE et al., Mol Cancer Ther., 2019*). We recently investigated the effect of 5 new MT4 analogues on OC cells behavior. We found that compound #3011 completely prevented spheroids formation, similar to *Tgm2* gene excision, while #3002 decreased cell adhesion onto FN. We used phosphoproteomics to analyze the adaptive signaling of OC cells treated with MT4 that escape treatment. New promising molecular TG2 co-targets for OC treatment have been identified as effectors of MT4 escape pathways: sirtuins and p21-activated kinases (PAKs). Combination of signaling inhibitors and TG2-directed SMIs induce OC cell death in both 2D and 3D conditions, with potential for future therapeutic use. Our multiplex IHC data shows that in OC tumors TG2 is expressed by cancer cells, as well as by α-SMA$^+$ CAF subset. Therefore, we next sought to test the effect of TG2 drug targeting on OC cells-fibroblasts 3D co-cultures. Our results showed that MT4 prevents heterospheroid self-assembly *in vitro*, which indicates potential disruption in tumor-stroma communication using TG2-directed therapies. In parallel, we developed new tools to test drug combinations, as well as radiation therapy regimens for their potential to inhibit cancer spreading. We produced a new glass microfluidic chip and adapted a miniaturized mesothelial clearance assay for live cell imaging. We obtained new SKOV3 and SKOV3-TG2KO LifeAct$^+$ stable cell lines to allow real time imaging measurements of OC cell behavior. Subpopulations were sorted based on actin-GFP expression level and characterized by cell motility quantification. Cell lines with behavior similar with parental cells were selected for further use. We currently use these tools to investigate the potential of TG2 targeting to enhance response of cancer cells to new FLASH radiation sources (*Orobeti S et al, Sci. Rep., 2024*), such as very high energy electrons generated with a high-intensity laser plasma accelerator system.

#3868 Decreased metastatic burden in a spontaneous metastatic melanoma model by inhibiting myeloid-derived suppressor cell function.

Kaitlyn M. Landreth[1], Angisha Basnet[1], Mary Garland-Kledzik[2], Brian A. Boone[2], Tracy W. Liu[1]

[1]Department of Microbiology, Immunology, and Cell Biology, West Virginia Univ. Health Sciences Ctr., Morgantown, WV, [2]Department of Surgery, West Virginia Univ. School of Medicine, Morgantown, WV

Metastatic melanoma is the leading cause of skin cancer-related deaths, with a five-year survival rate of only 30%. Myeloid derived suppressor cells (MDSCs) are considered a driver of metastatic progression, supporting pre-metastatic niche formation, facilitating tumor cell migration, invasion, and extravasation and promoting immune evasion. This study evaluates the contribution of myeloperoxidase (MPO) and extracellular trap formation in melanoma metastasis. Using a spontaneous melanoma metastasis model, we demonstrate metastases in the lungs, bone, and lymph node approximately 4 weeks post surgical resection of primary subcutaneous B16F10 tumors. Tumor growth and metastatic burden were quantified using a bioluminescent reporter stably expressed in B16F10 cells. Syngeneic myeloperoxidase-deficient and peptidyl arginine deiminase 4 (PAD4)-deficient mice, as well as C57BL/6J wild-type mice treated with an MPO-specific inhibitor or hydroxychloroquine (an extracellular trap inhibitor), exhibited significantly reduced lung and bone metastases compared to untreated wild-type controls. PAD4, a critical enzyme in extracellular trap formation, was essential for these effects. Targeting myeloperoxidase or extracellular traps significantly reduced MDSC populations while increasing B cells in the metastatic microenvironment, as assessed by flow cytometry. Ex vivo assays revealed diminished MDSC-mediated suppression of T and B cells when myeloperoxidase or extracellular trap formation was inhibited. Notably, depletion of B cells abrogated the anti-metastatic effects of targeting myeloperoxidase or extracellular traps, highlighting B cells as central mediators of metastasis suppression. These findings demonstrate that targeting myeloperoxidase or extracellular trap formation reduces metastatic burden and mitigates MDSC-mediated pro-metastatic and immunosuppressive functions. This work underscores the therapeutic potential of targeting myeloperoxidase and extracellular trap formation in combating melanoma metastasis.

#3869 Targeting anoikis resistance in ovarian cancer via metabolic enzyme inhibition.

Geeta Rao, Pallab Shaw, Shailendra Dwivedi, Priyabrata Mukherjee

University of Oklahoma Health Sciences Center, Oklahoma City, OK

Ovarian cancer (OvCa) is the deadliest gynecologic malignancy, with poor prognosis largely due to late-stage diagnosis and metastasis, which limit treatment options and contribute to high mortality. Anoikis resistance, a key mechanism enabling metastatic spheroids to survive in the peritoneal cavity, drives both metastasis and therapy resistance. Therefore, understanding the regulation of anoikis is critical for identifying novel therapeutic targets. Recent studies, including work from our group, have identified overexpression of cystathionine beta-synthase (CBS)—an enzyme that produces hydrogen sulfide (H2S)—as a contributor to OvCa progression. CBS has been shown to promote cancer progression through enhanced lipogenesis and mitochondrial fusion. Based on these findings, we hypothesize that CBS plays a central role in anoikis resistance in OvCa and propose that targeting CBS could inhibit metastasis and improve sensitivity to chemotherapy. To investigate the functional role of CBS in anoikis resistance, we utilized OvCa cell lines (COV318, OVCAR8, OVCAR4, and A2780_CP20) and achieved CBS silencing via siRNA-mediated knockdown. Cells were cultured in both monolayers (2D) and spheroids (3D) to better mimic in vivo tumor conditions. Our findings reveal that: (i) High CBS expression is associated with poor median survival in stage 3 and stage 4 high-grade serous ovarian cancer (HGSOC) patients, (ii)CBS expression directly correlates with elevated expression of stemness and epithelial-to-mesenchymal transition (EMT) markers, (iii) CBS silencing induces anoikis in both COV318 (ascites-derived) and A2780_CP20 (drug-resistant) OvCa cell lines, (iv) CBS silencing disrupts integrin-mediated downstream signaling through focal adhesion kinase (FAK) in both 2D and 3D cultures. These findings establish CBS as a critical regulator of anoikis resistance in ovarian cancer, underscoring its potential as a therapeutic target to suppress metastasis and enhance the efficacy of existing chemotherapy regimens.

#3870 Ruxolitinib diminishes cell proliferation and invasiveness in BRAF mutated metastatic melanoma.

Raza Ahmed[1], Jayesh Menon[2], Rishka Raj[3], Rakesh Singh[1], Philip Friedlander[4], Omar Aljitawi[1]

[1]University of Rochester Wilmot Cancer Institute, Rochester, NY,[2]University of Rochester School of Medicine and Dentistry, Rochester, NY,[3]IMC University of Applied Sciences Krems, Krems, Austria,[4]Icahn School of Medicine at Mount Sinai, New York City, NY

Introduction: Treatment with a BRAF inhibitor of unresectable or metastatic melanoma with a BRAF V600E or V600K mutation has an estimated 5-year survival rate of 34%, with 19% being progression free. To improve outcomes, we hypothesize that the blocking of the JAK/STAT pathway via the use of Ruxolitinib, a direct JAK2 inhibitor, may decrease proliferation and invasiveness in BRAF mutated melanoma in vitro.

Methods: *BRAFV600E* mutated melanoma cell line, SK-MEL-1, was obtained from ATCC. The effect of Ruxolitinib of proliferation was measured via a proliferation assay. Cells were treated with 0nM Ruxolitinib and 10nM Ruxolitinib for 24 hours, then counted with a hemocytometer. The effect of Ruxolitinib on invasiveness was evaluated through an invasion assay. After 24-36 hours, cells that invaded through a Matrigel coated membrane were fixed and stained with crystal violet, and then subsequently imaged by an inverted microscope. The number of invaded cells was quantified, and the total number of invaded cells was calculated by averaging the cell density (cells/um^2) for each image and then multiplying that by the total membrane area. All data was normalized to their respective controls and analyzed using the appropriate statistical analysis.

Results: In cells containing a BRAF mutation, Ruxolitinib caused a decrease in erythropoietin (EPO) induced proliferation. Modulation of *EPOR* with EPO, 10nM Ruxolitinib, and 10nM Ruxolitinib with EPO yielded a mean fold change over control in proliferation of 1.55 (95% CI: 1.16-1.94), 0.96 (95% CI: 0.84-1.09), and 1.24 (95% CI: 0.77-1.72), respectively (p=0.0254). In metastatic melanoma cells, Ruxolitinib significantly reduced invasiveness by a 0.48-fold change over control in proliferation. Additionally, Ruxolitinib blocked EPO induced increase in invasion, as EPO caused a 2.2-fold increase over control in invasiveness, however with the addition of Ruxolitinib there was a -0.04-fold change over control in invasion.

Discussion: Here we show that *JAK2* inhibition with Ruxolitinib diminishes erythropoietin (EPO) induced cell proliferation in an in-vitro model of invasive *BRAF*-mutated melanoma. We also show that Ruxolitinib decreases the invasive nature of metastatic melanoma, at baseline and when induced by EPO. Erythropoietin Receptor (EPOR) transduces signal through JAK/STAT and potentially contributes to a more aggressive phenotype. This provides initial evidence for future studies exploring the therapeutic role of Ruxolitinib in *BRAF* mutated melanoma. We further hypothesize that Ruxolitinib can be used in conjunction with BRAF inhibitors, such as Dabrafenib, to improve outcomes in metastatic melanoma.

#3871 Methyl jasmonate reduces proliferation and migration of head and neck cancer cell lines.

Jorge Viera-Vera[1], Maria M. Sanchez-Vazquez[1], Nicole J. Sariego-Hassan[2], Adianez Rosario-Vazquez[3], Magaly Martinez-Ferrer[1]

[1]University of Puerto Rico Comprehensive Cancer Center, San Juan, Puerto Rico,[2]Inter American University of Puerto Rico, Bayamon, Puerto Rico,[3]University of Puerto Rico, Mayaguez, Puerto Rico

Head and neck squamous cell carcinomas (HNSCC) arise from the mucosal lining of the oral and nasal cavities, pharynx, and larynx. The 2022 Global Cancer Statistics identify HNSCC as the sixth most common and seventh most lethal cancer globally, with 891,453 new cases and 458,107 fatalities. Over half of patients with HNSCC are diagnosed at an advanced stage, this has been associated with difficulties in early diagnosis, tumors with significant local invasion, and metastases in regional nodes. The Surveillance, Epidemiology, and End Results Program (SEER) estimates about 54,000 new HNSCC cases and 11,230 deaths in the United States during 2022, contributing to approximately 2% of all cancer deaths. The 5-year survival varies by stage at diagnosis, fluctuating from 86.3% for localized disease to 40.4% for metastatic disease. Current treatments for HNSCC include surgery, radiation, and chemotherapy, which can lead to serious side effects and may offer limited efficacy. Researchers have recently focused on exploring new complementary therapies. This study aims to analyze how methyl jasmonate (MeJa) affects HNSCC cell lines A-253 and SCC9 with special emphasis on cell migration. Methyl jasmonate is a plant-derived hormone that has been proven to exhibit highly selective cytotoxicity toward cancer cells that may prove to be a potentially less toxic therapeutic agent. The half maximal effective concentration (EC50) for MeJa was determined employing the MTS Assay Colometric Kit (Abcam, USA) and following the manufacturer instructions. Wound healing assays using the scratch method were performed and image data was collected with an Olympus LC35 camera attached to an CKX53 microscope. All experiments were performed in triplicates and data was analyzed using GraphPad Prism. MeJa inhibited the proliferation of A-253 and SCC9 HNSCC cell lines in a dose-dependent manner with estimated IC_{50} values of 2.23µM and 2.05µM respectively. Furthermore, the wound healing assay showed that MeJa significantly decreased cell migration in the A-253 cell lines at 12 and 24 hours after treatment. Our findings support the potential effects of methyl jasmonate on cellular events such as cell proliferation and migration during tumor progression of head and neck squamous cell carcinomas.

#3872 Fatty acid synthase inhibition to prevent metastatic colonization by pancreatic neuroendocrine tumor cells.

Piotr Rychahou[1], Benjamin M. Evers[1], Yekateriza Zaytseva[1], Courtney M. Townsend[2], B. Mark Evers[1]

[1]University of Kentucky, Lexington, KY,[2]University of Texas Medical Branch, Galveston, TX

Introduction: Pancreatic neuroendocrine tumors (pNETs) are among the most frequently occurring neuroendocrine neoplasms. At the time of diagnosis most patients have extensive metastatic disease and are not candidates for curative surgical resection. Therefore, identification of pathways that promote pNET metastasis is critical for developing targeted therapies to prevent cancer cells from colonization of parenchymal organs. The purpose of this study was to determine whether fatty acid synthase (FASN) inhibition can be used as a target against metastatic spread of pNETs.

Methods: Expression of FASN was evaluated in pancreatic neuroendocrine primary and metastatic tumors and scored by a pathologist. Neutral lipid droplet levels in BON, QGP-1 and NT-3 cell lines were determined with BODIPY 493/503 staining. Clonogenic and SRB assays were used to evaluate cytotoxic effects of FASN inhibition by TVB-3664 in QGP-1 and BON pNET cell lines. Western blot analysis was used to determine levels of cleaved PARP and cyclin D1 after FASN inhibition. FASN knockdown was used to delineate the effects of FASN downregulation on proliferation and lipid synthesis. Intravenous (iv) and intraperitoneal (ip) injections of BON GFP-Luc cells were used to establish lung metastasis and peritoneal carcinomatosis, respectively.

Results: FASN expression was determined at the maximum scores of 6 and 5 in 98% of patient samples. Highest levels of neutral lipid droplets were observed in the BON cell line. BON and QGP-1 cells were treated with TVB-3664 in either complete media with fatty acids (FA+) or complete media prepared with fatty acid depleted (FA-) FBS. Treatment with TVB-3664 resulted in significant inhibition of QGP-1 cell proliferation and cyclin D1 expression in both FA+ and FA- conditions. Depletion of extracellular FA enhanced the effect of TVB-3664 therapy on NET cells. FASN inhibition with TVB-3634 suppressed pNET cell proliferation and colony formation. Knockdown of FASN resulted in over 90% suppression of lung metastasis colonization. Pretreatment of BON GFP-luc cells with FASN inhibitor TVB-3664 before iv or ip injections also significantly suppressed lung metastasis colonization and peritoneal carcinomatosis.

Conclusions: Our study demonstrated high expression levels of FASN in pNET patient samples and cell lines. QGP cell viability was significantly decreased with FASN inhibition even in the presence of extracellular lipids. FASN inhibition alone in BON cells had only a minimal effect on cell viability and required depletion of extracellular lipids to achieve a significant decrease in cell viability. Despite minimal effects on BON cell viability, pretreatment with TVB-3664 in FA+ conditions had a pronounced effect on lung colonization and peritoneal carcinomatosis. These data demonstrate an essential role of de novo lipid synthesis in the pNET metastatic cascade - colonization of parenchymal organs.

#3873 Uncovering key regulators of OSCC invasion: A pathway to novel therapeutic strategies.

G. Flores[1], S. Uaroon[1], J. A. Raygoza Garay[1], M. R. Buchakjian[2];
[1]University of Iowa Hospitals and Clinics, Iowa City, IA, [2]University of Michigan, Ann Arbor, MI

Recurrence and metastasis are the primary drivers of poor prognosis in patients with Oral Squamous Cell Carcinoma (OSCC), even after aggressive surgical interventions. To uncover the genetic regulators of OSCC invasion and migration, we conducted a first-of-its-kind genome-wide CRISPR screen. This screen identified several key players involved in epithelial-mesenchymal transition (EMT), a critical process in tumor progression and metastasis. Among the top hits was *EDIL3*, a gene that codes for a secreted protein that promotes EMT and chemotherapy resistance by interacting with integrin receptors in the extracellular matrix. Though previously implicated in other cancers, EDIL3's role in OSCC has not been described. Additionally, our screen highlighted NISCH as the top anti-invasion hit, a gene whose expression is positively correlated with survival in head and neck cancers, including OSCC. NISCH appears to inhibit EDIL3-driven invasion by interfering with integrin-mediated signaling, potentially via a FAK and WNT5a-dependent mechanism. Current work is focused on characterizing *EDIL3* and *NISCH* knockouts to validate their roles in OSCC migration and invasion. Functional assays such as spheroid formation and wound healing experiments are being performed to assess the impact of these knockouts on tumor cell behavior. Additionally, we are investigating FDA-approved drugs targeting these pathways to explore immediate translational potential. This research represents a novel approach to identifying genetic drivers of cancer metastasis and offers promising avenues for the development of targeted therapies for OSCC to reduce recurrence and improve patient outcomes.

TUMOR BIOLOGY: Cancer Cell Heterogeneity, Evolution, and Trajectory Profiling
Poster Session

#3879 Threshold dynamics of EWS::FLI1 reveal drivers of tumor plasticity and prognosis in Ewing sarcoma.

Veveeyan Suresh[1], Christoph Hafemeister[1], Andri Konstantinou[2], Florencia Cidre-Aranaz[3], Sarah Grissenberger[1], Caterina Sturtzel[1], Karla Queiroz[4], Dorota Kurek[4], Andrea Wenninger-Weinzierl[1], Martha Zylka[1], Ana Sastre[5], Javier Alonso[6], Martin Distel[1], Anna Obenauf[2], Thomas G.P Grunewald[7], Florian Halbritter[1], Heinrich Kovar[1], Valerie Fock[1]

[1]St. Anna Children's Cancer Research Institute (CCRI), Vienna, Austria,[2]Research Institute of Molecular Pathology, Vienna Biocenter, Vienna, Austria,[3]German Cancer Research Center (DKFZ), Hopp-Children's Cancer Center (KiTZ), Heidelberg, Germany,[4]Mimetas, Oegstgeest, Netherlands,[5]Unidad Hemato-oncologia Pediatrica, Hospital Infantil Universitario La Paz, Madrid, Spain,[6]Unidad de Tumores Solidos Infantiles, Instituto de Investigacion de Enfermedades Raras, Instituto de Salud Carlos III, Centro de Investigacion Biomedica en Red de Enfermedades Raras (CB06/07/1009, CIBERER-ISCIII), Madrid, Spain,[7]German Cancer Research Center (DKFZ), Hopp-Children's Cancer Center (KiTZ), Institute of Pathology, Heidelberg University Hospital, Heidelberg, Germany

Ewing sarcoma (EwS) is a highly aggressive pediatric cancer driven by the EWS::FLI1 (EF) fusion oncoprotein. Emerging evidence suggests that variations in EF expression levels may contribute to tumor cell plasticity, promoting treatment resistance and relapse. Both tumor intrinsic and extrinsic influences such as microenvironmental and therapy related factors, may influence EF thresholds. However, the phenotypic consequences of EF fluctuations at different amplitudes and intervals remain poorly understood. To address this, we engineered a EF threshold modulation system in A673 cells by tagging endogenous EF with a fluorescent mNeonGreen (mNG) and a degron tag (dTAG). Using mNG fluorescence as a quantitative proxy for EF levels, we established a stable and highly reproducible EF gradient through treatment with graded concentrations of the dTAG^V-1 ligand, ranging from 3 hours to 4 weeks. Phenotypic assays revealed that partial EF depletion (35-65%), but not near-complete loss (90-95%), significantly enhanced migratory and invasive behaviors in vitro, while minor reductions (15-35%) were sufficient to impair anchorage-independent growth. In vivo studies corroborated these findings, as partially EF-depleted cells upon EF restoration exhibited increased extravasation in zebrafish models and enhanced lung metastasis in immunocompromised mice. To elucidate the molecular basis underpinning EF threshold-dependent phenotypes, we integrated acute transcriptional changes from nascent RNA sequencing (SLAM-seq) with EF chromatin occupancy (CUT&RUN) data following 3 hours of dTAG^V-1 gradient treatment. A 15% reduction in EF resulted in immediate downregulation of EF-activated genes enriched for GGAA microsatellites in enhancers and upregulation of EF-repressed genes associated with monomeric ETS binding sites in EF-bound promoter regions. Interestingly, the largest group of EF repressed genes, associated with epithelial-to-mesenchymal transition (EMT), showed graded upregulation in response to increasing dTAG^V-1 concentrations. For these genes, EF binding was predominantly localized to promoter regions enriched for Krüppel-related zinc finger protein binding sites, implicating novel EF coregulators in mediating the migratory phenotype. Strikingly, transient EF depletion (3 weeks) followed by rapid EF restoration led to sustained upregulation of EMT-related genes and migratory behavior for up to 2 weeks after dTAG^V-1 ligand washout. Supporting these observations, approximately 20% of these genes correlated with poor prognosis in a cohort of 196 EwS patients. Our findings underscore the importance of EF expression dynamics in driving EwS cell plasticity and highlight therapeutic challenges posed by insufficient direct EF inhibition. These insights provide a foundation for exploiting oncogene dynamics to uncover therapeutic vulnerabilities in single oncogene driven cancers.

#3880 Functional dissection of the highly plastic basal cell state in pancreatic cancer.

Anupriya Singhal[1], Hannah Styers[1], Jonathan Rub[1], Kate Ryan[1], Jung Yun Kim[1], Zeynep C. Tarcan[1], Jill Hallin[2], Olca Basturk[1], Rona Yaeger[1], James G. Christensen[1], Yan Yan[1], Elisa de Stanchina[1], Tuomas Tammela[1]

[1]Memorial Sloan Kettering Cancer Center, New York, NY,[2]Mirati Therapeutics, San Diego, CA

Pancreatic ductal adenocarcinoma (PDAC) is a treatment-refractory malignancy with a dismal prognosis. Targeting cancer cell plasticity—the capacity to adapt to cell-extrinsic pressure via non-genetic mechanisms—is a promising therapeutic strategy, yet cell states within PDAC harboring high plasticity remain poorly understood. Using genetically engineered mouse models driven by oncogenic KrasG12D and loss of p53 (“KPC” model), we profiled primary PDAC tumors using single-cell RNA sequencing and single cell ATAC sequencing. We find that the KPC model harbors a basal state, which harbors transcriptional signature that aligns with the "basal" signature in human PDAC and is linked to poor prognosis and chemoresistance. The basal state co-expresses both epithelial and mesenchymal programs and has a distinct epigenetic landscape with accessible chromatin across gene sets that mark cancer cell states with divergent phenotypes, suggesting that this cell state is primed for cell state switching. To functionally interrogate the basal state in PDAC progression, we developed a novel lineage-tracing and ablation system. Lineage-tracing experiments revealed that the basal state has a robust capacity for differentiation into epithelial and mesenchymal states, indicating that the basal state is functionally plastic and acts as a source of fixed malignant states. Strikingly, targeted elimination of the basal state over short time periods resulted in tumor collapse, underscoring a critical role in tumor maintenance. Further, we found that the basal state is highly dependent on KRAS activity, with acute KRAS inhibitor treatment leading to a selective depletion of the basal state. Taken together, our data indicates that PDAC harbors a striking dependency on the basal state and encourages therapeutic approaches aimed at eradicating this critical subset of cancer cells.

#3881 Comprehensive analysis of somatic mutagenesis in normal tissues across 19,643 samples reveals insights into clonal evolution and tumorigenesis.

Leonie Stockschlader[1], Eldar Abdullaev[1], Siddharth Annaldasula[1], Ino de Bruijn[2], Baby Anusha Satravada[2], Ritika Kundra[2], Rima Madupuri[2], Manuela Benary[3], Dieter Beule[3], Tim H. H. Coorens[4], Regine Armann[1], Nikolaus Schultz[2], Chris Sander[5], Kirsten Kubler[1]

[1]Department for Functional Diagnostics, Berlin Insitute of Health, Berlin, Germany,[2]Marie-Josee & Henry R. Kravis Center for Molecular Oncology, Memorial Sloan Kettering Cancer Center, New York, NY,[3]Core Unit Bioinformatics, Berlin Insitute of Health, Berlin, Germany,[4]Broad Institute of MIT and Harvard, Cambridge, MA,[5]CompBio Collaboratory, Harvard Medical School, Boston, MA

The detection of somatic mutagenesis in normal tissues has transformed our understanding of clonal evolution, revealing a mosaic of somatic mutations, including those in cancer driver genes, that influence cellular behavior and tissue homeostasis. However, key questions remain about how these mutational patterns contribute to tumorigenesis and the role of cancer driver mutations in the transition from normal to malignant states. To address this, we performed a combined computational analysis of the largest normal-tissue DNA-sequencing and clinical dataset to date, spanning 21 published datasets, 37 tissue types, 521 patients, and 19,643 samples, and integrated these with precancer and cancer tissue data to identify tissue-specific mutational changes potentially critical for tumorigenesis. We identified tissue-specific differences in mutation burden, with epithelial tissues exhibiting significantly higher mutation rates than other tissue categories ($p<2x10^{-16}$). These differences were further reflected in the mutational signatures: UV-light signatures dominated sun-exposed epithelial tissues, while gastrointestinal epithelial tissues showed mutation burden driven by intrinsic factors. Regression analysis revealed that, alongside aging, mutations in specific driver genes (e.g., *ARID2*, *ATM*, *TP53*), but not others, were selectively associated with higher mutation rates, highlighting nuanced contributions to tissue mutagenesis. Further MutSig2CV and dN/dS analyses identified positively selected driver genes with distinct patterns based on tumorigenic pathways: tumors that develop through normal-to-dysplastic sequences (e.g., colon cancer) shared driver genes across all progression stages, reflecting clonal evolution within the same tissue lineage. In contrast, tumors arising from metaplastic processes (e.g., esophageal adenocarcinoma) shared mutated driver genes between cancer and precancer metaplastic tissues, but not normal tissues, suggesting selective pressures emerge during metaplasia. Across the cohort, most driver genes were highly mutated in cancer and present at lower mutation frequency in normal tissues. In contrast, similar to *NOTCH1* mutations, *CHEK2* mutations were significantly enriched in the normal esophagus but absent in cancer, hinting at potential tumor-suppressive effects. Taken together, our large-scale analysis has allowed us to detect previously unrecognized phenomena through increased statistical power. The analysis of normal tissue mutagenesis underscores its potential for predicting tissue-specific cancer risks. In addition, our integration of normal tissue data into the cBioPortal platform provides an important resource to support and advance future cancer research.

#3882 Patterns of genomic patient-model conservation and evolution in the Human Cancer Models Initiative (HCMI) next-generation cancer model resource.

Andrew W. McPherson[1], Seongmin Choi[1], William F. Hooper[2], Jennifer M. Shelton[2], Timothy R. Chu[2], Dina ElHarouni[3], Mushriq Al-Jazrawe[4], Merve Dede[5], Toshinori Hinoue[6], Sean A. Misek[3], Heeju Noh[7], Luca Zanella[8], Moony Tseng[9], Hayley E. Francies[10], Priya Sridevi[11], Rachana Agarwal[12], Cindy W. Kyi[13], Julyann Perez-Mayoral[13], Megan J. Stine[14], Eva Tonsing-Carter[13], James M. Clinton[15], The HCMI Network, Peter W. Laird[16], Calvin J. Kuo[17], Olivier Elemento[18], David L. Spector[11], Andrew D. Cherniack[19], Kyle Ellrott[20], Martin L. Ferguson[21], Rameen Beroukhim[22], Katherine A. Hoadley[23], Nicolas Robine[2], Mathew J. Garnett[10], David A. Tuveson[11], Andrea Califano[24], Paul T. Spellman[25], Keith L. Ligon[26], Daniela S. Gerhard[13], Louis M. Staudt[13], Jesse Boehm[9]

[1]Computational Oncology, Memorial Sloan Kettering Cancer Center, New York, NY,[2]New York Genome Center, New York, NY,[3]Dana Farber Cancer Institute, Broad Institute of MIT and Harvard, Boston, MA,[4]Koch Institute for Integrative Cancer Research at MIT, Broad Institute of MIT and Harvard, Cambridge, MA,[5]Department of Bioinformatics and Computational Biology, University of Texas MD Anderson Cancer Center, Houston, TX,[6]Department of Epigenetics, Grand Rapids, Van Andel Institute, Grand Rapids, MI,[7]Computational Oncology, Vagelos College of Physicians and Surgeons, Columbia University Irving Medical Center, New York, NY,[8]Department of Systems Biology, Vagelos College of Physicians and Surgeons, Columbia University Irving Medical Center, New York, NY,[9]Broad Institute of MIT and Harvard, Cambridge, MA,[10]Wellcome Sanger Institute, Cambridge, United Kingdom,[11]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY,[12]Frederick National Laboratory for Cancer Research, Frederick, MD,[13]Center for Cancer Genomics, NCI, Bethesda, MD,[14]Information Management Services, Inc, Rockville, MD,[15]American Type Culture Collection, Manassas, VA,[16]Department of Epigenetics, Van Andel Institute, Grand Rapids, MI,[17]Department of Medicine, Division of Hematology, Stanford University School of Medicine, Stanford, CA,[18]Institute for Computational Biomedicine, Weill Cornell Medicine, New York, NY,[19]Broad Institute of MIT and Harvard, Dana Farber Cancer Institute, Harvard Medical School, Boston, MA,[20]Department of Biomedical Engineering, Oregon Health & Science University, Portland, OR,[21]Center for Cancer Genomics, National Cancer Institute, Bethesda, MD,[22]Departments of Cancer Biology and Medical Oncology, Department of Medicine, Broad Institute, Dana Farber Cancer Institute, and Harvard Medical School, Boston, MA,[23]Department of Genetics, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC,[24]Herbert Irving Comprehensive Cancer Center, Columbia University, Chan Zuckerberg Biohub, New York, NY,[25]Departments of Medicine and Human Genetics, University of California, Los Angeles, CA,[26]Dana Farber Cancer Institute, Boston, MA

Background: Patient-derived cancer models advance therapeutic development and are essential to studies of treatment resistance mechanisms. The Human Cancer Models Initiative (HCMI) has created 665 patient-derived models, including organoids, neurospheres, and conditionally reprogrammed cells, all paired with patient tumors and clinical annotations. This resource provides an opportunity to study genomic conservation and divergence during model development at scale in a large pan-cancer cohort of matched tumor-model pairs.
Methods: We characterized the genomes of models and parent tumors, computing allele specific Copy Number Variation (CNV), Single Nucleotide Variants (SNV), small Insertions and Deletions (InDels) and Structural Variations (SV) from bulk whole genome and whole exome Sequencing. We used pyclone to study changes in clonal abundances between tumor and model, MutationTimeR to understand the timing of model specific CNVs and Whole Genome Doubling (WGD), and AmpliconArchitect to identify ecDNA.
Results: HCMI models exhibited high concordance with matching tumors when evaluated on SNVs, SVs, InDels and CNVs, with 69% of SNV/InDel drivers and 43% of CNV drivers conserved between tumor and model. Only 2% of investigated models (9/405) showed significantly divergent patterns of aneuploidy and presence of distinct clonal outgrowth in the model. This included two pancreatic cancer models with oncogenic KRAS point mutations that differed from those in the parent tumor. The cohort included models and tumors with ecDNA affecting known oncogenes including EGFR in GBM samples, CCNE1/KRAS in esophageal cancer and MYC in pancreatic cancer. ecDNA were the least conserved aberration type; 20% (50/254) of tumor-detected ecDNA were retained in an associated model while 35% (50/143) of model-detected ecDNA were found in their parent tumors. While the tumors and models generally showed similar WGD states, 10% of models exhibited WGD private to the model, and this model specific WGD generally occurred later in estimated mutational evolution time than models for which the tumor and model were both WGD. Surprisingly, not all models were pure for cancer cells with 19 models, enriched for melanomas, containing more than 20% diploid presumably non-tumor cells.
Discussion: HCMI models exhibited high genomic concordance with their matching tumors across all cancer types. We catalogued classes of genomic divergence representing either selection of subclones or evolution during model development. SNVs and InDels exhibited greater conservation compared to CNVs, with ecDNA showing the highest variability between the tumor and the model. Our results provide full visibility into the genomic fidelity of each HCMI model, and enable researchers to select the most relevant HCMI model for their study.

#3883 Evolution of genomic and epigenomic heterogeneity in prostate cancer from tissue and liquid biopsies.

Marjorie Roskes[1], **Alexander Martinez-Fundichely**[1], Weiling Li[1], Sandra Cohen[1], Hao Xu[2], Shahd ElNaggar[3], Anisha Tehim[4], Metin Balabin[5], Chen Khuan Wong[6], Yu Chen[6], Ben Raphael[5], Ekta Khurana[1]

[1]Weill Cornell Medicine, New York, NY,[2]McGill University, Montreal, NY, Canada,[3]Barnard College, New York, NY,[4]Cornell University, Ithaca, NY,[5]Princeton University, New Jersey, NY,[6]Memorial Sloan Kettering Cancer Center, New York, NY

Castration resistant prostate cancer (CRPC) is an aggressive, highly plastic, late-stage disease. We previously showed that the two histological subtypes, adenocarcinoma (CRPC-Adeno) and neuroendocrine (CRPC-NE), show four epigenetic and transcriptomic subtypes: CRPC-AR depends on the androgen receptor pathway, CRPC-SCL is stem-cell like, CRPC-WNT is dependent on the WNT pathway, and CRPC-NE has high expression of neuroendocrine markers. Here, by analyzing data from 500 patients with tissue and/or liquid biopsies, we uncover the landscape of molecular heterogeneity in patient tumors. Analysis of whole-genome sequencing revealed genomic variants associated with CRPC-SCL and allowed development of a computational classifier which can predict presence of CRPC-SCL in patient tumors solely using genomic alterations with 81% accuracy. In particular, analysis of matched chromatin conformation data (Hi-C) showed a complex rearrangement on chromosome 4 disrupts enhancer - promoter contacts leading to downregulation of *MOB1B*, which can lead to upregulation of the *YAP/TAZ* pathway that is characteristic of CRPC-SCL. Joint computational inference of epigenomic state and genomic variants from cell-free DNA collected at multiple points during the evolution of resistance to AR signaling inhibitors allowed investigation of genomic and epigenomic co-evolution at an unprecedented resolution. We discuss the current limits of detection for tumoral epigenomic and genomic states using cell-free DNA for clinical application. Importantly, our study demonstrates the utility of liquid biopsies for discovery of basic biological mechanisms leading to treatment resistance, beyond their use for biomarkers.

#3884 Uneven inheritance and functional heterogeneity derived from extrachromosomal DNA enable rapid adaptation of cancer cells.

Shu Zhang[1], Aditi Gnanasekar[1], Ellis J. Curtis[1], Jun Tang[1], Vishnu Shankar[1], Ivy Tsz-Lo Wong[1], Xiaowei Yan[2], Rui Li[2], Lu Yang[1], Howard Y. Chang[2], Paul S. Mischel[1]

[1]Department of Pathology, Stanford University, Palo Alto, CA,[2]Department of Dermatology, Stanford University, Palo Alto, CA

The amplification of oncogenes on extrachromosomal DNA (ecDNA) is common in cancer and associated with poor outcomes. Uneven inheritance of ecDNA during cell division leads to intratumoral ecDNA copy number heterogeneity. However, the relationship between shifts in ecDNA copy number and the rapid environmental adaptation of cancer cells remains unclear. In this study, we utilized imaging and single-cell multi-omics analyses to demonstrate that the transcript and protein expression of ecDNA oncogenes, along with functional heterogeneity, correlate with ecDNA copy number heterogeneity. Using fluorescence-activated cell sorting (FACS) and live-cell imaging, we found that cells with “middle” ecDNA copy numbers display the highest growth rates compared to cells with the lowest or highest copy number statuses, indicating greatest fitness is achieved by the balance of promoting cell proliferation while minimizing heightened replication stress arising from increasing copy numbers of ecDNA oncogenes. Through simulations based on random segregation and optimal copy number models, we found that ecDNA-bearing cells can rapidly adjust their copy numbers to achieve optimal growth in new environments. Notably, compared to chromosomally amplified cells, those with ecDNA exhibited superior growth following xenograft injection and treatment with single drugs targeting replication stress or oncogenes, aligning with our simulation findings. Our results reveal that functional heterogeneity resulting from uneven ecDNA inheritance enhances cancer cells' adaptability to tumor microenvironments and rapid acquisition of drug resistance, warranting that the development of combinatorial therapies that synergistically target a wide range of copy numbers is critical for effectively treating ecDNA-containing cancers.

#3886 Decoding pediatric adrenocortical tumor heterogeneity through multimodal molecular profiling - pathways to improved risk stratification and therapy.

Victoria E. Fincke[1], Maurice Lo?ner[1], Marina Kunstreich[1], Irmengard Sax[2], Marlena Mucha[1], Felix Dorn[1], Maria D. Hernandez Ramirez[1], Stefan Wudy[3], Christian Vokuhl[4], Christoph Slavetinsky[5], Jorg Fuchs[5], Michael C. Fruhwald[1], Matthias Schlesner[2], Antje Redlich[6], Rainer Claus[7], Michaela Kuhlen[1], Pascal D. Johann[1]

[1]Pediatrics and Adolescent Medicine, Swabian Children's Cancer Center, University Hospital Augsburg, Augsburg, Germany,[2]Biomedical Informatics, Data Mining and Data Analytics, University of Augsburg, Augsburg, Germany,[3]Pediatric Endocrinology and Diabetology, University Hospital Giessen, Giessen, Germany,[4]Department of Pathology, Section of Pediatric Pathology, University Hospital Bonn, Bonn, Germany,[5]Department of Pediatric Surgery and Urology, University Children?s Hospital Tuebingen, Tuebingen, Germany,[6]Pediatric Hematology and Oncology, University Hospital Magdeburg, Magdeburg, Germany,[7]Molecular Oncology, University Hospital Augsburg, Augsburg, Germany

Pediatric adrenocortical tumors (pACTs) are rare neoplasms with significant mortality, presenting diagnostic challenges in distinguishing benign from malignant tumors. Here, we employed a comprehensive multimodal molecular profiling approach to delineate and characterize molecular subgroups and investigated their potential for risk stratification.

We profiled 109 pediatric adrenocortical tumors using Illumina methylation arrays and analyzed them together with 105 published samples. For 29 of the 109 tumors, fresh-frozen samples were analyzed with single-nucleus RNA sequencing and seven of these also with single-nucleus ATAC sequencing.

Our analysis revealed four molecularly distinct subgroups with differences in clinical presentation, cellular composition and molecular signatures. Importantly, the herein identified high-risk subgroup demonstrated substantially reduced overall survival, with a 5-year survival rate of merely 27% compared to 81-95% in other subgroups. Notably, these tumors showed increased CpG island methylation and aberrant activation of the canonical WNT signaling pathway in gene expression profiling.

Additionally, through integration of scRNA-Seq and scATAC-Seq, we uncovered complex transcriptional networks driving tumor biology. Tumor cells resembling the physiological adrenocortical zones exhibited unique gene expression patterns, with the high-risk subgroup characterized by an increased proportion of zona glomerulosa-like cells.

These zona glomerulosa-like cells showed dysregulated WNT/CTNNB1 signaling and a proliferative and cell cycle activation signature, which emerge as a critical driver of malignancy. In particular, the glomerulosa-like tumor cells seem to fuel tumor proliferation by a crosstalk with fasciculata/reticularis-like tumor cells, which is unique for the high risk pACT subgroup. Further key molecular alterations include upregulation of cancer associated genes like *PVT1* and *MDM2*, suggesting potential therapeutic vulnerabilities. Moreover, our analysis uncovered transmembrane glycoprotein BAMBI to be specifically upregulated in the high-risk tumors, highlighting its potential as a prognostic indicator.

This comprehensive characterization provides a robust framework for risk stratification in pACTs. Specifically, we have unraveled a previously undescribed molecular hub using canonical WNT signaling to drive proliferation in high risk tumors. These changes may open an avenue towards integrating WNT-directed therapies in the clinical setting.

#3887 Clonal expansion in the bile duct associated with primary sclerosing cholangitis.

Hirona Maeda[1], Nobuyuki Kakiuchi[2], Takashi Ito[3], Eri Ogawa[4], Masahiro Shiokawa[5], Norimitsu Uza[5], Koichi Watanabe[1], Yasuhito Nannya[6], Yuzo Kodama[7], Tatsuki Kataoka[8], Hatano Etsuro[3], Seishi Ogawa[1]

[1]Department of Pathology and Tumor Biology, Kyoto University, Kyoto, Japan,[2]The Hakubi Center for Advanced Research, Kyoto University, Kyoto, Japan,[3]Department of Surgery, Kyoto University, Kyoto, Japan,[4]Department of Pediatric Surgery, Kyoto University, Kyoto, Japan,[5]Department of Gastroenterology and Hepatology, Kyoto University, Kyoto, Japan,[6]Department of Hematology/Oncology, The University of Tokyo, Tokyo, Japan,[7]Division of Gastroenterology, Kobe University, Kobe, Japan,[8]Department of Pathology, Iwate University, Iwate, Japan

[Introduction] Chronic inflammation has been implicated in cancer development in many tissues and has recently been shown to promote clonal expansion in non-cancer tissues. Primary sclerosing cholangitis (PSC) is a chronic progressive disease of unknown etiology that affects the bile ducts and has been associated with an increased risk of cholangiocarcinoma. However, clonal dynamics in the normal bile duct and PSC epithelium remain poorly understood due to the limited accessibility to normal/inflamed bile ducts. In this study, we investigated somatic clonal events in the chronically inflamed bile duct epithelium and identified clonal expansions with and without cancer-associated driver mutations.

[Methods] Single cell-derived organoids were established from the bile and subjected to whole genome sequencing to estimate the mutation accumulation rate in the normal bile duct epithelium. Next to evaluate clonal expansion in the bile duct in PSC, we performed whole exome sequencing in bile duct-derived organoids and samples from the bile duct epithelium obtained from PSC patients using laser-capture microdissection (LCM) and detected somatic mutations and copy number alterations therein.

[Results] In total, 14 single cell-derived organoids were established from 2 PSC patients and 3 control donors. Based on the whole genome sequencing of these organoids, the number of mutations found in non-PSC bile duct epithelium increases with age at an estimated mutation rate of 34.3 mutations per genome per year. The mutation rate did not significantly differ from the rate in the PSC epithelium, suggesting that the inflammatory process in PSC does not substantially affect the mutation rate in the biliary epithelium. We next investigated the effect of chronic inflammation on clonal expansion in the biliary epithelium, in which we analyzed 120 bulk organoid samples from 3 PSC patients and 304 LCM samples from histologically normal bile duct and biliary intraepithelial neoplasia (BilIN) from 7 PSC patients. Excluding a case with hypermutation, we detected the presence of clones in 20 (16.7%) and 105 (47.1%) of organoid and LCM samples, where a median of 13 [1-30] and 19 [5-41] somatic mutations per exome were detected in organoid and LCM samples, respectively. Most mutations were detected in a single sample, but some were shared between multiple samples. In total, 51 clones were detected across multiple samples. These clones were observed regardless of the presence or absence of BilIN lesions and found in the samples separated up to 40mm. Mutations in known cancer drivers, such as ARID1A and KRAS, and chromosomal instability were detected in 11 clones in 64 samples, suggesting their roles in the clonal expansion in PSC bile ducts.

[Conclusions] Clonal expansion in the bile duct from PSC patients are common and accompanied by known cancer driver mutations, suggesting their role in biliary carcinogenesis associated with PSC.

#3888 Exploring intra-tumor heterogeneity in malignant peripheral nerve sheath tumors through single-cell multi-omics.

Yidan Pan[1], Yixiao Cheng[1], Chunxu Gao[1], Haixi Yan[2], Zhihui Zhang[1], Leah Weber[1], Tom Lesluyes[2], Cristina Cotobal Martin[3], Isidro Cortes-Ciriano[4], Nischalan Pillay[5], David T. Miller[6], Adrienne M. Flanagan[5], Maxime Tarabichi[7], Peter Van Loo[1]

[1]UT MD Anderson Cancer Center, Houston, TX,[2]The Francis Crick Institute, London, United Kingdom,[3]Wellcome Sanger Institute, Cambridge, United Kingdom,[4]EMBL's European Bioinformatics Institute, Hinxton, United Kingdom,[5]University College London, London, United Kingdom,[6]Boston Children's Hospital, Boston, MA,[7]Universite Libre de Bruxelle, Brussels, Belgium

Malignant peripheral nerve sheath tumors (MPNSTs) are a rare subtype of sarcomas arising from peripheral nerves, occurring either sporadically or in association with neurofibromatosis type 1 (NF1). Although comprising only 5-10% of soft tissue sarcomas, MPNSTs are highly aggressive and have limited treatment options, with 5-year survival rates of 34%-60%. Intra-Tumor Heterogeneity (ITH) is a key driver of tumor evolution and treatment resistance. However, prior genomic analyses of MPNSTs are often limited to a single sample per patient and have not explored ITH in detail. To fill this gap, we developed a multi-omics and single-cell integration pipeline to analyze bulk Whole-Genome Sequencing (WGS), single-nuclei DNA (snDNA) and RNA (snRNA) sequencing, and multi-regional shallow-coverage WGS derived from multiple Laser Capture Microdissection (LCM) spots within tissue sections. We applied this integrative multi-omics approach to analyze 63 samples collected from 17 patients. Somatic mutations were called in WGS using a two-out-of-three consensus approach (MuSE2, Strelka2, Mutect2), and Copy Number Aberrations (CNA) were analyzed using the Battenberg algorithm. Phylogenetic trees were reconstructed using Dirichlet Process-based methods and PyClone. We identified low-to-moderate mutational burden (median = 1.23 mutations/Mb, range: 0.52–2.34 mutations/Mb) but significant chromosomal aberrations and varied levels of genome instability. Whole genome doubling was highly prevalent (47/63 samples; 14/17 patients) and many samples showed widespread loss of heterozygosity (median = 26%, range: 4%-93%). Among the 17 patients, 5 exhibited a linear evolution pattern, while the majority demonstrated branching evolution. Cross-referencing CNA profiles across bulk WGS, snDNA (ASCAT.sc), LCM, and snRNA (inferCNV) data validated WGS-derived CNAs and demonstrated subclonal CNA co-occurrence within individual cells or subclones. Genotyping WGS-derived mutation clusters in snDNA revealed that co-occurrence of mutation pairs within single cells was exclusively observed when mutations belonged to the same cluster or lineage, further validating our bulk WGS-derived phylogenetic trees. Our findings reveal that MPNSTs are primarily driven by chromosomal instability, with ITH further elucidated through cross-referenced and refined phylogenetic tree reconstruction at single-cell resolution. Future work would focus on 3D phylogenetic tree reconstruction using spatial-genomics information derived from LCM and integrating single-cell transcriptomics. Additionally, incorporating spatial transcriptomics could provide deeper insights into the tumor microenvironment and its role in driving ITH. These efforts will further advance the understanding of MPNST biology and evolution, with potential implications for targeted therapeutic strategies.

#3889 Access and selection shapes peritoneal metastasis evolution in colorectal cancer.

Emma CE Wassenaar[1], **Alexander N. Gorelick**[2], Wei-Ting Hung[2], David M. Cheek[2], Emre Kucukkose[3], I-Hsiu Lee[2], Martin Blohmer[2], Sebastian Degner[2], Peter Giunta[2], Rene MJ Wiezer[1], Mihaela G. Raicu[1], Inge Ubink[3], Sjoerd J. Klaasen[3], Nico Lansu[3], Emma V. Watson[4], Ryan B. Corcoran[5], Genevieve Boland[6], Gad Getz[7], Geert JPL Kops[3], Dejan Juric[8], Jochen K. Lennerz[9], Djamila Boerma[1], Onno Kranenburg[3], Kamila Naxerova[2]

[1]Department of Surgery, St. Antonius Hospital, Nieuwegein, Netherlands,[2]Genetics, Harvard Medical School/Massachusetts General Hospital, Boston, MA,[3]Department of Surgical Oncology, Laboratory Translational Oncology, University Medical Center Utrecht, Utrecht, Netherlands,[4]University of Massachusetts Medical School, Worcester, MA,[5]Division of Hematology and Oncology, Department of Medicine, Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA,[6]Division of Surgical Oncology, Department of Surgery, Harvard Medical School/Massachusetts General Hospital, Boston, MA,[7]Cancer Program, Broad Institute of MIT and Harvard, Cambridge, MA,[8]Termeer Center for Targeted Therapies; Krantz Family Center for Cancer Research, Massachusetts General Hospital, Boston, MA,[9]Department of Pathology, Massachusetts General Hospital, Boston, MA

Whether metastasis in humans can be accomplished by most primary tumor cells or requires the evolution of a specialized trait remains an open question. To evaluate whether metastases are founded by non-random subsets of primary tumor lineages requires extensive, difficult-to-implement sampling. We have realized an unusually dense multi-region sampling scheme in a cohort of 26 colorectal cancer patients with peritoneal metastases, reconstructing the evolutionary history of on average 28.8 tissue samples per patient with a microsatellite-based fingerprinting assay. To assess metastatic randomness, we evaluate inter- and intra-metastatic heterogeneity relative to the primary tumor and find that peritoneal metastases are more heterogeneous than liver metastases but less diverse than locoregional metastases. Metachronous peritoneal metastases exposed to systemic chemotherapy show significantly higher inter-lesion diversity than synchronous, untreated metastases. Projection of peritoneal metastasis origins onto a spatial map of the primary tumor reveals that they often originate at the deep-invading edge, in contrast to liver and lymph node metastases which exhibit no such preference. Furthermore, peritoneal metastases typically do not share a common subclonal origin with distant metastases in more remote organs. Synthesizing these insights into an evolutionary portrait of peritoneal metastases, we conclude that the peritoneal-metastatic process imposes milder selective pressures onto disseminating cancer cells than the liver-metastatic process. Peritoneal metastases' unique evolutionary features have potential implications for staging and treatment.

#3891 Integrative single-cell tracking of genome evolution and tumor cell plasticity in small cell lung cancer (SCLC).

Marcel Schmiel[1], Laura Kaiser[2], Julie George[2], Roman K. Thomas[2]

[1]Pathology, University Hospital of Cologne, Cologne, Germany,[2]Translational Genomics, University Hospital of Cologne, Cologne, Germany

SCLC is the deadliest type of lung cancer, which is mostly diagnosed at late stages and accounts for 15% of pulmonary tumors. Chemotherapy is initially effective, but SCLC patients relapse quickly and develop resistant tumors. Large-scale studies uncovered key molecular patterns in SCLC, which include universal loss of TP53 and RB1, as well as transcriptional patterns based on the expression of 4 lineage transcription factors (TF). We aimed to dissect how genomic cues impact transcriptional phenotypes, and the dynamics of tumor and immune cell interactions, to thus decipher molecular mechanisms of phenotypic divergence in SCLC.

We performed genome and single cell transcriptome profiling on 56 SCLC patient tumors, including primary tumors, metastases and paired relapse tumors, including a patient sample after treatment with DLL3-targeted immunotherapy. Single cell RNA-seq was performed with the 10x Genomics platform to study cancer cells and the tumor micro-environment (TME), and tumor cell intrinsic heterogeneity. Data were processed with an in-house pipeline.

Single cell expression profiles were determined for > 200000 cells from 56 tumors. Transcriptome profiling at single cell levels revealed co-expression of at least two of the lineage TF *ASCL1*, *NEUROD1* or *POU2F3* for each SCLC tumor. Transcriptional profiles pointed to a marked intra- and interpatient, as well as to inter-metastatic heterogeneity. Cell-cell-interaction analysis across all samples uncovered recurrent patterns of intra-tumoral receptor-ligand interactions. The integrative analysis of genome and single cell transcriptome data pointed to somatic alterations and copy number alterations shaping transcriptional heterogeneity. Specifically, tumors with gene amplifications of *MYC* family members share similar expression profiles. We performed multi-regional and longitudinal studies of matched patient cases to project genomic patterns of clonal evolution to transcriptional profiles at single cell level, thus analyzing the effect of genome diversity on the transcriptional landscape. Additionally, tumor intrinsic profiles of heterogeneity were mapped to single cell data of patient tumors to elucidate the effects on tumor-immune cell interactions.

Altogether we provide a first comprehensive framework for the study of underlying genetic alterations that shape the transcriptional landscape and phenotypic plasticity in SCLC. Our data points to a concerted regulation of a multitude of factors including lineage TF, transcriptional programs and interaction networks in SCLC.

#3892 Multi-sample whole genome sequencing unveils complex mutational dynamics and clonal evolutionary patterns in young onset breast cancer from Nigeria.

Avraam Tapinos[1], Toshio Yoshimatsu[2], Ilona Siljander[2], Mustapha A. Ajan[3], Ayodele Sanni[4], Atara Ntekim[5], Abayomi Odetunde[6], Elisabeth Sveen[2], Jeffrey Mueller[7], Galina Khramtsova[2], Sulin Wu[2], Dorothy Nyamai[1], Mihai Giurcanu[8], Dezheng Huo[8], **Yonglan Zheng**[2], David C. Wedge[1], Olufunmilayo I. Olopade[2]

[1]Division of Cancer Sciences, School of Medical Sciences, University of Manchester, Manchester, United Kingdom,[2]Center for Clinical Cancer Genetics and Global Health, Department of Medicine, The University of Chicago, Chicago, IL,[3]Department of Pathology, University of Ibadan, Ibadan, Oyo, Nigeria,[4]Department of Pathology and Forensic Medicine, State University Teaching Hospital, Ikeja, Lagos, Nigeria,[5]Department of Radiation Oncology, Faculty of Clinical Sciences, College of Medicine, University of Ibadan, Ibadan, Oyo, Nigeria,[6]Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Oyo, Nigeria,[7]Department of Pathology, The University of Chicago, Chicago, IL,[8]Department of Public Health Sciences, The University of Chicago, Chicago, IL

Background: Breast cancer (BC) is a heterogenous disease, and the rising global epidemic of premenopausal BC remains poorly understood. Mutational landscape and evolutionary dynamics of BC from diverse geography and populations are largely unknown, preventing the global acceleration of progress towards precision prevention.

Methods: To examine etiology and heterogeneity of BC in indigenous Africans, 43 multi-region tumor samples and blood samples from 18 Nigerian women with BC (mean age 53 +/-12.7) were analyzed. Whole Genome Sequencing (WGS) was performed to identify somatic single nucleotide variants, insertions and deletions (ID), copy number alterations (CN), and structural variants (SV). Five mutational signature types (single base substitutions (SBS), double base substitutions (DBS), ID, CN, and SV), were analyzed and compared to COSMIC human cancer signatures. Multi-DPclust was used to classify mutations to clonal and subclonal cluster and create mutational phylogenetic trees.

Results: Driver gene analysis highlighted key driver genes, such as *TP53*, *GATA3*, and *PIK3CA*, corroborating prior findings. The most common signature was clock-like (CL) SBS5, followed by APOBEC-related SBS2 and SBS13. Other notable signatures included hypoxia-linked SBS18 and HRD-related DBS13. Signature profiles showed moderate heterogeneity across samples, with distinct patterns observed across mutational clusters. Intra-cluster correlation coefficients for signatures within samples from same patient range from 0.37 to 0.99 (median 0.84). *TP53* and *GATA3* mutations are common in clonal (early) clusters, with GATA3 often appearing in consecutive subclones. The CL SBS5 signature was prevalent early, while SBS18, linked to hypoxia, became prominent late, highlighting evolving mutational processes across disease progression. Distinct BC subtypes also displayed unique mutational profiles. HR+/HER2+ tumors exhibited higher levels of signature SBS91, while HR-/HER2- tumors exhibited higher levels of the HRD signature SBS3 compared to the rest of the tumors. HR-/HER2- tumors demonstrated lower levels of the mismatch repair-related signature ID1 but higher levels of the HRD signature ID6 and TOP2A signature ID8. Significant differences were observed in SV signatures, with higher SV9 signature activities in HR+/HER2+ tumors and elevated *BRCA*-related SV3 signature activities in HR-/HER2- tumors. Heterogeneity was also evident in the DBS and CN mutational signatures, further highlighting tumor complexity.

Conclusion: This analysis sheds light on the diverse mutational dynamics within and across molecular subtypes, providing insights into the mutational evolution of BC in a non-screen detected young onset population. Ongoing work integrating WGS and transcriptome data will be presented at the conference.

#3893 Enhanced detection of molecular subtypes in prostate cancer using saturation biopsy: Insights into tumor heterogeneity and racial disparities.

Wei Zhao[1], Pin Li[2], Shannon Carskadon[3], Jessica Ryba[3], Hristina Trpevski[3], Vishnav Ramesh[3], Dhananjay Chitale[4], Firas Abdollah[3], Wooju Jeong[3], Craig Rogers[3], Daniel Isaac[5], Nilesh Gupta[4], Nallasivam Palanisamy[3]

[1]Hematology/Oncology, Henry Ford Health System, Detroit, MI,[2]Public Health, Henry Ford Health System, Detroit, MI,[3]Urology, Henry Ford Health System, Detroit, MI,[4]Pathology, Henry Ford Health System, Detroit, MI,[5]Hematology/Oncology, Kamanos Cancer Institute at McLaren Greater Lansing, Lansing, MI

Prostate cancer is characterized by remarkable molecular heterogeneity associated with disease progression and clinical outcomes. We have identified distinct molecular subtypes between CA and AA in biomarker expression associated with altered clinical outcomes. This study compares the effectiveness of standard 12-core biopsy and saturation biopsy in identifying molecular subtypes of prostate cancer and evaluating biomarker expression patterns across racial groups. We analyzed 104 cases that underwent saturation biopsies (median min, max 23 [14, 27], cores with cancer (median 11 [5, 25] and compared them with 130 cases with standard 12-core biopsies (median min, max 12 [12, 16]; cores with cancer median min, max 4 [1, 16] using ERG, ETV1, ETV4 and SPINK1 by combined multiplex IHC and RNA ISH. Saturation biopsy demonstrated superior detection of molecular subtypes, identifying higher rates of SPINK1- and ETV4-positive tumor foci than standard biopsy ($p < .001$). It also reduced the frequency of negative cases for all four biomarkers, indicating a higher prevalence of ETS gene fusions in prostate cancer (94%) than was reported previously. Caucasian American (CA) patients exhibited higher frequencies of ERG- and ETV1-positive tumor foci, while African American (AA) patients showed higher frequencies of SPINK1- and ETV4-positive foci and presented with higher Gleason Grade Groups at the time of biopsy. ERG positivity was associated with lower Gleason Grade Groups in both racial groups (CA $p=.004$; AA $p < .001$), and ETV1 positivity was linked to higher Gleason Grade Groups (CA $p<.001$; AA $p<.001$). These findings concord with our previous observations on the association of ETV1 And ETV4 with worse recurrence-free survival in CA and AA, respectively, underscoring significant racial variations in prostate cancer biology and the prognostic implications of biomarker expression. Saturation biopsy identified 16 distinct molecular subtypes with one or more than one ETS gene fusion, compared with 13 detected by standard 12-core biopsy. By uncovering a greater diversity of tumor subtypes, saturation biopsy highlights the extent of complex tumor heterogeneity and prostate cancer biology. It reveals fewer cases classified as ETS fusions compared with standard biopsies. This study demonstrates that saturation biopsy is a more effective diagnostic tool for detecting molecular subtypes and evaluating tumor heterogeneity in prostate cancer. The enhanced detection of subtype diversity and its ability to identify biomarker expression patterns associated with racial disparities underscores the potential of saturation biopsy in guiding precision oncology. These findings have profound implications for improving diagnostic accuracy, addressing racial inequality, and informing tailored treatment strategies for prostate cancer patients.

#3894 Three-dimensional whole tumor genomic analysis redefine intratumor heterogeneity of gliomas, with implications for early tumorigenesis.

Lee H. Chen, Nicholas O. Stevers, Chibo Hong, Ivan V. Smirnov, Nadia R. Grishanina, Joshua S. Wu, Anny Shai, Susan M. Chang, Mitchel S. Berger, Shawn L. Hervey-Jumper, Joanna J. Phillips, Joseph F. Costello

Neurological Surgery, UCSF - University of California San Francisco, San Francisco, CA

Intratumor heterogeneity and evolution underlie treatment failures but are poorly understood due to a limited number of tumor biopsies. In this study, we examine IDH mutant gliomas, utilizing a 3D whole tumor sampling approach and exome sequencing (32 patients, 345 samples), along with RNAseq (12 of the 32 patients, 88 samples). We investigate and redefine intratumoral heterogeneity and evolution of insertion/deletion (indel) and single nucleotide variant (SNV) in driver genes on a whole tumor scale.

In the tumor suppressor capicua transcriptional repressor (CIC), a canonical driver in IDH mutant 1p/19q co-deleted (IDHmut-Codel) tumors, our exome and RNAseq data analysis highlights a wide spectrum of heterogeneity and inactivation by SNVs and indels in independent subclonal expansions. For instance, in nine samples from one patient's recurrent tumor, five showed distinct CIC SNVs and eight exhibited indels. Notably, all samples showed one or more CIC SNVs or indels. Among five samples harboring SNVs, three had 2 to 3 co-occurring SNVs, and among the 8 samples with indels, three had 2 and one had 5 indels. None had the same combinations of indels and SNVs, indicating extreme heterogeneity and subclonality. Additionally, TA-cloning of a long-range PCR from one sample suggested that each individual SNV and indel were in different tumor cells, raising the possibility of early diversification of subclonal expansions driven by independent CIC alterations. Similar patterns were observed in IDH-mutant and 1p/19q non-codeleted tumors involving tumor suppressor ATRX.

These findings demonstrate that lower grade IDH-mutant gliomas (LGGs) exhibit significant intratumoral heterogeneity, a trait typically associated with glioblastoma. To determine if CIC alterations were inactivating or simply passengers, we analyzed gene expression in those spatially mapped samples. Within the ETS-domain transcription factor family, ETS1, ETV1, ETV4, and ETV5 were upregulated in CIC mutant samples versus wild type samples. We also identified novel candidate genes potentially regulated by CIC, suggesting possible mechanisms contributing to tumorigenesis and new treatment targets for IDHmut-Codel tumors.

This study elucidates the substantial intratumor heterogeneity and evolutionary dynamics in the drivers of LGGs on a whole tumor scale, highlighting their important role in tumorigenesis. By utilizing spatially mapped samples within the same tumors, our research reveals convergence on CIC loss of function through many mutually exclusive SNVs and indels in IDHmut-Codel tumors. Our findings demonstrate the critical nature of CIC inactivation across entire tumors, emphasizing the importance of indels and SNVs in tumor progression. This research also identifies candidate genes upregulated by CIC inactivation as possible treatment targets for IDHmut-Codel tumors.

#3895 Clonal expansion in normal endometrium and its association with endometriosis-associated ovarian cancer.

Koichi Watanabe, Nobuyuki Kakiuchi, Kosuke Ieiri, Hirona Maeda, Tomonori Hirano, Mana Taki, Koji Yamanoi, Ryusuke Murakami, Ken Yamaguchi, Junzo Hamanishi, Masaki Mandai, Seishi Ogawa

Kyoto University, Kyoto, Japan

[Introduction] Recent studies have revealed pervasive mutations affecting cancer-related genes in many normal tissues, including the endometrium, suggesting their role in cancer development. Endometriosis-associated ovarian cancer (EAOC) is thought to develop from endometriosis lesions seeded from endometrium to the ovary, although no solid evidence supports this. In this study, we sought to identify the clonal origin of EAOC by demonstrating ancestral clones of EAOC in the endometrium by detecting shared mutations between EAOC and endometrial clones.
[Methods] We performed high-density sampling of endometrial tissues from 5 patients diagnosed with EAOC (5-32 sample/patient, 78 samples in total) and performed whole exome sequencing, and compared the somatic mutations with those of the EAOC tumors from the same patients.
[Results] The median number of somatic mutations in endometrial samples was 29/sample (range 6-74). In 74 of 78 endometrial samples (95%), one or more of known driver gene mutations such as *PIK3CA, PIK3R1, KRAS, PPP2R1A, ARHGAP35* and *FBXW7* were identified, with an average of 2.3/sample (range 0-6). *PIK3CA* mutations were the most common, identified in 42 of 78 samples (54%), followed by *ARHGAP35* mutations (25/78, 32%). Some of the adjacent endometrial samples shared multiple mutations, including driver mutations, indicating large clonal expansion over several millimeters. In one case with clear cell carcinoma in a 74-year-old woman, one of 32 endometrial samples shared 16 somatic mutations, including those affecting *PTEN, PIK3CA, KRAS* and *FOXA2* , with the ovarian tumor, indicating that this ovarian tumor originated from the endometrium. We confirmed that this endometrial tissue was histologically normal. Based on the phylogenetic analysis using whole genome sequencing, the most recent cancer founder was estimated to have diverged from the normal endometrial clones at approximately 20 years of age. No additional driver alterations were acquired in the main trunk except for multiple copy number alterations, suggesting their role in the progression from precancer to cancer.
[Conclusion] We demonstrated that at least some EAOC tumors originate from an ancestor in the endometrium, in which a number of driver events are acquired to contribute to carcinogenesis. Our findings provide new insight into early events during the development of EAOC.

#3896 Multi-omic and multi-region profiling of uterine leiomyoma reveals intra- and inter-tumor heterogeneity.

Chelsea De Bellis[1], Sujay Vennam[2], Christopher Eeles[3], Philippe Jolivet[1], Deirdre Lum[2], Benjamin Haibe-Kains[3], Matt van de Rijn[2], **Joanna Przybyl**[1]

[1]McGill University, Montreal, QC, Canada,[2]Stanford University, Stanford, CA,[3]Princess Margaret Cancer Centre, Toronto, ON, Canada

Objective: Molecular heterogeneity has been well-documented in malignant tumors but has yet to be thoroughly studied in benign non-metastasizing tumors. Uterine leiomyomas (LM) (fibroids) are benign tumors originating from the myometrium, commonly affecting women of reproductive age. Approximately half of patients present with multiple tumors, termed multifocal LM. LM are thought to be monoclonal proliferations based on their pattern of X chromosome inactivation. Mutations of the *MED12* gene are the genetic hallmark of LM. Treatment of LM includes surgery and hormone therapy. It is unknown whether multifocal LM exhibit inter-tumor heterogeneity, contributing to variable response to hormone therapy. Anecdotal reports suggest that LM may occasionally transform into malignant metastasizing tumor called leiomyosarcoma (LMS). Better understanding of intra-tumor heterogeneity of LM may identify cell clones that may contribute to oncogenic transformation. In this study, we sought to perform a comprehensive multi-omic investigation of the possible intra- and inter-tumor heterogeneity of uterine LM.
Methods: We performed multi-omic analysis of 62 specimens from 21 LM patients (median of 3 tumors per patient, range: 2-8). We analyzed multiple regions of a single tumor, and multiple concurrent LM from patients with multifocal disease. We profiled DNA methylation (EPIC microarrays), point mutations (whole exome sequencing) and gene expression (whole transcriptome RNA-seq) in these specimens.
Results: Multi-omic profiling showed a remarkable intra- and inter-tumor heterogeneity of genomic, epigenomic and transcriptomic patterns. Through reconstruction of phylogenetic trees based on single nucleotide variants, we identified novel clonal and subclonal somatic mutations in LM. We detected for the first time different *MED12* mutations in co-existing nodules in the same patient. DNA methylation and transcriptomic profiles appeared to have a similar degree of variability within individual tumors and between different tumors from the same patient. We also observed a significant enrichment of different molecular pathways between distinct regions of the same tumor.
Conclusion: Molecular heterogeneity is well-established in malignant tumors, and we report it here for the first time in histologically bland lesions like LM. Our study reveals that in multifocal LM, distinct tumors can acquire unique molecular alterations, meaning that single-tumor analysis may not capture the full spectrum of molecular changes. This highlights the importance of considering molecular heterogeneity in diagnosis and evaluation of response to hormonal treatment in LM. We also demonstrate for the first time the presence of intra-tumor heterogeneity of LM. It remains to be further investigated whether clonal evolution may contribute to occasional oncogenic transformation of benign LM into malignant LMS.

#3897 Investigating the prevalence of ecDNA in breast cancer.

Georgia Stavrou[1], Chris Bailey[2], Jeanette Kittel[1], Mariam Jamal-Hanjani[1], Charles Swanton[2], Nnennaya Kanu[1]

[1]University College London (UCL) Cancer Institute, London, United Kingdom,[2]Francis Crick Institute, London, United Kingdom

Extrachromosomal DNA (ecDNA) has been described in multiple cancer types. ecDNAs are circular and are usually greater than 500 base pairs in size. ecDNA structures lack centromeres and thus do not follow the Mendelian mode of inheritance, segregating unequally during mitosis. This can subsequently result in high copy number gene amplifications and enhance tumor heterogeneity, promoting tumor evolution. ecDNA structures have a dynamic nature whereby they can be maintained extrachromosomally in their circular form but can also reintegrate randomly back into the genome, in the form of Homogeneously Stained Regions (HSR). Earlier studies using TCGA reported that approximately 25% of breast samples contained ecDNA. We have recently reported that over 46% of HER2+ breast cancers have ecDNA amplifications. Moreover, ecDNA have been found in both pre-cancerous lesions, such as in tissues obtained from patients with Barret's esophagus, but also following both targeted treatment and chemotherapy. This highlights the ability of the structures to be detected at any disease stage and treatment phase although exact mechanisms of ecDNA formation and maintenance have not been described fully. In the present study we use a tumor-informed *in silico* approach in conjunction with *in vitro* readouts to elucidate these mechanisms. 2981 Whole Genome Sequencing data from the Genomics England breast cancer cohort and cell line data from the Cancer Cell Line Encyclopedia were analyzed using Amplicon Suite. In parallel, a panel of eight *ERBB2* amplified breast cancer cell lines was used to detect *in vitro* presence of ecDNA by metaphase FISH and Optical Mapping. Genes that were found to be differentially mutated according to the presence of *ERBB2* focal amplifications were silenced *in vitro*, then gene expression of *ERBB2* and target genes was evaluated using qPCR and the effect of *ERBB2* signal was assessed using FISH. In the cohort analyzed, 22.8% of breast tumors had ecDNA amplifications. Enrichment analysis was conducted to assess the presence or absence of specific mutations in *ERBB2*-amplified vs non-amplified tumors. Several genes were identified as being positively or negatively enriched in the cohort. Similarly, we identified 9 breast cancer cell lines containing up to 5 different species of ecDNA. In a subset of cell lines computationally predicted to harbor *ERBB2* on ecDNA, *ERBB2* amplification was in the form of HSR. We selected HCC1569 as our candidate model and silenced candidate epigenetic regulators. Our preliminary data suggests this leads to a measurable and significant change on *ERBB2* signal. At present, we highlight the propensity of *ERBB2* to be amplified on ecDNA in both breast cancer patients and breast cancer cell lines. These cell lines can serve as models for the further study of the mechanisms of maintenance of ecDNA *in vitro* which will shed light on some of the key unresolved questions in the field.

#3898 Integrating structural variants and single nucleotide variants to uncover evolutionary trajectories in melanoma.

Xiwen Cui[1], Ayse G. Keskus[1], Salem Malikic[1], Yuelin Liu[1], Anton Goretsky[1], Chi-Ping Day[1], Farid R. Mehrabadi[1], Mikhail Kolmogorov[1], Glenn Merlino[2], S. Cenk Sahinalp[1]

[1]Cancer Data Science Laboratory, Center for Cancer Research, National Cancer Institute, Bethesda, MD,[2]Laboratory of Cancer Biology and Genetics, Center for Cancer Research, National Cancer Institute, Bethesda, MD

Melanoma, a highly heterogeneous cancer, evolves through a complex interplay of genetic alterations, including both single nucleotide variants (SNVs) and structural variants (SVs). To study the evolutionary trajectory of melanoma, we established a model system composed of 24 single-cell-derived clonal sublines (C1-C24) from the M4 melanoma model, developed in a genetically engineered hepatocyte growth factor (HGF)-transgenic mouse. While SNVs have been extensively used to construct phylogenetic trees using Trisicell (Triple-toolkit for single-cell intratumor heterogeneity inference), a tool that analyzes intratumor heterogeneity and single-cell RNA mutations, the role and timing of SVs in melanoma evolution remain less well understood. This study integrates SV data with an SNV-driven phylogeny to investigate whether SV patterns align with SNV-based evolutionary trajectories in the mouse melanoma model, providing insights into the functional impact of SVs during tumor progression. We performed long-read sequencing on the 24 clonal sublines and detected SVs using Severus, a tool optimized for phasing in long-read sequencing. The SVs were mapped to the SNV-driven phylogeny using R and classified as either concordant (aligning with the SNV-based tree) or discordant (deviating from the SNV phylogeny). Gene ontology enrichment analysis revealed that concordant SVs were significantly enriched in genes associated with the hepatocyte growth factor receptor signaling pathway and the negative regulation of peptidyl-threonine phosphorylation, both of which represent core drivers of tumor progression. In contrast, discordant SVs were associated with a broader range of functional pathways, including the positive regulation of antigen receptor-mediated signaling and the regulation of natural killer cell-mediated cytotoxicity, though the exact mechanisms underlying these associations remain unclear. By integrating these SVs with an established SNV-driven phylogeny, this study highlights the distinct and critical roles SVs play in melanoma evolution. Concordant SVs appear to drive core oncogenic processes, while discordant SVs may contribute to other aspects of tumor evolution. These findings underscore the importance of considering SVs alongside SNVs to fully capture the complexity of melanoma evolution. Ongoing investigations will continue to explore the functional implications of these SVs and how the gene disruption patterns they cause shape the evolutionary trajectory of melanoma, offering potential targets for future therapeutic strategies.

#3899 Genetic insights into clonal evolution from normal prostate epithelium to cancer via HGPIN.

Kohsuke Hishiki[1], Nobuyuki Kakiuchi[2], Yuki Teramoto[3], Koichi Watanabe[4], Kosuke Ieiri[4], Hirona Maeda[4], Tomonori Hirano[4], Yuki Kita[5], Takashi Kobayashi[5], Seishi Ogawa[4]

[1]Department of Urology / Dept. of Pathology; Tumor Biology, Kyoto Univ. Graduate School of Medicine, Kyoto, Japan,[2]Dept. of Gastroenterology; Hepatology, Kyoto Univ. Graduate School of Medicine, Kyoto, Japan,[3]Department of Diagnostic Pathology, Kyoto Univ. Graduate School of Medicine, Kyoto, Japan,[4]Dept. of Pathology; Tumor Biology, Kyoto Univ. Graduate School of Medicine, Kyoto, Japan,[5]Department of Urology, Kyoto Univ. Graduate School of Medicine, Kyoto, Japan

<Backgrounds> Prostate cancer (PC) is one of the most prevalent cancers in the developed world, where its incidence is still increasing. Most PCs are thought to arise from high-grade prostatic intraepithelial neoplasia (HGPIN), a well-recognized precursor lesion. However, the genetic landscape of HGPIN and its relationship with normal prostate epithelium (NPE) and PC remains to be fully explored.
<Methods> To address these issues, we collected prostate tissue samples from five patients who underwent radical prostatectomy. Prostate tissue sections (3 mm thick) were fixed with an alcohol-based solution and prepared for dual immunostaining using AMACR/p63, in addition to hematoxylin-eosin staining, and subjected to laser-capture microdissection (LMD). Then, PC (n = 35), HGPIN (n = 71), and normal epithelium (n =244) samples were dissected and subjected to whole-exome sequencing (WES). Variant pathogenicity was assessed based on COSMIC and ClinVar databases. Furthermore, to investigate the mutation acquisition rate in the prostate epithelium, single cell-derived prostatic organoids established from three patients were analyzed using WES. Somatic single nucleotide variants (SNVs), indels, and copy number variations (CNVs) were identified.
<Results> A total of 10,047 somatic SNVs and indels were detected. The mutation rate was estimated to be 0.28 mutations/year/exon. Shared mutations were observed between NPE and HGPIN as well as between HGPIN and PC in some cases, suggesting a common clonal origin. Pathogenic *FOXA1* mutations were frequently identified in both HGPIN and PC. While pathogenic *FOXA1* mutations were also observed in NPE, none of these mutations were shared with HGPIN or PC, indicating that they likely represent independent mutational events. In one case, spatially separated multifocal cancers exhibited distinct *FOXA1* mutations, each of which was shared with the adjacent HGPIN. In other cases, mutations were also shared between PCa, HGPIN, and NPE samples, although no known driver mutations were detected therein. These observations suggest that these cancers were derived from their precancer lesions with apparently normal histology, which progressed through HGPIN and PC. Phylogenetic analysis of these samples revealed that HGPIN and PCa diverged recently from a common ancestor.
<Conclusions> We successfully characterized somatic mutations and CNVs in apparently normal prostate epithelium, which were shared between HGPIN and adjacent PC in some cases. These findings provide new insights into the early genetic events driving PC development. Further studies are needed to fully elucidate the clonal evolution from NPE to PC, which may offer novel targets for early detection and intervention.

#3900 Integrating single-molecule localization microscopy and computational pipelines to elucidate ecDNA dynamics.

Mesut Unal[1], Jessica Alsing[2], Gabrielle S. Dewson[2], Guy Nir[2], Kadir C. Akdemir[1]

[1]UT MD Anderson Cancer Center, Houston, TX,[2]University of Texas Medical Branch, Galveston, TX

Extrachromosomal DNA (ecDNA) is prevalent across various cancer types, notably Glioblastoma Multiforme (GBM), where it facilitates the rapid acquisition of high oncogene copy numbers by cancer cells. These ecDNAs exhibit self-replication and are randomly distributed to daughter cells during mitosis, resulting in uneven segregation. This leads to variations in oncogene copy numbers among subclones, contributing to significant intratumoral heterogeneity. Traditional sequence-based analyses are insufficient for addressing spatial heterogeneity. To overcome this limitation, we propose utilizing sequential OligoSTORM imaging combined with single-molecule localization microscopy (SMLM), an innovative hybridization technique, and a computational pipeline. Our hybridization method significantly enhances imaging speed: whereas imaging approximately 40 loci with typical sequential OligoSTORM requires 11 days to obtain data from about 20 cells, this method enables imaging of 10^4 loci within the same time frame. Additionally, we developed a computational pipeline to complement this technique. Together, these tools enable 3D imaging of chromosomes with a high resolution of 20 kb. Our hybridization approach allows imaging at both 100-kb and 20-kb resolution steps, organized hierarchically in a Matryoshka-like structure. Our pipeline incorporates advanced computational algorithms, including iterative closest point (ICP) and space-partitioning data structures, to precisely align each 20-kb sub-step to its corresponding 100-kb step. Using this methodology, we target the *EGFR* gene locus with high resolution. To validate our approach, we are applying it to a non-cancerous microglial cell line (HMC3) as a control and a patient-derived glioblastoma stem-like cell (GSC) line as our GBM model. The GSC line, rich in ecDNA content with an average of 18 ecDNAs per cell, demonstrates pronounced tumor heterogeneity, including significant *EGFR* amplifications among subclones. Through this methodology, we can elucidate the structural organization of ecDNAs containing oncogenes and their regulatory elements with fine precision. Furthermore, our approach seamlessly integrates these structural insights with matched single-cell genomic data. This combined strategy offers a comprehensive understanding of ecDNA dynamics in cancer, shedding light on tumor heterogeneity at both spatial and genomic levels and paving the way for novel insights into oncogene-driven cancer biology.

#3901 Charting copy number gain histories from mutational divergence among multi-samples.

Yunong Xia, Ruping Sun

Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN

In cancer, a somatic copy number gain (SCNG) on a tumor chromosomal region is often assumed to result from a series of minimum number of copy number events, but this parsimonious-trajectory assumption may be violated in reality. For example, an SCNG could develop through a complex trajectory involving a combination of gain and loss events, rather than exclusively through gains. While prior efforts have been made to infer the SCNG trajectories from patient data, the presence of complex trajectories remains to be validated. Furthermore, these methods are limited by either only utilizing SCNG profiles, or inferring trajectories from only a single sample. Here, we hypothesize that SCNG trajectories can be reflected by somatic single nucleotide variants (SSNVs) shared between samples from the same patient. By analyzing multi-regional whole genome sequencing data of tumor specimens, we found evidence where the multiplicities of shared SSNVs between samples pinpoint complex SCNG trajectories. Here, the SSNV multiplicity refers to the number of alleles harboring the corresponding SSNV in the dominating clone of a tumor sample. We also define the joint multiplicity distribution as the proportion of shared SSNVs that occur at different allelic states (multiplicities) across distinct tumor samples. We developed a probabilistic computational pipeline to automate the identification of non-parsimonious multi-sample SCNG trajectories utilizing shared SSNV multiplicities. Extending an existing method GRITIC from single sample to multi-sample setting, this pipeline enumerates all trajectories leading to SCNGs states in multiple samples, and characterizes the probability of observing the joint multiplicity distribution in the real patient data given each possible trajectory. We applied the method on prostate cancer and breast cancer datasets, and identified conclusive cases of complex trajectories on genomic regions affected by SCNGs. On regions showing different copy number states in multiple samples, we identified cases experiencing both gains and losses. On regions showing the same copy number states in multiple samples, we also identified a few of them experiencing independent acquisition of SCNGs in different samples. Our findings call for a stochastic modeling of SCNGs dynamics during tumor evolution.

#3902 Evolution of esophageal adenocarcinoma from precursor lesion stem cells.

Wa Xian[1], Frank McKeon[2], Jaffer Ajani[2], Caycel Desales[1], Ashley Hoffman[1], Amber Su[1], Crystal Nguyen[1], Rick Russell[3], William Bachovchin[4]

[1]University of Houston, Sugar Land, TX,[2]University of Houston, Houston, TX,[3]TractBio, Sugar Land, TX,[4]Tufts University, Boston, MA

Metastatic cancers arise from a decades-long succession of increasingly virulent precursor lesions, each of which represents prospective targets for therapeutic intervention. This evolutionary process has been particularly vivid in esophageal adenocarcinoma (EAC), as this cancer and associated precursor lesions, including Barrett's esophagus (BE), low-grade dysplasia (LGD), and high-grade dysplasia (HGD), co-exist in an accessible, two-dimensional pattern in esophageal mucosa. Given the durability of these precursor lesions, it is likely that they, like EAC, rely on stem cells for their regenerative growth. To assess the role of stem cells in the evolution of EAC, we applied technology that selectively clones stem cells from the gastrointestinal tract to patient-matched endoscopic biopsies from each of the precursor lesions implicated in EAC. Histologically validated, endoscopic biopsy series including EAC, HGD, LGD, BE, and normal esophageal mucosa were obtained from patients presenting with early (T1) EAC. Rare (1:1,000) cells from each of these lesions proved clonogenic and were assessed by *in vitro* differentiation, tumorigenicity in mice, and by molecular genetics. Each of the lesions in the evolution of EAC possess a discrete set of clonogenic cells marked by immaturity, enormous proliferative potential, and lesion-specific differentiation fate. DNA sequencing of these clones reveal intralesional heterogeneity and clonal resolution of the mutation progression within a given patient from BE, LGD, HGD, and EAC. High-throughput chemical screens against BE stem cells reveal drug combinations that are similarly effective against stem cells of LDG, HGD, and EAC.

#3903 Multi-omics and histological assessment to identify patient derived xenograft models of invasive lobular carcinoma.

Jagmohan Hooda[1], Jennifer M. Atkinson[2], Osama Shah[1], Megan Yates[1], Daniel D. Brown[3], Morgan DeBerry[4], Rohit Bhargava[4], Paolo Schiavini[5], **Stefano Cairo**[5], Mara Gilardi[5], Marianna Zipeto[5], Steffi Oesterreich[1], Adrian V. Lee[1]

[1]Hillman Cancer Center (HCC), Magee-Women's Research Institute, Women's Cancer Research Center, University of Pittsburgh Medical Center (UPMC), Pittsburgh, PA,[2]Department of Pharmacology & Chemical Biology, University of Pittsburgh, Pittsburgh, PA,[3]Institute for Precision Medicine, University of Pittsburgh, Pittsburgh, PA,[4]Department of Pathology, University of Pittsburgh, Pittsburgh, PA,[5]Champions Oncology, Inc., Hackensack, NJ

Breast cancer is a heterogeneous disease with various histological subtypes. Invasive Lobular Cancer (ILC) is the most common special subtype, accounting for 10-15% of all breast cancers. The pathognomonic feature of ILC is the loss of E-cadherin (CDH1), which leads to a unique single-file growth pattern of discohesive ILC cells. Although ILCs show better prognostic factors than No Special Type (NST), patients with ILC have worse long-term outcomes, which is not well understood. ILC has historically been understudied, in part due to the lack of appropriate research models. Since ILC affects "only" 10-15% of all breast cancers, most models have been generated from the more frequent subtype, NST. In this study, we characterized Patient-Derived Xenograft (PDX) models for Invasive Lobular Cancer (ILC) research. We collaborated with Champions Oncology and utilized the genomic data from their LUMIN portal to perform genomic characterization of PDX models. We identified potential ILC PDX models based on CDH1 mutation, low E-cadherin mRNA expression, or clinical annotation of ILC. We analyzed whole-exome sequencing (WES) and RNAseq data on the LUMIN portal to characterize these models. We also performed Immunohistochemistry (IHC) analysis for E-Cadherin, p120, ER, PR, and Her2 to further assess the PDX models. Our study characterized a total of 10 PDX models and identified 7 ILC models and 3 NST models. We showed that the identified ILC PDX models exhibited lower levels of CDH1 mRNA expression compared to NST, which is consistent with the characteristics of ILC. We also found that the top gene alterations in ILC PDX models included P53, CDH1, PIK3CA, FOXA1, and PTEN. The frequency and type of alterations varied between ILC and NST PDXs. Our study provides valuable tools for studying ILC biology and testing targeted therapies. The identification of potential ILC PDX models could provide insights into the molecular characteristics of ILC and provide tools for research for the development of new treatment strategies.

#3904 Clonal analysis of classical subtypes of pancreatic cancer stem cells in ascites.

Wa Xian[1], Frank McKeon[1], Paul Daniel[1], Saeed Khan[1], Caycel Desales[1], Ashley Hoffman[1], Jaffer Ajani[2]

[1]University of Houston, Houston, TX,[2]MD Anderson Cancer Center, Houston, TX

It is now established that pancreatic cancer often displays an intratumor heterogeneity at the levels of epithelial cell types, stromal activation, and immune signaling that is likely germane to therapeutic resolution. While the extent of epithelial subtypes is under investigation, there is general agreement that pancreatic ductal adenocarcinoma (PDAC) is comprised of p63+ "basal" and GATA6+ "classical" subtypes, as well as various "intermediate", hybrid, and immune subtypes. In this initial report, we have applied technology that clones stem cells of the human gastrointestinal tract (Wang et al., 2015) and of esophageal adenocarcinoma and its precursors (in review) to ascites samples from patients with PDCA. Libraries of clonogenic cells were sorted to CEACAM6+ and CEACAM6- populations that were cloned by single cell sorting. The majority of CEACAM6+ clones form colonies with morphologies distinct from the CEACAM6- clones, and yet both form aggressive tumors in immunodeficient mice. Both CEACAM6+ and CEACAM6- clones express high levels of GATA6 and LGALS4, suggesting their general inclusion into the classical subtype seen in PDCA. However, gene expression profiling and tumor histology indicate that the CEACAM6+ clones display an intestinal fate (high CDH17, CDX2, MUC17) whereas the CEACAM6- clones show a gastric profile (TFF1, TFF2, AQP5, EGR1, EGR2, SOX21, MUC5AC). In addition, the CEACAM6- clones showed high levels of interferon signaling and high levels of chemokine expression including CXCL1, 2, 3, and 8, CCL20. The stromal properties of tumors generated from CEACAM6+ and CEACAM6- clones were also distinct, with the CEACAM6- clones marked by tumors with a highly cellularized extracellular matrix including αSMA+ and FAP+ CAFs, and the intestinal CEACAM6+ clones largely devoid of these CAF subpopulations. Consistently, CEACAM6- clones expressing high levels of pro-fibrotic genes, including PLAU, FN1, and CCN1. Importantly, exome sequencing of the CEACAM6+ and CEACAM6- clones from the same patient reveal shared mutant alleles of the p53 and KRAS genes, suggesting that these phenotypically distinct classical subtypes arose from a post-transformation stage likely by epigenetic mechanisms. Ongoing studies aimed at cloning basal subtypes from ascites and resolving the epigenetic control of CEACAM6+ and CEACAM6- classical subtypes should help to define the stem cell heterogeneity in PDAC.

TUMOR BIOLOGY: Mouse and Other Models
Poster Session

#3908 Exploring CAR-T cell killing dynamics through live-cell fluorescence microscopy.
Skylar Dewees[1], Nathalie Opdam - van de Laar[2], Stacie Chvatal[1], Danielle Califano[1], Ben Streeter[1]

[1]Axion BioSystems, Atlanta, GA,[2]Axion BioSystems, Eindhoven, Netherlands

Introduction: Harnessing the potent cytotoxicity of chimeric antigen receptor T (CAR-T) cells has revolutionized cancer immunotherapy. A key factor influencing CAR-T cell efficacy is target antigen density on cancer cells. Human epidermal growth factor receptor 2 (HER2), overexpressed in various cancers like ovarian and lung carcinomas, is a promising target for CAR-T cell therapy. Traditional cytotoxicity assays generally provide only static endpoint measurements and often rely on 2D cell culture models, which fail to capture the structural complexity of tumors in vivo—a factor crucial to CAR-T cell efficacy. Live-cell imaging offers a solution by enabling non-invasive, real-time monitoring of cytotoxicity. Therefore, this study aims to explore the dynamics of CAR-T cell-mediated cytotoxicity against cancer cells with varying HER2 expression levels, comparing outcomes between 2D and 3D cell culture models using the Omni platform.
Methods: Monolayers and spheroids of SKOV3 (ovarian carcinoma) and A549 (lung adenocarcinoma) cells, tagged with GFP, were exposed to HER2 CAR-T cells at different Effector : Target (E:T) ratios. Using the Omni platform, hourly brightfield and fluorescent images were captured over 72 hours assess cancer cell growth and cytotoxicity and quantify the cytolysis of the fluorescent target cells. Percent cytolysis of the target cells was calculated by comparing the green fluorescent intensity of treated wells to no treatment control wells.
Results: The study demonstrated a dose-dependent cytotoxic effect of HER2 CAR-T cells that correlated with increasing amounts of CAR-T cells. Comparing SKOV3 cells with A549 cells showed higher sensitivity and earlier response for all E:T ratios. At 72 hours post HER-2 CAR T-cell addition, the 5:1 group demonstrated approximately 82.4% cytolysis of SKOV3 cells, while it demonstrated 40.3% cytolysis of A549 cells. In comparing the cytotoxic effect of HER2 CAR-T cells on SKOV3 spheroids versus monolayers, the 3D spheroid model exhibited a more rapid and efficient dose-dependent response. At 72 hours post HER-2 CAR T-cell addition, the 5:1 group demonstrated approximately 82.4% cytolysis of SKOV3 cells in spheroid, while it demonstrated 61.1% cytolysis of SKOV3 cells in monolayer.
Conclusion: This study underscores the importance of target antigen density in determining CAR-T cell efficacy, as SKOV3 cells, with higher HER2 expression, were more prone to CAR-T cell killing compared to A549 cells. Additionally, it also highlights the critical role of the assay model type, 2D versus 3D, in accurately assessing cytotoxicity. The Omni platform provided dynamic, real-time insights into cell viability and cytotoxicity, highlighting its potential to optimize immunotherapy strategies.

#3909 Defining Docetaxel's antitumor effects on Calu3 lung cancer cells using high-throughput live-cell imaging.

S. Dewees[1], L. Boekestijn[2], B. Streeter[1], S. Chvatal[1], D. Califano[1];
[1]Axion BioSystems, Atlanta, GA, [2]Axion BioSystems, Eindhoven, Netherlands

Introduction: Oncology focuses on understanding cancer's cellular mechanisms and developing effective treatments, with a key emphasis on evaluating drug toxicity to identify potential chemotherapies. Various assays, including cell viability, migration, and clonogenic assays, are used to assess the sensitivity and resistance of cancer cells to these drugs and define the drug's profile. Docetaxel, a chemotherapeutic agent inhibiting microtubule depolymerization, involved in migration and proliferation, is studied using these assays to evaluate its impact on cell proliferation, survival, and migration. The results of toxicity studies can vary significantly depending on the type of assay used, and combining multiple assays enhances the accuracy of drug profiling. In this study, the Omni Pro 12 high-throughput live-cell imaging platform was used to simultaneously perform multiple assays on Calu3 lung cancer cells.

Method: By conducting the assays simultaneously using the Omni Pro 12, variability was minimized, ensuring that differences in experimental conditions cannot affect the results. The aim was to evaluate the effects of Docetaxel on three key aspects of cancer progression: cell viability, migration, and colony-forming potential. These aspects were investigated using the cytotoxicity, scratch, and colony assay with Docetaxel concentrations between 0 - 1000 nM.

Results: First, real-time cell confluence measurements indicated that higher Docetaxel concentrations (500 nM to 1000 nM) significantly suppressed cell proliferation. Secondly, a wound-healing assay revealed a dose-dependent inhibition of cell migration, with higher drug concentrations resulting in slower and an overall decrease in scratch closure. A significant 70% decrease in scratch closure was seen when comparing the control group to 1000 nM Docetaxel. Lastly, a clonogenic assay showed a dose-dependent decrease in colony formation at 324 hours post-treatment, the time point was chosen right before the control group colonies clustered, with higher concentrations (100 nM and 250 nM) leading to inhibition of colony growth with less than 10 colonies formed compared to approximately 110 in the control groups.

Conclusion: These results demonstrate that Docetaxel effectively inhibits Calu3 cancer cell viability, proliferation, and migration in a dose-dependent manner. A comprehensive toxicity profile was obtained with the use of multiple 2D in vitro assays, which offers valuable insights into the potential therapeutic efficacy of Docetaxel in lung cancer treatment. This study underscores the importance of integrating multiple assays to better predict drug responses, helping to reduce the incidence of failures during in vivo and preclinical studies. Utilizing the Omni Pro 12 reduced the variance between assays as they are all performed simultaneously with similar experimental conditions.

#3910 Patient-derived conditionally reprogrammed cells in prostate cancer research.

A. Elbialy, A. Sood, S.-J. Wang, P. Wang, J. Li, **X. Liu**;
The Ohio State University, Columbus, OH

Prostate cancer (PCa) remains a leading cause of mortality among American men, with metastatic and recurrent disease posing significant therapeutic challenges due to a limited comprehension of the underlying biological processes governing disease initiation, dormancy, and progression. The conventional use of PCa cell lines has proven inadequate in elucidating the intricate molecular mechanisms driving PCa carcinogenesis, hindering the development of effective treatments. To address this gap, patient-derived primary cell cultures have been developed and play a pivotal role in unraveling the pathophysiological intricacies unique to PCa in each individual, offering valuable insights for translational research. This review explores the applications of the conditional reprogramming (CR) cell culture approach, showcasing its capability to rapidly and effectively cultivate patient-derived normal and tumor cells. The CR strategy facilitates the acquisition of stem cell properties by primary cells, precisely recapitulating the human pathophysiology of PCa. This nuanced understanding enables the identification of novel therapeutics. Specifically, our discussion encompasses the utility of CR cells in elucidating PCa initiation and progression, unraveling the molecular pathogenesis of metastatic PCa, addressing health disparities, and advancing personalized medicine. Coupled with the tumor organoid approach and patient-derived xenografts (PDXs), CR cells present a promising avenue for comprehending cancer biology, exploring new treatment modalities, and advancing precision medicine in the context of PCa.

#3912 Transcriptional heterogeneity between hemangiosarcoma and nodular hyperplasia using patient-derived cell lines and orthotopic xenograft model revealed new therapeutic targets.

Y. Liu, H. Yamamoto, M. Elbadawy, A. Abugomaa, T. Usui, K. Sasaki;
Tokyo University of Agriculture and Technology, Tokyo, Japan

Human angiosarcoma (AS) is a rare and aggressive subtype of soft tissue sarcoma of endothelial cell origin with a propensity for local recurrence and metastasis. The optimal treatment of metastatic AS is unclear and is associated with a generally poor prognosis unless diagnosed early. The lack of insight into the pathogenesis always causes treatment failure in the clinic. To discover new treatment strategies for AS, it is necessary to develop new and relevant preclinical models for AS. Dogs commonly suffer from a similar neoplasm, called hemangiosarcoma (HA), with breeds like the golden retriever carrying heritable genetic factors that put them at high risk. Angiosarcomas and HA were reported to share pathology, immunohistochemical characteristics, and molecular signatures. Therefore, HA is considered a closer preclinical model for advancing AS research than rodent models. In veterinary clinic, malignant HA always occurs with nodular hyperplasia (NH), a kind of benign tumor. However, the AS orthotopic xenograft mouse still needs standard guidelines for validation. In this study, we successfully generated 5 canine HA cell lines from fresh patient tumor tissues. We compared the RNA sequencing data between HA and NH patient-derived cell strains (5:5). Data revealed that pathways related to autophagy and lysosomal membrane were found upregulated in HA compared to NH. Additionally, *PIK3IP1*, *HIP1*, *PLAAT3*, and *MARVELD3* can be considered potential pharmacological targets and biomarkers for HA. Furthermore, we established an orthotopic xenograft model on mouse spleen using canine patient-derived HA cell lines by splenic injection. The results of xenograft spleen mass and drug screening test of toceranib, carboplatin, doxorubicin and cyclophosphamide using xenograft mouse HA cells showed corresponded sensitivity, supported that our model could maintain the original features of the patient tumor. In conclusion, the transcriptional difference between HA and NH might provide a better understanding of the progress of blood vessel sarcoma, and new pharmacological targets and biomarkers for diagnosis. Also, the xenograft model study may provide a reference for both AS and HA therapeutic predictions in basic medical science.

#3914 Leveraging canine osteosarcoma as a model to advance precision medicine and targeted therapies in human osteosarcoma.

Lucas Rodrigues[1], Kevin Wu[2], Garrett Harvey[1], Gerald Post[1], Aubrey Miller[1], Lindsay Lambert[1], Christina Lopes[1], James Zou[2]

[1]FidoCure, Palo Alto, CA,[2]Stanford University, Stanford, CA

Osteosarcoma (OS) is the third most prevalent tumor in adolescence, histologically characterized by the proliferation of abnormal osteoid-producing mesenchymal cells. Surgery remains the primary approach for OS treatment, but outcomes are poor without chemotherapy. Combining surgery, radiotherapy, and chemotherapy has improved results, yet 5-year survival rates persist at 60-70%.

Despite advances in molecular medicine, progress in the treatment of OS has been limited, no significant breakthroughs have been achieved in systemic therapy, particularly for relapsed OS. Additionally, there is no consensus on the optimal treatment for these cases, leaving a substantial unmet need for effective therapies.Precision medicine trials (PMTs) have gained traction due to the ability to map the genomic profiles of individual tumors and match them to targeted therapies, but is still a challenge for OS due to limited efficacy and low enrollment rate. This low enrollment rate is due to several challenges, including tumor heterogeneity, limited molecular targets, and logistical in conducting trials. These barriers hinder the full realization of the potential benefits of PMTs to improve patient outcomes.

One promising avenue is the use of naturally occurring canine OS, which share strong biological and molecular similarities with humans. Canine OS is estimated to be 10 times more common in pet dogs than in humans. Moreover, dogs develop these tumors spontaneously, with an intact immune system and comparable environmental exposures to humans, making them a valuable model for studying OS and improving the design of human trials.

This study leverages data from 202 spontaneous canine OS, aiming to bridge the gap between preclinical investigations and clinical application. We utilized real-world clinical-genomic data from the FidoCure® platform, a canine precision medicine initiative that uses next-generation sequencing (NGS) to screen for cancer-associated genomic alterations. We identified that the most common genomic alterations occurred in tumor suppressor genes TP53, SETD2, PTPRJ, PTEN, RB1, DMD, BRCA1/2, and oncogene PIK3CA. These findings are consistent with the genomic profile seen in humans, reinforcing the value of the canine model.

We also analyzed the correlation between small-molecule targeted therapies and genomic alterations. Through FidoCure®, veterinarians can leverage insights from the NGS panel to apply any of 11 human-approved therapies. Our analysis identified significant benefits from small-molecules when used as adjuvant treatment alongside chemotherapy with median survival of 354 days compared to 194 days when just chemotherapy is used (P=0.0011).

Our study demonstrates the potential of combining small-molecule targeted therapies with traditional treatments, emphasizing the value of cross-species models in advancing osteosarcoma research and treatment.

#3915 An orthotopic model for murine urothelial carcinoma with response to standard of care checkpoint inhibitor treatment.

E. Rainbolt, A. Wong, N. Ansari, I. Mick, E. Dubis, J. Crews, J. Neil, **P. Fadden**;
Charles River Laboratories, Inc., Durham, NC

MB49 is a commercially available cell line used to evaluate bladder cancer for syngeneic growth and potential therapeutic response in preclinical studies. Subcutaneous implantation provides a convenient and accessible location to monitor tumor growth and response to therapy; however, an orthotopic approach may provide a more clinically relevant model. Bladder cancer is the fourth most common cancer in men and current research is focusing on immunotherapy to reduce toxicity while improving efficacy. Current orthotopic bladder implant models include time consuming intralumenal installation methods, however we will focus on a faster more direct implant approach in the intramural space (for implant characterization and evaluation see Bitaraf 2022). To establish the orthotopic model, we implanted cells directly into the bladder wall or intramural space in syngeneic C57BL/6 mice. Two cell inoculums were evaluated: $2.5x10^5$ based on subcutaneous implant standards and $1x10^5$ after initial testing proved rapid tumor progression. Individual animals were evaluated for disease progression based on body weight and bladder palpation performed at regular intervals with the frequency increased as needed based on clinical observations. If enlarged, bladders were expressed to confirm bladder function and urine color. Humane endpoint was met if the bladder was unable to be expressed or if pigmenturia was present. Necropsies were performed to evaluate bladders for tumor and any abnormal observations were recorded. Both cell inoculums had fast growth with median time to endpoint at nine to twelve days post implant. The lower cell inoculum ($1x10^5$) was chosen to proceed with efficacy data. Responses to the checkpoint inhibitors anti-CTLA-4 and anti-PD-1 RMP1-14 are described in the poster figures and tables. For comparison, the anti-CTLA-4 and anti-PD-1 response data is provided for the MB49 subcutaneous model. In summary, the development of the MB49 bladder orthotopic model will provide a sensible alternative that recapitulates the difficulties of treating bladder tumors in addition to establishing a more clinically relevant model to evaluate cancer therapies compared to standard subcutaneous implanted models. Future testing with a luciferase labeled cell line will provide imaging capabilities to monitor tumor growth via in vivo bioluminescence imaging (BLI). Bitaraf , M., Muhammadnejad, S., Azimzadeh, A., Tanourlouee, S.B., Amini, E., Zolbin, M.M., Kajbafzadeh, A.M. Evaluation of direct intramural injection to the bladder wall as a method for developing orthotopic tumor models, Animal Model Exp Med (2022) Nov30; 5(6): 575-581.

#3916 Breast cancer brain metastasis development induced by intracarotid injection of MDA-MB 231 human breast cancer cells in mice.

Romain Roth Dit Bettoni, Nicolas Hoffmann, Jordan Longin, Peggy Provent, Ismahene Benzaid, **Marc Hillairet De Boisferon**

Oncodesign Services, Dijon, France

Breast cancer is the most common malignancy in women worldwide. Breast cancer can be divided into four types based on molecular subtypes: luminal A, luminal B, human epidermal growth factor receptor 2 (Her2) positive, and triple negative breast cancer (TNBC). These subtypes have very different prognoses and treatment methods.
Herceptin, a treatment specifically directed against Her2 (overexpressed in 20–25% of breast cancers), has been widely used and improves the prognosis for Her2-positive breast cancer. TNBC accounts for only 15–20% of breast cancers and is considered as the most aggressive breast cancer. This subtype does not express estrogen receptor (ER), progesterone receptor (PR), and Her2. Additionally, it is usually identified at an advanced stage of cancer progression and often results in metastases to the brain, bones, lungs, and liver.
Human subcutaneous tumors represent a small fraction of cancers that are easy to treat by surgery and chemotherapy and are technically easy to recapitulate in animals. In contrast, high grade breast cancer and metastases represent a leading cause of cancer-related mortality for which more relevant animal tumor models are required. Additionally, the poor prognosis of metastases is badly increased in the brain, particularly due to the difficulty for novel therapies to overcome the blood brain barrier. There are very few animal models exclusively focused on the development of brain metastases, and most are based on stereotactic intracranial inoculation of cells. This injection mode rather mimics the onset of primary tumors than metastases, as it leads to the formation of single tumors and does not recapitulate the extravasation and seeding events of the metastatic cascade.
In this poster we show a new TNBC mouse model of brain metastasis. We induced brain metastases by intracarotid injection of TNBC cells, thus recapitulating the tumor cells' pathway to the brain. We evaluated the metastases progression by morphological T2 weighted MRI imaging. In parallel, we assessed the blood brain barrier integrity by T1 weighted imaging after Gd-DOTA injections and followed edema formation by FLAIR MRI.

#3917 A functional precision medicine assay for immunotherapy and combination therapy testing in liver and pancreatic cancers (PEAR-PAL).

Eleonora Peerani[1], Farah Sangkolah[1], Niamh A. Leonard[1], Yann Bouremel[1], Demi Wiskerke[1], Thomas D. L. Richardson[1], Magda B. Martinez Espuga[2], Diana Visan[1], George R. T. B de Fraine[1], Jay Kearney[1], Francesco Iori[1], Duleek N. B. Ranatunga[1]

[1]Pear Bio, London, United Kingdom,[2]Biodiscovery Institute, University of Nottingham, Nottingham, United Kingdom

The PEAR-PAL study was aimed at developing a novel ex vivo 3D immune-tumor model with integrated AI-based image analysis to optimize precision therapies in hepatocellular carcinoma (HCC) and pancreatic ductal adenocarcinoma (PDAC). Both indications currently lack effective biomarkers to guide combination treatment clinical decision-making. Fresh samples from 46 patients (30 HCC, 16 PDAC) were analyzed to evaluate the feasibility of functional precision medicine (FPM) in high-unmet-need cancers.
The platform isolates viable tumor and immune cells, co-culturing them in a cancer-specific 3D hydrogel matrix, allowing subsequent testing of FDA-approved immunotherapies, chemotherapies, and tyrosine kinase inhibitors (TKIs) using a patented microphysiological immuno-oncology plate. After isolation, the 3D immune-microtumors were exposed to various drug combinations and monitored daily over five days through confocal imaging and a computer vision pipeline. Key metrics (e.g., immune cell infiltration, tumor cell viability, and microtumor volume) were evaluated to capture inter- and intra-patient variability in response to clinical treatment regimens. Therapies included chemotherapies (e.g., gemcitabine, nab-paclitaxel), TKIs (e.g., cabozantinib, sorafenib), and immunotherapies (e.g., pembrolizumab). In PDAC, further optimization focused on testing multi-drug chemotherapeutic combinations such as FOLFIRINOX.
Tumor processing was optimized, achieving mean viability of 73±19% for HCC and 80±17% for PDAC (N=36 and 12). Live/Dead and CyQuant assays determined the optimal hydrogels to maintain 3D cell viability over the five-day assay. PBMC infiltration in the optimized hydrogel formulation increased PBMC counts from 272 to 1,485 by day 4 compared to Matrigel. Dosing for each therapeutic class was optimized via 2D and 3D dose-response assays in cell lines, through molecular readouts (in-cell western, pRTK arrays, Live/Dead, CTG) and validated in primary samples. A microphysiological plate was developed for sequential treatment addition/removal and imaging without disrupting the 3D immune-microtumor. Functional assays showed patient-specific variability in responses to treatments, with pembrolizumab decreasing microtumor volume, enabling CD8- PBMC infiltration, and increasing T-cell infiltration 3-fold compared to controls (n=2).
Future trials will correlate ex vivo findings with clinical outcomes, positioning the PEAR-PAL platform as a promising tool for individualized treatment in liver and pancreatic cancers. This approach exemplifies the potential of FPM to address current limitations in precision oncology for complex cancers lacking actionable biomarkers.

#3918 Development and validation of a systemic human multiple myeloma model utilizing luciferase expressing H929 tumor cells.

B. John[1], D. Harris[1], S.-C. Chou[1], T. Rowe[2], N. Westrick[1], N. Nightenhelser[1], A. Wong[1], E. Rainbolt[1], Z. Ward[1], J. Neil[1];
[1]Charles River Laboratories, Inc., Durham, NC, [2]Charles River Laboratories, Inc., Durahm, NC

Multiple Myeloma (MM) is the second most common hematological malignancy of clonal plasma cells that originates in the bone marrow (BM). Despite significant advancements in the treatment of MM, this disease remains incurable with a poor prognosis in relapsed/refractory patients. MM has complex disease dynamics due to the genetically diverse clonal population of myeloma cells. Thus, pre-clinical models are needed to translate tumor heterogeneity for appropriate risk assessment and the development of safe and effective treatment regimens. We established a Multiple Myeloma in vivo model by systemic implantation of the luciferase-expressing tumor cell line H929 (H929-luc) into immunodeficient NCG mice. First, we generated a H929-luc monoclonal cell line from a polyclonal pool of stable luciferase-expressing cells by limiting dilution cloning. Single cells from the stable pool were expanded in selection medium and based on in vitro growth properties and bioluminescence intensity H929-luc monoclonal lines were selected for in vivo evaluation. The in vivo tumor growth profile was determined by testing the monoclonal lines at two different cell inoculums, and disease progression was monitored by in vivo serial bioimaging. For validation of the H929-luc in vivo model, we determined the efficacy and survival benefit in response to various clinically relevant standard of care agents including dexamethasone, daratumumab, cyclophosphamide, and vincristine. The quantification of tumor burden by bioluminescent imaging showed reduced tumor burden and prolonged survival with cyclophosphamide and vincristine. Taken together, we have developed a well characterized and robust systemic H929-luc model that recapitulates the disease dissemination and invasiveness of multiple myeloma for evaluating novel therapeutic approaches to treatment.

#3919 Identifying dual inhibitions of kinases to treat pancreatic ductal adenocarcinoma.

Masahiro Sonoshita

Hokkaido University, Sapporo, Japan

Significant advancements have been achieved in elucidating the pathogenesis of pancreatic ductal adenocarcinoma (PDAC) through the development and application of mammalian models. To accelerate drug discovery by identifying novel therapeutic targets on a systemic level, we established a *Drosophila* model recapitulating the genetic signature of PDAC, characterized by alterations in *KRAS*, *TP53*, *CDKN2A*, and *SMAD4*, which are associated with the poorest patient prognosis. The '*4-hit*' flies manifested tumorigenic phenotypes, such as epithelial transformation and reduced animal viability. Comprehensive kinome-wide genetic screening using this model identified potential therapeutic targets, including MEK, AURKB, and GSK3β. Notably, combined inhibition of these targets effectively suppressed the growth of human PDAC xenografts in mice. Moreover, the activity of these kinases correlated with adverse outcomes in PDAC patients. This *Drosophila*-based platform offers a robust whole-body assay to complement existing methodologies to determine therapeutic targets in PDAC.

#3920 The role of humanized monoclonal antibody ALT-100 in reducing cancer-induced bone pain: A murine animal model study.

N. C. Mathur[1], E. Villarroel[2], I. Villarroel[2], E. Bahramnedjad[2], S. Dickinson[1], J. G. N. Garcia[3], T. Largent-Milnes[1], T. W. Vanderah[1]; [1]University of Arizona College of Medicine, Tucson, AZ, [2]University of Arizona, Tucson, AZ, [3]The Herbert Wertheim University of Florida Scripps Institute for Biomedical Innovation and Technology, Gainsville, FL

Breast cancer commonly metastasises to the bone promoting a combination of inflammatory and neuropathic mechanisms. This leads to different perceptions of pain with a reported 30-50% of all cancer patients and 75-90% of late-stage patients having cancer-induced bone pain (CIBP). Thus, there is a need for therapeutic interventions to decrease pain caused by the mechanisms of CIBP. Extracellular Nicotinamide Phosphoribosyltransferase (eNAMPT) is a cytokine overexpressed in metastatic breast carcinoma that binds to Toll-like Receptor 4 (TLR4), triggering a signaling cascade through Nuclear Factor kappa beta (NFk-B) and resulting in inflammation, osteoclastogenesis, and severe bone pain. To assess the impact of eNAMPT on CIBP, ALT-100, a monoclonal antibody neutralizing eNAMPT, was tested in a murine CIBP model. The left femur of C57BL/6J female mice was inoculated with EO771 breast cancer cells and allowed 7 days for recovery from tumor growth and the development of cancer-induced pain. Behavioral data were collected to evaluate ALT-100's effectiveness in reducing pain. Four groups were used: sham + saline, sham + ALT-100, cancer + ALT-100, and cancer + saline, with ALT-100 dosed at 4 mg/kg. Pain was assessed on days 0, 7 (pre- and 2 hours post-injection of ALT-100), 10, and 14 using flinching and guarding behavior tests. A two-way ANOVA and Tukey's multiple comparisons test indicated that ALT-100 significantly reduced pain compared to the cancer + saline group. Bone scoring on days 0 and 14 showed no significant differences in bone lesions and fractures between cancer + ALT-100 and cancer + saline groups. To test ALT-100's efficacy in inhibiting osteoclastogenesis in vitro, RAW 264.7 macrophage cells were plated in five groups exposed to different drug combinations: media, eNAMPT, ALT-100, 1:1 eNAMPT + ALT-100, and 1:2 eNAMPT + ALT-100. After 5 days a marker for osteoclastogenesis, Tartrate-Resistant Acid Phosphatase staining, was measured demonstrating that cells treated with ALT-100 + eNAMPT had significantly fewer osteoclasts than those treated with eNAMPT alone. Together these results indicate that ALT-100 inhibits metastatic breast cancer pain and eNAMPT-induced osteoclast differentiation.

#3921 Development of a KRAS-G12C-driven pancreatic cancer mouse model.

Yongkun Wei, Minghui Liu, Er-Yen Yen, Jun Yao, Phuoc T. Nguyen, Xiaofei Wang, Zecheng Yang, Dean N. Pan, Jangho Park, Yiming Cai, Andrea Viale, Huamin Wang, Dan Zhao, Ronald A. DePinho, Wantong Yao, Haoqiang Ying

UT MD Anderson Cancer Center, Houston, TX

$KRAS^{G12C}$ inhibitor (G12Ci) has produced encouraging, albeit mild clinical benefit in pancreatic ductal adenocarcinoma (PDAC). Identifying and targeting resistance mechanisms to G12Ci treatment remains the major challenge due to the lack of faithful preclinical models. To better understand the tumor biology of the $KRAS^{G12C}$ allele, we established a mouse model engineered with the *LSL-*$Kras^{G12C}$, conditional *p53* knockout ($p53^{L}$), and pancreatic-specific *p48-Cre* alleles. The resulting *p48-Cre LSL-*$Kras^{G12C}$ $p53^{L/+}$ ($K^{C}PC$) mice exhibit decreased number and area of PanIN/ADM lesions compared to age-matched $Kras^{G12D}$-driven controls ($K^{D}PC$). The decreased neoplastic activity of $Kras^{G12C}$ aligns with decreased downstream signaling (phospho-ERK and phospho-AKT) and cell proliferation (Ki67) in premalignant epithelial cells, suggesting that $KRAS^{G12C}$ is a less potent oncogenic allele compared to $KRAS^{G12D}$. Compared to $KRAS^{G12D}$, $KRAS^{G12C}$ failed to induce complete ADM which is characterized with the transdifferentiating of acinar cells into progenitor-like ductal cells. The tumor microenvironment of $K^{C}PC$ mice is less immunosuppressive than that of $K^{D}PC$, which may partially explain the slower progression of $K^{C}PC$ tumor. The relatively weak tumor development in $K^{C}PC$ model results in significantly prolonged median overall survival compared to $K^{D}PC$ model. Histopathology analysis of advanced tumors from the $K^{C}PC$ model revealed similar histological features of ductal adenocarcinoma compared to $K^{D}PC$ model or human PDAC. As for KRAS downstream signaling, PDAC lesions from the $K^{C}PC$ and $K^{D}PC$ models exhibited similar phospho-ERK, phospho-MEK and phospho-S6K1. Both the total KRAS level and the amount of active form of KRAS are also comparable between the two groups. Interestingly, phospho-AKT level was lower in $K^{C}PC$ than in the $K^{D}PC$ tumor cells. Together, our data indicates that, while $KRAS^{G12C}$-driven PDAC premalignant lesions exhibit distinctive molecular features compared to the $KRAS^{G12D}$-driven ones, there is no obvious cellular or molecular difference between the two KRAS genotypes once the tumor progresses to an advanced stage.

#3922 A p53-deficient, PTK2/cMyc-driven ovarian cancer mouse model for preclinical drug discovery.

Ziping Liu[1], Wenjing Zhang[1], Xinxin Zhao[1], Yongshuai Li[1], Xiaoya Hou[1], Satyanarayana Pochampally[1], Shelby Waddell[1], Shiji Song[1], Yaohong Wang[2], Lawrence M. Pfeffer[1], Duane D. Miller[1], Junming Yue[1], **Wei Li**[1]

[1]University of Tennessee Health Science Center, Memphis, TN,[2]MD Anderson Cancer Center, Houston, TX

Background: Ovarian cancer (OC) remains a significant health issue for women due to its late diagnosis and chemoresistance. Currently, there is no relevant mouse OC model for preclinical drug screening and treatment.
Methods: We established a mouse OC cell line model, PC53K, by transforming primary ovarian surface epithelial cells through CRISPR/Cas9-mediated TP53 knockout and simultaneous overexpression of human PTK2 and cMyc. We further developed the paclitaxel-resistant PC53K/TxR cell line. PC53K and PC53K/TxR cell lines were validated in vitro and in vivo in an orthotopic mouse model. We also evaluated the efficacy of two novel tubulin inhibitors, the clinical drug VERU-111 and the preclinical compound SB-216, using the PC53K/TxR model in vitro and in vivo.
Results: Mice intrabursally injected with PC53K cells exhibited bloody ascites, aggressive primary tumor growth and widespread metastasis, which faithfully recapitulates the phenotypes of human ovarian serous adenocarcinoma. Additionally, the paclitaxel-resistant PC53K/TxR cell line shows significant higher IC50 for paclitaxel than PC53K cell line, indicating it is a valuable tool for studying OC chemoresistance. Compounds VERU-111 and SB-216 significantly inhibited tumor growth and metastasis in orthotopic OC mouse models by overcoming chemoresistance. This study demonstrates the effectiveness of the PC53K and PC53K/TxR models for preclinical drug screening and validates the potential of VERU-111 and SB-216 in overcoming chemoresistance and treating OC.
Significance: The development of the PC53K and PC53K/TxR mouse models represents a significant advancement in OC research. By mimicking the genetic profile, aggressive behavior and chemoresistance of human OC, these models provide a robust platform for preclinical drug discovery and development.

#3923 Lung cancer autochthonous model gene expression database.

Ling Cai

UT Southwestern Medical Center, Dallas, TX

Lung cancer, the leading cause of cancer mortality, exhibits diverse histological subtypes and genetic complexities. Numerous preclinical mouse models have been developed to study lung cancer, but data from these models remain disparate, siloed, and difficult to compare centrally. To address this, we established the Lung Cancer Autochthonous Model Gene Expression Database (LCAMGDB), an extensive repository encompassing 1,354 samples from 77 transcriptomic datasets, including 974 from genetically engineered mouse models (GEMMs), 368 from carcinogen-induced models, and 12 from a spontaneous model. Importantly, 85% of the data were reprocessed through standardized pipelines tailored to each platform, including RNA-seq and five microarray platforms, enhancing comparability across studies. LCAMGDB aligns 859 GEMM tumors with human lung cancer mutations, revealing the need to expand the diversity of genetic aberrations modeled. Our analysis highlights underrepresentation of key alterations such as ROS1, RET, and MET, which are pivotal in human cancers. Furthermore, we are actively updating LCAMGDB to include 989 additional samples from 44 studies, augmenting the current collection of RNA-seq data by nearly 80%. Complementing the database, we developed a web application at https://lccl.shinyapps.io/LCAMGDB/ offering researchers data review and download, as well as intuitive tools for dynamic gene expression analysis and cross-study comparisons. Future iterations aim to integrate pathology data through engagement with the lung cancer mouse model community, potentially complementing transcriptomic insights. LCAMGDB bridges gaps between mouse models and human lung cancer, laying the groundwork for translational research and collaborative advancements. By standardizing and expanding this resource, we aim to improve the fidelity and relevance of preclinical studies for understanding lung cancer biology and developing therapies.

#3924 *in vitro- in vivo* model correlations and cross-species comparisons in osteosarcoma.

Emma N. Vanderboon, Geoffrey A. Wood, Courtney R. Schott

Pathobiology, University of Guelph, Guelph, ON, Canada

Osteosarcoma (OS) is the most common primary malignant bone cancer of both humans and dogs, characterized by its high rate of metastasis. Despite decades of research, treatment strategies for OS have seen limited advancements in over 40 years. This stagnation highlights the critical need for accurate preclinical models to investigate metastasis and develop relevant therapeutic approaches. Understanding the utility and limitations of *in vitro* and *in vivo* OS models is paramount to deriving meaningful conclusions that can guide future research and inform clinical strategies. This study aims to develop an *in vitro* assay pipeline to investigate mechanisms of metastasis and to characterize a panel of human OS cell lines previously characterized using orthotopic amputation and intravenous murine metastasis models, allowing us to examine *in vitro- in vivo* correlations. Furthermore, the study seeks to characterize a diverse panel of canine OS cell lines, enabling cross-species comparisons since canine OS serves as an important model for human disease and vice versa. By correlating findings between species and *in vitro* and *in vivo* models, this investigation seeks to bridge gaps in understanding how metastatic behaviors observed in the laboratory translate to the complexities of metastasis in living organisms. The human panel includes eight patient-derived xenograft (PDX)-derived cell lines (5 primary, 3 metastatic) and two commercial OS cell lines (MG63, SJSA). The canine panel includes commercial cell line D17 and 13 primary cell lines (11 primary, 2 metastatic). The *in vitro* pipeline comprises six assays designed to mimic key steps of the metastatic cascade: migration (via scratch and transwell assays), invasion (using a spherical invasion assay), anchorage-independent survival (anoikis assay), and colony formation (clonogenic assay). Additionally, doubling-time will be measured to assess proliferation. Results show differences between *in vivo* and *in vitro* metastatic behavior for individual cell lines. By establishing a robust and reproducible cross-species *in vitro* assay pipeline, this study aims to enhance our understanding of OS metastasis and provide a platform for future mechanistic studies. The ultimate goal is to facilitate the accurate interpretation of *in vitro* and *in vivo* findings, ensuring that conclusions drawn from these models are relevant and actionable in the context of future OS research and therapeutic development.

#3925 *In vitro* and *in vivo* characterization of *Cdh1* CRISPR knockout in syngeneic ER+ SSM3 mouse mammary tumor cells.

Insa Thale[1], Dixcy J. S. John Mary[1], Sarah Wang[2], Lora Rigatti[3], Jagmohan Hooda[4], Peter Lucas[5], George C. Tseng[2], Adrian V. Lee[4], Steffi Oesterreich[4]

[1]University of Pittsburgh School of Medicine, Pittsburgh, PA,[2]Department of Biostatistics, University of Pittsburgh, Pittsburgh, PA,[3]Division of Laboratory Animal Resources, University of Pittsburgh, Pittsburgh, PA,[4]University of Pittsburgh, Pittsburgh, PA,[5]Mayo Clinic College of Medicine and Science, Rochester, MN

Invasive lobular carcinoma (ILC) accounts for 10-15% of all breast cancers, making it the most common special histological subtype of breast cancer. Initially, ILC is associated with a better response to adjuvant hormonal therapy compared to invasive ductal breast cancer (IDC). However, patients with ILC often experience a worse long-term outcome. Over 90% of ILCs are estrogen receptor positive (ER+) and do not express functional E-cadherin (CDH1). There are limited studies aimed at improved understanding of the tumor microenvironment (TME) in ILC. Therefore, we set out to generate and characterize a syngeneic mouse cell line model that mimics the genetic loss of *CDH1* seen in ILC.

We created an ER+ ILC-like mammary cancer model using the syngeneic SSM3 cell line, derived from an ER+ mouse mammary tumor in STAT1-deficient mice. To mimic the hallmark feature of ILC, we used CRISPR-Cas9 to knock out *Cdh1*.

In vitro assays were performed to evaluate the effects of *Cdh1* knockout (KO) in both 2D and 3D environments, focusing on cell proliferation and estrogen sensitivity. These experiments demonstrated increased growth of *Cdh1* KO SSM3 cells in both 2D and 3D environments, together with altered cell morphology and increased sensitivity to estrogen.For *in vivo* characterization, we focused on tumor growth and metastasis. SSM3 Scr and *Cdh1* KO cells were injected into the mammary fat pad of mice. Initial findings revealed increased tumor growth in mice injected with SSM3 *Cdh1* KO cells, while metastasis studies are ongoing.

RNA-seq analysis of both SSM3 cells grown *in vitro*, and *in vivo* tumor tissues is ongoing and will help to identify distinctive gene expression changes in the *Cdh1* KO model. We will also analyze changes in the TME, such as immune cells, ECM and fibroblasts.

In summary, we have successfully generated an ER+ syngeneic ILC model, and we expect that growing the cells *in vitro* and *in vivo* represents a valuable tool for studying ILC and holds promise for advancing the development of targeted immunotherapies and endocrine therapies for this breast cancer subtype.

#3926 The effect of *Pten* haploinsufficiency on genomic and transcriptomic profiles in combined genetically-engineered mouse models for colonic neoplasia based on *Apc* inactivation.

Haruki Sada[1], Hiroaki Niitsu[2], Hikaru Nakahara[2], Naoya Sakamoto[3], Hirotaka Tashiro[1], Hideki Ohdan[4], Takao Hinoi[2]

[1]Surgery, Kure Medical Ctr. & Chugoku Cancer Ctr., Kure, Japan,[2]Clinical and Molecular Genetics, Hiroshima University Hospital, Hiroshima, Japan,[3]Molecular Pathology, Hiroshima University, Hiroshima, Japan,[4]Gastroenterological and Transplant Surgery, Hiroshima University, Hiroshima, Japan

Background and Aim: We have reported genetically-engineered mouse models for colonic neoplasia by using our novel *CDX2P-Cre* mice, in which Cre is restrictedly expressed in colonic epithelial cells. Although colonic tumors are generated in *CDX2P-Cre;Apc$^{flox/+}$* (*CPC;Apc*) mice with highly penetrant rate, greater number and deeper invasion of colonic tumors were observed in *CDX2P-Cre;Apc$^{flox/+}$;Pten$^{flox/+}$* (*CPC;ApcPten*), possibly resulting from haploinsufficiency of *Pten* gene. We have also confirmed shorter lifespan in *CPC;ApcPten* than that in *CPC;Apc* (AACR Annual Meeting 2018). Here, we test if these phenotypes come from one copy loss of *Pten* itself or secondary genetic and/or transcriptomic alterations induced by *Pten* loss by using whole genome sequence (WGS) and RNA sequence from macroscopically-dissected colonic tumors (bulk RNA-seq).

Methods: WGS for five tumors and five livers as counterpart controls in each mouse model was performed by DNBSEQ platforms. Bulk RNA-seq was performed for three tumors in each model.

Results: In the analysis using B-allele frequency, copy-neutral LOH occurred in chromosome 18 including *Apc* loci specifically in colonic neoplasia in both *CPC;Apc* and *CPC;ApcPten* mice. In contrast, LOH was not observed in chromosome 19 including *Pten* loci in *CPC;ApcPten* mice. We also confirmed remarkably decreased coverage of reads in reference mapping in *Apc* locus, and there was a slight decrease of coverage in *Pten* locus. There were no additional driver gene mutations in *CPC;Apc* or *CPC;Apc+Pten* in WGS. All these results suggested just one copy loss of *Pten* resulting in aggressive tumor phenotype in *CPC;ApcPten* mice. In RNA-seq and subsequent GSEA comparing colonic tumors in *CPC;ApcPten* with those in *CPC;Apc* revealed upregulation of some signal pathways including mTORC1. Supporting this observation, number of colon tumors, especially adenocarcinomas, were decreased by rapamycin administration to *CPC;Apc+Pten*. Similarly, rapamycin was more effective in tumoroids from *CPC;Apc+Pten* those from *CPC;Apc*.

Conclusion: WGS reveals one copy loss of *Pten* locus is the only genetic alteration *in CPC;Apc+Pten* when compared with *CPC;Apc*. One copy loss of Pten is sufficient to upregulate mTORC1 pathway in a *CPC;Apc+Pten* mouse model, resulting in promotion of tumor formation and invasion.

#3927 Bone Metastasis Technology Platform: Establishing clinically relevant bone metastasis models for breast and prostate cancer and multiple myeloma.

Tiina E. Kahkonen[1], Jie Wen[2], Ru Yang[2], Yuyang Xu[2], Michael Zhang[2], Jussi M. Halleen[1]

[1]OncoBone Ltd, Kiviniemi, Finland,[2]PharmaLegacy LLC, Shanghai, China

Bone metastases are a significant clinical problem in many major cancers, especially in breast and prostate cancer where 70-90% of advanced patients develop bone metastases. Myeloma bone disease is associated with similar clinical problems than bone metastases, including increased risk of fractures and bone pain that decrease the quality of life. Current cancer therapies can only partially decrease tumor growth, resulting in only 5% of bone metastatic patients being alive 5 years after the diagnosis. Bone metastases are therefore a high unmet medical need with a high demand for effective therapies. Lack of appropriate preclinical bone metastasis models that would exhibit the same clinical features that are observed in bone metastatic patients has made it difficult to advance therapy development at early stages. This study describes the establishment of three preclinical bone metastasis models, a breast cancer model using 4T1 mouse triple-negative breast cancer cells in BALB/c mice, a prostate cancer model using RM-1 mouse androgen-insensitive prostate cancer cells in C57BL/6 mice, and a multiple myeloma model using human RPMI 8226 cells in immunodeficient NPG mice. The cancer cells were inoculated intratibially into the bone marrow to model tumor growth in bone. Tumor growth was monitored by bioluminescence imaging (BLI), cancer-induced bone changes by X-ray imaging, and bone pain by Von Frey filaments (mechanical allodynia).

In the 4T1 breast cancer model, 100% of the mice had bone metastases at day 7, and maximum study duration was 21 days. Osteolytic bone lesions were clearly observed and bone pain was detected at day 7. In the RM-1 prostate cancer model, 83% of the mice had bone metastases at day 7, and 100% of the mice at day 14, and maximum study duration was 28 days. Bone pain was observed at day 7, and osteolytic-mixed bone metastases were visible at day 14. In the RPMI 8226 multiple myeloma model, 100% tumor take rate was detected at day 7. Osteolytic bone metastases were visible at day 21, and maximum study duration was 100 days.

We have established a clinically relevant Bone Metastasis Technology Platform (BMTP©) that currently includes preclinical bone metastasis models for breast and prostate cancer and multiple myeloma. These models have clinical features that are similar to those observed in bone metastatic patients. In preclinical models established in BMTP, tumor burden is monitored by BLI, the type and extent of cancer-induced bone loss is visualized by X-ray imaging, and bone pain is analyzed to provide a clinically relevant readout about the quality of life. We conclude that BMTP is a clinically relevant translational tool for evaluating efficacy of cancer therapies on bone metastasizing cancers.

#3928 Analyzing treatment effects of chemo- and immunotherapy in the context of the TME in an *ex vivo* model of HNSC.

Mareike S. Haarmann, Philipp Zimmermann, Malte Suchan, Dominik Funken, Jens Peter Klu?mann, Johannes Bragelmann

University Hospital of Cologne, Cologne, Germany

Head and neck squamous cell carcinoma (HNSC) accounts for 450.000 deaths worldwide each year. Rising cases of HNSC have been predicted.

Understanding the tumor biology in patients is crucial to developing more efficient treatment strategies against HNSC. Especially in the context of immunotherapy, the interplay of immune, cancer and stroma cells in the tumor microenvironment (TME) strongly influences the biological characteristics and behavior of the tumor and its response to therapy. However, most HNSC *in vitro* models such as patient-derived cell lines and organoids do not recapitulate TME complexity.

Patient-derived tumor fragments (PDTFs) are a novel pre-clinical model to bridge this gap. Isolated directly from patients, they preserve the spatial and cellular tumor composition, allowing analysis of tumor behavior and reaction to treatment in the context of the TME. In this study, we adapted the PDTF methodology to study the effects of combined chemo- and immunotherapy on HNSC.

To generate PDTFs, tumors from patients with HPV-negative HNSC were extracted in routine surgery and immediately transferred to the lab. The tumors were mechanically dissected into 1 mm^3 fragments, termed PDTFs. Several PDTFs per tumor were distributed across wells of a cell culture plate and subjected to different treatments with cisplatin, nivolumab or caspase inhibition as well as combinations. After short-term *ex vivo* culture of max. 72h, we performed gene expression analysis via qPCR and RNA-seq as well as histological analysis of PDTFs. In total, PDTFs from 14 different HNSCs could be subjected to treatment, including 2 longitudinal biopsies from the same patient.

Histological analysis of freshly extracted PDTFs showed the intact TME *ex vivo*.

Transcriptomic analysis revealed a significant reaction to treatment that was consistent with previous *in vitro* results. HALLMARK gene sets involved in cell cycle progression (Mitotic spindle, G2M Checkpoint, E2F Targets), EMT as well as glycolysis were downregulated, while inflammatory signaling-related gene sets were enriched, especially in the combined treatment with immunotherapy. Additionally, increased IFNγ expression indicates specific effect of the immunotherapy on leucocytes in the TME.

Matched PDTFs from the same patient showed strong correlation of transcriptomic behavior.

Overall, we could show treatment-specific reaction of the PDTFs to both chemotherapy and immunotherapy, that included effects on cell cycle, metabolism and inflammation. The measurable reaction of the leucocytes within the TME sets this model apart from other *ex vivo* models.

Together with the patient-specific differences in transcriptomic behaviour, evident in the matched PDTFs, this makes PDTFs an interesting *ex vivo* model whose application for patient-specific treatment prognosis will be the subject of further studies.

#3930 Preclinical models of cancer cachexia: Bridging the gap to clinical applications.

Hongyan Sun[1], Dan Zhou[1], Yunlong Jiang[1], Jianming Xu[1], Jing Zhao[1], Xiang Gao[1], Jun Wang[2]

[1]GemPharmatech Co., Ltd, Nanjing, China,[2]GemPharmatech Co., Ltd, San Diego, CA

Cancer cachexia (CC) is a multifactorial syndrome affecting up to 80% of advanced cancer patients. Characterized by unintentional weight loss, skeletal muscle wasting, and fat depletion, CC significantly impairs quality of life, treatment tolerance, and survival. Despite its prevalence, effective therapeutic options remain limited, underscoring the urgent need for preclinical models that closely mimic clinical symptoms to support drug development.

Towards this end, we developed and validated a CC preclinical model with HT-1080 xenograft in NCG mice, which demonstrated hallmark CC features, including body weight loss, fat and muscle loss, as well as impaired physical performance. The tumor-bearing mice also displayed elevated hGDF15 levels (undetectable in non-tumor-bearing (NTB) mice), and significantly reduced cumulative food intake. In this study, we used Ponsegromab (anti-GDF15 monoclonal antibody) as a positive treatment for CC starting when mice had lost ~7% body weight. At the end of the study, mice in hIgG1-treated control group suffered over 15% body weight loss, while the Ponsegromab treatment restored body weight to baseline levels. Although Ponsegromab treated mice remained lighter than NTB PBS controls, our data suggested that Ponsegromab treatment could significantly improve food intake and increase survival rates. Fat and muscle tissue weights, including gonadal, peri-renal, and inguinal subscapular white fat, as well as gastrocnemius and soleus muscles, were significantly reduced in the hIgG1-treated control group compared to baseline controls. Ponsegromab treatment preserved fat and muscle weights to levels similar to the baseline group. Motor function assessed via rotarod performance, and grip strength showed coordination and strength impairments in tumor-bearing mice, while Ponsegromab significantly improved grip strength and physical performance in comparison to the hIgG1-treated control group.

In conclusion, our validated preclinical cachexia models closely align with clinical CC manifestations, providing a robust platform for the evaluation of novel therapeutic strategies. These models enable detailed exploration of CC mechanisms and support the development of effective interventions to mitigate cachexia and improve patient outcomes.

#3931 Establishment of an orthotopic intracranial luciferase labelled patient derived xenograft of glioblastoma in immunocompromised mice model.

Seth Hannah, Fu-Ju Chou, Ksenia Vulikh, Mara Gilardi, Stefano Cairo, Paul Heverly

Champions Oncology, Inc., Hackensack, NJ

Glioblastoma multiforme (GBM) is the most aggressive and fatal form of brain cancer, with a median survival time of less than 15 months. Currently, GBM patients have limited treatment options and poor efficacy outcomes, underscoring the urgent need for innovative research methods and more effective therapeutic strategies. Patient-derived xenografts (PDX) offer a promising approach to evaluate the side effects and potency of drugs in vivo. By implanting GBM patient tumor samples into immunocompromised mice, we gain invaluable insights into the unique mutations and genetic profiles of individual GBM models. PDX models provide a more accurate and personalized approach compared to traditional cell line-based studies. Intracranial xenograft techniques emulate the interaction of brain tumors with complex brain microenvironment and the blood-brain barrier, offering significant advantages for testing new regimens for brain tumors. Non-invasive imaging techniques like MRI and in vivo imaging systems (IVIS) have traditionally been used to monitor intracranial tumor growth and treatment response. However, challenges such as achieving the expected resolution with mouse MRI, long scan times, high costs, and technical barriers in labeling PDX material limit their application. This study aims to establish a pre-clinically relevant platform for the development and validation of potential pharmaceutical treatments for glioblastoma, utilizing patient-derived cells within an appropriate tumor microenvironment. To create this model, U87 and patient-derived glioblastoma cells were labeled with luciferase and cultured ex vivo to confirm bioluminescent signal integrity. Using a stereotaxic apparatus and established skull-to-brain mapping techniques, cell suspensions were implanted into predetermined cortical coordinates. The bioluminescent signal from the proliferating tumor was monitored, revealing consistent exponential growth of the orthotopically implanted tumor without adverse health effects over a 28-day period. Our findings demonstrate that this method allows for the orthotopic implantation of human patient-derived glioblastoma in immunocompromised mice, fostering tumor development within a relevant microenvironment. This model provides a robust platform for the accurate testing and development of glioblastoma therapeutics. Preliminary IVIS data indicate that the intensity of the U87-Luc tumor increased significantly over a designated time period. Similarly, the PDX-GBM Luc showed a remarkable increase as well. This new orthotopic intracranial platform enables us to monitor tumor growth through bioluminescent measurements, offering a valuable tool for advancing glioblastoma research and treatment.

#3932 Elucidating mechanisms of metastatic progression in bladder cancer with novel GEMMs and systems biology approaches.

Siyue Lin[1], Soonbum Park[1], Alexander Chui[1], John R. Christin[2], Andrea Califano[3], Michael M. Shen[4], Cory Abate-Shen[1]

[1]Department of Molecular Pharmacology and Therapeutics, Columbia University Irving Medical Center, New York, NY,[2]Department of Medicine, Columbia University Irving Medical Center, New York, NY,[3]Department of Systems Biology, Columbia University Irving Medical Center, New York, NY,[4]Herbert Irving Comprehensive Cancer Center, Columbia University Irving Medical Center, New York, NY

Metastasis is the leading cause of bladder cancer deaths. Despite recent advances in treatment, metastatic bladder cancer remains incurable. One of the major challenges in studying metastasis has been the lack of suitable *in vivo* models that recapitulate the natural evolution of metastatic progression within the native tumor microenvironment. To address this, we have generated genetically-engineered mouse models (GEMMs) of bladder cancer that have highly penetrant metastatic phenotypes. In particular, our new metastatic GEMMs build on our previously established GEMM, in which bladder-specific co-inactivation of *Pten* and *p53* (double knockout, DKO), leads to muscle-invasive bladder cancer (MIBC) with a low incidence of metastasis. By introducing loss-of-function mutations in *Arid1a*, an epigenetic regulator that is frequently mutated in high-grade human bladder cancer, we generated triple knockout (TKO) mice. These mice develop highly lethal MIBC with over 80% incidence of regional and distant metastasis. Histopathological characterization of TKO tumors revealed aggressive features, including aberrant differentiation and enhanced invasiveness, and demonstrated strong conservation with human MIBC, as shown by the expression of basal and luminal subtype markers. We implemented a systems biology approach to identify mechanistic determinants, or master regulators (MRs), of metastasis. To identify drivers of metastatic progression, we first performed RNA sequencing to compare metastatic tumors with pre-tumor bladder urothelium from TKO mice. Gene signatures from TKO tumors were highly conserved with human MIBC. Cross-species analysis integrating TKO mouse data with human bladder cancer datasets identified conserved MRs predictive of adverse outcome in several independent patient cohorts. Key regulators of bladder cancer, such as *Pparg* and *Shh*, and lineage plasticity drivers, like *Sox11*, emerged as candidates. To prioritize these MRs, we performed CRISPR-based functional screening, which highlighted promising targets that are now being evaluated in our mouse models. In summary, we developed GEMMs that faithfully model metastatic bladder cancer. Our cross-species analysis identified a panel of conserved MRs, including *Pparg*, *Shh*, and *Sox11*, as key drivers of lineage plasticity and metastatic progression. These findings offer insights into the mechanisms of bladder cancer metastasis and suggest novel therapeutic opportunities.

#3933 Modeling treatment naïve and castration-resistant metastatic prostate cancer in genetically engineered mouse models.

Jeannine Turgeon, Florencia Picech, Jie Zheng, Cory Abate-Shen

Department of Molecular Pharmacology and Therapeutics, Columbia University Irving Medical Center, New York, NY

Metastatic prostate cancer, the major cause of prostate cancer lethality, presents as several distinct subtypes. Treatment naïve metastatic prostate cancer, often called oligometastatic disease, develops within an androgen-intact context and, given its low frequency in the population, remains understudied. The more prevalent type of metastatic disease arises from castration-resistant prostate cancer (CRPC) as a consequence of treatment failure in response to androgen deprivation therapy (ADT). Both types of prostate cancer metastases have eluded curative treatments, and the lack of accurate molecular and phenotypic in vivo models has been a roadblock to developing effective treatments. We have developed genetically engineered mouse models (GEMMs) that recapitulate advanced prostate cancer through disruption of molecular processes that are typically altered in late-stage human prostate cancer. In particular, we have generated GEMMs that carry loss of function of the tumor suppressors, Nkx3.1, Pten and p53 alone or together with loss of DNA damage repair (DDR) genes Brca1, Brca2, or Atm. We have studied these GEMMs in parallel with mice that have both Nkx3.1 and Pten loss and gain of function of oncogenic Kras. The phenotype of these GEMMs were evaluated in androgen intact and deprived conditions to recapitulate treatment naïve and castration resistance contexts. Each of these GEMMs develop prostate cancer with distinct characteristics in the presence or absence of androgens. GEMMs with Pten loss displayed a non-metastatic indolent phenotype of prostatic intraepithelial neoplasia. When paired with p53 knockdown or mutation, the GEMMs showed phenotypic variety (adenocarcinoma, squamous cell, sarcomatoid) and had moderate metastasis incidence (~50%). GEMMs that additionally have loss of function of DDR genes developed more aggressive prostate cancer with increased penetrance to organs and bone, modeling the metastatic tropism observed in human patients. Most notably, the Brca GEMMs that underwent castration revealed higher metastatic incidences overall, with increases in the lung, liver, and bone, indicating increased CRPC penetrance. Additionally, constitutive activation of Kras resulted in an aggressive adenocarcinoma phenotype with fully penetrant metastatic disease and the highest incidence of bone metastasis (~40%). Interestingly, castration of Kras GEMMs led to a significant reduction in liver and bone metastases. To summarize, through the loss of function of tumor suppressor genes, DDR genes, and gain of function of Kras in murine models, we have successfully modeled treatment naïve and castration-resistant metastatic prostate cancer. These GEMMs are a valuable resource to identify therapeutic targets in advanced prostate cancer for various subtypes of metastatic prostate cancer in androgen intact and castrated contexts.

#3934 Establishing a non-invasive direct injection method to achieve an orthotopic murine model of cervical cancer in immunocompetent mice.

Marlene L. Campos Guerrero, Liyun Chen, Lori Strong, Stephanie Markovina

Washington University In St. Louis, St. Louis, MO

Current cervical cancer murine models consist of transgenic genetically engineered mouse models (GEMMS) that require multiple genetic mutations and/or hormone induction for spontaneous cervical cancer development. Other techniques used for studying cervical cancer in mice consist of subcutaneous injection of tumor cells and surgical implantation of tumor cells into the lower portion of the uterus. These approaches are useful for studying tumor cell behavior - growth and progression but are limited to their location and the use of immune-compromised mice models, and/or survival surgery. Attempts for tumor cell deposit into mice cervix have consisted of pretreating mice with hormones and irritating the cervix and vaginal canal prior to depositing tumor cells. Here, we focused on establishing a reproducible, reliable, non-surgical approach to orthotopic establishment of tumors directly into cervix of the immunocompetent mice. We hypothesized that this technique will not require hormone pre-treatment of mice and reliably establishes tumor cells within the cervix epithelium in mice. To test this hypothesis, we used TC1 murine cell line expressing E6 and E7 oncogenes and engineered to express luciferase to inject the cervix of mice through the urovaginal opening. We confirmed establishment of tumor cells in the cervix 3-hours post injection through bioluminescence imaging (BLI) and monitored tumor growth with BLI imaging every 3 days. Additionally, we confirmed tumor growth through micro-CT imaging every 7 days post injection. We identified that mice with cervix injection can be monitored up to 24-31 days post injection. Tumor histologic images confirm tumor establishment in cervix submucosa with local-regional spread, and lung metastasis, similar to the pattern of spread seen in human cervical cancers. While existing methods of orthotopic tumor establishment achieve a similar model of spread, this approach is minimally invasive and does not require breeding. Additional studies are ongoing to determine the success rate of desired cervical tumor establishment using this method and to discern molecular mechanisms of invasion and metastasis in cervical cancer.

#3935 Durable inhibition of ERK phosphorylation is required for tumor regression in novel human and mouse models of NF1-inactivated melanoma.

Olvania-Danyca Hilaire[1], Alexis M. Jones[1], Moriah H. Nissan[1], Sebastien Monette[1], Jordan Eichholz[1], Sandra Misale[2], Isabell Schulze[3], Naresh Vasani[1], Cailian Liu[1], Xia Yang[1], Elisa De Stanchina[1], Nikolaus Schultz[1], Michael F. Berger[1], Neal Rosen[1], Taha Merghoub[3], David B. Solit[1], Aphrothiti J. Hanrahan[1]

[1]Memorial Sloan Kettering Cancer Center, New York, NY,[2]Johns Hopkins University School of Medicine, Baltimore, MD,[3]Weill Cornell Medicine, New York, NY

Inactivating mutations in Neurofibromin 1 (*NF1*), a RAS-GTPase, have been identified in a significant subset of melanomas, both concurrent with and exclusive of oncogenic *BRAF* and *RAS* mutations. However, FDA-approved therapies targeting the MAPK pathway have been largely inactive in this molecular cohort. To address the underlying biology, we first leveraged sequencing data from 1,912 melanoma tumor/normal pairs generated as part of the MSK-IMPACT sequencing initiative and identified oncogenic mutations in *TP53* as the most significant event common to *NF1*-inactivated melanoma, with oncogenic *BRAF* mutations frequently co-occurrent. Given the lack of models for studying NF1 loss, we next generated a cohort of genetically engineered mice harboring conditional alleles for $Nf1^{fl/fl}$, $Trp53^{fl/fl}$, and/or oncogenic BRAF V600E ($Braf^{CA}$) and the transgene $Tyr::CreER^{T2}$, restricting induction to melanocytes. While melanocyte-specific, homozygous knockout of *Nf1* induced hyperpigmentation, it was insufficient for melanomagenesis. Combined *Nf1/Trp53* knockout induced tumor formation with an incidence of 55.56%; tumor onset and hyperpigmentation were hastened upon the addition of oncogenic BRAF V600E. Other genetic cohorts induced tumors with variable penetrance and latency ($Braf^{CA}$*/Nf1,* $Braf^{CA}$*/Trp53*). All tumors displayed features of human melanoma including pigmented melanocytic and amelanotic spindle-shaped neoplasms, with most staining positive for S100. To facilitate preclinical and functional studies, we generated 22 mouse tumor-derived, syngeneic cell lines. As anticipated, loss of *Nf1* conditioned the response to treatment with a BRAF monomer inhibitor (vemurafenib), while *Nf1*-inactivated cells retained sensitivity to MEK inhibition (trametinib) or the combination. Combined inhibition of BRAF/SHP2 inhibited ERK phosphorylation and cell proliferation in $Braf^{CA}$*/Nf1/Trp53*-mutant cells; however, inhibition of ERK signaling was transient. Combination treatments that resulted in more durable inhibition of ERK, including the triple combination of RAF/MEK/SHP2 inhibitors, achieved tumor regression in mice with established $Braf^{CA}$*/Nf1/Trp53*-null tumors as well as in a genetically similar human PDX of melanoma (SK-MEL-1273A). Overall, we find that addition of SHP2 to MEK or RAF inhibitors resulted in more durable MAPK pathway inhibition and greater tumor regression in mice bearing NF1-null tumors. The testing of additional combinations of novel agents that selectively target the MAPK pathway and other parallel pathways contributing to resistance and survival are ongoing.

#3936 CDK12 tumor suppressor function and therapeutic targeting in newly developed mouse models of serous ovarian cancer.

Jean Ching-Yi Tien, Yali Zhai, Rong Wu, Yuping Zhang, Yu Chang, Yunhui Cheng, Abigail J. Todd, Christina Wheeler, Shuqin Li, Rahul Mannan, Caleb Cheng, Somnath Somnath Mahapatra, Xuhong Cao, Yuanyuan Qiao, Lanbo Xiao, Marcin Cieslik, Ding Ke, Kathleen R. Cho, Arul M. Chinnaiyan

University of Michigan, Ann Arbor, MI

Adenocarcinoma of the ovary is the fifth leading cause of cancer death among American women. Most mortality results from high-grade serous ovarian carcinoma (HGS-OvCa)—a disease type resistant to conventional chemotherapy, and therein requiring urgent preclinical modeling for pharmaceutical testing. We generated a novel serous ovarian carcinoma model by employing *Ovgp1*-driven Cre recombinase to catalyze CRISPR-Cas9-medicated ablation of the *Trp53*, *Rb1* and *Nf1* tumor suppressor genes in the mouse ovary (sgPRN mice). The gene encoding cyclin dependent kinase 12 (CDK12) is among the most frequently inactivated in HGS-OvCa. Its loss corresponds with increased mortality, DNA damage (including tandem duplications) and tumor immunogenicity. Co-ablation of *Cdk12* in the sgPRN model ("sgPRN-C*dk12KO* mice") accelerated tumor progression leading to *bona fide* HGS-OvCa, and early mortality. In a conventional (Cre-lox-medicated) *Trp53*/ *Nf1*/ *Rb1* triple knockout model, *Cdk12*ablation ("PRN-*Cdk12KO* mice") effected immune infiltration characterized by CD3, CD8, CD4, and Granzyme B positivity like that seen in clinical HGS-OvCa. We observed recapitulation of this immunogenic phenotype—with corresponding sensitivity to immune checkpoint blockade—in a novel syngeneic allograft model derived from these animals. To screen for synthetic lethal interactions with *Cdk12* loss, we conducted a CRISPR-Cas9 screen in PRN-*Cdk12KO* cell lines. The gene encoding CDK12 paralog CDK13 emerged as the most depleted, suggesting CDK13 could be targeted to promote paralog-induced synthetic lethality as previously demonstrated in *CDK12*-mutant prostate cancer. Indeed, sgPRN-C*dk12KO* and PRN-*Cdk12KO* -derived cell lines exhibited enhanced sensitivity to the CDK13/12 degrader YJ1206. Our work defines CDK13-targeting as a novel treatment modality for *CDK12*-inactive HGS-OvCa while establishing biological resources for the *in vivo* modeling of this disease.

TUMOR BIOLOGY: Pediatric Cancer Models
Poster Session

#3940 Development of immunocomplex pediatric tumor organoids for onco-immunotherapy research.

Naiara Martinez Velez[1], Roel Polak[1], William Wang[1], Maria Fernanda Henao Echeverri[1], Diane Cruz Manjarrez[1], Moksha Desai[1], Yiyun Cheng[1], Peng Xu[1], Cara Coleen Rada[1], Bita Sahaf[1], Hiroyuki Yoda[2], William Weiss[2], Elena Sotillo[1], Calvin J. Kuo[1], Crystal L. Mackall[1]

[1]Stanford University, Palo Alto, CA,[2]UCSF, San Francisco, CA

Aggressive pediatric solid tumors urgently require effective and specific therapies. In the clinic, promising new therapies, including immunotherapies, have shown limited efficacy against pediatric solid tumors. One challenge is the lack of reliable preclinical models for the evaluation of new therapies. While patient-derived organoids recapitulate primary tumor heterogeneity, and some structural properties, they fail to preserve immune cells, limiting their utility to evaluate antibody-based or cell-based immunotherapies. To address this limitation, we aimed to generate tumor immune organoids (TIOs) using an air liquid interface culture system. By optimizing the media and matrix conditions, we successfully developed osteosarcoma (OS), medulloblastoma (MB) and neuroblastoma (NBL) TIOs from mouse models that mimic the architecture and composition of human primary tumors. Flow cytometric and confocal microscopy analyses demonstrated that TIOs were viable and preserved not only tumor cells but stromal cells including immune cells for more than 130 days in NBL, 14 days in MB and 30 days in OS. Singe-cell RNA sequencing of primary tumors and TIOs after several days in culture showed preservation of the immune landscape, including T cells, myeloid cells and NK population, as well as their specific phenotypes. To evaluate the feasibility of using TIOs to test onco-immune agents, we treated NBL TIOs with anti-CD47 and anti-GD2 antibodies alone or in combination, hoping to replicate our previous work showing that blockade of GD2 and CD47 exerted a synergistic antitumor effect (Theruvath et al, Nat Med 2021). Importantly NBL TIOs responded to single agents with less tumor cell killing than the combination treatment, confirming that the immune compartment within the TIOs is biologically functional, enabling the study of tumor-immune interactions. Next, we tested chimeric antigen receptor T-cell (CAR-T) targeting GD2 in NBL, GPC2 in MB and B7H3 in OS. First, we optimized the delivery of the CAR-T cells to the culture without disrupting the architecture of the TIOs, facilitating the study of CAR-T interplay with TME populations and CAR-T antitumor evaluation. Notably, we successfully established patient-derived MB TIOs from a pediatric patient, that preserved neoplastic, stromal and immune compartments, and co-cultured them with GD2.BBz CAR-T cells. Using confocal live imaging we tracked the antitumor activity of CAR-T cells in the native 3D MB microenvironment. Our findings highlight the invaluable potential of using patient-derived TIOs as a platform to predict clinical responses to immunotherapies. In the context of cellular therapies, co-culture with autologous CAR-T cells provides a unique opportunity to study the mechanisms of resistance and efficacy in pediatric solid tumors, facilitating the development and testing of more effective and personalized treatments.

#3941 CRISPR screen of human stem cell derived MYC-driven medulloblastoma tumors reveal the mitochondria as a vulnerability.

Kenya Pleitez Valle[1], Miriam Velez[1], Yang Zhao[1], Bo Cheng[1], Sophia Estrada[1], Linwei Li[1], Tom O'Loughlin[2], Balaraman Kalyanaraman[3], Ming You[4], Luke A. Gilbert[2], David M. Hockenbery[5], David B. Shackelford[6], Peter J. Mullen[7], Miller Huang[1]

[1]Pediatrics, Children's Hospital Los Angeles, Los Angeles, CA,[2]University of California San Francisco, San Francisco, CA,[3]Medical College of Wisconsin, Milwaukee, WI,[4]Houston Methodist Research Institute, Houston, TX,[5]Fred Hutch Cancer Center, Seattle, WA,[6]University of California Los Angeles, Los Angeles, CA,[7]University of Southern California, Los Angeles, CA

Background: Medulloblastoma (MB) is the most common malignant pediatric brain tumor and is derived from neuroepithelial stem cells (NESC). Amplification of each member of the MYC family of oncogenes (MYCN, CMYC, MYCL) has been found in MB tumors and amplified-MYCN or CMYC correlates with poor prognosis. Since transcription factors such as MYC are difficult to drug with small molecules, more druggable targets need to be identified. While genetic screens can be performed on human MB cell lines, there is a lack of a proper isogenic control to distinguish targets that also negatively affect healthy non-tumor cells. Here, we show the utility of a human induced pluripotent stem cell (iPSC) based model of MB to find therapeutic vulnerabilities.
Methods: Human iPSC derived from a healthy adult were differentiated towards NESC and transduced with empty vector (EV), MYCN, CMYC or MYCL. We analyzed how each MYC protein influences the molecular profile (RNAseq and methylation), proliferation in vitro (EdU assay), and transformation in vivo (orthotopic implantation into the hindbrain of immunocompromised mice) compared to EV in NESC. Whole genome CRISPR interference (CRISPRi) screen was performed on MYCN and CMYC NESC-derived tumors and EV NESC. Viability in response to 72 hours of inhibitor treatment was analyzed qualitatively by transmitted light images of cells and quantitatively by LIVE/DEAD stain followed by flow cytometry.
Results: MYCN and CMYC induced a higher percentage of NESC in S-phase (46%, 40%) compared to MYCL (35%) and EV (31%). Molecularly, MYCN and CMYC NESC had similar transcriptomic and methylation profiles compared to MYCL and EV. Gene ontology revealed the most enriched pathway in NESC with either MYCN or CMYC, but not MYCL, was mitochondria gene expression. In vivo, MYCN and CMYC were both 100% penetrant by day 98 while MYCL was only 30% penetrant and EV did not develop tumors. CRISPRi screen demonstrated MYCN and CMYC NESC-derived tumors, but not EV NESC, were dependent on subunits of Complex I, III, IV and V of the electron transport chain (ETC) in the mitochondria. In support of this, treatment with two different inhibitors of Complex I (Metformin, Mito-HNK) showed stronger toxicity against both MYC NESC-derived tumors than EV NESC. Strikingly, treatment with Mito-HNK resulted in higher cell death in CMYC-expressing MB cell lines D283 (30%), D425 (83%) and MB004 (82%) compared to healthy NESC (<10%).
Conclusion: MYCN and CMYC induce molecular changes distinct from MYCL in NESC that lead to upregulation of mitochondria gene expression, faster growth and transformation. Both MYCN and CMYC driven MB tumors are more dependent on the ETC for viability than non-tumor NESC suggesting MYC tumors could be specifically targeted by disrupting their metabolism. Thus, we show the combination of human iPSC-based model of MB and CRISPR screening can uncover vulnerabilities in MB cell lines.

#3942 3D models reveal novel drug synergies and mevalonate pathway activation in Ewing sarcoma.

Branka Radic Sarikas[1], Marica Markovic[2], Mathias Ilg[1], Caterina Sturtzel[1], Martha Zylka[1], Didier Surdez[3], Martin Metzelder[4], Martin Distel[1], Aleksandr Ovsianikov[2], Florian Halbritter[1], Heinrich Kovar[1]

[1]St. Anna Children's Cancer Research Institute, Vienna, Austria,[2]Institute of Materials Science and Technology, Technical University of Vienna, Vienna, Austria,[3]ZInserm U830, Equipe Labellise INCC, PSL Universite, Siredo Oncology Centre, Institut Curie, Paris, France, Paris, France,[4]Department of Pediatric Surgery, Medical University of Vienna, Vienna, Austria

Ewing sarcoma (ES) is a rare but aggressive cancer, with survival rates of only about 30% in metastatic cases. Its rarity and severity highlight the need for focused research, yet progress is limited by scarce patient samples, few animal models, and minimal preclinical testing platforms. Conventional 2D cell cultures often fail to replicate primary tumor gene expression and drug sensitivity, while patient-derived xenografts (PDXs), though more accurate, require extensive resources and tend to lose tumor-specific traits when cultured in 2D. We established and standardized a spheroid culture system for ES and developed a semi-automated drug-screening platform for tumor spheroids. This platform enables drug testing in a 3D environment, offering more relevant insights than 2D models and helping to define drug concentration ranges for testing on tumoroids and patient samples. Bulk RNA sequencing revealed significant transcriptional differences between 3D cell line models (including spheroids and encapsulated 3D constructs) and traditional 2D cultures. The 3D constructs were created using extrusion bioprinting, which ensures uniform cell distribution and high density. Notably, the mevalonate pathway was activated in all 3D models used. ES is among the tumors with the highest expression of mevalonate pathway genes, making its activation in 3D models a promising replication of *in vivo* tumor characteristics. This activation underscores the pathway's importance in ES metabolism and its potential as a therapeutic target. Building on these findings, we validated the encapsulated 3D models using PDX-derived cells, confirming consistent mevalonate pathway upregulation across these constructs. These models further demonstrated the spheroid platform's capacity to accurately predict effective drug concentration ranges, which proved optimal for testing PDX-derived cells and may extend to other primary materials. Next, we conducted synergy tests using concentration matrices in both spheroid and 2D cultures, systematically exploring different drug concentration combinations. Based on transcriptional data and earlier studies, we opted to test interactions between mevalonate pathway inhibitors (statins), and apoptosis inhibitors, specifically BCL2 family inhibitors. Remarkably, we observed a strong synergy between BCL-xL inhibitors and statins, but only within the physiologically relevant 3D environments. These findings underscore the value of 3D models as potent tools for uncovering therapeutic combinations missed by conventional approaches. By more accurately reflecting key aspects of tumor behavior, these models enhance the reliability of drug response predictions and enable targeted treatment strategies. Advanced 3D models could accelerate the translation of preclinical discoveries into clinical treatments for Ewing sarcoma, offering new possibilities for tackling this aggressive cancer.

#3943 SFPQ-TFE3 gene fusion reciprocally regulates mTORC1 activity and induces lineage plasticity in a novel mouse model of renal tumorigenesis.

Kaushal V. Asrani[1], Adrianna Amaral[1], Juhyung Woo[1], Sanaz Abadchi[1], Thiago Vidotto[1], Kewen Feng[1], Hans B. Liu[1], Mithila Kasbe[1], Masaya Baba[2], Yuichi Oike[2], Patricia Outeda[3], Terry Watnick[3], Avi Z. Rosenberg[1], Laura S. Schmidt[4], Marston Linehan[4], Pedram Argani[1], Tamara L. Lotan[1]

[1]Johns Hopkins University School of Medicine, Baltimore, MD,[2]Kumamoto University, Kumamoto, Japan,[3]University of Maryland School of Medicine, Baltimore, MD,[4]National Cancer Institute, National Institutes of Health, Bethesda, MD

The MiT/TFE family gene fusion proteins, such as SFPQ-TFE3, drive both epithelial (eg, translocation renal cell carcinoma, tRCC) and mesenchymal (eg, perivascular epithelioid cell tumor, PEComa) neoplasms with aggressive behavior. However, no prior mouse models for SFPQ-TFE3-related tumors exist and the mechanisms of lineage plasticity induced by this fusion remain unclear. Here, we demonstrate that constitutive murine renal expression of human SFPQ-TFE3 using Ksp Cadherin-Cre as a driver disrupts kidney development leading to early neonatal renal failure and death. In contrast, post-natal induction of SFPQ-TFE3 in renal tubular epithelial cells using Pax8 ERT-Cre induces infiltrative epithelioid tumors, which morphologically and transcriptionally resemble human PEComas. As seen in MiT/TFE fusion-driven human tumors, SFPQ-TFE3 expression is accompanied by the strong induction of mTORC1 signaling, which is partially amino acid-sensitive and dependent on increased SFPQ-TFE3-mediated RRAGC/D transcription. Remarkably, SFPQ-TFE3 expression is sufficient to induce lineage plasticity in renal tubular epithelial cells, with rapid down-regulation of the critical PAX2/PAX8 nephric lineage factors and tubular epithelial markers, and concomitant up-regulation of PEComa differentiation markers in transgenic mice, human cell line models and human tRCC. Pharmacologic or genetic inhibition of mTOR signaling downregulates expression of the SFPQ-TFE3 fusion protein and rescues nephric lineage marker expression and transcriptional activity in vitro. These data provide evidence of a potential epithelial cell-of-origin for TFE3-driven PEComas and highlight a reciprocal role for SFPQ-TFE3 and mTOR in driving lineage plasticity in the kidney, expanding our understanding of the pathogenesis of MiT/TFE-driven tumors.

#3944 Deciphering retinoblastoma indolence-malignancy transition with *RB1* knockout cone reporter retinal organoids.

Jinlun Bai[1], David S. Koos[1], Kayla Stepanian[1], Kevin Stachelek[1], Bhavana Bhat[1], Scott E. Fraser[2], Rex A. Moats[1], David Cobrinik[1]

[1]Children's Hospital Los Angeles, Los Angeles, CA,[2]Viterbi School of Engineering, University of Southern California, Los Angeles, CA

Purpose: This study aims to elucidate the molecular mechanisms driving the transition from pre-malignant lesions to malignancy using a human retinoblastoma retinal organoid (RBRO) model that faithfully recapitulates the cell-of-origin and multi-step retinoblastoma genesis.

Background: Retinoblastoma originates from maturing cone photoreceptor precursors with biallelic *RB1* inactivation. In explanted fetal retinae, pRB-depleted post-mitotic cone precursors proliferate, followed by a 3-5 month premalignant indolence phase before retinoblastoma-like masses emerge at tissue ages mirroring in vivo disease. Research on this transition is limited by fetal tissue availability, but CRISPR engineered retinal organoids (ROs) provide a promising alternative. This study introduces a robust organoid platform to model and investigate the retinoblastoma indolence-malignancy transition.

Methods: We generated cone-reporter iPSC lines through CRISPR knock-in of EGFP-P2A at the *GNAT2* locus. A second round of CRISPR editing produced homozygous *RB1* knockout. Chimeric $RB1^{+/+}$ ROs and $RB1^{-/-}$ RBROs were generated from edited iPSCs mixed with unedited parental iPSCs. ROs and RBROs were embedded in hydrogel and live-imaged episodically to track EGFP+ cone proliferation dynamics. Deep full-length scRNA-seq was carried out on FACS isolated EGFP+ $RB1^{+/+}$ and $RB1^{-/-}$ cones at key developmental and tumorigenesis stages, with results validated by immunofluorescent staining.

Results: In *RB1* WT ROs, EGFP specifically, robustly and innocuously labeled immature and mature cones. In RBROs, bi-weekly live confocal imaging revealed initial EGFP+ $RB1^{-/-}$ cone proliferation followed by a pre-malignant indolence phase starting at ~d150. The majority of the initially proliferating cones were Ki67-negative with some adopting mature cone morphology. Nascent retinoblastoma-like foci co-expressing EGFP, cone markers, and Ki67 formed after ~d280, a tissue age that equates to the first post-natal month when early retinoblastomas typically emerge. Single cell transcriptomics of $RB1^{-/-}$ cones from multiple tumorigenesis stages showed distinct molecular signatures of proliferation, differentiation and stress cell states and suggest a role of p53 pathway in indolence entry and escape. Immunofluorescent staining suggested post-transcriptional silencing of p14ARF underlying indolence escape.

Conclusion: We established a human retinoblastoma organoid model that recapitulates the cell-of-origin and timing of multi-step retinoblastomagenesis. This model revealed distinct molecular signatures at each tumorigenesis stage and highlighted altered expression of a p53 pathway regulator during the retinoblastoma indolence-malignancy transition.

#3946 VGLL2::NCOA2 structure-function analysis reveals domain requirements for fusion-driven infantile rhabdomyosarcoma.

C. S. Sankhe[1], D. Calderon[1], C. Taslim[1], M. Sammons[1], L. Ryan[1], A. C. Gross[1], O. Delattre[2], R. D. Roberts[1], G. C. Kendall[1];
[1]Center for Childhood Cancer Research, The Abigail Wexner Research Institute, Nationwide Children's Hospital, Columbus, OH, [2]Institut Curie, SIREDO Pediatric Center, Paris, France

Rhabdomyosarcoma (RMS) is the most common pediatric soft-tissue sarcoma and is associated with impaired skeletal muscle differentiation. RMS has significant diagnostic and therapeutic challenges, and the presence of fusion oncogenes contributes to the aggressiveness of the disease. There exists lack of mechanistic understanding of fusion-oncogene driven tumorigenesis. As a result, more efforts need to be directed towards defining fusion-oncogene biology to identify novel therapeutic strategies and improve clinical outcomes in patients. A new family of fusion-oncogenes, including VGLL2::NCOA2, has been identified in the infantile RMS subtype. Normally, vestigial-like family member 2 (VGLL2) and nuclear receptor coactivator 2 (NCOA2) independently regulate skeletal muscle differentiation as transcriptional co-activators. We found the VGLL2::NCOA2 fusion is sufficient for tumorigenesis in multiple systems. Here, we elucidate whether protein domains contribute to this activity. VGLL2::NCOA2 retains the vestigial (TDU) domain of VGLL2 and activating domains (AD1 and AD2) of NCOA2. We hypothesize that TDU, AD1 and AD2 domains of VGLL2::NCOA2 contribute to fusion-driven tumorigenesis. We implemented our C2C12 mouse myoblast cell culture model and a structure-function analysis of VGLL2::NCOA2 to define domain requirements for transformation. We transfected mouse myoblast cells with the full-length human VGLL2::NCOA2 fusion construct and truncated mutant constructs containing deletions of TDU, AD1, and AD2 domains. Using biochemical approaches, we found higher nuclear localization of VGLL2::NCOA2 in cells transfected with the full-length fusion and AD2 domain deleted mutant fusion; however, deletion of either the TDU and AD1 domain resulted in decreased nuclear localization. Furthermore, in testing the oncogenic potential of transfected cells via soft agar colony formation assay, we found higher number of colonies in cells transfected with the full-length fusion and the AD2 domain deleted construct. In the mutants with the TDU and AD1 domains deleted, there is a significant reduction in colony formation. We complemented our cell culture strategies with a mouse allograft approach. Compared to the full-length VGLL2::NCOA2 fusion, mice allografted with myoblast cells containing the VGLL2::NCOA2 deleted TDU and AD1 domains form significantly delayed tumors, with onset beginning 40 days after the full-length fusion. Overall, our results suggest that TDU and AD1 domains are required to drive oncogenic mechanisms. Targeting these domains will facilitate identification of novel molecular targets that can suppress VGLL2::NCOA2 activity, and potentially be used as therapeutic opportunities.

#3947 Defining VGLL2::NCOA2 and ARF6 cooperation in infantile rhabdomyosarcoma.

Delia Calderon[1], Chinmay Sankhe[1], Cenny Taslim[1], Olivier Delattre[2], Genevieve Kendall[1]

[1]Nationwide Children's Hospital, Columbus, OH,[2]Institut Curie, Paris, France

Rhabdomyosarcoma (RMS) is most common pediatric soft tissue sarcoma, is aggressive, and has characteristics of arrested skeletal muscle differentiation. Infantile RMS, a subtype of RMS, is genetically driven by a family of fusion-oncogenes involving key proteins essential for skeletal muscle development. One example is the fusion between transcriptional coactivators, VGLL2 and NCOA2. Recent VGLL2::NCOA2-driven RMS cases have shown that this fusion-oncogene can be lethal; however, there is a lack of significant knowledge surrounding the biological mechanisms of the fusion and an unmet need of effective targeted therapies. To assess VGLL2::NCOA2 tumorigenic mechanisms and therapeutic vulnerabilities, we generated two new VGLL2::NCOA2 driven tumor models, a C2C12 mouse allograft and transgenic zebrafish model. Both of these models histologically and transcriptionally recapitulate the human disease. Shared transcriptional features between patient data, mouse allograft, and transgenic zebrafish tumors revealed suppression of skeletal muscle specific genes and either reactivation or inappropriate developmental persistence of the small GTPase, ADP ribosylation factor 6 (ARF6). ARF6 is important for myogenic development and was significantly overexpressed across all cross-species tumors and is being pursued as a therapeutic opportunity in other cancer contexts. Knockout of *Arf6* in our C2C12-VGLL2::NCOA2 cell culture model suppressed VGLL2::NCOA2 colony formation and restored the expression levels of critical muscle differentiation specific genes. To define the role of ARF6 *in vivo,* we used CRISPR/Cas9 to generate a zebrafish arf6b germline knockout. Although arf6 is duplicated in the fish, the Arf6b homozygous null fish have a significant reduction in Arf6 protein levels and are viable as adults and fertile. We then injected wildtype and arf6b knockout fish with mosaic human VGLL2::NCOA2 to determine genetic cooperation in the disease. We found arf6b knockout fish still developed VGLL2::NCOA2 tumors; however, transcriptional analysis showed that these tumors downregulated VGLL2::NCOA2 expression and were more differentiated with a concomitant upregulation of skeletal muscle specific pathways. Given that infantile RMS displays features of arrested skeletal muscle differentiation, these results highlight the contribution of ARF6 with VGLL2::NCOA2 for oncogenesis. Our findings are consistent with our data that C2C12-VGLL2::NCOA2 cell models are unable to terminally differentiate, but that removing Arf6 ameliorates that suppression. We are also performing mouse allograft studies, to continue to delineate the *in vivo* oncogenic mechanisms of ARF6 cooperation in infantile RMS. We will assess tumor morphology and genetic features, and tumor incidence. Overall, our findings underscore the cooperative role of ARF6 in VGLL2::NCOA2 driven tumor highlighting its potential as a therapeutic target.

#3948 Enhancing immune checkpoint delivery to diffuse midline glioma (DMG) using focused ultrasound.

Amelia Claxton[1], Paul Cressey[1], David Thurston[1], Esther Hulleman[2], Antonios Pouliopoulos[3], Darren Hargrave[4], Maya Thanou[1]

[1]Institute of Pharmaceutical Sciences, King's College London, London, United Kingdom,[2]Pediatric Oncology, Princes Maxima Center, Utrecht, Netherlands,[3]Biomedical Engineering and Imaging Sciences, King's College London, London, United Kingdom,[4]Developmental Biology and Cancer Department, University College London, London, United Kingdom

Brain tumors, such as diffuse midline glioma (DMG), remain the leading cause of cancer-related death in pediatric oncology due to their universal fatality and limited treatment options. Treatment of DMG is hindered by the blood-brain barrier (BBB) presenting a roadblock in the clinical translation of therapies, particularly for larger therapeutic agents such as antibodies (e.g., immune checkpoint inhibitors). Focused ultrasound (FUS) and cavitating microbubbles provide a solution to this by transiently disrupting the BBB in a controlled and safe manner, allowing significantly enhanced drug delivery to a brain tumor whilst avoiding healthy tissue.

In this study, we investigated the use of immune checkpoint inhibitors in in vitro and in vivo (orthotopic murine) DMG models. An in vitro transwell model in combination with FUS was used to determine if immune checkpoint inhibitors can pass through an endothelial layer (BENd3) following FUS blood-brain barrier opening (BBBO) at therapeutic concentrations, using fluorescence imaging and flow cytometry. We further tested our immune checkpoint inhibitors in an in vivo orthotopic murine DMG model (mDMG-3.3). Uptake enhancement following FUS was determined using fluorescence imaging.

The results demonstrated a significant increase in the delivery of antibodies across the BBB following FUS in vitro. We also showed that FUS BBBO had no significant effect on endothelial cell viability. FUS-induced BBBO was performed in tumor-bearing mice using systemically administered SonoVue microbubbles (2 mL/kg), and FUS (peak-negative pressure = 320 kPa, cycle number = 500, pulse repetition frequency (Hz) = 5, number of pulses = 600). T1-weighted MRI was used immediately after FUS-induced BBB opening to confirm that the BBB had opened. Real-time passive cavitation detection was also used to record the harmonic signals of the cavitating microbubbles to control BBB opening, and for post-experiment analysis of microbubble behavior. Immediately following BBBO, mice were dosed with fluorescently labelled antibody (10 mg/kg) i.v., and then FUS BBBO was shown to cause a significant enhancement in uptake of the labelled antibody into the sonicated region.

These results suggest that FUS-BBBO is a suitable technology to safely enhance the uptake of immune checkpoint inhibitors into DMG tumors. Future work will include evaluation of the survival benefits of immune checkpoint inhibitors in our mouse DMG models.

#3950 Novel scaffold free method to establish 3D spheroid and 2D adherent primary neuroblastoma cell lines with adrenergic and mesenchymal-like phenotypes.

Min Huang, Robbie G. Majzner, Nathan Chiang, Alejandro Solis, Bill Chiu, Serena Tan, Emon Nasajpour, Kathleen M. Sakamoto, Norman J. Lacayo, Claudia K. Petritsch, Sheri L. Spunt, Raya Saab

Pediatrics, Stanford University, Stanford, CA

Neuroblastoma (NB) is the most frequent extracranial childhood tumor. The majority of NB tumors arise in the adrenal gland with bone marrow (BM) metastases present in 70.5% of Stage IV disease. A recent study using 23 most commonly used two-dimensional (2D) adherent NB cell lines found that expression of GD2, a target for immunotherapy, is significantly correlated with adrenergic (ADRN) and mesenchymal (MES) cell lineages through epigenetic regulation of ganglioside synthesis enzyme ST8SIA1. We aimed to establish primary NB patient cell line from adrenal NB or BM metastasis to determine whether ADRN and MES lineage specific phenotypes exist in primary NB patient cells and are also associated with distinct GD2 and ST8SIA1 expression. Using our *in vitro* primary cell culture conditions, all NB cells derived from four individual patients' BM metastases spontaneously assembled into three-dimensional (3D) spheroids in scaffold-free regular culture plates with a predominant GD2 high ADRN cell type as determined by flow cytometric and immunofluorescent analysis of ADRN marker genes. Cells maintained self-assembling ability for multiple passages (up to 30) within 5-8 months of prolonged *in vitro* cultures at a sustainable growth rate. In contrast to the BM-derived NB cells, a majority of the adrenal tumor-derived NB cells became 2D adherent within 7-10 days and displayed marked changes in immuno-phenotypes after several weeks, shifting from predominantly GD2-high to a mixture of high and low GD2 expression. Forcing the adherent NB cells with mixed GD2 level into suspension cultures using Corning's 3D Ultra-Low Attachment (ULA) plate resulted in a marked enrichment of NB spheroids with GD2-high, which regrew as 2D-adherent cells after re-seeding into regular culture plate, and retained the GD2-high and ST8SIA1-high ADRN phenotype for several months. In contrast, the NB adherent cells cultured in parallel without ULA-selection showed predominant GD2-low, ST8SIA1-low and vimentin (VIM)-high MES-like phenotype as characterized by GD2 flow and Real-time quantitative PCR of ADRN and MES marker gene expression. Our novel primary cell culture conditions allows long term *in vitro* spheroid growth of patient-derived NB cells from BM in regular culture plate, which is scaffold-free, faster, less expensive, less labor intensive, and mimics the *in vivo* NB cell clusters or aggregates seen in NB patient's BM aspirates with NB infiltration. Our culture conditions in combination with the 3D ULA-selection strategy enabled us to establish paired ADRN and MES-like NB sublines from two primary NB tumors derived from adrenal and BM sites. These patient-matched, paired NB cell lines in ADRN and MES-like cell states will be useful to study NB lineage specific biology and implications of cell states on an individual patient's response to targeted immunotherapy.

#3951 AKT inhibitor capivasertib combines with dexamethasone and venetoclax to enhance anti-tumor responses in the preclinical models of childhood acute lymphoblastic leukemia.

Brandon Willis, Nadezda Bryan, Justine Roderick-Richardson, Alan Rosen, Lori Chan, Simon T. Barry

AstraZeneca, Waltham, MA

Acute lymphoblastic leukemia (ALL) is the most common blood cancer of children and the most frequent cause of cancer related death before 20 years of age. Although the survival rate of pediatric patients with ALL is approximately 90% due to innovative chemotherapy regimens, finding treatment options for patients with relapsed/refractory ALL continues to be challenging. The PI3K/AKT/mTOR axis is often constitutively activated in relapse/refractory ALL. For example, loss of the tumor suppressor PTEN leads to hyperactivation of AKT signaling. Despite the importance of PI3K-AKT signaling in ALL, clinically effective treatment strategies to target PI3K-AKT signaling have had limited success. Inhibiting AKT signaling with capivasertib, a first in class pan AKT-inhibitor recently approved for the treatment of ER+ breast cancer, exhibits monotherapy anti-proliferative activity in pediatric T-ALL and B-ALL cell lines. However, only partial anti-proliferative activity was observed from monotherapy capivasertib treatment. To address if the activity of capivasertib in ALL could be improved by combining with other agents, we explored whether addition of the glucocorticoid, dexamethasone, and the Bcl-2 inhibitor, venetoclax could provide better therapeutic benefit in preclinical models of ALL. We conducted a capivasertib centric *in vitro* combination screen across a panel ALL cell lines. In contrast to monotherapy activities, the combination of capivasertib with dexamethasone and venetoclax showed greater cell killing in 4/5 B-ALL and 3/3 T-ALL cell lines. Interestingly, the combination enhanced antitumoral activity in most ALL cell lines regardless of the presence or absence known PI3K/AKT mutational pathway drivers. Importantly, capivasertib enhanced response to dexamethasone in a subset glucocorticoid refractory ALL cell line models that were insensitive to monotherapy glucocorticoid treatment. To further investigate whether the combination *in vitro* activity can translate *in vivo,* combinations of capivasertib, venetoclax and dexamethasone were evaluated in ALL CDX models and a disseminated pediatric PDX of T-ALL harboring known drivers of AKT hyperactivation. Using a clinically relevant dose and schedule of capivasertib, treatment combination groups led to 60% and 70% TGI in the MOLT4 and Karpas-231 xenograft models respectively. Whereas a 20% increase in survival relative to the vehicle group was observed in a disseminated T-ALL PDX model. Taken together, our results provide preclinical evidence for the rational combination of capivasertib, venetoclax and dexamethasone in the treatment of pediatric patients with relapsed/refractory ALL.

#3952 Noncoding elements within *MYCN* mRNA are autonomous drivers of oncogenesis in neuroblastoma.

Vishwaben Patel[1], Lorraine-Rana E. Benhamou[1], Dr. John T. Powers[2]

[1]The University of Texas At Austin, Austin, TX,[2]Department of Pediatrics, Dell Medical School at The University of Texas at Austin, Austin, TX

Neuroblastoma (NB) is a highly metastatic pediatric cancer originating from neural crest cells. Progression is frequently driven by *MYCN* amplified (*MA*) disease, a marker of poor prognosis presented in approximately 20% of cases. While *MA* NB is associated with increased *MYCN* protein expression, recent results suggest that non-coding RNA (ncRNA) elements within the *MYCN* mRNA independently contribute to its oncogenic potential. The *let-7* microRNA (miRNA) family, which targets the 3′ UTR of *MYCN*, is sequestered by *MYCN* mRNA itself in *MA* disease, thus impairing its function and establishing that ncRNA elements can contribute to disease pathology. To further explore the role of these ncRNA elements, we engineered cells expressing either *MYCN-ORF* (MYCN protein-coding region only), *MYCN-GL* (full-length *MYCN* mRNA from the intact genetic locus), and *Null-GL* (where *EGFP* replaces MYCN protein coding sequence but retains all ncRNA elements). *In vitro* growth analyses showed that *MYCN-GL*-expressing cells exhibited the most robust growth, followed by *Null-GL* and *MYCN-ORF*. *In vivo*, the *Null-GL* construct induced the highest incidence and largest tumors of all constructs, followed by *MYCN-GL* and then *MYCN-ORF*. Next-generation sequencing revealed that *MYCN-GL* and *Null-GL* cells were enriched for targets of microRNAs that also target *MYCN*, including *let-7*, *miR-101*, and *miR-34a*. This suggests that the *MYCN* 3'UTR may interact with these microRNAs, thus protecting other mRNA targets. Additionally, *MYCN-GL* and *Null-GL* constructs exhibited more dynamic differential gene expression than *MYCN-ORF* and *EGFP*-expressing cells. Gene ontology analysis showed that *MYCN-GL* and *Null-GL* cells were more enriched in cancer-related pathways, such as RNA metabolism and microRNA processing. Non-coding *MYCN* mRNA elements may thus regulate broader cellular functions that enhance oncogenesis. Exon usage analysis revealed that *MYCN-GL* and *Null-GL* constructs influenced splicing patterns, with increased exon inclusion and exclusion events observed across multiple chromosomes. KEGG pathway analysis of differentially spliced genes further confirmed non-coding RNA element influence on critical oncogenic pathways, including PI3K signaling, TNF signaling, viral carcinogenesis, cell survival, proliferation, and immune evasion. Whole genome sequencing revealed significant similarities in copy number variation (CNV) patterns between *MYCN-GL* and *Null-GL* constructs. These results demonstrate that non-coding elements within *MYCN* mRNA are potent drivers of tumorigenesis, influencing microRNA interactions, splicing patterns, genetic patterning, and overall activation of oncogenic pathways. Our findings provide new insights into the oncogenic mechanisms of *MA* NB, with broad implications for the genetic contribution of noncoding RNA elements in other oncogene-driven human malignancies.

#3953 FGF8 promotes non-canonical KRAS/MAPK pathway in favorable histology Wilms tumor.

Yongdong Su, Garrett Cooper, Katie Skinner, Hong Yin, Jason Yustein, Sumin Kang, Andrew Hong

Emory University School of Medicine, Atlanta, GA

Favorable Histology Wilms tumor (FHWT) is the most common pediatric kidney cancer and those with relapsed FHWT continue to have poor prognoses. A challenge in the field of FHWT research is the lack of faithful preclinical *in vitro* models for both primary and relapsed tumors. This prevents us from understanding the biology of FHWT and introducing new targeted therapies into FHWT clinical trials. We recently established 10 FHWT cell lines, including one from a relapsed case, which faithfully recapitulated the genome and transcriptome of the primary tumor. However, challenges persist in maintaining the long-term growth of these cell lines, limiting our insight into the functional study of FHWT.

Through comparison of the transcriptome of the FHWT primary tumor samples to their respective cell lines, we identified upregulation of fibroblast growth factor 8 (FGF8) expression in primary tumors. We then performed functional genomics to study the role of FGF8 in FHWT. We found that the splice variants, FGF8b or FGF8f, are required for the maintenance of FHWT cells. Re-introducing these two splice variants using either exogenous addition or endogenous overexpression led to increased viability and passaging of FHWT cell line models by an average of 100% and 30%, respectively. Further, we found that overexpressing FGF8b or FGF8f led to increased clonogenicity and invasion *in vitro* and led to tumor formation *in vivo*. Our mechanistic studies suggest that FGF8 drives a stemness and proliferation program through a non-canonical KRAS/MAPK pathway: FGF8b and FGF8f overexpression activates MEK1/2 without activating Erk1/2 while still promoting ETV1 and ETV4, leading to increased cell proliferation. Abrogation of MAPK pathway *in vitro* using MEK inhibitors led to decreased viability in cells overexpressing FGF8b and FGF8f.

Together, our study highlights the oncogenic effect of FGF8 in FHWT and links FHWT to an unexpected non-canonical MAPK pathway.

TUMOR BIOLOGY: The Tumor Immune Interplay as a Driver of Progression
Poster Session

#3957 Surface protein heterogeneity of acute myeloid leukemia is associated with distinct CD8 T cell landscape alterations.

Justin A. Cartailler, Y. Emily Chu, Chad R. Potts, Brent Ferrell

Department of Medicine, Vanderbilt University Medical Center, Nashville, TN

Acute myeloid leukemia (AML) is a heterogenous, aggressive hematologic malignancy with a poor response to immunotherapy. We applied non-negative matrix factorization (NMF) to cytometry by time-of-flight (CyTOF) data to classify AML into subtypes based on surface protein expression. We hypothesized that the resulting protein-defined subtypes of AML correlate with alterations to the CD8 T cell landscape, providing insight into the ineffectiveness of immunotherapy in AML.

Primary healthy and AML human bone marrows were stained with two CyTOF antibody panels to identify myeloid and T cell proteins. We performed NMF on the cell-protein counts matrix of the myeloid panel to determine latent protein factors present among AML samples, identifying five factors (NMF1 to NMF5) each enriched for proteins commonly associated with distinct phenotypic states. To compare to a supervised approach, the data were also clustered using the Leiden algorithm and annotated based on similarity to healthy phenotypes resulting in ten myeloblast clusters (HSC-like, monocyte-like, etc). This revealed an association among NMF factors and clusters identified through manual annotation. For example, NMF3 was defined by a primitive pattern whereas NMF5 had a pattern associated with mature AML cells. Thus, protein-defined NMF factors showed biological differences and correlated with myeloid differentiation states, allowing us to categorize AML protein heterogeneity into its predominant protein patterns.

We then used the Leiden algorithm to cluster CD8 T cells. Differential abundance analysis showed an enrichment of effector memory T cells re-expressing CD45RA (TEMRAs) in AML along a gradient with only some samples being significantly different (sFDR < 0.1). Suspecting this range was related to AML heterogeneity, we correlated each T cell subset with the respective sample's dominant AML NMF factors. We found several associations among NMF factors and enrichments of CD8 T cell subsets compared to healthy. NMF4, composed primarily of monocyte-like and dendritic-like cells, was significantly associated with an enrichment of CD57+ TEMRAs ($p = 2.8e-05$). These showed significantly increased expression of activation markers (CD95, $p_{adj} = 5.53e-39$; CD69, $p_{adj} = 1.5e-73$) and a significant reduction in GZMB ($p_{adj} = 1.3e-62$). We also observed significant marker differences among factors: CD57+ TEMRAs associated with NMF3 significantly upregulated activation markers (HLADR, $p_{adj} = 1.7e-73$; GZMB, $p_{adj} = 9.3e-39$) compared to those associated with NMF4. There was no significant difference in the abundance of naive T cells between healthy and promonocyte-like factor NMF1 ($p = 0.81$).

These data suggest that AML phenotype may directly or indirectly influence T cell phenotypes and function, which could hold implications for immunotherapeutic use in AML.

#3958 Targeting HSP70 enhances immunotherapy efficacy in triple-negative breast cancer by modulating tumor intrinsic and immunosuppressive pathways.

Ahmet B. Caglayan[1], Fulya Koksalar Alkan[1], Hilmi K. Alkan[1], Cordarryl Jones[1], Sukran Ozdatli Kurtulus[2], Elana Ishaya[1], Mahassen Elboraii[3], Hasan Korkaya[1]

[1]Oncology, Wayne State University, Detroit, MI,[2]Pharmaceutical toxicology, University of Health Sciences, Istanbul, Turkey,[3]University of Michigan-Dearborn, Dearborn, MI

Triple-negative breast cancer (TNBC) is an aggressive subtype characterized by resistance to cytotoxic therapies and immunotherapies due to the overexpression of survival proteins such as A20 and HSP70, which sustain the epithelial-to-mesenchymal transition (EMT) and cancer stem cell (CSC) phenotypes. We previously demonstrated that TNF-α stimulation in TNBC cell lines induces A20 expression, which upregulates HSP70 and protects cells from apoptosis. Luminal cell lines (MCF7 and ZR75-1), by contrast, exhibit approximately 70% apoptosis upon TNF-α treatment, and overexpression of A20 confers resistance and promotes aggressive metastatic properties in xenograft models. In vitro studies revealed that HSP70 secretion into the conditioned media is TNF-α-dependent in the EMT6 TNBC cell line, but no secretion was observed in the 4T1 TNBC cell line. LIMBO chaperone-binding assays confirmed A20 as a potential client protein of HSP70, and inhibition of HSP70 with the specific inhibitor JG-231 suppressed A20 expression in TNF-α-treated TNBC cells, reducing EMT/CSC phenotypes. Moreover, the A20/HSP70 axis was found to attract tumor-infiltrating lymphocytes (TILs) while promoting immunosuppressive myeloid-derived suppressor cells (MDSCs), as shown by increased Ly6G-positive cells in syngeneic mouse models. In vivo experiments using the EMT6 syngeneic mouse model demonstrated that JG-231, a small molecule HSP70 inhibitor, alone did not reduce primary tumor weight or metastasis after mammary fat pad injection. However, when combined with anti-PD-L1 therapy, tumor weight was significantly reduced, accompanied by a decrease in Ly6G-positive MDSCs in the tumor microenvironment. These findings suggest that the combination of JG-231 and immune checkpoint inhibitors reprograms the immunosuppressive tumor microenvironment and enhances therapeutic efficacy. In the 4T1 syngeneic model, JG-231 in combination with anti-PD-L1 and cyclophosphamide (CTX) achieved a greater than 10-fold reduction in primary tumor growth and complete elimination of metastases, while single-agent treatments showed no significant effect. Our study highlights the role of the A20/HSP70 axis in driving resistance to immunotherapy and shaping an immunosuppressive TME. By targeting HSP70, we demonstrate dual benefits: direct suppression of tumor-intrinsic survival pathways and reprogramming of the TME to reduce MDSCs and enhance TIL activity. These findings establish a strong preclinical rationale for combining HSP70 inhibitors like JG-231 with immune checkpoint inhibitors and standard chemotherapies to achieve durable therapeutic responses in TNBC. Further investigations are warranted to evaluate the clinical potential of this combinatorial strategy in aggressive breast cancers.

#3959 GAS-Luc2 reporter cancer cell lines demonstrating superior performance to the industrial standard interferon-gamma ELISA in 2-D and 3-D ex vivo immune activation and drug screening.

Hyeyoun Chang, John Foulke, Luping Chen, Catherine McManus, Fang Tian, Zhizhan Gu

American Type Culture Collection (ATCC), Manassas, VA

Interferon-gamma (IFN-γ), a cytokine critical for activating cellular immunity and facilitating anti-tumor responses, has become a key cytokine for assessing the efficacy of cancer immunotherapy drugs. While enzyme-linked immunosorbent assay (ELISA) is widely employed for the detection of IFN-γ, it has shown significant limitations in detecting low levels of IFN-γ during early-stage immune activation. Moreover, it presents serious challenges in accurately quantifying paracrine signaling of IFN-γ secreted by immune cells within 3-D co-culture models. To address the rapidly growing demand for more effective monitoring of immune activation for cancer immunotherapy research and better assessment of immunotherapy drug candidates, we developed immune activation reporter cancer cell lines engineered with a gamma-interferon activation site (GAS)-response element positioned upstream of luciferase gene. Upon activation of IFN-γ signaling pathway, these cells express luciferase, allowing for simple detection and quantification to measure immune activation. The cell lines were selected based on a comprehensive protein profiling to ensure endogenous expression of immune checkpoint ligands, such as PD-L1, CD155, or B7-H3, for additional benefit in application in immune checkpoint research. To evaluate the system, the reporter cells were stimulated with varying concentrations of IFN-γ, treated with conditioned media from primary T cells, or co-cultured with IFN-γ-producing primary immune cells. Additionally, the reporter cells were co-cultured in 2-D or 3-D systems for the comparison with ELISA. Our data revealed that bioluminescence intensity from the reporter cells increased by approximately 100- to 250-fold in a dose dependent manner following IFN-γ stimulation. Primary T-cell conditioned media stimulation resulted in a 50- to 100-fold increase in bioluminescence intensity. In co-culture assays with primary T cells or NK cells in the presence of immune checkpoint inhibitors, the reporter cell lines exhibited a 3- to 12-fold increase in bioluminescence intensity. Notably, in both 2-D and 3-D co-culture systems, the reporter cells generated a robust bioluminescent signal even at the IFN-γ concentrations below the detection range of conventional ELISA, highlighting their superior sensitivity and versatility. These reporter cell lines can serve as a valuable standard for early-stage monitoring of immune activation and offer a convenient and highly sensitive platform for evaluating cancer immunotherapy drug candidates.

#3960 Genome-wide CRISPR screens for genes inhibiting T cell-tumor cell interactions identify complex N-glycans.

Adriaan F. H. de Groot, Zowi R. Huinen, Juan Simon Nieto, Daniel S. Peeper

The Netherlands Cancer Institute, Amsterdam, Netherlands

The efficacy of immunotherapy fundamentally depends on effective T cell-tumor cell interactions, yet the molecular determinants orchestrating this engagement are not fully understood. Here, we systematically mapped and perturbed key regulators of the T cell-tumor cell interactome using innovative cell-cell interaction assays. We performed genome-wide CRISPR screens to identify genes critically required for physical and functional interactions between T cells and tumor cells. Using TurboID proximity labeling and T cell-tumor cell conjugates, we identified the complex N-glycan biosynthesis pathway as a crucial regulator of T cell-tumor cell interactions. Recognizing the role of N-glycans in protein trafficking and surface retention, we subsequently performed cell surface proteomics and TurboID-based interactome profiling on complex N-glycan-deficient T cells, revealing increased expression of cell adhesion and co-stimulatory molecules. T cells with perturbed N-glycans show a shift towards an effector memory phenotype with elevated granzyme and integrin expression. *In vivo*, both genetic and pharmacological interventions that decrease the number of complex N-glycans on the surface of T cells enhanced intra-tumoral infiltration. Our findings provide a comprehensive functional atlas of molecules modulating T cell-tumor cell interactions and position the complex N-glycan biosynthesis pathway as a potential therapeutic target to strengthen T cell-mediated cytotoxicity in cancer immunotherapy.

#3961 CBFβ-SMMHC directly activates immune regulatory genes to establish a global immune suppression in mouse AML model.

Hui Fang[1], Xiaocui Lu[1], Chang Liu[1], Xuexiu Fang[1], Stephanie Fay Mori[1], Bo Cheng[2], John K Cowell[1], Tianxiang Hu[1]

[1]Augusta University, Augusta, GA,[2]Zhongnan Hospital of Wuhan University, Wuhan, China

A recurrent inversion involved in chromosome 16 leads to the fusion of genes CBFB and MYH11, which encodes CBFβ-SMMHC fusion protein acting as a driving factor of acute myeloid leukemia (AML). Though breakthroughs have been made in the treatment of leukemia, drug resistance and relapse post treatment are still major causes of adverse prognosis of AML patients. It's found that an immune suppressive microenvironment usually affects the treatment response of cancer patients, therefore, a better understanding of the immune microenvironment holds the promise of developing novel therapy to improve outcomes. We investigated dynamic shifts in the immune landscape as leukemia progresses in the conditional Cbfb-MYH11 knockin mouse model. Accompanying the continuous increase of cKit+ leukemia cells, all the other immune cells dramatically dropped with the progression of AML. Notably, a significant reduction occurred in the levels of immune effector T, B, and NK cells. The presence of myeloid-derived suppressor cells (MDSCs) also decreased during leukemogenesis. This immune suppression phenomenon was consistently observed in both primary leukemia induction and following leukemia cell transplantation. When co-cultured with CD4 T cells, the cKit+ leukemia cells significantly inhibited the division of T cells. Mechanistically, RNA-Seq profiling revealed a global activation of the immunosuppressive pathway genes including PDL1, Tgfβ1, and Nfkb1 in these leukemia cells. Consistently, Cbfb-MYH11 inhibitor treatment can attenuate the inhibitive effects of AML cells on T cell proliferation, due to the reduced expression of immune suppressive genes. Correlative analysis in AML patient samples also confirmed the positive correlations between the immune suppression genes and MYH11. Furthermore, flow cytometry revealed that PD-L1 was exclusively upregulated in cKit+ leukemia cells, accompanied by increased CD4 and CD8 T cell exhaustion, suggesting that the PD-L1/PD1 pathway at least partially contributes to this global immune suppression. Indeed, transplantation of the same leukemia cells into PD1 KO mice led to delayed AML progression. Taken together, our results reveal the establishment of a global immune suppression in this AML mouse model by the immune suppressive AML cells expressing CBFβ-SMMHC, and immune suppressive pathways including PD-L1/PD1 are involved, justifying a combinational treatment of immunotherapy with current "7+3" regimen for treatment of AML carrying CBFβ-SMMHC.

#3962 Pan-cancer spatial characterization of tertiary lymphoid structures.

Kyung Serk Cho[1], Yunhe Liu[1], Guangsheng Pei[1], Jianfeng Chen[1], Tieling Zhou[1], Khalida M. Wani[1], Alejandra G. Serrano[1], Sharia D. Hernandez[1], Akshaya S. Jadhav[1], Daiwei Zhang[2], Hannah C. Beird[1], Jiahui Jiang[1], Diana Shamsutdinova[1], Xinmiao Yan[1], Yibo Dai[1], Enyu Dai[1], Ashley L. Wilson[1], Anne Waldrep[1], Fuduan Peng[1], Wei Lu[1], Yang Liu[1], Jimin Min[1], Paola A. Guerrero[1], Idania C. L. Julio[1], Tong Yin[3], Shin Hsu Shi[3], Sabitha Prabhakaran[1], Scott E. Kopetz[1], Dipen M. Maru[1], Alexander Lazar[1], Amir A. Jazaeri[1], Michael Green[1], Mingyao Li[4], Catherine Sautes-Fridman[5], Wolf H. Fridman[5], Andrew Futreal[1], Luisa M. S. Soto[1], Humam Kadara[1], Anirban Maitra[1], Suet Yi Leung[3], Jianjun Gao[1], Linghua Wang[1]

[1]UT MD Anderson Cancer Center, Houston, TX,[2]The University of North Carolina at Chapel Hill, Chapel Hill, NC,[3]The University of Hong Kong, Hong Kong, Hong Kong,[4]University of Pennsylvania, Philadelphia, PA,[5]University of Paris, Paris, France

Tertiary lymphoid structures (TLS) are frequently observed in various solid tumors, where they play a pivotal role in regulating anti-tumor immune responses. Despite their importance, our understanding of TLS and their interactions with cancer cells within complex tissue environments remains limited. Recent advances in spatial transcriptomics (ST) technology offer new possibilities for investigating the spatial and phenotypic heterogeneity of TLS.

We developed TLSphenotyper, a computational framework for automatic TLS segmentation, labeling, extraction, and comprehensive profiling of TLS using spot-level ST data. It enables reproducible and efficient large-scale analysis of TLS. To accurately map TLS and characterize their cellular composition, we created a robust TLS-specific gene signature and enhanced the resolution of spot-level ST data to near-single-cell resolution. TLSphenotyper computes cell type-specific gene signature scores to generate a detailed cell type probability map across the tissue section. This map facilitates segmentation of key cellular components within TLS, enabling precise spatial mapping and colocalization analysis of major cell types and their transcriptomic states. Additionally, TLSphenotyper automatically analyzes and classifies TLSs based on their maturation stages, offering insights into their phenotypic and functional heterogeneity. Its accuracy and precision were validated using single-cell ST data generated from adjacent tissue sections. Further benchmarking against established deconvolution methods demonstrated superior accuracy and granularity in spatial cell type inference, cell organization and co-location analysis within TLS regions.

To further investigate TLS and their interactions with cancer cells in a pan-cancer setting, we curated a comprehensive dataset of publicly available and in-house spot-level ST data, encompassing 340 tissue sections from 12 distinct cancer types. This represents the largest collection of ST data on human cancer assembled to date. Using TLSphenotyper, we identified and characterized 695 TLSs across 340 tissues. Our analysis revealed substantial variation in TLS presence, size, location, density, cellular composition, and maturation stage across cancer types. TLSs at different maturation stages exhibited distinct transcriptomic profiles. Notably, cancer cells in proximity to TLS demonstrated significantly different activity across key signaling pathways compared to those farther away, suggesting that TLS interaction can shape cancer cell expression profiles and cellular states. Additionally, patients classified based on the maturation stages of TLS demonstrated distinct survival outcomes. Together, this study provides novel insights into the spatial dynamics and phenotypic heterogeneity of TLS, identifies potential targets for modulating TLS, and establishes a valuable resource for the research community.

#3963 Tumor-intrinsic impact of PIP4K2C degradation drives preclinical efficacy in colorectal cancer.

Eva d'Hennezel[1], Morgan O'Shea[1], Guosen Ye[1], Kristin Brown[1], Andrew Good[1], Melvyn Chow[1], Julie Arnold[1], Lilly Ein[1], Vincent Giacalone[1], Robert Prenovitz[1], Sebastian Hayes[1], Sydney Alnemy[1], Erica Dube[1], oluwatimileyin A. Adigun[1], Kirti Khardenavis[2], Alyssa Martin[2], Michael Perricone[2], Krista Goodman[1], Emily Corse[1], Catherine Sabatos-Peyton[1]

[1]Larkspur Biosciences, Inc., Cambridge, MA,[2]Xsphera Biosciences, Boston, MA

Phosphatidylinositol 5-phosphate 4-kinase, type II, gamma (PIP4K2C) is a lipid kinase with critical roles in vesicular trafficking, autophagy-dependent catabolism and modulation of the immune system. PIP4K2 kinases regulate membrane localization and clustering of $PI(4,5)P_2$ and thus govern multiple aspects of receptor trafficking and signaling at the plasma and vesicular membranes, independently of their catalytic function (Wang et al.). Cancer cells maintain their fitness by developing survival adaptations that foster escape from intrinsic and extrinsic mechanisms of cell death in conditions of stress. Through its ability to modulate membrane lipid dynamics, PIP4K2C plays an important role in cancer fitness and has the potential to modulate key signaling pathways and cellular responses to exogenous and endogenous stress, in turn increasing the immunogenicity of the tumor. As germline PIP4K2C knockout mice develop subclinical autoinflammatory signs with age (Shim et al.), systemic modulation of PIP4K2C both enhances anti-tumor immunity and disrupts cancer cell fitness, both impacts synergizing to reduce tumor growth in cancer patients. Achieving potent targeting of PIP4K2C while sparing other family members or other lipid kinases has so far precluded the exploration of its potential as a therapeutic target. LRK-A was developed as a bifunctional degrader of PIP4K2C causing rapid and exclusive degradation of PIP4K2C in vivo and in vitro. Degradation of PIP4K2C in tumors leads to modulation of key pro-tumorigenic pathways and upregulation of immune activating cytokines, chemokines, and stress-associated receptors. Subsequently, tumors are more susceptible to immune cell killing by NK cells and phagocytosis by dendritic cells. Concurrently, PIP4K2C degradation in phagocytes increases PIP2 production and mediates an increased uptake of dead tumor material, enabling a synergistic, dose-dependent amplification of the immune response to cancer. Dosing of LRK-A as a single agent significantly reduces tumor growth including full regressions in a mouse syngeneic model of colorectal cancer. We further demonstrate that treatment of human primary spheroids derived from tumor resections of MSS-CRC patients leads to increased tumor cytotoxicity and upregulation of key immune activation markers. Collectively, our results show that PIP4K2C is a novel cancer fitness target at the critical convergence of tumor-intrinsic and tumor-immune pathways, and which can be specifically and effectively targeted in vivo using a bifunctional degrader. The reduced tumor growth we observed both in vivo in mice and ex vivo in human MSS-CRC patient samples upon targeting PIP4K2C alone suggests that PIP4K2C is a highly promising target for therapeutic intervention. Further explorations are ongoing to enable the development of PIP4K2C-targeted therapeutic strategies.

Wang DG et al. *Cell Rep.* 2019; Shim H et al. *PNAS* 2016

#3964 HLA typing and its association with TMB in a pan-cancer Chinese cohort study.

Ran Shu[1], Li Bian[2], Ying Yang[1], Jian Shen[1], Xiaoyu Tuo[1], Yanxi Li[1], Shiyue Liu[1], Feilong Zhao[3], Ding Zhang[4], Zhe Yang[1]

[1]The First Affiliated Hospital of Kunming Medical University, Kunming, China,[2]The First Affiliated Hospital of Kunming Medical University, Shannxi, China,[3]3D Medicine, Shannxi, China,[4]3D Medicine, Shanghai, China

Background: Previous studies have linked HLA-I heterozygosity to immunotherapy efficacy. Given that tumor mutational burden (TMB) is a well-established biomarker for immunotherapy, this study explores the relationships among HLA-I heterozygosity, HLA-I subtypes, and TMB.
Methods: Whole exome sequencing (WES) was performed on all samples. HLA-I typing was identified using HLAscan and clustered with HLAClustRView. Mutation analysis was performed with Maftools.
Results: We analyzed 810 patients, covering over 20 tumor types, with 163 in the any-homozygous group and 647 in the all-heterozygous group. No significant differences were observed between groups in age, gender, or MSI status. HLA-I subtype landscape revealed a predominance of A02 and A11 for HLA-A, B15 and B40 for HLA-B, and C03 and C01 for HLA-C. Approximately 50% of patients exhibited heterozygosity for both HLA-B and HLA-C loci, while none were heterozygous across all three loci. Utilizing the similarity distances of HLA-I gene subtypes, we categorized patients into six distinct clusters. Each cluster displayed a preference for specific tumor types, and for particular HLA-I gene subtypes. All-heterozygous patient proportion was notably higher in clusters #6, #2, and #3, and lower in cluster 4. Gene mutation analysis revealed significant enrichment of ADSS1, USP8, and MYOCD in cluster 3, as well as MUC22 in cluster 6, whereas the other clusters did not demonstrate significant mutation enrichment. HLA clusters also demonstrates a potential association with TMB ($p = 0.069$), where cluster 6 exhibited significantly lower TMB than the other clusters. TMB was slightly lower in the all-heterozygous group ($p = 0.13$). Stratified by cancer type, TMB was generally higher in the all-heterozygous group, particularly in liver cancer ($p = 0.005$), but lower in gastric cancer ($p = 0.027$). In gastric cancer, the any-homozygous group ($n = 39$) exhibited a higher proportion of MSI-H patients ($p = 0.024$), suggesting that MSI-H may contribute to TMB discrepancies. This finding also posits that HLA homozygosity may promote MSI-H occurrence. Comparative analysis of gene mutations revealed significant enrichment of 41 genes, including CSF2RB, NUP210L, and NUP214, in the any-homozygous group, with enrichment analysis linking these genes to reproductive system development, lipid localization, and HIV infection. In liver cancer, the any-homozygous ($n = 14$) and All-heterozygous groups ($n = 70$) showed no significant differences in age, sex, or MSI status. However, MUC16 and EPPK1 mutations were significantly enriched in the all-heterozygous group, while DSPP mutations were notably enriched in the Any-homozygous group ($p = 0.034$).
Conclusion: This study illustrated HLA-I subtypes in a large Chinese pan-cancer cohort, identifying six clusters. HLA heterozygosity and clusters were associated with TMB, suggesting potential for HLA-based personalized therapy development.

#3965 Targeting mTORC2 in lung squamous cell carcinoma improves anti-tumor immunity through the PSGL-1-VISTA axis.

Verra Ngwa, Yoonha Hwang, Wenqiang Song, Deanna Edwards, Jin Chen

Vanderbilt University Medical Center, Nashville, TN

Lung squamous cell carcinoma (LUSC) represents 30% of non-small cell lung cancer (NSCLC) cases and has a high mortality rate with limited targeted therapies. Unlike lung adenocarcinoma (LUAD), which has seen advancements in targeted therapies, LUSC has not benefited from similar breakthroughs despite having similar genetic abnormalities. While immune checkpoint inhibitor therapies have shown promise in some LUSC patients, a significant portion does not respond effectively, leaving limited treatment options. The metabolic landscape of tumors plays a crucial role in shaping the efficacy of immunotherapies, especially adoptive T cell therapies. However, identifying actionable metabolic vulnerabilities involved in T cell therapy is poorly understood. In this study, we investigated vulnerabilities in NSCLC and found that a substantial number of LUSC patients have genetic alterations in the PI3K-mTORC2-AKT signaling pathway. By using CRISPR/Cas9, we showed that disrupting mTORC2 function in LUSC cells reduced glycolysis and lactate secretion, which in turn reduced tumor growth in vivo. Remarkably, the loss of mTORC2 also enhanced the activity of cytotoxic T cells within the tumors. To further understand the metabolic and immune changes associated with mTORC2 loss of function, we performed RNA sequencing comparing wild-type (WT) and mTORC2-deficient cells. Our results confirmed a link between mTORC2 and HIF-2α, while regulating PSGL-1, a ligand of the VISTA/PD-1H checkpoint receptor. Furthermore, introducing a stable, non-degradable form of HIF-2α in mTORC2-deficient tumor cells restored PSGL-1 expression in vitro and in vivo. These results suggest that the interaction between PSGL-1 and VISTA plays a role in suppressing the anti-tumor immune response in LUSC tumors. Indeed, anti-VISTA treatment inhibited the growth of LUSC WT tumors in vivo, suggesting that mTORC2 plays a role in regulating PSGL-1 expression in the tumor microenvironment. Together, these data demonstrate that targeting mTORC2 or inhibiting VISTA checkpoint receptor may serve as a potential therapeutic strategy for improving immunotherapy in LUSC. Experiments are underway to perform an in vivo CRISPR screening to uncover metabolic genes that can modulate the immune response in lung squamous cell carcinoma (LUSC) while sensitizing this tumor subtype to T cell therapy.

#3966 Divergent immune responses in pancreatic cancer driven by KRAS mutations.

Andrew Wenger, Katherine Bacchi, Huanhuan Sun, Adrian Vega, Whitney Sisso, Erika Hissong, Lukas Dow, **Despina Siolas**

Weill Cornell Medicine, Queens, NY

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy with abysmal survival rates. The highly immunosuppressive microenvironment of PDAC poses a major barrier to effective therapy. KRAS mutations are near-ubiquitous in PDAC, and studies indicate clinical outcomes vary depending on the specific KRAS genotype. Given that tumor genotype can shape the immune cell composition of tumors, we examined the immune cell composition of pancreatic tumors with specific KRAS mutations, focusing on $KRAS^{G12D}$ mutations (representing 40% of PDAC cases which have the worst prognosis) and $KRAS^{G12R}$ mutations (which make up 20% of PDAC cases and have a better prognosis). Orthotopically implanted $Kras^{G12R/+}$;$Trp53^{KO}$ mouse pancreatic tumors have a distinct immune profile in comparison to $Kras^{G12D/+}$;$Trp53^{KO}$ tumors, characterized by an influx of intratumoral $CD3^{+}$ T cells and mature dendritic cells. Selective depletion of CD4+ T cells induces a rapid progression in KRASG12R, but not KRASG12D mouse tumors. RNA sequencing of $KRAS^{G12R}$ tumor cells revealed enrichment of Type I interferon (IFN) pathways accompanied by related chemokines, CXCL9 and CXCL10 in comparison to $KRAS^{G12D}$ tumors. Analysis of publicly available and real-world human PDAC datasets an enrichment of dendritic cells and other immune related cell pathways in $KRAS^{G12R}$ tumors. Collectively, these data link the $KRAS^{G12R}$ mutation to an unique immunogenic role.

#3967 Targeting Siglec-10 interactions enhances macrophage-mediated phagocytosis of pancreatic cancer.

P. Saini[1], G. Mirji[2], S. A. Bhat[2], R. S. Shinde[2], M. Abdel-Mohsen[1];
[1]Northwestern Univ. Feinberg School of Medicine, Chicago, IL, [2]The Wistar Institute, Philadelphia, PA

Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive and immunotherapy-resistant cancers. Tumor-associated macrophages (TAMs) contribute to this resistance by failing to phagocytose cancer cells. Cancer cells evade immune detection by upregulating sialic acid on surface glycoproteins like CD24, which bind to inhibitory Siglec receptors such as Siglec-10 on TAMs, triggering signals that block phagocytosis. While blocking Siglec-10/CD24 interactions with αCD24 antibodies has improved phagocytosis in ovarian and breast cancers, the role of Siglec-10 interactions with CD24 or other glycoproteins in PDAC immune evasion, as well as targeting Siglec-10 to enhance macrophage phagocytosis in PDAC, remains unexplored.
Methods: Flow cytometry with Siglec-10 protein and affinity pulldown, followed by mass spectrometry, were used to identify glycoprotein ligands interacting with Siglec-10 on various human PDAC cell lines (PANC-1, MIAPaCa-2, Hs766T, and AsPC-1). Surface plasmon resonance (SPR) assay validated binding of these ligands to Siglec-10. To assess the role of Siglec-10 interactions in PDAC cells' evasion of macrophage-mediated phagocytosis, live-imaging immune-killing assays were performed in vitro with αCD24 or Siglec-10 blocking antibodies. Finally, the efficacy of Siglec-10 blockade in enhancing TAM-mediated killing of PDAC cells in vivo was evaluated using a subcutaneous PDAC xenograft model in immunodeficient mice injected with human monocyte-derived TAMs and treated with an isotype control or Siglec-10 blocking antibody.
Results: Most PDAC cells (~95%) bind Siglec-10, indicating sialic acid ligands. However, unlike ovarian and breast cancers, most PDAC cells do not express CD24 (~81%), suggesting PDAC cells bind Siglec-10 through other glycoproteins. Mass spectrometry identified integrin alpha-3 (ITGA3) as a novel Siglec-10 ligand on PDAC cells. ITGA3 binds strongly to Siglec-10, as assessed by SPR, and this interaction was abrogated when sialic acid was removed from ITGA3 using sialidase enzyme. Analysis of PDAC tissue single-cell datasets confirmed that PDAC cells express significantly higher ITGA3 levels than normal cells ($p<0.0001$). Our newly developed antibodies against Siglec-10, but not αCD24, significantly increased macrophage phagocytosis of PDAC cells in vitro ($p<0.05$). In vivo, Siglec-10 blockade reduced tumor size by 36% compared to controls ($p=0.0002$).
Conclusion: We identified ITGA3 as a novel Siglec-10 ligand enabling PDAC immune evasion from macrophage-mediated phagocytosis. Glycan-based immunotherapies, such as Siglec-10 blocking antibodies, are promising for enhancing phagocytosis and countering PDAC's immunosuppressive environment.

#3968 Developing T cell and organoid co-cultures derived from breast cancer patients of African descent for drug screening.

Batoul Farran[1], Moses Kamita[1], Sylvester Antwi[1], Valerie Ofori Abohah[1], Harriet Larrious-Lartey[1], Jessica Bensenhaver[1], Haythem Ali[1], Eleonor Walker[1], Foster Amponsah[2], Josephine Nsaful[3], Rose Dampson[4], Patrick Kafui Akakpo[4], Evelyn Jiagge[1]

[1]Hematology/ Oncology, Henry Ford Health System, Detroit, MI,[2]Eastern Regional Hospital, Koforidua, Ghana,[3]Korle Bu Teaching Hospital, Accra, Ghana,[4]Cape Coast Teaching Hospital, Cape Coast, Ghana

Triple negative breast cancer (TNBC) is the most aggressive form of breast cancer. It is highly prevalent in women of West Sub-Saharan African ancestry (WSSA) and African American (AA) women, resulting in poorer survival outcomes compared to European American (EA) women. Studies have shown that immune cells in the tumor microenvironment (TME) contribute to the progression of TNBC by immune suppression, the reprogramming of macrophages into pro-tumorigenic subtypes and the release of inflammatory cytokines. A previous study by our laboratory revealed that the immune processes and markers associated with TNBC are different in TNBC patients of African ancestry compared to EA patients. We have established platforms consisting of T cells co-cultured with organoids derived from patients of diverse ancestries to investigate the interactions between immune cells and TNBC cells. Organoids from Ghanaian (GH), EA and AA patients were successfully established from PDXs and primary tumors. T cells were isolated from patient PBMCs (matched tumor and T-cell), activated and tested in co-cultures with organoids, derived from TNBC tumors. Flow cytometry was performed to assess the efficacy of T cell killing. The activated T cells successfully eradicated TNBC cells at conditions that replicate in vivo T cell infiltration in TNBC, as evidenced by decreased percentages of EpCAM+ cells. Concomitantly, we observed a reduction in the percentages of CD3+ CD137+ activated T cells, suggesting that the tumor cells in the co-culture also impacted T cells. Based on our initial results, we are evaluating the effects of co-culture on distinct populations of cytotoxic T cells as well as on markers of activation and exhaustion to determine potential differences between TNBC patients of diverse ethnicities. Characterizing the differences in tumor-immune cell interactions within the TNBC tumor microenvironment between EA and AA TNBC patients will hopefully inform the development of novel therapeutic strategies for women of African descent.

#3969 Quantitative spatiotemporal analysis of patient-derived organoid-based tumor-immune microenvironment model in lung cancer for immunotherapy response prediction.

M. Lee[1], J.-E. Lee[1], D. Lee[2], D. Kim[1], D. Kang[1], C. Chung[1];

[1]Chungnam National University Hospital, Daejeon, Korea, Republic of, [2]Chungnam National University, Daejeon, Korea, Republic of

The tumor-immune microenvironment (TiME) plays a crucial role in cancer progression and treatment response, yet establishing physiologically relevant ex vivo models that accurately recapitulate TiME remains challenging. Here, we present a novel multi-modal analytical platform using lung cancer patient-derived organoids (PDOs) and autologous lymphocytes to model TiME, aiming to predict immunotherapy responses and guide treatment strategies. We developed an integrated analytical platform combining: (1) PDO-lymphocyte co-culture system using autologous cells isolated from lung cancer patients, (2) multiplexed immunofluorescence imaging for spatial analysis of immune cell distribution, (3) label-free holotomography for real-time visualization of TiME dynamics, and (4) comprehensive genomic profiling. The formation and architecture of TiME were validated through quantitative spatiotemporal immunophenotyping. Machine learning algorithms were employed to integrate multi-modal data and identify predictive signatures for treatment responses. Our integrated platform successfully reconstituted patient-specific TiME models that maintained key molecular and cellular characteristics of original tumors. Real-time holotomographic imaging revealed distinct patterns of immune cell migration and tumor-immune cell interactions. Quantitative spatiotemporal analysis demonstrated specific immune response signatures correlating with clinical outcomes. Integration of multi-modal data through machine learning algorithms showed significant predictive value for immunotherapy responses. The platform successfully identified differential drug responses in patient-specific models, correlating with clinical treatment outcomes. We established a novel multi-modal analytical platform for studying tumor-immune interactions in patient-specific models. The integration of spatial, temporal, and molecular analyses provides comprehensive insights into TiME dynamics and treatment responses. This platform shows promise as a predictive tool for immunotherapy outcomes and could serve as a personalized drug screening system, presenting new opportunities for patient stratification and therapeutic strategy optimization in lung cancer treatment.

#3970 Advancing multiple myeloma research: patient-derived 3D Organ-on-a-Chip models to reveal immunological mechanisms influencing cancer growth and resistance.

Medbh A. Dillon[1], Kevin J. Lee[1], Cole G. Phalen[1], Aishwarya Chander[1], Imran McGrath[1], Love Tätting[2], Troy R. Torgerson[1], Melinda L. Angus-Hill[1]

[1]Allen Institute for Immunology, Seattle, WA,[2]Department of Hematology & Department of Biomedical and Clinical Sciences, Linkoping University, Linkoping, Sweden

Multiple myeloma (MM) is characterized by malignant plasma cells infiltrating the bone marrow. The standard induction therapy for MM includes combining bortezomib, lenalidomide and dexamethasone (VRd); this has recently expanded to include an anti-CD38 antibody, which has improved patient outcomes. Despite the recent advances in treatment, patients with MM commonly relapse and it is a disease that remains ultimately incurable. More advanced patient-derived 3D models would allow a greater understanding of the tumor microenvironment (TME) and how it changes during treatment. This is needed for the identification and validation of new biomarkers for this disease and could aid in predicting individual treatment responses.

Longitudinal Olink proteomic data, generated by our group, from the plasma and bone marrow interstitial fluid (BMIF) of patients undergoing VRd induction therapy, allowed us to glean key insights into the changes specific to the bone marrow tumor microenvironment (TME) throughout therapy that can be validated in our 3D MM model system.

Our longitudinal data shows elevated interleukin 16 (IL-16) in the BMIF of patients at diagnosis, which decreases following induction therapy. IL-16 is a proinflammatory cytokine that acts as a chemoattractant for CD4+ T cells, myeloid cells and eosinophils, and has also been shown to be essential for proliferation of MM cell lines. Extracellular IL-16 may contribute to tumor growth or influence immune cell recruitment to the bone marrow TME. Its increased presence in the BMIF compared to the plasma suggests a localized role within the bone marrow TME.

To further investigate the role of IL-16 in the MM TME we developed a novel, patient-derived organ-on-a-chip model of multiple myeloma bone marrow. Using bone marrow mononuclear cells (BMMC) and donor matched bone marrow stromal cells (BMSC) we developed a complex TME on-chip using primary human cells. Using this model we examined the role of IL-16 and other differentially expressed growth factors on tumor development, hematopoietic stem cell differentiation and cytokine production in the TME. Preliminary data shows an increase in proliferation of malignant MM cells in the presence of IL-16, highlighting IL-16 as a potentially promising therapeutic target.

#3971 Single-cell transcriptomic insights into tumor and immune dynamics driving resistance to sitravatinib, nivolumab, and ipilimumab in advanced clear cell renal cell carcinoma.

Kai Yu[1], Ying Yuan[1], Jianfeng Chen[1], Xinmiao Yan[1], Menuka Karki[1], Fei Duan[1], Rahul Annil Sheth[1], Priya Rao[1], Kanishka Sircar[1], Amishi Yogesh Shah[1], Amado Zurita-Saavedra[1], Giannincola Genovesa[1], Min Li[1], Chih-Chen Yeh[1], Minghao Dang[1], Guangchun Han[1], Yanshuo Chu[1], Max Hallin[2], Peter Olson[2], Rui Yang[2], Daniela Slavin[2], Hirak Der-Torossian[2], Curtis Chin[2], Nizar M Tannir[1], Linghua Wang[1], Jianjun Gao[1], Pavlos Msaouel[1]

[1]UT MD Anderson Cancer Center, Houston, TX,[2]Mirati Therapeutics, San Diego, CA

The combination of sitravatinib, nivolumab, and ipilimumab offers a novel approach to enhance immunotherapy outcomes in advanced clear cell renal cell carcinoma (ccRCC). To explore the immunomodulatory and resistance mechanisms underlying this triplet therapy, we performed single-cell RNA sequencing (scRNA-seq) on tumor samples collected longitudinally from 12 patients across treatment timepoints. Transcriptomic data from 70,526 cells, including 58,331 tumor microenvironment (TME) cells, revealed dynamic shifts in cellular composition and transcriptional states during therapy. Analysis of TME cells identified eight major cell types, with notable increases in T cells and reductions in myeloid and endothelial cells post-treatment. Baseline T cell abundance correlated with improved clinical response, while fibroblast enrichment predicted resistance. Tumor cells were classified into ten transcriptionally distinct clusters, including a treatment-resistant population (C8) enriched in epithelial-mesenchymal transition (EMT), hypoxia, and glycolysis signatures. These signatures correlated with shorter progression-free survival in the trial cohort and worse survival outcomes in an external TCGA ccRCC dataset. T cell states showed a transition from cytotoxic to exhausted phenotypes in non-responders and end-of-treatment samples, suggesting a role for T cell dysfunction in resistance. Similarly, myeloid cells exhibited a shift toward immunosuppressive phenotypes, characterized by M2-like macrophages and inhibitory signaling pathways. These findings elucidate resistance mechanisms to the triplet therapy, highlighting EMT-driven tumor cell states and immunosuppressive TME dynamics as key contributors. The identification of a tumor-specific C8 signature provides a potential biomarker for predicting poor responses and tailoring therapeutic strategies. Further validation in larger cohorts is needed to optimize patient selection and therapeutic outcomes.

#3972 The role of myeloid derived suppressor cells in organ-specific breast cancer metastasis.

Batul Al-zubeidy, Edgar Gonzalez, Aaron Baugh, Matt Jacobo, Evanthia Roussos Torres

USC - University of Southern California, Los Angeles, CA

The liver and lungs are the most common sites of breast cancer metastasis, associated with poor prognosis and limited response to systemic therapies. These tumor microenvironments (TMEs) exhibit distinct cellular compositions and molecular mechanisms that promote tumor growth and metastatic progression. Visceral organs differentially recruit specific immune populations that foster protumor interactions, contributing to the poor therapeutic response to immunotherapy. To explore the role of TMEs in tumor progression, immune evasion, and metastasis, we employed an orthotopic mouse model inoculated with an ERBB2/HER2-enriched breast cancer cell line. Flow cytometry analysis of primary breast tumor, and liver and lung metastases showed an early accumulation of $CD3^+$ lymphocytes in primary tumors shortly after inoculation, which significantly declined over time. Despite this overall reduction in lymphocytes within tumor microenvironments (TMEs), the lungs consistently maintained the highest levels of lymphocyte infiltration. Conversely, myeloid-derived suppressor cells (MDSCs) progressively infiltrated both primary and metastatic sites, exerting potent immunosuppressive effects that likely facilitate tumor progression and dampen immune responses. MDSCs derived from liver and lung metastases, but not from the primary tumor, suppressed $CD8^+$ T cell proliferation and significantly inhibited the production of cytotoxic cytokines, such as granzyme A. The most pronounced suppression was observed in breast-to-liver metastases. Flow cytometry analysis revealed that MDSC-mediated immunosuppression varies by microenvironment, with predominant arginase release in the liver, inducible nitric oxide synthase (iNOS) production in the lung, and interleukin-10 secretion in the primary tumor. Additionally, in the liver TME, $CD11b^+$ $F4/80^+$ tumor-associated macrophages (TAMs) exhibited higher arginase activity than MDSCs, further enhancing the suppressive microenvironment. These immunosuppressive cytokines likely impair the function of tumor-infiltrating lymphocytes (TILs) within their respective TMEs, highlighting the need to investigate organ-specific mechanisms of immunosuppression. Notably, liver metastases harbor the lowest levels of antitumor lymphocytes and the highest suppressive MDSC activity compared to lung metastases and the primary tumor. These immune alterations are likely initiated shortly after tumor seeding, emphasizing the importance of early intervention in metastatic disease.
In summary, the tissue-specific programming of MDSCs in breast-to-liver metastases, compared to primary tumors and breast-to-lung lesions, results in reduced T cell infiltration and impaired T cell function. These findings underscore the importance of developing niche-specific immunotherapeutic strategies to address organ-specific immune suppression.

#3973 Tumor-associated macrophages protect glioblastoma cells from ferroptotic stress in a sex-biased manner by inducing ferritin-bound iron release from cancer cells.

A. Sahu, K. Palsa, G. Shenoy, B. Slagle-Webb, J. R. Connor;
Penn State College of Medicine, Hershey, PA

Glioblastoma (GBM) is a highly aggressive and difficult-to-treat brain tumor originating from astrocytes. It is also sexually dimorphic, with females having a survival advantage and responding better to standard therapy. Sex-biased differences in iron metabolism play a significant role in making GBM a sexually dimorphic disease. Iron is a critical player in tumor metabolism, as cancer cells require iron to keep dividing rapidly; however, high cellular iron can make the cells vulnerable to ferroptosis. Macrophages play a pivotal role in tumorigenesis by regulating iron homeostasis in the tumor microenvironment. Here, we examine macrophage iron homeostasis as a potential driver of sex biases in glioblastoma using bone marrow-derived macrophages (BMDM) isolated from C57BL/6 mice. Immunoblot analysis shows that male macrophages have a higher storage (FTL expression) than female macrophages. We used a stable isotope of iron (Fe57) to determine macrophage iron uptake by ICPMS and observed that male BMDMs have a higher iron uptake. Flow cytometric analysis after ferro far red staining showed that male macrophages have a higher labile iron pool. These results indicate that macrophage iron metabolism is sexually dimorphic.

Next, we used a transwell co-culture system to study how macrophages regulate the iron status of GL261 cells. Immunoblot analysis showed that GL261 cells cocultured with BMDMs had a significant reduction in cellular FTH1 in a sex-biased manner. This reduction in cellular FTH1 correlated with decreased total cellular iron. Further immunoblot analysis of the condition media revealed increased secreted FTH1 levels from GL261 cells in coculture. Fe57 loading experiments showed that this increased secreted FTH1 levels correlated with an increased iron secretion. All these results indicate that macrophages induce FTH1-bound iron release in GL261 cells in a sex-biased manner. To understand if this cellular decrease in iron alters the GBM cells' susceptibility to ferroptotic stress, we induced ferroptosis by RSL3 treatment. Upon RSL3 treatment, GL261 cells in coculture with BMDMs had significantly higher cellular viability (Cell titer glo) and significantly less lipid peroxidation (Bodipy Staining). This indicates that tumor-associated macrophages protect GBM cells from ferroptotic stress in a sex-biased manner by inducing the cellular release of iron-containing FTH1.

#3974 Flexible and comprehensive phenotyping of tumor and peripheral blood mononuclear cells in endometrial carcinoma.

Deeqa Mahamed[1], **Jessica Elmore**[2], Geneve Awong[1], Gary Impey[1]

[1]Standard BioTools, Markham, ON, Canada,[2]Standard BioTools, South San Francisco, CA

Endometrial carcinoma (EC) is the most common form of uterine cancer, with increasing prevalence in developed countries due to population aging and rising obesity rates. Though prognosis is relatively good with an 80% survival at five years after diagnosis, the leading treatment is total removal of the uterus, ovaries and fallopian tubes. Thus, identifying biomarkers that may enable more personalized medicine or immunotherapy discoveries is important, both to create more treatment options as well as to tackle aggressive EC subtypes. To that end, we developed a 58-parameter mass cytometry (CyTOF™) panel that enables deep phenotyping of both immune and non-immune cells in tumors as well as in PBMC. In total, 48 markers were measured for each cell, allowing for deep profiling of cell identity, cytokine production, cytotoxicity, exhaustion and activation. This was achieved by combining pre-optimized panels for pan-immune cell phenotyping, T cell profiling and cytokine and cytotoxic mediator detection with nine drop-in antibodies selected for their relevance in EC as biomarkers or potential immunotherapy and drug targets. Cell proliferation was assessed using IdU incorporation and Ki-67 staining, while tumor cell and tumor-infiltrating leukocytes (TILs) were further defined with EpCAM, Her2, β-catenin, CD69, CD103, PD-L1 and FoxP3. Live-cell universal barcoding with a tellurium-based 7-choose-3 strategy allowed multiplexing of samples during cell staining and acquisition. Matched PBMC and dissociated tumor cells (DTCs) from EC patients and healthy donor PBMC (n=5 each) were stimulated with PMA and ionomycin, then barcoded and pooled. Cells were then stained for surface, cytoplasmic and nuclear targets before acquisition on a CyTOF XT mass cytometer. Data was normalized and debarcoded before analysis to yield sample-specific cell frequencies and marker expression. The tumor-derived T cells from EC patients exhibited a tissue residency phenotype (CD69+CD103+/-), robust staining of immune checkpoint markers TIGIT, LAG-3, TIM-3 and OX-40 and increased staining of CD95/Fas, PD-1 and 4-1BB compared with peripheral blood T cells. Intracellular cytokines were detectable on tumor-derived immune cells despite being rare, with some subsets producing IFNγ, TNFα, IL-2 and IL-17A in response to stimulation. This high-parameter panel allows for deep phenotypic and functional profiling of circulating immune cells, tissue and tumor cells and TILs. In addition to dissociated cells from tumor resections and biopsies, this panel could be used with liquid or fine needle aspirate biopsies from tumor samples when combined with live-cell universal barcoding to multiplex low-input samples. By utilizing a flexible and modular assay designed to characterize both immune and non-immune cells, this CyTOF panel can facilitate biomarker discovery and accelerate drug development in cancer therapeutics.

#3975 Regulating dendritic cells to promote mature tertiary lymphoid structures and enhance anti-tumor immunity.

Elizabeth E. Evans[1], Crystal Mallow[1], Terrence L. Fisher[1], Elaine Gersz[1], Holm Bussler[1], Maria Scrivens[1], Vuyani Matsenjwa[1], Ellen Giampoli[2], Saurabh K. Garg[3], Colin W. Snyder[3], Brian Czerniecki[3], Conor Steuer[4], Nabil Saba[4], Michael Lowe[4], Gregory B. Lesinski[4], Chrystal Paulos[4], Maurice Zauderer[1]

[1]Vaccinex, Inc., Rochester, NY,[2]University of Rochester, Rochester, NY,[3]Moffitt Cancer Center, Tampa, FL,[4]Winship Cancer Center of Emory University, Atlanta, GA

Mature tertiary lymphoid structures (TLS) characterized by the presence of germinal centers are strong predictors of response to immunotherapy. DCs play critical and decisive roles in determining the outcome of the immune responses in the TME and within TLS. Therefore, strategies to recruit and activate DC have the potential to induce TLS and enhance response to immunotherapy. Pepinemab, semaphorin 4D (SEMA4D) blocking antibody, reversed myeloid suppression and promoted infiltration of activated DC and CD8+ T cells into the TME in preclinical and clinical studies. New data demonstrates the presence of mature TLS following pepinemab treatment in combination with immune checkpoint inhibitors (ICI) in three separate clinical studies. KEYNOTE-B84 study (NCT04815720) evaluated the safety and efficacy of pepinemab in combination with pembrolizumab as a first-line treatment for immunotherapy-naïve patients with R/M HNSCC. Neoadjuvant studies in patients with surgically resectable metastatic melanoma (NCT03769155) and HNSCC (NCT03690986) received pepinemab treatment combined with nivolumab and/or ipilimumab. Screening and on-treatment tumor biopsies were evaluated using multiplex immunohistochemistry, RNAseq and spatial transcriptomics. TLS identified in biopsies of patients treated with pepinemab in combination with ICI were larger and more mature, containing high density of activated HLA-DR+ DCs, FDC, follicular B cells, and Tfh cells expressing CXCR5, characteristic of germinal centers. Importantly, comparison of matched biopsy pairs before and after treatment indicated that TLS were induced by the pepinemab combination treatment. Increased density and maturity of lymphoid aggregates, as well as significant changes in DC activation markers and the Flt3/Wnt pathway correlated with clinical benefit. We hypothesized that effects of pepinemab could be further enhanced by increasing the population of DC available in the TME. In preclinical studies, intra-tumoral vaccination with ex vivo expanded DC-1 enhanced effects of systemic treatment with SEMA4D-blocking antibody, resulting in improved DC trafficking to draining lymph nodes, and inducing tumor regression in both target and distant tumors. For in vivo expansion of DC, treatment with Flt3L improved anti-tumor activity when combined with SEMA4D-blocking antibody. Pepinemab represents a novel strategy to boost dendritic cell infiltration, activation, and organization of functional TLS to overcome limitations of immune checkpoint therapies. When combined with ICI, pepinemab induced de novo formation of TLS that correlated with clinical benefit, even in poorly immunogenic tumors that do not respond well to ICI, including HPV-negative and PD-L1 low HNSCC. When combined with modalities to further expand DC, SEMA4D blocking antibodies inhibited tumor growth in preclinical models, including in ICI-resistant models.

#3976 Disrupting CD44-SPP1 interaction between tumor cells and tumor-associated macrophages (TAMs) to overcome cisplatin resistance in muscle-invasive bladder cancer (MIBC).

Yuzhen Zhou[1], Jihyun Lee[2], Hiba Siddiqui[1], Martin Egger[1], Eliezer M Van Allen[1], Filipe De Carvalho[2], Kent Mouw[1]

[1]Dana-Farber Cancer Institute, Boston, MA,[2]Brigham and Women's Hospital, Boston, MA

Introduction: Cisplatin-based chemotherapy remains a mainstay of treatment for MIBC; however, cisplatin resistance is common and is associated with poor clinical outcomes. Tumor cells actively shape the tumor microenvironment (TME), including tumor-associated macrophages (TAMs)—which comprise 50-80% of stromal cells—to support tumor growth. While TAMs are critical determinants of pro- or anti-tumor TME characteristics, the mechanisms underlying tumor-macrophage crosstalk in cisplatin resistant tumors remain poorly understood.

Methods: We employed single-nucleus RNA sequencing (snRNA-seq) to analyze cell populations in cisplatin sensitive and resistant human bladder tumors. Spatial transcriptomic analysis was performed to assess the spatial organization of cell types within tumors. CRISPR-Cas9 was utilized to generate CD44-deficient bladder cancer cell lines and SPP1-deficient macrophages. Functional assays for tumor cells and macrophages were performed using direct and indirect tumor cell-macrophage co-culture systems. Phagocytosis was analyzed using live-cell imaging and flow cytometry. Finally, both immune-competent and immune -deficient mouse model was utilized to evaluate the therapeutic potential of targeting the CD44-SPP1 axis as a strategy to overcome cisplatin resistance.

Results: Our analysis revealed that high CD44 expression in tumor cells is positively correlated with cisplatin in human bladder cancer patients. SPP1-expressing macrophages were spatially localized near CD44 positive tumor cells in cisplatin resistant tumors. Deleting CD44 in tumor cells increased sensitivity to cisplatin, which was more significant in the presence of macrophage-conditioned media in co-culture with bladder cancer cell lines. CD44 loss also promoted the phagocytic activity of the mouse macrophage cell line Raw264.7. We are currently using an immune-competent mouse bladder cancer model to test whether genetic deletion or pharmacological blockade of CD44 sensitizes tumors to cisplatin. Furthermore, we will utilize immune-deficient nude mice to co-implant human bladder cancer cell with human macrophages with or without SPP1 expression to evaluate whether SPP1 deficient macrophage enhance cisplatin responses and promote tumor suppression.

Conclusion: Targeting the CD44-SPP1 interaction, either through genetic modification or antibody-mediated blockade, sensitizes bladder tumors to cisplatin. This approach represents a promising therapeutic strategy to overcome cisplatin resistance in MIBC.

#3977 Illuminating mesothelioma immune microenvironment in response to immune checkpoint inhibitors using spatial transcriptomics.

M. Alamgeer[1], D. Gough[1], V. Xue[1], B. Kumar[1], A. Pick[2], F. S. Yoosuf[1], S. Arulananda[2], J. Marquez[1], V. Ganju[1];
[1]Monash University, Clayton, Australia, [2]Monash Health, Clayton, Australia

Most mesothelioma patients either have primary refractory disease or develop acquired resistance to immune checkpoint inhibitors (ICIs). A more sophisticated understanding of the tissue microenvironment (TME) and intercellular communication will enable opportunities for overcoming ICI resistance. Here, we utilize the Visium 10x spatial transcriptomics (ST) technology to address this question. We conducted ST on a cohort of 12 mesothelioma patients (28 samples) treated with ICIs (Table 1). Nine patients had a response to ICIs with a median response duration of 9 months and three had primary ICI resistant disease. We show the transition from the immune infiltrated tumor in a pre-treatment sample transition to immune exclusion in the post-treatment (ICI-resistant) sample. In addition, we were able to map the immune cell sub-types in relation to cancer cells. In a responder, we identified CXCL12-CXCR4 mediated communication at the mesothelial-immune interface. We mapped a 48-gene prognostic signature to generate spatial heat maps showing specific regions within tissue with elevated poor prognosis signatures in treatment naïve samples that expanded in matched relapsed samples. We also identified remarkable heterogeneity showing pre- and post-ICI treatment samples to be transcriptionally diverse. In another patient, we assessed four samples from the primary tumor prior to treatment, two from PET-positive and two from PET-negative areas. Moreover, we complemented spatial transcriptomics with multi-region whole genome sequencing. This revealed that these sites are genomically stable but transcriptionally distinct. Further deconvolutional analysis to approximate cell composition, cell co-localization, and cell evolution/plasticity measures are underway. Together, this deep spatial investigation provides a key understanding of oncogenic communication in TME, before and after ICI therapy.

Table 1.

Patient No	Sex	Histological Subtype	Treatment	Pre-Treatment samples	Post Treatment samples	Response	Response duration
1	M	Sarcomatoid	PD-1	5	-	Yes	6
2	M	Epithelioid	PD-1	3	-	No	-
3	M	Biphasic	PD-1/CTLA-4	4	-	Yes	87
4	F	Biphasic	PD-1/CTLA-4	1	-	No	-
5	M	Epithelioid	PD-1/CTLA-4	2	1	Yes	12
6	F	Sarcomatoid	PD-1	1	-	Yes	52
7	M	Epithelioid	PD-1	1	1	Yes	17
8	M	Sarcomatoid	PD-1/CTLA-4	2	-	Yes	75
9	M	Epithelioid	PD-1/CTLA-4	1	1	No	-
10	M	Biphasic	PD-1	1	1	Yes	18
11	M	Sarcomatoid	PD-1/CTLA-4	1	1	Yes	22
12	M	Epithelioid	PD-1/CTLA-4	-	1	Yes	16

#3978 The downregulation of OGT1 reprograms tumor-associated macrophages and CD4 T cells fate in pancreatic adenocarcinoma tumor model.

Vira Chumak[1], Kuldeep Singh Attri[2], Pankaj Kumar Singh[1], Kamiya Mehla[1]

[1]Dep. of Onc. Science, University of Oklahoma Health Sciences Center, Oklahoma City, OK,[2]Baylor College of Medicine, Houston, TX

O-linked β-N-acetylglucosamine modification (O-GlcNAcylation) is a post-translational modification that plays a crucial role in development, normal physiology, and the pathophysiology of various diseases, including pancreatic cancer. This process is catalyzed by the enzyme O-GlcNAc transferase (OGT). The hexose biosynthesis pathway provides the necessary substrate, O-GlcNAc, for this reaction. However, the role of O-GlcNAcylation in tumor-associated macrophages has not been extensively studied. The hypoxic tumor microenvironment leads to increased glucose uptake and enhanced glycolysis. Additionally, macrophages undergo significant metabolic changes in this environment. The immunosuppressive nature of the tumor microenvironment promotes the polarization of macrophages toward the non-inflammatory M2-like phenotype, which is characterized by increased CD206, and Arg1 expression. To investigate the role of OGT1 in tumor-associated macrophages, we created a mouse strain, LysM-Cre; $Ogt^{fl/fl}$, which features OGT1 loss specifically in macrophages. We isolated bone marrow mononuclear cells from both mutant and control animals and differentiated them into bone marrow-derived macrophages (BMDM) in vitro. Following this, we cultured the BMDMs with pancreatic tumor cell-conditioned media (TCM) from pancreatic cancer cells and assessed arginase 1 (Arg1) levels using Western blotting. The BMDMs isolated from LysM-Cre; $Ogt^{fl/fl}$ mice expressed lower levels of arginase 1 compared to control mice after being cultured in TCM. Next, we investigated the effect of pharmacological inhibition of OGT1 using OSMI-1 on the BMDMs, which were treated with TCM or control media, and how these macrophages influenced CD4 T-cell subtypes. The BMDMs were cultured for 72 h in a medium containing 100 μM OSMI-1 under hypoxic conditions (1% O_2). Subsequently, the total CD4 T cells were co-cultured with the BMDMs for 62 h in hypoxia and stained to identify markers of Th1 and Th2 cell populations. We observed that macrophages stimulated with TCM reduced the number of IFNy+/IL-12+ CD4+ T cells compared to those treated with control media. In contrast, the inhibition of OGT1 in BMDMs resulted in an increase in the number of IFNy+/IL-12+ CD4+ T cells upon incubation with TCM. These findings suggest a potential role of OGT1 and O-GlcNAcylation in the interplay between macrophages and lymphocytes within the pancreatic adenocarcinoma TME. The data indicate that OGT1 may inhibit the immune components of the tumor microenvironment. This knowledge could pave the way for innovative strategies aimed at shifting the balance towards a more effective anti-tumor immune response.

#3979 Genomic determinants of canine tumor-associated macrophage polarization.

R. V. Brady, K. B. Farrell, E. P. Palmer, S. Das, D. L. Duval, D. H. Thamm;
Colorado State University, Flint Animal Cancer Center, Fort Collins, CO

There is poorly understood variability both between and within cancer types in their ability to maintain an immunosuppressive microenvironment and polarize tumor-associated macrophages (TAMs). Identifying genetic biomarkers of cancers that strongly polarize TAMs would enable the discovery of novel therapeutic targets and support the stratification of patients for appropriate immunotherapy. Our aim, therefore, was to correlate gene expression data from cancer cell lines with phenotypic changes in canine macrophages. We harvested standardized tumor-conditioned medium (TCM) from twenty-five canine cancer cell lines with bulk RNA-sequencing data available. Primary monocyte-derived macrophages were obtained from three canine donors and polarized with the twenty-five different TCMs. After a washout period, supernatants were collected, and polarization changes of the macrophages were assessed via secretory products using ELISA and a multiplex cytokine/chemokine assay. For each analyte measured, a modified z-score was used to rank each cell line's ability to stimulate the secretion of that analyte from macrophages. Multiple regression analysis and pairwise comparison analysis (composed of "strong" and "weak" stimulators) were performed against the full transcriptome. Many significantly differentially expressed genes were identified in the cancer cell lines with the strongest ability to polarize macrophages. For example, cell lines with MVB12A upregulated, a gene involved in selecting cargo for exosomes, had the highest ability to stimulate VEGF production from macrophages. This was subsequently confirmed by depleting the TCM of exosomes which resulted in a significant decrease in VEGF production. Multiple other genes involved in macrophage immunosuppression were upregulated, while those involved in cell adhesion and metastasis prevention were downregulated. In conclusion, canine cancer cell lines have varying abilities to polarize primary macrophages, irrespective of histologic type, as measured by secretory products. The cell lines capable of potent polarization had relevant differentially expressed genes and pathways. These are currently being explored to identify novel biomarkers and therapeutic targets.

#3980 Exploring the diversity and dynamics of the macrophage compartment in non-small cell lung cancer.

Eun Ji LEE[1], You Won Lee[2], Minyeop Kim[3], Seung Yeon Oh[1], YoungJoon Park[2], Su-Jin Choi[4], JuHyeon Lee[4], Kyoung-Ho Pyo[2], Jae Hwan Kim[5], Jii Bum Lee[6], Min Hee Hong[6], Sun Min Lim[6], Mi Ran Yun[5], Byoung Chul Cho[6]

[1]Graduated School of Medical Science, Brain Korea 21 FOUR Project for Medical Science, Yonsei University College of Medicine, Department of Biomedical Science Institute, Seoul, Korea, Republic of,[2]Yonsei New Il Han Institute for Integrative Lung Cancer Research, Yonsei University College of Medicine, Seoul, Korea, Republic of,[3]JEUK Institute for Cancer Research, JEUK Co., Ltd, Gumi, Korea, Republic of,[4]Department of Research Support, Yonsei University College of Medicine, Yonsei Biomedical Research Institute, Seoul, Korea, Republic of,[5]Severance Biomedical Science Institute, Yonsei University College of Medicine,, Seoul, Korea, Republic of,[6]Division of Medical Oncology, Yonsei Cancer Center, Yonsei University College of Medicine, Seoul, Korea, Republic of

Introduction: Although immune checkpoint inhibitors (ICIs) has improved the survival rate for some cancer, EGFR mutant (MT) non-small cell lung cancer (NSCLC) patients exhibit a poor response to ICIs than those with EGFR wild-type (WT). EGFR-MT cells contribute to immunosuppressive tumor microenvironment (TME) by secreting inhibitory cytokines and recruiting pro-tumor immunogenic cells, thereby diminishing the efficacy of ICIs. Tumor associated macrophages (TAMs) represent a major component of the TME, playing complex roles in tumor progression and metastasis. While single-cell RNA (scRNA) analyses have been performed in various cell types, studies on macrophage (MØ) diversity between EGFR-WT and -MT NSCLCs are limited. Therefore, we aimed to reveal the presence of multifaceted MØ populations, using scRNA sequencing.

Methods: Using the 10x Genomics, we generated scRNA expression data from 904,174 cells in primary lung tissues of 95 patients: EGFR-WT (n=35), treatment-naïve EGFR-MT (n=55), EGFR-TKI post-treatment (n=5), and matched normal (n=22). We annotated five distinct cell lineages—lymphocytes, epithelial cells, endothelial cells, fibroblasts, and myeloid cells—using canonical marker gene expression. We focused on MØs (n=158,640 cells) in myeloid, including markers such as *APOE, FCGR1A*, and *MARCO*.

Results: We characterized 13 transcriptional states of MØs: Four clusters of alveolar MØs (AMs; *PPARG*$^{+}$), two clusters of interstitial MØs (IMs; *MERTK*$^{+}$, *LYVE1*$^{+}$) and eight clusters of MØs expressing canonical markers (*CD68*$^{+}$, *C1QA*$^{+}$) with high monocyte derived MØ (MDM) signatures. To define significant clusters, we analyzed their proportions based on sample type, tumor stage, and recurrence status. MØ cluster compartment varied among normal, EGFR-WT, -MT, and -TKI samples. AMs were primarily found in normal tissues, while IMs and MDMs predominate in tumors. Some IM and MDM clusters showed opposing trends in proportion between EGFR-WT and -MT across tumor stages, with major recurrence-associated clusters differing between the two types. Next, we defined high proportion of four major TAM clusters identified in EGFR-WT, -MT, and -TKI, expressing the representative genes *SPP1, TREM2, IL1B*, and *FOLR2*, respectively. Gene ontology pathway analyses revealed the complexity of the TME. SPP1 TAMs, predominant in EGFR-MT, were associated with cellular stress response, while IL1B TAMs, mainly in EGFR-WT, were associated with response to external stimuli. FOLR2 TAMs were associated with chemoattraction and were prevalent in both EGFR-WT and -TKI. TREM2 TAMs, associated with lipid metabolism, were evenly distributed across all sample types.

Conclusions: We characterized the heterogeneity of MØ compartments between EGFR-WT, -MT and -TKI samples. These results highlight the need for selective treatment strategies based on EGFR mutation and treatment status in NSCLCs for successful immunotherapy.

#3981 The GZMK CD8 T cells cluster defined by single-cell transcriptome profiling with innate-like functions is a predictor of survival in hepatocellular carcinoma patients.

Wei-Ting Ku[1], Chien-Hao Huang[2], Jian-He Fan[2], Cheng-Heng Wu[2], Tsung-Han Wu[3], Wei Teng[2], Po-Ting Lin[2], Chung-Wei Su[2], Yung-Chang Lin[4], Chun-Yen Lin[5]

[1]Graduate Institute of Biomedical Sciences, Chang Gung University, Taoyuan, Taiwan,[2]Division of Gastroenterology and Hepatology, Linkou Chang-Gung Memorial Hospital, Taoyuan, Taiwan,[3]Department of General Surgery, Linkou Chang-Gung Memorial Hospital, Taoyuan, Taiwan,[4]Department of Hematology-Oncology, Linkou Chang-Gung Memorial Hospital, Taoyuan, Taiwan,[5]College of Medicine, Chang Gung University, Taoyuan, Taiwan

Hepatocellular carcinoma (HCC), the sixth most common cancer globally in 2020, is closely associated with hepatitis B or C infections and non-alcoholic steatohepatitis (NASH). Despite advancements in surgical and systemic therapies for early and advanced stages, tumor relapse and poor prognosis remain significant challenges, necessitating improved therapeutic approaches. This study highlights the role of GZMK-expressing $CD8^+$ T cells in HCC, identified through single-cell transcriptome profiling. These cells exhibit transcriptional profiles similar to hepatotoxic $CD8^+$ T cells in chronic hepatitis B and innate-like $CD8^+$ T cells in chronic hepatitis C. GZMK $CD8^+$ T cells are enriched with tissue-resident memory traits and are detectable in peripheral blood and tumor tissues. Furthermore, within the tumor microenvironment, GZMK CD8 cells expressed surface receptors resembling those of NK cells, allowing them to release IFN-γ and exert non-antigen specific innate-like cytotoxic effects. These GZMK $CD8^+$ T cells could be identified as $CD38^+$HLA-DR^+ $CD8^+$ T cells by flow cytometry. These $CD38^+$HLA-DR^+ $CD8^+$ T cells increased in the peripheral blood of HCC patients and accumulated in tumor tissue. Cytokines such as IL-12, IL-15, and IL-18 were shown to stimulate the proliferation of $CD38^+$HLA-DR^+ $CD8^+$ T cells, enhancing their production of IFN-γ and granzyme B, as well as their expression of NK-like receptors and effector molecules. These bystander-activated $CD38^+$HLA-DR^+ $CD8^+$ T cells, derived from effector memory (EM) and effector memory RA (EMRA) subsets, displayed cytotoxicity against K562 and THP-1 cell lines through DNAM-1, granzyme B, and perforin. A high GZMK $CD8^+$ T cell signature was strongly associated with improved overall survival (OS) and progression-free survival (PFS) in the TCGA-HCC database. These findings suggest that GZMK $CD8^+$ T cells have a significant impact on survival in HCC, possibly through their innate-like cytotoxic functions. This unique subset may serve as a biomarker and a potential therapeutic target in HCC management.

TUMOR BIOLOGY: Tumoroids, Organoids, and Cell Culture
Poster Session

#3985 Optimizing co-culture of tumoroid and immune cells for recapitulation of the tumor microenvironment.

Shyanne Salen, Sydney Hawkins, Sylvia Beam, **Colin D. Paul**, Chris Yankaskas, Pradip Shahi Thakuri, Matt Dallas, David Kuninger

Thermo Fisher Scientific, Frederick, MD

Immunotherapies for solid cancer treatment aim to potentiate immune cell recognition of cancer cells within the tumor microenvironment or recruit peripheral immune cells (native or engineered) to the tumor. Patient-derived tumoroids, or cancer organoids, are multicellular structures that closely mimic the architecture and genetic and transcriptomic landscape of tumors. When co-cultured with immune cells, these models enable a more comprehensive investigation of the tumor-immune microenvironment than traditional cancer cell lines. However, culture conditions to prolong survival of both epithelial cancer cells and immune cells remain poorly defined. Here, we explored methods to supplement Gibco™ OncoPro™ Tumoroid Culture Medium to promote immune cell survival for better recapitulation of the tumor microenvironment. In initial experiments, healthy donor PBMCs were thawed and cultured in suspension in OncoPro medium for 5 days. Total viable cells were counted at the end of the culture period and revealed loss of 80±2% of the cells initially present. Similarly, RNA sequencing comparing freshly dissociated colorectal and endometrial tumors with cells cultured for 7 days in OncoPro medium indicated downregulation of genes related to immune cell signaling pathways, and flow cytometry of tumoroid models showed loss of immune cell populations over time compared to the initial material. Therefore, designed experiments were used to screen and optimize cytokine cocktails that maximized PBMC survival while minimizing the change in the percentage of monocytes, B cells, NK cells, helper T cells, and cytotoxic T cells between the original (day 0) donor material and in vitro PBMC cultures. Cytokine addition led to an increase in PBMC cell number after 5 days in culture of 2.2±0.8x compared to culture in non-supplemented OncoPro medium across three independent donors, with <10% change in the relative proportion of cells in the immune compartments examined over the culture period. Follow up experiments are focused on determining conditions that support the survival of immune cells present in cryopreserved dissociated tumor cell (DTC) samples using cytokines identified during PBMC screening experiments. In preliminary work, the addition of cytokines to potentiate immune cell survival did not negatively affect the culture of epithelial tumoroid cells. Co-culture of immune cells with tumoroids will facilitate the examination of immune checkpoint inhibitors and other immunotherapy strategies in vitro to enable higher throughput and more controlled screening of immunotherapy candidates. We envision that this approach will provide critical insights into the mechanisms of immune evasion and resistance and potentially lead to more effective treatment options for cancer patients.

#3987 Implementation of advanced molecular imaging to evaluate probiotic L. lactis bacterium capability to preferentially target orthotopic colorectal tumor.

J. G. Gomez-Gutierrez[1], M. Ramos-Gomzalez[1], N. Rubio-Infante[1], J. Amos-Landgraf[1], S. Rachagani[1], L. McNally[2], H. Shirwan[3]; [1]University of Missouri - Columbia, Columbia, MO, [2]University of Oklahoma Health Science, Oklahoma, OK, [3]University of Missouri, Columbia, MO

Colorectal cancer (CRC) is the third most diagnosed cancer and has the second highest mortality of all cancers. CRC treatments include surgery, chemotherapy, and radiation. However, CRC patients suffer from significant toxic side effects, develop resistance to the therapies, and experience recurrence and metastasis. A new strategy for CRC treatment is immunotherapy, which uses the patients' immune system to attack cancer cells. Unfortunately, most CRC patients do not respond to immune checkpoint inhibitors (ICIs). To better treat CRC patients with immunotherapy, it is essential to improve tumor-targeting and tumor-specific uptake. Therefore, this study used advanced molecular imaging for real-time evaluation of the capability of probiotic facultative anaerobic bacterium *Lactococcus lactis* (*L. lactis*) to preferentially target orthotopic colorectal tumors. We combined bioluminescent (BL) and fluorescent imaging to simultaneously detect CRC tumors and *L. lactis* expressing infrared fluorescent protein (*L. lactis*-iRFP), respectively. To simultaneously detect BL and iRFP signals, we subcutaneously injected a CRC cell line expressing luciferase (CT26/luc) in the right flank of 6-week-old BALB/c mice. After the tumor reached a volume of 500 mm^3, *L. lactis*-iRFP was injected intratumorally. We found that BL and iRFP signals colocalized at the tumor site without directly overlapping without interfering one with another and *L. lactis*-iRFP signal was detected at the tumor site up to 5 days post-injection. Next, we proceeded to generate an orthotopic CRC mouse tumor model in 6-weeks-old BALB/c mice using the colonoscopy guided inoculation technique. We included two control mice without tumors, one administered with non-fluorescent *L. lactis* wild type, and the other with *L. lactis*-iRFP to compare the bacterium retention between tumor bearing mice. At day 11, when the orthotopic CRC tumor was well-established, the animals were administered with $1x10^{12}$ cfu/200 µL of *L. lactis*-iRFP or wild type via oral gavage, and BL and fluorescence signals were measured. The advanced molecular imaging revealed a strong iRFP signal in the tumor-bearing animals, up to 5 days post *L. lactis*-iRFP inoculation. The colons were scanned *ex vivo* and showed BL signal in the distal location of the CRC tumor. Interestingly, only the colons with tumors showed iRFP signal, suggesting that *L. lactis*-iRFP preferentially targets the CRC tumor microenvironment. However, additional experiments are required to reproduce this data and support a conclusion. We demonstrated that simultaneous detection of BL and iRFP signals is feasible and that this imaging technology could be potentially implemented for preclinical real-time evaluation of the capability of other bacteria to preferentially target solid tumors.

#3988 Patient-specific 3D engineered microphysiological systems to model triple-negative breast cancer chemotherapy using patient-derived xenograft (PDX) tissue.

Edgar A. Borrego[1], Linjie Luo[2], Janine Hagar-Montoya[1], Jose L. Perez[1], Paula Delgado[1], Khandan Keyomarsi[2], **Sylvia L. Natividad-Diaz**[1]

[1]Metallurgical, Materials, and Biomedical Engineering Department, The University of Texas at El Paso, El Paso, TX,[2]Department of Experimental Radiation Oncology, Division of Radiation Oncology, MD Anderson Cancer Center, Houston, TX

Chemotherapy efficacy and pharmacokinetics are influenced by a patient's unique characteristics, including gender, ethnicity, age, gene expression, and disease state. Three-dimensional (3D) tissue models integrated into microfluidic devices offer a controlled and reproducible platform to study cellular responses to drugs, leveraging fluid circulation for precise quantification. These models can be tailored to individual patients through the incorporation of patient-derived xenograft (PDX) tissues, which are purified and encapsulated within biomimetic hydrogels that replicate the tumor extracellular matrix (ECM). The objective of this study was to develop a patient-specific 3D model of triple-negative breast cancer (TNBC) cultured in a self-driven, closed-capillary microfluidic device to assess chemotherapy efficacy, toxicity, and tumor microenvironment interactions in vitro. We hypothesized that integrating human TNBC PDX tissue into customized microfluidic devices would create physiologically relevant systems to study disease pathology and treatment responses in vitro. TNBC-PDX tissues were isolated to establish a cell line expressing human EpCAM with a population doubling time of 48 hours. A CC50 value of ~10 µM was determined via flow cytometry viability assays following treatment with a 10-fold dilution series of Doxorubicin (DOX). The cells were encapsulated in 3D collagen hydrogels (0.2 wt%) and cultured in transwell (TW) dishes or microfluidic devices (DwP). These 3D TNBC-PDX models demonstrated viability, elongated morphology, and tumor-like clustering within DwP, reorganizing according to device microfeatures. When treated with 10 µM DOX, the 3D cultures in both TW and DwP conditions exhibited limited cell death, reflecting the original tumor's drug-resistant phenotype. These findings highlight the feasibility of integrating PDX tissues into microfluidic systems to create patient-specific 3D models, offering a physiologically relevant platform for evaluating chemotherapy responses. This approach may advance personalized cancer treatment strategies by enabling in vitro assessment of drug efficacy and resistance mechanisms.

#3989 Patient derived cancer organoids as a model to predict clinical response and drug discovery in colorectal cancer.

Alexa E. Schmitz, Shirsa Udgata, Maggie O. Smith, Rian Engeldinger, Yousef Gadalla, Xingqi Shen, Addison T. Zick, Alec B. Cornelio, Cheri A. Pasch, Dustin A. Deming

University of Wisconsin - Madison, Madison, WI

Background: Patient derived cancer organoids (PDCOs) have shown to preserve cell morphology and better recapitulate patients' cancers. Due to these advantages, PDCOs could be used as a model to better predict how a patient will respond to drug treatments. STX-478, a *PIK3CA*^{H1047R} mutant specific inhibitor, has shown promise to circumvent severe toxicities which could aid in the tolerability of novel combinations. Here, we examine the impact of PDCOs for predicting patient response to chemotherapy and in drug discovery.
Methods: PDCO lines were acquired from surgery and biopsies following consent and approved Institutional Review Board protocols. Seven PDCOs were imaged (Day 0) and treated for 48 hours based on the patient's clinical treatment. Media was subsequently replaced and PDCOs re-imaged 48 hours later (Day 4). Diameter analysis was performed using ImageJ and percent relative change (PRC) and Glass's Delta (GD) was determined for all treatments. Based on previous data, a Glass's Delta over 1.25 has been shown to correlate with partial response, and under 0.75 correlates with progressive disease. Clinical response was determined based on MRI scans and RECIST v1.1 guidelines. In the drug screen, a CRC PDCO line with a *PIK3CA*^{H1047R} mutation, was treated with 115 different inhibitors in combination with STX-478 for 48 hours. All plates were imaged on Day 0 and Day 2; diameter analysis was performed, and GD was determined for all treatment groups.
Results: Seven PDCO lines were treated with FOLFOX or FOLFIRI and a GD range of 0.01-1.61 with a median of 0.75 were observed. Based on previously described GD sensitivity thresholds, two PDCO lines were responsive, and five were nonresponsive to treatment. The five non-responsive PDCO lines were all collected from the residual tumor after treatment with FOLFOX. FOLFIRI treatment of PDCO line CRC1 caused a significant response compared to all treatment groups (PRC= 22.4%, GD= 1.26, control: 70.3%, 5FU: 33.5%, 0.97, SN38: 43.1%, 0.71). Clinically, the patient had a 42% reduction in cancer size per RECIST v1.1, which is considered a partial response. In the STX-478 drug screen, six different combinations resulted in an enhanced response compared to single agents, including enasartinib HCl (EHCL; STX-478 single agent PRC= 29.1%, EHCL= 38.6%, and combination= -2.06%, STX-478 vs combination GD: 1.57; EHCL vs combination: 2.07). Additionally, NSC23766, a Rac1 inhibitor, also demonstrated enhanced response in combination (STX-478 single agent PRC= 29.1%, NSC23766= 38.0%, and combination= 9.2%, STX-478 vs combination GD: 1.00; NSC23766 vs combination: 1.42).
Conclusion: PDCOs show exciting potential as a predictive model for patient response and drug discovery. These findings need to be validated in further studies.

#3990 Characterization of triple negative breast cancer organoids from diverse ethnic populations.

Moses Kamita[1], Batoul Farran[1], Sylvester Antwi[1], Forster Amphonsan-Manu[2], Josephine Nsaful[3], Patrick K. Akakpo[4], Jessica Bensenhaver[1], Kwabena Agbedinu[5], Michael Nortey[4], Nelson Affram[6], Mohammed Sheriff[7], Cassandra Mills[1], Alex Mremi[8], Theresia Mwakyembe[8], Ijeoma Aja[9], Moses Dokurugu[6], Haythem Ali[1], Eleanor Walker[1], Evelyn Jiagge[1]

[1]Henry Ford Health System, Detroit, MI,[2]Eastern Regional Hospital, Koforidua, Ghana,[3]Korle Bu Teaching Hospital, Accra, Ghana,[4]Cape Coast Teaching Hospital, Cape Coast, Ghana,[5]Komfo Anokye Teaching Hospital, Kumasi, Ghana,[6]Ho Teaching Hospital, Ho, Ghana,[7]Tamale Teaching Hospital, Tamale, Ghana,[8]Kilimanjaro Christian Medical Centre, Moshi, Tanzania, United Republic of,[9]Eastern Regional Hospital, Ho, Ghana

Introduction: Triple negative breast cancer (TNBC) among women with African ancestry has been shown to be more prevalent than in women of European ancestry. However, the root cause for this disparity is not fully understood. We have previously shown that the gene expression in the breast cancer stem cells of TNBC tumors differs based on Western sub-Saharan ancestry. Tumor organoids are three-dimensional structures that are derived from tumor cells and are able to mimic the organization and function of the organ of origin. Organoids are, therefore, valuable tools for studying complex interactions in cancer biology. To have a better understand of how differential gene expression based on ancestry affects aggressiveness of TNBC, we are developing and characterizing patient derived xenografts (PDXs) and organoids from TNBC tumors derived from African (Ghanaian), European American and AA populations.
Objective: The objective of the study is to develop and characterize PDXs from TNBC tumors that will serve as a renewable source for studying how differential gene expression based on ancestry affects aggressiveness of TNBC among AA.
Methods: Fresh tumors from continental Africa (Ghana), European American, and African American (Henry Ford Hospital) were collected from consenting patients during surgery. The tumors were then implanted in mice and organoids developed from the resulting PDX tumors. Tissues were processed using the Miltenyi Gentle MACS dissociator. The cells were seeded in a 12-well plate coated with Matrigel. Developed organoids are characterized using, immunohistochemistry, single cell RNA sequencing and 10X spatial sequencing
Results: We have successfully developed organoids using frozen tissues from West African European American, and African American TNBC breast patients. We have successfully established five (5) PDXs and their corresponding organoid lines. The organoids are passaged after every 7-14 days, with some organoid lines attaining 20 passages. We are in the process of characterizing the developed PDXs and organoids using immunohistochemistry, single cell RNA sequencing and 10X spatial sequencing and compare their different features.
Conclusion: This is an ongoing process, and the efforts will help us have a renewable source of tumor for studying how differential gene expression based on ancestry affects aggressiveness of TNBC among AA. We also aim to use the resources to identify tumor biomarkers for treating aggressive TNBC that disproportionately affects women of western sub-Saharan ancestry.

#3993 High-throughput screening of clinically relevant patient-derived 3D cultures to inform on the efficacy of antibody drug conjugate payloads in 10 distinct cancer indications.

Annamaria Rapisarda, Kayin Chan, Mara Gilardi, Stefano Cairo, **BanuPriya Sridharan**

Champions Oncology, Inc., Hackensack, NJ

High-throughput screening (HTS) enables rapid evaluation of the efficacy of anticancer agents alone and in combinations across diverse cell-based tumor models. However, the successful translation of these findings to clinical settings necessitates the use of tumor models with high biological fidelity. By integrating tumor cell heterogeneity and three-dimensional morphological features, patient-derived 3D cultures offer enhanced in vivo relevance compared to traditional tumor cell lines grown as monolayers. Nevertheless, the growth of these cultures within an extracellular matrix material and the use of complex media formulations represent challenges for culture scale-up and automated drug screening methods. To address these challenges, we leveraged our well-characterized Patient Derived Xenograft (PDX) bank and Champions TumorGraft3D platform (CTG3D), comprising over 165 well-characterized models, to develop an HTS solution. This platform is highly representative of the patient population and enables users to evaluate the activity of test agents in (i) expandable and ex vivo amenable 3D models from indications such as Colorectal and Non-Small Cell Lung Cancer (NSCLC); as well as in (ii) models freshly derived from PDXs, which present more heterogeneous and indication-specific material such as Prostate and Glioblastoma Multiforme (GBM). Champions Oncology's PDX-derived 3D platform (CTG3D) models are low passage and preserve the molecular and phenotypic signatures of parental PDX and hence original patient-derived material maximizing translatability and minimizing clonal drift. Here, we present work that supports the translation of our assays to a 384-well plate-based high-throughput format, using models representing more than 15 cancer indications. These models were evaluated for their recovery from cryopreservation, reproducible growth, and assay performance metrics in a miniaturized 384-well format using CellTiter-Glo® 3D luciferase assay-viability endpoint. PDX-derived and patient-derived 3D cultures from varied indications were screened with a library of agents used as Antibody-Drug-Conjugates (ADC) payloads. Over 4 days, our 3D cultures were exposed to eight concentrations of each drug in a 1/2-log dilution series. Assay performance metrics (such as Z' and S:N ratio) and pharmacological data demonstrated the robust performance of this organoid screening platform. Future initiatives will focus on developing additional PDX-derived 3D culture panels to expand our high-throughput screening (HTS) capabilities.

#3994 Comparing ATP-based and image-based viability readouts for assessing treatment responses in patient-derived organoids.

Claudia Beaurivage, Tao Yang, Anna Schepers, Mayke Doorn, Sumeyra Mucuk, Merel Derksen, **Sylvia F. Boj**

HUB Organoids B.V., Utrecht, Netherlands

High-throughput viability determination often relies on quantifying intracellular Adenosine TriPhosphate (ATP) levels. While this offers a robust option for assessing the efficacy of various compounds, it poses significant challenges when assessing compounds that interfere with metabolic or differentiation pathways, which affect intracellular ATP levels. HUB Organoid Technology is built on the discovery that adult stem cells proliferate and organize into three-dimensional organotypic structures when embedded into an extracellular matrix. Patient-Derived Organoids (PDOs) are generated from normal and malignant tissues and stored as high-quality biobanks that yield highly reproducible results. HUB Organoids® recapitulate complex characteristics of the parental tissue, including molecular heterogeneity and morphological and functional traits. Notably, PDOs can differentiate when subjected to the right conditions. This study compared ATP-based and image-based viability readouts to determine the best fit for quantifying treatment response in a panel of PDOs exposed to drugs with different modes of action. Herein, we show that an ATP-based viability readout fails to detect a viability shift in organoids treated with compounds known to modulate differentiation and metabolism, which instead becomes apparent when using an image-based readout. On the other hand, both readouts are equally effective at detecting changes in organoid viability following treatment with Oxaliplatin and 5-FU, which affect DNA damage and replication, respectively. Our results reveal the importance of consulting an expert organoid service provider who has an in-depth understanding of organoid biology and who can tailor your study based on your compound's expected mode of action, thus increasing your program´s chance of success.

#3995 Investigating the role of sphingosine kinase 2 and p53 in bioprinted head and neck cancer 3D models.

Thais More Milan[1], Luda Lin[2], Sara Sartini[2], Alberto Mendoza Valderrey[2], Alice Soragni[2], Andreia Machado Leopoldino[1]

[1]Department of Clinical Analyses, Toxicology and Food Sciences, School of Pharmaceutical Sciences of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, Brazil,[2]Department of Orthopaedic Surgery, David Geffen School of Medicine, University of California, Los Angeles, CA

Head and neck squamous cell carcinoma (HNSCC) presents a high incidence of recurrences and metastasis. Alterations in sphingolipids metabolism caused by deregulation of sphingosine kinase 2 (SK2) is related to tumor progression. Additionally, the interaction between SK2 and p53 has been found to promote aggressive behavior in HNSCC (Milan et al, 2024). However, SK2 deregulation in HNSCC and SK2/p53 crosstalk remains to be fully investigated. Our goal is to explore the interaction between sphingosine kinase 2 and p53 in HNSCC cell lines and patient organoids. We leverage 3D HNSCC models generated by bioprinting HNSCC cell lines expressing different p53 and SK2 levels with extracellular matrix components in high throughput-compatible mini-ring format (Phan et al, 2019; Tebon et al, 2023; Al Shihabi et al, 2024). We then investigated metabolic activity, organoids size, and quantified both SK2 and p53 protein levels. Given that p53 misfolding and aggregation has been reported as contributing to tumor progression in several cancers, we have determined its folding status by multiplexed immunofluorescence using conformation-specific antibodies in the presence or absence of SK2 activity and p53 aggregation inhibitors. Combined inhibition of SK2 activity by Opaganib and p53 aggregation by ReACp53 (Soragni et al, 2016) decreased organoid size, ATP levels, and p53 aggregation. Single agent and combinatorial treatment with ReACp53 and Opaganib also resulted in elevated γH2AX levels. Our studies highlight a complex interplay between SK2 and p53 in HNSCC, resulting in more aggressive disease. Combinatorial targeting of these proteins could offer a potential therapeutic strategy for HNSCC.

Funding: CAPES, CNPQ and FAPESP Grant n.2023/15046-8

#3996 Organoids and immune cells co-culture for evaluating the efficacy of enfortumab vedotin and pembrolizumab combination therapy..

Anne-Sophie Bajeot[1], Celine Rouget[2], Nadege Bidan[2], Claire Beraud[2], Sarah Pericart[1], Xavier Game[1], Mathieu Roumiguie[1], Philippe Lluel[2], **Emilie Decaup**[2]

[1]Toulouse Hospital, Toulouse, France,[2]Urosphere SAS, Toulouse, France

Significant therapeutic progress have been made for advanced bladder cancer (BC) particularly with the combination of Enfortumab Vedotin (EV) and the pembrolizumab (anti-PD1). This combination has shown a manageable safety profile and superior outcomes compared to chemotherapy, leading to FDA approval for patients with advanced BC. In clinical settings, decisions regarding PD1 immunotherapy are guided by PDL1 expression levels on tumor cells. We selected two organoid models (F659 and BLOU_191) out of 18 from our biobank based on their PDL1 and nectin-4 expression. An additional organoid model (F659R) with acquired-resistance to EV was also included. The objectives of this study were to highlight the advantages of a co-culture system, which combines organoids and immune cells, to evaluate the efficacy of EV and pembrolizumab combination. Furthermore, leveraging recent developments, effectiveness of this therapy was assessed in a context of EV resistance. Transcriptome sequencing of our biobank enabled the identification of mRNA levels expression for PDL-1 and nectin-4. Peripheral blood mononuclear cells (PBMCs) from healthy donors were introduced into organoid cultures at varying effector-to-target (E:T) ratios. Co-cultures were treated with pembrolizumab (200 nM) and EV (from 0.3 to 3 µg/mL), both individually and in combination. Organoid viability was assessed using the CellTiter-Glo® assay at intervals ranging from 48 to 96 hours post-treatment. The efficacy of the combination therapy was then compared to the effects of each compound alone. We have assessed the responses of the three models to pembrolizumab and EV, and correlated treatment efficacy with PDL1 and nectin-4 expression levels, respectively. Results demonstrated that pembrolizumab efficacy was related to PDL1 expression in organoid models, while EV efficacy did not show a strict correlation with nectin-4 expression. Indeed, despite its high Nectin-4 expression, BLOU_191 presented only a low response to EV. In co-culture systems using the EV-responsive organoid model (F659), the combination of EV and pembrolizumab exhibited significant 20% enhanced efficacy compared to the individual treatments whereas it did not in the low-responder model (BLOU_191). In the resistant model, neither EV nor pembrolizumab exhibited significant efficacy when used alone, and their combination did not enhance these effects. The co-culture system involving organoids and PBMCs demonstrated its utility in evaluating the combination of pembrolizumab and EV. Notably, this study highlighted a critical finding: while the combination has shown superiority over current treatment options, it does not overcome resistance to EV. Overall, this co-culture system is highly adaptable to other immunotherapeutic agents and presents a valuable tool for developing novel therapies, particularly in addressing resistance to EV.

#3997 Advancing precision cancer medicine with patient-derived organoids: an endometrial cancer case study.

Nikolina Radulovich, Pamela P. Soberanis, Molly Udaskin, Quan Li, Kevin C. J. Nixon, Irene Xie, Nhu-An Pham, Ming S. Tsao, Stephanie Lheureux

Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada

Background and Purpose: Endometrial cancer (EC) is one of the most common gynecologic cancer, comprising a group of complex, heterogeneous subtypes with distinct features and varying outcomes. While advancements in EC treatment have progressed rapidly, managing mixed endometrial carcinomas remains challenging due to the variability of subtypes and their impact on clinical and biological behavior. This study highlights the potential of patient-derived organoid (PDO) models through the case of a young patient initially diagnosed with grade 1 endometrioid endometrial cancer, treated with endocrine therapy. The disease later progressed into a biologically aggressive mixed carcinoma exhibiting three distinct patterns: grade 1 endometrioid, large-cell neuroendocrine, and undifferentiated carcinoma.
Methods: A PDO model (OPTO.85) and a corresponding organoid-derived xenograft (ODX) model were generated from the patient's surgical specimen. Patient tissue and OPTO.85 models underwent WES, RNAseq and ATACseq to profile genomic and epigenomic alterations. A high-throughput drug screen was performed using the ApexBio FDA-Approved and Epigenetic Drug Libraries, as well as the OICR Kinase Inhibitor and Tool Compound Libraries. Organoids were plated on 1536-well drug plates, drugs were added at 2.5 µM concentration, and cell viability was measured after 6 days using Alamar Blue. Drug sensitivity curves were performed on individual compounds, using a 21-point dose-response. To assess *in vivo* drug response, OPTO.85 was implanted into NOD SCID mice, and mice were treated with BKM120 (50 mg/kg) +/- Cediranib (6 mg/kg) daily via oral gavage.
Results: Histopathological and genomic analyses confirmed that the PDO model accurately reflected the tumor's biology. Sequencing revealed oncogenic alterations in PIK3CA, ARID1A, and CTNNB1 genes across patient tissue, PDO, and ODX models. OPTO.85 PDO demonstrated sensitivity to PI3K inhibitors. RNAseq and ATACseq analyses revealed enrichment in VEGF and Wnt signaling pathways, suggesting potential therapeutic vulnerabilities. High-throughput drug screening identified sensitivity to VEGF inhibition. The VEGF inhibitor Cediranib demonstrated synergy with BKM120, significantly reducing OPTO.85 organoid growth. This combination also showed *in vivo* efficacy in the OPTO.85 ODX model, where it significantly suppressed tumor growth.
Conclusion: We demonstrate the potential of PDO models in cancer research. By leveraging RNAseq, ATACseq, and high-throughput drug screening, this study identified actionable targets in the VEGF and PI3K pathways and validated the synergistic effects of Cediranib and BKM120 in an endometrial cancer with complex histology and genomic profile. These findings highlight the value of PDO models as innovative tools for personalizing cancer treatment and developing effective therapies for complex endometrial malignancies.

#3998 Exploring fibroblast's influence on chemoresistance in PDAC using patient-derived organoids.

Jonathan Barajas[1], Carolyn Ruiz[1], Zhi Yang[1], Edward Agyare[2], Saun-Joo Yoon[3], Ba Xuan Hoang[1], Bo Han[1]

[1]Surgery, University of Southern California, Keck School of Medicine, Los Angeles, CA,[2]College of Pharmaceutical Science, Florida A&M University, Tallahassee, FL,[3]Department of Behavioral Nursing Science, University of Florida, Gainsville, FL

Pancreatic adenocarcinoma (PDAC) remains one of the most lethal malignancies, with a 5-year survival rate below 13%. A critical factor in this poor prognosis is the tumor microenvironment (TME), characterized by dense stroma comprising cancer-associated fibroblasts (CAFs), immune cells, and epithelial cells. CAFs secrete inflammatory cytokines like IL-6 and TGF-β and deposit ECM components, particularly collagen, leading to immunosuppression and chemotherapy resistance. This study examines the role of fibroblasts in modulating the efficacy of gemcitabine in PDAC using patient-derived organoids (PDOs). PDOs were co-cultured with either human fibroblasts and tumor cells or tumor cells with fibroblast-conditioned medium. The PDOs were cultured in collagen based matrices, which allows for manipulation of the ECM stiffness, using either a soft or stiff gel condition to mimic the heterogeneity of in vivo environments. Conditioned Medium was collected from fibroblasts grown in either soft or stiff ECM gels. Patient-derived cells were obtained from two patients: one Black (G43) and one White (G46). Results reveal significant differences in response to treatment between the two cells used. G43 is shown to be more sensitive to treatment overall in co-culture and condition medium, when compared to G46. However, when cells are compared to themselves, either in co-culture or conditioned medium, there are differences seen. In particular, G43 cells are more responsive to treatment in conditioned medium, in soft ECM, when compared to co-cultures. However, in stiff ECM conditioned media, this effect is not seen. Indicating that the stiffness of the ECM, rather than just secreted soluble factors alone, is a key driver of tumor cell behavior. The difference between co-culture and conditioned media supports the observations that fibroblast induced mechano-stress, aids in chemotherapy resistance. The results suggest that therapeutic strategies aimed at modulating the physical properties of the tumor microenvironment, such as ECM stiffness, could potentially improve treatment responses in PDAC.

#3999 Single cell transcriptomic comparison of patient derived ovarian cancer organoids and primary tumor epithelial signatures.

Bisiayo Fashemi, Vijayalaxmi Gupta, Yukihidi Ota, Moreniola Akande, Mary Mullen, Dineo Khabele

Washington University School of Medicine in St. Louis, St. Louis, MO

High-grade serous ovarian cancer (HGSOC) is a rare but deadly disease. Platinum-based therapy along with PARPi maintenance therapy, is recommended to 80% of patients. However, acquired resistance to platinum and PARPi remains an ongoing clinical challenge, highlighting the need for the development of targeted therapies. To address this, there is a need for better in vitro models that mimic the tumor microenvironment (TME). Unlike traditional 2D culturing methods, patient-derived ovarian cancer organoids (PDOs) form 3D structures that more accurately recapitulate multilayered, multicellular tissue. The overall objective of this study is to examine how HGSOC PDOs can be used better understand tumor reprograming and how this relates to patient tumor behavior and response to therapy. Primary and metastatic tumor samples were collected from diagnosed HGSOC patients for organoid generation. Organoids were cultured for 1-5 passages before being submitted for sc-RNAseq. In addition to organoid samples, matched tumor FFPE samples were submitted for comparison. Our pilot study revealed that PDOs contain a mixture of epithelial, stromal, and immune cells. Using Monocle3, we assessed epithelial cell development and differentiation over time and identified distinct cell trajectory projections across all samples. We identified three distinct cell states: early, intermediate, and late. Our PDO sample exhibited multiple branch points within the intermediate cell state, which reflects different cell fate decisions. These findings suggest the presence of sub-populations within the intermediate cell state that are not detectable or are masked in whole tumor FFPE samples. Additionally, we examined cell-to-cell interactions using CellChat. Fibroblast cells were the most prolific producers of signaling messages across all cell types, while dendritic cells and macrophages received the highest volume of incoming signals. In conclusion, PDOs recapitulate key aspects of the TME, and can be used to investigate cell fate decisions of epithelial tumor cells, and cell-to-cell communications. This platform offers a promising tool for studying tumor behavior, therapeutic resistance, and developing more targeted and effective treatments.

#4000 The significance of GLP-1R and its agonist in neuroendocrine neoplasms.

Sophia A. Hueser, Reese E. Townsend, Casandro J. Chan, Leona Rupp, Leopaul J. Chan, Dawn E. Quelle, Joseph S. Dillon, Andrew M. Bellizzi, James R. Howe, **Po Hien H. Ear**

University of Iowa, Iowa City, IA

Neuroendocrine neoplasms (NENs) are rare cancers originating from neuroendocrine cells that are found throughout the body. NENs are subdivided into 2 categories: Well-differentiated neuroendocrine tumors (NET) and poorly differentiated neuroendocrine carcinomas (NEC). Both NET and NEC are highly metastatic and difficult to treat. NEN cases are on the rise and yet the etiology remains unclear. Commonly used drugs such as proton pump inhibitors can promote NET growth and result in poor prognosis for NET patients. Nowadays, the diabetes and weight loss drug semaglutide, a glucagon-like peptide 1 receptor (GLP-1R) agonist, has gain extensive popularity and are currently being taken by over 15 million people in the USA. Semaglutide is contraindicated for NET patients with medullary thyroid carcinoma (MTC) or multiple endocrine neoplasia syndrome type 2 (MEN2) since some of these cancers express the GLP-1R. However, this area of research remains understudied. Little is known about the expression levels of GLP-1R in NEN from different anatomical locations and their response to semaglutide since these are rare cancers and few NET models are available for drug testing. We recently identified 2 human NET cell lines (GOT1 and NT-3) that express the GLP-1R and showed that semaglutide promotes tumor cell growth both in vitro and in vivo. In this study, we aim to investigate the levels of GLP-1R expression in a large collection of NEN tissue microarrays and determine the effects of semaglutide in novel GLP-1R positive NET patient-derived spheroid cultures. We stained for GLP-1R expression by immunohistochemistry in 357 NET tissue microarrays covering 7 classes of NEN: 78 pancreas NET, 62 duodenum NET, 33 ileal NET, 42 lung NET, 10 small cell lung NEC, 12 extrapulmonary visceral NEC, 29 thyroid NET, 12 gastric NET, 6 appendix NET, 6 rectum NET, 22 pheochromocytoma, 22 paraganglioma, and 23 Merkel cell carcinoma. Furthermore, we generated GLP-1R positive pancreas, ileal, and duodenal NET spheroids for drug testing. Our data showed 45% of duodenum NET, 17% of gastric NET, and 14% of pancreas NET stained positive for GLP-1R expression. Less than 2% of other classes NET or NEC stained positive for GLP-1R expression. Using newly established NET patient-derived spheroid models, we demonstrated that 100 nM of semaglutide accelerates tumor cell growth by 1.4 to 2-fold. Surprisingly, duodenum NET is the class of NET that frequently express GLP-1R and should be highly cautioned for the semaglutide usage.

#4001 Patient avatar models for ovarian cancer: Predicting patient response to treatment.

Erin M. George[1], Yuxi Zhou[1], Alfonso Pepe[1], Athena Tsingelis[1], Angie Rivera[1], Diego Pedro[1], Katriana Johnson[1], Dominick Dag[1], Said Cifuentes Maury[1], Lucia Mazzacurati[1], Jack Famiglietti[2], Ryan Smolchek[2], Duy Nguyen[1], W. Gregory Sawyer[1]

[1]Moffitt Cancer Center, Tampa, FL,[2]Aurita Corporation, Gainesville, FL

Background: Despite recent advances in targeted therapies, ovarian cancer (OVCA) remains the deadliest gynecologic malignancy, with approximately 80% of cases diagnosed at advanced stages and an equally high recurrence rate despite high response rates to frontline chemotherapy. Current OVCA models fail to accurately replicate the intricate biological landscape of metastatic disease. In response, we developed an innovative 3D in vitro system that preserves the unique cellular complexity of patient's original tumor. This model features key advancements, including prolonged (>21 days) culture of patient-derived microtumors, a perfusable microgel medium (Liquid-Like Solids, LLS), specialized microtiter plates for controlled "pumpless" media perfusion (Darcy Plates), and integrated high-resolution multiphoton confocal microscopy for *in situ* imaging.

Methods: Tumors were collected at the time of OVCA surgery and fragmented into 1 µl sized microtumors, which were then suspended into a 3D Liquid Like Solid (LLS) medium and cultured in a perfusion (Darcy) plate. Cell viability and proliferation were assessed over 21 days using LIVE/DEAD™ Cell Imaging Kit and EDU staining, respectively. Immunofluorescence microscopy was used to identify epithelial OVCA cells and immune cell populations. Drug screens were performed in a 48-well perfusion plate using standard of care chemotherapy and targeted therapies and compared to patient response.

Results: Optimal conditions were established using HPLM media supplemented with 10% FBS, 1% Pen/Strep, 2.5 mg/L Amphotericin B, 10 mM HEPES, 0.5 µM A83-01, and ITS, along with a perfusion flow rate of 100 µl/day per well. PAX8 nuclear expression confirmed epithelial OVCA cells. EDU staining demonstrated sustained cell proliferation and CD45 markers showed immune cell retention. Drug testing with standard of care chemotherapies like carboplatin and targeted agents revealed differential microtumor responses and was comparable to patient response.

Conclusions: OVCA's complex biology and extensive metastatic potential present significant clinical challenges. This study highlights the potential of 3D patient-derived microtumors as a robust platform for drug screening, high-throughput assays, and biomarker discovery. By retaining tumor heterogeneity and microenvironmental interactions, this model offers a promising avenue for improving therapeutic strategies and personalized treatment approaches in OVCA management.

#4002 Development of an organoid coculture model to investigate tumor immune interactions and therapeutic strategies in prostate cancer.

Kinjal Bhadresha, Jessica L. Horner, Jessica D. Kindrick, Douglas K. Price, Cindy H. Chau, William D. Figg

Genitourinary Malignancies Branch, National Cancer Institute, NIH, Bethesda, MD

While immunotherapy has demonstrated remarkable success in other malignancies, its efficacy in prostate cancer remains limited. Organoid models, capable of recapitulating patient-specific therapeutic responses, offer a promising platform for studying tumor biology and treatment responses. However, their utility in modeling immune interactions remains underdeveloped. To address this, we established an in vitro co-culture system of tumor organoids and immune cells to investigate tumor-immune interactions and identify novel therapeutic targets for aggressive prostate cancer. Using publicly available LuCaP PDX microarray datasets (GSE199596 and GSE113743), we identified the downregulation of antigen processing and presentation (APP) pathways, indicative of immune evasion mechanisms. Moreover, our analysis revealed overexpression of macrophage-polarizing factors, such as the STAT signaling pathway, ARG1, and MALAT1, which are implicated in tumor progression, metastasis, and drug resistance. To experimentally validate these findings, we developed and optimized a macrophage co-culture model utilizing organoids derived from LuCaP167 and LuCaP167CR, effectively recapitulating distinct tumor microenvironment characteristics associated with castration resistance. We further assessed for drug response using targeted and Immunotherapeutics within this system to evaluate their therapeutic potential. This proof-of-concept study highlights the potential of this sustainable and scalable co-culture platform for studying cancer-immune interactions ex vivo. The model serves as a valuable tool for evaluating the efficacy of existing therapeutics and discovering novel agents that enhance immune responses in advanced prostate cancer.

#4003 Improved drug screening and resistance monitoring using cancer organoids derived from dissociated tumor cells and circulating tumor cells.

Yi-chun Han, David Hsieh, Shian-Jiun (SJ) Shih

Cellentia Inc., Foster City, CA

Cancer is a complicated disease, and despite recent breakthroughs in cancer treatment, cancer remains the second leading cause of death globally, with metastatic cancers responsible for the large majority. To develop effective cancer drugs, we need reliable *in vitro* models that represent cancers accurately, particularly for metastatic cancers. However, current experimental models of human cancers *in vitro* face several limitations. Well-established cell lines typically emerged from long-term exposure to simple tissue culture conditions, while genomic and epigenetic instability contribute to their drift away from the characteristics of the original tumors. Because of the significant heterogeneity both within each tumor and within each type of cancer, cell lines fail to represent the diversity of the disease. Even using a very large number of cell lines still restricts the results to cancers whose cells grow on plastic in relatively uncomplex media formulations.
Aiming to overcome these limitations, we are leveraging a novel system for culturing tumor organoids. We culture tumor organoids from dissociated tumor cells (DTCs) from primary tumors, or circulating tumor cells (CTCs) from the blood of patients with solid tumors, in our proprietary culture platform. We have successfully cultured a wide range of DTC and CTC samples into tumor organoids, including samples from breast, colon, renal, lung, pancreatic, and prostate cancers, and we continue to add to the list. The successful expansion of these samples enables us to overcome the limited availability of such material and to apply methods typically reserved for cell line-based drug screening. We tested the drug sensitivity of these organoids to standard-of-care chemotherapeutics and other approved anticancer drugs, by cell viability assay. We also monitored organoid morphology longitudinally by brightfield microscopy during the treatment period. To evaluate drug resistance, we plated tumor organoids into Corning Elplasia plates, separating organoids into individual microcavities and enabling longitudinal tracking of each organoid throughout treatment. We continued culturing the surviving drug-resistant organoids after the treatment period and evaluated their changes in phenotype, morphology, mutation status, and gene expression. To characterize the resistance of individual organoids, we tested whether single-organoid RNA-Seq could provide more useful insights than bulk RNA-Seq.
DTC- and CTC-derived organoids typically exhibit differing sensitivities in our drug sensitivity screening. Since CTC-derived organoids resemble metastatic cancers, this platform enables the evaluation of anti-metastatic cancer drug efficacy, representing a major improvement over the current system.

#4004 Identification of predictive biomarkers of USP1 inhibitor sensitivity using ovarian cancer organoid models.

Ozge Somuncu, Nicholas Ashton, Alexandre A. B. A. da Costa, Sirisha Mukkavalli, Ramya Ravindranathan, Benjamin Lamarre, Huy Nguyen, Lisa Moreau, Joyce Liu, Geoffrey I. Shapiro, Kalindi Parmar, Alan D. D'Andrea

Dana-Farber Cancer Institute, Boston, MA

BRCA1-deficient tumors are unable to efficiently perform DNA repair by homologous recombination (HR) and are dependent on alternative DNA damage response pathways. PARP inhibitors have improved the progression-free survival of HR-deficient ovarian cancer patients in clinical settings. However, PARP inhibitor (PARPi) resistance often develops, and new therapies are required for these patients. USP1 is a deubiquitinating enzyme that controls DNA repair pathways through the deubiquitination of PCNA-Ub and the activation of Translesion DNA Synthesis (TLS). Inhibitors of USP1 result in a strong upregulation of PCNA-Ub levels and a dysregulation of TLS repair. Importantly, USP1 is synthetically lethal in BRCA1-deficient tumors (Lim et al., Mol Cell, 2018), and USP1 inhibitors (USP1is) are currently undergoing clinical development for the treatment of these cancers as monotherapy or in combination with a PARPi. USP1is kill *BRCA1*-mutated patient-derived ovarian cancer organoids (PDOs) and overcome PARPi resistance in *BRCA1*-WT PDOs (da Costa et al, Cancer Research, 2024). Sensitivity to USP1i strongly correlates with the accumulation of single strand DNA (ssDNA) gaps in both PARPi-sensitive and PARPi-resistant PDOs. Moreover, knockdown of RAD18, the E3 ubiquitin ligase required for PCNA ubiquitination, results in a reduction in PCNA-Ub levels and a corresponding reduction in ssDNA gaps, and in USP1i resistance. These findings suggest that ssDNA gaps and RAD18 levels in the tumors can predict the sensitivity to USP1 inhibitors. As *BRCA1*-mutated tumors express high levels of USP1 mRNA, we also investigated the relevance of this biomarker in predicting the sensitivity to USP1 inhibitors. Interestingly, the USP1i sensitivity correlated with increased USP1 mRNA expression, at baseline, in ovarian cancer PDO and patient-derived xenograft-derived organoid (PDXO) models. Similar results were obtained in *BRCA1*-deficient breast cancer cell line models with acquired PARPi resistance. USP1i sensitive ovarian cancer organoid models retained high USP1 mRNA levels upon PARPi treatment. The specific organoid models that were resistant to PARPi or USP1i monotherapy exhibited up to 40-fold increase in expression of USP1 mRNA and they were sensitive to a combination of USP1i plus PARPi. Taken together, in addition to the accumulation of ssDNA gaps and RAD18 levels, the relative expression of USP1 mRNA is a useful prognostic biomarker for patients who may respond to USP1 inhibitors, either as monotherapy or in combination with PARP inhibitors. These predictive biomarkers will be useful in monitoring the response of ovarian cancer patients enrolled in USP1i plus PARPi clinical trials.

#4005 Integrative clinical and molecular characterization of an international community resource of organoid models of human cancer.

Dina ElHarouni[1], Mushriq Al-Jazrawe[2], Seongmin Choi[3], Merve Dede[4], Toshinori Hinoue[5], Sean A. Misek[6], Heeju Noh[7], Luca Zanella[7], Yuen-Yi Tseng[6], Hayley E. Francies[8], Priya Sridevi[9], Rachana Agarwal[10], Cindy W. Kyi[11], Julyann Perez-Mayoral[11], Megan Stine[12], Eva Tonsing-Carter[11], James M. Clinton[13], The Human Cancer Models Initiative Network, Peter W. Laird[5], Calvin J. Kuo[14], Olivier Elemento[15], David L. Spector[9], Andrew D. Cherniack[6], Kyle Ellrott[16], Martin L. Ferguson[11], Rameen Beroukhim[6], Katherine A. Hoadley[17], Nicolas Robine[18], Andrew McPherson[3], Mathew J. Garnett[8], David A. Tuveson[9], Andrea Califano[7], Paul T. Spellman[19], Keith Ligon[1], Daniela S. Gerhard[11], Louis M. Staudt[11], **Jesse S. Boehm**[2]

[1]Department of Oncologic Pathology, Dana Farber Cancer Institute, Boston, MA,[2]Koch Inst. for Integrative Cancer Research at MIT, Cambridge, MA,[3]Computational Oncology, Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY,[4]Department of Bioinformatics and Computational Biology,, University of Texas MD Anderson Cancer Center, Houston, TX,[5]Department of Epigenetics, Van Andel Institute, Grand Rapids, MI,[6]Broad Institute, Cambridge, MA,[7]Department of Systems Biology, Vagelos College of Physicians and Surgeons, Columbia University Irving Medical Center, New York, NY,[8]Wellcome Sanger Institute, Cambridge, United Kingdom,[9]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY,[10]Frederick National Laboratory for Cancer Research, Frederick, MD,[11]Center for Cancer Genomics, National Cancer Institute, Bethesda, MD,[12]Information Management Services, Inc, Rockville, MD,[13]American Type Culture Collection, Manassas, VA,[14]Department of Medicine, Division of Hematology, Stanford University School of Medicine, Stanford, CA,[15]Institute for Computational Biomedicine, Weill Cornell Medicine, New York, NY,[16]Department of Biomedical Engineering, Oregon Health & Science University, Portland, OR,[17]Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, NC,[18]New York Genome Center, New York, NY,[19]Departments of Medicine and Human Genetics, University of California Los Angeles, Los Angeles, CA

The discovery of novel cancer therapeutics requires evaluation in representative laboratory models. However, existing model collections do not represent the full spectrum and scale of molecular diversity required to support biological discovery and drive pre-clinical research. Recent technologies have enabled more efficient derivation of cell-based models (e.g., organoids). However, systematic pan-cancer comparisons of the integrity of genomic, transcriptomic and epigenomic states following long term ex vivo expansion remain elusive. Here, we report on a large-scale international program—the Human Cancer Models Initiative (HCMI), consented more than 2,500 donors resulting in the production and subsequent characterization of 665 organoid, neurosphere and cell line models by whole genome, exome, methylome, and transcriptome analysis. Critical properties of the donors and tissues included 71 derived from populations beyond European ancestry, primarily African (54%), Asian (45%) and Indigenous American (1%); 448 have therapeutic treatment profiles from donor medical records, and 45 represented rare cancers. Integrative analysis between models and source tumors shows that 92-96% of 417 analyzed models were highly concordant by molecular characterization. The small number of discordant behaviors were associated with either cell culture media influences in glioblastoma or stromal infiltration or rare cell differentiation state shifts, which were validated by single cell RNA sequencing. We validate the utility of the resource studying therapeutic resistance to chemo-, targeted-, and immuno-therapies by integrative analysis of extrachromosomal DNA-based gene amplification and post-treatment mutational signatures. This internationally accessible community resource, including data and accompanying integrative software tool packages, provides a roadmap for capturing an increasing spectrum of cancer diversity in preclinical models.

#4006 HER2-positive patient-derived xenograft organoids (PDXOs) as a novel preclinical tool to evaluate new therapies against HER2-driven cancers.

Fu-Ju Chou, Yamini Nallana, Hsiu-Wen Tsai, Mara Gilardi, Stefano Cairo, BanuPriya Sridharan

Champions Oncology, Inc., Hackensack, NJ

The human epidermal growth factor 2 receptor (HER2) is a key member of the receptor tyrosine kinase superfamily. Alterations in HER2 have been identified in various malignancies, including approximately 20% of non-small-cell lung cancer (NSCLC) and about 30% of breast cancer (BRCA). These HER2 alterations encompass gene mutations, gene amplification, and protein overexpression, all of which are associated with tumorigenesis and contribute to enhanced cancer cell proliferation. Cancers with HER2 alterations often exhibit more aggressive behavior, a higher recurrence rate, and a poorer prognosis. The introduction of HER2-directed therapies has significantly improved therapeutic outcomes. FDA-approved therapeutic options for HER2-positive cancers can be categorized into three major groups: tyrosine kinase inhibitors (TKIs), antibodies, and antibody-drug conjugates (ADCs). These treatments specifically target the HER2 receptor or its downstream signaling pathways. However, many studies show that nonselective TKIs and HER2 naked antibodies demonstrate limited therapeutic effectiveness in HER2-positive cancers. In contrast, HER2-directed ADCs selectively deliver cytotoxic compounds to HER2-positive tumor cells, resulting in better responses and fewer adverse effects, as evidenced by numerous preclinical and clinical studies. In our current work, to showcase our well-characterized PDX-O platform BRCA and NSCLC models were selected and assessed to be HER2 positive as evidenced by complete and intense membrane staining identified in patient-derived xenograft organoids (PDXOs) tissue blocks. Furthermore, from early low passages, assay-ready cells were seeded in a 96-well plate high throughput format at a density of 5000 cells per well. Well-defined 3D structures formed after 3 days, and the models were evaluated for morphology and proliferation assessment. Cytotoxicity controls: 10 µM Staurosporine and 0.1% DMSO were added to each plate to serve as plate controls and used in determining the Z factor of the assay. The models were tested for efficacy of Lapatinib (TKI); and Enhertu and Kadcyla (ADC) and their corresponding Isotypes. All models demonstrated a classic 3D morphology with > 150 µm diameter formed 72 hrs. after seeding; and were then dosed. The duration of drug incubation was 6 days after which Cell-Titer-Glo® reagent was added to all wells to determine half-maximal inhibitory concentration (IC50). The assay was robust with a Z factor > 0 and a high Signal: Noise ratio. Specifically for one model, the IC50 of Lapatinib was 69.15 µM. Both ADCs: Kadcyla and Enhertu, exhibited greater specificity and efficacy compared to their isotype controls. The ex vivo HER2-positive CTG3D platform represents a reliable and powerful tool for screening newly designed HER2 treatment regimens.

#4007 Building 3D ex vivo models as a powerful platform for anti-cancer drug and biomarker discovery.

Fu-Ju Chou, Annamaria Rapisarda, Mara Gilardi, Stefano Cairo, BanuPriya Sridharan

Champions Oncology, Inc., Hackensack, NJ

Patient-derived models have increasingly been adopted into target validation, lead-optimization and secondary screening campaigns to move translation ahead in drug discovery. While 2-dimentional cell line in vitro models continue to play a pivotal role in early screening studies, new advanced in 3D patient derived organoids (PDO) and patient derived xenograft organoids (PDX-O) technology have made it suitable to meet the rigor and reproducibility of traditional screening metrics. At Champions Oncology, we have built a successful pipeline of ex vivo models from pre-treated, metastatic, and genetically diverse cohorts across varied indications. PDX-derived cell cultures serve as (i) physiologically relevant high throughput platforms for early or clinical hypothesis testing, (ii) cellular systems to gain mechanistic and phenotypic insights into drug mode of action in 3D tumor microenvironment, and (iii) cost-effective translational bridge to well-informed in vivo studies. Ex vivo models have been widely established and characterized for non-small cell lung cancer (NSCLC), colorectal (CRC), and other epithelial tumor types. Indications such as renal cell carcinoma (RCC), Diffuse large B-cell lymphoma (DLBCL), gastric and bladder cancer, glioblastoma multiforme (GBM), prostate cancer, and sarcoma are less common and often a size-able cohort size rarely exists. In this poster, we showcase our recent efforts on the iterative media optimization process, growth kinetics, and morphology of models from these rare indications and characterization data that establish them front and center as valid preclinical screening tools. Through careful media formulation, the appropriate standard of care controls (SoC), and vigorous assay development criteria we show the versatility of these models for both efficacy and complex mechanistic readouts when probed with various modalities including antibody-drug conjugates (ADC) and anti-sense oligos (ASO). Our experience establishing 3D microstructures from various indications by iterative optimization of culture conditions is further validated by our comprehensive quality control (QC) procedures and deep molecular and pharmacological characterization. In summary, through a series of examples, we highlight the application of Champions TumorGraft3D Ex vivo models in enhancing the biological and clinical fidelity of novel modalities of drugs and/or novel drug combinations.

SCIENCE AND HEALTH POLICY: Cancer Survivorship and Patient Communication / Patient Advocacy and Engagement Poster Session

#4011 Health maintenance reframed: Insights from Black prostate cancer survivors on prioritizing wellbeing.

D. Fullwood, M. Young, F. Odedina;
Mayo Clinic Jacksonville, Jacksonville, FL

Background: Prostate cancer continues to disproportionately affect Black men, who face notonly higher incidence, but also more severe outcomes compared to other racial and ethnicgroups. While advancements in survivorship care have been made, significant disparities remain, highlighting the critical need for research that addresses the distinct challenges faced by Black men during their survivorship journey. In the face of these ongoing inequities, understanding the advocacy, resilience, and lived experiences of Black men post-treatment is essential. By exploring these factors, our study aims to uncover actionable strategies to enhance survivorship care and support, driving the development of targeted interventions that can close the gap in outcomes for this underserved population.
Methods: We conducted a secondary analysis of qualitative interviews with 31 Black prostatecancer survivors participated in the CaPCaS study, aiming to explore their perspectives onsurvivorship and advocacy. A Grounded Theory approach was employed to rigorously analyze the participants' narratives. Through open and axial coding, we systematically identified key themes from the transcribed, verified, and deidentified audio recordings, ensuring a comprehensive exploration of their post-treatment experiences.
Results: Our analysis revealed four central themes that highlight the evolving health behaviors and coping mechanisms of participants. First, Self-care and Health Management emerged, showcasing empowered decision-making around exercise, dietary improvements, and hydration. Second, Increased Awareness and Prioritization underscored the shift toward heightened health consciousness, with participants emphasizing the importance of personal health and prioritizing food quality. Third, the theme of Emotional and Social Support illustrated how shared experiences fostered comfort and perspective, reinforcing ongoing health maintenance. Finally, participants reframed Health Challenges, viewing them as minor nuisances rather than deterrents, bolstered by medical reassurance and a strong survivor mindset.
Conclusion: These findings emphasize the importance of holistic health strategies in improving the quality of life for Black men during their post-treatment prostate cancer survivorship journey. By fostering empowered self-care, prioritizing health awareness, and leveraging emotional and social support, survivors can navigate ongoing health challenges with resilience and confidence. The reframing of health issues as manageable aspects of their lives further underscores the potential for interventions to enhance long-term well-being and reduce disparities in prostate cancer survivorship.

#4012 Patient perspective on the prevalence and severity of adverse psychosocial outcomes amongst those treated for lymphoma or chronic lymphocytic leukemia: An analysis of the Lymphoma Coalition's 2024 Global Patient Survey.

Steve E. Kalloger[1], Amanda Watson[2], Shawn Sajkowski[3], Lorna Warwick[4]

[1]Lymphoma Coalition, Mississauga, ON, Canada,[2]Amanda Watson, Mississauga, ON, Canada,[3]Shawn Sajkowski, Mississauga, ON, Canada,[4]Lorna Warwick, Mississauga, ON, Canada

Patients who have received treatment for lymphoma or chronic lymphocytic leukemia (CLL) experience numerous side effects which can have a profound impact on health-related quality of life (HRQoL). Some of the most underreported are adverse psycho-social outcomes (APSO). We explored the prevalence and severity of APSO's amongst a globally derived sample from this patient population. Patient reported outcomes (PRO's) were collected from an anonymous global survey directed at patients with lymphoma and CLL. Nineteen different APSO's were pre-specified in the survey. Prevalence was captured with severity assessed using a 5-part Likert scale. Latent class analysis (LCA) was used to cluster patients with a propensity for experiencing multiple APSO's. Resultant clusters were investigated to determine if they were enriched for selected demographics. Responses were received from 5984 patients who had received treatment for lymphoma or CLL where the median age was 64, ranging from 18 - 94 years. Sixty percent were female. Fear of progression/relapse was the most prevalent APSO (49%; Table 1). Most APSO's had severity scores above 3 indicating a moderate level of severity (Table 1). LCA identified a cluster comprising 12% of the patients who experienced all APSO's at a higher rate than the remaining patients. Both younger age and female sex were significantly enriched in this cluster ($p<0.0001$). The results suggest that a segment of patients experience multiple APSO's which can compound to create a significant overall degradation in HRQoL. While younger age and female sex are enriched in this segment, further exploration is needed to identify those with heightened risk for multiple APSO's. The application of mitigation strategies in those suffering from APSO's may help to improve patient's HRQoL during treatment, recovery and rehabilitation.

Psychosocial Outcome	Prevalence (N = 5984)	Severity Score (N)
Fear of progression/relapse	49%	3.2 (2904)
Concern relating to being immunocompromised	38.4%	3.2 (2281)
Anxiety/nervousness	35%	3.1 (2067)
Worry	33%	3.2 (1939)
Concerns about physical appearance	27.4%	3.2 (1619)
Depression/sadness	26.6%	3.1 (1567)
Fear of dying	21.7%	3.3 (1279)
Isolation of loneliness	21.5%	3.3 (1273)
Loss of interest in usual activities	19.1%	3.3 (1125)
Anger	18.5%	2.8 (1087)
Loss of Self-Esteem	18.1%	3.2 (1071)
Changes in relationships	16.8%	3.2 (994)
Feelings of worthlessness	16.2%	3.3 (962)
Grief or loss	15.7%	3.2 (929)
Fear of being incapacitated	14.6%	3.4 (885)
Loss of sense of purpose	12.2%	3.4 (719)
PTSD	9.1%	3.2 (536)
Changes in faith or beliefs	4.4%	3.3 (257)
Conflicts between beliefs and cancer treatments	2.4%	3.3 (139)
Other	2.4%	3.6 (132)
None	18.4%	N/A

#4014 Prognostic evaluation of breast cancer long-term survival outcomes: contributions from a Brazilian hospital-based cohort.

Alessandra B. de Souza[1], Daniely R. Freitas-Alves[1], Taiana S. L. da Silva[2], Vanessa Indio-do-Brasil[1], Rosane Vianna-Jorge[3]

[1]Oswaldo Cruz Foundation, Rio de Janeiro, Brazil,[2]National Cancer Institute, Rio de Janeiro, Brazil,[3]Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Breast cancer is currently the second most common neoplasm worldwide and the leading cause of death by cancer among women. Treatment advances based on molecular classification of tumors have contributed to reduce mortality in many developed countries. Nevertheless, the growing incidence worldwide poses additional challenges to adjust prognostic evaluation to changing epidemiological profiles in distinct populations. In this regard, the present study evaluates a current prospective cohort of Brazilian women with primary unilateral and non-metastatic breast carcinoma, who were diagnosed and started treatment between 2009 to 2013 at the Brazilian National Cancer Institute (INCA #129/08). The study aims to characterize the contribution of well-known histopathological characteristics, in combination with clinical and sociodemographic aspects, for prognostic evaluation of survival outcomes. Here, we present the first analyses after completing ten years of follow-up. Survival curves were estimated using the Kaplan-Meier method, and the impact of individual variables on disease-free or overall survival rates was estimated by calculation of their hazard ratios (HR) and 95% confidence intervals (95% CI). Multivariate Cox proportional hazards regression models were used to calculate the adjusted HR (HRadj) and respective 95% CI. Total patient follow-up was 152,832 person-months, with a median follow-up time per person of 12.5 years (y). Disease-free survival (DFS) was 80.2% (5y) and 64.8% (10y), with a median of 99 months (95% CI 97.8 - 102.0). Overall survival (OS) was 83.1% (5y) and 69.6% (10y), with a median of 98 months (95% CI 96.0 - 100.7). Tumor staging ≥ IIb and molecular classification as HER-2 or triple-negative were prognostic of both DFS and OS, either for 5y- or 10y- models. In addition, obesity was significantly associated with worse DFS (HRadj = 1.554; 95% CI 1.021 - 2.365) and marginally contributing for OS (HRadj = 1.545; 95% CI 0.998 - 2.391), but only in 5y-models. In contrast, the 10y-models indicated worse outcomes for patients who self-reported as black or brown: HRadj = 1.365 (95% CI 1.048 - 1.777) for DFS or HRadj = 1.511 (95% CI 1.051 - 2.171) for OS. It is not clear how color/race is affecting survival in our cohort, as no significant differences were found for clinical or histopathological characteristics at diagnosis. We are currently including data from gene polymorphisms as an attempt to find new molecular biomarkers that might improve prognostic evaluation of breast cancer.

#4015 Mitigating Financial Toxicity b legal and financial navigation for individuals diagnosed with cancer, caregivers, and health care professionals.

Laura Beilke, Melanie Grant

Triage Cancer, Chicago, IL

Background and Purpose: After a cancer diagnosis, individuals are faced with a variety of legal and financial issues that leave individuals diagnosed with cancer and their caregivers with complex questions about insurance coverage, accessing government benefits, and employment. Without accurate information, individuals are unable to make educated decisions, which results in financial consequences, often referred to as financial toxicity. Triage Cancer's Legal & Financial Navigation Program (LFN) offers free expert guidance to individuals coping with cancer, their caregivers, and health care professionals on a wide range of legal and practical issues. The LFN provides the accurate information and resources needed within the patient's own community to help mitigate financial toxicity.

Methods: The LFN provides one-on-one guidance on practical strategies and referral resources that can empower individuals and caregivers facing cancer-related legal and financial challenges. Also, the LFN provides this information to health care professionals to guide their patients through these difficult decisions. Practical strategies are provided to help individuals understand complex legal and financial matters, enabling them to make informed decisions tailored to their unique circumstances. The efficacy of the program is measured through voluntary survey response. Callers receive a follow-up email with a one question survey within a day of the initial call. After three weeks, the caller also receives a detailed survey.

Results and Outcomes: In 2023, the LFN had 1,759 callers from 49 states, the District of Columbia, US territories, and other countries. After the call, individuals are provided with a one question survey asking if they feel better prepared after speaking with Triage Cancer. 95% of respondents say they "feel better prepared" after speaking with Triage Cancer. Callers receive a follow up email with a link to the survey three weeks after our initial email, which includes six follow up questions to measure the efficacy of the program. • 91% of callers strongly agreed or agreed that Triage Cancer answered their questions. • 88% of callers agreed or strongly agreed that they would use the program again in the future. • 88% of callers agreed or strongly agreed that they had actionable next steps. • 90% of callers agreed or strongly agreed that the follow up resources and materials were helpful. • 89% of callers agreed or strongly agreed that they were better prepared. • 85% of callers agreed or strongly agreed that their stress and/or anxiety levels decreased.

Conclusions: The LFN provides tailored education around cancer-related legal issues in order to improve the quality of life of individuals coping with cancer. The LFN provides individuals with the knowledge they need to address the practical and financial issues they encounter when faced with a cancer diagnosis.

#4016 Understanding clinician level barriers and facilitators to managing cancer survivors with chronic conditions at community health centers.

M. Museitif[1], B. Balasubramanian[1], G. H. Walton[1], L. A. Shay[1], H. Ma[2], S. Lee[3];
[1]University of Texas Health Science Center, Houston, TX, [2]MD Anderson Cancer Center, Houston, TX, [3]University of Kansas Medical Center, Kansas City, KS

Background: Primary care clinicians (PCCs) play a critical role in delivering comprehensive care to patients with chronic conditions, including cancer survivors. Community health centers (CHCs) serve as primary healthcare facilities, providing accessible and affordable care to underserved and vulnerable populations, including cancer survivors. Despite the essential role of CHCs, significant gaps remain in knowledge and research training regarding survivorship care within these settings. Given the rising population of cancer survivors in the United States, addressing these gaps is crucial to ensuring high-quality survivorship care. This study aimed to assess the barriers and facilitators PCCs encounter when providing survivorship care to cancer survivors with chronic conditions at CHCs.
Methods: Qualitative in-depth interviews were conducted with PCCs from two healthcare systems, encompassing four urban CHCs in Texas during two phases. The research team developed semi-structured interview guides to explore barriers and facilitators in survivorship care. Interviews were conducted via phone or WebEX, a virtual platform, and were audio-recorded and transcribed verbatim by a certified transcription service. A team member reviewed the transcripts to ensure accuracy. Data were analyzed using an adapted immersion and crystallization approach, guided by the Chronic Care Model (CCM), to identify key themes related to survivorship care.
Results: Ten PCCs participated in the study. Qualitative analysis identified five key barriers to delivering high-quality survivorship care: (1) role ambiguity between PCCs and specialists, (2) inaccessibility of survivorship clinical guidelines, (3) limited communication between PCCs and oncologists, (4) inconsistent documentation of medical history, and (5) patient socioeconomic challenges, including financial difficulties. Pragmatic solutions were proposed to mitigate these barriers, and a patient-centered approach emerged as a key facilitator in improving care delivery.
Conclusions: These findings highlight valuable opportunities to enhance survivorship care within CHCs by addressing identified barriers and leveraging facilitators to foster patient-centered, high-quality care for underserved cancer survivors. The utilization of the CCM framework provided deeper insights into the specific gaps and challenges PCCs face in CHCs.

#4017 The prevalence of comorbid chronic conditions among cancer patients in the All of Us Research Program.

Sue E. Kim, Crystal Rubalcava, Jennifer Tsui

Population and Public Health Sciences, USC - University of Southern California, Los Angeles, CA

Cancer survivors in racial/ethnic minority, low socioeconomic status, and aging populations are more likely to have other chronic conditions, including 30% of cancer survivors who have diabetes or cardiovascular disease, many of which were pre-existing before cancer diagnosis. We examined the prevalence of comorbid chronic conditions among cancer patients in the All of Us Research Program (AoURP) cohort (2016-2024) and disease code identification from the National Library of Medicine and compared demographic, social determinants, and health-related social needs across specific cancer diagnosis sites. Comorbidities were self-reported data from the personal health history survey and found in electronic health records. We assessed the socioeconomic status (SES) barrier as a composite score (0-5) that combined education (≤ high school), income (≤ 35K), uninsured, housing (rent/other), and employment status (unemployed).

We identified 12,415 breast cancer, 4,047 colorectal cancer (CRC), 3,126 lung cancer, and 7,951 prostate cancer cases among 574,000+ AoURP participants, as of Nov 2024. 54% of breast, 65% of colorectal, 76% of lung, and 57% of prostate cancer patients reported having at least 1 comorbid condition. About 20% had at least 2 comorbidities. Obesity and diabetes were the two most common conditions for breast and colorectal cancer, chronic pulmonary disease (CPD) and diabetes were the most common for lung cancer, and diabetes and moderate/severe kidney disease for prostate cancer. Among the other top 5 common comorbid conditions were rheumatoid arthritis, liver disease, and congestive heart failure.

About a quarter of lung cancer patients with diabetes had 3 or more SES barriers, compared to 23% of colorectal, 20% of breast, and 12% of prostate cancer patients. Also, about 28% of lung cancer patients with liver disease had 3 or more SES barriers. A smaller proportion of CRC patients (19%) with obesity had 3 or more SES barriers, compared to 18% of lung, 13% of breast cancer, and 8% of prostate cancer patients.

The highest proportion (>40%) of Black patients reported having diabetes for all cancer types, except prostate cancer (where Hispanic patients reported the highest rate). A higher proportion of Black patients with breast and colorectal cancer had obesity while similar proportions were reported between Hispanic and Black lung and prostate cancer patients.

Understanding comorbidity prevalence in this national sample of cancer survivors provides insight into care coordination, disease management, and team-based care strategies needed to improve cancer survivorship care quality and address inequities in cancer survivorship outcomes. Further assessment of All of Us Research Program data, including patterns of care through EHR data and lab and self-reported survey data, may provide opportunities to comprehensively assess and intervene in survivorship care improvements.

#4018 Primary gallbladder cancer association with cardiovascular mortality: A 20 year population-based retrospective study.

Oboseh J. Ogedegbe[1], Olanipekin Ntukidem[1], Asfand Y. Cheema[2], Sakshi Bai[3], Kai He[4], Utkarsh Acharya[4]

[1]Internal Medicine, Trinity Health System, Ypsilanti, MI,[2]Internal Medicine, Taussig Cancer Center, Cleveland, OH,[3]Internal Medicine, Henry Ford Health System, Jackson, MI,[4]Hematology/Oncology, The Ohio State University Comprehensive Cancer Center, Columbus, OH

Introduction: Primary gallbladder cancer (PGC) is the most common cancer of the biliary system. It is often late presenting, leading to a poor prognosis. While evolution and progress in medicine have resulted in reduced mortality rates in PGC, non-cancer deaths are at an increasing trend. Cardiovascular mortality (CVM) plays a significant role in this trend. We holistically evaluated the CVM risk in patients with PGC.

Methods: We utilized the Surveillance, Epidemiology, and End Results (SEER) database to retrieve PGC patients with CVM from 2000-2021. CVM was defined as death from diseases of the heart, hypertension without heart disease, cerebrovascular diseases, atherosclerosis, aortic aneurysm and dissection, and other diseases of arteries, arterioles and capillaries. We then obtained the standardized mortality rates (SMR) and absolute excess risk (AER).

Results: Total number of PGC patients who died from cardiovascular diseases was 706. Patients with non-cardiovascular causes of death and patients with unknown age, race and cancer stage were excluded. We compared the cardiovascular disease (CVD) mortality risk of each group to the general US population and noted significantly increased CVD SMRs. Regarding race, Asians or Pacific Islanders had the highest risk of CV mortality (SMR 6.80, CI 5.10-8.87, $p < 0.05$), followed by Blacks (SMR 6-15, CI 4.88-7.66, $p < 0.05$), then American Indians/Alaska Natives (SMR 5.89, CI 1.60-15.08, $p < 0.05$) and Whites (SMR 3.99, CI 3.67-4.33, $p < 0.05$). Based on cancer stage, distant had the highest risk of CV mortality (SMR 17.79, CI 14.62-21.44, $p < 0.05$) while localized had relatively the lowest risk (SMR 3.10, CI 2.59-3.69, $p < 0.05$) with regional being (SMR 4.52 CI 4.00-5.08, $p < 0.05$). In terms of age, patients aged 40-59 years had an SMR of 20.89 (CI 15.65-27.33, $p < 0.05$), 60-79 years had an SMR of 5.14 (CI 4.60-5.74, $p < 0.05$), while 80 years and above had an SMR of 3.34 (CI 2.99-3.72, $p < 0.05$).

Conclusion: Our analysis examines the magnitude of CVD-related deaths in PGC patients. Compared to the general United States population, patients with PGC have a higher risk of cardiovascular mortality. Therefore, patients with PGC should be screened for CVD to provide more holistic care in this patient population.

#4019 Effect of time interval from diagnosis to treatment on mortality in colorectal cancer by stage in Korea.

Ji Yoon Baek[1], Curi Park[1], Ji Won Park[2], Aesun Shin[1]

[1]Seoul National University, Seoul, Korea, Republic of,[2]Seoul National University Hospital, Seoul, Korea, Republic of

Background: Colorectal cancer (CRC) is one of the most common cancers worldwide and a major contributor to the disease burden. Treatment delay, defined as the time interval from diagnosis to treatment initiation, is an emerging public health concern. While treatment delays in cancer may not directly cause fatal health outcomes, some studies have reported associations though the evidence remains inconclusive. Therefore, we aimed to investigate the impact of treatment delays on all-cause mortality in CRC patients in Korea, stratified by cancer stage.

Methods: A retrospective cohort study was conducted using the sample cohort of CRC patients from the Cancer Public Library Database, which integrates data from four public sources in Korea. The study included patients diagnosed with CRC (ICD-O-3: C18-C20) between 2012 and 2019, with mortality follow-up until 2021 and health service utilization data until 2022. Treatment delay was categorized using two thresholds: 3 months and 6 months. Cumulative all-cause mortality was analyzed with the Kaplan-Meier methods, and group differences were assessed with log-rank tests. Adjusted hazard ratios (aHR) and 95% confidence intervals (CI) were estimated by Cox proportional hazard models, adjusted for sex (male/female) and age at diagnosis (<50, 50-59, 60-69, 70-79, ≥80). All analyses were stratified by SEER cancer summary stage (localized, regional, distant).

Results: Of 53,485 CRC patients, 28,520 were analyzed. Most had regional stage CRC (54.7%). The proportion of patients having treatment delays of ≥3 months was highest in the localized stage (5.5%), then in the distant (3.6%) and regional (1.7%) stages, with a similar trend observed for delays of ≥6 months (localized: 2.4%; distant: 1.7%; regional: 0.8%). Longer treatment delays were significantly associated with higher 5-year cumulative mortality across all stages (<3 months vs ≥3 months vs ≥6 months; localized: 12.7% vs 21.4% vs 37.6%; regional: 22.9% vs 35.4% vs 52.2%; distant: 77.4% vs 84.0% vs 84.1%; p<0.001 for all). Treatment delays of ≥3 months, compared to <3 months, significantly increased the risk of death, with the highest risk in the regional stage (aHR: 1.69 [95% CI: 1.42-2.02]), followed by localized (1.55 [1.24-1.93]) and distant (1.29 [1.09-1.53]) stages. For delays of ≥6 months (vs <6 months), the highest risk was examined in localized stage (2.91 [2.24-3.78]), followed by regional (2.63 [2.07-3.35]) and distant (1.47 [1.15-1.89]) stages.

Conclusion: Treatment delays of more than 3 months were significantly associated with higher all-cause mortality across all stages of CRC in Korea, with a more pronounced impact in the localized and regional stages. These findings emphasize the importance of timely treatment, especially for early-stage colorectal cancer.

#4020 Review of literature on the effect of stress and anxiety among African-American breast and endometrial cancer patients.

Gabrielle Ford, Zakirah L. Abdul-Hameed, Amirah Burton, Brian M. Rivers, Desiree Rivers

Morehouse School of Medicine, Atlanta, GA

African American women face disproportionately higher rates of breast and endometrial cancer recurrence compared to their Caucasian counterparts, highlighting an urgent need to explore the role of stress in cancer progression and recurrence. This study conducts a systematic review of the literature to evaluate the efficacy of stress and anxiety interventions in reducing cancer risk among African American survivors. By analyzing 25 articles from 2014-2024, this review examines the correlation between psychological stress, biological stress markers, and cancer outcomes. The findings emphasize the significant impact of stress on health outcomes, particularly in the context of cortisol's role in metabolism and immune response. The review also explores coping mechanisms, including social support, diet, and exercise, tailored to African American women's cultural and socioeconomic needs. The results underscore the critical need for targeted interventions to address stress in this population, with implications for improving cancer survivorship and advancing healthcare equity.

#4021 Science, prevention, and fun: The educational revolution of the project Cancer School.

Altair Filho M. Souza[1], **Talita Aguiar**[2], Andreia Juliana Rodrigues Caldeira[1]

[1]Instituto Academico de Ciencias da Saude e Biologicas, Universidade Estadual de Goias, Anapolis, Brazil,[2]Columbia University Irving Medical Center, New York, NY

Introduction: Cancer remains a leading cause of mortality worldwide, necessitating innovative strategies to enhance public understanding of its biology, risk factors, and prevention. The *Cancer School* project is part of the extension program "Laboratório de Educação Científica e Popularização da Ciência (LECPOP) from the State University of Goiás and seeks to bridge this gap by integrating science communication with educational tools that present complex topics like mutations and cancer in an accessible and engaging way. Leveraging social media (@cancerschooloficial) as a dissemination platform, the initiative has amassed over 10,000 followers and achieves consistent engagement through interactive posts, stories, and videos, fostering a virtual community for cancer education.

Methods: The project developed two essential educational tools: the *Cromocel* booklet and a board game. *Cromocel* features an engaging narrative led by characters Cromo, Alê, and Karcin to introduce concepts such as chromosome structure, mutations, and cancer progression. The board game, designed in the shape of a chromosome, includes themes like cancer biology, risk factors, and prevention. The development process involved 1) Defining educational objectives, 2) Conducting a literature review, 3) Designing prototypes, 4) Writing a question-and-answer script, and 5) Establishing gameplay rules. Both tools were piloted at the XIV National Research Meeting in Science Education (2023) in Caldas Novas, Goiás. Participants were invited to engage with the tools at an interactive stand and complete a short survey assessing the pedagogical effectiveness, engagement, and usability.

Results: The pilot study included 20 participants: 2 K-12 students, five teachers, and 13 undergraduates. All participants reported enjoying the tools, with 70% identifying "prevention" as the most straightforward concept to understand, likely due to their relevance to everyday life. The project's social media presence amplified these efforts, with posts about the educational tools achieving an average engagement rate of 15%, demonstrating strong interest from a broad audience. Followers frequently interacted with content, asking questions and sharing posts, further expanding the initiative's reach.

Conclusion: The *Cancer School* project demonstrates the potential of combining traditional educational tools with digital engagement to promote cancer literacy. The initiative successfully enhances public understanding of cancer biology and prevention strategies by integrating creative methodologies like booklets and games with social media outreach. These results highlight the value of active learning and digital platforms in science communication, offering a scalable model for addressing public health challenges.

#4022 Leveraging multidisciplinary partnerships to overcome barriers to prevent cancer disparities in healthcare.

Regina M. Sharma[1], Frankie M. Palazzolo[2], Shelley G. Skinner[3], Klementina Sula[4], Evelyn M. Jiagge[5]

[1]Detroit Justice Center, Detroit, MI,[2]Detroit Innovation Hub, Detroit, MI,[3]Genentech, Plymouth, MI,[4]Jewish Federation of Detroit, Bloomfield Hills, MI,[5]Henry Ford Health System, Detroit, MI

Background: Racial disparities in funding continue to be one of the most significant barriers to Black women research scientists progressing; impacting career trajectories and access to research grants. Because of this, there is a dire need to build connections and overcome barriers for funding opportunities for research in underrepresented communities. A multidisciplinary team of fund development professionals, research scientists, and pharmaceutical professionals came together with one intended goal; transition barriers into opportunities by navigating the complexities of a large healthcare organization and successfully build a leadership-driven model that demonstrates when people come together across a variety of entities and disciplines results will follow.
Method:

1. Navigated the complexities of a large healthcare organization
2. Successfully built a leadership-driven model to produce results
3. Engaged stakeholders by overcoming obstacles by leveraging educational opportunities to help the community and prospective donors understand the complexities of inequity in healthcare.
4. Created an elevator pitch that we could utilize in various public spaces - meetings, conferences, the community
5. Translated the work into ideas non-scientists could understand
6. Made connections across US and global Biotech, Pharma, and Precision Medicine organizations

Results: Over four years we have raised over 3.5 million dollars to support basic, translational, and qualitative research in Detroit and globally. With funding we received at Henry Ford Health, through our partnership, we generated needed preliminary data on the unique aggressive biology of African breast cancers, that was used for a successful NIH grant application. Through our initiative Precision Medicine for Aggressive Breast Cancer PMABC, we have supported research in Ghana, Tanzania, Rwanda, Uganda, Botswana, Nigeria, and South Africa. We have supported researchers in these countries with the training and education needed for cancer research in the country and have helped raise seed funding for local cancer research. We have ongoing community-based research in Detroit and SE Michigan to address barriers to Black/African Americans participating in cancer clinical trials.
Conclusion: In this session, we will review a framework that will highlight how to organize stakeholders, develop a plan of engagement, and leverage the necessary resources to execute a leadership-driven model.

#4023 ACCESS: Advancing childhood cancer experience, science and survivorship in Canada.

Stephanie A. Grover[1], **Christine J. Williams**[2], Adrienne Co-Dyre[3], David Malkin[1], James A. Whitlock[1], on behalf of the ACCESS network.

[1]The Hospital for Sick Children, Toronto, ON, Canada,[2]Ontario Institute for Cancer Research, Toronto, ON, Canada,[3]King's University College at Western University, London, ON, Canada

In Canada, roughly 1,500 children and adolescents are diagnosed with cancer each year. Advocacy for enhanced childhood cancer research and care in Canada by patients, families and the academic community led to a $23M investment through the Canadian Institutes of Health Research to establish a national pediatric cancer consortium (termed 'ACCESS'). ACCESS' mission is t*o ensure every child with cancer in Canada has access to the latest scientific advances, diagnostic tools, therapies, and supportive care leading to better outcomes and quality of life.* ACCESS is a unique collective of scientists, healthcare providers, industry leaders, partner organizations, advocates, patients, and their families who are conducting research, changing policy, and developing new tools to ensure better lives for children who experience cancer in Canada and for their loved ones. The goal is to implement transformative change in the delivery of fair, fast, effective, and safe cancer care for all children. ACCESS is driven and led by people with lived experience of pediatric cancer and framed by the patient journey. To date, ACCESS has unified a national community. It has: connected a community of more than 750 people and stimulated a culture of open communication in which diverse viewpoints, including those of people with lived experience of cancer, are sought and valued; built productive and effective partnerships to connect and leverage the work of organizations working to improve childhood cancer; initiated more than 20 new innovative pan-Canadian research studies that impact all aspects of the patient journey, including new Canadian-led clinical trials; developed an educational hub for all ACCESS members, and produced accessible communications materials such as webinars, a newsletter and a comprehensive website; developed national best practice standards and tools for research and care; started to address the complex policy, regulatory and practical barriers that prevent equitable access to cancer diagnosis and treatment for all children in Canada. Future priorities include achieving organizational sustainability, supporting collaborations and inclusivity, overcoming barriers that affect the access of children to diagnostics and treatment, enhancing data connectivity and sharing, and developing and implementing a national strategy for pediatric cancer.

#4024 Cancer research advocates: satisfaction, frustration, and opportunities.

Bob Riter[1], Hillary Andrews[2]

[1]Community Cancer Partnership, Cornell University, Ithaca, NY,[2]Friends of Cancer Research, Washington, DC

Introduction: Advocates have been involved in cancer research for several decades, but little is known about their opinions regarding what is satisfying and what is frustrating. This largely qualitative study gathered feedback from a range of advocates regarding their experiences with cancer research to identify common themes and to generate hypotheses for future research.
Methods: An online survey with 14 questions about the advocates' background and experiences with research advocacy was distributed in early 2024 to cancer research advocates through multiple organizations and research advocacy networks. Thirty individuals responded, half of whom were advocates with 10+ years of experience.
Results: Breast cancer was the most common cancer site of research activity (n=18/30, 60%), and clinical trials (n=29/30, 97%), and translational research (n=25/30, 83%) were the types of research of greatest interest. Respondents were generally enthused about their work and felt like they were making a meaningful contribution. The greatest frustration was being asked for letters of support at the last minute or otherwise "checking a box" for a researcher applying for a grant. Many respondents expressed difficulty in getting connected with research activities, especially at a local level. The advocates noted that the "career path" to becoming an established advocate is not always clear nor is it easy to find opportunities
Conclusions and Recommendations: Our survey results demonstrate that research advocates are very engaged and satisfied with their experiences. While excellent training programs provide a solid foundation for new advocates, there should be a greater focus on continued engagement and mentoring for "mid-career" advocates.

#4025 Improving collaboration and well-being among patient advocates: lessons from Escape to THRIVE.

Sherri Romanoski[1], Mindy Griffith[1], **Brandi Forbes**[1], Lisa Rezende[2]

[1]Bag It Cancer, Tucson, AZ,[2]Molecular and Cellular Biology, University of Arizona, Tucson, AZ

Background: Patient advocates play a crucial role in supporting individuals with cancer, but advocates often face challenges related to collaboration, work-life balance, and burnout. Professional development opportunities, such as conferences, may help address these challenges, yet limited research exists on their long-term impact on patient advocates.

Purpose: To evaluate changes in patient advocates' practices in three areas: 1) collaboration with other advocates, 2) work-life balance, and 3) self-care following participation in *Escape to THRIVE*, a leadership conference.

Methods: Patient advocates attending the *Escape to THRIVE* conference in three different years (2020 (n=27), 2023 (n=21), and 2024 (n=25)) were surveyed at three time points: pre-conference, immediately post-conference, and six or more months post-conference. Questions focused on collaboration with other advocates, workload management, and self-care behaviors. Responses were analyzed using both quantitative and qualitative methods.

Results: Following conference participation, advocates demonstrated meaningful changes in collaboration and personal well-being management. A trend toward increased collaboration, with advocates more frequently partnering with those supporting patients with different cancer types. Participating advocates also reported improved workload management and self-care behaviors.

Conclusion: Results suggest that targeted professional development at conferences can effectively promote positive changes in patient advocates' collaborative behaviors and personal well-being management practices, with benefits sustained over time. These findings support the value of professional development opportunities for patient advocates that promote professional collaboration and personal well-being management.

#4026 The Bench with Bedside Initiative™: an unconventional collaborative approach between scientists and research participants.

Audrey Saghian[1], Alex J. Kalfa[2], Robel Dagnew[2], Navid Golshan[1], Raed Jaffal[2], Dayanara Ruiz[3], Mary K. Falcone[4], Caryn Lerman[4], Bodour Salhia[1]

[1]Department of Translational Genomics, Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA,[2]Department of Translational Genomics, Keck School of Medicine, University of Southern California, Los Angeles, CA,[3]Department of Population and Public Health Sciences, Keck School of Medicine, University of Southern California, Los Angeles, CA,[4]Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA

Translational research (TR) relies on human specimens, yet traditional models often fail to engage diverse populations with ambiguity between researchers and research participants (RPs). This disconnect can hinder trust, diminish inclusivity, and reduce generalizability. To address this, we developed *The Bench with Bedside Initiative (TBBI)*, a trans-relational model of TR that integrates scientists at the bench with RPs at the bedside. TBBI fosters bidirectional communication, enabling researchers to understand invaluable RP perspectives while enhancing RP understanding of research to offer empowerment and inclusion. TBBI uses social media to expand regional recruitment, connect with underrepresented populations, promote researcher-RP rapport, and disseminate findings. TBBI also employs culturally tailored community engagement through webinars and in-person events to foster trust and participation among historically marginalized groups. We are currently using TBBI to recruit 1000 diverse RPs to validate a novel test for detecting minimal residual disease (MRD) in breast cancer survivors at the Norris Comprehensive Cancer Center (NCCC). RPs complete measures on social determinants of health, provide blood samples, and permit collection of clinical data. TBBI has attracted 2,723 entries via web-based eligibility surveys, primarily through social media advertising and email outreach. Of these, 63% met eligibility, and 1,357 RPs advanced to secondary screening. To date, 554 RPs have consented using TBBI. Concurrently, 135 RPs were recruited from the NCCC and Los Angeles General Medical Center clinics, representing 20% of recruitment. RP demographics include 66.3% White, 16.5% Hispanic, 6.7% Asian, 4.6% Black, 4.5% Multiracial, 0.4% Native Hawaiian, 0.4% American Indian or Alaskan Native, and 0.4% declined to disclose. Given NCCC catchment, 53.8% of RPs recruited in clinic identified as Hispanic and 21.9% identified as Asian. TBBI is transforming RP recruitment and the researcher-RP relationship by promoting transparency and collaboration to connect laboratory science and patient care. While long-term outcomes are being evaluated, TBBI demonstrates that trans-relational approaches to TR can diversify participant pools, improve recruitment efficiency, and foster sustained researcher-RP collaboration. By leveraging social media and community engagement, TBBI is paving the way for an inclusive and impactful future in translational science.

#4027 Self-confidence as a driver of community impact: Outcomes of the Community Genetic Navigation Engagement Specialist (CoGENES) train-the-trainer program.

Bianca Rosales[1], Janet Rodriguez[1], Joel Sanchez Mendez[1], Yaneth L. Rodriguez[1], Charite Ricker[2], Rosa Barahona[1], Heinz Joseph Lenz[1], John Carpten[3], Mariana C. Stern[1], **Lourdes A. Baezconde-Garbanati**[1]

[1]Keck School of Medicine of USC, Los Angeles, CA,[2]Department of Medicine, Division of Medical Oncology, Keck School of Medicine of USC, Los Angeles, CA,[3]Beckman Research Institute of City of Hope., Duarte, CA

Hispanic/Latino (H/L) populations are disproportionally affected by colorectal cancer (CRC). This disparity is driven by a lack of awareness about CRC prevention, genetic testing, and access to culturally appropriate resources. A CoGENES train-the-trainer program was develop for a workforce of community health workers (CHWs*)* as part of COPECC, a Cancer Moonshot NCI funded PE-CGS network program at USC. After development and testing with community input, the program was implemented in three phases. Phase 1: Twelve CHWs participated in six in-person sessions over three weeks in Spanish/English, which provided knowledge on CRC/genetic testing, and community education strategies. Phase 2: the trained CoGENES replicated the sessions within their respective H/L communities training 30 more CoGENES. Phase 3: all 42 CoGENES are disseminating information through community events using the CoGENES training manual, to sustain long-term community impact. Data was collected on the number of individuals educated, events attended, and CRC/genetic testing information disseminated. Demographic and acculturation information was collected through surveys. Self-confidence to train others was measured at baseline, after training completion, at a 6 and 12 month follow-up, using a team-developed nine-item tool. All p-values are two-sided from paired t-tests. Majority of the participants were female (phase 1 = 75%, phase 2 = 79%) Mexico-born (92% & 75%), and very Latino oriented (67% & 71%). We saw a statistically significant increase in the “self-confidence to train others” in 8/9 items and 7/9 items of the survey for Phase 1 and Phase 2 CoGENES after they received the training (p-values <0.05). The level of self-confidence was sustained at 6 and 12 months, with no difference in the mean values of the items when compared to post-training values in Phase 1 CoGENES (p-values >0.05). In Phase 2 CoGENEs, we saw a decrease in the mean values for 5/9 items in the survey for the 12-month follow-up when compared to after training (p-values <0.05). Despite this, all the values remained higher at 12-months than at baseline. The difference between baseline and 12-month follow-up was the confidence to “describe what genetic tumor testing is” (p = 0.016) in Phase 1 CoGENES, and the confidence to “describe what genetic counseling is” (p = 0.017) in Phase 2 CoGENES. The CoGENES train-the-trainer program, a culturally tailored linguistically specific approach to address disparities in CRC prevention and genetic testing within H/L communities. Results show a lasting increase in the self-confidence of participants to empower other individuals in their communities to disseminate CRC prevention knowledge. The CoGENES CHWs partnered with 20 community-based organizations, reaching over 3 million people through community events, media interviews, podcasts, and workshops.

#4028 Establishing optimal care coordination in localized bladder cancer in the United States: A modified Delphi consensus study.

Gautam Jayram[1], Monika Baskin[2], Gordon Brown[3], Mollie Deshazo[4], Michael Fabrizio[5], Balaji Hiremalagur[6], Angela Hunter[7], **Mukul Singhal**[6], Arveen Kaur[6], Michael Lattanzi[8], Lynn Redmond[9], Guru Sonpavde[10]

[1]Urology Associates, Advanced Therapeutics Center, Nashville, TN,[2]Department of Medicine, University of Pittsburgh, Pittsburgh, PA,[3]Department of Urologic Oncology, Summit Health, Moorestown, NJ,[4]Department of Hematology Oncology, University of Alabama, Birmingham, AL,[5]Department of Urology, Eastern Virginia Medical School, Nashville, VA,[6]Johnson and Johnson, Horsham, PA,[7]The Urology Group, Cincinnati, OH,[8]Department of Genitourinary Oncology, Texas Oncology, Austin, TX,[9]Pharmacy, Urology Associates, Advanced Therapeutics Center, Nashville, TN,[10]University of Central Florida, Orlando, FL

Introduction: Care for localized Bladder Cancer (BC) primarily begins with a urologist and generally requires multidisciplinary management. With emerging novel treatments and evolving standards, effective communication and coordination across specialties is crucial. This project aimed to build consensus among clinicians and supporting staff with expertise in managing bladder cancer care, to propose an outline for the optimal management and coordination of care in localized BC originating from the urology clinic.
Methods: The project used a modified Delphi method. A steering committee (SC) experienced in BC care coordination (including urologists, medical and radiation oncologists, advanced practitioners and administrators) convened to discuss issues in BC care, developing a series of consensus statements. A finalized set of 46 statements were used in a 4-point Likert scale survey, disseminated by a third-party to healthcare practitioners with active roles managing localized BC in the USA. A consensus threshold was set at 75% agreement. The SC then reconvened to discuss the results and formulate recommendations based the consensus achieved.
Results: 300 responses were received across the nation, 102 (34%) from urologists, 79 (26.3%) from medical oncologists, 31 (10.3%) from nurse/nurse navigators, 32 (10.7%) from radiation oncologists, 30 (10%) from advanced practitioner providers, and 26 (8.7%) from administration roles. Of the statements, 38/46 (83%) achieved ≥90% agreement, and 8 (17%) attained ≥75% but <90%. No statements fell below the consensus threshold. Sub-analyses showed there was disagreement between urologists in different practice settings, although there was overall concordance in agreement levels between responder roles. 95% agreed multidisciplinary teams and tumor boards need to be created to facilitate bladder cancer care. 97% agreed clear and ongoing provider to provider communication during care is essential to maximize outcomes. 98% agreed patients need to be educated on all treatment options to facilitate informed decision making. From the statements with highest agreement, a series of 10 recommendations were developed.
Conclusions: These consensus recommendations from 300 experienced healthcare practitioners and allied healthcare professionals from all major localized BC treating specialties provide a roadmap toward improving the quality of care of these patients.

#4029 Project Cure CRC: advancing colorectal cancer research and personalized treatment through a CRC platform clinical trial.
Kimberly Lynn Newcomer, David Fenstermacher

Colorectal Cancer Alliance, Washington, DC

Background: The Colorectal Cancer Alliance (Alliance) established Project Cure CRC to support critical research breakthroughs in colorectal cancer (CRC), driving advancements in treatment and improving patient outcomes. CRC is among the most prevalent and fatal cancers worldwide, accounting for over 1.9 million new cases and 935,000 deaths annually. A significant challenge in treating CRC is its high genetic variability among patients, leading to differing responses to standard therapies and underscoring the urgent need for personalized approaches. Many cases involve mutations linked to resistance to conventional treatments, resulting in poor prognoses and limited options for these patients.
Methods: Project Cure CRC initiated a targeted Request for Proposals (RFP) informed by insights from the previous Cure CRC Summit. This effort received 250 submissions, many aiming to fund clinical trials testing novel therapies targeting CRC directly or enhancing the tumor microenvironment to improve immunotherapy effectiveness. The Alliance intends to invest $100 million in research over the next three years; funding individual trials was deemed insufficient for achieving the desired impact. After extensive exploration of various strategies, the Alliance is establishing a multi-center colorectal cancer platform trial to focus on curative research in four key areas: Neoadjuvant therapy for CRC, Minimal residual disease-positive patients with no radiographic evidence of disease, Perioperative surgery of metastatic disease, Metastatic and oligometastatic disease.
Results: To advance treatment options, accelerate regulatory approvals, and increase the 5-year survival rate for metastatic CRC from 13% to 35%, the Alliance is leading an adaptive, multi-site platform trial tailored to high-risk CRC cases. Modeled after successful trials for breast cancer, AML, multiple myeloma, and PDAC, this innovative trial design enables researchers to test multiple therapies simultaneously through multiple trial arms. The trial will use real-time data and Bayesian statistical approaches to adaptively select and refine effective treatments. A synthetic cohort will also be created in collaboration with the FDA, minimizing the need for some patients to receive only standard-of-care therapies. By integrating cutting-edge biomarker research, the platform trial will identify the most effective treatments for specific CRC tumor molecular subtypes.
Conclusions: The CRC platform trial will establish a collaborative, data-driven framework to accelerate the translation of research into clinical application. By incorporating ongoing adjustments and emphasizing targeted therapies, the trial aligns with the Alliance's mission to deliver impactful research and offer personalized, effective treatments to CRC patients with limited options under current care standards.

#4030 Uncovering psychosocial and physical challenges in colorectal cancer: findings from a comprehensive patient and survivor survey.

Kimberly Lynn Newcomer, David Fenstermacher

Colorectal Cancer Alliance, Washington, DC

Background: Colorectal cancer (CRC) is the third most diagnosed cancer and the second leading cause of cancer-related deaths globally among men and women combined. While survival rates for CRC have improved, limited knowledge exists about the specific unmet needs of CRC patients and survivors. These needs include emotional support, information on treatment options, and strategies for coping. CRC significantly impacts patients' emotional well-being, quality of life (QoL), and healthcare experiences. Beyond the stress of a cancer diagnosis, patients endure the physical and emotional toll of rigorous treatments, even in cases where no signs of the disease remain. Methods: The Colorectal Cancer Alliance conducted a comprehensive survey of CRC patients, survivors, and caregivers to better understand their experiences and needs. This IRB-approved, cross-sectional study included over 150 questions addressing patient profiles, diagnosis, access to care, treatment experiences, resources, and QoL. The survey was distributed through the Alliance's social media, email, and web-based platforms, reflecting a collaborative effort to address the multifaceted challenges faced by the CRC community. Results: Insights from 283 CRC patients and survivors highlighted critical unmet needs. The median age group of respondents was 46-55. Key findings included 74% struggling to find someone who shared similar experiences, 41% reporting reduced support after treatment, 54% feeling fatigued, and 51% experiencing stress. Cancer significantly disrupted personal and social aspects of life: 64% reported adverse effects on careers, 51% on dating, 43% on family planning, 58% on spousal relationships, 80% on sexual health, and 65% on social participation. Although patients felt well-informed pre-treatment, 46% identified gaps in knowledge about complementary or alternative therapies. These findings underscore the importance of addressing psychosocial and physical needs to improve care outcomes. Conclusions: CRC patients and survivors face profound psychosocial and physical challenges that reshape their lives. By identifying these needs, healthcare providers can develop more personalized treatment plans and support systems, offering hope for better patient experiences and outcomes. The Alliance aims to use these findings to guide clinical practices, care programs, and survivorship support, driving transformative improvements in CRC care.

#4031 Project Cure CRC: advancing precision medicine in colorectal cancer through innovative research and targeted therapies.

Kimberly Lynn Newcomer, David Fenstermacher

Colorectal Cancer Alliance, Washington, DC

Background: The Colorectal Cancer Alliance founded Project Cure CRC to support breakthrough research in colorectal cancer (CRC) and advance precision medicine to improve patient outcomes. CRC remains one of the most common and deadly cancers, with over 1.9 million new cases and 935,000 deaths annually. A significant challenge in CRC treatment is the genetic variability among patients, leading to different responses to standard therapies and highlighting the need for personalized approaches. Many CRC cases involve mutations that resist conventional treatments, resulting in poor prognosis and limited options for affected patients.
Methods: Project Cure CRC launched a targeted Request for Proposals (RFP) to address these challenges based on insights from last year's Cure CRC Summit. The RFP attracted 250 proposals centered on novel precision medicine strategies, and nearly $7 million in funding has been allocated to support 18 high-impact research projects.
Results: Funded projects employ diverse and innovative strategies to tackle CRC. Critical approaches include enhancing intratumoral mRNA vaccines that target immune checkpoints to boost anti-tumor responses and combining targeted radiation with immunotherapies to create synergistic effects in advanced CRC. Additional studies focus on new drug combinations for BRAF-mutated CRC, which has low response rates to existing treatments. Other projects investigate ProAgio, a protein targeting cells that support cancer survival, with the potential to improve therapies for hard-to-treat tumors. Further research examines how fat tissue influences CRC growth and metastasis, particularly in patients with obesity, and leverages technology to target multiple genes simultaneously, identifying cancer cell vulnerabilities and enhancing immune response. The Alliance has also funded a theranostic project that is able to use the same drug molecule to determine the location of tumors and a target therapeutic approach, and the development of a drug pipeline platform that will accelerate the development of new targets and therapies.
Conclusions: These initiatives demonstrate the Alliance's commitment to advancing precision medicine and addressing key gaps in CRC care, particularly for patients with treatment-resistant profiles. By funding high-impact research and fostering collaboration, Project Cure CRC aims to accelerate the development of personalized, effective treatments for CRC patients, transforming the landscape of colorectal cancer care.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Gene Regulation and Transcription Factors 1
Poster Session

#4035 Elucidating the DNA binding dynamics of EWSR1::FLI1 using biochemical methods.

Jack Tokarsky[1], Blanche Chen[2], Michael G. Poirier[2], Emily Theisen[1]

[1]Nationwide Children's Hospital, Columbus, OH,[2]The Ohio State University, Columbus, OH

Importance: This work signifies a breakthrough in our ability to study Ewing sarcoma by developing a protocol to purify functional WT EWSR1::FLI1. This development changes the level of detail to which we can study EWSR1::FLI1 and potentially other difficult to purify fusion oncoproteins.
Objective: Ewing sarcoma is a pediatric bone and soft tissue sarcoma that arises in adolescents and young adults. The mutation that causes >85% of Ewing sarcoma cases is caused by the fusion oncoprotein, EWSR1::FLI1. Efforts to purify recombinantly expressed EWSR1::FLI1 have proven challenging for decades due to aggregation caused by the low complexity domain (LCD) of EWSR1. Our goal was to purify recombinantly expressed and functional EWSR1::FLI1 to quantitatively understand the nature of binding dynamics to various DNA sequences.
Methods: We successfully expressed and purified EWSR1::FLI1 using a newly developed protocol. We performed electrophoretic mobility shift assays (EMSAs) to test binding of EWSR1::FLI1 on naked- and nucleosome-wrapped DNA. Binding constants were determined with DNA containing either a canonical high affinity (HA) ETS DNA binding sequence or a 2X GGAA microsatellite repeat.
Results: Our laboratory has successfully developed a protocol to consistently achieve highly pure and functional EWSR1::FLI1. EWSR1::FLI1 has approximately 7-fold tighter binding to a 2X GGAA repeat, than to HA ETS sequence. For comparison, the minimal FLI DNA binding domain proteins (ETS85 and ETS102) bind to HA ETS sequences and GGAA microsatellites at similar affinities. These experiments suggest that the EWSR1 LCD alters the binding dynamics of the ETS DBD domain and that EWSR1::FLI1 preferentially binds to GGAA microsatellites. We tested the pioneer function of EWSR1::FLI1 by performing EMSAs with nucleosome-wrapped DNA. EWSR1::FLI1 bound to nucleosome-wrapped DNA containing GGAA repeats with only 3-fold less affinity than naked DNA. For comparison, when ETS85 and ETS102 bound nucleosome-wrapped DNA, this affinity was ~30-fold less, showing potentially weak pioneering function of the FLI DBD.
Conclusions & Relevance to Pediatric Care: These studies allow us to understand the physical nature of fusion oncoproteins such as EWSR1::FLI1 and will be the foundation for therapeutics that could be used to treat Ewing sarcoma patients.

#4036 Selective inhibition of the transcription factor Brachyury in chordoma and non-small cell lung cancer.

G. Mercenne, Y. Gao, B. McEllin, A. Gutierrez, J. Robbins, B. Hueffmeier, E. Broderick, D. Canzani, W. Fondrie, L. Pino, A. Federation; Talus Bioscience, Inc., Seattle, WA

Chordoma is a rare and slow-growing cancer arising from remnants of the notochord, a structure present during embryonic development. It most commonly affects the sacrum and cervical spine, accounting for about 20% of primary spinal tumors and 3% of all bone tumors. While surgery and radiation are the primary treatments, the tumor's location often makes complete removal challenging, and recurrence is common. There are no approved therapies at this time. A hallmark of chordoma is the aberrant expression of the transcription factor (TF) Brachyury (TBXT) which plays a key role in notochord development but is normally silenced in adult tissues. In chordoma, Brachyury is reactivated and contributes to tumor initiation and progression, making it both a diagnostic marker, and a potential therapeutic target.
Talus Bio has developed a groundbreaking technology, TF-Scan, the first cell-based global screening platform specifically designed to target TF. TF-Scan integrates functional proteomics, automated cell processing, and machine learning to evaluate a drug’s ability to modulate the activity of DNA-bound proteins in live human cells. Using our platform, we identified compounds that target TBXT. Hit validation and hit-to-lead optimization led to the development of a covalent small molecule, TAL61, that effectively modulates TBXT activity. In preclinical studies using a patient-derived xenograft (PDX) model for chordoma, TAL61 demonstrated significant efficacy in reducing tumor burden. TBXT has also been shown to be overexpressed in different cancers particularly lung cancer and breast cancer. We tested our lead compound in a model of NSCLC and were able to significantly decrease tumor burden by more than 50% and visualize additive effects while doing combination with a chemotherapy agent.
We are conducting other studies to further characterize TAL61 and uncover new cancer indications where TBXT plays a key role. The development of TF-Scan represents a paradigm shift in drug discovery, enabling the identification of compounds capable of disrupting targets that were previously inaccessible to conventional approaches.

#4037 Epigenetically-controlled CEBPB regulates kidney cancer tumorigenesis via GPD1L-mediated ether lipid synthesis.

Thi Ha Nguyen, Xuan Linh Mai, Tin Tin Manh Nguyen, Hoonsik Nam, Sunghyouk Park, **Ji Yun Lee**

Seoul National University College of Pharmacy, Seoul, Korea, Republic of

The VHL-HIF axis has been recognized as a major regulator of lipid accumulation and tumorigenesis in clear cell renal cell carcinoma (ccRCC), but a growing number of VHL-HIF-independent pathways suggests possible alternative regulators. By screening epigenetic drivers and transcription factors, we identified CCAAT/enhancer-binding protein beta (CEBPB) as a key VHL-independent transcription factor crucial to lipid-related ccRCC progression, which was confirmed through *in vitro/vivo* experiments. CEBPB levels, regulated by H3K27ac and H3K4me, were also correlated with various poor prognostic factors in ccRCC patients. Mechanistically, CEBPB transcriptionally repressed glycerol-3-phosphate dehydrogenase 1-like (GPD1L), causing substrate (dihydroxyacetonephophate) accumulation for ether lipid synthesis. This enhanced Akt activation through membrane recruitment, and the subsequent downregulation of carnitine palmitoyltransferase 1A (CPT1A) led to lipid accumulation. Connecting multiple elements of the Hallmarks of Cancer in an unbranched path, the CEBPB-GPD1L-ether lipid-Akt-CPT1A axis is proposed as a new VHL-independent mechanism of lipid-related ccRCC tumorigenesis.
Significance: The identification of CEBPB as a VHL-independent regulator of lipid metabolism and tumorigenesis shows how major cancer hallmarks (epigenetics, metabolites, and signaling) can be orchestrated by single factor. The proposed CEBPB-GPD1L-ether lipid-Akt-CPT1A axis provides new insights and therapeutic potentials for the regulation of lipid-related tumorigenesis in ccRCC

#4038 Oncogenic ETS transcription factors in prostate cancer and Ewing sarcoma are interchangeable when avoiding repression by a PRC2/FOXO1 complex.

Nicholas Frederick Downing[1], Katelyn M. Mills[1], Peter C. Hollenhorst[2]

[1]Indiana University Bloomington, Bloomington, IN,[2]Medical Sciences, Indiana University Bloomington, Bloomington, IN

ETS transcription factors play roles throughout development, aiding in hematopoiesis, blood vessel formation and cell fate. The ETS family is composed of 28 members, all of which share an ETS DNA-binding domain. Chromosomal rearrangements that lead to the overexpression of specific ETS promote oncogenic transformation and tumor development. More than half of prostate tumors are driven by aberrant expression of an ETS protein. Our lab has demonstrated that oncogenic ETS form essential interactions with a ubiquitous RNA-binding protein, EWS, to promote prostate tumorigenesis. A similar mechanism exists in Ewing sarcoma (ES). ES tumors are driven by the expression EWS/ETS fusion proteins. This project focuses on how ETS and EWS/ETS act as oncogenic transcription factors; furthermore, we identify a novel ERG/PRC2/FOXO1 regulatory complex that may be exploited for therapeutic benefit. We compared biological functions of oncogenic ETS found in prostate cancer (ERG, ERG(S96E), ETV1, ETV4, ETV5) and EWS/ETS fusions in ES (EWS/FLI1, EWS/ERG). All oncogenic ETS promoted migration and clonogenic survival in normal prostate epithelial cell lines, RWPE1 and PNT2. Knockdown of endogenous EWS/FLI1 in the ES cell line, A673, reduced anchorage-independent colony formation in soft agar. Exogenous expression of EWS/FLI1, ETV1, ETV4, and ETV5 rescued A673colony formation, however ERG and EWS fused to full-length ERG were unable to rescue this phenotype. Our lab has established that ERG can act as a transcriptional repressor through the formation of an ERG/PRC2 complex. Pulldown assays using purified proteins and A673 lysates revealed that ERG and EWS fused to full-length ERG interact with EZH2, a subunit of the PRC2, and FOXO1, a tumor suppressor that is thought to repress ERG transcriptional activity. Both the N-terminal truncation of ERG and phosphorylation of ERG at S96 disrupted these interactions and rescued colony formation in A673. Further immunoprecipitation assays suggest that a FOXO1/PRC2 complex can exist independent of ETS expression. ERG is thought to cooperate with mutations that promote prostate tumorigenesis through activation of the PI3K/AKT pathway. Our lab has observed increases in clonogenic survival and tumorigenesis upon expression of both ERG and a constitutively- active mAKT in RWPE1. AKT negatively regulates FOXO1 expression. We aim to evaluate the effects of FOXO1 expression in facilitating ERG/PRC2 interactions. Furthermore, we aim to explore the effects of Ipatasertib, an AKT inhibitor in clinical trials, on the ERG/PRC2/FOXO1 complex and ERG-dependent phenotypes in prostate cancer cell models.

#4039 An isogenic CRISPR screen identifies novel MYC-driven vulnerabilities.

P. Lin[1], C. Lourenco[1], J. Cruickshank[2], L. Palomero[3], J. E. van Leeuwen[1], A. H. Y. Tong[1], K. Chan[1], S. El Ghamrasni[2], M. Pujana[3], D. W. Cescon[2], J. Moffat[1], L. Z. Penn[2];

[1]University of Toronto, Toronto, ON, Canada, [2]University Health Network, Toronto, ON, Canada, [3]Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain

c-MYC (MYC) is a central regulatory protein that is dysregulated in >50% of all human cancers and is linked to aggressive disease. Developing MYC inhibitors would revolutionize cancer treatment; however, efforts to target MYC directly using small molecular inhibitors have historically failed. A promising approach is to identify and inhibit critical MYC partner proteins to inactivate MYC and trigger cancer cell death. Inhibiting these targets therapeutically can result in synthetic lethality (MYC-SL), which can be exploited in MYC-dysregulated cancers. To identify MYC-SL targets, we performed a genome-wide CRISPR knock-out screen using an isogenic pair of non-transformed and MYC-driven breast cancer cells. In contrast to other screens, this model is both dependent on MYC and recapitulates human disease at pathological and molecular levels *in vivo*. Finally, these hits were cross-referenced with our MYC protein-interactome data to reveal putative MYC partner proteins that are critical for MYC activity.

From our screen results, we performed gene set enrichment analysis to identify biological activities that may represent core functional dependencies in MYC dysregulated cancer cells. Using this approach, we identified and validated topoisomerase 1 (TOP1) as an actionable vulnerability that can be targeted with clinically approved inhibitors. Genetic and pharmacological inhibition of TOP1 in multiple orthogonal assays resulted in MYC-driven cell death. Finally, drug response to TOP1 inhibitors correlated with MYC levels and activity across panels of breast cancer cell lines and patient-derived organoids, highlighting TOP1 as a promising target for MYC-driven cancers.

The recent accessibility of large-scale datasets detailing functional dependencies across hundreds of cancer cell lines offers an unprecedented opportunity to prioritize targets with greater translational relevance, which is a major limitation of the synthetic-lethal approach for target discovery. As a secondary analysis of our hits, we utilized DEPMAP data to classify cancer cell lines as relatively MYC-dependent or MYC-independent, enabling the *in silico* evaluation of each MYC-SL hit's differential essentiality. MYC-SLs that exhibited selective essentiality in MYC-dependent cell lines were prioritized for further investigation. Results from this strategy revealed critical MYC cofactors that have been validated by us and others (e.g., CDK9), providing confidence in this approach. Excitingly, previously underexplored targets were identified with promising validation to-date. Together, this work features two successful strategies to prioritize hits from hundreds of synthetic-lethal genome-wide CRISPR screens to identify novel MYC-driven vulnerabilities in cancer.

#4040 p63 represses metabolic pathways in aggressive bladder cancer.

Artem Smirnov[1], Mariacristina Franzese Canonico[1], Damiano Barnaba[1], Maurizio Fanciulli[2], Alessandro Mauriello[1], Gerry Melino[1], Eleonora Candi[1]

[1]Departement of Experimental Medicine, University of Rome Tor Vergata, Rome, Italy,[2]Dept. of Research, Advanced Diagnostics, and Technological Innovation, IRCCS Regina Elena NCI, Rome, Italy

Over half a million of people get bladder cancer (BLCA) each year worldwide with a third of all cases leading to death. Recent breakthroughs significantly improved early detection of BLCA, however little progress has been done to manage advanced invasive cancers since 1980s. Regulation of gene expression by key transcription factors governs multiple molecular pathways determining tumour evolution and selection. Here, we screen the transcription factors based on changes of their essentiality throughout bladder cancer evolution and identify p63 is a key factor. p63 is an oncogene in multiple epithelial tumours, however its expression is dramatically reduced in advanced BLCA, furthermore the loss of p63 is associated with a worse prognosis. Yet, little is known about p63 targetome in bladder cancer. In this work, we use transcriptomics, genome occupancy and chromatin accessibility assays to characterise global p63 targetome in BLCA in vitro along with bioinformatic analyses of gene expression in BLCA patients. We identify metabolism as a key pathway repressed by p63 during BLCA evolution and we show that activation of this pathway is associated with poor prognosis. In vitro, targeting of specific metabolic pathways induces cell death. Altogether, we identify an unexpected tumour-suppressive role for p63 in bladder cancer and propose novel therapeutic strategies for treatment of invasive BLCA.

#4041 Overexpression of HMGA1 in gastric cancer cells is associated with reduced tumor cell malignant behavior and decreased YAP-TEAD signaling.

Dipti Athavale, Yan-ting Yann Zhang, Xiaodan Yao, Shumei Song

Coriell Institute for Medical Research, Camden, NJ

Background: The High Mobility Group A1 (HMGA1) is an architectural transcription factor and facilitates the interaction between transcriptional regulatory proteins and DNA in different cancers by remodeling chromatin structure. Hippo/YAP1 pathway is an evolutionary conserved signaling consisting of up-stream kinases, transcriptional co-activators and scaffold proteins. Unphosphorylated YAP and TAZ translocate to the nucleus, bind with TEAD transcription factor family and induce expression of a wide range of genes involved in cell malignant behavior. Silencing of HMGA1 downregulated YAP activity in breast cancer however, the functional role of HMGA1 and its interaction with Hippo/YAP1 signaling in mediating gastric cancer (GC) progression and metastasis remains unknown. We hypothesize that HMGA1 is essential to promote YAP activity in gastric cancer.
Methods: TCGA dataset and our own bulk RNAseq and single cell RNA sequencing (scRNAseq) will be analyzed to understand the expression pattern of HMGA1 in normal, primary tumors and metastatic tissues. HMGA1 will be overexpressed in GC cells; and YAP activity will be measured by its nuclear translocation, transcription of target genes (CTGF, CyR61, Birc5, Sox9) upon genetic modulation of HMGA1. *In vitro* functional assays such as proliferation, migration, colony formation and tumor sphere as well as *in vivo* tumorigenicity of GC cells with HMGA1 overexpression will be performed in SCID mice.
Results: HMGA1 expression was significantly increased in GC tissues compared to normal in TCGA datasets. Moreover, HMGA1 transcripts showed positive correlation with YAP1 and TEAD4 mRNA level. scRNAseq data from advanced GC patients indicated HMGA1 mRNA is upregulated in primary tumor, peritoneal and liver metastasis compared to normal in stomach. Tissue microarray of more than 200 GC indicated that HMGA1 is highly expressed in tumor tissue and associated with poor survival. Increased expression of HMGA1 was detected in cancer cells as compared to normal gastric epithelial cells by Western blot. Unexpectedly, overexpression of HMGA1 in GC cell lines (AGS, GA0518 and GT-5) reduced colony formation, and tumor-sphere size and number as compared to vector control. Invasion of AGS, GA0518 and GT-5 was significantly reduced upon HMGA1 overexpression. Mechanistically, nuclear localization of YAP, TAZ and SOX9 (YAP target) was decreased while Tead1-4 did not change in GC cells upon overexpressing HMGA1 compared to vector. Furthermore, YAP-TEAD luciferase reporter activity was significantly reduced along with transcript level of YAP target genes upon HMGA1 overexpression *vs.* vector in GC cells.
Conclusion: Although, endogenous expression of HMGA1 was increased in GC cells and tissues as compared to normal, its overexpression reduced tumor cell malignant behavior, migration and inhibited YAP-TEAD signaling.

#4042 Mitochondrial electron transport chain inhibition suppresses NRF2 expression in NSCLC.

Chang Jiang[1], Ryan Martin[1], Yun Huang[1], Nicolas Prieto[2], Yun Pyo Kang[3], Mingxiang Teng[1], Gina DeNicola[1]

[1]Moffitt Cancer Center, Tampa, FL,[2]University of South Florida, Tampa, FL,[3]Seoul National University, Seoul, Korea, Republic of

Lung cancer is the leading cause of cancer-associated death in the US. Non-small cell lung cancer (NSCLC) accounts for 85% of all diagnosed lung cancer cases, which has a high frequency of mutations in the KEAP1/NRF2 circuit (15-34%). NRF2 is a stress-responsive transcription factor that regulates transcriptional programs in response to oxidative stress. KEAP1 directs NRF2 degradation, and accordingly, disruption of KEAP1 increases the abundance and activity of NRF2. KEAP1/NRF2 mutations result in constitutive NRF2 activation and chronic induction of a battery of NRF2 target genes, which confer profound resistance to chemo/radiation therapy. Although direct inhibition of NRF2 holds great therapeutic potential, NRF2 inhibitors have not yet existed in the clinic. Instead, our attempt to address this challenge focuses on targeting upstream NRF2 regulators in NSCLC. To this end, we leveraged CRISPR/Cas9-based genetic screening approaches. We found that electron transport chain (ETC) complex I/III component deletion or pharmacological inhibition results in complete loss of NRF2 protein expression in KEAP1 mutant NSCLC cells. Our results indicate that NRF2 destruction by ETC inhibition is associated with post-translational ubiquitination and degradation facilitated by an alternative E3 ligase within the Cullin complex and is controlled by AMPK. We also translate these findings *in vivo* using novel NRF2 active genetically engineered lung tumor mouse models LSL-$Kras^{G12D}$; $Lkb1^{flox/flox}$; $Nrf2^{D29H}$ that mimic the common co-occurrence of KRAS and Lkb1 loss-of-function mutations in human lung adenocarcinoma. This work establishes the link between the mitochondria and NRF2 regulation in NSCLC and, more importantly, will lead to the discovery of novel mediators of this signaling, which can be targeted as a therapeutical strategy for NRF2 inhibition. Overall, our research has great implications in reversing the profound resistance of NSCLC with KEAP1/NRF2 mutations to chemo/radiotherapy, which can significantly improve treatment efficacy and patient survival.

#4043 MYB/AKT3 axis is a key driver of ovarian cancer growth and aggressiveness.

Kunwar Somesh Vikramdeo[1], Orlandric Miree[2], Shashi Anand[1], Sanjeev Kumar Srivastava[2], Seema Singh[1], Rodney P. Rocconi[3], Ajay Pratap Singh[1]

[1]Cell and Molecular Biology, University of Mississippi Medical Center, Jackson, MS,[2]University of South Alabama, Mobile, AL,[3]University of Mississippi Medical Center, Jackson, MS

Ovarian cancer (OC) is the most common and lethal gynecologic malignancy in the United States, primarily due to late-stage diagnosis and limited treatment options for the advanced disease. It disproportionately affects Black women at every stage, from initial diagnosis to treatment, resulting in higher mortality rates compared to non-Hispanic White women. In an effort to identify novel biomarkers and therapeutic targets for improved clinical management, we discovered *MYB* as a differentially-expressed gene in OC, exhibiting a race-specific prognostic association. *MYB* encodes for a transcription factor protein that drives oncogenesis by regulating the expression of a wide array of gene targets. We also found that MYB is expressed in OC cell lines, having elevated levels in aggressive (SKOV3-ip) and chemo-resistant (A2780-Cip) variants compared to their parental counterpart (SKOV3 and A2780) cell lines. Gain- and loss-of-function studies suggested that MYB plays a role in growth, aggressiveness, and chemoresistance. Gene expression profiling of low (SKOV3-Neo) and high (SKOV3-ip-Scr) MYB-expressing cells along with their forced MYB-expressing (SKOV3-MYB) and MYB-silenced (SKOV3-ip-shMYB) derivative lines using the nCounter® PanCancer Progression panel on a nanoString nCounter system, revealed several MYB-driven differentially expressed genes (DEGs). With a 1.5-fold-change cutoff, we identified 164 DEGs in the SKOV3-ip (Scr vs. shMYB) comparison and 87 DEGs in SKOV3 (Neo vs. MYB) comparison. Notably, the majority of DEGs were distinct between these comparisons, suggesting that MYB alters gene expression in a cell-specific manner. Validation of commonly altered genes in additional paired MYB-overexpressing and silenced cell lines by qRT-PCR identified *AKT3* as a significant downstream target of MYB. Moreover, KEGG pathway analysis of DEGs in MYB-modulated cells highlighted PI3K pathway as commonly altered. *In silico* analysis of *AKT3* promoter identified seven putative MYB binding regions (P1-P7), and chromatin immunoprecipitation confirmed strong MYB binding to P4 region of the AKT3 promoter. Inhibition of AKT3 through RNA interference resulted in reduced cell proliferation, clonogenicity, migration and invasion, and increased apoptosis in OC cells. These findings suggest that AKT3 serves as a key mediator in driving the oncogenic actions of MYB in ovarian cancer.

#4044 Hypomethylation and overexpression of transcription factor AP2B promote growth in ILC via cell cycle modulation.

Abdalla M. Wedn[1], Hunter Waltermire[2], Haley Arbore[3], Daniel Brown[4], Osama Shah[5], Fangyuan Chen[6], Sanghoon Lee[7], Jagmohan Hooda[2], Adrian Lee[2], Steffi Oesterreich[2]

[1]Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA,[2]Women's Cancer Research Center, Magee Women's Research Institute, UPMC Hillman Cancer Center, Pittsburgh, PA,[3]University of Pittsburgh School of Medicine, Pittsburgh, PA,[4]Institute for Precision Medicine, University of Pittsburgh, Pittsburgh, PA,[5]University of Nebraska Medical Center, Omaha, NE,[6]Dana-Farber Cancer Institute, Boston, MA,[7]New York University Langone Health, New York, NY

Invasive lobular carcinoma (ILC) is responsible for 10%-15% of all invasive breast cancers and is associated with worse long-term outcomes compared to the most common no special type (NST). Despite known differences between ILC and NST, the treatment for ILC is the same as stage matched NST. Therefore, identifying the biological molecules that are specific for ILC and can be therapeutically targeted is an urgent need. In this context, we recently identified the transcription factor AP2b (TFAP2B) as one of the most hypomethylated genes in ILC compared with NST and normal mammary tissues. Consistently, differential gene expression analysis in TCGA, METABRIC and SCAN-B datasets confirmed the upregulation of TFAP2B expression in ILC. Methylation of CG island in the first exon possesses the highest correlation with mRNA expression levels. Here, we examined the role of this transcription factor in ILC using various models including cell lines, patient derived organoids (PDOs) and tumor samples. Immunoblotting and RT-qPCR confirmed the specific upregulation of TFAP2B in ILC compared with NST cell lines and PDOs. Immunofluorescence showed a clear nuclear localization of TFAP2B and immunohistochemistry revealed a strong and uniform expression in tissues isolated from ILC patients. TFAP2B knockdown (using siRNA and inducible shRNA) significantly reduces the growth of ILC cell lines and PDOs in anchorage dependent and independent conditions as well as colony formation assay. The actions of TFAP2B seem to be mediated, at least in part, through cell cycle as knockdown of this factor decreases and increases the percentage of cells in S and G0/G1 phase, respectively. CDH1 knockout or inducible expression in NST or ILC cell lines failed to affect TFAP2B levels indicating that changes in E-cadherin levels, in the transformed cells, are not sufficient to alter TFAP2B expression. Preliminary data from CUT and RUN sequencing indicate possible binding sites for TFAP2B in growth factors and cell cycle related genes. Collectively, the data support the specific enhancement of TFAP2B in ILC and its importance for cell proliferation. Future studies will be conducted to uncover the interaction partners and pathways regulated by TFAP2B. This could serve as a basis for the development of novel therapeutic approaches that can be tailored specifically to patients with ILC tumors.

#4045 Leiomyosarcoma is characterized by two distinct subtypes driven by NFI or AP1 transcription factors.

Ryan A. Denu, Zhao Zheng, Yingda Jiang, Veena Kochat, Emre Arslan, Danh D. Truong, William Padron, Davis R. Ingram, Khalida M. Wani, Diana Shamsutdinova, Ravin Ratan, Neeta Somaiah, Christina L. Roland, Pamela T. Soliman, Larissa A. Meyer, Alexander J. Lazar, Elise F. Nassif Haddad, Emily Z. Keung, Kunal Rai

The University of Texas MD Anderson Cancer Center, Houston, TX

Objective: Leiomyosarcoma (LMS) is a smooth muscle-derived tumor documented to display significant heterogeneity. Although bulk transcriptome studies have been reported in LMS, single-cell characterization has been lacking to assess intra-tumoral cellular heterogeneity. We aimed to characterize heterogeneity, create a single-cell atlas, define molecular subtypes, and identify gene regulatory networks underlying the heterogeneity of LMS.
Methods: We performed single nucleus multiome sequencing with concurrent RNA sequencing (snRNA-seq) and assay for transposase-accessible chromatin (snATAC-seq) using 16 fresh frozen, untreated LMS samples (9 retroperitoneal/vascular, 4 uterine, and 3 extremity). We delineated malignant cells from benign cells using InferCNV and CytoTRACE analyses. Spatial transcriptomic analysis was performed on 12 LMS tissue microarrays (~250 total tissue cores, mostly pre-treated tumors) using 10x Xenium and a custom 480-gene panel informed by snRNA-seq data. Human LMS cell lines were employed to functionally validate findings from these data.
Results: We obtained high-quality data from 94,439 nuclei for snRNA-seq and 89,702 nuclei for snATAC-seq. Malignant cells were characterized by a less differentiated state by CytoTRACE and higher CNVs. UMAP analysis identified 15 different cell clusters including 12 malignant cell clusters and 3 non-malignant cell clusters. Some malignant cell clusters showed signs of aberrant differentiation with enrichment of osteoblastic and neural pathways. Malignant cells fell almost exclusively into 2 distinct subtypes: a dedifferentiated subtype with mesenchymal features (MES) and a differentiated subtype with enrichment of smooth muscle cell markers (SMC). Integration of chromatin accessibility data showed enrichment of nuclear factor I (NFI) transcription factor (TF) motifs in the MES and AP-1 (BACH2, FOSB, and FOSL2) motifs in the SMC group. Whole genome sequencing did not reveal an obvious genomic etiology for these subtypes. Expression of these TFs was observed in LMS cell lines. Depletion of NFI TFs, namely NFIX, reduced proliferation and invasion of LMS cell lines. Spatial transcriptomics identified spatial relationships between these subtypes and infiltrating immune cells. This revealed a greater tendency for immunosuppressive macrophages to cluster around MES cells compared to SMC cells.
Conclusion: We produced an atlas of the LMS single-cell transcriptome and epigenome. We demonstrate the presence of two predominant malignant cell clusters with dedifferentiated/mesenchymal or smooth muscle cell-like properties, a novel finding in LMS, and implicate NFI and AP-1 TFs in driving this heterogeneity.

#4046 Role of kaiso in androgen receptor signaling and castration resistance.

Oceane Kircha Nana[1], Huixian Lin[1], Isra Elhussin[1], Md. Abu Siddique[2], Omna Hayyat[1], Moray Campbell[3], Clayton Yates[1]

[1]Johns Hopkins University School of Medicine, Baltimore, MD,[2]Tuskegee University, Tuskegee, AL,[3]Cedars Sinai, Los Angeles, CA

Metastatic PCa patients are treated with androgen deprivation therapy (ADT) alone or ADT together with antiandrogens such as enzalutamide (Enza). ADT/Enza targets AR-expressing PCa cells; therefore, the mechanisms underlying ADT/Enza resistance involve AR genomic alterations, AR splice variants, and persistent AR signaling even under castrated conditions. Our lab recently identified Kaiso, a BTB-POZ methyl-binding transcriptional regulator, as a crucial player in AR signaling networks. However, it is known how Kaiso potentially influences progress and treatment outcomes. Firstly, we established that Kaiso expression levels increased, as well as a cytoplasmic to nuclear localization occurred during Enza treatment. Additionally, utilizing ChIP and Cut-n-Run assays we observed that Kaiso directly binds to the methylated regions within the AR promoter. Utilizing AR-specific methylation-sequencing, we further observed that CGCG-rich regions in the AR promoter can be modulated utilizing both Kaiso depleted (sh-Kaiso) and Kaiso (overexpression) OE cell lines. Furthermore, modulation of Kaiso expression levels influences both AR and PSA. RNA-seq of Kaiso OE cells demonstrate significant AR and PSA overexpression, as well as GSEA enrichments highlight that androgen receptor signaling, epithelial to mesenchymal transition, and MYC signaling as the most significant pathways. Finally, sh-Kaiso cells displayed increased sensitivity to both Enza and ABi, while Kaiso OE cell lines displayed a complete lack of response to both compounds. In summary, our findings suggest that Kaiso levels may significantly influence androgen sensitivity and highlight Kaiso as a therapeutic target to improve conventional AR-directed therapies.

#4048 The circadian gene Dec2 promotes pancreatic cancer dormancy by regulating tumor cell antigen presentation to facilitate immune evasion.

Lan Wang[1], Chris Harris[2], Orjola Prela[2], Wade Narrow[2], Jennifer L. Becker[3], Juliana Cazarin de Menezes[4], Darren R. Carpizo[2]

[1]Pathology, University of Rochester Wilmot Cancer Institute, Rochester, NY,[2]Surgery, University of Rochester Wilmot Cancer Institute, Rochester, NY,[3]Center of Advanced Research, University of Rochester Wilmot Cancer Institute, Rochester, NY,[4]Biomedical Genetics, University of Rochester Wilmot Cancer Institute, Rochester, NY

The mechanisms that regulate cancer dormancy remain poorly understood. Using an orthotopic mouse model of resectable pancreatic adenocarcinoma (PDAC), we identified the transcriptional repressor factor Dec2 (*Bhlhe41*) as a gene that was upregulated in metastatic dormant tumor cells from the liver. To understand how Dec2 regulates dormancy, we deleted it from the murine pancreatic cancer cells and implanted them in the model. Following resection, mice implanted with Dec2 KO cells demonstrated a striking improvement in survival compared to the mice with Dec2 WT cells. This difference in survival was abrogated when nude mice were used as hosts indicating the survival difference is from an immune mediated mechanism. Pancreatic cancer is a highly immunosuppressive tumor due to both a relatively low expression of major histocompatibility complex class I (MHC-I)-dependent antigen presentation and an abundance of myeloid-derived suppressor cells that lead to poor T cell trafficking and activation. We found that deletion of Dec2 overcame both of these mechanisms to restore anti-tumor immunity that inhibited tumor growth not only in primary tumors as well as metastases. Dec2 suppresses MHC-I antigen presentation by repressing multiple genes in the antigen presentation pathway including H2-k1, H2-d1, Tap1, and B2m as well as the source of tumor antigens from the proteosome by repressing multiple proteasome genes. Loss of Dec2 repolarized the tumor immune microenvironment by decreasing expression of multiple immunosuppressive cytokines such as Cxcl1, Ccl2 and Csf2 in tumors. We conclude that Dec2 facilitates both pancreatic tumor growth and dormancy by regulating tumor cell MHCI antigen presentation. Dec2 is known to function as part of a negative accessory arm of the molecular circadian clock. We found several components of the antigen presentation pathway oscillated in a circadian manner that was lost upon deletion of Dec2. Moreover, T-cell mediated tumor cell killing varied depending on the time of day. We suggest that lowered MHC-I presentation of antigens during rest phase is a natural effect of the circadian clock.

#4049 Transcriptional regulation of FOXA1 by GATA2/3 attenuates the antitumor effects of histone deacetylase (HDAC) inhibitors in basal-like breast cancer.

Congcong Tan, Hui Lyu, Yakun Wu, Margaret E. Larsen, Shou-Ching Tang, Bolin Liu

LSU Stanley S. Scott Cancer Center (SSSCC), New Orleans, LA

Introduction: Basal-like breast cancer (BLBC), an aggressive subtype of triple-negative breast cancer (TNBC), exhibits a high risk of recurrence and resistance to conventional treatments. Histone deacetylase inhibitors (HDACis), such as panobinostat and romidepsin, are approved for hematopoietic malignancies and have shown effectiveness against TNBC cells. Our study demonstrated that HDACis inhibited BLBC cell growth and induced apoptosis by suppressing HER3 expression via transcriptional downregulation of forkhead box protein A1 (FOXA1). However, the mechanisms by which HDACis regulate the expression of FOXA1 remain unclear.
Methods: Cell viability was evaluated using colony formation, MTS, and LIVE/DEAD cell staining assays. Apoptosis was assessed by flow cytometry. Gene and protein expression levels were analyzed via qRT-PCR, Western blotting, and immunohistochemistry (IHC). Co-immunoprecipitation was used to evaluate protein interactions. Lentiviral vectors carrying cDNA or shRNAs enabled overexpression or knockdown of target genes. Chromatin immunoprecipitation-quantitative PCR elucidated transcriptional regulatory mechanisms. *In vivo* experiments were conducted to examine therapeutic effects. Kaplan-Meier analysis evaluated clinical outcomes.
Results: FOXA1 was highly co-expressed with HER3 in BLBC specimens and cell lines, and FOXA1 induced HER3 expression. Panobinostat and romidepsin inhibited BLBC cell growth and induced apoptosis by suppressing FOXA1 expression and HER3 signaling. Gene expression profiling revealed a significant positive correlation between FOXA1 and transcription factors GATA2/3. High GATA2/3-FOXA1 expression correlated with poorer relapse-free survival (RFS) in BLBC patients. Mechanistic studies confirmed that GATA2/3 transcriptionally regulated FOXA1 by directly binding to its promoter. Overexpression of GATA2 in BLBC cells restored FOXA1 expression downregulated by HDACis and reduced their antitumor effects. Importantly, *in vivo* experiments showed that HCC1806 xenografts overexpressing GATA2 were less sensitive to panobinostat treatment. Consistent with these findings, IHC analyses revealed higher FOXA1 and HER3 expression in GATA2-overexpressing tumors.
Conclusion: HDACis exhibit potent antitumor effects in BLBC by suppressing the GATA2/3-FOXA1-HER3 axis. Our study highlights the critical role of GATA2/3 in transcriptionally regulating FOXA1 and mediating resistance to HDACis. These findings suggest that targeting the GATA2/3-FOXA1-HER3 axis may represent a promising therapeutic strategy for BLBC.

#4051 STK11-dependent NFkB localization drives characteristic transcriptional signatures in lung cancer.

Allison R. Racela[1], Sean M. Lenahan[2], Paula B. Deming[1], David J. Seward[1]

[1]University of Vermont, Burlington, VT,[2]Dana-Farber Cancer Institute, Boston, MA

Lung cancer is responsible for more deaths annually in the U.S. than any other cancer. Despite recent advances in lung cancer detection and treatment, the overall prognosis for patients remains poor since many are diagnosed at an advanced stage where the 5-year survival rate is less than 25%. Immune checkpoint inhibitors (ICI) have dramatically improved outcomes in patients with lung cancer by targeting mechanisms of immune evasion. Unfortunately, only ~30% of lung adenocarcinomas (LUADs) respond to anti-PD-1 therapy and we cannot reliably identify this group prior to treatment with ICIs. This underscores the need to identify which patients will respond to ICI therapies, while simultaneously highlighting the ~70% non-responder rate. Recent clinical studies have identified a subgroup of patients with KRAS-driven lung adenocarcinoma (LUAD) who exhibit anti-PD-1 therapy resistance. This group is characterized by tumors with the loss of function (LoF) of Serine/Threonine kinase 11 (STK11). STK11 operates in a heterotrimeric complex with the pseudo-kinase STRADα and the scaffolding protein MO25 where it regulates numerous intracellular signaling networks impacting metabolism, proliferation, transcription, and cell morphology. Why STK11 LoF correlates with anti-PD-1 resistance in the context of KRAS-driven LUAD remains unknown and represents a critical question in lung oncology. We performed whole transcriptome and pathway enrichment studies comparing KRAS-driven/STK11-compentent LUAD cell lines with KRAS-driven/STK11-null lines and identified STK11-loss-dependent differential gene expression profiles consistent with activation of the NF-kappa B (NF-kB) signaling pathway. Based on this analysis, we hypothesize that STK11 LoF leads to constitutive NF-kB-mediated transcriptional activation, including the upregulation of tumor-intrinsic, immune-evasion promoting cytokines, thereby connecting LUAD genotype with a putative mechanism explaining anti-PD1 resistance. Our ongoing studies aim to identify the downstream transcriptional effects of NF-kB activation and reveal the mechanism(s) linked to STK11 activity. To that end, we have established that NF-kB functions downstream of STK11. We demonstrate this relationship using the IKKB inhibitor PS1145. PS1145 blocks IKKB activation leading to NF-kB cytosolic sequestration and rescuing the STK11-loss-dependent transcriptional phenotype. In summary, our work highlights potential interventions to reduce ineffectiveness of immune-checkpoint inhibitor therapy by preventing tumor-intrinsic, genotype-dependent transcriptional changes contributing to immune-evasion. Understanding these molecular mechanisms could inform targeted therapies to overcome resistance and improve survival rates.

#4052 Biological Variations in NRF2 Hotspot Mutants.

Syed A. Ashraf[1], Anh Thi Thai[1], Jinghai Wu[1], McKenzie Kreamer[1], Katarina Benejova[2], Terry M. Williams[3], Martin Benej[2], Ioanna Papandreou[1], Nicholas C. Denko[1]

[1]Department of Radiation Oncology, The Ohio State University Comprehensive Cancer Center, Columbus, OH,[2]Department of Molecular Medicine and Therapeutics, The Ohio State University Wexner Medical Center, Columbus, OH,[3]Department of Radiation Oncology, City of Hope Cancer Center, Duarte, CA

Background: NRF2 is a transcription factor that is a master regulator for the cell's antioxidant response. Under normal conditions, NRF2 is rapidly degraded by cytoplasmic association with KEAP1 and subsequent ubiquitination and proteasomal degradation. Activating mutations in NRF2 contribute to lung cancer transformation. D29H, R34G, and E79Q are the most common mutations found in lung squamous cell carcinoma.
Methods: H520 lung cancer cells harbor an intact NRF2/KEAP system. H520 mutant cell lines were generated using lentivirus transduction of either WT or mutant NRF2 and confirmed by Western blot. Immunofluorescence was used to examine the effect of mutations on NRF2 levels and subcellular localization. Cells were tested for ROS levels by DCFFA fluorescence and subcellular NRF2 localization before and after treatment with tert-butyl hydroperoxide. Colony forming ability was tested after radiation with 0, 2, 4, or 6 Gy.
Results: D29H and R34G mutations result in increased NRF2 levels and stress-independent translocation to the nucleus compared to wild type NRF2. These mutant cells also showed higher expression of the NRF2 target gene G6PD. E79Q has little difference compared to wild type. All mutants showed decreased DCF fluorescence. With regards to colony formation ability, mutant cell lines were not statistically different from WT cells.
Conclusion: Our findings identified D29H and R34G as having increased nuclear levels and transactivation compared to WT cells. Despite the increased activity of NRF2 in these two mutations, there was little evidence for increased radiation resistance. These results indicate differential biological response of common NRF2 mutations.

#4053 Dynamic combinatorial p53 post-translational modifications regulate temporal transcription program and cell fates.

Dan Lu

Harvard Medical School, Boston, MA

The tumor suppressor p53 is activated by a range of cellular stresses including DNA damage, oncogenic signals, and nutrient deprivation. Upon activation, p53 transcriptionally upregulates an extensive repertoire of genes including those affecting cell fates such as cell cycle arrest, senescence, and apoptosis. What remains unclear is how information is conveyed through p53 to elicit the appropriate downstream responses according to the nature of the upstream stress. Changes in p53 temporal expression dynamics as well as specific p53 post-translational modifications (PTMs) are known to differentially regulate target gene induction. However, with over 100 known PTMs possible on p53, technical limitations have restricted the ability to detect the combinations of modifications on whole p53 molecules.
Using a state-of-the-art mass spectrometry method (I2MS) which enabled the first measurement of individual intact human p53 proteins, we have begun to uncover the landscapes of combinatorial PTMs from different p53 activation regimes in cancer cell lines. Together with complementary p53 modification specific antibodies we identified changes in PTM patterns which were both stimuli and time dependent. For example, we found that in response to double-strand DNA breaks, p53 exhibits pulses of expression. Furthermore, p53's post-translational modification state is altered between successive pulses, which we ascertained the most prominent temporal change in p53 PTMs in response to DNA damage to be a reduction of multiple p53 lysine acetylations. Inhibition of the deacetylase HDAC1, but not SIRT1, prevented the reduction in p53 acetylation, causing overexpression of senescence and apoptotic genes, and corresponding cell fates. Finally, we abrogated lysine acetylation by generating KR (lysine to arginine) mutations at single or multiple sites on p53, and found that acetylation sites are functionally non-redundant, with specific combinations exerting unique transcriptional effects on p53 target gene inductions. These results demonstrate an interplay between dynamics and modifications as a mechanistic strategy for cellular hubs like p53 to temporally organize multiple processes in individual cells. In addition, it highlights the importance of accounting for dynamical combinations of p53 PTMs in order to achieve the desired cellular response and efficacy of p53-mediated cancer therapies.

#4054 Heme oxygenase 1 and its nuclear interactors: a novel perspective on neuroendocrine prostate cancer.

Rocio A. Seniuk[1], Agustina Sabater[2], Juan Bizzotto[3], Gaston Pascual[1], Julia Lechuga[4], Estefania Labanca[5], Nicolas Anselmino[5], Elba Vazquez[1], Javier Cotignola[1], Pia Valacco[1], Geraldine Gueron[1], Ayelen Toro[1], Pablo Sanchis[2]

[1]IQUIBICEN (UBA-CONICET), Buenos Aires, Argentina,[2]Department of Genitourinary Medical Oncology, IQUIBICEN (UBA-CONICET), Universidad Argentina de la Empresa (UADE), Instituto de Tecnologia (INTEC), The University of Texas MD Anderson Cancer Center, Buenos Aires, Argentina,[3]IQUIBICEN (UBA-CONICET), Universidad Argentina de la Empresa (UADE), Instituto de Tecnologia (INTEC), Buenos Aires, Argentina,[4]University of Buenos Aires, Buenos Aires, Argentina,[5]MD Anderson Cancer Center, Houston, TX

Heme-oxygenase 1 (HO-1), the rate-limiting enzyme for heme degradation, has been implicated with a non-canonical antitumor role in prostate cancer (PCa). This study addressed the hypothesis that HO-1 exerts its antitumor effects through its protein interactome, aiming to identify HO-1's nuclear interactors and their potential association with specific PCa phenotypes. PCa cells were treated with hemin (80 µM, 24 h), a specific pharmacological inducer of HO-1, followed by HO-1 immunoprecipitation (IP) and LC-ESI MS/MS analysis, leading to the identification of 42 HO-1 interactors. Since we were interested in the nuclear functions of HO-1 we continue this work focusing in the 11 interactors that appeared only in the nucleui of hemin treated cells. Based on our hypothesis that nuclear HO-1 influences the activity of various transcriptional regulators, ChIP Atlas analysis identified four of 11 interactors—ILF3, DDX17, BCLAF1, and SAFB—as DNA-binding factors with regulomes significantly linked to neurological disorders. To explore the relevance of these interactors in PCa, we analyzed RNA-seq data from the MDA-PCa-PDX series (PCa Patient-Derived Xenografts Program, MD Anderson Cancer Center), which reflects the heterogeneity of PCa. Unsupervised clustering based on the expression of HO-1 interactors revealed that samples with high expression of these genes were predominantly associated with neuroendocrine prostate cancer (NEPC). Further bioinformatics analysis, comprising transcriptomic and clinical data from NEPC patients, demonstrated that the expression of HO-1 interactors distinctly clustered a subgroup of large cell neuroendocrine carcinoma samples characterized by elevated levels of *ILF3, DDX17, SAFB, BCLAF1,* and low expression of *HMOX1.* To functionalize these results *in vitro*, we established a protocol to transdifferentiate C4-2B cells to neuroendocrine (NE) cells using Forskolin (Fsk). Fsk (10uM, 48h) treated cells showed increased expression levels of NE markers namely *NSE, EZH2* and *SOX2.* Also, treatment with Fsk increased neurite-like processes formation, branch points formation and neurite-like processes length, consistent with a NE-like phenotype. In line with the previous bioinformatics analyses, we observed a significant suppression of *HMOX1 ($p<0.05$)* expression in NE-like C4-2B cells. Strikingly, this phenotype was accompanied by a significant induction of *BCLAF1 ($p<0.05$)* levels, reinforcing its implication in NEPC. Moreover, we confirmed by IP-Western blot HO-1/BCLAF1 physical interaction. In conclusion, our findings suggest that HO-1 exerts its non-canonical antitumor role in PCa by negatively regulating key nuclear interactors, including BCLAF1, ILF3, DDX17, and SAFB. This regulatory mechanism appears to suppress aggressive neuroendocrine phenotypes, particularly in NEPC samples, highlighting its potential as a therapeutic target.

#4055 Estrogen receptor beta suppresses melanoma progression through modulation of melanocyte master regulator activity.

Jocelyn Treneff, Amanda McCrary, Craig Burd

The Ohio State University, Columbus, OH

Female sex is associated with lower overall melanoma incidence and mortality, with estrogen signaling thought to protect against disease progression. Genomic estrogen signaling is mediated by estrogen receptors alpha (ERα) and beta (ERβ), which function as ligand-activated transcription factors. Notably, ERβ expression shows an inverse correlation with tumor thickness in the skin and is often lost in advanced melanomas, suggesting that ERβ activity may repress melanoma development. Here, we demonstrate that germline ERβ knockout leads to reduced melanoma-free survival in a murine model of $NRAS^{Q61R}$ spontaneous melanomas. While this implicates ERβ as a tumor suppressor, little is understood about the molecular activities of ERβ in melanomas and in melanocytes prior to transformation. To elucidate the genomic actions of the receptor, we performed ChIP-Seq in immortalized human melanocytes. We found that ERβ binding regions significantly overlap with those of MITF, a transcription factor that acts as a master regulator of melanocyte development. Changes in melanoma cellular phenotypes, such as increased proliferation, dedifferentiation, or invasiveness, are thought to be driven by differences in MITF activity. Therefore, we hypothesize that ERβ modulates MITF transcriptional activity in a way that suppresses melanoma progression. Using a reporter construct with activity driven by the tyrosinase (TYR) promoter, we showed that ERβ activation coincided with altered MITF regulation of reporter activity. As we have characterized overlapping ERβ and MITF recruitment in the TYR promoter, this result supports the hypothesis that ERβ co-binding can influence MITF transcriptional programs. Additionally, transcriptome analysis revealed that estrogen-responsive genes in melanocytes are enriched for migration and differentiation, biological processes that are directed by MITF and dysregulated in melanomas. Taken together, these findings indicate that ERβ functions as a tumor suppressor in melanoma by reprogramming MITF activity, identifying a new avenue through which melanoma may be better understood at the molecular level.

#4056 Determining the role of ZNF687, an uncharacterized zinc finger transcription factor, in lung adenocarcinoma (LUAD).

Xingyu Liu[1], Yixuan Xie[1], Zongtao Lin[1], Patrick Pribil[2], Benjamin Garcia[1]

[1]Washington University School of Medicine in St. Louis, St. Louis, MO,[2]Sciex Canada, Concord, MO, Canada

Introduction: ZNF687, a relatively understudied ZnTF, has been identified as a potential biomarker for lung adenocarcinoma (LUAD) through bioinformatic analysis of The Cancer Genome Atlas (TCGA) database, revealing its frequent upregulation in early-stage LUAD patients. This study aims to elucidate the molecular mechanisms that explain the role of ZNF687 in LUAD using liquid chromatography-mass spectrometry (LC/MS)-based proteomics approaches.

Methods: LUAD cell lines NCI-H1437 and Hcc2935 were obtained from ATCC. lentiviral system was employed to deliver shRNA targeting ZNF687 into the LUAD cells. An inducible expression system for tagged ZNF687 was generated in LUAD cells using the PiggyBac transposon system. Proteomics samples were processed with S-Trap and analyzed with LC/MS.

Results: We began by analyzing protein interactions of ZNF687. Compared to a control purification, several interaction candidates were significantly enriched in ZNF687 purifications. Among the top candidates identified were ZMYND8 and ZNF592, two ZnTFs previously reported to form the co-regulatory "Z3" complex with ZNF687. Other notable candidates included TSPYL2, a nucleosome assembly protein; KDM5C, a histone H3 lysine 4-specific demethylase; and the casein kinase 2 (CK2) complex.

Next, whole proteome profiling was conducted for each LUAD cell line, with or without ZNF687 knockdown via shRNA. Using a data-independent acquisition and label-free quantification approach, we quantified over 5,000 proteins. With a fold-change cutoff of 2 and a Q-value threshold of 0.05, more than 500 proteins showed significant abundance changes upon ZNF687 knockdown in Hcc2935 cells, while over 700 proteins were significantly altered in NCI-H1437 cells. Notably, the whole proteome analysis revealed an upregulation of epithelial-mesenchymal transition (EMT)-associated proteins following ZNF687 knockdown in both cell lines, suggesting an unexpected repressive role of ZNF687 in EMT. Further examination of common EMT markers, CDH1 and CDH2, in cells overexpressing ZNF687 revealed changes opposite to those observed with ZNF687 knockdown.

Conclusions: Given that both LUAD cell lines used in this study were derived from stage I patients and primarily exhibited epithelial characteristics, these findings suggest that the upregulation of ZNF687 in early-stage LUAD may play a role in preventing disease progression. Based on the identification of KDM5C as a potential ZNF687 interactor, we proposed the following model: ZNF687 recruits KDM5C to EMT related genes; subsequently, the expression of EMT genes are down regulated due to the loss of H3K4me3 from those chromatin regions, resulting in suppression of the EMT process.

#4057 Mechanism of TAL1 activation by alterations in the downstream enhancer in childhood T-lineage acute lymphoblastic leukemia.

Nadezhda V. Terekhanova[1], Xiaolong Chen[1], Wentao Yang[1], Ying Shao[1], Li Dong[1], Bensheng Ju[1], Yu Liu[1], John Easton[1], A. Thomas Look[2], Jinghui Zhang[1]

[1]St. Jude Children's Research Hospital, Memphis, TN,[2]Dana-Farber Cancer Institute, Boston, MA

The transcription factor (TF) TAL1 is a master regulator expressed at the early stages of normal thymocyte development. It is one of the major drivers in childhood T-lineage acute lymphoblastic leukemia (T-ALL) and is aberrantly activated in 30-40% of T-ALL. While somatic alterations in upstream and intronic cis-regulatory regions of *TAL1* are known mechanisms of activation, the role of downstream enhancer alterations (DEAs) remains less understood. We investigated somatic alterations in an intergenic region 11Kb downstream of *TAL1*, identifying a complex indel that created a *de novo* MYB transcription factor binding site (TFBS), and a 2.4Kb internal tandem duplication (ITD) encompassing a MYB TFBS. Alterations in this region were found to be present in 2% of the samples in a recent T-ALL landscape study involving 1,335 cases. Using the patient-derived xenograft (PDX) mouse models of the two T-ALLs harboring the complex indel and ITD DEA, we performed multi-omic profiling, including H3K27ac, H3K4me1, H3K27me3 and MYB chromatin immunoprecipitation sequencing (ChIP-seq); assay for transposase-accessible chromatin using sequencing (ATAC-seq), Hi-C, RNA-seq and full-length transcriptome sequencing (Iso-seq). Enhancer activity by DEA was validated by presence of H3K27ac peaks and cis-activation of *TAL1* which exhibits mono-allelic expression in both the primary tumors and PDX models. Involvement of MYB in enhancer activity is validated by MYB ChIP-seq peaks centered around the *de novo* and existing MYB TFBSs. Interestingly, we found that *TAL1* activation via DEA led to preferential initiation of transcription from the promoter located at the exon 4, resulting in predominant expression of the *TAL1*-short isoform in cases with DEA. Indeed, *TAL1*-short expression accounted for 78% of the *TAL1* expression in T-ALLs harboring DEA in contrast to the 37% frequency in other *TAL1*-activated T-ALLs (Wilcoxon two-sided test P=0.001). Furthermore, using the full-length transcripts generated by PacBio Iso-seq platform on the PDX samples, only *TAL1*-short transcripts were detected. By comparing Hi-C data generated from our PDX samples with those from Jurkat (TAL1+, with upstream enhancer region) and DND41 (TAL1-) cell lines, we identified a re-organization of chromatin looping structure showing distinct interaction between DEA region and *TAL1* promoter at exon 4. This study provides a detailed view of the regulatory mechanism of *TAL1* activation by DEA, indicating that preferential expression of *TAL1*-short isoform, protein product of which is known to bind more strongly to TAL1 E-protein partners than that of *TAL1*-long isoform, is active in initiating and maintaining the TAL1 core regulatory circuit in the transformation of pre-T cell progenitors in T-ALL.

#4058 CUT&RUN reveals E2F7 as a key transcriptional regulator of oncogenic functions in neuroendocrine small cell lung cancer.

Meijun Du[1], Jessica Schmeling[1], Angela Mathison[2], Victor Jin[3], Hui-Zi Chen[1]

[1]Division of Hematology & Oncology, Department of Medicine, Medical College of Wisconsin, Waukesha, WI,[2]Linda T. and John A. Mellowes Center for Genomic Sciences and Precision Medicine, Medical College of Wisconsin, Waukesha, WI,[3]Data Science Institute, Medical College of Wisconsin, Medical College of Wisconsin, Waukesha, WI

Small cell lung cancer (SCLC) is a molecularly heterogeneous disease, classified into neuroendocrine (NE) and non-neuroendocrine (non-NE) subtypes. The NE subtypes, including ASCL1 and NEUROD1, account for approximately 80% of SCLC cases. E2F7, a non-canonical E2F transcription factor family member, regulates cell cycle progression, and its dysfunction has been linked to cancers. A recent study identified E2F7 as a cofactor of ASCL1, enhancing its neurogenic activity. However, the role of E2F7 in SCLC subtypes is not yet clearly defined. The objective of this study is to profile both common and subtype specific E2F7 target genes and pathways that contribute to pathogenesis and may serve as targets for therapeutic intervention. To identify E2F7 targets in NE SCLC subtypes, we performed CUT&RUN (Cleavage Under Targets & Release Using Nuclease) in ASCL1 (DMS79, H146) and NEUROD1 (H82, H446) cell lines. Two antibodies against E2F7 and one against histone H3K4me3, marks of active gene promoters, were used in each cell line, with IgG as a negative control. Total 32 CUT&RUN next generation sequencing libraries (NGS) were generated and sequenced. NGS data was analyzed with a custom bioinformatics pipeline, using SEACR for peak calling and ChIPseeker for annotation. The identified E2F7 targets were then correlated with RNA-seq data from the CCLE database. In both ASCL1 and NEUROD1 cell lines, ~95% of E2F7-bound peaks were within 1 kilobase (kb) of gene promoters, most co-localizing with H3K4me3 peaks. Combining peaks from two E2F7 antibodies, we identified 872 E2F7 target genes in H146 (ASCL1) cell line, 818 in DMS79 (ASCL1) and 754 in H446 (NEUROD1). Fewer E2F7 peaks were called for H82 (NEUROD1) due to higher background. These targets included common and subtype-specific genes. Notably, E2F1 (regulator of G1/S transition) and EZH2 (mediator of the repressive H3K27me3 mark) were common targets, while DLL3 (target of multiple therapeutics in clinical development for SCLC) was ASCL1-specific, and CD82, a cell surface glycoprotein involved in cancer cell signaling, was NEUROD1-specific.Pathway analysis of E2F7-bound genes in ASCL1 and NEUROD1 cell lines revealed several shared pathways: cell cycle, spliceosome, ATP-dependent chromatin remodeling, and the Polycomb repressive complex. ASCL1-specific pathway was related to endocytosis, while NEUROD1 was linked to the HIF-1 signaling pathway. Correlation of E2F7 target genes with RNA-seq data revealed distinct gene expression patterns, specific to both the cell line and the subtype. These results offer new insights into E2F7-mediated transcriptional regulation in SCLC subtypes, providing a foundation for targeted therapeutic strategies. Our results support E2F7 as a master transcriptional regulator in SCLC and an important therapeutic target. Further analysis is needed to extend these findings to other subtypes, including POU2F3 and YAP1

#4059 CDK1-dependent hyperphosphorylation of NFIB governs its pro-metastatic function in SCLC.

Pawel Nowialis, Lina Song, Thi Na Nguyen, Thi Kim Ngan Bui, Wenjing Na, Ryan Clay, Kunyang Li, Lingtao Jin

UT Health Science Center at San Antonio, San Antonio, TX

Small cell lung cancer (SCLC) is a highly aggressive type of lung cancer. Its ability to form a metastatic spread in the early stages of disease accounts for high morbidity rates. Despite initial high rates of response to the most common line of treatment - conventional chemotherapy, majority of SCLC patients suffer from chemoresistant recurrence. Lack of full understanding of molecular basis driving metastasis and resistance are main obstacles in developing more efficient treatments. Several recent studies reported that nuclear factor I/B (NFIB) plays a role in promoting metastasis of SCLC through modulating chromatin accessibility. Moreover, NFIB is frequently found to be overexpressed or amplified in human SCLC and its high levels correlate with poor overall survival. Although oncogenic properties of NFIB have also been reported in studies on melanoma and triple negative breast cancer, other research, involving non-small cell lung cancer, glioblastoma, osteosarcoma and cutaneous squamous cell carcinoma suggest, that NFIB may also function as a tumor suppressor. The nature of this contradictions remains elusive and implies that the function of NFIB might depend on tissue specific regulation, e.g. post-translation modification (PTM). In this study we demonstrate that NFIB undergoes hyperphosphorylation during M-phase. Utilizing kinase specific inhibitors, siRNA and *in vitro* assays with recombinant proteins, we identified cyclin-dependent kinase 1 (CDK1) as a specific enzyme responsible for phosphorylation of NFIB during M-phase. Using Mass Spectrometry analysis of PTM of NFIB protein, we discovered specific amino acid targets of CDK1 and generated a quadruple NFIB mutant (Ser->Ala) construct, unable to undergo hyperphosphorylation during M-phase. qPCR analysis revealed that expression of phospho-incompetent form of NFIB in human SCLC cell lines resulted in altered expression of EMT-related genes in the fashion similar to NFIB knock-down. Altogether, these results suggest that phosphorylation of NFIB by CDK1 regulates its pro-metastatic function in SCLC. It also underlines the value of further research on targeting phosphorylation state of NFIB in aiding standard chemotherapeutic regimes.

#4060 The role of paraspeckles in EWS activated cancers.

Renee A. Kinne, Peter C. Hollenhorst

Medical Sciences, Indiana University School of Medicine, Bloomington, IN

The ETS family of transcription factors become aberrantly activated in multiple cancers. This activation is dependent on the co-activator EWS in both prostate cancer and Ewing sarcoma, an adolescent bone cancer. In prostate cancer, EWS is a binding partner necessary for oncogenic ETS, primarily ERG, activation. In Ewing sarcoma, the intrinsically disorder region (IDR) of EWS is fused to ETS members, primarily FLI1, and promotes aberrant expression and activation. The IDR of EWS promotes liquid-liquid phase separation (LLPS) and acts as a transcriptional activation domain. Current studies link LLPS with oncogenesis, making the LLPS ability of EWS a potential mechanism for oncogenic co-activation. In cells, EWS is found in phase separated bodies such as paraspeckles. Paraspeckles are nuclear bodies that have a role in transcriptional regulation, but their role in cancers is not well understood. I have shown that ERG and EWS/FLI1 colocalize with EWS granules in a purified protein droplet assay. This suggests that oncogenic ETS can participate in EWS LLPS. NEAT1, the essential RNA scaffolding of the paraspeckle, has a role in tumor progression in prostate cancer and I have shown that both ERG and EWS/FLI1 interact with NEAT1 through RIP. However, the potential role of paraspeckles in Ewing sarcoma or prostate cancer has not been further investigated. ETS positive prostate cancer and Ewing sarcoma lines showed paraspeckle presence through NEAT1 RNA-FISH. Knock down (KD) of two necessary paraspeckle components, NEAT1 or FUS, in Ewing sarcoma cells reduce paraspeckle presence, colony growth, and EWS/FLI1 activity at the transcriptional level. KD of FUS and NEAT1 also increased migration, which is consistent with EWS/FLI1 loss. Taken together, these data suggest that the paraspeckle has a role in EWS/FLI1 activity in Ewing Sarcoma and that components of the paraspeckle interact with ERG. Because ERG and FLI1 are homologous, there is potential that the paraspeckle acts as a mechanism of oncogenic transcription in prostate cancer as well as Ewing sarcoma.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: High-Throughput Sequencing / Microarrays / Next-Generation Sequencing Poster Session

#4064 Transcriptome-Wide Association Study of Alternative Polyadenylation Identifies Susceptibility Genes in Non-Small Cell Lung Cancer.

Xiaohang Xu, Sicong Wang, Hanyi Zhou, Qilong Tan, Zeyong Lang, Yun Zhu, Huadi Yuan, Zixiang Wu, Ling Zhu, Kejia Hu, Wenyuan Li, Dan Zhou, Ming Wu, Xifeng Wu

Zhejiang University, Hangzhou, China

Alternative polyadenylation (APA) plays a crucial role in cancer development and prognosis. However, the molecular characteristics of APA related to non-small cell lung cancer (NSCLC) susceptibility remain understudied especially in East Asian populations. In this study, we constructed an atlas of APA-regulated 3' untranslated region (3'UTR) and profiled its genetic regulation in 747 lung tissue samples (including tumors and paired normal tissues) from 417 Chinese NSCLC patients. We verified a significant global shortening of 3'UTRs in tumor samples compared to normal samples and underscored the value of APA-regulation as prognostic markers. The 3'UTR APA quantitative trait loci (3'aQTL) was identified by regressing the percentage of distal poly(A) site usage index (PDUI) value on genetic variants. We found that a significant proportion 3'aQTL are independent of genetic regulation of expression and are specific in Chinese. We also conducted a 3'UTR APA transcriptome-wide association study (3'aTWAS) by integrating the APA regulation atlas with a genome-wide association studies (GWAS) for NSCLC involving 7,035 cases and 185,413 cancer-free controls. We identified NSCLC-associated genes. Combining the consistent results from colocalization analysis, differential APA analysis, and survival analysis, we provide novel evidence for the role APA regulation in NSCLC and identified potential upstream regulators. Overall, our study profiled the APA regulation and highlighted the substantial role of APA in NSCLC carcinogenesis and prognosis.

#4065 Unveiling drivers of esophageal adenocarcinoma with CRISPR pooled screening and single-cell RNA-sequencing.

Ebtihal H. Mustafa, Julia Milne, Ka Meng Wu, Katherine Papastratos, Niko Thio, Wayne Phillips, Nicholas Clemons

Peter MacCallum Cancer Centre, Melbourne, Australia

Esophageal adenocarcinoma (EAC) is an aggressive malignancy with a rising incidence and limited treatment options. It develops from Barrett's esophagus, a pre-cancerous metaplastic condition, yet the molecular mechanisms driving its progression remain poorly understood. Genomic studies have identified ~76 candidate genes as potential drivers of EAC. In our previous work, we demonstrated that in the context of mutant TP53, the inactivation of one such candidate tumour suppressor, SMAD4, was sufficient to drive tumorigenesis in non-tumorigenic CP-B Barrett's esophagus xenografts. Building on this, we combined our EAC tumorigenesis model with Perturb-Seq (CRISPR-mediated gene editing coupled with single-cell RNA sequencing (scRNA-Seq)) to enable high-throughput functional characterization of EAC driver genes. The study specifically aimed to: (i) identify the molecular perturbations driving tumorigenesis in EAC, (ii) understand the transcriptional changes underlying the oncogenic effects of these perturbations, and (iii) tackle the genetic heterogeneity of EAC by categorizing driver genes according to shared transcriptional phenotypes. *In vivo* loss-of-function CRISPR screen was conducted using a boutique pooled CRISPR/Cas9 sgRNA library (6 sgRNAs per gene) targeting 57 known and putative tumour suppressor genes. To analyze the gene knockout-specific effects on the transcriptome at single-cell resolution, capture sequences compatible with 10X Feature Barcoding were incorporated into the sgRNAs. The library was transduced into two independent esophageal pre-malignant cell models, CP-B and CP-D, followed by xenotransplantation in NSG immunodeficient mice. We established that the knockout of 40 out of the 57 candidate tumour suppressors were independently sufficient to induce tumour formation in vivo. By integrating the scRNA-Seq data corresponding to each tumour suppressor knockout, we found that depletion of these tumour suppressor genes inhibited the proteasomal degradation of oncogenes and anti-apoptotic proteins, such as cyclin E, c-Myc, and Mcl-1. Additionally, this depletion led to the upregulation of genes involved in cell cycle progression, DNA repair, and lipid and carbohydrate metabolism. Perturb-seq also showed that driver-dependent transcriptional changes can be categorized into a smaller number of functional pathways allowing us to potentially consider groups of drivers as functional units. Collectively, the findings of this study enhance our understanding of the molecular events driving EAC tumorigenesis and offer a comprehensive view of the transcriptional phenotypes that can be targeted in future research. These insights lay the foundation for the development of novel therapeutic strategies for this highly heterogeneous disease.

#4066 High throughput single-cell TCR sequencing method identifies cancer-specific TCR clonotypes.

Efthymia Papalexi[1], Crina Curca[1], Bryan Hariadi[1], Alexa Suyama[2], Simone Marrujo[1], Gary Geiss[1], Charles Roco[1], Alexander Rosenberg[1]

[1]Parse Biosciences, Inc., Seattle, WA,[2]OHSU, Portland, OR

Cancer cells produce tumor-specific antigens and neoantigens that can be recognized by T cells and induce their clearance. Single cell profiling of T cell receptors (TCRs) and gene expression enables the identification and characterization of tumor infiltrating T cells that have antigen recognition and killing capabilities. Existing microfluidic-based single cell sequencing methods have limited cell and sample throughput making it hard to perform longitudinal studies to capture changes in TCR repertoire dynamics during tumor progression and therapeutic interventions. Understanding these dynamics will be key in designing the next generation of immunotherapies. To overcome these limitations, we developed a combinatorial barcoding approach to simultaneously characterize the TCRs alongside the full transcriptomes of up to 1 million fixed T cells from up to 96 unique samples in a single experiment. To demonstrate this approach, we profiled 1 million human T cells stimulated with a variety of antigens including the tumor antigens MART-1, NY-ESO-1, HER2/Neu, MAGE-A10, WT-1 and Tyrosinase. Using our technology, we recovered paired TCR alpha and beta clonotype information in 82% of all T cells. We estimated the clonotype diversity and found that the tumor antigen-stimulated samples had lower diversity than the controls. Specifically, when looking at the top three most expanded clonotypes for each sample we show that they account for 8-99% of all detected clonotypes. Next, we searched publicly available databases and showed our method not only detects previously annotated disease-specific CDR3 sequences but also uncovers previously unknown ones. Finally, we ran multiple sequence alignments and identified CDR3 sequence motifs unique to each antigen-stimulated population. In summary, we present a highly flexible and scalable method to single cell TCR profiling to allow researchers to profile up to 1 million T cells and up to 96 samples in a single experiment. We envision our approach to be invaluable in investigating T cell functional responses during infection, cancer, autoimmunity, or therapeutic interventions and pave the way for the development of new immune-based therapies.

#4067 Covaris truCOVER library prep kit: A streamlined PCR free WGS workflow for enhanced NGS library performance and data quality.

Vanessa Process[1], Martina Werner[1], **Madan Ambavaram**[1], Thuy Dao[1], Heidi Giese[1], Sushant Khanal[1], Luca Beker[1], Sameer Vasantgadkar[1], Maura Berkeley[2], Zachary Herbert[2], Greg Endress[1], Eugenio Daviso[1], Ulrich Thomann[1]

[1]Covaris, Woburn, MA,[2]DFCI, Boston, MA

Whole-genome sequencing (WGS) has become an indispensable tool in genomic research and clinical diagnostics, offering unprecedented insights into the underlying mechanisms of genetic diseases, cancer evolution, pharmacogenetics and pharmacology (1). As WGS increasingly transitions from research to routine clinical use, optimization of workflows for precision, scalability, and efficiency is essential. Addressing key challenges - including inconsistency in DNA fragmentation precision, suboptimal library conversion rates, cumbersome optimization to address sample input variation and its resulting sequencing biases - is essential for ensuring high-quality sequencing data. To this end, Covaris recently launched the truCOVER WGS PCR-free Library Prep Kit, a unique solution designed to streamline the whole-genome sequencing (WGS) library preparation process. This innovative, single-vessel workflow integrates Covaris Adaptive Focused Acoustics (AFA)-based DNA shearing, followed by end-repair and adaptor ligation, and adds a post-library size selection (2) that results in optimized fragment sizes. By optimizing fragment sizes for sequencing, this approach significantly reduces workflow complexity and turnaround time from sample to ready-to-pool libraries while lowering overall costs. The truCOVER library prep kit consistently delivers superior library conversion rates across diverse sample types, including DNA isolated from blood, saliva, and FFPE tissues. Sequencing analysis conducted in-house and in collaboration with leading institutions like the Dana-Farber Cancer Institute demonstrated improved genome coverage and enhanced variant detection accuracy with the Covaris workflow as compared to leading competitor workflows.

References: (1). Rosenquist et al., *Clinical Utility of Whole-Genome Sequencing in Precision Oncology.* Seminars in Cancer Biology, 84, 32-39 (2022).(2). Process et al., *Optimization of Insert Sizes for Illumina's WGS with Covaris AFA-Based Shearing and Bead-Based Size Selection.* Covaris Application Note (2024).

#4068 Development and evaluation of standardized samples for multiomic ctDNA assays across multiple cancer types.

Qiancheng You, Jiayue Xu, Jing Su, Xingyu Yang, Sa Zhang, Zeliang Deng, Xiaoling Li, Guangyou Li, Xinyue Kang, Si Zhang, Meifang Wu, **Bingsi Li**

Innovation Center, Burning Rock Biotech, Shanghai, China

Background: Multiomic circulating tumor DNA (ctDNA) assays offer several benefits over tumor-dependent ones, including being more rapid, less invasive, and cost-effective for monitoring tumor recurrence or therapy response through serial testing. Despite these advantages, identifying ultra-low frequency ctDNA alterations, whether genetic or epigenetic, from limited input material is highly challenging. Furthermore, complex assays are often more susceptible to experimental and computational variables and artifacts. This poses a pressing need for standardized samples to assess the analytical performance and enable unbiased comparisons between different technology platforms.

Methods: We have developed contrived standards using cell line, tissue, plasma, and PBMC samples that were either commercially purchased or provided with informed consent. The 'ground truth' for tumor fraction (TF) was defined by the dilution ratios and validated through *in silico* reads-mixing simulations. Subsequently, estimates of tumor allele fraction (TAF) using different biomarkers were analyzed and compared. (i) Mutation-based TAF: Both tumor-agnostic (somatic variants allele frequency, SVAF) and tumor-informed (circulating tumor allele fraction, cTAF) ones were characterized by a validated 520-/168-gene panel (10,000-35,000X) or an individualized mutation tracking panel (100,000X depth) following a WES scan on paired tumors. (ii) Methylation-based TAF: Both tumor-agnostic (tumor methylated fraction, TMeF) and tumor-informed (methylated tumor allele fraction, mTAF) ones were characterized by a validated targeted methylation assay.

Results: We have tested >400 contrived and clinical samples across different cancer types. Sample dilutions at pre-determined levels of TAF (0.001%-1%) are suitable for evaluating the limits of detection (LoDs) for most mainstream tumor-informed/-agnostic ctDNA assays. Samples with TAF near the LoD and background plasma were tested with replicates for precision and accuracy studies. Overall, reliable estimates of TAF were observed across different biomarkers, although the measurement of ultra-low fractions (i.e. TAF<0.01%) still remains a challenge. Notably, the number of unique patients/healthy donors is crucial for generating methylation-based standards, as demographic factors such as age, sex, and ethnicity have been shown to influence DNA methylation patterns.

Conclusions: With clinical adoption underway, it is critical to understand and evaluate the variables that impact the analytical performance of complex multiomic ctDNA assays. Here, we report a systematic evaluation of the analytical features and clinical relevance of standardized samples, thereby providing a versatile tool for assay optimization, validation, and cross-platform comparison.

#4069 Transcriptome-wide splicing analysis reveals changes in transcript usage of *MDM2* and *ESR1* in uterine leiomyomas.

Vilja Jokinen[1], Niko Valimaki[1], Kristiina Rajamaki[1], Auli Karhu[1], Emma Siili[2], Ralf Butzow[2], Oskari Heikinheimo[2], Annukka Pasanen[2], Lauri A. Aaltonen[1]

[1]University of Helsinki, Helsinki, Finland,[2]University of Helsinki and Helsinki University Hospital, Helsinki, Finland

Uterine leiomyomas (ULs) are smooth muscle tumors of the uterus. ULs are the most common tumors in women affecting up to 70% by the age of 50. ULs are often divided into molecular subtypes based on genetic driver alterations that are usually mutually exclusive. Transcriptome-wide aberrations in messenger-RNA (mRNA) splicing have been reported in many cancer types due to disturbed regulation of splicing. In addition, splice site mutations of individual driver genes, especially tumor suppressor genes, are common in cancer. Splice mutations of a few driver genes have been identified also in ULs. However, to our knowledge transcriptome-wide changes in splicing have not been studied in ULs.

Our aim was to study the potential role of mRNA-splicing alterations in UL genesis. We generated RNA-sequencing (RNAseq) data from 410 ULs and 151 corresponding normal myometrium samples and used LeafCutter for running splicing analyses.

The preliminary results suggest that splicing patterns differ between UL subtypes. Unsupervised hierarchical clustering based on splicing separates normals and molecular UL subtypes to their respective clusters. The different UL subtypes shared many splicing alterations compared to normal myometrium. mRNAs for components of extracellular matrix and contractile muscle fibers were enriched among differentially spliced transcripts in tumors compared to normals.

The only differentially spliced gene shared by all molecular UL subtypes was *MDM2*, a well-known oncogene that plays a role in the regulation of p53. Interestingly, *ZMAT3*, which is the third most overexpressed gene in ULs, is known to regulate *MDM2* splicing. Earlier GWAS studies have revealed a UL predisposition locus close to *MDM2*, affecting *MDM2* expression levels in ULs but not normal myometrium.

The UL subtypes with driver alterations in *MED12* or SRCAP complex genes showed differential usage of *estrogen receptor 1 (ESR1)* transcript isoforms. It is well known that excessive estrogen promotes UL genesis. The differential expression of *ESR1* isoforms may play a role in estrogen sensitivity of ULs.

ULs almost never progress to leiomyosarcoma. However, ULs share common driver genes and dysregulated signaling pathways with many cancer types. Better understanding of UL genesis broadens our knowledge on the common and distinguishing characteristics of benign and malignant tumors, perhaps shedding light on mechanisms preventing malignant degeneration in a benign neoplastic lesion.

#4070 Demonstrating analytical performance of Agilent Avida Duo utilizing novel multiomic reference standards.

Michael Ruvolo, Yingmin Wang, Grace Zhao, Heng Wang, Kyeong-Soo Jeong, Yun Bao, Shengrong Lin, Bram Herman, Mistuni Ghosh

Agilent Technologies, Inc., Santa Clara, CA

Measuring both genetic and epigenetic status by target enrichment NGS workflows is critical for high-sensitivity oncology applications using cell-free samples (cfDNA). Conventional workflows accomplish this by splitting a sample into multiple tests, potentially limiting sensitivity. The Agilent Avida target enrichment technology enables high-efficiency enrichment of native DNA fragments, preserving epigenetic markers such as methylation. This method, termed Avida Duo, measures both mutation and methylation status, maximizing the information that can be obtained from any single cfDNA sample. Multiomic assays have become increasingly important in research applications and determining their analytical performance is key to ensure they are fit for purpose. Currently, standards with known variants or methylation levels are available, however, there is a lack of multiomic standards for evaluation of more advanced dual modalities. We utilized commercially available mutation and methylation standards to create a combined reference to assess the performance of the Avida Duo workflow. To create this reference, the mutation status of the methylation-specific reference and the methylation profile of the mutation-specific reference was determined. We blended these fully characterized references in defined ratios to achieve known variant allele frequencies and methylation levels similar to real-world cfDNA samples. Using this combined reference standard, we demonstrated the analytical performance of the Avida Duo technology across an input range of 5-20ng using a 55.2kb mutational panel that combines hotspot and full-gene enrichment and a 173.9kb methyl panel that covering relevant differentially methylated regions (DMRs). From this, we were able to demonstrate robust detection of variants down to <0.5% VAF and 1% methylation of DMRs. The Avida Duo technology offers a single workflow for a combined interrogation of genetic and epigenetic information of cfDNA and as such, requires new tools to appropriately characterize the performance. Here we demonstrate an approach for generating reference samples for assay development, QC, and benchmarking the analytical performance of multiomic assays.

For Research Use Only. Not for use in diagnostic procedures.

#4072 Functional screen to optimize logic gate potency and selectivity.

Sara Martire, Michele Mcelvain, Vasantika Suryawanshi, Mark Daris, Tanveer K. Gill, Timothy P. Riley, Alexander Kamb, Chawita Netirojjanakul

A2 Biotherapeutics, Inc, Agoura Hills, CA

Background: Logic-gated cell therapy systems are considerably more complicated than conventional therapeutics and, therefore, more challenging to optimize. One type of logic gate, the NOT gate, responds to two inputs: the presence of antigen A and absence of antigen B. Perhaps the best studied NOT gate is called Tmod which consists of an activator (eg, a chimeric antigen receptor [CAR]) that is co-expressed with an inhibitory receptor, or blocker (DiAndreth B, et al. *Clin Immunol*. 2022). However, the complexity of the system limits the amount of structure-activity information that can readily be obtained. If further modules (eg, potency boosters) are added to the system, the behavior of these synthetic circuits can be even more complex.
Methods: A fluorescence-activated cell sorter (FACS)-based screening system was designed to rapidly select functional inhibitory receptors and inducible boosters from a pooled library of candidates. This screen incorporates an NFAT-responsive reporter cell line for iterative selection of reporter (+) and (-) cell populations by FACS after exposure to different stimuli. The selected populations were analyzed by sequencing to identify candidate blocker or booster sequences enriched during the process.
Results: Proof-of-concept experiments using a library of blocker variants demonstrated enrichment of active blockers. Moreover, selected features were used to successfully predict the blocker behavior with 70-80% accuracy by machine-learning algorithm. In another set of experiments, a library of almost 30,000 variants of a signal 1 booster that activate signaling in the presence of a small molecule (rimiducid) was screened. Signal 1 boosters with specific domain structures were enriched and validated in subsequent experiments.
Conclusions: The screening system described here identifies active blocker receptors and boosters from large libraries of variants. Its throughput is sufficient to generate large datasets and train machine learning models to better predict and optimize the function and control of logic-gated cell therapeutics.

#4073 Massively parallel barcoding as an alternative to genetic demultiplexing in large cohort scRNA-Seq studies.

Sanika Khare, Dominic Skinner, Austin Holmes, Felix Schlesinger, Keith Jackson

Scale Biosciences, Inc., San Diego, CA

Single-cell RNA sequencing (scRNA-Seq) studies with large sample cohorts are expensive, and prone to batch effects. Genetic demultiplexing allows for pooling of distinct samples beforehand and then using bioinformatic tools post sequencing to identify individual samples based on their genetic makeup. This allows for super-loading conventional scRNA-seq runs while reducing costs and batch effects. However, these strategies often lead to up to 30% doublets and are limited by the quality of the reference genome used, and the genetic diversity of the input. Additional bulk sequencing and computational analyses add on costs and times to identify individual samples. This problem becomes more acute in diseases with heavy mutational burden like cancer - tumor-driven mutations might overshadow natural genetic variation between samples and extra sequencing and analyses might be warranted. Scale Bio has pioneered the use of massively parallelized barcoding and novel multiplexing strategies (ScalePlex) to boost throughput and circumvent the need for additional sample demultiplexing strategies to identify individual samples. Typical doublet rates in a Scale Bio scRNA-Seq library are around 5% and the protocol allows for simultaneous profiling of up to thousands of samples with Scaleplex, with significantly lower per sample inputs and no additional bulk sequencing required. Here we demonstrate the utility of the Scale Bio scRNA-Seq with and without Scaleplex in sample demultiplexing 12 samples from diseased and non-diseased individuals with similar genetic backgrounds when compared to super loading a conventional scRNA-seq run. Furthermore, we compare our data with current on market, publicly available computational tools for genetic demultiplexing with ScalePlex readouts as the ground truth. We observe low doublet rates in the Scale Bio data along with maximum utilization of possible sequencing reads, and clear, unambiguous sample identification when compared to conventional scRNA-seq techniques and analyses tools. Thus, the Scale Bio scRNA-seq platform offers a viable, low cost and effort platform for conventional genetic demultiplexing techniques with better resolution and higher sample multiplexing capacity. Furthermore, ScalePlex provides an alternative to easily, distinctly barcode and multiplex samples without genetic demultiplexing where researchers can gain the same benefits of reduced cost and batch effects while removing sample heterogeneity considerations and bioinformatics barrier of genetic demultiplexing.

#4074 A 10 million cell atlas of cytokine-stimulated PBMCs via high-throughput combinatorial single cell RNA sequencing.

Joey Pangallo, Efthymia Papalexi, Simone Marrujo, Bryan Hariadi, Crina Curca, Alexa Suyama, Maria Nigos, Oliver Sanderson, Hoai Nguyen, Vuong Tran, Ajay Sapre, Olivia Kaplan, Sarah Schroeder, Alec Salvino, Ryan Koehler, Gokhan Demirkan, Gary Geiss, Charles Roco, Alexander Rosenberg

Parse Biosciences, Inc., Seattle, WA

Cytokines, secreted by immune cells, are essential for coordinating immune responses against pathogens and abnormal cells, such as those found in cancer. Despite their critical role, a comprehensive understanding of how many different types of immune cells respond to individual cytokines remains elusive. To begin to address this challenge, we created a comprehensive single cell atlas of immune responses to 90 cytokines, profiling the single cell transcriptomes of 10 million PBMCs from 12 donors after 24 hours of treatment. We identified 18 cell types, resulting in the analysis of 19,440 experimental conditions. Our analysis revealed gene expression changes that are both cell type- and donor-specific, uncovering responses even in rare immune cell populations that are difficult to capture in smaller datasets. Furthermore, we also identified a small number of cytokines that induced changes in cell type proportions after cytokine treatment. Given the limited number of publicly available large-scale single cell studies, we also provide an updated, detailed analysis workflow to encourage researchers to produce similarly scaled-up datasets. By sharing this comprehensive dataset and methodology, we aim to empower the scientific community to scale single cell experiments and drive advancements in immunology, immuno-oncology, cancer biology, and beyond.

#4075 GEM-X Flex: scalable and cost-effective whole transcriptome single cell analysis for cancer marker detection from FFPE tissue section slides.

Tingsheng Y. Drennon, Jawad Abousoud, Ryan T. Stott, Juan Pablo Romero, Paul Lund, Nico Sponer, Sarah Taylor, Peter Smibert, Andrew Kohlway

Biology, 10x Genomics, Inc., Pleasanton, CA

Formalin-fixed paraffin-embedded (FFPE) tissue blocks are the most widely used sample type in clinical research for preserving tissue morphology and supporting long-term storage. These samples can be particularly valuable for studying rare cancers or when sample procurement is challenging. The growing demand for comprehensive single cell gene profiling calls for methods that require less sample input, reduce costs and enable high-throughput analysis for large clinical studies.

Large-cohort studies are often limited by financial constraints. To overcome this challenge, we performed a cost-effective, high-throughput experiment with eight different FFPE blocks (glioblastoma, colorectal, lung, breast, skin, endocervical cancers, normal kidney, and reactive lymph node), with two replicates per sample. Using the 16-barcode multiplexing approach of GEM-X Flex, we processed 320,000 cells pooled from all 16 samples in a single lane (20,000 cells per sample). The distinct barcodes allowed us to obtain high quality single cell sequencing data from each individual sample with ~< 8% undetected multiples. We identified tumor markers specific to each cancer, including *PROM1* (glioblastoma), *BRCA1* (colorectal), *KRT19* (lung), *MUC1* (breast), *GPC3* (breast), and *MUC16* (endocervical). This method allows processing up to 2.56 million cells per chip across 8 lanes, reducing the cost per sample to just one cent per cell, making large-scale studies more cost-efficient.

We also extended this approach to tissue sections already mounted on slides and subjected to histological analysis, enabling the extraction of the most information from precious clinical samples. Using the GEM-X Flex assay, we generated whole transcriptome profiles from as few as 25,000 nuclei isolated from 5 µm FFPE tissue sections on standard superfrost slides. Gene expression profiles, cell type proportions, and assay sensitivity from these thin sections were comparable to those from 25 µm tissue scrolls from the same FFPE block, demonstrating that high-quality single cell RNA sequencing is achievable with minimal sample input. The ability to work with 5 µm tissue sections expands options for universal sample preparation across various assays. It also broadens sample procurement opportunities by using tissue sections instead of whole FFPE blocks, significantly lowering costs.

In summary, the GEM-X Flex assay enables whole-transcriptome gene profiling at single cell resolution from 5 µm FFPE tissue sections. By combining H&E image analysis with RNA sequencing data from sequential sections of the same cancer block, pathologists can achieve more precise diagnostics. The assay's multiplexing capability significantly lowers costs and supports high-throughput studies, enabling large-scale tumor marker screening for early detection, progression monitoring, and therapeutic target development.

#4077 Preserving and contextualizing in situ transcriptomes of hormone receptor-negative breast premalignancy.

Russell B. Hawes[1], Kristen A. Atkins[2], David R. Brenin[3], Kevin A. Janes[1]

[1]Biomedical Engineering, University of Virginia, Charlottesville, VA,[2]Pathology, University of Virginia, Charlottesville, VA,[3]Surgical Oncology, University of Virginia, Charlottesville, VA

Triple-negative breast cancer (TNBC) is a poor-prognosis molecular subtype of breast cancer that is thought to begin as an estrogen receptor-negative ductal carcinoma in situ (ER- DCIS). ER- DCIS is much less common than other types of DCIS, suggesting that it is a transient premalignant state before invasion begins. To better characterize the premalignant transcriptome, we use laser capture microdissection-guided stochastic 10-cell RNA sequencing to profile the transcriptome of ER- DCIS cases that present to the University of Virginia (n = 10 cases thus far). In early cases, RNA integrity was poor with RNA integrity numbers (RIN) near zero. To improve RNA integrity in future samples, we began transporting surgical excision material on ice to minimize RNase activity, and coordinated the pathological processing to minimize time to cryoembedding. We have reduced time to cryoembedding to 26 minutes on average and obtained usable RNA for 10-cell RNA sequencing, though RIN remains case dependent. We also optimized proteinase K and inhibitor concentrations during RNA extraction to ensure maximal reverse transcriptase activity after cell digestion. 10-cell transcriptomes will be combined with RNA sequencing data from the Human Tumor Atlas Network — a large, multisite patient dataset that includes ER- DCIS profiles acquired from whole lesions isolated by laser capture microdissection (LCM). These external LCM samples have been filtered to remove samples with low read counts across all genes. These additional samples in combination with our own will allow us to distinguish whether differences in handling and sample preservation give rise to tangibly higher quality transcriptomes when compared to existing archival samples.

#4078 Enabling single-cell RNA analysis of hundreds of samples and millions of cells through automated library preparation.

Rosary Nguyen, Swarnagowri Vaidyanathan, Zorine Hlathu, Xueying Zhao, Jamie Moskwa, Ricelle A. Acob, Chelsea Gordon, Larry Wang, Aruna Ayer

BD Biosciences, Milpitas, CA

Single-cell RNA-seq is a powerful method for studying complex cell populations. As opportunities grow for single-cell multiomics methods in translational research, there is an increased need for high-throughput approaches capable of efficiently capturing and analyzing large numbers of cells and samples simultaneously. Current assays on scRNA-seq platforms have limited throughput due to the low cell recovery and tedious hands-on library preparation steps.

Here we demonstrate the feasibility of automating single-cell RNA-seq library preparation with BD Rhapsody cell capture bead libraries on the Hamilton NGS STAR system. To show the range of cell input capabilities of the NGS STAR system, we input BD Rhapsody Cell Capture Beads containing captured molecules from 6,000 to 20,000 cells. To evaluate the repeatability and reproducibility of the platform, we generated a large pool of BD Rhapsody cell-captured beads to test the same sample library across multiple wells, instruments, and runs. Using in-process quality control and sequencing, we confirmed equivalent biological output in terms of library quality, sensitivity, and gene expression correlation ($R^2 > 0.9$) compared to manual bench controls. In addition, we loaded several BD Rhapsody HT Xpress lanes with 100,000 cells and used automated library preparation to compare with manual bench controls. This study demonstrates that BD Rhapsody cell captured beads can be utilized on the Hamilton NGS STAR to generate high quality libraries for sequencing and increase throughput of samples, while reducing hands on time. Processing 48 samples with 100K cells each allows users to prepare libraries for over 4 million cells in a single run. When combined with upstream sample multiplexing, hundreds of samples can be analyzed together, offering significant time savings and reducing variability between samples. This application could be readily extended to other existing multiomics single-cell technologies such as CITEseq assay.

For Research-Use Only. Not for use in diagnostic or therapeutic procedures.BD and the BD Logo are trademarks of Becton, Dickinson and Company. © 2024 BD. All rights reserved. NPM-5204.

#4080 A novel integrated target capture and rapid sequencing workflow for myeloid malignancy mutation detection within 12 hours.

Kyle S. Metcalfe, Benjamin J. Krajacich, Xiaodong Qi, Kelly Wiseman, Marina McCowin, Shawn Levy, Junhua Zhao

Element Biosciences, Inc., San Diego, CA

Hybrid capture technology is a powerful and cost-effective tool in precision oncology research to detect somatic mutations in hematologic malignancies. While target capture is important for efficient mutation detection, obtaining rapid sequencing results is critical for research in this field. Traditional hybrid capture workflows involve several time-consuming steps prior to sequencing, including post-hybridization capture, washes, and amplification, which can delay results. Here, we combine Trinity™ hybrid capture and a rapid AVITI™ sequencing protocol to deliver targeted sequencing results within 12 hours. We applied this rapid Trinity™ workflow together with a custom myeloid panel based on a design used by Genomics Medicine Sweden to detect mutations in reference DNA and patient blood/bone marrow/fresh frozen tissue. This custom panel builds on an earlier design, targeting genes included in contemporary guidelines and those with strong or potential clinical impact as well as genes reported in the literature but with less established clinical relevance. The final 7,901-probe panel spans a target region of 0.73 kb and provides uniform coverage across all targets, including challenging GC-rich regions. We were able to detect myeloid malignancy mutations between 0.5%-70% VAF in Horizon's Mimix™ Myeloid DNA Reference Standard, including internal tandem duplications observed in some patients with acute myeloid leukemia (AML). In sum, the Trinity™ workflow on the AVITI™ sequencer provides a rapid, accurate, and robust solution for targeted sequencing, offering significant advancements in turn-around time for hematology malignancy research.

#4081 Analysis of low-grade serous ovarian cancer by long-read full length transcripts sequencing.

Kwong-Kwok Wong, Yvonne Tsang, David M. Gershenson

UT MD Anderson Cancer Center, Houston, TX

Low grade serous ovarian carcinoma is a rare epithelial ovarian cancer that occurs more frequently in younger women. RNA sequencing (RNAseq) has been playing a pivotal role in understanding the molecular pathogenesis of the low-grade ovarian serous carcinoma (LGSOC). The quantification of gene expression with enough sequencing depth can be fairly accurate. However, the current short-read RNAseq approach is not very accurate in measuring individual transcript activity. This is because multiple transcripts from the same gene share high sequence similarity, which complicates the transcript mapping. As the accuracy of long-read sequencing technology is improving, long-read full transcript RNA sequencing is becoming an important tool for interrogating alternative transcripts and the discovery of gene fusion transcripts. In this study, we compare short-read RNAseq and long-read RNAseq from the same LGSOC patient samples. The RNA-Seq analysis for long-reads were performed by mapping sequencing reads to annotated reference human genome GrCh38 with minimap2. First, all annotated transcripts or genes were extracted including any annotated splice variants. Subsequently, the reads were mapped against all the transcripts as well as to the whole genome using minimap2. The distributing and counting the reads across genes and transcripts were calculated. We found that the expression level of transcripts in short-read in general is higher than that that of long-read. It is because short reads frequently could not be accurately mapped to the correct alternative transcripts from the same genes. When a read mapped to multiple transcripts, the short reads were randomly assigned to a transcript from the same gene. As a result, more than 100,000 transcripts had at least one assigned read in the short-read RNAseq analysis, but less than 50,000 transcripts had at least one assigned read in long-read RNAseq analysis. Long read with greater read-depth had better quantification accuracy for detecting specific transcripts. To identify potential gene fusions, the raw Oxford Nanopore long-read RNA-seq fastq files were corrected and polished with high sequencing quality short-read RNA-seq data by racon assembly tool. Those long-reads with unaligned ends of reads from a mapping generated by RNA-Seq analysis were re-mapped to the reference using the unaligned ends of reads. In this study, we identified several gene fusions including a gene fusion between *NSUN4* and *FAAH* genes with at least seven long reads covering the breakpoint. *FAAH* encodes the enzyme fatty acid amide hydrolase and has been shown to be a metastasis suppressor in breast cancer previously. It is possible that the truncated FAAH protein because of gene fusion is involved in the metastasis of the LGSOC that we have analyzed. This could be another mechanism involved in the progression of LGSOC. Additional analysis of the long-read full transcript sequencing of LGSOC is in progress.

#4083 Ancestral background and germline mutations in head and neck cancer: An analysis of disparities in genetic risk factors using the All of Us database.

Mayra S. Haedo-Cruz, Grace E. Velez-Crespo, Natalie Alamo-Rodriguez, Vivian Colon-Lopez, Julie Dutil, Josue Perez-Santiago

University of Puerto Rico Comprehensive Cancer Center, San Juan, PR

Background: Head and Neck Cancer (HNC) ranks as the seventh most common cancer globally, yet significant disparities persist in mortality rates across ethnic groups, especially in the United States (US). Hispanic and Non-Hispanic (NH) Black men exhibit higher cancer-specific mortality rates for HNC compared to NH White men, indicating gaps in cancer prevention, early detection, and genetic risk profiling for these groups. The influence of genetic ancestry on HNC risk remains understudied, particularly within Hispanics, who have a diverse ancestral composition. This study aims to elucidate the association between genetic ancestry and HNC, while also analyzing mutations in four genes associated with HNC: FAT1, POLB, TP53, and CDKN2A.
Methods: Demographic information, electronic health records, and microarray data for this study was sourced from the All of Us Research Program. A cohort of 2,552 individuals was identified, where half were of HNC cases, and half were demographically matched controls without a cancer diagnosis. Mann-Whitney U test was performed to analyze the differences between ancestry percentages within cancer and non cancer groups. Associations of genetic variants with cancer status or ancestral genetic makeup were assessed with Chi-square tests.
Results: Self-reported Hispanic or Latino participants in our cohort had an approximate average of 83% Indigenous American ancestry, 8% European ancestry, 6% African ancestry, 1% Eastern Asian ancestry, 0.6% Southern Asian ancestry, and 0.5% Middle Eastern ancestry. We identified that individuals with a higher percentage of Indigenous American ancestry were more likely to have a negative cancer diagnosis ($p<0.05$). Conversely, individuals with a higher percentage of European ancestry were more likely to have a positive HNC diagnosis ($p<0.01$). We did not identify significant results relating African ancestry to the diagnosis of HNC. Six single nucleotide polymorphisms (SNPs) within the four genes studied had a significant association to cancer diagnosis ($p<0.05$). There was a significant association in two of these SNPs between genotypes and predominant ancestry. Individuals with predominant Indigenous American ancestry were more likely to have the CT genotype for rs3136719 in the POLB gene ($p<0.05$). For rs3218009 in the CDKN2A gene, individuals with predominant European ancestry were associated with the CG genotype ($p<0.05$).
Conclusions: We identified that a high percentage of Indigenous American ancestry was linked to reduced HNC risk, while a high percentage of European ancestry was associated with increased risk, suggesting ancestry-related germline mutations may influence susceptibility. These findings indicate that the increased mortality rate related to HNC in Black and Latino populations in the US could be more significantly affected by social determinants of health.

#4085 Assessing RNA integrity in FFPE samples with the DV200 quality metric for automated electrophoresis systems.

I. Priester[1], A. Dorn[1], T. Butler[2];
[1]Agilent Technologies, Waldbronn, Germany, [2]Agilent Technologies, Ankeny, IA

Introduction:
Formalin-fixation and paraffin-embedding (FFPE) is a pivotal technique in pathology, enabling the long-term preservation of tissue specimens. This method not only maintains the cellular structure but also allows repeated analyses of the same sample without the need for additional collection. Despite its benefits, FFPE poses significant challenges for nucleic acid extraction and analysis, particularly for RNA, which is essential for gene expression studies. The fixation process can lead to RNA degradation and fragmentation, creating obstacles for researchers who require high-quality RNA for sequencing purposes. Implementing a quality control system to evaluate RNA integrity post-FFPE extraction is crucial for determining the viability of a sample for downstream analyses like next-generation sequencing.
Methods:
FFPE RNA samples extracted from different sources were analyzed on the Agilent 2100 Bioanalyzer system with the Agilent RNA 6000 Nano kit and the Agilent RNA 6000 Pico kit, and on the 4200 TapeStation system with the Agilent RNA ScreenTape and High Sensitivity RNA ScreenTape with corresponding reagents. Data analysis on the Agilent 2100 Bioanalyzer Expert software was completed using the DV_{200} assay configuration file. Analysis using the TapeStation was performed with the region mode, setting a region of 200 to 10,000 nucleotides to obtain the DV_{200} metric.
Results:
The obtained electropherograms from both systems exhibited similar overall patterns, with slight differences. Despite these differences in electropherogram appearance, the comparison of DV_{200} values demonstrated highly comparable results. The Bioanalyzer and TapeStation systems showed consistent DV_{200} scores across different FFPE RNA samples, demonstrating their interchangeability and reliability.
Conclusions:
The results demonstrate that each system is capable of similarly assessing the quality of FFPE RNA samples. Additionally, the data points out that a consistent measurement can be obtained regardless of the assay or kit being used within each system. This comparison allows users for confident use of the DV_{200} quality metric in their workflows.
For Research Use Only. Not for use in diagnostic procedures.
Keywords: RNA, DV200, quality control, FFPE, TapeStation system, Bioanalyzer system

#4086 A streamlined and modular workflow solution enhances coverage uniformity and achieves high library complexity from genomic DNA/total RNA using both short and long read sequencers.

Bahram Arezi[1], Adam Janssen[1], Nikki Liu[1], Sergey Shiryaev[1], Keith Chen[1], Bilan Hsue[1], Brandyn Clark[1], Jeff fox[1], Nedda Saremi[2], Inlora Jingga[2], Kyeong-soo Jeong[2], Khine Win[2], Gilbert Amparo[2], Neelima Mehendale[2], Jayati Ghosh[2], Cindy Hon[2]

[1]Agilent Technologies, Inc., La Jolla, CA,[2]Agilent Technologies, Inc., Santa Clara, CA

The application of NGS-based assays can be limited by the availability and quality of genomic DNA from certain sample sources. Practical factors such as turnaround time, workflow simplicity, target enrichment capability, and overall performance are crucial in determining whether NGS is the preferred method for germline or somatic mutation detection. In addition, due to certain short read sequencer limitations (e.g., mapping rate, repetitive regions, etc.), enabling long read capture and sequencing allows for better detection of structural variations, complex rearrangements, and variants in highly repetitive or polymorphic regions. To address these challenges, we have developed a flexible and automation-compatible workflow solution that supports both WGS (PCR-free and PCR-based, depending on the application) and target enrichment sequencing. Our modular workflow accommodates a wide range of DNA and RNA inputs (10-500 ng), standard and in-line molecular-barcoded adaptors, enzymatic and mechanical shearing methods, fast or overnight probe hybridization, and up to 384 unique dual sample indices to eliminate index hopping. Our enzymatic library preparation method combines fragmentation, end repair, and dA-tailing in a single step, allowing the use of samples with low concentrations. We demonstrate high base coverage uniformity and minimal bias across all GC content (Fold 80 of 1.12-1.13) using three bacteria of varying GC content (30%-67%) and human gDNA. Finally, our novel fast hybridization buffer formulation provides maximum complexity while maintaining high uniformity and base coverage, specifically addressing the issue of complexity loss when multiple libraries are pooled during bait hybridization. As we demonstrate, with small changes in the protocol, efficient long read capture (>5kb) and sequencing can be successfully accomplished. For Research Use Only. Not for use in Diagnostic procedures.

#4087 Panel-adjustable and user-friendly hybridization-capture technology delivers high performance demonstrated by different exome probe panels.

Alexandra Wang, Daniel Saatman, Ekaterina Star, Jinglie Zhou

R&D, Integrated DNA Technologies, Inc., Redwood City, CA

Targeted sequencing by hybridization-capture is a versatile and powerful way to interrogate selected regions of interest in large and complex genomes. However, the performance of a given hybridization-capture workflow is often optimized only towards a specific approach to probe panel design. Here we demonstrate a hybridization-capture enrichment system that delivers high performance on three commercially available Exome panels with short and overnight hybridizations. This is achieved using our new xGen Hybridization and Wash v3 (xGen v3), which features a simple user-friendly workflow without any pre-heating, hot-liquid handling, or time-and-temperature sensitive steps. Human genomic reference libraries were constructed with xGen™ cfDNA & FFPE DNA Library Preparation Kit using Coriell NA12878 gDNA. These equivalent libraries are hybridized to xGen™ Exome Hyb Panel v2, xGen™ Exome Hyb Panel v1, or a double-stranded-probe Exome panel. Hybridization incubations from 1-hour to overnight were compared, spanning total inputs from 100ng to microgram quantities per hybridization-capture. xGen v3 delivered consistent performance for all three Exome panels, without the increase in GC-bias that typically occurs for short hybridizations. Benchmarking using a currently available commercial kit is presented for performance comparison. Non-biased sampling reveals properties of the library as directed by the panel design. The demonstration here displays a panel-directed enrichment workflow that is reliable for wide range of total capture inputs and hybridization times for each given Exome panel, spanning different panel design approaches as well as single- and double-stranded probes. This behavior can be extrapolated to applications that requires multiplexing, low-pass or deep-sequencing needs, and custom panels. The panel-directed consistency over a large range of inputs and hybridization times makes xGen v3 adaptable to a large variety of applications, from quick surveys to high-throughput and/or in-depth interrogations as directed by the panel design.

#4088 Optimized workflow for isolating nuclei from FFPE tissue sections for snRNAseq.

Lea Eich, Svetlana Khorkova, Nojan Jelveh, Lilian Martinez Carrera, Olaf Hardt, Carsten Poggel, **Benjamin Theek**

Miltenyi Biotec B.V. & Co. KG, Bergisch Gladbach, Germany

Background: Formaldehyde fixed and paraffin embedded (FFPE) tissue sections are a valuable resource in cancer research due to the long-term preservation of clinical samples. However, these tissues present significant challenges for molecular analysis due to fixation-induced crosslinking. This study describes an optimized workflow that enables efficient isolation of nuclei from FFPE tissues, compatible with the Chromium Single Cell Gene Expression Flex Assay for downstream gene expression analysis.
Methods: FFPE tissue sections from different tissue types (i.e., breast-, lung-, and colon-cancer) were subjected to a deparaffinization and rehydration process, followed by a combined gentleMACS based mechanical dissociation and enzymatic digestion to obtain nuclei. Our new dissociation protocol was benchmarked against a Liberase TH based dissociation. Key modifications of our protocol include the selection of an optimized enzyme mix and a digestion buffer composition to enhance nuclei yield and purity. Nuclei purity and yield were quantified using flow cytometry and an automated cell counter, respectively, while RNA metrics, such as DV200, were assessed via the Agilent TapeStation. The isolated nuclei were then processed using 10x Genomics Chromium Single Cell Gene Expression Flex Assay to validate their suitability for RNA profiling.
Results: The optimized protocol successfully isolated nuclei from a variety of FFPE tissue types, often yielding sufficient nuclei from a single 25um FFPE tissue scroll. The following Chromium Single Cell Gene Expression Flex Assay confirmed the superior performance of our new dissociation workflow for RNA profiling. Cell Rangers QC metrics for median UMI counts, median genes, and median reads per cell were improved on average by 61%, 53%, and 59%, respectively. Furthermore, mitochondrial gene expression was reduced on average by 63% compared to the control, suggesting a cleaner nuclei sample.
Conclusion: The optimized workflow provides a significant advancement for utilizing FFPE samples in single-cell gene expression studies. By enabling efficient nuclei isolation from FFPE sections, this protocol allows for comprehensive transcriptomic analysis using the Chromium Single Cell Gene Expression Flex Assay, with minimal background, unlocking the potential of archived tissues in retrospective and translational research.

#4089 A novel NGS-based sample tracking system for robust sample chain of custody and quality control in fresh frozen and FFPE tumor samples.

Katherine Milligan, Yu Qiu, Andrea O'Hara, **Carrie Ziemniak**, David Corney, Haythem Latif

Azenta Life Sciences, South Plainfield, NJ

When processing large cohorts of samples, sample identity and functional quality are key to ensure the derived data is accurate and attributed to the appropriate sample. This is especially critical when working with precious and degraded material such as formalin-fixed paraffin-embedded (FFPE) samples. A potential sample swap or poor sample quality may result in incorrect data or delays due to re-processing. While traditional methods such as qPCR can be used for quality control, they may not be an effective predictor of sample success in Next-Generation Sequencing (NGS) based assays due to differential susceptibility to inhibitors and interfering substances. Performing a functional quality control assessment using the same methodology for data generation allows for a more accurate prediction of performance as it would be similarly affected by interfering substances or sample degradation. We have created a scalable, cost-effective sample tracking and functional quality control assay that may be assessed prior to performing more expensive downstream sequencing assays. This assay was designed to confirm consistency in sample identity during processing and confirm identity when multiple samples are submitted from the same subject. This amplicon panel targets autosomal SNPs with varying minor allele frequencies in different ethnic populations, limiting a bias towards variation in European ethnic populations. The average minor allele frequency for the panel developed for the general population is 0.45, more specifically 0.46 for European background, 0.44 for African background and 0.46 for Asian background based on data from the Allele Frequency Aggregator (ALFA) database. Most amplicons were designed to be less than 150 base pairs to increase the likelihood of success for samples with degraded DNA. Targets were included on the X and Y chromosome for sex determination. Tumor-normal paired samples were processed on the sample tracking assay, targeting regions throughout the exome to allow tracking of sample identity. When comparing “normal” samples extracted from blood to their paired FFPE tumor tissue, known matches had an average of 98.0% concordance, while known mismatches had an average of 40.4% concordance, with the highest known mismatch showing 61.9% concordance. Additionally, when comparing well-characterized samples to their corresponding data from the 1000 Genomes Project, samples had 99.4% concordance. This exemplifies a robust sample tracking assay that can ensure sample identity is correct. A strong quality control assay enables NGS laboratories to identify poor sample quality or degradation, allowing for re-extraction or new sample collection before processing on a more expensive assay. This newly developed sample tracking assay ensures both sample integrity and identity, leading to an overall increase in data quality for cancer research studies.

#4090 Analytical validation of PanTracer LBx performance, a comprehensive pan-solid tumor liquid biopsy assay.

Christodoulos Pipinikas[1], Faqiang Wu[2], Daniel Whang[2], Rebecca Hong[2], Samuel Koo[2], Qinqin Zha[2], Jieying Chu[2], Rachel Schell[2], Jiannan Guo[2], Giovanni Marsico[1], Tim Forshew[1], **Amber Chevalier**[3], Andrew Lukowiak[3], Heng Xie[2], Gregory Jones[4]

[1]NeoGenomics, Cambridge, United Kingdom,[2]NeoGenomics, Aliso Viejo, CA,[3]NeoGenomics, Fort Myers, FL,[4]NeoGenomics, Durham, NC

Introduction: Pan-solid tumor comprehensive genomic profiling (CGP) assays have gradually replaced single gene and smaller, tumor-specific panels to identify actionable somatic mutations in patients with advanced cancer. While tissue-based CGP remains important for clinical decisions, invasive biopsies can be challenging in terms of material quantity/quality and/or risk to patients. Plasma-based CGP assays provide the potential for rescuing cases where tissue fails, providing faster results to enable treatment decisions, monitoring treatment through repeated sampling, or capturing tumor heterogeneity. As such, CGP via liquid biopsy (LBx) represents an asset to patients and the options for such testing spans several to hundreds of genes. Here we describe initial results of the analytical validation (AV) process of a large deep sequencing panel aiming to establish the initial performance characteristics of the PanTracer LBx CGP assay. We also provide initial preliminary comparison data to an established highly sensitive targeted panel.

Methods: PanTracer LBx, a next-generation sequencing (NGS) assay based on TSO500 chemistry, is designed to detect key classes of somatic alterations across solid tumors, such as SNVs and small InDels (517 genes), CNVs (59 genes), fusions (23 genes) and key immune signatures. AV was performed in a CAP/CLIA certified laboratory across the above mutation classes to determine key assay performance characteristics: limit of detection (LoD), limit of blank (LoB), precision and analytical sensitivity/specificity using clinical samples with orthogonal data.

Results: LoD90, was assessed by measuring the detection of somatic variants present in SeraSeq complete ctDNA reference material at different %VAF levels (0.1%-2%) and DNA concentrations. Using a Probit regression model, LoD90 for SNVs and InDels was determined as 0.17% and 0.31%, respectively, and 1.23x for CNVs. LoB for SNVs/InDels and CNVs, assessed by calculating the cumulative per gene specificity across 21 healthy donors subjected to sequencing, was 0%. Analytical sensitivity and specificity were determined by sequencing late-stage cancer samples and comparing results to those obtained with an amplicon-based assay (InVisionFirst^{TM}-Lung). Positive and negative percent agreement with the orthogonal method was 96.9% and 99.3%, respectively. Precision (inter-assay, -lot and -operator) was >95%.

Conclusions: Performance evaluation of PanTracer LBx assay met all pre-specified AV acceptance criteria. Fusion LoD90 analysis is ongoing and will be presented during the meeting with additional results including input metrics and preliminary clinical validation. Based on a large panel targeting different classes of somatic variants across multiple genes, it offers results consistent with a smaller, established targeted panel with the addition of more biomarkers that may be informative or actionable.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Integrating Chromatin, Oncogenes, DNA Repair, and Biomarkers for Therapeutic Advancements
Poster Session

#4094 Allosteric chromatin activity transition mechanisms drive epigenetic dosage compensation in essential genes triggered by oncogene activation in TRACERx.

Francisco Gimeno-Valiente[1], Carla Castignani[2], Xiaohong Liu[1], Georgia Stavrou[1], Nana E. Mensah[1], Elizabeth Larose Cadieux[2], Ozgen Deniz[3], Mariam Jamal-Hanjani[1], Stephan Beck[1], Jonas Demeulemeester[4], Miljana Tanic[1], Charles Swanton[2], Peter Van Loo[5], Nnennaya Kanu[1]

[1]University College of London, London, United Kingdom,[2]Francis Crick Institute, London, United Kingdom,[3]Queen Mary University of London, London, United Kingdom,[4]Katholieke Universiteit Leuven, Leuven, Belgium,[5]MD Anderson Cancer Center, Houston, TX

Lung TRACERx is a prominent study employing multi-region and longitudinal multi-omics sequencing to unravel the evolutionary trajectories of cancer. While aberrant DNA methylation is described in most cancers, its interplay with genomic alterations and chromatin remodeling in non-small cell lung cancer (NSCLC) is less understood. We propose Allosteric Chromatin Activity Transition (AllChAT) as a framework to elucidate epigenetic dosage compensation mechanisms, focusing on essential genes impacted by oncogene-driven DNA methylation changes. The study utilized multi-region sampling from 59 NSCLC patients, encompassing 217 tumor regions and 59 adjacent normal tissue samples. RRBS was performed to profile DNA methylation patterns, with tumor-specific methylation rates determined using the CAMDAC tool. WES was conducted to identify somatic mutations and copy number alterations via ASCAT. Additionally, ChIP-seq for H3K4me3 and H3K27me3 was carried out on TRACERx patient-derived cell lines (PDCs) to investigate chromatin state changes associated with epigenetic transitions.We identified oncogenes such as RAC1, EGFR, and CDK4, whose expression scales with amplification and exhibited lower methylation levels in tumour regions where amplified compared to non-amplified tumor regions. We also discovered oncogene-proximal genes under dosage compensation by DNA methylation associated with amplification of CCND1 in LUSC and LUAD, as well as CDK4, KRAS, and GNAS, specifically in LUAD. These dosage-compensated genes were enriched for essential genes. Using chromatin immunoprecipitation on TRACERx patient derived cell lines (PDCs) from 2 tumors and one normal sample, we observed that oncogenes such as CDKN1B, FGFR1, and JAK2 were co-amplified and associated with chromatin opening and hypomethylation at amplified loci compared to non-amplified loci. Additionally, we found essential passenger genes, including NOP2, DCTN6 and FOXD4, were associated with closed chromatin and increased DNA methylation at the respective loci when amplified compared to when not amplified in tumor PDCs compared to the normal PDC. As observed in TRACERx tumor tissues, we observed evidence for AllChAT around LRRC34 when co-amplified with the PI3KCA oncogene in both tumor PDCs. Conversely, as expected, no evidence of AllChAT was observed in non-amplified control regions such as for the essential passenger genes TOMM40 and RPS9 located proximal to the ERCC2 oncogene when not amplified nor for the essential gene ECT2 located proximal to PIK3CA. Taken together, these data suggest that copy number alterations around amplified oncogenes might trigger an allosteric chromatin transition and DNA hypermethylation of co-amplified passenger genes, which might be crucial to the regulation of essential genes during tumor evolution.

#4095 Advancing cancer prognosis: FFPE-CUTAC for comprehensive and affordable transcription profiling in oncology.

Y. Zheng[1], K. Ahmad[2], S. Henikoff[2];
[1]UT MD Anderson Cancer Center, Houston, TX, [2]Fred Hutchinson Cancer Center, Seattle, WA

RNA-seq has been the method of choice for profiling patient samples. However, it predominantly detects genes expressed at moderate-to-high levels and is limited by the fragility of RNA, especially in Formalin-Fixed Paraffin-Embedded (FFPE) sections. We introduce Cleavage Under Targeted Accessible Chromatin (CUTAC) with an antibody to RNA Polymerase II (RNAPII) as a sensitive, cost-effective alternative for directly profiling transcription in FFPEs. Our findings reveal that FFPE-CUTAC libraries are significantly more comprehensive than RNA-seq libraries from both frozen and FFPE clinical sections, capturing low-level transcription of most active genes often missed by RNA-seq. Integrating RNAPII FFPE-CUTAC data from meningioma tumor samples with RNA-seq data from 1,298 patients, we demonstrate that RNAPII occupancy correlates strongly with transcriptional output. Embedding RNAPII FFPE-CUTAC datasets within a meningioma RNA-seq UMAP enhances the prediction of poor clinical outcomes, offering a powerful and economical replacement for RNA-seq in general cancer diagnosis.

Moreover, we observed widespread hypertranscription in human cancers, which correlates with poor prognosis. Using FFPE-CUTAC, we demonstrated genome-wide hypertranscription in transgene-driven mouse gliomas and various human tumors at active regulatory elements. Notably, hypertranscription challenges most normalization strategies that assume equal fragment volumes across samples. Tumor burden varies greatly across patients and tumor types, with significantly more cells in tumors compared to their corresponding normal samples. Within tumor cells, hypertranscription results in substantially higher FFPE-CUTAC fragment count. Assuming equal reads between tumor and normal samples can obscure hypertranscription signals. To address this, we developed a background-driven normalization strategy for FFPE-CUTAC data, accounting for the variations of cell numbers and fragment volumes to ensure accurate comparison across tumor and normal samples. We validated this strategy using both single-cell simulated data and specially designed experiments with known cell proportions and signal fold changes.

Our experimental and computational results affirm that FFPE-CUTAC provides a sensitive and affordable approach for detecting hypertranscription and classifying tumors using small tissue sections, advancing genome-wide strategies for personalized medicine.

#4096 Altered epigenetic aging in leukocytes with detectable mosaic chromosomal alterations.

Corey D. Young, Derek W. Brown, Charles Breeze, Rebecca L. Kelly, Kara M. Barnao, Aubrey K. Hubbard, Weiyin Zhou, Amy A. Hutchinson, Belynda D. Hicks, Aurelie L. Vogt, Wen-Yi Huang, Steven C. Moore, Stephen J. Chanock, Mitchell J. Machiela

NCI Div. of Cancer Epidemiology & Genetics, Rockville, MD

Background: Mosaic chromosomal alterations (mCAs), including autosomal mCAs, mosaic loss of chromosome X (mLOX), and mosaic loss of chromosome Y (mLOY) are types of age-related detectable clonal hematopoiesis (CH) in the blood and are associated with hematologic cancer risk. Epigenetic age, measured by DNA methylation-based clocks, can be used to estimate epigenetic age acceleration and could offer insights into the etiology of hematologic cancer; however, the relationship between mCAs and epigenetic age acceleration remains poorly understood.

Methods: DNA methylation was analyzed in 482 blood-derived samples from the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial using the Illumina EPIC array. Quality control and normalization were implemented using the Chip Analysis Methylation pipeline (ChAMP) and epigenetic aging metrics were derived using the DNA Methylation Age Calculator. T-tests were conducted to evaluate differences in age acceleration and age-adjusted logistic regression was used to investigate differences in estimated abundance of immune cells by mCA type.

Results: In total, 115 analyzed PLCO participants had autosomal mCAs, 68 had mLOX, 38 had mLOY, and 261 were mCA-free. Participants with mLOY exhibited the highest mean chronologic age (67.50 years) and the highest smoking prevalence (68.52% ever smokers). Across all samples, DNA methylation age demonstrated a moderate correlation with chronologic age ($r=0.57$, $p\ 8.38\times10^{-43}$, 95% CI:0.51-0.63). A trend toward higher epigenetic age acceleration was observed in individuals with detectable mCAs compared to mCA-free individuals (mean difference=1.17, p=0.05), with females having detectable mLOX displaying the highest age acceleration (mean difference=1.82, p=0.031). Notable alterations in methylation-estimated immune cell abundance were observed in individuals with detectable mCAs including elevations in CD4 T cells, natural killer cells, monocytes and granulocytes. Additionally, 2 probes, were differentially methylated in individuals with mCAs relative to mCA-free individuals, both located near the FOXI2 gene.

Conclusion: We observe epigenetic age acceleration in individuals with detectable mCAs, indicating epigenetic age acceleration could be a contributor to or consequence of mCAs in the hematopoietic compartment. Methylation-based evaluation of immune cell abundance identified increases in both lymphoid and myeloid cell types in individuals with mCAs, supporting prior findings of altered immune function in individuals with mCAs.

#4097 The ATAC histone acetyltransferase complex is a key oncogenic driver in sarcomas.

Souradip Sinha, Krishnendu Ghosh, Ali Khan, Yuliia Drebot, Gillian DeWane, Nicholas Scalora, Samuel Yu, Keith Garcia, Munir R. Tanas

Department of Pathology, University of Iowa, Iowa City, IA

Background: Sarcomas are heterogenous neoplasms originating in mesenchymal tissues such as muscles, fat, nerves, blood vessels, etc. Our lab has previously shown that the Hippo signaling pathway is perturbed in sarcomas, and TAZ/YAP, the end effectors of the Hippo pathway, are key oncoproteins driving sarcomagenesis. Epithelioid hemangioendothelioma (EHE), a vascular sarcoma, is predominantly driven by the fusion protein TAZ-CAMTA1, which promotes cancer hallmarks and alters gene expression patterns by interacting with YEATS2 and ZZZ3, which are key scaffolds of the Ada2A-Containing (ATAC) histone acetyltransferase (HAT) complex. YEATS2 is a histone reader and binds H3K27-acetyl, and ZZZ3 binds the histone H3 tail, and both YEATS2 and ZZZ3 are necessary for the proper assembly of the ATAC complex, which subsequently acetylates H3K9, a key histone modification necessary for gene transcription. Previously, we have utilized RNA-sequencing data from The Cancer Genome Atlas and shown that high YEATS2/ZZZ3 RNA levels correlate with a worse overall survival in multiple histological subtypes of sarcomas.

Methods: We performed western blot analysis to measure YEAST2/ZZZ3 protein levels in human sarcoma cell lines. We utilized siRNA/shRNA to knockdown YEATS2/ZZZ3 and measured H3K9-acetyl protein levels via western blot analysis. Apart from genetic inhibition, we utilized HAT-inhibitors and performed several *in vitro* cancer hallmarks, such as 3D soft agar assays and poly-HEMA assays to measure anchorage-independent growth and MTT-based assays to measure 2D proliferation. We also performed a cell-line-derived xenograft experiment where we injected immunocompromised NSG mice with SW872 cells expressing TAZ-CAMTA1 with or without stable knockdown of YEATS2.

Results: We observed that YEATS2/ZZZ3 protein levels are upregulated in multiple human sarcoma cells. We also observed that genetic and pharmacological inhibition of the ATAC complex in both fusion protein-positive (SW872 cells expressing TAZ-CAMTA1) and -negative (SKLMS; leiomyosarcoma and HT1080; fibrosarcoma) sarcoma cells decreases H3K9-acetyl protein levels. In the xenograft experiment, knockdown of YEATS2 significantly decreased tumor initiation and progression in NSG mice as compared to non-targeting cells. In SKLMS, YEATS2 knockdown significantly reduced 2D proliferation and colony formation on soft agar.

Conclusion: Our data show that in both fusion protein-positive and -negative sarcomas, the ATAC complex is a key histone acetyltransferase for H3K9. We show that the ATAC complex promotes tumorigenesis *in vivo* in a xenograft mouse model. In the leiomyosarcoma cell line SKLMS, the ATAC complex promotes 2D proliferation and anchorage-independent growth *in vitro*, which are key cancer hallmarks. Overall, our studies show that the ATAC complex is a potential therapeutic target in multiple sarcomas, such as EHE and leiomyosarcoma.

#4098 PARP1 and EZH2 independently limit 5'-3' end resection to promote rapid DNA repair after radiation.

Elena Efimova[1], Yue Liu[1], Sera Averbek[1], Isabelle Lomeli[1], SeokGyeong Choi[2], Sojung Ha[2], Woo-Young Kim[2], Stephen J. Kron[1]

[1]Ludwig Center for Metastasis Research and Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL,[2]Department of Pharmacy, Sookmyung Women's University, Seoul, Korea, Republic of

Cellular metabolism and epigenetic regulation have overlapping roles in cancer cell responses to radiation, with dynamic, reversible post-translational modifications including poly-ADP-ribosylation, O-GlcNAcylation and methylation emerging as key targets. This study explored crosstalk between poly-ADP-ribose polymerase 1 (PARP1), O-GlcNAc transferase (OGT), and the PRC2 subunit and histone methyltransferase EZH2 in the context of DNA double-strand break (DSB) repair. We focused on 5'-3' end resection, which determines repair pathway choice during S and G2 via guiding DSB repair away from non-homologous end joining (NHEJ) and toward homologous recombination (HR). DSB repair was examined in the human breast cancer cell line MCF7 using neutral comet assay and immunofluorescent detection of γH2AX, 53BP1, and BRCA1 foci. 5'-3' end resection was followed by tracking BrdU, RPA and RAD51 foci. We used CRISPR RNP to form pools of PARP1 and EZH2 knockout (KO) cells. After irradiation, both PARP1 KO and PARP inhibitor veliparib-treated cells displayed persistent DSBs and increased ssDNA, each restricted to S/G2 cells. While increased 5'-3' end resection may result from NHEJ defects, deregulation of resection may divert DSBs away from NHEJ to saturate cellular capacity for HR. Indeed, PARP inhibition enhanced HR repair based on a repair pathway reporter. Increasing protein O-GlcNAcylation in PARP1 KO or veliparib-inhibited cells restored DSB repair and suppressed hyper-resection, suggesting an independent, OGT-regulated determinant of pathway choice. Prior studies had implicated EZH2 and resulting histone H3 K27 trimethylation in promoting NHEJ and as a mediator of O-GlcNAcylation effects. Much like PARP1 KO, irradiated EZH2 KO cells displayed an S/G2 DSB repair defect associated with increased ssDNA. Hyper-resection in EZH2 KO was enhanced by PARP inhibition but modulating O-GlcNAcylation had no effect. Interestingly, along with increased nuclear BrdU foci, irradiated PARP1 KO and EZH2 KO each displayed cytosolic ssDNA foci. We infer that limiting NHEJ repair and/or promoting 5'-3' end resection in S/G2 can overwhelm HR, leading to hyper-resection, cytosolic DNA, and inflammation. Toward overcoming resistance to PARP inhibitors, O-GlcNac metabolism, OGT activity and its downstream mediator EZH2 offer attractive targets for therapeutic intervention. Overall, strategies aimed at deregulating 5'-3' end resection may help focus effects of genotoxic stress on proliferating cells and potentiate combinations with immunotherapies.

#4099 Leveraging the cell-free DNA hydroxymethylome as a prognostic biomarker in small cell lung cancer.

Janice J. N. Li, Dangxiao Cheng, Danielle B. Sacdalan, Luna J. Zhan, Sami Ul Haq, Vivek Philip, Gregory Schwartz, Scott V. Bratman, Geoffrey Liu, Benjamin H. Lok

UHN Princess Margaret Cancer Centre, Toronto, ON, Canada

Purpose: Small cell lung cancer (SCLC) is a highly aggressive neuroendocrine carcinoma characterized by rapid growth, early treatment resistance, and a dismal 5-year overall survival rate of <10%. Despite a high mutation burden, identifying robust biomarkers for SCLC remain a challenge due to the lack of actionable driver mutations and the scarcity of tumor tissue. Exploring DNA 5-hydroxymethylcytosine (5hmC) in plasma cell-free DNA offers a promising, minimally invasive opportunity for genome-wide profiling, however this remains understudied in SCLC. In this study, we hypothesize that the SCLC cell-free hydroxymethylome is distinct from healthy non-cancer controls and that it controls biological pathways that mediate prognosis.

Methods: Blood plasma samples were collected from a cohort of 49 de novo SCLC patients at pretreatment timepoints and 55 healthy non-cancer controls. Cell-free DNA (cfDNA) was isolated from plasma and examined using the 5hmC-selective chemical labeling (HMe-SEAL) assay, followed by next generation sequencing, to generate genome-wide 5hmC profiles. Global and gene-feature specific 5hmC patterns were compared between SCLC and control cfDNA to delineate SCLC-specific differentially hydroxymethylated regions (DhMRs). KEGG pathway analysis was performed on global DhMRs significantly enriched in SCLC. Kaplan-Meier and log-rank analyses were performed to determine the relationship between global DhMRs and survival outcomes. Overall survival (OS) was anchored from the time of SCLC diagnosis and progression-free survival (PFS) was anchored from the start of first-line treatment.

Results: Among the 49 SCLC patients, 69% had extensive-stage (ES-SCLC, n=34) and 65% were male (n=32). Global enrichment of 5hmC was observed in SCLC cfDNA compared to controls, particularly at intronic regions ($p<0.001$). Differential analyses revealed that global and gene feature-specific DhMRs, such as enhancer regions, could distinguish between SCLC and controls by principal component analysis. Pathway analysis of global DhMRs enriched in SCLC highlighted genes involved in cell proliferation (e.g. cAMP, Hippo, Wnt signaling pathways), axonal guidance, and stemness, which are commonly altered in cancer ($p<0.001$ for all mentioned pathways). When correlating 5hmC levels with survival data, SCLC patients presenting with high global 5hmC levels trended towards worse OS and PFS compared to those with low global 5hmC levels (median OS of 10.5 months vs 15.3 months, $p=0.13$; median PFS of 5.27 months vs 7.18 months, $p=0.07$).

Conclusion: Global cell-free 5hmC patterns distinguished between patients with SCLC and non-cancer controls and mapped to cancer-related pathways. Global 5hmC levels could also be leveraged for patient prognostic stratification, presenting a novel, minimally invasive biomarker for SCLC.

#4100 Aberrant translation regulated by METTL1/WDR4-mediated Val-AAC tRNA N7-methylguanosine modification promotes gastric carcinogenesis.

Yuan Li, Silei Sui, Rui Su, Ajay Goel

City of Hope Comprehensive Cancer Ctr., Duarte, CA

Background: Dysregulated epigenetic modifications in RNA are closely associated with the onset and progression of human tumors. Among cellular RNA modifications, transfer RNAs (tRNAs) are the most frequently modified type; however, the role of tRNA modifications in cancer remains largely unexplored. The RNA methyltransferase METTL1, which catalyzes N7-methylguanosine (m7G) modification of tRNAs, is often amplified and overexpressed in cancers and correlates with poor patient survival. In this study, we aimed to investigate the function and underlying mechanisms of m7G modification in tRNAs driving the development and progression of gastric cancer (GC).
Methods: We conducted a series of mechanistic studies to investigate the physiological functions of tRNA m7G modifications in GC tumorigenesis. Using tRNA modification and expression profiling, mRNA translation profiling, and rescue assays, we identified the molecular mechanisms underlying m7G modification in GC. Additionally, we analyzed the expression levels of the tRNA m7G methyltransferase complex components, methyltransferase-like 1 (METTL1) and WD repeat domain 4 (WDR4) in human GC samples.
Results: METTL1/WDR4 promoted GC progression and metastasis in cell-based and xenograft mouse models. Mechanistically, the loss of METTL1 reduced tRNA m7G levels, particularly in Val-AAC tRNAs, leading to decreased translation of mRNAs enriched with m7G tRNA codons and impairing the synthesis of a subset of oncogenic transcripts. Moreover, the tRNA m7G methyltransferase complex components METTL1 and WDR4 were found to be upregulated in GC and correlated with poor patient prognosis.
Conclusion: The tRNA m7G methyltransferase METTL1 was identified as a key driver of GC development and malignancy by regulating global mRNA translation through tRNA modifications. Our findings highlight METTL1 as a promising therapeutic target for GC patients.

#4101 Blood based biomarkers of DNA methylation associated with platinum resistance in high grade serous ovarian cancer.

Daniela Matei[1], Horacio Cardenas[1], **Elnaz Abbasi Farid**[2], Zhen Fu[3], Collin M. Coon[2], Adam Vieth[1], Kenneth P. Nephew[2]

[1]Feinberg School of Medicine, Northwestern University Chicago, Chicago, IL,[2]Indiana University School of Medicine, Bloomington, IN,[3]Van Andel Institute, Grand Rapids, MI

High grade serous ovarian cancer (OC) is initially a responsive tumor to platinum (Pt)-based therapy. Tumor suppressor genes (TSGs) are implicated in OC initiation and progression and can be inactivated through genetic or epigenetic events. DNA methylation has been shown to silence TSGs and other cancer-related genes and is detectable in cancer cells and in blood. Here we aimed to develop a blood-based methylation assay associated with cancer and cancer recurrence in OC. We evaluated genome-wide DNA methylation in de-identified peripheral blood mononuclear cells (PBMCs) from women: without cancer (controls, n=20), newly diagnosed OC (prior to treatment, Pt-sensitive, n=50), and platinum-resistant recurrent HGSOC before and after treatment with a hypomethylating agent (HMA, Pt-resistant, n=31). The Pt-resistant patients were enrolled on NCT02901899 clinical trial testing guadecitabine and pembrolizumab. DNA extracted from PBMCs was analyzed by using Infinium MethylationEPIC BeadChips. DNA methylation analysis used R package SeSAMe to derive beta (β) values and identify differential methylated loci (DMLs) and differential methylated regions (DMRs) between groups. Bioinformatics analysis was conducted to identify biological pathways and processes, determined using Ingenuity Pathway Analysis (IPA) and KEGG, GO:BP, REAC databases. Deconvolution analyses used the HEpiDISH algorithm to determine the cellular make-up of the samples. Comparing controls vs. Pt-sensitive groups, there were 30,369 DMLs (adj. $p<0.05$, $\beta>10\%$), with most loci being demethylated. Enriched pathways related to presence of cancer included: mechanisms of cancer, neutrophil degranulation, and cancer-related signaling pathways (PI3K/AKT, STAT3, HGF, interleukins). The number of DMLs was greater (880, adj. $p<0.05$, $\beta>10\%$) in Pt-resistant vs Pt-sensitive and enriched pathways associated with Pt-resistant OC included: pathways in cancer, metabolic pathways, platelet activation, ABC transporters and signaling pathways (calcium, PI3K/AKT, MAPK, Ras, ErbB, Hippo, Wnt). Massive genome-wide hypomethylation 5 days after treatment with the HMA was seen (13,742, adj. $p<0.05$, $\beta>10\%$), consistent with observed LINE1 hypomethylation in PBMCs, and genome-wide hypomethylation persisted 30 days after end of HMA treatment. Enriched pathways among hypomethylated genes included: Th17 cell differentiation, virus infection, focal adhesion, signaling (chemokine, cGMP-PKG, Rap1) and hormone (thyroid, parathyroid, oxytocin, growth hormone) pathways. Deconvolution analysis identified decreased B and NK cells, monocytes, and eosinophils and a trend towards decreased $CD4^+$ and $CD8^+$ cells in Pt-sensitive OC vs controls. In contrast, neutrophils were increased in cancer vs control patients. We propose new DMLs associated with Pt-sensitive vs. Pt-resistant OC. These findings can lead to new biomarkers for HGSOC.

#4102 N6-methyladenosine modification of FZR1 mRNA promotes gemcitabine resistance in pancreatic cancer.

Ziming Chen

Sun Yat-sen University Cancer Center (SYSUCC), Guangzhou, China

Background: Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive cancer with a 5-year survival rate of less than 10% and limited treatment options. Gemcitabine, a pyrimidine nucleoside analogue, is the standard first-line treatment for pancreatic cancer. However, drug resistance to gemcitabine remains a major challenge in PDAC. Dysregulation of N6-methyladenosine (m6A) modification has been linked to human disease regulation, and targeting aberrant m6A modifications or their key regulators may be beneficial to overcome drug resistance. However, the potential role and precise mechanism of m6A modification in the development of gemcitabine resistance in PDAC have not been fully elucidated.

Methods: In this study, we screened gemcitabine-insensitive and -sensitive PDAC cells through exposure to time- and dose-gradient gemcitabine. We then performed m^6A-sequencing analysis on these cells and identified the hyper-m^6A modification of FZR1 as a key molecular event contributing to gemcitabine insensitivity in PDAC. Additionally, we examined human PDAC samples using m^6A-qPCR assays and reaved that FZR1 m^6A modification had a significant clinical PDAC diagnosis and therapeutic value for PDAC.

Results: We found that PDAC cells with distinct gemcitabine sensitivity showed different m^6A profiles and that FZR1 had the most upregulated m^6A modification in gemcitabine-insensitive cells compared with gemcitabine-sensitive cells. Mechanistically, we discovered that RNA m^6A modification enhanced FZR1 translation in a GEMIN5-dependent manner, leading to a gemcitabine-insensitive phenotype by inducing a quiescent state. Importantly, targeting the m^6A modification of FZR1 increased the sensitivity of PDAC cells to gemcitabine both in vitro and in vivo.

Conclusions: This study identified the m^6A-FZR1 axis as an underlying mechanism contributing to gemcitabine treatment resistance in pancreatic cancer. The m^6A-FZR1 axis may serve as a predictor of gemcitabine treatment response in patients with PDAC. Overall, our discoveries interestingly link the dysregulated m^6A modification to gemcitabine insensitivity in PDAC, and provide new insights into the molecular mechanisms of gemcitabine resistance in this cancer type.

#4103 MLL4/COMPASS dysfunction in cancer and treatment.

Zibo Zhao[1], Sarah Gold[1], Yuki Aoi[1], Khyati A. Meghani[1], Luke St John[1], Rukkia Liaqat[1], Carolyn Moloney[1], Yanni Yu[1], Jun Qian[1], Issam Ben-Sahra[1], Rintaro Hashizume[2], Devalingam Mahalingam[1], Joshua Meeks[1], Ali Shilatifard[1]

[1]Biochemistry and Molecular Genetics, Northwestern Univ. Feinberg School of Medicine, Chicago, IL,[2]Pediatrics, University of Alabama at Birmingham, Birmingham, AL

Epigenetic status-altering mutations in chromatin-modifying enzymes are a feature of human diseases, including many cancers. We investigated cellular dependencies, or vulnerabilities, that arise when enhancer function is compromised by loss of the frequently mutated COMPASS family members MLL3 (KMT2C) and MLL4 (KMT2D). We performed a CRISPR dropout screen that revealed a targetable metabolic dependency arising from epigenetic factor deficiency, identifying potential therapies for cancers with epigenetic alterations secondary to MLL3/4-COMPASS dysfunction. We identified strong evidence that there is an internal balance between promoter and enhancer usage dictated by MLL1- versus MLL4-COMPASS, and this equilibrium is subject to disruption during cancer. Using an autochthonous carcinogen model of bladder cancer, we demonstrate that truncated, cytoplasmic MLL4 predicts response to targeted metabolic inhibition therapy for bladder cancer and could be developed as a biomarker for KMT2D-mutated cancers. We also highlight the broader potential for prognosis, patient stratification and treatment decision-making based on KMT2D mutation status in MLL4 truncation-relevant diseases, including human cancers and Kabuki Syndrome. Due to the high prevalence of loss-of-function mutations affecting MLL4 or its COMPASS partner UTX (KDM6A) in bladder cancer, we hypothesized that KMT2D mutation status may predict responsiveness to purine-targeting therapy, including methotrexate and pemetrexed. Our study found that KMT2D mutant bladder cancer cells were selectively dependent on TYMS and showed heightened sensitivity to pemetrexed, a drug that inhibits TYMS, DHFR, and GART. This suggests that targeting multiple enzymes within the one-carbon metabolism and de novo purine synthesis pathway could be promising for the treatment of MLL4/UTX-COMPASS mutant bladder cancer. Based on our previous and current findings, a clinical trial using pemetrexed in MLL4/UTX-COMPASS mutant solid tumors is being launched at Northwestern Medicine.

#4104 Adapter-anchored methylation specific amplification on genomic DNA using proximity primers (aMAPP).

F. Darbeheshti*[1], H. Zeggar*[1], H. Salmani[1], Y. Liu[2], V. A. Adalsteinsson[3], G. Makrigiorgos[1];
[1]Dana-Farber Cancer Institute, Boston, MA, [2]Wuhan University, Wuhan, China, [3]Broad Institute of MIT and Harvard, Cambridge, MA

Purpose: The epigenetic deregulation of CpG islands (CGIs) plays a crucial role in cancer initiation and progression. Genome-wide analysis of aberrant methylation patterns in CGIs offers a promising approach for the early cancer detection. However, the clinical utility of whole genome bisulfite sequencing is limited by the need for high sequencing coverage to achieve sensitive detection. To address this challenge, we have developed aMAPP, a method that enriches hypermethylated and/or hypomethylated CGIs. This approach enables the detection and tracing of aberrant methylation patterns, achieving high coverage in CGIs with ultra-low depth sequencing.
Methods: NEBNext Ultra II Library Prep. was used for adapter ligation. DNA was then subjected to APOBEC-mediated deamination and/or TET-Assisted Pyridine Borane Sequencing (TAPS) and pre-amplified. Methylation-specific amplification was then conducted using novel, customized proximity primers composed of an anchor, binding to the adapter, and a 6-10 bp probe, designed to bind tandem CpG dinucleotides downstream of the adapter. These primers amplify selectively DNA fragments containing methylated CpGs that remain intact after APOBEC deamination or unmethylated CpGs that remain intact after TAPS. Following Proximity Primer-PCR, MiSeq was conducted, generating approximately 500,000 reads per sample.
Results: Proximity primers were shown to amplify APOBEC-treated DNA fragments containing CpGs that match the primer probes, even if these CpGs are 100 bases downstream the adapter. While unmethylated fragments lack CpGs and are not amplified ($\Delta Ct_{meth\text{-}unmeth} \approx 18$). Applying aMAPP to APOBEC-treated methylated gDNA provided large amplification footprint of genome-wide methylated CGIs with remarkable efficiency, yielding 355-fold and 746-fold more CGIs with coverage of ≥5 and ≥10, respectively, compared to standard EM-Seq. Depending on conditions applied, aMAPP captures >80% of all CGIs with high coverage using ultra-low sequencing depth. Similarly, aMAPP applied to TAPS-treated unmethylated DNA captured selectively the unmethylated CGIs. aMAPP can be applied with very low starting material (10-100pg). Upon application to clinical samples, aMAPP detected thousands of CGIs and identified CpG island methylator phenotype (CIMP) and the top 20 hypermethylated CGIs in colorectal cancer. aMAPP could trace differentially methylated CGIs (DMR) in tumor samples present at 0.01% levels within adjacent normal tissue, or within circulating DNA of normal volunteers.
Conclusion: aMAPP enriches methylated and/or unmethylated portions of the genome with a unique efficiency, simplicity and flexibility that supersedes previously reported methylation enrichment approaches, enabling focused methylation sequencing at high coverage. Multiple applications in biomarker tracing and discovery can be envisioned in cancer, and beyond cancer.

#4105 ADAR1-high tumor-associated macrophages induce drug resistance and are therapeutic targets in colorectal cancer.

Kazuya Moriwake, Kunitoshi Shigeyasu, Hibiki Umeda, Toshiaki Takahashi, Masashi Kayano, Kaori Nitta, Shunsuke Nakamura, Masafumi Takahashi, Yuya Sakurai, Yuki Matsumi, Yoshitaka Kondo, Fuminori Teraishi, Hiroshi Tazawa, Toshiyoshi Fujiwara

Okayama Univ. Graduate School of Med., Dentistry & Pharm. Sci., Okayama, Japan

Background: Colorectal cancer (CRC) is one of the most prevalent cancers. In case of CRC with distant metastasis, it is initially treated by chemotherapy, and oxaliplatin is often used in first-line regimen. However, chemoresistance often becomes an issue and it remains unclear. We previously reported that RNA editing enzyme ADAR1 causes stemness (JCI Insight, 2018) and induces the poor prognosis in the liver metastasis of CRC (Scientific Reports, 2023). In this study, we cleared the role of RNA editing in chemoresistance of CRC.
Methods: We performed immunohistochemistry (IHC) of ADAR1 in 151 cases of CRC with distant metastasis. In vitro, we analyzed ADAR1 expression and RNA editing levels in both cancer cells and macrophages with the CRC cell lines and differentiated THP-1(dTHP-1). RNA-sequence was performed to compare wild type and ADAR1 knockdown dTHP-1 co-cultured with CRC cells respectively. In vivo, xenograft mouse models were made by mixing CRC cells and macrophages, and treatment experiment was conducted using oxaliplatin.
Results: IHC scoring showed that high expression of ADAR1 in CRC cancer cells relates the poor prognosis ($p<0.01$). Moreover, high expression of ADAR1 in the stromal mononuclear cells relates the poor prognosis ($p=0.032$) and resistance to oxaliplatin ($p<0.01$). Additionally, there was a positive correlation between the expression levels of ADAR1 in macrophages in tumor tissue and cancer cells ($\rho=0.74$, $p < 0.001$). In vitro, macrophages increased the intensity of ADAR1 expression when indirectly co-cultured with CRC cells ($p<0.01$). These results suggested that ADAR1 might be supplied from CRC cells to macrophages through some liquid factors. We focused on extracellular vesicles (EVs). Western blotting revealed that EVs from CRC cells contained ADAR1. When these EVs were added to macrophages, ADAR1 expression increased in the macrophages. ADAR1 increased RNA editing levels in macrophages, including AZIN1 and GLI1. Next, RNA-sequence analysis showed an enrichment of pathways associated with distant tumor metastasis in macrophages with high ADAR1 expression ($p<0.05$). Additionally, an increase in SPP1 gene expression, which is involved in tumor immune suppression, was observed. In vivo, xenograft mouse models were made, knockdown of ADAR1 in macrophages enhanced the effect of oxaliplatin to inhibit tumor growth ($p<0.01$). Treatment model mouse was made using oxaliplatin in combination with filgotinib, JAK inhibitor that reduces ADAR1 expression. The results showed a significant inhibition of tumor growth in the oxaliplatin plus JAK inhibitor group ($p=0.0197$).
Conclusions: We showed that ADAR1-high macrophages are induced by stimulation from CRC cells via EVs and it may cause chemoresistance. Cancer cells and macrophages dual-targeted RNA editing suppression therapy was considered a promising treatment option.

#4106 Glutamine deficiency drives epigenetic silencing of *GLS2* to promote survival and metastasis from the core region of colorectal cancer.

J.-Y. Lee[1], S. Kwon[1], S. Park[1], T. Yoon[1], J. Kim[1], H. Kim[1], E. Park[2], J. Lee[1];

[1]Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Korea, Republic of, [2]Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, Republic of

Rapid, uncontrolled proliferation is one representative hallmark of cancer. As tumor masses grow, the core regions receive inadequate blood supply, leading to severe nutrients and oxygen deprivation. Glutamine—regarded as non-essential for normal cells—has emerged as a critical nutrient that supports cancer cell metabolism. While glutamine deprivation typically induces cell death in the tumor core, some cancer cells survive by leveraging adaptive epigenetic mechanisms. However, the molecular mechanisms underlying this adaptation remain poorly understood. Using ATAC-seq and RNA-seq analyses, we revealed that glutamine deprivation induces the epigenetic silencing of *GLS2,* an enzyme that converts glutamine to glutamate, in colorectal cancer cells through increasing H3K27Me3 histone modification. This epigenetic silencing suppresses ferroptosis, an iron-dependent form of cell death, that enables cancer cells to survive and proliferate under glutamine-scarce conditions. With patient-derived colorectal cancer sample, we further confirmed a significant downregulation of *GLS2* within the tumor core, specifically in regions that remained actively proliferative despite nutrient deprivation. Additionally, tumor core cells deprived of glutamine exhibited increased invasion and migration abilities, indicating an enhanced metastatic potential towards nutrient-rich compartments or distal sites. Treatment with histone methylation inhibitors successfully reversed *GLS2* silencing, restored ferroptosis sensitivity, and suppressed metastatic behavior, which together emphasizes the potential of targeting epigenetic dysregulation as a therapeutic strategy. Combining epigenetic therapies to restore *GLS2* expression with ferroptosis-inducing drugs offers a promising, integrative approach to target resilient cancer cells in nutrient-deprived tumor cores. This strategy holds significant clinical potential for improving outcomes in patients with aggressive colorectal cancer, particularly those exhibiting resistance to ferroptosis-targeting anticancer drugs due to metabolism reprogramming.

#4107 CircZFR-YTHDF3 complex drives lymph nodes metastasis in cervical cancer via facilitating FASN translation.

Mingyi Zhou[1], Yong Zhang[1], Lian He[1], Bo Gao[1], Francois Claret[2], George Calin[2], Danbo Wang[1]

[1]Cancer Hospital of China Medical University, Liaoning Cancer Hospital & Institute, Shenyang, China,[2]the University of Texas MD Anderson Cancer Center, Houston, TX

Introduction: Lymph node metastasis is a key driver of poor outcomes in cervical cancer. Our study reveals that elevated fatty acid synthase (FASN) protein is closely linked to metastasis and reduced survival in cervical cancer, yet its regulatory mechanisms remained unclear.
Methods: We compared the expression of FASN in the cervical cancer samples with or without lymph nodes, long or short disease-free survival (DFS) via qRT-PCR, western blot assay, and tissue array. We investigated and validated the combination between circZFR and YTH N6-methyladenosine RNA binding protein F3 (YTHDF3) protein via circRNA pulldown assay, mass spectrometry, and RNA immunoprecipitation (RIP) assay. The m^6A sites on FASN mRNA were tested via MeRIP-qPCR. Protein co-immunoprecipitation (Co-IP) assay was used to validate the combination between YTHDF3 and eukaryotic translation initiation factor 4A3 (eIF4A3) protein. The siRNAs for YTHDF3 or FASN were transfected into HeLa, C33A, and Caski cells. Proliferation ability of cells was tested via CCK-8 and EdU assay. Migration and invasion ability of cells were tested via transwell assay. The function of circZFR on promoting lymph nodes metastasis was validated by animal assay.
Results: The test in clinical samples showed that the overexpression of circZFR and FASN is associated with lymph nodes metastasis and shorter DFS of cervical cancer patients. The results of circRNA pulldown and RIP assay demonstrated the combination between circZFR and YTHDF3 protein. The results of MeRIP-qPCR assay validated the m6A sites in FASN mRNA. The results of Co-IP assay showed that upregulated circZFR could increase the combination between YTHDF3 and eIF4A3 proteins. The results of proliferation, migration, and invasion assay suggested that knocking down FASN will inhibit the proliferation, migration, and invasion of cervical cancer cells. The results of animal assay validated the promotion of lymph nodes metastasis by circZFR.
Conclusions: We identified circular RNA circZFR as a crucial regulator, significantly enhancing FASN protein expression. CircZFR overexpression accelerates lymph node metastasis and shortens DFS in patients. Mechanistically, circZFR binds to the m^6A reader protein YTHDF3, facilitating m6A recognition on FASN mRNA and recruiting the translation initiator eIF4A3, thereby boosting FASN translation. These findings establish circZFR as a pivotal driver of cervical cancer progression and highlight its inhibition as a promising therapeutic strategy.

#4108 SMARCA4-driven oncogenic enhancer and transcriptional reprogramming in non-small cell lung cancer.

Hye-Ju Yang, Eun-Ju Kim, Sang-Hyeon Song, Tae-You Kim

Seoul National University, Seoul, Korea, Republic of

SMARCA4, an ATPase subunit of the SWI/SNF complex, is frequently mutated in cancers and plays a crucial role in chromatin remodeling and gene regulation. However, the mechanisms by which SMARCA4 drives oncogenesis in non-small cell lung cancer (NSCLC) remain poorly understood. In this study, we show that SMARCA4 restoration in SMARCA4-deficient NSCLC cells significantly enhances cell proliferation and migration. ATAC-seq and ChIP-seq analyses revealed that SMARCA4 restoration induces extensive epigenetic reprogramming, including altered chromatin accessibility, histone modifications, and activation of enhancers near genes linked to proliferation and migration. These changes were accompanied by increased expression of these genes, suggesting that SMARCA4-mediated enhancer reprogramming promotes oncogenic pathways. Motif analysis identified FOSL1 as a key transcription factor that collaborates with SMARCA4 by physically interacting with SWI/SNF subunits to activate enhancers. Enhanced FOSL1 binding correlated with the upregulation of proliferation- and migration-associated genes. Notably, FOSL1 knockout reduced the expression of these genes and suppressed cell growth, highlighting its critical role in SMARCA4-driven tumorigenesis. Furthermore, targeting SMARCA4 with small molecules such as BRM014 and ACBI1 selectively impaired cell viability in SMARCA4-restored cells, underscoring their potential as therapeutic agents for SMARCA4-dependent cancers. These findings reveal a central role for SMARCA4 in epigenetic regulation and enhancer activation, driven by the SMARCA4/FOSL1 axis, and offer insights into potential therapeutic strategies for NSCLC.

#4109 Accelerated aging in breast cancer survivors: the role of therapy-induced epigenetic modifications.

Nikita Nikita[1], Zhengyang Sun[2], Swapnil Sharma[2], Amy Shaver[2], Victoria Seewaldt[3], Ana Maria Lopez[2], Grace Lu Yao[2]

[1]Sidney Kimmel Comprehensive Cancer Center at Jefferson, Philadelphia, PA,[2]Thomas Jefferson University, Philadelphia, PA,[3]City of Hope Comprehensive Cancer Center, Duarte, CA

Breast cancer affects over 300,000 individuals annually in the United States, with survival rates improving due to advances in chemotherapy, radiation, hormonal, and targeted therapies. However, these treatments may inadvertently accelerate biological aging through epigenetic modifications, including DNA methylation, histone alterations, and changes in non-coding RNAs. This review explores how breast cancer therapies impact aging-related epigenetic pathways. Chemotherapy induces cellular senescence by altering DNA methylation patterns and histone modifications, potentially leading to the early onset of age-related diseases. Targeted therapies also affect epigenetic regulation, influencing gene expression related to cell cycle control, apoptosis, and DNA repair. Non-coding RNAs, such as microRNAs and long non-coding RNAs, are essential gene expression regulators affected by cancer treatments. They play significant roles in cellular senescence and therapeutic resistance, adding complexity to the aging phenotype observed in survivors. Clinical manifestations like cardiovascular dysfunction, endocrine disorders, and systemic inflammation are linked to therapy-induced epigenetic changes and in turn accelerated aging. Emerging epigenetic therapies targeting DNA methylation and histone modifications offer potential strategies to mitigate treatment-induced aging effects. DNA methyltransferase inhibitors and histone deacetylase inhibitors show promise in reactivating silenced genes and enhancing therapy sensitivity. Non-pharmacological approaches, including exercise, dietary modifications rich in bioactive compounds, and gut microbiota modulation, may also counteract therapy-exacerbated aging processes. In conclusion, existing evidence shows that cancer therapies are associated with decreased cardiorespiratory fitness, increased risk of cardiomyopathy, osteoporosis, and metabolic dysregulation, contributing to accelerated biological aging via diverse epigenetic mechanisms. Recognizing these changes is crucial for developing interventions that survivor’s health and quality of life. Further research is needed to fully understand therapy-induced epigenetic alterations and their long-term impacts, facilitating personalized survivorship care that addresses this population’s aging complexities.

#4110 Non-classical HLA epigenetic biomarkers associated with concordant cervical and oral HPV infection.

Y. Zheng[1], I. Morhason-Bello[2], Y. Qu[1], J. Wang[1], A. Fowotade[2], A. Daniel[2], A. Adisa[2], O. Awolude[2], J. Ogunbiyi[2], M. Pavon[3], R. Murphy[1], I. Adewole[2], D. Watson-Jones[4], L. Hou[1];

[1]Northwestern University Feinberg School of Medicine, Chicago, IL, [2]University of Ibadan, Ibadan, Nigeria, [3]Catalan Institue of Oncology, L'Hospitalet, Spain, [4]London School of Hygiene and Tropical Medicine, London, United Kingdom

Background: Human papillomavirus (HPV) is prevalent in mucocutaneous surfaces, with the persistence of infections causing approximately 5% of human cancers. While HPV presence at one site increases the risk at others and, possibly, increases the risk of cancer(s) in other anatomical sites, this risk varies among individuals despite similar sexual behaviors. Identifying women with high-risk cervical HPV who are also at risk for high-risk oral HPV could help reduce HPV-associated cancers. This study aims to identify methylation biomarkers explaining the risk of concordant high-risk oral HPV in women with cervical high-risk HPV infections.

Methods: As part of the Sexual Behavior and HPV Infections in Nigerians in Ibadan study, we conducted a cross-sectional analysis of sexually active women aged 18-45 years in Ibadan, Nigeria. Participants had interviews and clinical examinations and provided oral rinse and cervical swab samples. DNA was extracted and genotyped for HPV using the Anyplex™ II HPV28 assay. An epigenome-wide association study compared DNA methylation patterns between women with high-risk HPV types 16, 18, or 35 detected in both oral and cervical samples (concordant group) and those with high-risk HPV only in cervical samples (control group), matched by age, sexual behaviors, and lifestyle factors. Genome-wide DNA methylation profiling was performed on both sample types (total n=32) using the Infinium Methylation EPIC v2.0 BeadChip.

Results: We identified 61 differentially methylated CpG sites in the concordant group, with consistent associations in both cervical and oral samples (Pearson's $r = 0.91$, $p<0.001$). Notably, cg11201654 in the promoter region of a non-classical human leukocyte antigen (HLA) gene, *HLA-F,* was one of the top significant CpGs and showed approximately two-fold hypermethylation in the concordant group in cervical ($p = 0.00080$) and oral samples ($p = 0.00005$). Similarly, cg27020253 in the promoter of another non-classical HLA gene, *HLA-L*, exhibited ~1.7-fold hypermethylation in both cervical ($p = 0.00039$) and oral samples ($p = 0.00025$). These epigenetic modifications suggest that HPV may facilitate immune evasion and persistent infection by suppressing non-classical HLA genes, which are known for modulating immune responses through interactions with natural killer cells.

Conclusion: Hypermethylation of non-classical HLA genes may serve as novel biomarkers for identifying women at elevated risk for persistent high-risk HPV infections and related malignancies, offering insights into HPV-mediated immune evasion mechanisms. The consistent methylation patterns across oral and cervical tissues highlight the capacity of HPV to broadly influence host gene regulation. These epigenetic biomarkers could have applications in broader contexts of immunogenetics and cancer risk prediction, potentially refining the stratification of high-risk women.

#4111 Whole-genome methylation profiling enhances cancer detection and tumor fraction quantification in liquid biopsies.

Hang Dong, Shuang Gan, Haoran Tang, Yue Zhang, Feng Xie

Huidu (Shanghai) Medical Sciences, Ltd., Shanghai, China

Circulating tumor DNA (ctDNA) is a critical biomarker for non-invasive cancer diagnosis, prognosis, and therapeutic monitoring. However, accurately quantifying ctDNA content in liquid biopsy samples remains challenging due to its low abundance and interference from non-tumor DNA. Current approaches, such as targeted sequencing and mutation-based quantification, are limited by their dependence on known mutations and reduced sensitivity to low ctDNA fractions. Next-generation sequencing (NGS)-based methylation profiling offers a promising alternative by leveraging the distinct methylation patterns between tumor-derived and normal cell-free DNA (cfDNA). We developed a robust algorithm for the whole-genome methylation sequencing assay, PredicineEPIC^{TM}, as a multi-cancer detection solution enabling the quantification of ctDNA content in blood, urine, and cerebrospinal fluid samples. This approach focuses on comprehensive cancer-specific methylation markers to distinguish ctDNA fragments from background cfDNA molecules. We characterized methylation profiles of 800 simulated liquid biopsy samples with titrated tumor fractions (0.01%-50%) and applied machine learning models for analysis to evaluate ctDNA amounts. An independent set of 93 real-world samples from patients with lung, prostate, colorectal, ovarian, and bladder cancers was collected to validate the accuracy of the model. Performance was benchmarked against mutation panel-based reference tumor fractions. In the simulated dataset, our method achieved high accuracy ($R^2 = 0.92$) and sensitivity in detecting ctDNA at tumor fractions as low as 0.05%. The tumor fractions estimated by this approach correlated strongly with variant allele frequency (p-value < 0.01). Compared to conventional mutation-based methods, our approach achieved a higher detection rate in liquid samples with low tumor burden. Notably, the whole-genome strategy offered deeper insights into epigenetic alterations and fragmentomics features associated with gene expression activity, which remain undetectable by mutation-based assays. This study highlights the potential of whole-genome methylation profiling as a highly precise and comprehensive tool for tumor fraction quantification in liquid biopsy samples. The demonstrated accuracy and clinical relevance underscore its applicability for early cancer detection and real-time disease monitoring.

#4112 The epigenetic landscape identified through ATACseq reveals the NCAM1/FYN pathway as a potential therapeutic target in p53-/PTEN- transformed breast cancer stem cells.

Abigail G. Weatherford[1], Margaret V. Leonard[1], Maria Ouzounova[2], Hasan Korkaya[3], **Austin Y. Shull**[1]

[1]Department of Biology, Presbyterian College, Clinton, SC,[2]Cancer Research Center of Lyon, Lyon, France,[3]Karmanos Cancer Institute, Detriot, MI

Metastatic potential in basal-like breast cancers typically correspond with increased enrichment of aggressive progenitor-like populations called cancer stem cells (CSCs), typically identified by the loss of epithelial markers EpCAM and CD49f. In our MCF10A breast cell line model, deletion of p53 and PTEN lead to tumorigenic transformation and cancer stem cell expansion. With these observations that reflect cell dedifferentiation as a mechanism of oncogenic transformation, it was important to better detail the epigenetic events that correspond with breast CSC progression. With this in mind, we performed Assay for Transposase-Accessible Chromatin sequencing (ATACseq) on our isogenic panel of MCF10A breast cell lines where tumor suppressor genes TP53 and PTEN were silenced to drive breast CSC expansion. Furthermore, we performed gene expression analysis of isolated EpCAM-/CD49f- CSCs from MCF10A p53-/PTEN- cells to determine which genes differentially upregulated in CSCs corresponded with open chromatin in our ATACseq analysis. Based on this analysis, we identified 96 genes with open chromatin that were also significantly overexpressed within our isolated CSC populations. Several transcripts identified were potential metastatic drivers, including genes involved in signal transduction (PDE4B, PDE3B), cellular adhesion (MDGA2), transcriptional regulation (NPAS2), and oxidative stress responses (SMOX). Furthermore, many of these genes were also significantly upregulated clinically in basal-like breast cancers from The Cancer Genome Atlas (TCGA) breast cancer dataset. One key pathway of interest identified was the NCAM1/FYN pathway, which showed increased chromatin opening in the promoter regions of these two genes that are a part of the same signal transduction pathway and are of clinical relevance. Comparative analysis demonstrated that NCAM1/FYN pathway components were overexpressed in breast cancer samples, with statistically significant increased protein phosphorylation of MAP2K1 and RAF1 in the CPTAC proteogenomic TCGA samples. Collectively, this work provides an overview of open chromatin sites that contribute to oncogene overexpression in breast cancer and provide rationale for targeting these transcripts via chromatin targeting-based therapies (ex: BET bromodomain inhibition) to prime these CSC populations toward specific drug sensitivities.

#4113 α7 integrin is a critical co-regulator of chromatin accessibility and gene expression in HPV-negative head and neck cancer.
O. Borodins, A. Vehlow, N. Cordes;
Technische Universitat Dresden, Dresden, Germany

Epigenetic regulation and gene transcription, which are mainly restricted to the nucleus, are dynamically modulated by extracellular signals. In HPV-negative head and neck squamous cell carcinoma (HNSCC), genetic mutations and epigenetic alterations promote a complex and heterogeneous tumor microenvironment, often characterized by extensive remodeling of the extracellular matrix. This process affects the expression and function of integrins, making integrins central signaling mediators and key regulators of cancer progression and therapy resistance. While the 12 heterodimeric α/β1 integrin receptors are known to be key factors for therapy resistance in various malignancies, their direct influence on chromatin accessibility and gene transcription remains to be elucidated. Here, we systematically investigated 24 HNSCC cell models physiologically cultured in a 3D matrix for nuclear morphology (3D super-resolution microscopy), whole exome sequencing, euchromatic and heterochromatic markers, transcriptome (RNA sequencing), DNA accessibility (ATAC sequencing), and phosphoproteome (protein microarrays). Integrative, multiomic bioinformatic analyses, including random forest algorithms, provided comprehensive insights into integrin-mediated epigenetic and transcriptomic regulation. The HNSCC model panel showed substantial variability in nuclear architecture and protein-altering mutations. An RNAi screen for all integrin receptors identified α7 integrin as the most potent modulator of the selected histone modification markers. Mechanistically, α7 integrin depletion increased spatial aggregation of nuclear DNA. RNA- and ATAC-seq showed that knockdown of α7 integrin alters the expression of 3,257 genes, reducing chromatin accessibility at transcription start sites by 65%. This resulted in downregulation of genes involved in survival and chromatin regulation, while motifs for transcription factors such as JUN, FOSL1, and RELA were enriched in differentially accessible regions. Phosphoproteomics combined with artificial intelligence-based analyses showed significant inhibition of MAPK-dependent cell cycle progression, resulting in impaired proliferation, chromatin condensation, and reduced cell viability in all HNSCC models. In summary, this study provides substantial evidence that α7 integrin is an important co-regulator of gene transcription and chromatin accessibility. The discovery of the role of α7 integrin in these molecular mechanisms fundamentally supports the paradigm that cell adhesion dictates epigenetics and transcription. By increasing our understanding of these complex interactions, we may gain exploitable insights for the development of new, molecularly targeted drugs to treat patients with HNSCC.

#4115 Gene expression and bioinformatics profiles of four different cancer cells following treatment with HDAC inhibitor SAHA.

Umamaheswari Natarajan, Appu Rathinavelu

Rumbaugh Goodwin Institute for Cancer Research, Barry and Judy Silverman College of Pharmacy, Nova Southeastern University, Fort Lauderdale, FL

Background: Epigenetic modifications of chromatin play a crucial role in regulating gene expression and are often dysregulated in cancer. This study aimed to investigate changes in gene expressions following HDAC (Histone Deacetylase) inhibition in breast, ovarian, and lung cancer cells.
Aim: Our goal was to determine and compare the epigenetic alteration related gene expressions that may serve as diagnostic and/or predictive biomarkers for human cancers. To achieve this, we evaluated the effect of SAHA treatment on histone and DNA modification related gene expressions in human breast (MCF7), ovarian (A2780), and lung (HCC827 & H460) cancer cell lines.
Method: Using RT^2 Profiler PCR Array, we identified the differential expression of genes (DEGs) in four different human cancer cells following SAHA treatment in comparison to untreated controls. Independent quantitative real-time PCR (qRT-PCR) verified some of the most significant DEGs. We constructed a protein-protein interaction (PPI) network using STRING and Cytoscape to analyze the interactions of these DEGs.
Results: Our results showed that SAHA exposure significantly impacted histone and DNA modification related methyltransferases in the above listed human cancer cells. We identified three differentially expressed genes, including two commonly down-regulated (DNMT3A, NSD1) and one significantly up-regulated (HDAC3) genes in these cancer cells that were treated with SAHA. qRT-PCR and Western blot analyses confirmed the array results of DNMT3A, NSD1 and along with USP21, PAK1, and NEK6. All levels of all these five proteins were significantly down regulated following treatment with SAHA.
Conclusion: Our study demonstrates some commonality in the DEGs and the impact of HDAC inhibition with SAHA in four different human cancer cells compared with normal cells. Our findings provide new insights into the network and the interactions of significantly altered gene expressions that may eventually impact the growth abilities of cancer cells. Our findings also highlight some of the potential molecular targets and related signaling pathways. Altogether, these results may also contribute to our better understanding of the HDAC inhibition related mechanisms that may underly the growth regulation of breast, ovarian, and lung cancer cells.
Acknowledgements: This project was supported by the NSU’s President's Faculty Research and Development Grant (PFRDG), This research was partially funded by a Bankhead Coley Infrastructure Development Grant from the Florida Department of Health of the State of Florida that was awarded to A.R. through the Department of Pharmacology of the University of Miami, Coral Gables, Florida, USA, was used for this project, and also the authors would like to thank Royal Dames of Cancer Research, Inc. (Ft. Lauderdale, FL, USA) for their financial support in conducting this research.

#4116 A multi-omics study reveals an epigenomic subtype shapes a pro-metastatic niche in Epstein-Barr virus-associated nasopharyngeal carcinoma.

Kazi Anisha Islam, Larry Ka-Yue Chow, Kiu Wai Cheng, Dittman Lai-Shun Chung, Zhaozheng Hou, Yingpei Zhang, Anne Wing-Mui Lee, Wai Tong Ng, Roger Kai-Cheong Ngan, Dora Lai-Wan Kwong, Wei Dai

University of Hong Kong, Hong Kong, Hong Kong

Nasopharyngeal carcinoma (NPC) is a unique form of cancer that originates in the epithelial lining of the nasopharynx. It is often associated with Epstein-Barr virus (EBV) infection. NPC's etiology involves various factors, including genetic predispositions, environmental influences and particularly, epigenetic dysregulation. In an earlier study, it was discovered that there are two methylation subtypes with global hypermethylation (HyperNPC) and hypomethylation (HypoNPC) in NPC, representing 80% and 20% of patients, respectively. This study further investigates HypoNPC to characterize its biological features, association with EBV life cycles and to evaluate its clinical impact. Whole exome sequencing (WES), RNA-sequencing (RNAseq), and targeted bisulfite sequencing (BS) using the customized panel targeting the EBV genome and 23 selected human regions were performed for the tumor biopsies from 82 NPC patients. Patients were characterized as the HypoNPC subtype based on their methylation ratios across 13 selected regions using linear discriminant analysis (LDA). The WES data was utilized to evaluate the mutational profiles of the subtypes, while the RNAseq data was used to identify differentially expressed genes in both host and EBV. Chromosome instability (CIN) was quantified based on the extent of allelic imbalances in NPC tumors. The patients were stratified as metastatic (MET) NPC if they developed distant metastasis within 3 years post-treatment, and as non-metastatic (nonMET) NPC if they did not develop distant metastasis within the same time period. This multi-omics study revealed that HypoNPC patients are more likely to develop metastasis within 3 years post-treatment (OR=5.60, 95% CI=1.29-38.89) compared to HyperNPC patients. HypoNPC is enriched for loss-of-function mutations of KDM6A, which could lead to the hyperactivation of the mTORC1 signaling pathway. These patients also display elevated CIN, heightened DNA damage responses, replication stress and mitotic spindle assembly, alongside increased MYC activation. We found that pro-metastatic genes like SKP2 and PHF8 are upregulated, while anti-metastatic genes like PTPRD and ZNF154 are suppressed in these patients. Interestingly, the EBV genome in HypoNPC has a larger variation of DNA methylation levels, suggesting less stringent control of EBV gene transcription through DNA methylation. In addition, HypoNPC has a distinctive immunosuppressive tumor microenvironment, marked by a significant reduction in plasma B cells and suppressed complement function through macrophages. HypoNPC exhibits distinctive cancer cell features and the tumor microenvironment, which may form a pro-metastatic niche leading to early development of distant metastasis in these patients. This study highlights the needs for molecular subtyping in NPC to improve patient management and treatment.

#4117 ADAR1-mediated RNA editing of KCTD13s promotes pancreatic cancer metastasis by impeding RhoA ubiquitination.

Lingxing Zeng, Xiaoyu Wu, Ziming Chen, Jian Zheng

Sun Yat-sen University Cancer Center (SYSUCC), Guangzhou, China

Background: Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal disease with limited therapeutic options and a poor prognosis. Adenosine-to-inosine (A-to-I) RNA editing, a crucial post-transcriptional modification, introduces nucleotide changes in RNA sequences. However, few protein-recoding editing targets with functional significance have been identified. In this study, we propose that the ADAR1-KCTD13s-RhoA/ROCK1 signaling axis plays a critical role in PDAC progression.
Methods: We performed an in-depth analysis of A-to-I RNA editing using RNA-sequencing, validated by Sanger sequencing. In vitro phenotypic assays, including proliferation, colony formation, migration, and invasion tests, were conducted. In vivo studies included an orthotopic pancreatic tumor growth model and a lung metastasis model. Protein interactions were evaluated using immunoprecipitation, protein stability was assessed with cycloheximide (CHX) treatment, and ubiquitination was examined with MG132 treatment.
Results: Our analysis of A-to-I modifications in PDAC tissues and cell lines identified KCTD13s as a novel protein-recoding RNA editing target. Increased KCTD13s editing levels, driven by the overexpression of adenosine deaminase acting on RNA 1 (ADAR1), were associated with poor survival in PDAC patients. Functional experiments demonstrated that KCTD13s editing promotes pancreatic cancer metastasis. Mechanistically, this amino acid alteration stabilizes the KCTD13s protein by preventing its ubiquitination. The stabilized KCTD13s competes with KCTD13 for binding to CUL3, inhibiting RhoA ubiquitination. This results in elevated RhoA protein levels and activation of the RhoA/ROCK1 signaling pathway.
Conclusion: Our findings illuminate the malignancy-promoting role of the ADAR1-KCTD13s-RhoA/ROCK1 signaling axis in PDAC. This study reveals an epigenetic regulatory mechanism mediated by A-to-I RNA editing and highlights the potential of targeting epitranscriptomic pathways for therapeutic intervention in PDAC.

#4118 Decoding the noncoding: An insight into the noncoding genome of breast cancers.

A. Cuy Saques[1], A. White[1], K. Rabab[2], P. Scarry[3], F. Matra[4], C. Monger[5], A. Bracken[6], J. Crown[7], A. Eustace[1];
[1]Dublin City University, Dublin, Ireland, [2]Dundalk Institute of Technology, Dundalk, Ireland, [3]Bristol Myers Squibb, Dublin, Ireland, [4]ICON, Dublin, Ireland, [5]Pfizer, Dublin, Ireland, [6]Trinity College Dublin, Dublin, Ireland, [7]St. Vincent's University Hospital, Dublin, Ireland

Introduction: The PI3K/AKT pathway, a key oncogenic signalling pathway, is mutated in 70% of breast cancers (BC). PI3K/AKT coding mutations promote BC progression and are associated with poor survival. However, coding mutations represent <1% of the whole genome. Non-coding mutations (NCMs) can promote oncogenic gene expression in several cancers, including BC. Further study on NCMs can enhance our understanding of BC and help identify novel treatments targets.
Experimental procedure: NCMs surrounding kinome genes were identified from whole genome sequencing of 915 BC tumours. NCMs were prioritised based on recurrence and analysed with the Activity by Contact (ABC) bioinformatic model, which estimates their likelihood to interact with transcription start sites (TSS) and indicates their enhancer potential. Epigenetic data (Hi-C and H3K27ac, ChIP Seq) from BC cell lines (n=12) was screened to identify BC subtype-specific epigenetic changes surrounding the prioritised NCMs. The NCMs' regulatory effect over gene expression was confirmed by luciferase reporter assays.
Results: We identified 3,546 recurrent NCMs surrounding kinome genes in BCs and observed an enrichment of NCMs in gene loci of PI3K/AKT pathway members (p=0.005). We discovered subtype-specific enrichment of NCMs around oncogenes, like AKT2 in HR+ BCs (29/398, 7.2%) or AKT3 for TNBC (21/444, 4.7%). Of the 152 NCMs analysed, 17 (11.2%) reported positive by ABC analysis. 6/17 NCMs were identified in the gene loci of AKT2 or AKT3 (ABC scores 0.023-0.1) and were predicted to interact with genes up to 75kb from the NCM. Epigenetic analysis of ER+ and TNBC cell lines reported peaks of enhancer activity near the AKT2 and AKT3 NCMs, thus supporting the ABC analysis. Luciferase assays confirmed a 1.6-fold increase in luciferase expression for an AKT2 gene loci NCM (AC_A, chr19:41858248) and a 3-fold increase in luciferase expression for an AKT3 gene loci NCM (-_AT, chr1:244065861), whilst we observed a 3-fold decrease for a NCM in AKT2 gene loci (CAG_C, chr19:39686510).
Conclusions: We have identified that BCs have an enrichment of NCMs surrounding PI3K/AKT pathway genes. NCMs in the gene loci of AKT2 and 3 were predicted to interact with distal genes and luciferase reporter assays confirmed their regulatory effects *in vitro*. These results highlight the potential of NCMs to drive BC oncogenesis and potentially act as targets for drug development.

#4119 Epigenetic age as an early tumor biomarker for breast cancer.

Su Yon Jung

Translational Sciences Section; Department of Epidemiology, Fielding School of Public Health, UCLA - University of California Los Angeles, Los Angeles, CA

DNA methylation (DNAm)-based marker of aging, referred to as ‘epigenetic age’ or ‘DNAm age’ is a highly accurate multi-tissue biomarker for aging, associated with age-related disease risk, including cancer. Breast cancer (BC), an age-associated disease, is associated with older DNAm age and epigenetic age acceleration (age accel) at tissue levels. But this raises a question on the predictability of DNAm age/age accel in BC development, emphasizing the importance of studying DNAm age in pre-diagnostic peripheral blood (PB) in BC etiology and prevention. We included postmenopausal women from the largest study cohort and prospectively investigated BC development with their pre-diagnostic DNAm in PB leukocytes (PBLs). We estimated Horvath’s pan-tissue DNAm age and investigated whether DNAm age/age accel highly correlates with risk for developing subtype-specific BC and to what degree the risk is modified by hormones and lifestyle factors. DNAm age in PBLs was tightly correlated with age in this age range, and older DNAm age and epigenetic age accel were significantly associated with risk for developing overall BC and luminal subtypes. Of note, in women with bilateral oophorectomy before natural menopause experiencing shorter lifetime estrogen exposure than those with natural menopause, epigenetic age accel substantially influenced BC development, independent of obesity status and exogeneous estrogen use. Our findings contribute to better understanding of biologic aging processes that mediates BC carcinogenesis, detecting a non-invasive epigenetic aging marker that better reflects BC development, and ultimately identifying the elderly with high risk who can benefit from epigenetically targeted preventive interventions.

#4120 A systematic design of multiplex methylation-specific qPCR for CRC biomarkers.

Michelle Y. Lee[1], Joy Wang[2], Brian B. Lin[2], Katelyn Y. Chao[3], Annie X. Wu[4]

[1]Irvington HIgh School, Fremont, CA,[2]Basis Independent, Fremont, CA,[3]Menlo-Atherton High School, Atherton, CA,[4]Biochemistry and Biophysics, Rensselaer Polytechnic Institute, Fremont, CA

Analysis of circulating cell-free DNA (cfDNA) methylation abnormalities has emerged as a promising strategy for detecting various diseases, including cancer. While single-biomarker approaches may lack sensitivity, comprehensive next-generation sequencing (NGS) can be cumbersome and expensive. Multiplex methylation qPCR assays offer a practical intermediate solution by providing accurate, accessible, and affordable methylation biomarker detection. In this study, we demonstrated a process for developing a multiplex methylation-specific qPCR assay using colorectal cancer (CRC) methylation biomarkers as a case study. Starting with a set of CRC methylation biomarkers, we developed the Multiplex Methylation-specific qPCR (MMqPCR) algorithm to scan differentially methylated regions (DMRs) for methylation-specific PCR primer and probe design. We then established a systematic process to eliminate assay cross-reactions, reduce background noise, and assess multiplex compatibility. Using a low concentration of hypermethylated DNA (0.1%) in a digital analysis, we demonstrated a 95.8% detection rate with the multiplex strategy, significantly outperforming the singleplex approach (47.9%). The multiplex qPCR development principles and automated primer design algorithm presented here provide valuable tools for developing disease screening, detection or monitoring methods based on methylation analysis.

#4121 Correlation, interaction, and prognostic impact of SMC4 and NIPBL phosphorylation in endometrial carcinoma.

Jie Zhang[1], Qin Yang[1], Feilong Zhao[2], Ding Zhang[2], Yantao Li[1]

[1]The First People's Hospital of Yunnan Province. The Affiliated Hospital of Kunming University of Science and Technology, Kunming, China,[2]3D Medicine, Shanghai, China

Background: Uterine Corpus Endometrial Carcinoma (UCEC) poses treatment challenges due to limited options. Protein phosphorylation, a dynamic reversible process, emerges as optimal targets. Methods: We analyzed phosphoprotein, protein, and mRNA data of UCEC in the CPTAC dataset (PDC000126). Correlation network and hierarchical clustering were employed to identify core phosphoproteins and proteins. Protein-Protein Interaction network was constructed using the STRING database. Results:Data from 95 UCEC patients, mainly FIGO stage I, included 73,212 phosphoprotein sites (8,450 genes) and 9,099 protein expressions. Only genes expressed in ≥80% of samples were included. Generally, protein-mRNA expression correlation was high (median 0.51), while phosphoprotein-protein and mRNA correlations were weaker (median: 0). Using a correlation coefficient >0.4, we identified 259 phosphorylation sites that correlated with 91 proteins. Clustering identified 11 core phosphorylation sites that universally correlated with proteins and 15 core genes that universally correlated with phosphorylation sites. Further analysis found the phosphorylation sites that strongly associated with these core genes located at 72 genes. The interaction networks between the 14 core genes and 72 co-expressed genes revealed RFC4, TPX2, SMC4, and NCAPD2 as central nodes regulating at least 10 co-expressed genes. Survival analysis linked the 4 core genes to UCEC patient PFS and OS. By setting experimentally determined interactions >0.5, we identified previous confirmed phosphorylation counterparts of core genes: NCAPD2-NCAPG, RFC4-RFC1, SMC4-NCAPG, SMC4-NCAPD3, and SMC4-NIPBL. Notably, NIPBL harbored two phosphorylation sites (NIPBL_T599 and NIPBL_T592). Intriguingly, SMC4, NCAPG, and NCAPD3 are condensin complex subunits, while NIPBL is a cohesin-loading factor, suggesting coordinated regulation between the two protein complexes. Conclusion: The gene expression and protein phosphorylation network offer potential UCEC treatment targets. The SMC4-NIPBL interaction implies a harmonization between cohesin and condensin complexes, potentially impacting UCEC prognosis.

#4122 Epigenetic biomarker identification of each stage during the progression of prostate cancer.

S. Logotheti[1], K. Mesiakaris[1], K. Lazaros[2], E. Kontogianni[1], E. Panopoulou[1], S. Kontogiannis[1], M. R. Estecio[3], R. Soundararajan[3], C. J. Logothetis[3], A. G. Vrahatis[2], V. Tzelepi[1];

[1]University of Patras, Rion-Patras, Greece, [2]University of Ionion, Corfu, Greece, [3]UT MD Anderson Cancer Center, Houston, TX

Prostate cancer (PCa) heterogeneity is observed both across different patients and within the same patient during the progression of his disease. Although genetic profiling alone has not fully captured these complexities, epigenetic modifications may better characterize changes in bidirectional gene regulatory programs during PCa progression. In this study, we aimed to identify epigenetic biomarkers that may distinguish each stage of the disease. We thus focused our efforts on DNA methylation, the most extensively studied epigenetic mechanism, because it enables dynamic shifts in gene expression without altering the DNA sequence, often as a result of environmental and therapeutic pressures. To achieve our goal, we selected 24 formalin-fixed paraffin-embedded (FFPE) archival PCa specimens encompassing the full disease spectrum, from normal prostate tissue to aggressive states. This cohort includes indolent (N=8), high-grade non metastatic disease (N=4), aggressive/lethal disease (N=8), and adjacent normal tissues (N=4). DNA extracted from representative tissue sections was analyzed using the Infinium Methylation EPIC v2.0 array, followed by bioinformatic analyses: following standard beta-value calling and normalization, we applied unsupervised hierarchical clustering and found that the samples of each cohort clustered together. Then, differential methylation analysis identified 629 uniquely methylated CpG sites across the disease stages (Normal: 3 hypermethylated, 214 hypomethylated; Indolent: 4 hypermethylated, 1 hypomethylated; High-grade non-aggressive: 181 hypermethylated, 51 hypomethylated; Aggressive: 22 hypermethylated, 153 hypomethylated). We next took advantage that 422 of these identified differentially methylated sites (representing 67.1%) are also represented in previous versions of the bead array platforms (Illumina 450k and EPIC v1.0 arrays) and leveraged publicly available retrieved from TCGA and GEO databases to refine our pipeline, focusing on CpG sites consistently differentially methylated across disease stages. Based on our discovery set of well-characterized PCa specimens, ongoing analyses aim to incorporate over 100 additional public datasets to identify markers that reflect disease continuum. We anticipate that functional enrichment analysis of these datasets will also reveal candidate mechanisms involved in disease progression. Ultimately, this research seeks to lay the groundwork for a marker-informed approach to monitor PCa progression, establishing a basis for proactive strategies to manage and combat prostate cancer.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Kinases/Phosphatases, Ubiquitin, and Ubiquitin-like Proteins
Poster Session

#4126 Illuminating the dark kinome using proximity labeling and phosphoproteomics analysis.
I. Plutzer, D. P. Bhatt, E. M. Wilkerson, R. Williamson, D. Goldfarb, M. Major;
Washington University School of Medicine in St. Louis, St. Louis, MO

Kinases are highly druggable targets for cancer therapy due to their control over a variety of oncogenic processes via protein phosphorylation. While, large clinical phosphoproteomics datasets have expanded our understanding of how the phosphoproteome is altered in cancer, annotation of the phosphoproteome is significantly lacking. 95% of phosphorylation sites in the PhosphoSitePlus database have no associated kinase, and 80% of annotated substrates are assigned to just 20% of the most well-studied kinases. Of the ~650 kinases in the human genome, 162 are designated by the NIH as ‘dark kinases’ due to lack of information on their function and substrates. The primary aim of my research is to define the functions and substrates of the dark kinome and identify kinases with therapeutic potential.My lab has collected mass spectrometry (MS)-based proximity labeling data for 137 of the dark kinases. Probabilistic scoring of raw data yields functionally relevant proximity networks for each kinase that defines its cellular localization and protein complex membership. Enrichment analysis identifies functional protein domains and protein sequence motifs that drive kinase localization and substrate specificity via protein-protein interactions. Additionally, clustering of this data highlights the prevalence of kinase signaling hubs – distinct locations within the cell harboring diverse kinase populations – that regulate extracellular signal processing, protein trafficking, transcription, and translation.As a second branch of my work, I have developed kinase gain-of-function (GOF) phosphoproteomics experiments to identify substrates and pathways regulated by dark kinases. This medium-throughput method uses multiplexed deep phosphoproteomic profiling to measure changes in protein phosphorylation levels following transient overexpression of a kinase. Analysis of this data reveals signaling pathways regulated by a kinase, which can be validated using low-throughput western blots. When combined with proximity data, I am able to define high-confidence putative substrates that are both proximal to the kinase and significantly hyperphosphorylated following overexpression of the kinase. As an example of this workflow, I highlight the dark kinase BRSK2. In my BRSK2 GOF experiment, I identify 584 candidate sites that are significantly upregulated. Enrichment analysis of these sites reveals that BRSK2 is a positive regulator of AMPK and negative regulator of mTOR signaling. This is validated by western blotting of key AMPK and mTOR substrates. Mapping upregulated sites onto the proximity network for BRSK2 yields a small list of 39 high-confidence proximal substrates, an improvement on the 4 substrates in the PhosphoSitePlus database.

#4127 Oncogenic BRAF suppresses p53 function in melanoma through direct protein protein interaction.

Kayla T. O'Toole, Adamaris Martinez, Brandon Murphy, Deevya Baral, Anastasia Proveyeka, Fatima Al-Sudani, Joshua Andersen, Robert Judson-Torres, Martin McMahon

University of Utah Huntsman Cancer Institute, Salt Lake City, UT

Oncogenic BRAF is a driver of approximately 8% of all cancers, with a particular prevalence in lung cancer, thyroid cancer, and cutaneous melanoma. $BRAF^{V600E}$ is the most common oncogenic alteration, accounting for about 50% of cutaneous melanoma cases. Combined inhibition of $BRAF^{V600E}$ plus MEK1/2 is the current targeted therapeutic option approved for the treatment of $BRAF^{V600E}$-driven melanoma and lung cancer. Unfortunately, many patients only experience short-term therapeutic benefit with a mean progression-free survival of 7 to 14 months in non-small cell lung cancer and melanoma, respectively. Thus, this highlights the need to identify better therapeutic targets for BRAF-driven cancers. $BRAF^{V600E}$ is often referred to as a monomer as $BRAF^{V600E}$ signals independent of RAS-GTP and RAF dimerization. However, oncogeneic BRAF may still be dependent on other scaffolding proteins for optimal signaling. Thus, to evaluate this, we used gel filtration chromatography and immunoblotting analysis to visualize BRAF signaling complexes. These data revealed that $BRAF^{V600E}$ exists in a large multiprotein complex. Pharmacological inhibition of $BRAF^{V600E}$ shifted the elution of $BRAF^{V600E}$ complexes into lower molecular weight fractions. We further investigated this $BRAF^{V600E}$ signaling complex by implementing the proximity labeling technique, TurboID. Coupled with mass spectrometry, this technique enabled the unbiased identification of novel BRAF interactors and helped us understand how targeted therapy impacts this BRAF interactome. Here, we identified a multitude of unique wild-type BRAF and $BRAF^{V600E}$ interactors, with the tumor suppressor p53 being of particular interest. Intriguingly, TP53 alterations are infrequent in melanoma, prompting the hypothesis that by interacting with p53, $BRAF^{V600E}$ may influence p53 function. We show that $BRAF^{V600E}$ expression alters p53 subcellular localization, significantly reduces native p53 activity, and prevents p53 activation following UVB radiation and MDM2 inhibition. Ultimately, these findings provide unique mechanistic insight into the mechanisms by which $BRAF^{V600E}$ drives cancer development and may clarify why TP53 is infrequently altered in melanoma.

#4128 Mapping the hypoxic regulation of IFNγ and TNFα-mediated kinase pathways in melanoma.

Simar Pal Singh[1], Alex Miranda Rodriguez[2], Russell W. Jenkins[2]

[1]PamGene International B.V., 's-Hertogenbosch, Netherlands,[2]Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Mass General Cancer Center, Krantz Family Center for Cancer Research, Boston, MA

Introduction: Hypoxia can promote immunologic, transcriptomic, and metabolic alterations in the tumor microenvironment that contribute to immune evasion. Activation of hypoxia signaling pathways has been linked to cancer cell-intrinsic immune evasion and resistance. Hypoxic tumor cells show less susceptibility to direct killing by cytotoxic T lymphocytes and NK cells through several mechanisms, including upregulation of immune checkpoint ligands. IFNγ and TNFα are immune cell-derived effector cytokines critical for tumor immunity. However, the effect of these cytokines in the hypoxic tumor microenvironment is less well studied. Since, kinase signaling pathways are directly activated downstream of cytokine receptor, we explored the functional activity of kinases downstream of IFNγ and TNFα.
Methodology: Murine B16F10 melanoma cells were treated with 40 ng/ml of IFN-gamma and TNF-alpha under normoxia (21 % oxygen) and hypoxia (1% oxygen) conditions for 6 or 24 hours. Following treatment, cells were lysed with M-PER Mammalian Extraction Buffer supplemented with Halt Phosphatase Inhibitor Cocktail and Halt Protease Inhibitor Cocktail EDTA free and protein quantification was determined using Pierce Coomassie Plus (Bradford) Assay Kit. Tyrosine and Serine/threonine kinase activity profiles were generated using PamChip® comprising 340 different phosphosites that are known downstream substrates of kinases. Peptide phosphorylation and Upstream Kinase Analysis was performed with PamGene's tercen analysis pipeline.
Results: We found a global reduction of the cytokines induced kinome activation when cells were treated under hypoxia for 6h. However, after 24h a group of kinases associated mainly with the MAPK, RAS and Calcium signaling were increasingly activated in the hypoxia condition. A dynamic activation of the PI3K/AKT pathway was observed when comparing cells exposed to cytokines in normoxia or hypoxia conditions. At 6 h after IFNγ and TNFα treatment under normoxia, we observed an increased PI3K/AKT activity that decreased and became undetectable after 24h. However, when cells were exposed to the cytokines under hypoxia the reduced PI3K/AKT kinase activity at 6 h is reverted after 24h of treatment. Interestingly, the downstream mTOR pathway is predicted to have lower activity in the hypoxia conditions across both time points evaluated.
Conclusions: In the present work we unveil strong diversity on the kinome of melanoma cells when exposed to the inflammatory cytokines IFNγ and TNFα under different oxygen levels. These results provide deeper insight into the mechanisms by which hypoxia contributes to immune evasion and response to cytokines. Our data can help inform future therapeutic strategies targeting these pathways to enhance immune-mediated tumor suppression.

#4129 Phosphorylation of AMPKβ by EDIL3/Del-1 regulates the progression of triple negative breast cancer.

Seolhwa Jeong[1], Soo Jung Lee[2], Jeeyeon Lee[2], In Hee Lee[2], Byeongju Kang[2], Ho Yong Park[2], Eun Ae Kim[1], Jieun Kang[2], Yee Soo Chae[2]

[1]Kyungpook National University, Daegu, Korea, Republic of,[2]Kyungpook National University Chilgok Hospital, Daegu, Korea, Republic of

Background: Developmental Endothelial Locus-1 (Del-1), also known as EGF Like Repeats and Discoidin Domains 3 or Edil-3, is a secreted glycoprotein enriched in extracellular vesicles circulating in the plasma of breast cancer patients. Various papers have suggested that Del-1 might be important in Triple Negative Breast Cancer (TNBC), the subtype with the highest mortality rate, even though it only accounts for 15%-20% of all breast cancers. However, the precise role of Del-1 in TNBC regulation remains poorly characterized. AMP-activated protein kinase (AMPK), a heterotrimeric energy sensor, is composed of α (catalytic), β (scaffolding), and γ (nucleotide-binding regulatory) subunits. AMPKβ subunits, characterized by their carbohydrate-binding modules (β-CBM), facilitate glycogen binding and are often amplified in tumor cells. Although AMPKβ has been extensively studied for its metabolic functions, its contributions to oncogenic signaling pathways in TNBC remain poorly understood.
Methods: RNA sequencing of Del-1-suppressed MDA-MB-231 cells revealed reduced expression of AMPK subunits. Western blot assays confirmed that AMPKβ protein levels were significantly elevated in TNBC cell lines, and RT-PCR analysis further verified increased AMPKβ mRNA levels in breast cancer patient samples. CRISPR/Cas9 genome editing Del-1 knockout cell lines and pharmacological activator of AMPKβ, and tumor progression assays (MTT, MTS, BrdU and Matrigel transwell assays) were used to evaluate the effects of Del-1 and AMPKβ on TNBC cell proliferation, invasion, and survival. To understand how Del-1 positively activates AMPKβ, genetically mutated phospho-mimetic AMPKβ was overexpressed, and assessed the progression of TNBC cells.
Results: In TNBC cells (particularly TNBC mesenchymal stem-like cells), the expression levels of Del-1 and AMPKβ were significantly upregulated. While AMPKα remain unchanged, β subunit of AMPK was increased. Both overexpression of Del-1 and pharmacological activation of AMPKβ stimulated proliferation and invasion, whereas CRISPR/Cas9 genome editing Del-1 knockout inhibited AMPKβ and blocked proliferation and invasion. Notably, overexpression of Del-1 upregulated AMPKβ, but AMPKβ activation did not affect Del-1. Furthermore, DEL-1 phosphorylates AMPKβ at S108, and phosphor-mimetic AMPKβ overexpression significantly enhanced TNBC cell progression.
Conclusions: Our findings identify AMPKβ as a novel downstream target of Del-1 in TNBC mesenchymal stem-like cells. Elevated Del-1 and AMPKβ levels accelerate proliferation, invasion, and survival. These
results uncover a previously unrecognized Del-1-AMPKβ axis, offering new insights into the molecular mechanisms of TNBC progression and potential therapeutic targets.

#4130 Atypical protein kinase C iota (PKC-ι) regulates an oncogenic positive feedback loop between the MAPK/JNK signaling pathway, AP-1 complex and TNF-α in triple negative breast cancer.

Nuzhat Nowshin Oishee, Mahfuza Marzan, Khandker Mohammad Khalid, Abiral Hasib Shourav, Abigail Olatunji, Radwan Ebna Noor, Mildred Acevedo-Duncan

Chemistry, University of South Florida, Tampa, FL

Breast Cancer is the second most common cancer in the United States and consists of about 30% of all new female cancers each year. The average risk of a women developing breast cancer in her lifetime is about 13%. Atypical Protein Kinase C iota (PKC-ι) is over expressed in 88 of the 110 breast cancer cases (80.0%) and is highly active in the invasive progression of the disease. Therefore, considering PKC-ι as a novel therapeutic target is crucial. c-Jun is a Basic leucine zipper (bZIP) transcription factor and crucial component of activator protein-1 (AP-1) complex, which is also overexpressed in breast cancer and its oncogenic function regulates proliferation, apoptosis, metastasis and angiogenesis in breast cancer. c-Jun sits at the crossroad of the signaling network; hence, understanding the relationship between PKC-ι and c-Jun is fundamental. c-Jun is activated by the JNK pathway, which itself has its own oncogenic function and plays vital role in malignant transformation and metastasis in different cancer including breast cancer. In addition, the upstream markers of JNK are MKK7 and Tak1, which are part of the MAP2K and MAP3K families, respectively and these markers also play a pro-oncogenic role in breast cancer. The activation of these upstream markers further stimulates the phosphorylation and activation of JNK, which in turn activates c-Jun. Activated c-Jun forms homodimers and heterodimers in Activator Protein-1 complex (AP-1) complex, which also have oncogenic activity in cancer cell proliferation and apoptosis. The AP-1 complex further stimulates the pro-inflammatory cytokine TNF-α, which again stimulates the activation of the MAPK/JNKpathway. This indicates that there is an oncogenic positive feedback loop between these biomarkers, and it is hypothesized that PKC-ι regulates this positive feedback loop.This study examines the role played by PKC-ι in regulating the positive feedback loop between the MAPK/JNK pathway, AP1 complex, and TNF-α. Using ICA-1S, an inhibitor specific to PKC-ι, BT549 breast cancer cells were treated for 5 days (10μM dosage), and SDS-PAGE was conducted. Our results indicate that in BT549 cells, inhibition of PKC-ι with ICA-1S has significantly reduced the levels of Tak1, p-JNK, JNK, m-MKK7, MKK7, p-c-Jun, c-Jun, and TNF-α. These findings indicate that inhibiting PKC-ι can reduce the phosphorylation and activation of JNK and its upstream and downstream targets, and that inhibition of PKC-ι inhibits the oncogenic role of this positive feedback loop in breast cancer. Hence, it can be concluded that in BT549 triple negative breast cancer cells, PKC-ι regulates the oncogenic loop between MAPK/JNK pathway, AP-1 and TNF-α via the phosphorylation and activation of c-Jun, which further stimulates the activation of the entire loop.

#4131 Expanding the roles of 14-3-3 and Smad2/3 as coordinators of PKC-ι activity in medulloblastoma: a study based on PKC-ι specific inhibitor ICA-1S.

Luke Lajmi, Wishrawana Sarathi Ratnayake, Sloan Breedy, Aaron Todman, Mildred Acevedo-Duncan

Chemistry, University of South Florida, Tampa, FL

Medulloblastoma is one of the most aggressive and common pediatric cancers, second only to leukemia, and the most common childhood brain tumor, comprising 20% of all pediatric brain cancers. Current treatment options involving radiation and chemotherapy as well as surgical resection are extremely invasive and often lead to patients living with long term neurological and endocrinological consequences. This study focuses on exploring the PKC-ι specific small-molecule inhibitor 5-amino-1-((1R,2S,3S,4R)-2,3-dihydroxy-4-methylcyclopentyl)-1H-imidazole-4-carboxamide (ICA-1S) and its effects on medulloblastoma cell lines Daoy and ONS-76. Using the previously established IC_{50} values for both cell lines, this study demonstrates that PKC-ι inhibition by ICA-1S leads to significant downregulation of both 14-3-3/c-Raf/Erk and TGF-β/Smad2/3/β-catenin pathways. Our results showing the association and localization of PKC-ι with 14-3-3, Smad2/3, and Erk1/2 are likely driving this cross-talk communication between these two pathways. We also show that ICA-1S simultaneously induces apoptosis and pyroptosis in addition to reducing the migratory and invasive capabilities of medulloblastoma. Finally, as a proof of concept, we demonstrated that *in-vivo* treatment of ICA-1S decreased tumor volume in nude mice by approximately 50%. In summary, PKC-ι is likely an important oncogenic target in medulloblastoma and ICA-1S appears to be a promising potential medulloblastoma therapeutic that merits further research.

#4132 PTPRF negatively regulates c-MET signaling to inhibit cell migration in colon cancer.

Carolina Galeano-Naranjo[1], Haley Stanczyk[1], Warren Van Nort[2], Tianyan Gao[2]

[1]Molecular and Cellular Biochemistry, University of Kentucky, Lexington, KY,[2]Markey Cancer Center, University of Kentucky, Lexington, KY

The spatiotemporal control of cell signaling requires a balancing act of protein kinases and phosphatases. Hyperactivation of signaling downstream of receptor tyrosine kinases (RTKs) is one of the most common mechanisms leading to oncogenic transformation in numerous cancer types. Although the activation process of RTKs has been extensively studied, the inactivation mechanisms mediated by tyrosine phosphatase are less understood. Previously, we have determined the molecular mechanisms by which protein tyrosine phosphatase receptor type F (PTPRF) regulates the Wnt pathway. In this study, we investigated the functional importance of PTPRF in controlling c-MET signaling in colon cancer. Downregulation of PTPRF in colon cancer cells using CRISPR-mediated gene deletion or shRNA-mediated gene silencing activates c-MET signaling at the level of GAB1 scaffolding protein upon HGF-induced receptor activation. Co-immunoprecipitation experiments indicate that PTPRF interacts with the c-MET receptor/GAB1 complex. In addition, PTPRF downregulation prolongs the phosphorylation of GAB1 in colon cancer cells treated with c-MET inhibitor. Functionally, PTPRF-loss markedly increases cell migration. Moreover, bioinformatics analysis of TCGA colon cancer patient data reveals that the expression of PTPRF gene is significantly decreased in tumor samples compared to normal controls. Taken together, these results identify PTPRF as a novel phosphatase of GAB1 that negatively regulates c-MET signaling. Decreased expression of PTPRF may play an important role in promoting tumor progression in colon cancer.

#4133 Cyclin-dependent kinase signaling in oncology: assessing the effectiveness of a CDK9-targeting using no-wash immunoassays.

Jennifer Old[1], Najim Douayry[2], Milind Rajopadhye[3], Olivia J. Kelada[3]

[1]Revvity Inc., Waltham, MA,[2]Revvity Inc., Codolet, France,[3]Revvity Inc., Hopkinton, MA

Cyclins are regulatory subunits that bind to a cyclin dependent kinase (CDK), thus activating the kinase and controlling its activity. Most CDKs form a complex with a particular cyclin and are involved in the regulation of either cell cycle or cell transcription. Dysregulation of CDKs has been found to be a primary driver in tumorigenesis. Thus, CDK9 is an attractive target for cancer therapeutics due to its crucial role in transcription regulation, particularly of short-lived anti-apoptotic proteins such as MCL-1 and XIAP. Among the investigated CDK9-based therapeutic strategies, the degradation of CDK9 using specific compounds like proteolysis targeting chimera (PROTAC) is of high interest.

The purpose of our study was to determine whether homogeneous time-resolved fluorescence (HTRF) and a bead-based homogeneous immunoassay platform (AlphaLISA Surefire Ultra, ALSU) assays are effective solutions for identifying and characterizing PROTAC degradation of CDK9. Both assays were used to monitor the expression levels of CDK9 or GAPDH in cell lysates from HeLa or MCF-7 cells. Assessing CDK9 levels allowed the identification of a PROTAC degradation of CDK9 while the determination of GADPH levels was used as an internal control for potential changes to global protein levels in drug treatment.

Cells were treated for 4h either with 1µM of a reference CDK9 degrader, Thal-SNS-032 or with the same concentration of its two individual chemical components, Thalidomide and SNS-032 (as negative controls). Cells were then lysed, and the corresponding lysates were assessed in either CDK9 or GAPDH assays. Thal-SNS6032 induced a robust degradation of CDK9 (80 to 95%) while Thalidomide and SNS-032 did not affect the expression level of CDK9. Moreover, none of the tested compounds affected the GAPDH expression level suggesting that the changes of CDK9 levels is not due to changes in the global protein concentration. To further characterize the effect of Thal-SNS-032, a dose-response treatment was carried out on HeLa and MCF-7 cells. CDK9 and GAPDH levels were assessed in the corresponding cell lysates and a dose-dependent degradation of CDK9 was observed with half-maximal degradation values (DC50) ranging from 60 to 166nM while GADPH levels remained unchanged. These data suggest that HTRF and ALSU assays are efficient solutions to identify and characterize PROTAC targeting of CDK9. Results obtained for CDK9 can be translated to other CDK targets of interest in oncology research as a broad portfolio of total CDK detection kits is available using the two assay platforms.

#4134 An inhibitor of the atypical protein kinase C-ζ impede the growth of gastric cancer cells by inducing apoptosis.

Abiral Hasib Shourav, **Abigail Olatunji**, Mahfuza Marzan, Radwan Ebna Noor, Mildred Acevedo-Duncan

Chemistry, University of South Florida, Tampa, FL

The global statistics on gastric cancer suggest that it is the fifth most common type of cancer in terms of prevalence and third-most common type in terms of death rate. The considerably high mortality of gastric cancer is mainly because of the fact that it is mostly diagnosed when the disease has progressed to the advanced stage. While the bacterium Helicobacter pylori is known to be the most common cause of gastric cancer, other notable risk factors also include high salt intake, age and diets lacking in fruits and vegetables. Demographic data have revealed that the regions of East Asia, Eastern Europe and South America are notable hotspots of this type of cancer. Endoscopic resection is considered as the major treatment procedure for early detected gastric cancer while those diagnosed in later stages are treated with surgeries such as D2 lymphadenectomy. Previously, the Atypical Protein Kinases C (aPKCs) - ζ and ι - have been documented to play significant roles in elevated cell proliferation in various cancer types. Here, an inhibitor of the aPKC-ζ, known as ζ-stat (8-hydroxy-1,3,6-naphthalenetrisulfonic acid), that specifically inhibits the aPKC-ζ on a gastric cancer cell line (i.e., AGS) was used to observe its effects on cell proliferation. In separate trials, AGS cells were treated with ζ-stat for 72 hours (about 3 days). An assortment of drug concentrations for the inhibitor was tried and the analysis of the cell count demonstrated that the cell proliferation decreases were statistically significant for the drug in all concentrations. The highest decrease was observed at 10 μM ($P < 0.0001$) as the number of cells diminished to approximately 44%. Our previous research has revealed that ζ-stat downregulates the aPKC-ζ by interfering with established cancer pathways to cause cell deaths. Henceforth, Western Blot analysis of AGS cells treated with ζ-stat have shown evidence of apoptosis. There have been considerable decreases in the levels of total PARP, Caspase 3 and Survivin. Likewise, levels of cleaved-Caspase 3 and cleaved-PARP have also increased along with the mitochondrial apoptotic marker Cytochrome C. Current investigations include how ζ-stat play into some of the well-established gastric cancer pathways to lead to cell deaths with techniques such as Western Blot analysis, Immunoprecipitation Assays and Flow cytometry.

#4135 AKT1 mediates PTEN-loss induced intrahepatic cholangiocarcinoma development in mice.

Ielyzaveta Slarve, Yining Ding, Yushan Wang, Qi Tang, Zifei Xu, Lina He, Bangyan Stiles

USC - University of Southern California, Los Angeles, CA

Introduction: Liver cancer is the third most common cause of cancer-related mortality worldwide. Mice with liver-specific *Pten* loss (*Pten*$^{loxP/loxP}$; albumin [*Alb*]-*Cre*$^{-/+}$; LiPten) develop predominantly intrahepatic cholangiocarcinoma (ICC) and some hepatocellular carcinoma (HCC) by 12 months of age. *Pten* loss results in activation of the serine/threonine kinase AKT. Of the three AKT isoforms identified to date, AKT1 isoform is implicated in regulation of cell growth, proliferation, and survival. The present study investigated the role of AKT1 in mediating PTEN-loss induced liver cancer development.

Methods: To generate mice with liver-specific *Pten* and *Akt1* deletion (*Pten*$^{loxP/loxP}$; *Akt1*$^{loxP/loxP}$; *Alb-Cre*$^{-/+}$; LiPtenA1), LiPten mice were crossed with mice that have liver-specific *Akt1* loss (*Akt1*$^{loxP/loxP}$; *Alb-Cre*$^{-/+}$; LiA1). Whole livers from 12-month-old WT, LiA1, LiPten, and LiPtenA1 mice were analyzed for tumor prevalence and morphology via hematoxylin and eosin staining, and for markers of HCC and ICC via qPCR and immunofluorescence.

Results: All male LiA1 mice displayed WT-like phenotype. Like LiPten mice, all male LiPtenA1 mice develop steatosis followed by tumors by 12 months of age. Compared to LiPten mice, LiPtenA1 animals displayed predominantly HCC phenotype based on morphology, increased HCC markers (*Hnf4a*, *Alb*), and decreased ICC markers (*Sox9*, *Spp1*, *Hes1*, *Ck19*).

Conclusions: AKT1 loss in LiPten mice did not prevent development of steatosis and liver tumorigenesis but resulted in predominantly HCC rather than ICC phenotype, suggesting the role of AKT1 in mediating ICC development. These results contribute to the understanding of the role of AKT isoforms in regulating liver tumorigenesis, and further investigation into signaling mechanisms underlying these observations is required.

#4136 The causes and consequences of off-target activation of the GCN2-eIF2a-ATF4 axis in cancer therapy.

C. Ryland III[1], Nasreen Marikar[1], Vu Nguyen[2], Varuna Nangia[1], Alicia Darnell[3], Stacey Bagby[2], Kasey Couts[2], Matthew G. Vander Heiden[3], Philip Reigan[2], Sabrina L. Spencer[1]

[1]University of Colorado Boulder, Boulder, CO,[2]University of Colorado Anschutz, Aurora, CO,[3]Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA

Kinase inhibitors are notorious for their promiscuity and off-target effects. We discovered that multiple clinical inhibitors used in the treatment of cancer, including dabrafenib and encorafenib, activate the GCN2 kinase at patient-relevant concentrations in an off-target manner via direct interaction. GCN2 is an activating kinase of the integrated stress response (ISR) that leads to induction of the transcription factor ATF4. Cotreatment with a GCN2 inhibitor in BRAF-V600E melanoma cells significantly alters the rate of drug resistance and cell outgrowth. We thus propose that off-target activation of GCN2 affects development of drug-resistant and metastatic phenotypes. Finally, we perform an *in silico* inhibitor screen, identifying dozens of FDA-approved inhibitors used in the treatment of cancer that appear to share this off-target GCN2 interaction.

#4137 Investigating VRK1 inhibition as an anti-cancer therapy for pancreatic ductal adenocarcinoma.

K. Chu, Y. Xi, S. Schwarz, K. T. Vaughan;
University of Notre Dame, Notre Dame, IN

Pancreatic ductal adenocarcinoma (PDAC) is a aggressive and often a lethal form of exocrine pancreatic cancer with a 5-year survival rate of 12%. Current PDAC therapeutics are ineffective and highly toxic, leading to a variety of harsh side effects that greatly reduce patient quality of life. This low quality of life highlights the need for new approaches. This study investigates the potential of inducing nuclear envelope instability in PDAC cells through inhibition of Vaccinia-Related Kinase 1 (VRK1). Previous studies in the Vaughan lab have demonstrated that the knock down of VRK1 reduces BAF phosphorylation, reduces BAF/Lamin A interaction, and leads to nuclear blebbing. Evidence also suggests that nuclear blebbing leads to DNA leakage and activation of the cGAS-STING pathway and cellular senescence. However, it remains unclear what stage of the cell cycle is inhibited by VRK1 inhibition. To determine when this phenomenon occurs, we synchronized Cos7 cells using a thymidine and nocodazole block and treated with VRK1-IN-1, a potent VRK1 inhibitor. To determine if VRK1 inhibition affects a certain stage of mitosis, cells were synchronized and stained for tubulin and Lamin A. We observed that VRK1-IN-1 does not affect a specific stage of the cell cycle. Here we developed a protocol for measuring the effect of VRK1 inhibitors on mitosis and the cell cycle. This protocol will serve as a launching point to validate the discovery of VRK1 inhibitors from a 12,000-compound kinase assay screen. Elucidation and understanding of when VRK1 inhibition activate/induces the cGAS-STING pathway and cellular senescence, may provide the rational for to pursuing of VRK1 inhibitors as novel therapeutics for the treatment of PDAC.

#4138 Investigating the relationship between DUSP12 and ZPR9 in cell division and death.

Mai Abdusamad[1], Xiao Guo[1], Ivan Ramirez[1], Whitaker Cohn[2], Erick Velasquez[1], Ankur Gholkar[3], Julian P. Whitelegge[2], Jorge Z. Torres[1]

[1]Department of Chemistry and Biochemistry, UCLA - University of California Los Angeles, Los Angeles, CA,[2]Pasarow Mass Spectrometry Laboratory, UCLA - University of California, Los Angeles, Los Angeles, CA,[3]UCLA - University of California Los Angeles, Los Angeles, CA

Protein phosphatases regulate essential processes in human cells and have critical roles in many human diseases, including cancer. The dual-specific phosphatases (DUSPs) are a subset of protein phosphatases that have become increasingly implicated in human cancers over the past decade. DUSP12 is a poorly characterized atypical DUSP widely conserved throughout evolution. While DUSP12 has been shown to possess pro-survival attributes, it is unclear if this is related to its potential role in cell division. Specifically, DUSP12 has been shown to play a role in both cell death and proliferation, acting in a protective capacity against stressful conditions in both hepatic and myocardial ischemic-reperfusion injury. Interestingly, several studies have also linked aberrant expression of DUSP12 to the development of a wide range of cancers, highlighting the importance of investigating the biological activities of DUSP12 as it appears that dissecting its functions in detail can provide insight into new mechanisms supporting uncontrolled cell growth. More recent evidence further suggests that DUSP12 affects cell cycle progression by regulating DNA content at the G1/S and G2/M checkpoints, likely through its unique zinc binding domain. However, the lack of known DUSP12 targets has hindered our understanding of the biological significance of this unique enzyme. From preliminary proteomic analyses of DUSP12 using affinity- and proximity-based approaches coupled to mass spectrometry, we identified ZPR9 as a potential interactor of DUSP12 which has been validated by in-cell and *in-vitro* immunoprecipitation assays. Previous studies have shown that ZPR9 may play a role in apoptosis, but it is unclear if and how ZPR9 participates in cell division and, more so, how it promotes cell death. Using cancer cell lines as a model system and leveraging advanced imaging techniques, I am performing follow-up functional studies to determine if and how ZPR9 is modified by DUSP12 and, further, if modification of ZPR9 by DUSP12 is important in influencing improper cell division. Collectively, this work will provide insights into the mechanism and regulatory role of the phosphatase DUSP12 during cell division as well as form the basis for designing novel therapeutic approaches to address diseases associated with abnormal proliferation.

#4139 Targeting USP10 to enhance chemotherapy and immunotherapy in NSCLC.

S. Dorandish, K. Bhayekar, A. Samarbakhsh, B. Kushwaha, P. Parajuli, Y. Ge, N. S. Gavande;
Wayne State University, Detroit, MI

Non-small cell lung cancer (NSCLC) remains a leading cause of death from cancer. While platinum-based chemotherapy is initially effective for early-stage NSCLC, drug resistance often limits its long-term success. Combining platinum-based chemotherapy with immunotherapy has emerged as a first-line treatment for metastatic NSCLC. However, many patients still experience limited benefits due to various resistance mechanisms. One critical mechanism underlying drug resistance is the increased capacity of DNA damage response (DDR). The deubiquitinase USP10 plays a crucial role in regulating the DDR by stabilizing oncogene proteins such as HDAC6. Inhibition of USP10 disrupts the USP10-HDAC6 axis, leading to enhanced DNA damage and cell death. Further, HDAC6 inhibition enhances immunogenic cell death-inducing radiotherapy-induced immune responses. Furthermore, HDAC6 inhibition leads to decreased expression of CD47 that acts as a "don't eat me" signal to prevent immune cells from phagocytosing tumor cells. Based on these findings, we hypothesize that targeting USP10 leads to HDAC6 degradation and DNA damage, resulting in sensitization of NSCLC cells to chemotherapy. Additionally, we propose that USP10 inhibition can sensitize NSCLC cells to immune checkpoint inhibitors and immunotherapy targeting CD47. Our extensive computational and structure-activity-relationship (SAR) studies led to the discovery of potent USP10 inhibitors. Our preliminary data show that the lead compound, GL-320, selectively inhibits USP10 activity, as confirmed by in vitro deubiquitinase (DUB) and cellular thermal shift assays. GL-320 dose-dependently inhibited the in vitro cell proliferation of H1299, a p53 null lung cancer cell, whereas it was significantly less effective against A549 cells with w/t p53. We also show that by inhibiting USP10, we can disrupt the DDR pathway, leading to increased DDR and cell death, as confirmed by western blotting and flow cytometry analyses. Our preliminary data show that USP 10 is a validated drug target to enhance the efficacy of existing chemotherapy and immunotherapy in NSCLC.

#4140 Non-proteolytic regulation of oncogenic transcription factors by deubiquitinases.

Yunsik Choi

Sogang University, Seoul, Korea, Republic of

Oncogenic transcription factors (TFs) such as ETS2 and TEAD4 are critical regulators of genes driving cancer progression, including those involved in proliferation, survival, and metastasis. Despite their importance, the mechanisms modulating their activity through ubiquitination remain poorly understood. This study investigates deubiquitinases (DUBs) that interact with and regulate ETS2 and TEAD4, aiming to uncover novel insights into their transcriptional control.A DUB sub-library screening identified USP39 and OTUD6A as regulators of ETS2 and TEAD4, respectively. USP39 interacts with ETS2 via their amino-terminal regions, independent of the zinc finger and PNT domains, and deubiquitinates ETS2. This interaction reduces ETS2's nuclear localization and transcriptional activity without affecting its stability, indicating a negative regulatory role. In contrast, OTUD6A interacts with TEAD4 at its YAP-binding domain and deubiquitinates TEAD4. OTUD6A further enhances TEAD4 transcriptional activity and cofactor interactions, notably by promoting YAP binding. Similar to USP39 and ETS2, OTUD6A does not influence TEAD4 stability but positively regulates its activity.These findings highlight the distinct, non-proteolytic roles of USP39 and OTUD6A in modulating transcriptional activity of ETS2 and TEAD4, respectively. Our findings underscore the functional diversity and opposing regulatory mechanisms of DUBs in oncogenic transcriptional control.

#4141 PDE3A promotes ER positive beast cancer progression by preventing ERα ubiquitination degradation.

Shengyu Pu, Heyan Chen, Bohong Chen, Jianjun He, Na Hao

The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China

Background: ER+ breast cancer (ER+BC) is the most common subtype, which characterized by high risk of recurrence and metastasis after treatment. ERα is considered to be a key driver in the malignant progression of ER+BC, and it is necessary to understand the signaling mechanism of ERα. PDE3A, a member of the phosphodiesterases (PDEs) family, can hydrolyze the cAMP and cGMP, and is an important regulator of intracellular signaling. However, there are few studies on the effect of PDE3A on the malignant progression of ER+BC, and the specific mechanism is still unclear.
Aim: (i) To investigate the prognostic role of PDE3A in ER+BC and its links with breast cancer proliferation and metastasis; (ii) to explore the regulatory mechanism of PDE3Ain ER+BC biological process; (iii) to screen effective molecule inhibitor targetingPDE3A.
Methods: The expression level of PDE3A in ER+BC was determined by immunohistochemical staining and the mRNA expression profiles from the TCGA database. Roles of PDE3Ain cancer cell growth, metastasis were determined by molecular and cell biology methods. Co-Immunoprecipitation (CO-IP) was used to examine the binding of PDE3Aand ERα. Chromatin immunoprecipitation (ChIP) and promoter luciferase reporter assay were included for detecting the transcription of PDE3A in ER+BC. ER+BC xenograft mice were used to study the inhibition of tumor development.
Results: PDE3A is a novel ER+BC target, which is overexpressed in ER+BC tissues and significantly associated with clinical pathology of advanced TNM stage and poor prognosis of patients. In vitro and in vivo studies have demonstrated that PDE3Apromotes ER+BC proliferation and metastasis. Mechanically, PDE3A regulates ERαproteasome degradation through competitive binding to ERα with its E3 ligase SPOP, independently of the catalytic activity of PDE3A. In addition, ERα directly facilitates the transcription of PDE3A and up-regulate the expression level of PDE3A.Furthermore, we screened out brequinar, a small molecule inhibitor targeting PDE3A, can inhibit breast cancer cell proliferation and metastasis, and reverse endocrine resistance.
Conclusion: We identified an important role of PDE3A-ERα feedback loop in the ER+BC progress and resistance to endocrine therapy, representing a future biomarker and therapeutic target of ER+BC.

#4142 Modulation of kinase signaling by APLP2 in pancreatic cancer cells.

Kenadie Doty[1], Kaitlin J. Smith[2], Gabrielle L. Brumfield[1], Joyce C. Solheim[1]

[1]University of Nebraska Medical Center, Omaha, NE,[2]College of Saint Mary, Omaha, NE

Pancreatic cancer is a highly aggressive malignancy with few effective treatment options, highlighting the importance of understanding its underlying molecular mechanisms. The overexpression of amyloid precursor-like protein 2 (APLP2) in pancreatic cancer suggests that it may promote cancer progression. However, mechanisms by which APLP2 may influence critical signaling pathways in pancreatic cancer remain undefined. To address this, a membrane-based sandwich immunoassay was used to determine the relative phosphorylation levels of 37 kinase sites in S2-013 pancreatic cancer cell lysates following siRNA-mediated APLP2 knockdown. The results demonstrated that the downregulation of APLP2 led to significant changes in the phosphorylation of several key kinases, including a pronounced increase in the phosphorylation of the proline-rich Akt substrate of 40 kDa (PRAS40), which is involved in mTORC1 function, and decreases in the phosphorylation of CREB, ERK1/2, and JNK 1/2/3. Qiagen Ingenuity Pathway Analysis (IPA) identified that the pathways most affected by APLP2 knockdown involved MAPK targets and nuclear events mediated by MAP kinases. Together, these findings suggest that APLP2 may regulate key signaling pathways driving pancreatic cancer progression and provide insights into the molecular mechanisms by which APLP2 contributes to pancreatic cancer's aggressive nature. Our future studies will utilize chemical inhibitors, knockout experiments, and western blot analysis to validate and extend these new findings.

#4143 Analyzing BRAFV600E signaling complexes to better understand BRAF driven melanoma.

Adamaris M. Martinez, Kayla T. O'Toole, Martin McMahon

Huntsman Cancer Institute at the University of Utah, Salt Lake City, UT

In approximately 8% of all cancers, mutational activation of BRAF is an oncogenic driver, with a particular prevalence in lung cancer, thyroid cancer, and melanoma. Mutations in BRAF account for about 50% of the cutaneous melanoma cases in patients. The most common mutational alteration is the *BRAF*^{T1799A} encoding the BRAF^{V600E} oncoprotein kinase. Combined inhibition of BRAF^{V600E} and MEK 1/2 has been approved to treat BRAF^{V600E}-driven melanoma and lung cancer. There are several mechanisms of resistance to inhibitors of BRAF^{V600E} signaling, with one of the most intriguing being the emergence of tumors expressing p61-BRAF^{V600E} that is encoded by a variant mRNA that is aberrantly spliced to remove exons 4-8. Consequently, p61-BRAF^{V600E} is missing amino acid sequences that promote association with RAS.GTP (RBD) and the cysteine rich domain (CRD). Moreover, while full-length BRAF^{V600E} is widely considered to signal as a monomer, p61-BRAF^{V600E} is thought to require dimerization for activation. Here, we use gel-filtration chromatography and immunoblotting to show that both forms of BRAF^{V600E} are detected in a large molecular weight complex of 450-500 kDa. However, p61-BRAF^{V600E} containing complexes are smaller than those with full-length BRAF^{V600E}, which may reflect the difference in size of the BRAF^{V600E} proteins or subtle differences in the signaling complexes. Interestingly, the addition of a BRAF^{V600E} inhibitor, vemurafenib, shifted the elution of complexes with full-length BRAF^{V600E} into lower molecular weight fractions. In addition, we have utilized the proximity labeling technique, TurboID, to uncover more of the BRAF interactome.

#4144 PTPTF negatively regulates c-Src kinase through the dephosphorylation of the activation loop.

Haley Stanczyk[1], Carolina Galeano-Naranjo[1], Tianyan Gao[2]

[1]Department of Molecular and Cellular Biochemistry, University of Kentucky, Lexington, KY,[2]Markey Cancer Center, University of Kentucky, Lexington, KY

Kinases and phosphatases play a dynamic role in the regulation of signaling propagation. c-Src is an oncogenic kinase that plays multifunctional roles in the initiation and progression of many cancers, including breast, colorectal and lung cancers. The activity of c-Src kinase is tightly regulated by the phosphorylation status of two key tyrosine residues (Tyr419 and Tyr530 in human c-Src) that controls its propensity for entering an active conformation. Phosphorylation of Tyr530 at the C-terminus of c-Src locks the kinase in an inactive confirmation whereas phosphorylation of Tyr419 in the activation loop is required to activate c-Src. A number of tyrosine phosphatases have been identified to activate c-Src by dephosphorylating Tyr530. However, the phosphatases controlling the Tyr419 site remain elusive. In this study, we investigated the role of protein tyrosine phosphatase receptor type F (PTPRF) in regulating the phosphorylation of c-Src. PTPRF is characterized by an extracellular domain, a single transmembrane motif, and two tandem intracellular phosphatase domains (including a catalytically active D1 domain and a catalytically inactive D2 domain). We show that overexpression of PTPRF, but not a phosphatase inactive mutant, results in a decrease in Tyr419 phosphorylation of both wild-type and Y530F constitutively active c-Src in 293T cells. On the other hand, CRISPR-mediated knockout of PTPRF increases the phosphorylation of c-Src and signaling downstream. Co-immunoprecipitation experiments indicate that PTPRF interacts with c-Src through its D2 pseudo phosphatase domain. Consistent with the notion that the kinase activation reduces c-Src protein stability, we find that the expression of c-Src is increased when co-expressed with PTPRF, suggesting that PTPRF-mediated dephosphorylation stabilizes c-Src protein. Taken together, this study identifies PTPRF as a novel phosphatase that negatively regulates c-Src kinase activity through the dephosphorylation of Tyr419 in the activation loop.

#4145 TRIM21 functions as an oncogene in glioblastoma by transactivating FOSL1 and promoting the ubiquitination of p27 transactivating FOSL1 and promoting the ubiquitination of p27.

Vanajothi Ramar, Rajveer Sidhu, Osei Pako, Cheyenne R. Cisse, Shanchun Guo, **Mingli Liu**

Morehouse School of Medicine, Atlanta, GA

Background: Our previous studies demonstrated that FOSL1 promotes glioblastoma (GBM) progression and stemness through multiple pathways such as STAT3 and NF-κB signaling. Recently, we identified that FOSL1 physically interacts with the E3 ligaseTRIM21, which is localized in the nucleus of glioma cells. This study investigates the role of TRIM21 in GBM, its relationship with FOSL1, and its regulation of the TRIM21 ubiquitination target gene p27.
Methods: 1) Protein interaction: Immunoprecipitation assays were conducted to examine the physical interaction between TRIM21, FOSL1, and p27. 2) Regulation of p27: TRIM21 overexpression and knockdown (via siRNA) were used to analyze the effects on p27 protein levels. 3) Ubiquitination assay: TRIM21 knockdown followed by p27 immunoprecipitation and anti-ubiquitin probing was performed to assess ubiquitination. 4) Clinical data analysis: TCGA datasets were analyzed to correlate TRIM21 expression with glioma subtype and patient survival. 5) Functional assays: Glioma cell growth (MTT, colony formation) and invasion (transwell assay) were measured following TRIM21manipulation. 6) Histopathology: IHC was performed to evaluate TRIM21 expression in glioma patient tissues and its relationship with FOSL1 expression. 7) Nuclear TRIM21: The effects of nuclear-localized TRIM21 in regulating FOSL1 was assessed, including its ability to stimulate FOSL1 promoter activity via AP-1 binding sites.
Results: 1) TRIM21 physically interacts with FOSL1 and p27. 2) TRIM21 promotes p27 degradation through ubiquitination in a dose-dependent manner. 3) TCGA analysis reveals high TRIM21 expression in GBM patients, particularly in the mesenchymal subtypes. 4) TRIM21 enhances glioma growth and invasion in short-term and long-term assays. 5) IHC data show elevated TRIM21 levels in grade IV GBM tissues, correlating with FOSL1 expression. 6) nuclear-localized TRIM21 promotes FOSL1 transactivation via AP-1 sites and simultaneously mediates p27 degradation.
Conclusion: TRIM21 functions as an oncogene in GBM by driving FOSL1 transactivation and degrading the tumor suppressor gene of p27, thereby promoting GBM progression and aggressiveness.

#4146 USP36 associates with the rixosome complex and promotes ribosome biogenesis.

Yanping Li, Xiao-Xin Sun, **Mushui Dai**

OHSU, Portland, OR

Eukaryotic ribosome biogenesis is a highly regulated multistep process to generate the ribosome, including transcription of pre-ribosomal RNA (pre-rRNA), rRNA processing, ribosome subunit assembly and export. Dysregulation of ribosome biogenesis has been associated with development of various diseases, including ribosomopathies and cancer. Therefore, it is crucial to understand how ribosome biogenesis is regulated during normal cell homeostasis and how it is deregulated in human diseases. We previously reported that the ubiquitin-specific protease USP36 acts as a SUMO ligase to promote nucleolar protein SUMOylation. Using affinity purification, we found that USP36 associates with a number of nucleolar and ribosome biogenesis-related proteins, including the rixosome complex that consists of Las1L, Nol9, MDN1, Pelp1, WDR36, Tex10 and SENP3 and plays a critical function in ribosome maturation. We confirmed that that USP36 interacts with all the components of the rixosome complex. We further showed that USP36 promotes the SUMOylation of Las1L, Nol9 and Pelp1, suggesting that USP36 promotes rixosome protein group SUMOylation. As USP36 is a ubiquitin specific protease, we also test whether the stability of these rixosome proteins is regulated by USP36. We found that the levels of MDN1 and Las1L, Nol9 are reduced upon USP36 knockdown. Abolishing Las1L SUMOylation impaired rRNA processing and inhibited cell growth. Together, these results suggest that USP36 plays an important role in regulating ribosome biogenesis by post-translationally regulating the function of rixosome complex.

#4147 Role of MLK3-mediated phosphorylation of β-catenin in regulating cellular senescence and osteopontin expression in hepatocellular carcinoma.

Tanushree Kashyap, Rong Ke, Deepti Srivastava, Sunil K. Singh, Subhasis Das, Piush Srivastava, Sandeep Kumar, Ajay Rana, **Basabi Rana**

University of Illinois at Chicago, Chicago, IL

The canonical Wnt/β-catenin signaling pathway is important for normal liver growth and development, abnormal activation of which can lead to various liver pathologies, including hepatocellular carcinoma (HCC). Usually, β-catenin signaling is regulated by a degradation complex that includes adenomatous polyposis coli, Axin, Glycogen Synthase Kinase β, and Casein kinase 1α. Mutations of β-catenin itself and other genes regulating this pathway can cause aberrant activation of this axis. We have reported earlier that Mixed Lineage Kinase 3 (MLK3), a member of the MAPK kinase Kinase (MAP3K) family, can phosphorylate β-catenin and regulate its downstream pathway. The current studies were aimed to elucidate the biological consequences of MLK3-induced β-catenin phosphorylation in HCC. The experimental procedures included creating stable cell lines, RT^2 Profiler PCR Arrays, qPCR analysis, Immunofluorescence (IF), ELISA, IHC, MTT and β-Galactosidase (β-Gal) assays. Using Mass Spectrometry, we identified that MLK3 can phosphorylate the N-terminal region of β-catenin at Serine 129 amino acid residue. To understand the significance of β-catenin S129 phosphorylation, stable HCC cell lines overexpressing β-catenin-WT or its phospho-mutants (S129A, S129D) were generated. While MTT assays showed no significant differences between β-catenin-WT or mutant overexpression, β-Gal assays revealed that overexpression of β-catenin WT and S129D can promote a senescence phenotype, which was inhibited in the S129A mutant. These cells also showed increased expression of a set of unique senescent genes including COL1A1, COL3A1, and IGFBP7 which were dependent on S129 phosphorylation. Analysis by RT^2 Profiler PCR Array for cytokine and chemokine genes showed an increased expression of SPP1 (Osteopontin gene) in the WT compared to S129A cells. Further validation by qPCR, IF, and ELISA confirmed the induction and secretion of Osteopontin by β-catenin WT and S129D but not by S129A, suggesting a senescence-induced secretory phenotype (SASP) mediated by β-catenin-S129 phosphorylation. TMA analysis showed significant increase in Osteopontin expression in HCC patient samples compared to normal. Database analysis suggested that the SPP1 gene is induced in HCC, and high expression of SPP1 is associated with poor prognosis. In addition, there was a strong correlation between CTNNB1 (β-catenin gene) and SPP1 expressions in HCCs. Considering a well-established role of Osteopontin in HCC and other liver pathologies, these results suggest a potentially novel paracrine signaling network operating in liver cells mediated via a crosstalk of MLK3-β-catenin-Osteopontin axis. These also suggest MLK3 to be a viable drug target for antagonizing this axis and in clinical management of HCC.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Metabolic Pathways
Poster Session

#4151 Creatine and GAMT promote tumor stemness and malignancy and can be used as biomarkers for colorectal cancer recurrence.

Zhengran Zhou, Peishan Hu, Zerong Cai, Yufeng Chen, Xiaojian Wu, Xuanhui Liu

The Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou, China

BACKGROUND: The role of creatine, a common nutritional supplement, in tumorigenesis and development has gained increasing attention. Previous studies have shown that it has a dual effect on tumorigenesis and recent studies suggested that it promotes colorectal cancer (CRC) metastasis. However, the role creatine metabolism plays in tumor malignancy and recurrence is still unclear and identifying the mechanism could provide new strategies for the treatment of CRC.
METHODS: Targeted metabolomics analysis of creatine was dynamically performed on plasma samples of 160 CRC patients including 30 relapse cases. GAMT, the key enzyme in creatine synthesis, was investigated in TCGA and ICGC-ARGO database (a large multi-omics study of CRC in China conducted by the sixth affiliated hospital of Sun Yat-sen University). The effect of creatine and GAMT on CRC stemness and its mechanism were investigated in CRC cell lines, patient-derived organoids, and patient-derived xenografts. Limited proteolysis-mass spectrometry (LiP-MS) and RNA-sequencing was performed to illustrate creatine-protein interaction and the regulation of relative signal pathways.
RESULTS: In recurrent CRC cases, creatine level gradually increased over time after the first radical surgery until recurrence. Meanwhile, GAMT expression was found significantly higher in patients who were going to relapse, both in TCGA and ICGC-ARGO database. Patients with higher GAMT expression had worse prognosis. The addition of creatine inhibitor can effectively restrain CRC activity and proliferation both in vitro and in vivo. GAMT knockdown significantly reduced the self-renewal ability of CRC cells, and tumor stemness pathways were up-regulated, which can be reversed by adding creatine to the culture.
CONCLUSIONS: Creatine is associated with CRC recurrence through promoting tumor stemness and malignancy. Therapeutic strategies targeting creatine metabolism have the potential to reduce CRC recurrence.

#4152 Disrupting lipid elongation impairs prostate cancer progression in c-MYC driven mouse models.

Silvia D. Rodrigues[1], Isadora F. Teixeira[1], Giuseppe N. Fanelli[1], Caroline F. Ribeiro[1], Fabio Socciarelli[1], Jeanne Jiang[1], Debora C. Bastos[2], Hubert Pakula[1], Filippo Pederzoli[1], Pier V. Nuzzo[1], Alessandro Barberis[3], Lisa M. Butler[4], Johannes V. Swinnen[5], Massimo Loda[1]

[1]Pathology and Laboratory Medicine, Weill Cornell Medicine, NEW YORK, NY,[2]Departamento de Patologia, Faculdade Sao Leopoldo Mandic, Campinas, Brazil,[3]Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom,[4]South Australian Immunogenomics Cancer Institute, University of Adelaide, Adelaide, Australia,[5]Department of Oncology, KU Leuven, Leuven, Belgium

Background: Altered lipid metabolism is a hallmark of prostate cancer development and progression. Key pathways affected include *de novo* fatty acid synthesis and downstream fatty acid elongation, which are both activated by androgens and oncogenic signaling, including c-Myc, a downstream target of Fatty Acid Synthase. One of the enzymes that is upregulated in clinical PCa is ELOVL5 (Elongation of Very Long-Chain Fatty Acids Protein 5), a target of the androgen receptor (AR), which catalyzes the initial step of fatty acid elongation. However, its role in cancer progression remains unknown.

Methods: Prostate tumors from HiMyc transgenic mice were evaluated for growth, histopathological progression and lipidomics changes up to 48 weeks and compared to HiMyc mice with ELOVL5 homozygous deletion. 3D organoid model derived from the ventral prostate of these GEMMs were assessed for changes in cell proliferation, size, and response to anti-androgen therapy (Enzalutamide). Rescue experiments were performed by supplementation with cis-vaccenic acid, a key fatty acid product of ELOVL5 activity.

Results: ELOVL5 KO significantly inhibited progression from prostatic intraepithelial neoplasia to invasive adenocarcinoma in comparison to HiMyc mice. Lipidomic profiling confirmed reduced levels of ELOVL5 elongation products, characterized by shortened acyl chains in phosphatidylethanolamine (PE) and phosphatidylcholine (PC) species, in both serum and prostate tissues. The growth effect was recapitulated in organoids derived from these mice, was further enhanced by Enzalutamide and partially rescued by supplementation with cis-vaccenic acid. Gene expression profiling confirmed downregulation of fatty acid biosynthesis pathways and androgen receptor in HiMyc: ELOVL5 KO models.

Conclusion: ELOVL5 curtails c-Myc-driven prostate cancer, providing a direct link between fatty acid elongation and prostate cancer progression.

#4153 Biomarker-driven polyamine pathway targeting as a treatment approach for TNBC.

Chloe White, Caroline Liang, Thomas Velenosi

Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada

Triple negative breast cancer accounts for about 15% of all breast cancer diagnoses and represents the most aggressive breast cancer subtype. This study aims to use a polyamine biomarker-driven approach to treat TNBC. Polyamines are small cationic molecules important for cancer cell growth and spermidine/spermine N^1-acetlytransferase (SAT1) is the rate-limiting catabolic enzyme in the pathway. Our previous work demonstrated that doxorubicin induces SAT1 expression in moderate-SAT1 expressing TNBC models. We hypothesize that the effectiveness of using an SAT1 agonist to treat TNBC is dependent on baseline SAT1 expression and function. TNBC cell lines and patient-derived xenografts with low, moderate, and high baseline expression levels of SAT1 were used to evaluate the hypothesis. Cell lines were treated with TNBC standard-of-care doxorubicin, the SAT1 agonist N1, N11-diethylnorspermine (DENSpm), combination DENSpm-doxorubicin, or vehicle control. Adding DENSpm to sub-therapeutic doxorubicin treatments further decreased cell viability in moderate and high baseline SAT1 expressing cells ($p<0.05$). DENSpm depleted spermidine in moderate-SAT1 expressing TNBC-PDX ex vivo tumours slices, which was further depleted by combining with doxorubicin. In vivo, the addition of DENSpm to doxorubicin treatment further attenuated tumour growth in moderate-SAT1 expression TNBC-PDX, which corresponded to an increase in urine diacetylspermine as an indicator of treatment effectiveness. In conclusion, the results of this study suggest that combination doxorubicin and DENSpm treatment may be an effective treatment option for TNBC tumours with moderate baseline SAT1 expression, and that urine diacetylspermine production may be indicative of treatment effectiveness.

#4154 Cancer cells exhibit novel metabolic vulnerabilities when exposed to low lipid environments.

D. L. Ramesh[1], K. L. Abbott[1], R. Ferreira[2], M. G. Vander Heiden[1];

[1]Massachusetts Institute of Technology, Cambridge, MA, [2]Wyss Institute for Biologically Inspired Engineering and Harvard Medical School, Harvard University, Boston, MA

Cancer cell metabolism is significantly influenced by microenvironmental factors such as nutrient availability, which can vary across different tissues and introduce exploitable therapeutic vulnerabilities. Notably, lipid availability is particularly low in the brain compared to other tissues. To identify metabolic vulnerabilities of cells in lipid-poor environments, we performed a CRISPR/Cas9 screen of metabolic synthesis genes in human cancer cells cultured in lipid-rich versus lipid-poor media. Through this screen, we observed a novel metabolic dependency in the cells when grown in lipid-depleted conditions. We verified this differential dependency by knockout (KO) of this target gene, which resulted in slower cell proliferation in lipid-depleted media. Additionally, wild-type cells displayed heightened sensitivity to inhibitors of other proteins in the pathway in lipid-depleted conditions, thereby phenocopying the KO. Polar metabolomics analyses by LC-MS revealed depletion of several nucleotide species in the KO cells cultured in lipid-depleted conditions, and supplementation with purine nucleotides rescued the proliferation of KO cells cultured in lipid-depleted media. We hypothesized that this nucleotide depletion could in turn result in nucleotide imbalance and DNA damage, and Western Blot analyses confirmed elevated DNA damage levels in the KO cells in lipid-depleted media. Our model is that in low-lipid environments, the KO redirects metabolic resources toward lipid synthesis, causing nucleotide imbalance, DNA damage, and cell death. Overall, our findings indicate that cancer cells exhibit novel metabolic vulnerabilities when exposed to lipid-depleted environments, which could be leveraged for treating tumors growing in lipid-poor organs such as the brain.

#4155 Catabolism of extracellular glutathione supplies amino acids to support tumor growth.

F. Hecht[1], M. Zocchi[1], E. T. Tuttle[1], N. P. Ward[2], B. Smith[1], Y. P. Kang[2], J. Cazarin[1], Z. G. Soares[1], M. Emir Ozgurses[3], H. Zhao[3], C. Sheehan[4], F. Alimohammadi[1], L. Munger[1], D. Trivedi[1], G. Asantewaa[1], S. Blick-Nitko[1], J. Zoeller[5], Y. Chen[6], V. Vasiliou[6], B. M. Turner[1], A. Muir[4], J. L. Coloff[3], J. Munger[1], G. DeNicola[2], I. Harris[1];

[1]University of Rochester Medical Center, Rochester, NY, [2]Moffitt Cancer Center and Research Institute, Tampa, NY, [3]University of Illinois College of Medicine, Chicago, IL, [4]University of Chicago, Chicago, IL, [5]Harvard Medical School, Boston, MA, [6]Yale School of Public Health, New Haven, CT

Restricting amino acids from tumors is an emerging therapeutic strategy with significant promise. While typically considered an intracellular antioxidant with tumor-promoting capabilities, glutathione (GSH) is a tripeptide composed of cysteine, glutamate, and glycine that can be catabolized, yielding amino acids that could support tumor metabolism. However, the extent to which GSH-derived amino acids are essential to cancers is unclear. Here, we find that catabolism of extracellular GSH provides critical support for tumors by supplying cysteine. We show that disrupting the tumor's ability to synthesize intracellular GSH does not perturb its growth, highlighting the potential importance of extracellular GSH. Notably, we find GSH to be abundant in the tumor interstitial fluid, the primary environment surrounding cancer cells in vivo, where it far exceeds cystine levels. Our findings reveal that cancer cell survival and growth can be restored in cystine-deprived conditions through GSH supplementation. This process was dependent on the catabolic activity of γ-glutamyl-transferases (GGT) enzymes, which can break down GSH, yielding glutamate and cysteinylglycine, which can be further brown down to cysteine and glycine. GGT1 overexpression further facilitates this rescue by lowering the amount of GSH required for cell survival. Metabolomic analysis shows that GSH catabolism replenishes cysteine-related products depleted by cystine deprivation. Additionally, pharmacological screening revealed that when cancer cells acquire their cysteine from GGT-mediated GSH breakdown, they become resistant to inhibitors of cystine uptake and utilization. Finally, the systemic treatment of mice bearing breast tumor xenografts with a potent GGT inhibitor prevented circulating GSH breakdown, reduced tumor cysteine levels, and slowed down tumor growth. This effect was reversed by restoring cysteine levels with N-acetyl-cysteine, indicating that cysteine depletion drives the observed reduction in tumor growth. Our findings indicate a non-canonical role for GSH in supporting tumors by acting as a reservoir of amino acids. Depriving tumors of extracellular GSH or inhibiting its breakdown represents a potentially tractable therapeutic approach for cancer patients, as it could restrict tumors' access to critical amino acids. These findings offer a mechanistic explanation for the pro-tumorigenic role of GSH and reshape our understanding of how amino acids, including cysteine, are supplied to cancer cells, unveiling a novel therapeutic avenue for targeting cancer metabolism.

#4156 Differential effects of tumor metabolites on T cell activation and effector function.

D. Hua, S. Budhu, A. Assouvie, S. Verma, M. Bah, J. D. Wolchok, T. Merghoub;
Weill Cornell Medicine/Sandra and Edward Meyer Cancer Center, New York, NY

Over the past decade, immunotherapies have significantly improved clinical outcomes in cancer patients. The FDA approval of ICB for cancer treatment represents a clinical milestone, however, durable responses are observed in only ~20-40% of patients. The immunosuppressive nature of the tumor microenvironment (TME) presents a major challenge for effective anti-tumor responses. Tumor cells are highly metabolically active compared to other cells in the TME. Processes such as glycolysis and oxygen consumption (OXPHOS) lead to tumor hypoxia and the accumulation of metabolites in the TME. Metabolites found in the TME play key roles in immunosuppressive pathways including the adenosine-A2A receptor (A2AR) signaling pathway, and the Tryptophan-kynurenine-aryl hydrocarbon receptor (AHR) axis. Here we investigated how these metabolic pathways directly affect activation and effector function of T cells. In addition, we examined whether these pathways overlap functionally. To explore how TME metabolites affect T cell function, naïve mouse T cells were activated in the presence of metabolites including lactic acid (Lac), adenosine (Ado), kynurenine (Kyn). After 72 hours, T cells were analyzed via flow cytometry for proliferation and activation. We found that lactic acid decreased T cell frequencies and proliferation, but increased expression of CD39 and CD73, key enzymes involved in the conversion of ATP to Ado. Intriguingly, the combination of Kyn and Ado uniquely promoted T cell activation, showing markedly elevated expression of classical activation and effector markers such as CD44, CD25, and Granzyme B along with reduced CD39 and CD73 levels. Similar findings were observed in a clonogenic killing assay using tumor-specific T cells co-cultured with metabolites corroborating the functional relevance of our observations. Lactate negatively affected T cell killing function and the combination of Kyn and Ado showed the largest increase in killing efficiency. These findings suggest potential synergy between Kyn and Ado pathways in enhancing T cell function within the TME possibly through complementary pathways that bypass or counteract typical immunosuppressive signals. Further mechanistic investigation could unlock new strategies to overcome TME-induced immunosuppression and enhance existing immune based therapies.

#4157 Prodrug development of Cyclo-Creatine: Enhancing permeability and reducing toxicity for HER2+ breast cancer treatment.

Y. Li, Y. Huang, P. Dey, S. Hitosugi, S. Buhrow, J. Reid, T. Hitosugi;
Mayo Clinic College of Medicine and Science, Rochester, MN

HER2+ breast cancer is an aggressive subtype of cancer that often develops resistance to the current first-line therapy, Trastuzumab. In our previous work, we demonstrated that HER2 signaling activates mitochondrial creatine kinase 1 (MtCK1), which enhances mitochondrial energy metabolism for rapid proliferation of breast cancer cells. However, pharmacological blockage of MtCK1 with a creatine analog, Cyclo-Creatine (CCr), requires millimolar concentration to inhibit breast cancer cell proliferation and is associated with brain toxicity, limiting its clinical application. This brain toxicity may result from the inhibition of the creatine transporter SLC6A8 by CCr, which decreases to transport creatine into brain.

In this study, we took a prodrug development approach to identify and optimize protection groups for CCr, designed to bypass the SLC6A8 transporter, enhancing bioavailability and reducing toxicity. Liquid chromatography-mass spectrometry (LC-MS) was utilized to analyze drug uptake and creatine metabolites in cells treated with the prodrugs. Prodrug C, which is our lead prodrug candidate, demonstrated a seven-fold increase in cellular uptake and more than a hundred-fold improvement in cell-killing effects as compared to CCr in an MtCK1 specific manner in breast cancer cells. This approach offers a promising therapeutic strategy to inhibit HER2+ breast cancer cell by disrupting mitochondrial energy metabolism. Future investigations will focus on validating this prodrug's pharmacokinetics (PK) and pharmacodynamics (PD) through further preclinical studies.

#4158 PIKfyve inhibition drives lipogenic adaptation and reveals metabolic vulnerabilities in PDAC.

Ruya Pakkan[1], Caleb Cheng[1], Jasmine Wisniewski[1], Sydney Peters[1], Pietro Morlacchi[2], Li Zhang[1], Yuanyuan Qiao[1], Costas A. Lyssiotis[1], Arul M. Chinnaiyan[1]

[1]University of Michigan, Ann Arbor, MI,[2]Agilent technologies, Inc, Lexington, MA

PIKfyve plays a key role in autophagy and lysosomal functions, which are critical for pancreatic ductal adenocarcinoma (PDAC) cell metabolism and survival. Consistent with this, we found that PIKfyve perturbation suppresses autophagy and reduces PDAC cell viability. Prior studies show that autophagy and lysosomal processes are critical for maintaining iron homeostasis and mitochondrial respiration in PDAC. However, PIKfyve inhibition did not seem to significantly affect oxygen consumption rate (OCR) compared to other lysosomal inhibitors such as chloroquine and bafilomycin, suggesting that PIKfyve also regulates other metabolic demands in addition to iron homeostasis. To understand the role of PIKfyve in PDAC and detect genes essential for metabolic compensation to PIKfyve loss, we performed a CRISPR screen using the PIKfyve inhibitor apilimod as a selective pressure. This revealed that the most depleted sgRNAs targeted genes involved in de novo fatty acid synthesis, insinuating that de novo fatty acid synthesis acts as a functional adaption to loss of PIKfyve function. Consistent with these results, cells upon disruption of fatty acid synthesis were sensitized to PIKfyve inhibitors, confirming that PIKfyve perturbation makes cells more reliant on fatty acid synthesis for survival. Moreover, PIKfyve inhibition activated key lipogenic transcriptional factor SREBP1 and upregulated fatty acid synthesis genes such as FASN and ACACA. We further discovered that PIKfyve inhibition in PDAC cells changed cellular metabolism, with initial decreases in citrate levels and subsequent recovery, along with a decrease in upstream glycolytic metabolites. Targeted lipidomics and isotope tracing showed that PIKfyve inhibition rerouted glucose-derived carbon into de novo lipid synthesis, specifically sphingolipids, dependent on SREBP1 activity. An additional CRISPR screen using a more potent degree of PIKfyve inhibition further identified sphingolipid synthesis genes (SPTLC1, SPTLC2, and KDSR) as essential for cell survival under PIKfyve inhibition. These results suggest that PIKfyve inhibition drives an adaptive increase in lipid synthesis, specifically sphingolipid synthesis through the activation of SREBP1 as a survival mechanism for PDAC cells. These results show a metabolic vulnerability in PDAC, suggesting that PIKfyve inhibitors, in combination with the inhibition of fatty acid synthesis and specifically sphingolipid synthesis, represent promising therapeutic strategies.

#4159 Investigating the role of Akt isoforms in hepatocellular carcinoma and liver fibrosis.

Manahil Ghazi, Ahmed Abd El Razek, Nissim Hay

University of Illinois at Chicago, Chicago, IL

Liver fibrosis and hepatocellular carcinoma (HCC) are major global health issues, with HCC being the most common primary liver cancer and a leading cause of cancer-related death. Metabolic dysfunction-associated steatohepatitis (MASH) is an emerging risk factor for HCC. Theserine/threonine protein kinase Akt plays a central role in cell survival, growth, and metabolism, and its hyperactivation via the PI3K/Akt pathway is implicated in many cancers. Mammalian cells express three Akt isoforms, but the effects of isoform-specific Akt inhibition remain poorly understood. We previously showed that global deletion of Akt2 along with hepatic deletion of Akt1 surprisingly leads to early onset of spontaneous HCC. The current study investigates the mechanisms underlying spontaneous HCC in these mouse models and examines the roles of Akt1 and Akt2 in MASH-induced liver fibrosis and HCC. Two mouse strains were generated: $Akt2^{-/-}$; $Akt1hep^{-/-}$ and $Akt1^{-/-}$; $Akt2hep^{-/-}$. $Akt2^{-/-}$ mice survived hepatic Akt1 deletion, but all developed early onset HCC with elevated insulin and glucose levels, increased lipid droplet accumulation, and CD36 expression. In contrast, $Akt1^{-/-}$ mice survived hepatic Akt2 deletion without tumor development and had normal glucose levels. These findings suggest that HCC development in $Akt2^{-/-}$; $Akt1hep^{-/-}$ mice occurs despite the loss of Akt activity, likely due to compensatory mechanisms or alternative pathways in hepatocarcinogenesis. Given the high insulin and glucose levels in $Akt2^{-/-}$; $Akt1hep^{-/-}$ mice, we hypothesize that reducing insulin levels may prevent HCC. Additionally, we are examining whether CD36 is essential for HCC development in these mice. The second part of this study explores the impact of Akt isoforms on MASH-induced liver fibrosis and HCC. We have generated five mouse groups ($Akt1^{-/-}$, $Akt2^{-/-}$, $Akt1hep^{-/-}$, $Akt2hep^{-/-}$, and$Akt1hep^{-/-}$; $Akt2hep^{-/-}$), and these are fed with Western diet and subject to weekly CCl4 injections for MASH induced HCC. This model will help to understand the role of Akt1 or Akt2 upon hepatic versus systemic deletion and its effect on liver fibrosis and HCC progression in the MASH-induced model. Together, these studies will provide insights into the isoform-specific roles of Akt in liver carcinogenesis and fibrosis, with implications for targeted therapies in HCC.

#4160 Lipid metabolism is a metabolic vulnerability in LKB1 mutant, KRAS driven non-small cell lung cancer.

S. Flanagan[1], R. Stanley[2], B. Malacrida[3], D. O'Reilly[2], J. Naidoo[4], P. Murray[1], C. M. Dowling[2];
[1]University of Limerick, Limerick, Ireland, [2]Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland, [3]Barts Cancer Institute, Queen Mary University of London, London, Ireland, [4]Beaumont Hospital, Dublin, Ireland

Non-small cell lung cancer (NSCLC) accounts for 80-85% of all lung cancers worldwide, with KRAS-driven NSCLC representing 30% of these cases. The introduction of immunotherapy has improved outcomes in advanced lung cancer, however, many patients with lung cancer do not respond to this treatment, and some develop severe side effects. Patients with KRAS-driven NSCLC harbouring a co-mutation in the LKB1 gene (KL), have a significantly poorer response to immunotherapy compared to patients harbouring a KRAS and TP53 co-mutation (KP). However, the reason for this difference in response to immunotherapy is largely unknown. Recent studies demonstrate that metabolic vulnerabilities in KL genotypes can influence therapeutic response. Our research aims to identify and target these vulnerabilities in KL NSCLC, with the aim to enhance response to immunotherapy. Moreover, we will focus on creating a novel three-dimensional (3D) NSCLC co-culture model to study immunotherapy response. Using the cBioPortal mRNA database and publicly available patient protein data, we completed a bioinformatic analysis of key genes, proteins, and metabolites in major metabolic pathways. Notably, lipid metabolism pathways displayed significant differences between KP and KL genotypes, specifically the beta-oxidation of fatty acids and fatty acid synthesis pathways. Proteins involved in the beta-oxidation pathway showed significant upregulation in KL patients such as Acetyl-CoA Acetyltransferase 1, Acyl-CoA Dehydrogenase, Hydroxyacyl-CoA Dehydrogenase and Enoyl-CoA Hydratase, and proteins involved in fatty acid synthesis also displayed upregulation in KL patients. We conducted a drug screen using a series of inhibitors targeting proteins in the fatty acid synthesis and beta oxidation pathway, and observed strong efficacy in KL NSCLC cell lines. Moreover, we observed a reduction in cell migration, colony formation and 3D spheroid formation in response to fatty acid synthesis inhibitors in KL NSCLC. Separately, we have successfully established 3D co-culture models of NSCLC cell lines and human T-cells isolated from patient samples. We found the optimum NSCLC cell number to be 50,000 cells per gel. Using immunohistochemistry, we also observed positive Ki67 staining confirming cell proliferation within the cultures and low cleaved-caspase 3 staining indicating minimal cell death. In conclusion, we have identified fatty acid synthesis as a potential metabolic vulnerability in KL NSCLC, and targeting this pathway could be a potential therapeutic option for patients with this genotype of NSCLC. Further analysis, supported by our novel 3D co-culture model, aims to analyse the consequences of targeting this specific pathway in combination with immunotherapy in the KL genotype of NSCLC.

#4161 Hiding in plain sight: Understanding the role of tryptophan metabolism in hepatocellular carcinoma.

Shivani Krishnamurthy[1], Bavani Gunasegaran[1], Sharron Chow[1], Vincent Lam[1], Ken Liu[2], Fiona Guan[3], Joo-Shik Shin[4], Avik Majumdar[5], Geoffrey McCaughan[6], Seong Beom Ahn[1], Benjamin Heng[1]

[1]Macquarie Medical School, Faculty of Medicine, Health and Human Sciences, Macquarie University, Sydney, Australia,[2]AW Morrow Gastroenterology and Liver Centre, Royal Prince Alfred Hospital and Central Clinical School, Faculty of Medicine and Health, The University of Sydney, Sydney, Australia,[3]AW Morrow Gastroenterology and Liver Centre, Royal Prince Alfred Hospital, Sydney, Australia,[4]Central Clinical School, Faculty of Medicine and Health, The University of Sydney and Tissue Pathology and Diagnostic Oncology, Royal Prince Alfred Hospital, Sydney, Australia,[5]AW Morrow Gastroenterology and Liver Centre, Royal Prince Alfred Hospital, Central Clinical School, Faculty of Medicine and Health, The University of Sydney, Sydney, Australia,[6]AW Morrow Gastroenterology and Liver Centre, Royal Prince Alfred Hospital, Central Clinical School, Faculty of Medicine and Health, The University of Sydney and Centenary Institute, Sydney, Australia

Introduction: Tumour immune suppression has been identified as an emerging hallmark of cancer that facilitates cancer progression, including hepatocellular carcinoma (HCC). Studies suggest that cancer cells can remain undetected and proliferate by evading local immune surveillance. A major pathway in tryptophan metabolism, the kynurenine pathway (KP), has been suggested to be one of the key mechanisms mediating tumoral immune evasion. In a pro-inflammatory tumour microenvironment, the KP is highly activated which can suppress activation of tumour-targeting immune cells and enhance cancer cell growth. To elucidate the mechanisms underlying KP activation and its contribution to tumour progression in HCC, this study aims to characterize the KP profile and tumour secretome, including cytokines and proteins in HCC patients, to examine the relationship between changes in plasma KP activity and the inflammation-associated secretome.
Methods: We have acquired plasma samples collected from patients with HCC (n=30), cirrhosis only (n=30), and healthy control (n=30). The KP metabolomic analysis was carried out using high pressure liquid chromatography (HPLC), Ultra-HPLC and Gas-chromatography Mass-spectrometry. The cytokine and proteomics analysis was performed using flow cytometry and data-independent acquisition mass-spectrometry respectively.
Results/Discussion: Our findings demonstrate that the KP is highly activated in HCC, as inferred by the elevated enzymatic activities of indoleamine-2,3-dioxygenase/tryptophan-2,3-dioxygenase and kynurenine 3-monooxygenase in HCC patients. Activation of these KP enzymes implies that HCC patients have a suppressed immune profile to facilitate the progression of cancer. Cytokine profiling revealed a more inflamed systemic profile in HCC patients compared to controls and cirrhotic patients. Given that the KP is induced by inflammation, this provides a potential explanation for the elevated KP activity measured in HCC patients. Proteomics analysis further identified differentially expressed proteins linked to inflammation and immune system activation, highlighting that the immune system plays a major role in HCC pathophysiology, potentially driving inflammation and the observed KP profile in HCC patients.
Conclusion: Collectively, these results suggest that KP activation may potentially be one of the mechanisms used by HCC tumour to create an immune-tolerant environment, facilitating tumour progression and development.

#4162 Development of low-toxic targeted therapies for KRAS-mutant tumors: Harnessing synergistic effects of fasting-mimicking diet (FMD) and ascorbate.

Anastasiya Shmahala[1], Valter D. Longo[2]

[1]IFOM ETS - The AIRC Institute of Molecular Oncology, Milan, Italy,[2]Longevity Institute and Davis School of Gerontology, University of Southern California, Los Angeles, CA

Pharmacological doses of vitamin C (ascorbate) have been proposed as a potential anticancer therapy, according to several preclinical and clinical studies. However, the exact mechanisms of its function in cancer cells remains controversial. Our laboratory demonstrated that fasting and fasting-mimicking diet (FMD) delay tumor progression and sensitize a wide range of tumors to various types of treatment, such as chemotherapy, immunotherapy and tyrosine kinase inhibitors (TKIs). In addition, FMD can enhance the therapeutic effects of pharmacological doses of vitamin C across different cancer cell lines bearing mutations in KRAS oncogene. Namely, combination of pharmacological doses of ascorbate and FMD in preclinical models has displayed a significant anticancer activity on KRAS-positive colorectal carcinomas, delaying tumor progression, but not achieving cancer free survival because of cellular resistance mechanisms. According to our latest findings FMD has been shown to activate specific starvation-induced escape pathways (SEPs) in triple-negative breast cancer (TNBC) cells that can be identified and targeted by specific targeted treatments. For this reason, this study not only investigates the synergistic effects of short-term starvation (STS) and high-dose ascorbate on cancer cell viability, but aims as well at disentangling the resistance mechanisms of the cells not responsive to the proposed treatment. To unravel these resistance mechanisms, we performed bulk RNA-sequencing analysis on vitamin C and STS/FMD resistant cancer cells. Results unveiled a significant upregulation of different signaling pathways in pancreatic, colorectal and non-small cell lung cancer cell lines non responding to vitamin C and STS. Namely, targeting these pathways by specific inhibitors reactivated sensitivity to ascorbate and STS causing cell death in the resistant cell population. In this study we also highlighted the mechanism through which STS and ascorbate, if combined with inhibitors, induces cancer cell death. Reactive oxygen species (ROS) mediated the cytotoxicity of the combinatorial treatment, as antioxidants were able to reverse the cell death. Next, we also assessed the impact of FMD and ascorbate treatment on the tumor microenvironment: we observed a reshaping of collagen deposition and a normalization of vasculature inside the tumor stroma, which could improve drug delivery and immune cell infiltration. To conclude, this project provides evidence that FMD and ascorbate synergize to cause toxicity in different cancer cell lines. Furthermore, we identified a common escape mechanism toward the abovementioned treatment. All these data together suggest so far that a combinatorial low-toxicity treatment can be developed, thus possibly improving clinical outcomes for cancer patients.

#4164 Targeting glycogen metabolism as a potential therapy for Ewing sarcoma.

Yueying Liu[1], Pankaj K. Singh[1], Lyndsay E. A. Young[2], Harrison A. Clarke[1], Tara R. Hawkinson[1], Meagan L. McCall[3], Elias Sayour[1], Craig W. Vander Kooi[1], Li Chen[3], Ramon C. Sun[1], Matthew S. Gentry[1]

[1]University of Florida College of Medicine, Gainesville, FL,[2]Medical University of South Carolina, Charleston, SC,[3]University of Florida, Gainesville, FL

Ewing sarcoma (EWS) is a malignant bone tumor or soft-tissue tumor that mainly affects children, adolescents, and young adults. We recently demonstrated that the PAS+ accumulations in EWS indicate extensive deposits of glycogen, a macro-metabolite that provides an immediate source of glucose to support cellular energy needs. In this study, we sought to elucidate the role of glycogen and its related enzymes in EWS tumorigenesis and metastasis. Though complex carbohydrates like glycogen are traditionally difficult to study due to their biochemical and biophysical properties, the Gentry and Sun laboratories recently developed a robust workflow of matrix-assisted laser desorption/ionization (MALDI)-imaging of complex carbohydrates (MICC), a spatial technique to visualize glycogen *in situ*. Leveraging this technique, we profiled glycogen accumulation in a cohort of EWS patient tumors. This analysis revealed glycogen levels were dramatically higher in the intra-tumoral regions across all EWS samples. Additionally, through our comprehensive pan-cancer study encompassing 12 different cancer classes, we revealed that glycogen accumulation varies among tumor origins, signifying its unique role in EWS.

Lysosomal glycogen degradation is catalyzed by acid α-glucosidase (GAA). Preliminary data from our laboratory demonstrates that glycogen colocalizes with GAA in EWS patient tumors and that small-molecule inhibition of GAA significantly impairs EWS cell proliferation and migration. Transcriptomic analyses revealed a strong correlation between GAA expression and known EWS metastasis regulators, including TGF-β and Wnt/β-catenin signaling pathways. Further experiments revealed that while glycogen synthase 1 inhibitor reduced glycogen levels and impaired EWS cell viability, a small molecule promoting lysosomal glycogen degradation reduced glycogen but paradoxically enhanced cell viability. These findings highlight the critical role of lysosomal glycogen utilization in EWS cell viability, suggesting a potential link between GAA-regulated glycogen degradation, energy homeostasis, and apoptotic pathways in EWS.

Moving forward, we are defining the efficacy and mechanism of GAA*i* in the treatment of EWS preclinical models both as a single agent and in additive therapy with other chemotherapies. To assess the efficacy of GAA*i* in metastatic EWS, spontaneous lung metastatic mice models will be established by subcutaneous implantation of EWS patient-derived tumor in NSG mice. Our state-of-the art integrated spatial analysis of metabolome, lipidome, and glycome followed by high dimensionality reduction and spatial clustering, annotation, pathway enrichment analysis will provide in-depth spatial insights into tumor and stromal metabolism profile of EWS. Future potential of GAA*i* study is to conduct clinical trial to identify when and how to add GAA*i* to current standard care EWS therapy and improve survival outcomes.

#4166 The RNA demethylase FTO promotes glutamine metabolism in clear cell renal cell carcinoma through the regulation of SLC1A5.

M. Zhao[1], D. Zhang[1], H. Jiang[1], N. Kuganesan[1], S. Natarajan[1], L. Pu[1], K. N. Thakkar[1], H. Zhao[1], J. D. Brooks[1], D. M. Peehl[2], J. Ye[1], E. B. Rankin[1];

[1]Stanford University, Stanford, CA, [2]University of California, San Francisco, CA

Glutamine reprogramming is a hallmark feature of clear cell renal cell carcinoma (ccRCC). However, the regulation of glutamine reprogramming in ccRCC is incompletely understood. Here we demonstrate that the RNA demethylase enzyme, FTO, promotes glutamine reprogramming in ccRCC cells to support growth and survival. Genetic and pharmacologic inhibition of FTO reduced glutamine-derived reductive carboxylation, de novo pyrimidine synthesis and glutathione biosynthesis. FTO inhibition resulted in increased DNA damage and reduced growth and survival that were rescued by the administration of pyrimidines and the antioxidant N-acetylcysteine. Mechanistically, FTO demethylates the glutamine transporter SLC1A5 mRNA transcript to promote SLC1A5 expression and glutamine reprogramming in ccRCC cells. In FTO knockdown cells, restoration of SLC1A5 expression was sufficient to rescue glutamine-derived reductive carboxylation, glutathione levels, DNA damage as well as growth and survival. Further, we observed that the FTO inhibitor FB23-2 enhanced the efficacy of the PARP inhibitor talazoparib *in vitro* and *in vivo*. Our study reveals a novel role for FTO in glutamine reprogramming of ccRCC, highlighting the therapeutic potential of targeting the epitranscriptomic regulation of metabolic reprogramming in ccRCC.

#4167 CK2 inhibition restores IKAROS-mediated metabolic control and induces cell death in acute lymphoblastic leukemia.

Diwakar Bastihalli Tukaramrao[1], Arati Sharma[2], Dhimant Desai[2], Sinisa Dovat[1]

[1]Pediatrics, Penn State College of Medicine, Hershey, PA,[2]Pharmacology, Penn State College of Medicine, Hershey, PA

The transcription factor IKAROS functions as a critical metabolic gatekeeper in B cells by regulating glucose metabolism and the pentose phosphate pathway (PPP). In lymphoid leukemias, casein kinase 2α (CK2α) overexpression impairs IKAROS function, leading to dysregulated cellular metabolism. This study investigated the metabolic consequences of CK2α inhibition in B- and T-cell acute lymphoblastic leukemia (ALL). Treatment of ALL cell lines with the CK2 inhibitor CX4945 resulted in significant cell death, with IC50 values ranging from 4-10 μM. In NALM6 cells, CK2α inhibition led to increased reactive oxygen species (ROS), decreased NADPH/NADP+ ratio, and reduced glucose consumption by 40%. Additionally, CX4945 treatment significantly decreased both glycolytic activity and mitochondrial respiration in ALL cell lines (NALM6, 697, MOLT4 and CEM). Metabolomic analysis revealed 64 differentially expressed metabolites between CX4945-treated and vehicle-treated NALM6 cells, with notable reductions in glycolytic intermediates. These findings demonstrate that CK2α inhibition effectively restores IKAROS-mediated metabolic control, leading to energy deficiency and increased oxidative stress in ALL cells. This work provides evidence that targeting the CK2-IKAROS axis represents a promising therapeutic strategy for ALL through metabolic modulation.

#4168 NMN supplementation enhances proliferation and aggressiveness in UV-induced skin cancer.

Niti Kumari, Brian J. North

Creighton University School of Medicine, Omaha, NE

Nicotinamide adenine dinucleotide (NAD^+) is an important redox cofactor and substrate for key enzymes and is involved in several cellular processes such as, energy metabolism, cell signaling, and DNA repair. Disturbance in NAD^+ homeostasis is associated with multiple human diseases. NAD^+ boosting agents, such as, nicotinamide mononucleotide (NMN) is under investigation in clinical trials and is a subject of active research in various pathological conditions associated with declining NAD^+ levels including, Alzheimer's disease, diabetes, heart failure and overall health in aging. NMN administration in mice improved age-associated changes in body weight, energy metabolism, insulin resistance, mitochondrial respiration, and reversed gene expression changes. In human trials, NMN improved insulin sensitivity in pre-diabetic women, aerobic capacity in amateur runners and physical performance in older people. Although many diseases are negatively impacted due to a decline in NAD^+ levels, cancer cells are known to upregulate NAD^+ biosynthesis to support their growth and progression. For example, NAMPT, a rate limiting enzyme of NAD^+ salvage pathway, is frequently upregulated in cancer and is associated with cancer progression and stemness. As safety of prolonged NMN supplementation in humans are being evaluated, it is equally important to explore its potential impact, adverse or beneficial, in relation to cancer pre-disposition conditions. To evaluate the impact of NMN in skin tumorigenesis, SKH-1 mice (~11-17 weeks old) were supplemented with NMN in drinking water (vehicle) at 300mg/Kg/day. Mice were either sham treated or treated with UV at 18Kj/m^2 five times a week for spontaneous development of cutaneous squamous cell carcinoma (cSCC). Number of tumors per mice (tumor multiplicity) was evaluated once a week starting at week 23 post UV irradiation. Tumors from both groups, vehicle UV and NMN UV, were harvested on week 37 and were subjected to exome sequencing, bulk RNA-sequencing, and Ki67 immunofluorescence for assessment of differences in mutation burden, gene expression and proliferation, respectively. Effect of NMN and nicotinamide (NAM) on cell proliferation and cell migration of cSCC cells, Colo-16, and SRB12, was assessed *in vitro* via MTT assay and scratch assay, respectively. NMN supplemented group exhibited increase in tumor multiplicity upon UV treatment in male and female SKH-1 mice. NMN supplemented tumors had relatively high mutation burden and Ki67 expression. Additionally, increase in extracellular matrix organization, epithelial mesenchymal transition, and angiogenic gene signatures were also elevated in NMN UV group. Colo-16 and SRB12 exhibited increased proliferation and migration in the presence of NMN or NAM. Overall, this study indicates that continuous NMN supplementation in cancer pre-disposition conditions might enhance development and progression of skin cancer.

#4169 Coordination of malic enzyme 2 and the malate-aspartate shuttle supports growth and survival in triple-negative breast cancer.

Mark Daniel Slayton[1], Jin Heon Jeon[1], Zackariah Farah[1], Abhinav Achreja[1], Ben Krinkel[2], Yi-Hsien Eu[1], Alyssa Rosenfeld[1], Kerslee Kohagen[1], Liwei Bao[1], Xu Cheng[1], Kerry Loomes[2], Deepak Nagrath[1], Sofia Merajver[1]

[1]University of Michigan, Ann Arbor, MI,[2]University of Auckland, Auckland, New Zealand

Background & Hypothesis: Triple-negative breast cancer (TNBC) presents therapeutic challenges due to the lack of hormone-targeted treatments. Malic enzyme 2 (ME2), crucial for pyruvate and NADPH production, regulates cancer metabolism and redox balance. Elevated ME2 expression correlates with poor outcomes in multiple cancers. We hypothesized that ME2 knockdown (ME2kd) would impair TNBC growth by disrupting metabolic homeostasis, with the malate-aspartate shuttle (MAS) playing a complementary role.
Methods: TNBC cell lines (MDA-MB-468, HCC70, HCC1806, Hs578T, BT20) and a control line (MCF10a) were analyzed under ME2kd using siRNA and shRNA. Growth, migration, and metabolic flux were assessed in vitro. Metabolite profiling was performed using GC-MS and Metabolite-Glo assays. Xenograft models of MDA-MB-468 and HCC70 evaluated tumor growth in vivo. ME2kd effects on mitochondrial function were measured via Seahorse XF96 assays. Growth under serine, glycine, and glucose deprivation was also assessed. The role of MAS in supporting ME2kd cells was evaluated with double knockdown experiments.
Results: ME2kd reduced proliferation by over 50% in MDA-MB-468 and HCC1806 cells with minimal effects on MCF10a. Tumors derived from MDA-MB-468 ME2kd cells grew significantly slower, with extended survival in vivo. In contrast, ME2kd had no impact on HCC70 growth or migration. Metabolite profiling revealed elevated malate and reduced NADH/NADPH levels under ME2kd, with compensatory upregulation of serine biosynthesis genes. Deprivation of serine, glycine, or glucose with or without ME2kd impaired growth in 4 of 5 TNBC lines, with minimal effects on MCF10a. Combined ME2 and MAS knockdown further suppressed growth across TNBC lines, emphasizing MAS's role in maintaining cytosolic NAD+/NADH balance under ME2kd.
Conclusions: ME2 is essential for supporting the metabolic and proliferative demands in TNBC. Loss of ME2 disrupts key metabolic pathways, leading to significant reductions in cellular proliferation and migratory capacity. The observed increase in malate levels and altered NADH/NADPH ratios under ME2kd highlight its central role in maintaining metabolic homeostasis. Our data shows that ME2, de novo serine synthesis, and glycolysis are closely co-regulated in TNBC cell lines. The ability of the cell lines to compensate for ME2kd correlated with their inherent de novo serine synthesis capacity. Furthermore, the interdependence of MAS and ME2 suggests that MAS may act as a bridge between ME2 and de novo serine synthesis via regulation of cytosolic NAD+/NADH ratio. These findings confirm that ME2 is involved in TNBC metabolism and establish it as a compelling target for therapeutic intervention. Future studies could explore synergistic strategies combining ME2 inhibition with interventions targeting complementary metabolic pathways.

#4170 Glucose transporter 2-transported glucosamine inhibits glycolysis in cancer cells through competition with glucose for hexokinase 2.

Se Yong Park[1], Ki-Hoon Song[2], Ju-Hee Kang[1], Jung Ho Hwang[3], Seung Hyun Oh[1]

[1]Seoul National University, Seoul, Korea, Republic of,[2]ViroCure, Seoul, Korea, Republic of,[3]Gachon University, Incheon, Korea, Republic of

Anticancer effects of glucosamine, a glucose derivative with a similar structure to glucose, have been discovered, but the molecular mechanisms are not yet fully understood. Since glucosamine and glucose not only have similar structures but also are catalyzed by the same enzyme, hexokinase (HK), in this study, we delved into determining whether the anticancer effect of glucosamine involves the inhibition of glycolysis by competition with glucose. Whole-genome screening analysis showed that many of the gene pathways controlled by glucosamine were directly and indirectly involved in glycolysis. *In vitro* experiments revealed that as more glucose was added, the anticancer effect of glucosamine decreased. Also, we found that glucosamine was transported into cells mainly through glucose transporter (GLUT) 2 which was responsible for the anticancer effects of glucosamine. In addition, we found that cancer cells with low expression level of HKII show high sensitivity to glucosamine, and a HK inhibitor, 3-bromopyruvate, enhanced the anticancer effect of glucosamine. Under hypoxic conditions, activated hypoxia-inducible factor 1α (HIF-1α) inducing glucose uptake and glycolysis hampered glucosamine-induced cell death, and *HIF1A* knockdown or HK inhibitors restored glucosamine's anticancer effects. These findings demonstrate that glucosamine is an efficient glycolysis inhibitor and that GLUT2 and HKII play important roles as biomarkers for determining sensitivity to glucosamine. Moreover, our results suggest that the anticancer effect of glucosamine may be more efficient when administered in combination with other glycolytic agents or inhibitors targeting HIF-1α.

Keywords: glucosamine, cancer, glycolysis, GLUT2, HKII, HIF-1α

#4171 ADRM1 inhibitor, Up284 induces replication stress and suppresses cell proliferation in triple-negative breast cancer.

Iffat Jahan[1], Shacoya Mack[2], Ravi Anchoori[3], Balasubramanyam Karanam[2], Komaraiah Palle[1]

[1]Texas Tech University Health Sciences Center, Lubbock, TX,[2]Tuskegee University, Tuskegee, AL,[3]Up Therapeutics LLC, Frederick, MD, MD

Triple-negative breast cancer stands as the most aggressive and deadliest subtype of breast cancer associated with poor prognosis and limited treatment options due to the lack of important receptors; estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) used for targeted therapy. Relying exclusively on chemotherapy enhances the chances of resistance and recurrence and leaves TNBC challenging to treat. Thus, it mandates the need for novel drug compounds and combination therapies. Adhesion-regulating molecule 1 (ADRM1/RPN13), a ubiquitin receptor of 26S proteasome that supports recognition of polyubiquitinated substrates and their subsequent deubiquitination and degradation by unique deubiquitinase UCH37/UCHL5.Its inhibition causes a rapid and toxic accumulation of its substrate polyubiquitinated proteins in very high molecular weight (HMW) aggregate; disrupting the protein homeostasis. In response to that, the cancer cells halt the cell cycle and induce replication stress. Consequently, cells signal the survival mechanism by signaling the DNA damage repair (DDR) pathway activation. Using the Cancer Gene Genome Atlas and GEO datasets, our primary analysis demonstrated that ADRM1 is overexpressed in all types of breast cancer including TNBC. Higher expression in tumor cells compared to normal cells strengthens the stands for ADRM1 as a potent target for therapy. In vitro analysis has been performed in TNBC cell lines MDAMB 453 and MDAMB 231. The dose dependency assay demonstrated that the UP284 compound showed activation of CHK1 (Checkpoint kinase1) mediated DNA damage response and γH2AX foci formation confirms the double-stranded DNA breakage. Clonogenic assay confirms the suppression of cell proliferation and colony formation in a concentration-dependent manner in both BRCA positive and BRCA mutant cell lines. Up284 treated cells also showed cell cycle arrest at the G2/M phase. Together, our results indicate that ADRM1 is a promising target for TNBC therapy and suggest that this ADRM1 inhibitor, Up284, has the potential for clinical application in the treatment of TNBC. Our analysis will unravel the novel mechanistic insight into the therapeutic potential of this compound and provide preclinical evidence to develop further combination therapy.

#4172 Carnitine metabolism regulates ovarian cancer metastasis.

Medine Tasdemir[1], Shree Bose[1], Jason Locasale[2], Ernst Lengyel[1], Abir Mukherjee[1]

[1]University of Chicago, Chicago, IL,[2]North Carolina State University, Durham, NC

Ovarian cancer (OvCa), the most lethal gynecological malignancy, is often detected late when the cancer cells have metastasized throughout the peritoneal cavity. OvCa cells are readily shed into the abdominal cavity from the ovary or the fallopian tube and are disseminated throughout the peritoneal cavity via the peritoneal or ascetic fluid. Therefore, ascites function as a liquid tumor microenvironment intermediary between primary tumor and metastasis. It is unclear if the ascites is a mere vehicle for disseminating cancer cells or if it alters the metabolic machinery of OvCa cells to increase their metastatic capacity. Here, we performed systematic multi-site metabolomic analyses to characterize the metabolic milieu of ascites and identified the mechanisms that augment the metastatic capacity of OvCa cells. We analyzed patient-matched samples (comprised of primary, metastatic omental tumors, ascites, and plasma) collected from a cohort of 25 high-grade serous ovarian cancer patients using liquid chromatography-mass spectrometry. By performing multidimensional scaling, we show that each sample site has a characteristic metabolic profile. The metabolome of the ascites is unique, and a higher abundance of acylcarnitines distinguishes its metabolome from patient-matched tumor and plasma. Conjugation of fatty acids to carnitine (generating acylcarnitines) aids in transfer of fatty acids across the mitochondrial membrane for their oxidation and subsequent ATP generation. We found that ascites-driven growth of OvCa cells correlates with methionine uptake and accumulation of N^6-trimethyl-L-lysine (a key metabolite in the biosynthesis of carnitine). We also observed increased levels of acylcarnitines in metastatic tumors from the omentum compared to primary ovarian/fallopian tube tumors. Therefore, increased carnitine synthesis is a metabolic phenotype of OvCa cells growing in ascites and is associated with progression through the metastatic cascade. Gamma-butyrobetaine hydroxylase 1 and 2 (BBOX1 and BBOX2), are enzymes that regulate the rate-limiting steps of carnitine synthesis. Stable knockdown of BBOX1 and BBOX2 reduced the growth rate, and migratory and invasive capacities of OvCa cells in vitro. Colonization of the omentum (a visceral adipose tissue covering the bowel) marks an essential step in OvCa progression. We and others have shown that heightened fatty acid oxidation capacity in cancer cells aids in omental metastasis. We now show that increased carnitine biosynthesis in the OvCa cells precedes omental colonization and is a potential metabolic vulnerability.

#4173 Compensatory interactions between tryptophan catabolism and serine metabolism highlight potential vulnerabilities in TNBC cells with dysregulated *de novo* serine synthesis.

Jin Heon Jeon, Abhinav Achreja, Mark Slayton, Olamide Animasahun, Minal Nenwani, Fulei Wuchu, Sofia D. Merajver, Deepak Nagrath

University of Michigan, Ann Arbor, MI

Background: Despite the major advances people have achieved in treating breast cancer, triple-negative breast cancer (TNBC) remains a difficult cancer to conquer. TNBC is one of the most heterogeneous and aggressive forms of breast cancer and is characterized by the loss of ER, PR, and HER expression. Due to the lack of these receptors, TNBCs are especially hard to treat with traditional methods that target these receptors, necessitating a more targeted approach to tackling these cancers. Serine and tryptophan are both metabolites that play crucial roles in the production and balance of nucleotides, energy and redox. These overlaps in metabolic functions make it likely that these two metabolites play compensatory roles under certain conditions. Different TNBCs have been shown to have vastly varying levels of de novo serine synthesis capacity and IDO1 expression. In our study, we attempt to uncover the role of tryptophan catabolism, in relation to serine metabolism in TNBC cells.

Methods: LC-MS with [U-13C11]-Tryptophan and [U-13C3]-Serine were used to analyze their contributions to nucleotide synthesis, and energy metabolism via the tryptophan catabolism pathway and the folate/methionine cycle, respectively. [U-13C6]-Glucose was used to analyze its contribution to de novo serine synthesis under disrupted serine and tryptophan metabolism conditions. IDO1 inhibitor (Epacadostat), PHGDH inhibitor, and SHMT inhibitor (SHIN1) were used to inhibit tryptophan catabolism, de novo serine synthesis, and folate cycle, respectively. Cell growth was assessed via cell count imaging and changes in protein expression in relevant pathways were evaluated via western blot.

Results: IDO1 inhibition showed varying levels of efficacy in different TNBC cells relating to their ability to synthesize serine and the availability of extracellular serine. Growth was most affected in cell lines with dysfunctional de novo serine synthesis indicating a compensatory effect between tryptophan catabolism and serine synthesis. This was further corroborated with the changes observed in IDO1 and PHGDH expression under enzymatic inhibition and metabolic deprivation. LC-MS analysis on IDO1, PHGDH, and SHMT inhibition showed different methods of compensation in different cell lines, with some exhibiting larger changes in nucleotide synthesis, while others in energy (NAD/H and ATP) and redox metabolism (NAD(P)/H).

Conclusion: Our findings show serine and tryptophan metabolism are tightly co-regulated in TNBC cells. It also highlights the heterogeneity and complexity of how these cells utilize varying metabolic pathways to achieve these compensatory interactions. Our data supports that certain TNBC cells with dysfunctional de novo serine synthesis may be vulnerable to strategies that leverage their limited capacity to compensate between these pathways.

#4174 Regulation of metabolic programming in esophageal cancer by transcription factor p63.

Niamh McNamee[1], Keene Abbott[2], Aya Tal-Mason[1], Matthew G. Vander Heiden[2], Uma M. Sachdeva[1]

[1]Massachusetts General Hospital, Boston, MA,[2]David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Boston, MA

Background: Esophageal adenocarcinoma (EAC) and squamous cell carcinoma (ESCC) exhibit markedly different responses to treatment regimens, including chemotherapy, chemoradiation, and immunotherapy. The transcription factor TP63 is expressed in ESCC and notably absent in EAC and is associated with squamous differentiation and expression of the GLUT1 glucose transporter in squamous cell cancers. Given its homology to p53, a known tumor suppressor and regulator of cell metabolism, we sought to determine whether p63 expression may confer a distinct and targetable metabolic program in ESCC relative to EAC.

Methods: p63 and GLUT1 expression levels were established in ESCC (TE11, TE14) and EAC (OE19, OE33, and FLO-1) cell lines by immunoblot and qPCR. Associated metabolic programs in EAC vs ESCC cells were assessed using the Seahorse XF bioanalyzer, 2-(*N*-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl) Amino)-2-Deoxyglucose (2-NBDG) uptake, and cell viability in the setting glucose deprivation. Genetic knockdown of p63 was performed by siRNA transfection in ESCC cells and resulting effects on mitochondrial metabolism and expression of squamous and glandular differentiation markers were evaluated. The fate of glucose-derived carbon was tracked in ESCC cells with or without genetic knockdown of p63 by incubation with ^{13}C-glucose followed by gas chromatography mass spectrometry and correlated with metabolic gene expression by immunoblot.

Results: ESCC cells exhibited higher glycolytic metabolism and glucose uptake and reduced viability in response to glucose starvation, in the setting of increased p63 and GLUT1 expression, relative to EAC cells. ESCC cells also demonstrated lower mitochondrial respiration, intracellular ATP levels, and respiratory spare capacity. Genetic knockdown of p63 in ESCC cells resulted in increased mitochondrial respiration, ATP production, and respiratory spare capacity, as well as reduced sensitivity to glucose deprivation. This was associated with reduced expression of squamous differentiation markers and increased expression of glandular differentiation markers. Furthermore, knockdown of p63 expression increased glucose flux through the tricarboxylic acid (TCA) cycle and towards fatty acid synthesis. This was associated with enhanced pyruvate dehydrogenase activity, and as increased activation of acetyl-CoA carboxylase and fatty acid synthase.

Conclusions: Our data suggest that ESCC and EAC represent different metabolic programs, with ESCC more dependent on glucose and glycolytic metabolism and EAC exhibiting greater mitochondrial respiration and respiratory spare capacity. These differences are dependent on transcription factor p63, as loss of p63 in ESCC increased glucose shunting into mitochondrial metabolism and fatty acid synthesis pathways. These data suggest that differential metabolic targeting may be an effective approach for both ESCC and EAC.

#4175 Momordicine-I modulates head and neck cancer growth by altering key metabolic pathways.

Ellen T. Tran, Debojyoti Bandyopadhyay, Ruchi A. Patel, **Ratna B. Ray**

Saint Louis University, St. Louis, MO

One of the hallmarks of cancer is metabolic reprogramming which controls cellular homeostasis and therapy resistance. Here, we investigated the effect of momordicine-I (M-I), a key bioactive compound from *Momordica charantia*, on metabolic pathways in human head and neck cancer (HNC) cells and a mouse HNC tumorigenicity model. We found that M-I treatment on HNC cells significantly reduced the expression of key glycolytic molecules at the mRNA and protein levels. We further observed reduced lactate accumulation, suggesting glycolysis was perturbed in M-I treated HNC cells. Metabolomic analyses confirmed a marked reduction in glycolytic and TCA cycle metabolites in M-I-treated cells. M-I treatment significantly downregulated mRNA and protein expression of essential enzymes involved in *de novo* lipogenesis, including *ACLY*, *ACC1*, *FASN*, *SREBP1*, and *SCD1*. Using shotgun lipidomics, we found a significant increase in lysophosphatidylcholine and phosphatidylcholine loss in M-I treated cells. Subsequently, we observed dysregulation of mitochondrial membrane potential and significant reduction of mitochondrial oxygen consumption after M-I treatment. We further observed M-I treatment induced autophagy, activated AMPK and inhibited mTOR and Akt signaling pathways. However, blocking autophagy did not rescue the M-I-mediated alterations in lipogenesis, suggesting an independent mechanism of action. M-I treated mouse HNC MOC2 cell tumors displayed reduced *Hk1, Pdk3, Fasn,* and *Acly* expression. In conclusion, our study revealed that M-I inhibits glycolysis, lipid metabolism, induces autophagy in HNC cells and reduces tumor volume in mice. Therefore, M-I-mediated metabolic reprogramming of HNC has the potential for important therapeutic implications.

#4176 Targeting KRAS selectively induce metabolic reprogramming in pancreatic cancer.

Matthew Cheung, Laiba Shiekh, Don-Gerard Conde, Chiamaka Ezeh, Zeribe Nwosu

Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY

Pancreatic ductal adenocarcinoma (PDAC) is a lethal disease largely due to the lack of effective treatments that overcome the tumor drug resistance. Oncogenic KRAS mutations contribute to most PDAC and drive multiple metabolic pathways to promote tumor growth. Despite this predominant role of KRAS, the inhibition of KRAS/MAPK pathway often leads to resistance via mechanisms that remain unclear. Pharmacological inhibition of the KRAS/MAPK pathway produces varying and context-dependent responses in different phenotypes of pancreatic cancer cells. Specifically, while KRAS/MAPK inhibition using drugs such as MRTX1133 and Trametinib suppresses growth in some cell lines, the same treatment induces or does not alter the growth of other cell lines, and selectively impacted cell migration. Metabolic activity was suppressed in cells with inhibited growth but accelerated in cells where KRAS/MAPK inhibition promoted growth. Analysis of gene expression profile of human PDAC and KRAS-driven mouse tumors identified nutrient transporters that are upregulated upon KRAS induction, but further upregulated by KRAS inhibition. Our study reveals context-dependent response to KRAS inhibition in PDAC and suggests targeting nutrient transporters to overcome therapeutic resistance. Laiba Shiekh and Matthew Cheung contributed equally to this work.

#4177 Integrated assessment of alterations in coenzyme Q biosynthesis genes impacting cancer metabolism leading to critical vulnerabilities in tumors.

Nischal Mahaveer Chand, Eric M. Grund, Poornima K. Tekumalla, Gregory M. Miller, Niven R. Narain, Vijay Modur, Michael A. Kiebish

BPGbio, Framingham, MA

Coenzyme Q10 (CoQ) is critical in governing mitochondrial bioenergetics as a pivot point regulating the efficiency of the electron gradient, while balancing electron slippage leading to reactive oxygen species (ROS) generation. Alterations in cancer metabolism are a hallmark of oncogenesis and central is the Warburg Effect, which reflects a dependence on glycolysis due to inefficient mitochondrial function in the presence of oxygen. Alterations in CoQ biosynthesis can disrupt cellular respiration and energy homeostasis, leading to various metabolic disorders. In cancer, restoring proper CoQ function, by correcting its biosynthetic pathway and the levels of CoQ, could offer therapeutic benefits. Targeting CoQ alterations may enhance mitochondrial function and potentially inhibit the proliferation of cancer cells, presenting a novel avenue for cancer treatment. To understand the extent of CoQ biosynthesis imbalance in cancer, gene expression and mutation data for 33 cancers in the TCGA were analyzed. RNA-seq gene expression and SNP mutation data were evaluated for 13 genes in the CoQ biosynthesis pathway: COQ10A, COQ10B, COQ2, COQ3, COQ4, COQ5, COQ6, COQ7, COQ8A, COQ8B, COQ9, PDSS1, and PDSS2. The median expression level of transcripts was examined and 4 genes critical in the biosynthesis pathway demonstrated low median expression across cancers: PDSS1, PDSS2, COQ2, and COQ3. SNP mutations were aggregated across cancers and normalized to gene length for each of the 13 genes. Mutations were low across the 13 genes, with an average of 0.875 SNP mutations observed per 1000 base pairs. Additionally, COQ8A and COQ8B had no observed mutations in any TCGA samples. Kaplan-Meier analysis was performed for each cancer based on low expression (below 25th percentile) and high expression (above 75th percentile) for each CoQ gene. Low Grade Glioma was observed having 6 of 13 genes (COQ10B, COQ2, COQ8A, COQ8B, COQ9, PDSS1) with a significant change in overall survival based on expression levels (logrank test p-value < 0.001). Additionally, 3 genes (COQ5, COQ7, PDSS2) were identified with a significant difference in Kidney Renal Clear Cell Carcinoma, 2 genes each in Adrenocortical Carcinoma (COQ8A, PDSS1), Papillary Renal cell Carcinoma (COQ4, COQ9), and Uveal Melanoma (COQ2, COQ6). In conclusion, this comprehensive analysis of CoQ biosynthesis genes across multiple cancer types reveals critical insights into their expression and mutation profiles, as well as their potential impact on patient survival. The low mutational rates across the 13 genes underscore their importance in maintaining cellular energy homeostasis. Furthermore, the significant survival differences associated with the expression levels of these genes in specific cancers highlight the potential of restoration of CoQ pool as a therapeutic strategy for mitigating dysfunctional energy metabolism.

#4178 Investigating glucose utilization in cancer and immune cells using bioluminescent metabolite and enzyme assays.

Donna Marie Leippe, Natasha Karassina, Mike Valley, Kayla Sylvester, Anthony Lauer, Jolanta Vidugiriene

R&D, Promega Corporation, Madison, WI

Glucose metabolism is fundamental to the function and survival of both cancer cells and immune cells within the tumor microenvironment. Cancer cells often exhibit altered metabolic pathways to meet the elevated energy and anabolic demands of rapid proliferation, including increased glucose uptake for enhanced glycolysis and glycogen storage. This can deplete glucose availability for infiltrating immune cells, restricting their ability to execute an effective anti-tumor response and promoting an immunosuppressive environment. Understanding glucose metabolism in cancer cells and T cells is important for elucidating the role of nutrient competition in immunotherapy effectiveness. To investigate these dynamics, we developed sensitive bioluminescence assays capable of measuring multiple aspects of glucose metabolism in both cancer and immune cells, including glycogen synthesis and utilization. Glycogen serves as a glucose reservoir that can rapidly respond to glucose supply and demand to maintain cellular functions under fluctuating nutrient conditions. Changes in glycogen levels (both increases and decreases) were observed in cancer cell lines in response to medium composition (e.g., glucose concentration) and drug treatments. Glycogen was also measured in T cells upon activation, providing a useful method for studying requirements for glycogen synthesis in these cells and its relevance for T cell function. The increased sensitivity of the glycogen assay required low cell numbers and was amenable to reduced volumes, in 384-well plates, further facilitating these studies. In addition to glycogen synthesis and degradation, other glucose metabolic pathways, including glycolysis, oxidative phosphorylation, and the pentose phosphate pathway, were analyzed. We used assays to measure intermediates and products in these pathways, including lactate, pyruvate and malate, as well as assays for measuring dehydrogenase enzyme activity. Dehydrogenases representing all three pathways exhibited increased activity upon T cell activation, indicating enhanced engagement of these pathways. These assays should provide a valuable method for studying the impact of limited glucose on T cell activation and suggesting approaches to address it. To better understand the role of glucose availability and implications of nutrient competition in the tumor microenvironment for anti-tumor immune responses, we used sensitive bioluminescent assays to investigate metabolic profiles of cancer and immune cells. The assays can facilitate identification of potential approaches and targets to eliminate limited access to glucose and other nutrients as a barrier to efficient immunotherapies.

#4179 ADCK1 expression modulates pro-cancerous characteristics of hepatocellular carcinoma.

Noel A. Jacquet, Yunfeng Zhao

Pharmacology, Toxicology and Neuroscience, LSU Health Sciences Center - Shreveport, Shreveport, LA

The incidence of hepatocellular carcinoma (HCC) has steadily increased over the past few decades, with minimal progress in developing effective therapeutic agents. As the most common form of primary liver cancer, HCC is currently the third leading cause of cancer-related deaths. HCC originates from hepatocytes, the primary liver cell type responsible for vital metabolic functions, including the processing of amino acids, lipids, carbohydrates, and xenobiotics. Dysregulation of cellular metabolism can drive malignancies and lead to tumorigenesis. AarF Domain Containing Kinase 1 (ADCK1), a putative kinase, is implicated in regulating mitochondrial functions, which are essential for cellular metabolism. Defects in mitochondrial function are known to contribute to tumorigenesis. Although ADCK1 remains poorly studied in humans, early research suggests its involvement in the tumorigenesis of parathyroid, colon, and osteosarcoma cancers. Our lab aims to elucidate the role of ADCK1 in cellular metabolism and the behavior of HCC cells. Bioinformatics analysis conducted by our group revealed that high ADCK1 expression correlates with worse overall survival rates in HCC patients compared to those with low ADCK1 expression. To investigate the impact of ADCK1 loss, we generated ADCK1 knockout (KO) clones in HCC cells. Our findings indicate that the loss of ADCK1 modulates several malignant characteristics associated with HCC tumorigenesis, including alterations in cellular proliferation, cell cycle regulation, programmed cell death, colony formation, and mitochondrial respiration. Future studies will explore the mechanisms underlying these processes, to establish ADCK1 as a promising therapeutic target for HCC treatment. This abstract has been proofread and enhanced for clarity through the assistance of Grammarly and ChatGPT AI.

#4180 Insights into HuR as a modulator of aerobic glycolysis in triple-negative breast cancer.

Arundhathi Dev J R[1], Ajay Gogia[1], Sandeep R. Mathur[2], Ashutosh Mishra[3], Chandra Prakash Prasad[1]

[1]Medical Oncology, All India Institute of Medical Sciences (AIIMS), New Delhi, India,[2]Pathology, All India Institute of Medical Sciences (AIIMS), New Delhi, India,[3]Surgical Oncology, All India Institute of Medical Sciences (AIIMS), New Delhi, India

The RNA-binding protein Human Antigen R (HuR/ELAVL1) is ubiquitously expressed and plays a pivotal role in post-transcriptional gene regulation by stabilizing and enhancing the translation of mRNAs containing U/AU-rich elements. Its overexpression, particularly in the cytoplasm, has been implicated in various cancers, where it drives critical processes such as proliferation, survival, angiogenesis, and metastasis. As a key modulator of oncogenic pathways, HuR represents a significant target for understanding cancer progression and developing novel therapeutic strategies. The present study investigates the oncogenic role of HuR in TNBCS, an aggressive subtype of breast cancer. Meta-analysis with TNBC dataset (Liu-2014) correlated high HuR protein expression with reduced patient survival (p=0.028). Immunohistochemistry performed in 40 TNBC patient tissue showed an overexpression of HuR (77%) and its downstream target MMP9(55%). Disrupting the RNA binding activity of HuR with the HuR specific inhibitor CMLD2 resulted in the downregulation of HuR and its downstream targets CDK2, MMP9, and β-catenin in TNBC cell lines MDA-MB-231 and MDA-MB-468. Inhibition of cytoplasmic HuR was validated via immunofluorescence and cell fractionation followed by immunoblotting. HuR inhibition compromised proliferation, migration, invasion, extracellular acidification, clonogenicity (2D and 3D), and EMT (downregulated E-Cadherin, N-Cadherin, and Vimentin) in TNBC cells. Exploratory transcriptomics via nCounter XT showed that HuR inhibition in MDA-MB-231 altered the profile of glycolytic genes in addition to other pathways such as cell cycle, DNA damage repair, amino acid synthesis etc. In accordance, the expression of the glycolytic genes LDHA, PFKP and MCT4 was reduced upon CMLD2 treatment at mRNA and protein level in both the TNBC cell lines. Pretreatment with CMLD2 impaired the tumor forming ability of 4T1 *in vivo*. Consistent results were obtained on HuR siRNA knockdown. To our knowledge, this is the first report that explores HuR as a positive regulator of aerobic glycolysis in TNBCs. Inhibition of HuR by CMLD2 provides a rationale to consider it as a potential anticancer agent for TNBCs.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: DNA Damage Response and Modulation of DNA Repair 2
Poster Session

#4184 Development of a homogenous poly(ADP-ribose) assay for screening and profiling PARP inhibitors.

Mahbbat Ali, Ha Pham, Anibal Ramos-Martinez, **Robert Lowery**

BellBrook Labs, Madison, WI

Poly(ADP-ribose) polymerases (PARPs) initiate DNA damage repair (DDR) by binding to double strand breaks and creating a scaffold of poly(ADP-ribose) (pADPr) chains for assembly of other DDR proteins. The use of PARP inhibitors for ovarian tumors with BRCA-1 mutations was the first clinical exploitation of synthetic lethality, and PARP inhibitors are now used for breast, prostate, pancreatic, and ovarian cancers. New types of PARP inhibitors are being sought to broaden the scope of indications. To facilitate this effort, we developed a high throughput screening (HTS) assay for PARPs that enables quantitative detection of pADPr formation in a homogenous format. The assay relies on enzymatic conversion of pADPr to AMP, which is then detected using the Transcreener AMP/GMP assay with a fluorescence polarization (FP) or time-resolved Förster resonance energy transfer (TR-FRET) readout. To minimize interference from screening compounds, we first optimized the concentration of coupling enzymes to ensure that the pADPr will be completely converted to AMP even if the conversion reaction is inhibited by as much as 80%. Using this optimized system, the assay generated a robust signal (Z' > 0.8) using 0.8 nM PARP1 with sub-saturating NAD (100 µM) and saturated sheared salmon sperm DNA (0.25 mg/mL) under initial velocity conditions. The assay was validated for HTS using a 1,280 compound bioactive library, resulting in 18 hits, 5 of which proved to be interference. We also screened a 3,200 compound diversity library, resulting in 44 hits, 3 of which were identified as interference. Four known PARP1 inhibitors, Olaparib, PJ 34 HCl, Veliparib, and Rucaparib Camsylate, were detected in the bioactive screen and tested in dose response mode with both FP and TR-FRET readout; the measured IC_{50} values (0.45 nM, 29 nM, 1.4 nM and 0.4 nM, respectively) were consistent with published values. By enabling sensitive, quantitative detection of pADPr formation in a simple, mix-and-read format, the Transcreener pADPr assay should facilitate efforts to develop more effective PARP inhibitors.

#4185 A novel USP1 inhibitor enhances PARP inhibitor efficacy in elevated replication stress tumors: Evidence from BRCA-mutant and CCNE1-amplified models.

Ho-Jeong Lee, Hyerim Yoo, Jihye Kim, Sunkyu Kim, Sejeong Park, Sanghoon Lee, Kiwoong Yoo, Jin Kwan Kim, Kwang-Ok Lee, Jaewoo Kang

AIGEN Sciences, Seoul, Korea, Republic of

Synthetic lethality offers a promising strategy for cancers with elevated replication stress, including BRCA-mutant and CCNE1-amplified tumors. USP1, a deubiquitinating enzyme, stabilizes replication forks by regulating ubiquitinated PCNA (Ub-PCNA). Using the AIGEN ChemTailor AI platform, we developed a novel USP1 inhibitor, which exhibited selective anti-proliferative effects in BRCA-mutant cancer cells and enhanced efficacy when combined with PARP inhibitors *in vitro*. We evaluated the USP1 inhibitor's *in vivo* efficacy in BRCA-mutant breast cancer models and CCNE1-amplified ovarian cancer models.

In MDA-MB-436 breast cancer xenografts, the combination of the USP1 inhibitor with PARP inhibitor demonstrated significant tumor suppression in both subcutaneous and orthotopic models, with the orthotopic model better reflecting the cross-talk between tumor cells and the tumor microenvironment. In OVCAR3 ovarian cancer orthotopic metastasis models, the USP1 inhibitor enhanced PARP inhibitor's anti-tumor activity. Elevated Ub-PCNA expression in tumor tissues from MDA-MB-436 models further supports its potential as a pharmacodynamic marker.

These findings highlight the USP1 inhibitor's potential to enhance PARP inhibitor efficacy in BRCA-mutant and CCNE1-amplified cancers, with Ub-PCNA serving as a promising PD marker. IND-enabling studies, including GLP toxicology, are ongoing, with completion expected by late 2025.

#4186 Discovery of novel PARP1-selective inhibitors for treatment of brain tumors using artificial intelligence.

Sarah Truong[1], Beibei Zhai[1], Louise Ramos[1], Mona Marzban[1], Fariba Ghaidi[1], Fuqiang Ban[1], Jason Smith[1], Mohit Pandey[1], Ekaterina Manskaia[1], Kally Singh[2], Hans Adomat[1], Xiaoqi Chen[2], John Langlands[2], Dennis Brown[2], Jeffrey Bacha[2], Colin Collins[3], Artem Cherkasov[3], Mads Daugaard[3]

[1]Vancouver Prostate Centre, Vancouver, BC, Canada,[2]Rakovina Therapeutics, Vancouver, BC, Canada,[3]University of British Columbia, Vancouver, BC, Canada

Introduction: Brain tumors, including primary brain tumors and central nervous system (CNS) metastases, remain among the most challenging malignancies to treat, with therapeutic options often limited by the inability of drugs to penetrate the blood-brain barrier (BBB). Poly(ADP-ribose) polymerase (PARP) is a key enzyme in DNA repair, and inhibition of PARP1 specifically drives synthetic lethality in BRCA-mutated disease. While they have achieved commercial success, first generation PARP inhibitors are limited in their utility as they cannot readily pass through the blood-brain barrier and produce adverse side effects, likely driven by the collateral inhibition of PARP2. Development of a PARP1-selective CNS penetrant inhibitor could reduce toxicity, while providing a new therapeutic option for brain tumors. Traditional drug discovery methods are time-consuming and costly, necessitating innovative approaches. Here, we describe the application of Deep Docking combined with generative artificial intelligence (AI), to discover a novel, PARP1-selective inhibitor for use against brain tumors.
Methods: Deep Docking utilizes deep learning to accelerate the prediction of the binding affinity of a large number of compounds to target proteins, streamlining the virtual screening process and allowing for rapid docking of billions of compounds against the PARP1 protein. Additionally, state-of-the-art generative algorithms and deep learning techniques for predicting CNS penetrance can be combined with Deep Docking to rapidly perform hit-to-lead optimization of a CNS-penetrating PARP1-selective inhibitor.
Results: We will present Deep Docking screening results from billions of compounds. Validating in vitro and in vivo data, including PARP1 inhibition and selectivity, BBB permeability, metabolic stability, pharmacokinetic profile, and CNS penetration will be described.
Conclusion: The Deep Docking platform is being used in concert with generative AI approaches to discover and finetune a selective PARP-1 inhibitor for brain tumors. This approach will not only improve the efficiency of drug discovery but also enhance the specificity and efficacy of potential therapeutics.

#4187 Enhancing doxorubicin DNA-damaging therapy in neuroendocrine cancer via DNA-PK inhibition.

Subin Kim[1], Allisa Pack[1], Nathan L. Vanderford[1], Courtney M. Townsend[2], B. Mark Evers[1], Piotr Rychahou[1]

[1]University of Kentucky, Lexington, KY,[2]University of Texas Medical Branch, Galveston, TX

Introduction: Gastroenteropancreatic (GEP) neuroendocrine tumors (NETs) are neoplasms originating from the gastrointestinal track and pancreas. Unfortunately, most patients at the time of diagnosis have extensive metastatic disease and are not candidates for curative surgical resection of metastatic tumors. In such cases, chemotherapy can slow the progression of metastatic disease, but eventually tumors develop resistance to genotoxic agents through DNA repair pathways activation. Therefore, targeting DNA repair pathway can increase the tumor sensitivity to cancer therapies. In this study, we examined whether DNA-PK inhibition could sensitize pancreatic NETs to DNA damaging chemotherapy.
Methods: DNA double-strand break damage was induced with topoisomerase II inhibitor doxorubicin. DNA-PK activity was inhibited with peposertib. Clonogenic and proliferation assays were used to evaluate the efficacy of peposertib in combination with doxorubicin in BON and QGP-1 neuroendocrine cell lines. DSB-induced DNA repair was visualized with confocal laser scanning microscopy using γ-H2AX immunofluorescence. Induction of apoptosis in chemotherapy combination groups was visualized with confocal laser scanning microscopy using cleaved caspase-3 immunofluorescence and with western blot using cleaved PARP bands. To further validate the role of DNA-PK in the response to treatments, DNA-PK knockdown cell lines were established, and confocal microscopy and western blotting were used to assess DNA damage and apoptosis. Data was analyzed using GraphPad Prism and expressed as a mean ± standard deviation.
Results: DNA-PK inhibition resulted in enhancement of doxorubicin cytotoxic therapy (BON IC50 DOX = 194.73± 26.52 nM, DOX+Pep = 89.87± 17.6 nM; QGP IC50 DOX = 108.37± 23.97 nM, DOX+Pepo = 30.27± 4.80 nM) in both BON and QGP-1 cell lines. Immunofluorescence analysis detected γH2AX foci increase in the doxorubicin and peposertib combination groups. DNA-PK inhibition in combination with doxorubicin treatment significantly increased caspase-3 cleavage and PARP cleavage in BON and QGP-1 cell lines. Similar trends in DNA damage and apoptosis were observed across both drug combination and knockdown groups.
Conclusions: High DNA repair activity is commonly associated with cancer drug resistance. The combination of DNA-PK inhibition with DNA-damaging therapies impaired DNA damage response and enhanced neuroendocrine cancer cells apoptosis. These findings demonstrate that selective DNA-PK inhibition provides a potent therapeutic strategy for disruption of non-homologous end joining DNA double-strand break repair and may offer a novel therapeutic approach in advanced NET chemotherapy.

#4188 The role of NHEJ/HR mediated DSB repair in cancer cell sensitivity to payload of T-DXd ADC.

Len Katsumata[1], Manabu Abe[1], Misa Ikuta[1], Fumitaka Suto[2], Kumiko Koyama[1]

[1]Daiichi Sankyo Co., Ltd., Tokyo, Japan,[2]Daiichi Sankyo Inc., Basking Ridge, NJ

Introduction: Trastuzumab deruxtecan (T-DXd) is an innovative HER2-targeting antibody-drug conjugate comprising a HER2-specific antibody linked to an exatecan derivative (DXd) payload. This therapeutic agent has demonstrated substantial clinical efficacy across various HER2-expressing malignancies. The DXd payload functions as a topoisomerase I inhibitor, stabilizing the topoisomerase-DNA cleavage complex. This stabilization inhibits the re-ligation of DNA, leading to the accumulation of DNA double-strand breaks (DSBs) and ultimately inducing apoptotic cell death. DNA DSBs are primarily repaired through two distinct mechanisms: Non-Homologous End Joining (NHEJ) and Homologous Recombination (HR). However, the contributions of these DNA damage repair pathways to the sensitivity of cancer cells to T-DXd/DXd remain poorly understood.

Methods: In this *in vitro* study, we investigated the roles of NHEJ and HR in modulating the sensitivity of the HER2-expressing breast cancer cell line, MDA-MB-453, to T-DXd/DXd. This was achieved through siRNA-mediated knockdown of key components of the respective repair pathways, specifically TP53BP1 for NHEJ and BRCA1 for HR. Drug sensitivity was assessed through cell growth inhibition assays and caspase-3/7 activity assays. DNA repair machinery was evaluated using immunofluorescence to quantify RAD51 foci formation. Additionally, cell cycle dynamics in response to DXd treatment and the modulation of NHEJ/HR-mediated repair pathways were analyzed using flow cytometry and live cell imaging of MDA-MB-453 cells transduced with a Fluorescent Ubiquitination-based Cell Cycle Indicator.

Results: Our results demonstrated that the suppression of TP53BP1 led to a decrease in T-DXd/DXd sensitivity, which was associated with an increase in HR-mediated DSB repair, as evidenced by a higher incidence of RAD51 foci formation in the nuclei. Conversely, knockdown of BRCA1, which inhibits HR, substantially enhanced sensitivity to T-DXd/DXd. Notably, the alteration in DNA repair pathway balance resulted in distinct cell cycle phase shifts upon T-DXd/DXd treatment; specifically, BRCA1 knockdown increased the G2/M phase population while concurrently reducing DNA damage response (DDR)-related cell cycle arrest. Furthermore, the combination of Wee1 inhibitor with T-DXd and DXd enhanced cytotoxicity via aberrant cell cycle arrest.

Conclusions: Collectively, these findings point to the role of the mode of DNA damage repair pathways in modulating the activity of the DXd payload, suggesting the possibility of improved efficacy by the combination with DDR inhibitors. Further investigations are warranted to elucidate the contextual factors influencing these outcomes and to validate our findings in clinical samples.

#4189 CRISPR-edited cell lines targeting key molecular pathways: Accelerating the development of synthetic lethal therapeutics beyond PARP inhibitors.

Yue Huang, Yunpeng Zhai, Yan Feng, Xinyue Jia, Xiaohuan Zhang, Jinying Ning, **Feng Hao**

Kyinno Biotechnology Co., Ltd, Beijing, China

Synthetic lethality (SL) has emerged as a highly promising therapeutic strategy, gaining significant attention in the field of cancer treatment in recent years. The principle behind SL is that the simultaneous loss or inhibition of two genes results in cell death, while the inhibition of only one gene does not. SL drugs exploit this mechanism by targeting the partner gene of an already inactivated or mutated gene in cancer cells, inducing their death. This approach allows for the precise targeting of genetic vulnerabilities specific to cancer cells, sparing normal tissues from damage. Additionally, SL therapies expand the range of druggable targets, including proteins that are difficult to inhibit with traditional small molecules. By affecting both enzymatic and non-enzymatic protein functions, SL therapies reduce the risk of developing drug resistance, providing an effective option for treating cancers that are resistant to conventional therapies.Kyinno offers an extensive collection of knockout cell lines targeting a range of genes associated with synthetic lethality, including SHLD2, P53, T53BP1, AQR, ATRX, SETD2, ATM, WRN, BRCA1, and BRCA2. These cell lines, developed using the CRISPR/Cas9 genome editing technique and validated through sequencing and western blot analysis, are crucial for investigating the complex mechanisms underlying synthetic lethality and for identifying novel therapeutic strategies in cancer treatment. For example, our knockout lines for BRCA1 and BRCA2 enable researchers to investigate vulnerabilities in homologous recombination repair, paving the way for the development of innovative PARP inhibitors and targeted therapies. Additionally, lines with deletions in ATM and WRN provide essential insights into DNA damage response pathways, which can enhance the effectiveness of DNA-damaging agents. The diverse range of gene knockouts, including those involved in cell cycle regulation, DNA repair, and apoptosis, allows for comprehensive studies into the synergistic effects of dual gene inhibition. By utilizing our extensive library of synthetic lethality cell lines, researchers can effectively validate hypotheses, screen novel drug candidates, and optimize combination therapies to improve outcomes for patients with resistant cancers. We highly recommend these resources for advancing research in synthetic lethality and targeted cancer therapies.

#4190 CRISPR-CAS9 library screening identifies novel vulnerabilities in CRC drug-tolerant persister cells.

Martina Miotto[1], Simona Lamba[1], Gaia Grasso[1], Giorgio Corti[2], Julie Bonetto[1], Alberto Sogari[1], Alberto Bardelli[1], Mariangela Russo[1]

[1]Department of Oncology, Universita degli Studi di Torino, Turin, Italy,[2]Candiolo Cancer Institute, FPO-IRCCS, Candiolo, Italy

Introduction: Efficacy of targeted therapies in metastatic colorectal cancer (mCRC) is hindered by onset of resistance, therefore novel strategies are needed. A subpopulation of drug-tolerant persister cells (DTPs) survives to targeted therapies and initiates adaptive mutability response. DTPs experience an increased DNA damage and a shift to error-prone DNA damage repair, thus promoting mutability and resistance.

Aim: The purpose of this project is to explore key players modulating adaptive mutability to prevent DTPs' evolution to resistance, thus preventing tumor recurrence.

Methods: CAS9-expressing CRC cells were infected with a custom CRISPR library targeting over 500 DNA damage response (DDR) genes, divided in 12 subpopulations, and grown either for 4 weeks in normal cell culture conditions (untreated) or treated with specific oncogenic inhibitors until the development of resistance in a Time-To-Progression (TTP) assay. CAS9-expressing clones infected with empty library were grown in parallel, in absence or presence of targeted therapies, as control. Next Generation Sequencing-based analysis of sgRNA (single guides RNA) was then performed on resistant subpopulations grown in the presence of targeted therapies.

Results: We initially isolated CAS9-expressing clones from a microsatellite stable (MSS) CRC cell line and selected clones with growth rate and drug-sensitivity comparable to that of parental population. The selected CAS9-expressing clone was then infected with the CRISPR DDR library or empty library and, after the selection with puromycin, the selected populations were divided in 12 subpopulations, to exclude the possibility that pre-existing resistant cells might outgrow and interfere with the effect of DDR genes knock-outs (KO) on the development of drug-resistance. The subpopulations were then grown in absence or presence of oncogenic inhibitors for several weeks until the onset of resistance. Notably, while the 12 subpopulations infected with the empty library simultaneously developed resistance, the development of resistance in the DDR library-infected cells was spread over multiple weeks, thus highlighting that KO of DDR genes might either accelerate or delay the onset of resistance. sgRNA of resistant population were then analyzed by next-generation sequencing. A negative selection analysis will be applied to identify sgRNA that are lost in the resistant populations, thus targeting DDR genes whose depletion is potentially synthetical lethal with targeted therapies in mCRC.

Conclusion: A DDR CRISPR screening identified novel vulnerabilities of CRC DTPs able to improve efficacy of targeted therapies and prevent tumor relapse in mCRC.

#4191 Combining artificial intelligence and human expertise in pursuit of novel PolQ inhibitors.

Allan M. Jordan[1], Ageo Miccoli[2], Annabelle Charnock[2], Alexis Denis[3], Euan Fordyce[2], Cameron Franklin[2], Ennys Gheyouche[3], Ian Henderson[2], Christopher Housseman[3], Maxime Laugeois[3], Jean-Christophe Meillon[3], Stuart Onions[1], Quentin Perron[3], Clementine Pescheteau[3], Emilie Pihan[3], Mark Reeves[2], Kirsty Rooney[2], Hannah Rowlands[2], Kamaldeep Sumal[1], Franck Le Vaillant[3], Graeme Walker[2]

[1]Sygnature Discovery, Nottingham, United Kingdom,[2]Sygnature Discovery, Macclesfield, United Kingdom,[3]Iktos, Paris, France

DNA polymerase theta (POLθ or POLQ) recently emerged as a new promising drug target for the treatment of homologous recombination (HR) deficient tumours. This unique fusion protein comprises both DNA polymerase (Pol) and DNA helicase domains that function together to facilitate multiple DNA repair mechanisms. Small molecule approaches to target both domains have been described throughout the last decade and have shown potent and selective HR-deficient tumor cell death in vitro and in vivo. Despite the growing number of advanced PolQ polymerase inhibitors reported in the literature, significant ADME liabilities remain associated with the current reported scaffolds. To address this issue, machine-based learning methodologies were envisioned to act as a potential aid for medicinal chemistry efforts and help drive identification of chemical matter that might reside outside of the typical toolbox of small molecule design ideas.
Using the Makya generative AI platform, synergistically with traditional knowledge-based approaches, we have identified novel small molecule PolQ polymerase domain inhibitors. One chemotype, with an embedded oxime motif, has demonstrated potent biochemical activity and comparable cell activity to known tool compounds. A follow-up library of functionalised oxime derivatives, with varying compositions of donor and acceptor motifs, was synthesized to probe the chemical space further which demonstrated improved biochemical potency on the original hit matter. Initial ADME profiling suggests that these ligands possess properties that represent a good starting point for a lead optimisation campaign. Herein, we report key findings from the initial phase of development of our small molecule PolQ inhibitors.

#4192 Preclinical characterization of LAE122, a novel, potent and selective WRN inhibitor for the treatment of MSI-H tumors.

Ming Li[1], Minhua Zhang[1], Junyan Chen[1], Chaojun Cai[2], Xiaofen Lin[2], Justin Gu[2]

[1]Laekna Therapeutics Shanghai Co., Ltd., Shanghai, China,[2]Laekna Pharmaceutical Ningbo Co., Ltd., Ningbo, China

DNA mismatch repair (dMMR) deficiency could induce damage of genome, resulting Microsatellite instability (MSI-H), which is frequently observed in various tumor types, including colorectal, gastric, endometrial, and ovarian cancers. Although immune checkpoint inhibitors (ICI) have been approved for the treatment of MSI-H tumors, significant unmet medical need remains across several tumor types that fail to respond or relapse with ICI therapies. Werner syndrome ATP-dependent helicase (WRN) is a RecQ family helicase that plays important roles in DNA repair and in the maintenance of genome integrity. Recent studies have identified WRN as a promising synthetic lethal drug target for MSI-H cancers. LAE122 is a novel, potent and selective WRN inhibitor developed by Laekna through structure-based drug design strategy. LAE122 has highly differentiated structure from bench-mark molecules and displayed single digit nanomolar IC_{50} values in biochemical assay and cell anti-proliferation assay on MSI-H cells but had no effect on Microsatellite stability (MSS) cells up to 10 μM. LAE122 demonstrated favorable drug-like properties, including good in vitro ADMET and in vivo PK with low plasma clearance and high oral bioavailability across animal species. In SW48 and HCT116 CDX models, LAE122 showed robust anti-tumor growth effect at lower doses than bench-mark molecules with strong evidence on biomarker change. LAE122 didn't have off-target liability in in vitro assays and demonstrated good tolerability in preclinical toxicology studies. In conclusion, LAE122 is a novel, potent and selective WRN inhibitor for the treatment of MSI-H tumors.

#4193 Novel APE1 endonuclease inhibitors as precision oncology therapeutics in high grade serous ovarian cancers discovered and developed from crystallography-based fragment library screening.

D. Das[1], A. Deacon[1], M. Duncton[1], D. M. Wilson III[1], M. Algethami[2], A. Leshan[2], E. A. Rakha[2], C. Tosun[2], S. Madhusudan[2];
[1]XPose Therapeutics, San Carlos, CA, [2]University of Nottingham, Nottingham, United Kingdom

Our preclinical data show APE1 could be a synthetic lethality (SL) target in BRCA deficient ovarian cancers. *BRCA1*- and *BRCA2*-associated hereditary breast and ovarian cancer syndrome (HBOC) is characterized by increased risk for breast cancer, ovarian cancer (includes fallopian tube and primary peritoneal cancers) and other solid tumours (including pancreatic cancers and prostate cancers). About 10-20% of TNBC patients harbor germline BRCA mutations resulting in homologous recombination deficiency (HRD), and even amongst wild-type BRCA patients, somatic mutations occur more frequently producing a range of HRD phenotypes collectively termed as “BRCAness”. In epithelial ovarian cancers, germ-line BRCA mutations can be detected in up to 20% of high serous ovarian cancers, and up to 50% of somatic tumours can also manifest a HRD phenotype. PARP1 inhibitor (Niraparib, Olaparib, Rucaparib) maintenance therapy improves progression-free survival in BRCA germline deficient ovarian cancer or platinum sensitive ovarian cancers. Olaparib and Talazoparib are approved for use in BRCA germ-line deficient breast cancers. A response rate to PARPi of up to 60% has been described in clinical studies. Routine clinical use has shown that not all patients with BRCA deficient cancers respond to PARPi either due to intrinsic or acquired resistance. The search is on for other SL targets in BRCA/HRD deficient cancers that either bypass PARPi resistance or are more effective than PARP blockade. We evaluated APE1 protein expression in human epithelial ovarian cancers, correlated with clinicopathological features and survival outcomes and compared APE1 transcript levels in a panel of solid tumours and normal tissue. APE1 transcripts were evaluated in publicly available ovarian cancer clinical datasets (n=1257) for clinical outcomes. Highest levels of transcripts were observed in ovarian cancers compared to other solid tumours. When compared to normal ovarian tissue, there was striking elevation of transcripts in ovarian cancer tissues. High mRNA expression was associated with poor progression free survival (PFS) ($p=0.00019$). High nuclear APE1 protein expression was seen in 64% of tumours, a feature associated with high grade serous ovarian cancer, poor PFS and poor overall survival. XPTx-91 and XPTx-387 have biochemical IC50s of 250-500 nM. Optimized compounds were screened for selective toxicity in PEO1 (BRCA2 deficient, platinum sensitive, PARPi sensitive) and PEO4 (BRCA2 proficient, platinum resistant, PARPi resistant) cell lines. Several compounds screened in MTS cell proliferation assay showed preferential cytotoxicity in PEO1 cells compared to PEO4 cells. Further cell based (clonogenics, 3D spheroids) and functional assays (DNA double strand break accumulation, cell cycle progression and apoptosis) are ongoing.

#4194 Inhibiting PELP1 signaling enhances the therapeutic effectiveness of topoisomerase inhibitors in triple-negative breast cancer.

Khaled Mohamed Nassar[1], John R. Sanchez[2], Durga Meenakshi Panneerdoss[3], Behnam Ebrahimi[3], Xue Yang[2], Uday P. Pratap[3], Megharani Mahajan[3], Salvador Cardenas Alejo[3], Abhi Katabathina[3], Panneerdoss Subbarayalu[4], Daohong Zhou[5], Rajeshwar R. Tekmal[3], Gangadhara Reddy Sareddy[3], Manjeet K. Rao[4], Suryavathi Viswanadhapalli[3], Ratna K. Vadlamudi[3]

[1]Obstetric and Gynecology, UT Health Science Center at San Antonio, San Antonio, TX,[2]MD Anderson Cancer Center, Houston, TX,[3]Obstetric and gynecology, UT Health Science Center at San Antonio, San Antonio, TX,[4]Cell Systems and Anatomy, Greehey Children's Cancer Research Institute, UT Health Science Center at San Antonio, San Antonio, TX,[5]Biochemistry and Structural Biology, Greehey Children's Cancer Research Institute, UT Health Science Center at San Antonio, San Antonio, TX

Background: Triple-negative breast cancer (TNBC) is an aggressive tumor with a poor prognosis and high metastasis, accounting for 15-24% of breast cancer deaths. Proline-, glutamic acid-, and leucine-rich protein 1 (PELP1), an oncogene, is commonly deregulated in TNBC and serves as a biomarker of poor survival rates. The objective of this study is to establish the molecular mechanism of action of PELP1 in the progression of TNBC using IPTG-inducible PELP1 knockdown (PELP1-iKD) models and develop a novel combination therapy targeting PELP1.

Methods: TNBC models (MDA-MB-231, BT-549, HCC-1806, SUM-149) with PELP1-iKD were generated through lentiviral transduction. PELP1 signaling was further inhibited with the pharmacological inhibitor SMIP34. SMIP34's effectiveness was tested with 140 FDA-approved drugs using an MTT assay-based screen, and combination therapy was evaluated using colony formation and Annexin V/PI apoptosis assays. Mechanistic studies were conducted using RT-qPCR, Western blotting, and comet assays. The utility of SMIP34 + Topoisomerase inhibitors (TIs) combination therapy was assessed in cell line-derived xenografts (CDXs), patient-derived organoids (PDOs), and patient-derived xenografts (PDXs).

Results: Western blotting analysis confirmed that IPTG induction reduces PELP1 expression in a dose-dependent manner in all four TNBC PELP1-iKD cells. PELP1 KD significantly decreased cell proliferation and colony formation in TNBC cells while enhancing apoptosis. PELP1-iKD cells showed a delayed DNA damage response, characterized by elevated γ-H2AX levels and impaired activation of phospho-ATM, ATR, and DNA-PKcs following DNA damage. Treatment with SMIP34 also resulted in a delayed DNA damage response and accumulation of γ-H2AX, similar to the genetic approach. In an MTT-based screen, SMIP34 enhanced the sensitivity of TNBC cells to TIs. These results were validated in PELP1-iKD cells, where PELP1-iKD further increased sensitivity to TIs, providing genetic evidence of PELP1's role in the synergy between SMIP34 and TIs. The ability of SMIP34 to enhance the efficacy of TIs was confirmed in additional TNBC cell lines, with synergistic effects observed in cell proliferation, colony formation, and apoptosis assays. Mechanistic investigations demonstrated elevated levels of γ-H2AX in cells treated with the PELP1-iKD+TIs and SMIP34+TIs combinations, compared to monotherapy with TIs. Comet assay results indicated heightened DNA damage with these combination treatments. Furthermore, Combined SMIP34 and Mitoxantrone treatment significantly suppressed PDO proliferation *in vitro* and reduced CDX and PDX tumor growth compared to monotherapies or controls.

Conclusion: Collectively, these findings establish a robust preclinical foundation for the development of PELP1-targeted combination strategies for the treatment of TNBC.

#4195 Efficacy of tumor treating fields (TTFields) applied with cisplatin and paclitaxel in cervical cancer cells.

Roni Frechtel-Gerzi[1], Daria Gerasimova[1], Einav Zeevi[1], **Helena Mumblat**[1], Yara Eid Mutlak[1], Yuval Shmueli[1], Roni Monin[1], Hila Fishman[1], Inbar Schlachet-Drukerman[1], Naama Flint-Brodsly[1], Eyal Dor-On[1], Itai Tzchori[1], Adi Haber[1], Moshe Giladi[1], Uri Weinberg[1], Yoram Palti[1], Greg M. Palmer[2], Angeles Alvarez Secord[3]

[1]Novocure Ltd, Haifa, Israel,[2]Department of Radiation Oncology, and Duke Cancer Institute, Duke University Medical Center, Durham, NC,[3]Duke Cancer Institute and Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Duke University Medical Center, Durham, NC

Introduction: Most cervical cancer patients with advanced disease receive first-line cisplatin-based chemotherapy. For late-stage and recurrent cervical cancer, a second chemotherapy may be added, e.g. paclitaxel. Tumor treating fields (TTFields) are electric fields that disrupt cellular processes critical for cancer cell viability and tumor progression. TTFields concomitant with cisplatin or paclitaxel have shown benefit in several tumor types. This study explored the effects of TTFields together with cisplatin and paclitaxel in cervical cancer cells and the underlying mechanism of action of TTFields.

Methods: Ca Ski, HeLa and SiHa human cervical cancer cell lines were exposed to TTFields (0.5 or 1 V/cm RMS; 200 kHz; 72 h), in the presence or absence of cisplatin and/or paclitaxel. Cell count, colony formation, and apoptosis were evaluated; and overall effect was calculated as the product of the reductions in cell count and colony formation. TTFields-treated cells were also examined for formation of DNA damage using fluorescent microscopy for γH2AX and for reduced capacity to repair DNA damage via Western blot for proteins from the Fanconi Anemia (FA)-BRCA pathway. The abundance of mitotic spindle defects and chromosome miss-localization was determined by fluorescent staining for α-tubulin and phospho-histone 3 (PH3).

Results: TTFields treatment reduced cell count and colony formation in all cervical cancer cell lines tested and augmented the efficacy of cisplatin and paclitaxel. Cells treated with TTFields demonstrated increased levels of DNA damage, reduced expression of DNA repair proteins, and formation of abnormal mitotic figures and chromosome miss-segregation.

Conclusions: The preclinical findings suggest benefit for co-treatment of TTFields together with the DNA-damaging agent cisplatin and the anti-mitotic agent paclitaxel. The effects of TTFields are mediated via anti-mitotic and DNA repair impairment mechanisms. Downregulation of the FA-BRCA pathway, required for the repair of cisplatin induced DNA damage, creates conditional vulnerability of the cells that may account for the enhanced efficacy for application of TTFields together with cisplatin.

#4196 Alt-NHEJ inhibition leads to cGAS/STING-dependent immunogenic cell death in multiple myeloma.

Giada Juli[1], Domenica Ronchetti[2], Sara Squillacioti[1], Nicoletta Polera[1], Anna Maienza[1], Michelangelo Vocaturo[1], Emanuela Altomare[1], Katia Grillone[1], Maria Gaetano[1], Stefania Signorelli[1], Antonio Galvano[3], Niccolo Bolli[2], Pietrosandro Tagliaferri[1], Pierfrancesco Tassone[1], **Daniele Caracciolo**[1]

[1]Magna Graecia University of Catanzaro, Catanzaro, Italy,[2]Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy,[3]Department of Precision Medicine in Medical, Surgical and Critical Care (Me.Pre.C.C.), University of Palermo, Palermo, Italy

Background: Alternative Non Homologous End Joining (Alt-NHEJ) DNA repair is considered a major player in cancer genomic instability. Here, we investigated cGAS-STING pathway as crucial node in the interplay between Alt-NHEJ repair and immune response, in the aim to discover novel therapeutic vulnerability in Multiple Myeloma (MM).

Methods: *In silico* analyses were performed by consulting public MM dataset CoMMpass. Anti-proliferative activity was evaluated by CellTiter-Glo, while flow cytometry analysis was used to determine the apoptotic process, cell cycle, phagocytosis, micronuclei detection, Calreticulin and T-cell activation markers. Protein expressions were detected by western blot of whole or fractioned protein extracts.

Results: We first examined the prognostic role of a cGAS-STING signature described by Sokac et al, by interrogating public MM database. 767 MM patients were selected from CoMMpass dataset and divided into 4 groups according to cGAS-STING signature groups (CSG 1-4). Interestingly, 130 patients were classified into CSG2 group with non functional cGAS-STING pathway, 105 into CSG3 with active cGAS-STING and 532 patients into CSG4 group with abnormal activation of cGAS-STING pathway; no patients where classified as CSG1 with inactive cGAS-STING. Importantly, CSG3-4 groups showed significantly reduced PFS and OS as compared to CSG2, supporting the idea that cGAS-STING has a pivotal role in the progression of MM. Indeed, Gene Set Enrichment Analysis (GSEA) of CSG3 versus CSG2 revealed differential involvement of several pathways such as DNA repair, TNFA signaling, oxidative phosphorylation and MYC targets. In addition, gene expression analysis showed a higher mRNA expression DNA Ligase 3 (LIG3) and PARP1 in CSG3 and CSG4 group as compared to CSG2. On this basis, we evaluated the activity of Alt-NHEJ inhibitors Talazoparib (PARP1-inhibitor) and L67 (LIG3 inhibitor) on MM cell lines, focusing in their capability to modulate cGAS-STING pathway. We first detected a significant reduction of cell proliferation and the induction of apoptosis following Talazoparib and L67 treatment, which in turn induced DNA damage response and cell cycle blockade, and finally cGAS-STING pathway activation. Finally, by performing co-culture experiments with healthy donor's peripheral blood mononuclear cells (PBMCs), we demonstrated the increase of *i)* CD80 and CD83 maturation markers on dendritic cells, *ii)* CD69 and CD25 activation markers on T cells and *iii)* phagocytosis of tumor cells. Importantly, these results were confirmed also in HR-defective breast and ovarian cancer cell lines, further suggesting the onset of an immunogenic cascade upon Alt-NHEJ inhibition.

Conclusion: Taken together, our findings indicate that Alt-NHEJ inhibitors are potential immune-stimulating agents for MM with hyperactivation of cGAS-STING pathway, coherently with our working hypothesis.

#4197 Synergic antioxidant effect of cord blood stem cells derived exosomes and sulforaphane on CML.

Banan Majid Alkhatabeh[1], Shohreh Jafarinejad[1], Mohammad Isreb[1], Farshid Sefat[1], Saeed Heidari[1], Adi Baumgartner[2], Mojgan Najafzadeh[1]

[1]University of Bradford, Bradford, United Kingdom,[2]York St John University, York, United Kingdom

Despite the promising advances in the diagnostics and therapeutics of Chronic Myeloid Leukaemia in recent years, they are still the disease of the era. Conventional treatments cause severe side effects, including death. Investigating novel drugs and combination therapies is essential for improving and advancing leukaemia and treatment. In the context of haematological cancers, elevated levels of reactive oxygen species (ROS) can induce oxidative stress. This oxidative stress may be mitigated by the administration of increased antioxidant levels. sulforaphane (SFN) is an antioxidant compound found mainly in cruciferous vegetables. Many studies demonstrated that the biological properties of SFN make it a suitable candidate for blood cancer treatment, including the activation of several metabolic processes and induction of apoptosis in cancer cells. Also, In the last few years, the ability of human cord blood stem cells (CBSC)-derived exosomes to mediate cellular communication has been extensively investigated. CBSC exosomes have proved beneficial in treating various diseases. This study aimed to investigate the impact of CBSC exosomes and SFN *in vitro* on haematological disease in general and CML in particular, compared to healthy controls. The Comet assay was used to assess the impact of SFN and CBSC exosomes on DNA damage, as well as to evaluate the potential genoprotective effects of these treatments at the presence of H_2O_2 exposure. A high level of DNA damage was observed in blood cancer (such as lymphoma and multiple myeloma) PMBCs compared to healthy controls. In this study involving PMBCs from cancer patients (N=20) and healthy controls (N=20), treatment with two concentrations of sulforaphane (SFN) (10 and 15μM), along with the application of 12% v/v CBSC exosomes, demonstrated a significant reduction in DNA damage induced by hydrogen peroxide (H_2O_2), with a p-value($p<0.001$). The doses employed in this study had a minimal impact on the viability of healthy cells while demonstrating a noticeable reduction in cell viability among CML cells, as assessed by the CCK-8 assay. In this research we utilised qPCR and Western Blotting techniques to investigate the influence of SFN and hCBSC-derived exosomes, as well as SFN-CBSC exosomes, on the gene and protein expression related to the apoptosis pathway in CML cells. This study focused on key markers such as p53, p21, Bcl-2 genes and proteins, and Caspase-3, in addition to examining the BCR-ABL gene associated with tumorigenicity. We found that all previous treatments were able to induce apoptosis and reduce the oncogenicity of CML compared to negative control.

#4199 Defining roles of telomerase reverse transcriptase in DNA damage response.

Leon L. Zhou, Stephen J. Kron

University of Chicago, Chicago, IL

Telomerase Reverse Transcriptase (TERT), the catalytic subunit of telomerase, is essential for telomere maintenance, elongating the G-strand of telomeric DNA using its associated RNA template (TR). Recruitment of TERT to telomeres is mediated by the shelterin component TPP1. While TERT is highly expressed in cancer cells, efforts to target its telomeric function have shown limited clinical efficacy. Recent studies have identified noncanonical roles for TERT, particularly in the DNA damage response (DDR), suggesting alternative therapeutic opportunities. Notably, TERT inhibitors sensitize cancer cells to radiotherapy, though the mechanisms underlying this effect remain unclear. To address this gap, we established a mutant TERT phenotyping system to study how TERT variants affect the DDR. Using a tetracycline-inducible shRNA-miR knockdown system in MCF7 and HeLa cells, we targeted endogenous TERT and complemented with transfected wildtype or mutant TERT, validating expression by Western blot. Cells expressing only transfected TERT were irradiated with 6 Gy to induce double-strand breaks (DSBs), and DDR effects were assessed by 53BP1 or γH2AX foci resolution, cell survival, and senescence onset. Knockdown of TERT led to persistent DNA damage foci, confirming a role for TERT in DSB repair. Foci resolution was restored by WT TERT but not by the catalytic mutant TERT D712A, V713I. Similarly, the TERT R132E, K78E mutant, defective in TPP1 binding, exhibited persistent foci, implicating TERT-TPP1 interactions in DDR. Supporting this, TPP1 mutants unable to bind TERT also caused persistent DNA damage foci. Combined with evidence that shelterin complexes, including TPP1, localize rapidly to nontelomeric DSBs after DNA damage, these results suggest that TPP1 recruits TERT to DSBs, analogous to its role at telomeres. Ongoing studies aim to elucidate how TERT's catalytic activity promotes DSB repair and to explore TERT as a target for sensitizing cancer cells to therapy.

#4200 Elevated dNTP pools impair homologous recombination-mediated repair, promoting apoptosis in therapy-resistant glioblastoma.

Dominique Monroe, Mercy Kehinde-Ige, Apeksha Anand, Arilyn Williams, Edidiong Usoro, Matthew Kededa, Aman Kalsi, Christian Fahmy, Ramsha Khanam, Huidong Shi, Waaqo Daddacha

Medical College of Goergia, Augusta, GA

Glioblastoma (GBM) is a deadly brain tumor that often develops resistance to DSB-inducing therapeutics due to dysregulated DNA repair. Ionizing radiation (IR) targets these cancer cells by causing DNA double-stranded breaks (DSBs). However, GBM often has overactive genes involved in the error-free DSB-repair process known as Homologous Recombination (HR). This results in therapy resistance and poor patient outcomes. Here, we provide an alternative approach to target DSB-resistant GBM by modulating the dNTP pool instead of solely targeting DNA-repair proteins. Through a comet assay, we found that increasing the dNTP pool prior to IR resulted in impaired DSB-repair. Immunofluorescence analysis of key HR proteins revealed significant disruptions in end resection, a necessary phase of HR. Our RNA-seq results show that increasing the dNTP pool prior to IR treatment caused significant changes in gene expression when compared to IR or dN treatment alone. Pathways most affected include apoptosis and cell cycle arrest. Flow cytometry analysis confirmed the significant increase in apoptosis in irradiated cancer cells with elevated dNTP pools. Not only do these data suggest that HR efficiency is reduced due to end resection impairment, but they also show that as a result, cell viability is destabilized due to prolonged DNA damage, resulting in apoptosis. The identification of this impairment may be essential in finding novel therapeutic approaches to sensitize therapy-resistant GBM.

#4201 Combination of the PARP1-selective inhibitor saruparib with androgen receptor pathway inhibition provides combination benefit in preclinical models of prostate cancer with or without homologous recombination repair deficiency.

Mark R. Albertella, Giuditta Illuzzi, Anna D. Staniszewska, Sophie Cooke, Mark J. O'Connor, Josep V. Forment

AstraZeneca, Cambridge, United Kingdom

Recent phase 3 clinical trial readouts have shown benefit of the combination of poly(ADP-ribose) polymerase inhibitors (PARPi) with androgen receptor (AR) pathway inhibitors (ARPi) in metastatic, castration-resistant prostate cancer (mCRPC). While benefit was particularly evident in patients with tumors harboring mutations in homologous recombination repair (HRRm) genes, improved outcomes were also observed in patients with no such alterations in their cancers (non-HRRm). We evaluated the activity of the PARP1-selective inhibitor and trapper saruparib (AZD5305) in combination with the ARPi enzalutamide in a range of preclinical models in vitro and in vivo. Combination treatment of clinically relevant doses of 1mg/kg saruparib and 60mg/kg enzalutamide led to regressions or tumor control in BRCA2-mutant PDX models of prostate cancer, C901 and G266, and in the CDK12-mutant HRRm model PCU-012. Importantly, enhanced in vivo combination efficacy was also observed in non-HRRm prostate models LNCaP and C4-2, on treatment with saruparib and enzalutamide. Saruparib led to enhanced DNA damage after treatment in these models. We explored the mechanisms of action contributing to this observed combination benefit, focusing on the non-HRRm models where this was observed. The cell line C4-2 was not sensitive to saruparib alone, as expected, but showed enhanced sensitivity to the combination of enzalutamide with saruparib. Strikingly, this was not observed with the weaker PARP1 trapper veliparib, suggesting a requirement for trapping-induced DNA damage. The combination of enzalutamide and saruparib led to increased DNA double strand break formation, and enhanced accumulation of micronuclei, suggesting that this combination benefit is driven by an increase in unrepaired DNA damage. Enzalutamide treatment of C4-2 cells reduced the soluble nuclear fraction of the AR, as expected. Importantly, inhibition of PARP1 by saruparib reduced AR recruitment to chromatin in the presence of DNA damage. Combined treatment led to maximal reduction in AR recruitment, which may help explain the increased efficacy. These preclinical data demonstrate combination activity of saruparib and ARPi in HRRm as well as non-HRRm prostate cancer models and provide mechanistic evidence that combination benefit is driven by PARP1 trapping inducing DNA damage, and PARP1 inhibition and ARPi inhibiting the repair of this damage, leading to accumulation of cytotoxic genomic instability to the cancer cells. A clinical study ASCERTAIN (NCT05938270) has been initiated, where the focus is on collection of pre- and post- treatment biopsies from monotherapy and combinations of saruparib, and the ARPi darolutamide, with the aim of increasing further understanding of mechanisms of action between PARPi and ARPi.

#4202 PD-L1 promotes DNA double-strand-break repair and radioresistance in cancer.

Zhen Shu[1], Bhakti Dwivedi[2], Jeffrey Switchenko[3], David Yu[1], Xingming Deng[1]

[1]Radiation Oncology, Winship Cancer Institute, Emory University, Atlanta, GA,[2]Bioinformatics and Systems Biology Shared Resource, Winship Cancer Institute, Emory University, Atlanta, GA,[3]Department of Biostatistics and Bioinformatics, Rollins School of Public Health, Emory University, Atlanta, GA

Resistance to radiotherapy is a major barrier during cancer treatment. Using genome-scale CRISPR/Cas9 screening, we identified that the *CD274* gene, which encodes PD-L1, confers lung cancer cell resistance to ionizing radiation (IR). Depletion of endogenous PD-L1 retarded the repair of IR-induced DNA double-strand breaks (DSBs). Treatment of lung cancer cells with IR or glycosylation inhibitor tunicamycin resulted in translocation of PD-L1 from the membrane into nucleus in a manner that is dependent on deglycosylation of PD-L1 at N219. CMTM6 transports PD-L1 from the cytoplasm into the nucleus via direct interaction. PD-L1 loss downregulated non-homologous end joining (NHEJ) while overexpression of PD-L1 upregulated NHEJ. Mechanistically, after IR exposure, PD-L1 was recruited to DSB sites to form foci, and to interact with Ku in the nucleus. PD-L1 not only enhanced Ku binding to DSB DNA but also facilitated binding of Ku to other NHEJ factors. PD-L1 deglycosylation and its interaction with Ku occurred in a cell cycle-dependent manner. Interaction between the IgC domain of PD-L1 and the core domain of Ku is required for PD-L1 to accelerate NHEJ-mediated DSB repair and produce radioresistance. Thus, PD-L1, in addition to its immune inhibitory activity, also acts as a mechanistic driver for NHEJ-mediated DSB repair in cancer. Specifically targeting this novel function of PD-L1 may represent an attractive strategy for cancer therapy.

#4203 Discovery and development of a potent and highly selective ATR inhibitor IMP9064.

S. Cai, N. Ma, X. Wang, Y. Jiang, M. Guo, R. Zhou, M. Chen, Y. Tian;
IMPACT Therapeutics (Shanghai), Inc., Shanghai, China

The ataxia telangiectasia and RAD3-related (ATR) kinase is a crucial component of the DNA damage response (DDR) and functions in conjunction with ataxia telangiectasia mutated (ATM). Loss or functional deficiency of ATM may lead to increased reliance on ATR signaling pathways. Preclinical and clinical studies have indicated a potential synthetic lethality between ATR inhibition and ATM deficiency. We have identified a novel, potent, and highly selective ATR inhibitor IMP9064. Herein we present its discovery and IND enabling *in vitro* and *in vivo* studies. IMP9064 is highly activity inhibiting ATR, and is highly selective among kinases. IMP9064 also shows potent cytotoxicity to a wide range of cancer cell lines. In addition, IMP9064 has a desirable PK profile in preclinical species. IMP9064 has demonstrated anti-tumor efficacy in human colorectal cancer CDX animal models with good dose-response tumor growth inhibition and tolerability. The results of *in vitro* and *in vivo* studies suggest that used alone or in combination with PARP inhibitor, WEE1 inhibitor, PKMYT1 inhibitor or HER2 ADC, IMP9064 has good anti-tumor activity and synergistic effect. IMP9064 has entered a phase 1/2 study to evaluate the safety and efficacy either as monotherapy or in combination with PARP inhibitor Senaparib in patients with advanced solid tumors (ClinicalTrials.gov Identifier: NCT05269316). The recommended phase 2 dose (RP2D) has been determined and IMP9064 is currently in expansion studies for selected tumors.

#4204 A novel triple combination therapy in small cell lung cancer targeting DNA damage repair as a mechanism of resistance.
Ashley Sanchez Sevilla Uruchurtu, Kevin Kwon, Shengliang Zhang, Tyler Roady, Wafik S. El-Deiry

Brown University, Providence, RI

Small cell lung cancer (SCLC) accounts for approximately 15% of new lung cancer cases in the US every year. Treatment options remain limited and 5-year survival rates have languished at around 7% for the past decade. SCLC is notorious for its response to standard etoposide-platinum doublet therapy; initially responsive, it almost always relapses and no longer responds to first-line treatments. The DNA damage response (DDR) has been credibly posited as a targetable mechanism of resistance to DNA-damaging agents. For our experiments, we added prexasertib to our prior combination of imipridone ONC201 (dordaviprone) and small molecule lurbinectedin, previously shown to be synergistic. Prexasertib is a dual inhibitor of Chk1 and 2, protein kinases in the DDR pathway that regulate downstream cell cycle checkpoint (CCC) proteins particularly implicated in G1/S transition and G2 entry. Synergy scores and western blot data from experiments in four cell lines (H1048, H1882, H1105, H1417) point to the role of the DDR-CCC pathway in SCLC treatment resistance and offer a novel treatment modality for testing in vivo, with the ultimate view to progress into the clinic.

#4205 Direct measurement of TA repeat expansions significantly outperforms MSI-H status as a predictor of sensitivity to the novel WRN inhibitor MOMA-341.

Anthony Tubbs[1], Javad Golji[1], Meredeth McGowan[1], Theresa Baker[1], Giulia Bottoni[1], Erin Brophy[1], Xin Cindy Yang[1], Haley Amemiya[1], Momar Toure[1], John R. Butler[1], Haoxuan Wang[2], Cen Gao[1], Timothy Guzi[1], Peter Hammerman[1], Erica Evans[1], **Allison Drew**[1]

[1]MOMA Therapeutics, Cambridge, MA,[2]Insitro, South San Francisco, CA

MOMA-341, first disclosed herein, is a novel, potent, and selective clinical stage small molecule inhibitor of the Werner RecQ like helicase (WRN) that acts through an allosteric, ATP-competitive binding mechanism and covalent ligation of WRN at cysteine 727. Consistent with WRN genetic depletion in MSI-H models, MOMA-341 induces DNA damage, cell death, and tumor regressions in mismatch-repair deficient (dMMR)/microsatellite instability high (MSI-H) models but has no effect in MSS tumor models or cell lines derived from normal tissues. MSI-H status, defined by loss of MMR proteins or using short read sequencing algorithms, correlates with MOMA-341 single agent activity in preclinical models but is an imperfect predictor, with a 62% Positive Predictive Value (PPV) and a 38% False Positive Rate (FPR) (N=40 MSI, 8 MSS), consistent with rates reported with other WRN inhibitors. In contrast, direct measurement of genome-wide TA repeat expansions, the substrate of WRN helicase and a feature of many MSI-H tumors, by long read sequencing produces a near-perfect prediction of sensitivity to WRN inhibition across a large cohort of preclinical tumor models spanning colorectal, gastric, and endometrial lineages (N= 36 MSI-H, 8 MSS). Long-read sequencing provides optimal insight across the genome for this purpose as it can discriminate the location, total length, and allelic heterogeneity of TA repeat expansions, whereas the TA repeat expansions that dictate WRN sensitivity are largely invisible to commonly used short read sequencing methods. While MSI-H tumors with highly expanded TA repeat regions were quite sensitive to MOMA-341, incomplete single agent antitumor activity was observed in tumors with lower levels of TA repeat expansion. However, these tumor responses could be converted to regressions when MOMA-341 was combined with chemotherapies such as irinotecan. This work demonstrates clearly that direct and specific measurement of the TA repeats that serve as a WRN helicase substrate is feasible, quantifiable and results in near perfect prediction of MOMA-341 anti-tumor activity in preclinical models, thereby successfully matching the relevant biology with pharmacologic response. Guided by this work, TA repeat expansions will be assessed in patient tumors within the upcoming Phase 1 clinical trial of MOMA-341 in dMMR/MSI-H tumors to better inform patient selection and the choice of single agent or combination dosing regimens.

#4206 Investigation into the mechanisms and therapeutic potential of R-loop-induced DNA damage in ER+ breast cancer.

Alyssa Roberts[1], Nicole Traphagen[1], Anneka Johnson[1], Eugene Demidenko[2], Patricia Muskus[3], Xiaofeng Wang[1], Todd W. Miller[3]

[1]Dartmouth College, Hanover, NH,[2]Biomedical Data Science, Dartmouth College, Hanover, NH,[3]Medical College of Wisconsin, Milwaukee, WI

A potential therapeutic vulnerability of endocrine-resistant estrogen receptor alpha (ER)-positive breast cancer is R-loop-mediated DNA damage induced by 17b-estradiol (E2). This study aims to elucidate the mechanism of DNA damage and test combination therapies to exacerbate the anti-cancer effects of E2. ER+ breast cancer cells overexpressing exogenous ER, which induces sensitivity to the growth-inhibitory effects of E2, were exposed to E2 and inhibitors of DNA damage repair proteins including poly-ADP ribose polymerases (PARP) and CHK1/2. Experimental endpoints included immunoblotting, flow cytometry, immunofluorescence, DNA fiber analysis, and growth assays using cell lines and patient-derived xenografts (PDXs). Cells overexpressing ER showed R-loop-dependent DNA damage in replicating cells. Combination therapy of E2 and the PARPi olaparib increased DNA damage in replicating cells without negatively impacting the elevated replication rates induced by E2. E2 significantly increased single-stranded DNA in ER-overexpressing cells; ssDNA is known to be susceptible to damage. These effects of E2 were exacerbated by PARPi. E2 increased the proportion of cells replicating DNA, but E2 paradoxically interfered with replication fork progression as shown by DNA fiber analysis. These findings suggest that replication interference contributes to DNA damage and eventual cell death but does not impair the ability for the cell to replicate. An alternative therapeutic approach is combination therapy with E2 and Chk1/2 inhibitor. This combination successfully inhibits growth of both parental and ER overexpressing cells indicating that E2 and Chk1/2 inhibition may have clinical potential. E2 induced DNA damage mediated by R-loops can be amplified using combination therapies targeting DNA damage repair or cell cycle inhibition. While it is clear that replication conflicts plays an important role in DNA damage, the mechanism in which the DNA damage is occurring has yet to be elucidated.

#4207 Discovery of WRN helicase inhibitors that covalently engage a novel, induced allosteric site.

Francis W. Hunter[1], Lindsey DeRatt[1], Kenneth Maksimchuk[1], Tatsuya Maehigashi[1], Seong Joo Koo[2], Liesbeth Micholt[2], Ranjeet P. Dash[1], Faraz Kazmi[1], Zhengyu Jiang[1], Albi Francis[1], Neetu Shukla[1], Aaron Patrick[1], Trpta Bains[1], Romy Perez-Abraham[1], Nikit Kumar[1], Zhijie Liu[1], Brian Reiss[1], Caitlin Morgan[1], Inha Heo[2], Werner Eyckmans[2], Gerald Chu[1], Anna Kuhn[1], Lorraine Angelillo[1], Lin-Chien Huang[1], Yu Sun[1], Ashley M. Rouleau[3], Yongkang Kim[3], John J. H. Shin[3], Harris Bell-Temin[3], Victoria Wong[1], Ruth Steele[1], Paul Shaffer[1], Kurtis E. Bachman[1], David Pocalyko[1], Scott Kuduk[1]

[1]Johnson & Johnson Innovative Medicine, Spring House, PA,[2]Johnson & Johnson Innovative Medicine, Beerse, Belgium,[3]Johnson & Johnson Innovative Medicine, Cambridge, MA

The RecQ helicase WRN is a synthetic lethal dependency in microsatellite instability-high (MSI-H) cancer and a therapeutic target of interest in that context. The two clinical-stage WRN inhibitors, HRO761 and RO7589831, both bind in an allosteric pocket near the interface of the two helicase domains (D1 and D2). To discover novel WRN inhibitors, we conducted a high-throughput phenotypic screen using γH2AX immunofluorescence imaging in MSI-H HCT116 colorectal cancer cells and identified a singleton hit, Compound A. Compound A phenocopied WRN siRNA knockdown in multiparametric analysis and was confirmed to engage and inhibit purified WRN protein. X-ray co-crystal structures with and without ATP revealed that Compound A binds to a novel allosteric site in the D1 domain which is absent in apo-WRN. The allosteric site was induced by localized rearrangement, including displacement of the DEAH-box upon ligand binding. Electron density was indicative of covalent bond formation via addition of Cys672 to the imine C=N bond of Compound A. Mechanistically, Compound A was ATP-noncompetitive, exhibited time-dependent inhibition of WRN ATPase and helicase activity, and did not inhibit the paralogous RecQ helicases BLM, RECL and RECQL4. Exploration of structure-activity relationships resulted in Compound B and Compound C that were characterized for therapeutic pharmacology. Both Compounds B and C demonstrated high potency and selectively induced γH2AX phosphorylation, cell cycle arrest, apoptosis, and clonogenic killing in MSI-H cells. The cytotoxicity of Compound C in HCT116 cells was unaffected by isogenic, CRISPR-mediated knockout of *BLM*, *RECQL*, *RECQL4*, *RECQL5* or *BRIP1* (*FANCJ*). In global proteomics and cell signaling studies, Compound C caused concentration-dependent degradation of WRN and upregulation of DNA damage response pathways in HCT116 but not microsatellite stable (MSS) SW620 colorectal cancer cells. Compound B was highly selective for MSI-H models and recapitulated the profile of sensitivity to WRN perturbation in a PRISM screen of 906 cell lines. Co-administration of Compound C with a CYP inhibitor to mice bearing HCT116 xenografts produced dose-dependent γH2AX phosphorylation in tumor tissue. Whole-genome CRISPR modifier screens with Compound C and a close analog of HRO761 in HCT116 and KM12 cells showed generally concordant functional genomic profiles, with perturbations in *SMARCAL1*, *HELLS*, and *WDR76* enriched and *POLQ* and *RBM6* depleted by WRN inhibition. In conclusion, we report a series of WRN inhibitors that engage a novel cryptic binding site and show a distinct mechanism of inhibition to WRN inhibitors currently in clinical development.

#4208 Azenosertib is a potent and selective WEE1 kinase inhibitor with broad antitumor activity across a range of solid tumors.

Wen Liu[1], Jianhui Ma[1], Jiali Li[1], Daehwan Kim[1], Sangyub Kim[1], Alexandra Levy[1], Kimberly Blackwell[1], Alejandro Recio-Boiles[2], Jennifer M. Segar[3], Shirai Sen[4], Deborah Doroshow[5], Danielle Jandial[1], Olivier Harismendy[1], Stephan K. Grant[1], Ahmed Samatar[1], Mark R. Lackner[1], Kimberlee M. Fischer[1]

[1]Zentalis Pharmaceuticals, San Diego, CA,[2]Department of Medicine, Hematology and Medical Oncology, The University of Arizona Comprehensive Cancer Center, Tucson, AZ,[3]Department of Medicine, Hematology and Medical Oncology, University of Arizona Comprehensive Cancer Center, Tucson, AZ,[4]NEXT Oncology, Dallas, TX,[5]Thoracic Oncology, Early Phase Trials Unit, Icahn School of Medicine at Mount Sinai, New York, NY

Due to high proliferative rate, cancer cells experience significant stress during DNA replication and mitosis, leading to DNA damage and increased genomic instability. To mitigate this, cancer cells rely on checkpoint kinases like WEE1, which halt cell cycle progression and allow damaged DNA to be repaired before cells enter mitosis. Damage that is too extensive leads to apoptosis. Targeting WEE1 can exploit vulnerabilities created by replicative stress and genomic instability. Given these features are common in cancer, we hypothesized a WEE1-targeted therapeutic would have broad antitumor activity across various cancer types. Azenosertib (ZN-c3) is a novel, selective, orally bioavailable WEE1 inhibitor with optimized pharmacokinetic and pharmacodynamic properties. Preclinical studies demonstrate Azenosertib inhibits cancer cell proliferation through a WEE1-dependent mechanism, marked by a reduction of pY15-CDK1 levels and increases in DNA damage markers. Herein, we evaluated its antitumor activity in 54 mouse xenograft models representing 9 solid tumor subtypes and observed robust tumor growth inhibition (> 60% TGI) in 24 of these models, including breast, colorectal, lung, ovarian, pancreatic, prostate, soft tissue sarcomas, and endometrial cancers. We also explored various dosing schedules for Azenosertib, identifying regimens that maintain efficacy with minimal toxicity in preclinical models. To further understand the mechanisms underlying Azenosertib's antitumor activity, we analyzed cell cycle changes in four cancer cell lines with variable sensitivity to Azenosertib. Treatment with Azenosertib caused accumulation of EdU-negative cells in the S-phase, reduced cells in the G1 phase, and promoted premature entry into mitosis. These effects suggest Azenosertib bypasses the G1/S and G2/M checkpoints, leading to immature S-phase and Mitosis entry, consistent with WEE1 inhibition of CDK1 and CDK2. Further analysis of DNA repair revealed an increase in γH2AX-positive cells, primarily in G1, EdU-negative S, and G2/M, and the co-localization of mitotic marker pHH3 and DNA damage marker γH2AX. Notably, WEE1 plays a critical role in safeguarding the integrity of the cell cycle, and this regulation is disrupted by Azenosertib. As a result, cells with unresolved DNA damage are driven into premature mitosis, exacerbating DNA damage and leading to mitotic catastrophe. Azenosertib is currently being investigated as a single agent in a dose escalation Phase I clinical trial (NCT04158336) evaluating safety, tolerability, and efficacy in cancer patients with previously treated, advanced solid tumors. Early clinical data from heavily pretreated patients with different types of cancer show promising signs of activity. Our findings highlight the potential of WEE1 inhibition as a broad therapeutic strategy, for gynecological cancers and various malignant indications.

#4209 Targeting RNase H2: a dual mechanism strategy to elevate replication stress, DNA damage, and antitumor immunity in triple-negative breast cancer.

Thai Quynh Anh Nguyen[1], Daniel J. McGrail[2], Hui Dai[1], Aysegul Cifci[1], Yong Du[1], Funda Meric-Bernstam[3], Shiaw-Yih Lin[1]

[1]Systems Biology, The University of Texas MD Anderson Cancer Center, Houston, TX,[2]Center for Immunotherapy and Precision Immuno-Oncology, Cleveland Clinic, Cleveland, OH,[3]Department of Investigational Cancer Therapeutics, The University of Texas MD Anderson Cancer Center, Houston, TX

The inhibition of Ribonuclease H2 (RNase H2) activity offers a promising therapeutic approach for suppressing tumor growth in triple-negative breast cancers (TNBCs) by elevating DNA replication stress, inducing DNA damage, inducing cell death, and enhancing sensitivity to immunotherapy. RNase H2, a key enzyme responsible for removing misincorporated ribonucleotides from the genome, plays a pivotal role in maintaining genomic integrity. Overexpression of the RNASEH2A gene, which encodes the catalytic subunit of the RNase H2 complex, has been observed in breast tumors, with the highest levels detected in TNBC tumors and cell lines, making it a potential target for therapeutic intervention. This study explores the impact of RNase H2 inhibition on tumor proliferation, antitumor immunity, and its potential synergy with other DNA replication stress inhibitors. Our findings demonstrate that silencing RNASEH2A expression or pharmacologically inhibiting RNase H2 activity with the R14 inhibitor significantly increased levels of pRPA32 and γH2AX, indicating elevated DNA replication stress and DNA double-strand breaks. This was associated with substantial suppression of tumor growth and prolonged survival in TNBC models, while non-tumorigenic mammary epithelial cells remained unaffected. RNA sequencing analysis revealed that knockout of RNASEH2A in TNBC cell lines activated immune system signaling pathways, including increased production of T-cell-attracting chemokines such as CCL5, CXCL10, and CXCL11. This increase in immunostimulatory cytokine expression was attributed to the accumulation of cytosolic single-stranded DNA, which enhances innate immune responses. Moreover, pharmacological suppression of RNase H2 by the R14 inhibitor improved the efficacy of immune checkpoint blockade by promoting the infiltration of antitumor immune cells into the tumor microenvironment. This indicates that RNase H2 inhibition not only directly suppresses tumor growth but also modulates the tumor immune landscape, rendering TNBCs more responsive to immunotherapy. Additionally, the R14 inhibitor exhibited synergistic effects when combined with DNA replication stress inhibitors, including ATR inhibitors, PARP inhibitors, and their combination, consistent with the known role of RNase H2 deficiency in sensitizing cells to these therapeutic strategies. In conclusion, targeting RNase H2 activity represents a novel and dual-faceted therapeutic strategy in TNBCs, combining direct tumor growth suppression through increased DNA damage with enhanced antitumor immunity. This approach offers a promising avenue for combination therapy with immune checkpoint inhibitors and DNA replication stress modulators. These findings provide a rationale for the clinical development of RNase H2 inhibitors as part of an integrated treatment strategy for TNBC.

#4210 Replication stress detection using STRIDE technology platform.

Karolina Stępień, Kamila Schirmeisen, Jakub Lechowski, Magdalena Bartyńska, **Magdalena Kordon-Kiszala**, Kamil Solarczyk

intoDNA, Krakow, Poland

Replication stress, characterized by the slowing or stalling of replication forks, is a hallmark of many cancers and a key driver of genomic instability. Therapeutics targeting the ATR kinase, a central regulator of the replication stress response, have emerged as promising strategies for selectively exploiting the vulnerabilities of cancer cells. A reliable marker of replication stress is essential to better understand its dynamics and to optimize the development and application of DNA damage response (DDR) inhibitors. Such markers can provide critical insights into therapeutic mechanisms and enhance the precision of targeted treatments. Single-strand DNA breaks (SSBs) are both a cause and a consequence of replication stress, making them a potential biomarker. However, their occurrence during normal metabolism requires additional specificity, which can be provided by detecting replication stress-associated proteins. Here, utilizing our STRIDE technology platform, we combined direct *in situ* detection of SSBs with protein markers leading to the development of an assay aimed at measuring replication stress levels. The assay combined the detection of single-strand DNA breaks (SSBs) with replication stress-associated proteins: RPA, RAD17, and RAD9, evaluated separately in distinct assay variants. Each variant was assessed for steady-state readouts, negative control signal levels, and sensitivity to replication stress induced by known compounds. All variants effectively informed on replication stress induction, and baseline readouts of the assays correlated with the cellular response to replication stress-inducing agents. Importantly, the assays were also tested on FFPE tissue sections, demonstrating their feasibility for application to this type of material. The data collected thus far suggests that all assay variants hold potential as effective replication stress markers. Additional studies are required to further refine these assays, determine their optimal use in different contexts, and confirm their utility in identifying high replication stress backgrounds to predict responses to drugs targeting proteins involved in the replication stress response.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: HDAC and Methyltransferase Inhibitors
Poster Session

#4215 Targeting TNBC with a novel vorinostat conjugate that selectively inhibits and degrades HDAC3 and HDAC6.

Ping Gong[1], Min Huang[1], Haoling Wang[1], Xin Li[1], Linxiang Zhao[1], Samuel Waxman[2], Yongkui Jing[1]

[1]Shenyang Pharmaceutical University, Shenyang, China,[2]Icahn School of Medicine at Mount Sinai, New York, NY

Triple-negative breast cancer (TNBC) once metastatic is often a fatal disease with few molecular targets. HDAC3 is highly expressed in TNBC and regulates metastatic and cell death genes. The clinically approved pan-HDAC inhibitors exhibit limited efficacy with side effects in clinical trials. We have reported that the semisynthetic 18β-glycyrrhetinic acid analogues (10a-10e) bearing the 1-en-2-cyano-3-oxo substitution degrade HDAC3/6 proteins without direct enzymatic inhibition. We constructed AH3 to produce both HDAC 3/6 degradation and enzyme activity inhibition by 10a conjugation with the HDAC inhibitor vorinostat. AH3 has increased selectivity to HDAC3 and HDAC6 inhibition as compared to vorinostat. TNBC (MDA-MB-321, MDA-MB-468 and BT-459 cells) are more growth inhibited by AH3 (GI_{50}:0.6-1 μM) comparing to vorinostat (GI_{50}:1.9-2.9 μM) and exhibits selectivity for TNBC compared to normal mammary epithelial cells. Modification of the hydroxamic acid of AH3 with a methyl group (AH3-OCH3) loses HDAC inhibition and cell growth inhibition, confirming that HDAC3 and HDAC6 are the targets of AH3. AH3 inhibits colony formation, cellular invasion and migration in TNBC cells. AH3, but not AH3-OCH, induces apoptosis with downregulation of c-FLIP and upregulation of NOXA, associated with upregulation of acetyl-H3 and acetyl-tubulin as well as down-regulation of HDAC3 and HDAC6 proteins. AH3 has better tumor growth inhibition ability than verinostat in MDA-MB-231 xenograft model. AH3 is novel since both degrades and acts as an HDAC3/HDAC6 selective inhibitor with enhanced selectivity and antitumor ability against TNBC.

#4216 Advancing cancer immunotherapy through the development of a novel selective HDAC6 inhibitors.

Nithya Gajendran[1], Manasa Suresh[1], David Quiceno[1], **Sonia Sebaoui**[1], Xintang Li[1], Marie Durr[1], Sebastian J. Marquez Rodriguez[2], Mario A. Noboa[3], Sruthi Mohan[3], Barbora Havlinova[4], Julia Kudlacova[4], Annie Heuer[5], Satish Kumar Reddy Noonepalle[5], Cyril Barinka[4], Duncan J. Wardrop[2], Alejandro Villagra[1]

[1]Georgetown Lombardi Comprehensive Cancer Ctr., Washington, DC,[2]Department of Chemistry, University of Illinois at Chicago, Chicago, IL,[3]University of Illinois at Chicago, Chicago, IL,[4]Laboratory of Structural Biology, Institute of Biotechnology of the Czech Academy of Sciences, Czech Republic, Czech Republic,[5]Department of Oncology, Georgetown Lombardi Comprehensive Cancer Ctr., Washington, DC

Immune cells can significantly influence the therapeutic outcomes of anti-cancer therapies. Particularly, tumor-associated macrophages (TAMs) mainly exhibit an anti-inflammatory M2-like phenotype and are well recognized for their pro-tumoral activity, which supports tumor growth and suppresses anti-tumor immune responses. Nonetheless, TAMs exhibit remarkable plasticity, allowing for their reprogramming toward an anti-tumoral M1-like phenotype through targeted epigenetic modulations. Our group has previously reported several highly selective histone deacetylase 6 inhibitors (HDAC6is) that promote the polarization of TAMs toward the M1-like phenotype while suppressing the M2-like pro-tumoral phenotype. Despite this promising discovery, the current HDAC6is face several drawbacks, including efficacy at micromolar concentrations, which results in poor isoform selectivity and potential off-target effects. To address these limitations, we developed an advanced screening pipeline for HDAC6is on macrophage functions, and the antitumor benefits associated with their treatment, alone or in combination with anti-PD1 in the immunocompetent SM1 murine melanoma model. Based on an *in silico* screening of 980 HDAC6is derivatives and a macrophage-based screening described previously, we identified two candidates, SM-05-947 and SM-06-09, that showed superior properties compared to their parent molecules. The screening included the determination of cytotoxicity, specificity of HDAC inhibition, and the modulation of macrophage phenotype. Using bone marrow-derived macrophages, the treatment-associated changes in macrophage functions were characterized. Following dose determination, we studied their antitumor effects on SM1 melanoma tumors when administered as a single agent or combined with anti-PD1 antibodies. The tumor microenvironment was further characterized by immunotyping using flow cytometry, CyToF, and sc-RNA sequencing. The functional assays provided further evidence of increased phagocytosis of tumor cells and improved antigen cross-presentation with HDAC6 inhibition. Specifically, the presence of SIINFEKL peptide in the context of MHC class I in macrophages treated with HDAC6is showed a significant increase in antigen presentation by M1 macrophages. Administration of SM-06-09 and SM-05-947 as a single agent significantly reduced tumor growth when compared to the vehicle group. The two combination groups of HDAC6is and anti-PD1 antibodies further significantly improved these antitumor effects, as observed by flow cytometry, CyToF, and sc-RNA sequencing analysis. In conclusion, these findings provide valuable insights and pave the way toward the development of next-generation HDAC6is with maximum efficiency, minimal toxicity, and enhanced anticancer potential of TAMs.

#4217 Effect of HDAC3 targeting on endometrial cancer progression and therapeutic potential.

Keun Cheon Kim, Dunston Krystina, Hall Amanda, Hunter I Mark, Tae Hoon Kim, jaewook Jeong

University of Missouri, Columbia, MO

Endometrial cancer (EC) exerts a considerable burden on women's health worldwide. Epigenetic dysregulation is often reported in the development and progression of various cancers. A negative regulator of PI3K signaling and frequently found to be mutated in human EC is *PTEN* whereas *HDAC3* is critical in cell survival, proliferation, differentiation, and cancer development. HDAC inhibitors are being explored as an epigenetic treatment option, but their effects in EC are not well characterized. $Pgr^{cre/+}Hdac3^{f/f}Pten^{f/f}$ ($Hdac3^{d/d}Pten^{d/d}$) mice were generated and histopathologic analysis was performed to examine the effect of *Hdac3* ablation on EC with *Pten* deficiency. $Hdac3^{d/d}Pten^{d/d}$ mice revealed remarkably delayed tumorigenesis of EC compared to $Pten^{d/d}$ mice. To evaluate the effect of HDAC3 inhibitor (RGFP966), 6-week-old $Pten^{d/d}$ (Stage I EC) mice were treated with vehicle (sunflower oil) or RGFP966 (10mg/kg) twice per week for 5 weeks by intraperitoneal injection. The effect of EC progression was examined by immunohistochemistry using proliferation and apoptosis markers. In RGFP966-treated $Pten^{d/d}$ mice, uterine/body weight ratio (average 22.56 ± 2.99) was significantly ($p< 0.05$) decreased compared to vehicle-treated $Pten^{d/d}$ mice (66.78 ± 10.11). Immunohistochemistry revealed a significant reduction of proliferation marker and a significant increase of apoptosis marker in RGFP966 treatment compared to vehicle. Furthermore, the level of pAKT and pERK1/2 was substantially reduced in RGFP966 treatment group. These results demonstrate that HDAC3 targeting impedes the pathogenesis of EC and RGFP966 delays EC progression.

#4218 Discovery and in vitro characterization of FGFR/HDAC dual inhibitors for FGFR-driven cancer.

Chang-Hyun Yun[1], Sang-Heon Kim[1], Seunghwan Shim[1], Dongkyu Shin[2], Seonggu Ro[2], **Byoung-Gon Moon**[1]

[1]Handok Inc., Seoul, Korea, Republic of,[2]Pimedbio, Inc., Seongnam, Korea, Republic of

Gene alterations of Fibroblast growth factor receptors (FGFR) are frequently found in various solid tumors and are known as oncogenic drivers, particularly in bladder cancer and cholangiocarcinoma. Currently, pan-FGFR inhibitors are used for the treatment of FGFR2-driven intrahepatic cholangiocarcinoma and FGFR3-driven urothelial carcinoma. However, their efficacy is often limited by primary and acquired resistance mechanisms, including secondary FGFR mutation and alternative pathway activations. Secondary FGFR mutations are predominantly reported as an acquired resistance mechanism to pan-FGFR inhibitors. Additionally, substantial preclinical evidence suggests that alternative pathway activations, including EGFR, ERBB2, ERBB3, and c-MET, may significantly contribute to resistance. Interestingly, numerous preclinical studies have demonstrated that HDAC inhibitors can down-regulate their expression in certain tumor cell lines, suggesting the possibility of overcoming resistance through bypass activations. Furthermore, recent reports suggest that combining HDAC and FGFR inhibition may exhibit synergistic anti-tumor activity in both FGFR2 and FGFR3-driven tumors. Here we report the discovery and in vitro characterization of FGFR/HDAC dual inhibitors and their potential to overcome resistance via bypass activations. These compounds inhibited FGFR2 with an IC50 in the low nanomolar range in biochemical assay while sparing FGFR4. They also inhibited the proliferation of cancer cells harboring FGFR2 amplification with an IC50 in the low nanomolar range. Additionally, they inhibited HDAC1 with an IC50 in the low nanomolar range in biochemical assays and the HCT116 cell proliferation with an superior potency compared to vorinostat. To mimic drug resistance through bypass activations, SNU-16 cells were treated with EGF, HGF, or NRG1. In this model, the inhibitory efficacy of pemigatinib (an FDA approved FGFR 1-3 inhibitor) was significantly attenuated, whereas vorinostat (an FDA approved pan-HDAC inhibitor) maintained its efficacy. The greatest attenuation of efficacy was observed with EGF treatment, under which conditions some FGFR/HDAC dual inhibitors exhibited superior inhibitory efficacy compared to pemigatinib, correlating with their HDAC inhibition activity. In summary, these in vitro studies have resulted in several compounds that warrant further development for lead optimization. They also suggest that FGFR/HDAC dual inhibitors may be an option for overcoming bypass resistance in FGFR-driven tumors.

#4220 Anticancer effects of GEM144, a novel POLA1-HDAC11 dual inhibitor, on colorectal cancer models.

Rana Abdel-Samad[1], Susanna Noonan[1], Aileen Benitez[1], Teagan Mattox[1], Berthe Hayar[2], Sabrina Dallavalle[3], Claudio Pisano[4], Nadine Darwiche[2]

[1]Biology Depratment, Saint Mary's College, Notre Dame, IN,[2]Department of Biochemistry and Molecular Genetics, American University of Beirut, Beirut, Lebanon,[3]Department of Food, Environmental and Nutritional Sciences, Universita degli Studi di Milano, Milano, Italy,[4]Biogem, Institute of Molecular Biology and Genetics, Ariano Irpino (AV), Italy

Background and Objectives: Colorectal cancer (CRC) is the third most occurring cancer and the second cause of cancer-related deaths. Despite decades of research fighting the progression of CRC and the development of many therapeutic compounds, the 5-year survival rate remains at around 64%, with worse prognosis at advanced stages of the disease. The search for a fully efficacious treatment for CRC thus endures, in light of these suboptimal prognostic figures. GEM144 is a novel compound that was recently synthesized as a dual inhibitor of the DNA polymerase alpha (POLA1) and the histone deacetylase 11 (HDAC11). Initial investigations showed pronounced anti-proliferative potential of GEM144 when screened on a panel of hematological and solid cancer cells. Our study aims at examining the anticancer properties of GEM144 on CRC models.
Methods: We used two human CRC cell lines, HCT116 and HT29, derived from human colorectal carcinoma and adenocarcinoma, respectively, and one colon cell line, CCD-841-CoN, derived from the colon of a healthy subject. MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was used to assess cell growth, and trypan blue dye exclusion assay to evaluate cell viability. A wound healing assay was performed to investigate the effect of GEM144 on cell migration ability. Finally, the *in vivo* effect of GEM144 on tumor growth inhibition was analyzed in CRC xenograft nude mouse models.
Results: GEM144 decreased the growth of HCT116 and HT29 cells at 1 μM and 5 μM, respectively, starting at 24 hours post-treatment. This inhibition was more pronounced after 72 hours of treatment, where growth rate was less than 10% compared to control cells for both cell lines. Trypan blue exclusion assay showed comparable results for both cell lines, suggesting a reduction in cell viability. Interestingly, up to 10 μM of GEM144 had no effect on the growth of the normal-like CCD-841-CoN cells. GEM144 had an inhibitory effect on the migration of the HCT116 cells, evident by the reduction of wound healing in GEM144-treated cells compared to control ones. Finally, GEM144 showed around 20% relative decrease in the tumor volume of HCT116 xenograft mice treated with oral GEM144 compared to mice receiving the vehicle, without affecting body weight. Remarkably, GEM144 sensitized the tumor to cisplatin, a standard drug in the treatment of CRC. In fact, while cisplatin reduced tumor volume by 46%, a combination of cisplatin and GEM144 reduced tumor volume by 58%.
Conclusion: Our data show promising effects of GEM144 as an anti-cancer compound in the treatment of CRC cell lines and in mouse models. Our study will now further focus on understanding the mechanisms of action of GEM144 on cell cycle progression and on the expression of major players in colorectal carcinogenesis.

#4221 Precision targeting of HDAC3 in ovarian cancer.

Vijayalaxmi G. Gupta[1], Bisiayo Fashemi[1], Reni Akande[1], Preedia Babu[1], Yukihide Ota[1], Yufeng Xaio[2], Guangrong Zhang[3], Francisca Nathalia Vitorino[1], Benjamin Garcia[1], Mary Mullen[1], Dineo Khabele[1]

[1]University of Washington, School of Medicine, St. Louis, MO,[2]College of Pharmacy, University of Florida, Gainsville, FL,[3]University of Florida, Gainsville, FL

Introduction: Addressing chemo-resistant homologous recombination-proficient (HRP) ovarian cancers and CCNE1-amplified tumors, while minimizing treatment-related side effects, remains a significant clinical challenge. Our research demonstrated that HDAC inhibition suppresses tumors in PARP inhibitor (PARPi)-resistant ovarian cancers and enhances PARPi sensitivity in preclinical models. However, pan-HDAC inhibitors (HDACis) have shown high toxicity in clinical trials. To overcome this, we focused on selectively targeting specific HDACs, particularly HDAC3 and HDAC8, which have unique structures and co-repressor complexes. HDAC3 is frequently upregulated in ovarian cancers, making it a promising therapeutic target. Here, we explore HDAC3 genetic depletion/knockout (KO) and precision targeting using HDAC-PROTACs in five cell lines.

Methods: We employed siRNA-mediated depletion and CRISPR-Cas9 KO to study the impact of HDAC3 inhibition on CCNE1-amplified OVCAR3 cell proliferation, clonogenicity, and protein expression. Histone modifications were analyzed via mass spectrometry. The anti-proliferative efficacy of HDAC3 (XZ9002), HDAC8 (YL352), and dual HDAC3/8 (YX968) PROTACs was evaluated in OVCAR3, COV318, HRP and HRD mouse fallopian tube cells, and patient-derived primary cell lines.

Results: HDAC3 siRNA treatment in OVCAR3 cells led to dose-dependent reductions in HDAC3 protein levels and PCNA expression. HDAC3-KO cells exhibited increased histone acetylation and decreased histone methylation. PROTACs showed differential efficacy across cell lines. In OVCAR3 cells, IC50 values were 1.82 µM for HDAC3-PROTAC, 1.98 µM for HDAC8-PROTAC, and ~0.67 µM for HDAC3/8 dual PROTAC, indicating superior potency with dual targeting. In COV318 cells, the dual HDAC3/8 PROTAC exhibited an IC50 of 2.5 µM. HRD mouse fallopian tube cells were more sensitive to HDAC8 and HDAC3/8 PROTACs (IC50 ~6 µM), while HRP cells were less sensitive (IC50: 8 µM for HDAC3/8 PROTAC, 10 µM for HDAC8 PROTAC, >10 µM for HDAC3 PROTAC). Patient-derived primary cell lines demonstrated the highest sensitivity to the dual HDAC3/8 PROTAC (IC50 ~7 µM), with reduced response to single HDAC PROTACs. Pharmacodynamic analyses via Western blot and qPCR are ongoing.

Conclusion: Selective targeting of HDAC3, HDAC8, and HDAC3/8 using PROTACs provides a promising therapeutic strategy to address toxicity limitations of conventional HDACis. These agents hold potential for low-dose applications in ovarian cancers and broader therapeutic use in other malignancies and diseases.

#4222 N-acetyl-L-cysteine (NAC) sensitizes psammaplin A induced DNA damage in malignant hematologic cells by enhancing HDAC inhibition.

Bao Yu[1], Yumei He[1], Dan Liu[1], Ping Gong[1], Samuel Waxman[2], Yongkui Jing[1]

[1]Shenyang Pharmaceutical University, Shenyang, China,[2]Icahn School of Medicine at Mount Sinai, New York, NY

Psammaplin A (PsA) is a symmetrical bromotyrosine-derived disulfide dimer isolated from the *Psammaplysilla sponge*. PsA is a selective natural Class I HDAC inhibitor, that requires reduction into monomer with glutathione (GSH). Cell growth inhibition and apoptosis was determined in 12 malignant hematologic cell lines revealed 8 lines sensitive (more than 50% inhibition at 1 μM) and 4 lines insensitive (less than 20% inhibition at 1 μM). PsA induces acetylation of H3 and DNA damage detected with γ-H2A.X in the sensitive lines, but not in the four insensitive cell lines. The sensitivity of PsA in these lines correlates with HDAC inhibition and reduced GSH levels. The insensitive cell lines have lower GSH levels without HDAC inhibition. N-acetyl-L-cysteine (NAC) acts as a precursor of GSH synthesis and is used as an antioxidant. Pretreatment with 5 mM NAC following by PsA enhances cell growth inhibition 10 fold. NAC plus PsA is more effective than the HDAC inhibitor SAHA, which is not enhanced by NAC, to inhibit cell growth in vitro. Western blot analysis shows that NAC sensitizes PsA effect by increasing HDAC inhibition as measured by acetylated H3 and by DNA damage marker γ-H2A.X with upregulation of GSH. Chemical studies reveal that GSH, but not NAC, reduces PsA into a monomer. PsA is enhanced by NAC to inhibit tumor growth in MOML13 xenograft models without causing toxicity. These data support that NAC enhances PsA effect by increasing intracellular GSH which reduces PsA into a HDAC inhibiting monomer with increased cancer cell growth inhibition.

#4223 Mouse clinical trials (MCT) and other comprehensive pharmacology approaches highlight protein arginine methyltransferase 1 (PRMT1) targeting in various solid tumors with promising efficacy and combined mode-of-actions (MoAs) beyond epigenetic regulation.

Hui Shi, Xingnian Fu, Yilin Liu, Qiugeng Ouyang, Jiaxin Huang, Long Wang, Shuning Xing, Youzhen Wang, Yuan Mi, Haiping Wu

CytosinLab Therapeutics, Hangzhou, China

Arginine methylation is the most abundant post-translational modifications which provide docking site for protein binding and interaction (e.g. with the aromatic cage of the Tudor domain proteins), triggering a series of downstream epigenetic regulation and cellular functions or processes. PRMT1 is the major type-I PRMTs responsive for monomethylation and asymmetric dimethylation of arginines in histone tails or non-histone protein substrates. Highly expressed in various lineages of cancers with poor survival such as non-small cell lung cancer (NSCLC), pancreatic adenocarcinoma (PAAD), triple-negative breast cancer (TNBC), esophageal squamous cell carcinoma (ESCC), gastric cancer (GA), PRMT1 has been extensively reported for targeting in cancer with profound and broad tumor-intrinsic/extrinsic modulation to repress cancer growth, metastasis or refractory immunity. Here we showed that CTS2190, the only clinically active investigational drug of PRMT1 currently in phase I/II study (NCT06224387), could drive cancer specific mode-of-actions combining epigenetic gene expression, DNA damage response (DDR), cell cycle arrest, signal transduction and immunity activation. We applied an innovative, well-designed, patient-derived xenograft (PDX)-based mouse clinical trial (MCT) approach to evaluate CTS2190 efficacy in an all-comer solid patient panel. CTS2190 demonstrated excellent tumor growth inhibition and regression in GA, NSCLC, PAAD, ESCC, etc. We also explored and validated the efficacy and proposed MoAs of CTS2190 in multiple cellular or *in vivo* pharmacology approaches as well as the synergistic effects of CTS2190 in a couple of MoA-driven and biomarker-guiding combinatorial approaches with chemo-, targeted-, radio- and/or immune-therapies. These results indicate the comprehensive anti-cancer mechanisms of CTS2190 in targeting PRMT1. CTS2190 not only demonstrates synthetic lethality with epigenetic instability, DDR, or RNA splicing (RS) dysregulation, but also as an ideal partner for a wide range of cancer therapies, including combinations with PRMT5i, a type-II PRMT member in both *MTAPnull* and *wild-type* genetic context. Ongoing clinical and translational studies for CTS2190 will pioneer the breakthrough and pave the way for developing more next-generation epigenetic therapies in solid tumors.

#4224 The pharmacological intervention of protein arginine methyltransferase 1 (PRMT1) leads to metastatic castration-resistant prostate cancer (mCRPC) repression through unique and significant androgen receptor (AR) downregulation and epigenetic modulation.

Hui Shi, Yang Han, Xingnian Fu, Jiaxin Huang, Xinyue Duan, Yilin Liu, Youzhen Wang, Yuan Mi, Haiping Wu

CytosinLab Therapeutics, Hangzhou, China

AR based mechanisms drive resistance to first-line AR pathway inhibitor (ARPI) therapy and lead to poor survival in mCRPC, including AR amplification/mutations, and the emergence of novel splice variants such as AR-V7. The heterogeneity of AR genetic context and reactivation of AR-independent downstream genes, largely driven by cancer intrinsic epigenetic dysregulation further complicate mCRPC treatment. While AR degradation therapies such as proteolysis-targeting chimeras (PROTACs) show promise, they remain unsatisfied due to limited response and progression free survival (PFS) benefits in mCRPC patients with AR-V7 mutations or heterogeneous AR-resistant backgrounds. PRMT1 is the major type-I PRMT responsible for the asymmetric dimethylation of arginine residues on substrates such as H4R3 of histones or RG/RGG in proteins other than histones. Pharmacological intervention of PRMT1 can lead to cancer repression and benefit for long-term survival in multiple solid tumors in clinical trials, owing to its comprehensive mode-of-action (MoA) utilizing both epigenetic regulation of cancer gene expression and synthetic lethality to cancer intrinsic hallmarks such as DNA damage repair (DDR) and cell cycle arrest. Here we showed that PRMT1 is overexpressed in prostate cancer, particularly in advanced metastatic cases, well correlating with poorer prognosis of mCRPC. Inhibition of PRMT1 with CTS2190, a first-in-class (FIC), orally bioavailable, small molecular clinical investigational drug specific to PRMT1, results in significant AR degradation and tumor repression in various prostate cancer models, especially in AR-V7-positive mCRPC. CTS2190 not only decreases AR and asymmetric dimethylarginine (ADMA) levels, but also expression of prostate-specific antigen (PSA), MYC, and many downstream genes in AR signaling pathway in 22RV1 model, more effectively than docetaxel, enzalutamide, or even PROTAC targeting AR degradation. By co-modulating AR degradation and inducing DDR/cell cycle arrest, PRMT1 inhibition with CTS2190 shows promise as a potent monotherapy and as an ideal partner for combination with standard-of-care (SoC) treatments with different MoAs such as ARPI, chemo- or radio-therapies for mCRPC patients. CTS2190 could enhance antitumor activity with ARPI, docetaxel, topoisomerase I (TOP1) inhibitor or radiation, suggesting improved outcomes in enzalutamide-resistant mCRPC. Taken together, our findings highlight CTS2190 as a therapeutic agent, either alone or in combination, for a broader range of patients with ARPI-resistant mCRPC. CTS2190 is currently under evaluation in a phase I/II clinical trial, with an expansion cohort including mCRPC patients.

#4225 Targeting RNA splicing sensitizes MTAP-deleted cancers to MTA-cooperative PRMT5 inhibitors.

Katherine K. Slemmons[1], Chun Su[2], Siyuan Liu[1], Po-Wen Yu[1], Michele McEntee[2], Eric Yuan[2], Jasper Lee[2], Jiamiao Lu[2], Aaron Horning[2], Antreas Hindoyan[1], Brian Belmontes[1], Paul E. Hughes[1]

[1]Amgen, Thousand Oaks, CA,[2]Amgen, South San Francisco, CA

AMG 193, currently in Phase 1/2 trials, is an MTA-cooperative PRMT5 inhibitor that is showing promise as a targeted therapy in MTAP-deleted tumors. *MTAP* deletion, which occurs in ~10-15% of cancers, results in the accumulation of its substrate MTA leading to the partial inhibition of the arginine methyltransferase PRMT5. AMG 193, and its representative analog AM-9747, selectively bind PRMT5 in the presence of MTA resulting in preferential growth inhibition of MTAP-deleted tumor cells while sparing normal WT cells. Mechanistically, AMG 193 treatment induces DNA damage, cell cycle arrest, and a change in alternative splicing. We previously showed that in a large tumor cell line panel, elevated baseline levels of intron retention positively correlated with sensitivity to AMG 193 suggesting alternative splicing could potentially be utilized as a biomarker (Belmontes & Slemmons et al, 2024). As a result, we hypothesized that co-targeting pre-mRNA splicing could sensitize MTAP-deleted cells to PRMT5 inhibition.

To identify modulators of response or resistance to MTA-cooperative PRMT5 inhibitors, we performed a series of drug anchored genome-wide CRISPR knockout screens in engineered MTAP-deleted cell lines (HAP1, HCT116) and endogenous MTAP-deleted cell lines (A549, MIAPACA2). In line with our previous data that PRMT5 inhibition induces alternative splicing and intron retention is associated with AMG 193 sensitivity, these screens identified components of pre-mRNA splicing and RNA processing as modulators of AMG 193 response. One exemplary hit, AKAP17A, is involved in the regulation of alternative splicing. Here we demonstrate that AKAP17A knockout drives sensitivity to MTA-cooperative PRMT5 inhibition resulting in a 3-4X IC50 decrease in HAP1 MTAP-deleted cells and a 30% increase in max effect in A549 and MIAPACA2 cells. In the MIAPACA2 xenograft model, which has moderate sensitivity to PRMT5 inhibition (average tumor growth inhibition of ~50%), the addition of AKAP17A knockout drives tumor regressions. Knockout of other components of the spliceosome or splicing regulators similarly sensitizes MTAP-deleted cells to AM-9747 treatment in vitro. In a complementary pharmacologic approach, treatment with spliceosome inhibitors synergizes with PRMT5 inhibition in MTAP-deleted cell lines but not in MTAP WT cells.

In summary, our data suggests that alternative splicing is a vulnerability in MTAP-deleted cell lines that can be perturbed to augment the activity of MTA-cooperative PRMT5 inhibitors. Additional resistance and sensitization pathways identified from the CRISPR screens are currently being evaluated using in vitro and in vivo assays. Furthermore, clinical biomarker datasets from the ongoing early phase trials will be utilized to validate these preclinical findings.

#4226 Genome-wide CRISPRi screening identifies histone modification as a synergistic partner with JAK/STAT inhibition for enhancing anti-tumor activity in CTCL, validated in PDX-derived tumor cells.

Yan-Jin Liu[1], Haitao Wang[2], Laura Pincus[3], Frank McCormick[4], Weiyun Z. Ai[1]

[1]Department of Medicine (Hematology/Oncology), UCSF, San Francisco, CA,[2]Thoracic Surgery Branch, Center for Cancer Research, NCI, NIH, Bethesda, MD,[3]Department of Dermatology, UCSF, San Francisco, CA,[4]Helen Diller Family Comprehensive Cancer Center, UCSF, San Francisco, CA

Cutaneous T-cell lymphoma (CTCL) is a subtype of non-Hodgkin lymphoma and a counterpart to the nodal form of T-cell lymphomas, peripheral T-cell lymphoma (PTCL). Patients with advanced-stage CTCL have a median survival of 3-5 years, a result that has not significantly improved for decades, highlighting an unmet medical need. The JAK/STAT pathway is frequently dysregulated in PTCL and CTCL through point mutations and copy number changes. These genetic aberrations lead to uncontrolled signaling, cell proliferation, and disease progression. The JAK/STAT pathway has been established as a suitable therapeutic target in PTCL and CTCL, with several inhibitors, including ruxolitinib, demonstrating clinical activity. However, response rates remain modest, and the clinical benefits are often transient. To identify synergistic partners for ruxolitinib, we conducted a genome-wide CRISPRi screening in CTCL cell lines, HH and Hut78. The screen identified histone modification, particularly acetylation and methylation, as a key mechanism involving the sensitivity of CTCL cells to ruxolitinib. To validate these findings, we assessed the anti-tumor activities of ruxolitinib in combination with histone-modifying agents already in clinical use, such as romidepsin and 5-azacitidine. Combination studies demonstrated that romidepsin and 5-azacitidine synergistically enhanced the growth-inhibitory and apoptotic-inducing effects of ruxolitinib in CTCL cell lines, HH and Hut 78, as well as in primary tumor cells from CTCL patient-derived xenograft (PDX) models. Notably, similar synergy was observed with golidocitinib, a novel JAK1 inhibitor that demonstrated promising clinical activity in PTCL, indicating an on-target class effect of synergy between JAK/STAT inhibition and histone-modifying agents. Using primary human CTCL cells derived from PDX models, we observed synergistic induction of apoptosis with ruxolitinib or golidocitinib in combination with either romidepsin or 5-azacitidine, as demonstrated by caspase activation assays. In conclusion, histone modification agents significantly enhance the anti-tumor activity of JAK/STAT inhibitors, promoting both growth inhibition and apoptosis induction. Synergy was observed with two distinct JAK/STAT inhibitors, ruxolitinib and golidocitinib, both already in clinical use. These findings demonstrated in primary human CTCL cells from the PDX models—a clinically relevant experimental system—highlight the translational significance of this approach. Ongoing therapeutic trials with PDX animals will further investigate these promising findings.

#4227 Chromatin remodeling enhances the anti-cancer effects of estrogen therapy in ER+ breast cancer.

Anneka L. Johnson[1], Alexa Warren[1], Nicole Traphagen[1], Alyssa Roberts[1], Patricia Muskus[2], Barbara Karakyriakou[1], Eugene Demidenko[1], Fred W. Kolling[1], Owen Wilkins[1], Kimberly Birmingham[2], Brock Christensen[1], Todd W. Miller[2]

[1]Dartmouth College, Hanover, NH,[2]Medical College of Wisconsin, Wauwatosa, WI

Resistance to endocrine therapies in estrogen receptor alpha (ER)-positive breast cancer can be driven by ER amplification or overexpression. We hypothesized that the anti-cancer effects of the estrogen 17b-estradiol (E2) could be enhanced through combination with chromatin remodeling agents. We evaluated the effects of E2 and the histone deacetylase inhibitor (HDACi) entinostat in long-term estrogen-deprived (LTED) ER+ breast cancer cells, cells engineered to overexpress exogenous ER, and mice bearing endocrine-resistant patient-derived xenografts (PDX). Assayed endpoints included apoptosis, growth, DNA damage (gamma H2AX), histone post-translational modification, ER-inducible transcripts and proteins, cell cycle and replication status, chromatin accessibility by ATAC-seq, and transcriptomic changes. HDAC inhibition with entinostat increased histone H3 acetylation (H3K27ac) in cell lines and PDX models. Combination treatment with E2 and entinostat induced higher levels of apoptosis compared to single-agent treatments in LTED cells. In ER-overexpressing cells, single-agent E2 or entinostat inhibited growth that was increased with combination treatment. Entinostat and E2 each induced DNA damage and ER target gene expression. Pre-treatment with entinostat halted cell cycling while addition of E2 stalled cells in S-phase. ATAC-seq showed entinostat increases chromatin accessibility in regions containing ER target genes. In mice, entinostat slowed tumor growth rate, while single-agent E2 and the combination of E2/entinostat induced complete tumor regression. The drug combination synergized to promote more rapid tumor regression than E2 alone, and the combination delayed time to tumor recurrence compared to single-agent E2. We conclude that combination therapy with chromatin remodeling agents and E2 synergistically inhibits ER+ breast cancer cell growth and induces tumor regression in mice. Efficacy is a result of increased DNA damage, elevated chromatin accessibility, treatment-induced ER activation, cell cycle stalling, and subsequent cell death. The novel combination of an HDACi and E2 has the potential to be an effective therapy for patients with endocrine-resistant ER+ breast cancer.

#4228 PRISM cancer cell viability profiling enables insights into clinical candidate target selectivity: A case study revealing the most selective PRMT5 inhibitors for cancers with MTAP loss.

Laura Doherty, Colleen Harrington, Mustafa Kocak, Anthony Fazio, John Davis, Emily Reeves, Rachael Barry, Claudine Mapa, Dennie Frederick, Alvin Kalathungal, Jillian Eskra, Tenzin Sangpo, Melissa Ronan, William Sellers, Matthew Rees, Jennifer Roth

Broad Institute, Cambridge, MA

PRISM is a highly multiplexed, genomic barcode-based cell line viability technology of nearly 1000 solid tumor and hematopoietic cell lines, which enables profiling of cellular responses to small molecules or biologics to discover biomarkers of sensitivity and resistance and generate mechanistic hypotheses. In addition to the genomic characterization of PRISM cell lines, we have generated large pharmacologic perturbation reference datasets such as the ever-growing PRISM Oncology Reference dataset, in which hundreds of clinical stage agents are systematically profiled for cell viability effects. To highlight the capability of PRISM to uncover novel insights regarding agent selectivity, here we describe the results of profiling seven unique protein arginine methyltransferase 5 (PRMT5) targeting agents (six inhibitors and one degrader). PRMT5 is a methyl transferase capable of methylating arginines on histones and non-histone protein substrates involved in important cellular processes such as growth and migration. Homozygous deletions of the PRMT5 regulator, methylthioadenosine phosphorylase (MTAP) occur in 10% of all cancers, including 40% of glioblastomas, 25% of pancreatic adenocarcinomas, and 15% of NSCLCs. Loss of MTAP leads to accumulation of methylthioadenosine (MTA) and partial inhibition of PRMT5, creating a potential therapeutic window for further inhibition of PRMT5. Our results reveal that the MTA-cooperative PRMT5 targeting agents are more selective for this target patient population (cancers with MTAP loss) compared to earlier classes of PRMT5 inhibitors. Additionally, our results suggest the PRMT5 degrader we tested does have on-target activity, but its effectiveness may be dampened by MDR1-mediated efflux. Taken together, these results showcase the capabilities of the PRISM assay to characterize clinical candidates for selectivity within the patient population of interest and uncover potential liabilities (such as drug transporter efflux).

#4229 GTA182: A potentially best-in-class, MTA-cooperative PRMT5 inhibitor for MTAP-deleted advanced solid tumors.

Weifeng Mao[1], Bing Yao[1], Yanfeng Zhou[1], Wei Liu[1], Xiangping Zhang[1], Minghua Wang[1], Yingqi Hua[2], Wei Sun[2], Haoran Mu[2], Yining Tao[2], Qi Zhang[2], Mai Le[1], Corrine Zeng[1], Xiao-Hui Gu[1], Mingxi Li[1], James Joseph[1], **Fred Aswad**[1]

[1]Apeiron Therapeutics, Burlingame, CA,[2]Department of Orthopaedic Oncology, Shanghai General Hospital, Shanghai, China

Background: GTA182 is a second-generation, MTA-cooperative PRMT5 inhibitor developed to selectively target MTAP-null tumors, where MTAP deletion (often co-occurring with CDKN2A loss) creates a synthetic lethal vulnerability. Unlike first-generation inhibitors, GTA182 is optimized for high selectivity in MTAP-deleted versus MTAP wild-type cells, dose-proportional exposure, and CNS penetration, positioning it as a potential best-in-class treatment. GTA182 is currently in phase 1 clinical trials for advanced solid tumors with homozygous MTAP deletion.

Methods: Using structure-based design and advanced molecular modeling, GTA182 was optimized for enhanced ADME-PK properties, achieving broad systemic and CNS exposure. Preclinical selectivity and anti-tumor efficacy were evaluated in both MTAPwt and MTAP-null tumor models, with comparisons made against existing second-generation PRMT5 inhibitors.

Results: GTA182 demonstrated over 100-fold selectivity for MTAP-null cells, with strong anti-tumor efficacy at 3 mg/kg, substantially outperforming competitor compounds. Additionally, GTA182's optimized dose-proportional exposure profile resulted in a 30-fold multiple of exposure, with a therapeutic index up to 23-fold in murine mouse models, supporting its favorable safety margin and once-daily dosing. Effective blood-brain barrier penetration, exemplified by high Kp,uu and robust activity in the orthotopic U87 glioblastoma model, further supports its potential to treat CNS malignancies. Preliminary combination data with PD-1 inhibitors in MTAP-null osteosarcoma models suggest promising synergy, reinforcing GTA182's versatility and potential best-in-class status.

Conclusion: GTA182 represents a highly selective, MTA-cooperative PRMT5 inhibitor with significant preclinical efficacy and a broad therapeutic window. GTA182 is now advancing through phase 1 trials with the aim to establish safety, pharmacokinetics, and efficacy in MTAP-deleted advanced tumors. With the potential to safely address both CNS and non-CNS malignancies, GTA182 is positioned to emerge as a differentiated therapeutic in precision oncology.

#4230 Preclinical characterization of ATG-042, a novel MTAP ^{null}-selective PRMT5 inhibitor.

Ya Kong[1], Ge Lin[1], Ming Zhang[1], Peng Chen[1], Jay Mei[2], **Bing Hou**[2]

[1]Shanghai Antengene Corporation Limited, Shanghai, China,[2]Antengene Corporation, Shaoxing, China

Background: Targeting the PRMT5-MTA complex has become a promising strategy for treating MTAP^{null} cancer in a synthetically lethal manner, avoiding on-target-off-tumor hematological toxicity when using first-generation, non-selective PRMT5 inhibitors. The MTAP gene is deleted in around 10-15% of all human malignancies and the top3 human cancers with the highest frequency of MTAP deletion are GBM, pleural mesothelioma and bladder cancer with high unmet medical need (TCGA data). Herein, we report the preclinical feature of ATG-042, a novel MTAP^{null}-selective PRMT5 small molecule inhibitor with good brain penetration.

Methods: The in vitro activity and MTAP selectivity of ATG-042 on cell proliferation were profiled using HCT116 MTAP wild type (wt) cells, HCT116 MTAP knock out (ko) cells and multiple endogenous MTAP^{null} cell lines. The in vivo efficacy was tested in cell derived xenograft (CDX) mouse models with HCT116 MTAP wt cells, HCT116 MTAP ko cells, LU99 cells (MTAP^{null}) and U87MG-luc (MTAP^{null}). The pharmacokinetic and toxicological properties were assessed with corresponding assay methods.

Results: ATG-042 demonstrated potent and selectively inhibitory effect on HCT116 MTAP ko cells' proliferation (IC_{50}=52nM) and SDMA expression (IC_{50}=0.06nM), but very weak effect on HCT116 MTAP wt cells. Consistently, ATG-042 showed excellent anti-proliferative activities on multiple endogenous MTAP^{null} cell lines with IC_{50} values between 10nM and 100nM. ATG-042 demonstrated high permeability (Caco2 assay, A to B, P_{app} =12.2 x10^{-6}cm/s;ER=0.9), good metabolic stability, and low risk of drug-drug interaction. In vivo PK study shows that ATG-042 is well absorbed, with a dose-dependent increase in plasma distribution and high oral bioavailability in mice, SD rats and beagle dogs. Furthermore, ATG-042 is brain-penetrable (B/P ratio=51% in mice; $KPuu_{brain}$=0.73 in rats). ATG-042 showed robust in vivo efficacy in both subcutaneous CDX models (HCT116 -MTAP ko, LU99) and orthotopic CDX model (U87MG-luc) as a single agent. In addition, ATG-042 also exhibited the potential in combination with other drugs for antitumor therapy. ATG-042 was well tolerated in both SD rats and beagle dogs after four weeks of continuous administration.

Conclusions: In summary, ATG-042 is an oral MTAP ^{null}-selective PRMT5 inhibitor with potent efficacy against MTAP^{null} tumor. It also demonstrated good tolerability and brain penetrability.

#4231 Preclinical candidate RVU305, an MTA-cooperative PRMT5 inhibitor, shows activity in MTAP-deleted tumors resistant to immune checkpoint treatment.

Anna Bartosik, Adam Radzimierski, Aneta Bobowska, Aleksandra Więckowska, Kamil Kuś, Jacek Faber, Bożena Winnik, Agata Stachowicz-Wałaszek, Agnieszka Ludwig-Słomczyńska, Kamila Kozłowska-Tomczyk, Marta Wadowska, Szymon Woroszyło, Mateusz Stoszko, Aniela Gołas, Paulina Podkalicka, Adrian Zarębski, Agnieszka Piątek, Maciej Kujawa, Beata Kowalska, Julia Wirkijowska, Stefan Chmielewski, Jacek Stępniewski, Mateusz Ogorek, Lika Osugui, Agnieszka Szymula, Agnieszka Dorman, Kamila Białkowska, Marcelina Chmiel, Milena Mazan, Tomasz Rzymski, Karolina Gluza, Joanna Krawczyk, Marcin Kowiel, Adam Marciniak, Michał Combik, Oleksii Bryzghalov, Mateusz Nowak, Didier Pez, **Krzysztof Brzozka**

Ryvu Therapeutics, Krakow, Poland

Homozygous deletions of methylthioadenosine phosphorylase (MTAP), located on chromosome 9p21 are observed in 10 to 15% of all human tumors. This deletion leads to a substantial methylthioadenosine (MTA) build-up within cells. At high concentrations, MTA acts as a highly selective inhibitor of the PRMT5 methyltransferase, specifically competing with its substrate, S-adenosylmethionine (SAM). Hence, selectively targeting of residual PRMT5 levels, which are essential for survival of cancers, represents a promising strategy for eliminating cancer cells with homozygous MTAP deletion. There is growing evidence that MTA accumulation enhances immunosuppressive tumor microenvironment. Moreover, tumor-specific PRMT5 inhibition has been shown to enhance antitumor immune responses, opening the possibility of combining MTAP-deletion specific MTA-cooperative PRMT5 inhibitors with cancer immunotherapy. Ryvu has developed RVU305, a potentially best-in-class MTA-cooperative PRMT5 inhibitor, characterized by favorable drug-like physicochemical properties, low nanomolar potency, and differential efficacy in MTAP-null cancer cells. To evaluate the immunomodulatory activity of RVU305, we developed an MC38 syngeneic colon cancer model with homozygous deletion of MTAP and CDKN2A genes. The tumor growth inhibitory activity of RVU305 was assessed following oral administration, both alone and in combination with anti-PD-1 antibody. Effects on tumor-infiltrating lymphocytes were analyzed using flow cytometry. Changes in IFN-γ-secreting T cells were evaluated using ELISpot. PRMT5 inhibition in MTAP-null cancer cells, as well as in stromal and infiltrating lymphocytes, was assessed via SDMA IHC staining. RVU305 demonstrated robust tumor growth inhibition (TGI) in MC38 tumors with MTAP loss, with confirmed target engagement, while the growth of the MC38 variant with an intact MTAP gene was unaffected at pharmacologically active doses. Furthermore, co-treatment with RVU305 and anti-PD-1 antibody was well tolerated and resulted in antitumor activity. Immunomodulatory effects of RVU305, both alone and in combination with anti-PD-1, were supported by pharmacodynamic changes observed in tumor tissue. Taken together, these studies confirm that MTA-cooperative PRMT5 inhibitors induce a strong synthetic lethal phenotype in MTAP-deleted cancers, offering an exciting therapeutic opportunity for a large patient population and potentially addressing an unmet clinical need when combined with immunotherapy.

#4232 DW91170B: A novel potent EZH1/2 dual inhibitor for adrenocortical carcinoma treatment.

Doc-Gyun Jeong[1], **Yeonkyung Oh**[1], Woon Heo[1], DongHyuk Shin[1], Jung Hee Kim[2], Emilia Moonkyung Youm[2], Jihyun Youm[1], Kiyoon Kim[1], Sangho Lee[1], Yun-Ha Hwang[1]

[1]Dongwha Pharma, Yongin, Korea, Republic of,[2]Department of Internal Medicine, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Korea, Republic of

Adrenocortical carcinoma (ACC) is an aggressive cancer that occurs in the adrenal cortex with a poor prognosis, and its 5-year survival rate is less than 60%. Previous studies have characterized molecular and cellular alterations in ACC, including changes in p53, the WNT-β-catenin pathway, PKA, and chromatin remodeling pathways. EZH2 (Enhancer of zeste homolog 2), a catalytic subunit of PRC2 (polycomb repressive complex 2), also exhibits abnormal characteristics in ACC. EZH2 has been reported to be overexpressed in ACC patients and is associated with disease progression and survival rates. Here, we present DW91170B, a novel and potent dual inhibitor of EZH1/2, as a candidate for the treatment of adrenocortical carcinoma. DW91170B induces histone modification by reducing the trimethylation of H3K27, suppresses cell viability, and induces apoptosis in adrenal cancer cells. Additionally, DW91170B affects the molecular and cellular characteristics of adrenal cancer cells. Specifically, treatment with DW91170B reduces the expression of HSD3B2 and CYP11B1, genes involved in steroidogenic process. As a result, aldosterone and cortisol secretion induced by forskolin is inhibited by DW91170B. Long-term treatment with DW91170B decreases the expression of transcription regulators such as β-catenin and SF-1. These changes in transcriptional complex were confirmed through RNA sequencing, revealing alterations in gene expression. Among the differentially expressed genes, those related to epithelial-mesenchymal transition (EMT) and apical junctions were notably affected. Functional changes were further confirmed by EMT marker protein expression and a wound healing assay. The in vivo efficacy of DW91170B was assessed in two adrenal cancer models. NCI-H295R cells, which have high expression EZH2, and SW13 cells, which carry a SMARCA4 mutation that is susceptible to synthetic lethality from EZH2 inhibition, were used. DW91170B effectively inhibited tumor growth in both models. In summary, the novel EZH1/2 inhibitor DW91170B exhibits anti-tumor efficacy in adrenocortical carcinoma. These effects appear to be mediated by alterations in cancer cell characteristics and the inhibition of cell survival.

#4233 Ovarian cancer treatments: Using HSF1 and MYC gene amplification as a biomarker.

Matthew S. O'Malley[1], Imade Williams[1], Haddie DeHart[1], Bobby Walker[2], Vrushabh Ulhaskumar[2], Haimanti Ray[2], Pranav Jothirajah[3], Joe R. Delaney[4], Kenneth P. Nephew[5], Richard Carpenter[6]

[1]Medical Sciences, Melvin and Bren Simon Comprehensive Cancer Center, Indiana University School of Medicine - Bloomington, Bloomington, IN,[2]Medical Sciences, Indiana University School of Medicine - Bloomington, Bloomington, IN,[3]Medical Sciences, Indiana University Bloomington, Bloomington, IN,[4]Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, SC,[5]Melvin and Bren Simon Comprehensive Cancer Center, Anatomy, Cell Biology & Physiology, Indiana University School of Medicine - Bloomington, Bloomington, IN,[6]Melvin and Bren Simon Comprehensive Cancer Center, Biochemistry and Molecular Biology, Indiana University School of Medicine - Bloomington, Bloomington, IN

Ovarian cancer is one of the deadliest gynecological malignancies as approximately 1 in 87 women will die from this disease. One of the reasons for such a dismal prognosis for the disease is due to the limited treatment options and high rate of relapse from the first line treatment of platinum-based chemotherapy and taxanes. Therefore, patients with ovarian cancer would greatly benefit from new treatment options. To find new treatment options for ovarian cancer, we looked for ideal targets by analyzing the biology of high-grade serous ovarian cancer (HGSOC), the most common and most deadly form of ovarian cancer. This type of ovarian cancer is classified by high rates of amplification of oncogenes such as MYC, KRAS, and CCNE1, however this knowledge of oncogene amplification has been understudied as a genetic biomarker for efficacy of various treatments. Approximately 30% of ovarian cancers carry amplifications of both the MYC and Heat-shock Factor 1 (HSF1) oncogenes, which have both been shown to contribute to worse survival in multiple cancer types through mechanisms such as increased proliferation, metastasis, and immune evasion. Many drugs have been developed to directly bind to and inhibit these targets, however none have had success in clinical trials in part due to the difficulty in designing drugs to potently and specifically target transcription factors. To inhibit the activity of the MYC and HSF1 transcription factors in HGSOC, we tested alternative mechanisms of MYC and HSF1 inhibition through targeting a shared activating kinase, Polo-like Kinase 1 (PLK1), with the PLK1 inhibitor Volasertib (BI-6727). Since HSF1 and MYC are both transcription factors, we also aimed to find an epigenetic method of inhibiting their transcriptional activity. We performed a drug screen of epigenetic inhibitors in the background of HGSOC cells that carried the HSF1 and MYC co-amplification and a counter-screen of HGSOC cells that did not carry these amplifications. Results from this screen show that cells that are coamplified for the HSF1 and MYC oncogenes are more sensitive to treatment with class I HDAC inhibitors than HGSOC cells without this amplification. We show that treatment with the class I HDAC inhibitor Entinostat decreases the HSF1 and MYC expression at the mRNA and protein level. We show that loss of HSF1 and MYC decrease proliferation and colony formation ability of ovarian cancer cells using knockdown siRNAs and that the Volasertib and Entinostat inhibitors are more effective in treating ovarian cancers that carry amplifications of HSF1 and MYC than those with other driver amplifications. Work with both Volasertib and Entinostat aim to show that amplifications of oncogenes in high-grade serous ovarian cancer can be used as a biomarker for new treatment strategies in ovarian cancer.

#4234 Synergistic anti-proliferative effects of JIN-001, a novel HSP90 selective inhibitor, in combination with platinum-based drugs in ovarian cancer cell lines.

Jue Young Kim[1], Ha-Yeon Shin[1], Anna Jo[2], Ethan Seah[2], Choonok Kim[2], Jae-Hoon Kim[1]

[1]Gangnam Severance Hospital, Seoul, Korea, Republic of,[2]J INTS BIO Inc., Seoul, Korea, Republic of

Introduction: Heat shock proteins (HSPs) have been considered as a promising therapeutic target for ovarian cancer (OC), the most lethal gynecologic malignancy. HSPs facilitate tumor cell adaptation to short-term stress, promoting the development of more malignant phenotypes and the acquisition of resistance to therapeutic treatments over time. To date, Clinical trials of HSP90 inhibitors have shown modest benefits in advanced cancer patients, with no sufficient efficacy as monotherapy. Here, We investigated the anti-proliferative effects of JIN-001, a selective HSP90i, combined with platinum-based drugs in OC cell lines.

Material and methods: We examined the co-treatment effects of JIN-001 and paclitaxel (PTX) or cisplatin (Cis, platinum-based) on viability in OC cell lines (OV90, TOV21G, and OVCAR3) and drug-resistance sublines (OV90/PTX200, TOV21G/PTX100, and OVCAR3-CisR). Drug-resistant OC sublines were generated by gradually exposing OC cells to increasing concentrations of PTX or Cis. Each drug was tested either for monotherapy or in 1:1 ratio combination. Also, we assessed the synergistic anti-proliferative effects of JIN-001 (0.1 or 0.05 μM) with Cis on OVCAR3 and OVCAR3-CisR.

Results: Co-treatment of JIN-001 with PTX or Cis reduced IC_{50} in drug-resistant OC sublines compared to PTX or Cis alone. The IC_{50} of JIN-001 at a fixed dose in combination with Cis were lower in the OVACAR3 and OVCAR-CisR, compared to the single treatment. IC_{50} (μM) are shown below.

	Cell line					
Treatment	**OV90**	**OV90/PTX200**	**TOV21G**	**TOV21G/PTX100**	**OVCAR3**	**OVCAR3-CisR**
PTX	0.021	0.204	0.002	0.235	-	-
Cis	-	-	-	-	1.886	9.643
JIN-001+PTX	0.011	0.043	0.001	0.031	-	-
JIN-001+Cis	-	-	-	-	0.070	0.142
JIN-001 (0.1 μM)+Cis	-	-	-	-	0.022	0.282
JIN-001 (0.05 μM)+Cis	-	-	-	-	0.149	0.606

Conclusion: The combination of JIN-001 with PTX or Cis enhanced anti-proliferative effects and reduced IC_{50} in OC cell lines and drug-resistant sublines compared to monotherapy. This suggests JIN-001's potential as a combination therapy with platinum-based drugs in OC patients. Further studies are needed to examine whether the combination therapy of JIN-001 and the conventional drugs decelerates the tumor's ability to adapt and develop drug resistance.

#4235 PCNA and histone deacetylase targeted inhibitors in cutaneous T-cell lymphoma.

Caroline M. Li, Robert G. Lingeman, Long Gu, Robert J. Hickey, Linda H. Malkas

Beckman Research Institute of The City of Hope, Duarte, CA

AOH1996, a small molecule designed to bind proliferating cell nuclear antigen (PCNA), demonstrates selective inhibition of cancer cell growth across the NCI60 cell line panel with a mean GI50 of 300 nM, while exhibiting minimal toxicity to nonmalignant cells. Mechanistic studies reveal that AOH1996 influences DNA replication, cell cycle progression, DNA damage response, and transcription-replication conflicts. Inspired by phosphoproteomic analyses of AOH1996-treated cells, we investigated its potential in combination therapy with the histone deacetylase inhibitors (HDACi) vorinostat and belinostat. Notably, vorinostat, an FDA-approved treatment for cutaneous T-cell lymphoma (CTCL), exhibited synergistic effects with AOH1996 in cell viability assays with three CTCL derived cell lines. To better understand enhanced cell killing, we show combination drug effects on apoptosis, DNA damage and PCNA protein expression. Enhanced growth inhibition was also observed when AOH1996 was combined with other HDACi, such as domatinostat and panobinostat. These findings highlight the therapeutic potential of AOH1996 in combination with HDACi, particularly for CTCL, and pave the way for further exploration of this approach in cancer treatment.

#4236 Effects of a DNMT1-selective inhibitor on T-cell activity and tumor cells intrinsic pro-inflammatory response.

S. W. O'Brien, S. Rajapurkar, A. Della Pietra III, H. Sun, A. Fedoriw, M. McCabe, M. Pappalardi;
GSK, Collegeville, PA

Previous studies indicate that while combinations of DNA methyltransferase inhibitors, such as decitabine or 5-azacytadine, with immunotherapies has shown potential in activating anti-tumor activity, their limited clinical success could be attributed to the systemic delivery and toxic effects on the immune cells in the microenvironment, which may explain the lack of activity in clinical trials. To explore the potential of combining an epigenetic inhibitor with an immunomodulatory agent, the effects of a DNMT1 inhibitor on T-cell activity and intrinsic pro-inflammatory responses in tumor cells were examined. Human and mouse cancer cell lines or isolated T-cells were treated with DNMT1-selective inhibitors and assessed for gene expression, global DNA methylation, mass cytometry, fluorescent cytometry, and chemokine ELISA. DNMT1-selective inhibitors triggered a pro-inflammatory transcriptional response in human cancer cell lines similar to decitabine. In human T-cells, DNMT1 inhibition reduced proliferation and increased markers of both anti-inflammatory and pro-inflammatory phenotypes, with these changes being reversible upon compound removal. Mouse T-cells similarly exhibited temporary loss of proliferation and increased anti-inflammatory and pro-inflammatory phenotypes. Additionally, DNMT1 inhibition elevated the expression of anti-inflammatory cytokines IL-10 and IL-4 in both human and mouse T-cells. Notably, DNMT1 inhibition resulted in a lasting pro-inflammatory transcriptional response in malignant cancer cell lines, whereas the effect on normal cells was reversible. In summary, DNMT1-selective inhibitors may boost the efficacy of immuno-oncology therapies by inducing a pro-inflammatory response in cancer cell lines while minimizing impact on T-cells through alternative dosing schedules. Further research is required to compare DNMT1-selective inhibitors with traditional HMAs and to study the interaction between immune and cancer cells in vivo. Identifying biomarkers for patient stratification could help optimize treatment strategies. This study highlights the importance of evaluating the impact of small molecule inhibitors on both tumor characteristics and immune responses to optimize conditions for effective anti-tumor responses.

#4237 Homogenous detection of O-Acetyl-ADP-ribose as a generic HTS assay method for SIRTs.

Ha Pham, Mahbbat Ali, Anibal Ramos-Martinez, Robert Lowery

BellBrook Labs, Madison, WI

Sirtuins (SIRTs) are deacetylases that catalyze removal of acetyl groups from histones and other proteins; they are important components of the epigenetic regulatory landscape and have been targeted for diverse indications, including cancer. SIRTs deacetylate in an NAD-dependent reaction that yields O-acetyl-ADP-ribose (OAADPr) and nicotinamide as products along with the deacetylated protein. Screening and hit-to-lead efforts targeting SIRTs has been hampered by a lack of robust HTS assays methods for detection of SIRT enzymatic activity, a situation that is exacerbated by the diversity of deacetylated protein products and the inherent instability of the OAADPr product. To address this unmet need, we developed a generic activity assay for SIRTs based on enzymatic conversion of OAADPr to AMP in real time, which is then detected using the Transcreener AMP/GMP assay with a fluorescence polarization (FP) or time resolved Förster resonance energy transfer (TR-FRET) readout. Using recombinant full length Sirt1 and Sirt2, we determined optimal NAD, acetylated peptide substrate, and enzyme concentration for initial velocity detection of deacetylase activity. For both enzymes, a robust signal was observed using concentrations in the nanomolar range, and there was good concordance in activity measured using FP and TR-FRET readouts. The assays were validated for HTS using a 1,280 collection of bioactive compounds. Selected hits were further profiled in dose-response assays. The assay Z' value was above 0.7, supporting its robustness for screening and hit-to-lead/SAR for Sirt1 and Sirt2 antagonists. To our knowledge, the Transcreener OAADPr assay is the first SIRT activity assay that relies on detection of OAADPr rather than the deacetylated protein product. By enabling homogenous detection of SIRT activity with native acetylated substrates, it should simplify screening and profiling of inhibitors and facilitate drug discovery efforts targeting SIRTs.

#4238 DNMT1 inhibition by SGI-1027 promotes neuroblastoma cell death through a p53-independent pathway.

Farah Naz, Shyam Sundar Jaganathan, Umamaheswari Natarajan, Appu Rathinavelu

Rumbaugh Goodwin Institute for Cancer Research, Barry & Judy Silverman College of Pharmacy, Nova Southeastern University, Fort Lauderdale, FL

Introduction: Neuroblastoma is an aggressive childhood cancer originating from neural crest cells, accounting for 700-800 new cases annually and cuases15% of pediatric cancer deaths in the U.S. Treatment for high-risk neuroblastoma includes chemotherapy, stem cell transplants, radiation, and immunotherapy. Recent evidence suggests that DNMTs inhibitors such as SGI-1027 and CM-272 can potentiate apoptosis *via* epigenetic modulation of critical regulatory pathways in cancers. Hence, we hypothesized that SGI-1027 can induce apoptosis in SK-N-SH neuroblastoma cells by upregulating p21 through DNMT1 inhibition. Our study aimed to elucidate the underlying mechanisms and assess the potential of using SGI-1027 for treating high-risk neuroblastoma.

Methods: SK-N-SH cells were treated with 2 µM of SGI -1027 for 24 hrs. The cell viability was evaluated using Trypan Blue Dye Exclusion method, while apoptotic activity was quantified through a fluorescence assay using DEVD-*amc* substrate for measuring caspase-3/7 activation. qRT-PCR and Western Blot analyses were conducted to assess expression levels of the cell cycle, apoptotic, and epigenetic pathway-related markers such as p53, p21, PARP, BAX, BCL-XL, and DNMT1. Apoptotic changes in cells were further confirmed through Immunofluorescence studies.

Results: Our study results confirmed that SK-N-SH cells treated with SGI-1027 showed significant cell death and enhanced fluorescence in the DEVD-*amc*, indicating activation of apoptotic activity. Consistent with our hypothesis, we observed an elevation in p21 levels, an increased BAX/BCL-XL ratio, and a downregulation in the levels of DNMT1, suggesting that SGI-1027 may be facilitating neuroblastoma cell death through a p21-driven mechanism that is independent of p53 pathway.

Conclusion: This study highlights the potential of DNMT1 inhibitor SGI-1027 to induce apoptosis in neuroblastoma cells through upregulating p21 and modulating the BAX/BCL-XL ratio. Our findings suggest that targeting DNMT1 using SGI-1027 may offer an effective pathway for inducing cell death and controlling neuroblastoma growth. We are in the process of validating DNMT1 targeted therapies as a powerful addition for treating aggressive neuroblastoma.

Acknowledgments: This project was supported by the National Pediatric Cancer Foundation (NPCF) and the Royal Dames of Cancer Research Inc., Fort Lauderdale, Florida.

#4239 Effect of MTAP expression on PRMT5 inhibitor sensitivity in bladder urothelial carcinoma models.

Connor Purcell[1], Leiqing Zhang[1], Maryam Ghandali[1], Maximilian Pinho-Schwerrman[2], Paola Gyuliane[1], Brian K. Baker[3], Lanlan Zhou[1], Dinara Ryspayeva[1], Benedito A. Carneiro[1], Wafik S. El-Deiry[1]

[1]Legorreta Cancer Center at Brown University, Providence, RI,[2]Brown University Health, Providence, RI,[3]Morehouse School of Medicine, Atlanta, GA

Introduction: Methylthioadenosine phosphorylase (MTAP) is a metabolic enzyme integral to the methionine/adenine salvage pathway, whose expression is lost in 30% of bladder cancer (BCa). The cellular changes associated with MTAP loss, such as accumulation of its substrate, MTA, increase the dependence of cancer cells on other metabolic enzymes, opening opportunities for synthetically lethal targeted therapy. One such target is protein arginine methyltransferase 5 (PRMT5), which relies on the methionine-cycle intermediate SAM to methylate proteins. An emerging class of PRMT5 inhibitors exploits the increased dependence of MTAP-deficient tumors on PRMT5 to induce tumor-specific death. The present study investigates the sensitivity of genetically engineered BCa cell lines and patient-derived organoids (PDOs) to the PRMT5 inhibitor AZ14209703.
Methods: MTAP was knocked out of BCa cell line 5637 (MTAP+) by 48h reverse transfection with recombinant Cas9 and MTAP or CTRL sgRNAs. MTAP was overexpressed in MTAP-null BCa cell lines RT4 and UM-UC-3 and a patient-derived organoid by lentiviral transduction with a stable MTAP overexpression (OE) plasmid or empty vector (EV). Protein expression was determined by western blot (WB). For viability assays, cells were plated at 1000 cells/well in a 96 well plate and allowed to adhere overnight before treatment. Viability was assessed 7d after AZ14209703 treatment by Cell-TiterGlo® assay, and IC50 and area under the curve (AUC) were computed.
Results: WB analysis showed no detectable MTAP in 5637 cells transfected with the MTAP sgRNA-Cas9 complex and, conversely, robust overexpression in RT4, UM-UC-3, and the PDO following lentiviral transduction. In viability assays, MTAP expression conferred protection from PRMT5i in all models tested. 5637 MTAP-/- cells exhibited reduced area under the curve (AUC) and IC50 compared to the sgCTRL. MTAP OE in UM-UC-3 and RT4 increased the AUC and IC50 versus the EV.
Conclusions: The present findings suggest that PRMT5i is highly selective to MTAP-deficient BCa models. Future work involving these models will characterize the sensitivity of the MTAP-OE PDO to PRMT5i, study the effects of MTAP and PRMT5i on T and NK cell killing, and determine whether MTAP status affects the potency of PRMT5i in combination with other therapies.

#4240 Combining belinostat with red grape seed extract effectively inhibits cell proliferation, induces apoptosis, and reduces tumor resistance to doxorubicin by targeting mutant KRAS and p-ERK in human breast cancer.

Mariam Shaker Kerema, Sara Hassan Deghedy, Sofia Khalil, Ahmed Samir Sultan

Biochemistry, Alexandria University, Alexandria, Egypt

Breast cancer ranks as the second most prevalent type of cancer worldwide. Despite significant advancements in various therapeutic approaches, the incidence rates continue to rise due to the resistance caused by elevated levels of histone de-acetylating enzymes (HDACs). The current study aims to investigate a combination therapy involving red grape seed extract (GSE) and Belinostat (PXD101), a hydroxamate-type inhibitor of histone deacetylase (HDAC), as a novel treatment to overcome resistance in breast cancer. *In vitro*, Two breast cancer cell lines spanning from poorly to highly differentiated cell lines, MDA-MB-231 and ZR-75-1, were tested with PXD101, GSE, and their combined treatment with or without Doxorubicin treatments. The WST-1 assay was utilized to evaluate the potential synergies of this combination in reducing proliferation and inducing apoptosis in the tested cells. Additionally, the underlying molecular mechanisms responsible for cancer cell death were explored through western blotting, histone release assays, assessment of caspase-3 levels, and immunohistochemistry (IHC) to determine the localization of oncogenic markers. The Wst-1 combination assay revealed that the viability of PXD101/GSE treated cells significantly varied in a concentration-dependent manner compared to the control untreated cells or each compound after 48 h of treatment. The calculated combination index indicated a synergistic effect in MDA-MB-231 cells, although this was not observed in ZR-75-1 cells. Moreover, the combination treatment for up to 48h triggered apoptosis in both cell lines, as evidenced by morphological changes, histone release alterations, increased caspase-3 activity, and significant inhibition of mutant KRAS and p-ERK expression levels. Immunohistochemical analysis of β-catenin localization indicated its presence at cell-cell contacts and in the cytoplasmic compartment, contrasting with its localization in the nuclei of untreated control cells. In an *in vivo* study utilizing the MDA-MB-231 orthotopic xenograft model, the combined treatment exhibited remarkable anticancer activity compared to the control and individual drugs. Importantly, our findings demonstrated that the combination treatment had significant inhibitory effects on tumor growth and metastatic potential, with minimal toxicity relative to untreated control tumors. Additionally, this combination enhanced efficacy, reduced toxicity, and decreased tumor resistance to Doxorubicin after 48h of treatment. Overall, our findings propose a PXD101/GSE combination as a potential non-toxic fingerprint that exhibits promising anticancer activity against Doxorubicin resistance. This combination warrants further exploration in combination therapy for human breast cancer.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: New and Emerging Cancer Drug Targets
Poster Session

#4244 Application of a membrane interactomics (MInt) platform for novel surface target discovery.

Cody Scandore, Kendall Johnson, Chris May, Rebecca Howell, Jeffrey Guernsey, Tali Vittum, Noah Dephoure, Hengyu Xu, Heath Klock, Ertan Eryilmaz, Clare Malone, Francesca Nardi, Michael Warren, Danielle Zimmerman, Pamela Holland, Scott Lesley, Niyi Fadeyi, Rob Oslund

InduPro, Cambridge, MA

Despite advances in cancer therapeutics, there remains a critical need to identify new protein targets and targeting approaches for drug development, particularly those with improved efficacy and safety profiles. To address these challenges, InduPro leverages a high-resolution proximity proteomics technology that uses photocatalyst-generated reactive probes to label cell surface protein microenvironments (1,2). Combining this technology with quantitative mass spectrometry, we achieve high-throughput characterization of the plasma membrane protein interactome at an unprecedented detail. The integration of this specialized proximity proteomics data with clinical protein expression profiles through computational graphs and graph convolutional neural networks (GCNNs) provides powerful opportunities for surface protein target analysis and unique biological insight.

This approach enables the identification of surface antigens in close proximity to tumor associated antigens (TAAs) within tumor microenvironments. Here, we refer to these proximal antigens as tumor associated proximity antigens (TAPAs). The co-localization and interrelated biological functions of these antigens facilitate precise dual-targeting of malignant cells, while sparing healthy tissues. This approach addresses existing limitations in cancer therapeutics, particularly the off-target effects associated with TAAs expressed on normal cells, and the poor efficacy of some tumor-specific antigens as standalone targets. Through leveraging these TAPAs, we can explore a diverse range of bispecific therapeutic modalities, including but not limited to antibody-drug conjugates (ADCs), T-cell engagers (TCEs), and radioligand therapies (RLTs).

By applying our MInt platform to cancer cell lines, patient-derived tumor samples, and animal model xenografts, we have generated a database of protein interaction networks for many TAAs comprising millions of data points. This has resulted in the identification of novel TAA x TAPA bispecific pairings across multiple indications. This platform has led to multiple preclinical programs, including IDP-001, a bispecific ADC targeting EGFR and CDCP1 in solid tumors.

Here, we showcase our discovery engine for novel target identification as a powerful method for uncovering druggable protein targets with improved therapeutic windows. This approach has the potential to expand the repertoire of cancer targets and enable the next generation of bispecific therapeutics.

Ref:

(1) Oslund, RC et al., Nature Chem. Bio. 18 (2022) 850-858
(2) Geri, JB et al., Science. 367 (2020) 1091-1097

#4245 Targeting ERalpha disorder-function mechanisms in ER-positive breast cancer.

Zhanwen Du, Shuqi Luo, **Sichun Yang**

Case Western Reserve University School of Medicine, Cleveland, OH

Background: The estrogen receptor (ER or ERalpha) is commonly activated by phosphorylation at serine 118 (Ser118) within its intrinsically disordered N-terminal transactivation domain (NTD), enabling estrogen-independent function in breast cancer. While kinase inhibitors targeting this modification are in clinical trials, understanding the molecular mechanism of activation is essential for developing direct therapeutic strategies.
Methods: We combined small-angle X-ray scattering and NMR spectroscopy with functional assays to characterize conformational changes in the ER-NTD. We then conducted activity-guided screening to identify small molecules directly targeting the disordered NTD.
Results: Ser118 phosphorylation triggers an unexpected conformational expansion by disrupting specific hydrophobic clusters between aromatic-rich regions within the NTD - a mechanism extending beyond simple electrostatic effects (Nature 2024, in press). Leveraging this insight, we developed selective small molecules that directly bind the disordered NTD and potently inhibit growth of hormone-independent breast cancer models.
Conclusions: Our work establishes both the mechanistic basis of ER regulation and a therapeutic framework for targeting intrinsically disordered regions in treatment-resistant breast cancer. These findings open a new avenue for first-in-class drugs that modulate protein disorder in cancer.

#4246 Degradation of the ETS transcription factor ERG by stabilized helical peptide (Helicon™) degraders enables pharmacological validation in ERG-fusion prostate cancer models.

Amelia K. Luciano, Brian White, Graeme Lambert, Aaron Fulgham, Ian Wallace, Shelagh Fluharty, Miroslaw Lech, Dakota Hawkins, Pevindi Jayasuriya, Tara Travaline, Paula Ortet, Mallika Iyengar, Julia Bowers, Yusuf Vohra, Santosh Choudary, John Santa Maria, Jason Hoki, Claire May, Mark Ator, Brandon Nicolay, Diwakar Pattabiraman, Alexander W. Hird, Markus Haeberlein, Jonathan Hurov

Parabilis Medicines, Cambridge, MA

Approximately 300,000 new prostate cancer patient diagnoses and 35,000 deaths due to prostate cancer are documented annually in the United States. An estimated 40-50% of all prostate cancers harbor the TMPRSS2-ERG fusion, leading to androgen receptor (AR)-driven expression of the oncogene ERG. As a transcription factor, ERG induces tumorigenesis by increasing cell proliferation and both invasion and migration of tumor cells, promoting their metastatic ability. ERG and AR have interconnected functions in prostate cancer, where together they strike a balance between proliferative potential and luminal differentiation. ERG also enhances the stemness of prostate cancer cells by interacting with epigenetic modifiers such as EZH2, MAT2A, and the SWI/SNF complex, which may lead to increased therapeutic resistance after treatment with AR targeted therapies. While genetic deletion or knockdown demonstrates tumor dependence on ERG, the lack of small molecule ligandable pockets on ERG has limited preclinical target validation and pharmacological proof of concept. Using the Helicon™ (stabilized helical peptides) drug discovery platform, we can efficiently discover binders of traditionally undruggable targets lacking small-molecule binding pockets, like transcription factors. An example Helicon™ is our inhibitor of the β-catenin-TCF4 interaction, FOG-001, which is currently being evaluated in a Ph1/2 clinical trial (NCT05919264). ERG is a well-suited candidate for a Helicon™-based approach to targeting. Here we demonstrate the discovery of molecules that induce targeted ubiquitin-proteosome degradation of ERG by appending E3-ligase ligands to ERG Helicon™ binders. We demonstrate that ERG-degrader Helicons™ bind selectively and with high affinity to ERG in biochemical assays. In cells, ERG is potently degraded via an E3-ligase-dependent mechanism, reducing downstream expression of its target genes. Using both human cell line- (CDX) and patient-derived xenograft (PDX) models, we demonstrate deep and durable degradation of ERG with our Helicon™ degraders and have confirmed for the first time that pharmacological reduction of ERG protein levels leads to substantial tumor growth inhibition. Collectively these data demonstrate preclinical proof of concept for this therapeutic target and mechanism of action. Ongoing optimization of these Helicons™ may allow clinical application of ERG degraders in a patient population with high unmet need.

#4247 Discovery of GH3595: a novel allosteric DHX9 inhibitor for cancer therapy.

Faridoon Faridoon, Jiapeng Li, Jiyue Zheng, Xiang Wang, Guiping Zhang

Suzhou Genhouse Pharmaceutical Co., Ltd., Suzhou, China

DExH-Box helicase 9 (DHX9), also known as RNA helicase A (RHA), belongs to the DExD/H-Box superfamily II of helicases. Due to its fundamental roles in processes such as transcription and maintenance of genomic stability, DHX9 has emerged as a key regulator in various cancer types. Specifically, microsatellite instable (MSI) cancers, such as MSI-high (MSI-H) colorectal cancer, and tumors with defective homologous recombination exhibit a strong dependency on DHX9, suggesting that targeting DHX9 could offer a significant therapeutic opportunity. Employing fragment-based drug design, we have discovered GH3595, a novel allosteric DHX9 inhibitor that selectively targets the DHX9 protein. *In vitro*, GH3595 displayed a low nM potency in ATPase and helicase activity assays, which translated to specific low nM inhibition of MSI-H colorectal cancer and BRCA loss of function breast cancer proliferation. *In vivo*, GH3595 demonstrated favorable pharmacokinetic properties in different species and showed an acceptable safety profile. Furthermore, daily oral dosing of GH3595 led to the regression of MSI-H xenograft tumors without adverse effects on body weight. More importantly, GH3595 induced dose-dependent intra-tumoral circular RNA induction, showing a well-correlated PK/PD/Efficacy relationship. In summary, we report the discovery and characterization of GH3595, a potent and selective inhibitor of DHX9, which effectively disrupts DHX9-mediated signaling pathways. These findings highlight its significant therapeutic potential for the treatment of malignancies characterized by microsatellite instability or deficiencies in homologous recombination.

#4248 Statin-derived small-molecule inhibitors of metastasis (SIM) for high-risk epithelial cancers.

Moe Ishihara, Junhui Hu, Glen Brodie, Elizabeth Bowen, Shannon Shams, Diana Vaca, Jimin Kim, Stuart Conway, Robert Damoiseaux, Lily Wu

UCLA David Geffen School of Medicine, Los Angeles, CA

Background: Metastasis is the deadliest cancer stage, and there is no universal treatment. Different tumors have distinct tissue-specific signatures, but as cancer progresses, they follow a common evolutionary trajectory toward invasiveness and metastasis. Targeting this shared vulnerability in metastatic tumors holds significant translational potential. Recent insights from kidney cancer reveal a novel metastatic mechanism, leading to the discovery of small-molecule drugs, SIMs, that selectively inhibit metastasis across cancers. By understanding SIMs, we aimed to create safer, more effective treatments, potentially saving over 500,000 lives annually in the U.S.
Methods & Results: 1) New concept: Metastasis requires cooperation between VHL+ and VHL- cells, with VHL- cells driving growth and migration in VHL+ cells. 2) SIM candidates: In high-throughput screening of 2,530 FDA-approved drugs, small-molecule compounds selectively inhibiting VHL cells were identified, with fluvastatin being the most potent ("SIM-1"). Subsequent SAR studies identified SIM-2, four times more potent than fluvastatin (IC50 < 0.1uM). 3) Older statins (fluvastatin, simvastatin) were more cytotoxic than newer statins (e.g., atorvastatin and rosuvastatin) despite a lower affinity for their known target, HMG-CoA reductase (HMGCR). Thus, SIMs target a pathway distinct from cholesterol-lowering or HMGCR.4) Transcriptomics and proteomics analyses suggest that older statins may target a non-HMGCR molecule in their anti-metastatic activities, possibly explaining mixed clinical trial results regarding statin use as antineoplastics.5) SIMs are effective in treating various cancers, including kidney, breast (TNBC), and prostate (NEPC).
Conclusions: Critical steps to advancing SIM development toward clinical translation are (i) target identification and (ii) target and therapeutic validation. While our focus is on metastatic renal cell carcinoma (mRCC) to identify SIM's drug target, strategic findings from this project will be directly relevant to other high-risk urological cancers. By investigating this further, we could develop a novel pan-cancer anti-metastatic drug.

#4249 IBI3019, a first-in-class EGFR/CDH17/CD16A tri-specific antibody, demonstrated potent efficacy against CRC and an excellent safety profile in preclinical studies.

Tiongsun Chia, Weiwei Wu, Li Li, Shuaixiang Zhou, Jia Lu, Shuming Lin, Ming Wu, Jia Liu, Feifei Wang, Ya Liu, Chenjuan Zhu, Ying Zhang, Yayao Yan, Huisi Liu, Jian Guan, Junjie Deng, Ninghuan Li, Zhihai Wu, Bin Li, Enhong Zhong, Bingliang Chen, Kaijie He

Innovent Biologics (Suzhou) Co. Ltd, Jiangsu, China

Inhibition of EGFR signaling is clinically effective in colorectal cancer (CRC) as evidenced by the approval for Cetuximab in combination with chemotherapy or Encorafenib, a BRAF inhibitor, and the promising clinical results when combined with KRAS inhibitors, such as Adagrasib or Sotorasib. This underscores the clinical versatility of EGFR antibodies. However, these strategies are inevitably associated with high target-related toxicities, which limit the maximal potential of the drugs. Here, we designed IBI3019, a next-generation EGFR blocker that targets EGFR, CDH17 and CD16A. By targeting CDH17, an adhesion molecule overexpressed in CRC, we enhanced EGFR inhibition capabilities of the tri-specific antibody on the tumor while sparing normal tissues, particularly skin tissues, which are commonly associated with severe toxicities from EGFR blockers. Additionally, IBI3019 incorporates a high affinity CD16A single domain antibody that mediates stronger antibody-dependent cell cytotoxicity (ADCC) than IgG1 used in Cetuximab or the low fucosylated Fc of Amivantamab, a bispecific antibody targeting c-MET and EGFR, both in vitro and in vivo. IBI3019 demonstrated more potent in vitro efficacy than Cetuximab and Amivantamab in CRC cell lines, regardless of KRAS mutation status or the expression of CDH17, c-MET and EGFR. It exhibited strong synergy with Fulzerasib, a KRAS G12C inhibitor and MRTX1133, a KRAS-G12D inhibitor. In NCI-H508 and CCK81 CRC cell line-derived xenograft (CDX) models, IBI3019 suppressed tumor growth more effectively than Cetuximab and Amivantamab. Importantly, in a CRC patient-derived xenograft (PDX) model that is insensitive to Cetuximab, IBI3019 achieved near complete eradication of tumors in all mice. In pre-toxicity studies, cynomolgus monkeys were administrated 4 weekly doses of 50 mg/kg or 150 mg/kg of IBI3019 intravenously, and no mortality or serious toxicity was observed in either group. The highest non-severely toxic dose (HNSTD) of IBI3019 is 150 mg/kg and the calculated therapeutic index is 66. In contrast, previous reports indicated that 5 out of 10 monkeys treated with 75 mg/kg/week of Cetuximab exhibited early mortalities associated with excessive dermatologic toxicities. These results underscore the high safety profile of IBI3019. IBI3019 is a highly optimized FIC molecule with enhanced EGFR blocking capability and ADCC, maximizing tumor inhibition while minimizing toxicity. Its superior efficacy and safety profile support further clinical evaluation.

#4250 Tumor-wide RNA splicing aberrations generate immunogenic public neoantigens across cancers.

Darwin Kwok[1], Nicholas Stevers[1], Inaki Etxeberria[2], Takahide Nejo[1], Maggie Colton Cove[1], Lee Chen[1], Jangham Jung[1], Kaori Okada[1], Senthilnath Lakshmanachetty[1], Marco Gallus[1], Abhilash Barpanda[1], Chibo Hong[1], Gary Chan[1], Jerry Liu[1], Samuel Wu[1], Emilio Ramos[1], Akane Yamamichi[1], Payal Watchmaker[1], Hirokazu Ogino[1], Atsuro Saijo[1], Aidan Du[1], Nadia Grishanina[1], James Woo[1], Aaron Diaz[1], Susan Chang[1], Joanna Phillips[1], Arun Wiita[1], Christopher Klebanoff[2], Joseph Costello[1], Hideho Okada[1]

[1]UCSF - University of California San Francisco, San Francisco, CA,[2]Memorial Sloan Kettering Cancer Center, New York City, NY

Background: High tumor heterogeneity and low mutational burden in cancers pose significant challenges for immunotherapy. To address this, we developed a novel *in silico* pipeline to characterize cancer-specific splicing events (neojunctions; NJs) expressed ubiquitously across ten cancer types. This approach successfully identified tumor-wide, public, alternatively splicing neoantigens (ASNs) that elicit CD8+ T-cell-mediated cytotoxicity across multiple cancers.

Methods: Our SSNIP pipeline identified recurring public NJs expressed in TCGA RNA-seq data (positive sample rate (PSR) > 10%) but not in GTEx normal tissue RNA-seq data (PSR < 1%) across 10 cancer types. We utilized multi-site RNA sequencing data to characterize intratumorally conserved NJs. Our in-house glioma dataset included 56 patients with approximately 10 spatially distinct intratumoral biopsy sites per patient (n=535). In vitro sensitization (IVS) of CD8+ T-cells from healthy donors against high-confidence ASN candidates, followed by 10x VDJ scRNA-seq, identified ASN-specific TCR sequences. TCR-transduced donor-derived CD8+ T-cells were cultured against NJ-expressing glioma and melanoma cell lines to evaluate tumor-specific killing. CRISPRi KD of disease-specific splicing factors was performed to assess NJ modulation.

Results: Our pipeline identified 789 public NJs, with 32 NJs concurrently identified in transcriptomic and proteomic data and predicted to be presented by HLA-A*02:01 with high confidence. We captured TCR clonotypes reactive against NJs in *RPL22* (*n*=7) and *GNAS* (*n*=1), the latter being highly intratumorally-conserved (detected in > 90% of spatially-mapped biopsies across 17/56 patients (26.78%)). Mutant GNAS-derived ASN-specific CD8+ T-cell clones were detectable from glioma patient PBMC by IVS. TCR-transduced T-cells demonstrated recognition and tumor-specific killing against endogenously processed and presented ASNs in multiple glioblastoma and melanoma cell lines. Furthermore, *IDH1*-mutant oligodendroglioma samples demonstrated significantly elevated expression of NJs over *IDH1*-mutant astrocytoma and *IDH1*wt subtypes. Differential gene expression identified decreased expression of splicing factors due to oligodendroglioma-specific co-deletion of Chromosomes 1p/19q. CRISPRi KD of these splicing factors (e.g. *SF3A3*, *SNRPD2*) in *IDH1*wt glioma cells resulted in significantly increased expression of corresponding NJs. Performing DESeq2 on the remaining cancer types revealed similar splicing gene set-associated differences.

Conclusions: SSNIP identified novel public tumor-wide splice-derived neoantigen candidates and ASN-specific TCRs, offering a promising off-the-shelf immunotherapy approach for diverse cancer types. Characterization of intratumorally conserved neoantigens addresses the critical challenge of intratumoral heterogeneity in immunotherapy resistance.

#4251 Drugging of ACOD1 is associated with tumor regression in syngeneic tumor models.

Adonia E. Papathanassiu[1], Weixing Li[1], Yajie Zhong[2], Carmen Chak-Lui Wong[2]

[1]Ergon Pharmaceuticals, Washington, DC,[2]Department of Pathology, The University of Hong Kong, Hong Kong, Hong Kong

Aconitase decarboxylase 1 (ACOD1, also known as Immune Response Gene or IRG1) is a mitochondrial enzyme responsible for the synthesis of the metabolite itaconate in the TCA cycle of pro-inflammatory macrophages. Utilizing Acod1$^{-/-}$ mice and Acod1 KO cells, recent studies have assigned a cancer-promoting role for ACOD1 and demonstrated its expression in immunosuppressive macrophages, found in the TME of syngeneic tumors (Chen et al, Sci. Adv 2023), and in anti-PD-1 (aPD1)-resistant prostate cells (Schofield et al., Cell Rep 2024). To explore the druggability of ACOD1, we synthesized a series of small MW inhibitors and selected two compounds (ERG344 and its derivative ERG350) for further study. Here, we report that, while both compounds suppress synthesis of itaconate in vitro, ERG350 exhibited a 1000-fold improvement in inhibiting ACOD1 enzymatic activity (IC_{50} of ~200 nM) compared to ERG344. Furthermore, both molecules exhibited acceptable pharmacokinetic profiles with half-lives of 8h for ERG344 and 11h for ERG350. Administration of 3 mpk ERG344 as a monotherapy in mice bearing CT26 colon tumors led to complete tumor regression in ~20% of the animals and partial tumor regression in ~40% of the animals. Immunophenotyping of ERG344-treated CT26 tumors revealed an increase in the frequency of tumor infiltrating lymphocytes (TILs), especially those expressing the CX3C chemokine receptor 1 (CX3CR1), and an increase in the frequency of mature dendritic cells DC ($CD3^{-}CD11b^{+}F4/80^{-}MHCII^{+}CD206^{-}$) suggesting that ACOD1 inhibition promotes initiation of an anti-tumor immune response. Increased probability of survival was also observed in the CT26 colon cancer model after daily administration of ERG350 in doses ranging from 0.25 to 3 mpk. The study further reports the effect of ACOD1 inhibition in additional syngeneic models including models of hepatocellular carcinomas

#4252 Genetic and pharmacological disruption of the HBS1L/PELO complex impairs mRNA homeostasis and leads to in vivo tumor regressions in FOCAD-deleted cancers.

Lauren Catherine M. Martires, Matthew Tonini, Hongxiang Zhang, Minjie Zhang, Chengyin Min, Charlotte B. Pratt, Katherine Lazarides, Frank J. Bruzzese, Douglas A. Whittington, Patrick McCarren, Teng Teng, Madhavi Bandi, Shanchuan Zhao, Stephen Paik, Eden Gordon, Shangtao Liu, Alvin Lu, Maria Dam Ferdinez, Binzheng Shen, Hannah Stowe, Yi Yu, Samuel R. Meier, Ashley H. Choi, Lauren Grove, Alan Huang, Alice Tsai, William D. Mallender, Wenhai Zhang, Jannik N. Andersen, Ed Wu, **Hilary E. Nicholson**

Tango Therapeutics, Boston, MA

Loss of tumor suppressor genes in cancer often results in the co-deletion of nearby genes around the chromosomal locus, creating potential collateral damage contexts for targeted therapy. One such example is the loss of *MTAP*, which is frequently co-deleted with the tumor suppressor genes *CDKN2A/B* and creates a synthetic lethal dependency on PRMT5 and susceptibility to MTA-cooperative PRMT5 inhibitors. To identify additional novel drug targets for patients with chromosome 9p21 loss, we first conducted unbiased, combinatorial CRISPR screens evaluating the pairwise knockout of 'context' genes lost on Ch9p21 against a focused library of ~5,000 'target' genes. We identified loss of FOCAD, the gene encoding the SKI complex interacting exonuclease, as synthetic lethal with loss of PELO, a ribosome rescue factor. To expand on this genetic interaction, we conducted genome-wide CRISPR screens in FOCAD isogenic cell lines discovering both PELO and its binding partner HBS1L as synthetic lethal targets in FOCAD-null cells. In FOCAD-deleted xenografts, knockout of HBS1L eliminates tumor growth and results in protein loss of PELO, likely through destabilization of the HBS1L/PELO protein complex. The dependency of FOCAD-null cells on HBS1L/PELO could be rescued by exogenous cDNA expression of FOCAD, and conversely, knockout of FOCAD in WT cells rendered them dependent on HBS1L, demonstrating the robustness of this interaction and validating the HBS1L/PELO protein complex as a promising target for drug discovery. Through cellular phenotypic screens and a medicinal chemistry campaign, we identified pharmacological disruptors of the HBS1L/PELO complex that selectively kill FOCAD-deleted, but not FOCAD-proficient, cells across diverse models. Oral dosing of mice harboring FOCAD-deleted xenografts with our leading compound led to dose-dependent tumor regression and modulation of PD biomarkers. Mechanistically, HBS1L and PELO form a complex responsible for resolving stalled elongation complexes during protein translation. We propose a model in which loss of FOCAD (and/or other SKI complex members) leads to accumulation of aberrant mRNAs on which ribosomes become stalled, leading to hyperdependence on HBS1L/PELO for ribosome rescue. This model is supported by the observation of translational arrest and activation of the unfolded protein response upon HBS1L/PELO inactivation. Clinically, FOCAD loss occurs in ~30% of all MTAP deleted tumors, representing ~3% of all solid tumors (including ~7% of NSCLC). Our lead molecule provides preclinical validation for targeting the HBS1L/PELO complex as a novel strategy for the potential treatment of a large population of patients with FOCAD-deleted tumors.

#4253 Turning pro-tumor inflammation into target opportunity: Development of an IL1RAP ADC for solid tumor treatment.

Yufei Chen, Marlena Gray, Piper Meigs Treuting, Craig Miller, Sara Carbonetti, Katie Snead, Kaleb Smith, Robert Lawrence, Alyson J. Smith

Pfizer Inc, Bothell, WA

Chronic and dysregulated inflammation caused by activation of interleukin-1 (IL-1) signaling supports tumor growth and helps establish an immunosuppressive microenvironment. Interleukin 1 receptor accessory protein (IL1RAP, IL-1RAcP, IL1R3) serves as a co-receptor for interleukin 1 receptor type 1 (IL1R1) at the cell membrane and plays a key role in mediating IL-1 signaling. By combining multi-omics and immunohistochemistry analysis, we identified the upregulation of IL1RAP on many solid tumor indications, including melanoma, non-small cell lung cancer (NSCLC), head and neck squamous cell carcinoma (HNSCC) and esophageal cancer, while normal tissue expression is mainly restricted to the bone marrow. This expression pattern and tumor-supporting biological function makes IL1RAP an attractive target for an antibody-drug conjugate (ADC) therapy approach. Through a hybridoma antibody campaign, we generated a pool of candidate anti-IL1RAP monoclonal antibodies (mAbs) to evaluate as ADC candidates. The candidates all exhibited ability to disrupt the IL-1 signaling cascade but demonstrated variable potency to do so. All of the clones had similar ability to drive drug delivery and direct cytotoxicity to tumor cells as ADCs. These in vitro activities translated into strong in vivo anti-tumor activity in several CDX and PDX human tumor models, with clonal separation only apparent in low expressing models. While strong activity was seen in all CDX models with various IL1RAP expression levels, primary human sample PDX models demonstrated a much stronger expression:response correlation. To evaluate any potential normal tissue liabilities of an IL1RAP ADC, and assess how clone selection may impact this, a primary human bone marrow colony formation unit assay was employed. While several of the anti-IL1RAP antibodies demonstrated on-target myeloid progenitor cell lethality, not all clones were equipotent, despite all driving similar in vivo anti-tumor activity. Collectively this data demonstrates the potential to leverage IL1RAP as an effective and well tolerated ADC and how appropriate antibody selection could potentially aid in increasing the therapeutic index.

#4254 Development of DEM201, a blocking antibody against SMAGP, a novel "Don′t Eat Me" signal overexpressed in cancer.

Sarah E. Carden, Agnieszka Denslow, Mary Baumel, Jing Zhu, Lissette Garcia, Sarah Nesti, Kayla Belanger, Meghan Davis, Carmen Adriaens, Sandeep Kumar, Christophe Queva, Loise Francisco

DEM BioPharma, Inc., Cambridge, MA

Small Cell Adhesion Glycoprotein (SMAGP), a poorly characterized glycoprotein thought to function in cell adhesion, is highly expressed in gastrointestinal (GI) cancers and overexpressed in bladder cancer, hepatocellular carcinoma, kidney papillary cell carcinoma, pancreatic adenocarcinoma, thyroid carcinoma, and endometrial carcinoma. SMAGP was recently identified as a novel “Don’t Eat Me” (DEM) signal, and overexpression of human SMAGP suppresses tumor cell phagocytosis by primary human macrophages. Unlike the canonical DEM signal CD47, SMAGP is not detected in platelets or erythrocytes. Two cellular receptors for SMAGP have been identified, PILRα, an ITIM-containing transmembrane receptor broadly expressed in the myeloid lineage, and CD177, a GPI-anchored neutrophil marker also expressed in some epithelial cells and tumor-associated regulatory T cells (Tregs). To investigate the role of SMAGP in regulating anti-tumor responses, monoclonal antibodies were raised against SMAGP and humanized. DEM201 was selected as a clinical candidate and is specific for human SMAGP with sub-nanomolar (nM) affinity. DEM201 binds cell-associated SMAGP on cancer cell lines derived from GI, pancreatic and lung cancer and elicits potent ADCP and ADCC with sub-nM EC50. *In vivo*, DEM201 significantly inhibited the growth of SMAGP-expressing RKO tumor xenografts. To assess the potential of SMAGP inhibition in regulating both innate and adaptive immunity in vivo, we tested mAb255-mIgG2a, a SMAGP mAb with sub-nanomolar affinity to mouse SMAGP, in immunocompetent syngeneic tumor models. mAb255-mIgG2a potently inhibited the growth of H22 and Hepa1-6 liver cancer. A significant increase in CD8 T cells and macrophages was observed in H22 tumors after treatment with mAb255-mIgG2a. All SMAGP mAb treatments were well-tolerated, clearing the way for DEM201 IND-enabling activities.

#4255 Membrane interactomics (MInt) platform identifies EGFR and CDCP1 as effective co-targets for bispecific antibody drug conjugate IDP-001.

Clare F. Malone[1], Anna de Regt[2], Chris May[1], Lydia Vignale[1], Kendall Johnson[1], Cody Scandore[1], Kalee Hansen[1], Sohpia Romero[2], Hayley Ma[2], Brian Woodruff[2], Ben Setter[1], Hengyu Xu[2], Carol L. Farr[2], Noah Dephoure[1], Heath Klock[1], Ertan Eryilmaz[1], Rob Oslund[1], Niyi Fadeyi[1], Pamela Holland[2], Scott Lesley[2]

[1]InduPro, Cambridge, MA,[2]InduPro, Seattle, WA

EGFR is a biologically validated target which is overexpressed and activated in many human cancers and associated with poor prognosis. However, most current EGFR targeting therapies encompassing multiple modalities have shown limited therapeutic efficacy, frequently due to on-target toxicity in non-malignant tissues where EGFR is expressed. To improve selectivity via co-localization targeting over co-expression alone, we utilized InduPro's proprietary MInt platform across multiple cancer cell systems to identify CDCP1 as a tumor associated proximity antigen to EGFR on tumor cells. Like EGFR, CDCP1 is over-expressed in multiple cancers and plays key roles in oncogenic signaling and malignant transformation (1). Moreover, EGFR signaling induces upregulation and cell surface localization of CDCP1 as well as inhibition of its degradation (2-3). We further demonstrate that EGFR and CDCP1 are co-expressed in many cancer types by immunohistochemistry, including high levels of co-expression in lung squamous cell carcinoma. IDP-001 is a novel MMAE-conjugated EGFR-CDCP1 bispecific antibody with low DAR. We find that bispecific EGFR x CDCP1 antibodies display enhanced selective internalization and cell killing through co-binding. IDP-001 is effective in vitro against a panel of cancer cell lines of varying target expression levels, including Osimertinib resistant EGFR mutant NSCLC. Importantly, IDP-001 has minimal activity against normal cells expressing both targets. CDCP1 is known to undergo cleavage in some settings, and we present data that confirms IDP-001 binds both intact and cleaved isoforms. Finally, we demonstrate in vivo efficacy of IDP-001 in a subcutaneous xenograft model. Collectively, our data supports that EGFR and CDCP1 are inherently proximal to each other in cancer cell lines and that IDP-001 shows pre-clinical efficacy in a variety of cancer settings and warrants further development. Refs:

1) Khan T et al., Cancer Res 81 (2021) 2259-2978.

2) Adams MN et al., Oncogene 34 (2015) 1375-83.

3) Law ME, et al. Breast Cancer Res 18 (2016) 80.

#4256 HY0001a: A novel antibody-drug conjugate (ADC) targeting CUB domain containing protein 1 (CDCP1).

W. Yan, Y. Zhang, M. Zhang, B. An, Y. Zhang, Q. Xiong, G. Chen, A. Fu, D. Sun, X. Cai, H. Chen, M. Geng, H. Wang, C. Yan, Y. Yuan, T. Wei;
Sichuan Huiyu Seacross Pharmaceutical Technology Co., Ltd., Chengdu, China

Introduction: Current ADC development focus on a small number of tumor-associated antigens (TAA). In recognizing heterogeneity of cancers and acquired resistance evolved in response to therapies, we have strategized our ADC development by focusing on discovering and develop novel TAAs.
Methods: Integrative mining of large-scale omics databases was carried out to discover TAA. In-house antibody discovery and engineering platforms were employed for antibody discovery. In vitro assays were used to screen antibodies of high affinity, high specificity, efficient internalization and developability. A MMAE based ADC was evaluated *in vitro* for stability, cytotoxicity and bystander effect and *in vivo* in CDX models for anti-tumor efficacy. PK/TK and GLP toxicology studies were carried out in monkeys.
Results: CDCP1 was identified to be significantly up-regulated in large proportions of cancer cells across many solid tumors, which was comparable to or higher expression than targets of the marketed ADC drugs. It is associated with cancer progression, metastasis and contributed to resistance to cancer therapies. We discovered and humanized a CDCP1-binding antibody (HY0001) which shows high affinity and specificity, and displays high efficiency of internalization and yet no agonist activity. We developed an ADC preclinical candidate (HY0001a) by conjugating HY0001 with vcMMAE at a DAR=4. HY0001a was rapidly internalized upon binding to CDCP1+ cancer cells and colocalized with lysosomes in 1~2 hours after endocytosis. *In vitro* cytotoxicity assays with CDCP1+ cancer cell lines, HY0001a showed high cytotoxicity activities (IC50 = 0.05~10 nM) in a target- and dose-dependent manner with strong bystander effect. HY0001a demonstrated strong anti-tumor efficacy in a target- and dose-dependent (1-5 mg/kg) manner when tested across multiple CDX models, including prostate, breast, pancreatic, colon, ovarian and bladder cancers. Like MMAE, HY0001a, but not HY0001, could arrest proliferative cancer cells in their G2M phase and significantly increase the number of apoptotic cells. HY0001a significant induced the apoptotic pathway in the tumor tissue of MDA-MB-231 CDX model. HY0001a is stable in biomatrix and possesses desired PK profiles (T1/2= 2-4 days in the CDX models and 2-3 days in cynomolgus monkeys). HY0001a showed toxicity profiles, similar to the marketed MMAE-based ADC drugs. We have completed development of the CMC processes and production of lots to enable the IND submission and early clinical trials.
Conclusions: We identified CDCP1 as an excellent ADC TAA with great potential against multiple cancers. We discovered an antibody (HY0001) of high affinity, cancer cell-specific and efficiently internalization, and completed development of a preclinical candidate MMAE based ADC (HY0001a), which showed a great rationale for further clinical investigation to provide a novel cancer therapy.

#4257 Discovery of small molecules to target DDX5 unfolding of MYC promoter G-quadruplex for MYC inhibition.

Sarah D. Washburn, Danzhou Yang

Borch Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN

c-MYC (MYC) is one of the most deregulated oncogenes in human cancers and encodes a master regulator of cell proliferation. Direct targeting of MYC protein has proven to be challenging due to its disordered structure and lack of clear drug binding pocket. G-quadruplexes (G4s) are globular DNA secondary structures that form in guanine-rich regions of the genome with important regulatory roles. The G4 formed in the proximal promoter of the MYC gene (MycG4) acts as a transcriptional silencer. Our lab has previously discovered the DDX5 helicase, a founding member of the DEAD-box RNA helicase family, actively unfolds the MycG4 and transactivates MYC in human cancer cells. Therefore, the DDX5-MycG4 interaction and unfolding represents a promising target for effective MYC downregulation and anti-cancer therapeutics. Previously, our lab has shown for proof-of-principle that G4-ligand TMPyP4 can inhibit the DDX5-MycG4 interaction and downregulate MYC expression in cancer cells. However, TMPyP4 is not a good drug candidate due to its phototoxicity. Further, no inhibitors are available that directly bind DDX5 and specifically inhibit its G4 unfolding activity. Here, we report a high-throughput screen of ~20,000 diverse drug-like compounds to identify specific inhibitors of the DDX5-MycG4 interaction for MYC downregulation in human cancers. We adapted a FRET-based G4 unfolding assay for high-throughput screening in a 384-well plate format. Briefly, a 6-fluorescein fluorophore and a Black Hole Quencher 1 FRET pair is attached to the 3'- and 5'-end of the MycG4 DNA sequence, respectively, whose fluorescence is quenched in a formed G4. Unfolding of the G4 by DDX5 increases the fluorophore distance and increases the detected fluorescence. The Z' score of the adapted FRET assay was ~0.5 which provides sufficient assay range for screening inhibitors. Compounds that reduce DDX5 unfolding by more than 40% were defined as a hit. Out of 20,000 screened compounds, 31 initial hits were identified. Significantly, we identified five different classes of hit compound chemical structures. Several hits are substantially structurally different from classic G4 interacting compounds, suggesting a different mechanism of action for inhibition of the DDX5 protein-DNA interaction. We conducted hit validation and tested the identified compounds for DDX5 G4 and RNA unfolding, to better understand the mechanisms of action of different compound classes. The hits that specifically inhibit DDX5-MycG4 interaction were tested against MYC expression levels in cancer cells. In summary, we have developed a high-throughput screening method to discover inhibitors of the DDX5-MycG4 interaction and have identified several hit compounds with different structure classes. Our goal is to identify lead compounds to target this interaction for MYC downregulation for future anti-cancer therapeutics.

#4258 FL-501 is a potential best in class GDF-15 inhibitor with extended half-life and potent anti-cachexia activity in preclinical models.

Kelly Foster, Michael S. Haas, Walter Newman, Jason Baum, **Roma Kaul**

Leap Therapeutics, Inc., Cambridge, MA

Cancer cachexia is a devastating syndrome characterized by unintentional weight loss, muscle wasting, fatigue, and severely reduced quality of life. This condition affects up to 80% of cancer patients and represents a major contributor to cancer-related mortality. Despite its significant clinical impact, there are currently no approved therapies for cancer cachexia in most of the world. However, recent pre-clinical and clinical studies have identified Growth Differentiation Factor-15 (GDF-15) as a promising therapeutic target for treating this debilitating condition.FL-501, a novel GDF-15 antibody, was designed to neutralize GDF-15 with high affinity and extended half-life. FL-501 was engineered with specific mutations designed to optimize its pharmacokinetic and safety profiles. In studies using humanized FcRn mice, FL-501 demonstrated a 2-fold increase in half-life and 0.5-fold decrease in clearance compared to both ponsegromab and a wild-type precursor of FL-501.MC38 colorectal cancer (CRC) cells engineered to overexpress human GDF-15 (MC38_hGDF15) were used to assess the role of GDF-15 in driving cancer cachexia. Mice inoculated with these tumor cells exhibited significantly greater body weight loss compared to those bearing wild-type MC38 tumors. The weight loss was attributed to substantial reductions across multiple tissue types, including skeletal muscle mass, fat deposits, and cardiac muscle mass. Importantly, treatment with FL-501 fully restored body composition in mice bearing MC38_hGDF15 tumors to an extent similar or greater than other clinical stage antibodies including visugromab and ponsegromab. To further validate these findings in a clinically relevant setting, non-small cell lung cancer (NSCLC) patient-derived xenografts (Crown Bioscience, Model LU5176) were inoculated in immunocompromised mice. In this therapeutic model, mice were treated with cisplatin (5 mg/kg) alone or in combination with either FL-501 or ponsegromab. While untreated mice and those receiving cisplatin monotherapy experienced significant decreases in weight, the combination with either FL-501 or ponsegromab effectively restored their body weight. Moreover, the addition of anti-GDF-15 therapy significantly improved survival of the mice compared to cisplatin treatment alone. These results support GDF-15 as a key driver of cachexia. Further, the targeted inhibition of GDF-15 with FL-501 was able to effectively reverse characteristics of cancer cachexia in multiple pre-clinical models. Taken together, these findings support the continued evaluation of FL501 and its progression into the clinic.

#4259 Peroxiredoxin 3 is a key regulator of mesothelioma progression and a target for therapeutic intervention.

Victoria Gibson[1], Jan Rogel[2], Joanna Dzialo,[2], Terri Messier[1], Aida Habibovic[1], Charlotte Poile[2], Apostolos Nakas[3], Kudzayi Kutywayo[2], Aleksandra Bzura[2], George Naumov[4], Dean Fennell[2], **Brian Cunniff**[1]

[1]Larner College of Medicine at The University of Vermont, Burlington, VT,[2]University of Leicester, Leicester, United Kingdom,[3]University Hospitals of Leicester, Leicester, United Kingdom,[4]RS Oncology, Cambridge, MA

Tumor cells generate increased levels of reactive oxygen species (ROS) that promote tumor cell growth. To survive under elevated ROS levels, tumor cells must increase antioxidant expression, activity, and reliance on ROS scavenging pathways, including the mitochondrial matrix hydrogen peroxide (H_2O_2) scavenging enzyme peroxiredoxin 3 (PRX3). PRX3 expression is elevated in many cancers, including mesothelioma, and is associated with increased cell proliferation, resistance to apoptosis and chemotherapy. PRX3 is a novel molecular target of the first-in-human covalent inhibitor RSO-021. Recent phase 1 data demonstrated a favorable RSO-021 safety profile with early efficacy in patients with pleural mesothelioma, warranting transition to the ongoing phase 2 portion of the MITOPE trial (NCT05278975). RSO-021 inactivates PRX3 by covalently crosslinking active site cysteines, leading to increased levels of mitochondrial ROS, resulting in tumor cell death. In contrast normal cells remain unaffected by the same treatment. Tumor cells are vulnerable to pharmacological PRX3 inhibition; however, the specific role of PRX3 in tumor biology, particularly in mesothelioma, remains unclear. To investigate this, two PRX3 knockout (PRX3 KO) human mesothelioma cell lines were generated using CRISPR/Cas9 and compared to a mock CRISPR/Cas9 control cell line. In vitro characterization of the PRX3 KO clones showed that proliferation was significantly reduced, suggesting that PRX3 plays a critical role in promoting tumor cell growth. Assays using HyPer7, a genetically encoded H_2O_2-responsive fluorescent biosensor targeted specifically to the mitochondrial matrix, revealed a significant increase in HyPer7 oxidation in the mitochondrial matrix of PRX3 KO cells compared to control cells. Additionally, measurement of mitochondrial respiration by the Seahorse XF96 Cell Mito Stress Test showed PRX3 KO cells have a decrease in basal and maximal oxygen consumption further highlighting the role PRX3 plays in mitochondrial function. When injected into SCID mice PRX3 KO cells did not develop tumors, while the control mice presented with significant tumor burden over a four-week period. RNA-sequencing of PRX3 KO cells showed reduced expression of epithelial-to-mesenchymal transition (EMT) associated genes and TGFB signaling genes compared to controls. This correlates with RNA-sequencing run on 22 patient-derived primary mesothelioma explants following treatment with RSO-021. Results showed that the most sensitive explants had a reduction in EMT gene expression, a key signature associated with tumor cell migration and metastasis. Our results show that PRX3 supports mesothelioma tumor progression and survival through regulating redox metabolism and signaling while maintaining gene expression signatures supporting tumor aggressiveness, highlighting PRX3 as an important therapeutic target in oncology.

#4260 ADV-101: a novel ADC targeting IL-1RAP, leveraging tumor cell and the tumor microenvironment vulnerabilities for enhanced anti-tumor efficacy.

Edouard De Dreuzy, Soha El Sayed, Lucie Bouquet, Stephane Lameynardie, Delphine Genin, Amandine Barberot, Lucas Bourhis, Lucas Demontrond, Clementine Primus, Christophe Ferrand, Marina Deschamps, Alexis Delabriere, Laura Demolis, Erik Rutjens, Jens Hasskarl, Ksenija Pavletic, **Richard Charles Alfred Sainson**

Advesya, Paris, France

IL-1RAP is the common co-receptor of all IL-1-related cytokines, and constitutes a novel type of ADC target due to its strong expression on both tumor cells and immunosuppressive cells within the tumor microenvironment (TME). The increased value of this target is complemented by the dependency of certain tumors on IL-1RAP-dependent signaling arguing for a lower chance of antigen loss in response to anti-IL-1RAP therapies. IL-1RAP overall low expression in healthy tissues, also minimizes the risk of on-target off-tumor effects. While healthy tissues exhibit minimal plasma membrane IL-1RAP expression, tumoral tissues demonstrate high IL-1RAP expression, particularly in cancers with squamous histology such as HNSCC and ESCC, as well as other tumor types such as GBM, PDAC, Ewing Sarcoma (EWS) and AML.We developed ADV-101, an ADC consisting of a humanized anti-IL-1RAP IgG1 mAb conjugated to a clinically validated topoisomerase I inhibitor via a cleavable linker (DAR of 8). The anti-IL-1RAP antibody of ADV-101 was shown to be highly specific and selective to IL-1RAP, binding to both human and cynomolgus recombinant protein with EC_{50} values of 0.095 nM and 0.072 nM, respectively (ELISA), and with an EC_{50} of 6.47 nM to human IL-1RAP (by SPR). The antibody was also demonstrated to bind IL-1RAP-expressing cells from different cancer indications, with EC_{50} values ranging from 0.35 to 2.1 nM, and showed specific internalization in a panel of IL-1RAP-expressing cell lines, with EC_{50} values ranging between 0.34 and 4.44 nM. Notably, this binding and internalization were unaffected by the presence of excess IL-1β ligand. There was no impact of payload-linker conjugation on the ADC binding affinity to IL-1RAP protein and expressing cells. The ADC effectively killed IL-1RAP-positive cells *in vitro*, with IC_{50} values ranging from 0.02 to 78.5 nM. *In vivo*, ADV-101 demonstrated strong anti-tumor efficacy in established tumors derived from CDX models (including large tumors) and PDX models. An *in vivo* efficacy study in six EWS PDX models with varying levels of IL-1RAP expression and derived from both metastatic and primary disease demonstrated high anti-tumor efficacy in 5 out of 6 models, including full tumor regression, even in tumors known to be resistant to topoisomerase I/II inhibitors. ADV-101 safety was further established in a non-human primate (NHP) dose-range finding toxicity study, showing no severe adverse events up to the highest dose tested (50 mg/kg), together with robust pharmacokinetics data confirming exposure. Altogether, these findings warrant further development to bring ADV-101 to the clinic.

#4261 Synthetic lethality of targeting thymine DNA glycosylase (TDG) in p53-deficient lung cancer.

Jiaxin Zhou[1], Zhenyu Shao[1], Jiannan Guo[2], Yiqin Wang[1], Qing Xu[1], Yarui Du[1], Haiping Wu[2], Guoliang Xu[1]

[1]Center for Excellence in Molecular Cell Science, Chinese Academy of Sciences, Shanghai, China,[2]CytosinLab Therapeutics, Hangzhou, China

TP53 stands as the most frequently mutated gene in human cancers, with a strong correlation to poor prognosis across various cancer types. This makes it a critical target, representing the largest patient population and a substantial tumor drug market, with a pressing unmet medical need. Directly targeting p53 remains highly challenging, leaving a significant therapeutic gap. However, synthetic lethality presents a promising approach for developing treatments targeting tumors with *TP53* mutations. Thymine DNA glycosylase (TDG) is a multifaceted enzyme involved in DNA base excision repair (BER), transcriptional regulation, and DNA demethylation, playing critical roles in both embryonic development and tumor progression. In this study, we identified TDG as a synthetic essential effector in p53-deficient cancers. This identification was facilitated through an *in vitro* CRISPR-Cas9 screening assay conducted in *Trp53* knockout cells. Our finding reveals that knocking out *Tdg* selectively kills p53-deficient cells while sparing wild-type cells. Conversely, knocking out *Trp53* selectively kills Tdg-deficient cells. Furthermore, elevated TDG expression was observed to correlate with p53-deficiency in human cancers. TDG knockout significantly reduced the viability of the p53-deficient lung cancer cell line NCI-H1299, an effect was rescued by ectopic expression of wild-type p53, but did not affect the viability of p53 wide-type cell line A549. Using a genetically engineered mouse model of lung adenocarcinoma (LUAD), we further demonstrated that genetic inactivation of *Tdg* specifically suppressed tumor growth in the context of p53-deficiency. Mechanistically, TDG and p53 cooperate to regulate the transcription of the RNA helicase *DHX9*, which facilitates the resolution of double-strand RNA (dsRNA). Inhibition of TDG in p53-deficient cancer cells downregulated DHX9, leading to aberrant accumulation of dsRNA derived from SINEs (short interspersed nuclear elements). This accumulation triggered an anti-viral response via the RIG-I/MDA5-MAVS axis, resulting in tumor growth inhibition and an enhanced anti-tumor immune response. Notably, the dsRNA-mediated antiviral response induced by *Tdg* depletion facilitated the recruitment of tumor-infiltrating lymphocytes (TILs), enhancing response to immune checkpoint blockade (ICB) treatment. These findings reveal a novel therapeutic vulnerability in p53-deficient lung cancer and suggest that targeting TDG could synergize with immunotherapies to improve patient outcomes. Our study highlights the function of TDG in transcriptional regulation, extending beyond its proposed roles in active DNA demethylation and mismatch repair, and unveils TDG as a promising therapeutic target for p53-deficient lung cancer.

#4263 QX-001352 is a novel and specific inhibitor of PARG with potentially BIC in vitro and in vivo anti-tumor efficacy.

Dafydd Thomas, Xianhai Huang, Vijay Reddy, Wayne Tang

QuantX Biosciences Limited, Princeton, NJ

When DNA is damaged one of the critical steps is the addition of Poly-ADP-ribose (PAR) chains to target proteins. Poly ADP-ribose polymerase 1 (PARP1) is an enzyme that adds PAR chains to target DNA repair proteins including itself. This serves as a recruitment signal for other DNA repair enzymes to localize to the site of DNA damage. Poly ADP-ribose glycohydrolase (PARG) has a crucial role in DNA repair. This role is by virtue of its intrinsic enzymatic activity to catalyze the removal of PAR chains from target proteins. This removal leads to the resolution of DNA strand breaks. When PARG is inhibited or its expression is abrogated, PAR chains go unresolved and therefore DNA lesions also. This leads to genomic instability and cell death. Therefore, there is a critical balance of PAR chains which is reciprocally regulated by PARP1 and PARG and perturbing this balance may negatively affect tumor cell growth. This makes PARG a rational drug target in oncology, against which the compound QX-001352 has been developed as a potentially best in class (BIC) inhibitor.
QX-001352 is a potent inhibitor of PARG. QX-001352 was shown to inhibit the removal of PAR chains from PARylated PARP1 with an IC50 of 0.2 nM in a biochemical assay. This suggests QX1352 is a potent inhibitor of PARG.QX-001352 was able to inhibit the growth of two ovarian tumor cell lines as well as a breast and gastric tumor cell line. The IC50's ranged from 1.5 nM to 11.9 nM indicating potent cell growth inhibition which represents around 10 folds more potent activity than a benchmark compound in the clinic. QX-001352 blocked the removal of PAR chains in Kuramochi cells in dose dependent manner with an IC50 of 0.1 nM indicating excellent target engagement. In vivo in a Kuramochi cell xenograft model, QX-001352 caused robust tumor regression. Both low and high doses of QX-001352 were fully efficacious whereas no effect was seen with vehicle treated animals. QX-001352 was well tolerated in all the treated groups.
In conclusion, QX-001352 is a potent and specific inhibitor of PARG. This inhibition leads to PAR chain accumulation and prevents tumor cell growth both in vitro and in vivo. QX-001352 possesses highly desirable physicochemical properties and demonstrated favorable off-target profiles. Thus QX-001352 may be an effective PARG inhibitor in the clinic.

#4264 A novel, potent DNA polymerase theta inhibitor, QX-001280, demonstrates anti-tumor activity in combination with a PARP1 inhibitor.

Dafydd Thomas, James Duan, Xianhai Huang, Vijay Reddy, Wayne Tang

QuantX Biosciences Limited, Princeton, NJ

Microhomology-mediated end joining (MMEJ) is a repair mechanism that resolves double strand DNA breaks. It is considered a secondary double strand break repair mechanism behind homologous recombination (HR) but is often utilized by tumors that have lost the ability to perform HR. Critical to this process is DNA polymerase theta (Pol θ). Pol θ is a heterobifunctional protein that consists of an N-terminal helicase domain, a disorder central region and a C-terminal polymerase domain. Following DNA resection by the MRE11-RAD50-NBS-1 (MRN) complex Pol θ is recruited to the site of the double strand break where it adds the missing bases. The bases are then ligated to resolve the double strand break. The central role of Pol θ in MMEJ is highlighted by the fact that inhibition of Pol θ can be synthetic lethal with BRCA mutation and PARP-1 inhibitor treatment, with loss of BRCA1 or 2 resulting in non-functional HR. This loss was exploited with the development of the novel Pol θ inhibitor, QX-001280, and combining with a PARP inhibitor to elicit anti-tumor effect.
QX-001280 is a potent inhibitor which binds to the Pol θ Helicase domain. In vitro, QX-001280 had an IC_{50} for the inhibition of Polθ of 0.84 nM, indicating potent inhibition of the helicase domain of Pol θ. Potent inhibition was also observed in DLD-1 BRCA2 null cells with an IC_{50} of 1.03 nM. Combination with a PARP inhibitor, Olaparib, led to significant increase in phosphorylated H2AX indicating that double strand breaks are not being resolved inferring that Pol θ and MMEJ are being inhibited. In addition, cell viability was significantly reduced in DLD-1 BRCA2 null cells treated with increasing doses of PARP inhibitor in combination Pol θ inhibitor, with a reduction in IC_{50}. In vivo, single agent PARP inhibitor or Pol θ inhibitor had a modest effect on tumor growth. However, when Olaparib and QX-001280 are combined robust tumor regressions were observed. QX-001280 was well tolerated in all the dose groups.
In conclusion, QX-001280 is a potent and specific inhibitor of Polθ which exhibits anti-tumor activity both in vitro and in vivo. It is a high-quality candidate for clinical investigation in combination therapy with PARP inhibitors for cancer treatment.

#4265 The role of the mevalonate pathway in post-translational modifications of PD-L1.

Ruoheng Zhang[1], Emily J. Koubek[2], Jessie L. Reed[3], Jeffrey D. Neighbors[1], Joel M. Reid[4], Raymond J. Hohl[1]

[1]Penn State Hershey Cancer Institute, Hershey, PA,[2]USA Department of Neurology, University of Michigan, Ann Arbor, MI,[3]Department of Biology, Texas A&M University, Penn State Hershey Cancer Institute, College Station, TX,[4]Department of Oncology, Mayo Clinic, Rochester, MN

Introduction: The binding of Programmed Death-Ligand 1 (PD-L1) on the surface of tumor cells to PD-1 on T cells prevents T-cell-mediated anti-cancer immune responses. Immune checkpoint blockades that target the PD-L1/PD-1 interaction have improved survival rates but have several critical limitations. Post-translational modifications (PTMs) of PD-L1, such as glycosylation, are crucial for its stability and engagement with PD-1. Thus, targeting PD-L1 PTMs may be a novel method to improve immune checkpoint blockade efficacy. The mevalonate pathway (MVP) generates various substances involved in PTMs process. Here, we explore the effect of MVP in altering the PTMs of PD-L1 and its impacts on cell surface PD-L1 function.

Methods: The triple-negative human breast cancer (TNBC) cell lines MDA-MB-231 and BT549 were treated with the HMG-CoA reductase inhibitor lovastatin (LOV) with or without the addition of IFN-γ and/or MVP intermediates for 48 hours. PD-L1 transcription was evaluated by qPCR. Alterations in PD-L1 size were detected by Western blotting. PD-L1 surface levels were measured by flow cytometry. HMG-CoA reductase (HMGCR) knockdown was performed to validate the non-off-target effect of LOV. The binding of PD-1 protein to the cell surface PD-L1 was measured by PD-1/PD-L1 binding assay. PD-L1 glycosylation levels were measured by Concanavalin A (Con A) lectin binding assay with immunoprecipitated PD-L1 protein.

Results: LOV treatment caused a significant molecular weight shift of PD-L1 from approximately 45 kDa to 40 kDa and reduced the cell surface PD-L1 levels. However, PD-L1 transcription was not affected by LOV. HMGCR knockdown produced similar changes, indicating LOV's impact on PD-L1 was not due to off-target effects. The addition of MVP intermediates mevalonate (MEV) or geranylgeraniol, but not farnesol prevented LOV-induced changes in PD-L1 molecular weight and surface levels. Interestingly, the addition of 25-hydroxycholesterol, but not dolichol, partially prevented LOV-induced decreases in PD-L1 surface levels. Geranylgeranyl transferase inhibitor GGTI-286, which prevents protein geranylgeranylation, also altered PD-L1 molecular weight and decreased surface levels. In addition, LOV impaired the binding of PD-1 protein to tumor cells' surface PD-L1, which was reversed by the addition of MVP substrate MEV. Con A lectin-binding of immunoprecipitated PD-L1 showed that the LOV did not reduce the N-glycosylation level of PD-L1.

Conclusions: This study reveals the involvement of the MVP in regulating PD-L1 PTMs and highlights the MVP as a potential therapeutic target to modulate PD-L1’s molecular weight and function and improve the efficacy of immunotherapy interventions, presenting a promising avenue for further investigation.

#4266 Discovery and characterization of a novel LRRC15-targeting antibody drug conjugate (ADC) for the treatment of solid tumors.

Shanshan Yan[1], **Bing Wan**[1], Donghui Li[2], Yue Ge[1], Wilson Peng[2], Jiaqing Yi[2], Ziruo Wen[1], Xinchuan Dai[1], Leo Xu[2], Lina Wang[1], Qiuping Ye[2], Peter Huang[2], Linda N. Liu[2]

[1]Zai Lab (Shanghai), Co. Ltd, Shanghai, China,[2]Zai Lab (US) LLC, Cambridge, MA

Leucine-rich repeat-containing protein 15 (LRRC15) is a type I transmembrane protein involved in cell-cell and cell-extracellular matrix (ECM) interactions. It is overexpressed in various mesenchymal tumors such as sarcoma, glioblastoma and melanoma, where it promotes tumor metastasis. Additionally, LRRC15 is upregulated in cancer-associated fibroblasts (CAFs) across various cancer types, contributing to immune-excluded and immune-suppressive tumor microenvironment (TME). This makes LRRC15 an appealing target for cancer therapy. ZL-6201 is an innovative antibody-drug conjugate (ADC) targeting LRRC15, developed using the TME-activable linker-payload (TMALIN®) platform. This ADC comprises a humanized anti-LRRC15 antibody, a protease-cleavable linker, and a novel camptothecin derivative payload. It achieves a high drug-to-antibody ratio (DAR 8) with homogeneously conjugated, hydrophilic linker-payload combinations. ZL-6201 demonstrated strong and specific binding to human and cynomolgus LRRC15 without cross-reactivity to other LRRC family members. Its high affinity and specificity for LRRC15 on the cell surface led to target-mediated internalization and subsequent cytotoxicity in tumor cells upon payload release. Additionally, it exhibited potent bystander killing of LRRC15 negative tumor cells in an in vitro co-culture system. In in vivo efficacy studies, ZL-6201 was well-tolerated and effectively suppressed the growth of established human tumors in a dose-dependent manner in both tumor cell line-derived (CDX) and sarcoma patient sample-derived xenograft (PDX) models. In a human chondrosarcoma SA4109 PDX model, a significantly higher concentration of payload and ADC were detected in the tumors compared to mouse serum, indicating targeted delivery of the ADC. Collectively, these findings support advancing ZL-6201 into IND-enabling studies as a potential treatment for patients with sarcoma and other LRRC15-positive solid tumors.

#4267 Cancer cell-extrinsic effect of immune checkpoint IGSF8.

Xiangyang Wu[1], Flavian D. Brown[2], Jin Wang[1], Yixuan Tang[1], Chao Liu[1], X. Shirley Liu[2], **Xingfeng Bao**[2], Tengfei Xiao[1]

[1]Shanghai Xunbaihui Biotechnology Co., Shanghai, China,[2]Gv20 Therapeutics LLC, Newton, MA

Background: We recently discovered IGSF8 (Immunoglobulin superfamily member 8) as a novel innate immune checkpoint on cancer cells by interacting with KIR3DL2 on natural killer (NK) cells (Cell, 2024). Anti-IGSF8 antibody enhanced NK cell-mediated cancer cell killing and suppressed tumor growth in multiple syngeneic tumor models. In Phase 1 clinical trial, anti-IGSF8 antibody showed favorable safety profiles and promising monotherapy activities. While IGSF8 is overexpressed on cancer cells, it is also expressed on various immune cells within the tumor microenvironment. In this study, we investigated the cancer cell-extrinsic effects of IGSF8 in tumor immunity.
Methods: We generated germline IGSF8 knockout (KO) C57BL/6 mice using CRISPR-Cas9 technology. To evaluate the impact of IGSF8 deficiency on tumor immunity, we inoculated these KO mice with various syngeneic tumors and analyzed immune cell populations in tumor tissue and tumor draining lymph nodes by flow cytometry. To investigate antigen (Ag)-specific anti-tumor immunity, we implanted OVA-expressing B16-F10 melanoma in the KO mice, and quantified the Ag-specific CD8+ T cells with OVA257-264 tetramer and Ag-specific dendritic cells with antibodies specific for H-2Kb/SIINFEKL complexes. We also treated tumor-bearing wild-type (WT) mice with anti-IGSF8 antibody to compare the anti-tumor and immunoregulatory effects of IGSF8 blocking to those observed in IGSF8 KO animals.
Results: IGSF8 KO C57BL/6 mice were viable and fertile. Tumor growth was significantly suppressed in IGSF8 KO mice compared to WT controls across multiple syngeneic models, including B16-F10 melanoma, RM1 prostate cancer, MC38 colon cancer, and LLC non-small cell lung cancer. After tumor challenge, KO mice had a significantly higher frequency of activated $CD44^+$, $PD\text{-}1^+$ T cells from both tumor tissue and tumor draining lymph nodes. In the OVA-expressing tumor model, we observed elevated levels of $OVA_{257\text{-}264}$ $tetramer^+$ $CD8^+$ T cells and $H\text{-}2K^b$ bound $SIINFEKL^+$ dendritic cells in KO mice. Treatment of WT mice with anti-IGSF8 antibody recapitulated these findings, demonstrating enhanced activated T cell frequencies comparable to those observed in KO animals.
Conclusion: Genetic ablation of IGSF8 or its therapeutic blockade with anti-IGSF8 antibody significantly suppressed syngeneic tumor growth, associated with enhanced antigen-specific T cell and dendritic cell responses. These findings reveal a previously unknown cancer cell-extrinsic function of IGSF8 in regulating tumor immunity, further supporting IGSF8 as a promising target for cancer immunotherapy.

#4268 The allosteric MAT2A inhibitor IDE397 uniquely exploits metabolic liabilities associated with MTAP deletion to perturb DNA replication and repair.

Damien Garbett, **Arjun A. Rao**, Marcus M. Fischer, John Vivian, Khristina Magallanes, Natalie Bresnahan, Pari Lanham, Atul Rathore, Austin Li, Rebeca M. Choy, J. Carl Schultz, Scott R. Tyler, Angelica Gonzalez-Sanchez, Zhipeng Fang, Kelly Trego, Briana Marinoni, Yuchen Bai, Peter Teriete, Christian R. Frey, Michael A. White, Claire L. Neilan

Ideaya Biosciences, South San Francisco, CA

Methylthioadenosine phosphorylase (MTAP) is co-deleted with the tumor suppressor CDKN2A in approximately 15% of solid tumors. Loss of MTAP blocks cellular 1-carbon salvage pathway activity due to an inability to convert MTA into adenine and methionine. Accumulation of MTAP's substrate, MTA, and coincident depletion of the MTAP's products, adenine and methionine precursors, results in two distinct targetable vulnerabilities: direct S-Adenosyl methionine (SAM)-competitive inhibition of the essential methyltransferase Protein arginine methyltransferase 5 (PRMT5), and reduced capacity for sufficient nucleotide synthesis required to support tumor cell DNA replication and repair. MAT2A is the rate-limiting enzyme for SAM synthesis in tumor cells, and its position at the intersection of methyltransferase activity and 1-carbon metabolism indicates pharmacological suppression of MAT2A has the unique potential to exploit both vulnerabilities conferred by MTAP deletion (MTAPdel). IDE397 is an allosteric MAT2A inhibitor designed to deliver anti-tumor activity selectively in patients harboring MTAPdel tumors and has demonstrated clinical activity. Here, we have deployed label and label-free profiling of IDE397-dependent effects on tumor cell metabolism paired with genome-wide assessments of cellular methyltransferase activity to evaluate key determinants of IDE397 anti-tumor activity. IDE397 selectively perturbed de novo nucleoside/nucleotide biosynthesis in MTAPdel cells via depletion of homocysteine pools and disruption of methionine cycle/folate cycle integration. Global analyses of RNA splicing, chromatin accessibility, and DNA methylation indicated that IDE397 effectively suppressed PRMT5 methyltransferase activity, while other essential SAM-dependent processes were maintained. These observations indicate that MTAP loss confers a dual dependency on MAT2A activity for sufficient SAM biosynthesis to both support 1-carbon metabolism-associated DNA repair capacity in the absence of MTA recycling, and to ameliorate direct inhibition of PRMT5 by MTA. Furthermore, broad preservation of SAM-dependent methyltransferase activity in the presence of IDE397 suggests a hierarchy among methyltransferases for SAM consumption. Observations of adaptive compensation within distinct methyltransferase pathways, such as polyamine synthesis, suggest this hierarchy is dynamic and orchestrated. The metabolic liabilities arising from MAT2A inhibition in MTAP deleted cancer cells provide a mechanistic rationale for unique therapeutic opportunities for IDE397 as a single-agent and in combination which are now being evaluated in patients (NCT04794699 and NCT05975073).

#4269 Defining the impact of autophagy blockade on triple negative breast cancer and remodeling of the tumor-immune microenvironment in primary and lung metastatic settings.

Tao Li, Hatem Soliman, Ernst Schonbrunn, Andrij Marusyk, Marilyn Bui, **Conor C. Lynch**, Marilena Tauro

Moffitt Cancer Center, Tampa, FL

Background: Triple negative breast cancer (TNBC) cells have high basal rates of autophagy to meet metabolic demands. Our emerging *in vitro* data demonstrates that ULK3 is an important initiator of the autophagy program and is especially important in enhancing TNBC cell survival in response to stressors such as chemotherapy. Inhibiting autophagy can lead to TNBC cell death and potentially the subsequent induction of immunogenic cell death pathways. Here, we interrogate the effect of ULK3 ablation on TNBC migration, invasion and metastasis and whether there is an impact on the surrounding immune landscape.

Methodology: We investigated the impact of autophagy impairment on TNBC cells viability and migration by using CRISPR-Cas9 ULK3 knockout in MDA-MB-231Luc and 4T1Luc cells, halting downstream autophagic machinery. Cell proliferation, migration, and ATP metabolism were monitored using advanced imaging tools such as Incucyte and Opera Phenix. The effects of ULK3 ablation on the metastatic process were examined *in vivo* by injecting parental 4T1Luc and ULK3 knockout (KO) cells into syngeneic BALB/c mice (500,000 cells/mouse, n=10/group) and tracing metastasis to various organs (liver, bones) using bioluminescent imaging (BLI). Tissues were subsequently analyzed using 10x Genomics HD Visium Spatial Transcriptomics. Additionally, multiplex immunofluorescent analysis was performed on TNBC patient's tissue microarrays.

Results: Our *in vitro* data demonstrate that CRISPR-Cas9 ULK3 KO cells (MDA-MB-231Luc, 4T1Luc) exhibit reduced cell proliferation and migration, along with lower ATP metabolite levels, after 96 hours of incubation, compared to their parental counterparts. Our *in vivo* data confirmed reduced primary tumor growth and metastatic dissemination in BALB/c mice injected with 4T1Luc ULK3 KO cells compared to parental control. *Ex vivo* tissue analysis using spatial transcriptomics revealed distinct differences in the tumor microenvironment and immune infiltration between metastatic organs (liver vs bones), correlating higher tumor cell proliferation and higher T regulatory cell infiltration with poorer overall survival. Ongoing analysis of TMAs from Moffitt TNBC patients correlated similar immune e infiltration profiles with worse outcomes. Further analysis of autophagy markers in TNBC cells, along with their correlation to treatment regimens received, will help clarify the metabolic status of cells in patients.

Conclusions: Our experimental approach highlights the potential of targeting the autophagy metabolic pathway to influence TNBC progression. These findings support the use of pharmacological autophagy inhibitors at specific disease stages and offer insights into how patients might respond to immunomodulatory agents based on the localization of their metastases.

#4270 Targeting CNTN4 with exatecan-based antibody-drug conjugate for the treatment of solid tumors.

Mi Young Cha, **Mira Kim**, Seungmin Byun, Youngeun Ha, Kitae Park, Bu-Nam Jeon, Hyunuk Kim, Hyunkyung Yu, Hyunjin Yoo, Soojung Moon, Gyeong-Jin Cheon, Boeun Ju, Hansoo Park

Genome & Company, Suwon-si, Gyeonggi-do, Korea, Republic of

As a novel immune checkpoint, we previously confirmed that contactin 4 (CNTN4) expressed on tumors negatively regulates T cell activity by binding to amyloid precursor protein (APP) on T cells. Immunohistochemistry analysis revealed high expression of CNTN4 in various tumors, including gallbladder, pancreatic, gastric, endometrial, hepatic, prostatic, bladder cancers, melanoma, and others, while its expression remains low in normal tissues, including immune cells. Based on the tumor-specific expression profile of CNTN4, we have developed an antibody-drug conjugate (ADC) targeting CNTN4. This ADC utilizes GENA-104A16.hIgG1 (A16.1), an anti-CNTN4 antibody with reduced effector function of human IgG1, conjugated via cysteine-maleimide linkage to linker including hydrophilic moiety and the topoisomerase I inhibitor (exatecan) with high drug-to-antibody ratio (DAR)(A16.1-Exa). To evaluate its potential, the internalization performance of A16.1 was compared with trastuzumab (anti-HER2) using SK-BR3 cells engineered to overexpress CNTN4, which exhibited a higher HER2 target count than CNTN4 target count. A16.1-Exa demonstrated potent and highly specific cytotoxicity in CNTN4-positive cancer cells, accompanied by a robust bystander effect comparable to the Dxd conjugate. Additionally, A16.1-Exa exhibited high plasma stability and retained its immune checkpoint-blocking function, supporting T cell-mediated cytotoxicity alongside payload delivery. Treatment with A16.1-Exa demonstrated a very promising anti-tumor effect in a fibrosarcoma xenograft mouse model (HT1080/CNTN4). Investigation of the anti-tumor efficacy in a PDX model was conducted with GENA-104A16 (human IgG4 S228P) antibody in the IND-approved stage, conjugated with MMAF (low DAR) in a pancreatic cancer model. Once the tumor volume reached 195 mm^3, mice were randomly divided, and the A16-MMAF conjugate (5 mg/kg) was administered intravenously twice post-grouping. The treatment showed strong anti-tumor activity, leading to tumor regression. Future studies will expand the PDX models and optimize the therapeutic potential of exatecan conjugates to advance the development of CNTN4-targeted therapies, offering a promising approach for treating diverse cancers.

#4271 Discovery of NNC 05-2090, a small molecule antagonist of the neuromedin U receptor 2 for glioma treatment.

Yuna Roh, Taesang Son, Jang-Seong Kim, Tae-Su Han, Hyun Seung Ban, Seon-Kyu Kim

Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea, Republic of

Gliomas account for approximately 30% of all brain and central nervous system tumors, and 80% of all malignant brain tumors. Despite their high frequency, the causes of gliomas remain largely unknown. Temozolomide is the standard treatment for glioma, but its efficacy is limited due to the high rate of recurrence and the development of drug resistance in glioma patients. Therefore, discovering novel therapeutic targets and drugs is crucial to improving survival rate for glioma patients. This study aims to investigate the functions of neuromedin U receptor 2 (NMUR2) as a novel target for glioma and to identify NMUR2-specific regulatory drugs. NMUR2 is highly expressed in glioma tissue, and its overexpression promotes cell proliferation and migration, while its knockdown suppresses these effects. RNA sequencing analysis revealed that NMUR2 knockdown downregulates cell cycle-related genes. STAT5, a transcription factor, mediates the link between NMUR2 and cell cycle regulation. To discover an NMUR2-specific antagonist, we performed cell-based drug screening with 6,331 repurposing drugs and finally identified NNC 05-2090. The NNC 05-2090 treatment in glioma cells reduced cell cycle progression through its downstream signaling pathway. Additionally, we observed a synergistic effect between NNC 05-2090 and temozolomide in both in vitro and in vivo. In conclusion, this study suggests that NMUR2 is a promising novel target, and its antagonist, NNC 05-2090, combined with temozolomide, might offer a promising strategy to improve glioma treatment.

#4272 Cirtuvivint inhibits Ewing sarcoma (ES) growth by suppressing EWS-FLI-1 expression and inducing alternate EWS-FLI1 splice variants.

Sergei Vatolin, Gary K. Schwartz

Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH

Background: The transcription factor EWS-FLI1 is responsible for malignant transformation and ES cell survival. The breakpoints observed typically occur within introns of EWSR1 or FLI1, and expression of EWS-FLI1 requires the splicing machinery to generate an in-frame transcript. Cirtuvivint (SM08502, Biosplice Therapeutics Inc) has been shown to disrupt spliceosome activity by inhibiting Cdc2-like (CLK) and dual-specificity tyrosine phosphorylation-regulated (DYRK) kinases that regulate pre-mRNA splicing. In view of this, we elected to test its effect on ES cell growth, EWS-FLI-1 expression, and whether it would generate alternate splice variants that could suppress ES cell growth.
Results: Treatment of ES cells (A673, CHP100, and MHH ES1) with increasing concentrations of cirtuvivint, as well as its analog SM09419, significantly reduced cell viability (LD_{50} ~250 nM) both in vitro and in vivo, This treatment suppressed EWS-FLI-1 expression and generated multiple alternative EWS-FLI-1 variants within 4-6 hours, marked by the loss of whole exons. Mechanistic studies showed reduced β-catenin and c-myc expression, however, siRNA experiments targeting these genes did not affect cell growth. To investigate the role of these alternative EWS-FLI1 splice variants, transcripts generated by cirtuvivint treatment were cloned, sequenced, and inserted into eukaryotic expression vectors, including a Tet-inducible system and vectors for cell-free protein expression. A luciferase reporter vector containing the EWSR1-FLI1 binding site (GGAAx20) was constructed to assess the impact of wild-type and variant EWSR1-FLI1 on downstream gene expression. Results showed that the expression of specific alternative EWSR1-FLI1 variants in ES cells significantly reduced cell viability, whereas others had no effect. Moreover, cirtuvivint-treated cells transfected with the luciferase reporter exhibited decreased luciferase expression, indicating a loss of gene expression regulated by the oncogenic fusion protein. Comprehensive transcriptome analysis (RNAseq) 4-6 hours post-treatment exhibited significant changes in gene expression, including those associated with alternate splicing, offering new insights on the molecular shifts induced by splicing modulation in these cells.
Conclusion. These findings suggest that cirtuvivint, as a novel CLK/DYRK inhibitor, offers a new therapeutic strategy for targeting EWS-FLI-1 in ES. The induction of specific alternative splice variants has the ability to inhibit cell growth with the associated suppression of gene expression. A clinical trial in recurrent or refractory ES is planned.

#4273 Discovery of first in class, polyphenolic inhibitors of CKAP4 and their antitumor activity in ovarian and endometrial cancer.

Eugenio Gaudio[1], Viktor Ivasishin[2], Paulina Biniecka[1], Christian Kleusch[3], Cosimo Kropp[3], Benjamin Zimmer[3], Kamilla Pawlicka[4], Helena Almqvist[5], Alexey Chernobrovkin[5], Janneke Melis[6], Guido Zaman[6], **Dan F. Stoicescu**[1]

[1]Floratek Pharma, Aubonne, Switzerland,[2]Symeres B.V., Groningen, Netherlands,[3]2Bind GmbH, Regensburg, Germany,[4]Carcinotech Ltd, Edinburgh, United Kingdom,[5]Pelago Bioscience AB, Solna, Sweden,[6]Oncolines B.V., Oss, Netherlands

While some plant-derived polyphenolic compounds exhibit anticancer activity, their complex mechanism of action is generally hindering the efforts to produce clinically useful derivatives. Here we present several compounds that are, to the best of our knowledge, the first known inhibitors of cytoskeleton associated protein 4 (CKAP4) a transmembrane protein associated with resilient forms of cancer. Since CKAP4 is overexpressed in ovarian cancer the compounds were evaluated in the experimental models below as proof of concept.
Target deconvolution using CETSA was followed by spectral shift binding, TRIC and ITC validation. *In vitro* studies assessed anti-proliferative activity (ATPLite, Revvity), with Oncolines Profiler analysis comparing the IC_{50} fingerprints with those of 213 pre-profiled reference drugs. *Bioprinted* human tissue organoids tested the efficacy of the new chemical entities. Tumor microenvironment characterization was performed using immunofluorescence imaging.From our proprietary physical library of 400 new chemical entities SND 106, 108, 562, 581, 584 and 586 were selected based on their strong binding to CKAP4. They were highly active in the ovarian and endometrial cell lines: PA-1, ES-2, AN3-CA, OVCAR-3 with IC_{50}s in the low nanomolar range. Furthermore, the results of the organoid assays showed a significantly improved efficacy of the new compounds compared with the standard of care drug carboplatin.Novel, first-in-class small molecules inhibitors of CKAP4 were discovered, showing strong antitumor activity in ovarian and endometrial cancer. Future studies will determine whether they can be developed into new drugs.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Novel Cancer Therapeutic Targets 1
Poster Session

#4277 EP601: Agonism of GPR84 activates myeloid cells and augments the response to anti-CTLA4 to promote tumor growth inhibition and prolong survival in a syngeneic (CT26) tumor model.

Graeme L. Fraser, Catherine Sorlet, Nicolas Parmentier, Sarah Rorive, Guillaume Dutheuil

EPICS Therapeutics, Gosselies, Belgium

GPR84 is a G-protein coupled receptor predominantly expressed on myeloid cells such as macrophages, NK cells, monocytes and neutrophils. It has previously been reported that GPR84 expression is induced by acute inflammatory stimuli and furthermore that GPR84 may be an interesting target to reverse the immunosuppressive tumor microenvironment[1]. In the current study, induced expression of GPR84 is confirmed as it is demonstrated that there is a 5-fold (or greater) expression of GPR84 on human (M1) macrophages polarized with either IFN-gamma or muramyl dipeptide-and-LPS ("MDP+LPS"). Compound A (CpdA) is a proprietary, selective, small-molecule agonist at GPR84 with similar potency at both the human and mouse GPR84 receptor orthologs (cAMP assay, EC_{50} = 13 nM and 9 nM, respectively). CpdA promotes TNF-alpha secretion from MDP+LPS-activated human macrophages in a concentration-dependent manner and this response is blocked by the GPR84 antagonist GLPG1205. The pharmacokinetic profile of CpdA has been established in rodents ($t_{1/2}$ = 1h, oral bioavailability = 25%) and the compound is appropriate for *in vivo* testing. In the CT26 mouse tumor model, CpdA (30 mg/kg, PO, BID) significantly increased the response to anti-CLTA4 (2 mg/kg, IP, BIW) to >80% tumor growth inhibition and improved survival corresponding to altered immune cell infiltration into the tumor. These results demonstrate the potential of pharmacological activation of GPR84 as a novel approach relevant to cancer immunotherapy.[1]Li et al., Cancer Immunol Immunother (2024)

#4278 Targeting tumor pericyte-specific transcription factor heterodimer for anti-metastasis cancer therapy.

Junqiu Zhang[1], Wenqian Yin[1], Minfeng Chen[1], Yi Xue[2], Wencai Ye[1], Dongmei Zhang[1]

[1]State Key Laboratony of Bioactive Molecules and Druggabity Assesment, Jinan University, Guangzhou, China,[2]Tsinghua University, Beijing, China

Pericytes, mural cells that envelop capillaries, play a critical role in tumor metastasis. While current therapies targeting tumor pericytes (TPCs) effectively suppress tumor angiogenesis and growth, they paradoxically promote metastasis, highlighting the need for improved TPC-based strategies. In this study, we identified a TPC-specific transcription factor heterodimer, TCF21-TCF3, which is positively associated with hematogenous metastasis. TCF3 is essential for TCF21-mediated collagen hydroxylation, facilitating extracellular matrix deposition and accelerating tumor metastasis. Using hydrogen-deuterium exchange mass spectrometry (HDX-MS), transverse relaxation-optimized spectroscopy (TROSY) NMR, and mutational analysis, we characterized the interacting residues of the TCF21-TCF3 heterodimer. A peptide designed to disrupt the TCF21-TCF3 interaction inhibited the formation of the heterodimer, downregulated TCF21/TCF3-dependent gene expression, and suppressed malignant behaviors, effectively reducing metastasis. This study proposes that targeting TPC-specific transcription factor heterodimers offers a promising therapeutic approach to combat tumor metastasis.

#4279 FAK inhibitor APG-2449 induces immunogenic cell death and enhances anti-tumor immunity in cervical cancer through the PERK-eIF2α pathway.

Lin Zhang[1], Mingyue Zhang[1], Weihan Kang[2], Jing Xiao[1], Maple Tse[3], Yong Du[1], Dajun Yang[4]

[1]Sun Yat-sen University Cancer Center (SYSUCC), Guangzhou, China,[2]The First Clinical Medical College of Lanzhou University, Lanzhou, China,[3]University of Sheffield, Sheffield, United Kingdom,[4]Sun Yat-sen University Cancer Center (SYSUCC)/Ascentage Pharma (Suzhou), Guangzhou/Suzhou, China

The focal adhesion kinase (FAK) inhibitor APG-2449, a multi-target tyrosine kinase inhibitor that also targets ALK and ROS1, has demonstrated significant potential for inducing anti-tumor immunity. In this study, we explored the effects of APG-2449 on cervical cancer, emphasizing its capacity to induce immunogenic cell death (ICD) and stimulate immune responses via the PERK-eIF2α pathway. Our findings indicate that APG-2449 enhances ATP release and calreticulin (CRT) exposure on the surface of tumor cells, which are key indicators of ICD. Moreover, treatment with APG-2449 led to a notable increase in dendritic cell (DC) maturation and promoted their differentiation into classical dendritic cells (cDCs), which are essential for antigen presentation and the subsequent activation of T-cells.In vitro co-culture models, APG-2449 effectively boosted the activation of DCs and increased the frequency of tumor-specific CD8+ T cells. These observations underscore that APG-2449 elicits a robust immunogenic response, suggesting that inhibition of FAK/ALK/ROS1 could be a promising therapeutic approach for enhancing anti-tumor immunity in cervical cancer.These results highlight the potential of APG-2449 as an innovative therapeutic agent capable of modifying the tumor microenvironment to foster an effective anti-tumor immune response via the FAK/PERK-eIF2α axis. Future studies are needed to determine the clinical efficacy of APG-2449, particularly in combination with other immunotherapies.

#4280 DWP216, a novel TEAD1 selective inhibitor for NF2 mutant tumors and drug-tolerant persister cells.

Bora Yoo, YoungKu Kang, Won An, Seul Lee, Inyoung Lee, Ahreum Kwon, Eunji Ha, Hyoungrok Bak, Ji-Duck Kim, Joon Seok Park

Daewoong Pharmaceutical Company Ltd., Yongin-si, Korea, Republic of

Neurofibromatosis type 2 (NF2) controls the Hippo pathway signaling through YAP and TEAD axis. NF2 mutation leads to YAP and TEAD activation and is reported in malignant mesothelioma with 30-40% and meningioma with 50%. Furthermore, drug-tolerant persister cells to EGFR inhibitor or KRAS inhibitor show up-regulation of YAP and TEAD. Four isoforms of TEAD (1-4) are highly conserved and each TEAD isoform has the different biological function depending on organ and co-factors. DWP216, a TEAD1 selective inhibitor, shows the excellent in vitro activities. DWP216 also inhibits TEAD1 activity, TEAD1 palmitoylation and interaction between TEAD1 and YAP1 by binding to palmitate binding pocket of TEAD1. Pharmacokinetic parameter of DWP216 present good druglike properties with blood-brain barrier penetration. DWP216 has the superior in vivo efficacy compared to TEAD1 selective inhibitor and pan-TEAD inhibitor. Moreover, we found that TEAD1 is a key regulator for drug-tolerant persister cells to targeted therapy. Combination of DWP216 and EGFR inhibitor or KRAS inhibitor show synergistic anti-cancer effect in vitro and in vivo. In conclusion, DWP216 is a promising TEAD1 selective inhibitor for NF2 mutant tumor and may be a proper option for drug-resistance of targeted therapy. DWP216 is preparing toward to IND filing for Phase 1 in 2025.

#4281 Pharmacological inhibition of thymine DNA glycosylase (TDG) triggers an anti-tumor immunity to suppress p53-deficient cancer.

Jiannan Guo[1], Meng Wang[1], Jiaxin Zhou[2], Yao Cao[1], Yiqin Wang[2], Yilin Liu[1], Youzhen Wang[1], Yuan Mi[1], Yarui Du[2], Guoliang Xu[2], Haiping Wu[1]

[1]CytosinLab Therapeutics, Hangzhou, China,[2]Center for Excellence in Molecular Cell Science, Chinese Academy of Sciences, Shanghai, China

As the most frequently mutated or silenced tumor suppressor, *TP53* mediates cell-cycle arrest, apoptosis, and senescence in cancer development. Although patients with *TP53* alterations are unfortunately poor in prognosis and survival, targeting p53 directly is still largely difficult to activate or restore p53 normal function specifically in cancer. Recently, synthetic lethality approach has been successfully applied to treat patients with mutations in tumor suppressor genes (TSGs) like PARPi in BRCA1/2 mutated tumors, potentiating the future direction of next generation p53 therapies. However, it remains a huge challenge to identify the *bona fide* synthetic lethal partners to *TP53* mutations in cancer. In this study, we demonstrated that pharmacological inhibiting thymine DNA glycosylase (TDG), a multifunctional DNA modifying enzyme with essential roles in DNA base excision repair (BER), transcriptional regulation, and DNA active demethylation, can induce synthetic lethality specifically in various p53-deficient solid tumors including lung, breast, and skin cancer, etc. This offers a novel strategy to target mutated *TP53* for cancer therapies. We discovered and developed from CytosinLab's EpigenPLUS platform a series of small-molecule inhibitors that binds specifically and directly to TDG and disrupts its DNA-binding and modifying activities. Among the series, C-271 is an orally bioavailable front-runner with excellent pharmacokinetics (PK) properties and druggabilities and exhibits profound efficacy in suppressing growth of p53-deficient lung cancer cells both *in vitro* and *in vivo*, with minimal repression on p53 wide-type cells. In p53-deficient cancer cells, treatment with C-271 reduces transcription of the RNA helicase *DHX9*, thereby leading to the accumulation of SINE (short interspersed nuclear element) -derived dsRNA and subsequent activation of the anti-tumor immunity pathways. Additionally, TDG inhibition enhances anti-tumor immunity and displays significant synergy with anti-CTLA-4 antibody, the immune checkpoint blockade (ICB). Furthermore, we explored and validated the sensitivity of other p53-deficient cancer types to TDG inhibition, broadening the potential of TDG inhibition to treat a larger patient population. In conclusion, we identified inhibition of TDG as a brand-new synthetic lethality approach and an innovative synthetic immune activation anti-cancer mechanism in *TP53*-mutant tumors. It underscores the promise of profiling and developing C-271 or other preclinical candidate molecules of TDG into clinic study and extending the breakthrough first-in-class (FIC) therapy to be viable and beneficial for treating p53-deficient cancers. CytosinLab Therapeutics holds full intellectual property (IP) rights to C-271 and its series of TDG-targeting small molecules.

#4283 PLK4 inhibition: A therapeutic strategy to treat patients with TRIM37 amplified tumors.

Kristen Wong, Debashish U. Menon, Weichao Zhang, Boris Wilson, Jared Spletstoser

GlaxoSmithKline, Collegeville, PA

TRIM37 is a breast cancer oncogene and crucial regulator of pericentriolar matrix (PCM) components. Tumors with amplified TRIM37, an E3-ubiquitin ligase, undergo mitotic catastrophe when Polo-like kinase 4 (PLK4) is inhibited. PLK4 is essential for centriole duplication, a key process in cell division. While several PLK4 kinase inhibitors have been identified, their selectivity and in vivo pharmacokinetic properties are suboptimal. In this study, cellular proliferation and pharmacodynamic assays were developed to optimize the activity and selectivity of PLK4 inhibitors. PLK4 inhibition more effectively inhibited cell proliferation in TRIM37-amplified breast and neuroblastoma cell lines. Conversely, TRIM37 knockout breast cancer cells showed reduced sensitivity to PLK4 inhibition. Mechanistically, PLK4 stability is controlled by its auto-phosphorylation, which triggers rapid degradation via the proteasomal pathway. Tool compounds confirmed that inhibition of PLK4 leads to its stabilization, establishing PLK4 stabilization as a pharmacodynamic biomarker for PLK4 inhibition. These assays will enable evaluation of PLK4 inhibitor activity and selectivity in TRIM37 amplified cells.

#4285 Modulation of spliceosomal protein hnRNPH1 in BRAF, NRAS, and triple-wild type melanoma induces immunogenicity and in vivo immune response.

Maab Sultan[1], Sadeeshkumar Velayutham[1], Vladimir Beljanski[2], Keiran S.M. Smalley[3], Nhut Minh Nguyen[1], Shweta Shah[4], Tulsi Desai[4], Aveta Singh[5], Jun Yong Choi[5], Dmitriy Minond[1]

[1]Nova Southeastern University, College of Pharmacy, Fort Lauderdale, FL,[2]Cell Therapy Institute, Dr Kiran C. Patel College of Allopathic Medicine, Nova Southeastern University, Fort Lauderdale, FL,[3]Department of Tumor Biology, Moffitt Cancer Center, Tampa, FL,[4]Keiran Patel College of Osteopathic Medicine, Nova Southeastern University, Fort Lauderdale, FL,[5]Department of Chemistry and Biochemistry, Queens College, Flushing, NY

Background: We previously reported that treating WM266-4 with H1 siRNA, followed by RNAseq, resulting in increased immune related pathways. Additionally, we reported novel melanoma leads, 2155-14 and 2155-18, which act via downregulation of spliceosomal heterogenous nuclear ribonuclear proteins hnRNPH1/H2 (H1/H2) and demonstrated efficacy in an in vivo model of A375 BRAF mutation. This study aimed to determine the effect of H1 modulation on immune-related genes in vitro across melanoma cell lines with diverse mutational backgrounds and in vivo using a syngeneic mouse model.
Methods: To assess in vivo immune response, 2155-14 and 2155-18 were tested in the B16F10 C57BL/6J syngeneic mouse model (n=5). In vitro, we knocked down H1/H2 in BRAF-mutant, NRAS-mutant, and triple-wild type melanoma cell lines and treated BRAF and NRAS cell lines with both compounds to evaluate immune responses to spliceosomal modulation. Data from in vivo experiments were analyzed using one-way ANOVA to compare means, followed by Sidak's multiple comparisons for significant differences. Statistical significance was set at $p < 0.05$. For NanoString experiments, a 1.4-fold change and p-value <0.05 (n=3) were used as thresholds.
Results: Treatment of B16F10 syngeneic melanoma mouse model with 2155-14 and 2155-18 resulted in increased tumor infiltration of macrophages (p-value <0.01), dendritic cells (p-value <0.05), B cells (p-value <0.05), and CD4+ T cells (p-value <0.05) as determined by flow cytometry analysis. Moreover, NanoString gene expression analysis of both transfected and treated samples confirmed upregulation of interferon signaling and cellular immune responses, processes known to enhance cellular immunogenicity.
Conclusion: These findings highlight the therapeutic promise of targeting spliceosomal proteins H1 and H2, and they underscore the potential of 2155-14 and 2155-18 as promising drug candidates for melanoma treatment. Furthermore, the results suggest that spliceosomal inhibition can enhance immune system activation, presenting an opportunity to combine these therapies with FDA-approved immunotherapies to achieve improved treatment outcomes.

#4286 CHK1 as a novel therapeutic target for pleural mesothelioma.

K. Akagi[1], H. Taniguchi[1], H. Tomono[1], M. Matsuo[1], H. Gyotoku[1], S. Takemoto[1], Y. Nakao[1], S. Yano[2], K. Ashizawa[1], H. Mukae[1];
[1]Nagasaki University, Nagasaki, Japan, [2]Kanazawa University, Ishikawa, Japan

Background: Pleural mesothelioma (PM) is a rare thoracic malignant tumor with poor prognosis. First line nivolumab plus ipilimumab prolonged overall survival compared to standard chemotherapy, however its clinical benefit is limited and development of therapeutic strategy with novel mechanisms is strongly desired. In this study, we focused on CHK1, one of the major molecules involved in the DNA damage repair, as a candidate for novel therapeutic target since one of the hallmarks of PM is DNA damage repair, such as BAP1 loss or TP53 mutation.
Method: The prognostic impact of CHK1 mRNA expression on PM was analyzed using RNAseq data of The Cancer Genome Atlas (TCGA). The anti-tumor effect of CHK1 inhibitor using human PM cell lines were evaluated by MTT assay and annexin V assay. The protein expression was evaluated by Western blotting and the mRNA expression was evaluated by qRT-PCR. The anti-tumor effects of CHK1 inhibitor in vivo were evaluated with subcutaneous tumor model that was established by injecting mouse PM cells subcutaneously in immunodeficient Balb/c nu/nu mice, and the pleural carcinomatosis model that was also established with injecting the mouse PM cells into the intrapleural space of immunocompetent Balb/c mouse.
Results: The low CHK1 expression group demonstrated significantly longer overall survival than the high CHK1 expression group in PM cases of TCGA. CHK1 inhibitor had anti-tumor effect and induced apoptosis in a dose-dependent manner. CHK1 inhibitor increased micronuclei formation, activated STING pathway, and induced mRNA expression of type I and II interferons, CCL5, and CXCL10, suggesting CHK1 inhibitor activated anti-tumor immune response. PD-L1 expression was also increased by CHK1 inhibitor. CHK1 inhibitor demonstrated significantly suppressed tumor growth combined with cisplatin compared to CHK1 inhibitor alone in the subcutaneous mouse model. Furthermore, CHK1 inhibitor combined with anti-PD-L1 antibody significantly prolonged overall survival in the pleural carcinomatosis model.
Conclusion: The CHK1 inhibitor showed immunostimulatory effect on PM in addition to direct anti-tumor effect. Furthermore, the therapeutic benefit was enhanced when combined with cisplatin or anti-PD-L1 antibody.

#4287 GBC-11004: an AI-driven novel kinase target with potential to overcome osimertinib resistance in NSCLC.

Hat Nim Jeon, Ji Young Bae, Hyunsun Na, Ji Hwan Park, Seung Hyun Ahn, Bada Pyo, **Jihye Ryu**, Jinguen Rheey

Gradiant Bioconvergence, Seoul, Korea, Republic of

Osimertinib resistance poses a significant clinical challenge in the treatment of non-small cell lung cancer (NSCLC), with diverse mechanisms complicating patient outcomes. Conventional next-generation sequencing (NGS) analysis methods often fall short in identifying effective therapeutic targets due to the complexity and heterogeneity of resistance mechanisms. To address this issue, we have developed an artificial intelligence (AI)-driven target discovery platform designed to identify novel and effective drug target genes capable of overcoming Osimertinib resistance in NSCLC, thereby surpassing the capabilities of traditional NGS analysis. Our platform integrates three key components: deep learning (G-TAC), statistical significance testing (G-SET), and a large language model (G-LAT). G-TAC and G-SET evaluate and rank genes according to their responsiveness to Osimertinib and tumor-specific expression. G-LAT assesses these genes based on publications and clinical trials to ensure novelty and efficacy of the identified targets. We identified a novel kinase target named GBC-11004, as one of the top-ranked target genes that were found to be overexpressed in patient-derived organoids (PDOs) resistant to Osimertinib. To ascertain the functional impact of GBC-11004, target validation was conducted using PDOs and CRISPR/Cas9-based gene editing. Gene editing in Osimertinib resistant PDOs resulted in a significant decrease in cell viability corresponding to increased indel frequency. Furthermore, we have initiated lead compound optimization by preliminary IC50 analyses using compounds targeting GBC-11004 and observed significantly enhanced sensitivity in the combination therapy group (Osimertinib + GBC-11004 inhibitor) compared to the Osimertinib monotherapy group in resistant PDO models. Our results demonstrate the potential of GBC-11004 as a novel therapeutic target for overcoming Osimertinib resistance in NSCLC treatment and emphasize the capability of our PDO-based AI-driven target discovery platform in identifying high-priority novel targets.

#4288 Discovery of a small molecule inhibitor of ADAR1 for cancer immunotherapy.

Zijun Chen, Liqiang Shen, Qi Zhao, Jing Lu, Weiliang Li, Wen Jin, Xiang Ji, Mengchao Shi, Haowei Zhu, Lin Wang, Leduo Zhang, Xianqi Kong, Dawei Chen, **Tianlun Zhou**, Jiasheng Lu

Risen Pharmaceuticals Private Ltd., Shanghai, China

Adenosine deaminase acting on RNA 1 (ADAR1) is a critical enzyme that catalyzes the conversion of adenosine to inosine in double-stranded RNA (dsRNA). By modifying endogenous dsRNA, ADAR1 prevents erroneous recognition by cytoplasmic dsRNA sensors, including MDA5, PKR, and ZBP1. Inhibition of ADAR1's deaminase activity can activate MDA5-mediated innate immune responses and stimulate interferon production, while blocking ADAR1's RNA-binding activity can induce PKR-mediated translational shutdown and cell death. Emerging evidence suggests that silencing ADAR1 enhances tumor responsiveness to immune checkpoint inhibitors, highlighting ADAR1 as a promising target in cancer immunotherapy. In this study, we aimed to develop small-molecule inhibitors of ADAR1 as potential therapeutic agents for ADAR1-dependent cancers. A primary RNA-editing assay was established to screen a library of 265,000 compounds, leading to the identification of 505 initial hits. These hits underwent triage validation involving target engagement studies, cell-based editing assays, and selectivity profiling. Among the validated compounds, RP05129 emerged as a lead candidate for further investigation. RP05129 effectively inhibited ADAR1's RNA-editing activity in both biochemical and cellular assays. Treatment with RP05129 activated downstream interferon-β signaling in an MDA5-dependent manner, confirming its immunostimulatory potential. Detailed point mutation studies revealed the binding site of RP05129 on the ADAR1 protein, providing insight into its mechanism of action. The identification of RP05129 as a selective ADAR1 inhibitor underscores its potential to enhance immune-based therapies for cancers reliant on ADAR1 activity. Further preclinical evaluation of RP05129 may pave the way for novel therapeutic strategies in cancer immunotherapy

#4289 Discovery of potent RAD51 inhibitors with anticancer activity in preclinical models of non-small cell lung cancer.

Yifan Yu[1], Morgan Black[2], Nikolina Radulovich[1], Peter Ferguson[2], James Koropatnick[2], Ming Tsao[1], Mark D. Vincent[2], Geoffrey Liu[1], Samir H. Barghout[1]

[1]University Health Network, Toronto, ON, Canada,[2]Schulich School of Medicine & Dentistry, Western University, London, ON, Canada

RAD51 is a protein that plays a key, canonical role in double-strand break (DSB) repair through homologous recombination (HR) and is biologically important for maintaining genome stability. Given its additional, non-canonical role in mitigating the replication stress characteristic of tumor cells, RAD51 has emerged as an attractive therapeutic target in several malignancies including non-small cell lung cancer (NSCLC). Nonetheless, drug discovery endeavors for developing direct RAD51 inhibitors have had limited success. Here, we describe a series of novel RAD51 inhibitors and their cytotoxic activity in preclinical models of NSCLC. We accessed and improved upon a previously discovered series of phenylsulfonyl indolyl isoquinoline derivatives that bind to the hydrophobic pocket of RAD51 preventing its multimerization and interaction with BRCA2. Of these, JKYN-1 and its mesylate salt (patent application # CA3204011A1) displayed potent and selective RAD51 inhibition in cell-free assays. Given the biological importance of replication stress in a subset of NSCLC, we assessed the cytotoxicity of these RAD51 inhibitors in preclinical models of NSCLC. Using the CellTiter-Glo assay, JKYN-1 displayed cytotoxicity in A549 and H1975 cell lines with lower IC_{50} values compared to the classical RAD51 inhibitor B02 (A549, 4.9 vs >10 μM; H1975, 3.2 vs 8.3 μM). We subsequently assessed the cytotoxicity in a panel of 7 patient-derived organoids (PDOs) of NSCLC with diverse molecular, genetic, and clinical profiles (LPTO308, XDO137, LPTO357, LPTO318, LPTO366, LPTO362, LPTO221). In these models, JKYN-1 and JKYN-1-mesylate displayed a highly potent cytotoxicity with sub-micromolar IC_{50} values between 0.1-0.87 μM as opposed to B02 that had IC_{50} values between 3.7 to >10 μM. In conclusion, our data identify JKYN-1 and its mesylate salt as potent and selective RAD51 inhibitors with superior cytotoxic activity compared to classical inhibitors such as B02. These findings highlight the potential of these novel compounds as promising therapeutic agents in a subset of NSCLC.

#4290 Long chain fatty acid elongase 6 is a novel therapeutic target for breast cancer.

Linxiang Gong, Susumu Kohno, Chiaki Takahashi

Kanazawa University Cancer Research Institute, Kanazawa, Japan

Cancer cells alter the quality of lipids to grow and survive. De novo synthesized fatty acids need to be elongated to provide numerous varieties to lipids. Despite the critical functions of lipid metabolism in cell function, how specific elongase contributes to tumor progression remains incompletely understood. In breast cancer (BRCA), retinoblastoma 1 (RB1) mutation or deletion is considered as one of the key factors promoting tumor progression. We previously reported that loss of RB1 transcriptionally upregulates the elongation of long chain fatty acid family member 6 (ELOVL6). We observed that BRCA cells with lower RB1 expression tend to show high ELOVL6 expression. ELOVL6 inhibition reduced cell proliferation in all subtypes of BRCA cells in vitro and in vivo. Moreover, RB1 loss sensitized these cells to undergo apoptosis following ELOVL6 depletion. These data indicate that ELOVL6 may have synthetic lethal relationship with RB1. We tried to elucidate the mechanism whereby ELOVL6 inhibition causes anti-tumor effects from a metabolic perspective. We noticed that ELOVL6 depletion reduced p-AKT levels in BRCA cells. Using a lipidomic approach, we observed that ELOVL6 depletion caused significant increase in the amount of intracellular ceramide and glycosphingolipids and decrease in sphingomyelin. Cell death associated with enhanced accumulation of ceramide was observed in cells in which both RB1 and ELOVL6 depletion. Inhibition of serine palmitoyltransferase 1 (SPTLC1) restored the p-AKT level and cell proliferation. These results suggest that ELOVL6 inhibition reduces p-AKT level by enhancing ceramide accumulation thereby suppressing cell proliferation. Taken together, our observations suggest that ELOVL6 is a promising therapeutic target in BRCA.

#4291 Inhibition of FANCM helicase activity confers synthetic lethality in alternative lengthening of telomeres (ALT) positive cancers.

Youyou Zhang, Raghuvar Dronamraju, Tom Berrodin, Kevin Coleman, Benjamin Schwartz

GSK, Collegeville, PA

FANCM (Fanconi anemia, complementation group M) is a human gene that encodes a protein that plays a key role in DNA repair and genome maintenance. Alternative lengthening of telomeres (ALT) is a mechanism that allows cancer cells to maintain telomere length without using telomerase. A synthetic lethal relationship between ALT status and FANCM levels was uncovered by analyzing CRISPR Screens in DepMap database. By using telomerase (TERT) as a biomarker which is negative in ALT positive tumors, cell lines without TERT or ALT+ were found to be sensitive to FANCM knockdown. This synthetic lethal relationship was validated in cell lines with different status of ALT by targeting FANCM with siRNA pool and CRISPR-Cas9. Knock-in of K117K silent mutation, but not K117A/R mutations in catalytic domain of FANCM “rescued” the growth of ALT positive FANCM KO SAOS2 cell. Depletion or deletion of FANCM using siRNA/ CRISPR Cas9 led to the increase in the intensity/distribution of PML and TRF foci. ALT positive cell lines have significantly higher amount of C circle DNA compared to telomerase positive or ALT negative cell lines. Loss of FANCM leads to increase in the levels of C circles. Our results further validate FANCM as a target for ALT cancers and suggest inhibiting its helicase activity as a strategy to treat ALT cancer.

#4292 Targeting YB-1 as a treatment strategy for high-grade serous ovarian carcinoma.

D. Tailor[1], F. J. Garcia-Marques[2], A. Bermudez[2], W. Li[1], A. Dheeraj[1], A. Rolig[1], C.-F. Tsai[3], T. Liu[3], T. Pejovic[4], S. J. Pitteri[2], S. V. Malhotra[1];

[1]Knight Cancer Institute, Oregon Health & Science University, Portland, OR, [2]Stanford University, Palo Alto, CA, [3]Pacific Northwest National Laboratory, Richland, WA, [4]Providence OB/GYN Health Center, Medford, OR

Background: High-grade serous ovarian carcinoma (HGSC) is an aggressive type of ovarian cancer, accounting for 75% of ovarian epithelial cancers. It is often diagnosed at advanced stages and frequently develops treatment resistance, resulting in poor survival rates. A key protein associated with this resistance is Y-box binding protein 1 (YB-1), which binds to both RNA and DNA. We hypothesize that pharmacological inhibition of YB-1 may enhance HGSC treatment effectiveness and reduce the development of treatment resistance.
Method: We collected tumor tissue and adjacent normal tissue samples from HGSC patients whose disease relapsed after receiving four to six different therapies. These tissues were used for proteomics, multiplex immunohistochemistry (mIHC), and to create patient-derived xenograft (PDX) models. In mice, the PDX tumors were subjected to treatment with a combination therapy of YB-1 inhibition and chemotherapy agents. Additional proteome analysis was conducted on mouse HGSC cells.
Results: Proteome profiling of patient HGSC samples revealed that malignant tissues significantly overexpress YB-1 and related mRNA processing pathways in comparison to adjacent normal tissues. mIHC analysis of patient HGSC tissue indicated high YB-1 expression correlated with low infiltration of CD45-positive immune cells. *In vivo* mouse studies using the PDX models derived from HGSC samples indicated that treatment with a YB-1 inhibitor (monotherapy with SU056) slowed tumor progression and enhanced sensitivity to cisplatin and paclitaxel. Consistent with the analysis of patient tissues and PDX models, both *in vitro* and *in vivo* studies of mouse cell lines indicated that treatment with SU056 hindered tumor growth and the development of treatment resistance. Furthermore, proteome profiling of mouse HGSC cells treated with SU056 corroborated the patient tissue observations, demonstrating that YB-1 inhibition resulted in the downregulation of mRNA processing-associated pathways.
Conclusions: Combination therapy using a YB-1 inhibitor with chemotherapy enhances chemotherapeutic responses in HGSC PDX models.

#4294 SMCHD1 is a synthetic lethal strategy for cancers with a cancer-testis antigen gene expression signature.

V. Quereda[1], Y. Zhang[1], D. Klimaszewska[2], S. Tonin[2], T. Groth[1], Y. Rao[1], M. T. McCabe[1], B. Schwartz[3], M. Queisser[2], R. Buxton[2], T. J. Berrodin[1], S. Peace[2];

[1]GSK, Collegeville, PA, [2]GSK, Stevenage, United Kingdom, [3]GSK, Cambridge, MA

SMCHD1 is a component of the structural maintenance of chromosomes (SMC) protein family involved in epigenetic gene silencing of the X chromosome and other autosomal chromosome regions. SMCHD1 encodes an ATPase domain and binds chromatin at both N- and C-term domains to facilitate long-range chromatin interactions. However, the exact mechanism by which it elicits transcriptional silencing remains unknown. We have identified a selective dependency on SMCHD1 in cell lines exhibiting a gene expression signature characterized by high expression of multiple cancer testis antigen (CTA) genes. This CTA signature is observed in multiple cancers with >60% prevalence in Chronic Myeloid Leukemia. Importantly, SMCHD1's effect on cell growth was independent of its ATPase activity, and a knock-in degron tag phenocopied SMCHD1 KO indicating that the proliferative advantage offered by SMCHD1 might be due to structural SMCHD1 function unrelated with its enzymatic ATPase role. Finally, SMCHD1 depletion led to the upregulation of multiple genes, including a cluster of CTA genes, in sensitive cell lines with the CTA-positive signature, while CTA-negative cell lines remained unaffected. These data confirm that SMCHD1 is a potential therapeutic target for patients with elevated CTA expression, and provides insight to what therapeutic modality would be most effective.

#4295 Discovery of a novel class of androgen-competitive AR inhibitors that can combat drug resistance due to AR gene amplification.

A. Szempruch, T. Scott, J. Ernst, J. Hoffman, A. Klova, A. Liu, N. Lu, A. Milik, S. Niessen, G. Packard, J. Remsberg, R. Sipos, T. Soriano, G. O'Neill, S. Buchanan;
Belharra Therapeutics, Inc., San Diego, CA

In metastatic prostate cancer, most cases of resistance to direct AR inhibitors (ARi) are associated with a rebound in serum PSA implying AR reactivation. Resistance to first generation ARi, like bicalutamide, has been linked to AR amplification and is thought to be caused by increased levels of AR protein favoring the drug's partial agonist activity. This agonism counteracts inhibition with a net effect of incomplete antagonism. The phenomenon of anti-androgen withdrawal syndrome documented with early ARi supports this hypothesis and presumably reflects net agonist activity. Enzalutamide ushered in the current generation of approved ARi and was identified using prostate cancer cell line models of bicalutamide resistance with increased AR protein. It has recently become clear that a large majority of cases of mCRPC resistance to enzalutamide is associated with even higher copy number amplification of AR. Enzalutamide, and other latest generation ARi, including AR degraders in clinical development, all share a closely related ('lutamide) core chemical structure with the earlier generation partial agonists implying a similar mechanism of binding AR. This raises the possibility that high copy AR amplification-driven resistance to enzalutamide is due to an antagonism-to-agonism switch mechanistically identical to that of bicalutamide but requiring higher AR levels. However, AR levels exceeding those required to confer bicalutamide resistance are detrimental to standard cancer cell lines growing in culture. Here we describe new mechanistic models of androgen-stimulated AR activation across a wide range of AR levels. In these models early generation 'lutamides, but not enzalutamide, show a switch to agonist like behavior at moderate levels of AR overexpression. At higher levels of AR, enzalutamide, apalutamide, and darolutamide show the same agonist conversion and corresponding evidence of incomplete antagonism in enzalutamide-resistant prostate cancer cells with high copy AR amplification. We employed these assays in concert with a novel, in-cell, chemoproteomic screening approach to identify non-lutamide ARi capable of complete antagonism. We discovered a novel chemotype of ARi that lacks any propensity for an agonism switch, even at high levels of AR protein. These compounds completely suppress viability of VCAP prostate cancer cells with high copy AR amplification, have no effect on the viability of AR-independent cell lines, and show activity against all clinically common AR point mutants. These observations suggest that the potential for a switch to agonism is inherent, in the 'lutamide scaffold, and is exacerbated by increased AR levels leading to incomplete antagonism and resistance. This resistance can be effectively combated with a new class of complete AR antagonists.

#4297 Targeting metabolic dysfunction in prostate cancer: the anti-proliferative effects of ql47 through inhibition of dlat.

Grace Nirmala Jesuthankaraj, August John, Huanyao Gao, Arun Kanakkanthara, Richard Weinshilboum, Liewei Wang

Molecular Pharmacology and Experimental Therapeutics Department, Mayo Clinic Hospital-Rochester, Rochester, MN

Background: Prostate cancer is the most common cancer in men in the United States with an estimated 288,300 new cases diagnosed in 2023. The treatment strategies include androgen deprivation therapies (ADTs) for metastatic prostate cancer (mPC) but 10-20% of patients eventually progress to castration-resistant prostate cancer (CRPC). However, nearly all patients develop primary and acquired resistance overtime, and developing therapies and identifying novel molecular targets are essential in enhancing the clinical outcomes of patients with prostate cancer. In this study, we report a small molecule that belongs to a class of tricyclic quinolones, QL47 that was previously screened among various other compounds in prostate PDX organoid models and showed a potent anti-proliferative activity.
Methods: QL47 was tested on prostate cancer cell lines (22Rv1, 22RV1 abiraterone-resistant and DU145) for its anti-proliferative potential. Further, we used the biotinylated QL47 (QL47B) to pulldown its potential targets and we identified the targets through immunoprecipitation-mass spectrometry (IP-MS) technique followed by Western blot analysis. Cell cycle and cell death analyses were performed after staining the cells with propidium iodide and Annexin V followed by flow cytometry analysis. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) was investigated using the Seahorse assay.
Results: The initial screening of QL47 in prostate cancer cell lines showed that it inhibits the proliferation at an IC50 of 50-200 nM. Through IP-MS and Western blot analysis, we identified a member of the pyruvate dehydrogenase complex dihydrolipoamide S-acetyltransferase (DLAT) as a direct target of QL47. Mechanistically, we found that QL47 does not increase cell death or affect cell cycle, but inhibits metabolic function, with a decrease in both oxygen consumption rate (OCR) and extracellular acidification rate (ECAR).
Conclusion: We have identified a novel mechanism of action of QL47 on the pyruvate dehydrogenase complex, and we plan to further validate the safety and efficacy for the treatment of prostate cancer.

#4298 Investigating the potential of EO3001 as a therapeutic agent for clear cell ovarian cancers harboring ARID1A mutations.

Yuchen Ding[1], Tsz Yin (Jacky) Lam[2], Yuting (Shary) Chen[1], Longyijie (Grace) Wei[1], Yuqin (Lucy) Li[1], Yuhan (Joyce) Zhang[1], Farhia Kabeer[1], Forouh Kalantari[1], **Jeffrey Bacha**[3], Dennis Brown[3], Sarath Kanekal[3], Neil Sankar[3], Michael Lizardo[4], Michelle Woo[4], Amal M. EL-Naggar[1], David Huntsman[1]

[1]Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada,[2]Interdisciplinary Oncology, University of British Columbia, Vancouver, BC, Canada,[3]Edison Oncology Holding Corp, Menlo Park, CA,[4]Molecular Oncology, BC Cancer Research Centre, Vancouver, BC, Canada

Introduction: Clear cell ovarian cancer (CCOC) is a histological subtype of ovarian cancer that is resistant to standard chemotherapies. Although it accounts for only around 6% of all ovarian cancer cases, it is the second leading cause of mortality among ovarian cancers. ARID1A loss-of-function mutations have been identified as a hallmark (~65%) of CCOC. ARID1A, a key component of the SWI/SNF chromatin remodeling complex, plays a critical role in regulating gene expression. Loss of ARID1A disrupts the expression of SLC7A11, leading to decreased glutathione (GSH) levels and elevated reactive oxygen species (ROS) levels, while making cancer cells highly dependent on oxidative phosphorylation (OXPHOS). EO3001, a synthetic small molecule, can selectively transport extracellular Cu(II) to mitochondria, increasing mitochondrial ROS and inducing cuproptosis. Recent studies suggest that ARID1A-deficient CCOC cells are more vulnerable to EO3001. In this study, an ex vivo model, the pulmonary metastasis assay (PuMA), along with other in vitro models, was employed to assess the effectiveness of EO3001 in CCOC. PuMA enables the study of cancer cell growth in a more physiologically relevant microenvironment. In PuMA, fluorescently tagged cancer cells are inoculated into mouse lungs, which are then inflated with agarose gel, sectioned, and treated under different conditions. The growth of cells is quantified by fluorescence intensity from lung sections.
Methods: Isogenic ARID1A wild-type and mutant CCOC cell lines (RMG-1, OVCA429, and JHOC-5) were generated using CRISPR-Cas9. The effects of EO3001 were evaluated using in vitro assays and PuMA. Cell viability, proliferation, ROS levels, glycolysis and OXPHOS activity, migration, and invasion were measured with and without EO3001 treatment under various stress conditions. Drug efficacy was validated in both short- and late-harvest PuMA models.
Results: EO3001 demonstrated significant differential effects against ARID1A-deficient CCOC cells in both in vitro and ex vivo models. However, hypoxic conditions significantly diminished its efficacy, likely due to a metabolic shift between OXPHOS and glycolysis. Furthermore, Cu(II) concentration in the microenvironment remarkably impacts the efficiency of EO3001. Overall, EO3001 presents a promising therapeutic strategy for ARID1A-mutant CCOC as a single agent. Targeting the OXPHOS dependency in ARID1A-mutant CCOC cells and enriching Cu(II) concentration could further enhance the efficacy of EO3001.

#4299 Validation of the antitumor compound 3797 in human and murine glioblastoma models.

Lara Gonzalez-Rendo[1], David Moreiras-Alvarez[2], Martin Baleiron-Rodriguez[3], Laura Porres-Ventin[1], Jose A. Zumalave[1], Maria Isabel Loza-Garcia[3], **Victor M. Arce**[1], Jose A. Costoya[1]

[1]Molecular Oncology Laboratory, CIMUS, Departamento de Fisioloxia, IDIS, Universidade de Santiago de Compostela, Santiago de Compostela, Spain,[2]Kaertor Foundation, Santiago de Compostela, Spain,[3]Biofarma Research Group, CIMUS, Universidade de Santiago de Compostela, Santiago de Compostela, Spain

Multiple studies have highlighted the critical role of the RB-E2F pathway in the control of cell proliferation, therefore, decreasing E2F hyperactivity could be a valuable therapeutic strategy in cancer. Based on this, we propose a therapeutic approach in which E2F transcriptional activity is specifically reduced in cells that show hyperactivation of this pathway, in other words, tumor cells. Preliminary data obtained in the group show that poly (ADP-ribose) polymerase-1 (PARP1) is a transcriptional co-activator of E2F1 and, because of this, loss of PARP results in cell cycle regulation and reduced tumor growth. Furthermore, taking into consideration that most oncogenic processes are associated with cell cycle dysregulation, disruption of the PARP1-E2F1 interaction could provide a new therapeutic target for various tumor types. By searching for compounds that can interfere with the PARP1/E2F1 interaction, our group has obtained a library of 85 compounds generated by *in silico* screening of millions of commercially available compounds using a drug discovery platform. After initial *in vitro* screening, we have identified 12 molecules capable of inhibiting this interaction. The one that performed best was selected for validation using 2D and 3D models of human and murine glioblastoma. For this purpose, we carried out cell viability and migration assays to assess the effect of the compound on two murine and one human glioma cell lines. In all cell lines, cell growth was decreased by treatment, as revealed by violet crystal staining. In addition, wound closure response was inhibited in treated cells as compared with controls. Finally, the therapeutic efficacy of compound 3797 was tested in organoids originated from those tumor cell lines, using the lactate dehydrogenase (LDH) assay, which assess the cell metabolic activity. In keeping with results obtained in 2D cultures, LDH assay showed that compound 3797 decreases the growth rate in organoids derived from glioblastoma cell lines. This work was supported by Agencia Estatal de Investigación (AEI/10.13039/501100011033), Xunta de Galicia (GPC GI-1862, ED431B 2020/26; ED431G 2019/02) and European Regional Development Fund-ERDF. AIMS Awards Program - Project A19-513, Atomwise Inc.

#4300 AT1965, a novel mRNA cap 2′O methylation inhibitor, exerts potent antitumor activity and enhances chemotherapy efficacy.

Arshit Narang, Vinay S. Mahajan, Shiladitya Sengupta

Harvard Medical School/Brigham and Women's Hospital, Cambridge, MA

Cellular messenger RNA (mRNA) of higher eukaryotes and many viral RNAs are methylated at the N-7 and 2′-O positions of the 5′ guanosine cap by specific nuclear and cytoplasmic methyltransferases. N-7 methylation is essential for RNA translation and stability. Recent studies have highlighted the critical role of 2′O methylation of the cap in enabling the innate immune system to distinguish self-RNA from foreign RNA through pattern recognition receptors, such as RIG-I. Despite advances in understanding RNA modifications, the therapeutic potential of targeting RNA capping enzymes remains unexplored. Here, we report that AT1965, a novel mRNA cap 2′O methylation inhibitor, exhibits potent antitumor effects and significant synergy with cabazitaxel, an FDA-approved anticancer agent. Subcutaneous Lewis Lung Carcinoma (LLC) tumors were established in C57BL/6 mice, divided into three groups: untreated controls, those treated with cabazitaxel alone, and those treated with cabazitaxel in combination with AT1965. The combination therapy resulted in a mean tumor size reduction of 23.6%, while tumors in the other groups increased in size. Mechanistically, AT1965 impairs mRNA stability and translation of key oncogenic transcripts, sensitizing tumors to cytotoxic agents. These findings underscore the potential of RNA capping enzymes as therapeutic targets in cancer. Future studies should explore the application of AT1965 in other tumor models and its potential to synergize with diverse chemotherapeutics.

#4301 ENO1 as a potential target for anti-cancer therapy: Insights into its role in neuroendocrine prostate cancer cell growth and survival.

Krystal Rubi Santiago Torres, Alfonso Duran, Kristen Whitley, Shriya Roy, Frankis Almaguel, Carlos A. Casiano

Loma Linda Univ. School of Medicine, Loma Linda, CA

Prostate cancer (PCa) is the second most commonly diagnosed cancer and the leading cause of cancer-related death among men in the United States. Taxane-based chemotherapy with docetaxel (DTX) and cabazitaxel is used as last-line treatment for metastatic castration-resistant prostate cancer (mCRPC). However, these drugs often fail due to the development of chemoresistance. The prostate-specific membrane antigen (PSMA) has emerged as a critical target for theranostics (imaging + therapy) in mCRPC. Nonetheless, around 30% of patients show a limited response, particularly those with low or absent PSMA expression. Given the limitations of targeting PSMA in certain patients, exploring alternative molecular targets such as Enolase (isoforms ENO1 and ENO2) could enhance therapeutic options for patients with mCRPC. ENO1, an enzyme involved in glycolysis, demonstrates potential as an alternative target for mCRPC treatment as it is found on the surface of various tumors. We hypothesized that ENO1 is abundant on the surface of neuroendocrine-like prostate cancer (NEPC-L) cell lines, and that targeting this protein will disrupt key metabolic pathways, resulting in significant anti-cancer effects and reduced tumor growth. Western blot analysis revealed that DTX-sensitive PCa cell lines expressing NEPC markers also express both ENO1 and ENO2. In contrast, DTX-resistant NEPC-L cells expressed ENO1, with limited ENO2 expression, indicating a potential metabolic vulnerability in these cells due to ENO2 downregulation. Additionally, we observed that the cell surface localization of ENO1 in DTX-resistant NEPC-L cell lines varied according to glucose levels. High glucose levels, common in metabolically active metastatic tumors, promoted the cell surface localization of ENO1. By contrast, cells cultured in normal glucose conditions demonstrated lower levels of ENO1 cell surface abundance, likely due to inhibition of the c-MYC oncogene and upregulation of MBP1, a small variant of ENO1 that suppresses c-MYC mediated transcriptional induction of ENO1. We also determined if genetic silencing of ENO1 affects the proliferation and clonogenic ability of NEPC-L cells. Preliminary results following MTT and Clonogenic Assays indicated that ENO1 knockdown with specific siRNAs significantly reduced cell proliferation and clonogenic ability and altered cellular morphology, while preserving cell viability, compared to scramble negative control siRNA. This indicated that while genetic targeting of ENO1 may not necessarily impact tumor cell survival, it could have a dramatic inhibitory effect on tumor growth. Future experiments will explore if ENO1 knockdown re-sensitizes NEPC-L cells to DTX treatment, inhibits tumor-sphere formation, and modifies the stemness properties of these cells, supporting its potential as a novel therapeutic target in patients with drug-resistant NEPC.

#4302 Loss of carnitine acyl-carnitine carrier induced tumor regression by mTOR inactivation.

J. Kang[1], H. Lee[1], S. Woo[2], W. Choi[2], S. Sim[2], J. Chun[1], S.-Y. Kim[2];

[1]National Cancer Center Graduate School of Cancer Science and Policy, Goyang-si, Gyeonggi-do, Korea, Republic of, [2]National Cancer Center, Goyang-si, Gyeonggi-do, Korea, Republic of

Purpose: We found that most ATP production in cancer cells depends on fatty acid oxidation (FAO). However, the direct link between FAO and cancer promotion has not been interpreted. Therefore, we propose that blocking FAO in cancer may prevent pancreatic cancer growth promotion by blocking its ATP production.

Experimental Design: To block FAO, CAC (carnitine-acylcarnitine carrier, gene: *SLC25A20*) in the mitochondria inner membrane was knocked down in cancer cells, which was implanted for xenograft in mice. To compare cancer development, survival rates and histopathological differences were analyzed with KPC mice (*Kras*$^{G12D/+}$; *Trp53*$^{R172H/+}$; *Pdx1-Cre*) and KPC/Cac$^{+/-}$ mice.

Results: By CAC knockdown, the cancer cells reduced ATP production and decreased cell growth. Proteomic analysis showed that CAC knockdown significantly reduced cancer cell growth following mTOR inactivation by reduced ATP production, consequently leading to cell death. Xenografts using CAC knockdown PDAC cells showed up to 80% decrease of tumor growth compared to the control. Furthermore, the median survival of KPC/Cac$^{+/-}$ mice was increased by 3.1 weeks compared to KPC mice.

Conclusion: Blocking FAO by CAC knockdown significantly suppressed tumor growth by mTOR inactivation through ATP depletion in pancreatic cancer cells. mTOR inactivation by CAC knockdown, consequently, decreased levels of cyclin D and PARP, which were followed by cell cycle arrest and cell death. Thus, even if signal transduction is induced by various growth receptors, mTOR cannot be activated when ATP levels fall below the *Km* value.

Funding source: This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Science and ICT to SK (NRF-2019M3A9G1104345) and by a grant from the National Cancer Center of Korea to WC (NCC 2410891-1) and JWC (NCC 2410892-2).

#4303 AZD2284: A novel, alpha-particle emitting radioconjugate targeting STEAP2 in metastatic castration-resistant prostate cancer.

Darlene Monlish[1], Brigitte Theriault[2], Mel Ehudin[1], Vanessa Muniz-Medina[1], Dewald van Dyk[1], Lisa Godfrey[3], Dipal Patel[2], William Turnbull[2], Liang Zhang[4], Moditha Nawinne[3], Teresa Collins[3], Kenneth Thress[4], Douglas Ferguson[4], Rajiv Bhalla[2], Nicolas Giraldo[1], Melody Handali[1], Jeong Min Han[1], Crystal Cheung[1], Asurayya Worrede[1], Clare Hoover[4], Sujiet Puthenveetil[1], Edward Rosfjord[1], Frank Comer[1], Elaine Hurt[1], Darshan Dalal[5], Christopher Leamon[2], John Valliant[2], Puja Sapra[1]

[1]AstraZeneca, Gaithersburg, MD,[2]Fusion Pharmaceuticals, Hamilton, ON, Canada,[3]AstraZeneca, Cambridge, United Kingdom,[4]AstraZeneca, Waltham, MA,[5]AstraZeneca, New York City, NY

Background: AZD2284 is a novel, targeted alpha therapeutic directed against six-transmembrane epithelial antigen of prostate-2 (STEAP2), a receptor highly overexpressed in prostate cancer. AZD2284 is comprised of a humanized, STEAP2-targeting rodent cross-reactive IgG1 monoclonal antibody conjugated with a DOTA chelate and radiolabeled with the alpha-particle emitting radionuclide, actinium-225 [^{225}Ac]. Leveraging high STEAP2 expression in prostate cancer and the high linear energy transfer of the alpha emitter, AZD2284 is presumed to deliver the radioisotope to targeted tumor cells, generating clustered DNA double strand breaks and other cellular damage that ultimately leads to cell death.

Methods: Preclinical pharmacological assessments were conducted with the lutetium-177 [^{177}Lu] labeled analog of AZD2284, FPI-2281. In vitro binding and internalization assays with FPI-2281 were performed in STEAP2-positive prostate cancer cell lines with varying levels of target expression. In vivo biodistribution and uptake were evaluated ex vivo across multiple timepoints following FPI-2281 administration to tumor-bearing cell-line derived xenograft (CDX) models. To assess the radiotherapeutic efficacy of AZD2284, a single dose was administered intravenously to prostate cancer CDX and patient-derived xenograft (PDX) models.

Results: Binding and internalization studies demonstrated FPI-2281 effectively binds to the STEAP2-positive prostate cancer cell line models (LNCaP, C4-2, and 22Rv1) with over 30% cellular retention at 24 hours. Corresponding in vivo biodistribution profiles showed sustained tumor uptake in xenograft models, with peak tumor uptakes of 60% ID/g in the 22Rv1 model and 83% ID/g in the C4-2 model at 14 days, respectively, with limited normal organ uptake observed at all timepoints. AZD2284 elicited target- and dose-dependent anti-tumor efficacy and sustained tumor regressions in CDX and PDX models with varying levels of STEAP2 expression.

Conclusions: In preclinical models, AZD2284 effectively binds to and internalizes in prostate cancer cells, thereby delivering the radioisotope, [^{225}Ac]. Biodistribution studies confirm sustained tumor uptake of AZD2284 with low normal organ uptake. Single-dose administration of AZD2284 leads to durable anti-tumor efficacy in xenograft models. These data suggest AZD2284 is a promising therapeutic candidate in prostate cancer and support the initiation of clinical trials.

#4304 Novel small molecule targeting cancer metabolism by inhibiting SCAP/SREBP pathway.

Jeffrey N. Lindquist[1], Michael Green[2], Ravi K. Muttineni[3], Hirdesh Uppal[4]

[1]Research, Capulus Therapeutics LLC, San Francisco, CA,[2]Chemistry, Capulus Therapeutics, LLC, San Francisco, CA,[3]Immunocure Discovery Solutions Pvt Ltd, Hyderabad, India,[4]Capulus Therapeutics LLC, San Francisco, CA

Background: A critical component of oncogenesis and cancer progression is metabolic re-programming of cells to enable continuous growth. Oncogenic activated signaling pathways can drive *de novo* lipogenesis to provide ample lipids, e.g. cholesterol and fatty acids, to support the tumor cells heightened metabolic needs. The transcriptional regulation of lipogenesis is mediated by the sterol regulatory element-binding protein (SREBP) transcription factors, that are controlled by the regulatory SREBP cleavage activating protein (SCAP). Dysregulation of the SCAP/SREBP pathway has been documented in many tumor types, including breast, liver, and prostate cancer (Reviewed in Peck & Shulze, 2019). Activation of this pathway is inversely correlated with PFS and OS across tumor types, implicating the SCAP/SREBP pathway as a critical mediator of tumorigenesis and making it a good anti-cancer target. Historically, small molecule targeting of the SCAP/SREBP pathway has been difficult given the complexity of the pathway regulation and protein-protein interactions.
Results: Utilizing an integrated drug discovery AI (Artificial Intelligence)-based platform to model SCAP/SREBP complex interactions and identify druggable domains to target with small molecules. Screening a total of ~400 compounds across multiple scaffolds identified compounds with low nano-molar IC50 inhibition of SREBP pathway, and a pan-cancer cell line screen identified “responders” across cancer indications and subtypes. A comprehensive screening and SAR of *in vitro* ADME properties (LM stability, solubility) led to candidates for in vivo pharmacokinetics analysis. Several chemotypes with low clearance, long T1/2 in mice and good bioavailability were identified. Further testing of several Research Leads demonstrated robust tumor growth inhibition in breast (MCF7, 84.15%) and liver (HepG2, 45.5%) as a single agent, and synergistic activity with SoC in the HepG2 model (Sorafanib combination 76.1%). Probing publicly available datasets for glycolytic/lipogenic phenotypes across tumor types reveals heterogeneity across tumor types and the ability to stratify patients and indications by Glyco-/Lipo-genic score that may allow for patient selection in future clinical trials.
Conclusion: We report here a novel small molecule that interacts with SCAP to prevent activation of SREBP pathway that was identified by computational modeling of a multi-protein complex of SCAP/SREBP. This molecule, CAP-1628987 specifically targets SCAP/SREBP pathway, has good drug-like properties, robust efficacy in multiple tumor models and favorable CMC properties.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Oncogenes, Tumor Suppressor Genes, and Gene Products as Targets for Therapy 1
Poster Session

#4305 Targeting the exonuclease domain of POLE to induce synthetic lethality in highly aneuploidic tumor cells.

Meng He, Eun Jung Kim, Yangmeng Wang, Alejandro Chibly, Kristel Dorighi

Genentech, Inc., South San Francisco, CA

Aneuploidy is a hallmark of cancer evolution, it is associated with fast disease progression and drug resistance across many tumor types. The underlying molecular aberration that causes aneuploidy remains largely unknown. Currently, there are no established therapeutics for tumors with high levels of aneuploidy. We propose that inhibition of the exonuclease domain of the DNA polymerase, POLE, could induce synthetic lethality in tumor cells with high levels of aneuploidy. We observed that loss of function mutations in the exonuclease domain of POLE are mutually exclusive with high levels of aneuploidy in the Foundation Medicine database, the Cancer Genome Atlas (TCGA) as well as the cancer Dependency Map (DepMap). Our analysis and statistical modeling showed that the number and unique spectrum of mutations induced by loss of POLE's exonuclease activity have a high probability of hitting an essential gene with hemizygous loss in aneuploidic tumor cells, therefore leading to a synthetic lethality phenotype. Experimentally, we showed that specific inhibition of the exonuclease domain through genetic mutations can cause strong cellular fitness effects in highly aneuploidic cell lines. In summary, our data suggest that a specific inhibitor targeting the exonuclease domain of POLE might bring therapeutic benefits to patients with highly aneuploidic tumors.

#4306 KMT2A rearranged acute myeloid leukemia is vulnerable to the combinatorial inhibition of menin and MYC protein degradation.

Shreya Nair[1], Shayaun Khazaei[1], Lauren B. Ostermann[1], Hideaki Mizuno[1], Po Yee Mak[1], Bing Z. Carter[1], Steffen Boettcher[2], Liandong Ma[3], Miriam B. Garcia[4], Branko Cuglievan[4], Michael Andreeff[1], Yuki Nishida[1]

[1]Leukemia, UT MD Anderson Cancer Center, Houston, TX,[2]Medical Oncology and Hematology, University of Zurich, Zurich, Switzerland,[3]Oncobio Therapeutics, Radnor, PA,[4]Pediatrics, UT MD Anderson Cancer Center, Houston, TX

Background: Menin is the scaffolding protein part of the mixed lineage leukemia (MLL) histone methyltransferase complex and uniquely acts as both a tumor suppressor and oncogenic cofactor. KMT2A or MLL rearranged (KMT2Ar) leukemias are a distinct group with poor prognosis and a median overall survival of 0.9 years (Issa, 2021). This interaction has been targeted by menin inhibitors revunemib and ziftomenib with promising results in clinical trials (Aldoss & Issa, 2023; Erba, 2022), and revumenib is now FDA-approved. Menin directly interacts with the TAD domain of c-MYC (MYC) and amplifies MYC's transcriptional activities (Wu, 2017). We have investigated GT19715, the first-in-class MYC/GSPT1 degrader in MYC-driven hematological malignancies (Nishida, ASH 2022, 2023, 2024). We hypothesized that combined menin inhibition and MYC degradation induces synergistic cytotoxicity in KMT2Ar AML.
Methods: MOLM-13 CRISPR-generated isogenic cells with wild-type (WT), knockout (KO), or mutant TP53 (R175H, Y220C, M237I, R248Q, R237H, and R282W), MV411 and OCI-AML2 cell lines were exposed to menin inhibitor SNDX-50469 (SNDX) (selleckchem) in combination with GT19715 (GT). Cell death was determined by annexin V/DAPI assays. Time course experiments were conducted in MOLM13 WT, KO, Y220C and R282W cells. Bliss synergy scores (BSS) > 10 are considered synergistic. MOLM-13 cells WT and Y220C mutation labeled with GFP/luciferase were injected into NSG mice and SNDX and/or GT were administered.
Results: The combination treatment (100 or 400 nM SNDX and 2 or 4 nM GT) significantly induced cell death and reduced cell numbers by > 99% (> log10^2 difference), starting at day 2 (WT and KO) or 3 (Y220C and R282W) compared to SNDX or GT monotherapy. SNDX + GT induced synergistic cell death (BSS > 10) in all cell lines tested with BSSs up to 80, suggesting substantial synergistic effects by menin inhibition combined with MYC protein degradation. We injected MOLM-13 TP53 WT GFP/Luc and MOLM-13 TP53 Y220C GFP/Luc cell lines (0.5 e6/mouse through tail veins) into NSG mice. After confirming engraftment determined by bioluminescence imaging (BLI), we treated mice with vehicle, GT (3 times a week, every other day by IP injections), SNDX (PO, QD) or GT + SNDX combination for 6 weeks. The combination treatment reduced the total BLI signals at week 2 by 10^4, 10^4 and 10^2 compared to vehicle, GT or SNDX alone. The data suggest the combinatorial treatment of SNDX with GT synergistically reduced leukemia burden in vivo.
Conclusion: Combinatorial approach of menin inhibition and MYC degradation results in a profound synergistic reduction of KMT2Ar AML cells in vitro and in vivo, validating the underlying premise. Further mechanistic studies and efficacy in PDX models are ongoing.

#4307 A first-in-class EGFR-directed KRAS G12V selective inhibitor.

Lyla Stanland[1], Hayden Huggins[1], Alessandro Porrello[2], Yogitha Chareddy[2], Salma Azam[2], Jillian Perry[2], Pradeep Pallan[3], Kristina Whately[2], Lincy Edatt[2], Matthew Fleming[4], Jonah Im[2], Christina Gutierrez-Ford[2], Imani Simmons[2], Vandanaa Jayaprakash[1], Rani Sellers[5], Gabriela de la Cruz[5], Albert Wielgus[5], Martin Egli[3], Albert Bowers[2], Chad V. Pecot[2]

[1]EnFuego Therapeutics, Morrisville, NC,[2]UNC Lineberger Comprehensive Cancer Center, Chapel Hill, NC,[3]Vanderbilt Ingram Cancer Center, Nashville, TN,[4]UNC Eshelman School of Pharmacy, Chapel Hill, NC,[5]UNC Department of Pathology and Laboratory Medicine, Chapel Hill, NC

The KRAS proto-oncogene encodes a small GTPase that is crucial for the activation of intracellular signaling pathways that control cell proliferation, survival and differentiation. *KRAS* is frequently mutated in cancer resulting in its constitutive activation and dysregulation of downstream signaling pathways that drive oncogenic transformation. KRAS G12V is the second most common KRAS mutation in cancer, and occurs frequently in lung, colon and pancreatic cancers. However, while significant advancements have been made in developing KRAS G12C, G12D and pan-KRAS inhibitors, there remain no direct KRAS G12V inhibitors in the clinic. Due to challenges targeting oncogenes with traditional small molecule approaches, RNA interference (RNAi) has become an attractive therapeutic approach for many oncogenes. RNAi has faced many obstacles as an effective cancer therapeutic, including the lack of cancer-specific tissue targeting, rapid oligonucleotide nuclease degradation and clearance from circulation, the need for endosomal escape, and unwanted immune stimulation. However, recently, the use of targetable ligands conjugated to chemically modified siRNAs have shown promise in circumventing these barriers. Here, we describe the development of EFTX-G12V, a fully-modified, mutant-selective siRNA conjugated to an EGFR ligand that shows improved tumor delivery and significant anti-tumor activity in lung and colon cancer models. We used a structure-activity relationship screening approach to identify a highly mutant-selective fully chemically modified siRNA that inhibits KRAS G12V expression at both the mRNA and protein level while completely sparing KRAS WT. Furthermore, this siRNA inhibited cancer cell growth *in vitro* and showed no off-target effects. To address the issue of tumor-specific delivery, we conjugated a non-mitogenic EGFR ligand to the siRNA and found enhanced delivery to tumors with varying levels of EGFR expression. EFTX-G12V significantly inhibited KRAS G12V mRNA and protein *in vivo* and showed significant anti-tumor activity in lung and colon xenograft models. Importantly, EFTX-G12V showed no off-target effects on WT KRAS in somatic tissues including kidney, skin and bladder. Unexpectedly, we found that mutant-selective targeting showed improved efficacy in comparison to a pan-KRAS siRNA. EFTX-G12V significantly inhibited tumor angiogenesis while pan-KRAS targeting had inconsistent effects suggesting that mutant-selective targeting has advantages within the tumor microenvironment. Together our findings represent a technologic advance in ligand-directed, mutant-selective oncogene targeting using RNAi and reveal new biologic insights in KRAS targeting that may have broader implications with regards to safety and efficacy.

#4308 BRD8 is a therapeutic vulnerability for overcoming resistance to dual ER/HER2 blockade therapy in HR+/HER2+ breast cancer.

Ang Gao[1], Parth Khatri[1], Gui Ma[1], Peng Liu[1], Mark Burkard[1], Kari Wisinski[2], Charles Perou[3], Huy Dinh[1], Wei Xu[1]

[1]Univ. of Wisconsin Madison Sch. of Med. & Public Health, Madison, WI,[2]Univ. of Wisconsin Madison, Department of Medicine, Madison, WI,[3]University of North Carolina, Chapel Hill, Chapel Hill, NC

Hormone receptor (HR)-positive, HER2-positive breast cancers often develop resistance to endocrine and anti-HER2 therapies due to the heterogeneous expression of estrogen receptor (ER) and HER2 and the crosstalk of these growth-promoting pathways. However, how anti-HER2 agents activate ER and other growth-promoting pathways remains unknown. Single-cell RNA sequencing of BT474 breast cancer cells identified Bromodomain Containing Protein 8 (BRD8), an acetyl-lysine reader protein in the histone acetylase EP400 complex, as a pivotal mediator to activate ER in response to neratinib treatment. BRD8 expression was rapidly induced by various anti-HER2 agents (neratinib, lapatinib, and trastuzumab), and its depletion disrupted the crosstalk between ER and HER2 signaling pathways and rendered HR+/HER2+ cells and PDxOs more sensitive to anti-HER2 agents. BRD8, ER, and ER target genes are co-induced by neratinib in single-nucleus RNA and ATAC sequencing of a patient-derived xenograft (PDX). SnATAC-seq also reveals that the activated genes share open chromatin regions enriched in ER, forkhead box (FOX), and ETS family transcription factors (TF) binding motifs. FOX family TFs are well-known for regulating estrogen signaling, and ETS proteins have been shown to promote tumorigenesis. Since EP400 enhances H2AZ deposition and acetylation on chromatin, we performed H2AZ and H2AZac ChIP-sequencing in the presence or absence of BRD8 and neratinib treatment. We found that, in response to neratinib treatment, BRD8 activates ER, FOX, and ETS target genes through modulating H2AZac deposition and chromatin decompaction. This finding coincides with RNA-sequencing where BRD8 promotes cell growth in an ER-dependent and -independent manner. In line with these findings, patients who responded poorly to the anti-HER2 therapies exhibited higher levels of BRD8 target gene signature as compared to the responders. Furthermore, BRD8 knockout ablates the ER and HER2 signaling crosstalk and re-sensitizes neratinib-resistant HR+/HER2+ cells to neratinib. In summary, this work not only explains why ER signaling is activated upon anti-HER2 therapies but also identifies BRD8 as a druggable vulnerability for treating HR+/HER2+ breast cancer.

#4310 Novel analogues of indanone and tetralone-keto oximes as potent estrogen receptor beta agonists for treating GBM.

Uday P. Pratap[1], Michael Tidwell[2], Annabel Maciolek[2], Luca De Angelis[2], Sridharan Jayamohan[1], Jessica D. Johnson[1], Khaled Mohamed Nassar[1], Karinel Nieves-Merced[2], Henriette U. Balinda[1], Peter LoCoco[2], Joseph Boerma[2], Suryavathi Viswanadhapalli[1], Gangadhara Reddy Sareddy[1], Nicholas Clanton[2], Stanton McHardy[2], Andrew Brenner[1], Ratna K. Vadlamudi[1]

[1]UT Health Science Center at San Antonio, San Antonio, TX,[2]UTSA, San Antonio, TX

Background: Glioblastoma (GBM) is the most common and aggressive brain tumor, with a poor 5-year survival rate. Epidemiological studies suggest that estrogen receptor beta (ERb) may inhibit brain tumor growth, highlighting the potential therapeutic role of ERb. However, the lack of potent, selective, and brain-permeable ERb ligands has limited the clinical application of ERb-based therapies. We recently identified indanone and tetralone-keto oximes as promising ERb agonists. To further optimize these compounds, we employed a structure-based drug design strategy, utilizing existing X-ray structural data to guide the development of novel ligands targeting the ERb-ligand-binding domain. The objective of this study is to design and develop a new class of ERb agonists that can cross the blood-brain barrier and offer an effective treatment option for GBM.

Methods: To improve CIDD-0149897 potency and comprehend pharmacophores, we designed and synthesized 99 more analogues. The selectivity of CIDD-0149897 analogues was determined using ERb and ERa reporter assays. CIDD-0149897 analogues were examined for cell viability using CellTiter-Glo and apoptosis using Annexin V and Western blotting, ionizing radiation, RT-qPCR, comet assays, and mass spec analysis were used for mechanistic research. UTSA's Center for Innovative Drug Discovery (CIDD) pre-clinical pharmacology core performed in vitro ADME studies. The ERb agonist was tested *in vivo* using orthotopic patient-derived xenografts.

Results: Our analysis of new CIDD-0149897 analogues identified four optimized ERb agonists with improved specificity and potency. ADME studies showed these agonists have 20-50-times better aqueous solubility than CIDD-0149897, with excellent plasma stability. Microsomal and hepatocyte stability varied, with alcohol analogues being more stable. The MDCK-hMDR1 KO cell line shows low to moderate mono-directional permeability and minimal pGP efflux. Treatment with optimized ERb agonists dramatically reduced neurosphere formation, stemness marker expression, and promoted apoptosis in patient-derived GSC model cells. Mass spec data analysis showed that ERb binding proteins are involved in DNA repair and damage response, replication, and G1 checkpoint. In combination with Temozolomide, ERb agonists sensitized GSCs, increased DNA damage, and inhibited neurosphere formation. Comet assay demonstrated enhanced DNA damage in the combination group compared to single agent treatment. The lead compound CIDD-0169124 significantly reduced tumor volume in orthotopic model compared to the vehicle. CIDD-0169124 also improved TMZ-treated GSC tumor-bearing mouse survival relative to vehicle or TMZ monotherapy in an orthotopic GBM model.

Conclusion: These findings showed that CIDD-0169124 is a potent ERb agonist that can be used with existing chemotherapies to improve GBM patients' survival.

#4311 Exploiting the spontaneous intrahepatic cholangiocarcinoma mouse models to uncover MRTX1133's dual role in directly inhibiting KRAS^{G12D}-driven tumor progression and modulating the immune landscape.

X. Li, G. Wu, Z. Song, J. Xue, H. Zhang, C. Liu;
Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, China

Purpose: Mutations in the KRAS gene, particularly the G12D variant, are prevalent in intrahepatic cholangiocarcinoma (iCCA) and correlate with unfavorable patient outcomes. Currently, there is an urgent need for targeted therapeutic strategies against KRAS^{G12D} iCCA. This study aims to investigate the impact and mechanisms of KRAS^{G12D} mutation on iCCA progression and to conduct a preclinical evaluation of MRTX1133, a non-covalent inhibitor of KRAS^{G12D}.

Methods: We developed spontaneous tumorigenesis mouse models of iCCA harboring KRAS^{G12D} mutation (YAP^{S127A}+AKT+KRAS$^{WT/G12D}$ model and AKT+NICD+KRAS$^{WT/G12D}$ model) using hydrodynamic tail vein injection, aiming to study the oncogenic role and mechanisms of KRAS^{G12D} in iCCA. The impact of MRTX1133 on KRAS^{G12D} iCCA was investigated using human iCCA cell lines, patient-derived organoids (PDOs), cell line xenografts, patient-derived xenografts (PDXs), and spontaneous tumorigenesis mice. Single-cell RNA sequencing (scRNA-seq) and multiplex immunofluorescence were utilized to assess changes in the tumor microenvironment following KRAS^{G12D} mutation and MRTX1133 treatment.

Results: The KRAS^{G12D} mutation significantly increased oncogenicity in several iCCA mouse models, characterized by increased tumor proliferation, reduced survival, and robust activation of downstream ERK pathway, along with elevated Ki67 expression. MRTX1133 demonstrated potent antitumor activity against human KRAS^{G12D} iCCA cell lines, PDOs, and *in vivo* models, including PDX and iCCA spontaneous tumorigenesis mice, exhibiting inhibited tumor proliferation, suppressed ERK and AKT pathway activation, and extended mouse survival. The scRNA-seq analysis revealed a reduction in CD8$^+$ T cells and an increase in M2-type macrophages in the tumor microenvironment of KRAS^{G12D} iCCA mice, while these alterations were reversed by MRTX1133 treatment, as validated by multiplexed immunofluorescence. Notably, MRTX1133 showed a more pronounced antitumor effect in immunocompetent C57 mice compared to immunodeficient NSG mice (68% tumor volume reduction versus 36%), suggesting that immune system engagement enhances the efficacy of MRTX1133. The *in vivo* studies combining MRTX1133 with immune checkpoint blockade (ICB) are underway to determine their potential synergistic effects.

Conclusion: This study is the first to validate the oncogenic role and therapeutic vulnerability of the KRAS^{G12D} mutation in iCCA. It also suggests the potential benefits of combining KRAS^{G12D} inhibitor MRTX1133 with ICB immunotherapy with synergistic effects.

#4312 CTR9 controls the mammary luminal lineage and susceptibility to carcinogen/hormone-induced breast tumors.

Gui Ma, Ang Gao, Mingshan Hu, Ngai Ting Chan, Yidan Wang, Wei Xu

Univ. of Wisconsin Madison Sch. of Med. & Public Health, Madison, WI

CTR9 encodes a key component of the human RNA polymerase II (RNAPII)-associated factor complex (hPAFc). CTR9 loss-of-function variants are predisposed to rare myeloid malignancies and Wilms tumors. Although CTR9 promotes the growth of estrogen receptor (ER)-positive breast cancer cells and controls the expression of 90% of ER-target genes, the roles of CTR9 in mammary gland development and breast cancer etiology *in vivo* remain unknown. About 0.1 % of breast cancer patients were found to harbor loss of function CTR9 mutations based on TCGA data. Analyzing normal mouse mammary gland scRNA-seq data reveals that CTR9 is highly expressed in luminal cells, which is confirmed by immunofluorescence staining in human breast tumors. To study the effects of the loss-of-function of CTR9 on mammary gland development, we generated a mammary gland-specific CTR9 knockout (KO) mouse using MMTV-Cre and CTR9 loxP system. CTR9 depletion from mammary epithelial cells was validated using qPCR, Western blot, and IHC. Loss of CTR9 in C57B/6 female mice led to increased duct branching and elongation during puberty and increased alveologenesis during pregnancy. This observation coincides with the detection of expanded mammary stem cells by flow cytometry in the 6-week-old mouse mammary glands. When treated with DMBA and progestin (MPA) to induce mammary tumors, both wild-type (WT) and CTR9 null mice began to develop tumors 6 weeks after the last DMBA treatment, but CTR9 null mice exhibited a markedly enhanced susceptibility to carcinogen/hormone-induced mammary tumors as compared with the WT counterparts. To detect changes during the early stage of tumorigenesis, we profiled the stem and luminal progenitor cell populations in the mammary gland 4-week after the last DMBA treatment. Remarkably, CTR9 depletion enhanced the stem cell and luminal progenitor populations while decreasing the mature luminal cells. CTR9 KO mammary gland had elevated Ki67 levels, decreased ER and PR, decreased mature luminal cells, and increased expression of KRT15, a luminal progenitor marker. The CTR9 null mammary gland expressed lower levels of KDM5A and higher levels of H3K4me3 as compared to the wild-type mice. Taken together, our findings establish that CTR9 depletion blocks the terminal differentiation of luminal cell lineage possibly through epigenetic regulation of histone methylation, and renders the mammary glands more susceptible to carcinogen/hormone-induced tumors.

#4313 COPS5 inhibition synergizes the antitumor effect of trastuzumab by PTEN upregulation in HER2 amplified gastric cancer.

Sung-Hyun Hwang[1], Ji-Won Kim[1], Kuijin Kim[1], Haseseong Park[2], Andrew Aguirre[3], Keun-Wook Lee[1]

[1]Seoul National University Bundang Hospital, Seongnam-si, Korea, Republic of,[2]Dana-Farber Cancer Institute, Boston, MA,[3]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA

COP9 Signalosome Subunit 5 (COPS5), a deubiquitinating enzyme, contributes to chemoresistance, and its expression is closely correlated with poor prognosis in patients with solid tumors. However, the role of *COPS5* amplification in patients with gastric cancer (GC) and its inhibition in *HER2*-amplified GC models has never been elucidated. In the curated stomach adenocarcinoma cohort (n=294) from The Cancer Genome Atlas datasets, *COPS5* amplification significantly co-occurred with *HER2* amplification (10.5% vs. 2.7%, P=0.040) and was associated with worse disease-free survival (median 12.6 vs. 45.2 months, P=0.012) and overall survival (median 21.4 months vs. not reached, P=0.004) after surgery. In *HER2*-amplified GC cell lines, NCI-N87, SNU216, and OE19, COPS5 inhibition using CSN5i-3, a selective COPS5 inhibitor, synergistically enhanced the antiproliferative effect of trastuzumab. After CSN5i-3 treatment, *COPS5* knock-out (KO) cell lines demonstrated a uniform increase in *PTEN* transcription and translation levels. This effect was mediated by SNAIL ubiquitination, suppressing the AKT downstream signaling pathway. However, in *PTEN* KO cell lines, the synergistic effect of CSN5i-3 and trastuzumab was not apparent. Similarly, reduced PTEN expression caused by SNAIL overexpression also decreased the responsiveness of CSN5i-3 treatment. Moreover, *COPS5* KO cells exhibited increased sensitivity to the proliferative effect and downstream signaling inhibition of AKT inhibitors MK2206 and capivasertib compared to control cells. In a mouse xenograft model of NCI-N87 cells, CSN5i-3 treatment combined with trastuzumab yielded a synergistic anti-tumor effect compared to monotherapy without notable changes in body weight. This effect was accompanied by enhanced apoptosis and reduced SNAIL and AKT downstream signaling activity. In conclusion, the synergistic antiproliferative effect of COPS5 inhibition and trastuzumab was attributed to increased PTEN expression via SNAIL ubiquitination, resulting in the AKT downstream inhibition. These findings highlight the potential of COPS5 as a therapeutic target in combination with trastuzumab for *HER2*-amplified GC, warranting further clinical studies.

#4314 Understanding the mode of action and development of resistance towards HER2 targeting agents in preclinical in vivo models.

Eva Oswald[1], Philipp Meyer[1], Loreen Weichert[1], Roxana Redis[2], Dan Rocca[2], Louise Brackenbury[2], Justin Bryans[3], **Julia B. Schueler**[1]

[1]Charles River Laboratories, Inc., Freiburg, Germany,[2]Charles River Laboratories, Inc., Portishead, United Kingdom,[3]Charles River Laboratories, Inc., Saffron Walden, United Kingdom

Treatment options for HER2 positive cancer types have advanced significantly over recent years, focusing on targeted therapies that inhibit HER2 signaling and more recently using HER2 as the anchor-signal to bring tumor killing moieties or effector immune cells near the cancer cell. In our recent study we compared in vivo efficacy in tumor bearing immune-compromised mice using four different modalities in a proof-of-concept format in a HER2+ ovarian cancer xenograft model, SKOV-3. We then tested a panel of HER2 targeting agents in a broader range of clinically relevant PDX models of breast, ovarian and gastric cancer and correlated these results with different characteristics of the PDX models, such as HER2 expression (by IHC) and molecular phenotype (by WES and RNAseq). The range of modalities that were tested in this study comprised small molecules, antibodies, antibody-drug conjugates (ADC) and mRNA-LNP encoded antibodies. Antitumoral activity was measured as tumor growth inhibition (TGI) based on twice weekly tumor volume measurements. The sensitivity of SKOV-3 was heavily influenced by the modality of the respective drug. Whereas small molecule Lapatinib showed only mild efficacy (TGI 40%), Trastuzumab was moderately active (TGI 57%) compared to Trastuzumab-emtansine (TGI 68%). An mRNA-LNP encoding for trastuzumab was the most efficacious treatment with a TGI of 88%. Despite this, none of the treatments induced complete remission. The ongoing analysis of tumor tissue from these studies suggests an escape mechanism via epithelial-mesenchymal transition (EMT) as Vimentin is upregulated and E-Cadherin is downregulated in tumors after re-growth. The superior activity of the mRNA-encoded trastuzumab compared to the clinically approved therapeutic is likely to be due to the elevated and sustained trastuzumab protein levels in the serum of the tumor bearing animals. Broader screening across 33 PDX models revealed ADCs (trastuzumab-emtansin and trastuzumab-deruxtan) as the most efficacious modalities leading to higher TGI values with prolonged persistence of tumor remission. Two therapeutic antibodies (trastuzumab and pertuzumab) showed similar activity patterns despite their slightly different modes of action. HER2 expression correlated with the sensitivity of the PDX model, although this relationship was not strictly linear. HER2 has provided oncologists a tractable target for some cancer types. However, clinical as well as preclinical data indicate the further untapped potential of HER2 as a therapeutic target. Screening studies like those described are important tools to deconvolute sensitivity and possible mechanisms of resistance in a preclinical setting. This provides opportunities to discover biomarkers to better enable patient selection and to provide a better understanding of HER2 biology, further maximizing its therapeutic potential.

#4315 PELP1 oncogene targeted therapy for the treatment of ovarian cancer.

Gaurav Sharma[1], Behnam Ebrahimi[1], Arhan Rao[1], William C. Arnold[1], Hariprasad Vankayalapati[2], Rajeshwar R. Tekmal[1], Suryavathi Viswanadhapalli[1], Ratna K. Vadlamudi[1]

[1]UT Health Science Center at San Antonio, San Antonio, TX,[2]Biolexis Therapeutics, INC., Lehi, UT

Background: Ovarian cancer (OCa) is the most lethal of all gynecologic malignancies in the United States. Despite the initial responses observed in OCa patients to standard combinations of surgical and cytotoxic chemotherapy, roughly 80% of these patients will develop recurrence and ultimately succumb to chemotherapy-resistant disease. Novel targeted therapies for the treatment of OCa are needed. The oncogene PELP1 is highly expressed in a variety of cancers including OCa. The suppression of PELP1 is shown to reduce the proliferation of cancer cells and renders therapy-resistant cells more susceptible to chemotherapy. We have recently developed a novel inhibitor SMIP34 that binds and degrades PELP1. The purpose of this study is to evaluate the efficacy of SMIP34 in the treatment of OCa.
Methods: We have used multiple established and patient-derived OCa models that represent all 4 types of OCa. SMIP34 was used for the inhibition of PELP1 oncogenic functions. The effect of SMIP34 on OCa cell viability was examined by MTT assays. The efficacy of SMIP34 on cell survival and apoptosis was determined using clonogenic assays, Annexin V/PI labelling and caspase 3/7 assays respectively. Mechanistic studies were performed using western blotting, RTq-PCR and RNA-seq analyses. The effect of SMIP34 on STAT3 signaling was examined using reporter gene assays. The efficacy of PELP1 inhibitor on tumor growth was examined using *ex vivo* patient derived explants (PDEX), patient-derived organoids (PDO), and *in vivo* patient-derived xenograft models (PDX).
Results: Utilizing several established and primary OCa cells, we found that SMIP34 decreased cell viability, impaired colony formation capacity, and induced apoptosis. SMIP34 markedly diminished the viability of OCa cells resistant to carboplatin, cisplatin, and taxol. SMIP34 therapy reduced STAT3 reporter activity. Western blot analysis confirmed that treatment with SMIP34 decreased the phosphorylation of PELP1 regulated survival pathways including STAT3, mTOR and AKT. Mechanistically, SMIP34 interaction with PELP1 facilitates its degradation along with its associated protein complex, which includes TEX10, LAS1L, and SENP3. Biochemical analysis confirmed that SMIP34 decreased ribosome biogenesis and new protein synthesis. RT-qPCR demonstrated that SMIP34 therapy decreases the expression of PELP1 target genes. RNA-seq analysis indicated that SMIP34 therapy downregulated cell cycle genes and induced apoptosis. Utilizing PDEX and PDO *ex vivo*, assays we show that SMIP34 markedly reduced tumor proliferation as assessed by Ki67 staining. Moreover, SMIP34 (20 mg/kg/IP) administration *in vivo* markedly inhibited tumor progression in patient-derived xenografts.
Conclusions: Collectively our results using *in vitro*, *ex vivo*, and *in vivo* models suggest that SMIP34 may serve as potential therapeutic for treating OCa by inhibiting PELP1 oncogenic signaling.

#4316 Co-alterations in *PIK3CA* and *ARID1A* lead to greater sensitivity to PI3K pathway inhibition.

Xingqi Shen, Shirsa Udgata, Jordan N. Stoecker, Cheri A. Pasch, Dustin A. Deming

University of Wisconsin-Madison, Madison, WI

Introduction: Co-alterations in *PIK3CA* (PK*)* and *ARID1A* (AD) occur in ~1% of all cancers. Previously we have shown that patients with mutations in both genes are more sensitive to copanlisib (cop) treatment than those with just *PIK3CA*(PK) mutation. Here we evaluate the efficacy of PI3K/AKT and PI3K/mTOR dual inhibitors, compared to that of cop using PKAD patient-derived cancer organoids (PDCOs).
Methods: RSEM files from NCI repository were obtained for endometrial cancer (EC) PDCOs with PK, AD, PKAD and wild type for PK and AD. Expression data in RSEM format were processed using the tximport package in RStudio to import data into data frames. The DESeq2 package was used to create differential gene expression (DGE) files. Additionally, bulk-RNA sequencing was done by UW Bioinformatics Research Core on 2D colorectal cancer (CRC) cell lines, SW48 and SW48$^{PIK3CA\text{-}H1047R}$ (SW48PK), from which AD was deleted with CRISPR (SW48AD; SW48PKAD). Gene Set Enrichment Analysis was run to identify hallmark pathway alterations (| -log10(FDR q-val) | >= 1). PDCOs were obtained following surgery with consent and following UW IRB protocol. PDCOs were generated as described previously. AD was deleted from a CRC PDCO line, with a *PIK3CA*^{H1047R} mutation (CRC-PK) using CRISPR (CRC-PKAD). PDCO lines were treated with PI3K inhibitors (alpelisib (alp), pictilisib (pict), cop, capivasertib (cap), sapanisertib (sap) and everolimus (eve)). Relative change in diameter (ΔD) was measured at day 0 and day 2 using ImageJ. Normalized ΔD (nΔD) was calculated as the ratio of median ΔD of treatment and control.
Results: Bulk-RNA-seq analysis of EC PDCO samples showed significant downregulation in immune signature pathways like epithelial-mesenchymal-transition, Interferon-γ, and IL6-JAK9-STAT3 signaling, along with a significant upregulation in the WNT-beta catenin signaling pathway when comparing PKAD and PK. Comparison between PKAD and AD showed upregulation in p53, apoptosis, and WNT-beta catenin signaling pathways. RNA-seq of 2D CRCs showed significant downregulation of the p53 (-log10FDR = 10), MYC target v2 (2.366), and apoptosis (1.826) pathways when comparing SW48PKAD and SW48PK. PKAD CRC PDCO lines had greater sensitivity to cop treatment than those with PK alterations alone (PK: nΔD=0.175; PKAD: nΔD =-0.12; $p < 0.005$). An enhanced response was observed with some other PI3K inhibitors, including sapanisertib (PK=0.13; PKAD=0.014; $p < 0.005$) everolimus (PK=0.66, PKAD=0.36; $p < 0.005$) and capivasertib (PK=0.85; PKAD=0.47; $p < 0.05$). An enhanced response was not observed with alpelisib (PK=0.54; PKAD=0.61; $p = 0.77$) nor pictilisib (PK=0.64, PKAD=0.76; $p < 0.005$).
Conclusions: Cancers with PK and AD alterations have enhanced efficacy to PI3K pathway inhibition, especially those inhibitors with downstream activity against AKT or MTOR. Further mechanistic and in vivo studies are warranted in addition to further clinical validation.

#4317 Targeting KRAS in appendiceal cancer: promising results with MRTX1133 and RMC-6236 in organoids and PDX models.

Saikat Chowdhury[1], Ichiaki Ito[1], Vinay K. Pattalachinti[2], Abdelrahman MG Yousef[1], Mahmoud MG Yousef[1], Mohammad Mahdi Fanaeian[1], John Paul Shen[1]

[1]Department of Gastrointestinal Medical Oncology, The UT MD Anderson Cancer Center, Houston, TX,[2]The Joe R. and Teresa Lozano Long School of Medicine, University of Texas Health Science Center San Antonio, San Antonio, TX

Oncogenic *KRAS* mutations are frequent in mucinous appendiceal adenocarcinoma (AA) (63.4%), with G12D accounting for just over half (50.9%) and G12V representing 23.0%. There remains a significant unmet need for effective targeted therapies of AA. The advent of novel RAS inhibitors, such as $KRAS^{G12D}$ inhibitor MRTX1133 and Pan-RAS inhibitor RMC-6236, offer a promising opportunity to address this therapeutic gap by directly targeting this oncogene. Here, we investigate the therapeutic impacts of these two new inhibitors in preclinical models of AA tumors.

One AA organoid model with $KRAS^{G12D}$ and one with $KRAS^{G12V}$ mutation were each treated with MRTX1133 and RMC-6236. Additionally, the orthotopic AA PDX model TM00351 (Jackson Laboratory) harboring $KRAS^{G12D}$ was treated intraperitoneally (IP) with MRTX1133 (15 mg/kg) (N=5) or vehicle (N=5), administered BID for 4 weeks.

MRTX1133 was highly effective for $KRAS^{G12D}$ but not $KRAS^{G12V}$ organoids (IC50 4.1 nM vs 1.8 uM, sum-of-squares F test $p < 0.0001$). In contrast, both $KRAS^{G12D}$ and $KRAS^{G12V}$ organoids were sensitive to RMC-6236 (IC50 4.4 nM vs 0.5 nM, respectively). Both inhibitors significantly increased Caspase-3/7, consistent with induction of apoptosis (p-value < 0.01). IP treatment with MRTX1133 led to a marked decrease in tumor volumes (measured by MRI) at 4 weeks after treatment in orthotopic $KRAS^{G12D}$ PDX models (MRTX1133: 1.15 fold change from baseline vs Control: 2.39, $p = 0.04$). Furthermore, MRTX1133-treated mice showed better PFS than mice in the control group (median: 5 weeks vs unreached, p-value = 0.005). Pathologic evaluation showed that MRTX1133 treatment significantly reduced cellularity in tumor tissues, with only a few cells showing positive for Ki-67. pAKT expressions did not change. pERK expressions were reduced considerably in $KRAS^{G12D}$ mutant tumor cells but did not change in $KRAS^{WT}$ stromal cells, indicating MRTX1133 mediated RAS-MEK-ERK pathway inhibition is specific to tumor cells only. Gene Set Enrichment analysis revealed significant downregulations of hallmark gene set ‘E2F targets (NES = -1.9, p-adj = 0.06)’ and the newly developed ‘RAS/ERK upregulated (NES = -2.3, p-adj = 0.06)’ gene set after MRTX1133 treatment. Also, downregulations of RAS-MEK-ERK pathway proteins, such as pMEK (logFC = -3.3, p-adj = 2.9 X 10-5) in RPPA, confirmed robust inhibition of this pathway by MRTX1133.

This is the first report demonstrating the therapeutic efficacies of G12D and pan-KRAS inhibitors in preclinical models of AA. This data is consistent with the oncogenic effect of KRAS, which is mediated by RAS-MEK-ERK pathway, not by PI3K/AKT pathway. Single-agent MRTX1133 was highly potent and specific to $KRAS^{G12D}$ AA tumors, whereas pan-RAS inhibitor RMC-6236 inhibited both $KRAS^{G12D}$ and $KRAS^{G12V}$ organoids at nanomolar concentrations. MRTX1133 demonstrated robust suppression of the RAS-MEK-ERK pathway and prolonged survival in vivo models of AA.

#4318 Genome-wide in vivo CRISPR screen identifies the role of SRSF9 in immunotherapy response regulation in hepatocellular carcinoma.

Nanzhou Yu[1], Jie Luo[1], Beibei Ru[2], Renyue Ji[1], Lanqi Gong[1], Peng Jiang[2], Xinyuan Guan[1]

[1]Department of Clinical Oncology, The University of Hong Kong, Hong Kong, Hong Kong,[2]National Institutes of Health, Bethesda, MD

Hepatocellular carcinoma (HCC) is a leading malignant tumor globally and the second-highest cause of cancer-related mortality. The insidious nature and high recurrence rates of HCC largely necessitate systemic therapies for many patients, particularly those based on immune checkpoint inhibitors. For advanced HCC, the combination of atezolizumab and bevacizumab (Atezo+Beva) has become the preferred first-line treatment, providing superior survival benefits compared to sorafenib. However, the response rate to this therapy remains under 30%, with the underlying resistant mechanism still unclear. Therefore, identifying and characterizing predictive biomarkers associated with the response to Atezo+Beva therapy is essential for improving clinical prognosis in patients with advanced HCC. Here, we performed an in vivo genome-wide CRISPR screen model after anti-programmed death ligand 1 (anti-PD-L1) and anti-vascular endothelial growth factor a (anti-VEGFa) treatment to investigate genomic modulators of the immunotherapy response in HCC. Our screening identified serine and arginine rich splicing factor 9 (SRSF9) as a critical gene involved in the anti-immunotherapy regulation. Knockout of SRSF9 in mouse tumor cells significantly enhanced the sensitivity of primary tumors and the lung metastasis to anti-PD-L1 and anti-VEGFa treatment. This finding was further validated using SRSF9-overexpression mouse model. Functional assays indicated that without immunotherapy SRSF9 had a minimal direct effect on the proliferation of multiple HCC cell lines, aligning with the analytical results derived from the DepMap database. Clinically, SRSF9 expression was increased in multiple cancer types compared to normal counterparts and was positively correlated with poor survival in HCC patients following Atezo-Bev therapy. Furthermore, high SRSF9 expression could negate the prognostic advantage typically seen in patients with strong CD8+ T cell function. In addition, SRSF9 could decrease the secretion of interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) in tumor environment. These suggested a potential role for SRSF9 in downregulating CD8+ T cell activity. The underlying mechanism of how SRSF9 inhibits the response of HCC patients to Atezo+Beva therapy still needs further investigation. In conclusion, our study reveals the significant role of SRSF9 in modulating the response to immunotherapy in HCC, indicating its substantial potential for expanding the cohort of patients who may benefit from Atezo+Beva therapy and enhancing their prognosis. Further exploration of the underlying mechanisms will be undertaken in the future.

#4319 Loss of Golga7 suppresses oncogenic Nras-driven leukemogenesis without detectable toxicity in adult mice.

B. Jiao, L. Yan, R. Zhang, W. Huang, X. Wang, P. Liu, R. Ren;
Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China

NRAS mutations are prevalent in human hematological malignancies and are also common in certain solid tumors, including melanoma and colon cancer. Despite their crucial role in oncogenesis, no effective therapies targeting NRAS have been developed. Inhibiting NRAS localization to the plasma membrane (PM) represents a promising strategy for cancer therapy, as its oncogenic signaling relies on PM localization. Knocking out *Golgin subfamily A member 7 (Golga7)*, an accessory protein of RAS palmitoyltransferases, through a conditional gene editing approach drastically suppresses the development of myeloid leukemia induced by the activation of $Nras^{G12D/G12D}$ knock-in alleles in mice. The loss of *Golga7* disrupts $NRAS^{G12D}$ PM localization in bone marrow cells without altering the level of $NRAS^{G12D}$ palmitoylation. Notably, *Golga7* is dispensable for normal hematopoiesis in adult mice. While constitutive Golga7 knockout leads to embryonic lethality, the ubiquitous knockout of *Golga7* induced in adult mice does not manifest any measurable toxic effects. These findings indicate that GOLGA7 is an effective and safe therapeutic target for NRAS-driven leukemias.

#4321 Selective targeting of TBXT with DARPins identifies regulatory networks and therapeutic vulnerabilities in chordoma.

C. S. Umbaugh[1], M. Groth[1], C. Erkut[1], K.-S. Lee[1], J. Marinho[2], F. Iser[1], J. N. Kapp[2], P. Schroeter[1], S. Dolaner[1], A. Kayserili[1], J. Hartmann[1], P. Walch[1], T. F. E. Barth[3], K. Mellert[3], B. Dreier[2], J. V. Schafer[2], A. Pluckthun[2], S. Frohling[1], **C. Scholl**[1];
[1]German Cancer Research Center (DKFZ), Heidelberg, Germany, [2]University of Zurich, Zurich, Switzerland, [3]Institute of Pathology, Ulm University, Ulm, Germany

Aberrant expression of the embryonal transcription factor TBXT (also known as brachyury) drives chordoma, a rare spinal neoplasm with no effective drug therapies. The gene network regulated by TBXT is poorly understood, and strategies to disrupt its abnormal activity for therapeutic purposes are lacking. We developed TBXT-targeted designed ankyrin repeat proteins (T-DARPins) that selectively bind TBXT and inhibit its binding to DNA. In chordoma cells, T-DARPins reduced cell cycle progression, spheroid formation, and tumor growth in mice. Combining T-DARPin-mediated TBXT inhibition with transcriptomic and proteomic analyses, we determined the TBXT regulome in UM-Chor1 chordoma cells, which comprises in particular networks involved in cell cycle regulation, DNA replication and repair, embryonal cell identity, metabolic processes, and interferon response. The analysis of selected TBXT regulome components provided new insights into chordoma biology, such as the strong upregulation of IGFBP3 upon TBXT inhibition to fine-tune part of TBXT's downstream effectors. Finally, we assigned each TBXT regulome member a druggability status to create a resource for future translational studies and found high interferon response signaling in chordoma cell lines and patient tumors, which was promoted by TBXT and associated with strong sensitivity to clinically approved JAK2 inhibitors. These findings demonstrate the potential of DARPins to interrogate the function of nuclear proteins to understand the regulatory networks of cancers driven by aberrant transcription factor activity, including novel entry points for targeted therapies that warrant testing in patients.

#4322 NSC59984 is a radiosensitizer and cytotoxic agent in *in vitro* and *in vivo* models of *TP53mut* endometrial cancer.

Camilla Yu, Aaron Petty, Theresa D'Silva, Roberto Vargas

Cleveland Clinic, Cleveland, OH

Background: Endometrial cancer (EC) mortality is associated with high grade histologies harboring *TP53* mutations. NSC59984 is a small molecule drug hypothesized to restore p53 signaling in *TP53mut* cancers, but has not been explored in EC. We aimed to evaluate the cytotoxic and radiosensitizing potential of NSC59984 in *TP53mut* EC cell lines *in vitro* and *in vivo* and elucidate its mechanism of action.
Methods: *In vitro* experiments used 2 EC cell lines with differing *TP53* status: JHUEM2 (WT *TP53*) and Hec1B (homozygous R248Q). Dose response curves were generated by treating cells with NSC59984 for 24hrs and assessing viability with CellTiter-Glo after 9 days. To assess for mutation specific effects, we used CRISPR/Cas9 modified variants of JHUEM2 cells lacking WT p53 and harboring 1 of 5 *TP53* mutations (R248Q, R248W, R273C, R273H, Y220C). Western blot analysis assessed p53, p21, PUMA, GADD45, and NOXA signaling using whole cell lysates after treatment with 25μM of NSC59984 for 24hrs. Radiosensitizing effect was assessed using JHUEM2 *TP53* variants and Hec1B treated with increasing doses of NSC59984 and a single fraction of RT (0, 0.5, 1, 2, 4 and 8Gy.) *In vivo* experiments used HEC1B xenograft tumors generated in female NSG mice. 2 $x10^6$ cells were injected SQ in the flanks of 6 week old mice. 2 mice (4 tumors) were included in each arm: vehicle, RT, NSC59984, and NSC59984 + RT. Once tumors were 150mm^3, mice were treated with a 2Gy fraction of RT and NSC59984 (45mcg/kg) every 5 days for 23 days via IP injection. Synergy was tested using HSA and BLISS modeling.
Results: NSC59984 had a negative, dose dependent effect on cell viability. IC50s for JHUEM2 and Hec1B were 4.2μM and 5.7μM. IC50s for NTC, *TP53* KO and variants were similar (4.2-6.1μM). In Western blot analysis, NSC59984 restored p53 signaling via p21, PUMA, and NOXA with decreased mutant p53 signal across all variant cell lines. Radiosensitization assays demonstrated synergy of NSC59984 + RT in all cell lines with maximum synergy at 5μM of NSC59984 and 2Gy ($p<0.05$) based on HSA modeling. *In vivo* pilot experiments confirmed *in vitro* findings, showing tumor growth suppression with NSC59984 + RT compared to vehicle or single agent treatment. On day 20, mean tumor size in the NSC59984 + RT group was 103.4mm, significantly smaller than vehicle (384.5mm, $p=0.004$), RT (506.2mm, $p=0.03$), and NSC59984 (394.8mm, $p=0.02$) groups. Tumor growth inhibition synergy analysis using the Bliss model demonstrated synergy of NSC59984 + RT on days 20-23 with a mean synergy score of 101.6.
Conclusions: NSC59984 has cytotoxic and radiosensitizing effects in *in vitro* and *in vivo* models of *TP53mut* EC. These effects are independent of specific *TP53* mutations, suggesting it bypasses inactive p53 to drive p21, PUMA, and NOXA signaling. The synergistic effect is within biologically relevant doses and supports the need for further preclinical investigation in a disease with significant unmet need and poor outcomes.

#4323 CARM1-mediated methylation of ZNF703 regulates its oncogenic functions in estrogen receptor positive breast cancer.

Jingjing Zhou, Eui-Jun Kim, Gui Ma, Ang Gao, Wei Xu

UW Madison, Madison, WI

ER-positive (ER+) breast cancer, characterized by estrogen receptor expression on tumor cells, constitutes approximately 70% of all breast cancer cases. Identifying specific oncogenes and elucidating relevant oncogenic mechanisms are critical for developing effective therapies. *ZNF703*, a NET/N1Z family transcription factor, drives 8p12 locus amplification found in 10-20% of ER+ breast tumors. *ZNF703* has been reported as an oncogene that promotes ER+ breast tumorigenesis in vitro. However, its regulation and *in vivo* oncogenic function remain unclear. We showed that *ZNF703* expression is induced by estradiol in ER+ cell lines. ShRNA-mediated ZNF703 knockdown significantly reduced cell proliferation in MCF7 and BT474 cells. ZNF703 is post-translationally modified, which may affect its nuclear localization as well as its functions. Using mass spectrometry, we identified two asymmetrically di-methylated arginine residues (R544 and R580) on ZNF703 in human breast tumors. Protein arginine (R) methylation is catalyzed by protein arginine methyltransferases (PRMTs), which are classified into Type I, II, and III based on the type of methyl-R they produced. Among these, CARM1, a Type I PRMT, catalyzes asymmetric di-methylation of arginine residues. We showed that ZNF703 is methylated by CARM1 in *in vitro* methyltransferase assay. Furthermore, ZNF703 protein levels were reduced in CARM1 knockout MCF7 cells, accompanied by an increased degradation rate, suggesting that ZNF703 may be stabilized by CARM1. Given the lack of an ER+ breast cancer mouse model, we derived an ER+ MG1 rat cell line from estrogen-induced rat breast tumors. Consistent with our findings in human ER+ cell lines, ZNF703 was validated as an estrogen-induced gene in MG1 cells. ZNF703 knockout in MG1 cells impaired cell proliferation and colony formation. Functional rescue experiments demonstrated that re-expression of wild-type ZNF703 fully restored cell growth, whereas the methylation-deficient mutants ZNF703 R580K and R544K only partially rescued the proliferation defect. Our results implicate that CARM1-mediated ZNF703 methylation may regulate its stability and oncogenic functions. Given that patients diagnosed with 8p12 locus amplification caused by *ZNF703* typically have poor prognosis, our MG-1/ACI rat syngeneic model would enable the investigation of *ZNF703*'s oncogenic functions in vivo.

#4324 Inhibition of ERK reactivation by FGTI-2734: A strategy to overcome sotorasib resistance in KRAS G12C lung cancer.

Aslamuzzaman Kazi[1], Hitesh Kumar Kantilal Vasiyan[2], Deblina Ghosh[1], Dipankar Bandyopadhyay[3], Rachit D. Shah[4], Vignesh Vudatha[4], Jose Trevino[5], Said M. Sebti[1]

[1]Pharmacology and Toxicology, VCU Massey Comprehensive Cancer Center, Richmond, VA,[2]VCU Massey Comprehensive Cancer Center, Richmond, VA,[3]Biostatistics, Virginia Commonwealth University, Richmond, VA,[4]Surgery, Virginia Commonwealth University, Richmond, VA,[5]Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA

Discovery of sotorasib is a major breakthrough for KRAS G12C targeted therapies. However, overall response rates of patients with KRAS G12C lung cancer treated with sotorasib are modest due to resistance mediated by adaptive reactivation of ERK, which requires wild-type (WT) RAS membrane localization. In this study, we demonstrate that the dual farnesyltransferase (FT) and geranylgeranyltransferase-1 (GGT-1) inhibitor FGTI-2734 inhibits WT RAS membrane localization, and overcomes sotorasib adaptive resistance through inhibition of sotorasib-induced ERK feedback reactivation. The combination of FGTI-2734 and sotorasib is synergistic at inhibiting the viability and inducing apoptosis of lung cancer cell lines with KRAS G12C mutation, including those that are highly resistant to sotorasib. Anti-tumor activity of sotorasib is enhanced by FGTI-2734, leading to significant tumor regression of a patient-derived xenograft (PDX) from a KRAS G12C lung cancer patient as well as several xenografts from highly sotorasib-resistant KRAS G12C human lung cancer cells. Importantly, *in vivo*, FGTI-2734 inhibited sotorasib-induced ERK reactivation in the KRAS G12C PDX, and combinations of FGTI-2734 and sotorasib were also significantly more effective at suppressing the levels of P-ERK in sotorasib-resistant human KRAS G12C lung cancer xenografts. This study provides a rational strategy for overcoming sotorasib resistance in KRAS G12C lung cancer.

#4325 FXYD5 plays diverse roles in immune evasion and tumor progression in prostate cancer.

Ru Wen[1], G Edward W. Marti[1], Neeladrisingha Das[1], Zhengyuan Qiu[1], Nathan Lam[1], Eric E. Peterson[1], Zenghua Fan[2], Aram Lyu[2], Fernando Jose Garcia Marques[1], Abel Bermudez[1], Hongjuan Zhao[1], Lawrence Fong[3], Guillem Pratx[1], Donna M. Peehl[2], Sharon J. Pitteri[1], James D. Brooks[1]

[1]Stanford University, Stanford, CA,[2]UCSF, San Francisco, CA,[3]Fred Hutch Cancer Center, Seattle, WA

FXYD5 is involved in various biological processes, including inflammation, tumor progression, drug resistance, and hypertension. It has been suggested as a potential biomarker for several cancers, with elevated levels found in ovarian and colon cancer. However, the role of FXYD5 in prostate cancer has not yet been elucidated. Here, we found that the FXYD5 was expressed in tumor cells by sc-RNA sequencing of human metastatic prostate tumor tissues. The expression levels of FXYD5 were higher in androgen receptor (AR)-negative prostate cancer cells compared to AR-positive prostate cancer cells. Intrinsically, FXYD5 knockout promoted PC3 cell proliferation, migration, and invasion. *In vivo* studies showed that FXYD5 knockout promoted PC3 tumor growth, while overexpression of FXYD5 inhibited PC3 tumor progression. However, in AR-positive 22Rv1 cells, overexpression of FXYD5 promoted tumor progression. In vitro assays indicate that overexpression of FXYD5 in PC3, C42B, and 22Rv1 cells reduces mitochondrial membrane potential and reactive oxygen species. Additionally, FXYD5 overexpression reduces lactate dehydrogenase activity and lactate levels in PC3 cells but has no effect on these parameters in 22Rv1 cells. In PC3 cells, FXYD5 overexpression promotes the formation of tight cell-cell interactions, accompanied by downregulation of membrane markers and nuclear enlargement. In contrast, these effects are not observed in 22Rv1 or C42B cells. Further, FXYD5 is identified as a potential Siglec-7 ligand by CRISPRi screen. FXYD5 knockout decreased Siglec-7 Fc binding capacity and enhanced the NK-cell mediated cytotoxicity in prostate cancer cells. In a humanized mouse model, 22Rv1 tumor growth was inhibited with human immune cell modulation. However, when FXYD5 was overexpressed in 22Rv1 cells, tumor growth was not impacted by immune cell-based therapy. This was due to the interaction of FXYD5 on cancer cells with Siglec-7 on immune cells, thereby inducing immunosuppressive signals. These findings suggest potential therapeutic strategies targeting FXYD5-based mechanisms.

#4326 KRB-456: A novel KRAS G12D switch-I/II allosteric pocket binder that thwarts pancreatic cancer patient-derived tumors.

Aslamuzzaman Kazi[1], Alok Ranjan[1], Vasanth M. V. Kumar[2], Bogos Agianian[3], Martin Garcia Chavez[4], Vignesh Vudatha[1], Rui Wang[1], Rajani Kanth Vangipurapu[5], Liwei Chen [5], Perry Kennedy [5], Karthikeyan Subramanian[1], Quirke Jonathan Charles Kenneth [4], Francisca Beato[5], Patrick W. Underwood,[1], Jason B. Fleming,[5], Paul Hergenrother [4], Jose Trevino[1], Evripidis Gavathiotis[3], Said M. Sebti[1]

[1]VCU Massey Comprehensive Cancer Center, Richmond, VA,[2]Department of Biochemistry,, Albert Einstein College of Medicine, Richmond, NY,[3]Department of Biochemistry,, Albert Einstein College of Medicine, New York, NY,[4]University of Illinois, Champaigne Urnbana, IL,[5]Moffitt Comprehensive Cancer Center, Tampa, FL

Currently, there are no clinically approved drugs that directly thwart mutant KRAS G12D, a major driver of human cancer. Here, we report on the discovery of a small molecule, KRB-456, that binds KRAS G12D and inhibits the growth of pancreatic cancer patient-derived tumors. Protein nuclear magnetic resonance studies revealed that KRB-456 binds the GDP-bound and GCP-bound conformation of KRAS G12D by forming interactions with a dynamic allosteric binding pocket within the switch-I/II region. Isothermal titration calorimetry demonstrated that KRB-456 binds potently to KRAS G12D with 1.5-, 2-, and 6-fold higher affinity than to KRAS G12V, KRAS wild-type, and KRAS G12C, respectively. KRB-456 potently inhibits the binding of KRAS G12D to the RAS-binding domain (RBD) of RAF1 as demonstrated by GST-RBD pulldown and AlphaScreen assays. Treatment of KRAS G12D-harboring human pancreatic cancer cells with KRB-456 suppresses the cellular levels of KRAS bound to GTP and inhibits the binding of KRAS to RAF1. Importantly, KRB-456 inhibits P-MEK, P-AKT, and P-S6 levels *in vivo* and inhibits the growth of subcutaneous and orthotopic xenografts derived from patients with pancreatic cancer whose tumors harbor KRAS G12D and KRAS G12V and who relapsed after chemotherapy and radiotherapy. These results warrant further development of KRB-456 for pancreatic cancer.

#4327 Disruption of aggregated oncogenic mutant p53 as a novel therapeutic strategy for gynecologic cancers.

Geneva L. Williams[1], Jamie L. Padilla[1], Lane E. Smith[1], Jun-yong Choe[2], Doris M. Benbrook[3], Kimberly K. Leslie[1]

[1]University of New Mexico Health Sciences Ctr., Albuquerque, NM,[2]Chemistry, University of New Mexico, Albuquerque, NM,[3]School of Medicine, University of Oklahoma, Oklahoma City, OK

High grade serous ovarian and endometrial cancers possess *TP53* missense mutations that result in loss of the central tumor suppressive functions of wild type p53 (WT p53). In normal unstressed cells, p53 protein is regulated through ubiquitin-tagging and shuttling to the 26S proteasome by the E3 ubiquitin ligase MDM2 (Murine Double Minute 2). In cancer cells with mutant forms of p53, MDM2 is not adequately expressed in such cells to perform its regulatory role, thus leading to mutant p53 aggregates. Understanding mechanisms to target mutant p53 proteins for degradation in cancers has tremendous therapeutic potential. Recent discoveries in p53 refolding agents to reinstate WT p53 functionality for the treatment of gynecologic malignancies have shown promise. Treatment of cancer cells harboring p53 mutations with p53 reactivators HO-3867 and our novel curcumin analogue AKT-100 displayed an upregulated expression of *heat shock protein 70 (HSP70)* family member genes, as well as the autophagy-inducing co-chaperone *BAG3* (*Bcl-2-associated athanogene 3*) by RNA sequencing analysis. Western blot analysis showed a decrease in p53 protein expression after treatment with the curcumin analogues, while MDM2 levels were unchanged, indicating an alternative mechanism of mutant p53 degradation. *We hypothesize that the BAG3-HSP70 interaction varies with different mutations in p53 as well as the various HSP70 family members involved and can be therapeutically normalized to restore WT p53 regulation.* Using our cell models of high grade serous ovarian and endometrial cancer, we have determined through co-immunoprecipitation and mass spectrometry that p53 binds HSPA6 and BAG3 upon treatment only with AKT-100 in KLE (p53 R175H) and COV362 (p53 Y220C) cells. Furthermore, co-treatment of curcumin analogue AKT-100 plus HSP inhibitor SHetA2 (sulfur heteroarotinoid A2, NSC 726189) highly increased the expression of BAG3, HSPA1A, HSPA6 and ubiquitination while decreasing mutant p53 expression in KLE cells. Confocal microscopy in KLE cells also displayed a shift of mutant p53 and HSPA6 from nuclear to mostly cytoplasmic upon treatment with AKT-100. Co-treatment with AKT-100 and SHetA2 highly increased HSPA6 expression throughout the cell, while p53 returned to the nucleus to perform normal apoptotic functions based on CyQuant proliferation data. Our data indicates a novel mechanistic insight into therapeutically intervening to re-establish normal p53 levels and regulation to treat women with advanced, mutant p53 gynecologic malignancies.

#4328 Preclinical development of SCR-A008: A CDH17-targeted ADC for gastrointestinal cancer.

Guangcun Cheng, Hui Zheng, Zhiyong Yu, Chunlei Xia, Huipeng Guo, Xiyuan Wang, **Yayuan Fu**, Renhong Tang

Simcere Zaiming, State Key Laboratory of Neurology and Oncology Drug Development. Simcere Pharmaceutical Group, Shanghai, China

Background: Cadherin is a superfamily of cell adhesion molecules that depend on Ca^{2+} and play an important role in maintaining tissue architecture. Abnormal expression of Cadherins is often associated with malformation of tissues, tumor formation, and metastasis. Cadherin-17 (CDH17) also known as hepatocyte-enterocyte cadherin, is a non-classical member of the calcium-dependent cadherin superfamily. Unlike classical cadherins, the CDH17 sequence is formed by seven extracellular calcium-dependent cadherin domains and a very short cytoplasmic domain. CDH17 is a calcium-dependent transmembrane glycoprotein that mediates cell-cell adhesion in intestinal epithelial cells. Studies have found that CDH17 expression is limited to the intestinal and pancreatic duct epithelial cells in human normal tissues. However, in tumor tissues, CDH17 is highly expressed in various digestive tract tumors, such as gastric cancer (73%), colorectal cancer (100%) and pancreatic cancer (31%). Some studies have also reported positive staining for CDH17 in about 6% of lung cancer patients, 11% of ovarian cancer patients, and 2% of liver cancer patients. These results suggest that CDH17 may be a potential therapeutic target in related tumors, especially digestive tract tumors.

Methods & Results: We developed a novel CDH17-targeting antibody-drug conjugate (ADC) called SCR-A008, which is comprised of an anti-CDH17 humanized monoclonal antibody conjugated with novel topoisomerase 1 inhibitor (CPT116) via a hydrophilic cleavable linker at DAR 8. SCR-A008 specifically recognizes human and cyno CDH17 with high affinity. In vitro cytotoxicity experiment demonstrated that SCR-A008 induced tumor cell lysis in various tumor cell type. Compared to MMAE-based CDH17-ADC, SCR-A008 exhibits significantly stronger anti-tumor activity in colorectal cancer cells. Additionally, we utilized CDX and PDX model to evaluate the *in vivo* efficacy of SCR-A008. The results showed that SCR-A008 inhibited tumor growth of various tumors in a dose dependent manner. SCR-A008 also have good stability and favorable developability. Pharmacokinetics and tolerability were assessed in mouse and cynomolgus monkey. The pharmacokinetics experiment in mice showed better PK characteristic than the reference ADC.

Conclusion: These findings suggest that SCR-A008 is a potential Best-in-Class therapeutic drug for CDH17-positive solid tumor.

#4329 Developing oncogenic mutant-selective antisense oligonucleotide to treat PIK3CA-mutated cancers.

Peng Ning[1], Yamu Li[1], Clemens Burda[2], Zhenghe Wang[1]

[1]Department of Genetics and Genome Sciences, Case Comprehensive Cancer Center, Case Western Reserve University School of Medicine, Cleveland, OH,[2]Department of Chemistry, Case Western Reserve University, Cleveland, OH

Phosphatidylinositol 3-kinases are heterodimers consisting of a p110 catalytic domain and a p85 regulatory domain. PIK3CA, which encodes the p110α catalytic subunit, is frequently mutated in human cancer. The FDA approved a WT p110-specific inhibitor, alpelisib, to treat PIK3CA-mutant breast cancer patients a few years ago. However, alpelisib and other WT p110α inhibitors could cause severe hyperglycemia in patients because WT p110α inhibitors block the intracellular action of insulin on the liver and skeletal muscle, thereby creating a transient state of insulin resistance. Most PIK3CA/p110α oncogenic mutations occur at two hot spot regions, one in the helical domain and the other in the kinase domain. Recently, a couple of PIK3CA/p110α kinase domain mutant-specific inhibitors have been developed. Early-phase clinical trials showed that the kinase domain mutant-specific inhibitors did not cause severe hyperglycemia in patients. Half of all p110α mutations are in the helical domain, and the E545K is the most frequent recurrent helical domain mutation. However, developing small molecule inhibitors against the PIK3CA/p110α helical domain mutations remains challenging. To this end, we have developed antisense oligonucleotides (ASOs) that preferentially degrade PIK3CA/p110α helical domain mutant mRNAs. In a cell-based assay, our lead ASO (ASO18) potently and preferentially degraded the PIK3CA E545K mutant allele at low nM concentrations. In multiple subcutaneous xenograft models, intra-tumor injection of ASO18 significantly inhibits the growth of tumors that harbor a PIK3CA E545K mutation but not a PIK3CA WT tumor. Based on our previous findings that p85β dissociates from PIK3CA/p110α E545K mutant protein and translocates into the nucleus to stabilize EZH2, we further demonstrated that a combination of ASO18 with an EZH2 inhibitor tazemetostat synergistically suppress xenograft growth of tumors harboring a PIK3CA E545K mutation. To deliver the ASO systematically, we used an FDA-approved D-Lin-MC3-DMA lipid nanoparticle (LNP) formulation to load the ASO, showing that ASO capsulated efficiently (over 95%). Moreover, the dose of LNP-ASO in the nanomolar range could achieve the same potency as the micromolar level of free ASO in tissue culture cells. Experiments assessing the efficacy of the LNP-ASO in xenograft models are ongoing. To our knowledge, our study represents the first mutant-selective ASO against an oncogenic mutation, which may lead to the development of a mutant-selective ASO to treat patients whose tumors harbor a PIK3CA E545K mutation.

#4330 Employing synthetic mRNA therapeutic platform to restore p53 function to suppress growth of p53 mutant cancer.

Yong Ho Heo, Seung-Hyun Shin, Youngjin Han, Chang Gyu Lim, Innah Kim, Ji Hee Lee, Sol-Bi Shin, Soye Jeon, Aran Park, Jooyun Byun, Daejin Kim, In Young Choi

Hanmi Pharmaceutical Co., Ltd., Gyeonggi-do / Hwaseong-si, Korea, Republic of

TP53, a gene encoding p53, is a critical tumor suppressor gene, and its mutation serves as a major driver in various cancer types, deeply influencing tumor progression and patient prognosis. Efforts to restore wild-type p53 function have included targeting specific p53 mutants with small molecules, preventing p53 degradation through inhibition of its negative regulators, and employing adenoviral vectors to deliver wild-type p53. However, these strategies face challenges, including limited efficacy and the heterogeneity of p53 mutations. In this study, we present an optimized mRNA-based approach to restore p53 functionality, utilizing both in vitro and in vivo models of cancers with high p53 mutation frequencies. To enhance therapeutic efficacy, we optimized key elements of p53 mRNA, including the 5' and 3' untranslated regions (UTRs), the open reading frame (ORF), and Hanmi's 5' capping reagent. Using cancer cell lines derived from lung, ovarian, pancreatic, and head and neck cancers harboring p53 mutations, we evaluated the therapeutic potential of our p53 mRNA therapy. Selectivity was assessed by examining its effects on both normal and p53 wild-type cancer cells. Our findings demonstrate that Hanmi's p53 mRNA therapy selectively inhibits the proliferation of p53-mutant cancer cells by inducing apoptosis and cell cycle regulators such as PUMA, BTG2, and p21. Importantly, in vivo studies revealed significant tumor growth inhibition in xenograft models of lung, ovarian, and pancreatic cancers, with no notable impact on mouse body weight. Additionally, we tested the efficacy of our p53 mRNA therapy in a cancer orthotopic model, confirming its potent antitumor activity in a clinically relevant setting and demonstrating the selective efficacy against tumor. These results underscore the promise of p53 mRNA as a novel and efficient therapeutic strategy for selectively targeting p53 mutant cancers, paving the way for improved outcomes in precision oncology.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Gene Regulation and Transcription Factors 1
Poster Session

#4331 Collaboration between p63 and ZNF148 in regulating squamous cell carcinoma progression.

Artem Smirnov, Eleonora Candi

University of Rome Tor Vergata, Rome, Italy

p63, a member of the p53 family of transcription factors, is essential for the development and maintenance of squamous epithelium. In squamous cell carcinomas, including head and neck squamous cell carcinoma (HNSCC), p63 is the most frequently amplified transcription factor at the genomic level and is often overexpressed. HNSCC is a prevalent and aggressive form of cancer and treating advanced stages of the disease remains a significant challenge. Using a proteomics-based approach, we identified new binding partners of the oncogenic variant DNp63. Among these, the zinc finger protein ZNF148 was found to be co-expressed with DNp63 and to physically interact with it in carcinoma cells. Genome-wide binding studies revealed a functional enhancer-derived RNA (eRNA) located upstream of the *CCND1* gene, which is bound by both DNp63 and ZNF148. This interaction regulates the transcription of the eRNA, leading to increased cyclin D1 expression, thereby promoting tumor cell proliferation. Notably, this pathway is specific to cancer cells and remains inactive in normal epithelial cells. The expression levels of DNp63, ZNF148, and the eRNA are positively correlated in cancer, with higher levels linked to advanced disease and metastasis. Furthermore, ZNF148 also independently influences cell migration and inflammatory responses in HNSCC by regulating genes involved in actin cytoskeleton remodeling and type I and II interferon signaling pathways. Together, these findings uncover a novel molecular mechanism driving HNSCC progression and highlight potential targets for selective cancer therapies.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Oncogenes, Tumor Suppressor Genes, and Gene Products as Targets for Therapy 1
Poster Session

#4332 Concomitant therapy leveraging antibody-pan-*KRAS*^{mt} siRNA conjugate for PDAC and LUAD harboring *KRAS* mutations.

Aiyun Wen, Jing Guo, Zengyan Mu, Chengang Zhou, Junhao Wang, Tongtong Hu, Haibo He, Meijun Xiong, Cong Liu, Paul H. Song, Gang Qin

GeneQuantum Healthcare (Suzhou) Co., Ltd., Suzhou, China

Background: *RAS* is the most frequently mutated oncogene, with *KRAS* being the most mutated *RAS* isoform and accounting for 85% of *RAS* mutations observed in human cancers. *KRAS* mutation plays a crucial role in cancer initiation and progression and confers resistance to treatment. *KRAS* mutation is present in 90% of pancreatic ductal adenocarcinoma (PDAC) and 35% of lung adenocarcinoma (LUAD). With FDA's approval of the first-in-class *KRAS*^{G12C} inhibitor LUMAKRAS® for the lung cancer in 2021, the emergence of secondary mutations continues to pose challenges in the clinical treatment of *KRAS*-mutant (*KRAS*^{mt}) cancers via inhibition of the single mutations. The goal of the current study was to investigate the impact of *KRAS* oncogene silencing by antibody-pan-*KRAS*^{mt} siRNA conjugates (AOCs) on the pancreatic and lung tumor growth. A series of pan-*KRAS*^{mt} siRNA sequences that target a wide range of *KRAS* mutations were selected and ligated to a tumor targeting antibody utilizing GeneQuantum's (GQ) proprietary iGDC™ conjugation platform. The therapeutic potential of these AOCs with and without gemcitabine was examined in the cancer cell lines/patient derived organoid (PDO) models and subcutaneous CDX/PDX mouse models.

Results: GQ's iGDC™ conjugation process yielded unprecedented pure DAR1 pan-*KRAS*^{mt} AOCs, with over 98% purity confirmed by HPLC analysis. These candidate AOCs achieved potent target knockdown with reduced phosphorylation level of downstream effectors and significantly inhibited cell proliferation in multiple *KRAS* mutant cell lines/PDOs. The lead AOCs selected from the *in vitro* testing achieved enhanced tumor exposure compared to non-targeting isotype AOC in the CDX/PDX mice, and pharmacological reduction of oncogenic *KRAS* mutations suppressed the growth of tumor xenografts. Combined treatment of the lead pan-*KRAS*^{mt} AOC with gemcitabine demonstrated synergistic effects with prolonged survival rate and dramatically reduced mean tumor volume.

Conclusion: GQ's pan-*KRAS*^{mt} AOC demonstrated the benefit of the tumor enrichment and the positive impact on the tumor regression. The additive tumor reduction outcome via combining with gemcitabine suggested the potential sensitization effect of pan-*KRAS*^{mt} AOC on the conventional chemotherapy. Our findings in the present study unveiled a new path toward addressing *KRAS*-driven refractory cancers with tumor targeting siRNA therapy covering multiple *KRAS* mutations.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Pharmacokinetics and Pharmacodynamics of Cancer Therapeutics Poster Session

#4336 Translational PK/PD modeling of MEK inhibitors: Trametinib, cobimetinib and binimetinib.

Jake Dickinson, Hitesh B. Mistry

Seda Pharmaceutical Development Services, Stockport, United Kingdom

We propose a translational PK/PD framework that enables human dose estimation (HDE), compound ranking and to assess the clinical applicability of cancer cell-line-panels. Translational PK/PD simulations were performed to estimate the efficacious doses in humans. The translational approach was first calibrated using an approved therapy, Trametinib. Cobimetinib and Binimetinib were then used as test compounds. Translational simulations for Cobimetinib and Binimetinib were performed and benchmarked against the Trametinib simulations to aid in HDE. The modelling approach was qualified against reported efficacious doses for Binimetinib and Cobimetinib. The reported population PK (PopPK) models were used to simulate clinical PK. Drug effect was described by an E_{max} model. *in vitro* efficacy data for Trametinib, Cobimetinib and Binimetinib, were extracted from the PRISM screen within DepMap Public Access Portal which contained the results of pooled-cell line chemical-perturbation viability screens for 1448 compounds screened against 489 cell lines. EC50 distributions were derived from the PRISM screening for each compound. Only cell-lines that had the same biological profile as the target population, V600E mutated BRAF patients, were included in the EC50 distribution. We have developed a PopPK/PD model structure to simulate the dose-response, where response is the average drug effect (E_{avg}), by integrating in vitro efficacy cell line data and published PopPK models for MEK-inhibitors Trametinib, Cobimetinib and Binimetinib. Trametinib E_{avg} at the approved dose, 2mg once daily (QD), was simulated using the developed PopPK/PD model. The simulated median E_{avg} was 31% and 25th and 75th percentile were 13% and 57% respectively. Dose-response curves, where E_{avg} was the response, were simulated for Binimetinib and Cobimetinib. A dose that resulted in an E_{avg} that was matched to the median Trametinib E_{avg} was identified for both compounds. Expected, efficacious dose was predicted to be 10 mg QD 21/7 (50% Prediction Interval (PI); 6 - 15 mg QD 21/7) for Cobimetinib and 40 mg BID (50% PI; 15 - 95 mg BID) for Binimetinib. To qualify the modelling approach the predicted efficacious doses were compared to the reported maximum tolerable dose (MTD). The predicted efficacious dose for Binimetinib was similar to the reported MTD, 45 mg BID. The predicted efficacious dose for Cobimetinib was slightly lower than MTD, 60 mg QD 21/7. The translational PK/PD framework highlighted that the routine cancer cell-line-panels, in this instance the PRISM assay, may well be suitable for making HDE. The predictions may be improved further by using multiple different *in vitro* systems and more complex assays. Additionally, the translational framework enables compound ranking and selection and aids in quantifying a clinical therapeutic window. We are thankful for fruitful discussions with Heinrich Huber of Boehringer Ingelheim.

#4337 SYS6051, a next-generation tissue factor-targeted antibody-drug conjugates for the treatment of TF-expressing malignancies.

X. Wu[1], M. Shen[2], **M. Dan**[1], X. Hui[2], B. Yao[2], Y. Li[1], W. Yang[1], H. Xu[1], C. Yuan[2], Y. Sun[1], B. Kang[1], Y. Zhang[1], L. Xiao[1];
[1]CSPC Pharmaceutical Group Ltd., Beijing, China, [2]CSPC Megalith Biopharmaceutical Co., Ltd., Shijiazhuang, China

Tissue factor (TF) is an integral membrane protein, normally separated from the blood by the vascular endothelium, which plays a key role in the initiation of blood coagulation. However, TF is aberrantly expressed in solid tumors and its expression associated with poor prognosis. The first TF targeting antibody-drug conjugate (ADC) , a microtubule inhibitor based ADC(MTI-ADC), is approved for the treatment of cervical cancer. It had several prespecified adverse effects (AEs) including bleeding risks, ocular toxicity and perpheral neuropathy, the last two of which were known MMAE related AEs. In order to mitigate the safety concerns, we developed a novel ADC, SYS6051. It was composed of a humanized TF-targeting lgG1 antibody site-specifically conjugated to a topoisomerase I inhibitor (Exatecan) at a drug-to-antibody ratio (DAR) of 6 via a protease-cleavable linker. In addition, SYS6051 had improved resistance towards retro-Michael elimination reactions, resulting in improved stability compared to cysteine thiol-maleimide conjugated ADCs. The naked SYS6051-mAb did not interfere with the interaction among TF, Factor FVII and Factor X, and had no impact on coagulation cascade. SYS6051-mAb could quickly enter cells through endocytosis 3 hours after treatment, faster than the antibody of the MTI-ADC. Treatment with SYS6051 resulted in significant tumor growth inhibition in a panel of TF-positive cell line derived xenografts (CDXs), including pancreatic and cervical cancer xenografts. Furthermore, SYS6051 was well tolerated in a toxicity study in cynomolgus monkey at dosages ≤30 mg/kg. A stable PK profile of SYS6051 was also observed in NHP. Neither coagulation disorder nor skin rash was observed, superior to the historical toxicity data in NHP of the MTI-ADC. Taken together, SYS6051 has a wide therapeutic window because of promising efficacy, higher tolerability, stable PK profile, and could potentially provide benefits for the patients carrying TF positive malignancies.

#4338 SYS6042, a novel anti-TROP2 pH-sensitive antibody-drug conjugate (ADC) with improved therapeutic window.

Y. Wang[1], X. Gao[2], M. Dan[1], **X. Hui**[2], B. Yao[2], Y. Zhang[1], C. Niu[1], C. Yuan[2], X. Zhu[1], Z. Sun[2], B. Kang[1], M. Wei[1];
[1]CSPC Pharmaceutical Group Ltd., Beijing, China, [2]CSPC Megalith Biopharmaceutical Technology Shijiazhuang) Co., Ltd., Shijiazhuang, China

TROP2 is a ubiquitously expressed tumor-associated antigen. Its expression in normal tissues such as skin, oral mucosa, esophagus, liver, and cornea severely limits the development of anti-TROP2 ADCs. It is difficult to balance the efficacy and toxicity to obtain a larger therapeutic window. In clinical, Dato-DXd, Trodelvy and SKB264 showed obvious on-target toxicity or off-target toxicity, which affect clinical development and patient benefit. To address the unmet needs, we developed the SYS6042, an ADC composed of an anti-TROP2 pH-sensitive antibody, a tetrapeptide-based stable linker, and a topoisomerase I inhibitor payload, with a drug-to-antibody ratio (DAR) of 8. It binds TROP2 with nanomolar affinity at pH 6.0 but hardly binds the target at pH 7.4, thereby reducing on-target toxicity. In several cell line-derived xenografts (CDX) models, a single dose of SYS6042 at 0.4 - 4.0 mg/kg exhibited remarkable tumor growth inhibition. Notably, the antitumor activity of SYS6042 was superior to homemade Dato-DXd and SKB264. In exploratory toxicology study, SYS6042 showed favorable PK and good safety profiles with toxic findings unrelated to on-target toxicity(hematotoxicity) despite high levels of TROP2 expression in normal tissues of cynomolgus monkey. The highest non-severely toxic dose (HNSTD) for monkeys was defined as 30 mg/kg for repeating dosing. Based on these results, it demonstrates that the novel design of SYS6042 leads to an improved therapeutic window, which would help address unmet needs in the treatments of TROP2-positive tumors.

#4339 Glucosinolates selectively target cancer cell types and exhibit antineoplastic effects.

K. Carrozzi[1], A. Aggarwal[1], **K. W. Yip**[2];
[1]University of Toronto, Toronto, ON, Canada, [2]Anticancer.ca, University of Toronto, Toronto, ON, Canada

Introduction: Glucosinolates are organic compounds found in high concentrations within cruciferous vegetables. Plant tissue damage initiates the release of myrosinase, which breaks down glucosinolates into the biologically active compounds indole-3-carbinol (I3C) and sulforaphane. *In vitro* studies have suggested that I3C and sulforaphane can mitigate cancer progression via hormone regulation and antioxidant mechanisms, respectively. However, there has yet to be a systematic analysis of these two glucosinolates against a wide range of human cancer cell lines. We hypothesized that I3C would differentially affect cancer cell growth depending on cell line of origin, and that sulforaphane would induce a genetic signature similar to compounds and genetic perturbations with anticancer properties.

Methods: Cell survival data was obtained from the NCI-60 human tumor cell lines screen. 59 tumor cell lines were seeded into 96-well microtiter plates. 100 µL of 5 different I3C 10-fold dilutions (0.01 - 100 µM) were added to the wells and incubated for 48 hours. After staining with sulforhodamine, absorbance was measured, and percent growth was calculated relative to the no-drug control and number of cells at baseline. Genetic profiling data was obtained from the Broad Institute's L1000 assay. Sulforaphane was added to 9 cell lines in 384-well plates. mRNA was extracted and expression levels of 978 landmark genes were measured. This provided a transcriptomic signature that was compared to signatures from other compounds and genetic perturbations. A Connectivity Score was then developed to measure the similarity of each signature to the one induced by sulforaphane.

Results: I3C was most potent in the KM12 cell line (IC_{50} = 14.9 µM; colon cancer) where it inhibited ~90% of growth at a 100 µM concentration. Cancers of the colon and skin were most sensitive to I3C (median IC_{50} values of 28.3 µM and 36.1 µM, respectively), while breast and myeloid tissue cancers were least sensitive. Gene expression induced by sulforaphane was most similar to that induced by isoliquiritigenin, a flavonoid compound that exhibits antiproliferative and anti-inflammatory effects. Transcriptomic changes caused by sulforaphane were highly similar to NFE2L2 overexpression (tau score = +97.87), and SLC7A5 knockdown (tau score = +97.16). The NFE2L2 gene encodes a transcription factor (NRF2) that regulates antioxidant enzymes, and the SLC7A5 gene encodes an amino acid transporter that is often overexpressed in many cancer types.

Conclusion: Glucosinolates, specifically I3C and sulforaphane, demonstrate anticancer properties by inducing cell survival and gene expression changes in several cancer cell lines. In the future, identifying the genes and proteins targeted by I3C, as well as the pathway through which sulforaphane acts, will be crucial to further elucidate the effects of glucosinolates on cancer.

#4340 DM1 pharmacokinetics in combination with tucatinib in patients with HER2+ LA/M breast cancer.

Ariel R. Topletz-Erickson[1], Diqiong Xie[1], Flavia Storelli[1], Julie Meyer[1], Shawna Hengel[1], Wentao Feng[1], Lauren Farr[1], Amit Garg[2]

[1]Pfizer Oncology, Bothell, WA,[2]Pfizer Oncology, San Fransisco, CA

Background: HER2CLIMB-02 (NCT03975647) is a randomized, double-blinded, placebo-controlled, global phase 3 study designed to evaluate the efficacy and safety of tucatinib in combination with T-DM1 (ado-trastuzumab emtansine) in patients with HER2+ locally advanced/metastatic breast cancer (LA/mBC) who received prior treatment with trastuzumab and a taxane in any setting. Tucatinib is a strong cytochrome P450 3A4 (CYP3A4) inhibitor. The T-DM1 unconjugated payload DM1 is primarily metabolized by CYP3A4. Though T-DM1 is contraindicated for concomitant use with strong CYP3A4 inhibitors, no clinical drug-drug interaction studies have been conducted in patients to assess the impact of strong CYP3A4 inhibitors on DM1 PK. Thus, DM1 PK with and without tucatinib was characterized in HER2CLIMB-02.

Methods: Patients in HER2CLIMB-02 were randomized to either tucatinib+T-DM1 or placebo+T-DM1 arms. PK samples were collected on Days 1, 2, 3, and 5 in Cycle 2 (intensive PK substudy) and Day 1 of Cycles 3-6 (all patients). DM1 plasma concentrations were analyzed using validated mass spectrometry methods. DM1 PK parameters following T-DM1 administration, including plasma area under the concentration-time curve up to the last time point (AUC_{last}, Cycle 2 only) and maximal concentrations (C_{max}, end of T-DM1 infusion), were calculated using Phoenix (WinNonlin v8.2). Statistical summaries were produced using SAS version 9.4 or later.

Results: PK data were available for 196 (substudy: n=27) and 206 (substudy: n=20) patients in the tucatinib and placebo arms, respectively. Geometric mean (GM, CV%) Cycle 2 DM1 AUC_{last} values were 4.93 (34%) and 4.37 (23%) day*ng/mL in the tucatinib (n=11) and placebo (n=11) arms, respectively. Cycle 2 GM (CV%) DM1 C_{max} values were 2.38 (26%) and 2.75 (24%) ng/mL in the tucatinib (n=23) and placebo (n=20) arms. Geometric mean ratios for Cycle 2 DM1 AUC_{last} and C_{max} were 1.13 ($CI_{90\%}$ 0.92-1.4) and 0.86 ($CI_{90\%}$ 0.76-0.98), respectively, in the tucatinib versus placebo arms. Likewise, DM1 C_{max} GM values for Cycles 3-6 in all study patients were similar between tucatinib and placebo arms.

Conclusions: DM1 plasma PK was not impacted by tucatinib in patients with HER2+ LA/mBC.

#4341 Association of *HapB3 1129-5923C>G* and *1236G>A* with severe fluoropyrimidine toxicity: A systematic review.

Samantha J. Embley[1], Jaime L. Natoli[2], Pang F. Xiong[3], **Ryan S. Nelson**[1]

[1]ARUP Laboratories, Salt Lake City, UT,[2]SCPMG Department of Clinical Analysis, Kaiser Permanente Southern California, Pasadena, CA,[3]Children's Hospital of Colorado, Aurora, CO

Introduction: The *DPYD HapB3* haplotype includes a decreased-function intronic variant (*1129-5923C>G*) as well as a benign variant (*1236G>A*). Although it was thought that the variants were in complete linkage disequilibrium and testing for only *1236G>A* was an accurate proxy, new evidence suggests otherwise. In addition, evidence suggests that drug-related toxicity associated with *HapB3* may be less likely compared to no-function *DPYD* variants. The primary objective of this study was to evaluate the association between *HapB3* variants and severe fluoropyrimidine toxicity and to compare this to associations for other variants with functional impact.

Methods: We conducted a systematic review (date: Nov 2024) to identify studies that evaluated the association between *DPYD* variants and severe (grade ≥3) toxicity among patients undergoing fluoropyrimidine-based chemotherapy. Direct comparisons were made for each *HapB3* variant (*1129-5923C>G* and/or *1236G>A*) vs wild-type (WT) as well as for other decreased-function (*2846A>T*) and no-function variants (e.g., **2A*) vs WT. Findings were quantitatively combined using Excel to calculate an odds ratio (OR). Indirect comparisons of ORs made across variants using WT as a common comparator.

Results: *HapB3 vs WT:* Each *HapB3* variant was compared to WT data with results summarized in the included table. *Direct Comparison: HapB3* variants were directly compared to other decreased-function or no-function alleles within the same studies with results summarized in the included table. *Indirect Comparison: HapB3* variants were indirectly compared to other decreased-function or no-function alleles from populations outside of *HapB3* studies as summarized in the included table.

Conclusions: *HapB3* variants *1129-5923C>G* and *1236G>A* were not strongly associated with increased toxicity compared to wild-type. Both *HapB3* alleles showed less risk of grade 3 or higher toxicity compared to other decreased-function and no-function variants.

Association of DPYD variants with any fluoropyrimidine-related severe toxicity (grade ≥3)

Comparison Type	Variant	Studies	n	Odds Ratio	95% CI	P Value
Decreased Function vs WT	*1129-5923C>G* (*HapB3* Intronic Variant)	5	4315	1.28	0.99-1.65	0.06
	1236G>A (*HapB3* Benign Variant)	4	991	1.03	0.59-1.78	0.92
	2846A>T	4	2801	1.94	1.13-3.34	0.02
No Function vs WT	**2A*	6	3036	1.91	1.21-3.03	.01
	**13*	4	2659	1.16	0.35-3.87	0.81
Direct Comparisons: *HapB3* Variants vs Decreased Function	*1129-5923C>G* vs *2846A>T*	5	4315	0.57	0.32-1.03	0.06
	1236G>A vs *2846A>T*	4	991	0.55	0.26-1.18	0.13
Direct Comparisons: *HapB3* Variants vs Decreased Function	*1129-5923C>G* vs **2A*	5	4315	0.58	0.34-0.98	0.04
	1129-5923C>G vs **13*	5	4315	0.91	0.27-3.11	0.88
	1236G>A vs **2A*	4	991	0.56	0.28-1.14	0.11
	1236G>A vs **13*	4	991	0.88	0.24-3.30	0.85
Indirect Comparisons*: *HapB3* Variants vs Decreased Function	1129-5923C>G vs 2846A>T	NA	NA	0.59	0.40-0.88	0.01
	1236G>A vs 2846A>T	NA	NA	0.58	0.31-1.07	0.08
Indirect Comparisons*: *HapB3* Variants vs No Function	*1129-5923C>G* vs **2A*	NA	NA	0.62	0.43-0.90	0.01
	1129-5923C>G vs **13*	NA	NA	0.65	0.27-1.60	0.92
	1129-5923C>G vs *1475C>G*	NA	NA	0.46	0.06-3.29	0.44
	1129-5923C>G vs *1774C>T*	NA	NA	0.46	0.03-7.37	0.58
	1236G>A vs **2A*	NA	NA	0.60	0.33-1.10	0.1

	1236G>A vs **13*	NA	NA	0.64	0.23-1.75	0.38
	1236G>A vs *1475C>T*	NA	NA	0.44	0.06-3.37	0.43
	1236G>A vs *1774C>T*	NA	NA	0.44	0.03-7.44	0.57
*Blank cells for number of studies and sample size are not reportable for indirect comparisons; therefore, these cells are left blank.						

#4342 The lymphatic system plays a major role in the absorption of subcutaneously administered trastuzumab in a lymph-duct cannulated rat model.

Junyi Pei[1], Xiaoxuan Fei[1], Luojuan Hu[1], Huimin Zhang[2], Sifei Han[1]

[1]China Pharmaceutical University, Nanjing, China,[2]the First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China

Introduction: The lymphatic system plays a key role in the metastasis of a number of cancers, such as certain types of breast cancer, lung cancer, and colorectal cancer. Drug delivery to the lymphatics may co-localize high concentrations of therapeutic agents and the biological targets (cancer cells or immune cells) in a relatively confined area, thereby providing opportunities for the inhibition of lymphatic metastasis. However, such targeted delivery is usually difficult to achieve since drug molecules mainly circulate via the blood stream instead of the lymphatics. A number of preliminary studies on the pharmacokinetic behaviors of subcutaneously administered biologicals suggested that the lymphatic system served as a main conduit for the absorption of large molecule drugs. The current study aims to examine the absorption pathways (blood vs lymph) after subcutaneous administration of two marketed products of the same anti-HER2 monoclonal antibody drug trastuzumab (Herceptin for IV and Herceptin for SC).

Method: In vivo pharmacokinetic studies were conducted using a rat model with cannulas in thoracic lymph duct, carotid artery and jugular vein. Herceptin IV (intravenous formulation) and Herceptin SC (subcutaneous formulation) were administered to two groups of animals to the footpad at a dose of 2.5 mg/kg trastuzumab (both diluted to a concentration of 21 mg/ml using saline before injection). Lymph fluid from the thoracic duct and blood from the carotid artery were collected, with fluid loss compensated through jugular vein using saline.

Results: More than 70% of the subcutaneously administered dose was recovered in thoracic lymph for both formulations of trastuzumab in rats over 36 hours. Notably, trastuzumab concentration in the lymph of the cannulated group was approximately ten times higher than that in the plasma collected from the same animals. Furthermore, following subcutaneous administration, Herceptin SC formulation demonstrated faster absorption profile compared to the IV formulation, with trastuzumab $C_{max\ in\ lymph}$ 3 times higher (~150 μg/ml for Herceptin SC vs 50 μg/ml for Herceptin IV), and $T_{max\ in\ lymph}$ 1 hour earlier (1h for Herceptin SC vs 2h for Herceptin IV). The faster absorption and higher lymphatic transport are potentially due to the presence of rHuPH20 in the SC product that facilitated disperse of the antibody molecules in the subcutaneous interstitial spaces.

Conclusion: The study confirms the lymphatics is the main absorption pathway following subcutaneous administration of trastuzumab, regardless of the formulation type (IV or SC). This results in markedly high concentrations of trastuzumab in the draining lymphatics, and the co-localization of the drug molecules with potentially disseminated tumor cells and immune cells within the tumor-draining lymphatics may provide an opportunity for enhanced therapeutic outcomes.

#4343 Characterization and comparison of inhibitors of GPR68.

Piotr Stepien[1], Duncan Bellamy[1], Alice E. Berizzi[1], Alex Ceroni[1], Elena Fonfria[1], Major Gooyit[2], Mizuho Horioka[1], Katherine Lansu[1], Sam R. Lawrence[1], Manisha Naik[1], Maninder Panesar[1], Kostantinos Papachristos[1], Martin Redhead[1], Simon Richards[1], David A. Smith-Parkin[1], Jake Swadling[1], Diogo M. R. Tomaz[1], Bojan Vilagos[1], Andrew S. Walker[1], Michael Prime[1], Reid H. J. Olsen[3]

[1]Recursion Pharmaceuticals, Oxford, United Kingdom,[2]Recursion Pharmaceuticals, Chicago, IL,[3]Recursion Pharmaceuticals, Boston, MA

Background: GPR68 is a proton-sensing GPCR reported to play a role promoting tumor aggressiveness and immune-evasion, as well as in inflammatory disease. Most data supporting these findings come from genetic studies, and disclosed small molecule compounds targeting GPR68 are scarce. Nevertheless, two distinct chemical entities have recently been reported as GPR68 inhibitors. They have been used to define *in vitro* mechanisms by which GPR68 may affect disease biology. One of these, FT011, has completed a Phase 2 trial for scleroderma and is described as a GPR68 antagonist, however no pharmacological characterization at GPR68 has been reported to date. A chemical probe, ogremorphin, identified through a chemical genetic screen, is described as a GPR68 antagonist with anti-cancer efficacy *in vitro*.

Methods: Here we describe the identification of three series of novel, drug-like GPR68 antagonists from a screening campaign, which were further optimized via our proprietary generative AI design methods. We present the pharmacological characterization of these series and compare representative examples to FT011 and ogremorphin in primary pharmacology assays and benchmark their activity in fibroblast and glioblastoma (GBM) cell lines.

Results: Three unique chemical series were identified as potent antagonists of GPR68. Detailed characterization using the TRUPATH system suggests that all three scaffolds act as negative allosteric modulators. In comparison, in our hands, FT011 does not inhibit GPR68 activation when evaluated with classical signaling technologies, nor in TRUPATH. Ogremorphin does weakly antagonise GPR68 as reported, with the potency of these effects dependent on the assay technology. All three of our designed GPR68 antagonist series potently inhibit the release of GPR68-stimulated cytokines in a GPR68-expressing cancer associated fibroblast line, as does ogremorphin to a lesser degree. In our hands, FT011 does not antagonise GPR68-mediated cytokine release. We confirm the reported inhibition on GBM lines by ogremorphin, but this effect is absent in our three unique GPR68 antagonist series.

Conclusions: Here we describe multiple highly-potent chemical scaffolds targeting GPR68 as negative allosteric modulators with our data strongly supporting the characterization of our drug-like molecules as first-in-class antagonists of GPR68. In contrast, our profiling of FT011 does not support its purported activity at GPR68. Ogremorphin is confirmed as a weak antagonist of GPR68 which inhibits GBM cell proliferation but it is possible the effect of ogremorphin is due to activity against other targets, or polypharmacology not represented in our GPR68 antagonists. Our first-in-class compounds are currently in development for oncology indications.

#4344 Broader therapeutic window via combining sodium ascorbate for protecting radioactive antibodies from radiolysis and sodium perchlorate for blocking sodium iodide symporter in radioimmunotherapy with astatine-211.

Hiroki Takashima[1], Shino Manabe[2], Ryo Tsumura[1], Yoshikatsu Koga[1], Takahiro Anzai[3], Hiromitsu Haba[4], Anri Inaki[1], Masahiro Yasunaga[1]

[1]National Cancer Center, Kashiwa, Japan,[2]Hoshi University, Shinagawa-ku, Tokyo, Japan,[3]National Institute of Technology (KOSEN), Gunma College, Maebashi, Japan,[4]RIKEN, Wako, Japan

Background: Increasing attention has been paid to developing new cancer treatments with alpha particles characterized by a high linear energy transfer (LET) and a range of 50-100 µm in tissue, which is equivalent to 5-10 cells. Due to the short path length, tumor-selective accumulation of alpha particles is pivotal for achieving a reasonable therapeutic window. Previously, we demonstrated that reactive oxygen species (ROS) generated via the radiolysis of water in storage solution denature antibodies labeled with astatine-211, an alpha emitter, which results in disrupted specific binding activity of astatinated antibodies. On the other hand, free radical scavengers such as sodium ascorbate (SA) can quench ROS and protect radioactive antibodies from astatine-211-induced denaturation. The antibody denaturation significantly attenuates tumor accumulation and the antitumor activity of antibodies labeled with astatine-211, whereas SA protection successfully maintains them. On the other hand, stabilization with SA has no impact on distribution to normal organs, including the uptake in the stomach and thyroid caused by free astatine-211 released from antibodies. Thus, in this study, to reduce the accumulation in these organs, we combined sodium perchlorate (SP), a sodium iodide symporter (NIS) inhibitor, with SA protection and evaluated antitoxic activity. Although it is well known that NIS blockade can reduce halogen radionuclide accumulation in these organs, the antitoxic effect and tolerability in radioimmunotherapy (RIT) with astatine-211 under SA protection remain unclear.

Methods: Mice bearing tumor were pretreated with PBS or 1.2 µmol/g SP and intravenously administered 1 MBq of antibody labeled with astatine-211. We evaluated the impact of the NIS blockade on biodistribution. The accumulation in the thyroid was visualized by single-photon emission computed tomography/computed tomography (SPECT/CT). Toxicity after RIT was evaluated by body weight change and blood tests.

Results: Pretreatment with SP significantly reduced the percentage of injected dose per gram of tissue (%ID/g) for stomach and stomach contents without decreasing tumor accumulation (stomach, $P = 0.020$; stomach contents, $P = 0.013$). SPECT/CT images showed that the pretreatment resulted in lower signals in the thyroid and stomach contents. NIS blockade contributed to significantly more rapid recovery from body weight loss after the injection of astatine-211-labeled antibody ($P = 0.002$). Findings in blood tests were comparable between these groups, showing the tolerability of the combination.

Conclusion: Combining SA protection against radiolysis and NIS blockade with SP is tolerable in RIT with astatine-211, leading to a broader therapeutic window.

#4345 BH3120, a PD-L1x4-1BB bispecific antibody, exhibits favorable safety profiles in distinct in vivo and ex vivo liver toxicity evaluations.

Jing Wang, **Jiangcheng Xu**, Jun Wang, Jiangwang Liu, Kyoungwoo Lee

Beijing Hanmi Pharm. Co. Ltd., Beijing, China

BH3120 is a novel bivalent PD-L1/4-1BB bispecific antibody targeting both inhibitory (PD-1/PD-L1) and co-stimulatory (4-1BB clustering) signaling. In multiple non-clinical safety evaluations, BH3120 consistently showed minimal modulation of T cell functions in the blood or normal tissues resulting in favorable safety profiles. Clinical evaluation of BH3120 as a monotherapy and in combination with a PD-1 inhibitor is underway (NCT06234397).

While early stage clinical evaluations of 4-1BB agonists as a monotherapy or in combination with PD-1/PD-L1 inhibitors are generally associated with certain level of liver abnormalities, the potential biomarkers or patient's backgrounds that can pre-determine the risk of liver toxicity have not been discussed in details. In the meantime, treatment discontinuation or disruption, and administration of corticosteroid due to liver toxicity would be the limitations of 4-1BB agonists in tumor management, and the mode of action and impact of 4-1BB agonists on liver inflammation need to be further investigated. To understand potential risks of liver toxicity with BH3120, sensitive in vivo and ex vivo models for liver toxicity evaluations were established: a mice model with mild liver inflammation induced by Concanavalin A, and human liver organoids co-cultured with immune cells to mimic the histological and physiological changes in patient's liver tissues.

In both models, BH3120 was not associated with significant elevation of liver inflammation markers, indicating minimal risk of liver toxicity that is in line with the safety results we previously reported. The property of BH3120 to minimize systemic immune modulation enables de-coupling of anti-tumor efficacy from systemic toxicities, and would provide flexibility in combination with different anti-tumor treatments.

#4346 Improved pharmacokinetic properties of the purified S-enantiomer of the alkylphospholipid Edelfosine compared to the racemic mixture: Potential orally available novel anticancer agent active against pancreatic cancer.

Michael Ahrweiler, Wolfgang Meyer, Daniel Perrissoud, Axel Zander

RDP Pharma AG, Romanshorn, Switzerland

Edelfosine, an alkylphospholipid anticancer drug, has been extensively investigated, but the racemic mixture used in these studies has not been approved for human commercial use. This drug acts by integrating into lipid rafts in cell membranes, including mitochondria and the ER. The mechanism of action involves CD95-mediated apoptosis, AKT3/PI3K inhibition, phospholipase C and protein kinase C inhibition, as well as interference with cholesterol and phosphatidylcholine metabolism. Edelfosine is preferentially taken up by malignant cells, its efficacy in numerous xenograft studies, including metastatic breast and prostate cancer models, as well as in phase 2 clinical trials has been convincingly demonstrated. In patients, it is generally well-tolerated; the reported main adverse event was dose-dependent, but reversible gastrointestinal toxicity. We purified the S and R enantiomers from the racemic mixture and assessed the pharmacokinetic (PK) and pharmacodynamic (PD) differences between them (R-CP201 and S-CP201) in rodents using a validated LC-MS/MS bioanalytical method. Following a single-dose i.v. administration (20 mg/kg) in male rats (N=5), S-CP201 showed a favorable half-life ($t_{1/2}$ alpha and beta: 1.8 and 8.0 hours) vs R-CP201 (2.1 and 18.2 hours). C_{max} values were similar (~42 µg/mL); but AUC_{last} was higher for S-CP201 (172.6 µg*h/mL vs. 149.3 µg*h/mL for R-CP201). This trend persisted with oral administration (150 mg/kg), where AUC_{last} was 54.2 µg*h/mL for S-CP201 compared to 46.3 µg*h/mL for R-CP201. In another study, single doses of each enantiomer (10 mg/kg orally, 2.5 mg/kg i.v.) were given to male rats (N=8). Systemic exposure, expressed as AUC_{last}, was lower for R-CP201 (i.v.: 43.0 µg*h/mL; oral: 49.4 µg*h/mL) than S-CP201 (i.v.: 50.6 µg*h/mL; oral: 63.7 µg*h/mL). Absolute oral bioavailability (F) was 33.2% for S-CP201 and 29.1% for R-CP201. Daily doses of 10 mg/kg S-CP201 or R-CP201 were given to male rats (N=4) for 5 days. After 5 days, AUC_{last} values were 115.5 µg*h/mL for R-CP201 and 159.0 µg*h/mL for S-CP201, with respective C_{max} values of 1.9 µg/mL and 2.6 µg/mL. A single-dose PK study of Edelfosine (20 and 40 mg/kg orally; 5 mg/kg i.v.) and its enantiomers (10 and 20 mg/kg orally; 2.5 mg/kg i.v.) in male mice showed comparable relative bioavailability for Edelfosine and S-CP201 (~45%), but lower for R-CP201 (~35%). Also, no interconversion between the enantiomers was observed in rat plasma. S-CP201 demonstrated in vitro efficacy across diverse tumor cell lines and showed superior tumor reduction in a PDX pancreatic cancer study in mice (57% vs. 33%). No major toxicity was detected for S-CP201 in rats and mice. These results confirm PK and PD advantages and acceptable tolerability of S-CP201 and support its further development as an anticancer agent is warranted.

#4347 Preclinical evaluation of pharmacokinetic interactions among nebivolol, dasatinib and devimistat in mice.

Airong Li, Mikayla Skillman, Fatima Dagher, Amir Mohammad Gholizadeh, Emanuel Baltrip, Junghwa Park, Meghana Trivedi, Diana S.-L. Chow

University of Houston, College of Pharmacy, Houston, TX

Breast cancer ranks as the most frequently diagnosed cancer in 157 of 185 countries. It accounts for 30% of all new female cancers annually, with triple-negative breast cancer (TNBC) comprising 10-15% of the cases. TNBC is particularly aggressive, with rapid growth and spread, limited treatment options, and a worse prognosis. Due to the lack of markers, TNBC is challenging to treat, and monotherapies are often ineffective; instead, combination therapies with drugs with different mechanisms of action may offer more effective options. In the previous study, G protein-coupled receptor-targeting drugs were evaluated using high throughput screening. Nebivolol (NBV), a third generation β-blocker, was identified as a potential inhibitor against TNBC metastasis. This study investigated the pharmacokinetics (PK) of NBV in combination with dasatinib (DAS), a multi-kinase inhibitor, and devimistat (CPI), a mitochondrial tricarboxylic acid cycle inhibitor, respectively, in FVB healthy mice, focusing on the potential drug-drug interaction (DDI). In this study, FVB mice were randomly assigned to 5 groups (NBV, DAS, CPI, NBV+DAS and NBV+CPI, n=5 each). The compounds were administered via intraperitoneal injection at single doses of NBV at 4 mg/kg, DAS at 10 mg/kg, and CPI at 12 mg/kg, for each group. Blood samples were collected at 20 min, 1 h, 3 h, 6 h, 8 h, and 23 h post dose and quantified using LC-MS/MS assay (Sciex 5500). Data was analyzed using Phoenix (8.5.1) and GraphPad Prism (10). Statistical analysis of PK parameters was performed using ANOVA followed by Tukey post hoc test or t-test, for significance at $p<0.05$. The results indicated, in the NBV and DAS combination group, the half-life of NBV (Mean±SD) significantly increased to 5.32±1.72 hours, from 3.11±0.27 hours in NBV alone group, likely due to a higher distribution volume (V) and lower clearance (CL), which were not statistically significant due to large variations. The area under curve (AUC) remained comparable in combination treatment. The PK of DAS was unaffected by NBV. In the combination of NBV and CPI, the PK parameters of NBV were unaffected, whereas the CL of CPI significantly decreased in the combination group (70.29±30.32 L/h), from that in CPI alone group (142.32±49.35 L/h). CPI distribution volume decreased (124.51±90.18 L) in the combination group versus 1107.11±1379.40 L in the alone group. However, this change in V was not statistically significant due to a large variation. The AUC of CPI significantly increased to 201.72±92.25 h*ng/mL, compared to 87.62±22.21 h*ng/mL in CPI alone group. In summary, DDIs were observed among NBV, DAS, and CPI. Co-administration with DAS slowed the NBV elimination, suggesting that NBV accumulation would be considered when used alongside DAS. Additionally, CPI exposure significantly increased in the presence of NBV, indicating that dose adjustments may be necessary in combination therapies.

#4348 A translational pharmacokinetic-pharmacodynamic (PKPD) modeling framework of novel, highly selective, next-generation allosteric cyclin-dependent kinase 2 (CDK2) inhibitors.

Andrea Gruber[1], Sven Christian[2], Carsten Schmeck[3], Antje Rottmann[1], Hongyu Xue[2], Marine Garrido[4], Mariya Pavlyuk[4], Bart Ploeger[1], Peter N. Morcos[5]

[1]Bayer AG, Berlin, Germany,[2]Bayer AG, Cambridge, MA,[3]Bayer AG, Wuppertal, Germany,[4]Bayer AG, Basel, Switzerland,[5]Bayer AG, Whippany, NJ

Background: CDK2 plays a crucial role in regulating multiple oncogenic signaling pathways, and its activity is essential for the loss of growth control during cancer development. The amplification and overexpression of the CDK2 binding partner Cyclin E1 (CCNE1) is a key mechanism in various cancers. Selective CDK2/CCNE1 inhibition therefore offers a promising treatment for patients with CCNE1 overexpressing tumors. CDK2/CCNE1 inhibition has been shown to reduce phosphorylation of the retinoblastoma protein (pRB), a direct downstream target, which is associated with decreased tumor growth in preclinical *in vitro* and *in vivo* models. Reduced levels of pRB have also been observed in patients treated with CDK2 inhibitors. We have developed a pharmacokinetic-pharmacodynamic (PKPD) model for CDK2 inhibitors and examine the relationship between dose, exposure, pRB and tumor growth, using published and in-house *in vitro* and *in vivo* data from several compounds, to support preclinical and clinical phases.

Methods: Our PKPD model framework consists of *in vitro* and *in vivo* components. The *in vitro* model evaluates the relationship between compound concentrations and pRB levels as well as cell proliferation in CCNE1-overexpressing cell lines for multiple CDK2 inhibitors. The *in vivo* model describes compound exposure in plasma and tumor and the modulation of pRB, which is associated with changes in tumor growth. Model building and validation was informed by published literature on CDK2 inhibitors, *in vitro* data showing modulation of pRB and proliferation, and *in vivo* mouse data in CCNE1-overexpressing OVCAR3 xenografts, including plasma and tumor exposure, pRB measurements, and tumor growth inhibition data for different CDK2 inhibitors and dose levels. The model was developed using the MonolixSuite (Lixoft) software.

Results: For the investigated CDK2 inhibitors a direct nonlinear model quantitatively characterizes the association of compound exposure and pRB levels, allowing the derivation of an IC50 from *in vitro* data for use in the *in vivo* model. The *in vivo* model describes plasma PK by a two-compartment model and tumor PK by an additional tumor compartment. The modulation of pRB levels by compound concentration in the tumor is described using an indirect response model and the *in vitro* derived IC50. Further, the reduction of pRB is linked to tumor growth inhibition using a Simeoni model. Diagnostic plots and model parameter estimates show that the *in vitro* and *in vivo* models well capture the complete data of the investigated CDK2 inhibitors, increasing confidence in the established model and applied hypotheses.

Conclusion: We have established a translational PKPD modelling framework to support preclinical candidate selection, which will be evaluated in the clinical development of novel, next-generation CDK2 inhibitors.

#4349 Translational pharmacokinetic/pharmacodynamic (PK/PD) modeling for VVD-130037, a novel, first-in-class covalent small molecule Kelch-like ECH-associated protein 1 (KEAP1) activator.

Conrad Housand[1], Nil Roy[1], Tine Wyseure[1], Christie Eissler[1], Hauke Ruehs[2], Christoph Niederalt[2], Carsten Zieschang[3], Marielena Mata[1], Ramin Samadani[1], John Bothos[1], Lawrence Lu[1], Jenna Goldberg[1], **Peter N. Morcos**[4]

[1]Vividion Therapeutics, San Diego, CA,[2]Bayer AG, Wuppertal, Germany,[3]Bayer AG, Berlin, Germany,[4]Bayer AG, Whippany, NJ

Introduction: The KEAP1-nuclear factor erythroid 2 (NRF2) pathway provides homeostatic mechanisms for oxidative stress response which can be activated in cancers. Vividion Therapeutics has developed novel allosteric molecular glues of the Keap1-CUL3 E3-ligase complex. Preclinically, these agents covalently target KEAP1-CUL3 E3 ligase and allosterically increase the affinity between KEAP1 and CUL3, promoting the formation of active KEAP1-CUL3 E3 ligase complexes and enhancing NRF2 degradation (*Mol Cancer Ther* (2023) 22 (12_Suppl): PR011). We established a translational PK/PD model for KEAP1 target engagement (TE) for the clinical candidate, VVD-130037.

Methods: VVD-130037 PK/PD modeling leveraged an initially developed framework (*Mol Cancer Ther* (2023) 22 (12_Suppl): C126). Compartmental PK models are used to describe VVD-130037 plasma and tumor exposure and are coupled to a mechanistic TE model capturing intracellular covalent binding to KEAP1 and KEAP1/NRF2 synthesis, degradation and complex formation. The PK models were parameterized using mouse experiments; the predicted tumor concentration provided the input to the TE model. Predictive accuracy of the TE model was made by comparison to experimental TE. A physiologically-based PK (PBPK) model incorporating human physiology was linked to the TE model for human PK-TE predictions.

Results: The PK model captures mouse plasma and tumor PK across dose levels. Similarly, the mechanistic TE model adequately predicts in vitro and in vivo TE profiles at various drug concentrations. Sensitivity analyses reveal KEAP1 turnover as a key parameter influencing VVD-130037 TE. Biologically plausible ranges facilitate predictions of efficacious dose regimens to saturate TE. The VVD-130037 human PBPK model predicted a rapid plasma half-life, however when coupled with the TE model predict sustained peripheral and tumor TE with once and twice daily dosing regimens.

Conclusions: A translational PK/PD model is developed for VVD-130037; supporting efficacious doses during clinical development.

#4350 Pharmacodynamic biomarker modulation by OMX-0407, a novel, spectrum-selective kinase inhibitor for the treatment of angiosarcoma and renal cell carcinoma.

Marisa Stebegg-Wagner[1], **Ilona Petra Maser**[1], Bettina Bauer[1], Andreas Schirmer[1], Moritz Zulley[1], Carmen Amerhauser[1], Thorsten Meyer[2], Tiantom Jarutat[1], Hannes Loferer[1], Stefan Bissinger[1]

[1]iOmx Therapeutics AG, Martinsried/Munich, Germany,[2]Granzer Regulatory Consulting & Services GmbH, Munich, Germany

Background: OMX-0407 is an orally available spectrum-selective kinase inhibitor targeting a set of oncology-relevant tyrosine kinases (TK) as well as the salt-inducible kinase (SIK) family, demonstrating a dual mode of action (MoA) in cancer. This involves OMX-0407-induced cell cycle arrest in tumor cells by de-phosphorylation and inactivation of TKs such as Src family kinases (SFK) and the SIK family, and increased sensitivity of the tumor microenvironment to immune-mediated tumor cell killing. OMX-0407 is currently undergoing early clinical testing for the treatment of angiosarcoma (AS) and clear cell renal cell carcinoma (ccRCC) (NCT05826600).

Methods: Kinase activity profiling using the PamChip® kinase array was used to quantify OMX-0407 treatment effects. Moreover, a flow-cytometry-based “phosFlow” assay was developed to assess the phosphorylation status of SFKs. Both assays revealed dose-dependent inhibition of SFK phosphorylation and activity in peripheral blood mononuclear cells (PBMCs). Hence, they were implemented as pharmacodynamic biomarkers in the ongoing first-in-human trial (NCT05826600) of OMX-0407 in patients with previously treated unresectable solid tumors.

Results: As of 30th of October 2024, 24 patients have received continuous treatment with OMX-0407 administered orally in 28-day cycles in a dose escalation study at dose levels of 10, 30, 60, 90, 100, and 140 mg twice daily (BID). Treatment was well tolerated with the main safety finding being gastro-intestinal adverse reactions. One patient with secondary radiation-induced angiosarcoma achieved a complete response at doses of up to 60 mg BID, which remains ongoing at 14 months.

PhosFlow analysis confirmed OMX-0407 effects on the phosphorylation of SFKs in PBMCs at doses of 60mg and above. This was associated with the inhibition of oncology-relevant TKs as determined by functional kinase activity profiling. Treatment effects were significantly correlated with PK exposure. This confirms that both phosFlow and kinase activity profiling successfully resolve dose-dependent OMX-0407 activity in peripheral patient blood as pharmacodynamic biomarkers.

These findings, together with safety findings and PK profiling, contributed to the selection of 100mg BID as the recommended phase 2 dose (RP2D) for the dose expansion.

Conclusions & Outlook: OMX-0407 is a promising therapeutic candidate for the treatment of angiosarcoma and RCC with a potential dual MoA combining direct anti-tumor effects with modulation of the immune cell compartment towards a pro-inflammatory state. In September 2024, Ph1b expansion cohorts of the first-in-human trial (NCT05826600) were initiated for patients with unresectable/metastatic AS or ccRCC who have received at least one prior line of therapy, evaluating OMX-0407 at a dose of 100mg BID.

#4351 Clinical indenoisoquinolines strongly inhibit the global *MYC* transcriptional pathway.

Yichen Han[1], Sheng Liu[2], Mark Cushman[1], Jun Wan[2], Danzhou Yang[3]

[1]Borch Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN,[2]Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN,[3]Borch Department of MCMP, PICR, PIDD, Department of Chemistry, Purdue University, West Lafayette, IN

MYC, one of the most validated driver cancer oncogenes, is overexpressed in a majority of human cancers. However, the MYC protein is intrinsically disordered and considered “undruggable”. The *MYC* gene promoter forms G-quadruplex, a globular non-B DNA secondary structure. *MYC* G-quadruplex (MycG4) is a transcription repressor that silences *MYC* expression upon stabilization by small molecules. Targeting MycG4 for transcriptional inhibition is a promising approach for cancer therapeutics. Indenoisoquinolines were designed as topoisomerase I inhibitors with better physicochemical and biological properties than the clinical camptothecin drugs, with three compounds in phase I/II clinical trials. Recently, we found that indenoisoquinolines can bind and stabilize MycG4 and downregulate *MYC* expression in cancer cells, which significantly correlates with anticancer activities. Herein, we investigate the effects of the three clinical indenoisoquinolines on MYC downregulation and MYC gene pathways, with comparison to other well-known G4-ligands and the topoisomerase I inhibitor camptothecin (CPT). The clinical indenoisoquinolines strongly bind and stabilize MycG4, whereas CPT shows little binding and stabilization. We assessed global changes in gene expression by RNA sequencing in the MYC-dependent B-lymphoma Raji cells. We treated the tumor cells with the three clinical indenoisoquinolines (LMPs), other G4-ligands, and CPT, at subtoxic doses across various timepoints. Our enrichment analysis on gene ontology and pathway identified the primary cellular responses and pathways altered with MYC expression modulation. We also evaluated the treatment-induced effects on all hallmark biological pathways from the Molecular Signature Database using Gene Set Enrichment Analysis. The RNA-seq results indicate that the MYC pathway is the major cellular response of the clinical LMP compounds. We further examined the treatment-induced suppression of MYC-regulated genes and observed significant and durable inhibition of MYC-regulated gene pathways. Notably, we found that the clinical LMPs, especially LMP400, exhibited significantly stronger MYC suppression compared to other G4-ligands. We speculate that dual MYC-targeting and topoisomerase I inhibition is critical to the exceptional MYC inhibition and anticancer efficacy of indenoisoquinolines. In conclusion, we demonstrate that the clinical indenoisoquinolines effectively and persistently inhibit the global MYC transcriptional pathway, serving as a major mechanism of action. Because MYC is a common and essential oncogene, our study provides critical mechanistic understanding and a molecular basis for the future clinical developments and therapeutic applications of indenoisoquinolines in MYC-dependent cancers.

#4352 Safety and pharmacokinetics of tinengotinib monotherapy in different doses and dosing schedules in advanced solid tumors.

Sarina A. Piha-Paul[1], Sanjay Goel[2], Chih-Yi Liao[3], Nashat Y. Gabrail[4], Farshid Dayyani[5], Syed Kazmi[6], Sayeh Lavasani[7], Shumao Ni[8], Caixia Sun[8], Katie Hennessy[9], Hui Wang[8], Peng Peng[8], Jean Fan[9], Milind Javle[1]

[1]University of Texas MD Anderson Cancer Center, Houston, TX,[2]Rutgers Cancer Center Institute of New Jersey, New Brunswick, NJ,[3]The University of Chicago Medicine and Biological Sciences, Chicago, IL,[4]Gabrail Cancer Center Research, Canton, OH,[5]University of California, Irvine, Irvine, CA,[6]University of Texas Southwestern, Dallas, TX,[7]City of Hope National Medical Center, Duarte, CA,[8]TransThera Sciences (Nanjing), Inc., Nanjing, China,[9]TransThera Sciences (US), Inc., Gaithersburg, MD

Background: Tinengotinib is a novel multi-kinase inhibitor that targets cell proliferation, angiogenesis, and immune-oncology pathways by inhibiting Aurora kinases A/B, Janus kinases (JAK) and receptor tyrosine kinases (FGFRs and VEGFRs). Here we present the safety and pharmacokinetics (PK) of tinengotinib monotherapy in different doses and dosing schedules from a multicenter, open label phase Ib/II study in patients (pts) with advanced solid tumors.

Methods: Eligible pts received tinengotinib monotherapy in Arm A (12 mg once daily (QD)) and Arm C with QD doses of 5, 8, 10 and 12 mg, and twice daily (BID) doses of 4 mg and 6 mg every 28-day/cycle. Safety was assessed based on CTCAE v5.0. PK samples (plasma) were analyzed by liquid chromatography-tandem mass spectrometry. Preliminary efficacy including Objective Response Rate (ORR) was evaluated per RECIST 1.1.

Results: 197 pts were enrolled and received tinengotinib monotherapy at the following doses and schedules: 4mg BID (n=18), 6mg BID (n=18), 5mg QD (n=14), 8mg QD (n=18), 10mg QD (n=21), and 12mg QD (n=108). Treatment-related Adverse Events (TRAE) were reported in

81.7% (161/197) pts: 46.2% were Grade (G) 3-4, 0% G5. The most common G3-4 TRAE (>10%) was hypertension. Increased G3-4 TRAE rates were seen at higher dose exposures, but no significant differences were noted between the BID and QD schedules. The maximum concentration (T_{max}) was reached at ~2-4 hr. The terminal elimination half-life ($t_{1/2}$) was similar across all QD dose groups in the PK-run-in of Arm C, at ~26-34hr. The apparent volume of distribution (Vz/F) is much larger than 100L. Steady state was reached at C1D15, with mean accumulation ratios of approximately 2-5. Exposure of tinengotinib increased with increasing dose up to 10mg QD, reaching potential saturation of absorption between 10mg and 12mg QD. No notable difference in AUC was observed in QD vs. BID schedules. Sensitivity analyses were like those of the main analysis. Among 159 efficacy evaluable subjects (n=15 in 4 mg BID; n=18 in 6 mg BID; n=10 in 5 mg QD; n=13 in 8 mg QD; n=19 in 10 mg QD; n=84 in 12 mg QD), the best overall response was 13.2% (21/159) including PRs in 2 (11.1%) pts in 6 mg BID, 1(10.0%) in 5 mg QD, 4 (21.1%) in 10 mg QD, and 13 (15.5%) in 12 mg QD. 1 CR was achieved in 12 mg QD. The dose level demonstrating superior efficacy was 10mg QD, with an ORR of 21.1%.

Conclusions: There were no discernable trends in safety or PK between the BID and QD dose schedules. Although there was an increase in TRAEs with overall dose increase, the 10mg QD dose was shown to have an optimal PK profile and demonstrated superior efficacy as compared to the other dose levels. These results suggest that the combination of known PK and safety predictability with promising efficacy provides a basis of dose selection for further clinical development of tinengotinib.

Clinical trial Information: NCT04742959

#4353 A phase I open-label positron-emission tomography study to determine brain exposure of [^{11}C]savolitinib in healthy volunteers.

Kowser Miah[1], Zsolt Cselenyi[2], Jonas Svensson[3], Aurelija Jucaite[2], Ike Igwegbe[4], Caitlin Muldoon[5], Miriam Zanuy Porquet[6], Kenneth Dahl[2], Peter Johnstrom[2], Miguel A. Cortes Gonzalez[3], Antonia Hognasbacka[3], Andrea Varrone[3], Karthick Vishwanathan[1], Magnus Schou[2]

[1]Clinical Pharmacology and Quantitative Pharmacology, CPSS, AstraZeneca, Waltham, MA,[2]PET Imaging Centre, AstraZeneca, Stockholm, Sweden,[3]Department of Clinical Neuroscience, Centre for Psychiatry Research, Karolinska Institutet and Stockholm County Council, Stockholm, Sweden,[4]Global Clinical Program, AstraZeneca, Gaithersburg, MD,[5]Late Oncology Statistics, AstraZeneca, Cambridge, United Kingdom,[6]Patient Safety Oncology, AstraZeneca, Barcelona, Spain

Introduction: Savolitinib is a potent, oral highly selective small molecule MET kinase inhibitor and is capable of inhibiting tumor growth *in vivo* when used as a monotherapy or in combination therapy for multiple indications harboring MET aberrations (MET amplification or MET overexpression). The use of savolitinib in combination with osimertinib is currently being evaluated in NSCLC patients with EGFR mutations and MET amplification. Patients with EGFR mutations appear to have a higher incidence (50-60%) of brain metastasis[1]. Preclinical data suggest that savolitinib penetrates the blood-brain barrier (BBB) which is important to prevent or treat brain metastasis. This study was conducted to determine brain distribution and exposure of a microdose of carbon-11 labelled savolitinib using positron emission tomography (PET), in healthy adult volunteers under physiological conditions (i.e. when the BBB is intact).

Methods: This was an open-label, non-randomised, single-center study. The study was comprised of two site visits and follow-up call. The first visit included screening, the second visit involved two PET examinations. For the first PET examination (PET1), a single microdose (≤10 µg) of [^{11}C]savolitinib was administered. The second PET examination (PET2) using [^{11}C]savolitinib took place the same day, approximately 2 hours after the administration of a single oral 300 mg dose of savolitinib. Radioactivity in brain and arterial blood were measured. Kinetic PET parameters describing brain distribution and exposure were quantified: percent injected dose entering the brain, $\%ID_{max_brain}$; time to maximum concentration in brain, T_{max}; free brain to free plasma partition coefficient $K_{p,\,uu}$.

Results: Seven participants, 2 males, 5 females, 51-64 years of age were examined. [^{11}C]savolitinib entered the brain at a mean 0.80 $\%ID_{max_brain}$ (range: 0.65% to 0.93%, n = 7) and uniformly distributed in all brain regions. When the participants were pre-treated with a 300 mg oral dose of savolitinib the $\%ID_{max_brain}$ values were found to be similar (mean: 0.81 $\%ID_{max_brain}$, range: 0.69% to 0.99%, n = 6). The median T_{max_brain} values at PET1 and PET2 examination were 1.45 minutes (range: 0.22 to 10.95 minutes) and 1.72 minutes (range: 0.17 to 11.03 minutes) and the mean±SD for $K_{p,\,uu}$ was 0.29±0.08 (range: 0.21 to 0.46) and 0.35 ± 0.04 (range: 0.30 to 0.41), respectively.

Conclusion: PET brain imaging in healthy volunteers showed that savolitinib penetrates the BBB and distributes to the brain following IV injection of a microdose of [^{11}C]savolitinib. Reference: Makoto Nishino et al. 2019. Brain metastases in oncogene-driven non-small cell lung cancer. *Transl Lung Cancer Res.* S298-S307.

#4354 Safety and tolerability of NPX372, a novel B7-H7:CD3 bispecific T cell engaging antibody.

Silvia Ferrati, Matthew Rausch, Katalin Kis-Toth, Bijan A. Etemad-Gilbertson, Leena Gandhi, Tatiana Novobrantseva

NextPoint Therapeutics, Cambridge, MA

BACKGROUND: B7-H7 is a B7 family member upregulated on tumor cells of many histologic subtypes with a very restricted normal tissue expression and is, therefore, being explored as a novel tumor targeting antigen. NextPoint Therapeutics has developed a T cell engager bispecific antibody, NXP372, which simultaneously recognizes B7-H7 and CD3 from both human and non-human primates, providing T cell activation in the proximity of B7-H7. NPX372 induced T cell activation and T cell-mediated killing of B7-H7^{+} tumor cells *in vitro* and B7-H7 levels correlated with cytotoxic potency. In addition, NPX372 induced cytokine production in primary T cell cultures with B7-H7+ tumor cells. Finally, NPX372 demonstrated dose-dependent anti-tumor activity in a B7-H7+ tumor model in vivo at clinically relevant doses (1).
METHODS: Affinity to human and monkey B7-H7 and CD3 was evaluated by cell binding using flow cytometry and an Octet binding assay, respectively. Immunohistochemistry was used to evaluate human and monkey B7-H7 tissue expression. To evaluate the tolerability of NPX372, we performed a dose escalation toxicokinetic study in cynomolgus monkeys. Animals were evaluated for 4 weeks after a single or step-up intravenous dose(s) of NPX372. Clinical observations, hematology, chemistry, cytokine, immunophenotyping, and PK samples were collected. At completion, pathology evaluation of selected tissues was performed.
RESULTS: Expression of B7-H7 is predominant in the luminal epithelium of the gastrointestinal tract (GI) in both monkey and human. NPX372 affinity to NHP and human B7-H7 is similar as measured by on-cell binding. Octet binding confirmed cross-reactivity to NHP CD3. NPX372 was well tolerated up to 100 µg/kg after single IV dose and up to 300ug/kg in a step-up dosing. On-target GI toxicity was observed at the highest dose of 1000 µg/kg, lasting ~5 days. Pathological assessment of small and large intestinal epithelium was normal at time of scheduled evaluation 28 days after dosing, supporting reversibility of the adverse effects. Clinical chemistry, coagulation, and hematology lab parameters were normal, with minor increases in C-reactive protein in one animal each treated at 300 and 1000 µg/kg. No evidence of cytokine release syndrome was observed, although small increases in G-CSF, IL-1RA, IL-8, MCP-1 and MIP-1β cytokines were noted. One animal dosed at 1000 µg/kg showed a transient increase in IFNγ. Immunophenotyping data showed dose-proportional activation of T cells (by upregulation of CD25). PK exposure was also dose-proportional.
CONCLUSION: These data demonstrate on-target activity of NXP372 in monkeys, with doses tolerated up to 1000 µg/Kg, which covers a dose range that is effective in preclinical models. The totality of the data suggests that NXP372 may be a safe and effective drug to further develop toward human clinical studies. References 1) Rausch et al. Abstract SITC 2024

#4355 Investigating the role of CXCR6 signaling in sorafenib resistance in HCC.

Morgan Reeves, Satyamaheshwar Peddibhotla, Siobhan Malany

Department of Pharmacodynamics, University of Florida, Gainesville, FL

Introduction: Hepatocellular carcinoma (HCC), one of the most common liver cancers and a leading cause of cancer-related deaths globally, is often diagnosed at late stages limited to systemic treatment. Sorafenib, an FDA-approved first in line treatment, efficacy is hindered due to innate or developed resistance in patients. Hyperactivation of CXCR6/CXCL16 signaling has been detected and shown to contribute to a proinflammatory and pro-survival tumor microenvironment driving drug resistance in HCC. Wnt/β-catenin signaling pathway is dysregulated in 50% of HCC and has shown to be involved in sorafenib resistance due to accumulation of its core component, β-catenin. Our hypothesis posits that CXCR6 antagonism will restore potency of sorafenib in HCC cell lines via inhibition of β-catenin-mediated signaling.
Experimental Procedure: Basal sorafenib sensitivity assessed via MTT assay, and β-catenin and CXCR6 protein expression via western blot were compared across five HCC cell lines. An orally bioavailable small molecule antagonist, SBI-457, was used to probe CXCR6 signaling. We evaluated sorafenib-, SBI-457- and combination-induced modulation of β-catenin expression via western blot and IF imaging in SKHep1. CXCL16- and SBI-457-induced modulation of pCREB protein expression post forskolin activation in both SKHep1 and CXCR6-overexpressed CHOK1 cells were compared. Secretion of soluble CXCL16 was measured via ELISA in SKHep1 cells.
Results: Sorafenib dose-dependently increased β-catenin protein expression via immunofluorescence imaging and whole cell lysates of SKHep1 cells. SBI-457 in combination showed trends towards attenuating sorafenib-induced increases, yet lacked significance. We demonstrate that high CXCR6 protein expression levels match sorafenib growth inhibitory (GI50) values across all five HCC cells tested. 200ng/ml CXCL16 significantly attenuated forskolin-induced increase in pCREB expression and SBI-457 dose-dependently restored in a CXCR6 overexpressed CHOK1 cell line. However, both CXCL16 and SBI-457 were unable to clearly modulate forskolin-induced pCREB in CXCR6 endogenously expressed HCC cell lines. SKHep1 cells significantly cleave increasing levels of soluble CXCL16 as measured up to 24h, of which was blocked by ADAM 10 and 17 inhibition.
Conclusion: Sorafenib treatment increased total β-catenin levels in SKHep1 cells. Though we show clear CXCR6-mediated signaling in an overexpressed cell line, it is inconsistent in HCC cells, despite CXCR6 expression. SKHep1 cells secrete significantly increasing CXCL16 and this was attenuated by combined inhibition of ADAM10and17. This data suggests SKHep1 cleave soluble CXCL16 from its transmembrane form and potentially alter traditional forward signaling. Future studies aim to evaluate tmCXCL16 expression levels across HCC cells and evaluate the role this plays in CXCR6-mediated signaling via our small molecule inhibitor to further understand the therapeutic potential of CXCR6 inhibition in HCC.

#4356 OBI-902, a novel TROP2 targeted antibody-drug conjugate via GlycOBI® platform, has favorable pharmacokinetics and sustained antitumor activities in challenging solid tumors.

Ren-Yu Hsu, Chi-Huan Lu, Chi-Sheng Shia, Hsin-Shan Wu, Lu-Tzu Chen, Jhih-Jie Yang, Jyy-Shiuan Tu, Hui-Wen Chang, Shih-Ni Wen, Ming-Tain Lai, Ya-Chi Chen

OBI Pharma, Inc., Taipei, Taiwan

Trophoblast cell surface antigen 2 (TROP2) is overexpressed in a variety of solid tumors, including non-small cell lung cancer (NSCLC), pancreatic cancer (PC), gastric cancer (GC), and colorectal cancer (CRC), where high expression levels of TROP2 correlate with poor prognosis. OBI-902 is a novel site-specific antibody-drug conjugate (ADC) consisting of a humanized anti-TROP2 monoclonal antibody linked to a topoisomerase I (TOP1) inhibitor payload with drug-to-antibody ratio of 4 via OBI's proprietary glycan conjugation platform (GlycOBI®) and dual-action enzymatic technology (EndoSymeOBI®). This unique linker-payload design is expected to enhance the ADC stability and improve the efficiency of payload delivery in tumors. This study aimed to evaluate the therapeutic potential of OBI-902 in challenging solid tumors. *In vitro* linker-payload stability was evaluated in human serum albumin via LC-MS. Pharmacokinetics (PK) was assessed in the serum and tumor of NSCLC xenograft mouse model. The antitumor activity studies of OBI-902 were conducted in NSCLC, PC, GC, and challenging CRC xenograft mouse models with Dato-DXd, SG, or MK-2870 as benchmarks. The antitumor activities were evaluated in both tumor volumes of 200-250 mm^3 and 500-550 mm^3 (large tumor burden). Survival was analyzed with the log-rank (Mantel-Cox) test with *$p<0.05$, **$p<0.01$. Humane endpoint: Tumor volume was larger than 1500 mm^3. OBI-902 showed superior in vitro linker-payload stability than the Dato-DXd. In addition, OBI-902 exhibited favorable PK profiles with excellent ADC stability in circulation and efficient payload release in NSCLC xenograft model. The tumor-to-serum ratios of payload C_{max} and AUC_{0-t} were 33 and 86, respectively. OBI-902 achieved 4.2-fold higher intra-tumor payload exposure than that of MK-2870 at the same dosage. OBI-902 exhibited potent and durable antitumor activities. In the GC xenograft model, tumor volume reductions of OBI-902 were 3.6- to 6.2-fold better compared to benchmarks, by day 57. In the challenging CRC model, OBI-902 showed a sustained suppression of tumor growth by day 56 (329 ± 110 mm^3) compared to benchmark ADCs which reached over 1000 mm^3 before day 18. In the large-tumor NSCLC xenograft model, OBI-902 achieved superior antitumor activity, with 40% of treated mice surviving beyond day 70. In contrast, all mice treated with benchmarks were sacrificed early (tumor volume >1500 mm) at days between 29 and 42. Similarly, in the large-tumor PC xenograft model, OBI-902 prolonged survival beyond day 50, while all mice in the benchmark groups were sacrificed by day 28. OBI-902 can efficiently deliver payload to the site of action (tumor) to achieve desirable drug exposure and sustainable antitumor activities in various challenging solid tumors, pointing to its potential to be the best-in-class TROP2 ADC.

#4357 Disparities in neurotoxicities in cisplatin-treated cancer patients: a population pharmacogenomics approach.

Swetha Nakshatri[1], Paul C. Dinh[2], Darren R. Feldman[3], Robert J. Hamilton[4], David J. Vaughn[5], Chunkit Fung[6], Christian Kollmannsberger[7], Robert Huddart[8], Lawrence H. Einhorn[2], Nancy J. Cox[9], Lois B. Travis[2], M. Eileen Dolan[10]

[1]University of Chicago, Chicago, IL,[2]Indiana University School of Medicine, Indianapolis, IN,[3]Memorial Sloan Kettering Cancer Center, New York, NY,[4]Princess Margaret Cancer Centre, Toronto, ON, Canada,[5]Department of Medicine, University of Pennsylvania, Philadelphia, PA,[6]University of Rochester Medical Center, Rochester, NY,[7]BC Cancer - Vancouver Cancer Centre, Vancouver, BC, Canada,[8]Institute of Cancer Research and Royal Marsden NHS Foundation Trust, London & Sutton, United Kingdom,[9]Vanderbilt University Medical Center, Nashville, TN,[10]Department of Medicine, University of Chicago, Chicago, IL

Background: Disparities in peripheral sensory neuropathy (PSN) have been studied in taxane-treated breast cancer and vincristine-treated leukemia survivors, but not addressed in cisplatin. Both platinum compounds and environmental heavy metals are associated with vascular toxicity, and there is genetic variation in single nucleotide polymorphisms (SNPs) associated with metal burden, which may be due to adaptation to exposures across geographies. We hypothesize that disparities may exist in cisplatin-induced neurotoxicities related to differences in population allele frequency and variants' impact on gene expression.
Methods: In a study of 1663 genotyped testicular cancer survivors, logistic regression between multidimensional scaling-calculated geographic ancestry and neurotoxicities (400-450 mg/m^2 cisplatin) and clinical/lifestyle factors was assessed. SNPs with Fst > 0.25 in 1000 Genomes were filtered using GTEx and LDmatrix to find independent expression/splicing trait loci (eQTL/sQTL) in nerve/brain tissue or cisplatin-associated genes. Logistic regression was conducted between genotype and toxicity with age and 10 genetic principal components as covariates. Spearman correlation between gene expression (DepMap) and cisplatin sensitivity (GDSC) in cell lines was calculated for genes of interest from association analysis.
Results: Survivors of African (AFR) ancestry had significantly higher PSN incidence versus European (EUR) ($p=0.049$) and Asian (ASN)-axis ($p=0.034$) ancestry and higher vertigo incidence versus EUR ($p=5.2\times10^{-3}$) and ASN-axis ancestry ($p=0.04$), with significant differences in self-reported health. Clinical factors did not show disparities, although ASN-axis survivors were significantly less likely to be on antihypertensives. 19,992 SNPs passed filtering. While genotype-toxicity associations did not hit Bonferroni threshold, six SNPs had suggestively significant $p<1.0\times10^{-4}$. One SNP had increased frequency of the risk allele in the AFR population for PSN and four SNPs for vertigo. rs34904346 ($p=2\times10^{-5}$) was a RNF24 eQTL in the nerve, with 3 other RNF24 eQTLs also associated with PSN ($p<0.05$). For vertigo, rs3777909 ($p=3.1\times10^{-5}$) was an eQTL for MFSD4B in the nerve and REV3L in the brain, with other independent eQTLs associated with vertigo ($p<0.05$) and MFSD4B (3) and REV3L (4). For vertigo, rs56819906 ($p=7.6\times10^{-5}$) and rs73626678 ($p=1.8\times10^{-4}$) were DDX25 eQTLs in the nerve. MFSD4B ($p=0.0058$) and REV3L ($p=5.1\times10^{-5}$) expression in cancer cell lines were significantly correlated with cisplatin sensitivity, matching direction of effect in toxicity association analyses.
Conclusions: We show evidence for the contribution of differential risk allele frequencies to disparities in cisplatin-induced PSN and vertigo. If results are confirmed, genotyping risk alleles to identify at-risk patients may benefit cisplatin-treated populations.

#4358 Single nucleotide polymorphisms located in PK/PD pathway genes of standard AML chemotherapy regimen predicts outcome in pediatric AML.

Aneesha Nath[1], Richard Marrero[1], Xueyuan Cao[2], Jeffrey E. Rubnitz[3], Stanley Pounds[4], Jatinder K. Lamba[1]

[1]Pharmacotherapy and Translational Research, University of Florida, Gainesville, FL,[2]Health Science Center, University of Tennessee, Memphis, TN,[3]Department of Oncology, St. Jude Children's Research Hospital, Memphis, TN,[4]Department of Biostastics, St. Jude Children's Research Hospital, Memphis, TN

Acute Myeloid Leukemia (AML) is an aggressive hematologic cancer with poor treatment outcomes in pediatric patients, particularly due to high relapse rates and drug resistance. Standard treatment involves cytarabine (Ara-C), daunorubicin, and etoposide (ADE) but resistance to these drugs remains a significant challenge. To better understand the genetic factors influencing drug resistance and treatment outcomes, we conducted a pharmacogenomic analysis of genetic variants in AML-related and drug-metabolizing genes within the context of (ADE) chemotherapy regimen. We extracted SNPs in genes involved in pharmacological pathways of ADE as well as genes representative of myeloid panel from an existing set of genome-wide Illumina 2.5 Omni microarray data obtained from genomic DNA of 400 patients treated on multi-site AML02 [NCT00136084, n=167] and AML08 [NCT00703820, n=233] clinical trials. SNPs within +/-10 kb region of the gene locus were evaluated for associations with multiple clinical endpoints such as minimal residual disease after induction 1 (MRD1), event-free survival (EFS), and overall survival (OS), adjusting for genetic ancestry. Our analysis results showed that at a p value of <0.01,215 SNPs in 52 genes were predictive of at least one of the outcomes. SNPs in solute carrier (SLC) transporters, including SLC22A1, SLC28A1, and SLC28A3, were linked to increased MRD1 positivity, indicating poor prognosis. SLC28A1 and A3 have been implicated in uptake of nucleoside analogs such as Ara-C. In contrast, SNPs in SLCO1B1 were predictive of better EFS and OS, suggesting that variations in drug uptake influences treatment outcomes. Five SNPs within the cytidine deaminase (CDA), involved in inactivation of Ara-C were associated with poorer EFS and OS. These SNPs were linked to higher CDA mRNA expression in blood, as per the GTEx database, and showed regulatory potential in chromatin states. SNP rs1048977 has been associated with reduced CDA enzyme activity, further corroborating its role in poor treatment outcomes. Among the myeloid panel genes SNPs in the AML-related gene RUNX1, CSF3R and CDKN2A were associated with poor clinical outcomes. SNPs in CSF3R and CDKN2A genes were predictive of poor EFS and OS. RUNX1 is a critical factor for myeloid differentiation, CSF3R is involved in neutrophil biology and activates the JAK/STAT pathway, while CDKN2A functions as a tumor suppressor and regulates the cell cycle. Dual mutations in CSF3R and CEBPA have been linked to high relapse rates in pediatric AML. In conclusion, our comprehensive pharmacogenomic analysis of genes involved in drug metabolism and AML pathogenesis has identified key genetic variants that influence treatment outcomes. These findings, once validated in larger cohorts, could offer new opportunities for personalizing chemotherapy and improving prognosis prediction in pediatric AML patients.

#4359 Comprehensive pharmacokinetics of Re-phenformin as an anticancer agent for pancreatic cancer therapy in mice: a comparative study with phenformin.

Amir Mohammad Gholizadeh[1], Fatima Dagher[1], Chiyi Xiong[2], Chun Li[2], Diana S-L Chow[1]

[1]Department of Pharmacological and Pharmaceutical Sciences, College of Pharmacy, University of Houston, Houston, TX,[2]Cancer Systems Imaging, The University of Texas M.D Anderson Cancer Center, Houston, TX

Re-phenformin (Re-$(CO)_3$-phenformin, Re-phen (MW=476.07)), a derivative of phenformin (MW= 206.24), was previously synthesized, purified, and characterized to explore its anticancer potential in comparison to phenformin, a biguanide medication. It has demonstrated enhanced *in vitro* anticancer efficacy compared to phenformin with significantly lower IC_{50} values in MiaPaca-2 (0.75 mM) and Kras* cells (0.410 mM) versus phenformin (4.02 mM and 2.36 mM, respectively). The primary aim of this study is to compare the pharmacokinetic (PK) characteristics of Re-phenformin, with phenformin to evaluate their PK parameters. We conducted a PK study in healthy male FVB mice, administering equimolar intravenous doses of Re-phenformin (25 mg/kg, n = 5) and phenformin (12.69 mg/kg, n = 4) using a Saline: Ethanol: Cremophor EL (8:1:1) vehicle. Blood samples were collected at six time points (0.33-24 hours), and organs were harvested at 24 hours post-dose. Quantification was performed using a validated LC-MS/MS method with a Kinetex® 1.7 µm XB-C_{18} column (LLOQ: 0.25 ng/mL) following liquid-liquid extraction of 10 µL blood spiked with 10 µL internal standard (D_5-phenformin) and 200 µL acetonitrile (0.1% formic acid). The mixture underwent vortexing, sonication, centrifugation, and reconstitution in 100 µL mobile phase (ACN: water, 35:65, with 0.1% formic acid). The method yielded 93.91% recovery (CV = 2.10%, N=3) and minimal matrix effects (86.38%-90.96%). The PK profiles of Re-phen and phenformin were modeled using a two-compartment model in Phoenix® WinNonlin (version 8.0) for comparison. Statistical significance was evaluated using a two-tailed unpaired t-test at $p < 0.05$. Re-phen showed smaller systemic exposure (AUC = 15.9 hr.µg/mL, $p = 0.02$*) and lower peak concentration (C_{max} = 5.3 µg/mL, $p = 0.0062$**) than phenformin (AUC = 40.2 hr·µg/mL, C_{max} = 30.4 µg/mL). Despite its faster clearance rate (CL = 1.7 L/hr/kg vs. 0.3 L/hr/kg, $p = 0.0019$**), Re-phen exhibited a prolonged half-life (11.1 hours vs. 7.8 hours), primarily attributed to its significantly larger volume of distribution (Vss = 17.1 L/kg vs. 1.3 L/kg, $p < 0.001$ ****). These findings highlight distinct distribution and elimination differences between the two compounds. Ongoing co-modeling aims to elucidate the *in vivo* conversion of Re-phen to phenformin and their PK interplay. This first PK comparison in healthy mice extends previous research and supports further evaluation in an orthotopic PDAC model to explore therapeutic potential in pancreatic cancer.

#4360 Development of a novel mithramycin analogue with improved pharmacokinetics and therapeutic window for targeted inhibition of EWS-FLI1 in Ewing sarcoma.

Rajesh Yetirajam[1], Srijan Acharya[1], Kumar Kuldeep Niloy[1], Yasuda Kazuto[1], Jamie Horn[1], Thomas Prisinzano[2], Jon S. Thorson[2], Oleg Tsodikov[2], Jurgen Rohr[2], Markos Leggas[1]

[1]St. Jude Children's Research Hospital, Memphis, TN,[2]University of Kentucky, Lexington, KY

Background: Ewing Sarcoma (EWS) is characterized by aberrant activity of the oncogenic EWS-FLI1 transcription. Mithramycin (MTM) has been identified to be a specific inhibitor of transcription mediated by the EWS-FLI1. A phase I/II trial and PK study in Ewing sarcoma patients demonstrated that MTM had poor PK and dose-limiting hepatic and hematologic toxicities at subtherapeutic concentrations. Here, we are presenting a novel MTM analogue with significantly improved PK and a wider therapeutic window for targeted inhibition of.
Results and Discussion: MTMSA-Trp was prepared by chemical conversion of MTM to the MTMSA analogue and the subsequent conjugation of a tryptophan amino acid. This substitution appears to shift the ionization properties of the two hydroxy groups on the tricyclic core and to increase protein binding, which significantly improves PK. The cytotoxicity of MTMSA-Trp is in the order of low nM across several cell lines expressing EWS-ETS fusions, and the compound disrupts the expression of positively regulated proteins (NR0B1, ID2) and induces expression of negatively regulated ones (PHLDA1, LOX). MTMSA-Trp mediates apoptosis during which upstream caspases proteolytically processes for Caspase-7 activation. This effector caspase drives the cleavage of downstream substrates PARP and high secondary DNA damage (gH2A.X). Luciferase reporter assay demonstrates a dose-dependent effect of MTMSA-Trp on the attenuation of EWS-FLI1 transcription and DNA binding, and based on CETSA evidence, there is physical interaction between MTMSA-Trp and EWS-FLI1. MTMSA-Trp was found to modify the prototypical activity of EWS-FLI1 by disrupting R-loops. Evaluation of MTMSA-Trp effect on EWS-FLI1 mediated transcriptional activity by RNA Seq highlighted 287 genes among overlapping DEGs. ATAC seq analysis revealed that TEAD, ETS, CEBP, and NFKB TF families have divergent TF binding profiles between Ewing and non-Ewing cells treated with MTMSA-Trp. EWS-FLI1 modulation persists longer when cells are exposed to GI90 concentration for a short time compared to the GI50 for longer times, suggesting that daily dosing or continuous exposure is not required. MTMSA-Trp has improved PK in athymic nu/nu mice with a greater than 10-fold reduction in clearance and significantly reduced partition in the liver compared to MTM. Initial efficacy studies in TC32 EWS cells demonstrated tumor regression and improved survival at the maximum tolerated dose (MTD) and at of the MTD on a daily x 5 intravenous bolus injection. Significantly, a more protracted dosing schedule, which allowed higher doses every 3 days for 6 doses, led to an impressive reduction of tumors whose initial size was 1200-1600 mm3.
Conclusions: MTMSA-Trp demonstrate that improving the pharmacologic properties of mithramycin may lead to the development of an EWS-FLI1 inhibitor.

#4361 Development and application of a mechanistic pharmacokinetic pharmacodynamic (PKPD) model to predict anti-chronic myeloid leukemia (CML) effects of tyrosine kinase inhibitors.

Qi Wang[1], Andrew Santulli[2], Haili Ping[1], Scott van Wart[2], Damiette M. Smit[1], Donald Mager[2], Helen Collins[1], Samuel Kintz[1], Joseph P. Lyssikatos[1]

[1]Enliven Therapeutics Inc., Boulder, CO,[2]Enhanced Pharmacodynamics, Buffalo, NY

Introduction: Chronic myeloid leukemia (CML) is driven by BCR::ABL1 fusion resulting from t(9;22)(q34;q11) chromosomal translocation. Although tyrosine kinase inhibitors (TKIs) for BCR::ABL1 like imatinib, dasatinib, nilotinib, bosutinib, asciminib and ponatinib are available for CML treatment, novel treatments are needed for CML that is resistant or patients who are intolerant to available therapies. The objective of this study was to develop a mechanistic PKPD model to assess anti-CML activity of TKIs in patients with newly-diagnosed CML.
Material and Methods: The PKPD model consisted of 3 parts; Population PK (popPK), leukemic stem cell (LSC) PD, and TKI effect on PD. PopPK models and PK data for TKIs were obtained from the literature. The PD model was adapted from Fassoni et al. In vitro TKI potency was measured using a CRKL assay in the presence of 100% human serum and a STAT5 assay in the presence of 10% fetal bovine serum. Effects of TKIs on LSC growth were modeled using a hyperbolic model ($I = Imax*Cp^H/(IC_{50}^H+Cp^H)$). Key model parameters, Imax and H, were determined and fixed, and IC_{50} was fixed for each TKI. A virtual patient population using all key covariates and common demographic data was created to mimic patients with CML. The model was applied to simulate the BCR::ABL1 transcript values for patients with CML following TKI treatments over time, followed by calculating corresponding molecular response rates (MR2, MR3 [MMR], MR4, and MR4.5).
Results: PopPK models for TKIs were reproduced and validated using literature data. The relationship between plasma concentrations of each TKI and BCR::ABL1 transcript levels was described by the mechanistic population PKPD model. PD model parameters, their inter-individual variability, and inhibition parameters I_{max} and H were constant for all TKIs, with only IC_{50} values varying. The model-simulated mean BCR::ABL1 levels following imatinib 400 and 800 mg QD, nilotinib 400 mg BID and dasatinib 100 mg QD agreed with observed data over a treatment period of 60 months. Further, the model-estimated MMR agreed well with the observed data from Phase 3 studies of imatinib, nilotinib, dasatinib and bosutinib over a 60-month period. For example, the MMR rates by 24- and 48-month of dasatinib and imatinib were predicted to be 66% and 45 %, and 74.5% and 53% vs. observed 64% and 46%, and 73% and 56%, respectively. Interestingly, the model a priori predicted the asciminib12-month MMR rate of 72% in patients with newly-diagnosed CML agreed with the Phase 3 reported rate of 67.7% at week 48.
Conclusion: This generalizable PKPD model, using available PK and in vitro potency data, was developed and accurately predicted outcomes of five approved BCR::ABL1 TKIs in patients with newly-diagnosed CML, suggesting the mechanistic PKPD model may be used to predict efficacy for novel TKIs.

#4362 Body mass index influences imatinib exposure in CML patients: Evidence fromTDM with adaptive dosing in real-world patients.

P. Maroselli[1], R. Fanciullino[2], J. Colle[2], L. Farnault[2], P. Roche[2], G. Venton[2], R. Costello[2], **J. Ciccolini**[3];
[1]Aix-Marseille University, Marseille Cedex 05, France, [2]Assistance Publique Hopitaux de Marseille, Marseille Cedex 05, France, [3]Inserm and Aix Marseille Univ, Marseille Cedex 05, France

Imatinib remains a mainstay of treatment for elderly or frail patients with chronic myeloid leukaemia (CML). Trough levels of around 1000 ng/mL are associated with a major molecular response with acceptable tolerability. We present data from 60 adult CML patients treated with imatinib on therapeutic drug monitoring (TDM) and adaptive dosing. Mean trough levels after treatment initiation were 994.2 ±560.6 ng/mL and a large inter-patient variability was observed (CV: 56%). Only 29% of patients were in the therapeutic range. All parameters associated with corpulence (i.e. body weight, height, body surface area, body mass index (BMI)) and age were significantly associated with imatinib plasma levels on univariate analysis. Age and BMI remained the only parameters associated with trough levels on multivariate analysis. As severe toxicities have been previously reported in patients with low BMI treated with standard imatinib, we evaluated the extent to which low BMI may lead to plasma overexposure. We found a statistically significant difference in trough imatinib levels in patients with BMI<18.5, with exposure +61.5% higher than in patients with 18.5<BMI<24.9 and +76.3% higher than in patients with BMI>24.9 ($p<0.05$, ANOVA). After TDM with adaptive dosing, a statistically significant difference in dosing between patients was observed, with doses ranging from 200 to 700 mg ($p<0.001$, ANOVA). No difference in toxicity or efficacy was observed regardless of BMI after adaptive dosing ($p>0.05$, Chi-2 test). Our data suggest that low BMI has a significant impact on imatinib exposure, but that pharmacokinetically-guided dosing limits its clinical impact in patients.

#4363 Establishment of plasma inhibitory kinase assays (PIA) to detect functional drug levels in patient plasma.

Lena Pilgermayer, Eva M. Egenter, Katharina Schaich, Daniel Feger, Jan E. Ehlert

Cellular Pharmacology, Reaction Biology Europe GmbH, Freiburg, Germany

Anticipation of the in vivo activity of an investigational new drug (IND) is challenging. In the context of the living body, compounds are subjected to degradation, metabolic modification or binding to plasma proteins - mechanisms that are well described to potentially compromise the in vivo activity seen in the patient compared to its predicted potential to inhibit its target in specific in vitro assays. As such assays are performed in artificial cell-culture media, we had previously modified our proprietary cellular FLT3 kinase phosphorylation assay to measure the activity of FLT3 inhibitors directly in and from human plasma, similar as described by Levis et al. 1. We hence established a method to analyze the activity of kinase inhibitors in human or rodent plasma (plasma inhibitory activity assay, PIA) in ELISA-based cellular kinase assays. We have successfully applied this methodology to determine the pharmaco-kinetics of a newly developed inhibitor of FLT3 in the context of a clinical trial targeting FLT3. We have now expanded this methodology for the detection of further kinase targets of potential clinical relevance. We were able to compile in vitro PIA assay data for further kinases such as VEGFR2 and BCR-Abl which will allow us to better predict the in vivo effect of specific inhibitors of these kinases and to determine the amount of freely available active test substance in the blood/sera of treated cancer patients. 1: Levis et al. Blood. 2006; 108 (10):3477-3483

#4364 Preclinical pharmacokinetics and pharmacodynamics of MK-4464, a novel NK cell engager targeting CEACAM5.

T. Nayak[1], Y. Zheng[2], L. Li[2], S. Li[2], S. Mauze[2], M. Cai[3], R. Mangadu[2], K. Riffel[1], X. Meng[1], S. Hseih[2], K. Dobrenkov[4], A. Beebe[2]; [1]Merck & Co., Inc., West Point, PA, [2]Merck & Co., Inc., South San Francisco, CA, [3]Merck & Co., Inc., Boston, MA, [4]Merck & Co., Inc., Rahway, NJ

MK-4464 is a bispecific, trifunctional antibody designed to direct NK cells to a tumor with a CEACAM5-specific scFv arm, and to activate the NK cells to kill tumors by simultaneously binding to NK cell costimulatory receptors NKG2D and CD16A. To support clinical translation, preclinical pharmacokinetics and pharmacodynamics studies were performed. In-vitro characterization was performed using cell-based assays, in-vivo efficacy was performed in B16F10-hCEACAM5 tumor-bearing hCEACAM5 transgenic mice. PET imaging studies were performed with ^{89}Zr-labeled MK-4464 in CEACAM5+ve and CEACAM-ve tumor bearing hIL-15 transgenic NSG mice engrafted with CD34+ human hematopoietic stem cells. In vitro cytolytic activity assay demonstrated the ability of MK-4464 to kill CEACAM5-expressing tumor cell lines. The EC50 values for cytolytic activity in tumor cell lines MKN-45, SK-CO-1, and BxPC3 were 0.61 nM, 0.61 nM, and 4.45 nM, respectively. The cytolytic activity was higher in cell lines with higher CEACAM5 expression. MK-4464-mediated NK-cell activation was demonstrated by increased IFNγ production and CD107a expression. The activation was shown to be dependent on the binding to CEACAM5, NKG2D, and CD16A. The cytolytic activity of MK-4464 increased with a higher effector/target cell ratio. A mouse surrogate of MK-4464 induced significant tumor growth inhibition in B16F10-hCEACAM5 tumor-bearing hCEACAM5 transgenic mice, number of remaining animals was significantly greater in mice treated with murine MK-4464 at 15 mg/kg ($p<0.0001$), at 5 mg/kg ($p=0.0004$), and at 1.5 mg/kg ($p=0.0274$) compared with the isotype control group. PET imaging studies demonstrated high target-mediated localization in the tumor with relatively low uptake in normal tissues. [^{89}Zr]MK-4464 demonstrated 2.5-fold greater tumor uptake than ^{89}Zr-labeled control antibody in CEACAM5+ve tumors, while no significant difference was observed in CEACAM5-ve tumors. Dose-dependent tumor saturation was observed in CEACAM5+ve tumors with near complete saturation at 10 mg/kg. Accumulation in spleen and lymphoid tissues were significantly lower than CEACAM5+ve tumors. After IV bolus administration of MK-4464 on Study Days 1 and 15, mean AUC and Cmax values were approximately dose-proportional from 1 to 10 mg/kg/week and slightly greater than dose-proportional from 10 to 100 mg/kg/week. The MABEL approach was used to determine the first-in-human starting dose. These findings collectively demonstrate the potent and specific activity of MK-4464 in targeting and killing CEACAM5-expressing tumor cells through NK cell activation and cytokine release. These preclinical studies facilitated the clinical translation of MK-4464 and [^{89}Zr]MK-4464 (ClinicalTrials.gov ID NCT05514444).

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: RAS Inhibitors
Poster Session

#4367 ERAS-4001 is a pan-KRAS inhibitor with robust anti-tumor activity in KRAS altered solid tumors.

Jae Hyun Bae, Bingzhen Lin, Erin D. Lew, Alexei Brooun, Joanne Oh, Taylor Sullivan, Corine Holub, Alex Stromitis, Brian Qua, Robert F. Shoemaker

ERASCA, Inc., San Diego, CA

KRAS is one of the most frequently mutated proteins in cancer (approximately 16% of all cancers), including non-small cell lung (~30%), pancreatic (~90%), colorectal (~40%), and other cancers. Targeting both wild type (WT) KRAS and oncogenic KRAS mutant proteins within the tumor cell is a potentially more promising therapeutic strategy than targeting the mutant KRAS protein alone since WT KRAS activity can act as a resistance mechanism to mutant selective KRAS inhibition. Pan-KRAS inhibitors enable simultaneous mutant/WT KRAS inhibition and have the potential to show improved tolerability relative to pan-RAS inhibitors by sparing NRAS and HRAS. ERAS-4001 is a novel pan-KRAS inhibitor that binds to WT KRAS and KRAS G12X mutants and prevents KRAS from signaling downstream via effector proteins, such as the RAF protein family, with single digit nanomolar potency. ERAS-4001 was selective for KRAS G12X mutants and WT KRAS over WT HRAS and WT NRAS in surface plasmon resonance (SPR) studies, possibly providing an expanded therapeutic index. In the biochemical KRAS G12D-RAF1 RBD (RAS-binding domain protein) assay, ERAS-4001 inhibited complex formation when KRAS G12D was either in the active state (i.e., GMPPNP-bound) or inactive state (i.e., GDP-bound) with single digit nanomolar IC_{50}s. ERAS-4001 showed potent cellular activity in a panel of KRAS WT amplified and KRAS G12X mutant NSCLC, PDAC, and CRC cell lines in both ERK1/2 phosphorylation (pERK) inhibition and 3-dimensional Cell-Titer Glo (3D-CTG) assays with IC_{50}s in the range of 0.7-56 nM. In vivo, oral administration of ERAS-4001 at 100 mg/kg BID resulted in significant tumor growth inhibition in KRAS G12D and G12V mutant subcutaneous xenograft models from 77% TGI to 83% tumor regression. ERAS-4001 achieved a dose dependent increase in plasma concentrations and significant concomitant reduction in pERK. We hypothesize that the potency and selectivity profile of the pan-KRAS inhibitor ERAS-4001 will provide efficacy and tolerability for patients with tumors driven by oncogenic KRAS alterations. An IND submission is intended for the first quarter of 2025.

#4368 Selective targeting of oncogenic KRAS G12D using complementary peptide nucleic acid oligomers.

Jayati Mondal[1], Dennis Lam[1], Mary E. Gerritsen[2], Tilmann M. Brotz[3], Jodi G. Kennedy[4], Bruce Rehlaender[3], Arthur J. Ross[5], Daniel E. Levy[6], Christopher A. Bonagura[7], William Lanzilotta[8], Frank McCormick[9], Jeffrey H. Rothman[10], Andrew L. Wolfe[1]

[1]Biological Sciences, Hunter College, The City University of New York, New York, NY,[2]Gerritsen Consulting, San Mateo, CA,[3]PharmaDirections, Boston, MA,[4]Oncogenuity, Inc, New York, NY,[5]Fortress Biotech, Waltham, MA,[6]DEL BioPharma, LLC, San Mateo, CA,[7]Art Robbins Instruments, Sunnyvale, CA,[8]University of Georgia, Athens, GA,[9]Helen Diller Family Comprehensive Cancer Center, University of California San Francisco, San Francisco, CA,[10]Department of Medicine, Ichan School of Medicine at Mount Sinai, New York, NY

KRAS is a proto-oncogene that has activating mutations in ~15-30% of all cancers. Conventional therapies non-selectively target tumor and normal cells which may cause high toxicities, and programmable mutant-selective targeted inhibitors are lacking. Peptide nucleic acids (PNAs) have base sequences analogous to DNA, except they have modified peptide backbones instead of sugar-phosphate backbones, allowing them to hybridize with DNA with high avidity to suppress transcription. Here, we developed KRAS G12D-selective PNA oligomers with novel cell-penetrating peptides (CPP) flanking regions. Immunofluorescently labeled PNA-CPP oligomers had high uptake rates in cells and nuclei. Complementary PNA-CPP treatment resulted in repression of KRAS G12D RNA and protein expression within 2 hours, lasting up to 48 hours. Alternative end modifications and lengths of complementary KRAS sequence were tested using dose-response cell viability assays. These experiments identified modifications to PNA oligomer sequence composition and length that were most effective at selectively preventing growth of on-target KRAS G12D cells, while relatively sparing off-target KRAS G12C cells. These PNA-CPPs were effective against a panel of pancreatic ductal adenocarcinoma cell lines and patient derived xenografts *in vivo*. These results indicate that PNA-CPPs show promise as both a tool for studying tumors driven by oncogenic point mutations, and as a possible therapeutic strategy capable of sparing normal cells while selectively targeting mutant cancer cells.

#4369 Preclinical characterization of a dual-state pan-KRAS Inhibitor.

Kevin Gaida, Tao Osgood, Ying-Chu Chen, Deanna Mohn, Anne Y. Saiki, Gilbert Diaz, Monica Leavitt, Upendra Dahal, Amit Vaish, Jason DeVoss, Karen Rex, Ryan Wurz, Brian A. Lanman, Paul E. Hughes, **Rati Verma**

Amgen Oncology, Thousand Oaks, CA

Activating mutations in *KRAS* occur in greater than twenty percent of all cancers encompassing several solid tumors with significant unmet medical need, including lung, colon, pancreatic, endometrial and stomach cancers. While mutations of codon 12 of *KRAS* (encoding glycine) are the most common, activating missense mutations are also observed at codons 13 and 61. Guided by our experience in the successful development of the covalent *KRAS* G12C mutant-selective inhibitor, sotorasib, we leveraged structure-based design to identify a new class of reversible, pan-KRAS inhibitors capable of targeting an expanded set of hot spot mutations prevalent in the above tumor types (e.g., *KRAS* G12D, G12V, G13D and Q61H). These reversible inhibitors potently inhibit both the GDP(OFF)- and GTP(ON)-states of KRAS while sparing H/NRAS. Dual ON/OFF-state activity facilitates the inhibition of mutant alleles that rapidly cycle between ON and OFF states (e.g., *KRAS* G12C) as well as those demonstrating slower cycling (e.g., *KRAS* G12V & *KRAS* Q61H), which predominantly reside in the GTP-bound state. Mechanistically, these inhibitors block the interaction of KRAS with its downstream effectors, such as c-RAF, and potently inhibit ERK phosphorylation. In vivo profiling demonstrated promising preclinical efficacy in *KRAS* G12D and *KRAS* G12V xenograft models, and good tolerability, illustrating the potential of pan-KRAS inhibitors to extend therapeutic benefit to tumors beyond those with *KRAS* G12C mutations.

#4370 Synthetic cell penetrating RAS-binding peptide for the treatment of RAS mutated cancer.

Gookjin Yoon[1], Beom Soo Jo[2], Dong Woo Lee[2], Moonsil Choi[2], Eui Kyun Jeong[2], Hoo Cheol Lee[2], Yu-Bin Kim[2], Sanghui Seok[2], Yoon Shin Park[3], Jue-Yeon Lee[2], Chong Pyung Chung[2], Yoon Jeong Park[1]

[1]Seoul National University, Seoul, Korea, Republic of,[2]Nano Intelligent Biomedical Engineering Corporation (NIBEC), Seoul, Korea, Republic of,[3]Chungbuk National University, Cheongju, Korea, Republic of

The RAS-RAF-ERK1/2 signaling pathway plays a critical role in cancer progression and is a primary target for anticancer drug development, given its frequent activation in various human cancers. MAPK, a key molecule within this pathway, is vital for eukaryotic cells to process external stimuli, leading to cancer cell proliferation. The MDA-MB-231 cell line, which represents triple-negative breast cancer, carries a G13D KRAS mutation, rendering it resistant to numerous treatments. This mutation drives abnormal MAPK pathway activation, fueling tumor progression. To address this, a synthetic cell-penetrating peptide called RAS-binding peptide (RBP) was designed to selectively bind RAS with high affinity in MDA-MB-231 cells. Experimental results revealed that RBP effectively inhibited RAS-RAF interaction, leading to reduced cell proliferation and suppression of ERK1/2 phosphorylation in MDA-MB-231 cells. Additionally, RBP significantly decreased cell migration and invasion, indicating potential anti-metastatic properties. In both chicken egg and mouse xenograft models, RBP treatment markedly inhibited tumor growth and metastasis. Histopathological analysis further demonstrated enhanced immune cell infiltration, while immunohistochemistry confirmed reduced expression of Ki-67, a marker for tumor cell proliferation. Collectively, these findings highlight RBP as a promising therapeutic candidate for targeting RAS-driven cancer by inhibiting tumor growth and metastasis.

#4371 PHI-501 as a potent pan-RAF/DDRs inhibitor suppresses lung cancer cell proliferation and overcomes KRAS G12C inhibitor resistance.

An-Na Moon[1], Ji Young Kim[2], Hee-sun Hwang[1], Gi-Jun Sung[1], Jinsun Kwon[1], **Ky-Youb Nam**[1], Kyu-Tae Kim[1], June Han[1], Jeong Hyeok Yoon[1], Ki-Young Lee[2]

[1]Pharos iBio Co., Ltd., Anyang, Korea, Republic of,[2]Sungkyunkwan University School of Medicine, Suwon, Korea, Republic of

Background: Mutations of the KRAS are one of the most commonly mutated oncogenes in cancer. Oncogenic mutations in KRAS typically occur at hotspots in the protein, increasing the steady-state levels of KRAS proteins in the GTP-bound state that are capable of driving oncogenic signaling through downstream effector pathways, such as the MAPK and PI3K pathways. The recent development of direct $KRAS^{G12C}$ inhibitors(G12Ci) has improved outcomes in KRAS mutant cancer. Mechanisms of acquired resistance to G12Ci are heterogeneous including both on-target and off-target resistance. On-target acquired resistance includes secondary codon 12 KRAS mutations, but also acquired codon 13 and codon 61 alterations, and mutations at drug binding sites. Off-target acquired resistance can derive from activating mutations in KRAS downstream pathways including $NRAS^{Q61K}$ and $BRAF^{V600E}$, acquired oncogenic fusions, gene level copy gain, or oncogenic alterations in other pro-proliferative and anti-apoptotic pathways. PHI-501 is a potent and dual kinase inhibitor for pan-RAF and discoidin domain receptor (DDR). In this study, we investigated the anti-tumor effect of PHI-501 as monotherapy and addressed the molecular mechanism underlying overcoming G12Ci resistance.
Methods: RAF family kinase profiling was performed. Cell viability was measured using the WST assay in human lung cancer cell lines and Ba/F3 cell lines expressing $BRAF^{V600E}$, DDR1 and $BRAF^{V600E}$/DDR1. Western blot was used to investigate the effects of down-stream signals. In vivo anti-tumor activities of PHI-501 were evaluated in the A549 mouse xenograft model.
Results: In Ba/F3 cells carrying $BRAF^{V600E}$/DDR1, PHI-501 displayed 70-fold higher cellular potency (IC_{50} 0.012 μM) over control cells. PHI-501 demonstrated potent anti-proliferative activity (IC_{50} < 0.2 μM) in 7 lung cancer cell lines harboring KRAS wild-type, KRAS or NRAS mutation. PHI-501 profoundly inhibited the phosphorylation of MEK, ERK, and AKT proteins as the downstream signaling of EGFR and DDR1 in KRAS mutant lung cancer cells. The growth inhibitory activity of PHI-501 in $KRAS^{G12V}$, $KRAS^{G12S}$, $KRAS^{Q61H}$ and $NRAS^{Q61K}$ lung cancer cells was superior to that of G12Ci (sotorasib and adagrasib) and BRAF inhibitors (encorafenib and avutometinib). PHI-501 considerably suppressed the reactivation of both pERK and pAKT by inhibition of pDDR1 expression in lung cancer cells. Consistently, treatment of A549 ($KRAS^{G12S}$) with PHI-501 in mice resulted in dramatic tumor regression superior to control groups.
Conclusion: The treatment of PHI-501 alone showed robust growth inhibition in lung cancer cells harboring KRAS wild-type or KRAS and NRAS mutation. Our results suggest that PHI-501 as the pan-RAF/DDRs dual inhibitor is beneficial for the treatment of lung cancer and even helps overcome the resistance of previous G12Ci treatments.

#4373 Discovery and characterization of BAY 3498264: a small molecule inhibitor targeting the RAS-SOS1 interaction.

Nils Guthof[1], Hongyu Xue[1], Isabel Jerchel-Furau[1], Christoph Hethey[2], Jan-Erik Busse[2], Karsten Denner[2], Matthias Voemel[3], Marc Kunze[4], Yunlong Shi[1], Zebin Wang[1], Volker Schulze[2], Sangeeta Jethwa[5], **Shinghu Cheung**[1]

[1]Bayer U.S. LLC, Cambridge, MA,[2]Bayer AG, Berlin, Germany,[3]Bayer AG, Wuppertal, Germany,[4]Pharmaceuticals, Bayer AG, Berlin, Germany,[5]Bayer, Basel, Switzerland

RAS proteins are GTPases cycling between the active GTP-bound on-state and inactive GDP-bound off-state. *KRAS* is among the most frequently mutated genes in cancers including non-small cell lung cancer, colorectal cancer and pancreatic cancer. Recent regulatory approvals of KRAS G12C selective inhibitors (KRAS G12Cis) including sotorasib and adagrasib have offered clinical advances to patients with cancer harboring the *KRAS G12C* mutation. However, monotherapy with these inhibitors showed modest response rate with median duration of response of less than one year for both. It has been shown preclinically that there is a feedback activation of the MAPK pathway when the pathway is being suppressed. This on-treatment feedback reactivation of the MAPK pathway is hypothesized to play an important role in limiting the clinical efficacy of KRAS G12Cis. Therefore, rational combination approaches are needed to address the feedback reactivation.

The MAPK pathway is intricately regulated by negative feedback mechanisms, including ERK-mediated inactivation of SOS1. As a guanine nucleotide exchange factor, SOS1 interacts with RAS to promote the exchange of GDP for GTP, thus facilitating RAS activation. Inhibiting SOS1, a crucial node within the MAPK pathway negative feedback loop, could block the feedback reactivation of MAPK pathway via RAS, akin to the effect seen with SHP2 inhibitors as reported, yet potentially with better tolerability. Indeed, our preclinical data have demonstrated that inhibiting SOS1 enhances the duration and depth of response of KRAS G12Cis, suggesting that SOS1 inhibitors could improve the therapeutic outcomes of KRAS G12Cis in patients.

Here, we report on the preclinical characterization of BAY 3498264, a potent and selective small molecule oral inhibitor of the SOS1 and RAS interaction. In *KRAS G12C* mutant cells, BAY 3498264 inhibits the rebound of phospho-ERK induced by sotorasib, achieving a more prolonged ERK phosphorylation inhibition when combined. Additionally, in *in vitro* proliferation assays, combining BAY 3498264 with various MAPK pathway inhibitors demonstrates marked combination effect as compared to single agent MAPK inhibition. The combination of BAY 3498264 with sotorasib in *KRAS G12C* mutant CDX and PDX models *in vivo* is well tolerated and exhibits combination benefit, enhancing both the depth and duration of response over sotorasib alone. Furthermore, we have assessed combination of BAY 3498264 with additional MAPK pathway directed therapies *in vivo* and have demonstrated combination effect with multiple molecules in addition to KRAS G12Cis. Our findings support the clinical exploration of BAY 3498264 in combination with KRAS G12Cis, and other therapies targeting the MAPK pathway, potentially offering a more durable blockage of the pathway and greater benefit for patients. BAY 3498264 is now in the early stages of a novel phase I study (NCT06659341).

#4374 Discovery of a highly potent and efficacious SOS1 inhibitor targeting KRAS-driven cancers.

Yang Li[1], Yang Zang[1], Wenbing Hu[1], Yuan Zhou[1], Lifei Liu[1], Xuejun Zhang[1], Shaohua Chang[2]

[1]Humanwell Healthcare (Group) Co.,Ltd, Wuhan, China,[2]KinoTeck Therapeutics Co., Ltd, Guangzhou, China

SOS1 is a crucial protein that activates RAS by promoting its conversion from the inactive GDP-bound state to the active GTP-bound state, triggered by growth factor receptor signals. This process is further enhanced by a feedback mechanism between SOS1 and RAS, increasing the strength and duration of RAS signaling, making SOS1 a significant therapeutic target in the RAS-MAPK pathway. Genetic alterations in the RAS-MAPK pathway are frequently observed across a spectrum of cancer types. While therapies targeting KRAS G12C mutations has been approved for certain types of lung cancers, there remains a need for treatments addressing other mutations (e.g. KRAS G12D, G12V etc.). Inhibiting SOS1 has shown potential in reducing proliferation of cancer cells dependent on KRAS signaling. Here, we report the discovery of a highly potent and efficacious SOS1 inhibitor HW071021. It demonstrates an IC50 of 8.5 nM in SOS1 binding assay and modulates ERK1/2 phosphorylation (pERK) in DLD-1 cell assay. Oral administration of HW071021 as a monotherapy in mouse MIA PaCa-2 xenograft models harboring KRAS G12C mutations results in significant tumor growth inhibition (TGI:~78%). Additionally, HW071021 combination with the EGFRi Osimertinib demonstrated much greater antitumor activity than Osimertinib monotherapy (TGI: 97% vs 33%) in mouse NCI-H1975 (EGFR L858R/T90M) xenograft model. HW071021 has excellent ADME and physical properties with low risks of hERG-related cardiotoxicity and drug-drug interactions . These data support the clinical utility of HW071021 as a single agent or combination partner of Osimertinib for the treatment of human Kras-driven cancers. IND application in China has been approved in September.

#4375 LY4066434, an oral small molecule pan-KRAS inhibitor, demonstrates robust anti-tumor activity in KRAS-mutant models, including in the CNS.

H. Gao[1], Y. Zhang[1], C. Yu[2], W. Xu[2], B. Li[1], H. Bian[1], M. Tandon[1], T. Wang[1], T. R. Stewart[1], M. H. Bender[1], W. Ming[1], M. A. Johnson[1], L. M. Kays[1], M. Leonard[1], M. A. Castanares[1], A. Capen[1], A. You[3], W. Bian[2], L. Prieto[4], T. Kercher[5], L. LeBrun[5], A. Klippel[6], C. Iyer[1], X. Gong[1];

[1]Eli Lilly and Company, Indianapolis, IN, [2]Lilly (China) Research and Development Center, Shanghai, China, [3]Lilly (China) Research and Development, Shanghai, China, [4]Eli Lilly and Company, Alcobendas, Spain, [5]Eli Lilly and Company, Louisville, CO, [6]Eli Lilly and Company, New York, NY

KRAS is altered in ~16% of all cancers and is an oncogenic driver in NSCLC, pancreatic, colorectal, and other cancers. Next generation KRAS inhibitors, designed to target multiple oncogenic KRAS mutations, have the potential to improve outcomes for the large burden of KRAS-mutated disease. Importantly, preclinical data suggest that a pan-KRAS approach that spares wildtype HRAS and NRAS may avoid the skin toxicities associated with pan-RAS inhibition.[1] This is particularly important in the context of treating KRAS-mutated colorectal cancer in combination with EGFR inhibition, which also carries skin toxicity. Next generation KRAS inhibitors could treat a wide range of patients including the approximately 35% of those with NSCLC who are at increased risk of developing CNS metastases. LY4066434 is an orally bioavailable, highly potent pan-KRAS inhibitor that has high selectivity over HRAS and NRAS.[2] Here, we report the antitumor activity of LY4066434 in patient-derived xenograft (PDX) and intracranial cancer models. We evaluated the activity of LY4066434 in PDX models harboring different KRAS mutations (both common and less frequent) and representing diverse histologies. LY4066434 showed robust anti-tumor activity, ranging from significant tumor growth inhibition to strong regression, in KRAS-driven PDX models of NSCLC, pancreatic, colorectal, and gastric cancers. Furthermore, we analyzed the activity of LY4066434 in combination with standard-of-care therapies. LY4066434 when combined with cetuximab showed enhanced antitumor activity in PDX models of NSCLC, pancreatic, and colorectal cancers with various KRAS mutations. Additionally, the combination of LY4066434 with chemotherapies also demonstrated greater efficacy in KRAS-mutant PDX models of endometrial and ovarian cancers. Finally, evaluation of LY4066434 activity in two KRAS-mutant orthotopic brain tumor models of NSCLC showed that LY4066434 inhibited tumor growth and promoted survival in these brain tumor models. Taken together, these results demonstrate the potential of LY4066434 as a promising therapeutic option for a range of cancer types driven by KRAS mutations including NSCLC, pancreatic, and colorectal cancers. LY4066434 is currently being investigated clinically (NCT06607185).

1. Manousaridis I. et al. 2013. J Eur Acad Dermatol and Venereol, 27: 11-18. 2. 2. Prieto L. et al. AACR-NCI-EORTC. Oct 11-15, 2023. Boston, MA.

#4376 Preclinical characterization of SGR-4174, a potent and selective SOS1 inhibitor for the treatment of pan KRAS mutant cancers in combination with KRAS pathway inhibitors.

Hui Wang, Felicia Gray, Christian Atsriku, Adam M. Levinson, Andrew Placzek, Evelyne M. Houang, Abba E. Leffler, Anatoly Ruvinsky, Leah Frye, Jeremy Greenwood, Mats Svensson, Jeff Bell, Zhijian Liu, Roman Shimanovich, Matt Liu, Shaoxian Sun

Schrodinger, Inc., New York, NY

KRAS is the most frequently mutated oncogene in many tumor types including pancreatic (90%), colorectal (50%) and lung cancers (30%). KRAS mutation typically impairs the conversion of active GTP-bound KRAS to the inactive GDP-bound form, resulting in constitutively active KRAS. SOS1 is a predominant guanine exchange factor (GEF) that binds the inactive GDP-KRAS form and catalyzes conversion to the active GTP-KRAS. SOS1 inhibition has thus been described to be an effective approach for suppressing the KRAS signaling pathway and inhibiting tumor cell proliferation in KRAS mutant-driven cancers. Combination of SOS1 inhibitor with KRAS G12C inhibitor could be an attractive therapeutic approach to enhance the efficacy and overcome the resistance to KRAS G12C inhibitors driven by the reactivation of the RAS pathway observed in the clinic.

SGR-4174 is a potent and selective inhibitor of the SOS1-KRAS complex that was rationally designed using Schrödinger's computational platform. SGR-4174 binds to the catalytic domain of SOS1 (KD = 11 nM), disrupts the SOS1::KRAS interaction (SOS1::KRAS G12C FRET IC50 = 13 nM), and inhibits ERK activation (pERK IC50 = 28-61 nM) and KRAS mutant-driven tumor cell viability (3D CTG IC50 = 42-107 nM). SGR-4174 is effective in broad cancer types harboring KRAS G12C/D/V mutations and KRAS WT/EGFR mutations in drug sensitivity profiling of 49 tumor cell lines. In KRAS G12C tumor xenograft mouse models, SGR-4174 monotherapy demonstrated dose-dependent tumor growth inhibition and a decrease in the tumor PD biomarker pERK.

SGR-4174 was examined in combination with KRAS G12C inhibitor sotorasib and MEK inhibitor trametinib in vitro and in vivo. When combined with sotorasib, SGR-4174 demonstrated deeper and more sustained inhibition of the KRAS signaling pathway, stronger suppression of cell proliferation in 3D cell culture, and tumor regression in xenograft models compared to single agent activity. In addition, when combined with trametinib, SGR-4174 suppressed KRAS pathway reactivation by MEK inhibition and led to robust tumor cell death and tumor stasis. Compared to other published SOS1 inhibitors (BI-3406, MRTX0902), SGR-4174 is more potent, and demonstrated similar anti-tumor activity and target engagement at a lower dose both in monotherapy and combination therapy studies in xenograft tumor models. SGR-4174 was well tolerated as monotherapy and in combination with sotorasib or trametinib.

SGR-4174 demonstrated favorable PK properties across preclinical species and toxicity profile was well characterized. Our comprehensive preclinical efficacy and safety data support progressing SGR-4174 to clinical development to treat KRAS mutant cancers.

#4377 AN9025, an orally bioavailable pan-RAS(ON) inhibitor with potent, broad-spectrum anti-tumor activity.

Shuaishuai Liu, Meng Lv, Xiangyu Fu, Chenchen Zou, Hannah Yan, Tanya Yang, Feifan Li, Zhao Sun, Xiaoli Zhu, Hongyang Zhu, Meng Chen, Junying Li, Zhiyong Yu, Nanhai He

Adlai Nortye Ltd, Hangzhou, China

RAS mutations drive approximately 30% of human cancers, representing a critical unmet clinical need. AN9025, an oral RAS (ON) inhibitor, with best-in-class potential, demonstrates picomolar potency against RAS-dependent tumor cell lines, showing greater potency than a benchmark molecule. AN9025 binds to cyclophilin A (CypA) to form a binary complex which then engages the RAS (ON) protein to create a tri-complex that effectively inhibits downstream signaling pathways such as MAPK and PI3K/AKT. Preclinical characterization shows that AN9025 binds to CypA with high affinity (K_D=3.2 nM) and selectively engages with GMPPNP-bound RAS (ON), rather than RAS (OFF), to form a tri-complex with single-digit nanomolar affinity (K_D=8.8 nM). Consistent with its high binding affinity, cell-based assays showed that AN9025 disrupts the KRAS-RAF1 interaction and downregulates phospho-ERK1/2 levels in $KRAS^{G12D}$- and $KRAS^{G12V}$-mutated cells, with IC_{50} values of 0.068 and 0.013 nM, respectively. AN9025 also exhibited potent antiproliferative effects across a range of RAS-dependent tumor cell lines with various mutations ($KRAS^{G12C}$, $KRAS^{G12D}$, $KRAS^{G12V}$, $KRAS^{G12R}$, $KRAS^{G12A}$, $KRAS^{G13C}$, $NRAS^{Q61H}$, $NRAS^{Q61L}$, $NRAS^{Q61K}$), achieving IC_{50} values between 0.001 and 0.1 nM. Additionally, AN9025 demonstrated cytotoxic activity in cell models resistant to KRAS(OFF) G12C inhibitors sotorasib and adagrasib, while showing no cytotoxicity in BRAF-mutant, RAS-independent cell lines. In the $KRAS^{G12D}$-bearing HPAC xenograft model, oral administration of AN9025 at a dose of 0.125 mg/kg QD achieved ~95% intratumoral pharmacodynamic (PD) inhibition of DUSP6 and induced deep tumor regression compared to vehicle. Notably, AN9025 demonstrated sustained PD inhibition, with 60% inhibition persisting 14 days post-treatment cessation, suggesting extended therapeutic efficacy. In summary, AN9025 is a highly potent pan-RAS(ON) inhibitor that forms a tri-complex with CypA and RAS, effectively blocking downstream signaling and demonstrating robust antitumor efficacy, including in models that are resistant to mutant-selective RAS(OFF) inhibitors. Preclinical studies support its advancement into clinical development as a promising targeted therapy for RAS-driven cancers.

#4378 BBO-11818, an orally bioavailable, highly potent and non-covalent pan-KRAS inhibitor demonstrates robust anti-tumor activity in KRAS-mutant preclinical models.

C. E. Stahlhut Espinosa[1], K. Singh[1], Z. Zhang[1], K. Sullivan[1], N. Gitego[1], M. Rigby[2], R. Ma[2], P. Alexander[2], S. Setoodeh[1], J. Shu[1], K. Lin[1], D. Rabara[2], E. Larsen[2], A. Sharma[2], A. Chan[2], B. P. Smith[2], A. Stephen[2], Y. Yang[1], J. Pei[3], F. C. Lightstone[3], D. Simanshu[2], R. Xu[1], C. Ji[1], K. Wang[1], B. Wang[1], K. Sinkevicius[1], D. V. Nissley[2], E. Wallace[1], F. McCormick[2], A. E. Maciag[2], P. J. Beltran[1];
[1]BridgeBio Oncology Threapeutics, South San Francisco, CA, [2]Frederick National Laboratory for Cancer Research, Frederick, MD, [3]Lawrence Livermore National Laboratory, Livermore, CA

KRAS is the most commonly mutated oncogene, altered in approximately 15% of human cancers. While inhibitors of $KRAS^{G12C}$ have shown promising clinical efficacy, there are currently no approved targeted therapies against other KRAS variants, resulting in a significant underserved patient population across several major cancer types, including pancreatic carcinoma (PDAC), colorectal cancer (CRC), and non-small cell lung cancer (NSCLC). To address this unmet clinical need, we have developed BBO-11818: a potent, selective, orally bioavailable non-covalent pan-KRAS inhibitor with activity against multiple KRAS mutants, including $KRAS^{G12D}$ and $KRAS^{G12V}$, in both their inactive GDP-bound and active GTP-bound states. BBO-11818 binds mutant KRAS with high affinity and selectivity in its active and inactive conformations, locking it in the inactive State 1 to disrupt the association of GTP-bound KRAS with its key effector RAF1. Cell-based assays confirm that BBO-11818 potently inhibits several driver KRAS mutants, resulting in the suppression of MAPK signaling and inhibition of cell proliferation with low nanomolar EC_{50} values. The selectivity of BBO-11818 for KRAS is demonstrated by its >1000-fold lower potency against NRAS- and BRAF-mutant cell lines. BBO-11818 exhibits favorable pharmacokinetic (PK) and pharmacodynamic (PD) properties in mouse models of cancer. Single dose, oral administration results in strong dose- and time-dependent inhibition of pERK in $KRAS^{G12D}$ and $KRAS^{G12V}$ cell-derived xenograft (CDX) models. Importantly, BBO-11818 monotherapy induces strong anti-tumor responses, including regression at well-tolerated doses in CDX models of KRAS-mutant pancreatic, non-small cell lung, and colorectal cancer. In combination with either BBO-10203, a selective RAS:PI3Kα breaker that blocks RAS-mediated activation of AKT; or cetuximab, an anti-EGFR monoclonal antibody, BBO-11818 shows significantly enhanced efficacy in models harboring $KRAS^{G12D}$ or $KRAS^{G12V}$ mutations. BBO-11818 also shows a combination benefit with anti-PD-1 treatment, resulting in complete tumor regressions in the CT-26 syngeneic tumor mouse model. BBO-11818 is a potent pan-KRAS inhibitor with activity against both GTP and GDP forms of KRAS, presenting the opportunity to address, as monotherapy or combination, a large fraction of KRAS mutant tumors currently lacking targeted therapeutic options.

#4379 BPI-572270: An orally bioavailable and highly potent RAS (On) multi tri-complex inhibitor.

X. Yang, X. Zhu, **H. Han**, W. Wu, Y. Zhou, X. Kong, Y. Zhao, X. Liu, Y. Liu, T. Zhang, H. Chen, S. Li, C. Yang, J. Guo, Q. Zhou, H. Lan, L. Ding;
Betta pharmaceuticals, Co., LTD., Hangzhou, China

KRAS mutations are oncogenic drivers occur in approximately 20%-30% of all cancers with particularly high frequencies observed in pancreatic cancer (~90%), non-small cell lung cancer (~35%), and colorectal cancer (~45%). While approved $KRAS^{G12C}$ inhibitors have demonstrated clinical benefits in patients with $KARS^{G12C}$ mutation, other prevalent KRAS mutations, such as $KRAS^{G12D/G12V/G13D/Q61H}$, remain a significant therapeutic challenge. Here we present BPI-572270, a highly potent Cyclophilin A-dependent $RAS(On)^{multi}$ inhibitor with favorable oral bioavailability designed to target those difficult-to-treat RAS mutations. *In vitro,* BPI-572270 demonstrated exceptional potency by disrupting the GTP bound $RAS^{WT/Mut}$ and cRAF interaction in a cell-free assay, and by inhibiting tumor cell proliferation with sub-nanomolar IC_{50} values in a range of tumor cell lines harboring mutant KRAS, NRAS, or HRAS, as well as wild type RAS cell lines. *In vivo*, BPI-572270 achieved significant tumor regression in $KRAS^{G12V}$ human pancreatic Capan-2 xenograft mouse model at 0.3 mg/kg (PO, QD), and in $KRAS^{G12D}$ human pancreatic KP-4 xenograft model at 3 mg/kg (PO, QD), demonstrating potent efficacy. BPI-572270 also showed outstanding pharmacokinetic properties in mouse, rat and beagle dog. Safety evaluations in repeated-dose studies in rats and beagle dogs indicate a promising safety profile, reinforcing its potential for further clinical development. In conclusion, BPI-572270 emerges as a highly potent, orally bioavailable $RAS(On)^{multi}$ inhibitor, with favorable efficacy, pharmacokinetics, and safety profiles in pre-clinical studies.

#4380 HBW-016-K, a novel orally available pan-KRAS inhibitor targeting both “ON” and “OFF” states, is potent, selective, efficacious, safe, and high tissue-penetrant.

N. Lee, Y. Li, G. Liu, J. Li, M. Yang, z. Song;
Hyperway Pharmaceutical, Chengdu, China

Purpose: This study aimed to develop a novel, orally available pan-KRAS inhibitor capable of targeting both the “ON” and “OFF” states of KRAS, addressing drug resistance and expanding treatment options for KRAS-mutant cancers.
Methods: Through medicinal chemistry efforts, we designed and optimized a pan-KRAS inhibitor that selectively targets various KRAS mutant subtypes while sparing wild-type KRAS, NRAS, and HRAS. The lead compound was further evaluated for its pharmacokinetic (PK) properties and safety profile. Its anti-tumor efficacy was assessed in multiple KRAS mutant mouse models following oral administration.
Results: HBW-016-K demonstrated potent inhibition of various KRAS mutant cell lines with excellent selectivity for KRAS wild-type and other RAS isoforms. This compound effectively targets both the “ON” and “OFF” conformations of KRAS G12D. Additionally, HBW-016-K exhibits favorable PK properties and significant tissue penetration, particularly in lung, intestine, and pancreas. In a KRAS G12V mouse xenograft model, HBW-016-K exhibited dose-dependent anti-tumor activity. At the highest dose of 30 mg/kg (BID, PO), tumors shrank by 84.1% compared to baseline, with sustained tumor regression. HBW-016-K also demonstrated potent anti-tumor activity in KRAS G12D and G12C mouse models. Preliminary toxicity studies revealed a favorable safety profile for HBW-016-K, including minimal off-target effects, negative mutagenicity, and excellent tolerability in a 14-day rat toxicity study.
Conclusion: HBW-016-K is a promising novel pan-KRAS inhibitor with superior potency, selectivity, and efficacy. Its favorable PK profile and safety profile support its advancement into clinical development. HBW-016-K has the potential to significantly impact the treatment of KRAS-driven cancers, particularly those with limited therapeutic options, such as lung, colorectal, and pancreatic cancer.

#4381 Preclinical studies of HEC211909, an orally bioavailable, high potent pan-KRAS inhibitor.

H. Liu, H. Xie, F. Tan, N. Kang, M. Li, Y. Zhang;
Sunshine Lake Pharma Co. Ltd., Dong Guan Shi, China

Background: KRAS is the most commonly mutated oncogene, which is the driver in nearly 23% human cancers, and are especially prevalent in colorectal, lung, and pancreatic tumors. The KRAS signaling pathway is often hyperactivated in cancer cells due to KRAS gene mutations. This hyperactivation leads to uncontrolled cell proliferation, ultimately leading to the development and progression of cancer. There has been an urgent and unmet need to target KRAS mutations in KRAS-driven cancer.
Methods: Biochemical HTRF assay was used to measure the inhibition of HEC211909 to both GDP-bound and GTP-bound state of various KRAS mutant. CellTiter-Glo assay was performed to determine the effects on proliferation of tumor cell lines. Several KRAS dependent tumor models were employed to study the in vivo pharmacodynamic and anti-tumor effects.
Results: HEC211909 is a potent and orally bioavailable small molecule inhibitor of the KRAS oncogene, such as KRAS G12D, KRAS G12C, KRAS G12V, KRAS Q61H, KRAS G13D and KRAS wild-type amplifications. HEC211909 inhibits both active (GTP-bound) and inactive (GDP-bound) forms of multiple KRAS mutant protein with single-digit nanomolar IC50 values. HEC211909 has a strong anti-proliferative activity in a panel of KRAS mutant cancer cell lines and KRAS wild-type amplifications cancer cell lines in vitro with sub-nanomolar IC50 values, as well as significant inhibition of ERK1/2 phosphorylation with sub-nanomolar IC50 values. HEC211909 is highly selective for KRAS vs HRAS or NRAS, and is expected to be well-tolerated in normal tissues. In vivo, HEC211909 demonstrated dose-dependent anti-tumor activity with tumor regressions at 30 or 60 mg/kg given orally twice daily in KRAS-mutant xenograft models, such as PK59(KRAS G12D), HPAC(KRAS G12D), H358(KRAS G12C) xenograft models, and shows good tolerability during 21 days of treatment.
Conclusions: HEC211909 is a highly potent and selective orally pan-KRAS(ON/OFF) inhibitor against KRAS G12D, KRAS G12C, KRAS G12V, KRAS Q61H, KRAS G13D mutations and also displays high selectivity over WT HRAS and NRAS, thus providing an expanded therapeutic index.

#4382 Discovery and characterization of potent and orally bioavailable KRAS G12D inhibitors with therapeutic potential for KRAS G12D-mutated solid tumors.

Mijin Moon, Jiyoung Song, Soyoung Ki, Beomjin Hong, Ilkyoo Koh, Hyunho Cho, Seona Lim, Chuneung Park, Eunsung Jang, Gyeong Hi Cho, Hoiyun Jung, Minchang Cho, Sook-Kyung Park

Drug Research Division, SK Biopharmaceuticals Co., Ltd., Seongnam-si, Korea, Republic of

Background: KRAS G12D is the most prevalent KRAS mutation commonly found in pancreatic ductal adenocarcinoma (PDAC) and colorectal adenocarcinoma (CRC). This mutation drives tumorigenesis through aberrant downstream signaling, making it a critical therapeutic target. Tens of KRAS G12D inhibitors are currently under development, however many encounter challenges related to drug-likeness. Here, we present orally bioavailable KRAS G12D inhibitors designed to overcome these barriers.
Methods: Binding affinity to KRAS-mutant proteins was evaluated using thermal shift assays (TSA). The inhibitory effects on cancer cells were assessed by measuring ERK phosphorylation levels via ELISA. Cell proliferation assays were performed across various KRAS wild-type (WT) and mutant cell lines to determine compound selectivity and potency. *In vivo* efficacy was assessed using PDAC and CRC models harboring KRAS G12D mutation. Combination studies were evaluated using *in vitro* cell proliferation assays, and synergistic effects were confirmed with Combenefit software.
Results: A series of KRAS G12D inhibitors with novel and differentiated structures were identified through optimization of physicochemical properties to maintain inhibitory potency while achieving high plasma exposure following oral administration. These compounds exhibited strong binding affinity to KRAS G12D with remarkable selectivity over other KRAS mutant proteins. In KRAS G12D-driven cancer cell lines, our compounds demonstrated potent inhibition of pERK and cell proliferation at low nanomolar concentrations. In contrast, proliferation inhibition in cell lines expressing WT or other KRAS mutations was 50- to 1000-fold lower, highlighting exceptional KRAS G12D selectivity and minimal impact on normal cells. Additionally, our compounds exhibited favorable metabolic stability. Following oral administration at 30 mg/kg in mice, selected compounds achieved a C_{max} exceeding 1 μg/mL, surpassing plasma exposure levels reported for other KRAS G12D inhibitors. High plasma and tumor exposure led to robust inhibition of pERK in tumor tissues. In KRAS G12D-driven pancreatic (PDAC) and colorectal (CRC) cancer xenograft models, oral administration of the lead compound resulted in potent, dose-dependent tumor growth inhibition without notable body weight loss.
Conclusion: Our potent and selective KRAS G12D inhibitors demonstrate favorable ADME properties and significant antitumor activity in KRAS G12D-mutant xenograft models, addressing a critical unmet need for patients with KRAS G12D-driven cancers.

#4383 Discovery of ID12241161, an oral KRAS^{multi} inhibitor demonstrating potent and selective anti-cancer effects in KRAS-dependent cancers.

J. Kim[1], S. Lee[2], J.-H. Lee[3], C. Hong[4], Y. Song[4], D. Kim[4], W. Lee[1], M. Lee[1];

[1]Idience, Seoul, Korea, Republic of, [2]Chung-Ang Uviversity, Seoul, Korea, Republic of, [3]Korea Institute of Toxicology, Daejeon, Korea, Republic of, [4]Yunovia, Hwaseong, Korea, Republic of

Kirsten rat sarcoma virus (KRAS) gene mutations are prevalent in multiple cancers, contributing to approximately 89%, 44%, and 30% of pancreatic, colorectal, and lung adenocarcinomas, respectively. These mutations are associated with poor prognosis, and limited treatment options for all KRAS mutations, except for G12C, highlights the urgent need for oncogene targeted therapies. Despite ongoing development of KRAS pathway-targeting drugs, balancing safety and efficacy remains challenge owing to its essential role in cellular functions across various tissues. Through extensive computational analysis and structure-based drug design, we developed ID12241161, a novel, potent, and orally available small molecule inhibitor that favorably positions within the KRAS switch II pocket to target both “ON” and “OFF” states of diverse KRAS mutants. In this study, we demonstrated that ID12241161 exhibited robust anti-proliferative effects *in vitro* at picomolar to nanomolar concentrations (most IC50 values were lower than 10 nM) across a range of cancer cell lines with KRAS mutations or amplifications. Significant pharmacodynamic modulation of biomarkers was observed, including the inhibition of ERK1/2 phosphorylation, in these KRAS-dependent cell lines. In KRAS-independent cells, minimal cytotoxicity was observed, even at micromolar concentrations, suggesting that ID12241161 selectively targets KRAS-driven cancers. In mice, the pharmacokinetic profile of ID12241161 is characterized by a half-life of 6 hours with a high volume of distribution, which allows sustained effects for over 24 hours following once-daily dosing. Furthermore, *in vivo* studies demonstrated that a daily oral dose of 5-15 mg/kg ID12241161 was well tolerated and effectively induces tumor regression in KRAS-dependent xenograft models representing various cancer types. Our data indicated that ID12241161 possesses potent anticancer effects in KRAS-dependent cancers, with a wide safety margin and favorable pharmacokinetics, allowing for once-daily dosing. These findings support the potential use of ID12241161 as a viable treatment option for patients with KRAS-driven cancers.

#4384 SYNB021225: a potent oral pan-KRAS peptide inhibitor that can address unmet needs of small molecule inhibitors.

Michael Poss[1], Caihong Zhou[2], Cheng Lu[2], Ruochi Zhang[3], Yu Wang[3], Yuanpeng Xiong[3], Xin Gao[3], Xiao Zhang[3], Yan Degenhardt[4]

[1]Syneron Tech, Lawrenceville, NJ,[2]Syneron Tech, Shanghai, China,[3]Syneron Tech, Beijing, China,[4]Syneron Tech, Wynnewood, PA

KRAS mutations are present in 30% of cancer patients, underscoring the importance of KRAS as a critical target for drug development. Despite the advancements in small molecule inhibitors targeting specific KRAS mutations, such as G12C and G12D, significant challenges persist in addressing other prevalent mutations such as G12V. Furthermore, small molecule inhibitors predominantly target the GDP-bound form of KRAS due to accessibility of the SWITCH II pocket, even though most mutant KRAS proteins in cancer cells are in the activated GTP-bound form. Peptide drugs have emerged as promising alternatives due to their exceptional binding affinity and specificity, along with their reduced dependency on the presence of a well-defined binding pocket. Utilizing Syneron's innovative technology, we have successfully developed cyclic peptides capable of cell penetration and oral bioavailability, to overcome some of the limitations associated with small molecule KRAS therapeutics. SYNB021225 is a potent pan-KRAS inhibitor effective against multiple KRAS mutations, including G12V. With sub-nanomolar potency in both binding and *in vitro* cell killing, SYNB021225 induces tumor regression at oral doses of 10-20 mpk administered once daily. Besides expanding the treatable patient population, its broad targeting profile can also potentially address tumor heterogeneity and mitigate resistance. SYNB021225 inhibits both KRAS-ON and KRAS-OFF forms, directly disrupting the interaction between GTP-bound KRAS and RAF. Given that most mutant KRAS proteins in cancer cells are in the activated GTP-bound form, comparing to small molecules that mainly target the GDP-bound KRAS, this dual action offers potential advantage in enhancing efficacy as well as overcoming resistance from activated Receptor Tyrosine Kinases like EGFR. Indeed, in the *in vitro* proliferation assay, the potency of SYNB021225 remained unaffected when treated with EGF. Furthermore, SYNB021225 demonstrates efficacy against KRAS wild-type amplified tumors, which are resistant to KRAS-ON only molecular glue inhibitors. Current small molecule inhibitors often fail to maintain sustained p-ERK inhibition, impacting overall survival. In preclinical studies, SYNB021225 has shown prolonged tumor retention and p-ERK suppression lasting up to 72 hours, that may translate to improved clinical efficacy. Unlike small molecules, peptides such as SYNB021225 are metabolized by proteases and excreted via the kidneys, minimizing liver toxicity. This profile makes SYNB021225 more suitable for combination therapies, such as with anti-PD1 agents, without exacerbating the liver inflammation as seen in the clinic with small molecule inhibitors. Currently, SYNB021225 is being prepared for clinical development.

#4385 Preclinical evaluation of RAS pathway inhibitors in gastroesophageal adenocarcinoma.

Zachary Hammer[1], Jack Rifkin[2], Ralph Fleuranvil[1], Babita Pokharel[3], Stella Savone[4], Sandra W. Ryeom[1], Mariam El-Ashmawy[1]

[1]Surgical Sciences, Columbia University Irving Medical Center, New York, NY,[2]Sidney Kimmel Medical College, Philadelphia, PA,[3]Broad Institute, Cambridge, MA,[4]Greens Farms Academy, Westport, CT

Gastric cancer is the 4th leading cause of cancer-related deaths worldwide and up to 25% are driven by alterations in the RAS pathway. RAS activation in cancer is commonly a result of a mutation locking the protein in the ON state. However, KRAS amplification (KRAS^{AMP}), defined as >6 copies of wild type KRAS allele, occurs more frequently in gastroesophageal adenocarcinoma (GEA). Western blot analysis revealed differences in MAPK signaling amplitude between KRAS^{AMP} and KRAS^{MUT} cell lines after synchronization. KRAS^{AMP} cells show a sharp spike in signaling upon stimulation, followed by attenuation, we speculate due to negative feedback. Historically KRAS^{AMP} has been difficult to target due to adaptive signaling requiring MAPK inhibition at multiple signaling nodes, yet these combinations are clinically limited by toxicity. Here we test novel RAS pathway inhibitors in KRAS$^{AMP/MUT}$ GEA cell lines, to investigate toxicity, synergy with drug combinations, and efficacy of MAPK pathway target inhibition by quantifying levels of p-ERK. KRAS^{AMP} GEA cells ranged in copy number from 8-68; while all GEA mutant cell lines harbored KRAS^{G12D} mutations. GEA cells were seeded in 96 well plates, treated with drug for 5 days then assessed for viability via Cell titer glo. The half-maximal effective drug concentration (EC50) for p-ERK inhibition was determined by Western blot analysis after 24 hours of drug treatment. The pan-KRAS (OFF) Inhibitor, BI-2865 inhibits KRAS altered GEA cell viability with an IC_{50} ranging from 0.2 - 1.4 μM in KRAS^{AMP} and KRAS^{G12D} mutant cell lines. The RAF/MEK clamp VS-6766 demonstrated an IC_{50} range from 0.81 μM in KRAS^{AMP} in cell lines, while mutant KRAS^{G12D} lines exhibited 40-fold lower sensitivity. The allosteric SHP2 inhibitor RMC-4550 demonstrated an IC_{50} of 0.08-6.67 μM in KRAS^{AMP} and KRAS^{G12D} lines. BI-2865 suppressed p-ERK protein expression with an EC50 of 16 and 45 nM in KRAS^{AMP} and KRAS^{MUT} GEA cells respectively. There was no adaptive signaling through PI3K pathways based on suppressed p-AKT levels, in comparison to trametinib which resulted in increases in pAKT. The RAF/MEK clamp VS-6766 also inhibits signaling through MAPK as evidenced by decreased p-AKT, and an EC50 for p-ERK of 131.83 nM (KRAS^{AMP}) and 10.4725 nM (KRAS^{MUT}) GEA cells. Our data indicates a positive correlation between KRAS copy number and the IC50 of RAS-MAPK pathway inhibitors, suggesting cells with more KRAS copies require higher doses to achieve cytotoxicity; this is also reflected in the dose required for target engagement. This highlights the differential sensitivity of KRAS^{AMP} versus mutant cell lines, emphasizing the complexity of KRAS^{AMP} tumors and their reliance on high copy numbers. Our results suggest that combination strategies will be essential to overcome adaptive resistance and maximize therapeutic benefit in KRAS^{AMP} cells. Future *in vivo* studies will validate the potential of these inhibitors and combination approaches.

#4386 HJ-099, a novel, orally bioavailable pan-KRAS (ON) non-covalent inhibitor, demonstrates significant antitumor activity in multiple preclinical models with KRAS mutations.

Xiangyu Yang, Fengtian Du, Na Guo, Huiqiao Ling, Shuai Luo, Xiaorong Yu, Qingqing Qian, Ruisi Wu, Jingyu Tian, **Jianxin Ji**, Li Zhang

Chengdu Huajian Science Co., Ltd., Chengdu, China

Background: KRAS is one of the most common mutated oncogenes in cancer, which primarily stimulates tumor growth by activating the MAPK pathway. Tumors with non-$KRAS^{G12C}$ mutations, such as $KRAS^{G12D}$, $KRAS^{G12V}$, and other codon mutation subtypes, currently lack compelling precision medicines. Therefore, the development of pan-KRAS inhibitor is an unmet and pressing clinical need.
Methods and Results: Here, we report a novel, orally bioavailable pan-KRAS (ON) inhibitor HJ-099, designed to treat various KRAS mutation-driven tumors. HJ-099 exhibited significant anti-proliferative activity against multiple KRAS mutated cancer cell lines, including SW620, MAI PaCa-2, NCI-H358, AsPC-1, and NCI-H441, with IC_{50} <1.5 nM. In multiple KRAS mutated xenograft mouse models, HJ-099 showed significant inhibition on tumor growth. Tissue distribution study in tumor-bearing murine model showed that HJ-099, after oral administration, were mainly detected in the tumors, and the long-lasting accumulation of HJ-099 in tumor tissues resulted in more potent and durable antitumor activity in vivo.
Conclusions: The study indicates that HJ-099 is a novel, highly active pan-KRAS (ON) inhibitor that shows significant tumor suppression activity against major KRAS mutation subtypes, and merits clinical evaluation.

#4388 Novel small molecule on state inhibitor for GTP bound pan-KRAS (G12) mutations.

C. Gajendran, N. M. Sadhu, R. Gosu, M. Zainuddin, D. Sivanandhan, **S. Rajagopal**;
Jubilant Therapeutics Inc, Bedminster, NJ

Introduction: KRAS is part of the RAS family of small GTPases. They act as molecular switches by cycling between an active GTP-bound (ON) state and an inactive GDP-bound (OFF) state in response to extracellular signals. KRAS is frequently mutated in nearly 30% of cancers, with the G12, G13, and Q61 hotspots being the most common [1]. Among these, G12 mutations account for 83% of KRAS mutations, being one of the most prevalent drivers of cancers [2]. Active KRAS triggers effector pathways like MAPK and PI3K, promoting cellular proliferation and survival. Oncogenic KRAS remains constitutively active due to defective GTP hydrolysis [3]. Therefore, inhibitors that target the “ON” state can effectively interrupt the signaling pathways that promote cancer cell growth and survival. Recently pan-KRAS tricomplex inhibitor, which inhibits KRAS ON state, has entered the clinic. Therefore, targeting the KRAS in ON state could be of high clinical value in cancers with defects in G12 mutations and in overcoming resistance. In this regard, we are developing a series of novel pan-KRAS ON state inhibitors.

Methods: Fragment screening with SPR and structure-based drug design were used to identify novel pan-KRAS (G12) mutant “ON” state inhibitors. Over 20 scaffolds were synthesised and screened using the AlphaLISA and Nucleotide exchange assay. Binding was assessed using thermal shift assay. To assess *in vitro* potency and to drive the structure-activity relationship, an AlphaLISA assay that measures the disruption of active KRAS:C-Raf interaction and for the inactive GDP bound state Nucleotide exchange assay was used. The cell-based activity of these inhibitors was assessed by measuring the pERK levels and 2D/3D cell proliferation assays. PK-PD assessment was performed in A427 lung cancer and AGS gastric cancer xenograft models in mice.

Results: Based on in vitro screening, we identified a novel series of potent pan-KRAS ON inhibitors targeting (G12) mutations. These early hit compounds demonstrated differential anti-proliferative activity between wild type and G12 mutant KRAS cell lines under 2D and 3D culture conditions. Accordingly, significant reductions in PD biomarker, pERK was demonstrated in KRAS mutant cell lines. PK-PD correlation was also established in a tumor bearing mice models, showing dose-dependent reductions in pERK level. The early data with these compounds show good ADME properties, and oral bioavailability, making them suitable for in vivo profiling and further SAR exploration.

Conclusion: Our findings highlight the potential of these novel pan-KRAS ON state inhibitors as promising therapy approach for cancers harbouring KRAS (G12) mutations. Further SAR optimization, mechanistic studies, in vivo studies and toxicity evaluations are warranted to fully establish their efficacy and safety.

#4391 A novel dual inhibitor of RAS and β-catenin signaling with advantages over mutant-specific KRAS inhibitors to escape resistance has robust antitumor efficacy and activates antitumor immunity.

Gary A. Piazza[1], Dhana Sekhar Reddy Bandi[2], Ganji Purnachandra Nagaraju[2], Kristy L. Berry[1], Khalda Fadlalla[1], Md Yeashin Gazi[3], Upender Manne[2], Xi Chen[1], Jeremy B. Foote[2], Adam B. Keeton[1], Yulia Y. Maxuitenko[1], Donald B. Buchsbaum[2], Bassel F. El Rayes[2], Gang Zhou[3]

[1]Auburn University Harrison College of Pharmacy, Auburn, AL,[2]O'Neal Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL,[3]Georgia Cancer Center, Augusta University, Augusta, GA

Aberrant activation of RAS signaling due to oncogenic RAS mutations, constitutive activation of receptor tyrosine kinases, or gene amplifications are key drivers/dependencies of most human cancers. Co-occurring mutations in the APC/β-catenin pathway that often occur in gastrointestinal and other cancers also play a crucial role in tumorigenesis and likely contribute to resistance to KRAS inhibitors. Phosphodiesterase 10A (PDE10) is overexpressed in certain cancers compared with normal tissues, while known inhibitors or gene silencing selectively inhibit proliferation and induce apoptosis of PDE10-expressing cancer cell lines. In contrast, normal cells lacking PDE10 were remarkedly insensitive. A novel orally bioavailable PDE10 inhibitor, ADT-030, was identified by screening a proprietary indene library followed by chemical optimization with delivery advantages over conventional PDE10 inhibitors. PDE10 inhibition by ADT-030 was confirmed with enzymatic assays using recombinant PDE10 and in cancer cells by cellular thermal stability assays, as well as the ability of ADT-030 to activate cGMP/PKG signaling at concentrations that inhibit and bind PDE10. ADT-030 blocked RAS-MAPK/AKT signaling and destabilized β-catenin, suppressing the transcription of genes essential for cancer cell proliferation and survival (e.g., MYC, Cyclin D, Survivin). Cancer cells resistant to $KRAS^{G12C}$ and $KRAS^{G12D}$ inhibitors retained complete sensitivity to ADT-007. Oral administration of ADT-030 strongly inhibited tumor growth at doses without discernable toxicity in mouse tumor models of colon, lung, breast, and pancreatic cancer. Notably, ADT-030 caused tumor regression in patient-derived xenograft mouse models of pancreatic cancer and increased survival in orthotopic mouse models of lung cancer. ADT-030 also inhibited metastasis in the 4T1 orthotopic model of breast cancer and synergized with anti-PD1 in the CT26 mouse model of colon cancer to enhance survival. In addition, ADT-030 inhibited tumor formation in chemical-induced models of lung cancer and the MMTV/neu mouse model of breast cancer. Finally, deep immunophenotyping studies revealed a significant impact of ADT-030 treatment on the tumor immune microenvironment characterized by selective induction of apoptosis in myeloid-derived suppressor cells (MDSC) while increasing tumor infiltration of CD8+ T cells and natural killer cells. Together, these results support IND-enabling studies of ADT-030 as a monotherapy or in combination with immunotherapy for a broad range of KRAS-driven/dependent cancers.

#4392 A 1st in class pan-RAS inhibitor with robust antitumor activity in PDAC models and advantages over other RAS inhibitors to escape resistance.

Gary A. Piazza[1], Dhana Sekhar Reddy Bandi[2], Ganji Purnachandra Nagaraju[2], Kristy Berry[1], Junwei Wang[1], Sindu Ramesh[1], Chung-Hui Huang[1], Upender Manne[2], Xi Chen[1], Jeremy B. Foote[2], Adam B. Keeton[1], Yulia Y. Maxuitenko[1], Donald J. Buchsbaum[2], Bassel F. El Rayes[2]

[1]Drug Discovery and Development, Auburn University Harrison College of Pharmacy, Auburn, AL,[2]O'Neal Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL

ADT-007 is a novel reversible pan-RAS inhibitor with ultra-high potency and unique selectivity to inhibit the growth of cancer cells with mutant or activated RAS by blocking GTP activation of RAS. ADT-1004, an orally bioavailable prodrug of ADT-007, displayed robust antitumor activity in multiple mouse models of pancreatic ductal adenocarcinoma (PDAC), including PDX models harboring $KRAS^{G12D}$, $KRAS^{G12C}$, $KRAS^{G12V}$, and $KRAS^{G13Q}$ mutations, at dosages approximately 9-fold below the maximum tolerated dose. Analysis of treated tumors revealed that ADT-1004 suppressed GTP-RAS and p-ERK levels and activated antitumor immunity. As evidence of target selectivity for mutant RAS, ADT-1004 suppressed the growth of $KRAS^{G12C}$ MIA PaCa-2 tumors without affecting $KRAS^{WT}$ BxPC-3 tumor growth. ADT-1004 displayed superior antitumor activity over sotorasib and adagrasib in a PDAC model involving MIA PaCa-2 cells that developed resistance to mutant-specific KRAS inhibitors. *In vitro* experiments show that ADT-007 potently inhibited the proliferation of cancer cell lines harboring mutations in K/N/H RAS without affecting the proliferation of cells with downstream BRAF mutations or cells from normal tissues. Cancer cell lines with RAS^{WT} but amplified RAS or upstream mutations in RTK or NF-1 were also highly sensitive to ADT-007 with low nM potency. ADT-007 reduced GTP-RAS levels from mutant KRAS and co-expressed RAS^{WT} isoforms under serum-free and EGF-stimulated conditions while inhibiting MAPK/AKT signaling to suppress MYC levels. Growth assays involving direct comparison of ADT-007 with mutant-specific $KRAS^{G12C}$ inhibitors (sotorasib), pan-KRAS inhibitors (BI-2865), or other pan-RAS inhibitors (RMC-6236) revealed more complete cancer cell killing by ADT-007. In addition, colony formation assays showed near complete inhibition by ADT-007 but only partial inhibition by sotorasib, BI-2865, and RMC-6236. ADT-007 also inhibited the growth of RAS^{WT} cancer cells harboring amplified RAS with more extensive killing compared with BI-2865 and RMC-6236. Finally, cancer cell lines resistant to $KRAS^{G12C}$ and $KRAS^{G12D}$ inhibitors retained complete sensitivity to ADT-007 but showed resistance to MRTX849 and MRTX1133, respectively. These results show the unique advantages of ADT-1004 over mutant-specific KRAS, pan-KRAS, and other pan-RAS inhibitors to escape resistance that limits the efficacy of RAS inhibitors FDA-approved or in clinical trials.

#4393 RAF/MEK clamp avutometinib combined with a pan-RAF inhibitor induces nearly complete MAPK pathway inhibition with deep tumor regressions in NRAS or BRAF class III mutant models.

Silvia Coma[1], Poulikos I. Poulikakos[2], **Jonathan A. Pachter**[1]

[1]Verastem Oncology, Needham, MA,[2]Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY

Although there are targeted therapies currently approved for cancers with KRAS G12C and BRAF V600E (class I) mutations, there is still significant unmet medical need for patients harboring NRAS or BRAF class II/III alterations which are found in a range of cancers including melanoma and lung cancer. Avutometinib is a first-in-class orally available RAF/MEK clamp that potently inhibits MEK1/2 kinase activities and induces formation of dominant negative RAF/MEK complexes thereby blocking compensatory activation of MEK by RAF. The combination of avutometinib with the focal adhesion kinase (FAK) inhibitor defactinib has shown clinical activity for patients with low-grade serous ovarian cancer and is currently being investigated in a Phase 3 confirmatory study (RAMP 301; NCT06072781). Here, we tested the combination of avutometinib with a pan-RAF inhibitor (pan-RAFi) in cancer models harboring NRAS or BRAF class III mutations. In 3D proliferation assays, avutometinib inhibited cell proliferation across multiple MAPK pathway alterations including NRAS, and BRAF class II and III. Combination strategies of avutometinib with inhibitors of upstream MAPK pathway signaling, including pan-HER, SOS1 and pan-RAF inhibitors, were explored and striking synergy was observed between avutometinib and pan-RAF inhibitors in reducing viability of tumor cells *in vitro*. In a BRAF class III non-small cell lung cancer patient-derived xenograft (PDX) model (LU3057), the combination of avutometinib with the pan-RAFi exarafenib induced strong tumor regressions in all the mice, whereas avutometinib alone induced tumor stasis. In an NRAS mutant melanoma PDX model (ME9518), the combination of avutometinib and exarafenib showed deep tumor regression, whereas avutometinib or exarafenib single agents did not induce tumor regression. In contrast to the tumor regressions induced by avutometinib plus exarafenib in this ME9518 model, the combination of the MEK-only inhibitor binimetinib with exarafenib caused substantial body weight loss and failed to show tumor regression. Strong tumor regressions in all mice were also observed with the combination of avutometinib and the pan-RAFi belvarafenib in an NRAS mutant xenograft model (SKMEL2). Mechanistically, the deep tumor regressions observed with the combination of avutometinib plus exarafenib corresponded with virtually complete elimination of MAPK pathway markers such as pMEK, DUSP4 and pS6 in the tumors relative to avutometinib or exarafenib alone which conferred less complete inhibition. These results demonstrate that the combination of avutometinib with a pan-RAFi is especially efficacious and is well tolerated, indicating that clinical evaluation of avutometinib in combination with a pan-RAF inhibitor for treatment of patients with NRAS or BRAF class III mutations may be warranted.

#4394 GFH375 (VS-7375): An oral, selective KRAS G12D (ON/OFF) inhibitor with potent anti-tumor efficacy as single agent and in combination with other anticancer therapies in preclinical models.

Jonathan A. Pachter[1], Feng Yan[2], Silvia Coma[1], Cristina Caffarra Malvezzi[3], Xiuting Liu[4], Emilia Berardelli[3], Tao Jiang[2], Siyuan Le[2], Nathan Sanburn[1], Fusheng Zhou[2], Jiong Lan[2], David G. DeNardo[4], Chiara Ambrogio[3], Qiang Lu[2]

[1]Verastem Oncology, Needham, MA,[2]GenFleet Therapeutics, Shanghai, China,[3]Department of Molecular Biotechnology and Health Sciences,, University of Torino, Torino, Italy,[4]Department of Medicine, Washington University School of Medicine, St Louis, MO

KRAS G12D is the most prevalent KRAS mutation in human cancers, present in 37%, 12.5%, 8% and 4.9% of pancreatic, colorectal, endometrial and lung cancers, respectively. Currently there are no FDA approved KRAS G12D-selective inhibitors and thus, cancers with KRAS G12D mutations represent a significant unmet medical need. GFH375 (also known as VS-7375) is an orally available KRAS G12D-selective small molecule inhibitor that binds KRAS G12D in the active (ON; GTP-bound) and inactive (OFF; GDP-bound) states. By blocking the activities of the ON and OFF states of KRAS G12D, GFH375 has the potential to inhibit KRAS G12D signaling and tumor growth more completely than compounds that block KRAS G12D only in the OFF state or only in the ON state. Here we show that among a panel of human cancer cell lines, GFH375 potently and selectively inhibited proliferation of tumor cells bearing a KRAS G12D mutation relative to cell lines bearing wild-type KRAS or non-G12D KRAS mutations. Similarly, in a panel of mouse embryonic fibroblasts (MEFs) engineered to express various human KRAS mutation variants, GFH375 selectively inhibited proliferation of KRAS G12D mutant MEFs relative to MEFs expressing other KRAS G12X or Q61X mutations. Furthermore, GFH375 was more potent than other KRAS G12D inhibitors (e.g. RMC-9805, MRTX1133) in reducing the level of RAF1-bound active KRAS G12D-GTP (ON) and inhibiting cell proliferation in MEFs expressing human KRAS G12D. GFH375 also reduced pERK and pCRAF more potently than these other KRAS G12D inhibitors corresponding with greater increase in pro-apoptotic BIM. In vivo, oral administration of GFH375 single agent induced strong tumor regressions in multiple KRAS G12D colorectal and pancreatic cancer tumor models, including xenograft models and a transplanted pancreatic cancer model derived from KPC genetically engineered mice. GFH375 also demonstrated potent anti-tumor activity in an intracranial GP2D tumor model, suggesting potential to use for treatment of brain metastases. Combination of GFH375 with the anti-EGFR antibody cetuximab induced complete responses in all mice in a colorectal cancer xenograft model, and cetuximab also augmented the antitumor efficacy of GFH375 in pancreatic and lung cancer models. Combination with the RAF/MEK clamp avutometinib also enhanced the anti-tumor efficacy of GFH375. Additional combinations with GFH375 are currently being explored. Altogether, GFH375 is a potent and selective orally active inhibitor of KRAS G12D (ON/OFF) and demonstrated promising anti-tumor activity in multiple KRAS G12D tumor models in vivo as single agent and in combination with other anticancer therapies including cetuximab. These results support the ongoing clinical evaluation of GFH375 for treatment of patients with KRAS G12D mutant cancers (NCT06500676).

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Regulation of Gene Expression in Drug Resistance
Poster Session

#4398 Dysregulating transcription and epigenetic remodeling using dual BRD4 and CDK9 inhibition in pancreatic ductal adenocarcinoma.

Lucas J. Koeppel[1], Austin Stram[2], Robert J. Millikin[3], Md Shahadat Hossan[2], Ron Stewart[3], Jeremy D. Kratz[2]

[1]Department of Medicine, University of Wisconsin-Madison, Madison, WI,[2]Center for Human Genomics and Precision Medicine, Madison, WI,[3]Morgridge Institute for Research, Madison, WI

Background: Pancreatic ductal adenocarcinoma (PDAC) is the most common cause of mortality for pancreatic cancers with a five-year survival rate of only 13%. These malignancies acquire resistance to therapeutics through dysregulation of epigenomic and transcriptional signals, however few investigations have considered combination therapies to disrupt these pathways. BRD4 and CDK9 are both promising targets playing key roles in epigenomic and transcription regulation and are both upregulated in PDAC. Here, we evaluate the potential of dual BRD4 and CDK9 inhibition as a strategy to disrupt global transcriptional and epigenomic regulation in PDAC.
Methods: PDAC patient-derived cancer organoids (PCOs) were established from live tissue samples. PCOs were treated with single agents, VIP152 and ZEN3694, or the combination. ZEN3694 was treated continuously while VIP152 was removed after 24 hours to mimic *in vivo* pharmacokinetics. Western blotting was used to analyze phosphorylation of RNA polymerase II at serine 2 (pRPB1-s2) and anti-apoptotic proteins after 24 hours of treatment. Differential expression and peak calls were made on bulk mRNA and CUT&Tag sequencing (RNA-seq and CUT&Tag) performed on treated PCOs. PCOs were collected after 144 hours of treatment for RNA-seq and 48 hours for CUT&Tag. gProfiler was used for pathway analysis for both sequencing sets.
Results: Western blot analysis revealed combination therapy achieved downregulation of pRPB1-s2 and anti-apoptotic proteins compared to single agents. RNA-seq yielded no pathway dysregulation for VIP152 treated PCOs across 2 unique PDAC lines. The number of dysregulated pathways increased in the combination treated group compared to ZEN3694 in PDAC2 (ZEN3694 n=156, combination n=235), however this was not seen in PDAC1 (ZEN3694 n=69, combination n=37). Pathway analysis from CUT&Tag of PDAC1 for pRPB1-s2 revealed significant pathway dysregulation in the combination (n=186) with no single agent disruption. These pathways included biologically relevant gene sets such as ‘Pancreatic Cancer Subtypes’ (adj $p<0.001$), ‘response to stimulus’ (adj $p<0.001$), and ‘signal transduction’ (adj $p<0.001$) across both sequencing sets. CUT&Tag also revealed a significant decrease in pRPB1-s2 binding across all matched significant differential peak calls (VIP152=-0.21, ZEN3694=-0.23, combination=-0.74 $p<0.001$), and a decrease in open chromatin markers (H3K4me3=-0.64 $p<0.001$ and H3K27ac=-0.56 $p<0.001$) compared to both single agents (VIP152=-0.15 and -0.20; ZEN3694=-0.15 and -0.14).
Conclusion: Here, we show preclinical evidence that combination treatment of VIP152 and ZEN3694 synergistically dysregulates PDAC transcription and remodels the epigenomic landscape. Future directions will include exploring *Myc* amplified subtype specific selectivity and *in vivo* validation of toxicity and efficacy.

#4399 Systematic interrogation of cell states that drive inflammation tolerance and therapy resistance in cancer.

Srivatsan Raghavan[1], Evelyn Y. Tong[2], Aswanth H. Mahalingam[1], Zixin Chen[1], **Jacob Smigiel**[3], Tsukasa Shibue[3], Andrew Navia[3], Alex K. Shalek[2], Lorin Crawford[4], Ava Amini[4], Peter S. Winter[3]

[1]Dana-Farber Cancer Institute, Boston, MA,[2]Massachusetts Institute of Technology, Cambridge, MA,[3]Broad Institute of MIT and Harvard, Cambridge, MA,[4]Microsoft Research, Cambridge, MA

Cancers develop and evolve by overcoming numerous selective pressures including cell-intrinsic growth-inhibitory mechanisms, microenvironmental stresses, and therapeutic interventions. In addition to mutational mechanisms, cancer cell adaptation via transcriptional plasticity remains an important but poorly understood driver of cancer cell fitness. Specifically, when disease progression occurs after targeted therapy treatment, genomically-defined mechanisms of resistance are only identified in approximately 50% of patients, suggesting that non-genomic mechanisms play an equally important role. Thus, there is a pressing need to devise systematic and scalable methods to determine essential drivers of prognostically-relevant cancer cell states, and to engineer state-relevant models that can be applied to discover new therapeutic approaches that overcome these modes of resistance. Here, we present a strategy to systematically identify transcriptional drivers of inflammation- and therapy-resistant cancer cell states. We have successfully established a high-throughput screening pipeline to 1) over-express human transcription factor (TF) isoforms in a pooled format within cancer models; 2) perform positive-selection screens under a variety of selective pressures to define transcriptional mechanisms of inflammation tolerance and therapy resistance; 3) perform single-cell RNA sequencing to define families of TFs that drive specific cell state transitions and resistance phenotypes; and 4) conduct mechanistic studies to examine the downstream signaling networks that facilitate cancer cell growth in the setting of these selective pressures. We uncover TFs and states that drive resistance in a tissue- and perturbation-specific manner, as well as states that are robust to multiple different selective pressures. A subset of TFs and associated gene programs induce tolerance to chronic interferon exposure in our screens and are reflected *in vivo* in the setting of immunotherapy resistance. We also identify distinct TF families that drive resistance to pharmacologic KRAS inhibition. Notably, cancer cell states that confer resistance to KRAS inhibition also exhibit cross-resistance to other MAPK pathway inhibitors, suggesting that cell state adaption may contribute to multi-drug resistance as cancers progress. More broadly, our strategy enables the systematic identification and mapping of cell states that drive cancer cell fitness to *in vivo* clinical atlas datasets, providing a framework for interpretation of clinical phenotypes and potential predictive modeling of cell state evolution. Furthermore, the cell state-defined models developed here provide a substrate for new therapeutic discovery using cancer cell states as biomarkers, a key next step to developing combination strategies that overcome therapeutic resistance and improve outcomes for patients.

#4400 SRPK1: The dominant power of alternative splicing in tumor chemoresistance.

D. Duzgun, S. Oltean;
University of Exeter, Exeter, United Kingdom

Introduction: Abnormal pre-mRNA alternative splicing (AS) is critical in shaping tumor chemoresistance by modulating the function of genes involved in resistance mechanisms. The serine arginine protein kinase 1 (SRPK1) is a major regulator of AS by phosphorylating an important group of splice factors named SR-proteins. Our study aims to elucidate the molecular mechanisms by which SRPK1 promotes resistance to chemotherapeutic drugs.
Methods: Two cell lines resistant to cisplatin (CDDP) (breast cancer MDA-MB-231-CDDP-R and colon cancer HCT-116-CDDP-R) and one cell line resistant to docetaxel (DTX) (prostate cancer PC-3-DTX-R) were established by derivation from their parental using the pulse selection method. The CDDP and DTX resistance and the expression of SRPK1 of cells were verified by the IC_{50} measurements, MTT assay and western blotting (WB). The molecular mechanisms were determined using WB, siRNA, immunoprecipitation, the JC-10 apoptosis assay, and RT-PCR.
Results: The MTT assay revealed that resistant cell lines were about 5-17 times more resistant to chemotherapeutic agents than parental cell lines. SRPK1 protein was upregulated in MDA-MB-231-CDDP-R and PC-3-DTX-R cells; however, its expression in HCT-116-CDDP-R cells was markedly lower than that in parental cells. Using siRNA to knockdown SRPK1 expression lead to the re-sensitization of the MDA-MB-231-CDDP-R and PC-3-DTX-R cells to CDDP and DTX, respectively, but this effect was not observed in SRPK1-downregulated HCT-116-CDDP-R cells. Both WB and JC-10 assay demonstrated that co-treatment with CDDP, DTX and SRPK1 specific inhibitor, SPHINX31, remarkably upregulated pro-apoptotic markers (cleavage of caspase-8, caspase-3, and PARP) in MDA-MB-231-CDDP-R and PC-3-DTX-R cells, compared with CDDP, DTX and SPHINX31 alone conditions. Notably, in SRPK1 down-regulated HCT-116-CDDP-R cells, transient transfection of SRPK1 increased the sensitivity of the cells to the cytotoxicity of CDDP. JC-10 assay results also indicated an increase in apoptosis upon CDDP or a combination treatment with SPHINX31 in HCT-116-CDDP-R cells transfected with SRPK1. Moreover, immunoprecipitation assay showed that the level of SRSF1, phosphorylated by SRPK1, was elevated in CDDP and DTX-treated MDA-MB-231-CDDP-R and PC-3-DTX-R cells. Mechanistically, SRSF1 modulates various cancer-related splicing events, particularly the splicing of BCL2L1 and MCL-1. SPHINX31 dramatically promoted the pro-apoptotic Bcl-xS and Mcl-1S isoforms in MDA-MB-231-CDDP-R and PC-3-DTX-R cells as compared to control.
Conclusion: Our research reveals a key role for SRPK1 in chemoresistance by modulation of apoptotic genes splicing, suggesting a potential therapeutic avenue for alleviating challenges posed by chemoresistance.

#4401 Dual regulation of frizzled receptors (FZD1/7) by IGF2BP3: A novel oncogenic event promotes stem-like properties and reduces carboplatin chemosensitivity in Triple-negative breast cancer.

M. Cai, P. Yin, Z. Wang, Y. Huang, Y. Wang, K. Wu, X. Zhang, L. Shi, J. Wei, Q. Ding;
The First Affiliated Hospital with Nanjing Medical University, Nanjing, China

Background: Triple-negative breast cancer (TNBC) is primarily driven by cancer stem cells (CSCs), resulting in treatment resistance after chemotherapy. Emerging evidence suggests that TNBC-CSC transformation is strongly dependent on m6A modification. We aim to characterize the specific m6A regulatory factor in TNBC-CSCs and its underlying mechanism.
Methods: Transcriptome-based screening was used to determine the main m6A regulator in TNBC-CSCs. Mammosphere formation assay and flow cytometry were used to evaluate the role of insulin-like growth factor 2 mRNA-binding protein 3 (IGF2BP3) in regulating stemness in TNBC *in vitro*. Limiting dilution assay was used to assess the CSC frequency *in vivo*. Multi-omics data, including RNA immunoprecipitation sequencing (RIP-seq), Methylated RIP sequencing ((MeRIP-seq) and RNA sequencing data, were integrated to investigate the mRNA targets of IGF2BP3. RNA probes and IGF2BP3 truncations were generated to detect the direct binding location of IGF2BP3 and its target FZD1/7 mRNAs. Comet assay and homologous recomination (HR) assay were used to assess the effect of IGF2BP3 knockdown and FZD1/7 inhibitor, Fz7-21 in combination with carboplatin (CBP).
Results: We demonstrated that IGF2BP3 was the dominant m6A regulator in TNBC-CSCs. Its knockdown increased chemosensitivity and reduced tumor recurrence rate after CBP therapy *in vivo*. Furthermore, IGF2BP3-m6A-FZD1/7 axis might act as a pivotal oncogenic event in TNBC. Stem-like properties, including higher homologous recombination repair (HRR) efficacy and chemoresistance to CBP driven by frizzled receptors 1/7 (FZD1/7), were pharmacologically targeted by a small-molecule inhibitor, Fz7-21. Mechanistic studies unveiled that the RNA-recognition motif (RRM) domain of IGF2BP3 was the functional site to recognize m6A modification on the 3'-terminal of FZD1/7 mRNAs, leading to increased RNA stability. Enhanced stability elevated the expression levels of these two transcripts, leading to FZD1/7 heterodimerization and the activation of β-catenin pathway. This was marked by the nuclear translocation of non-phosphorylated (Ser37 and Thr41) β-catenin.
Conclusions: Our study highlights the critical oncogenic role of IGF2BP3 in promoting stem-like properties and reduces the TNBC sensitivity to CBP treatment. Targeting IGF2BP3 and FZD1/7 with inhibitors has the therapeutic potential to disrupt cancer stemness and reduce clinical drug dosage of CBP in TNBC patients.

#4402 A whole genomic CRISPR-Cas9 screen identifies the LAT3 neutral amino acid transporter as a major determinant of oxaliplatin resistance in colorectal cancer cells.

Nisha R. Pawar, Heidi M. Wade, Allison V. Mitchell, Connor Jewell, Lisa M. Jenkins, Robert W. Robey, Michael M. Gottesman

Center for Cancer Research, Laboratory of Cell Biology, NIH-NCI, Bethesda, MD

Colorectal cancer (CRC) is the second leading cause of cancer deaths, with a five-year survival rate of 65%. Oxaliplatin, developed as a derivative of the DNA-damaging agent cisplatin, is used as an adjuvant therapy for advanced CRC, yet 90% of patients develop resistance. Several mechanisms of oxaliplatin resistance have been proposed, but few are widely accepted. Genome-wide CRISPR activation and knockout screens were conducted to identify genetic changes that confer resistance to oxaliplatin in two CRC cell lines with distinct molecular backgrounds (SW620 and RKO). Guide RNA libraries covering approximately 18,000 human genes were utilized to identify several potential mechanisms of resistance. Guide RNAs corresponding to SLC43A1 (LAT3) were the most significantly enriched in knockout screens and depleted in activation screens, suggesting a potential role for the amino acid transporter LAT3 in oxaliplatin resistance. In vitro CRISPR knockout and overexpression of LAT3 in SW620 and RKO cell lines confirm increased resistance or sensitivity to oxaliplatin, respectively, and this correlated with levels of intracellular accumulation of oxaliplatin, suggesting its potential role as a novel transporter. Further studies to investigate the specificity of this mechanism of resistance to oxaliplatin in CRC and other cancers are ongoing to clarify the molecular basis of oxaliplatin resistance and uncover potential targeted therapeutic strategies for advanced CRC.

#4403 Enhancing chemotherapeutic activity against triple-negative breast cancer cells using induced electric fields.

G. Sarathy[1], S. Dhekni[1], A. M. Roy[1], M. Gatti-Mays[1], V. Subramaniam[2], **E. Shankar**[1];
[1]The Ohio State University Wexner Medical Ctr., Columbus, OH, [2]EMBioSys, Inc, Massachusetts., OH

Triple-negative breast cancer (TNBC), comprising approximately 15% of breast cancers, is particularly aggressive, often metastasizing to organs such as the lungs, brain, and bone marrow, leading to poor prognosis and resistance to therapy. Distant metastasis is responsible for most cancer-related deaths. Anthracyclines and taxanes remain the most active chemotherapeutic agents to treat TNBC, often in combination with immunotherapy. Of the taxanes, paclitaxel is widely used. In this abstract, we describe a novel, non-pharmacological, non-contact (i.e. electrode-free) approach that employs low-intensity, non-ionizing electromagnetic (EM) fields that potentiates standard of care (SOC) chemotherapeutic agents' paclitaxel and capecitabine. Using time-varying magnetic fields to penetrate cell interiors, these induced electric fields (iEFs) selectively alter the actin cytoskeletal machinery in TNBC cells, and significantly reduce their energy required for migration by hampering mitochondrial oxidative phosphorylation through the inhibition of succinate dehydrogenase activity. Importantly, this iEF treatment does not adversely affect normal breast epithelial cells. We show here that iEFs enhance the activity of paclitaxel and capecitabine in inhibiting TNBC growth. Furthermore, combination of anti-migration iEF with cytotoxic paclitaxel or capecitabine is found to hinder migration while also reducing cell viability. Moreover, iEF treatment was instrumental in significantly reducing the IC50 values (half-maximal inhibitory concentration) for paclitaxel (from 13 nM to 6 nM) and for capecitabine (from 1 mM to 0.7 mM) thus potentiating inhibition of cell viability by these cytotoxic agents. RNA sequencing was also performed in the presence and absence of iEF to determine the effect of paclitaxel or capecitabine on gene regulation. The sequencing data revealed that paclitaxel treatment in the presence of iEF upregulated 1058 genes and down regulated 1126 genes when compared to TNBC cells treated with paclitaxel in the absence of iEF. Collectively, our results show that iEF enhances the efficacy of chemotherapeutic agents' paclitaxel and capecitabine in inhibiting TNBC growth, viability, migration in addition to affecting the gene expression pattern. This work represents a critical step in improving current SOC for patients with TNBC for whom there are limited options. (This work is supported by DOD: W81XWH2010065, and Division of Medical Oncology award, OSU for Eswar Shankar)

#4404 BRPF1 inhibition restores chemosensitivity in triple negative breast cancer via ribosomal pathway modulation.

Ozlem Yedier Bayram[1], Ahmet Cingoz[1], Ebru Yilmaz[1], Ali Cenk Aksu[1], Beril Esin[1], Nareg Pinarbaşi-Degirmenci[1], Ayşe Derya Cavga[2], Beyza Dedeoglu[1], Buse Cevatemre[2], Hamzah Syed[3], MArtin Philpott[4], Adam P. Cribbs[5], Udo Opperman[5], Nathan A. Lack[6], Ceyda Acılan Ayhan[6], Tamer T. Onder[6], Tugba Bagci Onder[6]

[1]Koc University Research Center for Translational Medicine, Istanbul, Turkey,[2]Biostatistics, Bioinformatics and Data Management Core, Koc University Research Center for Translational Medicine, Istanbul, Turkey,[3]Department of Medical Biology, Biostatistics, Bioinformatics and Data Management Core, Koc University Research Center for Translational Medicine, Istanbul, Turkey,[4]Botnar Research Centre, Nuffield Department of Orthopedics, Rheumatology and Musculoskeletal Sciences, National Institute of Health Research Oxford Biomedical Research Unit (BRU), University of Oxford, Oxford, UK, Oxford, United Kingdom,[5]Oxford Centre for Translational Myeloma Research, University of Oxford, Botnar Research Centre, Nuffield Department of Orthopedics, Rheumatology and Musculoskeletal Sciences, National Institute of Health Research Oxford Biomedical Research Unit (BRU), University of Oxford, Oxford, United Kingdom,[6]Department of Medical Biology, Koc University School of Medicine, Koc University Research Center for Translational Medicine, Istanbul, Turkey

Triple-negative breast cancer (TNBC) is an aggressive cancer subtype lacking targeted therapies, leaving chemotherapy as the primary treatment option. However, acquired resistance to drugs like paclitaxel (Taxol) poses a significant challenge, often driven by complex genetic and epigenetic changes. Our study identifies bromodomain and PHD finger-containing protein 1 (BRPF1) as a critical epigenetic regulator of Taxol resistance in TNBC cells, linking it to ribosome biogenesis and ABCB1-mediated drug resistance. We developed Taxol-resistant TNBC cell lines using an escalating dose protocol. These cells exhibited sustained resistance to high doses of Taxol, accompanied by significantly elevated expression of ABCB1. Epigenome-wide CRISPR/Cas9 screens revealed that BRPF1 is required for the survival of Taxol-resistant cells. Genetic depletion or pharmacological inhibition of BRPF1 using small-molecule inhibitors sensitized resistant cells to Taxol. RNA sequencing and functional assays demonstrated that BRPF1 loss led to the downregulation of ribosomal biogenesis and translation-related genes, impairing global protein synthesis. Notably, BRPF1 was found to directly bind the ABCB1 promoter, regulating the elevated ABCB1 levels. These findings highlight BRPF1's dual role in maintaining translation machinery integrity and modulating ABCB1-driven chemoresistance. In conclusion, our work identifies BRPF1 as a novel therapeutic target for combating Taxol resistance in TNBC. Targeting BRPF1 disrupts ribosome biogenesis and reverses ABCB1-mediated drug resistance, offering a promising strategy to overcome chemotherapy failure in aggressive breast cancers. This work was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) 1003-216S461 and 1001-221S419.

#4405 Annexin A1 regulates lenvatinib resistance through mediating STAT3/S100A6 signaling cascade and M2 macrophage polarization in hepatocellular carcinoma.

Carmen Oi Ning Leung, Catherine Yu Jia Gu, Terence Kin Wah Lee

Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong, China

Hepatocellular carcinoma (HCC) is the sixth most commonly diagnosed cancer and third leading cause of cancer-related deaths worldwide. Lenvatinib has been approved by the FDA for front-line treatment of HCC patients at advanced stages. However, the survival benefit of lenvatinib is modest due to the development of acquired drug resistance. To understand its underlying mechanism, we have established lenvatinib resistant (LenR) HCC patient derived xenograft model (PDTX) via administration of four rounds of lenvatinib and the corresponding tumors from LenR and mock counterparts were analyzed by single cell RNA-sequencing (scRNA-seq). SCENIC analysis revealed that Annexin A1 (ANXA1) is enriched in LenR tumor. This finding was similar in patients with LenR HCC when compared to the sensitive group. An *in vitro* hepatocyte differentiation model showed that the expression of *ANXA1* peaked during the intermediate stages of liver development but was absent in mature hepatocytes but upregulated in HCC tumor tissues, indicating its role in the regulation of cancer stemness in HCC. Further functional analysis showed that ANXA1 drives cancer stem cell-driven lenvatinib resistance, as evidenced by *the in vitro* and *in vivo* limiting dilution and apoptosis assays. Through *in silico* analysis, SOX2 was predicted to be an upstream regulator of *ANXA1*, which was further validated using chromatin immunoprecipitation-qPCR and luciferase promoter assays in LenR and *SOX2*-silenced cells. To investigate potential downstream targets of ANXA1, differential gene expression (DEG) analysis was conducted on *the* $ANXA1^{High}$ and $ANXA1^{Low}$ groups within the LenR tumors. The results revealed S100A6 was the most significantly upregulated gene in the $ANXA1^{High}$ subpopulation, suggesting a possible regulatory relationship between ANXA1 and S100A6 expression. Further GESA analysis of DEGs of *the* $ANXA1^{High}$ to $ANXA1^{Low}$ group further showed that ANXA1 transcriptionally regulates S100A6 mediated through STAT3 activation. Consistently, a positive correlation between *STAT3* and *ANXA1/S100A6* levels was observed in the TCGA-LIHC cohort. Interestingly, ANXA1 secreted from HCC cells also drives cancer stemness and drug resistance in an autocrine manner. Analysis of the same set of scRNA-seq data revealed that tumor microenvironment (TME) in LenR tumors was altered by increasing the M2/M1 ratio of tumor-associated macrophages (TAM). Likewise, enhanced infiltration of M2 macrophages was observed in LenR PDTX and mouse HCC tumors. Using an *in vitro* assay with conditioned media, LenR-HCC-derived ANXA1 induced macrophage polarization towards the M2 phenotype. In conclusion, ANXA1 may regulate lenvatinib resistance intrinsically via the promotion of cancer stemness and extrinsically via reshaping immunosuppressive TME by inducing M2 macrophage polarization.

#4406 Temozolomide-resistant glioblastoma cells promote proteolytic activities of MMPs and PAI-1 to manifest invasive potentials.

H. T. Duong[1], T. H. Nguyen[1], S. Jeong[1], K.-S. Moon[2], C. Jung[1];
[1]Chonnam National University Medical School, Gwangju, Korea, Republic of, [2]Chonnam National University Hwasun Hospital and Medical School, Hwasun, Jeollanam-do, Korea, Republic of

Glioblastoma (GBM) is the most lethal and treatment-resistant tumor among brain cancers. Temozolomide (TMZ) is the standard chemotherapeutic agent for GBM, but the majority of patients experience recurrent tumors, leading TMZ-resistant cells to evade the cytotoxic effects of TMZ and continue to invade surrounding tissues. We have recently reported that transient treatment of high-dose TMZ in GBM cells upregulates matrix metalloproteinases 9 (MMP9), resulting in increased tumor cell migration and invasion through the activation of p38 and JNK MAPK pathways. To confirm potential adverse effects caused by TMZ, in this study, we constructed TMZ-resistant GBM cells through repeated culturing of cells by gradually doubling the TMZ concentration (12.5-300 μM). TMZ-resistant cells were analyzed for proteome profiling, revealing altered expression of several proteases including MMP2/9 and PAI-1. Compared to parental cells, resistant cells showed a significant increase in migration and invasion in vitro and either inhibition of JNK or suppression of MMP9 and PAI-1 dramatically counteracted the invasive potential of resistant cells. At the same time, media collected from TMZ-resistant cells significantly activated the migration and invasion potentials of normal fibroblasts. Further mechanistic studies demonstrated that TMZ-resistant cells retain higher promoter activities of MMP9 and PAI-1 through the regulation of MAPKs and β-catenin/TCF pathways. Since resistance to TMZ in GBM tumors leads to poor treatment outcomes and low survivability for patients, results from our study about potential adverse effects from ensuing TMZ-resistant cells may contribute to these malignant processes of GBM cells.

#4407 Taurine's potential role in tamoxifen resistant MCF-7 cells.

Yazan I. Hamadneh[1], Mohammad Alwahsh[2], Aya Hasan[2], Ala A. Alhusban[2], Osama M. Younis[1], Roland Hergenroder[3], Lama Hamadneh[4]

[1]School of Medicine, University of Jordan, Amman, Jordan,[2]Faculty of Pharmacy, Al-Zaytoonah University of Jordan, Amman, Jordan,[3]Leibniz-Institut fur Analytische Wissenschaften-ISAS-e.V., Dortmund, Germany,[4]Department of Basic Medical Sciences, Faculty of Medicine, Al-Balqa Applied University, Amman, Jordan

Taurine is a non-proteinogenic amino acid synthesized from cysteine. Although taurine does not contribute to protein synthesis, it regulates glutathione metabolism, upregulates proliferative and anti-oxidative pathways, downregulates pro-apoptotic pathways, and improves mitochondrial function in normal tissues. However, the role of taurine in tumorigenesis and resistance development in cancer, especially breast cancer, is not widely understood. We aim to study metabolic and cellular changes associated with the taurine metabolism pathway in tamoxifen resistant (TAM-R) MCF-7 breast cancer cell lines. Methods: Three TAM-R MCF-7 models were developed using two strategies. The first was achieved by incrementally increasing the dose until reaching a predetermined dose (TAM-P). The second was achieved by taking cell lines from the first method and continuously treating them with 1μM of tamoxifen (TAM-C). NMR was used to quantify the levels of intracellular metabolites, and HPLC/MS-MS was used to calculate taurine uptake by the cells from media. Gene expression levels of *ESR1, PI3KCA* and *PTEN* were measured by RT-PCR. GSCA: Gene Set Cancer Analysis platform was used to check the association of a gene set consisting of taurine metabolism pathway genes (*CDO1, ADO, GAD1, GAD2, CSAD*) with the regulation of cancer related pathways. Results: Taurine, choline, and glutathione concentrations were significantly higher in TAM-C compared to tamoxifen-sensitive control and TAM-P, while cystine was lower in TAM-C. Taurine uptake from the media in TAM-C and control were slightly higher than TAM-P. *ESR1* was 40 folds overexpressed in TAM-C and down regulated in TAM-P, while *PI3KCA* was overexpressed and *PTEN* was down regulated in both TAM-C and TAM-P. Moreover, there was a positive correlation between taurine metabolism pathway gene set and activation of ER pathway. Conclusion: The significant increase in taurine and decrease in cysteine in the TAM-C group cannot be explained by the marginal increase in taurine uptake alone, which necessities increased synthesis of the taurine. Overexpression of *ESR1* in TAM-C could be caused by upregulation of the taurine synthesis pathway. However, overexpression of PI3KCA and down regulation of PTEN indicates reliance on *PI3K/AKT/PTEN* pathway instead of estrogen signaling pathway. Moreover, increased glutathione and choline concentrations increased antioxidative capacity, mitochondrial function, and resistance to oxidative stress.

#4408 CRISPR/Cas9 knockout and AI-based drug screening for novel gene targets in ovarian cancer chemoresistance.

Jinjiang Wang[1], Jiaxi Li[2], Jiao Liu[1], Kit-Ying Chan[1], Ho-Sze Lee[1], Zhewei Zhang[1], Joseph Kwong[3], Tat-San Lau[1], Chi-Chiu Wang[1]

[1]The Chinese University of Hong Kong, Hong Kong, Hong Kong,[2]The University of Hong Kong, Hong Kong, Hong Kong,[3]Keele University, Keele, United Kingdom

Introduction: Ovarian cancer, characterized by its heterogeneity and resistance to conventional therapies such as cisplatin (CDDP), presents a significant clinical challenge. To address these challenges, we combined genome-scale CRISPR/Cas9 knockout screening, single-cell-CRISPR Purterb RNA sequencing (scRNA-seq), and AI-driven virtual screening to identify novel chemoresistance mechanisms and evaluate potential therapeutic strategies. This integrated approach aims to reveal genetic determinants and actionable therapeutic targets to improve treatment outcomes.

Methods: We performed genome-scale CRISPR/Cas9 knockout screening in ovarian cancer cell lines treated with CDDP, erastin, and a combination of elesclomol and copper (ES+Cu), identifying genes involved in drug resistance mechanisms. Differential gene candidates will be prioritized based on its strong association with resistance across the drug treatments. AI-based virtual screening will be employed to identify novel drugs targeting the candidates. Additionally, surviving cells from drug treatments will be subjected to scRNA-seq to reveal distinct cell cluster harboring resistance-related gene signatures. We also established ovarian cancer cell lines resistant to CDDP, erastin, and ES+Cu to validate the therapeutic targets and efficacy of the repurposed drugs in both resistant and wild-type cell lines.

Results: CRISPR screening identified several key genes associated with drug resistance, with YTHDC2 emerging as a central target. Pathway analysis highlighted its role in regulating survival and proliferation in the context of drug-induced stress. AI-driven virtual screening pinpointed Proguanil, Calcipotriol, and Sildenafil as high-affinity binders to YTHDC2, suggesting their potential as targeted therapeutics. scRNA-seq analysis of resistant cancer cells identified key gene cluster with GADD45A and ZFAS1 associated with cisplatin resistance. Gene Ontology and STRING pathway analyses revealed their involvement in DNA damage response and apoptosis regulation. Drug-response assays demonstrated that Proguanil, Calcipotriol, and Sildenafil effectively suppressed cell proliferation in the resistant cell lines. These findings support their potential to overcome the chemotherapy resistance.

Conclusion: This study integrates CRISPR-based screening, scRNA-seq, and virtual screening to provide the first comprehensive framework for addressing drug resistance in ovarian cancer. The identification of YTHDC2 as a key resistance-related gene and the validation of Proguanil, Calcipotriol, and Sildenafil as potential therapeutics underscore the translational potential. These findings offer new avenues for precision oncology, with the goal of improving outcomes for ovarian cancer patients.

#4409 Exploring PBX1's role in enzalutamide resistance in advanced prostate cancer.

Ephraim J. Gardner[1], Surendra Gulla[2], Leslie McClinton[2], Jonathan E. Bard[3], Remi Adelaiye-Ogala[2]

[1]Department of Pharmacology and Toxicology, University at Buffalo, Buffalo, NY,[2]Department of Medicine, University at Buffalo, Buffalo, NY,[3]Department of Biochemistry, University at Buffalo, Buffalo, NY

Aggressive prostate cancer (PCa) is often driven by upscaled activity of the androgen receptor (AR), and second-generation AR antagonists such as Enzalutamide have become standard therapeutics for men with advanced PCa. While patients initially respond to treatment, many develop resistance, with disease progression and poor prognosis. We have focused on a mechanism involving an AR cistrome switch from a canonical to a non-canonical cistrome as PCa becomes Enzalutamide-resistant. As collaborating protein partners drive transcriptional activity, we employed core regulatory circuitry analysis in Enzalutamide-resistant PCa cell line models to identify novel Transcription Factor (TF) candidates that may be involved in non-canonical AR cistrome reprogramming. One identified candidate is Pre B-Cell Leukemia Homeobox 1 (PBX1), a TALE homeodomain TF involved in embryonic development and cellular differentiation. PBX1 is known to work with other TFs to modulate gene expression and has been implicated as an oncogenic driver in hematologic and solid cancers, including PCa. Our group has shown that PBX1 is highly expressed in prostate cancer cell line models of castrate- and Enzalutamide-resistance compared to Enzalutamide-sensitive models. Through multi-ome single-cell ATAC + gene expression analysis of a heterogenous patient-derived xenograft (PDX) model, we identified clusters of cells that are enriched for PBX1, AR, and non-canonical AR target CREB5, while simultaneously expressing low levels of KLK3, a prominent canonical AR target. Based on these findings, we hypothesize that PBX1 works in tandem with AR to drive non-canonical AR cistrome and mediate Enzalutamide resistance in advanced PCa. To test this hypothesis, we first determined the impact of silencing PBX1 activity in Enzalutamide-resistant PCa models. By targeting PBX1 pharmacologically in our cell lines and PDX models, we have observed an anti-proliferative effect specifically in a castration-resistant, Enzalutamide-resistant cell line model. This effect was not observed when treating an Enzalutamide-sensitive cell line model, which has low PBX1 expression. Our observations were consistent across several models and our ongoing mechanistic studies will provide further insight into PBX1’s exclusive role in Enzalutamide-resistant CRPC. In addition, we are currently assessing the impact of inhibition or perturbation of PBX1 in Enzalutamide-resistant PCa cell lines and PDX models on canonical and non-canonical AR cistrome activity using multi-omic approaches.

#4410 Targeting MEIS2 in platinum resistant ovarian cancer.

Yinu Wang[1], Natalia Obrochta[2], Junzui Li[3], Daniela Matei[4]

[1]Northwestern University - Chicago, Chicago, IL,[2]Department of Neurobiology, Northwestern University, Chicago, IL, Northwestern University - Chicago, Chicago, IL,[3]Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Northwestern University - Chicago, Chicago, IL,[4]Department of Obstetrics and Gynecology, Northwestern University - Chicago, Chicago, IL

Introduction: Ovarian cancer has the highest mortality rate among gynecological cancers. Relapse of the disease accompanied by chemoresistance is common; however, the biological causes are still unclear. Although initial chemotherapy succeeds in debulking the burden of OC, it promotes emergence of cancer stem cells (CSCs), which are responsible for driving tumor relapse and developing chemo-resistant tumors in OC. *Myeloid Ecotropic Insertion Site 2 (MEIS),* is a homeobox transcription factor (TF), deregulated in various cancers, which plays critical roles in regulating stem cell identity and cell fate decision. Here, we examined the role of MEIS2 in regulating stemness features that promote chemoresistance of OC, and the impact of a MEIS2 blockade on OC initiation and progression.
Methods: Paired isogenic OC cell lines (OVCAR4/5 and COV362 platinum sensitive (Pt-S) and resistant (Pt-R)) were used. MEIS2 expression level in these cells were assessed by q-RT-PCR and Western Blotting. The effects of MEIS2 inhibition (by using a pharmacological inhibitor MEIS2i and biological knockdown (KD) on OC cell survival were assessed using colony formation and cell proliferation assays, and on stemness features by flow cytometry for analysis of ALDH+ cancer stem cell (CSC) population and measurement of stemness-related gene expression. MEIS2 direct binding targets in OC cells, were examined using ChIP-seq. The anti-tumor effects of MEIS2 inhibition on Pt-R OC cells were tested in vivo.
Results: MEIS2 expression levels were increased in Pt-R vs. Pt-S OC cells at mRNA and protein levels. Treatment with MEIS2i (500nM-1μM) reduced the number of colonies in OC cells by at least 2-fold change ($p<0.001$); however, proliferation of cells was not diminished. Treatment with MEIS2i (250nM-1μM, 48 hours) decreased ALDH+ CSCs population by at least ~2-fold ($p<0.05$), and inhibited the expression of stemness associated genes ALDH1A1, ALDH1A3, and Sox2 ($p<0.05$), compared to vehicle control. Knock down of MEIS2 decreased the CSC population from 29.4% to 14.3% ($p<0.05$) and inhibited stemness gene expression ($p<0.05$). MEIS2 knockdown inhibited tumor initiating capacity in vivo. OC xenografts derived from MEIS2 knockdown OVCAR5 cells were more responsive to carboplatin in vivo ($p<0.05$) compared with control xenografts. By integrating ChIP seq and RNA seq, we identified stemness-associated genes, ALDH1A2 and CD44 as direct MEIS2 targets contributing to maintaining stemness and chemo-resistance.
Conclusions: Overexpression of MEIS2 contributes to stemness in OC. MEIS2 blockade inhibited stemness traits that contribute to chemotherapy resistance and tumor relapse. Our results put forward MEIS2 as a potential new target.

#4412 Characterization and targeting of an androgen receptor pre-mRNA stem loop structure to inhibit AR-V7 splicing in prostate cancer.

Marek D. Zorawski, James P. Falese, Riley S. Spingler, Jason A. Somarelli, Andrew J. Armstrong, Amanda E. Hargrove

Duke University School of Medicine, Durham, NC

Alternatively spliced mRNAs can contribute to aggressiveness and resistance to androgen receptor pathway inhibitors (ARPIs) in castration-resistant prostate cancer (CRPC). Among these spliced mRNAs, the androgen receptor (AR) splice isoform AR-V7 participates in AR signaling independent of androgen levels or ARPIs, such as enzalutamide. AR-V7 detection correlates strongly with ARPI treatment resistance and poor patient outcomes in CRPC. AR-V7 incorporates cryptic exon 3 (CE3) within the *AR* gene that prematurely terminates the full-length transcript. While several proteins have been described that may promote splicing of CE3, the impact of the pre-mRNA structures in this region is unknown. Using selective 2′-hydroxyl acylation analyzed by primer extension with mutational profiling (SHAPE-MaP), we developed the first experimentally informed pre-mRNA secondary structure model of the CE3 3′ splice site from cellular RNA. In this structural model, we observe a novel stem loop structure, which we hypothesized could inhibit AR-V7 biogenesis. We used an AR-V7 minigene assay to evaluate structure-modulating mutations in AR-V7 biogenesis and observed concordant changes in AR-V7 abundance in response to stabilization of this repressive structure, leading us to term this structure the cryptic exon 3-splicing inhibitory stem loop (CE3-SISL). Protein binding site identification of the region through the Δ-SHAPE pipeline suggests a repressive protein interaction that may be lost in androgen independence and ARPI resistance. Together, these data suggest the importance of RNA structure in AR-V7 biogenesis and indicate a potentially targetable interface for the development of AR-V7-depleting agents, as we have exemplified using structure-guided antisense oligonucleotides (ASOs) that are able to inhibit AR-V7 splicing during enzalutamide treatment. These ASOs would represent the first RNA structure-targeted splicing modulators being developed for patients with AR-V7-driven CRPC.

#4414 Targeting CCL5 signaling pathway in endocrine resistant breast cancer therapy.

Dominick Lomonaco, **Kideok Jin**

Albany College of Pharmacy & Health Sciences, Albany, NY

Estrogen receptor (ER) is the primary transcription factor driving ~75% of all breast cancers and serves as the target for endocrine therapies. However, resistance to endocrine therapy remains a critical clinical challenge, particularly in metastatic ER-positive breast cancers harboring ESR1 point mutations in the ligand-binding domain, such as Y537S and D538G. These mutations are frequently identified in metastatic tumors and circulating tumor DNA (ctDNA) from patients undergoing endocrine treatment. Despite the efficacy of endocrine therapies combined with CDK4/6 inhibitors, resistance mechanisms and therapeutic vulnerabilities in ESR1-mutated metastatic breast cancer remain poorly understood. Our preliminary studies explored the crosstalk between endocrine-resistant breast cancer (ERBC) cells and stromal cells. By co-culturing seven ERBC cell lines, including genome-edited models of ESR1 mutations, with four stromal cell types, we screened 28 secretome combinations using cytokine antibody arrays. This analysis identified significant upregulation of CCL5 and endoglin in the tumor-stromal microenvironment. We hypothesize that CCL5 mediates endocrine therapy resistance and facilitates the metastatic transition of ERBC cells through interactions with stromal components. To test this, we used CRISPR-Cas9 to generate CCL5 knockout EO771 cells and validated the loss of CCL5 expression via qRT-PCR and ELISA. Functional assays revealed significantly reduced proliferation and migration in CCL5 KO cells compared to wild-type cells. In vivo studies using an orthotopic mouse model demonstrated decreased CCL5 KO tumor growth and metastasis, and treatment with maraviroc (a CCR5 inhibitor) selectively reduced wild-type cell viability. These findings establish CCL5 as a critical regulator of ERBC growth and metastasis, supporting its potential as a therapeutic target. This research provides a foundation for designing clinical trials targeting CCL5-mediated resistance mechanisms in endocrine-resistant breast cancer.

#4415 Nuclear receptor liver receptor homolog-1 (LRH-1) contributes to enzalutamide-resistance in castration-resistant prostate cancer.

Tiantian Gao, Franky Leung Chan

School of Biomedical Science, The Chinese University of Hong Kong, Hong Kong, Hong Kong

Introduction: Prostate cancer is the most common cancer in males worldwide and the second leading cause of cancer-related deaths. Targeting androgen signaling through androgen deprivation therapy (ADT) is an important treatment option for prostate cancer, although the majority of patients eventually progress to castration-resistant prostate cancer (CRPC). Several second-generation anti-androgen drugs including enzalutamide are conventionally used for CRPC patients. However, resistance to potent anti-androgen treatments inevitably develops in prostate cancer patients. Liver receptor homolog-1 (LRH-1, NR5A2) is a member of nuclear receptor super family, which is characterized to be involved in the regulation of steroids, bile acid and cholesterol homeostasis, glucose metabolism and cell proliferation. Our previous study has found that LRH-1 can promote the intratumoral androgen biosynthesis to support the growth of CRPC cells. Meanwhile, our unpublished results showed that LRH-1 was negatively regulated by androgen receptor (AR) signaling pathway, suggesting that LRH-1 expression may play roles in anti-androgen drug resistance, especially in enzalutamide-resistant prostate cancer.
Methods: Prostate cell line 22Rv1 is characterized as an enzalutamide-resistant prostate cancer cell line. CCK8 assay was performed to measure the viability of cells treated with enzalutamide and LRH-1 antagonist (ML180). ChIP-qPCR was performed to validate the potential binding sites of LRH-1 in AR promoter. Luciferase reporter gene assay using constructed reporters driven by different lengths of AR promoter and various LRH-1 variant mutants was performed to validate the activation of AR promoter by LRH-1.
Results: Public dataset analysis revealed that high LRH-1 expression was associated with poor disease-free survival rate. LRH-1 expression was elevated after long-term enzalutamide treatment. We found that knock-down of LRH-1 attenuated cell viability of enzalutamide-resistant cells and LRH-1 antagonist ML180 could reverse the drug resistance in prostate cancer cells. Furthermore, we demonstrated that LRH-1 could bind to and activate AR promoter region and induce AR expression, suggesting a possible mechanism on the occurrence of enzalutamide resistance in CRPC.
Conclusion: LRH-1 could contribute to the induction of enzalutamide resistance in prostate cancer via specific regulation of AR gene. Our results also indicated that targeting LRH-1 could be a potential therapeutic management for enzalutamide resistance in CRPC. (This work is supported by RGC-Granted Research Fund (Project number: 14107623), Hong Kong Research Grants Council.)

#4416 Impact of vaping on FoxM1-mediated chemoresistance mechanisms in lung adenocarcinoma.

Rizwana Begum[1], Shilpa Thota[1], Naveen Chintala[1], Biplov Sapkota[1], Dayanidhi Raman[2], Joseph Francis[1]

[1]Louisiana State University, Baton Rouge, LA,[2]University of Toledo, Toledo, OH

Background and Purpose: E-cigarette usage among students and adolescents is increasingly popular, attributed to appealing flavors, discreet usage, and the perception of being a safer alternative to traditional tobacco. However, this perception is misleading as e-cigarettes carry significant health risks, especially concerning cancer development due to the presence of carcinogenic compounds like formaldehyde, acrolein, nitrosamines, and polyaromatic hydrocarbons found in flavored e-liquids and their aerosols. These xenobiotics may impact DNA repair and cell cycle. FoxM1, a pivotal transcription factor within the forkhead box protein family, regulates genes crucial for DNA repair and cell cycle progression, such as BRCA1, BRCA2, and XRCC1. These genes are instrumental in mediating chemoresistance across various cancers via drug transporters, Wnt/β-catenin, and NOTCH signaling pathways. However, the specific impact of e-cigarette aerosol exposure on FoxM1 activity and subsequent chemoresistance is not well understood. Hence, we hypothesized that exposure to menthol-flavored e-cigarette aerosol would increase FoxM1 expression in lung adenocarcinoma cells, thus enhancing chemoresistance through Wnt/β-catenin and NOTCH signaling pathways.

Methods: The human lung adenocarcinoma cell line Calu-3 was exposed to menthol-flavored e-cigarette aerosol using a Vitrocell® VC10 system, following the CORESTA recommended method No. 81. We quantitatively assessed changes in FoxM1 and its downstream effectors in the Wnt/β-catenin and NOTCH pathways using qPCR, immunoblotting, and immunohistochemistry.

Results: Our results demonstrated a significant upregulation of FOXM1 at both mRNA (> 3-fold) and protein (> 1.5-fold) levels. This upregulation was correlated with increased expression of β-catenin (> 1.5-fold) and enhanced transcriptional activity of c-MYC (> 3-fold) and Cyclin D1 (> 2-fold), critical mediators of Wnt signaling. Furthermore, activation of the NOTCH signaling pathway was confirmed by the upregulation of Mastermind-like (MAML) protein (1.5-fold). Notably, siRNA-mediated knockdown of FOXM1 resulted in the downregulation of Wnt 3a (> 2-fold) and β-catenin (> 1.5-fold) levels, highlighting FoxM1's critical role in mediating the signaling pathways affected by vaping that are intricately linked to chemoresistance.

Conclusion: Our findings suggest that vaping can enhance cancer chemoresistance through the activation of FoxM1 and its downstream signaling pathways. This study contributes to a deeper understanding of how lifestyle choices like vaping can impact cancer treatment outcomes, underscoring the necessity to reevaluate the perceived safety of e-cigarettes.

#4417 Identification of signaling and apoptotic pathway alterations in oxaliplatin resistant HCT116 colorectal cancer cells.

Aliza Jafri[1], Sheerien Rajput[2], Areeb Ahmed[2], **Azhar Hussain Rajabali**[3], Kulsoom Ghias[3]

[1]University of Karachi, Karachi, Pakistan,[2]Centre for Regenerative Medicine and Stem Cell Research, Aga Khan University Hospital - Karachi, Pakistan, Karachi, Pakistan,[3]Biological and Biomedical Sciences, Aga Khan University Hospital - Karachi, Pakistan, Karachi, Pakistan

Background: Chemoresistance remains a significant barrier in colorectal cancer (CRC) treatment, particularly with oxaliplatin, a key first-line chemotherapy agent. This study is therefore aimed to develop an *in vitro* model of oxaliplatin-resistant HCT116 cells and investigate the associated morphological and molecular adaptations to identify potential dysregulated survival and apoptotic pathways that are playing a role in developing chemoresistance.
Methods: HCT116 CRC cells were cultured, and resistance was induced by stepwise oxaliplatin exposure, with recovery phases between treatments. Morphological changes during resistance development were observed using phase-contrast microscopy. Flow cytometry was performed to analyze cell cycle distribution and apoptotic cell populations. Gene expression of key survival pathways, including Wnt/β-catenin, NFκB, and PI3K/Akt, was assessed using qPCR to identify molecular alterations associated with resistance.
Results: Parental HCT116 cells displayed cuboidal morphology, high proliferation (Ki-67 positivity), and epithelial characteristics (moderate E-cadherin and ALDH1 expression). Resistant cells exhibited increased morphological heterogeneity, cell detachment, and features of epithelial-mesenchymal transition (EMT). Flow cytometry revealed a significant reduction in the sub-G1 population and an increase in the G2/M phase, suggesting diminished apoptosis and cell cycle dysregulation in oxaliplatin resistant HCT116 cells as compared to wild type. Gene expression analysis showed a marked upregulation of β-catenin, NFκB, and PI3K/Akt pathway components, coupled with increased expression of EMT markers (Snail, Vimentin) and drug efflux genes (ABCG2, ABCC1) in oxaliplatin resistant cells as compared to wild type HCT116 cells suggesting a role of these survival pathways and genes in induction of resistance.
Conclusion: Oxaliplatin resistance in HCT116 CRC cells is characterized by morphological plasticity, altered cell cycle regulation, and activation of survival pathways, including Wnt/β-catenin, NFκB, and PI3K/Akt. These findings highlight the complexity of chemoresistance and provide a basis for identifying novel targets to enhance treatment efficacy in CRC.

#4418 Epigenetic reactivation of estrogen receptor for treatment of triple-negative breast cancer.

Jer-Yen Yang, Chia-Hua Cheng, Wan-Chi Lu, Yu-Wa Lei, Alice CJ Chang

China Medical University, Taichung City, Taiwan

About 12% of breast cancers are invasive breast cancers, including triple negative breast cancer (TNBC), which manifest early metastasis and recurrence with limited treatment options. Therefore, a better understanding of the molecular mechanisms of TNBC holds the key for effective treatment of TNBC. Tamoxifen, a first line treatment for estrogen receptor (ERα) positive breast cancer, serves as an ERα antagonist by binding to ERα and blocking estrogen receptor signaling to suppress estrogen receptor dependent cancer cells. It is known that estrogen receptor gene (ESR1) is hypermethylated and hence ERα expression is deficient in TNBC. As a result, TNBC cells are highly resistant to tamoxifen, and the strategy used to resensitize tamoxifen response in TNBC has yet to be established.

We have discovered that a DNA demethylation enzyme tet methylcytosine dioxygenase 2 (TET2) plays a critical role in reversing DNA methylation of estrogen receptor gene (ESR1) and thereby enables to reactive estrogen receptor expression in TNBC, resulting re-sensitization of TNBC cells to tamoxifen. It is also reported that a clinically used anti-diabetic drug, metformin, can enhance TET2 protein stability and expression. Therefore, we hypothesize that through TET2-mediated reactivation of estrogen receptor, metformin combined with tamoxifen can synergistically suppress TNBC growth.

To test the hypothesis, we first investigated the effects of vehicle, metformin, tamoxifen, and the combination therapy (metformin + tamoxifen) on cell growth and migration/abilities of TNBC cell lines, MDA-MB-231 and BT549. We then analyzed the effects of the combination therapy on tumor growth and metastasis using a TNBC xenograft mouse model. Furthermore, immunohistochemical staining was used in patient tissue samples to analyze the correlation of TET2 and estrogen receptor expression levels to clinicopathological characteristics. Our data has revealed that metformin-tamoxifen combination therapy significantly inhibits TNBC cell growth compared to the single drug and vehicle treatment groups. Metformin-tamoxifen combination therapy more effectively suppresses TNBC cell migration compared to the single drug and vehicle treatment groups. Furthermore, using Combination Index (CI) and Dose Reduction Index analysis, we have demonstrated that metformin-tamoxifen combination therapy can synergistically inhibit TNBC cell growth compared to the single drug and vehicle treatment groups, where the metformin dose could be reduced by approximately 5-fold, and tamoxifen dose could be reduced by approximately 20-fold.

Together, these findings suggest that metformin-tamoxifen combination therapy could produce a significant synergy, allowing for lowered drug doses and thus reduced toxicity, and provide clinical implications for a novel, effective, and safe TNBC treatment regimen.

#4419 Cancer exosomal microRNAs promote global mRNA 3' end shortening, multidrug chemoresistance, and cancer stemness.
Kenneth K.W. To

School of Pharmacy, Chinese University of Hong Kong (CUHK), Hong Kong, Hong Kong

Background and Aim: Multidrug resistance (MDR) is a major cause of failure in cancer chemotherapy. It is commonly caused by efflux of anticancer drugs by ABC transporters including ABCG2. ABCG2 is also a cancer stem cell survival factor to drive cancer recurrence. Preferential production of mRNA isoforms with shortened 3'untranslated region (3'UTR) in cancer is associated with poor drug response. We previously reported that mRNA 3'UTR shortening could trigger ABCG2 overexpression in MDR cancer. Exosomes are cell-derived vesicles that are present in various biological fluids. Tumor-derived exosomes are emerging as important cell-to-cell couriers of oncogenic materials to mediate tumorigenesis. This study investigated the enrichment of specific microRNAs in MDR colon cancer derived exosomes to promote global mRNA 3'UTR shortening and spread chemoresistance.
Method: Exosomes were isolated from a sensitive (S1) or resistant (S1M1-80) colon cancer cell lines conditioned medium by differential centrifugation, denoted as exo/S and exo/R, respectively. The transfer of microRNAs from exo/S or exo/R to a few sensitive colon cancer cell lines was evaluated. The shortening of ABCG2 mRNA 3'UTR in the exosome-donor and -recipient cancer cells was investigated by 3'RACE assay. MicroRNA microarray was conducted to identify differentially expressed microRNAs in drug sensitive cells after incubation with exo/R. Western blot and qPCR analysis were conducted to measure relevant protein and transcript expressions.
Result: By 3'RACE assay, the shortening of ABCG2 3'UTR observed in the exosome donor resistant cells was also detected in the exosome recipient sensitive cells. MicroRNA profiling was conducted on 3 drug-sensitive and ABCG2^{low} colon cancer cell lines (HCT116, SW480, and SW620) incubated with exo/S or exo/R. MiR-1260b was found to be remarkably upregulated in all 3 sensitive cell lines after incubation with exo/R. Intriguingly, the polyadenylation factor PABPN1 was found to be remarkably downregulated in exo/R-treated cells, which was accompanied by higher expression of ABCG2 and a few cancer stem cell markers (DCLK1, LGR5 and BMI1), and shortening of their mRNA 3'UTR. PABPN1 was subsequently confirmed to be a miR-1260b target. MiR-1260b inhibitor was able to reverse the downregulation of PABPN1 in HCT116 cells treated with exo/R, which subsequently suppressed ABCG2 level and reversed the MDR phenotype. Furthermore, while exo/R treated HCT116 form tumorspheres readily *in vitro*, miR-1260b inhibitor effectively reduced tumorsphere formation.
Conclusion: MDR cells-derived exosomes may spread chemoresistance by transferring a miR-1260b/PABPN1/ABCG2 regulatory machinery to sensitive cells. We will set the stage to develop novel therapies aimed at interfering with the master regulators that mediate the microRNA sorting into exosome and mRNA 3'UTR shortening during MDR development.

#4420 Identification of a novel marker gene predicting recurrence after chemotherapy with DNA-damaging agents by using drug-resistant gastric cancer organoids.

Kenji Harada[1], Naoya Sakamoto[1], Shingo Sakashita[1], Motohiro Kojima[1], Kazuaki Tanabe[2], Hideki Ohdan[3], Kohei Shitara[4], Takahiro Kinoshita[5], Genichiro Ishii[6], Wataru Yasui[7], Atsushi Ochiai[8], Shumpei Ishikawa[1]

[1]Division of Pathology, National Cancer Center, Exploratory Oncology Research & Clinical Trial Center, Kashiwa, Japan,[2]Department of Perioperative and Critical Care Management, Hiroshima University, Hiroshima, Japan,[3]Department of Gastroenterological and Transplant Surgery, Hiroshima University, Hiroshima, Japan,[4]Department of Gastroenterology and Gastrointestinal Oncology, National Cancer Center Hospital East, Kashiwa, Japan,[5]Division of Gastric Surgery, National Cancer Center Hospital East, Kashiwa, Japan,[6]Department of Pathology and Clinical Laboratory, National Cancer Center Hospital East, Kashiwa, Japan,[7]Department of Molecular Pathology, Hiroshima University, Hiroshima, Japan,[8]Research Institute for Biomedical Sciences, Tokyo University of Science, Noda, Japan

Despite recent advances in gastric cancer therapy, chemotherapy resistance and lack of methods for selecting combination regimens remain major problems. Organoids, which recapitulate the *in vivo* micro-environment, can be established from surgical specimens with a high success rate, and are widely used for drug sensitivity assays. Cancer organoids are also known to contain abundant amounts of cancer stem cells (CSCs), a subpopulation of tumor cells with self-renewal and differentiation capabilities. CSCs are thought to contribute to tumor initiation, metastasis, and acquiring resistance to chemotherapy by altering their phenotype during the treatment.In this study, we aimed to discover a novel biomarker for predicting multidrug resistance using gastric cancer organoids (GCOs), which provide *in vivo*-like culture system that more closely resembles tumor cell organization than traditional cell lines, to understand the mechanism of multidrug resistance in cells, including CSCs, under *in vivo* conditions and to identify novel biomarkers to predict chemoresistance.We evaluated 5-fluorouracil or oxaliplatin-resistant GCOs to find novel biomarkers that reflect multidrug resistance in gastric cancer. To examine the resistance mechanisms, RNA-seq analysis and *ex vivo* drug sensitivity testing were performed. The association of biomarkers with patient prognosis and chemotherapy efficacy was evaluated using three original cohorts with a total of 230 cases. The results were also validated with two independent public cohorts and a single-cell RNA sequence data. Increased expression of *PI3*/Elafin was detected in all 5-fluorouracil or oxaliplatin-resistant GCOs. Our findings suggest a potential association of *PI3*/Elafin expression with ribosome biosynthesis and RNA metabolism under organoid conditions. We also found that *PI3*/Elafin overexpression promotes 5-fluorouracil/oxaliplatin/cisplatin resistance but not paclitaxel resistance. Immunohistochemical evaluation of Elafin expression revealed that the Elafin-positive gastric cancer group had a poorer outcome, especially in terms of time-to-recurrence. Elafin positivity was also an independent predictor of relapse after chemotherapy with DNA-damaging agents. *PI3*/Elafin promotes DNA-damaging drug resistance through multiple downstream regulations related to RNA and ribosomal metabolism. We demonstrated that *PI3*/Elafin contributes to the acquisition of resistance to 5-fluorouracil and platinum drugs in *in vivo*-like environments and identified GOs related to ribosome and RNA metabolism as novel *PI3*/Elafin downstream targets. Our results provide new insights into multidrug resistance mechanisms and highlight *PI3*/Elafin as a novel tool for precise stratification of patients with gastric cancer.

#4421 Chemotherapy-induced DNA damage triggers caspase-dependent degradation of N^6-methyladenosine reader YTHDF proteins, reshaping the cancer transcriptome.

S. Lee, S.-Y. Cho;
Seoul National University College of Medicine, Seoul, Korea, Republic of

N^6-methyladenosine (m6A) modification plays a crucial role in DNA damage response (DDR), which is the primary mechanism targeted by most chemotherapeutic agents. Here, we investigated the dynamic regulation of m6A-related molecules during chemotherapy-induced DNA damage. Anthracycline treatment led to a significant reduction in protein levels, but not mRNA levels, of m6A readers YTHDF1, YTHDF2, and YTHDF3. It was consistently observed with other chemotherapeutic agents, including 5-fluorouracil (5-FU) and oxaliplatin. Notably, this reduction occurred independently of p53 status, as YTHDF proteins remained unchanged upon p53 overexpression alone, while their reduction was still observed in p53-knockout cells treated with doxorubicin. Mechanistic studies showed that the decrease in YTHDF proteins was primarily associated with topoisomerase II inhibition rather than reactive oxygen species (ROS) generation, which are both known mechanisms of doxorubicin. Further investigation revealed decreased protein stability of YTHDFs upon doxorubicin treatment, which was primarily attributed to activated caspase-mediated degradation during apoptosis rather than proteasomal degradation or autophagy. RNA sequencing analysis of YTHDF2-overexpressing cells showed differential enrichment of MYC targets V1, V2 pathway upon doxorubicin treatment, indicating a potential role for YTHDF2 in modulating oncogenic pathways during chemotherapy response. Collectively, our findings unveil mechanisms of m6A reader regulation during chemotherapy and suggest potential therapeutic implications for cancer treatment through manipulation of m6A-mediated gene regulation.

#4422 Targeting MLK4 in herceptin-resistant breast cancer.

Subhasis Das[1], Jingjing Sun[2], Song Li[2], Basabi Rana[3], Ajay Rana[3]

[1]University of Illinois at Chicago, Chicago, IL,[2]Department of Pharmaceutical Sciences, University of Pittsburgh School of Pharmacy, Pittsburgh, PA,[3]Department of Surgery, University of Illinois at Chicago, Chicago, IL

Mixed Lineage Kinases play a significant role in cancer biology, including breast cancer, by influencing key cell proliferation, survival, and metastasis signaling pathways. Targeting MLKs offers a promising therapeutic strategy, especially in cancers with dysregulated MLK activity. We have reported that one of the members of the mixed lineage kinase (MLK) family, MLK3 kinase activity, was necessary to mediate the cytotoxic effect of anti-HER2 therapies (i.e., Herceptin and Lapatinib). Activation of MLK3 using C6-ceramide nanoparticles caused massive cell death and apoptosis in Herceptin-resistant breast tumors. Recently, we observed that another MLK family member, MLK4, was overexpressed in human HER2^{+} breast cancer Herceptin-resistant breast tumors and Herceptin-resistant cancer cell lines, suggesting that MLK4 could play a functional role in developing HER2-therapy resistance. Interestingly, MLK4 gene knockdown in Herceptin-resistant breast cancer cell lines significantly caused cell death and apoptosis. Knockdown of MLK4 in Herceptin-resistant SkBr3 & BT474 cell lines downregulated p-HER2 (Y1221/1222, Y1248), p-AKT (S473), pCDC2 (Y15) and arrested cells in G2-M phase suggesting MLK4 regulates HER2 activation, and cell cycle progression. Overexpressing MLK4 in Herceptin-sensitive cells further activated the HER2-PI3K-Akt pathway, leading to Herceptin resistance. Surprisingly, MLK4 and HER2 endogenously interacted, and an in vitro kinase assay showed that MLK4 phosphorylates the intracellular domain of HER2 at Tyrosine 1221 and 1222 sites (Y1221/2), indicating that MLK4-induced phosphorylation could activate HER2 downstream signaling. Finally, MLK4 knockdown using siMLK4-loaded nanoparticles in an *in vivo* mouse PDX model demonstrated significant tumor regression and apoptosis. These results suggest that MLK4 upregulation is necessary for Herceptin resistance in HER2-positive breast tumors. Considering our *in vitro* and *in vivo* data, we plan to develop a specific inhibitor of MLK4, which could be used clinically to treat Herceptin-resistant HER2^{+} metastatic breast cancer.

#4423 CLL drug resistance- A multifaceted mechanism.

Sutapa Sinha[1], Weiguo Han[1], Zhiquan Wang[1], Brianna M. Finke[1], Heather C. Darby[1], Kari G. Rabe[2], Susan L. Slager[3], Chantal E. McCabe[2], Daniel R. O'Brien[2], Sameer A. Parikh[1], Esteban Braggio[4], Yi Chen[5], Fenlai Tan[5], Stephen P. Anthony[5], Yu Chen[5], Bing Dai[5], Yue Shen[5], Neil E. Kay[6]

[1]Hematology, Mayo Clinic, Rochester, MN,[2]Computational Biology, Mayo Clinic, Rochester, MN,[3]Hematology & Computational Biology, Mayo Clinic, Rochester, MN,[4]Hematology and Oncology, Mayo Clinic, Phoenix, AZ,[5]Newave, Alameda, CA,[6]Hematology / Immunology, Mayo Clinic, Rochester, MN

Despite the advent of novel targeted therapies with high overall response rates, chronic lymphocytic leukemia (CLL) is still largely incurable due to the development of resistance. The bone marrow microenvironment (BMME) plays an important role in the survival and drug resistance in CLL. BM stromal cells (BMSCs) are considered a key component of BMME. Our long-term goal is to gain further insights of BMSCs-CLL crosstalk influencing both survival and drug resistance in CLL. To understand the global biologic changes that occur in leukemic CLL cells when in contact with BMSCs, we assessed RNA-seq in paired CLL cells isolated from untreated blood and BM and in untreated patients' CLL cells cultured alone or with BMSCs. CLL cells from blood/BM and cultured alone/with BMSCs were also examined by Immunoprecipitation (IP), Western blot (WB), untargeted metabolomics (LCMS+GCMS), and preclinical drug sensitivity assays. We detected only 13 upregulated genes that overlap between CLL cells from BM and cocultured with BMSCs (p<0.05, fold change >1.5). When we analyzed the expression of these 13 genes in CLL cells from a cohort of 162 untreated patients by RNA-seq, we found associations between 4 genes (*PNP*, *C16orf54*, *MOB3A*, *CDK2AP2*) and clinical outcomes including overall survival (OS), progression free survival (PFS), and time to first treatment (TTFT) [multivariable Cox proportional hazards models, p<0.05]. Of the 4 genes, purine nucleoside phosphorylase (*PNP*), an enzyme in the purine pathway, had the most significant association with OS, PFS, and TTFT. Metabolomic profiling indicated an increased level of purine pathway metabolites adenosine, adenine, inosine, and hypoxanthine in cocultured CLL cells. WB analysis showed variable baseline PNP protein expression with 2 cohorts: low /no (L) and high (H) PNP using CLL blood cells. An induction in PNP protein levels were noticed in CLL cells expressing only LPNP when cocultured. Given the association of HPNP with adverse clinical outcomes, we tested for L vs. HPNP cohorts drug sensitivity to known BTK inhibitors(is) or Bcl2is. Treatment of CLL cells from untreated patients with Bcl2is venetoclax, S55746, and LP-118 (Bcl2-Bclxli, Newave) showed increased sensitivity to the Bcl2/Bclxlis in HPNP but not in LPNP cells. In addition, BTKis showed no difference in drug sensitivity associated with CLL PNP levels. To explain this discrepancy, WB and IP analyses were conducted in L vs. HPNP leukemic cells for apoptotic profiles, where we found increased phosphorylated-Bcl2(S70) and an increased association of Bcl2 with Bax in LPNP-expressing CLL cells compared to HPNP. These findings support increased resistance to apoptosis afforded by inhibiting Bcl2 for LPNP vs. HPNP-expressing CLL cells. Our future goals are to continue to understand the prognostic significance of the 4 genes with adverse clinical outcomes and to investigate how best to exploit the peculiar, enhanced sensitivity to Bcl2/Bclxlis found in the HPNP CLL cells.

#4424 Heterogeneity in resistance mechanisms found in *KRAS* mutant and wild-type patient-derived organoids with anti-EGFR therapy panitumumab.

Jordan N. Stoecker, Shirsa Udgata, Xingqi Shen, Cheri A. Pasch, Dustin A. Deming

University of Wisconsin-Madison, Madison, WI

Introduction: Tumor heterogeneity plays a major role in the development of resistance to therapies in colorectal cancer (CRC). The epidermal growth factor receptor (EGFR) antibodies, panitumumab (pani) and cetuximab are commonly used for the treatment of CRC. At resistance, very low frequency *KRAS* mutations can be detected by circulating tumor DNA (ctDNA). This raises the question if rare *KRAS* mutant clones can lead to EGFR inhibitor resistance. Here we utilized patient-derived cancer organoids (PDCOs) to investigate the impact of low frequency *KRAS* mutant clones on the development of pani resistance.
Methods: PDCOs were obtained after surgery with patient consent under UW institutional review board protocol. Tissue samples were processed as described previously for PDCOs. Two CRC PDCO lines, with $KRAS^{G12V}$ mutation (KR) and without (WT) were mixed in culture at multiple concentrations (v/v) (100% KR, 100% WT, 20% KR+80% WT, 10% KR+90% WT, 5% KR+95% WT, 1% KR+99% WT, 0.5% KR+99.5% WT, 0.25% KR+99.75% WT). Diameter analysis from day 0 to 4 was completed with increasing concentrations of pani. Once complete resistance to treatment was reached (>20% median growth in diameter under treatment at 100% physiologic C_{max} for two consecutive weeks), PDCOs were collected for bulk RNA-Seq. Differential expression analysis was prepared by UW Madison Bioinformatics Research Core using edgeR. Gene Set Enrichment Analysis (GSEA) was performed on hallmark gene sets.
Results: Despite ≤1% of KR PDCOs present in some populations, mixed conditions escalated to ≥ 40% C_{max} within 10 weeks and reached complete resistance to pani by week 16, while 100% WT culture remained at 20% C_{max} for 22 weeks. GSEA revealed 0.5% KR+99.5% WT condition resulted in ($p < 0.05$) up and downregulation of eight and 22 hallmark pathways (HP), respectively, compared to 100% WT at baseline. Upregulated HP of interest include MYC Targets V1/2 and MTORC1 Signaling, while downregulated HP of interest were KRAS Signaling Up, Apoptosis, and P53 Pathway. Comparing baseline mixed populations to resistant counterparts, the significant upregulation in HP was heterogenous across conditions. Some HP of note were KRAS Signaling Up and Apoptosis. In contrast, the significantly downregulated HP ($p < 0.001$) was relatively homogenous across only the three HP of E2F Targets, G2M Checkpoint, and MYC Targets V1.
Conclusion: PDCO lines containing very low concentrations of resistant clones can lead to significant changes in HP regulation and treatment response. The heterogeneity in HP upregulation in fully resistant mixed populations indicate differing mechanisms of reaching resistance. To confirm these observations, further studies will evaluate the response of more PDCO lines to pani treatment and utilize patient ctDNA collected before and after treatment in clinic to see the effect of the presence of *KRAS* subclones on outcomes.

#4425 Quantum-inspired computing analysis of spatial omics uncovers immune pathways relevant to T-DXd resistance in metastatic breast cancer.

Glori Das[1], Wenjuan Dong[1], Matthew Vasquez[1], Ji-hoon Lee[2], Yuan Gao[3], Jenny Chang[1], Xiaoxian Li[3], Hong Zhao[1], Stephen T. C. Wong[1]

[1]Houston Methodist Neal Cancer Center, Houston, TX,[2]David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA,[3]Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA

Purpose: Trastuzumab deruxtecan (T-DXd) is a key therapy for HER2+/HER2-low metastatic breast cancer (mBC), but up to 50% of patients develop resistance. Resistance mechanisms have implicated dysfunctions in DNA repair, lysosomal activity, and immune responses. This study combines spatial omics and quantum-inspired regulatory network analysis to investigate tumor-immune-extracellular matrix interactions contributing to T-DXd resistance.

Methods: Using NanoString GeoMX, spatial proteomics were analyzed in 29 metastatic tissue sections from 15 responsive (R) and 14 non-responsive (NR) patients. Across 6-8 regions of interest per section, immune and stromal components were quantified. Additionally, a subset of samples (n = 2R, 2NR) underwent GeoMX whole transcriptomics profiling and analysis using both classical and quantum-inspired algorithms.

Results: NR tumors consistently exhibited immunosuppression, disorganized immune responses, upregulated fibronectin, and poor infiltration of granzyme B-producing cells. Organ-specific findings included:•NR bone and brain mets showed increased spatial correlations between dendritic cells and T-cell exhaustion markers (PD-L1, CTLA4), suggesting niches of immune suppression.•NR bone mets showed increased spatial correlation of fibronectin correlated with immune markers, indicating that stiffer ECM physically blocks immune infiltration. •R chest wall mets showed upregulated smooth muscle actin (SMA), possibly reflecting vascular remodeling and enhanced immune infiltration.

Additionally, spatial transcriptomics analysis revealed the following::•Tumor-associated B cells were identified in NR samples, interacting with exhausted T cells and the Wnt-catenin pathway. •QuantumGRN revealed interactions in immune cells involving RBPP4 with DNMT3A and TAF4, linked to altered chromatin remodeling and transcription regulation, respectively.

Conclusions: This work shows that T-DXd response is heavily influenced by microenvironment remodeling and immune infiltration. This work highlights the value of spatial omics for uncovering ADC-resistance mechanisms, as well as the value of quantum-inspired computing for identifying novel therapeutic targets. In addition to collecting more spatial and single-cell omics data, future directions include pathway validation in animal models and computationally simulating quantum and molecular dynamics of ADC resistance.

#4426 Chromatin constraints underpin adeno-to-squamous cell state transitions during acquired resistance to KRAS inhibition.

Ayushi S. Patel, Samuel Greene, Arianne Parvaresh-Rizi, Gustavo S. Franca, Kwok-Kin Wong, Itai Yanai

NYU Langone Health, New York, NY

The advent of single-cell RNA-seq has permitted a global view of the transcriptomic variation across cancer cells in individual tumors and revealed functionally-related sets of genes - a gene module - underpin a cancer cell state. While single-cell transcriptomic atlases have provided a descriptive characterization of cancer cell states across tumor types, we lack a mechanistic framework on the constraints of tumor heterogeneity. Therefore, we propose a 'developmental constraint' model - where *cancer cell state transitions are restricted by the organism's developmental map, wherein* from the cell-of-origin the malignant cells traverse states that are accessible by its developmental trajectory, both toward progenitor-like states and differentiated-like states of adjacent lineages. Our model posits a constraint on the emergence of states, even under evolutionary pressures such as therapeutic intervention. To test this framework, we modeled non-genetic mechanisms of acquired resistance to KRAS inhibitors - histological adeno-to-squamous transition (AST). Multi-omic analysis of 68 patients enrolled in the KRYSTAL-1 clinical trial revealed a squamous gene signature correlated with a poor response specifically in patients with co-occurring *KRAS*^{G12C};*STK11* (K_CL) mutations. We recapitulated AST upon resistance to KRAS^{G12C} inhibition in K_CL genetically engineered mouse models and tumor-derived organoids. Single-cell transcriptomic and epigenomic of tumor-derived organoid revealed that AST is mediated by a two-step transition - from adeno-like states to an intermediate highly plastic cell state expressing markers of mixed-identity to enriched for squamous-like states. These findings support the developmental constraint model where cell state transitions are mediated by progenitor states in contrast to a trans-differentiation model. Furthermore, we hypothesized that cancer cell state transitions are restricted by the cell's chromatin context and specific chromatin modifiers are required for cell state transitions analogous to development. Therefore, we leveraged a time-course Perturb-seq approach against 191 chromatin modifiers to identify functional requirements for the adeno-to-intermediate and intermediate-to-squamous transition. Using a module-based method, we found cells with perturbations of the SWI/SNF complex (Arid1a, Smarca4 and Smarce1) did not transition to the squamous cell state but were restricted to the intermediate highly plastic cell state, suggesting that the SWI/SNF complex is required for the intermediate-to-squamous transition. Together, we have showed evidence that cell state transitions in cancer may be explained by the developmental constraint model and revealed that these constraints may act at the chromatin level where chromatin modifiers and chromatin states enable AST plasticity during adaptation to KRAS inhibitors.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Reversal of Drug Resistance 2
Poster Session

#4430 Combination of IGF1R/InsR and proteasome inhibition shows strong antitumor activity in proteasome inhibitor resistant multiple myeloma.

Andrej Besse[1], Tiberiu Totu[2], Marianne Kraus[3], Max Mendez Lopez[3], Jana Veprkova[1], Ondrej Slaby[1], Marija Buljan[2], Leo Rasche[4], K. Martin Kortum[4], Christoph Driessen[3], **Lenka Besse**[1]

[1]Masaryk University, Brno, Czech Republic,[2]Swiss Federal Laboratories for Materials Science and Technology, St. Gallen, Switzerland,[3]Cantonal Hospital St. Gallen, St. Gallen, Switzerland,[4]University Hospital Wurzburg, Wurzburg, Germany

Background: Overcoming proteasome inhibitor (PI) resistance remains an unmet medical need in multiple myeloma (MM) therapy. Anaplastic Lymphoma Kinase (ALK) inhibitors co-target related tyrosine-kinases, such as InsR and IGF1R. Ceritinib, a second-generation ALK inhibitor, has anti-MM activity in monotherapy or in combination with PI carfilzomib (CFZ). MM cells do not express ALK, but are dependent on InsR/IGF1R to sustain their high metabolic demand. Here, we aimed to identify the mechanism of action of ceritinib and the mechanism of synergy between ceritinib and CFZ in MM cells in vitro, in vivo, and ex vivo.
Methods: PI-naïve and PI-resistant MM cell lines and primary cells were used. Genome-wide CRISPR/Cas9-based screening was performed to identify ceritinib resistance candidate genes. A pam-kinase screening, RNA sequencing and untargeted LC-MS/MS were used to elucidate mechanism of action of treatment combination. RNA-seq data from patients included in the CoMMpass study and from patients treated at the Wurzburg University Hospital were analyzed for InsR and IGF1R expression. An orthotopic in vivo mouse model with PI-resistant cells was used to determine the effect of the drug combination in vivo.
Results: Among several approved ALK inhibitors tested, ceritinib shows the highest synergistic cytotoxicity in combination with CFZ in MM cell lines and primary cells. The combination of ceritinib and CFZ is active in PI-resistant MM in vivo. MM cell lines and primary cells are negative for ALK but positive for InsR and IGF1R expression, which is increased in the relapsed/refractory MM cohort. Kinase screening identified InsR and IGF1R as major kinases inhibited by ceritinib in MM. CRISPR/Cas9-screening identified transcription factor FOXO1, a downstream target of InsR/IGF1R/Akt, as the top resistance candidate to ceritinib. Subsequently, FOXO1 inhibition protected MM cells from ceritinib-induced cytotoxicity. Ceritinib in combination with CFZ caused rapid translocation of FOXO1 to the nucleus, suggesting its activation. Consistently, ceritinib induced the expression of genes related to cell cycle arrest, which are regulated by FOXO1. Ceritinib, by targeting the InsR/IGF1R, impaired glycolysis and amino acid homeostasis, observed as an accumulation of aromatic amino acids. Collectively, ceritinib decreases Akt activity via InsR/IGF1R inhibition, leading to metabolic and proliferative shut-down. In combination with proteasome inhibition, this has a detrimental effect, impairing protein homeostasis and inducing starvation in MM cells.
Conclusion: Ceritinib in combination with carfilzomib represents a promising treatment option for MM to be tested in the clinic.
Support: Supported by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union - Next Generation EU.

#4431 RRM2-driven dNTP biosynthesis: a therapeutic vulnerability in temozolomide-resistant glioblastoma.

Atique U. Ahmed, Karan Dixit, Ella N Perrault, Jack M Shireman, Priya Kumthekar, Roger Stupp. David C James,

Northwestern Univ. Feinberg School of Medicine, Chicago, IL

Glioblastoma (GBM) remains one of the most aggressive and fatal cancers, with a median survival of only 12-18 months despite standard treatments, including surgical resection, temozolomide (TMZ) chemotherapy, and radiotherapy. Tumor recurrence and therapy resistance are significant barriers to improving patient outcomes. To address this, our lab investigated the molecular mechanisms driving resistance during TMZ therapy using a single-cell RNA-sequencing screen on a patient-derived xenograft (PDX) model. This approach enabled us to study tumor evolution pre-, during, and post-therapy. Our analysis identified 149 unique genes expressed during TMZ therapy, with the ribonucleotide reductase (RNR) family, particularly Ribonucleotide Reductase Regulatory Subunit 2 (RRM2), emerging as critical players. During TMZ treatment, GBM cells preferentially utilize RRM1-RRM2 interaction to support deoxynucleoside triphosphate (dNTP) biosynthesis, whereas post-therapy recurrent GBM shifts to RRM1-RRM2B complexes. Functional studies demonstrated that RRM2 knockdown sensitized GBM cells to TMZ, reducing DNA repair capacity, as evidenced by increased yH2AX fluorescence, while RRM1 or RRM2B knockdown conferred resistance ($p<0.001$). Metabolomic profiling revealed that RRM2 mediates dCTP and dGTP production during TMZ treatment, critical for chemoresistance. Supplementation with these dNTPs rescued TMZ susceptibility in RRM2-deficient cells, highlighting its pivotal role in DNA repair and therapeutic resistance. Recognizing the limitations of first-generation RNR inhibitors, we explored the second-generation inhibitor Triapine (3-AP). Preclinical studies showed that 3-AP effectively inhibits RRM2 activity, enhancing TMZ sensitivity and extending survival in PDX models ($p<0.0001$). Mice treated with a combination of 3-AP and TMZ demonstrated significantly longer survival compared to TMZ alone. These findings underscore the potential of targeting RRM2-mediated dNTP biosynthesis to overcome chemoresistance in GBM. We are conducting a Phase 1/1b clinical trial to assess the safety, toxicity, and maximum tolerated dose (MTD) of combining 3-AP with TMZ in recurrent GBM patients.

#4432 mRNA-based restoration of pomalidomide sensitivity in multiple myeloma.

J. U. Ogbede, M. S. Rogers, R. J. D'Amato, B. R. Zetter;
Boston Children's Hospital, Harvard Medical School, Boston, MA

Multiple myeloma (MM) remains an incurable hematologic malignancy, and immunomodulatory imide drugs (IMiDs) have been used as an effective treatment option. A significant challenge with IMiDs such as pomalidomide, as with other treatment options, is the development of drug resistance. This has become a substantial impediment to extending patients' lives. Currently, no effective strategy exists for overcoming IMiD resistance in MM patients. Exploring alternative approaches that could improve the efficacy of pomalidomide might help myeloma patients live longer. Genetic alterations in cereblon (CRBN) contribute to more than one-third of clinical cases of pomalidomide resistance. This study aims to restore sensitivity to pomalidomide-resistant cells through mRNA-based wildtype CRBN restoration. First, we repeatedly exposed multiple myeloma cell lines (MM1.S) to pomalidomide at increasing doses, starting from 2 nM to 10 μM. Following six months of pomalidomide exposure, we generated MM1.S cells that are resistant to pomalidomide and confirmed the absence of CRBN with RNA sequencing and western blotting. Through in vitro transcription, we synthesized CRBN mRNA and electroporated it to the resistant cells. We found restoration of wildtype cereblon and subsequent pomalidomide sensitivity compared to cells without CRBN mRNA treatment. As electroporation is impractical beyond laboratory studies, we are designing a polymer-lipid hybrid nanoparticle to efficiently deliver the mRNA to the cells. Additionally, to ensure targeted delivery of the nanoparticle-coated CRBN mRNA to plasma cells, we applied phage display peptide library screens and identified a cyclic peptide with a high affinity for B-cell maturation antigen (BCMA), a protein known to be almost exclusively expressed by plasma and myeloma cells. This peptide is being validated for BCMA binding, and will be conjugated with the nanoparticle-coated CRBN mRNA for targeted delivery to the malignant plasma cells. Findings from this work will advance mRNA-based therapeutics for overcoming drug resistance caused by genetic alterations in target proteins.

#4433 Effectiveness of USP1 inhibitor (VRTX531) in gynecological cancers with diverse BRCA mutation status.

Uday Kumar Surampudi[1], Prashant Kashinath Bhavar[1], Partha Pratim Sarma[2], Rishi Rahangdale[1], Anuj Ramesh Kshirsagar[2], Appaji Baburao Mandhare[2]

[1]VRise Therapeutics Inc., Cambridge, MA,[2]VeGen Therapeutics, Hyderabad, India

Triple-negative breast cancer and Ovarian cancer are aggressive malignancies often associated with poor prognoses due to their high recurrence rates and limited targeted treatment options. TNBC, which lacks expression of estrogen, progesterone, and HER2 receptors, accounts for 10-15% of breast cancers and disproportionately affects younger women, those with BRCA1 mutations, and certain ethnic groups, such as Black women. Similarly, ovarian cancer is often diagnosed at advanced stages and includes subtypes like high-grade serous carcinoma, which is strongly linked to BRCA1/2 mutations and homologous recombination deficiency. Both cancers share vulnerabilities in DNA damage repair pathways, making them promising candidates for treatments like PARP inhibitors and novel agents targeting DDR mechanisms. However, only 20-40% of the patients benefit from PARPi and are alive 5-years after diagnosis from these gynecological cancers. Ubiquitin specific protease 1 belongs to a group of deubiquitinating enzymes and is a key regulator in the DNA damage repair (DDR) pathway. A deficiency in USP1 results in compromised genomic stability, making it a potential therapeutic target in tumors that exhibit defects in the DNA damage repair mechanism. VRTX531 is a potent, selective, and allosteric oral inhibitor of USP1, specifically designed to target HRD Tumors. Treatment with VRTX531 resulted in decreased viability in cell lines with BRCA1/2 mutations and HRD+ tumors. USP1 inhibitors are anticipated to induce cell death via a pathway distinct from PARPi, and thus have the potential to overcome resistance to PARPi. The synergistic effect of VRTX531 combined with PARPi was demonstrated in cell-based assays representing these gynecological cancers representing BRCA mut, HRD+, MDA-MB-436 and BRCA null, HRD+, UWB1.289. Moreover, VRTX531 exhibited a significant synergistic effect in vitro when used in conjunction with a PARP inhibitor. These results indicate that VRTX531 not only provides substantial monotherapy benefits but also improves the effectiveness of PARP inhibition. The cell viability assay revealed a dose-dependent reduction in cell proliferation, with VRTX531 demonstrating potent anti-proliferative effects which was further confirmed by clonogenic assays. DNA damage was evaluated by γH2AX foci formation which showed a marked increase in DNA damage upon treatment with VRTX531. Our Previous work demonstrated that treatment with PARPi alone led not only to a delayed onset of response but also showed statistically significant variability between subjects, in contrast the combination of VRTX531 and PARPi led to rapid onset of response, followed by complete tumor regression with an TGI >98%, in a TNBC Xenograft of MDA-MB-436. USP1 inhibitor VRTX531, exhibits best-in-class pharmacokinetics, with exemplary safety profile, that supports its further development.

#4434 Ficerafusp alfa reverses acquired resistance to the KRAS-G12C inhibitor sotorasib in KRAS-G12C-mutated lung tumors.

Veena Somasundaram[1], Shiv Ram Krishn[1], Reshmi Nair[1], Pradip Nair[1], **Rachel L. Salazar**[2]

[1]Syngene International Ltd., Bengaluru, India,[2]Bicara Therapeutics Inc., Boston, MA

Approximately 15% of non-small cell lung cancer patients present with a KRAS-G12C mutation and do not respond to anti-Epidermal Growth Factor Receptor (anti-EGFR) therapy[1]. Recent approvals of KRAS-G12C inhibitors (G12Ci) such as sotorasib (AMG510) and adagrasib have improved treatment responses and overall survival rates[2]. One of the mechanisms associated with acquired resistance and subsequent relapse after prolonged treatment with G12Ci is linked to overexpression of TGF-β[3]. Increased TGF-β is associated with immune suppression and epithelial-mesenchymal transition (EMT), contributing to increased metastasis[3,4]. Ficerafusp alfa, a bifunctional antibody targeting EGFR and TGF-β within the tumor microenvironment, may enhance treatment efficacy when combined with G12Ci by overcoming drug resistance.

Lung cancer cell lines NCI-H1792 (KRAS-G12C$^{+/-}$) and NCI-H1373 (KRAS-G12C$^{-/-}$), both characterized by high EGFR and TGF-β levels, were cultured with sotorasib for over 6 weeks to induce resistance. Resistant NCI-H1792 cells were treated with sotorasib, cetuximab (anti-EGFR antibody), ficerafusp alfa, or their combinations. Treatments were assessed in 2D culture and 3D spheroid-PBMC co-cultures, measuring cytotoxicity and gene expression changes via Nanostring analysis (in 2D model). Additionally, sotorasib-resistant NCI-H1373 cells were used in a subcutaneous xenograft model to evaluate treatment efficacy *in vivo*.

Results demonstrated that combining sotorasib with ficerafusp alfa showed better efficacy over sotorasib alone and sotorasib in combination with cetuximab in sotorasib-resistant NCI-H1792 tumor cells *in vitro*. Additionally, in the 3D spheroid-PBMC co-culture assay, our combination reduced overall spheroid area. Nanostring analysis showed downregulation of TGF-β signalling, EMT, and metastatic pathways in the sotorasib and ficerafusp alfa group compared to sotorasib plus cetuximab. *In vivo*, TGF-β ELISA analysis revealed higher TGF-β levels in sotorasib-resistant NCI-H1373 tumors compared to the parental tumors. The combination of sotorasib with ficerafusp alfa resulted in a significant reduction in sotorasib-resistant NCI-H1373 tumors over sotorasib in combination with cetuximab.

This study reinforces the hypothesis that TGF-β plays an important role in acquired resistance to G12Ci. Our findings suggest that combining G12Ci with ficerafusp alfa could overcome G12Ci drug-resistance in KRAS-G12C-mutated lung cancer.

References 1.Lim TKH, et al., Lung Cancer. 2023. PMID: 37683526 2.Huang et.al., Signal Transduct Target Ther. 2021. PMID: 34776511 3.Tsai YS, et al., J Clin Invest. 2022. PMID: 34990404 4.Adachiet.al., Clin Cancer Res. 2020. PMID: 32900796

#4435 PRAME-mediated retinoid resistance is overcome by EZH2 inhibition in keratinocyte carcinoma.

Brandon Liam Ramchatesingh[1], Ivan V. Litvinov[2]

[1]Research Institute, McGill University Health Centre, Montreal, QC, Canada,[2]Division of Dermatology, McGill University Health Centre, Montreal, QC, Canada

Retinoids have exhibited efficacy as treatments and prophylactics for both types of keratinocyte carcinoma (KC): basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (cSCC). However, the practical efficacy of these compounds is limited by retinoid resistance mechanisms. Preferentially Expressed Antigen in Melanoma (PRAME) is a cancer-testis antigen ectopically expressed in a subset of BCC and cSCC tumors. In gametes, PRAME binds Enhancer of Zeste Homologue 2 (EZH2), an epigenetic silencer, selectively targeting transcriptional repression to retinoic acid response elements (RARE) in gene promoters. The functions and therapeutic relevance of PRAME expression in KC tumors have not been investigated. A combination of transcriptomic analyses, 2D and 3D cell culture approaches were undertaken to evaluate the contributions of PRAME to KC pathogenesis and treatment response. Pharmacological retinoid sensitization strategies were also investigated. Epidermal differentiation and cytokine signaling pathways were downregulated in high PRAME-expressing tumors and in PRAME overexpressing (OE) cell lines. Conversely, low-PRAME tumors and PRAME depleted cell lines exhibited an enrichment of epidermal differentiation and cytokine signaling gene signatures. PRAME OE impaired epidermal differentiation in 3D organotypic skin culture models. Additionally, PRAME OE impaired RARE activation and conferred resistance to retinoid-induced growth suppression. PRAME depletion restored RARE activation and restored retinoid-induced growth suppression. Mechanistically, PRAME was determined to abrogate the anti-keratinization effect of retinoid treatment in SCC cells and organoid models. PRAME also abrogated the cell death promoting effect of retinoid treatment in BCC cells. Targeting of EZH2, using pharmacological or genetic strategies, in combination with retinoid treatment, resulted in a selective repression of cell growth in KC cell lines. Similar effects were observed when performing co-treatments using cell lines representative of cutaneous melanoma and lung squamous cell carcinoma. This study illustrates potential roles for PRAME in modulating differentiation programs and demonstrates a role for PRAME in conferring resistance to retinoid-based therapeutics. Furthermore, this study offers a preclinical demonstration of a drug repurposing strategy including retinoid compounds and EZH2 inhibitors to suppress the growth of PRAME-expressing cells.

#4436 Mechanistic insights into CXCR7 activation and resistance pathways in EGFR-Mutant NSCLC with mesenchymal phenotype.

Kaajal Rengaraj[1], Laura C. Gunder[1], Ines Pulido[1], Jeffery H. Becker[1], Frida Pantoja[1], Jennifer Graeber[2], Malek G. Massad[2], Takeshi Shimamura[2]

[1]University of Illinois Chicago, Chicago, IL,[2]Surgery, University of Illinois Chicago, Chicago, IL

EGFR tyrosine kinase inhibitors (TKIs) have significantly improved outcomes for NSCLC patients with EGFR mutations. However, resistance inevitably develops, with the T790M mutation identified in over 50% of resistant tumors, alongside mechanisms like MET amplification, SCLC transformation, and Epithelial Mesenchymal Transition (EMT). Osimertinib, a third-generation EGFR TKI approved for first-line treatment, specifically targets the T790M mutation and mitigates T790M-related resistance. Despite this, non-T790M resistance mechanisms, including mesenchymal transformation, are increasingly prevalent. Our group previously reported that the atypical GPCR CXCR7 is critical for EGFR TKI resistance associated with a mesenchymal phenotype. CXCR7 activates the MAPK and PI3K pathways when mutant EGFR receptors are inhibited, offering alternative survival pathways. Although CXCR7 plays a pivotal role in drug resistance with a mesenchymal phenotype, the precise mechanisms underlying its activation remain unclear. Profiling cytokine-chemokine secretion from gefitinib-resistant HCC4006GeR cells identified elevated levels of CXCL12, MIF, and chitinase-3 like-protein-1 (CHI3L1). Exposure of HEK293 cells ectopically overexpressing CXCR7 to canonical CXCR7 ligands, CXCL12 and MIF, activated the MAPK pathway within 30 minutes. We found that CRISPR depletion of CXCR4 in HEK293 cells abrogated MAPK activation following exposure to CXCL12 and MIF. In contrast, HCC4006-CXCR7 cells lacking CXCR4 required eight hours for CXCL12 and MIF to activate MAPK in the presence of osimertinib. Notably, exposure of HCC4006-CXCR7 cells to CXCL12 and MIF in the absence of EGFR TKI failed to augment the MAPK signal. We discovered that CXCR7 co-immunoprecipitated with constitutively active mutant EGFR in NSCLC cells, and this association ceased upon EGFR inhibition. Thus, dissociation of CXCR7 from mutant EGFR appears to be a prerequisite for MAPK activation. Additionally, we identified CH3L1 as a previously unrecognized chemokine capable of activating MAPK in HCC4006-CXCR7 more effectively than CXCL12 or MIF. CXCR7 activation increased the expression of TWIST, ZEB1, and ZEB2, essential for maintaining the mesenchymal phenotype. Depletion of CXCR7 in HCC4006Ge-R cells with a mesenchymal phenotype led to the attenuation of these transcription factors, a mesenchymal-to-epithelial transition, and restoration of EGFR TKI sensitivity. Collectively, these findings reveal a unique ligand-induced mechanism of CXCR7 activation and downstream MAPK pathway modulation by putative ligands. This complex CXCR7 activation mechanism in EGFR-mutant NSCLC suggests that preventing receptor-ligand binding may not be a viable strategy for overcoming EGFR TKI resistance.

#4437 Pimitespib (TAS-116), an HSP90 inhibitor, overcomes resistance to androgen receptor pathway inhibitors in prostate cancer by simultaneously targeting several key resistance mechanisms.

Hiromi Muraoka, Kazuhisa Minamiguchi, Akihiro Hashimoto, Hiroshi Sootome, Shuichi Ohkubo

Taiho Pharmaceutical Co., Ltd., Tsukuba, Japan

Background: Resistance to androgen receptor pathway inhibitors (ARPIs) poses a significant challenge in the treatment of metastatic castration-resistant prostate cancer (mCRPC). Key mechanisms driving ARPI resistance include androgen receptor (AR) overexpression, AR mutations, AR splice variants such as AR-V7, alternative glucocorticoid receptor (GR) signaling, and activation of the AKT pathway. Heat shock protein 90 (HSP90), a molecular chaperone essential for stabilizing several of the key proteins involved in these resistance-related mechanisms, has emerged as a compelling therapeutic target. Pimitespib (TAS-116), an HSP90 inhibitor approved in Japan for the treatment of gastrointestinal stromal tumors, holds potential for addressing ARPI resistance by disrupting these mechanisms through the inhibition of nuclear translocation and protein destabilization of AR and related factors.
Materials and Methods: The effects of pimitespib were assessed in four human prostate cancer cell lines (LNCaP, VCaP, 22Rv1, and SAS MDV No.3). Cell proliferation was assessed using intracellular ATP measurements. Western blotting and quantitative reverse transcription polymerase chain reaction were used to analyze protein and gene expression levels of resistance markers, including AR, AR-V7, GR, AKT, as well as their downstream transcriptional targets. Subcellular localization of AR and GR was examined using Western blotting. Antitumor efficacy was evaluated in two castration-resistant prostate cancer (CRPC) xenograft models (LNCaP-Xeno-IL-6 and VCaP).
Results: Pimitespib enhanced the efficacy of three ARPIs (enzalutamide, darolutamide, and abiraterone) in the four prostate cancer cell lines. Mechanistic analysis revealed that pimitespib rapidly inhibited nuclear translocation of AR and GR within 1 h of treatment. Additionally, pimitespib reduced the protein levels of AR, GR, AR-V7, and AKT, effectively reversing ARPI resistance. Gene expression analysis showed that pimitespib downregulated AR, AR-V7, and GR target genes. In the CRPC xenograft models, pimitespib potentiated the antitumor effects of the ARPIs, resulting in enhanced inhibition of tumor growth. Combination therapy using pimitespib with enzalutamide was well-tolerated, with no significant increase in toxicity.
Conclusion: Pimitespib effectively addresses ARPI resistance in mCRPC by rapidly inhibiting AR and GR nuclear translocation and reducing the protein levels of AR, GR, AR-V7, and AKT. In addition, our findings highlight the potential of combining pimitespib with ARPIs for the treatment of mCRPC patients who have progressed on ARPI therapy.

#4438 Role of USP1 in overcoming chemoresistance Independent of BRCA mutation status.

Uday Kumar Surampudi[1], **Prashant Kashinath Bhavar**[1], Partha Pratim Sarma[2], Anuj Ramesh Kshrisagar[2], Rishi Rahangdale[1], Appaji Baburao Mandhare[2]

[1]VRise Therapeutics Inc., Cambridge, MA,[2]VeGen Therapeutics, Hyderabad, India

The efficacy of chemotherapeutic agents like Cisplatin is often limited by DNA repair mechanisms, including the PARP and USP1, that regulates DNA damage response proteins. In this study, we explore the effects of USP1 inhibition, both alone and in combination with a PARP inhibitor, on DNA repair and cell survival following Cisplatin treatment. Our experiments were conducted in a variety of cell lines, irrespective of their BRCA mutation status, to understand the broad applicability of this therapeutic strategy. Cells were treated with Cisplatin to induce DNA damage, followed by treatment with VRTX531 a USP1 inhibitor and/or a PARP inhibitor assessed by in vitro cell based assays such as cell viability, γH2AX foci formation, and comet assay. Inhibition of USP1 resulted in impaired repair of Cisplatin-induced DNA damage, leading to increased sensitivity to subsequent PARP inhibition, regardless of BRCA mutation status. The combination treatment synergistically enhanced cell death, as evidenced by a significant reduction in cell proliferation and an increase in DNA damage markers. These findings suggest that targeting USP1 in combination with PARP inhibition may be a promising therapeutic strategy to overcome chemoresistance, providing a potential treatment approach for tumors with or without BRCA mutations. Further investigation into the mechanistic underpinnings of this combination therapy is warranted to optimize its clinical application.

#4439 Adaptive rewiring after prolonged KRAS inhibition sensitizes PDAC cells to sequential targeting with ferroptosis inducers.

Tina Kos, Christian Schneeweis, Shinjini Bandopadhyay, Birgit Prinker, Chen Zhao, Dieter Saur

Chair of Translational Cancer Research and Institute of Experimental Cancer Therapy, Klinikum rechts der Isar, School of Medicine, Technische Universitat Munchen, Munich, Germany, Munich, Germany

Recent advances in direct targeting of oncogenic KRAS necessitate the need to understand and overcome intrinsic and extrinsic resistance mechanisms of cancer cells, especially in pancreatic ductal adenocarcinoma (PDAC) with KRAS as the main oncogenic driver. To investigate time-dependent KRAS resistance, we created a dual-recombinase PDAC mouse model that allows for inducible genetic deletion of $Kras^{G12D}$ *in vivo* and *in vitro*. A drug screen on primary PDAC cell lines derived from the model that were resistant to $KRAS^{G12D}$ inhibition identified a strong RSL3-induced ferroptosis sensitivity of $Kras^{G12D}$-depleted cell lines compared to their $Kras^{G12D}$-proficient counterpart. Inhibitors MRTX1133 and RMC-6236 also conferred ferroptosis sensitization in various mouse and human PDAC cell lines. Interestingly, ferroptosis sensitization was stronger after 7-day treatment with KRAS inhibitor, compared to acute inhibition of 1-3 days, indicating that exhaustive rewiring of cellular pathways is needed for complete sensitization. This was also evident in the transcriptome analysis of cells at different time-points after $KRAS^{G12D}$ depletion revealing temporary and chronic adaptations spanning various metabolic, proliferative and stress-associated pathways, several of which were previously linked to ferroptosis. $Kras^{G12D}$-depleted cells exhibited higher ROS, higher iron ion content, and lower capacity to reduce ROS based on redox ratio of intracellular glutathione. Intriguingly, the sensitized phenotype was maintained in resistant cells treated with MRTX1133 over a period of 30-45 days, suggesting a stable phenotype that could be exploited in a clinical setting using sequential therapy approach. To better understand which genes are essential for viability of $Kras^{G12D}$-depleted cells, we performed a whole-genome CRISPR knock-out screen on cell line with $Kras^{G12D}$-depletion (genomic and inhibitor-based), as well as with additional RSL3 treatment. Kras-depletion screen identified several ferroptosis-associated regulators among the top depleted and enriched genes, including redox balance regulators (Slc7a11, Txnrd1, Keap1, Nadk), iron homeostasis (Ttc7, Glrx5) and KRAS-resistance-associated pathways (Taz, Map3k7). Machine learning algorithm applied on RNAseq of differentially sensitized cell lines revealed a signature of genes whose expression is altered based on sensitivity to RSL3. These, together with global changes in transcriptome, suggest that multiple pathways likely contribute to ferroptosis sensitization, including KEAP-NRF2 redox balance pathway, iron and lipid metabolism, and also hint at involvement of TGFβ-EMT signaling axis. Our results suggest that a sequential two-hit therapeutic approach with KRAS inhibition followed by subsequent ferroptosis induction could efficiently mitigate resistance to KRAS-inhibitor based therapies in PDAC.

#4440 The efficacy of NST-628, a non-degrading pan-RAF/MEK molecular glue, and MEK inhibitors for overcoming acquired osimertinib resistance mediated by TRIM24-BRAF fusions in EGFR mutant NSCLC.

Alvaro Guimaraes Paula, Ximeng Liu, David Molkentine, Monique Nilsson, Junqin He, John V. Heymach

UT MD Anderson Cancer Center, Houston, TX

In patients with EGFR-mutant non-small cell lung cancer (NSCLC), acquired resistance to EGFR tyrosine kinase inhibitors (TKIs) can be driven by novel gene fusions. Among these, acquired fusions in the BRAF gene occur in approximately 2% of osimertinib resistant cases. Based on paired molecular profiling of biopsies from a patient with classical EGFR exon 19 deletion (del19) NSCLC, taken pre and post osimertinib treatment, we identified a novel BRAF fusion, TRIM24-BRAF, and hypothesized that this aberration played a causal role in osimertinib resistance. To test this hypothesis, we transfected HCC827 and HCC4006 EGFR mutant NSCLC cell lines to express TRIM24-BRAF fusion variants using lentivirus vectors and performed a 3 day cell viability assay to assess the impact of these fusions on sensitivity to osimertinib. Expression of TRIM24-BRAF fusions resulted in an IC50 of at least 100 times higher as compared to the parental cell lines, confirming that TRIM24-BRAF fusions are sufficient to induce EGFR TKI resistance. To further evaluate the mechanism, we performed Western blot analysis, which revealed that that osimertinib blocked EGFR phosphorylation in both parental cell lines and cells expressing the TRIM24-BRAF fusion variants. However, osimertinib inhibited MEK phosphorylation only in the parental cell lines, but not in those expressing the TRIM24-BRAF fusions, indicating that cells expressing the TRIM24-BRAF fusion had sustained activation of signal transduction pathways downstream of EGFR after osimertinib treatment. Next, we utilized a panel of compounds targeting the RAS/RAF/MAPK pathway including agents targeting pan-RAS (RMC-6236 and RMC-7977), SHP2 (RMC-4550), BRAF (dabrafenib, encorafenib, tovorafenib and vemurafenib), pan-RAF (TAK-632), MEK (binimetinib, cobimetinib, selumetinib and trametinib), pan-RAF/MEK (NST-628) and assessed whether targeting signal transduction molecule downstream of EGFR could overcome this adaptative resistance mechanism. Our data indicated that trametinib, a MEK inhibitor, and NST-628, a non-degrading pan-RAF/MEK molecular glue, were the most effective against cells expressing TRIM24-BRAF fusions. Moreover, cell viability assays combining osimertinib either with trametinib or with NST-628 showed an addictive effect among all the different TRIM24-BRAF variants and a synergistic effect in some. TRIM24-BRAF expressing cells showed minimal sensitivity to BRAF, SHP2 or RAS targeting single agents. Taken together, our findings indicate that expression of TRIM24-BRAF fusion induce osimertinib resistance in EGFR mutant NSCLC and targeting the MEK pathway using trametinib or NST-628 can overcome osimertinib resistance in this setting.

#4441 Specific OXPHOS inhibitors can overcome chemoresistance of triple negative breast cancer.

C. Uslu[1], E. Kapan[1], E. Abad[2], A. Lyakhovich[1];
[1]Sabanci Universitesi, Istanbul, Turkey, [2]Universitat Pompeu Fabra, Barcelona, Spain

Recently, we and others have shown that drug-resistant malignancies are characterized by higher levels of mitochondrial oxidative phosphorylation (OXPHOS) compared to chemotherapy-responsive tumors. Specifically, we used cisplatin- and cyclophosphamide-resistant triple-negative breast cancer cells and corresponding xenograft models to show that the chemoresistant phenotype is characterized by a higher oxygen consumption rate and higher metastatic potential compared to its chemosensitive counterpart. We hypothesized that OXPHOS inhibitors may resensitize chemoresistant cells to anticancer therapy. Given the endosymbiotic origin of mitochondria from alphaproteobacteria, we analyzed OXPHOS inhibitors among hundreds of antimicrobial agents and selected several that specifically target chemoresistant cancer cells. Experiments in nude mice confirmed the efficacy of these compounds against tumors derived from chemoresistant cells. In addition, we performed proteomic profiling and identified several mechanisms of action of these chemicals, including enhancement of oxidative stress, induced mitophagy, and suppression of metalloproteinase activity. Furthermore, we confirmed the synergistic effects of individual antibactericidal drugs and chemotherapeutic agents and suggest that this alternative approach should be considered for the treatment of OXPHOS-dependent chemoresistant tumors.

#4442 Drug resistant humanized PDX platform: Clinically relevant *in vivo* modeling of innate and acquired resistance within human immune environment.

Jie Wen, Hao Cheng, Cheng Zhang, Bingrui Han

PharmaLegacy LLC, Shanghai, China

Cancer treatment has been facing many hurdles, drug resistance is one of the most significant one among them. Over the years, treatment strategies have been evolving. A complex interaction between intrinsic (innate) and extrinsic (acquired) factors enables the survival of selected cancer cells, through progression under unfavorable conditions, ultimately develop resistance. Although numerous prospective drugs against cancer have been shown promising therapeutic efficacy on preclinical *in vitro* and *in vivo* models, only a few have been proven safe and effective in clinical validations. These problems are largely due to the lack of precise research tools to reveal the genuine status of cancer especially to recapitulate cancer resistance development in real-world patients. Patient derived xenograft (PDX) models have been shown superiority in reflecting the characteristics of cancer patients by retaining genomic information, diversified treatment backgrounds and clinical behaviors, all of these features make PDX an excellent *in vivo* system to study drug resistance, evaluate drug sensitivity and better treatment regimens. However, due to the low tumor engraftment rate and difficulty to reconstitute proper human immune system, only a few studies published about drug resistant humanized PDX model. The current study describes the successful establishment and validation of two drug-resistant humanized PDX models: a HER2 ADC resistant gastric PDX demonstrating clear innate resistance, and a multidrug resistant (MDR) melanoma PDX showing acquired resistance to targeted therapies, immune check point inhibitors (ICIs) and ionizing radiation. The gastric PDX model with HER2 expression confirmed by IHC (2+) shown limited response (TGI <10%) to trastuzumab deruxtecan and no response to Dxd, demonstrating a clear intrinsic resistance. In a separate study in hPBMC mice, significant tumor growth inhibition was achieved (TGI > 60%) when treating with anti-hPD1, implicating the clinical benefit of combining ICIs with ADC treatments. The MDR melanoma PDX model was derived from a patient with treatment history of initial surgical resections, followed by targeted therapies (VEM+COB) then ICIs when recurrence happened. The tumor shown limited response to BRAF inhibitor (TGI <15%) and completely abolished response to anti-hPDL1, anti-hPD1 and ionizing radiation in humanized mice with averaged hCD45% > 30%. We have successfully established and characterized several drug resistant PDX models particularly in humanized mice, making it possible to study interactions between human tumors and immune system, identify biomarkers and better clinical strategies to overcome drug resistance. This platform can be used to achieve personalized medication management, which is the ultimate translational goal.

#4443 Targeting the mutual regulation between EZH2 and FADS2 to suppress ovarian cancer by synergistic inhibition of Notch signaling and mitochondrial function.

Zhengyang Guo, Xiao Huo, Ying Song, Jiagui Song, Yang Bai, Tong Liu, Lixiang Xue

Peking University Third Hospital, Beijing, China

Ovarian cancer (OC) is the most aggressive and lethal gynecological cancer associated with abnormal lipid metabolism. Enhancer of zeste homolog 2 (EZH2), the catalytic subunit of the polycomb repressive complex 2, is overexpressed in OC and other various cancers. It functions as a tumor promoter through canonical H3K27me3 modification and non-canonical ways, and is considered as a potential anticancer target. Although numerous EZH2 inhibitors have been developed and tested in clinical trials, the therapeutic effect of EZH2 inhibitors on solid tumors is still very limited. Here, we report that EZH2 bi-directionally regulates transcription of fatty acid desaturases 2 (FADS2) transcript variant 1 (v1) and 2/3 (v2/3) in canonical and non-canonical ways respectively. FADS2 is considered the rate-limiting enzyme in the synthesis of long chain polyunsaturated fatty acids (PUFAs). The transcript v1 encodes the full-length isoform, and v2/3 encodes the shorter protein predicted to lack the desaturase activity. EZH2 deficiency increased FADS2 v1 and decreased v2/3 at mRNA and protein level. In fact, the transcriptional start site for FADS2 v2/3 is located about 12 kb upstream of v1. EZH2 occupied at the promoter of FADS2 v1 and v2/3, but the enrichment of H3K27me3 at v2/v3 promoter was significantly lower than that at v1 promoter. These data indicate that EZH2 inhibits FADS2 v1 transcription by catalyzing H3K27me3, and promotes FADS2 v2/v3 transcription in a non-canonical way. In most OC cell lines tested, FADS2 v2/3 was dominant at the protein level compared to v1, which may be due to the advantage in protein stability of v2/3. EZH2 inhibitors reversed the FADS2 expression pattern by especially upregulating FADS2 v1, and increased PUFA abundance in OC cells. In reverse, targeting FADS2 by shRNA or inhibitor impaired EZH2 level and Notch signaling, resulting in suppressed cell proliferation and increased sensitivity to EZH2 inhibition. Proteomics analysis revealed that SDHD (succinate dehydrogenase complex subunit D, a subunit of mitochondrial complex II) was one of the most down-regulated protein under the combined treatment of FADS2 and EZH2 inhibitors. The combined treatment suppressed mitochondrial function and activated AMPK signaling, leading to inhibition of protein synthesis and cell cycle arrest. Furthermore, we found that combined targeting EZH2 and FADS2 induced synergistic inhibition of Notch signaling and mitochondrial function, and enhanced the therapeutic effect of OC in vitro and in vivo. In conclusion, this study reveals the regulation between EZH2 and FADS2, and provides a novel potential therapeutic strategy for ovarian cancer.

#4444 Targeting eIF4F-mediated translation to reduce T cell exhaustion and enhance PD-1 blockade efficacy in melanoma.

Samad Muhammadnejad[1], Monireh Kazemimanesh[1], Naima Benannoune[1], Sandrine Agoussi[1], Cecile Badoual[2], Louis Vaquier[2], Helene Lecourt[1], stephan Vagner[3], **Caroline Robert**[1]

[1]INSERM U981 (Gustave Roussy), Villejuif, France,[2]Pathology, Gustave Roussy, Villejuif, France,[3]Institut Curie, Orsay, France

Background: The eukaryotic initiation factor 4F (eIF4F) complex is essential for the selective translational control of protein expression enabling cells to rapidly adapt to diverse stimuli. We previously identified an eIF4F/STAT1/PDL1 axis in melanoma cells. The effectiveness of cancer immunotherapy, particularly immune checkpoint inhibitors (ICIs) or tumor-infiltrating lymphocytes (TILs)-based adoptive therapy, is hindered by resistance mechanisms associated with the terminal exhaustion of T cells. Here, we investigate the role of eIF4F in the translational control of immune checkpoints (ICs) and associated transcription factors in both circulating T cells and TILs from melanoma patients, aiming to reveal potential connections between eIF4F-mediated translational regulation and T cell exhaustion.
Methods: Polysome profiling was conducted on both circulating lymphocytes and TILs isolated from 10 melanoma specimens. We examined the expression of various activation/exhaustion markers by FACS, assessed T cell functions and determined cytokine secretion profiles. These experiments were performed with and without inhibition of the eIF4F-dependent translation using three different inhibitors of the eIF4A helicase (eIF4A-i: silvestrol, EFT226 and RBX0901). The effects of eIF4A-i combined with PD1 blockade were assessed in ex-vivo co-cultures, organotypic models and three syngeneic murine cancer models of melanoma and colon cancer.
Results: Key negative immune checkpoints and transcription factors including TOX, NFAc2, CTLA-4, LAG3, and TIM3 are regulated by eIF4F in TILs and can be efficiently downregulated by eIF4A-i.. Notably, the factors controlled by eIF4F in circulating $CD8^+$ T cells differ from those in TILs emphasizing the importance of patients' tumor-derived samples. TILs proliferation is increased by eIF4A-I in vitro. Co-culture experiments with TILs and syngeneic melanoma cells along with organotypic melanoma models, showed enhanced melanoma killing by TILs when anti-PD1 was combined with eIF4A-i. Additionally a synergistic effect of eIF4A and anti-PD1 was observed in the three immunocompetent murine models tested.
Conclusion: Our findings unveil a previously unrecognized role of eIF4F in orchestrating the translational regulation of key proteins driving T cell exhaustion. This study is the first to demonstrate that targeting eIF4F with eIF4A inhibitors not only downregulates exhaustion markers but also synergistically enhances the efficacy of anti-PD-1 therapy across multiple models, including human TILs and murine cancer systems. This novel insight underscores the immense therapeutic potential of combining eIF4A inhibitors with ICIs to overcome resistance mechanisms and reinvigorate anti-tumor immunity.

#4445 Super-enhancers reorganization controls re-sensitization of oxaliplatin-resistant FBXW7-mutated colorectal cancer.

V. Tolotto[1], N. Gualandi[1], R. Picco[1], Y. Cortolezzis[1], F. D'Este[1], M. Colitti[1], W. W. Hancock[2], C. Brancolini[1], L. Xodo[1], E. Di Giorgio[1]; [1]University of Udine, Udine, Italy, [2]University of Pennsylvania, Philadelphia, PA

The resistance of cancer to therapies remains an unresolved issue. It may result from tumor evolution, which is characterized by epigenetic plasticity and large-scale chromatin remodeling. Catalytically inactive class IIa HDACs (HDAC4, HDAC5, HDAC7, HDAC9) control the targeted recruitment of chromatin remodeling complexes, making them attractive therapeutic targets in oncology. Recently, we have described the role of HDAC4 in monitoring the assembly of the end resection complex involved in homologous recombination (HR) repair. In cells defective for HR repair, HDAC4 is proteasomally degraded. Here, through *in silico* and *in vitro* screenings we identified FBXW7 as the E3 ligase involved in proteasomal degradation of HDAC4, and its role was confirmed by means of ubiquitin co-immunoprecipitation experiments and *in vitro* ubiquitylation assays. FBXW7 is a tumor suppressor gene encoding the substrate recognition component of an SCF ubiquitin ligase complex. It is one of the most frequently mutated genes in colorectal cancer (CRC), and loss of function of FBXW7 has been reported to induce resistance to chemotherapeutic agents such as oxaliplatin (OxPt), which is commonly used in adjuvant therapy for CRC. We confirmed that *FBXW7-/-* CRC cells were resistant to OxPt treatment and HDAC4 was not degraded under these conditions. Silencing or forced degradation of HDAC4 using a PROTAC-based compound restored OxPt sensitivity in *FBXW7*-mutated CRC cells, in patient-derived organoids (PDOs) and in a xenograft model in mice. By evaluating the transcriptomic landscape and super-enhancer (SE) repertoire status in FBXW7-mutated PDOs, we demonstrated the relevance of epigenetic resetting in controlling OxPt resistance. In particular, forced downregulation of HDAC4 resulted in dramatic loss of SEs in FBXW7-deficient tumors and restoration of certain SEs found in OxPt-sensitive tumors. Furthermore, patient profiling based on the epigenetic state of SEs controlled by HDAC4 identified a priori CRC patients resistant to OxPt. This study supports HDAC4 as a key mediator of OxPt resistance in FBXW7-mutated CRC and highlights the remodeling of a well-defined repertoire of SEs as part of the process of successful re-sensitization.

#4446 Collateral drug screening throughout the evolution of osteosarcoma drug resistance.

Gabriel Mandel, **Elizabeth Nowak**, Jarrel Imamur, Kristi Lin-Rahrdja, Mina Dinh, Masahiro Hitomi, Jacob Scott, Zachary Burke

Cleveland Clinic, Cleveland, OH

Introduction: Osteosarcoma is the most common primary malignant bone tumor in childhood. Advancements in therapy have been stagnant for over 40 years and the prognosis for osteosarcoma remains challenging. The 5-year relative survival rate of localized osteosarcoma is 75%. If disease is metastatic upon diagnosis or first line therapy fails, survival rate falls to approximately 25%. First line therapy consists of alternating cycles of methotrexate with doxorubicin and cisplatin (MAP). In contrast, second line therapy is not standardized, and outcomes after salvage therapy remain poor. Identifying better second line agents may be an avenue to successful salvage therapy. Cancer is a dynamic state and can be viewed through an evolutionary framework. When cancer cells are exposed to chemotherapy, resistant cells may be selected for and proliferate. These resistant cells may exhibit a different resistance/sensitivity profile to other therapy options, termed collateral changes. By correlating these collateral changes with genomic and transcriptomic data, we can theoretically develop a library of gene/gene expression markers that correlate with resistance or sensitivity to a panel of drugs.

Methods: We have exposed an osteosarcoma cell line, MG63.3, in cell culture to a clinically relevant sequential therapy that models MAP. 5 evolutionary replicates were exposed to combination cisplatin and doxorubicin, followed by methotrexate. 3 control replicates were exposed to solvent without drug. Once cells returned to confluency after each exposure to chemotherapy, we performed drug assays to a panel of 16 second-line drugs and drug combinations, in addition to radiation therapy, and extracted DNA/RNA from each replicate.

Results: As a result of this work, we created a heatmap displaying the development of sensitivity and resistance to this treatment panel, based on the change in half maximal effective concentration (EC50) over time and have found variable collateral changes indicating timepoints in which cells are significantly more sensitive or resistant to second line agents as our evolutionary replicates evolved resistance to frontline therapy.

Conclusions: This heat map can serve as a springboard for transcriptomic and genomic analyses to correlate the changes with resistance and sensitivity. This may allow future researchers to correlate our findings with patient derived genomics and transcriptomics to ultimately choose better second line therapeutics for patients who do not respond to MAP therapy.

#4447 Autophagy inhibition overcomes resistance to FLT3 inhibitors in *FLT3*-ITD acute myeloid leukemia models.

Manuela Albuquerque de Melo[1], Diego A. Pereira-Martins[2], Brunno G. S. Macedo[1], Priscila S. Scheucher[1], Joao A. Machado-Neto[3], Fabiola Traina[1]

[1]Department of Medical Images, Hematology, and Oncology, University of Sao Paulo at Ribeirao Preto Medical School, Ribeirao Preto, Brazil,[2]Department of Experimental Hematology, Cancer Research Center Groningen, University Medical Center Groningen, Groningen, Netherlands,[3]Department of Pharmacology, Institute of Biomedical Sciences, Sao Paulo, Brazil

Autophagy has recently been postulated as a potential mechanism that may contribute to the development of resistance to FLT3 inhibitors (FLT3i). Our aim was to investigate the role of pharmacologic autophagy inhibition in enhancing the efficacy of FLT3i in the *FLT3*-ITD acute myeloid leukemia (AML) resistant model and its impact on the survival and molecular response to FLT3i. In MOLM13 and MV4-11 (*FLT3*-ITD mutated cell lines), the administration of FLT3i first (midostaurin) or second (quizartinib) generation significantly elevates autophagic flux by inducing the formation of acidic vesicular organelles visualized by flux cytometry. We also observed a reduction in negative autophagy regulators, evidenced by the inhibition of AKT (p-Ser473), mTOR (p-Ser2448), and its downstream protein p70S6K (p-Thr421/Ser424), as well as a reduction of ULK1 (p-Ser757) by western blot assay. FLT3i treatment resulted in the conversion of LC3B-I to LC3B-II, accompanied by the degradation of LC3B-II and p62, indicating autophagy induction. The combination of FLT3i with autophagy inhibitors chloroquine (CQ), bafilomycin A1 and ROC-325 significantly reduced cell viability and survival in comparison with vehicle and monotherapies in vitro in MOLM13 and MV4-11 cells and ex vivo in primary cells from bone marrow of FLT3-ITD AML patients. To elucidate the role of inhibition of autophagy in FLT3i-resistant cells, we generated MV4-11 cells resistant to quizartinib (MV4-11 QR) trough selective pressure and we noted that MV4-11 QR cells exhibited a higher basal level of autophagy compared to conventional MV4-11 cells, as indicated by the increased formation of acidic vesicular organelles, and confirmed by the reduced expression of LC3B and p62 proteins. In MV4-11 QR cells, we observed that CQ effectively reduced the IC50 of quizartinib and increased FLT3i-induced apoptosis, suggesting its use to mediate resistance to FLT3i in AML. We also observed an increased molecular effect of quizartinib when associated with CQ, manifested by a significant increase in Cleaved-Caspase3 and a reduction of STAT5 (p-Tyr694) and P70S6K in comparison with vehicle and quizartinib monotherapy. The combination of quizartinib with CQ showed a strong synergistic effect in the resistant lineage demonstrated by a ZIP score value = 10.8, while in the non-resistant lineage it demonstrated an additive effect (ZIPscore = 8.4), suggesting that CQ is autophagy-dependent and has its effect potentiated in conditions of high autophagic flux, as observed in MV4-11 QR, being effective, mainly, in conditions of resistance generated by the induction of autophagy. In conclusion, FLT3i induces autophagy flux, limiting drug inhibitory activity, and autophagy inhibition can enhance the antileukemic effect of FLT3i and reverts cellular resistance. Analysis of the autophagic profile in AML can play a crucial role in individualized treatment.

#4448 Novel tyrosine kinase/MEK inhibitor combinations impair the viability and invasive potential of TNBC cell lines.

Celia Asdih[1], Ariane Lehmann[2], Jasmin Traichel[1], Eva Oswald[3], Julia Schueler[3], Rhena Klar[4], Patrick Metzger[4], Melanie Boerries[4], Tilman Brummer[1], Ricarda Griffin[1]

[1]Institute of Molecular Medicine and Cell Research, University of Freiburg, Freiburg, Germany,[2]Institute of Medical Bioinformatics and Systems Medicine, University of Freiburg, Freiburg, Germany,[3]Charles River Discovery Research Services Germany GmbH, Freiburg, Germany,[4]Institute of Medical Bioinformatics and Systems Medicine, Faculty of Medicine, Medical Center-University of Freiburg, Freiburg, Germany

This study demonstrates that the tyrosine kinase inhibitors ALW-II-41-27 and ponatinib enhance the efficacy of MEK inhibition in multiple TNBC cell lines, suggesting potential for combination therapy. This project aimed to identify compensatory mechanisms triggered by MAPK inhibitors (MAPKi) in TNBC and to develop novel treatment strategies to effectively block these mechanisms. To this end, we performed a conditioned medium (CM) experiment in which we identified secreted cytokines as potential mediators of drug resistance. We then performed a cytokine array and receptor tyrosine kinase (RTK) array to identify the cytokines and RTKs implicated in this process. Finally, we targeted some of these previously identified RTKs in combination with trametinib and tested the efficacy of the combination on the viability of 11 TNBC cell lines. Two combinations were tested for further experiments to characterize their efficacy and mechanism of action, including colony forming assay, transcriptomic analysis and wound healing assays. We identified several cytokines secreted in response to the pan-Raf inhibitor CCT196969, the MEK inhibitor (MEKi) trametinib and the ERK inhibitor (ERKi) ulixertinib such as FGF-basic, FGF-19, MIF and angiogenin. CM from drug treated cells induced the activation of several RTKs such as FGFR1, FGFR3, Insulin-R, ALK and DTK in untreated TNBC cells. To validate the significance of these upregulated RTKs, we used a panel of kinase inhibitors. This led us to identify two synergistic combinations: trametinib with the EPHA2 inhibitor ALW-II-41-27 and trametinib with ponatinib, which targets multiple RTKs, including EPHA2. These combinations demonstrated efficacy in other TNBC cell lines over a longer period and, notably, prevented cell re-growth even after an additional eight days without treatment. This was in contrast to trametinib single treatment, where cells resumed growth under similar conditions. Our transcriptomic analyses of the combination treatment with trametinib and ALW-II-41-27, either singly or in combination, across a panel of cell lines revealed significant pathway alterations. Notably, we observed increased *HER2* and *HER3* transcription following trametinib treatment, which we could confirm at the protein level by Western blotting. Lastly, the trametinib/ALW-II-41-27 combination revealed a significant reduction in cell migration and invasion compared to trametinib treatment alone. These findings indicate that ponatinib and ALW-II-41-27 combined with MAPKi could be a promising novel therapeutic approach for TNBC.

#4449 SIRT5 promotes chemoresistance through metabolic rewiring for nucleotide replenishing in refractory triple negative breast cancer.

Z. Ren[1], K. Kurmi[2], T. Bernasocchi[1], E. Zaniewski[1], S. Joshi[2], G. Lam[1], C. Guo[1], I. Smidt[1], P. Dave[1], A. Maccio[1], J. Kreuzer[1], G. E. Gioia[1], V. I. Bossuyt[1], B. N. Dontchos[1], G. X. Wang[1], S.-H. S. Chou[1], M. Lawrence[1], E. Rheinbay[1], L. M. Spring[1], W. Haas[1], R. Mostoslavsky[1], M. C. Haigis[2], L. W. Ellisen[1];

[1]Massachusetts General Hospital/Harvard Medical School, Boston, MA, [2]Harvard Medical School, Boston, MA

Drug resistance is the leading cause of neoadjuvant chemotherapy (NAC) failure to achieve pathological complete response (pCR) in more than 40% of patients with triple negative breast cancer (TNBC). Thus, understanding the potential drug resistance mechanisms in TNBC patients is a critical unmet clinical need. Using quantitative high-throughput metabolomic and proteomic approaches, we systematically analyzed clinicopathological, metabolic, and proteomic data profiled from NAC treatment-naive biopsy samples derived from primary TNBCs. Particularly, we identified that non-pCR TNBC primary tumors are uniquely and highly enriched in metabolic pathways for the TCA cycle and oxidative phosphorylation (OXPHOS) along with nucleotide metabolism relative to pCR TNBC tumors. In addition, sirtuin 5 (SIRT5) as a master regulator of mitochondrial metabolism is dramatically increased in non-pCR TNBC primary tumors. Of note, we found that SIRT5 is frequently overexpressed in TNBC tumors due to copy number gain and amplification. Furthermore, metabolomics and ^{13}C-glucose tracing uncovered that SIRT5 gain of function promotes chemoresistance by diverting TCA cycle intermediates into the pentose phosphate pathway (PPP) for nucleotide replenishing. Moreover, SIRT5 knockout restores chemosensitivity to TNBC cells. We anticipate that blocking SIRT5-mediated metabolic rewiring may reverse chemoresistance in refractory TNBC patients.

#4450 Targeting KEAP1/NRF2 signaling sensitizes KRAS-driven NSCLC to KRAS inhibitors.

Xianwen Yang, Peiling Ren, Guiping Li, Ying Yu, Guangxiu Dai

HUTCHMED, Shanghai, China

The emergence of KRAS G12C inhibitors has paved the way for new therapeutic approaches in NSCLC patients with KRAS mutations. However, the efficacy of these inhibitors has been limited in NSCLC based on clinical trial results. Numerous studies have uncovered that the co-occurrence of additional mutations, particularly in KEAP1 and STK11, is associated with a poorer prognosis for KRAS G12C-mutated NSCLC patients. KEAP1 is a negative regulator of the NRF2 signaling pathway. KEAP1 mutations lead to constitutive activation of NRF2, thus promoting tumor growth and drug resistance. In this study, we employed siRNA technique to specifically target NRF2, and found that targeting NRF2 could be a promising therapeutic approach to improve the efficacies of KRAS inhibitors in NSCLC. Firstly, we compared the basal mRNA and protein levels of NRF2 between cell lines with and without KEAP1 mutation. The results showed that the basal mRNA levels were comparable across 5 cell lines including A549, H2122, HEK293, H292 and LoVo. Only A549 and H2122, two cell lines with KEAP1 mutation, expressed high NRF2 protein levels. This observation aligns with the established mechanism that KEAP1 mutation leads to the accumulation of NRF2 through a protein degradation-repression manner. Furthermore, luciferase reporter assay demonstrated that NRF2 transcription was activated in A549 and H2122 in comparison to HEK293. Accordingly, the expression of NRF2 downstream genes, such as *AKR1B10* and *AKR1C1*, was relatively higher in KEAP1-mutated A549 and H2122 cells than in KEAP1-intact cells. To investigate the impact of NRF2 knockdown on cell proliferation, siRNA was performed in A549 and H2122 cells. The knockdown efficiency was verified through qRT-PCR and western blot analysis. The results revealed that knockdown of NRF2 could significantly inhibit cell growth in both A549 and H2122 cells. To ensure that the inhibitory effects of NRF2 knockdown were not limited to the two cell lines, we conducted an analysis of NRF2 dependency in NSCLC using the Depmap database. The results indicated that KEAP1/NRF2 pathway-activating mutations could predict NRF2 dependency in NSCLC, even in the broader context of lung cancer. Moreover, two downstream genes of NRF2, *AKR1B10* and *AKR1C1*, were observed down-regulated in A549 and H2122 cells post NRF2 knockdown. Finally, we assessed the combined effectiveness of NRF2 knockdown and KRAS inhibitors in the A549 and H2122 cells. The results demonstrated that silencing NRF2 could significantly improve the responsiveness of both the pan-KRAS inhibitor RMC-6236 and the KRAS G12C inhibitor Sotorasib in A549 and H2122 cells. Taken together, our study demonstrates that knockdown of NRF2 can enhance the effectiveness of KRAS inhibitors in treating NSCLC patients with KEAP1 mutations. This finding provides the scientific foundation for developing combination therapy strategies that target both NRF2 and KRAS to improve patient outcomes.

#4451 Stabilization of checkpoint kinase 1 by the lncRNA HOTAIR promotes PARP inhibitor resistance in high-grade serous ovarian cancer.

Yanchi Zhou, Kenneth P. Nephew

Indiana University Bloomington, Bloomington, IN

Poly (ADP-ribose) polymerase inhibitors (PARPis) are an important treatment modality for homologous recombination deficient (HRD) cancers, including high grade serous ovarian cancer (HGSOC). PARP mediates DNA single-strand break (SSB). PARPis trap PARP at the site of SSB and prevent DNA SSB repair, which eventually leads to double-strand DNA breaks (DSBs). DSBs are normally repaired through the HR pathway, and PARPis are highly efficient in HRD disease. However, the majority of HGSOC are HR proficient (HRP), and the use of PARPis for patients with HRP tumors represents an unmet therapeutic need. The long non-coding RNA (lncRNA) HOX transcript antisense RNA (HOTAIR) is frequently over-expressed in HGSOC and contributes to chemoresistance. We examined the role of HOTAIR in the response to PARPis in HRP and HRD HGSOC cell lines. Knockout or siRNA knockdown of HOTAIR sensitized ($p<0.05$) HGSOC cell lines to PARPis (olaparib, talazoparib). HOTAIR upregulated the expression of checkpoint kinase 1 (CHK1), a key kinase involved in DNA damage response. Treatment with talazoparib (5μM, 72hrs) increased ($p<0.05$) expression of CHK1, while HOTAIR depletion with paired CRISPR gRNAs decreased ($p<0.05$) CHK1 activation by PARPi. In contrast, overexpression of HOTAIR enhanced ($p<0.05$) talazoparib-induced activation of CHK1. It has been reported that CHK1 inhibition converted PARPi-insensitive cells to PARPi-sensitive cells and, furthermore, that CHK1 interacts with the DNA repair protein RAD51 which was necessary to promote the reversal of stalled replication forks and protect the reversed fork from degradation. Thus, we hypothesized that HOTAIR contributes to PARPi resistance in HGSOC cell lines by regulating CHK1 and CHK1-mediated replication forks stabilization and DNA damage response. We demonstrated that RAD51 expression was reduced ($p<0.05$) in HOTAIR depletion cells treated with talazoparib, while overexpression of HOTAIR increased ($p<0.05$) talazoparib-induced activation of RAD51. In addition to a role in replication stress, CHK1 and RAD51 contribute to DNA damage repair through the HR repair pathway. To further examine the role of HOTAIR in DNA damage repair, we examined the effect of PARPi on DNA damage using the comet assay. In HOTAIR overexpressed HGSOC cells, PARPi-induced DNA damage was reduced ($p<0.001$), based on comet tail moment. As no effect of HOTAIR on CHK1 transcription was observed, we investigated the mechanism of HOTAIR regulating CHK1 by using the cycloheximide chase assay and assessing CHK1 protein stability. HOTAIR depletion increased ($p<0.05$) degradation of CHK1, and HOTAIR overexpression stabilized CHK1 protein. Collectively, these findings suggest that HOTAIR contributes to CHK1 stabilization, promotes replication fork protection and DNA damage repair in HGSOC and in turn HOTAIR contributes to PARPi resistance, regardless of HR status.

#4452 Targeting matrix metalloproteinase-3 to reverse chemotherapy resistance in high-grade serous ovarian cancer.

Victor G. Reyes-Burgos[1], Marienid Flores-Colon[1], Eliud Hernandez-O'Farrill[2], Luis E. Vazquez-Quinones[3], Pablo E. Vivas-Mejia[1]

[1]Biochemistry, University of Puerto Rico School of Medicine, San Juan, Puerto Rico,[2]University of Puerto Rico School of Pharmacy, San Juan, Puerto Rico,[3]Sciences and Technology, Interamerican University, Arecibo, Puerto Rico

High-Grade Serous Ovarian Cancer (HGSOC) remains the most aggressive and lethal gynecological malignancy, with most patients developing resistance to platinum-based chemotherapies. Matrix Metalloproteinase-3 (MMP3), an enzyme involved in extracellular matrix remodeling, has been implicated in this resistance. Prior work in our lab demonstrated that MMP3 is overexpressed in chemotherapy-resistant ovarian cancer cell lines, including the cisplatin resistant cells, OVCAR3CIS and A2780CP20, compared to their sensitive counterparts, OVCAR3 and A2780, respectively. Our team previously showed that knockdown of MMP3 with small interference RNA (siRNA) reduce cell growth and invasion of cisplatin-resistant ovarian cancer cells. We have progressed toward therapeutic applications by developing small molecule inhibitors (SMIs) to target MMP3. Bioinformatics was used to select potential MMP3 inhibitor candidates starting with a library of 370,000 compounds. Cell viability assays showed that four out of 30 preselected SMIs significantly reduced cell viability in cisplatin resistant cell lines. Further evaluation in combination therapy revealed promising results, as pre-treating cisplatin-resistant HGSOC cells with SMIs resensitized the cells to cisplatin treatment. These findings provide compelling evidence that MMP3 inhibition can restore chemotherapy sensitivity in resistant HGSOC cells, offering a promising dual-therapy approach. This research represents a significant advancement in developing novel therapeutic strategies to overcome chemotherapy resistance and improve outcomes for HGSOC patients.

#4453 Glucagon-like peptide-1 receptor agonists target the HIF1α-PFKFB3 pathway to enhance Lenvatinib efficacy and overcome resistance in hepatocellular carcinoma through the reversal of the Warburg effect.

Takayuki Noma[1], Yuan Li[1], Yuma Wada[2], Katsuki Miyazaki[2], Yuji Morine[2], Mitsuo Shimada[2], Ajay Goel[1]

[1]Molecular Diagnostics and Experimental Therapeutics, Beckman Research Institute of The City of Hope, Duarte, CA,[2]Department of Surgery, Tokushima University, Tokushima, Japan

Background: Glucagon-like peptide-1 receptor agonists (GLP1RAs), originally developed for diabetes and obesity treatment, have recently gained attention for their potential anti-tumor effects. However, the mechanisms underlying these effects remain largely unexplored. Given their emerging role in improving metabolic conditions such as fatty liver disease and liver cirrhosis, we hypothesized that GLP1RAs could also target cancer-specific metabolic pathways, a potential that is yet to be fully understood. Hepatocellular carcinoma (HCC), characterized by metabolic reprogramming, including enhanced glycolysis, consequently contributes to chemotherapy resistance and malignancy. This study evaluates the anti-tumor potential of GLP1RAs in HCC, focusing on their ability to reverse the Warburg effect by inhibiting key glycolytic pathways, thereby improving therapeutic outcomes.
Methods: A comprehensive series of experiments was conducted using GLP1RAs and Lenvatinib, including cell proliferation, apoptosis, and glycolysis assays. Genes involved in metabolic pathways were screened using PCR analysis, with key targets identified and validated through Western blotting. Metabolic reprogramming was assessed using Seahorse XF analysis and lactate assays to measure extracellular acidification rate and lactate production. Additionally, the synergistic effects of GLP1RAs and Lenvatinib were evaluated to determine their combined therapeutic potential.
Results: The combination of GLP1RAs and Lenvatinib demonstrated synergistic effects, suppressing cell proliferation and enhancing apoptosis in Lenvatinib-resistant cell lines. PCR screening and subsequent validation revealed that Lenvatinib resistance was associated with enhanced glycolysis via the HIF1α-PFKFB3 pathway. GLP1RAs treatment significantly inhibited the HIF1α-PFKFB3 pathway, reducing ECAR and lactate production. By reversing the Warburg effect, GLP1RAs suppressed glycolysis and exerted potent anti-tumor effects.
Conclusion: GLP1RAs target the HIF1α-PFKFB3 pathway and significantly enhance the anti-tumor efficacy of Lenvatinib by reversing the Warburg effect in Lenvatinib-resistant HCC cells. These significant findings, which suggest that GLP1 can potentially overcome Lenvatinib resistance and improve therapeutic outcomes in advanced HCC, are a testament to the potential of GLP1RAs in cancer treatment.

#4454 Cancer cell permeability induced by tumor treating fields (TTFields) improves chemotherapy uptake and requires cells to transverse through the G2/M phase under TTFields application.

Bella Koltun, Tali Voloshin, Cfir David, Tal Kan, Yiftah Barsheshet, Alexandra Volodin, Shay Cahal, Catherine Tempel-Brami, Mai Shai, Sara Jacobovitch, David Lancry Abittan, Boris Brant, Noa Kaynan, Lilach Koren, Anat Klein-Goldberg, Efrat Zemer Tov, Rom Paz, **Naama Flint-Brodsly**, Adi Haber, Moshe Giladi, Uri Weinberg, Yoram Palti

Novocure Ltd, Haifa, Israel

Background: Multidrug resistance (MDR) poses a significant challenge in cancer treatment, and efforts to overcome it have not yet resulted in meaningful improvements in survival rates. Tumor Treating Fields (TTFields), a cancer treatment based on the application of electric fields, have recently been shown to increase the membrane permeability of glioblastoma cells. The current study aimed to characterize this phenomenon and examine whether it can be leveraged to facilitate uptake of anticancer agents and overcome MDR.
Methods: 4T1 breast mammary carcinoma cells were treated with TTFields (300 kHz; 1.7 V/cm). Intracellular 7-aminoactinomycin D (7-AAD) accumulation was measured by flow cytometry, adding the agent before or after treatment cessation. Cell cycle analysis using the Hoechst reagent was performed with or without G1/S blockage using thymidine. 4T1-MDR (doxorubicin (DOX)-resistant) cells were generated by repeated DOX exposure of the parental 4T1 (DOX-sensitive) cells. TTFields together with DOX were applied to the 4T1 and 4T1-MDR cells, followed by analysis of DOX accumulation and cell count. For testing drug accumulation in vivo, mice were orthotopically inoculated with 4T1 cells or with lung carcinoma LL/2 cells, followed by TTFields treatment and injection of DOX or paclitaxel (PTX), respectively. Drug accumulation was measured in single-cell tumor suspension and whole tumors, using flow cytometry or an in vivo imaging system (IVIS) for DOX, and LC-MS/MS for PTX. For the latter, accumulation was also measured in the healthy lung. For the efficacy studies, mice were orthotopically inoculated with 4T1 or 4T1-MDR cells. The mice were treated with TTFields or sham-heat continuously for 8 days, with DOX (1 mg/kg for 4T1 cells; 5 mg/kg for 4T1-MDR cells) or vehicle injections on days 2 and 6 of treatment. Tumor volumes were measured using caliper.
Results: 7-AAD accumulation tests demonstrated that TTFields-induced cancer cell permeability is reversible and requires that cells transverse through the G2/M phase under TTFields application. The application of TTFields allowed DOX accumulation to the same extent in both DOX-resistant and DOX-sensitive cells and sensitized both cell types to DOX cytotoxicity. Enhanced drug accumulation was observed in vivo, with DOX in breast tumors and PTX specifically in lung tumors. The application of TTFields concomitantly with DOX significantly decreased tumor growth in both 4T1 and 4T1-MDR tumor-bearing mice.
Conclusions: TTFields reversibly and in a G2/M dependent manner increased cancer cell permeability, allowing enhanced intracellular accumulation of chemotherapy, in vitro and in vivo. TTFields treatment concomitant with DOX improved treatment efficacy, in DOX-resistant and DOX-sensitive cells, in vitro and in vivo.

#4455 Involvement of the PI3K/AKT signaling pathway in the cancer cell response to tumor treating fields (TTFields).

Anat Klein-Goldberg, Tali Voloshin, Efrat Zemer-Tov, Rom Paz, Lilach Koren, Kerem Wainer-Katsir, Alexandra Volodin, Bella Koltun, Boris Brant, Yiftah Barsheshet, Tal Kan, Cfir David, Tharwat Haj Khalil, **Naama Flint-Brodsly**, Adi Haber, Moshe Giladi, Uri Weinberg, Yoram Palti

Novocure Ltd, Haifa, Israel

Introduction: Tumor treating fields (TTFields) therapy is an approved treatment for glioblastoma (GBM), pleural mesothelioma and non-small cell lung carcinoma (NSCLC), and is under investigation for the treatment of other solid tumors. The current study aimed to uncover cellular response mechanisms of cancer cells to TTFields treatment.
Methods: Changes in signaling pathways following TTFields application (150 or 200 kHz; 1.7 V/cm RMS) were analyzed using the Luminex multiplex assay and validated by protein expression in three cancer cell lines: A2780 ovarian cancer cells, H1299 lung cancer cells, and U87-MG GBM cells. The mechanism of action was tested with various tools, including immunofluorescence microscopy, inhibition assay, antibody neutralization assay, and immunoprecipitation. The efficacy of concomitant treatment with TTFields and a potential inhibitor were tested in cell lines and in an orthotopic LL/2 lung cancer mouse model.
Results: Increased PI3K/AKT and Focal Adhesion Kinase (FAK) phosphorylation were observed in the cancer cells following short term (2h) treatment with TTFields. Accordingly, FAK inhibition abrogated TTFields-induced AKT phosphorylation. For long term (72h) treatment, the PI3K/AKT pathway was activated via N-cadherin, as evident by the increased cell surface levels and reversal of the AKT phosphorylation when a neutralizing antibody was used to inhibit the N-cadherin-mediated cell-cell contacts. Additionally, pull-down assays with an anti-N-cadherin antibody demonstrated increased recruitment of the p85 regulatory subunit of PI3K to N-cadherin complexes. In accordance with the identified involvement of the PI3K/AKT axis in the response to TTFields, co-treatment with the PI3K inhibitor alpelisib enhanced the effectiveness of TTFields in vitro and in vivo.
Conclusions: These findings provide a molecular understanding of the cellular response to TTFields, with early PI3K/AKT activation through FAK cell-surface interactions, and late activation via N-cadherin cell-cell interactions. PI3K inhibition presents a potential therapeutic strategy to sensitize cancer cells to TTFields treatment.

CHEMISTRY: Computational Drug Discovery, Nanotechnology, and Therapeutics
Poster Session

#4459 Molecular docking and drug virtual screening of novel small molecule inhibitors of anti-apoptotic proteins for cancer treatment.

J. Shin[1], Y. Chen[2], B. Feldmeier[3], E. Lee[1], Q. Zhao[1];
[1]University of the Pacific, Stockton, CA, [2]The Quarry Lane School, Dublin, CA, [3]Amador Valley High School, Pleasanton, CA

Bcl-2, an anti-apoptotic protein, is involved in cancer development and progression and has been considered as a promising target in cancer therapy. ABT-199, a Bcl-2 inhibitor, has been approved, as a single agent or in combinations, for the treatment of adult patients with chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), or newly diagnosed acute myeloid leukemia (AML) patients with age of 75 years or older. Although most patients can initially benefit from ABT-199 treatment, patients eventually develop resistance majorly due to BCL2 mutations, such as G101V and D103Y, or overexpression of Bcl-xL. Therefore, inhibitors that can target both wild-type and mutant Bcl-2 as well as Bcl-xL have the potential to overcome the resistance mechanisms to ABT-199. LP-118 is a novel inhibitor targeting wild-type Bcl-2, Bcl-2^{G101V} and Bcl-2^{D103Y} mutants, and has moderate activity against Bcl-xL. In preclinical and clinical studies, LP-118 had excellent anticancer activity in animal models and patients with CLL or SLL. However, there is no co-crystal structure information available to show exactly how LP-118 binds to Bcl-2, Bcl-2^{G101V}, Bcl-2^{D103Y}, or Bcl-xL. Molecular docking is one of the most frequently used methods in drug design and discovery, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site. Using the molecular docking Software ICM-Pro, we are the first to illustrate how LP-118 binds to Bcl-2, Bcl-2^{G101V}, Bcl-2^{D103Y}, and Bcl-xL proteins in 3D. The molecule LP-118 is tightly tugged in the reception pocket of each protein where the interactions, including hydrogen bonding, electrostatic and π-stacking interactions, slightly vary in each case. These knowledge enables a better understanding of the mechanism of actions of LP-118 and provides a foundation for the rational design of future generations of Bcl-2/Bcl-xL inhibitor that will overcome the Bcl-2 mutation resistance. Based on the docking result, we designed over 1,000 analogues of LP-118 by fine-tuning the backbone and substituents on the molecule, used ICM-Pro/VLS software to virtually screen these analogues against Bcl-2, Bcl-2^{G101V}, Bcl-2^{D103Y}, or Bcl-xL, and successfully identified and prioritized 10 top-ranked analogues for chemical synthesis and anticancer activity testing.

#4460 OpencanSARchem: chemistry registration and standardization pipeline for FAIR integration of bioassay data.

Rezvan Chitsazi, Phillip W. Gingrich, James P. Long, Chong Wu, Bissan Al-Lazikani

UT MD Anderson Cancer Center, Houston, TX

Introduction: The rapid growth of medicinal chemistry and bioactivity data has driven AI advancements but inconsistencies in chemical representation across databases make bioactivity-focused data integration challenging. Tautomerism, a key challenge, complicates chemical registration due to the existence of multiple interchangeable molecular forms, significantly impacting database uniqueness and separating bioactivity data across tautomeric forms. We previously developed a standalone, fully deployable solution to address these challenges (Dolciami et al. 2022), now a core component of the canSAR (https://cansar.ai) knowledgebase. However, our solution fell short of achieving FAIR (Findable, Accessible, Interoperable, Reusable) data integration due to its reliance on commercial software. In addition, it was computationally intensive limiting the size of data and rapidity of analysis. We present OpencanSARchem, a fully open-source, RDKit-based, and computationally efficient tool for the drug discovery community. It is 100-fold faster, enabling analysis of larger databases, eliminates reliance on commercial software, and enhances scalability to advance data integration.
Methods: OpencanSARchem pipeline involves five steps: (1) assessing input structure for physical consistency, (2) removing irrelevant fragments, solvents, and counter-ions, (3) tautomerically canonicalizing compounds, (4) removing stereochemistry and isotopic labels, and (5) generating abstract representations for hierarchical generalization and grouping of related data across disparate data sources. Tautomeric canonicalization is the key difference between RDKit (OpencanSARchem) and ChemAxon (canSARchem), potentially leading to variations in canonical tautomer selection. We use quantum chemical methods to calculate the Gibbs free energy of tautomer pairs generated by both pipelines, followed by statistical analysis to evaluate the energetic consequences of our utility selection.
Results: The new pipeline can process 2.3 million compounds for standardization and generation of canonical tautomers in ~3 hours. We found that 20% of compounds resulted in different tautomers between ChemAxon and RDKit. The median difference in Gibbs free energy between ChemAxon and RDKit tautomers was found to be approximately 2 kcal/mol. In the context of drug discovery, small free energy differences allow tautomers to coexist in equilibrium, making either structure potentially available for therapeutic activity.
Conclusion: To our knowledge, OpencanSARchem is the only fully open-source pipeline addressing all aspects of chemical standardization, including tautomerization, and enabling FAIR data integration, democratizing this method to address the needs of the broader community.

Dolciami et al., *J. Cheminformatics, 2022,* 14, 28.

#4461 Utilizing steered molecular dynamics simulations for enhanced virtual screening: Active pharmacophore search by dynamic corrections to target MKK3-MYC interactions.

Muhammet Eren Ulug[1], Saima Ikram[1], Ehsan Sayyah[1], **Serdar DURDAGI**[2]

[1]Lab for Innovative Drugs (Lab4IND), Computational Drug Design Center (HITMER), Bahcesehir University (BAU), Istanbul, Turkey,[2]Molecular Therapy Lab, Department of Pharmaceutical Chemistry, School of Pharmacy, Bahcesehir University (BAU), Istanbul, Turkey

The complex interplay between mitogen-activated protein kinase 3 (MKK3) and the MYC proto-oncogene protein is critical for cancer progression, especially in triple negative breast cancer (TNBC). Despite substantial advancements, effective MYC-targeted drug discovery remains a challenge, underscoring the need for innovative approaches to manage MYC-dependent malignancies. One promising strategy involves disrupting the protein-protein interactions (PPIs) between MKK3 and MYC, as MKK3-mediated activation of MYC is pivotal in multiple cell types, highlighting a new target for therapeutic interventions in MYC-driven cancers. In this study, we employed a novel *in silico* approach to screen small molecule libraries targeting the MKK3-MYC interaction. Dynamic structure-based pharmacophore models were created to screen these libraries, identifying compounds that effectively aligned with the specified pharmacophore features. Physics-based simulations were then conducted on the selected hits. Steered molecular dynamics (sMD) simulations were employed to evaluate the correlation between the force required to dissociate the candidate ligands from the binding pocket and their corresponding average binding free energies. Comparative analysis of the average binding free energies of these identified hits, relative to the reference compound SGI-1027, indicates that the selected molecules have promising predicted binding affinities. These findings may represent a significant step forward in our ability to modulate MYC activation for cancer therapy.

#4462 Covalent inhibition of eIF4E: A computational approach.

X. Ma;
Portsmouth Abbey School, Portsmouth Abbey School, RI

Covalent docking has emerged as a promising strategy for targeting challenging protein residues. Specifically, lysine residues, which are often overlooked due to their lower nucleophilicity compared to cysteines, have shown potential in the development of covalent inhibitors for eukaryotic translation initiation factor 4E (eIF4E), a critical regulator of protein synthesis implicated in breast cancers. Leveraging aryl sulfonyl fluorides, recent studies had successfully docked to K162 within eIF4E and shown reduced protein activity, but the molecules had faced significant specificity and instability. In this study, I improved the molecules in the following aspects: specificity and stability. Regarding specificity, I found additional lysine residues, such as K106 and K108, as promising targets for covalent inhibition through structural analyses of eIF4E. I also designed bivalent molecules that covalently bind to both lysine residues simultaneously. This bivalent binding increases the specificity as there are only two regions of two lysine residues in three amino acids, whereas there are a total of 15 lysine residues. The differences in binding affinity and docking score between the bivalent and control groups were insignificant, suggesting that the potency of the bivalent molecule didn't drop. Finally, through molecular dynamic simulations, I verified that there is conformational changes at the cap-binding site after covalent inhibition, demonstrating the biological efficacy of the drug molecules.

#4463 A novel method for constructing diverse cyclic peptide libraries for efficient screening of PPI inhibitors.

Zhenyu Wu[1], Xiaoli Hou[2], Hongbo Zhang[3]

[1]The Ohio State University, Columbus, OH,[2]Beijing University of Chemical Technology, Beijing, China,[3]Nankai University, Tianjin, China

Cyclic peptides drug design and discovery has experienced a resurgence of interest due to their target specificity and metabolic stability. In this study, we developed a novel methodology for generating extensive, structurally diverse, and synthesizable virtual libraries of cyclic peptides. Such libraries are essential for the identification of hit compounds against a spectrum of therapeutic targets through screening assays. We introduce an innovative strategy for the creation of diverse cyclic peptide libraries leveraging a “hierarchical generation” approach combined with artificial intelligence (AI)-driven sampling. Our approach begins with the generation of a library of representative linear peptides which are subsequently subjected to cyclization via an array of cyclization reactions. The generation of linear peptides is executed via a hierarchical generation method wherein amino acids are initially categorized according to their physicochemical properties. Next, a sequence of these properties is formulated, and the similarity of these property sequences is calculated through sequence alignment algorithm. Low-similarity property sequences are then processed by AI models to generated multiple sequences of amino acids, while high-similarity sequences are judiciously excluded. The iteration of this process yields a set of representative linear peptides, which are then cyclized to produce the desired cyclic peptides. Employing this novel methodology, we have successfully generated a virtual cyclic peptide library encompassing nearly 6 million molecular cyclic peptides (covering approximately 4 thousand trillion peptides) within a single week. Over 1000 representative cyclic peptides were selected for synthesis via the solid-phase peptide synthesis (SPPS) technique, and a cyclic peptide library with high structural diversity was obtained. In conclusion, the structural diversity of our library, combined with the successful screening results, highlights the robustness of our method in designing diverse cyclic peptides, particularly for hit identification against traditionally undruggable targets. Our findings underscore the utility of this cyclic peptide library in discovering potential PPI inhibitors.

#4464 A large language model for diverse 2D molecular generation (2DMG) and its application in focused library generation and rational design.

Zihao Liu[1], Qingbo Xu[2], **Zhenyu Wu**[1], Hongbo Zhang[3]

[1]The Ohio State University College of Medicine, Columbus, OH,[2]China Agricultural University, Beijing, China,[3]Nankai University, Beijing, China

AI-driven large language models for molecular generation are gaining significant interest in structure-based drug design. However, challenges such as dataset quality, molecular diversity, and synthesizability still persist. To address these challenges, we introduce StoneWise 2DMG, an advanced model that sparks creativity in medicinal chemistry experts, accelerating the drug discovery process by generating diverse 2D molecular structures. The model is pre-trained on a vast dataset comprising 30 million commercial compound structures (covering most global commercial compounds), 8 million patent-derived compound structures (spanning from the 1960s to 2023), 11 million building block structures, and billions of easily synthesizable molecular structures. This large-scale training enables the generation of drug-like molecules suitable for focused library generation and rational design. The 2DMG model can be utilized for focused library generation. By analyzing the structures of molecular glues that have been published or advanced into clinical trials, we selected glutarimide as a seed molecule for focused library design. Utilizing the 2DMG model, we are able to rapidly generate a diverse, rational, synthesizable, and drug-like molecular library. This molecular glue library is currently under synthesis in the laboratory and will be employed for high-throughput screening in the future. We then extended the application of our model to tackle a more advanced challenge in rational drug design. In a case study involving ABBV-CLS-484 (a molecule targeting both tumor and immune cells; PDBID: 7UAD), we analyzed the key binding fragment, thiadiazolidin-3-one, in the crystal structure. The model then used this critical fragment as a seed to generate a focused library of over 100,000 compounds, which were docked and further selected by medicinal chemistry experts. Ten compounds were chosen for synthesis, and four showed acceptable activity, making them suitable for lead optimization. In summary, StoneWise 2D Molecular Generation model, trained on billions of data points, offers a robust platform for generating diverse and structurally rational molecules. The model supports focused library design for high-throughput or virtual screening and facilitates rational design to explore and expand chemical space, thus accelerating the drug discovery process.

#4465 Molecular docking studies on the factors affecting histone deacetylase inhibition: Design, rigidity, and chirality of the inhibitors.

Q. Zhao, J. A. Tang, K. Artero, A. Huang, R. Bosri;
University of the Pacific, Stockton, CA

Histone deacetylase (HDAC) has become one of the targets for cancer therapy since it was found overexpressed in cancer cells. New HDAC inhibitors were designed and investigated to demonstrate how the molecules interact with HDAC enzymes, using the molecular docking software ICM-Pro. Multiple families of HDAC inhibitors, derivatized from the FDA-approved HDAC inhibitors, were constructed and optimized for *in silico* screening. Detailed analyses of the docking results showed that the addition of an aromatic ring on the linker domain could boost or diminish the interactions of the molecules with the enzyme, depending on the location and size of the aromatic ring. The rigidity of the molecules was tuned by installing heterocyclic rings of various sizes at different positions. Molecules of specific rigidity could greatly enhance the dock scores and binding affinity, thus theoretically the enzyme inhibition. Modification of protein recognition and zinc-binding domains and chirality of the molecules were also explored to seek inhibitors for specific HDAC inhibitions. Drug candidates with the desired binding affinity and selectivity were selected for chemical synthesis and biological activity examination in the lab.

#4466 Machine-learning-driven optimization of kinase inhibitors to overcome CNS efflux and target breast cancer brain metastases.

Eneye D. Ajayi, Mostafa M. A. Aref, Rokaia Abdullah, Md Emran Hossain, Hamed I. Ali

Pharmaceutical Sciences, Texas A&M University, College Station, TX

Breast cancer (BC) is the most prevalent form of malignancy and the leading cause of cancer mortality in women. The fatality of BC is highly attributed to BC brain metastases (BCBM) which represent 30%, with a 1-year survival rate of 20%. About 15-20% of BC demonstrates significant overexpression of HER2, hence the development of anti-HER2 targeted therapies such as tucatinib which have prolonged survival rates and improved patient quality of life. Despite the improvements in HER2 targeted therapy to rapidly target BCBM, tucatinib and other quinazoline HER2 inhibitors (dacomitinib and gefitinib) possess poor CNS penetration due to P-gp and BCRP efflux. *In vitro,* BBB permeability assays such as the BBB-PAMPA are cost-intensive prompting the need for computational methods that can rapidly predict the BBB permeability potential of small molecules in the early phase of drug discovery. Herein, we used quantitative and qualitative machine learning (ML) algorithms trained with experimental data to direct structural optimization of in-house KIs to improve BBB penetration. We employed qualitative and quantitative ML models to identify the most promising compounds from our in-house library of 120 KIs previously designed for dual HER2/VEGFR targeting. The core of our approach lies in the structural simplification of starting compounds to drastically reduce molecular weight and topological surface area and the appropriate substitution of solubilizing residues to modulate pKa. Furthermore, we developed a benchmark of optimal physiochemical properties of molecules intended as CNS therapeutics using an approved CNS drug dataset. Optimized analogs possessing physicochemical properties within the benchmark range and a favorable ML prediction of BBB permeability were synthesized and tested on a kinase panel. BBB-PAMPA was also used to evaluate BBB penetration of both starting KIs and optimized analogs. Optimized analogs demonstrated HER2 kinase inhibitory activity alongside a significant increase in BBB permeability compared to the starting compounds. Our results suggest that structural optimization to modulate the physicochemical properties of small molecules is key to identifying potential drug candidates for targeting BCBM. *In vivo* studies using animal models will be conducted to validate these findings.

#4467 Receptor targeted delivery of the multi-kinase inhibitor F7/PIK-75 by organic-core templated lipid nanoparticles as cancer therapy.

Jonathan S. Rink[1], Andrea E. Calvert[1], David Kwon[2], Xu Hannah Zhang[3], Hongwei H. Yin[2], David Horne[3], SonBinh T. Nguyen[4], Adam Y. Lin[1], Steven T. Rosen[3], Leo I. Gordon[1], C Shad Thaxton[1]

[1]Northwestern Univ. Feinberg School of Medicine, Chicago, IL,[2]High Throughput Screening Core, City of Hope, Duarte, CA,[3]City of Hope, Duarte, CA,[4]Chemistry, Northwestern University, Evanston, IL

Introduction: PIK-75 is a potent multi-kinase inhibitor, targeting p110α, p38γ and DNA-PKs. We and others have demonstrated that PIK-75 is effective against a diverse range of malignancies, including prostate cancer, cutaneous T-cell lymphomas, acute myeloid leukemia (AML) and pancreatic cancer, among others. However, PIK-75 is hydrophobic and difficult to deliver to target tissues, which has limited clinical development. To address these limitations, we developed a bio-inspired delivery platform for PIK-75 that has some features of natural high-density lipoproteins (HDL). We employed an organic core (oc) template to synthesize HDL-like nanoparticles (ocHDL NP) with size, shape, surface composition and targeting functions similar to mature, spherical, naturally occurring HDLs. The ocHDL NPs target and bind to the high-affinity HDL receptor scavenger receptor class B type 1 (SR-B1). SR-B1 is often over-expressed in cancer, making it an ideal target for drug delivery. Therefore, we hypothesized that ocHDL NPs can deliver PIK-75 via SR-B1 to cancer cells to induce cell death in vitro and in vivo.

Methods: PIK-75 ocHDL NPs were synthesized by combining an oc with PIK-75, phospholipids and the HDL-defining apolipoprotein A-I. Following spontaneous self-assembly the PIK-75 ocHDL NP were purified by dialysis. PIK-75 ocHDL NPs were characterized for size, surface charge and drug loading. In vitro efficacy was assessed in prostate cancer cell lines (CWRR1, LnCaP), AML cell lines (HEL, KASUMI-1, BDCM, KG1a), and the NCI-60 panel + 6 pancreatic cancer cell lines. An SR-B1 blocking antibody and small molecule inhibitor of SR-B1 were used to demonstrate SR-B1-mediated delivery of PIK-75 by ocHDL NPs. In vivo efficacy was assessed in a prostate cancer tumor xenograft model.

Results: PIK-75 was successfully incorporated into the ocHDL NP platform. Data demonstrate PIK-75 ocHDL NP had 20 ± 1 PIK-75/ NP. PIK-75 ocHDL NPs specifically target SR-B1 and deliver PIK-75 to inhibit its kinase targets, as measured by flow cytometry and western blotting. PIK-75 ocHDL NPs potently induced cell death in all cell lines tested, with average IC50s for prostate cancer (1.58 nM), AML (2.50 nM), pancreatic cancer (10.56 nM) and NCI60 panel (3.09 nM) in the low nanomolar range. PIK-75 ocHDL NPs significantly reduced tumor growth in a murine model of castrate resistant prostate cancer and there were no obvious untoward side effects.

Conclusions: ocHDL NPs can be successfully loaded with hydrophobic drug cargo, such as PIK-75, to enable SR-B1 targeting and delivery of PIK-75 to cancer cells. PIK-75 ocHDL NPs potently induced cell death across a wide array of malignancies in vitro, and in an in vivo prostate cancer xenograft model. These results provide proof-of-principle that ocHDL NPs can be successfully leveraged to deliver hydrophobic small molecule drugs in a receptor targeted manner.

#4468 Modified 5-FU immunoliposome targeting EGFR-expressing pancreatic tumors: *In vitro* and *in vivo* evaluation.

Esther Frimpong[1], Raviteja Bulusu[1], Joy Okoro[1], Xue Zhu[1], Renee Reams[1], Bo Han[2], Saunjoo Yoon[3], Edward Agyare[1]

[1]Florida A&M University College of Pharmacy & Pharmaceutical Sciences, Tallahassee, FL,[2]Department of Surgery, Keck School of Medicine, University of Southern California, Los Angeles, CA,[3]Department of Biobehavioral Nursing Science, College of Nursing, University of Florida, Gainesville, FL

Purpose: Epidermal growth factor receptor (EGFR) is vital in tumor growth, invasion, and metastasis. Many pancreatic tumors express EGFR, making it important in targeted therapy. XYZ-I-73, a novel 5-Fluorouracil (5-FU) analog formed from conjugating lauroyl chloride to 5-fluorocytosine from our previous study, has shown cytotoxicity against pancreatic cancer (PCa) cells. The study aimed to develop an immunoliposome by conjugating pegylated XYZ-I-73 liposomal nanoparticles to EGFR monoclonal antibody and determining in vitro and in vivo anticancer effects in PCa.

Methods: EGFR expression in PCa cells was determined via Western blot analysis. XYZ-I-73 liposomes were prepared from dipalmitoyl phosphatidylcholine (DPPC),1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-Polyethylene glycol (DSPE-PEG 2000), and Labrasol as surfactant using a thin-film hydration method. We conjugated EGFR monoclonal antibody to XYZ-I-73 PEGylated liposomes (XYZ-I-73LnP) to produce an antibody-conjugated PEGylated liposome (Ab-XYZ-I-73LnP). Immunoliposomes conjugation was confirmed and characterized for size, entrapment, and stability. The in vitro cytotoxicity and apoptotic effects were determined in MIA PaCa-2 cells and PANC-1 cells. Pharmacokinetics and biodistribution studies were conducted on KPC mice.

Results: Characterization of XYZ-I-73LnP showed a mean particle size of 105±2.1nm, PDI (0.24), zeta potential of -34.3±0.9mV, and entrapment efficiency (EE) of 84.3±3.8%. Ab-XYZ-I-73LnP mean particle size was 143nm±2.3, PDI (0.37), and zeta potential -46.2±1.3mV. The coupling efficiency and conjugation of the antibody to the liposomes were confirmed by Bicinchoninic Acid Assay (BCA) and Fourier Transform Infra-Red (FTIR). The in vitro cytotoxicity of Ab-XYZ-I-73LnP treated MIA PaCa-2 cells (IC_{50} (2D) = 2.5±0.9μM and IC_{50} (3D) = 8.1±1.1μM) was significantly higher than 5-FU-treated cells (IC_{50} (2D) = 7.4±1.3μM; IC_{50} (3D) = 26.7±1.1 μM). Ab-XYZ-I-73LnP inhibition in PANC-1 culture was significantly higher (IC_{50} (2D) = 2.9±1.1 μM; IC_{50} (3D) = 11.6±1.9 μM) than 5-FU-treated culture (IC_{50} (2D) = 26.7±1.1 μM; IC_{50} (3D) = 37.1±0.9 μM). Ab-XYZ-I-73LnP showed a remarkable apoptotic effect as concentration increased (12.5, 25, and 50μM) in MIA PaCa-2 and PANC-1 3D spheroids compared to 5-FU. MIA PaCa-2-treated Ab-XYZ-I-73LnP showed a significantly reduced expression of EGFR than 5FU. The pharmacokinetic profile of Ab-XYZ-I-73LnP showed a longer half-life than 5-FU ($t_{1/2}$ = 1.62±0.03h vs 0.49±0.01h, $p < 0.001$). The area under the curve (AUC) of Ab-XYZ-I-73LnP was 2.5-fold higher than 5-FU. Ab-XYZ-I-73LnP demonstrated a significantly higher tumor accumulation than 5-FU.

Conclusion: The study demonstrated that modified 5-FU immunoliposome nanoparticles may enhance the therapeutic activity of 5-FU in treating PCa.

#4469 Multimodal liposomal organic nanoparticle for immunomodulation and photodynamic combinatorial therapy for triple negative breast cancer.

Sayantan Sinha[1], Dhruv Bhatnagar[2], Anne Frei[3], Heather Himburg[3], Amit Joshi[2]

[1]Biomedical Engineering, Medical College of Wisconsin, Wauwatosa, Milwaukee, WI,[2]Biomedical Engineering, Medical College of Wisconsin, Milwaukee, WI,[3]Radiation Oncology, Medical College of Wisconsin, Milwaukee, WI

Background: IL-12 is a potent cytokine with promising pre-clinical efficacy in solid tumors, including triple-negative breast cancer (TNBC), by enhancing anti-tumor immunity through Treg reprogramming and boosting $CD8^+$ T cell function. However, its clinical use is limited by systemic toxicity and challenges in targeted tumor delivery. Combining IL-12 with photodynamic therapy (PDT) represents a potential solution to these issues, optimizing therapeutic outcomes.

Concept & Novelty: We developed a cationic tetra-lipid organic nanoparticle to co-encapsulate IL-12 and HPPH (a photosensitizer; λ_{max} = 658 nm). Efficient IL-12 encapsulation has been challenging due to steric hindrance and hydrophilic nature. To overcome this, we employed a bipolar solvent system ($CHCl_3$:CH_3OH, 2:1), achieving 98% IL-12 loading. The self-assembly of IL-12 in methanol enables its complexation with chloroform, forming hydrogen-bonding through synergistic solvation that enhances encapsulation efficiency.

Methodology: (a) Liposomal nanoparticle (LNP) Synthesis: The cationic core lumen for IL-12 encapsulation in LNP was achieved by dissolving DPPC, cholesterol, and DOTAP in a 13:5:1 molar ratio in $CHCl_3$. IL-12 (2 mg/mL in CH_3OH) was added and stirred for 1 hour. Subsequently, DSPE-PEG-2000-NH_2 conjugated with HPPH and DiR in $CHCl_3$ added dropwise and stirred under inert conditions. Solvent removed to form lipid cake (15 mg/L total lipid concentration), and hydrated with PBS. LNP achieved with sonication and multiple freeze/thaw cycles. Purified with centrifugation-dialysis. (b) In-Vivo: 4T1 murine model of breast cancer was developed with 20 Balb/c mice. Double-blind segregation in 5 groups (1 test + 4 control) was done. The test group received intravenous (i.v.) injections of IL-12 and HPPH-loaded LNP (IL-12@LNP-DiR@HPPH) and treated with laser. The control groups received either/or free IL-12/IL-12@LNP-DiR/LNP/No treatment. The biodistribution of LNP was monitored through time-dependent IVIS imaging.

Results & Discussion: We synthesized 92 nm IL-12@LNP-DiR@HPPH nanoparticles with surface charge of 0-0.4 mV, indicating stability for biological use. After intravenous injection, maximum DiR fluorescence was observed at tumor site at 6 hours. At this time point, photodynamic therapy with IL-12@LNP-DiR@HPPH resulted in 80-94% tumor volume regression and increased cytolytic T cell presence in the tumor, compared to controls. No toxicity, ulceration, or metastasis was observed in the treatment group during the therapeutic window.

Conclusion: We present a novel platform that addresses the challenge of multiple drug loading including efficient loading of IL-12 and successful delivery to the tumor site. A crosstalk between IL-12-mediated immune response and photo-triggered ROS generation promises a potential therapeutic strategy for TNBC with interventions.

#4470 Development of a nanoparticle formulation of an MMP-activated, tumor-specific prodrug with an enhanced therapeutic index for the treatment of breast cancer.

Zubeda Khatoon, Hannah L. M. Spencer, Huw S. Jones, Goreti R. Morais, Amalia R. Estrada, Robert A. Falconer

University of Bradford, Institute of Cancer Therapeutics, Bradford, United Kingdom

Triple-negative breast cancer accounts for 20% of all breast cancer cases and has a poor prognosis due to the lack of targetable expression of specific hormone and growth factor receptors. Therefore, systemic chemotherapy is one of the main therapeutic options. However, a lack of tumor specificity results in systemic toxicity. A tumor-selective prodrug, such as ICT2588[1], could enhance the therapeutic index of the active drug. ICT2588 comprises a colchicine analogue as the active drug, which is conjugated to an MMP14-specific peptide sequence and an endcap to prevent non-specific degradation. MMP14 is widely expressed and functionally active in the tumor tissues and neo vasculature compared to healthy tissues where it is largely absent, thereby facilitating specific release of the active drug within the tumor microenvironment. Despite the therapeutic potential of ICT2588, a lack of aqueous solubility is a limitation. In this study we aim to optimize a nanoparticle formulation of ICT2588 using a lactic and glycolic acid (PLGA) based approach, to increase the aqueous solubility of ICT2588, while offering a controlled release system without additional toxicity.[2] ICT2588 was synthesized and purified by semi-preparative HPLC. Nanoparticles were synthesized via nanoprecipitation and the formulations were optimized in terms of hydrodynamic diameter, zeta potential, and encapsulation efficiency. The evaluation of the ICT2588-PLGA formulations in an MDA-MB-231 triple negative breast cancer model will be reported, with the MMP14 overexpression validated by SDS PAGE and western blotting. The improvements in aqueous solubility will increase the therapeutic potential of ICT2588 and facilitate its translation towards clinical evaluation. 1.Atkinson JM, Falconer RA, Edwards DR, et al. Development of a novel tumor-targeted vascular disrupting agent activated by membrane-type matrix metalloproteinases. Cancer Research 2010;70(17):6902-6912, doi:10.1158/0008-5472.CAN-10-1440 2.Alsaab HO, Alharbi FD, Alhibs AS, et al. PLGA-Based Nanomedicine: History of Advancement and Development in Clinical Applications of Multiple Diseases. Pharmaceutics 2022;14(12):2728, doi:10.3390/pharmaceutics14122728

#4472 Self-assembly of CD38-binding peptides into nanofibers for imaging multiple myeloma.

Jacqueline Godbe, Amit Kumar Sharma, Rui Tang, Han Jing, Alexander Zheleznyak, Monica Shokeen

Radiology, Washington University in Saint Louis, Saint Louis, MO

CD38 is overexpressed in multiple myeloma (MM) and the target of clinically approved therapeutic antibodies daratumumab and isatuximab. Our lab has previously developed a peptide-based radiotracer (LS1209) that targets CD38 for specific and sensitive imaging in MM. In this study, we demonstrate that the addition of lipid tails to LS1209 templates self-assembly of this peptide into multivalent nanofibers with morphology controlled by the length of the lipid tail. Morphology of the nanofibers was assessed via conventional and cryo-electron microscopy. Internal packing of the peptides was assessed via fluorescence. As the length of the lipid tail increases, the average spacing between residues increases. Additionally, there is a distinct morphologic change between cylindrical nanofibers and helical nanofibers as the lipid tail increases in length from a hexanoic acid tail to an octanoic acid tail. These nanofibers effectively multimerize LS1209 in order to take advantage of multivalent and cooperative binding to CD38, which was assessed via surface plasmon resonance (SPR) and cell surface binding. We demonstrate that binding activity is related to nanofiber morphology and the packing density/availability of peptides for binding interactions. Additionally, the nanofiber design co-localizes radiotracers to the site of interest without CD38 binding events, leading to additional increases in signal. The nanofibers demonstrate high efficiency radiolabeling with 64Cu and are stable at 24h in serum when assessed by radio thin layer chromatography. Preliminary animal experiments demonstrate no toxicity at expected imaging and high concentration (20x) doses.

#4473 Liposomal nanoparticle mediated gene modulation for the treatment of melanoma in murine models.

Poonam Yadav[1], Santosh K. Mandal[1], Bill Tang[2], Rahul A. Sheth[1]

[1]The University of Texas MD Anderson Cancer Center, Houston, TX,[2]Texas Tech University Health Sciences Center, El Paso, TX

Background: The Wnt/β-catenin pathway plays a significant role in melanoma progression, including the suppression of tumor-specific immunity. Inhibition of this pathway represents a promising therapeutic strategy. Our study investigates the potential of liposomal nanoparticles (LNPs) for delivering Wnt/β-catenin signaling pathway-inhibiting mIR-124 plasmids to melanoma cells.
Materials and Methods: Cationic liposomes were synthesized using the thin-film hydration method. Liposomes were mixed and incubated with a full-length cloned miR-124 plasmids vector (pDNA) at a 10:1 lipid-to-pDNA ratio to allow nanoparticle (NPs) formation referred to as lipoplex or (miR-124-NP). The size distribution and zeta potential of the LNPs were measured using dynamic light scattering (DLS). Nanoparticle morphology was assessed using transmission electron microscopy (TEM). B16-N2 melanoma cells were seeded and treated with miR-124-NP and blank liposomes (10 µg lipid). Cytotoxicity and transfection efficiency were evaluated at 24- and 48-hours post-treatment using immunofluorescence and flow cytometry. Female C57BL/6 mice, bearing B16-N2 tumors in both flank regions, were assigned to 1) Blank Liposomes B-NP, 2) miR-124-NP (n=12 tumors per group). On day 13 post-tumor inoculation, the mice received three intratumoral injections of miR-124-NP (40 µg DNA per injection) at 3-day intervals. Tumor volume was measured daily using caliper measurements. The delivery of nanoparticles to the tumors was monitored by an in vivo imaging system (IVIS), detecting GFP signals at multiple time points. At 24 hours post-injection, mice were euthanized, and tumors were harvested for further analysis via flow cytometry and RT-PCR to assess transfection efficiency.
Results: DLS and TEM analysis confirmed that the miR-124-NP exhibited proper size (180 nm), charge (16.66 mV), and structure. The miR-124-NP formulation (1:10 µg plasmid: lipid ratio) successfully transfected B16-N2 melanoma cells without causing toxicity. Immunofluorescence and flow cytometry data verified the transfection was successful, with 40% efficiency. In vivo imaging using IVIS demonstrated successful delivery of the plasmid to tumor cells. In vivo, miR-124-NP treatment significantly reduced tumor volumes compared to the blank liposome control, with the tumor growth curve showing a marked decreased total tumor burden to up to 50% through day 20 post-inoculation. RT-PCR analysis revealed that miR-124-NP treatment led to decreased expression of SLUG and SNAIL, along with a reduction in vimentin levels, indicating a modulation of epithelial-mesenchymal transition (EMT) processes.
Conclusion: LNPs showed high retention in B16-N2 cells and were non-toxic in vitro. In vivo miR-124-loaded LNPs effectively transfected tumor cells, modulating beta-catenin signaling and EMT gene expression, leading to reduction in tumor burden in C57BL/6 mice.

#4474 Turning up the heat: nanoimmunotherapy transforming outcomes in breast cancer treatment.

Ana I. Matos[1], Barbara Carreira[1], Liane I. F. Moura[1], Rita C. Acurcio[1], Ronit Satchi-Fainaro[2], **Helena F. Florindo**[1]

[1]Universidade de Lisboa, Lisboa, Portugal,[2]Tel Aviv University, Tel Aviv, Israel

Immune checkpoint blockade reaches remarkable clinical responses. However, even in the most favorable cases, half of these patients do not benefit from these therapies in the long term. This work hypothesized that the activation of host immunity by co-delivering peptide antigens, adjuvants, and regulators of the transforming growth factor (TGF)-β expression using a polyoxazoline (POx)-poly(lactic-co-glycolic) acid (PLGA) nanovaccine, while modulating the tumor-associated macrophages (TAM) function within the tumor microenvironment (TME) and blocking the anti-programmed cell death protein 1 (PD-1) can constitute an alternative approach for cancer immunotherapy. Nanoparticles (NP) prepared by the double emulsion solvent evaporation method were modified with mannose to target the mannose receptor (CD206) expressed at the dendritic cells (DC) surface, thus promoting receptor-ligand interaction and subsequently improving payload delivery. NP physicochemical properties were characterized, and the loading of antigens, adjuvants, immune modulators was quantified by fluorescence. The synergistic immunotherapeutic potential of our multifunctional nanovaccine, isolated and in combination with the TGF-β downregulation and Pexidartinib (colony-stimulating factor 1 receptor (CSF-1R) inhibitor that modulates TAM), was assessed in the immune-competent MC38 colorectal (CRC) mouse model. The added value of combining the immune checkpoint inhibitor anti-PD-1 monoclonal antibody to control the CRC (MC38 and CT26) and the highly aggressive melanoma (B16F10) growth and survival was also evaluated.NP presented a mean diameter close to 200 nm, low polydispersity index, neutral surface charge, homogenous spherical shape, and high loadings for antigens, immune adjuvants, and modulators. POx-Mannose (Man) nanovaccines generated stronger antigen-specific T-cell responses, which led to significant tumor growth inhibition when compared with poly(ethylene glycol) (PEG)-Man nanovaccines. This anti-tumor effect induced by the POx-Man nanovaccines is mediated by a $CD8^+$-T cell-dependent mechanism, in contrast to the PEG-Man nanovaccines. POx-Man nanovaccine combined with Pexidartinib restricted the MC38 tumor growth and synergized with PD-1 blockade, controlling MC38 and CT26 tumor growth and survival. This data was further validated in B16F10-bearing mice, a highly aggressive and poorly immunogenic melanoma mouse model for immunotherapy studies.This innovative approach discloses the synergy among the targeted cancer nanovaccine and immune modulatory and checkpoint therapies (αCSF-1R and αPD-1) within the immunosuppressive TME, which overall outcome may constitute a promising nanotechnology-enhanced immunotherapy for solid cancer patients.

#4475 Evaluation of targeted immunoliposomes encapsulating 4-N-hydroxylgemcitabine in EGFR-positive pancreatic cancer.

Joy Okoro[1], Raviteja Bulusu[1], Esther Frimpong[1], Saunjoo Yoon[2], Bo Han[3], Renee Reams[1], Xue Zhu[1], Edward Agyare[1]

[1]Florida A&M University, Tallahassee, FL,[2]University of Florida, , Gainesville, FL,[3]University of Southern California, Los Angeles, CA

Purpose: Pancreatic cancer (PCa) is a deadly malignancy that is challenging to treat. Gemcitabine (Gem) is a standard therapy in PCa treatment. Epidermal growth factor receptor (EGFR) is commonly overexpressed in PCa and contributes to tumor progression and metastasis. Gem is limited by its short half-life and metabolic instability. Our previous studies demonstrated that our modified Gem analog (GemAGY) had improved metabolic stability and anticancer activity compared to Gem. Targeted immunoliposomes have been developed to increase cellular uptake and accumulation in PCa. This study seeks to develop an EGFR-targeted immunoliposome with improved antitumor activity of Gem against PCa.
Methods: EGFR antibody (cetuximab) was conjugated to a liposome encapsulating 4-N-hydroxylGemcitabine hydrochloride (GemAGY) called GemAGY-LnP. Fourier-transform infrared spectroscopy (FTIR) was used to confirm the conjugation of the EGFR antibody to the liposome. GemAGY-LnP was characterized using the dynamic light scattering technique. MiaPaCa-2 spheroid cultures and patient derived xenograft (PDX) pancreatic tumor mouse models were treated with GemAGY-Lnp and Gemcitabine Hydrochloride (GemHCl). 3D cell viability assay was conducted using MiaPaCa-2 spheroids to evaluate the anticancer effects of GemAGY-LnP. Pharmacokinetics, biodistribution, and in vivo antitumor efficacy studies were conducted using PDX mouse models.
Results: FTIR confirmed the successful conjugation of the EGFR antibody to the liposome. The mean particle size and zeta potential of GemAGY-LnP were 141.6 ± 40nm (Poly Dispersity Index = 0.15± 0.01) and -25.2 ± 0.04mV, respectively. The entrapment efficiency of GemAGY-Lnp was 66%. The half-maximal inhibitory concentration (IC_{50}) value was significantly lower (6-fold lower) in GemAGY-LnP-treated MiaPaCa-2 spheroids (3.75±0.3μM) when compared to GemHCl-treated spheroids (24.12 ± 4.8μM) indicating that GemAGY-Lnp exhibited a higher cytotoxic effect when compared to GemHCl. The pharmacokinetics profile revealed that the area under the curve (AUC) of GemAGY-Lnp (52.70 ±0.05 μghr/mL) was over 15-fold higher than that of GemHCl (3.09±0.04) μghr/mL) while half-life of GemAGY-LnP (1.93 hr) was almost 4-fold higher than that of GemHCl (0.50 hr). In vivo studies showed that tumor growth inhibition in PDX pancreatic tumor mice treated with GemAGY-Lnp was significantly higher than in PDX mice treated with GemHCl (GemAGY-LnP vs GemHCl; $p < 0.0001$). The biodistribution studies revealed a significantly higher GemAGY-Lnp accumulation in tumor relative to GemHCl.
Conclusion: Our findings suggest that GemAGY-Lnp significantly increases the tumor accumulation of Gem, enhances the anticancer activity and improves Gem's pharmacokinetic profile compared to GemHCl. GemAGY-LnP may potentially inhibit tumor growth when treating EGFR-positive pancreatic cancer.

#4476 Curcumin gold nanoparticles significantly boost the effectiveness of docetaxel-induced apoptosis in breast cancer.

Santosh K. Singh, Rajesh Singh

Microbiology, Biochemistry & Immunology, Morehouse School of Medicine, Atlanta, GA

Breast cancer (BrCa) is the most diagnosed cancer in women; it develops locally and then metastasizes to other organs. Due to tumor heterogeneity, the prevalence of drug resistance, and disease recurrence, current therapy options are proving less effective for specific BrCa subtypes, such as triple-negative breast cancer (TNBC). Therefore, BrCa treatment has shifted from a one-size-fits-all strategy to a more focused approach. In this study, we demonstrated folate receptor-based targeted delivery of either curcumin (Cur), a natural compound isolated from *Curcuma longa*, or docetaxel (DTX), a chemotherapeutic drug, or a combination of the two, using gold nanoparticles (AuNPs) for successful BrCa therapy. We synthesized AuNPs coated with folic acid (FA)-conjugated with poly (ethylene glycol) copolymer, encapsulated with DTX and Cur, and then characterized these NPs for size, charge, shape, conjugation, and distribution using various techniques, including a Zetasizer, scanning electron microscope (SEM), transmission electron microscope (TEM), nuclear magnetic resonance (NMR), and nanoparticle tracking analysis (NTA). The results confirmed the successful conjugation of both drugs. To evaluate whether Cur enhanced the efficacy of DTX-AuNPs on BrCa cell lines (MCF-7 and MDA-MB-231), we treated the cells with Cur- or DTX-alone or in combination -AuNP followed by cell viability assessment using MTT assay and apoptosis assays. The live/dead cells immunofluorescence test confirms the effectiveness of combined drugs, showing an increase in dead nuclei in MCF-7 and MDAMB 231 cells treated with the combination (FA-Cur+ DTX-AuNP) for 48 hours. The combination was most effective in inducing apoptosis, inhibiting EMT (epithelial-mesenchymal transition), and BrCa cell proliferation. It showed the downregulation of the anti-apoptotic Bcl-xL and the upregulation of pro-apoptotic markers Bak and Bid. The EMT markers, E-cadherin, were upregulated, and the N-cadherin and Snail markers were downregulated in western blots and RT-PCR. Further, the combination of NPs increases the expression of $p27^{KIP1}$, inhibiting the CDK4, Cyclin D1 complex and the transition of cells into G_0/G_1 to the S phase in both cell lines. The NPs internalization, colony formation, and migration assays further confirm the synergistic effect of both NPs. These findings indicate that Cur may serve as a therapeutic contender for enhancing the effectiveness of DTX, representing a promising advancement in breast cancer treatment.

#4477 Biological evaluation of EGFR- conjugated liposomal drug delivery system for enhanced targeting of pancreatic cancer.

Raviteja Bulusu[1], Joy Okoro[1], Esther Frimpong[1], Saunjoo Yoon[2], Bo Han[3], Renee Reams[1], Zhu Xue[1], Edward Agyare[1]

[1]Florida A&M University, Tallahassee, FL,[2]University of Florida, Gainesville, FL,[3]University of Southern California, Los Angeles, CA

Purpose: The purpose of this study is to entrap and characterize a potential lead compound (heptyl (5-fluoro-2-oxo-1-(tetrahydrofuran-2-yl)-1,2-dihydropyrimidine-4-yl) carbamate (AGY_2) into antibody-conjugated liposomal drug delivery system (AGY_2-EGFR LDDS) to target pancreatic cancer.

Methods: The monoclonal antibody (Cetuximab) conjugated AGY_2 loaded liposome drug delivery system (AGY_2-LDDS) using DPPC, MPPC, and PEG_{-2000} lipids. The conjugation was confirmed by Fourier Transform Infrared Spectroscopy (FTIR) spectra. The particle size (PS), zeta potential (ZP), polydispersity index (PI), and entrapment efficiency (EE) of the formulation were determined. 3D cytotoxicity studies were performed on MiaPaCa-2 (MP-2) and PANC-1 (P-1) cells. Stability study was performed at different temperatures (4°C, 25°C, and 40°C). Pharmacokinetic (PK) and biodistribution studies were conducted. A tumor efficacy study was conducted by inoculating the KPCY PDX mice.

Results: The variations in the peaks for AGY_2, AGY_2 LDDS, and AGY_2-EGFR LDDS in the FTIR spectra confirmed the successful conjugation of EGFR to the lipids. The PS of the formulation was 115±7 nm with a ZP of -35 ±8 mV, and the EE was 80%, as analyzed by Stat-Ease360. In MP-2 spheroids, the IC_{50} values were as follows: AGY_2-EGFR LDDS at 7.7 ± 2.3 μM ($p < 0.0001$), AGY_2 LDDS at 16.5 ± 3.7 μM ($p < 0.001$), and AGY_2 at 27.5 ± 4.2 μM ($p < 0.0328$), compared to 5-Fluorouracil (5-FU) at 36.6 ± 4.8 μM. Similarly, in P-1 cells, the IC_{50} values were: AGY_2-EGFR LDDS at 9.6 ± 0.9 μM ($p < 0.0001$), AGY_2 LDDS at 18.5 ± 5.7 μM ($p < 0.0003$), and AGY_2 at 35.4 ± 5.2 μM, compared to 5-FU at 40.8 ± 5.9 μM. In MP-2 organoids, the IC_{50} values were: AGY_2-EGFR LDDS at 10.8 ± 3.3 μM ($p < 0.0001$), AGY_2 LDDS at 25.1 ± 3.9 μM ($p < 0.0004$), and AGY_2 at 35.2 ± 3.7 μM ($p < 0.0154$), compared to 5-FU at 47.9 ± 4.4 μM. Similarly, in P-1 organoids, the IC_{50} values were: AGY_2-EGFR LDDS at 12.2 ± 3.9 μM ($p < 0.0001$), AGY_2 LDDS at 29.09 ± 4.9 μM ($p < 0.001$), and AGY_2 at 41.56 ± 5.1 μM, compared to 5-FU at 45.96 ± 4.8 μM. Stability studies showed that at 4°C and 25°C, the formulations remained stable over 30, 60, and 90 days, with no significant changes in PS and EE. However, at 40°C, the formulations were stable for 30 and 60 days, but the 90-day samples showed reduced EE and increased PS. The PK study demonstrated that AGY_2-EGFR LDDS had a threefold increase in half-life (1.54±0.06 vs. $0.49±0.03_{5\text{-}FU}$ h), AUC (428±10 vs. $125±12_{5\text{-}FU}$ μg/L*h), and clearance (0.73 vs. $2.5_{5\text{-}FU}$ L/h). The biodistribution study showed a higher AGY_2-EGFR LDDS tumor accumulation compared to 5-FU. In the tumor efficacy study, the AGY2-EGFR LDDS treated group showed significant tumor growth inhibition compared to the 5-FU treated group.

Conclusion: This study shows that AGY_2-EGFR LDDS improves the anticancer activity 5-FU and may have the potential to significantly reduce tumor growth in EGFR-positive pancreatic cancer.

#4478 Persistent luminescent nanoparticles for autofluorescence-free tumor imaging: A novel approach to enhanced cancer diagnostics.

Bhupendra B. Srivastava[1], Swati Mohan[1], Murali M. Yallapu[2], Subhash C. Chauhan[2]

[1]School of Integrative Biological and Chemical Sciences, The University of Texas Rio Grande Valley, Edinburg, TX,[2]School of Medicine, The University of Texas Rio Grande Valley, Edinburg, TX

Background: Persistent luminescent nanoparticles (PLNs) represent a significant advancement in tumor imaging by providing autofluorescence-free imaging capabilities. Unlike conventional fluorescent probes, PLNs emit light for an extended period after excitation, enabling high-sensitivity imaging without interference from surrounding tissue autofluorescence. Despite their potential, commonly used PLNs suffer from low photoluminescence quantum yield (PLQY), limiting their brightness and imaging efficiency. This challenge necessitates the development of novel materials and synthesis strategies to enhance PLNs' optical properties and stability for bioimaging applications.
Method: PLNs were developed Mn^{2+}-doped Zn_2GeO_4 (ZGOM) nanorods through a modified hydrothermal synthesis method. The approach was designed to optimize crystal structure, doping efficiency, and luminescent properties. The nanorods were characterized using dynamic lightscattering (DLS), transmission electron microscopy (TEM), X-ray diffraction (XRD), andphotoluminescence spectroscopy to evaluate particle size, morphology, crystallinity, and opticalperformance. Their photoluminescence quantum yield (PLQY) was measured, and stability studies were conducted in biologically relevant media. Additionally, in vitro studies were performed to assess biocompatibility and imaging efficacy in cancer cell models.
Results: The synthesized ZGOM nanorods exhibited a highly uniform rod-like morphology with an average size below 100 nm. Under UV irradiation, the nanorods displayed intense green luminescence with a quantum yield exceeding 50%, demonstrating significant improvement over conventional PLNs. Stability studies confirmed excellent photostability and minimal degradation in aqueous and biological media. In vitro experiments showed efficient cellular uptake and bright, sustained luminescence, enabling high-resolution imaging of cancer cells. Furthermore, the absence of autofluorescence interference in surrounding tissues highlighted the potential of these nanorods as precise imaging agents for tumor targeting and cell tracking.
Conclusion: The Mn^{2+}-doped Zn_2GeO_4 nanorods synthesized in this study exhibit enhanced optical properties, including high PLQY and photostability, making them a promising candidate for autofluorescence-free tumor imaging. The combination of improved synthesis methods and superior material properties addresses the primary limitations of conventional PLNs, paving the way for their application in advanced bioimaging. These findings establish ZGOM nanorods as aviable platform for cancer diagnosis, with potential for further optimization and translation intoclinical imaging applications.

#4479 Optimization of ultrasound-responsive nanoparticles encapsulating cancer cells for enhanced immunotherapy.

Omer Aydin[1], Gizem Kursunluoglu[2], Munevver Akdeniz[1]

[1]Biomedical Engineering, Erciyes University, Kayseri, Turkey,[2]Drug Development and Research Center, Erciyes University, Kayseri, Turkey

Due to the limited effectiveness of current cancer treatment methods, numerous innovative therapeutic strategies are being developed. Among these, immunotherapy, which leverages the patient's immune system, has emerged as a highly investigated and promising approach for cancer therapy. Nanotechnology-based treatments enhance the efficacy of therapies, enabling innovative and advanced technological applications. Within these innovative systems, ultrasound therapy stands out as a technology particularly effective in eliciting and strengthening immune responses against cancer. Using ultrasound-active materials can enhance the mechanical ablation effect in ultrasound therapies, leading to more effective treatments. This study aims to develop biomimetic nanoparticles by synthesizing ultrasound-active gold nanocones (AuNCs), which facilitate tumor size reduction through mechanical ablation under therapeutic ultrasound. This process also releases tumor-specific molecules into the cancer microenvironment, promoting an increase in immune cells. The AuNCs were subsequently coated with B16F10 melanoma cancer cell membranes to create biomimetic nanoparticles (CCM@AuNCs). The membrane-coated nanoparticles were optimized using three distinct techniques: extruder, sonication, and microfluidizer methods. The CCM@AuNCs prepared by these methods were characterized using a Zetasizer (Malvern, Nano ZS, UK) to measure zeta potential, nanoparticle tracking analysis (NTA, Malvern, Nanosight NS300, UK) to determine size distribution, and scanning transmission electron microscopy (STEM) to examine their morphology. All methods produced nanoparticles approximately 200 nm in size with a zeta potential of -40 mV. STEM images clearly showed membrane coating around the particles. The efficiency of membrane coating on CCM@AuNCs prepared via the three methods was evaluated using the BCA Assay Kit (Thermo Scientific-23227). The extruder method demonstrated the highest coating efficiency (43.49%) and protein concentration in 100 µL (120 µg). Based on coating efficiency and characterization results, CCM@AuNCs prepared via the extruder method were selected for further studies. Anti-gp100 (Abcam, ab137078) antibodies were used to confirm membrane coating, and the fluorescence images of CCM@AuNCs were obtained. Cytotoxicity studies performed using the Resazurin assay demonstrated no cytotoxicity for CCM@AuNCs. The biomimetic nanoparticles exhibited homologous targeting, showing increased accumulation in melanoma cancer cells. Cellular uptake studies using fluorescence microscopy and flow cytometry revealed that CCM@AuNCs were internalized more efficiently by B16F10 melanoma cells than by 4T1 breast cancer cells. The study will be further extended to in vivo B16 murine models. This work is supported by the TÜBİTAK 2247-D program (#121C221).

#4480 Novel targeting API@API nanomedicines for synergistic molecular signaling modulation in head and neck cancer and the microenvironment.

Li-Xing Yang, Ru-Fen Chen, Yan-Wei Wu, Pei-Wen Wang, Chih-Chia Huang, Dar-Bin Shieh

National Cheng Kung University, Tainan City, Taiwan

Head and neck cancer (HNC) has become a prominent health concern in Taiwan, particularly among young males, with rising socioeconomic implications due to traditional treatments' limited success in preventing recurrence and metastasis. Our study introduces a novel API@API nanoformulation strategy that combines multiple active pharmaceutical ingredients (APIs) to synergistically target signaling pathways, aiming to overcome limitations of current HNC therapies and reduce toxicity. The API@API nanoformulation leverages natural nanoparticles derived from *Cordyceps sinensis*, incorporating key drugs such as FTY720 and Perifosine. The formulation optimizes amphipathic drug properties, allowing for higher bioavailability and targeted therapeutic action. By evaluating API@API nano cocktails in preclinical HNC models, we observed increased efficacy, with enhanced cytotoxicity and downregulation of critical pathways in HNC cells. The formulation demonstrated selective cytotoxicity across cancer stages, effectively targeting the tumor microenvironment (TME) through dual-drug-loaded nanoparticles. We also accessed the *in vitro* and *in vivo* efficacy of API@API nano cocktails in suppressing tumor growth, modulating immune responses, and reducing cancer cell migration. The platform demonstrated improved pharmacokinetics, enhanced tumor accumulation, and prolonged retention at tumor sites. Further immunological analyses indicated an immunosuppressive-to-immunomodulatory shift within the TME, with increased dendritic and M1 macrophage activity and reduced immunosuppressive cell populations. Spatial proteomic analyses confirmed enhanced immune cell distribution with reducing tumor associate neutrophiles, indicating that API@API formulations may improve HNC sensitivity to immunotherapy. In conclusion, the API@API nanoformulation platform has shown potential as an effective therapeutic strategy for HNC, offering significant advantages in efficacy, reduced side effects, and TME modulation. This platform supports broader biopharmaceutical applications, aligning with the global need for advanced cancer treatments.

#4481 Targeting Xkr8 via nanoparticles-mediated codelivery of siRNA and chemotherapy drugs for cancer immunochemotherapy.
Yuang Chen

Massachusetts Institute of Technology (MIT), Cambridge, MA

Activation of scramblases is one of the mechanisms that regulate the exposure of phosphatidylserine to the cell surface, a process that plays an important role in tumor immunosuppression. Here we show that chemotherapeutic agents induce overexpression of Xkr8, a scramblase activated during apoptosis, at the transcriptional level in cancer cells, both *in vitro* and *in vivo*. Given this finding, we developed a nanocarrier for co-delivery of Xkr8 siRNA and the FuOXP pro-drug to tumors. Intravenous injection of our nanocarrier led to significant inhibition of tumor growth in colon and pancreatic cancer models along with increased antitumor immune response. Targeting Xkr8 in combination with chemotherapy may represent a novel strategy for the treatment of various types of cancers.

#4482 Development of a stable and efficient therapeutic mRNA cancer vaccine utilizing RNA-plex technology.

Yongbin Liu, Dongfang Yu, Lingyi Huang, Junhua Mai

Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX

Background: Therapeutic mRNA vaccine is a promising approach for cancer immunotherapy. However, one of its major challenges is the inherent instability of mRNAs, as it is highly sensitive to ambient environments. Consequently, current mRNA vaccines require production, shipping, and storage at ultra-low temperatures, limiting their global application and increasing costs. Thus, enhancing mRNA stability is essential to develop stable mRNA-based therapeutic vaccines.
Methods: In this study, we developed a novel mRNA stabilization technology termed "RNA-plex", which prevents mRNA from hydrolytic degradation using polymeric macromolecules known as “carrier-base”, composed of a poly-glutamic/aspartic acid backbone conjugated with nucleobases.
Results: We demonstrated that unprotected (naked) mRNA was completely degraded after incubation at 95°C in only 5 minutes, while mRNA complexed with carrier-base remained intact, showing resistance to hydrolytic degradation at high temperatures. Additionally, RNA-plex stabilized mRNA in water at room temperature (RT) for over two weeks and at 4°C for more than two months, whereas naked mRNA degraded within 3 days at RT and within one month at 4°C. Moreover, the protective effect of carrier-base is independent of RNA sequence and structure, effectively safeguarding various linear mRNAs, circular RNAs, and single guide RNAs, and being compatible to commonly used RNA delivery systems. In addition to mRNA protection, RNA-plex effectively boosts mRNA translation in 293T cells and antigen presentation in dendritic cells, making it a potent tool for enhancing cellular responses. We prepared mRNA vaccines by encapsulating either naked OVA mRNA or OVA RNA-plex within *in vivo*-jetRNA (jetRNA), a liposomal *in vivo* mRNA transfection reagent, and tested its effectiveness in cancer immunotherapy in an ovalbumin (OVA) engineered B16 melanoma model (B16-OVA). The jetRNA-RNA-plex formulation demonstrated robust efficacy in inhibiting melanoma growth compared to the formulation containing naked mRNA only ($p<0.01$).
Conclusion: Our findings demonstrate that the RNA-plex technology effectively stabilizes mRNA, offering impressive potential for long-term storage in ambient environment. Moreover, RNA-plex enhances mRNA translation and significantly improves anti-cancer efficacy in cancer vaccines by eliciting a robust anti-tumor immune response. These promising results suggest that RNA-plex holds substantial potential for advancing mRNA-based therapeutics for cancers.

#4483 Photodynamic therapy in breast cancer by photosensitizer-chitosan particles inducing ferroptosis.

Marek Feith[1], Abhilash D. Pandya[1], Astrid Hyldbakk[2], Anders Hogset[3], Kirsten Sandvig[4], Tore Skotland[4], Tore-Geir Iversen[4], Gunhild Mari Malandsmo[1]

[1]Department of Tumor Biology, Oslo University Hospital, Institute for Cancer Research, Oslo, Norway,[2]Department of Biotechnology and Nanomedicine, SINTEF AS, Trondheim, Norway,[3]PCI Biotech AS, Oslo, Norway,[4]Department of Molecular Cell Biology, Oslo University Hospital, Institute for Cancer Research, Oslo, Norway

Triple-negative breast cancer (TNBC) is a subtype of breast cancer associated with poor prognosis and limited treatment options. While chemotherapy has traditionally been the main treatment, resistance frequently emerges, reducing its effectiveness. Recently, a range of immunotherapy strategies, including combinations of chemotherapy and immunotherapy, have expanded treatment possibilities. To further enhance the therapeutic efficacy, there is a desire to combine chemotherapy with other currently used modalities such as photodynamic therapy (PDT) or to encapsulate drugs into nanoparticles (NPs).

Ferroptosis has been described as a highly immunogenic type of cell death, characterized by increased ROS and lipid peroxidation. Mesenchymal cancer cells have been reported to be more sensitive to ferroptosis than epithelial cells. We have previously shown that tetraphenylchlorin-conjugated chitosan nanoparticles (TPC-CS NPs) loaded with mertansine and cabazitaxel induce ferroptosis in TNBC.[1] In this project, we investigated the effect of TPC-CS NPs together with free RSL3, a ferroptosis inducer. We show that such treatment induces cell death in a mesenchymal breast cancer cell line but not in an epithelial one. The combined treatment also significantly affects lipid peroxidation and mitochondrial function. Based on these results, we encapsulated RSL3 into TPC-CS NPs and used them in two different breast cancer cell lines. We explored the photodynamic effect of empty and RSL3 loaded TPC-CS NPs upon illumination on cell viability by using an MTS assay. Since we have not observed any beneficial effect of TPC-CS NPs-RSL3, we explored the uptake of only empty TPC-CS NPs by using fluorescence microscopy and flow cytometry in these two cell lines. Currently, a biodistribution study in immunocompetent animals bearing 4T1 tumors is being performed. We will perform the maximum tolerable dose experiments for illumination to determine the light dose for further experiments. Moreover, we will perform efficacy studies of TPC-CS NPs and investigate the immune cell composition of tumors and tumor microenvironment using a flow cytometry panel of 25 markers.

1.Pandya AD, Øverbye A, Sahariah P, et al. Drug-Loaded Photosensitizer-Chitosan Nanoparticles for Combinatorial Chemo- and Photodynamic-Therapy of Cancer. Biomacromolecules. 2020/04/13 2020;21(4):1489-1498.

This project has received funding from the Europe Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement 956544.

#4484 Targeting DNA repair mechanism that leverages current standard of care interventions with a novel agent in glioma.

Meser M. Ali[1], Hamid Suhail[1], Mahesh Yadab[1], Ataur Rahman[1], James Snyder[2]

[1]Wayne State University School of Medicine, Detroit, MI,[2]Henry Ford Hospital, Detroit, MI

Glioblastoma (GBM) is a malignant glioma characterized by molecular heterogeneity and poor prognosis. GBM remains a uniformly lethal malignancy, with patient survival rarely exceeding two years despite the best available treatments. In clinical practice, about 60% of GBM patients have shown overexpression of O^6-methylguanine-methyl-transferase (MGMT), an enzyme responsible for DNA repair, were found to receive little benefit from TMZ and represent a group of great unmet need with no chemotherapeutic agents that significantly improve survival. A key factor contributing to treatment resistance and disease relapse is the overexpression of MGMT. MGMT overexpression reduces the efficacy of standard therapies like temozolomide (TMZ) and radiotherapy (RT), leaving patients with active MGMT expressions in urgent need of new treatment strategies. The depletion of MGMT in tumors has been investigated as a strategy to increase TMZ and RT efficacies in resistant, MGMT-positive GBMs. Chemical depletion using O^6-benzylguanine derivatives as MGMT inhibitors have been actively tested, but these compounds are associated with high hematologic toxicity. Recent studies have shown that disulfiram (DSF), an alcohol aversion drug, combined with copper (Cu), can inhibit MGMT and improve the response to TMZ in GBM. However, phase II clinical trials using oral Cu/DSF demonstrated no survival benefit, primarily due to the instability of DSF *in vivo*. To address this limitation, we developed a novel delivery system using 2-hydroxypropyl beta cyclodextrin (HPßCD) encapsulating the Cu complex of DSF's active metabolite, diethyldithiocarbamic acid (DDC). This HPßCD-Cu$(DDC)_2$formulation stabilizes the complex, preventing degradation and reducing non-specific interactions in the bloodstream. *In vitro* studies revealed that HPßCD-Cu$(DDC)_2$ inhibited MGMT through the ubiquitin-proteasome pathway. Inhibition of MGMT activity in cell cultures vastly increased the alkylation-induced DNA double-strand breaks (gama-H2AX), that are hallmark of TMZ effectiveness as supported by cytotoxicity and the increased levels of apoptotic markers, like JNK-P and cleavage of PARP-1. Systemic delivery of HPßCD-Cu$(DDC)_2$ in MGMT overexpressed patient derived orthotropic xenograft resulted in depletion of MGMT with tumor size regression. Tumor burden has been evaluated within patient derived orthotropic rat models that represent high expression of MGMT followed by oral vs intravenous treatments.

#4485 Resistance to ferroptosis in high grade diffuse large B-cell lymphoma is mediated through the Nrf2 signaling pathway.

Meiying Yang[1], Jonathan S. Rink[1], Adam Y. Lin[1], Shuo Yang[1], Colby S. Thaxton[2], Leo I. Gordon[1]

[1]Medicine, Hematology/Oncology, Robert H. Lurie Comp. Cancer Ctr. of Northwestern Univ., Chicago, IL,[2]Urology, Robert H. Lurie Comp. Cancer Ctr. of Northwestern Univ., Simpson Querry Research Institute, Chicago, IL

Introduction: High-grade B-cell lymphomas (HGBCL) with *C-MYC* and *BCL-2* rearrangements are aggressive and resistant to standard treatment. Some B cell lymphomas, including HGBCLs and diffuse large B cell lymphomas (DLBCL), are susceptible to ferroptosis, a cell death pathway marked by toxic lipid peroxides. We have shown that some DLBCLs are sensitive to ferroptosis induced by gold high-density lipoprotein (HDL)-like nanoparticles (Au-HDL NPs), while others, including some HGBCLs, are more resistant. Differential gene expression (DEG) analysis of RNAseq data suggests that resistance to Au-HDL NP-induced ferroptosis correlates with increased expression of *Nrf2* associated genes. We hypothesized that *Nrf2* protects HGBCL cells against ferroptosis induced by Au-HDL NPs and other reactive oxygen species (ROS)-inducing chemotherapy agents and tested this by generating *Nrf2* knockout OCI-ly1 cells (*Nrf2*$^{-/-}$).
Methods: DLBCL (SUDHL-4) and HGBCL (RC, OCI-ly1, OCI-ly8, OCI-ly18) cell lines were studied. Au-HDL NPs were synthesized as previously described. RNA from RC and SUDHL4 cells treated with Au-HDL NPs or vehicle were subjected to RNA-seq. DEGs were validated by RT-qPCR and Western blot. *Nrf2* $^{-/-}$ OCI-ly1 cells were generated using CRISPR/Cas9. Viability was assessed by MTS and Annexin V-FITC/PI staining, while lipid peroxidation was quantified by C11 BODIPY.
Results: HGBCL cell lines (RC, OCI-ly1, OCI-ly8, OCI-ly18) were less sensitive to Au-HDL NP-induced ferroptosis (IC_{50} = 31.4, 27.2, 58.2, and 26.0 nM, respectively) compared with the DLBCL cell line SUDHL4 (8.1 nM). RNAseq, RT-qPCR and western blot revealed higher expression of *Nrf2* regulated genes, particularly *NQO1* in Au-HDL NP-treated RC (19.9-fold), OCI-ly1 (9.0-fold), and OCI-ly8 (36.5-fold) cells compared with SUDHL4 (4.2-fold). *Nrf2* $^{-/-}$ OCI-ly1 cells were less viable after treatment with Au-HDL NPs than SUDHL4 (30%, 14%, 6% vs. 104%, 75%, and 41% at doses of 10, 20, and 40 nM Au-HDL NPs, respectively). Annexin V^+/PI^+ was increased in *Nrf2* $^{-/-}$ OCI-ly1 cells (58%) compared to OCI-ly1^{wt} cells (32%), and lipid peroxidation levels were also higher in *Nrf2* $^{-/-}$ cells, suggesting that *Nrf2* mediates resistance to Au-HDL NPs by reducing oxidative burden. Compared to OCI-ly1^{wt} cells, *Nrf2* $^{-/-}$ cells increased sensitivity to other ferroptosis inducers, such as erastin (IC_{50} 1.1 vs 5.7 μM) and RSL3 (IC_{50} 26.5 vs 57.3 nM). *Nrf2* $^{-/-}$ cells exhibited increased sensitivity to ROS-related chemotherapy agents compared to OCI-ly1^{wt} cells, including doxorubicin (IC_{50} 1.2 vs 2.6 nM) and venetoclax (IC_{50} 21.5 vs 52.3 μM).
Conclusion: *Nrf2* signaling plays a crucial role in conferring resistance of aggressive lymphomas to ROS-dependent cell death mechanisms, including ferroptosis. Targeting *Nrf2* could be a promising strategy to overcome resistance to ferroptosis and enhance the efficacy of ROS-inducing chemotherapy agents.

CHEMISTRY: Proteomic Biomarkers and Therapeutics
Poster Session

#4491 HSP70 leverages STUB1 to modulate N-Myc protein turnover in lethal prostate cancer.
P. Xu, J. C. Yang, B. Chen, C. Nip, S. Ning, A. C. Gao, **C. Liu**;
UC Davis, Sacramento, CA

Background: Imbalances in protein homeostasis (proteostasis) are associated with a wide range of diseases, including neurodegeneration and tumorigenesis. Prostate cancer has proven to be a key model for understanding how proteostasis regulates tumor progression. Neuroendocrine prostate cancer (NEPC) is the most aggressive type of prostate cancer with no effective treatment. The amplification of N-Myc (encoded by *MYCN*) is a key modification of NEPC. However, it has proven challenging to identify therapeutic strategies that reduce N-Myc transcriptional activity or levels owing to the presence of intrinsically disordered functional domains and lack of enzymatically active sites. In this study, we sought to take a different approach and identify the molecular chaperones involved in N-Myc proteostasis.
Methods: N-Myc was knocked down by siRNA. Cell proliferation and N-Myc expression were assessed using cell viability assays and Western blotting, respectively. Rapid immunoprecipitation pull-down assays followed by label-free mass spectrometry were used to identify N-Myc-binding proteins. Co-immunoprecipitation (Co-IP) and Proximity Ligation Assay (PLA) were performed to detect the interaction between HSP70 and STUB1 with N-Myc. In vitro ubiquitination assays were conducted to detect the ubiquitination of N-Myc. Site-directed mutagenesis (SDM) was used to construct N-Myc, STUB1, and ubiquitination deletion or point mutation plasmids. Cycloheximide (CHX) chase assays were employed to examine the half-life of N-Myc.
Results: Knockdown of N-Myc by siRNA significantly inhibited the growth of H660, UCDCaP-CR, and CWR22Rv1 cells. A total of 779 proteins were identified as N-Myc-binding proteins. Among them, several HSP70 family proteins, such as HSPA1B (HSP70), HSPA6, and HSPA8 (HSC70), were the most enriched. The HSP70/STUB1 complex interacted with N-Myc and regulated N-Myc protein turnover. This interaction appeared to occur at a degron ‘SELILKR’ in N-Myc. The K416 and K419 of N-Myc seem to be sites for ubiquitination by STUB1, where it added K11-linked polyubiquitin chains for further proteasome degradation.
Conclusions: The HSP70 interacts with N-Myc at a conserved binding motif and regulates N-Myc protein turnover through STUB1.
Source of Funding: This work was supported in part by grants from National Institutes of Health R37CA249108 (C.L.), R01CA251253 (C.L.), R21CA277171 (C.L.), Department of Defense HT9425-23-1-0144 (C.L.), HT9425-23-1-0325 (C.L.)

#4492 Covalent ligands that selectively disrupt CDK4-CDC37/HSP90 interactions in cancer cells.

Z. E. Potter[1], B. Melillo[1], K. E. DeMeester[2], B. F. Cravatt III[1];
[1]The Scripps Research Institute, La Jolla, CA, [2]Lafayette, Easton, PA

The Heat-Shock family of Proteins (HSPs)—which are conserved in prokaryotic and eukaryotic organisms—are molecular chaperones that regulate protein folding and stability, and in turn cellular homeostasis. In human cancers and hematological malignancies, HSPs are often upregulated. Additionally, many oncogenic proteins are HSP90 client proteins. Thus, decades of research have focused on inhibiting HSP90's function as a potential anti-cancer therapeutic strategy. However, the clinical application of HSP90 inhibitors has been hampered by dose-limiting toxicity due to the systemic disruption and subsequent dysregulation of HSP90 client proteins. Hence, the selective targeting of individual cancer-relevant client:chaperone interactions could represent a more tractable therapeutic strategy. Using activity-based protein profiling (ABPP), we have identified a stereoselective and site-specific covalent liganding event at an isotype-restricted cysteine on CDK4 located in proximity to the interface for binding the HSP90 co-chaperone CDC37. Alterations to the CDK4/CDK6/Rb signaling pathway are common in almost all tumors, and typically arise via the overexpression of CCND1, or loss of the CDK4/CDK6-specific inhibitor p16INK4a—either event increases CDK4/CDK6 activity and leads to uncontrolled cell proliferation. Importantly, CDK4 stability is highly dependent on interactions with CDC37 and HSP90. We show that the covalent ligands selectively disrupt the CDK4:CDC37:HSP90 chaperone complex while sparing other kinase:CDC37:HSP90 interactions; the selective disruption of this chaperone complex leads to the partial degradation of CDK4 in breast cancer cells. The ligand-induced disruption of CDK4-chaperone interactions and CDK4 stability are both stereoselective (not observed with an inactive enantiomeric compound) and site-specific (not observed with a C135S mutant CDK4 protein). Given CDK4's validated role in regulating the growth of hormone-sensitive cancer cells, compounds that can selectively disrupt chaperone regulation and stability of this kinase may have therapeutic potential as precision medicines.

#4493 Exploring APE1-G4 interactions and FACT complex inhibition to overcome therapy resistance in pediatric medulloblastoma.

Ritesh Dey[1], Yingling Chen[1], Sutapa Ray[2], Kishor Bhakat[1]

[1]Genetics, Cell Biology and Anatomy, University of Nebraska Medical Center, Omaha, NE,[2]Pediatrics Hematology/Oncology, University of Nebraska Medical Center, Omaha, NE

Medulloblastoma or MB is the most common malignant brain tumor in children. Tumor recurrence and therapy resistance remain critical obstacles for the treatment of MB. Although surgical resection followed by high doses of chemotherapies and ionizing radiation IR has considerably improved the survival rates. Resistance of residual tumor to IR and chemotherapies often leads to tumor recurrence. Despite advances in standard therapies, survival rates for relapsed MB patients have remained stagnant for over three decades, leaving clinicians and families with limited options. Previous reports have demonstrated that APE1, a key protein involved in repairing AP sites and single-strand breaks SSBs in DNA, is overexpressed in MB, and its activity is associated with tumor progression. Knockdown of APE1 enhances sensitivity of MB cells to radiation and chemotherapy, suggesting that APE1 mediates IR/chemo resistance. We have recently demonstrated that IR or cisplatin treatment induced APE1 interaction which Facilitates Chromatin transcription FACT complex, and treatment with CBL0137, a FACT targeted small molecule, improves the potency of cisplatin and radiation in vitro and in intracranial orthotopic MB xenograft model. Our current study discovered that recombinant APE1 binds to non-canonical four stranded DNA secondary structure called G quadruplexes or G4 DNA in the genome of MB cells and interacts with G4 in cells. Inhibition of APE1 binding to G4 by small molecules affects damage repair in cells. Treatment with these small molecules dose-dependently affects MB tumor cell growth in vitro. Further, we have delineated a mechanistic link of G4 with APE1 mediated drug resistance. Our project unraveled a novel mechanism by which APE1 and G4 promote therapy resistance and identify a promising strategy to improve the efficacy of IR and cisplatin and prevent tumor recurrence.

#4494 Structure, dynamics and therapeutic potential of fatty acid synthase.

Naimul Hasan[1], Alexander Keszei[2], Matthew Waas[2], Thomas Kislinger[2], Razq Hakem[2], Mohammad Mazhab-Jafari[2]

[1]Medical Biophsysics, Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada,[2]Medical Biophysics, Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada

De novo lipid synthesis is facilitated by the multifunctional enzyme fatty acid synthase (FASN), a hallmark of cancer associated with chemotherapeutic resistance and poor prognosis. Human FASN (hFASN) is a dynamic protein that undergoes significant conformational changes during catalysis, posing challenges for structural characterization. To date, no structural information is available for full-length hFASN, hindering our understanding of its catalytic mechanism and complicating rational drug design.Here, we report the structure of hFASN resolved using electron cryo-microscopy (cryoEM). The modifying region of hFASN was determined at near-atomic resolution using a recombinant expression system bound to the first-in-class inhibitor Denifanstat, enabling structure-based small molecule discovery. Additionally, we solved the full-length structure of endogenous hFASN from MCF7 cells, revealing an interaction with the small heat shock protein HSP27. In some 2D classes of hFASN from cryoEM data, we found partial unfolding of the modifying region, evidence of potential protein degradation. However, HSP27 knockdown in breast cancer cell lines did not alter overall hFASN levels, indicating that this interaction may be transient or involve additional partners.Mass spectrometry-based post-translational modification analysis further identified dihydroxylation of proline, arginine, and tyrosine residues in hFASN from MCF7 cells, likely reflecting oxidative stress. These modifications may play a role in protein stabilization or regulation under stress conditions, highlighting a potential mechanism for hFASN activity modulation. Together, these findings provide critical structural and regulatory insights, paving the way for targeted therapeutic development.

#4495 Inhibition of OTUD4/CD73 proteolytic axis by novel small molecule inhibitor ST80 reinvigorates tumor immune response.

Kevin Li, Yueming Zhu, Yong Wan

Emory University, Atlanta, GA

Despite the widespread utilization of immunotherapy, the poor clinical response by immune-suppressive tumors is an emerging challenge in the use of current immune checkpoint inhibitors. Recent research has revealed that CD73, or Ecto-5′-nucleotidase, is an enzyme overexpressed in many cancers, and works in concert with CD39 to produce immunosuppressive extracellular adenosine, subsequently inhibiting CD8+ T cell responses. Our multi-omics analysis identified CD73 and OTUD4, a deubiquitinase that stabilizes CD73 by counteracting its that counteracts its E3 ligase-mediated ubiquitination and degradation, as potential targets for enhancing anti-cancer immune responses.

Using druggability and pharmacophore simulations, we developed ST80, a novel small molecule inhibitor that disrupts the OTUD4-CD73 interaction by binding to the hydrophobic OTUD4 binding site on CD73. Within in vitro models of triple-negative breast cancer, EGFR-mutant lung cancer, ovarian cancer, and colorectal cancer, ST80 demonstrates significant potential in overcoming immune evasion by reducing adenosine production in the tumor microenvironment, subsequently reinvigorating the tumor immune responses by enhancing CD8+ T cell proliferation and IFN-γ production. By targeting the CD73/OTUD4 proteolytic axis, ST80 presents a promising strategy for treating cancers with immune-suppressive properties, potentially enhancing the efficacy of current immunotherapies.

#4496 Small molecule inhibition of previously “undrubbable” transcription factors with AI-guided functional proteomics.

Alexander J. Federation, Daniele Canzani, Andrea Gutierrez, Julia Robbins, Brian McEllin, Anastasiya Prymolenna, Lillian Tatka, Bodhi Hueffmeier, Yang Gao, Tonibelle Gatbonton-Schwager, Erin Broderick, Gaelle Mercenne, William Fondrie, Lindsay Pino

Talus Bioscience, Inc., Seattle, WA

Transcription factors (TFs) are central regulators of gene expression and key drivers of diseases such as cancer, autoimmune disorders, neurological disease, and metabolic conditions. Despite their critical role, TFs have long been considered “undruggable” due to their intrinsic disorder outside of native cellular environments, complex multiprotein assemblies, and the absence of traditional binding pockets suitable for small molecule targeting.

To address these challenges, Talus Bio has developed TF-Scan, a live-cell platform that provides a proteome-wide view of drug-induced changes in TF DNA-binding activity. This technology integrates high-resolution functional proteomics with advanced machine learning to measure TF:DNA engagement directly in unmodified human cells. Data from TF-Scan feeds into Strategian, a deep tensor factorization model trained on Talus Bio’s proprietary TF-Scan database, enabling AI-driven discovery of novel small molecule modulators.

Strategian evaluates a chemical universe of billions of drug-like molecules to predict their effects on TF activity, prioritizing candidates by potency and selectivity. These predictions are validated experimentally using TF-Scan, which further refines lead selection. This approach has enabled the identification of novel scaffolds for TFs including TBXT, ARv7, and STAT3, demonstrating the ability to generate drug-like compounds tailored to specific TFs.

By combining live-cell proteomics with AI-driven modeling, TF-Scan and Strategian create a powerful, scalable platform for targeting TFs and other challenging regulators within the regulome. This work establishes a new paradigm for drug discovery, transforming previously intractable targets into actionable opportunities to address critical unmet medical needs.

#4497 Discovery and optimization of a covalent AKR1C3 inhibitor for evaluation in hormone-dependent cancers.

Robert J. Grams[1], Robert J. Seal[2], James Veccia[2], **Ku-Lung Hsu**[1]

[1]The University of Texas At Austin, Austin, TX,[2]The University of Virginia, Charlottesville, VA

Aldo-keto reductase family 1 member C3 (AKR1C3) is a member of the AKR superfamily of enzymes that metabolizes androgen, estrogen, and prostaglandin substrates to produce potent drivers of proliferation in hormone-dependent cancers. Interest in developing selective inhibitors have produced tool compounds for inactivation or degradation of AKR1C3 with varying degrees of selectivity among the 15 known AKR proteins. Selectivity of AKR1C3 inhibitors across the AKR family is critical since a clinical candidate failed due to hepatotoxicity from off-target inhibition of AKR1D1. Here, we report development of a sulfonyl-triazole (SuTEx) covalent AKR1C3 inhibitor (RJG-2051) that site selectively engages a non-catalytic tyrosine residue on AKR1C3 in cells. Importantly, RJG-2051 exhibited negligible cross-reactivity with detectable AKRs or other proteins across thousands of tyrosine and lysine sites quantified by chemical proteomics. The disclosure of a covalent and proteome-wide selective inhibitor for potent AKR1C3 inactivation in cells will expedite cell biological studies for testing the therapeutic potential of this metabolic target.

#4498 Drug screens identify new therapeutic targets that synergize with EZH1/2 inhibition in adult T-cell leukemia/lymphoma.

Noorhan Ghanem, Carman K.M. Ip, Kit Tong, Mehran Bakhtiari, Michael Y. He, Aaron Schimmer, Robert Kridel

University of Toronto, Toronto, ON, Canada

Adult T-cell leukemia/lymphoma (ATLL), a rare form of non-Hodgkin lymphoma, affects approximately 2-5% of individuals infected by the retrovirus Human T-cell Lymphotropic Virus- 1 (HTLV-1). The disease has a dismal prognosis and resistance to chemotherapy is commonly seen in most patients. A significant characteristic of ATLL pathogenesis involves the disruption of normal epigenetic regulation, which results in transcriptional repression of tumour suppressors, mediated by the polycomb repressor complex 2 protein. Within this protein complex, the histone methyltransferases EZH1 and EZH2 play vital roles in catalyzing the excessive trimethylation of histone 3 lysine 27 (H3K27me3). In light of these mechanisms, a phase II clinical trial evaluated the efficacy of Valemetostat (VAL), a dual EZH1/2 inhibitor, in ATLL patients. Half of the patients responded to VAL; however, the median progression-free survival was only 7 months.To address these challenges and unbiasedly identify agents that could synergize with VAL, we conducted extensive high-throughput drug screens across four ATLL cell lines (ATL1K, ATL43T-, ATL55, and ED41214+). We initially screened 3,113 FDA-approved drugs to assess their pharmacological relevance in the presence of VAL. Additionally, given the important role of epigenetic deregulation in the pathogenesis of ATLL, we also tested 68 epigenetic chemical probes (ECPs) from the Structural Genomics Consortium with and without VAL. From these screens, we narrowed down the top 98 candidates from the FDA-approved drugs, based on the average cell viability minus two standard deviations, and the top five synergistically effective ECPs based on Bliss syngery score calculated by Synergy Finder across all four cell lines. We next screened these hits from FDA-approved drugs and the synergistically effective ECPs along with the corresponding negative non-functional probes across a wider ten-point dose range(2.5nM to 1uM for FDA-approved drugs and 10nM to 5uM for ECPs), both in the presence or absence of VAL. Based on the observed synergy, we identified our top nine hits that included bromodomain and methyltransferase inhibitors. Finally, we tested these nine hits in 6x6 combination matrices to precisely determine the optimal dosing with VAL.Currently, we are expanding our validation across additional ATLL cell lines and optimizing dosage schedules for *in vivo* testing on our established ATLL models to ensure synergistic effective.This research aims to identify novel targets that will sensitize resistant ATLL to epigenetic therapies. These findings may contribute to developing strategies to address drug resistance and offer additional treatment opportunities for this difficult-to-treat disease.

#4499 Drug target ID and binding site mapping in complex cellular environments using LiP-MS.

M. Soste[1], P. Shichkova[1], R. Bruderer[1], M. Stefancic[1], D. Redfern[1], F. Cavallo[2], K. Bhalla[2], H. Burston[3], P. S. Baruah[3], R. Hjerpe[2], S. Thomson[2], A. Jordan[2], **Y. Feng**[1];

[1]Biognosys AG, Schlieren, Switzerland, [2]Sygnature Discovery, Nottingham, United Kingdom, [3]Sygnature Discovery, Saint-Laurent, QC, Canada

Identifying drug targets within cellular contexts is critical to advancing both target-based and phenotypic drug discovery, particularly for small molecule degraders, such as molecular glues (MG). In most common cases of MG, target identification involves two key mechanism of actions (MoA): determining specific protein target(s) that are engaged by the E3 ligase when a drug binds the E3 ligase, and, conversely, identifying the E3 ligase (adaptor) that is recruited to form the ternary complex when the drug directly targets the protein itself. Limited proteolysis combined with mass spectrometry (LiP-MS) has emerged as a robust, label-free method for deconvoluting small molecule and peptide targets directly within cell lysates, avoiding the need for compound modification or cell line manipulation. This approach leverages a non-specific protease under controlled conditions to detect conformational changes or steric hindrance induced by drug-protein interactions or protein-protein interactions.

Using quantitative MS, LiP monitors over 250,000 peptides, representing more than 8,000 proteins in a mammalian cell line, allowing for proteome-wide profiling of drug-target interactions. A comprehensive concentration-response study spanning seven concentrations, combined with machine learning-based LiP-scoring, provides a precise ranking of targets and predicts binding sites with peptide-level resolution. Beyond global target identification, we also established a high-throughput HR-LiP workflow that offers detailed peptide-level insights into small molecule-protein interactions directly in cell lysates from cell lines overexpressing target proteins or complexes. This workflow bypasses the need for protein purification, mitigating risks of protein truncation or misfolding.

To evaluate LiP-MS' performance on molecular glue MoAs, we performed global target ID experiments using two well-characterized models: the cyclin K degraders CR8 and SR-4835 and the GSPT1 degraders MRT-2359 and Eragidomide. While the cyclin K degraders bind directly to CDK12, inducing its interaction with DDB1 and leading to the degradation of cyclin K within the CDK12 complex, the GSPT1 compounds engage CRBN, which in turn recruits and degrades GSPT1 as a neosubstrate. Applying HR-LiP, we mapped the binding site of the BRD4 inhibitor JQ1 on full-length BRD4, a large protein challenging to study via standard methods, using a mammalian transient overexpression system. Similarly, we located binding sites for EGFR inhibitors gefitinib and afatinib on the membrane-bound EGFR protein. Additionally, we pinpointed the SMER28 binding site on the hexameric ATPase VCP, an autophagy enhancer, demonstrating the method's applicability to large protein complexes.

In conclusion, LiP-MS provides a versatile toolbox for identifying drug targets and mapping binding sites within complex cellular environments.

#4500 Exploring site-specific activation of kinase inhibitors via AKR1C3-mediated prodrug strategy.

Esther Elonga, Zhengnian Li, Michael Martinez, Anuradha Tathireddy, Stephen M. Hinshaw, Nathanael S. Gray

Chemical and Systems Biology, Stanford University School of Medicine, Stanford, CA

Targeted therapies such as kinase inhibitors have significantly advanced cancer treatment. However, targeting pan-essential kinases remains challenging due to the side effects associated with a narrow therapeutic window. To address this issue, we propose developing prodrugs that can be site-specifically activated by Aldo-keto reductase 1C3 (AKR1C3). Specifically, a kinase inhibitor prodrug will be selectively activated by AKR1C3 to release the active parent compound in tissues with overexpression of AKR1C3. In this study, we designed prodrugs of the kinase inhibitor OTS964, a potent inhibitor of the pan-essential kinase CDK11, modeled after the AKR1C3-activated prodrug OBI3424. Using rational design principles, our prodrugs were engineered for selective activation by AKR1C3. Enzymatic assays confirmed AKR1C3-mediated reduction of the prodrugs, liberating the parent compound OTS964. Subsequent in vitro assays demonstrated that these prodrugs retained comparable activity to the parent compound in cell viability, immunoblotting, and NanoBRET assays. This study introduces a promising strategy to enhance the therapeutic window of pan-essential kinase inhibitors by concentrating their effects in affected tissues while sparing normal cells.

#4501 Temporal Validation of Novel Therapeutic Targets in Non-Hodgkin Lymphoma.

Tudor I. Oprea[1], Mohammed Quazi[1], Suman Sirimulla[1], Cristian G. Bologa[2], Ruben Karapetian[1], Alexey Pushechnikov[1], Bill Farley[1], Nikolay Savchuk[1]

[1]Expert Systems, Inc., San Diego, CA,[2]Internal Medicine, University of New Mexico, Albuquerque, NM

In 2024, we reported the development of knowledge graph machine learning (KGML) models focused on 41 blood cancers (1), using several public resources. Our disclosure identified 19 drug targets that are likely to be druggable and fall into the "Tchem" category—that is, targets perturbed by small molecules (2). These 19 genes had limited or no known blood cancer associations, when evaluated via the DISEASES platform (3). Upon re-evaluation a year later, five genes (BLK, FCER2, FRK, CAMK2D, and EPHB1) were no longer investigated due to a growing body of literature. Of the remaining 14 genes (see Table 1), only two (IRAK3 and ITPKB) have marginally relevant associations with lymphoma, leukemia, or myeloma, according to the DISEASES platform (3).

Table 1 summarizes cytotoxicity assays for the 14 targets, using compounds specific for each target. Non-Hodgkin lymphoma cell lines were: Toledo (CL1), SU-DHL-6 (CL2), TMD8 (CL3), and JeKo-1 (CL4). CC50 (50% cytotoxic concentration) values, measured in micromolar concentrations, were collected at 3 (_3d) and 7 days (_7d), respectively; "nd" - not determined; "gt" - greater than value.

Except for GRM8, all targets exhibited at least one micromolar CC50 value. Five kinases (IRAK3, ITPKB, PRKCE, ACVR2B, and PIM1), one enzyme (ADCY1), and one ion channel (TRPV4) have at least one sub-micromolar CC50 value (25 values total). Some targets (e.g., GPR18, a G-protein coupled receptor; and ITPKB, a kinase) show low micromolar CC50s at 7 days, with gt 20 CC50 after 3 days, suggesting target engagement. Confirmatory target engagement experiments are in progress. Although preliminary, these data validate our KGML approach (1), as they implicate at least seven targets in non-Hodgkin lymphoma disease biology, with potentially novel therapeutic applications.

References

1. Quazi M, et al. *Cancer Res* (2024) 84 (6_Supplement): 3535.

2. Oprea TI, et al. *Nat Rev Drug Discov.* (2018) 17:317-32.

3. Grissa D, et al. *Database* (2022) baac019.

Table 1: Cytotoxicity results for four leukemia cell lines

Target	Compund ID	CL1_3d	CL2_3d	CL3_3d	CL4_3d	CL1_7d	CL2_7d	CL3_7d	CL4_7d
GPR18	ZF000001	8.1	gt 20	gt 20	gt 20	1.2	gt 20	16.2	2.5
IRAK3	ZF000004	2.2	8.6	2.7	4.2	0.7	8.6	1.1	1.5
LPAR5	ZF000008	gt 20	9.1	15.1	8.2	5.0	nd	5.5	nd
ADCY1	ZF000010	0.4	0.15	0.3	0.17	0.1	nd	0.06	nd
ITPKB	ZF000011	6.1	8.0	1.5	13.7	2.0	2.8	0.7	3.4
PLD1	ZF000012	10.3	4.4	14.1	4.2	1.1	nd	9.3	5.2
PRKCE	ZF000013	0.0004	gt 0.1	gt 0.1	gt 0.1	0.000086	gt 0.1	0.001	0.00053
TRPV4	ZF000017	2.4	3.3	2.5	4.0	0.8	2.6	1.0	1.5
ACVR2B	ZF000018	0.4	1.5	0.1	0.3	0.03	1.2	0.07	0.05
TRPC6	ZF000019	gt 20	gt 20	gt 20	gt 20	5.0	14.5	7.6	7.8
GRM8	ZF000021	gt 20	gt 20	gt 20	gt 20	gt 20	gt 20	gt 20	gt 20
CAMK2A	ZF000022	11.5	15.9	10.0	gt 20	3.5	7.4	3.3	5.5
ADORA3	ZF000023	gt 20	gt 20	gt 20	gt 20	3.8	gt 20	2.0	9.8
PIM1	ZF000024	0.7	15.3	8.1	2.6	0.04	8.5	6.1	0.14

#4503 Analyzing and expanding the druggable proteome from predicted models.

Phillip W. Gingrich, Ansuman Biswas, Bissan Al-Lazikani

Genomic Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX

Introduction: The number of experimentally solved protein structures is ever-growing. These are vital for informing early drug discovery, yet only 40% of the human proteome is structurally characterized experimentally. Newmodelling tools such as AlphaFold are heralded to plug the gap in structural knowledge for the drug discoverer. Importantly, what part of the human proteome is available to drug discovery using the collective data? Here, we report analyses at the residue, protein, and cluster levels to understand how much of the proteome is structurally enabled for drug discovery, and we present the first comprehensive, reliable druggability map of the human proteome.
Methods: We mapped experimental structures and AF models to canonical forms in the human proteome, mapping experimental coverage and pLDDT scores to each residue. As a proxy for useful domain-sized structures, the presence of at least 100 consecutive residues with experimental coverage or high prediction confidence was annotated per protein. MMSeqs2 clustering was used to explore proteins where AF struggled, both with and without experimental coverage in the PDB. Finally, protein pockets were found using SURFNET and classified by our draggability classifier.
Results: The PDB covers 2.9 million residues across the proteome. AF affords 4.2 million additional confident predictions, yielding 68% of the proteome as structurally enabled at the residue level. 7100 proteins of those in the PDB have >= 100 consecutive residues with experimental coverage. AF provides 7600 more proteins with >= 100 consecutive confident residues, resulting in 72% of the proteome being structurally enabled with a domain-sized structure. AF makes low confidence predictions for the last 5700 proteins, most of which no experimental coverage. Likewise, low confidence predictions are made for proteins typically in complex with other macromolecules, despite 75% of them having at least 50% experimental coverage. In total, 40% of the proteome was classified as druggable using experimental structures or high confidence models, with as much as 60% being druggable with loosened confidence criteria. We provide a detailed map and guidelines on the utility of AF for drug discovery.
Conclusion: Roughly 70% of the proteome has been made accessible and suitable for in silico drug discovery efforts based on experimental coverage and prediction uncertainty. This has expanded the druggable fraction of the proteome significantly over previous estimates. This is the first comprehensive analysis on the suitability of predicted models, highlighting where predicted models are practically useful to the drug discoverer.

#4504 The impact of metabolite kinetics on dysregulation of essential enzymes in cancers with MTAP deficiencies.

Oscar Aubi, Shannon Faris, Daniel M. Walden, Kai Yang, Zhipeng Fang, Mason J. Appel, Angelica M. Gonzalez-Sanchez, Kelly S. Trego, Briana E. Marinoni, Brittany Cruzan, Pari Lanham, Khristina Magallanes, Damien Garbett, Ivan G. Shabalin, Rebeca M. Choy, **Peter Teriete**

Ideaya Biosciences, South San Francisco, CA

Background: Methylthioadenosine phosphorylase (MTAP) deletion is a common genetic alteration in various cancers, leading to accumulation of methylthioadenosine (MTA) and dysregulation of protein arginine methyltransferase 5 (PRMT5), an essential enzyme that utilizes the MAT2A product S-adenosylmethionine (SAM). This study investigates the complex interplay between metabolite kinetics and enzyme inhibition in MTAP-deficient cancers, focusing on the efficacy of MTA-cooperative PRMT5 inhibitors and their combination with the MAT2A inhibitor IDE397.

Methods: We employed a multi-faceted approach including molecular mutagenesis, surface plasmon resonance (SPR), X-ray crystallography, molecular dynamics simulations, NanoBRET assays, and cell-based studies to elucidate the mechanisms of metabolite exchange and inhibitor binding in PRMT5. The effects of MTA/SAM ratios on PRMT5 inhibitor efficacy were examined, and combination studies with IDE397, an allosteric MAT2A inhibitor, were conducted.

Results: Our findings revealed that metabolite exchange in PRMT5 is possible even in the presence of MTA-cooperative inhibitors. Crystallographic and molecular dynamics studies identified a second access channel to the metabolite binding pocket, gated by Lysine 393, allowing for metabolite exchange in the presence of substrate-binding inhibitors. The efficacy of MTA-cooperative PRMT5 inhibitors in cell-based assays was significantly influenced by the ratio of MTA to SAM, not just by absolute MTA concentration. Co-treatment with IDE397 lowered SAM levels in cells, substantially enhancing the efficacy of MTA-cooperative PRMT5 inhibitors.

Conclusions: This study provides novel insights into the metabolic vulnerabilities of MTAP-deficient cancers and the mechanisms underlying the efficacy of targeted therapies. The discovery of the metabolite exchange channel in PRMT5 and the impact of MTA/SAM ratios on inhibitor efficacy offer new avenues for drug design and combination strategies. The synergistic effect observed with concurrent MAT2A and PRMT5 inhibition presents a promising approach with the potential to profoundly alter the treatment landscape for patients with MTAP deleted cancers.

Our novel findings in this study provide mechanistic support for the importance of understanding metabolite exchange in PRMT5, the crucial impact of MTA/SAM ratios on the potency of MTA-cooperative PRMT5 inhibitors, and the benefit of combination therapy with the MAT2A inhibitor IDE397.

#4505 Differential protein expression in platelets collected from glioma patients and healthy controls: A comparative study.

Vibecke Markhus[1], Katarina Fritz-Wallace[1], Olav Mjaavatten[1], Einar Kleboe Kristoffersen[2], Rupavathana Mahesparan[3], Hrvoje Miletic[4], Frode Selheim[1], Dorota Goplen[5]

[1]Institute of Biomedicine, University of Bergen, Bergen, Norway,[2]Immunology and Transfusion Medicine, Haukeland University Hospital, Bergen, Norway,[3]Neurosurgical department, Haukeland University Hospital, Bergen, Norway,[4]Experimental Neuro-oncology and Neuropathology, Haukeland University Hospital, Bergen, Norway,[5]Oncology, Haukeland University Hospital, Bergen, Norway

Background: Glioblastoma (GBM) is the most aggressive type of primary brain tumor in adults. Standard treatment fosters event free survival for approximately 7 months and extends overall survival to only 15 months. Due to the risk of biopsy complications, the verification of the tumor is not always possible. Therefore, in many cases the treatment is based on MRI findings and exclusion of other malignancies. A confirmative liquid biopsy would provide additional and useful support for clinical decision making and patient counselling. To date no liquid biopsy for GBM is available.
Platelets interact with tumor cells in multiple ways, including direct contact, release of cytokines, and uptake of tumor-derived extracellular vesicles. These interactions cause modifications in platelet RNA and protein profiles, yielding tumor educated platelets that reflect specific tumor characteristics.
Our study aimed to use proteomic analysis to identify differential protein expression in platelets collected from glioma patients compared to healthy controls, identifying potential biomarkers for glioma diagnosis and therapeutic monitoring
Methods: Platelet samples were collected from 14 glioma patients and 14 matching healthy controls. Platelets were isolated using differential centrifugation to minimize contamination with other blood components. Protein extraction and quantification were performed, followed by analysis using mass spectrometry-based proteomics. Protein identification and quantification were conducted with Label-Free Quantification techniques, and differentially expressed proteins were statistically validated. Bioinformatic tools were employed to analyze pathways and functional annotations related to identified proteins.
Results: In total 3444 proteins were identified and quantified. Of these, 463 proteins were differentially expressed between platelets in glioma patients and healthy controls.
Reactome Pathway, gene ontology enrichment and protein protein interactions analyses revealed proteins associated with pathways linked to Vesicle-mediated transport, Apoptosis, Metabolism of Lipids, Cell Cycle Checkpoints, Glutathione synthesis/recycling, Platelet Activation and Signal transduction.
Conclusion: The study identifies a distinct proteomic profile in platelets from glioma patients compared to healthy individuals, suggesting that platelets in glioma may harbor unique proteins characteristics related to tumor biology. These findings support the potential of platelets as a source of biomarkers for glioma detection, confirmation of diagnosis and monitoring. Future studies with larger cohorts are warranted to validate these proteins as diagnostic markers and explore their mechanistic roles in glioma progression.

#4506 Plasma proteomics reveals biomarkers of lung cancer in non-smoking women.

Guojin Si, Zhangyan Lyu, Ximin Gao, **Fengju Song**

Tianjin Medical Univ. Cancer Inst. & Hospital, Tianjin, China

Background: Lung cancer is the most prevalent cancer among women in China. The incidence of lung cancer in women is increasing, despite the fact that traditional risk factors such as smoking have decreased. The investigation of new protein markers may enhance the etiological network and further elucidate the pathogenesis of lung cancer in non-smoking women.
Methods: We selected three non-smoking female lung cancer patients from the Chinese Colorectal, Breast, Lung, Liver, And Stomach Cancer Screening Trial (C-BLAST) cohort. Three healthy controls were matched based on age (±3 years), sex, year of screening, and smoking status. The proteomic assays were conducted on the Somascan Assay 11k platform, utilizing plasma that had been collected at baseline. The differential proteins between lung cancer patients and healthy controls were analyzed using paired t-tests ($P < 0.05$). The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) platforms were employed to investigate the functions and pathways enriched by differential proteins. The STRING platform was utilized to develop the protein-protein interaction (PPI) network of these proteins, and the core proteins were determined based on their degree.
Results: The average age of the participants in the study was 55.8 years. Through the comparison of plasma proteomics between lung cancer patients and healthy controls, 224 differential proteins were discovered, including 116 up-regulated and 108 down-regulated proteins. GO analysis revealed these proteins were associated with some biological functions, including the response to hydrogen peroxide, nuclear-transcribed mRNA catabolic process, exonucleolytic, fatty acid homeostasis, immune effector processes, and T-helper 2 cell differentiation. KEGG analysis revealed that these proteins are enriched in pathways such as glycerolipid metabolism, aldosterone-regulated sodium reabsorption, transcriptional dysregulation in cancer, and NOD-like receptor signaling pathway. We constructed the PPI network based on these 224 proteins and identified the top ten core proteins: CD4, INS, MYD88, IFNA1, APOE, BECN1, CD86, MPHOSPH10, NLRP3, and C3. These core proteins are involved in biological processes, including immunology, inflammation, and antiviral responses, and may contribute to the development of lung cancer.
Conclusions: We found several plasma proteins by plasma proteomics that may assist in identifying lung cancer patients and offer new insights into the etiological network of non-smoking lung cancer in women.

#4507 Circulating plasma proteins and risk of multimorbidity of cancer and cardiometabolic diseases.

Heinz Freisling[1], Michael Stein[2], Vivian Viallon[1], Karl Smith-Byrne[3], David C. Muller[4], Antonio Agudo[5], Giovanna Masala[6], Salvatore Panico[7], Carlotta Sacerdote[8], Ruth C. Travis[3], Rosario Tumino[9], Martijn Kolijn[10], Roel Vermeulen[11], Monique Verschuren[12], Adam Butterworth[13], Nicholas Wareham[13], Marc J. Gunter[4], Elio Riboli[4], **Pietro Ferrari**[1]

[1]International Agency for Research on Cancer, Lyon, France,[2]University of Regensburg, Regensburg, Germany,[3]University of Oxford, Oxford, United Kingdom,[4]Imperial College London, London, United Kingdom,[5]Institut Catala d'Oncologia, Barcelona, Spain,[6]Institute for Cancer Research, Prevention and Clinical Network, Florence, Italy,[7]University of Naples Federico II, Naples, Italy,[8]University of Turin and CPO Piemonte, Turin, Italy,[9]Provincial Health Authority (ASP) Ragusa, Ragusa, Italy,[10]Erasmus MC, Rotterdam, Netherlands,[11]Utrecht University, Utrecht, Netherlands,[12]University Medical Center Utrecht, Utrecht, Netherlands,[13]University of Cambridge, Cambridge, United Kingdom

Background: Cancer, type 2 diabetes (T2D), and cardiovascular diseases (CVD) are the leading causes of death worldwide. These chronic conditions can also co-occur within individuals, a phenomenon known as multimorbidity. Other than exposure to common risk factors, multimorbidity could be the result of shared biologic pathways. Leveraging large-scale proteomic data, we investigated whether specific patterns of circulating proteins were common to the occurrence of combinations of cancer, T2D, and CVD, as well as to multimorbidity of these conditions.

Methods: In a case-cohort study of the European Prospective Investigation into Cancer and Nutrition (EPIC), participants from the sub-cohort (n=4,063), as well as pre-selected cases of cancer (n=5,577) were included. The SomaScan 7k Assay was used to acquire aptamer measurements on pre-diagnostic samples. Using Prentice-weighted multivariable-adjusted Cox regression, we assessed single aptamer associations with cancer, T2D, CVD, and multimorbidity. We also sought to showcase the potential of proteomic profiling to study exposure-disease associations by investigating a proteomics signature of weekly leisure-time physical activity and its association with disease outcomes.

Results: Common patterns of aptamers across multiple diseases (3,612 incident cancers, 435 T2D, 503 CVD, and 403 multimorbidity cases) are identified in ongoing statistical analyses. Weekly leisure-time physical activity was inversely associated with 6 proteins (e.g., sorbitol dehydrogenase and leptin) and positively with 10 proteins (e.g., COMP and laminin-2) after multiple-testing correction. After multivariable- and physical activity adjustment, the signature of those proteins per 1 SD increment was inversely associated with overall cancer (HR: 0.93, 95% CI: 0.86 to 0.99) and T2D (HR: 0.54, 95% CI: 0.45, 0.66), but not with CVD (HR: 0.91, 95% CI: 0.76, 1.09). Results of associations with multimorbidity will be presented.

Conclusions: Candidate biological pathways common to cancer, T2D, and CVD as well as to multimorbidity, and their link with major lifestyle factors, will be comprehensively investigated in this large-scale proteomics dataset.

#4508 Quantitation of PD-1, PD-L1, and CTLA-4 in human melanoma using SMC® high sensitivity immunoassays.

Anthony J. Saporita, Joseph Hwang, Matthew Lippold, Laila Faramarzi, Raad Gitan, Wen-Rong Lie, Brooke Gilliam, Rick Wiese

MilliporeSigma, Saint Louis, MO

Immune checkpoint proteins such as PD-1, PD-L1, and CTLA-4 play a central role in neoplastic cell immunosurveillance. Soluble forms of these cell surface proteins are currently being evaluated as potential biomarkers in the diagnosis, prognosis, and pathogenesis of several forms of cancer, such as melanoma. For this study, we first wanted to compare human plasma samples from 12 healthy donors and 12 individuals with melanoma using MILLIPLEX® Human Immuno-Oncology Checkpoint Protein Panel 1, a 17-plex immunoassay. Three immune checkpoint proteins (CD27, CD40, and TIM-3) exhibited statistically significant increases in melanoma samples versus healthy controls, despite the small sample size. While PD-1, PD-L1, and CTLA-4 trended toward increases in the disease state, differences were below the threshold of statistical significance when assessed by MILLIPLEX®. CTLA-4, in particular, was present in low abundance in circulation, with fifty percent of healthy plasma samples near the lower limit of quantification (LLoQ) for the MILLIPLEX® assay. To achieve greater sensitivity for this critical immune checkpoint protein, we developed a novel SMC® CTLA-4 high sensitivity immunoassay. SMC® technology delivers ultrasensitive biomarker measurements for low abundant proteins in comparison to the limits of traditional sandwich ELISA immunoassays. The SMC® assays for PD-1, PD-L1 and CTLA-4 consistently achieved LLoQs of 1.74 pg/mL, 0.78 pg/mL and 0.1 pg/mL, respectively. More importantly, these ultrasensitive SMC® kits allowed quantitation of these cell surface proteins in all plasma samples tested from both healthy donors and melanoma patients. Statistical differences in CTLA-4 (p-value <0.01) and PD-1 (p-value < 0.005) were observed with the SMC® kits in the melanoma cohort compared to the healthy samples. The integration of MILLIPLEX® multiplex assays and SMC® high sensitivity assays in a comprehensive workflow enables profiling of multiple analytes and a deeper understanding of immune response dynamics by accurately measuring low abundant proteins, thereby providing a powerful non-invasive tool for evaluating disease conditions.

CLINICAL RESEARCH: Biostatistics in Clinical Trials / Surgical Oncology
Poster Session

#4512 Impact of clinical response and treatment tolerability on HRQoL in patients treated with ponatinib or imatinib for newly diagnosed Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL).

Ajibade Ashaye[1], Ling Shi[2], Ibrahim Aldoss[3], Pau Montesinos[4], Pankit Vachhani[5], Vanderson Rocha[6], Cristina Papayannidis[7], Jessica T. Leonard[8], Maria R. Baer[9], Jose-Maria Ribera[10], James McCloskey[11], Yanyu Wu[1], Meliessa Hennessy[1], Alexander Vorog[1], Shien Guo[2]

[1]Takeda Development Center Americas, Inc., Cambridge, MA,[2]Evidera Inc., Bethesda, MD,[3]City of Hope National Medical Center, Duarte, CA,[4]Hospital Universitari i Politecnic La Fe, Valencia, Spain,[5]University of Alabama at Birmingham, Birmingham, AL,[6]Instituto do Cancer do Estado de Sao Paulo, University of Sao Paulo, Sao Paulo, Brazil,[7]IRCCS Azienda Ospedaliero-Universitaria di Bologna, Istituto di Ematologia "L. e A. Seragnoli,", Bologna, Italy,[8]Oregon Health and Science University, Portland, OR,[9]University of Maryland Marlene and Stewart Greenebaum Comprehensive Cancer Center, Baltimore, MD,[10]ICO - Hospital Germans Trias i Pujol, Josep Carreras Leukaemia Research Institute, Badalona, Spain,[11]Hackensack University Medical Center, Hackensack, NJ

In the PhALLCON trial (NCT03589326), ponatinib demonstrated superior minimal residual disease (MRD)-negative complete remission (CR) rate, patient-reported treatment tolerability, and health-related quality of life (HRQoL) over imatinib in combination with reduced intensity chemotherapy in adults with newly diagnosed Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL). To further substantiate the effect of treatment benefits on HRQoL, we assessed the effect of clinical response and treatment tolerability on changes in HRQoL over time in PhALLCON trial patients. HRQoL was assessed using prespecified primary domains from the Functional Assessment of Cancer Therapy-Leukemia (FACT-Leu) questionnaire and the EQ-5D-5L. Treatment tolerability was assessed using the FACT-GP5 item "bothered by treatment side effects". Linear mixed-effects regression models were used to examine changes in HRQoL over time, with CR status and treatment tolerability as time-varying predictors, while controlling for significant covariates. The analysis included 238 patients (ponatinib 159, imatinib 79). Achieving CR was linked to significant HRQoL improvement in all FACT-Leu domains and the EQ-5D visual analogue scale (EQ-VAS), with clinically meaningful improvements in the FACT-Leu Trial Outcome Index, FACT-G Total Score, and FACT-Leu Total Score domains (Table). Treatment-related side effects led to significant and clinically meaningful deterioration in all scales. Higher levels of "bother" were associated with greater worsening in HRQoL. Given the 2-fold higher MRD-negative CR rate of ponatinib compared to imatinib, these analyses further support the HRQoL benefit of ponatinib over imatinib in patients with Ph+ ALL.
1

Table. Changes in HRQoL over time[a]

		β (95% CI)						
Variable	**FACT-Leu PWB(MID=±2)[b,c]**	**FACT-LeuS(MID=±4)[b,d]**	**FACT-Leu TOI (MID=±5)[b,c]**	**FACT-G Total Score (MID=±3)[b,e]**	**FACT-Leu Total Score (MID=±6)[b,e]**	**EQ-VAS(MID=±7)[b,f]**	**EQ-5D-5L UKbased HUI(MID=±0.08)[b,g]**	**EQ-5D-5L USbased HUI(MID=±0.08)[b,g]**
Response status								
CR vs No CR	1.24(0.38, 2.09)	3.26(1.34, 5.18)	5.28(1.99, 8.57)	3.82(0.93, 6.71)	7.18(2.99, 11.38)	4.60(0.47, 8.73)	0.04 (0.00, 0.08)	0.02 (-0.01, 0.05)
p-value	0.005	<0.001	0.002	0.010	<0.001	0.029	0.081	0.182
Treatment tolerability								
"A little bit" vs "Not at all"	-2.53 (-3.09, -1.96)	-2.68 (-3.94, -1.42)	-6.16 (-8.32, -4.01)	-4.01 (-5.90, -2.12)	-6.26 (-9.02, -3.51)	-3.85 (-6.64, -1.07)	-0.07 (-0.09, -0.04)	-0.05 (-0.07, -0.04)
"Somewhat" vs "Not at all"	-5.50 (-6.24, -4.76)	-5.67 (-7.34, -4.00)	-12.75 (-15.60, -9.89)	-9.04 (-11.54, -6.55)	-14.11 (-17.77, -10.46)	-9.14 (-12.78, -5.50)	-0.09 (-0.12, -0.05)	-0.06 (-0.09, -0.04)
"Quite a bit"/"Very much" vs "Not at all"[h]	-9.90 (-10.84, -8.96)	-10.51 (-12.65, -8.37)	23.28 (-26.92, -19.63)	-16.82 (-20.00, -13.64)	-26.82 (-31.50, -22.14)	-15.09 (-19.76, -10.42)	-0.24 (-0.29, -0.20)	-0.18 (-0.21, -0.14)
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a]Patients completed the FACT-Leu and EQ-5D-5L at screening/baseline; on C1D1, C4D1, C7D1, C10D1, C13D1, C16D1, C19D1, and C21D1 and at least every 6 cycles thereafter; and at the end of treatment. [b]Prespecified MID thresholds were used to determine whether the differences were clinically meaningful. [c]Adjusted for baseline score and sex. [d]Adjusted for baseline score, sex, and time from initial diagnosis of Ph+ ALL to first dose date of prior anti-cancer regimen. [e]Adjusted for baseline score. [f]Adjusted for baseline score and age (years). [g]Adjusted for baseline score, age (years), sex,and time from initial diagnosis of Ph+ ALL to first dose date of prior anti-cancer regimen. [h]The FACT-GP5 categories "Quite a bit" and "Very much" were combined due to the small sample size at each response level.ALL, acute lymphoblastic leukemia; C, cycle; CI, confidence interval; CR, complete remission; D, day; FACT G, Functional Assessment of Cancer Therapy general score; FACT-GP5, Functional Assessment of Cancer Therapy–General Population Item 5; FACT-Leu, Functional Assessment of Cancer Therapy–Leukemia; HUI, health utility index; HRQoL, health-related quality of life; MID, minimal important difference; Ph+, Philadelphia chromosome-positive; PWB, physical well-being; TOI, trial outcome index; VAS, visual analogue scale

#4513 Impact of patient safety regulatory considerations on early oncology clinical investigations.

Baisong Huang[1], Cindy Lu[1], Danny Lu[2], Daniel Slade[3], Hazel Kurz[3], Thomas Jahn[4], Sergio Vicente[5], Bruno Medeiros[4], Jayne Marshall[3]

[1]AstraZeneca, Waltham, MA,[2]AstraZeneca, Mississauga, ON, Canada,[3]AstraZeneca, Cambridge, United Kingdom,[4]AstraZeneca, South San Francisco, CA,[5]AstraZeneca, Barcelona, Spain

Introduction: Model assisted approaches such as mTPI2 designs are widely applied in early phase oncology trials to guide dose escalation decisions. Recent health authority interactions have highlighted the importance of design parameters such as the target dose limiting toxicity (DLT) rate and the equivalence interval (EI), in situations where lower toxicity is desired. As the dose escalation strategy may vary with different parameter configurations, the shift of target DLT and EI motivated us to explore the optimal cohort size for a given design.
Methods: We simulated thousands of escalation trials using the mTPI2 design with five dose levels under five common scenarios with monotonal dose toxicity relationship, maximum total N=36-48 and maximum cohort size of n=12 in each simulated trial. The target DLT was set at 25% with an EI of (20%, 33%). Using simulation, we compared the mTPI2 performance with cohort sizes of three, four, and five, evaluating: 1) Reliability: Probability of identifying the true MTD; 2) Safety: Proportion of patients experiencing DLT or overdosed (receiving MTD or higher doses); 3) Dose Escalation Efficiency (DEE): Reliability adjusted by number of patients treated; and 4) Incorrect Dosing Decisions: Proportion of decisions escalating above or de-escalating below the true MTD.
Results: Preliminary results show that a cohort size of four increased the sample size by 1.4-4.9 but improved reliability by 4.6%-10.8% in absolute value compared to a cohort size of three, resulting in the highest DEE among all cohort sizes tested in all scenarios. A cohort size of five also increased reliability but marginally diminishing. A cohort size of four had the lowest likelihood of incorrect dosing decisions (an average ~4% absolute reduction) compared to a cohort size of three in all scenarios, which indicated that cohort size four generates the shortest dose escalation/de-escalation pathway to estimating MTD that ensures the highest DEE among the three cohort sizes tested. The simulation also indicated that, generally, safety performance improved when cohort size increased. When cohort size increased from three to four, the proportion of patients experiencing DLT reduced by 1-2% and proportion of overdosed patients reduced by 2.9-5.0% both in absolute values.
Conclusions: When using the mTPI2 dose escalation design in early clinical trials, we strongly recommend that trial statisticians conduct simulations to assess various configurations across all potential scenarios. This approach optimizes the dose escalation process, increasing the likelihood of identifying the true MTD and minimizing patient exposure to toxicity. Increasing cohort size can offer operational benefits, such as reducing the need to replace non-evaluable participants. Enhanced reliability may also mitigate risks in further development, at the cost of a small number of additional patients treated at early stages.

#4514 The impact of Project Optimus and FDA guidance on study design of early-phase clinical trials.

Deepak Bhamidipati[1], Suzanne Jones[2], David Spigel[1], Gerald Falchook[3], Manish Patel[4], Howard Burris[1], Vivek Subbiah[1]

[1]Sarah Cannon Research Institute at SCRI Oncology Partners, Nashville, TN,[2]Sarah Cannon Research Institute, Nashville, TN,[3]Sarah Cannon Research Institute at HealthONE, Denver, CO,[4]Sarah Cannon Research Institute at Florida Cancer Specialists, Sarasota, FL

Background: In recent years, the FDA has issued guidance on the design and conduct of clinical trials such as dose optimization (Project Optimus), increasing enrollment of participants in underrepresented communities, and reporting patient-centered outcomes. Specifically, the FDA recommends examination of multiple doses to determine the optimal dose but how this guidance affects the design of early-stage oncology clinical trials remains uncertain. The goal of this study was to evaluate trends in early phase trial design and incorporation of dose optimization into early phase protocols over time.

Methods: All early phase protocols activated at Sarah Cannon Research Institute between 2021-2023 were reviewed (n=286 protocols). Information such as class of therapy, study design, and dose optimization plan (if applicable) were extracted from review of each protocol. Fisher's exact test was used to compare proportions across categories and a p-value <0.05 was considered statistically significant.

Results: 268 unique protocols including 215 first in human trials (80%) were initiated with 179 different sponsors between 2021 and 2023. Trial classification by mechanism of action included 114 targeted therapies (43%), 109 immunotherapies (41%), 29 antibody drug conjugates (11%), 12 Immune effector cellular therapies (4%), and 4 chemotherapies (1%). Most trials employed a Bayesian dose escalation design (53%) versus rule-based (3+3), and this did not vary significantly year-over-year. 32% of trials (N=86) had a dose optimization plan. 47 trials (18%) included a dose optimization plan with randomization while 39 trials (14%) discussed optimization without randomization. The percentage trials with a dose optimization plan increased annually from 2021-2023 (22%, 29%, and 47% respectively; $p=0.002$). There was an increase in the proportion of trials which included backfill cohorts from 2021 (60%) to 2022-2023 (76%) ($p=0.007$) in addition to the proportion of protocols which allowed intra-patient dose escalation (45% in 2021 versus 57% in 2022-2023, $p<0.001$). The number of additional patients required in the trials with a dose optimization plan ranged from 12 - 200 with a median of 40. The inclusion of patient reported outcomes in early phase protocols was low at 12% from 2021-2023 and only 3 protocols (1%) addressed enrollment of underrepresented groups in the protocol.

Conclusion: Overall, there has been a trend towards increased implementation of a dose optimization plan within early phase protocols since the announcement of the FDA's Project Optimus initiative in 2021. Moreover, Bayesian designs, backfill cohorts, and intra-patient dose escalation are common in contemporary dose escalation studies. However, patient-centered outcomes and underrepresented populations are not routinely addressed in these protocols. Updated analysis including early phase clinical trials activated in 2024 is planned.

#4515 Epidemiological factors associated with breast density in Guam.

Gian M. Paras[1], **Rodney S. Teria**[1], Su Bin Jin[1], Grazyna Badowski[1], John Shepherd[2], Rose Grino[3]

[1]Pacific Island Partnership for Cancer Health Equity (PIPCHE), University of Guam, Mangilao, Guam,[2]Pacific Island Partnership for Cancer Health Equity (PIPCHE), University of Hawaii Cancer Center, Honolulu, HI,[3]FHP Health Center, Tamuning, Guam

Guam's breast cancer incidence is lower than the U.S. (86.6 vs. 126.8 per 100,000), but mortality rates are higher (27.3 vs. 20.3). This disparity underscores the importance of improving screening and understanding risk factors. Breast density, categorized using the Breast Imaging Reporting and Data System (BIRADS), complicates tumor detection and correlates with malignancy risk. This study investigates the demographic and reproductive factors associated with breast density in Guam. Data from 1,940 women undergoing mammography at FHP Health Center (Nov 2023-Aug 2024) in the HIPIMR study were analyzed. Associations between demographic factors (ethnicity, BMI) and reproductive factors (family history, age at menarche, menopausal status, parity, age at first live birth) with BIRADS categories were tested using chi-square tests. Reproductive factors were categorized by risk level. Multinomial logistic regression, adjusted for age and BMI, examined associations. Missing data (e.g., BMI, age, parity) were imputed using series means/medians. Women with extremely dense breast tissue (BIRADS D) were younger (mean age 57.13 vs. 58.32, $p<0.001$) and had lower BMI (74% vs. 37%, $p<0.001$). They more often experienced menarche after age 12 (58% vs. 51%, $p=0.002$), had fewer than two births (64% vs. 53%, $p<0.001$), and gave birth after age 30 (65% vs. 51%, $p<0.001$). Premenopausal (32%) and early menopause women (51%) had higher BIRADS D proportions ($p<0.001$, $p=0.005$). Regression showed BMI inversely associated with BIRADS D (OR = 0.815, 95% CI: 0.787-0.844), BIRADS C (OR = 0.875, 95% CI: 0.842-0.908), and BIRADS B (OR = 0.937, 95% CI: 0.907-0.968) ($p<0.001$). Family history increased odds of BIRADS D (OR = 1.64, 95% CI: 1.041-2.584, $p=0.033$) and BIRADS B (OR = 1.674, 95% CI: 1.042-2.69, $p=0.033$). Nulliparous women were 2.66 times more likely in BIRADS B (OR = 2.66, 95% CI: 1.157-6.114, $p=0.021$). Micronesians were less likely to belong to BIRADS D (OR = 0.419, 95% CI: 0.19-0.924, $p=0.031$) or BIRADS C (OR = 0.265, 95% CI: 0.092-0.766, $p=0.014$). BMI, parity, and childbirth age significantly influence breast density in Guam. Women with lower BMI, fewer births, or later childbirth were more likely to have extremely dense breast tissue, potentially complicating tumor detection. Ethnicity also played a role, with Micronesian women less likely in BIRADS D. While imputation mitigated missing data, bias remains possible. Future research should expand sample sizes and include malignancy risk via BIRADS outcomes for deeper insight. This research was funded by the National Cancer Institute: The University of Guam/University of Hawaii Cancer Center Partnership to Advance Cancer Health Equity (PIPCHE), Grant U54CA143728 (University of Guam, YCP)/Grant U54CA143727 (University of Hawaii Cancer Center).

#4516 Prognostic value of PET/CT in newly diagnosed multiple myeloma: A Bayesian analysis with missing data imputation.

E. A. Gonzalez Artunduaga[1], N. A. Cruz Gutierrez[2], S. Sandoval Alvarez[3], K. M. Galvez Cardenas[4];
[1]Universidad Nacional de Colombia, Bogota, Colombia, [2]University of the Balearic Islands, Balearic Islands, Spain, [3]Universidad de Antioquia, Medellin, Colombia, [4]Hospital Pablo Tobon Uribe, Medellin, Colombia

Introduction: PET/CT assessment in newly diagnosed patients with multiple myeloma is a significant predictor of long-term clinical outcomes. Incorporating this imaging technique into a Bayesian analysis that addresses data gaps allows for more accurate prognostic estimates. This methodology enhances our understanding of the disease and guides informed clinical decisions, ultimately improving patient care.
Objective: This study aims to evaluate the prognostic value of PET/CT in newly diagnosed patients with multiple myeloma, using Bayesian analysis to manage missing data imputation. We seek to determine how PET/CT findings predict long-term clinical outcomes, thereby enhancing treatment personalization.
Hypotheses:

1. A positive PET/CT at diagnosis may correlate with poor prognosis, indicating lower survival rates and worse disease control.
2. A negative PET/CT at diagnosis may be associated with better prognosis, suggesting higher treatment response rates and longer survival.

Study Design: An analytical study based on clinical and imaging data from PET/CT.
Study Population: Patients diagnosed with multiple myeloma between 2017 and 2024 at an institution in Medellín, Colombia, approved by the ethics committee of Pablo Tobón Hospital.
Variables of Interest:

- Clinical Variables: Age, sex, type of treatment.
- PET/CT Variables: Positive (+), negative (-).
- Other Variables: Cytogenetics, Beta 2 Microglobulin (B2M), Bone Marrow Biopsy, Albumin, Hemoglobin.
- Outcome Variables: Overall Survival, Progression-Free Survival, Treatment Response.

Data Imputation Methodology: Using advanced R tools, including:

- MCMC: For parameter estimation despite incomplete data.
- SMC: To optimize sequential inferences and improve precision.

Statistical Analysis:

- Kaplan-Meier survival curves for visualizing survival rates.
- Significance of covariates evaluated through 95% credibility intervals.

Results: The Bayesian model highlights PET/CT and cytogenetics as key outcome variables. A negative PET/CT correlates with better survival and more accurate treatment response prediction, while a positive PET/CT is linked to higher disease progression rates.
Limitations:

- Generalizability may be limited due to single-institution data.
- Missing data could introduce bias despite imputation methods.
- Short follow-up may restrict long-term outcome assessment.
- Variability in imaging protocols may affect result consistency.

Conclusions:

1. Prognostic Value of PET/CT: The study indicates that PET/CT evaluation is a significant predictor of long-term outcomes in multiple myeloma. A negative PET/CT is linked to better prognosis and treatment response.
2. Clinical Decision Making: Integrating Bayesian analysis and data imputation into PET/CT assessment enhances prognostic accuracy, providing clinicians with valuable insights for personalized treatment and optimized patient care.

#4517 Comprehensive intraoperative imaging of surgical margins with AI-driven open-top light-sheet microscopy.

D. Brenes[1], Q. Han[1], R. Wang[1], R. Kareem[1], M. C. Topf[2], E. L. Rosenthal[2], E. Marchiano[1], E. Palmquist[3], F. Mahmood[4], S. H. Javid[3], S. M. Dintzis[3], J. T. C. Liu[1];

[1]University of Washington, Seattle, WA, [2]Vanderbilt University, Nashville, TN, [3]University of Washington School of Medicine, Seattle, WA, [4]Harvard Medical School, Boston, MA

Surgeons rely on subjective visual and tactile feedback to differentiate tumors from adjacent normal tissue, which lacks accuracy and may make complete resection challenging. Over 20% of breast and 40% of head & neck cancer surgeries yield positive margins—cancerous cells at the edge of the resected specimen—requiring costly additional treatments with inferior outcomes. When pathologists assess margins, they quantify the tumor's proximity to the margin surface, where a margin of several millimeters is often desired. This is done by viewing formalin-fixed, paraffin-embedded (FFPE) tissue sections oriented perpendicularly to the specimen surface. FFPE sections are typically ~5 µm thick and are obtained at 3 - 5 mm intervals, meaning that < 0.1% of the specimen surface is evaluated. We propose that for solid continuous tumors, comprehensive surface microscopy — though unable to measure the tumor's proximity to the specimen surface — could more accurately identify positive margins and could be performed rapidly in the operating room to maintain specimen orientation. We are developing an intraoperative open-top light-sheet (OTLS) microscopy system that comprehensively assesses fresh specimen surfaces through an artificial intelligence (AI)-driven, time-efficient, multi-resolution workflow. The system resembles a flatbed scanner and images the bottom face of a specimen placed on a glass plate. Fresh specimens are rapidly stained (< 1 min) with a fluorescent agent for nuclear and stromal contrast. A profilometer quickly (< 1 min) obtains a height map of the specimen's bottom surface to guide the OTLS microscope as it captures a thin volume (50 – 100 µm in depth) encompassing the specimen's surface. The OTLS microscope operates at two resolution modes: low (~5 × 5 × 5 µm xyz) and high (~1 × 1 × 3 µm xyz). First, a comprehensive low-resolution scan is acquired rapidly (< 3 min per 10 x 10 cm area). An AI model then identifies high-risk regions to re-scan at high resolution for diagnosis. Our 3D AI-driven imaging strategy mimics the time-efficient workflow of pathologists, who first examine FFPE slides at low magnification before zooming into localized regions at high magnification. The ability to acquire a shallow amount of volumetric data (up to ~ 100 microns deep) can improve positive margin detection over a single 2D surface due to the greater amount of cellular information and the ability to avoid artifacts from surgical damage (e.g. cautery). To develop our 3D AI algorithm, we are building a dataset from freshly excised breast and head & neck specimens, with ground-truth diagnostic labels provided by pathologists. The model will be weakly supervised, using pre-trained models for 3D feature extraction.In summary, AI-guided multi-resolution OTLS microscopy has the potential to enable rapid, comprehensive intraoperative margin assessment, reducing positive margin rates and improving patient outcomes.

#4518 The use of surgical staging of inguinal lymph nodes in penile squamous cell carcinoma in the HIV era.

Victor Mapulanga, Owen Ngalamika, Edford Sinkala, Mumba Chibamba

University of Zambia, Lusaka, Zambia

Penile cancer is quite common in developing countries such as sub-Saharan Africa (SSA) and this high incidence in SSA has been attributed to high prevalence of Human Papilloma Virus (HPV). Additionally, Human Immunodeficiency Virus (HIV) prevalence is high in SSA and considering that HIV affects lymph nodes, this may potentially affect the clinical manifestation of draining lymph nodes in penile cancer. These lymph nodes are staged surgically by inguinal lymph node disection which is associated with high complications rates. Data on surgical staging of lymph nodes in penile cancer in SSA are scanty. We set to explore the use of surgical staging of inguinal lymph nodes in penile squamous cell carcinoma (PSCC) in a high HIV endemic setting.A prospective study was conducted from November, 2022 to January, 2024 at the University Teaching Hospital, Lusaka, Zambia. Patients with PSCC with surgically resectable tumor undergoing surgical treatment of primary tumor and inguinal lymph node dissection were recruited into the study. The questionnaire was administered to capture relevant information.Blood was collected pre operatively for HIV testing and viral load determination. The disected lymph node tissue and resected tumor were sent to the pathologist for histological determination of tumor involvement of lymph node and histopathological staging. Immunohistocehmistry was done on tumor tissue using p16 for HPV determination.Forty (40) were enrolled in the study with a median age was 53 years. Thirty-five (88%) were HIV seropositive with most HIV positive patients being virologically suppressed with antiretroviral therapy at the time of surgery. The prevelence of HPV among the participant was 65% with HPV types 16 and 35 being the most common. The prevalence of pathological lymph node metastasis (LNM) was 37.5% (12/32) in patients with clinically palpable (cN+) inguinal lymph nodes. In conclusion surgical staging of inguinal lymph nodes in patients with PSCC in the era of HIV has low yield of positive lymph node involvement with most being reactive adenopathy due do HIV infection and not the cancer

#4519 Lung carcinoma with air lucency: Possible classification and their prognosis.

Kazuya Hisamatsu[1], Kazuhiko Shien[2], Shuta Tomida[3], Daisuke Mizuno[1], Ryota Fujiwara[1], Kousei Ishimura[1], Ryunosuke Fujii[1], Atsushi Matsuoka[1], Ryo Yoshichika[1], Tomoaki Higashihara[1], Naohiro Hayashi[1], Yuma Fukumoto[1], Kazuhiro Okada[1], Fumiaki Mukohara[1], Mao Yoshikawa[1], Ken Suzawa[2], Shinichi Toyooka[2]

[1]General Thoracic Surgery, Okayama Univ. Graduate School of Med., Okayama, Japan,[2]Thoracic, Breast and Endocrinological Surgery, Okayama University Hospital, Okayama, Japan,[3]Center for Comprehensive Genomic Medicine, Okayama University Hospital, Okayama, Japan

Background: Lung cancer with air lucency (LCAL) represents a group of lesions in which a primary lung cancer manifests on CT images with a cyst or cystic airspaces. NSCLCs with adjoining cystic airspaces have been reported an increased risk of poor prognosis after surgical resection. However, the detailed classification of LCALs based on diagnostic imaging have not yet been established. We aim to classify LCALs into emphysematous and non-emphysematous groups objectively, and then analyze the prognosis of each group.
Methods: A total of 101 surgically resected LCAL cases in our institution between 2015 and 2022 (including all cancer stages and histological subtypes) were collected. Cases were separated into emphysematous and non-emphysematous groups based on Goddard's score, derived from LAA% (Low Attenuation Area percentages) analyzed in CT scans. A Goddard's score of 1 or more was considered emphysematous in this study. Then, clinicopathological features and prognosis for each group were analyzed.
Result: Sixty (59%) cases were classified as emphysematous, and forty-one (41%) cases as non-emphysematous. Higher pathological stages (worse than IIB) were more common in the emphysematous group (p-value<0.01). For respiratory performance (FEV1%), the emphysematous group had worse outcomes compared to the non-emphysematous group (p-value=0.01). Kaplan-Meier analysis revealed that the emphysematous group had a significantly worse overall survival (p-value=0.02). In multivariate analysis, a Goddard score of 1 or more, advanced TNM staging, and tumor histology subtypes other than adenocarcinoma were identified as independent risk factors for poor prognosis in LCAL patients.
Conclusion: LCALs can be categorized using Goddard's score into emphysematous and non-emphysematous groups, with the emphysematous group showing poorer overall survival. This result suggests that patients with emphysematous LCALs should be considered at high risk for poor outcomes following surgery. This classification may help identify patients who require alternative therapeutic strategies and/or more cautious post-operative management.

#4520 Assessing treatment strategies in high-risk metastatic nephroblastoma: A trinetx-based comparison of chemotherapy with surgery vs. primary nephrectomy.

Zayan Musa, **Javier Prieto**, Fatemeh Adabifirouzjaei

UC Riverside School of Medicine, Riverside, CA

Introduction:
Wilms Tumor, or nephroblastoma, is the most prevalent kidney cancer in pediatric populations. With a survival probability nearing 90%, the disease carries a 15% risk of recurrence. Wilms Tumor has a developmental origin, frequently involving mutations in genes such as WT1, WTX, CTNNB1, and TP53, which correlate with a poorer prognosis. The tumor can present unilaterally or bilaterally, often due to excess metanephric tissue cultures. Despite favorable survival rates, the potential for recurrence and malignancy calls for continuous evaluation of treatment strategies.
Methods:
A retrospective analysis was conducted on patients aged 0-18 diagnosed with Wilms Tumor and treated either by chemotherapy followed by surgery (Cohort 1) or surgery alone (Cohort 2). Cohort 1 included 289 patients who underwent chemotherapy prior to nephrectomy, while Cohort 2 consisted of 84 patients who received only a partial or full nephrectomy. Outcomes were compared over a 10-year follow-up period, focusing on secondary malignancies and survival rates.
Results:
Over the 10-year follow-up, Cohort 2 (surgery-only) demonstrated a significantly higher survival probability compared to Cohort 1 (chemotherapy plus surgery). The risk of a secondary malignancy was 97.23% in Cohort 1, compared to 73.8% in Cohort 2, indicating a 12.4% increase in malignancy risk with the addition of chemotherapy ($p < 0.0001$). This data suggests that chemotherapy prior to surgery may not be the safest option in terms of long-term outcomes for nephroblastoma patients.
Conclusion:
Current nephroblastoma treatment involves chemotherapy followed by surgical resection. Our study suggests that surgery alone may provide better outcomes, reducing the risk of secondary malignancies by 12.4% and significantly improving long-term survival. While surgical excision alone may not be feasible in all cases, it warrants consideration as an individualized treatment option. Further research should investigate nephrectomy-only treatment as a potentially safer and more effective alternative for pediatric Wilms Tumor patients.

#4521 Quilting of flaps reduces drain effluent without significantly increasing duration of surgery in mastectomy a low resource setting.

AbdulRazzaq O. Lawal, Jimoh O. Hassan, Adewumi Alabi, Oluwole A. Atoyebi

Lagos University Teaching Hospital, Lagos, Nigeria

Introduction: Mastectomy is the most common surgical procedure for breast cancer in low- and middle-income countries. Following mastectomy, patients usually require a drain to be inserted to prevent seroma formation. Several strategies are used to reduce the both the quantity of effluent and the duration a patient has to have the drain inserted.
Methods: We did a prospective trial where patients were randomized to either use of quilting with 2-0 vicryl sutures or lack of quilting in order to reduce the post operative drain volume and the post operative period during which the drain will be in situ. Consecutive patients who are scheduled for mastectomy were randomized to either quilting or non-quilting of the skin to the pectoralis muscle. Duration of surgery was recorded. Consecutive daily volumes of drain effluent were documented until removal of the drain. Number of days before drain removal as well as body mass index and other parameters were recorded.
Results: A total of 63 patients were recruited. Forty patients were randomized to non-quilting whilst 23 patients were randomized to quilting. The demographic data, stage of the disease and body mass index of the patients in the two categories were similar. The age range was 22 to 82 years. With mean age of 49.8 and median age of 48 years. The number of days before removal of drain ranged from 3 to 10 days with mean of 6.62 days and mode of 6 days. Mean daily drain effluent was 55 to 237mls with a mean of 134.5mls and median of 136mls while total drain effluent ranged between 166 to 2,173mls with mean of 938.1mls and median of 846 mls. Independent t-test revealed that there was a statistically significant difference in duration of drain insertion, mean daily drain effluent and total drain effluent between the two groups. There was however no statistically significant difference in the duration of surgery between the two groups.
Conclusion: It is recommended that surgeons should quilt between the skin and pectoralis muscle in order to reduce the duration of drain insertion, daily drain effluent and total drain effluent following mastectomy in patients with breast cancer.

#4522 Old dog, new tricks: The role of capecitabine in HPV-negative head and neck cancer.

Marco Antonio Mascarella, Nisha Suarez, Keith Richardson, Alex Mlynarek, Michael Hier, Nader Sadeghi, Khalil Sultanem, Derin Caglar, Marc Pusztaszeri, Livia Florianova, Nathaniel Bouganim, Khashayar Esfahani

McGill University Health Centre, Montreal, QC, Canada

Objective: Treatment for HPV-negative head and neck squamous cell carcinoma (HNSCC) has seen little innovation, particularly in advanced-stage disease, where outcomes remain suboptimal. The public healthcare system's delays in access to care can exacerbate this issue, allowing tumors to progress prior to treatment initiation. This study explores the use of capecitabine, an oral chemotherapy agent, in both preoperative and recurrent settings for HPV-negative HNSCC.
Study Design: We conducted a prospective cohort study of patients with stage III/IV HPV-negative HNSCC at two academic centers in Montreal, Canada. The study evaluated clinical and pathological responses in patients receiving capecitabine in a preoperative setting, with radiological and clinical outcomes assessed for those with unresectable recurrent disease.
Results: Among the 34 patients included in the study (23 oral cavity, 1 oropharynx, 5 skin, 5 larynx), 31 received capecitabine preoperatively, while 3 had recurrent, unresectable disease. Of the 31 patients undergoing surgery, 16 exhibited clinical tumor reduction, 13 remained stable, and 2 experienced tumor growth. Pathological responses were observed in 15 patients, including 1 complete response (skin) and 6 major responses based on the modified Ryan criteria. Two non-responders developed recurrences within one year. The mean follow-up for surgical patients was 6 months. In the non-surgical cohort, all three patients showed reduced pain and radiological improvements, with a mean follow-up of 8 months. No significant toxicity related to capecitabine was reported.
Conclusion: Capecitabine, used either in a preoperative window of opportunity or in the recurrent setting, demonstrated measurable clinical and pathological responses in patients with HPV-negative HNSCC. Given its efficacy and low toxicity, further investigation of this oral agent is warranted, particularly in resource-constrained settings where timely access to treatment may be a challenge.

#4523 Personalized risk prediction for treatment intolerance in operable HPV-negative head and neck cancer: Beyond the eyeball test.

Marco Antonio Mascarella[1], Keith Richardson[1], Nader Sadeghi[1], Alex Mlynarek[1], Michael Hier[1], Khalil Sultanem[1], Christina Tsien[1], Khashayar Esfahani[1], Marie-Jeanne Kergoat[2]

[1]McGill University Health Centre, Montreal, QC, Canada,[2]University of Montreal, Montreal, QC, Canada

Introduction: Head and neck cancer (HNC) treatment is highly complex, with substantial toxicity risks from curative therapies, often including surgery, radiation, and chemotherapy. Poor treatment tolerance can hinder optimal recovery, impacting patient outcomes. Current predictive tools lack precision in forecasting treatment intolerance for HNC patients undergoing curative surgery. Frailty and sarcopenia, especially muscle quantity biomarkers, are increasingly recognized as crucial predictive factors. This study aims to develop a comprehensive risk index incorporating sarcopenia, frailty, and patient-specific factors to better predict intolerance.
Methods: An ambispective observational study was conducted with 542 patients undergoing curative-intent HNC surgery from 2015 to 2024. Patient data on demographics, frailty, sarcopenia (via cervical paraspinal muscle index or CPSMI), tumor staging, and planned adjuvant therapy were collected. Using logistic regression, we evaluated predictive factors for treatment intolerance, defined as major adverse events (MAEs) or incomplete treatment. Risk model performance was validated internally and externally and compared against ASA, mFI, and RAI.
Results: In 542 patients, lower CPSMI (first tertile) correlated significantly with intolerance (OR 1.85, 95% CI 1.10-3.13). High-density muscle was protective, while low-density muscle predicted intolerance (OR 1.88, 95% CI 1.18-2.99). Sarcopenia and frailty were independently predictive, with the mFI also strongly correlating with intolerance (OR 4.92, 95% CI 1.88-12.9). The new risk index demonstrated superior predictive accuracy, showing a 0.04 increase in the C-statistic compared to existing indices (p=0.02).
Conclusion: The multimodal risk index effectively predicts treatment intolerance in HNC surgery patients. By integrating sarcopenia, frailty, and patient-specific factors, the tool enables better preoperative counseling and intervention planning, with particular utility for high-risk individuals. Further research on prehabilitation strategies may enhance treatment tolerance in vulnerable patients, supporting a proactive approach to HNC care.

#4524 The independent prognostic nomogram models for primary and recurrent retroperitoneal liposarcoma: A population-based cohort study.

Huan Deng, Zhenhau Lu, Binrui Wang, Yisheng Pan

Huan Deng (Individual), Beijing, China

Purpose: The aim of this study was to establish independent prognostic models for primary and recurrent retroperitoneal liposarcoma (RLS).
Experimental design: A total of 2429 patients confirmed to have RLS were extracted from the Surveillance, Epidemiology and End Results (SEER) database. The 245 patients collected from the same period at First Medical Center, Chinese People Liberation Army General Hospital (CPLAGH), were used for external validations. Nomogram were built on the basis of clinical practicability, univariate and multivariate Cox analyses.
Results: The simplified predictive models for primary RLS was mainly based on tumor size (median size, 162 mm [range, 90-230], $P < 0.001$) and pathological subtypes (WDL vs DDL, hazard ratio[HR]=2.11; 95% confidence interval [CI]=1.71-2.61; $P < 0.001$), which can be acquired in the outpatient clinics. However, TNM stage (Stage 1 vs Stage 2, HR=2.18; 95% CI=1.49-3.20; $P < 0.001$) as an extremely important postoperative indicator was enrolled in the model for recurrent RLS. The area under the time-dependent receiver operating characteristic curve (time-dependent AUC) and concordance index (C-index) of models almost approach 0.75 in the training and validation cohorts. These results indicated favourable discrimination of the models. The calibration curves of the model illustrated good consistency between the predicted and observed survival probabilities in both the training and two validation cohorts. Furthermore, decision curve analysis (DCA) revealed that the nomogram models were clinically useful and had considerable practicability.
Conclusion: Efficient and independent models for primary and recurrent RLS will providing clinical guidance for both prognostication and individualized therapeutic decision-making.

#4526 Safeguarding parathyroid glands during thyroidectomy using a real-time imaging agent.

Jing Huang, Jialun Ding, Zhishan Guo, Kuan Liu

Aisee Biotechnology, LLC, Hangzhou, China

Parathyroid gland (PG) inadvertent damage or removal is a frequent complication of thyroidectomy, largely due to the difficulty of distinguishing PGs from surrounding thyroid gland (TG) during surgery. To address this challenge, we developed a novel imaging agent (IO-100) for real-time intraoperative PG visualization. Our study demonstrates that locally administered dark-colored IO-100 preferentially diffuse into TG while remaining excluded from tissue-dense PGs, creating a visually distinct contrast observable by surgeons. Through systematic in vivo optimization, intraoperative PG/TG contrast has been significantly enhanced, and PGs have been well identified and preserved. Importantly, local IO-100 administration was well-tolerated, showed no adverse toxicities exploiting the unique biodegradable property. These findings establish IO-100 as a promising safe and effective imaging agent for enhancing PG identification and minimizing damage/removal risk during thyroidectomy, providing a valuable tool for improving surgical outcomes.

CLINICAL RESEARCH: Immune Responses to Therapies Including Chemotherapy and Radiotherapy
Poster Session

#4530 mTOR inhibition augments antitumor T cell response by modulating the expression of immune regulatory molecules.

Priyatosh Nath, Alok Khandelwal, Ivan Alvarez, Chun Li, Srivatsa Vasudevan, Tara Moore-Medlin, Cherie-Ann Nathan

Department of Otolaryngology- Head & Neck Surgery, LSU Health Sciences Center - Shreveport, Shreveport, LA

The mechanistic target of rapamycin (mTOR) is a protein kinase that plays a critical role in regulating immune responses by modulating the growth, differentiation, activation and migration of various immune cells. Dysregulation of mTOR signaling, which is common in various cancers, can potentially disrupt the immune cell function, leading to weakened immune surveillance. This study evaluates the antitumor activity of an mTOR inhibitor drug, everolimus, focusing on its impact on antitumor immunity. Treatment with everolimus inhibited the growth of PD-1 resistant and poorly immunogenic ROC-1 syngeneic grafts, potentially through the activation of T cell-mediated immunity. Flow cytometry analysis of single cell suspension of tumor cells revealed increased CD8+ T cell infiltration and increased expression of class-I MHC in tumor cells in mice treated with everolimus when compared to vehicle. Moreover, everolimus reduced the number of exhausted (PD1+) CD4+, CD8+ T cells in tumors, indicating a more potent immune response against the cancer cells. The CD8+ tumor infiltrated lymphocytes (TILs) magnetically sorted and cocultured with the tumor cells showed significantly higher T cell cytotoxicity for the group of mice who received everolimus in comparison to vehicle. These findings suggest the potential of everolimus in boosting anti-tumor immunity, providing a rationale for further investigation.

#4531 The effects of MRTX1133 on antitumor immunity in KRAS^{G12D}-mutant lung cancer.

K. Shono[1], S. Watanabe[1], T. Masuda[2], T. Wakabayashi[1], R. Yamazaki[1], Y. Ando[1], R. Suzuki[3], S. Tanaka[1], T. Tanaka[1], K. Nozaki[1], Y. Saida[1], N. Yanagimura[1], M. Arita[1], M. Sato[1], R. Ohashi[1], K. Shima[1], Y. Kimura[1], N. Aoki[1], Y. Ohshima[1], T. Koya[1], T. Kikuchi[1];
[1]Niigata Univ. Grad. School of Medical & Dental Sci., Niigata, Japan, [2]Niigata City General Hospital, Niigata, Japan, [3]Sado General Hospital, Niigata, Japan

Background: There is no effective treatment for KRAS^{G12D}-mutant tumors. Immune checkpoint inhibitors (ICIs) have improved survival rates for lung cancer patients, but they are less effective in non-small cell lung cancer (NSCLC) with KRAS^{G12D} mutations. MRTX1133 is a selective inhibitor of KRAS^{G12D} undergoing early clinical trials. This study aimed to investigate the immune landscape of a KRAS^{G12D}-mutant lung cancer mouse model and evaluate whether MRTX1133 could induce antitumor immunity. We also assessed whether MRTX1133 has anti-proliferative effects and can promote antitumor immunity in human KRAS^{G12D}-mutant lung cancer cell lines.
Methods: We generated conditional KRAS^{G12D}-mutant mice by crossing CCSP-rtTA/TetO-Cre and LSL-KRAS^{G12D} knock-in mice, inducing lung-specific tumors with doxycycline-containing food. MRTX1133 was administered intraperitoneally at 10 mg/kg once daily for 4, 8, or 12 days. After treatment, lungs and spleens were collected for flow cytometry analysis, and lung cells from the 4-day treatment group underwent RNA sequencing. Human KRAS^{G12D}-mutant lung cancer cell lines (A427 and SK-LU-1) were treated with MRTX1133 for RNA-Seq, differential gene expression analysis, and gene set enrichment analysis (GSEA). CCL20 protein levels were further validated by Western blotting and ELISA.
Results: Longer doxycycline exposure increased regulatory T cell (Treg) infiltration in lung tumors. Additionally, immune checkpoint molecules (ICMs) such as PD-L1, PD-1, TIGIT, TIM-3, and LAG-3 were upregulated in CD4+ and CD8+ T cells. MRTX1133 treatment inhibited KRAS^{G12D} tumor progression, reduced Treg percentages, and decreased PD-1 expression in CD4+ and CD8+ T cells. It also increased tumor-specific CD8+ effector T cell infiltration in lung tumors, and interferon-gamma was enriched in MRTX1133-treated lung cells. In human KRAS^{G12D}-mutant lung cancer cells, MRTX1133 increased immune-related gene expression and decreased cell division-related genes in both cell lines. GSEA showed enrichment of interferon-gamma and interferon-alpha in MRTX1133-treated SK-LU-1 cells, while DMSO-treated cells showed enrichment of cell division genes. Elevated CCL20 levels were confirmed in the SK-LU-1 MRTX1133-treated group.
Conclusion: KRAS^{G12D}-mutant lung tumors in mice exhibited T cell exhaustion. MRTX1133 showed therapeutic efficacy in the preclinical KRAS^{G12D}-mutant lung cancer model, possibly by enhancing antitumor immune responses along with oncogenic signaling inhibition. In human KRAS^{G12D}-mutant lung cancer cell lines, MRTX1133 reduced cell proliferation and increased antitumor cytokine expression, especially in SK-LU-1 cells. These findings may support the development of new immunotherapeutic strategies for patients with KRAS^{G12D}-mutant lung cancer.

#4532 Investigating the effects of radiation and anti-PD1 therapy on immune cell tumor infiltration in head and neck cancer.

Katelyn Jansen[1], Maria Lehn[1], Nicolas Nassar[2], Alexis Redmond[2], Mathieu Sertorio[3], Vinita Takiar[3], Dalia El-Gamal[1], Trisha Wise-Draper[1]

[1]Hematology and Oncology, University of Cincinnati College of Medicine, Cincinnati, OH,[2]Cincinnati Children's Medical Center, Cincinnati, OH,[3]Radiation Oncology, University of Cincinnati College of Medicine, Cincinnati, OH

Head and neck cancer (HNC) is predominantly comprised of head and neck squamous cell carcinoma (HNSCC), ranking as the 6th most common cancer worldwide. There are roughly 450,000 documented annual fatalities with predictions of annual increases in incidence. Despite advances in these intensive anticancer techniques, patient survival remains low. The current standard of care for local recurrence of HNSCC tumors is salvage surgery (SS) or combined modality with adjuvant re-irradiation using (X-ray)-based therapy (XRT). However, due to critical structure dose limits of the head and neck region, adjuvant XRT can cause excess morbidity. Proton radiation (PT), although less researched, may be advantageous to minimize the risk of injury to highly critical structures and minimize the radiation dosage outside of the tumor volume due to its energy course of the Bragg's Peak, which is thought to also have a higher relative biological effectiveness. While there may be high clinical relevancy of PT, there is currently a lack of translational and clinical data to confirm advantages of its use in HNSCC. Patients with HNSCC have impaired immune surveillance, and higher levels of inhibitory checkpoints such as PD-L1 permitting tumors to evade the immune system. Immunotherapy targeting PD-1, receptor to PD-L1, has greatly enhanced survival rates in these patients, but many patients fail to respond due to other immunosuppressive factors. Here we investigate the immune response effects of RT, with either XRT or PT combined with anti-PD1 antibody (αPD1) *in vivo* using an established murine oral cavity tumor model. Wild-type C57BL/6 mice were injected into the buccal region with 1.5 x 10^6 mouse oral cavity (MOC-1) cells. Once tumors reached 50-150mm^3, mice were treated over one week with XRT (3 treatments of 5 Gy) or sham. The following week the mice received αPD1 or IgG isotype control antibody. Here we present effects of XRT vs PT on immune infiltration by flow cytometry and immunofluorescence in three independent studies. Growth curves in the studies indicated that proton therapy and αPD1 in combination may be advantageous over αPD1 or PT alone with increased CD8 T cell infiltration, suggesting that PT in combination with αPD1 may offer significant advantages in eliciting a strong anti-tumor immune response.

#4533 Lymphotoxin-mediated tumor immunosuppressive regulation in ovarian cancer recurrence.

Zhewei Zhang[1], Kit Ying Chan[1], Ho Sze Jacqueline Lee[1], Joseph Kwong[2], Tat San Lau[1], Chi Chiu Wang[1]

[1]The Chinese University of Hong Kong, Shatin, Hong Kong,[2]Keele University, Keele, United Kingdom

Background: Recurrence is the major leading cause of death in patients with ovarian cancer. We previously report that inflammatory cytokine, lymphotoxin, derived from ovarian cancer cells, stimulates chemokine expressions in stromal fibroblasts via lymphotoxin-beta receptor, LTBR. These lymphotoxin-driven reciprocal tumor-stromal interactions in turn promote ovarian cancer progression via chemokine receptor CXCR3. However, the role of lymphotoxin in tumor immune microenvironment (TIME) and its contribution to recurrence remain poorly understood. This study aims to investigate the immunosuppressive regulation mediated by tumor-derived lymphotoxin in relation to ovarian cancer recurrence.

Methods: We determined the expression of a panel of immunosuppressive genes in a cohort of 202 primary ovarian cancer tissues, which included both high-risk (n=132) and low-risk (n=70) recurrence, using qPCR. Findings were validated by TCGA data analysis. Cell lines and syngeneic mouse models were used.

Results: Using multiplex immunofluorescence staining (mIF), we identified IFN-γ+ CD8+ T cell infiltration in ovarian tumors from patients following neoadjuvant chemotherapy with paclitaxel. While paclitaxel did not directly affect PD-L1 expression, IFN-γ significantly upregulated both PD-L1 gene expression and surface protein expression in most of ovarian cancer cell lines (14/17) as demonstrated by qPCR and flow cytometry. Primary tumors from high-risk recurrence patients showed elevated expression of IFN-γ signature genes (IFNG, STAT1, CCL5, CXCL9, CXCL10, HLA-DRA, CD8A, LAG3) and lymphotoxin-associated genes (LTA, LTB, CCL5, CXCL10, CXCL11). In our mouse models, lymphotoxin (Lta/Ltb) overexpression activated the NF-κB pathway and significantly reduced survival (p=0.0018). In situ immune profiling and mIF revealed that lymphotoxin overexpression specifically enriched regulatory B cells and LAG3+CD3+CD8+ exhausted T cells in the tumor microenvironment. Analysis of TCGA data confirmed that patients with high lymphotoxin expression showed significantly elevated levels of IFN-γ signature genes (IFNG, STAT1, CXCL9), lymphotoxin-stimulated chemokines (CCL5, CXCL10, CXCL11), and immunosuppressive cell markers (CD244, LAG3, FOXP3, TGFB1, EOMES, GZMB, ICOS, IL10, CCL2). These findings were further validated at the protein level using mIF in patient samples, which showed increased LAG3+CD3+CD8+ T cells and CD20+ B cells in recurrent tumors.

Conclusions: Our findings reveal that an immunosuppressive tumor microenvironment contributed to primary tumors from ovarian cancer patients with high recurrence risk. A distinct set of immunosuppressive regulators orchestrated by cancer-derived lymphotoxin through recruitment of B-regs and exhausted T cells suggests new therapeutic strategies targeting ovarian cancer recurrence.

#4535 Targeting YAP1 and DKK1 pathways to suppress peritoneal carcinomatosis in gastric adenocarcinoma.

Y. Fan[1], S. Song[2], G. Zou[1], L. Huo[1], A. Scott[1], R. Wang[1], E. Dai[1], S. Shao[1], L. Wang[1], S. Dhar[1], J. Ajani[1];
[1]UT MD Anderson Cancer Center, Houston, TX, [2]Coriell Institute for Medical Research, Camden, NJ

Peritoneal carcinomatosis (PC), characterized by malignant ascites cells or implants, affects approximately 45% of advanced gastric adenocarcinoma (GAC) patients and is associated with a poor prognosis, with survival rates often less than six months. Despite its prevalence and severe impact, the molecular mechanisms underlying PC in GAC remain poorly understood, highlighting a significant unmet need for targeted therapies. Our previous studies demonstrated that YAP is highly expressed in PC cells, promoting cancer stem cell (CSC)-like properties and correlating with decreased patient survival. Here, we observed that high YAP1 expression is inversely correlated with $CD8^+$ T cell infiltration in GAC. RNA sequencing analysis revealed a significant reduction in *DKK1* expression upon *YAP1* depletion, and both YAP and DKK1 were highly expressed in PC cells. High expression of these markers is significantly associated with poor survival outcomes in GAC patients. Further experiments showed that ectopic DKK1 expression in YAP1 knockout cells restored cell proliferation, tumor sphere formation, cell invasion, and impaired $CD8^+$ T cell function. Dual inhibition of YAP and DKK1 markedly reduced tumor growth and enhanced $CD8^+$ T cell responses within the TME. Combination therapy with YAP antisense oligonucleotides (ASO), DKN1 antibody, and anti-PD-1 further extended overall survival and bolstered immune response. Our findings reveal a critical role of YAP1 in maintaining an immunosuppressive microenvironment through DKK1 in GAC with PC. This combined inhibition approach holds promise and warrants further investigation as a therapeutic strategy for GAC patients with PC.

Key words: YAP1, DKK1, Immune response, Peritoneal metastasis, Gastric adenocarcinoma

#4536 Dissecting tumor-immune microenvironment in response and resistance to immune checkpoint blockade in metastatic melanoma.

Xinyu Cui[1], Giuseppe Tarantino[2], Yiwen He[3], Priyanka Solanky[1], Kathleen Pfaff[2], Aaron R. Thorner[2], Tyler J. Aprati[2], Boyang Zhang[4], Timothy Blosser[1], Emily Robitschek[4], Jiajia Chen[2], Junko Tsuji[5], Elliot Boblitt[5], Allison Frangieh[2], Hannah M. Faulkner[2], Marta Holovatska[2], Aleigha Lawless[6], Michael Manos[2], Karla Helvie[2], Tatyana Sharova[7], Dennie Frederick[8], James L. Fahey[9], Diego Villamarin[10], Sachi Krishna[5], Chanell Mangum[5], Ajit J. Nirmal[1], Domenic Abbondanza[11], Cai McCann[5], Bruce Johnson[2], Alex K. Shalek[12], Eliezer Van Allen[2], Xiaowei Zhuang[1], Ryan Sullivan[6], Barbara E. Engelhardt[4], Samouil L. Farhi[5], Scott Rodig[13], F. Stephen Hodi[2], Genevieve M. Boland[6], David Liu[2]

[1]Harvard Medical School, Boston, MA,[2]Dana Farber Cancer Institute, Boston, MA,[3]Northwestern University, Chicago, IL,[4]Stanford University, Stanford, CA,[5]Broad Institute of MIT and Harvard, Cambridge, MA,[6]Massachusetts General Hospital, Boston, MA,[7]Massachesetts General Hospital, Boston, MA,[8]Broad Institute of MIT and Harvard, Boston, MA,[9]Loyola University Chicago, Chicago, IL,[10]University of Rochester School of Medicine and Dentistry, Rochester, NY,[11]University of Chicago, Chicago, IL,[12]Massachusetts Institute of Technology, Cambridge, MA,[13]Brigham and Women's Hospital, Boston, MA

Background: Immune checkpoint inhibitor (ICI) therapies have markedly improved the prognosis for patients with stage III & IV metastatic melanoma by prolonging progression-free and overall survival rates. However, the variability in immune evasion and resistance mechanisms presents significant challenges to the clinical efficacy of ICIs. This project aims to define drivers of immunotherapy response and resistance by employing advanced genomic, single-cell mRNA analyses, and spatial profiling techniques on tissue biopsies from metastatic melanoma patients.

Methods: In this study, we developed a framework to analyze response and resistance, both intrinsic and acquired, via immune features in the tumor microenvironment in a standardized, uniformly processed, and deeply clinically annotated cohort of metastatic melanoma patients (n=61) treated with ICB as part of the human tumor atlas network (HTAN) initiative. From the tumor samples, we conducted single-nucleus RNA sequencing, and for a subset of the samples high-resolution spatial imaging (including protein mIHC, CODEX, and transcriptomics MERFISH). Standardized processing and data pipelines allowed for integrating genomic, transcriptomic, and spatial features to elucidate characteristics and mechanisms in tumor microenvironment and their relationships with resistance.

Results: Studies of the pretreatment samples demonstrated that CD4 and CD8 T cells, particularly TCF7+ CD8 T cells, are significantly more prevalent in responders to immunotherapy. Conversely, macrophages, especially Angio-TAMs, show higher levels of enrichment in non-responders. Moreover, the presence of B cells and follicular dendritic cells in non-lymph node biopsies supports the presence of tertiary lymphoid structures within the TME. Three levels of immune enrichment were identified through spatial analysis, and samples with more immune enrichment tend to have better responses. We also identified 10 recurrent cellular neighborhoods (RCNs) and found that RCN2,4, and 7 with high lymphocyte infiltration are significantly more enriched in responders than non-responders. In addition, RCN4 is associated with immune infiltration while RCN7 is TLS-like.

Conclusions: Our findings indicate that patients with low immune infiltration exhibited enrichment of macrophages associated with the hypoxia and angiogenesis phenotypes, while patients with high immune infiltrates displayed enrichment in lymphocytes, particularly TCF7+ CD8+ T cells, confirming previous findings and indicating a robust T cell-mediated immune response. This project integrates genomic, transcriptomic, and spatial features to elucidate shared tumor and microenvironmental states and their relationships with resistance, and guide more personalized and effective treatment strategies for metastatic melanoma.

#4537 Dynamics of B-cell repertoires following CAR-T and bispecific antibody therapy in multiple myeloma.

F. Jacob, **R. Garza-Morales**, J. Hentz, A. Wonnaparhown, J. Wiedmeier-Nutor, M. Arribas, G. Alatorre, S. Best, J. Poulignot, U. Yadav, S. Chhabra, P. Bergsagel, R. Fonseca, T. Hilal;
Mayo Clinic Arizona, Phoenix, AZ

Introduction: Multiple myeloma (MM) is a B-cell-derived malignancy associated with immune dysregulation. Anti-CD38 monoclonal antibodies (mAbs) therapies have shown improved outcomes in patients with MM. Silbermann *et al.* showed that anti-CD38 mAb-containing therapies lead to profound hypogammaglobulinemia and reduction in Ig gene repertoire. The effect of modern therapies such as CAR-T and bispecific antibodies (BsAbs) on B-cell repertoire has not been well described. In this study, we sought to investigate the effect of these therapies on B-cell repertoire in patients with MM.

Methods: This is a single-institution retrospective observational study evaluating MM patients treated with anti-BCMA CAR-T and BsAbs. Baseline characteristics, laboratory data, treatment history, were extracted from medical records. Total unique sequences (TUS) and total sequences of IgH, IgK, and IgL were identified and quantified using clonoSEQ. Patients were classified in two groups: CAR-T and BsAbs. The primary objectives were (1) characterize changes in TUS and total sequences within IgH, IgK, and IgL before and after treatment, and (2) examine how the sequences evolve over time following CAR-T.

Results: 76 MM patients received CAR-T or BsAbs. Among those, 22 CAR-T and 5 BsAbs patients had pre- and post-treatment sequences available. Median age at initiation of treatment was 66 years in the CAR-T group (42-81 y) and 65 years in the BsAbs group (48-71 y). Based on mSMART v3 criteria, 72% of CAR-T and 60% of BsAbs recipients were classified as high-risk. Median number of prior lines of therapy was 5 (2-8) in the CAR-T group and 6 (5-13) in the BsAbs group.CAR-T recipients were classified into early (<40 days) and late (>40 days), based on when the post-CAR-T treatment clonoSEQ assay was performed. Changes in TUS and total sequences of IgH, IgK, and IgL were calculated, and the averages were assessed independently. The mean early and late change in total unique IgH sequences were (-75,586 and 131,038, p=0.052), unique IgK (-57,797 and 52,586, p=0.020), and unique IgL (-22,354 and 25,056, p=0.011). A subgroup of 8 patients with sequential MRD reports post-CAR-T therapy were identified, six patients showed improvement in B-cell sequence dynamics and two patients showed a decrease over time. For patients who received BsAbs, results indicated a similar pattern, with an early decrease in B-cell repertoires, characterized by reductions in sequence diversity and quantity.

Conclusions: Our analysis of B-cell repertoires following CAR-T revealed changes before and after treatment. Patients receiving CAR-T therapy showed a decline in the number of TUS and total sequences of IgH, IgK, and IgL, likely due to the lymphodepletion regimen, followed by a gradual restoration reflecting immune recovery. Longer follow-up and a larger cohort will be necessary to assess the implications of these B-cell repertoire changes on patient outcomes.

#4538 T-cell immunogenicity and biomarker profiling in advanced melanoma patients receiving the personalized vaccine EVX-01 in combination with pembrolizumab.

Mads Lausen[1], Michail A. Pavlidis[1], Rasmus Ohrt Andersen[1], Thomas Trolle[1], Nikolas Thuesen[1], Christian Garde[1], Nicholas Willumsen[2], Morten Asser Karsdal[2], Nadia Viborg[1], Adnan Khattak[3], Georgina V. Long[4], Paolo A. Ascierto[5], Carolina Cimminiello[6], Michael J. Chisamore[7], Stine Friis Thorsen[1], Birgitte Rono[1], Daniela Kleine-Kohlbrecher[1]

[1]Evaxion Biotech A/S, Hoersholm, Denmark,[2]Nordic Bioscience A/S, Herlev, Denmark,[3]Hollywood Private Hospital, One Clinical Research, Nedlands, WA, Australia,[4]The University of Sydney, Royal North Shore & Mater Hospitals, Melanoma Institute Australia, Sydney, Australia,[5]Melanoma Cancer Immunotherapy and Development Therapeutics Unit, Instituto Nazionale Tumori IRCCS Fondazione "G. Pascale", Napoli, Italy,[6]Sarcoma e Tumori Rari, Divisione di Oncologia Medica del Melanoma, Milano, Italy,[7]Oncology Early Clinical Development, Merck & Co., Inc., New Jersey, NJ

Patients with advanced melanoma were treated with a personalized neoantigen vaccine, EVX-01, in combination with pembrolizumab in a phase 2 clinical study (NCT05309421). The peptide-based EVX-01 vaccine consists of multiple tumor-specific neoantigens (neoAg) identified by Evaxion Biotech's target discovery AI-Immunology™ platform. As reported in September 2024 at the one-year follow-up, the treatment was well tolerated, neoAg specific T-cell responses were observed, and the objective response rate was 11/16 (68.8%) in the overall cohort.To investigate potential markers associated with the immunogenic and therapeutic effect, several biomarker analysis technologies were applied. Baseline tumor biopsies as well as longitudinal blood samples before, during and after treatment were collected. Vaccine-specific T-cell responses were analyzed by ELISpot of peripheral blood mononuclear cells (PBMCs). Treatment-induced changes of circulating immune cell subsets were characterized using spectral flow cytometry.Baseline tumor samples were analyzed by genomic and transcriptomic analyses, immunohistochemistry (IHC), and AI-assisted digital pathology to understand the characteristics of the tumor and the immunological landscape of the tumor microenvironment (TME).Longitudinal plasma and serum samples were assessed for treatment-induced changes of circulating markers using the Olink proteomics technology and Nordic Bioscience's ProteinFingerPrint Technology™.
Immunogenicity analysis demonstrated vaccine-specific T-cells in all patients after EVX-01 priming (n=15) and repeated booster immunizations lead to sustained magnitude and broadness of T-cell responses. Overall, the analysis showed that 78% of the AI-Immunology™ selected neoAgs were immunogenic and 93% of the immunogenic peptides induced *de novo* responses.Quantification of functionally diverse immune cell subsets in patient PBMCs will further increase our understanding of the immunological changes induced by EVX-01. Immunohistochemistry analysis of baseline tumor biopsies showed PD-L1 expression and T-cell infiltration in 66% and 75% of tumor specimens, respectively. Additional analyses are being conducted to evaluate relevant features of TME, such as fibrosis and spatial distribution of different cell types in the TME.
Olink proteomic analysis and ECM turnover marker analysis of serum samples will provide further insights into tumor- and immune-related interactions during EVX-01 treatment. The immune and biomarker analyses enhance our understanding of the complex interactions between the immune and tumor compartments, while also shedding light on treatment induced adaptations. This new understanding can aid in refining therapeutic strategies and identifying predictive biomarkers that may guide treatment decisions in the future.

#4539 A new and standardized method to isolate viable, high-quality PBMCs from patient blood samples.

Eleonora Naimo, **Lotta Raty**, Stefanie Niehues, Migena Kodra, Svetlana Khorkova, Konstantina Kostadinovska, Gregor Winkels

Miltenyi Biotec, Bergisch Gladbach, Germany

With the rise of immunotherapy, monitoring patient immune responses during clinical trials has become increasingly critical. Peripheral blood mononuclear cells (PBMCs) serve as a reliable analyte for various cellular analyses, including flow cytometry, ELISpot, and transcriptomics. However, acquiring high-quality PBMCs is traditionally challenging due to long shipment times that compromise cell integrity. Reports indicate that a significant number of patient samples are rendered unsuitable for downstream applications due to these delays. Additionally, traditional gradient centrifugation methods are manual, time-consuming, and require skilled personnel. In this study, we present a novel, standardized, and automation-friendly method for generating PBMCs from whole blood samples. This innovative approach consistently yields high-quality, viable PBMCs, even from older samples, overcoming the limitations of traditional methods. Our method minimizes the need for extensive hands-on training and produces cells ready for immediate use in downstream applications. By streamlining the PBMC isolation process, our method enhances the reliability and efficiency of immune monitoring and biomarker discovery in clinical and research settings. This advancement holds significant potential for improving the quality and consistency of PBMC samples, thereby facilitating more accurate and reproducible immunological analyses.

#4540 Longitudinal analysis of multiple myeloma: Therapeutic response and immune microenvironment dynamics in the context of tumor heterogeneity.

Aishwarya Chander[1], Imran McGrath[1], Love Tatting[2], Yudong D. He[1], Emma L. Kuan[1], Marla C. Glass[1], Samir Rachid Zaim[1], Palak C. Genge[1], Medbh A. Dillon[1], Cole G. Phalen[1], Lauren Y. Okada[1], Zachary J. Thomson[1], Christian M. La France[1], Tao Peng[1], Peter J. Wittig[1], Alexander T. Heubeck[1], Julian Reading[1], Charles R. Roll[1], Veronica Hernandez[1], Vaishnavi Parthasarathy[1], Tyanna J. Stuckey[1], Blessing Musgrove[1], Elliott Swanson[3], Cara M. Lord[1], Morgan D. A. Weiss[1], Regina R. Mettey[1], Kevin J. Lee[1], John B. Johanneson[1], Erin K. Kawelo[1], Jessica Garber[1], Upaasana Krishnan[1], Xiaoling Song[4], Phillip D. Greenberg[5], Lynne A. Becker[1], Paul Meijer[1], Ernie M. Coffey[1], Thomas F. Bumol[1], Ananda W. Goldrath[1], Mackenzie S. Kopp[1], Evan W. Newell[4], Peter J. Skene[1], Damian J. Green[6], Troy R. Torgerson[1], Mary Kwok[7], Mikael Sigvardsson[8], Xiao-jun Li[1], Lucas T. Graybuck[1], **Melinda L. Angus-Hill**[1]

[1]Allen Institute for Immunology, Seattle, WA,[2]Department of Hematology in Linkoping, and Department of Biomedical and Clinical Sciences, Linkoping University, Linkoping, Sweden,[3]University of Washington, Seattle, WA,[4]Immunotherapy Integrated Research Center, Fred Hutchinson Cancer Center, Seattle, WA,[5]Immunotherapy Integrated Research Center, Immunology, Medicine,, Fred Hutchinson Cancer Center, University of Washington, Seattle, WA,[6]Translational Science and Therapeutics, Fred Hutchinson Cancer Center, Seattle, WA,[7]Clinical Research Division, Fred Hutchinson Cancer Center, Seattle, WA,[8]BKV, Linkoping University, Linkoping, Sweden

Multiple myeloma (MM) is a hematological malignancy characterized by an expansion of malignant plasma cells in the bone marrow. For newly diagnosed MM (NDMM), standard three-drug treatment regimens—such as the combination of bortezomib, lenalidomide, and dexamethasone (VRd)—and the more recent inclusion of a fourth drug, anti-CD38 antibody immunotherapy, have significantly improved patient outcomes by targeting multiple mechanisms of tumorigenesis. This treatment approach typically involves several cycles of VRd induction therapy, followed by high dose melphalan (HDM) and autologous stem cell transplant (ASCT), and maintenance therapy. Treatment responses vary widely, with some patients requiring longer and more intense therapy and others exhibiting more rapid responses and longer relapse-free remission. Relapse or treatment refractory multiple myeloma (RRMM) is driven by clonal evolution of malignant cells and tumor-supportive changes in the stromal and immune microenvironment. This cycle of remission, maintenance, and eventual relapse persists despite advancements in treatment modalities, including immunotherapy, leaving MM incurable. This study characterizes the tumor state and immune microenvironment in MM patients across variable graded patient responses. Using a multimodal, longitudinal approach, we investigate immune and microenvironmental cellular changes during treatment in NDMM patients and in a second cohort of RRMM patients. For the NDMM cohort, longitudinal blood and bone marrow aspirate samples were collected at diagnosis, throughout induction therapy, following ASCT, and at post-transplant intervals of 1 and 2 years. In the RRMM cohort, longitudinal blood and bone marrow samples were collected from patients who relapsed and were subsequently treated with anti-CD38 antibody in combination with a personalized three-drug regimen over a 2-year timeframe with regular collection of blood and bone marrow aspirate samples across timepoints. Multi-omic profiling of these samples magnifies the individual heterogeneity between tumor and therapeutic response. The longitudinal aspect of the study allowed us to focus on individual-level longitudinal responses instead of cross-patient effects, enabling detailed insights into tumor and microenvironment dynamics. Using Olink proteomic analysis of plasma and bone marrow interstitial fluid (BMIF), and single-cell analyses of peripheral blood mononuclear cells (PBMC) and bone marrow mononuclear cells (BMMC) we longitudinally characterized tumor, the immune microenvironment, and therapeutic responses. This revealed distinct immune states and pathways associated with therapeutic response, tumor survival, and relapse, highlighting potential targets and therapeutic strategies to improve patient outcomes.

#4542 B cells mediate antitumor immune response and predict pathological response in locally advanced NSCLC patients treated with perioperative chemoimmunotherapy (NADIM trials).

Belen Sierra-Rodero[1], Cristina Martinez-Toledo[1], Marta Molina-Alejandre[1], Angeles Gil-Gonzalez[1], Ernest Nadal[2], Alex Martinez-Marti[3], Bartomeu Massuti[4], Amelia Insa[5], Joaquin Casal[6], Javier de Castro[7], Rosario Garcia Campelo[8], Jose Luis Gonzalez-Larriba[9], Reyes Bernabe[10], Santiago Ponce[11], Manuel Domine[12], Noemi Reguart[13], Carlos Camps[14], Guillermo Lopez Vivanco[15], Manuel Cobo[16], Virginia Calvo[1], Ana Collazo-Lorduy[1], Mariano Provencio[1], Alberto Cruz-Bermudez[1]

[1]Medical Oncology, Instituto de Investigacion Sanitaria Puerta de Hierro-Segovia de Arana (IDIPHISA), Hospital Univ. Puerta de Hierro Majadahonda, Madrid, Spain,[2]Institut Catala d'Oncologia (ICO), IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain,[3]Vall Hebron Institute of Oncology (VHIO), Hospital Universitari Vall d'Hebron, Barcelona, Spain,[4]Hospital General Dr. Balmis de Alicante, ISABIAL, Alicante, Spain,[5]Fundacion INCLIVA, Hospital Clinico Universitario de Valencia, Valencia, Spain,[6]Hospital Universitario de Vigo, Pontevedra, Spain,[7]Hospital Universitario La Paz, Madrid, Spain,[8]Hospital Universitario A Coruna, A Coruna, Spain,[9]Hospital Universitario Clinico San Carlos, Madrid, Spain,[10]Hospital Universitario Virgen del Rocio, Sevilla, Spain,[11]Hospital Universitario 12 de Octubre, Madrid, Spain,[12]Hospital Universitario Fundacion Jimenez Diaz, Madrid, Spain,[13]Hospital Clinic and Translational Genomics and Targeted Therapies in Solid Tumors, Institut de Investigacions Biomediques, Barcelona, Spain,[14]Hospital General Universitario de Valencia, Universidad de Valencia and Centro de Investigacion Biomedica en Red Cancer, Valencia, Spain,[15]Hospital Universitario Cruces, Barakaldo, Spain,[16]Regional and Virgen de la Victoria University Hospitals, Instituto de Investigacion Biomedica de Malaga, Malaga, Spain

Perioperative chemoimmunotherapy (ChIO) has become a paradigm shift in the treatment of patients with locally advanced non-small cell lung cancer (NSCLC), considerably increasing complete pathological response (CPR) and survival rates. The role played by B lymphocytes in the antitumor response emerges as a crucial factor in the fight against cancer, although mechanisms remain to be elucidated. Therefore, the main objective of this study is the identification of B lymphocyte-related mechanisms and biomarkers of response to perioperative chemoimmunotherapy treatment in patients with locally advanced NSCLC. For this purpose, tumor tissue B-cell receptor (BCR) repertoire, gene expression profile by bulk RNA sequencing, and qualitative localization by spatial transcriptomics have been characterized in 110 NSCLC patients receiving ChIO from NADIM and NADIM II trials (NCT03081689 and NCT03838159). Results of this work show an association between CPR and neoadjuvant treatment with a more diverse (p=0.069) and less even (p=0.030) BCR repertoire in pre-treatment tissue, which appears to be dominated by a set of more frequent clones (top 10, p=0.043). In addition, neoadjuvant chemoimmunotherapy induced overexpression of B lymphocyte-related genes in surgical tissue, mainly localized at tertiary lymphoid structures (TLSs) identified by spatial transcriptomics. Those tumors with low BCR repertoire evenness (CPR response) exhibited a different gene expression profile compared to highly even tumors (non-CPR). TLSs area and density were higher in CPR/low evenness sample compared to NCPR/high evenness; and functional T helper lymphocytes, defined as T CD4 lymphocytes colocalized with antigen presentation genes, were detected only in CPR/low evenness sample. In conclusion, tumor BCR repertoire clonality is associated to CPR after neoadjuvant ChIO. Neoadjuvant ChIO induces expression of B cell related genes in the context of TLS, being their area and functionality dependent on baseline BCR repertoire clonality.

#4543 Low dose radiation enhanced antitumor immune response by reprogramming T cell and CAR-T cell metabolism in a tumor immune microenvironment.

Zahid Rafiq[1], Puebla Nahum Osorio[1], Weiqin Lu[2], James Welsh[1]

[1]Radiation Oncology, UT MD Anderson Cancer Center, Houston, TX,[2]Department of Pharmaceutical Sciences, School of Pharmacy, The University of Texas at El Paso, El Paso, TX

Background: The infiltration of T cells and chimeric antigen receptor T (CAR-T) cells into solid tumors is essential for robust antitumor response and therapeutic outcomes. While cell therapies succeeded in hematologic malignancies, their efficacy in solid tumors is limited due to poor tumor penetration and the immunosuppressive tumor immune microenvironment (TIME). Our previous studies showed that combining low-dose radiotherapy (LDRT) with T-cell therapy synergistically enhanced tumor control and survival. Nanostring metabolic gene analysis indicated that LDRT modulates T-cell metabolism within TIME. Therefore, we hypothesized that LDRT enhances T-cell metabolism, favoring anticancer potency.

Methods & Results: To investigate the role of LDRT in T-cell metabolism, we established a TIME using co-culture of cancer cells and PBMCs in xenograft NSG mice, followed by LDRT. Using nCounter Metabolic Pathways, we quantified the expression of genes associated with core metabolic and immune metabolic processes in intratumoral T cells. Nanostring analysis revealed key pathways and genes that regulate T-cell effector functions, further underscoring LDRT's impact on T-cell metabolism. Seahorse assays highlighted that LDRT positively influenced the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR), favoring improved metabolic and effector functions in T/ CAR-T cells. Mitotracker analysis revealed that LDRT-treated T/ CAR-T cells exhibited enhanced mitochondrial membrane potential (Mitotracker Deep Red) and mitochondrial mass (Mitotracker Green), suggesting robust mitochondrial health and functionality. Transmission electron microscopy (TEM) showed improved mitochondrial morphology of T cells following LDRT. Additionally, Incucyte live-cell analysis demonstrated that CAR-T cells treated with LDRT exhibited significantly enhanced cancer cell killing ($P \leq 0.001$) compared to controls. CAR-T cells conditioned with LDRT showed increased tumor cell killing in vivo, further validating the potentiating effect of LDRT on cell-based therapies. These findings collectively highlight the pivotal role of LDRT in modulating T-cell metabolism and enhancing antitumor immune responses.

Conclusion: The study highlights the translational potential of combining LDRT with cell therapies in solid tumors. By reprogramming T-cell metabolism within TIME, LDRT significantly enhanced tumor control and improved survival outcomes, establishing a promising foundation for metabolism-targeted therapies. These findings provide compelling evidence to support further exploration and clinical investigation of this synergistic approach, aiming to optimize the efficacy of cell-based immunotherapies and improve outcomes for patients with challenging solid tumor malignancies.

#4544 Immunotherapy with chemotherapy provides superb pathologic complete response rate in chemotherapy-resistant metaplastic carcinoma of breast.

I-Chun Chen[1], Shang-Hsuan Peng[2], Tsung-Che Wu[3], Ching-Hung Lin[4], Diana Hui-Min Lin[5], Li-Wen Huang[6], Chi-Han Cheng[7], Yen-Shen Lu[8]

[1]National Taiwan University Cancer Center (NTUCC), Taipei, Taiwan,[2]Department of Oncology, National Taiwan University Hospital Yunlin Branch, Huwei, Taiwan,[3]Department of Oncology,, National Taiwan University Biomedical Park Hospital, Hsinchu, Taiwan,[4]Department of Medical Oncology, National Taiwan University Cancer Center (NTUCC), Taipei, Taiwan,[5]Medical Solution Cluster, Roche Products Ltd., Taipei, Taiwan,[6]7. Medical Solution Cluster, Roche Products Ltd., Taipei, Taiwan,[7]University of Minnesota, Minneapolis, MN,[8]National Taiwan University Hospital, Taipei, Taiwan

Background: Metaplastic carcinoma of breast (MCB) is a rare subtype of breast cancer (BC) accounting for <1% of newly diagnosed BC. It is composed of co-existing sarcomatous and carcinomatous components. Because of its sarcomatous components, the response rate of chemotherapy in MCB is low both in early BC and advanced BC. The pathologic complete response rate (pCR%) after neoadjuvant chemotherapy in MCB is as low as 10%. Neoadjuvant immunotherapy plus chemotherapy is a new standard of care for early triple-negative BC providing improved pCR%, event-free survival, and overall survival. Pembrolizumab was also approved by the FDA in 2021 for neoadjuvant treatment in TNBC. We aim to examine the modern pCR% of immunotherapy and chemotherapy in MCB patients.
Methods: MCB patients and treatment regimens, including neoadjuvant immunotherapy, were retrospectively selected from Flatiron Health, a nationwide electronic health record database. Demographic data and neoadjuvant regimens were collected. pCR% was calculated for patients treated with neoadjuvant chemotherapy versus neoadjuvant immunotherapy plus chemotherapy.
Results: A total of 87 early stage MCB patients diagnosed between January. 2011 and May 2024 were included from the Flatiron Health. 20 and 5 of the MCB patients received neoadjuvant chemotherapy alone and neoadjuvant immunotherapy plus chemotherapy, respectively. 2 in the neoadjuvant chemotherapy group (pCR%=10%) and 3 in the neoadjuvant immunotherapy/ chemotherapy group (pCR%=60%) achieved pCR. The pCR% of neoadjuvant chemotherapy is as low as previously reported in MCB patients.
Conclusion: Neoadjuvant immunotherapy and chemotherapy provide comparably high pCR% in MCB patients as compared to other TNBC patients. We are validating the findings from additional cohorts from Taiwan. The high pCR% makes immunotherapy and chemotherapy an attractive strategy that worth further investigation in MCB patients.

CLINICAL RESEARCH: Liquid Biopsy: Circulating Nucleic Acids 2
Poster Session

#4548 The association between tissue genomic alterations and ctDNA shedding in non small cell lung cancer.

Robert Cameron[1], Anna Di Lello[1], Faith Abodunrin[1], Sulin Wu[1], Alessandra Esposito[1], Apameh Pezeshk[1], Valter Torri[2], Christine Bestvina[1], Marina Garassino[1]

[1]UChicago Medicine, Chicago, IL,[2]Institut Mario Negri, Milan, Italy

Introduction: Tumor burden, stage and metastatic extent are associated with the presence of circulating tumor DNA (ctDNA). However, the association between genomic alterations and the presence of ctDNA remains unclear.
Methods: We retrospectively analyzed patients with non-small cell lung cancer (NSCLC) with available Next-Generation Sequencing (NGS) analysis on both tissue and blood treated at the University of Chicago. NGS on tissue biopsies was conducted using the internal Oncoplus assay (168 genes). NGS on blood samples was conducted using the Guardant360 assay. Plasma ctDNA concentration was measured by the Variant Allele Frequency (VAF). Spearman rank correlation coefficient was used to assess the relationship between increasing number of mutations and VAF. Wilcoxon rank-sum test was used to compare the distribution of VAF between subgroups.
Results: A total of 221 patients were identified. Adenocarcinoma was the predominant histological subtype, accounting for 89.6% (n=199) of cases. The cohort was predominantly Caucasian (55.4%) and female (66.2%). The median age at diagnosis was 65 years. At the time of index liquid biopsy, 85.1% of patients had metastatic disease. Among the 221 patients, 129 (58.6%) harbored actionable mutations, with EGFR exon 19 or 21 mutations being the most prevalent (n=80, 36.2%). This study found a weak but significant association between VAF and mutation count in the tissue biopsy: for total mutations including both significant alterations and variants of unknown significance ($r = 0.146$, $p=0.032$) and for significant mutations only ($r = 0.160$, $p=0.018$). These associations were stronger in the KRAS G12C mutated sub-cohort for both total ($r=0.471$, $p=0.036$) and significant mutations ($r=0.573$, $p=0.008$). Significantly higher ctDNA VAF was observed in TP53 ($p=0.006$) and STK11 ($p=0.05$) mutant tumors.
Conclusions: Our results showed a correlation between increasing number of alterations and ctDNA shedding. The association was stronger for KRAS G12C mutated tumors. The relationship between genetic changes and ctDNA release is supported by the increased ctDNA VAF seen in TP53 and STK11 mutant tumors.

#4549 Impact of an ultrasensitive ctDNA test on MRD detection in real world clinical patient testing.

Rachel Marty Pyke[1], Steven Dea[1], Fabio C.P. Navarro[1], Charles W. Abbott[1], Sherif El-Refai[1], Yi Chen[1], Janet Lai[1], Gabor Bartha[1], John M. Lyle[1], Jason Harris[1], Georges Azzi[2], Sean M. Boyle[1], Richard O. Chen[1]

[1]Personalis, Inc., Fremont, CA,[2]Holy Cross Medical Group, Fort Lauderdale, FL

Circulating tumor DNA (ctDNA) is a non-invasive biomarker increasingly used to detect residual or recurrent disease and to monitor patient response to therapy. However, there is a need for more sensitive tests that can achieve earlier detection of disease. The NeXT Personal® Dx (NPDx) ctDNA MRD test uses paired tumor and normal whole genome sequencing (WGS) to design a bespoke panel of up to ~1800 variants and relies on advanced noise suppression to achieve ultrasensitive detection down to ~1 part per million (PPM) of ctDNA (1x10-6 tumor fraction) with high analytical specificity (>99.9%). Here, we describe the real world performance for a set of cancer patients (>15 cancer types) profiled using the NPDx test. For this study we have included 1693 plasma timepoints from 700 patients (range of 1 to 21 timepoints per patient), with the highest proportion of patients having breast, lung or colorectal cancer. The plasma timepoints have a median LOD of 1.93 PPM (IQR:1.55-2.78 PPM). Notably 41.6% of MRD+ samples were detected at low traces of ctDNA below 100 PPM, in the ultrasensitive range; moreover, a majority (50.4%) of the ultrasensitive detections are at or below 15 PPM, further highlighting the impact of a highly sensitive assay for MRD profiling.

We also examined performance in specific challenging situations. NPDx tests performed well with challenging FFPE tumor samples (low tumor DIN <=3 indicating some sample degradation or low tumor fraction <=30%), achieving low LODs (median = 2.97 and 2.56 PPM, respectively). The WGS-based panel design allowed NPDx to achieve ultrasensitivity on low TMB tumors (<=2 mutation/MB, median LOD = 2.90 PPM) through utilization of variants from intergenic regions (95% MRD panel variants were from non-exonic regions) that would largely be unavailable to exome-based tests. Testing was performed across a wide range of cell free DNA (cfDNA) inputs (range 2-30 ng, median = 14 ng). Even with low cfDNA inputs (<=3ng, 5.25% of cohort), NPDx still achieved a low median LOD of 2.63 PPM.

Two case studies demonstrate the impact of ultrasensitive MRD testing with patients. A 50-year-old woman with stage 1B endometrial cancer was monitored using NPDx following hysterectomy and brachytherapy, and ctDNA was detected in the ultrasensitive range (<50 PPM in this case). Reflex imaging revealed a new nodule leading to additional therapy and ctDNA clearance. In a second case, a woman in her 70s with stage 1 HER2+ breast cancer who underwent standard lumpectomy and adjuvant treatment was found with persistent low levels of ctDNA (between 25-30 PPM) detected with NPDx over a period of five months. Reflex imaging revealed a new nodule leading to additional radiation therapy, with plans for chemotherapy. These cases illustrate how NPDx's ultrasensitive detection capabilities can impact patient management in a real world setting.

#4550 Dried blood sot sampling as a minimally invasive and accessible method for investigating circulating tumor DNA in diverse cancer settings.

Amit Roshan[1], Fazlur R. Talukdar[1], Angela An[1], Emma-Jane Ditter[1], Ze Zhou[1], Paulius D. Mennea[1], Linfang Wu[1], Maria C. Neofytou[1], Anita Balakrishnan[2], Claire M. Connell[1], Gabriel Funingana[1], Giovanni Codacci-Pisanelli[2], Girma Mulisa[3], Tamrat Abebe[4], Constanza Linossi[5], Hui Zhao[1], Wendy N. Cooper[1], Huiqi Yang[2], Rajesh Jena[5], James D. Brenton[5], Pippa Corrie[2], Rebecca C. Fitzgerald[5], Simon Pacey[5], Richard Baird[5], Nitzan Rosenfeld[6]

[1]CRUK Cambridge Institute, Cambridge, United Kingdom,[2]Cambridge University Hospitals NHS Trust, Cambridge, United Kingdom,[3]Adama Hospital Medical College, Adama, Ethiopia,[4]Addis Ababa University, Addis Ababa, Ethiopia,[5]University of Cambridge, Cambridge, United Kingdom,[6]Queen Mary University of London, Lomdon, United Kingdom

Background: Proof-of-principle studies have identified circulating tumor DNA (ctDNA) in small volumes of whole blood, even dried blood spots (DBS) collected by finger-prick sampling from patients with advanced cancer. This minimally invasive sampling method offers ease, frequency and low cost of collection, simplified shipping and long-term room temperature stability. Adopting DBS for ctDNA collection and whole genome sequencing (WGS) analysis may support novel clinical trial design and increase real-world utility of liquid biopsies, especially where traditional phlebotomy is a barrier.

Methods: We collected sample quartets comprising serial DBS, and matching plasma, tumor, and buffy coat from ~125 patients with multiple cancer types including oesophageal, lung, head & neck, melanoma, ovarian, colorectal and pancreatic cancer. Collections were in diverse settings at hospitals in the UK and Ethiopia, and by patients at home in the UK. DNA isolated from DBS was size-selected using bead-based enrichment to remove genomic DNA. Libraries were generated from DBS size-selected DNA, and DNA extracted from plasma, tumor and buffy coat samples had paired-end WGS at depth of ~1X, 10X, and 50X respectively. Using bioinformatic pipelines for tumor-informed and tumor-agnostic detection, WGS data was analyzed for somatic copy number aberrations (SCNAs), fragment length, and tumor-specific single nucleotide variants (SNVs).

Results: We detected SNVs and SCNAs in DBS reflective of genomic changes in matching tumor DNA and plasma, from patients with Stage II-IV cancers. We observed concordant changes to the levels of those SNVs and SCNAs in plasma and DBS samples collected before and after systemic treatment compared with clinical and radiological measures. With tumor-informed analysis, utilizing patient-specific lists of 5,930-51,927 SNVs identified from tumor DNA, limit of detection for ctDNA was 34 ppm in plasma, and 95 ppm in DBS, comparable to commercially available assays for ctDNA. DBS collected in hospital by healthcare professionals, remotely in Ethiopia and at-home by patients in the UK with transportation at room temperature showed ctDNA can be extracted for analysis from diverse settings.

Conclusion: Our data highlights the promising role of DBS in advancing non-invasive cancer diagnostics and monitoring. The versatility of DBS in capturing ctDNA from various cancer types, coupled with its accessibility for collecting samples, is a significant step forward in blood-based cancer detection. This offers potential to enhance cancer monitoring and early detection strategies in both tumor-informed and tumor-agnostic settings. DBS is a cost-effective, user-friendly sampling strategy allowing at-home collection, enabling large-scale research and development programs needed to bring biomarker methods into widespread use.

#4551 Increased blood draws for ultrasensitive ctDNA and CTCs detection in early breast cancer patients.

I. Comino-Mendez[1], M. Quiros-Ortega[1], J. Velasco-Suelto[1], C. Gaona-Romero[1], A. Alba-Bernal[1], A. Godoy-Ortiz[1], M. Dominguez-Recio[1], B. Jimenez-Rodriguez[2], E. Lopez-Lopez[1], J. Peralta-Linero[1], M. Ortega-Jimenez[3], E. Bellagarza-Garcia[2], L. Troyano-Ramos[2], G. Garrido-Ruiz[3], A. Garrido-Aranda[1], R. Lavado-Valenzuela[1], M. Alvarez[1], N. Ribelles-Entrena[1], J. Pascual[1], E. Alba[1];
[1]Inst of Biomedical Investigation, Malaga., Malaga, Spain, [2]Hospital Regional Universitario de Malaga, Malaga, Spain, [3]Hospital Universitario Virgen de la Victoria de Malaga, Malaga, Spain

Relapse due to residual disease (RD) is a significant concern for early breast cancer (BC) patients following initial treatment. Liquid biopsy offers a non-invasive approach to detect tumor-derived components in blood; however, detection sensitivity remains limited at early disease stages due to low target concentrations. To improve this, we evaluated 282 high-volume plasma and blood-cell samples for the dual detection of circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs) using droplet-digital PCR. In pre-treatment samples, ctDNA and/or CTCs were detected in all cases. Post-treatment, positive samples achieved enhanced sensitivity, with a minimum variant allele frequency of 0.003% for ctDNA and a minimum CTCs concentration of 0.069 cells/mL, surpassing detection limits of prior studies. Dynamic ctDNA monitoring allowed accurate prediction of pathological response in the tumor, achieving an area under the ROC curve of 0.92. Moreover, blood detection of disease markers preceded clinical relapse by up to 34.6 months in relapsing patients, providing a significant lead time for intervention. This methodology was also applied to a study of 38 additional BC patients treated solely with surgery to further evaluate its capacity for RD detection in a surgically managed cohort. The findings and implications for RD detection are detailed and presented, underscoring the potential of this sensitive, high-volume approach for real-time monitoring in early-stage BC.

#4552 Quality assessment of cfDNA from urine and plasma using fragment size analysis.

Thorsten Voss, Daniela Mancarella, Christina Goebel

QIAGEN GmbH, Hilden, Germany

INTRODUCTION: The majority of liquid biopsy approaches are based on cell-free DNA (cfDNA) from plasma. In recent times, urine cfDNA is getting more attention as it has been shown to provide complementary information to blood cfDNA, which could increase sensitivity with respect to detection of pathogenic genetic variants. However, cfDNA degradation and genomic DNA (gDNA) contamination can impact the cfDNA profile and prohibit reliable molecular analysis of these analytes. Therefore, quality assessment of cfDNA eluates from both body fluids before use in sensitive downstream assays is essential. In this study, we present quality assessments based on capillary electrophoresis using samples stabilized and isolated with verified and standardized preanalytical workflows using the PAXgene Urine Liquid Biopsy Set (RUO) and the PAXgene Blood ccfDNA Tube (CE-IVD).

METHODS: Urine and blood samples from apparently healthy, consented individuals were collected and stabilized in the PAXgene Urine Liquid Biopsy Set and the PAXgene Blood ccfDNA Tube (PreAnalytiX). Unstabilized samples from the same individuals were collected and processed in parallel. The samples were stored for several days on the lab bench at room temperature (15-25°C) or incubated at varying temperatures mimicking real world storage and/or transport scenarios. After storage, urine and blood samples were centrifuged to remove cells and cfDNA was isolated from the urine supernatant or plasma either using automated or manual methods. The concentration of cfDNA was measured using the Qubit dsDNA HS Quantification Assay Kit on the Qubit Fluorometer (Thermo Fisher Scientific). Fragment profiles were analyzed using the QIAxcel DNA High Sensitivity Kit on the QIAxcel Connect instrument (both QIAGEN). For analysis of cfDNA from urine, the maximum fragment size shown was manually set to 5000 bp in the ScreenGel software (QIAGEN).

RESULTS: cfDNA isolated from the PAXgene Blood ccfDNA Tube showed the typical, predominantly mononucleosomal fragment length independent of whether the blood was stored or processed directly, while unstabilized blood showed contamination with gDNA. Urine cfDNA isolated from samples stabilized with the PAXgene Urine Liquid Biopsy Set showed that in addition to mono- and di-nucleosome DNA, longer fragments could be detected with and without storage. In unstabilized urine, DNA degrades rapidly, resulting in reduced fragment sizes compared to stabilized urine already on the day of urine collection without storage. After storage of unstabilized urine for three days, only degraded cfDNA could be detected.

CONCLUSIONS: In this study we could demonstrate that stabilization of cfDNA in urine and blood are essential to preserve the native profiles of this analyte. Standardized preanalytical workflows provided by PreAnalytiX and state of the art quality control via the QIAxcel instrument should be considered for development of reliable downstream analysis.

#4553 Evaluation of cfDNA release profiles in colorectal cancer patients during surgery.

Mailson Alves Lopes[1], Maria Elvira Ribeiro Cordeiro[1], Flavio de Alencar Teles Barreto[1], Lara de Souza Moreno[2], Andre Araujo de Medeiros Silva[3], Mayra Veloso Ayrimoraes Soares[2], Mariana Braccialli de Loyola[1], Joao Batista de Sousa[3], **Fabio Pittella-Silva**[1]

[1]Laboratory of Molecular Pathology of Cancer, University of Brasilia, Brasilia, Brazil,[2]Department of Radiology, Brasilia University Hospital, Brasilia, Brazil,[3]Department of Colorectal Surgery, Brasilia University Hospital, Brasilia, Brazil

Surgical intervention is one of the most employed treatments for colorectal cancer, especially in patients with localized disease. However, the dynamics of circulating cell-free DNA (cfDNA) release during surgical procedures remain poorly understood. This lack of understanding underscores a significant gap regarding the biological processes underlying tumor response to surgery. Here we examined surgical cfDNA release from 30 colorectal cancer patients. cfDNA was extracted from plasma samples at three key points: preoperative (just before surgery), intraoperative (during surgery), and postoperative (at the end of surgery). Automated electrophoresis was used to analyze cfDNA concentrations and fragment sizes, which were then correlated with different clinical variables. Our findings show a significant increase in cfDNA release during and after surgery in comparison to preoperative time (2.8- and 2.2-fold higher, respectively). cfDNA fragments of <400 bp were predominant at all surgical stages, with no significant release of genomic DNA detected in any stage. We found that cfDNA concentrations increase on an average of 2 to 3-folds during and after surgery in patients over 60 years old, in patients with comorbidities and in patients with CEA levels > 5 ng/mL. Importantly, cfDNA release during surgery was significantly higher in patients with adverse clinical characteristics. Patients with locally advanced tumors or metastasis had a 3.1-fold increase in cfDNA release intraoperatively and a 2.4-fold increase postoperatively ($p < 0.01$). cfDNA concentrations also increase intraoperatively in patients with high tumor bud scores (2.6-fold higher, $p < 0.02$), perineural invasion (3.4-fold higher, $p < 0.02$), and lymphovascular invasion (3.1-fold higher, $p < 0.05$). Overall, our findings indicate that factors such as advanced physiological age, existing comorbidities, adverse clinical characteristics, and extensive surgical manipulation may contribute to increased tissue damage and enhanced cfDNA release.

#4554 Advanced detection of *TMEM240* hypermethylation in circulating cell-free DNA as a novel biomarker for colorectal cancer monitoring.

Yao-Yu Hsieh[1], Ruo-Kai Lin[2]

[1]Taipei Medical University Shuang Ho Hospital, Ministery of Health and Welfare, New Taipei City, Taiwan,[2]Taipei Medical University, Taipei, Taiwan

Background: Carcinoembryonic antigen (CEA) is currently the most widely utilized biomarker for monitoring colorectal cancer (CRC) progression and treatment efficacy. With a sensitivity of approximately 80% and specificity of 70% at a cutoff level of 5 μg/L, CEA has demonstrated clinical utility in many cases. However, 20-30% of CRC patients fail to benefit from CEA monitoring due to its limited dynamic range and clinical irrelevance in specific cases, underscoring the urgent need for more accurate and reliable biomarkers. Recent advancements have identified the hypermethylation of the *TMEM240* gene as a promising biomarker for CRC detection, prognosis, and progression.

Results: We developed an advanced detection technology to analyze methylated circulating cell-free DNA (mcfDNA) specific to the *TMEM240* gene. A cohort of 30 colorectal cancer patients, including 5 with stage I and II, 3 with stage III, and 22 with stage IV, was monitored every 1-3 months over a follow-up period exceeding six months. The results revealed that elevated levels of *TMEM240* mcfDNA could be detected 1-3 months before image progression. Notably, patients with good prognoses exhibited no detectable *TMEM240* mcfDNA in their plasma, suggesting and good predictive marker of treatment response. In contrast, metastatic colorectal cancer patients with detectable *TMEM240* mcfDNA in plasma were identified as having a higher risk of cancer progression. Furthermore, high levels of *TMEM240* mcfDNA were strongly associated with the development of multiple metastases within three months, emphasizing its prognostic value in advanced disease stages. The method exhibited outstanding accuracy in predicting cancer progression, achieving an area under the curve (AUC) of 0.986, a sensitivity of 92.3%, and a specificity exceeding 99.0%. These results highlight the superiority of *TMEM240* mcfDNA as a biomarker compared to CEA, particularly in its ability to provide earlier and more precise detection of disease progression. Unlike CEA, which often fails to reflect subtle or early changes in CRC dynamics, *TMEM240* mcfDNA levels demonstrated robust applicability across all stages of CRC, from early detection in stages I and II to advanced monitoring in stage IV cases. *TMEM240* mcfDNA levels also provided real-time insights into treatment efficacy, particularly in metastatic colorectal cancer patients.

Conclusion: The hypermethylation of *TMEM240* mcfDNA in plasma represents a highly sensitive and specific biomarker for CRC monitoring, addressing the limitations of traditional markers such as CEA. The integration of this innovative assay into clinical practice could revolutionize CRC management by enabling earlier detection of progression, more accurate prognosis, and real-time evaluation of therapeutic efficacy.

#4556 Postoperative minimal residual disease detection using tumor-informed circulating tumor DNA analysis in stage II-III gastric cancer.

Bom Han[1], Hayoon Lee[1], Minkyu Jung[2], Bum Jun Kim[3], Yongjun Cha[1], Sunghoon Heo[1], Sun Young Rha[2], Hyung-Don Kim[4], Min-Hee Ryu[4], Dong-Hoe Koo[5], Jong Gwang Kim[6], Hye Sook Han[7], Sun Jin Sym[8], Jae-Joon Kim[9], Do-Youn Oh[10], Keun-Wook Lee[11], Hwang-Phill Kim[1], Duhee Bang[12], Sang-Hyun Song[1], Tae-You Kim[1], Dae Young Zang[3]

[1]IMBdx, Inc, Seoul, Korea, Republic of,[2]Division of Medical Oncology, Yonsei Cancer Center, Yonsei University College of Medicine, Seoul, Korea, Republic of,[3]Division of Hematology-Oncology, Department of Internal Medicine, Hallym University Sacred Heart Hospital, Hallym University College of Medicine, Anyang-si, Korea, Republic of,[4]Department of Oncology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, Republic of,[5]Department of Internal Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of,[6]Department of Hematology/Oncology, Kyungpook National University Chilgok Hospital, Kyungpook National University School of Medicine, Daegu, Korea, Republic of,[7]Department of Internal Medicine, Chungbuk National University Hospital, Cheongju, Korea, Republic of,[8]Department of Internal Medicine, Division of Medical Oncology, Gachon University Gil Medical Center, Incheon, Korea, Republic of,[9]Division of Hematology & Oncology, Department of Internal Medicine, Pusan National University Yangsan Hospital, Yangsan, Korea, Republic of,[10]Division of Medical Oncology, Department of Internal Medicine, Seoul National University Hospital, Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea, Republic of,[11]Department of Internal Medicine, Seoul National University College of Medicine, Seoul National University Bundang Hospital, Seongnam, Korea, Republic of,[12]Department of Chemistry, Yonsei University, Seoul, Korea, Republic of

Background: Circulating tumor DNA (ctDNA) analysis enables the detection of minimal residual disease (MRD), offering potential for improved risk stratification and guidance on decisions regarding adjuvant chemotherapy (ACT). We investigated the clinical utility of tumor-informed ctDNA analysis for postoperative MRD assessment in stage II-III gastric cancer (GC).

Methods: We plan to enroll 100 patients from the 976 expected in the ongoing EXODOX study (NCT04787354), a phase III randomized trial comparing reduced-duration XELOX with the standard 6-month regimen as ACT in patients with pathologic stage II-III GC after curative resection. In this sub-study, ctDNA MRD was assessed using the CancerDetect^{TM} assay (IMBdx, Inc.), a targeted sequencing method with a tumor-informed bespoke panel (BSP). To create the BSP, whole exome sequencing of tumor tissue and paired white blood cells identified up to 100 patient-specific variants. Whole blood (20 mL) was collected at five postoperative time points: 3-10 weeks (P0), 6 months (P1), 12 months (P2), 18 months (P3), and 24 months (P4). CtDNA MRD positivity was defined as the detection of two or more BSP variants in plasma.

Results: This analysis includes 42 patients enrolled from May 2022 to August 2024, with ctDNA MRD assessed at three time points: P0 (n = 42), P1 (n = 27), and P2 (n = 17). The median age was 66 years (range, 39-96); 27 patients (64.3%) were male, all had adenocarcinoma, and 30 patients (71.4%) had stage III disease. Overall, postoperative ctDNA (P0) positivity was 45.2% (19/42), with a median of 8 somatic mutations per patient (range, 2-94). CtDNA positivity was significantly higher in stage III than in stage II patients at P0 (56.7% vs. 16.7%, P = 0.037). CtDNA MRD clearance after ACT was achieved in 46.2% (6/13) of patients who were ctDNA-positive at P0. One initially ctDNA-negative patient converted to positive, resulting in an overall ctDNA positivity rate of 29.6% (8/27) at 6 months (P1). Twelve months postoperatively, ctDNA positivity (P2) was 35.2% (6/17). After a median follow-up of 12.0 months, 7 patients experienced recurrence. CtDNA positivity at P1 and P2 was significantly associated with worse recurrence-free survival (RFS) (P = 0.003 and P = 0.038, respectively), whereas it was not significantly associated at P0 (P = 0.101). RFS differed significantly based on changes in ctDNA positivity from P0 to P1 (P = 0.010), with the persistent ctDNA-positive group showing the poorest RFS. Notably, patients who achieved ctDNA clearance after ACT had RFS similar to those who were ctDNA-negative at both P0 and P1 (P = 0.480).

Conclusions: In patients with stage II-III GC, postoperative MRD was associated with recurrence risk. Temporal changes in ctDNA MRD status during monitoring reflect the response to ACT and may guide treatment decisions. To fully understand the impact of ctDNA MRD monitoring, further follow-up is planned.

#4557 Genome-wide cfDNA hypo-methylation landscape of 500 patients, a real-world study.

Chao Dai, Giancarlo Bonora, Kemin Zhou, Shidong Jia, Pan Du

Predicine Inc., Hayward, CA

Background: Liquid biopsies analyzing cell-free DNA (cfDNA) from plasma and urine can offer clinical diagnostic information in a non-invasive way. One major application of DNA methylation in liquid biopsies focuses on targeted panel regions, aiming to detect hypermethylation in cancer related regions. Recent studies have revealed that the loss of DNA methylation is significantly enriched in late-replicating loci compared with early-replicating loci in cancer cells. In this study, we profiled genome-wide DNA methylation of plasma and urine samples from various of cancer indications to detect DNA methylation loss in tumor DNA.
Methods: We used the PredicineEPIC assay for genome-wide DNA methylation profiling at 20× coverage. Unlike traditional differential methylation region (DMR) analysis, we identified fragment-level DNA methylation changes in CpG-rich genomic regions. For each fragment, we calculated the probability of the fragment being unmethylated versus methylated. This calculation is specific to genomic positions, with the background distribution built upon more than 100 healthy plasma samples.
Results: Compared with genome-wide DNA methylation profile of healthy plasma samples, we observed two distinct types of methylation loss in cancer patient samples. In the first case, hypomethylation occurs at fragments with significant methylation loss, where the methylation status of all CpG sites transitions from methylated to unmethylated. We observed that the magnitude of methylation loss is positively correlated with tumor fraction. Based on wet-lab titration experiment results, we can achieve tumor fraction detection limit of 0.1% for prostate, breast, and lung cancers. In the second case, hypomethylation at the fragment level is more subtle, with only a portion of CpG sites becoming unmethylated. We found that many samples in this category had CHIP mutations or other methylation enzyme deficiencies detected, with DNMT3A, which encodes DNA methyltransferase, being the most frequent. The same method can also be applied to urine cfDNA data for the detection of bladder cancer. We analyzed over 500 whole-genome methylation samples across different cancer indications, including prostate, colon, pancreatic, lung, breast and bladder cancers. Overall, genome-wide DNA methylation loss is highly prevalent in cancer samples and varies across cancer types. Some chromosome regions are consistently hypo-methylated across samples and cancer indications.
Conclusions: Our results indicate that global nuclear organization might be disrupted by methylation loss at closed chromatin compartments. The genome-wide methylation profile of circulating tumor DNA can measure cancer genome abnormality in terms of DNA methylation loss, CHIP mutations and copy number variations. Combination of all these features can provide a robust utility for cancer detection and estimating tumor fraction in liquid biopsies.

#4558 Comparative analysis of blood- and tissue-informed MRD studies in SCLC.

Haoran Tang[1], Wei Tan[2], Pu Sun[2], Yang Shi[2], Cancan Jia[1], Feng Xie[1], Yue Zhang[1], Qin Luo[1]

[1]Huidu Shanghai Medical Sciences, Ltd., Fengxian District, Shanghai, China,[2]BeiGene (Shanghai) Co., Ltd., Jing'an District, Shanghai, China

Introduction: Minimal residual disease (MRD) reflected by specific genetic alterations in ctDNA was considered to be closely related to disease status in solid tumors. MRD detection was reported to be promising to monitor therapeutic effects and identify recurrence risks. Next-generation sequencing (NGS) based MRD assays usually require tissue samples to identify tumor-specific mutations for subsequent monitoring (tissue-informed MRD). However, baseline tissue samples were not always available in clinical settings, limiting their applications. In this study, we explored using baseline blood samples to identify mutations for post-treatment ctDNA MRD detection (blood-informed MRD) as well as tissue-informed MRD in 62 limited-stage small cell lung cancer (LS-SCLC) patients receiving CRT combined with IO treatments (NCT04952597).

Methods: 62 patients with LS-SCLC underwent MRD testing using both tissue-informed and blood-informed PredicineBEACON assays during CRT treatment, either alone or in combination with IO. The assays utilized enhanced whole-exome sequencing (PredicineWES+) on initial tissue and blood samples to identify somatic mutations. Personalized MRD panels were developed for each patient, incorporating up to 50 mutations identified through bioinformatics analysis from either baseline tissue or blood, along with a standard panel of 500 key tumor mutations. The study conducted ultra-deep sequencing at 100,000X coverage using these tailored panels to detect MRD at two critical time points: cycle 1 day 1 (C1D1) and cycle 3 day 1 (C3D1).

Results: In this study, 62 patients were subjected to both tissue-informed and blood-informed MRD testing. PredicineWES+ at baseline identified 13,542 somatic mutations in tissue samples and 12,133 in plasma samples, with medians of 181 and 155 mutations per patient, respectively. There was a notable overlap where 10,503 mutations were recognized by both methods, resulting in a median concordance rate of 86.4% per patient, highlighting the WES assay's reliability in mutation detection across biopsy types. Upon MRD assessment at C3D1, a high concordance in MRD status was observed between the two methods: 35 patients tested MRD-positive and 26 MRD-negative in both assays. One patient displayed discordant results, being MRD-positive in the tissue-informed but negative in the blood-informed assay. Additionally, the study found a significant correlation in tumor fractions between the MRD tests at both C1D1 and C3D1 (R^2=0.96, p<2.2e-16), as established by linear regression, underscoring their robustness in evaluating tumor dynamics.

Conclusions: This study demonstrated high concordance of tumor-informed and blood-informed MRD assay in LS-SCLC patients treated with IO and CRT. These outcomes highlight the potential clinical applicability of using baseline ctDNA NGS assays for personalized MRD detection.

#4559 Paired liquid biopsies analysis reveals resistance mechanisms and prognostic factors in patients treated with KRASG12C inhibitors.

Kristi Beshiri, Damien Vasseur, Julieta Rodriguez, Smolenschi Cristina, Francesco Facchinetti, Antoine Italiano, Nicotra Claudio, Maud Ngo-Camus, Anas Gazzah, Capucine Baldini, Luc Friboulet, Yohann Loriot, Fabrice Barlesi, Christophe Massard, Antoine Hollebecque

Gustave Roussy Institute, Villejuif, France

Background: KRASG12C inhibitors (G12Ci) represent an emerging class of therapeutics. We aimed to explore resistance mechanisms and prognostic factors using liquid biopsy (LB) analyses.
Methods: We retrospectively assessed LBs from patients (pts) treated with G12Ci (February 2022 - November 2024), under the STING trial (NCT04932525). Paired LBs (baseline and progressive disease (PD)) were analyzed using the FoundationOne Liquid CDx assay. Survival outcomes were calculated, and univariate/multivariate analyses performed with R packages “survival” and “survminer” (v 3.3-1 and 0.4.9).
Results: A total of 18 pts treated with one or more G12Ci were included: NSCLC (n=9), colon cancer (n=8), and pancreatic cancer (n=1). All pts had a confirmed KRASG12C mutation in tissue samples. Three pts received two G12Ci sequentially. Overall, 21 LBs pairs were available: 13 included co-treatments (e.g., EGFR inhibitor (n=5), immunotherapy (n=4), and another small molecule (n=3)). At baseline LBs, KRASG12C was detected in 18/21 pairs (85.7%), and in 15/21 pairs at PD (71.4%). In three pairs the mutation was not detected at any timepoint. Overall response rate was 42.8% (9/21). At progression, acquired genomic alteration that may be associated with treatment resistance were identified in 6/21 pts (28.6%). All six pts exhibited at least one on-target KRAS alteration: KRAS amplification (n=2), KRAS R68S mutation (n=1), KRAS G12V (n=1), or KRAS mutations at G12A/D/F/S/Y, G13D, Q61H, R68S, Y96D (n=1). EGFR mutations (n=3),primarily associated with anti-EGFR resistance, a MET amplification (n=1), an ETV6 fusion (n=1), and a CDKN2A mutation (n=1) were also observed. Impact of the baseline variant allelic frequency (VAF) of the KRASG12C mutation on progression free survival (PFS) was assessed. The median PFS for pts with a VAF lower than the median (2.5%) was 7.5 months (95% CI: 5-NA), vs 4 months (95% CI: 2-NA) for those with a VAF higher, which was associated with worse PFS (p=0.0097). Univariate analysis revealed that the number of metastatic sites at baseline (HR: 2.00, 95% CI: 1.30-3.00, p = 0.0013) was associated with worse PFS, as well as higher baseline VAF (HR: 3.40, 95% CI: 1.30-8.90, p = 0.015). Multivariate analysis confirmed higher VAF at baseline as a predictor of worse PFS (HR: 2.79, 95% CI: 1.01-7.75, p = 0.0049), as did the baseline number of metastatic sites (HR: 1.84, 95% CI: 1.09-3.11, p = 0.022). The correlation between Tumor Fraction (cut-off 10%) and PFS was no statistically significant (p=0.081).
Conclusion: Paired LBs represent a potential method for idenfying up to 28.6% of the resistance mechanisms to G12Ci. Complementary molecular methods, such as tissue biopsy molecular analysis, are needed to comprehensively identify resistance mechanisms. Higher VAF at baseline LBs and a greater number of metastatic sites were both associated with worse PFS.

#4560 Use of ctDNA fragmentomics as a predictive biomarker in locally advanced non-small cell lung cancer.

Kishen R. Patel[1], Anisha Mistry[2], Robert Monfries[3], Florent Mouliere[4], John R. Conibear[3], James Wilson[5], Karim Keshwani[5], Wing K. Liu[6], Crispin Hiley[1]

[1]Hiley Lab, University College London (UCL) Cancer Institute, London, United Kingdom,[2]Cancer Research UK RADNET, London, United Kingdom,[3]Clinical Oncology, Barts Cancer Centre, London, United Kingdom,[4]Mouliere Lab, Cancer Research UK Manchester Insitute, Manchester, United Kingdom,[5]Oncology, University College London Hospital, London, United Kingdom,[6]Jamal-Hanjani Lab, University College London (UCL) Cancer Institute, London, United Kingdom

Introduction: Locally advanced non-small cell lung cancer (NSCLC) is a serious condition with a poor 5-year survival rate of approximately 30%. Standard treatment involves chemoradiotherapy (CRT) followed by immunotherapy (IO). However, predicting benefit prior to CRT and monitoring response post treatment are areas of unmet need. Cancers, like many tissues, release fragments of DNA into the bloodstream, which can be detected using whole genome sequencing (WGS). Research has shown that cancer cells tend to release shorter DNA fragments, typically between 90-150 base pairs, at higher rates than normal tissues. This study aims to analyze cell-free DNA fragment sizes (fragmentomics) as a predictive tool for assessing treatment response in patients with LA NSCLC. This tumor-uninformed approach is cost-effective and can be implemented using routine blood samples.
Methods: Low-pass WGS at 0.1x coverage was performed on 40 pre-treatment plasma samples from patients LA NSCLC obtained from the UCL biobank. The median follow-up for the cohort was 33 months. To investigate the cancer origin of short DNA fragments (90-150 bp), mutation calling was conducted using Mutect2. Mutation density was defined as the number of mutations per million bases (MPM). Tumor volumes were obtained using CT imaging, and the percentage quantity of short DNA fragments was plotted against tumor volume. Enrichment of short fragments (SFE) <20% was assessed and correlated with clinical outcomes, including relapse and overall survival.
Results: Short DNA fragments (90-150 bp) had a significantly higher mutation density (973.8 MPM) compared to unselected fragments (381.7 MPM; $p<0.0001$). This elevated mutational burden supports the hypothesis that shorter fragments are of cancer origin. Linear regression analysis showed no significant relationship between tumor volume and the percentage of short DNA fragments for the entire cohort ($R^2 = 0.035$, $p = 0.35$). However, in lung squamous cell carcinoma (LUSC), a strong positive correlation was observed ($R^2 = 0.6787$, $p = 0.003$). Suggesting a potential quantitative link between tumor burden and ctDNA quantity in this subtype. SFE was observed in 31 of 40 patients (78%) prior to treatment. SFE was found in similar proportions in LUSC (78%; 15/19) and lung adenocarcinoma (75%; 15/20). Mortality occurred in 45% (14/31) of patients with SFE, compared to 11.1% (1/9) of those without enrichment. Among patients for whom consolidation IO was indicated, relapse rates were higher in those with SFE (44%; 7/16) than in those without enrichment (25%; 1/4). Findings of the research were limited by sample size; however further centers have been recruited to address this issue. These findings suggest that fragmentomic patterns represent a cost-effective, non-invasive prognostic biomarker for LA NSCLC, with particular potential in LUSC. Ongoing research is exploring patterns following treatment.

#4561 ShieldingUltra: A novel approach for enhanced minimal residual disease detection through the integration of mutation, copy number variation, and fragmentomics.

Hao Zhang, Ningyou Li, Wanxiangfu Tang, Jinfeng Zhang, Xunbiao Liu, Peng He, Baihan Zhu, Shuang Chang, Zilin Wang, Zhili Chang, Dongqin Zhu, Rui Liu, Xiaoxi Chen, **Haimeng Tang**, Hua Bao, Xue Wu, Yang Shao

Geneseeq Research Institute, Nanjing Geneseeq Technology Inc., Nanjing, China

Introduction: Minimal Residual Disease (MRD) detection using circulating tumor DNA (ctDNA) has significant clinical value in cancer therapy. However, existing methods primarily focus on mutations, restricting their broader applicability. Personalized panels, while targeted, are limited by prolonged turnaround times (TAT) and reliance on central labs. Fixed-panel approaches provide standardized, efficient solutions with strong potential for in vitro diagnostic kit development.
Method: We present ShieldingUltra, a fixed-panel MRD solution targeting hotspot mutations across 2,365 genes using ultra-deep unique molecular identifier sequencing. Using over 1,000 clinical plasma samples, cutting-edge AI-driven models were designed to reduce sequencing background error and clonal hematopoiesis interference, and improve sensitivity by integrating mutations, copy number variations, and fragmentomics. ShieldingUltra demonstrates exceptional performance validated across diverse clinical settings.
Result: ShieldingUltra demonstrated exceptional performance, achieving over 70% and 90% presurgical plasma positivity in early- and late-stage pan-cancer samples, respectively, with >99% specificity in healthy plasma. We further evaluated ShieldingUltra in a few most challenging clinical scenarios, rarely studied before with poor outcomes, and achieved unparalleled performance. In a cohort of lung cancer patients, ShieldingUltra achieved the most sensitive MRD detection with 1-week postsurgical landmark plasma (61.5%, 16/26), with sensitivity rising to 96% (25/26) using longitudinal plasma. In another challenging and unresolved scenario aimed at further stratifying advanced non-small cell lung cancer (NSCLC) patients who exhibited a partial response upon completion of immunotherapy (IO), as well as identifying those unable to maintain prolonged progression-free survival, ShieldingUltra successfully identified over 50% (18/34) of individuals who quickly relapsed. This highlights ShieldingUltra's potential to pinpoint patients most likely to ultimately benefit from IO, which marks an innovative frontier in MRD research. Additionally, ShieldingUltra was explored for perioperative MRD tracking and the potential to guide the treatment strategies in an advanced stage ovarian cancer cohort, including evaluating neoadjuvant therapy for surgical decisions, assessing the need for adjuvant therapy post-surgery, and monitoring treatment efficacy and MRD status after surgery.
Conclusion: ShieldingUltra combines a fixed panel with multiple genomic features and AI modeling for exceptional MRD detection. Its standardized design minimizes TAT, eliminates custom setup delays, and ensures reliable, cost-effective results, making it ideal for time-sensitive clinical decisions and broad clinical adoption.

#4562 Bespoke sequencing of enriched and normalized amplicons increases on-target rate and reduces background noise for circulating tumor DNA detection and quantification.

Tania Contente-Cuomo[1], Devin Dinwiddie[1], Mojca Stampar[1], Adriana Marshall[1], Michael E. Berens[1], Stephanie J. K. Pond[1], Jeffrey M. Trent[1], Muhammed Murtaza[2]

[1]Translational Genomics Research Institute, Phoenix, AZ,[2]Surgery/Center for Human Genomics and Precision Medicine, University of Wisconsin-Madison, Madison, WI

Background: Analysis of circulating tumor DNA (ctDNA) using tumor-guided bespoke sequencing can achieve high accuracy for minimal residual disease detection. However, bespoke laboratory workflows are often complex, difficult to scale, and costly. Multiplexed PCR methods have lower costs and preserve input DNA but instill higher background noise, variable on-target rates and unpredictable relative representation of amplicons. Locus- and mutation-specific hybrid capture can overcome these challenges but with higher costs of patient-specific custom baits and greater loss of input DNA. Here, we describe a workflow that combines targeted amplification with fixed content exome-wide hybrid capture to overcome these challenges.

Methods: We performed tumor-guided plasma DNA analysis using a novel method based on enrichment and normalization of amplicons and targeted sequencing. Using tumor and germline exome sequencing, we identified somatic founder mutations, and designed primers for multiplexed amplification. Targeted sequencing libraries were prepared using a combination of single-stranded adapter ligation, targeted PCR and hybrid capture. Sequencing reads were grouped in read families based on fragment size and unique molecular identifiers, and analyzed to improve specificity for detection of known mutations. We evaluated assay performance using reference samples (Seracare Life Sciences) as well as plasma samples from patients with cancer.

Results: We analyzed 8 replicates of reference samples at 10 ng input each for variant allele fractions (VAFs) of 0.5%, 0.25% and 0.125%, using an 18 mutation panel, and detected mutated DNA in 8/8 samples for all VAFs. In addition, we prepared a dilution at 0.01% VAF and analyzed 16 replicates of this sample at 40 ng input each, and detected mutated DNA in 15/16 samples. Using the same analytical thresholds, 1/8 wild-type reference samples were called positive. A comparison of observed and expected tumor fractions across all samples showed a correlation coefficient of 0.97 ($p<0.001$, $n=48$). In addition, we developed 44 patient-specific panels targeting a median of 30 mutations per patient (range 5 to 125 mutations) for patients with pancreatic cancer, breast cancer and glioblastoma. Using a median input of 6.7 ng (range 1.1 to 24 ng), we analyzed 112 plasma DNA samples across 44 panels and observed a median on-target rate of 89% using the first round of panel design. ctDNA was detected in 62 plasma samples (55%) at a median tumor fraction of 0.013% (range 0.009% to 5.8%).

Conclusion: Our results demonstrate analytical performance and assay stability across 44 patients using bespoke sequencing of enriched amplicons for circulating tumor DNA (enACT-DNA) analysis. On-going studies are evaluating the clinical relevance of perioperative ctDNA detection using enACT-DNA in patients with cancer.

#4563 Tumor fraction estimation and tissue copy number inference using copy number signal from a liquid biopsy assay.

Patrick D. Cherry, David Tsao, Wen Zhou

BillionToOne, Menlo Park, CA

Purpose: Estimating tumor fraction and inferring copy number alterations (CNAs) from cell-free DNA (cfDNA) liquid biopsy is challenging due to SNV heterogeneity and occasional lack of SNV signal. About 90% of solid tumors exhibit chromosome arm level gains or losses [1-2], and this effect on copy number could potentially be used as a tumor load biomarker. This study aims to use aneuploidy signal in cfDNA to estimate tumor fraction and infer tissue copy number, addressing a limitation of variant-based cfDNA assays and providing a robust method for benchmarking against gold-standard techniques like FISH.

Methods: We employed Northstar Select for copy number quantification, BillionToOne's treatment-selection liquid biopsy assay, which is optimized for accurate plasma copy number measurement of genes and is resistant to technical and biological noise. A Gaussian mixture model (GMM) was applied to quantify liquid aneuploidy signal on measured genes. The periodicity pattern was used to estimate tumor fraction independently of SNV signal. From a databank of 3180 clinical samples, we analyzed 92 clinical samples (from 20 patients) for concordance with SNV-based methods over multiple timepoints, and 136 clinical samples (from 128 patients) with homozygous copy number loss calls for concordance with the tissue copy number estimate.

Results: Aneuploidy-based tumor fraction estimates showed robustness above 2% tumor fraction. GMM tumor fraction estimates demonstrated concordance with samples exhibiting clear SNV alterations (Pearson r = 0.65 across multiple accessions and patients), and the correlation can be as good as r = 0.96 in samples from patients with longitudinally trackable SNVs. In the retrospective analysis, 141 homozygous copy number losses were called by Northstar Select assay, and among those, aneuploidy-based tissue copy number estimation identified homozygous losses in 126 samples (89%). The algorithm also identified additional homozygous losses (both inside of and outside of the reportable gene set of Northstar Select). Analysis of serially tested clinical plasma samples revealed that—despite large tumor fraction fluctuations (≤5% to ≥50%)—tissue copy number estimates for the same patient remained stable.

Conclusions: Our aneuploidy-based approach provides a reliable alternative to SNV-based methods for tumor fraction estimation and tissue copy number inference from liquid biopsy data. These findings suggest potential improvements in the utility of liquid biopsy for cancer diagnostics and monitoring, particularly in cases with low/mixed SNV signals. The ability to accurately infer tissue copy number is crucial for predicting amplification levels, identifying driver mutations, and anticipating therapeutic responses.

References:1. Ben-David, U., Amon, A. Nat Rev Genet 21, 44-62 (2020).

2. Sansregret, L., Bakhoum, S.F., et al. Genome Medicine, 13(1), 93 (2021).

#4564 Enhancing circulating tumor DNA recovery and liquid biopsy sensitivity with precision liposomal priming agents.

Kasturi Chakraborty[1], Carmen Martin-Alonso[1], Daniel Kim[1], Savan Patel[1], Sahil Patel[1], Zhenyi An[2], Shervin Tabrizi[2], Claire Sullivan[2], Lily Gao[2], J. Christopher Love[1], Viktor A. Adalsteinsson[2], Sangeeta N. Bhatia[1]

[1]Koch Inst. for Integrative Cancer Research at MIT, Cambridge, MA,[2]Broad Institute of MIT and Harvard, Cambridge, MA

Liquid biopsies offer the potential for early cancer detection as well as a non-invasive, real-time window into the evolving tumor as the cancer progresses (*Turabi+, 2024 PMID: 39001494*). In this context, circulating tumor DNA (ctDNA) - the fraction of cell-free DNA (cfDNA) shed by tumor cells into the bloodstream, has emerged as a promising candidate. It carries the genetic and epigenetic alterations specific to the originating tumor, making it an essential source of information for cancer diagnosis and monitoring. While in patients with advanced cancer and a higher tumor burden the proportion of ctDNA is >10%, and can even exceed 40% of the total cfDNA, this fraction is less than 1% in early-stage tumors making early detections challenging. We hypothesized that manipulating ctDNA biology could improve its recovery and developed a SPE-liposomal “priming agent" which transiently inhibit macrophage mediated clearance (Martin-Alonso/Tabrizi/Xiong+, 2024 PMID: 38236959). The targeting of SPE liposomes to liver macrophages was predominantly size-dependent, utilizing particles with the average hydrodynamic diameter between 230 and 260 nm. This range was designed to match the size of murine liver capillary fenestrae, such that the nanoparticles would preferentially target liver-resident macrophages over hepatocytes. However, effective macrophage priming still required a significant lipid load to increase cfDNA levels in circulation. To improve on our priming agents, we developed three distinct SPE-modified nanoparticles to target macrophage subpopulations in the liver and tumor. These next-generation priming agents enabled a 60-fold reduction in lipid dose while achieving a more than 1500-fold higher recovery of cfDNA compared to untreated mice. Additionally, we show that these nanoparticles provided a marked increase in the detection of mutant duplexes in a transplantation model of colon cancer lung metastases, Luc-MC26 tumor-bearing mice. In summary, we optimized macrophage-targeted nanoparticle-based strategies that significantly improved the recovery of cfDNA, and that enhanced early tumor detection rate. These technologies have the potential to improve the accuracy and sensitivity of liquid biopsies, as well as to extend our fundamental understanding of the in vivo dynamics of circulating nucleic acid structures.

#4565 CANARy-TF: an improved copy number and tumor fraction estimator for liquid biopsy applications.

Diego Almanza, Takeshi Sugio, Emily Hamilton, David Kurtz, Maximilian Diehn, Mohammad Shahrokh Esfahani, Ash A. Alizadeh

Stanford University, Menlo Park, CA

Background: Accurate detection of copy number alterations (CNAs) and reliable estimation of tumor fraction from liquid biopsies are crucial for effectively triaging patient samples in large clinical trials. This is particularly vital in scenarios where variant allele frequency (VAF) data is unavailable, yet an estimation of tumor fraction remains necessary. We hypothesize that specific characteristics of circulating free DNA (cfDNA) can enhance the performance of algorithms aimed at detecting CNAs and estimating tumor fraction.
Methods: We introduce CANARy-TF (Copy number ANomaly Assessment and RecoverY Tumor Fraction), a novel algorithm designed to accurately identify CNAs and estimate tumor fraction from low-pass whole-genome and off-target sequencing. CANARy-TF utilizes sequencing coverage data to pinpoint amplifications and deletions by comparing samples against a panel of normals. Additionally, it incorporates unique cfDNA features, such as fragment size and shifts in the distribution of wildtype/mutant fragments, to improve tumor fraction estimation.
Results: The performance of CANARy-TF was evaluated on a cohort of 23 cfDNA samples from patients with non-small cell lung cancer (NSCLC), alongside 149 cfDNA samples from healthy individuals as a background population. For all samples, tumor-informed VAF data were available. Analysis revealed a strong correlation between true VAF and the estimated tumor fraction by CANARy-TF, with a Spearman's rho of 0.86 (p-value = 1.38e-07). In silico mixtures representing tumor prevalence from 0.001% to 5% were generated, demonstrating a limit of detection for the algorithm at around 1%.
Conclusion: CANARy-TF effectively estimates CNAs and tumor fraction from plasma cfDNA samples. Notably, this algorithm is robust when applied to low-pass whole-genome sequencing, offering an efficient solution for triaging samples in both clinical settings and liquid biopsy studies.

#4566 A methylation-based, tissue-independent blood test for recurrence monitoring and treatment assessment.

Jiayue Xu, Xingyu Yang, Qiancheng You, Jing Su, Zeliang Deng, Xinyue Kang, Guangyou Li, Si Zhang, **Bingsi Li**

Innovation Center, Burning Rock Biotech, Shanghai, China

Background: Circulating tumor DNA (ctDNA) has emerged as a promising biomarker of minimal residual disease (MRD) detection and therapy response assessment. However, the low tumor burden in early-stage cancer and interference from non-tumor variants (e.g. clonal hematopoiesis) often necessitate tumor tissue to achieve the desired sensitivity and specificity. This requirement can limit the clinical application, particularly in situations where tissue biopsy is challenging or rapid turnaround times are critical. We previously validated an tissue-free assay (OverC) that combines a novel methylation sequencing method and machine learning for sensitive detection of low-frequency ctDNA in multiple cancer types. Here we describe the expansion of this assay for recurrence monitoring and treatment assessment.

Methods: DNA extracted from 139 formalin-fixed paraffin-embedded tumor/normal tissues and 249 plasma samples subjected to whole-genome methylation sequencing (5-30X depth) for single-molecule pattern recognition. The targeted methylation panel of the refined version, known as OverC Monitor, covered 70, 546 CpGs (~1.0 Mb), representing promoters, introns and other epigenetic regulatory elements. The classifier was developed with a larger THUNDER training set and independently validated with 506 pre-treatment plasma samples from patients with cancer and age-matched non-cancer controls. A positive call is made upon the identification of a cancer signal exceeding a locked threshold. In addition, a quantitative measure of tumor allele fraction (TAF) was developed and reported for each sample to assess its prognostic and monitoring potential.

Results: The assay performance was tested using plasma samples from 104 patients with lung cancer (LC), 48 colorectal cancer (CRC) and 74 liver cancer (HCC) and 280 age-comparable non-cancer donors. For LC, the sensitivity was 69.2% (Stage I: 30.3%, Stage II: 60.0%, Stage III: 86.7%, Stage IV: 91.7%) at a specificity of 98.6%. The sensitivity for CRC was 81.3% (Stage I: 41.7%, Stage II: 90.0%, Stage III: 95.0%, Stage IV: 100%) at a specificity of 98.9%. For HCC, the sensitivity reached 95.9% (Stage I: 89.5%, Stage II: 100.0%, Stage III: 95.0%, Stage IV: 100.0%) at a specificity of 98.9%. Further validation with larger cohorts and additional tumor types is underway, and updated data will be presented as it becomes available.

Conclusions: By leveraging cancer-derived methylation signatures and machine learning, we have developed and validated a highly sensitive assay (OverC Monitor) for detecting trace amounts of ctDNA in blood. This innovative, tissue-free approach has demonstrated performance on par with cutting-edge tissue-dependent mutation tracking methods for certain cancer types, highlighting its potential for broader clinical use in recurrence monitoring and treatment efficacy assessment.

#4567 Circulating tumor DNA-based molecular residual disease guides treatment in metastatic colorectal cancer patients with no evidence of disease: An open-label, prospective, interventional phase II study.

Yaqi Li[1], Chengcheng Li[2], Peng Cui[2], Xiang Hu[1], Fangqi Liu[1], Wenhua Li[3], Guoqiang Wang[2], Kunli Zhao[2], Qi Pan[4], Jing Wang[2], Bing Li[2], Shangli Cai[2], **Junjie Peng**[1]

[1]Department of Colorectal Surgery, Fudan University Shanghai Cancer Center, Shanghai, China,[2]Burning Rock Biotech, Guangzhou, China,[3]Department of Medical Oncology, Fudan University Shanghai Cancer Center, Shanghai, China,[4]Department of Hepatic Surgery, Fudan University Shanghai Cancer Center, Shanghai, China

Patients with metastatic colorectal cancer (mCRC) can achieve no evidence of disease (NED) after curative treatment. However, the survival benefit of adjuvant chemotherapy (ACT) for these patients remains uncertain. Circulating tumor DNA (ctDNA)-based molecular residual disease (MRD) detection has shown to help predict recurrence risk in CRC. Recent retrospective studies have indicated that ACT offers no clinical benefit in surgically resected mCRC with negative ctDNA. In this study, we conducted a prospective, interventiaonal clinical trial (NCT05635630) to evaluate the efficacy of ctDNA-guided ACT in patients with mCRC who achieved NED. Eligible mCRC patients who achieved NED after curative treatment were enrolled. Surgical tissues or puncture tissues were sequenced by whole-exon sequencing, and up to 50 highly ranked patient-specific variants were selected for the personalized panel design. Post curative treatment blood samples was sequenced by the personalized panel to determine the MRD status. Patients with positive MRD post curative treatment were offered investigator-determined ACT, while those with negative MRD were under surveillance. During surveillance, blood samples were collected every 3 months and patients whose MRD turned positive would receive intensive image surveillance every 2 months. A total of 104 eligible patients were included in the current analysis, among them 7 patients did not receive assigned treatment strictly adhered to the study protocol and were excluded from the per protocol set (PPS). In the PPS, as date cutoff of October 2024, the median follow-up was 14 months with 58 patients (58/97, 59.8%) who experienced recurrence. MRD was detected in 53 patients (54.6%, 53/97) post curative treatment. The median recurrence-free survival (RFS) was 8.5 months and not reached for patients with positive or negative MRD post curative treatment, respectively (HR: 4.28, 95% CI: 2.36-7.77; P<0.001). Six patients with negative MRD received ACT and had non-superior RFS than those who did not receive ACT (HR: 1.60, 95% CI: 0.46-5.58; P = 0.46). During surveillance, the median RFS was 6.1 months, 11.8 months, 16.8 months and not reached in patients with persistently positive ctDNA, converted positive ctDNA, converted negative ctDNA, and persistently negative ctDNA, respectively (log-rank P for trend <0.0001). All recurrent patients had at least one positive ctDNA detection before imaging confirmed recurrence, and the median lead time interval of ctDNA positivity to radiographic recurrence was 7.8 months (range, 0-20.2 months). Our results demonstrated the feasibility of using ctDNA to guide ACT in mCRC patients with NED. Future randomized clinical trials are needed to further demonstrate the utility of ctDNA-guided ACT in mCRC patients with NED. The follow-up is still on-going.

#4568 Biochemical mitigation of technical artifacts improves performance of a ctDNA methyl sequencing assay.

James Yu, Peter George, Jason Close, Andy Eang, Neil Peterman, Yu Sun, Nafisa Bulsara, Eileen Dimalanta, Alexander Robertson, Yexun Wang, Nicole Lambert

Foundation Medicine, Inc., San Diego, CA

In addition to accumulating mutations that may drive tumor growth, cancer cells also show broad changes in DNA methylation patterns genomewide. For example, a colorectal cancer sample may have fewer than 10 driver mutations, fewer than 100 passenger mutations, but may have nearly 800 differentially methylated regions. In contrast to the traditional method of using sequencing to find these scarce mutations in the genome, probing for these relatively abundant aberrantly methylated regions in the genome can be a powerful alternative approach for the detection of cancer.

However, assessing DNA methylation via methyl sequencing is complicated by artifacts introduced during the traditional NGS library prep process. In vivo, CpG sites in mammals are typically methylated on both strands of the double stranded DNA; however, we observe that the majority of read fragments display some level of hemimethylation (only one strand displays methyl modifications), and roughly 10% of all reads are hemimethylated across the read. Furthermore, we observe that the fraction of methylated bases gradually decreases over the course of a sequencing read; the methylation state of DNA should not be expected to be impacted by the cycle number of a sequencing run. These artifacts are collectively known as M-bias and can be mitigated computationally, but these methods are an incomplete solution and may sacrifice valuable base coverage.

We have devised a strategy to correct M-bias biochemically, using a custom formulated end repair mix. We show that this novel library prep workflow reduces M-bias by ~50% in diverse ctDNA assays. This reduction in M-bias results in more accurate methylation status calls and reduces the number of reads needed to confidently cover a CpG region of interest. Finally, we show that biochemical M-bias mitigation methods lower healthy hypomethylation background signal by ~10x. These changes increase robustness of hypomethylation biomarker quantitation and utility in ctDNA detection assays.

#4569 MiRNA signatures in liquid biopsy for early detection of non-small cell lung cancer.

Alessandro Pascon Filho[1], Giovana M. Stanfoca Casagrande[1], Rodrigo Sampaio Chiarantano[2], Fabiana de Lima Vazquez[2], Rui Manuel Reis M. Reis[1], Leticia Ferro Leal[1]

[1]Molecular Oncology Research Center, Barretos Cancer Hospital, Barretos, Brazil,[2]Department of Medical Oncology, Barretos Cancer Hospital, Barretos, Brazil

Background: Liquid biopsy-based biomarkers present a promising approach for non-invasive risk assessment and early detection of lung cancer.
Aim: To identify a miRNA signature in sputum and plasma samples as a non-invasive biomarker for early detection of non-small cell lung cancer (NSCLC).
Methods: A case-control study was conducted within the Barretos Cancer Hospital Screening Program, utilizing a low-dose CT mobile unit. Sputum and plasma samples were collected from high-risk controls (NLST and/or PLCO criteria; n=61) and NSCLC patients (n=62), matched for age, sex, and smoking history. MiRNA expression was analyzed using the nCounter HumanV3 miRNA panel (Nanostring™). Counts were normalized by the top stable miRNAs, followed by differential expression analysis ($p<0.05$). MiRNA selection was conducted using Boruta and LASSO. Dataset was partitioned into training and validation sets (85/15 split). Machine learning models (ML) were conducted (Azure) and performance was assessed using AUC (Table 1).
Results: In plasma, 68 miRNAs were identified as differentially expressed, and 8 miRNAs were retained following filtering. Among the all-tested models (Table 1), the best ML (Voting Ensemble) for the 8-miRNA plasma signature showed a high accuracy in both training (80.3%, AUC = 0.931) and validation set (73.3%, AUC = 0.892). In sputum, 46 miRNAs were identified as differentially expressed, and 7 miRNAs were retained. The best ML (SVD,Logistic Regression) for the 7-miRNA sputum signature showed a high accuracy in both training (81.42%, AUC = 0.943) and validation set (78.57%, AUC = 0.916). The two defined signatures exhibited no overlap in miRNAs between fluids.
Conclusion: This study identified two distinct miRNA fluid-specific signatures in sputum and plasma as potential non-invasive biomarkers for the early detection of lung cancer, underscoring their potential to advance precision-based screening and improve clinical decision-making in lung nodules management.

#4570 ESR1-mutant circulating tumor DNA is associated with plasma interleukin-2 (IL-2) leading to circulating tumor cell proliferation in metastatic breast cancer.

Q. Zhang[1], J. W. Zhang[2], J. Jiao[3], A. A. Davis[4], L. Gerratana[5], Y. Zhang[1], X. Wang[1], P. D'Amico[6], W. Qin[7], L. Flaum[1], A. Chawla[1], J. Lu[1], L. C. Platanias[1], W. J. Gradishar[1];

[1]Robert H. Lurie Cancer Center, Feinberg School of Medicine, Northwestern University - Evanston, Chicago, IL, [2]Northwestern University and Whitney Young High School, Chicago, IL, [3]Xijing Hospital, Innovation Center for Tumor Immunocytology Therapy Technology, Xijing Innovation Research Institute, Fourth Military Medical University, Xi'an, Shaanxi, China, [4]Washington University in St. Louis, St. Louis, MO, [5]University of Udine, Udine, Italy, [6]Merck, Rahway, NJ, [7]Xijing Hospital, Innovation Center for Tumor Immunocytology Therapy Technology, Xijing Innovation Research Institute, Fourth Military Medical University, Chicago, China

Introduction: Immune suppression is one of the most important causes of metastasis in metastatic breast cancer (MBC). Monitoring circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs) in patients with MBC is predictive of a worse prognosis. We previously reported that ctDNA *ESR1* alterations are associated with disease resistance in MBC (2022-ASCO #1057). Here, we sought to examine if ctDNA *ESR1* mutations correlated with plasma IL-2 level, a key cytokine in the MBC immune microenvironment, leading to changes in CTCs levels following systemic treatment.

Methods: Whole blood samples (7.5ml/each) were collected from 50 ER^+ (12 HER^+ and 38 $HER2^-$) patients with MBC before (base line, BL) and 3 months (follow-up evaluation, FE) after systemic therapy at Northwestern Memorial Hospital (IRB-NU16B06). Plasma ctDNA was analyzed using the Guardant360 NGS-based assay and CTC enumeration was performed in the FDA-approved CELLTRACKS System (Menarini). The CTCs were classified as $CK^+/DAPI^+/CD25^-$. ELISA (Fisher) for IL-2 was analyzed by using patients' plasma. Kruskal-Wallis test was used for statistics.

Results: Of the 50 patients, CTCs were found in 26 patients at BL level and in 24 patients at the follow-up FE level, respectively. *ESR1* mutations were identified in 12 hotspots from 10 patients ($ESR1^{Mut}$, 20%), while 40 patients had no mutations ($ESR1^{WT}$, 80%). The median number of total CTCs (/7.5 ml) was significantly higher in the $ESR1^{Mut}$ group compared to the $ESR1^{WT}$ group, CTCs were 23.2 vs. 10.3 (P=0.006) at the BL level and 27.8 vs. 14.7 (P=0.014) at the FE level, respectively. Meanwhile, the corresponding IL-2 levels were significantly lower in the $ESR1^{Mut}$ group compared to the $ESR1^{WT}$ group, with levels of 71.9 pg/ml vs. 81.3 pg/ml (P<0.01) at the BL level and 68.3 pg/ml vs. 77.1pg/ml (P<0.01) at the FE level. In the $ESR1^{Mut}$ group after treatment, patients with decreased IL-2 levels (median decrease of 17.38 pg/ml) showed an average increase of 9.4 CTCs, while those with increased IL-2 levels (median increase of 19.8 pg/ml) showed an average decrease of 3.3 CTCs. The results indicate that *ESR1* mutations correlated with lower IL-2 levels and higher CTC counts both before and after treatment, and changes in IL-2 levels were inversely correlated with changes in CTC counts.

Conclusions: ctDNA *ESR1* mutations were associated not only with lower plasma IL-2 levels but also with significantly higher CTC counts, even after systemic treatment. Our new findings demonstrate that, in addition to contributing to endocrine therapy resistance, *ESR1* mutations may also suppress plasma IL-2 and weaken the immune environment in MBC, leading to an increase in CTCs escaping immune surveillance and promoting metastasis. The synergy between ctDNA *ESR1* mutations and IL-2 expands the predictive role of ctDNA monitoring in assessing metastatic prognosis for clinical decision-making.

#4572 Preliminary results of perioperative minimal residual disease monitoring using a tumor-informed ctDNA assay in stage II-III breast cancer.

Jongseong Ahn[1], Jiwoong Jung[2], Yongjun Cha[1], Sunghoon Heo[1], Eunhye Kang[3], Woosung Chung[4], Hwang-Phill Kim[1], Duhee Bang[5], Sang-Hyun Song[1], Han-Byeol Lee[6], Wonshik Han[6], Tae-You Kim[1]

[1]IMBdx, Inc, Seoul, Korea, Republic of,[2]Department of Surgery, Uijeongbu Eulji Medical Center, Eulji University, Uijeongbu, Korea, Republic of,[3]Department of Surgery, Seoul National University College of Medicine, Seoul, Korea, Republic of,[4]DCGen Co. Ltd., Seoul, Korea, Republic of,[5]Department of Chemistry, Yonsei University, Seoul, Korea, Republic of,[6]Department of Surgery, Biomedical Research Institute; Cancer Research Institute; Seoul National University College of Medicine, Seoul, Korea, Republic of

Background: Detection of minimal residual disease (MRD) via circulating tumor DNA (ctDNA) is a promising biomarker for risk stratification and treatment guidance in cancer. This study evaluated the clinical impact of tumor-informed ctDNA MRD monitoring during the perioperative period in stage II-III breast cancer (BC).

Patients and Methods: Patients with stage II-III BC were prospectively enrolled at Seoul National University Hospital (Feb 2023 - Jul 2024). In the neoadjuvant systemic therapy (NST) arm, 20mL of whole blood (WB) was collected at eight perioperative time points: baseline (N00), after one cycle of NST (N01), after completion of NST (N03), and at 1, 6, 12, 24, and 36 months post-surgery. In the adjuvant chemotherapy (AC) arm, WB was collected at seven postoperative time points: 1, 2 months post-AC, and at 6, 12, 24, 36, and 48 months post-surgery. Plasma samples were analyzed using the CancerDetect^{TM} assay (IMBdx, Inc), a personalized tumor-informed MRD detection test. Samples with ≥2 detected mutations were classified as ctDNA+.

Results: A total of 231 female patients were enrolled, and 189 met eligibility criteria. This analysis included 111 patients with analyzable WES data, focusing on 103 NST patients. Number of patients analyzed were 103 at N00, 100 at N01, 94 at N03, and 46, 52, 10 at 1, 6, 12 months post-op, respectively. Median age was 51 years (25-72). There were 48 (46.7%) HER2-positive (HER+), 31 (30.1%) triple-negative (TNBC), and 24 (23.3%) hormone receptor (HR)-positive/HER2-negative (HR+/HER2-) BCs. 58 (56.3%) were stage II and 45 (43.7%) were stage III. Median number of target variants from WES was 53 (8-100) and higher in TNBC (67) and HER2+ BC (55) than HR+/HER2- BC (31.5, $p = 0.006$). Baseline ctDNA positivity was 81.6% (84/103), and ctDNA+ rates by subtype were as follows: 87.1% for TNBC, 83.3% for HER2+ BC, and 70.8% for HR+/HER2- BC ($p = 0.281$). The positive rate by stage were 77.6% and 86.7% for stage II and stage III, respectively ($p = 0.356$). Median ctDNA fractions were higher in TNBC (0.186) than HER2+ BC (0.020) or HR+/HER2- BC (0.018, $p = 0.031$), and higher in stage III (0.125) than stage II (0.012, $p = 0.003$).After NST completion, the overall pathologic complete response (pCR) rate was 57.3% and ctDNA positivity dropped to 20.2% (19/94), and ctDNA negativity at N03 was associated with higher pCR rates (62.7% vs. 26.3%, $p = 0.010$). Among N00 ctDNA+ patients, 79.7%
achieved clearance after NST, which correlated with higher pCR rates (66.1% vs. 12.5%, $p < 0.001$). In this subpopulation (median follow-up 13.6, 6.7-19.9), only 4.9% (2/41) of the pCR patients were ctDNA+, no recurrences were reported.

Conclusions: Tumor-informed ctDNA assays correlated with NST response in stage II-III BC. Variations in target variants recovered from WES may explain differences in ctDNA positivity by subtype, which should be considered for clinical interpretation and application.

#4573 Verified preanalytical workflow for urine cfDNA including the PAXgene Urine Liquid Biopsy Set allows transport and storage at varying temperatures and is unaffected by blood interference.

Daniela Mancarella, Laura Sofie Witthaus, Moritz Meyer, Julia Rutsch, Moritz Rath, Lisa Erkelenz, Thorsten Voss

QIAGEN GmbH, Hilden, Germany

INTRODUCTION: Urine has become an important standalone source for biomarkers research using cell-free DNA (cfDNA). In addition, urine cfDNA has been shown to provide complementary information to blood cfDNA. However, cfDNA degradation, dilution by genomic DNA (gDNA) released from body cells and bacteria can impact the cfDNA profile and prohibit transport and storage of urine, making reliable urine molecular analysis impossible. Furthermore, interfering substances, such as blood, could affect the preanalytical workflow including the cfDNA isolation and hence, affect the research outcome. In this study, we tested the PAXgene Urine Liquid Biopsy Set (RUO) for urine collection and cfDNA stabilization in a standardized preanalytical workflow with challenging sample conditions and show that it enabled reliable analysis of cfDNA.

METHODS: Stabilized urine from apparently healthy, consented individuals was collected in the PAXgene Urine Collection Cup. Female urine specimens were spiked with cell-free male urine supernatant and, where indicated, with 1% v/v female blood. Urine was stabilized using the PAXgene Urine Liquid Biopsy Tube or was left unstabilized. Urine samples were stored for 3 days or 10 days at room temperature (RT, 15-25°C) on the lab bench or incubated for up to 10 days at varying temperatures mimicking real-world transport scenarios. Two temperature profiles were tested: Cold profile (4 hours (h) at RT , followed by 3 days (d) at 5°C, followed by 6 d at 12°C and 8 h at RT) and warm profile (3 d at 25°C, followed by 12 h at 37°C, followed by 1 d at 25°C, followed by 5 d at 5°C and 10 h at RT). Additional urine samples were transported via air from Germany to the USA. Where indicated, transported samples underwent dropping and vibration testing. cfDNA was isolated from the urine supernatant either using automated or manual QIAGEN kits. Autosomal and male-specific targets were quantified by qPCR on the QIAGEN RGQ instrument.

RESULTS: Stabilization with the PAXgene Urine Liquid Biopsy Tube increased the cfDNA yield isolated from urine. Furthermore, it minimized the loss of the cfDNA target of interest, minimized the dilution of the cfDNA target of interest by gDNA release and minimized bacterial growth during urine storage at challenging warm and cold weather temperature profiles and real-life urine transport. Blood concentration of 1% v/v in urine did not affect the yield of cfDNA isolated from the PAXgene Urine Liquid Biopsy Tube.

CONCLUSION: The optimized and standardized preanalytical workflow for cfDNA analysis from urine using the PAXgene Urine Liquid Biopsy Set enabled urine storage and transport and reliable analysis of urine cfDNA under challenging research settings. Furthermore, 1% v/v blood in urine did not interfere with cfDNA isolation and detection, underlining the robustness of the verified preanalytical workflow.

#4574 Epigenetic comparison of colorectal cancer tumors and patient-matched cfDNA.

Seeta Rajpara[1], Chris Gooden[1], Dan Weisenberger[1], Raed Jaffal[1], Hanna Jaynes[1], Carmen Chavez[1], Mariana Stern[1], Heinz-Josef Lenz[1], David Craig[2], John Carpten[2], Bodour Salhia[3]

[1]Keck School of Medicine of USC, Los Angeles, CA,[2]City of Hope, Duarte, CA,[3]Translational Genomics, Keck School of Medicine of USC, Los Angeles, CA

Colorectal cancer (CRC) is the third leading cause of cancer-related deaths in the U.S., with Hispanic/LatinX (HL) populations facing disproportionately higher mortality rates. Despite advancements in CRC screening and treatment, racial disparities in outcomes persist, partly due to limited access to early and precise detection tools. This study addresses these inequities by developing an innovative cfDNA methylation-based liquid biopsy for detecting minimal residual disease (MRD), a critical predictor of cancer recurrence that could improve outcomes in underrepresented populations. In this study, we performed methylation profiling using the EPIC v2 array on tumor tissue samples and cfDNA whole-genome bisulfite sequencing (WGBS) on 27 (16 females, 11 males; mean age 51.7 years) patient-matched blood plasma samples collected after tumor resection. Clinical data was abstracted to annotate each sample with tumor characteristics and the patient's disease status. Disease status was determined as either no evidence of disease (NED) for over 2 years or active disease (AD). Next, to identify markers potentially associated with recurrence, we performed differential DNA methylation analysis between patients with AD (N = 14) and who have been NED (N = 13) for greater than 2 years and examined the overlap of differentially methylated regions (DMRs) between tissue and cfDNA. These markers would essentially represent tumor burden in patients with AD. We identified 175 DMRs in tissue, (88% hypermethylated and 12% hypomethylated) and 350 DMRs in cfDNA (94% hypermethylated and 6% hypomethylated) between patients with AD versus NED status. Tissue DMRs were significantly more promoter-enriched (78.3% tumor vs. 55.1% cfDNA, $p=1.87x10^{-7}$) and had fewer gene body DMRs compared to cfDNA DMRs (10.9% tumor vs. 27.4% cfDNA, $p=1.03x10^{-5}$). Enhancer regions were notably more frequent in tissue than cfDNA (10.9% tumor vs. 0% cfDNA, $p=4.34\ x10^{-10}$), while intergenic regions were more enriched in cfDNA than tumors (17.4% tumor vs. 5.1% cfDNA, $p=5.94x10^{-5}$). Unsupervised clustering of showed clear separation of samples by disease status in both cfDNA and tissue samples. Furthermore, correlation analysis of beta values between matched tumors and cfDNA showed stronger correlations for AD samples ($R^2=0.68$) compared to NED ($R^2=0.61$), suggesting that tumor burden of AD samples are detectable in circulation to a higher degree and can have utility in biomarker development. The distinct methylation patterns observed between CRC tissue and cfDNA highlight the potential of liquid biopsies to capture tumor burden in circulation, particularly in active disease cases.

#4575 mRNA expression in paired saliva and blood samples from different cancer indices.

Srinivas V. Koduru, Mark Kidd, Abdel Halim

Wren Laboratories, LLC, Branford, CT

Introduction: Liquid biopsies have become gradually accepted, as a non-invasive alternative to traditional tumor biopsies in the field of oncology. The FDA and other regulatory bodies have already approved blood-based tests and researchers are working on validating urine, saliva and buccal swabs as alternative sample sources. This study's main goal was to compare mRNA expression levels, in matched blood and saliva samples from cancer patients, as well as apparently healthy individuals.
Methods: We collected matched saliva and blood samples from 35 individuals, including 27 patients diagnosed with cancers including Breast (4), Lung (4), Colon (5), Melanoma (3), Pancreatic (4), Prostate (7) cancer along with 9 healthy participants. The samples were collected in Wren's proprietary buffer kit and RNA was extracted via TRIzol-based method. RNA sequencing was carried out on an Illumina Novaseq 6000. The data was analyzed using high performance computing (HPC) through PartekFlow software with the BWA aligner (hg38).
Results: Nearly 94% of the genes found in saliva and 60% of the genes in blood were matched across all cancer types, with the exception of pancreatic cancer in blood, which showed only about 30% matching. We further evaluated both groups based on housekeeping genes (1003 genes) and the known cancer-associated genes as listed in the OncoKB database (1154 genes) for each cancer type. In addition, we investigated the expressions of 412 and 241 cancer genes priorly validated and used in IVD assays, respectively, in our lab. We found that 78-80% of the genes in blood were common across all cancer types, while saliva samples showed 64-73% commonality. Only 43% of genes detected in saliva from pancreatic cancer matched other cancer types. Out of the 241 internally validated cancer genes 68-71% genes were detected in blood and 57-65% in saliva in this cohort except pancreatic cancer where only 30% was detected. Out of the 412 genes, we found 67-69% in blood and 51-58% in saliva, again with the exception of pancreatic cancer, exhibits only 26% of genes. Additionally, 98% of housekeeping genes from blood and 71-87% of housekeeping genes from saliva were consistent, except for pancreatic cancer, which showed only 35%.
Conclusion: Our study indicates that the majority of mRNA (94%) detected in saliva from various cancer types was also detected in blood, compared to healthy controls. This suggests that exploring mRNA-based biomarkers development in saliva could be even a more convenient alternative to blood.

#4576 Sequential purification of cfDNA and cfRNA from blood stabilized in Nucleic Acid BCT using an automated workflow.

Nicholas M. George, Lisa Bartron, Jordan LaRue

Streck Laboratories, Inc.R, La Vista, NE

Liquid biopsies, which interrogate body fluids in search of analytes released by healthy or diseased cells, have become increasingly common in oncology. Foremost among the technologies used are those that employ nucleic acids, such as cfDNA and cfRNA. While cfDNA remains the primary choice for mutation detection, cfRNA is also informative, as it is released by both live and dying cells and represents the true disease state-mediated transcriptional output. Further, gene fusion events, which may be expressed at high levels in tumors, are easier to detect using cfRNA. As such, combined analysis of both cfDNA and cfRNA could provide a more comprehensive analysis. However, if blood samples are not stable, cell death or activation could lead to spurious increases in both analytes. To address this issue, Streck developed Nucleic Acid BCT (NAC), a stabilizing blood collection tube formulated to minimize changes to cfDNA and cfRNA concentrations during storage and shipping. To determine whether a combined cfDNA and cfRNA isolation protocol is compatible with NAC, blood from self-proclaimed healthy donors was drawn into EDTA and NAC and stored at ambient temperature for up to 7 days, plasma was isolated, and cfDNA and cfRNA were sequentially isolated using the Promega Maxwell RSC System with applicable kits. To mimic mutational events observed in lung cancer, synthetic 170-mer oligonucleotides encoding the EGFRL858R or EGFRdel19 events were spiked to an anticipated 1% MAF. Likewise, an RNA Ultramer encoding the EML4-ALK fusion was spiked at a copy number representative of that observed in clinical samples. Samples were then analyzed using ddPCR to determine concentrations of EGFRL858R and EGFRdel19 relative to the wildtype allele and EML4-ALK copy number relative to the housekeeping gene UBB. Our data support the combined use of the Promega Maxwell system with applicable extraction kits with plasma isolated from NAC. As expected, samples collected into NAC had minimal increases in cfDNA and cfRNA concentrations during storage compared to blood samples stored in EDTA. Baseline MAF of the spiked mutant cfDNA alleles was completely lost in samples stored in EDTA, while equivalent blood samples stored in NAC maintained MAFs reflective of draw time levels. A similar trend was observed when plasma RNA expression was investigated where samples collected in NAC had similar concentrations of cfRNA at draw and after storage, but those collected into EDTA did not. Specifically, EML4-ALK copy number relative to the UBB housekeeping gene fell precipitously in EDTA but maintained draw-time frequencies in blood samples stored in NAC. Taken together, these data indicate that the combined use of NAC and the sequential nucleic acid extraction protocol described herein can provide the liquid biopsy community with a robust workflow for both blood sample stabilization and multianalyte extraction and downstream analysis.

#4577 Ultra-sensitive detection of circulating tumor DNA using methylation anchor probes to enhance hepatocellular carcinoma (HCC) detection.

Nan Lin[1], Ying Mei[2], Nina Guanyi Xie[3], Yangjunyi Li[3], Liyuan Zhao[3], Zhihui Xu[3], Chen Zhu[3], Yujie Chen[3], Yalong Zhang[3], Bingqiang Fu[3], Jinlei Song[3], Min Li[3], Xiaohui Wu[3], Xueguang Sun[3]

[1]Department of Hepatobiliary Surgery, The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, China,[2]Health Medical Center, The Second Affiliated Hospital, Chongqing Medical University, Chongqing, China,[3]Shanghai Weihe Medical Laboratory Co., Ltd, Shanghai, China

Background: Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related death worldwide. Circulating cell-free DNA (cfDNA) methylation markers offer a promising approach for early cancer detection and therefore improve patient outcomes. Among the many CpG sites with altered methylation status, only specific methylation patterns (haplotypes) are disease-related and are often rare in clinical samples. Current cfDNA-methylation signal-based methods lack sufficient sensitivity, particularly in detecting low-abundance tumor-derived DNA with limited sequencing reads. Here, we introduce an experimental method to address this issue by using short hybrid capture probes specifically designed to bind tumor-derived DNA methylation haplotypes, while minimizing binding to wild-type fragments. This approach reduces the number of sequencing reads required and improves assay sensitivity.
Methods: Methylation haplotypes were selected based on in-house whole-genome bisulfite sequencing (WGBS) data from tumor tissue and paired normal tissues adjacent to the tumors (NATs) of HCC patients. Further filtering was applied using cfDNA data from a group of healthy individuals. Short hybrid capture probes were then rationally designed to specifically target HCC-related methylation haplotypes. The panel was tested on 118 clinical samples (57 HCC patients and 61 controls). Data processing included the quantification of haplotype frequencies, followed by mutual information-based feature selection to identify the most distinctive markers in the training data to enhance classification performance. Finally, a boosted tree model (XGBoost) was applied to predict cancer.
Results: All samples were divided into a training set (75%) and a test set (25%), ensuring a balanced distribution of controls and cancer stages. The top 128 features were selected for both early (Stage I & II) and late (Stage III & IV) HCC. Across all stages of HCC, the panel achieved 97.6% specificity and 97.2% sensitivity in the training set, and 94.7% specificity and 95.2% sensitivity in the test set.
Conclusions: We developed a novel and cost-effective method to specifically enrich HCC-related methylation haplotypes, enabling accurate and ultra-sensitive detection of HCC. In the future, we will test more HCC cancer, precancer, and benign samples to further assess our assay performance. Also, our method is versatile and holds potential for the early detection and monitoring of other cancer types, paving the way for broader applications in precision oncology.

CLINICAL RESEARCH: Molecular Diagnosis, Molecular Characterization and Theranostics of Tumors
Poster Session

#4582 Molecular and clinical characterization of claudin-18 expression in pancreatic cancer.

T. Satake[1], T. Shibuki[1], C. Morizane[2], M. Ueno[3], S. Boku[4], R. Sugimoto[5], S. Shimizu[6], Y. Kawamoto[7], Y. Horita[8], N. Matsuhashi[9], S. Itoh[10], N. Okano[11], T. Ohta[12], K. Ohtsubo[13], T. Hashimoto[1], T. Fujisawa[1], M. Imai[1], Y. Nakamura[1], H. Bando[1], T. Yoshino[1], M. Ikeda[1]; [1]National Cancer Center Hospital East, Kashiwa, Japan, [2]National Cancer Center Hospital, Tokyo, Japan, [3]Kanagawa Cancer Center, Yokohama, Japan, [4]Kansai Medical University Hospital, Osaka, Japan, [5]National Hospital Organization Kyushu Cancer Center, Fukuoka, Japan, [6]Saitama Cancer Center, Saitama, Japan, [7]Hokkaido University Hospital, Sapporo, Japan, [8]Saitama Medical University International Medical Center, Hidaka, Japan, [9]Gifu University Graduate School of Medicine, Gifu, Japan, [10]Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan, [11]Kyorin University Faculty of Medicine, Tokyo, Japan, [12]Kansai Rosai Hospital, Amagasaki, Japan, [13]Kanazawa University Hospital, Kanazawa, Japan

Background: Claudin-18 (CLDN18) is a major component of tight junctions located on the cell membrane surface. CLDN18.1 and CLDN18.2 are specifically expressed in normal lung tissues and gastric mucosa, respectively. However, CNDN18.2 is known to be frequently expressed in gastric cancer, and also overexpressed in pancreatic cancer (PC) in 30-60% of cases. Clinical features of PC due to CLDN18 expression have been reported in a limited number of cases, and molecular features remain unknown. Here, we evaluated molecular and clinical characterization of *CLDN18*expression in advanced PC.

Methods: This study used data from the MONSTAR-SCREEN-2 study, a nationwide molecular profiling project targeting solid tumors. Patients with advanced PC who received first-line systemic therapy and underwent tissue whole exome/transcriptome sequencing by Caris Life Sciences (Phoenix, AZ, USA) were included. Transcripts per million (TPM) for *CLDN18* expression were categorized into high and low groups based on the median value. The clinical and molecular characteristics of these groups were compared. Gene ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment studies were carried out in R language using the programs clusterProfiler, enrichplot, and ggplot2. Differentially expressed genes identified based on log2 fold change greater than 1 and p-values less than 0.05 were used for pathway analysis. Only results with p-values and q-values no more than 0.05 were deemed significantly enriched.

Results: A total of 104 patients were enrolled in the study. In the *CLDN18*-high and -low groups, the median age was 69 years/67.5 years; female was 57.7%/34.6%; tumor stage was recurrence 51.9%/30.8%, locally advanced 7.7%/9.6%, and metastatic 40.4%/59.6%; metastatic site was liver 48.1%/61.5%, lymph node 9.6%/11.5%, lung 17.3%/11.5%, and peritoneum 19.2%/9.6%; treatment regimens were FOLFIRINOX 21.2%/36.5% and gemcitabine + nab-paclitaxel 63.5%/51.9%, respectively. Objective response rate was 17.3%/19.2%, and disease control rate was 75.0%/63.5% (P = 0.29). The median overall survival was 16.5 and 18.7 months; hazard ratio (HR) 0.9 (95% confidence interval [CI] 0.5-1.7); P = 0.81. The median progression free survival was 7.3 and 5.3 months; HR 1.3 (95%CI 0.8-2.1); P = 0.28. The frequent gene alterations were *KRAS*, *TP53*, *SMAD4*, and *CDKN2A*, regardless of *CLDN18* high and low groups. Gene expression such as *GATA6* and *CTSE*, known as the characteristics of the classical type of Moffitt's classification, was significantly higher in *CLDN18*-high group, and gene expression such as *S100A2* and *KRT6A*, known as the characteristics of the basal-like type, was significantly higher in *CLDN18*-low group.

Conclusion: There was no significant difference in treatment efficacy and prognosis of systemic therapy between the *CLDN18*-high and -low groups. *CLDN*-high and -low were associated with known classical and basal-like molecular profiles.

#4583 *In-situ c*haracterization of small blue round cell tumor proteome and N-glycome.

C. Bollwein[1], K. Schwamborn[1], T. Tran[2], **A. Ly**[2], N. H. Patterson[2], J. P. L. Goncalves[1];
[1]Technical University of Munich, Munich, Germany, [2]Aspect Analytics, Genk, Belgium

Background: Small blue round cell tumors (SBRCT) is an umbrella term for different tumor types with similar histological presentation that can originate from entirely different tissues. Correct diagnosis is crucial, though challenging, as treatment strategies and prognosis vary significantly between tumor entities. Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) is a label-free analytical technique to analyze and visualize the spatial distribution of different classes of molecules, like peptides, glycans, lipids, and metabolites. In this study, MALDI-MSI was used to profile tryptic peptides and N-glycans from archival SBRCTs samples. The data was used to create classification algorithms to reliably distinguish the different entities and to identify discriminatory features between the tumor types.
Methods: SBRCT cases (n = 26) were assembled in a tissue microarray: Ewing sarcoma (n = 5), rhabdomyosarcoma (n = 5), neuroendocrine carcinoma (n = 5), acute lymphoblastic leukemia/lymphoma (n = 5), nephroblastoma (n = 3), and neuroblastoma (n = 3). Every case was represented by 3 to 4 tissue cores to account for tumor variability. One TMA section was subjected to on-tissue N-glycosidase F enzymatic digestions, and a second section with trypsin, followed by matrix application and measurement on a mass spectrometer (rapifleX, Bruker). Afterwards, the sections were H&E stained, digitized and underwent histopathological annotation (QuPath, version 0.4.3)Data analysis was performed using SCiLS Lab (Bruker, v.2024a), Weave (v.1.0, Aspect Analytics), and Python (v.3.9). Weave was used to co-register all datasets, combining the peptide and glycan data into a single dataset for comparison of analytes and histology, and interactive assessment of data analysis.
Results: MSI data was employed for the training and validation of different classification algorithms (gradient boosting, support vector machine, k-nearest neighbor, and linear discriminant analysis) using 10-fold cross-validation on pixel level. Multiclass classification was implemented as a one vs. rest (one entity vs. all other entities) and one vs. one (one entity vs. every other entity) approach. For the one vs. rest strategy, mean accuracy of all classifiers was 90.5 % with a mean accuracy of 88.7 % for peptide analysis, 92.4 % for glycan analysis and 95 % for the combined dataset. For the one vs. one approach, mean accuracy of all classifiers was 92.4 % with a mean accuracy of 88.8 % for peptide analysis, 96.1 % for glycan analysis and 97 % for the combined dataset.
Conclusions: Peptide and N-glycan mass spectrometry imaging, particularly in combined form, has huge potential to aid in distinguishing between different SBRCT entities. Future work will aim to expand the patient cohort and explore alternative statistical methods to further improve classification performance.

#4584 Advancing non small cell lung cancer detection and subtyping by MasSpec Pen technology.

Yasmin Shanneik[1], Bryan Burt[2], Maheshwari Ramineni[3], Livia S. Eberlin[1]

[1]Surgery, Baylor College of Medicine, Houston, TX,[2]Surgery, University of California, Los Angeles, CA,[3]Pathology, Baylor College of Medicine, Houston, TX

Introduction: Non-small cell lung cancer (NSCLC) is the most aggressive and frequently diagnosed form of lung cancer. Histological and molecular subtyping are critical in lung cancer treatment-decision making. However, despite recent advances, the 5 year survival rate remains low. Within the field of mass spectrometry (MS), the MasSpec Pen (MSPen) technology stands out as a nondestructive technique employed intra-operatively for cancer detection. The MSPen deploys a single droplet of sterile water onto tissue, facilitating the extraction of molecules upon contact in real-time within seconds. Here, we employ the MSPen for NSCLC diagnosis based on molecular signatures, with a specific focus on classifying major histological subtypes and identifying key proteogenomic markers. Furthermore, we tested the reproducibility of the MSPen, using samples collected across multiple centers and analyzed by different personnel.
Methods: 319 frozen human lung tissues (n=177 normal lung and n=142 NSCLC) were analyzed in negative ion mode using the MSPen coupled to a Q Exactive Orbitrap MS (Thermo Scientific). Following analysis, the analyzed region was marked with a surgical pen, frozen, and sectioned at 10µm. It was then histologically stained and evaluated by a pathologist to confirm diagnosis and subtype. Proteogenomic markers for lung cancer samples, such as EGFR and PD-L1, were obtained from clinical data or determined using biomarker and sequencing analysis. Mass spectra were processed and subjected to statistical classification using the least absolute shrinkage and selector operator (Lasso).
Results: MSPen analyses enabled the detection of hundreds of biomolecules in seconds with high sensitivity, specificity, and accuracy. Negative ion mode mass spectra presented rich molecular profiles characterized by multiple cancer-specific markers. Normal lung tissue showed high relative abundances of phosphatidylglycerol (PG)(34:1) and PG(36:2). Mass spectra of NSCLC showed a different profile with high relative abundances of phosphatidylethanolamine (PE)(38:4) and phosphatidylinositol (PI)(38:4). Samples with high PD-L1 expression also presented a distinct lipid pattern with a high relative abundance of PG lipids, whereas samples with high EGFR expression were enriched with PE lipids. Histological staining confirmed no macro- or microscopic tissue damage, highlighting the potential of the MSPen in clinical settings. Lasso classification of MSPen data allowed for the predictive performance of the molecular evaluation and diagnosis of the NSCLC samples. Lasso classification yielded exceptional performance, with an AUC=0.9 in the training set and an overall accuracy of 93% across training and test sets of samples compared with the pathological evaluation.
Conclusion: Our results highlight the robustness of MSPen technology and its utilization for lung cancer detection and subtyping in clinical procedures.

#4585 Exploiting intrinsic bioelectrical signatures for label-free immune cell sorting.

Dean E. Thomas[1], Ilyssa E. Ramos[2], Kyle S. Kinskie[1], Kyle M. Brown[1], James M. Cherry[3], **Alexandra R. Hyler**[1]

[1]CytoRecovery, Inc., Blacksburg, VA,[2]Miller School of Medicine, University of Miami, Miami, FL,[3]Center for Human Immunology, Autoimmunity, and Inflammation (CHI), National Institute of Allergy and Infectious Diseases (NIAID), Bethesda, MD

Immune cell separation from heterogeneous samples is crucial for various downstream research applications. Standard methods for cell purification are laborious and require additional fluorescent or magnetic labels to identify cells of interest. Approaches contingent upon the use of labels may interfere with downstream testing and analysis, like single cell sequencing or downstream oncology research. Label-free cell sorting by electrokinetic dielectrophoresis (DEP) has recently been used to characterize and separate target subpopulations from complex samples. Available literature describes the use of DEP for isolation and enrichment of limited cell types. However, technical challenges and uncertainty have restricted DEP's commercialization and widespread implementation for biological use. To address this pitfall, we have exploited the strength of the CytoR1 platform for label-free sorting of T-cells, B-cells, and monocytes from bulk sample preparations. Characterization and isolation of these cells will support the CytoR1 platform's capabilities. Variables like frequency, voltage, and flow rate are incorporated in the profiling of target cells, facilitating the development of custom workflows to enrich individual immune cell populations from heterogeneous samples without labels. Platform validation will involve downstream fluorescence-activated cell sorting (FACS) and potential RNA sequencing to confirm the accuracy of label-free sorting. Here, we introduce a workflow implementing a microfluidics-based, benchtop cell-sorting technology, the CytoR1, for precise enrichment and capture of unlabeled target cell populations.

#4586 Multi-scale multi-omic assessment of matched synchronous colorectal cancer liver metastases using multiple spatial transcriptomic tools.

Colin Wood, Luke McNickle, Andrew Cameron, Ritupam Sarma, Vaidehi Pandya, Joao Da Silva Filho, Colin steele, John Cole, Joanne Edwards, Paul Horgan, Campbell Roxburgh

University of Glasgow - Institute of Cancer Sciences, Glasgow, United Kingdom

Background: Primary colorectal cancer (CRC) is manageable with surgical and non-surgical therapies. However, high affinity for distant spread to the liver (CRLM) and lack of subsequent therapeutic options has maintained CRC as one of the most lethal malignant diseases worldwide. Tumors consist of malignant cells as well as a complex micro-environment (TME) of stromal and immune components, all of which have been implicated in disease severity and treatment resistance. Spatial transcriptomics (ST) is novel technology which allows molecular profiling of tissue while preserving tissue architecture. By studying matched CRC and CRLM with modern ST tools which allow scrutiny of the malignant cells and TME we aim to learn more about mechanisms of metastasis and why CRLM are resistant to treatment.
Methods: 41 patients (29 untreated) underwent synchronous resection of CRC and CRLM within our institution between April 2002 and June 2010 (64% 5 year mortality). Paired CRC and CRLM from 4 patients underwent single-cell ST assessment on the Nanostring CosMx Spatial Molecular Imager (23 fields of view (FOV)). Paired CRC and CRLM from 4 patients underwent regional ST assessment (PanCk+,PanCk-,aSMA+ comprtments) on the Nanostring GeoMx Digital Spatial Profiler (116 FOV). Paired CRC and CRLM from 2 patients underwent regional ST assessment on the Visium 10X Genomics platform (4 FOV).
Results/Conclusions: GeoMx offers entire slide visualization with bespoke and convenient FOV selection with reliable segmentation of TME components with whole transcriptome available. Visium 10X offers high resolution ST assessment of large pieces of tissue but slide preparation is difficult without specific tools. CosMx offers single-cell in-situ allowing the highest resolution with neighborhood and cellular interaction analysis possible but at lower plex and increased complexity. All technologies demonstrated differences between CRLM and their matched CRC. CRLM were generally more collagenous and chemokine rich. Furthermore, transcriptomic and morphological differences exist between CRLM from different patients. Specifically, CosMx demonstrated that epithelial cells and fibroblasts in CRC express MZTA compared to COL9A2, NEAT1 and IGF2 in CRLM. Neutrophils in CRLM under-express CXCL8. Desmoplastic CRLM were chemokine high whereas replacement CRLM over-expressed IGF2, DUSP5 and COL9A2. GeoMx identified MET and IL17 as potential drug targets in CRLM. These data suggest that CRLM evolve once metastasis is established and the immune response of the host to the metastasis is pivotal. Future therapeutic goals should emphasize personalized medicine and molecular staging of CRLM with specific targeted therapies.

#4587 Retrospective analysis of claudin-18.2 expression in ethnically diverse patients with gastroesophageal adenocarcinoma.

Sara Wallam, Jimyung Park, Ryan H. Moy

Department of Medicine, Columbia University Irving Medical Center, New York, NY

Background: Claudin-18.2 (CLDN18.2), a tight junction molecule, is a novel therapeutic target for patients with advanced gastroesophageal adenocarcinoma. Recent phase III trials demonstrated improved survival with the addition of an anti-CLDN18.2 antibody (zolbetuximab) to first-line chemotherapy. However, expression of CLDN18.2 and its association with other biomarkers, especially in racial and ethnic minorities, remains poorly defined. We evaluated CLDN18.2 expression, its association with demographic and clinicopathologic characteristics, and its prognostic potential in a small cohort of ethnically diverse patients.
Methods: We conducted a single-center retrospective cohort study among patients with gastroesophageal adenocarcinoma in whom CLDN18.2 immunohistochemistry had been performed. Positivity was defined as moderate-to-strong expression in ≥75% of tumor cells. We extracted demographic and clinicopathologic data from the electronic medical record. Associations with CLDN18.2 were investigated using the t-test and chi-square test. Survival curves were calculated using the Kaplan-Meier method and compared using the log-rank test. P-value <0.05 was considered statistically significant.
Results: Among 75 evaluable patients, 42.7% were CLDN18.2 positive. Mean age was 66.2 years (SD, 13.2 years), 34.7% were female, and 62.7%, 18.7%, and 18.7%, had primary gastric, gastroesophageal junction, and esophageal tumors, respectively. At diagnosis, 32% were metastatic. The cohort was 49.3% White, 12% Black, 8% Asian, and 30.7% Other/Unknown, and 20% identified as Hispanic. By ethnicity, 53.3% of Hispanic patients were CLDN18.2 positive compared to 38.2% of non-Hispanic patients. By race, 37.8% of White, 44.4% of Black, and 50% of Asian patients were CLDN18.2 positive. In univariate analyses, CLDN18.2 positivity was significantly associated with female sex (p=0.002) and HER2 negativity (p=0.040). TP53 mutations were found in 65.2% of patients with available next generation sequencing data, but there was no association with CLDN18.2. CLDN18.2 was also not associated with response to neoadjuvant therapy in non-metastatic patients, progression-free survival in metastatic patients, or overall survival in the total population.
Conclusions: This study provides new information on CLDN18.2 expression in an ethnically diverse population and suggests that Hispanic patients may have higher rates of CLDN18.2 positivity than non-Hispanic patients. However, this finding lacks statistical significance, likely due to the small sample size. We also demonstrate the association between CLDN18.2 and female sex and lack of association with survival, consistent with published data. Future research should further investigate differences in CLDN18.2 expression and identify subpopulations of patients who may benefit from CLDN18.2 targeted therapies.

#4588 Cell states and resistance in colorectal cancer: What can we learn from patient-derived organoids?.

M. Norkin[1], P. Hernandez Lopez[2], C. Esposito[3], G. Ramzy[4], M. A. Carbo[4], P. Ordonez-Moran[5], P. Nowak-Sliwinska[4], S. Piscuoglio[3], K. Homicsko[1], J. Huelsken[2];

[1]CHUV Lausanne University Hospital, Lausanne, Switzerland, [2]EPFL, Lausanne, Switzerland, [3]University Hospital Basel, Basel, Switzerland, [4]University of Geneva, Geneva, Switzerland, [5]University of Nottingham, Nottingham, Switzerland

Colorectal cancer (CRC) is among the three most commonly diagnosed cancers and one of the major causes of cancer-related morbidity and mortality. Despite the discovery of several predictive biomarkers, the 5-year survival rate for metastatic CRC is 15%. A hallmark of CRC is inter- and intra-patient heterogeneity. Moreover, chemo-radiation and targeted therapies often result in producing resistant clonal populations that are unresponsive to the ongoing treatment. Revealing a comprehensive landscape of primary and metastatic CRC cell states and their evolution during chemotherapy treatment provides the potential for tackling this problem. Patient-derived organoids are a valuable research tool to study the biology of the disease as well as for drug screening in personalized medicine. To that end, we have collected a biobank of 90 PDOs from primary and metastatic CRC patients, as well as autologous cancer-associated fibroblasts (CAFs) and tumor infiltrating lymphocytes (TILs). We have performed whole-exome sequencing (WES), scRNA-seq and bulk RNA-seq of original tumors and PDOs, with and without chemotherapy treatment. We have discovered specific and common transcriptional cell states within PDOs and tumors, as well as the multiple responses to treatment confirming high heterogeneity of CRC. Combination of these results with clinical data can predict new biomarkers.

#4589 Molecular subtypes identified in lung adenocarcinoma using a large-scale, real-world dataset.

Akul Singhania, Prerna Jain, Ezgi Karaesmen Rizvi, Radia M. Johnson, **Justin Guinney**

Tempus AI, Inc., Redwood City, CA

Introduction

Despite advances in targeted and IO therapies, lung adenocarcinoma (LUAD) remains a high unmet clinical need due to its complex molecular landscape. Previous studies have focused on early-stage disease with smaller patient populations to identify molecular subtypes in LUAD. This study characterized the molecular profiles and clinical features of LUAD patient tumors from a large real-world dataset (RWD) containing early and late-stage patients as well as primary and metastatic sites.

Methods

The analysis cohort consisted of de-identified clinical and genomic records of patients diagnosed with LUAD and profiled with Tempus' targeted DNA and whole-transcriptome RNA assays. Molecular subtyping was performed using non-negative matrix factorization on RNA-seq data from primary lung site tumors (n=3,975; Stage III and IV: 68%). A subtype classifier was trained on tumor-intrinsic features and applied to tumors from primary non-lung and metastatic sites (n=3,981; Stage III and IV: 99%). quanTIseq was used to estimate immune cell proportions. Progression free survival (PFS), defined as the time from the start of first metastatic therapy to the date of first progression or death from any cause, was assessed in LUAD patients with available data (17.6%).

Results

Six distinct molecular subtypes were identified: C1-C6. The C1 subtype (31.8%; median PFS 10.4 months, 95% CI 7.4-13.2) had the highest tumor mutation burden (22.1% with TMB≥10) and PD-L1 status (54.2% with TPS≥50), and was characterized by TP53 mutations (78.62%) with co-mutations in NF1 (log odds ratio 1.8, $p<0.05$). The C2 subtype (18.82%; PFS 8.8 months, 95% CI 6.6-10.7) showed high EGFR mutation frequency (33.31%), and lower immune cell proportion (41%). In contrast, the C3 subtype (12.63%) showed high prevalence of EGFR mutations (32.37%) with high immune activation (57%), and had the best prognosis (PFS 17.4 months, 95% CI 11.7-36). C3 comprised a large portion of primary lung samples (23%) and was overrepresented in early-stage primary tumors (Stage I and II: 61%) . The C4 subtype (7.03%; PFS 9.8 months, 95% CI 5.5-16.7) was enriched with a fibrotic tumor microenvironment (TME) and an increased proportion of immune cells (54%). The C5 subtype (23.15%) had the worst prognosis (PFS 5.6 months, 95% CI 4.3-7.4), lowest immune cell proportion (36%), and a mutational profile characterized by decreased EGFR mutations (5.6%) and increased STK11 (36.85%), KEAP1 (19.67%), and SMARCA4 (15.95%) mutations. Lastly, the smallest subtype, C6 (6.6%; PFS 9.7 months, 95% CI 6.4-15.4) was characterized with a fibrotic TME.

Conclusions

This study highlights the heterogeneity of LUAD, revealing patient states with distinct mutational landscapes, TME profiles, and clinical outcomes in a real-world setting. Future work is needed to explore how these subtypes may inform strategies for disease management and new treatment modalities.

#4590 Clinicopathological analysis of pulmonary high-grade neuroendocrine carcinoma with high expression of hepatocyte nuclear factor 4 alpha.

Kei Asayama, Ryota Matsuoka, Aya Shiba, **Daisuke Matsubara**

University of Tsukuba, Ibaraki, Japan

Pulmonary high-grade neuroendocrine carcinomas (HGNECs), including small cell carcinomas (SCLCs) and large cell neuroendocrine carcinomas (LCNECs), are highly aggressive and have a poor prognosis. The molecular subtyping of HGNECs has recently attracted attention, and Wang et al. recently proposed a cross-tissue classification framework of NECs, including the hepatocellular nuclear factor 4 alpha (HNF4A) subtype [*Cancer Cell*, 2024]. HNF4α, a transcription factor associated with gastrointestinal differentiation, and TTF-1 are mutually and exclusively expressed in lung adenocarcinomas; however, the characteristics of HNF4α-high HGNECs and TTF-1-high HGNECs have yet to be compared. We immunohistochemically examined the characteristics of HNF4α-high HGNECs in 83 surgically resected specimens (37 SCLCs and 46 LCNECs) and revealed that HNF4α-high and TTF-1-high HGNECs accounted for 15% (12/83) and 47% (39/83), respectively. Regarding morphology of HNF4α-high HGNECs, HNF4α-high SCLCs had a slightly more abundant cytoplasm and more obscured nuclear molding than HNF4α-low SCLCs, while HNF4α-high LCNECs were characterized by a coarse nuclear chromatin pattern and prominent nuclear atypia. The cytomorphological score referred to the previous study [Jimbo, *Am J Surg Pathol*. 2024] was significantly higher for HNF4α-high HGNECs than for HNF4α-low HGNECs (p<0.01). In SCLCs, HNF4α-high cases (n=3) and TTF-1-high cases (n=20) were almost confined to the neuroendocrine phenotype with high ASCL1 expression, and the expressions of HNF4α, TTF-1, and POU2F3 were mutually exclusive. Similar results were obtained for LCNECs; however, some HNF4α-high cases were positive for TTF-1 or YAP1, possibly due to the heterogeneity of LCNECs. Therefore, we investigated the heterogeneity of LCNECs and performed a spatial transcriptome analysis of one HNF4α-high LCNEC case, which revealed a mutually exclusive mixture of different subgroups characterized by *HNF4A* and *TTF-1* expression. A whole-genome analysis of 10 LCNECs showed that *NFE2L2/KEAP1* mutations were characteristic of HNF4α-positive LCNECs. A prognostic analysis revealed a significantly poorer prognosis in HNF4α-high LCNECs compared to HNF4α-low LCNECs (p<0.01). A cell line analysis showed that TTF-1-high-expressing (H446/Lu139/H889/H510A) and HNF4α-high-expressing (VMRC-LCD) lines were consistent with ASCL1-high-expressing lines. HNF4α knockdown/knock-in experiments in VMRC-LCD and SBC5 (HNF4α-negative) revealed that HNF4α promoted cell proliferation by inhibiting apoptosis. HNF4α subtype HGNECs are a unique subtype, belonging to a neuroendocrine phenotype with high ASCL1 expression and showing mutual exclusivity with the TTF-1/POU2F3 subtypes. *NFE2L2/KEAP1* mutations and HNF4α itself are potential therapeutic targets for this subtype.

#4591 Development and validation of a molecular classifier of meningiomas.

Alex Landry[1], Justin Wang[1], Jeff Liu[1], Vikas Patil[1], Chloe Gui[1], Andrew Ajisebutu[1], Yosef Ellenbogen[1], Qingxia Wei[1], Olivia Singh[1], Julio Sosa[1], Sheila Mansouri[1], Aaron Cohen-Gadol[2], Ghazaleh Tabatabai[3], Marcos Tatagiba[3], Felix Behling[3], Jill Banholtz-Sloan[4], Andrew Sloan[5], Lola Chambless[6], Serge Makarenko[7], Stephen Yip[7], Alireza Mansouri[8], Derek Tsang[1], Kenneth Aldape[4], Andrew Gao[1], Farshad Nassiri[1], Gelareh Zadeh[1]

[1]University of Toronto, Toronto, ON, Canada,[2]University of Southern California, Los Angeles, CA,[3]University of Tubingen, Tubingen, Germany,[4]National Cancer Institute, Bethesda, MD,[5]Piedmont Brain Tumor Center, Atlanta, GA,[6]Vanderbilt University, Nashville, TN,[7]University of British Columbia, Vancouver, BC, Canada,[8]Penn State, Hershey, PA

Background: Meningiomas exhibit considerable clinical and biological heterogeneity. We previously identified four distinct molecular groups (*immunogenic, NF2-wildtype, hypermetabolic, proliferative)* that address much of this heterogeneity. Despite the utility of these groups, the stochasticity of clustering methods and the use of multi-omics data for discovery limits the potential for classifying prospective cases. We sought to address this with a dedicated classifier.
Methods: Using an international cohort of 1698 meningiomas, we constructed and rigorously validated a machine learning-based molecular classifier using only DNA methylation data as input. Original and newly-predicted molecular groups were compared using DNA methylation, RNA sequencing, copy number profiles, whole exome sequencing, and clinical outcomes.
Results: We show that group-specific outcomes in the validation cohort are nearly identical to those originally described, with median PFS of 7.4 (4.9-Inf) years in *hypermetabolic* tumors and 2.5 (2.3-5.3) years in *proliferative* tumors (not reached in the other groups). Tumors classified as *NF2-wildtype* had no *NF2* mutations, and 51.4% had canonical mutations previously described in this group. RNA pathway analysis revealed upregulation of immune-related pathways in the *immunogenic* group, metabolic pathways in the *hypermetabolic* group and cell-cycle programs in the *proliferative* group. Bulk deconvolution similarly revealed enrichment of macrophages in *immunogenic* tumors and neoplastic cells in *hypermetabolic* and *proliferative* tumors with similar proportions to those originally described.
Conclusions: Our DNA methylation-based classifier, which is publicly available for immediate clinical use, recapitulates the biology and outcomes of the original molecular groups as assessed using multiple metrics/platforms that were not used in its training.

#4592 TERT expression predicts progression-free survival in meningiomas.

Chloe Gui[1], Justin Z. Wang[1], Vikas Patil[2], Andrew Ajisebutu[1], Jeff Liu[2], Zeel Patel[3], Rebeca Yakubov[4], Ramneet Kaloti[4], Yosef Ellenbogen[1], Christopher Wilson[5], Aaron Cohen-Gadol[6], Ghazaleh Tabatabai[7], Marcos Tatagiba[8], Felix Behling[8], Eric C. Holland[9], Jill S. Barnholtz-Sloan[10], Andrew E. Sloan[11], Craig Horbinski[12], Silky Chotai[13], Lola B. Chambless[13], Andrew Gao[14], Serge Makarenko[15], Stephen Yip[16], Kenneth Aldape[1], Farshad Nassiri[1], Gelareh Zadeh[1]

[1]Department of Surgery, Division of Neurosurgery, University of Toronto, Toronto, ON, Canada,[2]Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada,[3]Temerty School of Medicine, University of Toronto, Toronto, ON, Canada,[4]University of Toronto, Toronto, ON, Canada,[5]Oklahoma State University College of Osteopathic Medicine, Tulsa, OK,[6]Department of Neurological Surgery, Keck School of Medicine of the University of Southern California, Los Angeles, CA,[7]Department of Neurology and Interdisciplinary Neuro-Oncology, Center for Neuro-Oncology, Comprehensive Cancer Center, Hertie Institute for Clinical Brain Research, Eberhard Karls University Tubingen, Tubingen, Germany,[8]Department of Neurosurgery, Eberhard Karls University Tubingen, Tubingen, Germany,[9]Human Biology Division, Fred Hutchinson Cancer Center, Seattle, WA,[10]Central Brain Tumor Registry of the United States, Hinsdale, IL,[11]Case Comprehensive Cancer Center, Case Western Reserve University School of Medicine, Cleveland, OH,[12]Department of Pathology, Northwestern University, Evanston, IL,[13]Department of Neurosurgery, Vanderbilt University Medical Center, Nashville, TN,[14]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada,[15]Department of Surgery, Division of Neurosurgery, University of British Columbia, Vancouver, BC, Canada,[16]Department of Pathology & Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada

Background: TERT promoter mutation (TPM) is a rare but established biomarker in meningiomas associated with aberrant TERT expression, reduced progression-free survival (PFS), and reduced overall survival. While TERT is highly expressed in tumors with promotor mutations, its expression has also been observed even in tumors with wildtype TERT promoters (TP-WT). This study aimed to assess the prevalence of TERT expression and its association with clinical outcome in meningiomas. Methods: Bulk RNA sequencing (n=604), Sanger sequencing of the TERT promoter (n=1095), and methylation profiling (n=1218) of a multi-institutional cohort of meningiomas (total n=1241) were performed to determine TERT expression, TERT promoter mutation status, and TERT promoter methylation. A cohort of 380 meningiomas from Toronto was used for discovery, and 861 meningioma samples from external institutions were compiled as a validation cohort. Results: TERT expression was significantly higher in meningiomas with TPMs compared to TP-WT. However, TERT was still expressed in 30.4% of meningiomas that lacked TPM. TERT expression increased with higher WHO grades and was associated with shorter progression-free survival, even among TP-WT tumors. WHO grade 1 tumors that expressed TERT had PFS similar to those of WHO grade 2, while WHO grade 2 meningiomas expressing TERT had a PFS similar to those of WHO grade 3 meningiomas. Among grade 3 meningiomas, tumors expressing TERT had PFS similar to those with TPMs. Conclusions: Our findings highlight the prognostic significance of TERT expression in meningiomas, even in the absence of TPMs. Its presence may identify patients at greater risk of rapid progression. These results support the inclusion of TERT expression in risk stratification models and management strategies, including future WHO classification and cIMPACT-NOW molecular testing criteria.

#4593 Molecular and immunohistochemical classification of extrapulmonary small cell neuroendocrine carcinomas: A study of 181 cases.

Radoslav Matej[1], Pavel Dundr[2], Klara Pavlickova[1], Marian Svajdler[3], Jan Hojny[2], Nikola Hajkova[2]

[1]Pathology and Molecular Medicine, Thomayer University Hospital, Prague, Czech Republic,[2]Pathology, General University Hospital, Prague, Czech Republic,[3]Pathology, University Hospital Pilsen, Pilsen, Czech Republic

Extrapulmonary small cell neuroendocrine carcinoma (EP-SCNC) is a rare malignancy with a poor prognosis. Most patients with EP-SCNC have metastatic disease upon presentation, and their average overall survival (OS) is less than 12 months. Our study aimed to conduct a complex analysis of EP-SCNC. One hundred and eighty-one EP-SCNC tissue samples were subjected to a complex analysis. One hundred and fifty-five tumors were pure EP-SCNC, while 26 were combined tumors. Immunohistochemistry for ASCL1, NEUROD1, YAP1, POU2F3, Rb1, p53, cyclin D1, p16, PTEN, DLL3, PD-L1, CD56, synaptophysin, chromogranin A, and INSM1 was performed, and 128 samples were analyzed molecularly using next generation sequencing (NGS), comprising DNA and RNA analysis. Detailed results on immunohistochemical and molecular analyses were provided for each primary origin of EP-SCNC separately. Median survival for the whole cohort of patients was 8.94 months. Patient age (≥ 70 years), tumor mutational burden (TMB) < 15, and *TP53* and *BRCA2* mutations were negative prognostic factors. High expression of ASCL-1 was associated with shorter OS, whereas high expression of YAP1 was associated with longer OS. Patients with genitourinary tumors had significantly better OS than those with gastrointestinal tract EP-SCNC tumors. Rb1 expression loss was detected more often in genitourinary tract SCNCs. In contrast, p16 overexpression was found more often in genitourinary tract SCNCs. POU2F3 expression was detected more often in combined tumors, whereas NEUROD1 was detected more often in pure EP-SCNC. Regarding “druggable markers,” DLL3 was expressed in 66% of tumors and PD-L1 in 17.4%.Detailed analyses of different prognostic and predictive markers are needed to better understand EP-SCNC biology and create more personalized therapy to improve patient prognosis.

#4594 EGFR PACC mutations occur more frequently as compound mutations with better responses to EGFR TKIs.

Ximeng Liu[1], Moses Stamboulian[2], Maxime Borgeaud[3], Ashwin Ravichandran[4], Caroline Weipert[2], Monique B. Nilsson[1], Erjia Zhu[1], Hong Chen[1], Courtney Lewis[2], Simon Heeke[1], Junqin He[1], John Lawson[5], Jianjun Zhang[1], Addeo Alfredo[3], John V. Heymach[1], Xiuning Le[1]

[1]UT MD Anderson Cancer Center, Houston, TX,[2]Guardant Health, Inc., Redwood City, CA,[3]University Hospital Geneva (HUG), Geneva, Switzerland,[4]KBR, Inc., Intelligent Systems Division, NASA Ames Research Center, Moffett Field, CA,[5]Intelligent Systems Division, NASA Ames Research Center, Moffett Field, CA

EGFR mutations are key oncogenic drivers in NSCLC and can occur either as single or compound mutations. The frequency of classical (Ex19del and L858R) and non-classical PACC mutations in single and compound forms is not fully characterized, and the impact of single vs. compound EGFR mutations on drug sensitivity and clinical outcomes with EGFR TKIs remains unclear. We aimed to identify the effect of single and compound EGFR mutations to improve precision treatment and outcomes for EGFR-mutant NSCLC patients. We queried a circulating cfDNA sequencing database of de-identified NSCLC patients from Guardant Health and analyzed the incidences of EGFR mutations in single and compound forms. In this dataset, after excluding samples with T790M/C797S or Ex20ins, the most frequent classical mutations were Ex19del (6,670, 38.1%) and L858R (4,700, 26.9%), and the most frequent PACC mutations were G719X (712, 4.1%), S768X (335, 1.9%) and E709X (207, 1.2%). 84.22% of classical mutations occurred as single mutations, with Ex19del in 89.5% and L858R in 76.7% of samples, respectively. In contrast, 80.12% of PACC mutations occurred as compound mutations, with G719X in 73.2%, S768X in 86.9%, and E709X in 97.1% of samples. We engineered Ba/F3 cells to express single or compound PACC mutations and evaluated sensitivity to clinically available EGFR TKIs. Compound PACC mutants showed significantly increased sensitivity to 2nd-gen compared to 1st- and 3rd-gen TKIs. Cells with compound PACC mutations exhibited a tendency towards enhanced TKI sensitivity compared to cells with single mutations.

Cells with single or compound G719X/E709X, single V769L and compound S768I/V769L were most sensitive to 2nd-gen TKIs. Cells with single S768I had the highest sensitivity to 3rd-gen TKIs. In a retrospective clinical data analysis we assessed treatment outcomes for patients with single or compound PACC mutations (n=1268). Patients with either form of PACC mutations showed the best response rates (RR) (single: 54.67%, compound: 70.34%) and longest mPFS (single: 9mo, compound: 15mo) with 2nd-gen TKIs. Compound PACC mutations were associated with better RRs and longer mPFS than single forms across all TKIs. Specifically, patients with G719X or E709X, regardless of mutation form, had the longest mPFS with 2nd-gen TKIs (single vs. compound: G719X:11.2 vs. 18.5mo, E709X: 10.9 vs. 8.9mo). Patients with single S768X showed the longest mPFS to 3rd-gen TKIs (13.25mo), differing from those with compound S768X, whose mPFS was the longest with 2nd-gen TKIs (8.9mo). Collectively, our analysis indicates that EGFR PACC mutations predominantly occur as compound mutations while classical mutations most frequently present as single mutations. Compound and single PACC mutations were associated with improved clinical responses to 2nd-gen EGFR TKIs.

#4595 Transcriptomic and tumor microenvironment alterations in microsatellite stable rectal tumors: Comparison between neoadjuvant-treated and treatment-naive patients.

E. K. Hamalainen[1], K. Ojala[2], M. Marttinen[1], M. Nykter[1], T. Seppala[3];

[1]Tampere University, Tampere, Finland, [2]Helsinki University Hospital, Helsinki, Finland, [3]Tampere University Hospital, Tampere, Finland

Neoadjuvant therapy (NAT) for rectal cancer has markedly improved survival rates and the likelihood of complete therapeutic response. However, recurrence after curative intent therapy and resistance to primary oncological therapy remain, shortening the survival. The modulation of transcriptomic profiles by NAT and its association with therapeutic response remain unclear. Methods: The preliminary analyses of observational study of 680 colorectal cancer (CRC) patients focused on a sub-cohort of 233 primary rectal cancer patients, of whom 95 received NAT (chemoradiotherapy, radiation therapy with immediate surgery or wait period, or chemotherapy) and 138 were treatment-naive. Pathological responses varied from minimal to excellent. Transcriptomic, genomic, and immunohistochemical (IHC) profiling were performed on surgical resection samples. Mutation statuses of non-synonymous variants in CRC driver genes were compared between groups. Gene expression profiles were classified into consensus molecular subtypes (CMS) and intrinsic CMS (iCMS), with differentially expressed genes analyzed for hallmark pathway enrichment. Results: Transcriptomic analysis revealed a significant shift in molecular subtypes, with 66% of NAT tumors classified as iCMS3 (CMS4), compared to 63% of treatment-naive tumors classified as iCMS2. Pathway enrichment analysis of 4,817 differentially expressed genes showed significantly higher expression of hallmark pathways, particularly epithelial-to-mesenchymal transition and p53 signaling, in NAT tumors. Treatment-naive samples classified as iCMS3 (27%) displayed expression patterns similar to NAT iCMS3 tumors. The fraction of epithelial cells was significantly lower in NAT compared to treatment-naive, as determined by IHC. No significant differences in the overall mutation statuses of key CRC driver genes were observed between groups. Although, mutations in APC and TP53 were less frequent in the NAT tumors. Notably, KRAS mutations were mutually exclusive with TP53 in treatment-naive samples (36/95), whereas TP53 mutations co-occurred with APC in NAT tumors (48/22). Conclusion: NAT potentially induces a shift in gene expression from iCMS2 to iCMS3 in left-sided MSS rectal tumors, accompanied by increased epithelial-to-mesenchymal transition and altered driver gene interactions. Further investigations are needed to confirm whether NAT drives this gene expression shift, to elucidate its effects on tumor microenvironment modulation with available IHC data of T-cell, TMA, and CAF panels and to assess their impact on therapeutic outcomes and potential to inform adjuvant therapy strategies.

#4596 Spatial transcriptomics reveals subtype heterogeneity within the tumor epithelial compartment of pancreatic ductal adenocarcinoma.

James Topham[1], Jenny Chu[2], Maya Kevorkova[1], Steve Kalloger[1], Joanna Karasinska[1], Andrew Metcalfe[1], Hassan Ali[1], Dongxia Gao[2], Christine Chow[2], Jonathan Loree[3], Daniel Renouf[1], David Schaeffer[1]

[1]Pancreas Centre BC, Vancouver, BC, Canada,[2]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada,[3]BC Cancer Agency, Vancouver, BC, Canada

Molecular subtyping of pancreatic ductal adenocarcinoma (PDAC) has converged on basal-like and classical subtypes with the former being associated with worse prognosis. Sequencing-based tools have emerged to bring PDAC subtyping closer to incorporation into clinical practice. We sought to test the utility of PDAC subtyping using a cohort of patient tumor samples in a clinical setting. For 29 patients with resectable PDAC, two formalin-fixed paraffin-embedded (FFPE) blocks were sequenced from each patient tumor sample (total n=58 blocks) using Nanostring nCounter. 18/58 (31%) of tumors had inconsistent PurIST-based basal-like or classical labels between the two blocks. Two tumors were selected for digital spatial transcriptomics based on having either consistent or inconsistent subtype labelling. Regions of interest (ROI) were selected to capture a diverse range of cellular compartments within the tumor microenvironment including tumor, stroma, immune cells, tumor buds and neurons. ROIs were sequenced across two and three FFPE blocks from the subtype consistent and inconsistent tumors, respectively. Gene expression was segmented by PanCK positive (epithelial) and negative (non-epithelial) signal. Across all ROIs (n=35), PanCK+ segments showed higher expression of basal-like (p=1.9e-7) and classical (p=4.2e-9) genes compared to PanCK- segments. For the subtype-consistent (classical) tumor, all ROIs (n=13) across two blocks showed predominate classical gene expression in the PanCK+ segment. In the subtype-inconsistent tumor that originally had blocks labelled PurIST classical and intermediate, ROIs (n=22) taken across three blocks showed 50, 60 and 83% of ROIs having higher basal-like expression, while remaining ROIs had predominant classical gene expression, and $\log_2$ fold change basal-like vs. classical (median) gene expression ranged from -2.4 (T-cells) to 1.3 (tumor and stroma) across ROIs. Overall, the classical gene signature was positively correlated with *GATA6* expression (rho=0.40, p=3.2e-4) across all ROIs and PanCK segmentations. While these data support the notion of subtyping expression being specific to the tumor epithelial component, nearly one-third of patient samples had different subtype labels depending on which area of the tumor epithelium was captured. We demonstrate the ability for spatial transcriptomics to resolve subtype heterogeneity and the contributing cellular compartments, providing potential to better understand how aggressive disease develops and could be treated.

#4597 Globo H affects the immune tumor microenvironment in cholangiocarcinoma.

Chih Chieh Yen[1], Tzer Min Kuo[2], Chun Jung Lin[2], Hung Wen Tsai[3], Wen Lung Wang[1], Chien Jui Huang[4], Ping Jui Su[5], Ting Kai Liao[5], I Ting Liu[1], Li Chin Cheng[6], Ying Jui Chao[5], Yan Shen Shan[5], Chia Jui Yen[1], Ming Tain Lai[2]

[1]Department of Oncology, National Cheng Kung University Hospital, Tainan, Taiwan,[2]OBI Pharmaceuticals Inc., Taipei, Taiwan,[3]Department of Pathology, National Cheng Kung University Hospital, Tainan, Taiwan,[4]Division of Gastroenterology and Hepatology, Department of Internal Medicine, National Cheng Kung University Hospital, Tainan, Taiwan,[5]Department of Surgery, National Cheng Kung University Hospital, Tainan, Taiwan,[6]Department of Surgery, Chi Mei Medical Center, Tainan, Taiwan

Background: Cholangiocarcinoma (CCA), which is characterized by aberrant glycosylation on the surface, is a rapidly progressing cancer with an unsatisfactory response to chemoimmunotherapy. Globo H (GH) ceramide, a tumor-promoting glycosphingolipid, is expressed in various types of cancer and is correlated with poor prognosis. However, few studies have discussed the role of GH in CCA. Hence, the pathways involved and alterations in the immune tumor microenvironment (TME) have not been explored.
Methods: We aimed to reveal the clinical features and immune modulatory role of tumor GH and its correlation in CCA. We evaluated the signaling-associated proteins and those coexpressed with GH. Tumor-infiltrating immune cells (TIMCs) were compared between tumors with high vs. low levels of GH synthesizing enzyme (GH-se) using open-domain databases and single-cell RNA sequencing results.
Results: Surface GH expression correlated with poor prognosis and peritumor inflammation. High-GH CCA cells coexpressed GH with PD-L1, EGFR and EPCAM. While GH and its synthesizing enzymes affected immune modulation and survival, TIMCs such as neutrophils, tumor-associated macrophages, natural killer cells and foxp3 regulatory T cells were significantly altered in high-GH-se tumors.
Conclusion: Tumor GH expression correlates with a poor prognosis in patients with cholangiocarcinoma. GH modulates the immune TME by promoting an immune-evasive niche. The results provide insights for combining immunotherapy with GH-targeting therapy.

#4598 A novel systemic delivery approach for oncolytic adenovirus therapy using tumor-homing mesenchymal stem cells.

Yu Mikane, Nobuhiko Kanaya, Shinji Kuroda, Hitoshi Minagi, Makoto Matsumoto, Tetsuya Katayama, Daisuke Kadowaki, Shunya Hanzawa, Masashi Hashimoto, Yoshihiko Kakiuchi, Satoru Kikuchi, Shunsuke Kagawa, Hiroshi Tazawa, Toshiyoshi Fujiwara, Yasuo Urata

Department of Gastroenterological Surgery, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan

Oncolytic viral (OV) therapy is a promising approach for treating metastatic cancer; however, systemic delivery remains a significant challenge due to antiviral immune responses. We previously developed a telomerase-specific OV, Telomelysin (OBP-301), which utilizes the hTERT promoter to drive the expression of E1A and E1B. OBP-301 demonstrated an abscopal effect when combined with anti-PD-1 antibodies in distant metastases in vivo murine colorectal cancer (MC38) mouse models. A p53-armed variant, OBP-702, showed potent tumor-suppressive effects in mouse models of gastroenterological cancers via intratumoral injection. However, these therapies remain limited to accessible lesions. To address these challenges, we investigated three novel approaches for systemic OV delivery. First, we encapsulated the DNA plasmids of GFP-expressing OBP-301 (OBP-401) in liposomes, which effectively reduced the production of neutralizing antibodies compared to naked OVs. Second, we explored extracellular vesicles (EVs) derived from OBP-301-infected tumors as natural carriers. These EVs demonstrated the abscopal effects to non-injected metastatic lesions in preclinical models, achieving promising therapeutic efficacy. Finally, we utilized mesenchymal stem cells (MSCs), known for their tumor-homing properties, as carriers for OBP-702. When MSCs loaded with OBP-702 (MSC-OBP-702) were intraperitoneally injected into an MC38-bearing peritoneal metastasis model, they effectively migrated to metastatic sites, significantly suppressing tumor growth and improving overall survival compared to OBP-702 alone. These findings demonstrate that MSC-OBP-702 has significant potential to overcome the challenges of systemic OV therapy through the tumor-homing capability of MSCs, providing a foundation for future clinical applications.

#4599 Preclinical evaluation, and clinical translation of ^{68}Ga/^{177}Lu-JH04, a novel FAP-targeting radiolabeled agent.

Yun Yang[1], Hongfei Cheng[1], Yuxin Lai[1], Xiuting Lin[1], Yaoming Yu[2], Jian Zhan[2], Yueguan Sun[2], Eric Wang[2], Haihua Yu[2], Kevin Wang[2], Jinqiu He[2], Guochang Wang[1], Weibing Miao[1]

[1]the First Affiliated Hospital, Fujian Medical University, Fuzhou, China,[2]Bivision Pharmaceuticals, Inc, Shanghai, China

Introduction: Fibroblast activation protein (FAP) is an ideal target for tumor imaging and therapy. To enhance tumor uptake and prolong the retention of FAP-targeting agents, we developed JH04, a novel cyclic peptide composed of a FAP-targeting ligand. The objective of this study is to evaluate the specificity, biodistribution, pharmacokinetics, and dosimetry of ^{68}Ga/^{177}Lu-JH04 through preclinical and preliminary clinical investigations, and to compare these findings with those of ^{68}Ga/^{177}Lu-FAP-2286.

Methods: The FAP-positive cell line HT1080-FAP was employed in *in vitro* and *in vivo* studies. The affinity of JH04 precursor to recombinant FAP protein was measured using surface plasmon resonance (SPR) and radioligand binding assays. PET imaging with ^{68}Ga-JH04 was performed in xenograft mouse models to evaluate its *in vivo* specificity and preclinical pharmacokinetics. The tumor uptake and retention of ^{177}Lu-JH04 were assessed in xenograft mice model and compared with those of ^{177}Lu-FAP-2286. Furthermore, the biodistribution and radiation dosimetry of ^{68}Ga-JH04 were evaluated using data from five cancer patients, with head-to-head comparisons of imaging results against those of ^{68}Ga-FAP-2286. Lastly, the therapeutic efficacy of ^{177}Lu-JH04 was investigated in three patients with tumor.

Results: The dissociation equilibrium constant (K_D) of JH04 precursor to human FAP protein was 0.428 nM. The IC50 of JH04 for human FAP protein was 3.96 nM, which was superior to that of FAP-2286 (12.2 nM). ^{68}Ga-JH04 exhibited excellent stability both *in vitro* and *in vivo*. In cell binding studies, the uptake of ^{68}Ga-JH04 in HT1080 cells was approximately 2.83 times higher than that of ^{68}Ga-FAP-2286 after 30 min incubation. Small-animal PET imaging revealed that the tumor uptake of ^{68}Ga-JH04 was approximately twice as high as that of ^{68}Ga-FAP-2286. SPECT imaging demonstrated high tumor uptake and prolonged retention of ^{177}Lu-JH04, the area under the curve (AUC) for tumor uptake over 168 hours was 1.94 times higher for ^{177}Lu-JH04 compared to ^{177}Lu-FAP-2286. In a head-to-head clinical comparison with ^{68}Ga-FAP-2286, PET/CT imaging results revealed significantly higher tumor radiotracer uptake for ^{68}Ga-JH04 (SUVmax: 60 minutes, 14.58 ± 6.26 *vs.* 11.98 ± 6.43, $P = 0.025$; 150 minutes, 17.46 ± 9.07 *vs.* 12.00 ± 7.65, $P = 0.006$). Additionally, the retention of ^{68}Ga-JH04 in the kidneys and liver was 3.02 times and 3.80 times lower than that of ^{68}Ga-FAP-2286. In a preliminary clinical evaluation of ^{177}Lu-JH04 therapy, all three patients tolerated well. The mean effective whole-body dose of ^{177}Lu-JH04 was determined to be 6.18E-02 ± 6.85E-03 mGy/MBq.

Conclusion: ^{68}Ga/^{177}Lu-JH04, novel FAP-targeting agent with excellent binding affinity, high tumor uptake, prolonged retention, and robust tumor-suppressive effects. These promising results encourage us to conduct further clinical research.

#4600 Preclinical evaluation and clinical translation of ^{68}Ga/^{177}Lu-JH12, a novel CXCR4-targeting radioligand.

Tianrui Kang[1], Yuxin Lai[1], Hongfei Cheng[1], Xiuting Lin[1], Yaoming Yu[2], Jian Zhan[2], Yueguang Sun[2], Eric Wang[2], Haihua Yu[2], Kevin Wang[2], Jinqiu He[2], Guochang Wang[1], Weibing Miao[1]

[1]Department of Nuclear Medicine, the First Affiliated Hospital, Fujian Medical University, Fuzhou, China,[2]Bivision Pharmaceuticals, Inc., Shanghai, China

Introduction: C-X-C motif chemokine receptor 4 (CXCR4) is highly expressed by many kinds of tumors. Radiolabeled Pentixafor/Pentixather as typical targeting CXCR4 radioligands have been widely used in clinical practice, while there still remains barriers such as inadequate tumor uptake and high retention in blood or liver. Therefore, we designed a novel CXCR4-targeted agent, JH12. This research evaluated the specificity, biodistribution, pahrmacokinetics, and dosimetry of ^{68}Ga/^{177}Lu-JH12 in preclinical and preliminary clinical studies, using ^{68}Ga-Pentixafor/^{177}Lu-Pentixather as references.

Methods: HT1080-CXCR4 cell line was used in cellular and small animal experiments. The *in vitro* binding affinities to CXCR4 were evaluated and presented as IC50 values. The specificity of ^{68}Ga/^{177}Lu-JH12 was assessed using PET/SPECT imaging, compared with ^{68}Ga-Pentixafor/^{177}Lu-Pentixather. *Ex-vivo* biodistribution studies were performed to obtain tumor uptake of ^{177}Lu-JH12. Besides, the biodistribution and radiation dosimetry of ^{68}Ga-JH12 were evaluated in 5 patients with oligometastatic tumors. We also conducted a head-to-head comparison to assess the diagnostic performance of ^{68}Ga-JH12 and ^{68}Ga-Pentixafor PET/CT in 3 patients.

Results: ^{68}Ga-JH12 exhibited excellent radiochemical purity and stability both *in vitro* and *in vivo*. The lower IC50 values on cellular level was observed in JH12 (18.79 nM) than Pentixafor (400.3 nM) and Pentixather (148 nM). In small animal PET imaging, the tumor uptake of ^{68}Ga-JH12 was 3.29 times than ^{68}Ga-Pentixafor at 1 hour post-injection. In small animal SPECT imaging and the *ex-vivo* distribution, ^{177}Lu-JH12 showed significantly higher tumor uptake (approximately 2 times) while lower accumulation in liver (approximately 10 times) than those of ^{177}Lu-Pentixather. Furthermore, clinical PET/CT imaging of ^{68}Ga-JH12 demonstrated remarkably higher tumor radiotracer uptake than ^{68}Ga-Pentixafor (SUV_{max}: 60 minutes, 27.44 ± 14.75 *vs.* 12.98 ± 7.41, P<0.05). Ultimately, based on the results of ^{68}Ga-JH12 dosimetry in 5 patients with tumor, the mean dose of ^{68}Ga-JH12 in liver, kidney and red marrow was 2.31E-02 ± 3.25E-03 mGy/MBq, 7.57E-02 ± 2.12E-02 mGy/MBq and 3.03E-02 ± 1.34E-02 mGy/MBq respectively. The effective dose of ^{68}Ga-JH12 was 2.01E-02 ± 6.66E-03 mGy/MBq.

Conclusion: JH12 appears to perform better in tumor visualization and retention, which might be a promising biological radio-tracer in CXCR4-targeting imaging and treatment.

#4601 Development and clinical translation of a phage display derived MT1-MMP-specific bicyclic peptide for radiotheranostic applications.

Ann-Christin Eder[1], Mohamed A. Omrane[1], Anusha R. Regupathy[2], Mohamed El Fakiri[1], Nils Steinacker[1], Lisa-Charlotte Domogalla[1], Christoph-Ferdinand Wielenberg[1], Michael Mix[1], Johanna Lahdenranta[3], Ben Blakeman[2], Francesca Wood[4], Philip Huxley[4], **Gemma E. Mudd**[4], Matthias Eder[5], Philipp T. Meyer[1], Martin T. Freitag[1]

[1]Department of Nuclear Medicine, University of Freiburg Medical Center, Freiburg, Germany,[2]BicycleTx Limited, Cambridge, United Kingdom,[3]Bicycle Therapeutics, Cambridge, MA,[4]Bicycle Therapeutics, Cambridge, United Kingdom,[5]Radiopharmaceutical Development, German Cancer Consortium partner site Freiburg, Freiburg, Germany

Background: Membrane type 1 matrix metalloproteinase (MT1-MMP) is a pivotal enzyme involved in extracellular matrix remodeling, contributing to tumor invasion, metastasis, and poor prognosis in various cancers, including non-small cell lung, urothelial, pancreatic, gastric, and breast cancers. This study outlines the preclinical development and first in-human application of a phage display-derived MT1-MMP-specific bicyclic peptide, [^{68}Ga]Ga-BCY25286, as a radiotheranostic agent for PET/CT imaging.

Methods: The MT1-MMP-targeting bicyclic peptide BCY25286 was radiolabeled with either Ga-68 or Lu-177 and subsequently characterized for stability, binding affinity, and internalization. Preclinical evaluation included biodistribution and µPET/MR imaging in MT1-MMP^{+} HT1080 and MT1-MMP^{-} MCF-7 xenograft tumor-bearing nude mice. For clinical translation, a 65-year-old patient with advanced pulmonary adenocarcinoma underwent [^{18}F]FDG-PET/CT followed by [^{68}Ga]Ga-BCY25286 PET/CT imaging, with PET scans performed after 60 minutes for [^{18}F]FDG and up to 60 minutes for [^{68}Ga]Ga-BCY25286 (compassionate use).

Results: Radiolabeling achieved >99% radiochemical purity for both radionuclides. [^{68}Ga]Ga-BCY25286 demonstrated highly MT1-MMP-specific binding (7.2 ± 1.6 nM), proteolytic stability up to 72 hours, and rapid background clearance, thereby enhancing imaging contrast within 30 minutes. In mice, the tracer demonstrated high tumor uptake (10.6 ± 1.1 %ID/g at 1 h p.i.) with persistence up to 24 hours. In the clinical case, [^{68}Ga]Ga-BCY25286 PET/CT imaging revealed high uptake in both primary and biopsy-confirmed metastatic sites, corroborating the findings of [^{18}F]FDG-PET. SUV_{max}values were comparable for lymph node metastases but higher for bone metastases in MT1-MMP-PET compared to [^{18}F]FDG-PET, with significant kidney retention due to renal excretion.

Conclusion: This first-in-human application of MT1-MMP-targeting [^{68}Ga]Ga-BCY25286 demonstrates the feasibility for visualization of MT-1-MMP-expressing primary tumors and metastases, which is in line with the preclinical findings. These initial clinical results support further investigation of [^{68}Ga]Ga-BCY25286 as a diagnostic tool with potential to improve tumor characterization and patient management strategies in MT1-MMP-positive cancers.

#4602 GPRC5D alterations impact CD38 expression and provide proliferative advantage in multiple myeloma.

Umair Munawar[1], Seungbin Han[1], Elena Gerhard-Hartmann[2], Cornelia Vogt[1], Silvia Nerreter[1], Shilpa Kurian[1], Thomas Nerreter[1], Johanna Thurner[3], Julia Weingart[4], Patrick Eiring[4], Xiang Zhou[1], Nina Rein[1], Johanna Lehmann[1], Max Koeppel[1], Andreas Rosenwald[2], Ondrej Slaby[5], Michael Hudecek[1], Hermann Einsele[1], Leo Rasche[1], Markus Sauer[4], Johannes Waldschmidt[1], Bernhard Kuester[3], Klaus Martin Kortuem[1]

[1]University Hospital Wuerzburg, Wuerzburg, Germany,[2]Institute of Pathology, University of Wuerzburg, Wuerzburg, Germany,[3]Technical University of Munich, Freising, Germany,[4]University of Wuerzburg, Wuerzburg, Germany,[5]Masaryk University, Brnu, Czech Republic

Background: The treatment landscape in Multiple Myeloma (MM) shifts towards immunotherapies with the G protein-coupled receptor class C group 5 member D (GPRC5D) as a promising antigen for T cell engagers (TCE) and investigational CART constructs. Monoallelic genetic alterations in GPRC5D are present in up to 15% of MM patients prior GPRC5D directed therapy. The incidence of acquired biallelic GPRC5D events following treatment has to be determined. Recently, biallelic loss of GPRC5D was identified as the underlying mechanism for acquired resistance toward talquetamab, the first approved GPRC5D directed TCE. In this study, we modeled GPRC5D genetic alterations and studied their impact on the biology of the disease and responsiveness to anti-MM immunotherapies.

Methods: Mono- and bi-allelic *GPRC5D* knock-out (KO) cell models were created using the MM cell line OPM-2 via CRISPR-Cas9 technology. Surface receptor distribution and epitope quantification of common immunotherapeutic targets was performed via *Direct* stochastic optical reconstruction microscopy (*d*STORM). MM cells were engineered to perform cytotoxicity, functional and clonal competition assays using immunotherapeutic agents in presence of effector T cells from healthy donors.

Results: *d*STORM revealed a 2-fold reduction in GPRC5D surface expression on $GPRC5D^{Wt/Del}$ (0,16±0,02 clusters/μm^2) vs. WT cells (0,32±0,10 clusters/μm^2). $GPRC5D^{Del/Del}$ had barely detectable GPRC5D expression with 0,03±0,07 clusters/μm^2. CD38 expression was unexpectedly diminished in $GPRC5D^{Wt/Del}$ (5,74±0.43 CD38 clusters/μm^2) compared to WT parental cells (9,59±0,71 clusters/μm^2, p=0.04), with an even greater reduction detected in $GPRC5D^{Del/Del}$ clones (2,22±0.203 clusters/μm^2, p<0,0001). GPRC5D-altered cells with reduced CD38 expression depicted a significant resistance towards CD38 targeting antibodies (8.01% specific lysis in $GPRC5D^{Wt/Del}$ cells, 6.38 % in $GPRC5D^{Del/Del}$ compared to 31.50% in WT cells, p <0.0001). $GPRC5D^{Wt/Del}$cells depicted a significant resistance towards Talquetamab. Additionally, in presence of talquetamab $GPRC5D^{Del/Del}$ models showed a proliferation gain (2 fold, p<0,0001). High content chemokine profiling using the scioCyto microarray platform revealed significantly altered chemokine profiles, including growth and proliferation promoting cytokines. In addition, upregulation of MAPK and PI3K pathways involved in growth proliferation and downregulation of the RAP1 signaling pathway involved in cell adhesion was observed in GPRC5D altered cells via bulk RNA seq. To decipher potential mechanism of growth induction and a link to CD38, scRNA seq and phospho-proteomic based analysis are ongoing.

Conclusion: Our work suggest a link between CD38 and GPRC5D in MM and supports an anti-proliferative function of GPRC5D suggesting treatment over progression with talquetamab may favor tumor growth in GPRC5D deficient cells.

#4603 Monoclonal P40 antibody BC28 identifies neoplastic cells with basal-like differentiation in a subset of pancreatic ductal adenocarcinoma.

Sourat Darabi[1], Derek Cridebring[2], Daniel Von Hoff[2], Haiyong Han[2], Michael J. Demeure[1], F. Scott Heinemann[1]

[1]Hoag Cancer Institute, Newport Beach, CA,[2]Translational Genomics Research Institute, Phoenix, AZ

Background: Transcriptomic analysis of pancreatic ductal adenocarcinoma (PDAC) has identified classical and basal-like subtypes of PDAC as discrete entities with distinct outcomes that require transcriptomic analysis for accurate diagnosis. Adenosquamous carcinoma of the pancreas (ASPC) is a histologic variant of pancreatic cancer with a basal-like transcriptomic phenotype and a distinctly poor prognosis. Current guidelines for diagnosing ASPC require the presence of at least 30% squamous component. We hypothesized that classical and basal-like PDAC may exist along a spectrum with ASCP at one end. DeltaNp63 (P40) immunohistochemistry (IHC) is an excellent marker for diagnosing primary lung squamous cell carcinoma. The use of P40 IHC in pancreatic carcinoma is less well established. This study was performed to determine the percentage P40 positive cells in ASCP and PDAC.

Method: Archived tissue slides and blocks from patients with PDAC (N = 58) and ASPC (N = 3) were retrieved from the files of Hoag Hospital using Hoag tumor registry data. Tissue sections of selected blocks from each case were stained by IHC using monoclonal anti-P40 antibody clone BC28 (Abcam, Waltham, Mass). IHC staining was performed with the BOND III automated immunostainer (Leica Biosystems, Nusslock, Germany). Any neoplastic cells with strong nuclear P40 staining were considered P40+. For tumors with P40+ neoplastic cells, the percentage of tumor surface area that contained P40+ neoplastic cells was estimated and the percentage of P40+ cells within the P40 positive component was estimated. IRB approval was obtained to conduct the study with exemption for patient consent.

Results: Greater than 90% of the surface area and >90% of the tumor cells in each ASCP tumor studied were P40 positive. Within the 58 PDAC, 12 cases (21%) contained P40 positive tumor cells. The median percentage of P40 positive cells within the P40+ PDAC components was 3.5%. P40 positive cells were distributed focally in 10 cases and diffusely in 2 cases. In PDAC cases with focal P40 staining, the P40+ components represented between 5% and 50% of the total surface area of the tumor. Most P40+ neoplastic cells in PDAC were cytologically indistinguishable from P40 negative tumor cells, but a subset had a flattened shape and peripheral distribution within the neoplastic glands reminiscent of basal cells. The median length of follow-up/survival in the study cohort was 18 months. Thirty-one of the PDAC patients were alive and 24 were dead. The survival status of 3 patients was not recorded in the registry data. 42% of PDAC patients with P40+ cells were alive at the time of study whereas 60% of patients with P40 negative PDAC were alive, but this difference did not reach statistical significance.

Conclusions: These findings support the hypothesis that a subset of PDAC contains a minor population of neoplastic cells with basal-like differentiation.

#4604 Implications of genomic alterations in RNF43 and RSPO3 for the response of BRAFV600E metastatic colorectal cancer to anti-EGFR+BRAF combinatory therapy.

Jakob M. Stenersen[1], Max Z. Totland[1], Luis Nunes[1], Ina A. Eilertsen[1], Vilde C. W. Elster[1], Tuula A. Nyman[2], Ragnihild A. Lothe[1], Anita Sveen[1], Kushtrim Kryeziu[1], Edward Leithe[1]

[1]Department of Molecular Oncology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway,[2]Department of Immunology, Institute for Clinical Medicine, Oslo University Hospital, Oslo, Norway

The *BRAF*^{V600E} mutation, present in 8 - 10% of colorectal cancers (CRCs), is associated with poor patient outcome and has limited treatment options. Recent studies have shown that mutations in the E3 ubiquitin ligase RNF43 predict response to anti-EGFR/BRAF combinatory therapy in patients with *BRAF*^{V600E} metastatic CRC. Gene expression analysis of CRCs with the *BRAF/RNF43* co-mutation exhibit significantly higher expression levels of *MAP2K1* and *MAPK3* compared to their wild-type counterparts. This implies an unknown but clinically significant molecular relationship between RNF43 loss-of-function and the MAPK signaling pathway. RNF43 attenuates WNT signaling by promoting the endocytosis and lysosomal degradation of Frizzled receptors to desensitize the cells to WNT ligands. RNF43 itself is in turn negatively regulated by the R-spondin (RSPO) family of proteins. Activating fusions involving *RSPOs* and inactivating mutations in *RNF43* are found in ~2% and ~5% of CRCs, respectively. The aim of the present project is to better understand how gene alterations affecting the R-spondin/RNF43 signaling module influence the response to EGFR/BRAF inhibitor combination treatment. We use patient-derived organoids with different genomic alterations in *BRAF* and *RNF43*, *APC*, or *RSPO3* as preclinical models to elucidate signaling dynamics before and after treatment with EGFR and BRAF inhibitors alone or in combination. This analysis includes various omics approaches such as mass spectrometry, RNA sequencing and kinome profiling. Here, we present preliminary results from this study, showing how these treatments differentially affect MAPK and WNT signaling depending on the mutational profile of the organoids

#4605 Exploring resistance mechanisms in cancer using patient-derived organoids reveals replication stress and EMT as potential biomarkers.

Ruben M. Drews[1], Finnian Firth[2], Paul R. Barber[1], Anna Pasto[1], Tony T. Ng[1]

[1]Oncology Translational Research Organisation, GSK Plc, London, United Kingdom,[2]gsk.ai, GSK Plc, London, United Kingdom

Mutational processes such as chromosomal instability and replication stress are important molecular features of head and neck squamous cell carcinoma (HNSCC) and non-small cell lung cancer (NSCLC), contributing to poor patient survival. Their changing influences during cancer development creates challenges in identifying response biomarkers. Patient-derived organoid 3D cancer models (PDOs) have been shown to match their primary source material and to reflect tumour biology more faithfully than cell lines. In our companion abstract, we detail how we have built the Digital Biological Twin lab to study PDOs for drug testing and biomarker discovery. In this work, we used PDOs to investigate resistance to PARP inhibitors (PARPi), which are mechanistically linked to DNA repair and replication. 10 PDOs (6 HNSCC, 4 NSCLC) generated from untreated tumor resections were bulk RNA-sequenced and treated with Niraparib. IC50, cell viability measured by flow cytometry and protein expression levels were used to classify 3 PDOs as sensitive to treatment. Expression analysis, stratifying by PARPi sensitivity, resulted in 287 differentially expressed genes (DEGs). Enrichment analyses found involvement of the endothelial-to-mesenchymal transition (EMT) and small molecule transport pathways. Knowing that EMT is linked to multi-drug resistance, we treated the PDOs with Cisplatin and found a significant correlation to PARPi IC50 values. By examining the biology of the DEGs, we categorized them using MSigDB into five gene sets, each with 1 to 14 genes. We functionally validated the gene sets by assessing PARPi susceptibility in 45 cell lines pre and post a genome-wide CRISPR knockout, finding four significantly enriched in PARPi gene dependencies. For two replication stress-derived gene sets, we demonstrated significant concordance between PARPi sensitivity status of PDOs and cell lines from DepMap, and with progression free interval of HNSCC and NSCLC patients from TCGA. Leveraging Cox analysis on these patients, one replication stress gene set stratified the patients by showing a significant decrease in relapse probability. In summary, our results indicate that PARPi resistance is associated with the deregulation of genes related to small molecule transport, multi-drug resistance and EMT. The consistent findings around replication stress across disease models strongly suggests a conserved and central role in resistance mechanisms. We also identify a replication stress gene set that significantly correlates with resistance in cancer models and relapse in patients. Further work is needed to translate our findings into a predictive model for drugs linked to replication stress. Here we showed that PDOs can be successfully used for furthering our understanding of resistance mechanisms while improving molecular stratification of patients with direct clinical implications.

#4606 Digital biological twin platform: multiomics in patient derived organoids combined with machine learning.

Anna Pasto[1], Paul Barber[1], Halh Al-Serori[1], Maria Fankhaenel[1], Diianeira Maria Tsiridou[1], Shani Austin-Williams[1], Finnian Firth[2], Ruben M. Drews[1], Amro Ahmed-Ebbiary[1], Michael Finn[1], Mint Htun[1], Ellie N. McDowall[3], Annie-Rose Henry[3], Susan Ndagire[4], Eleni M. Karapanagiotou[3], Cheryl E. Gillet[5], Andrea Bille[3], Tony Ng[1]

[1]GlaxoSmithKline, Stevenage, United Kingdom,[2]gsk.ai, GlaxoSmithKline, Stevenage, United Kingdom,[3]Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom,[4]King's College of London, Comprehensive Cancer centre KHP Cancer Biobank., London, United Kingdom,[5]King's College of London, School of Cancer and Pharmaceutical Sciences, London, United Kingdom

Patient-derived organoids (PDOs) are in vitro 3D structures that mimic the complexity of tumors, including their molecular and phenotypic features. Recently, numerous studies have demonstrated their potential as preclinical model for drug screening and tailored treatment selection for patients.Non-small cell lung cancer (NSCLC) with its rapid growth and early metastasis onset, represents the most common cause of cancer-related deaths worldwide. Current treatments offer limited long-term survival, necessitating novel approaches. Immunotherapy, specifically anti-PD1 and anti-PD-L1, has transformed NSCLC treatment, but only a small percentage of patients respond. There is an unmet need to develop novel clinically relevant models of NSCLC to improve cancer patient risk stratification as well as to predict future targeted and/or immuno-therapy susceptibilities that may be met with PDOs.We have created the Digital Biological Twin lab, a platform that performs multi-omics analysis on PDOs combining advanced cellular, genomic, and molecular techniques with machine learning models, aiming to improve cancer patient risk stratification and predict future therapy susceptibilities. This platform, part of the Oncology translational research organization at GSK, will provide proof of concept data from patient samples collected in hospital biobank-linked organoid studies. In an ongoing study we are establishing PDO models from 200 NSCLC tissues collected at the Guy's and St Thomas' Biobank in London. The PDOs are treated with standard care, then co-cultured with pre-activated immune cells in the presence of IO drugs, and subjected to assays including exosome isolation, protein and DNA content analysis, imaging assays, flow cytometry analysis of tumor markers and immune phenotype, and transcriptomic analysis. This multi-omics approach allows for simultaneous analysis of different biological parameters after treatment to understand tumour cell interaction with immune cells and drug-induced changes. The results will be used to identify potential biomarkers of treatment response and generate hypotheses for drug resistance.Initial results in a pilot study with PDOs from NSCL and head and neck cancers have shown that several factors correlate with and can be combined in a linear elastic-net or robust Bayesian regression model that predicts resistance risk, as measured by PDO cell viability or IC50. These factors include the treatment induced change in CD45+ immune cell infiltrating, changes in tumour PD-L1 and immune PD-1 expression, changes in MYC, TIGIT and CD96 expression. More details of a genomic analysis will be presented in a companion presentation at this conference.

#4607 Pharmacokinetic analysis of palbociclib and association with safety/efficacy in patients with HR-positive/HER2-negative advanced breast cancer: Result from PATHWAY trial.

K. Sudo[1], s. Yagishita[2], E. Noguchi[1], N. Yamamoto[3], T. Yamanaka[4], H. Mukai[5], C.-F. Chung[6], Y.-S. Lu[7], J. Sohn[8], K. Lee[9], S. Lee[10], T. Iwasa[11], H. Iwata[12], K. Watanabe[13], K. Jung[14], Y. Tanabe[15], S. Kang[16], H. Yasojima[17], K. Aogi[18], E. Tokunaga[19], S. Sim[20], Y. Yap[21], K. Matsumoto[22], L.-M. Tseng[23], Y. Mori[24], Y. Umeyama[24], Y. Kojima[1], T. Hata[1], A. Kuchiba[1], T. Shibata[1], K. Nakamura[1], Y. Fujiwara[1], A. Hamada[2], K. Tamura[1], K. Yonemori[1];

[1]National Cancer Center Hospital, Tokyo, Japan, [2]National Cancer Center Research Institute, Tokyo, Japan, [3]Chiba Cancer Center, Chiba, Japan, [4]Kanagawa Cancer Center, Yokohama, Japan, [5]National Cancer Center Hospital East, Kashiwa, Japan, [6]Koo Foundation Sun Yat-Sen Cancer Center, Taipei, Taiwan, [7]National Taiwan University Hospital, Taipei, Taiwan, [8]Severance Hospital, Yonsei University Health System, Seoul, Korea, Republic of, [9]Seoul National University Hospital, Seoul, Korea, Republic of, [10]National University Hospital, Singapore, Singapore, [11]Kindai University Hospital, Osaka, Japan, [12]Aichi Cancer Center Hospital, Nagoya, Japan, [13]National Hospital Organization Hokkaido Cancer Center, Sapporo, Japan, [14]Asan Medical Center, Seoul, Korea, Republic of, [15]Toranomon Hospital, Tokyo, Japan, [16]Ajou University Hospital, Suwon, Japan, [17]National Hospital Organization Osaka National Hospital, Osaka, Japan, [18]National Hospital Organization Shikoku Cancer Center, Matsuyama, Japan, [19]Kyusyu Cancer Center, Fukuoka, Japan, [20]National Cancer Center, Goyang, Korea, Republic of, [21]National Cancer Centre Singapore, Singapore, Singapore, [22]Hyogo Cancer Center, Hyogo, Japan, [23]Taipei Veterans General Hospital, Taipei, Taiwan, [24]Pfizer R&D Japan, Tokyo, Japan

Background: We recently reported that the combination of palbociclib and tamoxifen significantly extended progression-free survival (PFS) in patients with HR+/HER2- advanced breast cancer, in both pre-, peri-, and post- menopausal Asian patients versus tamoxifen alone (PATHWAY trial, NCCH1607, NCT03423199). Here, we report on palbociclib pharmacokinetic (PK) analysis results and their relationship to adverse events and PFS.

Methods: In the PATHWAY study, 184 patients were randomized to palbociclib plus tamoxifen group (91 patients) or placebo plus tamoxifen group (93 patients). Blood samples were collected to assess steady-state trough plasma concentrations of palbociclib (at C1D15 and C2D15). PK data from patients who had been administered 125 mg of palbociclib or placebo once daily for seven consecutive days before sample collection, and for whom samples were collected within 24 hours ± 10% of the dose administered the day prior to sample collection, were used in the analysis. All the PK analyses were performed using LC/MS/MS. The study has been conducted as a Clinical Research Collaboration with the National Cancer Center Hospital as the regulatory study sponsor and Pfizer providing drug and financial support.

Results: A total of 171 patients were included in the analysis: 82 in the palbociclib group and 89 in the placebo group. In the palbociclib group, 82 patients had samples collected at C1D15, and 47 at C2D15, and the median palbociclib concentration (interquartile range) was 84.8 (62.45 - 104.75) ng/mL and 67.9 (51.75 - 80.05) ng/mL, respectively. In the quartile analysis of palbociclib C1D15 PK, the lowest quartile (Q1) group tended to have a higher rate of progressive disease than the other quartile groups (Q2-4) (Q1 vs Q2-4, 14.3% vs 1.6%). In terms of adverse events, the frequency of hematological toxicities such as neutropenia and thrombocytopenia increased in both cycle one and the entire treatment period, in correlation with the quartile of palbociclib C1D15 PK. On the other hand, the frequency of dose reductions also increased in correlation with the quartile. Finally, PFS in the Q1 group was comparable to placebo but significantly shorter than in the Q2-4 group (median PFS 95% confidence interval) Placebo; 11.1 (8.5 - 14.9) months, Q1; 9.5 (5.4 - NR) months, Q2-4; 29.5 (20 - 38.2) months, p=0.033).

Conclusion: In combination therapy with palbociclib and tamoxifen for HR+/HER2- advanced breast cancer, the palbociclib PK at C1D15 correlated with the frequency of hematological toxicity. At the same time, patients with lower exposures of palbociclib may not benefit in terms of PFS. Further accumulation of real-world PK data may lead to optimization of palbociclib dosing, which in turn could potentially reduce adverse events and improve survival outcomes.

#4608 Evaluation of the impact of homozygous MTAP truncations on the clinical activity of MTA-cooperative PRMT5 inhibitors.

Kimberly J. Briggs[1], Andre Mignault[1], Jordi Rodon[2], Victor Moreno[3], David Spigel[4], Capucine Baldini[5], Candace Haddox[6], Angela Hirbe[7], Ignacio Garrido-Laguna[8], Ibiayi Dagogo-Jack[9], Emiliano Calvo[10], Alexander Spira[11], Matthew R. Tonini[1], Douglas A. Whittington[1], Steven A. Lombardo[1], Binzhang Shen[1], Hannah Stowe[1], Shangtao Liu[1], Hongxiang Zhang[1], Satoshi Yoda[1], Brian Doyon[1], Wenhai Zhang[1], Minjie Zhang[1], Kevin M. Cottrell[1], Samuel Meier[1], Yi Yu[1], Teng Teng[1], Heidi Rego[1], Jennifer Morawiak[1], Heather DiBenedetto[1], Ellen Hooper[1], Adam Crystal[1]

[1]Tango Therapeutics, Boston, MA,[2]The University of Texas MD Anderson Cancer Center, Houston, TX,[3]START Madrid-FJD, Hospital Fundacion Jimenez Diaz., Madrid, Spain,[4]Sarah Cannon Research Institute, Nashville, TN,[5]Gustave Roussy, Paris, France,[6]Dana Farber Cancer Institute, Boston, MA,[7]Washington University School of Medicine in St/ Louis, St. Louis, MO,[8]Huntsman Cancer Institute - University of Utah Health, Salt Lake City, UT,[9]Massachusetts General Hospital, Boston, MA,[10]START Madrid-CIOCC, Madrid, Spain,[11]Next Oncology, Fairfax, VA

Homozygous deletion of the MTAP gene occurs in 10-15% of all human cancers. To benefit this large patient population, MTA-cooperative PRMT5 inhibitors, including TNG908, TNG462, and TNG456 were developed to leverage the synthetic lethal relationship between MTAP deletion and PRMT5 inhibition. Clinical trial eligibility for most MTA-cooperative PRMT5 inhibitors is restricted to patients with tumors with confirmed MTAP loss either detected by next-generation sequencing or immunohistochemistry. MTAP loss most commonly occurs as a co-deletion event with the proximal CDKN2A gene and the chromosomal 9p breakpoints are not uniform. Previously, we reported that homozygous loss of only the terminal exon (exon 8), an event reported to occur in only 0.5% of MTAP-deleted tumors, is insufficient for complete loss of MTAP activity in preclinical assays. Here, we report initial evidence for the clinical impact of MTAP truncations, as opposed to complete deletion, on the activity of MTA-cooperative PRMT5 inhibitors.

CLINICAL RESEARCH: Predictive Biomarkers 5
Poster Session

#4611 Microbiome-derived indole metabolites and response to immune checkpoint inhibitors in patients with hepatocellular carcinoma.

Shwetha V. Kumar[1], Iqbal Mahmud[2], Yehia I. Mohammed[3], Xiaotao Zhang[4], Philip L. Lorenzi[2], Jennifer A. Wargo[5], Ahmed O. Kaseb[3], Manal M. Hassan[6], Kristi L. Hoffman[7], Carrie R. Daniel[6]

[1]Section of Epidemiology and Population Sciences, Department of Medicine, Baylor College of Medicine, Houston, TX,[2]Department of Bioinformatics and Computational Biology, Division of Discovery Science, University of Texas MD Anderson Cancer Center, Houston, TX,[3]Department of Gastrointestinal Medical Oncology, Division of Cancer Medicine, University of Texas MD Anderson Cancer Center, Houston, TX,[4]Institute for Translational Epidemiology, Icahn School of Medicine at Mount Sinai, New York, NY,[5]Department of Genomic Medicine and Department of Surgical Oncology, University of Texas MD Anderson Cancer Center, Houston, TX,[6]Department of Epidemiology, Division of Cancer Prevention and Population Sciences, University of Texas MD Anderson Cancer Center, Houston, TX,[7]Alkek Center for Metagenomics & Microbiome Research, Department of Molecular Virology & Microbiology, Baylor College of Medicine, Houston, TX

Background: Immune checkpoint inhibitors (ICI) represent the most promising therapy for hepatocellular carcinoma (HCC) to-date; however, responses are heterogeneous and strategies to extend survival are urgently needed. Metabolites of the essential amino acid, tryptophan (Trp) are emerging as potential targets in immune tolerance, inflammation and ICI response. Trp metabolism occurs through: (1) the kynurenine pathway, (2) the serotonin pathway, and (3) the indole pathway—the latter of which is primarily mediated by the gut microbiota. Specific indole metabolites exhibit anti-cancer properties, such as inhibiting cell proliferation and activating CD8+ T cells to target tumor cells. In unresectable, neoadjuvant and adjuvant HCC patients treated with ICI, we aimed to identify metabolite biomarkers of durable response. We further explored microbial sources of these metabolites and validated the presence of relevant gut bacteria in a separate but overlapping cohort of HCC patients prior to ICI therapy.
Methods:Ten serum indole metabolites were measured by liquid chromatography-high-resolution mass spectrometry (LC-HRMS) in 38 HCC patients treated with anti-PD1/PL1 (nivo+/-ipi, atezo+bev). Kaplan-Meier analysis assessed time-to-progression (TTP) and recurrence-free survival (RFS) in patients with non-resectable (66%) and resectable disease, with Mann-Whitney U test in landmark analysis (TTP>8 mos, RFS>2 yrs). Finally, we characterized the fecal microbiome of 8 unresectable HCC patients using 16Sv4 rDNA sequencing and compared the profiles to predictions from TrpNet, a web-based tool that uses Bayesian logistic regression to identify potential microbial sources of Trp metabolites.
Results: Among unresectable patients with elevated vs. low indolepropionic acid (IPA), 50% vs. 10% reached TTP>8 mos. According to TrpNet, IPA is most likely produced by the Peptostreptococcaceae family, which was observed in our small microbiome cohort. However, the relative abundances of bacterial families Pasteurellaceae and Lachnospiraceae—producers of the IPA precursor indole pyruvate—were the most significantly elevated in patients that initially responded to IC1. No clear associations were observed in the small subset of resectable patients.
Conclusion: Elevated IPA levels were correlated with improved outcomes among unresectable HCC patients treated with ICI. These findings further highlight the potential role of circulating indoles and other microbial-derived metabolites in ICI response, warranting further validation in larger cohorts and investigation of their influence on tumor progression and treatment efficacy.

#4612 Development of an Integrated Microfluidic Chip for CAR T-Cell Therapy: A Preclinical Trial-on-Chip Tool.

Xuejia Kang[1], Lang Zhou[1], Weiqiang Chen[2], Zongliang Yue[3], Pengyu Chen[1]

[1]Department of Mechanical Engineering, Materials Research and Education Center, Auburn University, Auburn, AL,[2]Department of Biomedical Engineering, New York University, New York, NY,[3]Department of Drug Discovery and Development, Harrison College of Pharmacy, Auburn University, Auburn, AL

Chimeric antigen receptor (CAR) T-cell therapy has demonstrated promising outcomes in treating leukemia, yet it confronts significant hurdles due to severe side effects like cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). Furthermore, existing preclinical evaluation platforms often fall short in accurately replicating the human tumor microenvironment, leading to extended development durations and elevated costs. To overcome these challenges, we have developed an innovative, integrated microfluidic chip equipped with microarchitectural features that precisely mimic tumor niches, thus enhancing the modeling of CAR T-cell therapy. This chip incorporates an integrated biosensor for real-time cytokine detection, providing essential insights into cytokine dynamics throughout the therapy process. This advanced chip enables spatiotemporal monitoring of CAR T-cell functionality by observing key processes such as T-cell extravasation, target recognition, and leukemia cell destruction. Additionally, it supports the detailed examination of immune cell interactions, including those with monocytes and basophils, which are critical for understanding the side effects of CAR T therapy, particularly its impact on neuronal tolerance. By meticulously tracking these dynamics, our tool not only refines the precision of therapy modeling but also deepens our understanding of the immune response and potential toxicities associated with CAR T-cell therapy. Our investigations using the chip have yielded detailed profiles of cytokine levels across various therapy phases, including remission, resistance, and relapse. Moreover, the chip is capable of evaluating the effects of compounds in inhibiting cytokine release. This cutting-edge tool significantly shortens the development timeline, reduces costs, and enhances the physiological relevance of preclinical evaluations for CAR T-cell therapy. The implementation of this ‘(pre-)clinical-trial-on-chip’ platform is poised to revolutionize CAR T-cell therapy by facilitating the development of personalized therapies and informing clinical decision-making processes. Our findings represent a transformative step forward in the optimization and safety profiling of CAR T-cell therapies.

#4613 Differential TROP2 immunohistochemical expression between pleomorphic and classic invasive lobular carcinoma of the breast.

Elizve Barrientos-Toro[1], Harsh Batra[1], Pandurengan Renganayaki[1], Yasmeen Qamar Rizvi[2], Jason Aboudi Mouabbi[3], Funda Funda Meric-Bersntam[2], Qingqing Ding[4], Aysegul Sahin[4], Maria Gabriela Raso[5]

[1]Translational Molecular Pathology, UT MD Anderson Cancer Center, Houston, TX,[2]Investigational Cancer Therapeutics, UT MD Anderson Cancer Center, Houston, TX,[3]Breast Medical Oncology, UT MD Anderson Cancer Center, Houston, TX,[4]Anatomical Pathology, UT MD Anderson Cancer Center, Houston, TX,[5]UT MD Anderson Cancer Center, Houston, TX

Background:TROP2, a transmembrane glycoprotein implicated in tumor progression and metastasis, exhibits variable expression across breast cancer subtypes. The classic variant of invasive lobular carcinoma (ILC-C) and the pleomorphic variant (ILC-P) display distinct morphological and biological characteristics. This study aims to investigate differences in TROP2 expression between ILC-C and ILC-P, enhancing our understanding of its potential as a biomarker for aggressive subtypes.
Methods:TROP2 protein expression was evaluated in 14 samples (8 ILC-C and 6 ILC-P) using immunohistochemistry (IHC) and quantified with digital image analysis software. Samples were annotated and classified into tumor and stroma components. The percentage of TROP2-positive cells was assessed, and staining intensity was categorized as weak, moderate, or strong. H-scores were calculated to assess overall TROP2 expression. Statistical analyses included descriptive statistics and the Wilcoxon test to compare expression levels between ILC-C and ILC-P groups.
Results:TROP2 expression was significantly higher in ILC-P samples compared to ILC-C across multiple metrics. The percentage of positive cells was markedly elevated in ILC-P (mean: 73.04; median: 81.09; SD: 16.08) compared to ILC-C (mean: 17.97; median: 15.14; SD: 12.48). Similarly, H-scores were significantly higher in ILC-P (mean: 153.07; median: 146.79; SD: 54.31) than in ILC-C (mean: 24.97; median: 17.96; SD: 18.81). The Wilcoxon test for both parameters revealed statistical significance ($p = 0.002$). However, no significant difference was observed between molecular classification groups and TROP2 expression levels.
Conclusion:Our findings demonstrate distinct TROP2 protein expression profiles in ILC, with ILC-P samples exhibiting significantly higher expression levels. This elevated TROP2 expression in the aggressive ILC-P variant highlights its potential as a biomarker for identifying high-risk lobular carcinoma subtypes. Further studies are warranted to explore the clinical implications of TROP2 in ILC-P, potentially guiding targeted therapeutic strategies.

#4614 Differential PD-L1 expression by IHC between matched samples obtained from FFPE blocks versus frozen blocks.

Rebeeca Bascom[1], Paige Nelson[1], Jacob Harris[1], David Booker[2], Ken Dixon[3], Jared James Barrott[1]

[1]Brigham Young University, Provo, UT,[2]Specicare, Augusta, GA,[3]Specicare, Gainesville, GA

Introduction: Personalized cancer treatment is crucial for improving patient outcomes, yet the standard preservation methods for clinical tissue samples may not fully support this goal. Currently, most cancer tissue samples are preserved as formalin-fixed and paraffin-embedded (FFPE), which recent studies suggest compromise DNA quality and quantity. In this study, we extended previous research by comparing the protein expression of PD-L1, a significant biomarker associated with cancer cell evasion of immune response, between FFPE and cryopreserved (frozen) tissues from 37 patients with various types of cancer. We hypothesized that cryopreserved samples would exhibit higher and more accurate PD-L1 levels than FFPE samples.
Methods: The first step in our research was to confirm the presence of cancer in 37 patient tissue samples through standard hematoxylin and eosin (H&E) staining. Next, we stained both the FFPE and cryopreserved samples using immunohistochemistry (IHC) protocols to visualize PD-L1 expression. The FFPE protocol involved dewaxing, antigen retrieval, incubation with primary and secondary antibodies, and staining. The protocol for the cryopreserved tissues was similar, with slight changes by eliminating the dewaxing and antigen retrieval steps. DAB was used to stain PD-L1 (identified in brown) and hematoxylin was used to stain cell nuclei (identified in blue). Positive controls were included in each stain to ensure reliability and minimize overstaining. Once each sample was stained in both its FFPE and cryopreserved forms, samples were sent to a board certified pathologist for further analysis and comparison.
Results: Our research results indicated a trend towards increased PD-L1 expression in cryopreserved samples, supporting our hypothesis that FFPE tissue exhibits a weaker PD-L1 signal compared to cryopreserved tissue. We have seen a significant difference between the FFPE and cryopreserved tissue samples-a result that was corroborated by a board-certified pathologist. Among the FFPE samples, less than 6% were found to be positive for PD-L1. Conversely, the cryopreserved samples exhibited a 64% rate for positive PD-L1 expression.
Discussion: This study highlights the impact of tissue preservation methods on the accuracy of cancer diagnostics through an analysis of protein preservation. The personalization of treatment strategies in a clinical setting depends on data such as these, underscoring the need for more accurate analysis. This data, along with previous and future studies, will advocate for a shift towards cryopreservation in clinical settings, leading to better personalized treatment and results for the patient.

#4615 Comprehensive genomic profiling using VARIANT*Plex*™ and FUSION*Plex*™ panels along with Archer's new tumor-only homologous recombination deficiency algorithm.

Glen Bjerke, Taylor Patterson, Devin Tauber, Allison Hadjis, Ryan Rogge, Dhruti Legare, Mark Rogers, Laura Johnson, Brent Lutz

Integrated DNA Technologies, Inc., Boulder, CO

Comprehensive Genomic Profiling (CGP) for cancer research has arisen out of the need for Next Generation Sequencing (NGS) tests that can be used across tumor types and detect both discreet genomic changes and genome-wide biomarkers. Herein, we detail IDT's Archer CGP suite of assays, offering cutting-edge precision oncology research solutions designed to provide a thorough and accurate molecular characterization of tumors. This modular set of assays includes VARIANT*Plex* (DNA) and FUSION*Plex (*RNA) profiling with a full range of biomarkers that offer high concordance with established orthogonal methods, ensuring both reliability and reproducibility in genomic profiling. The newest component of Archer's CGP assays is Homologous Recombination Deficiency (HRD) analysis, which is based on Archer's tumor-only Copy Number Variation 2.0 (CNV2.0) and Allele Specific Copy Number (ASCN) calling along with an independent measure of Non-homologous End Joining (NHEJ). Archer's HRD solution comprises a modular set of chemistry targets that can be added to any VariantPlex panel and an HRD bioinformatics workflow within Archer Analysis software. Our HRD pipeline shows high concordance with orthogonal Genomic Instability Score (GIS) methods and consistent calling across various targeted panels and input masses. Additional CGP biomarkers for DNA analyses include Microsatellite Instability (MSI) and tumor mutational burden (TMB) calling. In addition to genome-wide biomarker analysis, all VARIANT*Plex* panels include Single Nucleotide Variant (SNV), Insertion/Deletion (InDel), and Internal Tandem Duplication (ITD) calling with panel sizes of up to 460 genes. The RNA components of Archer's CGP offering are FUSION*Plex* panels that allow fusion calling from low purity (>= 10% tumor cellularity) samples along with RNA variant calling, which we show to be consistent with DNA allele frequencies. Furthermore, all VARIANT*Plex* and FUSION*Plex* panels can be customized to allow users the flexibility to make a CGP solution according to their individual needs. Archer's CGP panels offer a complete end-to-end solution including automatable chemistry workflows, Archer Analysis bioinformatics software, integration with Molecular Heath's Guide Case Annotation Solution (CAS) tertiary analysis platform, and now include tumor-only HRD calling along with the full suite of biomarker testing for RNA and DNA panels.

#4616 Detection of phosphorylated biomarkers in clinical blood samples: a flow cytometry platform utilizing smart tube fixation.

Valerie Oberhardt, Philipp Metzger, Carla N. Castro, **Holger Weber**

Reaction Biology Europe GmbH, Freiburg, Germany

The identification and quantification of biomarkers in clinical blood samples play a pivotal role in personalized medicine, enabling disease monitoring and therapeutic assessment. Protein phosphorylation, a key post-translational modification, reflects cellular signaling in immune or tumor cells. To exploit this, we developed a platform detecting phosphorylation states of key signaling proteins from clinical blood samples via flow cytometry. Our approach analyzes phosphorylated STAT5 (pSTAT5), ERK1/2 (pERK1/2), and p38 (p-p38), vital components in immune regulation and cancer progression.

A major challenge in the analysis of phosphorylated proteins is the rapid degradation or change in phosphorylation state ex vivo, which requires immediate stabilization of samples after collection. To solve this problem, we use Smart Tubes, which enable rapid fixation of clinical blood samples and preserve the phosphorylation state upon collection, ensuring signal integrity and minimizing variability. Sample collection with Smart Tubes requires no technical equipment and can be easily implemented and standardized at any clinical trial site.

Our test system employs flow cytometry, enabling the analysis of linage-specific markers (e.g., T/B cells, monocytes) and phospho-specific antibodies to quantitatively detect pSTAT5, pERK1/2, and p-p38 in peripheral blood mononuclear cell (PBMCs) subpopulations. Validation experiments with specific inhibitors demonstrate sensitivity and specificity in detecting these phosphorylated targets, even in heterogeneous cell populations within blood samples. The method allows for its application across a range of clinical conditions, from cancer immunotherapy monitoring to autoimmune disease characterization.

By integrating rapid sample fixation with advanced analytical techniques, this platform addresses key challenges in analyzing phosphorylated biomarkers in clinical samples. The use of flow cytometry as the central analytical tool enables single-cell resolution, allowing detailed investigation of individual cell populations within complex samples by differential marker expression. This precision makes it possible to differentiate and study specific subpopulations of immune cells or circulating tumor cells, within a heterogeneous sample. The ability to preserve and accurately measure phosphorylation states directly in patient-derived samples provides invaluable insights into real-time cellular signaling and facilitates the optimization of therapeutic strategies targeting dysregulated signaling pathways.

In summary, our system can detect phosphorylated proteins in clinical samples, with Smart Tubes ensuring sample stability and reliable biomarker analysis, advancing translational research and precision medicine.

#4617 Blood-based biomarkers for predicting treatment response to immune checkpoint inhibitors after EGFR-TKI resistance.

Jun Hyeok Lim, Min Seok Park, Nuri Park, Yejin Cho, Sehan Kwak, Beom Seok Han, Minseo Lee, Donghyun Seo, Woo Kyung Ryu, Jeong-Seon Ryu, Soon-sun Hong, Kyung Hee Jung

Inha University College of Medicine, Incheon, Korea, Republic of

Background: Immune checkpoint inhibitors (ICIs) improve survival in advanced non-small cell lung cancer (NSCLC), but their benefits are limited in EGFR-mutant NSCLC. ICIs are often used after resistance to EGFR tyrosine kinase inhibitors (EGFR-TKIs). Since EGFR-TKI treatment alters the tumor microenvironment, predictive biomarkers for ICI response should ideally be identified post-EGFR-TKI resistance. However, obtaining re-biopsy samples is challenging. This study explores predictive biomarkers for ICI response using plasma samples collected before and after EGFR-TKI therapy.

Methods: We retrospectively analyzed 28 EGFR-mutant NSCLC patients treated with ICIs at Inha University Hospital (2016-2023). All patients received first-line EGFR-TKI therapy and transitioned to ICIs after resistance. Plasma samples were collected before EGFR-TKI initiation and post-resistance. Ninety-six plasma proteins were quantified using the Olink Immuno-Oncology panel. PD-L1 expression in tumor tissues was assessed at diagnosis with SP263 or 22C3 assays. Progression-free survival (PFS) was defined as time from ICI initiation to progression. Responders were defined as PFS ≥6 months, and non-responders as PFS <6 months.

Results: Among 28 patients, 25 had adenocarcinoma, 1 squamous cell carcinoma, 1 adenosquamous carcinoma, and 1 poorly differentiated NSCLC. EGFR mutations included exon 19 deletion (n=14), L858R (n=10), and uncommon mutations (n=4). First-line EGFR-TKIs included erlotinib (n=15), afatinib (n=9), and gefitinib (n=4). T790M mutations were identified in 11 patients, of whom 10 received osimertinib and 1 received lazertinib. ICIs included atezolizumab (n=15), pembrolizumab (n=5), and nivolumab (n=8). PD-L1 expression was evaluated in 23 patients using SP263 (5 negative, 15 with 1-49%, 3 with ≥50%) and in 22 patients using 22C3 (7 negative, 11 with 1-49%, 4 with ≥50%). Median PFS for ICIs was 2.7 months (95% CI: 1.7-3.7), with 6 responders and 22 non-responders. Plasma levels of IL-4, IL-6, GZMH, and Gal-9 differed significantly between responders and non-responders post-EGFR-TKI resistance. These findings were validated using tissue samples obtained post-resistance. Progression-free survival (PFS) showed significant differences according to plasma biomarker expression.

Conclusions: Plasma samples collected after EGFR-TKI resistance can predict ICI responses in EGFR-mutant NSCLC. Post-resistance plasma biomarker levels may serve as a minimally invasive approach to guide therapeutic decisions in this challenging patient population.

#4618 Baseline and dynamic cytokines as biomarkers for first-line therapy in advanced hepatocellular carcinoma.

X. Zhao[1], H. Wilbur[2], M. Yarchoan[2];

[1]Johns Hopkins University School of Medicine, Baltimore, MD, [2]Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University, Baltimore, MD

Background: Hepatocellular carcinoma (HCC) causes significant cancer mortality. The preferred treatment for patients with advanced HCC is immune checkpoint inhibitors (ICIs), with or without the VEGF-inhibitor bevacizumab. The immune effects of bevacizumab are not well understood, and biomarkers are needed to guide therapy selection in advanced HCC. We serially evaluated circulating cytokines in patients with HCC treated with ICIs with or without bevacizumab.

Methods: Circulating cytokines were evaluated in 57 advanced HCC patients treated with ICI-based therapy as standard of care at Sidney Kimmel Cancer Center from 2000-2024. A total of 36 patients received ICIs without bevacizumab (ICI-Only group), and 21 patients received the combination of an ICI with bevacizumab (ICI+Bev group). Serum was collected prospectively at baseline and at approximately 2 months after starting therapy. Cytokines were assessed using a Luminex panel of 33 cytokines. Patients were stratified by above- or below-median baseline cytokine levels and fold-change post-treatment. We evaluated the relationship between treatment group (ICI-Only versus ICI+Bev), cytokine changes, and clinical outcomes.

Results: Across all patients, the median age was 65, 26% were female, and 60% were White. Baseline demographic characteristics were balanced between the ICI-Only and ICI-Bev treatment groups, except for a higher proportion of patients receiving prior systemic therapy in the ICI-Only group (75%) compared to the ICI+Bev group (14%). Compared to patients receiving ICI-Only, those receiving ICI+Bev exhibited reduced fold changes in multiple cytokines after 2 months of treatment, including sCD40L ($p = 0.017$), MIP-1b ($p = 0.015$), and MIG ($p = 0.049$). Across all patients, lower baseline levels of IL-8 ($p = 0.017$) and lower on-treatment levels of IL-1Ra ($p = 0.008$), IL-4 ($p = 0.049$), IL-8 ($p = 0.021$), IL-9 ($p = 0.028$), and IL-18 ($p = 0.004$) were associated with longer survival. In the ICI-Bev group, higher baseline IL-6 ($p = 0.008$) was associated with improved progression-free survival, while smaller post-treatment increases in IL-6 were associated with both improved PFS ($p = 0.028$) and objective response rate ($p = 0.010$). In contrast, in ICI-Only patients, lower baseline IL-8 ($p = 0.012$) and smaller post-treatment increases in IL-1Ra ($p = 0.021$) were associated with extended overall survival.

Conclusion: Adding in bevacizumab to ICIs in advanced HCC may influence systemic cytokines, particularly those associated with innate immune populations. These findings suggest that bevacizumab may modulate innate immune responses and highlight the potential role of specific myeloid-related cytokines as biomarkers to identify patients who could benefit from bevacizumab-based combination therapy. These exploratory results warrant prospective validation in independent patient cohorts to confirm their clinical significance.

#4619 SEPT9 isoform expression and response to neo-adjuvant taxane treatment in breast cancer patients.

Jacob Nathaniel Essif, Kate Lathrop, April Risinger

UT Health Science Center at San Antonio, San Antonio, TX

Drugs that disrupt the microtubule cytoskeleton, such as the microtubule stabilizing taxanes, are regularly used in the treatment of breast cancer in the neoadjuvant setting. However, there are currently no molecular biomarkers that are used to guide which patients will respond to taxane treatment. Septins, a class of small GTPases, interact with both microtubules and actin to functionally link these cytoskeletal components and regulate cell biological processes shown to be critical for the invasion and migration of cancer cells. Septin 9 (SEPT9) directly interacts directly with microtubules through an N-terminal domain on the longest isoform, SEPT9 isoform 1, which is sufficient to promote migration and invasion of breast cancer cells *in vitro.* Additionally, both SEPT9 isoform 1 and SEPT9 isoform 3 have been shown to play a role in taxane resistance *in vitro*, but the possible role in clinical taxane resistance had not been studied. We hypothesized that the relative expression of SEPT9 isoforms in breast tumors could play a role in their response to treatment taxanes in the neoadjuvant setting. We performed a prospective clinical trial to correlate the expression of SEPT9 isoforms in breast tumors with pathological response to taxanes in the neoadjuvant setting. Pretreatment levels of SEPT9 isoform mRNA, as well as mRNA from other genes associated with clinically relevant drug resistance mechanisms, were determined from FFPE biopsy samples by multiplexed PCR, while protein levels of SEPT9 isoforms were determined by IHC. Patients were treated in the neoadjuvant setting with either paclitaxel (dose dense or weekly), docetaxel or nab-paclitaxel. Expression was reevaluated in post-treatment surgical samples of patients who did not have a pathological complete response (pCR). We found that the expression of SEPT9 mRNA variants and drug resistance genes differed amongst the pre-treatment patient samples. Initial SEPT9v2 mRNA levels are higher in patients that have a pCR. Additionally, expression of SEPT9v4, MDR1 and ABCC11 mRNA levels are statistically significantly reduced post-taxane treatment in patients that did not have a pCR. We are currently determining the mRNA expression levels of a panel of 96 clinically relevant genes in the pre-treatment samples to identify a genetic profile of response to taxane treatment. We are performing *in vitro* studies to determine if elevated levels of SEPT9v2 correspond with response to other chemotherapeutic drugs. Overall, the data support the hypothesis that the relative expression of SEPT9v2 in breast tumors plays a role in their response to treatment with to taxanes in the neoadjuvant setting and supports the use of SEPT9v2 expression levels in determining whether to use taxane treatment or other drug treatment in the neoadjuvant setting, facilitating the use of microtubule targeted chemotherapy in a more personalized manner.

#4620 Assessing the impact of germline and somatic variants in breast cancer treatment.

Jonathan V. Wooten[1], Santiago Avila[2], Becca Pollard[3], Sheila Rajagopal[4]

[1]National Cancer Institute, Rockville, MD,[2]Pritzker School of Medicine, University of Chicago, Chicago, IL,[3]Harvard University, Cambridge, MA,[4]National Cancer Institute, Bethesda, MD

Germline genetic variants are hereditary DNA alterations, whereas cancer somatic variants originate in the tumor. For patients with breast cancer, treatment approvals focus on either germline or somatic data with no consideration of co-occurrence. As such, most clinical datasets lack either germline or somatic variant designation, and most molecular oncology datasets contain limited treatment information, focusing primarily on survival. Datasets that contain germline and somatic sequencing and treatment information are typically from single institutions, limiting their statistical power. This project seeks to aggregate datasets inclusive of germline and somatic sequencing and treatment information with the goal of starting to assess the effect of co-occurring variants on treatment response in breast cancer.

We reviewed breast cancer datasets in CBioPortal and papers published in the past 15 years for germline and somatic sequencing with reported variants, treatment information, and associated responses. We identified 3 datasets: The Cancer Genome Atlas, The Metastatic Breast Cancer Project, and Fudan University Shanghai Cancer Center (BC cohort). We evaluated a total of 7 co-occurring gene pairs obtained from literature review: *BRCA1* and *BRCA2*; *BRCA2* and *RB1*, *BRCA1* or *2* and *TP53*, *PTEN*, *PIK3CA, MYC*; and *PALB2* and *NOTCH2*. We used the NIH Integrated Data Analysis Platform to harmonize variant calls across these datasets. For early-stage disease, we assessed disease-free survival (DFS) in response to the classes of chemotherapy or hormone therapy. For metastatic disease, we assessed duration of treatment response for specific treatments.

We were able to assess one gene pair using our dataset – *BRCA1/2* and *TP53*. Other gene pairs had 0-2 patients with co-occurring variants, limiting statistical analysis. We observed statistically significant differences in DFS among early-stage patients who received chemotherapy (Kruskal-Wallis test: <0.05), but observed patients with *BRCA1/2* alone having shorter DFS relative to those with *TP53* regardless of co-occurrence:No variants (n=212): Mean=33.32 months (SD=26.7 months)*BRCA1/2* alone (n=141): Mean=18.32 months (SD=13.91 months)Co-occurring (n=10): Mean=49.17 months (SD=38.14 months)*TP53* alone (n=95): Mean=41.45 months (SD=31.88 months)

In the context of treatment, significantly more publicly available, better annotated data will be required to understand the influence of co-occurring variants, germline origin, and somatic origin on treatment response. In this preliminary analysis, we observed differential patterns of changes in treatment response based on the presence or absence of *BRCA1/2* and *TP53*. These genes commonly co-occur – where co-occurrence is less common, we lacked power for our analysis. This question is under-addressed and may potentially influence treatment response and decision-making.

#4621 Beyond tumor biomarkers: Multiparametric biophysical assessment of T cells from peripheral blood to inform checkpoint blockade therapy.

Robert Kimmerling[1], Selim Olcum[1], Mark Stevens[1], Rachel LaBella[1], Madeleine Vacha[1], Katelin Katsis[1], Reginald Aikins[1], Steven Wasserman[1], Tatyana Sharova[2], Aleigha Lawless[2], Sonia Cohen[2], Genevieve Boland[2]

[1]TRAVERA, Medford, MA,[2]MGH, Boston, MA

Clinical decisions for immune checkpoint blockade (ICB) treatment currently rely on tumor-based biomarkers such as mutational burden, microsatellite instability, and PD-L1 expression. While these markers serve as valuable proxies for tumor antigenicity and the immune status of the tumor microenvironment, they fail to account for the fitness of a patient's T cells, a critical factor in anti-tumor immunity. Direct assessment of T cell functional capacity could complement existing biomarkers to improve patient stratification and efficacy prediction for ICB therapies. Our previous work showed that single-cell mass measurements serve as a label-free means of assessing immune cell activation and ICB response. T cells from patients with various malignancies displayed significant variability in both their activation capacity and ICB response ex vivo, indicating that mass measurements capture key functional differences in T cells across patients.Here we present a next-generation platform that measures cell mass alongside linked measurements of volume, density, and morphological features. This platform incorporates fluorescence exclusion measurements to determine single-cell volume which, when combined with cell mass, enables precise determination of single-cell density. Additionally, we utilize inline brightfield imaging to capture images from individual cells and extract morphological features using an autoencoder-based reconstruction model.Our results show that multiparametric measurements significantly enhance the ability to characterize T cell functional states. In activation experiments using healthy donor T cells, these combined signatures reveal notable phenotypic changes as early as six hours after activation—changes that remain undetectable with mass measurements alone.In a cohort of peripheral blood samples from patients with advanced melanoma, we observed substantial heterogeneity in T cell biophysical signatures at baseline and following stimulation, with and without immune checkpoint blockade. These signatures were distinct from those observed in T cells derived from healthy donor PBMCs.These findings underscore the potential of a blood-based functional precision medicine assay to assess patients' baseline immune fitness. When integrated with existing clinical biomarkers, these unique functional signatures offer orthogonal insights that could improve patient stratification for immune checkpoint inhibitor therapies. Moreover, the multiparametric nature of these measurements aligns well with emerging multimodal AI approaches, paving the way for more effective identification of patients most likely to benefit from these treatments.

#4622 Proteomic insights into anti-CTLA4 therapy resistance in metastatic melanoma: Pathway-specific biomarkers for treatment response.

Katherine Bronson, Nathan Avaritt, Erin Taylor, Alan Tackett

University of Arkansas for Medical Sciences, Little Rock, AR

The current standard of care for metastatic melanoma includes immune checkpoint inhibitor (ICI) therapy that, although revolutionary for melanoma treatment, is ineffective for most patients long-term. According to clinical data, patients have a limited response to anti-PD1 and anti-CTLA4 immunotherapy treatment, with overall response rates of roughly 40% and 15% respectively, and increasing to 60% with combination therapy. However, with half of patients not responding to combination therapy or acquiring resistance to therapy as the treatment progresses, there is an evident need for identifying and vetting more reliable biomarkers for treatment response or resistance. Although up-to-date ICI treatment often includes both anti-PD1 and anti-CTLA4 effectiveness, the different treatment regimens have independent factors of patient responsiveness. Anti-PD1 therapy directly mediates tumor-immune interactions, permitting immune system activation; however, protein levels of PD1 or its ligand is a poor indicator of responsiveness. Recent data suggests an enrichment in oxidative phosphorylation and lipid metabolism in anti-PD1-responsive patients. Identifying key biomarkers for response to CTLA4 therapies is more nuanced, as its mode of action occurs outside of the tumor microenvironment. Due to this, the approach to identifying tumoral patterns or biomarkers that indicate anti-CTLA4 responsiveness is more nuanced. To identify anti-CTLA4 response-specific biomarkers, we collected metastatic melanoma tumors from patients prior to ICI treatments and sorted them as responders or non-responders depending on patient response. The tumor proteome from patients treated with anti-CTLA4 therapy was analyzed via tandem mass tag (TMT) mass spectrometry to mark differentially expressed proteins between responder and non-responder groups. Comparative pathway analyses on platforms such as Ingenuity Pathway Analysis (IPA) and GSEA found several distinct protein and pathway enrichments, including that anti-CTLA4 non-responding patient tumors were enriched for pathways such as oxidative phosphorylation and fatty acid metabolism. This anti-CTLA4-specific enrichment is distinct from anti-PD1-treated patient tumors. These differences between the ICI treatment responses could be used clinically to inform patient treatment plans and improve the likelihood for response to initial treatments. Future studies will elucidate the function of specific proteins and pathways in ICI resistance as well as identify potential novel neoadjuvant therapies to increase patient responsiveness to ICI therapies.

#4623 The relationship of KRAS mutation and clinical outcomes in patients treated with consolidation durvalumab for locally advanced non-small cell lung cancer.

W. J. Phillips, M. Pradier, D. J. Stewart, S. Moore;
University of Ottawa, Ottawa, ON, Canada

Introduction: *KRAS* G12C is associated with a favorable response to immunotherapy in patients with advanced non-small cell lung cancer (NSCLC), while other *KRAS* variants show mixed effects. In addition, there is little known on how *KRAS* mutations affect immunotherapy responsiveness in the curative setting. This study aims to evaluate the impact of *KRAS* mutations on clinical outcomes of patients treated with consolidation durvalumab for unresected locally advanced NSCLC.

Methods: Clinical data were gathered from the medical records of patients seen at The Ottawa Hospital Cancer Centre from June 1, 2019 to Mar 1, 2024. Adult patients with non-squamous NSCLC who completed chemoradiation therapy and received at least 1 dose of consolidation durvalumab were included. Patients were grouped by KRAS mutation as follows, (1) G12C, (2) non-G12C, or (3) wild type/unknown. Real world progression free survival (PFS) was the primary outcome. Univariable analysis was performed using the Cox Proportional Hazards Model.

Results: 78 patients were included. The median age was 68 years old and 64% of patients were female. PDL1 expression was <1% in 14 (18%) patients, ≥1% in 51 (65%), and missing in 13 (17%). 50 cases were tested for KRAS and 31 (62%) harbored a KRAS mutation. Of those, 16 (32%) were G12C and 15 (30%) were non-G12C. Non-G12C point mutations included G12V (n=6), G12A (n=3), G12D (n=3), G13D (n=2), and G13V (n=1). The median PFS of the entire cohort was 40.6 months (95% CI: 23.1-58.2), G12C mutated was 32.1 months (95% CI: 26.0-38.1), and non-G12C was 20.5 months (95% CI: 12.7-28.3). Compared to KRAS wild type/unknown, there was a trend to improved PFS in patients with G12C mutations (hazards ratio (HR) 0.48, p=0.17), and shortened PFS in patients with non-G12C (HR 1.47, p=0.35). Furthermore, PDL1≥1% was associated with improved PFS (HR 0.21, p<0.01).

Conclusions: This study demonstrates the potential prognostic value of both *KRAS* G12C and non-G12C mutations in locally advanced unresectable NSCLC treated with consolidation durvalumab. It also supports PDL1 expression as an established biomarker of immunotherapy sensitivity in the real world. Further studies to prospectively validate KRAS as a predictive marker are warranted.

#4624 Analysis of the characteristics of immune microenvironment in non-small cell lung cancer patients with EGFR amplification and exploration of immunotherapy.

Zhi Xian Jin, Min Chen, Xu He, Hong Bi, Chao Yang, Jing Shao, Zhaoming Huang, Yuyan Zhou, Cuilin Yang

Department of Pulmonary and Critical Care Medicine, Affiliated Calmette Hospital of Kunming Medical University and The First People's Hospital of Kunming City, Kunming, China

Background: EGFR is the most common driver gene in Asian NSCLC patients, and its association with tumors and the efficacy of targeted therapy for sensitive mutations are well-established. According to current guidelines, first-line immunotherapy is not recommended for patients with EGFR-sensitive mutations. However, the treatment strategy for EGFR variants with limited sensitivity to TKIs remains unclear. EGFR amplification has been shown to exhibit resistance to TKIs in several studies, and its relationship with immunity has yet to be definitively established. Therefore, exploring the immune microenvironment to assess the efficacy of immunotherapy in this context holds significant clinical value.

Methods: Tumor tissue samples from 210 Chinese lung adenocarcinoma (LUAD) patients were analyzed using next-generation sequencing (NGS) and multiplex immunohistochemistry (mIHC). The mIHC analysis, performed with the Akoya Detection Kit targeting 13 markers, quantified immune cell percentages among total cells, offering insights into the distribution of cell subpopulations within the tumor and stromal compartments.

Result: Among the enrolled patients, 114 had EGFR point mutations and insertion-deletion mutations (54.3%), with 83 cases of L858R and 19del mutations, and 31 cases of EGFR amplification (14.8%), which is consistent with previous studies. An analysis of the immune microenvironment characteristics among patients with EGFR wild-type (EGFRwt), EGFR-sensitive mutations (EGFRm), and EGFR amplification (EGFRa) revealed that the levels of CD8+PD-1+ cells in the tumor stroma were significantly higher in the EGFRwt (P=0.008) and EGFRa (P=0.025) groups compared to the EGFRm group. Additionally, the NK cell levels in the tumor stroma were significantly higher in the EGFRwt group (P=0.037) compared to the EGFRm group, while no significant difference was found between the EGFRa and EGFRm groups (P=0.071). Although there were no significant differences in the infiltration levels of macrophages, T cells, and B cells between the three groups in the tumor parenchyma and stroma, the numbers were generally slightly higher in the EGFRwt and EGFRa groups compared to the EGFRm group.

Conclusions: The results of this study suggest that the level of immune cell infiltration in the EGFR amplification group is higher than in the EGFR-sensitive mutation group. This may indicate that EGFR amplification patients could have better outcomes with immunotherapy compared to those with EGFR mutations. Therefore, immunotherapy as a first-line treatment may offer greater benefits for EGFR amplification patients, providing a potential mechanistic basis for the treatment strategy of this patient population.

#4625 The optimal tumor mutational burden cutoff value for predicting the efficacy of the PD-1 checkpoint inhibitor combined with chemotherapy in advanced gastric cancer.

Jaeyeon Jang[1], Youngkyung Jeon[2], Sun Young Jeong[2], Sung Hee Lim[2], Jeeyun Lee[2], Seung Tae Kim[2]

[1]Division of Hematology-Oncology, Department of Internal Medicine,, Wonju Severance Christian Hospital, Yonsei University, Wonju College of Medicine, Wonju-si, Korea, Republic of,[2]Division of Hematology-Oncology, Department of Internal Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of

Purpose: Chemotherapy is associated with an increase in tumor mutational burden (TMB). When receiving the combination of programmed cell death-1 (PD-1) checkpoint inhibitor and chemotherapy, TMB can be a better option for predicting the response, especially for tumors with initially low TMB. We aimed to investigate the optimal TMB cutoff value that could predict the efficacy of PD-1 checkpoint inhibitors combined with chemotherapy in advanced gastric cancer. **Materials and Methods:** Patients with AGC who received nivolumab with capecitabine and oxaliplatin as the 1st line palliative treatment, between September 1, 2021, and July 15, 2023, at Samsung Medical Center in Korea were retrospectively analyzed. The TMB levels were measured using a next-generation sequencing assay. Based on receiver operating characteristic curve analysis, the TMB cutoff value was determined. **Results:** A total of 101 patients were analyzed. The TMB cutoff value for predicting the overall response rate (ORR) to nivolumab combined with capecitabine and oxaliplatin was defined as 10.2 mutations per megabase (mt/Mb) with 97.7% sensitivity and 22.4% specificity. Based on this definition, 14 (13.9%) patients were TMB-high (TMB-H). The ORR differed between the TMB-low (TMB-L) and TMB-H groups (51.7% vs. 92.9%, P=0.004). The progression-free survival (PFS) and overall survival (OS) for 101 patients were 11.8 (95% confidence interval [CI], 8.46-15.14) and 27.9 months (95% CI, 20.34-35.45), respectively. The median PFS tended to be longer in the TMB-H group (22.0 months; 95% CI, 11.86-32.14) than in the TMB-L group (11.1 months; 95% CI, 8.56-13.64; P=0.217). The median follow-up duration of the TMB-H group was 23.7 months, and the median OS was not reached. The median OS of the TMB-L group was 27.9 months (95% CI 20.50-35.23; P=0.241). **Conclusions:** The TMB cutoff value for predicting treatment response in AGC patients who received the PD-1 checkpoint inhibitor combined with capecitabine and oxaliplatin as the 1st line palliative treatment was 10.2 mt/Mb. When applying the programmed death ligand-1 status to TMB, patients who would benefit from PD-1 checkpoint inhibitors can be selected.

#4626 A machine learning model for predicting hyperprogressive disease risk in cancer immunotherapy: Integrating clinical and molecular data.

Haitao Wang[1], Lili Wang[1], Yu Zhang[1], Hongzheng Li[1], Nan Li[1], Qijie Jian[2], Yaxian Wang[2], Zhijian Song[2], Fei Pang[2], Kai Wang[2]

[1]The Second Hospital of Tianjin Medical University, Tianjin, China,[2]OrigiMed, Shanghai, China

Background
Immunotherapy has revolutionized cancer treatment, offering significant efficacy across various cancer types. However, hyperprogressive disease (HPD)—a severe and unexpected acceleration of tumor growth—poses a critical challenge, as some patients experience this adverse effect. Early and accurate identification of HPD risk is essential for optimizing immunotherapy strategies and improving patient outcomes. To address this, we developed a machine learning-based predictive model for HPD risk across cancer types, integrating clinical and molecular data.
Methods
This study analyzed data from 1,005 patients with solid tumors treated with at least one dose of immune checkpoint inhibitors between 2018 and 2024. After stringent data preprocessing, feature selection was performed using chi-square and Fisher's exact tests to identify significant variables associated with HPD. Logistic regression evaluated feature importance, accounting for multicollinearity, correlation, and interpretability. A random forest algorithm was then employed to construct a predictive model based on selected features.
Results
From an initial pool of 44 variables, eight features were identified as significant predictors of HPD, including HPD-associated genes, immunotherapy resistance genes, cancer type, bone metastasis, the number of metastatic sites, lactate dehydrogenase levels, neutrophil-to-lymphocyte ratio, and treatment regimen. Patients lacking pre-immunotherapy genetic and blood test data for the selected features were excluded, resulting in a final cohort of 363 patients across 17 cancer types, including 52 HPD cases and 311 non-HPD cases. The model demonstrated strong predictive performance, achieving a cross-validated AUC of 0.87, sensitivity of 0.90, and specificity of 0.78. On an independent test set, the model attained an AUC of 0.71, sensitivity of 0.70, specificity of 0.73, and overall accuracy of 73%. Using the same eight features, a traditional logistic regression model was constructed (AUC = 0.66) and found to be less effective than the machine learning model.
Conclusions
We identified eight statistically and clinically significant predictors of HPD and developed the first high-sensitivity pan-cancer predictive tool combining molecular and clinical features. This machine learning-based approach enhances HPD risk stratification, assisting clinicians in tailoring immunotherapy and mitigating risks, ultimately improving patient outcomes across cancer types.

#4627 Correlation of early circulating tumor DNA dynamics with whole body FDG PET/CT parameters during immune checkpoint inhibitor therapy in advanced stage melanoma.

Vincent T. Ma[1], Fauzia Hollnagel[2], Tomas A. Romero[3], Janmesh D. Patel[1], Alexander Birbrair[4], Robert J. Jeraj[5], Mark R. Albertini[1], Steve Y. Cho[3], Nandakumar Menon[3]

[1]Medicine; Division of Hematology, Medical Oncology, and Palliative Care, University of Wisconsin Carbone Cancer Center, Madison, WI,[2]Medicine, University of Wisconsin Carbone Cancer Center, Madison, WI,[3]Radiology, University of Wisconsin Carbone Cancer Center, Madison, WI,[4]Dermatology, University of Wisconsin Carbone Cancer Center, Madison, WI,[5]Medical Physics, University of Wisconsin Carbone Cancer Center, Madison, WI

Background: Evaluation of tumor volume change and [^{18}F]Fluoro-2-deoxy-2-D-glucose (FDG) uptake using whole-body PET/CT is frequently used to evaluate treatment response with immune checkpoint inhibitors (ICIs) in patients with advanced stage melanoma. We previously reported that early circulating tumor DNA (ctDNA) dynamics after 3-4 weeks of ICI initiation appears to be a candidate biomarker to predict treatment response. This study explores the correlation between early ctDNA dynamics and various FDG PET/CT parameters in ICI-treated patients with advanced melanoma.

Methods: A prospective study was conducted for patients with unresectable stage III/IV melanoma who underwent personalized, tumor-informed ctDNA analysis (Natera) and whole-body FDG PET/CT imaging at baseline and at 3-4 weeks after treatment initiation (prior to the second dose of ICI therapy). Assessed FDG PET/CT parameters included: largest target lesion (TaL) volume, TaL SUV_{max}, TaL SUV_{max} to liver mean ratio, total lesion (ToL) SUV_{max}, ToL SUV_{max} to liver mean ratio, ToL SUV_{mean}, and ToL SUV_{mean} to liver ratio. Correlation coefficients were calculated to evaluate the relationship between early ctDNA dynamics and FDG PET/CT parameter changes.

Results: Nine patients were evaluated, with a median age of 65 and a median baseline total number of metastatic lesions per patient of 6. Primary sites of melanoma included cutaneous (59%, 5/9), primary unknown (22%, 2/9), uveal (11%, 1/9), and mucosal (11%, 1/9). Disease stages included M1d (33%, 3/9), M1c (11%, 1/9), M1b (33%, 3/9), and M1a (22%, 2/9). Treatment regimens were split between ipilimumab/nivolumab (56%, 5/9) and nivolumab/relatlimab (44%, 4/9). All subjects with ctDNA decrease at 3-4 weeks had an overall partial response (6/9, 67%) or stable disease (1/9, 11%); and all other subjects with ctDNA increase had progressive disease (2/9, 22%) per RECIST v1.1. Strong correlations were observed between early ctDNA dynamics and changes in largest TaL volume ($r = -0.9541$), ToL SUV_{max} ($r = 0.8697$), and ToL SUV_{max} to liver mean ratio ($r = 0.8414$). Weak to moderate correlations were identified for other parameters.

Conclusions: This study demonstrates very strong correlations between early ctDNA dynamics and several FDG PET/CT parameters, including largest TaL volume, ToL SUV_{max}, and ToL SUV_{max} to liver mean ratio. These findings highlight the potential of using ctDNA and FDG PET/CT as multifaceted early biomarkers of response to assess ICI efficacy. Ongoing patient enrollment and validation efforts are essential to establish their role in response-adapted treatment studies.

#4628 Exploring the differences in immunotherapy efficacy and immune microenvironment characteristics among colorectal cancer patients with different notch receptor gene subtypes.

Junyi Chen[1], Jiahui Fang[2]

[1]Department of Gastrointestinal Surgery, Shanghai Fourth People's Hospital, School of Medicine, Tongji University, Shanghai, China,[2]General Practice, Gaojing Town Community Health Service Center Shanghai, Shanghai, China

Background: The NOTCH signaling pathway is a highly conserved signaling system regulated by close-range cell-to-cell interactions. The interaction between NOTCH receptors (NOTCH1-4) and "classical" ligands, or the activation of other non-classical pathways, is associated with the occurrence and progression of various tumors. Although some studies have confirmed a correlation between the efficacy of immunotherapy and NOTCH gene mutations, no definitive conclusion has been reached in colorectal cancer. Exploring the tumor immune microenvironment may help us find better answers.
Methods: An independent cohort (MSKCC) comprising next-generation sequencing (NGS) data from 109 pan-cancer colorectal cancer (CRC) patients on immunotherapy outcomes. Tumor tissue samples from 249 Chinese CRC patients were analyzed using NGS and multiplex immunohistochemistry (mIHC). The mIHC were stained using the Detection Kit (Akoya), targeting 13 markers Results, showing immune cell percentages of total cells, provided insights into cell subpopulation distributions in tumor and stroma.
Result: In the MSKCC cohort revealed that CRC patients with NOTCH3 mutations had significantly longer overall survival (OS) following immunotherapy (11.5 m VS 8 m; P = 0.008). In contrast, although patients with mutations in other NOTCH pathway genes showed numerically longer OS compared to the wild-type group, the differences were not statistically significant. In Chinese patients, Among the 249 Chinese CRC patients included in the study, 21.7% (54/249) carried NOTCH gene mutations, with mutation rates of 12.0% for NOTCH1, 7.6% for NOTCH2, 11.6% for NOTCH3, and 7.2% for NOTCH4. Further analysis of the immune microenvironment characteristics revealed that patients with NOTCH1 (P=0.002), NOTCH2 (P=0.003), and NOTCH3 (P=0.0192) mutations had significantly higher levels of CD8+ T-cell infiltration in the tumor parenchyma compared to the wild-type, while no significant differences were observed for the other mutations. An additional analysis of differences between the tumor parenchyma and stroma showed that the percentage of CD8+ T cells in the tumor stroma was significantly higher than in the parenchyma for patients with NOTCH3 mutations (P=0.0307), whereas no significant differences were observed for the other three mutation subtypes. Similarly, patients with NOTCH3 (P=0.0446) and NOTCH4 (P=0.0285) mutations exhibited significantly higher levels of NK cell infiltration in the tumor parenchyma compared to the stroma.
Conclusions: NOTCH pathway genes have a better immune microenvironment, which may be the reason for the better immune efficacy of patients with Notch pathway mutations. In particular, NOTCH3 has more CD8+ T cell reserves in stroma and more NK into the tumor parenchyma, which shows significantly better immune efficacy.

#4629 Plasma exosomal miRNA and disease progression in metastatic breast cancer on CDK4/6 inhibitors: A feasibility study.

Ding Quan Ng[1], Julia Trudeau[1], Casey Hudson[2], Khai Kober[1], Kord M. Kober[3], Munjal M. Acharya[2], Alexandre Chan[1], Ritesh Parajuli[4]

[1]Clinical Pharmacy Practice, University of California, Irvine, Irvine, CA,[2]Anatomy & Neurobiology, University of California, Irvine, Irvine, CA,[3]Physiological Nursing, University of California, San Francisco, San Francisco, CA,[4]UCI Chao Family Comprehensive Cancer Center, Orange, CA

Background: Treatment resistance is common among metastatic breast cancer patients receiving CDK4/6 inhibitors (CDKi). Exosomal miRNA play a key role in intercellular communication and gene regulation that may predict treatment outcomes. We hypothesize that patient's blood exosomal miRNA can uncover biological pathways critical for CDKi response and resistance.
Methods: Plasma samples from metastatic breast cancer patients were prospectively collected every 12 weeks during CDKi treatment until discontinuation. Next generation sequencing was performed on isolated plasma exosomes for miRNA profiling. MiRNA independently associated with treatment response (hazard ratio [HR] < 1) and resistance (HR > 1) after Bonferroni correction were identified with mixed-effects Cox regression with time-to-disease progression as the outcome. We generated a miRNA-gene network using miRNet and performed pathway enrichment analysis of target genes using hypergeometric test.
Results: Eleven patients provided 26 samples during CDKi treatment. Five (45%) progressed; they were more likely to be hormone receptor negative (40% vs 0%) and a race-ethnic minority (80% vs 17%). After adjustment, 54 and 18 miRNAs were associated with treatment response and resistance, respectively. A total of 48 pathways (false discovery rate [FDR] < 0.05) were identified in relation to treatment outcomes (Table 1). Of these, 25 pathways (52%, e.g. TGF-β, insulin, Jak-STAT) were associated exclusively with response, 2 (4%, long-term depression and oocyte meiosis) were associated with resistance, and 21 (44%, e.g. cell cycle, p53, neurotrophin) were shared by both. The study's feasibility is demonstrated by the identification of numerous cell-cycle and cancer-specific pathways.
Conclusion: This study highlighted the potential of exosomal miRNA as gene expression modulators to uncover key pathways underlying CDKi treatment outcomes. Findings warrant validation in independent studies.

Table 1: Select significant biological pathways († FDR<0.05)

FDR<0.5 for response, resistance, or both	Pathways	Response: P-value	Response: FDR	Resistance: P-value	Resistance: FDR
Response	Apoptosis	2.19E-02*	***4.76E-02†***	1.79E-01	2.45E-01
Response	Fc epsilon RI signaling pathway	1.59E-02*	***3.98E-02†***	5.5E-02	1.28E-01
Response	Insulin signaling pathway	3.9E-04***	***2.17E-03†***	1.73E-02*	6.18E-02
Response	Jak-STAT signaling pathway	4.88E-04***	***2.44E-03†***	1.21E-01	2.05E-01
Response	Leukocyte transendothelial migration	1.99E-02*	***4.49E-02†***	2.96E-01	3.22E-01
Response	MAPK signaling pathway	1.47E-02*	***3.77E-02†***	2.77E-02*	8.15E-02
Response	mTOR signaling pathway	1.76E-02*	***4.09E-02†***	3.46E-02*	9.35E-02
Response	Regulation of actin cytoskeleton	5.43E-04***	***2.59E-03†***	3.63E-02*	9.55E-02
Response	RNA transport	1.81E-03**	***6.7E-03†***	2.18E-02*	7.03E-02
Response	TGF-beta signaling pathway	1.88E-04***	***1.11E-03†***	6.68E-02	1.36E-01
Response	Tight junction	1.27E-02*	***3.53E-02†***	6.12E-02	1.33E-01
Response	Wnt signaling pathway	4.71E-04***	***2.44E-03†***	1.25E-01	2.07E-01
Resistance	Long-term depression	2.25E-01	2.37E-01	6.62E-03**	***3.15E-02†***
Resistance	Oocyte meiosis	5.07E-02	8.5E-02	7.05E-03**	***3.2E-02†***
Both	Adherens junction	2.83E-06***	***4.72E-05†***	3.43E-05***	***4.29E-04†***
Both	Axon guidance	7.05E-05***	***6.41E-04†***	2.43E-03**	***1.52E-02†***
Both	Cell cycle	2.34E-10***	***1.17E-08†***	3.34E-09***	***2.13E-07†***
Both	Endocytosis	8.03E-04***	***3.42E-03†***	3.02E-03**	***1.78E-02†***
Both	ErbB signaling pathway	8.23E-05***	***6.48E-04†***	4.46E-05***	***4.96E-04†***
Both	Focal adhesion	1.33E-03**	***5.32E-03†***	1.15E-04***	***9.58E-04†***
Both	Neurotrophin signaling pathway	2.55E-05***	***3.19E-04†***	6.77E-05***	***6.54E-04†***
Both	p53 signaling pathway	3.18E-07***	***6.36E-06†***	7.19E-05***	***6.54E-04†***
Both	Pathways in cancer	2.66E-12***	***2.66E-10†***	4.26E-09***	***2.13E-07†***
Both	Protein processing in endoplasmic reticulum	5.85E-05***	***6.41E-04†***	4.14E-03**	***2.18E-02†***

#4630 A novel approach to define ctDNA molecular response to immunotherapy.

John Guittar[1], Sandra Hui[1], Terri Driessen[1], Wei Zhu[1], Christine Lo[1], Akash Mitra[1], Michelle Stein[1], Halla Nimeiri[1], Rotem Ben-Shachar[1], Wade T. Iams[2]

[1]Tempus AI, Inc., Chicago, IL,[2]Tennessee Oncology, Nashville, TN

Molecular biomarkers that quantify changes in circulating tumor DNA (ctDNA) can predict clinical outcomes. However, there is no consensus on classification of molecular responders (MRs) and molecular non-responders (nMRs), who may benefit from early treatment intervention. We explored the classification of MRs vs. nMRs in a real-world pan-cancer cohort of patients treated with immune checkpoint inhibitor (ICI) therapy in two ways. (1) As previously described, a ≥ 50% decrease in ctDNA tumor fraction (TF) between baseline and on-treatment time points (MR50), and, (2) hypothesizing that consistently low TF may represent a favorable subgroup with prolonged outcomes despite small increases in TF, MRlow, we evaluated a "low TF" threshold, above the TF detection rate, classifying patients as MRlow if TF remains below the TF threshold at both timepoints, regardless of magnitude change.

The cohort consisted of advanced cancer patients from the Tempus de-identified clinicogenomic database who received a liquid biopsy at pre-treatment baseline and within 21-180 days after starting ICI therapy. TF was quantified via an ensemble algorithm, xM for treatment response monitoring, that incorporates pathogenic variant allele frequencies, copy number information, and germline information. Evaluable patients had TF ≥ limit of blank in at least one blood sample. Real-world overall survival (rwOS) was defined as the time from on-treatment testing to death or last known clinical record in event-free patients. Cox proportional hazards models were used to estimate the hazard ratio (HR) for MR status (MR vs. nMR) and tests for significance were conducted using 1-sided Wald tests at a 5% significance level.

The evaluable cohort consisted of 71 advanced pan-cancer patients with >10 cancer types; most commonly NSCLC (38.0%, n=27) and small cell lung cancer (19.7%, n=14). Twenty-five patients (35.2%) were treated with ICI monotherapy and 46 (64.8%) were treated with ICI-chemotherapy combination.

Using a definition of MR of a 50% decrease in TF alone, 44 patients were MRs and 27 patients were nMRs, with MRs having significantly longer rwOS than nMRs (HR=0.44, P=0.012). We next evaluated various low TF thresholds and found that a 1% low TF threshold further improved the association between MR and rwOS (HR = 0.32, P = 0.0008). 53 patients were classified as MRs [18 MRlow + 35 MR50] and 18 patients were classified as nMRs. Of the MRlow patients, 6 had increasing TF. Median rwOS for nMRs was seven months and not reached at 18 months for both MRlow and MR50.This study suggests that patients with consistently low TF below 1% while on ICI therapy (MRlow), regardless of TF changes, represent a unique, biologically differentiated, prognostic group. Our study suggests that MRlow patients have prolonged rw-survival, similar to molecular responders based on a 50% decrease in TF (MR50). Prospective validation for this threshold is planned.

#4631 Deciphering immunotherapy response variability and immune-related toxicities through longitudinal multimodal ctDNA analysis in advanced melanoma.

Z. Cheng[1], S. McDonald[1], H. Thomas[1], Z. Zhou[1], P. Mennea[1], H. wang[1], D. Shcherbo[1], C. Zhang[1], G. McAndrew[1], F. Talukdar[1], W. N. Cooper[1], H. Zhao[1], N. Rosenfeld[2], P. G. Corrie[1], A. Roshan[1];

[1]University of Cambridge, Cambridge, United Kingdom, [2]Queen Mary University of London, London, United Kingdom

Background: Immune checkpoint inhibitors (ICIs) have revolutionized treatment of advanced cutaneous melanoma by leveraging the host immune system to target and eliminate tumour cells. However, predictive biomarkers for clinical response to ICIs remain elusive, limiting the ability to stratify patients effectively. The heterogeneity in treatment outcomes, coupled with the limited sensitivity and timeliness of radiographic assessments, particularly in patients with stable disease, underscores the urgent need for molecularly guided, minimally invasive approaches for real-time monitoring of therapeutic efficacy. Biomarkers, such as circulating tumour DNA (ctDNA) and T-cell clonal dynamics, offer promise not only in assessing tumour burden and dynamics but also potentially in providing early indicators of immune-related adverse events (irAEs).

Methods: We analysed 70 longitudinal plasma samples from 13 patients with resectable or unresectable, Stage III melanoma from the MELResist study. These patients were treated with either pembrolizumab or a combination of ipilimumab and nivolumab. PD1/PDL1 expression in FFPE tumour samples was quantified using digital pathology, and deep whole-genome sequencing (around 30x coverage) was performed on tumour tissue to profile genomic alterations. By integrating ctDNA analysis (>10x WGS coverage) with matched tumour samples using the cfDNAPro, ichorCNA and INVAR2 pipelines, we conducted serial WGS-based monitoring to evaluate molecular response dynamics as measured by copy number alterations, fragmentomics and patient-specific tumour mutations respectively. Additionally, irAEs were assessed through longitudinal tracking of the peripheral T-cell repertoire (TCR) using TCR-sequencing of buffy coat samples, alongside with plasma protein expression profiling via the Olink Explore panel.

Results: In evaluating ctDNA molecular response, we found a copy number fraction ranging from 0 to 42% by ichorCNA analysis, with higher fractions at later timepoints correlating with poorer overall survival. Fragmentomic analysis revealed changes in 4-mer end motif frequencies and the ratio of short-to-long fragments (100-150 bp vs. 151-220 bp). Specifically, a decreased CCCA motif frequency and an increased short-to-long fragment ratio at later timepoints were associated with poorer overall survival.

Conclusion: ctDNA dynamics during immunotherapy for advanced melanoma reflect clinical outcomes. Changes in ctDNA can be identified using tumour informed methods with high sensitivity but at additional cost or tumour agnostic methods with lower logistical burden. Together with our assessment of T-cell clonal dynamics and proteomic changes, these approaches will offer valuable insights for monitoring therapeutic benefits and immune-related adverse events (irAEs) during immunotherapy.

#4632 Molecular determinants to neoadjuvant chemotherapy resistance in breast cancer patients: Insights from a Latin-American cohort.

H. M. Guevara-Nieto[1], C. A. Orozco[1], R. S. Parra-Medina[1], J. Zabaleta[2], L. Lopez-Kleine[3], A. L. Combita[1];
[1]Instituto Nacional de Cancerologia, Bogota, Colombia, [2]Louisiana State University Health Sciences Center, New Orleans, LA, [3]Universidad Nacional de Colombia, Bogota, Colombia

Neoadjuvant chemotherapy (NAC) is vital in treating primary breast cancer, yet the mechanisms behind its success are not fully understood. Understanding transcriptomic changes associated with NAC resistance is crucial for improving treatment. This study sought to identify transcriptomic alterations linked to NAC resistance, enhance comprehension of treatment resistance, and lay the groundwork for developing targeted strategies for patients unresponsive to standard NAC protocols.We analyzed the transcriptomes of 44 matched breast cancer specimens from nonresponder patients to NAC at the Colombian National Cancer Institute. Samples were collected before and after NAC, representing luminalA, luminalB/HER2-positive, luminalB/HER2-negative, and TNBC subtypes. We performed differential gene expression and enrichment analyses and compared hallmark gene sets. Furthermore, we used deconvolution algorithms to estimate tumor microenvironment (TME) infiltration and assess immune population activity within the TME. Our findings were compared with publicly available GEO database data.Gene expression patterns before and after treatment were distinct across subtypes, with FOS and NR4A1 common to all, and ZBTB16 unique to luminalB. FOSB was found in all luminal samples. ATF3, DUSP1, EGR1, and JUN were shared among HER2-negative subtypes. GEM was common to luminalA and LuminalB/HER2-negative subtypes. Pathway variations included complement and coagulation cascades, and IL-17, TNF, MAPK, and NF-κB signaling pathways among subtypes and post-NAC specimens. Post-treatment LuminalA samples had increased CD4 memory cells, Tregs, and neutrophils. The LuminalB/HER2+ group showed higher CD8+ and CD4+ memory-activated cells post-treatment. In LuminalB/HER2- post-treatment samples, B cell CD4 memory resting, Macrophages M2, and neutrophils were elevated. TNBC post-treatment samples had higher resting mast cells and CD4+ memory cells. MSIGBD comparison of gene sets linked to cisplatin, docetaxel, and doxorubicin resistance identified several shared genes. GeoDatabases confirmed FOS and NR4A1 across all groups, with other DEGs shared among subtypes.Our research indicates that NAC affects gene expression, cell function, and interactions within the TME, potentially contributing to treatment resistance. Assessing TME components is crucial for predicting NAC effectiveness and reducing the risk of recurrence. These results highlight the importance of studying diverse patient populations to gain fresh perspectives and address disparities in treatment outcomes. This investigation emphasizes the need to understand how TME influences chemotherapy resistance, paving the way for the development of more precise and efficient therapeutic strategies.

#4634 Development of novel monoclonal antibody detection of ERα36 in breast cancer.

Alexandra C. Selico Dunn[1], Hogyoung Kim[1], DeZha A. Robinson[1], Sihyoung Kim[2], Clark J. Perkins[2], Sichan Kim[2], Emily L. S. Smith[1], Emma Elkins[1], Christopher Williams[1]

[1]Division of Basic Pharmaceutical Sciences, Xavier University of Louisiana, New Orleans, LA,[2]Biology and Applied Health Sciences, Xavier University of Louisiana, New Orleans, LA

Approximately 75% of breast cancer tumors express the estrogen receptor alpha steroid receptor. Canonical estrogen receptors ERα66 and ERβ1 are the main mediators of estrogen-dependent pathophysiology in mammals. However, numerous isoforms have been identified, stimulating unconventional estrogen response pathways leading to complex cellular and tissue responses. Estrogen receptor alpha plays essential roles in the human body, such as the reproductive organs and metabolic and inflammatory systems. Recent studies suggest that a 36 kD variant of ERα (ERα36, ERα isoform 4) may play a role in endocrine therapy resistance in breast carcinoma (BCa). However, a key limitation to discovering the precise role of ERα36 in BCa is the lack of reliable, commercially available antibodies. Most ERα polyclonal antibodies show poor antigen specificity, hindering the evaluation of ERα36 as a clinical biomarker in BCa. Our goal is to develop a reliable monoclonal antibody targeting ERα36, which could facilitate its development as a clinical biomarker in BCa. Here, we describe the development of hybridoma producing an ERα36 monoclonal antibody (34F11) followed by rigorous enrichment (ultrafiltration), purification (fast protein liquid chromatography), and affinity (spectral shift assay) characterization. We assessed the utility of 34F11 antibody in several applications (immunoblot, immunohistochemistry, and fluorescence cytometry). Here, we provide the first comparative characterization of a monoclonal antibody directed toward ERα36 for the potential implementation of biomarker detection in BCa.

#4635 Reg4-induced CD44ICD expression: A potential biomarker of human pancreatic cancer.

Tashvi Anand[1], Matthew A. Ciorba[2], **Kumar S. Bishnupuri**[1]

[1]Internal Medicine - Division of Gastroenterology, Washington University School of Medicine in St. Louis, St. Louis, MO,[2]Internal Medicine - Division of Gastroenterology, Matthew Ciorba, St. Louis, MO

Background and Aims: Regerating Gene 4 (Reg4) is highly upregulated in gastrointestinal (GI) malignancies including pancreatic cancers (PC). Numerous studies have demonstrated an association between higher Reg4 expression and PC tumor aggressiveness. However, the underlying mechanism remained unknown. Our recent studies identified CD44 intracytoplasmic domain (CD44ICD), a γ-secretase-mediated cleavage product of CD44, as a key downstream mediator of Reg4-signaling to activate genes involved in the regulation of cancer cell proliferation. We accordingly aimed this study to determine a link between Reg4/CD44ICD expression and PC patient survival and define its importance as an early biomarker for metastatic PC.

Methods: A closed IRB-approved human study with PC patient linked survival and invasion data was used to construct 3 tissue microarray (TMA) slides of resected specimens. TMA slides were immunostained using Reg4-specific rabbit polyclonal and CD44ICD-specific goat polyclonal antibodies. Spearman's correlation was used to study the association between individual proteins using percent mean expression (0-100%) and mean intensity (0, 1, 2, 3) as scored blindly by the pathologist. Product-limit survival estimates were obtained to estimate survival probability and log-rank tests were used to compare survival probability between groups. Patient-linked tumor grades were used to determine correlation between Reg4/CD44ICD expression and PC tumor grades.

Results: Assessing immunostaining scores in PC specimens, cytoplasmic Reg4 (R^2 = 0.121) and CD44ICD (R^2 = 0.1168) expression along with nuclear expression of CD44ICD (R^2 = 0.0261) showed a positive correlation with increasing PC grades. Analysis of product-limit survival estimates revealed that cytoplasmic expression of Reg4 and CD44ICD, and nuclear expression of CD44ICD in PC specimen was directly associated with poor patient survival irrespective of tumor grades. To determine the association of Reg4/CD44ICD expression with PC invasiveness, we extended our study and analyzed the patient linked lymphatic, venous and perinuclear invasion data. Following analysis, we observed almost universal presence of venous and perineural invasion in most of the PC patients without a direct correlation with cytoplasmic expression of Reg4 and CD44ICD, and nuclear expression of CD44ICD. However, we observed a significantly higher expression of nuclear CD44ICD in PC patients positive for lymphatic invasion, and data demonstrated a positive correlation between nuclear CD44ICD expression and lymphatic invasion (R^2 = 0.0699).

Conclusion: These results suggest that higher expression of Reg4 and CD44ICD constitute an important event responsible for poor survival of PC patients and identify CD44ICD as a key downstream mediator of Reg4 signaling, a potential therapeutic target and a critical biomarker of metastatic PC with lymphatic invasion.

#4636 Keratin 6 and SOX2 regulate growth, stemness, basal/squamous expression, and chemotherapy response in muscle-invasive urothelial carcinoma.

N. Nargis, A. Mehus, D. A. Sens;
University of North Dakota, Grand Forks, ND

Background and purpose: Urothelial carcinoma (UC) is prevalent globally and it is well-established that arsenic exposure is associated with development of UC. Muscle-invasive UC (MIUC) is aggressive and accounts for 25% of all diagnosed UC cases. Cisplatin-based treatment is the standard treatment for MIUC. Patients with MIUC often have a dismal prognosis and frequently acquire chemoresistance, especially if the tumors exhibit squamous differentiation (SD). UC can be further subtyped as being either basal or luminal based on gene expression signatures of the tumors. The basal tumors are considered more aggressive and are enriched with expression of genes associated with SD (KRT1, KRT5, KRT6, KRT14, and KRT16). The UROtsa cell line is derived from normal human urothelium and our lab has malignantly transformed these cells using arsenite (As^{3+}). The tumors derived from As^{3+}-transformed cells resemble clinical cases of basal MIUC displaying focal areas of SD and expression of basal keratins (KRT1, 5, 6, 14, and 16). Additionally, SOX2 was found to be overexpressed in these As^{3+}-transformed UROtsa cells. SOX2 expression in UC is correlated with reduced survival and promotes malignant stemness and proliferation. SOX2 is also associated with the development of cisplatin resistance in other cancers. The goal of this study was to define the role of KRT6 and SOX2 in regulating basal/squamous expression, regulating growth, and regulating sensitivity to cisplatin.

Methods: We performed stable lentiviral knockdown of KRT6 or SOX2 in the As^{3+}-transformed UROtsa cells (basal MIUC with SD), along with the commercially available UC cell lines 5637 (basal, invasive) and RT4 (luminal, non-invasive). After successful knockdown KRT6 or SOX2 in these cells, we assessed cellular proliferation and colony formation. Additionally, the gene/protein expression of the basal keratins along with other genes/proteins involved with growth and stemness were analyzed. Migration was evaluated using a wound healing assay and drug sensitivity was tested after KRT6 or SOX2 knockdown.

Results: Knockdown of KRT6 or SOX2 in the As^{3+}-transformed cells attenuated basal keratin expression, reduced cell proliferation, decreased colony forming abilities, and increased sensitivity to cisplatin compared to control cells. Furthermore, cell migration was reduced in SOX2 knockdown cells.

Conclusion: These results indicate that both KRT6 and SOX2 play critical roles in basal/squamous expression, growth, migration, and cisplatin sensitivity in MIUC. However, further *in vivo* studies are required to confirm the impact of KRT6 and SOX2 expression on tumor growth, SD, invasion, and chemotherapy response.

#4637 Nanomechanical signatures: novel class of clinically translatable biomarkers to predict response to immunotherapy in advanced solid tumors.

Sara Nizzero[1], Nahum Puebla-Osorio[2], Gitika Srivastava[3], **Reinier Oropesa Nunez**[3], Kathleen Graham[1], Papa Diogop Ndiaye[3], Joylise Mitchell[2], Ailing Huang[2], Thomas Riad[2], Carolina Ortiz-Velez[3], Sienna Welsh[3], Philipp Oertle[3], Tobias Appenzeller[3], Vittorio Cristini[4], Marko Loparic[3], Marija Plodinec[3], James W. Welsh[2]

[1]ARTIDIS INC, Houston, TX,[2]University of Texas MD Anderson Cancer Center, Houston, TX,[3]ARTIDIS AG, Basel, Switzerland,[4]Houston Methodist, Houston, TX

Background: Advancements in immunotherapy have opened new avenues for the treatment of solid tumors. However response remains poor, especially in case of advanced tumors where resistance to immunotherapy after initial response remains a significant challenge. In recent years it has become evident that mechanical properties of tumor and immune cells as well as of the tumor microenvironment (TME) pose an upstream challenge to efficacy of a variety of therapeutics, including immunotherapy. Approaches such as pretreatment with low-dose radiation therapy (LD-RT) to remodulate the physical tumor microenvironment in combination have shown promise in clinics, reporting as effective as fully extinguishing detectable tumors in patients with previously stage IV tumors in lung and liver. Accurate prediction of benefit by such strategies is of outmost importance. In this work, we report on a novel class of predictive, tumor-agnostic biomarkers for combination immunotherapies: nanomechanical signatures.

Methods: To characterize the nanomechanical signature, the clinically-embedded, tissue-preserving AFM-based Automated and Reliable Tissue Diagnostics (ARTIDIS) platform is used. ARTIDIS multimodal analysis includes integration with available histopathological and clinical data. Two preclinical models are used: PD1-resistant lung adenocarcinoma on 129Sv/Ev mice, and gastric cancer on NSG mice, treated with anti-CTLA and anti-PD1 in combination with LD-XRT and anti-mesothelin CAR-T cell therapy in combination with LD-XRT, respectively. Case reports from patients with advanced metastatic solid cancers resistant to immunotherapy enrolled under the on-going ARTIDIS Umbrella protocol at MDACC are also included.

Results: Our preclinical studies identify the nanomechanical signature of response to low-dose radiation and immunotherapy with high sensitivity, specificity and AUC (90%-99%). Furthermore, we can identify the unique nanomechanical signature of T cell infiltration. Our clinical studies confirm that a unique nanomechanical signature reflects TME remodulation in different therapeutic regiments among similar clinical and histopathological cases.

Conclusions. In this work we demonstrated across different therapeutic strategies, preclinical tumor models and clinical cases that the nanomechanical signature can quantitatively describe the TME remodulation upon LD-XRT that mediates restored response to immunotherapy. This work supports the use of nanomechanical signature as predictive biomarker of response to immunotherapy to be used to support clinical decision making.

#4638 Investigations on association of prognostically important pan-cancer genes with disease progression and acute transformation in CML indicate involvement of diverse cellular mechanisms.

Sabreen Alanazi[1], Sarah Alfayez[1], Maryam Butuwaybah[1], Nawaf Alanazi[1], Abdulkareem Algarni[1], Kanza Adeel[1], Yaqob Taleb[1], Aamir Aleem[2], Aemir Mohammed[2], Zafar Iqbal[1]

[1]King Saud bin Abdulaziz University for Health Sciences (KSAU-HS), Al Mubarraz, Saudi Arabia,[2]King Saud University, Riyadh, Saudi Arabia

Chronic myeloid leukemia (CML) is caused by the abnormal fusion of the bcr-abl gene which leads to the overexpression of tyrosine kinase function. Tyrosine kinase inhibitors (TKI) are targetable drugs that inhibit the kinase activity of the bcr-abl oncoprotein. They have been used over the last two decades and have shown tremendous efficacy in treating CML, specifically in chronic phase (CP-CML) and accelerated phase CML patients (AP-CML). However, disease progression to advanced stages, specifically blast crisis CML (BC-CML), leads to TKI resistance, morbidities and subsequently death, with a median overall survival of less than 2 years, despite all innovations in diagnosis, risk stratification and treatment modalities. Therefore, further investigations of gene mutations associated with CML progression is primarily essential in accurate prognostic stratification and patient-tailored treatment of advanced phase CML. As cancer is a molecular disease, different types of cancers have recently reported to share many common genomic variants. Therefore, this study aimed to investigate the association of prognostically-important pan-cancer genes with advanced phases in CML patients. The study included a total of 73 CML patients at different CML Phases (CP=41, AP=20, BC=12). The study was approved by Institutional Review Board of the participating centers and was carried out per regulations of the Declaration of Helsinki. Whole exome sequencing (WES) was conducted using Illumina NextSeq500 platform using compatible kits per manufacturer's instructions. Healthy controls were included from genomic databases. All study subjects were screened for prognostically important pan-cancer gene mutations. Genes exclusively and commonly shared with each CML phase were subjected to further analyses. The data was analyzed using SSPS Ver. 26. The first data a total of 64 genes with 2531 mutations were detected in BC-CML patients that were absent in chronic phase CML (CP-CML) as well as healthy controls, indicating that these genes/mutations are exclusively associated with BC-CML. RPTOR was the most common gene mutated (7.35%) and chromosome 17 was the most encountered (16.32%) while chromosome 12 was the least involved (0.04%). The second data 55 genes in the accelerated phase while 83 genes in BC. Therefore, we conclude that many gene mutations associated with BC-CML are associated with different types of leukemias and solid tumors. Moreover, these genes were found to have resistance causing mutations with various underlying. Mechanisms in cell signaling, which could potentially impact treatment and prognosis of advanced phases CML patients.

#4639 Predictive clinical-pathological features for CDK 4/6 inhibitors: Outcomes in hormone receptor-positive, HER2-negative metastatic breast cancer patients.

Amer N. Radwi[1], Anwar Khan[1], Mohammed Essam Mahroos[2], Fares Alhejaili[1], Saleh Alsaiari[1], Ali Nasfan[1], Ahmad Almuslim[1], Mohammed Alasri[1], Yousef Alraddadi[1]

[1]King Saud University for Health Sciences, Jeddah, Saudi Arabia,[2]Oncology, King Abdulaziz Medical City-WR,NGHA, Jeddah, Saudi Arabia

Background: Breast cancer is the most common cancer among Saudi women and around the world, accounting for 30.9% of all Saudi female cancers in 2020. Metastatic breast cancer (MBC) represents a significant health burden. There has been a recent increase in hormone receptor-positive (HR+) breast cancer in young women. When used with endocrine therapy, CDK4/6 inhibitors have become a big step forward in the treatment of hormone receptor-positive breast cancer. There are sparse studies on their response prediction. We aim to determine predictive clinical-pathological features for CDK4/6 inhibitors response in HR+, Her2 negative metastatic breast cancer by getting their impact on the response rate and progression-free survival. The study looked at the medical records of women with HR+, Her2 negative breast cancer who went to the Princes Noora Oncology Centre (PNOC) in King Abdulaziz Medical City-WR between January 2016 and December 2023. The study was approved by the Institutional Review Board (IRB). We collected data for patients' demographics, clinical features, peripheral blood inflammatory cells, pathology, treatment, and follow-up details from patients' files in Electronic Medical Records. We included 18 years or older, histopathology-confirmed, advanced unresectable, and systemic therapy-naïve MBC. We excluded intracranial metastases and nonassessable responses. We cleaned and explored the data using tables, histograms, box plots, and bar charts. We applied the chi-square test, Fisher exact test, and Kaplan-Meier survival curves for survival.
Results: We found 3084 breast cancer patients and 1250 hormone receptor-positive patients. We analyzed 162 cases after applying inclusion-exclusion criteria. The median age was 51 years; PFS was 72.5 months. Menopause status, estrogen receptor positivity percentage, body mass index (BMI), tumor grade, and adenocarcinoma histology were clinical factors that could not predict the outcome. A high KI67 index of more than20%, high monocyte lymphocyte ratio (MLR), and a low lymphocyte count before the second cycle predicted the response of the CDK4/6 inhibitors, as shown by a higher response rate (RR) and better progression-free survival (PFS).
Conclusion: While clinical features were not predictive, factors such as elevated KI67 by more than 20%, elevated peripheral blood monocytes (PBMC), monocyte lymphocyte ratio (MLR), and a low lymphocyte count before the second cycle were predictive of the response to CDK4/6 inhibitors.

#4640 Identifying novel drivers of drug sensitivity using an AI-enabled composite biomarker - osimertinib sensitivity beyond EGFR.

Maayan Baron, Felicia Kuperwaser, Colin Tang, Sunil Kumar, Dillon Tracy, Zong Miao, Olena Marchenko, Sepideh Foroutan, Amy Sheide, Nathaniel Tann, Samantha Pindak, Jacob Kaffey, Jordan Wolinsky, Sean Klei, Andrey Chursov, Brandon Funkhouser, Nick Lee, Patrick Bohan, Fahad Khan, Ripple Khera, Jean Michel Rouly, George Komatsoulis, Jeff Sherman, Emily Vucic, Rachael Brake

Zephyr AI, McLean, VA

Background: Precision medicine (PM) has transformed cancer treatment but remains effective for only a subset of patients. Most tumors profiled with comprehensive genomic testing lack actionable biomarkers, and even when present, many patients fail to benefit from corresponding therapies. Some without recognized biomarkers respond in off-label contexts, highlighting the need for better biomarkers to capture drug response biology and deliver on the promise of PM. Osimertinib, a third-generation EGFR inhibitor, is used in conjunction with the single gene biomarker, EGFR. Response rates vary by EGFR variant and off-target activity has been reported. We hypothesized that integrating multi-modal data with machine learning could enhance osimertinib response predictions beyond the single-gene EGFR biomarker.

Methods: We developed an AI/ML-enabled drug response prediction model that integrates clinical and molecular features with drug structure and functional data to generate drug response predictions. The model produces an AI-enabled composite biomarker (AI-CB) based on a small amount of clinical and molecular patient input data. Each prediction is paired with a proprietary Vulnerability Network™ (VN), providing insights into biological dependencies underlying response predictions. Osimertinib response predictions were evaluated retrospectively across multiple real-world (rw) NSCLC cohorts, including clinicogenomic data from liquid biopsy. The AI-CB was also validated in EGFR-negative NSCLC patient-derived xenografts (PDX).

Results: Across multiple osimertinib-treated EGFR+ rw-NSCLC cohorts, the AI-CB accurately stratified patients by sensitivity to osimertinib, despite the presence of EGFR alterations in all patients. EGFR+ patients predicted to be insensitive showed a significant reduction in rw-overall survival compared to those predicted to be sensitive, indicating that osimertinib sensitivity may be impacted by more than just EGFR alterations. Compared to EGFR+ osimertinib insensitive patients, post-hoc and VN analysis across these two cohorts linked sensitivity to upregulation of pathways downstream of EGFR including NOTCH and TP63 and downregulation of RAS/RAF/MEK activity. The AI-CB was successfully validated in human PDX models without activating EGFR alterations. AI-CB application to AACR Project GENIE cohorts suggest that sensitivity to osimertinib could extend to a novel group of patients with NSCLC and beyond, e.g., colorectal cancer.

Conclusion: ML enables the integration of diverse data types relevant to drug response, overcoming limitations of single-dimensional approaches. Incorporating AI-driven patient stratification models into drug development and clinical practice could greatly expand the utility of targeted therapies and create effective stratification strategies for drugs lacking adequate biomarkers.

CLINICAL RESEARCH: Prognostic Biomarkers 3
Poster Session

#4643 The role of subcellular localization of interleukin-1alpha in head and neck cancer.

Ishrat Nourin Khan[1], M M Hasibuzzaman[2], Andrean L. Simons-Burnett[3]

[1]Interdisciplinary Graduate Program in Human Toxicology, University of Iowa, Iowa City, IA,[2]Free Radical and Radiation Biology Program, Department of Radiation Oncology, University of Iowa, Iowa City, IA,[3]Holden Comprehensive Cancer Center, University of Iowa, Iowa City, IA

Interleukin-1α (IL-1α) is a pro-inflammatory cytokine upregulated in multiple solid malignancies, including head and neck squamous cell carcinomas (HNSCCs) and has been associated with tumor growth, metastasis, immunosuppression, and therapy resistance. Unfortunately, targeting circulating IL-1α in clinical trials has shown disappointing results for cancer patients. Our previous research revealed that high nuclear IL-1α expression is associated with perineural invasion and poor progression free survival. However, little is known about the importance of subcellular localization of IL-1α. This study aims to explore the differences in nuclear and cytoplasmic IL-1α expression on tumor aggressiveness. Cal-27 HNSCC cells were transfected with four plasmid constructs of different forms of IL-1α: 1 - precursor/full length (FL) IL-1α (1-271 AA) which localizes in both the nucleus and cytoplasm; 2 - N-terminal/propiece (N-tern) IL-1α (1-112 AA) which localizes in the nucleus; 3 - C-terminal/matured (C-term) IL-1α (113-271 AA) which localizes in the cytoplasm; and 4 - pGenLenti control (CON) construct. Intracellular IL-1α expression was validated using PCR and western blot, subcellular localization was validated using subcellular fractionation kits prior to western blot, and secreted IL-1α and IL-1α-signaling endpoints (e.g. IL-6, IL-8) were analyzed by ELISA. Differences in cell growth and epithelial-mesenchymal transition (EMT) markers were assessed among the IL-1α-expressing cell lines. IL-1α gene expression was significantly increased in all IL-1α construct-expressing cell lines (FL, C-term, N-term) compared to CON. Whole cell lysates showed significantly increased precursor and matured IL-1α protein expression in the FL cells; and matured IL-1α in C-term expressing cells compared to CON. Subcellular fractionation showed increased precursor IL-1α in FL and matured IL-1α in C-term expressing cells in the cytoplasmic fraction; while higher precursor IL-1α was found in nuclear fraction of FL expressing cells. IL-1α secretion was significantly increased from both FL and C-term expressing cells while the IL-1 signaling endpoints IL-6 and IL-8 were increased in the FL expressing cells only. C-term expressing cells grew significantly more rapidly compared to the other cell lines and demonstrated “mesenchymal-like” morphology. To confirm, western blot revealed an increase in the expression of the mesenchymal markers N-cadherin and vimentin, and a complete loss of epithelial markers E-cadherin and claudin. Overall, these findings suggest that the subcellular localization of IL-1α may play a complex role in tumor growth and EMT. Future work is needed to understand the significance of IL-1α subcellular localization to improve more efficient targeting of the IL-1 pathway for cancer therapy.

#4644 Real-world outcomes in dual checkpoint inhibitor therapy: A single community center perspective.

Victoria Moe[1], **Rachel Elsey**[2], Tobias Meissner[2], Bing Xu[2], Yuliang Sun[2], Crystal Hattum[2], Casey Williams[2]

[1]University of South Dakota Sanford School of Medicine, Vermillion, SD,[2]Avera Cancer Institute, Sioux Falls, SD

Introduction: Immunotherapy using dual checkpoint inhibitors is used to induce an antitumor response towards cancer. The varied response in treatment outcomes for those who receive dual checkpoint may be influenced by many factors. We attempted to interrogate factors impacting response to dual checkpoint inhibitor therapies in a single-center retrospective analysis, with the primary objective of examining how these factors may influence cancer progression.

Methods: This single-center retrospective analysis examines patients with documented treatment histories of dual immunotherapy between 2015 and 2023. Additional data collected included: demographics, disease characteristics, additional treatment history, imaging, and next generation sequencing results. The primary endpoint of this study was progression free survival, with the secondary endpoint of overall survival. Patients were excluded from the study if they passed within one month of starting dual immunotherapy, were being treated for more than one form of cancer or had insufficient documentation.

Results: In this study, the median age at the start of dual immunotherapy was 65 years old (range 30 to 84). Of the 144 patients examined, 20% were diagnosed with stage II or stage III cancer, 76% were diagnosed with stage IV cancer, and 3.5% had an undetermined stage at the start of dual immunotherapy. The most common diagnoses were melanoma (42%), kidney cancer (29%), and lung cancer (9%). 47% of the patients received 4 doses of dual immunotherapy and the next most common group received 2 doses (22%). 51 of the patients had their tumor mutation burden (TMB) assessed with 31% of the patients in this subgroup determined to have a high TMB. 33 patients had PD-L1 assessed, with 69% having a positive PDL-1 status. 46% of patients had not received prior lines of immunotherapy or chemotherapy while 22% received a single line. There was no significant change in survival found for PDL1 status ($p=0.49$), TMB ($p=0.48$), or age ($p=0.68$). The relationship between doses of dual checkpoint immunotherapy to survival was found to be significant ($p < 0.001$) when assessed over 8000 days but showed insignificant ($p=0.18$) when assessed over 2500 days. The number of prior lines to survival also was found to be significant ($p=0.0014$) when assessed over 2500 days.

Conclusions: An increase in cancer survivorship was found to be higher in patients that received more doses of dual checkpoint immunotherapy which may suggest that doses of dual immunotherapy received may increase the lifespan of some individuals with cancer. The small sample size of this study is a limitation to this analysis as well as the limited results for PD-L1 and NGS testing results, including TMB.

#4645 Dynamic post-translational events designate muscle invasion of non-muscle invasive bladder cancer.

Sabir Salim[1], Sreenidhi Mohanvelu[1], Sheeja Aravindan[2], Poorvi Subramanian[1], Loganayaki Periyasamy[1], Natarajan Aravindan[1]

[1]Physiological Sciences, Oklahoma State University, Stillwater, OK,[2]OU Health Stephenson Cancer Center, Oklahoma City, OK

Bladder cancer (BC), the second most prevalent genitourinary malignancy where majority (~70%) of the patients present with non-muscle invasive BC (NMIBC) while the rest (~30%) present with muscle invasive BC (MIBC) at diagnosis. Despite the extensive clinical management, 50% of NMIBC relapses and progress to anew MIBC. Owing to the poor prognosis for progressive MIBC, it is indispensable to understand and mitigate the evolution of NMIBC to MIBC. In particular the intratumoral heterogeneity pre- and post-therapy in NMIBC, is crucial to pin the genetic, molecular and/or post-translational events and the mechanism(s) involved in this critical transformation. Studies have identified Promyelocytic leukemia factor (PML) as a defined proapoptotic protein that regulates BC cell growth and progression. Further, studies have identified that loss of PML-nuclear bodies (PML-NB) corresponds to a 'low-is-worse' association with BC clinical outcomes. Herein, we investigated the mechanism on how the PML-NB derails and its significance in disease prognosis. In this study, we identified a unique site specific post-translational modification (SSPT) in PML that leads to its destabilization and loss. Further, significance of this PML-SSPT in clinical outcomes were investigated in a test cohort of (n=112) BC patients. For this, MIBC and NMIBC tissues in triplicates were custom archived in tissue microarrays and were examined for PML-SSPT with Opal immunohistochemistry utilizing our custom synthesized antibody. The spatio-temporal PML-SSPT modification was analyzed in QuPath v 0.5.1 bioimage analysis platform with a modified fluorescence image analysis module. We observed a significantly higher PML-SSPT modification in MIBC compared to NMIBC and a relative increase in PML-SSPT modification associated with advanced disease stage defining its crucial role in cancer progression. More importantly, higher PML-SSPT in patients with metastatic disease as well as with relapsed disease highlighted its critical role in mediating disease dissemination and evolution to aggressive MIBC. Together, the outcomes identified a crucial post-translation modification event in PML that corresponds to the coordination of de novo MIBC from NMIBC. Further, these outcomes identified a novel molecular target and paves way to develop novel molecular tumor targeted maintenance therapeutic strategies for the cure of BC. Funding: This work was funded by the Department of Defense, DoD CA-210339; Oklahoma Center for the Advancement of Science and Technology, OCAST-HR19-045; and the National Institutes of Health P20GM103639 awarded to Dr. Aravindan. The work was also supported by the NCI Cancer Center Support Grant [P30CA225520], NIGMS [P30GM154635], and a grant from the Oklahoma Tobacco Settlement Endowment Trust [R23-03] awarded to the OU Health Stephenson Cancer Center.

#4646 Technical, intratumoral and interpatient variability of a novel assay that quantifies fractional occupancy of PD1 by PDL1 in clinical biospecimens.

Suresh Veeramani, Chaobo Yin, Kristen L. Coleman, George J. Weiner

University of Iowa, Iowa city, IA

Background: New biomarkers are needed to predict clinical response to anti-PD1 therapy. We have been evaluating whether quantifying fractional occupancy of PD1 by PDL1 (PD1 FO) within a tumor may be of value as a predictive biomarker. The Ligand-Receptor Complex-binding Aptamer (LIRECAP) assay used to quantify PD1 FO is based on the preferential binding of one RNA aptamer to unoccupied PD1 (P aptamer) and the other to the PD1/PDL1 complex (C aptamer). Here, we report the technical, intratumoral, and inter-patient variability of the PD1 LIRECAP assay using clinical biospecimens.

Methods: Biospecimens: Formalin Fixed Paraffin Embedded (FFPE) sarcoma biospecimens were selected based on their availability with varying expression of PDL1. All samples had significant T cell infiltration. Ten-µm-thick scrolls were cut, deparaffinized, rehydrated and used for analysis. To assess technical variability, scrolls were macerated, aliquoted, and repeat LIRECAP assays were performed on the same sample. To assess intratumoral variability, multiple scrolls were cut from the same biospecimen. To assess inter-patient variability, biospecimens from different patients with high (tumors 1, 2 and 3) or low PDL1 (tumors 4, 5 and 6) were evaluated.

PD1-PDL1 LIRECAP assay: An equimolar mix of a “P aptamer” and a “C aptamer was added to a macerated scroll. After extensive washing, bound P and C aptamers were quantified using a TaqMan-based RT-qPCR with a set of common PCR primers (against their constant regions) and two unique TaqMan probes (against their unique central regions). The ratio of bound aptamers (C:P ratio) reflected PD1 FO.

Results: Technical variability. The PD1 LIRECAP assay showed high technical reproducibility, with low coefficient of variation (CV) for C:P ratio between the aliquots from the same scroll (e.g. Tumor #1: 10%; Tumor #5: 7%).

Intratumoral variability. Intratumoral variability was greater than technical variability demonstrating regional variations in PD1 FO within an individual tumor (CV: Tumors #1 - 71%; #2=84%; #3=44%; #4=45%; #5=76%; #6=79%).

Interpatient variability. $PDL1^{high}$ tumors showed a higher C:P ratio, indicating higher PD1 FO, when compared to $PDL1^{low}$ tumors ($p=0.017$) with higher interpatient variability in $PDL1^{low}$ patients (CV: $PDL1^{high}$=15%; $PDL1^{low}$=32%).

Conclusions and Significance: Evaluation of clinical FFPE biospecimens using the PD1 LIRECAP assay is technically feasible and reproducible. There is intratumoral variability in PD1 FO indicating regional differences within a tumor. Nevertheless, PD1 FO was highest in the $PDL1^{high}$ biospecimens. Studies assessing the value of PD1 FO as a predictive biomarker as determined by the PD1 LIRECAP assay are ongoing with the understanding that further evaluation of PD1 FO as a biomarker for response to anti-PD1 therapy will need to take intratumoral variability into account.

#4647 Systematic review and meta-analysis on the prognostic value of circular RNAs in triple-negative breast cancer.

Gessika Lanzillo de Almeida Nunes[1], Thoa Thi Kim Vu[1], So-Youn Jung[2], Yoon-Jung Choi[3], Sun-Young Kong[4]

[1]Department of Cancer Control and Population Health, Graduate School of Cancer Science and Policy, National Cancer Center, Goyang, Korea, Republic of,[2]Center for Breast Cancer, National Cancer Center Hospital, Goyang, Korea, Republic of,[3]National Cancer Control Institute / Center for Cancer Prevention & Detection, National Cancer Center - National Cancer Center Hospital, Goyang, Korea, Republic of,[4]Department of Laboratory Medicine / Targeted Therapy Branch, Research Institute, National Cancer Center, Goyang, Korea, Republic of

Background: Triple-negative Breast Cancer (TNBC) is known for its aggressive nature, high recurrence rate, poor prognosis and limited treatment options. Currently, the use of non-coding RNAs as diagnosis or prognosis biomarkers or even target treatment options for TNBC are being studied. Among the non-coding RNAs, recent studies show that circular RNAs (circRNAs) levels are differentially expressed in TNBC tumor tissues, they also interact with micro RNAs and regulate multiple intracellular mechanisms involved in tumorigenesis. Furthermore, their distinct shape makes them more stable and resistant. However, the transition from laboratory research to their clinical application as biomarkers is still in progress. In this meta-analysis we compare multiple studies on prognostic values of circRNAs.
Methods: After detailed literature search on EMBASE, PubMed, and Web of Science, eligible studies were meta-analyzed to estimate the pooled hazard ratio (HR) and 95% confidence intervals for overall survival (OS) and disease-free survival (DFS) in TNBC patients based on their levels of expression of circRNAs.
Results: A total of 1991 patients had their serum or TNBC tumor samples analyzed across 15 different studies and the levels of expression of certain circRNAs were found to be upregulated or downregulated in TNBC compared to normal breast tissues. High expression of upregulated circRNAs also significantly impacts the outcomes of TNBC patients with poor OS (adjusted: HR=2.85, 95%CI: 2.02 - 4.00) and reduced DFS (HR=5.43, 95%CI: 1.82 - 16.21). Moreover, circCRIM1, circEIF6, circTRIO, circCFL1, circGNB1, circRAD18, circ_0001006, circSIPA1L3, circ_0000199, circTBC1D14, circIFI30, circSEPT9, circ_0003528 were upregulated and their high level was associated with worse outcomes. Conversely, circFBXW7 was downregulated in TNBC samples and low levels were significantly associated with bad prognosis.
Conclusion: This review showed that circRNAs are abnormally expressed in TNBC tumors, their levels significantly impact the outcome of the patients and their distinct molecular structure is more stable and less prone to degradation which makes them good candidates for prognosis biomarkers and potential therapeutic targets.

#4648 Circulating tumor DNA analysis demonstrates newly emerging alterations in previously treated IDH1 mutated cholangiocarcinoma patients enrolled in the ClarIDHy study.

Hatice Duygu Saatcioglu[1], Haley Ellis[2], Jinkai Wan[2], Sam Lubner[3], Milind Javle[4], Ghassan K. Abou-Alfa[5], James M. Cleary[6], Robin Kate Kelley[7], Mitesh J. Borad[8], Teresa Macarulla[9], Do Youn Oh[10], Camelia Gliser[1], Susan Pandya[1], Sung Chloe[1], Nabeel Bardeesy[11], Adriana Tron[1]

[1]Servier Pharmaceuticals, Boston, MA,[2]Massachusetts General Hospital Cancer Center/Harvard Medical School, Boston, MA,[3]University of Wisconsin, Madison, WI,[4]MD Anderson Cancer Center, Houston, TX,[5]Memorial Sloan Kettering Cancer Center, New York, NY,[6]Dana-Farber Cancer Center, Boston, MA,[7]University of California, San Francisco, San Francisco, CA,[8]Mayo Clinic Arizona, Phoenix, AZ,[9]Vall d'Hebron University Hospital, Vall d'Hebron Institute of Oncology, Barcelona, Spain,[10]Seoul National University Hospital Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea, Republic of,[11]Massachusetts General Cancer Center Harvard Medical School, Boston, MA

Background: Ivosidenib (IVO) is a first-in-class oral inhibitor of the IDH1 mutant (mIDH) enzyme. In the ClarIDHy phase 3 study of previously treated patients with mIDH cholangiocarcinoma (CCA), IVO treatment led to improved progression-free survival (PFS) and overall survival (OS) vs placebo (median PFS: 2.7 vs. 1.4 months and OS 10.3 vs. 5.1 months, when adjusted for the 70% crossover rate; HR, 0.49 [95% CI, 0.34-0.70]; 1-sided *P*<0.001. Here, we studied the dynamics of mutations emerging after 6 months of IVO treatment using ctDNA analyses.

Methods: Prior to enrollment, all subjects in ClarIDHy had *IDH1* R132 mutations confirmed by a central NGS panel utilizing tumor biopsy tissue. Since post-progression tissue samples were unavailable, discovery of emerging mutations was limited to plasma ctDNA assessments. To explore molecular changes associated with acquired resistance, an analysis was performed on 18 subjects treated with IVO for ≥6 months (4 partial response; 12 stable disease, 2 NA) using matched baseline and end of treatment (EOT) samples (PGDx elio^{TM} plasma complete, 521 gene panel). A subset of the oncogenic alterations identified were selected for confirmation by ddPCR analysis in ctDNA samples collected longitudinally. Longitudinal plasma 2HG levels were also evaluated.

Results: This analysis identified several EOT mutations not present at baseline. The most common mutations were in the MAPK pathway, including activating KRAS, NRAS, and MAP2K1 mutations and loss-of-function NF1 mutations (6/18 patients, 33%). In 2/6 patients, multiple MAPK pathway mutations were detected. There was a range of variant allele frequencies (VAFs) of RAS alterations, including some that had comparable levels to the truncal mIDH, suggesting that MAPK activation may have made a major contribution to the burden of resistant disease. ddPCR analyses from longitudinal plasma samples from selected cases confirmed these findings. In addition, 2 patients had mutations affecting transcription/epigenetics (WT1 and KMT2C). Finally, only 1 patient had acquired mutations in IDH genes (IDH2-R172K and IDH1-G131V). The WT1, KMT2C, and IDH variants had a low VAF. Accordingly, EOT plasma 2HG levels were reduced vs pre-treatment levels in the patient with secondary mIDH, indicating a limited role in the overall emergence of resistance.

Conclusion: Analyses of paired baseline/EOT samples suggest that multiple acquired mutations could contribute to IVO resistance, with oncogenic MAPK pathway alterations being the most common. Consistent with previous clinical studies conducted in AML, IDH1 second site mutations and isotype switching to IDH2, rarely emerge during IVO treatment. The lack of detectable mutations in the majority of patients could reflect the sensitivity of the NGS assay or a role for non-genetic mechanisms of resistance.

#4649 Proposal of the concept of "*p53* mutational spectrum": Its clinical implication in oral squamous cell carcinoma.

T. Hyodo[1], N. Kuribayashi[2], C. Fukumoto[1], Y. Komiyama[1], T. Hasegawa[1], K.-i. Nakashiro[2], D. Uchida[2], H. Kawamata[1];
[1]Dokkyo Medical University School of Medicine, Tochigi, Japan, [2]Ehime University Graduate School of Medicine, Ehime, Japan

The p53 gene is the most frequently mutated gene in malignant tumors. We found that p53 abnormalities were observed in most cases of oral squamous cell carcinoma (OSCC). Therefore, it is necessary to search for type of the functional abnormality (complete loss of function, partial loss of function, or gain of oncogenic function) of the p53 gene rather than the presence or absence of gene mutation. Here we would like to propose the concept "p53 mutational spectrum". In this study we performed whole-exome sequencing of p53 using clinical specimens from OSCC and attempted to clarify the implication of "p53 mutational spectrum" with clinical manifestation. Sixty-seven patients with OSCC who visited the Department of Oral and Maxillofacial Surgery, Dokkyo Medical University or Ehime University were subjected to the study. Genomic DNA was extracted from biopsy and surgical specimens, and a custom panel was created for OSCC. p53 and other driver gene mutations were analyzed by NGS. p53 mutation pattern was classified into 5 categories, wild type without p16 expression, wild type with p16 expression, missense mutations in the DNA binding domain, missense mutations out of the DNA binding domain and truncation mutations. Patients with several p53 mutational patterns were statistically analyzed in relation to biological and clinical grade, and overall survival (OS) and disease-free survival (DFS) were also analyzed. The patterns of p53 gene was wild type without p16 expression in 7 patients, wild type with p16 expression in 6 patients, missense mutations in the DNA binding domain in 31 patients, missense mutations out of the DNA binding domain in 2 patients and truncation mutations in 7 patients. Thus, the p53 gene was mutated in 54 patients, 33 with missense mutations and 24 with truncation mutations. p53 mutation distribution was concentrated in the DNA binding domain and missense mutations were common, while truncation mutations were common in other domains such as the transcriptional activation domain. In univariate analysis, p53 truncation mutations were identified as a risk factor for DFS and were associated with poorer prognosis compared with other mutation groups. p53 mutational spectrum was not significantly associated with other driver gene mutations. p53 missense mutation group. The p53 missense mutationp group was also analyzed in the same way, but the results were not statistically significant. The p53 truncation mutation (complete loss of function) was associated with poorer prognosis than other gene status, wild type with/without p16 and missense mutation. The p53 missense mutation may contain functional diversity (complete loss of function, partial loss of function, almost wild, or gain of oncogenic function). We are currently constructing a platform for p53 mutational spectrum database.

#4650 The impact of GLI3 mutations and expression on prognosis in gastric cancer.

Wanfu Zhang[1], Wendi Jin[1], Hongwei Wan[1], Chao Niu[1], Chu Yan[1], Chunxiao Wang[1], Juanjuan Liu[1], Xiaogang Li[1], Qinglan Zong[2], Ning Gao[2], Ding Zhang[2]

[1]Department of General Surgery, The Affiliated Hospital of Yunnan University, Kunming, China,[2]3D Medicines Inc., Shanghai, China

Background: GLI3, a member of the GLI zinc finger family, has been extensively investigated in various cancers, including gastric cancer. It has been observed that GLI3 is significantly upregulated in solid cancer types. These findings suggest the involvement of GLI3 in the malignant progression of different cancers. However, the precise impact of GLI3 mutations and expression on the prognosis of gastric cancer patients remains unclear. Further investigation is warranted to elucidate the prognostic significance of GLI3 alterations in gastric cancer.
Methods: This study included 269 gastric cancer patients from TCGA and 2903 patients from China. The mutation rate of GLI3 was analyzed using Excel. Additionally, the survival rates of gastric cancer patients with GLI3 mutations were compared to those with wild-type using KM survival curves in the TCGA cohort. In addition, the impact of high and low expression of GLI3 on survival was analyzed using a 50% threshold. The expression of GLI3 in tumor tissues were evaluated using Mann-Whitney analysis to compare with those in normal tissues.
Results: The mutation rate of GLI3 was 13.55% in the TCGA cohort, whereas it was significantly lower at 7.79% in the Chinese cohort. KM curve analysis demonstrated that patients with GLI3 mutations in the TCGA cohort had a significantly higher survival rate than those with the wild group ($p < 0.05$). Additionally, GLI3 expression was significantly higher in tumor tissues compared to normal tissues ($p < 0.01$). Furthermore, based on GLI3 expression analysis, patients with a 30% higher expression of GLI3 had a significantly worse prognosis compared to the remaining 70% of patients ($p < 0.05$). The analysis of GLI3 mutations and expression revealed that patients with GLI3 mutations had significantly lower RNA expression compared to those with the wild-type.
Conclusion: The mutation rate of GLI3 was found to be higher in the TCGA cohort compared to the Chinese cohort. Both GLI3 mutations and expression were closely associated with the prognosis of gastric cancer patients. These findings highlight the significance of GLI3 as a potential prognostic indicator in gastric cancer. Further research is needed to elucidate the underlying mechanisms and validate these observations in larger and more diverse patient populations.

#4651 Long non coding RNA LINC01224 is prognostic marker and regulates cancer stemness in the NSCLC.

Ji Woong Son, Wan Jin Hwang, Minhyeok Lee, Daeun Kang, In Beom Jeong, Sun Jung Kwon

Konyang Univ. College of Medicine, Daejeon, Korea, Republic of

Long noncoding RNA is also a molecule that is attracting attention these days. Only about 1.5% of the genome is protein-coding, while the other 98.5% consists of non-coding elements, which are transcribed by RNA polymerase II (polII) into non-coding RNA (ncRNA). Among them, ncRNAs with a size of 200nt or more are called long non-coding RNAs (lncRNAs). To date, lncRNA have been recognized to have regulatory roles in gene expression through various mechanisms. They are involved in diverse biological processes such as cell differentiation, apoptosis, development, inflammatory response, and immune response. Cancer stem cells (CSCs) identified in lung cancer exhibit resistance to chemotherapy, radiotherapy, and targeted therapy. Therefore, a technology for controlling CSCs is needed to overcome such resistance to cancer therapy. The authors planned this study to discover lncRNAs involved in cancer stemness. We analyzed TCGA data to search for lncRNAs that predict the prognosis of NSCLC and selected LINC01224 as a candidate lncRNA involved in cancer stemness. Treatment of LINC01224 with siRNA reduced colony formation ability in cell lines with stem cell characteristics. Next, we performed whole-genome expression in lung cancer cell line after siRNA treatment to identify gene controlled by LINC01224. This experiment revealed that lncRNA is involved in apoptosis, cell cycle, cell death, cell differentiation, cell migration, and DNA repair. LINC01224 is a prognostic factor and an attractive treatment target for non-small cell lung cancer. LINC01224 possibly regulates various biological phenomena and specific mechanism studies are needed.

#4652 Prevalence and prognostic role of KDM6A deficiency and overexpression in surgically treated prostate cancer.

Neele Heckmann[1], Natalia Gorbokon[1], Maximilian Lennartz[1], Martina Kluth[1], Eike Burandt[1], Till S Clauditz[1], Waldemar Wilczak[1], Stefan Steurer[1], Christian Bernreuther[1], Elena Bady[1], Lisa Hornsteiner[1], **Ronald Simon**[1], Hans Heinzer[1], Alexander Haese[1], Sarah Minner[1], Guido Sauter[1], Thorsten Schlomm[2], Markus Graefen[1], Florian Viehweger[1]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany,[2]Charite - Universitatsmedizin Berlin, Berlin, Germany

KDM6A, also known as UTX (ubiquitously transcribed X chromosome tetratricopeptide repeat protein) is a critical epigenetic regulator which is often subject to truncating mutations and subsequent expression loss in cancer. To interrogate the clinical significance of aberrant KDM6A expression, a tissue microarray containing prostate cancers from patients treated by prostatectomy was analyzed by immunohistochemistry. KDM6A immunostaining was weak in 33.5%, moderate in 52.9%, and strong in 7.3% of 13,747 interpretable tumors while 880 (6.4%) tumors completely lacked KDM6A staining (KDM6A deficiency). The KDM6A status depended on the *TMPRSS2:ERG* fusion status. Strong KDM6A staining occurred in only 4.5% of ERG IHC negative, but in 11.1% of ERG IHC positive cancers while KDM6A deficiency occurred in 10.5% of ERG negative, but in only 1.4% of ERG positive cancers ($p<0.0001$ each). High KDM6A expression was linked to advanced pT, high Gleason grade, nodal positivity, and early PSA recurrence ($p<0.0001$ each). The prognostic role of high KDM6A expression was independent of pre- (Gleason score from biopsy, cT, PSA) and postoperatively (Gleason score from prostatectomy, pT, pN, R status) available parameters in all tumors ($p<0.0001$) and in ERG negative ($p<0.0001$) and positive subgroups ($p=0.0049$). In contrast, the outcome of KDM6A deficient cancers varied substantially between ERG negative (good prognosis) and ERG positive (bad prognosis) subgroups. In conclusion, the striking independent association of high KDM6A expression with poor prognosis suggests an important role of KDM6A expression in cancer cells which may have clinical utility in prostate cancer. The reciprocal role of KDM6A expression losses in ERG positive and negative cancers indicates a variable, cancer subtype dependent clinical effect of KDM6A deficiency. The clinical effects of anti-EZH2 drugs remains may depend on the ERG status in prostate cancer.

#4653 Platelet to lymphocyte ratio and platelet count: new frontiers as prognostic factors in metastatic colorectal cancer patients receiving anti-angiogenic drugs.

Eleonora Lai, Francesco Loi, Enrico Palmas, Claudia Codipietro, Andrea Pretta, Dario Spanu, Stefano Mariani, Alessandra Pia D'Agata, Pina Ziranu, Valeria Pusceddu, Marco Puzzoni, Mattia Cucca, Andrea Cadoni, Maria Letizia Ledda, Francesca Semonella, Veronica Dell'Utri, Marta Pozzari, Daiana Rizzo, Giulia Deias, Giulia Papalexis, Giorgia Sanna, Giusy Moledda, Benedetta Congiu, Fabio Bardanzellu, Fabiana Contu, Emanuela Piseddu, Erika Cimbro, Antonio Maccioni, Elisa Aresu, Sara Murgia, Gianluca Pannella, Clelia Donisi, Giorgio Astara, Elena Massa, **Clelia Madeddu**, Mario Scartozzi

Medical Oncology Unit, University Hospital and University of Cagliari, Cagliari, Italy

Background: Anti-angiogenic drugs represent a milestone of metastatic colorectal cancer (mCRC) pts) treatment. To date, no validated prognostic biomarkers are available. Recently, hematological parameters became of particular interest in solid tumors, including mcRC. Here, we present the results of our research in order to identify potential prognostic factors among clinical and laboratory parameters in mCRC pts receiving anti-angiogenic agents at our Centre.
Patients and Methods: We retrospectively collected clinical and laboratory data of mCRC pts treated with anti-angiogenic drugs at the Medical Oncology Unit of Cagliari University Hospital (2018-04/2024) in order to identify a potential prognostic tool. Statistical analysis was performed with MedCalc (survival distribution: Kaplan-Meier; survival comparison: log-rank test; cut-off: ROC curves).
Results: Globally, 42 mCRC pts were included in our study. 25 were male, 17 female; 17 had a RAS wild-type tumor, 30 had a left-sided primary). 14 pts received anti-angiogenic agents in the the first-line, 15 in the second-line (4 bevacizumab and 11 aflibercept) and 13 in the first and further lines. Median OS was 31.4 months (95% CI: 18.3 -39.5). When assessing the correlation between clinical and laboratory parameters and prognosis of the study population, a statistically significant improvement in overall survival (OS) was found in patients with platelet to lymphocyte ratio (PLR) equal or lower than 179.23 (31.4 months [95% CI: 20.3-47.1] versus 10.5 months [95%: CI 6.8 - 39.5], p=0.0315, HR= 0.27) and platelet count equal or lower than 360/µl (31.4 months [95% CI: 20.3-41.8] versus 10.3 months [95%: CI 6.8-10.5], p<0.0001, HR=0.0002). Then, according to these findings, we separated pts in two prognostic groups: good prognostic group (no unfavorable variables) and poor prognostic group (≥1 unfavorable variables). We observed a trend for longer OS in patients belonging to the good prognostic group (31.4 months [95% CI: 20.3-41.8] versus 10.5 months [95% CI: 6.8 - 39.5], p=0.0521, HR=0.3166).
Conclusions: Our research showed a promising prognostic role of baseline platelet count and PLR in mCRC patients receiving with anti-angiogenic drugs in a limited population at our center.

#4654 Clinical significance of tumor infiltrating lymphocytes and neoadjuvant chemotherapy in colorectal cancer liver metastases.

Yasaman Rezaie[1], Kerryan Ashley[1], Janie Zhang[2], Thejal Srikumar[1], Jassim DiPalermo[1], Kurt Schalper[1], Michael Cecchini[1]

[1]medicine, Yale University, New Haven, CT,[2]medicine, UPMC, Pittsburgh, PA

Background: The effects of neoadjuvant chemotherapy (NAC) and the survival impact of tumor infiltrating lymphocytes (TILs) in the colorectal cancer liver metastases (CRCLM) tumor microenvironment (TME) remain poorly understood.
Methods: We studied the clinicopathologic characteristics and the levels of different TIL subpopulations using multiplexed quantitative immunofluorescence (mQIF) in paired primary/liver metastasis in two independent CRCLM cohorts from Yale-New Haven Hospital between 2000-2024 using tissue microarrays. The first cohort included 84 patients (Cohort 1) and the second cohort comprised 95 patients (Cohort 2). The mQIF analysis included the markers DAPI for all cells/nuclei, cytokeratin (CK) for tumor epithelial cells, CD4 for helper T-cells, CD8 for effector T-cells, and FOXP3 for regulatory T-cells (Tregs). The levels of different cell populations were measured in specific tumor tissue compartments including the CK+ cancer-cell nests, the CK-negative peritumoral stroma and the total tissue area. The association between TILs and treatment-specific overall survival (OS) were analyzed.
Results: The OS was comparable between cases treated with or without NAC with a median OS after stage 4 diagnosis in NAC treated and not treated of 84 vs. 65 months, $p = 0.31$ and 59 vs. 115 months, $p = 0.23$ in Cohorts 1 and Cohort 2, respectively). NAC was associated with significantly higher CD8+ TILs in liver metastases in all studied tumor tissue compartments ($p < 0.05$ in Cohort 1 and $p < 0.003$ in Cohort 2). Elevated CD8+ TILs in the cancer-cell compartment of CRC liver metastases were associated with significantly improved survival irrespective of NAC (Hazard Ratio (HR) = 0.39, $p = 0.022$ in Cohort 1 and HR = 0.25, $p = 0.025$ in Cohort 2), a pattern not observed in primary tumors of both cohorts. In contrast, Tregs were consistently lower in liver metastases compared to primary tumors across all compartments ($p < 0.0001$ in both cohorts). Patients who did not have disease recurrence after NAC and hepatectomy had higher levels of CD8+ T-cells in liver lesions compared to those with tumor recurrence ($p = 0.05$ and 0.04 in Cohort 1 and 2 respectively). Microsatellite instability-high (MSI-H) tumors exhibited higher CD4+ and CD8+ TILs in primary tumors, but not in liver metastases (n = 5, $p < 0.05$ and < 0.01 for CD4+ and CD8+ T cells, respectively, across all compartments).
Conclusion: NAC did not show survival benefit in CRCLM patients but was associated with increased effector TILs in liver metastases. Elevated CD8+ TILs in CRCLM are prognostic and this highlights the potential for immunostimulatory strategies to leverage TME changes induced by NAC. Liver metastatic MSI-H CRCs show prominent inter-tumor heterogeneity with increased TILs only in the primary lesion.

#4655 Identification of non-small cell lung cancer metabolic subtypes and targeting glucose pathway.

Seo-Young Lee[1], Youngjoo Lee[1], Park Seog Yun[1], Beom K Choi[2], Sohyun Park[1], Jaemin Kim[1]

[1]National Cancer Center, Goyang-si, Korea, Republic of,[2]InnoBationbio, Seoul, Korea, Republic of

Background - NSCLC accounts for approximately 80% of all lung cancer cases. Despite significant treatment efforts, overall survival rates for NSCLC patients remain low. The NSCLC microenvironment and metabolism are also not fully understood, prompting ongoing research to better understand lung cancer progression and metastasis. To address tumor heterogeneity and define precise metabolic clusters, we applied non-negative matrix factorization (NMF) and discovered that the NMF clusters were significantly associated with clinical outcomes (progression, survival).

Method - To investigate metabolic functions in 73 patients who received immunotherapy, we performed NMF clustering to identify subgroups based on significantly differentially expressed genes from NanoString data. After performing NMF, we found 5 subgroups then defined the metabolic function of each cluster using Gene Set Variation Analysis (GSVA) and identified the top 10 marker genes for each cluster using the Wilcoxon test from the Seurat package.

Result - We found that clusters 1 and 2 had a higher proportion of immune cells compared to other clusters. Interestingly, while cluster 3 showed a higher proportion of immune cells, it had a lower overall survival rate and progression-free survival rate compared to cluster 1. We identified the key difference between these two clusters as glucose uptake. Moreover, glucose-related genes (*SLC2A1, SLC2A3, SLC2A6, SLC2A14, SLC2A8*, and *SLC2A5*) were highly expressed in cluster 2, which exhibited a higher proportion of Tregs and a stronger correlation between the glucose transport pathway and Tregs. Additionally, we found that *SLC2A3* expression was significantly associated with overall survival and progression-free survival. To validate our findings and demonstrate their robustness, we corroborated them using GSE161537 data. This study may help identify metabolic pathways and biomarkers that could be targeted in precision oncology.

#4656 Fatty acid β-oxidation enzyme ECI1: a biomarker of poor survival outcome in prostate cancer patients after transurethral resection of the prostate (TURP).

Karl-Philippe Guerard[1], Elie Fadel[1], Ryan Antel[1], Fadi Brimo[2], Tarek Bismar[3], **Jacques Lapointe**[1]

[1]Department of Surgery, Divion of Urology, McGill University, Montreal, QC, Canada,[2]Department of Pathology, McGill University, Montreal, QC, Canada,[3]Department of Pathology, University of Calgary, Calgary, AB, Canada

Deregulated mitochondrial fatty acid β-oxidation is known to drive prostate cancer (PCa) pathogenesis. ECI1 (Δ3, Δ2-Enoyl-CoA Delta Isomerase 1) is a key mitochondrial fatty acid β-oxidation enzyme whose role in PCa remains to be explored. We recently showed the impacts of ECI1 on PCa phenotype by modulating its expression in cell lines and assessed the clinical implications of its expression in human prostate tissue samples. ECI1 overexpression increased PCa cell growth and enhanced colony formation, cell motility, and maximal mitochondrial respiratory capacity. PCa cells stably overexpressing ECI1 injected orthotopically in nude mice formed larger prostate tumors with higher number of metastases. Immunohistochemistry analysis of a tissue microarray representing radical prostatectomy cases revealed a stronger ECI1 staining in prostate tumors compared to corresponding benign tissues. ECI1 expression was higher in cases with high tumor grade and advanced tumor stage. ECI1 overexpression was a strong independent predictor of biochemical recurrence after adjusting for known clinicopathologic parameters. ECI1 overexpression was also associated with increased risk of distant metastasis and reduced overall survival, suggesting a key role in disease progression. The purpose of this study was to assess the prognostic value ECI1 expression in PCa tissue on overall survival of patients who underwent palliative transurethral resection of the prostate (TURP). ECI1 immunohistochemistry was performed on a tissue microarray representing 257 TURP cases. ECI1 staining of malignant epithelial cells was assessed using the H-score method and patients were dichotomized into two groups based on ECI1 expression (high vs low). ECI1 overexpression was associated with an increased risk of death, which remained significant after adjusting for age and tumor grade group (hazard ratio=1.57, P=0.01). Moreover, a subgroup of this cohort considered of favorable survival outcome according to their PTEN positive and ERG negative tumor expression statuses (n=121) were further risk stratified by ECI1 even after adjusting for age and tumor grade group (hazard ratio =2.26, P=0.004). Overall, this study supports the contribution of ECI1 to PCa progression and highlights its potential as biomarker to identify patients at high risk of death among those who underwent a palliative TURP procedure. Future studies should explore whether the ECI1 metabolic pathway may serve as therapeutic target to improve patient survival and quality of life.

#4657 Investigating the role of circulating bone cells in melanoma patients on immune checkpoint inhibitor therapy.

Nicholas Rozza, Catalin Mihalcioiu, Richard Kremer

McGill University, Montreal, QC, Canada

Bone metastasis is a frequent progression of metastatic melanoma affecting 20-40% of patients with metastatic melanoma. While immune checkpoint inhibitors have been revolutionary in the treatment of advanced melanoma, certain patient populations do not respond to immunotherapy. Clinical data from our own treated melanoma population at the MUHC has shown that patients with bone metastases respond poorly to immune checkpoint inhibitors and have worse outcomes when compared to patients without bone metastases. These findings have prompted the investigation into this observed phenomenon of treatment resistance. The bone microenvironment is known to be a key player in cancer progression and metastasis and has been shown to form a vicious cycle where cancer and bone cells interact symbiotically to promote tumor growth and enhance bone degradation. Bone cells that are released into the circulation during the dynamic process of bone metastasis can easily interact with immune cells and may disrupt the ability of the immune system to mount an effective anti-cancer response. In our cohort of 185 metastatic melanoma patients treated with immune checkpoint inhibitors, patients with bone metastasis have a 43% response rate versus 65% in patients with visceral metastases alone. In terms of the complete response (CR) rate, only 18% of patients with bone metastasis achieved a complete response compared to 52% of patients with visceral metastasis alone. The treatment resistance appears to be systemic in patients with bone metastasis; the response rate in lesions outside the bone is also reduced. In a subset of these patients with serial prospective blood collections, we profiled PBMCs to identify certain immune and bone cell populations in the circulation at a pre-treatment baseline, early on treatment 2-3 cycles and after 4-6 cycles depending on the regimen. Patients were grouped based on objective response to determine the relationship between clinical outcome and immune/bone cell profiles. Patients with bone metastasis and a poor response had reduced immune effector cells and increased osteocalcin positive bone cells in the circulation when compared to patients that achieved partial and complete responses. Immune and bone cell profiling is a novel method of evaluating melanoma patients undergoing immunotherapy and may serve as an effective predictor of response to immune checkpoint inhibitors.

#4658 Clinical characteristics, molecular subtypes, and prognosis of 176 surgically resected esophageal small cell carcinoma patients.

Ruijie Ma, Peng Wu, Chaoqi Zhang, Nan Sun, Jie He

Department of Thoracic Surgery, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Introduction: Small cell carcinoma of the esophagus (SCCE) is an aggressive neuroendocrine gastrointestinal malignancy with poor prognosis, and accounts for 0.8%-2.4% of all histological types of esophageal malignant tumors worldwide. The pathological characteristics of SCCE are similar to those of small cell lung cancer (SCLC), and SCLC subtypes can be defined by differential expression of four key transcription regulators: ASCL1, NeuroD1, YAP1 and POU2F3. However, there is limited research on the molecular subtypes of SCCE. Here, we conducted the study to classify the molecular subtype of SCCE, and explored the relationship between SCCE molecular subtypes and patients' clinical pathological characteristics and prognosis.
Methods: Patients with surgically resected SCCE at the Cancer Hospital of the Chinese Academy of Medical Sciences between 1985 and 2019 were included in the study. Clinicopathologic data of SCCE patients were collected, including gender, age, alcohol abuse, tobacco abuse, BMI, family history, location of tumor, length of tumor, TNM stage, lymphovascular invasion, and treatment. Immunohistochemical staining was performed to calculate the H-scores of ASCL1, NeuroD1, YAP1, and POU2F3 of FFPE sample. Data processing and analyses were performed using SPSS 27, R (4.4.1), and the R package survival [3.7.0].
Results: A total of 176 patients were included in the study. The median overall survival (OS) for all SCCE patients included in the study was 21 months, and the median disease-free survival (DFS) for all SCCE patients included in the study was 13 months. Four subtypes of SCCE samples were categorized by H-score: high ASCL1 (SCCE-A), high NeuroD1 (SCCE-N), high YAP1 (SCCE-Y) and high POU2F3 (SCCE-P). Univariate Cox analysis indicated that the SCCE-P subtype, adjuvant therapy, T stage, TNM stage, lymphovascular invasion, tumor length, T stage, and TNM stage were significantly associated with patient DFS ($p < 0.05$), while adjuvant therapy, T stage, TNM stage, lymphovascular invasion, tumor length, T stage, and TNM staging were significantly associated with patient OS ($p < 0.05$). Multivariate COX analysis indicated that adjuvant therapy, tumor length, and the SCCE-P subtype were independent significant predictors of patient DFS ($p < 0.05$), while adjuvant therapy and tumor length were independent significant predictors of patient OS ($p < 0.05$). The subtypes of SCCE had no significant relationship with clinicopathologic factors of patients.
Conclusion: This study indicate that the SCCE-P subtype is an independent prognostic factor for DFS in SCCE patients, providing a reference for the molecular classification of SCCE and helping to more accurately identify the disease status and prognosis of SCCE patients. Additionally, the study observed that adjuvant therapy after surgery for SCCE patients can significantly improve both OS and DFS.

#4659 AI-powered assessment of morphologic likeness to small cell lung cancer (SCLC) predicts progression to SCLC and TKI response in EGFR-mutant NSCLC.

Soohyun Hwang[1], Hyukjung Kim[1], Yeong Hak Bang[2], Jun-Gi Jeong[3], Chang Ho Ahn[1], Seungeun Lee[1], Sanghoon Song[1], Aaron Valero Puche[1], JaeWoong Shin[1], Sehhoon Park[2], Hyun Ae Jung[2], Jong-Mu Sun[2], Yoon-La Choi[4], Jin Seok Ahn[2], Myung-Ju Ahn[2], Siraj Ali[1], Chan-Young Ock[1], Se-Hoon Lee[2]

[1]Lunit Inc., Seoul, Korea, Republic of,[2]Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center, Seoul, Korea, Republic of,[3]Department of Digital Health, Samsung Advanced Institute for Health Sciences and Technology, Sungkyunkwan University, Seoul, Korea, Republic of,[4]Department of Pathology and Translational Genomics, Samsung Medical Center, Seoul, Korea, Republic of

Background: A subset of *EGFR*-mutant non-small cell lung cancers (NSCLC) progresses to small cell lung carcinoma (SCLC) during tyrosine kinase inhibitor (TKI) therapy, particularly in cases with inactivating *RB1* mutations. As *RB1* mutations are likely foundational events in SCLC transformation, we hypothesize that tumors susceptible to this transformation exhibit morphologic features resembling SCLC even at the time of the initial diagnostic biopsy.

Methods: We developed a predictive model for identifying the SCLC phenotype using H&E-stained slides from cases with confirmed SCLC histological diagnosis. Morphological features of individual cells were extracted through a grid-based image retrieval method, enhanced by subcellular compartmental analysis with an emphasis on nuclear and cytoplasmic characteristics. These features were used to quantify the morphological SCLC-likeness for each case. The SCLC-like phenotype was defined as the top 25% of cases exhibiting the highest SCLC-likeness scores. The model was then applied to 106 real-world advanced-stage *EGFR*-mutant NSCLC cases to validate the extracted features and assess clinical correlations. The primary endpoints were progression-free survival (PFS) after TKI therapy according to SCLC-like phenotype versus others, along with the rate of SCLC transformation. Secondary endpoints were *RB1* mutations confirmed by targeted panel sequencing or whole exome sequencing.

Results: The SCLC-like phenotype case images correlated with pathologist-interpretable features of SCLC, including a significantly smaller nuclear area (56 μm^2 vs. 102 μm^2) and increased nuclear optical density (18.01 vs. 15.68) reflecting hyperchromasia, consistent with typical SCLC morphology. Among the 106 patients with *EGFR*-mutant NSCLC, 26 were classified as having the SCLC-like phenotype. Patients with the SCLC-like phenotype demonstrated significantly worse PFS after TKI treatment (HR 1.71, CI 1.08-2.71, P = 0.02). Among the cases with secondary tissue biopsies (n = 68), SCLC transformation was observed in 4 cases, with a numerically higher frequency in the SCLC-like phenotype group compared to others (15.8% [3/19] vs. 2.0% [1/49], P = 0.06). No significant difference in *RB1* mutation frequency was found via panel or whole exome sequencing.

Conclusion: This is the first study to demonstrate the potential of morphologic assessment in identifying features pertinent to SCLC transformation and in optimizing prognostication in *EGFR*-mutant NSCLC treated with TKI. Given the heterogeneity in advanced-stage *EGFR*-mutant NSCLCs and the need to optimize therapeutic regimens, further and prospective studies are warranted.

#4660 Epstein-Barr virus transcriptomic signatures of tumor subpopulations predict relapse in nasopharyngeal carcinoma.

Eymon Ka Chun Wu[1], Jun-Yi Chen[2], Ken Hung On Yu[2], Nelson Su[2], Esther Hyunji Won[1], Hazel Yap[1], Hin Kwok[3], Raymond King-Yin Tsang[4], Joseph Chun-Kit Chung[4], Anthony Wing-Ip Lo[5], Stephanie Nga Sze Wong[4], Jack Kuipers[6], Jeffery Yan Ho Lau[1], Horace Cheuk-Wai Choi[7], Ka-On Lam[1], Sum-Yin Chan[1], To-Wai Leung[1], Xin Yuan Guan[1], Anne Wing Mui Lee[1], Chi Ming Che[8], Joshua Wing Kei Ho[2], Victor Ho Fun Lee[1]

[1]Department of Clinical Oncology, Centre of Cancer Medicine, School of Clinical Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China,[2]School of Biomedical Sciences, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China,[3]Centre for PanorOmic Sciences, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China,[4]Department of Surgery, School of Clinical Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China,[5]Department of Pathology, School of Clinical Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China,[6]Department of Biosystems Science and Engineering, ETH Zurich, Zurich, Switzerland,[7]Division of Epidemiology and Biostatistics, School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China,[8]Laboratory for Synthetic Chemistry and Chemical Biology Limited, Hong Kong, China

Nasopharyngeal carcinoma (NPC), originating from nasopharyngeal epithelium and frequently metastasizes to cervical lymph nodes and distal organs, is endemic in Southeast Asia and highly associated with Epstein-Barr virus (EBV). Radiation therapy with or without concurrent chemo-radiation is the most common treatment, but 30-50% of treated patients still relapse. Plasma EBV DNA test is the gold standard in screening and monitoring relapse. However, 20-50% NPC relapse are plasma EBV DNA-negative. In situ hybridization (ISH) of EBV-encoded small RNAs (EBER) of formalin-fixed paraffin-embedded nasopharyngeal biopsy can identify EBV-positive tumors regardless of plasma EBV DNA, but remains unable to predict relapse at initial diagnosis.

Recently, single-cell RNA sequencing (scRNA-seq) technology has emerged as a long-sought solution to low resolution in bulk sequencing. We launched the largest and most comprehensive scRNA-seq NPC study. 74 patients were recruited. Matched biopsies from normal adjacent tumor, primary tumor and lymph node were scRNA-seq profiled. 1298414 cells had been successfully profiled. Our study spanned 5 years and 5 patients relapsed. By unsupervised clustering, cell type classification by canonical markers, and copy number variation, differential abundance and survival analyses, we identified a number of relapse-predicting neoplastic and non-neoplastic subpopulations. For the first time, we also attempted to unfold EBV transcriptomic signature and its association with relapse at single-cell resolution using Vulture developed by our team.

Our results confirmed EBV transcripts were highly enriched in neoplastic cells. Surprisingly, EBV transcripts could also be detected in both non-neoplastic epithelial and tumor micro-environment cells especially T cells and B cells. Neoplastic subclones carrying mutations predictive of relapse revealed by our modified Mutect2 pipeline and other relapse-predicting tumor subpopulations were also found to possess higher EBV positivity. Cox regression analysis identified both cell and tissue type-specific EBV transcriptomic signatures implicated in initial attachment and entry during infection, viral gene regulation, immune evasion and cellular transformation that predicted relapse. Receiver operating characteristic analysis suggested EBV transcriptomic signatures outperformed both nasopharyngeal EBER ISH and plasma EBV DNA in relapse prediction at initial diagnosis.

To conclude, we successfully discovered previously overlooked relapse-predicting EBV transcriptomic signatures with cell and tissue type specificity that may be implicated in (1) tumorigenic transformation and (2) progression evolution. EBV transcriptomic signatures outperforming current strategies in relapse prediction at initial diagnosis may serve as new diagnostics and therapeutic targets for relapse prevention.

#4661 PTPRT mutations in gastric cancer: prognostic implications and functional domain analysis.

Jianbiao Xu[1], Lei Zou[2], Yun Shang[1], Tongmin Wang[1], Jianlin Song[1], Wenchuan Zhu[1], Yunjun Zeng[1], Jianxun Wang[1], Chuntao Li[1], Qinglan Zong[3], Ning Gao[3], Ding Zhang[3]

[1]Department of General Surgery II, The First People's Hospital of Yunnan Province, the Affiliated Hospital of Kunming University of Science and Technology, Kunming, China,[2]Department of Gastrointestinal and Bariatric Metabolic Surgery, The First People's Hospital of Yunnan Province, the Affiliated Hospital of Kunming University of Science and Technology, Kunming, China,[3]3D Medicines Inc., Shanghai, China

Background: The PTPRT gene is recognized as a tumor suppressor in human cancers. However, prior investigations have predominantly concentrated on PTPRT mutations in colon cancer, leaving a dearth of information regarding gastric cancer. Existing studies have proposed that mutations in the PTPRT phosphatase domain are infrequent in common human cancers and have no bearing on prognosis. Conversely, our research on gastric cancer has produced conflicting outcomes, thus presenting an alternative perspective.
Methods: This study included a cohort of 369 gastric cancer patients from TCGA database and 234 gastric cancer patients from real-world settings. The mutation rate of the PTPRT gene in gastric cancer patients and the mutations at different functional domain sites were analyzed using EXEL. Furthermore, the impact of mutations in different functional domain sites on survival was assessed using Kaplan-Meier survival curves.
Results: The mutation rate of PTPRT was 10.84% (40/369) in the TCGA cohort and 11.54% (27/234) in the real-world cohort. The predominant mutations were observed in the Tyrosine-protein phosphatase domain, accounting for 52.50% (21/40) of total mutations in the TCGA cohort and 37.04% (10/27) in the real-world cohort. Additional mutations were also detected in domains such as Fibronectin type-III and HAM. Survival analysis of the TCGA cohort demonstrated that patients with PTPRT mutations had significantly higher survival rates compared to those with wild-type PTPRT ($p=0.0197$). Specifically, patients with mutations in the Tyrosine-protein phosphatase domain exhibited significantly improved survival outcomes compared to the remaining patients ($p=0.0325$). However, there were no significant differences in survival among patients with mutations in other functional domains ($p>0.05$).
Conclusion: In summary, our study elucidated a mutation rate of approximately 10.84% (TCGA cohort) and 11.54% (real-world cohort) for the PTPRT gene in gastric cancer. The Tyrosine-protein phosphatase domain emerged as the primary hotspot for these mutations. Notably, patients harboring PTPRT mutations, particularly in the Tyrosine-protein phosphatase domain, exhibited significantly improved survival outcomes compared to those without mutations in this domain. These findings underscore the potential prognostic significance of PTPRT mutations in gastric cancer and emphasize the need for further investigations to explore their therapeutic implications.

#4662 Tracking gene expression in the tumor microenvironment throughout breast cancer progression.

O. S. Ahmed[1], h. Morsy[1], H. Ateya[1], M. Medhat[2], A. Zekri[1];
[1]National Cancer Institute, Cairo University, Cairo, Egypt, [2]faculty of pharmacy- Russian university, Cairo, Egypt

Background Genetic profiling of the tumor microenvironment involves looking at a lot of genes to figure out what roles they play in the progression of cancer and in how well treatments work. The tumor microenvironment is responsible for tumor genesis, metastasis, and resistance to standard therapy. Characterizing the tumor microenvironment through gene expression, such as ABCB1, FOXA, SFRP1, EGF, and XPB1, can provide valuable insights into the mechanisms driving cancer behavior. By understanding the interactions between these genes and their surrounding environment, researchers can develop more targeted and effective therapeutic strategies that enhance patient outcomes. This study design indicates the systematic procedure of genetic profiling, including patient enrollment and data analysis, with the objective of enhancing our comprehension of the tumor microenvironment in the specific context of Egyptian patients.
Materials and Participants This study involved 60 patients, evaluating the expression levels of specific genes and identifying patterns linked to disease characteristics. RT2 Profiler PCR Arrays PASH-131Z were used to determine the gene expression of 84 genes for tumor cells and surrounding normal cells in each individual..
Results: The FOXA1 gene is up-regulated significantly ($p = 0.03$). It is involved in the laminal classification of breast cancer and the control of transcription for genes related to estrogen hormone and estrogen receptor activity. On the other hand, genes like ABCB1, ATM, BRCA1, CCNA1, CCND2, CDK2, CDKN1A, EGF, EGFR, GATA3, IGF1R, IL6, TP53, and HPRT1 show significant down-regulation ($p < 0.02$). These genes are involved in DNA damage and repair, cell cycle regulation, and apoptosis.
Conclusion :FOXA1 and XBP1 are more important for luminal A breast cancer as a connect with hormone receptor signaling. On the other hand, SFRP and ABCB1 are more important for triple-negative breast cancer because they affect Wnt pathway activation and treatment resistance, respectively. These genes underscore the unique biological pathways present in breast cancer subtypes and demonstrate the problems and potential in their therapeutic targeting.

#4663 Human papillomavirus circulating tumor DNA for risk stratification in cervical cancer.

Emma M. Collier[1], Lucas Penny[1], Jinfeng Zou[2], Zhen Zhao[2], Yangqiao Zheng[2], Pamela Soberanis Pina[3], Michelle McMullen[4], Eric Y. Stutheit-Zhao[5], Michael M. Hoffman[2], Sarah E. Ferguson[6], Kathy Han[7], Eric Leung[8], Stephanie Lheureux[2], Scott V. Bratman[5]

[1]Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada,[2]UHN Princess Margaret Cancer Centre, Toronto, ON, Canada,[3]Medical Oncology, UHN Princess Margaret Hospital, Toronto, ON, Canada,[4]Department of Medical Oncology, Sir Charles Gairdner Hospital, Nedlands, Australia,[5]Department of Radiation Oncology, University of Toronto, Toronto, ON, Canada,[6]Obstetrics and Gynecology, UHN Princess Margaret Cancer Centre, Toronto, ON, Canada,[7]Radiation Medicine, UHN Princess Margaret Cancer Centre, Toronto, ON, Canada,[8]Department of Radiation Oncology, Sunnybrook Health Sciences Centre, Toronto, ON, Canada

Background: Human papillomavirus (HPV) DNA is detectable in the peripheral blood plasma from patients with locally advanced and metastatic cervical cancer. Levels of HPV circulating tumor DNA (ctDNA) in pretreatment plasma have weak associations with prognosis, but the significance of detecting HPV integration into the host genome or fragmentation features within HPV ctDNA has not been explored. We hypothesized that these molecular features of HPV ctDNA may serve as prognostic biomarkers and reflect HPV biology, independent from total ctDNA abundance.

Methods: Plasma cell-free DNA was collected at baseline from 57 patients with locally advanced cervical cancer (stage IB-IVA) and 21 patients with metastatic cervical cancer. Whole viral genome sequencing was performed following hybrid capture. HPV ctDNA levels were expressed in copies/mL plasma. HPV integration sites were detected using SearcHPV. Progression-free survival (PFS) was evaluated using Kaplan-Meier analysis and log-rank tests. The normalized fragment midpoint coverage across the HPV-16 genome was calculated for individual samples and averaged across patient groups. NuPoP, was used to calculate the expected nucleosome occupancy at each base pair of the HPV-16 genome. Peaks corresponding to the observed nucleosome occupancy within our cohorts were called using pracma, and occupancy probabilities were calculated for each peak.

Results: Baseline HPV ctDNA levels were detected in 57/57 locally advanced patients (median=283 copies/mL) and 20/21 metastatic patients (median=299 copies/mL). Unique HPV integration sites were detected at varying levels in 21/57 locally advanced patients (median sites:1, range:0-23) and 12/21 metastatic patients (median sites:2, range:0-45) (Wilcoxon p=0.8). No significant differences in PFS were observed in locally advanced or metastatic patients when stratified by median ctDNA levels (p=0.7 and p=0.8 respectively). However, high confidence integration detected at baseline was associated with inferior PFS in locally advanced patients (p=0.01), but not metastatic (p=0.28). HPV ctDNA fragment coverage varied across the HPV-16 genome with the number of observed merged peaks being 32 in the locally advanced cohort (n=36) and 31 in the metastatic cohort (n=14). The median expected nucleosome occupancy probabilities across all peaks were 98.6% and 97.2%, respectively.

Conclusion: Detection of viral integration within baseline HPV ctDNA was associated with significantly inferior PFS in locally advanced patients. Peaks in the average HPV-16 ctDNA coverage profiles corresponded to high expected nucleosome occupancy in both the locally advanced and metastatic cohorts, suggesting that ctDNA may reflect chromatin accessibility of the virus. These findings suggest that quantitative and qualitative features of HPV ctDNA from baseline plasma may reflect HPV biology associated with cervical cancer.

#4664 Differential transcript usage reveals SERINC2 as a prognostic biomarker in clear cell renal cell carcinoma (ccRCC).

Chinaza Nnam, Minghui Zhang, Brock Christensen, Lucas Salas

Dartmouth College, Lebanon, NH

Background: Clear cell renal cell carcinoma (ccRCC), the most common subtype of renal cell carcinoma, accounts for approximately 75% of cases[1]. While differentially expressed gene (DEG) analyses have provided valuable insights, they often neglect alternative splicing (AS), a key driver of transcriptome diversity and functional protein complexity. AS is essential in modulating tumor biology and can impact disease progression and survival outcomes[2]. To address this gap, we performed a differential transcript usage (DTU) analysis in ccRCC to explore the role of AS in shaping survival-associated signatures.

Methods: A total of 172 tumor samples from the Dartmouth Renal Tumor Biobank were analyzed. Following quality control, transcripts expressed in > 0.1 TPM in at least 25% of tumor samples were retained, yielding 31,850 transcripts. DTU was assessed using DRIMSeq, while functionality was evaluated with IsoformSwitchAnalyzeR. Cox proportional hazards analysis and machine learning-based Cox-LASSO regression was applied to identify survival-associated features, followed by log-rank analysis to explore their prognostic relevance.

Results: DTU analysis revealed significant transcript-level changes linked to survival outcomes. Among these, *SERINC2* emerged as the most statistically significant survival-associated gene, with its transcript NM_001199039.2 exhibiting a hazard ratio (HR) of 0.5 (95% CI: 0.39-0.69). Kaplan-Meier analysis confirmed that elevated expression levels of this transcript were significantly associated with better survival outcomes ($p < 0.05$). *SERINC2* is linked to membrane lipid synthesis and may be associated with lipid metabolic reprogramming, a hallmark of ccRCC pathogenesis. Notably, isoform NM_001199039.2 has the shortest N-terminus regions compared to other validated isoforms, possibly conferring unique functionality. Finally, a prognostic model based on the Cox model demonstrated AUC values of 0.68 for 3-year survival and 0.78 for 5-year survival, indicating strong predictive potential for long-term outcomes.

Conclusions: Our findings identify the *SERINC2* splice variant (variant 5), NM_001199039.2, as a potential biomarker for favorable outcomes. We will conduct further validation in independent datasets and experimental systems to delineate the role of this splice variant on survival outcomes in ccRCC.

Key words: Differential Transcript Usage, variant, survival.

References. 1. Jonasch E, Walker CL, et al. Clear cell renal cell carcinoma ontogeny and mechanisms of lethality. *Nat Rev Nephrol*. 2021;17(4):245-261. doi:10.1038/s41581-020-00359-22. Marques-Coelho D, Iohan L da CC, Melo de Farias AR, et al. Differential transcript usage unravels gene expression alterations in Alzheimer's disease human brains. *NPJ Aging Mech Dis*. 2021;7(1). doi:10.1038/s41514-020-00052-5

#4665 Real-world data of resected early-stage non-small cell lung cancer (NSCLC) harboring copy number variations.

Jun Wang[1], Ning Gao[2], Ding Zhang[2]

[1]Department of Thoracic Surgery, Jiangsu Province Hospital and The First Affiliated Hospital of Nanjing Medical University, Nanjing, China,[2]3D Biomedicine Science & Technology Co., Ltd., Shanghai, China

Introduction: Copy number variations (CNVs) in several genetic regions are associated with cancer susceptibility and poor clinical outcomes. Currently, the role of CNVs in Non-Small Cell Lung Cancer (NSCLC) is relatively vague. This study investigates the CNVs profile in early-stage NSCLC.

Methods: We retrospectively analyzed the next-generation sequencing (NGS) data of tumor tissue specimens from NSCLC patients over the past 3 years in our center. All samples were obtained by surgical resection, and clinical characteristics were collected for further analysis.

Results: A total of 102 early-stage NSCLC performed NGS in our study, the mean age was 60.5 years, with 51% female. Genomic analysis revealed that 85.3% (87/102) patients had driver gene mutations, predominantly *EGFR* mutations (72/102) and *ALK* fusions (6/102). CNVs were observed in 29 NSCLC patients, including 22 with *EGFR* mutations, 1 with *ALK* fusion, 1 with *BRAF* V600E, and 5 without driver gene mutations. Among patients with CNVs, 9p21 (*CDKN2A/CDKN2B*) copy number losses were most frequent (24.1%, 7/29), followed by *EGFR* copy number gains (20.7%, 6/29), *MYC* copy number gains (13.8%, 4/29), and *GATA1* copy number losses (13.8%, 4/29). There was no significant difference in age (median: 63 vs 61 years, *P*=0.504, Mann-Whitney U test) and sex (male: 66% vs 34%, *P*=0.221, chi-square test) between patients with and without CNVs. A significant trend of increased CNVs was observed in smokers compared to non-smokers (CNVs: 45.5% vs. 23.8%, P=0.046, chi-square test). Patients with small tumors accompanied by lymph node metastasis showed a significantly higher incidence of driver gene fusions than those without lymph node metastasis ($T_{1-2}N_{1-2}$ vs. $T_{1-2}N_0$ = 50.0% vs. 7.7%, P<0.001, chi-square test). However, no significant difference in CNVs incidence was found between these groups. The prognostic value of CNVs requires further validation through follow-up.

Conclusions: Our study reveals a high prevalence of copy number losses of 9p21 (*CDKN2A*/*CDKN2B*) exists in early-stage non-small cell lung cancer. And we find that NSCLC patients with a history of smoking are more likely to have copy number variations.

#4666 Unraveling the role of Nrf2 in the acute myeloid leukemia: implication for therapeutic targeting.

Avanish Kumar Pandey[1], Neha Thukral[1], Jay Singh[1], Mercilena Benjamin[1], Anmol Gaur[1], Pranay Tanwar[1], Jayantha Kumar[2], Sameer Bakhshi[3], Surender K. Sharawat[3], Deepam Pushpam[3], Akash Kumar[4], Karthikeyan Pethusamy[2], Anita Chopra[1]

[1]Laboratory Oncology, All India Institute of Medical Sciences (AIIMS), New Delhi, India,[2]Department of Biochemistry, All India Institute of Medical Sciences (AIIMS), New Delhi, India,[3]Medical Oncology, All India Institute of Medical Sciences (AIIMS), New Delhi, India,[4]NCI Jhajjar, All India Institute of Medical Sciences (AIIMS), New Delhi, India

Background: Acute myeloid leukemia (AML) is a hematologic malignancy characterized by uncontrolled proliferation of myeloid precursor cells, presenting significant therapeutic challenges. *NRF2* (Nuclear factor erythroid 2-related factor 2) is a transcription factor that regulates cellular defense mechanisms against oxidative stress, which may contribute to AML progression and chemoresistance.
Aim: This study aims to investigate the expression and regulatory role of *NRF2* in AML, identify its downstream target genes, and evaluate its contribution to disease progression and chemoresistance.
Methods: *NRF2* expression was analyzed in de novo AML bone marrow samples, including adult (n=268) and pediatric (n=173) cases, alongside healthy controls (n=45), using qPCR. AML cell lines (MOLM13 and MV411) were also evaluated for *NRF2* mRNA and protein expression. Protein levels of *NRF2* in AML patient samples and cell lines were assessed via flow cytometry. ChIP sequencing was conducted to identify *NRF2* downstream targets, with ChIP-PCR used for target validation. The cytotoxic effects of a chemotherapeutic agent (Daunorubicin) and an *NRF2* inhibitor (Brusatol) were assessed using MTT assays to determine the IC50 values. Drug assays and inhibition studies were performed to evaluate the effects of *NRF2* inhibition on target gene expression. Statistical analyses, including univariate and multivariate Cox regression, were conducted to correlate *NRF2* expression and clinical parameters with patient survival.
Results: *NRF2* mRNA expression was significantly elevated in de novo AML samples compared to controls (p=0.0024 in adults, p=0.001 in pediatrics). Similarly, high *NRF2* expression was observed in MOLM13 and MV411 cell lines. Flow cytometry analysis revealed higher *NRF2* protein levels in CD33-positive AML blast cells than in lymphoid cells. ChIP sequencing identified 95 enriched regions associated with *NRF2*, including genes involved in the HALLMARK Reactive Oxygen Species Pathway, such as *NQO1*. ChIP-PCR confirmed the significant enrichment of *NRF2* target genes, including *NQO1, HO1, DAD1, STAT2*, and *CYP1B1*. Treatment with Daunorubicin significantly increased *NRF2* expression, while Brusatol significantly reduced *NRF2* and its target gene expression (e.g., *DAD1, STAT2, and CYP1B1*). Survival analyses showed that higher *NRF2* mRNA expression was associated with poorer overall survival (p=0.044 in adults, p=0.037 in pediatrics). Multivariate Cox regression analysis indicated that elevated *NRF2* expression (HR=1.49, 95% CI=1.10-2.12, p=0.05) and higher WBC counts (HR=1.55, 95% CI=1.06-2.25, p=0.02) were independently associated with poor survival outcomes.
Conclusion: This study underscores the role of *NRF2* in AML progression and its potential as a biomarker of poor prognosis. Targeting *NRF2* and its downstream pathways could provide a novel therapeutic approach to improve outcomes in AML patients.

#4668 Prognostic significance and therapeutic potential of TOP2A expression in rare cancers.

Jifa Li, hua Ye, xiaoqiong Bao, wenwen Yu, xiangxiang Chen, yangyang Ni, jincong Wang, yubo Shi

Yueqing People's Hospital, Yueqing, China

Background: TOP2A (Topoisomerase IIα) is a crucial nuclear enzyme involved in various processes of DNA metabolism, including replication, transcription, and chromosome segregation. Aberrant expression of TOP2A leads to chromosomal instability, increased cell proliferation and migration, and enhanced tumor invasiveness. Its high expression has been associated with poor prognosis in several common cancers, but its relationship with prognosis in rare cancers remains unclear. This study aims to investigate the expression, prognosis, and mechanisms of TOP2A in rare tumors using the TCGA (The Cancer Genome Atlas) bioinformatics database. The findings of this study will contribute to the understanding of targeted therapy and the molecular mechanisms underlying prognosis in these rare tumors.
Methods: We utilized the TCGA datasets, comprising 80 cases of ACC, 516 cases of LGG, 120 cases of THYM, and 259 cases of SARC, to investigate the expression levels of TOP2A. A comparison between normal and tumor tissues was conducted using the Mann-Whitney test. Based on the expression levels of TOP2A, the patients were categorized into two groups: high and low. The prognostic significance of TOP2A expression in these cancer types was evaluated using Kaplan-Meier Plotter. Furthermore, the correlation between TOP2A expression and BRCA1, BRCA2, and MMR genes was assessed using the Pearson correlation coefficient.
Results: Kaplan-Meier survival analysis revealed that in the overall population, the group with high expression of TOP2A exhibited significantly lower survival rates compared to the group with low expression ($p < 0.05$). This trend was consistently observed in ACC, LGG, and THYM cohorts. In the SARC cohort, when the threshold for TOP2A expression was set at 50%, there was no significant difference in survival between the two groups. However, a significant survival difference was observed between the top 25% and bottom 25% of TOP2A expression ($p = 0.022$). According to the Mann-Whitney analysis, the expression of TOP2A was significantly higher in these four cancer types compared to normal tissues. The correlation analysis between TOP2A expression and mismatch repair genes as well as homologous recombination repair genes revealed a strong association between TOP2A expression and BRCA1 and RAD51 (correlation coefficients of 0.59 and 0.8, respectively).
Conclusion: The elevated expression of TOP2A in rare cancers, along with its significant correlation with tumor progression and poor prognosis, suggests its potential as a promising therapeutic target and prognostic marker for these rare malignancies. These findings provide valuable insights and a theoretical foundation for further elucidating the mechanistic role of TOP2A in the pathogenesis of these cancer types.

#4669 Prognostic value of postoperative circulating tumor DNA in patients with resectable hepatocellular carcinoma.

Van-Anh Nguyen Hoang, Dao Nguyen Vinh, Giang Hoang Vu, Duy Sinh Nguyen, Hoa Giang, Hoai-Nghia Nguyen, Lan N. Tu

Medical Genetics Institute (Vietnam), Ho Chi Minh, Viet Nam

Background: Hepatocellular carcinoma (HCC) is endemic in Asia, particularly Vietnam due to the prevalence of hepatitis B virus infection. Liver resection is the first-line treatment but prognosis remains poor with a high relapse rate. Current methods including imaging and serum tumor biomarkers have suboptimal accuracy to monitor tumor burden and predict relapse.
Method: In this prospective study, sixty HCC patients who were eligible for curative-intent liver resection were recruited. Genomic DNA isolated from primary tumor tissues and paired white blood cells were subjected to targeted sequencing of 95 cancer-related genes to profile tumor-derived mutations. Top-ranked mutations specific to each individual were then used to track the presence of circulating tumor DNA (ctDNA) in serial plasma samples.
Results: The most frequently mutated genes in our cohort were *TP53* (42.4%), *TERT* promoter (39.0%), *CTNNB1* (23.7%), and *AXIN1* (22.0%). *TP53* R249S was the major hotspot found in 8.5% of the patients. Mutations in *TERT* promoter were significantly more prevalent in hepatitis C- than hepatitis B- infected patients, while *CTNNB1* was not mutated in those with no virus infection. On average, 4 mutations were selected to track ctDNA in plasm samples. Pre-operative detection rate of ctDNA was 96.6%, higher than AFP (57.6%). Post-operative ctDNA positivity, both at the landmark time point and during surveillance, was the strongest independent predictor of disease-free survival (HR: 17.9, 95% CI: 5.9 - 54.1, $p<0.001$). ctDNA detection could predict clinical relapse with sensitivity and specificity of 82.8% and 96.0% respectively, and the lead time of up to 7.3 months. This outperformed all serum tumor biomarkers: AFP, AFP-L3, and PIVKA-II.
Conclusion: Our simplified and cost-effective assay enabled longitudinal ctDNA monitoring for early relapse detection and predict disease-free survival in HCC patients. This study also provided the first mutation spectrum of Vietnamese HCC that could be helpful to uncover the multifactorial etiology of the disease.

#4670 The expression of HERV-K subfamilies indicated improved prognosis in small cell lung cancer.

Xingyuan Wu[1], Jingwei Zhang[2], Yan Ju[1], Xiao Li[2]

[1]Peking University Health Science Center, Beijing, China,[2]Thoracic Surgery, Peking University People's Hospital, Beijing, China

Background: The abnormal activation of human endogenous retroviruses (HERV) in tumor tissues has been proven implicated in cancer development and progression. Prior research demonstrated that antibodies against the HERV-K envelope protein could be produced by B cells in non-small cell lung cancer (NSCLC), enhancing immunotherapy efficacy. However, the role of HERV-K in small cell lung cancer (SCLC) remains largely unexplored.

Methods: Bulk RNA sequencing data from the IMpower-133 cohort (n = 271) was analyzed to detect the expression of HERV transcripts using the TELESCOPE algorithm. Single-cell RNA sequencing data from SCLC patients (n = 20) were employed for cell annotation. Carcinoma ecotypes (CEs) and cell-type proportions in SCLC were identified using EcoTyper, CIBERSORTx, and BayesPrism software. Immunohistochemistry (IHC) was performed to validate HERV-K expression in tumor samples collected at Peking University People's Hospital (n = 48). Kaplan-Meier survival analysis and multivariable Cox regression were used to evaluate clinical outcomes.

Results: By applying TELESCOPE in IMpower-133 dataset, we detected the expression of 468 HERV subfamilies and depicting the landscape of HERV subfamilies in SCLC. Survival analyses revealed that high expression of HML-2 (HERV-K), HERV-K11, and HERV-K13 subfamilies was associated with prolonged overall survival. Using CIBERSORTx and EcoTyper, bulk RNA sequencing data from IMpower-133 was deconvoluted into cell types, and five carcinoma ecotypes (CEs) were identified based on the co-occurrence of cell states. Notably, high expression of ERVK7, a locus-specific transcript of the HML-2 subfamily, was significantly correlated with higher abundance of CE1 across all samples (r = 0.43, p < 0.0001). CE1, the most prevalent ecotype in SCLC, was primarily composed of macrophages, CD38+ CD8+ T cells, CD4+ T cells, mature B cells, and plasma cells. CE1 abundance was associated with improved progression-free survival in SCLC patients receiving chemotherapy, as supported by multivariable Cox regression (HR = 0.32, CI: 0.13-0.77, p = 0.011). This finding was consistent with progression-free survival analysis based on ERVK7 expression (p = 0.036). The association between ERVK7 and macrophages was further validated through deconvolution of single-cell RNA sequencing data from SCLC using BayesPrism. Additionally, IHC of SCLC patient tumor samples confirmed HERV-K envelope protein (27/48) and ERVK7 (5/8) expression in tumor tissue. Higher HERV-K envelope protein expression was also associated with better disease-free survival, as supported by Kaplan-Meier analysis (p = 0.028).

Conclusions: The elevated expression of HERV-K indicated the better prognosis of SCLC patients, potentially due to the immune-hot tumor environment. These findings suggest that HERV-K may serve as potential biomarkers or co-stimulatory therapeutic targets for SCLC immunotherapy.

CLINICAL RESEARCH: Radiation Sciences 1
Poster Session

#4674 Pathological outcomes of short course vs. long course neoadjuvant radiation for stage II/III rectal cancer.

Alyssa Habermann, Patrick Chizek, Xiaoyan Deng, Dipankar Bandyopadhyay, Adam Khader, Stephen P. Sharp

Virginia Commonwealth University - VCU, Richmond, VA

Background: Total neoadjuvant therapy for rectal cancer has emerged as a favorable treatment plan. However, a definitive treatment algorithm has not been identified. Our study compares pathological response rates for neoadjuvant induction therapy regarding short and long course radiation across various demographic and disease-specific criteria.
Methods: Using the National Cancer Database (NCDB), data were collected for patients with clinical stage II and III rectal cancer who underwent neoadjuvant induction chemoradiation with either short or long course radiation from 2004-2021. Chi-squared test was used to compare the proportion of pathologic downstaging, pathologic compete response, positive surgical margin, and decreasing T and N stages individually for short course versus long course radiation. Logistic regression was used to perform multivariable analysis of factors impacting pathologic downstaging. Kaplan-Meier survival curves were used to compare overall survival between groups.
Results: A total of 1,470 patients underwent induction chemoradiation for stage II or III rectal cancer; 293 underwent short course radiation, while 1,177 underwent long course. Patients with stage II/III rectal cancer who underwent long course radiation were more likely to achieve pathologic downstaging (OR 1.83 [1.13, 2.97]) and decreasing T stage (OR 1.82 [1.14, 2.95]). On multivariate analysis, patients who received care at a community cancer program were less likely to achieve pathologic downstaging than those who received care at an academic program (OR 0.35 [0.14, 0.87]), patients with initial cN1 and cN2 tumors were more likely to achieve pathologic downstaging than those with cN0 tumors (OR 2.86 [1.94, 4.22] and OR 3.20 [1.80, 5.68], respectively), and patients who underwent long course radiation were more likely to achieve pathologic downstaging than those who underwent short course (OR 2.04 [1.19, 3.47]). Overall survival was improved for the long course group compared to the short course group (p=0.02). Median overall survival was 165 months for patients who underwent long course radiation and 104 months for those who underwent short course radiation.
Conclusion: Based on a retrospective review of NCDB data from 2004-2021, patients who underwent induction therapy with long course radiation for stage II/III rectal cancer were more likely to achieve pathologic downstaging than those who underwent short course radiation. Furthermore, overall survival was improved in patients who underwent long course radiation. This leads us to recommend long course over short course neoadjuvant radiation in induction therapy of patients with stage II/III rectal cancer. Given the extended median overall survival reported here, we call for long-term follow-ups from the randomized clinical trials reporting on long course vs short course radiation before the controversy can be settled.

#4675 Radiation therapy utilization and outcomes in primary and secondary central nervous system lymphoma.

Brianna Hostler[1], Jona A. Hattangadi-Gluth[2], Parag Sanghvi[2], Kathryn R. Tringale[2]

[1]Department of Psychology, San Diego State University, San Diego, CA,[2]Department of Radiation Medicine and Applied Sciences, University of California, San Diego, San Diego, CA

Background: Radiotherapy (RT) is an established consolidation strategy for primary CNS lymphoma (PCNSL). However, the role of RT in secondary CNSL (SCNSL) is unclear. Here, we describe RT doses, disease failure patterns, and survival outcomes in an RT-treated CNSL cohort.

Methods: Patients treated with CNS-directed RT for CNSL at UCSD were identified by ICD-10 codes, RT course description, or radiation oncologist. Site of CNS disease failure was local (involving initial site) or distant (outside of initial site). Overall survival (OS) and CNS progression-free survival (CNS PFS) were evaluated from time of RT start using Kaplan-Meier. Outcomes were stratified by PCNSL vs SCNSL.

Results: 30 patients (13 [43%] PCNSL, 17 [57%] SCNSL) and 39 total CNS-RT courses were included. Most received RT for relapsed/refractory disease (23, 77%) as opposed to definitive (6, 20%) or consolidative (1, 3%) intent. At the time of RT, 1 (3%) patient had CSF, 13 (43%) had ocular, and 12 (40%) had extracranial disease (6 progressive, 6 stable). Median RT dose was higher for PCNSL (40.5 Gy vs 30 Gy for SCNSL, p=.031). First course RT targets were whole-brain RT (WBRT; 17, 57%; 6 included orbits [3 electively]), orbits alone (10, 33%), focal (2, 7%), and spine (1, 3%).

17 patients relapsed, most only distantly (9 [53%] vs 5 [29%] locally). Only SCNSL patients had synchronous local and distant failures (3, 18%). 2 patients received focal RT to dura and did not progress. Of the 4 (36%) who relapsed after WBRT, 2 were local-only SCNSL failures, and 2 were distant-only PCNSL failures. Of the 4 (67%) who relapsed after WBRT + orbits, 3 failed in the parenchyma. Most of the 8 who relapsed after orbits RT alone had distant (5, 63%) failures, most (3, 60%) parenchymal. Those with ocular involvement received WBRT + orbits or orbital RT alone, most relapsing in the parenchyma (8, 62%). No ocular failures occurred in those without ocular disease at RT start.

Median OS was longer for PCNSL vs SCNSL (18.6 months [95%CI 0.0-42.4] vs 6 months [95%CI 3.0-8.9]), respectively; log-rank p=.058).

Median CNS PFS was similar between PCNSL vs SCNSL (2.83 months [95%CI 0.0-19.8] vs 4.41 months [95%CI 1.4-7.4], respectively; log-rank p=.256). 3 patients had systemic relapse preceding CNS relapse.

Conclusions: Different disease failure patterns were seen in mostly relapsed/refractory SCNSL vs PCNSL receiving CNS-RT. Only SCNSL patients failed both distantly and locally, suggesting the inclusion of more comprehensive treatments alongside RT (e.g., systemic or intrathecal). As SCNSL received a lower RT dose and most patients suffering local failure were SCNSL, further dose optimization should be considered. Future studies will examine MRI predictors of disease failure patterns.

#4676 MRI-based radiomics ensemble model for predicting radiation necrosis in brain metastasis patients treated with stereotactic radiosurgery and immunotherapy.

Yijun Chen[1], Corbin A. Helis[1], Christina K. Cramer[1], Michael T. Munley[1], Fei Xing[2], Qing Lyu[3], Christopher T. Whitlow[3], Jeffrey Willey[1], Michael D. Chan[1], **Yuming Jiang**[1]

[1]Radiation Oncology, Wake Forest University School of Medicine, Winston Salem, NC,[2]Wake Forest University School of Medicine, Winston Salem, NC,[3]Radiology, Wake Forest University School of Medicine, Winston Salem, NC

This study aims to apply an ensemble model integrating MRI-based radiomics and clinical information as a reliable tool for precisely predicting radiation necrosis, a severe consequence of radiation therapy for brain metastases that requires accurate early prediction to improve patient management. We retrospectively collected and analyzed MRI images and clinical information from 209 stereotactic radiosurgery sessions involving 130 patients with brain metastasis. Radiomic features were extracted from MRI using PyRadiomics and selected via L2 regularization and coefficient analysis. An ensemble model integrating gradient boosting, random forest, decision tree, and support vector machine as base regressors was developed using a soft voting approach to generate the final prediction of the likelihood of necrosis. Performance was assessed and compared with other machine-learning algorithms using metrics including the area under the curve (AUC), sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV). SHapley Additive exPlanations (SHAP) and local interpretable model-agnostic explanations (LIME) analyses were applied to explain the model's prediction. The soft-voting ensemble model demonstrated strong performance in the validation cohort, achieving the highest AUC of 0.873 (95%CI: 0.672 - 1.000). It consistently outperformed individual models and the stacking ensemble model, exhibiting superior accuracy, generalizability, and reliability in predicting radiation necrosis. Both univariate and multivariate logistic regression analyses were performed and confirmed the model as the strongest predictor. SHAP and LIME analyses were employed to interpret the predictive model for radiation necrosis, identifying metastasis volume and the radiomic feature, log-sigma-1-mm_glcd_ldmn, as key predictors. Both analyses highlighted similar significant factors, enhancing the understanding of prediction dynamics. This MRI-based radiomics ensemble model exhibited high accuracy and robustness in predicting radiation necrosis. It has the potential to serve as a novel and valuable tool to facilitate radiotherapy for patients with brain metastasis.

#4677 Combating radioresistance in lung cancer: S6K1 signaling as a therapeutic target through metabolic modulation.

Ali Jose Calderon-Aparicio, Nicole L. Simone

Radiation Oncology, Thomas Jefferson University, Philadelphia, PA

Background: Locally advanced non-small cell lung cancer (NSCLC) is treated with a combination of chemotherapy, immunotherapy, and radiation but is unfortunately still associated with poor cancer survival. Improving radiation efficacy is essential to enhance tumor control and reduce resistance, ultimately improving outcomes for patients. Metabolic reprogramming could play a key role in this by targeting cancer cell metabolism to increase sensitivity to radiation and reduce tumor growth. S6K1 has emerged as a metabolic regulator that promotes chemo and radio-resistance in several cancers. However, the associated mechanisms to this phenomenon are unclear. We sought to determine if pharmacological or metabolic inhibition of S6K1 modulates radioresistance in lung cancer.
Methods/Results: The intrinsic radiosensitivity of cells lines originating from NSCLC was determined by clonogenic and proliferation assays. Four cancer cell lines of varying intrinsic radiation sensitivity were chosen as a model to investigate the role of S6K1. The A549 cells were the most resistant followed by H661, H226 and the most sensitive as H23. The phospho-activation of S6K1 and its downstream target S6 increased in more resistant cells, associating S6K1 activation with radioresistance. TCGA analysis showed that S6K1 is overexpressed in lung cancer patients compared to healthy controls ($p<0.001$). Inhibition of S6K1 by pharmacologic means using a highly specific inhibitor (PF-4708671), or by genetic deletion of S6K1 by Crispr-Cas9, in combination with radiation, significantly decreased the colony formation compared with controls. Using *in vivo* murine models, $S6K1^{-/-}$ tumors had notably decreased tumor progression than the $S6K1^{+/+}$ tumors ($p<0.001$). To determine the role of metabolic reprogramming on S6K1 activation and its effects on radioresistance, *in vitro* models of caloric restriction were tested and demonstrated a decreased S6K1 activation, and radiosensitivity trough reduction of transcriptional activation of STAT3, a target involved in radio-resistance. In NSCLC murine models, caloric restriction significatively decreased the tumor burden in mice.
Conclusion: Our findings demonstrate that targeting S6K1, through pharmacological inhibition or metabolic interventions like caloric restriction, significantly enhances the therapeutic efficacy of radiotherapy in NSCLC. Studies are underway to elucidate the detailed mechanisms linking S6K1 activity and caloric restriction to radiosensitivity, with the potential to inform novel therapeutic strategies for improving outcomes in lung cancer patients.

#4678 Identifying and exploiting a novel role of FTO in promoting cysteine metabolism for NSCLC therapy.

N. Kuganesan, S. Melemenidis, E. E. Graves, E. B. Rankin;
Stanford University, Stanford, CA

The RNA demethylase enzyme FTO is emerging as an important oncogenic factor and therapeutic target in a variety of solid tumors. However, its effect on cancer metabolism remains largely unknown. Here we show that FTO promotes cysteine metabolism, a hallmark of metabolic rewiring in non-small cell lung cancer (NSCLC) cells. Mechanistically, FTO inhibition reduces the expression of multiple targets in the cysteine metabolism pathway including the cystine transporter SLC7A11 involved in exogenous cystine uptake as well as two key enzymes cystathionine β-synthetase (CBS) and cystathionine γ-lyase (CTH) involved in de novo cysteine synthesis. Functionally, FTO inhibition reduces cystine uptake and NSCLC growth and survival in a homocysteine-dependent manner. As cysteine plays a crucial role in glutathione biosynthesis and maintenance of cellular redox homeostasis, FTO inhibition decreased glutathione levels and increased reactive oxygen species (ROS) levels in NSCLC cells. To therapeutically exploit the increased oxidative stress in NSCLC cells treated with FTO inhibitors, we combined FTO inhibition with radiation therapy. FTO inhibition enhanced the radiosensitivity of NSCLC cells. Our findings reveal a novel role for FTO in NSCLC cysteine metabolism and oxidative stress, highlighting the therapeutic potential of FTO inhibition as a strategy to enhance the efficacy of radiation therapy for the treatment of NSCLC.

#4679 FTO inhibition enhances the therapeutic index of radiation therapy in head and neck cancer.

L. Ji[1], L. Pu[1], H. Cao[1], S. Melemenidis[2], S. Sinha[2], L. Guan[1], E. E. Laseinde[1], S. Richter[1], R. Eyben[2], K. Casey[2], C. Kong[1], E. Graves[2], Q. Le[2], E. Rankin[1];

[1]Stanford University School of Medicine, Stanford, CA, [2]Stanford University, Stanford, CA

Despite the use of intensive combined modality therapy such as chemoradiation or surgery and radiation, the overall survival rate for patients with HPV-negative head and neck squamous cell carcinoma (HNSCC) remains poor. This underscores the unmet clinical need for the development of more effective drug-radiotherapy combinations to enhance the therapeutic index of radiation therapy (RT). The RNA demethylase enzyme FTO has emerged as a promising target for cancer therapy. However, its role as a radiosensitizer in HNSCC remains unknown. Here, we investigated the potential of targeting FTO to augment the effectiveness of RT in HPV-negative HNSCC. We found that genetic and pharmacologic inhibition of FTO enhanced the efficacy of RT in both human and mouse HNSCC tumor xenografts. Importantly, FTO inhibition did not increase radiation-induced oral mucositis. Mechanistically, FTO inhibition radiosensitizes HPV-negative HNSCC cells through amplifying DNA damage while reducing RAD51 foci formation, providing a molecular rationale for exploiting FTO inhibitors as radiation sensitizers in HNSCC. Collectively, our results suggest that therapeutic targeting of FTO may be an effective strategy to enhance the therapeutic index of RT for the treatment of HNSCC.

#4680 Simultaneous DDR1/FGFR inhibition elicits adaptive EGFR/MAPK pathway activation to counteract susceptibility to radiochemotherapy in preclinical glioblastoma models.

Anne Vehlow, Irina Korovina, Olegs Borodins, **Nils Cordes**

OncoRay, Dresden, Germany

GBM cell survival critically depends on cell adhesion molecules like discoidin domain receptor 1 (DDR1) and receptor tyrosine kinases like fibroblast growth factor receptors (FGFR), both of which represent potential therapeutic vulnerabilities. Based on the radiochemosensitizing potential of DDR1 mono-inhibition, we investigated the adaptive response induced by DDR1 deactivation using broad-spectrum kinome analyses. Since FGFR were identified most hyperactive, we examined the efficacy of simultaneous pharmacological DDR1/FGFR inhibition and found enhanced cytotoxicity and radiochemosensitization in a human gliomasphere panel. Subsequent evaluation of DDR1 and FGFR single and dual inhibition in orthotopic, radiochemotherapy-treated GBM mouse models revealed a profound reduction in efficacy for dual as opposed to single inhibition. This was mechanistically underpinned through RNA-seq analyses uncovering strong activation of EGFR/MAPK signaling under DDR1/FGFR deactivation relative to monotherapies. In conclusion, our data evidently show that particularly DDR1 monotherapy effectively augments radiochemotherapy in GBM, while simultaneous DDR1/FGFR inhibition requires more in-depth investigation.

#4681 Multiomic insights into mechanisms of androgen receptor-driven radioresistance in triple-negative breast cancer.

Benjamin Hauk[1], Breanna McBean[2], Anna Michmerhuizen[2], Douglas Gurdak[1], Savannah Tidmore[1], Michelle Paulsen[2], Lynn Lerner[2], Connor Ward[2], Kassidy Jungles[2], Daniel Spratt[1], Lori Pierce[2], Mats Ljungman[2], Corey Speers[1]

[1]Radiation Oncology, Case Western Reserve University School of Medicine, Cleveland, OH,[2]Radiation Oncology, University of Michigan, Ann Arbor, MI

Background: Due to the lack of molecular-targeted therapies for triple-negative breast cancer (TNBC), there have been efforts to use multimodal strategies to enhance the efficacy of existing therapies such as radiation. The androgen receptor (AR) has been identified as a radiosensitizing target and is expressed in 25-50% of TNBC tumors. The combination of AR inhibitors, such as enzalutamide (enza), and radiation therapy (RT), leads to radiosensitization in AR+ TNBC models but the mechanism by which AR mediates radioresistance is not known, and targeting this radioresistance could provide a novel strategy to improve outcomes in this aggressive breast cancer subtype. Following RT, AR is translocated to the nucleus suggesting that AR may drive transcriptional changes in response to RT.
Methods: Bulk RNA-seq with and without R1881 stimulation and RT was used to assess transcriptional changes at 24 hours. RPPA analysis evaluated protein and phophoprotein changes. AR ChIP-seq was used to identify changes in AR binding and Bru-seq to profile changes in nascent RNA. These experiments were performed in two AR+ TNBC models, MDA-MB-453 and MFM-223 cells, with and without enza and RT. Clonogenic survival assays were performed to assess for radiosensitivity.
Results: RNA-seq and RPPA analysis demonstrated numerous changes in gene and protein expression after enza-, RT-, and combination treatment. Gene set enrichment analysis (GSEA) of these changes nominated alterations of MAPK pathway genes with R1881 and R1881+RT compared to controls. To assess the MAPK/ERK pathway, constitutively active ERK was overexpressed in AR+ TNBC models which induced radioresistance, confirming this relationship. As expected, AR ChIP-seq data showed enza treatment induced a significant reduction in AR peaks compared to DMSO-treated controls. Radiation induced fewer changes in AR binding than enza treatment. Bru-seq GSEA showed significant upregulation of MYC target gene sets in response to RT in control conditions. Conversely, with enza treatment, there was no significant MYC target gene set upregulation. While there were no changes in MYC expression or AR binding to known MYC enhancers, there was increased binding of AR to known MYC binding sites in control samples in response to RT. This RT-induced binding to MYC sites was not seen in the enza-treated samples suggesting that AR inhibition may dampen the RT induction of MYC signaling leading to radiosensitization.
Conclusions: These findings provide new insights into the molecular underpinnings of AR-mediated radioresistance and identify MAPK and MYC pathways as potential therapeutic targets to enhance radiosensitivity in AR+ TNBC. Ongoing studies aim to translate these findings into clinically actionable strategies. This work expands our understanding of AR-mediated radioresistance which may uncover resistance mechanisms to combination AR inhibition and RT.

#4682 Elucidating the mechanisms of 2-deoxy-D-glucose (2-DG) and 2-deoxy-D-glucose phoaphate (2-DG-P) in maintaining gastrointestinal homeostasis.

Rao V. Papineni[1], Shahid Umar[2], Prashanthi Karyala[3], Kavya Ramesh[3], Badal Roy[2]

[1]University of Kansas Medical Center, Branford, CT,[2]University of Kansas Medical Center, Kansas, KS,[3]MS Ramaiah University, Bangalore, India

2-Deoxy-D-glucose (2-DG), a polypharmacological agent, has shown promise in treating various pathological conditions, including cancer, bacterial and viral infections, and as an adjuvant to radio-chemo-therapy. 2-DG exerts its effects by inhibiting glycolysis, disrupting protein N-glycosylation, inducing endoplasmic reticulum stress, autophagy, and apoptosis. Our recent studies demonstrated that 2-DG mitigates bacterial infection-induced inflammation and colitis, and proposed its potential as a radiation countermeasure agent. Utilizing a colonoid model, we investigated the mechanisms underlying 2-DG's maintenance of gastrointestinal homeostasis. Specifically, we explored its interaction with Doublecortin-like kinase 1 (DCLK1), a protein with two major isoforms, DCLK1-L and DCLK1-S.Here, 2-DG-Phosphate (2-DG-P), the non-metabolizable product of 2-DG, is also implicated in this process. Combining in silico molecular docking of 2-DG and 2-DG-P with DCLK1, we propose that 2-DG-P, along with 2-DG, may serve as potential candidates for radioprotection and mitigation of irradiation-induced gastrointestinal acute radiation syndrome (GI-ARS).

#4684 An in vivo comparison of ^{137}Cs and orthovoltage irradiation for murine bone marrow transplantation.

Danielle M. Burgenske, Brett L. Carlson, Matthew J. Rajcula, Michael J. Shapiro, Virginia M. Shapiro, Jann N. Sarkaria

Mayo Clinic, Rochester, MN

Total body irradiation for bone marrow transplantation (BMT) conditioning is commonly used in immunologic research. Historically, cesium-137 (^{137}Cs) irradiators were predominantly used as a radiation source for these studies. However, ^{137}Cs has a moderate half-life (30 years), is highly water soluble, and poses significant environmental and radiological safety concerns. As a result, the Department of Energy is promoting the replacement of ^{137}Cs irradiation systems with orthovoltage x-ray cabinet systems, which pose no such concerns. Because the physical characteristics of x-ray and ^{137}Cs photon beams have slightly different energy deposition characteristics, the relatively biological effectiveness (RBE) for x-rays is slightly higher than ^{137}Cs in cell survival studies. This study aims to compare the RBE of orthovoltage and ^{137}Cs irradiators with regards to BMT efficiency. Total body irradiation was performed on C57BL6 mice using ^{137}Cs or 320 kVp x-rays across six dose levels to establish a RBE for lethality. Cohorts of five mice were irradiated using split-dose radiation delivered four hours apart. Daily observations and body weights were captured over a 30-day period. Mice with poor body condition scores or weight loss ≥20% were euthanized. With x-rays, 50% lethality was observed ~750 cGy while ^{137}Cs required ~850 cGy, which results in an RBE of 750 cGy/850 cGy = 0.88. Bone marrow ablation and subsequent engraftment efficacy was compared in a parallel study. Cohorts of five C57BL6 CD45.1 recipient mice received total body irradiation with an established single dose 1000 cGy ^{137}Cs regimen or a range of x-ray doses (910-1030 cGy single-dose) and then were infused with CD45.2 donor bone marrow. Ten weeks after BMT, bone marrow, spleen, thymus, and peripheral blood were analyzed via multi-color flow cytometry and the percentage of CD45.1 and CD45.2-positive cells were analyzed to determine marrow engraftment efficiency across various immune compartments. A clear dose-response relationship with bone marrow ablation and engraftment was observed in the stem cell and progenitor compartments. LSK (Lineage$^-$ Sca-1$^+$ c-Kit$^+$) cells and other hematopoietic progenitors in the bone marrow were efficiently engrafted (>99%) except for the lowest dose of 910 cGy (around 98%). Similarly, in the spleen, B cells, myeloid, megakaryocyte/erythrocyte, and granulocyte/macrophage lineages were also efficiently engrafted similar to that of ^{137}Cs. Developing thymocytes were efficiently replaced by all radiation regimens, while replacement of peripheral T cell populations varied but were similar to traditional ^{137}Cs at the highest doses. Taken together, these data demonstrate comparable BMT efficiencies with orthovoltage irradiation after appropriate dose-optimization.

#4685 Targeting fatty acid oxidation improves radiation sensitivity in glioblastoma.

Tingting Huang[1], Kenneth Austin[1], Todd W. Miller[2], **Kelli B. Pointer**[1]

[1]Dartmouth Cancer Center, Lebanon, NH,[2]Medical College of Wisconsin, Milwaukee, WI

Background: Glioblastoma (GBM) is the most aggressive primary brain tumor, with a median survival of less than two years. A significant barrier to effective treatment is GBM's resistance to standard therapies, including radiation. This resistance is partially attributed to glioblastoma cancer stem cells (GSCs), a subpopulation with self-renewal capacity and the ability to regenerate all tumor components. Alterations in metabolic pathways, including fatty acid metabolism, are critical to GSC-mediated resistance. While fatty acid metabolism is known to be upregulated in GBM, its specific role in radiation-resistant GSCs remains poorly understood. Here, we investigated the impact of fatty acid metabolism inhibition on GSC radiation sensitivity.

Methods: Two human GBM cell lines were treated for 72 hours with perhexiline, a carnitine acyltransferase 1a/b inhibitor, or vehicle control. The IC25 was determined using the SRB assay and validated with cell counts. Cells were subsequently treated with the IC25 concentration of perhexiline and irradiated with a single dose of either 0 Gy or 8 Gy. Sphere formation assays and immunoblotting for stemness markers were performed. All experiments were conducted in triplicate for statistical rigor.

Results: The IC25 for perhexiline was determined to be 3 μM. Sphere formation assays using 3μM perhexiline combined with radiation revealed a significant reduction in both sphere number ($p < 0.0001$) and sphere size (350 μm vs. 125 μm). Immunoblotting demonstrated a decrease in stemness markers CD44 and nestin following drug treatment compared to vehicle control.

Conclusion: These findings suggest that perhexiline, an FDA-approved drug, enhances radiation sensitivity in GBM by targeting GSCs. The observed reduction in sphere formation and stemness marker expression highlights its potential as a GSC-directed therapy. Further studies are warranted to explore the therapeutic potential of perhexiline in overcoming radiation resistance and improving outcomes for GBM patients.

#4686 Efficacy and safety of efbemalenograstim alfa as primary prophylaxisfor concurrent chemo-radiotherapy induced neutropenia.

Yuanyuan Chen[1], Qiwen Li[1], Zunbei Wen[2], Yi Ouyang[1], Shuzhen Lai[3], Rong Huang[4], Minying Li[5], Xuefang Zhang[6], Yongling Ji[7], Chen Wang[8], Ming Chen[1]

[1]State Key Laboratory of Oncology in South China, Guangdong Key Laboratory of Nasopharyngeal Carcinoma Diagnosis and Therapy, Guangdong Provincial Clinical Research Center for Cancer, Sun Yat-sen University Cancer Center, Guangzhou, China,[2]Gaozhou People's Hospital Cancer Center, Gaozhou, China,[3]Department of Radiation Oncology, Yue Bei People's Hospital, Shaoguan, China,[4]The First People's Hospital of Foshan, Foshan, China,[5]Department of Oncology, Zhongshan People's Hospital, Zhongshan, China,[6]Department of Radiotherapy, Dongguan People's Hospital, Dongguan, China,[7]Zhejiang Key Laboratory of Radiation Oncology, Hangzhou, China,[8]Department of Oncology, Ganzhou People's Hospital, Ganzhou, China

Background: Concurrent chemo-radiotherapy (CCRT) occupies a pivotal position in the therapeutic management of various malignant tumors and frequently causes significant hematologic toxicity, particularly leukopenia and neutropenia. Nonetheless, granulocyte-macrophage colony-stimulating factor (GM-CSF) is not recommended for preventing CCRT, while granulocyte colony-stimulating factor (G-CSF) is seldom investigated in this setting. This study aims to evaluate the effectiveness and safety of efbemalenograstim alfa, a novel long-acting G-CSF, as a primary prophylaxis during CCRT.

Method: This multicenter, open-label, randomized controlled trial will enroll 120 patients who are scheduled to undergo definitive CCRT for malignancies, including non-small cell lung cancer, small cell lung cancer, esophageal cancer, nasopharyngeal carcinoma, head and neck squamous cell carcinoma, or cervical cancer. Patients will be randomized 1:1 to study group or control group. In the study group, patients will receive efbemalenograstim alfa approximately 48 hours post-chemotherapy. In the control group, patients will receive rhG-CSF upon an absolute neutrophil count (ANC) drop below 1.0×10^9/L. Patients who developed febrile neutropenia (FN) or dose-limiting neutropenic event in the control group will receive efbemalenograstim alfa in the subsequent chemotherapy cycles. The primary endpoint is the incidence of 3/4 grade neutropenia (ANC < 1.0×10^9/L) throughout the CCRT period.

Result: Efbemalenograstim alfa administration significantly reduced the incidence of grade 3/4 neutropenia during the CCRT period. Four patients (12.90%) in the efbemalenograstim alfa group experienced grade 3/4 neutropenia, compared to 14 (45.16%) in the control group, resulting in a difference of -32.26% (90% CI: -49.20%, -13.66%, p= 0.0035). The incidence of grade 3/4 neutropenia was 9.68% (study group) vs 45.16% (control group) (p=0.0009) across first chemotherapy cycle, and 3.23% (study group) vs 16.13% (control group) (p=0.0881) across second cycle. Efbemalenograstim alfa administration also resulted in shorter duration of grade 3/4 neutropenia, with a median duration of 0 day (IQR: 0-0) in study group and 0 day (IQR: 0-5) in control group (p=0.008). The depth of ANC nadir is higher in study group than that in control group (3.19 (0.26-8.45) $\times 10^9$/L vs 1.00 (0.06-4.43) $\times 10^9$/L, p < 0.001), the mean difference was 2.10 (1.39, 2.71) $\times 10^9$/L. Efbemalenograstim alfa was well-tolerated, as no≥3 grade treatment-related adverse event (TRAE) was reported in both group. The most common treatment-emergent AEs in study group included thrombocytopenia (48.39%), anemia (45.16%) and hypokalemia (35.48%).

Conclusion: Primary prophylaxis with efbemalenograstim alfa provide effective neutrophil support to patients undergoing CCRT, with good safety.

#4687 Clonal hematopoiesis of indeterminate potential may protect against radiation-induced toxicities in breast cancer patients.

Tirthankar Ray[1], Madison Hossack[2], Pierre-Antoine Bissey[1], Justin Williams[1], Wei Shi[1], Sagi Abelson[3], Steven Chan[4], Kenneth W. Yip[5], Fei-Fei Liu[6]

[1]University Health Network, Toronto, ON, Canada,[2]Institute of Medical Science, University of Toronto, Toronto, ON, Canada,[3]Ontario Institute for Cancer Research, Toronto, ON, Canada,[4]Medical Biophysics, University of Toronto, Toronto, ON, Canada,[5]Cell and Systems Biology, University of Toronto, Toronto, ON, Canada,[6]Radiation Oncology, University of Toronto, Toronto, ON, Canada

Introduction: Radiation therapy (RT) is administered to ~50% of all cancer patients yet can lead to toxicities such as radiation-induced fibrosis and/or lymphedema. Detailed understanding of the underlying pathogenesis of these post-RT complications remains to be elucidated. One potential factor might relate to clonal hematopoiesis of indeterminate potential (CHIP); an age-related clonal expansion of hematopoietic stem cells (HSCs) harboring characteristic somatic mutations (e.g., *Tet2*). CHIP is implicated in cardiovascular disease and leukemia progression, as well as several chronic fibrosis states. The current hypothesis is that breast cancer patients with CHIP at the time of radiation therapy (RT) are at higher risk of radiation-induced toxicities. To investigate this hypothesis, we utilized patient samples and mouse models with Tet2 mutations.

Methods: Blood samples were collected from breast cancer patients undergoing adjuvant RT prior to treatment. DNA was extracted, and CHIP mutations were identified using next-generation sequencing. We then examined the association between CHIP status and clinical data related to radiation-induced fibrosis and/or lymphedema. In addition, a CHIP mouse model was generated by transplanting myelo-ablated mice with HSCs heterozygous for *Tet2* null mutations. Mouse hindlimbs were irradiated at 20 Gy to induce fibrosis, evaluated through joint angle contracture (measured weekly), dermal thickness, and collagen. Smaller joint angles indicated increasing contracture/fibrosis. At 16 weeks post-RT, skin samples were collected to assay dermal thickness and collagen deposition.

Results: Amongst 138 breast cancer patients, 28 had CHIP mutations at baseline, of which only 1 patient developed radiation-induced fibrosis/lymphedema. Amongst the remaining 110 patients without CHIP, 24 developed fibrosis/lymphedema, demonstrating a significant association between toxicity outcomes with no-CHIP patients (Fisher's exact test, $p<0.05$). This was reflected in the *in vivo* model, wherein CHIP-positive mice (carrying *Tet2* mutant HSCs, n=9) exhibited reduced fibrosis, thinner skin (27% decrease), and less collagen (51% decrease) post-RT, compared to control mice with wild-type *Tet2* HSCs (n=9).

Conclusion: Our findings disproved the null hypothesis and unexpectedly demonstrated that breast cancer patients with CHIP displayed a lower risk of fibrosis/lymphedema compared to patients without CHIP. These findings need to be validated in other clinical cohorts and additional pre-clinical models to further understand the apparent protective effects of CHIP with development of radiation fibrosis.

#4688 Focused ultrasound for the treatment of osteosarcoma in a canine comparative oncology model: Findings from veterinary clinical trials.

Elliana Vickers[1], Alayna Hay[2], Lauren Ruger[3], Ny Luong[4], Sheryl Coutermarsh-Ott[5], Gregory Daniel[6], Steven Soliman[7], Gunjan Malhotra[7], Timothy Ziemlewicz[8], Adam Maxwell[3], Eli Vlaisavljevich[3], Joanne Tuohy[6]

[1]Virginia Tech, Blacksburg, VA,[2]Animal Cancer Care and Research Center, Virginia-Maryland College of Veterinary Medicine, Roanoke, VA,[3]Department of Biomedical Engineering and Mechanics, Virginia Tech, Blacksburg, VA,[4]Department of Biomedical and Veterinary Sciences, Virginia Tech, Blacksburg, VA,[5]Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA,[6]Department of Small Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA,[7]Department of Radiology, University of Michigan/Michigan Medicine, Ann Arbor, MI,[8]Department of Radiology, University of Wisconsin-Madison, Madison, WI

Osteosarcoma (OS) is the most common primary bone tumor in dogs and humans, and the similarities between human and canine OS make the dog a valuable comparative oncology model. Histotripsy, a non-invasive ablation modality using pulsed focused ultrasound to mechanically disintegrate tissue, shows promise for the treatment of bone tumors. Previous canine clinical trials have shown the safety and feasibility of ablating canine OS tumors, followed by limb amputation. The present study is the first use of histotripsy without standard-of-care tumor resection in OS, allowing for evaluation of long-term clinical outcomes. Pet dogs with spontaneous bone tumors were assessed at baseline and over time with MRI, gait analysis, pain & quality of life surveys, and lameness assessments. Histotripsy treatments were fractionated (separate treatments applied on different days) with a plan to ablate >50% of the tumor in 1-5 treatments. Additional treatments were applied if residual tumor was remaining or if untreated portions of tumor continued to grow on contrast-enhanced T1-weighted MRI. Histotripsy was delivered by a 700 kHz transducer designed for targeting musculoskeletal tumors, with coaxial ultrasound imaging to guide treatment planning and execution. 9 dogs with primary bone tumors were treated, and 6 dogs received long-term follow-up. Dogs generally tolerated histotripsy treatment well and were able to return home within 1-3 hours post-treatment. The maximum volume targeted in a single session was 29.2 cm^3. The mean total ablation volume was 35.6 cm^3, which equated to a mean percent ablation of 50.2% when compared to the pre-treatment tumor size on MRI. For each treatment, an area closely matching the targeted region was observed as a non-enhancing area on MRI. Whole tumor signal intensity as well as enhancing tumor volume decreased immediately after treatment but tended to return to baseline or increase by 6 weeks after the initial treatment. Considering clinical outcomes, 4/6 dogs with follow-up (all patients with >50% ablation coverage of their tumor) showed a clinically significant reduction in pain at the end of their follow-up period (mean 8 weeks) as compared to baseline. Across those 6 dogs, there was a significant increase in peak pressure on the tumor-bearing limb at the end of follow-up as compared to baseline. these results highlight the promise of histotripsy as a non-invasive limb salvage treatment for OS. This work also shows the potential of fractionated histotripsy treatments for the ablation of large tumors, which may expand the patient populations that can be treated with histotripsy. Ongoing studies are optimizing treatment strategies to achieve safe, rapid, and complete ablation of OS, including combination with immunotherapy.

#4689 Poly ADP ribose polymerase inhibitors potentiate proton therapy end-of-range relative biological effective dose by increasing replication stress.

Yiqun Han[1], Qin Zhou[1], Anna Olson[1], Cameron M. Callaghan[1], Bin Chen[1], Nicholas B. Remmes[1], Meng Xu Welliver[1], Zheming Wu[1], Daniel K. Ebner[1], Jake A. Kloeber[2], Zhenkun Lou[2], Robert W. Mutter[1]

[1]Radiation Oncology, Mayo Clinic, Rochester, MN,[2]Oncology, Mayo Clinic, Rochester, MN

Poly ADP ribose polymerase inhibitors (PARPi) are being investigated in combination with photon and proton radiotherapy in clinical trials of pediatric and adult malignancies. The precise mechanisms of PARPi radiosensitization, and whether differences in photon and proton-mediated relative efficacy exist, have not been fully elucidated. To study mechanisms of PARPi radiosensitization we investigated the effects of PARPi in combination with photons and two different ionizing densities of protons delivered at the entrance (ENT) or the Bragg peak (BP) of the profile (dose-averaged LET [LETd] 2.2 and 7.0 keV/µm, respectively) of a 76.8 MeV proton beam after observing an extreme tumor and normal tissue response in an 18-year-old female patient with high grade glioma treated veliparib and proton therapy in a Children's Oncology Group clinical trial. BRCA1/2 wild-type hTERT RPE-1, U2OS, and MDA-MB-231 cells were hypersensitive to the combination of veliparib and proton BP, with enhanced relative biological effectiveness (RBE) at 2 Gy values of 1.7, 1.4, and 1.3, respectively. Similar findings were observed with talazoparib and olaparib, suggesting that PARP trapping efficiency was not a major influence on the proton BP RBE. Further, we did not observe differences in transcription replication conflict between the different proton ionizing densities. Unexpectedly, DNA fiber assays revealed more elongated replication fork tracks in PARPi treated cells 60 minutes after proton BP compared with proton ENT, indicative of accelerated replication fork progression. To assess gap formation in the vicinity of nascent DNA we treated cells with the ssDNA-specific S1 nuclease and found that the lengths of CldU-labeled tracks were significantly reduced in cells treated with the PARPi/proton BP combination, suggestive of greater gap formation at replication forks. We postulated that more ssDNA gaps were being converted to DSBs during replication as we further observed that PARPi/proton BP resulted in increased γH_2AX foci at 4- and 24-hours after treatment. Consistent with increased DSB formation, a higher proportion of cells arrested in the G2/M phase of the cell cycle at 24 hours following PARPi/proton BP. Despite being arrested in G2/M when homologous recombination is active, there was a significant increase in 53BP1 and a decrease in RAD51 recruitment, indicative of upregulated repair by more error-prone non-homologous end-joining (NHEJ) repair pathway. The enhanced proton BP RBE in PARPi treated cells was abrogated upon knockdown of *XRCC4*, a critical component of NHEJ repair pathway, suggesting that toxic NHEJ may be contributing to the enhanced proton BP RBE. In conclusion, PARPi renders cells hypersensitive to high LET BP proton irradiation. Enhancing the LET in combination with PARPi warrants further investigation to overcome therapeutic resistance.

#4690 Radio-sensitizing activity in apoptosis pathway in HPV+ and HPV- head and neck squamous cell carcinoma (HNSCC).

Loren D. Lopez Rivera[1], Elizabeth Boeree[2], Kwang P. Nickel[1], Zafer Gurel[1], Randall J. Kimple[1]

[1]Human Oncology, University of Wisconsin-Madison, Madison, WI,[2]Cancer Biology, University of Wisconsin-Madison, Madison, WI

Head and Neck Squamous Cell Carcinoma (HNSCC) is the sixth most common cancer worldwide, presenting as two distinct diseases: HPV-positive HNSCC driven by high-risk human papillomavirus (HPV) infections; and HPV-negative HNSCC associated with heavy tobacco and alcohol use. Poor prognosis in HNSCC is partly attributed to therapy resistance, which differs between the two subtypes and is influenced by their underlying mechanism. Accumulating evidence points to dysregulation of the apoptotic pathway in HNSCC tumors via overexpression of Inhibitor of Apoptosis Proteins (IAPs). This results in evasion of cell death and makes these proteins critical therapeutic targets. Second mitochondria-derived activator of caspases (SMAC) mimetics, or IAP antagonists, are being explored as anti-cancer agents, especially in combination with radiation therapy. This study evaluates the efficacy of the IAP inhibitor AT406, alone and with radiation, in both HPV positive (UM-SCC47 and UD-SCC2) and HPV-negative (Cal27 and DET562) HNSCC cell lines. Preliminary findings suggest AT406 reduces cell viability in both subtypes, with HPV-positive cells exhibiting greater sensitivity, particularly in combination with radiation. On the other hand, HPV-negative cells show less pronounced reductions in cell viability, suggesting subtype-specific resistance mechanism. This trend underscores the potential of AT406 as a radio-sensitizing agent and highlight differences in therapeutic response between HPV-positive and HPV-negative HNSCC. Ongoing studies aim to confirm these findings and elucidate the molecular mechanism driving these differential responses. Apoptotic activity will be assessed through Caspase-3/7 activity, and expression of IAP proteins (cIAP1, cIAP2 and XIAP) will be evaluated using western blotting. In addition, Liquid Chromatography- Mass Spectrometry (LC-MS/MS) is being utilized to profile changes in protein expression under different treatment conditions to identify molecular contributors to resistance mechanisms. These studies aim to provide mechanistic insights into IAP inhibition and inform the development of combination therapies outcomes in HNSCC.

#4691 Concurrent EGFR inhibition and radiotherapy potentiates radioresistance in *TP53*-mutated endometrial cancer.

Gabriella Smith[1], Aaron Petty[2], Roberto Vargas[2]

[1]Division of Gynecologic Oncology, Cleveland Clinic Foundation, Cleveland, OH,[2]Department of Translational Hematology and Oncology Research, Cleveland Clinic Foundation, Cleveland, OH

Introduction: Endometrial cancers (EC) that harbor *TP53* mutations portend a worse clinical prognosis. EGFR inhibition has emerged as a potential radio-sensitizing strategy, particularly in NSCLC and HNSCC, however, its effect in *TP53*-mutated tumors is mixed. As emerging data demonstrates that *TP53* mutations may confer radioresistance in endometrial cancer, we explored the role of EGFR inhibition as a novel radio-sensitizing strategy in this disease site.

Methods: To evaluate the effect of EGFR inhibition on radiation response, we employed the parental EC cell line, Hec1B (*TP53* R248Q mutation). Cells were treated with increasing doses of the EGFR inhibitor, erlotinib, while concurrently exposed to varying doses of radiation. Cells were treated for 48 hours with erlotinib, and viability was assessed with CellTiter-Glo. IC_{50} values were determined using GraphPad Prism software. Drug synergy was determined using the Bliss independence model. A MeanR test was also used to compare the predicted response (additive) of the Bliss model to our data. All experiments were carried out in triplicate.

Results: The IC_{50} value of erlotinib alone was 14.4uM. At 6Gy of radiation, the IC50 of erlotinib was 23.1uM. Per the Bliss independence model, erlotinib was not observed to have a synergistic effect with radiotherapy. However, the MeanR test of the deviation from additivity demonstrated antagonism with an F-statistic of 6.46, $p = 0.02$.

Conclusion: Concurrent use of erlotinib potentiates radioresistance. Novel therapeutic and sequencing radio-sensitizing strategies remain to be elucidated in *TP53* mutated EC.

#4692 Multimodal cancer therapy consisting of antibody-drug conjugates and radiotherapy in cancer treatment.

Allegra Gerharz, Julian Schlegel, Christiane Rutenberg, Claudia Rittmuller, Mahmoud Moustafa, Ivana Dokic, Jurgen Debus, Amir Abdollahi

German Cancer Consortium (DKTK) Core-Center, German Cancer Research Center (DKFZ); Divisions of Molecular & Translational Radiation Oncology, Heidelberg Faculty of Medicine and Heidelberg University Hospital, Heidelberg Ion-Beam Therapy Center, Heidelberg, Germany

Targeting tumor associated antigens (TAA) with highly potent cytotoxic payloads by Antibody Drug Conjugates (ADC) technology is revolutionizing cancer treatment. Whether ADC could replace current chemotherapy as the standard partner of radiotherapy (RT) remain elusive. We sought to systematically decipher the impact of different payload classes and ADC combinations on tumor response to RT. A broad matrix of frequently targeted TAAs including EGFR, HER2, HER3, TROP2, Nectin4 and CEACAM in prototypic cancer cell lines representing different entities, including squamous cell carcinoma (A431), breast cancer (AU565, BT474, MDA-MB-361 and 231, MCF-7), lung cancer (NCI-H1975, A549), gastric cancer (NCI-N87) and pancreatic cancer (BxPC3) were evaluated. Moreover, representative payloads classes, i.e., tubulin inhibitors (MMAE and DM1/4), topoisomerase I inhibitors (DXd and SN-38), and the alkylating agent duocarmycin were examined. Accordingly, combination of RT with ADCs targeting HER2: trastuzumab emtansine/deruxtecan/duocarmazine (T-DM1/T-DXd/T-Duo) and disitamab vedotin, TROP2: datopotamab deruxtecan and sacituzumab govitecan, EGFR: depatuxizumab MMAE, HER3: patritumab deruxtecan, Nectin4: enfortumab vedotin and CEACAM5: tusamitamab ravtansine were evaluated. A broad heterogeneity in sensitivity to payload classes was found e.g., for topoisomerase inhibitor Deruxtecan a low nM (3,3 nM) IC50 was observed in sensitive A431. In contrast, 1 µM Deruxtecan led to only 20% loss of viability in resistant BT474 cells. In line, BT474 showed resistance to HER2 targeting ADC with topoisomerase inhibitor payload (T-DXd) but was sensitive to the same antibody conjugated with tubulin inhibitor payloads (T-DM1). These data indicate that not only TAA presence and density but also intrinsic payload sensitivity maybe relevant for assessment of ADC efficacy. Consequently, in tumors sensitive to both tubulin and topoisomerase inhibitors like AU565 breast cancer cell line similar ~ 65% cytotoxicity was found at 1 nM to both T-DM1 and T-DXd, regardless of the payload substance class. The relevance of TAA targeting, payload and drug antibody ratio (DAR) was further demonstrated by comparing different ADCs vs. antibody vs. payload alone. Enhanced radiosensitivity was exploited for different ADC and substance classes e.g., MMAE and SN-38 IC50 concentrations led to >2-Fold reduction of the survival fraction (SF) of A431 cells when combined with 2Gy RT. Together**,** this study demonstrates that inherent sensitivity of tumor cells to different payload classes, DAR and TAA dynamic are of relevance for the efficacy of ADC. Informed selection of ADC exhibited synergistic effects with RT providing a novel multimodal and promising strategy for efficient cancer eradication.

#4693 Impact of pelvic radiation on vaginal microbiome and pelvic health: Findings from the GEORGIA Trial (NCT04713618).

Yogita Mehra, Elizabeth LaPlante, Laura Flora, Caroline Dravillas, January Kim, Jessica Aduwo, Naa Korley, Alexandra Diaz, Eric Miller, Kenneth Pitter, Nyelia Williams, Rebecca Hoyd, Xiaokui Mo, Daniel Spakowicz, Laura Chambers, Elizabeth K. Arthur, Allison M. Quick

The Ohio State University, Columbus, OH

Radiation therapy (RT) is a common treatment for gynecological and lower gastrointestinal cancers with negative side effects on pelvic health and sexual function. These effects significantly impact patients' quality of life, yet research on mitigating such toxicities remains limited. RT has been shown to alter the vaginal microbiome, enriching opportunistic pathogens, which may contribute to vaginal toxicity and treatment resistance. This study aims to assess how pelvic health, and the microbiome change over the course of and after treatment, and whether pre-treatment microbiome composition can predict post-treatment outcomes. Here we describe the results of the GEORGIA trial (NCT04713618) a longitudinal study of women undergoing pelvic radiation for anal, rectal, cervical, endometrial, vaginal, or vulvar cancer. We assessed the effect of RT on the metatranscriptome (metaT), evaluating microbial abundance, activity, and human gene transcription by sequencing total RNA with human ribosomal depletion to a depth of >50M reads per sample on an Illumina NextSeq 2500. Samples were collected at baseline and at 1, 3, 6-, 12-, 18-, and 24-months post-treatment. Longitudinal mixed-effects models were used the assess the relationship between clinical variables and microbes, with a random intercept by patient, using lme4 in R. Vaginal length changes pre/post-treatment were analyzed via paired t-tests, while vaginal diameter changes were assessed using the Wilcoxon rank-sum test. A total of 72 patients were recruited and of those 68 (94%) participants completed the sociodemographic questionnaire. The median age was 56.5 ranging from 28 to 90 years old. Most participants were white (90%), postmenopausal before treatment (62%), and married or partnered (65%). Vaginal swab RNAseq reads aligned to human and microbe reference genomes in approximately equal numbers. Deconvolving the human reads to immune cells showed increased neutrophils following RT ($p = 0.05$). We observed a significant change in vaginal diameter over the course of treatment (Wilcoxon rank sum, $p = 0.04$). Women with *Candida*-positive microbiomes experienced a significant reduction in vaginal diameter over time, compared to women with *Candida*-negative microbiomes ($p = 0.03$). The findings suggest a potential role for fungi, and particularly *Candida,* in treatment-associated vaginal changes and sexual health. These results imply a therapeutic target to improve patient outcomes via modulation of the vaginal microbiome.

#4694 (R)evolutionary approach to predicting individual response to radiation therapy in breast cancer.

Naheel Khatri, Raafat Chalar, Saiful Samad, Alexander Stessin, **Mehdi Damaghi**

Stony Brook University, Renaissance School of Medicine, Stony Brook, NY

Despite increasing incorporation of gene panel testing into radiotherapeutic decision-making, there is no established method to tailor prescribed radiation regimens to individual tumor radiosensitivity. We propose a novel approach to assessing evolutionary determinants of radioresistance from patient-derived tumor samples. We hypothesized that pre-existing resistance to oxidative damage in tumor microenvironment (TME) can define a cell's evolutionary trajectory and its radiosensitivity. In contrast with the current genetic panels, we use a panel catered toward heterogeneity within the tumor and the TME niches, which may harbor radioresistant populations from collateral TME mechanisms of resistance. The aim is to develop an AI-based algorithm using these eco-evolutionary results to individualize radiation dose in patients, thus minimizing the percentage of non-responders. 3D spheroids of several breast cancer cell lines were grown and collected at multiple time points after 10 Gy of radiation. MALDI data was acquired in negative and positive ionization mode for spatial lipidomics and metabolomics analysis. Additionally, spatial single cell multiplexed immunofluorescence was performed on the sequential cut to MALDI using markers associated with hypoxia, acidosis, ROS, and epithelial-to-mesenchymal transition. Then we grew patient derived organoids (PDO) of breast cancer and irradiated them and analyzed them as described above. Based on a previously validated oxygen diffusion model, spheroids were categorized according to size, with those <240 micrometers in diameter designated as O-spheroids (oxygenated) and those ≥ 240 microns designated as H-spheroids (hypoxic core). While the irradiated O-spheroid growth curves plateaued upon irradiation, H-spheroids grew at the same rate as the non-irradiated controls. To investigate this phenomenon, habitats and cell phenotypes were defined using spatial multi-omics data from MALDI and multiplexed-IF, respectively. We found differential distributions of habitats in radiated spheroids as determined by the cluster size and abundance spatio-temporally. We discovered a switch in the sphingolipid metabolism pathway. Using principles of ecology and evolution, we have developed a novel approach to identifying radiosensitivity markers. Detecting these cell states and ecological niches in patients' pre-treatment biopsies can inform a more individualized approach to radiotherapy.

#4695 Post-pandemic evaluation of hypofractionated radiotherapy in preventing acute skin toxicity in multiracial/ethnic breast cancer populations.

Veronica Zhang Paley[1], Cristiane Takita[2], Isildinha M. Reis[1], Jean L. Wright[3], Jennifer J. Hu[1]

[1]University of Miami Miller School of Medicine, Miami, FL,[2]Radiation Oncology, University of Miami Miller School of Medicine, Miami, FL,[3]Department of Radiation Oncology and Molecular Radiation Sciences, Johns Hopkins University, Baltimore, MD

Background: Acute skin toxicity is a prevalent and debilitating side effect of radiotherapy (RT) for breast cancer, disproportionately impacting minority populations. Hypofractionated radiotherapy (HF-RT), which involves delivering higher doses of radiation over fewer treatment sessions, has emerged as a preferred approach during the COVID-19 pandemic due to its efficiency and convenience. This study evaluates the effectiveness of HF-RT compared to conventional fractionated RT (CF-RT) in preventing grade 2+ RT-induced skin toxicity among diverse racial and ethnic groups post-pandemic.

Objective: To investigate the severity and frequency of RT-induced skin toxicity among breast cancer patients treated with HF-RT and CF-RT, as well as the associations between toxicity and patient demographics or clinical characteristics.

Methods: A retrospective analysis was conducted using data from 105 breast cancer patients treated at the Sylvester Comprehensive Cancer Center. Patients were grouped based on RT type: HF-RT (42.5-50 Gy over 3-5 weeks) or CF-RT (50+ Gy over 5-7 weeks). Skin toxicity was evaluated using the Common Terminology Criteria for Adverse Events (CTCAE v5.0). Patients were categorized into HF-RT (n=84), CF-RT (n=15), and partial RT (n=6) groups. Patient demographics, including age, BMI, race/ethnicity, and tumor characteristics, were analyzed for their association with toxicity grades. Statistical analyses were conducted using chi-square tests and multivariate logistic regression.

Results: 12.4% of patients experienced grade 2+ skin toxicity at follow-up, with no grade 3 or higher observed. HF-RT patients showed lower grade 2+ toxicity rates (10.7%) compared to CF-RT (26.7%), although the difference was not statistically significant (p=0.143). Age was significantly associated with skin toxicity, with patients aged ≥50 years exhibiting higher rates of grade 2+ toxicity (17.4%) compared to those <50 years (2.8%, p=0.031). Other variables, including BMI, race/ethnicity, tumor stage, and treatment location, were not significantly associated with skin toxicity. HF-RT outcomes were consistent with prior studies, demonstrating safety and feasibility across all racial/ethnic groups, including 55% Hispanic and 11% Black patients.

Conclusion: Our findings suggest that HF-RT is feasible and safe for reducing acute skin toxicity in early-stage breast cancer patients across multi-racial/ethnic populations, with outcomes comparable to those reported in prior research conducted predominantly in Caucasian populations. These findings emphasize the importance of tailoring RT regimens to improve survivorship outcomes and address disparities in cancer care.

#4696 Targeting CSF1R+ macrophages to synergize immune checkpoint therapy in SCCA-mediated radioresistant cervical cancer.

Liyun Chen, Eric Liu, Victoria Shi, Marlene L. Campos Guerrero, Jasmin Yang, Abhay Singh, Matthew Inkman, Jin Zhang, Julie Schwarz, Stephanie Markovina

Washington University in St. Louis, St. Louis, MO

Tumor-associated factors released by cancer cells can promote immune suppression and tolerance to radiation or immune therapy. Squamous cell carcinoma antigen (SCCA) is a tumor-associated antigen released by cancer cells. In cervical cancer, elevated pre-treatment SCCA in the serum is seen in 28-88% of patients and these patients are more likely to experience treatment resistance and recurrence. The poor prognosis for patients with elevated SCCA shows the need for improve treatment strategies. Here, we aim to understand the mechanism of action of circulating SCCA to tumor progression and response to radiotherapy. Using sequencing analysis of paired pre- versus on-treatment biopsies, we found that patients with elevated serum SCCA had increased macrophages expressing immunosuppressive features and T-cell immune checkpoints, inversely correlated with inflammatory signaling and radiation-induced interferon-stimulated gene expression. The supplement of SCCA protein (rSCCA) to the media during in vitro macrophage differentiation promotes anti-inflammatory cytokines and gene expression. Peritoneal macrophages isolated from mice treated with intra-peritoneal rSCCA expressed high M2-associated markers and functional inhibition of T cell activation. Moreover, we found that mouse tumors engineered to express SCCA with a signal peptide promoting extracellular secretion displayed increased CSF1R+ TAM infiltration and decreased T cell activation, associated with reduced response to combined radiation and anti-PDL1 treatment. Giving the findings, we hypothesized that targeting TAMs in tumors with elevated SCCA is imperative. We further demonstrated that tumor-bearing mice showed improved response to radiation and immune checkpoint therapy when receiving concurrent CSF1R blockade, abolishing the protective effect of high circulating SCCA. Our results suggest that blockade of CSF1R signaling or other myeloid-targeting therapeutics will optimize response to radiation and PD-L1 blockade in cervical cancer, particularly those with high circulating SCCA.

#4697 FDA approved non-cardiotoxic hERG1 inhibitor ketoconazole enhances radiation sensitivity in glioblastoma.

Johnathan Rodgers Gochicoa[1], Tingting Huang[2], Kelli B. Pointer[2]

[1]Dartmouth College, Hanover, NH,[2]Dartmouth Cancer Center, Lebanon, NH

Glioblastoma (GBM) is the most aggressive primary brain tumor in adults, with a median survival of less than two years despite multimodal therapy, including surgery, radiation, chemotherapy, and tumor treating fields. GBM's dismal prognosis is largely due to its high recurrence rates. Emerging evidence implicates ion channels in promoting tumor progression and treatment resistance across various malignancies. Specifically, the potassium voltage-gated channel hERG1, encoded by the KCNH2 gene, has been associated with worse patient outcomes in GBM. However, the mechanisms underlying hERG1's contribution to treatment resistance, particularly radiation resistance, remain poorly understood. Here, we investigated the role of hERG1 in driving radiation resistance in GBM. RNA-sequencing data from 350 GBM patient tumors in The Cancer Genome Atlas (TCGA) were analyzed to assess KCNH2 expression and its correlation with overall survival. Patients were stratified into quartiles by KCNH2 expression, and Kaplan-Meier survival analyses were performed using R Studio (v4.3.1). The human GBM cell line LN-229 was used for in vitro studies. A drug screen determined the IC25 for an FDA-approved, non-cardiotoxic hERG1 inhibitor ketoconazole. Cell viability was assessed after 72 hours of drug treatment. Cells were treated with IC25 concentrations of inhibitors for three days, with radiation (0 Gy or 8 Gy) administered on day two, followed by two additional days of drug treatment. All experiments were performed in triplicate for statistical rigor. KCNH2 expression in the upper quartile was 9.7 times higher than in the lower quartile. Patients in the upper quartile exhibited worse median overall survival compared to those in the lower quartile (11.8 months vs. 12.9 months, $p = 0.00053$). The IC25 for ketoconazole was 28μM. Ketoconazole combined with radiation decreased cell viability by 54.4% compared to radiation alone ($p = 0.01$) and by 78.8% compared to vehicle control ($p < 0.0001$). High KCNH2 expression is associated with worse survival outcomes in GBM patients. FDA-approved, non-cardiotoxic hERG1 inhibitor ketoconazole decreased cell viability and enhanced radiation sensitivity in vitro. These findings suggest that combining a non-cardiotoxic hERG1 inhibitor with radiation may represent a promising therapeutic strategy to improve GBM patient outcomes.

#4698 Towards treating prostate cancer using an engineered sodium/iodide symporter (NIS) protein.

Haswitha Sabbineni[1], Alejandro Llorente[1], Todd D Giorgio[2], Nancy Carrasco[1]

[1]Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN,[2]Biomedical Engineering, Vanderbilt University, Nashville, TN

The sodium/iodide symporter (NIS) is the theranostic plasma membrane protein that mediates the active transport of iodide (I^-) ions and various clinically approved radiotracers used for imaging [such as pertechnetate ($^{99m}TcO_4^-$), radioiodide ($^{123}I^-$), and tetrafluoroborate ($[^{18}F]BF_4^-$)] and for therapeutic purposes [e.g., radioiodide ($^{131}I^-$) and preclinically approved perrhenate ($^{186/188}ReO_4^-$)]. For over 80 years, targeted internal radiation therapy (TIRT) has been used successfully to treat thyroid cancer: administered $^{131}I^-$ is selectively accumulated in the thyroid cancer cells by NIS.

NIS-mediated TIRT is a promising approach to treating prostate cancer, the second deadliest cancer in men in the United States and Europe. About 1 in 7 men will be diagnosed with prostate cancer at some point in their life. Currently available therapies, including prostate surgery and targeted irradiation are often ineffective against advanced/recurrent and metastatic prostate cancer, so new treatments are urgently needed.

In order for NIS-based TIRT to be successful, three challenges must be overcome: a) the thyroid must be protected from β-radiation; b) the immune response triggered by the viral vectors used for NIS delivery must be mitigated; and c) tissue specificity must be ensured.

To tackle these challenges, we have engineered a NIS protein (PF-NIS) by replacing two amino acids that are close to the substrate cavity (the substitutions are L253P and V254F). PF-NIS differentiates between two substrates, namely I^- and oxyanions (such as $^{186}ReO_4^-$ and $^{99m}TcO_4^-$): it transports oxyanions but virtually no I^-. We have shown in *in vitro* experiments that cells expressing wild-type NIS (WT NIS) do not die when treated with $^{186}ReO_4^-$ and saturating concentrations of non-radioactive iodide (I^-) (100 μM), because WT NIS does not transport oxyanions in the presence of excess I^-.

To decrease immunogenicity, we will use polyplexes which are nanocomplexes composed of DNA and polyvalent molecules such as polyethyleneimine (PEI). Polyplexes were formed using in vivo jetPEI and PF-NIS cDNA at an N/P ratio of 1:8. Dynamic light scattering analysis revealed an average polyplex size of approximately 100 nm. Morphological analysis using scanning electron microscopy and atomic force microscopy confirmed that the polyplexes were spherical.

Tissue specificity was achieved by suing the prostate-specific antigen (PSA) promoter, a well-characterized regulatory element that drives gene expression specifically in prostate epithelial cells and is highly active in prostate cancer cells.

We conclude that polyplexes containing the PF-NIS cDNA under the PSA promoter will render prostate cancer cells susceptible to being treated with $^{186/188}ReO_4^-$ even in the presence of non-radioactive I^-, which will protect the thyroid.

#4699 Intratumoral adenosine: Contributions from host versus tumor and interaction with radiotherapy.

Shruti Bansal, Samantha Sarti, Namita Sen, Chelsea Rahiman, Brian Ragaishis, Lindsay Caprio, Benjamin Izar, Catherine Spina

Columbia University Irving Medical Center, New York, NY

Purpose: Tumor irradiation generates high levels of extracellular ATP that is metabolized to suppressive adenosine that is implicated in cancer progression. The relative contribution of host and cancer cell-derived adenosine and downstream signaling to radioresistance is unclear.

Methods: In a murine model of breast cancer (EO771), we measured the expression of adenosine signaling proteins: CD73 that metabolizes AMP to adenosine and adenosine receptors, A2aR and A2bR by flow cytometry. We measured adenosine concentration by LC-MS in supernatants of WT tumor cells and those that lack CD73 ($CD73^{-/-}$) grown *in vitro*. To understand the non-tumor cell-derived contribution to adenosine, we measured adenosine concentration in breast EO771 tumors grown in $CD73^{-/-}$, $A2aR^{-/-}A2bR^{-/-}$ or WT mice. Tumor growth studies were performed in the untreated and irradiated (8 Gy x 3) settings. In comparison, we conducted tumor growth experiments in $CD73^{-/-}$ & WT EO771 tumors grown in WT mice to understand the contribution of tumor-derived CD73 to tumor control.

Results: In murine EO771 tumors, the proportion of viable non-immune ($CD45^{-}$) cells that express CD73 was significantly higher than in the $CD45^{+}$ immune compartment (18.3% vs 5.7%). In contrast, the proportion of viable immune ($CD45^{+}$) cells that express A2aR was significantly higher than the $CD45^{-}$ compartment (7.8% vs 4.4%), suggesting complementary roles of the two compartments. *In vitro*, adenosine production was abrogated in $CD73^{-/-}$ EO771 tumor cells compared to WT controls (0.14μM vs 2.4μM). Tumor cell irradiation caused a significant increase in adenosine in WT EO771 but not $CD73^{-/-}$ EO771. When grown in WT mice, tumor growth was delayed in $CD73^{-/-}$ tumors compared to WT controls (306 mm^3 vs 1165 mm^3 on day 22). Loss of CD73 in tumor cells significantly improved radiosensitivity and increased overall survival on 150-day by 50%. Quantification of adenosine in EO771 tumors grown in WT, $CD73^{-/-}$ & $A2aR^{-/-}A2bR^{-/-}$ mice revealed that absence of host CD73 caused 3.5-fold decrease in intertumoral adenosine, while loss of A2aR and A2bR caused no significant change. Furthermore, tumor control in both $CD73^{-/-}$ & $A2aR^{-/-}A2bR^{-/-}$ mice improved by 50% in the absence of radiation. However, after tumor irradiation, loss of host A2aR and A2bR but not CD73 improved tumor control.

Conclusions: Results suggest that tumor cell expression of CD73 is integral for production of adenosine and loss of expression significantly improves radiosensitivity. Loss of CD73 from non-tumor cells did not influence radiosensitivity, whereas expression of A2aR and A2bR does. These data suggest that tumor-derived adenosine contributes to tumor growth and radioresistance and non-tumor cells in the TME are important for A2aR/A2bR-mediated effects of adenosine. CD73 & A2aR/A2bR offer an exciting therapeutic strategy to mitigate radiation-induced suppressive changes for improved radiosensitivity and tumor control.

CLINICAL RESEARCH: Targeted Therapies and Combinations 2
Poster Session

#4703 EGFR is a promising therapeutic target in NF1 mutant melanoma.

Milad Ibrahim[1], Irineu Illa-Bochaca[1], George Jour[2], Eleazar Vega-Saenz de Miera[1], Kelly Ruggles[3], Jeffrey Weber[4], Markus Schober1[1], Iman Osman[1]

[1]The Ronald O. Perelman Department of Dermatology, NYU Langone Health, New York, NY,[2]Department of Pathology, Molecular Pathology and Diagnostics, NYU Langone Health, New York, NY,[3]Department of Medicine, New York University School of Medicine, New York, NY,[4]NYU Langone Health Perlmutter Cancer Center, NYU Langone Health, New York, NY

Background: Molecularly targeted therapies and immunotherapy improved treatment outcomes for melanoma patients. However, patients with driver mutations in the Neurofibromin 1 (*NF1*) tumor suppressor gene, which represent 20-30% of melanomas, have not benefitted from these treatment advances, underscoring an unmet clinical need.
Methods: We compared 13 *NF1* mutant ($NF1^{Mut}$) and 19 *NF1* wild-type ($NF1^{WT}$) patient-derived short-term cultures (STCs) integrating multi-omic analysis of DNA mutations (Whole Exome Sequencing), RNA expression, and gene regulation (RNA-Seq, ATAC-Seq, H3K4Me3 and H3K27Ac). Independent melanoma patients' specimens (n= 40) were used for immunohistochemical validation studies. We then used STCs for functional studies defining the role of *NF1* loss in melanoma and testing potential therapeutic targets *in-vitro* and in PDX models.
Results: Comparing $NF1^{Mut}$ to $NF1^{WT}$ uncovered gene regulatory elements (ATAC $P = 0.024$, H3K27Ac $P = 0.011$) controlling a significantly increased transcription of EGFR in $NF1^{Mut}$ ($P = 0.005$). The significant upregulation of EGFR expression was confirmed by western blotting ($P = 0.003$) and IHC in an independent melanoma patient cohort ($P = 0.0007$). Functional studies revealed that EGF rapidly activates EGFR, AKT and ERK in $NF1^{Mut}$ but not $NF1^{WT}$ melanoma cells ($P < 0.0001$). Moreover, *NF1* knockdown in $NF1^{WT}$ *in-vitro* resulted in an increased melanoma cell proliferation ($P < 0.0001$) as well as, EGFR, AKT and ERK activation. Genetic or immunological EGFR inhibition blunted cell proliferation, cell survival, and tumor growth in $NF1^{Mut}$ but not $NF1^{WT}$ melanoma STCs ($P < 0.0001$) and PDX models ($P < 0.0001$) independent of *BRAF* or *NRAS* mutation status.
Conclusion: Our study uncovers a stereotypical and transcriptionally controlled activation of the EGFR-AKT signaling axis in $NF1^{Mut}$ melanoma, with EGFR inhibition limiting tumor growth in $NF1^{Mut}$ but not $NF1^{WT}$ melanomas. Data suggest a possible role of targeting EGFR as therapeutic target in melanoma patients with $NF1^{Mut}$ tumors.

#4704 Development of a novel zebrafish patient-derived xenograft (zPDX) platform for functional precision cancer medicine for immune checkpoint (ICIs) and tyrosine kinase inhibitors (TKIs) of renal cell carcinoma (RCC).

N. Pawar, J.-S. Chung, V. Cantrell, I. Dougherty, N. Bingham, V. Margulis, P. D. Cruz, Jr., H. Hammers, K. Ariizumi;
UT Southwestern Medical Center, Dallas, TX

Predictions of cancer treatment outcomes are critical to personalized therapy. Despite extensive studies of genome-based precision medicine, we still lack reliable markers to predict patient-specific response to ICIs and TKIs for RCC. zPDX has been used to develop *in vivo* preclinical models using mouse PDX, primary cultured cells or dissociated tissues (without T cell analysis). These samples do not reflect enough of the intratumor heterogeneity. To create assays better representative of their natural state, we used freshly isolated tumor cells for zPDX and tested their ability to display patient-specific response to ICIs and TKIs. Within 24h after nephrectomy, the RCC mass (0.8-1.9 g) was extracted and purified for whole tumor cells (WTCs) via Ficoll gradient centrifugation and dead cell removal (viability >85%; yield 0.3-33 x 10^6 cells) and analyzed by FACS for % CD45+ or CD8+ cells (or PD1+CD8+). RCC-WTC was rich in CD45+ immune cells (65% ± 10, n=8) and CD8+ T cells (38% ± 10, n=5), among which PD1 expression was low (4.7% ± 2.2, n=5). WTCs were labeled with Red Cell-Tracker, mixed with Nivolumab or control IgG (0.5 ng/fish), injected into the perivitelline space (PVS) of 2dpf zebrafish embryos (~200 cells/fish, n=10/group), and zPDX cultured individually in a microwell for 2-5d at 34°C. For TKIs, WTC-injected zebrafish were cultured in media with Cabozantinib (0.1 μM) or Sunitinib (0.3 μM) (n=8/group). 2-4d post-injection, fluorescence images were taken and analyzed for tumor area (TA; procedural defined units) around PVS. % reduction in TA was recorded as response and RECIST-categorized into PR (>30% reduction), PD (>30% increase) and SD (<30% reduction). To date, we have performed zPDX assays for 8 cases (none with pre-determined treatment plans). For ICIs, 6 cases displayed PR (53% ± 1.6, $p<0.003$) and the rest (2 cases) did SD (11% ± 10) with weak association of ICI-effects with PD1 expression, but PR responders (5 out of 6) showed significant upregulation of hIL-2 mRNA (median 6.2 x10^{-5} to hGAPDH) by nested qRT-PCR of total RNA from Nivolumab-treated zPDX. This tumor inhibition was T cell-mediated, shown by co-applied anti-CD3 Ab that reversed tumor regression by ICI (n=3, 82-100% reversal) and the presence of hT-cells in zPDX treated with Nivolumab (but not with control IgG) shown by IHC staining of whole body FFPE with anti-CD3. TKIs inhibited TA, with 50% PR response rate for Sunitinib and Cabozantinib (38% ± 5.5, $p<0.05$ & 40% ± 7.7, $p<0.02$, respectively) with significant individual differences in % inhibition. We showed our zPDX model with freshly isolated WTC can quantify patient-specific responses to ICIs and TKIs before onset of actual treatments. Thus, this zPDX platform will establish a fast, accurate, and clinically relevant system for functional precision medicine for RCC immunotherapy.

#4705 FreezeO organoid biobank: A translational pipeline for precision oncology at the University Medical Center Freiburg.

Rhena F. U. Klar[1], Georg Vladimirov[2], Tanja Werner[3], Tamara Sloboda[3], Patrick Metzger[4], Maria E. Hess[5], Andreas Blaumeiser[4], Jasmin Traichel[6], Linus Schomig[7], Lioba Klaas[8], Maryam Barsch[8], Heiko Becker[9], Christopher Berlin[10], MTB Freiburg Network[11], Alexandra Nieters[12], Michael Quante[13], Tilman Brummer[14], Melanie Boerries[15]

[1]German Cancer Consortium (DKTK), Partner site Freiburg; Freeze0 - Biobank for Human Tumor Organoids, Institute of Medical Bioinformatics and Systems Medicine, Medical Center - University of Freiburg, Faculty of Medicine, Freiburg, Germany,[2]German Cancer Consortium (DKTK), Partner site Freiburg; Faculty of Biology, University of Freiburg, Institute of Medical Bioinformatics and Systems Medicine, Medical Center - University of Freiburg, Faculty of Medicine, Freiburg, Germany,[3]German Cancer Consortium (DKTK), Partner site Freiburg; FreezeO - Biobank for Human Tumor Organoids, Institute of Medical Bioinformatics and Systems Medicine, Medical Center - University of Freiburg, Faculty of Medicine, Freiburg, Germany,[4]Institute of Medical Bioinformatics and Systems Medicine, Medical Center - University of Freiburg, Faculty of Medicine, Freiburg, Germany,[5]Faculty of Biology, University of Freiburg, Freiburg, Germany, Institute of Medical Bioinformatics and Systems Medicine, Medical Center - University of Freiburg, Faculty of Medicine, Freiburg, Germany,[6]German Cancer Consortium (DKTK), Partner site Freiburg; Faculty of Biology, University of Freiburg, Institute of Molecular Medicine and Cell Research, University of Freiburg, Freiburg, Germany,[7]Faculty of Biology, University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany Clinic for Internal Medicine II, Gastroenterology, Hepatology, Endocrinology, and Infectious Disease, University Hospital Freiburg, Freiburg, Germany,[8]Faculty of Medicine, University of Freiburg, Freiburg, Germany Clinic for Internal Medicine II, Gastroenterology, Hepatology, Endocrinology, and Infectious Disease, University Hospital Freiburg, Freiburg, Germany,[9]German Cancer Consortium (DKTK), Partner site Freiburg; Comprehensive Cancer Center Freiburg (CCCF), Department of Medicine I, Medical Center-University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany,[10]German Cancer Consortium (DKTK), Partner site Freiburg, Department of General and Visceral Surgery, Medical Center - University of Freiburg, Faculty of Medicine, Freiburg, Germany,[11]Comprehensive Cancer Center Freiburg (CCCF), Medical Center-University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany,[12]FreezeO - Biobank for Human Tumor Organoids, Medical Center - University of Freiburg, FREEZE Biobank - Center for Biobanking, Medical Center - University of Freiburg, Freiburg, Germany,[13]FreezeO - Biobank for Human Tumor Organoids, Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany Clinic for Internal Medicine II, Gastroenterology, Hepatology, Endocrinology, and Infectious Disease, University Hospital Freiburg, Freiburg, Germany,[14]German Cancer Consortium (DKTK), Partner site Freiburg; FreezeO - Biobank for Human Tumor Organoids, Institute of Molecular Medicine and Cell Research, University of Freiburg, Freiburg, Germany,[15]German Cancer Consortium (DKTK), Partner site Freiburg; Comprehensive Cancer Center Freiburg (CCCF), Institute of Medical Bioinformatics and Systems Medicine, Medical Center - University of Freiburg, Faculty of Medicine, Freiburg, Germany

The FreezeO Organoid Biobank at the University Medical Center Freiburg represents an innovative resource and an integral extension of the Molecular Tumor Board (MTB) that enables patient-specific drug testing for precision oncology. The focus of this biobank is to isolate and culture patient-derived organoids (PDOs) from fresh tumor biopsies, particularly from metastatic sites, to address an unmet need for therapy recommendations in advanced cancer. Our workflow begins when the MTB decides to obtain new tumor biopsies for whole exome sequencing (WES) or RNA sequencing (RNA-Seq). Whenever possible, PDO cultures are established in parallel to generate living tumor models. Once molecular profiling is complete, the targeted therapies recommended by the MTB are tested on the PDOs using an established semi-automated drug testing platform. Response to therapy is assessed and validated using complementary methods such as Western Blotting and transcriptomic analysis of the treated PDOs. The results will be rapidly integrated into MTB discussions and provide important data to refine and personalize treatment decisions. To date, we have successfully established and biobanked PDOs from nine different cancer types, including colorectal cancer (CRC), non-small cell lung cancer (NSCLC) and rare cancers such as jejunum carcinoma. Remarkably, 60% of our biobank consists of PDOs derived from metastatic lesions, highlighting a clear benefit for late-stage cancer patients in the future. Drug tests were carried out on the PDOs within a clinically relevant timeframe and the results were included in the MTB decision-making process. Among the cases we will present, we report a metastatic CRC case featuring a PDO from a lung metastasis harboring a KRAS G12V mutation. Based on the oncologist’s recommendation, the drug testing strategy focused on the combination of Lapatinib and Trametinib. This approach reflects the ongoing clinical interest in overcoming intrinsic and acquired resistance to EGFR-targeted treatments in CRC. The inhibitor combination demonstrated promising efficacy in the PDO model. The robustness of these findings was validated through Western Blot analysis and RNA sequencing, providing critical data to inform the MTB panel. Ultimately, this evidence was instrumental in tailoring a more informed treatment strategy. The FreezeO Organoid Biobank demonstrates the translational potential of PDOs in precision oncology, particularly in metastatic disease. By seamlessly integrating rapid organoid culture, molecular profiling and targeted drug testing, our pipeline supports the MTB in making the most informed, data-driven treatment recommendations. This approach not only reinforces therapeutic decision making, but also underscores the utility of PDOs as a bridge between research and clinical application, paving the way for broader adoption in personalized oncology.

#4706 Evaluating the efficacy of RAS(ON) inhibitor RMC-6236 combined with chemotherapy and other targeted therapies in 3D models involving patients with KRAS-mutated pancreatic cancer.

Taylor Bargenquast[1], Cherie Wesley[2], Janith Don[3], Guangfa Zhang[3], Tithi Ghosh Halder[4], Serina Ng[4], Alexis Weston[1], Trason Thode[4], Erkut Borazanci[1], Mohan Kaadige[4], Sara A. Byron[2], Nicholas Schork[3], Raffaella Soldi[4], Sunil Sharma[1]

[1]HonorHealth Research Institute, Scottsdale, AZ,[2]Bioinnovation and Genome Sciences Division, Translational Genomics Research Institute (TGen) of City of Hope, Phoenix, AZ,[3]Clinical Genomics and Therapeutics Division, Translational Genomics Research Institute (TGen) of City of Hope, Phoenix, AZ,[4]Applied Cancer Research and Drug Discovery, Translational Genomics Research Institute (TGen) of City of Hope, Phoenix, AZ

Background: RAS mutations, particularly $KRAS^{G12X}$, are critical oncogenic drivers in pancreatic cancer, with existing $KRAS^{G12C}$ inhibitors unable to target other mutations and often leading to resistance. RMC-6236, a multi-selective RAS(ON) inhibitor, demonstrates potent activity in $KRAS^{G12X}$ models, but resistance mechanisms that increase mutant RAS(ON) may reduce its effectiveness. The fibrotic tumor microenvironment in pancreatic cancer exacerbates therapy resistance, and combining RMC-6236 with other therapies could overcome both intrinsic and acquired resistances. This study evaluates RMC-6236 efficacy in patient-derived pancreatic tumors harboring KRAS mutations, and the impact of cancer-associated fibroblasts (CAFs) on its potency as a single agent and in combination with other therapies. We also assess its impact on downstream RAS signaling and apoptosis using pro-apoptotic markers.
Methods: Patient-derived tumor cells and fibroblasts were treated with RMC-6236 in 2D and 3D viability assays. Patient-derived organoids (PDOs) were generated by combining tumor and fibroblasts in a 1:1 ratio and allowing them to form in media for 72 hours. Synergy studies explored combinations of RMC-6236 with gemcitabine and abraxane chemotherapies, along with other targeted therapies, including CDK7, WNT, and mTOR pathway inhibitors. Samples were stained for α-SMA via immunofluorescence for CAF detection. Western blot analysis assessed RAS pathway inhibition and apoptotic markers.
Results: These results demonstrate the efficacy of RMC-6236 in a pancreatic cancer PDO model when combined with other therapeutic agents. Sensitivity to RMC-6236 was assessed in tumor cells, fibroblasts, and tumor-fibroblast co-cultures, yielding IC50 values ranging from 0.1 to 11 nM, 1 to 3.5 nM, and 0.2 to 5 nM, respectively. These results highlight that the presence of CAFs in KRAS-mutated pancreatic cancer PDOs does not affect sensitivity to RMC-6236 treatment. Notably, synergy studies assessing RMC-6236 in combination with chemotherapy agents (gemcitabine and abraxane), as well as WNT inhibitors (WNTi) and CDK7 inhibitors (CDK7i), revealed a significant reduction in IC50 values, with a more than 100-fold decrease (ranging from 0.0001 nM to 0.001 nM). This substantial enhancement in drug potency underscores the synergistic effects of combining RMC-6236 with these agents, particularly with WNTi.
Conclusion: The combination of RMC-6236 with standard chemotherapy and targeted therapies enhances its antitumor activity, suggesting a promising strategy for improving therapeutic outcomes in pancreatic cancer. The observed synergistic interactions, especially with WNTi, warrant further investigation in clinical trials to evaluate the potential of this combination approach in improving treatment responses.

#4707 Electrochemotherapy for the treatment of head and neck squamous cell carcinoma.

F. H. Maglietti[1], S. D. Michinski[2], L. A. Evans[3], R. Lertora[4];

[1]Instituto Universitario de Ciencias de la Salud. Fundacion HA Barcelo-CONICET, Buenos Aires, Argentina, [2]INFINA-CONICET, Buenos Aires, Argentina, [3]Mayo Clinic, Jacksonville, FL, [4]Hospital Zonal General de Agudos "Petrona V. de Cordero", Buenos Aires, Argentina

Squamous cell carcinoma (SCC) is the second most prevalent type of skin cancer after basal cell carcinoma, with the highest incidence occurring in sun-exposed regions of the body, particularly the head and neck. Approximately 300,000 new cases are diagnosed each year, imposing significant financial burdens on healthcare systems due to treatment costs and on the economy due to productivity losses. Surgery remains the primary treatment option; however, patients, particularly those with advanced-stage disease, may decline surgery due to concerns about functional and cosmetic outcomes. Other effective treatments include cryosurgery, radiotherapy, topical therapies, photodynamic therapy, and Mohs micrographic surgery. Recently, electrochemotherapy (ECT) has emerged as a novel treatment option. ECT involves administering bleomycin followed by electric pulses targeted at the tumor and surrounding margins, inducing transient cell membrane permeabilization. This enhances drug uptake and amplifies cytotoxic effects by over 1,000-fold. Since 2006, ECT has been a standard therapy in Europe and was introduced in Argentina in 2020, offering a new approach for patients who are unsuitable for or decline other treatments. In this study, we present outcomes from six patients with head and neck SCC treated with ECT on an outpatient basis. Most patients required only a single 60-minute treatment session. Observed side effects included grade 1-2 edema, pain, and flu-like symptoms per CTCAE 4.0. Tumor response was assessed using RECIST criteria, with results showing 71.4% complete response (5 of 7 lesions), 14.3% partial response (1 lesion), and 14.3% progressive disease, yielding an objective response rate of 85.7%.In conclusion, electrochemotherapy proved to be a safe and effective treatment for select head and neck SCC patients who are not candidates for surgery, providing a promising alternative in the therapeutic landscape.

#4708 FGFR gene amplification and response to HER2-directed therapy in patients with HER2+ metastatic breast cancer (MBC).

Diana Jaber[1], Natalie K. Heater[1], Sarah Marini[1], Surbhi Warrior[1], Yangruijue Ma[1], Patricia Robinson[1], Lisa Flaum[1], Regina Stein[1], Claudia Tellez[1], Huiping Liu[1], Qiang Zhang[1], Youbin Zhang[1], Massimo Cristofanilli[2], William Gradishar[1], Janice Lu[1]

[1]Robert H. Lurie Comprehensive Cancer Center, Northwestern Memorial Hospital, Chicago, IL,[2]Department of Hematology and Medical Oncology, Weill Cornell Medicine, New York, NY

Introduction: Amplification of fibroblast growth factor receptor (FGFR) signaling has been implicated in driving resistance to endocrine therapies and, more recently, HER2-directed therapies in vitro. While FGFR pathway dysregulation is well-established as a poor prognostic marker in hormone-positive BC, its impact in HER2+ BC is less clear. This study aims to investigate the association between FGFR amplification and clinical response to HER2-directed therapy in patients with HER2+ MBC.
Methods: This study retrospectively analyzed 73 patients with HER2+ MBC by IHC/FISH and/or HER2+ CTCs who received HER2-directed therapy between 2016-2024 under an IRB-approved trial (NU16B06) at Northwestern University Robert H. Lurie Cancer Center. CTC enumerations were performed via CELLTRACKS (Menarini). All patients completed NGS testing. FGFR1 and FGFR2 amplifications were identified via plasma ctDNA (Guardant 360) and tissue NGS (TempusX or FoundationOne®). FGFR gene amplification was considered positive for copy number values > 3 copies per cell.
Results: FGFR amplification was detected in 21 (28.8%) patients with HER2+ MBC. The most common co-mutations included TP53 (52.4%), MYC (33.3%), PIK3CA (28.6%), and ESR1 (28.6%). Prior to FGFR detection, 17 (80.9%) FGFR+ patients had received endocrine therapy, 13 (61.9%) had received CDK4/6 inhibitors, and 10 (47.6%) had received HER2-directed therapy. Median progression-free survival (PFS) after detection of FGFR amplification was 3.4 months (range 0.5-19.2). FGFR+ and wild-type (WT) patients did not differ in age at metastatic diagnosis (median age 50 vs 56; p = 0.8), de-novo MBC presentation (28.6% vs 25.0%; p = 0.8), or having received endocrine therapy (85.7% vs 67.3%; p = 0.2). FGFR+ patients were more likely to have ER-positive MBC (85.0% vs 50.0%, p = 0.012) and to have received a CDK4/6 inhibitor (61.9% vs 28.8%; p = 0.009). FGFR+ patients demonstrated significantly shorter median PFS in all HER2-directed therapy lines compared to WT patients, as follows: first- or second-line therapy (4.53 vs 8.25 months; p = 0.0053), third- or fourth-line therapy (3.03 vs 8.17 months; p = 0.0023), and in fifth-line or later therapies (2.20 vs 9.57 months; p < 0.0001). FGFR+ patients had significantly worse median overall survival compared to WT patients (HR: 2.13, 95% CI: 1.19-3.83; 4.47 vs 6.19 years, p = 0.011).
Conclusion: FGFR amplification in HER2+ MBC is associated with shorter PFS in all lines of HER2-directed therapies, as well as worse overall survival. These findings suggest that FGFR amplification may drive poor outcomes in patients with advanced HER2+ MBC and highlight its potential as a therapeutic target. Future studies are needed to confirm these findings and evaluate the efficacy of combining HER2 and FGFR directed therapies in this population.

#4709 Enhancing chemotherapy efficacy in pancreatic cancer synergistic effects of ONC206 and ONC212 with 5-FU through inhibition of p-ERK.

Vida Tajiknia, Claire Lin, Maximilian Pinho-Schwermann, Connor Purcell, Sean Lawler, Wafik El-Deiry

Legorreta Cancer Center at Brown University, Providence, RI

Background: Pancreatic cancer is a lethal malignancy projected to become the second leading cause of cancer deaths by 2030, with a 5-year survival rate of just 12%. Resistance to chemotherapy, particularly 5-fluorouracil (5-FU), remains a major challenge in treating pancreatic ductal adenocarcinoma (PDAC). ONC206 and ONC212, imipridone-class agents targeting DRD2/ClpP and GPR132/ClpP, show promise in disrupting tumor growth and inducing apoptosis. Phosphorylated ERK (p-ERK), a key driver of PDAC aggressiveness via the MAPK pathway, is a critical therapeutic target. We hypothesize that combining ONC206 or ONC212 with 5-FU could enhance efficacy by synergistically inhibiting p-ERK, supporting their potential in PDAC treatment.

Materials & Methods: The synergy of ONC206 or ONC212 with 5-FU was tested in PDAC cell lines (PANC1, CFPAC-1, HPAF-II, CAPAN-2, ASPAC1) using CellTiter-Glo for cell viability at 72 hours. Western blotting assessed p-ERK, ATF4, and other key proteins for pathway inhibition.

Results: Synergy between 5-FU and ONC212, or 5-FU and ONC206, was observed across all cell lines, with IC50 values for ONC206 ranging from 300-500 nM and for ONC212 from 70-100 nM. At the 48-hour time point in the HPAF-II cell line, 100 nM ONC212 significantly inhibited p-ERK levels compared to 300 nM ONC206. Both treatments as monotherapies resulted in reduced p-ERK levels compared to control. When combined with 25 µM 5-FU, ONC212 caused a more pronounced reduction in p-ERK. In the PANC-1 cell line, the strongest reduction in p-ERK was observed with the combination of ONC212 and 5-FU at 48 hours. In the ASPAC1 cell line, p-ERK inhibition occurred only in conditions containing ONC212, with a more significant effect when combined with 5-FU.

Conclusions: Our data demonstrate that combining 5-FU with ONC206 or ONC212 results in the inhibition of p-ERK, a critical node in the PDAC growth pathway. This synergy between 5-FU and the imipridone compounds ONC206 and ONC212 is a universal phenomenon in the tested human PDAC cell lines. Notably, ONC212 is more potent than ONC206, allowing for lower dosages, and in cases where ONC206 does not effectively inhibit p-ERK, ONC212 can be a more effective option Further studies are needed to elucidate the exact mechanism of this synergy, but these findings provide a promising foundation for developing novel combination therapies to improve PDAC treatment outcomes.

#4710 Heterogeneous efficacy patterns of KRAS inhibitors in digestive cancers.

Gustave Ronteix[1], Jerome Caron[1], Rafal Pietrzak[1], Gizem Altay[1], Fabiana Graziosi[1], Lelia Polit[1], Anna-Rose Gryspeert[1], Jacques RR Mathieu[2], Jerome Cartry[2], Sabrina Bedja[2], Alice Boileve[2], Michel Ducreux[2], Sophie Postel-Vinay[2], Fanny Jaulin[1]

[1]Orakl Oncology, Le Kremlin-Bicetre, France,[2]Gustave Roussy, Villejuif, France

Introduction: KRAS is a well-studied oncogenic driver in a variety of solid tumors, playing a pivotal role in cell proliferation and survival. Its involvement in digestive cancers, including colorectal cancer (CRC) has made it an attractive target for therapeutic development. However, efforts to expand KRAS-targeted assets in these cancers are hindered by the complex and heterogeneous expression patterns of KRAS in solid tumors, which complicate effective targeting and the translation of therapeutic efficacy across patient populations. Furthermore, clinical studies have shown that resistance mechanisms compensate KRAS inhibition and require the development of efficient combination therapies.

Methods: To address these challenges, we collected 24 patient-derived organoids (PDO) representing the molecular diversity of KRAS-driven CRC and PDAC with 5 KRAS G12C variants, 8 KRAS G12D variants and 6 KRAS G12R variants. In-depth molecular and transcriptomic characterization was performed with a particular focus on co-mutation patterns for each KRAS variant. Then the PDOs were screened against a KRAS drugs with and without combination with an EGFR inhibitor. The efficacy of the drugs were systematically compared among different variant populations.

Results: Our findings demonstrate that the PDO models faithfully recapitulate the KRAS heterogeneity observed in primary tumors, providing a robust preclinical platform for testing KRAS-targeted therapies. Strong synergistic effects were identified when using KRAS and EGFR inhibitors simultaneously, matching prior clinical trial results. This model system allows us to dissect the impact of KRAS expression patterns on therapeutic response and resistance mechanisms.

Conclusion: Our study emphasizes the utility of PDOs as a precision tool for advancing KRAS-targeted strategies in complex solid tumors and highlights the potential for individualized approaches in the treatment of KRAS-driven digestive cancers.

#4711 HER3 promotes triple-negative breast cancer progression by upregulating PHF8 via miR-34b-dependent mechanism.

Hui Lyu[1], Congcong Tan[1], Yakun Wu[1], Margaret E. Larsen[1], Shou-ching Tang[1], Guobin Kang[1], Qingzhao Yu[2], Bolin Liu[1]

[1]LSU LCMC Health Cancer Center, School of Medicine, New Orleans, LA,[2]School of Public Health, LSU-Health New Orleans, New Orleans, LA

Introduction: Triple-negative breast cancer (TNBC) is a highly aggressive and heterogeneous malignancy with poor prognosis and limited treatment options, presenting a significant clinical challenge. Current therapeutic strategies are insufficient to address the complexities of TNBC. In a previous study, we identified the human epidermal growth factor receptor 3 (HER3) signaling as a key driver of TNBC progression and a potential therapeutic target. However, the exact mechanisms through which HER3 signaling contributes to TNBC remain poorly understood.

Methods: To explore the molecular mechanisms underlying HER3 signaling in TNBC, we performed RNA sequencing (RNA-seq) to identify transcriptomic changes following HER3 knockdown in HCC1806 TNBC cells. Colony formation assays were used to assess cell viability, while Western blotting and quantitative real-time PCR (qRT-PCR) were employed to analyze protein and mRNA expression levels, respectively. Gene expression was modulated using lentiviral vectors carrying specific cDNAs or shRNAs. Flow cytometry was used to analyze cell cycle distribution. In vivo tumor xenograft models were established by subcutaneously inoculating HCC1806 cells into nude mice to evaluate the role of HER3-PHF8 axis in tumor progression. Immunohistochemistry (IHC) was performed to assess protein expression in both mouse tumor models and clinical TNBC specimens.

Results: RNA-seq analysis revealed a significant reduction in the expression of the histone demethylase PHF8 (also known as KDM7B) following HER3 knockdown in HCC1806 cells. Activation of HER3 by Heregulin (HRG) increased PHF8 expression, while HER3 knockdown decreased PHF8 levels and upregulated p27. PHF8 was shown to be critical for HER3-driven TNBC cell proliferation and progression. Notably, overexpression of PHF8 reversed the G1-phase cell cycle arrest induced by HER3 knockdown. In vivo, increased PHF8 expression rescued tumor growth inhibition resulting from HER3 signaling blockade. Analysis of The Cancer Genome Atlas (TCGA) data and clinical TNBC samples revealed a positive correlation between HER3 and PHF8 expressions at both the mRNA and protein levels. Further investigation identified miR-34b, which was negatively regulated by HER3 signaling, as a microRNA targeting PHF8. Luciferase reporter assays confirmed that miR-34b suppressed PHF8 expression, and MiR-34b mimics and inhibitors significantly downregulated or upregulated PHF8 protein levels, respectively, compared to the negative control in TNBC cells.

Conclusion: Our findings demonstrate that activated HER3 signaling suppresses miR-34b, leading to increased PHF8 expression and promoting TNBC progression. Targeting the HER3/PHF8 axis or restoring miR-34b levels offers promising therapeutic strategies to improve outcomes for TNBC patients.

#4712 ELVN-002; a potent, selective HER2 inhibitor with a differentiated binding mode conferring the potential for enhanced efficacy in combination with HER2-targeting antibody-drug conjugates.

A. J. Broad, M. Aujay, S. D. Gross, L. Ren, S. Kintz, J. P. Lyssikatos;
Enliven Therapeutics, Boulder, CO

Overexpression, amplification or mutation of human epidermal growth factor receptor 2 (HER2) is observed in up to 30% of solid tumors resulting in increased downstream signaling and oncogenic transformation. While trastuzumab deruxtecan (T-DXd), a HER2-targeting antibody-drug conjugate (ADC) recently approved for HER2 altered cancer types, has resulted in improved outcomes there remains a substantial unmet need for patients who either progress on T-DXd or discontinue treatment due to adverse events. The advent of small molecule HER2 inhibitors represents an opportunity to provide meaningful therapeutic benefit to these patients either as a single agent or in combination. However, given the structural similarities between EGFR and HER2, most small molecule HER2 kinase inhibitors are dual EGFR/HER2 inhibitors. These inhibitors often face significant dose-limiting toxicities related to EGFR, which hinder their clinical utility. ELVN-002 is a potent irreversible HER2 inhibitor with > 100-fold selectivity over EGFR currently undergoing clinical evaluation in patients with HER2 alterations both alone and in combination with approved standard of care therapies. We evaluated the effect of ELVN-002 and other clinically-relevant HER2 small molecule inhibitors on HER2 cell surface expression and internalization in HER2 positive cell lines derived from patients with either gastric cancer or breast cancer. We determined that ELVN-002 reduces HER2 expression on the cell surface but had no effect in total HER2 expression. Further, HER2 degradation was quantified by measuring ubiquitinated HER2. We discovered that upon ELVN-002 treatment, HER2 ubiquitination and internalization was significantly increased compared to control cells. In contrast, treatment with other clinically-relevant HER2 inhibitors did not increase HER2 internalization. Reduced cell surface HER2, in conjunction with enhanced HER2 degradation suggest that ELVN-002 induces greater HER2 receptor flux at the cell surface. ADCs bind their target and are subsequently degraded in the lysosome, resulting in the intracellular release of their toxic payload. However, some intact ADCs escape degradation via endocytic recycling. The increase in ubiquitinylated HER2 upon treatment with ELVN-002 suggests ELVN-002 enhances the amount of HER2 targeted to the lysosome and would therefore increase the amount of payload released relative to untreated cells. In contrast, treatment with other HER2 inhibitors results in increased cell surface HER2 retention and decreased internalization suggesting a decrease in cytotoxic payload deposition if combined with an ADC. The differentiated profile and mechanism of ELVN-002 has the potential to be a superior treatment in HER2 expressing cancers in combination with ADCs, other modalities, and as a single agent.

#4713 Elucidating the mechanism of clinical Teliso-v (cMet-MMAE ADC) and osimertinib combination: A CAV1 and EGFR multi-protein complex controls endolysosomal trafficking of Teliso-V.

Izhar Batth[1], Ronald Pandoy[1], Lisa Roberts-Rapp[2], Athan Vasilopoulos[2], Peter Ansell[2], Vincent Blot[2], Gregory K. Potts[2], Janice Y. Lee[2], Hua Tang[2], Jon D. Williams[2], Tamar Uziel[2], PK Epling-Burnette[2]

[1]AbbVie Inc., South San Francisco, CA,[2]AbbVie Inc., North Chicago, IL

Introduction: Telisotuzumab Vedotin (Teliso-V), a first-in-class c-Met-MMAE antibody-drug conjugate (ADC), achieved FDA breakthrough therapy designation in c-Met overexpressing (OE), 2nd Line+ EGFR wild-type, non-squamous, non-small cell lung cancer (NSCLC) patients. In addition, in a phase 1/1b trial, Teliso-V demonstrated 50% response in combination with the EGFR inhibitor, osimertinib, in *EGFR* mutant (*EGFR*-MT) NSCLC patients who had progressed on osimertinib. Here, we present mechanistic insights into the combinatorial effects of Teliso-V with osimertinib in this patient population.

Methods: EGFR-MT NSCLC cell lines were exposed to osimertinib and subjected to RNA expression, mutation, EGFR-interactome, and ADC intracellular localization and endolysosomal (EL) trafficking analysis.

Results: While no mutation gains or losses were detected, chronic and acute treatment with osimertinib enhanced Teliso-V sensitivity in *EGFR*-MT NSCLC cells. In addition to enhanced Teliso-V cytotoxicity, we observed enhanced accumulation of Teliso-V in a low pH intracellular compartment, consistent with increased endolysosome (EL) trafficking. Chronic osimertinib exposure induced gene expression changes related to increased lysosomal membranes, lytic vacuole membranes, and endoplasmic reticulum as well as reduced EGFR signaling. In addition to osimertinib, other EGFR inhibitors including erlotinib, gefitnib, or afatinib also enhanced Teliso-V EL trafficking which is consistent with the effect of *EGFR*-MT signaling in suppressing c-Met ADC EL trafficking. Cells harboring *EGFR*-MT are known to form large multimeric protein complexes to regulate EGFR trafficking and cell survival. EGFR-MT immunoprecipitation, followed by mass spectrometry analysis identified 84 proteins associated with an osimertinib-sensitive complex including c-Met, and caveolin-1 (CAV-1), a structural protein involved in signaling and trafficking. Confocal microscopy imaging showed that osimertinib treatment altered the subcellular localization of these proteins and their interaction with CAV-1. CAV-1 knockdown disrupted the multimeric protein complex, suppressed EGFR and c-Met activation, reduced downstream AKT/ERK signaling, and enhanced Teliso-V-associated EL trafficking, phenocopying osimertinib effect.

Conclusions: Chronic and acute treatment with osimertinib displaces EGFR/c-Met/CAV-1 multimeric signaling complexes leading to improved lysosomal processing of c-Met-targeted ADCs and enhanced tumor cytotoxicity. This study provides mechanistic insights into the combination of Teliso-V and osimertnib in 2L+ *EGFR*MT NSCLC patients in the clinic.

#4714 Elucidating mechanisms of acquired resistance to mobocertinib in non-small cell lung cancer harboring EGFR exon 20 insertion mutations.

G. Yamamoto[1], Y. Tanaka[1], Y. Zenke[1], J. Liu[1], T. Sakai[1], H. Izumi[1], E. Sugiyama[1], S. Umemura[1], S. Matsumoto[1], K. Yoh[1], K. Goto[1], S. S. Kobayashi[2], H. Udagawa[1];

[1]National Cancer Center Hospital East, Kashiwa, Chiba, Japan, [2]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA

Mobocertinib (TAK-788) is an oral irreversible tyrosine kinase inhibitor specifically developed to target *EGFR exon 20 insertion (ex20ins)* mutant non-small cell lung cancer (NSCLC); however, acquired resistance inevitably develops, limiting its clinical efficacy. This study aims to elucidate mechanisms of mobocertinib resistance through multiple clinical and preclinical approaches. Through targeted Next-Generation Sequencing on tumor samples from a patient with *EGFR ex20ins* (A767_S768insSVD) mutant NSCLC treated with mobocertinib, we identified secondary *KRAS Q61H* mutation as a potential resistance mechanism. To recapitulate clinical resistance, we successfully established a cell line (TH937) derived from this patient's pre-treatment tumor. *In vivo* mobocertinib-resistant tumors (mobo-R) were generated by continuous oral administration of mobocertinib to TH937 xenograft nude mice. Although no actionable secondary *EGFR* or *KRAS* mutations were identified in whole exome sequencing, transcriptomic analyses revealed upregulation of MAPK and RAS-related signaling in mobo-R tumors. *In vitro* analyses also showed reactivation of ERK signaling after mobocertinib treatment, while phosphorylation of 49 major receptor tyrosine kinases (RTKs), including EGFR and other ErbB family members, was not observed. These finding indicates that RTK-independent MAPK reactivation, potentially driven by RAS signaling, may serve as a resistance mechanism. In conclusion, we found that genomic or transcriptomic alterations of MAPK/RAS signaling mediate mobocertinib resistance. Further validation and exploration are needed to improve the therapy of *EGFR ex20ins*mutant NSCLC.

#4715 Depletion of mMDSCs using a CCR2-directed cytotoxicity targeting chimera (CyTAC) leads to remodeling of the tumor microenvironment and immune mediated tumor regression.

Brandon J. Turunen[1], Beth Knapp-Reed[2], Craig Leach[1], Peiling Chen[3], Matthew Sender[4], Joseph Marino[2], Cunyu Zhang[3], Yao Chen[5]

[1]Solu Therapeutics, Boston, MA,[2]Medicinal Chemistry, GSK, Collegeville, PA,[3]Chemical Biology, GSK, Collegeville, PA,[4]AntibodyChem Biosciences, Boston, MA,[5]Merck, Rahway, NJ

mMDSCs (monocytic myeloid derived suppressor cells) are recruited to solid tumors and serve as potent inhibitors of T-cell mediated antitumor immunity. Therapeutic depletion of mMDSCs presents an exciting opportunity to increase the frequency and durability of anti-tumor clinical responses achieved with immune checkpoint inhibition. Currently, no marketed or investigational agents function by specific mMDSC depletion and no specific markers for therapeutic targeting of these cells have been clinically validated. We demonstrate that mMDSCs express high and homogeneous levels of the chemokine receptor CCR2. Because of this, we have developed a CCR2 targeting Cytotoxicity Targeting Chimera, which potently binds CCR2 on cells, and when combined with the CyTAC platform antibody, eliminates target cells via enhanced effector mediated killing. We demonstrate that the CCR2 CyTAC can deplete CCR2+ monocytes and mMDSCs from the periphery and intratumorally both ex vivo and in vivo. In the MC38 syngeneic tumor model this depletion leads to T-cell dependent anti-tumor activity and increases the levels of CD8 T-cells within the tumor microenvironment. We show across multiple additional syngeneic tumor models that depletion of CCR2+ cells remodels the tumor microenvironment causing an increase in tumor infiltrating CD8 T-Cells and a decrease in regulatory T-cells and mMDSCs. These PD changes were observed in both responding and non-responding tumor models where response can be enhanced with anti-PD1.
Significance: We have applied our CyTAC technology to specifically target CCR2 expressing cells and depletion of mMDSCs and demonstrate that this can lead to remodeling of the tumor microenvironment from a pro-tumor to pro-inflammatory phenotype. This agent has potential for treating tumors with high mMDSC content regardless of tumor type. Note: Human biological samples were sourced ethically, and their research use was in accord with the terms of the informed consents under an IRB/REC approved protocol. All studies conducted according to GSK's Policy on the Care, Welfare and Treatment of Lab Animals and reviewed by the Institutional Animal Care and Use Committee at GSK or by the ethical review process at the institution where the work was performed.

#4716 Functional precision medicine: Uncovering high actionability in rare cancers beyond genomics.

Rachele Rosati[1], Payel Chatterjee[1], Alex C. Rajewski[1], Marwah Al-Aloosi[1], Lauren R. Appleyard[1], Vaishnavi Pallem[1], Shalini Pereira[1], Robert L. Diaz[1], Christopher J. Kemp[2], Ryan Jones[3], Brady Bernard[3], Pashtoon M. Kasi[4], Kiran Turaga[5], Michael Castro[6], Tara Seery[7], Arun Singh[8], Sameek Roychowdhury[9], Melind Javle[10], Jill Alldredge[11], Soledad Jorge[12], Kathryn Pennington[12], Barbara A. Goff[12], Heidi J. Gray[12], Elizabeth M. Swisher[12], Carla Grandori[1]

[1]SEngine Precision Medicine, a Subsidiary of Tempus AI, Seattle, WA,[2]Fred Hutchinson Cancer Center, Seattle, WA,[3]Tempus AI, Inc., Chicago, IL,[4]City of Hope, Irvine, CA,[5]Yale School of Medicine, Chicago, IL,[6]Beverly Hills Cancer Center, Beverly Hills, CA,[7]Hoag Medical Group, Irvine, CA,[8]UCLA Health, Santa Monica, CA,[9]Ohio State University, Columbus, OH,[10]MD Anderson Cancer Center, Houston, TX,[11]University of Colorado, Highlands Ranch, CO,[12]The University of Washington, Seattle, WA

Background: Precision medicine relies on mutational profiling, but clinical actionability remains limited for many cancer patients. We evaluated the feasibility and actionability of ex-vivo drug sensitivity testing across solid tumors, focusing on rare cancers. This study uses the PARIS® CLIA-certified test, assessing patient-derived tumor cells (PDTCs) responses to a broad oncology drug panel.

Methods: Tumor specimens (biopsies, fluids, or surgical samples) were shipped to our laboratory to arrive within 48h and cultured in either 3D or 2D with cancer type specific protocols to generate short term tumor cell cultures. PDTCs were tested with a panel of oncology drugs (up to 42 drugs /sample), customized for each patient based on cancer type, oncologist recommendations, and genomic features. Reports, ranking drug responses, were sent to oncologists within ~3 weeks on average.

Results: 86 patients with diagnosis of rare cancers (occurring in fewer than 15 out of 100,000 people each year) were consented to the SEngine IRB protocol and had a successful PARIS® test. The overall actionability was 85%, defined by one or more drugs exhibiting exceptional/good responses based on absolute and relative metrics of drug responses in our database. The cohort included biliary tract cancers (n=26), sarcomas (n=15), low grade serous ovarian cancers (n=15), cancer of unknown primary (n=4), appendiceal cancers (n=4), esophageal and gastric cancers (n=6), brain and nervous system cancers (n=2). Notably, targeted therapies showed the strongest drug sensitivities, often revealing actionable insights beyond genomic predictions. In the LGSOC cohort in the recurrent setting the results demonstrated that the PARIS® test recapitulates the clinical resistance of LGSOC to most chemotherapies but can identify multiple targeted treatments for nearly all patients. Importantly, few of these agents wouldn't have been chosen based on standard molecular testing, and multiple patients have demonstrated months to years of disease stabilization, biomarker remission and good quality of life with PARIS® -informed therapies (Gray et al., NPJ 2023, and unpublished data). As an example, one LGSOC case, which did not present any actionable biomarkers and had progressed after standard chemotherapy, achieved disease stabilization with 29 months duration, no hospitalizations, following a PARIS®-informed therapy with regimens including Lapatinib, Everolimus, Trametinib and Letrozole. Currently, the patient has biomarker remission demonstrated by ctDNA and CA-125. Cases from other cancer types will be presented.

Conclusions: The PARIS® test offers an unbiased ex-vivo measurements of oncology-drug responses and can identify responders to targeted therapies also in cases lacking biomarker-drug matches, particularly in rare tumors with complex genomics or gene fusions without targeted drugs and limited on-label treatment options.

#4717 Glycosylation inhibition in multiple myeloma and its impact on treatment.

Heni Patel, Sharnett Riley, Hiral Kapadia, Robert B. Campbell

Department of Pharmaceutical Sciences, Massachusetts College of Pharmacy and Health Sciences, Worcester, MA

Background: Multiple myeloma (MM) is a rare and incurable blood cancer that predominantly affects older adults. It is characterized by the uncontrolled proliferation of malignant plasma cells within the bone marrow, which often exhibit abnormal glycosylation patterns that may influence their interactions with chemotherapy. Notably, MUC1-C is aberrantly expressed in MM cells, playing a pivotal role in their growth and survival, making it a promising target for therapeutic intervention. Research has demonstrated that the inhibition of MUC1-C with benzyl-α-GalNAc enhanced the susceptibility of these cells to CTL-mediated immune responses and the effects of cytotoxic drugs. Modifying the glycosylation of MUC1-C has the potential to increase the susceptibility of MM cells to clinically relevant treatment approaches. Building on this, the current study investigated the use of glycosylation inhibitors as a strategy to improve drug efficacy in MM. We hypothesized that the glycosylation levels normally observed in MM cells functions as a cellular barrier to therapeutic agents.
Method: The RPMI-8226 human plasmacytoma cell line (ATCC CCL-155) was cultured aseptically in RPMI-1640 growth medium supplemented with 10% fetal bovine serum (FBS). The cells were subsequently seeded into 24-well plates and pre-exposed to the glycosylation inhibitor, benzyl-α-GalNAc. Following a 48-hour incubation, the plates were next exposed to various agents for an additional 24-hr period. To assess the extent to which glycosylation influences drug uptake, one study involved the use of a fluorescently tagged anti-MUC1 antibody. To examine the influence of glycosylation on drug effects, the clinical agents imatinib and cyclophosphamide were also employed.
Results: The pre-exposure of RPMI-8226 to glycosylation inhibitor cells resulted in greater uptake of the anti-MUC1 antibody compared to the untreated cells ($P = 0.0008$). Furthermore, the combination of glycosylation inhibitor and imatinib improved the drug effects when compared to imatinib alone ($P = 0.0012$). A similar observation was observed with cyclophosphamide ($P = 0.0329$). These findings emphasize the promising potential of combining therapeutic agents with glycosylation inhibitors to improve the effectiveness and precision of drug therapy.
Conclusion: The application of a glycosylation inhibitor significantly enhanced drug uptake and subsequent drug effects. The promising findings suggest that the alteration of glycosylation patterns in MM may improve drug treatment efforts.

#4718 Evaluation of the potential use of glycosylation inhibitors for the treatment of chronic myeloid leukemia: A transformative approach to drug therapy.

Norymar Ayala, Binyaz Ilavia, Zein Dib, Najlaa Alsini, Robert B. Campbell

Department of Pharmaceutical Sciences, Massachusetts College of Pharmacy and Health Sciences, Worcester, MA

Background: Chronic Myeloid Leukemia (CML) is a clonal disorder of hematopoietic stem cells characterized by excessive production of neutrophils. The disease is driven by the Philadelphia chromosome (BCR-ABL1), caused by the translocation of chromosomes 9 (ABL - pro-oncogene) and 22 (BCR). The genetic alterations lead to heightened tyrosine kinase activity, thereby promoting the proliferation of myeloid cells and decreased levels of apoptosis. Leukemic cells have been shown to display aberrant glycosylation, particularly involving O-glycans, referred to as TACAs (Tumor Associated Carbohydrate Antigens). The relationship between glycosylation and drug effects has been shown for solid tumors, such as pancreatic cancer, but not yet for hematological malignant types, such as CML. For this reason, the following study investigated the influence of type O-glycosylation on drug uptake and activity against a model CML cell line (K-562-GFP) *in vitro*.
Method: The CML cell line K-562-GFP was cultured in RPMI + 10% fetal bovine serum (FBS). To assess the cytotoxic effects, the glycosylation inhibitor (benzyl-α-GalNAc) was employed to establish the maximum non-toxic concentration. In general, the K-562-GFP cells were seeded in a 24-well plate and subsequently exposed to a non-toxic concentration of Benzyl-α-GalNAc for a required length of time. A fluorescently tagged anti-MUC1(SPM533) monoclonal antibody was initially employed to evaluate the glycosylation drug barrier effect and functional activity of imatinib in the presence and absence of the glycation mesh.
Results: Preliminary studies demonstrated a significant increase in the uptake of Anti-MUC1 (SPM533) antibodies by K-562-GFP cells following exposure to the O-glycosylation inhibitor ($P < 0.0001$). Imatinib resulted in significantly higher cellular death for K-562-GFP cells pre-exposed to glycosylation inhibitor than in imatinib treatment alone ($P < 0.0001$). Early preliminary findings suggest that glycosylation may play an important role in regulating cytotoxic drug entry into target cells and in the subsequent functional activity in vitro. Additional studies involving other relevant drug agents are currently underway.
Conclusion: Initial findings support the notion that targeting glycosylation could represent a promising therapeutic strategy, as O-glycosylation may serve as a barrier limiting drug effects in CML. Ongoing studies will evaluate the potential impact of glycosylation on FDA-approved agents used for the treatment of CML.

#4719 Discovery of potential synergistic targets for PKMYT1 inhibitor through AI-assisted drug combination screening in an MPS-based vascularized metastatic gastric cancer model.

Yeongmin Choi, Sumin Kim, Yangji Kim, Youngsook Song, Jinhwan Jang, Delaney Donnelly, Bushra Rajput, Tsung-Li Liu, Kyusuk Baek, Aneesh Sathe, **Sanghee Yoo**

Qureator, Inc., San Diego, CA

Introduction: Many approvals of advanced drugs, such as Antibody-Drug Conjugates (ADCs) or immunotherapies, have not provided significant treatment benefits for patients with metastatic gastric cancer (GC). There remains an urgent need to develop novel therapies. However, the lack of well-defined oncogene targets has hindered progress in therapy development. A patient-like disease model was established using patient samples and a 3D co-culture platform, the Microphysiological System (MPS). Patient cells in this model preserved key properties and successfully replicated clinical phenotypes in the model. Promising therapeutic targets were identified through chemogenomic screening. To address resistance to single-target inhibition, potential combination targets were predicted using AI tools enhanced with target-based knowledge graphs (KG) and active learning algorithms. The top 20 combination targets were selected and tested, resulting in promising combination hits that demonstrated significant efficacy for this challenging and heterogeneous disease. The integration of AI-assisted combination discovery with the patient-like disease model has uncovered new opportunities for developing novel therapies and combination treatments.

Methods: For the vascularized GC model, patient cells, isolated from surgical specimens or ascitic fluid, were expanded and formed into spheroids. These spheroids were co-cultured with Human Umbilical Vein Endothelial Cells (HUVECs) in Curiochips by embedding them in a hydrogel. The chemogenomic library was developed based on pathway analyses of RNAseq and proteomics studies of advanced gastric cancer. Additionally, the combination library was designed by the process utilized AI for image feature extraction, supplemented by a target-based knowledge graph (KG) that integrated data from over 20 public databases, encompassing genomics, proteomics, drug interactions, and pathway information. All screens were performed using high-throughput screening (HTS) instruments, including high-content confocal microscopy, leveraging the HTS compatibility of Curiochips.

Results: Metastatic GC patient samples exhibited clinical phenotypes of aggressive migration and angiogenesis when co-cultured with endothelial cells in 3D. From pathway analysis, 200 targets were identified, and their inhibitors were screened in the vascularized model. The top targets were prioritized based on image analysis of GC phenotypic landscape. Among these, PKMYT1 was selected as a test case for identifying potential combination targets. Using the target-based KG, 20 top targets for combination with the PKMYT1 inhibitor were identified and screened in the disease model. The combination demonstrated promising efficacy and significantly delayed the onset of drug resistance.

#4720 Evaluation of lorlatinib efficacy using plasma DNA.

Yasushi Goto[1], Tadaaki Yamada[2], Akehiro Nishiyama[3], Naoko Aragane[4], Minehiko Inomata[5], Hiroki Nagai[6], Satoshi Takahashi[7], Kazuhito Miyazaki[8], Yukari Tsubata[9], Hiroaki Akamatsu[10], Tetsuya Mitsudomi[11]

[1]National Cancer Ctr. Japan, Tokyo, Japan,[2]Department of Pulmonary Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan,[3]Department of Medical Oncology, Kanazawa University Hospital, Kanazawa, Japan,[4]Takagi Hospital, Okawa, Japan,[5]Toyama University Hospital, Toyama, Japan,[6]Kobe City Medical Center General Hospital, Kobe, Japan,[7]Department of Surger, Tokyo Medical University, Tokyo, Japan,[8]Respiratory Medicine, Yokohama Municipal Citizen's Hospital, Tokyo, Japan,[9]Shimane University, Izumo, Japan,[10]Internal Medicine III, Wakayama Medical University, Wakayama, Japan,[11]Izumi City General Hospital, Izumi, Japan

Background: Lorlatinib is approved globally as a second-line treatment for ALK-positive NSCLC following resistance to alectinib, with Japan being the first country to approve its use. This study is the first to analyze lorlatinib's efficacy in such patients by integrating ctDNA analysis to explore predictive markers and resistance mechanisms.
Methods: This multi-center prospective study enrolled patients with ALK-rearranged NSCLC resistant to alectinib, with no prior exposure to ALK-TKIs other than alectinib and brigatinib. Patient eligibility required documented ALK translocations, CT imaging within 56 days, and written consent. Blood and pathology samples were collected pre- and post-treatment. Clinical data were integrated using an Electronic Data Capture (EDC) system. ctDNA analysis was conducted via Guardant Health, focusing on mutation emergence, persistence, and correlation with treatment outcomes.
Results: Among 27 evaluable patients, partial response (PR) was observed in 37% of cases, while stable disease (SD) was noted in 14.8%. The median progression-free survival (PFS) was 6.5 months (95% CI: 1.8-23.5), consistent with previously reported outcomes for lorlatinib-treated patients. Disease progression commonly involved the CNS (18.5% of cases). Genetic analysis of ctDNA revealed that patients with TP53 mutations had a reduced PFS of 2.9 months compared to 6.7 months in wild-type TP53 cases, suggesting a potential role as a poor prognostic marker. Additionally, persistent ALK fusions or new rearrangements were observed and correlated with reduced efficacy, indicating coactivation of other oncogenic pathways. Notably, two patients exhibited ALK secondary mutations (L1196M) following alectinib treatment, both of whom responded with a partial response (PR) to lorlatinib.
Conclusions: This exploratory study highlights the complexity of resistance mechanisms in ALK-rearranged NSCLC treated with lorlatinib, demonstrating ctDNA's potential as a predictive biomarker for treatment outcomes. The persistence and emergence of specific mutations, such as in TP53 and ALK, underscore the need for further investigation into combination or personalized therapeutic strategies. Despite the study's limited sample size, these findings offer critical insights into the genomic landscape influencing lorlatinib efficacy following alectinib resistance.

#4721 Glycosylation mediated cellular uptake and functional activity of conventional chemotherapy in acute lymphoblastic leukemia.

Kenny D. Pham, Ghada Alhafez, Daniel Muteba, Robert B. Campbell

Department of Pharmaceutical Sciences, Massachusetts College of Pharmacy & Health Sciences, Worcester, MA

Background: Acute lymphoblastic leukemia (ALL) is a heterogeneous hematologic disease characterized by the proliferation of immature lymphoid cells in the bone marrow, peripheral blood, and other organs. Conventional chemotherapeutic approaches are associated with toxicities leading to reduced efficacy over time due to its systemic approach to drug delivery. O-linked glycosylation patterns on lymphoid cells are common modifications on hydroxyl groups of serine or threonine residues that are associated with key functions including cell adhesion and migration. Aberrant expression of O-glycans have been associated with leukemic malignancies making it an attractive biomarker to explore in vitro. These studies aim to provide insights into the influence of O-glycosylation on cellular access of ALL target cells to model therapeutic agents, including antibody and relevant therapeutic agents currently employed for the treatment of ALL.
Methods: The target cell line employed for use in this study was CCRF-CEM. Cells were plated at 20,000 cells/mL in 24-well plates or 500,000 cells/mL in 6-well plates. Cells were exposed to Benzyl-α-GalNAc, an O-linked glycosylation inhibitor, for 48 hours prior to exposure to FITC-tagged Anti-MUC1 antibodies or conventional chemotherapy at various time points. Conventional chemotherapeutic agents were selected based on the National Comprehensive Cancer Network guidelines for the treatment of ALL. Drug agents employed were, cyclophosphamide, imatinib, and doxorubicin hydrochloride. Cell viability was assessed via electronic cell count, Caspase 3, and SRB (Sulforhodamine) assay procedures.
Results: Following inhibition of glycosylation a statistically significant decrease in the uptake of FITC-tagged Anti-MUC1 antibody by CCRF-CEM cells compared to control cells ($P<0.001$) was observed at 4 hours, with no difference reported at 24 hours. The finding suggests that the diminished glycosylation levels surrounding CCRF-CEM cells decreased the access of anti-MUC1 antibody to the membrane core protein. The studies showed that inhibition of glycosylation in CCRF-CEM cells decreased the overall effectiveness of chemotherapeutic agents compared to control *in vitro*. A diminished cytotoxic drug effect was reported for doxorubicin hydrochloride ($P<0.0001$), Imatinib ($P<0.0001$) and Cyclophosphamide ($P<0.001$), when compared to the effects of each agent alone. These findings were further supported by the cytotoxicity results from the SRB and Caspase 3 assay.
Conclusions: Our preliminary findings support the notion that the inhibition of O-linked glycosylation in ALL cells decreased, rather than increased, the entry of drug agents into ALL cells. The data are supported by diminished general effects of conventional chemotherapeutic agents against the target cells. Additional confirmation studies are currently underway.

#4722 Crucial prognostic biomarkers for resistance to KRAS-G12C inhibitors.

Y. Wang, L. Tian, C. Liu, S. Zheng, H. Shi, F. Wei, W. Jiang, Y. Dong, H. Xu, E. Yin, N. Sun, J. He;
National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Objective: KRAS-G12C inhibitors are promising in targeted therapeutic strategies. However, it remains uncertain to search for biological reminders of clinical outcomes and early resistance.
Methods: Systematic review has been performed up to September,2024 to evaluate treatment efficacy and beneficial patients. We constructed KEAP1 and STK11 knockout mutants with CRISPR/Cas9 technology and PD-L1 overexpression mutants with lentiviral vectors. The sensitivity of cells to different KRAS-G12C inhibitors was assessed using CCK-8 assays. Potential pathways of resistance were identified through comprehensive bioinformatics analyses.
Results: 13 clinical trials with 1,132 enrolled patients indicated remarkable clinical efficacy and benefit the senior and the female. Patients with liver and brain metastases also tends to benefit from KRAS-G12C inhibitors. KEAP1 mutation serves as a critical negative biomarker for early progression and resistance. Validation studies indicated a notable increase in IC50 values for different KRAS-G12C inhibitors after KEAP1 knockout ($P < 0.0001$). Differentially expressed genes in KEAP1 knockout cells versus contrast groups were conducted with GO, KEGG, and Reactome pathway analyses, suggested evident enrichment in extracellular matrix organization and cell adhesion processes. In addition, STK11 mutations and an increasing PD-L1 expression level reminds of worse prognosis, despite absence of significant difference.
Conclusion: This research considered KRAS-G12C inhibitors as optional therapy and selected some subgroups who are more likely gain benefits from targeted therapy. As a key adverse biomarker, KEAP1 mutation tends to affect extracellular matrix organization and cell adhesion and promotes early resistance.

#4723 Development and preclinical activity of BSI-721, a novel antibody-drug conjugate (ADC) targeting cadherin 17 (CDH17) in gastrointestinal cancers.

Xiaoyao Hao[1], Jingyu Liu[1], hongyan li[1], jun li[1], jialong wang[1], **Hui-Han Hu**[2], frank liu[1], mingjiu chen[1], Qun Lv[1]

[1]Biosion, Inc., NanJing, China,[2]Biosion, Inc., Taipei, Taiwan

Background: Cadherin 17 (CDH17) is a cell adhesion protein that belongs to the cadherin superfamily. CDH17 is highly restricted to tight junctions in normal gastrointestinal (GI) tissues. In contrast, CDH17 is frequently overexpressed and becomes accessible to targeted therapeutics in several GI malignancies, including colorectal, gastric, pancreatic, and esophageal cancers. This differential expression profile makes CDH17 a promising therapeutic target for antibody-based therapeutics, including ADCs. Here, we described the development and preclinical activity of BSI-721, a novel ADC that combines a fully humanized anti-CDH17 monoclonal antibody (mAb) with monomethyl auristatin E (MMAE) via a cleavable peptide linker.

Methods:The anti-CDH17 mAb was generated from AceMouse™ mice immunized with 293T cells overexpressing human full-length CDH17 and purified CDH17 extracellular domain recombinant protein, sequentially. Binding affinity of the anti-CDH17 mAb was assessed by surface plasmon resonance and flow cytometry. CDH17 expression as cell surface density on cancer cells was determined by quantitative flow cytometry. The anti-CDH17 mAb was conjugated to MMAE via a protease-cleavable valine-citrulline linker. Cytotoxicity of BSI-721 was evaluated in cell lines with varying endogenous expression levels of CDH17. In vivo, efficacy of BSI-721 was assessed in CDH17-high gastric and CDH17-moderate/low pancreatic CDX models.

Results: The anti-CDH17 mAb showed strong binding affinity to both human and cynomolgus monkey CDH17 and cell lines with varying expression levels of CDH17. HIC-HPLC analysis revealed that MMAE was conjugated to the anti-CDH17 mAb in a drug-to-antibody ratio (DAR) of 4 with a high yield of (among which, more than 50% conjugates carrying a DAR of 4). BSI-721 exhibited potent cytotoxicity against cell lines with varying expression levels of CDH17 (subnanomolar IC_{50}). In CDH17-high gastric cancer model (SNU-5), treatment with BSI-721 resulted in significant tumor regression (with achievement of partial response) and tumor growth inhibition (TGI). BSI-721 also demonstrated a significant TGI in CDH17-moderate/low pancreatic cancer model (AsPC1). Additionally, candidate antibodies have been identified with potential to be developed into immunohistochemistry (IHC) companion diagnostic assay.

Conclusion: These data highlight the potential of BSI-721 as an effective targeted therapy for GI malignancies with varying expression levels of CDH17. Further, pharmacokinetics, toxicity, and IND-enabling studies of BSI-721 are underway.

#4724 Evaluating immunotherapy and combination therapy efficacy in kidney cancer using a multicellular 3D immune-microtumor model.

E. Peerani[1], K. Nan[1], J. Ient[1], P. Maximchik[1], N. K. Bah[1], J. Kearney[1], J. A. Hudson[1], R. K. Hare[1], F. Sangkolah[1], G. R. T. de Fraine[1], K. L. Loughrey[1], E. Tham[1], F. Iori[1], M. H. Williams[1], M. G. B. Tran[2], D. N. B. Ranatunga[1];

[1]Pear Bio, London, United Kingdom, [2]Royal Free Hospital, University College London, London, United Kingdom

Advanced renal cell carcinoma (RCC) presents a significant treatment challenge, as response rates to immunotherapies, receptor-tyrosine kinase inhibitors (TKIs) and combination therapies remain unpredictable. A functional precision medicine approach that models tumor-immune interactions can help tailor patient-specific therapy selection, reducing ineffective treatment cycles. Pear Bio's 3D immune-microtumor platform recreates the RCC tumor immune microenvironment (TiME), retaining relevant cell subpopulations to evaluate drug efficacy.

Using fresh RCC tumor tissues and matched blood, the Pear Bio platform generates immune-microtumor cultures within a custom hydrogel optimized to sustain tumor, immune, and stromal cells (N=20). Tumor cells are co-cultured with activated peripheral blood mononuclear cells (PBMCs), with CD8+ T cells and CD8- PBMCs tracked via fluorescent dyes. FDA-approved RCC therapies, including immunotherapies (e.g. ipilimumab, nivolumab, pembrolizumab) and TKIs (e.g. axitinib, lenvatinib), are administered as monotherapies or in combination. Over 4 days, 3D confocal imaging assesses functional metrics including immune cell infiltration and tumor cell death, quantified through a proprietary computer vision pipeline.

Immunofluorescence (IF) of 3D immune-microtumors (N=20) demonstrated retention of diverse TiME cell types—including tumor, endothelial, epithelial, immune, and cancer-associated fibroblasts—at day 0 and day 4 of culture. RNAseq confirmed transcriptional similarity of cells pre- and post-culture, with cell deconvolution further supporting immune compartment retention. Therapy dosing was optimized through molecular assays (IF, Luminex, flow cytometry, in-cell western) and functional screens on immune-microtumors (N=45).

Post optimization, 3D immune-microtumors were used to test clinical treatment regimens and assess combo therapy responses in RCC in our trial (PEAR-TREE N=20 ISRCTN10001405). Pembrolizumab monotherapy increased PBMC and CD8+ cell infiltration by 26% and 14%, respectively, and boosted tumor cell death by 15% compared to controls (N=5). When combined with lenvatinib or axitinib, pembrolizumab further reduced tumor cell viability by 10% and 19%, respectively. This functional analysis reveals intra-patient response variability, underscoring the immune-microtumor model's potential to capture personalized RCC responses to combination therapies.

This 3D immune-microtumor model allows for precise ex vivo testing of combination therapies in RCC, capturing patient-specific interactions within a functional TiME. Current clinical validation in the PEAR-TREE2 RCC trial (NCT06264479) aims to further correlate ex vivo findings with in vivo outcomes, holding promise to guide optimized, personalized RCC treatment.

#4725 Engineering a novel serine-degrading enzyme for targeted cancer therapy.

Ladan Mashouri[1], Ebru Cayir[1], Sarita Bhetawal[1], Daniel J Diaz[1], Vipin Rawat[2], Jonathan Coloff[2], George Georgiou[1], Everett Stone[1]

[1]The University of Texas At Austin, Austin, TX,[2]University of Illinois at Chicago, Chicago, IL

The non-essential amino acid L-serine (serine) is the second most consumed amino acid by cancer cells, supporting their survival and proliferation by contributing to the biosynthesis of nucleotides, lipids, and proteins. Serine-auxotrophic tumors such as luminal breast cancers have compromised serine synthesis pathways, thus rely on extracellular serine, making them vulnerable to serine depletion therapies. While dietary serine restriction, the only current method to reduce serine availability for tumors *in vivo*, has shown limited success in reducing tumor growth, its application is hindered by poor patient compliance and an inadequate reduction of circulating serine. To overcome these limitations, we developed an engineered human serine/threonine dehydratase (eSDH) enzyme that irreversibly depletes over 95% of circulating serine and threonine without requiring dietary changes. Preclinical studies show that eSDH treatment significantly inhibits the growth of serine-auxotrophic tumors. To enhance the therapeutic potential of eSDH and reduce off-target effects, particularly the depletion of the essential amino acid threonine, we focused on improving the specificity of eSDH for serine. Through iterative structure-based engineering, we developed a new SDH variant with over 20 times higher selectivity for serine toward threonine. Both *in vitro* and *in vivo* studies have confirmed that this novel SDH variant depletes serine while preserving threonine in cell culture media and serum. Current efforts are focused on improving the stability of the new SDH variant to extend its half-life in serum and optimize dosing for greater therapeutic efficiency. This advancement allows us to target serine-auxotrophic cancers more precisely while minimizing the impact on normal cells. The broader impact of this work lies in establishing enzymatic serine depletion as a more effective and feasible alternative to dietary restriction for cancer therapy. This approach could provide a new therapeutic strategy for treating various serine-auxotrophic tumors, offering better patient compliance and fewer side effects.

#4726 ETC-501, a novel brain penetrant MNK kinase inhibitor, potentiates TMZ-induced senescence and sensitizes glioblastoma cells to senolytic therapy.

Giselle Nah[1], Yuk Kien Chong[2], Fatin Nasha Sulaimi[1], Kian Leong Lee[1], See Wee Lim[2], Kassoum Nacro[3], Jeffrey Hill[4], Alex Matter[1], Henry Yang[5], Beng Ti Ang[2], Carol Tang[2], Sin Tiong Ong[1]

[1]Duke-NUS Medical School, Singapore, Singapore,[2]National Neuroscience Institute, Singapore, Singapore,[3]Experimental Drug Development Centre, A*STAR, Singapore, Singapore,[4]BeiGene Innovation Center, Guangzhou, China,[5]Cancer Science Institute, National University of Singapore, Singapore, Singapore

Glioblastoma (GBM), the most lethal primary brain tumor in adults, has a median survival of only 15 months, with fewer than 10% of patients surviving beyond five years. Despite aggressive multimodal therapies, including maximal surgical resection, radiation, and temozolomide (TMZ) chemotherapy, recurrence is almost inevitable. Contributing factors include incomplete resection, elevated levels of O6-methylguanine DNA methyltransferase that repair TMZ-induced DNA damage, therapy-resistant glioma stem cells, intratumoral heterogeneity, and the restrictive blood-brain barrier. Additionally, therapy-induced senescence from chemoradiation poses further challenges, as senescent glioma cells secrete pro-tumorigenic senescence-associated secretory phenotype (SASP) factors and may escape senescence, potentially facilitating recurrence. To evaluate the clinical relevance of MNK kinases in gliomas, we analyzed data from The Cancer Genome Atlas (TCGA), showing that elevated MNK1 and MNK2 expression correlates with increased tumor aggressiveness and poor prognosis. Genetic knockdown experiments confirmed that MNK1/2 are essential for GBM growth in vitro and drive aggressive behavior in vivo. To exploit their therapeutic potential, we developed ETC-501, a potent, selective, brain-penetrant MNK inhibitor. ETC-501 inhibited critical GBM processes, including MYC-driven transcriptional programs, DNA replication, ribosome biogenesis, inflammatory responses, and coagulation. Importantly, in combination with TMZ, ETC-501 significantly enhanced TMZ-induced senescence and reduced tumor-promoting SASP factors in GBM cells, acting both as a senescence inducer and senomorphic. Leveraging this enhanced senescence, we increased the susceptibility of GBM cells to the senolytic agent navitoclax, enabling targeted clearance of residual senescent cells. This "one-two punch" therapeutic strategy effectively eradicated cells that could otherwise act as reservoirs for recurrence. Our findings underscore the therapeutic potential of MNK inhibition in GBM, advancing our approach to managing GBM and potentially other malignancies characterized by senescence and SASP.

#4727 Exploring glycosylation as a barrier to drug therapy in lymphoma: unlocking new therapeutic potential.

Shayan Mosaffa, Thao Nguyen, Nasr Issa, Robert B. Campbell

Department of Pharmaceutical Sciences, Massachusetts College of Pharmacy & Health Sciences, Worcester, MA

Background: Lymphoma, a cancer more common in younger males, poses challenges in developing treatments with precise target selectivity. Mucin, a glycosylated protein overexpressed in tumor cells, forms a barrier shown elsewhere to impede drug effects. For example, we previously demonstrated in pancreatic cancer models that the inhibition of mucin glycosylation improved treatment outcomes *in vitro* and *in vivo*. This approach remains unexplored in lymphoma. This study examines the extent to which mucin glycosylation impacts the intracellular uptake and effectiveness of lymphoma therapeutics *in vitro*. We hypothesize that glycosylation functions as a cellular barrier limiting cellular access to model agents and subsequent drug effects.
Methods: The U-937 human lymphoma cell line (ATCC CRL-1593.2) was cultured in RPMI-1640 medium supplemented with 5% fetal bovine serum (FBS). Cells were seeded in 24-well plates and exposed to a glycosylation inhibitor (benzyl-α-GalNAc). After a required incubation period, the cells were exposed to a fluorescently tagged anti-MUC1 antibody (used to probe the degree of cell access to molecular entities in the presence and absence of the glycation mesh). Antibody uptake was analyzed using a fluorescent microplate reader. The functional drug effects of pre-exposing U-937 cells to the glycosylation inhibitor were initially assessed through cell viability experiments (using Quadcount™), with follow-up studies using a fluorometric assay.
Results: At 1 hour, cells that were pre-exposed to the glycosylation inhibitor demonstrated significantly higher uptake of the anti-MUC1 antibody compared to untreated cells ($p = 0.0266$), suggesting enhanced drug delivery in the early stages. Future studies will investigate whether the uptake is time-dependent, favoring prolonged cellular exposure to the antibody. Preliminary data shows that the pre-exposure of U-937 cells to benzyl-α-GalNAc enhanced the effect of imatinib significantly when compared to imatinib alone ($P= 0.0240$). The impact of cellular glycosylation on the effect of additional therapeutic agents is currently under investigation.
Conclusions: Targeting mucin glycosylation with benzyl-α-GalNAc enhanced anti-MUC1 antibody uptake. The improved cell kill observed with the inhibitor-imatinib combination further underscores the promise of this strategy in overcoming drug resistance in lymphoma.

#4728 The selective Yap/Tead inhibitor TY-1054 re-sensitizes acquired resistance to multiple tyrosine kinase-based targeted therapies.

Shengli Dong*,#[1], Apeng Liang*,#[1], Zhengfei Guo#[1], Meihua Li[1], Huan Wang[2], Shuaibo Han[1], Hongqiang Li[1], Jing-Lan Mu[2], Yakun Qi[2], Chao Zhou[1], Yu Yu[1], Xinlong Yang[1], Lian Fang[1], Zhiyong He[1], Haoyun Li[1], Chengshan Niu[3], Mingyu Jiang[2], Xiugui Chen[2], Shaoqing Chen[2], **Jun Li**[2], Yusheng Wu[3]

[1]TYK Medicines, Inc., Changxing county, Huzhou City, China,[2]TYK Medicines, Inc. (Shanghai), Xuhui District, Shanghai, China,[3]TYK Medicines, Inc. (Zhengzhou), Zhengzhou, Henan, China

Introduction: Dysregulation of Hippo/YAP signaling is widespread in human tumors. YAP/TEAD activation is one of the major reasons for resistance in response to targeted therapies. Therefore, the Hippo/YAP signaling pathway is an emergent therapeutic target for inhibiting tumor progression and drug resistance. Small molecules that block auto-palmitoylation of TEADs, such as VT3989 (NCT04665206) and BPI-460372 (NCT05789602), have been successfully to enter the clinical trials. TY-1054 is a potent TEAD inhibitor fully developed by TYK Medicines Inc.

Results: TY-1054 had excellent efficacy in the H226 CDX and HNSCC PDX mouse models. TY-9591 is a third-generation EGFR inhibitor developed by TYK Medicines and is currently under pivotal Phase III clinical investigation in China. Our studies showed that TY-9591 had excellent efficacy in the PC9 CDX mouse model, but tumors gradually recurred, as Osimertinib did. Drug-tolerant persister (DTP) and Yap/Tead activation are believed to cause drug resistance to Osimertinib. We found that TY-1054 significantly inhibited the expansion of DTP cells that emerged in PC9 and H-1975 cells after TY-9591 treatment. To investigate whether combined YAP inhibition would result in an extension of responses to EGFR therapy, we treated EGFR-driven NSCLC cell lines with TY-1054 and TY-9591. The results showed that the combination treatment not only offered synergistic effects, but also enhanced apoptosis, compared to single-drug treatment. Furthermore, TY-1054 in combination with TY-9591 significantly delayed tumor regrowth in the PC9 CDX mouse model. The renal safety concerns associated with Yap/Tead inhibitors have attracted significant attention. Since the chemical structures of VT3989 and BPI-460372 have not yet been disclosed, we examined the renal toxicities of TY-1054 with reference compounds VT-105, an analog of VT3989, and TY-2225, which is a Tead inhibitor protected by a polymorph patent of Betta Pharma (WO2023155760A1). In a rat renal toxicity experiment, TY-1054 at a dose of 2.5 mg/kg caused much less albuminuria than VT-105 and TY-2225. To address the differences in renal toxicity between species, we performed a mouse renal toxicity study using TY-1054, VT-105, and TY-2225. In terms of preclinical efficacy and safety, the data suggest that TY-1054 is potentially the best-in-class compound compared to its major competitors.

In summary, has a favorable PK profile, good drug exposure, manageable tolerance at high drug exposure levels, and a potentially wide therapeutic window. IND clearances from the US FDA and CN NMPA have received in 2024. This study provides a strong rationale for testing TY-1054 in human clinical trials. The successful execution of the TY-1054 clinical trial will provide a powerful solution for future precision oncology. # contributed equally to this work. * To Whom Correspondence should be addressed.

#4729 The reversible and selective FGFR2/3 inhibitor CGT4859 has superior target coverage of resistance mutations missed by leading FGFR inhibitors.

John Fischer, Karyn Bouhana, Richard Brizendine, Mark Chicarelli, Brad Fell, Jennifer Fulton, Anna Guarnieri, Leyla Haygood, Ravi Jalluri, Amber Johnson, Keith Koch, Erika Koslov-Davino, Macedonio J. Mejia, Rob Rieger, John Robinson, Mareli Rodriguez, Francis Sullivan, Yang Wang, Shannon Winski, Silas Wood, Yeyun Zhou

Cogent Biosciences, Boulder, CO

FGFR2 and FGFR3 alterations are oncogenic events across many solid tumors, often through chromosomal fusion or activating mutations. FGFR2 translocations are prevalent in intrahepatic cholangiocarcinoma, while FGFR3 mutations and fusions are prevalent in bladder cancer. Both indications have approved pan-FGFR inhibitors that demonstrate responses in patients that harbor these genetic alterations, however, responses are short-lived or show reduced activity in patients with on-target resistance mutations. In addition, efficacy of these agents has been hampered by inhibition of FGFR1, which leads to dose-limiting hyperphosphatemia. To address these issues, we present our clinical candidate CGT4859, a novel reversible FGFR2/3 inhibitor with activity against clinically relevant mutations and >140-fold selectivity over FGFR1. In vivo characterization of CGT4859 demonstrates robust efficacy in target-altered models at tolerated doses. Together, these data demonstrate CGT4859 as a potential best-in-class FGFR1-sparing pan-mutant FGFR2/3 inhibitor.

#4730 The selective WEE1 inhibitor azenosertib shows synergistic anti-tumor activity with encorafenib + cetuximab in multiple BRAF^{V600E} models.

Nathan M. Jameson, **Harshit Shah**, Blake Skrable, Mona Abed, Hooman Izadi, Nam-phuong Nguyen, Olivier Harismendy, Mark R. Lackner

Zentalis Pharmaceuticals, San Diego, CA

BRAF^{V600E} is a potent oncogenic driver which results in downstream hyperactivation of MAPK signaling and unchecked oncogenic growth, while simultaneously increasing replication stress (RS) and accumulation of DNA damage. The BRAF^{V600E}-specific inhibitor encorafenib in combination with the anti-EGFR monoclonal antibody cetuximab (E + C) are approved in the second line plus (2L+) setting in metastatic colorectal cancer (mCRC) resulting in 20% overall response rate (ORR) in the confirmatory Phase 3 study. However, many patients do not respond, and alternative therapeutic options are still needed in this challenging patient population. WEE1 is a kinase involved in cell cycle progression, allowing cells to repair damaged DNA before entering the next cell cycle phase. Consequently, inhibition of WEE1 leads to premature mitotic entry, inducing replication stress (RS) and unchecked accumulation of DNA damage, ultimately resulting in mitotic catastrophe and cell death. Paradoxically, inhibition of oncogenic drivers like BRAF^{V600E} have been demonstrated to drive high levels of chromosomal instability (CIN) and RS. We hypothesized that high levels of CIN and RS in *BRAF^{V600E}*-mutant tumors before and after treatment with E + C present a vulnerability to WEE1 inhibition and provide rationale for combining azenosertib, a novel, selective, and orally bioavailable WEE1 inhibitor, with E + C. Here, we report the effectiveness of azenosertib combined with E + C as an anticancer treatment in *BRAF^{V600E}* CRC models. *In vitro* combination of azenosertib with E + C increased cell growth inhibition over monotherapy azenosertib or doublet E+ C across multiple *BRAF^{V600E}* cell lines, and was accompanied by a decrease in activity of MAPK and mediators of cell cycle checkpoints, highlighting possible mechanisms for synergy. *In vivo* treatment of *BRAF^{V600E}* CRC cell-line derived xenograft (CDX) models with azenosertib demonstrated significant monotherapy tumor growth inhibition (TGI) in the LS411N model (69%) as well as synergistic TGI and tumor regression when combining azenosertib with E + C compared against E + C alone in two CDX models (LS411N: 108% vs 63%; HT-29: 101% vs 88%). Similarly, treatment of multiple *BRAF^{V600E}* patient-derived xenograft (PDX, N=3) models of CRC resulted in synergistic triplet TGI (80%, 102%, 103%) and some tumor regression compared against monotherapy azenosertib (43%, 19%, 51%) or the E + C doublet (49%, 65%, 87%). Taken together, our results suggest that the *in vitro* and *in vivo* combination of azenosertib with E + C enhances tumor growth inhibition over single agent azenosertib or doublet E + C therapy, and may be an effective treatment option for patients with *BRAF^{V600E}* mCRC.

ENDOCRINOLOGY: Molecular, Preclinical, and Clinical Endocrinology
Poster Session

#4734 Alteration of tumor immune environment in metastatic and non-metastatic papillary thyroid microcarcinoma.

Rhitajit Sarkar[1], Shilpa Thakur[1], Gus Fridell[2], Noemi Kedei[3], Maria Hernandez[3], Sonam Kumari[1], Maria Merino[3], Joanna Klubo-Gwiezdzinska[1]

[1]NIH National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), Bethesda, MD,[2]Department of Biology, Johns Hopkins University, Baltimore, MD,[3]NIH National Cancer Institute (NCI), Bethesda, MD

Introduction: Papillary thyroid microcarcinomas (PTMC), defined as tumors ≤1 cm in size, are mostly indolent and warrant conservative management. However, up to 10% of PTMC present with clinically significant lymph node metastases or very rarely with distant metastases (M1). Deciphering molecular signatures of metastases-prone PTMCs may help strategize management options. Therefore, the goal of this study was to analyze the transcriptome profiles and tumor microenvironment in metastatic and non-metastatic PTMC.
Methods: Patients diagnosed with indolent non-metastatic (N0/Nx) PTMCs and with tumors metastatic to the lateral neck lymph nodes or distant sites (N1b/M1) were selected by reviewing medical records and pathology slides. RNA sequencing was done on 85 tumor samples and adjacent normal tissues. An R package pathfinder was used to do pathway enrichment analysis utilizing active subnetworks. A subset of samples (n=9) was subjected to multiplex immunofluorescence (MxIF) staining using CD8a and CD20 antibodies. Image analysis of the whole tissue, tumor, and three 400 µm regions around the tumor to count cytotoxic T cells, B-cells, and macrophages was done in a single-blinded manner using HALO (Indica Labs). Differential spatial expression was tested using t-test for normally distributed data and Mann-Whitney test for skewed data, with two-sided $p \leq 0.05$ rendered statistically significant.
Results: The study cohort consisted of 17 patients from N0/Nx group and 19 presenting with N1b/M1 disease. No difference in the clinical characteristics, viz. age (39y vs 46y, p=0.64), gender (female 82.4% vs. 94.7%, p=0.24), median tumor size (0.6cm vs 0.8cm, p=0.27) was found between the groups. There was a significant downregulation of pathways involved in the regulation of B cell activation (FDR-adjusted p-value<0.001), activation of immune response (p<0.001), antigen receptor-mediated signaling pathway (p<0.001), humoral immune response (p<0.001), B cell receptor signaling pathway (p<0.001), pathways activating and regulating cell surface receptor and signal transduction (p<0.001) in primary tumors derived from the N1b/M1 compared to N0/Nx group. However, MxIF revealed no difference in B cell ($CD20^+/CD8a$) infiltration in the primary tumors (p=0.43) nor in the tumor rims (p=0.92) between the groups. In contrast, there was a significantly higher number of cytotoxic T cells ($CD8a^+/CD20^-$) in the rims of the primary tumors from N1b/M1 group compared to N0/Nx (p=0.007).
Conclusion: Metastasis-prone PTMC is characterized by a different immune landscape as compared with the classic indolent variant, with increased cytotoxic T cell infiltration in tumor edges and decreased activation of infiltrating B-cells. GeoMx DSP experiments are undergoing to identify immune cells and their crosstalk with the cancer cells that might be involved in transducing metastatic potential for a subset of PTMCs.

#4735 In silico reconstruction of primary and metastatic estrogen receptor positive breast tumor architecture using geographic information science-augmented spatial transcriptomics.

Jin Young Yoo[1], Sabrina Akter[1], Qianying Zuo[1], Mahima Goel[1], Audrey Lam[2], Debapriya Dutta[2], Betsy Barnick[2], Maria Grosse-Perdekamp[2], Aiman Soliman[1], Zeynep Madak-Erdogan[1]

[1]University of Illinois Urbana-Champaign, Urbana, IL,[2]Carle Foundation Hospital, Urbana, IL

The tumor microenvironment consists of different cell populations, whose interactions contribute to tumor heterogeneity and plasticity and pose challenges to therapy efficacy and prognosis prediction. Breast cancer is one of the highly heterogeneous diseases, and estrogen receptor (ER) positive subtype, the most common one, shows worse prognosis once metastasized. Spatial transcriptomics (ST) is emerging as a powerful tool that offers valuable insights into spatial complexity of tumor microenvironment as well as the cell interactions underpinning tumor heterogeneity. In this study, we aimed to examine tumor heterogeneity of ER positive metastatic breast cancer by applying our geographic information science (GIS)-augmented ST analysis pipeline. Visium datasets were prepared from four metastatic liver tumors from xenograft mouse model and twelve primary and metastatic bone tumors from human patients, including three matching pairs. We integrated geospatial tools, such as multivariate spatial association analysis, with spatial omics data structures to check degree of auto-correlations and visualize localizations of single gene or gene sets. We further compensated for multi-cellular resolution of our spatial data via cell type mapping and deconvolution of cell type proportion (STdeconvolve). We identified hotspot regions displaying extensive co-localization between estrogen response gene set and lipid metabolism gene sets in the fulvestrant-treated metastatic liver tumors from xenograft mouse model. These hotspots were not concentrated in the same area within the tumor and differed by gene sets, suggesting intra-tumor metabolic heterogeneity of the endocrine responsive cells. Our primary samples from human patients showed similar pattern of co-occurrence between estrogen response and metabolic programs as well as cell-cycle related gene sets. On the other hand, mutual exclusivity was observed for estrogen response with immune-related gene sets and metastasis-related ones (epithelial mesenchymal transition, angiogenesis). Compared to primary tumors, metastatic bone tumors showed inter-tumor heterogeneity as such consistency in spatial pattern was obscured. Deconvoluting cell type proportions mapped a higher proportion of mature luminal cells in endocrine responsive hotspots, whereas cancer associated fibroblasts and pericytes were prevalent in the hotspots of metastasis-related gene sets. Our study fully utilized the potential power of ST data by delineating the tumor microenvironment in relation to intra-tumoral heterogeneity as well as cell type proportions. Findings from our study support the development of strategies to target different endocrine resistant cell populations with a combination of metabolic and metastasis-related signaling inhibitors.

#4736 A highly selective CDK4/9 dual inhibitor reverses tumor resistance to CDK4/6 inhibitors.

Jinwen Huang, Zhongyuan Li, Zeng Song, Yunli Liu, **Steve Shen**

Convalife (Shanghai) Co., Ltd., Shanghai, China

Background: CDK4/6 inhibitors have been approved for the treatment of metastatic ER+ and HER2- breast cancer. However, patients will become resistance due to various mechanisms, resulting in high unmet need. Literature suggests that CDK9 activity may be involved in the mechanism of resistance to CDK4/6 inhibitors, and targeting CDK9 could be an effective strategy to overcome breast cancer resistance.
Methods and Materials: Using the Convalife "Right 6D+AI" molecular design platform, AI-based screening and multi-angle evaluation were conducted on over 8,000 virtual molecules, ultimately yielding candidate molecule N049 with selectivity for CDK4 and CDK9 that were >30-fold and >80-fold higher than for CDK6, respectively. To explore the therapeutic potential of N049 in palbociclib-resistant tumors, effects on cell viability were evaluated in both palbociclib-sensitive and -resistant cell lines. Additionally, patient-derived organoids (PDOs) and PDX models were used to assess the impact of CDK4/9 inhibitors on cell viability.
Results: The CDK4/9 inhibitor N049 targeting CDK9 significantly impaired the viability of both palbociclib-sensitive and palbociclib-resistant LTED cell lines. Furthermore, in patient-derived organoids (PDOs) and PDX models, the CDK9 inhibitor drove tumor regression in palbociclib-resistant PDXs.
Conclusion: Our findings suggest that simultaneous inhibition of CDK4 and CDK9 may exert potential synergistic effects to overcome CDK4/6 inhibitor resistance. The use of CDK4/9 inhibitors N049 could be an effective strategy to address resistance to endocrine therapy and CDK4/6 inhibitors in ER+ breast cancer. CDK4/9 inhibitors N049 represent a promising therapeutic approach for overcoming breast cancer resistance.

#4737 IGF and insulin system in regulating hallmarks of cancer.

Bhumika Rajoria, Xihong Zhang, Rick Jansen, Deepali Sachdev, Douglas Yee

University of Minnesota, Minneapolis, MN

The Insulin-like growth factor (IGF) and insulin system is implicated in various cancers and is known to regulate cell growth, proliferation, and metabolism. Type 1 IGF receptor (IGF-1R) targeting strategies have been unsuccessful, probably due to its characteristic to form heterotetrameric hybrids with the homologous insulin receptor (IR). This introduces multiple IGF-1 binding sites and draws focus on IR and its isoforms - IR-A and IR-B, as potential therapeutic targets. A high IR-A to IR-B ratio is observed in tumors including breast cancer. IR-A regulates fetal growth and development while IR-B is predominantly found in the liver and skeletal muscle where glucose uptake is regulated via glucose transporter GLUT4. Non-specific IR targeting likely leads to metabolic imbalance and thus necessitates delineating IR isoform roles to develop efficient targeted strategies. Ideally, targeting IR-A specifically, and leaving IR-B unaffected, might be an effective IGF and insulin system targeting approach. Our group developed IR K/O MCF-7L (ER+ breast cancer cell line) and introduced IR-A or IR-B to generate isoform-specific cell lines. The real-time ATP production rates suggest IGF-1 promotes glycolytic ATP production in MCF-7L, MDA MB 231, MCF-7L IR K/O, and MCF-7L IR-B K/I while insulin at physiological concentration doesn't affect ATP production. Therefore, IR-B may maintain the metabolic demand of ER+ breast cancer cells when activated by IGF-1. Additionally, IR inactivation and metabolic stimulation via IGF-1 suggest investigating alternative fuels to glucose. Our group previously showed IGF-1 activates cystine/glutamate transporter: xC-, in ER+ cells through PI3K/Akt signaling, sequentially regulating ROS. This further supports proliferation, metastasis, and drug resistance. Glutamine is the rate-limiting step for this transporter exchange and hence a potential candidate driving the observed glycolytic phenotype. Preliminary data suggests MCF-7L maintains the metabolic demand through glucose and glutamine when stimulated with IGF-1. IGF-1 also stimulated migration in MCF-7L and MDA MB 231 cell lines while insulin promoted this phenotype in MDA MB 231 only. This suggests that the IGF and insulin system maintains the metabolic demand of cancer cells to promote cancer aggressiveness. Further investigation will elucidate IR isoform roles in cellular hallmarks of cancer.

#4738 Androgen regulation of HER3 in prostate cancer differs by duration of treatment.

Reem Belal Zalloum, Robert A. Holt, John Smetana, Candice Loitz, Lillian Perez, Larisa Nonn

Department of Pathology, University of Illinois at Chicago, Chicago, IL

Background: Prostate cancer (PCa) is the second most common cause of cancer-related death in men and occurs in 1 out of every 8 men. Normal prostate and PCa are responsive to androgens. The interplay between androgens and the ERBB3/HER3 receptor tyrosine kinase has recently been shown to differ by race. ERBB3 was higher in Black/African American patients and correlated with the androgen response pathway despite stable expression of the androgen receptor (AR) transcript. HER3 protein expression was negatively correlated with intraprostatic androgen in Black/African American men. Here we examined the effects of androgen dose and treatment duration on the HER3 pathway in vitro.

Methods: Three PCa cell lines were used; MDA-PCa-2b, derived from an African American patient bone metastasis, LNCaP and LaPC4 derived from white patient lymph nodes. Cells were treated for 48h or 6 months with low or high dose R1881 (synthetic androgen). Endpoints include proliferation (Incucyte), gene expression (RT-qPCR), and protein levels (Western blot). Neuregulin (NRG1) was used to stimulate the HER3 pathway.

Results: PCa treated for 48h with R1881 showed a biphasic effect on proliferation, whereas those treated for 6 months showed a dose-dependent increase. HER3 protein levels were increased after 48h of R1881 but decreased during the 6-month exposure. NRG1 treatment slightly increased cell proliferation. Ongoing experiments will examine NRG1 signaling in the R1881-treated cell lines.

Conclusions: The crosstalk between the HER3 pathway and androgens, two key pathways known to drive aggressive prostate cancer, has been shown to play a more pronounced role in PCa in African American men compared to their White counterparts. Moreover, short-term exposures in vitro may not mimic the effects of androgens in vivo.

#4739 Development of a prognostic ovarian cancer gene signature based on glucocorticoid receptor transcriptional activity.

A. V. Panchamukhi[1], L. Huang[2], E. P. Gray[3], Y. Liu[4], M. Taya[3], J. T. Veneris[5], X. Hou[4], S. Weroha[4], B. Győrffy[6], G. F. Fleming[7], C. Wang[4], S. D. Conzen[3];

[1]Baylor College of Medicine, Houston, TX, [2]Kite Pharma, A Gilead Company, Santa Monica, CA, [3]UT Southwestern, Dallas, TX, [4]Mayo Clinic, Rochester, MN, [5]GSK, Boston, MA, [6]Semmelweis University, Budapest, Hungary, [7]The University of Chicago, Chicago, IL

Background: Epithelial ovarian cancer (EOC), commonly known as ovarian cancer (OvCa), is the deadliest gynecological malignancy. High tumor expression of either *NR3C1* mRNA (encoding the glucocorticoid receptor or GR) or GR protein (IHC) are associated with poor overall survival (OS) in patients (pts) with advanced stage OvCa. We hypothesized that high GR expression and associated GR transcriptional activity favor OvCa cell survival and chemotherapy resistance, thereby reducing OS. Here we investigated whether a GR transcriptome-based gene signature (GR-sig) would be a better prognosticator than tumor *NR3C1* mRNA expression alone.

Methods: mRNAs up- or downregulated >1.5 fold after glucocorticoid treatment (Dex) in one or both cultured OvCa cell lines (OVSAHO and HEYA8) were defined as differentially expressed genes (DEGs). DEGs were then identified in n=1247 unique OvCa microarrays from 15 publicly available Gene Expression Omnibus (GEO) datasets with matched pt OS (training set). We then used the OS data to train a Cox proportional hazards model. The "best subset selection" (BeSS) algorithm and bootstrap sampling identified those GR-regulated DEGs whose tumor mRNA expression correlated most significantly with pt OS, creating a GR-sig "risk score." Subsequently, all n=522 microarray and n=374 RNA-seq publicly available samples from the OvCa TCGA were used as two independent validation sets. Microarray and RNA-seq data were normalized using the MAS5 and FPKM-UQ algorithms respectively. The two validation sets were independently stratified relative to the median training set risk score into low- and high-risk score groups. To examine the prognostic ability of *NR3C1* mRNA expression alone, the median training set *NR3C1* expression value was used to stratify validation set pts into low- and high-expression groups. In all cases, OS was estimated by the Kaplan-Meier method.

Results: We identified n=2288 protein-coding DEGs in one or both cell lines. Of these genes, n=1251 were expressed in each GEO microarray sample (training set). A 10-gene GR-signature (GR-Sig) was then identified using the BeSS algorithm and bootstrap sampling, yielding a median training set risk score of 1.02. In both the microarray and RNA-seq validation datasets, the 10-gene GR-sig risk score was associated with significant OS differences between low- and high-risk score groups (microarray $p = 0.018$; RNA-seq $p = 0.00049$). In contrast, the OS difference between low- and high- *NR3C1* mRNA expression groups did not reach significance (microarray $p = 0.12$; RNA-seq $p = 0.28$).

Conclusion: In this retrospective analysis, a 10-gene GR-sig derived from OvCa GR-regulated DEGs performed better as a prognosticator than *NR3C1* mRNA expression alone. Prospective application of the GR-sig may be useful in stratifying patients for treatment response with novel agents in OvCa clinical trials.

#4740 Decipher androgen receptor condensate-mediated chromatin remodeling in gastric cancer.

Ben Liu, Huahuan Liu, Mengyue Qiu, **Kexin Chen**

Tianjin Medical University Cancer Institute and Hospital, Tianjin, China

Liquid-liquid phase separation (LLPS) is an intracellular phenomenon where biomolecules, including proteins and nucleic acids, interact to form dense complexes in the cytoplasm or nucleus. This process is crucial for regulating biological functions, including gene expression, signaling pathways, and the cell cycle. Recent studies have revealed a strong association between abnormal LLPS activity and diseases, such as neurodegenerative disorders and cancer. There is a sex difference in the incidence and mortality of gastric cancer (GC), with males exhibiting a higher incidence than females. Sex hormones are among the biological factors contributing to this difference, and the androgen receptor (AR) serves as the key mediator of androgen action. However, its role in transcriptional regulation in GC remains unclear. To address this, we conducted multi-omics assays, including ATAC-seq, CUT&Tag, HiChIP, and RNA-seq, to investigate the AR-related transcriptional regulatory network in GC. Results revealed that chromatin accessibility increased following DHT treatment, and AR target genes were predominantly enriched in pathways related to proliferation, stemness, and metabolism. Furthermore, CUT&Tag analysis indicated that AR binding peaks were primarily located in non-promoter regions, suggesting that AR might bind to enhancer regions. We identified AR-associated typical and super-enhancer (TE/SE) target genes in GC. HiChIP results further confirmed spatial proximity interactions between promoters and enhancers at AR binding sites. In this study, we further investigated the ability of AR to form condensates and observed that AR translocates into the nucleus and forms condensates in the presence of DHT. The mobility of AR-formed foci was confirmed through both in vivo and in vitro experiments. Mutational analysis of key amino acids in AR's structural domains revealed that hydrophobic amino acids, specifically proline and leucine, are critical for AR phase transition. Proximity labeling and IP-MS assays identified SWI/SNF complex subunits and transcriptional coactivators, including BRD4 and MED1, as potential components of AR condensates. To explore the relationship between AR condensation and transcriptional regulation, the AR inhibitor enzalutamide was employed. Enzalutamide disrupted AR condensation and led to the downregulation of genes enriched in proliferation- and stemness-related signaling pathways. The combination of enzalutamide and AU-15330 effectively suppressed cell proliferation, colony formation, and organoid viability. These findings suggest that AR's LLPS capacity plays a critical role in regulating its transcriptional activity. Furthermore, combining SWI/SNF inhibitors with AR antagonists showed synergistic effects in inhibiting GC cell proliferation.

#4741 HER2 status correlated with interleukin-2 (IL-2) levels after systemic therapy in hormone receptor (HR)-positive metastatic breast cancer.

Justin W. Zhang[1], **Qiang Zhang**[2], Andrew A. Davis[3], Lorenzo Gerratana[4], Jianhua Jiao[5], Weijun Qin[5], Youbin Zhang[2], Paolo D'Amico[6], zheng Cai[7], Akhil Chawla[8], Lisa Flaum[2], Janice Lu[2], Leonidas C. Platanias[9], William J. Gradishar[2]

[1]Department of Surgery, Division of Surgical Oncology, Northwestern University and Whitney Young High School, Chicago, IL,[2]Department of Medicine, Division of Hematology/Oncology, Circulating Tumor Cell (CTC) core facility, Robert. H. Lurie Cancer Center, Feinberg School of Medicine, Northwestern University, Chicago, IL,[3]Department of Medicine, Division of Hematology/Oncology, Washington University in St. Louis, st. Louis, MO,[4]Department of Medicine, University of Udine, Udine, Italy,[5]Department of Urology, Xijing Hospital, Innovation Center for Tumor Immunocytology Therapy Technology, Xijing Innovation Research Institute, Fourth Military Medical University, Xi'an, China,[6]Merck, Rahway, NJ,[7]Department of statistics, University of Washington, Chicago, IL,[8]Department of Surgery, Division of Surgical Oncology, Robert. H. Lurie Cancer Center, Feinberg School of Medicine, Northwestern University, Chicago, IL,[9]Department of Medicine, Division of Hematology/Oncology, Robert. H. Lurie Cancer Center, Feinberg School of Medicine, Northwestern University, Chicago, IL

Introduction: The immune response plays a crucial role in prognosis in metastatic breast cancer (MBC). Detecting plasma immune components, including cytokines and circulating tumor cells (CTCs), can help predict prognosis. We previously identified Interleukin-2 (IL-2) as a biomarker associated with disease progression in MBC. Similarly, HER2 is a critical tumor marker linked to therapeutic outcomes and poor prognosis in advanced breast cancer (2018-ASCO #1090, 2022-ASCO #1057). Here, we report a novel finding: HER2 status is correlated with plasma IL-2 levels following systemic therapy in MBC patients.

Methods: Whole blood samples (7.5 mL each) were collected from 42 patients with MBC, including 25 ER+, 17 ER-, 12 HER2+, and 30 HER2- patients, both before systemic therapy (baseline, BL) and 3 months after therapy (follow-up) at Northwestern Memorial Hospital (IRB-NU16B06). CTC enumeration was performed using the FDA-approved CELLTRACKS System (Menarini). Plasma Interleukin-2 (IL-2) levels were analyzed using ELISA (Fisher). Estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki67 status were assessed in biopsy tissue (prior to surgery) by NU PathCore. Statistical analyses were conducted using Causal Inference with Ensemble Learning.

Results: Based on pathological diagnoses, patients were categorized into 4 groups: 17 HR+/HER2- (Group 1), 8 HR+/HER2+ (Group 2), 4 HR-/HER2+ (Group 3), and 13 HR-/HER2- (Group 4). Ki67 expression was found in 9 patients in Group 1, 6 in Group 2, 1 in Group 3, and 6 in Group 4. There was no significant difference in baseline mean IL-2 levels among the groups, with values of 82.1 pg/mL, 78.6 pg/mL, 80.5 pg/mL, and 81.2 pg/mL for Groups 1, 2, 3, and 4, respectively ($P > 0.05$). After therapy, the mean IL-2 levels changed to 80.8 pg/mL, 69.3 pg/mL, 70.2 pg/mL, and 77.6 pg/mL at follow-up. A significant difference was observed between Groups 1 and 2 ($P = 0.037$), showing that IL-2 levels were notably higher in HR+/HER2- patients compared to HR+/HER2+ patients. There was no significant correlation between IL-2 and Ki67. Additionally, 20 patients (46%) had CTCs ≥5 at baseline, with 15 having HER2-negative CTCs (Group 5) and 5 having HER2-positive CTCs (Group 6). The mean IL-2 level was significantly higher in Group 5 (88.17 pg/mL) than Group 6 (66.81 pg/mL), indicating that patients with HER2-positive CTCs had significantly lower IL-2 levels ($P = 0.02$).

Conclusion: The finding that HER2 status in CTCs correlates with lower plasma IL-2 levels, and that HER2 status in biopsy tumor tissue is associated with IL-2 levels after systemic treatment in HR-positive MBC patients, suggests that HER2 may contribute to immune suppression and weaken the immune response through its interaction with IL-2 during therapy. Exploring the HER2-IL-2 relationship could provide valuable insights into assessing therapeutic efficacy in hormone receptor-positive MBC.

#4742 Novel (Z)-endoxifen-related chemical entities exhibit potent anti-cancer activity in ERα+ breast cancer.

Lena Batoon[1], Rajeev S. Muthyala[1], Sandra S. Hammer[2], Natalie Farris[2], Steven C. Quay[2], John R. Hawse[1]

[1]Mayo Clinic, Rochester, MN,[2]Atossa Therapeutics Inc, Seattle, WA

Approximately 80% of breast cancers express estrogen receptor alpha (ERα, ESR1), which drives cancer progression. Despite the array of targeted therapies, ERα+ breast cancer exhibits the highest recurrence and late distant relapse rates among subtypes. (Z)-Endoxifen (ENDX), an active metabolite of tamoxifen, is a potent, orally bioavailable selective estrogen receptor modulator with a favorable safety profile now under development for breast health applications. Its synthesis yields endoxifen-related byproducts that are new chemical entities (NCEs), including AT416E, AT416Z, AT402E and AT402Z. This study compared the anti-estrogenic effects of these compounds to ENDX in ERα+ breast cancer cell lines (MCF7, T47D, UCD12), including those with ESR1 mutations (Y537S, D538G) linked to endocrine resistance. In 2D cultures with estrogen (E2), ENDX and all NCEs showed significant anti-proliferative effects across all lines, achieving 20-90% inhibition at 0.02-5 μM ($p<0.0001$). ENDX was the most effective in MCF7; ENDX, AT416Z, and AT402Z in T47D; and ENDX, AT416E, and AT416Z in UCD12. Under E2-deficient conditions, all NCEs had lower IC50 values than ENDX in MCF7 cells, while AT416Z and AT402Z outperformed ENDX in UCD12. In T47D D538G mutants, ENDX, AT416E, and AT402Z exhibited the highest potency, while all NCEs showed comparable efficacy in Y537S mutants. In MCF7 cells, ENDX, AT402E, and AT402Z demonstrated similar activity against Y537S mutants, while AT402Z was the most potent in D538G mutants. In 3D cultures at a clinically relevant concentration (~1 μM), all compounds significantly reduced proliferation (~75-90%; $p<0.0001$), with ENDX and AT402Z showing superior potency. AT416E outperformed ENDX in wound-healing assays ($p<0.0001$). All NCEs induced comparable G1 cell cycle arrest in MCF7 ($p<0.0001$), while AT402E and AT402Z showed stronger promotion of G1-to-S phase arrest and S/G2/M inhibition compared to ENDX in T47D cells ($p<0.05$). These NCEs also significantly induced apoptosis in MCF7 cells at 1-5 μM ($p<0.0001$), with AT416Z, AT402E, and AT402Z surpassing ENDX ($p<0.0001$). In T47D cells, apoptosis was significantly induced by ENDX, AT416E, and AT416Z ($p<0.0001$ vs. vehicle), with ENDX showing superior activity. RT-qPCR analysis at 0.1 μM revealed ENDX and AT416Z as the most potent inhibitors of E2-induced ERα target gene expression in MCF7 cells, with ENDX surpassing all NCEs in T47D cells. Synergy assays indicated that the NCEs do not synergize with ENDX, supporting their potential use as stand-alone agents. These findings highlight four NCEs with notable anti-cancer activity, with some matching or surpassing ENDX in several cancer-relevant cellular readouts, marking them as novel candidates for ERα+ breast cancer therapy. Further studies will advance one or more of these NCEs into *in vivo* models to confirm their therapeutic efficacy and potential for clinical application.

#4743 Defining the impact of anti-androgen pathway inhibitors on ABI1, dendritic spines and mouse behavior.

Anna Seidl, Kevin M. Lin, Maria A. Ortiz, Leszek Kotula

Urology, Biochemistry and Molecular Biology, Upstate Cancer Center, SUNY Upstate Medical University, Syracuse, NY

Prostate cancer (PCa) is the most commonly diagnosed cancer and the second leading cause of cancer-related deaths among American men. Following radiation therapy and surgery, patients are often treated with androgen deprivation therapy (ADT) or anti-androgen drugs to suppress tumor growth, as prostate cancer is highly androgen-dependent. However, emerging evidence highlights significant cognitive side effects, including dementia and behavioral changes, associated with anti-androgen treatments. While clinical trials are investigating these 'treatment-induced' cognitive deficits, the underlying mechanisms remain poorly understood. Our previous work revealed that the Androgen Receptor (AR) regulates Abelson Protein 1 (ABI1) expression in prostate tumors. ABI1, a key stabilizer of the WAVE complex, plays a critical role in actin polymerization and cytoskeletal reorganization. In dendritic spines, ABI1 supports the dynamic remodeling of the cytoskeleton essential for synaptic plasticity, a fundamental process underlying learning and memory. Disruption of ABI1 in hippocampal neurons results in abnormal dendritic spine morphology and impaired synapse formation, but there are no genetic studies on the role of Abi1 in cognitive performance in mouse models. Androgen deprivation, through surgical castration or ADT, has been shown to impair memory and behavior in mice. Given that ABI1 expression is downregulated by anti-AR therapy in prostate tumors, we hypothesize that ABI1 is similarly regulated by androgens in dendritic spines. We propose that AR suppression by ADT leads to reduced ABI1 expression in dendritic spines, contributing to cognitive and behavioral impairments in patients undergoing ADT. To test this hypothesis, we utilized a novel Abi1-selective knockout mouse model developed in our lab to explore the effects of ABI1 disruption on dendritic spines and behavior. Additionally, we compared dendritic spine morphology and ABI1 levels in the hippocampi of mice treated with anti-AR drugs, such as enzalutamide, or subjected to surgical castration. These results are being analyzed alongside findings from mice with genetic ABI1 disruption. This study provides critical insights into the role of ABI1 in dendritic spine dynamics and synaptic plasticity, uncovering a potential mechanistic link between ADT and cognitive dysfunction. Our findings aim to define the molecular basis of treatment-related cognitive deficits in PCa patients and may guide the development of strategies to mitigate these side effects.

#4744 State-gating cancer to prevent chemotherapy-resistance.

Christine Louise Chaffer[1], Heloisa H. Milioli[1], Beatriz P. San Juan[1], Rachel F. Dear[2], Jia (Jenny) Liu[2]

[1]Garvan Institute of Medical Research, Darlinghurst, Australia,[2]St Vincent's Hospital, Sydney, Australia

New approaches are urgently required to treat advanced-stage cancers that are therapy resistant and incurable. Furthermore, salvage treatment protocols rely on cytotoxic chemotherapies that actively induce cancer cells to transition into therapy-resistant states via non-genetic mechanisms. We show in large clinical cohorts of triple-negative breast cancer (TNBC) that chemotherapy-induced cell state changes are mediated by androgen receptor (AR) signaling and are associated with poor patient outcome. AR inhibition blocks canonical and non-canonical AR signaling to lock cancer cells in therapy-sensitive states, and synergizes with a broad spectrum of cytotoxic chemotherapies to improve survival in pre clinical TNBC models. Seviteronel, a dual CYP17 lyase and AR inhibitor, faithfully mimics genetic AR ablation and is the most effective AR inhibitor in combination with chemotherapy. A Phase 1 clinical trial (NCT04947189) confirmed the safety of combining seviteronel with taxane chemotherapies in metastatic breast cancer. Early signs of clinical efficacy in patients with advanced disease led to a Phase 2 expansion study for AR-positive TNBC. These data provide proof-of-concept for AR inhibition as a ‘state-gating’ strategy to lock cancer cells in therapy-sensitive states and prevent the emergence of chemotherapy-resistance in TNBC. This paradigm may be widely applicable to other cancers.

#4745 Comprehensive screening of drugs targeting neural signaling identifies a neuropeptide receptor as a potential therapeutic target in small cell lung cancer.

Masakatsu Tokunaga, Natsuki Nakagawa, Mirei Ka, Yuriko Sugiura, Takahiro Iida, Hiroaki Ikushima, Takahiro Ando, Kousuke Watanabe, Akiko Kunita, Hidenori Kage, **Masanori Kawakami**

Graduate School of Medicine and Faculty of Medicine, The University of Tokyo, Tokyo, Japan

Small cell lung cancer (SCLC) is characterized by poor prognosis with limited therapeutic agents. SCLC can be classified into subtypes based on the expression levels of four transcription factors: ASCL1, NEUROD1, POU2F3, and YAP1. The former two subtypes are neuroendocrine (NE)-type tumors. Recently, neural activity has been noted as a key regulator in the field of oncology. We hypothesized that some neurotransmitters and their receptors can be therapeutic targets regulating the biology of NE-type SCLC. We created a drug library consisting of 560 drugs known to target various neural signaling and examined their growth-inhibitory effects comprehensively on SCLC cells. From this library, 56 drugs exhibited more than 50% inhibition of growth of NE-type SCLC cell lines at 10 μM. After excluding drugs with possible nonspecific effects, the IC50 values of 24 drugs were examined in an expanded panel of SCLC cell lines including non-NE-type cells. The inhibitor of neuropeptide receptor X exhibited distinctly lower IC50s in NE-type SCLC cells relative to non-NE-type cells, suggesting that this receptor is potentially a specific therapeutic target for NE-type SCLC. Neuropeptide receptor X expression was high in the brain but not detectable in the lung when normal tissues were analyzed *in silico* with NCBI database. Interestingly, when cancer cells were analyzed with CCLE database, this receptor expression was substantially high in NE-type SCLC cells, compared with other cancer cells. Furthermore, the growth-inhibitory effects of the receptor X inhibitor shown in the drug screening were correlated with receptor X expression levels. Using multiple NE-type SCLC cell lines with high receptor X expression, knockdown/knockout of this receptor by siRNAs and CRISPR/Cas9 inhibited cell proliferation. Furthermore, addition of the ligand for receptor X promoted cell proliferation of NE-type SCLC cells, suggesting the significance of signaling by this neuropeptide receptor for NE-type SCLC. Inhibition of receptor X also induced apoptosis in these NE-type SCLC cells, and cell cycle assays showed an increase in sub G1 phase. These effects were not seen in non-NE-type cells. Phospho-protein array analysis followed by Gene Ontology analysis after treatment with the receptor X inhibitor suggested that MAPK and PI3K-Akt signaling pathways were involved in the antineoplastic effects of this inhibitor. Finally, *in vivo* effects of the receptor X inhibitor were examined in NCI-H146 (NE-type SCLC) xenograft mouse model. The receptor X inhibitor was well tolerated and suppressed the *in vivo* tumor growth statistically significantly. Collectively, we identified one of the neuropeptide receptors as a potential therapeutic target for NE-type SCLC. This finding warrants further exploration to establish the novel therapeutic strategy for SCLC by targeting neural signaling.

#4746 PLB-001, a novel anti-dll3 ADC with topoisomerase 1 inhibitor-based linker-payload for treatment of SCLC.

Yingdong Lu, xin li, Ganyuan Xiao, Kia J. Puan, Ling xu, Mao Yin

PrimeLink BioTherapeutics, suzhou, China

Delta-like ligand 3 (DLL3) is a promising target for antibody-drug conjugates (ADCs) in the treatment of small cell lung cancer (SCLC) due to its high expression on the cell surface of SCLC cells and minimal expression in normal tissues. With highly specific, fast internalized mAbs from antibody screening, various types of payloads including tubulin and TOPI inhibitors were conjugated with different platforms, linkers, conjugation methods available from our linker-payload arsenal to obtain the optimal ADC. Here, we present a novel DLL3 targeted ADC, PLB-001, which using a highly potent topoisomerase I inhibitor exatecan, conjugated to a humanized IgG1 antibody, through a highly hydrophilic, enzyme-cleavable linker with a DAR of 8. PLB-001 demonstrated strong *in vitro* efficacy in several different SCLC cell lines correlating with DLL3 expression levels. This ADC also exhibited potent and durable antitumor effects in cell line-derived and patient-derived xenograft models, and exhibited favorable pharmacokinetic profiles. These preclinical result support PLB-001 as a potential candidate for the treatment of DLL3-positive SCLC

#4747 Dopamine receptor D2 and dopamine antagonism modulate the progression of small cell lung cancer.

Parvez Khan[1], Aatiya Ahmad[1], Md Arafat Khan[1], Mahek Fatima[1], Asad Ur Rehman[1], Laiba Anwar[1], Sanib Chaudhary[1], Imayavaramban Lakshmanan[1], Anish Thomas[2], Surinder K. Batra[1], Varun V. Prabhu[3], Apar K. Ganti[4], Mohd Wasim Nasser[1]

[1]University of Nebraska Medical Center, Omaha, NE,[2]Developmental Therapeutics Branch, National Cancer Institute, Bathesda, MD,[3]Chimerix, Inc, Durham, NC,[4]Division of Oncology-Hematology, Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE

Small cell lung cancer (SCLC) is an aggressive neuroendocrine (NE) cancer and is one of the most lethal types of solid tumors. Although platinum-based therapy forms the backbone of systemic therapy for SCLC, resistance, and recurrence occur rapidly, and most patients die within a year of diagnosis. Despite the approval of immune checkpoint inhibitors for SCLC, the survival benefit of these combinations in an unselected patient population is modest. The majority of SCLCs (~70-75%) are initiated from pulmonary NE cells; therefore, exploiting NE drivers/modulators is crucial for developing effective therapeutic strategies. To identify novel therapeutic molecules, we performed unbiased RNA-seq expression analysis of NE modulators in SCLC gene expression datasets. Our data showed an atypical expression profile of dopamine receptor D2 (DRD2) in various cell lines of SCLC belonging to NE, non-neuroendocrine, brain metastatic (BrM), and chemotherapy-resistant phenotypes. DRD2 is a G-protein coupled receptor that governs multiple physiological functions, including modulation of the synthesis, storage, and secretion of dopamine (DA); and seems to play a role in neuropsychiatric disorders, brain tumors, and promotes oncogenesis. We studied the role of DRD2 in cell lines representing multiple subtypes of SCLC and tumor tissues compared to their matched normal controls. DRD2 expression was also high in murine SCLC cell lines derived from RPM (*Rb1*$^{flox/flox}$; *TP53*$^{flox/flox}$; *LSL-Myc*^{T58A}), a spontaneous SCLC mouse model. High expression both in murine and human cell lines derived from brain metastatic lesions (BrM), suggests that DRD2 expression may play an essential role in brain metastasis. Additionally, a substantially high expression of DRD2 was observed in chemotherapy- and immunotherapy-resistant cell lines derived from SCLC patient-derived xenografts. To assess the therapeutic potential of DRD2, we utilized ONC206, a highly specific, orally bioavailable, and blood-brain barrier penetrating DRD2 antagonist. ONC206 treatment attenuated SCLC growth and metastasis, including brain metastasis both in preclinical in vitro and in vivo models. Integrated gene expression studies coupled with pathway analysis showed that DRD2 inhibition reduces SCLC growth and metastasis by inhibiting the cholesterol biosynthesis pathway. Altogether, our study provides strong preclinical rationale for targeting DRD2 via dopamine antagonism to attenuate SCLC growth and metastasis.

#4748 Understanding the RSAD2/CMPK2 signaling axis in PVT1 exon 9 overexpressed neuroendocrine prostate cancer.

Rachel E. Bonacci, Meghan McGill, Nu Thuy Anh Le, Murtaza Barkarar, Chinedum Udekwu, Olorunseun O. Ogunwobi

Michigan State University, East Lansing, MI

Introduction/Background: Plasmacytoma variant translocation 1 (PVT1) is overexpressed in prostate cancer (PCa) and alters gene expression directly or by acting as a noncoding RNA sponge. Our group previously identified overexpression of PVT1 exon 9 in aggressive PCa tissues and cell lines and overexpression led to transformation of non-tumorigenic prostate epithelial cells to PCa with neuroendocrine prostate cancer (NEPC) phenotype when implanted *in vivo*. RNA-sequencing analysis of our PVT1 exon 9 overexpression PCa model revealed upregulation of Radical S-Adenosyl Methionine Domain Containing (RSAD2) downstream of PVT1 exon 9. We have uncovered RSAD2 overexpression as a common characteristic of NEPC and have elucidated two novel molecular pathways at play in this disease; PVT1 exon 9 independent and dependent RSAD2 upregulation. These pathways lead to differences in Type II interferon signaling, immune signaling and AR sensitization. RSAD2 is located upstream of and directly interacts with CMPK2 in a variety of cellular models and some of their functions are interdependent.
Objectives/Hypothesis: We hypothesize that RSAD2 and CMPK2 work together to contribute to NEPC pathogenesis regardless of PVT1 exon 9 overexpression status.
Methods: We tested this hypothesis with a variety of genetic and biochemical approaches.
Results: RNA-sequencing analysis of our PVT1 exon 9 overexpression NEPC model (RWPE1, RWPE1_ev, RWPE1_ex9) revealed significant (p-value < 0.05) upregulation of RSAD2 and CMPK2, in RWPE1_ex9 as compared to RWPE1_ev and RWPE1. In a second NEPC model (that also models circulating tumor cells in metastasis) derived from 22RV1 castrate-resistant prostate cancer (CRPC) parental cells implanted *in vivo* and isolated from blood, we found overexpression of PVT1 exon 9, RSAD2 and CMPK2 in isolated circulating tumor cells (C22OH) we have previously validated as having neuroendocrine features. In NEPC models that do not overexpress PVT1 exon 9, such as DU145 and NCI-H660, we found only overexpression of RSAD2 but not CMPK2. Knockdown of CMPK2 led to ~35% decreased cell viability in all models suggesting that CMPK2 may be a cancer promoting aberration in NEPC. Both RSAD2 and CMPK2 are clinically relevant and significantly overexpressed in neuroendocrine prostate cancer human cohorts. Overexpression models of CMPK2 revealed alterations in cell proliferation, colony formation and PD-L1 immune signaling and localization suggestive of a larger role in prostate cancer pathogenesis.
Discussion/Conclusions: Based on these results, PVT1 exon 9, RSAD2 and CMPK2 are promising therapeutic targets in PVT1 exon 9 overexpressed NEPC whereas RSAD2 targeting alone may be more useful in NEPC without PVT1 exon 9 overexpression. Further investigation is ongoing to understand therapeutic strategies to use alone or in combination with current clinically approved therapeutics.

#4749 Evaluating NUAK2 as a potential target in neuroendocrine prostate cancer.

Umar Mehraj, Shaista Manzoor, Julie Parks, Emily Cordova, Everardo Macias

Department of Pathology, Duke University School of Medicine, Durham, NC

Background: Neuroendocrine Prostate Cancer (NEPC) is an aggressive and lethal prostate cancer subtype. Due to Androgen Receptor-independence, NEPC lacks targeted therapeutics, with platinum-based chemotherapy serving as the primary treatment modality. This highlights the need for new molecular targets in NEPC. NUAK2, also known as sucrose non-fermenting (SNF1)-like AMPK-related kinase (SNARK) is an unstudied kinase. Yet, pan-cancer analysis shows increased expression of NUAK2 across several malignancies. Analysis of prostate cancer and NEPC datasets revealed a significant increase in NUAK2 expression levels correlating with disease progression. The objective of our study was to determine if NUAK2 is essential for NEPC tumor growth.
Methods: To investigate the therapeutic potential of NUAK2 in NEPC, we conducted genetic and pharmacological modulation of NUAK2 both in vitro and in vivo. We conducted label-free quantitative phospho-proteomic and proximity ligation interactome analyses of NUAK2 using LC-MS/MS to elucidate its role in NEPC.
Results: NUAK2 knockdown significantly decreased proliferation and 3D spheroid growth in NEPC preclinical models. CRISPR-Cas9-mediated knockout of NUAK2 in NCI-H600 cells led to reduced tumor growth *in vivo*. Furthermore, NUAK2 overexpression accelerated NEPC growth in a kinase-dependent manner. Pharmacological inhibition of NUAK2 significantly reduced the growth of NEPC cell-line and patient-derived xenografts. Phosphoproteome and interactome studies by LC-MS/MS and pathway enrichment analysis revealed the role of NUAK2 in chromatin remodeling, mRNA metabolic processes, and cytoskeleton organization.
Conclusions: Collectively, our study highlights NUAK2 is essential for NEPC tumor growth and is an actionable target in NEPC.

#4750 Epigenetic approaches to deliver a targeted radiotherapy for triple negative breast cancer.

Khalid B. Mir[1], Katie Brookes[2], Martin L. Read[2], Daniel G. Stover[3], Mathew D. Ringel[3], Christopher J. McCabe[2], Moray J. Campbell[1]

[1]Cedars-Sinai Medical Center, West Hollywood, CA,[2]University of Birmingham, Birmingham, United Kingdom,[3]The Ohio State University, Columbus, OH

The sodium iodide symporter (NIS, encoded by *SLC5A5*) is exploited in thyroid cancer to uptake radioiodide. NIS appears to function in mammary epithelial cells, being upregulated during lactation, and offers the possibility of a novel breast cancer (BrCa) radiotherapy. Unfortunately, NIS appears repressed in BrCa, and therefore we are examining mechanisms to restore its expression and function. Retinoic Acid Receptor alpha (RARα) upregulates *SLC5A5*, and pre-clinical studies suggest this is epigenetically corrupted in BrCa. To investigate RARα-dependent epigenetic control of *SLC5A5* levels we identified differentially expressed RARα coregulators in TNBC tumors in the TCGA BrCa cohort. This identified upregulation of PRAME, a known RARα co-repressor that interacts with histone deacetylase (HDAC) enzymes. We modulated PRAME expression using CRISPR methods in a panel of luminal A, luminal B and TNBC BrCa cell lines (MCF-7, T47-D, ZR-75-1, AU565 and MDA-MB-231). Elevated PRAME expression significantly repressed the antiproliferative response to ATRA (a pan-RAR ligand) and AM580 (a RARα-selective ligand). RNA-Seq confirmed that increased PRAME expression reduced by approximately half the number of significantly regulated genes in response to ATRA and AM580. For example *SLC5A5* and a significant number of RARα-canonical target genes were upregulated in MCF-7 and AU565 cells and the magnitude was significantly reduced by elevated PRAME. In MDA-MB-231 cells elevated PRAME expression led to transrepression of numerous genes including *SLC5A5* in response to ATRA and AM580. Gene set enrichment analyses also supported a significant role for PRAME to uniquely shape ATRA signaling by increasing inflammatory networks (MCF-7 and AU565) and decreasing estrogen (MCF-7) and TNFA (MDA-MB-231) signaling. Ongoing studies are repressing PRAME and examining its impact on activating and repressing histone marks. In a parallel orthogonal approach we have sought to stabilize NIS expression by targeting valosin-containing protein (VCP) that induces NIS proteolysis. We tested VCP inhibitors in combination with the HDAC inhibitor SAHA or the transcriptional potentiator, disulfiram metabolite Cu(DDC)2 in the same cell lines. Remarkably, in MDA-MB-231 cells, $Cu(DDC)_2$ combined with the VCP inhibitor CB5339, significantly increased NIS expression and radioiodide uptake leading to a prominent decrease in cell proliferation and survival. These studies, along with ongoing drug strategies in TNBC xenograft and PDX tumors, combine to support the concept that SLC5A5/NIS expression can be restored and offer the possibility of promoting radioiodide as a novel BrCa radiotherapy.

#4751 CS5005: A novel SSTR2-targeted antibody-drug conjugate (ADC) with robust antitumor activity in preclinical studies.

C. Wang, X. Zhang, N. Zhang, Y. Wang, Y. Li, X. Liu, M. Zhu, Y. Qian, Y. Yang, J. Sun, X. Dai, F. Ma, Y. Sun, J. Yang;
CSTONE Pharmaceuticals, Suzhou, China

Background: Somatostatin receptor 2 (SSTR2) is overexpressed in neuroendocrine tumors (NETs) and small cell lung cancer (SCLC). SSTR2-targeted treatment options for NETs include somatostatin analogs and radionuclide conjugates (RDCs). In SCLC, SSTR2-targeted therapies, including RAZ101 (225Ac bearing RDC), PEN221 (peptide drug conjugate) etc., have shown encouraging clinical results. Unfortunately, such therapies with peptide (SSA analogs like octreotide) suffer from short half-lives and high toxicities due to poor selectivity. Moreover, RDCs are often restricted by limited availability. To tackle these, a highly selective and potent antibody against SSTR2 could offer a promising therapeutic alternative. Here, we present CS5005, a novel SSTR2-targeted ADC developed to treat SSTR2-positive tumors, including NETs and SCLC.

Methods: SSTR2-targeted antibodies were generated from immunized mice, followed with rounds of screening, maturation and humanization. The lead antibody of CS5005 was selected based on the binding, internalization capacity, and selectivity for SSTR2. CS5005 is composed of our lead SSTR2-targeted humanized antibody, a proprietary CSL linker and a well-validated topoisomerase 1 inhibitor exatecan (EXd). The plasma-stable, hydrophilic, beta-glucuronide CSL linker enables effectively tumor-selective toxin release. The antitumor activity of CS5005 was evaluated *in vitro* and *in vivo* using cell line-derived xenograft (CDX) models originated from multiple carcinomas. Tolerability and PK were assessed in rodents and cynomolgus monkeys.

Results: The lead humanized SSTR2-targeted antibody, 221.3.1, demonstrated high-affinity binding to SSTR2-positive cells (e.g. SSTR2-overexpressing HEK, H524, and H446) and high-rate internalization on tumor cells. Importantly, 221.3.1 selectively bound SSTR2 without cross-reactivity to other somatostatin receptors. 221.3.1's cross-species recognition of SSTR2 supported the selection of cynomolgus monkey for nonclinical toxicology studies. CS5005 retained the antibody features of binding affinity, internalization and selectivity, showing excellent *in vitro* stability in serum incubation assays and a mAb-like PK profile in the *in vivo* rodent studies. *In vitro* cytotoxicity assays confirmed the ability of CS5005 to induce effective, target-dependent killing of SSTR2-expressing tumor cells. CS5005 also demonstrated robust *in vivo* antitumor activity in CDX models originated from multiple carcinomas, with minimal effective dose (MED) of 5 mg/kg. Pilot toxicity studies in non-human primates demonstrated a favorable tolerability and PK profile of CS5005.

Conclusion: CS5005 represents a promising SSTR2-targeted ADC for the treatment of advanced solid tumors. The preclinical findings support further IND-enabling studies and clinical investigations of CS5005 as a novel therapeutical option.

#4752 The combination of GLP1-R agonists and progestins enhances endometrial cancer cell killing through membrane receptor crosstalk.

Lane E. Smith[1], Jamie L. Padilla[1], Geneva L. Williams[1], Kristina Thiel[2], Kimberly K. Leslie[1]

[1]University of New Mexico Health Sciences Ctr., Albuquerque, NM,[2]University of Iowa, Iowa City, IA

Obesity is a major risk factor for endometrial cancer, and glucagon like peptide-1 receptor (GLP-1R) agonists such as semaglutide may be helpful to achieve weight loss. Conservative treatment of endometrial cancer includes hormone therapy with progestins for women who wish to preserve their uterus. Our previous work showed a significant reduction in viability of multiple endometrial cancer cell line and organoid models in response to the combination treatment of semaglutide and levonorgestrel, and identified a positive feedback loop between GLP-1R and progesterone receptor (PR) signaling. Here, we further explored mechanisms underlying the enhanced signaling, using confocal microscopy to show the co-localization of membrane PR (Progesterone Receptor Membrane Component 1; PGRMC1) and GLP1-R in response to semaglutide + progesterone combined treatment. We also found that semaglutide alone (100nM) induces the translocation of PR to the nucleus within 30 minutes. We are undergoing microarray analyses to assess specific phosphorylation events as well as RNA sequencing occurring downstream to identify the signaling molecules and transcripts activated by dual treatment. These studies highlight the potential of semaglutide to improve the anti-cancer effects of progestins, and, importantly, may reduce recurrences that can occur with progestin therapy alone.

#4753 ACSS2 inhibition alters epigenetic landscape to improve endocrine therapy outcome in ER+ metastatic breast cancer cells.

Ayca N. Mogol, Jin Young Yoo, David J. Dutton, Zeynep Madak-Erdogan

University of Illinois at Urbana-Champaign, Urbana, IL

Endocrine therapies target the hormone dependencies of the cancer cells in various ways. One primary target is estrogen receptor alpha (ERα), which is expressed in approximately 70% of human breast cancers (ER-positive or ER+). Despite treatment, recurrence and metastasis are still observed in 30-40% of ER+ patients, and the 5-year relative overall survival rate for these patients is only 24%. Local Acetyl-CoA availability plays a crucial role in regulating histone acetylation and gene expression, thereby supporting tumor progression. Our previous research demonstrated that ERα antagonist Fulvestrant (Fulv) treatment increased ACSS2 (acetyl-CoA synthetase 2) levels in vivo in metastatic breast tumors. Combining the Fulv with a highly specific ACSS2 inhibitor (ACSI) synergistically reduced cell viability in ER+ metastatic breast cancer (MBC) cells in vitro. In the present study, we employed multiple analytical approaches—including isotope tracing assay with labeled acetate, CUT&RUN sequencing, immunofluorescence, western blot, and RNA sequencing—to characterize the epigenetic and metabolic alterations induced by Fulv treatment and ACSS2 enzyme and how they contribute to endocrine resistance.

To investigate how ACSS2 drives endocrine resistance, we examined ACSS2 expression and acetate utilization in response to Fulv. We showed that the ACSS2 expression and acetate utilization by ACSS2 to synthesize Acetyl-CoA increase with Fulv treatment. Isotope tracing assay with labeled acetate indicated that Fulv treatment decreased the acetate flux to the TCA cycle and drove it toward fatty acid synthesis. However, inhibition of fatty acid synthesis did not impact Fulv-induced cell viability, suggesting an additional role for ACSS2-mediated acetyl-CoA production in metastatic breast cancer cells. Immunofluorescence assays showed that ACSS2 was almost exclusively located in the nucleus, supporting a role in epigenetic regulation. We then examined chromatin enrichment of ACSS2, ERα, and H3K27ac using the CUT&RUN sequencing method in ER+ MBC cells. Fulv treatment resulted in an increased number of global ACSS2 binding sites as well as overlapping binding sites of ERα, ACSS2, and H3K27ac. These sites were lost with the ACSI and Fulv combination. Global gene expression profiling using RNA sequencing assay showed that the gene expression is increased for genes involved in cell proliferation with Fulv treatment.

In conclusion, our findings suggest a nuclear role for ACSS2 in endocrine therapy resistance by providing a substrate for epigenetic alterations. Targeting these alterations caused by cancer cell adaptations is a novel approach that could potentially lead to new therapies. This strategy may help reduce metastasis-related mortality in breast cancer patients, offering additional opportunities for improved treatment outcomes in the future.

#4754 discovery of a casein kinase 2 small-molecule inhibitor that antagonizes wildtype and mutant estrogen receptor signaling in breast cancer.

Anil Kumar Devakrishnan, Yong Wang, Suzanne AW Fuqua, Martin Matthew Matzuk, **Murugesan Palaniappan**

Baylor College of Medicine, Houston, TX

Introduction: Activating somatic mutations in ESR1, the gene encoding estrogen receptor (ER) α, are frequently found in metastatic breast cancers. The somatic mutations Y537S and D538G mutants drive estrogen-independent activities and diminish the potency of the current standard-of-care agents. Consequently, there is an unmet clinical need to develop a novel drug that inhibits ER mutant signaling. Casein kinase 2 (CK2) is a serine/threonine protein kinase that is abnormally elevated in various cancers, including breast cancer. CK2 activates ER signaling and is considered a promising therapeutic target. Here, we seek to investigate mechanisms of CK2-mediated activation of ER signaling in breast cancer and to use DNA-encoded chemical libraries (DELs) to identify and validate potent, selective, and efficacious CK2 inhibitors that will serve as lead compounds for novel therapeutics in ESR1 mutant breast cancer.
Methods: The catalytic subunits of CK2 were screened with our in-house collection of 3.98 billion barcoded compounds. Hits enriched with CK2 were synthesized off DNA and tested in a biochemical assay. The most potent hits were analyzed for intracellular target engagement using NanoBRET tracer displacement assays. The top hit was extensively studied in WT, Y537S, and D538G mutant breast cancer cells. Metabolic stability was determined in mouse and human liver microsomes, and pharmacokinetics studies were performed in mice. Primary and metastatic tumors were harvested and used to develop ex vivo organoids and a WHIM20 patient-derived xenograft (PDX) model carrying the *ESR1* mutation Y537S. The antitumor activity of the top hit was tested in the WHIM20 PDX model.
Results: Leveraging our multibillion small molecule collection of DELs against catalytic subunits of CK2, we identified compound CDD-2007, which has low nanomolar cellular potency and good metabolic stability. CDD-2007 abolishes estrogen-induced gene programs in both wild type and mutant (Y537S and D538G) ER-positive breast cancer cell lines. In cellular assays, CDD-2007 inhibits Ser129 phosphorylation of AKT with double-digit nanomolar potency in WT and mutant breast cancer cells, but no significant loss of AKT Ser129 phosphorylation is seen in normal human breast epithelial cells (MCF10A). CDD-2007 potently inhibits primary and metastatic tumor-derived organoid growth in an *ESR1* Y537S PDX model. CDD-2007 inhibits primary tumor growth in PDX models of breast cancer and synergizes with the ER antagonist fulvestrant in the *ESR1* Y537S model without toxicity.
Conclusion: Together, our data show that CDD-2007 is a selective and potent inhibitor of CK2 with good drug-like properties that inhibit ERα signaling in endocrine-resistant metastatic breast cancer. Therefore, blocking CK2 signifies a promising therapeutic strategy for *ESR1* mutant breast cancer.

#4755 Preclinical mechanistic PK/PD-efficacy modeling for AZD9750, a novel oral androgen receptor degrader (PROTAC), to support dose selection during early clinical development.

Pablo Morentin Gutierrez[1], Ana Quiroga Campano[1], Antonio Ramos Montoya[1], Claire Crafter[1], Chrysiis Michaloglou[1], Aaron Smith[1], Nuria Galeano Dalmau[1], Jamie Scott[1], Michael Niedbala[2]

[1]AstraZeneca, Cambridge, United Kingdom,[2]AstraZeneca, Waltham, MA

Objectives: The androgen receptor (AR) is highly expressed in prostate cancers and is a clinically validated target in oncology. AZD9750 is a novel potent oral selective AR Proteolysis-targeting chimera (PROTAC) due to start clinical testing in 2025. We present here the preclinical PK/PD modeling work used to understand the required target modulation and concentration required to see anti-tumor efficacy and therefore help support the dose selection during the early clinical development of AZD9750.
Methods: We developed a novel mechanistic mathematical model applied to in vivo preclinical mouse PDX models (MR041 and C901, primary prostatic adenocarcinoma, hormone sensitive and AR-wt) linking the compound pharmacokinetics with the magnitude of target modulation expressed as relative levels of AR measured by Western Blotting. These changes at the biomarker level were subsequently linked to inhibition of tumor cell proliferation resulting in macroscopic dynamic effects on tumor size. All model parameters were derived from internal studies; some were estimated using Non-Linear Mixed Effect modeling of individual longitudinal PK, PD biomarker and tumor size data taken from several studies.
Results: The model described well the relationship between plasma concentration of AZD9750 and modulation of AR. In vivo AZD9750 concentration required to see 50% of the maximal target modulation in both animal models was on the region of 0.2-0.7 µM. Population tumor size patterns, for all treatment regimens ranging from 0 to tumour regressions across both tumor models were very well described with the model proposed. 55 - 80% reduction of AR levels was required to induce tumor regression in both models.
Conclusions: This study provides quantitative mechanistic insights into the links between compound exposure, biomarker modulation (AR) and anti-tumor responses, supporting our understanding of the required target modulation needed for maximal anti-tumor effects in these two hormone sensitive patient-derived tumor models. This mechanistic understanding is valuable to contextualize compound-induced PD modulation in patients, for given doses and schedules.

#4756 Novel anaplastic thyroid carcinoma patient-derived xenograft models and cell line.

Ahmad Abubaker[1], Ying Henderson[1], Marie-Claude Hofmann[2], Naifa Busaidy[2], Maria E. Cabanillas[2], Michelle Williams[3], Anastasios Maniakas[1], Stephen Y. Lai[4]

[1]Department of Head and Neck Surgery, The University of Texas MD Anderson Cancer Center, Houston, TX,[2]Department of Endocrine Neoplasia and Hormonal Disorders, The University of Texas MD Anderson Cancer Center, Houston, TX,[3]Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX,[4]Department of Head and Neck Surgery, Radiation Oncology, Molecular and Cellular Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX

Introduction: Anaplastic Thyroid Carcinoma (ATC) is the deadliest thyroid malignancy with a historic median overall survival of 5 months. Its highly aggressive nature limits our ability to test novel targeted therapies in patients. Patient-Derived Xenograft (PDX) models and cell lines are considered reliable preclinical models to test novel therapies as well as drug combinations.
Aim: Our group has previously reported ATC PDX model development, although certain key genetic mutations frequently found in the clinical setting are lacking. Our goal is to report our experience developing and characterizing three novel PDX models and one paired cell-line that have pathologically and clinically relevant mutations that may be used for drug development and testing.
Methods: Fresh tumor from ATC patients was harvested at the time of surgery and cut into 4x4x4 mm pieces and implanted subcutaneously in 5-8-week-old nu/nu female nude mice. Tumor was measured with a calibrated tool every 2 weeks. Mice were euthanized when the tumor reached 1000 mm3. Tumor was then harvested and transferred into a new generation of mice. Tumor samples were used to confirm genotyping by Short Tandem Repeats DNA fingerprinting as well as interspecies contamination testing to compare the fractional abundance of human versus mouse DNA. H&E staining, immunohistochemistry (IHC), and PCR were done to confirm microscopic, IHC, and genetic similarity to the original patient tumor, respectively. Successful PDX establishment was defined by successful engraftment (F0), tumor passaging through 4 consecutive generations (F1-F4), and successful tumor growth from a cryopreserved sample. A cell line was developed by collagen and extracellular DNA degradation, RBC removal, followed by incubation in RPMI medium. A total of 20 passages and at least 6 months in culture were considered adequate to deem a cell line as established.
Results/Discussion: Three novel ATC PDX models were successfully established. MDA-ATC8 is a BRAF wild type (WT) and TP53, NRAS_Q61K, TERT, ASXL2_G853S, ERBB4_D243N, INHBA_I410T mutant ATC model, from a patient previously treated with dabrafenib, trametinib and pembrolizumab (DTP). This is the only established model with a paired cell line (MDA-T317). MDA-ATC9 is a BRAF, RAS, and TERT WT tumor with TP53, FGFR2, LRP1B, and MEN1 mutations. Tumor was harvested from a previously untreated patient. MDA-ATC11 is a BRAFV600E, P53, TERT, ABL1, KMT2C, LRP1B, RICTOR mutant derived from a patient previously treated with DTP.
Conclusion: We report the development and establishment of three new ATC PDX models with one paired cell line. These models show excellent compatibility with their human tissue counterparts, making them highly reliable as preclinical models. Furthermore, with novel drugs being developed for RAS_Q61 mutation, MDA-ATC8 may play a major role in testing potential candidates for this untreatable patient population in the near future.

#4757 Elucidating novel pro-apoptotic consequences of ceramides in endocrine therapy-resistant breast cancer.

Purab Pal[1], Godwin K. Sarpey[1], G. Ekin Atilla-Gokcumen[2], Jonna Frasor[1], Jonathan L. Coloff[1]

[1]Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, IL,[2]Department of Chemistry, University at Buffalo, Buffalo, NY

Endocrine Therapy (ET)-resistant breast cancer (BC) remains a clinical problem, with approximately 40% of patients experiencing disease relapse. Previously, we demonstrated that preclinical models of ET-resistant BC have an altered sphingolipidome, which is characterized by low levels of ceramides across multiple models of ET resistance. ET-resistant models are also more sensitive to ceramide-induced death compared to their ET-sensitive counterparts suggesting a functional significance of the low levels of ceramides. This study is designed to identify mechanisms by which ceramides preferentially kill ET-resistant BC models to identify novel therapeutic strategies to improve ET-resistant BC patient outcomes.

We performed total RNA-seq of multiple ET-resistant BC cells with or without ceramides and found that ceramide exclusively induces genes associated with endoplasmic reticulum stress (EnRS), particularly in the PERK/peIF2A/ATF4 pathway, in multiple models of resistance but not in the parental ET-sensitive cells. Inhibitors of EnRS, PERK, and peIF2A partially reverse ceramide-induced cell death suggesting that ceramide-induced EnRS is lethal for ET-resistant cells. Since ceramides are well-known bioactive lipids, we hypothesized that ceramides interact with specific proteins that are instrumental in ET-resistant cell survival. To test this, we used a photoactivable-and-clickable ceramide probe and quantitative proteomics to identify differentially expressed ceramide-interacting proteins (CIPs) in ET-resistant BC cells. We found that ceramide interacts with a group of clinically relevant CIPs which are located in the endoplasmic reticulum (EnR) membrane. While most of these CIPs have yet to be implicated in ET resistance, a subset of the CIPs is known to be involved in attenuating EnRS. Knocking down one of the top CIPs, i.e., TRAM1, phenocopies ceramide treatment, suggesting that ceramides, by interacting with EnR-membrane proteins, such as TRAM1, may disrupt their function and induce a lethal level of EnRS in ET-resistant cells. Together, we find that ceramide induces preferential cell death in ET-resistant cells through interactions with a novel array of proteins leading to the inhibition of key survival proteins and a consequential activation of lethal levels of EnRS. Understanding how ceramides interact with and affect these proteins could lead to the development of novel strategies to selectively treat ET-resistant BC and to improve patient outcomes.

#4758 AZD9750, a novel androgen receptor proteolysis targeting chimera (AR-PROTAC) for the treatment of prostate cancer.

Claire Crafter[1], Antonio Ramos-Montoya[1], Chrysiis Michaloglou[1], Ziyanda Sholugu[1], Nuria Galeano-Dalmau[1], Gemma Hardman Fowler[1], Thomas Jones[2], Ana Quiroga[3], Pablo Morentin-Gutierrez[3], Andy Pike[3], Laura Evans[4], James S. Scott[4], Michael Niedbala[5]

[1]Oncology Targeted Discovery, Bioscience, AstraZeneca, Cambridge, United Kingdom,[2]Biopharmaceuticals R&D, Discovery Sciences, Cambridge, United Kingdom,[3]Oncology Targeted Discovery, DMPK, AstraZeneca, Cambridge, United Kingdom,[4]Oncology Targeted Discovery, Chemistry, AstraZeneca, Cambridge, United Kingdom,[5]Oncology Targeted Discovery, AstraZeneca, Waltham, MA

The androgen receptor (AR) is a member of the nuclear hormone receptor superfamily and a key driver of prostate tumor growth. Several androgen receptor pathway inhibitors (ARPIs) have been developed and approved for the treatment of locally advanced and metastatic prostate cancer, including the androgen synthesis inhibitor, abiraterone and the AR antagonists, enzalutamide, apalutamide and darolutamide. Despite initial success resistance does occur, often involving alterations in the receptor itself, such as AR amplification or point mutations in the ligand binding domain (LBD). Therefore, there remains an urgent need to develop alternative strategies to target the AR. Here we describe the characterization of AZD9750, a novel AR PROTAC that potently degrades AR (DC50 in LNCaP cells = 9.9nM) and shows good cross species oral bioavailability. In a panel of prostate cancer (PC) cells expressing different altered forms of the receptor, AZD9750 was shown to degrade wild type, amplified and LBD mutant AR. The reduced AR levels were confirmed to be a consequence of increased AR degradation with AZD9750 reducing the half-life of AR from 4.9 to 1.0 hr in LNCaP and from 4.6 to 0.4 hr in VCAP PC cells. Degradation was also shown to be mediated via a PROTAC mechanism that required CRBN binding and proteasomal activity. Additionally, AZD9750 decreased AR-regulated gene expression and inhibited the proliferation of AR-dependent PC cell lines with GI50s in the low nM range, which represents a 10-fold increase in potency compared to enzalutamide in the same cell lines. In vivo, in the C901 patient derived xenograft (PDX) model, AZD9750 caused a dose-dependent reduction in tumor AR levels with oral doses of 3, 10 and 30 mg/kg giving a statistically significant reduction of 72, 88 and 95%, respectively. Consistent with this, the mRNA expression of a panel of AR-regulated genes was also decreased. Anti-tumor efficacy studies demonstrated that AZD9750 reduced tumor growth and in some cases caused tumor regression in a range of AR positive PDX models that represent both hormone-sensitive and castration-resistant prostate cancer. The anti-tumor effect was dose-dependent and accompanied by reduced AR levels and AR-regulated gene expression, consistent with the observed efficacy being driven by AR degradation and inhibition of AR signaling. Furthermore, in several models, the efficacy achieved with AZD9750 was superior to the efficacy achieved with a clinical exposure matched dose of enzalutamide. These data confirm that AZD9750 is a potent oral degrader of AR that leads to anti-tumor efficacy in a range of hormone-sensitive and castrate-resistant PDX models. Through degradation of AR, including mutant and amplified forms of the receptor, it has the potential to overcome key resistance mechanisms arising to current standard of care therapies and provide benefit to patients with prostate cancer.

#4759 Novel treatment approach for efficacy outcome in androgen dependent and independent prostate cancer orthotopic mice models.

Balaji Ramachandran, Satheeshkumar Rajendiran, Rajendran Venkidusamy, Raghu Patil, Sachin Joshi, Krishnappa Haladasappa

Pharmacology, Adgyl Lifesciences Private Limited, Bangalore, India

Prostate cancer is one of the leading cause of deaths worldwide. Orthotopic injection of cancer cells into mouse prostate remains significant challenge and orthotopic mouse models resemble human pathophysiology. In the current study Androgen dependent (LNCaP VCaP and VCaP-EnzR) and Androgen independent (PC3) cells were orthotopically inoculated (1x10^6 cells) into anterior prostate of male athymic nude mice. Seven to eleven days post implantation, the preclinical efficacy of Enzalutamide (15mg/kg, PO QD x 6 weeks) and Docetaxel (DTX; 7.5mg/kg, i.p Q7D x 6 weeks) as standalone and combination treatment was evaluated. Body weight, clinicals signs, mortality was monitored up to 7 weeks. Terminally prostate was harvested, weighed, tumor volume (TV) was measured. Prostate, distant organs (lung, liver, and bone) were fixed in 10% NBF for metastatic evaluation by histopathology. In LNCaP and VCaP model standalone Enzalutamide treatment significantly reduced prostate weight and TV compared to vehicle control. However standalone DTX treatment resulted in moderate reduction of prostate weight and TV. Whereas combination of Enzalutamide with DTX demonstrated superior efficacy compared to respective standalone treatments. In the VCaP-EnzR model, docetaxel (DTX) treatment demonstrated significant antitumor activity both as standalone treatment and in combination with Enzalutamide. In contrast, Enzalutamide treatment did not reduce tumor volume and results were similar to control group. In PC3 model, the standalone DTX treatment exhibited highest antitumor activity followed by Enzalutamide therapy. The combination of Enzalutamide with DTX demonstrated superior efficacy compared with respective standalone treatments. Histopathological examination of prostate, lung, liver, and bone tissues in all treatment groups for LNCaP, VCaP & PC3 models revealed a significant reduction in organ metastasis with a clear progression of metastasis observed in the vehicle group.In conclusion, standalone hormone therapy slowed down primary tumor growth of androgen dependent prostate cancer by reducing androgen levels or blocking androgen receptors. The lack of response to enzalutamide in the VCaP-EnzR model likely reflects resistance mechanisms. In contrast, docetaxel targeting cell division, is effective regardless of AR signaling, allowing it to overcome enzalutamide resistance. In condition where prostate cancer became resistance to hormonal therapy, chemotherapy plays critical role in attacking prostate cancer cells irrespective of androgen status. This combination offers advantageous, multifaceted approach in treating metastatic resistant prostate cancer and improves treatment effectiveness, delay progression and potentially extending survival.

Key words: Prostate cancer, orthotopic, hormone therapy, metastatic

#4760 Oxysterol sulfotransferase interferes with prostate cancer progression.

Bobae Park[1], Okunola Igbekoyi[1], Mukund Bhandari[2], Bandana Chatterjee[1]

[1]Molecular Medicine, UT Health Science Center at San Antonio, San Antonio, TX,[2]Pathology, UT Southwestern Dallas, Dallas, TX

Background: Oxysterol sulfotransferase (SULT2B) mediates enzymatic sulfation of oxysterols. Oxysterols are agonist ligands for the nuclear receptor LXR (liver X receptor) and upon sulfation they become LXR inert. Poor overall survival is linked to low-SULT2B tumors in primary PC (TCGA data) and in esophageal carcinoma, evident for post-surgery patients[1]. We have shown that clinical samples of metastatic castration-resistant prostate cancer (CRPC) lack SULT2B, and it is reduced or undetectable for a subset of primary PC. In a CRPC model, SULT2B ablation led to faster xenograft growth; increased cell migration and invasion; EMT-like activation; upregulation of AKR1C3, which is a key enzyme for androgen biosynthesis; and activation of ERK - a kinase that regulates cell survival[2]. LXR is inactivated in SULT2B-overexpressed cells[3], and we observed AKR1C3 upregulation in LXRα-silenced cells. Herein we report impacts of high SULT2B on CRPC cells in culture and xenografts.

Results: PC3 cells, transduced to express SULT2B and Akaluciferase reporter (PC3-SA cells) or reporter only (PC3-VA cells) were isolated under antibiotic selection and flow sorted to >98% enrichment for the transduced genes on the basis of fluorescence from the Venus fluorescent protein. PC3-VA and PC3-SA cells showed similar proliferation rates *in vitro*. In contrast, the growth of PC3-SA xenografts showed marked and statistically significant reduction compared to PC3-VA xenografts. The reduction was calculated from differences in tumor volumes and tumor-emitted luminescence. Apoptosis, evident from caspase3 cleavage, was detected in the SA, not VA xenografts. Cleaved caspase3 in tumors was detected by Western Blot and IHC analyses. Xenograft growth was also reduced for SULT2B-high tumors of castration-resistant CWR22Rv cells which, unlike PC3 cells, express androgen receptor. The inhibition of SULT2B-high tumors is consistent with our earlier data showing escalated growth of SULT2B-null xenografts. Further, SULT2B-expressing CRPC cells showed enhanced sensitivity to an anti-cancer biologic for the inhibition of cell proliferation and invasion *in vitro*. Bioinformatic probing of single cell RNA sequences is ongoing to identify genes expressed differentially and pathways impacted in response to SULT2B expression.

Conclusion: SULT2B interferes with CRPC xenograft growth and causes enhanced sensitization of CRPC cells to an anti-cancer biologic. Factors extrinsic to cancer cells are likely to play a role in the growth suppression of high-SULT2B tumors to account for the result showing a lack of impact of SULT2B on cell proliferation *in vitro*. Our study of allograft tumors in syngeneic mice may identify extrinsic factor(s) at the tumor niche that are involved in the growth suppression of SULT2B-positive prostate tumors.

Funding: DoD-W81XWH-21-1-0307 & NIH-R21CA289167 Refs (PMID): 1) 34730281; 2) 94239; 3) 17189208

#4761 The ERBB4 exon skipping isoform JMA-CYT2 is the dominant isoform of ERBB4 gene fusions.

Shivani Jagannathan Murali, Bharath Kumar Karre, Cristina Ivan, Chao Sima, Asfetaw Abera, David Spetzler, Milan Radovich, Heather O'neill, Ravi Chakra Turaga

Department of Precision Medicine, Target and Drug Discovery, Caris Life Sciences, Inc., Chandler, AZ

Background: ERBB4, a member of the ERBB family of receptor tyrosine kinases, has received less attention than its counterparts, ERBB1 (EGFR), ERBB2 (HER2) and ERBB3(HER3), despite its established involvement in oncogenesis and its potential as a therapeutic target. While the role of ERBB4 and its isoforms in neuronal development is well understood, mutations and fusions of ERBB4 have been linked to several types of cancer, however they have not been fully explored. Further research into ERBB4 fusions and their oncogenic mechanisms is essential for advancing targeted therapies.
Methods: We performed a comprehensive analysis of ERBB4 fusions using next-generation sequencing (NGS) data from a total of 216,176 formalin-fixed paraffin-embedded (FFPE) tumor samples across various solid tumors. NGS was conducted on DNA isolated from these samples and RNA fusions were detected using whole transcriptome sequencing (WTS). We analyzed patient demographics, fusion partners, ERBB4 isoforms, and co-occurring mutations. Additionally, tumor mutational burden (TMB) was assessed, and data from public bioinformatic resources were used for comparison.
Results: ERBB4 fusions were detected in 0.25% of the 24 solid tumor types analyzed, with the highest prevalence observed in OSEC, BRCA, BLCA, UTNE, and NSCLC. Notably, 63.1% of ERBB4 fusions involved the 3' end of the gene and included various fusion partners. The most common fusion partner was IKZF2:ERBB4, accounting for 58.2% of cases. Exon 2 was identified as the most frequent breakpoint in ERBB4 fusions. As a result, 5' ERBB4 fusions retain only the signal peptide, while 3' fusions lose the signal peptide, which may impair translocation to the cell membrane. The most common isoform observed in ERBB4 fusions was the JMa-Cyt2 isoform, seen in over 83% of cases. This isoform skips exon 26, which contains a PI3K phosphorylation site critical for ubiquitination and degradation. Further analyses revealed a significant upregulation of ERBB4 expression in ERBB4 fusion positive cases in BLCA($P>0.001$), OSEC($P>0.001$), and NSCLC($P>0.001$). Pathway analyses revealed upregulation of Myc targets indicating ERBB4-ICD-STAT5A complex in promoting cell proliferation. Co-occurring mutations are enriched in TP53, PIK3CA, and TERT in ERBB4 fusions.
Conclusion: Our study highlights the frequent occurrence of ERBB4 fusions across various cancer types, with IKZF2 being the most common fusion partner. The prevalence of the ubiquitination-resistant JMa-Cyt2 isoform and the retention of cytoplasmic (kinase) domain in many fusions suggest that ERBB4 fusions may contribute to oncogenesis through a STAT5A-dependent mechanism, independent of PI3K. These findings underscore the potential of ERBB4 fusions as therapeutic targets and suggest that gamma-secretase inhibitors could be a promising treatment option for patients with these fusions.

IMMUNOLOGY: Antibodies and Antibody-Drug Conjugates
Poster Session

#4765 Suppression of triple-negative breast cancer metastasis by a new glyco-humanized polyclonal antibody.

Carine Ciron, Ophelie Dauphouy, Pierre-Joseph Royer, Pierre Morice, Odile Duvaux, **Firas Bassissi**

Xenothera, Nantes, France

Context: Triple-negative breast cancer (TNBC) is an aggressive subtype accounting for 15-20% of breast cancer cases, characterized by poor prognosis, high recurrence rates, and limited treatment options. The lack of hormone receptors associated with a high level of molecular heterogeneity of TNBC hinders the development of effective therapies. Chemotherapy remains the standard of care but is often compromised by resistance and toxicity, emphasizing the urgent need for innovative treatments. Here, we assess the efficacy of XON7, a first-in-class glyco-humanized polyclonal antibody (GH-pAb), in preclinical models of TNBC.
Methods: The anti-tumor efficacy of XON7 was assessed in TNBC cell lines for its capacity to induce complement-dependent cytotoxicity (CDC) and apoptosis, compared to paclitaxel and the ADC Sacituzumab Govitecan. Apoptotic mechanisms were explored by evaluating caspase 8/9 activation, reactive oxygen species (ROS) levels, and mitochondrial membrane potential. In vitro 3D cultures, XON7's ability to inhibit tumor growth, spheroid formation, and metastasis was tested. In vivo antitumor efficacy was evaluated in orthotopic and metastatic models in SRG rats inoculated with MDA-MB-231-Luc cells, using bioluminescence imaging.
Results: XON7 demonstrated potent and selective cytotoxicity across human TNBC cell lines (80-100% cytotoxicity), sparing healthy PBMCs. Apoptotic induction involved both intrinsic and extrinsic pathways, evidenced by caspase 8/9 activation, ROS generation, and mitochondrial membrane potential loss. In 3D cultures, XON7 reduced spheroid size by 50% after two treatments (300 µg/ml) and significantly decreased cancer cell viability (28% remaining). Pre-treatment with XON7 prevented spheroid formation, outperforming paclitaxel and sacituzumab govitecan. XON7 also inhibited metastasis in transwell invasion assays. In vivo, bi-weekly XON7 treatments reduced tumor growth by 50% and eliminated lung metastases in an orthotopic rat model. In a metastatic model, tri-weekly XON7 treatments reduced total metastases (lung, liver, etc.) by 90%.
Conclusion: XON7 is a multi-target antibody currently under clinical evaluation, it represents a promising candidate for advancing treatment paradigms in TNBC.

#4766 Characterization of XON9, an effective glyco-humanized polyclonal antibody against hepatocarcinoma.

Pierre-Joseph Royer, Carine Ciron, Ophelie Dauphouy, George Graur, Odile Devaux, **Firas Bassissi**

Xenothera, Nantes, France

Context: Hepatocarcinoma is the second leading cause of cancer-related death, and its incidence is increasing. Multikinase inhibitors (Sorafenib or Lenvatinib) are the conventional first line treatment for unresectable HCC, with limited response rates and serious side effects. Although immune base therapies such as checkpoint inhibitors have recently showed promising results, they remain applicable to a small fraction of patients. Thus, new treatment and strategies are desperately needed to improve the management of hepatocarcinoma. We evaluate here the in vitro and in vivo efficacy and safety of XON9, first-in-class glyco-humanized polyclonal antibody (GH-pAb) against hepatocarcinoma.

Material and methods: Cytotoxic activity of XON9 against HUH7, HepG2 and Hep3B cell lines was investigated and compared to the activity of standards of care Sorafenib and Lenvatinib. Primary hepatocytes were used as control. Mechanisms of apoptosis were further explored. Activity of caspases 8 and 9, level of reactive oxygen species (ROS) and mitochondrial membrane potential was investigated after treatment with XON9. The *in vivo* efficacy of XON9 was then assessed in NMRI nude engrafted with HUH7 cancer cells while pharmacokinetics and safety parameters were evaluated in a non-human primate after once weekly dosing at 50mg/kg over 2 weeks.

Results: XON9 shows a potent complement dependent cytotoxicity (CDC) against HUH7, HepG2 and Hep3B cells (EC50<10µg/ml). By contrast, primary hepatocytes were relatively spared by XON9 (EC50>50µg/ml). Moreover, XON9 treatment induced apoptosis of the three cell lines tested with activation of caspases 8 and 9, increase of ROS levels and drop of mitochondrial membrane potential. Overall, *in vitro* lytic activity of XON9 towards HUH7, HepG2 and Hep3B was superior to that of Sorafenib and Lenvatinib, the current standards of care for hepatocarcinoma. *In vivo,* XON9 significantly reduced tumour progression in a mouse xenograft model (40% reduction at day 25). No toxicity was observed after repeated injections of 50mg/kg of XON9 in a non-human primate.

Conclusion: Although recent immunotherapy advances have been made, hepatocarcinoma remains an incurable disease with a real need for innovative therapies. XON9 represents a promising and selective immunotherapy against refractory hepatocarcinoma.

#4767 XON11, a novel multi-target antibody in pancreas cancer.

Carine Ciron, Ophelie Dauphouy, Pierre-Joseph Royer, Gwenaelle Evanno, Odile Devaux, **Firas Bassissi**

Xenothera, Nantes, France

Background: Pancreatic cancer continues to be one of the most lethal cancer types, with a 5-year overall survival rate of only 12%. It poses one of the toughest challenges in oncology, as chemotherapy and immunotherapy have not significantly improved patient outcomes. Consequently, there is an urgent need for new therapeutic approaches. XON11 is a new polyclonal antibody targeting several pancreatic cancer antigens, including KrasG12D. The aim of these studies was to assess the tolerance and efficacy of XON11 in non-clinical pancreatic cancer models.
Material and methods: XON11 ability to induce Complement Dependent Cytotoxicity (CDC) and apoptosis compared to gemcitabine was tested in a panel of human pancreas cancer cell (MiaPaca, Aspc1, Capan, Panc1) after 24h of incubation. The capacity of XON11 and gemcitabine to impair tumorsphere formation and growth was investigated in pancreatic cancer cells for 11 days. In vivo XON11 and gemcitabine tolerance and efficacy was evaluated in NMRI nude mice after intraperitoneal dosing at 40 mg/kg tri-weekly for 4 weeks.
Results: XON11 showed potent and selective anti-proliferative activity in a panel of human pancreatic Kras muted cancer cell lines, including those resistant to gemcitabine. It induced specific tumor cell cytotoxicity (Mean EC50 = 25.85 µg/mL) without affecting healthy cells (PBMC) at this concentration. XON11 displayed significantly higher potency against pancreatic cancers compared to gemcitabine. It was able to kill up to 97% of the cancer cells, whereas gemcitabine's maximum effect reached 30%. XON11 induced both caspase 8 and caspase 9 (suggesting activation of both apoptosis pathways). Furthermore, it induced a dose- response loss of mitochondrial membrane potential and ROS generation in different pancreatic cell lines. In contrast to gemcitabine, XON11 significantly decreased Aspc1 tumorspheres viability after treatment (>75% of cytotoxicity). In addition, XON11 completely inhibited sphere formation from 33µg/ml. XON11 was significantly effective against tumor growth in two xenograft mouse models and well tolerated in mice after repeated intraperitoneal dosing at 40 mg/kg over 28 days.
Conclusion: Based on its high potency and tolerance, XON11 can provide a novel and promising therapy for fighting recurrent pancreatic cancer.

#4768 Development of fully human TCR-mimic antibodies targeting intracellular tumor antigens using humanized RenTCR-mimic transgenic mice.

Han Zhang, Wanbo Tang, Jun Du, Yue Zhang, Limin Zhao, Xin Jiao, Baihong Liu, Yi Yang

Biocytogen, Waltham, MA

T cell receptors (TCRs), a promising therapeutic strategy for targeting intracellular antigens, can specifically recognize antigen peptide presented by human lymphocyte antigen (HLA) and mediate T cell responses to eliminate tumor cells. However, the low affinity of TCRs presents a great challenge to their broader clinical application. TCR-mimic (TCRm) antibodies, with similar antigen recognition capabilities as TCRs but higher affinity, offer a solution to these limitations, enabling the targeting of previously undruggable cancer antigens. Neverthelss, specificity remains a major challenge to ensure therapeutic accuracy and patient safety, as TCRm antibodies need to precisely target a single tumor-specific peptide antigen presented by HLA rather than other similar peptides.
Using RenTCR-mimic transgenic mice, we describe the generation and preclinical evaluation of fully human TCRm antibodies by fusing them with a humanized anti-CD3 scFv to form T cell engager or assembled on T cell surface to build TCRm-T cells, further to specifically redirect T cells to lysis tumors by our proprietary RenTCR-mimic platform. We determine cross-reactive peptide sequences predicted by in silico or known off-target sequences. We further confirm the specificity and safety of TCRm antibodies. Despite low density antigen copies, TCRm antibodies elicited antibody-mediated T-cell recognition and cytotoxicity against antigen peptide-HLA complexes-restricted cell lines. Based on these methods, we perform screens to validate TCRm antibodies specificity. These experiments led us to discriminate between the target peptide and the potential off-target peptides presented by HLA and highlight the therapeutics potential of TCRm antibodies in tumor immunotherapy, offering a novel approach to expanding the repertoire of druggable cancer targets.

#4769 Characterization of novel humanized FGFR2b antibody-based ADCs site-specifically conjugated with topoisomerase I inhibitor payload in preclinical tumor models.

Fei Teng, Huanhuan Guo, Hongjun Li, Lizhi Qin, Xinlai Yao, Lei Shi, Yi Gu, Xueming Qian

Suzhou Transcenta Therpeutics Co., Ltd, Suzhou, China

Background: Fibroblast growth factor receptor 2 IIIb (FGFR2b), one of the four FGFR family members that encode transmembrane receptor tyrosine kinases, is overexpressed in a variety of cancers, such as gastric/GEJ (29%), esophageal (41%), squamous NSCLC (31%), TNBC (13%), ovarian (40%), endometrial (86%), cervical (80%), colorectal (62%), and cholangiocarcinoma (22%). We have developed a FGFR2b-targeting ADC (TST105) with a novel topoisomerase I inhibitor payload by using glycotransferase mediated site-specific conjugation and characterized its *in vitro* and *in vivo* anti-tumor activity in preclinical tumor models.

Methods: A novel anti-FGFR2b humanized antibody 38D4 was generated by hybridoma platform. The binding affinity was analyzed by Fortebio Octet, and the selectivity and species cross-reactivity were assessed by ELISA. Antibody internalization was conducted by using Fluoresence Image. Cytotoxicity of TST105 was tested in tumor cell lines with different levels of FGFR2b expression. The bystander effect was assessed in co-culture experiments with the FGFR2b-negative and -positive tumor cells. Anti-tumor efficacy was evaluated in FGFR2b-positive tumor cell line-derived xenograft (CDX) models in mice.

Results: 38D4 specifically binds to FGFR2b with a higher affinity than the Bemarituzumab-analog. 38D4 binds with comparable affinity to human, cynomolgus, rat and mouse FGFR2b. Significant internalization of 38D4 was observed after 4 hour and 24 hour-culture at 37℃. In the gastric (SNU-16) and colorectal (SW480-FGFR2b) tumor cell lines, TST105 demonstrated specific killing activity with a potency between 0.3 nM and 0.4 nM *in vitro*, which was weaker than both 38D4-MMAE and Bema-MMAE (stochastic conjugated). However, a higher potent bystander effect of TST105 than them was observed, indicating the potential to treat tumors with heterogeneous target expression. Furthermore, in SNU-16 xenograft model, which endogenously expresses FGFR2b at a high level, TST105 induced a better anti-tumor efficacy than Bema-MMAE. TST105 had a tumor growth inhibition of 91.25% and overall response rate (ORR, 50% reduction of tumor volume from baseline) of 70% at 3 mg/kg QW, while Bema-MMAE only reduced tumor growth of 48.32% with no ORR. The similar superior tumor regression efficacy of TST105 was also observed in colorectal tumor model SW480-FGFR2b. This differentiation may be contributed by the glycotransferase site-specific conjugation, which can eliminate Fc function, thus reduces the non-specific endocytosis in normal cells and leads to ADC enrichment in the FGFR2b-positive tumors.

Conclusions: FGFR2b ADC TST105 demonstrated the high potency, strong anti-tumor activity and bystander effect in the preclinical studies, which support further investigations of TST105 in FGFR2b positive solid tumors.

#4770 First-in-class anti-TRPV6 antibody as a new therapeutic agent in cancer.

Lindsay B. Alcaraz[1], Vincent Roux-Portalez[1], Aurelien Haustrate[2], Gautier Robin[1], Alexia Parmentier[1], Clement Cordier[1], Florence Boissiere-Michot[3], Amelie Gudin-de-Vallerin[3], Evelyne Lopez-Crapez[3], Gaelle Fromont-Hankard[4], Tristan Mangeat[1], Margaux Maurel[1], Marianne Le Gall[1], Thierry Chardes[5], Bruno Robert[5], Yoann Sottejeau[6], Diego Tosi[7], Natacha Prevarskaya[2], V'yacheslav Lehen'kyi[2], Bernard Pau[1], **Johanna Marines**[1]

[1]Mabqi, Montpellier, France,[2]PhyCell Laboratory, Lille, France,[3]Translational Research Unit, Montpellier Cancer Institute Val d'Aurelle, Montpellier, France,[4]Universite de Tours, Tours, France,[5]Institut de Recherche en Cancerologie de Montpellier (IRCM), Inserm U1194, Montpellier, France,[6]SATT Nord, Lille, France,[7]ICM, Montpellier, France

Context: The involvement of calcium signaling in tumor progression has been well documented in the literature. In particular, the calcium channel TRPV6 has been identified as a key player in the physiopathology of solid tumors. At the transcriptomic level, TRPV6 is overexpressed in many cancers such as prostate, pancreatic and ovarian cancers underscoring its potential as a new therapeutic target. While some TRPV6-targeting antagonistic peptides have reached clinical stage, no antibody candidate is currently in development. Leveraging its phage display technology and unique proprietary antibody libraries, Mabqi (France), in collaboration with SATT Nord and PhyCell laboratory (France), has discovered and characterized a first-in-class human anti-TRPV6 antibody "20H3" with high clinical potential.

Methods: A comprehensive series of *vitro* and *vivo* preclinical studies demonstrated the safety profile, efficacy and clinical relevance of 20H3 antibody, as a new candidate for the treatment of prostate cancer.

Results: *In vitro* studies demonstrated the high affinity and specificity of 20H3 antibody against TRPV6 target. Exploration of antigen-antibody interaction was performed through epitope and paratope mapping and antagonistic activity of 20H3 antibody was validated via calcium flux modulation assays. The excellent developability properties were also demonstrated through thermostability, aggregation and stability assays.

In vivo, the 20H3 safety profile was confirmed in mice model. After repeated doses up to 64 mg/kg, no effect on mice body weight and no sign of toxicity in main organs collected were detected. The antitumoral potential of 20H3 antibody was evaluated in three *vivo* cancer models. As initial positioning is prostate cancer indication, efficacy studies in two xenografted prostate models (LNCaP and VCaP) were performed. In both models, specific tumor targeting and tumor penetration were confirmed and a strong antitumoral efficacy was demonstrated at 4-8 mg/kg with a tumor growth inhibition superior to 60 % and an increased mice survival of 62 %. Interestingly a strong efficacy was also shown in MCA205 sarcoma model. To further elucidate antibody's mode of action, a comparison between Fc-WT and Fc-silencing format was carried out in immunocompetent and immunodeficient mice models. All together, these results demonstrated that antitumor efficacy is mediated by both paratopic region and Fc region of the antibody that potentializes the effect of the treatment. Finally, the 20H3 clinical relevance was demonstrated by immunohistochemistry studies with positive immunoreactivity in a large proportion of prostate cancer patient samples.

Conclusion: The 20H3 antibody is the first anti-TRPV6 antibody under development, with a strong potential to advance rapidly into clinic. The CMC process of 20H3 drug candidate is planned in 2025 and Phase Ia clinical trial is expected to start in early 2027.

#4771 A novel immunotherapy for metastatic prostate cancer: A monoclonal antibody that can bind both STEAP1 and STEAP2.

Minzhi Sheng, Boyang Su, Amanda Sparkes, Gobi Thillainadesan, Esther Matus, Jessica Wright, Stanley Liu, Jean Gariepy, Hon S. Leong

Sunnybrook Research Institute, Toronto, ON, Canada

Prostate cancer (PCa) is the most common cancer among Canadian men, with metastatic PCa (mPCa) patients having a 5-year survival rate of only 30%. However, the prostate-specific membrane antigen, a key target in PCa therapy, is expressed in many normal tissues and shows heterogeneity among PCa patients. Recently, Six Transmembrane Epithelial Antigens of the Prostate 1 and 2 (STEAP1 and STEAP2) have gained attention as promising therapeutic targets due to their high expression in most PCa cases compared to normal tissues and essential organs. This study aimed to develop a novel immunotherapy targeting STEAP1/2 to elicit PCa-specific immune responses in cellular and animal mPCa models. Monoclonal antibodies were generated against the extracellular region of STEAP1, which shares 60% sequence similarity with STEAP2 but has minimal homology with other human proteins. The specificity and binding strength of these antibodies were validated through techniques such as immunofluorescence, flow cytometry, immunoprecipitation, and ELISA. Results from immunofluorescence and flow cytometry using STEAP1 overexpression and knockdown PCa cell lines demonstrated the specificity of certain clones for human STEAP1. Furthermore, one antibody clone successfully pulled down STEAP2 in immunoprecipitation-mass spectrometry experiments and showed binding to STEAP2 in cell lines overexpressing the antigen. ELISA results revealed binding affinities in the low nanomolar range for STEAP1 and the low micromolar range for STEAP2.These findings highlight the potential of the monoclonal STEAP1/2 antibody developed in this study, providing a promising platform for future immunotherapy strategies and expanding treatment options for mPCa patients.

#4772 AI-guided engineering and preclinical development of biparatopic anti-DLL3 antibody-drug conjugates (ADCs).

Chuan Chen, Yue Wu, Liang Tian, Chenpeng Su, Zhaohui Chen, Jiyuan Tian, Dandan Liu, Yang He, Xiaoqian chen, Yongxin Shang, Jian Peng, Zhenping Zhu

Helixon US Inc., New York, NY

Delta-like ligand 3 (DLL3) is often overexpressed in small cell lung cancer (SCLC) and several other neuroendocrine tumors, with no or very low expression in normal issues. An anti-DLL3 x CD3 bispecific T-cell engager, tarlatamab, has demonstrated significant clinical benefits and was recently approved by the US FDA for the treatment of DLL3-expressing SCLC. On the other hand, Rova-T, the first antibody-drug conjugate (ADC) targeting DLL3 entered advanced clinical development, failed in phase III studies due to insufficient efficacy and an unfavorable safety profile. In this study, via AI-guided engineering and optimization, we produced a novel biparatopic anti-DLL3 antibody and generated a number of ADCs utilizing various linkers and cytotoxic payloads. The ADCs demonstrated efficient DLL3-mediated cell binding and rapid internalization at a much higher efficiency than the mono-epitope targeting ADCs. The ADCs showed selective cytotoxicity towards multiple tumor cell lines with varying levels of DLL3 expression in vitro, and potently inhibited the growth of several DLL3-expressing tumor xenografts in animal models. Taken together, our findings underscore the significance of AI-guided antibody engineering and optimization, and provide support for the further development of the biparatopic anti-DLL3 ADCs in patients with DLL3-expressing tumors such as SCLC and other neuroendocrine tumors.

#4773 AM E3-SG3249, a first-in-class α-CRLF2 antibody-drug conjugate for the treatment of CRLF2-rearranged Ph-like B-ALL.

Claudia Dall'Armi, Valentina Di Biasio, Chantal Paolini, Daniela Natale, Francesco Scalabri, Antonino Missineo, Nadine Alaimo, Maurizio Nuzzo, Anna Demartis, Pamela Di Pasquale, Roberto Benoni, Fulvia Caretti, Elisa Beghetto, Fabrizio Colaceci, Romina Sasso, Vincenzo Pucci, Carlo Toniatti, **Alessandro Carugo**

IRBM S.p.A., Pomezia, Italy

Background: Patients affected by Ph-like B-cell acute lymphoblastic leukemia (B-ALL) have a high mortality rate and elevated levels of disease relapse upon chemotherapy treatment. More than 60% of Ph-like B-ALL is characterized by genomic rearrangements resulting in high expression levels of the CRLF2/TLSPR receptor on the cell surface. While significant advances have been made in developing targeted agents for B-ALL treatment, the CRLF2-rearranged subpopulation is lacking selective and effective therapies. Here, we report the development and preclinical validation of AM E3-SG3249, a first-in-class α-CRLF2 Antibody-Drug Conjugate (ADC) for the treatment of CRLF2-rearranged Ph-like B-ALL.

Materials and Methods: AM E3, a fully human α-CRLF2 monoclonal antibody with single-digit nanomolar affinity for the target (5.9 ± 1.0 nM), was identified leveraging our proprietary phage display platform. Due to its rapid internalization rate by CRLF2-expressing cells, AM E3 emerged as an ideal candidate for the development of an ADC targeting CRLF2. Upon conjugation with the cytotoxic payload tesirine (SG3249), a clinically-validated cross-linking agent, AM E3-SG3249 demonstrated a potent anti-tumor effect on both CRLF2-rearranged *in vitro* cell lines and *in vivo* orthotopic xenograft models. We also developed an ADC surrogate against murine CRLF2, which has low homology with the human counterpart (<35%), to further prove tolerability of the ADC therapy in preclinical mouse models.

Results: AM E3-SG3249 was potently cytotoxic (EC_{50} = 40pM) against a panel of hCRLF2-rearranged B-ALL cell lines *in vitro*. Upon single injection in xenograft models, AM E3-SG3249 showed strong anti-tumor activity proportional to receptor density. Moreover, AM E3-SG3249 treatment extended survival of hCRLF2-rearranged B-ALL orthotopic xenografts over standard induction therapy combining vincristine, dexamethasone, and L-asparaginase (VXL). The ADC surrogate mB4 AM23-SG3249 was also efficacious and well-tolerated in xenograft models engineered to overexpress murine CRLF2.

Conclusions: AM E3 is a novel monoclonal antibody with ideal features for the development of a first-in-class α-CRLF2 ADC therapy. AM E3 conjugated with the cytotoxic agent tesirine (SG3249) demonstrated remarkable anti-tumor efficacy and a reasonable therapeutic window for the treatment of CRLF2-rearranged Ph-like B-ALL.

#4774 Synergistic enhancement of immunogenic cell death: Combining AWT020 with antibody-drug conjugates for superior anti-tumor activity.

Fan Ye, Jianing Huang, Ella Li, Wen-Chin Huang, Fang Huang, Xiaoli Cheng, Jenny Jiang, Shih Chieh Chen, Botong Hua, Hanna Lin, Joyce Kwan, Eugene Liu, Ziyang Zhong

Anwita Biosciences, San Carlos, CA

Antibody-Drug Conjugates (ADCs) are targeted cancer therapies delivering cytotoxic agents selectively to cancer cells. Despite clinical success across various malignancies, resistance to ADC therapy limits long-term efficacy. A key ADC mechanism is inducing immunogenic cell death (ICD), releasing damage-associated molecular patterns (DAMPs) and tumor antigens to stimulate an immune response. This provides a rationale for combining ADCs with immunotherapies to overcome resistance and improve survival rates.AWT020 is a novel fusion protein currently in Phase I clinical trials. It combines a humanized antibody targeting programmed cell death protein 1 (PD-1) with a potency attenuated interleukin-2 (IL-2) mutein. This design blocks the PD-1 pathway while preferentially activating IL-2 signaling on PD-1^{high} tumor-infiltrating T cells (TILs), minimizing peripheral PD-1^{low} lymphocyte activation and reducing systemic toxicity. In preclinical tumor models, a murine surrogate of AWT020 (mAWT020) demonstrated superior anti-tumor activity over a PD-1 monoclonal antibody by selectively expanding TILs.Both ADCs and AWT020 selectively target the tumor microenvironment and promote tumor cell killing through distinct mechanisms. We hypothesized that ADC-induced ICD could be further amplified by AWT020. To test this, we developed two syngeneic tumor models, $EMT6^{B7H3}$ and $EMT6^{Her2}$, expressing human B7H3 and human Her2, allowing specific ADC targeting. In the $EMT6^{B7H3}$ model, combination of B7H3 ADC (B7H3-Exa) and mAWT020 eradicated all tumors after a single dose, outperforming monotherapies. Similar synergy between Her2-Exa ADC and mAWT020 was observed in the $EMT6^{Her2}$ model. In a subsequent tumor rechallenge study, most mAWT020-treated mice rejected the replantation of $EMT6^{B7H3}$, indicating that mAWT020 had induced long-term immunological memory in these animals. As a comparison, combination of anti-mPD-1 with B7H3-Exa did not enhance the antitumor activity of ADC. Characterization of ICD markers and TIL phenotypes in the $EMT6^{B7H3}$ model showed that B7H3-Exa treatment significantly upregulated calreticulin (CRT) expression in $EMT6^{B7H3}$ tumor cells. The combination of B7H3-Exa and mAWT020 further increased CRT expression. Compared to B7H3-Exa monotherapy or the combination of B7H3-Exa and anti-mPD-1, mAWT020 treated groups significantly increased $CD3^{+}$ T cell infiltration within the tumor, as well as tumor-specific T cells, as detected by ELISpot assay.These preclinical findings demonstrate that ADC-induced ICD is amplified by AWT020, but not by anti-mPD-1, resulting in greater tumor regression and enhanced immunological memory. These results support the continued clinical development of AWT020 and to explore its potential in combination with ADCs, aiming to improve long-term survival and complete response rates in cancer patients.

#4775 Combination therapy with TB511 and anti-nectin-4 antibody drug conjugate in a human pancreatic cancer mouse model.

Ik-Hwan Han[1], Ilseob Choi[1], Soyoung Kim[1], Juwon Yang[1], Heekyung Lee[2], Geumseong Yun[1], Hyunju Lee[1], Hyunsu Bae[1]

[1]Department of Physiology, Kyung Hee Univ. College of Oriental Medicine, Seoul, Korea, Republic of,[2]R&D Center, Twinpig Biolab Inc., Seoul, Korea, Republic of

Pancreatic cancer is a type of cancer that occurs in the pancreas and occurs in the pancreatic duct in more than 90% of cases and is known as the 10th most common cause of cancer deaths worldwide. Recently, tumor-associated macrophages (TAMs) in tumor microenvironment (TME) have been known to closely associate with progression of pancreatic cancer. Among TAMs, M2 type macrophages constitute the majority within the TME and are associated with tumor progression and drug resistance. Targeting M2 macrophages represents a promising therapeutic strategy to reprogram the TME and enhance anti-tumor immunity. In our previous study, we developed TB511, a peptide drug conjugate that preferentially binds to M2 macrophages and induces apoptosis through mitochondrial targeting by conjugating with dKLA. Furthermore, in pancreatic cancer, the nectin-4 expression has been known to contribute to tumor proliferation, angiogenesis, and patient prognosis. Nectin-4 has been considered a potential new target for the treatment of pancreatic cancer. However, therapeutic efficacy of anti-nectin-4 antibody has not been established in pancreatic cancer. This study aims to evaluate the efficacy of the combination therapy of TB511 and anti-nectin-4 antibody drug conjugate (ADC) in reducing tumor growth and modulating the immune cells of tumor microenvironment in a humanized pancreatic cancer mouse model. In this study, humanized mice bearing subcutaneous pancreatic cancer using the PANC1 cell line were treated with TB511, ADC, their combination, or PBS as a control. As a result, TB511, PADCEV, and their combination significantly reduced tumor growth and Ki67 expression. EMT marker analysis revealed increased E-cadherin and decreased vimentin expression in combination therapy groups. TB511 and combination therapy reduced M2 macrophages (CD163, CD18, Kim127, CD11b) and increased CD8 T cells in the TME, while ADC alone showed no significant immune modulation. Spatial transcriptomics highlighted decreased epithelial cancer cells, increased M1 macrophages, and elevated CD8 T and NK cells in the TB511 and combination groups. Immune checkpoint markers (CTLA4, FOXP3, LAG3) were downregulated, and cytotoxic markers (GZMB, IFNG) were upregulated with TB511 and combination therapy. No significant off-target effects were observed in the spleen, liver, lung, or brain. In conclusion, combination therapy with TB511 and ADC effectively suppresses pancreatic tumor growth and reprograms the immune microenvironment by decreasing M2 macrophages and enhancing cytotoxic T and NK cell responses, offering a promising strategy for pancreatic cancer treatment.

#4776 Preclinical study of bispecific antibody drug conjugates (bsADCs) targeting DLL3 and SEZ6 demonstrated potent anti-tumor activity in small cell lung cancer (SCLC) xenograft models.

Kaiqing Zhang, Ting Yi, Liu Yang, Kunying Hao, Wenpeng Cao, Yu Qi, Jushi Gai, Luheng Du, Gao An, Chengzhang Shang, Yi Yang, Frank An

Biocytogen, Waltham, MA

Lung cancer is the most common type of cancers and the leading cause of cancer-related mortality worldwide with 2.5 million new cases and 1.8 million deaths in 2022. Small-cell lung cancer (SCLC) accounts for ~15% of all lung tumors, representing the most aggressive high-grade neuroendocrine carcinoma with a five-year survival rate less than 4%. Majority of patients are diagnosed with extensive stage SCLC (ES-SCLC) with brain metastases identified in ~80% of cases and the prognosis is dismal with 5-year survival under 2%. Patients often relapse from front line therapies and current treatments in the second-line setting are very limited.

Delta-like ligand 3 (DLL3) expression is highly restricted to SCLC, neuroendocrine tumors, and glioblastoma with minimally or no expression in normal tissues, making it a very specific and attractive tumor associated target (TAA) for SCLC. Like DLL3, seizure-related homolog protein 6 (SEZ6) is also expressed in SCLC, neuroendocrine tumors, and glioblastoma. Tarlatamab, a T cell engager targeting DLL3, was recently approved for SCLC. ADCs targeting DLL3 have shown robust anti-tumor activities in clinical trials. Similarly, anti-SEZ6 ADCs have also shown promising clinical activities.

We constructed a bispecific ADC (bsADC) that targets both DLL3 and SEZ6 (DLL3xSEZ6) for SCLC and other tumor types where both DLL3 and SEZ6 are co-expressed. We hypothesized that dual targeting of these clinically validated targets would provide more selective and more potent anti-tumor activity, help overcome tumor heterogeneity in tumoral DLL3 and SEZ6 expression levels and allow a broader patient population for treatment. The bispecific antibody (bsAb) moiety of the bsADC was 1+1 format and was conjugated to a novel TOP1 inhibitor payload with potent bystander effect (DAR 8) and a highly hydrophilic, enzyme-cleavable linker. In vitro, the bsAb showed robust internalization capability in multiple double positive SCLC cell lines. In vivo, the bsADC exhibited robust anti-tumor efficacy in SCLC xenograft models. In summary, our DLL3xSEZ6 bsADC showed promising in vitro and in vivo preclinical activities with first-in-class potential for treatment of SCLC.

#4777 GDF-15 neutralization enhances the therapeutic activity of antibody-drug conjugates.

Neha Vashist, Daniel Schatzlein, Amelie Kohler, Sabrina Genssler, Jose Medina-Echeverz, Eugen Leo, Thorsten Ross, Christine Schuberth-Wagner

CatalYm GmbH, Planegg-Martinsried, Germany

Background: Antibody-drug conjugates (ADCs) target cytotoxic payloads like microtubule and topoisomerase I inhibitors to the tumor, thereby inducing selective tumor cell death. ADCs can also trigger anti-tumoral immune responses via induction of immunogenic cell death (ICD). Recent advances in treatment of urothelial cancer demonstrate the high potential of ADCs, e.g. also in combination with PD-(L)1 immune checkpoint inhibitors (ICI). However, significant challenges still remain such as (partial) non-response, high relapse rates and notable toxicity, all highlighting the need to further improve the treatment. GDF-15 acts as a local immunosuppressant, disrupting immune cell infiltration and inhibiting myeloid cell differentiation. GDF-15 blockade in anti-PD1/PD-L1 relapsed/refractory, last-line cancer patients has shown durable anti-tumor responses when combined with PD-1 ICI (NCT04725474). Beyond tumoral overexpression, GDF-15 is also induced by various chemotherapeutic agents. Whether GDF-15 is induced by ADC payloads and could mediate resistance to therapy is currently unknown. Here, we investigated if ADC treatment can induce GDF-15 release from tumor cells and if GDF-15 neutralization may enhance ADC activity.
Methods: Human tumor cell lines were treated with ADCs or unconjugated payloads. Cell viability, induction of ICD, and GDF-15 levels were measured *in vitro* by quantifying intracellular ATP, ecto-Calreticulin (ecto-CRT), and release of HMGB1, extracellular ATP (eATP), and GDF-15 in cell culture supernatants, respectively. Induction of tumoral and serum GDF-15 levels by ADCs was assessed *in vivo* using xenograft tumor models. The effect of GDF-15 neutralization on the anti-tumoral activity of ADCs was evaluated in syngeneic tumor models by monitoring tumor growth and animal survival.
Results: ADCs and unconjugated payloads (MMAE, SN-38, calicheamicin, and PBD dimers) induced concentration-dependent lysis of tumor cells, releasing eATP and HMGB1 and translocating ecto-CRT, indicative of ICD induction. Concomitant with cell death, ADCs and their payloads induced GDF-15 release from tumor cells in vitro. ADC-induced GDF-15 release was confirmed in vivo by increase in tumoral and circulating GDF-15 levels in human tumor xenografted mice. Lastly, combining anti-GDF-15 with ADC treatment improved tumor growth delay markedly in a syngeneic tumor model, accompanied by enhanced immune cell infiltration and activation.
Conclusion: ADC treatment induces GDF-15 which contributes to reduction of ADC activity. Blockade of GDF-15 enhances ADC activity and immune cell number and activation in the tumor. Therefore, GDF-15 blockade may hold significant potential as therapeutic strategy to enhance ADC activity in cancer patients.

#4778 ADCE-T02: A first-in-class topoisomerase-1 inhibitor based antibody drug conjugate against tissue factor demonstrates excellent preclinical efficacy and tolerability.

Thomas Tuxen Poulsen[1], Yue Zhang[2], Jianjian Zhang[2], Huiyan Shi[2], Shu-Hui Liu[3], Xun Meng[2], Mette Gillberg[1], Dominik Mumberg[1]

[1]Adcendo ApS, Frederiksberg, Denmark,[2]Multitude Therapeutics, Shanghai, China,[3]Multitude Therapeutics, Redwood City, CA

Tissue factor (TF, F3, coagulation factor III, thromboplastin, or CD142) is a 47-kDa transmembrane glycoprotein. In normal tissues TF expression is limited to the perivascular compartment where it is the cell surface receptor for the serine protease factor VIIa (FVIIa). Functionally, the TF/FVIIa complex converts the inactive zymogen Factor X (FX) to FXa triggering initiation of the extrinsic coagulation protease cascade. TF is overexpressed in a wide range of solid tumors, including pancreatic, cervical, and colorectal cancer as well as non-small cell lung cancer (NSCLC) and head and neck squamous cell carcinoma (HNSCC), which in combination with efficient receptor internalization makes TF an ideal antibody-drug conjugate (ADC) target. The TF-ADC tisotumab-vedotin (Tivdak®) has been approved for treatment of cervical cancer but is associated with serious side effects including ocular toxicities, peripheral neuropathy, and bleeding. ADCE-T02 is a first-in-class Topoisomerase-1 inhibitor TF-ADC composed of a humanized anti-TF IgG1 monoclonal antibody (Ab-754), conjugated via a novel T1000 linker moiety to the Topoisomerase-1 inhibitor Exatecan payload at a drug-to-antibody ratio (DAR) of 4. ADCE-T02 binds with high affinity to human recombinant TF protein and multiple TF positive cell lines. Binding of ADCE-T02 to TF on the cell surface triggers internalization, resulting in intracellular exatecan release and cancer cell death. In addition, ADCE-T02 induces potent bystander cytotoxicity in TF negative cancer cells when cocultured with TF positive cells *in vitro*. In contrast to tisotumab, Ab-754 does not inhibit FXa activation and demonstrates reduced effect in coagulation assays *in vitro*. ADCE-T02 induces antibody-dependent cellular cytotoxicity but not antibody-dependent cellular phagocytosis or complement-dependent cytotoxicity. *In vivo*, ADCE-T02 shows strong anti-tumor activity in a wide range of solid tumor models including pancreatic cancer, esophageal squamous cell carcinoma, NSCLC and HNSCC and is well tolerated in non-human primate toxicology studies with no evidence of ocular toxicity, peripheral neuropathy, or bleeding.
In summary, ADCE-T02 constitutes a novel first-in-class Topoisomerase-1 inhibitor ADC targeting TF with a potentially enhanced therapeutic and safety profile. A phase I clinical study of ADCE-T02 in patients with advanced solid tumors is currently ongoing (Clinical Trial ID NCT06597721).

#4779 Preclinical studies of BB-1705: An EGFR-targeting eribulin-containing ADC with an affinity optimized antibody to minimize potential toxicities to normal tissues.

B. Xia[1], L. Cao[1], J. Yang[1], C. Feng[1], F. Jiang[1], C. Li[1], L. Gu[1], Y. Yang[1], J. Tian[1], J. Geng[1], L. Gong[2], T. Liu[2], Q. Qin[2], Z. Wei[1], Y. Zhou[1]; [1]Bliss Biopharmaceutical (Hangzhou) Co., Ltd., Hangzhou, China, [2]Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China

Epidermal Growth Factor Receptor (EGFR) is a type I receptor tyrosine kinase critical for the development and normal function of epithelial cells. However, its overexpression or hyperactivity in certain cancers can lead to tumor growth and metastasis. While existing EGFR-targeting therapies can effectively inhibit tumor progression, they often cause significant toxicity due to EGFR expression in normal tissues. To mitigate these side effects, we have developed BB-1705, an antibody-drug conjugate (ADC) that utilizes eribulin as its cytotoxic agent. Eribulin is an FDA-approved treatment for metastatic breast cancer and advanced liposarcoma. BB-1705 employs an affinity-optimized anti-EGFR antibody to selectively deliver eribulin to tumor cells, sparing normal tissues by not effectively blocking the EGFR signaling pathway in those areas. This strategy aims to enhance the therapeutic index of EGFR-targeted treatments while minimizing adverse effects on normal tissues.

In vitro studies demonstrated that BB-1705 exhibits potent cytotoxicity against a range of cancer cell lines with medium to high levels of EGFR expression, while showing reduced efficacy against normal human keratinocytes, likely due to their lower EGFR expression and the antibody's affinity profile. Notably, BB-1705 also induced a significant bystander effect on EGFR-low/null cells when co-cultured or co-inoculated with EGFR-high cells.

In vivo experiments using xenograft models revealed that a single dose of BB-1705 resulted in substantial antitumor activity. When administered repeatedly via a clinically relevant route and schedule, BB-1705 demonstrated a favorable pharmacokinetic profile and safety in cynomolgus monkeys with manageable hematological side effects and minimal skin toxicity observed even at the highest tested dose (12 mg/kg, Q3W x 3).

In summary, our preclinical findings suggest that BB-1705 has the potential to optimize the efficacy and safety profile of EGFR-targeting therapies for cancers characterized by medium to high EGFR expression levels. A clinical trial evaluating BB-1705 is currently underway, aiming to assess its therapeutic potential in treatment of EGFR-expressing human cancers including TNBC, NSCLC and head and neck cancers.

#4780 Engineered ICAM-1 refractory trastuzumab improves its therapeutic activity against HER2-positive cancers in native and antibody-drug conjugate (ADC) format.

J. Bradford Kline, Luigi Grasso, **Nicholas C. Nicolaides**

Navrogen, Glen Mills, PA

The ICAM-1 protein has recently been found to bind the CH3 domain of IgG1-type antibodies and suppress their immune effector activity and internalization against ICAM-1 over-expressing cancer cells. Immune effector suppression involves reduced binding of IgG1 to CD16a Fc-γ-activating receptors on NK cells leading to reduced antibody dependent cellular cytotoxicity (ADCC), as well as binding of the complement initiating C1q protein leading to reduced complement dependent cytotoxicity (CDC) via allosteric alteration of the Fc domain. The effect of ICAM-1 on reduced antibody internalization, a requisite for ADC cytotoxicity, due to the physical interaction of the ADC antibody component with cell surface ICAM-1 has shown significant suppression of ADC activity against antigen-positive target cells. To overcome ICAM-1/IgG1 cytotoxic suppression, we identified a consensus motif within the IgG1 CH3 domain when modified leads to ICAM-1 refractory antibodies, thereby restoring maximal immune effector and ADC cytotoxicity of target cells. Here we present the development of an ICAM-1 refractory trastuzumab and its efficacy against HER2 cancer cells over-expressing ICAM-1 in native and ADC format. These data demonstrate the importance of how tumor-produced proteins may negatively impact the cytotoxic effects of tumor-targeting antibodies and offers a novel platform to improve upon the efficacy of antibody-based therapeutics in the presence of immunosuppressive proteins such as ICAM-1.

#4781 Generation and in vivo characterization of a novel antibody targeting Prostatic Acid Phosphatase (ACP-3).

Claudia Comacchio[1], Stefanie Pfister[2], Domenico Ravazza[3], Samuele Cazzamalli[2], Ettore Gilardoni[3], Emanuele Puca[2], Tobias Weiss[1], Michael Weller[1], Dario Neri[2], Roberto De Luca[2]

[1]Department of Neurology and Clinical Neuroscience Center, University Hospital Zurich, Zurich, Switzerland,[2]Philochem AG, Zurich, Switzerland,[3]Philochem AG, Otelfingen, Switzerland

Prostate cancer is the second deadliest tumor in men globally, despite the advent of novel drugs. Therapeutic modalities targeting Prostate Specific Membrane Antigen (PSMA) have provided benefit to patients but suffer from on-target off-tumor liabilities responsible for the side effects observed in clinical practice. These liabilities are a result of the expression of PSMA in healthy tissues, such as salivary glands, lacrimal glands, intestine, and kidneys. Innovative targeting ligands against better prostate cancer targets are therefore needed.
Here, we present the generation and characterization of a first-in-class fully human antibody targeting ACP-3, a tyrosine phosphatase produced by prostate epithelial cells. Compared to PSMA, ACP-3 is expressed at significantly higher levels in the majority of prostate cancer lesions (both primary and metastatic), while being nearly undetectable in most healthy organs, including salivary glands, intestine and kidneys. A novel anti-ACP3 antibody was isolated from a phage display synthetic library containing 3 billion human single chain variable fragment clones. The antibody was thoroughly characterized, and its binding properties for the cognate target were studied *in vitro* using ELISA, flow cytometry and immunofluorescence on human prostate cancer specimens. Two orthogonal *in vivo* biodistribution studies were conducted in mice bearing HT1080 tumors stably transfected with human ACP-3. The first method relied on mass spectrometry to quantify the uptake antibody in tumors and healthy organs. The second method relied on the use of the immunofluorescence imaging to evaluate the microscopic distribution of the anti-ACP-3 antibody in tumors and healthy organs. The analyses were performed 24 hours post intravenous injection of the antibody. The results confirmed that the novel anti-ACP-3 antibody can selectively bind to its target *in vitro* and can preferentially localize to the tumor when injected intravaneously in tumor-bearing mice.
In conclusion, given the exceptional binding selectivity and tumor-targeting performance, this novel anti-ACP-3 antibody promises to be an attractive scaffold for the generation of new best-in-class tumor targeted pharmaceuticals for the treatment of prostate cancer.

#4782 Inducing abscopal effect in colorectal cancer via antibody-conjugated photothermal nanoparticles in combination with immunocytokine.

Myeung Ryun Seo[1], Jinwon Park[2], Hee Geon Park[3], Jae Choi[3], Sang Moo Lee[1], Sangin Lee[3], Sae Hyung Lee[3], Hobin Yang[4], Sungyoul Hong[5], Yu-Kyoung Oh[2], Young Kee Shin[1]

[1]Department of Molecular Medicine and Biopharmaceutical Sciences, Seoul National University, Seoul, Korea, Republic of,[2]College of Pharmacy and Research Institute of Pharmaceutical Science, Seoul National University, Seoul, Korea, Republic of,[3]ABION Inc., Seoul, Korea, Republic of,[4]College of Pharmacy, Kyungsung University, Busan, Korea, Republic of,[5]Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul, Korea, Republic of

Colorectal cancer is characterized by high rates of recurrence and metastasis, making it particularly challenging to manage due to multidrug resistance after treatment and/or the difficulty of detecting metastatic lesions. Photothermal therapy (PTT) has emerged as a promising strategy to overcome the drug resistance, with ongoing studies investigating its potential to induce the abscopal effect—an immune-mediated response targeting metastatic cancer. However, current research indicates that PTT alone is insufficient to trigger a significant abscopal effect and requires combination with immune modulators, such as immune checkpoint inhibitors or immune stimulating cytokines. Interferon-β (IFN-β), a cytokine capable of directly inducing tumor cell death, also exhibits anticancer effects through immune cell modulation. We hypothesized that combining PTT with IFN-β could induce a synergistic response, enhancing the abscopal effect. In a previous study, we developed antibody-conjugated polydopamine (PDA) nanoparticles targeting Claudin-3 (CLDN3), a protein overexpressed in various cancers, including colorectal cancer, to serve as a material for PTT. In addition, we utilized a CLDN3-targeting antibody-IFN-β mutein fusion protein (ABN202, αCLDN3) to enhance the abscopal effect. The objective of this study was to evaluate whether combining PTT with ABN202 (αCLDN3-IFN-β) could synergistically enhance immune cell activation compared to each treatment alone. Moreover, in human interferon receptor 1/2 knock-in mice, the combination effectively inhibited tumor growth at distant sites. It also demonstrated that the efficacy in preventing recurrence when tumor was re-challenged after the treatment. Therefore, these findings suggest that PTT in combined with ABN202 (αCLDN3-IFN-β) is a promising a novel therapeutic strategy for preventing cancer metastasis and recurrence in colorectal cancer.

#4783 Development and characterization of an antibody-drug conjugate (ADC) utilizing PB-223, a novel monoclonal antibody (mAb) specifically targeting core 2 O-glycans on human carcinomas.

Kwong Y. Tsang, Massimo Fantini, Anjum Zaki, Sharon A. Mavroukakis, Philip M. Arlen

Precision Biologics, Inc., Bethesda, MD

Introduction: The three essential components of an ADC include the mAb, the linker, and the cytotoxic payload. The mAb is designated to target specific tumor-associated antigens that are overexpressed on the surface of cancer cells. The mAb PB-223 was developed through the affinity maturation of mAb NEO-102, a chimeric human IgG1 mAb that specifically targets truncated Core-2 O-glycans, found in colorectal and pancreatic cancers. The binding affinity of PB-223 for its target was improved, compared to NEO-102, by optimizing its VH and VL sequences through Fast Screening for Expression Biophysical Properties and Affinity. PB-223 demonstrated a binding affinity (KD) at least 4-fold lower than NEO-102, indicating stronger tumor binding. IHC analysis also revealed that PB-223 binds to a wider spectrum of tumor tissues compared to NEO-102, but not to normal tissues. PB-223 can be internalized into human cancer cell lines expressing its target. The objective of this study is to develop an ADC utilizing PB-223 for treatment of various human malignancies.
Experimental Design: PB-223 was used for the development of the ADC. Monomethyl auristatin E (MMAE) was used as payload, with mc-vc-PABc serving as the linker. PB-223 was conjugated to the linker-payload through a cysteine-based conjugation method. Drug-to-antibody ratio (DAR) was measured using Size Exclusion Chromatography (SEC)-MS. Flow cytometry was used for binding assessment of the ADC using the ovarian cancer cell line OV90 as the target. Cytotoxicity of the ADC was evaluated through cell viability assays using CellTiter-Glo cell viability Kit using OV90.as target.
Results: Three ADCs were developed: PB-MMAE-2, PB-MMAE-5 and PB-MMAE-6. The DAR for these ADCs were 3.72, 3.92 and 4.15 respectively. We analyzed their target binding capabilities in comparison to PB-223. The results indicated that all three ADCs exhibit similar binding affinity to PB-223. The cytotoxicity of the ADCs was evaluated using the breast cancer cell line SK-BR-3 with Trastuzumab deruxtecan ADC (DS-8201) as positive control. At a concentration of 10nM, DS-8201 inhibited cell proliferation by 82.39%. All three PB-223 ADC effectively killed OV90 cells. At a concentration of 333nM, the percentage of cell killing for PB-MMAE-2, PB-MMAE-5 and PB-MMAE-6 was 82.91%, 84.04% and 83.16%, respectively. In contrast, both human IgG1 and naked PB-223 mAb showed no killing of OV90 cells.
Conclusions: In this study, we developed PB-223 ADCs and evaluated their anti-tumor efficacy using an *in vitro* model. We assessed three PB-223 ADCs: PB-MMAE-2, PB-MMAE-5 and PB-MMAE-6. Our findings highlight the potential of these ADCs as innovative options for treating human carcinomas expressing core 2 O-glycans. Further *In vivo* studies to evaluate the pharmacokinetics and pharmacodynamics are ongoing.

#4784 ADCE-D01: a first in class antibody drug conjugate against urokinase plasminogen activator receptor-associated protein (uPARAP) demonstrates excellent preclinical efficacy and tolerability.

Jonathan H. Wardman[1], Anne Bie[1], Christophe Come[1], Pernille Barkholt[1], Sander van Putten[1], Cecillie Lokke[1], Ida Gregersen[1], Jette Lange[1], Mette Gillberg[1], Mikkel Krogh-Madsen[1], Karen Bannister[1], Thomas Tuxen Poulsen[1], Christoffer Nielsen[1], Lars Engelholm[2], Niels Behrendt[2], Carmel Lynch[1], Pernille Hemmingsen[1], Dominik Mumberg[1]

[1]Adcendo Aps, Copenhagen, Denmark,[2]Copenhagen University, Copenhagen, Denmark

The urokinase plasminogen activator receptor-associated protein (uPARAP, endo180, CD280) is a type-1 membrane protein which acts as an endocytic receptor for collagen. uPARAP binds and internalizes both intact and partially degraded collagens. uPARAP-bound collagen is internalized via clathrin-mediated endocytosis and routed to the lysosome for further degradation, while uPARAP is rapidly recycled to the cell surface. In healthy tissues, uPARAP is expressed in a restricted number of cell types, mostly mesenchymal cell types in tissues characterized by an active matrix turnover such as skin during wound healing and in bone. In contrast, high expression levels of uPARAP are found on malignant cells of various non-epithelial cancers, including soft tissue sarcoma (STS) and osteosarcoma, glioblastoma multiforme and mesothelioma. Recent publications on immunohistochemistry (IHC)-based expression analysis with tumor samples from more than 900 patients[1,2] demonstrate that uPARAP is widely expressed at high levels across most sarcoma subtypes. The efficient internalization of uPARAP combined with the highly prevalent expression in non-epithelial tumors makes it a very attractive antibody-drug conjugate (ADC) target for the treatment of sarcomas. ADCE-D01 is a first-in-class uPARAP-ADC composed of a humanized anti-uPARAP IgG1 monoclonal antibody conjugated via a GFGG tetra-peptide linker moiety to the Topoisomerase I inhibitor P-1021 payload[3] at a drug-to-antibody ratio (DAR) of 8. ADCE-D01 binds with high affinity to human recombinant uPARAP protein and multiple uPARAP positive cell lines. Binding of ADCE-D01 to uPARAP on the cell surface triggers internalization, resulting in intracellular payload release, induction of DNA damage and cell death. In addition, ADCE-D01 induces potent bystander cytotoxicity in uPARAP negative cells. *In vivo*, ADCE-D01 shows strong anti-tumor efficacy in a range of mesenchymal solid tumor models including soft-tissue sarcoma and is well tolerated in non-human primate toxicology studies with no evidence of target-specific toxicity. In summary, ADCE-D01 constitutes a novel First-in-Class ADC targeting uPARAP with an excellent therapeutic as well as safety profile. A phase I/II clinical study of ADCE-D01 in patients with advanced STS is imminent. [1]Barkholt et al., *Cancer Res* (2024), (6_Supplement): 6355; [2]Evans et al., *Mol Cancer Ther* (2023); [3]Lin et al., J Transl Med (2024)

#4785 Preclinical characterization of the anti-tumor activity of the investigational anti-B7-H3 antibody-drug conjugate (ADC), vobramitamab duocarmazine (vobra duo), in pediatric sarcomas (pSC).

G. Bianchi, G. Rolandi, F. Pastorino, D. Segalerba, N. Pedemonte, V. Tomati, E. Ciampi, **M. Ponzoni**, C. Brignole;
G. Gaslini Children's Hospital, Genova, Italy

Background: pSC are a heterogeneous group of rare bone and soft tissue malignancies. Vobra duo (MGC018, MacroGenics, Inc., Rockville, MD, USA) is a duocarmycin-based ADC directed against B7-H3 that showed clinical anti-tumor activity in adults as well as preclinical activity in neuroblastoma.

Materials and methods: A panel of human pSC cell lines expressing B7-H3, as assessed by flow cytometry, was used. Cytotoxicity, in response to vobra duo, was evaluated in 2D and 3D models. Proliferation was assessed by using the Livecyte instrument. Apoptosis was investigated by annexin V assay. Cell signaling focused on PI3K and its downstream effectors involved in cell proliferation, AKT and S6K, was investigated by phospho-specific western blot assays. The expression of ULK-1, ATG13, LC3A-I, LC3A-II, and LAMP-1, all genes involved in autophagy and lysosomal metabolism, was also studied. An *in vivo* experiment was carried out in athymic nude mice inoculated sub cutaneously with a rhabdomyosarcoma cell line.

Results: *In vitro,* treatment with vobra duo induced a time-dependent reduction in cell viability in both monolayer and 3D cell culture models. Automated tracking of single-cell proliferation showed that vobra duo led to a significant increase in the doubling time of treated cells compared to untreated ones. A change in cell morphology, underlined by a significant increase of the cell area of vobra duo-treated cells, was observed. Following treatment with vobra duo, a down-modulation of phospho-AKT and phospho-S6K proteins was observed in both A204 (rhabdomyosarcoma) and U2OS (osteosarcoma) cells, the most treatment-sensitive and resistant lines, respectively, suggesting their potential involvement in the vobra duo-mediated anti-proliferative effect. In response to vobra duo, all cell lines underwent apoptotic cell death, although to a different extent and with different kinetics. A significant increase in executioner cleaved caspase-3 was observed, and a partial, although significant, reversion of apoptotic cell death was noted following pre-treatment with the pan-caspase inhibitor, Q-VD-OP-h. The expression of autophagic machinery's proteins involved in induction, nucleation (ULK-1 and ATG13), elongation and maturation (LC3A-I and LC3A-II) phases, was significantly down-regulated after vobra duo treatment. Moreover, vobra duo significantly affected the expression of the glycosylated form of LAMP-1 protein, a marker of lysosomal membrane integrity and functionality. A single intravenous injection of 3 mg/Kg vobra duo induced a significant tumor growth delay in a mouse rhabdomyosarcoma model.

Conclusions: Vobra duo shows anti-tumor activity toward *in vitro* and *in vivo* models of pSC. Further in depth pre-clinical assessment to support potential clinical application is warranted.

#4786 A humanized monoclonal antibody targeting the Ig-like V1-C1 domains of B7-H3 suppresses the growth of breast cancer.

Fengjuan Zhang, **Qi Zhao**

University of Macau, Taipa, Macao

Breast cancer, characterized by its heterogeneity and complexity, poses a significant threat to life. Current monoclonal antibody drugs for breast cancer primarily target HER2, which is only present in 15%-25% of all breast cancer cases. Recently, immune checkpoint therapy has emerged as a groundbreaking approach to reactivating anti-tumor immune responses. B7-H3 (CD276), a newly identified member of the B7 family, is expressed in various tumor tissues, making it an attractive candidate for therapeutic intervention. Utilizing advanced antibody discovery platforms, we have developed a humanized monoclonal antibody targeting B7-H3. This antibody exhibits specific binding affinity to epitopes in the Ig-like V1-C1 domain and demonstrates significant antibody-dependent cellular cytotoxicity effects on triple-negative breast cancer and trastuzumab-resistant HER2+ breast cells. Furthermore, both HER2+ cell-derived xenograft (CDX) and patient-derived xenograft (PDX) mouse models indicated that this antibody displayed potent in vivo anti-tumor activity. RNA-seq analysis of mouse tumor tissues revealed that the B7-H3 antibody downregulates the MAPK and NF-κB signaling pathways, leading to reduced tumor cell metabolism and migration, as well as diminished inflammation and cytokine release. Our findings indicate that B7-H3 is a viable therapeutic target, supporting the continued development of related therapeutic agents in both preclinical and clinical settings. This work is supported by FDCT/009/2023/RIC.

#4787 BGB-A3055, an afucosylated anti-CCR8 antibody, preferentially depletes intratumoral regulatory T cells and inhibits tumor growth in preclinical models.

Xitao Wang, Yannan Xu, Ming Fang, Ming Jiang, Haolan Li, Mingchao Kang, Xiaomin Song

BeiGene (Beijing) Co., Ltd., Beijing, China

Background: Regulatory T cells (Tregs) is a well-known key immune suppressive cell population enriched in tumor microenvironment (TME) that inhibits the anti-tumor immunity. Accordingly, specific depletion of Tregs in TME is considered as an attractive strategy to enhance the efficacy of immunotherapy such as PD-(L)1 treatment. C-C motif chemokine receptor 8 (CCR8), a G-protein coupled receptor, is predominately upregulated in intratumoral Tregs while CCR8 level in peripheral Tregs is relatively low. Therefore, CCR8 is widely explored as a promising therapeutic target for intratumoral Treg depletion. BGB-A3055 is a novel humanized afucosylated immunoglobulin G (IgG) 1 monoclonal antibody against human CCR8 (hCCR8). Here, we present the in vitro and in vivo data of BGB-A3055 in preclinical models.
Methods: The cellular binding of BGB-A3055 was determined by fluorescence-activated cell sorting (FACS) on 293T cells that overexpress hCCR8 or primary human Treg cells. The blocking activity of BGB-A3055 on CCL1-CCR8 induced signaling was determined by Path Hunter eXpress HuCCR8 CHO-K1ß-Arrestin GPCR Assay kit. Antibody-dependent cellular cytotoxicity (ADCC) effect was evaluated on primary Treg cells. In vivo intratumoral Treg depletion and anti-tumor efficacy was assessed in GL261 and MC38 syngeneic models using hCCR8 knock-in mice.
Results: BGB-A3055 exhibited a potent cellular binding to hCCR8 overexpressing cells and primary human Treg cells. BGB-A3055 can also efficiently block CCL1-CCR8 signaling and induce potent ADCC effect against CCR8 expressing primary Treg cells. Furthermore, in preclinical in vivo models, BGB-A3055 preferentially depleted Tregs in TME, demonstrated potent single agent and synergistic anti-tumor effect in combination with PD-1 antibody.
Conclusions: BGB-3055 is a novel fully humanized afucosylated antibody that has high binding affinity to hCCR8, strong CCL1-CCR8 blocking capacity and potent ADCC effect. BGB-A3055 demonstrated strong anti-tumor activity as single agent or in combination with PD-1 antibody. A Ph1 study evaluating BGB-A3055 alone and in combination with Tislelizumab in participants with solid tumor is ongoing (NCT05935098).

#4788 Discovery of HX035, an OX40-bi-epitope bi-specific antibody (BsAb), for the treatment of inflammatory/autoimmune diseases.

H. Ke[1], T. Yang[1], F. Peng[1], J. Li[1], C. Chen[1], P. Song[2], L. Zhang[1], F. Zhang[1], **H. Q. Li**[1];
[1]Hanx Bio, Hangzhou, China, [2]Crown Bioscience, Taicang, China

With hyperactive $OX40^+CD4^+$ T-cells implicated as the pathogenic cells in various inflammatory/autoimmune disorders, we hypothesized that OX40-antagonistic and/or depleting antibody are both important candidate therapeutics for these diseases. To test this hypothesis, we designed and tested a novel bi-specific antibody (BsAb), HX035 and a 1+1 asymmetric IgG1 mAb, against two different OX40 epitopes. Epitope-1 is featured with non-ligand blocking but strong ADCC (depleting antibody), while epitope-2 is characterized by ligand blocking and antagonistic activity. We found that HX035 binds to recombinant OX40 proteins with the similar binding affinity as the respective parental mAbs, with EC_{50} 0.056 nM (avidity) as opposed to 0.027 nM (epitope 1-mAb) and 0.036 nM (epitope 2-mAb), respectively. Although the binding avidity of HX035 to $OX40^+$ cells is similar to its parental mAbs, HX035 showed more binding to $OX40^{lo}$ and even more to $OX40^{hi}$ cells, as anticipated. HX035 is also an OX40 ligand blocker slightly less potency than the epitope 2-mAb (IC_{50} 5.0 nM vs. 2.6 nM). HX035 also showed similar extent of antagonistic activity to epitope2-mAb in an *in vitro* reporter bioassay. Remarkably, HX035 induced significantly enhanced ADCC over both parental mAbs, making it a stronger depleting antibody than both parental mAbs. We have also further engineered an ADCC-enhanced (5 times stronger) HX035 molecule, so making it an even stronger depleting-antibody. A human PBMC acute graft-versus-host disease (aGVHD) mouse model indeed confirmed the superior anti-aGVHD activities of HX035, over both parental mAbs in the preclinical setting, accompanied with significant depletion of $CD4^+$ T-cells in vivo. All these suggested that HX035 may be a potential “best-in-class” (FIC) candidate for the treatment of autoimmune diseases.

#4789 Development of IMC-002, a next-generation anti-CD47 mAb: an affinity optimized antibody with enhanced safety and therapeutic efficacy in preclinical models.

Jiyea Choi, Ji-Eun Park, A-Ra Jeon, Heewook Shin, Yeonsun Yu, HyeHyeon Jang, Hyesun Lee, Jiyoung Yoon, Sung Ho Kim, Heung Tae Kim

ImmuneOncia Therapeutics, Seoul, Korea, Republic of

CD47, a broadly expressed transmembrane glycoprotein, interacts with SIRPα to inhibit macrophage-mediated phagocytosis. Its overexpression on cancer cells is associated with poor prognosis, making the CD47-SIRPα axis a promising target for cancer immunotherapy. While blocking this pathway enhances macrophage-driven anti-tumor responses, clinical translation is hindered by challenges such as hematological toxicity and rapid drug clearance due to CD47 expression on normal cells, including red blood cells.IMC-002, a fully human IgG4 monoclonal antibody targeting CD47, was screened for an optimized affinity to balance therapeutic efficacy and safety. Comparative studies with Hu5F9 and 13H3 demonstrated IMC-002's enhanced selectivity and unique binding profile. In a tumor-RBC co-culture system, IMC-002 exhibited superior binding to CD47+ tumor cells while showing significantly reduced binding to normal cells, including RBCs, platelets, and CD3+ immune cells, compared to Hu5F9. The tumor selectivity and lack of binding to CD47 on RBCs by IMC-002 appear to result from differences in its binding site. Studies investigating the roles of CD47 clustering, Rh complex, Integrin β1, lipid rafts, and glycosylation revealed that O-glycosylation significantly influences IMC-002's selective binding to CD47. Antibody structure simulations and antigen binding docking analyses confirmed that IMC-002 engages distinct epitopes and adopts a unique binding orientation compared to Hu5F9. Supporting this mechanism, enzymatic removal of O-glycans on RBCs in vitro increased IMC-002 binding, and in-silico epitope mapping identified an O-glycosylation site within the predicted binding region. We evaluated the therapeutic potential of IMC-002 in various solid tumor models through both in vitro and in vivo studies. In vitro functional assays demonstrated that IMC-002 significantly enhanced macrophage-mediated phagocytosis of tumor cells, both as a monotherapy and in combination with standard of care (SoC) treatment. In vivo, IMC-002 exhibited dose-dependent anti-tumor activity in mouse models of triple-negative breast cancer (TNBC), hepatocellular carcinoma (HCC), and biliary tract cancer (BTC). Furthermore, in non-human primates, IMC-002 was well-tolerated, with no hematological toxicity observed at doses up to 100 mg/kg, and exhibited a pharmacokinetic profile consistent with typical therapeutic antibodies. In conclusion, IMC-002 demonstrated exceptional efficacy and safety, attributed to its unique binding site and mechanism of action. These findings provide strong support for its clinical development as a cancer therapeutic. Phase 1a trials confirmed IMC-002's superior safety profile, and ongoing Phase 1b trials are evaluating its efficacy in combination with standard-of-care therapies in patients with HCC and TNBC.

#4790 Immunostimulatory effects of GENA-104A16, a monoclonal anti-CNTN4 antibody, on tumor-infiltrating lymphocytes in a colon cancer liver metastasis model.

Hyunkyung Yu[1], Taebum Lee[2], Gyeongyeon Kim[1], Sanghoon Song[2], Youngeun Ha[1], Soo Ick Cho[2], Kitae Park[1], Hyunuk Kim[1], Mi Young Cha[1]

[1]Genome & Company, Sungnam-si, Gyeonggi-do, Korea, Republic of,[2]Lunit, Seoul, Korea, Republic of

Introduction: Immune checkpoint inhibitors (ICIs) have significantly improved response rates and survival, becoming a cornerstone in the treatments for various cancers. ICIs are effective when their target immune checkpoints, most notably PD-L1, are expressed in tumor cells, and are less effective when they are not. CNTN4 is a novel immune checkpoint expressed independently to previous targets of ICIs. GENA-104A16, a humanized anti-CNTN4 IgG4 (S228P) antibody, binds to CNTN4 on tumor cells and blocks its interaction with amyloid precursor protein (APP) on T cells. This disruption neutralizes immune-suppressive activity and enhances anti-tumor immune responses. This study aimed to further investigate the immunostimulatory effects of GENA-104A16 in a colon cancer liver metastasis model, which is known to recapitulate the resistance of ICIs.

Method: CT26 mouse colon cancer cells overexpressing mCNTN4 (CT26/Cntn4) were inoculated into the spleen. One day later, mice were treated with either isotype control (n=10), GENA-104A16 (n=10), or atezolizumab (n=9) via intraperitoneal injection (3 mg/kg) on a q3d schedule. On Day 11 post-inoculation, animals were sacrificed, and livers were harvested for formalin fixation and paraffin embedding preparation. Tumor-infiltrating lymphocytes (TILs) within hepatic tumor nodules were analyzed using an artificial intelligence (AI)-powered immunohistochemistry (IHC) analyzer applied to CD4 or CD8 stained liver sections containing tumor nodules. $CD4^+$ or $CD8^+$ TILs within the hepatic tumor area were quantified on whole slide images both as a percentage (%) of total cells and by cell density.

Results: GENA-104A16 treatment significantly increased $CD4^+$ T cells as well as $CD8^+$ T cells in both metrics ($CD4^+$ %: 17.5, $CD4^+$ density: 1470; $CD8^+$ %: 12.6, $CD8^+$ density: 1124) compared to isotype control ($CD4^+$ %: 10.0, $CD4^+$ density: 760; $CD8^+$ %: 7.0, $CD8^+$ density: 615). Meanwhile, atezolizumab treatment did not significantly increase TILs (CD4+ %: 14.0, $CD4^+$ density: 1056; $CD8^+$ %: 6.7, $CD8^+$ density: 587) in the current model, which showed similar expression levels of CNTN4 and PD-L1. Notably, an inverse correlation trend was observed between TILs quantification and tumor nodule count across all samples, regardless of the treatment group.

Conclusions: Our findings demonstrate a significant increase in $CD4^+$ and $CD8^+$ TILs following treatment with GENA-104A16 in a clinically relevant model, suggesting a potential for enhanced anti-tumor immunity. These results support the clinical evaluation of GENA-104A16 and underscore the potential of TILs quantified by an AI-powered IHC analyzer as a predictive biomarker for its therapeutic efficacy.

#4791 Immunosuppressive myeloid cells can be modulated with NRP2-targeting antibody ATYR2810 leading to enhanced anti-tumor immunity.

Christoph Burkart[1], Clara Polizzi[1], John Hyunkuk Choi[2], Max Pastenes[1], Alison Barber[1], Michael Lim[2], Leslie Nangle[1]

[1]aTyr Pharma Inc., San Diego, CA,[2]Department of Neurosurgery, Stanford School of Medicine, Stanford, CA

Introduction: A major obstacle for many cancer immunotherapies is intrinsic or acquired resistance to treatment. Myeloid-derived immune cell populations such as myeloid-derived suppressor cells (MDSCs) and tumor associated macrophages (TAMs) are promoting an immunosuppressive tumor environment. This limits the effectiveness of current cancer immunotherapies. When activated, myeloid lineage immune cells express high levels of Neuropilin2 (NRP2), a single pass transmembrane protein. NRP2 forms heterodimeric complexes with other membrane receptors such as VEGF receptors, plexins and integrins. In experimental models of immune disease, NRP2 has demonstrated anti-inflammatory effects. However, the role of NRP2 on immune cells in the context of tumor biology is largely unknown.
Results: NRP2 expression is upregulated on myeloid cells in an experimental model of acute lung injury and can be detected on myeloid cells in syngeneic tumor models. In experimental models of immune diseases like atopic dermatitis and collagen-induced arthritis, NRP2-deficient mice show a stronger immune response and worse pathology. Blocking NRP2 with a monoclonal antibody (ATYR2810) in a syngeneic, orthotopic mouse model of glioblastoma (GBM), resulted in anti-tumoral polarization of myeloid cells and a significant increase in overall survival. Additionally, in syngeneic tumor models with a large immunosuppressive myeloid population, ATYR2810 treatment improved the effectiveness of checkpoint inhibition via anti-PD-1.
Conclusions: Here we demonstrate that NRP2 is a negative regulator of the inflammatory response and is highly expressed on immunosuppressive myeloid cells in the tumor environment. Blocking NRP2 with ATYR2810 was effective as a single therapy and when combined with checkpoint inhibition in syngeneic tumor models with a high prevalence of MDSCs. This suggests that modulating NRP2 offers a new approach to targeting and reversing the immunosuppressive function of myeloid cells in the tumor microenvironment.

#4792 Highly specific GLUT-1 inhibiting antibodies for the treatment of pancreatic cancer.

Siret Tahk[1], Paule Hermet[1], Kyumhyuk Kim[2], Takefumi Morizumi[2], Anu Ustav[1], Kai Virumae[1], Robin Pau[1], Korneelia Anton[1], Alastair J. King[3], Francisca Neethling[3], Andres Mannik[1], Joan Teyra[1], Oliver Ernst[2], **Mart Ustav**[1]

[1]Icosagen Cell Factory OU, Kambja, Estonia,[2]University of Toronto, Toronto, ON, Canada,[3]Eurofins Discovery, St. Charles, MO

Background: Cancer cells often exhibit upregulated glucose metabolism to support their abnormal growth and division. Targeting the cancer-specific glucose transporter GLUT-1 offers a promising strategy to limit glucose uptake and disrupt tumor metabolism. Unlike small molecule inhibitors, monoclonal antibodies provide a unique opportunity to selectively target complex multi-pass membrane transporters. This study presents the development of highly specific antibodies that recognize GLUT-1 glycovariants, effectively blocking GLUT-1 function in cancer cells while preserving glucose transport across the blood-brain barrier.

Methods: GLUT-1-specific monoclonal antibodies were generated using GLUT-1-displaying virus-like particles (VLPs) as immunogens. Chickens were chosen for immunization due to their ability to produce antibodies against highly conserved multi-transmembrane proteins. Antibody discovery was conducted using HybriFree B cell cloning technology and phage display. Functional screens included assays for 2-deoxyglucose uptake inhibition and cancer cell proliferation.

Results: The identified antibodies bind GLUT-1 with nanomolar EC50 values and exhibit no cross-reactivity with other glucose transporters. The lead candidate, ICO-33, inhibits glucose uptake and reprograms GLUT-1-dependent cancer cells to rely on oxidative phosphorylation (OXPHOS). This metabolic shift synergizes with OXPHOS inhibitors, resulting in significant cancer cell growth inhibition at doses subtherapeutic as monotherapies. In vivo, the combination of ICO-33 and an OXPHOS inhibitor was well-tolerated and led to substantial tumor growth suppression in colorectal and pancreatic cancer models. Importantly, ICO-33 preserves glucose uptake in the brain, minimizing systemic toxicities.

Conclusions: This study highlights the discovery of GLUT-1-targeting monoclonal antibodies with high specificity and efficacy. The lead antibody, ICO-33, effectively restricts glucose uptake in cancer cells and enhances sensitivity to OXPHOS inhibitors, demonstrating its potential as a candidate for clinical development in cancer therapy.

#4793 BCG033: a first-in-class PTK7×TROP2 bispecific ADC demonstrating potent and broad efficacy in various preclinical models.

Lillian Ma, Na Zhang, Chengzhang Shang, Yi Yang

Biocytogen, Waltham, MA

TROP2 is frequently overexpressed in a number of epithelial cancers, and therapeutics targeting TROP2 has demonstrated clinical efficacy in multiple indications, including triple-negative breast cancer (TNBC), HR+/HER2- breast cancer, and non-small cell lung cancer (NSCLC). However, its broad expression in normal tissues raises the risk of on-target and off-tumor toxicity, potentially limiting the therapeutic effectiveness. PTK7 has emerged as an alternative target, exhibiting high prevalence and overexpression in a variety of tumors, such as TNBC, NSCLC, ovarian, oesophageal and colorectal cancers.

BCG033 is a bispecific antibody-drug conjugate (ADC) that targets both PTK7 and TROP2, enhancing the therapeutic efficacy while reducing the off-target effects. Developed using Biocytogen's fully human common light chain RenLite® mice, BCG033 is conjugated with BLD1102--a novel linker/payload containing a DNA Topoisomerase I inhibitor paired with a highly hydrophilic, protease-cleavable linker, achieving an optimal drug-to-antibody ratio (DAR) of 8.

Structurally, the backbone of BCG033 consists of a bivalent antibody with two monovalent components that separately target PTK7 and TROP2, which are engineered to minimize internalization and improve tumor selectivity. BCG033 backbone antibody demonstrates strong binding activity under varying PTK7 and TROP2 expression levels in tumors, and exhibits promising developability under stress force degradation.

In proof of concept studies, BCG033 AB-vcMMAE showed synergistic effect and enhanced activity compared to the parental ADCs in both cell line-derived xenograft (CDX) and patient-derived xenograft (PDX) models of TNBC, even with low PTK7 expression. When conjugated with Biocytogen's linker/payload BLD1102, BCG033 demonstrated superior efficacy across varying PTK7 and TROP2 expression levels in PDX models and consistently outperformed benchmark ADCs in multiple tumor models, including NSCLC, TNBC, gastric cancer, and colorectal cancer.

In summary, BCG033 demonstrates promising potential for delivering potent and broad anti-tumor activity across a range of solid tumors, offering broad clinical application opportunities.

#4794 Anti-VEGF x 4-1BB bifunctional AND-Body combines VEGF blockade and allosteric 4-1BB mechanism for sustained activation of adaptive and innate anti-tumor immunity.

Xiaofei Zhou, Michelle Yen, Joseph R. Brady, Oliver Yvon, Marwa Bsat, Kyriakos D. Economides, Daniel Blom

Ampersand Biomedicines, Boston, MA

Background: Bifunctional therapeutics that combine synergistic mechanisms in a single molecule (e.g., anti-PD-1/VEGF) have recently shown great promise in the clinic. The potent co-stimulatory receptor 4-1BB represents a powerful pathway to apply these bifunctional mechanisms, where growing evidence supports a unique ability to drive sustained activation of both adaptive and innate immunity. We leveraged our AND-Body platform to develop a novel anti-VEGF x 4-1BB bifunctional therapeutic, which simultaneously blocks VEGF and triggers allosteric activation of the 4-1BB pathway in the tumor, thereby increasing the therapeutic window. In preclinical models, tumor VEGF drives potent 4-1BB agonism in a cis/paracrine mechanism, enhancing tumor killing and mitigating systemic activity in tissues where VEGF levels are low. This represents a new approach to bifunctional cancer therapeutics.

Methods: The VEGF x 4-1BB bifunctional was evaluated in humanized 4-1BB transgenic mice harboring MC38 or B16F10 tumors and compared to aflibercept and urelumab. Tumor growth, survival rates, immune cell infiltration, and adverse events were assessed, including scRNA sequencing of tumor-infiltrating lymphocytes. The molecule was also tested in combination with anti-PD-1.

Results: The AND-Body robustly activated 4-1BB signaling only at high concentrations of VEGF *in vitro*. Monotherapy induced durable, localized anti-tumor immunity in MC38 and B16F10 models, leading to enhanced survival, and 90% of animals were tumor-free upon re-challenge with MC38 vs. 47% in the urelumab group. Combination therapy with anti-PD-1 showed additive benefit with 100% tumor-free survival in the MC38 model and extended survival in B16F10 tumor-bearing mice. AND-Body treatment mitigated hepatotoxicity and dermatitis compared to urelumab. Mechanistically, VEGF x 4-1BB bifunctional promoted progenitor exhausted T cell expansion and enhanced M1 macrophage differentiation distinct from other immune checkpoint inhibitors. Treatment normalized the TME, promoting aerobic respiration, preventing T cell exhaustion, and supporting sustained anti-tumor immunity.

Conclusions: Anti-VEGF x 4-1BB AND-Body represents a novel bifunctional agent, combining established benefits of VEGF blockade with an allosteric 4-1BB agonist. Potent anti-tumor activity with high therapeutic index was achieved in preclinical models by localizing immune responses via a unique mechanism of action. These studies support advancement to the clinic as a potentially first-in-class candidate for cancer immunotherapy, both as a monotherapy and in combinations.

IMMUNOLOGY: CAR-T Cells
Poster Session

#4798 In vivo generation of universal CAR T cells for targeting heterologous antigens in solid tumors.

Suilan Zheng[1], Bo Huang[1], Sudarshan R. Kasireddy[1], Yashapal Singh[1], Haiyan Chu[2], Madduri Srinivasarao[1], Laurie Beitz[2], Byoung Ryu[2], Andrew Scharenberg[2], Philip Low[1]

[1]Chemistry, Purdue University, West Lafayette, IN,[2]Umoja Biopharma Inc., Seattle, WA

The successes of autologous CAR T cell therapies in treating hematologic malignancies has spurred interest in applying the same approaches for treating solid tumors. However, challenges associated with antigen heterogeneity, CAR T cell exhaustion, cytokine release syndromes, high production costs, and manufacturing complexities have limited their use in solid tumors to date. To address these issues, we have developed a universal CAR expressing a single ScFv against fluorescein (FL) that together with fluorescein-containing bispecific adapters (tumor tags, TTs) can mediate engagement of the universal CAR T cell with multiple other cell types. The TTs that we currently use include UB-TT170 that targets folate receptors (FR) on cancer cells and tumor infiltrating myeloid cells, UB-TT4xx (prostate-specific membrane antigen [PSMA]), UB-TT2xx (carbonic anhydrase IX [CA IX]), and UB-TT5xx (fibroblast activation protein [FAP] on cancer-associated fibroblasts [CAFs]). In this study, we first test various combinations of TTs in a KB tumor model, where FRα and CA IX are expressed on the cancer cells, and FAP is expressed on CAFs. Results from these studies reveal that concurrent administration of two TTs significantly enhances tumor regression relative to injection of only a single TT. Considered together with previous data showing that optimization of TT dosing frequency and concentration can avoid problems associated with CAR T cell exhaustion and excessive cytokine release, we conclude that universal CAR T cells warrant serious consideration as viable alternatives to fixed specificity CAR T cells for treatment of solid tumors. Another challenge limiting CAR T therapies is their high cost of production. To address this limitation, we have employed a surface-engineered lentiviral particle, VivoVec^{TM}, that is currently undergoing development for production of the CAR T cells in vivo to assess whether they can be exploited to generate human CAR T cells by simple incubation in vitro. For this purpose, we have incubated the viral particles for only 1 hour with unmodified human PBMCs and then immediately injected PBMC suspension into MDA-MB-231 tumor-bearing mice. Importantly, injection of these modified PBMCs into NSG tumor-bearing mice led to eradication of their MDA-MB-231 tumors, but only when the mice were subsequently injected with an appropriate TT. Taken together, the data demonstrate that our universal anti-FL CAR T cell can be produced by brief co-incubation of a lentiviral particle with unfractionated PBMCs and that the resulting unprocessed PBMCs can be immediately exploited for eradication of MDA-MB-231 tumors in NSG mice when combined with the appropriate bispecific adapter (TT). The data further suggest that with additional improvements heterogeneous solid tumors might be treated with in vivo manufactured universal CAR T cells if they are co-administered with appropriately selected tumor tags (TT).

#4799 MSLN and MUC1 dual-targeting CAR-T with conditional activation and tumor specificity to improve clinical safety and efficacy.

Jiaguo Li[1], Yan Sun[1], Yong Xia[2], Jinxing Lou[2], Jun Guo[1], Lijie Rong[1], Dan Sun[1], Zhicai Lin[1], Yi Liu[1], Xinhao Wang[3], **Wenfeng Xu**[3], Qijun Qian[1]

[1]Shanghai Cell Therapy Group Co., Ltd, Shanghai, China,[2]Shanghai Mengchao Cancer Hospital, Shanghai, China,[3]Chantibody Therapeutics Inc., Menlo Park, California, Armenia

Background: MSLN and MUC1 are highly expressed in numerous solid tumors, presenting them as potential targets for solid tumor treatment. Nevertheless, these two targets also display varying degrees of expression in important human tissues, at risk of triggering the "on-target off-tumor" effect in CAR-T therapy. Additionally, the tumor immune microenvironment (TME) has a strong immunosuppressive impact on CAR-T cells, making them prone to exhaustion and consequently difficult to exert a long-lasting anti-tumor effect.
Methods: In this study, a dual-targeting CAR-T (designated as BZE2203) for MUC1 and MSLN was generated, validated in pre-clinical and clinical studies. The anti-MSLN VHH was engineered with a pH-dependent characteristic, endowing CAR-T cells with cytotoxicity only at acidic pH in the TME. The anti-MUC1 VHH was selected based on antigen-density dependence to ensure that it has no cytotoxic effect on cells with low MUC1 expression in normal tissues. Meanwhile, the CAR-T was armed with secreting anti-PD1 VHH to alleviate the inhibitory effect of TME on CAR-T cells and tumor infiltrating lymphocytes. Cao-V3 xenograft animal model was utilized to evaluate the safety and efficacy of BZE2203 CAR-T *in vivo*. In an investigator-initiated trial (IIT), patients including two with platinum-resistant ovarian cancer were recruited and infused with a low-dose of BZE2203 CAR-T cells to preliminarily assess the safety and efficacy of this product.
Results: *In vitro* experiments showed that BZE2203 CAR-T cells exerted cytotoxicity on MSLN-positive target cells only in the acidic pH but not neutral pH, and on MUC1-positive tumor cells in a density-dependent manner, but no cytotoxic effect on normal human primary lung cells with low MUC1 expression. After being activated by target cells, CAR-T cells secrete high level of anti-PD1 VHHs. BZE2203 CAR-T showed substantial anti-tumor effect in the Cao-V3 animal model. In the IIT clinical trial, patients did not experience severe safety issues such as cytokine storm and neurotoxicity caused by CAR-T treatment, and two ovarian cancer patients achieved partial remission.
Conclusions: Our results indicate that pH-dependent and antigen-density-dependent CAR-T can mitigate on-target off-tumor toxicities, demonstrating significant potential for improved safety and efficacy in clinical applications of solid tumors.

#4800 Modulation of Th1/Th17 axis through IFNγ-blockade alleviates Anti-CD19 CAR-T associated toxicities in a pre-clinical immune competent model.

Payal Goala[1], Marco Davila[1], Yongliang Zhang[2], Nolan Betty[1], Shannon McSain[1], Sailer Cooper[1], Muhammad Junaid Tariq[1], Showkat Hamid[1]

[1]Roswell Park Comprehensive Cancer Center, Buffalo, NY,[2]Iovance Biotherapeutics, Tampa, FL

CD19-targeted chimeric antigen receptor T cell (CAR-T) therapy for B cell malignancies has led to tremendous improvement in patient survival, yet a subset of patients experience high-grade cytokine release syndrome (CRS) and prolonged cytopenias leading to poor clinical outcomes. Owing to IL2Rα's role in moderating inflammation and supporting Treg survival, we used IL2Rα knockout (KO) mice to emulate the inflammatory conditions that drive CAR-T associated CRS and neutropenia (NP). KO mice injected with a B cell leukemia cell line, followed by CAR-T show elevated levels of IL6 **(p=0.046),** IFNγ **(p=0.006)**, and TNFα **(p=0.048)** in serum compared to WT, which coincided with increase **(p<0.0001)** in CRS causing M1 macrophages (Mϕ) in bone marrow (BM). Onset of CRS was accompanied by a reduction in peripheral blood neutrophils (NEUT) **(p=0.004)** due to a disruption of BM NEUT homeostasis characterized by an increase in apoptotic NEUTs (**p=0.0002)**, and a decrease in proliferative (**p=0.0052)** and mature (**p<0.0001)** NEUTs. Due to the established role of IFNγ in monopoiesis (Mϕ production) over granulopoiesis (NEUTs production), we treated KO mice with IFNγ-/-CD19-CART (IFNγKO-CART) that resulted in restoration of CRS-NP symptoms. Interestingly, IFNγ-blockade restored levels of NEUT regulating cytokine IL-17. In a Th0 to Th1/Th17 in vitro differentiation assay, we noted an increase (**p=0.008**) in Th17 cells and a decrease (**p<0.0001**) in Th1 cells in the presence of IFNγKO-CART compared to CAR-T. KO mice treated with CAR-T and adoptively transferred Th17 cells displayed a significant recovery of CRS-NP symptoms with improved survival **(p=0.002)**. Conversely, KO mice treated with IFNγ KO-CART and adoptively transferred Th1 cells reversed the benefit of IFNγ blockade on CRS-NP by triggering polarization of M1 Mϕ **(p=0.048)** that significantly reduced their survival **(p<0.0001).** Serum analysis of 27 patients diagnosed with hematologic malignancies and treated with standard of care CAR-T products showed that CRS-NP patients had a decline in IL-17A levels post CAR-T therapy **(p=0.049),** compared to non-CRS-NP patients. Furthermore, CRS-NP patients had higher baseline levels of IL-17A, typical of KO mice, putatively serving as a biomarker for CRS (Area under the curve of receiver operator characteristic curve, **ROC-AUC=0.773**) and prolonged NP (**ROC-AUC=0.821**). Our study shows that Th1-Th17 imbalance under inflammatory conditions triggered by CAR-T cells drive CRS-NP in an IFNγ-dependent manner. Finding ways to fine-tune IFNγ release during CAR-T therapy will benefit B-ALL patients by reducing their chances of developing toxicities without compromising their anti-tumor efficacy. To this end, single-cell sequencing analyses are being conducted of BM mononuclear cells of KO mice treated with CAR-T, IFNγKO-CART, CAR-T+Th17, and IFNγKO-CART+Th1 to identify sources of IFNγ production.

#4801 T-bet overexpression promotes CAR T cell potency and responsiveness to low antigen density leukemia.

Jennifer M. Cimons, Kole R. Degolier, Samuel D. Burciaga, M. Eric Kohler, Terry J. Fry

The University of Colorado School of Medicine, Aurora, CO

Data from our lab and others have demonstrated that CAR T cells, contrary to canonical T cell subsets, form populations that appear to be a mixed Th1/Th2 phenotype and produce both IFNγ and IL-4. IL-4 has been shown to have immune-suppressive functions, whereas CAR T cell-derived IFNγ has been shown to promote anti-tumor responses by bolstering CAR8 cytotoxicity and promoting the activation and function of endogenous immune cells. We sought to diminish the Th2-like phenotype and boost CAR T cell potency by overexpressing T-bet, a transcription factor that is essential for Th1 differentiation of CD4 T cells and promotes effector functions in CD8 T cells. We tested overexpression of T-bet in 2nd generation CD19 CAR T cells (1928z and 19BBz) and found that T-bet reduced production of IL-4, demonstrating successful redirection to a Th1/Tc1 fate. Production of IFNγ, TNFα, and IL-2 were enhanced with T-bet overexpression, especially against leukemia cells expressing low levels of CD19 (CD19lo). T-bet overexpression also improved polyfunctional cytokine secretion from these CAR T cells. Furthermore,1928z and 19BBz CAR T cells showed increased effector functions, and degranulated and proliferated at higher rates against CD19lo leukemia when T-bet was overexpressed. To determine the impact of T-bet overexpression on CAR T cell efficacy *in vivo*, we adoptively transferred CAR+ T cells co-transduced to express a 1928z or 19BBz CAR with T-bet-EGFR or an EGFR only control into NSG mice engrafted with WT NALM6 leukemia expressing high levels of CD19. T-bet overexpression negatively impacted leukemia burden and survival of mice treated with 1928z CAR T cells. However, T-bet overexpression had no significant impact on the *in vivo* efficacy of 19BBz CAR T cells against WT leukemia. Since CD28-containing CAR T cells are known to signal with a higher intensity than 4-1BB-containing CAR T cells, we hypothesized that T-bet overexpression may be beneficial only in the context of suboptimal antigen receptor signaling. To this end, we tested the impact of T-bet overexpression on CAR T cell responses against CD19lo leukemia, as downstream CAR signaling is reduced against low-antigen target cells. We found that T-bet overexpression did not significantly affect the leukemia clearance or survival of mice engrafted with CD19lo leukemia treated with 1928z CAR T cells. However, T-bet overexpression enhanced the leukemia clearance and survival of mice engrafted with CD19lo leukemia treated with 19BBz CAR T cells. Together, this data supports a model in which the impact of T-bet overexpression on CAR T cell efficacy is dependent on the CAR signal intensity, with T-bet enhancing responses of CAR T cells receiving a low-intensity signal. Thus, we demonstrate T-bet overexpression as a viable therapeutic strategy to enhance 4-1BB- co-stimulated CAR T cell responses to low-density antigens, overcoming a well-known obstacle limiting CAR T cell efficacy.

#4802 CAR-T cell therapy workflow for discovery and development.

David Cobeta Lopez[1], Benita Quist[1], Sara Compte Sancerni[1], Sophie Vermond[1], Namrata Jayanth[1], Monika Hiller[1], Lauren Schewitz-Bowers[2], Daniel Rocca[2], Robert Nunan[2], Louise Brackenbury[2], Rhiannon Griffiths[3], Mark Aspinall O'Dea[3], Eva Oswald[4], Julia Schueler[4], Renny Feldman[5], Sabrina de Munnik[1], **Gemma Moiset**[1]

[1]Charles River Laboratories, Inc., Leiden, Netherlands,[2]Charles River Laboratories, Inc., Portishead, United Kingdom,[3]Charles River Laboratories, Inc., High Peak, United Kingdom,[4]Charles River Laboratories, Inc., Freiburg, Germany,[5]Charles River Laboratories, Inc., South San Francisco, CA

Engineered T cell therapies such as Chimeric Antigen Receptor (CAR) T cells and T cell receptor (TCR)-engineered T cells have emerged as a promising therapy. To date, seven CAR-T cell products have been approved by the FDA for the treatment of hematological malignancies and many more products are currently being explored for other therapeutic areas such as autoimmune diseases. Due to its innovative nature and the complex biology involved, CAR-T cell therapy product development can face many challenges. While most CAR programs rely on precursor molecules (scFv or VHH) affinity screening, this has proven to not always correlate best to the derived CAR-T activity observed in vivo. However, screening many CAR constructs in primary T cells is associated with lengthy timelines and high costs for their production. To overcome current limitations and streamline the lead selection, Charles River Laboratories offers alternative screening methodologies. Using Jurkat cells as a reporter cell line, plasmids encoding any number of CAR constructs can be transfected and transiently expressed. Afterwards, the CAR-Jurkat cells activation can be measured by the increased expression of CD69 upon co-culture with cells bearing the target of interest. This approach better mimics the cell-to-cell interactions critical for the CAR-T cell therapy function and allow for a more physiologically relevant ranking of the CAR constructs, aiding the selection of the lead candidate with higher success chances. Furthermore, the lack of pharmacologically relevant animal models emphasizes the need to evaluate the safety of CAR-T cell therapy products in vitro. An important step is the identification of potential off-target binders, where the Retrogenix cell microarray technology can be used for the selection and characterization of leads. Additionally, we have developed in vitro co-culture assays using a wide variety of primary or iPSC-derived human cells (representing various tissues including vital organs) to assess potential off-target and on-target/off-tumor effects. A different safety concern to take into consideration is to determine the CAR vector copy number (VCN) and insertional sites and to identify potential insertional mutagenesis effects after introducing the CAR cassette. Lastly, the cells can be tested in vivo to assess their efficacy in a more representative system and gain insights in their bio-distribution, homing sites, prevalence, and differentiation. In conclusion, we have developed a complete workflow to screen CAR candidates in a surrogate model reducing the costs and timelines for CAR-T cell therapies development funneling down the number of CARs to be tested in the in vitro safety and in vivo studies, that would support Investigational New Drug application in accordance with the regulatory agencies' requirements.

#4803 Adaptation of headless CAR T cells to tumor microenvironment induces enhance proliferation and anti-tumor activity against solid cancers.

Archana Thakur[1], Amy Schienschang[1], Sarah Whitaker Amrhein[1], Yelena Bronevetsky[2], Shannon Eaker[2], Lawrence G. Lum[1]

[1]University of Virginia, Charlottesville, VA,[2]Xcell Biosciences Inc, San Francisco, CA

Cell therapy in solid tumors faces significant challenges due to the suppressive tumor microenvironment (TME), characterized by hypoxia and high interstitial pressure. These conditions affect gene and protein expression, contributing to tumor metastasis and drug resistance. Hypoxia dampens the anti-tumor T cell response by altering immune cell activation and function by modulating metabolic activity and the presence of immune suppressive cytokines, growth factors, and immune checkpoint molecules. The success of chimeric antigen receptor (CAR) T cell therapy in treating several hematopoietic malignancies has been challenging to replicate in solid tumors, in part because of T cell exhaustion and eventual T cell dysfunction. T cell reprogramming to adapt to TME is likely to represent a novel and essential aspect of enhancing anti-tumor activity in the TME. We used the Avatar and Avatar AI incubators (Xcell Bio) to precisely control oxygen concentration and pressure to simulate TME conditions to reprogram the headless CAR T cells (hCART). Bispecific antibody armed (ahCART) or unarmed (hCARTs) headless CAR T cells were acclimated under normal environment (NE) or TME conditions before coculturing with target cells. The effect of NE or TME conditions was determined on T cell proliferation, phenotypic changes, and functional activity of hCARTs. Functional readouts were measured by real-time cytotoxicity assay and cytokine profiles under NE or TME-like conditions (1-5% O_2 + 2-5 *PSI* pressure). The phenotypic changes of ATC and hCARTs showed no changes in co-stimulatory receptor expression (41BB, CD40L, GITR, HVEM, ICOS, NFATc1, and TBET) on CD4 or CD8 T cells and co-inhibitory receptors (LAG2, LAIR, PD1, TIGIT, CTLA-4, FOXP3, GATA3 and BTLA) on CD8 T cells, except for reduced expression of certain co-inhibitory receptors on CD4 T cells under TME condition compared to NE condition. Acclimation of hCARTs under TME showed a delayed, but 100% cytotoxicity prolonged up to 7 days at low effector to target ratios of 1:1 or 2:1; non-acclimated hCART showed 100% cytotoxicity initially but declined in long-term or serial killing assays. There was no effect of TME preconditioning on cytokine profile (IL-2, GM-CSF, IFN-γ, TNF-α, and IL-6) compared to NE on hCART. These data suggest that TME reprogramming of hCARTs could be a promising approach to improve its effectiveness, especially in solid tumors. TME acclimation has the clinical potential to enhance the anti-tumor activity of cellular therapeutics.

#4804 RNA binding proteins in CAR-T armoring: Reprogramming T cells for tumor killing.

Augustin B. Biswas[1], Martin Turner[2], Romain Lara[1]

[1]Discovery Sciences, BioPharmaceuticals R&D, Astrazeneca PLC, Cambridge, UK, United Kingdom,[2]The Babraham Institute, Babraham Research Campus, Cambridge, UK, United Kingdom

Background: CAR T cell therapy uses genetically engineered T cells to express a chimeric antigen receptors (CARs) that trigger detection and killing of target tumor cells. It has been previously shown that a functional screen of mouse RNA binding proteins (RBPs) identifies genes that regulate the abundance of activated plasma cells. Deletion of specific RBP genes was shown to increase the potency of effector CD8 T cell functions. We hypothesized that these observations can be translated as an armoring approach to improve human CAR-T cell functions.
Methods: We performed cytotoxicity assays based screening using Her2 CAR T cells with or without 46 RBP knockouts in co-culture with Her2 expressing target cells. The co-culture were set up at a low E:T ratio of 1:32 to evaluate the effect of RBP KO on CAR-T cell exhaustion through engagement in multiple rounds of target cell killing. We validated the modulation of killing with time-course experiments using several Her2 cell lines as HCC-1954, SKOV-3 as well as a control Her2-KO HCC-1954 cell line. We further evaluated the relevance of these findings using patient derived organoid models co-cultured with CAR-T cells bearing specific RBP KOs.
Results: Our screen identified several RBPs which upon deletion enhanced Her2 CAR T killing similarly to Med12 deletion which was previously identified as a CAR-T cell armouring candidate. NGS sequencing and FACS analysis validated the KO efficiencies in CAR Ts used for co-culture. Intracellular flow cytometry prior to co-culturing showed that certain CAR T KOs exhibited higher expression of Interferon gamma, Granzyme B and Perforin 1. RBP KOs in CAR Ts also showed increased killing of patient derived organoids in co-culture.
Conclusion: Our findings suggest that KO of RBPs may be a good strategy to enhance CAR-T cell cytotoxic function and persistence. They suggest the importance of RBPs as a novel class of armoring targets for CAR-T cell enhancement.

#4805 Dual-target CAR-T strategy for MSLN and CLDN18.2: Advancing cancer treatment with superior efficacy and safety.

Shasha Yang, Kai Wu, Zhongjun Shi, Jiantao Wang, Xuefeng Kong, Huajing Wang, **Xiaowen He**

Oricell Therapeutics Co., Ltd., Shanghai, China

Background: CAR-T-cell therapy has achieved great success in the treatment of hematological malignancies. However, due to the characteristics of solid malignant tumors, CAR-T for solid tumors is still in the exploration stage. The restricted expression of MSLN and CLDN18.2 in normal tissues, combined with their abnormal overexpression in various tumor tissues, makes them highly promising targets for CAR-T therapy. Although targeting CLDN18.2 demonstrates significant therapeutic potential, its application in CAR-T therapy carries the risk of gastrointestinal (GI) toxicities, due to on-target/off-tumor adverse effects. MSLN and CLDN18.2 are not co-expressed in normal human tissues but are simultaneously overexpressed in various tumor tissues, including gastric and pancreatic cancers,both with limited treatment options and poor prognoses.

Methods: Considering that CLDN18.2-BBZ exhibited gastrorrhagia toxicity in our preclinical mouse models, we designed OriC613 using “AND” logic gate, aiming to improve safety while maintain anti-tumor efficacy. OriC613 employing an anti-CLDN18.2 scFv with high binding activity and an anti-MSLN V_HH with moderate binding affinity, eliciting completely activation only targeting MSLN&CLDN18.2 double positive tumor cells.

Results: OriC613 exhibits more potent *in vitro/vivo* cytotoxicity efficacy against MSLN&CLDN18.2 double positive cells than MSLN-CD3Z, and IFN-γ and IL-2 released by OriC613 were positively dependent on MSLN and CLDN18.2 expression levels. Notably, OriC613 demonstrated no cytotoxicity towards cells expressing CLDN18.2 alone, both *in vitro* and *in vivo*, indicating mitigate the toxicity toward CLDN18.2 positive normal stomach cells. OriC613 exhibited efficacy, prolonged survival and no GI toxicities in tumor stress model (NUGC-4 cells, $MSLN^{low}CLDN18.2^{med}$), even when targeting relatively large (976 mm^3) tumor sizes, indicating its potential effectiveness in patients with high tumor burden. OriC613 showed efficacy, re-enhanced production of IFN-γ and T cell expansion in a tumor re-stress model for pancreatic cancer (AsPC-1-hCLDN18.2 cells, $MSLN^{low}CLDN18.2^{high}$), with T cells homing to bone marrow and spleen (at Day 115, endpoint). These findings demonstrate that OriC613 enhance both the safety and the effectiveness and persistence of its anti-tumor activity.

Conclusions: The impressive antitumor effects and promising safety of OriC613 highlight the need for continued clinical trials in pancreatic and gastric cancer patients.

#4806 CAR binders affect CAR T cell tonic signaling, durability, and sensitivity to target.

Divanshu Shukla[1], Khatuna Gabunia[1], Shannon E. McGettigan[1], Prachi R. Patel[1], Shannon Christensen[1], Ting Jia Fan[1], Decheng Song[1], Yanping Luo[2], Yanling Wang[2], Huaishan Wang[2], Regina M. Young[1], Carl H. June[1], John Scholler[1], James L. Riley[1]

[1]University of Pennsylvania, Philadelphia, PA,[2]Tmunity Therapeutics/Kite Pharma, Philadelphia, PA

Patients can develop human anti mouse immune responses against CD19- specific CAR T cells (CARTs) due to the use of a murine scFv to redirect T cells. To mitigate immunogenicity, a humanization process of the FMC63- based binder was undertaken that resulted in a CAR (119) that when introduced into T cells showed improved activity in a humanized mouse model. To further characterize how binding domains affect CART function, we screened a yeast display library to identify an array of fully human CD19 scFv binders. We saw significant differences in the ability of CARs employing these CD19 binders to be expressed on the cell surface, induce tonic signaling, redirect T cell function, mediate tumor killing, recognize lower levels of CD19 antigen, and maintain function upon continuous antigen exposure. From this in vitro analysis, CARTs using binders 42 and 52 were selected to be evaluated in a humanized mouse model. Here, we observed that CARTs using both 42 and 52 binders controlled the initial tumor burden and prevented tumor establishment upon rechallenge. We advanced a CAR construct using binder 42 for more advanced preclinical testing due to its greater similarity to FMC63 based binders and ability to robustly respond to tumors expressing lower levels of CD19. We found that this binder uniquely bound CD19 using distinct contact residues than FMC63 with ~40-fold lower affinity. Combined with their potential for less immunogenicity and comparable in vivo activity, CARTs using the 119 or 42 binder should be considered for clinical use.

#4807 Claudin-3-targeting CAR-T using C-terminal domain of Clostridium perfringens enterotoxin for TKI-resistant EGFR-mutated NSCLC.

O. Kwon[1], Y. Joo[1], Y. Lee[1], S. Jeon[1], J. Kim[1], H. Lee[1], M. Yun[2], B. Cho[3];

[1]Yonsei University College of Medicine, Department of Biomedical Science institute, Seoul, Korea, Republic of, [2]Severance Biomedical Science Institute, Yonsei University College of Medicine,, Seoul, Korea, Republic of, [3]Yonsei University College of Medicine, Seoul, Korea, Republic of

Introduction: Epidermal growth factor receptor (EGFR) mutations (MT) are oncogenic drivers in non-small cell lung cancer (NSCLC). However, acquired resistance to EGFR tyrosine kinase inhibitors (TKIs) remains a significant challenge for the treatment of NSCLC. Claudin-3 (CLDN3), a tight junction protein involved in maintaining epithelial integrity, has been reported to be overexpressed via EGFR downstream pathways or mislocalized in NSCLC, contributing to its malignant potential and making it a promising therapeutic target. The Clostridium perfringens enterotoxin (CPE), with its receptor-binding C-terminal domain (cCPE), offers a capability to specifically target CLDN3. This study aims to develop a novel CAR-T therapy utilizing cCPE to target CLDN3, focusing on overcoming resistance in post-TKI NSCLC.
Methods: CLDN3 expression was evaluated through scRNA sequencing (scRNA-seq) of primary lung tissues from 95 patients: EGFR-WT (n=35), treatment-naïve EGFR-MT (n=55), post-EGFR-TKI (n=5), and normal tissues (n=22). Protein expression was validated using ATCC and patient-derived cell lines (PDCs), including NCI-H1703 (EGFR-WT, CLDN3-negative) and EGFR-MT cell lines: HCC827 (E19del), HCC827_AR (E19del/MET amplification), YU1092 (L861Q), YU1157 (L858R), YU1162 (E19del), YU1150 (L858R/T790M), YU1182 (L858R/C797S), and YU1097 (E19del/T790M/C797S). CLDN3-overexpressing cell line (H1703Cld3) was engineered via viral transduction. CAR constructs were designed with various cCPE lengths (amino acids 116-319 [C3L], 184-319 [C3M], 194-319 [C3S]) and linked to 4-1BB and CD3ζ intracellular signaling domains via CD8α hinge and transmembrane regions. CAR expression was analyzed using flow cytometry. Antitumor activity was assessed through RTCA, LDH assays, and ELISA. In vivo efficacy was evaluated in xenograft models using H1703, H1703Cld3, and CLDN3-positive PDCs.
Results: scRNA-seq analysis revealed significantly elevated CLDN3 expression in post-EGFR-TKI patients compared to EGFR-WT and -MT. H1703 was selected as a CLDN3-, and HCC827, HCC827_AR, YU1092, YU1157, YU1162, YU1150, YU1182 and YU1097 as CLDN3+ target cells. Among the CARs, C3M showed optimal transduction efficiency (46.2%) and balanced activity, producing high IFN-γ (70,000pg/mL) against H1703Cld3 without auto-reactivity observed in C3S. C3M-CAR-T cells showed specifically higher secretion of IL-2, IFN-γ, and TNF-α against CLDN3+ target cells compared to CLDN3-. LDH assay and RTCA confirmed robust tumor lysis of CLDN3+ tumor cells than mock T cells. In vivo, C3M-CAR-T treatment led to significant tumor regression in xenografts of H1703Cld3 and PDCs.
Conclusions: This study highlights CLDN3 as a promising therapeutic target using cCPE-based CAR-T in NSCLC for overcoming resistance to EGFR-TKI. The C3M-CAR-T demonstrated robust anti-tumor efficacy across in vitro and in vivo models.

#4808 Physiological manufacturing conditions enhance in vivo efficacy of CD19 CAR T cells in mice.

Anita Zaitouna[1], **Candy Garcia**[2], Lauren Kucharczyk[1], Michael Steffey[1], Abriel Czachorowski[1], Natalie Czeryba[1], Ningchun Liu[1], Sheri Barnes[1], Yelena Bronevetsky[2], Shannon Eaker[2], James Lim[2], Scott Wise[1]

[1]Labcorp Early Development Laboratories Inc., Ann Arbor, MI,[2]Xcellbio, San Francisco, CA

Since the first chimeric antigen receptor T-cell therapy was approved in 2017, significant progress has been made in manufacturing to improve patient access to these life-saving therapies. For CD19 CAR T cells, complete response rates of 40-54% have been reported in patients with relapsed or refractory aggressive B cell lymphomas, a staggering result considering the historically poor outcomes associated with this disease. However, roughly half the treated patients did not exhibit the same profound response, owing to the complexity associated with the production of potent autologous cell therapies. In this study, we examined whether manufacturing conditions can impact the in vivo efficacy of CAR T cells in tumor-bearing mice. Utilizing the AVATAR manufacturing platform from Xcellbio, we expanded healthy donor-derived CD19 CAR T cells under physiological conditions mimicking the human vasculature system. We achieved this by tightly regulating oxygen and hyperbaric pressure levels during the expansion process. We then compared tumor-bearing mice treated with AVATAR expanded cells against conventionally manufactured CD19 CAR T cells derived from a conventional CO_2 incubator (21% O_2 + 0 PSI). CD19 expressing NALM6-Luc-mCh-Puro tumor cells were implanted intravenously in 48 mice, across 6 groups (female NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/Sz*J*, Jackson Laboratories, Bar Harbor, ME), and tumor burden was measured via bioluminescence across 29 days post-implant. We report that CD19 CAR T cells manufactured under conditions mimicking the vasculature environment (15% O_2 + 5 PSI) exhibited superior tumor control compared to cells expanded in a conventional CO_2 incubator (1.8e8 vs 6.4e6 photons/second, mean BLI signal on day 29). Furthermore, we observed significant increases in plasma IFNg levels in animals treated with AVATAR expanded cells (mean: 111 pg/mL vs 32 pg.mL; day 19 post dose) indicating potent anti-tumor activity compared to animals treated with conventionally manufactured CD19 CAR T cells. Blood analysis revealed similar trends, with AVATAR expanded CD19 CAR+ cells present in higher numbers (21 cells/uL of blood vs. 7 cells/uL; mean, day 28 post dose). Also noteworthy was the increased trafficking of AVATAR-manufactured CD19 CAR+ T cells to the spleen and bone marrow compared to conventionally expanded CAR T cells. In summary, physiological culture conditions mimicking the oxygen and pressure levels of the vasculature system significantly enhanced the potency of CD19 CAR T cells in tumor-bearing mice, as measured by decreased tumor burden, higher effector cytokine production, and increased trafficking of CAR T cells to lymphoid organs. Manufacturing therapeutic cells in the AVATAR system has the promise to boost the efficacy of CD19 CAR T therapy, and may enable solid tumor-targeting cell therapy which has long suffered from poor potency.

#4809 Preclinical characterization of an iPSC-derived allogeneic CD19/CD20 CAR-T cell designed to address multiple limitations of current CAR-Ts.

Justin Yoo[1], Jen F. Lai[1], Laura Prochazka[2], Elisa Martinez[2], Chris Saxby[1], Michael H. Cadell[1], Sommer Apelu[1], Libin Abraham[1], Dylan Lee[2], Zahra Sahaf[2], Charlie Liu[1], Laxsha Thaya[2], Elizabeth Csaszar[2], Siddarth Chandrasekaran[1], Emily Titus[2], Chris Bond[1]

[1]Notch Therapeutics, Inc., Seattle, WA,[2]Notch Therapeutics, Inc., Toronto, ON, Canada

In this work, we present the differentiation and *in vitro* characterization of iPSC-derived dual antigen targeting CD19/CD20 CAR-T cells as a milestone towards enabling iPSC-derived allogeneic cell therapies. While autologous CAR-T cell therapy has become an established model in treating B cell malignancies, its application is limited by manufacturing constraints, the necessity of bridging therapy, and heterogeneity of cell product. Allogeneic CAR-T cells derived from iPSCs present an opportunity to overcome these challenges through the scalable production of homogeneous cell products that are genetically engineered and precisely differentiated. Additionally, tumor antigen loss or downregulation remains a driver of disease relapse in patients treated with CD19 CAR T-cells. Accordingly, multi-antigen targeting CAR strategies are being developed to overcome these challenges and elicit more complete and durable responses to CAR-T cell therapy. Multiple expression strategies have been employed to express CARs for multi-targeting applications, including use of bicistronic and tandem formats. We demonstrate strong functional CAR expression from both formats dependent upon the composition of the CAR hinge and transmembrane domains. Based on expression level and antigen binding of dual-targeting CAR formats and designs in Primary T cells, lead CARs were nominated for screening via iPSC-T cell differentiation and *in vitro* functional testing. These lead CARs were site-specifically integrated into the genome of iPSCs using a streamlined workflow for identification of appropriate insertion site and promoter. iPSCs engineered with CD19/CD20 CARs were successfully differentiated while preserving the anticipated CD8 T cell phenotype. The dual-targeting CAR-T cells successfully eliminate disease-relevant tumor cells expressing both or single antigen targets *in vitro*. The development of dual-targeting iPSC-derived allogeneic CD19/CD20 CAR-T cells using a readily scalable manufacturing platform represents a formidable advance in overcoming multiple hurdles faced by established CAR therapies and increasing accessibility of potent and persistent off-the-shelf treatments for cancer and autoimmune disease patients.

#4810 Metabolic reprogramming enhances IL15-GPC3-CAR T cell therapy for hepatocellular carcinoma while mitigating cytokine release syndrome.

Kevin Song[1], Yue Hu[2], Abhijit Sarkar[2], Andras Heczey[3], Xiaotong Song[2]

[1]University of Houston, Houston, TX,[2]Texas A&M University, Houston, TX,[3]Baylor College of Medicine, Houston, TX

Background: This study aims to develop a safer and more effective CAR T cell therapy for hepatocellular carcinoma (HCC). While recent clinical trials have shown promise, IL15-GPC3-CAR T cells demonstrated a 33% partial response rate but were associated with a high incidence of cytokine release syndrome (CRS), occurring in 75% of patients (9/12) compared to 8% (1/12) in GPC3-CAR T cells. Thus, strategies to mitigate CRS while enhancing efficacy are urgently needed. We previously demonstrated that inosine could serve as an alternative energy source for CAR T cells in the glucose-deprived tumor microenvironment (TME) (Nat Metabolism, 2020). More recently, we provided proof of concept that a metabolic reprogramming strategy involving adenosine deaminase 1 (ADA1) enhances T cell survival by converting adenosine to inosine while reducing pro-inflammatory cytokine production (Cell Rep Med, 2024). In this study, we aim to improve IL15-armed GPC3-CAR T cell therapy for HCC by leveraging ADA1 signaling to enhance CAR T cell persistence in the TME while mitigating inflammatory responses, potentially reducing the risk of CRS.
Methods: Human PBMCs were transduced with retroviral vectors encoding IL15-GPC3-CAR and ADA1 (designated IL15-GPC3-MRCAR). Antitumor efficacy and toxicity were evaluated in a Huh7 xenograft mouse model. Cytokine levels and mouse survival were measured to assess immune activation and CRS risk.
Results: IL15-GPC3-MRCAR T cells demonstrated enhanced proliferation and reduced exhaustion following repeated HCC stimulation in vitro. They also exhibited superior cytotoxic activity compared to IL15-GPC3-CAR or GPC3-MRCAR T cells both in vitro and in vivo. Notably, in the mouse study, IL15-GPC3-CAR T cells resulted in mortality rates comparable to the PBS-treated group, likely due to severe CRS. In contrast, IL15-GPC3-MRCAR T cells significantly extended survival, with outcomes similar to those observed in GPC3-CAR T cells and GPC3-MRCAR T cells groups.
Conclusions: IL15-GPC3-MRCAR T cell therapy enhances metabolic fitness and reduces CRS risk, representing a promising advancement in GPC3-targeted therapy for HCC. Further clinical validation is warranted.

#4811 Enhanced potency of BCMA/CD19 dual-targeting allogeneic CAR-T cells for relapsed/ refractory multiple myeloma with 4-1BB/TACI intracellular domains.

Steven Wang, Iris Pang, Claudia Chang, Michelle Burrascano, Nick DeMarco, Danny Mendoza-Reyes, Sonia Reyes, Arturo Barcenas, Pastor Nieto, Jose Diaz, Garret Arauz, Chris Lynn, Tony Nguyen, Connor Reed, Mona Connerney, Quy Le, Julia Coronella, Devon J. Shedlock

Poseida Therapeutics, Inc., San Diego, CA

B-cell maturation antigen (BCMA)-directed chimeric antigen receptor (CAR) T cell therapy has shown high initial response rates in relapsed/refractory multiple myeloma (RRMM), but most patients eventually relapse with progressive disease. Previous studies have identified a less differentiated, CD19-positive progenitor subpopulation of MM cells that contributes to resistance and poor survival. Furthermore, anti-CD19 CAR-T cells given after high-dose melphalan and stem cell transplantation have shown to improve patient survival and can eliminate MM progenitor cells that are resistant to BCMA-directed CAR-T cells.

P-BCMACD19-ALLO1 is a fully allogeneic CAR-T therapy designed to target BCMA and CD19. Additionally, P-BCMACD19-ALLO1 is a T stem cell memory (T_{SCM})-rich product derived from healthy donor T cells using a nonviral, transposon-based system for transgene delivery, with knockout of *TRBC1/2* and *B2M* to prevent graft-versus-host disease and reduce host T-cell mediated rejection, respectively. T_{SCM} -rich CAR-Ts, including our BCMA-targeting allogeneic P-BCMA-ALLO1 CAR-T for RRMM (NCT04960579), have shown favorable efficacy and safety in the clinic.

To develop P-BCMACD19-ALLO1, we constructed the BCMA CAR using two fully human, single-domain, heavy-chain variable domains (VHs) that independently bind BCMA and are joined together by a G_4S linker in tandem. The tandem anti-BCMA VHs binds to all clinical escape mutants, including teclistamab-resistant R27P mutation. The anti-CD19 CAR binder comprises of a fully human VH against CD19. Both CARs are expressed from a single, multi-cistronic transgene that also contains an inducible Caspase-9 safety switch and a dihydrofolate reductase mutein.

Herein, we show enhanced in vivo potency of P-BCMACD19-ALLO1against BCMA/CD19-double positive RPMI-8226 MM xenograft model using an optimized ICD combination that incorporates a novel co-stimulatory domain derived from the tumor necrosis factor receptor family member transmembrane activator and CAML interactor (TACI) in the CD19 CAR and the standard 4-1BB in the BCMA CAR. P-BCMACD19-ALLO1 demonstrates target-specific, in vitro cytotoxicity against tumor cells expressing BCMA, CD19, or both antigens but lacks activity against antigen-negative tumor cells. P-BCMACD19-ALLO1 also demonstrates potent, in vitro killing of tumor cells expressing R27P, P33Del, P33S and S30Del escape mutants. Lastly, P-BCMACD19-ALLO1 effectively eliminates primary bone marrow CD81+CD19+ progenitor cells as well as colony-forming cells derived from MM patient samples, suggesting that P-BCMACD19-ALLO1 may increase the depth and the durability of response.

Taken together, our data underscore the therapeutic potential of P-BCMACD19-ALLO1 and support its application for treating RRMM to be further evaluated in clinical trials.

#4812 Intracranial delivery of MC9999 CAR T-cell therapy: a novel strategy to simultaneously eliminate GBM tumors and immunosuppressive components in the tumor microenvironment.

Yan Luo, Yaqing Qie, Maria J. Ulloa-Navas, Jesus E. Sanchez-Garavito, Vanessa K. Jones, Martha E. Gadd, Mieu M. Brooks, Tanya Hundal, Andrew D. Brim, Isas K. Vazquez-Rosario, Haidong Dong, Alfredo Quinones-Hinojosa, Hong Qin

Mayo Clinic Florida, Jacksonville, FL

Immunosuppression within the tumor microenvironment (TME) poses a significant challenge to the efficacy of Chimeric Antigen Receptor (CAR) T-cell therapy in solid tumors, including glioblastoma (GBM), the most common and deadly primary brain tumor. Tumor and TME-residing immunosuppressive cells can hijack program death 1(PD1) pathway to evade antitumor T-cell immunity by upregulating its ligand, PD-L1. Thus, targeting PD-L1 emerges as a promising therapeutic strategy to simultaneously eliminate tumor cells and immunosuppressive components within the TME. We developed a novel PD-L1-targeting CAR T-cell therapy, MC9999, and investigated its therapeutic effects against GBM. The antigen-specific cytotoxicity of MC9999 CAR T-cells was firmly validated using a LN229 GBM cell line and its PD-L1-deficient variant, as well as patient-derived brain tumor-initiating cells (BTICs). Furthermore, in vivo therapeutic antitumor effects were confirmed in orthotopic GBM models through intracranial administration of MC9999 CAR T-cells. To pave the way to the translation of MC9999 CAR T-cell therapy to patient bedside, we established a unique experimental system using patient-specific CAR T-cells against their own tumor and immunosuppressive cells. High-quality, patient-specific MC9999 CAR T-cells were manufactured using T cells isolated from peripheral blood, while paired autologous BTICs were generated to serve as target cells. Autologous immunosuppressive cells include monocyte-derived M2 macrophages from patient's peripheral blood and tumor-associated macrophages (TAMs) isolated from patient's tumor. Data from multiple patients' samples consistently demonstrated that these patient-specific MC9999 CAR T-cells elicited robust cytotoxicity against autologous GBM tumor and TME-residing immunosuppressive cells, supporting our hypothesis that targeting PD-L1 effectively address both tumor and its TME. Immunohistochemistry (IHC) staining of our GBM patients' tissues showed that PD-L1 expression was restricted to tumor cells and macrophages /microglia within the tumor, with no expression detected in neurons, astrocytes, oligodendrocytes, or blood vessels. These findings suggest that MC9999 CAR T-cells can be safely delivered to the brain without compromising normal cell populations. Ongoing studies are underway to further validate the safety profile of MC9999 CAR T-cell therapy. Collectively, the data obtained from this preclinical study support the effectiveness, feasibility and safety of local administration of MC9999 CAR T-cells as a promising new approach for GBM treatment. These encouraging results have motivated us to prepare an Investigational New Drug application to initiate a phase I clinical trial.

#4813 Multi-armored allogeneic CD5 γδ CAR T cells enhance anti-tumor efficacy in T cell malignancies.

Y. Seo, H. Lee, S. Yu, H. Lee, J. Lee, H. Kim, Y. Lee;
Curocell Inc., Daejeon, Korea, Republic of

T cell malignancies, such as T cell lymphoma and leukemia (T-NHL/T-ALL), present substantial challenges to conventional first-line therapies, highlighting an urgent need for novel therapeutic approaches. CAR-T therapies targeting T-cell-specific antigens have emerged as promising strategies against these cancers. However, the risk of tumor contamination by patient-derived T cells underscores the necessity for developing allogeneic, blast-free CAR-T therapies. Healthy donor-derived γδ T cells are attractive candidates for allogeneic CAR-T cell therapy due to their blast-free nature and the minimal risk of inducing Graft-versus-Host Disease (GvHD). Nevertheless, the difficulties in large-scale expansion and limited in vivo efficacy remain substantial obstacles to clinical application. To overcome these limitations, we aimed to enhance both the expansion and *in vivo* efficacy of anti-CD5 γδ CAR-T (γδCAR5) cells by incorporating “signal-3” supplement. A membrane-bound cytokine approach was employed to ensure targeted “signal 3” supplement specifically to CAR-T cells, promoting controlled expansion and activation. Among various cytokines engineered in membrane-bound forms (IL-2, IL-12, IL-15, IL-18, IL-21, and IL-36γ), only membrane-bound IL-18 (mbIL-18) induced a more than 10-fold increase in γδCAR5 cell expansion *in vitro* and demonstrated robust anti-tumor efficacy in a T-ALL xenograft model. Furthermore, knockdown of CD5 and PD-1, which are associated with fratricide and immunosuppression, respectively, further maximized the anti-tumor activity of γδCAR5-mbIL-18. Consequently, this multi-armoring strategy, which integrates enhanced proliferative capacity, fratricide resistance, and immune checkpoint evasion, represents an innovative approach to maximizing the therapeutic efficacy of allogeneic cell therapies for T cell malignancies. This study provides a promising framework for the advancement of more effective CAR-T therapies against challenging T cell cancers.

#4814 Development of a novel claudin-6-targeting CAR-T cell.

Ana Shahpazir, Siret Tahk, Paule Hermet, Anu Ustav, Robin Pau, Gaily Kivi, Mario Plaas, Andres Mannik, Joan Teyra, Mart Ustav, **Tanel Mahlakoiv**

Icosagen Cell Factory OU, Kambja, Estonia

Chimeric antigen receptor (CAR) T cell therapies have achieved remarkable efficacy in treating B cell malignancies but have shown limited clinical success in patients with solid tumors. A key challenge in advancing these therapies lies in the identification and targeting of highly tumor-specific antigens to ensure both safety and efficacy. In this study, we employed HybriFree B-cell cloning technology and phage display to discover antibodies targeting Claudin-6 (CLDN6), an oncofetal protein selectively expressed in subsets of ovarian, lung, endometrial, and testicular cancers, with minimal or no expression in healthy adult tissues.

To generate a robust immune response, chickens were immunized with virus-like particles (VLPs) displaying a high-density presentation of transmembrane CLDN6. Monoclonal antibodies specific to CLDN6 were subsequently screened for their specificity towards CLDN6 using VLPs bearing other Claudin family members. The most promising specific binders that were able to discriminate between binding of CLDN6 and CLDN9 were humanized via the Qumanize platform and incorporated into a second-generation CAR construct with a 4-1BB costimulatory domain and CD3ζ signaling domain.

CLDN6-specific CAR T cells were then generated and evaluated for their cytotoxicity in tumor models of ovarian, lung, and gastric cancers. Two CLDN6-specific CARs exhibited highly specific recognition and efficient lysis of cancer cells endogenously expressing CLDN6. Within 24 hours of co-culture, CLDN6 CAR T cells eliminated 80% of PA-1 cells and 60% of OV-90 cells. To assess T cell immune activation, cytokine secretion was measured upon co-culture with various target cells. Notably, IFN-γ secretion was observed exclusively when CLDN6 CAR T cells were co-cultured with CLDN6-positive targets, confirming their antigen specificity.

This study underscores the utility of HybriFree technology and the Qumanize platform in generating highly specific and high-affinity CARs for tumor targeting. These findings present a promising avenue for developing next-generation immunotherapies for CLDN6-expressing solid tumors.

#4816 Enhancing the persistence of universal CAR-T against DLL3 positive small cell lung cancer using the epigenetic regulation system mediated by CRISPR.

Zichong Peng[1], Zhuoran Yao[1], Linglu Yi[2], Kai Kang[1], Ren Luo[1], Shanghai Liu[1], Yufeng Zhang[1], Shuangsi Liao[1], Feifei Na[1], Lisa Xiang[1], Ruizhan Tong[2], You Lu[1]

[1]Division of Thoracic Tumor Multimodality Treatment and Department of Radiation Oncology, West China Hospital of Sichuan University, Chengdu, China,[2]Laboratory of Clinical Cell Therapy, West China Hospital of Sichuan University, Chengdu, China

Background: Chimeric antigen receptor T-cell (CAR-T) therapy faces challenges in solid tumors. Long-term exposure of CAR-T to antigens and the immunosuppressive tumor microenvironment leads to T cell exhaustion, which impairs T cell antitumor persistence. Moreover, universal CAR-T (UCAR-T) based on knockout of TRAC, HLA or CD52 genes often has the problem of diminished function. Previous studies have shown that stem-like T cells are essential for offsetting undesirable effects of exhaustion, and both exhaustion and stemness-related genes are epigenetic regulated, which offer a safer alternative to genetic modifications in CAR-T therapy. In this study, we designed a system to turn off or turn on epigenetic regulation genes by CRISPR, named as Epi-off and Epi-on, to enhance the persistence of CAR-T and UCAR-T therapy. And the exhaustion-related epigenetic regulation gene ARID1A was chosen to test the system function.
Methods and Results: The ARID1A KO DLL CAR-T cells were constructed by an Epi-off system mediated by CRISPR using lentiviral and electrical transduction. Continuous repetitive chronic stimulation was performed to induce T cell exhaustion in vitro. The ARID1A KO DLL CAR-T cells exhibited better antitumor effects and less exhaustion, with a less exhausted T cells population (TIM3+ or LAG3+). Meanwhile, they maintained higher proportion of TCF-1+, CD62L+Slamf6+ subsets and faster expansion, which indicated ARID1A KO led to more stem-like characteristics and longer persistence. In vivo, ARID1A KO DLL CAR-T exhibited more effective and long-lasting antitumor effect. The longer survival, smaller tumor volume and more tumor infiltrating lymphocytes (TILs) were observed in tumor mice who received ARID1A KO DLL CAR-T. Moreover, we also assessed the anti-tumor effect between ARID1A KO and SUV39H1 KO CAR-T which was another epigenetic regulation gene against exhaustion. ARID1A KO CAR-T showed a comparable anti-tumor effect at reinfusion dose of 1×10^7 CAR-T cells per mouse but better than SUV39H1 KO CAR-T at comparatively low reinfusion of 5×10^6. Furthermore, ARID1A KO was implemented on UCAR-T. In vivo, ARID1A KO improved DLL UCAR-T therapy (like CAR-T) with a limited toxicity (like conventional UCAR-T) in SCLC mouse model. In addition, we also established an Epi-on system to enhance the persistence and safety of CAR-T, which had a gated structure so that the CRISPR activation component would be released into nucleus by protease to up-regulate expression of certain gene when CAR-T recognized DLL3+ SCLC.
Conclusion: We established an epigenetic regulation system (Epi-off and Epi-on) to enhance CAR-T/UCAR-T function. And the ARID1A KO DLL3 CAR-T and UCAR-T based on this system exhibited better persistence, reliable anti-tumor effects and low toxicity in both in vitro and vivo.

#4817 CAR negative T cells predominate during xenogeneic graft versus host disease after CAR T cell treatment in NSG mice.

Wahed Firoz, Michael K. Sheng, Logan Vick, Craig P. Collins, Ryan Nielsen, Sean J. Judge, William J. Murphy

UC Davis School of Medicine, Sacramento, CA

Chimeric antigen receptor (CAR) T cells are currently FDA-approved and provide a curative solution for patients with select B-cell malignancies in the relapsed or refractory setting. However, there have been multiple reports of long-term toxicities associated with CAR T cell treatment, such as second primary malignancies, highlighting the importance of assessing the long-term effects of CAR T cells on the host in preclinical models. Since the study of CAR T cell efficacy is performed in *in vivo* xenograft mouse models, long-term study is limited by xenogeneic graft-versus-host disease (xenoGVHD) in which human T cells recognize foreign murine major histocompatibility complex, leading to immune-mediated pathology and eventual death of the animal. On the other hand, allogeneic CAR T cells are being increasingly investigated in the aims of lowering cost and increasing access of treatment, though poses the risk of GVHD. In this project, we aimed to investigate the cellular dynamics of CAR T cells in mediating xenoGVHD. To accomplish this aim, we utilized a tri-specific human CAR T cell product which targets CD19, CD20, and CD22 against the Raji human B-cell lymphoma cell line in the immunodeficient NSG mouse model. During the initial tumor clearance, the CAR-positive T cells preferentially expand and constitute upwards of 90% of CD3+ T-cells in the bone marrow of treated mice. However, after approximately 40 to 180 days post tumor clearance, we observed weight loss and outward signs of xenoGVHD. We found that the variability in xenoGVHD onset and occurrence is dependent on the human T cell donor used to make the CAR T cells. Additionally, we found that the CAR-negative T cells predominated in the spleen and bone marrow of Raji-bearing NSG mice treated with human CAR T cells. Using flow cytometry, we observed upwards of 85% CAR-negative T cells of CD3+ T-cells in the spleen and bone marrow of mice experiencing xenoGVHD across multiple experiments. This was an increase from the frequency of CAR-negative T cells at both the pre-infusion baseline timepoint and during tumor clearance, suggesting a preferential expansion of CAR-negative T cells in response to xenoantigens. In addition, we observed upregulation of markers associated with T-cell activation, such as PD-1 and HLA-DR, among the CAR-negative T cells during xenoGVHD, indicating TCR-mediated activation by xenoantigens. Overall, our findings suggest that the CAR-negative T cells predominate during xenoGVHD and play a role in mediating pathology in our tested model. The occurrence of xenoGVHD in these preclinical xenograft models of CAR T cell efficacy are a limitation and prevent the study of potential long-term effects of treatment. Our findings also give insights into the generation of allogeneic CAR T cell products, especially regarding the role of CAR-negative T cells in mediating allogeneic GVHD with off-the-shelf allogeneic CAR T cell products.

#4818 CSPG4-targeting CAR-macrophages inhibit melanoma growth.

Daniel Greiner, Minna Roh-Johnson

University of Utah School of Medicine, Salt Lake City, UT

Macrophages are abundant in the tumor microenvironment of solid tumors like melanoma, and while often pro-tumorigenic, macrophages also possess inherent anti-tumor capabilities. Recent studies have highlighted the potential of CAR-Ms, or chimeric antigen receptor expressing macrophages, to recognize and kill cancer cells. As both phagocytes and antigen-presenting cells, macrophages offer a unique toolset compared to cytotoxic CAR-T cells, potentially improving efficacy in solid tumors. We engineered primary human blood monocyte-derived macrophages to express CAR constructs with an scFV targeting chondroitin sulfate proteoglycan 4 (CSPG4), an antigen commonly overexpressed in solid tumors including melanoma and glioblastoma. Using melanoma as an initial model, we evaluated the impact of CSPG4-targeting CAR-Ms on melanoma growth *in vitro* and *in vivo*. We found that CSPG4-targeting CAR-Ms lead to increased phagocytosis of melanoma cells and increased melanoma cytotoxicity compared to control CAR-Ms. Furthermore, CSPG4-targeting CAR-Ms inhibit melanoma spheroid growth in 3D and inhibit melanoma growth in mice. Interestingly, priming CSPG4-targeting CAR-Ms with CSPG4-expressing cancer cells leads to increased phagocytosis and infiltration of melanoma spheroids compared to control CAR-Ms or unprimed CSPG4-targeting macrophages. Together, these results suggest that CSPG4-targeting CAR-M immunotherapy is a promising solid tumor immunotherapy approach for treating melanoma.

#4819 Pulmonary neutrophil-CAR T cell crosstalk amplifies antigen-specific off tumor toxicity.

Robert L. Eil, Alexandra Bartlett, Matthew McWhorter, Chelsea Jenkins, Jessica Briones

Knight Cancer Institute, Oregon Health & Science University, Portland, OR

Despite the potential for tumor-specific T cells to mediate durable complete responses of advanced disease, no CAR-T cell treatment is currently approved for use against a solid cancer. While nearly all solid cancers express a tumor-associated antigen (TAA) vulnerable to CAR T cell recognition, the same TAAs are also present on healthy tissues. CAR T cells recognize and attack healthy tissues that express their target, even at low levels, provoking lethal and dose-limiting 'on-target off-tumor' toxicities, most commonly the lungs, resulting in vanishingly small therapeutic index. Genetically engineered and immunocompetent mouse models continue to inform our understanding of the immunostimulatory and inhibitory roles of myeloid populations in cancer development and antitumor immunity. However, insights into the role of myeloid populations in constraining or promoting CAR T cell function in the tumor and sites of toxicity in cancer-free organs has been limited due to the prominent use of xenograft models when evaluating CAR T cell function. CAR T cell xenograft mouse models (i.e. transfer of human T cells into immunocompromised mice bearing human tumors) fail to capture off-tumor toxicities (due to the lack of host antigen expression) and are compromised in their ability to recapitulate cytokine crosstalk between the transferred and endogenous populations (due to un-even cross-reactivity of heterologous cytokine-receptor interactions).We develop two immunocompetent murine models (C57BL/6) of CAR T cell transfer targeting TAAs abundant in multiple epithelial cancers (EpCAM and CEA). EpCAM and CEA CAR T cells affected moderate control of pancreatic and metastatic colon cancer but also infiltrated and damaged non-cancerous organs that expressed the TAA (lung & colon). The respiratory system was the site of greatest macro and microscopic pathology in both models. Flow cytometry and immunohistochemical analysis of sites of off-tumor toxicity revealed a marked accumulation of macrophages and neutrophils, along with transferred CAR T cells, in the lungs of host mice, along with immune crosstalk mediated activation and proliferation of endogenous CD4 and CD8 T cell populations. To assess their functional significance in mediating off-tumor toxicity, we depleted endogenous macrophage and neutrophil accumulation in the lungs of CEA and EpCAM CAR T cell recipients using clondronate liposomes or anti-Ly6G depleting antibodies (clone 1A8). In both CEA and EpCAM CAR T cell models, we found that depletion of host neutrophils, but not macrophages, prevented CAR T cell accumulation, activation of endogenous T cells, and pathologic tissue destruction in the lungs of host mice. These findings provide strong justification for targeting host myeloid populations for depletion or reprogramming in the setting of CAR T cell transfer to expand their therapeutic index.

#4820 Anti-CD72 nanobody-based CAR-T affinity maturation leads to improved in vitro efficacy versus low antigen density lymphoma models, but limited benefit in vivo.

Adila Izgutdina[1], Tasfia Rashid[1], Bonell Patino-Escobar[1], Sujata Walunj[1], Huimin Geng[1], Amrik Kang[1], Szu-Ying Chen[1], Haley Johnson[1], William C. Temple[2], Matthew A. Nix[1], Akul Naik[1], Emilio Ramos[1], Hiroyuki Takamatsu[3], Daniel Gil-Alos[4], Joaquin Martinez-Lopez[4], Antonio Valeri[4], Constance Yuan[5], Hao-Wei Wang[5], Sarah Aminov[6], Srabani Sahu[6], Christopher Carpenter[7], Fernando Salangsang[8], Paul Phojanakong[8], Juan Antonio Camara Serrano[8], Isa Tariq[8], Veronica Steri[8], Samir Parekh[9], Amit Verma[6], Nirali N. Shah[10], Arun P. Wiita[1]

[1]Department of Laboratory Medicine, UCSF - University of California San Francisco, San Francisco, CA,[2]Department of Pediatrics, Division of Hematology/Oncology, UCSF Benioff Children's Hospital, UCSF - University of California San Francisco, San Francisco, CA,[3]Department of Hematology, Kanazawa University, Kanazawa, Japan,[4]Department of Hematology, Hospital Universitario 12 de Octubre, Madrid, Spain,[5]Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, Bethesda, MD,[6]Department of Oncology, Blood Cancer Institute, Montefiore Einstein Comprehensive Cancer Center, Bronx, NY,[7]Arc Institute, Palo Alto, CA,[8]Preclinical Therapeutics Core, Helen Diller Family Comprehensive Cancer Center, UCSF - University of California San Francisco, San Francisco, CA,[9]Hematology and Medical Oncology, Icahn School of Medicine at Mount Sinai, New York, NY,[10]Pediatric Oncology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD

Introduction: CD19 CAR T-cells have markedly improved the survival of patients with B-cell malignancies. However, over half of patients relapse after this therapy, many due to CD19 antigen loss. Our laboratory discovered CD72, a B cell specific surface antigen widely expressed on B-cell malignancies. We developed an effective humanized nanobody-based CAR-T (“H24 nanoCAR”) against this target (Nix et al. Cancer Discov 2021, Temple et al. JITC 2023) that we are moving to clinical translation. However, we observed reduced efficacy of H24 nanoCAR versus tumor with low CD72 antigen density. We hypothesized that affinity matured CD72 nanoCAR-Ts could target lower antigen thresholds in lymphoma models.

Methods: Affinity maturation and humanization of CAR-Ts was described in Temple et al. 2023. Humanized affinity matured NbD4.13-H24 CAR was generated by altering the framework region of clone NbD4.13 (K_D = 0.8 nM) to that in clone H24 (K_D = 33 nM). We previously described engineered JeKo1-CD72 low cell line (Izgutdina et al. ASH 2023).

Results: Flow cytometry confirmed ubiquitous CD72 expression on 20 primary B-NHL samples. Two B-ALL patient samples relapsed after CD19 CAR therapy showed downregulation of both surface CD19 and CD22, but surface CD72 was retained, raising the hypothesis that CD72 is a preferential second-line antigen after CD19 relapse. To evaluate efficacy versus CD72 antigen low model, we tested affinity matured NbD4.13 CAR and humanized affinity matured NbD4.13-H24, in comparison to H24 (clinical candidate), against an engineered JeKo1-CD72 low cell line. NbD4.13 and NbD4.13-H24 demonstrated enhanced cytokine secretion compared to H24. Upon repetitive restimulation with JeKo1-CD72 low, NbD4.13 CAR showed more rapid elimination of tumor than H24. However, NbD4.13 and H24 demonstrated equal efficacy in vivo versus mantle cell lymphoma PDX. In an in vivo experiment with JeKo1-low, NbD4.13 showed improved early tumor control than H24, but no survival benefit, while NbD4.13-H24 showed a modest survival benefit (p=0.0442 by Log-rank test). As an alternate strategy to address low antigen density, we showed that the protein kinase C inhibitor bryostatin can lead to increased surface CD72 when applied to the CD19 resistant model Nalm6-R. To understand the mechanism of action, we performed bulk RNAseq of CD72 antigen-low lymphoma cell line SC1 treated with bryostatin. GSEA showed that bryostatin broadly increases B cell lineage specific expression programs.

Conclusion: CD72 is a potential second line antigen post-CD19 relapse in B-cell malignancies. We found that affinity maturation did not demonstrably improve CD72 nanoCAR efficacy. Our results support findings demonstrated for scFv-based CARs, that moderate affinity for surface antigen may lead to optimal CAR performance in vivo, even when nanobodies are used as the recognition element.

#4821 ICAM1-specific chimeric costimulatory receptor enhances CAR T cell activity against HER2-expressing gastric cancer.

Yanping Yang, Arshiya Parveen, Yogindra Vedvyas, Sydney J. Trumper, Irene M. Min, Moonsoo M. Jin

Houston Methodist Research Institute, Houston, TX

Background: Human epidermal growth factor receptor 2 (HER2) is amplified or overexpressed in approximately 15-30% of breast and gastrointestinal cancers, leading to rapid cell proliferation and metastasis through HER2-generated cell signaling. HER2-specific chimeric antigen receptor (CAR) T cells constructed with scFv derived from the FRP5 antibody have been proven safe against advanced sarcoma and brain cancer indications but have shown limited clinical response. In this study, we engineered HER2-specific CAR T cells that co-express a chimeric costimulatory receptor (CCR) targeting intercellular adhesion molecule-1 (ICAM1). The CCR is designed to provide complementary costimulation through OX40 and CD28 endodomains, which can synergize with the 4-1BB incorporated in the CAR construct. The combination of a CAR and a CCR aims to enhance T cell activation, expansion, and persistence, particularly when CAR activation alone is insufficient due to low or heterogeneous antigen expression in target cells. ICAM1 is selected as the target for costimulation because it is highly inducible by inflammatory cytokines such as interferon-gamma, which is released by activated T cells.
Methods: HER2 CAR/ICAM1 CCR T cells (designated as HMJ01) were manufactured using the fully automated CliniMACS Prodigy system at cGMP and FACT accredited facility at Houston Methodist Research Institute. The antitumor activity and toxicity of HMJ01 were evaluated in a GLP/non-GLP hybrid study using HER2-positive human gastric cancer xenograft models.
Results: Four batches of HMJ01 CAR T cells were successfully manufactured from healthy donor leukopak, achieving a transduction efficiency of 44.0 ± 8.7%. Compared to second-generation HER2 CAR T cells, HMJ01 exhibited increased sensitivity to recognize and lyse target cells with low levels of HER2 expression. Additionally, HMJ01 CAR T cells enabled the in vivo eradication of HER2-low tumor xenografts, which were otherwise resistant to treatment with conventional second-generation HER2 CAR T cells. We also observed increased cytokine secretion and clonal expansion of HMJ01 cells in vivo compared to conventional HER2 CAR T cells. Furthermore, HMJ01 CAR T cells were found to be safe in the toxicology study, which evaluated mortality, clinical signs, hematology and clinical chemistry, and histopathology at both intermediate and final time points, warranting further clinical evaluation.
Conclusions: Our studies demonstrate the significant potential of ICAM1-specific CCR to improve the therapeutic outcomes of CAR T cells by enhancing cytotoxic efficacy and persistence. We plan to open a first-in-human phase I study to assess the safety and preliminary efficacy of HMJ01 cells in patients with HER2-positive or HER2-low unresectable or metastatic gastric cancer.

#4822 Efficacy of 4-1BB-costimulated NKG2D CAR-T cells for synovial sarcoma in comparison with CAR-T cells targeting other antigens.

Naoki Oike[1], Tomohiro Miyazaki[1], Yudai Murayama[2], Akira Ogose[3], Takashi Ariizumi[1], Hiroyuki Kawashima[1], Chihaya Imai[4]

[1]Orthopedic surgery, Niigata Univ. School of Medicine, Niigata, Japan,[2]Orthopedic surgery, Tochigi cancer center hospital, Utsunomiya, Japan,[3]Orthopedic surgery, Uonuma-kikan hospital, Minamiuonuma, Japan,[4]Pediatrics, Toyama Univ. School of Medicine, Toyama, Japan

Although the development of multimodal therapy consisting of surgery, radiotherapy, and chemotherapy has significantly improved the survival rate for patients with localized synovial sarcoma (SS), the prognosis of metastatic or recurrent SS remains poor. This has not changed over the past three decades, and a novel approach including immunotherapy is urgently needed. Clinical studies of CAR-T cell therapies against sarcomas have been performed. However, the clinical outcomes for patients with sarcomas are still limited. We have previously reported cytotoxicity of HER2-targeted CAR-T cells against SS in vitro. Here, we demonstrated that NK2GD-based CAR-T cells showed significantly higher cytotoxicity than that of HER2-targeted CAR-T cells for SS cell lines.Surface expressions of HER2 and NKG2D ligands (NKG2DLs) on three SS cell lines (SYO-I, HS-SY-II, and Yamato-SS) were evaluated by flow cytometry. Gene expression level of HER2 and NK2GDLs of SS patients were also evaluated by RNA-sequencing using public data base. Then, we generated 4-1BB-type second generation CAR-T cells targeting HER2 or NKG2DLs and examined their cytotoxicity and specific responses against SS cells. Furthermore, we compared the differences between HER2-targeted CAR-T cells and NKG2D-based CAR-T cells by co-culturing with SS cells using short-term assay (WST-8 assay), long-term assay (Real-time Cell Analysis), intracellular cytokine assay, and CD107a assay. Finaly, animal experiments were also performed to evaluate antitumor effect of CAR-T cells against SS. CAR-T cells were injected one week after the implantation of human SS cell lines.Flow cytometry revealed that surface expression HER2 and NKG2DLs were observed in all three SS cell lines. In vitro, NKG2D-based CAR-T cells showed antitumor effects against SS cells with degranulation and cytokine production. Furthermore, NKG2D-based CAR-T cells showed much stronger cytotoxicity than HER2-targeted CAR-T cells in both short- and long-term assay. Higer expression level of cytokines was found in NK2GD-based CAR-T cells than that of HER2-targeted CAR-T cells.In this study, NKG2D-based CAR-T cells demonstrated much stronger antitumor effects than HER2-targeted CAR-T cells. Although CAR-T cell therapy has been shown to be promising in blood malignancies, the efficacy in solid tumors has been disappointing due to problems such as low penetrance to tumors, the immunosuppressive microenvironment, and immune evasion of tumor cells. While further validation of clinical application of CAR-T cells against SS is necessary, we demonstrated that CAR-T cell therapy would be promising immunotherapy for SS patients.

#4823 CDH3-targeted ADC and CAR-T immunotherapies for metastatic double-negative prostate cancer and triple-negative breast cancer.

Guoqiang Liu, Shiyu Wang, Loan Duong, Xin Lu

University of Notre Dame, Notre Dame, IN

Double-negative prostate cancer (DNPC) and triple-negative breast cancer (TNBC) are two challenging malignancies characterized by frequent metastasis and resistance to current therapies. Among them, DNPC may share similar traits with basal-like prostate cancer. These aggressive cancer subtypes demand new treatment strategies. This study investigates CDH3 (P-cadherin), a cell adhesion molecule, as a new target for antibody-drug conjugate (ADC) and chimeric antigen receptor (CAR) T-cell immunotherapeutics. We conducted comprehensive in silico analysis and confirmed the enriched expression of CDH3 in DNPC and metastatic TNBC. We hypothesize that CDH3-positive ($CDH3^{+}$) prostate and breast cancer cells will be effectively targeted and eradicated using anti-CDH3 immunotherapeutics in vitro and in vivo. Both anti-CDH3 ADC and CAR-T cell therapies were developed and they both demonstrated significant target-specific cytotoxicity against $CDH3^{high}$ DU145 and MDA-MB-468 cell lines, while sparing $CDH3^{low}$ and $CDH3^{-/-}$ cell lines, highlighting their selectivity in vitro. In vivo efficacy was further validated using NOD-SCID mice engrafted with prostate and breast cancer cell lines in an experimental metastasis setting, where treatment with ADC or CAR-T therapy significantly reduced metastasis burden. Notably, CAR-T therapy, in combination with anti-PD-1 therapy, exhibited enhanced therapeutic potential.This study establishes the robust target-specific cytotoxicity of anti-CDH3 ADC and CAR-T therapies against the most virulent subtypes of metastatic prostate and breast cancers in vitro and in vivo, paving the way for potential clinical development of these therapeutic agents in patients with metastatic DNPC and TNBC.

#4826 Tyrosine kinase inhibitor combination therapies with AZD5851, dominant-negative TGFβRII armored anti-GPC3 CAR-T, for the treatment of hepatocellular carcinoma.

Nina J. Chu[1], Ashley A. Merlino[1], John Stone[2], Niresh Hariparsad[3], Scott Rata[3], Gordon Moody[1], Letizia Giardino[4]

[1]Early Oncology, Cell Therapy Unit, AstraZeneca US, Gaithersburg, MD,[2]Clinical Cell Therapy, AstraZeneca, Cambridge, United Kingdom,[3]Oncology Targeted Discovery, Drug Metabolism and Pharmacokinetics, AstraZeneca US, Waltham, MA,[4]Early Oncology, Cell Therapy Unit, AstraZeneca, Cambridge, United Kingdom

Background: AZD5851 is a glypican-3 (GPC3)-targeting CAR-T armored with a TGFβRII dominant-negative receptor that protects the CAR-T from the immunosuppressive cytokine TGFβ. AZD5851 is currently being tested in a Phase I/II clinical trial (NCT06084884) for patients with advanced/recurrent hepatocellular carcinoma (HCC). The multi-target tyrosine kinase inhibitors (TKIs) sorafenib and lenvatinib are licensed for first line treatment of advanced/recurrent HCC. Sorafenib inhibits tumor cell proliferation and angiogenesis by blocking the Ras/Raf pathway, vascular endothelial growth factor receptors (VEGFR), and platelet-derived growth factor receptor (PDGFR). Lenvatinib mediates its antiangiogenic functions by targeting VEGFR, PDGFR, and fibroblast growth factor receptors (FGFR). Additionally, lenvatinib can reduce immunosuppression in the tumor microenvironment by decreasing infiltration of tumor-associated macrophages, myeloid-derived suppressor cells, and T regulatory cells. Due to these anti-tumor and immunomodulatory effects, we evaluated whether sorafenib or lenvatinib can enhance in vivo efficacy in combination with AZD5851, and if either TKI impacts CAR-T function.
Results: Therapeutic concentrations of sorafenib and lenvatinib did not affect AZD5851 viability, antigen dependent proliferation, activation, effector cytokine secretion, and target cell killing after co-culture with GPC3+ target tumor cells in vitro. In the PLC/PRF/5 HCC xenograft model, efficacious doses of both TKIs did not impair CAR-T anti-tumor function. High efficacious dose of sorafenib in combination with AZD5851 only subtly enhanced anti-tumor efficacy, whereas coadministration of high dose lenvatinib significantly increased tumor control compared to AZD5851 alone. To further explore the combination of lenvatinib with AZD5851, pharmacokinetic modeling for lenvatinib was performed to determine doses that would achieve exposures in mice equivalent to those observed in human patients. Combination of AZD5851 with the clinically relevant dose of lenvatinib significantly enhanced the anti-tumor efficacy and survival compared to CAR-T or lenvatinib alone.
Conclusions: The TKI lenvatinib enhances TGFβRII dominant-negative receptor armored GPC3 CAR-T (AZD5851) anti-tumor efficacy in vivo without affecting acute CAR-T viability, proliferation, activation, effector cytokine production, and cytotoxicity. Therefore, combining AZD5851 with lenvatinib may be a promising combination therapeutic regimen for patients with HCC.

#4827 Cell state targeting CAR T cell therapies for glioblastoma.

Christopher Mount[1], Emily Boxer[2], Sophia Kovatsis[1], Demi Gerovasilis[1], Sydney Dumont[1], Jun Zhong[1], Jack Lu[1], Itay Tirosh[2], Mario Suva[1]

[1]Massachusetts General Hospital, Boston, MA,[2]The Weizmann Institute, Rehovat, Israel

Despite decades of concerted research and clinical efforts, patient outcomes in glioblastoma (GBM) remain dismal. The emergence of chimeric antigen receptor (CAR) T cell therapies offer great promise as immunotherapies in this disease. To date, efforts to utilize CAR T cell therapies in GBM have resulted in isolated reports of impressive efficacy in individual patients, but these have yet to generalize to larger trial populations. It is thought that inter and intra patient tumor heterogeneity contributes to the variability of these responses. Single cell RNA sequencing has demonstrated that this heterogeneity can be organized along axes representing cellular states. The ability of existing CAR T cell therapies to target these cell states is unclear, and the dynamics of this heterogeneity under the selective pressure of therapy is uncertain. We present the development of a panel of tool CAR T cells targeting cell state associated surface markers with the objective of selective targeting of these cellular states in GBM. We show that these CAR T cells have target specific activity against GBM model systems representing these cellular states. Using single cell RNA sequencing, we model the dynamics of these treatment effects, with the aim of understanding the mechanisms of GBM dynamics in response to the selective pressure of CAR T cell therapies. We demonstrate global shifts in the transcriptional landscape at the single cell level and identify state dependent and state independent effects. We anticipate that these cell state targeted CAR T cells have great potential to elucidate mechanisms of GBM adaptation to the selective pressure of CAR T cell activity and will inform foundational biology necessary for rational design of multi target constructs for therapeutic application.

IMMUNOLOGY: Epigenetic Regulation of Tumor Immunity
Poster Session

#4831 DNA methylation of immune synapse genes drives tumor immune evasion.

Imene Hamaidi[1], Anders Berglund[2], Matthew Mills[2], Ryan M. Putney[2], James J. Mule[2], Sungjune Kim[1]

[1]Mayo Clinic Florida, Jacksonville, FL,[2]Moffitt Cancer Center, Tampa, FL

Background: While cancer immunotherapies have revolutionized treatment, their efficacy remains limited for most patients, highlighting tumors' capacity to evade immune detection. Epigenetic modifications, particularly DNA methylation, play a pivotal role in silencing immune-related pathways, thereby facilitating immune escape. By modulating gene expression, methylation disrupts immune activation, contributing to reduced tumor immunogenicity. This study examines the methylation landscape of co-stimulatory and immune checkpoint genes across various cancers to elucidate how these changes influence immune responses.
Methods: Using The Cancer Genome Atlas (TCGA), we analyzed methylation and gene expression profiles across diverse cancer types. Data from 8,186 solid tumors and 745 adjacent normal tissues were processed via t-distributed stochastic neighbor embedding (t-SNE) with 247 probes targeting 20 immune-related genes. Principal component analysis (PCA) was applied to capture methylation variability across 8,931 tumor and normal samples. To explore the effects of methylation on immune gene expression, we analyzed data from 26 epithelial cancer cell lines treated with 5-azacitidine, a demethylating agent, using the GSE57342 dataset. Post-treatment changes in gene expression were assessed, and survival analyses from prior studies were used to explore clinical correlations.
Results: We identified distinct methylation patterns in immune synapse genes, differentiating tumors from adjacent normal tissues. Co-stimulatory genes such as CD40 were hypermethylated in tumors, correlating with decreased expression, whereas immune checkpoint genes like PDL1 and HHLA2 were hypomethylated, correlating with increased expression. This inverse relationship between methylation and gene expression underscores the transcriptional regulatory role of DNA methylation. Treatment with 5-azacitidine reversed hypermethylation in co-stimulatory genes, including CD40, potentially enhancing immune recognition. Methylation levels of these genes were also associated with T cell infiltration into the tumor microenvironment and predicted better outcomes in melanoma patients, underscoring their clinical relevance.
Conclusion: This study demonstrates that DNA methylation of immune synapse genes contributes to tumor immune evasion by modulating immune gene expression. Targeting these epigenetic alterations represents a promising strategy to restore tumor immunogenicity and improve immunotherapy outcomes, particularly when combined with immune checkpoint inhibitors. Reversing methylation-driven immune suppression could strengthen the immune response and enhance clinical outcomes for cancer patients.

#4832 Differential roles of histone lactylation in regulating CD8 T cell effector functions and exhaustion.

Deblina Raychaudhuri, Pratishtha Singh, Bidisha Chakraborty, Aminah Tannir, Swadhin Meher, Sangeeta Goswami

The University of Texas MD Anderson Cancer Center, Houston, TX

CD8 T cells undergo metabolic reprogramming upon activation to meet the increased energy demands associated with their cytotoxic functions. A hallmark of CD8 T cell activation is the upregulation of glycolysis, accompanied by increased lactate production. Lactylation, a post-translational modification of histones, derived from lactate, has garnered attention, though its specific role in CD8 T cell biology remains unclear. We have recently demonstrated the enrichment of histone lactylation marks, specifically H3K18-lactylation (H3K18la) and H3K9-lactylation (H3K9la), in human and murine CD8 T cells. Integrating ChIP-sequencing and RNA-sequencing data, we delineated the role of histone lactylation as transcriptional regulators of key genes essential for CD8 T cell-mediated cytotoxicity and effector functions. Importantly, our results, obtained from both *in vitro* experiments and *in vivo* disease models demonstrated that H3K18la and H3K9la exert differential effects across various CD8 T cell populations intricately tied to the metabolic profiles of distinct CD8 T cell subsets. Effector CD8 T cells, characterized by elevated glycolytic activity, exhibited correspondingly high levels of histone lactylation. In contrast, terminally exhausted CD8 T cells, where both glycolysis and mitochondrial metabolic pathways are impaired, displayed a marked reduction in histone lactylation. These cells were instead predominantly regulated by histone acetylation. Notably, co-immunoprecipitation assays revealed distinct epigenetic readers that specifically recognized histone lactylation marks, differentiating them from those binding to histone methylation or acetylation modifications. Modulating histone lactylation marks through metabolic and epigenetic interventions significantly altered CD8 T cell effector functions, including anti-tumor responses in preclinical models. Cumulatively, our findings demonstrated that histone lactylation, driven by intrinsic metabolic processes, regulates key aspects of CD8 T cell metabolism and function. Further, we identified distinct epigenetic mechanisms by which selective targeting of H3K18la and/or H3K9la can be leveraged to modulate CD8 T cell phenotype and functionality.

#4833 Targeting DOT1L to enhance the immune response in ovarian cancer.

Aisha Jamil, Shraddha Gupta, Sangeeta Kumari, Deyana Tabatabaei, Sergey Karakashev

Department of Cancer and Cellular Biology, Temple Univ. Lewis Katz School of Medicine, Philadelphia, PA

Epithelial ovarian cancer stands as the second most prevalent cause of death among all gynecological cancers. Current treatment protocols for patients incorporate platinum-based therapy regimens along with PARP inhibitors tailored for HR-deficient high-grade serous ovarian cancers (HGSOC). Despite initial efficacy, patients inevitably develop disease progression as tumor cells acquire resistance. HGSOC is characterized by low immune cell infiltration and reduced anti-tumor immune response. MHC class I mediated antigen presentation by cancer cells to the immune cells is one of the steps required to activate the anti-tumor immune response. Therefore, HGSOC often downregulates MHC class I expression to evade immune surveillance. To identify epigenetic factors negatively regulating MHC class I expression, we conducted a comparative analysis of HGSOC patient cell samples and HGSOC cell lines from the TCGA and DepMap. Our findings indicate that the epigenetic regulator DOT1L is overexpressed in ovarian cancer and negatively correlates with MHC class I expression. DOT1L is an enzyme responsible for the methylation of lysine79 on histone3 that regulates gene expression. However, the role of DOT1L in regulating MHC class I or antitumor immune response has not been previously studied. We hypothesized that DOT1L might regulate the expression of MHC class I in ovarian cancer and block the immune response. To investigate the role of DOT1L in the regulation of MHC class I, we generated a DOT1L knockout in two DOT1L-high EOC cell lines, OVCAR3 and ID8. Genetic inhibition of DOT1L led to enhanced expression of MHC class I on the surface of both cell lines. This suggests that DOT1L inhibition might be a valuable strategy to upregulate MHC class I expression, which could lead to an increased anti-tumor immune response. Importantly, a small-molecule DOT1L inhibitor, Pinometostat, is currently in late-stage clinical trials for other types of cancer. We then tested the effect of Pinometostat on MHC class I expression. In accordance with our hypothesis, DOT1L pharmacological inhibition also promoted upregulation of MHC class I expression in both cell lines. This discovery could potentially pave the way for the development of DOT1L inhibitors for HGSOC to increase anti-tumor immune response in ovarian cancer. The present research indicates that DOT1L inhibitors have the potential to be utilized in the precision medicine strategy for addressing DOT1L-high ovarian cancer patients.

#4834 Genomic and metabolic effects of KDM6A loss regulates differential responses to immune checkpoint therapy and chemotherapy in bladder cancer.

Pratishtha Singh, Deblina Raychaudhuri, Bidisha Chakraborty, Aminah J. Tannir, Swadhin Meher, Anurag Majumdar, Jessalyn Hawkins, Yun Xiong, Philip L. Lorenzi, Padmanee Sharma, Patrick G. Pilie, Sangeeta Goswami

The UT MD Anderson Cancer Center, Houston, TX

Bladder cancer often harbors driver mutations in genes regulating epigenetic processes, yet their influence on therapeutic perturbations is understudied. One key example is *KDM6A*, a gene frequently mutated in bladder cancer, which encodes a histone demethylase that confers transcription permissive chromatin state. Retrospective analysis of patient cohorts demonstrated that *KDM6A* mutations correlate with improved response to immune checkpoint therapy (ICT) and decreased sensitivity to cisplatin-based chemotherapy. Mechanistic studies reveal that CRISPR-Cas9 deletion of Kdm6a reduced expression of critical genes including *Msh2, Msh6*, and *Exo1* involved in DNA Mismatch repair (MMR) and double-stranded base repair (DSBR) in tumor cells with improved response to anti-PD-1 therapy. Further, KDM6A deficiency downregulated genes involved in cisplatin resistance and attenuated sensitivity to cisplatin-based chemotherapy *in vitro* and *in vivo*. Additionally, loss of KDM6A was associated with metabolic shift from glycolysis to oxidative phosphorylation. CyTOF analyses show decreased abundance regulatory T cells (Tregs) in Kdm6a-knockout tumors. Importantly, reduced lactate in the Kdm6a-knockout tumor microenvironment impaired histone lactylation (H3K9la/H3K18la) in Tregs with concurrent attenuation of expression of key immunosuppressive genes including *Foxp3, Tgfß1 and Pdcd1*. Collectively, this study provided insights into the role of KDM6A-mediated epigenetic regulation of DNA repair and metabolic reprogramming which could potentially govern response to chemotherapy and ICT aiding patient stratification and development of personalized treatment algorithms.

#4835 The role of ARID1A and ARID1B in melanoma.

Pei-Yu Chen, George P. Souroullas

Washington University in St. Louis, St. Louis, MO

ARID1A and ARID1B are highly homologous and mutually exclusive subunits of the chromatin remodeler SWI/SNF complex. In melanoma, *ARID1A* and *ARID1B* are mutated in about 13% and 10% of melanoma patients, respectively. Interestingly, mutations in *ARID1A* or *ARID1B* are also associated with a better response to immunotherapy in many cancers, highlighting their potential clinical relevance. The molecular mechanisms that drive this response, or their oncogenic mechanisms, remain largely unknown. What is also unknown are their unique and redundant properties within the SWI/SNF complex, and how that affects cancer progression. To study the function of ARID1A and ARID1B in melanoma, we crossed conditional knockout *Arid1a* and *Arid1b* mouse alleles to the tamoxifen-inducible tyrosinase Cre driver for melanocyte-specific deletion, in the presence of *Braf* ^{V600E} and *Pten* loss. We found that deletion of *Arid1a* or *Arid1b* did not change melanoma initiation; however, deletion of *Arid1b* accelerated nevi formation. Since melanoma initiation and progression can be two distinct processes, we next assessed the effect of Arid1a/1b deletion on melanoma progression. Using stable cell lines we generated from the primary tumors, we assessed melanoma tumor growth over time. We found that deletion of *Arid1b* dramatically decreased tumor growth compared to controls. Because deletion of Arid1a/1b did not affect *in vitro* cell growth, we hypothesized that cell extrinsic variables, like anti-tumor immunity, might be contributing to these phenotypes. Analysis of immune cell infiltration in the melanoma tumor revealed increased infiltration of CD8 T cells, particularly effector T cells in the absence of Arid1b, but not Arid1a. These results suggest that Arid1a and Arid1b are functionally distinct on regulating tumor immunity in melanoma.To mechanistically understand how Arid1a/1b affect melanoma progression, we performed gene expression profile analysis using RNA-seq. GSEA of RNA-seq data revealed enrichment in several immune-related signatures after Arid1b deletion, including IFN-α response, IFN-γ response, and MHC I antigen presentation and processing signatures. More specifically, we identified a dramatic upregulation of Stat1, an upstream regulator of MHC I antigen presentation genes that promote the cytotoxic activity of CD8 T cells. However, Stat1 remains largely unphosphorylated, so it remains unclear whether its upregulation results in increased canonical Stat signaling or whether it functions via other mechanisms. Overall, we show that Arid1a and Arid1b have distinct functions in a Braf/Pten-driven model of mouse melanoma and affect melanoma progression via alteration of pathways that control cytotoxic CD8 T cell activity. Finally, ARID1B may potentially serve as a predictive marker for immune checkpoint blockade treatment in melanoma patients.

#4837 NSD2 modulates immune surveillance in multiple myeloma.

Amin Sobh[1], Daphne Dupere-Richer[1], Emilie Bonilla[1], Guya Avshara[1], Karthik Vasan[2], Monica Clerio[1], Richard Bennett[1], Navdeep S. Chandel[2], Jonathan D. Licht[1]

[1]University of Florida, Gainesville, FL,[2]Northwestern University, Chicago, IL

Background: The histone methyltransferase NSD2 is overexpressed in 15% of multiple myeloma (MM) patients due to the translocation (4;14). NSD2 overexpression disrupts chromatin methylation promoting oncogenic gene expression. Despite advancements in MM treatments, the t(4;14) translocation remains a poor prognostic marker. Interestingly, NSD2 disruption in t(4;14) MM cell lines does not affect cellular fitness *in vitro*, suggesting its oncogenic effects might involve mechanisms beyond intrinsic cancer cell survival. Emerging evidence indicates a role of NSD2 in tumor immune response. *In vivo* CRISPR screens showed that NSD2 is selectively crucial when tumor cells are engrafted into immunocompetent mice, suggesting that NSD2 contributes to immune evasion. Targeting NSD2-mediated immune suppression shows potential therapeutic benefits in t(4;14) MM.
Aim: To investigate the role of NSD2 in modulating immune surveillance in MM.
Methods: Multi-omics analyses were performed on isogenic t(4;14) MM cell lines with varying NSD2 expression. These analyses were supported by targeted assays, including flow cytometry and multiplex immunoassays. We utilized both immunocompetent and immunodeficient MOPC315.BM myeloma mouse models to study NSD2's role in immune modulation *in vivo*.
Results: RNA-seq analysis revealed that NSD2 regulates several immune-related pathways, with a particularly strong suppression of MHC-II gene expression in NSD2-overexpressing cells. Unbiased proteomics confirmed reduced MHC-II protein levels in these cells. Flow cytometry showed that disrupting NSD2 in t(4;14) MM cells increases MHC-II expression, an effect reversed by conditioned media from NSD2-high cells. This suggests that NSD2 promotes secretion of factors that suppress MHC-II expression, reducing antigen presentation and ultimately the immune response. NSD2 inhibitor treatment increased MHC-II expression, indicating potential for immunotherapy. Cytokine and chemokine profiling revealed that NSD2 overexpression alters the secretion of CCL2, CXCL9, MCP-1, IL-16, and IL-10, with implications for immune modulation. Metabolomics showed that NSD2-high cells accumulate the immunosuppressive metabolite spermidine. Proteomics further revealed marked reduction of adenosine deaminases ADA1 and ADA2 by NSD2, suggesting an accumulation of the immunosuppressive metabolite adenosine in the tumor microenvironment.
Conclusion: NSD2 overexpression in MM suppresses immune surveillance potentially contributing to the aggressiveness of t(4;14) myelomas. These findings highlight the potential of NSD2 as a therapeutic target to enhance immunotherapy in MM.
Future directions: The effect NSD2 on MM eradication by immune cells will be investigated by immune cell killing assays. The immunocompetent MOPC315.BM MM model will be used to evaluate how NSD2 regulates immune cell composition.

#4838 Epigenetic modulation of polyamine biosynthetic pathways rectifies T cell dysfunction to enhance anti-tumor immunity in lung cancer.

Hsing-Chen Tsai[1], Yi-Chieh Wu[1], Yu-Ting Lee[1], Meng-Wei Chou[1], Shu-Yung Lin[2], Sheng-Yao Su[1], Chia-Lang Hsu[3], Nai-Wen Chang[3], Yi-Jhen Huang[1], Yi-Hsiu Juan[2], Hsuan-Hsuan Lu[2], Pei-Shan Wu[4], Miao-Hsia Lin[4], Li-Chung Hsu[5], Yen-Ling Chiu[6], Shih-Yu Chen[7], Chong-Jen Yu[8]

[1]Graduate Institute of Toxicology, National Taiwan University College of Medicine, Taipei, Taiwan,[2]Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan,[3]Department of Medical Research, National Taiwan University College of Medicine, Taipei, Taiwan,[4]Department of Microbiology, National Taiwan University College of Medicine, Taipei, Taiwan,[5]Graduate Institute of Molecular Medicine, National Taiwan University College of Medicine, Taipei, Taiwan,[6]Far Eastern Memorial Hospital, New Taipei City, Taiwan,[7]Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan,[8]National Taiwan University Hospital Hsin-Chu Branch, Hsinchu City, Taiwan

T cell exhaustion, a dysfunctional state caused by prolonged antigen exposure, presents a major challenge in cancer immunotherapy. While immune checkpoint inhibitors show therapeutic promise, they fail to rejuvenate T cells in a terminally exhaustion state, potentially due to epigenetic barriers. We hypothesized that targeting epigenetic regulators may provide novel therapeutic strategies to overcome these barriers, thereby rescuing terminally exhausted T cells. In this project, we used malignant pleural effusions from advanced lung cancer patients as a source of primary exhausted T cells. Through high-throughput screening of an epigenetic compound library, we identified several bromodomain inhibitors (BETis) as potent enhancers of T cell polyfunctionality in primary human T cells. Treatment with BETis, like JQ1, reduces inhibitory receptor expression and increases effector cytokine production. Transcriptomic and ATAC-seq profiling revealed that JQ1 modulates key metabolic pathways and induces chromatin accessibility changes in primary effusion-infiltrative T cells. Untargeted metabolomics showed that JQ1 expands the polyamine spermidine pool in T cells. Inhibition of ornithine decarboxylase (ODC1), a key enzyme in polyamine biosynthesis, diminishes JQ1's reinvigorating effects on these T cells. scRNA-seq analysis revealed that JQ1 reduces terminally exhausted T cells and promotes the expansion of progenitor exhausted T cells, which are more responsive to checkpoint inhibitors, by upregulating ODC1 and its upstream regulator, MYC. Notably, adoptive transfer of JQ1-treated T cells significantly reduced tumor-associated MPE in a syngeneic mouse model. Our study demonstrates that BET inhibitors enhance T cell polyfunctionality and modulate terminally exhausted T cells through metabolic reprogramming, notably by upregulating the polyamine biosynthesis pathway. These findings highlight the potential of epigenetic-based cancer immunotherapy for novel therapeutic strategies.

#4839 Harnessing the cGAS-STING signaling and immune checkpoint pathways to enhance the tumor microenvironment for breast cancer immunotherapy.

Shu-Ping Wang[1], Marvin Angelo E. Aberin[1], Saurabh Singh[2], Soumya Chatterjee[2], Guang-Zhi Sui[2], Joy Khag[1], Weng-Lang Yang[2], Chen-Yang Shen[1], Yao-Ming Chang[1], Shih-Yu Chen[1], Chandan Guha[2]

[1]Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan,[2]Department of Radiation Oncology, Albert Einstein College of Medicine, Bronx, NY

Immune checkpoint blockade (ICB) therapy has revolutionized cancer treatment, but resistance remains common with only some patients showing lasting responses. This underscores the need to understand resistance mechanisms and develop strategies to enhance responsiveness. In this study, we focus on breast cancer, particularly estrogen receptor-positive (ER$^+$) breast cancer, which is generally poorly immunogenic and responds unfavorably to ICB. Upon analyzing the METABRIC cohort's immunogenic biomarkers and tumor mutational burden (TMB), we found that relapsed ER$^+$ patients treated with endocrine therapy (ET) exhibit activated immune pathways. However, high levels of TMB and interferon-stimulated gene (ISG) expression correlated with worse outcomes in these patients, suggesting that ET modulates an active but clinically ineffective immune response. To explore the ET-dependent immune resistance mechanisms, we developed chronically tamoxifen-treated T47D and MCF7 cells (cTMX-T47D and cTMX-MCF7) and found increased type I interferon (IFN-I) signaling and innate immunity. This was confirmed in vivo with T47D xenograft showing enhanced IFN-I signaling. Further analyses revealed that tamoxifen's DNA-damaging ability modulates the TME through mitochondrial DNA (mtDNA) damage-induced STING upregulation. Depleting mtDNA in tamoxifen-treated cells reduced STING upregulation, IFN-I pathway activation, and ISG expression. Furthermore, we identified that the RACK7/KDM5C H3K4 demethylase complex is crucial for such regulation. Tamoxifen induces the reduction of RACK7/KDM5C protein stability via its ER inhibitory ability, leading to the upregulation of STING and ISG expression. Surprisingly, we discovered that tamoxifen can also induce CEACAM1 upregulation via a RACK7/KDM5C-USF1/IRF2-CEACAM1 transcriptional regulatory axis. These findings highlight the tamoxifen-RACK7/KDM5C-CEACAM1 axis's role in creating a tumor-favorable TME conducive to immune evasion and robust tumor growth. Preclinical TS/A (ER$^+$) syngeneic tumor mouse model showed that combining tamoxifen treatment with RACK7 knockdown enhances innate immunity effects compared to either treatment alone. Blocking CEACAM1-TIM3 interaction with an anti-TIM3 antibody significantly inhibited TS/A tumor growth upon the combined tamoxifen and RACK7-knockdown treatment, underscoring the CEACAM1-TIM3 pathway's role in tamoxifen resistance. In conclusion, our study provides direct evidence for tamoxifen-mediated ICB resistance mechanisms. Prolonged tamoxifen treatment reduces RACK7/KDM5C levels, increasing STING and CEACAM1 expression, creating an immunosuppressive TME via TIM3-dependent T-cell exhaustion. This fosters an immunosuppressive yet ready-to-fire TME, augmenting the efficacy of anti-CEACAM1/TIM3 immunotherapy.

#4840 Investigation of the potential immunomodulatory effect of vitamin D supplementation in vitamin D deficient cancer patients.

Alexandra Kalmar[1], Zsofia Brigitta Nagy[1], Liza Dalma Sumegi[1], Barbara Kinga Bartak[1], Sandor Spisak[2], Istvan Takacs[1], Bela Molnar[1]

[1]Department of Internal Medicine and Oncology, Semmelweis University, Budapest, Hungary,[2]Institute of Enzymology, Research Centre for Natural Sciences, Eotvos Lorand Research Network, Budapest, Hungary

Background: Advantageous effects of vitamin D supplementation have been studied in certain diseases, but the underlying chromatin remodeling processes remains to be explored in detail during the treatment of cancer patients.
Aim: We aimed to assess the molecular effects of high-dose vitamin D supplementation in patients diagnosed with various cancer types.
Methods: High-dose (30.000 NE) vitamin D per week was supplemented to 8 patients including 5 lung cancer, 2 colorectal cancer, and 1 urothelial cell carcinomas for two months. Following the blood collection before and after the supplementation period, Histopaque density gradient cell separation was applied to retrieve peripheral mononuclear cells (PBMCs). To examine alterations in the chromatin accessibility after the treatment in PBMCs, assay for transposase-accessible chromatin with sequencing (ATAC-Seq) was performed using the 10X Genomics on a NextSeq 550 instrument with a High Output Sequencing kit (Illumina Inc.). DNA integrity was assessed by the alkaline Comet-assay and telomere qPCR. To analyze further potential epigenetic alterations, global DNA methylation level was estimated on the basis of LINE-1 bisulfite-pyrosequencing experiments on cfDNA and PBMC cells.
Results: Vitamin D serum levels elevated 2,5 fold on average after 2 months of supplementation in all patients. Based on ATAC-Seq, the cellular ratio among PMBC cells altered with an elevated number of NK cells following vitamin D supplementation. DNA integrity was significantly better after treatment in mononuclear cells with decreased Comet assay DNA tail average % ($p=0.01$), but not in granulocytes. Supplemented samples showed significantly altered telomere length in mononuclear cells ($p=0.007$). However, no significant difference was observed in cfDNA levels between the sample groups. Slight, but not significant LINE-1 methylation alteration was detected in PBMCs and cfDNA after vitamin D supplementation.
Conclusion: Vitamin D supplementation caused significant changes in immune cells that might have an influence on the response to the tumors in cancer patients.

#4841 DNA methylation status classifies pleural mesothelioma cells according to their immune profile: implication for precision epigenetic therapy.

Maria Fortunata Lofiego[1], Rossella Tufano[2], Emma Bello[1], Laura Solmonese[1], Francesco Marzani[1], Francesca Piazzini[1], Fabrizio Celesti[1], Francesca Pia Caruso[2], Teresa Maria Rosaria Noviello[3], Roberta Mortarini[4], Andrea Anichini[4], Michele Ceccarelli[3], Luana Calabro[5], Michele Maio[1], Sandra Coral[1], Anna Maria Di Giacomo[1], Alessia Covre[1]

[1]Center for Immuno-Oncology - University of Siena, Siena, Italy,[2]BIOGEM Institute of Molecular Biology and Genetics, Ariano Irpino, Italy,[3]Sylvester Comprehensive Cancer Center, Miller School of Medicine, University of Miami, Miami, FL,[4]Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy,[5]University of Ferrara, Ferrara, Italy

Background: co-targeting of immune checkpoint inhibitors (ICI) CTLA-4 and PD-1 has recently become the new first-line standard of care therapy of pleural mesothelioma (PM) patients, with a significant improvement of overall survival (OS) over conventional chemotherapy. The analysis by tumor histotype demonstrated a greater efficacy of ICI therapy, compared to standard chemotherapy, in non-epithelioid (non-E) *vs.* epithelioid (E) PM, although some E PM patients also benefit from treatment. This evidence suggests that molecular tumor features, beyond histotype, could be relevant to improve the efficacy of ICI therapy in PM. Among these, tumor DNA methylation emerges as a promising factor to explore, due to its potential role in driving the immune phenotype of cancer cells. Therefore, we utilized a panel of cultured PM cells of different histotype to provide preclinical evidence supporting the role of the tumor methylation landscape and of its pharmacologic modulation, to prospectively improve the efficacy of ICI therapy of PM patients.
Methods: the methylome profile (EPIC array) of distinct E (n=5) and non-E (n=9) PM cell lines was analyzed, followed by integrated analysis with their associated transcriptomic profile (Clariom S array), before and after *in vitro* treatment with the DNA hypomethylating agent (DHA) guadecitabine. The most variable methylated probes were selected to calculate the methylation score (CIMP index) for each cell line at baseline. Genes that were differentially expressed and methylated were then selected for gene ontology analysis.
Results: the CIMP index stratified PM cell lines into two distinct classes, CIMP (hypermethylated; n=7) and LOW (hypomethylated; n=7), regardless of their E or non-E histotype. Integrated methylome and transcriptome analyses revealed that CIMP PM cells exhibited a substantial number of hypermethylated, silenced genes, which negatively impacted their immune phenotype compared to LOW PM cells. Treatment with DHA reverted the methylation-driven immune-compromised profile of CIMP PM cells and enhanced the constitutive immune-favorable profile of LOW PM cells.
Conclusion: the study highlighted the relevance of DNA methylation in shaping the constitutive immune classification of PM cells, independent of their histological subtypes. The identified role of DHA in shifting the phenotype of PM cells towards an immune-favorable state supports its potential role in clinical trials of precision epigenetic therapy combined with ICI.

#4842 Inhibition of BPTF enhances the antitumor effects of doxorubicin through mitochondrial disfunction.

Manjulata Singh[1], Nasser AlAbdulaly[1], Madison Isbell[1], Dipankar Bandyopadhyay[1], Mikhail Dozmorov[1], Rebecca Martin[1], Will Pomerantz[2], **Joseph W. Landry**[1]

[1]Virginia Commonwealth University, Richmond, VA,[2]University of Minnesota, Minneapolis, MN

Epigenetics has established itself as a hallmark of cancer having essential roles in its initiation, establishment, and progression. Because of their reversibility, epigenetics has been aggressively targeted pharmacologically to correct abnormal cancer biology in the hopes of reversing cancer phenotypes. To develop novel epigenetic based therapeutic strategies for triple negative breast cancer (TNBC) we combined loss of function approaches (shRNA - KD, CAS9 - KO, and small molecule inhibitors) to the chromatin remolding protein Bromodomain PHD-finger Transcription Factor (BPTF) with standard of care chemotherapies doxorubicin (Doxo), etoposide (Eto) and paclitaxel (Pac). Using the 4T1 and E0771-LMB mouse TNBC models we show that BPTF inhibited cells are more susceptible to the anticancer effects of Doxo, Eto and Pac. However, in tumor bearing mice sensitivity to Doxo, but not Eto or Pac is observed. A 30 color spectral flow cytometry analysis of both intratumor and splenic immune cells does not observe significant differences in immune cell populations from mice bearing BPTF inhibited tumors treated with Doxo. Using immune compromised mouse backgrounds, we observe that the in vivo sensitivity to Doxo requires NK cells, but not CD8 or CD4 T cells. Ex vivo NK cell coculturing assays discovers selectivity for NK cell killing after treatment with Doxo, but not Eto or Pac. RNA-Seq analysis of genetic and BPTF small molecule inhibited cells treated with Doxo discovers the activation of several pro-apoptotic pathways including TNF signaling, apoptosis, mitophagy, and ferroptosis. In vitro analysis shows that BPTF inhibited cells increase apoptosis selectively after exposure to Doxo over Eto and Pac, and the increase in apoptosis coincides with increased mitophagy (Mitotracker QC), but not autophagy (LC3B-GFP-RFP conversion) and is preceded by enhanced cytochrome c release. Depletion of mitophagy regulators reduces the observed enhanced apoptosis in vitro and tumor growth control in vivo with the combination of BPTF KO and Doxo exposure. Analysis of NK cell receptor binding (NKG2D, NCR1, DNAM1) to control and BPTF KD Doxo treated cells did not reveal significant differences and analysis of cytokine, and eicosanoid release with Doxo, Eto and Pac show minor significant differences with BPTF KD. However, enhanced sensitivity to select pro-apoptotic molecules produced by NK cells was observed, and this sensitivity was reduced with suppression of mitophagy. These results suggest that BPTF inhibition results in enhanced mitochondrial disfunction with Doxo exposure which results in enhanced activity of NK cell proapoptotic molecules which results in enhanced tumor growth control.

#4843 TET2 oxidizes chromatin-associated regulatory RNA m^5C to potentiate anti-tumor immunity.

Ying Qing[1], Dong Wu[1], Yangchan Li[1], Keren Zhou[1], Keith Leung[1], Wei Li[1], Le Xuan Truong Nguyen[2], Lei Dong[1], Chao Shen[1], Meilin Xue[1], Zhenhua Chen[1], Kitty Wang[1], Lillian Sau[1], Mike Fernandez[1], Zhaoxu Xu[1], Yuan Che[1], Tingting Tang[1], Miao Sun[3], Li Han[1], Zhicong Zhao[1], Meiling Chen[1], Mingjiang Xu[4], Lili Wang[1], Guido Marcucci[2], Rui Su[1], Xiaolan Deng[1], Jianjun Chen[1]

[1]Department of Systems Biology and Center for RNA Biology and Therapeutics, Beckman Research Institute of The City of Hope, Duarte, CA,[2]Department of Hematologic Malignancies Translational Science, Beckman Research Institute of The City of Hope, Duarte, CA,[3]Keck School of Medicine of USC, Los Angeles, CA,[4]The University of Texas Health Science Center at San Antonio, San Antonio, TX

Current immunotherapies have shown limited success in treating acute myeloid leukemia (AML) due to immune evasion, which often stems from epigenetic dysregulation of immune-related pathways. TET2, a well-studied DNA 5-methylcytosine dioxygenase, is frequently mutated and recognized as a tumor suppressor in AML. While *TET2* deficiency in hematopoietic stem/progenitor cells (HSPCs) has been associated with elevated inflammation, TET2 has also been reported to promote inflammatory interferon (IFN) signaling in solid tumors, enhancing tumor responses to immunotherapies. Despite these findings, the precise role of TET2 in the response of established AML to anti-tumor immunity and the underlying molecular mechanisms remain poorly understood. By use of both syngeneic and xeno-transplantation AML mouse models, including patient-derived xenotransplantation (PDX) models, as well as *in vitro* co-culture systems with primary AML cells and T cells, our results revealed that TET2 sensitizes AML cells to T cell-mediated immunity by promoting the transcription of IFN-stimulated genes (ISGs). Mechanistically, we employed UHPLC-QQQ-MS/MS and optimized bisulfite-mediated 5-methylcytosine (m^5C) sequencing and demonstrated that chromatin-associated regulatory RNAs (carRNAs) are modified with m^5C and TET2 removes m^5C from target carRNAs such as LINE1, which subsequently prevents carRNA degradation mediated by SRSF2. Loss of *TET2* in AML cells leads to increased m^5C levels on carRNAs and decreased LINE1 abundance, which in turn suppresses ISG transcription, facilitating immune evasion. Collectively, this study clarifies the immune-promoting role of TET2 in established AML and elucidates a previously unappreciated molecular mechanism by which TET2 promotes ISG transcription through carRNA m^5C oxidation and thereby facilitates immune response. Our findings underscore the therapeutic potential of targeting the TET2-carRNA m^5C-ISG pathway to overcome AML immune evasion and improve immunotherapy outcomes in AML.

#4844 Epigenetic reprogramming of the STING pathway as a combination strategy in high grade serous ovarian cancer.

Saranya Rajendran[1], Elnaz Abbasi Farid[1], Kaushlendra Tripathi[2], Ishani Chattopadhayay[1], Shu Zhang[1], Stephen B. Baylin[3], Feyruz V. Rassool[2], Kenneth P. Nephew[1]

[1]School of Medicine, Indiana University, Bloomington, IN,[2]Marlene and Stewart Greenebaum Comprehensive Cancer Center, University of Maryland, Baltimore, MD,[3]Department of Oncology, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD

High-grade serous ovarian carcinoma (HGSOC), accounting for 70% of ovarian carcinoma cases, is often diagnosed at an advanced stage and associated with a poor prognosis. A significant clinical challenge is the lack of effective treatments for platinum-resistant OC and non-BRCA-mutated, homologous recombination proficient (HRP) tumors, which represent over 50% of HGSOC. Furthermore, although cancer immunotherapies can produce complete and durable responses, response rates in OC are modest with most patients experiencing only transient therapeutic benefit, underlining the urgent need for novel approaches to enhance antitumor immunity. Our prior research demonstrated that DNMT inhibitors (DNMTis) and PARP inhibitors upregulate ZNFX1, a zinc finger protein that facilitates STING-dependent interferon signaling and antitumor immune responses through a mechanism of pathogen mimicry. We hypothesize that in addition to upregulating ZNFX1 and immune responses, DNMTis restore STING through epigenetic reprogramming, leading to tumor cell intrinsic STING activation mediating anti-tumor response. In this study, we investigated the role of STING in HGSOC and the mechanisms underlying STING pathway activation by DNMTi. Analysis of the TCGA and GTEX database revealed reduced basal STING expression in HGSOC patient tumors, predominantly in TP53 mutant HGSOC. Furthermore, higher STING expression was linked to improved overall and progression free survival. Analysis of STING methylation in normal and HGSOC samples from TCGA database identified 6 hypermethylated sites near the promoter region/transcriptional start site. Our Infinium MethylationEPIC Array v2.0 BeadChip profiling of the HGSOC cell line OVCAR3 showed hypermethylation of these 6 sites. To determine whether this promoter-driven silencing could be reversed, we treated HRP and HR-deficient (HRD) HGSOC cell lines with increasing concentrations of DNMTi (0-100 nM decitabine, DAC). This treatment increased ($P<0.01$) STING expression in a dose-dependent manner and upregulated ($P<0.001$) downstream STING targets, CXCL10, TNFα, IL6. To further evaluate STING agonist could restore functional activation of STING signaling in addition to demethylation, HGSOC cells were treated with DAC (10nM), STING agonist (CRD3874; 1μM) and combination and evaluated for downstream STING targets by western and qPCR. The DAC-STING agonist combination increased pSTING1, pTBK1, and pIRF3 and apoptosis-dependent cell death. Additionally, combination treatment markedly reduced proliferation, colony and spheroid formation compared to single drug treatments. Together, these results demonstrate epigenetic regulation of STING expression in HGSOC, regardless of HRD status. Combining a DNMTi with a STING agonist has the potential to enhance the antitumor immune response and represents a promising therapeutic strategy for HGSOC patients.

#4845 The MLL3/GRHL2 complex modulates early malignant development and anti-tumor immunity in upper aerodigestive squamous cell carcinoma.

Chehyun Nam[1], Guowei Huang[2], Yueyuan Zheng[3], Hua Zhao[1], Ethan Pan[1], Boyan Hu[1], Talia Wenger[1], Hieu Van[4], Liyan Xu[5], En-min Li[5], H.Phillip Koeffler[3], Kai Ge[4], Yali Dou[6], Uttam K. Sinha[7], Young Min Park[8], De-Chen Lin[1]

[1]Center for Craniofacial Molecular Biology, USC - University of Southern California, Los Angeles, CA,[2]Institute of Oncologic Pathology, Shantou University Medical College, Shantou, China,[3]Cedars-Sinai Medical Center, Los Angeles, CA,[4]National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD,[5]Shantou University Medical College, Shantou, China,[6]USC - University of Southern California, Los Angeles, CA,[7]Keck school of medicine, USC - University of Southern California, Los Angeles, CA,[8]Department of Medicine, Yonsei University, Seoul, Korea, Republic of

Upper aerodigestive squamous cell carcinoma (UASCC), including head and neck squamous cell carcinoma (HNSCC) and esophageal squamous cell carcinoma (ESCC), poses substantial challenges in clinical care due to its aggressive behavior and poorly understood mechanisms of early malignant transformation. This study investigates the role of MLL3 mutations as critical, clonal genomic events driving UASCC tumorigenesis. Using CRISPR-edited organoid models across species, we demonstrate that MLL3 loss facilitates early squamous neoplastic evolution. Additionally, we uncover an MLL3/GRHL2 protein complex that orchestrates epigenomic regulation, particularly influencing immune-related pathways. Strikingly, we identify a novel MLL3/GRHL2-IRF1 axis that induces the expression of Th1 chemokines, such as CXCL9 and CXCL10, enhancing anti-tumor immunity by promoting T cell infiltration within the tumor microenvironment. In syngeneic mouse models, including both subcutaneous and orthotopic settings, MLL3 was shown to modulate the efficacy of immune checkpoint blockade (ICB) therapy. Consistently, clinical data from human patients demonstrate a robust correlation between MLL3 expression and responsiveness to ICB therapy. This study underscores the pivotal role of MLL3 in UASCC pathogenesis and highlights the therapeutic potential of targeting the MLL3/GRHL2-mediated immune response pathways to improve outcomes in UASCC treatment.

#4849 MYC-mediated resistance to immune activation by SWI/SNF mutation or chemical inhibition.

Shitian Li, Diana Hargreaves

Molecular and Cell Biology Laboratory, Salk Institute For Biological Studies, La Jolla, CA

ARID1A is a critical subunit of the SWI/SNF chromatin remodeling complex, frequently mutated in several cancers. We have shown that loss of ARID1A induces an accumulation of R-loops, leading to cytosolic release of RNA:DNA hybrids and single stranded DNA (ssDNA) and cGAS/STING-dependent activation of Type I interferon (IFN-I) stimulated genes (ISGs). SWI/SNF ATPase inhibitors (FHD-286, BRD98, and BRM014) and degraders (ACBI1) similarly induce ISGs through a cGAS/STING-dependent mechanism. Consequently, ARID1A mutant tumors are more highly infiltrated with cytotoxic CD8+ T cells and exhibited enhanced tumor immunity and sensitivity to immune checkpoint blockade (ICB)[1]. These results provide a molecular mechanism for the enrichment of ARID1A mutations among ICB responders. However, co-occurring oncogenic mutations in ARID1A mutant cancers, such as MYC, KRAS, and β-catenin, have been associated with immunosuppression in some cases through suppression of IFN-I signaling and ISG expression. It is not known how such co-occurring mutations affect the ARID1A mutant ISG response or whether such mutations would counteract ISG activation by SWI/SNF inhibitors. In this study, we investigate the impact of MYC overexpression on IFN-I responses induced by ARID1A loss or SWI/SNF inhibition in cancer cells. Our data reveal that MYC overexpression impairs the ability of these cells to induce IFN-I response genes (ISGs) when cells are directly stimulated with IFN-I or treated with inhibitors targeting the SWI/SNF complex, including FHD-286 and BRM014. Through bulk RNA-seq, we characterize the molecular mechanisms underlying this resistance, with a focus on IFN-I related genes. This study highlights the suppressive effect of MYC overexpression on IFN-I responses in ARID1A-deficient cancers and suggests a potential link to therapeutic resistance in MYC-high tumors. While the precise molecular mechanisms remain to be fully elucidated, these findings provide a foundation for future studies aimed at understanding the interplay between oncogenic drivers and immune signaling pathways, with implications for developing more effective therapeutic strategies.

Reference: (1). Maxwell, M. B., et al. ARID1A Suppresses R-Loop-Mediated STING-Type I Interferon Pathway Activation of Anti-Tumor Immunity. Cell 2024, S0092867424004513. https://doi.org/10.1016/j.cell.2024.04.025.

IMMUNOLOGY: Immune Fitness and Metabolic Regulation of Cancer Immunity
Poster Session

#4851 Disrupting redox homeostasis in pancreatic ductal adenocarcinoma initiates anti-tumor immune responses.
Vishal Pandya[1], Lucie Malbeteau[2], Emily Poulton[3], Marianne Koritainsky[2]

[1]Institute of Medical Science, University of Toronto, Toronto, ON, Canada,[2]UHN Princess Margaret Cancer Centre, Toronto, ON, Canada,[3]Medical Biophysics, University of Toronto, Toronto, ON, Canada

Immunotherapy has improved cancer treatment by ameliorating outcomes for patients with aggressive cancers. However, pancreatic ductal adenocarcinoma (PDAC) remains resistant to various immunotherapies. This resistance can be attributed to the immunosuppressive PDAC tumor microenvironment. Immunosuppressive cells populate the tumor microenvironment in the early neoplastic stages of the disease, suggesting that neoadjuvant therapies may be needed to overcome immune suppression. New therapeutic strategies that prime the immune response against PDAC are needed to improve the efficacy of immunotherapy. We aim to overcome immune suppression by leveraging redox vulnerabilities in PDAC. Our lab identified the antioxidant protein peroxiredoxin-4 (PRDX4) as essential for PDAC cell survival but dispensable in normal tissue, making it a promising therapeutic target. PRDX4 neutralizes reactive oxygen species in the endoplasmic reticulum and its depletion leads to decreased tumor growth and substantial DNA damage. From this, we hypothesize that PRDX4 depletion causes immunogenic DNA damage and initiates immune responses against PDAC. We generated stable doxycycline-inducible PRDX4 knockdown in the human PANC-1 and mouse KPC (LSL-Kras^{G12D};LSL-p53^{R172H};Pdx1-CreER) PDAC cell lines. Using immunofluorescence imaging, we show that PRDX4 depletion leads to cytosolic DNA in the form of micronuclei and dispersed DNA. We also show using RT-qPCR and ELISA that PRDX4 depletion leads to an increase in chemokine production, like CCL5, CXCL10 and CCL20. Using siRNA targeting both NF-κB and the DNA sensor cGAS, we show that this chemokine production is activated through the cGAS-STING pathway. Finally, using DNase to clear the cytosolic DNA, we show that cGAS-STING activation and chemokine production can be attributed to PRDX4 depletion-induced cytosolic DNA. To extend these findings *in vivo*, we created an immunocompetent KPC-PRDX4 mouse model by crossing KPC mice with PRDX4^{KO} mice. This model develops spontaneous and staged cancer progression, from neoplastic lesions to PDAC. It also recapitulates the immune infiltrate seen in the human disease, making it a good model to study immune dynamics in the tumor microenvironment. Based on the strong chemokine response seen *in vitro*, we are now measuring the effects of PRDX4 depletion on the immune cell composition in the tumor microenvironment. Ongoing studies include immunofluorescence imaging and flow cytometry using tumor samples from our model. The aggressive growth and treatment resistance of PDAC demand research towards novel treatment strategies. This project contributes supporting evidence for targeting cancer metabolism, particularly PRDX4, to enhance immune responses in PDAC.

#4852 Carbonic anhydrase IX and hypoxia-induced metabolic adaptations in tumor immune microenvironment: Insights into enhancing anti-PD1 efficacy in lung cancer.

Mariel Alejandra Fusco[1], Maria Florencia Mercogliano[2], Marco Aurelio Diaz Gutierrez[1], Carlos Rafael Picon[3], Paula Rosello[4], Mara Stinco[1], Flavia Piccioni[1], Constanza Paula Arriola Benitez[1], Mauricio Cesar De Marzi[5], Manglio Miguel Rizzo[3], Mariana Malvicini[1]

[1]Cancer Immunobiology Lab, Instituto de Investigaciones en Medicina Traslacional (IIMT), Universidad Austral-CONICET, Pilar, Argentina,[2]Tumor Immunology Lab, Instituto de Biologia y Medicina Experimental (IBYME-CONICET), CABA, Argentina,[3]Servicio de Oncologia, Hospital Universitario Austral, Pilar, Argentina,[4]Instituto de Investigaciones en Medicina Traslacional (IIMT), Universidad Austral-CONICET, Pilar, Argentina,[5]Universidad Nacional de Lujan - CONICET, Lujan, Argentina

In solid tumors, cancer cell survival is largely driven by hypoxia-induced metabolic changes, including the enhancement of glycolysis and extracellular acidification. This process is facilitated by enzymes such as carbonic anhydrase IX (CAIX), which is upregulated under low oxygen tension to regulate pH by exchanging H+ ions and releasing lactate. These changes contribute to an acidic tumor microenvironment (TME) that alters immune cell functionality, particularly by recruiting tumor-associated macrophages (TAMs) and stimulating immune checkpoints like PD-L1. Our analysis of The Cancer Genome Atlas (TCGA) lung cancer data reveals that CAIX and hypoxia-inducible factor 1a (HIF1a) expression levels are significantly elevated in lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) subtypes of non-small cell lung cancer (NSCLC) ($p<0.001$), with higher CAIX expression correlating with reduced overall survival. Strong correlations between HIF1a and CAIX, HIF1a and PD-L1, and PD-L1 and TAM markers (CD68, CD163) underscore a complex hypoxia-driven immunosuppressive mechanism. As we have reported that coumarin 4-methylumbelliferone (4Mu) modifies HIF1a tumor levels, we aim to test the effect of 4Mu in vitro under hypoxia/normoxia and in vivo combined with antiPD-1 therapy, using Lewis Lung Carcinoma cells (LLC). Hypoxia induces an increase in CAIX levels compared to normoxia ($p<0.05$), reduces extracellular pH ($p<0.05$), and increases levels of lactate. However, under hypoxic conditions, 4Mu restored CAIX, pH, and lactate levels to near-baseline levels. Moreover, LLC cells conditioned under hypoxia + 4Mu induced an M1 cytokine profile in macrophages, contrasting with the M2 polarization observed with hypoxia alone. In vivo, 4Mu reduced CAIX expression, and combination of 4Mu with anti-PD1 therapy increased the infiltration of CD8+ and CD4+ T cells and significantly improved anti-tumor efficacy ($p<0.01$). These findings suggest that targeting CAIX can modulate the TME, enhancing immune responses and potentially improving clinical outcomes for NSCLC patients receiving immunotherapy.

#4853 Knockout of hypoxia inducible factor 1 subunit alpha (HIF1a) augments the antitumor activity of EGFR CAR-NK cells.

Fanxiang Gao[1], Tingting Liu[1], Qin Wang[1], Yanxue Gong[1], Chao Wang[1], Feng Zhou[2], **Zhuoxiao Cao**[1]

[1]Shanghai Simnova Biotechnology Co., Ltd., Shanghai, China,[2]State Key Laboratory of Neurology and Oncology Drug Development, Simcere Zaiming Pharmaceutical Co, Ltd., Shanghai, China

Chimeric antigen receptor T-cell (CAR-T) therapies have gained remarkable success with durable remissions in hematologic malignancies. However, the potential adverse effects, including cytokine release syndrome (CRS), graft-versus-host disease (GVHD), neurotoxicity, and off-tumor toxicity, and high production cost have limited the clinical application of CAR-T cells in oncological treatment. Recently, natural killer (NK) cells has emerged as a promising alternative immunotherapy with high biosafety and universal application. Nevertheless, the persistence and efficacy of CAR-NK cells remains to be solved. Knockout of immune checkpoint molecules (ICPs) has been proved to be an effective way to enhance the potencies of CAR-NK cells, such as cytokine inducible SH2-containing protein (CISH) and Cbl proto-oncogene B (CBLB). In solid tumors, hypoxic microenvironment can drive tumor-infiltrating NK cells dysfunction. Cellular response to hypoxia is mediated by hypoxia-inducible factors. Hypoxia inducible factor 1 (HIF1) is a master regulator and inhibits activation markers and effector molecules of tumor-infiltrating NK cells in hypoxic conditions. In this study, we disrupted HIF1a, alpha subunit of HIF1, in NK cells derived from peripheral blood mononuclear cells (PBMCs) to evaluated the anti-tumor effects. Then EGFR CAR molecules were transduced into the NK cells with retroviral vector. Successful knockout was performed by CRISPR/Cas9 and confirmed by TIDE, ICE and qPCR analysis. Our results showed that the NK cell viability was slightly affected by electroporation and gradually returned, leading to a slight reduction in knockout NK cell expansion. However, the freeze-thaw viabilities viability of knockout NK cells was comparable to that of control cells. Flow cytometry assay demonstrated that several stimulatory receptors were up-regulated in HIF1a-/- NK cells, such as CD57, CD62L, CD69, and NKp44, and some inhibitory and activating receptors were down-regulated, including NKG2A, CD38, NKp30 and NKp46. Using luciferase-based serial killing analysis, the knockout group exhibited greater in vitro cytotoxicity against HCT116 and SHP77 tumor cells compared to that of unedited CAR-NK. Moreover, HIF1a knockout significantly enhanced the anti-tumor activity of CAR-NK cells in SHP77 subcutaneously inoculated xenograft mouse model. Taken together, our data suggested that HIF1a deficiency could enhance the cytotoxicity and persistence of CAR-NK cells, and supported the further exploration for clinical application.

#4854 Alleviation of low pH induced immunosuppression through antagonism of the pH sensor GPR65 in highly glycolytic NSCLC tumors.

Prajna Behera[1], Samantha Buongervino[1], Kayla Guidry[1], Kyle Krois[1], Yanah Hossain[1], Victor Du[1], Tamara Ouspenskaia[1], Hongyu (Annie) Liang[2], Shu-Yun Zhang[2], Laura Johnston,[2], Abby Lin[2], Tolu Aweda[2], Ramona Plant[2], Paul Bojczuk[2], Marica Speranza[1], Chris Hopson[2], Charles McHugh[2], Stacey Kendrew[2], Bradley Spencer-Dene[3], Susanne Moore[3], Deon Hildebrand[3], Jonathan Lea[3], Kerena Norris[3], Antonia Lewis[3], Jacob Brett[3], Paru Rao[2], Carol Harris[3], Joseph Pero[2], Laura Ator[2], Jing Fang[2], David washburn[2], Lisa Sloan[3], Catherine Alder[3], Richard Lonsdale[3], Nick Barton[3], Sue Griffin[3], Paul Redford[3], James Smothers[2]

[1]GSK, Cambridge, MA,[2]GSK, Collegeville, PA,[3]GSK, Stevenage, United Kingdom

Out of more than 5683 clinical trials testing anti-PD1/PDL1 mAbs, on average only 20% of patients achieved an objective response, leaving an unmet need for patients who do not respond to immune checkpoint inhibitors (ICIs)(Zhao *et al*, 2020, Upadhyaya *et al*, 2022). Independent reports in NSCLC and melanoma suggest that non responders to ICI exhibit elevated levels of tumor “aerobic” glycolysis (Renner *et al*, 2019, Silva *et al*, 2022), a phenotype associated with solid and metastatic tumor cells (Hanahan, 2022). Aerobic glycolysis results in accumulation of lactate and acidification of the tumor microenvironment (TME), resulting in immune evasion through inhibition of pro-inflammatory/ anti-tumorigenic responses in surrounding immune cells (Li *et al*, 2022). GPR65 is a proton (pH) sensing GPCR, expressed on immune cells, signals via cAMP to activate anti-inflammatory pathways in multiple immune cell types (Robert and Mackay, 2018; Wang *et al*, 2023). In the context of solid tumors, GPR65 activation promotes the increase in anti-inflammatory TAMs, in addition to suppressing anti-tumor responses by NK and cytotoxic-T cell (Raker *et al*, 2016). GSK analysis of Tempus RW data conducted with TEMPUS LENS software indicated high glycolytic gene signature in NSCLC cohorts is associated with worse prognosis to both chemo and ICI therapies, providing a window of opportunity to target pH sensor GPR65. Ex vivo experiments demonstrated that low pH dampens the proinflammatory phenotype of monocyte-derived macrophages (MDMs), while knockout of GPR65 under these acidic conditions restores secretion of the proinflammatory cytokines CXCL9/10/11 in M1-like polarized MDMs. Compound A, an exemplar compound from our lead series, is a potent GPR65 antagonist with low nanomolar *in vitro* functional activity (pIC50=8.2) and excellent lipophilic ligand efficiency (6.9). It also features strong correlation between its functional and binding (nanoBRET) activities along with selectivity at GPR65 over GPR4 and GPR68. In vitro translational and cell-based assays demonstrated dose-dependent attenuation of proinflammatory cytokine release by primary CD8+T cells, M1 macrophages at pH 6.8 and in a NSCLC patient-derived tumor fragmentation assay. In vivo, compound A displays attractive pharmacokinetic (PK) properties in rodent with robust oral bioavailability. A preliminary estimate of projected human dose (PHD) is ~245 mg qd based on liver blood flow scaling of its rat PK properties and a targeted 24 h free drug coverage over functional *in vitro* IC50. Additional *In vivo* PD and/or efficacy data in translational models would further refine this calculation. In summary, we hypothesize that restricting immune cells from sensing acidification in the TME via antagonizing GPR65 will prevent the activation of anti-inflammatory programs in immune cells and promote proinflammatory anti-tumor effector mechanisms.

#4855 Metabolic and functional dynamics of T cell activation: bioluminescent assays for immune monitoring and modulation.

Kim Haupt, Kayla Sylvester, Tony Lauer, Natasha Karassina, Jolanta Vidugiriene

Promega Corporation, Madison, WI

Advancing our understanding of T cell activation and its regulatory pathways is critical for developing effective cancer immunotherapies. This study characterizes T cell health and functional dynamics under diverse activation conditions, highlighting key molecular mechanisms that influence immune responses within the tumor microenvironment. We investigate mTOR and AMPK signaling pathways, as well as the metabolic balance between glycolysis and oxidative phosphorylation, which are integral to T cell function in immunotherapeutic contexts. Using selective inhibitors, we identify modulators that regulate cytokine secretion and T cell metabolic states, revealing media composition as a significant variable affecting immune function. Our methodology leverages a suite of bioluminescent Lumit and Metabolite-Glo assays for sensitive quantification of cytokines and metabolites, enabling precise, non-invasive monitoring while preserving cell integrity. This approach is scalable and adaptable for analyzing diverse activation conditions and high-throughput screening applications. Additionally, our findings correlate these metabolic and functional profiles with cell surface markers assessed by traditional methods, providing a comprehensive framework for immune monitoring. These insights underscore the importance of metabolic and signaling interplay in T cell activation, offering a robust platform for studying immune dynamics and uncovering novel immunomodulatory targets. Our work supports the development of precision strategies to modulate T cell activity and optimize immune responses in cancer therapy.

#4856 Chronic anti-CD3/CD28 stimulation during cell expansion generates exhausted T cells with a distinct metabolic and cytotoxic phenotype.

G. Wang[1], X. Zhang[2], N. Romero[1];

[1]Agilent Technologies, Inc., Lexington, MA, [2]Agilent Technologies, Inc., La Jolla, CA

T cell exhaustion is one of the major causes of cancer immune evasion and contributes to the failure of immunotherapies targeted to solid tumors. Reversing T cell exhaustion is a promising strategy in cancer immunotherapy, as it can improve patients' responses to immune checkpoint inhibitors and adoptive T cell therapies. Recent publications showed that impairment of mitochondria function plays a critical role in developing and maintaining T cell exhaustion. This prompted us to explore the possibility of using metabolic profiling of T cells to assess relative T cell exhaustion as a tool to optimize T cell therapy product development. In our study, we induced exhaustion in previously expanded T cells via chronic stimulation with anti-CD3/CD28 Dynabeads and used briefly stimulated (24-hour) counterparts as controls. Across all three donors tested, chronically stimulated T cells exhibited reduced net proliferation, increased percentage of $PD1^{+}Tim3^{+}$ population, and decreased IL-2 secretion upon restimulation, while TNFα and IFNγ secretion varied by donor. We performed Xcelligence RTCA impedance-based, acute killing assays using Epcam-BiTEs to engage T cells to the Epcam expressing T47D cells. Surprisingly, we found that in some donors, cells exposed to chronic anti-CD3/CD28 Dynabeads, despite secrete significantly less cytokines upon antigen restimulation, display similar acute cytotoxicity compared to its non-exhausted counterparts. Importantly, when assessed using Seahorse XF assays, these exhausted T cells displayed a very distinctive metabolic phenotype, including decreased maximal respiration and spare respiratory capacity (SRC), increased glycolytic activity, and reduced capacity to further increase glycolytic activity upon acute reactivation. These findings suggest that metabolic profiling can represent a valuable tool for identifying metabolically fit T cells and is a great addition to the current matrix of parameters for assessing T cell exhaustion.

#4857 The role of tumor microenvironment lactic acid in the cancer cell resistance to anti-PD-L1 and anti-PD-1 blockade therapy.

Alyssa Kim[1], Wonkyung Oh[1], Deepika Dhawan[2], Deborah W. Knapp[2], Seung-Oe Lim[1]

[1]Borch Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN,[2]Department of Veterinary Clinical Science, Purdue University, West Lafayette, IN

Immune checkpoint blockade therapy targeting the PD-1/PD-L1 axis has shown remarkable clinical impact in multiple cancer types. However, despite its recent success, such impact has been shown to be limited to tumors encompassing specific tumor microenvironment characteristics. Furthermore, a significant proportion of initial responders eventually develop resistance. Combining PD-1/PD-L1 blockade with chemotherapy, radiotherapy, or targeted therapy have been suggested to overcome resistance, yet have been shown to be insufficient in fully accounting for resistance. Unlike normal, differentiated cells, most cancer cells produce large amounts of lactic acid. This metabolic property is often referred to as “aerobic glycolysis,” a well-known metabolic reprogramming of cancer cells to sustain cell proliferation and a hallmark of cancer. Such property as well as others of the altered metabolism of cancer cells and its byproducts affect the anti-tumor immune response. Notably, glycolytic metabolites, such as lactate, regulate T cell proliferation and function. However, the mechanism behind how such metabolic alterations impact the cancer cells’ resistance to PD-1/PD-L1 blockade therapy remains unclear. Thus, we sought to decipher the role of tumor-cell derived lactic acid in PD-1/PD-L1 therapy resistance and propose new immunotherapeutic strategies to improve the efficacy of PD-1/PD-L1 blockade therapies. Here, we found that tumor cell-derived lactic acid renders the immunosuppressive tumor microenvironment in the PD-1/PD-L1 blockade-resistant tumors by inhibiting the interaction between the PD-L1 protein and anti-PD-L1 antibody. Furthermore, we showed that the combination therapy of targeting PD-L1 with our PD-L1 antibody-drug conjugate (PD-L1-ADC) and reducing lactic acid with the MCT-1 inhibitor, AZD3965, can effectively treat the PD-1/PD-L1 blockade resistant tumors. Altogether, the findings in this study uncover a new mechanism of how lactic acid induces an immunosuppressive tumor microenvironment and suggest a potential combination treatment strategy to overcome the tumor resistance to PD-1/PD-L1 blockade therapy and improve clinical outcomes.

#4858 NKG2D co-stimulation via anti-sMIC/MIC immunotherapy reprograms intratumoral CD8 T cells toward TCF-1$^+$ stemness to enhance anti-tumor immunity.

Tyler A. Smith[1], Sizhe Liu[1], Ryan J. Brown[2], Weiguo Cui[2], Jennifer D. Wu[1]

[1]Urology, Northwestern University Feinberg School of Medicine, Chicago, IL,[2]Pathology, Northwestern University Feinberg School of Medicine, Chicago, IL

Effective co-stimulation of CD8 T cells is critical for maintenance of TCF-1$^+$ stem-like CD8 T cells necessary for long-term tumor control. NKG2D co-stimulation of CD8 T cells via binding of stress ligands such as membrane-bound MIC enhances CD8 T cell effector functions and is important for promoting memory development. Cancer cells often shed MIC into a soluble form (sMIC) that inhibits CD8 T cell activation. TRAMP prostate tumor mice expressing human MICB (TRAMP/MICB) treated with anti-sMIC/MIC antibody elicited potent anti-tumor responses with re-invigorated CD8 T cell immunity. However, mechanisms of CD8 T cell reprogramming by NKG2D co-stimulation in the tumor microenvironment are poorly understood. In this study, we used scRNA-seq of CD8 TILs from TRAMP/MICB mice treated or untreated with the anti-sMIC/MIC antibody to investigate the hypothesis that anti-sMIC/MIC therapy reprograms CD8 T cells via NKG2D pathway co-stimulation, optimizing TCR-dependent effector and memory responses. Adult male TRAMP/MICB mice were treated with 200µg anti-sMIC/MIC antibody intraperitoneally twice per week for 4-6 weeks. Prostate tumors were collected, and CD45$^+$ cells were flow sorted for scRNA-seq. scRNA-seq analyses were validated using flow cytometry. Tumors from mice treated with anti-sMIC/MIC therapy revealed enhanced functional heterogeneity of CD8 T cell subtypes. In contrast to tumors from untreated mice that were populated with primarily effector and/or exhausted CD8 T cells, tumors from treated mice were enriched in NKG2D$^+$ TCF-1$^+$ stem-like CD8 T cells. This effect of anti-sMIC/MIC therapy was then recapitulated in the syngeneic TRAMP-C2-MICB tumor model. TCF-1$^+$ CD8 T cells expressed higher levels of NKG2D compared to TCF-1$^-$ CD8 T cells, and displayed differential utilization of PI3K/Akt signaling pathways as well as upregulation of epigenetic regulators of CD8 T cell function such as *Kdm6b* and *Setd2*. Antigen-specific CD8 T cells chronically stimulated *ex vivo* with anti-sMIC/MIC antibody in complex with sMICB maintained greater proportions of TCF-1$^+$ stem-like CD8 T cells compared to antigen stimulation alone or in combination with sMICB. The results suggest that NKG2D co-stimulation via anti-MIC/sMIC therapy reprograms CD8 TILs to promote their development of TCF-1$^+$ stem-like function necessary for persistence of the anti-tumor response, and provides proof-of-concept for the translational potential of NKG2D signaling modulation in CD8 T cells to treat cancer.

#4859 TMEM33 loss improves CD8$^+$ T cell fitness and tumor control.

M. T. Jackson[1], T. Zhao[1], I. Pedroza-Pacheco[1], A. Grover[2], D. Gabrilovich[3], D. Withers[1], J. Rehwinkel[1], E. Honore[4], J. C. Christianson[1], E. E. Parkes[1];

[1]University of Oxford, Oxford, United Kingdom, [2]AstraZeneca, Cambridge, United Kingdom, [3]AstraZeneca, Gaithersburg, MD, [4]Institut de Pharmacologie Moleculaire et Cellulaire, Valbonne, France

The Endoplasmic Reticulum (ER) is a critical immunomodulatory hub governing multiple processes relating to anti-tumor immunity. TMEM33 is an ER-resident membrane protein implicated in various ER-associated functions including cholesterol metabolism, calcium oscillations, and proteostasis. We previously reported TMEM33 as a negative modulator of the innate immune signaling protein STING (STimulator of INterferon Genes). Here we identify a central role of TMEM33 in tumor immunology where its deficiency profoundly impacts infiltrating immune populations and T cell phenotype. STING-mediated ISG (interferon-stimulated gene) expression was augmented in *Tmem33*$^{-/-}$ bone marrow-derived macrophages and TMEM33-depleted Flp-In T-REx 293 cells. Strikingly, *Tmem33*$^{-/-}$ mice exhibited significantly attenuated B16F10-OVA tumor growth and enhanced CD8$^+$ T cell tumor infiltration compared to wild-type (WT) controls, while immunosuppressive immune populations including Treg cells, monocytic myeloid-derived suppressor cells and M2-like macrophages were diminished. CD8$^+$ T cell enrichment was also observed in MC38 tumor-bearing *Tmem33*$^{-/-}$ hosts. Unchallenged mice displayed no overt phenotypic or immunological differences compared to WT, however. High dimensional spectral flow cytometry was leveraged to further examine effects of *Tmem33*$^{-/-}$ on T cell compartments in B16F10-OVA tumors and draining lymph nodes (DLNs). Progenitor exhausted (Tpex) TCF-1$^+$PD-1$^+$ tumor infiltrating lymphocytes (TILs) were significantly enriched in *Tmem33*$^{-/-}$ hosts, notable given that Tpex are responsive to checkpoint blockade therapy and associate with improved patient prognosis. Compared to WT counterparts, OVA-specific *Tmem33*$^{-/-}$ TILs exhibited increased cytotoxicity and effector function, expressing elevated granzyme-B and TNF-α. Moreover, EOMES^{lo}T-bet^{hi} TILs were more frequent among TCF-1$^+$PD-1$^+$ and TCF-1$^+$ populations in *Tmem33*$^{-/-}$ hosts further suggestive of a more persistent CD8$^+$ precursor pool, while inhibitory receptor (LAG-3, TIGIT) abundance was blunted, indicating reduced exhaustion. In DLNs of *Tmem33*$^{-/-}$ mice, antigen-specific CD8$^+$ populations demonstrated enhanced effector memory (CD44$^+$CD62L$^-$) phenotypes alongside elevated CXCR3 expression compared to WT, suggesting increased tumor-migratory potential. In summary, here we identify TMEM33 as a novel immunoregulatory factor, the loss of which in hosts majorly reshapes the tumor-immune landscape and ameliorates multiple aspects of CD8$^+$ T cell fitness including stemness, exhaustion, memory, cytotoxicity and migratory capacity. Further analysis of T cell populations from tumor-bearing mice and *ex vivo* analysis will enable mechanistic refinement of the role of TMEM33. Moreover, our study supports the importance of ER-resident proteins in fine-tuning immune cell function, underlining their potential as promising targets for therapeutic intervention.

#4860 Pseudohypoxia induced by iron chelator activates tumor immune response in lung cancer.

Toshiaki Ohara[1], Yusuke Hamada[1], Yuehua Chen[1], Manato Terada[1], Hotaka Kawai[2], Masayoshi Fujisawa[1], Kazuhiro Noma[3], Hiroshi Tazawa[3], Toshiyoshi Fujiwara[3], Akihiro Matsukawa[1]

[1]Pathology and Experimental Medicine, Okayama Univ. Graduate School of Med., Okayama, Japan,[2]Oral Pathology and Medicine, Okayama Univ. Graduate School of Med., Okayama, Japan,[3]Gastroenterological Surgery, Okayama Univ. Graduate School of Med., Okayama, Japan

Hypoxia-inducible factor (HIF) plays a critical role in cellular to adaptation low oxygen levels and has been extensively studied as a biomarker of hypoxia. In cancer cells, HIF upregulation is believed to facilitate survival under hypoxic conditions, contributing to poor prognosis. However, recent studies have demonstrated that HIF signaling is crucial for immune cell function. Oxygen or iron depletion elevates HIF expression by inhibiting HIF-prolyl hydroxylase (HIF-PH) and preventing HIF degradation. Pseudohypoxia, characterized by normoxic HIF upregulation, is facilitated by iron chelators. In this study, we investigated whether pseudohypoxia induced by an iron chelator suppresses tumor growth by activating tumor immune responses, and elucidated the underlying mechanisms. Iron chelators, including Super-polyphenol 10 (SP10) and deferoxamine (DFO), were utilized to induce iron deficiency and pseudohypoxic condition. These compounds reduced the viability of human NSCLC cell lines (A549 and PC-3) but had no significant effect on murine NSCLC cell lines (LLC). Furthermore, SP10 and DFO did not impair the viability of murine CD8+ T cells isolated from normal spleen, while upregulating HIF-1α expression in these cells. Pseudohypoxia by iron chelators stimulated the secretion of IL-2, a cytokine with immunostimulatory properties, from lymphocytes (CD3+ T cells) and NSCLC cell lines (A549, PC-3, and LLC). In contrast, although hypoxic conditions promoted IL-6, a pro-inflammatory cytokine, pseudohypoxia did not induce IL-6 secretion in these cells. Oral administration of SP10 suppressed tumor growth in C57BL/6 mice with implanted tumors, a phenomenon absent in immunodeficient RAG1-deficient C57BL/6 mice. Western blot analysis revealed SP10-induced upregulation of HIF-1α expression within tumors. Immunohistochemistry and flow cytometry demonstrated that SP10 treatment enhanced IL-2 expression in both tumor and spleen tissues. Moreover, SP10 synergistically amplified the efficacy of PD-1 antibody therapy in lung cancer by increasing the infiltration of tumor-infiltrating lymphocytes (TILs). Iron chelation-induced pseudohypoxia directly upregulated HIF-1α, enhanced T cell functionality, and stimulated IL-2 secretion from CD4+, CD8+ T cells, and cancer cells, thereby enhancing the immune response and augmenting the therapeutic efficacy of PD-1 blockade in lung cancer.

#4861 INCA33890 increases CD8⁺ T-cell effector function compared with pembrolizumab as assessed by single-cell RNA sequencing in human PD-1xTGFβR2 knock-in mouse model.

Michelle Kinder[1], Jin Lu[1], Justine Carl[1], Lifeng Zhang[1], Paul Collier[1], Paul Waeltz[1], Rodrigo Hess[1], Natalia Pardo[2], Maria Terradas[2], Liang-Chuan Wang[1], Cynthia Timmers[1]

[1]Incyte Research Institute, Wilmington, DE,[2]Discovery and Translational Science (DTS), Clarivate Plc, Barcelona, Spain

INCA33890, a novel PD-1xTGFβR2 bispecific Biclonics® antibody, is designed to selectively inhibit TGFβR2 on PD-1⁺ cells, leveraging a higher binding affinity for PD-1 over TGFβR2. In preclinical studies using the MC38 colorectal tumor model in human PD-1xTGFβR2 Knock-in mice, INCA33890 had a superior antitumor efficacy compared with pembrolizumab, anti-TGFβR2 or isotype control. This study aimed to uncover the differential mechanism of tumor regression by applying single-cell TCR sequencing (scTCRseq) coupled with single-cell RNA sequencing (scRNAseq) on T cells derived from treated MC38 tumor-bearing mice. Intratumoral T cells were enriched and profiled using 10X Genomics scTCRseq and scRNAseq from MC38 tumor-bearing mice treated with INCA33890, pembrolizumab, anti-TGFβR2, or control antibodies. Results revealed that CD8⁺ T cells in INCA33890-treated mice exhibited a robust increase in RNA transcripts associated with cytotoxicity, proliferation, and T-cell exhaustion compared with pembrolizumab or control antibody treatments, signifying a potent effector response within the tumor microenvironment. Sub-grouping of CD8⁺ T cells into 13 distinct clusters based on marker expression and gene enrichment analysis demonstrated a notable expansion of proliferating, activated, and cytotoxic CD8⁺ T cell populations following INCA33890 treatment—an outcome not observed with pembrolizumab or control treatments. INCA33890 treatment also reduced the proportion of intratumoral-naïve CD8⁺ T cells, transforming their gene expression profile toward an effector-like phenotype. In intratumoral CD4⁺ T cells, treatment with INCA33890 resulted in signatures of enhanced activation, proliferation, and exhaustion compared with pembrolizumab and control treatments, suggesting broad engagement of the adaptive immune response. scTCRseq demonstrated a marked increase in the clonality of intratumoral T cells, further underscoring the impactful immune modulation induced by INCA033890. In summary, bispecific Biclonics® targeting of TGFβR2 and PD-1 with INCA33890 promotes the expansion and activation of effector CD8⁺ T-cell populations within the tumor microenvironment, driving enhanced antitumor activity in vivo.

#4862 Targeting *fli1* improves antitumor response of CD8 T cells by promoting their activation and limiting exhaustion.

Reza Alimohammadi[1], Yongxia Wu[1], Allison Pugel[1], Gustavo Leone[2], Xue-Zhong Yu[1]

[1]Microbiology & Immunology, Medical College of Wisconsin, Milwaukee, WI,[2]Cancer Center, Medical College of Wisconsin, Wauwatosa, WI

Fli-1 (Friend leukemia integration 1) is a transcription factor critical to cell proliferation and differentiation, and its overexpression has been identified in various cancers, including leukemia, Ewing sarcoma, and certain breast and prostate cancers. Emerging evidence links Fli-1 to T cell activation and exhaustion. In our recent study, we demonstrated that Fli-1 plays a key role in regulating the gene transcription for CD8 T-cell activation and their differentiation into effector and memory subsets after allogeneic hematopoietic cell transplantation. We hypothesize that targeting *fli1* in T cells will potentially improve the effectiveness of cellular immunotherapy by enhancing the persistence and function of CD8 T cells. To study how Fli-1 regulates CD8 T cell-mediated antigen-specific response, we utilized OT-I and Pmel TCR transgenic mice in which T cells are specific to ovalbumin (OVA) and melanoma-associated gp-100 antigen, specifically respectively. We generated *fli1*-deficient OT-I and Pmel mice by crossing them with $fli1^{fl/fl}$ $CD4^{cre}$ micestrain. Upon stimulation of OT-I T cells with OVA SIINFEKL peptide, $fli1^{-/-}$ OT-I T cells had increased cytolytic activity against peptide-pulsed tumor targets compared to wild-type (WT) OT-I T cells in *vitro*. Additionally, we observed enhanced cytokine secretion and reduced exhaustion in $fli1^{-/-}$ OT-I T cells after repetitive activation stimulation by OVA peptide *in vitro*. Following adoptive cell therapy *in vivo*, $fli1^{-/-}$ OT-I T cells had significantly improved ability to control $MC\text{-}38^{OVA}$ growth as compared with WT counterpart cells. Deficiency of Fli-1 also enhanced antitumor response of Pmel T cells against B16 melanoma *in vivo*. The $fli1^{-/-}$ Pmel or OT-I CD8 T cells had significantly increased effector activity, persistence, and central memory differentiation in spleen and tumor. ScRNA-seq analysis revealed that *fli1* was mainly expressed in exhausted, but not in progenitor-like subsets of CD8 T cells in tumor. Increased Granzyme B and decreased CTLA-4 gene transcription were observed in $fli1^{-/-}$ OT-I T cells compared to WT cells. To investigate the role of *fli1* in regulating human T cells, we used CRISPR/CAS9 nucleofection-based gene silencing approach to knock down *fli1* in hPBMCs. Preliminary data suggest deficiency that knock-down of *fli1* in human CD19 CAR-T cells, improve their killing ability against Raji cells human B cell lymphoma *in vitro*. In conclusion, Fli-1 plays key roles in regulating the differentiation and anti-tumor function of CD8 T cells and *fli1* silence in T cells may improve cancer immunotherapy.

#4863 IOV-5001, autologous tumor-infiltrating lymphocytes (TIL) armored with inducible membrane-tethered IL-12, shows enhanced antitumor efficacy with an improved cellular state.

Patrick Innamarato[1], Joe Dean[2], Mohammed Alkhouli[2], Marcus Machin[2], Nathan Gilbert[2], Sadie Johnson[2], Gerard Sapena[2], Nino Mtchedlidze[2], Behzad Damirchi[2], Robyn Oldham[2], Andrew Yuhas[2], Judy Fang[2], Jamie Blauvelt[3], Shari Pilon-Thomas[3], Rongsu Qi[2], Hequn Yin[2], Sean Hall[2]

[1]Patrick Innamarato (Individual), Tampa, FL,[2]Iovance Biotherapeutics, Inc., San Carlos, CA,[3]H Lee Moffitt Cancer Center and Research Institute, Tampa, FL

Introduction: Autologous tumor-infiltrating lymphocytes (TIL) engineered to secrete IL-12 have shown robust clinical efficacy in metastatic melanoma. However, systemic toxicity from elevated circulating IL-12 has limited its application. IOV-5001 is a next-generation TIL therapy engineered to inducibly express membrane-tethered IL-12 (TeIL12) upon antigen engagement, restricting IL-12 activity to the tumor microenvironment. This study evaluated the antitumor efficacy and molecular phenotype of IOV-5001.
Methods: TIL were derived from solid tumor tissues, cultured, and transduced with a lentiviral vector encoding inducible TeIL12 bicistronically with a growth factor. Antitumor activity of IOV-5001 was tested in vitro via a tumor antigen-directed TCR killing assay or against autologous patient-derived endometrial cancer organoid (PDO), and/or tumor digests. Transcriptional profiling (bulk and single-cell) was used to characterize the molecular features of IOV-5001.
Results: IOV-5001 TIL were successfully generated and effectively expressed TeIL12 upon T cell receptor (TCR) engagement. The expression of TeIL12, soluble IL-12, and IFN-gamma was lacking in the absence of stimulation, indicating that the activity of IOV-5001 TIL is dependent on TCR engagement. IOV-5001 demonstrated superior killing and effector cytokine production compared with unmodified TIL in a tumor antigen-directed TCR killing assay and against autologous tumor, irrespective of aldesleukin support. The antitumor activity was further enhanced when IOV-5001 was expanded in an optimized media. Analysis of bulk gene expression profiling revealed transcriptional profiles for IOV-5001 characterized by upregulation of genes involved in cell cycle, telomere maintenance, mitochondrial metabolism, IL-12, and TCR signaling. Downregulated genes exhibited an enrichment for IL-10 and IL-5 signaling, and prostanoid/eicosanoid ligand binding receptors. Single-cell analysis confirmed these patterns were enriched within tethered cytokine-positive TIL. Furthermore, single-cell analysis of preREP cells demonstrated that proportions of putative tumor-reactive CD8 T cells were similar in IOV-5001 TIL among tethered cytokine-positive and -negative TIL, indicating unbiased transduction.
Conclusions: These findings demonstrate that IOV-5001 with inducible and membrane-tethered IL-12 confers superior antitumor activity compared with unmodified TIL. The enhanced molecular and functional characteristics highlight IOV-5001 as a promising product for enhancing TIL efficacy in solid tumors.

#4864 The dysregulated YY1-EZH2-RKIP axis in cancer and immune evasion.

Benjamin Bonavida, **Talia Festekdjian**

UCLA - University of California Los Angeles, Los Angeles, CA

Background: The current treatment strategies for cancer have evolved with novel effective approaches, particularly immunotherapy. These have resulted in significant tumor regression and prolongation of survival in a subset of cancer patients. However, there is a subset that is initially unresponsive and a responsive subset that becomes subsequently unresponsive leading to tumor progressions and deaths.
Rationale: To develop a new immune-therapeutic approach to reverse resistance.
Hypothesis: We hypothesized that the unresponsive cancers have in common pivotal gene factors that regulate the resistance and, therefore, are amenable for targeting. These gene factors are the over-expressed Yin Yang1 (YY1) and EZH2 tumor promoters and the under-expressed Raf kinase inhibitor protein
(RKIP) tumor suppressor. Altogether, they constitute the dysregulated YY1-EZH2-RKIP axis in cancers and immune evasion.
Findings: Analyses of the regulation of expressions of these three gene products revealed that they exhibit cross-talk signaling pathways (eg the Raf/MEK/ERK, NF-kB, PI3K/Akt). YY1 and EZH2 activate pathways involved in immune resistance and evasion (e.g., inhibition of T cell effector functions, upregulation of T-cell inhibitory receptors and ligands on cancer cells, infiltration of MDSCs, etc.). In contrast, RKIP reverses YY1 and EZH2 immune inhibitory signaling pathways and enhances the anti-cancer immune response.
Conclusion: The findings revealed that the dysregulated YY1-EZH2 -RKIP axis in cancer is involved in the resistance of cancer to various immuno-therapeutics and induces an immune-suppressive TME and immune evasion. Various approaches are presented targeting each of the three gene products, individually or in combination, to reverse immune resistance and restore the effectiveness of anti-cancer immunotherapy. We suggest that further investigations are necessary to validate the targeting strategies, preclinically and then clinically. However, there remain challenges to develop agents that specifically target the cancer cells in vivo with minimal side effects.

#4865 Inhibition of PD-1 signaling during TCR stimulation promotes increased T cell activation, but upregulates compensatory inhibitory molecules and induces activation induced cell death.

Logan V. Vick[1], Michael K. Sheng[1], Wahed Firoz[1], Craig Collins[1], Lam T. Khuat[2], Bruce Blazar[3], Sean Judge[1], William J. Murphy[1]

[1]UC Davis Comprehensive Cancer Center, Sacramento, CA,[2]University of Southampton, Southampton, United Kingdom,[3]Department of Pediatrics, University of Minnesota, Minneapolis, MN

The PD1/PDL1 axis acts as a potent immune regulator for T cells. Inhibition of the PD1/PDL1 pathway has demonstrated success in the context of checkpoint immunotherapy, yet the biological consequences of the long-term effects of PD1 inhibition on T cells remain unclear. In murine models we have observed that inhibition of the PD1 signaling either through genetic ablation or PD1 checkpoint blockade results in increased activation and function. This has been demonstrated through in vitro stimulation of PD-$1^{-/-}$ T cells in which increased percentages of activation markers including CD25, CD69, and IFNγ were observed. Similar effects of increased activation markers MFI and percent were illustrated in vivo both through adoptive transfer of PD-1 -/- cells into allogeneic NSG recipients and in NSG recipients receiving allogenic wild type cells in combination with anti-PD-1. However, within both in vitro and in vivo models we observed decreases in T cells overtime. We hypothesized that due to increased early activation T cells with inhibited PD-1 signaling underwent increased apoptosis via activation induced cell death (AICD). We observed that apoptosis was increased in PD-$1^{-/-}$ T cells after culture indicated by elevated levels of Annexin V. Further, in vivo following anti-PD-1 treatment we observed increases in other inhibitory markers such as TIM3 and LAG3 via flow cytometry, and confirmed via PCR, suggesting an upregulation of other inhibitory markers following blocking of PD-1. The role PD-1 plays in CAR T cell therapy still requires additional study. Thus, to assess the role of PD-1 and its role in AICD on human cells we next implemented a CAR T xenograft model in which NSG mice were challenged with Raji cells transduced with PD-L1 and then treated with CAR T cells with and without Pembrolizumab. We observed that human CAR T cells transplanted into tumor bearing NSG mice had increased percentages of activation markers(HLA-DR and CD69) when treated with Pembrolizumab, but we also observed elevated flow percentages of inhibitory markers including TIGT, TIM3, and LAG3. These cells in addition to increased activation and upregulation of inhibitory molecules also had decreased numbers overtime and increased apoptosis identified by Annexin V staining. These data suggest a complex interplay in which PD-1 regulates T cell activation as a means of protection, but when this balance is manipulated, T cells can experience increased activation and effector function, followed by an upregulation of inhibitory molecules such as TIM3, LAG3 and TIGIT to compensate, but ultimately still resulting in AICD. Thus, targeting PD-1 unlocks a “short term gain” of activation and function of T cells at the cost of a “long term loss” in T cell numbers due to apoptosis in which cells undergo AICD.

#4867 Enhancing TCR-T with a Fas-based switch receptor boosts T cell engraftment, persistence, and anti-tumor activity in models of hard-to-treat PRAME solid tumor indications.

Laure Twyffels, Alvaro Haroun-Izquierdo, Friederike Knipping, Mikhail Steklov, Panagiota A. Sotiropoulou, **Marleen M. Van Loenen**

T-knife Therapeutics Inc., San Francisco, CA

Background: Adoptive cell therapies have shown promising efficacy in melanoma and synovial sarcoma, but success in other tumor types remains limited. A significant challenge is the hostile tumor microenvironment (TME) that restricts T cell infiltration, function and persistence. Here, we developed a switch receptor (SwR) tailored to PRAME positive solid tumors.

Methods: Analysis of TIGIT, Fas and PD1 ligand expression was performed using the Tempus^{TM} bulk RNA database and publicly available single-cell RNA sequencing (scRNAseq) data. T cells were retrovirally transduced to express different SwRs in combination with an HLA-A*0201 restricted PRAME-specific TCR and a CD8 co-receptor, and tested for proliferation, viability, cytotoxicity and cytokine secretion in models reflecting the TME of PRAME positive tumors.

Results: Analysis of the ligands of the common inhibitory receptors PD1, Fas, and TIGIT in (1) 4,622 stage III/IV patient samples across 10 PRAME-expressing indications, (2) scRNAseq of 7,000 - 176,000 cells covering 98 patients over 5 tumors, and (3) healthy tissue scRNAseq data, demonstrated high expression of TIGIT ligands in tumors and healthy tissue, raising potential safety concerns. PD-L1 and FasL had moderate expression across all target indications, with FasL having the most promising expression profile. Expression in tumors is generally stable across tumor sites and lines of treatment and increased upon CPI treatment. In healthy tissue, FasL is expressed mainly in the lymph nodes (LNs), enabling a Fas-SwR to support TCR-T engraftment and persistence during trafficking. Eight SwR candidates were designed combining the extracellular domain of Fas receptor with different intracellular activating motifs. The SwR combining CD40 and OX40 motifs (Fas-TNFR) was selected based on high cell surface expression and superior protection against FasL-induced apoptosis. When testing in models reflecting the TME of PRAME positive indications, PRAME TCR-T co-expressing Fas-TNFR showed improved T cell fitness as demonstrated by earlier memory phenotype, higher cytokine production and long-term cytotoxic capacity. Notably, Fas-TNFR significantly outperformed a Fas-41BB SwR currently in the clinic. To mimic trafficking through the draining LNs before tumor homing, PRAME TCR-T cells expressing Fas-TNFR were pre-stimulated with FasL. This led to improved T cell fitness and a trend for increased cytotoxic activity upon encountering cancer cells.

Conclusions: Extensive characterization of the TME of PRAME-expressing solid tumors guided the development of a best-in-class switch receptor. Fas-TNFR increased fitness of PRAME TCR-T cells before encountering cancer cells, and boosted long-term persistence and anti-tumor activity, indicating the potential of deeper clinical responses at lower doses.

#4868 Optimal activation of TCR T cells in solid tumors through addition of best-in-class single chain CD8 coreceptors results in CD4 engagement and improved T cell fitness and persistence.

Paul Najm, Alvaro Haroun-Izquierdo, Mikhail Steklov, Panagiota A. Sotiropoulou, Marleen M. van Loenen

T-knife Therapeutics Inc., San Francisco, CA

Background: Clinical response rates in solid tumors have been improved and/or broadened by including either CD8α or wild type CD8αβ co-receptors (CoR) in T cell receptor (TCR) T cell therapy constructs, enabling engagement of CD4 T cells. We designed a small, chimeric, single-chain CD8 CoR maintaining all functional elements of the CD8αβ CoR and used it as a scaffold to add intracellular costimulatory signaling motifs (co-stimCD8 CoR). By providing activating signals simultaneously to TCR engagement, co-stimCD8 CoR prevents TCR-T cell exhaustion caused by the lack of costimulatory molecule expression in tumors. TCR-T cells expressing co-stimCD8 CoR fully engaged CD4 T cells and mediated enhanced and durable TCR-T anti-tumor responses, superior to commonly used wild type CD8 CoRs.
Methods: The ActiviT platform consists of different single-chain CD8 CoRs designed by combining building blocks from CD8αβ and CD4 CoRs. In silico 3D modeling using the Rosetta software suite was used to model interactions between TCR-CD8CoR with HLA-I-peptide complexes. HLA-A*02:01-restricted PRAME or MAGE-A1 targeting TCR-T cells with or without different CD8 CoRs were tested for cytokine production (LEGENDplex) and long-term and serial cytotoxicity (Incucyte). Flow cytometry was used to analyze phenotype, activation and exhaustion markers.
Results: Structural evaluation by in silico 3D modeling showed that the single-chain CD8 CoR scaffold retains HLA-I binding interface identical to that of the wild type CD8 CoR. The co-stimCD8 CoR was generated by adding tumor necrosis factor receptor associated factor (TRAF) binding motifs. Assessment of cytotoxicity and cytokine secretion showed that co-stimCD8 CoR conferred higher functionality to CD4 T cells incorporating an HLA class-I restricted TCR compared to a wild type CD8 CoR. To evaluate the impact of co-stimCD8 CoR on the anti-tumor activity of TCR T therapies, functional comparison of CD4/CD8 PRAME TCR-T cells co-expressing different CD8 CoRs, including versions currently tested in the clinic, was performed. PRAME TCR-T cells expressing co-stimCD8 CoR improved T cell fitness, resulting in a less exhausted and earlier memory phenotype and enhanced functionality in serial killing experiments at low effector-to-target ratios. Notably, enhanced activity of co-stimCD8 CoR was demonstrated in 3D tumor spheroid models recapitulating hard-to-treat indications.
Conclusions: Co-stimCD8 CoR is a chimeric CD8 CoR with built-in co-stimulation. Combination of co-stimCD8 CoR with a PRAME TCR resulted in improved T cell fitness and higher functional activity compared to common PRAME TCR-T approaches, including current clinical development candidates. Inclusion of the co-stimCD8 CoR in TCR-T constructs has the potential to further deepen and broaden responses of hard-to-treat solid tumors.

#4869 Chimeric costimulatory receptor targeting ICAM1 augments functional efficacy of antitumor T cell.

Yanping Yang[1], Dessislava Stefanova[2], Yogindra Vedvyas[1], Diella S. Babu[1], Yago Alcaina[2], Maria Cristina Riascos[2], Janusz Puc[3], Kevin Chen[2], Juan Gonzalez[2], Jose Thaiparambil[1], Thomas J. Fahey[2], Rasa Zarnegar[2], Moonsoo M. Jin[1], **Irene M. Min**[1]

[1]Houston Methodist Research Institute, Houston, TX,[2]Weill Cornell Medicine, New York, NY,[3]AffyImmune Therapeutics, Inc., Natick, MA

Background: Adoptive T cell therapy has evolved with innovations such as chimeric antigen receptor (CAR)- and engineered T cell receptor (TCR)-T cell therapies that target tumor surface antigens or tumor-associated antigens, respectively. Although remarkable antitumor response has been demonstrated in engineered T cell therapy, tumor recurrence still occurs partly due to tumor antigen escape. Low affinity of endogenous TCR for tumor antigens has been an obstacle to the success of TCR-therapy. We hypothesized that tumor-specific T cell function in an immunosuppressive tumor microenvironment could be enhanced by an expression of ICAM1-specific chimeric costimulatory receptor (ICCR). ICAM1 is overexpressed in many solid tumor cell membranes and is greatly induced by T cell cytokine IFNγ, which may enable reinforcement of specificity and efficacy of those TCRs engaged with the target tumor antigens.
Methods: We genetically engineered primary T cells with an ICCR, comprised of a single chain fragment variable sequence fused to a costimulatory molecule CD137. We first tested an ICCR in combination with CTL16, an HLA-A2-restricted, MAGE-C2-specific TCR, in cocultures with A375-MA2 melanoma cells. We measured levels of cytotoxicity and cytokine secretion when the antigen density is low or high. Next, we genetically modified allogeneic and autologous T cells with an ICCR and tested their functional efficacy in xenograft models of anaplastic thyroid cancer. Finally, TCR sequencing, signaling reporter assay, and gene expression analysis were performed to gain insights into the ICCR activity.
Results: Jurkat T cell line expressing both ICCR and CTL16 showed elevated NFκB and NFAT activities following TCR engagement when compared to cells expressing CTL16 alone. Cytotoxicity induced by the ICCR/CTL16 primary T cells were specific to target tumor cells, and functional efficacy was augmented even when antigen presentation is at a subthreshold level. The ICCR expression increased allogeneic TCR activity, leading to improved control of anaplastic thyroid cancer growth. When autologous peripheral T cells were genetically modified with the ICCR, $PD1^+$ T cells showed a superior response in controlling the growth of anaplastic thyroid cancer patient-derived tumor. ICCR expression promoted selective clonal expansion, enabling isolation of tumor-specific TCRs.
Conclusion: ICCR improves functional efficacy of the TCR activated at a subthreshold level, facilitating a robust activation of polyclonal tumor-specific T cells that may have been restricted in an immunosuppressive tumor microenvironment.

IMMUNOLOGY: Modulation of Tumor Microenvironment: Modulation of Lymphocyte Influx
Poster Session

#4873 Characteristics of TIM-3 in regulating T cell activity in the brain tumor microenvironment.

mingi song[1], Su Bin Won[2], Hyung-Seok Kim[2], Dae Yeon Kim[1], Ki Sun Kim[1], Su Jeong Gu[2], Chanju Lee[2], Hee Jung Yoon[2], Eun Jung Park[2]

[1]Department of Cancer Biomedical Science, Graduate School of Cancer Science and Policy, National Cancer Center, Ilsan, Korea, Republic of,[2]Immuno-oncology Branch, National Cancer Center, Ilsan, Korea, Republic of

T-cell immunoglobulin and mucin domain-containing molecule 3 (TIM-3), a member of TIM family, has been shown to exhibit diverse characteristics in a context-dependent manner. In this study, we investigated the characteristics of TIM-3 on T cells in the brain tumor microenvironment. First, using an intracranial syngeneic mouse model, we examined the expression of TIM-3 in the brain tumor microenvironment. Immunohistochemistry (IHC) revealed that TIM-3 as well as PD-1 was expressed on various cell types, including CD3-positive cells in brain sections of tumor-bearing mice. Similar expression patterns were observed in tumor-infiltrating lymphocytes (TILs) by using flow cytometry analysis. To investigate the role of TIM-3 in T cells within the brain tumor microenvironment, we utilized T-lymphocyte specific TIM-3 conditional knock-out (TIM-$3^{fl/fl}$ Lck-cre, TIM-3 cKO). TIM-3 cKO mice exhibit a higher $CD8^+/CD4^+$ TIL ratio in the ipsilateral brain regions compared to tumor-bearing wild-type (WT) mice after GL26 cell injection into the right hemisphere. Moreover, *in-vivo* experiments with TILs demonstrated the influence of TIM-3 on T cell functional activity within the brain tumor microenvironment. Collectively, these results suggest that TIM-3 of T cells have an influence on the immune microenvironment of brain tumors.

#4874 Anti-CCR8 mediates long-lasting antitumor immunological memory and enhances anti-tumor immunity in immune-cold cancer through the combination with chemotherapy.

Shuiqing Hu[1], Minjuan Deng[2], Wei Tan[1], Rui Liu[1], Amin Zhang[1], Lu Tan[1], Mengqian Chen[1], Pu Sun[1], Li Luo[1], Wen Li[1], Juan Yang[1], Jiyuan Zhang[1], Wei Jin[2], Zhirong Shen[2]

[1]BeiGene (Shanghai) Biotechnology Co., Ltd., Shanghai, China,[2]BeiGene (Beijing) Co., Ltd., Beijing, China

Tumor infiltrating regulatory T cells (Tregs) suppress CD8+ T cells and facilitate tumor progression. Tumor Tregs are elevated in a variety of malignancies and have been associated with poor prognosis. Targeting Tregs presents an appealing strategy in cancer immunotherapy. Recent research has identified the chemokine receptor CCR8 is preferentially expressed by tumor Tregs and labels highly suppressive Tregs. Thus, using anti-CCR8 mAb to deplete tumor Tregs could be an efficient and safe strategy that is widely being explored. Although the anti-tumor efficacy of CCR8 mAb was reported, the dynamics and specific contributions of Tregs and CD8+ T cells to anti-tumor activity are still not fully elucidated. We found that tumor Tregs were rapidly depleted within two days, and CD8+ T cells increased after one week following treatment with anti-CCR8 in both the MC38 and CT26 tumor models, which coincided with the observed tumor growth inhibition. Tumor volume was found to have a strong negative correlation with the percentage of tumor-infiltrating CD8+ T cells, suggesting that the increased tumor CD8+ T cells may be indicative of an active anti-tumor immune response. Furthermore, treatment with CCR8 mAb has shown dose-dependent efficacy in controlling the growth of CT26 tumor. In the 1 mg/kg dosage group, 3 out of 10 mice exhibited a complete response (CR), while in the 3 mg/kg dosage group, 4 out of 10 mice achieved a CR. Rechallenging the mice with 50-fold more tumor cells on the contralateral side of the CR mice resulted in the expulsion of tumor growth without any treatment, suggesting that the use of CCR8 mAb induces a long-lasting antitumor immunological memory. This is crucial for maintaining persistent tumor immune surveillance and achieving durable responses to cancer therapy. This finding underscores the potential of CCR8 mAb not only in directly targeting tumor growth but also in bolstering the host's immune memory, contributing to a more comprehensive and enduring antitumor defense. In addition, further improved tumor growth inhibition was observed when combining anti-CCR8 with docetaxel, oxaliplatin, or gemcitabine in immune cold tumor models. PD analysis revealed that the combination of CCR8 mAb therapy with gemcitabine induced the depletion of Tregs, increase of CD8+ T cells, decrease of tumor-associated macrophages, and the activation of DCs, which are instrumental in treating immune-cold tumors. This suggests that the combined therapy may enhance the antitumor immune response by modulating the tumor microenvironment, thereby improving the efficacy of cancer treatment. Collectively, these findings advance our understanding of CCR8 as a promising target for Treg depletion in anti-cancer drug development and therapy.

#4875 B7-H3 (CD276) regulates the migration and infiltration of tumor-associated macrophages.

A-Ram Kang[1], Da Hyun Yun[1], Eun Jung Lee[1], Jong Dae Ji[2], **Serkin Park**[1]

[1]Biochemistry and Molecular Biology, Korea University College of Medicine, Seoul, Korea, Republic of,[2]Internal Medicine, Korea University College of Medicine, Seoul, Korea, Republic of

B7-H3 (CD276), a member of the B7 family of immune checkpoint proteins, is expressed on tumor cells as well as various immune cells within the tumor microenvironment (TME), including macrophages. While evidence from numerous in vitro, in vivo, and ongoing clinical studies highlights its potential as a promising therapeutic target, the function and molecular mechanisms of B7-H3 in distinct stromal cell populations within the TME remain unclear. In this study, we investigated the roles of B7-H3 in tumor-associated macrophages (TAMs) using B7-H3 knockout mice, macrophage-tumor co-culture spheroids, and in vitro experiments. B7-H3 expression was significantly upregulated during the progressive stages of bone marrow-derived macrophage (BMDM) differentiation in vitro, supporting its functional role in macrophages. For in vivo studies, we generated B7-H3 knockout mice. Immunohistochemical staining and flow cytometric analysis of B16F10 melanoma tumor tissues subcutaneously implanted in B7-H3 knockout mice revealed a significant reduction in $F4/80^+$ TAMs compared to tumors in wild-type mice. Similarly, anti-B7-H3 antibody treatment in 4T1 breast cancer-bearing mice reduced $F4/80^+$ TAMs compared to control IgG treatment. However, tumor growth and the number of $PCNA^+$ proliferating tumor cells were unaffected by either knockout or antibody-dependent inhibition of B7-H3 in vivo. Subsequent in vitro studies demonstrated that migration of BMDMs from B7-H3 knockout mice was impaired in both two-dimensional scratch-wound migration assays and a three-dimensional macrophage-tumor co-culture spheroid model. Furthermore, interferon-gamma treatment significantly enhanced the migration of BMDMs from wild-type mice, an effect that was completely blocked by a pharmacologic STAT1 inhibitor (fludarabine). Interestingly, BMDMs from B7-H3 knockout mice exhibited a significantly attenuated response to both interferon-gamma and STAT1 inhibition, indicating that B7-H3-dependent macrophage migration is mediated through the interferon-gamma and STAT1 signaling pathway. Collectively, our data indicate that B7-H3 regulates the migration and infiltration of TAMs into tumor tissues via the interferon-gamma/STAT1 signaling pathway. These findings suggest that B7-H3 is a promising therapeutic target for solid tumors such as melanoma and breast cancer.

#4876 Avidity engineered multispecific antibodies that are highly efficacious in immune cell stimulation in patient tissue and in mice: significantly improved tumor control and turning cold tumors hot.

Sophie Poznanski[1], Erik Slinger[1], Jarek Juraszek[1], Fatemeh Vahedi[2], Marcelo Pereira[3], Loreto Parga-Vidal[1], Dean Lee[3], Ali Ashkar[2], **Robert Friesen**[1]

[1]Avidicure, Oegstgeest, Netherlands,[2]McMaster University, Hamilton, ON, Canada,[3]Nationwide Children's Hospital, Columbus, OH

Introduction: Antibodies and ADCs are mainstays in the treatment of cancer. However, given difficulties in achieving a deep and sustained response, significant improvements are desirable. We report on first in class “Booster” molecules, based on clinically validated ADCC-competent antibodies, equipped with two immunomodulatory domains that are affinity engineered to be functional only when in contact with a tumor cell. We see strong expansion and increased cytotoxicity of immune cells in the presence of cancer cells *in vitro*, activation of relevant immune cell types *ex vivo*, and reduction of tumor burden *in vivo*, with significantly better activity than adoptive cell therapy or control antibody without fusion domains.

Methods: NRG mice were engrafted with luciferase expressing SKOV3 cells via intraperitoneal injection 7 days prior to treatment. Mice received 1 million NK cells isolated from healthy donors. Compounds were administered biweekly, and low dose IL2 thrice weekly. Blood was collected weekly, and tumor burden monitored weekly *via* bioluminescence. *ex vivo*: *in situ* activation of tumor infiltrating immune populations was evaluated by nanostring in freshly isolated tumor tissue. *in vitro*: cytotoxicity was measured by quantifying the number of alive tumor cells using automated microscopy. Expansion was performed by stimulating NK cells weekly with tumor cells that were opsonized with Booster or antibody, NK cells were counted weekly to determine expansion.

Results: Mice treated with a HER2 targeting Booster demonstrated superior tumor control than trastuzumab treated mice, with near tumor remission by day 35. High NK counts were observed in the blood of Booster treated mice, and no NK cells were detected in that of trastuzumab treated mice. In the peritoneal cavity, NK counts were up to 600x higher in Booster treated mice than in trastuzumab treated mice. Our Booster reprograms the immune microenvironment *ex vivo* in freshly isolated tumor tissue, transforming a cold tumor into a hot tumor. It activates multiple cytotoxic and IFN-γ pathways, stimulates CD8+ T cell activation, downregulates pro-tumor pathways in Tregs and induces a phenotypic shift in macrophages from the immunosuppressive M2 to the pro-inflammatory M1 phenotype. In separate *in vitro* assays we saw sustained expansion and enhanced cytotoxicity of NK cells for at least 6 weeks. Cells stimulated with HER2 Booster showed prolonged tumor control, whereas trastuzumab stimulated cells failed to sustain tumor control beyond 21 days.

Conclusion: In correlation with extensive *in vitro* and *ex vivo* data, we observe a prolonged and significant improvement in tumor control in mice treated with Boosters compared to mice treated with trastuzumab. Work is ongoing to develop these molecules, with the first clinical trial expected to start in 2026.

#4878 Ablation of neutrophil-specific CXCR2 and PD-L1 alters neutrophil phenotype and can reduce disease burden in a murine model of advanced colorectal cancer liver metastases.

Alistair S. McLaren, John Falconer, Sarah Ressel, Leo M. Carlin, Owen J. Sansom, Colin W. Steele

CRUK Scotland Institute, Glasgow, United Kingdom

Introduction: We have previously shown that as colorectal liver metastases (CRCLM) develop, there is a pressure that drives phenotypic development of neutrophils within the metastatic niche. Through transcriptional and protein analysis, different neutrophil transcriptional states can be delineated. Combination therapy with CXCR2 and PD-1/PD-L1 checkpoint inhibition has been shown to be a strategy to reduce disease burden in mouse models of hepatocellular carcinoma. Through loss of neutrophil-specific target proteins CXCR2 and PD-L1 in mouse models of CRCLM, we show modification of neutrophil phenotype and changes in disease burden.

Methods: *Kras*$^{G12D/+}$*, Trp53*$^{fl/fl}$*, Rosa*$^{26N1CD/+}$ (KPN) tumour cells modelling CMS4 pMMR colorectal cancer generated from murine CRCLM organoids were injected orthotopically into the spleen of male and female C57BL/6 mice with loss of neutrophil-specific proteins using the Cre-loxP system. Mrp8CreCxcr2$^{fl/fl}$ and Mrp8CrePd-l1$^{fl/fl}$ genetically engineered mouse models with neutrophil-specific loss of CXCR2 and PD-L1 respectively were sampled at 14 days and 35 days, and neutrophils isolated using MACS prior to bulk sequencing to assess the impact on neutrophil transcriptional phenotype. Neutrophil protein expression, phenotype and functional role was assessed using flow cytometry and ELISA.

Results: Loss of neutrophil-specific CXCR2 does not alter CRCLM burden at a late timepoint following intrasplenic injection but leads to an altered neutrophil phenotype with reduced PD-L1 checkpoint inhibitor expression, and increased Lactoferrin expression, a neutrophil granule protein with known anti-cancer properties. These neutrophils are strongly suppressive of T cell proliferation when co-cultured *ex vivo*. Loss of neutrophil-specific PD-L1 leads to a reduction in CRCLM tumour size but not number, suggesting an important role for neutrophil PD-L1 in growth of CRCLM without affecting tumour seeding. *Ex vivo* assays will examine the effect of loss of neutrophil-specific CXCR2 on NETosis and ROS generation, and the effect of lactoferrin upon these neutrophil functions in neutrophils with and without CXCR2 expression.

Conclusions: We speculate that inhibition of CXCR2 and PD-L1 and loss of these neutrophil proteins alters neutrophil phenotype to be more “anti-tumour”. This may be a strategy to alter the immunosuppressive microenvironment of CRCLM and allow for downstaging of CRCLM allowing for resection and preventing recurrence.

#4879 Unveiling a key obstacle to effective antitumor immunity in T-cell-restricted tumors.

D.-C. Chiu[1], X. Zhang[1], B.-L. Cheng[1], K. Hayashi[1], Q. Liu[1], B. Yu[2], R. Lee[1], C. Zhang[1], J. Rajadas[1], N. Reticker-Flynn[1], E. Rankin[1], E. Engleman[1];

[1]Stanford University School of Medicine, Palo Alto, CA, [2]ImmunEdge Inc., Mountain View, CA

Endogenous antitumor immune responses in cancer patients can lead to the formation of "inflamed" tumors, characterized by a significant infiltration of tumor-reactive T cells. However, the majority of patients fail to mount such robust immune responses, resulting in immune-evasive tumors. To explore the mechanisms underlying this failure, we established and compared spontaneous mouse models of hepatocellular carcinoma (HCC) with either an inflamed, T-cell-rich or non-inflamed, T-cell-deprived tumor microenvironment (TME). Intriguingly, non-inflamed tumor models exhibited splenomegaly and elevated plasma erythropoietin (EPO) levels despite the absence of anemia, and these effects were independent of tumor size. This finding raised the possibility that tumor-derived EPO might dictate tumor immunotype. Our investigations revealed that EPO ablation in non-inflamed HCC models led to a substantial increase in effector memory CD8 T-cell infiltration, reduced tumor burden, and even induced tumor regression. Conversely, enforced EPO expression in inflamed HCC models suppressed T-cell infiltration and promoted tumor progression. Further analysis of both human and mouse HCC tissues demonstrated that the erythropoietin receptor (EPOR) is predominantly expressed on tumor-associated macrophages (TAMs). Tumor-derived EPO interacts with EPOR on TAMs to autonomously establish a non-inflamed, immunosuppressive TME, effectively evading T-cell surveillance. Mechanistically, EPO-EPOR signaling reprograms TAMs toward an immunoregulatory phenotype via NRF2-mediated heme depletion. Disruption of this axis—through either tumor-derived EPO ablation or EPOR deletion on TAMs—transformed the TME into an inflamed, T-cell-rich TME, driving tumor regression regardless of tumor genotype. Notably, this immune reactivation synergized with immune checkpoint blockade therapy. In summary, the EPO/EPOR axis functions as a critical immunosuppressive switch, enabling tumors to escape immune surveillance. Its inactivation reprograms macrophages to foster antitumor immunity, offering a promising strategy to enhance the efficacy of immunotherapy in HCC and potentially other cancers.

#4880 A novel heparan-sulfate (HS) oligosaccharide on tumor-blood vessels is a target for anti-immune vascular therapy in renal cell carcinoma (RCC).

J.-S. Chung, N. Pawar, P. D. Cruz, Jr., H. Hammers, K. Ariizumi;
UT Southwestern Medical Center, Dallas, TX

In ccRCC, tumor cells are arranged in nests surrounded by capillary endothelial cells (EC) abundant in HS polysaccharide proteoglycan receptors (HSPGs). HS structure is highly heterogeneous, due to HS sulfotransferases (HSST) that randomly sulfate at different positions of the core disaccharides. Specific sulfation pattern determines the avidity of HS to soluble factors, which regulates the interaction between EC and leukocytes. We identified 6-*O*-highly sulfated HS 8-mer saccharides (6O-HS) to be the ligand of GPNMB receptor that inhibits T cell activation and induces angiogenesis. Using our 6O-HS-specific 1A7 mAb, we examined the expression of 1A7 epitope in RCC. IHC staining of RCC specimens (n=4) showed 1A7 mAb stained some but not all EC and nearly no staining in tumor cells. We examined this EC selectivity by identifying 6O-HS+ HSPGs using mouse SVEC line expressing HSPGs (syndecans (SD), CD44). Surprisingly FACS analysis showed very little 1A7 expression on these cells. To ask whether this is due to low expression of HS6ST1 (for 6-*O*-sulfation), HS6ST1-transfected SVEC cells were stained with 1A7 and HSPG Ab and found all HSPGs positive for 1A7. To examine whether binding of GPNMB to 1A7+ HSPG induces specific signaling, SD4/HS6ST1-transfected 293T cells were treated with GPNMB-Fc and measured Tyr-phosphorylation (p-Tyr) on SD4 protein. Western blotting showed GPNMB-Fc induced p-Tyr on SD4 in 10 min post-incubation and the signaling was completely blocked by pretreatment of transfectants with 1A7 mAb. We assayed the effects of 1A7 mAb on T cells and EC. For T cells, we assayed *in vitro* suppressor function of GPNMB+ myeloid-derived suppressor cells (MDSC from RCC patients, n=3) to autologous T cells. In coculture of T cells/MDSC, 1A7 mAb inhibited IFN-γ T cell response by 50-84% (p>0.01) and 40-60% T cells were 1A7+. For EC, we used *in vivo* angiogenesis assays: 1A7 mAb was injected into GPNMB-Fc-matrigel plugs in mice and 2 dpf zebrafish embryos. This mAb (n=5, 10 μg/ml) reversed GPNMB's angiogenic activity completely in mice and inhibited growth of the subintestinal vessels (SIV) (n=9, 50% reduction in SIV # by 2 μg/ml, p<0.001). To evaluate the efficacy of 1A7 mAb to treat RCC, BALB/c mice bearing RenCa tumor were treated with 1A7 or PD1 mAb; 200 μg *i.p.*/mouse, 2x/wk. 1A7 mAb inhibited tumor growth as greatly as PD1 Ab (64% inhibition by 1A7 vs. 61% by PD1 Ab, p<0.01, n=6) with a reduction in tumor density of microvessels and myeloid cells (50-60% reduction) and a rise in T cells (~5-fold). Similarly, 1A7 mAb inhibited human RCC growth in Caki-2-derived xenograft NSG mice injected with human PBMC (49% inhibition by 1A7 vs. 37% by Nivolumab, p=0.005). 6O-HS+ renal EC may regulate T cell activation/ trafficking into RCC and facilitates tumor angiogenesis via the high avidity to signaling factors. Thus, 6O-HS-targeting may be a promising anti-angiogenic immunotherapy for RCC.

#4881 A PD1-IFNα fusion protein, with an attenuated IFNα fused to a clinically validated PD1 mAb, elicited PD1-dependent IFNα signaling and superior anti-tumor efficacy.

Jinyang Li, Yi Wang, Mengqiang Di, Min Wu, Xueying Feng, Yiyi Li, Weiwei Wu, **Xiguang Zhang**, Shuaixiang Zhou, Huizhong Xiong

Innovent Biologics (Suzhou) Co. Ltd, Jiangsu, China

Immune checkpoint blockade (ICB) therapies blocking PD1/PDL1 signaling have shown unprecedented clinical efficacy in multiple indications. However, the overall response rate for ICB-sensitive tumors are only 10-50%. Moreover, many types of cancer, e.g. pancreatic, ovarian, and MSS colorectal cancer, remain highly refractory to ICB therapies. Thus, it remains a significant unmet medical need to improve the responsiveness and durability of current ICB therapies. Based on emerging clinical evidence, it is possible to address this challenge by combining PD1 or PDL1 mAbs with drugs targeting complementary mechanisms. Indeed, next-generation ICB therapies, e.g. PD1 x IL2 fusion protein, PD1 x CTLA4 BsAb, and PD1 x VEGF BsAb, have shown promising clinical signals and improved efficacy compared to PD1 mAb. This underlines the potential of incorporating two clinically validated anti-cancer mechanism-of-action into one drug. IFNα has shown clinical efficacy and potential synergistic anti-cancer effect with PD1 mAb. Indeed, soluble IFNα has been approved to treat cancer. Similar to the development of other cytokine-based therapies, it is necessary and challenging to overcome off-tumor activity of IFNα to achieve efficacy-safety balance, limit systemic toxicity, and improve pharmacokinetics profile and drug exposure. Here, we engineered a PD1-IFNα fusion protein composed of a functionally attenuated IFNα and a clinically validated PD1 mAb, Sintilimab, aiming to specifically stimulate PD1-positive immune cells. This PD1-IFNα fusion protein utilized bivalent PD1 binders and induced potent PD1/PDL1 blockade. To minimize systemic toxicity induced by IFNα and improve developability, we engineered human IFNα-2b to decrease the affinity between IFNα and IFNα receptors. The PD1-IFNα fusion protein elicited highly PD1-dependent IFNα signaling, selectively activated in PD1-high cells compared to PD1-low cells. Systemic administration of a mouse surrogate molecule showed superior anti-tumor efficacy compared to PD1 mAb, with undetectable toxicity, in several mouse syngeneic tumor models. This PD1-IFNα fusion protein showed favorable pharmacokinetics profile in mice and drug developability. Together, our pre-clinical data demonstrated that a bifunctional PD1-IFNα fusion protein elicits robust PD1/PDL1 signaling blockade and PD1-dependent IFNα signaling activation to achieve anti-tumor efficacy, with potential to expand the benefits of ICB therapies to more cancer patients.

#4882 LCB39, a novel systemically deliverable STING agonist with strong preclinical activity and tolerability.

Jihye Oh, Hyun Joo Bae, Ye Seul Jang, Sung Min Kim, Ho Young Song, **Sook Kyung Chang**, Chul-Woong Chung

LigaChem Biosciences Inc., Daejeon, Korea, Republic of

Direct STING activation with STING agonists is very effective in tumor control through Type I IFN responses in preclinical animal models. However, STING agonist therapeutics have faced major hurdles in clinical success due to limited bioavailability and suboptimal efficacy within tolerable doses. Here, we introduced LCB39, a novel non-CDN STING agonist small molecule currently at the preclinical development. LCB39 was intentionally generated toward the characteristics of lower cell permeability while maintaining its direct STING binding potency to improve systemic tolerability. Compared to diABZi compounds, such as GSK#3 or XMT-1616, LCB39 had about 100-fold lower THP-1 reporter activity with about 5-fold lower direct binding potency. As expected, LCB39 dramatically improved normal cell cytotoxicity to hPBMCs and HSCs. In addition, LCB39 did not induce stromal cell activation nor cell death. In contrast to the in vitro potency, LCB39 strongly promoted tumor growth suppression in a dose-dependent manner in several syngeneic mouse models. Mechanistically, LCB39 had a tumor-targeting and retention activity and promoted immune priming of tumor tissue including the activation of myeloid and lymphoid cells and induction of anti-tumor memory CD8 T cells in mice. In summary, systemically delivered LCB39 targeted tumor tissue and promoted robust anti-tumor activity with high tolerability in preclinical studies. LCB39 is a highly differentiated potent STING agonist with a safety profile, which suggested that LCB39 may have the potential to be the best-in-class STING agonist to treat solid cancers. LCB39 plans to enter first-in-human clinical study in early 2026.

#4884 Reversing immune desertification for effective cancer therapy.

Alessandra Araujo[1], Max Rodnick-Smith[2], Ranya Al-Khaledy[1], Mark Badeaux[1], **Everett Stone**[2]

[1]Chemical Engineering, The University of Texas at Austin, Austin, TX,[2]Molecular Biosciences, The University of Texas at Austin, Austin, TX

Patients with tumors that have a “cold, or “immune desert” phenotype typically have poor outcomes on immune checkpoint therapy (ICT). Aberrant sphingosine-1-phosphate (S1P) signaling in tumors and in their draining lymph nodes (TDLN) appears to causes a common form of immune desertification by disabling or stalling lymphocyte egress from the TDLN, ultimately restricting immune trafficking to tumors. Clinically, tumor expression levels of the S1P producing enzyme sphingosine kinase 1 (SPHK1) strongly and negatively correlate with survival of patients after immune checkpoint inhibitor (ICI) therapy) and pre-clinically correlate with reduced tumor infiltrating lymphocytes (TILs). While there are five known S1P receptors (S1PR1-5), the expression of S1PR1 is required for lymphocyte egress from lymphatic organs. Abnormal S1P signaling or S1PR1 agonists functionally downregulate S1PR1 levels, trapping lymphocytes in the lymph node and is the mechanism of action of certain immunosuppressants (e.g., fingolimod) used to treat autoimmune diseases such as multiple sclerosis. In preliminary studies we have demonstrated in murine cancer models treated with pharmacologically optimized S1P degrading enzymes (SGPL1), that depleting extracellular S1P dramatically increases the trafficking of lymphocytes out of TDLNs, increases tumor T cell infiltration and strongly or completely inhibits tumor growth when administered as a single agent. Data from our lab suggests that deeply lowering extracellular S1P without eliminating internal stores of S1P needed for lymphatic egress is a powerful modality for increasing the level of S1PR1 on lymphocytes, releasing tumor specific T cells from TDLNs, and ultimately increasing tumor immune infiltration. Essentially the exact opposite mechanism as observed with immunosuppressants such as fingolimod. Current small molecule inhibitors of S1P production can only lower extracellular S1P levels by ~ 50% via decreasing intracellular pools and only show limited anti-cancer activity. A cold tumor microenvironment is a common phenomenon that limits ICT efficacy and therapeutics that could enhance lymphocyte trafficking to the tumor would represent a significant advance for improving patient outcomes.

#4885 Targeting heterogeneity to improve non-small cell lung cancer treatments.

Avery Childress[1], Xiulong Song[1], Dave-Preston I. Esoe[1], Christian M. Gosser[1], Harrison Yang[1], Kassandra J. Naughton[1], Daniel R. Plaugher[1], Zhonglin Hao[2], Christine F. Brainson[3]

[1]Department of Toxicology and Cancer Biology, University of Kentucky, Lexington, KY,[2]Department of Internal Medicine, Markey Cancer Center, University of Kentucky, Lexington, KY,[3]Department of Toxicology and Cancer Biology, Markey Cancer Center, University of Kentucky, Lexington, KY

Non-small cell lung cancer (NSCLC) is currently the leading cause of cancer related death in the United States. Despite improvements in treatment, success is extremely varied due to tumor heterogeneity. Our laboratory hypothesis that targeting heterogeneity could improve treatment responses for NSCLCs. Our first method to accomplish this is to inhibit the histone methyltransferase EZH2, which tri-methylates histone 3 lysine 27 to silence genes. Using a syngeneic lung cancer model, we observed that the EZH2 inhibitor valemetostat combined with anti-PD1 immunotherapy drives tumor regression, increased activated T cells and increased antigen expression on the tumor cells. This switch to an MHC class II high state makes the tumors susceptible to immunotherapy-mediated cell killing. To closely study tumor-immune cell interactions ex vivo, our lab has optimized 3D tumoroid model that recapitulates the liquid air interface seen within the lung and immune cells. With this model, we have observed that bone marrow from valemetostat-treated mice led to a depletion of tumoroids, and an increase in CD8+ T cells. In addition to EZH2 inhibition that is currently in clinical trial, we are also exploring methionine restriction (MR) as an alternative way to alter heterogeneity. Methionine is a pre-cursor to S-adenosyl methionine, the methyl donor for EZH2. Our previous studies showed that dietary MR improved carboplatin response and slowed tumor progression in an aggressive KRAS/*Lkb1* mouse model1. Given some controversy around MR and immunotherapy, we next performed a syngeneic graft assay with the mixed lineage KRAS/*Lkb1* tumor cells. We observed that MR coupled with carboplatin and or anti-PD1 lead to improved response compared to control arms. We have also created “multi-culture” experiments that mirror the dietary intervention approach by reducing methionine within the media and treating with chemotherapy + anti-PD1. We observed that tumoroid growth in low methionine was stalled, particularly for KRAS/*Lkb1* tumoroids co-treated with chemo + anti-PD1. We also show that with the low methionine media there were significantly more T-cells present. Together these studies show that modulating EZH2 or methionine levels in NSCLC are effective ways to increase treatment efficacy. We also demonstrate use of cutting edge “multi-culture” experiments that can successfully model in vivo environments to better predict cancer response to treatment. 1 doi: https://doi.org/10.1101/2024.06.25.599795. This work was funded by: Markey STRONG Scholars Program through the American Cancer Society IRG-22-152-34 (HY), T32 CA165990 (DRP), R01 CA237643 (CFB), R01 HL170193 (CFB), P30 CA177558 (Markey Shared Resources)

#4887 TIM-3 signaling modulates brain tumor growth in an orthotopic mouse model.

Su Bin Won[1], Hyung-Seok Kim[1], Dae Yeon Kim[2], Ki Sun Kim[2], Su Jeong Gu[1], Chanju Lee[1], Hee Jung Yoon[1], Eun Jung Park[1]

[1]Immuno-oncology Branch, National Cancer Center, Ilsan, Korea, Republic of,[2]Cancer Biomedical Science, Graduate School of Cancer Science and Policy, National Cancer Center, Ilsan, Korea, Republic of

T-cell immunoglobulin and mucin domain-containing molecule 3 (TIM-3) is a transmembrane protein originally identified as a T helper type 1 (Th1)-specific protein. While there is growing interest in the roles of diseases, including tumors, the properties of TIM-3 in brain tumors remain unexplored. In this study, we investigated the functional properties of TIM-3 in the brain tumor microenvironment using TIM-3 mutant mice with intracellular signaling defects (*Tim-3^{mut}*). To determine whether defects in TIM-3 signaling could affect brain tumor, we examined tumor growth using an intracranial mouse brain tumor model with glioma. EGFP-GL26 glioma cells were injected into the right hemisphere of mice with *Tim-3^{mut}* mice and WT mice, and tumor growth was monitored using MRI. Interestingly, TIM-3 singling defects lead to reduced glioma growth and improved survival in the orthotopic mouse glioma model. Next, we performed experiments to investigate how TIM-3 signaling defects influence brain tumor growth and identified alterations in several signaling molecules, including CBP35, a lectin family member in *Tim-3^{mut}* mice with GL26 tumor cells. Collectively, Our findings suggest that modulating TIM-3 signaling affects the brain tumor microenvironment, thereby influencing tumor growth and survival.

POPULATION SCIENCES: Advancing Cancer Health Equity Through Screening, Diagnostics, Genetics, and Social Determinants Poster Session

#4890 Cancer health awareness through screening and education, CHANGE: evidence of improved screening rates at 6 months post-test.

Marlo M. Vernon, **Mina Tsai**, Alma Carrillo, Ara Idun, Martha Tingen

Augusta University, Augusta, GA

Purpose: Incidence and mortality rates for breast, colorectal, and prostate cancers all exceed national rates in Georgia, and are higher among African Americans. The goal of the CHANGE initiative is to provide a sustainable model of evidence-based cancer awareness through education - with an emphasis on prevention and early detection behaviors. Thus, we examine whether participation of CHANGE initiative was related to referral follow-up rates for cancer screening and greater screening uptake for breast, colorectal, and prostate cancers.
Methos: Residents of five Augusta Housing Authority communities participated. At each site, four education sessions were provided by trained facilitators on cancer risk factors and etiology, racial disparities, eligibility for cancer screening, and participation in clinical trials. Attendance, eligibility for breast, prostate and colorectal screening were measured at baseline and 1- and 6-months post-CHANGE sessions.
Results: There were 110 residents (47.5% 65 and older years old, 45% male, 62.5% High School education, 90% AA) participated in this initiative. Around 95% completed post-intervention evaluation. Participants (n=67) were eligible for a cancer screening; breast (n=21), prostate (n=14), and colorectal screening (n=30) based on American Cancer Society guidelines. CHANGE participants who have attended 75% or above sessions had significantly higher referral rates (93.6%) and subsequent follow-up (receipt of care) (90% vs. 10% those who have attended < 75% sessions) at 6 months post-sessions. Those with higher attendance also had higher follow up with breast cancer (84.6%), colorectal, (100%), prostate cancer (100%), screening uptake when compared those without attending 75% or above sessions, respectively. Majority of those who completed follow up screening were aged 65+ years, were female, were Black, had high school or lower education, and had an insurance (either public or private).
Conclusions: Findings from our study demonstrated the participation of CHANGE initiative was related to greater referral rates and follow-up screening uptake for breast, prostate, and colorectal cancers. CHANGE participants demonstrated improved access and receipt of care/resources by attending CHANGE sessions. Participants reported being motivated and confident in seeking preventive care and satisfaction with community engagement efforts. Our study suggests that replication of this initiative in similar communities may improve knowledge for cancer risk and health equity simultaneously among underserved populations.

#4891 Genome-first approach identifies shelterin complex gene variants and associated cancers.

Akanksha Nagarkar, Hasset Nurelegne, Jung Kim, Kelvin C. de Andrade, Sharon A. Savage

NCI Div. of Cancer Epidemiology & Genetics, Rockville, MD

Telomeres, nucleotide repeats and a six-protein complex called shelterin at chromosome ends, are critical for genomic integrity. Rare heterozygous pathogenic or likely pathogenic (P/LP) germline variants in shelterin complex genes have been associated with long telomeres and risk of certain cancers, including melanoma, lymphoproliferative diseases, glioma, papillary thyroid cancer, and sarcoma. Traditional phenotype-first studies of P/LP germline variants and disease are often limited by ascertainment of the most severely affected individuals. Genome-first approaches can reduce this bias by first assessing germline variants and then evaluating the associated diseases. Since most studies, to date, have focused on variants in *POT1* and cancer, we sought to estimate associations between cancer and rare P/LP variants in the five less well studied shelterin genes (*ACD*, *TERF1*, *TERF2*, *TERF2IP*, and *TINF2)* in the UK Biobank (UKB) to understand their role in cancer etiology at the population level. Exome sequencing data on 469,578 participants were retrieved from the UKB DNA Nexus platform. Variants with minor allele frequency <1%, exonic or splice site, genotype quality >30, depth >20, and allele fraction >0.3 were retained. Variant classification used Automated Germline Variant Pathogenicity (PMID: 38426335). Logistic regression analyses adjusted for age, sex, alcohol use, smoking, and BMI were used to calculate odds ratios (OR) and 95% confidence intervals (CI). There were 177 participants (97 female, 80 male, median age = 57.0 years, range = 40.0 - 69.0) with 59 unique P/LP germline variants (30 *ACD*, 9 *TERF2*, and 20 *TINF2*). Strikingly, there were no rare variants in *TERF1* or *TERF2IP.* The population prevalence of individuals with P/LP germline variants in these five shelterin genes is estimated at 1:2,514 (174/437,486 unrelated individuals, 95% CI 2,189-2,953). Forty-eight individuals with P/LP germline variants were diagnosed with at least one cancer with a median age at first cancer of 59.3 years (range 19.4 - 80.6). Overall, P/LP variants were significantly associated with melanoma (OR 2.93, 95% CI 1.20-7.15, p = 0.018) and cancers of the urinary system (OR 2.78, 1.02-7.57, p = 0.045). By gene, P/LP variants were associated with cancers of the oral cavity/pharynx (*ACD*, OR 4.78, 95% CI 1.18-19.4, p = 0.030), soft tissue including heart (*ACD*, OR 8.90, 95% CI 1.24-63.9, p = 0.030), colon excluding the rectum (*TERF2*, OR 8.44 , 95%CI 1.05-68.0, p= 0.045), female genital system (*TERF2*, OR 12.98, 95% CI 1.43-117.8, p = 0.023), and melanoma (*TINF2*, OR 7.63, 2.35-24.75, p = 0.001).Rare P/LP germline variants in *ACD*, *TERF2*, and *TINF2* may be associated with increased risk of certain cancers in the UKB. Analyses applying the genome-first approach other population-based cohorts and assessment of telomere length are underway to better understand the extent to which variation in shelterin genes contributes to cancer etiology.

#4892 Tools for screening for social determinants of health in head and neck cancer: a scoping review.

Rebecca A. Zasloff[1], Alexandra Hunter[1], Ayush Khanna[2], Huda Haque[2], Elizabeth R. Blackwood[3], Nosayaba Osazuwa-Peters[4]

[1]Duke University School of Medicine, Durham, NC,[2]Duke University, Durham, NC,[3]Duke University Medical Center Library, Duke University School of Medicine, Durham, NC,[4]Department of Head and Neck Surgery and Communication Sciences, Duke University School of Medicine, Durham, NC

Background: Racial/ethnic disparities in head and neck cancer (HNC) outcomes are well documented. HNC has the third worst Black vs White five-year survival gap of all cancers. Despite these disparities in mortality, access to care remains an issue for minoritized individuals and lead to decreased cancer health care utilization. In recent years, there has been a growing recognition of social determinants of health (SDoH) as contributors to barriers to care and drivers of health disparities for cancer patients. Among patients with HNC, SDoH have been linked to later-stage presentation and treatment delays leading to adverse health outcomes for patients with increased social needs. Despite calls by the National Cancer Institute to address SDoH in advancing equitable cancer care delivery, there is limited research on the SDoH screening tools in the context of HNC care. This study aims to report on screening tools to assess SDoH among HNC patients.
Methods: We conducted a systematic literature search of Medline, Embase, and Web of Science databases on July 24, 2024. The included studies were US-based, written in English, and examined a validated tool to assess SDoH among HNC patients. Tools were categorized by the five primary SDoH domains as defined by the Healthy People 2030 Initiative: Economic Stability, Education Access and Quality, Social and Community Context, Health Care Access and Quality, and Neighborhood and Built Environment. Subdomains within each primary domain were used to further categorize the included tools.
Results: After screening 272 articles, 5 studies that evaluated 4 unique SDoH screening tools were included in the analysis. The number of questions in any given SDoH assessment tool ranged from 2 to 33 (mean: 16). Economic Stability (n = 3, 75%) and Health Care Access and Quality (n =2, 50%) were the only 2 SDoH domains assessed by the tools. Most tools evaluated one domain (n =3, 75%). Poverty (n = 3, 75%) and Access to Health Services (n = 2, 50%) were the most commonly assessed SDoH sub-domains. Only one study proposed a specific intervention to address SDoH.
Conclusion: This review found that most SDoH screening tools in HNC care focus on assessing Economic Stability, but do not capture several other Healthy People 2030 domains. Ultimately, the limited number of validated SDoH tools fail to assess the wide-reaching spectrum of HNC patient experiences, making it difficult to comprehensively address the social needs of HNC patients. In addition to the development of tools to screen other SDoH domains, future studies are needed to integrate adequate public health interventions in order to address social need particularly among racial/ethnic minority and marginalized populations.

#4893 Who falls through the cracks?: Characteristics of women successfully and unsuccessfully navigated to breast cancer screening from the emergency department.

Sara Heinert[1], John Hemphill[2], Gabrielle Gracias[3], Maya Iglesias[3], Jessica Kim[4], Hrithika Ravuri[5], Antonia Sames[5], Alexandra Sobocinski[5], Ana Natale-Pereira[6], Erin Muckey[7], Pamela Ohman-Strickland[8], Vince Silenzio[9], Jonathan McCoy[1], Amanda Esposito[1], Ethan Halm[2], Mary O'Dowd[2], Humaira Chaudhry[10]

[1]Department of Emergency Medicine, Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ,[2]Office of Population Health, Rutgers Health, New Brunswick, NJ,[3]Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ,[4]Rutgers University, New Brunswick, NJ,[5]Rutgers New Jersey Medical School, Newark, NJ,[6]Division of General Internal Medicine, Rutgers New Jersey Medical School, Newark, NJ,[7]Department of Emergency Medicine, Rutgers New Jersey Medical School, Newark, NJ,[8]Department of Biostatistics and Epidemiology, Rutgers School of Public Health, Piscataway, NJ,[9]Department of Urban-Global Public Health, Rutgers School of Public Health, Piscataway, NJ,[10]Department of Radiology, Rutgers New Jersey Medical School, Newark, NJ

Screening mammography has contributed to decreased breast cancer mortality, but screening disparities exist among racial/ethnic minority populations. Emergency departments (EDs) serve in a safety net capacity for underserved patients and are a novel place for cancer screening education and navigation. We implemented an intervention at two EDs in New Jersey where students recruited patients overdue for breast cancer screening and connected them to patient navigators. This analysis describes participants who were reached by a navigator to make a mammogram appointment versus those not reached, as well as women who attended their appointment versus not. Of 415 ED patients eligible for breast cancer screening (women 40-74 years old), 152 (37%) were eligible for the study (last mammogram >1 year ago), of which 101 agreed to participate. When contacted, 14 (14%) participants were reached and made a mammogram appointment. Women reached were more likely to: be in their 40s (71% vs. 49%), Hispanic (77% vs. 47%), Spanish-speaking (64% vs. 38%), not have private insurance (33% vs. 49%), and have Medicaid (22% vs. 10%) than women not reached. Nine women (9% total, 64% of those reached) attended a mammogram appointment. Women who attended their appointment had similar characteristics as women reached but were more likely to have a primary care provider (PCP) (78% vs 64%) and Medicaid (33% vs 22%) compared to all women who made an appointment. (See table.)Follow-up care is vital to an ED cancer screening intervention. Surprisingly, English speaking and patients with private insurance were less likely to be reached for a mammogram appointment. Patients without a PCP were less likely to attend their appointment. Adapting interventions to target women who “fall through the cracks” is imperative to improving breast cancer equity. More work is needed to strengthen the follow-up process for breast cancer screening for ED patients.

Characteristics of Study Participants by Follow-Up Status					
	%	Attended Appointment (n=9)	Made Appointment (n=14)	Was Not Reached (n=87)	All (n=101)
Age	Mean (SD)	48.7 (7.4)	47.8 (6.2)	51.1 (8.1)	50.6 (8.0)
	40-49 years	67	71	49	53
	50-59 years	22	21	31	29
	60-69 years	11	7	19	17
	70-74 years	0	0	1	1
Race/ Ethnicity	Asian	0	0	6	5
	Black	22	23	30	29
	Hispanic	78	77	47	51
	White	0	0	10	8
	Other	0	0	7	6
Language	English	33	36	60	56
	Spanish	67	64	38	42
	Other	0	0	2	2
Insurance Type	Private	33	33	49	47
	Medicaid	33	22	10	12
	Medicare	0	0	8	7
	No Insurance	33	33	27	28
	Other	0	11	6	7
PCP (Y/N)	Yes	78	64	52	54
Highest Level of Education	Less than HS degree	38	42	38	38
	HS degree or GED	38	33	40	39
	Associate’s degree	13	8	5	5
	Bachelor’s degree	13	17	10	11
	Master’s degree	0	0	7	6

#4894 Cervical cancer screening disparities among the deaf and hard-of-hearing women.

Roxana Soto-Abreu, Francisco J. Munoz-Torres, Vivian Colon-Lopez

Cancer Control and Population Sciences Division, University of Puerto Rico Comprehensive Cancer Center, San Juan, Puerto Rico

Background: Recent studies have highlighted persistent disparities in cancer screening among deaf populations[1-4]. Deaf women show lower adherence rates for cervical and breast cancer screenings compared to hearing women, with significant disparities remaining for cervical cancer screening after adjusting for correlates[1,5]. Barriers to cancer screening for deaf individuals include ineffective patient-physician communication and a lack of culturally and linguistically aligned resources[6].
Objective: Examine the association of up-to-date cervical cancer screening in deaf and hard-of-hearing women and investigate if disparities in cancer screening among this population persist.
Methods: A secondary analysis of the 2022 Behavioral Risk Factor Surveillance System (BRFSS) data set was conducted. Logistic regression was used to evaluate up-to-date cervical cancer screening, by disability status and covariates (age, race, education, income, regular provider, health insurance, and urban residency). Up-to-date cervical cancer screening was defined using the recommendation from the US Preventive Services Task Force guidelines and considered females ages 21 to 65 years who received a Papanicolaou (Pap) test in the previous 3 years to be up to date with screening[7]. The main independent variable (deafness and hard-of-hearing) was self-reported using the Guide to Disability Questions from the Centers for Disease Control and Prevention[8].
Results: A total of 13,937 females between 21 years and 65 years old reported to have a cervical cancer screening. From this sample, 3.3 % (n=455) of respondents identified as deaf and hard-of-hearing. The likelihood of being up-to-date in cervical cancer screening was lower in deaf women (AOR: 0.82; $p<0.05$) when compared to hearing women. Non-Hispanic black women have lower odds of being up-to-date with cervical cancer screening (AOR: 0.84; $p<0.05$) when compared to non-Hispanic white women. Uninsured women showed lower odds (AOR: 0.73; $p<0.001$) for being up-to-date on cervical cancer screening, compared with women with health insurance coverage. Women with an annual household income of less than $25,000 had an adjusted odds of 0.62 ($p<0.001$) being up-to-date on cervical cancer screening, compared with women with an annual household income of $75,000 or more. Additionally, those women who reported not having a personal healthcare provider had half the odds of not being up-to-date on cervical cancer screening when compared to women having 2 or more health providers (AOR: 0.50; $p<0.001$).
Conclusion: Findings from this study support that disparities among this population of deaf and hard-of-hearing women persist regarding adherence to cervical cancer screening. Social determinants of health (economic stability, healthcare access, and quality) play an important role in access to cancer care prevention, especially in people with disabilities.

#4897 Self-reported experiences of discrimination and inflammatory-related biomarkers among Black breast cancer survivors.

Nur Zeinomar[1], Marley Perlstein[1], Bo Qin[1], Hari S. Iyer[1], Jesse J. Plascak[2], Tengteng Wang[1], Kitaw Demissie[3], Christine B. Ambrosone[4], Elisa V. Bandera[1], Chi-Chen Hong[4]

[1]Rutgers Cancer Institute, New Brunswick, NJ,[2]The Ohio State University College of Medicine, Columbus, OH,[3]SUNY Downstate School of Public Health, Brooklyn, NY,[4]Roswell Park Comprehensive Cancer Center, Buffalo, NY

Purpose: Racial discrimination disproportionately affects Black individuals and is associated with adverse mental and physical health outcomes. Black individuals also exhibit higher levels of systemic inflammation compared to other racial and ethnic groups, which may contribute to disparities in cancer outcomes and comorbid conditions. Despite this, the relationship between racial discrimination and inflammatory biomarkers in cancer survivors remains understudied. This study examines the association between experiences of discrimination and inflammatory biomarker levels in a cohort of Black breast cancer survivors.
Methods: We analyzed data from the Women's Circle of Health Follow-up Study, a population-based longitudinal cohort of Black breast cancer survivors in New Jersey. Self-reported lifetime racial discrimination was assessed during baseline home visits (approximately 12 months post-diagnosis) using the validated "Experiences of Discrimination (EOD)" instrument, which captures experiences of interpersonal and institutional discrimination across nine domains, including work, education, health care, and public settings. Inflammatory biomarkers, including high sensitivity C-reactive protein, Interleukin-6 (IL-6), Tumor Necrosis Factor (TNF)-α, and IL-1β, were assayed in blood samples collected 24-36 months post-diagnosis. Multivariable linear
regression models were used to evaluate the associations between EOD (ever vs. none) and biomarker concentrations, adjusting for potential confounders.
Results: Among the 156 women included in this analysis, 74% reported experiencing any discrimination. Self-reported EOD was not significantly associated with inflammatory biomarker levels. However, women reporting discrimination had non-significant elevations in TNFα and IL-1β compared to those reporting no EOD. In age-adjusted models, the mean for TNFα among women reporting any EOD was 5.94 pg/mL (95% CI: 5.50, 6.42) compared to 5.57 pg/mL (95% CI: 4.91, 6.32) among those reporting no EOD. Similarly, the age-adjusted mean value for IL-1β was 1.37 pg/mL (95% CI: 1.21, 1.56) for women reporting any EOD versus 1.28 pg/mL (95% CI: 1.03, 1.58) for those reporting none. Fully adjusted models accounting for age at diagnosis, sociodemographic factors (marital status, income), lifestyle factors (smoking, alcohol use, physical activity, body mass index), and chemotherapy yielded comparable results.
Conclusion: While we did not observe statistically significant associations, our findings suggest a potential link between EOD and elevated levels of inflammatory biomarkers in Black breast cancer survivors, pointing to a possible biological impact of discrimination-related stress. Future studies with greater statistical power are needed to better understand these relationships and their potential relevance to cancer prognosis and survivorship.

#4898 Factors influencing implementation of a breast cancer screening intervention for women with food insecurity in a community setting: An application of the Implementation Research Logic Model.

Charlotte L. Kerber, Julie H. T. Dang, Daniel J. Pasillas-Pablo, Jocelyn T. Canty, Laura Fejerman

UC Davis, Davis, CA

Among individuals and communities with low-income, the experience of food insecurity is associated with greater delays in recommended mammography screening and late-stage breast cancer at diagnosis. Community-based interventions that address barriers to promoting breast cancer screening among diverse communities of women with food insecurity in real-world safety-net settings have been limited. In partnership with Sacramento Food Bank and Family Services (SFBFS), the largest non-profit provider of social services in Sacramento County, this study aimed to (1) identify factors influencing implementation of a breast cancer screening intervention in a food distribution setting and (2) identify mechanisms through which factors impact implementation outcomes. As part of a larger participatory-based mixed methods project with SFBFS, we conducted semi-structured interviews (n=10 staff; n=10 clients) and collected observations from direct food distribution events during the pre-implementation phase. The Consolidated Framework for Implementation Research (CFIR) guided the rapid iterative content analysis to identify barriers and facilitators of implementation. Then as part of the Implementation Research Logic Model (IRLM), we developed a matrix using CFIR, Expert Recommendations for Implementing Change (ERIC), and the Behavior Change Wheel (BCW) to identify mechanisms linked to these factors and to assess implementation outcomes of acceptability, appropriateness, and feasibility. Overall, there was consensus that promoting breast cancer screening in the food distribution setting was beneficial and welcomed by all stakeholders. Key determinants related to the general theme of sustainability were identified including financing, the availability of resources, and the process of engaging with clients while they are at the food distribution event. Characteristics of individuals, specifically the clients' capability and motivation to receive a breast cancer screening intervention in this context, must also be considered. An active and sustained partnership with food bank leaders is crucial to facilitate these outcomes. Intervention format should include tailored messaging that is culturally appropriate and empowers individuals to obtain recommended screening. Content of the intervention should provide interactive assistance that facilitates local support for screening eligibility and services. This IRLM can be adapted for future breast cancer screening interventions in novel community-based food distribution settings.

#4899 Colorectal cancer screening among deaf and hard of hearing individuals in Puerto Rico.

Francisco Munoz-Torres, Roxana Soto-Abreu, Vivian Colon-Lopez

University of Puerto Rico Comprehensive Cancer Center, San Juan, Puerto Rico

Background: Colorectal cancer (CRC) screening rates among Deaf and Hard of Hearing (DHH) individuals are often lower than those of the hearing population. This disparity is driven by factors including communication barriers, limited access to healthcare services, and insufficient support systems. As a result, DHH individuals face unique challenges in accessing timely screening, leading to delayed diagnoses and poorer cancer outcomes.
Methods: We analyzed data from the 2022 Behavioral Risk Factor Surveillance System (BRFSS) to examine colorectal cancer (CRC) screening rates among Deaf and Hard of Hearing (DHH) individuals. The sample included 3,496 individuals aged 40 years and older, residing in Puerto Rico. Prevalence ratios (PR) for non-endoscopic colorectal cancer screening methods were calculated. Subgroup analyses were performed to assess the impact of transportation access, social and emotional support, and the ability to manage daily errands on CRC screening rates.
Results: Compared to hearing individuals, DHH individuals had an 11% lower prevalence of colorectal cancer (CRC) screening (PR = 0.89, 95% CI: 0.89, 0.90). After adjusting the model for age, sex, education, income, access to personal healthcare providers, and health insurance status, the prevalence ratio (PR) decreased further to 0.84 (95% CI: 0.83, 0.84).In subgroup analyses, the PR remained stable for individuals with reliable transportation. However, it decreased by 13% for those facing transportation challenges (PR = 0.78, 95% CI: 0.77, 0.79). The PR dropped by 15% for individuals without social and emotional support (PR = 0.76, 95% CI: 0.74, 0.77) and by 11% for those struggling with daily errands on their own (PR = 0.80, 95% CI: 0.79, 0.80).
Discussion: This study reveals a disparity in CRC screening rates between DHH individuals and the hearing population, driven by factors such as limited transportation, lack of social support, and difficulties managing errands. The lack of culturally and linguistically appropriate materials, including PRSL, worsens these barriers. Training healthcare providers to improve communication with DHH individuals is essential for increasing screening.
Conclusion: Addressing these barriers requires targeted research and interventions, focusing on accessible educational resources (including PRSL translations), improving healthcare provider training, and enhancing screening access. Future research should examine how social factors, such as socioeconomic status and geography, affect CRC screening access in DHH populations. These strategies are key to reducing disparities and improving health outcomes for DHH individuals in Puerto Rico and beyond.

#4900 Barriers and facilitators influencing the implementation of a mHealth (MeMoSA) oral cancer screening program in rural communities in a low- and middle-income country: Preliminary findings of a qualitative study.

Senthilmani Rajendran[1], Nur Dinie Junaidi[1], Davinna Satguna Rajah[1], Aliya Nabil[1], Nurshaline Kipli[2], Thaddius H. Maling[3], Roslina Mah[2], Ignatius Niap[2], Shirley Hui Hui Hu[2], Suzanne Scott[4], Rafdzah Ahmad Zaki[5], Sok Ching Cheong[1]

[1]Cancer Research Malaysia, Subang Jaya, Malaysia,[2]Ministry of Health, Malaysia, Kuching, Malaysia,[3]Ministry of Health, Malaysia, Kota Kinabalu, Malaysia,[4]Queen Mary University of London, London, United Kingdom,[5]Univeristi Malaya, Kuala Lumpur, Malaysia

Introduction: Sixty-five percent of oral cancer cases in Malaysia are detected at late stages due to poor awareness and difficulties in accessing healthcare in remote communities. The early detection of oral cancer (OC) could be overcome through teleconsultation using mHealth applications that enable remote review of clinical data and oral lesion images. We determined the feasibility of healthcare volunteers (HCVs) using a mHealth application MeMoSA for oral cancer screening in rural communities in Malaysia by identifying the barriers and facilitators of implementation.

Methods: This qualitative study was part of a larger mixed-methods single-arm feasibility study. HCVs were trained to capture oral images of villagers using MeMoSA. Dentists at the nearest public clinic reviewed images remotely to give a referral decision. Suspicious high-risk oral lesions were also reviewed by specialists via MeMoSA and where warranted, make a referral directly to the nearest tertiary center for biopsy. To identify implementation barriers and facilitators, in-depth interviews were conducted with HCVs in the Malay language, which were recorded and then transcribed and translated verbatim into English. The Consolidated Framework for Implementation Research (CFIR), was used to guide interviews, coding and data analysis.

Results: Forty-seven HCVs screened 1865 individuals in 12 rural villages with 93% uptake. Thirty-one HCVs were interviewed. Main themes related to the innovation and implementation process domains of CFIR. Barriers encountered by the HCVs included limited telecommunications infrastructure for internet access and community perceptions. For instance, the community's poor oral symptom knowledge, fear of knowing and lack of motivation to engage in preventive behaviors that influenced uptake of screening and adherence to follow-up appointments. HCVs overcame issues with internet access by traveling to areas with an internet connection to transmit information for teleconsultation. Facilitators included the avoidance of travelling long distances and incurring high transportation costs to be checked at the clinic, and the simplicity and user-friendly design of MeMoSA which motivated HCVs to conduct screening. In addition, HCVs' relationship and trust towards village leaders was a major facilitator for a well-planned implementation.

Conclusion: This novel approach to oral cancer screening has good reach, with motivated HCVs and could be feasible in low- and middle-income countries. However, strategies to improve barriers such as adherence to follow-up appointments are required and facilitators should be leveraged for sustained implementation.

#4901 Investigating whether TXNRD2 is a therapeutic target against triple negative breast cancers using African American-derived models.

Keacha Chang, Brenna Flowers, Abigail Rullo, Irida Kastrati

Loyola University Chicago, Chicago, IL

African American women are twice more likely to be diagnosed with triple negative breast cancer (TNBC) compared to Caucasian women; they are diagnosed with TNBC at a younger age and exhibit a more aggressive disease. TNBC is a heterogenous subtype of breast cancer that does not benefit from targeted therapies thus prognosis remains poor. The increased TNBC incidence and its poor prognosis exacerbate the disparate mortality experienced by African American women diagnosed with breast cancer. Thioredoxin reductases (TXNRDs) are key antioxidant enzymes within the thioredoxin pathway. Most tumors, including TNBCs, express higher levels of antioxidants to cope with higher redox stress resulting from the tumor's deregulated metabolism and mitogenic stress. This presents a therapeutic vulnerability and TXNRD1, the cytoplasmic isoform, is proposed as a cancer therapeutic target. TXNRD2 is the mitochondrial isoform; its contribution to cancer progression is understudied and unclear. Our investigation into breast cancer patient databases (Clinical Proteomic Tumor Analysis Consortium, CPTAC) demonstrated that African American patients have elevated levels of TXNRD2. Based on these observations, our goal is to (i) investigate whether TXNRD2 is a therapeutic target against TNBC using African American-derived cell models, and (ii) determine whether a new class of TXNRD inhibitors in development by us, TXNRD(i)s, can be effective drugs against aggressive TNBC. If successful, our work will set the stage for developing new drugs that are highly needed for treating TNBC in the clinic and may reduce the racial disparity gap in breast cancer. A panel of African American derived TNBC cell lines were characterized by western blot for expression levels of TXNRD1 and 2. HCC1806 cells, which had elevated levels of TXNRD2, were transfected with siTXNRD2 or control and cell growth was monitored over 5 days. Silencing of TXNRD2 significantly attenuated cell growth. Treatment of these cells with TXNRD(i)s inhibited cell viability *in vitro* and *in vivo* using a xenograft model. To determine to what extent, the inhibition of TXNRD2 vs TXNRD1 by TXNRD(i)s contributes to the anti-cancer activity we are optimizing a fluorescent probe and live cell imaging by microscopy. HCC1806 cells were incubated with TRFS-Green which fluoresces upon reduction by TXNRDs and MitoTracker-Red which stains for mitochondria where TXNRD2 is located. Colocalization between TRFS-Green and MitoTracker-Red indicates TXNRD2 specific activity. Novel TXNRD(i)s are being catalogue for TXNRD specific inhibition. Together our data support the development of TXNRD2 inhibitors to treat African American women with TNBC.

#4903 Short chain per- and polyfluoroalkyl substances associate with elevated alanine aminotransferase: cross-sectional analysis results from the STRIVE cohort.

Bojung Seo[1], Hongxu Wang[1], Rachel Maguire[2], Yukun Liu[2], Beatrice Smith[2], Kristin Haller[3], David Conti[1], Heinz-Josef Lenz[4], Antonio Planchart[2], Giorgio Roccaro[3], Lesley S. Miller[3], Andrew Muir[5], Susan K. Murphy[6], Andrew Moon[7], Melissa Troester[8], Cynthia A. Moylan[5], Joellen Schildkraut[3], Cathrine Hoyo[2], Jesse A. Goodrich[1]

[1]Department of Population and Public Health Sciences, Keck School of Medicine, University of Southern California, Los Angeles, CA,[2]Department of Biological Sciences, North Carolina State University, Raleigh, NC,[3]Department of Medicine, Emory University School of Medicine, Atlanta, GA,[4]Division of Medical Oncology, Keck School of Medicine, University of Southern California, Los Angeles, CA,[5]Division of Gastroenterology, Duke University Health System, Durham, NC,[6]Department of Obstetrics and Gynecology, Duke University Medical Center, Durham, NC,[7]Division of Gastroenterology and Hepatology, University of North Carolina, Chapel Hill, NC,[8]Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC

Background: Although long chain, high molecular weight per- and polyfluoroalkyl substances (PFAS) (≥8 and ≥6 carbons for carboxylic and sulfonic acids; molecular weight >400 g/mol) have been associated with elevated ALT, the association of short chain, low molecular weight PFAS with elevated ALT or cirrhosis, a risk factor for hepatocellular carcinoma, remains unclear.
Objectives: To examine associations of short or long chain PFAS mixtures with elevated ALT or cirrhosis and baseline factors related to PFAS.
Methods: We included 349 adults aged 40-75 years from the Southern Liver Health Study (STRIVE), an ongoing longitudinal observational study. At enrollment, 135 out of 196 participants had a confirmed diagnosis of cirrhosis. We used logistic regression to examine associations between individual PFAS serum levels and elevated ALT or cirrhosis, at baseline. Quantile g-computation was used to test the associations of short or long chain PFAS mixtures with the outcomes. Linear regression examined baseline factors associated with PFAS.
Results: Increasing all short chain PFAS by one quartile was associated with higher odds of elevated ALT (odds ratio: 2.20 [1.32-3.67]); no association was observed with the long chain PFAS mixture. Perfluoroheptanoate, a short-chain PFAS, was associated with both elevated ALT and cirrhosis (odds ratios: 1.53 [1.01-2.33]; 1.51 [1.01-2.27], respectively) at unadjusted *P*. Long chain PFAS were higher in males and linked to higher reported levels of alcohol consumption. Both long and short chain PFAS were lower in bottled water users, non-water drinkers, and current smokers compared. Perfluoropentanoic acid, a short chain PFAS, was higher in females.
Conclusions: In the STRIVE cohort, higher short-chain PFAS mixture concentrations were significantly associated with elevated ALT and varied by sex and lifestyle behaviors.

Table 1. Associations of PFAS mixtures with outcomes

	C	Molecular Weight (g/mol)	Geometric Mean (95% CI) (ng/mL)	PFAS mixture on Elevated ALT		PFAS mixture on Cirrhosis	
				Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*
Long-chain, high molecular weight PFAS							
PFOS	8	500.1	3.97 (3.59, 4.39)	0.84 (0.38, 1.83)	0.66	0.95 (0.86, 1.05)	0.34
PFOA	8	414.1	0.997 (0.91, 1.09)				
PFHxS	6	400.1	0.94 (0.85, 1.04)				
PFNA	9	464.1	0.40 (0.37, 0.43)				
PFDA	10	514.1	0.16 (0.15, 0.18)				
PFHpS	7	450.1	0.14 (0.12, 0.15)				
PFUnA	11	564.1	0.08 (0.07, 0.09)				
PFDoA	12	614.1	0.010 (0.009, 0.012)				
Short-chain, low molecular weight PFAS							
PFHpA	7	364.1	0.051 (0.047, 0.054)	2.20 (1.32, 3.67)	0.003	1.02 (0.93, 1.12)	0.62
PFPeA	5	264.1	0.044 (0.043, 0.046)				
PFBS	4	300.1	0.032 (0.029, 0.034)				
PFPeS	5	350.1	0.019 (0.018, 0.021)				

#4904 Say it ain't psoas: addressing edema in pancreatic cancer body composition analyses.

Evan W. Davis[1], Margaret A. Park[2], Toni L. Basinski[3], J. Pablo Arnoletti[4], Francisca Beato[3], Mark Bloomston[5], Tiffany L. Carson[6], Tiago Biachi de Castria[3], Dung-Tsa Chen[7], Elena M. Cortizas[8], Sylvia L. Crowder[6], Maria Genilo-Delgado[3], Wade G. Douglas[9], Kevin L. Huguet[10], Kun Jiang[11], Pamela J. Hodul[3], Aleksandra Karolak[12], Dae Won Kim[3], John Koomen[13], Anjana A. Menon[3], Kenneth Meredith[14], Qianxing Mo[7], Shaffer R. Mok[3], Lina Moreno-Urazan[3], Sabeen Ahmed[12], Nathan Parker[6], Jose M. Pimiento[3], Ghulam Rasool[12], Lauren M. Sparks[15], Paul Stewart[7], Alexandra F. Tassielli[3], Jamie K. Teer[7], Dan Viet Tran[8], Jose G. Trevino[16], Vic Velanovich[17], Xuefeng Wang[7], Christopher J. Whelan[18], Sarah M. Judge[19], Andrew R. Judge[19], Jason B. Fleming[20], Daniel Jeong[21], Jennifer B. Permuth[1], on behalf of the Florida Pancreas Collaborative

[1]Departments of Gastrointestinal Oncology & Cancer Epidemiology, H. Lee Moffitt Cancer Center, Tampa, FL,[2]Departments of Gastrointestinal Oncology & Biostatistics and Bioinformatics, H. Lee Moffitt Cancer Center, Tampa, FL,[3]Department of Gastrointestinal Oncology, H. Lee Moffitt Cancer Center, Tampa, FL,[4]Orlando Health Cancer Institute, Orlando, FL,[5]South Florida Surgical Oncology, Fort Myers, FL,[6]Department of Health Outcomes and Behavior, H. Lee Moffitt Cancer Center, Tampa, FL,[7]Department of Biostatistics and Bioinformatics, H. Lee Moffitt Cancer Center, Tampa, FL,[8]University of Miami Miller School of Medicine, Miami, FL,[9]Department of Clinical Sciences, Florida State University College of Medicine, Tallahassee, FL,[10]Department of Surgery, St. Anthony's Hospital, St. Petersburg, FL,[11]Department of Anatomic Pathology, H. Lee Moffitt Cancer Center, Tampa, FL,[12]Department of Machine Learning, H. Lee Moffitt Cancer Center, Tampa, FL,[13]Department of Molecular Medicine, H. Lee Moffitt Cancer Center, Tampa, FL,[14]Sarasota Memorial Hospital, Sarasota, FL,[15]Translational Research Institute, AdventHealth, Orlando, FL,[16]Department of Surgical Oncology, Virginia Commonwealth University & Massey Cancer Center, Richmond, VA,[17]University of South Florida & Tampa General Hospital, Tampa, FL,[18]Department of Metabolism and Cancer Physiology, H. Lee Moffitt Cancer Center, Tampa, FL,[19]Department of Physical Therapy, University of Florida, Gainsville, FL,[20]Department of Surgery HCSCC, University of Texas Southwestern Medical Center, Dallas, TX,[21]Department of Diagnostic Imaging, H. Lee Moffitt Cancer Center, Tampa, FL

Background: Image based body composition is the gold standard for assessing skeletal muscle area and quality in cancer patients. In patients with pancreatic ductal adenocarcinoma (PDAC), the decrease in muscle area and quality can be particularly profound. Yet image-based muscle assessment in these patients may be impeded by the presence of edema at follow up caused by disease burden and/or treatment. Edema in superficial muscle can affect the Hounsfield units of surrounding tissue leading to overestimation of muscle area. Despite this, to our knowledge no studies have examined alternative methods of assessing muscle loss in PDAC patients in the context of edema. To address this gap, we examined the prevalence of edema in PDAC patients, its impact on change in total skeletal muscle area (SMA) between diagnosis and six month follow up, and the use of psoas muscle area (pSMA), which is less impacted by edema, as an alternative method of assessing muscle loss.

Methods: The study population included sixty-nine PDAC patients from the multi-center Florida Pancreas Collaborative study with serial computed tomography (CT) scans obtained at diagnosis and six month follow up. Using the CT scans, we assessed edema by identifying opacification in the subcutaneous fat defining those with opacification as edematous. We examined the prevalence of edema at six-month follow up in the overall PDAC population and tested for differences by race and sex using Fisher's Exact test. SMA and pSMA were quantified as surface area (cm2) at L3 with sliceOmatic software. SMA and pSMA percent change and mean change between diagnosis and follow up were calculated in the overall population and by edema status at follow up. Spearman correlation was used to test the interrelationship of SMA and pSMA according to edema status.

Results: Prevalence of edema at follow up was 53.6% and did not significantly differ by race ($p>0.76$) or sex ($p>0.05$). In the overall population, SMA increased by 0.472% between diagnosis and follow up. When stratified by edema status, SMA increased in patients with edema (+3.06%) whereas SMA in patients without edema decreased (-2.52%). Conversely, pSMA decreased by 6.32% and this was consistent across edema status (-5.69% for present and -7.05% for absent). Finally, we observed that SMA and pSMA at follow up are strongly correlated in both the presence ($r=0.72$) and absence of edema ($r=0.77$).

Conclusions: In this study, we observed that edema is prevalent in most PDAC patients at follow up contributing to an overestimation of SMA. This overestimation may introduce bias into understanding the impact of PDAC on muscle wasting, an important factor in cancer cachexia. Encouragingly, findings suggest pSMA may be a suitable proxy for total SMA and limit the impact of edema on SMA estimates. These findings have important implications for enhancing our understanding of muscle changes over the PDAC cancer care continuum and should be considered in future studies.

#4905 Mosaic chromosomal alterations overlapping hotspot and coldspot sites of somatic structural variation are associated with increased odds of hematological malignancy.

Jasmine Ryu Won Kang, Vanessa Bruat, Kimberly Skead, Mawusse Agbessi, June Kim, Elias Gbeha, Marie-Julie Fave, Philip Awadalla

Ontario Institute for Cancer Research, Toronto, ON, Canada

BACKGROUND
Clonal hematopoiesis (CH) occurs when hematopoietic cells acquire somatic mutations and proliferate to generate clones in blood. CH can be driven by mosaic chromosomal alterations (mCAs), which are large somatic structural variants, and is associated with increased risk of blood malignancy. We conducted a genome-wide search for sites significantly more or less impacted by mCA events, termed hotspots and coldspots. We tested whether the odds of blood malignancy in participants harboring mCAs overlapping hotspots or coldspots differed from the overall association between mCAs and blood malignancy.
METHODS
This study utilized two cohorts of the Canadian Partnership for Tomorrow's Health, including the Ontario Health Study (OHS; n=7070) and CARTaGENE (n=28,639). Participants were genotyped with the UK Biobank Axiom or Infinium Global Screening Array and completed a baseline cancer questionnaire. All mCA calling was performed using MoChA software. We identified hotspot and coldspot sites of autosomal mCA accumulation using binomial tests scaled by chromosome and array type. We determined whether the number of overlapping mCAs at each query site was greater or less than would be expected under the null expectation. Then, we calculated the prevalence of cancer in participants with or without mCAs overlapping hotspots/coldspots and conducted Fisher's exact tests to generate odds ratios.
RESULTS
In OHS, participants with an mCA had a significantly greater odds of having a hematological malignancy at baseline (OR=7.29, 95% CI=3.72-13.38, p=7.84e-08). They were also more likely to have a cancer diagnosis of any type (OR=1.63, 95% CI=1.14-2.30, p=5.59e-03). Participants with an mCA overlapping a hotspot site had a further increased odds of harboring hematological malignancy (OR=9.79, 95% CI=3.94-21.29, p=5.05-e06). For participants with an mCA overlapping a coldspot site, the OR reached 20.52 (95% CI=3.68-76.85, p=7.36e-04). In CARTaGENE, participants with an mCA were also at increased odds of hematological malignancy (OR=11.31, 95% CI=6.78-18.17, p=9.77e-16), and any cancer diagnosis (OR=1.93, 95% CI=1.53-2.41, p=5.95e-08). Those carrying an mCA overlapping a hotspot site were 14.6 times more likely to have a hematological malignancy (95% CI=7.94-25.29, p=3.30e-13), and those with an mCA overlapping a coldspot site were 14.2 times more likely (95% CI=5.0-32.9, p=7.63e-06).
CONCLUSIONS
These results suggest that there may be genomic locations at which somatic structural variation has a larger impact on the development of blood malignancy, relative to other regions. Further work is needed to characterize the functional consequences of somatic mutation at these key regions and explore whether these associations of hotspots, coldspots, and cancer incidence are conserved across a range of tissues.

#4906 A prospective analysis identified mismatch repair genes as predisposing for uveal melanoma.

M.-H. Stern, A. Le Ven, M.-C. Villy, A. B. Silveira, A. Houy, M. Warcoin, M. Le Mentec, F. Simaga, A. De Pauw, B. Buecher, M. Gauthier-Villars, T. Verrier, K. Merchadou, V. Renault, A. Vincent-Salomon, J. Sauge, S. El Zein, C. Dubois d'Enghien, S. Piperno-Neumann, A. Matet, D. Malaise, N. Cassoux, L. Lumbroso-Le Rouic, D. Stoppa-Lyonnet, M. R. Rodrigues, L. Golmard, C. Colas;
Institut Curie, Paris, France

Background and Aim: Uveal melanoma (UM) is the most common intraocular malignancy in adults. Thus far, germline mutations of two genes have been identified to be predisposing to UM with a high penetrance: *BAP1*, responsible for the *BAP1* tumor predisposition syndrome (*BAP1*-TPDS), and *MBD4*, which germline alterations are associated with tumor predisposition, specific tumor mutational signature and response to immunotherapy. However, only a fraction of familial and bilateral/multifocal forms of UM can be explained by these germline alterations, suggesting the existence of other predisposing factors.

Methods: We analyzed a panel of 122 candidate cancer predisposing genes by target sequencing on germline DNA from a prospective unbiased cohort of 381 patients diagnosed with UM at Institut Curie between July 2021 and February 2023.

Results: We identified 29 pathogenic variants (PVs) in genes that are relevant for patient monitoring and genetic counselling (6 in *MBD4,* 3 in *BAP1,* 3 in *BRCA2,* 1 in *ATM,* 3 in *FAN1,* 2 in *FANCL,* 3 in *FANCM,* 1 in *MLH1,* 1 in *MSH2,* 2 in *MSH6* and 4 in *PMS2*). Two patients had PVs in *MSH6*, 4 in *PMS2*, 1 in *MLH1* and 1 in *MSH2*. The frequencies of the 3 MisMatch Repair (MMR) genes *MLH1*, *MSH2* and *MSH6* were significantly higher than in the gnomAD (v2.1.1) non-Finnish European database as a matched control sub-cohort (p=0.00073, OR: 10.4 [2.8-27.5]). *PMS2* was excluded from this statistical analysis because its pseudogene prevents the interpretation of public data. One UM tumor sample was further characterized from the patient carrying the germline *MLH1* PV. This UM displayed biallelic inactivation of *MLH1,* loss of MLH1 and PMS2 expression by immuno-histochemistry, a prominent Substitution Mutational Signature 6 (SBS6) associated with MMR deficiency from whole Genome Sequencing (WGS) and an MSI-H phenotype.

Conclusions: This study confirms MMR genes as predisposing factors in UM, as suggested by two previous case reports identifying *MLH1* germline PVs and biallelic inactivation in UM cases (Lobo et al. Virchows Arch 2017;470:347; Abdel-Rahman et al. Ophthalmology 2020;127:668). Further analyses will be needed to define the risk of developing UM for patients with a Lynch syndrome.

#4907 Availability and characteristics of energy balance programs offered by NCI-designated cancer centers.

Jean Miki Reading[1], Alexandra Rich[2], Aarifah Bandealy[3], Payton Solk[4], Julia Starikovsky[4], Allegra Frank[5], Kristina Hasanaj[4], Shirlene Wang[4], Siobhan M. Phillips[4]

[1]Family and Community Medicine, University of Illinois Chicago College of Medicine, Chicago, IL,[2]Quinnipiac University, Hamden, CT,[3]Kansas City University, Kansas City, MO,[4]Preventive Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL,[5]Northwestern University, Chicago, IL

To broaden our understanding of energy balance programs (diet, physical activity, weight control available for cancer survivors in NCI-designated cancer centers across the United States, we reviewed the characteristics (program components, personalization, delivery mode) of programs using publicly available information on cancer center websites. All NCI-designated cancer centers (n=72 as of 4/24/2024) were included, and two coders reviewed their websites. Each cancer center's website was searched and reviewed from June 2023 to April 2024. We applied the RE-AIM (Reach, Effectiveness, Adoption, Implementation, Maintenance) framework, an implementation framework used for planning and evaluation of programs, to evaluate each cancer center's energy balance programming. Information was extracted by each reviewer independently and then checked by a third study staff member for discrepancies. A total of 55 (76%) cancer centers had information on at least one energy balance program or resource posted on their website. These programs included diet/nutrition (n=41), physical activity (n=23), and weight control programs (n=3). Personalized programming was offered in 68% of diet/nutrition programs, 30% of physical activity programs, and 33% of weight control programs. Across all energy balancing behaviors, most programs offered individual sessions (76% diet/nutrition; 57% physical activity; 67% weight control). Group-based support was offered in less than half of programs across all behaviors (29% diet/nutrition; 40% physical activity; 33% weight control). In-person delivery was offered in 56% of diet/nutrition programs, 74% of physical activity programs, and 33% of weight control programs. Most of the programming for cancer survivors addressed diet/nutrition. Weight control programs were limited. Most physical activity programs were delivered in-person, which could limit reach and increase barriers to participation since the median of NCI-designated catchment areas is 26,500 square miles. There is a need for more consistency and availability of personalized support, particularly concerning exercise and weight control. Comprehensively addressing all energy balance components as part of cancer survivorship care is needed.

#4908 Exploring the role of algorithms in accessing culturally tailored nutrition information to improve survivorship in women with reproductive cancers.

Zakirah L. Abdul-Hameed, Gabrielle Ford, Amirah Burton-Obanla, Desiree Rivers

Morehouse School of Medicine, Atlanta, GA

Background/Significance: African American/Black (AA/B) women experience a 46 percent higher mortality rate from reproductive cancers compared to White women. While dietary interventions have shown effectiveness in improving survivorship, barriers such as limited access to culturally and linguistically relevant nutrition information hinder healthy dietary behaviors. Digital platforms are key sources of cancer nutrition information for AA/B women, yet algorithmic biases often limit the visibility of culturally relevant, reliable resources in underserved communities. Optimizing algorithmic frameworks to prioritize culturally tailored content could enhance survivorship outcomes for AA/B women.

Methods: An environmental scan of 25 sources evaluated the accessibility and cultural alignment of online cancer nutrition information for AA/B women. Focus groups with reproductive cancer survivors explored trust in digital health platforms and barriers to accessing culturally adapted nutrition resources. Interviews with SEO specialists, bio-nutritionists, and food industry leaders examined how algorithms impact access to culturally specific cancer nutrition information.

Results: The environmental scan revealed a gap in culturally adapted cancer nutrition content for AA/B women in national guidelines. Focus groups emphasized the reliance on digital platforms for dietary guidance but noted challenges in accessing trustworthy, culturally relevant information. Interviews revealed algorithmic biases that limit visibility of culturally tailored content, particularly in underserved areas. These findings stress the need for culturally competent nutrition education to address disparities and improve cancer survivorship outcomes.

Conclusion: Optimizing search engine algorithms and integrating trustworthy, credible, and culturally relevant nutrition content into digital platforms are essential to addressing access barriers for AA/B cancer survivors. By advancing algorithmic design and promoting reliable, culturally tailored online resources, technology can build trust and improve survivorship outcomes. This research stresses the critical role of digital innovation and health equity-focused strategies in promoting equitable access to cancer nutrition information, supporting the continuum of innovation in cancer care.

Acknowledgement of Funding: The project described was supported by Award Number CHERC-MSI-21-166-01-CHERC-MSI. from the American Cancer Society (ACS). The content is solely the responsibility of the authors and does not necessarily represent the official views of the ACS.

#4909 Artificial intelligence (AI) chatbots and their reponses to most searched Spanish cancer questions.

E. Cheng[1], J. D. Anampa[2], C. Bernabe-Ramirez[2], J. Lin[1], X. Xue[1], A. B. Moadel-Robblee[1], E. Chu[2];
[1]Albert Einstein College of Medicine, Bronx, NY, [2]Montefiore Einstein Comprehensive Cancer Center, Bronx, NY

Background: AI chatbots are predominantly trained on English contents. They perform well in answering English cancer questions, but their performance in other languages (such as Spanish) is unknown. Spanish-speaking patients are also concerned that they must use the paywall versions to get better responses, which may exacerbate existing cancer disparities.
Methods: We evaluated the responses of AI chatbots to most searched Spanish cancer questions. Using Google Trends (1/1/2020-1/1/2024), we identified the top 5 most searched Spanish cancer questions related to the top 3 common cancers in US Hispanics/Latinos. We selected 6 popular AI chatbots (free and paywall versions of ChatGPT, Claude, and Gemini) and then generated 90 Spanish responses. Board-certified oncologists speaking native Spanish assessed the quality using DISCERN Instrument (score from 1 [low quality] to 5 [high quality]), actionability using Patient Education Materials Assessment Tool (score from 0 [no clear action suggestions] to 100% [clear action suggestions]), readability using Fernández Huerta Reading Grade Level (score from 1 [1st grade] to 13 [college]).
Results: The quality of overall AI chatbot responses was moderate (mean [95% CI]: 3.5 [3.4-3.6]). The actionability was low (mean [95% CI]: 35.6% [30.8%-40.3%]), and the readability was high-school level (mean [95% CI]: 9.2 [8.8-9.6] grade). The performance of quality, actionability, and readability did not differ by free and paywall versions ($P > 0.05$).
Conclusions: AI chatbots provided moderately accurate information for most searched Spanish cancer-related questions. The responses were not readily actionable and written at the high-school level, which was not concordant with the American Medical Association's recommendation (6th grade or lower). The performance did not improve by using the paywall versions.
Relevance: To reduce cancer disparities in health literacy, AI chatbots need improvement in responding to Spanish cancer questions.

The Performance of AI Chatbots in Responding to Most Searched Spanish Cancer Questions

Performance	Overall	Free Versions	Paywall Versions	*P*
Quality (points)	3.5 (3.4-3.6)	3.4 (3.2-3.7)	3.6 (3.3-3.8)	0.22
Actionability (%)	35.6 (30.8-40.3)	36.0 (27.3-44.7)	35.1 (26.4-43.8)	0.76
Readability (grade)	9.2 (8.8-9.6)	9.2 (8.6-9.8)	9.2 (8.7-9.8)	0.99

#4911 Cancer disparities based on seven years of sexual orientation and gender identity collection at an NCI-Designated Comprehensive Cancer Center.

Shahrzad A. Zamani[1], Jaileene Perez-Morales[2], Min-Jeong Yang[1], Matthew B. Schabath[1]

[1]Moffitt Cancer Center, Tampa, FL,[2]OHSU Knight Cancer Institute, Portland, OR

Background: Sexual and gender minority (SGM) populations are underserved and minoritized. To aid in identifying real world cancer disparities among SGM patients, in 2016 our institution began collecting sexual orientation and gender identity (SOGI) on all patients. Leveraging these valuable data, this study analyzed differences between SGM and non-SGM cancer patients for clinical characteristics and quality-of-life (QoL).

Methods: Data were acquired on >118,000 patients who completed the standard-of-care Electronic Patient Questionnaire which collects self-reported SOGI. Patients were categorized as SGM or non-SGM based on SOGI. Clinical covariates were obtained from cancer registry for a subset of 51,189 patients. Descriptive statistics and survival analyses were used.

Results: ~3.2% of patients self-identified as SGM. *Cancer diagnoses*: Breast cancer was the most common diagnosis in SGM and non-SGM patients, followed by melanoma, hematologic, prostate, and lung cancers. Notably, SGM patients exhibited higher-than-expected rates of anorectal (6.8% vs. 0.7%, p<0.001) and uterine cancers (5.4% vs. 3.6%, p<0.001) compared to non-SGM patients. *Age:* Among the cancers analyzed, the mean age at diagnosis was significantly (p<0.001) lower for SGM compared to non-SGM: breast (54.8 vs. 60.5), melanoma (57.8 vs. 65.5), hematologic (62.0 vs. 67.1), prostate (65.4 vs. 68.1), lung (63.4 vs. 68.9), anorectal (55.1 vs. 64.4), and uterine (55.1 vs. 63.6). *Stage:* Among anorectal cancers, SGM were more likely to be diagnosed *carcinoma in situ* compared to non-SGM (45.8% vs. 4.8%, p < 0.001). For uterine cancers, SGM were more frequently diagnosed at stage II compared to non-SGM patients (18.2% vs. 4.8%, p = 0.008). No significant differences were observed for breast, lung, and hematologic cancers. *Survival:* Cancer site specific analysis revealed no significant differences. *QOL*: Concerns about barriers to seeking care were significantly higher (p<0.001) among SGM patients with breast (27.0% vs. 15.3%), lung (14.3% vs. 27.2%), and anorectal (35.9% vs. 15.5%) cancers. SGM patients with prostate cancer showed statistically significant lower social support (8.0% vs. 2.9%, p<0.001) and higher rates of living alone (28.4% vs. 10.8%, p<0.001), with similarly higher rates of living alone among SGM patients with anorectal cancer (33.9% vs. 16.3%, p=0.007). Worse mental health was reported by SGM patients with breast, hematologic, melanoma, prostate, uterine cancers (p<0.001), and SGM breast cancer patients had lower physical health scores (47.2% vs. 45.3%, p=0.042).

Conclusions: SGM patients were diagnosed at younger age, diagnosed at higher rates of some cancer types, experienced elevated psychosocial challenges, reported worse mental health, and higher barriers to obtaining care. These findings highlight the value of SOGI data in advancing health equity in oncology.

#4912 Assessing transcriptomic profiles of visceral adipose tissue in colon cancer patients.

Patricia A. Erickson[1], Victoria M. Bandera[1], Bettina Katalin Budai[2], Tengda Lin[1], Caroline Himbert[1], Sheetal Hardikar[1], Jeffrey T. Yap[3], Elaine M. Glenny[4], Aik Choon Tan[5], Jennifer Ose[1], Christy A. Warby[1], Olena Aksonova[1], Tobias Nonnenmacher[2], Chris Stubben[6], David Nix[6], Kenneth Boucher[7], Peter Schirmacher[8], Ildiko Strehli[1], Alejandro Sanchez[9], Jolanta Jedrzkiewicz[10], Lyen C. Huang[11], Jessica N. Cohan[11], Alexander Brobeil[8], Hans-Ulrich Kauczor[2], Christoph Kahlert[12], Victoria Damerell[12], Erin M. Siegel[13], Doratha A. Byrd[13], Adetunji T. Toriola[14], David Shibata[15], Christopher I. Li[16], Jane C. Figueiredo[17], Jatin Roper[18], Biljana Gigic[12], Stephen Hursting[19], Cornelia M. Ulrich[1]

[1]Huntsman Cancer Institute, Salt Lake City, UT,[2]Department of Diagnostic and Interventional Radiology, Heidelberg University Hospital, Heidelberg, Germany,[3]Center for Quantitative Cancer Imaging, Huntsman Cancer Institute, Salt Lake City, UT,[4]Department of Nutrition, University of North Carolina at Chapel Hill, Chapel Hill, NC,[5]Department of Biomedical Informatics, Huntsman Cancer Institute, Salt Lake City, UT,[6]Cancer Bioinformatics Shared Resource, Huntsman Cancer Institute, Salt Lake City, UT, Salt Lake City, UT,[7]Division of Epidemiology, University of Utah, Salt Lake City, UT,[8]Institute of Pathology, Heidelberg University Hospital, Heidelberg, Germany,[9]Department of Surgery, Division of Urology, Huntsman Cancer Institute, Salt Lake City, UT,[10]Department of Pathology, University of Utah, Salt Lake City, UT,[11]Department of Surgery, University of Utah, Salt Lake City, UT,[12]Department of General, Visceral and Transplantation Surgery, Heidelberg University Hospital, Heidelberg, Germany,[13]Department of Cancer Epidemiology, Moffitt Cancer Center, Tampa, FL,[14]Division of Public Health Sciences, Department of Surgery, and Siteman Cancer Center, Washington University School of Medicine, St. Louis, MO,[15]Department of Surgery, University of Tennessee Health Science Center, Memphis, TN,[16]Fred Hutchinson Cancer Center, Seattle, WA,[17]Department of Medicine, Samuel Oschin Comprehensive Cancer Center, Cedars Sinai Medical Center, Los Angeles, CA,[18]Department of Medicine, Division of Gastroenterology, Duke University, Durham, NC,[19]Nutrition Research Institute, University of North Carolina at Chapel Hill, Kannapolis, NC

Background: Obesity is a major risk factor for colon cancer (CC) with a number of potential biologic mechanisms. Visceral adipose tissue (VAT) has been identified as a key contributor, partly due to its secretion of adipokines and proinflammatory cytokines. VAT has also been associated with worse cancer-related outcomes. In this study, we evaluated transcriptomic differences in patients with high versus low amounts of VAT among people with CC.

Methods: Tumor-adjacent VAT was collected in 60 patients diagnosed with stage I-III CC who underwent primary surgery as part of the ColoCare Study at Huntsman Cancer Institute (HCI), University of Utah, and University of Heidelberg, Germany. Bulk transcriptomic analysis (RNAseq) was performed on VAT at the HCI genomics core. Computed tomography (CT) scans were used to measure visceral fat area (VFA) via body composition analysis performed using the Data Analysis Facilitation Suite (DAFS). The “DESeq2” R package was used to identify differentially expressed genes stratified by VFA (low VFA: <175 cm2, high VFA ≥ 175 cm2), adjusting for age at diagnosis, sex, study site, and tumor stage. Ranked/sorted genes were entered into gene set enrichment analysis (GSEA) software to identify enriched gene sets and pathways.

Results: Among the 60 patients included the study, 53% were male, the mean age at diagnosis was 63±13 years, 45% had stage III CC, and the mean VFA was 182±105 cm2. Among patients with high VFA, 173 genes were differentially expressed compared to those with low VFA. GSEA identified 17 significantly upregulated and 20 significantly downregulated gene sets (p-adj. < 0.05). Upregulated gene sets among patients with high VFA included pathways were related to extracellular matrix signaling, pyrimidine metabolism, and cytokine-cytokine receptor interactions, while downregulated gene sets included pathways related to branched-chain amino-acid degradation, propanoate metabolism, and pyruvate metabolism.

Discussion: VAT in patients with high levels of visceral adiposity was characterized by the overexpression of pathways that are thought to be associated with CRC development and progression. Our next steps involve further evaluation of these findings in a larger cohort, along with an assessment of VAT gene expression in relation to survival.

#4913 PREDICT Breast v4: Advancing equity in breast cancer prognosis for diverse populations.

Yi-Wen Hsiao[1], Gordon C. Wishart[2], Paul D.P. Pharoah[1], Pei-Chen Peng[1]

[1]Cedars-Sinai Medical Center, West Hollywood, CA,[2]Anglia Ruskin University, Cambridge, United Kingdom

Breast cancer, the most common cancer among women globally, remains a significant public health challenge. Prognosis, influenced by factors such as tumor characteristics, patient demographics, and treatment choices, is a major concern for patients. Tools like the PREDICT Breast v3 model (https://breast.v3.predict.cam/) help weigh treatment benefits against short- and long-term risks, enabling more personalised prognostic predictions. However, its performance disparities among non-Hispanic Black and non-Hispanic Asian patients in the US highlight the need for more equitable and inclusive tools. Therefore, this study aims to develop a breast cancer prognostic model that improves predictive accuracy across diverse populations and reduces disparities in prognostic outcomes. Here, we analysed data from 273,918 breast cancer patients in the Surveillance, Epidemiology, and End Results (SEER) database, each with clinical records and socioeconomic status (SES) information. The data were split 7:3 for model training and validation, with 10-fold cross-validation applied for parameter tuning. We first evaluated both conventional methods (PREDICT v3) and advanced machine learning models, including survival XGBoost, survival random forest, DeepHit, and DeepSurv, using calibration and discrimination as evaluation metrics. After comparing the models, we selected the DeepHit neural network as the best-performing approach due to its lowest calibration error and highest discrimination. We then examined whether incorporating SES improved performance by comparing SES-adjusted and unadjusted DeepHit models. Our results showed that the SES-adjusted DeepHit model achieved strong calibration (within 1.3% of observed deaths) and reduced racial disparities compared to PREDICT v3, which exhibited calibration errors of 30% for non-Hispanic Asians and 20% for non-Hispanic Blacks. These findings emphasise the importance of incorporating SES factors to enhance model accuracy and equity, underscoring the critical need for fairness in prognostic prediction. This work advances the application of machine learning in cancer prognosis and provides a foundation for addressing health disparities in clinical prediction tools.

#4914 Association of *HOXB13* G84E with prostate cancer among 592,158 men.

Taylor B. Crawford[1], Tyler Nelson[2], Roshan Karunamuni[3], Heena Desai[1], Ryan Hausler[1], Craig Teerlink[2], Hannah Carter[4], Meghana S. Pagadala[5], Patrick R. Alba[6], Scott L. DuVall[6], Morgan E. Danowski[7], Charles A. Brunette[7], Dmitry Ratner[7], Isla P. Garraway[8], Brent S. Rose[4], Kathleen A. Cooney[9], Jason L. Vassy[7], Richard L. Hauger[4], Julie A. Lynch[2], Tyler M. Seibert[4], Kara N. Maxwell[1]

[1]University of Pennsylvania, Philadelphia, PA,[2]VA Salt Lake City Health Care System, Salt Lake City, UT,[3]University of California, San Diego, San Diego, CA,[4]University of California San Diego, San Diego, CA,[5]VA San Diego Healthcare System, San Diego, CA,[6]University of Utah, Salt Lake City, UT,[7]VA Boston Healthcare System, Boston, MA,[8]University of California, Los Angeles, Los Angeles, CA,[9]Duke University School of Medicine, Durham, NC

Background: The *HOXB13* c.G251G>A:p.G84E variant was identified as a prostate cancer (PCa) risk allele in individuals of European descent approximately ten years ago. Several studies from high-risk cohorts have demonstrated a 3-5-fold increased risk of developing PCa with this variant. However, whether this variant confers risk of more aggressive PCa remain controversial. We report age-specific PCa risks and clinical pathological associations in a population-based *HOXB13* p.G84E cohort of heterozygous individuals (n=1660) from the VA Million Veterans Program.

Methods: *HOXB13* p.G84E heterozygotes were identified by SNP genotyping in patients from the Million Veterans Program (MVP). Cox proportional hazard models were used to determine the association of the variant with patient outcomes. Multivariable logistic regression models were used to determine the association of the variant with risk of PCa diagnosis on first biopsy in a sub-cohort of Veterans diagnosed with PCa in the VA.

Results: This MVP study included a total of 592,158 males with 1,660 (0.28%) *HOXB13* p.G84E heterozygous individuals. There was a significant increase in overall incidence of PCa in *HOXB13* p.G84E heterozygous individuals (31.3% (n=519) vs 12.1% (n=71,500), p<0.001) compared to homozygous wild-type individuals. Additionally, *HOXB13* p.G84E heterozygotes were more likely to be diagnosed with PCa at a younger age group (p=0.001). In a Cox proportional hazards model, *HOXB13* p.G84E heterozygous individuals had a significantly increased risk of any PCa (HR 3.23, 95% CI 2.97-3.53, p<2.0E-16), metastatic PCa (HR 2.96, 95%CI 2.30-3.81, p<2.0E-16), and PCa related death (HR 2.65, 95% CI 1.67-4.21, p=3.8E-05). At age 60, the fraction of heterozygotes with any PCa (8.5%) and metastatic PCa (0.7%) was higher than that of wild-type individuals (any PCa: 2.8%, metastatic PCa: 0.1%). At age 90, a larger fraction of heterozygotes had any (59.5%) or metastatic PCa (12.4%) compared to wild-type individuals (any PCa: 26.9%, metastatic PCa: 5.0%). The fraction of *HOXB13* p.G84E heterozygotes who died of PCa at age 80 and 90 was 1.2% and 7.5% respectively, compared to 0.6% and 2.2% of wild-type individuals. To validate our results, we analyzed 36,321 males genotyped in MVP who underwent first prostate biopsy in the VA, among whom 202 (0.56%) were *HOXB13* p.G84E heterozygous individuals. *HOXB13* p.G84E heterozygotes were more likely to be diagnosed with PCa on first biopsy (83.2% (n=168) vs 62.7% (n=22,639), p<0.001) and had higher risk of PCa diagnosis on first biopsy (OR 2.60, 95%CI 1.94-3.52, p<0.001) compared to homozygous wild-type individuals.

Conclusions: In the largest cohort of heterozygotes with the *HOXB13* p.G84E variant to date, we report that this variant confers a 3-fold increased risk of developing prostate cancer. Further work is needed to determine if initiation of early PCa screening in *HOXB13* p.G84E heterozygous individuals would improve patient outcomes.

#4915 A multi-protein score to predict hepatocellular carcinoma occurrence.

Eleanor Watts, Steven Moore, Belynda Hicks, Wen-Yi Huang, Erikka Loftfield, Mitchell Machiela, Katherine McGlynn, Jianxin Shi, Rachael Stolzenberg-Solomon, Cody Watling

National Cancer Institute, Rockville, MD

Background: Hepatocellular carcinoma (HCC) is the most common type of liver cancer, frequently developing on a background of chronic liver disease. Our understanding of the totality of HCC etiology remains incomplete. Early detection of HCC remains a critical challenge in clinical practice as prognosis remains poor for individuals diagnosed at advanced stage. Recent advances in proteomic technologies enable systematic analysis of protein-disease associations, which has potential to improve mechanistic understanding of HCC etiology and aid in the discovery of novel biomarkers for early detection.
Aims: We aimed to investigate the associations of serum proteins with subsequent risk of HCC and create a multi-protein score to predict disease occurrence.
Methods: We conducted a nested case-control study within the PLCO cohort, analyzing 118 incident HCC cases individually matched to 118 controls (median time to HCC diagnosis:10 years). Using the Olink HT platform, we measured 5,400 proteins. Multivariable conditional logistic regression models were used to estimate protein-HCC risk associations, and we developed a multi-protein risk score using LASSO regression, which was evaluated using the area under the curve (AUC). All analyses underwent Bonferroni correction for multiple testing. We tested for replication our findings in the UK Biobank, a prospective cohort of 50,000 participants with protein data from the Olink Explore 3072 platform. During a median follow-up of 11 years, 28 participants developed HCC. We estimated protein associations using Cox proportional hazards regression and evaluated the multi-protein score's predictive performance in the overall cohort and among participants with liver disease—a population at elevated risk of HCC—by calculating the C-index.
Results: Among PLCO participants, we identified 127 proteins significantly associated with HCC risk. Of these, 126 proteins were positively associated, with the strongest association observed for TNFRSF11B (OR per 1 SD: 2.85, 95% CI 1.90-4.28). IGFBP-3 was the only protein inversely associated with risk (0.36, 0.24-0.54). Notably, 118 of the 127 proteins were available in the UK Biobank, and 117 of these associations were successfully replicated. LASSO regression revealed 8 key protein predictors. The resulting protein score exhibited high discriminatory performance in the PLCO cohort (AUC = 0.90) and generalizability in the UK Biobank (C-Index = 0.89 overall, 0.92 among high-risk individuals).
Conclusion: Our findings highlight strong potential of a multi-protein predictive score for HCC, evident years before diagnosis across 2 distinct cohorts.

#4916 Co-occurring clonal hematopoiesis in UK Biobank participants with prior cancer.

Kara Marie Barnao[1], Aubrey K. Hubbard[1], Irenaeus Chan[2], Weiyin Zhou[1], Corey D. Young[1], Rebecca L. Kelly[1], Derek W. Brown[1], Giulio Genovese[3], Duc Tran[2], Yin Cao[2], Stephen J. Chanock[1], Kelly L. Bolton[2], Mitchell J. Machiela[1]

[1]National Cancer Institute, Rockville, MD,[2]Washington Univerisity School of Medicine, St. Louis, MO,[3]Broad Institute of MIT and Harvard, Cambridge, MA

Clonal hematopoiesis (CH) describes the age-related clonal expansion of hematopoietic stem cells, driven by either acquired mutations in driver genes (referred to as clonal hematopoiesis of indeterminate potential (CHIP)), or by large-scale mosaic chromosomal alterations (mCAs). Evidence from previous studies indicate anti-cancer agents, such as chemotherapy and radiation therapy, may promote the expansion of clones with DNA damage response mutations (e.g., *TP53, PPM1D,* and *CHEK2).* However, the co-occurrence of CHIP and mCAs in participants with prior cancer has not been extensively characterized. We therefore examined the frequency of co-occurring CHIP and mCAs to further evaluate the landscape of CH within individuals with prior cancer diagnoses. We analyzed sequencing and genotyping array data from 34,339 UK Biobank participants with a prior cancer diagnosis. Among these, 2,756 CHIP events were detected in 2,470 (7.2%) of participants, at a frequency 1.5 times greater than those participants with no prior cancer diagnosis (N = 453,807). *TP53, PPM1D, MYD88, ATM, JAK2,* and *CALR* CHIP mutations in particular were detected at increased rates in those with prior cancer (ORs ranging from 1.9 for *TP53* mutations to 6.1 for *CALR* mutations, $p < 6.5 \times 10^{-4}$). Autosomal mCAs were also detected at a higher rate compared to those with no prior cancer diagnoses; 2,100 events were detected in 1,665 participants (4.8% vs 3.0% in cancer-free participants). Specifically, mCAs on 10 chromosomes (chr22, chr11, chr12, chr17, chr1, chr19, chr20, chr9, chr7, chr13, respectively) were more frequent (OR = 1.3 - 2.6, $p < 6.4 \times 10\text{-}3$). Co-occurring CH was characterized in three groups: CHIP-CHIP, mCA-mCA, CHIP-mCA. Among CHIP-CHIP events, 4 enriched co-occurrences ($p < 2.7 \times 10^{-5}$) were observed, all of which included *TET2* or *SRSF2* mutations. For mCA-mCA events, enrichment was observed for whole chromosome gains of chr3, chr12, and chr18. Approximately 1% of participants had both detectable CHIP and mCA events. A total of 12 specific CHIP-mCA co-occurrences were enriched; 3 of which were overlapping and also identified in the population with no prior cancer diagnosis (e.g., *TP53* CHIP and chr17p loss) and 4 of which were not observed in cancer-free individuals. *DNMT3A*-mLOY CH was observed less frequently than expected in prior cancer cases. These observations suggest potential instances of both mutual exclusivity and cooperation among CH types in participants with prior cancer diagnoses. The increased detection rate of some co-occurrences in this population, compared to those with no prior cancer, may reflect selective pressures of cancer treatment.

#4917 The association between MGUS and CHIP and their impact on multiple myeloma.

Nicholas J. Boddicker[1], Cristine Allmer[1], Danelle H. Moonen[1], Aaron D. Norman[1], Angelica Macauda[1], Alyssa Clay-Gilmour[2], Janet E. Olson[1], Mrinal S. Patnaik[1], Vincent Rajkumar[1], Esteban Braggio[3], David L. Murray[1], Shaji Kumar[1], Susan L. Slager[1], Celine M. Vachon[1]

[1]Mayo Clinic, Rochester, MN,[2]University of South Carolina, Columbia, SC,[3]Mayo Clinic, Phoenix, AZ

Monoclonal gammopathy of undetermined significance (MGUS) is a precursor to multiple myeloma (MM). Clonal hematopoiesis of indeterminate potential (CHIP) is a precursor to either myeloid or lymphoid malignancy, depending on whether the mutation is in a gene associated with lymphoid (L-CHIP) or myeloid (M-CHIP) outcome. Little is known about the association between CHIP and MGUS and their combined effect on risk of MM. We examined this question within Mayo Clinic Biobank cohort of participants aged 50 years or older with no prior history of hematological malignancies. Using whole blood, individuals were screened for MGUS using a matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry assay. M-CHIP and L-CHIP were defined based on mutations in 56 genes associated with myeloid malignancies or mutations in 235 genes associated with lymphoid malignancies, respectively, using whole-exome-sequencing. Incident MM was identified using International Classification of Diseases (ICD) codes and confirmed via medical record review. Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals for the association of CHIP and MGUS. Cox proportional hazards regression was used to estimate hazard ratios (HR), with time defined as date between blood sample and the first incident MM, date of death, or 12/31/2023. All models were adjusted for age and sex. Of the 16,479 individuals (median age 65 years, 45% male) screened, 12.3% were positive for MGUS, 9.8% were positive for M-CHIP, and 1.5% were positive for L-CHIP. There was no evidence of an association between M-CHIP and MGUS (OR=0.97, 95% CI:0.83-1.12) nor L-CHIP and MGUS (OR=1.12, 95% CI:0.78-1.57), including with any MGUS isotype (IgA, IgG, or IgM; P-values>0.22). Next, we investigated the association of these precursors on risk of MM. Median follow-up was 11.7 years (range 0.1-15.1), and 46 individuals developed incident MM. Both MGUS (HR=29.7, 95% CI: 14.1-62.4) and L-CHIP (HR=4.1, 95% CI: 1.3-13.2) were significantly associated with incident MM, but not M-CHIP (HR-1.0, 95% CI: 0.4-2.5). Investigating the combined effect of MGUS and L-CHIP, we found those with MGUS alone had a 27.5-fold (95% CI: 13.0-58.2) increased risk of incident MM compared to those with no precursors. No individuals with L-CHIP only developed MM, but those with both L-CHIP and MGUS had a 129.4-fold (95% CI: 34.4-486.4) increased risk of incident MM. In the largest cohort with screened MGUS and CHIP, we found no evidence of an association between either definition of CHIP with MGUS. However, individuals with both L-CHIP and MGUS were at the highest risk of developing MM.

#4918 Impact of social drivers of health on clinical trial knowledge among Hispanic/Latino and Black cancer survivors.

Hilmaris Centeno-Girona[1], Marievelisse Soto-Salgado[1], Stephanie Cameron-Maldonado[1], Arnethea L. Sutton[2], Katherine Y. Tossas[2], Robert A. Winn[2], Marcia R. Cruz-Correa[3]

[1]University of Puerto Rico Comprehensive Cancer Center, San Juan, Puerto Rico,[2]Virginia Commonwealth University Massey Comprehensive Cancer Center, Richmond, VA,[3]School of Medicine, University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico

Introduction: Despite significant advancements in cancer treatment, disparities in clinical trial (CT) participation persist among minority populations, such as Hispanic/Latino (H/L) and Black women (BW). Social drivers of health (SDoH) can influence health outcomes and health care decisions, like CT participation. This study assessed the association between SDoH and CT knowledge among H/L and BW cancer survivors in Puerto Rico and in Virginia, United States.
Methodology: This cross-sectional study included H/L gastrointestinal (GI) cancer survivors from Puerto Rico and BW with breast or gynecological cancers from Virginia, aged 21+ years. Participants completed an interviewer-administered questionnaire to assess their awareness, knowledge, and participation in cancer-related CTs and SDoH. A logistic regression model was used to estimate the odds ratios and 95% confidence intervals (CI) for CT knowledge and associated factors. As reported in the literature, significant variables ($p < 0.05$) and other relevant variables were included in the multivariate logistic regression model.
Results: As of November 11, 2024, 285 H/L and BW cancer survivors completed the questionnaire. Women accounted for 78% of the sample, and the mean age of study participants was 55 years (SD: 11.8). Over half of the participants (52%) had breast or gynecological cancer, whereas 47% reported GI cancer. While most study participants were aware of CTs (64%) and 80% accurately identified the correct definition, only 26% reported ever participating in a cancer-related CT. Logistic regression models showed that participants with lower educational attainment, those unemployed, and those with another type of transportation rather than their own transportation were significantly more likely to have lower CT knowledge than their counterparts ($p<0.05$). However, participants who reported an income between $20,000-$74,999 and those who perceived discrimination in medical settings were more likely to have higher CT knowledge than their counterparts ($p<0.05$). After adjusting for age, sex, educational attainment, employment, and health literacy, participants who reported an income between $20,000-$74,999 (OR: 3.43, 95%CI: 1.39-8.50) and those who perceived at least one type of discrimination in medical settings (OR: 2.44, 95%CI: 1.17-5.09) were more likely to have higher CT knowledge than their counterparts.
Conclusion: While CT awareness was relatively high, low CT participation was observed among H/L and BW cancer survivors. Understanding factors that impact CT participation is important for developing targeted interventions to improve CT participation and reduce health disparities among these underserved populations.
Acknowledgments: Research supported by an AACR grant, Social Determinants of Health, Grant Number: 23-01-SDoH.

#4919 Evaluation of lung cancer risk and screening strategies for individuals who quit smoking more than 15 years ago.

H. A. Park, K. Alcala, J. McKay, M. Johansson, H. A. Robbins, X. Feng, Lung Cancer Cohort Consortium;
International Agency for Research on Cancer, Lyon, France

The U.S. Preventive Services Task Force (USPSTF) recommends lung cancer screening for individuals who currently or formerly smoked with at least 20 pack-years of smoking and quit less than 15 years ago. However, the number of former smokers now surpasses that of current smokers in the U.S., recently, the American Cancer Society (ACS) recommended removing the quit-year exclusion criterion for lung cancer screening eligibility. Little is known for about lung cancer risk in long-term former smokers, and the optimal criterion to identify high-risk long-term former smokers is uncertain. Our analysis included 424,483 long-term former smokers (>15 quit years) aged 40 to 80 years from the Lung Cancer Cohort Consortium (LC3) with detailed risk factors information and lung cancer incidence data from 24 prospective cohorts. We evaluated the validity of the PLCOm2012 risk model by estimating model calibration (E/O: ratio-of-expected to observed number of cases) and discrimination (AUC) in long-term former smokers. We considered individuals with six-year-risk above 1.0% as high risk, using the PLCOm2012 model. The 1.0% risk threshold was defined to identify a similar number of LDCT screening eligible individuals as the USPSTF2021 criteria. We further compared the effectiveness of the ACS criteria (i.e. USPSTF2021 without 15-year quit year exclusion) and the PLCOm2012 model in identifying future lung cancer cases and deaths in long-term former smokers. Among long-term former smokers, the PLCOm2012 model showed good risk discrimination with AUC of 0.78 (95% CI: 0.77-0.79) but underpredicted the overall lung cancer risk (E/O: 0.72; 95% CI: 0.70-0.74). The PLCOm2012 model indicated that 20.0% of long-term former smokers (N=85,041) had six-year-risk above 1.0%, which identified 62.0% (N=2,035) of lung cancers and 64.0% (N=1,169) of lung cancer deaths within 5 years in this population. The ACS criteria identified 31% of long-term former smokers as screening eligible (N=131,225), which identified 69.0% (N=2264) of lung cancers and 70.9% (N=1,294) of lung cancer deaths occurring within 5 years. When using the PLCOm2012 model to define eligibility, applying a threshold that selected a population of equal size to ACS criteria (6y risk ≥0.63%), 75.2% (N=2,469) of lung cancers and 76.4% (N=1,395) of lung cancer deaths would be identified in this group. Our findings suggest that at least 20% of long-term former smokers are at high risk and may benefit from LDCT screening. Removing the 15-year quit-year exclusion would identify over two-thirds of lung cancer cases, by screening one-third of long-term former smokers. A risk-based approach to prioritize a subset of long-term former smokers for screening may be more efficient than age and pack-year criteria. These results provide insight into the benefits of expanding current screening criteria to include long-term former smokers.

POPULATION SCIENCES: Environmental, Social, and Biological Determinants of Cancer Risk and Survival
Poster Session

#4923 The impact of HPV on oropharyngeal cancer tumor microenvironment and survival.

Esther Lam[1], Jia Y. Wan[1], Rachel Bolanos[2], Brenda Y. Hernandez[3], Charles F. Lynch[4], Robert A. Edwards[1], Anil K. Chaturvedi[5], Adrian J. Correa[2], Arjan Diepstra[6], Wendy Cozen[1]

[1]UCI Health, Orange, CA,[2]University of Southern California, Los Angeles, CA,[3]University of Hawai'i Cancer Center, Honolulu, HI,[4]University of Iowa, Iowa City, IA,[5]National Cancer Institute, National Institutes of Health, Bethesda, MD,[6]University Medical Center Groningen, Groningen, Netherlands

The increased incidence of oropharyngeal squamous cell carcinoma (OPSCC) is attributed to increased human papillomavirus (HPV) infections. HPV-positive (HPV+) OPSCC has a better prognosis than HPV-negative (HPV-) OPSCC, highlighting the need to understand the determinants of this difference. The tumor microenvironment (TME) is associated with OPSCC prognosis, but differences due to HPV infection in OPSCC and the impact on survival are not well understood. To address this, de-identified formalin-fixed paraffin-embedded (FFPE) tumor blocks from 191 multiethnic OPSCC patients diagnosed from 1984-2004 were collected from three population-based cancer registries in the Surveillance, Epidemiology, and End Results Residual Tissue Repositories (Los Angeles, Hawai'i, and Iowa). HPV16 status was extracted from an earlier study (Chaturvedi et al, 2011) using in-situ hybridization (ISH) and line probe assays (LiPA), and HPV- tumors were further confirmed using p16 INK4a immunohistochemistry (IHC). Specimens that were ISH/LiPA negative and p16 positive were excluded (n=24). Sections were stained for CD3, CD8, CD163, B2M (HLA-I), and HLA-DP/DQ (HLA-II), using IHC. Counts of positive cells (CD3+, CD8+, and CD163+) were quantified using Visiopharm digital image analysis, while HLA stains were manually scored in tumor cells and used to identify dendritic cells (DC). Analysis of HPV16 tumor status and TME biomarker positive counts were compared using the nonparametric Mann-Whitney U test, and associations between the TME biomarkers and overall survival (OS) were analyzed using multivariable Cox regression to estimate the hazard ratio (HR) of TME biomarkers adjusted for age at diagnosis, sex, race/ethnicity, and SEER stage. There were 53 HPV+ and 114 HPV- patients included in the study after exclusions. Patients were primarily non-Hispanic white (56%) and male (80%); median age at diagnosis was 60. There were significantly higher levels of CD3+ ($p<0.0001$) and CD8+ ($p<0.0001$) T-cells in HPV+ compared to HPV- OPSCCs, and HPV+ tumors had reduced HLA-I expression ($p<0.0001$). High CD8+ T-cell levels were associated with better OS in HPV+ OPSCC (HR=0.21, p=0.036) but worse OS in HPV- OPSCC (HR=2.4, p=0.035). In HPV- tumors, HLA-II expression was associated with worse survival (HR=2.4, p=0.034), but HLA-II expression on DC in HPV+ tumors was strongly associated with improved survival (HR=0.07, P=0.025). In a subgroup (n=75; 36 HPV+, 39 HPV-), CD3+ and CD8+ T-cell infiltrations were higher in both tumoral ($p<0.007$; $p<0.0001$, respectively) and stromal areas ($p=0.002$; $p<0.0001$, respectively) of HPV+ compared to HPV- tumors. Higher stromal CD163+ macrophage infiltration was associated with worse OS in HPV+ tumors (HR= 4.3, p=0.030). These findings provide evidence that HPV+ OPSCC tumors have distinct TME profiles and associations with OS compared to HPV- OPSCC tumors and that the immune cell location within the TME is also important.

#4924 Geographic and racial/ethnic differences in stage at diagnosis and survival among adolescents and young adults with thyroid cancer in the United States: An analysis of 2000-2021 SEER data.

Emma Hymel, Krishtee Napit, Josiane Kabayundo, Amelia Nichols, Shinobu Watanabe-Galloway

University of Nebraska Medical Center, Omaha, NE

Objectives: Relatively few studies have focused on thyroid cancer in the adolescent and young adult (AYA) population. The objective of this study was to examine racial and geographic predictors of thyroid cancer outcomes in the AYA population.
Methods: This population-based longitudinal study uses data from the Surveillance, Epidemiology, and End Results (SEER)-22 registries database. All primary cases of malignant thyroid cancer (including papillary, follicular, medullary, and anaplastic thyroid cancers) from 2000 to 2021 diagnosed among individuals aged 15-39 were included. Multinomial logistic regression models were used to compute the association between race/ethnicity and rurality (measured using Rural Urban Continuum codes) with stage at diagnosis (measured using SEER summary stages: localized, regional, or distant). Cox proportional hazards regression models were used to compute the association between race/ethnicity and rurality and both cancer-specific and all-cause mortality. Directed acyclic graphs were used to identify variables to adjust for in the multivariable analyses to control for confounding.
Results: Our study included 74,215 AYAs with thyroid cancer (93.04% papillary, 5.89% follicular, 1.03% medullary, and 0.04% anaplastic). Approximately 8% of the cases were diagnosed in rural counties. Compared to those diagnosed in rural counties, those in urban counties were more likely to be diagnosed with regional vs. localized cancer (aOR=1.17, 95% CI: 1.10-1.25). Compared to Non-Hispanic White AYAs, Hispanic AYAs are more like to be diagnosed with regional (aOR=1.48, 95% CI: 1.41-1.52) and distant (aOR=2.00, 95% CI: 1.74-2.30) cancer vs localized cancer. Among AYAs with thyroid cancer, living in a rural county at diagnosis was associated with an increased risk of all-cause mortality (aHR=1.41, 95% CI: 1.13-1.76). Additionally, compared to non-Hispanic White AYAs, the risk of thyroid cancer-specific mortality was highest among non-Hispanic Black (aHR=3.66, 95% CI: 2.37-5.65) and Hispanic (aHR=1.85, 95% CI: 1.32-2.60) AYAs.
Conclusion: We observed racial and geographic differences in thyroid cancer stage at diagnosis and survival among AYAs in the United States. Future research should explore the underlying causes of these disparities and identify potential opportunities for intervention.

#4925 Social determinant predictors of lung cancer incidence in diverse urban populations.

Vijeth Narra, Alex Krule, Abdul J. Zakkar, Mason Burrage-Burton, Farah Yousef, Vijayakrishna Gadi, Frank Weinberg, Mark Korpics, Yamile Molina, Sage J. Kim, Margaret Geise-Wright, Zhengjia Chen, Elisabeta Marai, Ryan H. Nguyen, Ameen A. Salahudeen

Medicine/Hematology Oncology, University of Illinois College of Med. at Chicago, Chicago, IL

Background: Lung cancer is the leading cause of cancer-related mortality globally. While screening and predictive analytics provide insights into its pathogenesis, the role of social determinants of health (SDOH), particularly in urban U.S. populations, is unexplored at the individual level. This study examines associations between SDOH factors and lung cancer within Chicago.
Methods: An IRB-approved protocol was used to build a retrospective cohort of 11,154 patients (60% African American [AA], 30% White, 10% Other) from the University of Illinois Health System. Inclusion criteria followed the U.S. Preventive Services Task Force guidelines. Twenty-three zip code-level SDOH indices (measured between 2016-2023) and patient data were analyzed using statistical analyses, including hypergeometric testing and principle component analysis. Benjamini-Hochberg correction was used for multiple hypothesis testing.
Results: Non-cancer patients had less green space access than lung cancer patients ($mean_{diff}$=0.93, P=0.048). Lung cancer incidence correlated with increased fine particulate matter (PM2.5) levels ($mean_{diff}$=0.03, P=0.030). Men with lung cancer had lower PM2.5 exposure ($mean_{diff}$=0.06, P=0.007), poverty rates ($mean_{diff}$=1.73, P=0.003), and Medicaid coverage ($mean_{diff}$=1.89, P=0.017) compared to women, suggesting increased sensitivity to these factors. Patients with low/normal BMI and lung cancer experienced higher food insecurity ($mean_{diff}$=0.64, P=0.019) compared to those with high BMI. Among non-cancer patients, low/normal BMI individuals showed lower area deprivation scores ($mean_{diff}$=1.38, P=0.001) but higher lack of vehicle access ($mean_{diff}$=1.34, P=4.202e^{-7}), lifetime inhalation cancer risks ($mean_{diff}$=0.29, P=0.003), and respiratory hazards ($mean_{diff}$=0.01, P=0.003) compared to high BMI individuals. AA patients experienced disproportionately adverse SDOH impacts, regardless of cancer status (P<0.001). AA lung cancer patients had higher PM2.5 exposure than non-cancer AA patients ($mean_{diff}$=0.03, P=0.016). AA women without lung cancer had higher PM2.5 exposure ($mean_{diff}$=0.02, P=0.010), green space access ($mean_{diff}$=1.02, P=0.001), and respiratory hazard risks (meandiff=0.004, P=0.028), but lower high school graduation rates ($mean_{diff}$=0.401, P=0.046) compared to AA men.
Conclusions: Our preliminary findings highlight the need to integrate environmental and social variables, such as PM2.5 and SDOH, into lung cancer risk frameworks.. Additional studies are also merited to investigate a mechanistic link between SDOH factors, $PM_{2.5,}$ and lung cancer disparities.
Limitations: Patient zip codes may not reflect long-term residential histories. Minimal PM2.5 variability at the zip code level warrants higher-resolution mapping. Racial comparisons were limited to AA and White patients, prompting broader demographic inclusion in future studies.

#4926 African American patients with stage III laryngeal squamous cell carcinoma have poorer survival despite similar definitive treatment and follow-up care.

Pranay Adavelly[1], Helen Gandler[1], Jessica Bauman[2], Jeremy Price[3], Curtis Miyamoto[4], Parth Anil Desai[3]

[1]Medicine, Lewis Katz School of Medicine, Philadelphia, PA,[2]Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA,[3]Fox Chase Cancer Center, Philadelphia, PA,[4]Radiation Oncology, Fox Chase Cancer Center, Philadelphia, PA

Background: Laryngeal squamous cell carcinoma (LSCC) represents a significant subset of head and neck squamous cell carcinomas (HNSCC), contributing to substantial morbidity and mortality. While disparities in outcomes and care delivery for HNSCC and LSCC have been documented, there is a lack of contemporary literature exploring these disparities in the modern era of widespread chemoradiation therapy and advancements in radiation therapy (RT), chemotherapy, and supportive care.
Methods: We conducted a retrospective review of our single-institution database, identifying patients diagnosed with LSCC between January 1, 2014, and December 31, 2022. The study focused on assessing disparities in care and outcomes between African American (AA) and European American (EA) patient populations. Patients were staged according to the AJCC 8th edition criteria.
Results: Data from 251 patients with LSCC were analyzed. Focus on subgroup analysis of AA and EA ancestries (n=202) revealed a higher incidence among AA women (35% vs. 22% in EA), similar smoking rates in both groups (>94%), and a higher incidence of government insurance (Medicare/Medicaid) among AA patients(85% vs. 75% in EA). Compared to EA patients, AA patients presented with higher Stage IV disease (43% vs. 32%), predominantly due to higher rates of T4 tumors (18% vs. 9%) and more frequent N2 nodal involvement (29% vs. 21%). Rates of distant metastases at presentation were low in both groups (3.1% in EA and 3.8% in AA). For patients with Stage III/IV disease, the majority (90%) received combined modality chemoradiation therapy, with a median definitive RT dose of 7,000 cGy delivered in both populations (p=0.52). Upfront or salvage laryngectomy rates for Stage IV non-metastatic disease were similar between AA and EA patients (20%(AA) vs. 19% (EA)). Stage-specific overall survival (OS) analysis demonstrated significantly poorer survival for AA patients with Stage III LSCC (median OS = 30 months) compared to EA patients (median OS = 95 months) (p=0.01; hazard ratio [HR] 2.741, 95% confidence interval [CI] 1.229-6.116), despite similar median follow-up times for surviving patients (AA mean = 67 months [95% CI 56-79 months]; EA mean = 65 months [95% CI 54-77 months]; p=0.81). This difference in survival was not observed in Stage I, II, IV, or stage-agnostic analyses.
Conclusion: AA patients with Stage III LSCC exhibit significantly poorer survival compared to EA patients, despite receiving similar definitive treatment and follow-up care. These findings suggest that biological factors may contribute to outcome disparities beyond socioeconomic factors, highlighting the need for further research into underlying mechanisms and the development of tailored interventions.

#4927 Tumor tissue microbiome in the high-grade serous ovarian cancer: a pilot investigation within the African American Cancer Epidemiology Study.

Tengteng Wang[1], Lauren C. Peres[2], Jeffrey R. Marks[3], Courtney E. Johnson[4], Elisa V. Bandera[1], Joellen M. Schildkrau[4]

[1]Rutgers Cancer Institute, New Brunswick, NJ,[2]Moffitt Cancer Center, Tampa, FL,[3]Duke University, Durham, NC,[4]Emory University, Atlanta, GA

Background: High-grade serous ovarian cancer (HGSOC) is the most common histological subtype of ovarian cancer. Notably, Black and African American (AA) women face a ~40% higher mortality risk from this disease compared to White women. Recent studies have suggested that intratumor microbes may play a key role in shaping the tumor microenvironment and influencing the prognosis of ovarian cancer. However, to date, no studies have investigated the tumor microbiome in Black/AA women diagnosed with HGSOC using formalin-fixed paraffin-embedded (FFPE) samples and a whole metagenome sequencing approach.
Methods: We analyzed tumor FFPE samples from 20 HGSOC cases who self-identified as Black/AA and participated in the African American Cancer Epidemiology Study (AACES). Tumor DNA was extracted using a standardized protocol, and microbial profiling was performed using 2bRAD sequencing for microbiome (2bRAD-M), a high-precision, reduced-metagenome sequencing technique that enables species-level resolution. To assess tumor microbial differences by cancer stage and age at diagnosis, we compared tumor tissue microbial diversity, taxonomic composition, and functional pathways. Conventional statistical analyses (two-sample t-test and analysis of variance) were employed to evaluate significant differences across groups.
Results: 2bRAD-M sequencing successfully detected tumor microbial DNA in all 20 participants of this pilot study. A total of 285 microbial species were identified, including 160 genera and 11 phyla, from results that passed quality control assessments. The mean alpha diversity, as measured by the Shannon index, was 2.41, and the Chao1 index was 15. Compared to women with localized (stage 1 and 2, n=8) HGSOC, those with stage 3 HGSOC (n=12) exhibited higher Shannon (mean 2.45 vs. 1.57, p=0.12) and Chao1 indices (mean 21 vs. 13, $p < 0.001$). Stage 3 tumors also showed a higher relative abundance of microbial species from the phyla Pseudomonadota, Bacteroidetes, and Bacillota, compared to localized stage tumors ($p < 0.001$). Pathway analysis identified secondary bile acid biosynthesis as the most significantly enriched pathway in stage 3 tumors, with over 1.5-fold higher abundance of this pathway ($p < 0.0001$) than stage 1 and 2 tumors. Age at diagnosis was also associated with microbiome composition; women diagnosed at a younger age (<50) had higher Shannon and Chao1 indices compared to older women. Additionally, women with younger age at diagnosis vs. ≥50) were more likely to have a lower relative abundance of Bacteroidetes and a higher abundance of the prolactin signaling pathway.
Conclusion: Our pilot findings suggest that HGSOC tumor microbiome features measured among Black/AA women differ by stage and age at diagnosis, and thereby may influence the progression of HGSOC. Further studies with larger sample size are warranted as the next step.

#4929 Differences in cancer risk by environmental disparities in Puerto Rico: findings from 2016-2020 cancer data.

Nancy Raquel Cardona[1], Ana P. Ortiz[1], Carlos R. Torres[2], **Polaris Torres-Rodriguez**[3], Karen Ortiz-Ortiz[2], Noreen Michael[4], Pablo Mendez-Lazaro[5], Marievelisse Soto-Salgado[1]

[1]Cancer Control and Population Sciences, UPR - Comprehensive Cancer Center, San Juan, PR,[2]Puerto Rico Central Cancer Registry, UPR - Comprehensive Cancer Center, San Juan, PR,[3]Cancer Control and Population Sciences, UPR - Medical Sciences Campus, San Juan, PR,[4]University of the Virgin Islands, St Thomas, Virgin Islands, U.S.,[5]Environmental Health, UPR - Medical Sciences Campus, San Juan, PR

Background: Chemical contaminants have been established as risk factors for certain types of cancer including breast and prostate, which are the most commonly diagnosed cancers in Puerto Rico among women and men, respectively. By law, industries report their chemical emissions to air, water, and soil to the Environmental Protection Agency's Toxic Release Inventory Sites (TRI) program. In 2006, Puerto Rico had 148 TRI facilities across 49 of the 78 municipalities, releasing 518 chemicals, including 170 carcinogens such as arsenic, benzene, radon, and trichloroethylene. This ecological study assesses the strength of the association between cancer risk and municipalities with environmental disparities (contaminated vs. non-contaminated sites) in Puerto Rico.
Methods: Cancer incidence data from 2016 to 2020 were obtained from the Puerto Rico Central Cancer Registry. Contaminated sites, defined as municipalities with TRI facilities, were identified using data from the TRI program in 2006 (10 years before the estimation of cancer risk). To estimate cancer risk between contaminated and non-contaminated sites, we calculated the standardized incidence relative risk (RR = standardized rate of contaminated sites / standardized rate of non-contaminated sites) with 95% confidence intervals (95% CI) from 2016 to 2020 by type of cancer and sex. All analyses were conducted using SEER*Stat v8.4.4.
Results: Between 2016 and 2020, 71,814 new cancer cases were diagnosed in Puerto Rico, 37,582 among males and 34,232 among females. The RR for residents of contaminated sites showed a significant excess risk of 7% (95%CI: 1.05-1.09, p<0.0001) for all types of cancer compared to residents of non-contaminated sites; similar findings were observed in both men (9%, CI 1.06-1.12, p<0.0001) and women (6%, CI 1.03 -1.09). When evaluating the risk of female-related cancers, excesses of 6% (95%CI: 1.01-1.11, p=0.01) and 24% (95%CI: 1.04-1.49, p=0.02) were observed for breast and ovarian cancers, respectively, among residents of contaminated sites compared to residents of non-contaminated sites. For male-related cancers, an excess risk of 10% (95%CI: 1.06-1.14, p<0.0001) was observed for prostate cancer among residents of contaminated sites compared to residents of non-contaminated sites.
Conclusions: Our findings suggest a significant excess in cancer incidence among residents in municipalities with TRI facilities overall and for certain cancer types. These results underscore the need for targeted environmental interventions and public health actions to mitigate exposure to carcinogens in Puerto Rico.
Acknowledgments: This study was supported by the Caribbean Climate Change Adaptation, Cancer, and Health Disparities Research Center (CARIB-CARES) funded by the National Cancer Institute (NCI, award # 1P20CA294096-01).

#4930 Age and sex differences in the prevalence of specific comorbidities at diagnosis among pediatric acute lymphoblastic leukemia and lymphoblastic lymphoma patients.

Xin Yang[1], Maua Mosha[2], Dave Bell[1], Jennifer Dean[2], Jennifer Mayer[2], Ernest K. Amankwah[3]

[1]Medical College of Wisconsin, Milwaukee, WI,[2]Johns Hopkins All Children's Hospital, St. Petersburg, FL,[3]Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD

Introduction: Comorbidities affect treatment outcomes in adult cancer. However, limited knowledge exists on the prevalence of comorbidity among pediatric patients diagnosed with acute lymphoblastic leukemia (ALL) or lymphoblastic lymphoma (LL). The aim of this study was to determine the prevalence of comorbidities at diagnosis among pediatric ALL/LL patients, and to examine if the prevalence varied by age, biological sex at birth, and race/ethnicity.

Methods: We analyzed data on patients diagnosed with ALL/LL at ≤21 years of age from 1/1/2005 to 6/30/2020 (n=5,455), using data from the TriNetX Research Network that has de-identified patient-level data from electronic health records. All diagnoses were identified based on ICD-9-CM and ICD-10-CM. Comorbidities were categorized as pulmonary disease; cardiac disease; developmental disorder; vascular disease; cerebrovascular disease; immune disorder; metabolic disorder; infectious disease; genitourinary disease; digestive tract disease; muscle and connective tissue disease; and nervous system disease. Only comorbidities documented within three months prior to the initial ALL/LL diagnosis were included in the analysis.

Results: Overall, 34.1% (n=1,904) of patients had at least one comorbidity at diagnosis and 4.01% of the entire cohort (n=219) had ≥4 comorbidities. Females (36.6%) had a higher prevalence of overall comorbidity than males (33.6%) (p=0.024) and Non-Hispanic White patients (37.5%) had a higher prevalence of overall comorbidity than other racial/ethnic groups (33.1%) (p< 0.001). The most common comorbidities identified were digestive tract disease (n=667, 12.2%; 95%CI:11.4%-13.1%), muscle and connective tissue disease (n=523, 9.6%; 95%CI:8.8%-10.4%), metabolic disorders (n=513, 9.4%; 95%CI:8.6%-10.2%), nervous system diseases (n=466, 8.5%; 95%CI:7.8%-9.3%), and infectious diseases (n=446, 8.2%; 95%CI:7.4%-8.9%). Analyses of specific comorbidities revealed notable patterns based on sex at birth and age at ALL/LL diagnosis. Females exhibited a higher prevalence of infectious diseases (9.7% vs. 7.0%, p<0.001), digestive tract diseases (13.7% vs. 11.1%, p=0.005), and musculoskeletal conditions (11.0% vs. 8.6%, p=0.004) compared to males. Additionally, younger patients (≤10 years) had a higher prevalence (13.8%) of digestive diseases compared to those diagnosed after 10 years (10.4%, p<0.001).

Conclusion: The overall prevalence of comorbidity among pediatric patients diagnosed with ALL/LL varies by biological sex at birth. Additionally, disparities exist for specific comorbidities, with females showing a higher prevalence of infectious diseases and younger patients having a higher prevalence of digestive tract diseases. Future studies are warranted to determine if these differences contribute to disparities in treatment outcomes.

#4931 Pan-cancer proteomic profiling of the EPIC cohort identifies novel plasma biomarkers of cancer risk.

James Yarmolinsky[1], Vivian Viallon[2], David C. Muller[3], Antonio Agudo[4], Pietro Ferrari[2], Giovanna Masala[5], Salvatore Panico[6], Carlotta Sacerdote[7], Karl Smith-Byrne[8], Ruth C. Travis[8], Rosario Turmino[9], P.Martijn Kolijn[10], Roel C. H. Vermeulen[11], Monique Verschure[11], Nicholas Wareham[12], Elio Riboli[3], Marc J. Gunter[3]

[1]James Yarmolinsky (Individual), London, United Kingdom,[2]International Agency for Research on Cancer, Lyon, France,[3]Imperial College London, London, United Kingdom,[4]Catalan Institute of Oncology, Barcelona, Spain,[5]Institute for Cancer Research, Prevention and Clinical network, Florence, Italy,[6]University of Naples Federico II, Naples, Italy,[7]University of Turin and CPO Piemonte, Turin, Italy,[8]University of Oxford, Oxford, United Kingdom,[9]Hyblean Association for Epidemiology Research, AIRE ONLUS, Ragusa, Italy,[10]Erasmus MC, Rotterdam, Netherlands,[11]UMC Utrecht, Utrecht, Netherlands,[12]University of Cambridge, Cambridge, United Kingdom

Plasma proteins play critical roles in various cancer-related processes, are frequently dysregulated in cancer, and represent a key source of druggable targets. The integration of high-throughput proteomic assays into large-scale population-based studies may provide important insights into mechanisms of cancer pathogenesis, identify biomarkers for cancer risk and early detection, and prioritize novel therapeutic targets for cancer prevention. Here, we describe initial findings from proteomic profiling of baseline plasma samples from a case-cohort of 6,073 incident cancer cases and 4,115 sub-cohort members within the European Prospective Investigation into Cancer and Nutrition (EPIC) using the SomaScan 7K assay. Participants at baseline were 53.8 (SD 8.7) years of age and 57.3% were women. Prentice weights and robust variance were used to account for the case-cohort design of the study. Models were stratified on sex, center, and age at recruitment and adjusted for education status and cancer type-specific risk factors. Pre-diagnostic plasma concentrations of 7,596 protein aptamers (representing 6,432 proteins) were evaluated for association with 24 cancer types. In total, 2,264 protein aptamer-cancer associations were observed at FDR $P < 0.05$, including 53 for overall cancer risk. The majority of aptamers (80%, 1,422/1,770) were associated with one cancer type with 11 associated with 4 or more types, including aptamers binding to GDF15, MMP12, and TNFRSF1B. Proteins represented among FDR-significant aptamers map to a diverse set of biological processes including the epithelial-mesenchymal transition, coagulation, and inflammatory signaling pathways. Around half of all aptamer-cancer associations (52%, 1,173/2,264) remained FDR-significant after excluding the first 5 years of follow-up, supporting the potential role of proteins in etiological processes, as opposed to being markers of latent/undiagnosed cancer at baseline. The number of FDR-significant associations per cancer type varied substantially, ranging from 885 for hepatocellular carcinoma and 403 for chronic lymphocytic leukemia to 3 for both pancreatic and thyroid cancer and 2 for endometrial cancer (median 23 aptamers per cancer type, IQR: 6-65). Our plasma-based proteomic profiling of a large case-cohort analysis established within the EPIC study has identified potentially novel biomarkers across a range of cancers, with evidence of both shared and distinct proteomic profiles across cancer types. Replication of findings in independent cohort studies and further interrogation of mechanistic pathways will help to validate proteins identified in this analysis and clarify their roles as potential markers for the early detection, prediction, and prevention of cancer.

#4932 Modeling uncertainty in personalised breast cancer risk prediction.

Bethan L. White, Lorenzo Ficorella, Xin Yang, Douglas F. Easton, Antonis C. Antoniou

Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom

Breast cancer risk prediction models use a range of predictors to estimate an individual's chance of developing breast cancer in a given timeframe. These can facilitate risk stratification, to identify individuals who would benefit most from screening or preventive options. The BOADICEA breast cancer risk model, implemented in the CanRisk tool (www.canrisk.org), uses genetic, lifestyle, hormonal, family history and anthropometric data to estimate an individual's risk. When implementing risk prediction models, risk predictor data are often incomplete. Point-estimates calculated when some risk factor data are missing can hide considerable uncertainty.

We developed a methodological approach for quantifying uncertainty and the probability of risk-reclassification in the presence of missing data. We employed Monte-Carlo simulation methods to estimate the distribution of breast cancer risk for individuals with missing data. Multiple imputation by chained equations (MICE) with UK Biobank as a reference dataset was used to sample missing covariates. We developed a framework for estimating uncertainty, that can be applied to any given individual with missing risk factor data. We used exemplar cases to assess the probability that collecting missing data would result in a change in risk categorisation, on the basis of the 10-year predicted risk from age 40, using the UK National Institute for Health and Care Excellence (NICE) guidelines.

For example, a woman known to have a pathogenic variant in *CHEK2*, but with all other information unmeasured, will be categorised as at "moderate risk" by the BOADICEA model. Measuring lifestyle and hormonal risk factors would result in reclassification to either the "population-" or "high-risk" group in around 24% of cases (respectively 23% and 1%). Measuring only a polygenic score (PGS) would reclassify the individual about 43% of the time into either the "population-" or "high risk" group (respectively 34% and 9%); measuring a PGS and lifestyle and hormonal risk factors would reclassify the individual to the "population-" or "high-risk" group in around 51% of cases (respectively 43% and 8%).

The results demonstrate that there may be a considerable likelihood of reclassification into a different risk category after collecting missing data. The methodology presented here can identify situations where it would be most beneficial to collect additional patient information, and enable better informed clinical decision making.

#4933 Leveraging chromosome-scale length variants for ovarian cancer risk prediction.

Sarvenaz Fatapour, James P. Brody

Biomedical Engineering, University of California, Irvine, Irvine, CA

Introduction: Polygenic risk scores are tools used to predict outcomes based on germline genetics. Typically, human germline genetics are described by millions of SNPs. These scores often rely on linear combinations of SNPs to estimate risk, though nonlinear interactions may also contribute. Advanced machine learning techniques are capable of detecting non-linear patterns between groups, but they require a dataset with far more subjects than features, making it infeasible to analyze genetic differences between populations without millions of participants. To address this, we developed a genome representation using a small set of values that summarize chromosome-scale length (CSLV) variations. Using this method and machine learning, we tested whether there are genetic distinctions between women who develop ovarian cancer and those who do not.

Methods: To test our hypotheses, data from the NIH All of Us Research Program was used to determine whether CSLV can predict the occurrence of ovarian cancer among women from diverse racial backgrounds. CSLVs were computed from germline DNA for each participant, and we constructed a dataset comprising women diagnosed with at least one of the following conditions: malignant neoplasm or tumor of the ovary, malignant epithelial tumor of the ovary, cell adenocarcinoma of ovary, ovarian cystadenocarcinoma, or endometrioid carcinoma of the ovary (TRUE). An equal number of matched controls without any of these conditions (FALSE) were randomly selected using an exact matching method based on age, race, and sex at birth. Machine learning models were developed using the H2O AI platform to differentiate TRUE/FALSE groups. Model performance was evaluated by calculating the area under the receiver operating characteristic curve (AUC) on unseen test data, with scrambled labels as controls.

Results: CSLV parameters were calculated for 312,944 patients from the NIH All of Us V7 controlled tier with genetic microarray data. We then identified a study cohort that included 1,120 patients diagnosed with ovarian cancer at least once during the study period. The average age at the time of first diagnosis with ovarian cancer within the cohort is 55 years. Most ovarian cancer patients self-identified themselves as White (736), None Indicated (168), Black or African American (125), Asian (29), and multiracial (14). Using CSLV values, we evaluated the ability of our model to differentiate between patient groups by assessing the AUC. The AUC analysis provided insight into whether CSLV-based models could reliably predict ovarian cancer risk and occurrence, offering a potential tool for stratifying patients based on genetic risk.

Conclusion: A CSLV can serve as an effective genomic representation for predicting ovarian cancer risk and occurrence. By leveraging CSLV and machine learning, this approach has the potential to improve early detection and personalized risk assessment for ovarian cancer patients.

#4934 Achieving the goal of reducing cancer mortality by 50% through early detection: Insights from simulation modeling of multi-cancer screenings.

Jiheum Park[1], Stephanie Shue[1], Francesca Lim[1], Matthew Prest[1], Jenniffer Ferris[1], Jeong Yun Yang[1], Stella Kang[2], Chin Hur[1]

[1]General Medicine, Columbia University Irving Medical Center, New York, NY,[2]Radiology, New York University Langone Health, New York, NY

The Cancer Moonshot aims to reduce cancer mortality by at least 50% (~4 million cancer deaths) over the next 25 years. While cancer incidence has slightly increased from 2014 to 2018, particularly among young adults aged 15-39, cancer mortality has decreased by 33% from 1991 to 2021. This decline is largely attributed to advances in early detection, improved treatments, and reduced smoking rates, especially for lung cancer. However, the approach and feasibility of meeting the Moonshot goal remain uncertain. This study explores the potential for achieving the Cancer Moonshot goal by focusing on early detection as a primary strategy. We developed a simulation model to evaluate multi-cancer early detection/screening scenarios, using data from the NCI's SEER database. Our model focused on the five leading cancers by mortality—lung, colorectal, pancreatic, breast, and prostate—since these cancers account for the majority of cancer-related deaths in the U.S. and represent the most significant opportunity for mortality reduction through early intervention. Using a discrete event simulation model, we simulated a cohort of U.S. individuals aged 0 to 100 years in 1994 and tracked them over a 25-year period. The model was calibrated to reproduce U.S. population cancer incidence and mortality trends from 1994 to 2019, based on SEER data. To examine the impact of early detection, the model incorporated sojourn time—the period during which preclinical cancer is detectable. Sojourn times for each cancer type were randomly sampled from a triangular distribution based on values derived from a comprehensive literature review. We modeled a “perfect” scenario where cancers were detected at the earliest point in the sojourn period, at localized stages, to estimate the maximum benefit of early detection. We then compared the number of cancer deaths averted in this scenario to a natural history model with no screening. Our preliminary findings suggest that screening for all five cancers could achieve the Cancer Moonshot goal of reducing cancer deaths by 50%. Lung cancer screening provided the greatest benefit, reducing cancer deaths by ~20%, followed by breast cancer screening (~5%). Pancreatic cancer screening offered the smallest reduction, at about 2%. While these results underscore the potential of early detection in reducing cancer mortality, the model assumes perfect screening sensitivity and specificity and patient adherence, conditions that do not reflect real-world implementation. To refine these estimates and develop actionable strategies, future research should focus on addressing practical limitations, including variability in screening uptake and accuracy. Improving early detection methods, expanding access to screening, and increasing public awareness are essential to making the Cancer Moonshot goal a realistic and achievable outcome.

#4935 Disentangling the association between age and overall survival in an international cohort of patients with advanced prostate cancer.

Hannah E. Guard[1], Michelle O. Sodipo[1], Colleen B. McGrath[1], Anna Siefkas[1], Lauren E. Howard[2], Konrad H. Stopsack[1], Christopher M. Sauer[3], Robert Dreicer[4], Emilio Esteban[5], Hassan M. Dogo[6], Ademola Popoola[7], Charles Waihenya[8], Anders Bjartell[9], Kim N. Chi[10], Sebastien Hotte[11], Richard Cathomas[12], Deborah Enting[13], Scott Tagawa[14], Michael Ong[15], Michael Kolinsky[16], Vincent Khoo[17], Joaquin Mateo[18], Chidiebere N. Ogo[19], Rana R. McKay[20], Stefanie Fischer[21], Heather H. Cheng[22], Russell Szmulewitz[23], Young E. Whang[24], Anand Sharma[25], Frederic Pouliot[26], Simon Crabb[27], Monica S. Chatwal[28], Miguel A. Climent[29], Raymond McDermott[30], Ian D. Davis[31], Camille Ragin[32], Folakemi T. Odedina[33], Simon Anderson[34], Simone Badal[35], Natalie Greaves[36], Karen A. Autio[37], Laurel Cannon[38], Alyssa Chan-Cuzydlo[38], Marie Grant[38], Travis Gerke[38], Hannah D. McManus[2], Philip W. Kantoff[39], Daniel J. George[2], Lorelei A. Mucci[1], on behalf of the IRONMAN investigator team

[1]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA,[2]Duke Cancer Institute, Durham, NC,[3]West German Cancer Center, University Hospital Essen, Essen, Germany,[4]University of Virginia, Charlottesville, VA,[5]Department of Medical Oncology, Hospital Universitario Central of Asturias, ISPA, Oviedo, Spain,[6]University of Maiduguri Teaching Hospital, Maiduguri, Nigeria,[7]University of Ilorin Teaching Hospital, Ilorin, Nigeria,[8]Department of Surgery, Faculty of Health Sciences, University of Nairobi, Nairobi, Kenya,[9]Department of Urology, Skane University Hospital, Malmo, Sweden,[10]British Columbia Cancer Agency, Vancouver, BC, Canada,[11]Juravinski Cancer Centre, Hamilton, ON, Canada,[12]Kantonsspital Grabunden, Chur, Switzerland,[13]Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom,[14]Weill Cornell Medical Center, New York, NY,[15]Ottawa Hospital Cancer Centre, Ottawa, ON, Canada,[16]Cross Cancer Institute, Edmonton, AB, Canada,[17]The Royal Marsden NHS Foundation Trust, London, United Kingdom,[18]Vall d'Hebron Institute of Oncology, Barcelona, Spain,[19]Federal Medical Centre Abeokuta, Abeokuta, Nigeria,[20]University of California San Diego, San Diego, CA,[21]Kantonsspital St. Gallen, St. Gallen, Switzerland,[22]University of Washington Medical Center and Fred Hutchinson Cancer Center, Seattle, WA,[23]University of Chicago, Chicago, IL,[24]University of North Carolina, Chapel Hill, NC,[25]Lister Hospital, Chelsea, United Kingdom,[26]CHU de Quebec-Universite Laval, Quebec, QC, Canada,[27]University Hospital Southampton NHS Foundation Trust, Southampton, United Kingdom,[28]Moffitt Cancer Center, Tampa, FL,[29]Instituto Valenciano de Oncologia, Valencia, Spain,[30]Tallaght University Hospital, Dublin, Ireland,[31]Monash University Eastern Health Clinical School, Melbourne, Australia,[32]Fox Chase Cancer Center, Philadelphia, PA,[33]Mayo Clinic, Jacksonville, FL,[34]George Alleyne Chronic Disease Research Centre, The University of the West Indies, Cavehill, Barbados,[35]University of the West Indies, Mona, Jamaica,[36]Faculty of Medical Sciences, The University of the West Indies, Cave Hill, Barbados,[37]Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY,[38]Prostate Cancer Clinical Trials Consortium, New York, NY,[39]Convergent Therapeutics, Cambridge, MA

Introduction: Most people who die from prostate cancer are older than 75 years. However, prostate cancer diagnosed at younger ages is often more aggressive. While epidemiologic studies have examined the association between age and survival in patients with prostate cancer overall, the clinical drivers of this relationship in patients with advanced disease remain unclear. We leveraged the International Registry of Men with Advanced Prostate Cancer (IRONMAN, *NCT03151629*) to (1) quantify the association between age and overall survival and (2) explore whether this association is explained by treatments.

Methods: We included 3,734 patients enrolled from 15 countries with new diagnoses of metastatic hormone sensitive (mHSPC, N=2,642) or castration resistant prostate cancer (CRPC, N=1,092). Age at enrollment was categorized as <55, 55-59, 60-64 (reference), 65-69, 70-74, 75-79, 80-84, 85+ years. We computed age-specific death rates (95% confidence intervals [CIs]) by disease state (mHSPC, CRPC) at enrollment. To explore whether this relationship was explained by treatment, we fitted disease state-stratified Cox models and sequentially adjusted for (1) *de novo* metastatic status and country, and (2) baseline treatment (androgen deprivation therapy [ADT] alone, ADT + androgen receptor pathway inhibitors [ARPI], ADT + chemotherapy, ADT + ARPI + chemotherapy, other).

Results: Over 5.5 years of follow-up (median: 3.2 years, IQR: 1.7, 4.8), 995 patients died from any cause. Younger patients were more likely to have *de novo* metastatic disease. Among those with mHSPC at enrollment, the age-specific death rates per 1,000 person-years followed a J-shaped pattern: 80 (95% CI: 54, 117) in patients under 55 years, 58 (46, 74) in patients aged 60-64, 102 (78, 133) in patients aged 80-84, and 172 (125, 236) in patients aged 85 and older. The death rates among those with CRPC followed a similar pattern, albeit rates were higher in magnitude: 212 (95% CI: 140, 323) in patients under 55 years, 153 (115, 202) in patients aged 60-64, 200 (155, 258) in patients aged 80-84, and 204 (142, 291) in patients aged 85 and older. After adjusting for disease state, country, and the timing of diagnosis of metastases, death rates were substantially higher for ages 75-79 (HR 1.4; 95% CI 1.1, 1.7), 80-84 years (HR 1.6; 95% CI 1.2, 2.1), and 85 years and older (HR 2.0; 95% CI: 1.5, 2.7) compared to ages 60-64 years. Patients aged under 55 years had 1.4-fold higher death rates (95% CI: 1.0, 1.9) compared to those 60-64 years. After additionally adjusting for baseline treatment, overall survival remained worse for the older patients and the youngest age group with hazard ratios remaining meaningfully unchanged.

Conclusion: Age at enrollment is associated with overall survival in people with advanced prostate cancer enrolled in IRONMAN in a J-shaped pattern. This relationship is not explained by differences in baseline treatment.

#4936 The association between plasma IGFBP7 and cancer incidence, cancer mortality, and all-cause mortality in ARIC.

Vernon A. Burk[1], Michael N. Pollak[2], Corinne E. Joshu[1], Anna Prizment[3], Elizabeth A. Platz[1]

[1]Johns Hopkins Bloomberg Sch. of Public Health, Baltimore, MD,[2]McGill University, Montreal, QC, Canada,[3]University of Minnesota, Minneapolis, MN

Background: Insulin-like Growth Factor Binding Protein 7 (IGFBP7) may modulate IGF signaling and influence non-IGF molecular mechanisms. We analyzed whether IGFBP7 concentration in plasma is associated with risk of cancer incidence, cancer mortality, and all-cause mortality.

Methods: We conducted a prospective cohort analysis of 10,834 participants (54.8% female, 24.3% Black) with no cancer history for cancer incidence and mortality analyses, and 11,761 participants (55.6% female, 23.6% Black) for the all-cause mortality analysis in the Atherosclerosis Risk in Communities study. The median follow-up time was 22.8, 23.5, and 25.6 years for the respective analyses. Participants were aged 46-70 years old at start of follow-up. Plasma IGFBP7 was measured using SomaScan® 5K assay, an aptamer-based technology for protein profiling. Incident cancer diagnoses were ascertained by state cancer registry linkage supplemented with medical records and death certificates. Mortality was ascertained by death certificates, including via the National Death Index. We estimated the association between IGFBP7 plasma levels (log_2transformed) and outcomes including cancer incidence, cancer mortality, and all-cause mortality using Cox proportional hazards regression models adjusting for age, sex, race, and other potential risk factors for cancer and/or mortality.

Results: The association between IGFBP7 and total cancer incidence (3,347 cases, 200,279 person-years) was null (HR=0.99, 95% CI 0.85-1.16). However, IGFBP7 was positively associated with lung cancer incidence (479 cases, 199,823 person-years; HR=1.40, 95% CI 0.93-2.10), unlike for other common cancer sites. The association between IGFBP7 and total cancer mortality (1,420 deaths, 222,320 person-years) was statistically significant (HR=1.39, 95% CI 1.10-1.76). Of the common cancers, IGFBP7 was positively associated with lung cancer mortality (HR=1.69, 95% CI 1.10-2.60) and breast cancer mortality (HR=2.09, 95% CI 0.88-4.97). The association between IGFBP7 and all-cause mortality (6,981 deaths, 262,734 person-years) was statistically significant and the strongest among the three main analyses (HR=1.72, 95% CI 1.55-1.91). Stratifying by age, the strongest association between IGFBP7 and all-cause mortality was in the youngest age quartile (ages 46.9-52.6 years; HR=2.69, 95% CI 2.01-3.59). IGFBP7 is not strongly correlated with IGF-1 and other family proteins.

Conclusions: High circulating IGFBP7 levels may be a risk factor for cancer and all-cause mortality, especially among those in middle adulthood. High circulating IGFBP7 levels may also be a risk factor for both lung cancer incidence and mortality. Additional research and laboratory testing will be necessary to determine whether IGFBP7's influence on IGF signaling or other mechanisms contribute to tumor progression and mortality, in general.

Support: NHLBI, NCI, NPCR

#4937 Circulating leukocyte composition and risk of colorectal cancer recurrence: Findings from the ColoCare Study.

A. Richards[1], E. Jean-Baptiste[1], T. Barrow[2], S. Hardikar[3], B. Gigic[4], J. Figueiredo[5], C. Li[6], D. Shibata[7], A. T. Toriola[8], D. Byrd[1], C. M. Ulrich[3], P. Stewart[1], E. M. Siegel[1], **J. K. Kresovich**[1];

[1]H. Lee Moffitt Cancer Center, Tampa, FL, [2]University of Essex, Essex, United Kingdom, [3]Huntsman Cancer Institute, Salt Lake City, UT, [4]Heidelberg University Hospital, Heidelberg, Germany, [5]Samuel Oschin Cedars-Sinai Medical Center, Los Angeles, CA, [6]Fred Hutchinson Cancer Center, Seattle, WA, [7]University of Tennessee Health Science Center, Memphis, TN, [8]Washington University School of Medicine, St. Louis, MO

Introduction: Disease recurrence occurs in approximately 20% of colorectal cancer (CRC) patients and is a major driver of CRC-specific mortality in those with non-metastatic CRC. Interactions between the immune system and malignant tumors can select for immune-resistant cell populations, increasing the risk of recurrence. Although immune cell profiles of the tumor microenvironment are associated with CRC recurrence, less is known about how recurrence risk is influenced by the composition of circulating leukocytes. Circulating leukocyte profiles reflect systemic immune activation and may provide unique mechanistic insights and predictive value for recurrence risk that can personalize treatment strategies and improve patient outcomes. However, the relationship between circulating leukocyte profiles and CRC recurrence risk has not been examined in detail.

Methods: The ColoCare Study is an international, multi-center, prospective cohort of newly diagnosed CRC patients. For this study, we selected a nested case-control sample of 137 patients enrolled at Moffitt Cancer Center, matching recurrence cases 1:3 to recurrence-free controls by age, sex, body mass index, stage, and follow-up time. Pre-treatment whole blood DNA methylation profiles were assayed and deconvolved to estimate proportions of twelve circulating leukocyte subsets (naïve and memory B cells, CD8+ T cells, and CD4+ T cells, Tregs, natural killers, monocytes, eosinophils, basophils, and neutrophils). Absolute counts were calculated by multiplying cell proportions by total leukocyte count at the time of blood draw. Logistic regression models, adjusted for matching factors and tumor location, estimated odds ratios (ORs) for associations between each leukocyte subset (count and proportion; per 1-standard deviation [SD] change) and risks of CRC recurrence and all-cause mortality.

Results: Patients had a mean age of 62.7 years at diagnosis (SD= 13), with a majority being male (57%), overweight (78%), and White (90%). During follow-up, 35 (25%) patients experienced disease recurrence, and 47 (34%) died. Higher proportions and counts of circulating memory B cells were positively associated with CRC recurrence risk (proportions, OR: 1.84, 95% CI: 1.20, 2.84, P= 0.006; counts, OR: 2.33, 95% CI: 1.33, 4.06, P= 0.003). Higher proportions and counts of circulating neutrophils were positively associated with all-cause mortality (proportions, OR: 1.52, 95% CI: 0.95, 2.44, P= 0.08; counts, OR: 1.59, 95% CI: 1.06, 2.40, P= 0.03).

Conclusions: The composition of circulating leukocyte in pre-treatment blood samples is associated with CRC recurrence risk and mortality. These findings suggest that specific leukocyte subsets may be novel risk factors for CRC recurrence, providing insights into mechanisms underlying disease progression.

#4938 Socioeconomic position and survival after lung cancer diagnosis: Comparisons in 4 continents.

Justina Ucheojor Onwuka, Karine Alcala, **Hilary A. Robbins**, Mattias Johansson, The Lung Cancer Cohort Consortium

Genomic Epidemiology Branch, International Agency for Research on Cancer, Lyon, France

Background: The incidence of lung cancer is lower among higher socioeconomic position (SEP) groups in most settings. It is important to assess whether this translates to disparities in survival by SEP after lung cancer diagnosis. We sought to describe the relationship between SEP and survival after lung cancer diagnosis across different countries.
Methods: We analyzed data from the Lung Cancer Cohort Consortium (LC3) and included 18 prospective cohorts from 14 countries in North America, Europe, Asia, and Australia. The total sample size included 48,482 lung cancer cases with pre-diagnostic risk factor information, amongst whom 37,559 died during the follow-up (median, IQR= 10.8, 6.5-15.0 years). Information on education was harmonized using the International Standard Classification of Education and then modeled as an ordinal variable in 4 categories. We evaluated the relationship between educational level and survival after lung cancer diagnosis using Cox proportional hazards models. Models were adjusted for age at diagnosis, sex, and (where applicable) cohort, smoking duration, cigarettes per day, and time since cessation.
Results: We observed a clear trend toward better survival in participants with higher educational level in lung cancer cases diagnosed in North America, Europe and Asia, but not in Australia. After accounting for detailed smoking history, the hazard ratios were partly attenuated (North America: hazard radio per 1-unit increase in educational level [HR]=0.94, 95%CI=0.93-0.96; Europe: HR=0.94, 95%CI: 0.92-0.95; Asia: HR=0.81, 95%CI=0.77-0.85), but not in Australia. When additionally adjusting for stage at diagnosis, the inverse relationship between educational level and survival was further attenuated in Asian lung cancer cases, but not in European or North American study participants. We estimated that differences in tobacco exposure mediated approximately 20% of the relationship between education and lung cancer survival, and that disease stage mediated an additional 14% of this relationship in Asian lung cancer cases.
Conclusion: Higher educational attainment is consistently associated with better lung cancer survival in North America, Europe, and Asia. Differences in tobacco exposure may partly, but not entirely mediate this relationship, and differences in stage at diagnosis may be additionally important in mediating the survival differences seen in Asian lung cancer cases. This study highlights the importance of socioeconomic position in the continuum of lung cancer disparities.

#4939 Social determinants disadvantage score and risk of liver cancer in the All of Us Research Program.

Xinyuan (Cindy) Zhang[1], Longgang Zhao[2], David Vlahov[2], Yun Chen[2], Tamar Taddei[3], Xuehong Zhang[2]

[1]Harvard Medical School/Brigham and Women's Hospital, Boston, MA,[2]Yale University School of Nursing, Orange, CT,[3]Yale University School of Medicine, New Haven, CT

Background: Social determinants of health (SDOH) are crucial in shaping health outcomes, yet comprehensive studies that span all key SDOH domains (economic stability, education, healthcare access and quality, neighborhood and built environment, and social and community context) to examine their associations with liver cancer risk are sparse. We developed and validated a Social Determinants Disadvantage Score (SDDS) to address this knowledge gap.
Methods: We conducted a cross-sectional analysis of 117,783 participants from the All of Us Research Program. The SDDS was systematically constructed using validated questionnaires covering five domains recognized by the World Health Organization and U.S. Department of Health and Human Services. Each domain was dichotomized into advantage (0) or disadvantage (1), with the SDDS ranging from 0 to 5. Prevalent liver cancer, especially hepatocellular carcinoma (HCC), was identified by the Electronic Health Record. We used logistic regression models to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the associations of SDDS with HCC, adjusting for age, sex, race, ethnicity, birthplace, marital status, smoking status, and alcohol intake.
Results: We identified 110 HCC cases in the participants who completed the SDOH questionnaires. Disadvantage in SDOH, as assessed by higher SDDS, was associated with a higher risk of HCC. Per unit increase in SDDS, the adjusted OR (95% CI) was 1.21 (1.02, 1.43) for HCC. The OR was 5.62 (1.95, 16.22) comparing the most disadvantaged to the most advantaged SDDS group. Strongest associations were observed for disadvantage in economic stability (OR = 2.37; 95% CI = 1.22, 4.63) and education (OR = 1.87; 95% CI = 1.08, 3.24).
Conclusion: The SDDS provides a comprehensive, validated tool for assessing SDOH. Our findings highlight significant associations between social disadvantage and HCC prevalence, emphasizing the need for future longitudinal studies to inform targeted interventions.

Social Determinants Disadvantage Score (SDDS) structural components and criteria for scoring.

Domain and variables	**Disadvantage level scored 1, otherwise 0**	**Total score summed up five domains**
Economic stability		Score 1 if 1 in any subdomain
Annual household income	Less than 25k	
Employed	No	
Current Home Own	Rent	
Food insecurity	Yes	
Housing quality problem	Yes	
Education		Score 1 if 1 in any subdomain
Education Year	Under twelve or GED	
Health care access and quality		Score 1 if 1 in any subdomain
Covered by health insurance	No	
Discrimination in health	≥ 80th percentile	
Neighborhood and built environment		Score 1 if 1 in any subdomain
Neighborhood physical disorder	≥ 80th percentile	
Neighborhood social disorder	≥ 80th percentile	
Neighborhood walkability	≤ 20th percentile	
Neighborhood safety	≤ 20th percentile	
Social and community context		Score 1 if 1 in any subdomain
Social cohesion	≤ 20th percentile	
Social support	≤ 20th percentile	
Loneliness	≥ 80th percentile	
Perceived discrimination	≥ 80th percentile	
Perceived stress	≥ 80th percentile	

#4940 Demographic trends in chronic myelogenous leukemia in adults in the United States between 1999 and 2020.

Saad Rashid[1], Zeeshan Muzammil[2], Taqi Khaja[1]

[1]Mercyhealth Graduate Medical Education Consortium, Rockford, IL,[2]Rosalind Franklin University of Medicine and Science, North Chicago, IL

Chronic myeloid leukemia (CML) represents about 15% of new leukemia cases annually in the United States (US), predominantly affecting older adults with a median diagnosis age of 64. Despite advances in treatment leading to a 5-year survival rate exceeding 70%, disparities in CML mortality persist. This study examines demographic differences and trends in CML mortality from 1999 to 2020 in the US.

We accessed mortality data for CML (ICD-10 code C92.1) from the Centers for Disease Control and Prevention (CDC) Wonder Database from 1999 to 2020. Age-adjusted mortality rates (AAMR) were calculated per 100,000 deaths and stratified by sex (male, female), race (White, African American), and adult age groups. Temporal trends were analyzed using Joinpoint regression software to calculate the annual percent change (APC) and average annual percent change (AAPC) to identify shifts over time. Statistical significance was set at $p < 0.05$.

Between 1999 and 2020, CML accounted for 26,329 deaths in the US, with the overall AAMR decreasing by 50%. Gender-specific trends revealed reductions in mortality for males and females. Among females, the AAMR declined from 0.5 in 1999 to 0.2 by 2007 (APC of -10.6% from 1999 to 2008, $p < 0.01$). For males, the AAMR decreased from 0.8 in 1999 to 0.4 by 2007 (AAPC of -8.9%, $p < 0.01$).

Racial comparisons show that African Americans' AAMR decreased from 0.7 in 1999 to 0.35 by 2008 (APC of -8.5%, $p < 0.01$). White Americans saw a larger reduction, with AAMR declining from 0.65 in 1999 to 0.3 by 2007 (APC -10.0%, $p < 0.01$). Age-specific analyses highlighted decreases in mortality across adult age groups. For individuals aged 45-54, AAMR declined from 0.5 in 1999 to 0.35 by 2020 (APC of -21.2% from 1999 to 2004, $p < 0.05$). For the 55-64 age group, AAMR decreased from 1.0 in 1999 to 0.4 by 2020 (APC of -11.9%, $p < 0.05$). The 65-74 age group experienced a decline in AAMR from 2.25 in 1999 to 0.8 by 2008 (APC of -11.0% from 1999 to 2007, $p < 0.05$). In contrast, the 75-84 age group initially saw an increase in AAMR from 2.4 in 1999 to a peak of 3.8 in 2006, followed by a decrease to 2.25 by 2011. This group exhibited an APC of +19.5% from 2003 to 2006 ($p < 0.05$), followed by a decline of -10.4% from 2006 to 2011 ($p < 0.05$). The oldest group aged 85 years+, had an AAMR of 5.25 in 1999, which decreased to 4.7 by 2020 but remained higher than other age groups.

While mortality from CML in the US has decreased, disparities persist among demographic groups. Older individuals, males, and African Americans exhibit higher mortality rates compared to younger individuals, females, and White Americans. These disparities may result from age-related comorbidities, differential treatment responses, and barriers to timely care among males, and limited healthcare access and socioeconomic disadvantages faced by African Americans. Addressing these disparities through targeted interventions and equitable healthcare access is crucial for further reducing CML mortality.

#4941 Psychosocial factors in association with overall survival after prostate cancer diagnosis among predominantly low-income Black and White adults.

Douglas DeMoulin[1], Loren Lipworth[1], Melinda Aldrich[1], Heather Munro[1], Ronald Fisa[2], Kabisa Mwala[2], Martha J. Shrubsole[1], Wei Zheng[1], Xiao-Ou Shu[1]

[1]Vanderbilt University Medical Center, Nashville, TN,[2]University of Zambia, Lusaka, Zambia

Background: Prostate cancer is the most common cancer and the second leading cause of cancer death among men in the US. Psychosocial factors may influence prostate cancer survival, but this association has not been investigated among a low-income population, particularly in Black men.

Methods: This study used resources of the Southern Community Cohort Study (SCCS), a prospective cohort study of ~85,000 individuals aged 40-79, from predominantly low-income backgrounds, enrolled between 2002-2009 across 12 southeastern states. A total of 1,367 men (309 white and 1,058 black) newly diagnosed with prostate cancer during follow-up of 34,313 SCCS male participants were included. At recruitment, information was obtained on psychosocial variables (including depressive symptoms, religious and spiritual beliefs and practices, and social support) along with sociodemographic, behavioral, and lifestyle factors and medical history. The Kaplan-Meier method was used to generate 5-year survival rates. Statistical significance was assessed using the log-rank test. Multivariable Cox proportional hazard models were used to estimate adjusted hazard ratios (aHR) and 95% confidence intervals (CI) for mortality in association with psychosocial variables with adjustment for potential confounders.

Results: Over 18 years of follow-up among participants with prostate cancer, 291 Black (27.5%) and 59 White patients died (19.1%) (P = 0.03). White men with prostate cancer self-reporting major depressive symptoms had an 11% lower 5-year survival ($P_{survival}$ = 0.01) and were at higher risk of dying (aHR = 2.26, 95% CI = 1.02-5.03) compared to White men reporting no depressive symptoms. There was a 41% higher risk of death per standard deviation increase of depression score (95% CI = 1.05-1.88). The depression-mortality association was attenuated after adjusting for inability to control important things in life (aHR = 1.61, 95% CI = 0.62-4.23) and marital status (aHR = 1.97, 95% CI = 0.87-4.46). Additionally, White men self-reporting inability to control important things in life as "*much, most, or all of the time*" had a 12% lower 5-year survival ($P_{survival}$ = 0.01) and were at increased risk of dying (aHR = 2.30, 95% CI = 1.07-4.95) compared to White men reporting "*rarely or none of the time*". No statistically significant associations were found among Black men with prostate cancer. Testing for multiplicative interaction was significant for race-depression ($P_{interaction}$ < 0.01) and race- inability to control for important things in life ($P_{interaction}$ = 0.01).

Conclusion: The associations between psychosocial factors and mortality among prostate cancer patients are complex and may not affect all individuals equally. Programs aiming to reduce mortality for individuals with prostate cancer need to take into consideration their psychosocial needs and demographic background

#4942 Associations of smoking with the risk of second primary cancer in adults with a history of various cancer types.

H. Sung, C. C. Newton, W. Diver, E. Mitchell, E. Deubler, K. Kelly, J. Westmaas, L. Teras, A. Jemal, A. Patel, C. Bodelon;
American Cancer Society, Atlanta, GA

Importance: Smoking increases the risk of developing second primary cancers (SPCs) among individuals with a history of cancer. However, most evidence comes from survivors of smoking-related first primary cancers (FPC) with limited data available for a range of FPC types.
Objective: Examine the association between smoking and the risk of developing SPC by FPC types.
Design, Setting, and Participants: In the Cancer Prevention Study II Nutrition Cohort, we identified 28,272 participants who were diagnosed with first primary invasive, non-metastatic cancer between January 1, 1992, and June 30, 2015, and survived for 1+ year, and followed them for SPC diagnoses until June 30, 2018.
Exposures: Smoking status and years since quitting, assessed shortly (mean=1.6 years; SD=1.4) before FPC diagnosis.
Main Outcome: Self-reported SPCs (excluding those at the same site as FPC), defined as smoking-related cancers by the IARC Working Group on the Evaluation of Carcinogenic Risks to Humans
Measures: Multi-variable adjusted hazard ratio (HR) using Cox proportional hazards model
Results: Approximately 59% were male and 49% were in their 70s at their FPC diagnosis (mean=72.2; SD=7.08). The most common FPCs were prostate (32.8%), breast (18.5%), colorectal (9.0%), and non-Hodgkin lymphoma (8.3%). Smoking prevalence was highest among those with FPC of the lung (88.2%), esophagus (78.3%), and bladder (75.3%) and lowest among those with uterine corpus (38.5%) and ovarian (38.2%) cancers. During a median follow-up of 7.0 years, 1,926 individuals (7%) developed smoking-related SPCs. In multivariable models, former (HR=1.62, 95%CI=1.46-1.80) and current (HR=3.77; 95%CI=3.22-4.41) smokers were more likely than never-smokers to develop smoking-related SPCs. The HRs for current smokers varied across FPC types: 5.84 (95%CI=2.51-13.6) for uterine corpus, 4.93 (95%CI=2.60-9.33) for colorectum, 4.07 (95%CI=3.12-5.31) for prostate, 3.87 (95%CI=1.41-10.6) for melanoma, 3.27 (95%CI=2.24-4.75) for breast, 3.11 (95%CI=1.65-5.89) for non-Hodgkin lymphoma, and 2.77 (95%CI=1.50-5.15) for bladder. Among former smokers overall, HRs declined with years since quitting from 2.56 (95%CI=2.17-3.02) for <10 years to 1.30 (95%CI=1.14-1.48) for 30+ years before FPC diagnosis. The risk reduction was most marked for uterine cancer survivors after 10+ years (HR=0.80, 95% CI=0.37-1.74) and breast cancer survivors after 20+ years since quitting (HR=0.88, 95% CI=0.63-1.22), although a lower-level risk persisted for several other FPCs.
Conclusions and Relevance: Associations between smoking and the risk of SPC are evident across various FPC types, even when the first cancer is not smoking-related. The finding highlights the importance of smoking cessation efforts in reducing the burden of second primary cancers in the expanding population of cancer survivors.

#4943 A prospective study of the association between statin use and the risk of overall and advanced bladder cancer among men.

Megan R. Shanahan[1], LeeAnn Lucas[1], Colleen B. McGrath[1], Jane B. Vaselkiv[1], Chaoran Ma[2], Mark Preston[3], Edward Giovannucci[4], Eva Schernhammer[5], Lorelei A. Mucci[1]

[1]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA,[2]Department of Nutrition, University of Massachusetts Amherst, Amherst, MA,[3]Brigham and Women's Hospital, Boston, MA,[4]Department of Epidemiology and Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA,[5]Department of Epidemiology, Center for Public Health, Medical University of Vienna, Vienna, Austria

Background: High cholesterol has been linked to the risk of several cancers, including bladder cancer. Previous research is not consistent as to whether statin medications, which reduce cholesterol levels and have other anti-tumor effects, impact bladder cancer risk. We explored this question in a large prospective cohort study of men, for whom bladder cancer is the fourth leading cause of cancer incidence in the United States.

Methods: Our analysis included 44,116 cancer-free participants of the Health Professionals Follow-Up Study, who were followed for bladder cancer incidence from 1990 through 2018. Statin use was self-reported and updated every two years on questionnaires. Bladder cancer diagnosis was validated with medical or death records. We used multivariable Cox regression to investigate the association between statin use (ever, current, former, and never) and duration of statin use (never, less than 4 years, 4-8 years, and 8 or more years) and the risk of overall bladder cancer, bladder cancer by stage at diagnosis (localized, advanced), and fatal bladder cancer. Models estimating hazard ratios (HR) and 95% confidence intervals (CI) were adjusted for demographic, medical, and lifestyle confounders.

Results: During a median of 13.5 years of follow-up, 1,248 bladder cancers were documented, including 562 advanced and 143 fatal cancers. By mid-way through follow-up, 34% of men reported statin use. In multivariable models, there was a suggestive increased risk of overall bladder cancer comparing current statin users to former or never users (HR 1.13; 95% CI 1.00-1.27), yet no association was observed for localized, advanced, or fatal cancers. Ever use of statins (including former and current use) was not associated with overall, localized, nor advanced bladder cancer, compared to never use. However, there was a suggestive decreased risk of fatal bladder cancer (HR: 0.77; 95% CI 0.58-1.02) among ever users of statins compared to never use. Long-term statin use of 8 or more years was associated with a lower risk of advanced (HR 0.78; 95% CI 0.60-1.00) and fatal bladder cancer (HR 0.59; 95% CI 0.40-0.88), compared to never use. No association was observed for long-term statin use and overall bladder cancer (HR 0.99; 95% CI 0.85-1.16).

Conclusion: Results of this study, which investigated overall and clinical subtypes of bladder cancer incidence, suggest that long-term statin use may be protective against the development of advanced and fatal bladder cancer among men. Together with data from clinical trials supporting benefits of statins, this warrants further investigation into whether statins can be used as a preventive tool. Further research is also needed on the potential mechanism by which statins act in bladder carcinogenesis, particularly advanced forms of the disease.

#4944 Role of a planetary health diet in survival following breast cancer diagnosis.

Andrea Romanos-Nanclares[1], Michelle D. Holmes[1], Wendy Y. Chen[2], Walter C. Willett[3], Bernard A. Rosner[4], A. Heather Eliassen[1]

[1]Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA,[2]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA,[3]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA,[4]Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA

Introduction: Whilst the current trend recommendation for breast cancer survivors to follow a healthy plant-centric diet seems prudent, evidence supporting this remains limited. Furthermore, diets that address both human health and environmental sustainability remain a research priority, and the EAT-Lancet Commission's 2019 "Planetary Health Diet" has offered a framework for this approach. Our study aimed to investigate whether a Planetary Health Diet Index (PHDI), quantifying adherence to these targets, is associated with overall and breast cancer-specific mortality.

Methods: For 7,639 women with stage I-III breast cancer from the Nurses' Health Study (NHS) and Nurses' Health Study II (NHSII), we calculated the cumulative average PHDI, using repeated measures of post-diagnostic diet assessed by food frequency questionnaires (FFQ). The PHDI index included a variety of high-quality plant-based foods (whole grains, fruits, vegetables, nuts and legumes) and low amounts of animal-based foods, refined grains, added sugars, and unhealthy fats. Deaths were ascertained by report from family members or by searching the National Death Index. Hazard ratios (HRs) and 95% confidence intervals (CI) were estimated using Cox proportional hazards models and adjusted for multiple time-varying and fixed-time covariates (measured prior to or at the time of diagnosis) and clinical factors.

Results: Participants were followed up for a median of 12 years after breast cancer diagnosis. We documented 2,983 deaths, including 788 due to breast cancer. In the multivariable-adjusted model, we observed a statistically significant inverse association for cumulatively averaged post-diagnosis PHDI and all-cause mortality ($HR_{Q5vs.Q1}$ 0.85 [0.73, 0.99], p-trend <0.01), but not breast cancer specific mortality. Findings for overall mortality were similar for PHDI measured at other timings/settings, including the first post-diagnosis dietary assessment ($HR_{Q5vs.Q1}$ 0.88 [0.76, 1.01], p-trend <0.01).

Conclusions: High adherence to a diet consistent with both human and planetary health after a breast cancer diagnosis is associated with lower risk of all-cause, but not breast cancer-specific, mortality. As the number of breast cancer survivors grows and environmental sustainability becomes more urgent, strategies to reduce mortality should focus on the win-win benefits of dietary changes for both population and planetary health.

#4945 Exposure to per and poly fluoroalkyl substances and cancer risk: A systematic review and meta-analysis.

M. Seyyedsalehi, P. Boffetta;
University of Bologna, Bologna, Italy

Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals that humans can encounter through occupational or environmental exposure. Recent evidence suggests that exposure to PFAS may increase the risk of different types of cancer. In our comprehensive review and meta-analysis, we investigated the association between PFAS exposure and the risks of breast, female genital, bladder, kidney, prostate, testis, liver, esophageal, gastric, colorectal, pancreatic, head and neck, lung, thyroid, and lymphatic cancers. We systematically reviewed literature from IARC Monographs, ATSDR Reports, PubMed, and Scopus (up to January 2024) for cohort, case-control, and ecological studies. Four reviewers independently screened studies, and data extraction included study design, patient characteristics, and effect size measures. The quality of studies was assessed using the modified version of the Newcastle-Ottawa Scale (NOS). We constructed forest plots and random-effects meta-analyses of relative risks (RR) for PFAS-cancer associations with at least 3 independent risk estimates and conducted stratified, dose-response, and publication bias analyses. A total of 41 articles reported results on PFAS exposure and cancer. The pooled RR for the associations between exposure to different types of PFAS and cancer incidence and mortality are reported in Table 1. Our findings suggest that overall PFAS exposure may be associated with kidney, ovary, and lung cancer and high doses of PFAS with testicular cancer. Further prospective studies with an appropriate assessment of co-exposure with other carcinogens and detailed exposure assessment among study participants are needed to establish causality of observed associations.

Table 1: The combined relative risk (RR) of occupational and environmental PFAS exposure and various types of cancer.

Cancer type	**PFAS type**	**RR (95%CI), I2, p value**	**Number of risk estimates**	**Publication bias**
Breast	PFOA	1.08(0.97-1.20), I^2=85.2%, p<0.001	21	0.30
	PFOS	1.00(0.85-1.18), I^2=0.81.5%, p<0.001	12	
	PFNA	0.95(0.81-1.12), I^2=83%, p<0.001	10	
	PFDA	1.29(0.73- 2.25), I^2=91.3%, p<0.001	6	
Cervix	PFAS overall	0.94(0.79-1.12), I^2=0.0%, p=86	3	0.55
	PFOA	1.00(0.90-1.11), I^2=0.0%, p=0.58	4	
Ovary	PFAS overall	**1.07(1.04-1.09), I^2=99.3%, p<0.001**	12	
	PFOA	1.05(0.89-1.24), I^2=22.6%, p=0.27	4	
Kidney	PFAS overall	**1.18(1.05-1.32), I^2=52.6%, p=0.02**	11	0.31
Bladder	PFOA	0.93(0.86- 1.01), I^2=1%, p=0.43	11	0.79
	PFOS	0.94(0.78- 1.13), I^2=45.1%, p=0.14	4	
Prostate	PFOA	0.95(0.91-1.00), I^2=34%, p=0.08	17	0.71
	PFOS	1.04(0.98-1.11), I^2=34.4%, p=0.14	9	
	PFNA	0.96(0.83-1.12), I^2=92.7%, p<0.001	6	
Testis	PFAS overall	1.12(0.94-1.34), I^2=0.0%, p=0.52	8	0.53
Colorectal	PFOA	0.83(0.65-1.06), I^2=81.6%, p<0.001	6	0.37
	PFOS	0.71(0.22-2.27), I^2=81.5%, p<0.001	4	
Liver	PFAS overall	1.03(0.91-1.16), I^2=47.9%, p=0.02	16	0.51
Pancreas	PFOA	1.01(0.89-1.15), I^2=0.0%, p=0.79	9	0.44
Lung	PFAS overall	**1.20 (1.12-1.28), I^2=0.0%, p=0.95**	9	0.57
Head and neck	PFAS overall	1.15 (0.96-1.37), I^2=0.0%, p=0.72	3	0.79
Thyroid	PFAS overall	1.54 (0.86-2.78), I^2=69.0%, p=0.02	4	0.08
Hodgkin lymphoma	PFAS overall	1.23(0.90-1.70), I^2=0.0%, p=0.38	3	0.47
Non-Hodgkin lymphoma	PFAS overall	1.01(0.90-1.13), I^2=31.8%, p=0.19	6	0.64

#4946 Liver disease risk: Cyanotoxins in water and food sources in Guam.

Ella Marie Macatugal

U54 Pacific Islander Partnership for Cancer Health Equity Grant, University of Guam, Mangilao, Guam

Liver cancer is the third most common cancer among males in Guam, where incidence rates are 2.6 times higher than the U.S. average. Rates of liver cancer among Pacific Islanders are even higher in comparison with incidence among Micronesians and CHamorus at 4.6 and 2.8 times greater respectively. Chronic liver disease, including non-alcoholic fatty liver disease (NAFLD), cirrhosis, which serve as risk factors for hepatocellular carcinoma (HCC), account for a significant health burden in Guam's Asian American and Pacific Islander (AAPI) population. This health disparity has consistently plagued the community for decades and its cause remains unknown. Emerging evidence implicates microcystins (MCs), liver-damaging toxins produced by cyanobacteria, as a potential environmental contributor to liver disease and HCC prevalence. MCs are widespread in tropical freshwater and marine ecosystems and can bioaccumulate in human biospecimens (serum, saliva, and urine). In preliminary studies, MCs have been detected in residents of Guam and Hawaii. This study served to more comprehensively quantify sources of MCs in food and water sources on Guam while also examining the correlation between MC levels in human biospecimens and primary sources of drinking water and produce. This study analyzed water and produce samples from residents of Guam who participated in a previous University of Guam Liver Study and consented to follow-up research (CHRS#: IRB-21-110). Participants completed detailed surveys regarding their drinking water sources (tap, filtered, or store-bought), village of residence, and the top three fruits and vegetables they consumed. Each participant provided samples of their tap and drinking water, while produce consumption patterns guided the collection of local and imported produce. MCs in water and produce samples were quantified using the Abraxis Microcystins-ADDA SAES ELISA, a sensitive assay for detecting microcystin congeners. Tap water samples were more frequently MC-positive compared to filtered water. Detectable levels of MCs were found in both locally sourced produce and imported produce, highlighting multiple routes of exposure. A moderate positive correlation was also observed between participants' serum MC levels and the MC content of their water samples. Although MC concentrations in water and produce were generally minute, the potential for bioaccumulation is significant, particularly given the elevated levels detected in serum samples. These findings suggest that chronic exposure to MCs through tap water and produce consumption may contribute to liver disease disparities in Guam's AAPI communities. Further research is warranted to examine how these environmental toxins may contribute to the etiology of liver disease in this community, providing critical insights to inform public health interventions aimed at reducing exposure and mitigating health disparities.

#4947 Objectively measured individual light exposures and risk of colorectal cancer.

Melania Boker[1], Xiaoyu Zong[1], Jushawn Macon[1], Nick Jeschke[1], Charlie Bowe[1], Yin Cao[2]

[1]Division of Public Health Sciences, Department of Surgery, Washington University School of Medicine, St. Louis, MO,[2]Division of Public Health Sciences, Department of Surgery, Alvin J. Siteman Cancer Center, Washington University School of Medicine, St. Louis, MO

Introduction: The global rise in early-onset colorectal cancer underscores the urgent need to explore emerging risk factors like circadian disruption, which have become increasingly common in recent generations. Mechanistic studies showed that individual nighttime light exposure disrupts melatonin secretion and circadian rhythms, contributing to tumorigenesis. However, human studies are scarce and rely on proxies of personal light exposures. We assessed the prospective association between objectively measured individual nightlight and daylight exposures and colorectal cancer risk.

Methods: A total of 80,709 participants without prior history of shift-work and cancer in the UK Biobank who wore Axivity AX3 wrist accelerometers that contained a silicon photodiode light sensor for up to seven days between 2013-2015 were followed up to 2021. Nighttime and daytime light exposures (measured in lux) were quantified using factor analysis of 24-hour light profiles. Relative amplitude (RA) was calculated by the difference in mean activity during the highest 10-hour and lowest 5-hour active periods. Cox models were used to estimate relative risk (RRs) and 95% confidence intervals (CIs).

Results: During 471,456 person-years of follow-up, a total of 569 incident colorectal cancers occurred (378 colon cancer; 191 rectal cancer). Personal nighttime light exposure but not daytime light exposure was associated with increased risk of colorectal cancer (P_{trend} = 0.07), primarily driven by rectal cancer. Compared to the lowest tertile (T1), individuals in the highest tertile (T3) of nighttime light exposure had 53% increased risk of rectal cancer (RR 1.53; 95% CI: 1.05-2.22; Ptrend = 0.003). This association was independent of putative risk factors of colorectal cancer and remained robust after adjusting for daytime light exposure as well as RA, a commonly used measure of 24-hr rest-activity rhythm. In joint analysis, individuals with the highest nighttime light exposure (T3) and high level of circadian disruption (below median RA) had the highest risk of rectal cancer (RR 2.15, 95% CI: 1.20-3.86).

Conclusion: In a large prospective cohort, objectively measured individual nightlight exposure was linked to increased risk of rectal cancer, which has been rapidly increasing in younger adults. These findings underscore the need for integrative epidemiologic and mechanistic studies to validate the associations and dissect the biological pathways driving site-specific tumorigenesis, and to better inform tailored prevention and intervention strategies in younger generations.

#4949 Late-stage breast cancer risk and five-year survival by stage at diagnosis among top 5 Asian ethnic subgroups in California.

Michelle Tran, Kimberly A. Miller, Sue E. Kim, Kali Zhou, Myles Cockburn, Lihua Liu

USC - University of Southern California, Los Angeles, CA

Introduction: Breast cancer (BC) incidence over the past decade has been rapidly increasing among Asian American (AA) women compared to non-Hispanic White (NHW) women. AA women are more likely to be diagnosed with BC at an earlier stage and have better survival compared to Hispanic and Black women. However, the aggregation of AA may conceal significant population heterogeneity and health disparities among ethnic subgroups, as the risk profile in one subgroup differ from another. Thus, we aimed to understand AA ethnic disparities in BC by assessing AA subgroups and their relationship with late-stage diagnosis and all-cause mortality by stage at diagnosis using a cohort of AA BC patients from the California Cancer Registry (CCR).

Methods: We identified BC cases among NHW and among the top 5 AA subgroups (Chinese, Japanese, Filipino, Korean, and Vietnamese) diagnosed in California between 1988 to 2020 using the CCR. We conducted multivariable logistic regression to evaluate the association between the odds of being diagnosed with late-stage BC (regional and distant stages) and AA subgroups. Additionally, 5-year observed survival rates across each AA subgroup were calculated at each stage at diagnosis (in situ or localized, regional, and distant). Lastly, we conducted multivariable Cox proportional hazards regression to assess all-cause mortality by AA subgroups. All models were adjusted for year at diagnosis, age at diagnosis, nativity, socioeconomic status (SES), comorbidity, insurance status, and BC subtype. We used NHW as the reference group.

Results: Our sample contained a total of 557,551 BC cases (NHW N=493,227; Chinese N=19,380; Japanese N=8,893; Filipino N=24,938; Korean N=5,075; Vietnamese N=6,038). We found that Chinese, Japanese, Korean, and Vietnamese women were less likely to be diagnosed with late-stage BC compared to NHW ($p<0.01$ for Chinese, Japanese, and Vietnamese; $p=0.02$ for Korean), while no difference was observed for Filipino women ($p=0.72$). When comparing the 5-year observed survival rates from all causes of death by stage at diagnosis, all 5 AA subgroups had higher 5-year survival compared to NHW across all stages. However, when adjusted for year at diagnosis, age at diagnosis, nativity, SES, comorbidity, insurance status, and BC subtype, Chinese women diagnosed at the distant stage were 25% more likely than NHW women to die from all causes ($p<0.01$).

Conclusions: Most AA subgroups were at lower odds of being diagnosed with late-stage BC compared to NHW, except for Filipino women. Survival outcomes also differed by subgroups and by stage at diagnosis. Differences in AA subgroups could be due to several factors (i.e., social factors, environmental factors). Thus, different cancer strategies in terms of cancer control and care improvement for each group are needed.

#4950 Exploring the impact of neighborhood-level conditions: Geographic disparities in breast cancer mortality in Maryland, 2000-2019.

Katherine L. Ho, Kassandra Alcaraz, Avonne Connor, Michael Desjardins

Johns Hopkins Bloomberg Sch. of Public Health, Baltimore, MD

Purpose: To observe the geographic distribution of breast cancer (BC) mortality in Maryland by differences in neighborhood-level social determinants of health
Background: Cancer surveillance efforts indicate that BC mortality is unevenly distributed geographically in Maryland; however, it is unknown how area-level socioenvironmental disadvantages might be driving these neighborhood-level disparities.
Methods: Utilizing data from the Maryland Cancer Registry (MPCR), we identified women aged 18+ diagnosed with first primary, invasive BC between 2000-2019. We used census tract-level Social Vulnerability Index (SVI) as a proxy measure for neighborhood-level built and social environments. We calculated the 20-year average SVI percentile ranking and categorized each census tract as having low (least disadvantaged), low-medium, medium-high, or high (most disadvantaged) SVI. We computed age-adjusted (<50, 50+) standardized mortality ratios (SMRs) for each SVI level using the 2000 standard female population and BC-specific referent mortality rates for Maryland from 2018-2022 (age <50: 4.4 deaths/100k; age 50+ 60.8 deaths/100k). We used spatial epidemiology methods to estimate age-adjusted SMRs by census tracts among women of all ages (referent mortality rate: 20 deaths/100k). We mapped this data to visualize the geographic distribution of SVI and BC mortality.
Results: Our study population included 79,145 women (n=17,027 [2.5%] aged <50; n=62,118 [78.5%] aged 50+), of which 13,005 (16%) died from their BC diagnosis. We observed the largest differences in SMRs by age at diagnosis. Across all SVI categories, BC mortality among women diagnosed at ages 50+ was higher than expected relative to older women in the general female population (SMR range: 19.1-22.1). Results were similar for women diagnosed at ages <50 (SMR range: 48.2-53.3). Census tracts with low-medium/medium-high SVI had higher SMRs (low-med: 50.2, med-high: 53.3) than low/high SVI areas. After calculating the SMRs by census tract for all ages, we observed that census tracts in the Eastern Shore and western Maryland showed a strong spatial correlation between higher SVI and higher SMRs. Similarly, most of the Capital region and central Maryland experienced low SVI and correspondingly lower SMRs, but there was considerable spatial variation in the distribution of SVI and BC mortality around Baltimore City/County and Greater Washington region.
Conclusion: The uneven geographic distribution of BC mortality in Maryland may be due to the underlying heterogenic spatial distribution social vulnerability. Younger women diagnosed in census tracts with low-medium or medium-high SVI disproportionately experienced the highest SMRs. Our findings suggest a differential mortality impact of neighborhood-level conditions and socioenvironmental factors among younger vs. older women.

PREVENTION / EARLY DETECTION / INTERCEPTION: Health Disparities across the Cancer Continuum
Poster Session

#4954 Age- and race/ethnicity-specific values of prostate-specific antigen levels: Implications for prostate cancer screening.

Pranoti Pradhan[1], Timothy R. Rebbeck[2], William K. Oh[3], Sigrid Carlsson[4], Kevin H. Kensler[5]

[1]Harvard T.H. Chan School of Public Health, Boston, MA,[2]Dana-Farber Cancer Institute, Boston, MA,[3]Icahn School of Medicine at Mount Sinai, New York, NY,[4]Memorial Sloan Kettering Cancer Center, New York, NY,[5]Weill Cornell Medicine, New York, NY

Background: A significant limitation in prostate cancer screening is the limited diversity and scope of data that have defined prostate-specific antigen (PSA) values. The use of PSA in prostate cancer screening could be enhanced with knowledge of the distribution of PSA in diverse groups of men. These data may inform triggers for clinical actionability, including biopsy.
Objective: This study aims to evaluate the distribution of PSA values among healthy men of different ethnicities and age groups to derive age- and ethnicity-specific low, middle, and upper PSA distribution values.
Methods: We meta-analyzed studies that reported PSA reference values in healthy men by age and self-identified race or ethnicity. The analysis was restricted to full text articles published in English. Participants were healthy men, aged 40-79 years with no history of prostate cancer, prostate disease, or prostate surgery. PSA values were not age-adjusted. We included data for either observed or estimated PSA values. Studies were excluded if they reported PSA in men with prostate cancer diagnosis, prostate disease, history of prostate surgery, suspicious DRE/TRUS values, age-adjusted results, or a small sample size (N<50). The primary outcomes of interest were to estimate PSA values to construct age- and race-stratified PSA values. Estimates were calculated for the upper limits of the following percentiles: 5th, 10th, 25th, 50th, 75th, 90th, and 95th for 16 race/ethnicity groups. Results were stratified by the original exclusion criteria used by the individual studies, including 1) PSA>4.0 ng/ml, Suspicious DRE/TRUS; or 2) history of prostate disease, surgery, or cancer. Forty-two studies met our criteria and were included in this report.
Results: Men of African descent had higher PSA values followed by men of Asian and European descent. Within the 95th percentile, African descent men had PSA estimates of 4.78 ng/ml, 5.47 ng/ml, 8.93 ng/ml. White men had PSA values of 1.90 ng/ml, 2.06 ng/ml, and 3.31 ng/ml respectively for 40-49 years, 50-59 years, and 60-69 years. When stratified, the distribution of PSA was overall higher among those studies which excluded based on PSA levels greater than 4.0 ng/ml compared to studies which excluded based on history or prostate disease, surgery, or cancer; for both race and age. However, Japanese men had higher a higher distribution compared to African descent men for all ages in the 95^{th}percentile among those with no history of prostate disease, surgery, or cancer.
Conclusion: We report an association of PSA levels with race and age. PSA was elevated among men of African descent and increase with age. Our data may inform the use of PSA in screening for prostate cancer in diverse populations

#4955 Somatic mutation frequency in prostate cancer patients of different ancestries and age of onset.

Tara S. K. Jennings[1], Anton N. Nguyen[1], Weiping Chu[2], Dan Mercola[1], Farah Rahmatpanah[1]

[1]Department of Pathology and Laboratory Medicine, University of California, Irvine, Irvine, CA,[2]Department of Microbiology and Molecular Genetics, University of California, Irvine, Irvine, CA

Background: Prostate cancer (PCa) varies significantly in outcomes based on age of onset and genetic ancestry. Early-onset PCa (EoPCa), diagnosed in men under 55, exhibits aggressive behavior, poor differentiation, and resistance to hormonal therapies, resulting in worse outcomes compared to late-onset PCa (LoPCa). African American (AA) men face more aggressive disease and higher incidence rates than White men with European heritage (EA), even after accounting for socioeconomic factors. Investigating somatic mutations in PCa is essential to understanding disease progression, as the biology of EoPCa remains poorly defined. Comparing mutational frequencies in AA and EA men with EoPCa and LoPCa provides key insights into these disparities and informs targeted interventions.
Methods: We analyzed the mutational status of EoPCa and LoPCa in AA and EA patients. We isolated DNA in tumor tissue from FFPE prostatectomy tissues using Qiagen's FFPE DNA Purification Plus Kit. DNA samples from three patients in each cohort (AA LoPCa, AA EoPCa, EA LoPCa, EA EoPCa) were investigated for 81 somatic mutations across 13 genes using real-time PCR. Mutational analysis was performed with Qiagen's qBiomarker Prostate Cancer Gene Panel, an assay designed to detect PCa specific sequence alterations. DNA and qBiomarker Probe Master Mix were prepared following the manufacturer's protocol. Relative fold gene expression was calculated using the 2-ΔΔCt (delta-delta Ct) method, with DNA from normal epithelial prostate tissue serving as the control. Values exceeding a threshold of 4 were considered indicative of mutations.
Results: The analysis of somatic mutation frequencies, with their position and substitution, revealed notable differences across race and the combined factors of race and age of onset. Our analysis revealed higher mutation rates in AA patients for CTNNB1 98 C>T (83.3% AA vs. 66.7% in EA) and KRAS 34 G>A (66.7% AA vs. 33.3% in EA), mutations not previously reported as elevated in AA PCa. When examining race and age of onset together, distinct patterns emerged. Among LoPCa patients, the TP53 827 C>T mutation was found in 100% of AA patients compared to 66.7% of EA patients, while the HRAS 35 G>T mutation was present in 33.3% of AA patients but absent in EA patients. In EoPCa, AA patients exhibited higher frequencies of the CTNNB1 101 G>T mutation (33.3% AA vs. 0% in EA) and KRAS 35 G>C mutation (33.3% AA vs. 0% in EA).
Conclusion: These mutations, previously unexamined in the context of race and age of onset, highlight disparities in the mutational landscape of PCa.

#4956 Exploration of sub-Saharan African immigrants' reactions to prostate cancer diagnosis.

Kaitlin Van Voorhis, Ernie Kaninjing

Georgia College & State University, Milledgeville, GA

Background: About 300,000 men will hear "you have Prostate Cancer" annually in the United States. Black men are 1.7 times more likely to be diagnosed than White men, and sub-Saharan African Immigrants (SSAIs) are 1.5 times more likely than Black men. These 4 words spark various reactions as the journey towards treatment and survivorship begins. Emotions influence the decision-making process, mental health, adherence to treatment, and treatment outcomes. An exploration of the initial reactions to diagnosis displayed by SSAI will better inform future interventions aimed at improving health outcomes.
Methods: In depth interviews were conducted with 22 SSAI Prostate Cancer (CaP) survivors, guided by Grounded Theory. Purposeful sampling was employed to ensure the study population reflected the cultural diversity among SSAIs. Interviews were transcribed, verified, and analyzed in NVivo 13.
Results: Participants expressed a range of reactions, unique to their individual experiences. These reactions were categorized into 3 over-arching categories: positive (showing signs of agreement or acceptance with the diagnosis), negative (showing signs of resistance to the diagnosis), or neutral (showing no strong pull to positive or negative, often revolving around action-based desires). Participants often experienced multiple reactions across this spectrum, but analysis showed potential correlation among various demographic variables such as age and time passed from diagnosis resulting in more neutral recollections of reactions.
Conclusions: The wide range of initial reactions to CaP diagnosis are reflective of the individual and their unique experiences as an SSAI. These common reactions and mindsets immediately following diagnosis are insightful to a patient's current headspace, focus, personal outlook, and overall mental health. All these emotions impact decision making with regards to treatment, treatment adherence, and even treatment regret as the survivorship journey continues. The National Institute of Health's Cancer Control Continuum includes focus areas for etiology, prevention, detection, diagnosis, treatment, and survivorship. However, there remains a lack of focus on the initial reaction to diagnosis. Future studies should focus on further illuminating the relationship between a patient's initial reaction to diagnosis and their treatment decisions, adherence, and overall survivorship.

#4957 Oncogenic role of stress survival gene MCM10 in colorectal cancer progression and Hispanics health disparities.

Md Zahirul Islam Khan, Urbashi Basnet, Sourav Roy

Biological Sciences, The University of Texas at El Paso, El Paso, TX

Background: Colorectal cancer (CRC) remains the second leading cause of cancer mortality in the U.S., with significant racial and ethnic disparities. This study investigates the role of a stress-survival pathway (SSP) gene MCM10 in CRC, particularly focusing on disparities between Hispanics and Non-Hispanic Whites (NHWs) in late-stage carcinogenesis. Despite comprising over 17% of the U.S. population, Hispanics face significant healthcare inequities, particularly in cancer diagnosis, leading to delayed CRC detection and reduced survival rates. Since CRC is highly treatable when identified early, addressing these disparities is therefore very crucial. Establishing MCM10 as a diagnostic biomarker and therapeutic target could significantly improve CRC management and outcomes in underserved populations.
Methods: Our in-silico analysis previously identified MCM10 as a differentially expressed SSP gene associated with CRC progression. To validate this finding, we analyzed MCM10 expression in CRC cell lines and tissue samples using qRT-PCR. The stage-specific expression patterns were further assessed through cDNA microarray analysis, while protein-level expression was confirmed via immunohistochemical assay of tissue microarrays. Functional studies were conducted in vitro after MCM10 knockdown to investigate the role of MCM10 in critical cancer-related processes such as proliferation, invasion, and survival. RNA sequencing and mass spectrometry were subsequently employed to identify the molecular mechanisms underlying MCM10-driven CRC progression.
Results: Our results showed significant upregulation of MCM10 in CRC cell lines and tissues at both transcript and protein levels. Notably, Hispanic CRC samples exhibited differential expression of MCM10 compared to NHWs, with marked upregulation of MCM10 in late-stage Hispanics. These results are in line with their traditional late-stage diagnosis. The functional study demonstrated that MCM10 knockdown in CRC cell lines significantly reduced proliferation, growth, invasion and migration of cells by inducing cell cycle arrest, apoptosis and reactive oxygen species pathways. In addition, our integrative RNA-sequencing and proteomics study reported two novel genes from zinc finger family along with 17 additional proteins which could be associated with MCM10 mediated carcinogenesis.
Conclusion: This study identifies MCM10 as a key oncogenic driver in CRC, highlighting its potential as a diagnostic biomarker and therapeutic target to enhance disease management and reduce ethnic disparities in outcomes among Hispanics and NHWs.

#4958 Mutational disparities in colorectal cancers of White Americans, Alabama African Americans, and Oklahoma American Indians revealed ANGEL2 as an African American CRC proneness candidate gene.

Hiroshi Y. Yamada[1], Madhusmita Rout[2], Chao Xu[3], Gary Sanghera[2], **Surya Singh**[4], Venkateshwar Madka[4], Farrukh Afaq[5], Katherine T. Morris[6], Dharambir K. Sanghera[7], Upender Manne[5], Chinthalapally V. Rao[4]

[1]Medicine, OU Health Stephenson Cancer Center, Oklahoma City, OK,[2]OU Health Sciences Center, Oklahoma City, OK,[3]Public Health, OU Health Sciences Center, Oklahoma City, OK,[4]Medicine, OU Health Sciences Center, Oklahoma City, OK,[5]Department of Pathology, Heersink School of Medicine, University of Alabama at Birmingham, Birmingham, AL,[6]Surgery, OU Health Sciences Center, Oklahoma City, OK,[7]Department of Pediatrics, College of Medicine, OU Health Sciences Center, Oklahoma City, OK

Introduction: The high incidence and mortality rates of colorectal cancer (CRC) in Alabama African Americans (AAs) and Oklahoma American Indians (AIs) are recognized as cancer disparities, yet the underlying causes have been poorly demonstrated. Our previous study of transcriptomic profiles of CRCs of Alabama AAs, Oklahoma AIs, and white people from both states revealed molecular disparities and differentially expressed genes (DEGs) in the racial groups.
Methods/Procedures: By evaluating CRC whole-exome sequencing and mutational profiles, we report sets of mutated genes whose frequencies differed significantly ($p<0.05$) in a race-specific manner. Secondary screening with a CRC database (cBioportal database for bowel cancer) showed 42, 38, and 35 “survival-critical genes (SCGs)” (i.e., genes whose mutations/alterations are associated with significant differences in the patients’ survival rates) among the differentially mutated genes, suggesting that the mutations have a functional impact on cancer. Notable SCGs with race-pronounced variants were different from DEGs and their involved pathways included nucleotide catabolism (GDA, NT5M, XDH) and cell cycle checkpoints (CLIP1, PSMB2, PSMD3, TP53) for AAs, and extracellular matrix organization (COL4A4, LAMA1, LAMA2, NRXN1) for AIs (Benjamini-Hochberg adjusted $p<0.05$). Most of the SCGs with race-pronounced variants (35 SCGs out of 42 in whites, 29 of 38 AAs, and 30 of 35 AI) are under-investigated with less than ten CRC-related publications in PubMed.
New results: We considered multitudes of factors to select gene(s) for further validation, such as (i) statistical significance, (ii) being a SCG, (iii) type of variant/mutation (variants occurring in open reading frame may be prioritized with an assumption of direct effects on the protein function, compared with likely indirect effects of intron variants), (iv) amount and quality of existing work, and (v) available resources, then proceeded to validate the SCGs with race-pronounced variants. Among the candidate genes, ANGEL2 with Alabama African American-pronounced variant is involved in 3'-UTR-mediated mRNA stabilization and negative regulation of mitotic cell cycle. ANGEL2 expression increases in rat colon tumor (IHC) and in human CRCs in TCGA (vs normal; <0.0001), implicating a role in regulating CRC development.
Prospective: Cancer-influencing genes with minority race-pronounced variants have evaded discovery by being diluted in all-inclusive tumor data analysis. The inclusion of these SCGs with race-pronounced variants in the clinical CRC next-generation sequencing panels and the development of targeting drugs will serve as refinements for precision medicine to overcome racial disparities in health outcomes of CRC.

#4959 Hearing Status and Colorectal Cancer Screening: Identifying Access Barriers.

Dt Bruno[1], Erika Bergeron[1], Regina Wang[2], Poorna Kushalnagar[1]

[1]Gallaudet University, Washington, DC,[2]Department of Family Medicine, University of California, San Diego, CA

Background: Early detection through colorectal cancer screening is critical for effective treatment, improved survival rates, and reduced mortality. However, adherence to screening is influenced by various factors, including education and communication barriers. These barriers can be particularly pronounced for the deaf, deafblind, and hard of hearing (DDBHH) population using American Sign Language (ASL), potentially hindering preventive care. Education is widely known to be associated with cancer screening; however, the contributory role of a person's functional ability to understand spoken language (e.g., lipreading, speechreading, combination with gestures) in a quiet room has not been explored.

Methods: Using the NCI Health Information National Trends Survey in ASL as well as USPSTF guideline for colorectal cancer screening, we surveyed 566 DDBHH adult participants between July 2023 and October 2024. Binary logistic regression was used to assess the association between functional hearing ability (understand well vs cannot understand well in a quiet room) and colorectal cancer screening adherence, after adjusting for age and education. A two-sided $p<=.05$ was considered significant.

Results: After adjusting for age and education, results did not indicate a significant association between functional hearing status and cancer screening adherence [β=1.14 (95% CI=0.75,1.73)], suggesting that a person's functional hearing ability does not independently influence screening rates. In this model, education level remained as a significant predictor, with lower educational attainment associated with a decreased likelihood of adhering to colorectal cancer screening guidelines [β =.51(95% CI=0.31,.84)].

Discussion: These findings highlight that functional hearing status alone is not a significant predictor of cancer screening adherence within the DDBHH community, underscoring the need for providers to look beyond a single moment of effective communication. While sign language interpreters ensure effective healthcare communication, they alone cannot address the educational disparities affecting DDBHH populations. Our study shows that DDBHH individuals with fewer years of education are less likely to undergo cancer screening. To achieve equitable health outcomes, providers should consider adding trained community health workers or patient navigators who have lived experience to the interpreter team. These navigators have potential to help overcome educational barriers and improve cancer screening rates among DDBHH patients.

#4960 Knowledge, attitudes, and practice patterns of primary care physicians towards cervical cancer screening in Yerevan, Armenia.

N. Chu[1], R. Shahhosseini[2], M. Hovhannisyan[3], S. Shekherdimian[4];

[1]University of California, Irvine School of Medicine, Irvine, CA, [2]Western University College of Osteopathic Medicine, Pomona, CA, [3]Yerevan State Medical University, Yerevan, Armenia, [4]University of California, Los Angeles, Los Angeles, CA

Background: In Armenia, over 60% of cervical cancer is diagnosed at stages 3 or 4. Cervical cancer screening significantly reduces mortality and has been covered by Armenia's national cervical cancer screening program since 2015. As of 2020, only 41% of women aged 30 to 60 have been screened by Papanicolaou test (Pap smear) in the past three years, which is below the screening rate of similarly resourced countries. This study aimed to understand the knowledge and practices of primary care physicians (PCPs) in Yerevan, Armenia towards cervical cancer screening.

Methods: Following institutional review board exemption from University of California, Los Angeles and Yerevan State Medical University, a survey of PCPs was administered in Yerevan, Armenia. Yerevan is the capital and largest city in Armenia. Population proportional sampling throughout all 12 administrative districts to include all 31 adult policlinics was utilized. Consenting physicians who worked as family physicians and "therapists" (internists) and practiced at least half time were included. Pediatricians, gynecologists, and specialists were excluded. A previously developed survey to assess provider knowledge and attitudes regarding cervical cancer screening was utilized and edited for local relevancy. The survey was translated to Eastern Armenian and back translated to English to assure accuracy.

Results: 140 PCPs completed the survey, of which 98% were female and 89% were internists. 91% of respondents were over the age of 40 and 86% had practiced for over 10 years. 98% of PCPs recognized human papillomavirus as a risk factor for cervical cancer. Over 85% of PCPs correctly identified foul-smelling discharge and heavy bleeding as common symptoms for cervical cancer. Almost all PCPs (99%) believed that cervical cancer screening is effective in early detection of cervical cancer. However, most PCPs (98%) disagreed that cervical cancer screening is harmless. Less than half (49%) of PCPs surveyed were trained in performing a Pap smear and only two (1%) performed the procedure. Of those PCPs who did not perform Pap smears, most (97%) believed that it was the role of the gynecologist. A majority (73%) also believed that there was no benefit for PCPs to perform Pap smears. Most respondents (65%) did not believe that patients would have Pap smears if performed by PCPs.

Conclusions: Knowledge regarding risk factors for cervical cancer and benefits of screening is high among PCPs in Yerevan. However, lack of training and hesitancy to perform Pap smears are key barriers to cervical cancer screening delivery and uptake. Inclusion of Pap smears in primary care medical curriculum and redefining the role of PCPs in providing cervical cancer screening may be useful in improving screening access. Furthermore, developing greater trust within the physician-patient relationship may encourage patients to receive Pap smears from their PCPs.

#4961 Bridging gaps in cervical cancer screening: Prevalence and key predictors among women aged 25-59 years in Eswatini.

A. M. Tafese[1], B. D. Dirbaba[2], M. T. Fentie[2], C. Zeigler-Johnson[3];
[1]Thomas Jefferson University, Philadelphia, PA, [2]Drexel University, Philadelphia, PA, [3]Fox Chase Cancer Center, Philadelphia, PA

Background: Cervical cancer is the most prevalent cancer and the leading cause of cancer-related deaths among women in Eswatini, which reported the world's highest cervical cancer incidence (95.9 per 100,000) and mortality (64.3 per 100,000) rates in 2022. Despite this significant burden, cervical cancer screening coverage was limited to 19% as per WHO's 2019 report. This study assessed progress in cervical cancer screening prevalence and identified predictors of screening uptake among women aged 25-59 in Eswatini.
Method: Data were obtained from the 2021 Eswatini Population-based HIV Impact Assessment Survey, a cross-sectional household survey designed to assess the coverage and impact of HIV services among individuals aged 15 years and older. In this study, women aged 25-59, the national target group for cervical cancer screening, were included. Survey weights were applied to adjust for the study design. Bivariate analyses identified factors associated with screening uptake, with significant variables ($p < 0.05$) entered into multivariate logistic regression models. Screening predictors were identified using stepwise backward elimination, with results reported as adjusted odds ratios (OR) and 95% confidence intervals (CI).
Result: Among 3,193 women aged 25-59, the lifetime cervical cancer screening prevalence was 48.2%. HIV-positive women had significantly higher odds of screening compared to HIV-negative women (59% vs. 39%; OR=2.22, 95%CI:1.90-2.59). Women aged 36-45 were more likely to undergo screening than those aged 25-35 (OR=1.35, 95%CI: 1.12-1.62). Higher economic status (fourth vs. lowest wealth quintile) was associated with increased screening uptake (OR=1.41, 95% CI: 1.06-1.88). Conversely, lower odds of screening were observed among women with no formal education (OR=0.58, 95%CI: 0.41-0.84), no healthcare visits in the past 12 months (OR=0.67, 95% CI: 0.55-0.83), and no use of family planning services (OR=0.81, 95% CI: 0.68-0.96). Women with no sexual partners were also less likely to be screened compared to those with two or more partners (OR=0.08, 95% CI: 0.01-0.62). Although rural women had lower odds of screening than urban women, the difference was not statistically significant (OR=0.86, 95% CI: 0.70-1.05).
Conclusion: While cervical cancer screening in Eswatini has shown significant progress, disparities persist among women with lower socioeconomic status, limited education, and fewer healthcare visits.

#4963 The role of education level in cervical cancer screening adherence among deaf, deafblind, and hard of hearing women.

Ciara Luttrell, Hiruni Hewapathiranage-Mayadunne, Erika Bergeron, Poorna Kushalnagar

Gallaudet University, Washington, DC

Background: Deaf, deafblind, and hard of hearing (DDBHH) women using American Sign Language (ASL) experience disparities in cancer screening and cancer-related health outcomes. Barriers to cancer screenings and cancer care have been well documented, owing to social determinants of health ranging from communication barriers to systemic inequalities within the healthcare system. There is a need to further clarify the roles of education status and English proficiency in DDBHH women's adherence to the U.S. Preventive Services Task Force (USPSTF) screening guidelines for cervical cancer.
Methods: Using the NCI Health Information National Trends Survey in ASL, we surveyed adult participants to assess the USPSTF guidelines' age-appropriate adherence to cervical cancer screening. Associations between pap smear adherence (screened vs not screened), self-reported English proficiency (well vs not well), and education status (HS degree or less, some college, college) were assessed using Pearson Chi-squared tests. A two-sided p<=.05 was considered significant.
Results: Over 475 DDBHH adult participants (21-65 years), recruited in-person and virtually between July 2023 and October 2024, answered screening questions for cancer screening in ASL and English. Within this group, 67% self-reported screening adherence for pap smear. In a published study, similarly aged hearing women reported 73.9% adherence in the English version of the National Health Interview Survey (NHIS). Chi-square results indicated that more years of education was significantly associated with screening adherence $[X^2 (2, 484) = 7.613, p= 0.02]$. Self-reported English proficiency was not associated with cervical screening adherence $[X^2 (1, 477) = 1.104, p= 0.30]$.
Discussion: With a 67% adherence rate, the DDBHH community has lower adherence to the recommended screening guidelines compared to the general hearing population. Within this group, those who self-report as HS graduates and/or have left college demonstrate lower adherence to cervical cancer screening guidelines. These findings show that education is key in preventive health behaviors, highlighting the need for targeted interventions to address educational gaps and achieve the Healthy People 2030 target of 79.2% screening adherence. Such research will also aid the Center for Deaf Health Equity in actively working with this DDBHH group to improve strategies for increasing cervical cancer screening adherence.

#4964 Exploring women's healthcare barriers: impacts on adolescent HPV vaccination in Latino communities.

Anju F. Kane, Lourdes Baezconde-Garbanati

Population and Public Health Sciences, USC - University of Southern California, Los Angeles, CA

Objective: This study examines how barriers to healthcare faced by women influence HPV vaccination uptake among Latino adolescents in Los Angeles.
Background: Human papillomavirus (HPV)-induced cervical cancer (CC) is a significant public health concern. Although routine HPV vaccination has been recommended in the United States for girls since 2006 and boys since 2011, disparities in vaccine initiation and completion persist, particularly among Latino adolescents in Los Angeles.
Methods: Using data from the Es Tiempo campaign (2016-2018), this cross-sectional study analyzed responses from mothers of 353 Latino adolescents aged 9 to 26 years. Crosstabs were used to examine differences in vaccine initiation and completion by child sex and birth order. Logistic regression models were used to assess the association between parental healthcare barriers, such as lack of health insurance and access to a primary care provider (PCP), and HPV vaccination initiation.
Results: Among the sample, 44% initiated the HPV vaccine, and 23% completed the series. Girls and firstborn children had higher vaccination rates compared to boys and later-born siblings. Lack of health insurance was associated with lower odds of vaccine initiation, while access to a PCP showed a modest positive effect on vaccination uptake.
Conclusion: Women's healthcare barriers significantly contribute to HPV vaccination disparities among Latino adolescents in Los Angeles. Addressing issues such as limited health insurance access and implementing targeted interventions—especially for boys—could improve vaccination rates. Future research should explore how intersecting factors, including gender, socioeconomic status, education, and healthcare access, shape vaccination behaviors and outcomes.
Keywords: HPV, cervical cancer, women's health, health equity, minority health disparities

#4965 Cooperativity of HIV and HPV co-infection contributes to cervical carcinogenesis and cervical cancer health disparity.

Vivek Kumar Kashyap[1], Namita Sinha[2], Nadeem Zafar[3], Santosh Kumar[2], Murali M. Yallapu[1], Everardo Cobos[1], Subhash C. Chauhan[1]

[1]University of Texas Rio Grande Valley, McAllen, TX,[2]University of Tennessee Health Science Center, Memphis, TN,[3]University of Washington, Seattle, DC

Clinical study show that cervical cancer is the most frequently detected cancer in women living with human immunodeficiency virus (HIV) which has been defined as an acquired immunodeficiency syndrome (AIDS). Women living with HIV are 6 times more likely to develop cervical cancer compared to the general population, and an estimated 5% of all cervical cancer cases are attributable to HIV. Studies suggests that several genes are involved in different molecular pathways which can influence vulnerability, severity, or advancement of cervical cancer in the presence of HIV and human papillomavirus (HPV) co-infection. Clinical study have also shown levels of cervical COX-2 are increased in HIV-infected and HIV/HPV-coinfected women. However, the precise molecular level interactions between HPV and HIV co-infection is not well defined. Further, smoking is well documented among HIV-positive women, but very little is known about the smoking induced molecular mechanism of HPV-HIV co infections. In this study, we investigated the interplay between smoking and HIV/HPV co-infection infectivity and their cooperativity. To check the influence of HIV-HPV co-infection at molecular level, cervical cancer cells, CaSki, were treated with U1(HIV-1-infected U937 cells) cell culture supernatant (UCS) and U937 (HIV-1 noninfected cells) culture supernatant (U9CS) every day for 4 days. To differentiate the U1 cells into macrophages, U1 cells were treated with 100 nM phorbol 12-myristate 13-acetate (PMA) for 3 days. For the treatment of smoking components, both U937 and U1 cells were treated with benzo[a]pyrene (B[a]P) at 100 nM every 24 hours for 4 days. Similarly, we treated HIV infected (U1) and noninfected (U937) cells were treated with HPV infected (CaSki) cells supernatant to determine if HPV infection can influence HIV replication related molecular pathways. Our study demonstrated that HIV-HPV-co-infection and/or B[a]P enhanced cell proliferation, colony formation, migration, and invasion as demonstrated by MTS, wound healing, boyden chamber assay respectively in CaSki cells. HIV-HPV-co-infection and/or B[a]P enhanced also induced the expression of HPV16 E6/E7, HIF-1α, COX-2 and EMT markers in CaSki cell as determined by qRT-PCR and immunoblotting. Furthermore, our qRT-PCR results demonstrated that cytokine IL-6, IL-8, IP-10, MCP-1, IL-1α, IL-10, MIP-1β TNF-α, IFN-γ and G-CSF were significantly increased in CaSki cell treated with UCS and B[a]P. We have also demonstrated that the supernatant of cervical cancer cells exacerbate HIV-1 replication in differentiated U1 cell line via transferring CYPs and HPV oncoproteins through extracellular vesicles. Taken together, our results demonstrate that a molecular link between smoking, HIV infections in HPV cooperativity in cervical carcinogenesis that might also be responsible for cervical cancer disparity among underserved populations.

#4966 Understanding social capital and health care treatment delays for cervical cancer survivors: a cross-sectional study using All of Us data.

Samantha Garcia[1], Angel Arizpe[1], Albert J. Farias[1], Brittany N. Morey[2], Brenda Y. Hernandez[3], Lawrence A. Palinkas[4], Lourdes Baezconde-Garbanati[1], Jennifer Tsui[1]

[1]USC - University of Southern California, Los Angeles, CA,[2]University of California Irvine, Irvine, CA,[3]University of Hawai'i, Manoa, HI,[4]University of California San Diego, San Diego, CA

Black and Latina women are more likely to report heath care delays when receiving treatment for cervical cancer compared to other racial and ethnic groups. Although studies have found higher social capital to be associated with higher cervical cancer screening, it is unclear if social capital is associated with health care delays among women diagnosed with cervical cancer. The purpose of this study was to examine the role of social capital (resources rooted in social networks) on treatment delays in a national sample of cervical cancer survivors from the All of Us dataset. We assessed the relationship between neighborhood social capital and healthcare delays (i.e., experienced at least one health care delay) among women self-reporting they have ever been diagnosed with cervical cancer. We conducted multivariable logistic regression to assess the association between social capital and health care delays while accounting for potential confounding variables (i.e., socioeconomic status, race, marital status, nativity status). Approximately 20% of participants (N=1,009) encountered health care treatment delays. Higher social capital was associated with lower odds of experiencing a health care treatment delay among women diagnosed with cervical cancer after adjusting for confounders (OR = 0.67, CI: 0.60, 0.79). Compared to their White counterparts, Black (OR = .63, CI: 0.50, 0.80) and Hispanic (OR = 0.74, CI: 0.60, 0.90) women had lower levels of self-reported social capital. Social capital was unrelated to nativity status among sample participants. Findings suggest that the development of social capital may help to reduce health care treatment delays among racially and ethnically diverse communities. Understanding how healthcare delays impact cancer care follow-up is an important step towards addressing cancer equity.

#4967 Identifying prognostic biomarkers and risk factors contributing to racial disparity and high mortality in pancreatic cancer at Sentara-EVMS-VOA.

Shoba Abraham[1], Fatima Chaudhry[1], Zakary Kolkey[2], Richard Hoefer[3], Amy Tang[1]

[1]Department of Biomedical and Translational Sciences, Macon & Joan Brock Virginia Health Sciences at Old Dominion University, Norfolk, VA,[2]Department of Internal Medicine, UPenn Health System, Philadelphia, PA,[3]Sentara Surgery Specialists, Sentara Healthcare, Norfolk, VA

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is the most aggressive and tthe deadliest form of human cancer. Early detection and curative intervention regimens are unmet needs in the clinic. The PDAC incidence and mortality have doubled from 1990-2017. Oncogenic K-RAS mutation is universally activated in PDAC tumors, that drive tumor malignancy, early relapse, chemo-resistance, metastasis, and dismal survival. In this study, we examine whether SIAH, an evolutionarily conserved E3 ligase that is the most downstream signaling gatekeeper of the EGFR/K-RAS pathway, has prognostic value in PDAC.
Methods: A cohort of 1,156 patients with PDAC, diagnosed at Sentara-VOA between 2001-2016 was analyzed to determine the association of SIAH expression with TNM staging, treatment efficacy, and survival prediction. SIAH expression was calculated by the average immunohistochemical (IHC) scores from two clinical pathologists in 148 operable PDAC patients, including 34 neoadjuvant chemotherapy (NACT)-treated residual tumors and 114 untreated primary tumors.
Results: Among 1,156 PDAC patients, 354 of them were operable and 802 of them are inoperable. A comparison of the 5-year survival rates showed that our local cohorts were underperforming to that of the national average from 2001 to 2016. Following the PDAC clinical procedural standardization at Sentara Cancer Network, our local 5-year survival rates have started to improve steadily, and it has approached the national standards after 2010. Analysis of the SIAH expression in 34 NACT-treated residual tumors showed that low SIAH expression (< 5%) correlated with a longer survival than those with higher SIAH expression (>5%).
Discussion: Among 1,156 PDAC patients, 32.8% of the white patients and 27.4% of Black/AA patients received surgical resection. A 5.4% difference of the two-race groups in the operable cohort suggested that Black/AA patients are more likely to present with advanced and unresectable PDAC. The narrowing of the survival gap between the local and national cohorts reflects a better PDAC management plan, treatment standardization, and timely implementation of effective treatment modalities, including the Whipple procedure, and NACT regimens for all the eligible operable PDAC patients, at Sentara Hospitals after 2010. We found that persistent high SIAH expression in residual tumors post-NACT is indicative of chemo-resistant tumor cells at the 1st-line neoadjuvant setting, and SIAH may be prognostic to identify high-risk PDAC patients who are prone to develop early relapse and suffer poor survival clinically.

#4968 Cardiovascular disparities amongst pancreatic ductal adenocarcinoma patients.

Arunima Punjala, Vignesh Vudatha, Vashti Bandy, Maria Gergoudis, Leopoldo Fernandez, Wendy Bottinor, Jose Trevino

Virginia Commonwealth University Health System, Richmond, VA

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is a lethal malignancy associated with skeletal muscle cachexia which results in chemotherapy intolerance, nutritional deficiencies, physical debilitation, and increased mortality. Survival time is significantly lower for those with cardiovascular-associated mortality, and Black patients face a disproportionately higher risk of overall and cardiovascular-associated mortality. Minimal literature exists on the impact of PDAC on cardiovascular function and the implications on disease course. The aim of this study is to utilize cardiac magnetic resonance imaging (CMR) to quantify changes in cardiovascular structure and function that can potentially identify patients with PDAC who are at increased risk for cardiovascular events.

Methods: In this prospective observational study, treatment-naïve PDAC patients underwent non-contrasted CMR prior to initiation of therapy and six months after. Left ventricular mass was calculated using short axis cine images at end diastole. The maximal and minimal descending thoracic aortic areas were measured on 4-chamber cinematic sequences. Resting blood pressure was measured during the exam. Aortic distensibility (AD) was calculated using a validated equation and compared with age-appropriate reference values[1]. Variables were analyzed using a Mann-Whitney U Test with $\alpha = 0.1$.

Results: 22 patients underwent baseline CMR with 32% (7/22) receiving a follow-up scan. In total, 59% (13/22) were female, 41% (9/22) identified as Black, and 46% (10/22) were sarcopenic at baseline. Amongst all participants, 64% (14/22) demonstrated abnormal AD with Black participants demonstrating a significantly higher prevalence compared to White participants (89% vs. 50%, p=.087). Left ventricular mass index (LVMI) of 57 g/m^2 or lower is a known predictor of major adverse cardiovascular events amongst cancer patients. All participants had a low LVMI with sarcopenic patients demonstrating lower LVMI relative to non-sarcopenic patients (50.7 g/m^2 vs. 54.8 g/m^2, p=0.623). There were no significant differences between baseline and follow-up scans.

Conclusions: PDAC patients, particularly those who are Black or sarcopenic demonstrate abnormalities in cardiovascular function and structure prior to cancer therapy. These changes are known to be associated with adverse cardiac events and advocates for close cardiac monitoring, particularly in susceptible populations. Further studies are necessary to identify specific risk factors associated with these initial changes and interventions to optimize cardiovascular health.

1. Nethononda RM, Lewandowski AJ, Stewart R, Kylinterias I, Whitworth P, Francis J, Leeson P, Watkins H, Neubauer S, Rider OJ. Gender specific patterns of age-related decline in aortic stiffness: a cardiovascular magnetic resonance study including normal ranges. J Cardiovasc Magn Reson. 2015 Feb 19;17(1):20.

#4969 Ethnic disparities in breast and cervical cancer screening in Guam: a cross-sectional analysis (2012-2022).

Aurienne Cruz[1], Grazyna Badowski[1], Grace Garces Bordallo[2]

[1]University of Guam, Mangilao, Guam,[2]Guam Department of Public Health and Social Services, Mangilao, Guam

Background: In Guam, breast and cervical cancer late-stage diagnoses and mortality rates exceed U.S. averages. From 2013-2017, 30.7% of breast and 28.6% of cervical cancers were diagnosed late. Micronesians had the highest late-stage breast cancer rate (59.1%); Filipinos had the highest for cervical cancer (33.3%). Breast cancer mortality was 27.3 per 100,000 in Guam vs. 20.3 in the U.S., with Micronesians at 104.6. Cervical cancer mortality was 6.7 in Guam vs. 2.3 in the U.S., with Micronesians at 22.3.
Methods: A cross-sectional study analyzed 6,625 female adults from Guam using Behavioral Risk Factors Surveillance System data from 2012 to 2022. We examined current mammogram screenings for women aged 40 and older and Pap tests for women aged 21 to 65. Binary logistic regression models assessed trends and associations, adjusting for age, education, income, and survey year.
Results: Overall, 64.4% of women ≥40 were current with mammograms, and 67.7% of women aged 21 to 65 had recent Pap tests. White women had the highest screening rates (70.7% for mammograms; 79.7% for Pap tests), while Micronesian had the lowest (44.2% for mammograms; 49.1% for Pap tests). Mammogram rates increased by 1.4% bi-annually, with the highest increases among Filipino (2.9%) and White (1.3%) women, but declined among Micronesians (-0.8%). Pap test rates declined slightly (-0.3%), with CHamoru women having the biggest decrease (-2.1%). Despite Micronesian women having the largest increase in Pap test rates (3.7%), their screening rates (54.1%) remained significantly below the overall rate (68%). Adjusted odds ratios showed higher mammogram screening among Asians (OR = 1.3; 95% CI: 1.1-1.6) and lower among Micronesians (OR = 0.6; 95% CI: 0.6-0.8) compared to White women. Age groups 50-59 (OR = 2.2; 95% CI: 2.0-2.6), 60-69 (OR = 2.8; 95% CI: 2.6-3.0), and 70+ (OR = 2.0; 95% CI: 1.8-2.2) had higher odds than ages 40-49. Higher education and income levels were associated with increased screening. For Pap tests, Filipinos (OR = 0.6, 95% CI: 0.6-0.7), Asians (OR = 0.7, 95% CI: 0.6-0.8), and Micronesians (OR = 0.5, 95% CI: 0.5-0.6) had lower odds compared to White women. Age group 60-69 had lower odds (OR = 0.6; 95% CI: 0.5-0.7) compared to ages 18-29. Higher education and income were also associated with increased Pap test screening.
Conclusion: Despite some improvements, Guam's cancer screening rates remain below U.S. medians. Significant ethnic disparities exist, especially among Micronesian and CHamoru populations. Targeted interventions are needed to improve screening rates and reduce late-stage cancer diagnoses among these groups.
Acknowledgement: This research was funded by the National Cancer Institute: The University of Guam/University of Hawaii Cancer Center Partnership to Advance Cancer Health Equity (PIPCHE), Grant U54CA143728 (University of Guam, YCP)/Grant U54CA143727 (University of Hawaii Cancer Center).

#4970 Inpatient outcomes and racial differences in male breast cancer: insights from the 2021 National Inpatient Sample.

Raj Shah[1], Anas Alqam[1], Elie Chalhoub[2], Khalil Choucair[3]

[1]Internal Medicine, University of Kansas Medical Center, Wichita, KS,[2]Cancer Center of Kansas, Wichita, KS,[3]Medical Oncology, Barbara Ann Karmanos Cancer Institute/Wayne State University, Detroit, MI

Introduction: Male breast cancer (MBC) accounts for 1% of all breast cancer patients. In the US, 2800 MBC new cases are diagnosed yearly. MBC patients have high mortality due to late and advanced stages at diagnosis. Understanding demographics and racial disparities in MBC is crucial, and may significantly impact morbidity and mortality.
Method: We analyzed data from the National Inpatient Sample and identified patients admitted to the hospital with MBC diagnosis in 2021 using STATA BE 18.0 software. To accurately identify metastatic disease to the bone, lung, and meninges, we excluded patients with any of these concurrent diagnoses: melanoma, primary central nervous system (CNS) tumors, and prostate and lung cancer. We investigated baseline patient characteristics and stratified the results based on race. The primary endpoint was in-hospital mortality (IHM). Length of stay (LOS) and total charge (TOTCH) were secondary endpoints. Results were adjusted for age, race, insurance type, smoking, alcohol, liver disease (LD), diabetes (DM), chronic kidney disease (CKD), and congestive heart failure (CHF). Other factors including metastases to the bone, lung, meninges, history of chest radiation, protein energy malnutrition (PEM), and palliative care utilization were also assessed and adjusted for.
Results: A total of 1,915 patients met criteria for inclusion. Of those, 75% were Caucasians,16% African Americans (AA), and 9.2% of other races. AA patients were significantly younger (mean age 64 vs. 69 years in non-AA, $p<0.05$), and more commonly in the lowest income quartile (49% vs. 22% for non-AA; $p<0.05$). Clinically, 24% had estrogen receptor-positive status, 10% had prior chest radiation, and 32% had metastatic disease (24% to the bones, 16% to lung, 11% to liver, and 5.4% to CNS). Comorbidities included smoking (40%), obesity (20%), type 2 DM (35%), coronary artery disease (27%), CHF (26%), and PEM (12%), Overall IHM was 5% with a mean 6-day LOS. Compared to non-AA, AA patients had significantly higher mortality rates (11% vs. 4 %, $p<0.05$) and longer LOS (9 vs 5.4 days; $p< 0.05$). TOTCH was higher in AA ($87,937 vs. $68,852) but did not reach significance ($p=0.48$). LD, CKD, and liver metastases were independent predictors of inpatient mortality. Palliative care utilization was 10% overall, and similar in both races (AA-16% vs non-AA 10%, $p=0.15$).
Conclusion: Significant socio-economic and racial disparities impact MBC outcomes, with AA patients presenting at younger ages, experiencing longer LOS, and higher mortality rates. The high prevalence of comorbidities and metastatic disease at presentation highlights the need for earlier detection through screening and education programs, and for interventions to address racial disparities, namely in AA patients. Future research should focus on understanding potential barriers underlying these disparities, to develop strategies to mitigate them.

#4971 Predicting lung cancer screening adherence among underserved and minoritized communities using social determinants and neighborhood factors.

Kun-Han Lu, Yi Xiao, Aamna Akhtar, Jazma Tapia, Calvin Tribby, Jay Yang, Marta Jankowska, Chi Wah Wong, **Loretta Erhunmwunsee**

City of Hope National Medical Center, Duarte, CA

Purpose: Lung cancer screening (LCS) lowers cancer mortality if individuals adhere to annual scans. Minoritized communities have significantly lower adherence to LCS than non-Hispanic whites. The influence of social determinants of health (SDOH) on these disparate adherence rates are rarely evaluated. The aim of this study is to develop a machine learning (ML) model that integrates individual and neighborhood level SDOH to predict which minoritized individuals are at risk of LCS non-adherence.
Methods: A total of 168 participants with high-risk smoking histories who underwent their first low dose computed tomography (LDCT) between 2017 and 2021 were enrolled. Surveys on SDOH and perceived discrimination were completed. Participants' addresses were joined to neighborhood level SDOH data from various publicly available sources. These indices captured neighborhood socioeconomic status and segregation. Adherence rates were calculated by assessing electronic medical records to determine if participants had a second LDCT within 15 months. Participants were temporally split into training (N=125) and testing (N=43) sets with a cut-off date of June 30, 2021. A gradient boosting classifier, XGBoost, was trained to predict LCS non-adherence, incorporating variables such as sociodemographic status, perceived discrimination, previous screenings, smoking status, and geospatial indices. The SHapley Additive exPlanations (SHAP) method was used to determine the relative importance of each feature in the model's predictions.
Results: The participants represented a diverse group: Asian (26%), Black/African American (32%), and Hispanic/Latino (32%). The LCS non-adherence rates were 66% in the training set and 63% in the testing set. The XGBoost classifier achieved an Area Under the Receiver Operating Characteristic Curve (AUROC) of 0.79 and an Area Under the Precision-Recall Curve (AUPRC) of 0.90, outperforming a simple logistic regression model (AUROC=0.63 and AUPRC=0.79). The XGBoost classifier's performance metrics were Accuracy=0.77, Recall=0.85, Specificity=0.62, Positive Predictive Value=0.79, and Negative Predictive Value=0.71. The SHAP analysis indicated that participants with no prior history of colorectal cancer screening, who lived in areas with lower quality school systems, and with a higher percentage of individuals below the poverty line, were at a higher risk of non-adherence to LCS.
Conclusions: LCS reduces lung cancer survival, but LCS adherence is significantly lower in minoritized groups. Our ML model strongly predicted LCS non-adherence based on SDOH and geospatial factors. Specific neighborhood level SDOHs predicted LCS non-adherence more strongly than individual level factors. These findings support interventions that address both individual and neighborhood-level barriers to increase LCS adherence.

#4972 Changing landscape of liver cancer disparity due to the impact of social determinate of health in Georgia.

Amit Kumar Srivastav[1], Manoj Kumar Mishra[2], Shailesh Singh[1], Brian Rivers[3], James W. Lillard[1], Rajesh Singh[1]

[1]Department of Microbiology, Biochemistry and Immunology, Morehouse School of Medicine, Atlanta, GA,[2]Department of Biological Sciences, Alabama State University, Montgomery, AL,[3]Morehouse School of Medicine, Atlanta, GA

Liver cancer (LC) is one of the most devastating malignancies. Low socioeconomic status (SES), especially poverty, negatively affects HCC outcomes. Poverty is a risk factor in cancer incidence, late-stage diagnosis, and mortality for all racial/ethnic groups. This study aims to evaluate the correlation between LC incidence and Social Determinants of Health (SDOH), focusing on major ethnic groups (African American (AA) and European Americans (EA)) in Georgia (GA). We utilized Geospatial Technology (GT) to analyze the connection between SDOH and LC prevalence in AA and EA populations in GA. This study incorporated county-level LC prevalence and association with SES and health disparities. The robust data mining (All of Us, NCI Cancer Atlas, and SEER) enabled us to explore the association between SDOH and LC in the spatial context. Integrating spatial and non-spatial data on LC prevalence to SES will help predict and formulate LC prevention methods. The GT's secondary data analysis revealed that LC affects minority communities in GA. The GT analysis further demonstrates poverty, uninsured rates, and food insecurity were positively correlated with higher LC incidence, reflecting the impact of economic instability on health outcomes. Limited access to exercise and higher crime rates in some counties further compounded risks, likely through indirect effects on stress and health behaviors. Racial disparities were prominent, with AA populations experiencing a disproportionately higher burden of LC compared to EA. The analysis highlighted that GA counties with elevated poverty rates (20-33%) and higher uninsured percentages (18-26%) exhibited increased LC incidence, particularly within AA populations. In these communities, the LC rates reached 20-24 cases per 100,000 for AA males and 15-20 for AA females, which is significantly higher compared to the rates for EA males at 10-16 cases and EA females at 6-12 cases. The study underscored notable racial disparities, revealing that AA populations bear a disproportionate burden of LC, linked to a complex interplay of socioeconomic, behavioral, and systemic influences. Additionally, the findings indicated that AA individuals had lower levels of educational attainment, with 10-20% holding a bachelor's degree, alongside higher rates of smoking (20-30%) and alcohol use (15-25%). Conversely, EA populations demonstrated more favorable SES indicators, including lower unemployment rates (6-12%) and a greater percentage with higher education (20-25%). These findings underscore the intricate interplay of socioeconomic, behavioral, and systemic factors driving LC disparities. Our finding demonstrates that SES is an essential factor that modulates LC incidences in underserved minority populations in GA. Hence, understanding the association between socioeconomic disparities will help predict and prevent LC more effectively.

#4973 Assessing urban-rural differences in the outcomes of the most common cancers in Georgia: Incidence rates and stage at diagnosis.

Amelito Manuel Andaya[1], A. Miguel Andaya[2], Stanley Atencah[1], Jason Holligan[1], Chinenye Egwuonwu[1]

[1]Piedmont Athens Regional Medical Center, Athens, GA,[2]University of Santo Tomas Faculty of Medicine and Surgery, Manila, Philippines

Introduction: Lung, pancreatic, colon, prostate and breast cancers are the leading causes of cancer-related deaths in Georgia. Mortality is highest in rural counties which have higher poverty rates and larger composition of minority population. Our study aims to compare the proportion of the five most common cancers in Georgia that were diagnosed at late stages between urban and rural county locations.
Methods: The National Institutes of Health State Cancer Profiles database was examined for incidence rates of breast, colon, lung, prostate and pancreatic cancers from 2017 to 2021 in Georgia. Age-adjusted incidence rates were calculated and stratified by county name, location and percentage at late stage of diagnosis. Late stage was defined as the presence of regional or distant metastases at the time of diagnosis.
Results: Among women diagnosed with breast cancer, rural counties had a statistically significant higher percentage of late-stage diagnoses compared to urban counties. In contrast, no significant differences were observed in the incidence of late-stage diagnosis for colon, lung, prostate, or pancreatic cancers between rural and urban populations.
Conclusion: The study demonstrated a statistically significant disparity in late-stage breast cancer diagnoses between rural and urban counties in Georgia. This emphasizes the need to improve early detection of this cancer that is alarmingly becoming more common. More attention should be given to these underserved communities given these findings. While no statistically significant rural-urban differences were found for other cancer types, further observational studies with more robust methodologies are needed in order to understand the observed incongruence in stage at diagnosis of breast and the other leading cancers in Georgia.

Comparison of Mean Percentage of Late-stage Diagnosis of the Top Five Cancers in Georgia

Type of Cancer	Rural	Urban	p-value
Breast	36.43	33.45	0.0007
Colon	57.86	58.24	0.7137
Lung	70.97	70.11	0.4418
Prostate	19.31	19.86	0.6224
Pancreas	78.67	79.44	0.6217

#4974 Leveraging partnerships through a Florida Innovation Grant: A collaborative approach by the Care2 Health Equity Center.

Nazleen Del Rio[1], Brooke Hensel[1], Ileana Guzman[1], Sandra Suther[2], Fern J. Webb[3], Paul Okunieff[4], Diana Wilkie[4]

[1]University of Florida, Orlando, FL,[2]Florida A&M University, Tallahassee, FL,[3]University of Florida, Jacksonville, FL,[4]University of Florida, Gainesville, FL

The Florida-California CaRE^2 Health Equity Center advances its mission to eliminate cancer health disparities through a multifaceted approach that promotes innovation in cancer research. Funded by a Florida Department of Health Innovation Award to UF, the Florida-based CaRE^2 research team is directly collaborating to implement this innovative project. One of the first activities was to develop materials designed to increase awareness of the RadTox test - a test using small amounts of blood to assess whether one's tumor is responding to the current cancer treatment. Having educational materials in Spanish is essential for enabling Spanish-speaking individuals to consider participating in cancer-related research. Recognizing the importance of language access, CaRE^2 bi-lingual team members translated materials into Spanish with CaRE^2 tri-institutional investigators indirectly involved back-translating information to ensure accurate information transfer. The other key collaborative effort is information sharing to reach a broader community beyond clinical populations in collaboration with the industry partner, DiaCarta. The CaRE^2 Health Equity Center's is leveraging several resources to inform the community about this project, including our community contact registry, community events direct outreach, and collegial connections. With more than 1,408 adults enrolled from CaRE^2's communities, the contact registry enhances the capacity to inform more people about this test and involvement opportunities. Information also is shared at English and Spanish-speaking community events held in CaRE^2's immediate catchment areas. Additionally, CaRE^2 investigators continue to leverage institutional, community, and industry partnerships, resulting in a network filled with varying perspectives and expertise partnering on this innovative project. Since October 2024, 1,116 CaRE^2 contact registry members who are Florida residents and 20 people attending three community events received CaRE^2 and IRB approved materials about this new test. So far 1,200 individuals have responded to seek and give additional information from the study REDCap site. CaRE^2 investigators also partner to assist with sharing this information through mass media in local and statewide outlets, leading to additional opportunities to collaborate on this innovative research study. CaRE^2 continues to leverage our capacity by involving our tri-institutional team and co-investigators not directly involved with this project to review materials for population-relevance and fidelity and suggest additional strategies to inform the broader population about this novel test. Given our continued success, CaRE^2 recommends using multifaceted approaches to address cancer health disparities and create community-centered solutions that leverage existing human capacity and resources.

#4976 Examining the association between chronic inflammation, stress, and resilience factors among diverse breast cancer survivors using the NIH All of Us dataset.

Bathsheba Aklilu, Erica Tate, Cathy Samayoa

Biology, San Francisco State University, San Francisco, CA

Breast cancer disparities exist by race and ethnicity, including disparities in age at diagnosis, quality of life, and health outcomes. These disparities may be influenced by chronic stress, exacerbated by unique challenges that extend beyond disease management, such as the effects of psychosocial stress and social determinants of health (SDOH) on chronic inflammation. Elevated C-reactive protein (CRP) levels, a marker of chronic inflammation, are implicated in worse health outcomes. Understanding how stress and SDOH interact to influence CRP and other inflammatory biomarkers is critical for addressing disparities in survivorship care. However, most studies lack adequate representation of racial and ethnic minorities; thus, factors associated with chronic inflammation among diverse breast cancer survivors remain unknown. This study aims to investigate factors associated with chronic inflammation among diverse breast cancer survivors using the NIH All of Us research dataset. Using Python, a programming language available within the NIH All of Us research program dataset, we examined CRP and additional inflammatory biomarkers and their relationships with stress, economic, social, and environmental factors. Economic and environmental factors included income, housing security, and neighborhood characteristics. Social and psychological factors included social support, the Perceived Stress Scale (PSS), and the Everyday Discrimination Scale (EDS). Multivariate analyses were conducted to evaluate associations between stress, resilience, SDOH, and inflammation, with stratification by age, BMI, and allostatic load. Preliminary findings reveal significant racial and ethnic disparities in CRP levels. Black and Hispanic breast cancer survivors exhibited the highest CRP levels. We identified 3,110 participants with breast cancer in the All of Us research program dataset. Of those, 7.2% were African American or Black, 2.2% were Asian, and 6.7% were Hispanic. We hypothesize that inflammation will be higher among racial and ethnic minorities, as well as among women with higher levels of stressors, such as low income and increased PSS and EDS scores. Furthermore, we expect inflammation to be negatively associated with resilience factors, such as increased social support and greater neighborhood safety. This study will identify factors associated with chronic inflammation among diverse breast cancer survivors and provide a deeper understanding of the biological mechanisms driving breast cancer health inequities.

#4977 Knowledge and acceptance of the human papillomavirus vaccine in parents from the Colombian Caribbean.

Ines Benedetti, Laura Mendoza Carmona, Valeria Duran Cordero, Sebastian Payares Florez, Ariel Marrugo Ortiz, Luis Alvis Estrada

School of Medicine, Universidad de Cartagena, Cartagena, Colombia

Introduction: cervical cancer ranks eighth in global cancer incidence and fourth among women worldwide. Its development is strongly associated with infection by the Human Papillomavirus (HPV) making vaccination a critical preventive measure. In 2012, Colombia's Ministry of Health launched a free HPV vaccination program, positioning the country as one with the highest coverage rates globally. However, vaccination rates began to decline in 2014, with coverage for the second dose reported at 27% that year. This decline has been attributed to an incident occurred in 2014 in the municipality of El Carmen de Bolívar. The reasons behind this decrease and the current perceptions of parents regarding the vaccine remain unclear. The objective of this study was to identify factors influencing HPV vaccine acceptability among parents of girls under 13 in two Colombian Caribbean municipalities.
Methods: a cross-sectional study was conducted among parents of girls under the age of 13 residing in two Colombian Caribbean municipalities. Data were collected using a structured survey administered, focused on exploring sociodemographic characteristics and knowledge related to the HPV vaccine.
Results: a total of 238 participants were surveyed, with a mean age of 33 years old, 87.39% of whom were women. 78.1% belonged to the lowest socioeconomic stratum. All participants reported having daughters aged 13 years or younger. Vaccine acceptance was 76.8% (88% among those under 30 years old, n=84) and 70% among parents 30 years of age and older (n=154). Despite this, a significant proportion of participants lacked knowledge about how HPV is transmitted or prevented (38.6%, n= 92),and misconceptions about severe adverse effects, such as seizures, were common (between 32.79% and 35.34%).
Conclusion: vaccine acceptance was associated with knowledge about HPV, and influenced by sociodemographic factors. In both communities, distrust regarding adverse effects remains a significant barrier to vaccination. Tailored educational interventions that address the specific needs and characteristics of each community are essential to address these challenges, improve HPV vaccine acceptance, and meet coverage goals, contributing to cervical cancer prevention and enhancing women's quality of life.

#4979 Effect of allostatic load (AL) and neighborhood socioeconomic status (NSES) on cancer detection and false positive rate after screening mammography.

Niam Abeysiriwardena[1], Braelyn Wekwerth[1], Nathaniel Mercaldo[1], Sarah Bell[2], Ruth Carlos[1]

[1]Massachusetts General Hospital, Boston, MA,[2]University of Michigan, Ann Arbor, MI

Purpose: AL refers to the cumulative physiological dysregulation on the body's systems due to chronic stress. These cumulative stressors have been hypothesized to increase the risk of breast cancer (BC). AL has been associated with increased risk of high-risk benign breast pathology among women with a false positive (FP) biopsy. Separately, NSES has also been associated with BC outcomes. However, the impact of AL and NSES on cancer detection and FPs rate in a screening mammography population has not been evaluated.
Materials and Methods: We retrospectively reviewed the Mass General Brigham Biobank to identify women aged ≥40, who underwent screening mammography from Jan 1, 2021 to Dec 31, 2021. We collected age, self-reported race/ethnicity and zip code, which were geocoded to their corresponding census tract. The census tract-assigned NSES were calculated using U.S. Census Bureau American Community Survey 5-Year Estimates for 2015-2019 and summarized to the ZIP code level. We collected the following biomarkers obtained within two years before the index screen: cardiovascular [heart rate, systolic, and diastolic blood pressure], metabolic [body mass index, alkaline phosphatase, glucose, albumin], immunologic [white blood count], and renal [blood urea nitrogen, creatinine]. AL was assigned one point for each lab value in the worst quartile. The sum of all assigned points represented the composite AL, treated as a continuous variable. We collected diagnostic breast imaging and biopsy encounters that occurred in the 12 months after the index screen. To account for missing data, we performed multiple imputation. Separate multivariable logistic regression models assessed age, race, NSES, and AL associations with cancer detection and with FP rates. Rubin's rules were applied to estimate overall odds ratios, confidence intervals, and p-values for all covariates.
Results: Of 13,754 women assessed, 1.2% (n=169) women received a cancer diagnosis. Of the women, 87.6% were White, 2.6% Asian and 5.3% were Black; 1.5% self-identified as Hispanic; the mean age was 64.4±11.3 SD. AL increased risk of cancer detection after screening mammography by 16% (odds ratio OR=1.16, 95% CI:[1.06, 1.26], p=0.001). NSES was marginally associated with cancer detection (OR=1.13[0.99-1.29], p=0.067). After adjusting for AL and NSES, age and race were not significant correlates of cancer detection. We did not detect an association between FPs and either AL or NSES, after adjusting for race and age.
Conclusion: In our sample, allostatic load was associated with increased risk of cancer detection after screening mammography after adjusting for age, race, and NSES. A similar association between AL and FPs was not detected. Further work is needed to confirm these observations.
Clinical Relevance Statement: AL may represent a potential biomarker for personalized mammographic screening for BC.

#4980 Association of allostatic load (AL) and neighborhood socioeconomic status (NSES) and breast biopsy outcomes after screening mammography.

Braelyn Wekwerth[1], Niam Abeysiriwardena[1], Nathaniel Mercaldo[1], Sarah Bell[2], Ruth Carlos[1]

[1]Massachusetts General Hospital, Boston, MA,[2]University of Michigan, Ann Arbor, MI

Purpose: AL represents the physiological consequences of repeated or chronic exposure to exogenous and endogenous stressors, including NSES, which are hypothesized to increase the risk of breast cancer (BC). We showed AL correlation with BC and high-risk benign breast disease after biopsy after a palpable mass or abnormal diagnostic breast imaging. However, the effect of AL and NSES on cancer detection and false positive (FP) biopsy results after a screening mammogram has not been evaluated.

Methods: We retrospectively reviewed the Mass General Brigham Biobank to identify women aged ≥40 who underwent screening mammography between 1/1/2021-12/31/2021 and subsequent breast biopsy. We collected age, self-reported race/ethnicity, and zip code and then geocoded to their corresponding census tract. The census tract assigned socioeconomic index scores (SES) were calculated using U.S. Census Bureau American Community Survey 5-Year Estimates for 2015-2019 and summarized to the ZIP code level. We collected the following biomarkers obtained within two years before the index screen: cardiovascular [heart rate, systolic, and diastolic blood pressure], metabolic [body mass index, alkaline phosphatase, glucose, albumin], immunologic [white blood count], renal [blood urea nitrogen, creatinine]. AL was assigned one point for each lab value in the worst quartile. The sum of all assigned points represented the composite AL, treated as a continuous variable. We collected diagnostic breast imaging and biopsy encounters occurring within 12 months after the index screen. Multiple imputation accounted for missing data. Multivariable logistic regression assessed age, race, SES, and AL associations with cancer detection and FP rates. We applied Rubin's rules to estimate overall odds ratios, confidence intervals, and p-values for all covariates.

Results: Of the 418 eligible women, 59.6% had an FP biopsy, and the remainder had BC, including ductal carcinoma in situ. Women, on average, were 62 years old (SD=13), and 85.6% were White. Each unit increase in AL was associated with a 14% increase in risk of cancer detection (OR 1.14(1.01,1.30),p=0.034) after adjusting for age, race, and NSES. Both age (OR 1.21(1.11,1.321),p<0.001) and marginally NSES (OR 1.19(0.99,1.43),p=0.068), also correlated with cancer detection. We did not detect a significant association between AL and high-risk FP pathology.

Conclusion: AL, age, and NSES are associated with cancer detection after screen-indicated biopsy but not high-risk FP pathology. These results underscore the importance of considering biological markers and demographic factors in refining biopsy strategies after a positive screening mammography.

Clinical Relevance Statement: AL represents a potential biomarker for precision selection of women after screen-detected mammographic abnormalities to increase cancer yield for biopsy.

#4981 Implementation of a community-based HPV vaccination intervention supported by a cross-sector community coalition.

Ruth Rechis[1], Haley Gardiner[1], Mayra Aquino[1], Katherine Oestman[2], Efrat Gabay[3], Paula Cuccaro[3]

[1]Cancer Prevention and Control Platform, The University of Texas MD Anderson Cancer Center, Houston, TX,[2]Daybreak Consulting, Las Vegas, NV,[3]UT Health Houston, Houston, TX

Human papillomavirus (HPV) vaccination is a successful strategy to prevent HPV related infection and cancer. However, vaccination uptake remains suboptimal in the U.S., with Texas having the largest number of unvaccinated adolescents. Be Well Communities™, MD Anderson's place-based approach for cancer prevention and control, works with communities to promote wellness and address modifiable cancer risk factors. Initially, the initiative in Baytown, TX focused on healthy eating and active living interventions. Over time, the community's trust and interests expanded to more traditional cancer prevention interventions (e.g., HPV vaccination, screening). In this project, UTHealth Houston's All for Them (AFT) program was funded for three years to work with Goose Creek Consolidated Independent School District (GCCISD) to reduce adolescent vaccination barriers by providing community-based mobile vaccination clinics. GCCISD's students are 90% minority and 56% economically disadvantaged. The Be Well Communities team facilitated the relationship and supported GCCISD staff to ensure optimal implementation, assisted with mobile clinic site location, and provided educational sessions at Steering Committee meetings, of which the AFT team was a steadfast member. AFT included development and implementation of an educational messaging campaign for adolescents and their parents, school nurse continuing education to improve HPV vaccination knowledge, confidence in talking with parents about vaccination and data recordkeeping, and mobile vaccination clinics at school- and community-based venues. Program strategies resulted in increased parental engagement and trust of the team and project, improved parental acceptance of HPV vaccination, and excellent feedback from GCCISD staff about services provided. School nurses (n=31) participated in multiple professional development and training opportunities. HPV vaccines (n=1,154) were provided at 57 vaccination clinics. The number of students in GCCISD who received vaccines at multiple clinics was 37% higher than the largest school district in which AFT intervenes (outside of this initiative). With two years of available data, HPV vaccination rates increased 16.9 percentage points (46.3%) in initiation and 11.1 percentage points (77.1%) in completion among students in the participating schools. Due to their active participation on the Steering Committee, the AFT team formed relationships with all 21 organizations and fostered formal partnerships with 4 organizations. The relationships developed through participation with the Steering Committee built community collaborations in Baytown and facilitated alternative ways to bring awareness to and host vaccination-related education and activities. The project's success led to additional grant funding and sustained partnerships.

#4982 A randomized controlled trial of financial incentives to improve electronic health record (EHR)-driven recruitment among underrepresented communities.

M. A. Fiala, W. Gema, A. Davis, F. Ademuyiwa;
Washington University School of Medicine in St. Louis, St. Louis, MO

Background: Disparities in EHR portal use have been documented based on age, race, ethnicity, and other factors. Thus, EHR-driven research recruitment may worsen representation of these groups. Monetary incentives can increase response rates, but their impact on improving inclusivity in research is unknown. In this study, we aimed to determine if a financial incentive improves response rates to recruitment messages in an equitable fashion.
Patients and Methods: A random sample of 1,200 patients with diagnoses of either breast, colorectal, prostate, or lung cancer treated at Washington University in St. Louis received a recruitment message for a fictitious cross-sectional survey study “The Social Aspects of Cancer Care” via their EHR portal. Two strata of patients were sampled, those who identify as non-Hispanic White (n = 600) and those who identify as another race or ethnicity (n = 600). Participants were randomized to receive an offer of a $20 monetary incentive (n=400) or no incentive (n=800) for their enrollment. Patients were debriefed following their participation, and all participants received the incentive regardless of randomization assignment. The primary endpoints include the comparison of response rates by 1) race and 2) financial incentive.
Results: The sample consisted of 1200 patients with a history of breast (31%), prostate (28%), lung (25%), and colorectal cancer (16%). The mean age was 65.7 years (SD 12.3) and 46% were male. Fifty percent identified non-Hispanic White, 38% African American or Black, and 12% as another race or ethnicity. Overall, 39% of participants viewed the recruitment message and 13% responded that they were interested. Incentives did not improve response rates to a statistically significant degree. The response rate for the incentive cohort was 15% compared to 12% for no incentive ($p = 0.206$). Response rates were higher ($p < 0.001$) among non-Hispanic White patients (18%) than for African American or Black (7%) and members of other racial/ethnic groups (13%). This was true for patients who did not receive an offer of an incentive (18% compared to 9% and 10%; $p <0.001$) and for those that did (19% compared to 9% and 20%; $p = 0.028$). There was a trend for greater improvement in response rate for patients of racial and ethnic minorities, but it was not statistically significant ($p = 0.129$). We performed a secondary analysis of the 471 patients who viewed the message and found similar results regarding the differences in response rates based on race and ethnicity and the lack of impact of incentives.
Conclusions: People with cancer who identify as a racial and ethnic minority are less likely to view and respond to recruitment messages for research studies through their EHR patient portal. The impact of a $20 financial incentive was modest, if any, but may be greater on people who identify as a racial and ethnic minority.

#4983 Allostatic load and prevalence of monoclonal gammopathy of undetermined significance.

M. A. Fiala, T. Lan, M. Slade, R. Vij, S.-H. Chang;
Washington University School of Medicine in St. Louis, St. Louis, MO

Background: Repeated exposure to stress can result in dysregulation of the endocrine system and excess secretion of hormones, even when stress is not present. The degree of dysregulation has been coined allostatic load (AL), and higher AL has been associated with increased risk of cancer. We explored the relationship of AL with the development of monoclonal gammopathy of undetermined significance (MGUS), the asymptomatic precursor to multiple myeloma, and the potential mediating effect of AL on racial disparities in MGUS prevalence.
Methods: Nationally representative data from National Health and Nutrition Examination Survey (NHANES) were used. In 1999-2004, MGUS testing was performed on a subtset of participants who were 50 years or older and had available serum samples. AL was scored 0-7, based on markers of metabolic, renal, and immune physiologic systems, as previously described (Obeg-Gyasi, et al, 2022). The association of AL with MGUS was assessed using multivariable odds ratio (aOR). A mediation analysis was performed to determine the relationship of AL in the observed disparities in MGUS incidence.
Results: Among the 5616 participants sampled, 2.8% had MGUS, suggesting a MGUS prevalence of 2.2% of the U.S. population above the age of 50. The characteristics of the sample with and without MGUS are compared in Table 1. African American and Blacks were twice as likely to have MGUS compared to non-Hispanic Whites (aOR 2.00; 95% Confidence Interval [CI] 1.42-3.31; *P*<0.01). After adjusting for age, race, and sex, each one unit increase in AL was associated with 30% increase in odds of having MGUS (aOR=1.30; 95% CI 1.14-1.47; *P*<0.01). AL mediated 9.6% (95% CI -1.1%-20.3%, *P*=0.08) of the disparities between African American and Black and non-Hispanic White participants.
Conclusions: Higher AL was associated with increased odds of MGUS. AL may also explain, at least in part, the higher incidence of MGUS among the African American and Black population in the US. Table 1

Characteristic	MGUS (n=155, N=1,364,360)	No MGUS(n = 5461, N= 60,016,515)	*P*
Age, mean (SD)	68.65 (1.01)	62.92 (0.22)	<0.01
Male (%)	53.2	46.8	0.20
Non-Hispanic White (%)	79.4	79.9	0.03
African American or Black (%)	13.4	7.9	
Hispanic (%)	3.6	8.2	
Other (%)	3.6	4.0	
Allostatic Load, mean (SD)	2.65 (0.11)	1.97 (0.04)	<0.01
Overweight or obese (%)	80.4	71.1	0.03
Elevated Alkaline phosphatase (%)	22.1	20.7	0.68
Elevated Creatinine (%)	29.9	20.3	0.01
Reduced Creatinine Clearance (%)	31.6	16.1	<0.01
Elevated C-reactive protein (%)	32.2	22.7	<0.01
Elevated White Blood Count (%)	23.4	24.1	0.87
Reduced Albumin (%)	45.2	22.3	<0.01

PREVENTION / EARLY DETECTION / INTERCEPTION: Survivorship Research
Poster Session

#4987 Impact of treatment on cognitive function and quality of life in head and neck cancer patients: A prospective study in Indian population.

Saravanan Sampoornam Pape[1], Delfin Lovelina Francis[2]

[1]Kalinga Institute of Industrial Technology (KIIT), Bhubaneshwar, India,[2]Public Health Dentistry, Saveetha Dental College and Hospitals, SIMATS, Chennai, Tamil Nadu, India, Chennai, India

Aim and Objectives:
•To assess cognitive function in patients with head and neck malignancies post-treatment using cognitive ability scales.
•To evaluate the impact of treatment modalities on cognitive function.
•To assess the quality of life in head and neck cancer patients post-treatment using validated QoL scales.
Study Design: A prospective observational cohort study on patients diagnosed with head and neck malignancies in the North Indian population, post-surgical and oncological treatment.
Study Population
•Inclusion criteria: 1. Age 18-70 years diagnosed with malignancies in the head and neck. 2. Undergone surgery, chemotherapy, or radiotherapy for their malignancy. 3. Follow-up of 6 months post-treatment.
•Exclusion criteria: 1. Pre-existing neurocognitive disorders. 2. Psychiatric disorders or active neurological diseases.
Cognitive Assessment
•Montreal Cognitive Assessment (MoCA)
•Mini-Mental State Examination (MMSE)
•Trail Making Test (TMT)
Quality of Life Assessment: European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30)
Data Collection and Statistical Analysis: A minimum sample size of 150 patients was selected to detect changes in cognition and QoL post-treatment with a 95% confidence level and 80% power. Changes in cognition and QoL was analyzed using paired t-tests and ANOVA. Correlations between cognitive function and QoL was analyzed using Pearson's correlation coefficients.
Results:
Cognitive Function Assessment- MoCA scores showed a mean decrease of 3.5 points in areas of memory, attention, and visuospatial functioning. Patients receiving chemoradiation experienced greatest cognitive decline, with reduction of 4.8 as assessed by the TMT. Patients receiving surgery alone showed minimal cognitive decline, with reduction of 1.2 points on MoCA. MMSE scores mirrored these findings, with a decline of 2.5 points in the chemoradiation group and 1 point in the surgery-alone group. Quality of Life (QoL) Global health score decreased by 20%, with most affected domains being emotional functioning (30% decline) and cognition (25% decline). Physical functioning was impaired, with a 15% decline, largely due to the adverse effects of treatment. The EORTC QLQ-H&N35 module revealed that pain & swallowing were prominent concerns.
Conclusion: The results of this study indicate that head and neck cancer patients experience significant cognitive decline and QoL deterioration post-treatment, with those receiving chemoradiation being most affected. Persistent cognitive deficits correlate strongly with reduced QoL, emphasizing the importance of integrating cognitive assessments and QoL evaluations into standard care practices. Future research should explore targeted interventions to mitigate these adverse effects and improve the overall survivorship experience.

#4988 Port flushes in oncology - Does frequency matter?.

Shabbir Haiderbhai[1], Julia Parrish[2], Grace Lavandowska[3], Megan Kelly[3], Aarabhi Gurumoorthy[4], Anu Gaba[4]

[1]Internal Medicine, University of North Dakota, Fargo, ND,[2]Hematology and Oncology, University of North Dakota, Fargo, ND,[3]University of North Dakota, Fargo, ND,[4]Sanford Roger Maris Cancer Center, Fargo, ND

Background: Ports, a type of central venous access device, are frequently used for the administration of intravenous medications, such as chemotherapy. These devices can remain in place for extended periods of time, from several months to years. To reduce the risk of complications, manufacturers recommend routine maintenance, often through monthly heparin flushes which leads to frequent visits to health care settings, adding to the burden and cost of care for both the patient and the healthcare system. The COVID-19 pandemic necessitated decreasing the frequency of port flushes to every 3 months when possible. The aim of this study was to evaluate if extending the interval between heparin flushes impacted safety outcomes.

Methods: A retrospective chart review study was performed utilizing patient data from a single cancer center. Medical records were abstracted for patients with the following characteristics; a diagnosis of malignancy, ≥ 18 years of age, not on current chemotherapy, port placement greater than 30 days prior to enrollment, at least 6 months of follow-up data, and at least one successful port flush within the 6-month study period. The control group (CG) consisted of patients with a standard port flush interval of 4 weeks vs the experimental group (EG) that received an extended interval of every 12 weeks. The primary outcome was port removal, and the secondary outcome was complications of any kind (discomfort, erythema, use of tPA, thrombus, and infection). Descriptive statistics were used to summarize data, with Pearson's chi-square or Fisher's exact tests utilized to calculate group differences.

Results: Of the 1,162 patients who were initially screened for eligibility, 215 patients were included in the analysis: 69 in the CG and 146 in the EG. The average port flush interval was 30.97 days in the CG and 86.21 days in the EG. In the CG, 4 patients (5.8%) required port removal due to complications, compared to 3 patients (2.05%) in the EG (p=0.21). Twelve (17.4%) patients in the CG had port-related complications as compared to 3 patients (2.05%) in the EG (p=0.001). The complications evaluated were discomfort (n=1 in CG), erythema (n=1 in CG, n=1 in EG), infection (n=1 in CG), port malfunction (n=5 in CG, n=2 in EG), thrombus (n=2 in CG), and use of tPA (n=5 in CG).

Conclusion: Our findings suggest that extending the interval between port maintenance flushes from 4 to 12 weeks may lead to lower port-related complications. While the results are promising, the study was limited by the relatively small sample size due to strict inclusion and exclusion criteria. Adopting a 12-week flushing interval may improve the quality of life of cancer patients by reducing the frequency of clinic visits and may also lower healthcare costs for patients and hospitals alike. This extended interval approach is a promising step towards patient-centered care, improving overall healthcare efficiency while maintaining safety.

#4989 Sex disparity in cancer-related fatigue, depression, and impact on quality of life among cancer survivors: Insights from NHANES.

S. Du[1], R. Memon[1], S. Han[2], Y. Wang[3], H. Yusuf[1], J. Anampa Mesias[3];

[1]Jacobi Medical Center - Albert Einstein College of Medicine, Bronx, NY, [2]Mount Sinai Hospital, New York City, NY, [3]Montefiore Medical Center, Bronx, NY

Introduction: Cancer-related fatigue (CRF) and depression are prevalent and debilitating conditions among cancer survivors, significantly impacting their quality of life (QoL). CRF affects up to 80% of patients during or after treatment, while depression affects 15-25%, often co-occurring with CRF. Female survivors are disproportionately affected due to hormonal, psychosocial, and societal factors. However, the extent of these disparities and their impact on QoL remain understudied. This study aims to address the gap by investigating sex differences in CRF and depression, with a focus on their effects on work and recreational activities as key surrogates for QoL

Methods: We analyzed data from the 2015-2016 and 2017-2020 cycles of the National Health and Nutrition Examination Survey (NHANES), including 1,555 cancer survivors representative of over 25 million U.S. survivors. Sampling weights were applied to account for NHANES' complex survey design. Three-step sequential logistic regression models were used to examine sex disparities in CRF and depression: (1) unadjusted, (2) adjusted for demographic variables (age, race), and (3) fully adjusted for demographics, socioeconomic factors (education, insurance), and comorbidities (e.g., hypertension, diabetes). CRF and depression were assessed using the validated Depression and Anxiety Screening Questionnaire (DPQ). Outcomes included vigorous or moderate work activities and recreational activities. Statistical significance was set at $\alpha = 0.05$.

Results: After adjusting for age, race, socioeconomic status (SES), and comorbidities, female cancer survivors had a 58% higher likelihood of experiencing depression compared to male survivors (OR = 1.58, 95% CI: 1.10-2.26, $p = 0.014$). Similarly, female survivors were 69% more likely to experience CRF than their male counterparts (OR = 1.69, 95% CI: 1.24-2.31, $p < 0.001$). CRF was not significantly associated with work-related activity limitations (OR = 1.17, 95% CI: 0.79-1.73, $p = 0.431$) but was strongly associated with reduced moderate recreational activities (OR = 1.83, 95% CI: 1.26-2.66, $p = 0.002$). Depression was also associated with reduced recreational activities (OR = 1.65, 95% CI: 1.05-2.58, $p = 0.02$).

Conclusion: Female cancer survivors are disproportionately affected by higher rates of CRF and depression, with both conditions reducing engagement in recreational activities and adversely affecting QoL. These findings highlight the importance of targeted interventions to address sex disparities in CRF and depression, with the ultimate goal of improving QoL among cancer survivors.

#4990 A discovery of novel methylation profiles in head and neck squamous cell carcinoma in relation to pretreatment pain by cancer subtype.

Monica A. Wagner, Yanning Wu, Naji Ayyash, Fredrick R. Schumacher, Quintin Pan

Case Western Reserve University, Cleveland, OH

Pain is the initial symptom reported by nearly 75% of individuals diagnosed with head and neck squamous cell carcinoma (HNSCC), yet effective management remains elusive, with up to 80% of patients reporting inadequate pain management despite opioid use. Pretreatment pain is a key predictor of survival and recurrence, but the molecular mechanisms underlying pain in HNSCC are not well understood. This pilot study aims to explore the epigenetic mechanisms underlying pain perception in HNSCC patients by cancer subtype and identify potential biomarkers to improve pain management strategies.

DNA methylation levels were generated for individuals newly diagnosed with HNSCC of oral cavity (OCSCC; n=18) or oropharynx (OPSCC; n=8) prior to definitive treatment using the Illumina Infinium MethylationEPIC v2.0 array. Perceived worst pain score was measured using the Brief Pain Inventory-Worst Pain item prior to treatment, categorized according to NCCN guidelines, with patients reporting no pain serving as the reference group. After quality control, M-values for 26 samples and 913,962 methylation probes were evaluated. Differentially methylated positions (DMPs) associated with pain severity were identified combining subtypes, with hypo- (fold change (FC) < -2) and hypermethylated (FC > 2) sites determined after adjusting for age. Stratified analyses were performed by cancer type. All analyses set a significance level with a false discovery rate < 5%.

Pain phenotype and blood collection occurred on the same day, after diagnosis but prior to definitive treatment. The cohort had a mean age of 65.1 ± 11.3 years, 42.3% of those reporting any level of pain were male. Pre-treatment pain was reported by 94.4% (N=17) of OCSCC and 25% (N=2) of OPSCC patients. DMP analysis revealed probe cg08573204_TC21 in *LMO1* gene was hypermethylated in OCSCC patients with mild pain versus no pain (p=0.02, Log_2FC=1.11). While *LMO1* has not been previously linked to pain, its role in adrenergic cell identity suggests potential involvement in pain pathways. In patients with moderate/severe pain, cg18566479_BC21 located in *CHDH* gene was hypermethylated in OCSCC (p=1.09E-04, Log_2FC=1.98). *CHDH*, associated with metabolic signatures in HNSCC and neuroinflammatory activation in neuropathic pain, showed significant hypermethylation, suggesting its potential as a biomarker for pain management in HNSCC.

Differential DNA methylation may be associated and predictive of the development and severity of pain in HNSCC. With future work in larger samples, this research may provide insight into the biological underpinnings of pain and guide cancer symptom management by using DNA methylation profiles to identify patients at risk for pain who may benefit from preventative measures.

#4991 Temporal trends in health-related quality of life (HRQoL) outcomes in fourth line or higher treatment for relapsed/refractory multiple myeloma (RRMM): A systematic literature review (SLR) and meta-analysis.

Ken Hasegawa[1], Xavier P. Leleu[2], Taha Itani[1], Monique Giordana[1], Enrique Granados[1], Rebecca J. Chan[3], Ana Kostic[3], Jie Ting[3], Sean P. Harrigan[4], Michael J. Zoratti[4], Rahul Banerjee[5]

[1]Kite, A Gilead Company, Santa Monica, CA,[2]Centre Hospitalier Universitaire de Poitiers, Poitiers, France,[3]Arcellx, Inc., Redwood City, CA,[4]RainCity Analytics, Vancouver, ON, Canada,[5]Fred Hutchinson Cancer Center, Seattle, WA

Background: The HRQoL of patients with RRMM is highly impacted by their disease and is typically only maintained with conventional treatments before deteriorating. We conducted an SLR and meta-analyzed temporal trends in HRQoL to contextualize findings from emerging immunotherapies for 4L+ RRMM such as chimeric antigen receptor T-cell (CAR T) therapies and bispecific antibodies (BsAb).

Methods: MEDLINE, EMBASE, CENTRAL and the grey literature were systematically searched (Jan 2012 to Mar 2024) for studies of patients with 4L+ RRMM where HRQoL was measured according to either the EORTC QLQ-C30 or QLQ-MY20. Changes from baseline were described, by treatment category (CAR T, BsAb, and Other), using fixed-effect meta-analyses over one year of follow-up. Estimates were compared to minimally important differences (MIDs) to determine clinical meaningfulness.

Results: We included 14 studies with QLQ-C30 (n=14) or QLQ-MY20 (n=11) outcomes at timepoints of interest (Table). In patients treated with CAR T, sustained clinically meaningful improvements were observed from 3 months onwards on the Physical functioning and Pain domains and from 4 months onwards on Global health status and the Social functioning, Fatigue, and Future perspective domains. Treatment with a BsAb tended to maintain baseline HRQoL over time, with some meaningful improvements in Emotional functioning, Physical functioning, Fatigue, and Pain. Meaningful deteriorations were seldom observed on any domain.

Conclusions: This evidence base is evolving, and some findings are limited by few studies and small sample sizes. Nevertheless, our study demonstrated earlier and sustained improvement in key HRQoL domains among patients with 4L+ RRMM who receive CAR T. These findings are similar to the results of CAR T in other hematological settings and reflect improved general well-being post-CAR T.

*Negative changes on symptoms show improvement; Bold: Clinically meaningful change from baseline

Domain (MID for improvement/deterioration)		Treatment category	Mean change from baseline (95% CI)							
			Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 9	Month 12
QLQ-C30	Global Health Status +5/-5	CAR T	+2.5 (-0.3, +5.2)	+5.8 (+2.6, +9.0)	+6.7 (+2.8, +10.6)	**+10.3 (+6.3, +14.3)**	**+12.0 (+8.0, +15.9)**	**+11.6 (+7.9, +15.3)**	**+13.2 (+9.1, +17.4)**	**+12.1 (+7.9, +16.3)**
		BsAb	-2.1 (-4.2, -0.0)	-0.2 (-2.4, +2.0)	-3.4 (-4.0, -2.8)	+4.1 (+1.6, +6.7)	+4.2 (+1.3, +7.1)	+5.6 (+3.1, +8.1)	+2.1 (-3.3, +7.5)	--
		Other	-1.9 (-3.5, -0.3)	-0.2 (-1.9, +1.5)	-2.5 (-4.5, -0.4)	-0.9 (-3.3, +1.5)	-0.6 (-2.7, +1.5)	+0.4 (-2.3, +3.1)	-1.3 (-6.1, +3.5)	-3.9 (-9.8, +2.0)
	Physical Functioning +2/-5	CAR T	-1.0 (-4.3, +2.4)	+2.5 (-0.8, +5.7)	**+5.6 (+2.5, +8.8)**	**+9.1 (+5.4, +12.7)**	**+8.7 (+4.8, +12.6)**	**+11.4 (+8.2, +14.6)**	**+9.5 (+6.1, +12.9)**	**+8.8 (+4.7, +12.8)**
		BsAb	-4.9 (-7.4, -2.4)	-1.3 (-3.6, +1.0)	+0.6 (-1.6, +2.8)	+2.8 (+0.3, +5.3)	+1.6 (-1.7, +4.9)	**+5.3 (+2.6, +8.0)**	--	--
		Other	-1.4 (-3.0, +0.2)	-0.7 (-2.4, +0.9)	-1.5 (-3.3, +0.3)	-0.8 (-2.8, +1.1)	-0.1 (-2.3, +2.0)	+0.4 (-2.0, +2.8)	-1.7 (-6.1, +2.7)	+0.6 (-4.8, +6.0)
	Social Functioning +3/-6	CAR T	-5.5 (-9.5, -1.5)	+4.0 (+0.6, +7.4)	+5.6 (+0.4, +10.9)	**+12.3 (+7.4, +17.1)**	**+15.7 (+11.0, +20.3)**	**+11.1 (+6.5, +15.7)**	**+12.6 (+7.7, +17.5)**	**+8.4 (+3.6, +13.2)**
		BsAb	-4.9 (-9.9, +0.1)	--	+1.0 (-4.4, +6.4)	--	+1.5 (-4.8, +7.8)	--	--	--
		Other	+2.9 (-6.5, +12.3)	+3.0 (-3.1, +9.1)	-4.2 (-15.2, +6.8)	-6.6 (-20.5, +7.3)	+6.8 (-2.8, +16.4)	-6.9 (-19.5, +5.7)	--	--
	Fatigue* -4/+5	CAR T	+3.6 (+0.3, +6.9)	-4.1 (-7.6, -0.6)	-7.1 (-11.3, -2.9)	**-10.1 (-14.4, -5.8)**	**-12.7 (-16.8, -8.5)**	**-13.2 (-17.7, -8.6)**	**-18.3 (-23.2, -13.5)**	**-15.7 (-20.4, -11.0)**
		BsAb	+4.7 (+1.6, +7.7)	+1.3 (-1.4, +3.9)	-0.9 (-3.8, +2.0)	-5.0 (-8.1, -1.8)	-2.9 (-6.2, +0.5)	**-7.4 (-10.8, -4.0)**	--	--
		Other	+2.7 (+0.8, +4.5)	+1.6 (-0.3, +3.5)	-0.4 (-2.9, +2.1)	-0.7 (-3.4, +2.0)	-0.8 (-3.2, +1.6)	-1.1 (-4.3, +2.1)	-3.0 (-8.3, +2.3)	-0.5 (-7.9, +7.0)
		CAR T	-8.1 (-12.0, -4.3)	-7.0 (-11.7, -2.4)	**-10.3 (-14.8, -5.7)**	**-14.5 (-18.9, -10.1)**	**-14.1 (-19.0, -9.2)**	**-13.2 (-17.9, -8.5)**	**-17.3 (-21.8, -12.8)**	**-16.4 (-22.2, -10.6)**

	Pain* -5/+3	BsAb	+1.4 (-1.2, +4.1)	-6.3 (-9.2, -3.4)	-6.9 (-10.1, -3.8)	**-9.9 (-13.2, -6.6)**	**-12.0 (-15.6, -8.3)**	**-10.0 (-13.2, -6.7)**	-9.8 (-18.1, -1.5)	--
		Other	-2.2 (-4.1, -0.3)	-2.7 (-4.9, -0.5)	-2.8 (-5.6, -0.0)	-2.0 (-4.9, +0.9)	-2.8 (-5.5, -0.0)	-4.3 (-7.7, -0.8)	-5.0 (-12.3, +2.4)	-8.3 (-16.1, -0.5)
QLQ-MY20	Future Perspective +9/-9	CAR T	+8.0 (+6.1, +9.9)	+11.7 (+8.2, +15.1)	+11.8 (+8.3, +15.4)	**+15.1 (+10.7, +19.4)**	**+16.2 (+12.1, +20.3)**	**+15.0 (+11.0, +19.1)**	**+14.5 (+10.8, +18.2)**	**+17.2 (+13.0, +21.4)**
		BsAb	+4.5 (+0.9, +8.1)	+10.2 (+6.0, +14.4)	+5.6 (+3.5, +7.8)	+9.8 (+5.0, +14.5)	+11.2 (+6.6, +15.7)	+9.7 (+5.6, +13.7)	+14.7 (+7.2, +22.1)	--
		Other	+5.7 (-0.8, +12.1)	+2.6 (-3.1, +8.4)	-2.0 (-11.8, +7.8)	+3.8 (-8.0, +15.5)	+7.7 (-0.8, +16.1)	+3.0 (-10.2, +16.3)	--	--

#4992 Results of a survey of US Ayurvedic medicine professionals regarding their work with people with cancer.

Julia T. Arnold[1], Oluwadamilola Olaku[1], Anand Dhruva[2], Gabriel Lopez[3], Bhaswati Bhattacharya[4], Manohar Gundeti[5], Anupama Kizhakkeveettil[6], Benmei Liu[7], Frank Ninivaggi[8], Sophie King[1], Jeffrey D. White[1]

[1]Office of Cancer Complementary and Alternative Medicine- OCCAM, National Cancer Institute, Bethesda, MD,[2]University of California, San Francisco, San Fransisco, CA,[3]MD Anderson Cancer Center, Houston, TX,[4]Weill Cornell Medical College, New York, NY,[5]Central Ayurveda Research Institute, CCRAS, Mumbai, India,[6]Southern California University of Health Sciences, Los Angeles, CA,[7]Division of Cancer Control and Population Sciences, National Cancer Institute, Bethesda, MD,[8]Yale University School of Medicine, New Haven, CT

INTRODUCTION: The goal of this study was to understand how Ayurveda, the traditional medicine of India, can be incorporated into the management of patients with cancer in the United States (US). Patients with cancer often seek Ayurvedic treatments to complement conventional care for prevention, to reduce symptoms/ toxicity, support recovery, improve quality of life, or palliative care. Ayurveda is a whole system personalized approach to health incorporating multiple modalities including herbal-natural products, diet, routines, behavior, detoxification, and mind/body techniques for restoring health and preventing disease. The authors surveyed the opinions of Ayurvedic professionals on the management of cancer patients using Ayurveda including its benefits for the treatment of cancer side-effects and symptoms and communications and collaborative research with allopathic practitioners.

METHODS: This Survey was a collaboration of the co-authors led by the Office of Cancer Complementary and Alternative Medicine (OCCAM), National Cancer Institute (NCI). Authors contacted key leaders of the US Ayurvedic professional community including directors of Ayurvedic colleges/academies, and leaders of state, regional and national Ayurvedic associations. An invitation letter with a web-link and QR code for the survey website was distributed by the US Ayurveda Professionals leadership (on behalf of OCCAM) to the alumni and association members of these groups. The survey had 321 respondents, of which 199 (62%) have practiced Ayurveda on patients/clients in the US. (Demographics and educational background of this population will be included in the presentation).

RESULTS: Brief highlights of the Ayurvedic professionals' responses to the 110 questions are:1- Reasons why patients sought Ayurveda care, [improve their quality of life (QoL) (94%), reduce allopathic treatment-related side effects (87%), prevent recurrence of their cancer (77%)], 2--Recommended targets of Ayurvedic consultations, [daily routine/lifestyle (97%), specific food counseling (94%), pranayama (breathing exercises; 88%), yoga and meditation (86%)], 3- The most common goals of practitioners for clients with cancer, [to improve diet and digestion (94%), increase QoL (90%), and stress reduction (90%)], 4- Practitioners agreed that combined Ayurveda and allopathic medicine is the best approach for cancer symptom and side effect management (87%), support during cancer treatment (84%), QoL (80%) cancer prevention (75%), and cancer detection (65%).

CONCLUSION: This Survey aims to increase awareness among oncologists and researchers regarding the integration of Ayurveda with conventional cancer care to enhance patient QoL and treatment outcomes. Research collaborations between oncologists and Ayurvedic professionals could support efficacy and mechanistic research on the use of Ayurveda or its components in cancer care.

#4993 A longitudinal examination of nutritional-inflammatory biomarker associations with cancer related cognition, treatment side effects and quality of life in newly diagnosed older adults undergoing chemotherapy.

Jennifer M. Crook, Michael Owings, Victoria Loerzel

Nursing, University of Central Florida, Orlando, FL

Intro: Chemotherapy-induced cognitive impairment affects 16-75% of cancer patients with neither a clear understanding of the underlying causes nor assessment guidelines for intervention. A potential consideration may be found in nutritional-inflammatory biomarker ratio panels of neutrophil-albumin ratio (NPAR), neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), and monocyte-lymphocyte ratio (MLR). These calculated proportions may be obtained from readily available and normally scheduled chemotherapy labs and have been implicated for their prognostic value in cancer treatment effectiveness and/or mortality risk with sensitivity to multiple cancer types and stages. However, the relationships between these and cognitive, chemotherapy related symptoms and quality of life (QOL) evaluations have not yet been explored.

Methods: This study examined data from a RCT of 144 newly diagnosed adult participants (≥ 60 years of age) undergoing first-time chemotherapy recruited from community cancer centers in central and south Florida. Various cancer types and stages were included with treatment cycles of 1-6 timepoints captured. NPAR, NLR, MLR, and PLR were calculated and ran as continuous variables for nonparametric Friedman analysis of variance and Wilcoxon signed rank tests and categorized into three equal groups of low, medium, and high for Pearson chi-square analyses. Cognitive and symptom information were collected through Mini-Mental State Examination (MMSE), EORTC - Quality of Life (QLQ-C30), and Symptom Representation Questionnaire (SRQ) instruments.

Results: NPAR, NLR, PLR, and MLR were significantly associated with MMSE scores ($p < 0.001$). Substantial changes were identified in individuals with moderate dementia and normal cognition between baseline through final chemotherapy treatments. Cognitive and emotional assessment (EORTC-QLQ) revealed significant associations between NPAR, NLR, PLR, MLR and feeling tense ($p < 0.01$), difficulty remembering, depression, pain, nausea, and needing assistance with ADLs ($p < 0.05$). Significant treatment-related symptom associations with NPAR, NLR, PLR, MLR were identified in hair loss, numbness ($p < 0.001$), nausea ($p < 0.01$), drowsiness, lack of appetite, and hot flashes ($p < 0.05$).

Discussion: Nutritional-inflammatory lab ratios of NPAR, NLR, PLR, and MLR were significantly associated with cognitive, side effects, and QOL indicators during chemotherapy treatments of newly diagnosed older adult cancer patients. These easily obtained lab calculations may provide prognostic value in assessing and intervening in high-risk individuals to mitigate treatment-related sequalae.

BIOINFORMATICS / COMPUTATIONAL BIOLOGY / SYSTEMS BIOLOGY / CONVERGENT SCIENCE: Multi-modal Analysis and Multi-omics Characterization
Poster Session

#4997 The ROSALIND Study: Identifying multi-omic signatures in long-term cancer survivors for therapeutic target discovery.

Julieta Rodriguez[1], Olivia Le Saux[2], **Paloma Cejas**[3], David Gentien[1], Nick Riddiford[4], Naouel Zerrouk[4], Josep Tabernero[5], Olivier Elemento[6], Adrien Paix[4], Alexandre Yazigi[4], Nicolas Wolikow[4], Simon Istolainen[4], Remi Nicolle[4], Cecile Badoual[7], Elisabeth Auzias[4], Ludovic Lacroix[1], Nathalie Droin[1], Gina Dorpholz[8], Rodrigo Dienstmann[9], Jean-Yves Blay[10], Ulrich Keilhoz[11], Fabrice Andre[1]

[1]Gustave Roussy, Villejuif, France,[2]Centre Leon Berard, Lyon, France,[3]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA,[4]Cure51, Paris, France,[5]Vall d'Hebron Institute of Oncology, Barcelona, Spain,[6]Englander Institute for Precision Medicine, Weill Cornell Medicine, New York, NY,[7]Department of medical biology and pathology, Gustave Roussy, Villejuif, France,[8]Institute of Chemistry and Biochemistry, Charite Cancer Comprehensive Center, Berlin, Germany,[9]Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain,[10]Centre Leon Berard, Lyon, France,[11]Charite, Berlin, Germany

Background: Long-term survival in cancers with poor prognosis, such as metastatic pancreatic ductal adenocarcinoma (mPDAC), glioblastoma (GBM-IDHwt), and extensive-stage small cell lung cancer (ES-SCLC), is exceedingly rare. The ROSALIND study is designed to explore the biological factors that contribute to exceptional survival in these cancers by comparing multi-omic and clinical profiles of long-term survivors (outliers) with those of patients who exhibit standard survival outcomes. By building a comprehensive clinical and molecular database, the study aims to identify novel therapeutic targets that could benefit a broader cancer population.
Methods: ROSALIND is a retrospective, multi-center, case-control study conducted across three cohorts: mPDAC, GBM-IDHwt, and ES-SCLC. Cases (long-term survivors) are defined as patients with survival exceeding 5 years for mPDAC and ES-SCLC, and 3 years for GBM-IDHwt. Controls are matched to cases based notably on tumor stage, age, sample type and treatment history, with overall survival in the control group centered around the median overall survival reported in pivotal clinical trials for each tumor type. Tumor samples from over 500 long term survivors and matched controls are undergoing multi-omic analysis, including genomics, transcriptomics, proteomics, microbiomics and radiomics, with centralized sample processing at Gustave Roussy Cancer Campus / Paris Saclay Cancer Cluster. The molecular profiling pipeline includes 10X Visium HD spatial whole transcriptomics, whole-genome sequencing (WGS) or Whole-exome sequencing (WES), bulk RNA sequencing (RNAseq), microbiomics (16s rRNA) and proteomics.
Results: As of October 2024, 135 patients have been enrolled in the ROSALIND study across three cohorts: 38 with ES-SCLC, 62 with GBM-IDHwt, and 35 with mPDAC. These patients were drawn from a global network of over 50 centers in 37 countries, and a total of 1211 outliers have been identified for potential inclusion. Molecular data collection is ongoing, with tumor and clinical data being curated into a centralized, multimodal database. The study aims to uncover key differences in molecular signatures between outliers and standard survivors, focusing on dysregulated pathways and proteins that may serve as novel therapeutic targets.
Conclusions: The ROSALIND study is pioneering the creation of an extensive, multi-omic and multimodal database of cancer outliers worldwide and at scale. By examining at the spatial and single cell level the biological underpinnings of exceptional survival in some of the most aggressive cancers, this study holds the potential to discover novel therapeutic targets. Future findings from ROSALIND could lead to the development of precision oncology treatments aimed at replicating the biology of long-term survivors, thereby improving outcomes for patients with poor-prognosis cancers.

#4998 Deriving patient similarity networks in myeloid neoplasms using multi-modal representation learning.

Georgios Asimomitis[1], Jake Sauter[1], Darin Moore[1], Himanshu Bhurtel[2], Katherine Lopez[2], Armaan Kohli[1], Kevin Boehm[1], Christopher Fong[1], Arfath Pasha[1], Luke Geneslaw[2], Anika Begum[1], Tom Pollard[1], Jacob Glass[3], Gregory M. Goldgof[2], Nicholas Barasch[2], Ahmet Dogan[2], Mikhail Roshal[2], Elli Papaemmanuil[1]

[1]Halvorsen Center for Computational Oncology, Memorial Sloan Kettering Cancer Center, New York, NY,[2]Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, NY,[3]Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY

Myeloid neoplasms (MNs), such as Myelodysplastic Syndromes (MDS) and Acute Myeloid Leukemia (AML), are clinically overlapping hematologic malignancies. Their assessment includes the evaluation of bone marrow (BM) morphology, immunophenotyping by flow cytometry, cytogenetics, and gene mutation profiling. Established diagnostic criteria between the MN subtypes are primarily guided by empirical unimodal thresholds (i.e. 20% blasts differentiating MDS from AML) that may not always reflect the underlying disease biology. This study integrates different data modalities and produces patient-specific multi-modal views aiming to uncover associations between molecular subtypes and clinical phenotypes at the cellular and morphological level, show distinct biological and clinically relevant subgroups in MNs, and help inform future guidelines for disease classification.

The study cohort consists of 523 MSKCC patients across the spectrum of MNs. The dataset includes mutation and cytogenetic profiles, raw hematoxylin and eosin (H&E) BM whole slide images (WSIs), raw single-cell flow cytometry measurements, and differential cell type counts. Data for all modalities are present for 269 patients.

The flow cytometry and imaging data views were the products of in-house computational workflows. In flow cytometry, single cells were grouped in clusters by the Leiden algorithm. Then, the cell abundance distributions across these clusters were used to form patient-level representations. Our imaging framework first identified the cell-rich regions of the WSIs by training a tile-level vision transformer (ROCAUC: 0.95). After obtaining the encodings of the cell-rich regions from the QuiltNet foundation model (Ikezogwo et al. NeurIPS 2023), we trained a graph neural network (SlideGraph+, Lu et al. Medical Image Analysis 2022) to predict diagnostic labels (ROCAUC: 0.79), and we subsequently retrieved the WSI deep embeddings.

Different combinations of the unimodal views (genomics, flow cytometry, WSIs, differential cell types) were integrated into multi-modal embeddings through the Multiple Similarity Network Embedding (Xu et al. Methods 2021) method. Low dimensional projections and transformation of these embeddings to patient similarity graphs show the formation of homogeneous subgroups driven by somatic mutation status (i.e. TP53 mutated MNs), clusters defined by contributions of features across modalities, and the presence of out-of-group patients indicative of disease heterogeneity.

In summary, this work produces multi-modal patient embeddings in MNs and constructs patient networks indicative of both inter-patient similarity and heterogeneity. Characterizing the data-derived representations of disease profiles and the underlying inter-modality relationships will be critical for the future definition of diagnostic criteria that incorporate multi-modal measurements.

#4999 Multi-omics insights into the molecular signature and prognosis of hypopharyngeal squamous cell carcinoma.

Yanxin Ren[1], Wei Xiong[2], Chun Feng[3], Dan Yu[4], Xiaoyan Wang[5], Qing Yang[6], Siting Yu[2], Hongjiang Zhang[2], Bangyun Huo[3], Honglu Jiang[7], Zuli Li[8], Junlin Wang[4], Yu-Xiong Su[9], Ping Yang[10], Yong Liao[8], Qi Zhong[5], **Junwen Wang**[4]

[1]Department of Head and Neck Surgery, Yunnan Cancer Hospital, The Third Affiliated Hospital of Kunming Medical University, Kunming, Yunnan, China,[2]Department of Radiotherapy, Yunnan Cancer Hospital, The Third Affiliated Hospital of Kunming Medical University, Kunming, Yunnan Province, China,[3]Department of Otolaryngology, The First People's Hospital of Yunnan Province, The Affiliated Hospital of Kunming University of Science and Technology, Kunming, Yunnan Province, China,[4]Division of Applied Oral Sciences and Community Dental Care, Faculty of Dentistry, The University of Hong Kong, Hong Kong Island, Hong Kong,[5]Department of Otolaryngology Head and Neck Surgery, Beijing Tongren Hospital, Capital Medical University, Beijing, China,[6]Department of Head and Neck Surgery, Yunnan Cancer Hospital, The Third Affiliated Hospital of Kunming Medical University, Kunming, Yunnan Province, China,[7]Department of Otolaryngology, The First People's Hospital of Yunnan Province, The Affiliated Hospital of Kunming University of Science and Technology, Kunmimg, Yunnan Province, China,[8]Institute for Viral Hepatitis & Department of Infectious Diseases, Second Affiliated Hospital, Chongqing Medical University, Chongqing, China,[9]Division of Oral and Maxillofacial Surgery, The University of Hong Kong, Hong Kong Island, Hong Kong,[10]Department of Quantitative Health Science, Mayo Clinic, Scottsdale, AZ

Approximately two-thirds of hypopharyngeal squamous cell carcinoma (HPSCC) are diagnosed in advanced stage, with the worst prognosis among head and neck squamous cell carcinomas (HNSCCs). Identifying biomarkers for high-risk patients who need aggressive treatment is crucial. We present mutational, transcriptomic and proteomic studies of 103 Chinese HPSCC patients, observing a higher prevalence and poorer prognosis in males comparing to females. Genes in estrogen response pathways are up-regulated and proteins phosphorylated by protein kinase C (PKC) and Cyclin-dependent kinases (CDKs) are aberrantly regulated. We identify aberrant copy number regions including *SOX2* (3q26.33),*FGFR* (8p11.23), *CCND1* (11q13.3), *CDKN2A/2B* (9p21.3), and *MYC* (8q24.21). HPV status combined with highly mutated genes as predictive markers was assessed, including *SYNE1* in HPV(-), *MUC4* in HPV(+) patients. We confirm the presence of four known gene expression (GE) subtypes of HNSCC in our HPSCC cohort and present the genomic alterations, patient characteristics and survival status of each subtype. A predictive model involving clinical factors and transcriptomic expression of six genes was established and cross-site validated. The findings open new opportunities for stratifying high-risk patients and molecular targets for novel therapeutic strategies.

#5000 Single cell and spatial mapping of the fallopian tube with insight into origin of high grade serous carcinoma.

Sahar Nasr[1], Jacob Gutierrez[2], Caleb Iareau[3], Brooke Howitt[1]

[1]Pathology, Stanford University, Palo Alto, CA,[2]Cancer Biology, Memorial Sloan Kettering Cancer Center, New York, NY,[3]Memorial Sloan Kettering Cancer Center, New York, NY

High-grade serous “ovarian” carcinoma (HGSC), the most lethal gynecologic cancer, typically presents at advanced stages owing to lack of symptoms in early-stage disease. Additionally, no effective screening strategies exist. Despite the misnomer, most HGSC arise from fallopian tube epithelium (FTE). Better understanding early steps in pathogenesis of serous tubal intraepithelial carcinoma (STIC) and cell of origin for HGSC will inform early detection strategies and lead to new insights for effective screening approaches. HGSC and STIC are characterized by TP53 mutation and chromosomal instability. Earlier lesions in the FTE, such as “p53 signatures,” benign lesions harboring TP53 mutation, are less well understood. We aimed to study STIC, p53 signatures, and the benign FT at the single cell and spatial level, considering not only epithelial and lesional cells but also the surrounding stromal and immune microenvironment.
We employed single-cell RNA sequencing (scRNA-seq) in combination with single-nuclei RNA sequencing using 10x flex and single-cell spatial transcriptomics (scST), on formalin-fixed paraffin-embedded (FFPE) tissues to analyze normal and neoplastic fallopian tubes. scST data from 84 fields of view representing 8 patients (5 benign, 3 STIC) were used to define epithelial subtypes within the FTE, considering both gene expression profiles and spatial distributions correlating to histopathological findings. Furthermore, scRNA-seq data from fresh non-neoplastic FT and FFPE STIC samples were integrated to construct comprehensive atlases. The epithelial subtypes within the scST data were mapped on to the integrated atlas using gene module scores.Seven distinct epithelial subtypes were identified, with transcriptional diversity linked to HGSC precursors. Subtype-specific gene expression profiles were linked to histological features, revealing cellular heterogeneity in normal epithelium in contrast to clonal expansions and STIC. In the microenvironment, we found epithelial subtype-specific stromal and immune interactions. Furthermore, four subclusters of macrophages were identified with distinct expression programs; some subtypes were only abundant in association with STIC. We implemented scST in clinical FT samples including STIC, and integrated ST features with a large atlas of scRNA-seq data to refine single cell characterization of benign FT, p53 signatures, and STIC. Our spatially informed analyses recognized distinct subsets of epithelial, immune, and stromal cells that change over the course of STIC/HGSC development. Additionally, the trajectory of epithelial subtypes demonstrates the most likely epithelial cell subtype from which STIC may form. Our integrated FT atlas provides a critical resource for others seeking to understand early events in HGSC pathogenesis, advancing early detection and therapeutic strategies.

#5001 Multimodal topic modeling for single-cell multiomics.

Cigdem Ak, Nicole Szczepanski, Aaron Doe, Gurkan Yardimci

Oregon Health & Science University, Portland, OR

Single-cell (sc) multiomics assays simultaneously measure transcriptomic, epigenomic, and proteomic modalities in single cells. These multimodal assays enable deeper characterization of cells compared to single-omic assays by offering a more comprehensive measurement of the cell state. Additionally, they can identify cross modality associations, such as promoter-enhancer linkages. Recently, numerous computational methods for sc-multiomics data have been released; however, there is a dearth of interpretable methods While matrix factorization and deep learning approaches, such as MOFA+ and scVI, are effective at cell type clustering; however, these methods are difficult to interpret, masking the underlying biology that defines cell states. Our solution, EpiConfig, is a multimodal topic model that can accurately cluster cells based on cell types and yields biological insights by jointly modeling transcriptomic and epigenetic states combinations. Unlike other sc-multiomics topic models, EpiConfig explicitly models cross modality associations. Furthermore, we describe important cell filtering and subsetting approaches to speed up and improve the convergence of topic model. Benchmarking EpiConfig on publicly available scRNA+ATAC datasets to healthy and cancerous cell populations, we show that EpiConfig outperforms competing methods, such as Cobolt and MOFA. Furthermore, EpiConfig cross modality associations cell type capture 3D genome interactions. Modeling sc-multiomics data from lymphoma, EpiConfig identifies topics for cancerous cells. Coupled with our model interpretation R Shiny app, EpiConfig allows for the interpretation and identification of associations between transcriptomic and epigenomic features that define cell types and states.

#5002 Multi-omics insights into the role of clonal hematopoiesis in multiple myeloma.

Jeongmin Park[1], Ja Min Byun[2], Dong-Yeop Shin[2], Junshik Hong[2], Sung-Soo Yoon[2], Siddhartha Jaiswal[3], Youngil Koh[2]

[1]Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea, Republic of,[2]Seoul National University Hospital, Seoul, Korea, Republic of,[3]Department of Pathology, Stanford University School of Medicine, Stanford, CA

Multiple myeloma (MM) is a cancer involving the abnormal proliferation of malignant plasma cells in the bone marrow (BM). Clonal hematopoiesis (CH), a condition involving the clonal expansion of blood cells due to somatic mutations, is commonly observed with aging. However, its precise role in the pathogenesis of MM remains unclear. This study hypothesizes that CH influences MM cells and the microenvironment through paracrine mechanisms, thereby contributing to disease progression and treatment resistance. Using UK Biobank data, somatic CH variants were called, excluding those with a variant allele frequency (VAF) < 0.02, and analyzed them against ICD-10 codes. Hazard ratios (HR) were computed through Cox proportional regression, adjusting for covariates. Single-cell multi-omics (scDNA) sequencing was performed on BM aspirates from two MM patients, followed by cell type annotation and CH mutation subtyping using the MissionBio Mosaic pipeline. Exosomes were extracted from BM samples of 30 MM patients, and exosome small RNA sequencing was conducted. Differential expression of miRNAs was identified, and a computational tool was used to predict and integrate the target genes and pathways regulated by the differentially expressed miRNAs. Single-cell RNA (scRNA) sequencing was performed on BM sample from 12 MM patients, with data analyzed using the Seurat pipeline. Proteomics analysis using UK Biobank data was performed on a cohort of 190 MM patients identified based on ICD-10 codes. Differentially expressed protein and pathway analyses were conducted using OlinkAnalyze R package. Survival analysis conducted using the UK Biobank cohort revealed that the presence of CH was significantly correlated with MM diagnosis (HR: 1.64, CI: 1.35-2, $P < 0.001$), and CH in MM impacted EFS (HR: 1.61, CI: 1.25-2.1, $P < 0.001$). The results of the scDNA analysis revealed that approximately 88% of CH-mutated cells were distributed within myeloid cells, suggesting potential indirect effects on MM through paracrine interactions. Exosomal miRNA analysis of 30 MM patients revealed a significant reduction in miRNAs targeting the MAPK and JAK-STAT pathways (KEGG, $P < 0.005$), indicating dysregulation of oncogenic and inflammatory signaling in individuals with CH. scRNA analysis identified the activation of inflammatory pathways in CD14+ monocytes of individuals with *TET2* and *DNMT3A* mutations, including the MAPK pathway (GOBP, adj$P < 0.05$) and cytokine interactions (KEGG, adj$P < 0.05$). Proteomics analysis using Olink data from 190 MM patients in the UK Biobank further validated the activation of inflammatory and oncogenic pathways, including MAPK pathway (GOBP, adj$P < 0.05$), STAT pathway, and cytokine-cytokine receptor interactions (KEGG, adj$P < 0.05$), in CH-positive MM cases. These findings highlight the potential for CH mutations, particularly in *TET2* and *DNMT3A*, to exacerbate MM progression through inflammatory paracrine mechanisms.

#5003 Machine-learning informed multifactorial biomarkers as an approach to stratify survival outcomes among ADC treated patients.

Anna Shcherbina, Yajas Shah, Zachary McCaw, Yang Su, Serra Elliott, David Light, Angela Oliveira Pisco, Sanjana Narayanan, Chaya Wurman, Vincent Luckzow, Christopher Probert, Sacha Holland, Virginia Donovan, Peter Szabo

Insitro, South San Francisco, CA

As oncology treatments increase in molecular complexity, the need for biomarkers continues to grow. This becomes critical in treatments such as antibody-drug conjugates (ADC's), which are a promising targeted anti-cancer therapy, that not all patients respond equally well to. Though prior work suggests that patients who over-express ADC targets relative to those that do not are more likely to benefit from ADC therapy, a robust biomarker signature that predicts response has yet to be defined for most ADCs. To address this unmet need, we developed an approach to impute composite warhead-target ADC biomarkers from digitized histopathology (H&E) slides.

The first component of this biomarker signature is a machine learning model that imputes ADC target RNA expression from histopathology slides. Flash frozen and FFPE histopathology slides from 11,115 patients in The Cancer Genome Atlas (TCGA) and 2,732 patients from Aster across 32 tumor types were embedded to a lower dimensional representation via the DINO ViT model. A multilayer perceptron (MLP) was trained within each tumor type to predict transcriptome-wide gene expression (19,421 protein-coding genes) from these embedding vectors, achieving a mean spearman r = 0.20+/-0.17 across all genes (mean pearson r = 0.21+/-0.18), and a mean spearman r = 0.32 +/-0.17 across the top 1000 genes across cohorts. Improved performance was observed when imputing a weighted sum of expression for transcriptomically correlated genes (as ascertained via differential expression analysis) for a suite of 346 known candidate ADC targets.

The second component of the biomarker signature focuses on stratifying longitudinal outcomes in patients treated with topoisomerase 1 (TOP1) inhibitors. We developed a gene signature (weighted mean expression of 52 genes) based on treatment response in cell lines (GDSC2) and therapy-agnostic patient gene expression profiles (TCGA, Aster). Additional weighting based on cross-modality evidence was employed. The resulting signatures were associated with longitudinal endpoints in primary and metastatic colorectal cancer patients. An MLP was subsequently trained within the TCGA and Aster cohorts to impute the TOP1 signature from H&E datasets, achieving cross-validation spearman correlation of 0.42 within the COAD-READ cohort and a spearman R of 0.29 in a held-out MRC FOCUS test set (N=73).

In summary, the combined warhead-target imputation signature shows promise in stratifying likely ADC treatment responders from non-responders. Given that assays directly measuring gene expression at the bulk and single cell level are still seldom used in the clinic, this imputation model may provide a useful tool to leverage H&E imaging routinely performed as part of patient diagnosis as a tool to inform treatment selection.

#5004 Application of a comprehensive multi-omic immune profiling strategy achieves superior checkpoint immunotherapy response prediction in lung cancer.

Helen M. McGuire[1], Natalie Smith[1], Michael Cohen[2], Julien Hedou[3], Gregoire Bellan[3], Xavier Durand[3], Erika L. Smith-Mahoney[4], Julie Alipaz[2], Jennifer Snyder-Cappione[4], Brice Gaudilliere[3], Christina Loh[2], David King[2], Michael Hinterberg[2], Clare Paterson[2], Barbara Fazekas de St Groth[1]

[1]The University of Sydney, Sydney, Australia,[2]Standard BioTools, San Francisco, CA,[3]SurgeCare, Paris, France,[4]Boston University Chobanian, Boston, MA

Immune checkpoint inhibitors (ICIs) are currently the most effective treatment for late-stage lung cancer. However, most patients fail to mount a durable response, and the mechanisms underlying non-responsiveness remain elusive. Here, we apply a universal omics approach to identify correlates of non-responsiveness to ICIs in lung cancer. In-depth characterization of the plasma proteome was performed using the SomaScan™ platform on pretreatment and longitudinal blood samples from 40 ICI-treated patients, resulting in quantification of 10,000 unique proteins. In parallel, comprehensive immune phenotyping was achieved with matched pretreatment PBMC for 90% of patients (36/40) through CyTOF™ technology, with a 38-plex panel describing over 150 immune populations in circulation. To enhance clinical outcome predictability of cellular and plasma-based immune relationships, cross-platform data integration was achieved with Stabl, a sparse, reliable omic biomarkers analysis strategy. Stabl identified key predictive features from both CyTOF and SomaScan technology, providing insight into immune deficits present in non-responsive patients. The combined model demonstrated a stronger capability to predict non-response from a pretreatment blood sample (AUROC = 0.79, p-value = 1.7e-2, Mann-Whitney non-parametric test) compared with individual platforms alone. Additionally, this multi-omic strategy is compatible with Imaging Mass Cytometry™ technology, unlocking another layer of clinical investigation via spatial biology. This study ultimately demonstrates both the clinical impact and utility of blood- and imaging-based CyTOF technology and the benefit of combining SomaScan and multi-omic-appropriate analysis approaches. Overall, robust treatment prediction, attributed to biomarker features, provides insights into mechanisms for non-response and highlights the potential for better treatment options in lung cancer.

#5005 Integrative multi-omics characterization of syngeneic tumor models.

Zihan Xu, Binchen Mao, Wubin Qian, Xiaobo Chen, Sheng Guo

Crown Bioscience, Inc., Suzhou, China

Background: Mouse syngeneic models are widely used to study tumor-immune interactions and evaluate the efficacy of immunotherapies. Proteins serve as a link between the genetic code and phenotype, and can better reflect the dynamic state of a cell. However, the large-scale profiling of proteomics and phosphoproteomics data using syngeneic models has been limited. This study aims to reveal molecular mechanisms underlying tumor development and therapeutic response using multi-omics data generated from syngeneic models.
Methods: Untreated fresh tumor tissues (around 500 mm^3) from 12 mouse syngeneic models (H22, EMT6, Hepa16, CT26, MC38, Pan02, A20, Renca, RM1, LL2, B16F10 and B16BL6) were collected. Tissues were analyzed using RNA-seq, proteomics and phosphoproteomics. Efficacies of immune checkpoint inhibitors (ICIs) including anti-PD-1 (RMP1-14), anti-PD-L1 (10F.9G2) and anti-CTLA4 (9D9 and 9H10) were tested in each model over a period of 2-3 weeks. Tumor growth inhibition (TGI) values were calculated as: $TGI = 1 - \Delta T(t)/\Delta C(t)$, where $\Delta T(t)$ and $\Delta C(t)$ represent average changes in tumor volume from the start of experiment to time t in the treatment and vehicle group, respectively. Models with TGI>=0.45 for at least two ICIs were defined as “responder”, while models with TGI<=0.40 for all ICIs were defined as “non-responder”. Tumor purity scores were inferred with the ESTIMATE software. Differential Expressed Genes (DEGs), Differentially Expressed Proteins (DEPs) and Differentially Phosphorylated Sites (DPSs) were calculated with the limma package. Gene Set Enrichment Analysis (GSEA) was performed using the clusterProfiler package. Kinase Substrate Enrichment Analysis (KSEA) was finished using the KSEAapp package. Network analysis was performed with the STRINGdb package.
Results: Estimation of stromal and immune cells using specific genes with transcriptomics and proteomics data revealed that B16BL6 and B16F10 models had the highest tumor purity and low percentage of immune and stromal cells, while A20 model had more immune cell infiltration, and other solid tumor models had relatively higher stromal cell percentage. Next, at RNA and protein levels, genes positively correlated with the drug efficacy were enriched in interferon related pathways and immune response pathways for all ICIs. Furthermore, Dnmt3a and Igfr2 were identified as potential resistant biomarkers for immunotherapy efficacy. Finally, a user-friendly website was designed for both direct acquisition of results generated in this study and deep exploration by simply clicking to choose combinations of parameters and generate new results.
Conclusion: This study presents a comprehensive integrated multi-omics analysis using transcriptomics, proteomics and phosphoproteomics data collected from 12 widely used mouse syngeneic models, which provides insights into molecular mechanisms and pathways involved in tumor development and therapeutic response.

#5006 Multi-modal large language models for metastatic breast cancer prognosis.

Raphael Pelossof, Mark Carty, Talal Ahmed, Stanislas Lauly, Alberto Purpura, Erik Mueller, Justin Guinney

Tempus AI, Chicago, NY

Inputs into cancer prognostic models are primarily structured data such as demographic and clinicopathological features, and lack richer and temporal context often found in unstructured clinical notes. We hypothesize that creating a temporal clinical patient note from structured data that preserves longitudinal and clinical contextual information, and coupling it with a large language models (LLM) that is trained to prognosticate overall survival (OS), may improve model accuracy with an interpretable embedding space.

We developed the Patient Chronological Note (PCN), an algorithm that converts structured data elements into textual strings, mirroring physician notes of patient histories. A bidirectional large language model, was pre-trained using PCNs from breast cancer patients (N=580,000), allowing the LLM to learn a representation of the patient journey. The resulting embeddings were fed into a fully-connected 2-layer network that was fine-tuned using Cox survival loss. Fine tuning was performed on PCNs derived from mBC patients (N=28,500), where the model was trained to predict OS from the time of first metastatic diagnosis. A held-out validation dataset of mBC patients (n=28,800) was used to validate survival prediction accuracy.

Our LLM-Cox model achieved a prediction performance of 0.66 (concordance index) on the validation cohort, outperforming a standard linear cox model that achieved 0.62. A marginal effect analysis showed that features associated with metastasis, medications, and patient demographics were most important in prediction performance while hormone receptor status and sequencing information were less important.

Clustering the LLM embeddings revealed 10 distinct patient groups enriched for key mBC traits, including a high-risk cluster enriched for triple negative status, TP53 mutations, and African American race, a low-risk cluster enriched for ESR1 mutations and CDK46 treatment, and a cluster enriched for low-risk early-onset patients. The different clusters showed different prognostic risk levels.

LLM-Cox model learning from PCNs can improve prediction performance over standard models. The internal embedding representation of the LLM was interpretable, and yielded distinct clinical-molecular subtypes that also showed distinct levels of prognostic risk. Furthermore, creating LLM-based clinical-molecular groups of patients with similar journeys and similar prognostic risk presents an opportunity to identify novel stratifications within each group that are associated with treatment-specific responses and not prognostic risk.

#5007 Integrated histology-genomic multimodel deep learning to predict prognosis in non-small-cell lung cancer.

Yang Xu, **Haimeng Tang**, Xue Wu, Qiuxiang Ou

Nanjing Geneseeq Technology Inc., Nanjing, China

Background: Deep learning (DL) provokes a paradigm shift in deciphering high-dimensional data for various clinical applications, and multimodal DL approaches show significant potential to integrate diverse datasets and enhance prediction accuracy. Despite these advancements, multimodal DL remains underutilized in prognostic assessment, particularly for non-small-cell lung cancer (NSCLC) patients.
Methods: We analyzed whole-slide imaging (WSI; approximately 6,000 slides and ~40,000,000 image tiles), whole exome sequencing (WES), and overall survival (OS) data from 2,366 NSCLC patients sourced from various online databases. A Sequential-Adapted Attention (SeAttn) model was developed to process WSI data, while multiple DL models were trained to analyze WES data and clinical features. Multimodel DL was then used to combine these learned features to predict long-term OS outcomes. The model was further confirmed using a validation (N=100) and a held-out test (N=101) cohort.
Results: The SeAttn model exhibited strong predictive power for NSCLC histological subtypes, achieving an area under the curve (AUC) of 0.98 in the test cohort (P<0.0001). SeAttn-derived WSI features, baseline clinical characteristics, as well as WES-based genetic features were used individually to predict OS, yielding concordance indices (C-indices) ranging from 0.55 to 0.67. The combination of these multimodal features using a COX-based DL approach further improved the test cohort C-index to 0.71 (95% CI 0.14, P=0.002), with WSI, mutation gene-sets, and baseline clinical data as the top contributors. Notably, our multimodal DL model accurately predicted prognosis for up to 5 years post-diagnosis (P<0.05 for all time-dependent AUCs within 5 years in both validation and test cohorts), and our model could significantly stratify patients based on their OS (P=0.012 in the test cohort). Additionally, we identified and validated novel prognostic markers, including some genetic mutations, co-deletions, and amplification of two microRNA clusters.
Conclusion: Our multimodal deep learning model demonstrates substantial predictive capacity for prognosis in NSCLC patients, providing a robust framework for integrating diverse clinical, genetic, and imaging data to yield accurate survival estimates and uncovering clinically relevant biomarkers to guide risk stratification. Our findings underscore the broader promise of multimodal DL, offering a scalable and data-driven approach that can be adapted for other cancers.

#5008 A multi-dimensional vision-language deep learning model for radiology and pathology imaging.

Marta Ligero, Tim Lenz, Jakob Nikolas Kather

Technische Universitat Dresden, Dresden, Germany

Multimodal vision-language models (VLM) have shown promising biomarker classification performance in individual data modalities such as radiology and pathology. By learning joint representations of visual and textual information, these methods have shown to provide better features from medical images. However, current methods are limited to the multimodal landscape of 2D images in radiology and pathology, leaving a substantial gap for the creation of models with 3D data. In this study, we introduce a novel, multimodal VLM that can process both radiology and pathology images of varying dimensionalities. Our approach processes 3D CT reconstructions and 2D digitized hematoxylin- and eosin-stained histology slides simultaneously, learning a joint representation which is applicable for biomarker classification in either modality. Our zero-shot approach was validated using two distinct datasets, eliminating the need for model fine-tuning. The first evaluation assessed whole-body CT lesion classification across multiple organs (lung, liver, kidney, and soft tissue) in 839 patients. The second evaluation focused on distinguishing between lung adenocarcinoma and squamous cell carcinoma in 974 patients. The model achieved an average Area Under the Receiver Operating Characteristic (AUROC) curve of 0.70 for lesion detection and 0.60 for lung cancer subtyping in a zero-shot setting. Our findings demonstrate the potential of multimodal vision-language models to perform medical image analysis without task-specific training, which offers a promising direction for developing more flexible and generalizable image processing and multimodal integration towards an artificial intelligence-driven molecular tumor board.

#5010 High-resolution multi-omic dissociation of brain tumors with deep multimodal autoencoder.

Jiao Sun, Yue Pan, Tong Lin, Kyle Smith, Arzu Onar-Thomas, Giles W. Robinson, Wei Zhang, Paul A. Northcott, Qian Li

St. Jude Children's Research Hospital, Memphis, TN

Single cell technology measures multi-dimensional molecules at cellular resolution in cancer biology with challenges of low coverage and cell annotation. The inherent heterogeneity and complexity of central nervous system (CNS) tumors may hinder single-cell multi-omic profiling in large population-based studies. An alternative and efficient approach is digital dissociation, i.e., cell abundance quantification and high-resolution prediction of cell-type-specific (CTS) bulk samples. Many tools were developed to resolve mixture bulk transcriptomes by integrating scRNA-seq reference, not immediately transferable to other omics (e.g., chromatin accessibility, DNA methylation) because of ambiguous cell marker identification.

We develop MODE - a novel multimodal autoencoder neural network with parallel decoders - to purify CTS multi-omic profiles and estimate the multimodal cellular compositions. MODE is trained on the large-scale pseudo bulk multiomes constructed by probabilistic data generation with an external scRNA-seq reference and individualized non-RNA reference panel recovered from the target tumors. The CTS differentially expressed genes (ctsDEGs) in purified transcriptomes were detected by Wilcoxon rank-sum test with false discovery rate corrected, while survival analysis was performed by log-rank test.

MODE was benchmarked in an extensive simulation study generating realistic multiome from normal and tumor brain tissues, showing outperformance and superior replicability over the competing deconvolution pipelines. The purified CTS multiomes exhibited high resolution and fidelity with enhanced robustness in detecting ctsDEGs. We applied MODE to the bulk methylome-transcriptome in two independent brain tumor studies, recovering modality-specific cellular landscape for pediatric medulloblastoma (MB) and adult glioblastoma multiforme (GBM). The embryonal lineage cells for MB tumor groups were accurately recovered, i.e., Group 3 (G3) MB tumors having the origin of rhombic lip (RL) stem cells ($P < 10^{-5}$) and Group 4 (G4) arising from unipolar brush cells ($P < 10^{-5}$). Notably, hundreds of marker genes for RL or granule neuron progenitor cells identified in human fetal cerebellum were differentially expressed among (n=253) Sonic Hedgehog, G3, G4 MB patients in the corresponding lineage-specific tumor transcriptomes predicted by MODE. The results in TCGA GBM cohort showed higher abundance of myeloid cells in either methylation (P=0.002) or mRNA (P=0.019) being associated with poorer event free survival, while increased tumor purity delayed (P=0.009) the relapse or progression of GBM tumor.

Overall, the high-resolution multimodal dissociation by MODE elucidates the origins, evolution, and prognosis in pediatric and adult CNS tumors, without the need for large-scale single cell multimodal profiling. MODE is freely available at https://github.com/jsuncompubio/MODE.

#5011 Integrating multimodal data with survival-based variational autoencoders to predict recurrence-free survival in breast cancer.

Sydney Grant, Aritro Nath

Medical Oncology & Therapeutics, City of Hope, Duarte, CA

Breast cancer is the most commonly diagnosed malignancy globally and responsible for over 670,000 deaths each year. Accurate prediction of recurrence-free survival (RFS) is crucial for clinical management and personalized care in breast cancer, enabling improved surveillance, risk stratification, and optimized therapy selection. Although extensive multi-omic data from next generation sequencing tests have been collected, there is a notable gap in clinically relevant models that leverage advanced artificial intelligence-based approaches that integrate multiple data modalities. Variational autoencoders (VAEs) have been used to compress omics datasets into lower dimensionality, however the latent dimensions extracted from VAEs typically lack biological significance, hindering interpretation and the performance of downstream predictive modeling. To address this pitfall, we have developed a VAE with a built-in survival model. Our approach uses the performance of the survival model to iteratively optimize the VAE, generating latent dimensions which are relevant for predicting RFS. We developed our model by extracting latent features from approximately 800 breast cancer cases from METABRIC split into training and test datasets. Gene expression, MSigDB pathway enrichment scores, CNAs, and SNV/Indels were used to train four separate VAEs. The model's encoder compressed high-dimensional omic datasets into a set of latent dimensions, while the decoder then used the latent dimensions to reconstruct the original data; simultaneously, linear representations of the latent dimensions were used as inputs to a cox regression model. The reconstruction loss, Kullback-Leibler divergence, and negative partial log likelihood based on the survival model were used to reparametrize the VAE during each training iteration. Final models for each modality were selected based on survival prediction on the test dataset with c-index ranging from 0.61-0.65. A cox regression model was fit based on a combined dataset of the latent dimensions from each model, and clinical data from each patient. To assess the ability of our model to stratify high- and low-risk patients, an optimal cut-off was established based on the linear predictors of the cox regression model in the training dataset. There was a significant difference in survival between high- and low-risk groups in both the training ($p < 0.0001$) and the test ($p < 0.0001$) cohorts. The 5-year survival rates of high- and low-risk groups were 30.7% vs 88.9% in the training dataset and 60.5% vs 87.8% in the test dataset. Overall, our survival-based VAEs successfully extracted relevant latent dimensions for prediction of recurrence-free survival in breast cancer patients. Further implementation of our model may be used to predict treatment-specific response, helping clinicians make informed treatment decisions for cancer patients.

#5012 Multi-omics signature for estimating survival time in breast cancer patients.

Srinivasulu Yerukala Sathipati[1], Manya Reddy Elugoti[2], Rachel Tillman[3], Scott Hebbring[4], Rohit Sharma[5]

[1]Marshfield Clinic Research Institute, Marshfield, WI,[2]Edison Academy Magnet School, Edison, NJ,[3]Department of General Surgery, Marshfield Clinic Health System, Marshfield, WI,[4]Center for Precision Medicine Research, Marshfield Clinic Research Institute, Marshfield, WI,[5]Department of Surgical Oncology, Marshfield Clinic Health System, Marshfield, WI

Introduction: Predicting survival outcomes for breast cancer patients is critical for tailoring personalized treatments. However, effectively integrating multi-omics datasets for survival estimation is a persistent challenge due to the high dimensionality and complexity of the data. To address this, we developed the Multi-Omics Survival Time Estimator (MOST), a novel machine learning framework designed to accurately predict survival time by leveraging multi-omics data.
Methods: MOST employs a multi-step pipeline combining stringent feature selection, evolutionary learning, and survival estimation. Using datasets encompassing copy number variations (CNV), microRNA (mRNA), mRNA, and reverse-phase protein array (RPPA) data, the platform identifies a concise yet informative set of biomarkers that contribute to survival time prediction. Feature selection was optimized to ensure relevance and robustness, while evolutionary algorithms enhanced predictive accuracy. The model was rigorously validated to ensure reliability and generalizability.
Results: MOST identified a 38-feature multi-omics signature capable of predicting survival time with a correlation coefficient (R) of 0.90 and mean absolute error (MAE) of 1.06 year between predicted and actual survival times. The top 10 features contributing to the prediction included ENSG00000145826.7 and ENSG00000101624.9 from CNVs; hsa-miR-3928 and hsa-miR-326 from miRNAs; KIAA1199, SYT10, PSG3, ACTG1, MYPN from mRNAs; and GUSP4 from RPPA data. These results emphasize the role of integrated genetic, epigenetic, and proteomic markers in defining patient survival. MOST demonstrated superior predictive performance compared to existing methodologies.
Conclusions: MOST offers a machine learning framework for integrating multi-omics data to predict survival outcomes in breast cancer patients. Its ability to identify a robust multi-omics signature establishes a foundation for future exploration of multi-omics data across other cancers, reinforcing its clinical relevance and potential translational impact.

#5013 The interplay of genomic aberrations, transcriptional states, and therapeutic responses in lung cancer.

Hanbyul Cho[1], Noshad Hosseini[1], Yi Hsiao[1], Rahul Mannan[1], Chandan Kumar-Sinha[1], Yuping Zhang[1], Gabriel Cruz[1], Yamei Deng[1], Taiwan Cancer Moonshot Program and Clinical Proteomic Tumor Analysis Consortium, Natalie Clark[2], Shankha Satpathy[2], Gilbert S. Omenn[1], Yu-Ju Chen[3], Michael A. Gillette[2], Marcin P. Cieslik[1], Saravana M. Dhanasekaran[1], Alexey I. Nesvizhskii[1], Arul M. Chinnaiyan[1]

[1]University of Michigan, Ann Arbor, MI,[2]Broad Institute of MIT and Harvard, Cambridge, MA,[3]Academia Sinica, Taipei, Taiwan

Lung cancer leads cancer-related deaths worldwide, with 2024 estimates of 234,580 new cases in the United States. This study explores the roles of tumor driver aberrations, epithelial transcriptional states, and microenvironment composition in lung cancer outcomes using proteogenomic data from 965 patients across the Clinical Proteomic Tumor Analysis Consortium (CPTAC) and International Cancer Proteogenome Consortium (ICPC). We examined oncogenic alterations, epithelial and stromal cell type enrichment distributions, transcriptional modules activities, and drug sensitivities in lung adenocarcinoma (LUAD), squamous carcinoma (LUSC) and adenocarcinoma with no conventional drivers (NCD-LUAD).LUAD tumors displayed mutual exclusivity in RAS/Receptor Tyrosine Kinase (RTK) pathway aberrations, complemented by significant patterns of co-occurrence and exclusivity in tumor suppressor genes like TP53, STK11, RBM10, and CDKN2A. Our integrative analysis used gene expression from single-cell RNA-seq (scRNA) derived cell types reference and CPTAC bulk RNA/proteomic data for correlation-based estimation of epithelial/stromal cell type enrichment. We assembled scRNA references from 3 different studies that profiled normal tissues containing, 142 cell types from 38 tissue types, 20 stromal cell types from 11 tissues, and lastly exclusive lung cell types reference respectively. We found significant enrichment of AT2 and Club cells in LUAD tumors (p-value < 0.0001), in addition heterogeneity in epithelial cell type enrichment within each driver group. Patients enriched for AT2 and AT1 cells demonstrated better survival outcomes (p-value < 0.0001) while, secretory Goblet and Basal cells enrichment was associated with poor survival (p-value < 0.0001), underscoring the potential link between tumor transcriptional / differentiation states and prognostic implications. NCD-LUAD showed similar epithelial cell type enrichment patterns like LUADs with known drivers while LUSC tumors show predominant basal cell enrichment. Importantly we saw specific transcriptional modules (up: E2F1,MYBL2, down: NKX2-1, NFE2L2) and cell type enrichment differences in genome unstable (high copy number burden) tumors associated with poor survival. Finally, enrichment of a myofibroblast subtype associated with survival and immune infiltration status, warrants further investigation. Cell line drug sensitivity analysis showed that the RTK-altered group with poor response to targeted therapy was enriched with undetermined differentiation transcriptional status compared to more defined cell type enrichments in the good response group (p-value = 0.016). Our study underscores the importance of multi-dimensional integrative analysis approaches of molecular, cellular, and pharmacological data to understand and advance lung cancer biology, potential cell of origin, and therapeutic strategies.

#5014 Integrative molecular and spatial analysis to reveal tumor intrinsic and extrinsic drivers of immunotherapy response in mucosal melanoma.

Yingxiao Shi[1], Tuulia Vallius[2], Aikaterini Dedeilia[3], Roxanne Pelletier[2], Jia-Ren Lin[2], Mariana Lopez Leon[2], Christine Lian[4], Shishir Pant[2], Giuseppe Tarantino[5], Annette Wang[6], Jiajia Chen[5], Elizabeth I. Buchbinder[7], Peter K. Sorger[2], David Liu[1], Genevieve M. Boland[3]

[1]Computational Oncology, Dana-Farber Cancer Institute/Harvard Medical School, Boston, MA,[2]Laboratory of Systems Pharmacology, Harvard Medical School, Boston, MA,[3]Department of Surgery, Massachusetts General Hospital, Boston, MA,[4]Department of Pathology, Brigham and Women's Hospital, Boston, MA,[5]Computational Oncology, Dana-Farber Cancer Institute, Boston, MA,[6]Massachusetts General Hospital, Boston, MA,[7]Medical Oncology, Dana-Farber Cancer Institute/Harvard Medical School, Boston, MA

Background: Mucosal melanoma (MM) is a rare subtype of melanoma that responds poorly to immune checkpoint blockade (ICB), exhibiting low response rates and few durable responses. The biological mechanisms underlying ICB response and resistance in MM remain poorly understood, highlighting the need for novel therapeutic targets and biomarkers to improve patient outcomes.
Hypothesis: While most MM patients respond poorly to ICB, a subset of durable responders (DRs) exists. We hypothesize that DRs have distinct biological and genomic features compared to rapid progressors.
Methods: We performed multimodal analysis - including whole exome sequencing, bulk transcriptomics, H&E, and multiplexed Cyclic Immunofluorescence (CyCIF) imaging - on pre-treatment and post-progression tumor samples from 126 MM patients, with a focus on a subset of 80 patients treated with ICB to identify features associated with response.
Results: Our genomic analysis of 80 MM patients revealed that DRs (PFS 1 year) had significantly fewer copy number alterations (CNAs) compared to those with progressive diseases (PFS 6 months). The most common MM driver mutations were identified including *SF3B1*, *KIT*, *NF1*, and *NRAS*. Notably, *SF3B1* mutations were primarily found in anorectal primary tumors (10/17) and had fewer CNAs, a feature also observed in DRs, suggesting it may be associated with better ICB responses in MM. Bulk RNAseq analysis (n = 67) identified a tumor cluster enriched for epithelial-mesenchymal transition and hypoxia stress pathways, suggesting increased invasiveness and potential ICB resistance. These signatures were observed in 37% of our MM primary tumors, underscoring the aggressive nature of MM. CyCIF analysis of primary and metastatic MM lesions revealed increased $CD8^+$ T cells and $CD11C^+$ myeloid cells in metastatic sites. By defining the tumor/stromal border, we found fewer antitumorigenic $CD68^+$ macrophages in the tumor center but more at the tumor border at the metastatic site compared to the primary sites, suggesting immune exclusion. In sinonasal primary tumors, non-metastatic patients had fewer protumorigenic $CD163^+$ macrophages in both tumor center and border compared to those with distant metastases. Multimodal analysis of a longitudinally sampled MM patient treated with ICB showed distinct resistance clones associated with different microenvironments. Two lymph node metastases collected simultaneously after ICB showed clonal differences, with one (harboring a *SOX17* mutation) exhibiting lower $CD8^+$ T cell infiltration, suggesting the evolution of diverse resistance mechanisms.
Conclusion: Our preliminary findings suggest that distinct genomic features, tumor states, and microenvironments differentiated ICB response and disease progression in MM, highlighting key tumor-intrinsic and microenvironmental factors associated with patient prognosis.

#5016 Enhancing prostate pelvic multimodality data generating with conditional generative models: A Pix2Pix-based approach for MRI-to-PET synthesis.

Ruobing Liu[1], Shuo Wang[2], Shibiao Wan[3], **Jieqiong Wang**[1]

[1]Department of Neurological Sciences, University of Nebraska Medical Center, Omaha, NE,[2]Department of Radiation Oncology, University of Nebraska Medical Center, Omaha, NE,[3]Department of Genetics, Cell Biology & Anatomy, University of Nebraska Medical Center, Omaha, NE

Prostate cancer remains the second leading cause of cancer-related mortality among men globally, underscoring the critical need for early detection of treatment failure and effective assessment of metastatic risk. Positron Emission Tomography (PET), particularly with prostate-specific membrane antigen (PSMA) tracers, has demonstrated superior sensitivity for identifying prostate lesions, including metastases. However, the accessibility of PET imaging is often limited by its high costs and associated radiation exposure. To overcome these challenges, we developed a deep learning model to synthesize PET images from Magnetic Resonance Imaging (MRI) image, facilitating treatment response evaluation.High-resolution T2-weighted MRI and PSMA PET images acquired within a close timeframe were retrieved from 10 prostate cancer patients, who underwent definitive radiotherapy. The PSMA-PET scans were registered to the MRI images within the Eclipse (Varian Medical Systems), and the PET images were cropped to match the same size and resolution of MRI images, resulting in 321 pairs of MRI-PET 2D images. Preprocessing included grayscale transformation, z-score normalization, and pixel value inversion to enhance model learning. A Pix2Pix framework was implemented, employing a U-Net generator and a PatchGAN discriminator. The loss function used consisted of a combination of adversarial loss, to ensure the realism of the generated images, and L1 loss, to maintain pixel-wise consistency between the generated and target images. Model evaluation was performed using leave-one-out-cross-validation (LOOCV), where all slices from one patient was used for testing and the remaining 9 patients for training.The model achieved an average Peak Signal-to-Noise Ratio (PSNR) of 14.55 and a Structural Similarity Index Measure (SSIM) of 0.648. Although these quantitative metrics were moderate, qualitative evaluation demonstrated precise and clinically meaningful localization of lesions, offering utility for aiding physician visualization of high-risk regions.This study highlighted the feasibility of leveraging Pix2Pix-based conditional generative models for synthesizing PET-equivalent images from MRI data as a cost-effective alternative to enhance prostate cancer imaging. Future efforts will focus on expanding the dataset and investigating advanced architectures, including 3D-to-3D generative models and diffusion techniques, to further improve the accuracy of prostate lesion localization and clinical applicability.

#5017 A multimodal deep learning approach to predict survival in never smoking lung cancer patients.

M. Saha[1], T. Zhang[1], P. M. Bhawsar[1], W. Zhao[1], J. Shi[1], S.-R. Yang[2], J. S. Almeida[1], M. Landi[1];
[1]National Cancer Institute, Rockville, MD, [2]Memorial Sloan Kettering Cancer Center, New York, NY

Purpose: Lung cancer in never-smokers (LCINS) is the fifth most common cause of cancer deaths worldwide. Identifying subjects who need more aggressive treatment could greatly help improve survival rates. In this study, we investigated whether a multimodal deep learning approach could be used to predict overall survival in a large cohort of never smoking lung adenocarcinoma patients from the Sherlock-*Lung* study. We leveraged multiple datasets containing different modalities, including clinical, digital pathology (WSIs: whole slide images), and genomic (driver gene mutations in EGFR, KRAS, TP53, RBM10, as well as CDKN2A deletions, ALK fusions, and mutation patterns like Kataegis) data.
Method: The sample set contained 495 LCINS cases which were randomly split into training (60%), validation (15%), and testing (25%) sets. Tiles of 256 x 256 pixels were extracted from the WSIs and processed through a customized VGG-19 convolutional neural network. The output of this network was high-dimensional feature vectors representing morphological patterns in the tiles. Genomic and clinical data were modeled separately and then concatenated with the output of the CNN to generate a shared feature space, which was then modeled using a simple densely connected neural network. The final feature vector was then used to predict the risk score using Cox partial likelihood as the loss function. The risk predictor was then validated on the held-out test set. The above procedure was conducted with and without genomic data. We also conducted a gene-specific survival study across LCINS with different driver genes as well as restricting the analysis to stage I cases only.
Results: We achieved an index of 0.86 for the concordance between predicted mortality risk and actual survival time on the test set using both approaches, with and without genomic data. The Kaplan-Meier curves for low- and high-risk groups, with the cutoff for the risk score determined by the median value, showed distinct survival patterns - patients predicted to have low risk exhibited a higher survival probability compared to those with higher scores. The log-rank test yielded a p-value of ≤ 0.05 for overall and gene-specific survival, adjusting for stage and age. Heatmaps generated using the activation map of the image model highlighted non-tumor regions as low-risk regions and tumor regions as high-risk regions.
Conclusion: The multimodal approach strongly predicted overall survival across all stages and within stage I in LCINS. Specific driver gene mutations do not significantly enhance survival prediction, highlighting the benefits of modeling on multimodal information over using only molecular data. Such approaches for LCINS prognostication could be used in the future to guide effective treatment modalities.

#5018 Multiomic integration of DNA methylation, DNA hydroxymethylation, and gene expression in clear cell renal cell carcinoma.

Yiping Li, Brock C. Christensen, Lucas A. Salas

Dartmouth College, Hanover, NH

Background

DNA methylation (5-methylcytosine -5mC) and DNA hydroxymethylation (5-hydroxymethylcytosine -5hmC) are critical epigenetic modifications for understanding gene expression in clear cell renal cell carcinoma (ccRCC). While 5mC and 5hmC modifications may influence promoter and enhancer regions differently, their distinct roles in gene regulation remain unclear. Integrating RNA sequencing (RNA-seq) data with 5mC and 5hmC analyses enables a multiomic understanding of gene regulation, revealing critical changes associated with ccRCC progression. This study integrates 5mC, 5hmC, and RNA-seq data to identify genes and transcriptional regulators driving ccRCC by comparing tumor and normal samples.

Methods

We analyzed 188 ccRCC samples from the Dartmouth Renal Tumor Biobank using the Infinium MethylationEPIC BeadChip to differentiate 5-methylcytosine (5mC) from 5-hydroxymethylcytosine (5hmC) via bisulfite and oxidative bisulfite tandem conversion. Whole RNAseq data was obtained from the same samples. Briefly, we used ENmix to process and perform quality control of the 5hmC/5mC data. RNA-seq data was processed using Nextflow rnaseq using the STAR/Salmon alignment option. The distal and proximal genomic associations between 5mC/5hmC and gene expression were analyzed using Enhancer Linking by Methylation/Expression Relationship (ELMER). The supervised mode was used because the two groups of comparisons were specified. For each differentially methylated or hydroxymethylated probes, the CpGs were mapped to nearby genes (10 upstream and 10 downstream), and enriched motifs and associated transcription factors (TFs) were identified.

Results/Discussion

Motifs associated with transcription factors ZBTB14 and ZNF148 were enriched in regions of both hypo-hydroxymethylation and hypo-methylation at enhancers, implicating their roles in kidney cancer development[1,2]. Both ZBTB14 and ZNF148 have been associated with negative regulation of transcription by RNA polymerase II, while ZNF148 has also been involved in negative regulation of gene expression[1,2].

Conclusion

The initial analysis of DNAm and DNAhydroxym after integrating RNAseq in ccRCC allows us to find the essential genes that could contribute to kidney cancer development. Next, we hope to integrate a robust analysis at the transcript level to explore the implications of epigenetic regulation on alternative splicing in ccRCC. Further research is warranted to explore additional regulatory genes and epigenetic alterations in ccRCC progression.

Reference

1) doi:10.7554/elife.807602
2) doi:10.1158/0008-5472.can-15-3150

#5019 Genomic landscape, tumor heterogeneity and immune-related post-translational regulation in cutaneous melanoma.

Jianhui Gong[1], Gabriel Cruz[1], Yamei Deng[1], Maria Virginia Ruiz Cuevas[2], Chandan Kumar-Sinha[1], Rahul Mannan[1], Yi Hsiao[1], Francesca Petralia[3], Weiping Ma[3], Shrabanti Chowdhury[3], Noshad Hosseini[1], CPTAC Clinical Proteomic Tumor Analysis Consortium[4], Gilbert Omenn[1], Arul Chinnaiyan[1], Paul Harms[1], Raghav Jain[5], Jennifer Kyle[5], Avi Ma'ayan[3], Saravana Dhanasekaran[1], Karl Clauser[2], Hui Zhang[6], Alexey Nesvizhskii[1], Steven Carr[2], Tao Liu[5], Pei Wang[3], Marcin Cieslik[1]

[1]University of Michigan, Ann Arbor, MI,[2]Broad Institute, Boston, MA,[3]Icahn School of Medicine at Mount Sinai, New York, NY,[4]National Cancer Institute, Bethesda, MD,[5]Pacific Northwest National Laboratory, Richland, WA,[6]Johns Hopkins University, Baltimore, MD

Melanoma, the deadliest form of skin cancer, accounts for an estimated 100,640 new diagnoses and 8290 deaths in the United States in 2024. This study leverages 13 multi-omics data types from 164 melanoma samples provided by the Clinical Proteomic Tumor Analysis Consortium (CPTAC), uniquely featuring ubiquitinome and HLA peptidome profiling, to investigate the genomic aberrations, molecular subtypes, tumor microenvironment heterogeneity and post-translational modifications (PTMs).Key findings include mutual exclusivity between hotspot mutations affecting the kinase activity of BRAF and RAS, as well as between alteration types impacting TERT expression. High ultraviolet (UV) impact melanoma is characterized by NF1 loss and TERT promoter mutations. Metastatic melanoma shows higher whole genome duplication (WGD) prevalence (58% vs 46%), with early focal loss of CDKN2A and PTEN preceding WGD. A chr5-12 translocation involving CDK4 and TERT and significant clonal heterogeneity were identified, with single-clone tumors linked to better prognosis ($p = 0.029$). Transcriptional states derived from single-cell RNA sequencing (melanocytic, transitory, invasive) and proteome clustering based on immune checkpoint inhibitor (ICI) features highlight immune response as a key determinant of melanoma heterogeneity. HLA peptidome analysis identified 830 tumor antigen candidates, including 23 non-coding antigens shared across >6 patients, predominantly sourced from introns and MAGEA family genes. Metabolomic and lipidomic data provide insights into immune-metabolic interactions, while ubiquitinome and acetylome profiling are being further investigated to understand immune differences in melanoma. These findings underscore the critical role of post-translational regulation and immune regulation in melanoma progression and therapeutic targeting.

#5020 Quantum algorithms improve feature selection for multimodal cancer classification.

Siddhi Ramesh[1], Teague Tomesh[2], Ryan Robinett[3], Dmitry Karpeyev[1], Colin Campbell[2], Bharath Thotakura[2], Frederic Chong[2], Samantha Riesenfeld[3], Alexander Pearson[1]

[1]The University of Chicago Medicine Comprehensive Cancer Center, Chicago, IL,[2]Infleqtion, Chicago, IL,[3]The University of Chicago Pritzker School of Molecular Engineering, Chicago, IL

Introduction: Cancer machine learning research is often limited by overparameterization and overfitting, which arise because cancer 'omic' variables significantly outnumber patient samples. Traditional feature selection methods for multimodal cancer data fail to completely capture complex biological relationships across genomic, transcriptomic, and pathomic data due in part to computational intractability—interaction information for 3rd-order and higher terms scale exponentially, infeasible for classical methods. We developed a novel quantum-classical hybrid algorithm to identify small, informative feature sets that incorporate higher-order correlations, potentially revealing previously undetected biological signals to improve cancer classification.

Methods: We implemented a quantum-classical hybrid algorithm that frames feature selection as a polynomial constrained binary optimization problem (PCBO-Tournament). Our approach leverages quantum resources through the Recursive Quantum Approximate Optimization Algorithm to explore higher-order feature correlations. We evaluated the algorithm on The Cancer Genome Atlas (TCGA) pan-squamous cell carcinoma cohort (lung, cervical, and head and neck) (1,427 patients) with DNA mutational data, mRNA expression data, and pathomics data for tissue-of-origin classification. To reduce noise and enable efficient data loading, we binarized DNA single-nucleotide polymorphisms (2,720 loci), discretized normalized mRNA expression values into quantiles (18,879 genes), and processed pathomics data with the Slideflow platform, using the Prov-GigaPath foundation model to generate slide-level features (2,048) that we then discretized into quartiles. Feature sets were assessed in terms of the balanced accuracy of logistic regression models in cancer sub-type classification.

Results: On the TCGA pan-squamous dataset, our quantum-enhanced algorithm selected feature sets of size 14 that achieved higher balanced accuracy in cancer classification (97%, $P<0.01$), compared to both models containing all features and those with classically selected feature sets. The algorithm identified biologically relevant features, including tumor suppressor genes such as *TP53*, along expression of tissue-specific markers (*SFTPA1/A2/B*), developmental regulators (*TBX4/5*), and epigenetic factors (*HIRIP3*). The selected features demonstrated robust performance across data modalities, with a variant of our full-qubo PCBO outperforming classical approaches in the small feature set regime ($N \leq 20$).

Conclusion: Our quantum-enhanced PCBO feature selection method identifies small, biologically meaningful feature sets that outperform classical approaches, particularly in terms of incorporating higher-order correlations. This work illustrates the potential for quantum computing to advance multimodal biomarker discovery and precision oncology.

#5021 Multi-omics integration reveals interplay among intratumoral microbiome, host metabolism, and immune response in non-small cell lung cancer.

J. Yang[1], H. Woo[1], S. Yang[2], S.-i. Eyun[1];
[1]Chung-Ang University, Seoul, Korea, Republic of, [2]Sungkyunkwan University, Seoul, Korea, Republic of

The lung, constantly exposed to diverse microorganisms, harbors a microbiome that can influence disease development, including cancer, through mechanisms such as altered immune function and host metabolism. While intratumoral microbiota have been linked to cancer progression and therapy response, their specific role in lung cancer remains poorly understood. Microbial metabolites play a crucial role in mediating the host signaling and metabolic pathways. However, the precise connection between intratumoral microbiota and cancer metabolism is still unresolved. To address this, we comprehensively analyzed the relationship between intratumoral microbiota and host metabolism in 1,111 NSCLC patients using data from TCGA and single-cell RNA sequencing samples. We identified 17 microbial genera associated with host metabolism through gene set variation analysis (GSVA) and Spearman correlation, further validated these associations through survival and differential abundance analyses. To uncover key pathways associated with specific microbes, we applied gene set enrichment analysis (GSEA) and weighted correlation network analysis (WGCNA). Drug sensitivity was assessed based on microbial profiles, while Scissor method identified microbe-associated immune cell subpopulations and their functional roles within the tumor microenvironment. Our findings revealed that *Succinimonas* and *Marichromatium* act as risk factors, while *Luteibacter* serves as a protector in NSCLC patients. High abundance of *Succinimonas* was associated with upregulated glycolysis, downregulated immune responses, enhanced imuune evasion and poorer survival outcomes. Additionally, *Succinimonas* abundance was associated with mutations in key tumor progression genes, such as TP53, SPTA1, and SYNE1. Single-cell analysis demonstrated that *Succinimonas* influenced T cell subpopulations, notably by downregulating Treg Helios- cells, potentially inhibiting antitumor immunity. Furthermore, *Succinimonas* abundance was associated with sensitivity to specific cancer drugs. This study uncovers the complex relationship among intratumoral microbiota, host metabolism, and immune responses in lung cancer. *Succinimonas* emerges as a critical microbial player influencing cancer metabolism, immune evasion, and treatment responses. Our findings provide a preliminary understanding of these interactions, further in vivo and in vitro studies exploring the impact of intratumoral microbes on cancer could potentially lead to novel therapeutic strategies for cancer treatment.

#5022 iS^{3}RGs: Discriminating diverse medulloblastoma subtypes by leveraging heterogenous transcriptome data with batch effects.

M. Sun, J. Wang, S. Wan;
University of Nebraska Medical Center, Omaha, NE

As the most common malignant pediatric brain cancer, medulloblastoma (MB) accounts for around 20% of all pediatric central nervous system (CNS) neoplasms. MB includes a complex array of distinct molecular subtypes characterized by morphological, molecular, and genetic alterations, mainly including SHH, WNT, Group3 and Group4. Although the overall cure rates for MB are around 70%, the survival rate varies significantly across different molecular subtypes. Conventional experimental methods for identifying MB subtypes are usually costly, time-consuming, and labor-intensive. Accurate identification of MB subtypes enables improved downstream risk stratification and tailored therapeutic treatment design. Previous studies indicate that identifying MB subtypes based on transcriptome data is an efficient and feasible way. However, their performance may be poor due to limited cohorts and severe batch effects if combining data in different sequencing platforms and various data sources. To overcome these limitations, we propose a novel computational approach based on intra-sample subtype-specific ranked genes (iS^{3}RGs) analysis to effectively integrate heterogeneous transcriptomic data with batch effects for accurate identification of MB subtypes across diverse cohorts. For a baseline microarray dataset comprising 763 samples as training part, we specifically converted the absolute gene expression to the intra-sample rank values to capture reliable gene patterns by reducing bias from fluctuations and measurement errors. By comparing gene pairs within cohorts rather than across cohorts, we could avoid batch effects and successfully identified those gene pairs that significantly contributed to the identification of MB subtypes. Subsequently, we performed differential ranking analysis for iS^{3}RGs identification. These genes were organized into gene ranking ratios and the subtype-specific gene ratios were filtered using Fisher's exact test and LASSO regression, based on which 1035 gene ratios (corresponding to 738 unique genes) were identified. Then by testing on 14 independent datasets, our proposed method significantly outperformed state-of-the-art MB subtyping approaches with a median accuracy of 99.02%. We believe that our proposed approach has direct positive impacts on downstream MB risk stratification and tailored treatment design. We also expect that our proposed approach has great potential to address many biomedical problems with batch effects for various types of pediatric, adolescent, and young adult (AYA) cancers.

#5023 Machine learning prediction of perineural invasion in hepatobiliary cancers.

J. A. Yasin[1], A.-a. Obeid[2], L. Chen[3], M.-A. A. Tamimi[1], F. A. Qtaishat[1], O. M. Younis[1], A. E. Saeed[1], Y. A. Ateiwi[1], R. M. Odat[4], M. M. Alghaniem[1], N. Al-Awamleh[1], A. Saeed[5], A. Saeed[3];

[1]University of Jordan, Amman, Jordan, [2]World Islamic Sciences and Education University, Amman, Jordan, [3]University of Pittsburgh Medical Center, Pittsburgh, PA, [4]Jordan University of Science and Technology, Amman, Jordan, [5]University of Vermont, Burlington, VT

Background: Hepatobiliary cancers, which affect the liver, gallbladder, and bile ducts, are aggressive malignancies with a poor prognosis. Factors such as perineural invasion (PNI) can further complicate these cancers, as it can facilitate the spread of cancer cells to distant organs, reducing treatment effectiveness and overall survival. In this study, we aimed to develop machine learning (ML) models to predict PNI in patients with hepatobiliary cancers.

Methods: After data processing and cleaning, our dataset included profiles of 433 samples of hepatobiliary cancers from the Memorial Sloan Kettering Cancer Center 2024 HCC dataset. Binary logistic regression (BLR) was used to investigate predictors of PNI. Various ML models were developed by python libraries, such as “scikit-learn” and “TensorFlow”, then trained and tested on 80-20 split. Ten classification models were built with several features including demographic, clinicopathological, and genomic variables such as fraction genome altered, microsatellite instability, and tumor mutational burden. GridSearch cross-validation was used for hyperparameter tuning. Model performance was evaluated on test sets and SHapley Additive exPlanations (SHAP) analysis was applied to interpret feature contributions.

Results: Of the 433 samples, 25.9% (n = 112) were extrahepatic cholangiocarcinoma, 24.7% (n = 107) were gallbladder cancer, 13.6% (n = 59) were hepatocellular carcinoma, 32.6% (n = 141) were intrahepatic cholangiocarcinoma, and 3.2% (n = 14) were biphenotypic tumors. Cancer subgroup was a significant predictor in BLR analysis, with extrahepatic cholangiocarcinoma having the highest proportion of PNI. Among the ML models, Decision Tree demonstrated the highest precision at 0.902. On the other hand, according to area under the curve (AUC), Logistic regression (LR), Gradient Boosting (GB), and Random Forest (RF) were the top three performing models (AUC = 0.892, 95% CI [0.845, 0.939]; AUC = 0.889, 95% CI [0.841, 0.936]; and AUC = 0.888, 95% CI [0.841, 0.936], respectively). Cancer subgroup was the most influential feature among these top three models, and primary tumor site ranked second in the RF model, according to SHAP analysis. Multilayer perceptrons were the least-performing, achieving AUC values of 0.797 (95% CI: 0.736-0.858) for the single-hidden-layer model and 0.758 (95% CI: 0.693-0.823) for the four-hidden-layer model.

Conclusion: This study demonstrates the use of ML in predicting PNI in hepatobiliary cancers, with LR, GB, and RF models achieving acceptable performance. These models can improve disease management and evaluation, allowing for personalized treatment strategies by predicting tumor aggressiveness and invasive potential.

#5024 Multiomics signature reveals network regulatory mechanisms in colorectal cancer progression: Going beyond the scope.

Juan Carlos Higareda-Almaraz[1], **Francesco Mattia Mancuso**[1], Pol Canal-Noguer[1], Kristi Kruusmaa[2], Arianna Bertossi[2]

[1]Universal Diagnostics SA, Seville, Spain,[2]Universal Diagnostics d.o.o., Ljubljana, Slovenia

Background: Colorectal cancer (CRC) progresses from normal tissue to advanced precancerous lesions (APLs) and ultimately to malignancy, driven by stepwise genetic and epigenetic alterations. A key challenge in managing CRC is the risk of overdiagnosis in APLs, which can lead to unnecessary interventions. Identifying molecular signatures that accurately distinguish between low-grade dysplasia (LGD) and high-grade dysplasia (HGD) is crucial for optimizing risk assessment and clinical decision-making. This study integrates methylation, gene expression, and mutation analyses to uncover transcription factor (TF)-driven regulatory networks, with the aim of developing signatures that could support risk-optimization scores and reduce diagnostic uncertainty.
Methods: A differentially methylated signature (DMS), previously identified from HGD and LGD samples using EM-seq and bioinformatics, was applied to the TCGA CRC methylation dataset. Unsupervised hierarchical clustering based on this DMS was performed to identify methylation-driven clusters, followed by a survival and a mutational burden analysis to assess potential associations between clusters and patient outcomes. Cluster-dependent regulatory patterns were used to construct transcription factor (TF) target networks, with hub TFs identified through network topology analysis. Functional enrichment analyses, including Gene Ontology (GO) and pathway analyses, of hub TF target genes were conducted to reveal biological processes specific to each cluster.
Results: The DMS effectively distinguished 4 Clusters (CLs), one with Normal samples and 3 tumoral CLs. A continuum in methylation patterns correlated with mutational burden ($p = 7.2e-4$) and patient survival probability, with Tumoral CL1 showing the highest impact compared to Tumoral CL3 ($p = 2.9e-2$). TF enrichment and networks analysis revealed by variations in network topology metrics ($p = 4.2e-10$), shows unique regulatory networks CLs-dependent. Functional enrichment analysis highlighted cluster-specific pathways, such as PI3K-Akt and AMPK signaling, besides cancer specific pathways in CL1; and JAK-STAT, TNF, and IL-17 signaling in Cl3.
Conclusions: This multiomics analysis reveals heterogeneity in CRC by identifying stratified regulatory mechanisms and biological processes driving progression from APLs to different CRC subtypes. By integrating these insights into risk-optimization scores, the identified signatures could enhance diagnostic accuracy, mitigate overdiagnosis, and refine patient management strategies. These findings pave the way for the development of precision diagnostic tools that optimize clinical decision-making while minimizing unnecessary interventions. Further validation is required to translate these signatures into robust clinical applications.

#5025 Applying deep learning transformers and expression quantitative trait loci to interrogate immunogenetic determinants of cancer risk.

Sophie Herszterg[1], Gustavo Arango-Argoty[2], Etai Jacob[2]

[1]Oncology Data Science, Oncology R&D, Astrazeneca PLC, Cambridge, United Kingdom,[2]Oncology Data Science, Oncology R&D, Astrazeneca PLC, Waltham, MA

The immune system plays a pivotal role in suppressing cancer development through a process known as cancer immunosurveillance. However, substantial immune variation among healthy individuals suggests that the effectiveness of the immune system in eliminating cancer is very likely to vary. This study aims to identify genetic immune characteristics that either protect against or increase cancer risk and to understand how these immune factors interact with known environmental and lifestyle-related risk factors. To achieve this, we have employed a deep learning approach, leveraging transformer models. Transformers can capture complex, non-linear interactions between features, accommodate multimodal input data and incorporate prior knowledge through self-supervised pre-training, which are all relevant properties for our application. As a discovery dataset, we have utilized the UK Biobank, a comprehensive longitudinal cohort consisting of half a million individuals with over 15 years of follow-up. Immune characteristics were derived from plasma proteomics as well as whole genomic sequencing data, in the form of immune cell-specific expression quantitative trait loci (eQTLs). Immune-cell specific eQTLs have been associated with multiple diseases and can provide mechanistic insight into the genetic basis of cancer risk. Here, we share our analysis in exploring immunogenetic factors modulating cancer risk by integrating advanced deep learning techniques with extensive genomic and environmental data. We hope this work will contribute to enhancing our understanding of individual susceptibility to cancer and thereby inform risk stratification strategies and personalized approaches to cancer prevention and intervention, leading to better health outcomes.

#5026 Discovery of oncogenic mediator genes in rectal cancer chemotherapy response using gene expression data from matched tumor and patient-derived organoids.

Hanchen Huang[1], Wini Zambare[2], Chao Wu[2], Antonio Colaprico[1], Janet Alvarez[2], Min Jung Kim[2], Aron Bercz[2], Paulina Bleu[2], Lily Wang[1], Philip B. Paty[2], Paul B. Romesser[3], J. Joshua Smith[2], X. Steven Chen[1]

[1]Department of Public Health Sciences, University of Miami Miller School of Medicine, Miami, FL,[2]Colorectal Service, Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY,[3]Department of Radiation Oncology, Colorectal and Anal Cancer Service, Memorial Sloan Kettering Cancer Center, New York, NY

Rectal cancer (RC) remains a significant clinical challenge, in part due to chemotherapy resistance encountered in neoadjuvant treatment approaches. To address this, we utilized a unique platform integrating gene expression data from RC tumor tissues and matched patient-derived organoids (PDO). Gene expression data were analyzed using the computational tool Moonlight to investigate the molecular mechanisms underlying chemotherapy response. We hypothesized that combining genomic data from RC PDOs and matched tumors would implicate molecular targets predicting chemotherapy response and clinical outcomes better than targets identified using RC tumor tissue or RC PDO input alone. We analyzed 18 tissue samples and 32 matched PDOs, capturing a comprehensive representation of tumor biology. Our integrative approach involved differential expression analyses (DEAs) and gene regulatory network (GRN) studies, identifying 5,199 genes regulating at least one potential regulon. Focusing on cancer-associated biological processes curated in Moonlight, we highlighted 2,118 regulator-regulon pairs with potential roles in oncogenic processes. Further integration with DEA results revealed 334 regulator-regulon pairs significantly enriched in both tissue and PDO samples, designating these regulators as oncogenic mediators (OMs). Among them, five genes—BTK, CDK1, LAX1, NAT2, and RAD51AP1—were associated with FOLFOX response. Survival analysis across four independent cohorts further validated CDK1, LAX1, NAT2, and RAD51AP1 as indicators of prolonged recurrence-free survival (RFS), demonstrating consistent patterns associated with good prognosis. These findings provide new insights into the molecular mechanisms of chemotherapy response in RC, and validation of these targets is ongoing. Our integrated approach underscores the translational relevance of PDOs and demonstrates the utility of Moonlight in identifying potential therapeutic targets, setting a new benchmark for precision medicine in rectal cancer.

BIOINFORMATICS / COMPUTATIONAL BIOLOGY / SYSTEMS BIOLOGY / CONVERGENT SCIENCE: Sequence Analysis and Genome Landscapes
Poster Session

#5031 Analyzing *CHEK2*'s role in breast cancer via *CHEK2*-specific genomic profiles.

Shuoying Qu[1], Marcel Smid[1], John Martens[1], Antoinette Hollestelle[2]

[1]Erasmus Medical Center, Rotterdam, Netherlands,[2]Amsterdam UMC, Amsterdam, Netherlands

Background: *CHEK2* is a tumor suppressor gene in multiple types of cancers, notably estrogen receptor-positive (ER+) breast cancer (BC). We previously revealed that *CHEK2*-derived BC genomes have specific genomic features that are dissimilar from *BRCA1/2*-initiated BC genomes suggesting a different underlying biology. In the study, we aimed to identify estrogen receptor-positive (ER+) samples that are wild-type for CHEK2 but which exhibit similar properties to *CHEK2* mutated tumors to reveal additional genetic drivers of this phenotype.
Methods: Using whole genome sequencing data of primary and metastatic BC from different subtypes, we categorized the size distribution of structural variants (SVs) such as inversions (INV), tandem duplications (DUP), and deletions (DEL) separately, and established *CHEK2*-specific SV profiles. We calculated cosine similarity (CS) scores for all samples comparing the SV profile of a sample to that of the baseline SV profile of *CHEK2* samples. We labeled ER+ samples without *CHEK2* mutation as ER+ *CHEK2*-like samples if their CS scores were higher than the average + 1 SD of the actual *CHEK2* samples. We also studied the location of SVs, defined CHEK2-specific sizes of SVs, and investigated the genes with SVs of these sizes. Lastly, we compared germline mutation frequencies of ER+ *CHEK2*-like and ER+ *CHEK2*-unlike tumors.
Results: The SV size distributions of 926 primary BCs were established for INV, DUP, and DELs. The INV SV profile of *CHEK2* tumors proved very similar in all ER+ tumors and was not used in the classification. Using the CS scores of ER+ tumors to the *CHEK2*-specific DUP and DEL size distributions, 31 and 47 samples, respectively, were classified as ER+ *CHEK2*-like tumors. Interestingly, DUPs in *CHEK2* and ER+ *CHEK2*-like tumors were localized, mainly on recurrently affected chromosomes 11 and 17 for ER+ BC. However, no regions or genes specific for *CHEK2* tumors were observed in the ER+ *CHEK2*-like tumors compared to ER+ *CHEK2*-unlike tumors. Evaluating germline genetic variations in ER+ *CHEK2*-like and -unlike tumors, we found 7 genes, *EBLN2, TAAR2, FAM166B, PNPLA7, IL1RAPL1, ABCC12, and SLC23A2*, with increased mutation frequency in ER+ CHEK2-likes ($p<0.05$), but none of them remained significant after correction for multiple testing.
Conclusion: Our method of selecting ER+ samples with similar SV profiles as *CHEK2* tumors appeared successful and will likely facilitate the identification of potential *CHEK2*-associated pathways. Tandem duplications in *CHEK2 and CHEK2*-like cancers mapped to specific chromosomes but did not pin-point recurrent genes. Only four potential genes with increased germline mutation frequency in ER+ CHEK2-likes (*TAAR2, PNPLA7, ABCC12, SLC23A2*) have been associated in literature with BC or its subtypes, but none of them connected to *CHEK2*-related biology.

#5032 Discovery of XPO1 as a therapeutic target in TNBC: Mechanistic insights and preclinical validation.

A. L. Paulson, R. F. Gruener, A. M. Lee, R. Huang;
University of Minnesota, Minneapolis, MN

BACKGROUND: Triple-negative breast cancer (TNBC) is an aggressive form of breast cancer with limited treatment options. The nuclear export protein XPO1 has emerged as a potential therapeutic target in cancer, but its role in TNBC hasn't been fully characterized.
OBJECTIVES: This study screens, nominates, and investigates the potential of repurposing selinexor, an FDA-approved XPO1 inhibitor, as a novel therapeutic option for TNBC.
METHODS: We utilized a computational drug repurposing pipeline to predict breast cancer patient tumor responses to hundreds of drugs. We then stratified patient tumor's predicted sensitivities by breast cancer subtype and focused on drugs preferentially sensitive towards TNBC. We identified XPO1 inhibitors as the top candidate drug and its efficacy validated in an independent patient dataset and across various TNBC cell line. Additionally, we conducted RNA-sequencing after longitudinal XPO1 inhibition in a panel of TNBC cell lines to explore the mechanism contributing to the preferential sensitivity of TNBC cells to XPO1-inhibition mediated cell death. Further mechanistic studies were conducted to validate our RNA-sequencing findings.
RESULTS: Selinexor was found to significantly reduce the viability of a variety of TNBC cell lines. Mechanistically, we reveal that selinexor induces TNBC cell death by inhibiting the NF-kB pathway through nuclear retention of *NFKBIA*, a key regulator of this pathway. This effect was consistent across multiple TNBC cell lines of differing TNBC subtypes, confirming the broad applicability of XPO1 inhibition in TNBC treatment.
CONCLUSION: This study identifies XPO1 inhibitors as a promising therapeutic candidate for TNBC patients by inhibiting the XPO1-mediated export of *NFKBIA* and blocking the NF-kB pathway. These findings pave the way for more personalized treatment strategies and provide a potential alternative to conventional chemotherapy.
IMPLICATIONS FOR PRACTICE: Repurposing selinexor for the treatment of TNBC could accelerate the availability of targeted therapies for patients with this aggressive cancer. New mechanistic insight into the causes leading to TNBC cell specific sensitivity to XPO1-inhibition mediated cell death warrant further clinical trials to evaluate the safety and efficacy of selinexor in TNBC patients.

#5033 Secondary findings from genome sequencing are prevalent in pediatric cancer patients.

Safa Majeed Grant[1], Brianne Laverty[1], Stephenie Prokopec[2], Christian Marshall[3], Trevor Pugh[2], Adam Shlien[1], Anita Villani[4], Stephen Scherer[1], Yvonne Bombard[5], David Malkin[1]

[1]Genetics and Genome Biology, The Hospital for Sick Children, Toronto, ON, Canada,[2]Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada,[3]Division of Genome Diagnostics, Department of Pediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, ON, Canada,[4]Division of Hematology/Oncology, The Hospital for Sick Children, Toronto, ON, Canada,[5]Institute of Health Policy, Management, and Evaluation, University of Toronto, Toronto, ON, Canada

Purpose: Genome sequencing in cancer care can reveal secondary findings (SFs) - genetic variations unrelated to the primary condition - yielding potential impacts on patient health and reproductive decision-making. While the American College of Medical Genetics (ACMG) recommends investigating a specific list of actionable SFs during clinical diagnostic sequencing, the prevalence of these findings in pediatric patients with cancer remains unknown. Germline genome sequencing is becoming standard in cancer care, yet no evidence exists to guide providers on the frequency and clinical relevance of SFs in pediatrics.
Methods: To understand the landscape of SFs in pediatric cancer, we analyzed germline variants from genome sequencing across pediatric patients from the SickKids Cancer Sequencing Program (KiCS) and the Canadian COVID-19 Genomics Network (HostSeq). Germline pathogenic (P) and likely-pathogenic (LP) variants with allele frequencies <0.03 in gnomAD populations were considered SFs (including carrier status). Variants associated with primary clinical indications were removed. We compared the prevalence of SFs (including ACMG SF v3.2) across cancer and non-cancer populations of diverse genetic ancestry to inform clinical decision-making in pediatrics.
Results: The median age of KiCS participants (n=396) was 15 years (SD=10.6), compared to 7 years for HostSeq participants (SD=5.3, n=158), with 55% and 56% male participants respectively. Cancer diagnoses for KiCS participants included 51 different tumor types. On average, KiCS patients had 3.96 P or LP SFs from germline genome sequencing, similar to the HostSeq COVID-19 cohort (3.73). Across the combined cohort (n=454), P and LP variants were most common in CFTR (n=39), ABCA4 (n=32), NPHS2 (n=32), FLG (n=31) and BCHE (n=21) genes. Moreover, 5.7% of participants across cohorts had ≥1 clinically actionable ACMG SF (36 patients, n=454). Hereditary breast and ovarian cancer were the most common ACMG conditions identified from ACMG SFs in the Hostseq cohort.
Significance: Our study advances the understanding of germline variants in pediatric oncology, highlighting the potential SFs can have on cancer care in pediatrics. Ongoing work in our lab will categorize SFs by their inheritance model and reverse phenotype autosomal dominant conditions. The findings from our study will help providers understand what SFs to expect when their patients undergo genome sequencing and their clinical significance.

#5034 Comparative whole genome sequencing between matched cryopreserved and FFPE tumor samples.

Paytin Curran[1], DeLaney Anderson[1], Jeffrey Okojie[1], Ryan Miller[1], Sangmoon Lee[2], Ken Dixon[3], Jared James Barrott[1]

[1]Brigham Young University, Provo, UT,[2]Inocras, San Diego, CA,[3]Specicare, Gainesville, GA

Background & Aims: Next generation sequencing, specifically whole genome sequencing (WGS), plays a key role in precision medicine. Currently, the main source of cancer biospecimens for WGS is formalin-fixed, paraffin-embedded (FFPE) tissues. This is partly because cryopreserved (CP) tissues have not been routinely stored in real-world clinical settings. This study aimed to compare the outcomes of WGS by using matched CP and FFPE samples. We hypothesized that CP samples would demonstrate superior quality metrics before and after sequencing the DNA.

Methods: A total of 50 matched pairs of CP and FFPE tumor samples were obtained from patients with various types of cancers, primarily representing gynecological cancers. DNA was extracted and quality and quantity were determined. WGS was performed using 200 ng of DNA extracted from each sample. We conducted a comparison between CP and FFPE samples in terms of the sequencing quality control metrics and the number of identified variants.

Results: Our samples consisted of 18 ovary, 5 liver, 6 kidney, 7 uterus, 4 colon, and 10 other cancers. Metrics were obtained before sequencing, and it was found that gDNA concentration in ng/ul was significantly higher in CP (85.2) than FFPE (12.5) tissue. Additionally, DIN number (FFPE: 4.7; CP: 8.4) and fragment size in base pairs (FFPE: 444.1; CP: 644.6) were also significantly higher in CP tissue. WGS was then performed, and it was found that both mean read depth (FFPE: 34.6; CP: 54.2) and insert size (FFPE: 243.6 CP: 365.7) were significantly higher in CP than FFPE tissues. The percentage mapped over 15x was similar and not statistically different with FFPE being 99.3% and CP being 99.7%. To determine concordance between the matched samples, 94,147 variants were found in FFPE, and 80,036 variants were found in CP. The median point mutation overlap (VAP>=5.0%) was used to find a concordance percentage, with that being 43.5% between FFPE and CP tissues (shared: 51,619; FFPE: 38,690; CP:28,434). Tumer mutation burden was determined on 8 samples because germ line sequencing was performed on available blood samples. FFPE had a TMB of 13.7 and CP exhibited a TMB of 6.4, demonstrating a statistical difference with a p-value = 0.02. Lastly, structural variance was evaluated and FFPE averaged 21 and CP averaged 38.

Conclusion: WGS utilizing CP samples provides superior quality indices compared to FFPE samples. Despite matched samples, there was significant discordance in variants identified in the cancers. Therefore, CP sample collection should be prioritized when performing WGS.

#5035 Barcode-free demultiplexing of HiFi reads using natural genetic variation.

Deniz Guney Olgun[1], Simai Wang[2], Saeid Parast[3], Ali Shilatifard[3], Hani Goodarzi[4], Vijay Ramani[2]

[1]University of Virginia, Charlottesville, VA,[2]Gladstone Institute for Data Science and Biotechnology, San Francisco, CA,[3]Department of Biochemistry and Molecular Genetics, Northwestern University Feinberg School of Medicine, Chicago, IL,[4]Arc Institute, Palo Alto, CA

Cleverly exploiting the natural genetic variation among cells from unrelated individuals enables high throughput, highly parallel droplet single-cell RNA sequencing. We sought to lift this principle and bring analogous improvements to the setting of single-molecule long read sequencing. Toward this end, we present an error-robust method for matching individual long genomic reads to their genetic background based on a Naive Bayes Classifier. As a case study, we apply this to explore allele-specific splicing variation in the colorectal adenocarcinoma cell line DLD1 using Iso-Seq long-read RNA sequencing. Surprisingly, we uncover many allele differentially-expressed isoforms even across genes with no allele-specific expression bias in aggregate. Further, we investigate RNA-binding proteins and functional pathways potentially involved in mediating this allele-specific isoform expression pattern. In future work, our method will be applied to pooled single-molecule epigenomic screens of cancer cell line responses to treatment.

#5036 CAPTATe: a novel tool for enhanced artifact filtering and precise variant detection in single-cell DNA sequencing data.

Luigi Ferraro, Fulvio D'Angelo, Anna Lasorella, Antonio Iavarone, Michele Ceccarelli

Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine, Miami, FL

Bulk sequencing has proven inadequate in capturing the full complexity of cancer heterogeneity, shifting focus toward cellular resolution. Single-cell DNA sequencing (scDNA-seq) technologies offer an effective approach for analyzing subclonal molecular architecture of tumor with greater granularity, facilitating the identification of somatic genetic mutations (single nucleotide variants, SNVs, and short indels) and copy number variants (CNVs) at single-cell resolution. This capability holds multiple promises, such as the characterization of timing and hierarchy of molecular events driving tumor initiation and progression. However, traditional scDNA-seq methods are hindered by significant artifacts during genome amplification, causing issues like allelic dropout and imbalance that compromise variant detection accuracy. The introduction of the Primary Template-directed Amplification (PTA) addressed these limitations by providing higher genome coverage and uniformity; however, further refinement in filtering amplification-induced artifacts remains necessary for accurate variant calling.
Here, we present CAPTATe (Characterize Artifacts in PTA Technology), a novel tool developed for improved artifact filtering and enhanced detection of SNVs, indels, and CNVs in scDNA-seq data. CAPTATe employs allele-specific CNV calling by addressing fluctuations in the read-depth ratio (RDR) through a multi-channel joint segmentation approach at the subclonal level. The tool leveraged an extension of the variational framework recently developed for single-cell RNA-seq (de Falco et al. Nature Communications 2023) and integrates both B-allele frequency (BAF) and RDR for accurate CNV detection. Additionally, SNV and indel artifact filtering are performed through a machine learning-based approach. This model accounts for allelic imbalance by evaluating the ratio between observed variant allele frequency (VAF) and expected BAF. Genetic tumor subclones can be accurately identified by clustering cells based on their genomic alteration profiles.
The increased precision and accuracy in single-cell genomic variant identification using CAPTATe overcome the main limitations of PTA scDNA-seq profiling, enabling precise characterization of genetic tumor heterogeneity at individual cell resolution.

#5037 Accelerated somatic mutation calling for whole-exome sequencing of tumor samples.

Jessica Castrillon Lal[1], Shuangxi Ji[2], Ruonan Li[2], Scott Kopetz[1], Wenyi Wang[2]

[1]Gastrointestinal Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX,[2]Department of Bioinformatics and Computational Biology, The University of Texas MD Anderson Cancer Center, Houston, TX

Background: Integrating next-generation sequencing (NGS) data into clinical practice faces several challenges. High-throughput sequencing generates massive amounts of data that need to be processed quickly to deliver timely clinical insights. Additionally, formalin-fixed paraffin embedded (FFPE) tissue is the primary resource for tumor molecular profiling in clinical settings. However, nucleic acids extracted from FFPE are prone to chemical alterations and technical artifacts during sequencing. Therefore, distinguishing tumor-specific variants from artifacts and germline variation with a low false discovery rate rapidly with high accuracy remains challenging.
Methods: We recently developed MuSE2, an advanced somatic mutation caller for cancer genomics that integrates a nucleotide-specific Markov substitution model for molecular evolution, a tumor-specific error model to determine tier-based cutoffs for selecting SNVs, and an optimized multi-threaded processing pipeline. It was demonstrated significantly enhanced accuracy and up to 8-80 folds faster performance compared to other common variant callers. Next, we evaluated the performance of our variant calling pipeline on FFPE derived whole exome sequencing data as part of a liquid biopsy test from patients with colorectal cancer at MD Anderson (n=808). We benchmarked our consensus calls from two out of three of the following callers, MuSE2, Strelka2, and MuTecT2, using a patient matched Medical Diagnostic Lab genetic panel sequencing as the ground truth. Our consensus calls performed well in both panel 1 (n=126 genes, F1=0.726) and panel 2 (n=146 genes, F1=0.773).
Results: MuSE2 achieves high sensitivity when combined with existing callers for both whole exome sequencing (WES, F1=0.88 0.04) and whole genome sequencing (WGS, F1=0.93 0.02). Furthermore, the calls had optimal performance when benchmarking the top driver genes in colorectal cancer (TP53, F1=0.95, KRAS, F1=0.99, PIK3CA, F1=0.74, and BRAF, F1=0.88). However, for less frequent genes the performance drops indicating additional measures are required to remove potential technical artifacts.
Conclusion: Our results suggest that a consensus mutation calling strategy including MuSE2 achieves high efficiency without compromising accuracy for clinical tumor sequencing. This is an urgent matter as genetic screening is considered a fundamental aspect of translational cancer research and is entering cancer care standard practice.
2,134 characters (max allowed = 2,600)

#5038 Study on acquisition timing of *KRAS* mutations in the pancreatic ductal epithelium.

Tomonori Hirano[1], Nobuyuki Kakiuchi[1], Yasuhide Takeuchi[1], Sachiko Minamiguchi[2], Takayuki Anazawa[1], Kazuyuki Nagai[1], Hiroshi Seno[1], Seishi Ogawa[1]

[1]Kyoto Univ. Graduate School of Medicine, Kyoto, Japan,[2]Fujita Health University, Aichi, Japan

Introduction: Pancreatic cancer is a highly aggressive malignancy with a poor prognosis, emphasizing the urgent need for early detection to improve outcomes. In the multistep carcinogenesis of pancreatic cancer, *KRAS* mutations are acquired at the earliest stage. However, the exact timing of acquiring *KRAS* mutations in the pancreatic ductal epithelium remains insufficiently understood.
Methods: Non-cancerous regions of the pancreas, distant from macroscopic tumors, were obtained from surgical specimens. The main pancreatic ducts were isolated, spread open manually, and excised into pieces ~1 mm in size to create pancreatic duct organoids. DNA was extracted and subjected to whole-exome sequencing (WES) with matched control DNA. Phylogenetic trees were reconstructed based on shared mutations. Single-cell-derived organoids were established from some bulk organoids and analyzed via WES to determine the mutation accumulation rate of pancreatic ductal epithelium. The timing of *KRAS* mutation acquisition was estimated based on the mutation rates and the height of the branches to which *KRAS* mutations were allocated.
Results: A total of 430 organoid samples were analyzed from 25 pancreatic cancer patients and 23 subjects without pancreatic cancer (1-21 samples per case; median: 8). A median of 33 mutations per sample (range: 8-141) was identified. 18 *KRAS* mutations were detected in 16 cancer patients, while 4 mutations in 4 non-cancer subjects, suggesting a higher prevalence of *KRAS*-mutated clones in the pancreatic ductal epithelium of cancer patients. In the phylogenetic analysis, 13 out of 22 *KRAS* mutations were allocated to the trunk, and KRAS mutant clones expanded to 6 samples on average (2-20). In contrast, *KRAS*-negative clones rarely expanded across multiple samples. We analyzed 56 single-cell-derived organoids from 19 cases, including 7 *KRAS* mutant cells. *KRAS* wild-type cells showed a positive correlation between mutation number and age, with a rate of 0.29 mutations per exome per year. *KRAS* mutant cells displayed a significantly higher mutation number compared to *KRAS* wild-type cells, suggesting an increased mutation rate of *KRAS* mutant cells. *KRAS* mutations were acquired at various ages (21-44), with some occurring as early as the early 20s.
Conclusions: In this study, we elucidated the evolutionary history of clones in non-cancerous pancreatic ductal epithelium. Our findings will contribute to a better understanding of early events of pancreatic carcinogenesis.

#5039 Machine learning enhances histology-agnostic detection of PTEN deletions in panel sequencing of advanced cancers.

Irina A. Kerle[1], Oleksandr Husak[2], Thomas Gross[3], Andreas Hartig[4], Daniela Richter[1], Sascha Bruckmann[4], Andreas Mock[5], Anja Kogler[3], Rebecca Prause[3], Alexander A. Wurm[1], **Maximilian Werner**[1], Dorothea Hanf[1], Lino Mohrmann[1], Doreen William[6], Daniela E. Aust[4], Evelin Schrock[6], Christoph Heining[1], Hanno Glimm[1]

[1]Translational Medical Oncology, National Center for Tumor Diseases Dresden, Dresden, Germany,[2]Institute of Software and Multimedia Technology, Dresden, Germany,[3]Core Unit for Molecular Tumor Diagnostics (CMTD), National Center for Tumor Diseases Dresden, Dresden, Germany,[4]Institute of Pathology, Carl Gustav Carus University Hospital, Dresden, Germany,[5]Translational Medical Oncology, Institute of Pathology, Ludwig-Maximilians-Universitat Munchen, Munich, Germany,[6]Institute for Clinical Genetics, University Hospital Carl Gustav Carus at TU Dresden, Dresden, Germany

Targeted panel sequencing of known genetic driver alterations is an established part of the initial clinical diagnostic routine for many tumors. However, exploratory sequencing for advanced and rare cancers remains experimental. MASTERsg is a prospective clinical broad panel sequencing program that utilizes the TSO500 and TST170 DNA/RNA sequencing assays to identify therapeutically relevant alterations in patients with advanced cancers. The TSO500 pipeline detects copy number variations (CNVs) by dividing the CNV caller's raw data into gene-specific bins, each consisting of 50-250 base pairs. This approach enables the detection of both amplifications and deletions through bin count averaging. However, it may miss focal CNVs that do not meet the standard caller's threshold fold change. This study aimed to enhance the CNV calling capabilities of the TSO500 pipeline to include focal CNVs. To achieve this, we developed a graphical representation of each gene's bins through plotting, enabling the visual identification of focal CNVs. Additionally, we sought to automate this identification process using machine learning, exemplified by detecting focal deletions of Phosphatase and Tensin homolog (PTEN), a tumor suppressor gene whose functional loss often serves as the basis for recommending mTOR/Akt inhibition in molecular tumor boards. For this purpose, we implemented an Extreme Gradient Boosting (XGBoost) model utilizing normalized PTEN bin counts, enhanced with feature transformations such as polynomial line fitting and derivative to capture read depth variations. Our dataset included 59 patients representing 20 advanced solid tumor entities: 29 PTEN deletions identified by the TSO500 pipeline with a gene-specific fold change cutoff of <0.65 (FC range: 0.05-0.64), five focal PTEN deletions detected only by visual bin plot analysis (FC range: 0.66-1.051), and 25 wildtype cases (FC range: 0.65-1.096). A balanced test set of 16 patients was selected for evaluation. To verify the results, all sequenced tumor samples underwent PTEN immunohistochemical (IHC) staining, with staining results quantified using the H-Score, which ranges from 0 (completely negative) to 300 (strongly positive).Our best-performing model achieved a Receiver Operating Characteristic Area Under the Curve (ROC AUC) of 0.97, accurately identifying all five focal PTEN deletions missed by the TSO500 pipeline. The biological relevance of these deletions was confirmed by IHC, with H-Scores of 0 in 4/5 cases and 35 in 1/5 cases. This study demonstrates that machine learning can enhance PTEN deletion detection in TSO500 panel sequencing, with potential applications for other genes. Prospective validation on additional tumor samples is currently ongoing.

#5040 Transformer-based foundation model for clinically relevant DNA alterations: Filling gene status gaps and enhancing classification of rare cancer types.

Dmitrii Chebanov, Quaid Morris

Computational and Systems Biology, Memorial Sloan Kettering Cancer Center, New York, NY

Differences in target panels, data collection strategies, and in data processing among clinical genomic profiling platforms, including MSK-IMPACT and FoundationOne, results in incomplete and incompatible data. For example, other GENIE panels lack the comprehensive genomic features provided by IMPACT. To address this challenge, we developed a Transformer-based foundation model, called CanBART, that generates “plausible patients” to harmonize patient profiles collected on different platforms. To illustrate the value of these generated patients, we adapted the pre-trained CanBART model for a tumor classification task to predict cancer types across 39 tumor types. The results demonstrated strong performance, showing that these plausible patients significantly enhance the robustness of classification, particularly for underrepresented cancer types.

CanBART leverages a BART-based transformer architecture to predict missing genomic data. Patient profiles were represented in a “sentence-like” format, for which they were tokenized using a word-level approach and sorted by chromosomal location, capturing clinically relevant alterations, including mutations, hotspots, copy number variations, and structural variants. We used genomic data from the MSK-IMPACT and GENIE databases, encompassing a total of 144,000 patient profiles. Missing gene statuses were addressed by generating plausible patients, producing a range of possible alterations grouped in profiles that could be sorted by their probability of presence in a particular patient. To validate these profiles, we fine-tuned the model for a classification problem to predict cancer types across the dataset. CanBART was applied to harmonize genomic profiles across patient genomic profiling platforms, producing biologically plausible patient profiles that augmented the dataset for classification tasks. Adding these patients to the training dataset improved tumor-type classification, with a notable 20% enhancement in accuracy in rare cancer predictions compared to using real patient data alone. The enriched dataset demonstrated the benefits of the proposed data representation and transformer-based architecture in addressing the challenges of cancer classification when data is scarce.

CanBART is a Transformer-based foundation model that provides an innovative solution for harmonizing patient genomic profiling platforms and generating plausible patients to train clinical prediction tools. CanBART not only addresses the challenge of incomplete data but also offers a robust method for enhancing tumor classification accuracy, particularly for rare cancers. This approach demonstrates potential for transformative advancements in genomic profiling and precision oncology, facilitating research and clinical decision-making where data scarcity has been a barrier.

#5041 OncoTagger, a novel tool for accelerated and accurate literature-based curation of cancer related genes and variants.

Chenlian Fu[1], Pia Droop[2], Xing Wang[2], Hongxin Zhang[1], Nicole Fernandez[1], Calvin Lu[1], Abhilash Anand[3], Umair Ayub[3], Saahil Jain[4], Kritik Seth[3], Debyani Chakravarty[1], Ulf Leser[2], Quaid Morris[1], Nikolaus Schultz[1]

[1]Memorial Sloan Kettering Cancer Center, New York, NY,[2]Humboldt-Universitat zu Berlin, Berlin, Germany,[3]Center for Data Science, New York University, New York, NY,[4]New York University, New York, NY

Cancer is a genetic disease, and a tremendous number of papers are published each year describing the role of individual genes and specific genomic variants in different cancer types, on cancer progression, and response to therapy. Databases such as OncoKB, COSMIC, and ClinVar effectively catalogue these variants. However, building such databases currently requires laborious manual curation, which does not scale to the speed at which new findings are published: curators need to first identify relevant papers to identify novel associations, and then read through the selected papers word-by-word to ascertain such associations.

To support and accelerate this process, we developed OncoTagger, a first-of-its-kind natural language processing (NLP) powered tool that identifies cancer-related genes and variants from published abstracts and classifies them according to their oncogenicity. OncoTagger provides an abstract-level summary to identify important papers containing cancer genes and variants. Within each abstract, OncoTagger further provides a word(token)-level assessment, predicting words that are cancer genes or variants and their oncogenicity to facilitate curation.

OncoTagger consists of two BioMedBert models finetuned and evaluated on OncoKB's cancer biomarker summary data (3381 annotated abstracts with 22219 mentions of cancer genes, and 1291 abstracts with 3446 cancer variant mentions). One model performs the gene classification task: a token can be assigned to be (part of) the name of an oncogene, tumor suppressor gene, both, or none of the above; the other model carries out similar classifications for variants.

To evaluate abstract summary accuracy, we determined that OncoTagger's most frequently predicted class for each known cancer gene and variant token across the held-out test abstracts achieved 95.2% and 82.1% sensitivity, respectively. We then evaluated accuracy on the task of token classification. OncoTagger significantly outperformed LSTM and key-term matching models in identifying cancer genes and variants (overall accuracy 97.4%/99.4%, F1=72.3%/73.6%, 5.8pp/5.1pp better than the second-best model). OncoTagger achieved high sensitivity in detecting never-seen-before genes and variants (67.7%, 83.3% in oncogenes and tumor suppressor genes, respectively; 60.2% in oncogenic variants). Manual reviews of OncoTagger predictions are ongoing to further confirm OncoTagger's ability to discover new cancer genes and variants currently not included in OncoKB.

In summary, the NLP-powered OncoTagger tool accurately extracts cancer biomarkers. The scaled application of OncoTagger will help identify and curate cancer driver genes and variants from the literature, accelerating the translation of research into clinically implementable targets.

#5042 The Paipu pipeline for pan-mammalian cancer genomics.

Bria S. Smith[1], Geesa V. Daluwatumulle[1], Leslie A. Smith[1], James A. Cahill[2], Kiley Graim[1]

[1]Computer and Information Science and Engineering, University of Florida, Gainesville, FL,[2]Environmental Engineering Sciences, University of Florida, Gainesville, FL

The emerging field of comparative oncology may provide a novel solution to rare human cancer studies, as genomic resources, reference genomes, and non-human model cancer sequencing data are proliferating rapidly. Studying cancer across a diverse array of species provides a unique opportunity to interrogate factors underpinning cancer, facilitating disease modeling in the setting of spontaneous tumors complicated by comorbidities and metastases. Unfortunately, spontaneously developed tumors in non-human mammalian models have been largely overlooked, leaving a foundational resource underutilized. Several key barriers prevent the adoption of these critical data. Extensive reporting of the frequency and diversity of animal tumors has not been systematically linked to the plethora of genomic resources available. Additionally, complications in integrating data from hundreds of genomes have prevented large-scale analysis. Fortunately, recent advances in comparative genomics allowing for joint alignments of hundreds of species have bridged this gap, enabling us to integrate the rapidly expanding genetic resources for non-traditional model species and presenting an enormous potential resource for oncology.

To facilitate the transition of comparative oncology studies from human plus one or two other species to pan-mammalian analyses, we developed the Paipu computational pipeline to systematically identify and process data from hundreds of mammalian species, creating a pan-mammalian tumor compendium. Paipu consists of three phases: reference genome preparation, SRA metadata retrieval and harmonization, and FREYA for RNA-seq data processing and harmonization. Paipu identified and harmonized data from over 100,000 non-human mammalian cancer RNA-seq samples from NCBI, spanning 52 of the 241 mammalian species in the Zoonomia multi-species genome alignment. We demonstrate Paipu's potential via a comparative analysis of human cancers and a rapidly emerging model system, the dog. Using the Paipu cancer data compendium, we analyzed ten cancers in human and dog. We identified a subset of the cancers as strong models of human cancer, which we validated through literature analysis. Additionally, our comparative cancer analysis results parallel the original TCGA pan-cancer analysis findings.

Paipu's harmonization of data from numerous mammalian species facilitates downstream comparative genomics analysis. Increasing the diversity of genomes used in comparative studies can reveal shared and lineage-specific variation across species, leading to the identification of novel mechanisms of cancer risks and resistance. Our approach provides a unique opportunity to harness significant advances in the rapidly developing fields of machine learning and genomics for human cancer research by identifying reciprocal links between evolution and cancer resistance.

#5043 reVUE: repository for variants with unexpected effects.

Xiang Li[1], Ino de Bruijn[1], Thomas Y. Cong[2], Walid Chatila[1], Hongxin Zhang[1], Moriah Nissan[1], Amanda Dhaneshwar[1], Sara E. DiNapoli[3], Erika Gedvilaite[1], Bryan Lai[1], Selcuk Onur Sumer[1], Aditi Gopalan[4], Tonatiuh Gonzalez[5], Madelaine Rangel[6], Trevor J. Pugh[7], Rose Brannon[1], Michael F. Berger[1], Jianjiong Gao[1], Nikolaus Schultz[1], Debyani Chakravarty[1]

[1]Memorial Sloan Kettering Cancer Center, New York, NY,[2]Massachusetts Institute of Technology, Cambridge, MA,[3]The Melanoma Research Alliance, Washington, DC,[4]Sage Bionetworks, Seattle, WA,[5]Joan & Sanford I. Weill Medical College of Cornell University, New York, NY,[6]Cornell University, Ithaca, NY,[7]Ontario Institute for Cancer Research, Toronto, ON, Canada

Clinical sequencing of tumor samples is now a component of routine cancer care. By identifying genomic alterations that contribute to tumor initiation or progression, clinical cancer genomic sequencing may be used to identify predictive biomarkers of drug response, refine patient cancer diagnoses, assess heritable cancer risk, or inform patient prognosis. Most genomic alterations are accurately annotated with tools such as the Variant Effect Predictor (VEP) that infer the effects of these alterations on the mRNA and protein by following basic rules of transcription, mRNA post-transcriptional processing, and translation. However, a select subset of variants' effects cannot be predicted as easily by these rules. While many of these "variants with unexpected effects (VUE)" are functionally characterized and documented in the literature**,** these VUEs are often mis-annotated during routine clinical cancer genomic sequencing. Importantly, certain VUEs may have therapeutic implications, which, if mis-annotated may lead to suboptimal treatment decisions for individual patients with cancer.

To address this unmet clinical need, we created a centralized database resource, the **re**pository for **V**ariants with **U**nexpected **E**ffects (reVUE - cancerrevue.org), which curates and programmatically stores VUEs to enable the annotation of these variants during routine clinical cancer genomic sequencing. The reVUE resource consists of (1) an intuitive website listing curated VUEs with their observed effects as demonstrated by functional characterization in peer-reviewed literature and (2) an application programming interface (API) for programmatic annotation of variants.

We successfully curated 109 VUEs spanning 22 genes from 31 articles, and continue to expand this database. Several curated VUEs were associated with clinical treatment implications, including *KIT*, *MET*, *ATM*, *EGFR*, and *BRCA1/2.* The reVUE database has also been integrated into the publicly available bioinformatic ecosystem of cancer variant annotation and interpretation tools that currently includes Genome Nexus, OncoKB, and cBioPortal.

By addressing the critical challenge of the accurate annotation of genomic variants with unanticipated protein effects, reVUE enhances our understanding of complex variant interpretation and contributes directly to improved patient care. Notably, the software and all annotated variants are publicly available, allowing for community contributions, and enabling seamless integration into other genomics tools, clinical workflows, and research pipelines.

#5044 Whole-genome investigation of transcription factors that promote UV damage and mutation.

Bo Chi[1], Raluca Gordan[2]

[1]Duke University, Durham, NC,[2]Department of Biostatistics and Bioinformatics, Duke University, Durham, NC

Understanding the spatial distribution of ultraviolet light (UV)-induced damage and mutation across the genome is an important question due to their oncogenic role in melanoma and other cancers. Previous research suggests UV-induced Cyclobutane Pyrimidine Dimer (CPD) damage lesions and mutations are enriched at transcription factor (TF) binding sites, genomic locations where TFs bind to regulate gene expression. However, current studies of the links between observed enrichment and TF activity revolve around individual qualitative studies with a control sample (often naked DNA) representing sequence effects. With many TFs, damaged sites, and other contributory factors, we seek to quantitatively assess the genome-wide relationship between TF activity, damage formation, and mutagenesis. To accomplish this, we designed a distribution-free simulation pipeline to model the baseline effects of the underlying nucleotide sequence on damage and mutation accumulation by connecting TF binding sites, ATAC-seq chromatin accessibility, CPD lesions in cellular and naked DNA, and mutations from melanoma patients. This analysis reveals that CPDs exhibit varying patterns at the binding sites of TFs relative to what would be expected based on sequence context alone. While some TFs show no additional association with CPD damage, others demonstrate either an enrichment or a reduction in CPDs at their binding sites. Specifically, the top TF clusters associated with UV damage enrichment at a position are ETS, NFY, SPI, and MBD2. ETS, in particular, is consistent with previous studies as a suggested damage driver. In contrast, the top TF clusters associated with reduced damage are AP1, CCAAT_CEBP, NR (including NR1H3 and NR1I2), NFY, and IRF. Using a similar simulation approach, we identified that the most damaged positions in most of the top TF clusters had corresponding mutational enrichment. However, NFY, with damage enrichment at a non-mutagenic TT dimer, showed no mutational enrichment. These clusters can serve as a preliminary list for experimental validation of the modulation of UV-induced damage and mutagenesis driven by TF binding. Our findings and further study will reveal novel insights into the role of TFs in DNA damage accumulation and mutagenesis, which may be vital in reducing cancer incidence.

#5045 Cancer multiomics: Whole-genome DNA, methylation, RNA-seq, and direct RNA characterization of cancer genomes using Oxford Nanopore sequencing.

Sergey Aganezov[1], Philipp Rescheneder[2], Aino Jarvelin[3], Sean McKenzie[1], Ella Kennett[3], George Dodd[3], Sissel Juul[1]

[1]Oxford Nanopore Technologies Inc, New York City, NY,[2]Oxford Nanopore Technologies GmbH, Munchen, Germany,[3]Oxford Nanopore Technologies plc, Oxford, United Kingdom

Cancer progression is characterized by the accumulation of both genomic and epigenomic alterations as well as transcription changes, leading to the formation of tumors that are highly heterogeneous. This heterogeneity arises because the tumor consists of a diverse population of cells, each with distinct genomic, epigenomic, and transcriptomic profiles. These differences influence the tumor's behavior, its response to therapy, and its ability to evolve. To better understand the mechanisms driving cancer, it is crucial to adopt a multi-omics approach.

Here we describe an approach that leverages Oxford Nanopore Technologies' sequencing platform to achieve a comprehensive multi-omics analysis, enabling the detection of a wide spectrum of haplotype-specific somatic genomic variants (ranging from single nucleotide changes to large chromosomal rearrangements), epigenetic modifications such as 5mC and 5hmC, chromatin accessibility changes, and detailed transcriptomic profiles. Through both cDNA and direct RNA (dRNA) nanopore sequencing, it captures transcriptional alterations, isoform diversity, and RNA modification landscapes, providing a holistic view of the molecular changes driving cancer progression.

We highlight several notable examples of identified somatic genomic mutations in an example tumor vs matching normal sample, including loss of heterozygosity and exon deletion in PTEN gene, as well as apparent promoter 5mC hypermethylation in the tumor as well as genome wide changes in 5hmC levels. We further explore changes in isoform-level transcript expression with both cDNA and dRNA nanopore reads. We demonstrate high concordance between the two approaches with dRNA reads allowing for additional examination of RNA base modifications, including m6A and pseU. We also illustrate how the treatment with artificially induced 6mA in both normal and tumor DNA samples allows for the identification of differences in chromatin accessibility across the whole genome, while preserving the ability to detect somatic variations using a single dataset.

Lastly, we highlight the ability of Oxford Nanopore sequencing to produce telomere-2-telomere diploid assemblies of the matching normal sample enabling analyses relative to individualized assembled references, plotting a path towards truly personalized cancer genome analysis.

In summary, our approach highlights how Nanopore sequencing facilitates multi-omics analysis, providing a comprehensive characterization of diverse genomic, epigenomic, and transcriptomic alterations in tumor samples using a single platform.

#5046 Three-dimensional genome landscape of primary human cancers.

Y. Zhao[1], K. E. Yost[1], K. L. Hung[1], K. Zhu[2], D. Xu[3], R. Corces[4], S. Shams[1], B. H. Louie[1], S. Sarmashghi[5], L. Sundaram[1], J. Luebeck[2], S. Clarke[3], A. S. Doane[3], J. M. Granja[1], H. Choudhry[6], M. Imieliński[3], A. D. Cherniack[5], E. Khurana[3], V. Bafna[2], I. Felau[7], J. C. Zenklusen[7], P. W. Laird[8], C. Curtis[1], Cancer Genome Atlas Analysis Network, W. J. Greenleaf[1], H. Y. Chang[1];

[1]Stanford University School of Medicine, Stanford, CA, [2]University of California at San Diego, La Jolla, CA, [3]Weill Cornell Medicine, New York, NY, [4]Gladstone Institute, San Francisco, CA, [5]Broad Institute of MIT and Harvard, Cambridge, MA, [6]King Abdulaziz University, Jeddah, Saudi Arabia, [7]NIH, Bethesda, MD, [8]Van Andel Research Institute, Grand Rapids, MI

Genome conformation underlies transcriptional regulation by distal enhancers, and genomic rearrangements in cancer can alter critical regulatory interactions. Here we profiled the 3D genome architecture and enhancer connectome of 69 tumor samples spanning 15 primary human cancer types from The Cancer Genome Atlas (TCGA). We discovered three archetypes of enhancer usage for over one hundred oncogenes across human cancers: static, selective gain, or dynamic rewiring. Integrative analyses revealed the enhancer landscape of non-cancer cells in the tumor microenvironment at genes related to immune escape. Deep whole genome sequencing and enhancer connectome mapping provided accurate detection and validation of diverse structural variants across cancer genomes, and revealed distinct enhancer rewiring consequences from noncoding point mutations, genomic inversions, translocations, and focal amplifications. Extrachromosomal DNA promoted more extensive enhancer rewiring of oncogenes among several types of focal amplification mechanisms. These results suggest a systematic approach to understand genome topology in cancer etiology and therapy.

#5047 Enhancing germline variant calling: Adjustment with tumor samples to filter low-confidence variants from clonal hematopoiesis.

Maria Shumilova, Danil Stupichev, **Gleb Khegai**, Irina Bulusheva, Sevda Nuralieva, Miryam Spektor, Daria Melikhova, Georgy Sagaradze, Michael Goldberg, Alexander Bagaev

BostonGene, Corp., Waltham, MA

Background: High-quality germline variant calling is crucial for accurate genetic reports; yet, certain factors can lead to false identifications. Clonal hematopoiesis (CH), caused by somatic mutations in blood cells, can mimic germline variants, while pseudogenes and mosaic mutations generate low variant allele frequency (VAF) signals that can be misinterpreted. Current methods for addressing these artifacts use only non-tumor samples, limiting their use in clinical settings for generating genetic reports for tumor samples. To expand the capability of germline variant calling, we developed a filtering method to identify and eliminate low-confidence variants from CH, pseudogenes, and mosaicism within both non-tumor and tumor samples.

Methods: We analyzed whole-exome sequencing (WES) data from 2,078 patients (55.2% females and 44.8% males; mean age 58.7 years, range: 6-99) with 175 unique oncological diagnoses. From each patient, a tumor biopsy and non-tumor samples, including whole blood (71.4%), saliva (25.3%), and buccal swabs (3.3%), were obtained. Strelka v2.9.10 was used for variant calling; precise VAF values were calculated using SAMtools mpileup v1.10. The VAF values were compared between tumor and non-tumor samples. Copy number alterations (CNAs) detected with Sequenza v2.1.2 were used to evaluate expected VAF values in tumor samples adjusted for gene dosage. Mappability metrics were estimated using bigwig reference files for hg38 with various read lengths (75bp, 100bp, 150bp, 200bp) and pyBigWig v0.3.22. The binomial distribution model was calculated based on CNAs and sample purity to determine expected VAF range and identify low-confidence variants. Variants with VAFs outside the 99.9% confidence interval were flagged as potential artifacts.

Results: Overall, 3,591 unique low-confident variants were found in 1,428 patients, with about 29% being associated with low mappability (≤0.95). In total, 64 unique variants were found in 42 CH-associated genes in 121 patients (5.8%), including actionable genes *ATM*, *BRCA1*, *TP53*, and *RB1*. The mean VAF for CH mutations was 30% (standard deviation, SD 5%) in non-tumor samples, compared to 6% (SD 3%) in tumor samples. The mean patient age for filtered-out variants was 61, consistent with higher CH probability in older individuals. In non-tumor samples, CH artifacts were found mostly in whole blood (78%). Breast cancers and sarcomas were the main diagnoses with observed CH artifacts.

Conclusion: Our filtering strategy integrates WES data from both tumor and non-tumor samples, including the copy number alteration (CNA) profiles to improve the accuracy of germline reports. This unique method reduces CH-associated errors and other artifacts, consequently streamlining quality control of genetic reports for cancer patients to guide their therapeutic decision-making.

#5048 MyInformatics®: Invivoscribe's end-to-end NGS analysis software system for streamlined variant calling and reporting.

J. Burke[1], J. Wemmer[1], J. D'Angelo[1], J. Velaquez-Muriel[1], T. Johnson[1], P. Sanchez[2];
[1]Invivoscribe, San Diego, CA, [2]LabPMM, San Diego, CA

Introduction: MyInformatics® is an NGS software system that streamlines the entire sample processing workflow for Invivoscribe's CAP/CLIA-certified MyAML® and MyMRD® Assays—from data creation and entry into a laboratory information system (LIS), through comprehensive and standardized bioinformatics analyses, to annotated variant reports. The system automates processes including quality control and sample tracking through integration with LIS and web display components, enhancing data transparency, and decreasing turnaround times. These seamlessly interlinked processes also eliminate many sources of potential human error.

Methods: Following specimen accessioning and sequencing in the lab, a MyInformatics® gene-panel analysis begins when an operator executes the main NextFlow script, which orchestrates the entire workflow by calling multiple Docker containers in a single workflow. Each individual Docker container includes a bioinformatics system/tool component. Each component is responsible for a specific bioinformatics task such as variant calling, artifact identification, sample QC, or variant annotation. Different components can be used by the bioinformatics workflow via a configuration file, allowing for flexible reuse of software. Following the bioinformatic analyses, the MyInformatics® system communicates with the LIS to perform sample QC based on each panel's established quality thresholds. The system also communicates with Invivoscribe's MyInformatics Annotation Portal (MAP®), a web interface for reporting clinically significant variants. Invivoscribe's Clinical Genomics scientists use MAP® to generate final sample reports.

Results: Invivoscribe developed the MyInformatics system with a modular software architecture based on containerization and automated workflow management for bioinformatics components. This reduces code complexity, allows for efficient integration of new versions of tools, and decreases manual overhead and operator errors. The system seamlessly integrates with other software components developed by Invivoscribe, such as the LIS and a web-based annotation tool and can handle multiple assays with a single user-specified parameter.

Conclusions: MyInformatics® connects all key components of a modern NGS assay analysis workflow, from the sequencing instrument to the annotated sample reports. The containerization makes deploying the software in any computational infrastructure easier, and the automation through connecting multiple software components dramatically reduces overall turnaround times for sample reporting while improving data transparency and limiting the potential for errors. MyInformatics® also facilitates the standardization of assay workflows across our global LabPMM laboratories, promoting consistency in results.

#5050 Comparative analysis of somatic copy number variations and allelic imbalance in whole-genome bisulfite sequencing: challenges and insights.

Yiru Gu, Jian Gu, Paul Scheet, Justin Wong

Epidemiology, UT MD Anderson Cancer Center, Houston, TX

Goal: To assess the feasibility, reliability, and limitations of using Whole Genome Bisulfite Sequencing (WGBS) for detecting somatic copy number variation (SCNV) and tumor-specific allelic imbalance (AI). Background: In cancer genomics, the use of WGBS for SCNV and AI detection enables simultaneous analysis of genetic variations and methylation landscapes, potentially providing insights into the interplay between somatic alterations and DNA methylation in tumorigenesis. However, WGBS data is inherently noisier compared to whole-genome sequencing (WGS) or microarray data due to artifacts introduced during bisulfite conversion. These challenges, including difficulties in read alignment, genotype calling, and phasing, the lack of strand complementarity, and the need to distinguish true C>T SNPs from bisulfite-induced changes, collectively complicate SCNV and AI detection. *Method:* We analyzed WGBS data from The Cancer Genome Atlas (TCGA), including 39 tumor samples and 8 adjacent normal tissues across 9 tumor types. SCNV detection was performed using GATK-CNV, while AI callings were conducted with BisSNP, BISCUIT, Revelio (with Unified Genotyper), and BSsnp. Confusion matrices were used to compare GATK-CNV (WGBS) and hapLOH (microarray) at the base, gene, and arm levels. F1 score, specificity, and sensitivity were calculated to evaluate their performance across different genomic scales. Additionally, AI identified from WGBS data using BisSNP, BISCUIT, Revelio (with Unified Genotyper), and BSsnp were compared to those detected from array and WGS data using hapLOH/seq. To determine detection thresholds and refine results, sequencing data were downsampled, with filters applied to exclude low-quality data and retain high-confidence SNPs and genotypes. *Result:* GATK-CNV exhibited variable performance across samples, with most samples showing high true positives and low false positives, indicating good sensitivity for detecting SCNVs and capturing a genome burden similar to hapLOHseq data. However, for samples with a high number of events, normalization disrupts event callings, resulting in inconsistencies in SCNV identification compared to array data. For AI detection, Revelio (with Unified Genotyper) showed the best overall performance among the tools evaluated. Downsampling analysis showed that reducing sequencing coverage impaired event detection, becoming less effective at lower coverage levels. BSsnp, BISCUIT, and BisSNP produced widespread and inconsistent variant calls, and initial filtering for high-quality SNPs showed limited improvement in precision. This study highlights the potential and challenges of WGBS as a tool for analyzing SCNV/AI alongside DNA methylation with phased data, offering insights into tumorigenesis and advancements in cancer diagnostics and treatment.

#5052 Large-scale genomic analysis of mutation signatures reveals pathway alternations linked to breast cancer prognosis.

Mate Posta, **Balazs Gyorffy**

Semmelweis University, Budapest, Hungary

Introduction: Advancing breast cancer treatment demands a thorough understanding of the genetic and molecular mechanisms that drive disease progression. Our study aims to explore pathway-wide mutational patterns to uncover molecular interactions that influence survival outcomes.

Methods: We integrated mutation data from three major breast cancer databases, comprising a total of 4,586 samples and over 25,000 genes. We considered disruptive mutations for survival analysis. We applied Cox proportional hazard regression to link gene mutations and altered molecular pathways with patient outcomes.

Results: Our analysis identified 17 genes with significant associations with relapse-free survival, with the most impactful genes being TP53 (HR: 2.04, p: 4.65×10^{-33}), CARD11 (HR: 2.59, p: 1.54×10^{-5}), and PIK3R1 (HR: 2.27, p: 3.66×10^{-5}). The most significant KEGG pathways affecting relapse-free survival were *MicroRNAs in cancer* (hsa05206, HR:2.48, p: 2.69×10^{-32}), *Hepatocellular carcinoma* (hsa05225, HR: 2.39, p: 8.88×10^{-30}), and *Breast cancer* (hsa05224, HR:2.38, p: 1.78×10^{-28}). We also identified significant patterns of mutual exclusivity and co-mutation. Pairs such as *GATA3-TP53* (OR: 0.201, p: 1.42×10^{-37}), *CDH1-TP53* (OR: 0.336, p: 3.66×10^{-21}), and *AKT1-PIK3CA* (OR: 0.199, p: 4.97×10^{-13}) were mutually exclusive, while *MAP3K1-PIK3CA* (OR: 2.83, p: 4.62×10^{-23}), *CBFB-GATA3* (OR: 4.67, p: 3.37×10^{-18}), and *AHNAK2-MUC16* (OR: 3.39, p: 1.35×10^{-15}) emerged as co-mutated gene pairs. Finally, an online platform (https://server2.kmplot.com/breast) has been developed to facilitate future studies designed to validate pathway-specific results.

Conclusions: In summary, by compiling a large-scale breast cancer database, we identified disruptive mutation patterns associated with altered survival outcomes. Out new tool provides a valuable resource for future research and treatment development.

#5053 Targeted sequencing panel for comprehensive MTAP deletion detection in diverse cancer types.

Hua Bao, **Haimeng Tang**, Rui Liu, Ningyou Li, Min Wu, Xunbiao Liu, Zhili Chang, Xue Wu

Nanjing Geneseeq Technology Inc., Nanjing, China

Objective: Methylthioadenosine phosphorylase (MTAP) deletion is a prevalent genomic alteration across various malignancies, often correlated with unfavorable clinical outcomes. The detection of MTAP deletions offers opportunities for personalized therapeutic strategies aimed at exploiting specific metabolic vulnerabilities. However, existing detection methodologies are frequently costly and labor-intensive, highlighting the necessity for an efficient and economical detection panel. Developing such a tool is essential for the timely identification of MTAP deletions, facilitating prompt initiation of targeted therapies and improving patient prognoses.
Methods: We retrospectively analyzed 5,787 tissue samples from patients diagnosed with 11 distinct cancer types, including colorectal, lung, liver, gastric, bladder, pancreatic, and esophageal cancers. A targeted sequencing approach (approximately 1,000X coverage) was employed using the GeneseeqPrime™ panel, which encompasses 437 cancer-related genes. Additionally, an independent cohort of 29 cancer samples was analyzed to assess the concordance of our sequencing panel with Fluorescence In Situ Hybridization (FISH) and Immunohistochemistry (IHC).
Results: The evaluation of MTAP deletions across a spectrum of cancer types revealed significant variations in both total and homozygous deletion rates. Bladder cancer exhibited a total deletion rate of 43.43%, with homozygous deletions accounting for 11.11%. Esophageal squamous cell carcinoma indicated a total deletion rate of 46.15%, with homozygous deletions at 15.73%. Glioblastomas displayed the highest prevalence of total deletions at 58.33%, including 30.21% homozygous deletions. Furthermore, non-small cell lung cancer (NSCLC) and pancreatic cancer had total deletion rates of 35.84% and 42.55%, respectively, with homozygous deletions at approximately 8.21% and 15.55%. Our panel demonstrated perfect concordance with both FISH and IHC (100% agreement).
Conclusion: This study establishes the variable prevalence of MTAP deletions across multiple cancer types, underscoring its significance as a prognostic biomarker associated with poor outcomes. The GeneseeqPrime™ panel provides a rapid and cost-effective means for identifying MTAP deletions, facilitating timely diagnosis and personalized therapeutic strategies. Further validation in larger cohorts and clinical settings is warranted to enhance its applicability in routine oncological practice.

#5054 Understanding the genetic heterogeneity within clonal spheroids and its interaction with the tumor microenvironment.

A. Yadav, R. Chaturvedi, R. P. Singh;
Jawaharlal Nehru University, New Delhi, India

Introduction: Head and neck squamous cell carcinomas (HNSCC) exhibit significant inter- and intra-tumor heterogeneity, complicating diagnosis and treatment. Our previous work demonstrated that spheroids developed from patient-derived cells and HNSCC cell lines display distinct proliferative and migratory behaviors. We classified them as hyperproliferative and hypoproliferative spheriods. To uncover the molecular basis of this functional heterogeneity, we utilized single-cell RNA sequencing (scRNA-seq) and advanced computational tools like CellChat. Our analysis revealed key signaling pathways, cellular interactions, and surface markers defining spheroid phenotypes, offering insights into their genetic and functional diversity.

Material and Methods: scRNA-seq was performed on hyperproliferative and hypoproliferative spheroids derived from the UMSCC-22B cell line. Spheroids were cultured, dissociated into single cells, and subjected to high-throughput scRNA-seq. Bioinformatic analyses identified differentially expressed genes, signaling pathways, and cellular states. CellChat was employed to analyze intercellular communication networks within spheroids. Ligand-receptor interactions were modelled to identify pathways mediating cross-talk between distinct cell clusters.

Results: scRNA-seq revealed distinct transcriptional profiles between the two spheroids. Hyperproliferative spheroids expressed genes related to cell division and growth, while hypoproliferative spheroids expressed genes linked to migration and invasion. Pathway analysis identified differences in angiogenesis, metastasis, and tumor microenvironment interactions. Midkine signalling(MDK-SDC) through basal cells was found to be the principal contributor to the proliferative advantage in hyperproliferative spheriods, supporting their role in tumor expansion and progression. While, Desmosomal signaling (DSC2-DSG2) through keratinocytes and fibroblasts was found to play a critical role in maintaining cellular adhesion and promoting migratory capacity in hypoproliferative spheroids. TCGA data linked MDK to advanced tumor stages, poor survival, and therapy resistance, while DSG2 and DSC2 were overexpressed in metastatic tumors. These findings highlight the distinct functional states of spheroids contributing to tumor heterogeneity.

Conclusion: The balance between proliferation and migration in spheroids reflects tumor heterogeneity's dual role in cancer progression. Midkine-driven hyperproliferative spheroids anchor tumor growth, while desmosome-mediated hypoproliferative spheroids drive metastasis. Targeting Midkine or modulating desmosomal remodeling could inhibit growth and prevent dissemination, enabling patient-specific therapies.

#5055 Efficient detection of chromosomal instability in diverse cancers using a targeted sequencing approach.

Hua Bao, Rui Liu, **Haimeng Tang**, Ningyou Li, Min Wu, Xunbiao Liu, Zhili Chang, Xue Wu

Nanjing Geneseeq Technology Inc., Nanjing, China

Objective: Chromosomal instability (CIN), characterized by frequent chromosomal alterations, plays a crucial role in tumorigenesis, significantly influencing cancer progression and therapeutic resistance. Targeting vulnerabilities associated with CIN, particularly in DNA repair pathways, presents promising therapeutic strategies. However, existing CIN detection methods are often costly and labor-intensive. Thus, the development of efficient and cost-effective CIN detection panels is essential for optimizing cancer treatments and enhancing patient outcomes while reducing healthcare costs.

Methods: We conducted a retrospective study analyzing 4,941 tissue samples from patients diagnosed with 14 distinct cancer types, including ovarian, uterine, breast, pancreatic, prostate, cervical, liver, lung, colorectal, and gastric cancers. A targeted sequencing panel, GENESEEQ GENAVISTA, assessing 437 cancer-associated genes with an approximate depth of coverage of 1,000X, facilitated detailed mutational profiling. CIN assessment utilized read depth and allele frequency evaluation, alongside variant calling algorithms to identify gene duplications and genomic rearrangements. Additionally, a prospective cohort of 33 cancer patients was analyzed to compare the efficacy of our sequencing panel against the gold standard of whole genome sequencing (WGS, ~30X).

Results: Our analysis revealed significant CIN prevalence across various cancer types, particularly elevated in ovarian cancer (73.26%) and breast cancer (76.80%). Lung cancer exhibited a CIN rate of 78.57%, while melanoma demonstrated an 80.00% prevalence. Endometrial cancer presented a moderate CIN rate of 40.62%, whereas pancreatic cancer displayed an equal distribution between CIN and genomically stable tumors, each accounting for 50.00%. Prostate cancer showed a CIN incidence of 45.16%, and cervical cancer recorded a rate of 62.30%. The findings illustrate the heterogeneous nature of CIN among malignancies, suggesting its utility as a prognostic marker and therapeutic target. The panel demonstrated a high correlation with WGS results in the prospective cohort (Spearman rank correlation = 0.91), confirming its effectiveness in identifying CINs in cancer patients.

Conclusion: Our study establishes the GENESEEQ GENAVISTA targeted sequencing panel as an effective tool for accurately detecting CIN across various cancer types. The strong correlation with whole genome sequencing highlights its potential as a reliable, cost-effective alternative for CIN assessment, facilitating optimized cancer treatment strategies and improving patient outcomes while minimizing healthcare costs.

#5056 Full spectrum of somatic structural variations (SVs) detection in COLO829 with long-read sequencing.

Zishan Peng, Zechen Chong

Department of Biomedical Informatics and Data Science, University Of Alabama At Birmingham, Birmingham, AL

Somatic SVs significantly contribute to cancer development and progression. Characterizing these variations has traditionally been difficult due to the limitations of short-read sequencing technologies and the diverse types and lengths of SVs. The advent of long-read sequencing has enabled a more comprehensive analysis of germline SVs, highlighting its potential applications in cancer genomics. The melanoma cell line COLO829, along with its normal counterpart COLO829BL, is a standard reference for somatic SV detection. Valle-Inclan et al. recently validated 68 somatic SVs combining short-read, long-read, and linked-read sequencing data. However, the sensitivity may be compromised as only a limited number of alignment-based callers were employed, such as NanoSV, Sniffles (for Oxford Nanopore Technologies (ONT)), and pbsv for (PacBio HiFi). As more alignment-based SV callers are being developed, it is crucial to integrate a broader range of tools to construct a comprehensive call set of somatic SVs. Moreover, the performance of assembly-based methods in somatic SV detection within cancer remains largely unexplored. In this study, we aimed to establish a comprehensive spectrum of somatic SVs in the COLO829 melanoma cell line by employing both alignment-based and assembly-based methods with long-read whole-genome sequencing data from PacBio and ONT aligned with Minimap2, SVs were identified and integrated from 7 alignment-based callers: cuteSV, NanoVar, DeBreak, Sniffles2, Svision-Pro, NanoSV, and pbsv. In addition, genome assemblies were constructed via Hifiasm and Verkko, incorporating HiFi reads with Hi-C and ultra-long ONT integration, followed by SV detection using four assembly-based callers: Dipcall, PAV, SVIM-asm, and cuteSV. A dedicated pipeline was developed to identify and filter somatic SVs from germline events, which considers mapping quality, sequencing depth of SV regions and their flanking regions, and 50% reciprocal overlap. Compared to the truth set established by Valle-Inclan et al., we discovered 19 novel somatic SVs, including 10 deletions, 4 insertions, 3 duplications, and 2 inversions. As a comparison, assembly-based methods detected all the deletions, insertions and duplications found by the alignment-based methods. However, it missed 20 inversions and 6 translocations. Our findings highlight the superior sensitivity of alignment-based techniques in somatic SV detection and underscore the complementary nature of assembly-based approaches. This work enhances the benchmark for somatic SV characterization in cancer genomics and confirms the utility of integrated long-read sequencing analyses.

#5057 Enhancing clinical applications by evaluation of sensitivity and specificity in whole exome sequencing.

Chunghwan Hong[1], Youngbeen Moon[1], Hye Won Choi[1], Eun-Kyung Kang[1], Seo-Hyeon Ye[1], Jong-Kwang Kim[1], Young-Ho Kim[1], Hyeri Cho[1], Hana Choi[1], Dong-eun Lee[1], Yongdoo Choi[1], Tae-Min Kim[2], Seong Gu Heo[3], Namshik Han[4], Kyeong-Man Hong[1]

[1]National Cancer Center - Korea, Goyang-si, Korea, Republic of,[2]The Catholic University of Korea, Seoul, Korea, Republic of,[3]Dana Farber Cancer Institute, Boston, MA,[4]University of Cambridge, Cambridge, United Kingdom

The cost-effectiveness of whole exome sequencing (WES) remains debated, primarily due to variability in variant calls. Evaluating the sensitivity and specificity of WES results may clarify this issue. WES was performed by three companies (AA, BB, and CC) using reference standards composed of DNA from Hydatidiform mole and individual blood at various ratios. Sensitivity was assessed by the detection rate of null-homozygote (N-H) alleles at expected variant allelic fractions, while false positive (FP) errors were counted for unexpected alleles. Sensitivity was approximately 20% for in-house results from BB and CC and around 5% for AA. Dragen analyses identified 1.34 to 1.71 times more variants, detecting over 96% of in-house variants, with sensitivity for common variants increasing to 5%. In-house FP errors varied significantly among companies (up to 13.97 times), while Dragen analyses minimized this variation. Despite Dragen showing higher FP errors for BB and CC, the increased sensitivity highlights the importance of effective bioinformatic conditions. We also assessed the potential effects of target enrichment and proposed optimal cutoff values for read depth and variant allele fraction in WES. Optimizing bioinformatic analysis based on sensitivity and specificity from reference standards can enhance variant detection and improve the clinical utility of WES.

#5058 Evaluation of false positive and false negative errors in targeted next generation sequencing.

Chunghwan Hong[1], Youngbeen Moon[1], Hye Won Choi[1], Eun-Kyung Kang[1], Jong-Kwang Kim[1], Young-Ho Kim[1], Dong-eun Lee[1], Tae-Min Kim[2], Seong Gu Heo[3], Namshik Han[4], Kyeong-Man Hong[1]

[1]National Cancer Center - Korea, Goyang-si, Korea, Republic of,[2]The Catholic University of Korea, seoul, Korea, Republic of,[3]Dana Farber Cancer Institute, Boston, MA,[4]University of Cambridge, Cambridge, United Kingdom

Background: Next-generation sequencing (NGS) has emerged as a vital diagnostic tool for various diseases; however, errors associated with NGS present significant challenges to its clinical implementation. Specifically, in cancer diagnostics, the analysis of low-level mutations is complicated by contamination from normal cells and tumor heterogeneity.
Results: In targeted NGS (T-NGS) analyses involving reference-standard samples consisting of mixtures of homozygous Hydatidiform mole DNA and blood genomic DNA at varying ratios from four certified NGS service providers, we observed considerable variability in both the lower detection limits of variants (16.3-fold difference, ranging from 1.51% to 24.66%) and false positive (FP) error rates (4280-fold difference, ranging from 5.814 × 10^-4 to 1.359 × 10^-7). The commercially available Dragen system for bioinformatics analysis reduced FP errors in results from companies BB and CC; however, inherent errors from the raw NGS data remained. Adjustments to bioinformatics parameters aimed at increasing sensitivity (by less than two times) substantially elevated FP error rates (by 610 to 8200 times). Additionally, we identified issues such as biased base calling during bioinformatics analysis and a high frequency of false negative (FN) errors in HLA regions.
Conclusion: T-NGS results from certified NGS service providers reveal considerable variability in sensitivity and FP error rates, underscoring the necessity for enhanced quality control measures in the clinical application of T-NGS. Furthermore, our study suggests that mixtures of homozygous and heterozygous DNA can serve as effective reference-standard materials for T-NGS quality control.

#5059 Self-supervised representation learning of somatic mutational data.

Marzieh Haghighi, Gustavo Alonso Arango Argoty, Etai Jacob

AstraZeneca US, Waltham, MA

Traditional approaches for learning representations from somatic mutation data often rely on the distribution of motif counts, which have limitations, including the use of small sequence contexts and the exclusion of critical information, such as mutation location and interactions among mutations. Recent efforts have leveraged advances in natural language processing (NLP) to improve representation learning from somatic mutations by incorporating larger sequence contexts into shallow networks. More recently, studies have demonstrated the feasibility of leveraging attention mechanisms to learn meaningful representations. However, these methods primarily use supervised or weakly supervised learning paradigms, which are inherently task-specific. The development of general-purpose representations, by harnessing the vast amount of somatic mutation data through self-supervised techniques, remains underexplored. Such representations could drive progress in applications including tumor subtype classification, biomarker discovery, patient stratification, drug response prediction, and pathway enrichment analysis. This study aims to develop a deep, unsupervised learning framework that leverages abundant somatic mutation data to generate robust, general-purpose representations. By adapting recent advances in self-supervised learning, the proposed approach seeks to establish a foundation model capable of capturing meaningful patterns in mutation data for diverse downstream tasks. The framework employs novel tokenization and embedding strategies to encode mutation sequences and utilizes transformer-based architectures to model contextual dependencies. The process of inputting mutation data into a transformer architecture, particularly the tokenization step, is critical for this problem, and we explore various tokenization strategies. Benchmarking these representations will include evaluating their utility across tumor subtype classification, biomarker discovery, patient stratification, drug response prediction, identification of mutational signatures. Although the model is under development, experiments from prior work—limited to mutation identifiers without leveraging sequence motifs, mutation location, or other contextual information—have demonstrated the feasibility of encoding mutational data into meaningful representations using transformer-based architectures and self-supervised learning. Building on these findings, this framework will benchmark its performance on multiple downstream tasks, including tumor subtype classification and patient stratification.

BIOINFORMATICS / COMPUTATIONAL BIOLOGY / SYSTEMS BIOLOGY / CONVERGENT SCIENCE: Single-Cell and Spatial Molecular Analysis
Poster Session

#5063 Hepatomap: An interactive shiny app for spatial transcriptomics insights in hepatocellular carcinoma.

Wei Lin Tang[1], Teresa J. Y. Tye[2], Menaka Priyadharsani Rajapakse[1], Solomonraj Wilson[1], Olaf ROtzschke[1], David Tai[3], Darren Wan-Teck Lim[3], Juha P. Vayrynen[4], Joe Poh Sheng Yeong[3], **Mai Chan Lau**[1]

[1]Singapore Immunology Network, Agency for Science, Technology And Research, Singapore, Singapore,[2]Department of Life Sciences, Imperial College London, London, United Kingdom,[3]Department of Medicine, SingHealth Duke-NUS Academic Medical Centre, Singapore, Singapore,[4]Translational Medicine Research Unit, Medical Research Center Oulu, Oulu University Hospital, Finland, Finland

Introduction: Hepatocellular carcinoma (HCC) is a heterogeneous cancer where spatial context and cellular microenvironments influence disease progression and therapeutic responses. Spatial Transcriptomics (ST) enhances immune analysis by mapping gene expression spatially, revealing cell organization and interactions. However, interpreting ST data in HCC is challenging due to high data dimensionality, unestablished bioinformatics pipelines, and fragmented knowledge in the literature. We introduce HepatoMap, a code-free interactive tool designed to streamline ST analysis for HCC research. It enables in situ visualization of expression patterns for curated HCC-associated signatures, identification of cellular interactions through cell type annotation, and uncovers molecular heterogeneity in HCC samples.
Methods: A cohort of 13 HCC patients included baseline and radiotherapy-nivolumab treated samples (with 9 pairs), 2 super responders (SRs), 2 progressors (PRs), and 9 non responders (NRs). HepatoMap (R shiny v1.9.1) supports Visium data in Seurat object and spot annotation text files. Gene enrichment scores were computed via Seurat's AddModuleScore. It includes 2 cell type annotation methods: MCP counter (marker-based) and SPOTlight (reference-based deconvolution method, with 2 pre-trained models built on TICAtlas and GSE149614 single-cell RNA references). It also provides a curated list of HCC-associated modules (cancer stem cell (CSC), driver genes), and allows users-upload gene sets , where Seurat's AddModuleScore is used to compute enrichment scores. ggplot2 (v.3.5.1) is used for box plots, pie charts, and tissue visualization; ComplexHeatmap (v2.10.0) is for heat maps. The tool offers multi-tissue visualization for cross-sample comparison and downloadable publication-ready graphics.
Results: To characterize immune response, the SPOTlight models revealed an increased T cells in treated SRs, with TICAtlas model providing finer resolution by identifying CD4 effector memory cells. SPOTlight offers resolution flexibility but assumes reference cell types represent the entire microenvironment, which may be limiting. MCP counter complements this by computing scores for predefined gene markers, revealing B cell increases in SRs and identifying additional cell types like endothelial cells and neutrophils for more comprehensive immune profiling. Additionally, contrasting to literature, SRs showed elevated CSC scores in tumor epithelium upon treatment, while a decrease was seen in NRs and PRs after treatment, adding depth to immune cell analysis.
Conclusion: We presented HepatoMap, a tool streamlining ST analysis for HCC, uncovering molecular heterogeneity and cell-type dynamics through robust cell type annotation and tumor-specific profiling. The tool is adaptable to ST technologies like Xenium and Stereo-seq, expanding its relevance in spatial immunology.

#5065 Single-cell transcriptomic profiling of longitudinal patient tumors and PDX models reveals resistance mechanisms in ALK- and EGFR-mutant LUAD.

Naomi Mfonfu[1], Floriane Braye[1], Galina Boldina[2], Matteo Cesaroni[2], Luc Fibroulet[1], Sergey Nikolaev[1], Andrey Yurchenko[1]

[1]Gustave Roussy, Villejuif, France,[2]Sanofi, Vitry-sur-Seine, France

Resistance to EGFR and ALK tyrosine kinase inhibitors (TKIs) in lung adenocarcinoma (LUAD) remains a major barrier to effective treatment. Tumor adaptation frequently involves dynamic cellular state transitions, altered signaling networks, and interactions within the tumor microenvironment (TME). Understanding the mechanisms driving resistance in EGFR- and ALK-mutant tumors is critical to developing strategies to improve patient outcomes. This study investigates transcriptomic changes underlying TKI resistance and persistance, combining patient data with functional insights from PDX models. Tumor biopsies from 11 LUAD patients were collected across three treatment stages: baseline (n=6), minimal residual disease (MRD, n=4), and relapse (n=1). Patient-derived xenograft (PDX) models were generated from pretreated tumors with EGFR and ALK mutations. Tumors were grafted into five mice per model, with three receiving treatment until stable residual disease emerged and two serving as untreated controls. Single-nucleus RNA sequencing was performed on both patient tumor samples and PDX models to comprehensively profile transcriptomic changes. Transcriptomic changes were observed in both patient tumor samples and PDX models during TKI treatment. In patients, at MRD, a novel subpopulation expressing mesenchymal and neuronal markers was identified, accompanied by altered cell-cell communication involving neuronal signaling (RELN), stromal remodeling (FGFRs), and survival pathways (MAPK). At relapse, signatures of epithelial-mesenchymal transition (EMT), ECM remodeling, and immune evasion were enriched. In PDX models, most persister cells were arrested in the G1 phase and showed suppression of canonical cancer pathways, while notable upregulation of the ciliated pathway was observed across models. This study provides a high-resolution transcriptomic map of TKI-treated LUAD tumors. Resistance mechanisms in EGFR- and ALK-mutant tumors include neuronal, EMT, MAPK signaling, ECM remodeling, and activation of the ciliated pathway. These findings offer novel therapeutic targets to combat resistance, leveraging the integration of patient-derived data and functional insights from PDX models.

#5066 Single-cell characterization of airway field alterations in patients with lung squamous premalignant lesions.

Regan D. Conrad[1], Conor Shea[1], Lukas Kalinke[2], Kitty De Jong[3], Sherry Zhang[1], Zhihan Li[1], Gang Liu[1], Erin Kane[1], Emily Siedlecki[3], Kelsey Simon[3], Kate E. J. Otter[2], Kate Gowers[2], Mark Hennon[3], Sai Yendamuri[3], Steven Dubinett[4], Avrum Spira[1], Mary Reid[3], Sam Janes[2], Marc Lenburg[1], Sarah Mazzilli[1], Joshua Campbell[1], Jennifer Beane[1]

[1]Section of Computational Biomedicine, Boston University School of Medicine, Boston, MA,[2]Lungs for Living Laboratory, University College London, London, United Kingdom,[3]Roswell Park Comprehensive Cancer Center, Buffalo, NY,[4]David Geffen School of Medicine, UCLA, Los Angeles, CA

Lung cancer is the leading cause of cancer-related mortality worldwide, and there is a critical need for early detection strategies to reduce its burden. In high-risk individuals, low-dose computed tomography (LDCT) reduces lung cancer mortality by detecting cancer at earlier stages. Gene expression obtained from bronchial and nasal brushings effectively discriminates benign from malignant lung nodules detected via LDCT. Lung premalignant lesions (PMLs), precursors to cancer, represent an opportunity for further mortality reduction, but detection methods remain underexplored. This study uses single-cell RNA sequencing (scRNA-seq) to investigate molecular and cellular alterations in the airway field of patients with PMLs.

Endobronchial biopsies from lung sites with suspect PMLs and bronchial and nasal brushes from normal-appearing epithelium were collected from high-risk patients (n=46). Epithelial and immune cells were profiled using scRNA-seq via the CEL-Seq2 protocol (n=9405 biopsy, n=3416 bronchial brush, and n=2002 nasal brush cells). Cell and gene clustering were performed via Celda. Co-expressed gene modules and cell-type abundance differences by sample type, smoking status, and lesion histology were identified with differential composition analysis and linear mixed models. Gene module associations with phenotypes were validated in independent bronchial brushes profiled by bulk mRNA-seq (n=89 samples, n=30 patients).

Clustering of epithelial cells (40 cell clusters, 70 gene modules) showed nasal brushes are enriched in cells from 9 cell clusters ($p<0.001$) with high expression of detoxification and mucous production gene modules. Nine gene modules associated with smoking status ($p<0.001$) across sample types were enriched for xenobiotic metabolism genes. The effect of smoking on these gene modules was strongest in bronchial biopsy samples, decreased in bronchial brushes and weakest in nasal brushes. This stepwise decrease was significant for each pairwise comparison ($p <= 0.001$). Five basal cell clusters enriched with carcinoma in situ lesion or tumor cells (termed high-grade basal cells) expressed 7 gene modules linked to keratinization and inflammation ($p<0.01$). Cells from a bronchial brush sample clustered with high-grade basal cells. The expression of a gene module upregulated in high-grade basal cells was associated with the worst lesion histology sampled during the procedure ($p=0.006$) in bronchial brushes profiled by bulk RNA-seq.

This study identifies smoking and lesion severity associated molecular and cellular alterations in the airway fields of patients with PMLs. These findings suggest that airway field brushings may be useful in detecting the presence of high-grade PMLs.

#5067 Concurrent spatial transcriptomics and metabolomics map differential effects of drug across brain cancer tissues.

Tuan Vo[1], Cedric Cui[1], Andrew Causer[2], Xiao Tan[2], Eunju Kim[1], Zherui Xiong[2], Hani Vu[2], Mark Hodson[2], Tianyao Lu[3], Saskia Freytag[3], Jenny Fung[1], John Lee[1], **Quan Nguyen**[2]

[1]The University of Queensland, Brisbane, Australia,[2]QIMR Berghofer Medical Research Institute, Brisbane, Australia,[3]WEHI, University of Melbourne, Melbourne, Australia

The spatial heterogeneity of tumors poses significant challenges for effective treatment, resulting in varied therapeutic responses across different regions of the same tumor. This challenge is especially pronounced in brain cancers, where uneven drug distribution—largely due to biological barriers like the blood-brain barrier—complicates treatment. Techniques that overlay drug distribution with transcriptional profiles would enable a comprehensive, unbiased investigation of drug effects.

In this study, we present a spatial multiomics approach that integrates spatial metabolomics and spatial transcriptomics within the same tissue section of mouse brain samples treated with Palbociclib, a selective CDK4/6 inhibitor with potential for targeting various cancers, including breast cancer and medulloblastoma (MB). Our high-resolution (2-55 µm) concurrent multiomics maps reveal that the differential distribution of Palbociclib results in heterogeneous effects, promoting differentiation and reducing proliferation in specific tumor regions. Through this approach, we identified tissue regions exhibiting distinct transcriptional and metabolic signatures, including areas unaffected by drug treatment, which could contribute to recurrence.

To support this analysis, we developed SpaMTP, a state-of-the-art software package providing a comprehensive pipeline for in-depth analysis of spatial metabolomics for integrating spatial multiomics data. This package bridges processing functionalities for metabolomic data from Cardinal with user-friendly cell-centric analyses implemented in Seurat. SpaMTP's comprehensive analysis pipeline covers (1) automated mass-to-charge ratio (m/z) metabolite annotation, (2) a wide range of downstream statistical analyses including differential metabolite expression and metabolic pathway analysis, and (3) integrative spatial-omics analysis. For broad implementation and interoperability, SpaMTP includes various functions for data visualisation, data object conversion and data import/export.

Overall, our work describes novel experimental and analytical methods to map drug distribution and its transcriptional impacts directly, offering critical insights into the heterogeneous effects of Palbociclib across brain tumor tissues.

#5068 Spatial architecture and epigenetic programs in IDH-mutant and wildtype glioma.

Jingxian Liu[1], Song Cao[1], Wagma Caravan[1], Xiangwei Fang[1], Preet Lal[1], Andrew Houston[1], Yuting Zhao[1], Faria Anjum Simin[1], Michael C. Jin[1], Jennifer Ponce[2], Robert Fulton[2], Michael Heinz[1], Yang Li[3], Sonika M. Dahiya[4], Dimitrios Mathios[3], Tao Liu[5], Albert H. Kim[3], Milan G. Chheda[1], Li Ding[1]

[1]Department of Medicine, Washington University in St. Louis, St. Louis, MO,[2]McDonnell Genome Institute, Washington University in St. Louis, St. Louis, MO,[3]Department of Neurosurgery, Washington University in St. Louis, St. Louis, MO,[4]Department of Pathology & Immunology, Washington University in St. Louis, St. Louis, MO,[5]Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA

Gliomas account for over 80% of primary malignant brain tumors. Among the most aggressive of these is IDH-wildtype high-grade glioma (HGG), known as glioblastoma, which is resistant to treatment and carries a median survival of 15 months. Genomic and transcriptomic characterization of HGG demonstrate strong inter- and intra-tumor heterogeneity. Distinct from IDH-mutant glioma which notably exhibit diffuse infiltrative histology with hypermethylated molecular phenotype, IDH-wildtype HGG circumferentially organize around a necrotic core with aggressive tumor expansion and macrovascular proliferation. Both IDH mutation status and tumor grade are significant prognostic factors. While IDH inhibitors have had success in IDH-mutant low-grade glioma (LGG), prognosis remains poor for HGG regardless of IDH status. Hence, a comprehensive multi-omic and spatial dataset spanning IDH status and tumor grades is crucial to further delineate similarity and heterogeneity along the disease course. In this study, we present a cohort of 85 specimens from 68 patients with high- and low-grade glioma, as well as comparisons with metastases and normal brain. To comprehensively investigate the epigenetic programs and spatial architecture of glioma driven by IDH status and tumor grade, we performed multiome single-nucleus sequencing, Xenium spatial in-situ gene expression assay, whole exome sequencing and pathological reviews. Concurrent snATAC and snRNA, in the same cell, identified differential epigenetic programs and downstream transcriptional changes. Global ATAC and RNA similarity shows IDH-mutant tumors, regardless of grade, are most closely related to oligodendroglial precursor cells (OPCs) whereas IDH-wildtype tumors show similarity to neurons, astrocytes and OPCs. To understand the spatial context differences between these tumors, we performed Xenium in-situ gene expression assays on 9 IDH-wildtype and 8 IDH-mutant HGG samples. We observe not only an increased density of OPCs in IDH-mutant HGG, but also a distinct tumor niche organized around OPCs that is more frequently observed in IDH-mutant tumors. This niche demonstrated upregulation of neurogenesis pathways and reduced hypoxia signature compared to tumor cells spatially discontiguous from OPC communities. Taken together, multimodal assessment of single-cell epigenetics, transcriptomic trajectory, and spatial organization provide further insight on glioma pathogenesis.

#5070 Tumor spatial configuration predicts pancreatic cancer recurrence risk after total neoadjuvant therapy.

Andrea V. Maraone[1], Zafar Siddiqui[1], Luis H. Cisneros[1], Merih D. Toruner[2], Mark Truty[1], Cornelius Thiels[1], Robert R. McWilliams[1], Steven R. Alberts[1], Rondell P. Graham[1], Carlo Maley[3], Qian Shi[1], Martin E. Fernandez-Zapico[1], Ryan M. Carr[1]

[1]Mayo Clinic, Rochester, MN,[2]Brown University, Providence, RI,[3]Arizona State University, Tempe, AZ

Despite advances in surgery and chemotherapy, patients with resectable pancreatic ductal adenocarcinoma (PDAC) face high recurrence rates (40-60%). Total neoadjuvant therapy (TNT) seeks to enhance margin-negative resections (R0) and address micrometastatic disease, with potential survival benefits. Yet, TNT still results in high recurrence rates, with 1-, 2-, and 3-year disease-free survival (DFS) at 65%, 48%, and 32%, respectively. Residual cancer extent is a key prognostic factor in PDAC after TNT. Tumor response scores show favorable outcomes only for patients achieving near-complete or complete pathologic response. In a cohort of 511 PDAC patients treated with TNT and surgery (2015-2022), improved DFS was only found in those with a complete pathologic response (pCR, 7.8%). This highlights the limited use of current response scoring in stratifying post-TNT patient risk, underscoring the need to better understand disease persistence and relapse. Here we developed a quantitative method to assess residual cancer. Using a digital pathology H&E library from 2180 PDAC cases (2000-2022), an AI platform segmented tissue into cancer epithelium, stroma, and normal regions. For 202 TNT-treated cases without pCR, we calculated a ratio of cancer area to stromal area from representative slides. Modeled after the histologic tumor regression grade (HTRG) system, cases were classified into <5%, 5-50%, or >50% cancer-to-stroma ratios. However, this measure alone showed no difference in DFS, suggesting tumor burden cannot fully explain differences in clinical outcomes. We hypothesized that the spatial organization of the tumor microenvironment (TME) is a determinant of patient outcomes. Drawing from landscape ecology, which studies spatial properties of ecosystems, we applied similar principles to analyze tissue architecture. Using an AI-based two-class segmentation of whole-slide images, we assessed spatial features like patch area, perimeter, density, and Shannon diversity. Python's 'pylandstats' package calculated 21 spatial metrics, reduced via stepwise Cox regression using the Akaike information criterion (AIC). The final model included mean Shannon diversity, mean and standard deviation of stromal patch areas, and stromal patch density. Kaplan-Meier analysis of 202 samples stratified model predictions into quartiles, correlating with high (mean DFS 10.85 months), medium (19.32 months), and low risk (25.92 months) groups. These findings aligned with model expectations, underscoring the potential of spatial TME analysis to predict outcomes.

#5071 Spatially resolved gene expression analysis of primary and metastatic fibrolamellar hepatocellular carcinoma (FLC).

Waqar Arif[1], Luciane Kagohara[2], Robert A. Anders[1], Kiyoko Oshima[1], Mark Yarchoan[2]

[1]Department of Pathology, The Johns Hopkins Hospital, Baltimore, MD,[2]Department of Oncology, The Johns Hopkins Hospital, Baltimore, MD

Fibrolamellar carcinoma (FLC) is a rare and aggressive malignancy of the liver that predominantly affects adolescents and young adults. These tumors are distinct from conventional hepatocellular carcinomas and are characterized histologically by thick fibrous collagen bands. The transcriptome of FLC is characterized by a recurrent oncogenic fusion between DNAJB1 and PRKACA. Liver resection is a potentially curative treatment option for early-stage FLC, but there are no standard systemic therapies available for advanced FLC. Most patients present with unresectable disease or relapse after surgery; for these individuals, outcomes are dismal with a median survival of only 12 months. This highlights the urgent need to understand the natural evolution of FLC, which in turn will support the development of effective targeted therapies. To investigate the evolution of FLC, we leveraged FLC tissue samples from n=5 patients with paired primary and distant metastatic surgical biopsies. Among these patients, 4/5 received systemic therapy including immunotherapy in the interval between primary resection and resection of the distant metastatic lesions. A histological view of the cases revealed notable morphological differences between the primary and metastatic tumors. Primary tumors showed the characteristic oncocytic tumor cells separated by thick cords of collagen fibrosis. However, the metastatic lesions exhibited reduced fibrosis or lacked it entirely, suggesting malignant progression of the tumor and loss of a key histological hallmark of FLC over time. To date, most transcriptomic analysis of FLC has been performed on bulk tissue which neglects the heterogeneity of the tumor. To capture the complexity of the lesion, we performed spatial transcriptomic analysis of our paired samples (n = 10, primary and metastatic lesions) using the VisiumHD platform by 10x Genomics. Furthermore, we succeeded in labeling the neoplastic cells using a custom-designed probe against the DNAJB1-PRKACA chimeric transcript which we included in the standard probe library. Spatially resolved gene expression analysis and neighborhood analysis using the Seurat workflow revealed marked differences in transcriptional networks coinciding with the progression of the tumor. Here we present a spatially and temporally resolved transcriptome atlas of FLC. This rich and valuable resource will be critical for understanding FLC biology and ultimately developing novel therapeutics.

#5072 Single cell-based elucidation of molecularly distinct glioblastoma states and drug sensitivity.

L. Tomassoni[1], W. Zhao[1], H. Ding[2], K. Egbuji[1], P. Laise[1], M. J. Alvarez[1], J. L. Furnari[1], M. G. Argenziano[1], M. A. Banu[1], J. N. Bruce[1], P. D. Canoll[1], P. A. Sims[1], A. Califano[1];

[1]Columbia University Irving Medical Center, New York, NY, [2]University of Arizona, Tucson, AZ

Glioblastoma (GBM) heterogeneity and plasticity represent critical challenges to the development of successful treatment options, because molecularly distinct subpopulations present different drug sensitivities, leading to resistance [Yuan et al., 2018; Neftel et al., 2019; Ding et al., 2019]. In fact, clinical outcomes for GBM, which accounts for >80% of all primary gliomas [4], have not improved over the last 20 years. Unfortunately, due to the sparse nature of single cell RNA-seq (scRNA-seq) profiles, prediction of subpopulation-specific dependencies and drugs has been difficult. To overcome this problem, we leveraged network-based algorithms, single cell versions of the ARACNe [Margolin et al., 2006], VIPER [Alvarez et al., 2016], and OncoTreat algorithms [Alvarez et al., 2018] (NY and CA Dept of Health approved, CLIA compliant algorithm, currently used in several clinical trials), to analyze a collection of scRNA-seq profiles from 16 GBM patients to elucidate Master Regulator (MR) proteins, representing molecular determinants and thus non-oncogene dependencies, of distinct, co-existing GBM cell states and to prioritize compounds capable of inverting their activity. OncoTreat prioritizes drugs by assessing their ability to invert the activity of subpopulation-specific MR proteins, based on the analysis of large-scale drug perturbation assays, thus supporting the identification of small molecule inhibitors targeting distinct tumor compartments within the same patient for potential combination therapy. Specifically, we used a library of genome-wide RNASeq profiles representing the response of proneural (PN) (HF2597 neurosphere) and mesenchymal (MES) (U87 cell line) cells at 24h following treatment with >350 clinically relevant drugs generated by the Califano Lab. The top six drugs predicted to target MES and PN subpopulations were tested in acute slices from GBM resections allowing direct characterization of the drug-mediated depletion of subpopulations predicted to be sensitive in adjacent drug vs. vehicle control treated slices [Zhao et al., 2021].Confirming our predictions, we observed statistically significant depletion of the MES and PN compartments by 3 of 3 (Dasatinib, Bexarotene and Prednisone) and 2 of 3 (Givinostat and Ixazomib) OncoTreat-predicted drugs, respectively. Given its high validation rate, this study paves the road and provides significant insight into the future development of combination trials where these drugs may be administered either concomitantly or sequentially. Moreover, the computationally inferred differential activity of subtype-specific MR proteins (CD44, OLIG2, KDM2B, EPHB3, TOP2B and others) was experimentally validated by FACS in cultured patients-derived GBM neurospheres.

#5073 Whole transcriptome spatial molecular imaging in matched pre and post treatment specimens to identify treatment-associated effects in pancreatic adenocarcinoma.

Beatrice W. Awasthi[1], Dennis Gong[1], Nicole Lester[1], Jung Woo Bae[1], Xunqin Yin[1], Ashley Heck[2], Elizabeth Stirling[2], Maximilian Walter[2], Megan Vandenberg[2], Margaret Hoang[2], Prajan Divakar[2], Joe Beechem[2], David T. Ting[3], William L. Hwang[1]

[1]Center for Systems Biology, Department of Radiation Oncology, and Center for Cancer Research, Massachusetts General Hospital, Boston, MA,[2]Bruker, Seattle, WA,[3]Mass General Cancer Center, Harvard Medical School, Charlestown, MA

Pancreatic ductal adenocarcinoma (PDAC) is among the deadliest cancer types with a poor therapeutic response. The molecular heterogeneity of PDAC has been well-studied using dissociated single-cell approaches, but clinically relevant spatial architecture remains poorly understood. Innovations in spatial profiling approaches over the years have facilitated *in situ* annotation and mapping of cell types in PDAC tumors. Nevertheless, spatial imaging at subcellular resolution has been limited to only a few thousand targets at a time, due to the challenges in attaining acceptable resolution as target panel sizes increase. Moreover, increased data sparsity with higher panel sizes greatly increases the challenges of cellular annotation. Consequently, the analysis of cellular programs has been limited to preselected targets. A whole-transcriptome (WTx; 18,985 targets) panel for spatial molecular imaging has recently been developed that enables unbiased discovery and spatial annotation of novel gene programs that may be missed with smaller panel sizes. These signatures could parse out nuanced clinically relevant transcriptional features, including subtype and intercellular interactions. We previously applied a 1000-plex (1K) spatial transcriptomics panel to evaluate post-treatment tissue remodeling in 13 unmatched primary PDAC specimens. Here, we analyzed five matched pre-treatment core biopsies and post-treatment resection specimens at subcellular resolution at both 1K panel and whole transcriptome levels on consecutive sections. This analysis provides the first direct window into treatment-induced effects in PDAC. With the WTx panel, we identified a mean of >1000 unique genes per cell, and up to 1,700 transcripts, with up to 125,000 cells analyzed per matched pair. To overcome the challenge of cellular annotation in the context of increased sparsity in the WTx data, we first annotated cells using the 1K panel, then used these labels to guide the WTx annotations. Merging our transcriptional annotations with hematoxylin and eosin-staining of FFPE sections annotated by a board-certified pathologist gave insight into the cellular composition of pre-and post-treatment samples and potential intercellular interactions around specific features and cell populations of interest. This study highlights new strategies for analyzing whole-transcriptome spatial transcriptomics at subcellular resolution and the potential thereof to identify novel treatment-associated effects.

#5074 Estimation of homologous recombination deficiency map from spatial transcriptomics.

Jeongbin Park, **Yuchang Seong**, Dongjoo Lee, Hongyoon Choi

Portrai, Inc., Seoul, Republic of Korea, Seoul, Korea, Republic of

Background: Homologous recombination deficiency (HRD) is a predictive biomarker for various anti-cancer drugs such as poly(ADP-ribose) polymerase inhibitors (PARPi) and platinum-based chemotherapeutic agents. The HRD score has been estimated using genomic and molecular assays, even though due to cancer heterogeneity, HRD could vary in the tumor microenvironment. Here, we developed a model to predict HRD score by estimating copy numbers and calculating loss of heterogeneity (LOH), large-scale state transitions (LST), and telomeric-allelic imbalance (TAI) scores resulting in exhibiting both spatial coherence and heterogeneity, making them suitable for clinical biomarkers addressing tumor heterogeneity. To estimate these scores with counts from ST data, STARCH algorithm was utilized and the copy number for each gene across spots within cancer clones was obtained. HRD scores are determined by adding together LOH, TAI, and LST. We applied these analyses to Visium and VisiumHD data from breast cancer samples and visualized the spatial distribution of the HRD score within the cancer cell map in the tumor microenvironment.
Result: This method was applied to both Visium and Visium HD data to show the heterogeneity in the HRD score. The HRD scores effectively reflected spatial patterns and demonstrated high heterogeneity, highlighting their potential as clinical biomarkers. Furthermore, when applied to direct Visium data (Visium FF), the HRD scores predicted using only gene expression data yielded similar conclusions to those calculated considering both gene expression and allele counts. Notably, the concordance between the two approaches was higher when normal spots were not separately input. Additionally, running the analyses separately or collectively produced comparable conclusions.
Conclusion: By developing a model to predict HRD scores from ST data, it becomes possible to analyze the relationship between pathophysiological mechanisms and HRD across various cancer types. This approach not only enhances our understanding of tumor biology but also enables the prediction of HRD score maps while accounting for spatial heterogeneity in predictive biomarkers. Such advancements could improve personalized treatment strategies and clinical decision-making for patients.

#5075 Detection and spatial analysis of mRNA delivered by lipid nanoparticles through spatial transcriptomics.

Jeongbin Park[1], **Yuchang Seong**[1], Dongjoo Lee[1], Jinyeong Choi[1], Hanna Jung[2], Somin Lee[3], Hongyoon Choi[1], Hyungjun Im[1]

[1]Portrai, Inc., Seoul, Korea, Republic of,[2]Department of Applied Bioengineering, Graduate School of Convergence Science and Technology, Seoul National University, Seoul, Korea, Republic of,[3]Department of Molecular Medicine and Biopharmaceutical Sciences, Graduate School of Convergence Science and Technology, Seoul National University, Seoul, Korea, Republic of

Background: Lipid nanoparticle (LNP) platforms are widely used for mRNA delivery, but the precise distribution of mRNA within tissues remains poorly understood. Spatial transcriptomics technologies have enabled detailed mapping of RNA localization in tissues. In this study, LNPs targeting the spleen were used to encapsulate tdTomato mRNA, and spatial transcriptomics was employed to analyze its distribution and interactions within the spleen microenvironment.
Methods: Spleen-targeting LNPs were developed to deliver codon-optimized tdTomato mRNA. Space Ranger and advanced alignment algorithms were used to distinguish endogenous and externally delivered mRNA. Overlapping sequences with mouse hemoglobin genes were excluded to ensure accurate analysis. After mapping tdTomato mRNA onto the ST data, we performed colocalization analysis with the ST data of the mouse spleen to demonstrate the precise distribution of tdTomato mRNA within the tissues.
Results: The transcriptomic analysis of mouse spleen tissues revealed distinct red pulp, white pulp, and marginal zone morphologies. Similar gene expression profiles between control ('Con') and experimental ('Exp') samples indicated comparable tissue conditions. tdTomato mRNA was observed exclusively in the 'Exp' sample, predominantly localized in the red pulp, aligning with the expected LNP delivery pathway via blood circulation. Spatial correlation analysis in the 'Exp' sample revealed that hemoglobin genes were highly correlated with tdTomato mRNA, while ribosome-related genes showed the lowest correlation. RNA degradation analysis demonstrated intact tdTomato mRNA in the medulla, whereas significant degradation was observed in the cortex. Compared to fluorescence imaging, the platform used provides distinct advantages, such as absence of autofluorescence, offering PCR-level sensitivity, and enabling absolute quantification, thereby enhancing the precision of these findings.
Conclusion: Using spatial transcriptomics, the study successfully mapped the distribution of externally delivered tdTomato mRNA within tissue and analyzed its spatial interactions with the surrounding microenvironment. RNA degradation patterns revealed distinct microenvironments affecting mRNA stability. These findings provide insights into the pharmacokinetics of mRNA-loaded LNPs and support the development of optimized nucleic acid delivery systems.

#5076 Unravelling melanoma brain metastasis tumor microenvironment characteristics using spatial transcriptomics.

Clemence J. Belle[1], Sandra Brosda[1], Vanessa F. Bonazzi[1], Victor Bulteau[1], Thomas Stuart[1], Zherui Xiong[2], Duncan Lambie[3], Peter A. Johansson[2], Lauren G. Aoude[1], Kalpana Patel[1], Samantha J. Stehbens[4], Mitchell S. Stark[1], Nikolas K. Haass[1], Wen Xu[3], Mark B. Pinkham[3], Matthew C. Foote[3], Sarah Olson[3], Victoria Atkinson[3], Arutha Kulasinghe[1], Quan H. Nguyen[2], Andrew Barbour[1]

[1]Frazer Institute, The University of Queensland, Woolloongabba, Australia,[2]QIMR Berghofer Medical Research Institute, Herston, Australia,[3]Princess Alexandra Hospital, Woolloongabba, Australia,[4]Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, St Lucia, Australia

Background: Advanced melanoma is an aggressive cancer with a high metastatic potential often resulting in melanoma brain metastases (MBM). The complexity of the MBM tumor microenvironment (TME) impacts tumor progression and therapy response, leading to poor outcomes. Our study integrates spatial transcriptomics profiles with tissue morphology annotations of MBM and clinical data to investigate the TME landscape in relation to treatment and patient outcomes.
Methods: We performed spatial transcriptomics on 21 MBM samples collected after various treatment strategies. All samples were preserved in formalin-fixed paraffin-embedded (FFPE) blocks and profiled using the Visium Spatial Gene Expression kit (10X Genomics) with a 6.5x6.5mm Visium Capture slide. All sequencing was performed on a NovaSeq6000 platform (Illumina) with an average of 71,391 reads per spots. H&E slides were annotated by our study pathologist (DL) to enable pathology-guided cluster analysis. Spatial transcriptomics data analysis was conducted using Seurat R pipeline which involved data quality check, normalization, integration, cell type annotation and deconvolution, and clustering. Further differential gene expression analyses were performed between different identified tumor clusters or between clinical groups, leading to the identification of enriched pathways and cell-cell interaction patterns.
Results: Our phenotyping of 25,208 spots revealed the presence of diverse cell populations, including neurons, melanoma, plasma, endothelial, stromal, and myeloid cells. We identified 8 distinct functional clusters characterized by specific gene expression and activated pathways. These include 5 tumor clusters with different immune infiltration or expression profiles, identifying differences in cell metabolism and heterogeneity in the TME. Importantly, we found MBM from patients exposed to radiation were significantly enriched for epithelial-to-mesenchymal transition pathway and genes related to cell adhesion. We further showed a reduced immune response in patients received radiation compared those who did not, and we noted that an active immune response was generally positively correlated with survival.
Conclusions: Our study is the largest spatial analysis of MBM reported to date. Advances in spatial technologies provide novel insights into the structure and composition of MBM TME. Our findings highlight the importance of spatial context in understanding MBM biology and the potential of spatial transcriptomics in advancing precision oncology.

#5077 Single-cell and bulk RNA sequencing reveal SPINK1 and TIMP1 as epithelial cell marker genes linked to colorectal cancer survival.

N. N. Al-Bzour[1], Z. N. Abu-Rjai'[2], A. N. Al-Bzour[1], A. Qasaymeh[1], L. Chen[3], A. Saeed[4], A. Saeed[5];
[1]Jordan University of Science & Technology, Irbid, Jordan, [2]Yarmouk University, Irbid, Jordan, [3]University of Pittsburgh, Pittsburgh, PA, [4]University of Vermont Medical Center, Burlington, VT, [5]UPMC Hillman Cancer Center, Pittsburgh, PA

Introduction: Colorectal cancer (CRC) is a leading cause of cancer-related mortality, with tumor microenvironment and gene expression playing critical roles in disease progression and prognosis. Identifying specific epithelial cell marker genes linked to survival may enhance prognosis and guide targeted therapies.
Methods: Single-cell RNA (scRNA) data for CRC patients was obtained from TISCH database (n=23176 cells), in which we identified EMGs using differential expression clustering analysis. Bulk RNA data was downloaded from The Cancer Genome Atlas (TCGA-COAD, n = 375). Using TCGA-CRC data, EMGs were analyzed using univariate Cox model, followed by Least Absolute Shrinkage and Selection Operator (LASSO) to reduce overfitting. Significant genes from LASSO were then fitted in a multivariate Cox model to screen independent predictors of survival. A survival-related EMG signature was developed to calculate a risk score, classifying patients into low- and high-risk groups based on the median risk score. We validated the risk score using GSE17536 CRC dataset (n=177). xCELL was used to perform immune deconvolution analysis.
Results: 142 EMGs were obtained from scRNA and analyzed for prognostic significance in the TCGA bulk-RNA dataset. Univariate Cox analysis retrieved 34 survival-associated genes and was screened using LASSO-cox resulting in 6 genes: *CA2, CLCA1, ITLN1, SPINK1, SPINK4,* and *TIMP1.* Multivariate Cox analysis showed that SPINK1 and TIMP1 were independent predictors of survival with SPINK1 associated with better outcomes (HR: 0.87, p-value: 0.009), and TIMP1 with worse outcomes (HR: 1.81, p-value <0.001). The 2 survival-related EMG risk score was calculated, and patients were grouped into high (n = 187) and low-risk (n = 188) groups. The low-risk group showed significantly higher (overall survival) OS and (disease free survival) DFS compared to the high-risk group (Median OS: 23 vs. 21.8 months, p-value = 0.0027; Median DFS: 20.5 vs. 20.3 months, p-value = 0.044). The immune microenvironment analysis showed significant enrichment of dendritic cells, CD8+ central-memory T-cells, class-switched memory B-cells, and macrophages in the high-risk group, while the low-risk group was significantly enriched in gamma delta T-cells, T-helper 1 and T-helper 2 cells, plasma cells, and natural killer cells. In a validation cohort, the low-risk group (n=89) showed better survival outcomes compared to the high-risk group (n=88) (Median OS: 50.2 vs. 35.2 months, Median DFS: 43.8 vs. 24.5 months, p-value = <0.001).
Conclusion: Our results showed a significant role of EMGs in CRC prognosis. Among them, SPINK1 and TIMP1 emerged as independent predictors of survival. Differential immune enrichment patterns between risk groups show the potential of integrating EMGs with immune landscape analysis to refine risk stratification and inform personalized therapeutic strategies.

#5078 Single-cell characterization of a carcinosarcoma patient-derived xenograft model.

Alida Palmisano[1], Yuri Kotliarov[2], Parimal Kumar[3], Ting-Chia Chang[3], Li Chen[3], Brandie Fullmer[3], Alyssa Chapman[3], Amanda Peach[3], Biswajit Das[3], Tomas Vilimas[3], Thomas Forbes[3], Shahanawaz Jiwani[3], Melinda G. Hollingshead[4], Yvonne A. Evrard[5], Sarah Shin[6], Hari Sankaran[2], Julia Krushkal[2], Alice Chen[6], Yingdong Zhao[2], Chris A. Karlovich[3], Mickey Williams[3], Lisa M. McShane[2], James H. Doroshow[6]

[1]General Dynamics Information Technology, Falls Church, VA,[2]Biometric Research Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Rockville, MD,[3]Molecular Characterization Laboratory, Frederick National Laboratory for Cancer Research, Frederick, MD,[4]Biological Testing Branch, Developmental Therapeutics Program, National Cancer Institute at Frederick, Frederick, MD,[5]Advanced Development Research Directorate, Frederick National Laboratory for Cancer Research, Frederick, MD,[6]Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, MD

Carcinosarcoma (CS) is a rare aggressive biphasic tumor characterized by the presence of both carcinomatous (epithelial) and sarcomatous (mesenchymal) components. It can occur in various organs, including uterus and ovaries, and often exhibits rapid progression. The biphasic nature of CS makes it challenging to treat. In this study, we characterized a patient-derived xenograft (PDX) model developed from a 27 year-old female with CS of unknown primary origin in the NCI Patient-Derived Models Repository (https://pdmr.cancer.gov) to understand the transcriptomic features of these different components.

PDX samples of the tumor were iteratively passaged in separate NSG mice, and samples from different lineages across passages P0-P2 were collected for analysis. Twenty-five separate carcinoma/sarcoma regions from 7 samples through laser capture microdissection were obtained for bulk RNA-seq. Fourteen samples were sequenced through single-cell RNA-seq using 10x Genomics Single Cell Chromium, including 2 samples in P0, 8 in P1 and 4 in P2. Mouse reads were excluded using XenoCell. Human-only reads were processed through standard QC filtering, integration and annotation with Cell Ranger, Seurat and other R packages.

After quality-based filtering, we obtained 24,648 single cells from the integrated tumor samples. Through unsupervised clustering, cells separated into 16 Seurat-identified clusters, with consistent percentages of carcinoma and sarcoma components in P0, P1 and P2. Based on gene expression signature scores, each cluster was given a label descriptive of the cluster status. Five clusters had more epithelial/carcinoma characteristics (including one with a distinct ciliated signature), 2 clusters displayed a clear mesenchymal/sarcoma phenotype, 4 had low reads or gene counts, 2 had high cell cycle signature score and 3 clusters did not have a distinct carcinoma or sarcoma status.

We identified differentially expressed genes in each annotated cluster relative to the other clusters and performed gene set enrichment analysis. Individual carcinoma clusters demonstrated upregulation of pathways related to apoptosis, hypoxia and inflammation (p-value < 0.05), while sarcoma clusters demonstrated upregulation of WNT signaling, EMT and angiogenesis (p-value < 0.05). Clusters with unclear carcinoma/sarcoma phenotype showed patterns of expression similar to carcinoma clusters, with the additional activation of oxidative phosphorylation and DNA repair pathways.

Single cell data for a CS PDX model show that the biphasic nature of this histology is stable through passaging. Using single cell data, we identified the presence of clusters that are not fully committed to either the carcinoma or sarcoma phenotype. These clusters could represent transitional cell populations which may provide insight into biological drivers of cell plasticity in this tumor histology.

*The first four authors contributed equally

#5079 Spatial transcriptomics analysis of early-stage ER+/HER2- breast cancer reveals unique prognostic immune-fibroblastic subtypes.

Hannah N. Nyarko[1], Zak Kinsella[1], Daria Kalinska-Lysiak[1], Niamh M.C. Connoly[2], Jochen Prehn[2], John Crown[3], Catherine Kelly[4], William Gallagher[5], Darran O'Connor[1]

[1]Pharmacy and Biomolecular Sciences, Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland,[2]Physiology and Medical Physics, Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland,[3]St. Vincent's Private Hospital, Dublin, Ireland,[4]Mater Private Network, Dublin, Ireland,[5]Conway Institute, University Colege Dublin, Dublin, Ireland

Introduction: ER+ breast cancers have the best prognosis among all known breast cancer subtypes. Nonetheless, a substantial proportion of these patients, approximately 41%, experience late recurrence after at least 10 years after diagnosis. Numerous studies have highlighted the pivotal role of the tumor microenvironment (TME) in ER- breast cancer prognosis. However, this has not been thoroughly explored in the ER+ breast cancer subtype. In this study, we investigated immune and stromal cell diversity in the tumor microenvironment in hormone-dependent breast cancers and their association with survival.
Methods: Using NanoString GeoMx Digital Spatial Profiling (DSP) with the Whole Transcriptome Atlas (WTA), we conducted spatial transcriptomics profiling on formalin-fixed, paraffin-embedded tissue samples (FFPE). Regions of interest (ROIs) were selected from a cohort of 410 early-stage ER+/HER2- patients' tissue microarray (TMA). Guided by Pan-cytokeratin immunostaining (panCK), ROIs were segmented into neoplastic epithelial cells (panCK+) and TME (panCK-). Cell deconvolution was performed to elucidate immune and stromal cell abundances, using Nanostring's Spatialdecon and CIBERSORT tools.
Results: Cell abundances dichotomized into high and low groups were assessed for their association with survival. While the overall lymphocyte abundance and its subpopulations (TCD4 and TCD8 abundances) were not significantly associated with survival, they were associated with increasing Oncotype risk score. On the other hand, high fibroblast abundance was significantly associated with decreasing Oncotype risk score and a better survival outcome as a categorical and continuous variable. Based on an unsupervised clustering of patients by their immune and stromal cell proportions two distinct subgroups of patients were identified; lymphocyte-rich and lymphocyte-deficient. The lymphocyte-deficient group was characterized by high infiltration of fibroblasts and endothelial cells. Survival analysis in the subgroups yielded a significant result with the lymphocyte-rich group being associated with poorer outcomes ($HR = 1.87$, $p = 0.04$). Pathway analyses of differentially expressed genes in the lymphocyte-rich subgroup showed increased activity in immune activation, while the lymphocyte-deficient group was associated with increased activity in the extracellular matrix organization and angiogenesis.
Conclusion: While this finding challenges the conventional understanding of the role of lymphocytes and fibroblasts in cancer research, this complexity throws light on the heterogeneity of the tumor microenvironment in ER+ breast cancers, and can be harnessed to improve risk stratification, for more accurate, individualized therapies.

#5080 Investigation of exome-wide tumor heterogeneity on colorectal tissue-based single cells.

N. Szakallas[1], A. Kalmar[2], B. Bartak[2], B. Molnar[2], I. Takacs[2];
[1]Eotvos Lorand Tudomanyegyetem, Budapest, Hungary, [2]Semmelweis University, Budapest, Hungary

The progression of colorectal cancer is highly influenced by various environmental and genetic conditions, however, we can highlight tumor heterogeneity presenting serious resistance to targeted therapies and having crucial effects on disease outcomes. Motivated by this issue, we aimed to develop an end-to-end single-cell sequencing workflow starting from morphology-derived sample isolation from different regions of a tissue slide continue with exome sequencing to investigate the patterns of tumor heterogeneity.
Colonic biopsy specimens were collected during the endoscopic procedure, then cryosections (15 µm) were prepared and mounted on MMI MembraneSlides. Hematoxylin-eosin staining was applied to visualize the tissue morphology. Single-cell samples were obtained from 12 individual regions of negative control (NEG), tumor-associated normal (NAT), and colorectal cancer (CRC) tissues. Each sample had a diameter of 30-40 µm and a height of approximately 10-20 µm and was cut from a membrane frame slide by laser microdissection method. Following the single-cell DNA extraction and library preparation (Qiagen GmbH, CA, US), we performed paired-end next generation sequencing using NextSeq High Output kit on a NextSeq 500/550 instrument (IIllumina Inc., San Diego, CA, USA). Finally, we implemented bioinformatics analysis focusing on the patterns of tissue heterogeneity in the samples illustrated by CRC-specific mutated genes.
We obtained sequencing data with an average of 30X coverage. We investigated the mutually exclusive and co-occurring gene pairs and found that the co-occurrence of genes corresponding to near-tumor tissues was more significantly detected (such as *TTN-ADGRE1* in CRC with $p<0.05$). Our observations on single-cell tumor heterogeneity revealed that the different regions associated with the tumor - including negative control tissues - displayed altered mutation and heterogeneity profiles. Eventually, we presented the onco-mutation motifs of tissue regions which showed that the occurrence of mutations in genes BRAF (4/12), FBXW7 (2/12), and PCDH17 (1/12) in CRC samples are linked to resistance to EGFR inhibitor therapy. Additionally, the non-zero mutation rate of COL5A1 (1/12) gene in NAT indicates poorer survival outcome of patients. Supplementary, we aimed to present additional therapy resistant mutations characteristic to antiangiogenic, 5-fluorouracil (5-FU), chemo- and cetuximab therapies.
In our study, we identified region-specific heterogeneity motifs and demonstrated the individual and cumulative heterogeneity patterns of CRC and NAT single-cell-like samples. Additionally, we successfully detected high impact actionable mutations that influence therapy response and survival chances.

#5082 Deep learning model for cancer diagnosis in frozen section sentinel lymph nodes with limited annotations.

Joonho Lee, Joonyoung Cho, DoKyung Kim, Yoon-La Choi, Kyungsoo Jung, **Tae-Yeong Kwak**, Sun Woo Kim, Hyeyoon Chang

Deep Bio Inc., Seoul, Korea, Republic of

Introduction: Frozen section (FS) rapidly examines suspected tumor tissue microscopically during surgery, aiding diagnosis and surgical decisions. However, when applying AI or digital pathology to FS examination, stain and scanner variations pose significant challenges in developing robust deep learning-based diagnostic models. This study proposes a method that reduces stain variation to detect cancer in H&E-stained FS images of breast sentinel lymph nodes.
Design: We utilized 19,881 FS WSIs and 11,985 FFPE WSIs. The dataset included FS and FFPE WSIs from 33 organs collected from The Cancer Genome Atlas (TCGA), additional FS and FFPE WSIs from a domestic hospital and the Camelyon16 dataset. We reserved 20% of the FS dataset for evaluation; the remaining data were split into training (75%) and validation (25%). We employed instance-based Multiple Instance Learning (MIL) to identify cancerous patches (512×512 pixels at 10× magnification) in each WSI. After achieving sufficient feature discrimination, we fixed the backbone network. We obtained rough annotations from pathologists on 12 lymph node FS WSIs and trained a classifier with various data augmentation.
Result: Our method shows that the MIL plus classifier-only approach consistently outperforms MIL with full fine-tuning across diverse datasets (Camelyon16 FFPE and domestic FS). By reducing scanner variation, we significantly improved the model's generalization, which is a primary contribution of this study.
Conclusion: These findings suggest that mitigating stain and scanner variations with limited annotations can substantially enhance the performance and generalizability of deep learning models in cancer diagnosis.

Method	Dataset	Scanner	AUC/ROC	Sensitivity	Specificity	F1-score
MIL + Full fine-tuned	Domestic (FS)	Pannoramic 1000(3DHISTECH)	0.9546	0.9337	0.8043	0.9415
	Camelyon 16 (FFPE)	Pannoramic 250 Flash II(3DHISTECH)	0.9223	0.9286	0.7000	0.7879
	Camelyon 16 (FFPE)	NanoZoomer-XR C12000-1(Hamamatsu)	0.9482	0.9400	0.6875	0.7705
MIL + classifier only	Domestic (FS)	Pannoramic 1000(3DHISTECH)	0.9536	0.9337	0.8696	0.9494
	Camelyon 16 (FFPE)	Pannoramic 250 Flash II(3DHISTECH)	0.9241	0.8929	0.8000	0.8197
	Camelyon 16 (FFPE)	NanoZoomer-XR C12000-1(Hamamatsu)	0.9380	0.8800	0.8875	0.8544

#5083 Investigating the impact of T cell receptor repertoire diversity on cancer outcome.

Naja Lange, Asbjorn Kjar, Randi Istrup Juul, Iver Nordentoft, Lars Dyrskjot, Nicolai Juul Birkbak

Department of Molecular Medicine (MOMA), Aarhus University Hospital, Aarhus, Denmark

T cells are key effector cells in the adaptive immune system, playing a central role in the defense against pathogens. However, as we age, T cells undergo functional decline due to immunosenescence, the gradual decay of the immune system with age. This decline negatively affects the function and composition of immune cells, leading to an increased susceptibility to age-related diseases, such as cancer. We hypothesized that characterization of the peripheral blood T cell receptor (TCR) repertoire has the potential to provide insights into the current state of the immune system and serve as a biomarker for cancer progression. To investigate this, we analyzed the peripheral blood TCR repertoire in 5441 non-cancer patients and 450 cancer patients, utilizing sequencing data of the TCR-β chain from multiple cohorts obtained locally and from public sources. In both cancer and non-cancer patients, we observed a decline in the percentage of non-expanded clones, averaging approximately 4.6% ($P = 2.15 \times 10^{-8}$) and 3.5% ($P < 2 \times 10^{-16}$) for every 10-year increase in age, respectively. This decline was accompanied by a proportional increase in the expanded T cell clones with advancing age. Analyses of TCR-β diversity across age groups revealed that, among older individuals, males exhibited lower diversity than females in both non-cancer ($P = 4.9 \times 10^{-5}$) and cancer patients ($P = 5.3 \times 10^{-5}$). Additionally, investigating the relationship between tumor stage and TCR-β diversity showed that lower diversity correlates with more advanced tumor stages. Our results suggest that low TCR diversity represents reduced immune competency and a limited ability to prevent cancer progression.

#5084 Evaluation of single-cell foundation models for cancer outcome predictions.

Haitham Elmarakeby, Ahmed Roman, Shreya Johri, Eliezer M. Van Allen

Dana-Farber Cancer Institute, Boston, MA

Introduction: Tumor single-cell data provide unique insights into the cellular dynamics of cancer progression and therapeutic outcomes. Inspired by advancements in natural language processing (NLP), foundation models are increasingly used in single-cell analyses to uncover biological insights and identify clinical biomarkers. However, the performance of these models on cancer-specific tasks remains underexplored. We present an extensive evaluation of foundation models trained on millions of single-cell profiles for cancer-focused investigations.

Methods: We evaluated nine predictive models-three traditional methods and six foundation models (four Geneformer and two scGPT models), across various downstream tasks. Traditional models used embeddings from high variable genes (HVG), principal components (PCA), and generative model embedding (scVI), each paired with a random forest classifier for downstream classification. The evaluated foundation models varied in architecture sizes, pretraining dataset sizes (30M to 95M cell profiles), and training datasets, which were either generic or cancer-specific. We assessed the quality of representations by measuring their predictive performance on lung cancer treatment status (TKI treated vs. treatment-naive patients) and breast cancer subtypes (ER+ vs. TNBC). We examined how model characteristics influenced their performance in these domains for all tasks.

Results: Zero-shot evaluations of the foundation models showed superior single-cell data clustering, measured by average silhouette width (ASW), compared to traditional methods (HVG, PCA, scVI), suggesting that pre-trained embeddings from millions of single-cell profiles may offer enhanced representations. We thus evaluated the learned representation on downstream predictive tasks, showing that foundation models with a larger pretraining dataset (95M cells), cancer-specific training, larger input size (4096 genes), and deeper model (12 layers) achieved the best performance among foundation models (F1=0.97) for predicting treatment status in lung cancer. In contrast, smaller and generic foundation models had significantly lower performance, however, additional self-supervised training on task-specific data rescued their performance to match larger foundation models. Similar trends were observed in predicting breast cancer subtypes. Notably, the best foundation model's performance was not significantly different from the best traditional method (PCA) in predicting breast cancer subtypes.

Conclusions: These results highlight key factors driving foundation model performance on cancer-specific tasks. While current foundation models show inconsistent contributions to cancer-specific questions, observed performance trends highlight their potential to enhance single-cell data representation and drive significant advancements in cancer research and precision medicine.

#5085 Network toxicology and single-cell RNA sequencing to reveal the mechanism of ethylene oxide inducing blood cancers.

Bitian Zhang[1], **William C. Cho**[2], Dongjie Wang[1]

[1]ICM & State Key Lab of Research on Bioactivities & Clinical Applications of Medicinal Plants, The Chinese University of Hong Kong, Hong Kong SAR, China,[2]Department of Clinical Oncology, Queen Elizabeth Hospital, Hong Kong SAR, China

Introduction: During the COVID-19 pandemic, ethylene oxide (EO) was extensively utilized for mask disinfection to curb viral transmission. Despite its efficacy as a disinfectant, EO is a recognized carcinogen associated with an increased risk of leukemia and lymphoma. Understanding the carcinogenic dangers of EO exposure, especially in large-scale mask sterilization, is crucial for public health.
Methods: This study investigated the molecular mechanisms linking EO exposure to the development of leukemia and lymphoma. We employed RNA sequencing data from The Cancer Genomes Atlas to construct prognostic models using LASSO Cox regression, Kaplan-Meier survival analysis, and multivariate survival regression. Key genes associated with each type of cancer were identified. Single-cell RNA sequencing was also utilized to examine gene expression within the tumor microenvironment. Enrichment analyses were conducted to identify the biological pathways involved, and cell-cell interactions, along with transcription factors, were analyzed to elucidate EO-induced oncogenic processes.
Results: Our prognostic models identified two key genes for leukemia - ACKR3 and CCZ1B, and one for lymphoma - DECR2. Survival analysis revealed that high expression levels of ACKR3 (HR = 5.9, p = 1.5e-4) and CCZ1B (HR 0.15, p = 6.1e-5) are significantly associated with prognosis in leukemia patients. For lymphoma, DECR2 (HR = 19.85, p = 1.7e-4) similarly correlated with poorer survival rates. Single-cell RNA sequencing data demonstrated that ACKR3 and CCZ1B are predominantly expressed in monocytes/macrophages within the leukemia microenvironment, while DECR2 is highly expressed in advanced-stage lymphoma. Enrichment analyses indicated that apoptosis and cell cycle regulation pathways were significantly disrupted in both malignancies. Furthermore, critical cell-cell interactions involving ligand-receptor pairs (e.g., CXCL12-CXCR4) and transcription factors SPI1 (p = 0.001) and STAT1 (p = 0.003) were identified as potential mediators of EO-induced oncogenic processes.
Conclusions: Prolonged EO exposure is associated with significant alterations in immune cell function and apoptotic mechanisms, thereby increasing the risk of developing leukemia and lymphoma. The identified prognostic genes and disrupted pathways provide insights into the molecular underpinnings of EO-induced carcinogenesis. These findings highlight the necessity for alternative disinfection methods and the implementation of stricter regulatory measures to mitigate the long-term carcinogenic risks posed by EO.

#5086 Machine learning prediction and single-cell landscape of distant organ metastasis in non-small cell lung cancer.

Zaid Nassar Abu Rjai'[1], Ahmad Majid Bani-Ata[2], Ayah N. AL-Bzour[2]

[1]Yarmouk University, Irbid, Jordan,[2]Jordan University of Science and Technology, Irbid, Jordan

Introduction: Management of non-small cell lung cancer (NSCLC) depends on the presence and site of distant metastasis. Machine learning (ML) models have shown promise in analyzing clinical and molecular data to predict cancer outcomes and prognosis. Advancements in single-cell RNA sequencing (scRNA) provide deeper understanding of the tumor microenvironment (TME) and in metastasis. We aim to analyze NSCLC metastasis across different organs, investigate the clinical and molecular predictors of distant organ metastasis, and the single-cell landscape driving tumor metastasis.

Methods: We used the cBioportal and the Gene Expression Omnibus (GEO) databases to retrieve clinical and scRNA data from the MSKCC Metastasis Cohort and GSE131907. The MSKCC dataset included 4,601 NSCLC patients with distant organ metastasis to adrenal, bone, CNS/brain, liver, lung, mediastinum, and pleura. Seven random forest classifiers (RFC) were built for each organ metastasis and trained on clinical and genomic features like age, sex, race, histology, overall survival, fraction genome altered (FGA), microsatellite instability (MSI), mutation count, tumor mutational burden (TMB), and tumor purity. Models were evaluated using area under the receiver operating characteristics curve (AUC/ROC), 10-fold cross-validation (CV), accuracy score, and feature importance. Single-cell RNA data was filtered to include primary tumor samples (n=65,702) and brain metastasis (n=28,429) for cell distribution analysis.

Results: Distant metastasis occurred in 3,784 (82%) patients. The most common metastatic sites were lung (41%), bone (38%), pleura (35%), CNS (26%), liver (18%), adrenal gland (12%), and mediastinum (8.7%). The RFC-Mediastinum model showed the highest accuracy (0.89), AUC (0.61), and 10-fold CV (0.90), followed by RFC-Adrenal (0.86, AUC=0.62, 10-fold CV=0.86). The lowest performance was seen in RFC-Lung (0.57, AUC=0.56, 10-fold CV=0.52). The most important predictive features across all models were FGA, MSI, TMB, and mutation count, while histology was the least important. Single-cell analysis revealed that brain metastasis had a predominance of stromal cells, particularly epithelial, compared to primary tumor samples. Primary tumor samples consisted mostly of CD8+ T-cells and macrophages. COL1A1/2 genes were significantly expressed in brain metastatic stromal cells, suggesting extracellular matrix remodeling in the TME.

Conclusion: Our study highlights the potential of ML models in accurately predicting distant organ metastasis in NSCLC. Variations in TME cellular compositions may be driven by complex intercellular communications in metastatic disease.

#5087 Single cell AI-prediction of protein biomarker on novel whole slide unstained blood smear images.

F. Wee[1], W. W. Yim[1], J. Meng[1], J. Lim[1], L. Chong[1], C. R. Joseph[1], L. Song[2], J. Iqbal[2], M. Lau[3], D. W. Lim[4], J. Yeong[1];
[1]A*STAR - Inst of Molecular and Cell Biology (IMCB), Singapore, Singapore, [2]Singapore General Hospital, Singapore, Singapore, [3]A*STAR - Bioinformatics Institute (BII), Singapore, Singapore, [4]National Cancer Centre Singapore, Singapore, Singapore

Introduction

Identification of immune cell types within bodily fluids, including blood samples, plays a critical role in early detection and disease monitoring within public health initiatives. Although protein biomarkers present in blood samples are useful for clinical assessment, the process of sample preparation for immunostaining is time-consuming and costly. In this study, we present an efficient and rapid preparation procedure for affixing cells on a glass slide for downstream histological staining. Furthermore, using $CD3^+$ T cells as a paradigm, our study concurrently investigates if deep learning models can predict these cells from unlabeled brightfield images of these cell smears. The prediction models could potentially aid in the initial screening process of blood samples and identify samples that require further testing, therefore increasing the throughput of obtaining cell identification results.

Material and Methods

Buffy coat samples were separately processed and spread as monolayers across positively charged glass slides, where they were subjected to fluorescent IHC (fIHC) staining (CD3 and DAPI) using the automated clinical stainer Leica BondMax (Leica Biosystems, Australia). Whole slide fIHC and brightfield differential interference contrast (DIC) images were acquired concurrently with Axioscan 7 Slide Scanner (Zeiss, Germany).

Cell segmentation and guided thresholding of $CD3^+$ cells were performed on the fIHC image using HALO (Indica Labs, U.S.A.) to obtain cell centroids and cell-level ground truth labels. Next, each cell was extracted from the DIC image as a 56×56-pixel image patch, yielding 200,000 image patches (equal ratio of $CD3^+$:$CD3^-$ cells) that were split 80:20 for training and validation respectively. A held-out dataset consisting of 56,000 image patches was used for testing. The model architecture used is a custom ResNet18 convolutional neural network, and its performance was evaluated with metrics such as F1 score and area under the receiver operating characteristic curve (AUROC).

Results

Cell smear slides were successfully stained and imaged, where intact cell morphology was observed across all images. The $CD3^+$ prediction model attained an F1 score of 0.638 and AUROC of 0.87 when applied to the held-out test dataset, indicating that it can differentiate between the unstained brightfield images of $CD3^+$ and $CD3^-$ cells.

Conclusion

Our cell smear preparation procedure is straightforward and rapid, where the slides could be further processed in an automated staining and imaging pipeline. The acquired images can then be used for identification of protein biomarker expression via IHC. If prediction via DL models is applied, then immunostaining could be omitted. Future works include applying this workflow to all other samples of bodily fluids from patients to examine other disease-related biomarkers, and to build prediction models on other protein biomarkers.

#5088 Single cell-based elucidation and pharmacologic targeting of master regulator dependencies in small cell lung cancer (SCLC) subpopulations.

Lucas ZhongMing Hu[1], Anish Thomas[2], Andrea Califano[1]

[1]Columbia University Irving Medical Center, New York, NY,[2]National Cancer Institute, Bethesda, MD

Small Cell Lung Cancer (SCLC) is a highly aggressive and deadly neuroendocrine lung cancer with no major effective treatments. SCLCs are usually detected after wide metastasis, which leads to extremely low five-year survival rate (around 7%). To address this challenge, we leveraged network-based computational approach to first dissect the heterogeneity of SCLC tumors and to discover Master Regulator (MR) proteins representing pharmacologically accessible, mechanistic determinants of molecularly distinct SCLC cell populations. Specifically, we first profiled transcriptomes of 182,189 cells from 41 fresh SCLC human tissue samples, acquired from 14 patients. Samples are collected from both primary site (lung) and metastases (Mediastinum, liver and adrenal gland). Built upon this dataset, we reverse-engineered single-cell based SCLC-specific regulatory networks through ARACNe algorithm. Using these networks, we then measured protein activity of over 6,000 regulatory and signaling proteins for all the cells via VIPER. To dissect intra-tumor heterogeneity of SCLC, protein activity-based analysis of tumor cells identified cell subpopulations representing distinct differentiation and proliferation stages. Our analysis helped identify cell populations presenting highly aberrant activity of proliferative MRs, which are termed as “Tumor Checkpoint” regulatory module. We then identified SCLC cell lines showing high protein activity similarity with the target tumor cell populations using rank-based statistics. Following perturbation of the selected cancer cell lines with hundreds of oncology drugs, we identified potent drugs that could invert the activities of the MRs of target tumor subpopulations using OncoTreat algorithm. The identified drugs are subsequently validated using *in vivo* mouse model. The outcome of this project is four-fold: First, the patient derived SCLC single-cell RNA-Seq dataset generated in this project represents the largest cohort of this kind up to date; Second, our analysis helps study SCLC intra-tumor heterogeneity from the protein activity angle, which is complementary to gene expression measurement alone; Third, our drug perturbation dataset is the first whole-genome based drug perturbation gene expression profile in SCLC cell lines. Fourth, our comprehensive analysis identifies drugs effectively induce tumor population demise in SCLC. Currently, we are working on the drug perturbation experiment on SCLC cell lines to finalize potent drug predictions.

#5089 Cross-platform comparison of snRNAseq, Visium, and Xenium on lung and breast cancer samples.

Mariia Bilous[1], **Daria Buszta**[2], Jonathan Bac[1], Yixing Dong[1], Sylvie Andre[2], Marina Alexandre-Gaveta[2], Senbai Kang[1], Krisztian Homicsko[2], Raphael Gottardo[1]

[1]Data Science Center, CHUV Lausanne University Hospital, Lausanne, Switzerland,[2]Oncology, CHUV Lausanne University Hospital, Lausanne, Switzerland

Background: Spatial transcriptomics is revolutionizing biology and pathology of cancer by enabling the spatial analysis of RNA profiles in formalin-fixed tissues. However, the comparative performance of different platforms and their potential complementarity remain unclear.
Methods: We conducted a comprehensive analysis of single-nuclei RNA sequencing (snRNAseq, n=15), full-transcriptome Visium v2 (n=10), and Xenium (n=40) on lung and breast cancer samples. This allowed a direct comparison between Visium and Xenium and an assessment of how snRNAseq can complement these spatial platforms.
Results: Our findings highlight that Xenium provides high-resolution spatial data with single-cell accuracy and robust performance on FFPE tissues. We demonstrate that snRNAseq enhances Xenium analysis by facilitating automated cell annotation, quality control, and imputing missing gene expression profiles. Although Visium's full transcriptome capabilities offer broader coverage, its resolution is limited and remains a challenge for interpretation, even when refined with super-resolution modeling (e.g., BayesSpace).
Conclusion: Xenium excels in resolution and single-cell spatial mapping, seamlessly integrating with snRNAseq without requiring additional normalization. While Visium offers full transcriptome data, its resolution restricts biological interpretation. Together, Xenium and snRNAseq provide a powerful framework for spatial transcriptomics, advancing insights into tissue biology and pathology.

#5090 Bioinformatics analysis of the role of asymmetric self renewal associated genes in the cancer progression.

S. Hwang, M. Lee, J. Cho, M. Kim, S. Lee, M. Noh;
Seoul National University, Seoul, Korea, Republic of

Adult stem cells possess a unique ability for asymmetric self-renewal, enabling the simultaneous production of non-stem cell progenitors and self-renewing stem cells, distinguishing them from symmetrical cell division. In cancer cells, the regulation of cell division is often disrupted to sustain proliferation. However, specific patterns might be essential for evading cell death and adapting to the tumor microenvironment. In this study, transcriptomic data from engineered p53-expressing and p53-null mouse fibroblasts were re-analyzed to identify expression signatures of asymmetric self-renewal associated (ASRA) and p53/IMPDH2-related self-renewal patterns associated (SRPA). Pathway analysis revealed that ASRA signatures were enriched in immune regulation and angiogenesis pathways, in addition to cell cycle regulation. Genomic analysis using The Cancer Genome Atlas (TCGA) database demonstrated correlations between ASRA signatures and survival outcomes, as well as molecular properties across various cancer types, including stomach adenocarcinoma (STAD). Additionally, public single-cell RNA sequencing (scRNA-seq) and clinical dataset analyses revealed the role of ASRA genes in the interactions between tumor cells and surrounding immune and stromal cells. These findings suggested that targeting ASRA signatures is a promising strategy for developing novel cancer therapies.

#5091 Exploration of conversion therapies target in pancreatic cancer with liver metastasis in single cell spatial transcriptomic levels.

Zijin Xu[1], Qiang Zhou[1], Ying Ying[1], Zeyuan Cao[2], Wenquan Wang[1], Huaxiang Xu[1], Liang Liu[1]

[1]Department of Pancreatic Surgery, Zhongshan Hospital Fudan University, Shanghai, China,[2]State Key Laboratory of Genetic Engineering, Fudan University Human Phenome Institute, Shanghai, China

Objectives: Multidisciplinary treatment drives conversion surgery of pancreatic cancer with liver metastatic clinical cases with unresectable features. The adjuvant regime included AG and FOLFIRINOX and partial cases exhibited no poor response for the treatment. We analyzed single cell spatial transcriptome data to explore biomarkers of therapy.
Materials and Methods: The GSE202051 and GSE199102 cohorts derived from GEO database. The quality control, analysis, and plotting of single cell spatial transcriptome rely on the R packages including R packages ISCHIA, robustbase, data.table, ggplot2, Seurat, dplyr, factoextra, cluster, showtext, gridExtra, and pdftools.
Results: Malignant biomarkers in liver metastatic samples We drew dim plots to display spatial distribution of various types cells in primary and liver metastatic pancreatic cancer samples. We selected subset of tumor epithelial cells to analyze clusters and displayed c0-c26 tumor epithelial clusters in primary and liver metastatic two groups with dim plots and bar plot. The clusters among c0(59.2%), c5(32.8%), c6(32.4%), and c10(21.9%) possessed more enrichment distribution in liver metastatic region. We used the function FindAllMarkers to analyze top differentiation expression genes in clusters and found three prominent genes including TNFSF14, CYP2E1, and LCN2(Fig1).Stromal microenvironment evolution driven liver metastatic biomarkers gene We calculated and settled each cell type proportion data in each sample based on spatial transcriptome information. Subsequently, we drew the correlation heatmap to discover the positive correlation of TNFSF14 expression levels in tumor epithelial cells with endothelial cells(R=0.64, pvalue=0.032) and negative correlation of CYP2E1 with CAF(R=-0.91, pvalue<0.001) in primary cell type proportion. We identify CYP2E1 driving myCAF changes in stromal microenvironment. For the purpose of exploring extinct myCAF subtypes in volution, we also displayed the correlation heatmap to discover the correlation results of c0-c14 myCAF clusters with CYP2E1 gene expression. Interestingly, only positive result demonstrated positive correlation between c5 cluster and CYP2E1 gene(R=0.76, pvalue<001). The heatmap displayed that CYP2E1 expressed prominently in c5 cluster myCAF. Simultaneously, we identified the expression tendency in samples with neoadjuvant therapy and displayed the positive correlation between CYP2E1 and poor response(Fig2).
Conclusion: Our study used single cell spatial transcriptome data with in-depth explaining spatial distribution and expression levels and explored vital biomarker CYP2E1 in pancreatic cancer with liver metastasis. A series of experiments and clinical trials would be designed and conducted by our team in future.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Pharmacokinetics and Pharmacodynamics of Cancer Therapeutics Poster Session

#6393 Patient selection, target engagement and pharmacodynamic markers of WRN inhibitor GSK4418959 (IDE275).

Yanhua Rao[1], Gabriele Picco[2], Diana M. Munoz[3], Susan Merrihew[1], Yang Lee[4], Anjana Srivatsan[3], Marya Liimatta[3], Yang Peng[1], Thomas E. Angel[1], Robert Silvers[1], Joshua E. Cottom[1], Matthew G. Rees[5], Melissa M. Ronan[5], Jennifer A. Roth[5], Raynold Shenje[1], Brian T. Jones[3], Edward J. Brnardic[1], Michael P. DeMartino[1], Geeta Sharma[4], Mathew J. Garnett[2], Joshua P. Taygerly[3], Michael A. White[3], Benjamin Schwartz[4]

[1]GSK, Upper Providence, PA,[2]Wellcome Sanger Institute, Cambridge, United Kingdom,[3]IDEAYA Biosciences, South San Francisco, CA,[4]GSK, Cambridge, MA,[5]Broad Institute of MIT and Harvard, Cambridge, MA

GSK4418959 (IDE275) is a clinical-stage WRN helicase inhibitor designed to treat MSI-H cancer. This compound phenocopies WRN genetic inactivation, with exquisite MSI-H selectivity in large panel of cell lines. Results from extensive cell line profiling indicate a strong correlation between MSI score and sensitivity to the compound. Interestingly, sensitivity to the compound also correlates with the TA-repeat prevalence of individual cell lines. There appears to be no bias toward specific genetic mutations in the dMMR panel, and the compound's sensitivity is not affected by the driver mutation status of the tested cell lines or models. These findings support the use of dMMR and MSI-H as patient-selection biomarkers for GSK4418959 (IDE275). A mass spectrometry-based target occupancy assay was developed to measure WRN target engagement by GSK4418959 (IDE275), using a covalent WRN inhibitor as a probe. GSK4418959 (IDE275) demonstrated dose-dependent target occupancy in cells, showing that over 90% target occupancy is required to inhibit growth. This assay was successfully applied to xenograft tumors, demonstrating dose-dependent WRN occupancy by GSK4418959 (IDE275) in vivo. GSK4418959 (IDE275) induced significant DNA damage selectively in MSI-H cells, but not in MSS cells. DNA damage markers such as gH2AX and pKAP1 were strongly induced both in vitro and in vivo. Immunohistochemistry (IHC) results suggested dose- and time-dependent induction of these markers in MSI-H xenograft models by GSK4418959 (IDE275), providing evidence to support their use in monitoring drug response in clinical settings.

IMMUNOLOGY: CAR-T Cells
Poster Session

#6395 Synthetic pathway activators enhance CAR-T functional persistence and prevent T cell exhaustion.

Lan Chen[1], Thomas Gardner[1], Beatriz Millare[1], Anzhi Yao[1], Josephine Giles[2], Ashley Cass[1], Suchismita Mohanty[1], Jeremy Chen[1], Alma Gomez[1], David DeTomaso[1], Aaron Cooper[1], Levi Gray-Rupp[1], John Wherry[2], Nicholas Haining[1]

[1]Arsenal Biosciences, South San Francisco, CA,[2]University of Pennsylvania, Philadelphia, PA

The emergence of T cell exhaustion in CAR-T cells used to treat solid tumors remains a barrier to clinical efficacy. T cell engineering approaches are increasingly used to enhance CAR-T cell function, but to date few approaches directly modulate the development of T cell exhaustion. We have used large scale screening of synthetic transmembrane signaling molecules to identify a class of SPA (Synthetic Pathway Activators) that result in constitutive STAT1/STAT3 activity with marked gains of CAR-T cell anti-tumor potency, expansion, and persistence both in-vitro and in-vivo. In vitro, CAR-T cells expressing SPA displayed higher proliferation in response to both TCR and tumor antigen stimulation, and secreted more Granzyme B and IL-10 cytokines, but did not show cytokine-independent proliferation. SPA-expressing CAR-T cells also retained superior function relative to control CAR-T cells in a repetitive stimulation assay that recapitulates T cell exhaustion. In vivo, SPA-expressing CAR-T cells showed greatly enhanced potency in both 786-O and A498 xenograft models of Renal Cell Carcinoma, and demonstrated increased T cell expansion followed by rapid contraction after tumor clearance. Mechanistic studies utilizing temporal RNAseq data and pseudotime analysis of SPA expressing cells throughout the 14-day RSA assay revealed a divergent trajectory of T cell differentiation in comparison to control CAR-T cells, with greater maintenance of memory marker expression. Importantly, SPA-expressing CAR-T failed to induce TOX - a canonical transcriptional regulator of exhausted T cells - compared to control CAR-T cells. ATAC-Seq analysis further confirmed that the TOX locus remains inactive in SPA cells following chronic antigen stimulation, while unmodified CAR-T cells develop characteristic open chromatin regions surrounding the TOX locus. Thus the SPA module is a T cell intrinsic enhancement that boosts CAR-T potency and persistence by limiting the development of exhaustion. Moreover, it suggests that persistent STAT3/STAT1 activation may provide a general approach to modulate the emergence of T cell exhaustion.

#6401 *In vivo* mRNA CAR-T cell engineering using peptide based targeted nanoparticles.

Gilles Divita[1], Audrey Grunenberger[2], Lea Cabrera[2], Julien Vollaire[3], Veronique Josserand[3], Neil Desai[1]

[1]Aanastra, Pacific Palisades, CA,[2]Divincell, Nimes, France,[3]Plateforme OPTIMAL, IAB, Grenoble, France

The Chimeric Antigen Receptor (CAR) T cell therapy has emerged as a ground-breaking immunotherapeutic approach in cancer treatment and demonstrated encouraging clinical results in autoimmune disease indications. However, personalized CAR T cell production is a bottleneck due to challenges in cell manufacturing, scalability, high production costs and the use of viral transduction. *In vivo* CAR T cell generation has been proposed to make this treatment immediately accessible to patients and applicable for broader use. However, efficient and highly selective gene delivery to target cells *in vivo* is challenging. To that end, we are developing a new peptide based (non-lipid) delivery platform (PEP-NP) to enable *in vivo* delivery of CAR mRNA to T-cells. The PEP-NP platform is based on short amphipathic peptides that form stable, scalable nanoparticles capable of efficiently packaging and delivering RNA to the target site. PEP-NPs have been designed to bypass the liver and to enhance mRNA expression in the spleen and T-cells *in vivo*. PEP-NPs were selected based on their efficiency to deliver functional Fluc-mRNA on Jurkat or THP-1 cells and primary human T cells from different donors. Efficiency of PEP-NP to deliver a mRNA encoding for αCD-19 protein was evaluated on human primary T cells. The cytotoxicity of CAR-PEP-NPs transfected T cells was evaluated by co-cultured with either Nalm6 (CD19+) or K651 (CD19-) cells. *In-vivo* efficacy of PEP-NP (0.5 and 1.0 mg/kg) to deliver mRNA in the spleen and in specific immune cells, was evaluated in C57BL/C6 mice using luciferase or eGFP encoding mRNA and analyze by flow cytometry. PEP-NPs carrying mRNA encoding luciferase showed a dose responsive transfection starting at 6 hr post treatment with more than 80% of treated cells expressing Luciferase after 72 hr. We validated transfection, translation and surface expression of a functional CD19 CAR protein by transfecting T cells with PEP-NPs mRNA encoding for αCD19 CAR protein. CAR expression was observed in more than 80% of the transfected T cells from different donors. CAR-PEP-NPs transfected T Cells showed a robust dose response specific cytotoxic effect, resulting in 65% to 92% killing of CD19+Nalm6 cells, while in contrast, no cytotoxicity was observed on K561 (CD19-) cells or with T cells transfected with PEP-NP eGFP mRNA . Then we demonstrated that IV administration of T cell targeted PEP-NPs resulted in a high Luciferase or GFP mRNA expression in the spleen. PEP-NPs carrying GFP mRNA showed a dose-dependent mRNA delivery to T cells in the blood as well as splenic T cells with 15-22% of GFP positive T cells after a single injection. No toxicity of PEP-NP was detected after single or repeat dosing. Our study provides a proof-of-concept that PEP-NP platform can be applied to directly target T cells for *in vivo* CAR T cell engineering. PEP-NP is a novel strategy for specific T cell reprogramming providing redosable, off the shelf T cell gene therapies for patients.

TUMOR BIOLOGY: Cancer Cell Heterogeneity, Evolution, and Trajectory Profiling
Poster Session

#6713 RB1 and TP53 restrict lineage plasticity in prostate cancer by regulating chromatin accessibility.
M. R. Flores, J. Jacobi, D. W. Goodrich;
Roswell Park Comprehensive Cancer Center, Buffalo, NY

Lineage plasticity has been widely recognized in various cancers as a driver of therapeutic resistance, but the molecular basis of this plasticity remains incompletely defined. Evidence from the literature reveals that the inactivation of RB1 and TP53 in prostate cancer (PC) results in the emergence of alternative lineage states that are resistant to androgen receptor (AR)-targeted treatment. How RB1 and TP53 loss cause this lineage plasticity is not clear. This study proposes that RB1 and TP53 help maintain heterochromatin in order to restrict chromatin accessibility and maintain the fidelity of lineage specific gene expression. To test this hypothesis, changes in chromatin accessibility (ATAC-seq) and lineage gene expression (RNA-seq) are monitored in single prostate organoid cells over time following deletion of PTEN (SKO) or PTEN/RB1/TP53 (TKO). Analysis of resulting data indicates that TKO organoids have increased transcriptional diversity and elevated expression pathways like JAK-STAT that have been functionally linked to lineage plasticity and resistance to AR-targeted therapies. These gene expression changes are preceded by increased chromatin accessibility at relevant gene promoters. Enrichment of neuroendocrine PC gene signatures is found in both SKO and TKO organoids likely driven by histone methyltransferase EZH2 as well as DNA methyltransferase DNMT1. TKO organoids also exhibit increased ability to adapt to changing growth conditions, likely facilitated by their increased transcriptional plasticity. Future directions for this study will assess the influence of EZH2 and DNMT1 on chromatin accessibility, gene expression, lineage plasticity, and therapeutic resistance following tumor suppressor deletion. Overall, results are consistent with the hypothesis that RB1 and TP53 loss in PC cells creates a more accessible chromatin state permissive for transcription. Findings may also reveal approaches to suppress therapeutic resistance in PC by therapeutic targeting of lineage plasticity.

TUMOR BIOLOGY: A Role for the Tumor Microenvironment in Progression
Poster Session

#5096 G2.2, a synthetic mimetic of heparan sulfate hexasaccharide, inhibits chemotherapy-induced dormancy.

Shoja M. Haneefa[1], Rio Boothello[2], Bharath Villuri[1], Rawan Fayyad[1], Priyanka Mishra[1], Sajni Vora[3], Umesh R. Desai[1], Bhaumik B. Patel[1]

[1]Virginia Commonwealth University, Richmond, VA,[2]Strand Therapeutics, Boston, MA,[3]Rutgers UMDNJ, New Brunswick, NJ

Background: Tumor cells can enter distant sites and persist as disseminating tumor cells (DTCs). DTCs are known to remain dormant, exhibiting reversible cell cycle arrest/quiescence and anti-cancer therapy resistance. We and others have shown that Insulin-like Growth factor 1 (IGF1) - Receptor (IGF1R) is critical in therapy resistance and cancer stem cell (CSC) phenotype. We have recently discovered a novel role of sulfated glycosaminoglycan (sGAG) oligosaccharides, particularly heparan sulfate hexasaccharide (HS06), in regulating IGF1R signaling through direct binding to its extracellular domain in competition with IGF1. A structurally specific non-saccharide mimetic of HS06, called G2.2, potently inhibits CSC phenotype in various cancers. These observations led us to explore the possible involvement of IGF1R in dormancy phenotype and G2.2 in targeting dormant cells.

Methods: HT-29, as well as HCT-116 (wild type, IGF1R KO (CRISPR), and IGF1R KO + GAG binding site mutant IGF1R (R107H) expressing) cells were studied. Cells were treated with clinically relevant oxaliplatin and 5-fluorouracil (FUOX) (5μM and 125μM, respectively) for 48 hours. They were reseeded in the presence and absence of IGF1 (20 ng/ml) or G2.2 (0→100 μM) to monitor growth and characterize dormancy - DEC2, p27, and Ki-67 (Flow and IF) as well as sGAG expression. Furthermore, FUOX-treated HT-29 cells were harvested on day 7 and implanted subcutaneously in nude mice (10^6 cells/mouse). Two weeks following s.c. injection, the animals were randomized to Vehicle or G2.2 (100 mg/kg, three times a week) for 10 weeks and monitored till euthanasia criteria were met.

Results: The growth curve showed three phases- the initial dormancy induction (phase 1) followed by a growth arrest/dormancy maintenance (phase 2), which was promptly followed by an exponential growth/dormancy escape (phase 3) representing the dormant cell cycle. Indeed, DEC2, p27 expression, and % of Ki-67 negative cells were higher during phase 2 and decreased before phase 3. Interestingly, sGAG and pIGF1R levels were significantly higher in Phase 1 and Phase 2. Consequently, in the presence of IGF-1, cells failed to enter phase 2. On the other hand, G2.2 treatment resulted in a prompt entry into phase 2 but failure to exit phase 3 in cells expressing wild-type IGF1R but not R107H mutant IGF1R. In the HT-29 DTC-induced xenograft model, we observed 100% inhibition of tumor recurrence in the G2.2 treated group, but all the animals in the treated group eventually succumbed to tumor regrowth (with a latent phase).

Conclusions: Our *in vitro* model is representative of therapy-induced tumor arrest followed by spontaneous recurrences observed in the clinic. sGAG-IGF1R interaction may be a critical regulator of DTC survival, and G2.2 represents a novel therapy for targeting DTC*, partially* through IGF1R inhibition.

#5097 Single cell analysis reveals upregulation of immune stimulatory pathways in quiescent metastatic castration resistant prostate cancer cells.

Kristina G. Ibrahim, Julie Boerner, Steven Zielske, **Frank C. 1394557 Cackowski**

Oncology, Wayne State University School of Medicine, Detroit, MI

Quiescent, or G_0 cell cycle phase, cells are common in most prostate cancer (PCa) cases, yet are relatively resistant to many treatments, especially cytotoxic chemotherapy. They also play key roles in tumorigenesis, and dormancy and recurrence after curative intent therapy, but are difficult to identify when viable. To identify characteristics of quiescent prostate cancer cells, we used previously published FACS methods to isolate cancer cells from bone or bone marrow of eight metastatic castration resistant prostate cancer (mCRPC) patient samples and generated single cell RNA sequencing (scRNA-seq) gene expression data of individually sorted cells. We also generated scRNA-seq data from six PCa cell lines and two normal (cancer free) bone marrow samples as positive and negative controls for cell identification. We eliminated *a priori* any potentially contaminating cells based on tSNE cluster location, expression of a 12 gene panel of PCa markers, and lack of expression of bone marrow markers (*PTPRC* / CD45, and *GYPA* / CD235a), leaving 297 high confidence PCa cells. To identify characteristics of G_0 cells, we separated the patient PCa cells into quiescent and cycling groups based on expression of *MKI67* (Ki67). GSEA analysis of KEGG pathways revealed enrichment of primarily immune related pathways in quiescent cells with the top 3 pathways; “allograft rejection,” “autoimmune thyroid disease,” and “intestinal immune network for IgA production.” Similarly, for GSEA analysis using gene ontology (GO) groups, “beta-2-microglobulin binding” was the second most enriched group in quiescent cells. Type 1 HLA genes, were common among multiple pathway groups suggesting potential differences in antigen presentation. In subsequent validation experiments, induction of quiescence in LNCaP, C4-2B, PC3, and RM1 PCa cell lines increased cell surface HLA class 1 or Mhc-1. Overall, these results suggest that antigen presentation is increased in quiescent PCa cells, which may be important for their elimination by the immune system, or useful for future immunotherapy approaches.

#5098 Targeting fatty acid dependency in hormone receptor positive HER2 negative drug tolerant persister bone metastases.

Abigail E. Goen[1], Jonathan D. Marotti[2], Todd W. Miller[3]

[1]MCB Cancer Biology, Dartmouth College, Lebanon, NH,[2]Molecular and Systems Biology, Pathology and Laboratory Medicine, Dartmouth College, Lebanon, NH,[3]Medical College of Wisconsin, Milwaukee, WI

The HR + subtype constitutes 65% of breast cancer (BC) cases with a 1/3 of patients eventually developing metastatic disease, most often in bone. This implicates a drug-tolerant persister cancer cell (DTP) population as a source of disease recurrence. Mouse models of primary and metastatic dormancy along with human tumor transcriptional profiles suggest preferential use of fatty acids (FA) by DTPs, offering fatty acid inhibition as a potential therapeutic vulnerability. HR+ BC cells were treated ± 1 nM 17b-estradiol (E2) for 14 d to provide growing and DTP cell populations. Cells were then treated ± inhibitors of fatty acid transporter CPT1a or fatty acid synthase (FASN). Assay endpoints included levels of proteins and transcripts associated with fatty acid metabolism. CD36 over-expressing, shRNA knock-down, and control cell lines were cultured with exogenous FA adipocytes, or treated with adipocyte-condition medium; assay endpoints included fatty acid uptake, growth response, and levels of fat metabolism proteins and stored lipid. Luciferase-labeled xenografts were induced in NSG mice with E2, which was removed for 90 d to induce tumor regression and yield DTP populations both orthotopically and intra-tibially. Mice were randomized to treatment with vehicle or CD36 mAb and assayed for DTP burden by imaging. Tumors were harvested and molecular endpoints were assessed. DTPs had increased lipid uptake compared to E2-treated controls. Estrogen deprivation increased levels of fatty acid metabolism-associated proteins, and FASN inhibition induced CD36 upregulation, indicating a potential compensatory mechanism between sources of fatty acids. In DTPs, culture with FA supplementation, adipocyte-conditioned media, or adipocyte co-culture rescued growth to levels observed with E2, suggesting the abundance of FA may push DTP cells out of dormancy. Functional metabolic assays showed inhibition of FASN, CPT1a, or CD36 reduced the ability of DTPs to use fatty acid oxidation to drive respiration. Xenografts showed model-specific upregulation of FASN or CD36 after 90 d of E2 deprivation compared to baseline. Treatment of mice bearing MCF-7 primary and metastatic xenografts with a CD36-neutralizing mAb decreased DTP burden compared to vehicle control. Preclinical modeling suggests that DTPs have an increased dependence upon fatty acids (e.g., mammary fat pad, bone associated adipocytes, co-culture of adipocytes, or FA supplementation) than their proliferative counterparts, offering fatty acid inhibition as a therapeutic vulnerability, particularly for bone-tropic DTPs.

#5099 Notch inhibition as a therapeutic approach to eliminate dormant cancer cells in multiple myeloma.

Hayley M. Sabol[1], Aric Anloague[1], Japneet Kaur[1], Sharmin Khan[1], Bethany Paxton[1], James Smith[2], Michelle McDonald[3], Peter Croucher[2], C. Lowry Barnes[1], Elena Ambrogini[1], Alison Frontier[4], Frank H. Ebetino[4], Jesus Delgado-Calle[1]

[1]University of Arkansas for Medical Sciences, Little Rock, AR,[2]Garvan Institute of Medical Research, Sydney, Australia,[3]University of Sydney, Sydney, Australia,[4]University of Rochester, Rochester, NY

Multiple myeloma (MM) remains incurable due to disease relapses, thought to stem from the activation of dormant MM cells. Preventing the awakening or eliminating dormant MM cells is crucial to avoiding disease relapse. In this study, we sought to identify targetable vulnerabilities to eliminate or control dormant cells. First, we used a scRNAseq dataset of dormant MM cells and found that dormant MM cells exhibit upregulation of Notch pathway genes compared to proliferating MM cells. Based on this, we hypothesized that inhibiting Notch signals in the tumor niche should decrease dormant MM cells. To test this notion, we injected immunocompetent mice i.v. with murine 5TGM1 MM cells double-labeled with a fixed genetic reporter and a membrane dye (DiD) retained by non-dividing cells, allowing us to distinguish dormant (GFP^+DID^+) from proliferating cells (GFP^+DID^-). After 2wks, we randomized mice to groups receiving vehicle or a bone-targeted Notch inhibitor (BT-GSI). We detected a population of GFP^+DiD^+ dormant cells that expressed high levels of Axl and were growth arrested throughout the life span of the mice. Short-term treatment with BT-GSI (1wk) reduced tumor burden (75%) and decreased the number of dormant MM cells (70%) via apoptosis. Prolonged BT-GSI treatment (7 wks) eliminated all dormant MM cells and further reduced proliferating cells. These effects were replicated in immunodeficient mice injected with RFP^+DID^+ human OPM2 MM cells and in human bones infiltrated by OPM2 RFP^+DID^+ cultured ex vivo. Next, we explored if modulating the tumor niche could influence MM cell dormancy. Since dormant MM cells colocalize with osteoblasts in the endosteal bone surface, we assessed if increasing osteoblasts numbers and their activity prevents the awakening of MM dormant cells or enhances their engagement in dormancy programs. To test this, we injected GFP^+DiD^+ MM cells and, after two weeks, randomized mice to groups receiving vehicle or Scl-Ab, an anabolic agent that rapidly increases osteoblasts. Scl-Ab (2wks) increased osteoblasts but did not affect the proportion of proliferating or dormant MM cells. Similar results were observed in human bones infiltrated with human MM cells cultured ex vivo treated with Scl-Ab. Next, we pre-treated mice with Scl-Ab (2wks) to generate a microenvironment loaded with osteoblasts before MM cell injection. Despite the increase in osteoblasts, Scl-Ab did not impact the number of dormant or proliferating MM cells. Further, co-administration of Scl-Ab did not interfere with BT-GSI's ability to eliminate proliferating or dormant MM cells in any of these models. In conclusion, our findings show that 1) Notch signals promote the survival of dormant MM cells, 2) manipulating osteoblasts in the tumor niche does not affect MM cell dormancy, and 3) bone-targeted Notch inhibition is a promising approach to eradicate dormant tumor cells and potentially delay/prevent disease relapse in MM patients.

#5100 Mediator subunit *Med4* enforces metastatic dormancy in breast cancer.

S.-Y. Bae[1], H.-H. Ling[1], Y. Chen[1], H. Chen[2], D. Kumar[1], J. Zhang[1], A. Viny[1], R. A. DePinho[2], F. G. Giancotti[1];
[1]Columbia University Irving Medical Center, New York, NY, [2]The University of Texas MD Anderson Cancer Center, Houston, TX

Long term survival of breast cancer patients is limited due to recurrence from metastatic dormant cancer cells. However, the mechanisms by which these dormant breast cancer cells survive and awaken remain poorly understood. Our unbiased genome-scale genetic screen in mice identified *Med4* as a novel cancer-cell intrinsic gatekeeper in metastatic reactivation. *MED4* haploinsufficiency is prevalent in metastatic breast cancer patients and correlates with poorer prognosis. Syngeneic xenograft models revealed that *Med4* enforces breast cancer dormancy. Contrary to the canonical function of the Mediator complex in activating gene expression, *Med4* maintains 3D chromatin compaction and enhancer landscape, by preventing enhancer priming or activation through the suppression of H3K4me1 deposition. *Med4* haploinsufficiency disrupts enhancer poise and reprograms the enhancer dynamics to facilitate extracellular matrix (ECM) gene expression and integrin-mediated mechano-transduction, driving metastatic growth. Our findings establish *Med4* as a key regulator of cellular dormancy and a potential biomarker for high-risk metastatic relapse.

#5103 Digital spatial profling with GeoMx to identify differential protein expression in Non-Hispanic/Latino and Hispanic/Latino Patients with metastatic hormone sensitive prostate cancer.

Peter D. Zang, Joe Bonner, Alexander Chehrazi-Raffle, Lixin Yang, Natasha Garg, Swapnil Rajurkar, James Shen, Naveen Gupta, Amartej Merla, Sumanta Pal, Raju Pillai, Stephen Gruber, Tanya B. Dorff

City of Hope National Medical Center, Duarte, CA

Background: Emerging evidence suggests that there is a complex interplay between ethnicity and disease biology in metastatic hormone sensitive prostate cancer (mHSPC). We examined digital spatial profiling with GeoMx to evaluate differential protein expression between non-Hispanic/non-Latino (NHL) and Hispanic/Latino (HL) patients with mHSPC.
Methods: Pre-treatment prostate biopsy specimens were obtained from patients on a clinical trial as part of an IRB approved protocol. The GeoMx Digital Spatial Profiler (DSP) was used to evaluate protein expression in selected regions of interest (ROIs). Differential expression analysis was performed using R (v 4.4.2). To determine significant differences in protein expression, a false discovery rate (FDR)-adjusted q-value was applied and a Log2Fold change (Log2FC) with a q-value < 0.01 was considered significantly differentially expressed.
Results: A total of 8 patients (4 NHL and 4 HL) were enrolled at City of Hope including network sites and who had pre-treatment biopsy specimens that were available for digital spatial profiling analysis. We examined the Log2FC between protein expression in NHL and HL patients and filtered for q-values < 0.01. We identified multiple proteins that were significantly differentially expressed. Proteins that were more highly expressed (Log2FC > 1) in NHL patients included CD44, fibronection, and Ki-67. Proteins that were more moderately expressed (Log2FC < 1) in NHL patients included beta-2 microglobulin and. In contrast, HL patients were found to have higher expression in BCL6, CD68, CD80, Foxp3, PARP, and STING (Log2FC < 0).
Conclusions: Digital spatial profiling with GeoMx reveals significant differences in protein expression in NHL vs. HL patients with mHSPC. Notably, these include markers of cancer stem cells (CD44), DNA damage pathway proteins (PARP), and both immunoregulatory (Foxp3) and immunostimulatory (STING) proteins. This may have both prognostic and therapeutic implications.

#5104 Fibrosis activates pancreatic cancer Schwann cells in a non-canonical pathway through a nuclear mechanosensing mechanism.

Pavel Stupakov[1], Golbahar Sadatrezaei[1], Ines Velazquez Quesada[2], Lillian Boe[1], Efsevia Vakiani[1], Bojana Gligorijevic[2], Boris Reva[3], Richard J. Wong[1], **Sylvie Deborde**[1]

[1]Memorial Sloan Kettering Cancer Center, New York, NY,[2]Temple University, Philadelphia, PA,[3]Mount Sinai, New York, NY

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive malignancies, with only 12% of patients surviving 5 years after diagnosis. Several elements of the PDAC tumor microenvironment (TME) including fibrosis and tumor innervation contributes to the malignancy, but how they affect each other is unstudied. Here we report a relationship between the stiff desmoplastic environment of PDAC and Schwann cell (SC) activation in nerves of the tumor. The activated SCs reprogram into cells that induce cancer cell spread via activation of the transcription factor c-Jun and the formation of tracks for cancer cells. We show how mechanical forces encountered by SCs in the stiff TME activate SC in a non-canonical manner. We use in vivo and in vitro live imaging, mechanobiology and computational analysis of transcriptomic data, as well as several models including human PDAC specimens, mouse models of pancreatic fibrosis and cells expressing live reporter for c-Jun phosphorylation. We report a positive correlation between c-Jun phosphorylation in SCs of nerves and stiffness of the stroma surrounding these nerves, suggesting a role for stromal stiffness in SC activation. To monitor c-Jun phosphorylation in live SCs, we express a c-Jun kinase translocation reporter (KTR), a fluorescent biosensor that translocates from the nucleus to the cytoplasm upon phosphorylation of a c-Jun motif, in the cell line HEI-286 SCs. Imaging of HEI-286 KTR SCs injected in murine fibrotic and non-fibrotic pancreas shows an association of activated cells with fibrosis. Intravital 4D multiphoton microscopy allows the observation of HEI-286 KTR SCs along collagen fibers and shows a concomitant change of nuclear shape with KTR translocation. Using three different in vitro models, we show that mechanical forces on HEI-286 KTR SCs induce KTR translocation. We show that this activation is associated with a cPLA2 activity and an increase of the AP1 transcription factor network molecules, but not with the expression of the conventional Jun kinase molecules. The results support a nuclear mechanical sensing mechanism inducing SC activation in PDAC through a non-canonical mechanism of c-Jun activation. Furthermore, we show that the SC cellular stress response differs from the cancer cell response. We propose that SCs is activated by two different mechanisms localized at the nucleus and at the plasma membrane. The findings reveal therapeutic opportunities to treat PDAC by indirect targeting of specific component of the TME.

#5105 Tumor treating fields (TTFields) alter nanomechanical attributes of cell-ECM complex in pancreatic ductal adenocarcinoma.

T. Kulkarni, H. Babiker, D. Mukhopadhyay, **S. Bhattacharya**;
Mayo Clinic Florida, Jacksonville, FL

Tumor treating fields (TTFields), a noninvasive anticancer treatment modality, has received FDA approval for pleural mesothelioma, metastatic NSCLC following platinum-based therapy, newly diagnosed and recurrent glioblastoma multiforme. TTFields have also demonstrated potential benefits in other cancer types, including pancreatic ductal adenocarcinoma (PDAC). Although the PANOVA trial yielded positive results in PDAC patients, the mechanisms underlying these beneficial effects remain unclear. Severe desmoplasia is a hallmark characteristic of PDAC due to accumulation of excess extracellular matrix (ECM) caused by activated fibroblasts and cancer cells. This dense ECM contributes to an immunosuppressive tumor microenvironment (TME) and drug resistance, which ultimately leads to poor 5-year survival in PDAC patients. Our previous work with atomic force microscopy (AFM) showed that the nanomechanical properties of the ECM change dynamically based on its composition and the presence of PDAC cells, and vice versa. Herein, we prepared an ECM complex comprising of collagen, fibronectin and laminin at physiological concentrations. Following which, we investigated the alteration of stiffness of both PDAC cells and the ECM in presence of TTFields using an AFM. A frequency-dependent analysis of TTFields showed that a frequency of 150 KHz led to consistent decreases ECM stiffness at 48 and 72 hours. Next, we evaluated the influence of TTFields in PDAC cells alone. In Panc-1 cells exposed to TTFields for 48 and 72 hours, we observed a more than 6-fold increase in membrane stiffness compared to untreated controls. Additionally, AsPC-1 cells subjected to TTFields exhibited a greater than 2-fold increase in membrane stiffness at these time points. Furthermore, we have investigated influence of TTFields on PDAC cells and ECM components in a co-culture model. In this co-culture scenario for Panc-1 cells, TTFields increase membrane stiffness by 5-fold compared to untreated at these time points. Also, AsPC-1 cells demonstrated a 1.5-fold increase in membrane stiffness in the presence of TTFields compared to the untreated. Lastly, we also evaluated the influence of cells on the ECM stiffness in the presence of TTFields. It significantly increased the ECM stiffness in presence of Panc-1 co-culture by 1.3-folds compared to control. We also observed 3- and 4-fold increase in ECM matrix stiffness when co-cultured with AsPC-1 for 48 and 72 hours from TTFields, respectively compared to control. In conclusion, TTFields significantly modulate the stiffness of PDAC cells and the ECM, both individually and in co-culture.

#5106 Cell crowding-induced TRPV4 inhibition and relocation to plasma membranes: Implications for a pro-invasive mechanotransduction pathway in DCIS progression.

Xiangning Bu[1], Nathanael Ashby[1], Teresa Vitali[1], Sulgi Lee[1], Ananya Gottumukkala[1], Kangsun Yun[1], Sana Tabbara[2], Patricia Latham[2], Christine Teal[3], **Inhee Chung**[1]

[1]Anatomy and Cell Biology, The George Washington University, Washington, DC,[2]Pathology, The George Washington University, Washington, DC,[3]Surgery, The George Washington University, Washington, DC

Cell crowding is a common microenvironmental factor influencing disease progression, yet its role in promoting cell invasiveness remains poorly understood. Here, we demonstrate that cell crowding enhances the invasiveness of high-grade ductal carcinoma in situ (DCIS) cells through pro-invasive cell volume reduction. High-grade DCIS cells showed significant volume reduction compared to hyperplasia-mimicking or normal cells under crowded conditions. Mass spectrometry revealed selective relocation of TRPV4, a calcium-permeant ion channel, to the plasma membrane in high-grade DCIS cells, a response absent in less aggressive or normal cells. We found that cell crowding inhibits TRPV4 activity in high-grade DCIS cells, reducing intracellular calcium levels and inducing volume reduction. This inhibition triggers TRPV4 relocation to the plasma membrane, priming it for subsequent activation to counterbalance calcium loss. Analyses of patient-derived breast cancer tissues confirmed TRPV4 association with the plasma membrane specifically in high-grade DCIS, correlating with increased cell invasiveness and motility. Moreover, hyperosmotic stress and pharmacologic TRPV4 inhibition replicated crowding-induced pro-invasive volume reduction, while TRPV4 activation reversed these effects. These findings uncover a novel, selective mechanotransduction pathway in high-grade DCIS that links cell crowding to enhanced invasiveness, shedding light on the biophysical mechanisms driving breast cancer progression. Our results highlight TRPV4 as a potential diagnostic and prognostic biomarker for high-risk DCIS and provide insights into therapeutic interventions targeting cell volume regulation.

#5107 Alterations in cancer-associated fibroblasts are associated with a dysfunctional T cell phenotype and antioxidant response in LKB1/KEAP1-mutant lung adenocarcinoma.

Amirali Karimi[1], Yu Qian[1], Ana Galan-Cobo[2], Fuduan Peng[3], Monique Nilsson[1], Linghua Wang[3], Ferdinandos Skoulidis[1], John V. Heymach[1]

[1]Department of Thoracic, Head and Neck Medical Oncology, UT MD Anderson Cancer Center, Houston, TX,[2]Department of Molecular Diagnostic, UT MD Anderson Cancer Center, Houston, TX,[3]Department of Genomic Medicine, UT MD Anderson Cancer Center, Houston, TX

Background: LKB1/*STK11* and *KEAP1* are the second and third most-commonly mutated tumor suppressor genes in lung adenocarcinoma. *STK11* and *KEAP1* mutations often co-occur together and with *KRAS* mutations and have been associated with an immunosuppressive microenvironment, lack of response to anti-PD-(L)1 therapy, and enhanced antioxidant response. Cancer-associated fibroblasts (CAFs) have been associated with tumor progression and worse survival in lung adenocarcinoma. However, the role of this cell population in promoting the phenotype of LKB1/KEAP1-mutant lung adenocarcinoma remains undefined.

Methods: We analyzed single-cell RNA sequencing (scRNAseq) data from syngeneic *Kras*^{MUT} models (LKR10 and LKR13) of lung adenocarcinoma with or without LKB1 or KEAP1 deficiency. *Stk11* and *Keap1* genes were knocked-out from LKR10K and LKR13K cell lines using CRISPR-Cas9 technology, and mice were injected subcutaneously with *Kras*^{MUT} (K), *Kras*^{MUT}/*Lkb1*^{MUT} (KL), *Kras*^{MUT}/*Keap1*^{MUT} (KK), and *Kras*^{MUT}/*Lkb1*^{MUT}/*Keap1*^{MUT} (KLK) tumor cells. CAF populations were analyzed by scRNAseq using R software packages.

Results: CAF proportion among non-tumor cells was increased in KL and KK populations compared to K, with the highest percentage observed in the KLK tumors. In the LKR10 model UMAP, KK CAFs clustered distinctly from other samples, showing their different phenotype from other three groups. This was not observed in the LKR13 model where differentially expressed genes (DEGs) from KK-vs-K and KL-vs-K comparisons had considerable overlap, indicating similarities in this population. Twelve genes overlapped in DEGs from LKR10 KL-vs-K and KK-vs-K, of which four were related to MHC class I antigen presentation (*B2m*, *H2-D1*, *H2-Q7*, *Psmb8*), suggesting a possible role of CAFs in antigen-presentation to CD8 T cells and their exhaustion, a phenomenon previously described in colorectal carcinoma. *Npc2*, a gene involved in CD1d-mediated lipid antigen presentation to NKT cells, was also upregulated in both groups. In the LKR10 model, classical and non-classical MHC class I genes had the highest expression in KK CAFs, and this was also observed for KL CAFs in the LKR13 model. However, MHC class I upregulation was not replicated in KLK models. *Sod3*, a secretory extracellular superoxide dismutase that generates an antioxidant response in the microenvironment, was upregulated in both LKR10 KL and KK CAFs. Concurrently, LKR13KLK CAFs also demonstrated increased *Sod3* expression compared to LKR13K CAFs.

Conclusions: Our analysis demonstrated that CAFs display distinct phenotypes in LKB1 and KEAP1 deficient tumors, indicating that these cells may in part contribute to the immunosuppressive tumor microenvironment and enhanced antioxidant response, hypothetically partly through the upregulation of MHC class I molecules and secretion of antioxidant protein *Sod3*.

#5108 Decoding tumor intrinsic and stroma-related drug resistance in EGFR mutant NSCLC.

Radhika Koranne[1], Aimee Gleason[2], Emily Hensley[2], Xiaodong Wang[3], Alba Font-Tello[3], Brian Rabe[3], Fayaz Seifuddin[3], Eugene Drokhlyansky[3], Kartik Subramanian[3], Martino Colombo[3], Jason Dobson[3], Mavrakis J. Konstantinos[3], Leila Dardaei[3], Aaron Hata[1]

[1]Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA,[2]Cancer Center, Massachusetts General Hospital Cancer Cente, Boston, MA,[3]Bristol Myers Squibb, Cambridge, MA

The presence of oncogenic driver mutations such as EGFR, ALK, or KRAS renders non-small cell lung adenocarcinomas sensitive to molecularly targeted therapies, however the inevitable emergence of acquired drug resistance remains an unsolved clinical problem. Osimertinib, a third-generation EGFR inhibitor that was initially developed to overcome the EGFRT790M resistance mutation, is now the standard first-line therapy. Although various mechanisms of acquired resistance to osimertinib have been identified, the specific drivers of resistance remain unexplained in over half of patients. Recent work from our group and others has suggested that cancer-associated fibroblasts may play a role in drug resistance to tyrosine kinase inhibitors (TKIs). Notably, we have observed that patient-derived cancer-associated fibroblast (PDF) cell lines established from TKI-resistant tumors display heterogeneous phenotypes with ability to rescue cancer cells from TKIs. To enable parallel discovery of both tumor cell-intrinsic and stroma-driven resistance mechanisms to osimertinib, we developed a genome-wide CRISPR sensitizer screening approach that incorporates osimertinib-resistant patient-derived EGFR-mutant NSCLC cell lines and conditioned media from matched immortalized PDFs. These studies aim to uncover intrinsic and extrinsic resistance pathways and nominate targets for next-generation therapies to overcome acquired resistance to osimertinib

#5109 ATF4 and SCD: Co-conspirators in promoting the progression of metastatic prostate cancer in bone.

Alexis R. Wilson, Shane Mecca, Mackenzie Herroon, Shrila Rajendran, Laimar Garmo, Izabela Podgorski

Wayne State University School of Medicine, Detroit, MI

Prostate Cancer (PCa) is predisposed to metastasize to bone, a hospitable environment abundant in adipocytes. Our previous studies have highlighted the critical role of marrow adipocytes in PCa progression and chemoresistance in bone. Bone marrow adipocytes are metabolic cells that interact with neighboring cells through the secretion of lipids and adipokines. Specifically, we have shown that interaction with adipocytes promotes lipid uptake by PCa cells and activates ER/oxidative stress pathways; however, the mechanisms behind the tumor-promoting effects of adipocytes are not clearly understood. *The main objective of this study* was to investigate the molecular mechanisms underlying lipid-induced stress during tumor cell-adipocyte crosstalk and its functional relationship to tumor progression in bone. Previously, we have demonstrated that adipocyte exposure increases expression of lipogenic enzyme stearoyl-CoA desaturase (SCD) and ER stress regulator ATF4 in PCa cells. Here, we show that SCD expression decreases with transient knockdown of ATF4. Also, through dual transient knockdown of SCD and ATF4, we determine that SCD deficiency leads to an increase of ER stress genes and demise of PCa cells only when expression of ATF4 is present. We also observe this phenomenon in PCa cells with a stable knockdown of ATF4 (shATF4), suggesting an interplay between SCD and ATF4. Additionally, we reveal that shATF4 PCa cells have decreased proliferation *in vitro,* reduced ER stress signaling, and increased lipid peroxidation levels. RNAseq analyses of shATF4 cells demonstrate the downregulation of inflammation-related pathways, such as NFkB. Notably, we observe that shATF4 cells have decreased expression of *protein tyrosine phosphatase receptor type D* (*PTPRD*) compared to control cells. PTPRD has been implicated in regulating inflammatory pathways but, to our knowledge, has not been shown to be regulated by ATF4. Curiously, shATF4 cells that undergo knockdown of SCD cannot induce pathways involved in apoptotic signaling and ER stress signaling compared to control cells with SCD knockdown. We also observe the induction of the NOTCH signaling pathway and increased expression of *p53 apoptosis effector* (*PERP*), a gene regulated directly by NOTCH, in shATF4 cells grown in co-culture with adipocytes. This is in contrast to control cells with SCD knockdown, where we observe a decrease in *PERP* expression. These results indicate that the downregulation of inflammatory pathways during ATF4 knockdown may be occurring through PTPRD. Also, the increase in NOTCH signaling, which may be due to the reciprocal activation of NFkB and NOTCH pathways, may be counteracting the anti-tumor effects of SCD inhibition on PCa cells. Overall, we demonstrate an interplay between ATF4 and SCD and the critical role of ATF4 in mediating the anti-tumor effects of SCD, which may have therapeutic complications for bone metastatic PCa.

#5110 Solid stress reduces synaptic density and neuronal activity via hypoxia-induced reactive gene expression programs in glioblastoma.

Maksym Zarodniuk, Anna Wenninger, Julian Najera, Jihaeng Lee, Jack Markillie, Cameron Mackenzie, Bianca Batista, Megna Panchbhavi, R'nld Rumbach, Alice Burchett, Hailey Sallaberry, Bradley Smith, Meenal Datta, Christopher Patzke

University of Notre Dame, Notre Dame, IN

Mass effect in central nervous system pathologies poses a significant clinical challenge. This phenomenon, characterized by the compression and deformation of neural tissue from space-occupying lesions, can lead to debilitating neurological symptoms. In glioblastoma, we have shown previously that compressive solid stresses from the growing tumor are correlated with significantly worse neurological performance and survival in patients. While edema is often measured as a prognostic biomarker, it fails to consistently predict neurological deficits; conversely, solid stress correlates with functional impairments and reduced cerebral blood flow. In this study, we explored the direct effects of solid stress on human induced pluripotent stem cell (hiPSC)-derived neurons using an in vitro compression system. Our results reveal that solid stress decreases synaptic puncta and neuronal excitability by activating hypoxia response and pro-apoptotic programs in neurons. We further show that compression upregulates reactive gene expression programs in astrocytes and microglia, contributing to neuroinflammation. These findings illuminate the critical role of solid stress in glioblastoma, offering new insights into its potential contribution to neurodegeneration.

Equal contribution: M.Z. & A.W.; Joint supervision: C.P.& M.D.

#5111 Targeting the dysadherin/CA9 axis inhibits colorectal cancer adaptation and growth within the acidic tumor microenvironment.

Jeong-Seok Nam, Choong-Jae Lee, Hyeon-Ji Yun

School of Life Sciences, Gwangju Institute of Science & Technology, Gwangju, Korea, Republic of

The tumor microenvironment (TME) has a significant impact on colorectal cancer (CRC) progression by promoting tumor survival and aggressive behavior. A key player in this process is the tumor-associated glycoprotein dysadherin, which is highly expressed in aggressive CRC cells and facilitates their adaptation to the TME. In this study, we conducted a comprehensive bioinformatics analysis of clinical genomic data from CRC patients, integrated with studies on dysadherin-knockout CRC cells. We also examined the downstream effects of dysadherin in human CRC cells, patient tissue samples, and a xenograft mouse model. Through bioinformatics and pathological analysis, we found that increased tumor acidity is a prominent feature of CRC progression and is positively associated with high dysadherin expression. Overexpression and silencing studies of dysadherin in CRC cells further confirmed that high dysadherin levels enhance malignant traits, especially under acidic conditions within the TME. Mechanistically, dysadherin activates the Integrin/FAK/STAT3 signaling pathway, leading to upregulation of carbonic anhydrase 9 (CA9). CA9 helps cancer cells survive in acidic environments by maintaining intracellular pH balance. By buffering the acidic TME, CA9 enhances CRC cell adaptation and resilience in an otherwise hostile environment. Notably, the deletion of dysadherin in xenograft models led to reduced tumor growth, while the reintroduction of CA9 restored malignancy, underscoring the essential role of the dysadherin/CA9 axis. This pathway is crucial for CRC cell survival in acidic TME, positioning dysadherin and CA9 as promising prognostic markers and therapeutic targets. Targeting this axis could disrupt CRC progression, offering new therapeutic avenues and potentially more effective interventions for inhibiting disease advancement

#5112 Tumor cell expression of a distinct TME gene signature defines differential drug sensitivities to EGFR/MEK/SRC targeted agents.

Mingli Yang[1], Michael V. Nebozhyn[2], Lance Pflieger[3], Ramani Soundararajan[1], Michelle Maurin[1], Heiman Wang[1], Andrey Loboda[2], W. Jack Pledger[4], Timothy J. Yeatman[1]

[1]University of South Florida, Tampa, FL,[2]Merck Research Laboratories, Boston, MA,[3]Phenome Health, Seattle, WA,[4]Tampa General Hospital Cancer Institute, Tampa, FL

Recently, we serendipitously identified a pair of distinct 10-gene signatures, one associated with epithelial cells (EPI^S) and one the tumor microenvironment(TME^S). When we classified 2373 colorectal cancer (CRC) tumors into the consensus molecular subtypes (CMS1-4), the EPI^S signature score was predominantly related to the TME-poor CMS2/CMS3 classes and was highly predictive of EGFRi outcomes in two clinical trial datasets and a large, real-world clinico-genomics dataset. By contrast, the TME^S signature score was strongly associated with the immune/stromal CMS1/CMS4 subtypes and was highly correlated with the gene expression signature scores portending EMT, SRC activation and MEK inhibitor resistance (MEKi-R). To understand the cellular features underpinning the TME^S correlation, we further analyzed the cellular origins of 61 SRC signature genes and 13 MEKi-R signature genes, as well as 8 EMT genes using an independent public scRNASEQ dataset (n=62). All of the 13 MEKi-R genes and all of the 8 EMT genes, as well as most of the SRC activation genes, were predominantly expressed in various immune/stromal TME cells, supporting a key role of the TME gene expression. Remarkably, when the signature scores were analyzed in a dataset of ~150 heterogenous CRC cell lines that do not have a TME, a similar strong correlation among the TME^S, EMT, SRC activation and MEKi-R scores was also observed. These data suggest that the CRC epithelial cells may have adopted the TME gene expression features important in modulating the drug sensitivities. Spatial transcriptomics of 8 CRC samples confirmed that the EPI^S genes were predominantly expressed in epithelial cells. By contrast, the signature genes of the TME^S linked to EMT/SRC activation/MEKi-R were diffusely expressed across epithelial/invasive cancer mixed with the TME, indicating an interactive tumor-TME cellular feature. Our data suggest that the TME through its impact on epithelial cell gene expression may define drug sensitivities to targeted therapies.

#5113 Elucidating the biological effects of platinum adduct-containing cell-free DNA.

Sri Meghana Yerrapragada, M. Alexandra Carpenter, Michael G Kemp

Pharmacology and toxicology, Wright State University, Dayton, OH

Cisplatin is a widely used chemotherapeutic agent for cancers such as lung, breast, esophageal, ovarian, and pancreatic. However, its effectiveness is constrained by its cytotoxicity to healthy tissues and the rapid development of cancer cell resistance. Cisplatin binds to the N7 position of purines in DNA, forming intra- and inter-strand crosslinks, which are potentially mutagenic and lethal if not removed by the nucleotide excision repair (NER) system. When DNA repair mechanisms are overwhelmed, cisplatin-DNA adducts can trigger caspase-dependent apoptosis, leading to degradation of the damaged DNA. Interestingly, the ultimate fate of this damaged DNA remains unclear. Our study, using differential centrifugation and DNA immunoblotting, has identified that cisplatin-adduct-containing DNA are released into extracellular spaces upon treating with lethal doses of cisplatin. Notably, caspase inhibition blocks this release, while inhibition of NER enhances it. Further, our research demonstrates that non-damaged bystander cells can uptake the cisplatin-DNA adducts, potentially activating DNA damage checkpoints or innate immune responses. This process can also alter the sensitivity of bystander cells to cisplatin. Given the role of cell-free DNA (cf-DNA) in intercellular communication, our findings suggest that damaged DNA could be transferred to distant organs, contributing to cisplatin resistance, toxicity, and systemic side effects. Understanding the mechanism by which cisplatin-DNA adducts influence cell signaling and treatment outcomes could open new avenues for using cf-DNA as biomarkers to monitor therapeutic responses and minimize long-term genomic risks associated with chemotherapy.

#5114 Investigating the novel role of the protease A Disintegrin And Metalloprotease (ADAM)-17 in small cell lung cancer.

M. I. Saad[1], K. Hon[1], J. Solujic[2], T. Weng[3], A. Badiei[2], C. Young[4], S. Dusterhoft[5], D. Gough[6], S. Rose-John[7], P. Hoffmann[4], P. Nguyen[2], B. Jenkins[1];

[1]University of Adelaide, Adelaide, Australia, [2]Royal Adelaide Hospital, Adelaide, Australia, [3]Monash University, Victoria, Australia, [4]University of South Australia, Adelaide, Australia, [5]RWTH Aachen University, Aachen, Germany, [6]Hudson Institute of Medical Research, Victoria, Australia, [7]Kiel University, Kiel, Germany

Lung cancer is the most lethal and common cancer worldwide, which comprises two main subtypes, non-small cell lung cancer and small cell lung cancer (SCLC), accounting for ~85% and ~15%, respectively, of all diagnoses. SCLC is a highly aggressive neuroendocrine malignancy which is detected in two thirds of SCLC patients at a late stage with a dismal 5-year survival rate of less than 5%. This poor outcome is largely due to the stagnated clinical management of SCLC over the last 3 decades, comprising first-line platinum-based chemotherapy with etoposide, which, despite initial high response rates, is associated with rapid disease relapse due to acquired chemoresistance. Therefore, there is a pressing clinical need to identity new molecular drivers of SCLC which can serve as therapeutic targets. In this regard, A Disintegrin And Metalloprotease 17 (ADAM17) is the major “sheddase” for >80 membrane-tethered pro-tumourigenic substrates, including epidermal growth factor receptor ligands, notch receptor and tumour necrosis factor-α, which control many cellular processes (e.g. inflammation, proliferation) in immune and non-immune cells. Therefore, ADAM17 modulates key physiological and pathophysiological cellular processes. Despite the large body of data implicating ADAM17 in lung cancer, the pathological role of ADAM17 in SCLC is unknown. Using our unique highly relevant and well-established clinical and preclinical models, including SCLC patient biopsies, the *Rb1*$^{fl/fl}$*;Trp53*$^{fl/fl}$ (RP) and *Rb1*$^{fl/fl}$*Trp53*$^{fl/fl}$*Myc*$^{LSL/LSL}$ (RPM) genetic mouse models, patient-derived xenografts and human SCLC cell lines, as well as proteomics pipeline and a suite of biochemical and molecular biology techniques, we aim to define the role of ADAM17 in SCLC. ADAM17 expression, interactome and substrate repertoire was investigated while the anticancer activity of ADAM17 inhibitors in SCLC was assessed. This research therefore could pave the way for the development of novel therapeutic and biomarker avenues for SCLC.

#5115 Engineered TIMP-1 for selective MMP-9 inhibition: A novel strategy to suppress breast cancer invasiveness.

A. Shoari, A. Hockla, M. A. Coban, A. M. Dimesa, D. C. Radisky, E. S. Radisky;
Mayo Clinic, Jacksonville, FL

Tissue inhibitors of metalloproteinases (TIMPs) are endogenous inhibitors that regulate the function and activity of matrix metalloproteinases (MMPs), enzymes that are known for their involvement in cancer progression. MMP-9, also known as gelatinase B, plays a crucial role in tumorigenesis of several cancers including triple negative breast cancer (TNBC); tumor cell-produced MMP-9 promotes malignant progression and metastasis of basal-like TNBC. Here, we have used full-length TIMP-1 as a scaffold for engineering inhibitors that bind with higher selectivity to MMP-9, for potential therapeutic applications. We previously developed a yeast surface display library of human TIMP-1, with diversity incorporated at 17 residues within five loops; mutation burden was tuned to 3-5 mutations per clone. Here, we have screened the TIMP-1 library for affinity toward recombinant MMP-9 by using fluorescence-activated cell sorting (FACS). Yeast cells were incubated with an incrementally decreasing concentration of biotinylated MMP-9 (400 nM to 25 nM) in successive rounds of sorting. Samples were analyzed using flow cytometry and highest affinity populations were selected using FACS. Among the TIMP-1 variants with highest enrichment, we identified the TIMP-1-C15 variant that showed 12-fold enhanced binding with improved selectivity toward MMP-9 compared with wild type TIMP-1 (WT TIMP-1). We next expressed and purified soluble recombinant TIMP-1-C15 and conducted inhibition studies using a fluorogenic substrate to evaluate inhibitory potency towards MMP-9 activity. These experiments showed that TIMP-1-C15 inhibits MMP-9 activity with an inhibition constant (K_i) of 0.61 nM, more than 5-fold stronger than WT TIMP-1; these studies also corroborated the improved selectivity towards MMP-9 over other MMPs. Additionally, we further evaluated the functional impact of the TIMP-1-C15 variant on cancer cell invasiveness using TNBC cell lines in cellular invasion assays. The TIMP-1-C15 variant markedly reduced the invasive capabilities of TNBC cells to a greater extent than WT TIMP-1. Currently, we are working to isolate additional TIMP-1 variants from our library that exhibit enhanced selectivity for MMP-9, with MMP-8 included in the sorting process as a competitive target to ensure specificity. Selective inhibition of MMP-9 over MMP-8 is particularly important in breast cancer therapy, as MMP-8 has been shown to have anti-tumorigenic properties, and its inhibition could counteract therapeutic efforts aimed at reducing cancer invasiveness. Our findings suggest that engineered TIMP-1 variants could serve as potent inhibitors of MMP-9, offering a novel approach to reducing the invasiveness of breast cancer cells and potentially limiting the progression of metastatic disease.

#5116 Alpha-2-macroglobulin in the tumor microenvironment of pancreatic ductal adenocarcinoma to regulate versican proteolysis.

Somak Chaudhuri, Yousef Gadalla, Katherine A. Johnson, Philip B. Emmerich, Sean G. Kraus, Cheri A. Pasch, Dustin A. Deming

University of Wisconsin-Madison, Madison, WI

Background: Versican (VCAN), a large chondroitin sulfate matrix proteoglycan is upregulated in the pancreatic ductal adenocarcinoma (PDAC) stroma and plays an immunosuppressive role. In contrast, the VCAN V1 isoform can be cleaved by ADAMTS proteases 1, 4, 5, 9, 15 and 20 into the fragment versikine which is known to be immunostimulatory. PDAC displays poor VCAN proteolysis and CD8+ T-cell infiltration, but interestingly overexpresses ADAMTS 1, 4, 5 and 9. Alpha-2-macroglobulin (A2M) has been shown to inhibit ADAMTS 4 and 5 and may be overexpressed in the PDAC tumor microenvironment (TME). This complex interplay is currently not well understood and is investigated here.

Methods: The PDAC TCGA PanCancer Atlas dataset was used to compare relative levels of ADAMTS protease expression at the bulk RNA seq level. A TNM plot was used to compare levels of ADAMTS proteases between normal adjacent (NA) and tumor tissues. Patient-derived tissue microarrays (TMAs) (n=66) were used to correlate VCAN proteolysis, ADAMTS 4 and 5 as well as A2M expression. To explore the causative link between A2M and VCAN proteolysis, a major drawback of the well-studied Pan02 injectable model of PDAC is the lack of intense desmoplasia seen in the TME of human PDAC. To overcome this limitation, KPC tumor organoids and cancer-associated fibroblasts (1:10) were co-allografted into the flanks of B6 mice, followed by validation of cytokeratin-17/19 and alpha-smooth muscle actin expression using immunohistochemistry.

Results: ADAMTS 1, 4 and 9 have similar relative levels of expression in PDAC; ADAMTS 5 and 15 slightly lower [median RSEM $\log_2$(value+1) = 10.2, 9.91, 9.54, 7.25, 5.33] and ADAMTS 20 is almost absent. ADAMTS 4, 5, 9 and 15 have significantly higher expression in PDAC tumor tissue versus (vs) normal adjacent (NA) pancreas tissue ($\log_2$FC= 2.16, 1.9, 1.57, 1.4, p-values <0.05). In patient-derived TMAs, PDAC tumors show higher levels of ADAMTS 4 expression vs. NA pancreas tissue (58.42% vs 44.12% of samples are ADAMTS 4^{hi}). ADAMTS 5 expression is high in both tumor and NA tissue (90.5% vs 90%) which may be attributed to residual tumor tissue in the NA samples. Both ADAMTS 4 and 5 correlate with poor VCAN proteolysis. A2M RNA ($\log_2$FC= 2.09, p-value <0.05) and protein levels are significantly higher in tumor tissue vs. NA pancreas (67.41% vs 33.33% of samples are $A2M^{hi}$) and correlate with poor VCAN proteolysis. The murine co-allograft model resembles the human PDAC TME closely as evidenced by the pattern of cytokeratin-17/19 staining on epithelial cells and alpha-smooth muscle actin on cancer-associated fibroblasts.

Conclusions: High A2M expression correlates with high ADAMTS 4/5 expression and poor VCAN proteolysis, indicating a potential for A2M to aid in VCAN accumulation in the TME. The co-allograft model will be used to explore the role of A2M further in the PDAC TME.

#5118 Ectopic expression of cathepsin G in mammary tumor cells and its role in tumor growth and osteolytic bone metastasis.

Ridhi Bhola, Reegan Sturgeon, Rakesh K. Singh

University of Nebraska Medical Center, Omaha, NE

Breast cancer has a high propensity to metastasize to the bone microenvironment. The resulting lesions are predominantly osteolytic and are associated with patient morbidity and mortality. We have demonstrated that tumor-bone interaction plays an important role in osteolytic bone metastasis mediated by the dysregulation of a unique set of genes. Among these genes, several proteases are up-regulated at the tumor-bone interface, including Cathepsin G, a serine protease. These proteases act as mediators between the tumor and stroma. Previously, we have shown that both malignant cells and osteoclasts at the TB interface express higher levels of Cathepsin G, which is critical for osteolytic bone metastasis. The precise role of tumor and/or host-derived Cathepsin G in osteolytic bone metastasis is unknown. In this report, we examined the contribution of tumor-derived Cathepsin G by testing the hypothesis that ectopic expression of Cathepsin G modulates malignant cell phenotype, osteoclast recruitment and activation, and osteolytic metastasis. We generated stable mammary tumor cell lines constitutively expressing Cathepsin G (Cl66-CTSG). Cathepsin G-expressing tumor cells showed diminished *in vitro* cell proliferative potential and *in vivo* mammary fat pad tumor growth as compared to vector control transfected Cl66-CMV cells. We demonstrated that Cathepsin G-overexpressing tumor cells induced osteoclast activation and differentiation *in vitro* and tumor-induced osteolysis *in vivo*. To observe the morphological changes in tumor cells by CTSG, we performed phalloidin staining and live cell imaging using CTSG-treated tumor cells. We observed that CTSG induced the formation of homotypic aggregations of tumor cells. We found that increasing the concentration of CTSG decreases tumor cell proliferation and increases cell cycle arrest. Our data suggest that tumor-derived Cathepsin G might have distinct tumor-modulating activities in the bone microenvironment by regulating cell proliferation and osteoclast recruitment and activation.

#5119 Serine proteases modulate neutrophil survival and contribute to immunosuppression.

R. Sturgeon[1], S. Maram[2], A. Meyerson[3], R. Bhola[1], E. Johnson[1], R. K. Singh[1];
[1]University of Nebraska Medical Center, Omaha, NE, [2]Millard North High School, Omaha, NE, [3]University of Michigan, Ann Arbor, MI

Tumor-promoting inflammation is a hallmark of cancer, contributing to malignant cells' survival and proliferation. Infiltrating leukocytes and pro-inflammatory cytokines released into the tumor microenvironment (TME) often cause this inflammation. We have previously shown that increased infiltrating neutrophils are associated with pancreatic ductal adenocarcinoma (PDAC) disease progression, which correlates with increased inflammation and neutrophil accumulation. Prolonged neutrophil accumulation has been linked to chronic inflammation, which in turn is thought to contribute to tumor progression, therapy resistance, and metastasis. In the present study, we examined how neutrophil-PDAC interaction enhances the survival of neutrophils dependent on serine proteases and their inhibitors. Previous reports suggest that serine proteases, such as cathepsin G, can contribute to inflammation and induce caspase-independent neutrophil apoptosis. We examined the role of serine proteases and their inhibitors on neutrophil survival using defined cellular models. We observed an association between increased serine protease and neutrophil survival. Moreover, this survival was modulated by cellular aggressiveness and therapy resistance. In addition, we observed increased survival of neutrophils associated with inhibition of serine proteases and increased expression of serpins to proteases in neutrophils upon PDAC cancer cell conditioned media treatment. In summary, these studies point to neutrophil serine proteases and their inhibitors as versatile mediators that modulate neutrophil survival, leading to an immunosuppressive microenvironment and identifying them as potential targets for therapeutic interventions.

TUMOR BIOLOGY: Molecular Regulation of EMT
Poster Session

#5123 Up-regulation of KRT14 promotes EMT in basal muscle-invasive bladder cancer through IGF2BP1/FTO dependence on methyladenosine-modified SNAI1.

C. Huang;
Wenzhou Medical University, Wenzhou, China

Basal muscle-invasive bladder cancer (BMIBC) is considered one of the subtypes of BC with the highest metastatic rate and the poorest prognosis. Therefore, elucidating the mechanisms underlying BMIBC metastasis and identifying novel precision therapeutic targets are current research hotspots and challenges to the cancer researchers. Through a series of *in vitro* and *in vivo* functional experiments, we have identified the crucial role of KRT14 in the high invasiveness and adverse prognosis of BMIBC. We found that the K294 site within the IGF2BP1-KH2 domain is responsible for reading the conserved genetic information carried by D226/E227 in the KRT14 nuclear export signal (NES). Activation of the KRT14-IGF2BP1 signaling axis is essential for IGF2BP1-mediated stabilization of SNAI1 mRNA through FTO modification. Additionally, IGF2BP1 forms a positive feedback loop by stabilizing its own mRNA, thereby accelerating the invasion and metastasis of BMIBC. Collectively, our study identifies the KRT14/IGF2BP1/FTO/Snail signaling axis as an essential regulatory mechanism associated with poor prognosis in BMIBC, providing a theoretical basis for KRT14 and its downstream regulated molecules as therapeutic targets for BMIBC and the development of corresponding targeted therapies.

Keywords: Basal muscle invasive bladder cancer; KRT14; IGF2BP1; m^6A methylation; Nuclear translocation

#5124 Dynamic subcellular localization of the EMT-associated lncRNA lncX in pancreatic cancer.

Shu Hirako[1], Keiko Shinjo[1], Miho Suzuki[1], Tatsunori Nishimura[1], XingXing Wang[1], Yoshiteru Murofushi[1], Shinji Mii[1], Reo Maruyama[2], Kohei Kumegawa[2], Atsushi Enomoto[1], Yutaka Kondo[1]

[1]Nagoya University, Nagoya, Japan,[2]Japanese Foundation for Cancer Research, Tokyo, Japan

Long non-coding RNAs (lncRNAs) are non-coding RNAs that are longer than 200 nucleotides. These RNAs play essential regulatory roles in processes such as chromatin modification, transcriptional regulation, and protein localization, depending on their subcellular distribution in the nucleus or cytoplasm. These functions have attracted considerable attention in cancer research. LncX, highly expressed in various cancers, has been implicated in promoting tumor malignancy by influencing processes such as proliferation, epithelial-mesenchymal transition (EMT). It has been reported that lncX regulates mRNA by acting as a competing endogenous RNA through binding to miRNAs. However, the subcellular localization of lncX and its interactions with specific proteins in pancreatic ductal adenocarcinoma (PDAC) remain poorly understood. We investigated the localization of lncX in the PDAC cell lines using RNA fluorescence in situ hybridization (RNA-FISH) and super-resolution microscopy. Under conditions where cells were tightly adhered to one another, lncX was localized at the cell membrane. Interestingly, when cell adhesion was disrupted and the cells entered a suspended state, lncX predominantly localized in the nucleus and cytoplasm. A similar localization pattern was observed in PDAC samples from a PDAC mouse model (KPC) and human PDAC tissues. Notably, lncX co-localized with CDH1 at the cell membrane and translocated to the nucleus following specific stimuli. These findings suggest that lncX may play distinct roles depending on its localization, potentially contributing to cancer malignancy.

#5125 Low-density lipoprotein receptor-related protein 1 as a negative regulator in epithelial-mesenchymal transition and metastasis of colorectal cancer: Targeting the Wnt/β-Catenin signaling pathway.

Zhihong Zhang[1], Dingwen Zhong[2], Tanxing Cai[1], Ze Li[1], Yufeng Chen[1], Xiaojian Wu[1], Xiaobin Zheng[1]

[1]the Sixth Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong, China, China,[2]Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, Guangdong, China, China

Background and aim: Low-density lipoprotein receptor-related protein 1 (LRP1) is a multifunctional receptor involved in various cellular processes, including cancer progression. Its role and the underlying mechanisms in the metastasis of colorectal cancer (CRC) remain to be fully elucidated.

Methods: LRP1 expression in CRC was assessed by immunohistochemistry in a cohort of 400 tissue samples, with subsequent Kaplan-Meier survival analysis. In vitro studies included real-time PCR, western blot, colony formation assays, immunofluorescence assays, wound healing assays, and migration and invasion experiments to explore LRP1's impact on epithelial-mesenchymal transition (EMT), cell migration, and invasion. In vivo, mouse xenograft and metastasis models were employed to assess tumorigenicity and metastatic potential, with immunohistochemistry, real-time PCR, and western blot used to evaluate protein expression levels. Luciferase reporter assays were conducted to investigate LRP1's influence on the Wnt/β-catenin signaling pathway.

Results: LRP1 was found to be highly expressed in 68.2% of the CRC specimens and was significantly associated with membranous E-cadherin expression and cytoplasmic β-catenin localization. Elevated LRP1 levels, in conjunction with reduced Wnt/β-catenin signaling activity, correlated with decreased nuclear accumulation of β-catenin and predicted better prognosis. LRP1 suppressed cell proliferation, migration, and invasion both in vitro and in vivo, and hindered the nuclear translocation of β-catenin. Wnt/β-catenin signaling could counteract LRP1's effects on EMT and enhance the expression of vimentin, a mesenchymal marker. Restoring vimentin expression mimicked the effects of LRP1 knockdown without altering Wnt/β-catenin activity.

Conclusions: LRP1 appears to be a significant negative regulator in the EMT, invasion, and metastasis of CRC, potentially acting upstream of the Wnt/β-catenin signaling pathway. Therefore, the combined targeting of LRP1 and the Wnt/β-catenin pathway may offer a novel and promising therapeutic strategy for CRC patients.

#5126 Time-series single-cell RNA sequencing uncovers PLAU enhancing early lung metastatic seeding of esophageal squamous cell carcinoma.

Yingchen Lyu, Ching Ngar Wong, Hongyu Zhou, Songna Wang, Xinyuan Guan

The University of Hong Kong, Pokfulam, Hong Kong

Background: Metastasis continues to be the primary cause of cancer death even with recent advancements in cancer treatment. The initiating ability of metastatic growth bottlenecked in cancer development and could be a promising target. We have successfully captured the distinctive features of early metastatic cells through time-series scRNA-seq using the pulmonary metastatic model.
Methods: To characterize the early metastatic tumor microenvironment (TME), we looked into the highly expressed genes encoding the secreted protein in early metastasis performing GO and KEGG pathway analysis. Clinical implication of PLAU overexpression was studied in ESCC patients. Multiplex immunohistochemistry staining of PLAU and previously identified metastatic initiating genes in ESCC patient samples were completed. Functional assays, including cell migration, invasion, wound healing, and foci formation were undertaken to evaluate the metastatic ability. PLAU knockdown ESCC cells were injected intravenously and subsequent in vivo lung imaging was obtained.
Results: Through GO and KEGG pathway analysis of the single-cell profiling, the genes highly expressed in early metastatic lung exhibited a significant enrichment in processes related to coagulation and fibrinolysis (F3, THBS1, PLAU, SEPRINE1 and ANXA2). Among these genes, PLAU increased greatly in 6 hours and was maintained at a relatively high level during the early metastatic phase. We further explored the implications of PLAU. The analysis of the TCGA database reveals that PLAU is significantly upregulated in tumor tissues compared to non-tumor tissues .Multiplex immunohistochemistry staining of both ESCC cell lines and clinical samples demonstrated co-expression of PLAU and the previously identified metastatic initiating signature CD44 and TACSTD2. Knockdown of PLAU greatly impaired the ability of migration, invasion, wound healing and foci formation ($p<0.05$). In addition, the knockdown of PLAU led to significantly fewer surviving cancer cells in mice lungs in the early phase of metastasis ($p<0.05$).
Conclusions: In all, the finding indicated that PLAU co-expressed with previously identified metastatic initiating genes and increased early dissemination of ESCC lung metastasis. Targeting PLAU could offer a promising approach to limit early metastatic progression in ESCC.

#5127 Metastatic organotropism in small cell lung cancer.

M. Krishnamurthy[1], A. Dhall[2], C. W. Schultz[2], M. Baird[3], P. A. Desai[2], J. Odell[4], S. Sahoo[5], N. Takahashi[6], M. Nirula[2], S. Zhuang[2], Y. Huang[2], B. Schroeder[2], Y. Zhang[2], C. Redon[2], C. Robinson[2], L. Thang[2], L. Ileva[2], N. L. Patel[2], J. D. Kalen[2], A.-A. Varlet[4], N. Zuela-Sopilniak[4], A. Jha[3], D. W. Zong[7], D. Butcher[2], T. Morgan[2], A. Afzal[2], R. Chari[2], K. Baktiar[2], S. Kumar[2], L. Pongor[8], S. Difilippantonio[2], M. I. Aladjem[2], Y. Pommier[2], M. K. Jolly[5], J. Lammerding[4], A. K. Sharma[2], A. Thomas[2];

[1]Icahn School of Medicine at Mount Sinai, New York, NY, [2]National Cancer Institute, Bethesda, MD, [3]National Heart, Lung, and Blood Institute, Bethesda, MD, [4]Cornell University, Ithaca, NY, [5]Indian Institute of Science, Bengaluru, India, [6]National Cancer Center East Hospital, Kashiwa, Japan, [7]National Center for Advancing Translational Sciences, Bethesda, MD, [8]Cancer Genomics and Epigenetics Core Group, Szeged, Hungary

Metastasis is the leading cause of cancer-related deaths, yet its regulatory mechanisms are not fully understood. Small-cell lung cancer (SCLC) is the most metastatic form of lung cancer, with most patients presenting with widespread disease, making it an ideal model for studying metastasis. However, the lack of suitable preclinical models has limited such studies. We utilized well-annotated rapid autopsy-derived tumors to develop xenograft models that mimic key features of SCLC, including histopathology, rapid and widespread development of metastasis to the liver, brain, adrenal, bone marrow, and kidneys within weeks, and response to chemotherapy. By integrating in vivo lineage selection with comprehensive transcriptomic, epigenomic, and single cell multi-omic analyses, we identified critical cellular programs driving metastatic organotropism to the liver and brain, the most common sites of SCLC metastasis. Our findings reveal the key role of nuclear-cytoskeletal interactions in SCLC liver metastasis. Specifically, the loss of the nuclear envelope protein lamin A/C, encoded by the *LMNA* gene, increased nuclear deformability and significantly increased the incidence of liver metastasis. Human liver metastases exhibited reduced *LMNA* expression compared to other metastatic sites, correlating with poorer patient outcomes and increased mortality. This study introduces novel preclinical models for SCLC metastasis and highlights pathways critical for organ-specific metastasis, offering new avenues for the development of targeted therapies to prevent or treat metastatic disease.

#5128 Role of adherent-to-suspension transition in melanoma metastatic dissemination and circulating tumor cell dynamics.

Dong Ki Lee[1], Jongwook Oh[1], Soyeon Lee[1], Hyun Woo Park[2], Heon Yung Gee[1]

[1]Department of Pharmacology, Yonsei University College of Medicine, Seoul, Korea, Republic of,[2]Department of Biochemistry, Yonsei University College of Life Science and Biotechnology, Seoul, Korea, Republic of

Background: Melanoma is the most fatal form of skin cancer, presenting significant therapeutic challenges due to its high metastatic potential. Once melanoma spreads to distant sites, prognosis declines sharply, underscoring the need to uncover the mechanisms driving metastasis. This study investigates the role of transcriptional regulators in melanoma metastasis, focusing on how primary tumor cells undergo “Adherent-to-Suspension Transition” (AST) to reprogram their anchorage dependency and disseminate into the bloodstream.
Methods: Using fluorescent protein-tagged melanoma cells, we developed an orthotopic melanoma metastasis mouse model and performed single-cell RNA sequencing (scRNA-seq) on melanoma cells isolated from the primary footpad tumor, blood, and lung metastases. Publicly available spatial transcriptomics data were analyzed to identify the cells undergoing AST within the primary tumor. Doxycycline-inducible AST-overexpressing melanoma cell lines were generated for functional validation.
Results: We identified three AST factors—IKZF1, IRF8, and NFE2—known hematopoietic transcriptional regulators that reprogram adherent cells into suspension cells. Induction of these AST factors upregulated hemoglobin genes while downregulating extracellular matrix (ECM)-integrin genes, leading to cell-matrix dissociation and anoikis resistance. During metastasis, AST factor expression was induced in circulating tumor cells (CTCs) but reversed in metastatic niches. Notably, AST factor expression was independent of epithelial-mesenchymal-like transition (EMT-like transition). Phylogenetic analysis of scRNA-seq data revealed that AST-positive clones exhibited greater aggressiveness and metastatic potential.
To evaluate AST factors as therapeutic targets, we performed an intervention study using lenalidomide, an IKZF1 inhibitor. Treatment significantly reduced the number of CTCs and metastatic burden without affecting the primary tumor. Spatial transcriptomics analysis demonstrated an inverse correlation between AST factor expression and proximity to blood vessels, further implicating AST in early metastatic processes.
To explore AST-mediated regulation of anchorage dependence, we subjected B16F10-3AST^{TetR} cells to repeated cycles of doxycycline treatment. Doxycycline induction caused cell detachment, which was reversed upon withdrawal, highlighting the dynamic nature of AST. RNA sequencing revealed that AST-induced cells were depleted of the Hallmark EMT pathway, suggesting that AST reprograms anchorage dependency through distinct mechanisms.
Conclusion: AST represents a novel, EMT-independent mechanism driving melanoma metastasis by reprogramming anchorage dependency. These findings position AST factors as actionable therapeutic targets for preventing metastatic progression in melanoma.

#5130 DNA methylation profiling reveals epigenetic dynamics across epithelial-mesenchymal transition states in the triple-negative breast tumor microenvironment.

Hanxu Lu[1], Fred W. Kolling[1], Lucas A. Salas[1], Diwakar R. Pattabiraman[1], Todd W. Miller[2], Brock C. Christensen[1]

[1]Dartmouth Geisel School of Medicine, Lebanon, NH,[2]Medical College of Wisconsin, Milwaukee, WI

Epithelial-mesenchymal transition (EMT) is a dynamic, reversible process that facilitates tumor invasion and metastasis. Tumors often exhibit intermediate EMT states with co-expression of epithelial and mesenchymal markers. DNA methylation can regulate the expression of EMT-related genes, influencing the stability of epithelial and mesenchymal cell states. However, DNA methylation changes during the dynamic process as cells transition through intermediate EMT states are not well understood. This study integrated single-cell RNA sequencing (scRNA-seq) and DNA methylation profiling to investigate dynamic DNA methylation changes across the EMT spectrum. Single-cell-derived clonal populations from the heterogeneous 4T1 murine triple-negative breast cancer cell line were isolated for five states representing the spectrum of EMT: an epithelial-like clone (E), three intermediate clones (EM1, EM2, EM3), and a mesenchymal-like clone (M). These clones were orthotopically transplanted into the mammary fat pads of mice, and tumors were harvested to isolate CD45- and CD45+ cell populations for DNA methylation profiling at over 285,000 CpG sites throughout the mouse genome using an Illumina mouse methylation array. Previous scRNA-seq revealed significantly higher expression of *Epcam* in the epithelial-like clone and *Gpnmb* in the mesenchymal-like clone. Consistent with the changes in gene expression, methylation shifts were observed across EMT states in the *Epcam* and *Gpnmb* promoter regions, both *in vitro* and *in vivo*. Notably, immune cell proportions estimated using DNA-based deconvolution exhibited trends consistent with scRNA-seq data, highlighting the potential of multi-omics approaches to comprehensively study the EMT process. To further investigate the progressive increase in mesenchymal transition, we employed a linear model using the CD45- fraction. Hypomethylated CpGs in mesenchymal states were enriched in EMT-related transcription factors, such as FOXC1 and HOXA6. Compared with DNA methylation data from the tumor CD45+ cell fraction, principal component analysis (PCA) of CD45- samples showed separation by EMT states, suggesting fewer DNA methylation changes in tumor infiltrating immune cells. This study highlights epigenetic alterations as cells transition from epithelial to mesenchymal states, with methylation patterns aligning closely with gene expression profiles. Furthermore, these findings emphasize the opportunity to use DNA-based cell type deconvolution to accurately assess and investigate immune cell infiltration within the tumor microenvironment in association with cancer cell plasticity and immune response in tumor progression.

#5131 Dysregulated cholesterol metabolism facilitates epithelial-to-mesenchymal transition and tumor progression in colorectal cancer.

Blake M. Arciga[1], Van Nguyen[2], Jussuf T. Kaifi[3], Satyanarayana Rachagani[4]

[1]Roy Blunt NextGen Precision Health Institute, University of Missouri-Columbia, Columbia, MO,[2]Department of Clinical Pathology and Anatomical Sciences, University of Missouri-Columbia, Columbia, MO,[3]Department of Surgery, University of Missouri-Columbia, Columbia, MO,[4]Department of Veterinary Medicine and Surgery, University of Missouri-Columbia, Columbia, MO

Introduction: Colorectal cancer (CRC) is a leading cause of cancer-related death in the United States, and its development is closely correlated with lifestyle factors, including a high cholesterol “Western” diet. In our novel study, we investigated the etiology of dysregulated cholesterol homeostasis in CRC and characterized its systemic effects on tumor progression.

Methods: Expression of DHCR7, the rate-limiting enzyme of cholesterol biosynthesis, in CRC was analyzed *in silico* using TCGA-COAD data. A human tissue microarray (n=208 cores) with samples from normal colon, local colon tumors, and lymph node metastases was stained for DHCR7 using IHC. Stained tissue cores were scored blindly by a clinical pathologist based on our histological scoring system, which accounted for the percentage of positive cells and the intensity of staining. We generated an APCCDX-Cre^{ER} CRC mouse model and collected colonic tissue samples for analysis. Tissues were stained for DHCR7 and scored. To assess the impacts of high diet cholesterol on epithelial-to-mesenchymal transition (EMT) *in vivo*, we developed an APC model which was divided into three diet groups of equal size: a control group (fed a 0 ppm cholesterol diet), a low cholesterol group (186 ppm), and a high cholesterol group (880 ppm). Colonic tissue samples were stained for claudin-1, a classical marker of epithelial barrier integrity. MTT assays were used to measure the *in vitro* proliferation of SW620 human CRC cells incubated in 0, 5, 10, 15, 20, and 25 µM cholesterol.

Results: *In silico* data analysis indicated that DHCR7 is broadly overexpressed in CRC patients. Histological scoring of human patient samples showed upregulation of DHCR7 in local and metastatic CRC tumors compared to normal colonic tissue. Surprisingly, DHCR7 expression was significantly higher in lymph node metastases than in the primary tumor. DHCR7 overexpression was also observed in tumor-bearing APC mice relative to tumor-free controls. Claudin-1 expression was found to be significantly downregulated in mice fed a high cholesterol diet compared to mice fed a low cholesterol or control diet. Finally, SW620 cells grown in cholesterol proliferated at a faster rate over 96 hours than control cells incubated in 0 µM cholesterol media, although a dose-dependent effect was not observed.

Conclusions: DHCR7 is systemically overexpressed in CRC primary tumors, and expression is even further elevated in metastatic disease. Therefore, *de novo* cholesterol synthesis is dysregulated in tumor cells, causing a profound disruption to cholesterol homeostasis which may potentiate EMT and metastasis. High cholesterol uptake also promotes a mesenchymal phenotype in tumor cells and stimulates proliferation. Our findings indicate that dysregulation of cholesterol homeostasis increases the tumorigenicity and metastatic potential of CRC cells and may be a target for therapy.

#5132 DNMT1-associated EMT promotes KRAS escape in pancreatic cancer.

Loan Duong, Xin Lu

Biological Sciences, University of Notre Dame, Notre Dame, IN

Pancreatic adenocarcinoma (PDAC) is presently the 3rd leading cause of cancer death in the United States and is further projected to overcome colorectal cancer to become the 2nd leading cause by 2030. The genetic landscape of PDAC is characterized by pervasive oncogenic mutations in KRAS, observed in over 90% of PDAC tumors. KRAS is considered essential for maintaining tumor progression and so targeting it has long been considered crucial to improving outcomes for PDAC patients. For preclinical studies of KRAS in our lab, we are using an inducible KRAS (iKRAS) model to model KRAS activation and inhibition. This model is based on iKRAS cell lines derived from transgenic tetO_LSL-KrasG12D; p53L/+; p48-Cre mice and upon doxycycline treatment, express oncogenic KrasG12D. Orthotopic implantation of iKRAS cells results in the formation of doxycycline-dependent pancreatic tumor growth in both immune-deficient and immunocompetent models. Withdrawal of doxycycline results in tumor regression. However, we have observed instances of tumor relapse despite the absence of doxycycline and established cell lines from these relapsed tumors. In vitro analyses confirm that these cells are independent of both doxycycline and KRAS, unlike the parental cell line. In vivo, upon reimplantation in the pancreas, they maintain the ability to grow independently of doxycycline. Transcriptome and reverse phase protein array analysis reveal an enrichment of markers associated with epithelial to mesenchymal (EMT) with western blot analyses further confirming increased expression of EMT markers, including Snail, Slug, and αSMA. The KRAS-independent cell line further demonstrated elevated DNMT1 expression, a previously described driver of EMT. Targeting DMNT demonstrated more pronounced effects on cell viability in the KRAS-independent cell line than the parental KRAS-dependent cell line. Deeper insight into the ability of these cells to escape KRAS addiction will enhance our understanding of resistance to KRAS inhibition and suggest further avenues for co-targeting.

#5133 End-stage breast cancer metastases manifest as two subtypes with distinct dissemination patterns, proliferation/EMT signatures, and immune microenvironments.

Isaac Bishara[1], Xuan Liu[2], Jason I. Griffiths[1], Patrick A. Cosgrove[1], Jasmine R. McQuerry[1], Jiayi Liu[2], Kena K. Ihle[1], Eliza R. Bacon[1], Feng Chi[1], Pierre Wallet[1], Vince Grolmusz[1], Diana Simons[1], Benjamin Copeland[1], Lance Pflieger[1], JinFeng Chen[1], Sumana Majumdar[1], Terron T. Crowder[1], Rena Emond[1], Rachel Factor[3], David D. L. Bowtell[4], Adam L. Cohen[5], Daniel D. Schmolze[1], Peter P. Lee[1], Lusine Tumyan[1], James R. Waisman[1], Andrea Bild[1], Aritro Nath[1], Jeffrey T. Chang[2]

[1]City of Hope National Medical Center, Duarte, CA,[2]University of Texas Health Science Center at Houston, Houston, TX,[3]University of Utah, Salt Lake City, UT,[4]Peter MacCallum Cancer Center, Melbourne, Australia,[5]Inova Schar Cancer Institute, Fairfax, VA

End stage breast cancer often presents with oligometastatic tumors and lacks effective treatment options. Previous studies leveraged post-mortem tissue procurement (also called rapid autopsy) programs to collect tumors shortly after death for profiling by bulk whole exome sequencing (WES). This has revealed substantial heterogeneity in the genomes of metastatic estrogen receptor-positive (ER+) and triple-negative breast cancers. In order to understand the heterogeneity of both the genotypes, transcriptional phenotypes, and evolution of these tumors, we collected multi-site metastatic tumors from two end-stage ER+ breast cancer patients enrolled in the LEGACY rapid post-mortem tissue procurement trial. Metastatic tumors were profiled using WES and single-nuclei RNA sequencing (snRNA-seq). We reconstructed high resolution phylogenies for each patient using the mutation called from the snRNA-seq reads to determine the subclonal architecture of cancer cells. Based on the mutation patterns, the gene expression profiles, and the subclonal architecture, we found that the tumors in each patient were divided into two distinct subtypes. A majority of the tumors were grouped into a subtype characterized by a high intra-tumor heterogeneity, with many cell lineages shared widely across metastasis, indicating extensive and undirected dissemination. The cancer cells of this subtype exhibited an EMT gene expression signature. In contrast, the second tumor subtype observed was rare and demonstrated little intra-tumor heterogeneity, a proliferative gene expression signature, and an interferon response signature present widely across cells in the microenvironment. Across all tumors, macrophages comprised the majority of the immune cells. Low diversity tumor macrophages exhibited strong activation of multiple interferon signaling pathways compared to high diversity tumor macrophages. Interestingly, these macrophages expressed a mixture of M1 and M2 polarization markers as STAT1, SOCS1, ISG15, ISG20, MX1, MX2, OAS1, OAS3 that did not correspond to classical M1 or M2 polarization states. This contrast demonstrates that multi-site metastatic tumors and their microenvironment adopt dichotomous states, which may contribute to the difficulties in treating progressive disease. It also underscores the need for a comprehensive and personalized late-stage therapeutic strategy to target the diversity of existing tumor phenotypes.

#5134 Cross-talk of EZH2/PRC2 and Gata5/Foxa2/miR-200 regulates EMT in lung cancer metastasis.

Cesar Seigi Fuziwara[1], Guilherme A. Gois[1], Pedro L. S. Oliveira[1], Joice M. Menezes[1], Bin Xue[2], Lin He[2], Edna T. Kimura[1]

[1]Cell and Developmental Biology, University of Sao Paulo, Sao Paulo, Brazil,[2]University of California Berkeley, Berkeley, CA

Introduction: Lung cancer is the most lethal type of cancer. Part of this lethality is due to late diagnosis when more than 60% of cases present distant metastases at bones, brain and liver, leading to an overall survival of less than 5%. Thus, investigating the biology of metastatic process is mandatory as a public health policy. One molecular mechanism involved in lung cancer progression is the repression of miR-200 family of miRNAs that regulate the epithelial-mesenchymal transition (EMT). Our previous study showed that miR-200 deletion accelerated the formation of lung adenocarcinoma and progression in KP transgenic mice model. Objective: Investigate the transcriptional regulation of miR-200 cluster and its impact in lung cancer metastasis.
Methods: For in silico analysis of promoter region, we used the FANTOM 5, genome browser and transcription factor (TF) prediction tools. We cloned several fragments of miR-200c/141 and miR-200b/miR-200a/miR-429 into the luciferase reporter plasmid pGL4-20, and transfected into a panel of murine lung cancer cell lines (metastatic: 889PF, 404M1 and KPT4-LM and non-metastatic: 368T1, 394T4 and KPT4). Gene expression analysis of TFs and miR-200s was performed for comparisons among lung cancer lines, including. For overexpression experiments, we cloned the coding sequence of TFs (Gata5 and Foxa2) into MSCV-puro plasmid and transfected into metastastic lines 889PF and 404M1. Modulation of Ezh2 was performed with CRISPR/Cas9 in 889PF cells.
Results: we defined the minimal promoter region of miR-200c/141 and miR-200b/miR-200a/miR-429 as the fragments -1246 to 0bp and -4363 to -4027bp from transcription start site, respectively. In silico search for TFs revealed a list of several TFs related to lung biology, such as Nkx2-1, Gata5 and Foxa2. Gene expression analysis showed that reduced levels of miR-200s expression in metastatic lines (889PF and 404M1) inversely correlated with TFs Gata5 and Foxa2 levels. The reactivation of Gata5 and Foxa2 in the metastatic lines reverted the mesenchymal phenotype to an epithelial phenotype while restored miR-200s expression and blocked EMT factors. Further investigation of Gata5 and Foxa2 genes promoter region revealed a common signature for polycomb gene Ezh2/PRC2. Overexpression of Ezh2 in non-metastatic cancer lines reduced TFs and miR-200 expression. Moreover, KPT4-LM, another metastatic line, showed activation of EMT and reduction of miR-200s and TFs, while Ezh2 and PRC2 components were upregulated. Ezh2 gene editing in 889PF induced epithelial phenotype in some clones.
Conclusion: mesenchymal phenotype is controlled by the loop between EZH2/PRC2 and TFs Gata5-Foxa2/miR-200s. Moreover, Gata5 and Foxa2 are pioneer TFs that can restore epithelial phenotype in lung cancer metastatic cell lines.

#5135 Effect of EZH2 inhibition in anaplastic thyroid cancer epithelial-mesenchymal transition revealed by transcriptome analysis.

Diego Claro de Mello, Kelly Cristina Saito, Marcella Maringolo Cristovao, Edna Teruko Kimura, Cesar Seigi Fuziwara

Institute of Biomedical Sciences, Departament of Cell and Devolpmental Biology, University of Sao Paulo, Sao Paulo, Brazil

Introduction: Anaplastic thyroid cancer (ATC) is a rare but highly aggressive endocrine malignancy characterized by rapid tumor growth, early metastasis (>40%), and resistance to standard therapies like radioiodine. The epithelial-mesenchymal transition (EMT) is a critical process driving ATC progression and metastasis, underscoring the urgency of identifying its molecular regulators. In that sense, EZH2, the catalytic subunit of the Polycomb Repressive Complex 2, promotes transcriptional silencing by deposition of trimethylation of histone H3 at lysine 27 (H3K27me3) at target genes. Previously, we demonstrated that CRISPR/Cas9-mediated EZH2 inhibition suppresses ATC cell dedifferentiation, growth, migration, and invasion by downregulating EMT markers. However, the mechanisms through which EZH2 regulates EMT in ATC are not fully understood.
Objective: To investigate the impact of EZH2 inhibition in the transcriptomic landscape of coding genes and miRNAs involved in EMT in ATC biology.
Methods: RNAseq analysis was performed on EZH2-inhibited SW1736-ClE cells to identify differentially expressed genes (DEGs), with sequencing conducted on an Illumina Nextseq 2000 (paired-end 2 × 100 bp), yielding up to 42 million reads for SW1736-CTR and SW1736-ClE. Functional enrichment and protein-protein interaction (PPI) analyses were carried out using Enrichr and STRING web tools, respectively. EZH2/H3K27me3 targets were predicted using the Cistrome Database. A miRNome analysis (Qiagen miScript Arrays) assessed the expression of 1,008 human miRNAs, and predicted miRNA targets were overlapped with DEGs for enrichment analysis. To explore the EZH2/Wnt/β-catenin/miR-200 axis in EMT regulation, Wnt signaling and miR-200 promoter activity were evaluated using β-catenin and miR-200 family overexpression models.
Results: RNAseq analysis identified 1,024 downregulated and 939 upregulated DEGs in SW1736-ClE. EZH2 inhibition suppressed EMT programs by downregulating canonical Wnt signaling genes, including *CTNNB1, FZD4*, and *ZEB1*, as shown by Enrichr and PPI analyses. Using Cistrome database, we observed that EZH2/H3K27me3 enrichment was observed at loci of negative Wnt signaling regulators (*WNT5A/7A, FZD2/7*), which were upregulated in SW1736-ClE cells. Suppressing canonical Wnt signaling reduced EMT-promoting factors and increased epithelial markers. miRNome analysis revealed significant upregulation of tumor suppressor miRNAs, notably the miR-200 family. The overexpression of the miR-200c or miR-200a alone in SW1736 cells did not independently inhibit Wnt signaling activity. Conversely, β-catenin overexpression impaired miR-200 promoter activity.
Conclusions: EZH2 is a key regulator of ATC oncogenesis, driving EMT through a mechanistic link between Wnt signaling and miR-200 family regulation, highlighting potential therapeutic targets in ATC.

#5136 Unraveling SMARCA1's role in cancer progression, drug resistance, and muscle differentiation through EMT-related signaling, TGF-β pathway, and key transcription factors SNAI1 and EGR1.

Ashwaq K. Aljabri[1], Yuliya Kriga[2], Juan Manuel Caravaca[2], Jyoti Shetty[2], Bao Tran[2], Matthew Geisler[3], Judith K. Davie[4], Marielle E. Yohe[1]

[1]Laboratory of Cell and Developmental Signaling Department, National Cancer Institute, National Institute of Health, Frederick, MD,[2]Sequencing Facility, Cancer Research Technology Program, Frederick National Laboratory for Cancer Research, Frederick, MD,[3]Department of Plant Biology, Southern Illinois University, Carbondale, IL,[4]Department of Biochemistry and Molecular Biology, Southern Illinois University School of Medicine, Carbondale, IL

SMARCA1, a member of the ISWI family of ATP-dependent chromatin remodelers, plays a crucial role in organizing DNA structure and regulating gene expression. The role of SMARCA1 in either normal skeletal muscle or rhabdomyosarcoma (RMS) development has not previously been explored. RMS, an aggressive soft tissue cancer, arises from cells resembling skeletal muscle precursor cells and exhibits invasive behavior. In this study, we conducted an integrated analysis of RNA-seq, ATAC-seq, and CUT&RUN data from RH30 cells, a PAX fusion-positive alveolar RMS line, with and without SMARCA1. This comprehensive data integration revealed that SMARCA1 knockout (KO) cells showed significant changes in gene expression and chromatin accessibility. Key genes such as TGFBR1, MYOG, and SMARCD3, critical for muscle differentiation, were highly active in normal RH30 cells but markedly reduced in SMARCA1 KO cells, underscoring SMARCA1's role in supporting genetic pathways essential for muscle growth, survival, and differentiation. Additionally, SMARCA1 influenced regulatory factors, including SNAI1, EGR1, PTEN, TBX2, and P53. Interestingly, SMARCA1 appears to interact with HDAC2, and combined inhibition of SMARCA1 and HDAC2 has shown promise in overcoming drug resistance in these models. Further cell survival assays revealed that SMARCA1 KO cells are particularly sensitive to the HDAC2 inhibitor Mocetinostat and the MEK inhibitor Trametinib, proposing that targeting SMARCA1 in combination with HDAC2 or MEK inhibition could be an effective therapeutic approach for RMS. Our integrated analysis also links SMARCA1 to regulatory genes and transcription factors such as SNAI1, EMC1, PPP2R5C, EGR1, EYA2, SMARCA4, MYCN, CDK4, and NFKB2, with SMARCA1 enriched on the DNA of these genes. These factors are essential for EMT, cell adhesion, and key cancer-related pathways, including MAPK, WNT, PI3K, and TGF-β. Additionally, SMARCA1 KO cells responded differently to both canonical and non-canonical TGF-β pathway inhibitors, including SB505124 (TGFBR1 inhibitor), the PI3K-mTOR inhibitor Dactolisib, and Withaferin A (NF-κB inhibitor). Notably, these TGF-β pathway inhibitors effectively overcame resistance in ARMS cells with SMARCA1 expression, exposing specific vulnerabilities in these otherwise resistant cells. Together, our findings suggest that targeting both canonical and non-canonical TGF-β pathways, alongside SMARCA1, presents promising therapeutic strategies for overcoming drug resistance and potentially limiting RMS progression.

#5137 Epithelial-to-mesenchymal transition coordinates REEP2 to drive pro-metastatic membrane trafficking program in lung cancer.

Kevin Fulp, Oluwafunminiyi Obaleye, Guan-Yu Xiao

University of Kentucky, Lexington, KY

Membrane trafficking governs the transport of molecules to both intracellular and extracellular locations, thereby maintaining cell homeostasis. During cancer progression, alterations in membrane trafficking are frequently observed. Nevertheless, no effective drugs target membrane trafficking currently and the many mechanisms underlying the dysregulation of membrane trafficking in cancer progression remain a significant unresolved question. Our recent research discovered that the epithelial-to-mesenchymal transition (EMT) employs a membrane trafficking program to coordinate cancer cell invasion and immunosuppression in the tumor microenvironment (TME) in lung adenocarcinoma (LUAD). Importantly, blocking the EMT-driven membrane trafficking program re-sensitizes LUAD to immune checkpoint inhibition, suggesting significant therapeutic potential for this pathway. To dissect the pro-metastatic membrane trafficking program, we initiated a CRISPRi *in vivo* screen to assess more than 2,000 trafficking-related genes in syngeneic mouse LUAD models for their requirements for tumorigenesis. Our preliminary studies identified REEP2 as a novel regulator of the EMT-dependent membrane trafficking program, which is linked to poor prognosis and an immunosuppressive TME in LUAD patients. Our findings here show that the EMT-activating transcription factor ZEB1 upregulates REEP2 by relieving its silencing mediated by miR-193a, thereby facilitating endoplasmic reticulum (ER) polarization to the Golgi apparatus. The REEP2-driven ER polarization promotes pro-metastatic secretory trafficking. Blockade of REEP2 suppresses LUAD cell proliferation, migration, invasion *in vitro*, and tumor metastasis in the syngeneic LUAD mouse tumor model. Our findings elucidate the molecular mechanisms underlying LUAD metastasis and support the development of REEP2 inhibitors to prevent EMT-driven cancer metastasis.

#5138 NRAS overexpression influences EMT in claudin-low triple negative breast cancer.

Jackson Marlon Rodney Thomas[1], Stephanie Dance-Barnes[2]

[1]DePaul University, Chicago, IL,[2]College of Science and Health, DePaul University, Chicago, IL

This study investigates Nras-mediated Ras-Mapk in epithelial-mesenchymal transition and the therapeutic potential of RAS-MAPK inhibitors in Claudin-low(CL) triple-negative breast cancer (TNBC). The K14TRT line represents a potential model for CL and metastatic TNBC research due to its genomic similarities to human CL, TNBC. Research at the Perou Lab indicated that the K14TRT cell line exhibited great expression of the NRAS gene, which may influence the EMT characteristics of the cell line. NRAS influences EMT by altering the expression of signaling proteins involving stem-cell characteristics, cell-to-cell attachment, and cell growth regulators. Subsequent knockout of NRAS via siRNA transfection and use of the MEK inhibitor Trametinib were used to observe changes in transcriptome expression. These changes in the transcriptome will then be analyzed to identify future vulnerabilities in the K14TRT cell line using significance analysis of microarray (SAM) in R-Studio. TNBC lacks targeted treatments that other subtypes of breast cancer have, leading to significant disparities in the 5-year survival rate of breast cancer (78.0% for TNBC vs. 91.2% AVG). In addition, triple-negative breast cancer disproportionately affects young Black American and pre-menopausal women. This study highlights the potential influence of NRAS-mediated RAS-MAPK on EMT in Claudin-low, TNBC. Highlighting the RAS-MAPK pathway for its potential use as a primary target in CL, TNBC.

#5139 PP2A-B56a as a critical regulator of cellular plasticity and epithelial-to-mesenchymal transition.

Brittany N. Heil[1], Claire M. Pfeffer[1], Garima Baral[1], Mei B. Bahler[1], Anna K. Darling[1], Sydney J. Clifford[2], Brittany L. Allen-Petersen[1]

[1]Purdue University, West Lafayette, IN,[2]Brandeis University, Waltham, MA

Metastasis is the major cause of death in cancer patients including lung cancer, the leading cause of cancer-related deaths in the United States. Fifty percent of patients have metastatic disease at the time of diagnosis, plummeting the 5-year survival rate from 26% to just 9%, demonstrating a critical gap in our understanding of the contributing cell signaling pathways. Epithelial-to-Mesenchymal Transition (EMT) is an important initiating step in metastasis, allowing cells to become more migratory and invasive to establish secondary tumors in distant locations. Previous studies have identified transcriptional reprogramming as the main driver of EMT. While transcription factors are important in this cell state switch, there are many factors that contribute to EMT outside of transcriptional regulation, such as post-translational modifications (PTMs). PTMs, such as phosphorylation, play a large role in cell signaling that can impact cellular identity and plasticity, yet these modifications are largely understudied, particularly in the context of EMT. The serine/threonine phosphatase, Protein Phosphatase 2A (PP2A), is a vital phosphatase, accounting for 50% of serine/threonine phosphatase activity in the cell. PP2A is a heterotrimeric complex consisting of a scaffolding subunit (A), a catalytic subunit (C), and regulatory subunit (B) which determines substrate specificity. Though PP2A has previously been shown to play a role in cell state regulation and EMT, the contribution of the individual B subunits is unknown. PP2A-B56α has previously been implicated in tumor suppression and the regulation of cellular plasticity. Through the use of 2D immunofluorescence, western blot analysis, and 3D invasion assays, our *in vitro* studies show that loss of PP2A-B56α leads to a significant alteration in cell identity, demonstrated by a reduction in epithelial markers (E-cadherin) and an increase in mesenchymal markers (Vimentin). These shifts in cell markers also correlate with enhanced migratory and invasive capabilities *in vitro* and are recapitulated *in vivo* via tail vein invasion assay. Collectively, these findings highlight PP2A-B56α as a key regulator of cellular plasticity and EMT in lung cancer.

#5140 Phenotypic plasticity and cellular decision-making: role of SLIT2 in epithelial-to-mesenchymal transition.

Sravani Ramisetty[1], Ayalur Raghu Subbalakshmi[1], Madhu krishna B[1], Atish Mohanty[1], Aditya Agarwal[2], Mathias C Emery[3], Andrew Mc Carthy[3], Dana Do[1], Mari Ida[4], Tamara Mirzapoiazova[1], Siddhika Pareek[1], Deric L Wheeler[4], Sharad S Singhal[1], Prakash Kulkarni[1], Ravi Salgia[1]

[1]Medical Oncology, City of Hope National Medical Center, Duarte, CA,[2]Indian Institute of technology, Varanasi, India,[3]European Molecular Biology Laboratory (EMBL), Grenoble, France,[4]Human Oncology, University of Wisconsin-Madison, Wisconsin, WI

Background: Epithelial-to-mesenchymal transition (EMT), a key driver of cancer progression, is influenced by cell-specific factors. SLIT2, a ligand for ROBO1, regulates tumor growth and migration through a non-canonical ROBO1/EPHA2 pathway in lung and head and neck cancers. Modeling of the SLIT2-EPHA2 EMT circuit revealed SLIT2 stabilizes the epithelial phenotype, while EPHA2 coupling diminishes this suppression, inducing a tri-stable system with epithelial, hybrid, and mesenchymal states. In vitro, recombinant SLIT2 increased E-cadherin and decreased SNAIL in NSCLC and HNSCC cells. SLIT2 knockdown elevated mesenchymal markers, and recombinant SLIT2 suppressed migration in zebrafish xenografts, highlighting its anti-metastatic potential. These findings demonstrate SLIT2's critical role in EMT regulation and its therapeutic promise for controlling metastasis.

Introduction: Phenotypic plasticity, including epithelial-to-mesenchymal transition (EMT), drives cancer progression and resistance. SLIT2, signaling through ROBO1, has dual roles in cancer. In lung and head and neck cancers, SLIT2 inhibits tumor growth via a non-canonical ROBO1/EPHA2 pathway, though its role in EMT remains unclear. This study models SLIT2's effect on EMT and confirms its potential to inhibit hybrid/mesenchymal transitions, offering therapeutic promise.

Methods: SLIT2's role in EMT was assessed in lung and head and neck cancers. A mathematical EMT model incorporating SLIT2 analyzed epithelial, hybrid, and mesenchymal states. Recombinant SLIT2 was tested on NSCLC and HNSCC cells, with EMT markers analyzed. SLIT2 knockdown was used to assess mesenchymal markers. Zebrafish xenografts quantified migration post-SLIT2 treatment.

Results: SLIT2 expression was inversely correlated with EMT scores in LUSC and HNSCC. Epithelial subtypes had higher SLIT2 levels, while mesenchymal subtypes had lower levels. SLIT2 suppressed SNAIL expression, maintaining epithelial characteristics and reducing mesenchymal markers. Recombinant SLIT2 treatment decreased cell proliferation, migration, and spheroid growth. Zebrafish xenografts showed reduced tumor growth and metastasis. Patient tumor analysis indicated that high SLIT2 expression correlated with better survival.

Conclusion: SLIT2 inhibits EMT in lung cancer cells, suppressing mesenchymal markers and maintaining the epithelial phenotype, thus limiting metastasis. Future studies will validate the therapeutic potential of the SLIT2 D2 fragment as an anti-metastatic treatment.

#5141 ADAR1 maintains cancer stem cell proliferation in a mouse model of TNBC-derived breast cancer brain metastases.

Teresa Sposito[1], Emma Klacking[2], Jessica Pham[2], Roman Sasik[2], Inge van der Werf[2], Najla Kfoury-Beaumont[3], Alexander Khalessi[3], Thomas Whisenant[2], Catriona Jamieson[2]

[1]University of California San Diego - UCSD, La Jolla, CA,[2]Medicine, University of California San Diego - UCSD, La Jolla, CA,[3]Neurological Surgery, University of California San Diego - UCSD, La Jolla, CA

Background: Essential for preventing autoinflammation, adenosine deaminase acting on double-stranded RNA (ADAR1) is also a recognized driver of epi-transcriptomic remodeling and tumor immune silencing. High levels of ADAR1 have been associated with a worse prognosis and reduced survival in patients with high-grade breast cancer (BC), especially in the $HER2^+$ and triple-negative BC (TNBC) subgroup, which are the most likely to develop brain metastasis (BrM), arising in up to 30% of TNBC and $HER2^+$ cases. Nevertheless, the role of ADAR1 in the metastatic context is only partially understood.
We have hypothesized that ADAR1 activation and upregulation of the p150 isoform play a pivotal role in promoting BC-BrM invasion by maintaining BC stem cell metastatic niches. We focused on the brain, one of the most aggressive and less studied metastatic sites.
Methods: We developed a BC-BrM model based on intracerebroventricular implants of MDA-MD-231 TNBC and primary BC-BrM patient cells in $Rag2^{-/-}\gamma c^{-/-}$ mice. Before implantation, tumor cells were transduced with a nanoluc-GFP reporter for tracing ADAR1 enzymatic activity in the brain niche, or shRNA vectors for ADAR1 down regulation studies.
Results: Upon establishment of BrM, scRNA sequencing and qRT-PCR revealed that ADAR1 p150 was upregulated, alongside WNT/β-Catenin pathway activation, a switch in the CD47 isoforms, and extra cellular matrix remodeling. Preliminary results from FFPE imaging of patient samples further suggested a correlation between ADAR1 and WNT/β-Catenin activation, showing that high levels of ADAR1 are associated with nuclear translocation of β-Catenin.
Additionally, sc-RNA sequencing of ADAR1 knocked-down TNBC-derived BrM compared to scrambled controls, identified a cluster with increased protein stress and arrest of growth-related processes, predominantly represented by the ADAR1 KD cells. These findings suggest that ADAR1 is essential for maintaining cancer cell proliferation in the brain.
Future Directions: We are now further validating the role of ADAR1 and its molecular interactors in maintaining the metastatic brain niche through scRNA-sequencing of BC-BrM patient samples collected from UCSD hospitals. Overall, this study provides new insights into the molecular role of ADAR1 deaminase activity in establishing and maintaining metastatic niches.

#5142 Role of UCA1 in Warburg effect during anoikis resistance in colorectal carcinoma.

Ricardo Pequeno Bracho, Salique H. Shaham, Yamile Abuchard Anaya, Sophia Leslie, Kyle Doxtater, Bilal B. Hafeez, Tamer Oraby, Subhash C. Chauhan, Manish K. Tripathi

Medicine and Oncology ISU, University of Texas Rio Grande Valley, McAllen, TX

Colorectal carcinoma (CRC) remains the second leading cause of cancer-related deaths in the United States. The survival rate for CRC patients drops precipitously from 90% for localized disease to only 14% upon metastasis. Approximately, 40-50% of CRC cases progress to metastasis, a multifaceted process requiring the acquisition of anoikis resistance as one of the critical steps. This anoikis or anchorage-independent resistance enables cancer cells to survive detachment from the extracellular matrix, migrate through vascular and lymphatic systems, and establish secondary tumor sites. A deeper understanding of the molecular mechanisms underlying anoikis resistance, particularly in the context glucose metabolism, is crucial for advancing therapeutic strategies in CRC metastasis. Cancer cells frequently exhibit the Warburg Effect, a metabolic adaptation characterized by preferential glycolysis over oxidative phosphorylation, even under normoxic conditions. This metabolic reprogramming supports proliferation and may promote survival under anchorage-independent conditions. Our preliminary data suggests that the long non-coding RNA (lncRNA) urothelial carcinoma-associated 1 (UCA1), which is overexpressed in CRC and correlates with poor prognosis, may play a pivotal role in metastasis and glucose metabolism. Utilizing isogenic a CRC cell line model SW480 (primary tumor-derived) and SW620 (metastasis-derived), we have developed stable UCA1-overexpressing (SW480+UCA1) and UCA1-knockdown (SW620+shUCA1) experimental models. In vitro characterization of the cell lines via phenotypic assays showing invasion, migration, and cell proliferation by xcelligence and colony formation to be enhanced through UCA1 overexpression and reduced by its knockdown. Furthermore, our data indicates that UCA1 overexpression enhances glucose uptake, lactate production, and anchorage-independent survival, suggesting its involvement in promoting anoikis resistance through metabolic reprogramming. Moreover, these cell lines will be subjected to anchorage-independent growth conditions to assess alterations in cell cycle, pro-survival, and stemness-related markers. Furthermore, glucose metabolism will be evaluated using Seahorse XFp metabolic analysis parameters.

#5143 Using a multipronged approach to characterize C-terminal domain polybasic motif STK11 variants.

Gopika Nandagopal, Peyton Jenkins, Olivia Morton, Shannon Prior, David Seward, Paula Deming

University of Vermont, Burlington, VT

KRAS-driven lung adenocarcinomas (LUADs) with concurrent STK11 loss of function (LoF) mutations are associated with aggressive tumors with increased risk of metastasis and lower overall and progression free survival. In LUAD, STK11 LoF impacts many aspects of coordinated cell motility and promotes alterations characteristic of metastasis. STK11 is a serine-threonine kinase that is comprised of an N-terminal, kinase, and C-terminal domain (CTD). With the goal of developing a method to identify pathogenic STK11 mutations, we previously evaluated functional impact of variants identified from the University of Vermont Medical Center on STK11 kinase activity using in-vitro kinase assays, and luciferase reporter assays. Here, we saw that CTD variants retained kinase activity and the ability to form the STK11 heterotrimeric complex. Although the CTD has no catalytic activity, it is thought to be integral for STK11 localization to the plasma membrane and cytoskeleton, where it negatively regulates the activity of focal adhesion kinase (FAK). Recent work by others has shown that a region of the CTD, the polybasic motif (PBM), (aa403-426) is key for localization of STK11 to the plasma membrane. Therefore, we reasoned that STK11 variants in the CTD would demonstrate altered subcellular localization and cytoplasmic functions. Indeed, we show in cell lines through immunofluorescent microscopy that expression of a truncated construct encoding STK11 with a CTD deletion results in full nuclear sequestration. Additionally, point mutants in 4 of the polybasic motif residues (R409W, K416E, A417S and K423E) retain kinase activity, but lead to partial nuclear sequestration despite their ability to bind heterotrimeric complex partners STRAD-α and MO25. This suggests a novel mode of dysregulated localization, independent of the known mechanism of STK11 export into the cytoplasm through binding of STRAD-α. This phenotype is exacerbated in a double mutant of STK11 PBM sites K416E and K423E. As FAK activity is known to be negatively regulated by STK11 in the cytoplasm, we used a KRAS-driven LUAD cell model system with (STK11-WT) and without STK11 (ΔSTK11) to monitor pY397 levels via SDS-Page and western blot analysis. This is a known FAK autophosphorylation site and was used as a proxy for active FAK. Under glutamine starved conditions known to activate STK11, the STK11 WT cells demonstrate a decrease in pY397-FAK levels. However, the levels of pY397-FAK in ΔSTK11 cells were significantly elevated. To determine if this elevated pY397-FAK phenotype is recapitulated in PBM mutants, we are generating a stable K416E_K423E double mutant cell line which will allow us to ascertain the ability of this mutant to regulate FAK activity. Ongoing experiments examine the migratory and invasive ability of the WT-expressing vs. PBM mutants. Additionally, we are utilizing the TurboID system to identify PBM-dependent STK11 interacting proteins.

#5144 Prevalence and clinical significance of CBP deficiency and disturbed CBP expression in human cancer.

Viktoria Chirico[1], Seyma Buyucek[1], Katharina Moller[1], Florian Lutz[1], Florian Viehweger[1], Martina Kluth[1], Claudia Hube-Magg[1], Elena Bady[1], Lisa Hornsteiner[1], Christian Bernreuther[1], Guido Sauter[1], Andreas H Marx[2], Till Krech[1], Stefan Steurer[1], Christoph Fraune[1], Sarah Minner[1], Natalia Gorbokon[1], Maximilian Lennartz[1], Eike Burandt[1], Anne Menz[1], Soren Weidemann[1], Waldemar WIlczak[1], Morton Freytag[1], Viktor Reiswich[1], Ronald Simon[1], Simon Kind[1], Ria Schlichter[1], **Nina Schraps**[1]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany,[2]Academic Hospital Fuerth, Fuerth, Germany

CBP (CREB-binding protein) is a ubiquitously expressed multidomain protein with histone acetyltransferase and transcriptional co-activator activity. As a major histone modifier, it is involved in the regulation of thousands of genes. CBP has a complex role in cancer where both loss-of-function and overexpression can occur. To better comprehend the role of CBP expression in cancer, a tissue microarray containing 14,966 samples from 134 different tumor entities and 608 samples of 76 different normal tissue types was analyzed. In normal tissues, a strong nuclear CBP staining was ubiquitously seen except few cell types. Among 12,255 evaluable tumors, CBP staining was completely absent (CBP deficiency) in 226 (1.84%) while 232 (1.89%) showed only a faint to moderate staining of a minority of cancer cells (substantial reduction, SR), 384 (3.13%) a 1+, 3,079 (25.12%) a 2+, and 8,334 (68.00%) a 3+ CBP positivity. CBP deficiency or SR was particularly common in pancreatic acinar cell carcinoma (23,5%), endometrioid endometrial carcinoma (22,8%), hepatocellular carcinoma (19.0%), urothelial carcinoma of the renal pelvis (17,4%), medullary carcinoma of the breast (14.3%), serous carcinoma of the endometrium (12.5%), neuroendocrine carcinoma of the pancreas (11.1%), and chromophobe renal cell carcinoma (10.7%). Less than 10% CBP deficient or SR cases were seen in 57 additional tumor categories. Among tumors with detectable CBP expression, low CBP expression levels were linked to advanced pT stage ($p<0.0001$), high UICC stage ($p=0.0005$), poor ISUP ($p=0.0001$), Thoenes ($p=0.0001$) and Fuhrman ($p<0.0001$) grades in clear cell renal cell carcinoma (ccRCC). In papillary RCC (pRCC), low CBP was associated with high UICC grade ($p=0.0409$) and distant metastasis ($p=0.0022$). Low CBP was furthermore associated with advanced pT stage ($p=0.0003$) in colorectal carcinoma and reduced overall survival ($p=0.0236$) in muscle-invasive urothelial carcinoma. High CBP expression was statistically related to nodal metastasis in pancreatic ductal adenocarcinoma (p=0,0201). CBP expression was not unequivocally related to clinico-pathological parameters in breast, ovarian, endometrial and gastric cancer. It is concluded, that CBP deficiency - as a rare event - occurs in many different tumor types and that a reduced or absent CBP expression is linked to aggressive cancer in multiple tumor entities.

#5145 E-selectin binding is enriched within B-cell acute lymphoblastic leukemia compared to healthy lymphoid precursors.

Chase M. Weizer[1], Rachel A. Kraan[2], Evan Ales[2], Stella L. Jaeckle[1], Julia H. Keating[3], Marlise R. Luskin[1], Robert Sackstein[2], Mark A. Murakami[1]

[1]Medical Oncology, Dana-Farber Cancer Institute, Boston, MA,[2]Florida International University Herbert Wertheim College of Medicine, Miami, FL,[3]Data Science, Dana-Farber Cancer Institute, Boston, MA

Background: Adhesion molecules within the bone marrow vascular niche govern the self-renewal, differentiation, and proliferation of hematopoietic progenitors. Among these, E-selectin, a lectin expressed by endothelial cells which binds to the tetrasaccharide known as sialyl Lewis X (sLeX), promotes dormancy, self-renewal, and chemoresistance in native hematopoietic stem cells (HSCs). However, leukemic blasts hijack these signals, fueling their proliferation. While therapeutic disruption of E-selectin binding to its ligands has demonstrated clinical activity in acute myeloid leukemia, its role in B-cell acute lymphoblastic leukemia (B-ALL) remains underexplored. Identifying E-selectin ligands and associated downstream molecular effectors in B-ALL holds potential to reveal targetable mediators of leukemia-initiating capacity, drug tolerance, and persistence.

Methods: Fresh peripheral blood and bone marrow from appropriately consented healthy donors (n=9) and clinically annotated patients (n=11) with BCR::ABL1 B-ALL identically treated on a clinical trial (NCT03595917) were profiled for sLeX expression and E-selectin binding by flow cytometry using HECA-452 monoclonal antibody and E-selectin-Ig chimera (E-Ig) respectively, along with glycoproteomic analyses. Expression of canonical E-selectin ligands (HCELL/CD44, CLA/PSGL-1, CD43E/CD43) and B cell developmental markers (CD45, CD34, CD10, CD19, CD20, CD22) were also assessed.

Results: SLeX expression and E-selectin binding varied between native antigen-independent B cell developmental stages ($p < 0.05$). Malignant CD19+ B cells from the bone marrow of treatment-naïve B-ALL patients exhibited increased sLeX expression ($p < 0.05$) and a nominal increase in E-selectin binding compared to counterparts from healthy donors. E-Ig-reactive CD19+ B-ALL cells display higher CD43 expression and lower CD44 and PSGL-1 expression than E-Ig-reactive CD19+ healthy donor B cells ($p < 0.05$). Treatment-naïve leukemic progenitors displayed greater sLeX expression and E-selectin binding than patient-matched non-malignant populations ($p < 0.05$). Leukemic progenitors isolated from serial bone marrow specimens exhibited a nominal increase in sLeX expression and E-selectin binding at progression versus baseline.

Conclusions: Within the lymphoid lineage, E-selectin binding is developmentally determined and enriched in malignant compared to native cells. Interrogation of downstream effectors and functional phenotypes is ongoing.

#5146 E-cadherin expression as a prognostic biomarker in colorectal cancer.

Anika Bhandare[1], Anjana Saxena[2], Mintoo Patel[1]

[1]South Florida State College, Avon Park, FL,[2]Brooklyn College and the Graduate Center, City University of New York, New York, NY

Colorectal cancer (CRC) represents the third most common cancer in the United States, with increasing incidence among younger populations despite remaining predominantly prevalent in individuals over 65 years of age. E-cadherin, a critical cell-cell adhesion molecule, plays a significant role in the development and progression of colorectal cancer by regulating intricate cellular mechanisms including polarity, differentiation, proliferation, and migration through complex cytoskeletal network associations. Loss of E-cadherin expression fundamentally disrupts intercellular adhesion, triggering enhanced cellular motility and facilitating the epithelial-to-mesenchymal transition (EMT)—a critical transformative process that enables metastatic potential. This molecular mechanism represents a crucial turning point in cancer progression, where cellular architectural integrity becomes compromised. This study investigated E-cadherin expression across different stages of colorectal cancer using immunohistochemical analysis of de-identified formalin-fixed paraffin-embedded biopsy tissue samples categorized as precancerous, cancerous, and normal noncancerous groups. Complementing the primary immunohistochemical analysis, a rigorous systematic meta-analysis was conducted through an extensive PubMed literature search to validate and contextualize the findings. Immunohistochemical staining revealed a robust E-cadherin expression in the mucosal epithelial cells of the normal tissues, whereas cancerous tissue samples exhibited reduced E-cadherin levels that correlated directly with advanced cancer staging. Low E-cadherin levels correlate significantly with adverse clinicopathological features and potentially more aggressive disease progression. The comprehensive meta-analysis substantiated these immunohistochemical observations, reinforcing the potential diagnostic and prognostic significance of E-cadherin as a sophisticated molecular biomarker. This study underscores the potential of E-cadherin as a supplementary biomarker in routine histopathological examinations. By providing more nuanced molecular insights into cancer stage and progression, E-cadherin analysis could facilitate further personalized, targeted clinical treatment strategies for colorectal cancer patients.

#5147 Prognostic role of MTAP expression is reversed by the ERG status in prostate cancer treated by radical prostatectomy.

Natalia Gorbokon[1], Seyma Buyucek[1], Henning Plage[2], Neele Heckmann[1], Ronald Simon[1], Maximilian Lennartz[1], Martina Kluth[1], Elena Bady[1], Lisa Hornsteiner[1], Claudia Hube-Magg[1], Sarah Minner[1], Eike Burandt[1], Till S Clauditz[1], Waldemar Wilczak[1], Guido Sauter[1], David Dum[1], Andrea Hinsch[1], Hans Heinzer[1], Alexander Haese[1], Thorsten Schlomm[2], Andreas Lubke[1], Markus Graefen[1], Stefan Steurer[1], Christian Bernreuther[1], Luca Roma[3], Lukas Bubendorf[3], Sarah Weinberger[2]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany,[2]Charite - Universitatsmedizin Berlin, Berlin, Germany,[3]University Hospital Basel, Basel, Switzerland

Loss of S-methyl-5′-thioadenosine phosphorylase (MTAP) expression offers an important therapeutic option through synthetic lethality with inhibitors targeting related pathways, and has been linked to resistance to immune checkpoint inhibitors in several tumor types. To better understand the prevalence and clinical relevance of MTAP expression in prostate cancer, a tissue microarray with 17,747 prostate cancer samples and a molecular and clinical database was analyzed for MTAP expression by immunohistochemistry. Normal prostate glands showed a weak to moderate, predominantly cytoplasmic MTAP positivity of epithelial and stromal cells. In 13,189 interpretable cancers, a complete loss of MTAP staining as compared to adjacent non-neoplastic cells was seen in 33 (0.3%) tumors, while MTAP staining was considered 1+ in 14.8%, 2+ in 42.2%, and 3+ in 42.7% of tumors. Fluorescence in situ hybridization analysis of 9 MTAP negative cancers confirmed homozygous *MTAP* deletion in all of these tumors. MTAP staining was significantly stronger in cancers harboring the *TMPRSS2:ERG* fusion than in *TMPRSS2:ERG* fusion negative tumors ($p<0.0001$). A comparison with clinico-pathological features revealed inverse correlations depending on the ERG fusion status: In ERG negative carcinomas, high (3+) MTAP expression was linked to advanced pT stage, high classical and quantitative Gleason grade, and early PSA recurrence in uni- and multivariate analysis ($p<0.0001$ each). In ERG positive cancers, MTAP expression decreased with advanced pT stage ($p<0.0001$), classical ($p=0.0004$) and quantitative Gleason grade ($p=0.0005$), and low (1+) MTAP expression was significantly linked to early PSA recurrence ($p=0.0012$). Comparison with 11 previously analyzed chromosomal deletions identified ERG-status-dependent positive or negative associations between MTAP expression and deletions of *PTEN* and 12p13 ($p\leq0.0274$) suggesting possible functional interactions with PTEN and one or several 12p genes. Taken together, the results of our study demonstrate that MTAP deficiency is exceedingly rare in prostate cancer, while high MTAP expression is a strong and independent marker for poor prognosis in ERG negative cancers.

#5148 Plakophilin 3 and ovarian cancer progression: Functional transition from early expression to advanced-state regulation through RB and STAT3 pathways.

Ha-Yeon Shin, JueYoung Kim, Jae-Hoon Kim

Department of Obstetrics and Gynecology, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea, Republic of

Background: Ovarian cancer (OC) is a heterogeneous disease with a high mortality rate and poor prognosis, primarily due to late-stage diagnosis. The identification of reliable biomarkers is crucial for improving patient outcomes. Plakophilin 3 (PKP3) is often overexpressed in cancers, yet its role in OC remains poorly understood.
Methods: We performed immunohistochemistry with tissue microarray, including normal (n=39), benign (n=66), borderline (n=44), and ovarian cancer (n=187), and analyzed clinical and survival rates according to the expression of PKP3. Either lacking or overexpressing PKP3 in immortalized normal cell and OC cell lines, we measured cell proliferation, cell cycle, and migration. Molecule change associated them checked western blot and qRT-PCR. Additionally, cellular localization of PKP3 in cancer cells was confirmed via cell fraction and immunofluorescence.
Results: The expression levels of PKP3 in OC are higher than in normal and benign tissues; notably, PKP3 shows the highest expression in stage I compared to other stages. Analysis of overall and disease-free survival revealed that patients with high PKP3 expression had a better prognosis than those with low PKP3 expression. Overexpression of PKP3 in immortalized normal cell lines led to increased cell growth. The depletion of PKP3 in OC cells triggered G1 cell cycle arrest. This was achieved through the attenuation of retinoblastoma (RB) protein phosphorylation and suppression of E2F1 target gene expression, ultimately resulting in reduced cell growth. Although PKP3 depletion decreased OC cell growth, it paradoxically increased cell migration. This increase in migration was mediated by the activation of STAT3. Regulation of RB and STAT3 activation by PKP3 was confirmed to occur in the nucleus. We found that alteration of PKP3 expression in normal and OC ultimately regulates the progression of OC.
Conclusion: PKP3 promotes the initiation of OC by driving cell growth, and in advanced stages, its reduced expression is associated with increased epithelial-mesenchymal transition (EMT), leading to a worse prognosis. This study suggests that PKP3 could serve as a biomarker for early diagnosis and that targeting the PKP3-STAT3 axis may offer new therapeutic opportunities for OC.

#5149 Sialyation of desmosomal protein DSG2: A potential regulator of colorectal cancer cell junctions.

Teresa Chiyanne Beamon[1], Collin Ainslie[2], Regina Irwin[1], Jeremie M. P. Lever[1], Alexa L. Mattheyses[2], Karin M. Hardiman[1]

[1]Surgery, University Of Alabama At Birmingham, Birmingham, AL,[2]University Of Alabama At Birmingham, Birmingham, AL

Introduction: Colorectal cancer (CRC) remains one of the most common and deadly cancers in the US. Despite advances in treatment, once CRC is metastatic (spread from the original tumor site to other parts of the body), the 5-year survival rate is 20%. Metastasis requires a complex series of events, including the release of tumor cells from the origin site. Tumor cells adhere to each other via adhesive cell-cell junctions, one family of these is called desmosomes. A key desmosomal protein is Desmoglein-2 (DSG2), which is a cell surface protein. DSG2 function could influence cancer cell detachment and migration, essential steps in metastasis, which has not been fully studied. Upregulation of ST6GAL1, a sialyltransferase that adds sialic acid to proteins in the Golgi, is a hallmark of progression of certain types of cancer. High levels of ST6Gal1 have been linked to worse cancer outcomes. We hypothesized, that increased sialylation of the desmosome protein DSG2 by ST6GAL1 in CRC enhances its surface localization, possibly altering its function.
Methods: Human SW620 CRC ST6GAL1 control (CV) and ST6GAL1 knockdown (KD) cells were cultured and utilized to evaluate the presence of DSG2 through three methods: SNA- lectin pull-down assay (SNA recognizes α2,6-sialylation caused by ST6GAL1), immunofluorescence (IF), and western blotting (WB). The pull-down samples were evaluated for presence of sialylated DSG2 by WB and further analyzed and quantified using densitometry. For IF staining, cells were fixed, permeabilized, and stained with a DSG2 antibody and DAPI to assess differences in location and presence between KD and CV cells. Quantitative image analysis was performed, utilizing 5 high powered fields per experimental group. Further, to assess the relationship between cell density and DSG2 expression, cells were plated at increasing densities and analyzed by both WB and IF techniques.
Results: SNA pull-down assay and WB analysis revealed that DSG2 is α2,6 sialylated by ST6GAL1. ST6GAL1 CV cells had 2.31 times more sialylated DSG2 than ST6GAL1 KD cells. This was corroborated by DSG2 IF staining. We observed an average surface DSG2 to DAPI ratio in KD cells of 0.09 (SEM ± 0.01) and CV cells' ratio of 0.20 (SEM ± 0.03) (p=. 0.0353, n=3) such that CV cells had 2.7 times more DSG2.
Conclusions: Based on these findings, we hypothesize conclude that ST6GAL1 sialylates DSG2 and that this appears to alter its increased sialylation of the desmosome protein, DSG2, in CRC by ST6GAL1 may enhance DSG2's surface localization which likely alters its function. Further study is needed to investigate how this may alter CRC progression and metastasis.

TUMOR BIOLOGY: Mouse Models of Cancer and Mechanisms
Poster Session

#5153 Synergistic efficacy of PARP and DNMT inhibition in ATM and CHEK2 CRISP-engineered in vitro and in vivo models.

Fateme Karimi Dermani, Sibapriya Chaudhuri, Luika Timmerman, Scott Thomas, Pamela Munster

UCSF - University of California San Francisco, San Francisco, CA

Introduction: More than a decade of work has demonstrated the benefit of using poly (ADP-ribose) polymerase inhibitors (PARPi) in patients with BRCA1 and BRCA2 mutant tumors, based on their compromised ability to repair DNA damage by homologous recombination (HRD). While the benefits of PARPi monotherapy can be limited, our laboratory is working to identify co-therapeutics to improve therapeutic efficacy. Homologous recombination requires the function of many other proteins besides BRCA1 and BRCA2, and PARP sensitivity of tumors with these mutations has not been well explored. Of particular interest are tumors with mutations in the ATM and CHK2 proteins. Here in preclinical studies, we explore the sensitivity of these mutants to PARP directed therapeutics, and to our current therapeutic combination with a DNA methyltransferase inhibitor (DNMT1; decitabine) that we now have in Phase 1 clinical trials. We hypothesize that tumors with pathogenic ATM and CHK2 mutations may also show PARP inhibitor sensitivity, which might also be potentiated in combination with decitabine.
Methods: To test this hypothesis, we generated inducible RNA knockdown variants of the HR proficient triple negative breast cancer cell line MDA-MB-231, targeting *ATM* and *Chek2* transcripts under doxycycline control via dCAS9 technology. Responses to the PARP inhibitor talazoparib, with and without decitabine were assessed with and without doxycycline induction. We evaluated colony formation, cell death, cell cycle fraction alterations, DNA damage and measured PARP1-DNMT1 adduct formation using proximity ligation assays.
Results: Preliminary findings show that the combination of talazoparib and decitabine resulted in greater synergistic efficacy at lower doses than talazoparib alone. In the ATM dCas9, and CHEK2 dCas9 cell lines, the combined treatment led to substantial DNA damage, prolonged trapping of PARP1-DNMT1 complexes on DNA, and significant induction of cell death. Accordingly, potent growth inhibition was also seen in xenografts models with clinically relevant *ATM* and *CHEK2* mutations.
Conclusion: These results support further investigation into the combined use of PARP and DNMT inhibitors in tumors with these mutations. The synergistic effects observed in the MDA-MB231-derived models suggest that this combination could provide a more effective treatment option for HRD cancers, particularly in overcoming resistance to PARPi monotherapy.

#5154 Modeling luminal urothelial carcinoma in the bladder and the upper tract.

D. Xu, K. Wieczorek, L. Wang, Y. Zhang, J. Wang, B. Xu, D. W. Goodrich, A. A. Hussein, K. Guru, Q. Li;
Roswell Park Comprehensive Cancer Center, Buffalo, NY

Introduction: Pan-urothelial carcinoma (panUCC) involves synchronous or metachronous urothelial carcinoma of the bladder (UCB) and upper tract urothelial carcinoma (UTUC). We sought to model panUCC using a triple knockout (*TKO: Trp53; Rb1; Pten*) genetically engineered mouse model (GEMM).

Methods: Using a urothelium-specific *Uroplakin 2-Cre* ^{ERT2} (*Upk2-Cre*^{ERT2}), we generated novel *TKO* mice (*Upk2-Cre* ^{ERT2}; *Trp53*$^{f/f}$; *Rb1*$^{f/f}$; *Pten*$^{f/f}$). The mice were monitored for hematuria, urine retention, tumor formation, and survival. PCR primers were designed to amplify the genes after recombination. Tumor histology and metastasis were examined by hematoxylin and eosin (HE) and immunohistochemistry (IHC). The subtype of tumors was classified using luminal (Upk3, CK8, GATA-3) and basal markers (CK5, p63), and a neuroendocrine marker (synaptophysin, Syp).

Results: *Upk2-Cre*^{ERT2}; *TKO* mice (n = 34, 18 males and 16 females) developed non-muscle invasive urothelial carcinoma tumors located in the bladder and/or the renal pelvis/ureter with a median survival of 294 days. PCR results validated *Trp53/Rb1/Pten* mutations in UC tumors. Mice with UCB or mixed UCB/UTUC tumors exhibited a longer survival compared to mice with UTUC only [median survival 393 days (n = 7) VS 278 days (n = 27), P = 0.006]. No obvious evidence of distant metastases was detected. IHC of both UCB and UTUC tumors demonstrated luminal UC tumors with strong expression of luminal markers (Upk3, CK8, GATA-3) and no expression of basal and neuroendocrine markers (CK5, p63, and Syp).

Conclusion: We developed a novel *TKO* GEMM that faithfully recapitulates human panUCC. This GEMM provides a useful tool for investigating the molecular and therapeutic differences between UCB and UTUC tumors.

Funding: This research work was supported in part by NIH Grants, K08CA252161(Li, Q), P30CA016056 (NCI Cancer Center Core Support Grant), and the Roswell Park Alliance Foundation.

#5155 A novel lipid nanoparticle for delivering sgRNA and mRNA to develop lung cancer model mice.

Bingxin Liu[1], Yutaka Maeda[2]

[1]Materials Science and Engineering Program, University of Cincinnati, Cincinnati, OH,[2]Pulmonary Biology, Cincinnati Children's Hospital, Cincinnati, OH

Background: Genetically engineered mouse models (GEMMs) that develop autochthonous lung tumors are suitable to study lung tumor initiation and the tumor microenvironment involved in immune cells. However, GEMMs require lengthy mouse breeding with multiple crossing of genetic lines to develop mice carrying multiple alleles of specific genes. A more convenient approach to develop these GEMMs will facilitate lung cancer research.

Methods: We have recently developed a novel lipid nanoparticle PEG-PBAE/4A3-SC8/DOPE/Cholesterol/DOTAP (hereafter, LNP) that can deliver mRNA into lung epithelial cells in mice by intratracheal (IT) injection (Liu et al., bioRxiv 2024; https://doi.org/10.1101/2024.08.22.608643). Using this LNP, we delivered different sgRNAs and/or Cre mRNA into *Cas9* mice that constitutively express Cas9 (or *Kras*$^{LSL\text{-}G12D/+}$*;Cas9* mice) by IT injection and sought to develop lung cancer model mice.

Results: The LNP delivery of sgRNAs targeting *Eml4* (sgEml4) and *Alk* (sgAlk) into *Cas9* mice produced autochthonous lung tumors induced by the driver oncogene EML4-ALK two months after IT injection. The LNP delivery of Cre mRNA along with sgRNA targeting the lung lineage transcription factor *Nkx2-1* (sgNkx2-1) into *Kras*$^{LSL\text{-}G12D/+}$*; Cas9* mice produced autochthonous invasive mucinous adenocarcinomas of the lung (IMA) that carry *Kras*^{G12D} with loss of NKX2-1 and *de novo* expression of the gastrointestinal transcription factor HNF4A while the LNP delivery of only Cre mRNA into *Kras*$^{LSL\text{-}G12D/+}$*; Cas9* mice produced autochthonous non-mucinous adenoma/adenocarcinoma two months after IT injection.

Conclusion: Delivery of our novel LNPs encapsulating sgRNAs and/or Cre mRNA into *Cas9* or *Kras*$^{LSL\text{-}G12D/+}$*;Cas9* mice by IT injection successfully resulted in producing autochthonous lung tumors driven by EML4-ALK or KRAS^{G12D} similar to traditional GEMMs. This approach using our LNPs will allow for agile development of lung cancer mouse models for investigating novel lung cancer therapeutics.

#5156 Combining the RCAS/tv-a retrovirus and CRISPR/Cas9 gene editing systems to generate primary mouse models of midline glioma.

Sophie R. Wu[1], Julianne Sharpe[2], Joshua Tolliver[1], Abigail J. Groth[1], Reid Chen[1], Maria E. Guerra Garcia[3], Vennesa Valentine[4], Nerissa T. Williams[1], Sheeba Jacob[1], Zachary J. Reitman[1]

[1]Radiation Oncology, Duke University, Durham, NC,[2]Biology, University of Kentucky, Lexington, KY,[3]Biomedical Engineering, Washington University, St. Louis, MO,[4]Pharmacology & Cancer Biology, Duke University, Durham, NC

Diffuse midline gliomas (DMGs) are fatal pediatric brain tumors that commonly arise in the brainstem and other midline brain structures, with a median overall survival of less than two years. Genetically engineered mouse models (GEMMs) are key to studying tumorigenesis and tumor-immune interactions, which may inform the development of urgently-needed treatment approaches. However, current midline glioma GEMM approaches are limited in their ability to model multiple gene perturbations and to target specific cell lineages in the brain. Here, we establish a platform that combines the RCAS/tv-a avian retrovirus system with CRISPR/Cas9 gene editing to drive midline glioma formation in mice. To express endonuclease Cas9 and the avian retrovirus receptor TVA in mouse neural stem cells, we generated mice of genotype Nestin-TVA; Nestin-Cre; LoxP-STOP-LoxP-Cas9-EGFP (NNC). RCAS retrovirus plasmids were cloned containing guide RNAs (gRNAs) targeting various DMG-relevant genes for perturbation, including *Trp53, Cdkn2a, Atm, Pten,* and a non-targeting control (Cntl). Two types of plasmids were generated for each gene target, one with a platelet-derived growth factor beta ligand (PDGFB) oncogene driver and another with a blue fluorescent protein (BFP) marker.To confirm if the combined system could generate high grade midline gliomas in mice, the *Trp53* tumor suppressor that is frequently disrupted in midline gliomas was targeted for CRISPR/Cas9 based disruption. We observed high grade tumors with moderate latency (median time to tumor formation of 12.57 weeks). Immunohistochemistry (IHC) and next-generation sequencing (NGS) confirmed tumor features that model human DMG, expression of Cas9 and PDGFB, and CRISPR-mediated loss of p53. Next, we disrupted multiple tumor suppressor genes (*Trp53, Atm, Cdkn2a, Pten)* in a mini-pooled *in vivo* experiment, generating midline gliomas with decreased tumor latency (median time to tumor formation 3.6 weeks, $P < 0.0001$, log-rank test compared to a single-plex gRNA). Quantification of gRNA barcodes and CRISPR editing events using NGS revealed that all tumors contained cells with disruption of all target genes, suggesting a multiclonal origin for the tumors and stronger selection for *Trp53* disruption compared to other genes. This mouse modeling approach may streamline future GEMM DMG research by enabling complex experiments in which new genes of interest can be rapidly perturbed by CRISPR/Cas9. Sophisticated experiments such as pooled *in vivo* genetic experiments may allow for screens to identify genetic determinants of tumorigenesis or treatment response.

#5157 The novel CD34 huNSG-FLT3-IL15 mouse strain, which expresses human IL15 and FLT3 ligand, enhances the development T, myeloid, dendritic, and natural killer cells.

Peter Deng, Pali Kaur, Shuang Hu, Brian S. Soper, Voot Yin

The Jackson Laboratory, Sacramento, CA

Cell humanized mouse models that reconstitute the human immune system are valuable in the field of immuno-oncology (IO) and IO drug development. Importantly, the development of cell humanized mouse models that recapitulate a comprehensive immune system is critical for the study of the interplay between T, myeloid, dendritic, and natural killer cells in the context of cancer. In the present study, we have developed a novel NOD-scid IL2rγ^{null} (NSG) mouse variant (NSG-FLT3-IL15) that transgenically expresses both human IL-15 and human FLT3 ligand to enhance maturation of functional innate immune cells including human CD56+ CD16+ NK cells, CD33+ myeloid cells, CD141+ and CD1c+ conventional DC subsets, CD123+ pDCs, and CD14+ monocytes alongside adaptive immune cells such as CD4+ regulatory T cells and CD4+ and CD8+ effector T cells over a 28-week period.
NSG-FLT3-IL15 mice, along with control strains NSG, NSG-IL15, and NSG-FLT3, were engrafted with human umbilical cord blood-derived CD34^{+} hematopoietic stem cells from three different donors, and blood samples were collected until week 28 post-transplantation. Flow cytometry revealed that hCD45^{+} cell engraftment and T cell subpopulations (CD3+, CD4+, and CD8+) in NSG-FLT3-IL15 mice were comparable to parental control strains. Strikingly, NSG-FLT3-IL15 mice exhibited enhanced CD56$^{+/dim}$ CD16+ NK cells compared to the control strains. Furthermore, levels of myeloid sub-lineage cells such as monocytes, macrophages (M1), granulocytes, dendritic cells, and neutrophils, were higher than in NSG or NSG-IL15 strains but comparable to NSG-FLT3 strain mice. Current growth kinetic studies with human patient derived tumors will assess human immune cell infiltration across NSG variants. In vivo efficacy studies are underway to demonstrate therapy induced tumor killing by the enhanced innate human immune cells developed in NSG-FLT3-IL15 mice.
Overall, these results demonstrate that the NSG-IL15-FLT3 model supports enhanced development of functional human innate immune cells and serves as a novel comprehensive immune model for studying human IO.

#5158 Establishing novel patient-derived xenograft models for peritoneal metastasis in a racially diverse population.

Vashti Bandy[1], Adam Khader[1], Praveen Bhoopathi[1], Vignesh Vudatha[1], Arunima Punjala[1], Jose Trevino[1], Bhaumik Patel[1], Leopoldo Fernandez[1], Savannah Gregg[2]

[1]Virginia Commonwealth University, Richmond, VA,[2]Central Virginia VA Health Care System, Richmond, VA

Colorectal cancer (CRC) is highly treatable when localized, but survival drops sharply with metastasis, especially to the peritoneum (PM). CRC PM is particularly resistant to chemotherapy, emphasizing the need for models that replicate disease biology for use in drug discovery. Our study establishes reproducible CRC PM patient-derived xenografts (PDX) from a racially diverse patient population.

Patients with CRC PM were recruited for a tissue collection study at a single academic tertiary care center. Tumor samples were collected under sterile conditions and prepared for subcutaneous propagation into NOD.Cg-Prkdcscid/J (NSG) mice. Next, tumor was harvested and reimplanted intraperitoneally into new NSG models. Peritoneal metastasis was induced via 1) Intraperitoneal injection of a single cell suspension or 2) Intraperitoneal tumor implantation via laparotomy. Post-necropsy, peritoneal carcinomatous index (PCI) was calculated and pictures were taken using a cross-polarization technique to allow for PCI scoring by an independent investigator. Collected tumor was processed for histological analysis.

We developed four successful intraperitoneal PDX models using CRC PM derived from a 62-year-old non-Hispanic Black female with KRAS G12V mutation and a 60-year-old non-Hispanic White female with KRAS wildtype. Both tumors had microsatellite stability (MSS). All four PDX were representative of the human clinical condition as diffuse peritoneal metastases were found in each. The laparotomy group exhibited a higher tumor burden and PCI compared to the tumor injection group. In histological comparison of PDX to patient tissue, we observed that tumor gland formation and architecture were maintained.

Early findings show that CRC PM PDX models can be successfully established from a racially diverse patient population. Intraperitoneal tumor implantation into NSG mice via laparotomy resulted in a higher PCI compared to tumor injection, though the latter may be more reproducible. Cross-polarization imaging optimized tumor visualization and allowed PCI scoring by an independent investigator. These models mimic gross and histologic characteristics of the original patient tumor. Future directions include extensive multi-omic profiling to further characterize each PDX. We aim to expand our PDX library to better represent CRC PM heterogeneity with a focus towards drug development.

#5159 From subcutaneous to orthotopic: Bridging the gap to human disease in lung cancer research.

Daniel Orive, Benat Picabea, Maeva Houry, Ane Alava, Covadonga Rodriguez-Suarez, Alfonso Calvo, Luis M. Montuenga, Karmele Valencia

Center for Applied Medical Research (CIMA), Pamplona, Spain

Subcutaneous (SC) models are commonly used in preclinical lung cancer research. However, differences in outcomes of mouse-tested therapies suggest that SC models fail to accurately mimic the complexity of the tumor microenvironment, limiting their reliability for evaluating therapeutic responses, tumor-immune interactions, and metastasis. To overcome these limitations, we developed and analyzed novel orthotopic lung cancer (LuO) models using syngeneic and xenograft approaches with human and murine lung cancer cell lines. Tumors were implanted into the left lung via thoracotomy, forming a single nodule per lung. Depending on the cell line, the model generates metastasis and allows for organotropism studies, closely resembling key traits of human lung cancer.

Our study compares molecular and functional properties of SC and LuO models. Tumor microarrays (TMAs) were generated from 20 human and mouse lung cancer cell lines implanted either subcutaneously or orthotopically in mice. SC tumor cores were subcategorized into central and peripheral regions, while LuO cores were classified by parenchymal or pleural location when pleural seeding occurred. Two fluorescence-based multiplex (VECTRA®) panels characterized the tumor microenvironment (TME), profiling lymphoid and myeloid immune populations. Cancer hallmark-related proteins were also analyzed. Compared to SC tumors, LuO models showed significantly higher vascularization (CD31 area, $p<0.0001$), reduced hypoxia (HIF1-α, $p<0.0001$), and lower senescence marker expression (p16 and p21 H-scores, $p<0.0001$). Immune profiling revealed increased $CD4^+$ T-cell infiltration ($p=0.0128$) and elevated $CD4^+PD1^+$ populations ($p=0.0372$), indicating enhanced T-cell exhaustion in LuO tumors. LuO tumors also exhibited significantly higher PD-L1 expression ($p=0.0002$), more M2 macrophages ($p<0.0001$), and an increase in MDSCs ($p<0.0001$), suggesting a reliance on immune suppression mediated by M2 macrophages and MDSCs rather than the PD-1/PD-L1 pathway. Proliferative, apoptotic, and metabolic activity were also compared between LuO and SC tumors.

Although SC models are widely used for lung cancer research and therapy, our results show biological differences depending on the transplant location. Molecular features and TME profiles of SC tumors differ substantially from the clinically relevant LuO context. Our findings suggest that LuO models are more suitable for translational lung cancer studies. Therefore, beyond generating TMAs, we developed additional tools from our LuO models to support research into tumor biology and the TME. These resources are available to the scientific community for deeper understanding of lung cancer pathophysiology. In summary, LuO models improve the recapitulation value of mouse-transplanted models for human disease, offering a more robust platform for preclinical research and therapeutic development.

#5161 Preclinical evaluation of CD3/DLL3 bispecific antibody in syngeneic and humanized mouse models for solid tumor treatment.

Ravneet Chhabra, Chong Li, Shuangshi Liu, Qiang Liu, Liu Yang, Hui Xu

Biocytogen, Waltham, MA

In solid tumors, the number of T cells is limited, and immunosuppressive cells in the tumor microenvironment, such as cancer-associated fibroblasts (CAFs), myeloid-derived suppressor cells (MDSCs), and regulatory T cells (Tregs), can inhibit T cell activity. Additionally, T cells in solid tumors often exist in a state of exhaustion, reducing the effectiveness of treatments.

Delta-like 3 (DLL3) is abnormally highly expressed in various tumor cell lines and primary tumor tissues, particularly showing a significant increase in neuroendocrine tumors (NETs) and small cell lung cancer (SCLC). In SCLC, the expression of DLL3 is closely associated with poor prognosis in patients.

Tarlatamab is the first approved bispecific T cell engager (BiTE) therapy for the treatment of solid tumors and is the bispecific antibody (BsAb) that targets DLL3. This BiTE works by directing T cells to the vicinity of small cell lung cancer cells and activating them to eliminate tumor cells. While the powerful therapeutic effects of Tarlatamab are being highlighted, there is also a consideration for more effective assessment of bispecific antibodies. Appropriate preclinical evaluation models can provide critical data support for subsequent clinical trials of CD3/DLL3 bispecific antibodies, aiding in the understanding of their potential and mechanisms in therapy.

Biocytogen successfully developed a cell line B-hDLL3 B16-F10 that expresses human DLL3. B-hDLL3 B16-F10 cell line can bind to CD3/DLL3 BsAb Tarlatamab analog (in house) and is capable of forming tumors in C57BL/6 mice and B-hCD3E/hCD28 mice after injected. When B-hDLL3 B16-F10 cells are subcutaneously implanted in B-hCD3EDG mice, the Tarlatamab analog (in house) significantly inhibits tumor growth. In PBMC-reconstituted B-NDG mice implanted with human tumor cell line SHP-77, Tarlatamab analog (in house) almost completely suppresses tumor growth. These results indicate that both preclinical animal models can be used to evaluate the in vivo efficacy of the CD3/DLL3 BsAb treatment.

#5162 Decreases in blood pressure in a patient-derived xenograft model of pancreatic ductal adenocarcinoma.

Amelia R. Malicki[1], Leslie M. Ogilvie[1], Alexa N. King[1], Patrick Sanosa[1], Jana Michaud[1], Bridget Coyle-Asbil[1], Praveen Bhoopathi[2], Vignesh Vudatha[3], Jose G. Trevino[3], Keith R. Brunt[4], Jeremy A. Simpson[1]

[1]Human Health and Nutritional Sciences, University of Guelph, Guelph, ON, Canada,[2]Human and Molecular Genetics, Virginia Commonwealth University, Richmond, VA,[3]Department of Surgery, Virginia Commonwealth University, Richmond, VA,[4]Department of Pharmacology, Dalhousie University, Halifax, NS, Canada

Introduction: Chemotherapy and radiation treatment cause cardiac injury and dysfunction. It remains unclear how cancer affects cardiac structure and function in therapy-naïve mice. Emerging data shows that epithelial ovarian cancer affects hemodynamics by causing cardiac atrophy, intrinsic cardiac dysfunction and arterial hypotension in mice. As pancreatic cancer is associated with heart disease risk, we wanted to explore if impaired hemodynamics occurs in other types of cancer. Methods: We utilized a patient-derived xenograft (PDX) model of pancreatic ductal adenocarcinoma (PDAC), established by implanting fresh tumor fragments from a human PDAC patient into NSG mice. Cardiovascular hemodynamic parameters were analyzed at 28-weeks post-tumor implantation, which coincided with an advanced stage of tumor development. Results: Invasive hemodynamic assessments showed a decrease in systolic and diastolic blood pressure. Conclusion: Independent of chemotherapy, pancreatic cancer causes arterial hypotension, impairing perfusion and overall recovery. Monitoring cardiovascular health is crucial to prevent further deterioration, optimize outcomes, and improve the quality of life for pancreatic cancer patients throughout their journey.

#5163 Characterization of endometrial cancer xenograft humanized mouse models.

Sung Wan Kang[1], Ji-Young Lee[1], Min-Seo Lee[1], Ok-Ju Kang[1], Yong-Man Kim[1], Chong Jai Kim[2], Shin-Wha Lee[1]

[1]Obstetrics and Gynecology, Asan Medical Center, University of Ulsan, Seoul, Korea, Republic of,[2]Pathology, Asan Medical Center, University of Ulsan, Seoul, Korea, Republic of

Background: Recently, immunotherapy with immune checkpoint inhibitors (ICIs) has shown promising efficacy in endometrial cancer (EC), particularly in patients with mismatch repair deficiency (dMMR). Preclinical models of EC are essential for evaluating the efficacy of immuno-oncology (IO) drugs and investigating strategies to enhance the antitumor responses to immunotherapy. Humanized (Hu) mouse models can contribute to a better understanding of the underlying mechanisms associated with the improved efficacy of ICIs in treating patients with EC.
Methods: We established EC xenograft Hu-mouse models by subcutaneously transplanting dMMR HEC-1A and Ishikawa human EC cells into $CD34^+$ Hu-mice. Tumor growth was monitored by periodic measurements with a caliper. The immunophenotypical characterization of EC xenografts in Hu-mice was performed using immunohistochemical staining and flow cytometry analysis.
Results: Tumor development and growth were observed in both EC cell lines within $CD34^+$ Hu-mice. In the Hec-1A xenograft Hu-mouse model, tumor growth was significantly inhibited compared to the immunodeficient mouse model. Flow cytometry analysis of tumors, spleen, and blood from Hu-mice injected with HEC-1A cells revealed a high frequency of human immune cells, exhibiting various subtypes, including $CD4^+$/$CD8^+$ T cells, NK cells, NKT cells, B cells, and myeloid cells. In contrast, no significant difference in tumor growth was observed for Ishikawa xenografts between Hu- and the immunodeficient mouse model. While the spleen and blood of Hu-mice harboring Ishikawa tumors exhibited a high frequency of human immune cells similar to the HEC-1A model, the xenografts demonstrated an immune-desert phenotype, characterized by a low frequency of tumor-infiltrating immune cells. Notably, both HEC-1A and Ishikawa EC xenografts in Hu-mice exhibited a significantly higher proportion of PD-1/$PD\text{-}L1^+$ T and B cells compared to those found in the spleen and blood.
Conclusion: Two models of EC xenograft were successfully established in Hu-mice, demonstrating contrasting tumor immune phenotypes. These models might serve as valuable tools for evaluating the efficacy of immunotherapy in EC.

#5164 Deleterious germline *DDX41* variants drive solid tumors in conjunction with second cancer risk alleles and a pro-inflammatory environment.

Sophia C. Korotev, Joshua Y. Strauss, Salina Dominguez, Ashwin Koppayi, Hina Usman, Kehan Ren, Honghao Bi, Peng Ji, Lucy Godley

Hematology and Oncology, Northwestern Univ. Feinberg School of Medicine, Chicago, IL

The presence of solid tumors in those with deleterious germline *DDX41* variants in addition to the expected myeloid malignancies suggests that these alleles could contribute to solid tumor development. To test if *DDX41* cancer risk alleles contribute directly to solid tumor development, we analyzed somatic mutations in solid tumors from people with these variants. We did not detect any somatic *DDX41* mutations (n=18) in contrast to myeloid malignancies in which somatic *DDX41* mutations are common. Moreover, we observed that among families with hematopoietic malignancies and solid tumors, 30% had second germline deleterious variants in other cancer-associated genes. Together, these data suggest that *DDX41* may contribute to solid tumor development indirectly. Since DDX41 regulates innate immunity, we hypothesized that *DDX41* cancer risk alleles could contribute to solid tumor development via inflammation. We measured inflammatory cytokine levels in conditioned media from lymphoblastoid cell lines with deleterious germline *DDX41* variants ($DDX41^{var/+}$) and found elevated levels of some cytokines in $DDX41^{var/+}$ versus controls. Western blots confirmed elevated NF-kB p65 subunit levels and increased nuclear NF-kB localization (p=0.008), suggesting that the DDX41-related inflammatory milieu may be partially characterized by dysregulated NF-kB-driven cytokine expression. To investigate the possibility that *DDX41* variants drive an inflammatory milieu, we performed flow cytometry on peripheral blood of germline $Ddx41^{+/-}$ versus WT mice. We found a 20% increase in T-cells (p=0.0069) in the context of Ddx41-deficiency, suggesting that Ddx41-deficiency contributes to inflammation in this mouse model. To test our hypothesis that DDX41 is a modifier of solid tumor pathogenesis, we have bred $Ddx41^{+/-}$ mice to $Apc^{Min/+}$ mice, which develop intestinal adenomas and are a model of human colon cancer. At three months of age, our preliminary data suggest there are more adenomas in Ddx41-deficient versus WT mice. We are now testing whether Ddx41-deficiency in the hematopoietic compartment drives the elevated adenoma levels, allowing us to delineate the role of immune cells in solid tumor development in the context of Ddx41-deficiency. Overall, our findings suggest germline deleterious *DDX41* variants contribute indirectly to solid tumor development in the context of other germline cancer risk disorders, possibly through elevated inflammation.

#5165 Development of a syngeneic mouse model to study small cell lung cancer (SCLC).

Aarushi Garg[1], Diego A. Velarde[2], Mario A. Pulido[2], Daniel J. Mullen[2], Chunli Yan[2], Matthew A. Gladstone[2], Ite A. Offringa[2]

[1]Biochemistry and Molecular Medicine, USC - University of Southern California, Los Angeles, CA,[2]Department of Surgery, USC - University of Southern California, Los Angeles, CA

Small cell lung cancer (SCLC) is an aggressive neuroendocrine carcinoma with limited treatment options and a 5-year survival rate of 8% (American Cancer Society, 2023). Preclinical models are essential for studying SCLC, yet traditional models either lack immune system relevance (when using nude mice with human SCLC samples) or have lengthy tumor development times (when using genetically-engineered models). The genetically-engineered mouse model originally developed in the Berns lab is the homozygously floxed *Tp53*fl/fl;*Rb1*fl/fl mouse. SCLC is induced through instillation of adenovirus carrying Cre recombinase into the lungs. It requires 6-8 months for proper SCLC development, limiting the model's utility for rapid therapeutic testing. This model has been combined with additional genetic hits to accelerate cancer development, but this narrows the diversity of developed SCLC tumors. Here, we aim to create a fast, immune-competent SCLC mouse model using cell lines derived in-house from three *Tp53*fl/fl;*Rb1*fl/fl SCLC-induced mice. Two types of cell lines were derived from each of three SCLC mouse lungs: cell lines that grow in suspension (S) or adhere (A) to the dish (477A, 477S, 494A, 494S, 625A, 625S, where the number indicates the mouse and A/S indicates attached/suspension growth, respectively). The different growth types mirror that seen in human SCLC cell lines, which can grow suspended in spheres ("classic" SCLC cell lines) or in loose aggregates that attach to plates ("variant" SCLC cell lines). We performed RNA sequencing to gain insight into the molecular characteristics of the six cell lines. Suspension cell lines express neuroendocrine-related genes and the adherent cell lines express epithelial to mesenchymal transition (EMT)-related genes. We tested different injection modes of the mSCLC cell lines (subcutaneous *vs.* tail vein) and different numbers of injected cells to optimize tumor formation. 477S and 625S cell lines induced subcutaneous tumors in several weeks. 477, 494, 625 (S) cell lines generated liver metastases in mice when injected via the tail vein. Adherent cell lines did not efficiently form tumors, though they did exhibit deletion of *Tp53* and *Rb1*. We are lentivirally-transducing the cell lines with a luciferase construct to allow *in vivo* imaging of tumor growth and metastasis. We will use the newly developed mSCLC cell lines to test the effects of immunotherapy following cisplatin-etoposide (CE) chemotherapy. We will focus particularly on the immune response to ELAVL4, which in human patients is associated with improved survival. The mSCLC cell line model will provide a new tool to accelerate the evaluation of novel therapeutic strategies and provide a robust platform for studying SCLC progression in mice. Funding: This work was supported by a Wright Foundation Transformative Cancer GrantAward and the Norris Comprehensive Cancer Center core grant, award number P30CA014089 from the NIH/NCI.

#5166 Increasing translatability of syngeneic mouse models of cancer through the application of human-validated, clinically relevant gene expression-based measurements of the tumor immune microenvironment.

Zachary D. Wallen[1], Kyle C. Strickland[1], **Sheri Barnes**[2], Sarabjot Pabla[1], Simon Brooks[1], Taylor J. Jensen[1], Brian Caveney[3], Marcia Eisenberg[3], Eric A. Severson[1], Scott C. Wise[2], Shakti Ramkissoon[1]

[1]Oncology, Labcorp, Durham, NC,[2]Pre-Clinical Oncology, Labcorp, Ann Arbor, MI,[3]Labcorp, Burlington, NC

Background: Syngeneic mouse models serve as invaluable tools in preclinical research. Implanting cancer cells into immunocompetent mice allows immunotherapy responses to be assessed in a physiologically relevant setting. To further improve the translatability of syngeneic models, we (1) characterized the immune landscape of different syngeneic models using human-validated immune gene expression (iGEX)-based measurements of the tumor immune microenvironment and (2) investigated the feasibility and utility of using these measurements to differentiate variable responses to treatment in mice.

Methods: Our study analyzed tumor fragments from 15 models covering 10 cancers. Tumor fragments were taken from treatment naïve tumors of representative mice from each model and subjected to whole-transcriptome RNA-seq. Non-implanted cell lines from each model were also subjected to RNA-seq for comparison to tumor fragments. Data for immune-related genes were extracted and normalized to gene expression ranks for analysis. Previously validated iGEX signatures for monitoring the tumor immune microenvironment were calculated including tumor immunogenicity (TIGS), cellular proliferation (CP), and cancer testis antigen burden (CTAB) signatures. RNA-seq and tumor growth data from 1,839 mice treated with 1 of 8 treatments were paired to observe iGEX-based tumor growth differences among treatment conditions.

Results: Tumor fragments and cell lines had distinct iGEX profiles with tumor fragments being highly enriched for genes involved in immune activity. Measures of iGEX in mouse tumor fragments recapitulated values in human patient tumors, providing confidence in the translatability of our results. Various associations were detected between iGEX signatures and response to treatment, most notably TIGS and CP. When testing mouse orthologs of 64 clinically relevant immune genes, almost all were associated with variable responses to therapy in at least one treatment condition. Robust positive associations were seen for *Gm5751* (ortholog of *SSX1/2* genes in humans), where high-expressing models had slower tumor growth overall and better responses to TNF receptor-based agonists (anti-CD137, -GITR, -OX40) and anti-CTLA4.

Conclusions: We characterized the immune landscape of different syngeneic models using human-validated iGEX-based measurements. We detected variable responses to treatment in mice treated with various immunotherapies and radiation based on the high or low expression of these measurements. Our work shows that human-validated iGEX-based measurements can be implemented in syngeneic models, which enhances the translatability of syngeneic models in pre-clinical research and reiterates their important role in informing therapeutic strategies.

#5167 Development of advanced preclinical models for an in-depth characterization of cardiac angiosarcoma.

Rosalba Minelli, Luigi Perelli, Benjamin J. Bivona, Sanjana Srinivasan, Desiree Catania, Bianca E. Amador, Frederick S. Robinson, Johnathon L. Rose, Christopher J. Terranova, Parth P. Shah, Alejandro Hernandez Martinez, Charles E. Deckard, Courtney Le, Kuo-Shun Hsu, Michael Peoples, Aparna Subramaniam, Hannah C. Beird, Ningping Feng, Joseph R. Daniele, Christopher Vellano, Joseph Marszalek, Vinod Ravi, Alexander J. Lazar, Andrew Futreal, Timothy P. Heffernan, Giannicola Genovese, Virginia Giuliani

UT MD Anderson Cancer Ctr., Houston, TX

Cardiac angiosarcoma (CAS) is a rare and highly aggressive tumor that originates from the endothelial cells lining the heart's blood vessels, with the right atrium being the most common site of occurrence. There are no effective treatments, and the five-year survival rate is under 10%. The absence of reliable preclinical models has also slowed progress in understanding its biology. To address this gap, we have developed a somatic mosaic genetically engineered mouse model (smGEMM) that faithfully recapitulates angiosarcoma features, including lung dissemination. Using an endothelial-specific CRE recombinase and CRISPR/Cas9, we engineered endothelial cells to express tdTomato and spCas9, enabling tissue-specific fluorescence and targeted mutation of CAS drivers. Primary endothelial cells from the cardiac tissue of our smGEMM were isolated and transduced with specific AAV particles *in vitro* and then transplanted orthotopically via ultrasound-guided injection. Primary and metastatic tumors were extensively characterized and closely mimic the features observed in CAS patients. Additionally, cell lines derived from both primary and metastatic tumors were established and comprehensively analyzed. Whole genome and RNA-seq were performed in all CAS samples and integrated with available patient data. Copy number profiling revealed whole genome duplication and loss of heterozygosity (LOH), further highlighting the complex genetic landscape of our CAS models in tumor tissues and cell lines. The comparison between our CAS samples and patient data displayed a similar mutation profile, highlighting the relevance of our model in replicating key genetic features of human cardiac angiosarcoma. To identify novel context-specific dependencies we conducted *in vitro* genome-wide CRISPR-Cas9 screens which revealed both common and unique hits across preclinical models. Several key pathways were identified and validated using different pharmacological tools. The results revealed critical therapeutic dependencies, not only when targeted with single agents but, more significantly, when these targets were evaluated in combination. This underscores the potential for developing novel, more effective therapeutic strategies for treating this rare and aggressive disease.

#5168 Mouse models accelerating cancer research at The Jackson Laboratory.

Jason Beckwith, Jen Merriam, Darcy Pomerleau, Crystal Davis, Jennifer Kelmenson, Hiroaki Onda

The Jackson Laboratory, Bar Harbor, ME

The Jackson Laboratory (JAX) provides genetically defined mutant and transgenic mice to support the scientific research community. Available are mouse models for specific cancers, xenograft recipients, immunodeficient platforms for PDX studies and multi-purpose tool strains having CRISPR utility, conditional/inducible expression (Cre-lox, FLP-frt, Tet-On/-Off), optogenetic function and calcium-sensing technologies. This poster will highlight newly developed models designed for cancer research. The portfolio of strains useful for transplantation and humanized mouse research continues to expand and complement the NSG (NOD scid IL2rγc-/-) model as premier tools for engraftment with a wide range of malignant or non-malignant human or mouse tissues. Novel fluorescent strains include Gene Editing Reporters for visualizing homology-directed repair (HDR) and non-homologous end joining (NHEJ) repair events, as well as several CRISPR Editing, Bxb1 Integrase Editing, Base Editor, Prime Editor and DNA repair pathway tools. Furthermore, new strains enable researchers to achieve extensive barcode diversity within targeted cell populations. Models utilizing the auxin-inducible degron system allow degradation of tagged proteins following administration of 5-Ph-IAA. In addition to cryopreserving each strain, JAX employs a genetic quality control program that confirms mutation identity and genetic background, and screens for unwanted alleles (such as stray GFP, Cre, neo, etc.). Cancer researchers can query the collection of innovative mouse models using the JAX Mouse Search site (mice.jax.org). This resource includes models created by many generous donating institutions. Researchers are encouraged to donate their mouse lines via a very short Strain Submission form (jax.org/donate-a-mouse). Please also visit the JAX Oncology webpage for cancer research related strains and resources (jax.org/jax-mice-and-services/solutions-by-therapeutic-area/oncology). JAX is supported by the NIH, The Howard Hughes Medical Institute and other private charitable foundations.

#5169 Validation of genetically defined Oncopig hepatocellular carcinoma cell lines for *in vitro* evaluation of targeted therapeutic efficacy.

Mahmoud Khalafalla[1], Navin Viswakarma[1], Lobna Elkhadragy[2], Theresa K. Kelly[3], Mark Eccleston[3], Lawrence B. Schook[1], Ron C. Gaba[2], Kyle M. Schachtschneider[1]

[1]Sus Clinicals, Inc., Chicago, IL,[2]Radiology, University of Illinois at Chicago, Chicago, IL,[3]Volition Rx, San Diego, CA

Hepatocellular carcinoma (HCC) is the second leading cause of cancer related death worldwide with a 5-year survival rate of 18%. Development of novel targeted therapeutics to advance HCC precision medicine approaches is challenging due to a lack of malleable large animal HCC models that replicate human genetics, anatomy, metabolism, and physiology. We previously demonstrated our ability to develop HCC cell lines and tumors that recapitulate clinically relevant characteristics of human HCC utilizing the Oncopig cancer model (capable of forming tumors through induced $KRAS^{G12D}$ and $TP53^{R167H}$ expression). Here, we demonstrate our ability to develop Oncopig HCC cell lines with differential therapeutic susceptibilities through introduction of clinically relevant HCC driver mutations ($PTEN^{KO}$, $AXIN1^{KO}$, or $ARID1^{KO}$) via CRISPR-Cas9 gene editing. Specifically, we demonstrate that $PTEN^{KO}$ increases susceptibility to PI3K inhibitors currently in clinical trials for treatment of *PTEN* null HCC and other cancer types. Therapeutic susceptibility of the established Oncopig WT, $PTEN^{KO}$, $AXIN1^{KO}$, and $ARID1^{KO}$ HCC cell lines was assessed for PI3K inhibitors (GSK2636771, BAY 80-6946, and BKM120). Consistent with human studies, Oncopig $PTEN^{KO}$ HCC cell lines displayed significantly increased sensitivity to GSK2636771 and BAY 80-6946, but not BKM120, compared to WT and PI3K inhibitor non-responsive $AXIN1^{KO}$ and $ARID1A^{KO}$ cells (see Table). In addition, Oncopig $PTEN^{KO}$ HCC cells possessed significantly increased proliferation and migration rates compared to WT Oncopig HCC cells (1.6 and 2.8 fold, respectively; $p < 0.01$). These results validate the utility of the Oncopig HCC model for preclinical evaluation of novel targeted therapeutics *in vitro*. Future work demonstrating consistent results *in vivo* will enable the genetically defined Oncopig HCC model to be an ideal animal system to test precision medicine therapeutics prior to entry into the clinic.

Therapeutic Susceptibility (Log IC50 (μM))

PI3K Inhibitor	Oncopig WT HCC	Oncopig $AXIN1^{KO}$ HCC	Oncopig $ARID1^{KO}$ HCC	Oncopig $PTEN^{KO}$ HCC	Human *PTEN* Null Cells
GSK2636771	1.46	1.17	0.66	0.07	Responsive
BAY80-6946	11.5	5.6	4.5	2.38	Responsive
BKM120	2.05	0.95	NA	2.3	Non-Responsive

#5170 A xenograft mouse model of virus-positive Merkel cell carcinoma directly links germ cell theory to somatic cancer.

Sara Contente, Sarah Rahman, **Wendy Yang**

USUHS - Uniformed Services University of the Health Sciences, Bethesda, MD

While mutation theory has dominated the somatic cancer arena for decades, germ cell theory has been the necessary alternative for germ cell tumors (GCTs) with their unique (epi)genomic profiles. However, all cancers are unified by a set of hallmarks and the paradigm division between somatic versus GCTs has been challenged by accumulating paradoxes. Merkel cell polyomavirus (McPyv) positive Merkel cell carcinoma (MCC), a very rare and highly malignant skin cancer, is one such striking paradox contradicting the cancer mutation theory. MCC is a small cell neuroendocrine carcinoma (SCNC) with 80% containing McPyv, a tiny ubiquitous DNA virus of normal skin flora. In common with GCTs, virus-positive (VP)-MCC exhibits striking genomic stability in dichotomy to its virus-negative counterparts and other SCNCs. Intriguingly, the SCNC phenotype is also the pan-cancer convergent end state of tumor progression/dedifferentiation, implicating closeness to somatic cancer stem cells. It is thus rational to hypothesize VP-MCC being an extra-gonadal GCT with oncovirus driven malignant somatic transformation (MST) in an adult cutaneous niche. GCT research has supported human primordial germ cells (hPGCs) being the cell of origin for GCTs. hPGCs are considered unipotent that cannot form teratomas in adult mice but are readily converted in vitro to pluripotent human embryonic germ cells (hEGCs) that can form teratomas in adult mice, like pluripotent human ESCs (hESCs) or human iPSCs (hiPSCs) which may rarely spontaneously differentiate to hPGCs in vitro. To validate our hypothesis, we performed a mouse study by injecting hPGC-like cells (hPGCLCs) with or without McPyv transfection to NSG mouse flank skin, as well as McPyv transfected hiPSCs and hEGC-like cells (hEGCLCs). We then monitored tumor formation and growth over 4 months. All tumors were analyzed for histology and gene/protein expression by H&E, immunohistochemistry, flow cytometry and/or RT-qPCR. 2 out of 3 mice injected by McPyv transfected hPGCLCs exhibited MST to typical MCC-like tumors from a background of teratoma. All mice injected with McPyv transfected hiPSCs or hEGCLCs formed teratomas as expected, but only tumors derived from very high number hiPSC injections showed MST to atypical MCC-like tumors. Our findings thus demonstrated McPyv transfection enabled MST to MCC-like tumors from hPGCLCs and high dose hESCs. Per literature search, this is the first MST model to directly link germ cell theory to somatic cancer, with its simplicity to enable easy tractability of molecular events.

Disclaimer: The opinions and assertions expressed herein are those of the author(s) and do not reflect the official policy or position of the Uniformed Services University of the Health Sciences or the Department of Defense.

#5172 Advanced models of breast cancer brain metastasis and imaging protocols for preclinical efficacy studies.

Sigrid Cold[1], Maria Z. Alfsen[2], Mark U. Juul[2], Camilla Malec[2], Camilla Christensen[3], Michael J. Wick[4], Lene Jessen[2], Trine B. Engel[2], Lotte K. Kristensen[2], Andreas Kjaer[1], **Carsten H. Nielsen**[2]

[1]Cluster for Molecular Imaging; Department of Biomedical Sciences, Copenhagen University Hospital - Rigshospitalet; University of Copenhagen; and Minerva Imaging ApS, Copenhagen, Denmark,[2]Minerva Imaging ApS, Oelstykke, Denmark,[3]Department of Clinical Physiology and Nuclear Medicine, Copenhagen University Hospital - Rigshospitalet; Minerva Imaging ApS, Copenhagen, Denmark,[4]XenoStart, San Antonio, TX

Introduction: Approximately 30% of breast cancer (BC) patients eventually develop metastases in distant organs and especially breast cancer brain metastases (BCBM) are associated with poor prognosis and high mortality. Translatable animal models mimicking and predicting clinical disease and outcomes are urgently needed to increase the success of novel anti-cancer therapies. In this study, we generated advanced orthotopic BCBM models established by three different inoculation methods. Furthermore, we optimized imaging protocols to monitor tumor growth, BBB permeability and efficacy in preclinical studies.

Methods: BCBM was established by stereotactic inoculation of human BC cells lines (BT474 and MDA-MB-231.Luc2) and cells isolated from patient derived xenografts (PDX) (ST1339, ST3338, ST941, ST340, ST1616B and ST1360B). To mimic metastatic spread, BCBM was also established by intracardial (ICar) and intracarotid (ICt) inoculation (BT474 and MDA-MB-231.Luc2). Metastatic spread and tumor growth were monitored using T2-weigthed MRI and bioluminescence imaging while BBB permeability was evaluated *in vivo* by Gd-contrasted MRI. Transport of HER2 targeting trastuzumab across the BBB was confirmed by PET/MRI, resulting in a subsequent study evaluating efficacy of Trastuzumab emtansine (T-DM1) (ST1339 and ST3338). IHC and light sheet microscopy (LSM) were performed to validate *in vivo* findings of tumor characteristics including metastatic spread and BBB permeability.

Results: BCBM were successfully established by stereotactic inoculation with high tumor take rates (>80%) and consistent tumor location and number (n=1 per animal). The BCBM presented with varying brain infiltration profiles and BBB permeability. ^{64}Cu-trastuzumab uptake in animals bearing ST1339 tumors (HER2 3+) confirmed delivery across the BBB. Animals treated with T-DM1 experienced tumor growth delay with ST1339 having the greatest response. ICar inoculation gave rise to metastatic spread, but <25% of animals inoculated developed BCBM prompting the need for a more targeted delivery of BC cells to the brain. ICt inoculation resulted exclusively in the formation of BCBM with a take rate of >65%. MRI imaging revealed 1-7 metastases per animal with intrasubject and intersubject variation in BBB permeability. *Ex vivo* analysis validated the *in vivo* imaging of metastases and showed multiple metastases of varying sizes and BBB permeability.

Conclusion: We've successfully developed multiple complex orthotopic models for BCBM mimicking different aspects of BCBM. ICar and ICt models especially resemble the pathophysiology of BCBM to a large extent with extravasation and micro seeding in the brain. Together with the developed imaging protocols allowing longitudinal evaluation of compound delivery, tumor growth and BBB permeability this serves as a great platform for preclinical studies with novel anti-cancer agents.

#5173 Heterozygous *Ppp2r1a* mutations with concurrent loss of Tp53 drive invasive high-grade uterine cancer.

Caitlin M. O'Connor[1], Kelsey Barrie[1], Kaitlin Zawacki[1], Ingrid L. Bergin[2], Gabrielle Hodges Onishi[1], Analisa DiFeo[3], Kathleen Cho[3], Thom Saunders[1], Goutham Narla[1]

[1]Internal Medicine: Genetic Medicine, University of Michigan, Ann Arbor, MI,[2]In Vivo Animal Core Facility, University of Michigan, Ann Arbor, MI,[3]Pathology, University of Michigan, Ann Arbor, MI

Uterine cancer is the only cancer type for which survival has fallen in the past four decades. This year in the United States, over 13,000 women will die from this disease, surpassing ovarian cancer for the first time: making uterine cancer the deadliest gynecological cancer. Mortality from uterine cancer is primarily due to the aggressive, non-endometrioid subtypes of the disease which include uterine serous carcinomas and uterine carcinosarcomas (high-grade subtypes). Molecularly, high-grade uterine tumors harbor few mutations, however, almost every high-grade uterine tumor harbors a *Tp53* mutation, and 30-40% of tumors also harbor a heterozygous *PPP2R1A* mutation, most commonly - P179R, S256F, or R183W. PPP2R1A encodes for the Aα scaffolding subunit of the heterotrimeric protein phosphatase 2A (PP2A). Previous literature from our group and others has shown that these mutations occur very early in uterine cancer development and that these mutations modulate PP2A function in a manner that impairs the formation of tumor-suppressive PP2A holoenzymes. However, a lack of models, particularly transgenic mouse models, of high-grade uterine cancers has limited our understanding of the disease as well as the preclinical testing of targeted therapeutics to the underlying drivers of disease development and progression. Knockout of p53 in mouse uterine epithelium has been shown to result in the formation of uterine tumors (uterine serous and carcinosarcomas) at 16-20 months of age. Our group has generated novel conditional Ppp2r1a-P179R and R183W knock-in mice. Because these mutations exclusively co-occur with Tp53 alterations, we crossed these mice to the $Tp53^{fl/fl}$ and Ksp1.3-Cre mouse to generate uterine epithelium-specific knockout of Tp53 and knock-in of either Aα-P179R or R183W PP2A mutant alleles. Our preliminary data demonstrate that these mice develop advanced high-grade uterine cancers as early as 6 months, compared to 16 months with Tp53 KO alone, showing that *PPP2R1A* mutations significantly accelerate uterine tumor formation. Pathological analysis of these tumors has shown that they are high-grade and resemble high-grade uterine tumors in humans. Combined, our data definitively show that *PPP2R1A* mutations are drivers of high-grade uterine cancers for the first time and provide a novel mouse model of high-grade uterine cancer that can be used to develop a better understanding of this deadly cancer.

#5174 Exploring extinction therapy for advanced cutaneous squamous cell carcinoma using UV-induced mouse models.

Karol M. Prieto-Sarmiento[1], Stanislav Avdieiev[1], Omar Chavez Chiang[1], Sofia Cordero[1], Irmak Aksit[1], Derek Weimer[2], Christopher J. Whelan[1], Joel S. Brown[1], Kenneth Y. Tsai[1]

[1]H. Lee Moffitt Cancer Center, Tampa, FL,[2]Nova Southeastern University, Davie, FL

Advanced cutaneous squamous cell carcinoma (cuSCC) remains a major therapeutic challenge, with an estimated 50.000 cases annually in the United States. While anti-PD1 immunotherapy—the only FDA-approved therapy—yields response rates of approximately 50%, responses to chemotherapy and radiotherapy are typically transient, and no comprehensive strategy for immunotherapy-refractory disease exists. We developed a novel K14-CreERT2 Confetti mouse model to investigate cuSCC progression, from UV-induced mutagenesis to invasive tumors. This model enables lineage tracing of keratinocytes and their progeny through fluorescent labeling, allowing precise monitoring of tumor evolution. Using this platform, we conducted a preclinical study across multiple tumor development stages to compare continuous therapy with extinction therapy—a novel first-strike-second-strike approach hypothesized to exploit vulnerabilities in fragmented cancer populations. Fifty-four mice were exposed to UV radiation on one side of their backs to induce mutagenesis, mimicking human cuSCC progression. The animals were divided into early-stage (tumors ~3mm) and late-stage (tumors ~6mm) cohorts based on tumor size at enrollment. Mice were assigned to either continuous or extinction therapy regimens, receiving combinations of anti-PD1 immunotherapy and the MEK inhibitor trametinib. Continuous therapy involved initial treatment with either immunotherapy or targeted therapy until the tumor grew 20% larger than its enrollment size. Then, the therapy switched to the alternate modality and continued until the endpoint (tumors >10mm). Extinction therapy began with either targeted therapy or immunotherapy, with treatments alternating under specific conditions: when tumors failed to respond after two weeks or when a different tumor exceeded the initial tumor's enrollment size. This adaptive strategy aimed to enhance efficacy by targeting dynamic tumor vulnerabilities. UV-irradiated versus non-irradiated skin was monitored through confocal microscopy for eight months post-irradiation to evaluate resistance evolution and underlying cellular and clonal changes. As observed previously, tumors only developed on UV-irradiated skin, with significant variability in tumor numbers (3-25 per animal). Tumor burden was assessed twice weekly to evaluate therapy efficacy. Preliminary results suggest that extinction therapy significantly reduced tumor burden and delayed resistance compared to continuous treatment, particularly in advanced disease stages. We provide valuable insights into therapeutic strategies for cuSCC management with our approximation of treatment regimen combinations and their efficacy across distinct tumor progression stages.

#5175 Investigating the cooperative effects of the *BRAF* oncogene and inflammation in *BRAF*-mutant CRC development.

Caleb Green[1], Catherine Andreadi[1], Emma Parrott[1], Hong Cai[1], Karen Brown[1], Abdolrahman Shams-Nateri[2], Alessandro Rufini[3]

[1]University of Leicester, Leicester, United Kingdom,[2]Biodiscovery Institute, University of Nottingham, Nottingham, United Kingdom,[3]Universita degli Studi di Milano, Milan, Italy

Colorectal cancer (CRC) currently represents the third most commonly diagnosed cancer worldwide, with its tumorigenesis associated with multiple risk factors, including the presence of inflammation. However, the pro-tumorigenic mechanisms of inflammation remain ill-defined, with poor understanding of the roles of individual inflammatory pathways. Mutations in the *BRAF* oncogene drive a CRC subtype with serrated histology, typically co-occurring with high levels of microsatellite instability. Unpublished gene set enrichment analysis revealed increased inflammatory signaling in human serrated colonic lesions from publicly available human transcriptomic data. Paradoxically, in-house RNA-sequencing analysis from mice harboring an inducible oncogenic mutation in *Braf* showed repression of inflammatory pathways in intestinal tissue. In addition, *in vivo*, *Braf* mutant mice exhibit long latency and low frequency of tumor formation, which may be due to repressed inflammatory signaling following $Braf^{V600E}$ induction. Our work aims to further investigate the impact of the *Braf* oncogene on specific inflammatory pathways through analysis of phosphorylated (p)STAT1 and (p)STAT3 expression, biomarkers for IFN-γ and IL6/JAK/STAT pathways respectively. This will be assessed by western blot using an *in vitro* mouse intestinal organoid (MIO) model, and through multiplex immunofluorescence (mIF) on *in vivo* murine intestinal tissue slides. To elucidate whether *Braf* mutations employ cell autonomous mechanisms to alter inflammatory signaling, inducible *Braf* mutant intestinal crypts were isolated and grown *in vitro* to form MIOs before treatment with IFN-γ. Western blotting of MIO lysates revealed unchanged phosphorylation status of STAT1 and STAT3 irrespective of Braf mutational status in our experimental conditions. Additionally, mIF revealed the expression and localization of phosphorylated STAT1/STAT3 in intestinal tissue were comparable in wildtype control and $Braf^{V600E}$ mice, 3 days and 6 weeks after induction of the mutant oncogene (n=9). However, in mice expressing $Braf^{V600E}$ for 6 months, there was significantly restrained signaling for both pSTAT1 ($p<0.05$) and pSTAT3 ($p<0.05$) in the mutant compared to the wildtype control animals (n=4). Overall, our data indicate temporal control of inflammatory signaling by the $Braf^{V600E}$ oncogene *in vivo*. With short term Braf induction leaving IFN-γ and IL-6 signaling unperturbed, assessed through pSTAT1/3, whereas mice with prolonged *Braf* expression showed modulation of both IFN-γ and IL6/JAK/STAT pathways. Further investigation is required into the repression of inflammation induced by the *Braf* oncogene, and whether subsequent restoration of inflammatory signaling would lead to increased potency in tumor formation. Knowledge of these mechanisms could prove crucial in understanding the development of human BRAF-mutant CRCs.

#5176 NaJa cells - the first syngeneic cell line model to study BRAF^{V600E}-driven colorectal cancer.

Jasmin Traichel[1], Ariane Lehmann[2], Nadine Wohlfeil[3], Ricarda Griffin[3], Maria Walz[3], Reinhild Feuerstein[4], Celia Asdih[3], Rebecca Kesselring[4], Melanie Boerries[2], Tilman Brummer[3]

[1]Institute for Molecular Medicine and Cell Research, University of Freiburg, German Consortium for Translational Cancer Research DKTK, Freiburg, Germany,[2]Institute of Medical Bioinformatics and Systems Medicine (IBSM), Medical Center University of Freiburg, Freiburg, Germany,[3]Institute for Molecular Medicine and Cell Research, University of Freiburg, Freiburg, Germany,[4]Department of General and Visceral Surgery, University of Freiburg, Freiburg, Germany

Background: Due to the lack of suitable syngeneic murine models to study malignant BRAF^{V600E}-driven colorectal cancers (CRC), the aim of this study was to establish new murine models that recapitulate this aggressive CRC subtype both *in vitro* and *in vivo* in an immunocompetent syngeneic context.

Methods: A murine colonic organoid model with VillinCreER^{T2}-inducible BRAF^{V600E}, TRP53^{R172H} and APC$^{\Delta}$ mutations (designated BPAC) was generated. Orthotopic transplantations with BPAC organoids resulted in tumor growth and spontaneous metastasis into the peritoneal cavity of immunocompetent syngeneic C57/Bl6 recipient mice. Through subsequent peritoneal lavages, three new murine cell lines (NaJa-D, NaJa-F and NaJa-G) originating from the same mutational background were established. These cell lines were thoroughly characterized by RNA- and whole exome sequencing. Furthermore, their response to kinase inhibitors was tested and validated by colony formation assays and Western blotting. To test their invasive potential *in vivo*, the NaJa cells were injected via the portal vein into immunocompetent syngeneic mice and the tumor microenvironment was subsequently analyzed by immunohistochemistry (IHC).

Results: The three established NaJa cell lines all expressed the aforementioned driver mutations and microsatellite stability was confirmed through whole exome sequencing. They differed greatly in their morphology despite the common genetic background, with NaJa-D displaying an epithelial morphology, NaJa-G adopting a mesenchymal phenotype and NaJa-F presenting an intermediate phenotype. Furthermore, they exhibited differential responses to treatment with clinically relevant kinase inhibitors, resulting in differentially altered signaling pathways and overall survival. Upon re-transplantation via the portal vein into immunocompetent syngeneic mice, all three NaJa cell lines induced fast and aggressive tumor growth in the liver while retaining their distinct morphological characteristics, and promoted metastasis, particularly to the peritoneal cavity and surrounding tissues. The tumor microenvironment was analyzed in terms of immune cell composition and distribution, revealing notable differences between the cell lines. These findings establish the NaJa cell-induced tumor model as a valuable tool for exploring the combinatory use of targeted therapies with immune checkpoint inhibition in future studies.

Conclusion: Given the general lack of murine cell lines to model BRAF^{V600E}-driven CRC, the NaJa cells provide a unique and valuable tool to research cancer heterogeneity, test clinically relevant kinase inhibitors and research the immune cell environment and immune escape of BRAF^{V600E}-driven metastatic CRC.

#5177 Modeling 6q deletion and MYD88^{L265P}-driven B-cell lymphomas in mice reveals early lymphomagenesis mechanisms and identifies the putative cell of origin.

Filip Garbicz[1], Johany Penailillo[1], Senthilkumar Muthiah[2], Fernanda Cuevas[1], Tuyet Nguyen[1], Elisa Mandato[3], Eleonora Calabretta[3], Tomasz Sewastianik[1], Elena Ivanova[4], Aisha Saldanha[4], Moataz Noureddine[4], Rafael Irizarry[2], Margaret A. Shipp[3], Zachary Hunter[5], Steven P. Treon[5], Ruben D. Carrasco[1]

[1]Department of Pathology, Dana-Farber Cancer Institute, Boston, MA,[2]Department of Data Science, Dana-Farber Cancer Institute, Boston, MA,[3]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA,[4]Belfer Center for Applied Cancer Science, Dana-Farber Cancer Institute, Boston, MA,[5]Bing Center for Waldenstrom's Macroglobulinemia, Dana-Farber Cancer Institute, Boston, MA

Chromosomal aberrations often represent early events in cancer development and might play a critical role in establishing malignant clones. Large chromosomal deletions typically involve the loss of multiple cooperating tumor suppressor genes, which makes it challenging to study their contribution to cancer pathogenesis. Recent genomic studies have identified the MYD88^{L265P} mutation and chromosome 6q deletion as frequent alterations in Waldenstrom's macroglobulinemia (WM)/lymphoplasmacytic lymphoma (LPL). These genetic abnormalities are considered to be key drivers of oncogenic NF-κB signaling and are also observed in diffuse large B-cell lymphoma (DLBCL). Previous studies, including our own, have demonstrated that neither human MYD88^{L265P} nor murine Myd88^{L252P} mutations alone are sufficient to induce neoplastic transformation in mouse models. Chr6q deletion, containing multiple genes involved in B cell biology, might constitute a critical "second hit" necessary for sustaining MYD88^{L265P}-induced signaling. This study aimed to elucidate the roles of MYD88^{L265P} and Chr6q (syntenic to murine Chr10q) deletion in B-cell lymphoma pathogenesis. We generated the following genetically engineered mouse models: *Myd88$^{L252P/+}$;Cd19$^{Cre/+}$*, *10q$^{+/-}$;Cd19$^{Cre/+}$* and *Myd88$^{L252P/+}$;10q$^{+/-}$;Cd19$^{Cre/+}$*. We have observed spontaneous development of low- and high-grade B cell lymphomas in aged cohorts of *10q$^{+/-}$;Cd19$^{Cre/+}$* and *Myd88$^{L252P/+}$;10q$^{+/-}$;Cd19$^{Cre/+}$* mice. Pathological analysis showed an expansion of lymphoplasmacytic B220^{-} SOX5+ PAX5+ IgM+ B cells, which infiltrated the bone marrow, spleen and lymph nodes. To investigate the progression of B-cell changes during premalignant and malignant stages, we performed scRNA-seq on murine lymphoid organ samples. There was an early clonal expansion of B1 and memory B cells, potentially representing lymphoma precursor cells. Later on, these populations were replaced by larger cells frequently positive for Ki67, IRF4, and IgM, suggesting a transition from a low-grade WM-like disease to a high-grade DLBCL. Both early- and late-stage lymphomas exhibited elevated expression of therapeutic targets, including *Bcl2*, *Ms4a1*, *Btk* and *Hck*, offering potential avenues for intervention and therapy response studies. Additionally, profound alterations were observed in the T-cell compartment, with an increased frequency of PD1^{+} LAG3^{+} TIM3^{+} cells, suggesting immunotherapy targets that merit further validation. The mouse models provide valuable platforms for investigating the mechanistic roles of these genetic alterations and testing therapeutic strategies. In the future, we plan to validate the models as preclinical tools for studying MYD88 and Chr6q deletion-driven lymphomagenesis. This project was supported by the Robert A. Kyle Career Development Award from the IWMF and NIH grant R01CA273123.

#5178 Ascites conditioning reduces latency in vivo for progression of ID8 TP53 KO murine ovarian cancer model.

Taylor Barnhisel, Zari Dehdashti, Stephen Kron

Ludwig Center for Metastasis Research and Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL

High-grade serous ovarian carcinoma (HGSOC), an aggressive subtype of epithelial ovarian cancer (EOC) characterized by peritoneal metastasis at diagnosis and poor prognosis, is almost universally associated with p53 mutations. Mouse models of EOC and particularly HGSOC remain unsatisfactory for therapy development. Underscoring its low-grade behavior, intraperitoneal injection of the C57BL/6-derived EOC cell line ID8 from tissue culture is followed by a 9-to-10-week latency before onset of peritoneal carcinomatosis, associated with development of ascites and abdominal distention. Toward overcoming limitations of ID8 cells as a mouse model for HGSOC, Walton et al. (2018) performed CRISPR/Cas9-mediated knockout and isolated the F3 Trp53-/- cell line. While the latency period was reduced upon Trp53 deletion, 5-to-6-weeks may pass before ascites can be observed after injection of plastic-grown F3 cells. Prior studies have ascribed the delay to a dearth of cancer stem cells and/or a low propensity for colonizing the omentum. Indeed, ID8 cells isolated after 11 or 12 weeks from ascites fluid were found to be enriched for stem cells and to display immediate omental colonization and ascites onset. We confirmed that transplanting even small volumes of F3 ascites fluid into naive mice led to rapid carcinomatosis and ascites. Strikingly, passaging F3 ascites cells in tissue culture did not restore latency. These observations suggested that ascites-derived Trp53-/- ID8 cells might provide superior HGSOC models. To form an ascites-conditioned F3 cell line, CF3, plastic-grown F3 cells were injected into a C57BL/6 mouse, allowed to progress to carcinomatosis and ascites, and the ascites cells isolated and passaged four to six times. Within 14 days after being injected with CF3 cells, mice display ascites. Examining the viscera reveals visible tumors studding the abdominal mesentery and omentum. At this timepoint, mice injected with F3 display no visible signs of engraftment. CF3-injected mice reach a humane endpoint within approximately 30 days, compared to about 54 days for F3. Underlining the primary effect of ascites conditioning as reduced latency, CF3 and F3 mice display similar ascites volume, size, and distribution of metastases at their endpoints. Despite the rapid onset of carcinomatosis, CF3 cells maintain similar sensitivity to carboplatin in vivo. We suggest that ascites-conditioned cells provide a rapid and robust preclinical HGSOC model that may streamline experimental timelines and facilitate evaluation of immune and other therapies. Supported by Roswell Park-University of Chicago Ovarian Cancer SPORE P50 CA159981 and the University of Chicago Medicine Comprehensive Cancer Center.

#5179 Investigating the tumor suppressive role of RanBP9 in mice deficient in p53.

Brianna Gonga-Cave[1], Gabriel Onea[1], Xu Wang[2], Sean Cregan[3], Patti Kaiser[4], Caroline Schild-Poulter[1]

[1]Biochemistry, Western University, London, ON, Canada,[2]Robarts Research Institute, Western University, London, ON, Canada,[3]Physiology and Pharmacology, Western University, London, ON, Canada,[4]Pathology and Laboratory Medicine, Western University, London, ON, Canada

RAN binding protein 9 (RanBP9) is a scaffolding protein that is essential for the formation a multi-subunit E3 ubiquitin ligase termed the C-terminal to Lis-1 homology (CTLH) complex. Multiple groups have identified the CTLH complex in regulating a broad range of biological processes including cell cycle, cell proliferation, and metabolism. Various studies have demonstrated that RanBP9 functions as a tumor suppressor, but none have shown whether the loss of RanBP9 can initiate spontaneous tumor formation. Here, we investigated the tumor suppressive role of RanBP9 in C57BL/6 mice. Bulk RNA sequencing (RNA-seq) analysis of 1-month-old *RanBP9*$^{-/-}$ mice liver identified an upregulation of several oncogenic signaling genes. Although *RanBP9*$^{-/-}$ mice displayed a shortened lifespan, these mice did not develop spontaneous tumors suggesting that additional genetic perturbations are required for tumor development. Next, we conducted an observational study in mice with *RanBP9*$^{+/-}$*: Trp53*$^{+/-}$ (*R9; p53*$^{+/-}$) and *RanBP9*$^{-/-}$*: Trp53*$^{-/-}$ (*R9; p53*$^{-/-}$) genotypes. These mice were monitored over 17 months for the development of spontaneous tumors. The primary endpoint was defined as the development of a visible mass (>1.5 cm) or symptoms of cancer. *R9; p53*$^{+/-}$ mice (n=23) developed sarcomas at an earlier time compared to control *p53*$^{+/-}$ mice (n=18; $p<0.05$). *R9; p53*$^{+/-}$ mice displayed altered tumor spectrum with increased frequency in soft tissue sarcoma (STS) development. H&E analysis of tumor biopsies revealed increased mitotic activity of sarcomas derived from *R9; p53*$^{+/-}$ mice compared to control *p53*$^{+/-}$ mice. We observed no significant difference in tumor incidence and altered tumor spectrum in *R9; p53*$^{-/-}$ mice compared to control *p53*$^{-/-}$ mice. Analysis of TCGA sarcoma database revealed a correlation between low gene expression of CTLH complex genes and reduced survival in sarcoma patients. Our data indicates that loss of RanBP9 alone is insufficient to initiate tumor development. However, the heterozygous loss of RanBP9 and p53 promotes increased STS development. Ongoing efforts are to further characterize *R9; p53*$^{+/-}$ tumors and identify the mechanism to understand how the loss of CTLH activity contributes to sarcoma development in the absence of p53. Our findings may support the development of anti-cancer therapeutics that activates RanBP9-CTLH complex activity.

#5180 CXCR7 mediates an immune-suppressive tumor microenvironment and promotes prostate cancer progression.

Hongshun Shi[1], Ziqi Zhang[2], Lourdes Brea[3], Zhuoyuan Lin[3], Viriya Keo[3], Sambhavi Senthil[1], BaoHan Thi Vo[1], Galina Gritsina[3], Xiaojun Kong[1], Jonathan Zhao[4], Haydn Thomas Kissick[1], Xiuwei Zhang[2], Jindan Yu[1]

[1]Department of Urology, School of Medicine, Emory University, Atlanta, GA,[2]School of Computational Science and Engineering, College of Medicine, Georgia Institute of Technology, Atlanta, GA,[3]Division of Hematology/Oncology, Department of Medicine, Northwestern University, Chicago, IL,[4]Department of Human Genetics, School of Medicine, Emory University, Atlanta, GA

Background: Genetic inactivation of Pten and/or TP53 are common in human cancers including advanced prostate cancer (PCa). We previously reported CXCR7 up-regulation in castration resistant prostate cancer (CRPC), following androgen-receptor-targeted therapies. However, how CXCR7 regulates PCa and the tumor immune microenvironment (TIME) is poorly understood.
Methods: We generated PbCre:Pten/TP53/Cxcr7 (TKO) mice, which were compared to the PbCre:Pten/TP53 (DKO) mice. We performed histology analyses of the mice aged from 12 to 24 weeks and utilized scRNA-seq to analyze prostate samples collected from transgenic mice of 12- and 18-week age. Multiplexed error-robust fluorescence in situ hybridization (MERFISH) platform was used to further confirm our results and demonstrate a spatial relationship. In addition, Cy-Tek and FACS analysis were employed to explore the tumor microenvironment focusing on immune cells. We then analyzed publicly available human PCa datasets to explore the correlation of CXCR7 with immune cell subsets using CIBERSORT.
Results: Prostate-specific deletion of Cxcr7 in the Pten/TP53-DKO mice resulted in significant delay in prostate tumor progression. We found differential enriched lymphoid cluster and decreased Spink1+ luminal and Tff3+ luminal clusters after Cxcr7 knockout. Cxcr7 deletion was associated with profound TIME remodeling characterized by increased CD8+ T cells and reduced Gr-1+CD11b+ myeloid-derived suppressor cells (MDSC). In addition, Cxcr7 knockout facilitated the invasion of CD4+ and CD8+ T cells into the tumors, while these immune cells were mainly located in the stroma of the prostates of the DKO mice. TKO mouse also exhibited enhanced cell-cell interactions with PCa, compared to the DKO mouse, characterized by a decrease of IL6 signaling and an increase of IFN-II and MHC-I signaling. Critically, examination of human PCa datasets confirmed that high levels of CXCR7 were indeed correlated with lower CD8+ T cell infiltration in the tumors.
Conclusion: Cxcr7 deletion delayed prostate cancer progression caused by Pten/TP53 loss. Combining scRNA-seq with a spatial framework of gene expression analyses of transgenic mouse models, we demonstrated a critical role of CXCR7 in remodeling the tumor microenvironment. Our result indicates CXCR7 as a promising therapeutic target for Pten/TP53-deficient prostate cancer.

#5181 SPTBN1$^{D1089Y/+}$ aggravates liver carcinogenesis via activating SIRT6 and TGF-beta signaling.

Kazufumi Ohshiro, Xiyan Xiang, Xiaochun Yang, Krishanu Bhowmick, Lopa Mishra

The Feinstein Institute for Medical Research, Manhasset, NY

Background: Obese-driven liver cancer (HCC) is alarmingly rising in the US. Yet, key signaling mechanisms underlying this obesity-driven HCC remain unclear. SIRT6 is a NAD^+-dependent deacetylase that limits the activity of the lipogenic transcription factors SREBP1/2 and TGF-β signaling. Reduced SIRT6 is associated with human fatty liver and HCC. We found that TGF-β/SMAD3 signaling pathway and SREBP1/2 are modulated by βII-spectrin (encoded by *SPTBN1*), and a mutation in human HCC, D1089Y abrogates SMAD3 binding (*Gastro 2018; 154:195-210*). In stressed hepatocytes, βII-spectrin promotes lipogenesis through interactions between Caspase-3-cleaved forms of both SREBP1 and βII-spectrin *(Sci TM 2021;13:624)*. Liver-specific knockout of βII-spectrin blocks fatty liver and HCC. Here, we examined the extent of SPTBN1-D1089Y regulation of SIRT6 expression through SMAD3, and the combined roles of SIRT6 and SPTBN1 in human HCC.

Methods: We generated a SPTBN1-D1089Y mutant mouse and examined SIRT6 expression in liver tissues and MEFs. Four-month-old Sptbn1$^{D1089Y/+}$ mutant and WT male mice were injected with DEN/CCI4 and fed on WD.TGF-β/SMAD3 pathway activity was analyzed by Western blotting and RNA-seq. SIRT6 expression was examined in mouse MASLD/MASH. Structure-based molecular docking simulations using structural coordinates of human SIRT6 and SPTBN1 from the AlphaFold database.

Results: SIRT6 expression is significantly decreased in liver macrophages from mice with MASLD/MASH. Sptbn1$^{D1089Y/+}$ mice receiving DEN/CCI4 plus WD displayed significantly more tumor nodules with severe liver injury, steatosis, and inflammation. TGF-β/SMAD3 pathway activity is suppressed in SPTBN1-D1089Y mutant MEFs with decreased phosphor-SMAD3 levels. SIRT6 levels are decreased in Huh7 cells overexpressing SPTBN1-D1089Y and the D1089Y-mutant mouse liver tissues. Structural modeling predicts that SIRT6 binding sites of SPTBN1 (R429-S439) are close to the region mediating SREBP1/SPTBN1 interaction. In HepG2 cells, overexpression of SIRT6 deacetylates βII-spectrin; SMAD3 and βII-spectrin were found to interact with SIRT6 upon TGF-β treatment by co-immunoprecipitation; SIRT6 knockdown increased nSREBP1, SCD1, and βII-spectrin. SPTBN1-WT overexpression increased slightly HCC cell viability, whereas SPTBN1-D1089Y mutant increased approximately more than two times of the WT viability. Exogenous SPTBN1-WT increased the expression of transcriptional repressor CTCF and SIRT6, and SPTBN1-D1089Y mutant decreased both expressions.

Conclusions: Our study indicates a SMAD3 dependent as well as a SMAD3 independent role of SPTBN1 in regulating SIRT6. Sptbn1-D1089Y heterozygous loss aggravates liver carcinogenesis. Sptbn1-D1089Y together with SIRT6 deficiency may increase the susceptibility to MASH/HCC.

#5182 Transcriptomic analysis of mouse models of lung adenocarcinoma with different metastatic phenotypes.

Naomi Mitchell-Hutchinson[1], Paige Ramkissoon[2], Julie Wells[1], Rick Maser[1], Tim Stodola[1], Brian Hoffman[1], Anne Marchini[1], Elaine Bechtel[1], Rosalinda Doty[1], Carol Bult[1]

[1]The Jackson Laboratory, Bar Harbor, ME,[2]Cincinnati Children's Hospital, Cincinnati, OH

Introduction: Lung cancer is the leading cause of cancer deaths worldwide with most deaths attributed to metastatic disease. Existing mouse models of lung adenocarcinoma (LUAD) rarely become metastatic, limiting their usefulness for basic and pre-clinical research aimed at identifying mechanisms of metastatic disease. We have developed two novel mouse models of LUAD, KPDT-1 and KPDT-2, that feature cell type-specific inducible oncogenic $Kras^{G12D}$, loss of *Trp53* function, and deleted/truncated *Dicer1*. The survival times, rate of tumor progression, and metastatic phenotypes of these models depend on the cell types that harbor the genetic alterations. We performed transcriptomics (mRNA and microRNA) to investigate the molecular and cellular drivers underlying the observed differences in the models.

Methods and Results: KPDT-1 and KPDT-2 models were generated by adding mutations in *Dicer1*, an RNAse III enzyme within the microRNA (miRNA) biosynthesis pathway, to a mouse model of *Kras*-driven LUAD. For KPDT-1, tumorigenesis was induced by conditional expression of an oncogenic $Kras^{G12D}$ allele, deletion of both alleles of *Trp53* and deletion of one allele of *Dicer1* in club cells and the expression of a truncated *Dicer1* allele in alveolar type II (ATII) cells. In KPDT-2 animals, the cell types were reversed. We observed decreased survival, accelerated tumor progression, and enhanced lymph node metastasis only in KPDT-1 animals. Induction of tumorigenesis in ATII cells and truncation of *Dicer1* in club cells (KPDT-2) did not accelerate tumor progression. Differential expression analysis using DESeq2 revealed 206 differentially expressed genes and 45 differentially expressed microRNAs between the two models. Term enrichment analyses showed that genes upregulated in KPDT-1 animals are associated with microtubule movement and organization. The upregulated genes of KPDT-2 animals are enriched in functions related to metabolism. Six microRNAs were upregulated in the KPDT-1 model. These miRNAs were reported previously as being differentially expressed in other cancers, and 4 of them (miR-92a-3p, miR-542-5p, miR-449a-5p, and miR-296-5p) have been linked previously to metastasis.

Conclusions: Through cell type-specific truncation/deletion of *Dicer1*, we have generated a new mouse model that rapidly develops *Kras/Trp53*-driven LUAD and metastatic disease. Our findings suggest that tumorigenesis and metastasis are influenced by miRNA-mediated communication between different cell types. Further investigation into the pathways involved in these models will give more insight into miRNA-mediated tumorigenesis and the underlying mechanisms of metastasis. We will continue to develop these models to support investigation of molecular mechanisms underlying metastasis.

TUMOR BIOLOGY: Organoids
Poster Session

#5187 Characterization and prediction of drug responses in NF1 patient-derived tumor organoids through multiplex phenotypic profiling.

Alberto Mendoza-Valderrey[1], Huyen T. L. Nguyen[1], Jenna Tomkinson[2], Cameron Mattson[2], Michael J. Lippincott[2], Summer Swearingen[1], Sara Gosline[3], Gregory P. Way[2], Alice Soragni[1]

[1]Department of Orthopaedic Surgery, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA,[2]Department of Biomedical Informatics, University of Colorado School of Medicine, Aurora, CO,[3]Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory, Richland, WA

Neurofibromatosis Type I (NF1) is a tumor-predisposing genetic disorder driven by pathogenic variants in the *NF1* gene. The development of neurofibromas, including cutaneous neurofibromas (cNFs) and plexiform neurofibromas (pNFs), constitutes a major challenge of NF1 management. Although cNFs do not carry a risk of malignant transformation, they are associated with significant comorbidities. Furthermore, benign pNFs have a risk of evolving into malignant peripheral nerve sheath tumors (MPNSTs), a rare type of soft tissue sarcoma, in ≈15% of patients. Current therapies for NF1 are limited. To date, there are no systemic treatment options for cNF lesions other than desiccation or surgical removal. For pNF management, selumetininb (a MEK1/2 inhibitor) is the only approved treatment for pediatric patients with inoperable, symptomatic lesions. However, treatment is not curative and requires continuous administration, with toxicity concerns. Lastly, MPNSTs are resistant to most systemic therapies, with surgery being the only available approach for treating localized lesions. NF1-associated tumors present a complex microenvironment that comprise different cell types including Schwann cells, fibroblasts, and mast cells. The identification of effective non-surgical treatments has been severely limited by the rarity of these tumors, their pronounced cellular heterogeneity, and the lack of patient-derived models that accurately replicate these complex characteristics. We have developed patient-derived organoids of both neurofibromas (*Nguyen et al, Cell Reports Methods 2024*) and MPNSTs (*Al Shihabi et al, Cell Stem Cell* 2024) that maintain cellular and molecular features of parental tumors, as novel tractable models for drug discovery studies. Here, we develop a modified, 3D-compatible Cell Painting assay (*Bray et al, Nature Protocols 2016*) to stain cellular structures and provide unbiased phenotypic readouts of NF1 patient-derived tumor organoids. We combined Cell Painting with our high throughput mini-ring organoid screening platform (*Phan et al, Communications Biology 2019; Al Shihabi et al, Science Advances 2022*) to rapidly screen FDA-approved drugs. We are developing a deep-learning based microscopy analysis framework to extract phenotypic features, which we will integrate with omic-based readouts to prioritize candidate therapeutic agents and establish phenotypic biomarkers that predict clinical outcomes, enabling precision medicine approaches for NF1 patients.

#5188 Recapitulating the initiation, progression and functional heterogeneity of Barrett's esophagus using a comprehensive patient-derived organoid model platform.

Lizhe (John) Zhuang, Daniel H. Jacobson, Emily L. Black, Ginny Devonshire, Ahmad Miremadi, Massimiliano Di Pietro, Rebecca C. Fitzgerald

Early Cancer Institute, University of Cambridge, Cambridge, United Kingdom

Barrett's esophagus (BE) is a common condition that increases the risk of esophageal adenocarcinoma (EAC). It originates from gastric cells at the gastroesophageal junction (GEJ) and can progress stepwise from BE to dysplasia and eventually to EAC. However, most BE cases do not progress, suggesting involvement of the tissue microenvironment and other extrinsic factors. BE is a mosaic tissue consisting of diverse clonal units of gastric and/or intestinal phenotypes, but the association between this heterogeneity and progression risk remains unclear. Further investigation is challenging due to a lack of human-based models. Here, we established a comprehensive organoid platform covering the full disease spectrum to study clonal and phenotypic heterogeneity, extrinsic factors, and other cellular components. Methods: We derived organoid cultures from endoscopic biopsies and organ donor tissues and developed a microscopic method to generate single-clonal organoids (sc-organoids) lines, serving as functional proxies of single clones in the original tissue. We comprehensively characterized the molecular features of all organoids and their parental tissues. We also established two models: an aggregated model that co-cultures esophageal fibroblasts with gastric/BE organoids under various pathological conditions; and a labeling-tracking model where BE/EAC organoids were genetically modified with fluorescent nuclear tags for live imaging and tracking. A mathematical model was developed to quantify organoid growth and morphological kinetics. Results: We established 48 organoid lines: 13 non-dysplastic BE, 7 dysplasia, 2 early EAC, and 26 gastric covering the full disease spectrum, recapitulating key molecular hallmarks of stepwise initiation, development, and progression. Transcriptomic analysis indicated that gastric cells at the GEJ are predisposed to BE development due to higher oncogenic stress. We developed 31 sc-organoids from 2 non-dysplastic BE cases and 1 dysplasia case, which captured phenotypic diversity and clonal heterogeneity with some displaying advanced-stage genomic features. The aggregated co-culture model revealed that BE cells promote polarization of fibroblasts into cancer-associated fibroblast-like phenotypes when exposed to refluxate, further enhancing BE's expansion. The labelling-tracking model showed increased BE organoid size and number when co-cultured with EAC, suggesting that cell competition and/or secreted factors alter the balance between precancerous lesions and established tumor. Conclusion: The organoid models provide a valuable platform to study mechanisms underlying precancerous initiation, progression, and neoplastic susceptibility in normal tissues, potentially informing risk stratification and new therapeutic strategies.

#5189 Exploring the clinical utility of HER2-targeted antibody-drug conjugate in urothelial carcinoma using patient-derived models.

Xinran Tang[1], Ziyu Chen[1], Jasmine Thomas[1], Karan Nagar[1], John Christin[2], Sizhi Gao[1], Jiaqian Luo[1], Elisa de Stanchina[1], Jonathan A. Coleman[1], Michael M. Shen[2], Hikmat Al-Ahmadie[1], Gopa Iyer[1], Kwanghee Kim[1], David B. Solit[1]

[1]Memorial Sloan Kettering Cancer Center, New York, NY,[2]Columbia University Irving Medical Center, New York, NY

Introduction. The FDA granted accelerated approval for trastuzumab deruxtecan (T-DXd), a HER2-targeted antibody-drug conjugate (ADC), for the treatment of patients with advanced solid tumors with HER2 3+. However, the role of HER2 in bladder cancer pathogenesis and its utility as a therapeutic target for this disease remains unclear. Here, we characterized a growing biobank of patient-derived organoid (PDO) and xenograft (PDX) models for HER2-targeted kinase inhibitor and ADC sensitivity, with the goal of guiding future therapeutic strategies for patients with bladder cancer.

Methods. We generated and characterized the mutational status and HER2 expression levels of 19 urothelial cancer PDOs and 34 PDXs. A subset of these models was further characterized using a multiplatform approach, including single-cell DNA and RNA seq. We leveraged these models to study the biologic determinants of PDX sensitivity to neratinib and T-DXd. We also studied sensitivity to T-DXd, as well as to its cytotoxic payloads (exatecan) *in vitro* using paired PDO models. To understand the real-world clinical outcomes of T-DXd in metastatic urothelial carcinoma, we analyzed 28 patients treated with T-DXd at Memorial Sloan Kettering to evaluate the therapeutic outcomes and to identify potential biomarkers of sensitivity to HER2-targeted therapies.

Results. Single-cell RNA and DNA seq analysis of 4 PDOs demonstrated high interpatient and intratumoral heterogeneity of HER2 expression and *ERBB2* copy number. Our bladder cancer PDX models also demonstrated greater sensitivity to T-DXd than neratinib, consistent with clinical data in bladder cancer patients. Sensitivity to exatecan, as measured by inhibition of cell proliferation and induction of apoptosis, was more predictive of T-DXd sensitivity than HER2 expression level. 3 patients remained on the treatment for more than 1 year, all of whom had an IHC score of HER2 3+ and *ERBB2* amplification, suggesting HER2 3+ and *ERBB2* amplification are predictive of but not required for clinical benefit with T-DXd.

Conclusion. Multi-omic single-cell analysis demonstrated significant interpatient and intratumor heterogeneity of HER2 expression and *ERBB2* copy number gain. Sensitivity to the T-DXd payload was more predictive of PDX response than HER2 expression levels, with significant tumor regression observed with T-DXd in models with low HER2 expression. Our data suggest that clinical trials of HER2-targeted ADCs should not be restricted to patients with HER2 overexpression and that novel biomarkers will be needed to identify those urothelial cancer patients most likely to benefit from FDA-approved and investigational ADCs. Real-world data from metastatic urothelial carcinoma patients treated with T-DXd suggested that HER2 3+ and *ERBB2* amplification are associated with exceptional response to T-DXd treatment.

#5190 Leveraging the International Space Station to study glioblastoma-immune interactions in microgravity-grown organoids.

Alice Burchett[1], Maksym Zarodniuk[1], Fionn Lay[1], Ina Satpathy[1], Wenjun Zhao[2], Karyme Hernandez Torrens[3], Haley Marco[1], Maria Mendez[1], Julian Najera[1], Shelby Giza[4], Jason Rexroat[4], Paul Kuehl[4], Twyman Clements[4], Meenal Datta[1]

[1]University of Notre Dame, Notre Dame, IN,[2]Smith College, Northampton, MA,[3]University of Puerto Rico Mayaguez, Mayaguez, PR,[4]Space Tango, Lexington, KY

Glioblastoma (GBM) is the most aggressive primary brain cancer with current treatments achieving an average overall survival of less than 2 years. This is due in part to the highly immunosuppressive tumor microenvironment (TME), a characteristic of many solid tumors that thwarts immunotherapy. However, the complex interactions between cancer cells and immune cells are difficult to recapitulate in culture models. Microgravity offers key advantages for cancer organoid development, including the ability to generate more robust, larger, complex, uniform, and reproducible spheroids that recapitulate cell-cell interactions more accurately than ground-based 3-D models, which are subject to gravitational forces leading to spheroid settling, disassociation, and heterogeneity. We sent individual small-scale, static experiments contained within Space Tango's High Sample Capacity CubeLab to the International Space Station (ISS) in March 2024 aboard the SpX-30 commercial resupply mission. We first developed tumor-myeloid organoids to recreate the 3D spatial interactions in the TME. These combine human GBM cells with monocytes and macrophages and can be treated to polarize the macrophages into pro-inflammatory (M1-like) or anti-inflammatory (M2-like) phenotypes. Spheroids of uniform size and tunable cell content were generated using the hanging drop method. We then optimized spheroid size and media conditions to support long-term spheroid growth in the absence of gas or media exchange to be compatible with the carrier system. We observed intact spheroids with viable cells beyond two months in sealed vials. Over time, the organoids reside in an acidic and hypoxic environment, with live cells at the organoid edges and a necrotic central core. This recapitulates the microenvironment of GBM and other tumors, which is known to be acidic, hypoxic, and necrotic. The launched experiment contained spheroids with GBM cells alone, GBM cells + macrophages, GBM cells + monocytes, and GBM + macrophages with M1-like or M2-like polarizing agents. These were maintained in microgravity aboard the ISS for 40 days and were processed within 24 hours of their return to Earth. We collected RNA for bulk sequencing, media for secreted proteomics, and fixed intact spheroids for spatial sequencing, spatial proteomics, and histology. While analysis is ongoing to determine the impact of microgravity on GBM-immune interactions, this experiment has already laid the groundwork for future flights by revealing successful organoid culture conditions for future experiments. We anticipate that these microgravity immune-tumoroids will provide valuable insight into the role of macrophages in GBM biology that would not be possible to obtain in Earth’s gravity. Upcoming microgravity experiments will include more complex organoids from patient-derived glioma cells and the potential for drug screening.

#5191 Modeling the metastatic tumor microenvironment: A co-culture platform for cancer and vascular organoids in drug screening.

M. Lee, Y. Yoon, Y. Lee, J. Rheey;
Gradiant Bioconvergence, Seoul, Korea, Republic of

Patient-derived organoids (PDOs) have emerged as a promising tool for drug screening, as they can closely mimic the genetic profile and heterogeneity of real tumor tissues. Despite these advantages, PDOs cultured in isolation fall short in replicating the complex *in vivo* tumor environment, particularly the critical interactions with the vascular network. Research that neglects these tumor-vascular interactions provides only partial insights into drug responses and the metastatic potential of cancer cells. To address these limitations, we developed a co-culture system of cancer and vascular organoids to better simulate the authentic tumor microenvironment (TME). After four days of vascularized tumor organoid (vTO) culture, RFP-positive lung cancer organoids (LCO) were centrally positioned, encircled by blood vessels, creating an interconnected structure between the LCO and blood vessel organoids (BVO). As the culture period progressed, the LCO began to spread along the vascular network, and by day 10, RFP-positive LCO cells were observed in the peripheral BVO regions. To investigate strategies for limiting metastasis, we targeted the TGF-β pathway, known to play a key role in metastatic progression, within this novel metastatic platform. On day 5, we administered the TGF-β/Smad inhibitor A83-01. Image-based analysis revealed a reduction in LCO dissemination, while quantitative PCR analysis showed a dose-dependent decrease in the expression of genes associated with epithelial-to-mesenchymal transition (EMT) following A83-01 treatment. Additionally, to assess anti-cancer drug responses, we treated the vTO with the AXL inhibitor Bemcentinib. Image analysis indicated that the vTO exhibited higher drug resistance compared to LCO alone, and qPCR analysis confirmed a reduction in metastasis-associated gene expression. Taken together, this innovative co-culture platform, which incorporates metastatic cancer organoids, offers a structurally advanced model that more closely resembles the *in vivo* TME than previous drug screening methods. Therefore, this platform serves as a powerful new tool for studying metastatic cancer and provides a novel approach to evaluating metastasis-inhibiting drugs, new therapeutics targeting metastatic tumors, and combination therapies.

#5192 Next-generation NSCLC drug development powered by PDCs and PDOs: Mimicking real responses.

Sewon Park[1], Min Hak Lee[2], Ju-hyeon Lee[2], Mi Ran Yun[1], Min Hee Hong[3], Sun Min Lim[3], Jii Bum Lee[3], Byoung Chul Cho[3]

[1]Yonsei University College of Medicine, Seoul, Korea, Republic of,[2]JEUK Institute for Cancer Research, JEUK Co., Ltd., Seoul, Korea, Republic of,[3]Division of Medical Oncology, Department of Internal Medicine and Yonsei Cancer Center, Seoul, Korea, Republic of

Purpose : Patient-derived cells (PDCs) and organoids (PDOs) derived from pleural effusion or ascites closely mimic the biological characteristics of individual patients. In cancer research, PDCs and PDOs serve as powerful tools for replicating tumor-specific mutations, overcoming the limitations of traditional cell lines, which fail to capture the heterogeneity and complexity of human tumors. Due to their ability to predict preclinical drug efficacy more accurately, PDCs and PDOs are increasingly utilized in therapy development. This study aims to demonstrate the potential of PDCs and PDOs as effective platforms for screening novel therapeutic strategies for non-small cell lung cancer (NSCLC).

Experimental design : Malignant effusions were samples were tested for malignancy, processed, observed by light microscopy, and cultured. The establishment of a PDC was considered complete when it was free of normal cells, maintained known patient tumor mutation, and recapitulated patient's response. Also, mutations that cell harbors are confirmed by Sanger sequencing, Whole exome sequencing, or RNA-sequencing.

Results : Total of 54 PDCs and 171 PDOs were established from NSCLC patients, including models harboring various EGFR, KRAS and BRAF mutations and EML4-ALK or ROS1-CD74 fusions. Particularly the 8 models of PDC and 7 models of PDO harbor mutations which are well known for resistance mechanisms to EGFR-TKIs (T790M, C797S/C797G, MET amplification). Also, some models of PDCs were harboring uncommon EGFR mutations(EGFR G719S, L861Q, S768I etc.).For example, YUO-139 and YU-1092 are patient derived models that harbors uncommon EGFR mutation G719S and L861Q respectively. The models response to afatinib sensitively. IC_{50} of YUO-139 and YU-1092 to afatinib were 2.1 nM and 23.8 nM respectively. These models were evaluated for novel strategies for targeting uncommon EGFR mutations. Another example is YUO-143, which harbors EGFR mutations E19del/T790M/C797S and was derived from a patient resistant to gefitinib and mavelertinib. YUO-143 was used in the development of the 4th gen TKI, BLU-945, and showed an IC_{50} of 43 nM.

Conclusions : Patient-derived models could serve as a crucial tool in developing novel therapeutic strategies and next-generation drugs for NSCLC.

#5193 Optimization of tumoroid size and payload delivery methods for the engineering of patient-derived tumoroid models.

Isha Dey[1], Xiaoyu Jenny Yang[2], Jacob Delgadillo[2], Garrett Wong[2], **Colin D. Paul**[3], Chris Yankaskas[3], Brittany Balhouse[3], Shyanne Salen[3], Sylvia Beam[3], Amber Bullock[3], Anthony Chatman[3], Pradip Shahi Thakuri[3], Kanika Singh[1], Jason Sharp[2], Matt Dallas[3], David Kuninger[3]

[1]Thermo Fisher Scientific, Bangalore, India,[2]Thermo Fisher Scientific, Carlsbad, CA,[3]Thermo Fisher Scientific, Frederick, MD

Tumoroids, also known as cancer organoids, are three-dimensional (3D) in vitro cell cultures established from primary tissue samples. These next-generation cancer cell models largely recapitulate the spatial organization, gene expression, and mutational profiles of patient tumors. It may be beneficial to engineer tumoroids to generate reporter lines, model specific mutations involved with tumor progression, or modulate gene expression to help understand the mechanism of action of therapeutics. Here, we explore methods for genetic engineering of tumoroids established and cultured in Gibco™ OncoPro™ Tumoroid Culture Medium to facilitate these studies. We first examined the impact of tumoroid size on transfection efficiency via electroporation with the Neon™ NxT Electroporation System. Colorectal tumoroids were transfected with enhanced green fluorescent protein (eGFP) immediately after dissociation or after 4 or 7 days in culture in OncoPro medium, at which point tumoroid size had reached approximately 400 µm in diameter. Nearly 80% of dissociated tumoroids were successfully transfected, while efficiency dropped to 35-40% when using intact 4- or 7-day old tumoroids. Therefore, dissociated tumoroids were used for further experiments. We then compared lipofection, electroporation, and lentivirus-based transduction in a difficult-to-transfect model, focusing on reporter line generation. While lipofection is easy-to-use and cost effective, delivery of enhanced green fluorescent protein (eGFP) by lipofection led to transfection efficiencies of only 15%. Electroporation of eGFP was more efficient, transfecting 30% of tumoroid cells for the tumoroid line used. Given the importance of preserving the cellular heterogeneity found in patient samples and recapitulated in tumoroid models, these lower delivery efficiencies indicate that lipofection- and electroporation-based methods are not ideal for the generation of stably engineered tumoroid models. In contrast, lentiviral transduction at high MOI was the most efficient method for eGFP overexpression, initially transducing 67% of cells and enabling stable pool reporter line generation with nearly identical mutational profile and gene expression compared to the parental population. In follow up experiments, transduction also enabled generation of eGFP-expressing breast and endometrial reporter tumoroid lines. Overall, our results indicate that electroporation may be more suited for transient transfection across a wide range of payloads, and siRNA, gRNA, and Cas9 protein were able to be delivered by electroporation in follow up experiments. Lentivirus-based transduction is more suitable for generation of stable tumoroid pools. The insights gained from this study can be applied to tumoroids derived from various tissues, potentially accelerating advancements in cancer research and personalized medicine.

#5194 Establishment and characterization of tumoroids from patient derived xenograft tissue for in vitro applications.

Jannik Scherer[1], Kanstantsin Lashuk[1], Julia Thiel[2], Meng Dong[2], **Julia B. Schueler**[1]

[1]Charles River Laboratories, Inc., Freiburg, Germany,[2]Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology and University of Tubingen, Stuttgart, Germany

Lung cancer remained the most frequently diagnosed cancer in 2022 and the leading cause of cancer-related deaths worldwide. Oncology drug development attrition is highest compared with other therapeutic areas, and success rates in clinical development range from 3.4-5%. Tumoroids as sophisticated three-dimensional *in vitro* cultures serve as an advanced near-physiological model but lung cancer tumoroid development remains challenging. The treatment strategy for lung cancer patients is currently determined based on histology, stage, and biomarker status, thus we aimed to establish a panel of complex *in vitro* tumoroids covering a broad range of different subtypes of the disease. Two methods of primary cell isolations, five different media formulations and two types of culture methods were assessed to find optimal conditions for tumoroids. Tumoroids were kept in culture for up to 10 passages including freeze-thaw tests. Characterization involved determination of morphology by brightfield microscopy, histology (H&E staining), biomarker expression using multiplex IHC staining and QuantiGene for murine stroma content assessment. Multiple commercial systems that allow uniform organoid formation using microwell technologies were compared. The models in question were mostly of lung adenocarcinoma origin, either “hot” or “cold” in terms of immune cell infiltration status. Tumoroid growth was dependent on the niche growth factor formulation. The highest success rates were achieved with a custom medium formulation (7/8 models). Feasibility of biobanking the organoids either as single cell suspension or whole organoids was confirmed. Different media formulations led to distinct phenotypes and retention of biomarkers in each model. Tumoroids showed signs of cellular plasticity by epithelial to mesenchymal transition characterized by simultaneous expression of EPCAM and aSMA in a subpopulation of cells. The process of de-differentiation during the culture phase has been explored in detail. After model establishment, the models will be tested in a viability assay with several SoC small molecules and in an immune cell co-culture with activated PBMCs and checkpoint inhibitors, to fully establish a platform for drug-screening and evaluation of immunotherapies. This study showcases a complete workflow in tumoroid establishment, highlighting the importance of adequate quality control and validation. Substantial variations of histological characteristics and biomarker expression depending on the culture condition, show the lineage-specific differences and the heterogeneity of the tumoroid. Organoid equivalence to the parental tissue is rarely addressed and this work highlights the impact of standardized culture conditions and thorough characterization of tumoroids to unfold their full potential as drug development platform.

#5195 Development of paired PDX-PDXO preclinical models for HER2-targeting ADC efficacy evaluation.

Jinxi Wang, Jun Zhou, Limei Shang, Qingzhi Liu, Binchen Mao, Yi Sun, Peng Han, Lin Zhang, Likun Zhang, Xiaolong Tu, Wubin Qian, Peng Wang, Leilei Chen, Marten Hornsveld, Marrit Putker, Ludovic Bourre, Jingjing Wang

Crown Bioscience, Inc., San Diego, CA

Introduction: Tumor organoids replicate cancer lesions and capture tumor heterogeneity *in vitro*, and are invaluable for cancer drug development. Combining Patient-Derived Xenograft (PDX) models with corresponding Patient-Derived Xenograft Organoid (PDXO) models offers a comprehensive approach, enabling high-throughput, scalable *in vitro* data collection and providing insights into tumor behavior and drug responses *in vivo*. HER2 amplification is frequent in cancers like breast and gastric cancer, and HER2-targeted ADCs have shown exceptional efficacy. To support preclinical and mechanistic investigations that develop next-generation HER2-targeted ADCs and therapies, we established a preclinical panel of paired PDX-PDXO models with varying HER2 expression levels.

Methods: Gastric (GA) and breast cancer (BR) PDXOs were generated from PDX tissue and authenticated by SNP assay. Morphology, histopathology, and genomic profiling were performed and compared with its corresponding PDX. *ERBB2* expression and HER2 levels in PDX-PDXOs were detected by RNA sequencing and IHC. *In vivo* efficacy of 10 mpk DS8201 was studied in the PDXs, and *in vitro* IC50 was assessed in the PDXOs using CellTiterGlo (CTG) and High Content Imaging (HCI), allowing differentiation between cytostatic and cytotoxic responses. Correlation analysis of DS8201 response were performed based on the efficacy data from PDXOs and PDXs.

Results: 34 GA and 24 BR PDXOs were developed from PDX tumors originally established from patients' biopsies. Hematoxylin-eosin (H&E) staining showed distinct features within PDXOs, similar to the corresponding PDXs. High correlation (>95%) in mutation and gene expression demonstrated that PDXOs largely preserve the genomic and transcriptomic features of the originating tumors. *In vivo* and *in vitro* efficacy studies of DS8201 were performed in 14 pairs of GA and BR PDX-PDXOs, among which 12 pairs display consistent HER2 IHC scores between PDX and PDXO. Overall, 6 out of 7 DS8201-sensitive PDXOs were also sensitive in PDXs and showed tumor regression, indicating high positive predictive value (PPV: 0.857) of PDXOs. The average TGI of PDXs classified for the corresponding PDXO as sensitive is much higher than TGI of PDXs classified for the corresponding PDXO as non-sensitive (97% vs. 69%, p=0.13). Besides, DS82001 sensitivity was observed in both HER+ and HER2- models, which might relate to the bystander effect of the payload and heterogeneity of PDX.

Conclusion: The paired PDX-PDXO models were successfully established and provided a novel preclinical platform for investigating mechanisms of action (MOA) and resistance in patient-relevant models matched in both *in vitro* and *in vivo* stages for developing next-generation HER2-targeting therapies.

#5196 Large scale in vitro expansion of primary tumors from metastasis cancer patients with different mutations for preclinical drug screening.

Dylan Bryda[1], Megan Groves[2], Anthony Tolcher[3], Alex Armento[1], **Seyoum Ayehunie**[1]

[1]MatTek Corporation, Ashland, MA,[2]Next Oncology, San Antonio, TX,[3]NextOncology, San Antonio, TX

The use of patient derived primary tumors (PDPT) is becoming a powerful vitro tool in candidate cancer drug screening for safety and efficacy. Compared to 2D cell lines, the PDPTs can 1) better recapitulate human tumor microenvironment, 2) predict drug responses in humans, 3) be used in high throughput drug screening platforms to test dose responses and drug-drug interactions, and 4) ultimately, be used in precision medicine. However, adoption of PDPTs has been hindered by challenges related to in vitro tumor cell growth and expansion without inducing phenotypic changes. Here, we present a novel system of in vitro PDPT expansion method and biobanking of tumoroids that can be used in high throughput drug screening for metastasis disease. We have combined a specialized matrix coated plates with that of microwell technology to scale-up in vitro tumor cell growth and expansion. In this study we introduced two assay systems for screening of drugs/compounds: a) expanded colorectal cancer (CRC) tumoroids as a stand-alone test system and b) reconstructed complex 3D tumor containing tissues with matrix, fibroblasts, endothelial cells, and immune cells that mimics the tumor microenvironment (TME). To examine the utility of both testing platforms, single tumoroids or tumoroids embedded in 3D tissues were exposed to different doses of 5 chemotherapeutic drugs Cisplatin, Doxorubicin, Oxaliplatin, Fluorouracil, and Cetuximab. Treatment was initiated on day 10 of the culture period. Thereafter, tumoroids were dosed with drugs every other day for 11 days. After each treatment cycle, tumoroids or tumoroid containing tissues were fixed for H&E and live/dead cell staining. The results showed that: 1) large scale expansion of PDPT is achievable, 2) PDPT can be cultured with multiple cell types to simulate the TME, 3) histological evaluation of the tumor models showed a glandular-like tumoroid structure, 4) immunohistochemical staining revealed epithelial origin of the CRC (CK20+), and 5) live-dead cell staining of tumoroids showed differential drug responses in a dose and time dependent manner. Additionally, a by-standard cell death effect was noted for some of the drugs. In summary, large-scale in vitro tumoroid expansion is critical to scale up metastasis cancer drug screening. The developed assay systems are more physiological, cost effective, less time-consuming, better predictive of drug responses in humans, can lead to precision medicine, and reduce animal use for experiments.

#5197 Establishing patient-derived xenograft organoids (PDXOs) from renal cell carcinoma for drug screening and identification of treatment-induced vulnerabilities.

Stefano Piatto Clerici[1], Adriano O. Beserra[1], Stephania M. Bezerra[2], Luciana M. Leite[3], Stenio C. Zequi[4], Tiago G. Santos[1], Vilma R. Martins[5]

[1]International Research Center, A.C. Camargo Cancer Center - Brazil, Sao Paulo, Brazil,[2]Department of Anatomic Pathology, A.C. Camargo Cancer Center - Brazil, Sao Paulo, Brazil,[3]Department of Medical Oncology, A.C. Camargo Cancer Center - Brazil, Sao Paulo, Brazil,[4]Reference Center of Urology, A.C. Camargo Cancer Center - Brazil, Sao Paulo, Brazil,[5]Aljava Biotech, A.C. Camargo Cancer Center - Brazil, Sao Paulo, Brazil

Clear Cell Renal Cell Carcinoma (ccRCC) is the most prevalent urinary kidney malignancy, presenting significant challenges in disease control, particularly for patients with locally advanced or metastatic disease. The urgent need for more effective therapies remains unmet. Patient-derived models, including 3D patient-derived organoids (PDOs) and *in vivo* patient-derived xenografts (PDXs), faithfully recapitulate tumor histological morphology, genetic landscape, and therapeutic responses. The ACCamargo Cancer Center PDX Biobank includes several ccRCC cases and comprehensive patient clinical data supporting the generation of PDX organoids (PDXOs) for high-throughput drug screening and The present work aims to a) establish and validate ccRCC PDXO as predictive models for standard-of-care treatments and b) investigate drug vulnerabilities and synthetic lethality approaches in BAP1-mutated tumors. Twelve PDX samples (fresh and cryopreserved) were enzymatically digested, resuspended, seeded in growth factor-reduced Geltrex®, and cultured in a kidney organoid medium. PDXOs were assessed using histology and immunohistochemistry. Cell viability assays evaluated six PDXOs' responses to Sunitinib (one of the stand-of-care drugs in RCC). Results were correlated with patients' clinical outcomes for three cases of PDXOs with BAP1 mutations ($BAP1^{MUT}$) and wild-type BAP1 ($BAP1^{WT}$) were treated with Olaparib (Poly(ADP-ribose) polymerase (PARP) inhibitor) and Vorinostat (Histone deacetylase (HDAC) inhibitor). The success of PDXOs' growth and expansion efficiency, at approximately 60%, proves our research's reliability. Histology and immunohistochemistry c confirmed that five PDXOs and PDX matched the characteristics of the corresponding patient tumors. PDXOs treated with Sunitinib exhibited varied responses. In three out of the five cases, the sensitivity of PDXOs to Sunitinib aligned with the clinical outcomes of the respective patients. Preliminary data indicated that $BAP1^{MUT}$ PDXOs were more sensitive to Olaparib and Vorinostat treatments than $BAP1^{WT}$ PDXOs. The study demonstrated the predictive power of PDXOs in correlating therapeutic responses to Sunitinib with clinical outcomes. BAP1 mutations in PDXOs also revealed drug vulnerabilities, highlighting increased sensitivity to Olarapib and Vorinostat. These findings underscore the value of PDXOs as robust tools for predicting patient responses to therapy and exploring target treatments for ccRCC.

#5198 Semi-automated, scaffold-free organoid workflow for the T cell screening assay using AI.

Zhisong Tong[1], Angeline Lim[1], Oksana Sirenko[1], Jia-Yang Chen[2], Hao-Wei Han[2], Ying C. Chang[2]

[1]Molecular Devices, LLC (Moldev), San Jose, CA,[2]AcroCyte Therapeutics Inc., New Taipei, Taiwan

Among the therapies for treating cancer, immunotherapy—especially chimeric antigen receptor (CAR) T-cell therapy—is increasingly popular. CAR T cells are genetically altered T cells that when injecting back into patients, they help arm the immune system to target and destroy cancer cells. Though it has achieved much success with immunotherapy in treating blood cancers, it remains challenging in targeting solid tumors due to the intrinsic solid tumor microenvironment (TME) which suppresses the tumor-killing ability of T cells. Thus, TME plays an important role in the treatment of solid tumors and finding an approach of mimicking TME in vitro is essential for Car-T cell screening. Studies show that the 3-dimensional (3D) patient-derived organoids (PDO) model more of the physical and chemical cues present within the TME that are lacking in traditional 2D cell line cultures. They also show similar responses to drugs as original tumors, suggesting the value of using PDOs to improve therapeutic outcomes. Despite the benefits associated with the use of PDOs, there are significant barriers that hinder the widespread adoption of PDOs in drug discovery, i.e., the costly and highly labor-intensive processes associated with their growth and maintenance. To address the challenges associated with the use of PDOs in large-scale applications, a semi-automated bioreactor was developed for the large-scale expansion of assay-ready organoids. Further, to free the burden of using Matrigel for organoid culture in the assay, we developed a Matrigel-free organoid workflow to quantify the efficacy of the cells in solid tumors, automatically seeding and culturing PDOs in a novel nano film coated cell culture plate enabling spontaneous scaffold-free 3D organoid formation using a fully integrated cell culture system. Using bioreactor-expanded patient-derived colorectal cancer organoids (CRCs), activated human peripheral blood mononuclear cells (PBMCs) were added to CRCs in a 96-well plate and monitored every 1 hour for 3 days. To quantify the morphological changes induced by T cells, we first segment each organoid with the transmitted light (TL) channel using a deep-learning model and extracted morphological features from each organoid. The resulting features were then classified using a trained Random Forest model that differentiates the morphologically modified organoids from the intact organoids. Using this approach, we found that—compared to control wells—the percentage of modified organoids in stimulated PBMC-treated wells increases more rapidly, suggesting a potential live T cell efficacy assessment approach with or without labeling. The results demonstrate the utility of the scaffold-free organoids with this analysis approach in large-scale T cell screening.

#5199 Enhancing precision medicine in advanced lung, gastric and rectal cancer: Exploring the reliability of Patient-derived organoids.

Hye Ryeong Jun[1], Shin Young Chang[1], Hyoyoung Kim[2], Hyunki Kim[3], Minkyu Jung[4], Hye Ryun Kim[4], Tae Il Kim[5], Jung Eun Kim[1]

[1]Department of precision medicine, PODO Therapeutics, Seongnam, Korea, Republic of,[2]Department of Internal Medicine, Yonsei Cancer Center, Yonsei University College of Medicine, Seoul, Korea, Republic of,[3]Department of Pathology, Yonsei University Health System, Yonsei University College of Medicine, Seoul, Korea, Republic of,[4]Division of Medical Oncology, Department of Internal Medicine, Yonsei Cancer Center, Yonsei University College of Medicine, Seoul, Korea, Republic of,[5]Division of Gastroenterology, Department of Internal Medicine, Yonsei Cancer Center, Yonsei University College of Medicine, Seoul, Korea, Republic of

Introduction: A significant challenge in cancer treatment is the development of effective therapies, particularly for patients with recurrent and advanced-stage cancers. Major barriers to successful treatment include the identification of druggable targets and the exploration of novel drug modalities. Investigating strategies for effective combination regimen with standard therapies or immuno-oncology (IO) agents is crucial for optimizing treatment sequences. Traditional clinical trials are resource-intensive and time-consuming; in contrast, patient-derived organoids facilitate the efficient testing of tailored combination therapies specifically for recurrent and metastatic patients.
Method: We employed organoid cultures derived from patients with advanced lung, gastric and rectal cancer to assess their responses to a range of anticancer drugs, including standard agents, IO therapies, and radiotherapy. Drug responses and radiosensitivity data were correlated with matched clinical data, and metrics such as predictive accuracy, sensitivity, and specificity were calculated. The organoids were validated as reliable models reflecting clinical cancer behaviors, ensuring their relevance for evaluating treatment responses.
Results: We investigated drug and radiation responses, molecular marker expression, and dynamic caspase imaging of patient-derived organoids, validating these findings in conjunction with clinical data. Our analysis revealed a significant correlation between organoid based in vitro responses and clinical observations, suggesting potential pathways for personalized treatment strategies in advanced cancer settings. The distinct in vitro response patterns observed in organoids align with clinical experiences, underscoring the importance of optimizing treatment sequences and evaluating novel drug modalities alongside immunotherapy.
Conclusion: This research highlights the efficacy of patient-derived organoids (PDOs) in evaluating treatment responses, thereby contributing to the advancement of precision oncology for patients with advanced lung, gastric and rectal cancer. The findings underscore the importance of using PDOs as reliable preclinical models to tailor therapeutic strategies based on individual patient profiles. Future studies should focus on leveraging organoid models to optimize combinations of targeted therapies, radiotherapy and immuno-oncology agents, ultimately aiming to enhance clinical outcomes and personalize treatment approaches for advanced stage cancers.

#5200 Patient-derived esophageal squamous cell carcinoma organoids and analysis of MDR1 expression levels in clinical specimens for promoting personalized medicine.

Yasushige Takeda[1], Kazuhiro Noma[1], Hajime Kashima[1], Akito Shimizu[1], Tasuku Matsumoto[1], Seitarou Nishimura[2], Tatsuya Takahashi[1], Hijiri Matsumoto[1], Kento Kawasaki[3], Tomoyoshi Kunitomo[1], Naoaki Maeda[1], Satoru Kikuchi[1], Shunsuke Tanabe[1], Toshiaki Oohara[1], Hiroshi Tazawa[1], Hiroshi Nakagawa[3], Toshiyoshi Fujiwara[1]

[1]Gastroenterological surgery, Okayama Univ. Graduate School of Med., Dentistry & Pharm. Sci., Okayama, Japan,[2]Surgery, Fukuyama City Hospital, Fukuyama, Japan,[3]Herbert Irving Comprehensive Cancer Research Center, Columbia University Irving Medical Center, New York, NY

Background
Esophageal cancer is the 7th leading cause of cancer-related mortality as of 2022, with esophageal squamous cell carcinoma (ESCC) comprising 90% of cases in Japan. The Japanese Clinical Oncology Group (JCOG) reported that preoperative DCF therapy (Docetaxel + Cisplatin + 5-Fluorouracil) improves prognosis over CF therapy (Cisplatin + 5-Fluorouracil). However, some patients exhibit resistance to DCF therapy, leading to recurrence or metastasis post-surgery. Tumor heterogeneity contributes to these outcomes. MDR1, a multidrug resistance protein, shows variable expression levels, and cancer tissues are composed of tumor cells alongside diverse microenvironmental components. To address this, accurate models are essential. Recently, three-dimensional patient-derived organoids (3D-PDOs) have gained attention as cost-effective platforms replicating cancer heterogeneity.
Method
3D-PDOs were derived from pre-treatment ESCC biopsy specimens. Their histological morphology was compared with clinical specimens, and responses to Docetaxel, Cisplatin, and 5-Fluorouracil were assessed. Additionally, 78 ESCC cases treated with preoperative DCF therapy followed by surgery (2012-2018) were analyzed. Immunohistochemistry (IHC) was performed on biopsy specimens to evaluate MDR1 expression and its association with prognosis.
Results
3D-PDOs formed three-dimensional epithelial aggregates. ESCC PDOs exhibited irregular edges compared to the spherical, well-defined shapes of normal PDOs. IHC revealed protein expression variability in 3D-PDOs, reflecting clinical specimen heterogeneity. Expression trends in PDOs matched those in clinical samples, demonstrating their histological accuracy. In one case, a therapeutic experiment with Docetaxel, Cisplatin, and 5-Fluorouracil showed significant efficacy for Docetaxel and Cisplatin but resistance to 5-Fluorouracil. MDR1 analysis in biopsy specimens indicated MDR1-positive cases had significantly shorter recurrence-free survival than MDR1-negative cases, though no significant difference was observed in 5-year overall survival. MDR1 IHC on 3D-PDOs used in treatment experiments confirmed MDR1 expression, consistent with observed 5-FU resistance and suggesting a high recurrence risk in this case.
Conclusion
3D-PDOs are valuable for analyzing histological features and therapeutic resistance in heterogeneous ESCC cases. MDR1 analysis demonstrated potential utility in predicting postoperative recurrence risk, supporting its role in personalized treatment strategies.

#5201 Modeling human mammary epithelial lineages using patient-derived organoid cultures.

David A. Rowbotham, Samantha Rothwell, Sharon A. Louis, Allen C. Eaves, John Stingl

Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada

Organoids have emerged as a powerful model for studying normal and tumor cell biology; however, the application of organoid technology for culturing human breast epithelial cells has been challenging due to the difficulty in maintaining estrogen receptor (ER)-expressing cells in culture. To address this problem, we have developed MammoCult™ Organoid Kit (Human), a modular serum- and phenol red-free cell culture kit that promotes the growth of multilineage organoids, as well as organoids composed solely of ER+ and ER- luminal or basal cells. Primary human breast cells from reduction mammoplasty tissues from both pre- and postmenopausal donors were dissociated and seeded in 3D extracellular matrix domes, as either single cells or tissue fragments. Organoids were typically dissociated and passaged every 10 - 20 days. Cultures were analyzed by flow cytometry and immunocytochemistry for the expression of the pan epithelial marker EpCAM, the luminal markers Keratin 8 (K8), KIT, ER, MUC1, progesterone receptor (PR), and the basal markers CD49f, smooth muscle actin (SMA), and K14. Organoid cultures initiated from single cell suspensions generated distinct organoid morphologies reflective of their cell of origin. Luminal cells generated small hollow cystic organoids composed predominantly of cells that expressed K8, MUC1, and variable levels of expression of ER, PR, and KIT (n = 6 - 12). Notably, ER expression was maintained in luminal organoids for a minimum of 5 passages when split at a 1:3 to 1:5 split ratio (n = 3). Basal cells seeded in Corning® Matrigel® supplemented with collagen I (2 mg/mL) formed terminal ductal lobular unit (TDLU)-like branching structures that were composed solely of K14+SMA+ basal cells and could be passaged for a minimum of 2 passages when co-seeded with human mammary fibroblasts (n = 3). Multilineage organoids composed of both luminal and basal cells could be generated when cell aggregates or tissue fragments were initially seeded (n = 5). These results demonstrate that the MammoCult™ Organoid Kit (Human) offers a robust, in vitro model system for studying human mammary gland biology.

#5202 Clinical, molecular, and functional characterization of a diverse collection of patient-derived colorectal cancer organoids from the Human Cancer Models Initiative.

Matthew Graziano, Stephen Friend, James Clinton, Changsuk Moon, **Carolina Lucchesi**

Microphysiological System Department, ATCC, Manassas, VA

Background: In the United States, colorectal cancer (CRC) is the third most common cause of cancer-related mortality and is second in mortality rate. There is a need for clinically representative, in vitro CRC models that reflect the genomic and phenotypic diversity of disease seen in patient populations. The Human Cancer Models Initiative (HCMI) is a collaboration between the National Cancer Institute, Cancer Research UK, Wellcome Sanger Institute, Hubrecht Organoid Technology, and ATCC that has generated hundreds of novel patient-derived cancer models that are supported with clinical and molecular annotation. These models were manufactured, characterized, and validated at ATCC and are available for academic and commercial use to support cancer research and drug discovery.
Methods: CRC organoids available in the ATCC collection of HCMI organoid models (n=70 unique models, n=67 unique donors) were subjected to whole-exome sequencing. Models were derived from primary tissues including colon, rectosigmoid junction, and rectum across 10 different anatomical sites. Data were compared to publicly available clinical and genotyping results from the tumor of origin and large cancer datasets such as The Cancer Genome Atlas (TCGA). A subset of models was tested for sensitivity to a bespoke panel of compounds (n=24) that included drugs in clinical use, chemotherapeutics, and experimental compounds with a variety of mechanisms of action and molecular targets. Models were tested with a 12-point, half log dilution dose curve and drug sensitivity was calculated as the half-maximal inhibitory concentration (IC50). Post-treatment cultures were stained with live- and dead-cell fluorescent dyes and a luminescent ATP viability assay to assess response.
Results: CRC organoids from the HCMI were predominantly derived from primary tumors (83%) with an adenocarcinoma histological type (94%). Models had mutations in disease-relevant driver genes including APC, TP53, KRAS, PIK3CA, BRAF, ARID1A, GNAS, and SMAD4 at frequencies similar to the TCGA COAD cohort. Drug sensitivity testing revealed donor to donor variability in response to targeting compounds, which supplemented with WES data may suggest variant specific sensitivity to compounds of interest.
Conclusion: Patient-derived CRC organoids from the HCMI recapitulate genotypes seen in patient populations and respond to therapeutic treatment in vitro.

#5203 Patient derived organoids capture heterogeneity and uncover therapeutic opportunities in esophageal adenocarcinoma.

Dylan Peter McClurg[1], Kayli Bolton[1], Christopher M. Jones[1], Daniel Jacobson[1], Ahmad Miremadi[2], Ginny Devonshire[3], Xiaodun Li[1], Sriganesh Jammula[1], Adrienn Blasko[1], Rebecca C. Fitzgerald[1]

[1]Early Cancer Institute, University of Cambridge, Cambridge, United Kingdom,[2]Department of Histopathology, Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom,[3]Cancer Research UK Cambridge Institute, Cambridge, United Kingdom

Introduction: Esophageal adenocarcinoma (EAC) is a hard-to-treat cancer that contributes to considerable morbidity and mortality. A major treatment challenge is the inherent disease heterogeneity. We sought to recapitulate this *in vitro* using a comprehensive organoid platform to allow correlation with genomic driver events, including extrachromosomal circular DNA (ecDNA), and response to systemic therapies and radiation.

Methods: A total of 48 patient-derived organoids (PDOs) and their matched parental tissue were characterized using clinical annotations, whole-genome sequencing (WGS), and RNA sequencing. Additional phenotypic characterization was undertaken via immunohistochemistry (IHC) for key protein markers. Ten EAC organoids with distinct genomic driver events were selected for in-depth analysis, including evaluation of ecDNA events using IHC, Fluorescence in situ hybridization (FISH), and AmpliconArchitect tool, as well as characterization of sensitivity to chemotherapeutics and radiation. PDO cultures were treated with five anticancer compounds as single agents based on genomic drivers and GI_{50} values were calculated. Radiation sensitivity was assessed by exposure to a single fraction of 0-8Gy x-ray irradiation and subsequent evaluation of organoid forming efficiency (OFE) using an IncuCyte.

Results: The organoid platform successfully captured the heterogeneity of EAC, replicating key histological and molecular features observed in matched patient samples. WGS analysis demonstrated that EAC organoids reflect the heterogeneous genomic landscape of their primary tumor including point mutations, copy number alterations, structural variations, mutational signatures, and driver alterations. FISH analysis and AmpliconArchitect confirmed the presence of ecDNA events leading to focal amplification of up to 41 copy numbers of functionally relevant genes with corresponding RNA expression. HER2 amplified organoids were confirmed through IHC and FISH analysis. In addition, IHC staining for CDK6 demonstrated biomarker potential of ecDNA events. Therapeutic sensitivities were observed to CDK4/6 (GI_{50} < 10 μM) CDK2 (GI_{50} < 0.03 μM), HER2 (GI_{50} 1-10 μM), MEK (GI_{50} 0.3 - 10 μM) and mTOR (GI_{50} 0.3-10 μM) inhibitors. The most sensitive of responses were seen in the CDK2 inhibitor, with most organoids responding (n = 6). Putative ecDNA events were found to confer sensitivity to predicted targeted compounds and, in some cases, showed a greater response than nuclear amplification of the same oncogenic driver. Significant variation in OFE was seen in response to x-ray irradiation, highlighting novel molecular determinants of radiation response.

Conclusion: Our organoid platform captures the molecular and phenotypic heterogeneity of EAC and provides a platform in which to identify novel molecular determinants of therapeutic response.

#5204 Morphology-guided classification of oral cancer using patient-derived organoids and its therapeutic implications.

Mi Rim Lee[1], sumin Kang[2], Jonghyun Lee[3], Sun-Young Kong[4], Yu-Sun Lee[1], Hye Won Shon[1], Gyeongmin Kang[2], Jiyoung Lee[1], Suk Min Youn[5], Da Woon Kwack[5], Joo Yong Park[5], Soung Min Kim[6], Wonyoung Choi[7], Jong-Ho Lee[5], Dongkwan Shin[3], Ik-Jae Kwon[6], Sung-Woen Choi[5], Yun-Hee Kim[1]

[1]Molecular Imaging Branch, Division of Convergence Technology, National Cancer Center - Korea, Goyang-si, Korea, Republic of,[2]Department of Cancer Biomedical Science, National Cancer Center Graduate School of Cancer Science and Policy, Goyang-si, Korea, Republic of,[3]Bioinformatics Branch, Division of Cancer Data Science, National Cancer Center - Korea, Goyang-si, Korea, Republic of,[4]Department of Laboratory Medicine, National Cancer Center - Korea, Goyang-si, Korea, Republic of,[5]Oral Oncology Clinic, National Cancer Center - Korea, Goyang-si, Korea, Republic of,[6]Department of Oral and Maxillofacial Surgery, Seoul National University Dental Hospital, Seoul, Korea, Republic of,[7]Center for Clinical Trials, National Cancer Center - Korea, Goyang-si, Korea, Republic of

Oral cancer is an aggressive malignancy with a survival rate below 50% and a high rate of locoregional recurrence in advanced stages. The treatment method for patients with oral cancer is highly dependent on surgery and radiation, because there is still no therapeutic target due to the low mutation rate and the absence of molecular subtypes due to high tumor heterogeneity. Patient-derived organoid (PDO) is a good model that can overcome the limitations in translational study of oral cancer, as they reflect the characteristics of patient tissues at a high level and serve as a valuable preclinical model.In this study, we classified oral cancer into three subtypes based on patient-derived organoid (PDO) morphology and proposed new treatment strategies based on subtype-specific mutations and -transcriptional characteristics. First, we classified OSCC organoids into three distinct morphologies from successfully established 77 cancer and 81 normal oral cancer PDO libraries: normal-like, dense, and graph-like. Morphological subtypes were consistent with genomic mutations and transcriptome characteristics. Morphological subtypes were correlated not only with genomic mutations and transcriptomic characteristics, but especially with clinical recurrence prognosis. Patients with normal-like had a low mutation burden and a good prognosis, whereas patients with dense or grape-like had many mutations and a poor prognosis for recurrence. As a result of evaluating 14 drugs by organoid subtype in a high-throughput drug evaluation system using a 384-well plate, normal-like or grape-like organoids showed a good response to single drug treatment. We observed that dense organoids, which exhibited resistant responses to single drugs, showed an increased synergistic effect when co-treated with the first-line standard chemotherapy drug, cisplatin, and the ATR inhibitor (bay1895344), compared to other subtypes.

#5205 ECM-free patient-derived organoids preserve diverse prostate cancer lineages and uncover in vitro-enriched cell types.

Robin Dolgos[1], Romuald Parmentier[1], Jing Wang[1], Raphaelle Servant[1], Arnoud J. Templeton[2], Tobias Zellweger[3], Alastair Lamb[4], Kirsten Mertz[5], Svetozar Subotic[6], Tatjana Vlajnic[7], Helge Seifert[8], Ashkan Mortezavi[8], Cyrill A. Rentsch[8], Lukas Bubendorf[7], Clementine Le Magnen[1]

[1]Institute of Pathology, Department of Urology, Department of Biomedicine, University Hospital Basel, University of Basel, Basel, Switzerland,[2]Division of Medical Oncology, Faculty of Medicine, St. Claraspital, University of Basel, Basel, Switzerland,[3]Department of Urology, St. Claraspital, Basel, Switzerland,[4]Nuffield Department of Surgical Sciences, Department of Urology, University of Oxford, Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom,[5]Institute of Pathology, Kanton Baselland Liestal Hospital, Liestal, Switzerland,[6]Clinic for Urology, Kanton Baselland Liestal Hospital, Liestal, Switzerland,[7]Institute of Pathology, University Hospital Basel, University of Basel, Basel, Switzerland,[8]Department of Urology, University Hospital Basel, Basel, Switzerland

Background: Patient-derived organoids (PDOs) offer new opportunities to model various cancers. However, their application in prostate cancer (PCa) has been hampered by poor success rates and overgrowth of cell types which are not representative of the patient samples. Here, we aimed at modulating culture conditions and investigating *in vitro*-associated cellular heterogeneity to identify refined PCa PDO culture conditions.

Methods: A cohort of 164 diverse samples was obtained from 162 PCa patients undergoing radical prostatectomy, prostatic biopsies, transurethral resection of the prostate or the bladder or metastasis biopsy/resection. Out of this cohort, PDOs were established in various extracellular matrices (ECM: Matrigel, Collagen I, Collagen IV, Laminin-I, or no ECM) and medium compositions. PDOs were characterized using IF, IHC, and FISH. Single-cell RNA sequencing (scRNA-seq) was performed on 11 tumor and organoid samples, followed by unsupervised clustering, trajectory inferring, cell type annotation, and CNV analysis. Published spatial and bulk transcriptomic data were re-analyzed to validate specific markers. A prostate PDO scRNA-seq atlas was generated by integrating three published PDO datasets (Huang 2023, Song 2022, McCray 2019) together with our newly-generated data.

Results: Out of 5 ECM conditions, an ECM-free culture system increased the take-rate of PDOs with luminal-like ($CK5^-$, $CK8^+$, AR^+) and PCa features ($AMACR^+$, $PTEN^-$), while standard Matrigel-based culture conditions yielded basal-like benign organoids ($CK5^+$, AR^-, $AMACR^-$). Cellular composition of organoids generated in ECM-free conditions differed significantly from those generated in Matrigel, with minimal overlap observed in their transcriptomic profiles. ECM-free PDOs comprised cell populations associated with known PCa signatures, exhibited transcriptomic resemblance with their respective parental tumors, and maintained patient-specific epithelial populations. Furthermore, we defined organoid cell type signatures and identified markers discriminating between several types of benign cells (basal, hillock, club, transitioning) *versus* tumor cell populations *ex vivo* and *in situ*. Finally, our integrative single-cell atlas highlighted that Matrigel-based organoids derived from primary PCa are essentially composed of benign epithelial cells, irrespective of the dataset or the malignant nature of the tissue of origin. In contrast, ECM-free conditions preserved heterogenous tumor-like cell populations with higher inferred copy-number variations and were enriched in intermediate club-like cell populations.

Conclusions: ECM-free PCa PDOs exhibit improved fidelity and *in vitro*-enriched cell populations, while aberrant benign lineage programs are associated with Matrigel culture. Our work contributes to significantly enhancing the potential of PDOs in PCa research.

#5206 ADC efficacy and safety evaluation based on organoid models.

Yuhui Wang, Junwen Zhang, Jiawen Xu, Lin Feng, Junjie Zhu, Pengwei Pan, Fang He

Pharmaron Beijing Co., Ltd., Beijing, China

Introduction: Antibody-drug conjugates (ADCs) are effective cancer therapies that selectively deliver cytotoxic payloads to tumors. For the discovery of new ADCs, *in vitro* efficacy testing with organoids is crucial, as they replicate the 3D structure of tumors, providing a more accurate model for assessing the complex mechanism of action of ADCs. In this poster, we present an organoid-based ADC testing platform suitable for high-throughput screening. We demonstrate its application through a case study targeting human epidermal growth factor receptor 2 (HER2) to predict ADC efficacy and safety.

Method: We established multiple PDX-derived organoid (PDXO) models from different cancer types with low, medium or high expression levels of HER2, which we verified by RNA-seq and immunohistochemistry. Responses to four groups of HER2-targeted ADC drugs were evaluated in these models by assessing cell viability, including Trastuzumab deruxtecan, Trastuzumab duocarmazine, Trastuzumab emtansine, Trastuzumab MMAE, and their corresponding payloads (with or without linker). Furthermore, we conducted additional safety studies to assess the intestinal toxicity of these drugs using human intestinal organoids (HIO) derived from normal intestinal tissues.

Results: The HER2 expression levels in the *in vitro* cultured PDXO models were almost consistent at both RNA and protein levels, and they accurately reflected the expression status of the original tissues. The ADC screening revealed a differential sensitivity of organoids based on HER2 expression levels, with high HER2 expression correlating with increased sensitivity to ADC treatment, while low expression indicated resistance. Interestingly, normal organoid models were insensitive to ADC treatment, suggesting a potential for minimal side effects. However, the payloads alone demonstrated strong, unbiased cytotoxic activity against all models. In addition, the cytotoxic efficacy of payloads connected to linkers was significantly reduced, likely because the linkers hindered the payloads from entering the cells.

Summary: Our organoid-based ADC screening platform offers a rapid and reliable method for early high-throughput screening, pharmacology, and efficacy research that help bridge the gap between in vitro and in vivo studies. The translational results enhance the development process of ADCs through more accurate assessments of their potential effectiveness and safety.

#5207 Organoid-based drug screening identifies CDK9 as a target for stratified treatment of high-grade serous ovarian cancer.

Wojciech Senkowski[1], David Fontaneda-Arenas[1], Yilin Li[2], Kari A. Lavikka[2], Aura Crispino[1], Jakub Červinka[1], Francesca Gorini[1], Kyriaki Driva[1], Laura Gall-Mas[1], Lidia Moyano-Galceran[1], Jaana Oikkonen[2], Yan-Jun Chen[1], Karolin Vo?grone[1], Elin Pietras[1], Jutta Huvila[3], Kaisa Huhtinen[2], Johanna Hynninen[4], Sampsa Hautaniemi[2], Krister Wennerberg[1]

[1]University of Copenhagen, Copenhagen, Denmark,[2]University of Helsinki, Helsinki, Finland,[3]University of Turku, Turku, Finland,[4]Turku University Hospital, Turku, Finland

High-grade serous ovarian cancer (HGSC) is the most common and lethal ovarian cancer type. Despite favorable response to first-line therapy, most HGSC patients eventually relapse. HGSC presents few recurrent mutations that could be used as therapeutic biomarkers and hence, the progress with genomic-based precision HGSC treatment has been limited. We therefore took a functional precision medicine approach and asked whether we could identify patient-specific vulnerabilities of refractory HGSC using patient-derived organoids. We have previously established a collection of HGSC organoids[1], which is currently being expanded to a publicly accessible biobank of >100 stable and expandable cultures (OvaCure Collection, ovacurecollection.com). We also demonstrated that organoid drug responses correlate with clinical treatment outcomes[1].

Herein, in organoids from several HGSC patients, we performed a large-scale drug-response profiling of a library of 370 compounds. Of these, we found that inhibitors of CDK9 (CDK9i), but not other CDKs, demonstrated pronounced activity in subset of patients. CDK9i have not yet been thoroughly assessed for the treatment of HGSC, and only *MYC* overexpression has been considered as a sensitivity biomarker[2]. To explore the CDK9i responses in HGSC, we profiled 18 organoid cultures against selective CDK9i (AZD4573, VIP-152, NVP-2 and KB-0742). We observed a range of CDK9i-sensitivities in the patient cohort, from resistant cultures to exceptional responders. As all organoids have been previously characterized with whole genome- and RNA sequencing, we assessed genetic and transcriptomic differences between models to identify potential biomarkers of CDK9i sensitivity. *MYC* expression, biomarker of sensitivity in previous CDK9i clinical trials, did not correlate with CDK9i sensitivity in organoids (Pearson r = 0.244, p=0.346), suggesting its poor predictive value. However, we identified a set of genes, expression of which closely correlated with sensitivity (36 genes with Pearson r>0.7) or resistance (84 genes with Pearson r<-0.7) to CDK9i. For instance, expression of phosphodiesterase 3B (*PDE3B*) was correlated with CDK9i resistance (Pearson r = -0.744, p<0.001), while expression of *ERBB3* was correlated with increased CDK9i sensitivity (Pearson r = 0.841, p<0.0001). Taken together, we demonstrate the application of an HGSC organoid biobank for identifying patient-specific targets, exceptional responder groups and potential CDK9i sensitivity biomarkers in HGSC. Results of this project could serve as a basis for designing a molecularly stratified CDK9 inhibitor-focused clinical trial in refractory HGSC patients.

1. Senkowski et al. A platform for efficient establishment and drug-response profiling of high-grade serous ovarian cancer organoids. *Developmental Cell* (2023)

2. https://clinicaltrials.gov/study/NCT04718675

#5208 The use of human small intestinal organoids as a preclinical screening tool to assess gastrointestinal toxicity induced by CDK inhibition.

Frida Ponthan, Thomas Kedward, Valentina Ubertini, Gary Beale, Cath Booth

Epistem Ltd., Manchester, United Kingdom

Dysregulated cell division is one of the most important hallmarks of cancer. Cyclindependent kinases (CDKs), involved in cell cycle regulation, have been identified to be overexpressed in many cancers. The development of CDK inhibitors has emerged as a promising and novel approach for cancer treatment in both solid and hematological malignancies. However, first generation pan-CDKIs, which initially showed reliable results *in vitro* and in Phase I trials, lacked selectivity. Many were discontinued due to adverse effects, including Gastrointestinal (GI) toxicity, and reduced efficacy *in vivo*. The development of specific CDKIs, which can provide optimum therapeutic outcomes with good efficacy and reduced off-target effects are therefore needed.

In this study, we have used a human small intestinal organoid model (adapted from Sato *et al*. 2009), as a screening tool to predict GI toxicity and subsequent mucosal regeneration. The culture conditions have been designed to mimic the stem cell niche and allow cell differentiation and proliferation to occur. All intestinal lineages were present in the organoids and resembled the frequency of linage observed *in vivo*.

The effect of several clinically available CDKIs, such as Dinaciclib, Palbociclib and Abemaciclib, some of them known to induce GI toxicity were assessed. Treatment was applied on Day 3 after plating of fragments, when the organoid structures had formed. On Day 5, the number of dead, viable unbranched and branched organoids were counted and IC50 values were calculated. Furthermore, morphometric analysis of acquired images were performed. To compare the toxicity profiles on a molecular level, organoids were harvested for RNA-seq.

The second generation pan-CDK inhibitor Dinaciclib was the most toxic. Whereas Palbociclib was the least toxic compound within the human organoid assay, reflecting clinical data that shows the lowest incidence of severe diarrhea.

We conclude that organoids are a predictive preclinical model that can be used to identify potential on-target, off-tissue GI toxicities induced by novel therapeutics such as CDKIs. Toxicity and mechanism of action (MOA) can all be addressed *in vitro* to potentially reduce *in vivo* experimentation.

#5209 Sequential orthogonal assays for longitudinal and endpoint characterization of three-dimensional cell and tissue culture models.

Olivier De Wever, Eva Blondeel

Ghent University Hospital, Gent, Belgium

Spheroids are near-spherical reaggregated multicellular three-dimensional (3D) structures generated from cells or cell cultures from healthy as well as pathological tissue. Basic and translational spheroid application across academia and industry led to the development of multiple set-ups and analysis methods which mostly lack the modularity to maximally phenotype spheroids. This protocol presents the self-assembly of single-cell suspensions into spheroids by the liquid overlay method followed by a modular framework for a multifaceted phenotyping of spheroids. Cell seeding, supernatant handling, and compound administration are elaborated by both manual and automated procedures. The phenotyping modules contain a suite of orthogonal assays to analyze spheroids longitudinally and/or at an endpoint. Longitudinal analyses include morphometry with/out spheroid or cell state specific information, and supernatant evaluation (nutrient consumption and metabolite/cytokine production). Spheroids can also be used as starting point to monitor single and collective cell migration and invasion. At an endpoint, spheroids are lysed, fixed, or dissociated into single cells. Endpoint analyses allow to investigate molecular content, single-cell composition and state, and architecture with spatial cell and subcellular specific information. Each module addresses time requirements and quality control indicators to support reproducibility. The presented complementary techniques can be readily adopted by researchers experienced in cell culture and basic molecular biology. We anticipate that this modular protocol will advance the application of 3D biology by providing scalable and complementary methods.

TUMOR BIOLOGY: Shaping Tumor Stromal Interactions in Cancer
Poster Session

#5213 Understanding how IDVCs (IDO1-dependent vascularizing cells) respond to and shape the inflammatory milieu to promote neovascularization.

S.-C. Shen[1], J. B. DuHadaway[2], E. Sutanto-Ward[2], G. C. Prendergast[2], A. J. Muller[2];
[1]Drexel University College of Medicine, Philadelphia, PA, [2]Lankenau Institute for Medical Research, Wynnewood, PA

Inflammation and angiogenesis are considered fundamental processes within the TME to the extent that they have been designated “hallmarks of cancer”. In studying tumor development in the lungs, we discovered that the tryptophan catabolizing enzyme IDO1 interconnects these two processes by realigning local inflammation to promote neovascularization. Specifically, the inflammatory cytokine IFNγ elicits neovascular regression, however, it is also a primary inducer of IDO1, which acts in a negative feedback capacity to counter the anti-neovascular activity of IFNγ through the GCN2/ISR dependent induction of an opposing inflammatory cytokine IL6. At the heart of these studies has been the identification of a unique, IDO1-expressing myeloid cell population designated IDVCs (IDO1-dependent vascularizing cells) based on their functional capacity to support neovascularization in an IDO1 dependent manner. A powerful tool used in these studies to analyze the biological activity of isolated IDVCs is the Matrigel plug assay in which FACS sorted cells are incorporated into a subcutaneously implanted Matrigel plug, which is subsequently evaluated by confocal microscopy for blood vessel density.
An actionable breakthrough in addressing the fundamental question of how the inflammatory cytokines IFNγ and IL6 respectively promote and mitigate neovascular regression has emerged from subsequent studies that have identified the NOS2 enzyme, previously implicated in facilitating the IFNγ mediated anti-tumor response, as playing a downstream effector role in driving neovascular regression. Based on aggregate evidence obtained from analyzing 4T1 metastatic tumors and Matrigel plugs, we have constructed a basic conceptual framework for how inflammatory neovascularization is orchestrated by IDVCs. The likely source of IFNγ appears to be NK cells, though how they are recruited and activated by IDVCs remains to be determined. In response, the IFNγ inducible chemokine CXCL10, also apparently produced by IDVCs and signaling through its receptor CXCR3, serves a bridging role leading to the induction of NOS2. However, through induction of IDO1 in IDVCs, IFNγ also drives IL6 trans-signaling, which counteracts the CXCL10/NOS2 neovascular regression pathway. Underlying all this, the initial process of establishing the neovasculature, which is also orchestrated by IDVCs but independently of IDO1, appears to involve vasculogenesis (vessels formed by recruited endothelial progenitor cells, EPCs) as it requires signaling by CXCL12, a key chemokine for EPC attraction. As we continue to interrogate how inflammatory neovascularization, autonomously nucleated by IDVCs, unfolds, we anticipate these ongoing investigations will continue to yield even deeper insights into the regulatory crosstalk between these complex biological processes involved in cancer and other diseases.

#5214 Nuclear APC maintains colon homeostasis and mitigates inflammation.

A. James, K. Neufeld;
University of Kansas, Lawrence, KS

Adenomatous polyposis coli (APC) is critical for maintaining intestinal homeostasis. Although widely recognized for its cytoplasmic tumor suppressor functions, the roles of APC in other subcellular compartments and inflammation are less defined. To investigate nuclear APC functions, we developed a mouse model with compromised nuclear Apc import ($Apc^{mNLS/mNLS}$ mice). $Apc^{mNLS/mNLS}$ mice displayed lower levels of mucin-2 (*MUC2*) RNA, the primary component of the intestinal mucus barrier, and had significantly thinner colonic mucus layers. These mice were more susceptible to experimentally induced colitis than their wild-type littermates. Importantly, male $Apc^{mNLS/mNLS}$ mice exhibited significantly increased weight loss and higher colitis scores compared to female mice. Therefore, we hypothesize that nuclear APC promotes gut barrier integrity by upregulating *MUC2* expression. In cultured human colon cells, APC positively regulates *MUC2* RNA levels and inhibits NF-κB signaling. Overall, this study provides evidence that nuclear APC upregulates colonic *MUC2* expression, increases the mucus barrier, and inhibits colonic inflammation. Furthermore, our results reveal notable sex-specific differences in colitis susceptibility.

#5215 IL-17RA expression by NSCLC is vital for restraining homeostatic tumor cell proliferation and baseline cytokine production.

Dongpu Li, Jun Wang

Microbiology and Immunology, Dalhousie University, Halifax, NS, Canada

Background: Lung cancer is the leading cause of cancer-related death worldwide. The Cancer Genome Atlas shows over 30% of non-small cell lung cancer (NSCLC) samples carrying genetic alterations in the *IL17RA* gene, and deletion of *IL17RA* is linked to poor overall survival rates. IL17RA is a transmembrane protein with multiple intracellular signaling motifs. The SEFIR domain is required for IL17-induced signaling, which is negatively regulated by A20 (TNFAIP3) via a self-feedback loop. The objective of our study is to examine the impact of IL17RA-deletion in NSCLC and the underlying molecular mechanisms.
Methods: Two complementary approaches were used in our study. IL17RA knockdown subclones were created in murine lung cancer cell lines (LLC and CMT167) via shRNA. Human NCIH23 cells carry homozygous deletion of *IL17RA* gene, whereas human A549 cells possess normal copy number of *IL17RA*gene. We expressed IL17RA in both human cell lines via a lentiviral vector. The effects of IL17RA expression on the tumor cell proliferation and the production of soluble mediators were compared in paired cell lines, with and without IL17RA expression. Domain-specific IL17RA mutants were constructed in NCIH23 cells to dissect their functional contributions. Western blots and human phosphorylation pathway profiling arrays were used to study how signaling pathways were affected by IL17RA-deletion.
Results: The knockdown of IL17RA significantly augmented the proliferation of cancer cells in LLC and CMT167 cells. Conversely, the expression of full-length IL17RA in both NCIH23 and A549 cells diminished proliferation rates. Furthermore, IL17RA knockdown increased the production of multiple proinflammatory cytokines, chemokines, and growth factors, whereas IL17RA expression in NCIH23 markedly inhibited their production. We showed that the SEFIR domain was not required for the effects of IL17RA expression on restraining tumor cell proliferation, while it is known to be vital for IL17A-induced cytokine production. The reduced expression of IL17RA was consistently associated with diminished A20 expression in all tested cell lines. Finally, we demonstrate that IL17RA-deletion caused a marked increase of Glycogen synthase kinase 3 beta (GSK3β) Serine-9 phosphorylation, an inactivated form of GSK3β, in NCIH23 cells.
Conclusion: Collectively, our data clearly demonstrate that IL17RA expression is critically required for restraining homeostatic tumor cell proliferation and baseline cytokine production via a previously undefined IL17RA-A20-GSK3β axis.

#5216 RPS19-C5AR1 axis in tumor-associated macrophages drives glioblastoma progression: Insights from single-cell transcriptomics.

Chan-Chuan Liu[1], Gangga Anuraga[2], Cheng-Lin Wu[3], Jian-Ying Chuang[4], Chih-Yang Wang[5], Pin-Yuan Chen[6], Kwang-Yu Chang[1]

[1]National Institute of Cancer Research, National Health Research Institutes (NHRI), Tainan, Taiwan,[2]PhD Program for Cancer Molecular Biology and Drug Discovery, Taipei Medical University, Taipei, Taiwan,[3]Department of Pathology, National Cheng Kung University Hospital, National Cheng-Kung University, Tainan, Taiwan,[4]Research Center for Neuroscience, International Master Program in Medical Neuroscience, Taipei Medical University, Taipei, Taiwan,[5]PhD Program for Cancer Molecular Biology and Drug Discovery, Taipei Medical University, Tainan, Taiwan,[6]Department of Neurosurgery, School of Medicine, Chang Gung Memorial Hospital, Chang Gung University, Taoyuan, Taiwan

Glioblastoma (GBM) is a prevalent and lethal disease with limited treatment options. Tumor-associated macrophages (TAMs), which comprise up to 30% of the GBM mass, play a pivotal role in shaping the TME by supporting tumor growth and establishing an immunosuppressive and inflammatory environment. To investigate how tumor cells affect TAMs during TME evolution, we analyzed single-cell transcriptomics (SCT) datasets from GBM patient-derived tumor mass (Core) and peri-tumoral adjacent tissues (Margin), alongside murine datasets from healthy and GL261-bearing brains. This comparison aimed to clarify the immunological landscapes of GBM and identify specific interactions between tumor cells and TAMs. Margin SCT data revealed increased cellular heterogeneity compared to the Core, with immune cells, tumor cells, and brain stromal cells contributing 57%, 3%, and 40% of the total cell population, respectively. UCell analysis of GBM subtype signatures demonstrated a strong association between TAMs in the Margin and mesenchymal signatures, which are linked to poor prognosis. Further reclustering of TAMs in Margin and the comparison with murine SCT datasets showed that these cells were predominantly derived from peripheral monocytes and macrophages, with M1-like TAMs representing the majority. Pseudotime trajectory analysis revealed that M1-like TAMs serve as precursors to protumoral M2 TAMs. Cell-cell communication mediated by ligand-receptor interactions among tumor cells, TAMs, T cells, and NK cells identified a critical interaction between tumor-derived ribosomal protein S19 (RPS19) and C5AR1 on TAMs. C5AR1 was highly expressed in M1-like TAMs across patient and murine datasets, while RPS19 was ubiquitously expressed by all cell types. *In vitro* models confirmed that M1 macrophages and microglia exhibited higher C5AR1 expression than their M2 counterparts. Analysis of public datasets (The Cancer Genome Atlas-GBM and Chinese Glioma Genome Atlas) showed elevated C5AR1 expression in GBM tissues, particularly in recurrent tumors, and a strong correlation between high C5AR1 expression and poor patient survival. Additionally, tumor-derived RPS19 was released extracellularly under growth stress or therapeutic interventions, promoting an inflammatory TME through M1 TAM polarization and driving GBM progression. These findings suggest that targeting the RPS19-C5AR1 axis, in combination with current therapies, may represent a promising strategy to improve GBM outcomes.

#5217 Interaction of cancer associated fibroblasts and apoptotic cancer cells reduces macrophage survival and promotes reprograming of tumor associated macrophages.

Jihee Lee Kang, Kyungwon Yang, Kiyoon Kim

Ewha Womans University, Seoul, Korea, Republic of

Recent studies highlight the critical role of cancer-associated fibroblasts (CAFs)- tumor-associated macrophages (TAMs) interactions in tumorigenesis. In prior work, we demonstrated that the interaction between CAFs and apoptotic cancer cells can inhibit tumor growth and metastasis. However, the molecular mechanisms underlying the crosstalk between CAFs reprogrammed by apoptotic cancer cells and TAMs are incompletely understood. In this study, we show, using immunohistochemistry in primary tumors, that intratumoral injection of conditioned medium (CM) from lung CAFs exposed to apoptotic cancer cells reduces the density of tumor-associated macrophages (TAMs) and the proportion of M2 TAMs. Additionally, this treatment shifts M2 TAMs toward an M1-like phenotype within the tumor microenvironment (TME). Furthermore, CM from apoptotic cancer cell-exposed CAFs promotes STAT1 phosphorylation in M2 TAMs. Depletion of WISP-1 from the CM reversed these effects, emphasizing its pivotal role. These findings suggest that CM from apoptotic cancer cell-exposed CAFs may provide a promising therapeutic strategy for targeting TAMs in the TME.

#5218 RGS4+aSMA+CAF subsets promote tumor immune escape by regulating the formation of immune-exhaustive mesenchymal networks.

Huiping Chen, Wenfeng Zeng

Sun Yat-sen University, Guangzhou, China

Solid tumors consist of tumor cells and the tumor microenvironment (TME), a dynamic system comprising various immune cells, mesenchymal cells, cytokines, microvesicles, extracellular matrix, and vascular lymphatic networks. The recruitment of lymphocytes into tumors is essential for effective anti-tumor immunity and successful immunotherapy. The tumor stroma serves as the initial site for effector immune cell infiltration, and the mesenchymal network directly influences immune cell function. Cancer-associated fibroblasts (CAFs) are the largest proportion of TME intrinsic stromal cells, with significant heterogeneity and multiple origins, and perform different biological functions. CAFs can not only induce PD-L1 expression in tumor cells, but also promote PD-L1 expression in TME immune cells through the release of cytokines and vesicles, thereby facilitating tumor immune escape. In this study, we found that in some breast cancer patients, PD-L1 in tumor stroma was abundantly expressed and associated with immunosuppression and poor prognosis, and the distribution of tumor stromal PD-L1 was also closely related to the activation status of CAFs. Furthermore, we performed high-throughput RNA sequencing on PD-L1^{low} and PD-L1^{high} stroma cells, respectively. We found a CAF subset in high PD-L1 expression group, which was characterized by immune exhaustion and hyperactivation. Notably, Regulator of G protein signaling 4 (RGS4) pathways were significantly upregulated in this subset. Furthermore, we confirmed that RGS4 induces fibroblasts to adopt a PD-L1 immunodepleting phenotype and establishes an immune-exhaustive mesenchymal network that modulates anti-tumor immunity. In addition, we validated in humanized murine models that targeting RGS4 significantly enhances the efficacy of immunotherapy in animal models. The spatial heterogeneity of PD-L1 expression in TME is a primary factor contributing to the limited efficacy of PD-L1/PD-1 antibody therapies. Currently, numerous studies aim to enhance the immune response by inhibiting or degrading PD-L1. A comprehensive understanding of the molecular mechanisms governing PD-L1's spatial distribution could fundamentally enhance its clinical application. This study explored the mechanism governing the spatial distribution difference of PD-L1, and provided more possibilities for the development of targeted intervention in CAFs.

#5219 Taxanes induce interleukin 6 cytokine in women's cancer and stromal cells in vitro.

Samantha S. Yee, Jacky Gomez-Villa, Sonia L. Hernandez

University of Chicago, Chicago, IL

Background: Taxane microtubule stabilizers, including paclitaxel (PTX), docetaxel (DTX) and nab-paclitaxel (Nab-PTX), are standard of care in the treatment of women's cancer alone or in combination with other therapies. The effects of specific taxanes on cancer cells have been well studied such as disruption of cell division and inhibition of cellular trafficking, inducing cancer cell death; however, their effect on the tumor microenvironment is less understood. Cytokines released by cancer cells into the tumor microenvironment such as interleukin 6 (IL-6) can contribute to therapeutic resistance. Specifically, IL-6 binds to the IL-6 receptor (IL-6R) consisting of alpha and beta subunits, and activates the JAK/STAT signaling pathway, promoting chemoresistance. Taxane effects on tumor microenvironment, specifically tumor vasculature, have been less studied. We hypothesized that PTX, DTX, and Nab-PTX, including pericytes, and human dermal lymphatic endothelial cells (HDLECs), result in IL-6 secretion unique to each taxane, promoting chemoresistance.
Methods: Primary human pericytes and HDLECs, 5 human women's cancer cell lines, and 2 human breast epithelial cell lines were grown in vitro. The CCK-8 cell viability/cytotoxicity assay determined IC_{50} potency for the taxanes in primary human cells and cancer cells. Quantitative-PCR and cytokine arrays were used to evaluate IL-6 transcript levels and protein secretion, and flow cytometry to determine the abundance of IL-6 receptors at the cell surface.
Results: DTX had the lowest IC_{50} values with a range of 0.3 - 2.1 nM, followed by PTX with 1.0 - 4.0 nM, and Nab-PTX with 2.9 - 64.5 nM after 72h treatment across 5 different cancer cell lines. DTX and PTX increased IL-6 transcript levels more than 2-fold following 24h treatment, $p<0.05$), whereas Nab-PTX showed a slight increase ($p > 0.05$). MDA-MB-231 cells had the highest basal IL-6 transcript levels compared to cancer and stromal cells. All cells tested expressed both IL-6R subunits at varying levels, suggesting taxane chemoresistance via IL-6R activation is possible in cancer cells and tumor associated cells. Primary human pericytes or HDLECs did not reach 50% survival following 24h treatment of 10 nM taxanes. DTX increased pericyte IL-6 transcript levels by two-fold following 24h treatment, $p<0.05$. Preliminary cytokine array data revealed IL-6 was increased in 10 nM DTX-treated pericytes following 24h treatment compared to DMSO (2-fold change).
Summary: We show that DTX is the most potent taxane following 72h treatment. DTX also induces IL-6 production in both women's cancer cell lines and stromal cells, potentially contributing to chemoresistance and cancer progression. All cells express varying levels of IL-6R subunits, suggesting that combining DTX treatment with IL-6 inhibitors may prevent DTX resistance in women's cancer patients.

#5220 Regulating tumor-associated macrophage polarization via inhibition of hedgehog signaling for treating colorectal cancer liver metastasis.

Bu Gyeom Kim[1], Bo Ram Kim[1], Dae Yeong Kim[1], Hyemi Yun[2], Okhyeon Kim[2], Yoon Namgung[1], Jun Woo Bong[3], Sanghee Kang[3], Sun Il Lee[3], Sang Cheul Oh[1]

[1]Department of Oncology, Korea University Guro Hospital, Seoul, Korea, Republic of,[2]Biomedical Sciences, Korea University College of Medicine, Seoul, Korea, Republic of,[3]Surgery, Korea University Guro Hospital, Seoul, Korea, Republic of

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related mortality worldwide. The liver is the most frequent site of metastasis in patients with CRC. Approximately 15-25% of patients with CRC have synchronous liver metastasis (LM). Despite the development of surgical techniques and targeted therapy, the prognosis of colorectal cancer liver metastasis (CRLM) remains poor. The tumor microenvironment (TME) is composed of cancerous and non-cancerous cells, including fibroblasts, endothelial cells and immune cells, as well as non-cellular components. As a major component of immune cells in the TME, tumor-associated-macrophages (TAMs) account for up to 50% of cells in the tumor mass and are generally relevant to worse prognosis in cancer patients. TAMs are an important component of the immunosuppressive TME, but the precise mechanisms of the interactions between TAMs and tumor cells are still not completely understood. The hedgehog (Hh) signaling pathway is aberrantly activated in multiple types of human cancer. Hh signaling pathway plays an important role in tumorigenesis, including promoting tumor invasion, metastasis, and multi-drug resistance. However, it remains to be elucidated how Hh ligands ac on stromal cells within the TME to promote tumor growth and what cell types are involved in this process. In this study, we established an irinotecan-resistant colorectal cancer cell lines to represent liver metastatic colorectal cancer. We then found that irinotecan-resistant colorectal cancer cells significantly increased SHH secretion using quantitative reverse transcription PCR (qRT-PCR) and an ELISA assay. Subsequently, we used a THP-1 and bone marrow-derived monocytes (BMDMs) to determine whether Hh signaling and metastatic colorectal cancer cells affect the polarization and activity of macrophages. We found that tumor cells-derived SHH induced M2 polarization of TAMs under tumor cells-derived conditioned medium and co-culture systems using Western blot and Flow cytometry analysis. Moreover, phagocytosis ability of macrophages was decreased with irinotecan-resistant colorectal cancer cells compared with irinotecan-parent colorectal cancer cells under co-culture systems. To further investigate whether inhibition of Hh signaling affects macrophage polarization, we treated with Vismodegib, a Hh signaling inhibitor. M2 macrophage polarization increased by SHH was reduced by Hh signaling inhibitor treatment. Furthermore, TISIDB revealed that SHH was strongly negatively correlated with macrophage abundance and CD80, CD86 in COAD patients In conclusion, our study identified a novel mechanism for macrophage polarization through Shh signaling pathway and provided insights into developing macrophage-targeted therapeutic strategies for CRLM patients.

#5221 Immune-organoid cocultures to investigate the role of herpesvirus serology in oncolytic viral therapy for glioblastoma.

Ethan Chen[1], Alexander L. Ling[1], Michael C. Prabhu[2], Connor C. Bossi[3], Keith L. Ligon[4], E Antonio Chiocca[1]

[1]Neurosurgery, Brigham and Women's Hospital, Boston, MA,[2]West Virginia University School of Medicine, Morgantown, WV,[3]UMass Chan Medical School, Worcester, MA,[4]Pathology, Dana-Farber Cancer Institute, Boston, MA

Background: Complex relationships between treatment and intratumoral immunity are challenging to study in glioblastoma patients owing to the dynamic nature of these relationships and the difficulty in obtaining serial tissues from brain tumors. The importance of these processes necessitates the development of robust model systems that enable researchers to study immune dynamics in controlled and clinically relevant ways. To this end, we have generated a panel of patient derived organoids from glioblastoma patients with paired PBMCs and known clinical features. We are leveraging these models to more deeply study our recently published finding that patients with pre-existing seropositivity to herpesvirus 1 (HSV1) survive significantly longer than seronegative patients when treated with an engineered oncolytic HSV1 agent.

Methods: Resected tumor tissues were freshly obtained and cultured to establish glioblastoma organoids alongside paired PBMC and plasma samples. To assess differential immune activation between HSV1 seropositive and seronegative models, PBMCs were expanded for all antigen-experienced immune cells using IL-21, IL-15, and IL-7 and cocultured with their paired organoid in the presence or absence of oncolytic HSV1 infection. Cocultures were observed for spatial dynamics and viral infectivity under time lapse microscopy over 72 hours, followed by flow cytometric analysis at endpoint.

Results: Viable organoid cultures were successfully generated for 8 patients over a 7 month period, with paired PBMCs/plasma being available for 5 of these patients, of which two were HSV1 seropositive. Optimization experiments confirmed that expanded T cells and organoids could be cocultured for 72 hours, enabling interrogation of tumor-immune interaction dynamics. Initial results from our cocultures revealed serology-dependent T cell activation in the context of HSV1 infection, with effector CD4 T cells increasing with virus infection in seropositive but not seronegative coculture models.

Conclusion: Our results demonstrate that we can rapidly generate glioblastoma organoids with a high success rate. By coculturing these models with paired patient PBMCs, we were able to study dynamic tumor-virus-immune interactions with a high degree of spatiotemporal resolution. We believe this approach holds significant promise for enabling novel insights into the dynamic impact of immunotherapy on the tumor-immune microenvironment-potentially providing critical insights into why these therapies often show limited efficacy in glioblastoma and how they can be improved.

#5222 Establishment of a patient-derived organoid-PBMC co-culture model to investigate immune responses and immunotherapy sensitivity in advanced gastric cancer.

Han Byeol Mun[1], Woo Sun Kwon[2], Tae Soo Kim[2], Sun Young Rha[3]

[1]Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul, Korea, Republic of,[2]Song-Dang Institute for Cancer Research, Yonsei University College of Medicine, Seoul, Korea, Republic of,[3]Division of Medical Oncology, Department of Internal Medicine, Yonsei Cancer Center, Yonsei University Health System., Seoul, Korea, Republic of

Advanced gastric cancer (AGC) exhibits significant molecular heterogeneity with aggressive behavior and treatment resistance. Immunotherapy (IO), such as PD-1 or PD-L1 inhibitors, has emerged as an important treatment for AGC, but only a small subset of patients responds to these therapies. Therefore, it would be of great benefit to understand mechanisms of anti-tumor immune response in AGC patients. This study aims to establish a co-culture model of patient-derived GC organoids and PBMCs that can recapitulate tumor-immune interactions PBMCs were isolated from healthy donors, pooled equally, and cryopreserved. They were activated with anti-CD3/CD28 Dynabeads at ratios of 1:1 (Bead : Cell) for 48 hours and stained with 1 μM CellTrace Far Red. Organoids with a diameter of ≥50 μm were harvested, filtered through a 50 μm strainer, and seeded (50 per well) into 96-well plates at ratios of 1:1 to 200:1 (PBMCs : Organoids). Pembrolizumab was treated in mono-culture and co-culture assays at a ratio of 50:1 for 48 and 72 hours, and sensitivity was measured with CCK-8. PBMC recruitment was analyzed using the Operetta CLS system and Harmony 4.9 software. Supernatants were collected post-co-culture and analyzed for cytokines (IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ, and IL-17A) using BD Cytometric Bead Array, with values below the detection limit excluded from the analysis. In co-culture conditions, the organoids exhibited variable responses depending on the ratio in the co-culture conditions. YCC-GO10-1 showed 63.1% viability with a 5-fold increase in PBMC recruitment, while YCC-GO63 showed 14.8% viability with a 1.6-fold increase. This indicates that increased PBMC recruitment is not necessarily associated with organoid death. In the co-culture supernatant of YCC-GO10-1, higher levels of inflammatory cytokines were observed compared to YCC-GO63, including IL-6, a cytokine that promotes tumor progression, as well as IFN-γ, TNF and IL-2. Pembrolizumab had no impact on the viability of organoids or PBMCs under monoculture conditions. However, under co-culture conditions, it exhibited variable effects on organoid viability, reducing the viability of YCC-GO63, while YCC-GO10-1 remained resistant to the treatment. Even under identical immune environments, organoids displayed heterogeneous responses, resulting in distinct immune reaction patterns and cytokine secretion profiles. The established organoid-immune cell co-culture model demonstrates the potential to investigate tumor-immune interactions and study the mechanisms underlying sensitivity to IO, antibody-drug-conjugates, and other anticancer agents *in vitro*.

#5223 Deciphering cellular heterogeneity and therapeutic responses in NSCLC through scRNA-seq analysis.

Hwajin Choi[1], Kyoung-Ho Pyo[2], Sun Min Lim[2], Byoung Chul Cho[2], Seunghee Hong[1]

[1]Yonsei University, Seoul, Korea, Republic of,[2]Yonsei University College of Medicine, Seoul, Korea, Republic of

Programmed cell death protein and its ligand (PD-1 / PD-L1) blockades have been approved for the treatment of metastatic non-small cell lung cancer (NSCLC). PD-L1 binds to PD-1 and functions as a co-inhibitory factor. It is well known that in cancer, PD-L1 is highly expressed hence cancer cells can evade the host immunity in the tumor microenvironment (TME). Although PD-1 / PD-L1 blockades have been clinically utilized, the biological factors determining treatment outcome remain convoluted. An in-depth investigation of cellular heterogeneity including both cell subtypes and states (defined by cellular biomolecular programming) will provide foundational evidence for clinicians and researchers to improve PD-1 / PD-L1 immunotherapeutic practice. The recent decade has shown an explosive growth of single-cell RNA sequencing (scRNA-seq) to study biomedical phenomena. In contrast to bulk RNA-seq, scRNA-seq enables researchers to properly decipher and analyze the complex cellular heterogeneity. Our scRNA-seq data spans 674,609 cells populating early, late, metastatic stage lung cancer and the normal lung tissues from 100 subjects. Especially, part of our metastatic samples harbor classification based on the existence of immune checkpoint blockade (ICB) treatment and therapeutic efficacy of the treatment. Our scRNA-seq analysis results thoroughly provide an intensive comprehension of dysregulation in T cells. This single-cell analysis potentially reveals pathways targeted by immunotherapy and discovers possible therapeutic targets which could be combined with the current immunotherapy for the synergistic effects.

#5224 Systemic microenvironment determines the disease outcome in murine models of breast cancer.

Fulya Koksalar Alkan[1], Ahmet Burak Caglayan[1], Hilmi Kaan Alkan[1], Cordarryl Jones[1], Adriana Langer Gramer[2], Roni Bollag[2], Eric Sebzda[1], Kristen S. Purrington[1], Max Wicha[3], Huidong Shi[2], Hasan Korkaya[1]

[1]Wayne State University, Detroit, MI,[2]Augusta University, Augusta, GA,[3]University of Michigan, Ann Arbor, MI

The systemic immune microenvironment plays a critical role in determining disease outcomes in breast cancer, yet its molecular and functional characteristics remain largely unknown. Using single-cell RNA sequencing (scRNAseq), CyTOF, and functional assays, we investigated immune dynamics in metastatic (4T1) and non-invasive (EMT6) murine models of triple negative breast cancer (TNBC). While primary tumors in both models exhibited similar infiltration by macrophages and myeloid-derived suppressor cells (MDSCs), profoundly distinct immune profiles in target tissues of metastatic mice. In the metastatic 4T1 model, metastatic tissues showed an expansion of neutrophil progenitors and immature neutrophils ($CD11b^{+}CD33^{+}Ly6G^{+}CD115^{-}Ly6C^{low}$) at the expense of natural killer (NK) cells, T cells, and B cells. These immature neutrophils mediated systemic immune suppression, contributing to the establishment of pre-metastatic niches where they regulate cancer stem cell (CSC) proliferation. Recombinant S100A8/A9 (calprotectin) proteins induced a similar expansion of these immunosuppressive neutrophils in EMT6 models, driving metastasis and immune evasion. In contrast, S100A9 knockout mice showed reduced metastatic burden and enhanced anti-tumor immune responses. Depletion of $Ly6G^{+}$ immature neutrophils using anti-Ly6G antibody significantly restored systemic anti-tumor immunity and eliminated metastasis, while having no effect on primary tumor growth. Conversely, depletion of $CD115^{+}$ macrophages selectively inhibited EMT6 tumor growth without altering systemic immune profiles. Our findings highlight the critical influence of systemic immune dynamics in shaping metastatic progression and therapeutic resistance. The identification of neutrophil progenitors and inflammatory S100A8/A9 proteins as key mediators of immune suppression offers novel insights into the interplay between systemic immunity and tumor biology. Targeting these components may provide a promising approach to mitigating metastasis and improving therapeutic outcomes in aggressive breast cancer subtypes.

#5225 Engineered signaling reporter immune cell lines for monitoring immune activation and multidirectional interactions among cancer cells, immune cells, and tumor microenvironmental cells.

Hyeyoun Chang, Alicia Walker, John Foulke, Luping Chen, **Fang Tian**, Zhizhan Gu

ATCC, Gaithersburg, MD

While immunotherapies targeting T cells have achieved remarkable success, a significant subset of patients remain unresponsive or experience relapse, often attributed to the immunosuppressive nature of the tumor microenvironment. Emerging evidence suggests that other immune cell types, such as B cells and myeloid cells, play crucial roles in modulating the efficacy of cancer immunotherapies. However, there remains a clear lack of widely accessible immunological models capable of accurately representing the intricate multidirectional interactions among cancer cells, adaptive and innate immune cells, and other tumor microenvironmental cells. To address this gap, we engineered signaling reporter cell lines of T cell, B cell, or myeloid origin to express luciferase reporter protein in response to the activation of nuclear factor of activated T cells (NFAT) or nuclear factor kappa B (NF-κB) signaling pathway. The cell lines also endogenously express high level of T cell checkpoints (PD-1, TIGIT, and/or GITR) or myeloid checkpoints (SIRPα, Siglec-10, LILRB1, and/or B7-1) for additional application in immune checkpoint research. To validate luciferase expression, NFAT luciferase reporter T cell lines were stimulated with phorbol 12-myristate 13-acetate (PMA) and ionomycin, while NF-κB luciferase reporter myeloid cell lines were activated with tumor necrosis factor alpha (TNF-α) or conditioned media from T cells. A B cell-derived NF-κB reporter cell line (BDCM-NFκB-Luc2) with high basal luciferase activity was treated with an NF-κB signaling inhibitor. These reporter cells were subsequently evaluated in various co-culture conditions with primary immune cells, cancer cells, and/or other tumor microenvironmental cells to study immune cell crosstalk. Luciferase assays were performed for convenient quantification of luminescence intensity under all conditions. The results demonstrated that stimulations leading to NFAT or NF-κB signaling activation yielded dose-dependent increase in luciferase expression. In contrast, inhibition of the signaling led to dose-dependent decrease in luciferase expression. Furthermore, co-culture of reporter cell lines with various other immune and cancer cells significantly enhanced luminescence signals, with increases of up to 200-fold. In summary, these newly developed luciferase reporter immune cell lines provide a robust ex vivo model for evaluating cancer immunotherapies. These cell lines enable sensitive and reproducible monitoring of complex interactions among cancer cells, innate and adaptive immune cells, and other tumor microenvironmental cells, offering a valuable standard for the assessment of combinatorial immune responses in the tumor microenvironment.

#5226 CD47-Sirpαblockade enhances antitumor activity against mouse liver cancer by boosting cDC2 functions.

Guan-Sian Low[1], Cheng-Zhe Jian[2], Yi-Chiao Tseng[1], Da-Liang Ou[2], Chiun Hsu[3], Chien-Kuo Lee[1]

[1]Graduate Institute of Immunology, National Taiwan University College of Medicine, Taipei, Taiwan,[2]Graduate Institute of Oncology, National Taiwan University College of Medicine, Taipei, Taiwan,[3]Department of Medical Oncology, National Taiwan University Cancer Center, Taipei, Taiwan

The CD47-SIRPα axis functions as an immune checkpoint, transmitting a “don’t eat me” signal to prevent macrophage-mediated phagocytosis of tumor cells. While CD47 is expressed on most immune cells and is highly induced in tumor cells, SIRPα is expressed on phagocytes, including macrophages and type II conventional dendritic cells (cDC2s). Blocking the binding between CD47 and SIRPα is shown to promote antitumor immunity. However, the mechanism of how CD47-Sirpα blockade regulates DC functions is still unclear. Here we demonstrated that anti-CD47 and/or anti-Sirpα antibodies increased the phagocytic activity and antigen presentation of MutuDC2, a cDC2 cell line, against Hepa1-6, a mouse hepatocellular carcinoma cell line expressing LCMV-GP protein. Moreover, CD47 blockade also enhanced BM-derived cDC2 functions in boosting the proliferation and cytotoxic activity of P14 CD8 T cells in vitro. More importantly, anti-CD47 antibodies reduced tumor growth in vivo and cDC2s and IFNγ+ functional CD8 T cells were enriched in the tumor microenvironment compared to isotype control. In conclusion, CD47-Sirpα blockade boosts cDC2 functions, including phagocytic activity, antigen presentation, and cross-presentation ability, eliciting a better antitumor activity in antigen-specific CD8 T cells and emphasizes the therapeutic benefits of immune checkpoint blockade through cDC2.

#5227 Extrachromosomal MYC-paralogs amplification shapes suppressive tumor microenvironment in small cell lung cancer.

Jingwei Zhang[1], Yueqi Jin[2], Ence Yang[2], Xiao Li[1]

[1]Peking University People's Hospital, Beijing, China,[2]School of Basic Medical Sciences, Peking University Health Science Center, Beijing, China

Introduction: Although immunotherapy has demonstrated promise in small cell lung cancer (SCLC), certain patients encounter limited benefits, highlighting the need for identifying immunosuppressive biomarkers. Extrachromosomal circular DNA (ecDNA) promotes amplification of MYC-paralogs, which drive cross-resistance in SCLC. Here, we aim to investigate whether ecDNA-mediated MYC-paralogs amplification (ecMYC+) represents immunosuppressive features in SCLC.

Methods: Bulk RNA sequencing (RNA-seq) data were retrieved from public database and paraffin-embedded samples (n = 40). The copy number of MYC-paralogs was investigated using real-time polymerase chain reaction. RNA-seq, flow cytometry and metaphase fluorescence in situ hybridization (FISH) were performed in SCLC cell lines. The overexpression and amplification of MYC-paralogs in SCLC clinical samples (n = 42) were identified using immunohistochemistry (IHC) and FISH. The spatial distribution of tumor immune microenvironment (TIME) was characterized by multiplex immunofluorescence and imaging mass cytometry (IMC).

Results: The mean copy number of ecDNAs was higher in SCLC than in the other cell lines (15.3 vs 8.4, $p = 4.0\times10^{-3}$), and the frequency of ecMYC+ cell lines was higher in SCLC than that in the other lineages (SCLC 22/47 vs others 15/282, $p = 3.9\times10^{-12}$). In ecMYC+ SCLC, multiple immune-related pathways were down-regulated, while nucleotide metabolism processes were up-regulated. Inhibition of nucleotide metabolism by hydroxyurea and gemcitabine resulted in the reduction of ecDNA, along with the activation of antigen presenting pathways. Immunohistochemical analysis revealed that overexpression of MYC-paralogs was observed in 19 out of 42 resected SCLC samples. FISH identified 8 samples as MYC-paralogs copy number gain or amplification, and 3 samples exhibited a scattered signals distribution resembling ecDNA amplification. Through the resolution of 103,341 cells from 24 pathological regions, we observed higher expression of MKI67, VEGFA, FAP and FOXP3 and reduced T cell infiltration in ecMYC+ samples. Moreover, ecMYC+ samples exhibited elevated cellular neighborhoods dominated by Ki67+ tumors, with reduced spatial interaction with immune cells.

Conclusions: Extrachromosomal amplification of MYC-paralogs shapes suppressive tumor microenvironment, identifying potential subgroup of immunotherapy resistant patients.

#5229 From the patient to the culture plate and beyond: Challenges and opportunities in cancer-associated fibroblast research.

F. De Vuyst, S. Ernst, S. Vermeulen, Z. Xia, S. Dedeyne, O. De Wever;
Ghent University, Ghent, Belgium

Cancer-associated fibroblasts (CAFs) are a key component of the microenvironment of various solid tumor types. Due to their critical role in tumor progression and therapy resistance, a surge in CAF-related publications is observed exploring the functionality, heterogeneity and potential as therapeutic targets. To facilitate community wide data consolidation and sharing, we initiated a crowdsourcing data-repository together with both national and international collaborators. With this initiative we mapped 85 general and CAF-specific experimental, biological and functional parameters from more than 1000 CAF-related publications from the past decade. This unique set of data indicates a general heterogeneity and underreporting of crucial experimental parameters, related to isolation procedures, culture conditions and CAF characterization. Using single-cell RNAseq we were able to show a phenotypic shift in the CAF population going from the *in vivo* situation to an *in vitro* culture. Additionally, we empirically investigated the impact of *in vitro* aging and hTERT immortalization on the stability of this obtained *in vitro* CAF phenotype. At the molecular level, single cell RNAseq, bulk proteomics, flow cytometry and Luminex cytokine assay revealed a specific gene/protein and pathway rewiring upon passaging of primary CAF cultures, while immortalization resulted in a loss of heterogeneity in the culture but with gain of phenotype stability. Functional assays evaluating collective migration and type I collagen invasion confirmed the increased phenotypic stability in hTERT immortalized CAF cultures. In conclusion, CAF literature mining combined with empirical evaluation will further support interpretable, reproducible and transparent experimental reporting in the CAF research field.

#5230 Co-culture with glioblastoma stem-like cells induces neuron death.

Komal Nalinkant Rawal, Charlotte Degorre, Philip J. Tofilon

Radiation Oncology Branch, National Cancer Institute, National Institutes Of Health (NIH), Bethesda, MD

The vast majority of GBMs recur by 2 years after standard of care treatment, which includes radiotherapy. As a model for investigating the biology of recurrent GBMs, we used brain tumor xenografts initiated from human glioblastoma stem-like cells (GSCs). In this system brain tumors were irradiated (10Gy) on Day 35 after implantation into right striatum of nude mice and allowed to regrow for an additional 21 days. In untreated mice tumor cells are shown to invade throughout the right hemisphere with a high degree of cell-cell contact with mouse neurons as determined by immunohistochemical staining for NeuN, a marker for neuronal soma. In mice irradiated with 10Gy, tumors that regrow are less invasive with a loss of neurons. These data suggest that GBM cells may have a detrimental effect on neuronal survival. Of note, while recent literature suggests neurons contribute to GBM growth, seizures are a frequent occurrence in GBM patients, which may reflect neuronal toxicity. As an in vitro model for investigating the mechanisms mediating the loss of neurons in the recurrent GBM xenografts, neurons differentiated from iPSC derived Neural Progenitor cells (NPC) were directly cocultured with GFP-positive GSCs in stem cell growth medium. After the initiation of coculture the number of neurons and GSCs were determined from 24-96h. As compared to GSCs cultured alone, there was no difference in the number of GSCs in cocultures out to 96h. However, as compared to neurons cultured alone, there was a significant reduction in the number of neurons in cocultures beginning at 48h with further decreases out to 96h. These data suggest that GSCs induce neuron toxicity. As an initial step towards defining the mechanism through which GSCs induce neuronal death, cell culture supernatants from neurons, GSCs and cocultures were subjected to cytokine profiling after 48h. As compared to cell-free media, neuron culture media predominantly contained Osteopontin, TIMP-1 and TIMP-2, which have been implicated in neuron differentiation and functional development, while GSC culture media contained FGF-4, FGF-7 and TGFβ-3, which have been associated with GBM growth. However, in media isolated from cocultures MCPs, Angiogenin and IL-8 were uniquely detected suggesting an interaction between GSCs and neurons resulted in the production of these cytokines. IL-8, the most abundant cytokine detected in the coculture media, has previously been associated with neuron toxicity. These data suggest that interactions between GSCs and neurons alter cytokine levels, which then leads to neuron death. This effect may account for the loss of neurons observed in the GBM xenografts that regrow after irradiation.

#5231 Follistatin shapes the tumor microenvironment in head and neck squamous cell carcinoma through COL1A1-CD44-mediated tumor-stroma crosstalk.

Jennifer Sosa, Satrajit Sinha

Biochemistry, University at Buffalo, State University of New York, Buffalo, NY

Head and Neck Squamous Cell Carcinoma (HNSCC) is a common malignancy with limited therapeutic options. The initiation and progression of HNSCC is closely tied to epithelial cell fate decisions influenced by the tumor microenvironment (TME). The Transforming Growth Factor-β (TGF-β) Superfamily of ligands are pleiotropic growth factors and key mediators of cell-cell communication within the TME. Although TGF-β has been extensively studied for its critical role in cancer progression, therapeutic strategies targeting TGF-β have been largely unsuccessful. Such efforts have often overlooked the potential contribution of myriad factors that influence TGF-β signaling such as follistatin (FST), a secreted glycoprotein and potent inhibitor of TGF-β. FST is frequently upregulated in solid tumors where it is associated with dismal survival outcomes and reduced therapeutic responses. However, the molecular mechanisms by which FST counterbalances TGF-β and potentially other signaling pathways to modulate the TME is not well understood. This underscores the critical need for a more nuanced and molecular understanding of the FST driven networks to develop more effective therapeutic approaches. Here we examined the tumor cell intrinsic and extrinsic roles of FST within the TME of HNSCC by leveraging single cell RNA-seq and spatial transcriptomics datasets from HNSCC patients and analyzing transcriptional changes following depletion of FST in human HNSCC cell lines. We find that while fibroblasts and epithelial cells both express FST, tumor epithelial cells are the primary source of heightened FST levels in HNSCC. Notably, we discover that FST expression is intrinsically linked to a transcriptional program promoting a basal stem-like state in cancer epithelial cells, suggesting a role for FST in tumor plasticity. Consistently, tumor cells that express FST exhibit increased signaling and molecular attributes of the epithelial-to-mesenchymal transition (EMT) program and reduced differentiation. Since cancer stem cells are enriched at the tumor leading edge, where they leverage EMT processes for dissemination, we investigated the spatial distribution of FST-enriched tumor cells and their interactions with the surrounding extracellular milieu. Our analyses show that FST is enriched within the tumor leading edge invasive niche, and further, that tumor-stroma crosstalk via COL1A1-CD44 interactions is spatially localized within FST-rich domains. Finally, we find CD44 positively correlates with FST expression in patient tumors and cancer cells at the transcriptional and protein level. Collectively, our findings define the functional role of FST within the HNSCC TME and supports a model in which FST counterbalances TGF-β signaling and facilitates distinct epithelial-stroma interactions at the dynamic leading edge to enhance tumor aggression.

#5232 Tumor-adipose microenvironment drives progression in advanced oral squamous cell carcinoma.

Gyeongmin Kang[1], Mi Rim Lee[2], Sumin Kang[1], Yu-Sun Lee[2], Hye Won Shon[2], Jiyoung Lee[2], Sung Weon Choi[2], Sun Il Choi[2], Yun-Hee Kim[2]

[1]Graduate School of Cancer Science and Policy, National Cancer Center, Goyang, Korea, Republic of,[2]Research Institute and Hospital, National Cancer Center, Goyang, Korea, Republic of

Advanced oral squamous cell carcinoma (OSCC) is an aggressive cancer with a recurrence rate of 40-60%, and lymph node metastasis (LNM) occurs in 50% of cases, serving as a critical prognostic factor. Recent evidence suggests that fatty acid oxidation (FAO) enhances cancer cell survival, invasiveness, and therapeutic resistance. OSCC's histological proximity to dense adipose tissue provides a unique opportunity for cancer cells to acquire energy from the adipose environment, underscoring the importance of elucidating the tumor-adipose interactions in OSCC progression and metastasis. In this study, we investigated the impact of the tumor-adipose microenvironment on OSCC progression, focusing on interactions between adipocytes and cancer cells. Human adipose-derived stem cells (ADSCs) were differentiated into adipocytes and co-cultured with the OSCC cell line YD10B for three weeks to induce cancer-associated adipocytes (CAAs). Co-cultured adipocytes exhibited fibroblast-like phenotypic changes, reduced expression of adipogenic differentiation markers, and increased free fatty acid release. Inflammatory cytokines (e.g., IL-6) and tumor-promoting proteins (e.g., IGFBP-2) were upregulated. YD10B cells exposed to co-cultured adipocytes showed enhanced proliferation, invasion, and epithelial-mesenchymal transition (EMT) signaling. Adipocytes and cancer cells both showed increased expression of matrix metalloproteinases (MMP-2 and MMP-9), indicating mutual reinforcement of tumor aggressiveness. Collectively, these findings indicate that adipocytes, through their interaction with OSCC cells, undergo changes to acquire a CAA-like phenotype , which support tumor progression by providing fatty acids as an energy source and promoting aggressive phenotypes, including proliferation, invasion, and LNM. Targeting tumor-adipocyte interactions could offer novel therapeutic strategies to mitigate OSCC progression and improve outcomes.

#5233 Increased galectin-3 in tumor-stromal cells enhances gemcitabine resistance in pancreatic cancer via suppressing oxidated phosphorylation.

Jia Tong[1], Guo An[2], Jingtao Liu[2], Chunxiang Ye[3], Huachiong Ma[3], **Wei Zhao**[2]

[1]Shandong Provincial Hospital Affiliated to Shandong First Medical University, Shandong, China,[2]Key laboratory of Carcinogenesis and Translational Research (Ministry of Education), Peking University Cancer Hospital & Institute, Beijing, China,[3]Beijing Chaoyang Hospital, Capital Medical University, Beijing, China

Pancreatic cancer accounts for nearly about 3% of all cancer cases, which represents a relatively common malignant tumor with rising incidence and mortality rates. Our previous studies have proved that Gal-3 could increase the malignancy of tumor cells and activate collagen and fibrin crosslinking in the stroma. Besides, Gal-3 promoted stromal cells releasing a large number of inflammatory cytokines, such as IL-6 and IL-8, which accelerated tumor progression through the tumor microenvironment deterioration. Additionally, we found the modified citrus pectin (MCP), a natural inhibitor of Gal-3, could inhibit tumor growth in the mouse xenograft model of pancreatic cancer. It is providing a new idea for tumor treatment using dietary interventions. However, there are still few reports on the role of Gal-3 in chemotherapy resistance in pancreatic cancer, so in-depth exploration of this area is urgently needed. This study aims to further elaborate on whether inhibition of Gal-3 expression in tumor cells or stromal cells can be combined with gemcitabine to effectively prevent pancreatic cancer progression. We evaluated Gal-3 levels in various types of pancreatic cancer samples and subsequently established stable-transfected pancreatic cancer cell lines. Based on our previous findings, gemcitabine sensitivity in different cell lines was determined to assess the relationship between Gal-3 expression and drug resistance. The results have indicated that inhibition of Gal-3 expression may reduce the dose-dependence of pancreatic tumors on gemcitabine chemotherapy, thereby enhancing treatment efficacy. Furthermore, we explored the effects of Gal-3 on the mitochondrial oxidative phosphorylation and reactive oxygen species (ROS) levels, during the substrate end of the respiratory chain in the mitochondrial membrane in PAAD cells, and its associated pathways in gemcitabine chemotherapy. It has been demonstrated that fibroblast-induced drug-resistant tumors exhibit a significant reduction through the CCL2-CCR2 axis. This reduction occurs in a multifactorial manner, involving calcium-dependent PPIA-BSG (CD147) pathway and dephosphorylation of NFAT1 intracellular mechanisms. The consequence of such a phenomenon is that it thereby enables these cells to effectively counter the DNA damage caused by gemcitabine. Our research provides significant insights for future dietary interventions and the development of novel agents, opening new avenues for improving patient prognosis.

#5234 A novel strategy to inhibit colorectal cancer liver metastasis by targeting plasminogen activator inhibitor-1 in liver stromal cells.

Tomokazu Ohishi[1], Yukinari Kato[2], Mika K. Kaneko[2], Shun-ichi Ohba[1], Akiko Harakawa[1], Hiroyuki Inoue[1], Junjiro Yoshida[3], Daisuke Tatsuda[3], Tomoko Nakanishi[4], Masanori Hatakeyama[5], Manabu Kawada[3]

[1]Numazu branch, Institute of Microbial Chemistry (BIKAKEN), Numazu, Japan,[2]Department of Antibody Drug Development, Tohoku University Graduate School of Medicine, Sendai, Japan,[3]Laboratory of Oncology, Institute of Microbial Chemistry (BIKAKEN), Shinagawa, Japan,[4]Center of Biomedical Research Resources, Juntendo University, School of Medicine, Bunkyo, Japan,[5]Laboratory of Microbial Carcinogenesis, Institute of Microbial Chemistry (BIKAKEN), Shinagawa, Japan

Liver metastasis significantly contributes to mortality in colorectal cancer (CRC) patients, highlighting the urgent need for more effective therapeutic strategies. CRC liver metastasis develops within a complex tumor microenvironment that promotes cancer cell colonization and growth. The liver's supportive role is well described in Paget's "seed and soil" hypothesis. It illustrates how metastatic cancer cells (the "seeds") spread and proliferate in a compatible microenvironment (the "soil"), which includes liver stromal cells. These stromal cells release various factors that aid tumor progression, making it crucial to understand these molecular interactions.This study aims to elucidate the contribution of liver stromal cell-derived factors in CRC metastasis and identify potential therapeutic targets. Using both in vivo and in vitro coculture systems, we found that plasminogen activator inhibitor-1 (PAI-1), a protein produced by liver stromal cells, significantly promotes CRC cell proliferation. Mechanistically, PAI-1 enhances CRC cell survival and proliferation by activating the Akt signaling pathway, which regulates key cellular processes. In xenograft models, therapeutically inhibiting PAI-1 markedly reduced liver metastasis. Furthermore, experiments using PAI-1 knockout mice showed a substantial decrease in metastatic burden, underscoring PAI-1's pivotal role in CRC progression.These results establish PAI-1 as a promising therapeutic target. Its inhibition could potentially reduce CRC liver metastasis and improve patient outcomes. Given the complex nature of tumor-stromal interactions, our findings provide critical insights into targeting liver stromal proteins for effective CRC metastatic treatment.

#5235 Cancer associated fibroblasts-derived SULF1 facilitated carcinogenesis and metastasis of esophageal squamous cell carcinoma.

Hongyu Zhou, Beilei Liu, Licheng Tan, Baifeng Zhang, Yingchen lyu, Xin-Yuan Guan

The University of Hong Kong, Hong Kong, China

Cancer-associated fibroblasts (CAFs) play a significant role in creating a supportive environment for tumor growth and progression. Esophageal squamous cell carcinoma (ESCC) ranks as the seventh leading cause of cancer-related deaths globally, characterized by poor clinical outcomes and limited treatment options. CAFs-based therapeutic strategies might benefit ESCC patients. In this study, we utilized single-cell RNA sequencing (scRNA-seq) data from four integrated ESCC cohorts to identify a unique protein, SULF1, secreted by CAFs. Primary human CAFs were isolated from six clinical samples of ESCC and various functional assays, such as transwell migration/invasion and co-culture/co-injection experiments, were conducted to assess the oncogenic or pro-metastatic effects of SULF1-secreting CAFs. Neutralizing antibodies against SULF1 was proposedly used for treatment. Here, we revealed that CAFs exhibited the highest expression levels of SULF1 compared to other cell types based on scRNA-seq data. Immunohistochemistry and immunofluorescence staining confirmed the unique expression of SULF1 in CAFs, particularly in αSMA-rich CAFs within ESCC patient samples. Furthermore, extracellular secretion of SULF1 was elevated in primary human CAFs compared to tumor cells and normal fibroblasts. Co-culture or co-injection of SULF1-knockdown CAFs mixed with tumor cells significantly inhibited tumor growth and metastasis both in vivo and in vitro. Mechanistically, RNA-seq data suggested that CAF-derived SULF1 might activate the TGF-beta and PI3K-AKT signaling pathways, thereby mediating the aggressive behavior of tumor cells. Notably, blocking SULF1-secreted CAFs resulted in suppression of ESCC tumor growth and progression in vivo. Collectively, CAF-secreted SULF1 emerged as a critical mediator in educating the ESCC tumor microenvironment and promoting tumor aggressiveness. Targeting SULF1-expressing CAFs could be viewed as a potential therapeutic strategy for ESCC treatment.

#5237 Optimizing multiple myeloma treatment approaches by elucidating tumor-stroma interactions in the bone marrow microenvironment.

Panteha Behboodi[1], Angelica Castano[2], Iriana Colorado[2], Mauricio Rigo[2], Martha Salas[2], Mariam Tariq[2], Woo Lee[1], Benjamin Tycko[2], Rena Feinman[2]

[1]Stevens Institute of Technology, Hoboken, NJ,[2]Hackensack Meridian Health Center for Discovery and Innovation, Nutley, NJ

Multiple Myeloma (MM) is a plasma cell malignancy arising in the bone marrow. Despite advancements in therapies, this disease becomes refractory such that relapse and death is inevitable. Immune evasion and drug resistance are partly due to MM-osteoblast cell interactions in the bone marrow microenvironment (BME). We conducted complementary in-vitro and in-vivo studies, each providing insights that converge to a treatment-relevant hypothesis. Several human MM cell lines (MM.1S, U266, NCI-H929, L363) were co-cultured with osteoblasts for two days to assess proliferation in response to two proteasome inhibitors, bortezomib, and carfilzomib. Osteoblasts, represented by the hFOB fetal osteoblast-derived cell line, markedly enhanced MM cell growth and conferred significant chemoresistance determined by a luciferase-based viability assay. We then performed RNA sequencing of FACS-sorted MM.1S and hFOB cells following 1 day of coculture. We found a significant upregulation of Type I Interferon (IFN) signaling pathway target genes (e.g. IRF7, ISG15, $p<0.001$) in co-cultured MM.1S cells compared to MM.1S cells alone. Two pro-inflammatory chemokines, CXCL1 and CXCL8, were markedly increased in hFOB co-cultured cells compared to hFOB monocultures. A 9-fold increase of CXCL1 ($p<0.0001$) and CXCL8 ($p<0.0001$) was validated by qPCR and both chemokines were elevated in co-culture supernatants in a cytokine array. Parallel studies in vivo, using the 5TGM1 MM immunocompetent mouse model, revealed that low-dose decitabine (DAC), a DNA hypomethylating agent known to induce a Type I IFN response significantly reduced MM burden ($p<0.0001$) and prolonged survival by 9 days ($p<0.001$), compared to MM-bearing mice treated with PBS vehicle. From these two lines of work, we hypothesize that low-level IFN signaling, mediated by MM cell cross-talk with osteoblasts, is supportive of MM cell proliferation and survival, while hyper-induction of the IFN pathway by DAC may contribute to the therapeutic efficacy of this epigenetic-modifying agent, both alone and in combination with immunotherapies, such as immune checkpoint inhibitors. This postulated biphasic response to Type I IFN signaling in MM cells is consistent with prior reports from other experimental systems. Our findings highlight the utility of epigenetic therapy in MM and suggest that hyper-induction of IFN signaling in MM cells, which already have low-level activation of this pathway from their interactions with osteoblasts, should be further investigated as a therapeutic mechanism.

TUMOR BIOLOGY: The Immune Microenvironment in Cancer
Poster Session

#5242 YTHDF2 is a radiotherapy checkpoint in dendritic cells.

Dapeng Chen, Hua Liang, Ralph Weichselbaum

Department of Radiation and Cellular Oncology, University of Chicago, Chicago, IL

Dendritic cells (DCs) play a central role for initiating anti-tumor immunity of T cells in radiotherapy (RT), yet its generalized success to cancer RT has been hampered by insufficient antigen presentation and induction of suppressive regulators from tumor microenvironment. Here, we reveal that ionized radiation (IR) induces the expression of m^6A reader YTHDF2 in DCs with clinical significance, and the depletion of Ythdf2 in DCs augments the anti-tumor effect of RT. We observed that following IR, SPI-1 promotes the transcription of YTHDF2 in DCs. The IR-induced YTHDF2 in DCs directly targets and degrades m^6A-binded *Mfng*, *Aph-1b*, and *Aph-1c,* which are essential for notch activation. YTHDF2 blockades in DCs activates notch signaling to promote the expression of MHC-I family gene *Gm8909*, thus enhancing cross-priming of $CD8^+$ T cells to afford amplified anti-tumor effect of RT. Pharmacological inhibition of YTHDF2 in DC vaccines contributes to RT efficacy in both local tumor control and distant metastasis suppression. Our findings identify IR-induced YTHDF2 as an immunosuppressor in DCs, reveal the mechanism for regulating anti-tumor immunity, and open a new path for the development of m^6A-mRNA targeting therapies

#5243 Peripheral Foxp3^{high} regulatory T cells develop into tumor-associated counterparts by proinflammatory cytokines and act as prognostic biomarker in patients with hepatoma.

Wei-Ting Ku[1], Chien-Hao Huang[2], Jayashri Mahalingam[2], Cheng-Heng Wu[2], Tsung-Han Wu[3], Jian-He Fan[2], Chung-Wei Su[2], Po-Ting Lin[2], Chien-Wei Peng[2], Wei Teng[2], Wen-Juei Jeng[2], Wen-Juei Jeng[2], Wei-Ting Chen[2], Chen-Chun Lin[4], Shi-Ming Lin[2], I-Shyan Sheen[2], Yung-Chang Lin[5], Chun-Yen Lin[6]

[1]Graduate Institute of Biomedical Sciences, Chang Gung University, Taoyuan, Taiwan,[2]Division of Gastroenterology and Hepatology, Linkou Chang-Gung Memorial Hospital, Taoyuan, Taiwan,[3]Department of General Surgery,, Linkou Chang-Gung Memorial Hospital, Taoyuan, Taiwan,[4]Division of Gastroenterology and Hepatology, New Taipei Municipal TuCheng Hospital (Built and Operated by Chang Gung Medical Foundation), New Taipei City, Taiwan,[5]Department of Hematology-Oncology, Linkou Chang-Gung Memorial Hospital, Taoyuan, Taiwan,[6]College of Medicine, Chang Gung University, Taoyuan, Taiwan

CD4$^+$ regulatory T cells (Tregs) play a critical role in the progression of hepatocellular carcinoma (HCC). However, indiscriminate depletion of Tregs risks triggering autoimmunity. While previous research highlights the phenotypic and functional heterogeneity of Treg subpopulations, a unified classification system remains elusive. This study aims to characterize Treg subtypes in HCC patients using single-cell CITE-seq and validate their clinical relevance and prognostic potential. CITE-seq profiling of 51,067 CD4$^+$ T cells from eight HCC patients identified distinct Treg populations in tumor, non-tumor, and peripheral blood samples. Validation studies conducted on 96 HCC patients and 53 healthy donors using flow cytometry, functional assays, and clinical data revealed a peripheral Foxp3^{high} Treg subset that preferentially migrates to tumor sites. Upon infiltration, these Tregs undergo terminal differentiation, exhibiting heightened activation, immunosuppressive functions, and increased expression of LAYN and TRM-associated genes. Proinflammatory cytokines within the tumor microenvironment further amplify Foxp3 expression and the suppressive capacities of these cells. Mechanistic analysis implicated the CCL5/CCR5 signaling axis as essential for the recruitment of Foxp3^{high} Tregs from peripheral blood to the tumor microenvironment. Peripheral Foxp3^{high} Tregs strongly correlated with their tumor-infiltrating counterparts, suggesting their potential as a non-invasive biomarker. A threshold of peripheral Foxp3^{high} Tregs/CD4$^+$ T cells > 3.5% was found to predict overall survival and early recurrence, achieving AUROCs exceeding 0.75. In conclusion, peripheral Foxp3^{high} Tregs transition into tumor-associated counterparts via the CCR5-CCL5 axis and proinflammatory cytokines, contributing to immunosuppression in HCC. Their abundance in peripheral blood correlates with tumor infiltration and clinical outcomes, underscoring their utility as a prognostic biomarker for HCC.

#5244 Functional γδ T cells in humanized mice.

Florent Creusat, Siham Hedir, Alexis Gonon, Amelie Marguier, Yacine Cherifi, Fabiane Sonego, Gaelle Martin, **Kader Thiam**

genOway, Lyon, France

Development of immunotherapies has been a major landmark in the field of oncology, leading to numerous effective treatments. From the first use of an immunomodulatory agent in clinical studies to modern immunotherapies, these were mainly focused on modulating adaptive immune response. However, only a fraction of patients can respond to these treatments, and the complexity of the tumor microenvironment requires other players to be targeted. γδ T cells are unconventional T cells, as they recognize antigens mostly in a MHC-unrestricted fashion. They show a high diversity of effector functions, from cytotoxicity to mediators' production and wound healing. Their preactivated state allows a quick immune response, and their role in tumor development, both in beneficious or deleterious manner, was demonstrated in numerous types of cancer. Current therapeutic approaches involving γδ T cell include adoptive cell transfer, *in vivo* stimulation and combined therapies. While preliminary results are promising, investigation of such therapies in preclinical models is challenging, because γδ T cells are not developed at satisfactory levels in most of the humanized mouse models. Here we describe the presence and functionality of γδ T cell in BRGSF (Balb/C $Rag2^{-/-}$, $IL2R\gamma^{-/-}$, SIRPαNOD and $Flt3^{-/-}$), a highly immunodeficient mouse featuring reduced murine myeloid cells. BRGSF mice reconstituted with human cord blood $CD34^+$ cells (BRGSF-HIS) develop functional lymphoid and myeloid compartments. This engraftment is stable for over a year (Labarthe *et al.*, 2019) and mice do not develop GvHD. At steady-state, BRGSF-HIS mice show a diverse population of γδ T cells in their blood and peripheral organs, expressing Vδ1, Vδ2 or Vδ3 chains, although their ratio is different in blood from BRGSF-HIS mice and human PBMC. These cells can be expanded using zoledronate with human IL-2, allowing *ex vivo* or *in vivo* assays for γδ T cells targeting compounds. This specific activation, as well as the activation with a Vδ1-targeting agent, leads to an increase in γδ T cells activation state (expression of CD25, CD69, CD137) and their effector function (cytokine production or CD107a-associated degranulation). Similarly, an "Expander" targeting Vγ9 significantly increased Vγ9 expressing cells frequencies, and serum levels of perforin and INF-γ in treated mice. Interestingly, γδ T cells were recruited into the tumor microenvironment of the triple negative breast cancer line MDA-MB-231 and the colorectal adenocarcinoma cell lines HT29 and SW480, suggesting that therapies designed to expand and activate γδ T cells could be assessed in BRGSF-HIS tumor bearing mice. Therefore, the development of functional γδ T cells in BRGSF-HIS mice brings a new perspective on the assessment of therapies targeting this cell population in humanized mouse models.

#5246 Chromosomal instability promotes colorectal cancer progression through manipulation of gamma delta T cells.

Paulino Tallon de Lara[1], Aviad Ben-Shmuel[1], Wen-Hao Hsu[2], Chaohao Li[1], Kyle Labella[3], Chi Wut Wong[1], Yonghong Liu[1], Shan Jiang[1], Xiaoying Shang[1], Scott Kopetz[1], Ronald DePinho[1]

[1]The University of Texas MD Anderson Cancer Center, Houston, TX,[2]Stanford University, Stanford, CA,[3]Genentech, South San Francisco, CA

Colorectal cancer (CRC) can be divided into 2 genetically different subtypes: Microsatellite instable CRC (MSI-CRC; ~15% of CRC), and microsatellite stable (MSS-CRC; ~85%). While MSI-CRC develops point mutations that increase neoantigen burden and enhanced response to immunotherapy, MSS-CRC develops chromosomal instability (CIN) and resistance to immunotherapy. Current mouse models do not recapitulate the key genetic defect of MSS-CRC: CIN. This limits our understanding of CIN's role in regulating tumor immunity in CRC, and therefore our capacity to generate new immunotherapies for MSS-CRC.

To address this gap, we developed the first genetically engineered mouse model of MSS-CRC with human-like CIN. To recapitulate human CIN caused by telomere erosion, we incorporated the conditional null *Apc* and *Trp53*, and inducible Lox-Stop-Lox (LSL) mouse telomerase reverse transcriptase (*mTert*) alleles, referred to as iTAP. Due to LSL-*mTert*, mice lack *mTert* expression and therefore telomerase activity, which leads to telomere erosion upon successive generational intercrosses, evolving from G0 mice (LSL-*mTert*+/-) that have no CIN (CIN- iTAP) to G3 mice after 3 breeding generations with the mTert+/+ allele that develop CIN (CIN+ iTAP). Colonoscopy-guided submucosal administration of 4-OHT activates Villin-driven Cre-ER in colonocytes leading to *Apc* and *Trp53* deletion and deletion of the stop cassette of LSL-*mTert* causing activation of telomerase in cancer cells. Oncogenesis in cells with telomere erosion leads to CIN.

Consistent with worse OS in human MSS CIN^{high}, CIN+ iTAP tumors were more aggressive than CIN- iTAP. Single-cell RNA sequencing (scRNA-seq) confirmed that CIN+ iTAP tumors developed more copy number variations. Subclustering of the infiltrating immune populations revealed that γδ T cells expanded and changed their phenotype in CIN+ iTAP tumors, which was confirmed by flow cytometry. Treatment with a blocking antibody towards γδ TCR in mice with established tumors lead to increased survival in CIN+ iTAP mice but not in CIN- iTAP mice. ScRNA-seq of primary tumors of patients with MSS-CRC revealed that γδ T cells also exhibit phenotypic changes upon different levels of CIN in human MSS CRC.

#5247 Comprehensive characterization of pseudocapsules in clear cell renal cell carcinoma reveals unique invasion mechanisms: A multi-omics investigation.

Xiyi Wei[1], Yitong Pan[2], Weiyu Kong[1], Da Zhong[1], Zijie Yu[1], Yang Wang[1], Longjun Ma[3], Zhou Yang[1], Yize Li[4], Chenxi Tian[2], Bing Yao[3], Ninghong Song[1], Chao Qin[1]

[1]Department of Urology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China,[2]Key Laboratory of Genomic and Precision Medicine, Beijing Institute of Genomics, Chinese Academy of Sciences (CAS), Beijing, China,[3]Department of Medical Genetics, School of Basic Medical Sciences, Nanjing Medical University, Nanjing, China,[4]Division of Oncology, Department of Medicine, School of Medicine, Washington University in St. Louis, St. Louis, MO

Clear cell renal cell carcinoma (ccRCC) represents the most common subtype of renal cell carcinoma, accounting for approximately 70% of cases. Notably, the majority of ccRCC tumors are surrounded by a prominent pseudocapsule tissue, highlighting its potential diagnostic, prognostic, and therapeutic relevance. To comprehensively understand the implications of pseudocapsules in ccRCC, we constructed an extensive multi-omics atlas. The cohort included single-cell RNA sequencing, spatial transcriptomics, proteomics, and extracellular matrix (ECM) proteomics, derived from 36 ccRCC cases. These analyses were further augmented with functional assays and animal studies, enabling a multi-dimensional exploration of pseudocapsule biology. Our cohort analysis demonstrated that pseudocapsules are predominantly composed of fibroblasts and a rich diversity of immune cells, a finding corroborated through immunofluorescence (IF) and immunohistochemistry (IHC). The tissue was also characterized by a substantial accumulation of extracellular matrix components. Importantly, pseudocapsule invasion revealed significant alterations in the tumor microenvironment (TME). During tumor invasion, CD8+ T cells and dendritic cells (DCs) within the pseudocapsule exhibited increased activation, accompanied by pronounced exhaustion. Concurrently, tumor-associated fibroblasts underwent functional reprogramming, transitioning from a myofibroblast-like state (myoCAF) to an inflammatory CAF phenotype (iCAF). These iCAFs primarily interacted with T cells and promoted their exhaustion, which was critical to ccRCC progression. Additionally, our investigation identified a loss of chromosome 14q during tumor cell invasion. Notably, the FOS gene on chromosome 14 was significantly downregulated and identified as a key gene. Clinical data from large patient cohorts revealed that low FOS expression was associated with advanced tumor stage, higher histological grade, and poorer overall survival. Functional assays demonstrated that FOS overexpression reduced the invasive potential of ccRCC cell lines in vitro, whereas FOS knockdown enhanced lung metastasis in vivo. Transcriptomic analysis further indicated that FOS is involved in regulating extracellular matrix dynamics and immune-related pathways. These findings provide novel insights into the role of pseudocapsules in ccRCC and highlight critical molecular mechanisms underlying tumor progression. By uncovering key invasion biomarkers and therapeutic targets, this study lays the groundwork for precision medicine approaches in ccRCC management.

#5248 Identification of a chromatin-remodeling peptide in diffuse midline glioma by immunopeptidomics.

Niloofar Khairkhah[1], Ali Namvar[1], Arun Everest-Dass[2], Seeta Nyayapathy[3], Richard Jones[4], Julie M. Rumble[3], Christopher E. Whitehead[5], Judith S. Sebolt-Leopold[5], Stefanie Galban[6]

[1]Department of Radiology, University of Michigan Medical School, Ann Arbor, MI,[2]Institute for Glycomics, Griffith University, Gold Coast Campus, Gold Coast, Queensland 4222, Australia,[3]Immunology Services, Cayman Chemical, Ann Arbor, MI,[4]MS Bioworks, Ann Arbor, MI,[5]Department of Radiology (UMMS), University of Michigan Medical School and MEKanistic Therapeutics, Inc., Ann Arbor, MI,[6]Departments of Radiology and Surgery, Center for Molecular Imaging, Rogel Cancer Center, University of Michigan Medical School, Ann Arbor, MI

The identification of peptides presented by major histocompatibility complex class I (MHC-I) molecules on cancer cells is crucial for developing targeted immunotherapies, particularly for malignancies like pediatric diffuse midline gliomas (DMG), where therapeutic options remain limited. In this study, we applied advanced immunopeptidomics and mass spectrometry techniques to characterize the immunopeptidome of DMG H3K27M-altered. To identify DMG specific peptides, we included comparative studies to the immunepeptidome of adult glioblastoma (GBM). We identified a total of 454 peptides in DMG and U87 (glioblastoma) xenografts pertaining to 229 genes. Among these, 42 peptides were uniquely expressed in DMGs and 11 were shared with U87 xenografts, highlighting distinct antigen presentation profiles between these tumors. Notably, the peptide RSRKESYSVVYYKVLKQVHPD, which stems from the Histone H2B type 1-M (H2B1M) gene, is presented by the MHC-I molecule HLA-B*57:01, and is associated with chromatin remodeling, was enriched in DMG cells compared to GBM, revealing a potential immunotherapeutic target specific to DMG. To explore whether targeted therapies alter the identified immunopeptidome, we utilized MTX-241F, a previously described experimental compound that inhibits key proliferative pathways (EGFR/PI3K) in DMG and GBM. Comparative analysis of MTX-241F-treated DMGs and U87 xenografts revealed both overlapping and unique regulatory patterns of peptides, underscoring the tumor-specific effects of MTX-241F. MTX-241F selectively upregulated peptides in DMG stemming from the NCOA4, VATF and THOC2 genes that are linked to MHC-I presentation. In summary, our findings provide the first evidence of dynamic treatment dependent modulation of antigen presentation in DMGs and offer new insights into the tumor immunopeptidome. The distinct peptide profiles of DMG and U87 and their different responses to MTX-241F suggest potential therapeutic avenues for precision immunotherapy targeting DMG. In addition, the identification of a shared immunopeptidome presents an opportunity to develop a therapeutic for both pediatric and adult glioma.

#5249 *In vitro* co-culture CRISPR screen identifies SMG1 to potentiate T cell-mediated cytotoxicity in hepatocellular carcinoma.

Ka Hei Lam[1], Tin Lok Wong[1], Kai Yu Ng[1], Vincent Tano[2], Jia Jian Loh[1], Ianto B. Huang[1], Guang Sheng Ling[1], Terence KW Lee[3], Lei Lei Chen[2], **Stephanie KY Ma**[1]

[1]School of Biomedical Sciences, The University of Hong Kong, Hong Kong, Hong Kong,[2]Cancer Science Institute of Singapore, National University of Singapore, Singapore, Singapore,[3]Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong, Hong Kong

The overall response rate to immune checkpoint blockade (ICB) in hepatocellular carcinoma (HCC) patients remains suboptimal, highlighting the need for immediate efforts to identify resistance factors and develop synergistic strategies to enhance the antitumor activity of ICB. Cytotoxic T lymphocytes (CTLs) are the central effectors of anti-tumor immune response. Understanding the relationship between tumor intrinsic kinases and CTL-mediated cytotoxicity could reveal novel mechanisms in promoting immunotherapy efficacy. Here, we conducted a kinome knockout (KO) CRISPR screen in an *in vitro* tumor-T cell co-culture system to identify kinases in HCC cells that contribute to immune escape from cytotoxic T cell killing. Within the top depleted candidates, Tumor Immune Dysfunction and Exclusion (TIDE) analysis of The Cancer Genome Atlas (TCGA) HCC dataset revealed that higher suppressor of morphogenesis in genitalia 1 (SMG1) expression in patients predicted ICB resistance. Further bioinformatics analysis showed that SMG1 expression is inversely associated with CTL signature, and patients with low SMG1 expression and high CTL signature have more favorable survival outcomes. *In vitro* proliferation assay and orthotopic transplantation of murine HCC cells in mice demonstrated that SMG1 depletion elicited immune-dependent tumor suppressive effect. Using single-cell RNA sequencing (scRNA-seq), we observed an increased proportion of effector CD8+ T cells in SMG1-depleted tumors, which exhibited enhanced activation of T cell receptor (TCR) signaling pathway along pseudotime, indicating an enhanced T cell-mediated immune response in tumors following SMG1 inhibition. Additionally, we showed that SMG1 loss promoted transcription of major histocompatibility complex class I expression (MHC-I) genes and enhanced antigen presentation on the surface of tumor cells. On the other hand, further investigation of the transcriptome profile following SMG1 knockout indicated dysregulation of alternative splicing (AS) in HCC cells. We also demonstrated that nonsense-mediated mRNA decay (NMD) inhibition can enhance T cell-mediated cytotoxic response, and NMD signature is inversely associated with CD8+ T cells signature in HCC. This suggested the potential of inhibiting SMG1 to promote antigen presentation, as well as disrupt AS and NMD coupled events to drive the generation of splicing-derived neoantigens, thereby augmenting anti-tumor immunity.

#5250 Re-activating suppressed tumor-infiltrating lymphocytes from melanoma brain metastases.

Mark W. D. Sweep[1], Imke van Wandeloo[1], Mark A. J. Gorris[2], Guido van Mierlo[2], Benno Kusters[3], Kiek Verrijp[2], Alfred C. Koetsveld[4], Mark ter Laan[4], I. Jolanda M. de Vries[2], Carla M. L. van Herpen[5], Kalijn F. Bol[5]

[1]Medical Oncology & Medical BioSciences, Radboudumc, Nijmegen, Netherlands,[2]Medical BioSciences, Radboudumc, Nijmegen, Netherlands,[3]Pathology, Radboudumc, Nijmegen, Netherlands,[4]Neurosurgery, Radboudumc, Nijmegen, Netherlands,[5]Medical Oncology, Radboudumc, Nijmegen, Netherlands

Brain metastases (BrMs) occur in up to half of patients with metastatic melanoma and are a major cause of death. The response of BrMs to immunotherapy can be different from extracerebral metastases (ECMs). Hence, in this study the immune microenvironment of BrMs is compared with that of ECMs. Bulk RNA sequencing was performed on a retrospective cohort of 38 melanoma samples from 14 patients, consisting of 15 matched BrMs and 23 ECMs. This cohort was expanded to perform multiplex immunohistochemistry on a total of 107 matched BrM and ECM samples from 44 patients. Fresh BrM material was prospectively obtained from a total of 12 patients with melanoma BrM, who underwent surgical resection of their BrM. From this material, tumor-infiltrating lymphocytes (TILs) and corresponding tumor cells were cultured. These cells were characterized by flow cytometry. TIL cultures were expanded and tested for reactivity against autologous tumor cell lines using flow cytometry and ELISA. In a patient-matched comparison, gene set enrichment analysis showed that brain-specific pathways are enriched in BrMs, whereas immune activation pathways, especially pathways related to TILs, are suppressed in BrMs compared to ECMs. However, the computationally inferred abundance of TILs did not differ between BrMs and ECMs, but rather showed an increase of anti-inflammatory M2 macrophages in BrMs. This indicates that TILs are equally present in BrMs and ECMs, but that they are less activated in BrMs. Interestingly, M2 macrophages seemed reduced in patients who received prior treatment with BRAF/MEK inhibitors. When taken out of their native environment*, ex vivo* cultures of BrM-derived TILs and tumor cells are successful in 7/10 (70%) and 7/12 (58%) patients, respectively. In 3/6 (50%) patients with matched TIL and tumor cell cultures, tumor reactivity was indicated, without external stimulation, by production of TNFα, IFNγ, perforin, and expression of CD107a and CD137. Our data suggest that TILs are suppressed in melanoma BrMs as opposed to ECMs, possibly due to increased presence of anti-inflammatory M2 macrophages. Nevertheless, these TILs can efficiently be expanded *ex vivo* and show tumor reactive potential, providing opportunities for TIL-based and other immunotherapeutic treatments in BrM. Combined with macrophage targeting, TILs could synergize to provide effective targeting of the tumor.

#5251 Anergic response of NK cells mediated by killer cell lectin-like receptor C1 and tumoral HLA-E interaction in pleural metastasis of lung adenocarcinoma.

Ying-Ming Tsai[1], Kuan-Li Wu[2], Ya-Ling Hsu[3]

[1]Pulmonary and Critical Care Medicine, Gangshan Hospital, Kaohsiung Medical University, Kaohsiung City, Taiwan,[2]Pulmonary and Critical Care Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung City, Taiwan,[3]Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung City, Taiwan

The presence of effusion is negatively correlated with prognosis in lung cancer. Despite the advent of novel therapies, clinical outcomes remain unsatisfactory. Pleural metastasis and malignant pleural effusion (MPE) result from tumor-host interactions involving mechanisms such as pleural inflammation, angiogenesis, and vascular hyperpermeability. Natural killer (NK) cells are potent immune effectors capable of responding to cancer. However, several aspects of NK cell biology remain under investigation. Understanding their role will aid in identifying effective therapeutic targets. Effusion samples from patients with congestive heart failure (designated as HP) and lung cancer pleural metastasis (LCP) were collected. Cells were harvested to delineate the functional phenotype of NK cells using single-cell RNA sequencing (scRNA-seq). Additionally, changes in NK cells were validated using immunohistochemistry and cell marker identification. The results revealed six subsets of NK cells: $CD16^+/CD56^-$ NK cells, $CD16^-/CD56^+/XCL2^+$ NK cells, $CD16^-/CD56^+/RUNX2^+$ NK cells, $CD16^+/CD56^+/BCL11B^+$ NK cells, $CD16^+/CD56^+/CCL3^+$ NK cells, and $CD16^-/CD56^-$ NK cells. Most NK cell subclusters dominated in the LCP group, except for $CD16^+/CD56^-$ NK cells, which were prevalent in the HP group. These NK cell subsets exhibited different expression patterns of inhibitory receptors (KLRC1 and KLRD1) and activating receptors (HCST and KLRK1). Notably, $CD16^-/CD56^+/RUNX2^+$ NK cells were present exclusively in MPE and absent in effusions from HP. Compared to other NK cell subsets, $CD16^-/CD56^+/RUNX2^+$ NK cells exhibited lower expression of activating receptors and higher expression of inhibitory receptors, particularly KLRC1, suggesting an immunosuppressive phenotype. Cell-cell interaction analysis using CellChat indicated that HLA-E in pleural tumors interacted with $RUNX2^+/CD16^-/CD56^+$ NK cells, resulting in decreased NK cytotoxicity. This interaction was further validated through higher expression of HLA-E in pleural tumor cells by immunohistochemistry and the presence of $CD16^-/CD56^+/RUNX2^+$ NK cells in malignant effusions of lung cancer by a flow cytometry. These findings imply that the presence of the inhibitory ligand HLA-E from tumor cells supports the formation of pleural metastasis by evading NK cell-mediated cytotoxicity. Further investigation into these cell groups may aid in the prevention and treatment of MPE in lung cancer.

#5252 2D and 3D spatial characterisation of tertiary lymphoid structures in head and neck cancer patients receiving immune checkpoint blockade therapy.

Meg L. Donovan[1], Habib Sadeghirad[2], Naomi Berrell[1], James Monkman[2], Vahid Yaghoubi Naei[3], Chin Wee Tan[4], Joseph Yunis[2], Zoe West[5], Majid E. Warkiani[3], Rahul Ladwa[6], Brett G. M. Hughes[7], Jessica Da Gama Duarte[8], Arutha Kulasinghe[2]

[1]Queensland Spatial Biology Centre, Wesley Research Institute, Brisbane, Australia,[2]Frazer Institute, Faculty of Medicine, The University of Queensland, Brisbane, Australia,[3]School of Biomedical Engineering, University of Technology Sydney, Sydney, Australia,[4]The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia,[5]UQ Centre for Clinical Research, Faculty of Medicine, The University of Queensland, Brisbane, Australia,[6]The Princess Alexandra Hospital, Brisbane, Australia,[7]The Royal Brisbane and Women's Hospital, Brisbane, Australia,[8]Olivia Newton-John Cancer Research Institute, and School of Cancer Medicine, La Trobe University, Melbourne, Australia

Tertiary lymphoid structures (TLS) play an important role in the tumor microenvironment (TME) of Head and Neck Cancer (HNC) and have been linked to improved response to immunotherapy. Moreover, HNC patients with human papillomavirus (HPV)+ tumors have better survival compared to those with HPV- tumors, although both groups have better clinical outcomes when the tumors are TLS-rich. How TLS composition, characteristics, and signatures differ between response to treatment in relation to HPV status is still unclear. To investigate this, whole-tissue samples from 55 HNC patients were collected across two separate sites in Brisbane, Australia (The Princess Alexandra Hospital and The Royal Brisbane and Women's Hospital) prior to immune checkpoint therapy (ICI). Using a multi-pathway panel, covering the 'hallmarks of cancer' and 'TLS-associated proteins,' we profiled 53 spatially resolved biomarkers using both a PhenoCycler-Fusion panel and 7-plex PSP panel (Akoya Biosciences). Using spatial proteomics, the TLS were assessed for their composition, activity, maturity, and distance metrics from the tumor. Using spatial transcriptomics, we profiled 96 TLS using whole transcriptome profiling (Bruker Spatial Biology). In addition, in patient samples abundant with TLS, we profiled 50 serial sections to obtain and profile the volumetric 3D data. Our study identified various degrees of TLS maturity, proteomic, and transcriptomic profiles associated with TLS localisation within the TME and proximity to the tumor. In addition, we identified TLS signatures associated with clinical endpoints such as response and resistance to ICI therapy. Taken together, this research highlights the multifaceted role of TLS in modulating the immunogenic landscape of HNC, influencing immunotherapy efficacy, and potentially serving as a biomarker for patient stratification and therapeutic strategies.

#5253 Immune landscape analysis of solid tumors *via* spatially guided cell isolation for enhanced immunotherapeutic development.

Elie Abi Khalil[1], Yi-Chien Wu[1], Jingtian Zheng[1], Joshua Plank[2], Alexander Lippert[2], Steve Lee[1]

[1]University of Illinois at Chicago, Chicago, IL,[2]Southern Methodist University, Dallas, TX

The tumor-immune microenvironment (TIME) in solid tumors is a complex ecosystem consisting of diverse interactions between cellular populations. Deeper investigation into the TIME reveals a prominent role of immune cells in regulating tumor progression- a novel phenomenon that has encouraged the evaluation of the crosstalk between cell types. The development of spatial omic technologies has thus enabled the exploration of these nuances at previously inaccessible resolutions, but the currently available platforms come at the cost of introducing bias, excluding molecular information, and incompatibility with single cell technologies. Here, we leverage a photoactivatable (PA) dye Janelia-Fluor-646 in combination with a digital light processing (DLP) microscope to address these issues by developing a platform that enables the spatially guided isolation of cell populations from a region of interest (ROI) for downstream multiomic and single cell analyses. As proof of concept, we show that ROIs identified via immunofluorescence could successfully be barcoded in mouse spleen and tumor sections using the dye and distinguished from the background by fluorescence activated cell sorting (FACS). We also demonstrate the ability to extract high quality DNA, RNA, and protein- as well as maintain high cell viability post-sorting. We have also developed a pipeline for the analysis of the sequenced RNA to identify differential patterns of gene expression present in immune cells from spatially distinct regions. Moving forward, we hope to establish this as a platform for immune landscape analysis within the TIME to uncover unique targets that could be explored within the context of tumor-immune interactions as well as immunotherapeutic development. Our findings will also help us generate the first single-cell atlas of niche-specific immune cells.

#5254 Multi-plexed spatial interaction analysis of the tumor microenvironment in head and neck cancers.

Vahid Yaghoubi Naei[1], Sara Bodbin[2], Habib Sadeghirad[1], Rafael Tubelleza[1], James Monkman[1], Agata Wicher[2], Subham Basu[2], **Arutha Kulasinghe**[1]

[1]University of Queensland, Brisbane, Australia,[2]Navinci Diagnostics AB, Uppsala, Sweden

Head and neck cancers are the 7th most common cancer type globally accounting for over 900,000 cases annually. With increasing numbers of systemic and targeted therapies, including immune checkpoint inhibitors (ICI), it has become increasingly important to understand the complex tumour microenvironment (TME) milieu dictating clinical responses to therapy. In this study, we profiled 40 whole slide, head and neck cancer tissues from patients prior to ICI therapy and profiled the TME for spatial interactions using the NaveniPlex in situ proximity ligation assay (isPLA, Navinci). Using the Leica BOND RX, formalin-fixed, paraffin-embedded (FFPE) tissue sections were prepared by baking, deparaffinization, and epitope unmasking. Staining was then carried out using the NaveniPlex isPLA technology on the Leica BOND RX instrument. To assess interactions between PD1/PD-L1, CD8/MHCI, and CD3+ T cells, three pairs of Navenibodies were employed simultaneously. Briefly, the NaveniPlex isPLA protocol involves tissue blocking, followed by incubation with primary Navenibodies, ligation, and rolling circle amplification (RCA). Each Navenibody pair generates a distinct RCA product. The RCA products for PD1/PD-L1, CD8/MHCI, and CD3+ T cells were visualized using oligonucleotides conjugated to Atto647N, Atto550, and AlexaFluor488, respectively.Our spatial interaction analysis for PD1/PDL1, CD8/MHCI and CD3+ T-cells showed distinct TME interactions associated with responders and non-responders to ICI therapy. Interactions mapped with cellular phenotypes revealed the cellular neighbourhoods and communities at which these interactions may be useful prognostically. Taken together, this study provides a methodological framework to profile pre-ICI treatment tissues to identify spatial interactions associated with clinical endpoints.

#5255 Systemic immunosuppression in pancreatic cancer.

Damian Matysniak, Chunbo He, Pankaj Kumar Singh, Kamiya Mehla

Oncology Science Department, University of Oklahoma Health Sciences Center, Oklahoma City, OK

Background: Natural killer (NK) cells play a critical role in cancer immunosurveillance and are considered a potent immunotherapeutic tool for many cancers, including pancreatic ductal adenocarcinomas (PDACs). Increased number of ongoing clinical trials investigating CAR-T/NK cell therapies in PDAC patients (NCT04390399, NCT02839954, NCT03634501, NCT03941457, NCT03323944, NCT05275062) driving the need for research understanding of how systemic immune perturbations regulate the effector function of adoptively transferred immune cells is important for development of these therapies. The immunosuppressive tumor microenvironment contributes to shaping antitumor immunity and can dramatically diminish the efficacy of immunotherapies. However, the effects of PDAC tumor burden on systemic NK cells remain poorly understood. Emerging evidence suggests that cancer can induce systemic immune perturbations, including an increase in immunosuppressive myeloid populations in lymphoid organs. Understanding the interplay between these systemic factors and local anti-tumor immunity is crucial for developing effective immunotherapies against PDAC.
Methods: To investigate the systemic effects of PDAC on the NKs, we orthotopically implanted immunocompetent C57BL/6 mice with syngeneic pancreatic tumor cell lines derived from *LSL-KrasLSL.G12D/+; LSL-p53R172H/+; Pdx1-Cre* (KPC mice) PDAC mouse model. Next, we evaluated the functional, transcriptional, and metabolic profiles of endogenous and adoptively transferred syngeneic NK cells.
Results: Our study showed remarkable impairment in the effector function of endogenous and adoptively transferred NKs obtained from tumor-bearing mice compared to the healthy mice. Endogenous and adoptively transferred NK cells displayed attenuated cytotoxic killing capabilities against pancreatic tumor cells in the *ex vivo* assay. Besides, our metabolomics data demon stared an altered metabolism in the NK cells from the pancreatic tumor mice compared to healthy control cohort. Depleting myeloid cells rescued the proportion and cytotoxic function of systemic NK cells in pancreatic tumor-bearing mice. Also, myeloid cells alter the metabolism of NK cells, which underlie the attenuated cytotoxic function of these cells in pancreatic cancer.
Conclusion: Our study proves the critical role of the systemic immunosuppressive in impairing the function of endogenous and adoptively transferred NK cells in pancreatic cancer. We demonstrate that inhibiting myeloid cells enhances the anti-tumor function of NK cells. Our findings also show the importance of considering the systemic immune microenvironment in cancer patients when utilizing CAR T/NK-based therapies.

#5256 Tertiary Lymphoid Structures as Predictors of Immune Checkpoint Inhibitor Efficacy in NSCLC and the Development of an Ex Vivo Model for Precision Medicine.

Jean-Philippe Guegan[1], Imane NAFIA[1], Florent PEYRAUD[2], Lucile VANHERSECKE[3], Christophe Rey[1], Lucie Lefevre[1], Assia CHAIBI[1], **Alban Bessede**[1], Antoine ITALIANO[1]

[1]Explicyte Immuno-Oncology, Bordeaux, France,[2]Department of Medicine, Institut Bergonie, Bordeaux, France,[3]Department of Pathology, Institut Bergonie, Bordeaux, France

Background: Tertiary lymphoid structures (TLS) emerge as essential players in modulating anti-tumor immune responses and shaping the outcomes of immune checkpoint inhibitor (ICI) therapies. Here we explored, on the one hand, the impact of TLS maturity on clinical outcomes in non-small cell lung cancer (NSCLC), and introduce, on the other hand, a novel ex vivo tool model to investigate TLS-mediated immune synapses and test potential therapeutic strategies.
Methods: In this study, 509 NSCLC patients from the BIP cohort (NCT02534649) - treated with ICI-based regimens - were retrospectively analyzed and assessed by multiplex IHC (Ventana Discovery, Roche) and digital pathology (PhenoImager HT, Akoya). TLS status and maturity were evaluated histologically in pre-treatment FFPE samples, with mature TLS (mTLS) defined by the presence of CD23+ follicular dendritic cells. Correlations with overall survival (OS), progression-free survival (PFS), and response rates were analyzed. Interestingly, an ex vivo model was developed using T follicular helper (Tfh) and memory B cells isolated from human tonsils. Tfh cells (CD4+/CXCR5+/PD1high/ICOShigh) and memory B cells (CD19+/CD27+) were co-cultured in the presence of staphylococcal enterotoxin B (SEB). Effects of ICIs (e.g., anti-PD1, anti-TIGIT, anti-CTLA4), anti-CD40, and kynurenine (IDO metabolite) were evaluated on IgG produced levels - quantified by ELISA - as a surrogate of synapse function.
Results: TLS were detected in 49.5% of cases, of which 28.7% were classified as mTLS. mTLS were significantly associated with improved OS and PFS, independent of PD-L1 expression and molecular status. Long-term survivors (≥24 months) had a higher frequency of mTLS (41.3% vs. 22.9%; $p < 0.001$). Response rates to ICIs were notably higher in mTLS-positive patients than in mTLS-negative patients (56.8% vs. 33.6%; $p < 0.001$). On the other hand, the ex vivo model demonstrated robust IgG production in Tfh-B cell co-cultures, modulated positively by ICIs (Nivolumab, Atezolizumab, Tiragolumab, Ipilimumab) and negatively by anti-CD40 and kynurenine.
Conclusions: This study highlights the critical role of TLS maturity in predicting response to ICIs and improving survival outcomes in NSCLC. The ex vivo Tfh-B cell model provides a novel platform to evaluate therapies targeting TLS function. Combining TLS stratification via histology with Tfh-B cell synapse-enhancing agents represents a promising approach in precision medicine.

#5257 Functional profiling of γδ T cell subsets in malignant pleural effusion and their prognostic value in advanced lung cancer.

Tsai-Tsen Shih[1], Yi-Chieh Wu[2], Hsing-Chen Tsai[3]

[1]Yanping High School, Taipei, Taiwan,[2]Institute of Toxicology, National Taiwan University, Taipei, Taiwan,[3]Department of Internal Medicine, National Taiwan University Hospital, Institute of Toxicology, National Taiwan University, Taipei, Taiwan

The tumor immune microenvironment reflects a patient's immune reserve and offers valuable insights for clinical decision-making. It has the potential to stratify late-stage cancer patients, identifying those likely to benefit from aggressive therapies and those better suited for palliative care. However, obtaining detailed immune profiling from tumor tissues often requires invasive biopsies and poses additional risks. Malignant pleural effusion (MPE), a common complication in advanced lung cancer, provides a minimally invasive alternative for evaluating microenvironmental immune dynamics via thoracentesis. In this study, we analyzed the immune cell composition of malignant pleural effusions (MPE) from 73 patients with late-stage lung adenocarcinoma using high-dimensional spectral flow cytometry and a comprehensive panel of immunophenotypic and functional markers. Our analysis focused on cytotoxic immune cell populations, including NK cells, NKT cells, CD8+ T cells, and γδ T cells. Notably, γδ T cells constituted approximately 1.4% of viable cells, with the Vδ1 and Vδ2 subpopulations representing 32.3% and 46.6% of the γδ T cell compartment, respectively. Higher γδ T cell abundance was associated with shorter overall survival, while no correlation was observed for other immune cell populations. Further analysis revealed that a higher Vδ2/Vδ1 ratio correlated with poorer clinical outcomes. Conversely, elevated expression of the activating receptor DNAM-1 (CD226) on Vδ1 cells was observed in long-term survivors (>2 years) compared to short-term survivors (<6 months), independent of mutation type or treatment modality. These findings were validated using the TCGA lung cancer datasets, confirming a strong link between high DNAM-1 expression and extended survival. In conclusion, γδ T cell subpopulations in MPE, particularly the Vδ1 and Vδ2 subsets, are promising biomarkers for immune fitness and prognosis in advanced lung adenocarcinoma. These results highlight the potential of γδ T cells to identify late-stage patients likely to benefit from aggressive therapies and emphasize the need for larger-scale studies to confirm these findings.

#5258 The immunomodulatory effects of daratumumab-based therapy on functional high-risk relapse/refractory multiple myeloma (fHRMM) patients enrolled in the MyDRUG trial.

Chaitanya R. Acharya[1], Darwin D'souza[2], Reyka Jayasinghe[3], Mark Hamilton[1], Zhihong Zhihong[2], Seunghee Kim-Schulze[2], Hearn J. Cho[1], Shaji Kumar[4], George Mulligan[1]

[1]Multiple Myeloma Research Foundation, Norwalk, CT,[2]Human Immune Monitoring Center, Icahn School of Medicine at Mount Sinai, New York, NY,[3]Washington University in St. Louis, St. Louis, MO,[4]Department of Internal Medicine, Mayo Clinic, Rochester, MN

Background: The goal of this study is to evaluate the immunomodulatory effects of daratumumab-(anti-CD38)-based therapy on fHRMM patients enrolled in the MMRF sponsored MyDRUG umbrella clinical trial (NCT03732703) over the duration of therapy and to correlate immune changes with patient response.
Methods: fHRMM who had received 1-3 prior therapies (exposed to at least one proteasome inhibitor and an IMiD) were given a combination of daratumumab (D), ixazomib (I), pomalidomide (P) and dexamethasone (d) (D-IPd) quadruplet therapy. Bone marrow (BM) aspirates were then collected from patients prior to therapy (BL), after 2 cycles (EOC2), after 4 cycles (EOC4) of the quadruplet therapy and at the end of treatment or disease progression (EOT). Immune and tumor fractions were enriched by CD138 selection. Here, CD138- bone marrow mononuclear cells were isolated and transcriptionally profiled by scRNAseq and scTCRseq.
Results: Single-cell RNA and TCR profiles were generated from ~90,000 cells across 41 samples from 16 subjects. scRNAseq identified CD3+ T cells, NK cells, classical and non-classical monocytes and B cells. CD8+ T cells were further resolved into their subsets including precursor (T_{PEX}) and terminally exhausted T cells (T_{EX}) within the bone marrow microenvironment. Within the CD8+ T cell compartment, an expansion of the effector memory (T_{EM}) cell population was observed following 2-cycles of therapy (EOC2). However, this T_{EM} expansion was not sustained at timepoint EOC4. Towards the end of therapy, enrichment of precursors to exhausted and terminal dysfunctional effector memory cells (T_{EMRA}) was observed. Subjects with partial (PR), very good partial (VGPR) and complete (CR) best overall responses showed an initial increase in T_{EM} cells followed by a slow expansion of T_{EX} and T_{EMRA} cells over time. Longitudinal tracking of T cell clonality showed preferential progressive expansion of T_{EMRA} cells after the therapy. We mapped hyperexpanded (more than 16 copies) clonotypes, presumed to be tumor-reactive, to various CD8+ T cell subsets. These hyperexpanded clonotypes mapped to the T_{EMRA}, T_{EX} and T_{EM} CD8+ T cells. Analysis of TCR repertoires clonality and diversity also demonstrated a reduced richness and increased clonality of CD8+ TCRs in responders.
Conclusions: Paired single cell RNA and TCR sequencing of BM T cells from RRMM patients receiving D-IPd quadruplet therapy revealed a selective expansion of clonotypic CD8+ T cells with a clonal replacement of BM exhausted clonotypes by dysfunctional circulating CD8+ T cells in responders over time.

#5259 Unraveling the impact of smoking behavior on the immune landscape of prostate cancer.

Amod Sharma[1], Mohammad Aslam Khan[2], Muhammad Tahir[3], Ajay P. Singh[1], Seema Singh[1]

[1]Department of Cell and Molecular Biology and Cancer Center and Research Institute, University of Mississippi Medical Center, Jackson, MS,[2]Department of Pharmacy and Pharmaceutical Sciences, St. Jude Children's Research Hospital, Memphis, TN,[3]Department of Pathology, University of South Alabama, Mobile, AL

Smoking is a significant modifiable risk factor for prostate cancer (PCa) diagnosis, recurrence, and mortality. Immune cells play crucial role in cancer-associated inflammation, having a significant impact on tumor fate from early neoplastic transformation to metastatic spread and influencing treatment outcomes. We hypothesize that, in addition to causing carcinogenic mutations, tobacco smoke alters the tumor microenvironment to promote tumor progression. In this study, we examined the effect of smoking behavior on the immune landscape of prostate tumors by performing multiplex gene expression analysis of various immune cell types using the nanoString nCounter® PanCancer immune profiling panel. A total of 24 prostate tumor tissues (Gleason's score: 7-8) were analyzed, including 12 from never smokers, 7 from current smokers, and 5 former smokers. Unsupervised hierarchical clustering based on the immune scores from different immune cell types grouped PCa samples into two major clusters, representing low and high immune cell infiltration. A higher proportion of current smokers were grouped in the high immune infiltration cluster, whereas never smokers and former smokers predominantly fell into low immune infiltration cluster. In direct comparisons, significantly higher infiltration of tumor-associated macrophages (TAM) ($p=0.037$) and regulatory T cells (Tregs) ($p=0.046$) was observed in current smokers compared to never smokers, with a trend for lower mast cell infiltration ($p=0.055$). Additionally, a significant positive correlation ($r=0.42$; $p=0.036$) was observed between TAM with Tregs infiltration in all samples, with a stronger correlation ($r=0.88$; $p=0.008$) in current smokers. A total of 89 differentially expressed genes (DEGs) at a 1.5-fold change cutoff were identified between current and never smokers, with 77 genes being upregulated and 12 downregulated in current smokers. Interestingly, only 17 DEGs were identified between never smokers and former smokers. Pathway analysis of the DEGs in current smokers revealed activation of 'primary immunodeficiency' and 'PD-L1 expression and PD-1 checkpoint' pathways, while the 'antigen processing and presentation' and 'NF-kappa B signaling' pathways were repressed. Collectively, these findings suggest that current smokers have a relatively compromised tumor immune microenvironment compared to never and former smokers with PCa, potentially facilitating rapid tumor growth and aggressive progression. These findings also have implications for developing novel immune-based therapeutic interventions for PCa patients based on their smoking behavior.

#5261 The significance of CD200R1 on NK cells in the endometrial cancer tumor microenvironment.

Youssef Youssef[1], Caprice D. Eisele[1], Joel Cesar[1], Noah Koenigs[2], Micheal Ruesch[1], Courtney J. Riedinger[1], Paulina J. Haight[1], Ankur Sheel[1], Bethany L. Mundy-Bosse[1], Bradley L. Blaser[1], Casey M. Cosgrove[1], Aharon G. Freud[1]

[1]The Ohio State University Wexner Medical Ctr., Columbus, OH,[2]Biomedical Sciences Graduate Program, The Ohio State University Wexner Medical Ctr., Columbus, OH

Endometrial cancer (EC) is the most common gynecologic cancer in the USA and is expected to surpass ovarian cancer in mortality. Advanced-stage EC has poor outcomes with high relapses (>35%) and low chemotherapy response (50%). Immune checkpoint blockade (ICB) therapies are often ineffective in EC, indicating a need for better understanding the tumor microenvironment (TME). In many cancers, cytotoxic NK cell (cNK) function is suppressed in the TME through dysregulated differentiation toward an immune-suppressive tissue-resident (trNK) state. Single cell RNA-sequencing (scRNA-seq) data on treatment naïve primary EC samples (n=6, unpublished; n=11; Regner MJ et al), revealed trNK populations expressing the inhibitory glycoprotein CD200 receptor 1 (CD200R1). CD200R1+ trNK cells correlated with high expression of CD200 (the corresponding ligand on EC). CD200 is known to promote NK cell-mediated immunotolerance in physiological settings such as pregnancy to prevent abortion and ectopic endometrial implantation (endometriosis). In cancers, CD200 mediates inhibition of CD200R1+NK cells and promotes an immunosuppressive TME through TGF-β. Given that TGF-β is known to drive cNK-to-trNK polarization, we investigated their patterns in the scRNA-seq data revealing correlation between CD200 expression and TGF-β. Immunohistochemical staining of EC tissue showed trNK cells were enriched within tumor glands compared to stroma (6 vs. 2 cells per 400× high-power field; n=6, p=0.046), suggesting an enrichment of non-cNK cells within EC glands. *Hypothesis: CD200+ ECs promote a TME conducive to dysfunctional trNK phenotypes with impaired differentiation into effector cNK cells, enabling immune evasion and reduced ICB efficacy.* To address this, we performed flow cytometry on EC primary samples (n=17) and confirmed CD200R1 expression on trNK compared to benign endometrium (n=8) (32% vs 5% respectively; p=0.006). CD200R1 expression on trNK correlated with low/undetectable levels of NK cell cytotoxic/maturity markers (granzymes, perforin, CD94, KIRs, CD16, and EOMES). Functionally, co-culturing CD200R1+ NK precursor cells with stromal feeder cells overexpressing CD200 impaired NK cell maturation (increased innate lymphoid cells and decreased cNK). Further, co-culturing immature NK cells with human stromal cells in the presence of TGF-β promoted a trNK phenotype with elevated CD200R1 expression. This trNK polarization was prevented by adding anti-TGF-β. Altogether, our findings suggest that a CD200/CD200R1 axis modulates NK cell differentiation and function within the EC TME, potentially downstream of TGF-β signaling. Ongoing studies seek to determine whether blocking this axis enhances ICB efficacy, and potentially support clinical trials using Samalizumab (humanized monoclonal anti-CD200 antibody).

#5262 A zinc transporter drives pancreatic cancer cachexia via the crosstalk between tumor cells and immune microenvironment.

Zhijun Zhou[1], Mingyang Liu[1], Yu Ren[1], Jingxuan Yang[1], Xiuhui Shi[1], Yang Cai[1], Alex X. Arreola[1], Wenyi Luo[2], Kar-Ming Fung[1], Chao Xu[1], Ryan D. Nipp[1], Michael S. Bronze[1], Lei Zheng[3], Yi-Ping Li[4], Courtney W. Houchen[1], Yuqing Zhang[1], Min Li[1]

[1]The University of Oklahoma Health Sciences Center, Oklahoma City, OK,[2]Yale School of Medicine, New Haven, CT,[3]Johns Hopkins University School of Medicine, Baltimore, MD,[4]The University of Texas Health Science Center at Houston, Houston, TX

Background: Cachexia is a systemic disease manifested with muscle atrophy and appetite loss, especially in patients with pancreatic cancer. Emerging evidence showed that cachexia is an inflammation-associated disease. But the role of tumor immune microenvironment in cachexia remains elusive. ZIP4, a zinc transporter, plays a critical role in promoting pancreatic cancer cachexia. We aimed to study how ZIP4 promotes cancer cachexia via interacting with immune microenvironment.
Methods: We evaluated the association between cachexia and several major immune cell types in the tumor tissue of patients with cancer. Genetically engineered mouse models and clodronate treatment were used to establish the mouse models with macrophage depletion. Flow cytometry, western blotting, chromatin immunoprecipitation assay and luciferase assay were used to study the underlying mechanism.
Results: We found that macrophage infiltration level in tumor tissue is associated with the prevalence and severity of cancer cachexia.

Depletion of macrophage using *Ccr2* knockout mice (*Ccr2*$^{-/-}$ mice) or clodronate treatment ameliorated cancer cachexia. Cytokine array identified TWEAK (also known as TNFSF12) as a key player in cancer cachexia. The basal level of TWEAK is low in macrophages and tumor cells. However, TWEAK level drastically increased in tumor cells when cocultured with macrophages. Mechanistically, macrophage-derived CCL5 upregulates TWEAK expression in cancer cells through p65 signaling axis. On the other end, ZIP4 induced CCL2 secretion from cancer cells through ZXDC, a zinc dependent transcription factor, leading to the recruitment and activation of macrophages. Overexpression of TWEAK restored the cachexia phenotype of cancer cells with low cachexia potential. Blocking ZIP4-TWEAK signaling axis restored appetite and muscle mass in pancreatic cancer mouse models.
Conclusion: This study identified an uncharacterized role of tumor immune microenvironment in promoting cancer cachexia through increasing TWEAK secretion from tumor cells. Specifically, ZIP4 increased CCL2 secreting from tumor cells, which recruits and activates macrophages. The activated macrophages secreted CCL5, upregulating TWEAK expression in cancer cells through TRAF6/NF-κB axis, thus forming a feedforward loop to promote cancer cachexia.

#5263 The spatial landscape of tumor-infiltrating lymphocytes in stage III colorectal cancers by single-cell spatial analysis.

HoJoon Lee[1], Andrew Su[1], Minh Tran[2], Richard Delacruz[3], Anuja Sathe[1], Xiangqi Bai[1], Ignacio Wichmann[1], Lance Pflieger[3], Bryce Moulton[3], Tyler Barker[3], Derrick Haslem[3], David Jones[3], Lincoln Nadauld[3], Quan Nguyen[2], Hanlee P. Ji[1], Terence Rhodes[3]

[1]Stanford University, Stanford, CA,[2]University of Queensland, Brisbane, Australia,[3]Intermountain Healthcare, Saint George, UT

Colorectal cancer (CRC) is a leading cause of cancer-related deaths. An important contributor to CRC development, maintenance and metastasis is the tumor microenvironment (TME). This cellular milieu contains neighboring tumor epithelial cells, normal epithelium, fibroblasts, endothelial cells, immune cells and many others. The characterization of the TME is critical for understanding tumor biology as well as determining specific features with clinical implications. Representing a subset of the TME, the tumor immune microenvironment (TIME) has specific cellular characteristics that are important predictors for the response to treatments such as immunotherapy. However, the accurate interpretation of complex spatial cell patterns poses significant challenges. To address these issues, we developed a novel computational framework that systematically analyzes spatial data. We conducted a spatial analysis using imaging mass cytometry applied to stage III colorectal adenocarcinomas. This study used multiplexed markers to distinguish individual cells and their spatial organization from 52 colorectal cancers. We determined the landscape features of cellular spatial features in the CRC tumor microenvironment. This spatial single-cell analysis identified 10 unique cell phenotypes in the tumor microenvironment that included stromal and immune cells with a subset which had a proliferative phenotype. These special features included spatial neighborhood interactions between single cells as well as different tissue niches, especially the tumor infiltrating lymphocyte regions. We applied a robust statistical analysis to identify significant correlations of cell features with phenotypes such as microsatellite instability or recurrence. We determined that microsatellite stable (MSS) colorectal cancers had an increased risk of recurrence if they had the following features: 1) a low level of stromal tumor-infiltrating lymphocytes, and 2) low interactions between CD4+ T cells and stromal cells. Our computational framework precisely defined two types of tumor-infiltrating lymphocytes (TILs) based on their spatial properties in the local tumor microenvironment: 1) intra-tumoral TILs within cancer CNs and 2) sTILs within stromal CNs. These spatial characterizations of TILs provide deeper insights into their role, beyond simple lymphocyte abundance. Moreover, our results point to the utility of spatial single-cell interaction analysis in defining novel features of the tumor immune microenvironments and providing useful clinical cell-related spatial biomarkers.

#5265 Single cell and spatial analysis unravels the immunosuppressive tumor microenvironment, HPV-associated features, and potential immunotherapy response in vulvar squamous cell carcinoma.

Athena Golfinos-Owens, Taja Lozar, Evie Carchman, Paul F. Lambert, Megan Fitzpatrick, Huy Q. Dinh

University of Wisconsin-Madison, Madison, WI

Vulvar squamous cell carcinoma (VSCC) is a rare gynecological cancer with a projection of a 35% increase in the next 20 years in North America. HPV is a known risk factor in VSCC, but HPV+ and HPV- subtypes of VSCC have previously been reported to be biologically but not clinically distinct. In addition, VSCC could be categorized by HPV and TP53 status with different prognoses. However, the VSCC tumor microenvironment (TME) and its contributions to patient clinical outcomes are not well understood. Here, we use single-cell and spatial omics to interrogate VSCC TME transcriptional and cellular heterogeneity as a foundation for understanding the tumor, stromal, and immune cell compartments of HPV+ and HPV-VSCC. Our integrated bioinformatics analysis of 8 scRNA-seq samples (5 HPV+ and 3 HPV-) from vulvar cancer patients identified diverse stromal and immune compartments with immunosuppressive features (high presence of Tregs, pro-tumoral macrophages and myofibroblasts) presenting “immunologically cold” tumor phenotypes. In addition, we identified HPV+ epithelial cells and analyzed HPV-associated cell-cell interactions. Furthermore, we used scRNA-Seq gene signature to deconvolute a bulk 800-gene dataset from an independent patient cohort of 48 samples with different HPV subtypes, including integrated and beta-HPV, to identify TME features associated with different HPV risk subtypes. Bioinformatics deconvolution results showed a new way to subtype VSCC, including a subset of “immunologically hot tumors” (increased CD8 T cells and dendritic cells) with high expression of known and emerging immunotherapy response markers (PD-L1 high, CXCL9 high). Lastly, we integrate scRNA-Seq with patient-matched spatial transcriptomics to identify cellular neighborhood and Ligand-Receptor interactions that may be associated with HPV, subtypes, and clinical outcomes. This study reports a comprehensive single-cell and spatial VSCC TME atlas, highlighting the immunosuppressive features of the TME, differences between HPV+ and HPV- samples, and identification of a subset of patients with “immunologically hot” tumors that may respond favorably to immunotherapy. These findings provide a foundation and resource to further study VSCC TME in both HPV+ and HPV- subtypes. Future studies include validating and studying potential biomarkers of clinical outcomes and immunotherapeutic targets of VSCC treatment.

#5267 Unveiling associations between Duffy-null status and circulating chemokines among a diverse breast cancer patient cohort.

Stevens M. Patino[1], Kenya Bynes[1], Jason White[1], Danielle S. Doucette[1], Emma Guyonnet[2], Julie Sahler[3], Brian Stonaker[2], Avery August[3], Lisa Newman[2], Rachel Martini[1], Melissa Davis[1]

[1]Institute of Translational Genomic Medicine, Morehouse School of Medicine, Atlanta, GA,[2]Weill Cornell Medicine, New York, NY,[3]Cornell University, Ithaca, NY

Breast cancer (BC), the most diagnosed cancer and the leading cause of cancer-related mortality among women worldwide, includes triple-negative breast cancer (TNBC), its most aggressive subtype, which is disproportionately prevalent among individuals of West African ancestry. Our previous studies have demonstrated a correlation between West African ancestry-specific expression of the Duffy Antigen Receptor for Chemokines (DARC) and TNBC incidence. DARC regulates CC and CXC chemokines, maintaining homeostatic chemokine levels. However, the Duffy-null polymorphism (rs2814778), fixed in individuals of West African descent as an evolutionary defense against Plasmodium vivax malaria, results in the absence of DARC on erythrocytes. We aim to understand how this absence may influence chemokine regulation and contribute to ancestry-specific differences in the tumor microenvironment. We hypothesize that individuals with the Duffy-null genotype exhibit higher circulating chemokine levels than those with the DARC allele among BC cases.

Since 2006, the International Center for the Study of Breast Cancer Subtypes (ICSBCS) has recruited patients from sites across the United States and Africa, collecting blood and tumor tissue samples. We utilized the Luminex multiplex assay to analyze plasma from the ICSBCS cohort, quantifying 50 biomarkers using the bead-based ELISA platform. Plate/batch effect normalization was done with MDimnNormn, and statistical tests were performed using JMP Pro 16. Genotyping for the Duffy-null allele (C) was conducted using TaqmanTM GTXpressTM Master Mix and specific probes on an Applied Biosystems 7500 Fast Real-Time PCR system.

Our cohort included Duffy-null (CC, n = 183), Duffy-null carriers (CT, n = 40), and non-Duffy-null (TT, n = 294) individuals. Univariate analysis of chemokine levels revealed significant associations between Duffy-null status and the following biomarkers: MCP-1/CCL2 ($p = 0.0116$), Gro- b/CXCL2 ($p = 0.0137$), MIP1a/CCL3 ($p = 0.0316$), GCP2/CXCL6 ($p = 0.0423$), I-TAC/CXCL11 ($p = 0.0008$), BCA- 1/CXCL13 ($p = 0.0252$), MIP3b/CCL19 ($p = 0.0023$), 6Ckine/CCL21 ($p = 0.0066$), and MPIF1/CCL23 ($p < 0.0001$).

These findings highlight significant associations between Duffy-null status and circulating chemokine levels, introducing novel BC biomarkers that characterize the relationship between Duffy-null status, BC, and West African ancestry. Moreover, these findings have important prognostic and therapeutic implications, particularly for addressing ancestry-specific factors in BC. Understanding these associations advances precision medicine efforts to address BC disparities affecting individuals of West African ancestry globally. Multivariate analyses to control for co-variates are ongoing to support these findings further.

#5268 Impede cancer brain metastasis by enhancing innate immunity with CCL2 targeting.

Yimin Duan, Lin Zhang, Yutao Qi, Xiangliang Yuan, Dihua Yu

UT MD Anderson Cancer Center, Houston, TX

Among the more than 300,000 newly diagnosed breast cancer patients every year, 5~20% of them will develop symptomatic brain metastasis (BrM) with a lower than 20% of 1-year survival rate. Our group previously reported that brain tumor microenvironment (TME)-mediated *PTEN* downregulation in BrM tumor cells leads to increased secretion of the CCL2 chemokine, which enhances the infiltration of $CCR2^+$ bone marrow derived myeloid cells to promote brain metastasis outgrowth. Here, we further assessed whether targeting CCL2 can effectively inhibit BrM progression. Indeed, targeting CCL2 through either knocking-down CCL2 or with Ccl2-blocking antibody exhibited significant inhibition of BrM growth. Mass cytometry analysis of the brain TME demonstrated dramatically decreased immune suppressive myeloid populations in the CCL2-targeted group, which is consistent with our previous findings. Remarkably, natural killer (NK) and $CD8^+$ T cells were dramatically increased in the TME of CCL2-knockdown BrMs. To test the contribution of the increased NK cells and/or $CD8^+$ T cells on BrM inhibition by CCL2-targeting, we specifically depleted NK cells or $CD8^+$ T cells with anti-NK1.1 or anti-CD8 antibody. Our data showed that depleting NK cells, but not $CD8^+$ T cells, blocked BrM inhibition effect of CCL2-targeting, indicating that the increased infiltration of NK cells significantly contributed to the BrM inhibition under CCL2 knockdown. These findings led us to postulate that CCL2 inhibits NK cell infiltration into BrM TME to facilitate BrM growth. Our in vitro NK cell migration assay with high- versus low-CCL2 tumor cells showed that high CCL2 level inhibited NK cell migration. In summary, our data indicate the potential of CCL2 targeting for controlling breast cancer BrM progression and highlight the pivotal role of NK cell-mediated innate immunity in combating BrM.

#5269 CXCL10 promotes melanoma angiogenesis and tumor growth.

J.-H. Lee;
Dong-A University, Busan, Korea, Republic of

Upregulation of CXC motif chemokine 10 (CXCL10) in melanoma patients has been found to be associated with melanoma progression. However, the role of endogenous CXCL10 from the host in melanoma tumor growth remains unclear. In the present study, we found that host-derived endogenous CXCL10 production was dramatically augmented during subcutaneous B16F10 melanoma tumor growth and that host ablation of CXCL10 in Cxcl10-/- mice showed a decrease in both angiogenesis and tumor growth of B16F10 melanoma in vivo. Several signaling pathways involved in production of pro-angiogenic factors and tumor growth were activated by CXCL10 in B16F10 melanoma cells. CXCL10 increased expression of pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), platelet-derived growth factor subunit-B (PDGF-B), fibroblast growth factor 2 (FGF2), hepatocyte growth factor (HGF), and angiopoietin 2 (Angpt2), in B16F10 melanoma cells, resulting in enhanced tube formation and proliferation of human umbilical vein endothelial cells in vitro. In addition, CXCL10 directly enhanced B16F10 melanoma tumor growth in an in vitro three-dimensional cell culture system. Together, our findings reveal that amplified host-derived endogenous CXCL10 is critical for B16F10 melanoma angiogenesis and tumor growth. Therefore, CXCL10 might represent a therapeutic target for melanoma.

#5270 Novel dual-stain IHC assay enables discovery with enhanced clarity.

Scott Anderson[1], Stephen Pyper[1], **Jack Heath**[2], Julio Poveda[3]

[1]Leica Biosystems, Newcastle Upon Tyne, United Kingdom,[2]Leica Biosystems, Vista, CA,[3]University of Miami Miller School of Medicine, Miami, FL

Background: Immunohistochemistry (IHC) analysis has become a powerful and integral component in the diagnostic workup of tumors. It has been essential in biomarker discovery, with a growing demand for high-output methods that maximize data from limited tissue samples. Chromoplex II, an advanced technique that enables the simultaneous detection of dual stains on a single slide, permits the selection of primary antibodies to be stained in either color, aiding tissue differentiation while minimizing the interference often seen with endogenous pigments or slide debris. The duplex approach also reduces slide usage compared to single-stains and individual slides.
Methods: Using the BOND-III Automated IHC and in-situ hybridization (ISH) stainer from Leica Biosystems, Chromoplex II was evaluated in three dual-marker applications. At least five cases with known tumors of distinct tumor histology/classification were utilized to examine differences between the distinct cellular histology (i.e., adenocarcinoma and squamous carcinoma) as well as classical Hodgkin lymphoma (cHL) versus nodular lymphocyte predominant Hodgkin lymphoma (NLP). Another commonly encountered scenario is the difficulty in separating lung adenocarcinoma and bystander macrophages, so these tissues were tested as well. Stained slides were scanned using the Aperio GT 450 scanner from Leica Biosystems and evaluated by a clinical pathologist for specificity and comparability to single staining. The marker combinations and tissues tested were the following:•CD20/CD30: Lymph node•CK5/Napsin A: Lung •CD163/PD-L1: Lung
Results: In all three marker combinations, the Chromoplex II kit produced distinct red and green stains, providing a clear visualization of target markers. Importantly, it minimized background interference, significantly enhancing clarity in challenging tissue sections. The slides exhibited comparable signal and reproducibility compared to single marker staining, and many examples were noted in which the dual stain provided vital context about the nature of the relationship between the two markers studied.
Conclusions: Chromoplex II represents a promising tool for high-throughput biomarker studies in research, offering benefits in both clarity of staining and operational efficiency by reducing slide volume. Furthermore, additional studies and/or combinations are necessary to explore expanded marker combinations for broader cancer biomarker research applications.

TUMOR BIOLOGY: Tumor Microenvironment and Metastasis
Poster Session

#5273 Changes in the tumor immune microenvironment during disease progression in gynecological carcinosarcoma: Exploratory analysis from the randomized ROCSAN GINECO trial.

Sonia Canjura-Rodriguez[1], Cassandra Assaf[2], Victor Heurtier[3], Annalisa Pesavento[4], Justine Berthet[2], Audrey Bellesoeur[3], Coriolan Lebreton[5], Sheik Emambux[6], Dominique Berton[7], Magali Provansal[8], Guillaume Bataillon[9], Lucas Michon[1], Jessie Auclair[1], Alexandra Laine[1], Valery Attignon[1], Christophe Caux[2], Anthony Ferrari[1], Ivan Bieche[3], Bertrand Dubois[2], Isabelle Ray-Coquard[1], **Pierre Saintigny**[1]

[1]Centre Leon Berard, Lyon, France,[2]Centre de Recherche en Cancerologie de Lyon, Lyon, France,[3]Institut Curie, Paris, France,[4]Universite de Bourgogne Franche-Comte, Besancon, France,[5]Institut Bergonie, Bordeaux, France,[6]CHU de Poitiers - Hopital de La Miletrie, Poitiers, France,[7]ICO - Centre Rene Gauducheau, Saint-Herblin, France,[8]Institut Paoli Calmettes, Marseille, France,[9]IUCT - Oncopole Toulouse, Toulouse, France

Background: Gynecologic carcinosarcomas (CS) are highly aggressive/rare tumors. Most patients relapse after first-line therapies and responses to systemic therapy remain low. ROCSAN (NCT03651206) is a multicentric randomized phase II evaluating dostarlimab (anti-PD1) in combination with niraparib (PARPi) compared to standard of care in the treatment of metastatic/recurrent (R/M) CS. Objective: Mandatory tumor tissue archival from naïve initial disease (ND) & baseline biopsies at relapse (R/M) were used to decipher the biological changes of CS in R/M disease vs. ND.
Methodology: bulk transcriptomic profiling was performed on paired samples from 60/63 included patients. Differential expression, gene ontology (GO), and immune cell deconvolution were performed. Our previously reported HOT signature was computed (GSVA) (PMID36038492). Additionally, a 7-color multiplex immunofluorescence (mIF) analysis of 52 paired samples (CD20, Ki67, CD3, CD8, FoxP3, panCK, DAPI) was done to evaluate total T cells, CD8 and CD4 Teff, Treg, and B cells cell densities and tertiary lymphoid structures (TLS).
Results: Differential expression analysis adjusted for FIGO stage, primary site and number of previous systemic therapies identified 1,502 and 584 genes up and down regulated respectively in R/M disease vs. ND. Among 13 immune checkpoints (ICP) and 22 of their ligands (ICPL), 8 ICP (TIGIT, HAVCR2, CTLA4, VSIR, CD40LG, TNFRSF9, CD27, ICOS) and 4 ICPL (PD-L1, PDCD1LG2, CD80, CD86), were upregulated in R/M disease vs. ND. Top GO enriched pathways in R/M vs. ND included immune activating pathways, regulation of lymphocyte activation and positive regulation of cytokine production. Among 50 biological hallmarks (GSEA), R/M showed a significant enrichment in inflammatory response, TNFA signaling via NFKB, and IFNG response vs. ND. Deconvolution analysis found an increase in NK cells (P=0.024), Macrophage/Monocyte (P=0.001) and Neutrophils (P=0.023) in R/M disease vs. ND. The HOT signature score was found to be increased in R/M disease vs. ND (P=0.01). The proportion of HOT CS (score>0) was 33/60 (55%) and 23/60 (38%) in R/M and ND respectively. Among the 37/60 (62%) COLD CS (score<0) in ND, 18/37 (49%) became HOT in R/M. Among the 23/60 (38%) HOT CS at ND, 8/23 (35%) became COLD at R/M. mIF analysis showed that total T cells (P=0.037), CD8 Teff (P=0.039), CD4 Teff (P=0.034), and CD4 Treg (P=0.003) increased in R/M disease vs. ND. Intriguingly, TLS density decreased in R/M disease vs. ND (P=0.035).
Conclusion: When compared with ND, R/M CS host a greatest immunologically active microenvironment, as suggested by the changes observed in the HOT score and HOT/COLD phenotype, implying potentially more activity for immune therapy in relapse than for localized initial therapy. Whether this could be used to select patients for immunotherapy should be investigated.

#5275 A dynamic equilibrium of innate immune deaminases and deregulation in hematopoietic malignancies.

Jane Marie Isquith[1], Inge Van der Werf[1], Jessica Pham[1], Thomas Whisenant[2], Ludmil Alexandrov[3], Catriona Jamieson[1]

[1]Sanford Stem Cell Institute, UC San Diego, San Diego, CA,[2]Center for Computational Biology and Bioinformatics, UC San Diego, San Diego, CA,[3]Department of Bioengineering, Department of Cell and Molecular Medicine, UC San Diego, San Diego, CA

Inflammatory cytokine responsive apolipoprotein B mRNA-editing enzyme, catalytic polypeptide (APOBEC3) cytosine deaminases have been shown to be deregulated during cancer evolution, presenting as gene expression changes and inclusion of distinct C-to-T mutation patterns. However, the exact mechanisms by which APOBEC3 enzymes promote cancer initiation and progression remain to be investigated, especially in hematopoietic malignancies. Building on our advanced sequencing data of healthy, pre-leukemic, and myeloproliferative neoplasm patient samples, we seek to uncover normal and malignant patterns of APOBEC3C overexpression in hematopoietic cells. Overexpression of lentiviral APOBEC3C in normal cord blood, healthy bone marrow, and myeloproliferative neoplasm (MPN) patient hematopoietic stem/progenitor cells (HSPCs) allows us to study the effects of deaminase dysregulation in the hematopoietic niche. These overexpression studies show novel differential gene expression changes, repetitive element alterations, RNA hyper-editing sites, and DNA mutation signatures induced by APOBEC3 mutagenesis. In addition to APOBEC3, we are exploring the upregulation of adenosine deaminase acting on RNA1 (ADAR1), as we have previously shown that these two innate immune deaminases are synchronously upregulated in the high-risk myelofibrosis (MF) stem cell population compared to normal aged bone marrow. We have discovered a co-regulatory relationship dependent on a delicate equilibrium between APOBEC3C and ADAR1, indicating a correlative association between the dysregulation of these essential deaminases and the initiation and progression of hematopoietic malignancies as indicated by survival statistics and gene expression changes. We will use this comprehensive study of innate immune deaminase deregulation to uncover predictive biomarkers and hallmarks of malignant transformation.

#5276 Comprehensive spatial mapping uncovers immune ecotypes linked to tumor heterogeneity in HCC lung metastasis.

J. Zhu[1], G. Cai[1], B. Guo[1], X. Chen[1], H. Zhang[1], H. Zhu[2], W. Zhang[1];

[1]Huazhong University of Science and Technology, Wuhan, China, [2]the First Affiliated Hospital of Soochow University, Suzhou, China

Tumor immune microenvironment (TME) heterogeneity, shaped by diverse immune and stromal cells, significantly influences tumor metastasis, yet the specific immune ecotypes involved and their relationship to intra-tumor heterogeneity remain unclear. In this study, we present a comprehensive spatial and cellular multi-omics analysis of 44 primary and 54 lung metastatic lesions from 34 HCC patients. By integrating public single-cell RNA sequencing data from 187,290 tumor cells across 146 HCC cases, we define HCC cellular states and uncover their organization in high-dimensional spatial expression profiles from paired HCC primary and metastatic lung lesions. Our integrated analysis, incorporating data with three independent cohorts, uncovers three pro-metastatic modules—Cell cycle(G2/M), Cellular Stress and Hypoxia —that contribute to early HCC lung metastasis. Notably, the first two modules are specific to lung metastasis, while the latter one is common across all extrahepatic organs. We show how tumor cells with distinct modules interact with various immune cell types, facilitating metastasis through different mechanisms. Additionally, these tumor cells form multi-layered structures of varying sizes, with specific state pairs preferentially clustering across multiple scales, all within a 55 μm limit. In vivo studies further reveal that interactions between G2/M-high tumor cells and Angio-macrophages and T3-neutrophils accelerate lung dissemination. Treatment with the G2/M inhibitor paclitaxel effectively disrupts this process, reducing metastasis. These findings provide critical insights into how tumor cell heterogeneity reshapes the TME to drive metastasis, underscoring the importance of spatially informed features for personalized cancer therapies. Our work emphasizes the potential of tailored strategies to prevent and manage metastasis based on tumor-immune interactions.

#5279 Utilizing intravascular MHC-I antibody labeling to investigate regulation of tumor cell extravasation by ACKR1.

Rishi Patel, Samuel Tanner Roach, Serena Thomas, Braulio Aguilar, Hsun Wang, L.A. Naiche, Jan Kitajewski

Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, IL

Background: Endothelial Ackr1 is a major regulator of immune cell extravasation. Tumor cells can co-opt mechanisms of leukocyte extravasation to exit the vasculature at distant metastatic sites. Detection of intravascular vs. extravasated tumor cells in the lung has previously relied on time-consuming confocal imaging with 3-D reconstruction. We optimized an intravascular labeling procedure to easily and quantitatively detect tumor cell extravasation via flow cytometry, and used it to investigate the effect of endothelial Ackr1 expression on tumor cell extravasation into the lung metastatic niche.
Methods: To investigate the effect of endothelial Ackr1 on tumor cell extravasation, we employed an experimental metastasis model. Metastatic breast cancer cells (E0771.LMB) expressing mCherry were intravenously injected into Control or endothelial-specific Ackr1 knock out (Ackr1 ECKO) mice and allowed to circulate and extravasate into lung tissue over 24 hours. 3 minutes before sacrificing the mice, we intravenously injected a fluorescently labeled anti-MHC I antibody to label tumor cells remaining within the vascular compartment. Harvested lungs were dissociated, and tumor (mCherry+) cells were classified as intravascular or extravascular depending on the presence of the MHC I antibody signal. To confirm that inflammation from the tumor did not create extravascular antibody leakage, we treated wildtype mice intratracheally with either PBS (control) or 300 ng of TNF-α. After 24 hours, we administered 15 µg of anti-Type IV Collagen (Col4) antibody, which does not bind within the vascular lumen but will bind endothelial basement membrane if extravasated. Antibody leakage was evaluated via Col4 staining in lung tissue and in the liver, which served as a positive control due to its fenestrated endothelial barrier.
Results: We were successfully able classify intravascular versus extravascular tumor cells in the lung metastatic niche via flow cytometry using intravascular MHC I antibody labeling. We detected no leakage of antibody from the vascular compartment of the lung in naïve or inflamed settings. Despite the well-characterized role of Ackr1 in extravasation of other cell types, we observed that endothelial Ackr1 expression does not affect the ability of tumor cells to extravasate into lung tissue after 24 hours in circulation in an experimentally induced metastasis model.
Conclusions/Future Directions: Our results highlight an efficient intravascular antibody labeling technique to assess tumor cell extravasation at distant metastatic sites. While endothelial Ackr1 did not impact tumor cell extravasation in the lung metastatic niche, we are using this labeling technique to evaluate the impact of endothelial Ackr1 expression on neutrophil extravasation into the lung pre-metastatic niche.

#5280 A strategic framework for modeling abnormal quiescence that drives cancer evolution, and for preclinical development of therapies to prevent recurrence and metastasis in high-risk patients.

Balraj Singh, Anthony Lucci

Breast Surgical Oncology, UT MD Anderson Cancer Center, Houston, TX

Our goal is to develop a cell culture model of cancer cells that persist under all challenges including therapies, mostly in quiescence, and evolve further to cause recurrence. The rationale for the choice of model as cell culture is that it is ideal of evaluating therapeutic strategies that may inhibit highly abnormal but adaptable cells. Body imposes a variety of bottlenecks ranging from different components of immunity to metabolic challenges to enforce quiescence and influence cancer evolution. As it is not possible to replicate all such bottlenecks in cell culture, our approach is to apply as severe a bottleneck as possible, thus eliminating all proliferative (non-adaptable) cells, leaving behind rare cells surviving in deep quiescence. We hypothesize that if the quiescent cell state being modeled can survive a severe bottleneck, it can also survive other bottlenecks of similar or lower severity. We have arrived at a prolonged lack of glutamine in cell culture medium as a bottleneck for selecting evolutionarily fit rare cells after trying several alternatives, including a lack of glucose, a partial lack of oxygen (hypoxia), or a lack of serum on surface that is unsuitable for cell attachment. While investigating glutamine dependence in aggressive triple-negative breast cancer cell lines, we serendipitously discovered that some rare cells could survive in deep quiescence not only this but variety of other metabolic and therapeutic challenges. Since glutamine and its metabolites are important in protein synthesis, nucleic acid synthesis, epigenome and epitranscriptome regulation, and in maintaining redox status, its sudden disappearance creates a catastrophe killing 99.99% cells; only rare cells survive in deep quiescence and evolve further to proliferate (an important feature of cancer recurrence/metastases). The adaptable cells selected in this manner have many desirable functional and molecular features that are relevant in resistant cancers with an elevated risk of recurrence. We will discuss our observations with rare cells (0.01% in population) surviving in quiescence for several weeks in aggressive breast cancer and melanoma cell lines and their functional characteristics. Our results obtained with adaptable cells from SUM149 TNBC cell line suggest that they are stem-like quiescent cells. Our gene expression analyses and phenotypic changes during quiescence suggest that like a model proposed for stem cell quiescence, cancer stem cells are not dormant; instead, they represent states of poised potential and active restraint as cells “idle” in anticipation of activation, proliferation, and differentiation. We will also discuss how we choose and evaluate therapies that would likely inhibit quiescent and slow-growing cancer cells. This framework may significantly improve anti-cancer drug discovery.

#5281 Analysis of tumor cell and neutrophil distribution relative to ACKR1 expressing venules during breast cancer metastasis.

Serena Thomas, S. Tanner Roach, Rishi Patel, Lauren Raasch, L. A. Naiche, Jan K. Kitajewski

University of Illinois at Chicago, Chicago, IL

Background: Metastasis is the leading cause of breast cancer-related mortality. However, the success of metastatic colonization is not solely determined by tumor cells' ability to disseminate; it is also influenced by the establishment of premetastatic niches within distant organs. Neutrophils promote lung metastasis by priming the premetastatic niche to make it a more hospitable environment for disseminating tumor cells. Atypical Chemokine Receptor 1 (ACKR1) is expressed on endothelial cells and is critical for neutrophil migration in inflammatory conditions, but its role has not previously been characterized in pre-metastatic niche formation.
Methods: To study the role of endothelial ACKR1 in tumor cell and neutrophil extravasation, we analyzed the distribution of disseminating tumor cells and neutrophils relative to ACKR1-positive versus ACKR1-negative vessels in the lungs of orthotopic mammary tumor-bearing mice. To analyze disseminating tumor cells and extravasated neutrophils, we performed immunofluorescent staining of lung vasculature from immunocompetent mice implanted with E0771.LMB mammary tumor cells 28 days post-tumor injection. Lung samples were stained with ACKR1, CD31, and either Pan-CK (to label tumor cells) or Ly6G (to label neutrophils). We developed a semi-automated workflow to analyze the distance between disseminated tumor cells, neutrophils, and the closest venule within the lung. Images were blinded and target cells (tumor cells and neutrophils), ACKR1-positive venules, and ACKR1-negative venules were manually annotated in QuPath. A custom script was developed to outline venules and calculate the minimum distance between each target cell and the nearest vessel wall within 150 microns of a venule. These results were binned to categorize target cells based on their proximity within 50, 100, or 150 microns of an ACKR1-positive or -negative venule.
Results: Our analysis revealed a significant increase in the average number of tumor cells within 50 microns of ACKR1-positive venules compared to ACKR1-negative venules. Similarly, neutrophil analysis showed a significant increase of neutrophils within 50 microns of ACKR1-positive venules. In lung tissue more than 50 microns from a venule, no differences in the number of tumor cells or neutrophils were detected.
Conclusion/Future Directions: These findings demonstrate that both disseminated tumor cells and neutrophils preferentially localize near ACKR1-positive lung venules during breast cancer metastasis, suggesting that both cell types extravasate preferentially through ACKR1-expressing venules. Ongoing studies aim to define the role of ACKR1 in regulating neutrophil and tumor cell extravasation, phenotype, and survival in the premetastatic niche.

#5282 New insights into the roles of transcriptional regulation and differentiation state in rhabdomyosarcoma cell migration.

Andrea Largent, Yadong Wang, Abraham Hengyucius, Eleanor Chen

University of Washington, Seattle, WA

The differentiation state of a tumor cell has been known to impact the cell's pro-tumor and pro-metastatic abilities; for example, in epithelial tumors like lung cancer, epithelial-mesenchymal transitions (EMT) involve a conversion from a differentiated phenotype to a stem-like state, allowing for increased tumor cell motility and successful metastasis. In rhabdomyosarcoma (RMS), a pediatric soft tissue sarcoma, tumors are heterogeneous for cells at different stages of myogenesis or muscle cell differentiation, but it is not fully clear whether certain myogenic cell types have a propensity towards metastatic migration than others. In both development and RMS, master regulatory factors (MRFs) are essential for transcriptional regulation of genes involved in myogenesis. One of these MRFs is myogenin, a transcription factor expressed during mid-differentiation that leads to a more committed myocyte state. Myogenin-positive RMS cells in a zebrafish model have been shown to exhibit many metastasis-promoting abilities, such as increased migratory velocity compared to stem-like RMS cells, the ability to enter the bloodstream, and the capacity to seed tumor-free areas surrounding the tumor. This indicates that metastasis in RMS may rely heavily on transcriptional regulation and likely involves cells of a myocyte-like state. Knowing this, our lab carried out a human cell line knockdown (KD) scratch assay screen of multiple epigenetic factors to investigate if any may also be involved in RMS cell movement. From this screen, BRD1, a bromodomain-containing transcriptional regulator, emerged as a driver of RMS cell migration, and subsequent quantitative PCR (qPCR) analysis of BRD1 KD cells additionally indicated a significant role in MRF and stem cell marker expression. Immunofluorescent staining of untreated RMS cells 24 hours post-scratch revealed that migratory cells exhibit increased BRD1 and myogenin expression as well as decreased MyoD expression (an earlier MRF), suggesting that migratory RMS cells are at a later differentiation state. These results and future experiments would significantly impact the field, as the metastatic functions of BRD1 and myogenin were previously unknown and understudied, respectively.

#5283 Decoding and disrupting the coupled cellular plasticity and myeloid cell instigation in metastatic prostate cancer.

Tianhe Huang[1], Yaoyin Li[1], Jun Wan[2], Liang Cheng[3], **Xin Lu**[1]

[1]Biological Sciences, University of Notre Dame, Notre Dame, IN,[2]Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN,[3]Department of Pathology and Laboratory Medicine, Brown University Warren Alpert Medical School, Providence, RI

Prostate cancer (PCa) metastasis significantly worsens patient prognosis, particularly when it spreads to the lungs. Understanding the mechanisms underlying PCa metastasis is crucial for developing effective therapies. Here, we developed a genetically engineered mouse model by activating MAPK pathway in the PB-Cre Pten p53 Smad4 mice, creating a model that mimics the aberrant cell signaling associated with human metastatic PCa and exhibits strong metastatic phenotype. We observed dynamic changes in epithelial-to-mesenchymal transition (EMT) and mesenchymal-to-epithelial transition (MET) during metastasis: circulating tumor cells (CTCs) displayed mesenchymal characteristics, while lung metastases regained epithelial features.

Transcriptomic profiling of primary tumors, CTCs, and lung metastases revealed that lung metastatic cells (LM) upregulated pro-inflammatory cytokines, notably IL-1α. This cytokine profile correlated with increased recruitment and activation of neutrophils in the premetastatic lung niche, as confirmed by single-cell RNA sequencing and flow cytometry. Functional experiments demonstrated that depleting neutrophils using anti-Ly6G antibodies or inhibiting their recruitment and activation via a CXCR1/2 antagonist (currently in clinical trials) significantly reduced lung metastasis and extended survival in mice. Similarly, IL-1 receptor knockout mice showed decreased neutrophil infiltration and fewer metastatic nodules, indicating the pivotal role of IL-1 signaling in neutrophil-mediated metastasis.

Mechanistically, we identified the transcription factor KLF4 as the master regulator of both the EMT-to-MET plasticity and IL-1α expression in metastatic cells. Chromatin immunoprecipitation confirmed KLF4 binding to the IL-1α promoter, leading to the upregulation of neutrophil-recruiting cytokines. Knockdown of KLF4 in LM cells reduced IL-1α levels and metastasis, while overexpression enhanced them. Co-culture of tumor cells with neutrophils increased KLF4 expression and cytokine production, suggesting a feedback loop promoting metastasis.

Clinical data analysis showed that high levels of KLF4 and IL-1α correlate with increased neutrophil infiltration and poorer disease-free survival in PCa patients. Our findings identify the crucial role of the KLF4/IL-1α axis and neutrophil recruitment in PCa lung metastasis. Targeting neutrophil activation or the KLF4/IL-1α signaling pathway may offer new therapeutic strategies for preventing or treating metastatic prostate cancer.

#5284 Reduced occludin expression is related to unfavorable tumor phenotype and poor prognosis in many different tumor types: a tissue microarray study on 16,870 tumors.

Seyma Buyucek[1], Florian Viehweger[1], Viktor Reiswich[1], Natalia Gorbokon[1], Viktoria Chirico[1], Christian Bernreuther[1], Florian Lutz[1], Simon Kind[1], Ria Schlichter[1], Soren Weidemann[1], Till S Clauditz[1], Andrea Hinsch[1], Ahmed Abdulwahab Bawahab[2], Frank Jacobsen[1], Andreas M Luebke[1], David Dum[1], Elena Bady[1], **Lisa Hornsteiner**[1], Claudia Hube-Magg[1], Martina Kluth[1], Katharina Moller[1], Anne Menz[1], Andreas M Marx[3], Till Krech[1], Patrick Lebok[1], Christoph Fraune[1], Guido Sauter[1], Ronald Simon[1], Eike Burandt[1], Sarah Minner[1], Stefan Steurer[1], Maximilian Lennartz[1], Morton Freytag[1]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany,[2]University of Jeddah, Jeddah, Saudi Arabia,[3]Academic Hospital Fuerth, Fuerth, Germany

Occludin is a key component of tight junctions. Reduced occludin expression has been linked to cancer progression. To study the role of occludin in cancer, a tissue microarray containing 16,870 samples from 148 different tumor types and 608 samples of 76 different normal tissue types was analyzed by immunohistochemistry. Occludin immunostaining was observed in 10,746 (76.6%) of 14,017 analyzable tumors, including 18.9% with weak, 16.2% with moderate, and 41.6% with strong staining intensity. Occludin positivity was found in 134 of 148 tumor categories and was most frequent in adenocarcinomas (37.5-100%) and neuroendocrine neoplasms (67.9-100%), less common in squamous cell carcinomas (23.8-93%) and in malignant mesotheliomas (up to 48.1%) and rare in Non-Hodgkin's lymphomas (1-2%) and most mesenchymal tumors. Reduced occludin staining was associated with advanced pT stage ($p<0.0001$), L1 status ($p=0.0384$), and absence of microsatellite instability ($p<0.0001$) in colorectal adenocarcinoma, advanced pT stage in pancreatic adenocarcinoma ($p=0.005$), high ISUP grade ($p<0.0001$), advanced pT stage ($p<0.0001$), high UICC stage ($p<0.0001$), distant metastasis ($p=0.0422$), and shortened overall ($p=0.0045$) or recurrence-free survival ($p=0.0116$) in clear cell renal cell carcinoma (RCC), high pT stage ($p<0.0001$) and UICC stage ($p=0.0228$), distant metastasis ($p=0.0338$), and shortened recurrence-free survival ($p=0.006$) in papillary RCC, and advanced pT stage ($p=0.0133$) in serous high-grade ovarian cancer. Occludin staining was unrelated to parameters of tumor aggressiveness in breast, gastric, endometrial, and thyroidal cancer. Our data demonstrate significant levels of occludin expression in many different tumor entities and identify reduced occludin expression as a potentially useful prognostic feature in several tumor entities.

#5285 Activation of endothelial ACKR1 in the pre-metastatic niche regulates neutrophil recruitment and tumor metastasis in breast cancer.

Samuel Tanner Roach, Serena Thomas, Rishi Patel, Hsun Wang, Braulio Aguilar, Chinwe Ewenighi-Amankwah, Lauren Raasch, William Muller, L.A. Naiche, Jan Kitajewski

University of Illinois at Chicago, Chicago, IL

Background: Extravasation of neutrophils into the pre-metastatic niche promotes metastatic breast cancer cell survival. ACKR1 is a non-signaling cytokine receptor expressed on endothelial and erythroid cells and acts as a critical regulator of neutrophil extravasation in multiple inflammatory contexts. Our work has shown that endothelial ACKR1 is upregulated in the lung pre-metastatic niche during primary breast tumor progression. Therefore, we tested the requirement for lung endothelial ACKR1 expression in neutrophil recruitment to the pre-metastatic niche and for breast cancer metastasis to the lung.
Methods: We investigated the role of endothelial ACKR1 in the lung pre-metastatic niche using a novel endothelial-specific ACKR1 knockout mouse model (ACKR1 ECKO). Pre-metastatic niche formation was induced in control and ACKR1 ECKO mice via daily intraperitoneal injections of tumor-conditioned media. Immunohistochemistry and flow cytometry were employed to analyze the percentage of neutrophils in the pre-metastatic niche and quantify neutrophil extravasation into the lung parenchyma. Intravenous tumor cell injections were performed following pre-metastatic niche formation to assess the impact of endothelial ACKR1 on tumor cell extravasation and colonization in the lung. Lung tissues were collected at 24 hours and 2 weeks post-tumor cell injection, and tumor cell extravasation and overall metastatic burden were analyzed using flow cytometry and whole-mount immunofluorescent imaging. The effect of endothelial ACKR1 expression on neutrophil adhesion and transendothelial migration under tumor inflammatory conditions was tested using monolayer transmigration assays *in vitro*.
Results: We found that endothelial ACKR1 expression was activated in the lungs of mice bearing mammary tumors or injected with tumor-conditioned media. After pre-metastatic niche induction, the lung parenchyma of ACKR1 ECKO mice had significantly fewer neutrophils than control mice, with no reduction in circulating neutrophils. Tumor cell extravasation and seeding of the lung 24 hours after tail vein injection were unaffected by endothelial ACKR1. However, at the 2-week timepoint, we found that ACKR1 ECKO mice had significantly decreased lung metastatic nodules compared to controls. Finally, we observed that tumor-conditioned media can induce high ACKR1-expressing HUVEC cells *in vitro* and promote neutrophil adhesion and transendothelial migration.
Conclusion: Our study shows that secreted factors from the primary tumor upregulate endothelial ACKR1 in the lung pre-metastatic niche and facilitate neutrophil recruitment, ultimately promoting lung metastasis. We are now investigating which steps of neutrophil extravasation are regulated by endothelial ACKR1 and identifying the chemokines that allow ACKR1 to regulate this process during metastasis.

#5286 The acquisition of CD11a via trogocytosis by gastric cancer cells after contact with T cells increases adhesion to endothelial cells.

Hideyo Miyato, Eri Suizu, Rei Takahashi, Misaki Matsumiya, Hirofumi Sonoda, Hideyuki Ozawa, Hironori Yamaguchi, Naohiro Sata, Joji Kitayama

Jichi Medical University, Shimotsuke, Japan

It has been revealed that plasma membrane and membrane molecules are frequently transferred upon cell-to-cell contact, which is named as trogocytosis, and that this phenomenon between immune cells plays an important role in the immune responses. However, the physiological roles of trogocytosis between cancer cells and immune cells are poorly understood. In this study, we hypothesized that the transfer of membrane molecules from immune cells to cancer cells arrows cancer cells to acquire the functions involved in extravasation, which results in increased metastatic ability. Gastric cancer cell line, OCUM-1 was co-cultured with T cells whose plasma membrane was stained with PKH26. Flowcytometric analysis revealed that OCUM-1 became positive for PKH26 at 3 hours and the positive rate increased over time. The acquisition of PKH26 was dependent on cell-to-cell contact and suggested to be attributed to trogocytosis. Among various immunological synapse molecules of T cells, OCUM-1 came to express CD45, CD3, CD4 and CD11a after 8 hours of coculture (positive rate, CD45: 64.0%, CD3: 36.0%, CD4: 11.3%, CD11a: 16.8%). We focused on CD11a, which belongs to β2 integrins and aids immune cells to adhere endothelial cells and extravasate. After co-culture OCUM-1 with T cells on which CD11a was fluorescently labelled with anti-CD11a antibody, CD11a was transferred from T cells to OCUM-1. The expression level of CD11a on OCUM-1 was accelerated after co-culture with activated T cells (positive rate, with T cells: 12.6% vs with activated T cells: 65.9%) and the adhesion rate on endothelial cells increased in a CD11a dependent manner (adhesion rate, monoculture: 2.0±0.64%, co-culture: 6.3±2.0%, co-culture (pre-treat with CD11a antibody): 2.3±1.4%, n=10, monoculture vs co-culture: $p<0.0001$, co-culture vs pre-treat: $p<0.0001$).These results suggest that after escape from attack of T cells, cancer cells could acquire CD11a from T cells and increase the adhesive property on endothelial cells of target organs, which might result in promotion of intravenous metastasis.

#5287 Decoding tumor-immune interactions in colorectal cancer liver metastases: Insights from single-cell analysis.

Ji Hye Choi[1], Yeong Hak Bang[2], Kyung Yeon Han[2], Woong-Yang Park[2]

[1]Sungkyunkwan University, Seoul, Korea, Republic of,[2]Samsung Genome Institute, Samsung Medical Center, Seoul, Korea, Republic of

Background: Liver is the most common site of colorectal cancer (CRC) metastasis. As liver microenvironment differs significantly from the colon microenvironment surrounding the tumor, we hypothesized that both cancer cells and liver microenvironment undergo changes that promote tumor growth and suppress the immune response. Here, we aimed to identify distinct cancer cell clusters in the liver and evaluate the upregulated interactions between immune cells in the liver.
Method: We conducted single-cell RNA sequencing of primary and liver metastasis (CRLM) samples, along with paired normal samples from colon and liver, respectively. We conducted non-negative matrix factorization to define multiple epithelial cell clusters and evaluate proportional enrichment and interactions between immune cells.
Results: To investigate colorectal epithelial cells and identify those contributing to liver metastasis, we analyzed a total of 273,711 cells derived from 78 CRC samples (primary tumor from localized CRC, N = 44; primary tumor from CRLM, N = 21; metastatic tumor from CRLM, N = 13), and 33 normal samples (25 from the colon and 8 from the liver). Excluding five epithelial cell clusters that clustered with normal tissues, we defined 14 tumor-associated epithelial cell clusters. Three dominant clusters in primary colorectal cancers (EPI_PC) showed upregulation of hypoxia, stress, and MHC pathways. On the other hand, two dominant clusters in CRLM (EPI_LM) exhibited enriched hyperkeratosis and cell junction pathways, and these cells were found to be in a later stage of differentiation in trajectory analysis. Furthermore, these clusters exhibited an increase in the activation of angiogenesis and epithelial-mesenchymal transition (EMT) pathways, from localized CRC (without metastasis) to primary tumor from CRLM, and metastatic tumor from CRLM. To examine differences in interactions between epithelial cells and the tumor microenvironment in the colon and liver, we performed cell-to-cell interaction analysis focusing on CRLM and matched primary CRC samples. The analysis revealed upregulation of semaphorin 6, semaphorin 7, CD137, and TRAIL pathways between these epithelial cells and C1QC+ macrophage and CD8+ effector T cells. Notably, immune checkpoint signaling, including TIGIT and PVR pathways, was upregulated between epithelial cells and CD8+ exhausted T cells in the CRLM. Spatial transcriptomics showed distinct showed distinct distributions of EPI_PC and EPI_LM, presenting specific immune-cell interactions in some CRLM samples.
Conclusions: Our study highlights that the CRLM-associated clusters were featured in a later stage of differentiation and high expression of angiogenesis and EMT pathways. Considering the upregulated pathway between EPI_LM and immune cells, treatment strategies targeting these interactions may be helpful for CRC patients with liver metastasis.

#5288 Identification of cancer cells with metastatic potential in nasopharyngeal carcinoma through spatial multi-omics analysis.

Songran Liu[1], Zhaozheng Hou[1], Kazi Anisha Islam[1], Yingpei Zhang[1], Larry Ka-Yue Chow[1], Ying Wang[2], Ping Feng[3], Michael King Yung Chung[1], Kiu-Wai Cheng[1], Dittman Lai-Shun Chung[1], Yunfei Xia[2], Wei Dai[1]

[1]Clinical Oncology, University of Hong Kong, The - Li Ka Shing Faculty of Medicine, Hong Kong, China,[2]Radiation Oncology, Sun Yat-sen University Cancer Center, Guangzhou, China,[3]Department of Experimental Research, Sun Yat-sen University Cancer Center, Guangzhou, China

Background: Metastasis is the leading cause of nasopharyngeal carcinoma (NPC) mortality. The understanding of the process of metastasis is inadequate.
Objectives: To identify the high-metastatic-potent (HMP) cluster in NPC using a set of key genes based on spatial multi-omics analysis.
Method: 238 NPC samples, 110 from Hong Kong (HK) and 128 from Guangzhou (GZ), were classified into the metastatic (MET) group if the patient developed a remote metastasis within 5 years after the diagnosis of NPC, and into the non-metastasis (nonMET) group vice versa. Two HK samples (MET vs. nonMET: 1 vs. 1) were profiled by single-cell spatial transcriptome sequencing (scST) to explore the HMP cluster and its signature genes. Twenty-eight GZ samples were utilized for spatial transcriptome (GZ_ST: 14 vs. 11) and spatial proteome (GZ_SP: 17 vs. 11) profiling to streamline the signature genes into a final gene list. Validation at the transcriptome level and protein level was accomplished by bulk RNA sequencing (HK: 39 vs. 38; GZ: 41 vs. 59) and spatial proteome sequencing (HK_SP: 19 vs. 12), respectively. Independent validation was performed by multiplex immunohistochemical staining (mIHC).
Results: Ten epithelial clusters from tumor cells were defined by cell segmentation and deconvolution in scST. Three clusters exhibited significant enrichment in the MET samples generating 69 signature genes for HMP. Gene set enrichment analysis (GSEA) in GZ_ST indicated a substantial activation of HMP signature in the MET group (two-sided $p = 0.044$). Thirty-three leading-edge genes in HMP signature were selected to evaluate the expression levels in scST, GZ_ST, and GZ_SP. Nineteen genes were included in the final gene list as their expressions were higher than average, upregulated in the MET group, and specific to HMP clusters. GSEA analysis in bulk RNA sequencing showed that the 19 HMP genes were activated in the MET group (one-sided $p = 0.032$). The lactate dehydrogenase A (LDHA)/enhancer of zeste homolog 2 (EZH2)/insulin-like growth factor 1 receptor (IGF1R) regulatory axis may play a key role in tumor metastasis by affecting tumor invasion. Pearson correlation analysis in bulk RNA sequencing displayed a positive correlation between HMP cluster and several lytic EBV genes transcribed from the BART regions ($r > 0.4$, $p < 0.05$), suggesting the potential role of EBV lytic reactivation in promoting metastasis.
Conclusions: The high-metastatic-potent cluster was identified through this spatial multi-omics study. The selected biomarkers well define this cluster and enlighten the underlying metastatic mechanisms and its relevance of EBV lytic reactivation.
Acknowledgement: This work was supported by General Research Fund (17102619) and Theme-based Research Scheme (T12-703/22-R; T123-70323-N) from Research Grant Council, and Technology Program of Guangdong Province in China (2021A0505110010).

#5289 Mechanisms of metastasis revealed through single-cell spatial transcriptome analysis in patients with liver metastatic colorectal cancer.

Jongwon Lee[1], Yeseul Kim[2], Hyo Seon Ryu[3], Jongmin Sim[2], Chungyeul Kim[2], Hyun Woo Kwon[4], Hyun Je Kim[5], Jong Min Park[6], Ah-Reum Lim[6], Jung Sun Kim[6], Hwa Jung Sung[6], Xingyi Guo[7], Jungmin Choi[1], **Jungyoon Choi**[6]

[1]Department of Biomedical Sciences, Korea University College of Medicine, Seoul, Korea, Republic of,[2]Department of Pathology, Korea University College of Medicine, Seoul, Korea, Republic of,[3]Division of Colon and Rectal Surgery, Department of Surgery, Korea University College of Medicine, Seoul, Korea, Republic of,[4]Department of Nuclear Medicine, Korea University College of Medicine, Seoul, Korea, Republic of,[5]Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea, Republic of,[6]Division of Oncology/Hematology, Department of Internal Medicine, Korea University College of Medicine, Seoul, Korea, Republic of,[7]Division of Epidemiology, Department of Medicine, Vanderbilt Epidemiology Center, Vanderbilt University School of Medicine, Nashville, TN

Background: The efficacy of treating colorectal cancer (CRC) liver metastases is hindered by significant tumor heterogeneity, both within and between tumors. This diversity stems from varied cell populations and complex interactions in the tumor microenvironment, necessitating a deeper understanding of spatial organization and cellular communication patterns.
Methods: We examined 23 formalin-fixed paraffin-embedded tissue samples from 11 patients, comprising primary lesions and matched liver metastasis (6 with synchronous and 5 with metachronous metastases) using NanoString 6K CosMx Spatial Molecular Imaging (SMI). Pathologists selected optimal fields of view at tumor invasive fronts. Image processing, cell segmentation, and feature extraction utilized an in-house CosMx SMI pipeline with the Cellpose algorithm. Following quality control, the remaining cells underwent unsupervised clustering with differential gene expression analysis and cell-to-cell interaction analyses performed using the Seurat R package.
Results: Analysis of 105,624 high-quality cells revealed 23 distinct clusters, categorized as ten epithelial tumors, three cancer-associated fibroblast (CAF), two endothelial cells, five immune cells (T cell, Monocyte, Macrophage, SPP1+ Macrophage, and Plasma cell), alveolar cells, hepatocytes, and low complexity cell populations. We identified significant differences in gene expression between colon and liver CAFs, with liver CAFs displaying upregulation of 92 genes associated with epithelial-mesenchymal transition (EMT). Enhanced communication between CAFs, T cells, and macrophages was observed in liver metastases, with specific ligand-receptor pairs (SPP1-ITGA4/ITGB1, LGALS9-P4HB, LGALS9-CD44, CXCL12-CXCR4) enriched in the metastatic microenvironment.
Conclusion: Our findings demonstrate distinct spatial heterogeneity and intercellular communication networks in CRC liver metastases. The identification of liver-specific CAF gene expression profiles and unique ligand-receptor interactions provides new insights into metastatic mechanisms and potential therapeutic targets.

#5290 Spatial multi-omics defines a shared glioblastoma infiltrative signature at the resection margin.

Balagopal Pai[1], Susana Ramos[1], Wan Sze Cheng[1], Tanvi Joshi[1], Gabrielle Price[1], Jessica Tome-Garcia[1], Kristin Beaumont[1], Robert Sebra[1], Raymund Yong[1], Elena Zaslavsky[1], **Nadejda M. Tsankova**[2]

[1]Icahn School of Medicine at Mount Sinai, New York, NY,[2]Pathology and Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY

Glioblastoma (GBM) remains an untreatable disease. Understanding of GBM's infiltrative biology at the resection margin is limited, despite causing disease recurrence and progression. To address this, we generated a high-throughput single-nucleus (sn)RNA-seq and snATAC-seq multi-omic dataset from six tumors with distinct genomic drivers and combined it with spatial transcriptomics to characterize the unique molecular phenotype of GBM near the margin. By contrasting GBM-specific biology in matching "Core" vs. "Margin" dissections, we define unique, shared "GBM infiltration" and chromatin accessibility signatures near the margin. We prioritize *EGFR* as a top differentially expressed and accessible "Margin" marker across GBM subtypes, show its dynamic expression along a core-to-margin infiltration trajectory, and validate its role in migration through CRISPR/Cas9 deletion in two patient-derived models. ChIP-seq studies further corroborate preferential TEAD1 binding at EGFR's accessible regulatory elements. This validated multi-omic dataset enables further studies into tumor and microenvironment biology in the context of residual GBM disease.

#5294 Prevalence of KDM6A deficiency in human cancer: a tissue microarray study on 14,814 cancers from 153 different tumor types.

Florian Viehweger[1], Paul Wirth[1], Natalia Gorbokon[1], **Maximilian Lennartz**[1], Viktor Reiswich[1], Till Krech[2], Claudia Hube-Magg[1], Elena Bady[1], Lisa Hornsteiner[1], Guido Sauter[1], Ronald Simon[1], Stefan Steurer[1], Martina Kluth[1], Andreas H Marx[3], Andrea Hinsch[1], Andreas M Luebke[1], Anne Menz[1], Clara von Bargen[1], Frank Jacobsen[1], Piero Caneve[1], Ria Schlichter[1], Sebastian Dwertmann Rico[1], Simon Kind[1], Soren Weidemann[1], Maria Christina Tsourlakis[1], Waldemar Wilczak[1], Till S Clauditz[1], Christian Bernreuther[1], Sarah Minner[1]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany,[2]Clinical Center Osnabrueck, Osnabrueck, Germany,[3]Academic Hospital Fuerth, Fuerth, Germany

KDM6A (lysine demethylase 6A) is an epigenetic regulator and part of the COMPASS-like complex. Truncating KDM6A mutations commonly occur in cancer and may result in a dependency on EZH2, a potential therapeutic target. Because only one copy of X chromosome genes is normally active in human cells, truncating KDM6A mutations result in a complete expression loss which can be seen by immunohistochemistry. To assess the prevalence of KDM6A expression loss and its role in cancer, a tissue microarray containing 14,814 samples from 153 different tumor entities was analyzed. In normal tissues, KDM6A staining was ubiquitously seen in nuclei. At least one case with a complete KDM6A expression loss was observed in 58 of 153 tumor categories. KDM6A deficiency predominated in urothelial carcinomas (17.3-42.0%). Tumor entities with a KDM6A deficiency rate of 4-10% included adenocarcinomas of the gallbladder (9.3%), the stomach (up to 8.3%), the pancreas (7.7%), and the prostate (5.9%), endometrioid and serous endometrial carcinoma (up to 7.8%), squamous cell carcinomas (up to 7.5%), papillary renal cell carcinoma (5.1%), hepatocellular carcinoma (4.4%), and malignant melanoma (4.2%), and serous high-grade carcinoma of the ovary (4.1%). Reduced KDM6A expression was associated with advanced pT stage ($p=0.0233$), high grade ($p=0.0002$), distant metastasis ($p=0.0152$), and absence of ER ($p<0.0001$) and PR ($p=0.0045$) expression as well as HER2 amplification ($p=0.0005$) in breast cancer, nodal metastasis in squamous cell carcinomas ($p=0.0498$), advanced pT stage ($p=0.0002$), nodal metastasis ($p=0.0112$), and microsatellite instability (MSI; $p=0.0147$) in colorectal adenocarcinoma, and with MSI ($p=0.0033$) in gastric adenocarcinoma. Among gender agnostic cancers, KDM6A deficiency was markedly more common in tumors from males (4.3%) than from females (1.9%; $p<0.0001$). KDM6A sequencing of 78 bladder cancers revealed truncating mutations in 48.7% and non-truncating mutations in 5.1% of tumors. Truncating but not non-truncating KDM6A mutations were significantly associated with KDM6A expression loss ($p<0.0001$). All 15 (100%) male patients and 17 of 23 (73.9%) female patients with a truncating KDM6A mutation had a complete KDM6 expression loss. It is concluded that KDM6A deficiency predominates in urothelial neoplasms although it occurs in many different tumor entities, and that both reduced and absent KDM6A correlates with increased aggressiveness. Whether KDM6A deficient cancers are susceptible to EZH2 inhibitors in vivo remains to be seen.

TUMOR BIOLOGY: Where's Waldo: Spatial Complexity of the Tumor Microenvironment 2
Poster Session

#5296 Hepatocellular carcinoma resistance and recurrence to anti-PD-1 therapy is associated with tumor heterogeneity and plasticity driven by MYC.

S. Zhang, L. Danilova, J. T. Mitchell, I. Kwon, A. S. Popel, M. Yarchoan, E. M. Jaffee, E. J. Fertig, L. T. Kagohara;
Johns Hopkins University School of Medicine, Baltimore, MD

Hepatocellular carcinoma (HCC) is the most prevalent type of liver cancer and a major cause of cancer-related deaths worldwide. HCC is characterized by low survival rates and a high incidence of recurrence. Systemic therapy responses are observed in only a small proportion of HCC patients. Our spatial transcriptomics (ST) analysis of HCC tumors from patients treated with anti-PD-1 and cabozantinib (a multi-tyrosine kinase inhibitor) suggested that immunotherapy resistance is driven by cancer cell heterogeneity, tumor microenvironment (TME) dynamics, and cancer cell plasticity. These mechanisms were detected in an HCC sample collected from a responder who later experienced recurrence. To understand the genomic drivers of resistance and recurrence in HCC, we performed new bioinformatic analyses to investigate the molecular mechanisms associated with HCC plasticity that result in anti-PD-1 resistance. Across our ST samples, we observed up-regulation of oncogenic and immune evasion mechanisms following anti-PD-1 treatment. Interestingly, these signaling pathways share a common upstream modulation by MYC. Gene set enrichment analysis identified a significant upregulation of the MYC pathway in immune-poor cancer regions. Spatially weighted correlation analysis revealed that MYC upregulation is colocalized with cancer cell-intrinsic pathways, such as proliferation, metabolism, and cell plasticity, and is spatially exclusive with immune-related pathways, including antigen presentation and interferon-gamma response. Moreover, intercellular interaction analysis showed that MYC-upregulated cancer cells engage in crosstalk with cancer-associated fibroblasts (CAFs) via PDGF-PDGFR signaling, which is associated with the epithelial-mesenchymal transition (EMT) process, a hallmark of cell plasticity. Our findings were validated in additional anti-PD-1-treated HCC single-cell and ST public datasets, confirming that therapy-reinvigorated lymphocytes neutralize only MYC-downregulated HCC cells, leaving MYC-high HCC cells to survive. These residual HCC cells potentially become the source of tumor recurrence. Our findings highlight MYC as a key regulator of therapeutic resistance and immune suppression in HCC, potentially serving as a target for future interventions to delay anti-PD-1 treatment relapse and improve patient outcomes.

#5297 Immune profiling of CRC identifies a potential signature associated with immunosuppression.

Dongyu Han, Yong Qin

The University of Texas at El Paso, El Paso, TX

Colorectal Cancer (CRC) shows limited response to immunotherapies due to 85% of cases involving proficient mismatch repair. The immune profile of CRC remains poorly understood, making it critical to investigate immune infiltration patterns in the tumor microenvironment (TME) of CRC to improve therapeutic outcomes. Adenosine accumulates at high levels in the TME, suppressing immune responses via the A2A receptor (A2AR) on immune cells, a crucial pathway in immune suppression. This study aims to characterize the immune profile of CRC and identify the immune signature associated with adenosine-mediated immunosuppression. Multiplex immunofluorescence staining was performed on 170 CRC samples from 85 patients on 12 immune markers, including CD4+ and CD8+ for T cells, CD56+ NK cells, CD68+ macrophages, CD11b+ dendritic cells, FOXP3 for Treg cells, Glut-1 (hypoxia), Ki67, IDO and PD-1 (immune checkpoints), and CD73 and A2AR of the adenosine pathway. These markers provided a comprehensive assessment of immune suppression mechanisms. Our findings showed CRC tumors with specific immune infiltrates, including high levels of CD4+ and CD8+ T cells, CD11b+ dendritic cells, and CD68+ macrophages, alongside elevated Glut-1 and A2AR expression, marking A2AR's role in the adenosine-mediated suppression in CRC. Conversely, low levels of infiltrates were observed for CD56+, CD73+, FOXP3+, IDO+, Ki67+, and PD-1+ cells. Subdividing patients into A2AR-high and A2AR-low groups revealed that A2AR-high tumors exhibited significantly higher CD68+ macrophage infiltration, suggesting A2AR may mediate adenosine effects primarily through macrophages. Notably, A2AR was predominantly expressed on CD8+ T cells in CRC tumors, suggesting that infiltrating CD8+ cells are suppressed by adenosine signaling. Markedly, high A2AR expression was observed despite significant CD73 deficiency in CRC tumors. On average, only 0.09% CD73+ cells. with 39% of tumor cores showing no CD73 expression. Moreover, 40% of CD73-deficient tumors retained high A2AR expression, indicating the presence of alternative adenosine-producing pathways independent of CD73. Significant differences in immune infiltrates were also noted between CRC subtypes. Colon adenocarcinomas (n=50) exhibited higher A2AR+ infiltrates and lower CD4+ T cell infiltration compared to rectal adenocarcinomas (n=35), suggesting stronger adenosine-mediated immunosuppression in colon adenocarcinomas. These findings provide valuable insights into the role of adenosine-driven immunosuppression in CRC and highlight potential therapeutic targets to counter immune suppression. The observed CD73 deficiency suggests the need to explore alternative adenosine-producing pathways in CRC. Continued investigation into CRC immune signatures will aid in identifying new biomarkers and developing improved immunotherapy strategies for CRC patients.

#5298 Impact of targeted therapy on the tumor immune microenvironment in *EGFR*-mutated NSCLC.

Daisuke Shibahara[1], Satoshi Nakamura[1], Yasuyuki kishikawa[1], Keita Nakatomi[2], Noriaki Nakagaki[3], Kohei Otsubo[1], Yoshimasa Shiraishi[1], Yasuto Yoneshima[1], Eiji Iwama[1], Kentaro Tanaka[4], Isamu Okamoto[1]

[1]Kyushu University, Fukuoka, Japan,[2]Fukuoka Higashi Medical Center, Fukuoka, Japan,[3]Fukuoka Red Cross Hospital, Fukuoka, Japan,[4]Kagoshima University, Kagoshima, Japan

Background: In *EGFR*-mutated lung cancer, osimertinib, a third-generation EGFR tyrosine kinase inhibitor, has demonstrated durable efficacy in many cases. However, early resistance arises in some patients during treatment. The tumor immune microenvironment is believed to play a pivotal role in shaping treatment outcomes, though the detailed molecular mechanisms are not fully understood.
Methods: This study analyzed four cases with paired tissue samples collected both before and after osimertinib treatment. Using Visium-HD, we performed analyses comparing the pre-treatment samples between the early resistance and long-term efficacy groups, as well as paired samples collected before and after treatment. We converted the Visium-HD spot data into single-cell data using StarDist, a deep learning-based method for nuclei/cell detection and segmentation. Furthermore, by adding the calculated coordinate data derived from StarDist to the single-cell data, we performed spatial transcriptome analysis using single-cell coordinates instead of spot coordinates. This enabled spatial transcriptomic analysis at the single-cell level while preserving the actual spatial relationships between cells.
Results: In the post-treatment samples from early resistance cases, the proportion of macrophages, particularly SPP1-positive macrophages, was increased compared to that in long-term efficacy cases. In the comparison of pre-treatment samples between early resistance and long-term efficacy cases, the former exhibited a high degree of intermixing among diverse cell populations (e.g., lymphocytes, macrophages, and fibroblasts), whereas the latter showed minimal intermingling between tumor cells and other cell types. Furthermore, in early resistance cases, the expression levels of CXCR4 and CD74 were higher compared to long-term efficacy cases. Analysis of cell-cell interactions suggested potential effects of tumor cells on tertiary lymphoid structures.
Conclusions: *EGFR*-mutated lung cancer is typically characterized by reduced immune cell infiltration, often referred to as “cold tumor.” However, early resistance cases exhibited increased immune cell infiltration and the presence of tertiary lymphoid structures, indicating a “hot tumor” phenotype. This finding suggests that *EGFR*-mutated lung cancer cases prone to early resistance might possess a tumor immune microenvironment resembling that of *EGFR* wild-type tumors.

#5299 *BZW2*: a novel therapeutic target for liver metastasis in lung cancer.

Kuan-Li Wu[1], Ying-Ming Tsai[2], Ya-Ling Hsu[3]

[1]Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan,[2]School of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan,[3]Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

Introduction: Liver metastasis (LM) occurs in approximately 13% of patients with lung cancer at the time of diagnosis and is associated with poor prognosis. Currently, there are no effective preventive or therapeutic strategies specifically targeting LM. Investigating the LM microenvironment can not only elucidate the mechanisms underlying its development but also provide opportunities to identify novel therapeutic targets.
Methods: An LM-prone mouse model was established by repeatedly injecting and harvesting Lewis Lung Carcinoma (LLC) cells from LM lesions. The spatial transcriptomics (Visium, 10X Genomics) was applied to explore the microenvironment and intratumoral heterogeneity of LM. Validation was conducted using single-cell RNA sequencing and spatial proteomics (PhenoCycler System, Akoya Bioscience) on human and murine LM samples, respectively. Bioinformatics analyses, including Gene Set Enrichment Analysis (GSEA), were applied to infer gene function and associated behaviors. Gene set variation analysis (GSVA) was performed to evaluate lung cancer patient survival in relation to the expression of the *BZW2*-associated gene set. Functional validation of *BZW2* was conducted *in vitro* by assessing cell migration using transwell and wound healing assays, and by examining epithelial-to-mesenchymal transition (EMT) markers via immunoblotting.
Results: *Bzw2* is the significantly upregulated differential expressed gene in LM, particularly at the invasive front, based on the murine spatial transcriptomic data. The finding was further validated in murine LM samples using spatial proteomics as well. In human single-cell RNA sequencing data, *BZW2* exhibited high expression in LM subclusters characterized by aggressive phenotypes, including enhanced proliferation, hypoxia, mesenchymal traits, invasiveness, and cancer stemness. GSVA analysis demonstrated that elevated expression of the *BZW2*-associated gene set was associated with poorer patient survival and enrichment of EMT-related pathways using the TCGA LUAD dataset. Furthermore, *in vitro* functional assays showed that *BZW2* inhibition suppressed cell migration and reduced the expression of EMT markers, as confirmed by immunoblotting.
Conclusion: By integrating findings from spatial transcriptomics, proteomics, and single-cell RNA sequencing, this study elucidates the role of *BZW2* in promoting the aggressiveness of LM in lung cancer through its influence on cell migration and metastasis. These results suggest that *BZW2* could serve as a promising therapeutic target worthy of further advanced research for LM in lung cancer.

#5300 Spatially resolved integrated omics profiling for ADC targets in urothelial carcinoma.

Pranauti Panshikar[1], Eleanor O?Roberts[1], David Krantz[2], Tony Ullman[1], Carolina Oses Sepulveda[1], Fernanda Svedman[3], Paivi Ostling[4], Anders Ullen[3], Charlotte Stadler[1]

[1]Dept of Protein Science, KTH- Scilifelab, Stockholm, Sweden,[2]Department of Oncology and Pathology, Karolinska Institute, Stockholm, Sweden,[3]Department of Oncology & Pathology, Department of Pelvic Cancer, Genitourinary Oncology and Urology, Karolinska Institute, Stockholm, Sweden,[4]Department of Oncology and Pathology, Karolinska Institute- Scilifelab, Stockholm, Sweden

Background: Urothelial Carcinoma (UC) is the tenth most common cancer worldwide, accounting for more than 200 000 deaths annually (Ferlay, 2020, Sung, 2021). Despite advances over the past decade, particularly with the introduction of immunotherapy (ICI) in various disease settings alongside conventional platinum-based chemotherapy, the prognosis for metastatic UC (mUC) remains poor.
Antibody drug-conjugates (ADC) have emerged as a novel treatment option for mUC, offering selective and targeted delivery of cytotoxic agents to cancer cells. ADCs targeting tumor-associated surface proteins including Nectin-4, trophoblastic cell surface antigen 2 (Trop-2) and tyrosine kinase receptor ERBB-2 (HER2), have demonstrated promising efficacy and are all approved for mUC (Wong, 2021, Goodson, 2023). However, the response rate to these ADCs in mUC is limited 25-40% and with a growing portfolio of accessible ADCs, identifying predictive biomarkers to optimize treatment selection remains an unmet medical need.
The use of multiplexed immunofluorescence (mIF) offers deeper insights into tissue microenvironment and can potentially be used to better stratify patients for the various ADC targets in cancer. Recently, an integrated assay combining the sequential IF for protein detection with RNAscope was developed for Lunaphore's COMET platform. This allows for simultaneous detection of several RNA targets and their corresponding proteins to be analyzed within the same cells. While the ADC target proteins have been shown to be highly expressed in many UC patients, there are limited studies characterizing the spatial expression of these protein targets, especially in combination with their corresponding transcripts.
Methods: The integrated assay of the COMET platform offers a fully automated workflow that includes iterative cycles of staining, imaging and elution facilitating the simultaneous detection of RNA and proteins at single cell resolution. We used custom designed RNAscope probes (ACDBio), a SPYRE antibody panel (Lunaphore) and additional antibodies to detect the ADC-targets Nectin-4, Trop-2 and HER-2, and to spatially map the tumor microenvironment of human FFPE bladder cancer tissues from a cohort of mUC patients.
Results & Discussion: We have optimized the assay for bladder cancer tissues on the COMET platform to detect multiple ADC targets at transcript and protein levels. We aim to employ the integrated multi-omics setup to explore the expression of the ADC targets in sections from a retrospective cohort of mUC patients treated by monotherapy with Enfortumab Vedotin (EV) in the third line. We have already observed differences across patients with regard to Nectin-4, Trop-2 and HER-2 protein expression and aim to better characterize the spatial distribution and expression of these tumor-associated antigens with the inclusion of target transcripts and the association to treatment benefit from EV.

#5301 Visium HD 3' enables unbiased whole transcriptome spatial profiling of tumor microenvironment in fresh frozen cancer tissues at single cell-scale resolution.

Debashish Chitnis, Marco Serra, Josh Gu, Anushka Gupta, Nancy Conejo, Aarushi Kalaimani, Govinda Kamath, Zixue Ma, Monica Nagendran, Joey Arthur, Julia Cowen, Anuj Patel, David Sukovich, Augusto M. Tentori

RnD, 10X Genomics, Pleasanton, CA

Spatial transcriptomic technologies demonstrate the ability to comprehensively investigate the complexity of the tumor microenvironment, providing crucial insights into the interplay of different cellular and acellular components that orchestrate the tumor progression, angiogenesis, immune evasion, and metastasis. Here, we introduce Visium HD 3', a novel assay for unbiased spatial gene expression profiling of fresh frozen tissue sections mounted on a standard glass slide. The Visium HD slide's array has a gapless design that enables integration of unsupervised gene expression clustering data with high-resolution H&E images from the same tissue section, allowing precise profiling of finer anatomical features with high tissue coverage at single cell-scale resolution. Furthermore, this novel reverse transcription-based assay generates cDNA products compatible with both short reads and long reads sequencing, extending its utility beyond gene expression analysis to enable immune profiling and the discovery of isoforms and novel transcripts on a spatial level unlocking deeper insights into cancer diversity.

Our research focused on deciphering the spatial heterogeneity of gene expression patterns within the tumor microenvironment, shedding light on the interactions between cancer cells, stromal cells, and vasculature. Visium HD 3' was performed on fresh frozen human breast, pancreatic, liver, and lung cancer tissues. Spatial profiles of clinically-relevant cancer biomarkers such as AR, MUC1, CDH1, and ERBB2 were able to highlight tumor-prone regions in the tissues. Expression of known prognostic angiogenic markers such as VEGF, FGFR, PDGF was inline with the tumor region, indicating tumor growth and metastasis. Additionally, targeting stromal markers such as POSTN, LUM, MMP1, ACTA2, and SFRP4 revealed a distinct boundary between the tumor and stromal layer. The high resolution of this technology further enabled profiling of macrophage and T cell infiltration in cells surrounding the tumor by identification of immune markers like CD4, CD68, CD3E, PTPRC and CCL5.

Our study highlights the importance of exploring the spatial organization of the entire transcriptome in cancer tissues and demonstrates that Visium HD 3' platform is a powerful tool for unraveling the complexities of the tumor microenvironment. These findings lay the groundwork for the development of innovative therapeutic strategies and precision medicine approaches, ultimately contributing to improve outcomes for cancer patients.

#5302 Mapping the tumor microenvironment at single-cell resolution with Stereo-seq v1.3.

Jiajun Zhang[1], Xing Liu[1], Zexian Zeng[2], Rui Zhang[2], Tibby Tianbi Duan[1], Jing Guo[1], Liang Wu[3], Sha Liao[1], Ao Chen[1], **Weijia Su**[4]

[1]STOmics Tech Co., Ltd., Shenzhen, China,[2]Center for Quantitative Biology, Academy for Advanced Interdisciplinary Studies, Peking University, Peiking, China,[3]BGI Research, Shenzhen, China,[4]Complete Genomics, San Jose, CA

Tumor cell heterogeneity and the tumor microenvironment are fundamental questions that heavily influence the process of cancer development, disease progression, and the response to treatments. Knowledge of cancer biology facilitates understanding of pharmacological effects and mechanism of action to improve clinical outcomes. Spatial transcriptomics technology, particularly Stereo-seq, makes the groundbreaking advancement in cancer research with its outstanding performance with high capture efficiency and single cell resolution. It allows scientists to visualize and analyze the complexity of tumors at an unprecedented level of detail. In this study, we mapped tumor heterogeneity and microenvironment of fresh frozen section from colorectal cancer tissue (CRC) at a single-cell resolution with Stereo-seq v1.3 kit. We identified10 cell populations using cell2location, including B cells, fibroblasts, CD4+ T cells, CD8+ T cells, dendritic cells, endothelial cells, epithelial cells, macrophages, mast cells and NK cells. In situ mapping and portion analysis of cell identification revealed a B-cell dominated area which was confirmed by H&E staining of adjacent section. To comprehensively understand the immune activities, we quantified the interactions among different cell types (by StereoSite framework), revealing biologically meaningful intercellular communications mediated by GZMA-PARD3 and CCR10- CCL28 between B cells and epithelial cells. Further pathway enrichment analysis indicated cooperated molecular activities and underlying functions in CRC, which showed interaction activities significantly associated with EGF or BMP signaling pathways. Patteren recognition method identified how functional units assocatied with pathways. Take EGF pathway as an example, results revealed CD4+ T cells and macrophages may coordinated to impact EGF pathway by communication activities. Analysis in this study showed that spatial transcriptomic data at single-cell resolution is vital for exploring intercellular communications, molecular activities, and functional units by providing spatial context. Stereo-seq possess the ability to efficiently gathering evidence of mode of action and resistance mechanims by deeply mapping the spatial profiling in TME. It paves the ways for drug development by removing uncertainties and reducing time and cost. Implementation of Stereo-seq workflow could not only offer impetus to solve unmet needs in area of oncological research, but also bring new findings from lab to patients faster.

#5303 Temporal dynamics of transcriptional and spatial landscape in tumor microenvironment of esophageal squamous cell carcinoma during chemotherapy treatment.

H. Yamashita[1], C. Mashima[1], M. Narahara[2], R. Kamata[1], K. Watanabe[2], R. Nakamura[2], S. Muramatsu[2], A. Tezuka[2], E. Otsuka[2], C. Wada[2], Y. Ishimoto[2], D. Kotani[3], T. Kojima[3], N. Wada[2], H. Sumi[2], T. Yano[3], **G. Takayama**[4], A. Ohashi[1];

[1]National Cancer Center, Chiba, Japan, [2]Daiichi Sankyo Co. Ltd., Tokyo, Japan, [3]National Cancer Center Hospital East, Chiba, Japan, [4]Daiichi Sankyo Inc., Basking Ridge, NJ

Although esophageal squamous cell carcinoma (ESCC) is a highly aggressive malignancy, there is little knowledge about its tumor heterogeneity affecting treatment response. To decipher transcriptomic heterogeneity of tumor microenvironment (TME) from a therapeutic perspective, we conducted CITE-seq and immunostaining by Hyperion in ESCC with or without chemotherapeutic stimuli.
Tumor biopsies of ESCC were sequentially collected by endoscopy from 3 patients during chemotherapy treatments. CITE-seq samples were processed with Chromium Next GEM Single Cell 5′ Reagent Kit v2 (10 x Genomics) and TotalSeq Universal Cocktails (BioLegend) as per the manufacturer's recommendations, respectively. Cell-to-cell interaction analyses in CITE-seq and neighborhood analyses in Hyperion were performed.
Clinical responses of 3 patients for ESCC tumor biopsies were PD, SD, and PR, respectively. Cell annotation analyses in TME components revealed that populations of myeloid cells exhibited an overall decrease during the treatment across three patients, while the decrease in myeloid cell populations was more pronounced in the PR patient. The cluster analyses in epithelial cells exhibited 6 unique subclusters, which were transcriptionally characterized in proliferation, EMT, inflammation, and metabolic pathways. In spatial analyses, the epithelial cells in the PD patient adhered tightly to each other, while the epithelial cells in the SD or PR patient clustered relatively mixed with immune and stromal cells. In SD or PR patients, furthermore, varieties of T cell populations were more accumulated in response to the chemotherapy treatments compared to the PD patient. Neighborhood analysis and cell-cell interaction analysis together identified cell type pairs that showed consistent changes in physical and molecular perspectives. In PD patient, dendritic cell (DC)-macrophage pair was enriched in the proximity of each other, and also increased molecular interactions after chemotherapy. In contrast, activated T-Treg pair was depleted and decreased molecular interactions. Interestingly not just activated T-Treg interaction decreased, but proliferating T cells were also depleted from the tumor.
We conducted integrative analyses with cutting-edge technologies to determine temporal dynamics of TME landscape in ESCC during chemotherapy treatment. Our observations may indicate that chemotherapy increased DC-macrophage interaction to suppress antigen presentation by DC, which suppressed T cell activation and proliferation. The mechanism might contribute to the chemotherapy resistance. These findings will shed light on the molecular insights to deeply understand complexity of the TME networks for chemotherapy resistance in ECSS.

#5304 Profiling the tumor- immune landscape with a same-section multiomics assay.

Anushka Dikshit[1], Cansaran Saygili Demir[2], Rose Delvillar[1], Emerald Doolittle[1], Alice Comberlato[1], Arec Manoukian[2], Maria-Giuseppina Procopio[2], Pino Bordignon[2], Saska Brajkovic[3], Maithreyan Srinivasan[1]

[1]Bio-Techne Corporation, Newark, CA,[2]Bio-Techne Corporation, Tolochenaz, Switzerland,[3]Bio-Techne Corporation, Tolochenez, Switzerland

Checkpoint therapies and engineered immune cell therapies are increasingly becoming the mainstay when it comes to cancer treatments. Continued and improved success of these therapies relies on our deep understanding of the checkpoint marker and immune cell biology, cell-cell interaction in the tumor microenvironment (TME), and immune cell activation. Spatially visualizing the expression of soluble factors such as cytokines and chemokines along with immune cell markers can provide information about the immune cell composition and enable a comprehensive understanding of mechanisms underlying immune cell recruitment, infiltration and exclusion. To address this, we have developed a fully automated spatial multiomics protocol on the COMET™ platform that enables RNA detection using the RNAscope™ HiPlex Pro assay combined with protein detection using sequential immunofluorescence (seqIF™, (Rivest et al, 2023)) to integrate same-section sequential detection of up to 12 RNAs followed by up to 24 proteins. This workflow allows the detection of any RNA and protein target of interest by utilizing existing RNAscope probes or generating a custom design for any RNA target and use standard, non-conjugated primary antibodies for protein detection. We used Lunaphore's HORIZON™ software to analyze this multiomics dataset. Here, we have demonstrated the precise spatial profiling of FFPE solid tumors through the detection of key cytokines indicative of activated T cells and macrophages by using cytokine RNA probes targeting *IFNG*, *IL-1B*, *TNFA*, *TGFB1, IL-4, IL-6, IL-8, IL-10* among others in combination with cell marker antibodies detecting CD3,CD8,CD4, FOXP3 and CD68.In addition, we were also able to visualize T cell recruiting chemokines and receptors such as *CXCL10, CXCL9, CXCL13, CXCL12* and *CXCR3*, and spatially map tumor-infiltrating T cells within tissue context and with subcellular resolution. To that end, we successfully demonstrated same-slide RNA-protein detection for profiling the TME. Using single-cell spatial multiomics we were able to identify unique phenotypes within the tumors that might directly correlate to immunosuppressive or immunostimulatory microenvironments. Detailed mapping of the cellular components of the TME has the potential to better predict therapeutic response for checkpoint and immune-based therapies.

#5305 Tumor cell lineages and immune remodeling of *RHOA*^{Y42C}-driven diffuse gastric cancer.

Kyung Pil Ko[1], Jinho Jang[1], Gengyi Zou[1], Yuanjian Huang[2], Jie Zhang[1], Jae-Il Park[1]

[1]UT MD Anderson Cancer Center, Houston, TX,[2]Nanjing Medical University, Nanjing, China

Diffuse gastric adenocarcinoma (DGAC) is a highly aggressive cancer characterized by poor prognosis and distinct molecular subtypes. Among these, *CDH1* inactivation and *RHOA* mutations, particularly *RHOA*^{Y42C}, define separate oncogenic pathways with distinct biological and clinical implications. To elucidate the functional consequences of *RHOA*^{Y42C} mutation, we established genetically engineered murine gastric organoid models carrying genetic mutations frequently observed in human DGAC. Organoids harboring *RhoA*^{Y42C} in combination with *Kras*^{G12D} and *Trp53* loss (RKP: *RHOA*^{Y42C}, *Kras*^{G12D}, *Trp53*$^{-/-}$) and Cdh1 loss (EKP: *Cdh1*$^{-/-}$, *Kras*^{G12D}, *Trp53*$^{-/-}$) exhibited hyperproliferative and multilayered epithelial architectures, indicative of neoplastic cell growth. Transplantation of RKP organoids into immunocompetent mice displayed features of mucinous DGAC, marked by abundant mucin production and signet-ring cell morphology. By contrast, EKP resulted in tumor architectures with invasive features consistent with non-mucinous DGAC.O ur multiomic approaches combining single-cell RNA-sequencing and spatial transcriptomics (Xenium In Situ) revealed divergent tumor cell lineages and transcriptional programs between EKP and RKP models. EKP tumors displayed enrichment of Runx1-dependent epithelial lineages, whereas RKP tumors exhibited Aqp5-driven lineages with high transcriptional plasticity and robust activation of the Hippo signaling pathway. RKP tumors featured prominent MUC1/4+ mucinous cell populations, whose differentiation was mediated by YAP1, the Hippo pathway effector. Pharmacologic inhibition of Hippo signaling effectively abrogated these mucinous cell lineages, highlighting potential therapeutic vulnerabilities in *Rhoa*^{Y42C}-driven tumors. Additionally, immune profiling revealed significant differences in the tumor microenvironments (TMEs) of EKP and RKP tumors. RKP tumors exhibited an immune-suppressive microenvironment characterized by peripheral enrichment of exhausted T cells expressing markers such as *Pdcd1* and *Ctla4*. Consistently, cell-to-cell interaction analyses revealed enhanced interactions mediated by PDCD1 and CTLA4. Further analyses revealed that differentially secreted cytokines in EKP and RKP induced immune-suppressive genes expression in T cells of RKP tumor, suggesting that *Rhoa*^{Y42C} mutation not only drives tumor-intrinsic changes but also remodels the immune landscape to favor immune evasion. By contrast, the TME of EKP tumors exhibited lower immune suppression, with a reduced presence of T cell exhaustion markers. Together, these findings identified the genotype-specific nature of tumor cell differentiation programs, transcriptional plasticity, and immune microenvironmental remodeling in DGAC. This study highlights Hippo signaling and immune checkpoint pathways as promising therapeutic targets in *RHOA*^{Y42C}-driven mucinous DGAC.

#5306 Dissecting the formation mechanisms of tertiary lymphoid structures and their impact on prognosis and immunotherapy.

Ben Liu, Wenbo Zhu, Mengyue Qiu, Yanxin Yao, **Kexin Chen**

Tianjin Medical University Cancer Institute and Hospital, Tianjin, China

Background: The mortality from gastric cancer is high due to its frequently advanced stage at diagnosis. The standard treatment is mainly surgical, including a comprehensive treatment model of chemotherapy, radiotherapy, targeted therapy and immunotherapy. However, the existing classification of gastric cancer cannot fully guide the precise treatment. TLSs, sometimes also referred to as tertiary lymphoid organs or ectopic lymphoid structures, are organized aggregates of immune cells that arise postnatally in nonlymphoid tissues. The presence of TLSs in tumor environment often indicates a better prognosis.

Method: Identifying the expression of TLSs and its maturation status on pathological sections of gastric cancer surgical specimens via HE staining, immunohistochemistry staining and multi-color fluorescence staining. Surgical tumor specimens were collected and analyzed using single-cell RNA sequencing and spatial single-cell transcriptomics sequencing. To identify TLSs artificially by establishing the artificial intelligence machine learning models of TLSs. To explore the mechanism of TLSs formation in the tumor environment in vivo by humanized PDOX mice and in vitro by co-culture systems of gastric organoids and immune cells.

Results: HE staining, immunohistochemistry staining and multi-color fluorescence staining of tumor sections identified the expression and mature status of TLSs. Single-cell sequencing and spatial single-cell transcriptomics sequencing found the specific markers to identify the TLSs and analyzed the cell composition and cell communication in the tumor environment. Artificial intelligence machine learning models of TLSs identified the presence of TLSs and its maturation status. Furthermore, in vitro co-culture systems of organoids and immune cells and humanized PDOX mice found that organoids of patients with high TLSs score increased apoptosis of tumor cells while using PD-1 antibody and CTLA-4 antibody.

Conclusions: The high expression of mature TLSs indicates a better survival. We establish the artificial intelligence machine learning models of TLSs to identify the presence of TLSs and its mature status. We establish the TLS score to predict the TLS expression and prognosis of patients. Patients with high TLSs score have a better survival and are more sensitive to PD-1 antibody and CTLA-4 antibody.

#5307 New developments in spatial transcriptomics unlock insights into the tumor microenvironment.

Manisha Ray, Justin He, Bin Wang, Bing Yang, Sudhir Tattikota, Timothy Wiggin, Hao Wang, Jichuan Zhang, Lizi Maziashvili, Alexander Genshaft, Peter Reinhold, Brittany Auclair, Robert Mathis, Shawn Wang, Jiang He, George Emanuel

Vizgen, Cambridge, MA

The emergence of tools enabling researchers to perform high-plex spatial transcriptomics with single-cell resolution has revolutionized our understanding of tumor development. However, tissue samples with degraded RNA or extensive crosslinking present challenges for gene expression measurements. For decades, cancer tissue samples have been collected and preserved using formalin-fixation and embedding in paraffin (FFPE), which is not optimal for preserving RNA integrity. This has limited the depth of insights researchers can obtain from archival samples.

The Multiplexed Error-Robust Fluorescence In Situ Hybridization (MERFISH) technology facilitates direct RNA profiling in situ with high sensitivity and resolution. The high accuracy and high plexity are enabled by tiling probes along the length of a transcript, which is particularly challenging in samples with fragmented RNA, such as in FFPE. The MERFISH 2.0 chemistry and sample preparation workflow was developed specifically to improve transcript detection efficiency in measurement of up to 1000 genes in low quality tissues. These improvements can be applied to tissues of up to $3cm^2$ using the MERSCOPE® Ultra™ Platform. Here we applied the improved capabilities of MERFISH 2.0 to profile human breast cancer and human lung cancer tumors over a range of sample qualities.

Breast and lung tumor samples, both whole sections and tissue microarrays (TMA), were run on the MERSCOPE Ultra Platform using a panel of 815 immuno-oncology genes with both MERFISH 1.0 and MERFISH 2.0. The resulting data was used for spatial and single-cell analyses. We show that in all tissues, particularly in lower quality tissues, MERFISH 2.0 increases the counts and subsequent quality of data analysis, such as increased detection of cell types and increased neighborhood associations. Inter- and intra-tumor immune profiling showed improved detection of low-expressing markers, enabling deeper study of the tumor microenvironment.

Spatially resolved transcriptomic profiling of low-quality samples at single-cell level offers significant opportunities for understanding how cancers develop in situ. These improvements will enable new genomic inquiries into how tumors develop and modulate their environment, which in turn will open up new areas of therapeutic research.

#5308 RePROBE: A simple and customizable multiplex immunohistochemistry technique.

Takashi Semba, Atsuko Yonemura, Huaitao Wang, Yilin Tong, Masaya Yamazaki, Takatsugu Ishimoto

Division of Carcinogenesis, The Cancer Institute, Japanese Foundation for Cancer Research, Tokyo, Japan

Background: Multiplex immunostaining techniques have been actively developed and have recently become more prevalent in cancer research. However, most of these techniques require specialized instruments and customized primary antibodies. Here, we established a simple and versatile workflow for multiplex immunohistochemistry (IHC) named RePROBE (Repetitive Primary antibody Replacement and Overlaying images from a Broad range of fluorescence imaging Equipment) that can be readily adopted in many laboratories, providing deeper insight into tumor organization and spatial molecular biology in the tumor microenvironment (TME).
Methods: Formalin-fixed paraffin-embedded (FFPE) sections of various tissue types, such as resected tumors of cancer patients, patient-derived xenografts, and syngeneic mouse tumors,, were subjected to the RePROBE workflow. Commercially available primary and secondary antibodies were used for fluorescence immunostaining with routine indirect IHC methods. Images were acquired by multiple imaging platforms, including fluorescence microscopes, confocal microscopes, and whole slide image scanners. Antibody complexes were removed by heat-based stripping after imaging, and the slides were subjected to the next cycle. All image processing and analysis were performed by open-source software.
Results: We confirmed that the RePROBE workflow worked across different types of tissue sections and imaging modalities and achieved up to 36-plex on human gastric cancer samples and more than 20-plex on syngeneic mouse tumors. The multiplex IHC images derived from the RePROBE workflow were able to be analyzed in downstream pipelines, such as QuPath, Cellpose, and MCMICRO. Heat-based stripping successfully removed most antibodies with some exceptions. Although some loss of cells was observed (less than 7%) after cycle 1, significant cell loss was not observed after cycle 2 up to cycle 10 in most of the samples. We also found that the cell loss was dependent on tissue type and samples.
Conclusions: The RePROBE workflow enabled up to 36-plex whole-slide single-cell resolution imaging and spatial phenotyping analysis, revealing the cell population and spatial organization of tumor cells, CAFs, and immune cells in TME.

#5309 Single-cell-based analysis of tumor microenvironment heterogeneity in human MASLD/MASH-associated HCC: Classifications by inter-cellular network variations.

Naoko Ohtani[1], Yoshiki Nonaka[1], Kanae Echizen[1], Yi Cheng[1], Takahiro Kodama[2]

[1]Pathophysiology, Graduate School of Medicine, Osaka Metropolitan University, Osaka, Japan,[2]Gastroenterology and Hepatology, Graduated School of Medicine, Osaka University, Osaka, Japan

Immune checkpoint inhibitor (ICI) therapy has demonstrated limited efficacy in MASLD/MASH-associated hepatocellular carcinoma (HCC), suggesting distinct anti-tumor immune responses within the tumor microenvironment (TME) of this cancer type compared to other cancers. Understanding the mechanisms driving MASLD/MASH-HCC progression and identifying novel therapeutic targets are therefore critical. While ICI therapy alone may not be sufficient, combining it with drugs targeting key cancer progression pathways may hold promising enhancement of anti-tumor efficacy in this context. In this study, we sought to elucidate the tumor-promoting pathways in MASLD/MASH-associated HCC by characterizing the comprehensive transcriptomic landscape of cancer cells and stromal cells within the tumor microenvironment (TME). We conducted single-cell RNA sequencing on the samples from 31 HCC patients and 8 healthy donors. Using this data, we classified the samples based on the cellular composition of the TME and performed interactome analysis with CellChat version 2. This analysis stratified the samples into five distinct groups: Group 1, TME with stem cell-like cancer cells and immune cell infiltration; Group 2, TME with Wnt signaling-activated cancer cells regardless of immune cell infiltration; Group 3, TME containing cancer cells not classified in the categories mentioned above with immune cell infiltration; Group 4, TME containing cancer cells not classified in the categories mentioned above without immune cell infiltration; and Group 5, TME with normal hepatocytes, as observed in healthy donor livers. Detailed analysis highlights several notable features of the tumor stroma. For example, “senescent” cancer-associated fibroblasts (CAFs) exhibiting a senescence-associated secretory phenotype (SASP) are frequently observed in the TMEs of Groups 1-3. Interactome analysis revealed a VEGFA-VEGFR2 interaction between endothelial cells and various other cell types, consistent with the hypervascularity characteristic of HCCs and supporting the clinical efficacy of VEGF inhibitors. Furthermore, group-specific interactome analysis uncovered distinct novel interactions, indicating molecular network variations among TME groups. We will present spatial distributions of each cell type in TME and TME-dependent inter-cellular interactions. We discuss their potential as biomarkers and therapeutic targets for MASLD/MASH-HCC treatment.

#5310 Spatially resolved cell profiling of testicular diffuse large B-cell lymphoma unveils recurrent cellular neighborhoods associated with survival.

S.-K. Leivonen[1], M. Autio[1], M. Pollari[2], M.-L. Karjalainen-Lindsberg[3], T. Pellinen[4], S. Leppa[1];

[1]University of Helsinki and Helsinki University Hospital Comprehensive Cancer Center, Helsinki, Finland, [2]Tampere University Hospital, Tampere, Finland, [3]Helsinki University Hospital, Helsinki, Finland, [4]Institute for Molecular Medicine Finland (FIMM), Helsinki, Finland

Introduction: Testicular diffuse large B-cell lymphoma (T-DLCBL) is a rare and aggressive lymphoid cancer, accounting for 1% of all lymphomas. In recent years, the impact of the tumor microenvironment (TME) on DLBCL pathogenesis and survival has been recognized. We have previously shown that in T-DLBCL, the T cell-inflamed TME is associated with favorable survival. In this study, we aimed to dissect the T-DLBCL TME in more detail and characterize its spatial architecture at the single-cell resolution.

Methods: We used a tissue microarray (TMA) containing formalin-fixed paraffin-embedded diagnostic T-DLBCL samples from 79 patients. TMA was stained with a 12-plex antibody panel to characterize B cells, T cells, macrophages, and immune checkpoint molecules. Image processing and quality control were performed by combining Ilastik and CellProfiler softwares, and nuclei segmented by a pretrained deep learning segmentation model nucleAIzer. Single cell features from the images were extracted using histoCAT, and cell phenotyping and cell neighborhood analyses were performed utilizing Python package SCIMAP.

Results: We identified over 900,000 single cells, which were classified into 16 distinct phenotypes, including various subpopulations of B cells, T cells, and macrophages. In line with our previous findings, higher T cell proportions were associated with better outcomes. In addition, a subgroup of PD1+ B cells (13% of the whole cell population) was associated with worse outcomes independent of the treatment or international prognostic index (IPI). Neighborhood analysis revealed 10 recurrent cellular neighborhoods (RCNs) with differential cellular compositions, including B cell, macrophage, T cell, or immune checkpoint-rich RCNs. The most abundant was an RCN rich in B cells and macrophages (25% of all cells), followed by an immune-depleted RCN (17% of all cells) and an RCN rich in PD1+ B cells (16% of all cells). Interestingly, a subset of T-DLBCLs was characterized by a very high PD1+ B cell-rich RCN content (>50% of the total cellularity), which translated to poor survival independent of the treatment or IPI. In contrast, a T cell-rich RCN was associated with better outcomes. Finally, when we calculated the minimum average distance between the RCNs, we noticed that a shorter distance between the immune-depleted RCN and an RCN rich in T cells and non-immune cells was associated with better survival.

Conclusions: In summary, the study provides detailed insights into the spatial architecture of the T-DLBCL TME, highlighting the significance of specific cell populations and their neighborhoods in predicting patient outcomes.

#5311 Characterization of ADC and TCE targets in patient tumors using high-parameter spatial proteomics.

M. A. Conner, F. Xie, L. Zhou, E. McKinley, J. Tarrant, T. Sato, Y.-F. Chang, D. Poore, S. McKearnan, K. Hance, J. Waight;
GSK, Philadelphia, PA

Target heterogeneity and intensity of expression in patient tumors are two of many critical factors in understanding the clinical potential of antibody drug conjugates (ADCs) and T cell engagers (TCEs) in oncology.1,2 Despite extensive clinical usage, conventional immunohistochemistry (IHC) is often restricted to a handful of markers per section, limiting its capacity to identify complex patterns of antigen expression in patient samples. In contrast, high-parameter, spatially resolved, proteomic analysis has the potential to not only provide widespread characterization of tumor-associated antigens (TAAs) but also to reveal impactful cell-cell and protein-protein neighborhoods for bispecific pairings and/or novel biomarkers.

Here, we utilized imaging mass cytometry (IMC) complemented by multiplex immunofluorescence (mIF) to spatially evaluate the expression of greater than 30 protein markers simultaneously in 35 tumor specimens from 7 indications. Using whole slide imaging and a bespoke analysis pipeline, coupled with statistical modeling, we profiled multiple TAAs, including HER2, TROP2, B7-H3, and B7-H4 for expression in tumor and adjacent non-tumor tissues.3 Notably, whole-slide mIF was used in tandem with IMC on the same tissue section to enable both region of interest (ROI) selection and image co-registration. With this approach, we observed a high level of concordance between the two modalities for markers of interest such as B7-H3 and B7-H4, helping to further validate our findings. Stromal and immune markers such as SMA (cancer-associated fibroblasts [CAFs,]) CD3/CD8/FoxP3 (T cells,) and CD206 (macrophages) were also included to help characterize the tumor microenvironment and cellular distribution of each TAA. The panel was also incorporated with markers of sensitivity to common ADC payloads, such as SLFN11 and TOP1. In addition to differences in target prevalence and level of expression, we describe the spatial relationship between various TAAs across tumors including such parameters as the average distance between cytotoxic T cells and a given TAA+ cell.

Altogether, these results provide a framework to apply dual-modality imaging of mIF and IMC on the same tumor samples for comprehensive characterization of TCE and ADC targets. In the future, the total number of markers which can be characterized by both mIF and IMC are expected to increase, allowing for an even deeper characterization of tumor biology which may inform bispecific pairings and more precise treatment strategies for ADCs and TCEs.

References:

1 - Cancers(Basel). 2023 Mar 19; 15(6):1845. doi: 10.3390/cancers15061845 2 - Cancers(Basel). 2020 Apr 26; 12(5):1075.doi: 10.3390/cancers12051075 3 - Cell Rep Methods. 2023 Oct 23;3(10):100595. doi: 10.1016/j.crmeth.2023.100595

#5312 Murine syngeneic tumor model characterization by ultrahigh-plexed immunofluorescence imaging.

Jaimee Wong, Ngan Doan, Andrew Woolley, Anandi Sawant, Shilpa Keerthivasan, Sunitha Bachawal, Jennifer Sahni, Nicholas Wilson, Amanda Mikels Vigdal, Xiaoyun Liao

Bristol Myers Squibb Company, Redwood City, CA

Murine syngeneic tumor models are widely used tools to demonstrate efficacy and the mechanism of novel anticancer immunotherapies. Each model is likely to have a unique tumor microenvironment (TME) with immune cells, fibroblasts, endothelial cells and extracellular matrix that may reflect a variety of TME profiles in humans. Baseline characterization of tumor TME features of each model paired with therapy-based mechanistic studies can confirm pharmacodynamic (PD) biomarker hypotheses and inform combination strategies. However, a comprehensive dataset of TME compositions and spatial information is unavailable. PhenoCycler is an ultrahigh-plexed spatial biology imaging platform that can detect up to 100 markers on formalin fixed paraffin embedded (FFPE) sections at a single cell resolution using cyclic detection of DNA-conjugated antibodies. Very few publications have used Phenocycler technology to image murine FFPE tissues. In this study, we constructed a FFPE tissue microarray (TMA) block that includes 12 commonly used preclinical murine models of breast, colon, lung, pancreatic ductal, renal and bladder carcinomas with duplicate cores, and built a 29-marker panel to characterize the complexity of the TME of these models. Our data showed distinct TMEs in the different models and cell-cell interactions that differed depending on the tumor type examined. For instance, Py2T and Pan02 are cancer associated fibroblast (CAF) and macrophage enriched tumors whereas MC-38 tumors have low levels of CAF and T cell infiltration. Based on these analyses, the EMT6 tumor model, an immunocompetent triple negative mammary carcinoma with adequate extracellular matrix, was selected for an in vivo pharmacology study for a novel anti-stroma immunotherapy. Evaluation of whole tissue tumor samples from the study revealed significant increases in CD8+ T cell infiltration and a reduction of COL1A1 expression in treated tumors compared to vehicle group tumors consistent with antitumor efficacy. Our study demonstrated that ultrahigh-plexed immunofluorescence imaging is a powerful technology that can efficiently assist in vivo model selection and support biomarker discovery, especially in combination with a TMA section that can include multiple tumor models.

#5313 Spatial transcriptomics reveals clone-specific features of primary and metastatic tumors in triple-negative breast cancer.

Yu-Ting Kang, Tzu-Hung Hsiao

Taichung Veterans General Hospital, Taichung, Taiwan

Breast cancer has become the most commonly diagnosed malignancy, with metastasis remaining the leading cause of mortality, particularly in triple-negative breast cancer (TNBC). In this study, we applied spatial transcriptomics with an 8 μm resolution to analyze the primary tumor and lung metastasis, occurring six months post-recurrence, of a TNBC patient, encompassing a total of 1,033,074 bins. Through this analysis, six tumor clusters (T0-T5) with distinct gene expression profiles were identified. Among these, T0 and T3 were the predominant clusters observed in the metastatic tumor. T0 was primarily distributed in the tumor core, while T3 localized at the tumor boundary. Both clusters exhibited high expression of *VIM*, a marker associated with epithelial-mesenchymal transition (EMT). Differentially expressed gene (DEG) analysis revealed that T3 displayed elevated levels of genes associated with extracellular matrix (ECM) remodeling pathways. Trajectory analysis indicated that T3 represented the most advanced differentiation stage as a subclone. Our findings provide insights into the dynamic spatial heterogeneity and metastatic progression of primary and metastatic TNBC tumor subtypes.

#5314 Prostate club-like cells resist treatment and carry prognostic value in lung and pancreatic adenocarcinoma.

Antti Kiviaho[1], Sini K. Eerola[1], Heini M. L. Kallio[1], Miina Hoikka[1], Aliisa M. Tiihonen[1], Iida Salonen[1], Alexander Giesen[2], Ebrahim Afyounian[1], Sini Hakkola[1], Charles T. A. Parker[3], Mikael Marttinen[1], Mazlina Ismail[3], Wout Devlies[2], Teuvo L. J. Tammela[1], Teemu J. Murtola[1], Ian G. Mills[4], Steven Joniau[2], Gerhardt Attard[3], Frank Claessens[5], Tapio Visakorpi[1], Alfonso Urbanucci[1], Kirsi J. Rautajoki[1], Matti Nykter[1]

[1]Tampere University, Faculty of Medicine and Health Technology, Tampere, Finland,[2]Department of Urology, University Hospitals Leuven, Leuven, Belgium,[3]University College London Cancer Institute, London, United Kingdom,[4]Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom,[5]Department of Cellular and Molecular Medicine, Laboratory of Molecular Endocrinology, Leuven, Belgium

Introduction: The prostate cancer tumor microenvironment (TME) contains various cell type identities. We recently described club-like epithelial cells as a constitutive part of the TME present in tissue regions with increased myeloid-derived suppressor cell infiltration. The origin, exact function, and identity of club-like cells remain unresolved.
Methods: Here we used bulk, single-cell, and spatial transcriptomics to characterize club-like cells and their relation towards androgen deprivation therapy. We additionally analyzed multiplex immunofluorescence data for interactions between club-like cells and myeloid-derived suppressor cells. Furthermore, we investigated club-like expression in lung and pancreatic tumors.
Results: In the spatial transcriptomics data, club-like enriched regions comprise a mixture of club, hillock, and senescent epithelial cells. Prostate cancer tumors from men treated with androgen deprivation therapy display elevated expression of hillock and senescence, but not the club signature. A high club signature score is associated with improved overall survival in TCGA lung adenocarcinoma (LUAD, HR = 0.59, $p = 5.1x10^{-4}$), while a high hillock gene signature score indicates worse overall survival in both LUAD (HR = 1.80, $p = 9.3x10^{-5}$) and pancreatic ductal adenocarcinoma (PDAC, HR = 1.94, $p = 1.4x10^{-3}$). In PDAC, club-like expression is associated with IL-1β^{+} myeloid cell interaction. We define IL-1β^{+} myeloid cells in the prostate single-cell data and demonstrate co-localization with club cells in the spatial transcriptomics data. Finally, we show that epithelial cells from castration-resistant prostate cancer samples have an elevated hillock signature compared to normal prostate and treatment-naïve prostate cancer, suggesting hillock expansion or phenotypic plasticity during disease progression.
Conclusion: Our results indicate that the canonical club phenotype arises in response to inflammatory signaling, while closely related hillock cells survive androgen deprivation in prostate cancer and indicate poor overall survival in LUAD and PDAC tumors.

#5315 Mapping tumor infiltrating lymphocytes in whole prostatectomy specimens to visualize and quantify the immune microenvironment of prostate cancer.

Yuwei Zhang[1], Rajarsi Gupta[1], Joel Saltz[1], Tahsin Kurc[1], Jakub Kaczmarzyk[1], Erich Bremer[1], Veronica Wallaengen[2], Amanda Galvez[2], Abigail Bartley[2], Leonard Salcedo[2], Alan Pollack[2], Sanoj Punnen[2], Oleksandr Kryvenko[2], Radka Stoyanova[2], Sandra M. Gaston[2]

[1]Stony Brook University, Stony Brook, NY,[2]University of Miami Miller School of Medicine, Miami, FL

Background: Prostate cancer (PCa) frequently presents as multifocal lesions with histologic and molecular heterogeneity. A comprehensive understanding of tumor immune responses within the context of the complex PCa microenvironment remains elusive. Through the use of Digital Pathology and innovative Pathomics image analysis tools, tumor infiltrating lymphocytes (TILs) can be mapped, visualized, and quantified in whole slide images (WSIs) of H&E stained PCa slides. We have developed a novel workflow to map and quantify TILs by Gleason histologic grade (Gleason-TIL maps) in whole radical prostatectomy specimens.
Approach: WSIs from 211 H&E tissue sections from eight whole radical prostatectomy specimens containing Gleason histologic patterns 3 and 4 were used in the development of the PCa Pathomics workflow. Pathomics tools generated PCa Tumor-TIL and Gleason-TIL maps for each corresponding H&E WSI for all eight prostatectomy specimens. To support tissue sampling for correlative biomarker studies and genomic testing in PCa clinical trials, each Tumor-TIL and Gleason-TIL map was partitioned to quantify total PCa content, Gleason grade (3 vs 4/5) content, and intratumoral and peritumoral TILs per 0.5 x 0.5 cm area tile of tissue in the WSI.
Results: Both Tumor-TIL and Gleason-TIL maps reveal complex and heterogeneous tumor immune interactions across multifocal lesions within each radical prostatectomy specimen. Our novel workflow added the ability to quantify PCa, Gleason grade, and TILs to specific 0.5 x 0.5 cm tissue tiles to quantify TILs relative to Gleason grade that varies within and across each case of multifocal PCa. Quantification of the TIL tile maps showed that in 6 of the 8 prostatectomies the “hot spot” tile with the most intratumoral TILs had more TILs in the Gleason 4/5 patches than in the adjacent Gleason 3 patches.
Conclusions and Future Studies: This novel workflow was developed to generate Tumor-TIL and Gleason-TIL maps for every PCa H&E WSI to serve as powerful visual tools for real world laboratory tissue sampling for downstream genomics, immunohistochemistry, and other correlative studies for PCa clinical trials. Adding a 0.5 x 0.5 cm tile partitioning function increases the efficiency of identifying and quantitatively mapping TILs in the complex and heterogeneous PCa microenvironment. The preliminary association of TILs with higher grade Gleason 4/5 PCa appears provocative as potential actionable information as an immune biomarker in PCa.

#5316 Multi-platform spatial profiling reveals intra-tumor heterogeneity in intrinsic subtypes and immune markers in breast tumors from Kenyan patients.

Mustapha Abubakar[1], Shahin Sayed[2], Hela Koka[1], Scott Lawrence[1], Karun Mutreja[1], Xing Hua[1], Viviane Oluoch[2], Veronica Ngundo[2], Zaitun Ajuoga[2], Difei Wang[1], Chad Highfill[1], Petra Lenz[1], Maria Brown[1], Aaron M. Rozeboom[1], Kristine Jones[1], Amy Hutchinson[1], Belynda Hicks[1], Paul S. Albert[1], Francis Makokha[3], Stefan Ambs[4], Jonine Figueroa[1], Jianxin Shi[1], **Xiaohong Rose Yang**[1]

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD,[2]Aga Khan University Hospital, Nairobi, Kenya,[3]Mount Kenya University, Thika, Kenya,[4]Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD

Breast cancer is a highly heterogeneous disease at the clinical, population, and molecular levels. Using key molecular markers, breast cancer can be classified into at least four major intrinsic subtypes: luminal A, luminal B, HER2-enriched, and basal-like or triple-negative. These subtypes are associated with distinct risk factors, clinical behaviors, treatment responses, and outcomes. Recent research highlights the importance of the breast tumor microenvironment (TME) in shaping tumor evolution and cancer progression. Both tumor and TME cells within an individual patient often exhibit substantial intratumoral heterogeneity (ITH), the extent of which may impact accurate tumor classification and treatment response, but has not been well characterized especially in women of African Ancestry. The goal of this study was to comprehensively investigate ITH of intrinsic subtypes and immune TME markers in breast cancer patients from an East African population. Specifically, we employed the Nanostring GeoMx^{TM} platform to quantify 44 key breast cancer intrinsic and immune subtyping markers in spatially defined regions of breast tumors from 31 Kenyan breast cancer patients. We found that all 31 tumors exhibited some degree of ITH, with a third of them showing substantial ITH. Cell composition (epithelial vs. stromal), spatial location of TME immune cells (tumor-leading edge, proximity to tumor or stroma), and intrinsic subtype (specifically, basal-like vs. luminal A) all contributed significantly to ITH of these markers. When comparing results to those obtained from an *in-situ* multiplex staining platform InSituPlex (ultivue) and bulk Nanostring nCounter RNA expression, all generated from the same set of cases, we found that intrinsic or immune subtyping by bulk approaches, which is heavily influenced by the predominant subtypes within a tumor, may lead to inaccurate classification when substantial ITH exists. Similarly, sampling-based approaches like tissue microarrays (TMAs) or region-of-interest (ROI)-based assays are also likely to be subjective to inaccurate and inconsistent measurements of immune markers due to sampling bias. In low-resource settings, *in-situ* assays combined with multi-region scoring may offer a more accurate yet practical alternative to bulk or single-cell assays, which are often costly and technically challenging.

#5317 Multimodal spatial analysis of colon cancer tissue reveals emergence of an immunosuppressive tumor maintenance mechanism.

Gaurav Joshi[1], Nicholas Sciascia[1], Michael Yang[1], Brian Falkenstein[2], Raymond Yan[2], Shannon Quinn[2], Brenna Fearey[1], Junya Yoshioka[1], Arif Burak Tosun[2], S. Chakra Chennubhotla[2], Filippo Pullara[2], Fumiki Yanagawa[1]

[1]Nikon Instruments, Inc., Lexington, MA,[2]PredxBio Inc, Pittsburgh, PA

Background: Colorectal cancer is the second most fatal cancer worldwide, accounting for 9% of all reported cancer deaths, and is the number one cause of death among people under the age of 50. Rapid technical advances in the field of spatial biology are allowing us to gain new insight into colorectal cancer biology and patient heterogeneity, which can help to guide the development of immunotherapies. Using a combination of modalities such as sequential multiplex immunofluorescence (mIF) and spatial transcriptomics (STx) on a colon cancer sample we determined the spatial distribution, phenotype, function, and a cytokine and chemokine profile within the colon cancer tissue.

Experimental Procedure: 5 um FFPE sections were prepared from a colon cancer sample exhibiting moderate to poorly differentiated adenocarcinoma of T3, N0 staging. mIF was performed using Lunaphore COMET with a 20-antibody panel, followed by H&E staining and imaging. An adjacent section was used for STx with the 10x Genomics Visium CytAssist platform. Data was analyzed with Lunaphore Horizon®, 10x Genomics Loupe Browser software and PredxBio SpaceIQ™ unbiased cell typing, microdomain discovery and network biology platform that co-analyzes mIF and STx datasets.

Results: The tumor showed an aberrant growth pattern and a highly proliferated crypt region. Three distinct microdomains in the tumor tissue were discovered: one with high levels of PD-L1+ cells, a second with high levels of NK cells and a third with low levels of infiltrating cells in the lumen and crypt. There was significant presence of VISTA+ and IDO1+ cells throughout the lumen. These cells interacted with CD4+ and CD8+ cells which were found to be present in both lumen and crypt regions. PD-L1 was prominent in the cells found in both lumen and crypt. Finally, NK cells were abundant throughout the lumen and in regions adjacent to the crypt.

Conclusions: Using multimodal spatial imaging and spatial co-analysis of mIF and STx data, we were able to elucidate relationships between different cell types in a complex tumor tissue and assign context to their activities. Despite high levels of NK cells, indicative of a possible active anti-tumor immune response, the tumor has developed an immune evasion mechanism due to the presence of cells expressing high levels of the anti-tumor immune response markers such as PD-L1, IDO1, and VISTA. Based on these results, therapies targeting more than one immune checkpoint protein may yield improved response due to an abundance of the existing NK cells. The transcriptomic profiling reveals a complex relationship between various cytokines secreted by different cell populations which alter the landscape of the tumor microenvironment, facilitating the emergence of an immunosuppressive phenotype.

#5318 Identifying immune cell phenotypes and their function by using high throughput spatial multiomics.

Debia Wakhloo[1], Julia Yu[1], Sonali Deshpande[1], Donald Allen[2], Anne Hellebust[2], Anushka Dikshit[1]

[1]Bio-Techne Corporation, Newark, CA,[2]Indica Labs, Albuquerque, NM

Advances in our understanding of the tumor microenvironment (TME) will help inform the development of next generation immunotherapies and engineered immune-cell therapies. Spatially characterizing immune cells with cell marker protein and activation marker RNAs is crucial to determine therapeutic efficacy and potential therapeutic toxicity. To address this, we have developed a high throughput RNAscope™ assay on the BOND RX to spatially visualize RNA and protein markers on the same slide. We utilized the new RNAscope™ Multiomic LS assay that can detect up to 6 RNA and protein targets on the same slide. The TSA-based amplification strategy offers signal boost for both RNA and protein targets. To optimize protein detection, the workflow is completely protease-free there by preserving antigen integrity and tissue morphology. The assay can be customized to include any target RNA and proteins of interest. Here, we demonstrate the use of our pre-conjugated antibody panel which includes CD8, CD4, FoxP3 and PanCK to visualize tumor infiltrating lymphocytes (TILs). We also utilized unconjugated primary antibodies for CD68 and CD163 to detect tumor macrophages in multiple tumor tissues. Using the HALO® image analysis software from Indica Labs, we performed RNA and protein quantification as well as cell phenotyping and spatial analysis. Infiltrating Cytotoxic T cell lymphocytes were detected using CD8, *GZMB* and *IFNG* co-expression. Regulatory T cells were detected using CD4, FOXP3 co-expression. PanCK was used as a tumor marker to delineate tumor from stroma. Similarly, tumor associated macrophages were detected using CD163, CD68 , *IL10* and *IL-1B* expression. We were also able to identify distinct M1 and M2 macrophage populations in the tumor samples. The protease-free RNAscope Multiomic LS assay was successfully able to interrogate different subpopulation of immune cells with high fidelity RNA and protein signal while maintaining tissue morphology. The higher plex capability provides detection of more markers simultaneously, in turn enabling immune phenotype profiling while maintaining the sensitivity and specificity. Finally, the assay affords a very high throughput on the BOND Rx allowing large-scale pre-clinical and patient sample analysis.

#5319 Simultaneous spatial epigenomic and transcriptomic analysis of human breast cancer reveals regulatory patterns governing tumor and microenvironment architecture at the cellular level .

Katelyn Noronha, Molly Wetzel, Gumaro Rojas, Jennifer Garbarino, James McGann, Jose Perez, Machele Riccio, Danijela Pavic, Jaden Joaquin, Joshua Barnett, Jeffrey Sabina, Colin Ng

AtlasXomics, New Haven, CT

Recent advances in spatial transcriptomics and spatial proteomics have enabled increasingly complex questions on the nature of gene regulation and expression in cellular subtypes in tumor tissue and the tumor microenvironment. However, most spatial omics techniques do not profile the epigenomic landscape responsible for downstream gene expression. Furthermore, current spatial technologies have yet to profile the epigenome and transcriptome simultaneously, and thus it remains a challenge to correlate multi-omics data across sections of extremely heterogenous tumor tissue. Co-profiling of spatial epigenomics and transcriptomics using principles of Deterministic Barcoding in Tissue for spatial omics sequencing (DBiT-seq) has been demonstrated on normal brain tissue. Joint spatial profiling of chromatin states and whole transcriptome in tissue allows for parallel characterization of gene regulation programs across all cell types, while preserving the tissue architecture for greater understanding of the cellular environment. Here we present the first application of spatial ATAC-seq and spatial transcriptomics on the same tissue section to characterize the tumor microenvironment of an infiltrating ductal carcinoma, the most common type of breast cancer, and adjacent normal tissue. Distinct spatial clusters representing different cell subtypes were identified via both spatial chromatin accessibility and spatial transcriptomics. Spatial ATAC-seq profiling of accessible regulatory elements correlated well with RNA expression. Spatial patterns of transcription factor motif accessibility also correlated well with the observed transcriptional program of tumor tissue. When compared to adjacent normal tissue, spatial co-profiling of chromatin accessibility and the transcriptome revealed that the epigenetic landscape is significantly altered in the tumor. Future work will focus on development of co-profiling of histone modifications and the transcriptome to enable the study of another layer of the epigenomic landscape, especially as targeting epigenetic modifiers such as HDACis in breast cancer is being tested in clinical trials with other standard of care therapeutics. Overall, we present a solution to profile multiple layers of gene regulation and expression with spatial context, which can be applied to most tumor types for better understanding of tumorigenesis and the consequences of new targeted therapies.

#5320 Integrative spatial omics reveals distinct tumor-promoting multicellular niches and immunosuppressive mechanisms in African American and European American patients with TNBC.

Qian Zhu[1], Akhila Balasubramanian[1], Jaya Ruth Asirvatham[2], Badrajee Piyarathna[1], Jaspreet Kaur[3], Nada Mohamed[2], Ling Wu[1], Megha Chatterjee[1], Stacy Wang[1], Niloufar Pourfarrokh[2], Uttam Rasaily[1], Yitian Xu[4], Junjun Zheng[4], Deborah Jebakumar[2], Arundathi Rao[2], Shu-hsia Chen[4], Yi Li[1], Eric Chang[1], Xiaoxian Li[5], Ritu Aneja[6], Xiang H.-F. Zhang[1], Arun Sreekumar[1]

[1]Baylor College of Medicine, Houston, TX,[2]Baylor Scott and White Hospital, Temple, TX,[3]The University of Alabama at Birmingham, Birmingham, AL,[4]Houston Methodist Research Institute, Houston, TX,[5]Emory University, Atlanta, GA,[6]Georgia State University, Atlanta, GA

Racial disparities in triple-negative breast cancer (TNBC) outcomes have been reported. However, the biological mechanisms underlying these disparities remain unclear. We integrated imaging mass cytometry and spatial transcriptomics, to characterize the tumor microenvironment (TME) of African American (AA) and European American (EA) patients with TNBC. The TME in AA patients was characterized by interactions between endothelial cells, macrophages, and mesenchymal-like cells, which were associated with poor patient survival. In contrast, the EA TNBC-associated niche is enriched in T-cells and neutrophils suggestive of an exhaustion and suppression of otherwise active T cell responses. Ligand-receptor and pathway analyses of race-associated niches found AA TNBC to be “immune cold” and hence immunotherapy resistant tumors, and EA TNBC as ‘inflamed’ tumors that evolved a distinctive immunosuppressive mechanism. Our study revealed the presence of racially distinct tumor-promoting and immunosuppressive microenvironments in AA and EA patients with TNBC, which may explain the poor clinical outcomes.

Statement of Significance We have uncovered distinct tumor-promoting and immunosuppressive microenvironments that may contribute to the observed disparities in patient outcomes. Our findings lay the groundwork for developing race-specific therapeutic interventions, potentially improving outcomes for patients with TNBC. Given that chemotherapy and anti-PD1 therapy are currently available treatments for TNBC, our finding of race-specific spatial multicellular niche-associated cell-cell interactions suggest that these treatments may have racially distinct response profiles among TNBC patients. Hence, our novel insights on molecular and cellular interactions in TNBC stratified by race has the potential to inform personalized treatment strategies.

#5322 Spatial profiling of the tumor microenvironment in colorectal cancer: Insights into immune, tumor, and stromal interactions using high-dimensional protein analysis.

Enrique Velazquez Villarreal[1], Ei-Wen Yang[2], Fernando C. Diaz[3], Maria Ninova[4]

[1]City of Hope Comprehensive Cancer Ctr., Duarte, CA,[2]Poliagent, San Francisco, CA,[3]Lineberger Comprehensive Cancer Center, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC,[4]Department of Biochemistry, University of California Riverside, Riverside, CA

Introduction: Colorectal cancer (CRC) is the second leading cause of cancer-related deaths globally, claiming over 900,000 lives annually. In the US, CRC disproportionately affects certain populations, with higher prevalence among racial and ethnic minorities. The tumor microenvironment (TME) plays a pivotal role in CRC progression, therapeutic response, and overall patient outcomes. However, the spatially intricate interactions within the TME that distinguish effective from ineffective tumor control remain poorly understood. Addressing these gaps is essential for enhancing our understanding of spatial heterogeneity and advancing immunotherapy development.
Methodology: To investigate these dynamics, we employed a multi-modal approach using re-engineered co-detection by indexing (CODEX) on publicly available data (Schürch et al., 2020) from 35 late-stage CRC patients. Our systematic workflow classified cells, analyzed protein expression, and spatially represented the data. Cells were grouped into three main classes—stroma, tumor, and immune—based on structural characteristics and functional roles. Protein expression analysis was conducted by integrating data for individual surface protein expression, enabling direct comparisons between the stroma, tumor, and immune classes. Through this framework, 56 protein markers were profiled to identify conserved cellular neighborhoods (CNs) that characterize the spatial and functional organization within the CRC TME.
Results: Protein expression analysis revealed distinct patterns across the stroma, tumor, and immune classes for the biomarkers profiled. These markers exhibited differential expression profiles across the three classes, highlighting their unique roles within the TME. For instance, immune markers such as CD3, CD8, CD68, and PD-1 showed significant variation between the tumor and immune classes, underscoring their spatial organization and functional importance within the TME. Similarly, functional markers such as MMP9 and Ki-67 demonstrated distinct expression dynamics, reflecting their roles in tumor progression and immune responses. Auxiliary markers, including cytokeratins, highlighted structural differences unique to the stroma class. These results provided a comprehensive understanding of the spatial and functional interplay between immune, tumor, and stromal components in the CRC TME.
Conclusion: This study provides a deeper understanding of protein expression within the CRC TME, enhancing the classification and spatial characterization of TME. These results provide key insights into spatial heterogeneity within the TME through distinct protein expression profiles. These findings provide valuable guidance for future research in drug discovery, with the potential to significantly advance the field of precision oncology.

#5323 Spatial heterogeneity of tumor and stromal cells in desmoplastic small round cell tumors.

Jiaqian Fan, Adewale A. Adebayo, Diana Shamsutdinova, Davis Ingram, Clement Agyemang, Roberto Cardenas-Zuniga, Alexander Lazar, Danh Truong, Joseph Ludwig

UT MD Anderson Cancer Center, Houston, TX

Desmoplastic small round cell tumor (DSRCT) is a rare, aggressive sarcoma subtype that harbors a pathognomonic EWSR1::WT1 fusion protein (FP). DSRCT is usually incurable and FP-targeted agents are nonexistent. The five-year survival rate is below 20%. Tumor nests are classically surrounded by desmoplastic stroma in DSRCT, yet the interactions between the tumor cells and the stromal cells are poorly understood. Given this context, our study aims to understand the spatial heterogeneity within DSRCT and the tumor-stroma interactions. We utilized a 20-panel marker to study the cellular constituents of the tumor microenvironment (TME) in DSRCT and to characterize its multilineage expression, including epithelial and neural (NE) signatures. The markers included: DSRCT (ST6GALNAC, pan-Cytokeratin), Fibroblasts (Collagen, α-smooth muscle actin), Endothelial Cells (CD31, α-smooth muscle actin), T-cells (CD45, CD4, CD8), Macrophages (CD45, CD68, CD163, CD11c), and AR/NE markers (androgen receptor, neural-specific enolase, synaptophysin). Marker expression was explored in a nine-patient cohort using 9 x 9 mm² tissue sections imaged by the Lunaphore COMET system. A deep learning model, Visiopharm, extracted high-quality regions and segmented cells based on nuclear staining. Spatial data were transformed into matrix format for analysis in Python, where UMAP and Leiden clustering identified distinct clusters consisting of tumoral cells, fibroblasts, and endothelial cells. Our analysis revealed two distinct fibroblast clusters—one with high α-SMA expression and the other with high collagen expression. These two clusters exhibited distinct spatial distributions and proportions across patient samples, indicating heterogeneity in stromal composition within the DSRCT microenvironment. Our previous study identified three DSRCT subtypes: AR+/NE-, AR-/NE+, and Hybrid AR+/NE+. The current spatial analysis suggests that AR+/NE- tumor nests are consistently surrounded by α-SMA-high fibroblasts, hinting at subtype-specific stromal interactions. Preliminary findings from co-culture experiments of DSRCT cells with human mesenteric fibroblasts suggest that fibroblasts enhance the proliferative capacity of DSRCT cells. These findings provide novel insights into the stromal heterogeneity of the DSRCT microenvironment and its association with tumor subtypes. Our future work will focus on quantifying spatial relationships and patterns, which includes how tumor nest characteristics are associated with fibroblast cell type or DSRCT subtype. Proximity and neighborhood analysis in python and Visiopharm analysis will be applied. Ultimately, our results might reveal novel drug targets that stop oncogenic tumor-stroma signaling.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: CDK Inhibitors
Poster Session

#5327 CDK2 inhibition produces persistent polyploid cancer cells with a survival advantage.

Liliya Tyutyunyk-Massey[1], Zibo Chen[1], Xiuxia Liu[1], Masanori Kawakami[1], Adam Harned[2], Yeap Ng[3], Brian Luke[4], Sameul C. Okpechi[1], Blessing Ogunlade[1], Yair Alfaro[1], Roberto Weigert[3], Kedar Narayan[2], **Xi Liu**[1], Ethan Dmitrovsky[1]

[1]Molecular Pharmacology Program, Frederick National Laboratory for Cancer Research, Frederick, MD,[2]Center for Molecular Microscopy, Frederick National Laboratory for Cancer Research, Frederick, MD,[3]Laboratory of Cellular and Molecular Biology, Frederick National Laboratory for Cancer Research, Frederick, MD,[4]Advanced Biomedical Computational Science, Frederick National Laboratory for Cancer Research, Frederick, MD

Aneuploidy, a hallmark of cancer, drives chromosomal instability and antineoplastic drug resistance. Common in hematologic and solid tumors, centrosome amplification leads to supernumerary centrosomes, causing chromosomal missegregation in dividing cancer cells. This imbalance produces progeny with abnormal chromosome counts, leading to cell death, resistance to antineoplastics, and clinically-aggressive tumors. This study examined the effects of CDK2 inhibition on aneuploid lung cancer cells and tumors. It revealed that CDK2 antagonism triggers anaphase catastrophe through centrosome clustering disruption and multipolar mitosis. Unexpectedly, this also leads to the formation of a persistent population of polyploid, apoptosis-resistant cells. Using independent CDK2 inhibitors and shRNAs, we observed that some cancer cells underwent multipolar division and subsequent cell death. Others exhibited cytokinesis failure, resulting in multinucleated, and polyploid cancer cells that continued to survive and proliferate despite continuous CDK2 inhibition. Through time-lapse imaging with transfected FUCCI cell cycle probes and RNA-seq analysis of polyploid versus diploid populations, we identified upregulated CDK1-related pathways and kinesin (KIF) family members in the polyploid cellular population. This likely contributes to the persistent polyploid population, despite CDK2 antagonism. These findings were independently validated *in vivo* using intravital microscopy and patient-derived xenograft (PDX) lung cancer models. Intriguingly, polyploid and multinucleated cancer cells were found to proliferate post-CDK2 inhibitor treatments. In marked contrast, a polyploid population was not found in CDK2 inhibited primary human alveolar epithelial cells, indicating that this is a pathway that is preferentially activated in aneuploid cancer cells. Analysis of gene expression in TCGA lung cancer datasets linked CDK1 and KIF family overexpression to an unfavorable patient survival, implicating this persistent polyploid population as contributing to poor survival outcomes in lung cancer. These findings are relevant to the clinical development of CDK2 inhibitors. They indicate that despite the pro-apoptotic effects of CDK2 antagonism in aneuploid lung cancer cells, a population of drug-resistant, polyploid cells persists. We propose that a combinatorial therapy targeting CDK2 alongside polo-like kinase 4 (PLK4), CDK1 or kinesin family members would further disrupt centrosome clustering, eliminate polyploid cancer cells, and enhance tumor cell apoptosis and antineoplastic effects. This dual approach would enhance the anti-tumor efficacy of CDK2 inhibition, potentially overcoming resistance mechanisms. This provides a promising strategy to eradicate persistent lung cancer cells following CDK2 inhibition.

#5328 p16 confers sensitivity to CDK2 inhibitors in CCNE1-amplified ovarian cancers.

Chance Sine[1], Lotte Watts[1], Brianna Fernandez[1], Jianxin Wang[2], Erik S. Knudsen[2], Agnieszka K. Witkiewicz[2], Sabrina L. Spencer[1]

[1]University of Colorado Boulder, Boulder, CO,[2]Roswell Park Comprehensive Cancer Center, Buffalo, NY

Blocking the cell cycle is a promising avenue for cancer therapy, with Cyclin-Dependent Kinase 2 (CDK2) emerging as a key target. However, in multiple cell types, CDK4/6 activity compensates for CDK2 inhibition and sustains the proliferative program, enabling CDK2 reactivation. Thus, we hypothesized that sensitivity to CDK2 inhibition is linked to the absence of this CDK4/6-mediated compensatory mechanism. Here we show that Cyclin E1-driven ovarian cancers often co-express the tumor suppressor p16, which inhibits CDK4/6. We show that ovarian cancer cells expressing p16 exhibit heightened sensitivity to CDK2 inhibitors and that depletion of p16 renders them significantly more resistant. Multiplexed immunofluorescence of 225 ovarian patient tumors reveals that at least 18% of tumors express high Cyclin E1 and high p16, a group that we expect to be particularly sensitive to CDK2 inhibition. Thus, p16 may be a useful biomarker for identifying the patients most likely to benefit from CDK2 inhibitors.

#5329 Development of a novel selective CDK4 inhibitor for HR+/HER2- breast cancer.

Mei Hua Li*,#,[1], Apeng Liang*,#,[1], Kai Wang[1], Yi Long[1], Rui Wu[1], Hui Xu[1], Jian Zhu [1], Guangbin Liu[1], Ling Fang[1], Xi Wang[1], Chenjie Pan[1], Yiming Cai[1], Xiang Zhang [1], Shengli Dong[1], Yu Yu[1], Xinlong Yang[1], Chao Zhou[1], Wei Wu [1], Chengshan Niu[2], Mingyu Jiang[3], Shaoqing Chen[1], Jun Li*.1,2,[3], Yusheng Wu1,2, [3]

[1]TYK Medicines, Inc., Huzhou, Zhenjiang, China,[2]TYK Medicines, Inc. (Zhengzhou), Zhengzhou, China,[3]TYK Medicines, Inc. (Shanghai), Xuhui District, Shanghai, China

Introduction: FDA approved CDK4/6 inhibition medicines has demonstrated significant clinical benefits in patients with HR+/HER2- breast cancer. However, the dual CDK4/6 inhibitors also present severe on-target toxicity leading to severe adverse effects (SAE) in the clinic, such as neutropenia. Clinical studies with palbociclib reported grade 3-4 neutropenia events in 60-66% of patients receiving the treatment. To dissect the cause of the clinical toxicity of CDK4/6, it has been found that CDK6, not CDK4, is directly involved in the differentiation of myeloid cell in which CDK6 expression is up-regulated. Thus, we sought to develop a mono-CDK targeting agent, a CDK4 inhibitor, to grant similar pharmacological potency against HR+/HER2- breast cancer while diminishing or even abolishing CDK6 on-target toxicity to improve the efficacy, and more importantly, the safety of the hallmark cell cycle therapies. To this end, we at TYK Medicines have developed lead CDK4 inhibitor candidates (TY-2072) with high selectivity and potency.

Results: TY-2072 demonstrates high selectivity against CDK4, and more than 17-fold selectivity against CDK6 compared to palbociclib, proving that TY-2072 is a CDK4 inhibitor rather than a CDK4/6 dual inhibitor. Western blot assays showed a dose-dependent reduction in phosphorylated RB (Ser780) in breast cancer cell lines after 48 hours TY-2072 treatment. The anti-proliferation effects of CDK4 inhibitors were assessed with MCF7 (breast cancer, CDK4 sensitive), JEKO-1 (mantle cell lymphoma), and MOLM13 (acute myeloid leukemia, CDK6 sensitive) through a CTG cell viability assay after 5-day incubation. Palbociclib shows a strong inhibitory effect on MCF7/T47D cells due to dual CDK4 and CDK6 kinase activity. TY-2072 strongly inhibited JEKO-1, but had no inhibitory effect on CDK6 high-expression MOLM13. In MCF7-xenograft mouse model, TY-2072 showed significant suppression of tumor growth in a dose-dependent manner at 20 and 40 mg/kg BID, TY-2072 exhibited synergetic effects with Fulvestrant on anti-tumor growth inhibition with good tolerance, evidenced by the maintenance of body weight of the animals throughout the study. We conducted preliminary *in vitro* combination studies with lead CDK4 compounds, and the combination of a selective CDK4 inhibitor with a CDK2 inhibitor or a PI3K inhibitor demonstrated a significant synergistic inhibitory effect on MCF7 cells and MCF7 Palbociclib-resistant cells. Positive results were also observed when TY-2072 was combined with AMG510/MRTX849 in the NSCLC H2122 (KRAS G12C) cells.

In summary, the optimization of the lead compounds is continuing with goal to identify a PCC compound in the near future for IND-enabling studies.

#Meihua Li and Apeng Liang contributed equally to this work.

*To Whom Correspondence should be addressed to: Jun Li Meihua Li and Apeng Liang.

#5330 Antitumor activity of potent and selective CDK2 inhibitors as monotherapy and in combination with chemotherapy in models of small cell lung cancer.

Nealia House, Liang Yuan, Christophe Lemetre, Maxine Chen, Philip Ramsden, Scott Ribich, Lakshmi Muthuswamy, Kerrie Faia

Blueprint Medicines Corporation, Cambridge, MA

Background: A molecular hallmark of small cell lung cancer (SCLC) is the high frequency of inactivating mutations in the retinoblastoma 1 (Rb1) gene, leading to loss of functional Rb expression and dysregulated G1-S phase progression. Analysis of publicly available DepMap data shows that Rb-mutant SCLC cell lines exhibit a genetic dependency on cyclin-dependent kinase 2 (CDK2), suggesting aberrant CDK2 activity may be essential in regulating cell cycle progression in the absence of CDK4/6-Rb signaling. BLU-222 is an investigational, potent, highly selective, orally bioavailable CDK2 inhibitor currently in Phase I/II clinical trials (NCT05252416). Here, we report on the activity of BLU-222 and BLU-956, a next-generation CDK2 inhibitor, in pre-clinical models of SCLC. Using *in vitro* cell line screening, we identified specific biomarkers predictive of response to BLU-222 and BLU-956.

Methods: The *in vitro* antiproliferative effect of BLU-222 and BLU-956 alone or in combination with cisplatin was assessed by CyQuant in a panel of SCLC cell lines. *In vivo* antitumor activity of BLU-222 or BLU-956 as single agents or in combination with cisplatin was measured in biomarker positive cell line-derived xenograft (CDX) models.

Results: Both BLU-222 and BLU-956 demonstrated strong anti-proliferative activity in Rb-null SCLC cell lines. Biomarker analysis identified high levels of *SKP2* expression as a predictive biomarker for CDK2 inhibitor sensitivity. Further, co-expression of high *SKP2*, high *CDKN2A*, and high *CCNE1* expression was associated with the strongest responses to BLU-222 or BLU-956 in Rb-null cell lines. BLU-222 and BLU-956 were well tolerated as single agents and in combination with cisplatin in immunodeficient mice. CDK2 inhibitor monotherapy achieved tumor stasis in pre-clinical SCLC CDX models, including NCI-H526. Responses were enhanced in combination with cisplatin, superior to cisplatin monotherapy, and led to tumor regression.

Conclusions: CDK2 inhibition represents a novel therapeutic approach in SCLC, an aggressive cancer with high unmet medical need. In pre-clinical models of SCLC, BLU-222 and BLU-956 demonstrated substantial anti-tumor activity as single agents, with enhanced effects in combination with cisplatin. These findings provide rationale for exploring targeting CDK2 in combination with cisplatin in SCLC and suggest that specific biomarkers could be useful to predict response.

#5331 Orthosteric CDK2 kinase inhibitors have a distinctive profile when compared to genetic perturbation of CDK2 in CCNE1-amplified and non-amplified tumor cell lines.

Joanne M. Munck[1], Susan J. Tudhope[2], Kleopatra Papa[2], Alex Howard[1], Sam Hogan[2], Suzanne Kyle[2], Jessica Watt[2], Luke Gaughan[3], Maria Ahn[1], Stephen R. Wedge[2], John F. Lyons[1]

[1]Astex Pharmaceuticals, Cambridge, United Kingdom,[2]Cancer Research Horizons Therapeutic Innovation, Newcastle University, Newcastle, United Kingdom,[3]Translational and Clinical Research Institute, Newcastle University, Newcastle, United Kingdom

Aim: There has been renewed interest in pursuing cyclin-dependent kinase 2 (CDK2) as a therapeutic target for cancer treatment, given its essential role in driving the survival of *CCNE1*-amplified tumors and mediating resistance to CDK4/6 inhibitor treatment in estrogen receptor positive breast cancer. This has resulted in the identification of a next generation of orthosteric inhibitors which have increased selectivity for CDK2 kinase versus other CDK-family members. This study aimed to contrast genetic and chemical perturbation of CDK2 in human tumor cell lines with and without *CCNE1*-amplification. Methods: Cellular responses were assessed using assays for proliferation (CyQUANT), clonogenicity, cell cycle (flow cytometry), and western blotting. NanoBRET tracer displacement assays were used to examine orthosteric kinase inhibition in cells. Genetic perturbation of CDK2 used siRNA and CRISPR-Cas9 editing with sgRNA. Four CDK2 inhibitors currently undergoing clinical trials were profiled. Results: CDK2-targeting siRNA conferred a >75% decrease in the clonogenicity of *CCNE1*-amplified OVCAR-3, Kuramochi and FUOV1 cells, thus confirming dependence of the cells on CDK2. The non-*CCNE1* amplified cells TYK-nu and PEA2 were not sensitive to CDK2-siRNA. The orthosteric CDK2 inhibitors inhibited cellular CDK2 with IC_{50} values in the range of 1-20 nM. As expected, the compounds inhibited the proliferation of *CCNE1*-amplified cell lines but they also demonstrated activity against non-*CCNE1* amplified TYK-nu cells (one example had GI_{50} values of 89 ± 8 nM in OVCAR-3 cells, and GI_{50} values of 122 ± 13 nM in TYK-nu cells, respectively). Consistent with the role of CDK2 in controlling the G1 checkpoint, we observed G1 arrest in the *CCNE1*-amplified cell line OVCAR-3 following CDK2 CRISPR knock-out. As expected, CDK2 gene deletion did not significantly impact the cell cycle in the TYK-nu non-*CCNE1* amplified cell line. In contrast, an orthosteric CDK2 inhibitor induced a G2 arrest in the TYK-nu cell line at doses 2 - 5-fold greater than the compound GI_{50}. Evidence of cyclin E1 accumulation was observed in both *CCNE1*-amplified and non-amplified cells following orthosteric CDK2 kinase inhibitor treatment (48 - 72 hours). It was also observed following generation of an F80G CDK2 mutant TYK-nu cell line and treatment with the bulky purine analog 3MB-PP1. In contrast, gene-silencing, or gene-editing of CDK2, had no effect on cyclin E1 levels. Conclusion: We demonstrate that the profiled orthosteric CDK2 inhibitors do not reproduce genetic perturbation of CDK2 in *CCNE1*-amplified and non-amplified cell lines. We propose the need for more selective CDK2 inhibitors and those that inhibit CDK2 in a manner that more closely reflects the genetic perturbation of CDK2.

#5332 Targeting CDK2 in estrogen receptor-positive/RB-deficient breast cancer.

Chang-Ching A. Lin[1], Pamela Luna[1], Khushi Ahuja[1], Dan Ye[1], Yisheng V. Fang[1], Alec Trub[2], John Bisi[2], Jay Strum[2], Patrick Roberts[2], Jeon Lee[1], Ariella B. Hanker[1], Carlos L. Arteaga[1]

[1]UT Southwestern Medical Center, Dallas, TX,[2]Incyclix Bio, Durham, NC

The estrogen receptor-positive (ER+) subtype continues to be the leading cause of breast cancer-related mortality worldwide. Although CDK4/6 inhibitors (CDK4/6i) have extended survival of patients with ER+ breast cancer, resistance to these therapies is inevitable. *RB1* loss-of-function alterations are a well-established mechanism of resistance to CDK4/6i, occurring in approximately 10% of ER+ breast cancer patients who progress on CDK4/6i. With the broad use of CDK4/6i as the standard treatment, the population of patients with ER+/RB-deficient breast cancer is expected to rise, highlighting an urgent need for effective targeted therapies and therapeutic strategies for this drug-resistant subtype. Using a genome-wide CRISPR screen in ER+ *RB1* knockout (RBKO) T47D breast cancer cells, we identified CDK2 as an essential gene. Additionally, analysis of the DepMap dataset further confirmed *CDK2* as a top essential gene in cancer cells with low *RB1* mRNA expression, suggesting an RB-independent role for CDK2 and its potential as a therapeutic target in cancers with RB deficiency. Supporting this hypothesis, siRNA-mediated silencing of *CDK2* (siCDK2) in MCF7 RBKO cells and in lung (H596 and H1155), prostate (Du-145), and triple-negative breast (MDA-MB-468) cancer cells harboring somatic *RB1* mutations or deletions led to the accumulation of cells in the G1 phase and a reduction of cells in the S phase, indicating an overall delay in cell cycle progression. Similarly, treatment of MCF7 RBKO cells with a CDK2-specific small molecule inhibitor, INX-315, also resulted in suppression of G1-to-S cell cycle progression. In line with the cell cycle analysis, gene set enrichment analysis (GSEA) of RNA-seq data revealed a significant downregulation of the E2F Hallmark gene signature ($q<0.0001$) in MCF7 RBKO cells transfected with siCDK2 vs. control siRNA. Notably, the therapeutic combination of INX-315 and fulvestrant (a selective ERα degrader) synergistically suppressed the growth of MCF7 RBKO xenografts and ER+/*RB1*-deleted patient-derived xenografts in mice. Treatment with this combination reduced Ki67 levels more potently than each monotherapy or vehicle. These findings collectively identify CDK2 as a promising therapeutic target for ER+/RB-deficient breast cancer and support clinical testing of CDK2- and ERα-tagerted combination therapies in this refractory population.

#5333 CDK4/6 inhibitors in patients with bone-only metastatic breast cancer, the role of immune microenvironment.

Mahta Mardani[1], Suleyman Noordeen[1], Katherine Clifton[1], Cynthia Ma[1], Jingyu Xiang[1], Jingqin Luo[1], Takayuki Kobayashi[1], Jing Xi[2], Nusayba Bagegni[1], Foluso Ademuyiwa[1], Rama Suresh[1], Ashley Frith[1], Andrew A. Davis[1], Ron Bose[1], Lindsay Peterson[1], Shana Thomas[1], Yu Tao[1], Kaylee O'Donnell[1], Netra Navadkar[1], Kristin Kwakwa[1], Will Mattock[1], Thomas Walsh[1], Yalin Xu[1], Deborah J. Veis[1], Xinming Su[1], Katherine Weilbaecher[1]

[1]Washington University School of Medicine in St. Louis, Saint louis, MO,[2]Rocky Mountain Cancer Centers, Littleton, CO

Background: CDK4/6 inhibitors (CDK4/6i) have revolutionized HR+ metastatic breast cancer treatment. Trials like PALOMA, MONALEESA, and MONARCH showed improved progression-free survival (PFS) with endocrine therapy (ET), establishing it as first-line treatment. Bone metastases are common in HR+ breast cancer, with 40% of patients having bone-only disease (BoD), which has a distinct course and better prognosis. However, CDK4/6i trials showed less significant PFS benefits in BoD subgroups. This study explores BoD survival outcomes and CDK4/6i efficacy.

Methods: Patients with HR+, $HER2^-$ metastatic breast cancer from a retrospective study at Washington University School of Medicine were included. All the patients received CDK4/6i in the first- or second-line settings from June 2014 to March 2020. Outcomes were collected through June 2023. The Kaplan-Meier with log-rank test and multivariable Cox model were used for survival analysis. We used murine triple negative 4T1 and ER+ luminal B PyMT Bo1 breast tumor cell line-derived models in immunocompetent mice.

Results: We included 167 female patients (131 first, 36 second line) treated with palbociclib plus ET for at least two months. Median age was 62 years (28-87), and 70 (41%) were in the BoD group. Median PFS was significantly shorter in the BoD group compared to the BV (Visceral +/- Bone) group (16.8 vs. 25 months, p=0.04). Multivariate analysis, adjusted for age and race, showed BoD had an HR of 1.65 (95% CI: 1.05-2.68) for PFS.The results of preclinical models showed that single-agent ribociclib decreased the primary mammary gland tumor burden. This effect was abolished when CD8+ T-cells were depleted. However, in the bone colonization model, ribociclib had a nonsignificant effect on bone tumor burden despite decreases in tumoral Ki-67. Arg1+ immune suppressive macrophages were significantly decreased in primary tumors but not in bone tumors after ribociclib administration, suggesting a relative treatment resistance and immune suppression in the bone tumors. Next, we analyzed baseline bone biopsies from 44 patients, categorized as responders (PFS >1 year) or non-responders (PFS <6 months). Ki67 expression was not different between groups but was decreased in 4 patients with paired pre-and post-treatment biopsies. Using Hyperion imaging mass cytometry, we profiled a patient's bone tumor before and after Palbociclib; we found increased PD-L1, CD8+ T-cells, and CD206+ myeloid cells post-treatment. Immune profiling for all 44 patients is ongoing, with future efforts to understand different immune cell populations' role in CDK4/6i response and identify targets to enhance their effects in bone metastases.

Conclusion: Our retrospective study and preclinical data suggest CDK4/6i may be less effective in the bone microenvironment, underscoring the need for research into the role of the bone tumor immune microenvironment in treatment response.

#5334 Proliferative signaling through the ras-erk pathway can be inhibited by the overexpression of dual-specific phosphatase genes in collaboration with other negative regulators of cell cycle progression in the acute myeloid cell line HL-60.

Michael Roberts, Amelia Harper, Kishan Mangru, Katy Meta, Sophia Kovatsis, Jeffrey Forrester, Grace Crossland, Cuong Nguyen, Sher Bahadur

Dickinson College, Carlisle, PA

Reversing or reprogramming malignant cells toward their normal gene expression patterns, without repairing their oncogenic mutations, can be accomplished in culture as demonstrated by the treatment of certain acute myeloid leukemia (AML) cell lines with phorbol esters, which through the activation of PKC results in cell cycle arrest, myeloid differentiation, and ultimately programmed cell death. Gene therapy continues to show promise in the development of new and effective cancer treatments. At its inception, replacing deficient tumor suppressor gene functions was an attractive goal and with the development of gene editing strategies, the repair of activated oncogenes is possible. Here we report the delivery and overexpression of negative regulators of oncogenic RAS signaling, through transfection, that provide tumor suppressor functions in the NRAS-driven cell line, HL-60, that inhibit their proliferative competency. Genes belonging to the dual-specific phosphatase family (DUSPs), including DUSP1, 5 and 6, along with RAS-ERK pathway inhibitors RASA1 and SPRY2, when transfected and overexpressed in HL-60 cells, affects decreases in phospho-ERK and Ki67 levels resulting in reduced proliferation. In combination with the overexpression of genes encoding the cyclin-CDK inhibitors, CDKN1A and CDKN2B, these anti-proliferative alterations are enhanced. While efficient strategies wait for the delivery of lacking tumor suppressor functions to cancer cells through targeted gene therapies, we propose candidate genes for reversing the proliferation program of RAS-driven AML cells.

#5335 A comparative study of CDK2 inhibitors.

Lotte P. Watts[1], Lisa Nguyen[2], Marissa Baker[2], John Chionis[2], Ryan Vong[2], Advait Nagle[2], Stephen Dann[2], Sabrina L. Spencer[1]

[1]University of Colorado Boulder, Boulder, CO,[2]Genesis Therapeutics, San Diego, CA

CDK2 is a key Cyclin-Dependent Kinase that is responsible for driving cells through the cell cycle. CDK2 is both hyper-activated in a variety of cancers and is a mechanism of resistance to CDK4/6 inhibitors, making it an attractive therapeutic target. Using PF3600 and PF4091 (tagtociclib), we previously showed that upon CDK2 inhibition, cells exhibit a rapid drop in substrate phosphorylation that rebounds within several hours ('drop-rebound'). We further showed that CDK4/6 activity compensates for inhibition of CDK2 and sustains the proliferative program by maintaining Rb hyper-phosphorylation and E2F transcription, allowing many cell types to continue cycling in drug. With the advent of numerous new CDK2 inhibitors in development and in clinical trials, we sought to compare five CDK2 inhibitors side-by-side. We first show that all five CDK2 inhibitors elicit the drop-rebound in CDK2 activity. Thus, the drop-rebound response to CDK2 inhibition is not drug-specific, but instead reflects how the cell cycle is wired for robustness. Second, we find that GTAI-664 is ten times more potent than other CDK2 inhibitors. This potency advantage paired with improved selectivity may result in greater target coverage at attainable plasma concentrations in preclinical models. In agreement, several xenograft tumor models appear to respond with better tumor growth inhibition when treated with GTAI-664 relative to PF4091. Finally, we test the combinability of CDK2 inhibitors with CDK4/6 inhibitors *in vitro* and *in vivo.* We hypothesize that durable cell-cycle arrest requires forcing Rb kinase activity below a specific threshold where the Rb-E2F positive feedback loop is broken. Our results are timely given the increasing interest in CDK2 inhibitors across a variety of cancer types.

#5336 A RAS(ON) multi-selective inhibitor combination therapy triggers long-term tumor control through senescence-associated tumor-immune equilibrium in preclinical models of PDAC.

Caroline Broderick[1], Riccardo Mezzadra[1], Exequiel Sisso[1], Almudena Chaves-Perez[1], Ho Yu-Ji[1], Andrea Schietinger[1], Mallika Singh[2], Scott W. Lowe[1]

[1]Memorial Sloan Kettering Cancer Center, New York, NY,[2]Revolution Medicines, Redwood City, CA

Pharmacological inhibition of oncogenic RAS represents an attractive strategy to target pancreatic ductal adenocarcinoma (PDAC), an almost ubiquitously RAS-driven disease. However, initial responses to targeted monotherapy inhibition of active RAS can be followed by relapses, potentially driven by the persistence of drug-tolerant tumor cells. To target these ‘persister’ cells, we investigated strategies to increase their immune visibility in mouse models of PDAC. We show that combining a RAS(ON) multi-selective inhibitor with the CDK4/6 inhibitor palbociclib drives persister cells into a senescent-like state, which coincides with improved tumor control and substantial remodeling of the tumor microenvironment. Combining RAS(ON) and CDK4/6 inhibition with a CD40 agonist results in durable regressions and CD4 T cell-dependent tumor-immune equilibrium. Our studies reveal a combinatorial approach that circumvents resistance to RAS(ON) inhibitor monotherapy in preclinical models and demonstrate a mechanism by which therapy-induced senescence can be reinforced by the immune system, resulting in durable tumor control.

#5337 Homogeneous bioluminescent immunoassay for hKi-67 provides a simple and robust screening tool for antiproliferative agents.

Dan F. Lazar, Kevin R. Kupcho, Andrew L. Niles, James J. Cali

Promega, Madison, WI

We present a robust, homogeneous, and luminescent microplate-based immunoassay utilizing Lumit® technology for fast and quantitative detection of human Ki-67, a marker of cell proliferation. This assay enables efficient screening of antiproliferative agents, demonstrating exceptional performance in assessing antiproliferative activity across multiple mechanisms of action.

We evaluated the Lumit® hKi-67 immunoassay in HCT116 colorectal cancer cells treated with increasing concentrations of four agents known to inhibit proliferation via different mechanisms: palbociclib (CDK4/6 inhibitor), samuraciclib (CDK7 inhibitor), nutlin-3A (MDM2/p53 interaction inhibitor), and thymidine (dNTP pool perturbation). With all four test agents, the Lumit® hKi-67 immunoassay detected a marked, dose-dependent 63-82% reduction in Ki-67 after only 24 hours, and an even greater 79-98% reduction at 48 hours, demonstrating a fast and reliable measure of antiproliferative effects. A same-well multiplex with a cell-impermeant fluorogenic DNA dye demonstrated no cell death provoked by these compounds at the doses and times tested.

In contrast to the large decrease in the proliferation marker Ki-67 elicited within 24 h of treatment by these antiproliferative agents, the response windows achieved with assays of viable cell number were significantly smaller at the early time point. Only 25-40% less viable-cell DNA was observed after 24 h compound treatment vs. untreated controls, with 48 h required to produce response windows of 57-89%. Similar observations were obtained with two other viability assays, one detecting ATP levels and the other live-cell protease activity. However, ATP levels were reduced more than expected by 24 h treatment with thymidine, likely due to perturbation of metabolic activity. Equally concerning, at 48 h, the live-cell protease assay and, especially, detection of ATP levels dramatically underreported the decrease in viable cell number (relative to time-matched untreated controls) in response to palbociclib and nutlin-3A. This latter effect is likely due to the substantial increase in cell size induced by palbociclib and nutlin-3A even as they inhibit cell proliferation. The strong antiproliferative effects induced by palbociclib and nutlin-3A and detected with the hKi-67 immunoassay, however, were further validated by two additional assays: Ki-67 immunofluorescence and BrdU ELISA. Notably, the Lumit® assay provides faster, more robust data compared to these more labor-intensive alternatives.

This study highlights the Lumit® hKi-67 immunoassay as an attractive, high-throughput alternative for screening antiproliferative agents, offering a simpler, faster, and more consistent approach than traditional methods. It is poised to be an invaluable tool in drug discovery and preclinical screening.

#5338 TY-1781, a potent and highly selective CDK2 inhibitor, demonstrates superior anti-tumor activity in CCNE1 high-expression solid tumor models.

Chengshan Niu*,#,[1], Meihua Li*,#,[2], Shaoqing Chen*,[3], Maolin Zheng[1], Mingtao Chen[1], Hui Xu[2], Kaige Ji [1], Yuanjie Li[1], Rui Wu[2], Shengli Dong[2], Yu Yu[2], Xinlong Yang[2], Chao Zhou[2], Apeng Liang[2], Wei Wu[2], Mingyu Jiang[2], Jun Li*,1,2,[3], Yusheng Wu1,2,[3]

[1]TYK Medicines, Inc. (Zhengzhou), Zhengzhou, China,[2]TYK Medicines, Inc. (Changxing), Changxing, China,[3]TYK Medicines, Inc. (Shanghai), Shanghai, China

Background: The common prescription of Palbociclib and other approved CDK4/6 inhibitors in clinic have greatly benefitted breast cancer patients worldwide. The cell cycle regulation therapy however has been found that the long-term use of the CDK4/6 inhibition agents eventually causes primary or acquired resistance to the therapy, and patients eventually relapse from the treatment. Approximately 20% of patients treated with CDK4/6 inhibitors show initial treatment failure. Based on the finding that abnormal activation of CDK2/Cyclin E1 due to CCNE1 gene amplification is determined the key resistant mechanism to CDK4/6 inhibition. Amplification of CCNE gene is prevalent in multiple tumor types, particularly in high-grade ovarian, uterine, and gastric cancers. CDK2 activity is important for survival of CCNE aberrant cancer cells and is also associated with potential resistance to targeted therapies, such as CDK4/6 dual inhibitors. Therefore, CDK2 is a promising therapeutic target in cancer. However, due to the structural homology and functional conservation of CDK family members, as well as the toxic effects associated with the inhibition of other CDKs, the development of selective CDK2 inhibitor is challenging. Here, we present preclinical data of TY-1781, a novel CDK2 inhibitor for the treatment of CCNE1 high-expression tumors.

Results: TY-1781 demonstrates excellent CDK2 kinase inhibitory activity (IC_{50} 0.7 nM), and high selectivity against CDK1, CDK4, CDK6, CDK7, and CDK9 in a panel screening of CDK kinases. *In vitro* cell proliferation data shows that tumor cell lines bearing CCNE1 amplification, are highly sensitive to the TY-1781 treatment. TY-1781 inhibits cellular Rb phosphorylation in Cyclin E1 amplified OVCAR3 cells in a potent yet selective manner, but not in Cyclin E1 wild type TOV21G cells; TY-1781 blocks the G1/S transition in CCNE1 overexpression OVCAR3 cells; and the tumor inhibitory activity of TY-1781 is further underscored when combined with a CDK4/6 inhibitor (Palbociclib), as demonstrated in a cell colony formation assay with ER+/Her2- breast cancer cells. Consistent with the *in vitro* results, TY-1781 demonstrated a potent inhibitory effect in immune-defect mouse models of CCNE1-amplified cancer cells. When combined with a chemotherapy agent (gemcitabine) or PARP inhibitor, TY-1781 robustly suppressed tumor growth in the examined ovarian cancer models.

Conclusion: Taken together, we identified a highly selective and potent CDK2 inhibitor through medicinal chemistry screening and rounds of refining. The data suggest that TY-1781 may be eligible for further clinical development for Cyclin E1 high-expression patients who relapse from previous standard of care treatments.

# Chengshan Niu and Meihua Li contributed equally to this work.

*To Whom Correspondence should be addressed to Shaoqing Chen, Chengshan Niu, Meihua Li and Jun Li.

#5339 Single cell analysis enables tracking the evolution of resistance to CDK4/6 inhibitors in ER+ breast cancer.

Yuki Matsunaga[1], Emma Aleksandrovic[2], Hima M. Patel[1], Dhivya R. Sudhan[1], Khushi Ahuja[3], Dan Ye[1], Chang-Ching A. Lin[1], Lei Guo[4], Cynthia X. Ma[5], Jeon Lee[3], Siyuan Zhang[2], Carlos L. Arteaga[1], Ariella B. Hanker[1]

[1]Simmons Comprehensive Cancer Center, UT Southwestern Medical Center, Dallas, TX,[2]Department of Pathology, UT Southwestern Medical Center, Dallas, TX,[3]Lyda Hill Department of Bioinformatics, UT Southwestern Medical Center, Dallas, TX,[4]Peter O'Donnell Jr. School of Public Health, UT Southwestern Medical Center, Dallas, TX,[5]Washington University School of Medicine in St. Louis, St. Louis, MO

The combination of CDK4/6 inhibitors (CDK4/6i) with endocrine therapy prolongs survival of patients with ER+/HER2- metastatic breast cancer. Nearly all patients with advanced disease treated with this combination eventually progress, highlighting a need for therapeutic interventions that prevent the emergence of drug resistance. Drug tolerant persister (DTP) cells represent a reservoir of surviving cells from which drug-resistant clones eventually emerge. We hypothesized that targeting breast cancer cells in a DTP state that emerge under selective pressure from CDK4/6i would prevent the emergence of drug resistance. First, we aimed to delineate genomic and epigenomic features of DTPs under continuous CDK4/6i + antiestrogen therapy. We treated MCF7 and T47D ER+ breast cancer cells with palbociclib + estrogen deprivation long-term (4 weeks). Cells that survived long-term treatment exhibited characteristics of DTPs, such as reversible sensitivity to CDK4/6i + estrogen deprivation, reversible cell cycle arrest, and reversible transcriptional programs associated with the DTP state. To translate these findings to patients, we examined gene expression data from residual tumors after neoadjuvant treatment with palbociclib and the aromatase inhibitor anastrozole in the NeoPalAna trial and found that these tumors showed a similar enrichment of gene signatures associated with DTPs compared to tumors before treatment. To elucidate with DTPs give rise to drug resistant clones, we barcoded MCF7 and T47D cells with a lineage and RNA recovery (LARRY) barcode library with ~315,000 diverse barcodes, such that we can track them during long-term treatment. Barcoded cells were treated with palbociclib plus estrogen deprivation for 0 h, 72 h, and 4 weeks and then subjected to single-cell RNA-seq; 101 and 15 clones that were initially arrested in G0/G1 eventually expanded at 4 weeks, while 376 and 27 clones were eliminated during treatment of MCF7 and T47D cells, respectively. Comparing the expanding clones with the exhausted clones, we identified 50 marker genes for DTP cells in each cell line. We are currently using a CRISPR-Cas9 targeted knockout screen to identify which of these genes are causal to DTP survival. We will then compare results from these single cell approaches with RNA microarray analysis from residual tumors after neoadjuvant treatment in the NeoPalAna trial. In summary, a small subset of ER+ breast cancer cells survive long-term treatment with CDK4/6i plus estrogen deprivation and exhibit reversible transcriptomic features of DTPs. Single-cell RNA-seq analysis in barcoded breast cancer cells revealed genes significantly upregulated in expanding clones that show features of the DTP state. We proposed therapeutic targeting of these genes may reduce survival of drug-tolerant cells and prevent the emergence of drug resistance.

#5340 Simultaneous targeting of CDK4/6 and BETs is independent of RB status in osteosarcoma.

Rada Malko[1], Pankita H. Pandya[2], Harlan E. Shannon[3], Erika A. Dobrota[3], Barbara J. Bailey[3], Niknam Riyahi[3], Keiko E. Kreklau[3], Ryli E. Justice[3], Melissa A. Trowbridge[4], Kathryn L. Coy[4], Anthony L. Sinn[4], Khadijeh Bijangi-Vishehsaraei[2], M. R. Saadatzadeh[3], Micheal J. Ferguson[2], Kyle W. Jackson[2], Brian D. Weiss[2], Karen E. Pollok[3]

[1]Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN,[2]Department of Pediatrics, Hematology/Oncology, Indiana University School of Medicine, Indianapolis, IN,[3]Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN,[4]Preclinical Modeling and Therapeutics Core, IU Melvin and Bren Simon Comprehensive Cancer Center, Indiana University School of Medicine, Indianapolis, IN

Osteosarcoma (OS) is the most prevalent primary bone cancer in children and adolescents, characterized by genetic instability and aggressive progression leading to poor survival. Despite the use of multimodal therapies, survival rates have stagnated around 60% highlighting a critical need for the identification of effective drug targets to improve outcomes. Sequencing efforts reveal cell-cycle dysregulation which may be targeted via CDK4/6 inhibitors (CDK4/6i) such as palbociclib and abemaciclib. While Retinoblastoma proficiency (RB+) has been used as a biomarker of response to CDK4/6i clinically, over 70% of OS patients exhibit RB deficiency (RB-). Therefore, it is imperative to understand to what degree RB- affects response to CDK4/6i in OS. Based on in vitro screening data, we hypothesize a combination of CDK4/6i with bromodomain and extraterminal domain inhibitors (BETi) will potentiate the CDK4/6i anti-tumorigenic effects in both RB+ and RB- OS. A hallmark of OS is high levels of genomic instability and BETi may further induce DNA damage to kill DNA repair-deficient cells. In-vitro combination studies identified dose ratios of CDK4/6i and bivalent BETi (AZD5153) that induce synergistic growth inhibition in a panel of monoallelic and biallelic RB OS cell lines. CRISPR-mediated RB-knockout (KO) clones were developed in human (143B, MG63.3) and murine (K7M2) metastatic OS cell lines. In comparison to wildtype (WT) clones, KO clones were 2-10-fold more resistant to palbociclib and at most 2-fold more resistant to abemaciclib; with differences attributed to broader selectivity profile of abemaciclib. However, dual CDK4/6i and BETi therapy resulted in synergistic growth inhibition in both WT and KO clones. To track emergence of therapeutic resistance, a longitudinal growth assay indicated that dual therapy induced cytotoxicity over time with greater effects observed in KO clones, presumably due to increased genome instability. CDK4/6i therapy with abemaciclib, but not palbociclib, increased C-PARP expression by 2-fold in WT cells and 50-fold in KO cells. Annexin V flow cytometry corroborated these findings, showing apoptosis in 25% of WT cells and 75% of KO cells. Limiting dilution assays revealed that dual CDK4/6i and BETi therapy reduced clonogenic cell frequency, with a more pronounced effect in KO clones. Additionally, RB monoallelic OS PDX models TT2 (pretreated) and PDX96 (naïve), were treated for 6 weeks with CDK4/6i (palbociclib, 40mg/kg, BETi (AZD5153,1 mg/kg), or their combination and significantly reduced tumor growth compared to single agents in both models ($p<0.05$) and was well tolerated. A trend for increased probability of survival was observed in PDX96. Future studies will extend dosing duration and focus on metastatic and RB- variant models. These findings provide rationale for further study of novel therapeutic options which may expand the clinical utility of CDK4/6i regardless of RB status.

#5341 Duration of CDK4/6 inhibitor (CDKi) treatment in metastatic HR+ HER2- breast cancer (mHRBC) is associated with changes in AKT pathway protein expression.

Brie Chun[1], Allison Creason[1], Shaun M. Goodyear[1], Laura Heiser[2], Zahi Mitri[3]

[1]Knight Cancer Institute, Oregon Health & Science University, Portland, OR,[2]Biomedical Engineering, Oregon Health & Science University, Portland, OR,[3]BC Cancer Agency, Vancouver, BC, Canada

Background: First-line CDKi and endocrine therapy has improved survival for patients with mHRBC. Upon CDKi progression, the optimal treatment strategy for mHRBC without a targetable mutation in *PIK3CA, AKT, PTEN* or *ESR1* is unclear. The CAPItello-291 trial showed that targeting the PIK3CA pathway after CDKi improved progression-free survival, including mHRBC without identifiable genomic alterations in *PIK3CA, AKT* or *PTEN*. Protein expression analysis may identify whether signaling pathways such as PIK3CA/AKT are upregulated after CDKi and proffer rational targets even without genomic alterations. We used digital spatial profiling (DSP) to perform protein expression analysis of oncogenic pathways on mHRBC samples collected from patients before and after CDKi treatment.
Methods: Patients with mHRBC treated with a CDKi between 2015 and 2022 with available archival tissue were included in this study. Samples were obtained <2.5 years prior to CDKi start and <1 year after CDKi discontinuation. The sample cohort included 6 paired biopsies, which enable direct assessment of CDKi changes. A single FFPE slide per sample was stained with an 82-protein cocktail of antibodies conjugated to UV-photocleavable DNA barcodes and analyzed using the NanoString GeoMx platform. For each sample, three 660-micron regions of interest (ROI) were selected to maximize tumor cell content and avoid necrosis or sectioning artifacts. Protein expression data was normalized by geometric mean, ROI expression was averaged per patient, and means comparisons were made between groups. This study was approved by the OHSU Institutional Review Board
Results: Thirty-five samples from 29 patients were analyzed (23 pre-CDKi, 12 post-CDKi). AKT and INPP4B expression was greater post-CDKi in patients treated for >6 months compared to those treated for ≤6 months (AKT, >6 months: 2254, ≤6 months: 1194, p = 0.032; INPP4B, >6 months: 1135, ≤6 months: 390, p=0.032). BCL-2 expression was greater and Ki-67 expression was lower post-CDKi in patients treated for >6 months compared to those treated ≤ 6 months (BCL-2, >6 months: 1547, ≤6 months: 563, p=0.016; Ki-67, >6 months: 421, ≤ 6 months 1807, p=0.016).
Conclusion: Patients treated with CDKi for longer durations had increased expression of PIK3CA/AKT pathway proteins; suggesting that the pathway could become upregulated during CDKi treatment. Higher BCL-2 expression is associated with reduced apoptosis, and lower Ki-67 expression is associated with slower proliferation rates. Whether these observations are a result of CDKi treatment or are related to indolent tumor behavior merits further study. Future studies will evaluate RNA sequencing and multiplex immunohistochemistry of immune microenvironment on samples from the same cohort to more deeply characterize the effects of CDKi on mHRBC tumors.

#5342 Inhibition of glutaminase elicits senolysis in therapy-induced senescent melanoma cells.

Justin Kim[1], Bryce Brunetti[1], Ayanesh Kumar[1], Ankit Mangla[2], Kord Honda[3], **Akihiro Yoshida**[1]

[1]Dermatology, Case Western Reserve University School of Medicine, Cleveland, OH,[2]Hematology and Oncology, University Hospitals Cleveland Medical Center, Cleveland, OH,[3]Dermatology, University Hospitals Cleveland Medical Center, Cleveland, OH

The cyclin D1-Cyclin-Dependent Kinases 4 and 6 (CDK4/6) complex is crucial for the development of melanoma. We previously demonstrated that targeting CDK4/6 using small molecule inhibitors (CDK4/6i) suppresses $Braf^{V600E}$ melanoma growth *in vitro* and *in vivo* through induction of cellular senescence. However, clinical trials investigating CDK4/6i in melanoma have not yielded successful outcomes, underscoring the necessity to enhance the therapeutic efficacy of CDK4/6i. Accumulated research has shown that while senescence initially suppresses cell proliferation, a prolonged state of senescence eventually leads to tumor relapse by altering the tumor microenvironment, suggesting that removal of those senescent cells (in a process referred to as senolysis) is of clinical necessity to facilitate clinical response. We demonstrate that glutaminase 1 (GLS1) expression is specifically upregulated in CDK4/6i-induced senescent $Braf^{V600E}$ melanoma cells. Upregulated GLS1 expression renders $Braf^{V600E}$ melanoma senescent cells vulnerable to GLS1 inhibitor (GLS1i). Furthermore, we demonstrate that this senolytic approach targeting upregulated GLS1 expression is applicable even though those cells developed resistance to the $Braf^{V600E}$ inhibitor vemurafenib, a frequently encountered substantial clinical challenge to treating patients. Thus, this novel senolytic approach may revolutionize current CDK4/6i mediated melanoma treatment if melanoma cells undergo senescence prior to developing resistance to CDK4/6i. Given that we demonstrate that a low dose of vemurafenib induced senescence, which renders $Braf^{V600E}$ melanoma cells susceptible to GLS1i and recent accumulated research shows many cancer cells undergo senescence in response to chemotherapy, radiation, and immunotherapy, this senolytic therapy approach may prove applicable to a wide range of cancer types once senescence and GLS1 expression are induced.

#5343 Proximity labeling reveals role of 14-3-3 protein in CDK4/6 inhibitors resistance for hormone positive breast cancer.

Wei-Ting Chung[1], Yi-Ru Tseng[1], Tsan-Jan Chen[2], Wenching Wang[2], Muh-Hwa Yang[1], Chun-Yu Liu[3], Yi-Fang Tsai[4], Chi-Cheng Huang[4], Ling-Ming Tseng[4], Ta-Chung Chao[4], **Jiun-I Lai**[1]

[1]National Yang Ming Chiao Tung University, Taipei, Taiwan,[2]National Tsing Hua University, Taipei, Taiwan,[3]taipei veterans general hospital, Taipei, Taiwan,[4]Taipei Veterans General Hospital, Taipei, Taiwan

CDK4/6 inhibitors are currently standard of care for advanced hormone positive (HR+) breast cancer, and CDK6 overexpression induces resistance against CDK4/6 inhibitors. We incidentally discovered that overexpression of a CDK6 ^{D224Y} mutant mitigated this resistance. Mechanistic studies revealed that CDK6 overexpression resulted in suppressed apoptosis and increased G1/S progression after treatment with CDK4/6 inhibitors; these features were abolished when CDK6 ^{D224Y} was overexpressed. APEX2- based proximity labeling proteomics revealed CDK6 overexpression, but not CDK6 ^{D224Y}, induced proximity of the 14-3-3 protein YWHAB. Co-immunoprecipitation experiments confirmed YWHAB bound overexpressed wild type CDK6 but not CDK6 ^{D224Y}, and YWHAB knockdown decreased CDK6 protein in a proteosomal dependent manner, suggesting that YWHAB served as a chaperone role for CDK6 stablization. Knockdown of YWHAB reversed the resistance conferred by CDK6 amplification. Molecular modeling studies predicted that the aspartate-to-tyrosine mutation of CDK6 ^{D224Y} would result in destabilizing binding between CDK6 and YWHAB. Xenograft studies confirmed that CDK6 ^{D224Y} overexpression reversed the resistance conferred by CDK6 overexpression *in vivo*. Analysis of independent clinical cohorts revealed an association of 14-3-3 proteins with survival in HR (+) breast cancer. Both 14-3-3 beta (YWHAB) and zeta (YWHAZ) were associated with activation of G1/S pathway genes, suggesting a role for 14-3-3 proteins in CDK4/6 inhibitors treatment outcome. In a clinical cohort, high YWHAZ expression was associated with worse outcome to CDK4/6 inhibitors treatment. Transcriptomic studies revealed that high YWHAB/YWHAZ expression was associated with upregulated G1/S pathway signature, confirming a role of 14-3-3 proteins in CDK4/6-cyclinD-Rb-E2F axis. In summary, by studying CDK6 ^{D224Y}, we uncovered a role for the 14-3-3 protein YWHAB in CDK4/6 inhibitor resistance.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Genomic Profiling of Tumors 1
Poster Session

#5347 Transcriptome analysis for Merkel cell carcinoma patients testing positive (+) and negative (-) for polyomavirus.

M. Perez Salva[1], N. Munoz[1], F. Srividya[1], M. Pirhoushiaran[1], D. Wang[1], M. Delgado Velez[2], J. A. Lasalde Dominicci[2], P. P. Aung[1], L. Y. Ballester[1];
[1]MD Anderson Cancer Center, University of Texas, Houston, TX, [2]University of Puerto Rico, School of Medicine, San Juan, PR

Merkel cell carcinoma is a rare, aggressive primary cutaneous malignancy with neuroendocrine differentiation. Approximately 3,000 new cases are diagnosed annually in the United States, with incidence rates rising partly due to advancements in diagnostic methods. The exact cause of MCC is unknown, but several potential etiologies have been proposed, including exposure to ultraviolet radiation (UVR) and integration of Merkel cell polyomavirus (MCPyV). MCPyV has been detected in up to 80% of MCC tumors in the United States, and its presence is associated with a better prognosis, with a five-year overall survival (OS) rate of approximately 50%. This study aims to investigate the transcriptomic and methylomic profiles of skin samples from 27 patients with MCC, comparing those positive and negative for MCPyV. The objective involves identifying potential genetic biomarkers that could be targeted for therapeutic intervention for both MCPyV (+) and MCPyV (-) patients.

#5348 Gene alterations in breast tumors reveal significant association with patient and tumor characteristics.

M. Al Dalali[1], B. Al Amri[1], N. Al Zeheimi[1], R. Al Busaidi[1], M. Islam[1], M. Al Riyami[1], A. Al Jarrah[1], Y. Tamimi[1], C. Crozier[2], J. Bartlett[3], J. Bayani[2], S. A. Adham[1];

[1]Sultan Qaboos University, Muscat, Oman, [2]Ontario Institute for Cancer Research, Toronto, ON, Canada, [3]University of Edinburgh, Edinburgh, United Kingdom

Background: Profiling breast tumor gene alterations gives insight into the nature of the disease and will eventually lead to better diagnosis and personalized treatments. Ethnicity plays a role in the behavior of breast cancer etiology. In this study, we aimed to verify gene mutations in the context of single nucleotide variation (SNV) and copy number variations (CNV) in a patient group from The Sultanate of Oman. We correlated the gene alterations with age and tumor characteristics simply because breast cancer in this population happens at an earlier age with an aggressive nature compared with Western women.

Materials and Methods: The DNA extracted from formalin-fixed paraffin-embedded FFPE tissues was analyzed using the Oncomine Comprehensive Assay Plus targeted cancer genes panel using a next-generation sequencing platform. The data was analyzed using the Oncomine informatics software, and statistical analysis was further employed to analyze the significant gene alterations with the patient and tumor characteristics.

Results and discussion: 40 Patients' tumors were collected and classified based on patient age and tumor-specific parameters, including tumor size, grade, lymph node status, hormone receptor status, and molecular subtypes. Remarkably, SNVs in NTRK1 (*p=0.003*) and *PIK3CA* (*p=0.008*) were more associated with the tumors of older patients. SNVs in *TP53* (*p=0.007*) and *CBFB* (*p=0.037*) exhibited significance with the molecular subtype of patients. Additionally, copy number amplifications in *FGF 3,4* and *19* were significant based on the tumor molecular subtype (P ≤0.05). *TSC1* with SNV was exclusively mutated significantly in smaller tumor sizes (*p=0.023*). Besides, the CNVs (gain) in *NBN* (*p=0.010*) and *RECQL4* (*p=0.028*) were also found in smaller tumors. Regarding tumor grade, five genes showed more CNV-gain in higher-grade tumors: *BRIP1* (*p=0.015*), *RPS6KB1*(*p=0.019*), *SOX9* (p=*0.015*), *CHEK2* (*p=0.013*), and *NOTCH1* (*p=0.045*). *CDK12* copy number gain led to a significant decrease in the overall survival of the studied patients (*p=0.048).* Conclusion: Like the international distribution of BC mutations, *TP53* and *PIK3CA* are still the most mutated in the studied cohort. The analysis indicated a few unique gene mutations in this study, such as *NTRK1* and its mutations in older breast cancer patients. Further analysis of the transcriptome data will confirm the highlighted gene activity and correlation with the characteristics of the disease.

#5349 Ferroptosis as a determinant of prognosis and response to immunotherapy in liver cancer.

Sowon Park[1], Hyewon Park[2], Yun Seong Jeong[3], Bo Hwa Sohn[3], Sun Young Yim[4], Sung Hwan Lee[5], Ju-Seog Lee[3]

[1]Bioengineering, Rice University, Houston, TX,[2]Elkins High School, Missouri City, TX,[3]UT MD Anderson Cancer Center, Pearland, TX,[4]Korea University, Seoul, Korea, Republic of,[5]CHA Bundang Medical Center, Seongnam, Korea, Republic of

Background & aims: Ferroptosis, a regulated cell death mechanism driven by iron-dependent lipid peroxidation, is important for survival of cancer cells due to the increased vulnerability of fast-growing cancer cells to oxidative stress. However, its impact on clinical outcomes and treatment responses is not well understood. Our goal is to explore ferroptosis' contribution to liver cancer, focusing on its association with clinical outcomes, specifically incidence rates in high-risk cirrhotic patients, overall survival, and responsiveness to immunotherapy in HCC patients.

Methods: Utilizing gene expression signature reflecting ferroptosis from liver cancer cells, we integrated gene expression profiles from patients with cirrhosis and liver cancer and from liver cancer cells under ferroptosis. To categorize patients, we developed a genomic predictor and applied it to gene expression data from a cirrhosis cohort (n=216) and large liver cancer cohorts (n=1390). Comprehensive statistical and informatics analyses were conducted to evaluate clinical significance, including overall survival and responses to standard treatments such as sorafenib and immunotherapy with atezolizumab and bevacizumab.

Results: In the cirrhosis cohort, the low ferroptosis baseline in cirrhotic liver was significantly associated with higher liver cancer development. Among liver cancer cohorts, patients with the low ferroptosis baseline exhibited characteristics such as poor survival, increased hepatic stem cell features, and increased genomic instability. Notably, when liver cancer patients undergoing systemic treatment with sorafenib or the combination of atezolizumab (anti-PD-L1) and bevacizumab (anti-VEGF) were stratified using our genomic predictor, those with high ferroptosis baseline derived significant benefits from the combination of atezolizumab and bevacizumab treatment. Conversely, such benefit of treatment was not visible in patients treated with sorafenib. This suggests that ferroptosis activity may influence liver cancer response to immune checkpoint inhibitor therapy. Gene network analysis of the ferroptosis signature using ingenuity pathway analysis revealed that statin signatures are strongly correlated with high sensitivity to the combination treatment with of atezolizumab and bevacizumab, indicating that statins may be used for sensitizing liver cancer to the combination treatment.

Conclusions: This study identifies clinically distinct subtypes of liver cancer associated with baseline ferroptosis, biomarkers associated with these subtypes, and potential mechanisms underlying ferroptosis-mediated sensitization of liver cancer cells to the combination of immunotherapy. This work emphasizes the potential of ferroptosis-targeted approaches in optimizing liver cancer treatment.

#5350 Establishment and validation of a prognostic signature based on thirteen hub genes in colorectal cancer.

H. Zhang#[1], Y. Lang#[1], Z. Tang#[2], J. Lin[3], L. Yang[4], J. Kang[5], C. Yang*[6], Q. Lin*[1], Q. Li*[7], F. Ye*[1], W. Tang*[1];
[1]Department of Medical Oncology, The First Affiliated Hospital of Xiamen University, School of Medicine, Xiamen University, The School of Clinical Medicine of Fujian, Medical University, Xiamen, China, [2]Department of Pharmacy, Zhongshan Hospital (Xiamen), Fudan University, Xiamen, China, [3]Department of Medical Oncology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, [4]Department of Medical Oncology, Weifang People's Hospital, Weifang Medical University, Weifang, China, [5]Department of Medical Oncology, Zhangzhou Municipal Hospital, Zhangzhou Municipal Hospital Affiliated of Fujian Medical University, Zhangzhou, China, [6]Department of Surgical Oncology, Fujian Provincial Hospital, Fuzhou, China, [7]Department of Hematology, The First Affiliated Hospital of Xiamen University and Institute of Hematology, School of Medicine, Xiamen, China

Background: The survival rate for late-stage colorectal cancer (CRC) is extremely low (4%-12%). The prognosis assessment for CRC pose numerous challenges. Recently, the role of the immune microenvironment in CRC progression has increasingly garnered attention, with immune cell infiltration and molecular expression closely related to patient prognosis. Therefore, this study aims to deeply analyze the molecular mechanisms underlying CRC and identify novel immune-related biomarkers for prognosis evaluation.
Methods: High-throughput sequencing technology was utilized to compare and analyze the differential gene expression between CRC tumor tissues and normal tissues, as well as between patients with long and short survival durations. Univariate Cox analysis and Least Absolute Shrinkage and Selection Operator (Lasso) regression analysis were combined to screen hub genes closely related to CRC prognosis. A prognostic signature based on these hub genes was constructed to predict the prognosis of CRC patients. RNA sequencing (RNA-Seq) data from five paired CRC patients were collected to validate the expression patterns of the hub genes in CRC samples and their relationship with the immune microenvironment.
Results: We successfully identified 13 hub genes (HOXC6, IGF2BP3, SGCG, HTR2C, ADCY5, SHC2, SUCLG2, PDHB, TSLP, CCR9, TNFRSF19, TGFB2, and LEP) that compose the prognostic signature. In the Cancer Genome Atlas (TCGA) dataset, this signature accurately classified patients into high- and low-risk groups with high precision ($p < 0.001$). Similar results were obtained in the Gene Expression Omnibus (GEO) dataset ($p < 0.001$ and $p = 0.036$). The study also found that when patients were stratified into high-risk and low-risk groups using the prognostic signature, there were significant differences in the immune environment between the two groups, including the infiltration degree of immune cells ($p < 0.001$) and the expression levels of immune molecules($p < 0.01$). Additionally, when validated using RNA-Seq data from five paired CRC patients, the expression patterns of these hub genes in the five CRC samples were consistent with those observed in the TCGA analysis, further confirming the important roles of the 13 hub genes in CRC patients and their association with the immune microenvironment.
Conclusion: In summary, this research has developed a novel signature that can predict both the survival outcomes and the immune infiltration status of CRC patients. The clinical application of this signature has the potential to enhance survival rates and individualize therapy approaches for CRC based on their risk score.#These authors contributed equally: Hui Zhang, Yu Lang, Zhaoqi Tang.*Correspondence to:Dr. Weiwei Tang, weiweitang008@xmu.edu.cn;Dr. Feng Ye, yefengdoctor@xmu.edu.cn;Dr. Qiyuan Li, qiyuan.li@xmu.edu.cn;Dr. Qin Lin, linqin05@163.com;Dr. Changshun Yang, 282483331@qq.com

#5351 The landscape of somatic mutations and copy-number alterations in the Indian Cohort of Prostate Cancer Patients.

T. Biswas[1], P. Vats[2], V. Bhatia[3], A. Singhai[4], A. Srivastava[5], A. Chauhan[5], M. Ahmad[6], P. Tripathi[7], S. Mehrotra[7], A. Tewari[7], N. Husain[5], A. Goel[4], N. Palanisamy[8], B. Ateeq[1];

[1]Indian Institute of Technology Kanpur (IIT Kanpur), Kanpur, India, [2]University of Michigan, Ann Arbor, MI, [3]University of California Los Angeles Jonsson Comprehensive Cancer Center, Los Angeles, CA, [4]King George's Medical University, Lucknow, India, [5]Dr. Ram Manohar Lohia Institute Of Medical Sciences, Lucknow, India, [6]Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, India, [7]Regency Hospital, Kanpur, India, [8]Vattikuti Urology Institute, Detroit, MI

In India, prostate cancer (PCa) has the third highest incidence rate among males and is often diagnosed at the advanced stage. PCa is known to be molecularly heterogeneous, and an in-depth characterization of its subtypes by geographical ethnicities may aid in the understanding of its etiology and disparities. Hence, we perform molecular characterization of PCa patients from India. Integrative genomics studies categorized PCa into molecular subtypes based on genetic aberrations (e.g., somatic mutations, INDEL, etc.). Although genetic factors are known to impact PCa pathobiology, genomic profiling of Indian PCa patients and its clinical utility remain largely unexplored. Thus, in a prospective study, we performed whole exome sequencing (WES) of treatment-naive primary PCa (N=90) and benign prostatic hyperplasia specimens (N=14) along with matched blood. Our findings reveal distinct genetic alterations unique to the Indian cohort, wherein an elevated somatic tumor mutational burden and an increased percentage of clinically actionable alterations were noted. About 80% of the somatic variants (called using Mutect2) were found to be missense mutations with a high recurrence of C>T transitions, a signature of age-related human malignancies. Several novel pathogenic alterations affecting critical domains of the cancer-associated genes (e.g., *APC, BRCA2, PI3KCA,* etc.) were found, which have not been reported in the dbSNP or COSMIC databases. Systematic comparison of our findings with primary PCa data available at The Cancer Genome Atlas Prostate Adenocarcinoma (TCGA-PRAD) revealed distinct genomic alterations in Indian PCa patients: specifically, 35% of the patients showed presumed actionable lesions in the RTK-Ras pathway genes, while 10% had mutations in the PI3K pathway genes, and 21% of all the specimens harbored putative inactivating mutations of genes involved in the homologous recombination repair pathway. We also found major differences in the mutational frequency of some driver genes, such as *ATRX*, *PIK3C2G*, *TAL1*, etc., compared to the TCGA-PRAD cohort. Furthermore, GISTIC2 analysis revealed significant arm-level amplification at 8q (*MYC*, *GLI4*), 7q (*GNB2*, *PDIA4*), and 16p (*TELO2*, *TSC2*) regions and deletion at 8p (*NKX3.1*, *MSR1*), 9p (*CDKN2A*, *CDKN2B*), and 13q (*RB1*, *BRCA2*) regions. We also identified recurrent focal amplifications at 9q, 14q, and 11q, and deletions at 16q, 10q, and 5q loci. Further, integrating WES data with transcriptome data would be helpful in categorizing PCa patients based on gene fusion status and prioritizing functionally impactful and actionable variants. Collectively, our findings reveal molecular heterogeneity among PCa patients from India, leading to the identification of clinically relevant candidates that need to be further validated and would help in strategizing therapies and clinical management of this disease.

#5354 Single-cell sequencing resolves prevalence of PD-L1/2-expressing tumor-associated endothelial cells in human solid tumors.

C. Yan[1], F. T. H. Wu[1], J. Laskin[2], C. Ho[2], M. A. Marra[1];

[1]University of British Columbia, Vancouver, BC, Canada, [2]BC Cancer Vancouver Centre & University of British Columbia, Vancouver, BC, Canada

BACKGROUND: The PD-1 ligands (PD-L1 and PD-L2) are immune checkpoint proteins that can be expressed by stromal cells including endothelial cells (ECs) within the tumor microenvironment (TME). Pe-clinical studies have implicated endothelial PD-L1 as a contributory therapeutic target of immune checkpoint inhibitor drugs especially when combined with anti-angiogenic drugs. However, the clinical prevalence and implications of endothelial PD-L1/2 expression in human solid tumors remain unexplored.
HYPOTHESES: Endothelial PD-L1/2 expression occur at low frequencies in the TME overall, but may be upregulated in specialized EC phenotypes, e.g.,tumor-associated high-endothelial venules (TU-HEVs) or tip-like ECs of angiogenic sprouting blood vessels.
OBJECTIVES: (1) To explore the utility of single-cell profiling as a means to survey the levels of PD-L1 and PD-L2 transcription in ECs from clinical solid tumor specimens. (2) To identify potential EC subtypes in the TME that may be enriched for PD-L1/2 expression.
METHODS: We performed single-cell RNA-seq (scRNA-seq) and single-cell ATAC-seq (scATAC-seq) on a pan-cancer cohort of 33 clinical core biopsies from 25 patients using the 10X Chromium Multiome kit. After performing quality control and normalization using Seurat (v4.3.0), there was a median of 4367 cells per sample (IQR: 2548-6025).
RESULTS: Two orthogonal cell type classification methods - SingleR (v2.0.0) using the Human Primary Cell Atlas [PMID 24053356], and Seurat's reference mapping function using the annotations from Bassez et al., 2021 [PMID 33958794] - identified 3,829 ECs from 31 samples (median 102, IQR 43-141) with 99.7% concordance. The overall prevalence of ECs expressing PD-L1 or PD-L2 was low (1.4% and 2.6%, respectively). ECs were then classified into subtypes (arterial, venous, lymphatic, tip-like, TU-HEVs) by first clustering cells based on RNA expression using Seurat, and then assessing the expression of marker genes in each cluster against a published reference dataset Hua et al., 2022 [PMID: 36423635]. After subtyping, PD-L1 was enriched in lymphatic ECs (3.5%), and PD-L2 was enriched in lymphatic ECs (4.1%) and TU-HEVs (5.6%).
CONCLUSIONS: This study suggests that endothelial PD-L1/2 expression can be found in clinical core biopsies of human solid tumors, and that the role of endothelial PD-L1/2 within the TME warrants further investigation especially in the context of combined anti-PD-1/PD-L1 plus anti-angiogenic therapy. Ongoing work aims to confirm these results at the protein level using spatial imaging in larger cohorts of specific cancer types.

#5355 Whole genome landscape of small cell lung carcinoma and large cell neuroendocrine carcinoma of the lung.

Hisashi Hashimoto[1], Yosuke Tanaka[1], Jun Takahashi[1], Toshihide Ueno[1], Shinya Kojima[1], Kouya Shiraishi[2], Takashi Kohno[2], Yuichi Shiraishi[3], Yasushi Yatabe[4], Takeshi Kaneko[5], Hiroyuki Mano[1]

[1]Division of Cellular Signaling, National Cancer Center Research Institute, Tokyo, Japan,[2]Division of Genome Biology, National Cancer Center Research Institute, Tokyo, Japan,[3]Division of Genome Analysis Platform Development, National Cancer Center Research Institute, Tokyo, Japan,[4]Department of Diagnostic Pathology, National Cancer Center Hospital, Tokyo, Japan,[5]Department of Pulmonology, Yokohama City University Graduate School of Medicine, Yokohama, Japan

Small cell lung carcinoma (SCLC) and large cell neuroendocrine carcinoma (LCNEC) are the most deadly forms of lung cancer. The advent of numerous molecular targeted therapeutics, including immune checkpoint inhibitors, has improved outcomes in non-small cell lung cancer. However, the prognosis for SCLC and LCNEC has shown slight improvement, and the development of novel therapeutics is craved. In addition, because SCLC frequently presents at advanced stages and resected samples are scarce, large-scale genomic analysis has rarely been performed. In particular, while molecular biological classification based on neuroendocrine markers has been proposed so far for SCLC, no classification has been fully established for LCNEC. To refine the classification and to identify vulnerabilities of SCLC and LCNEC, we performed comprehensive multi-omic analyses on 154 samples (107 SCLC and 47 LCNEC) using whole-genome sequencing with both short-read and long-read sequencers, RNA sequencing, and spatial transcriptomics. Integrated transcriptome clustering of SCLC and LCNEC allowed us to classify our cases into three clusters. Each cluster was characterized by distinct molecular features, with distinct profiles of expression patterns, mutations, structural variations, and copy number alterations. We also identified novel driver mutations and recurrent structural variations that were not previously reported. Importantly, we have successfully identified several gene mutations and structural abnormalities which may be functionally significant. *Gene X*, which we detected as a novel driver gene, is presumed to function as a tumor suppressor gene, and increased TMB was observed in cases with *Gene X* mutations, regardless of MSI status. In addition, *Splicing Factor Y*, identified as a novel recurrent structural variation, is a gene that functions as a splicing regulator, and these structural variations affect alternative splicing of genes related to cell morphology, suggesting that they may affect the phenotype of these cancers. Analysis of structural variation using long-read sequencing identified structural variations that could not be detected by short-read sequencing. In addition, spatial transcriptomics elucidated the differences in the tumor microenvironment between SCLC and LCNEC or among the molecular biological classifications. The findings of novel technology-based analyses suggest a new therapeutic strategy for SCLC and LCNEC.

#5357 Mutational landscape and tyrosine kinase inhibitor sensitivity in *EGFR* L833 and H835 mutated non-small cell lung cancer.

Nong Yang[1], Long Huang[2], Dou Ren[1], Fanxu Zeng[1], Chengchuan Jiang[1], Weiwei Zhang[1], Weifeng He[1], Wei Yin[1], Peng Huang[2], Hanlin Chen[3], Xiaoying Wu[3], Jiaohui Pang[3], **Haimeng Tang**[1], Xue Wu[3], Qiuxiang Ou[3]

[1]The Second People's Hospital of Hunan Province (Brain Hospital of Hunan Province), Changsha, China,[2]The Second Affiliated Hospital of Nanchang University, Nanchang, China,[3]Nanjing Geneseeq Technology Inc., Nanjing, China

Background: *EGFR* L833 and H835 mutations account for 0.5% of *EGFR*-mutated non-small cell lung cancer (NSCLC) cases and are not classified as sensitive mutations to EGFR tyrosine kinase inhibitors (TKIs) according to NCCN guidelines, while chemotherapy remains the standard of care for patients with these mutations. Previous case reports have shown favorable EGFR-TKIs efficacy in patients with EGFR L833 and H835 mutations, this has yet to be validated in larger cohorts.

Methods: DNA sequencing data from 240 NSCLC patients with *EGFR* L833 and H835 mutations were analyzed, including 57 with documented EGFR-TKI treatment records. Additionally, an external cohort of 346 NSCLC patients with *EGFR* L858R mutations and wild-type *EGFR* L833 and H835 was evaluated for comparative analyses of molecular landscapes. Kaplan-Meier survival curves were generated, and the log-rank test was employed to compare survival times between subtypes.

Results: In the study cohort, 98.3% (235/239) of patients with *EGFR* L833 mutations and 100% (82/82) with *EGFR* H835 mutations had concurrent *EGFR* mutations. Interestingly, 97.5% (78/80) of patients with *EGFR* H835L mutations had concurrent L833V mutations, while the most frequent co-mutations of *EGFR* L833V being L858R (44.1%, 93/211) and H835L (37.0%, 78/211). Compared to *EGFR* L858R patients in the external cohort, those with *EGFR* L833V+H835L and L833V+L858R mutations had lower frequencies of *LRP1B*, *RB1*, *TP53* mutations, as well as *CD4K* amplifications and mutations in the RTK-RAS and cell cycle signaling pathways. Tumor mutational burden (TMB), chromosomal stability, whole-genome duplication rates, and age-related and APOBEC-associated mutational signatures were also lower in patients with *EGFR* L833V+H835L and L833V+L858R mutations compared to those with *EGFR* L858R, with significant differences in TMB ($P = 0.110$ and $P = 0.001$), chromosomal instability ($P = 0.036$ and $P = 0.042$), whole-genome duplication rates ($P = 0.008$ and $P = 0.028$), age-related signatures ($P = 0.006$ and $P = 0.027$), and APOBEC-associated signatures (both $P < 0.001$). Notably, EGFR-TKI efficacy data revealed that patients with EGFR L833 and H835 mutations can benefit from EGFR-TKI treatment, with a median progression-free survival (PFS) of 16.4 months. PFS was comparable across mutation subtypes (L833V+H835L vs. L833V+L858R vs. others, $P = 0.096$) and across different generations of EGFR-TKIs received ($P = 0.533$).

Conclusion: This study demonstrates that patients with *EGFR* L833 and H835 mutations can benefit from EGFR-TKIs, with comparable median PFS to those with classical EGFR L858R mutations in previous literatures. Additionally, patients with L833 and H835 mutations displayed distinct molecular features compared to L858R, characterized by lower TP53 mutation frequencies, lower TMB values, and lower chromosomal instability.

#5358 A comparison of tumor microenvironment immune profiles using CIBERSORTx in breast cancer patients from Kenya and Hong Kong.

Li Feng[1], Shahin Sayed[2], Hela Koka[1], Viviane Oluoch[2], Veronica Ngundo[2], Alfred M. Githuka[2], Zaitun Ajuoga[2], Kristine Jones[3], Belynda Hicks[3], Amy Hutchinson[3], Maria Brown[3], Petra Lenz[3], Aaron M. Rozeboom[3], Difei Wang[3], Francis Makokha[4], Stefan Ambs[5], Jonine Figueroa[1], Xiaohong R. Yang[1]

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD,[2]Aga Khan University Hospital, Nairobi, Kenya,[3]Cancer Genomics Research Laboratory, Frederick National Laboratory for Cancer Research, Frederick, MD,[4]Research and Innovation, Mount Kenya University, Thika, Kenya,[5]Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD

Background: Tumor-infiltrating immune cells in the tumor microenvironment (TME) are critical for breast cancer (BC) progression and treatment outcomes, with notable variations observed across populations. Unique BC risk factors, such as germline genetics, mammographic density, infectious exposures, and lifestyle factors, may shape distinctive immune profiles and lead to distinct tumor characteristics and clinical outcomes in different populations. Comparing immune cell compositions across populations can improve our understanding of BC racial disparity and guide personalized treatment strategies.
Aims: This study aims to characterize and compare immune composition in the BC TME between East Africa (Kenya) and East Asia (Hong Kong) when accounting for age, intrinsic subtype, and analytical methods.
Methods: We collected formalin-fixed paraffin-embedded (FFPE) tumor blocks from BC patients in Kenya and Hong Kong, processed them for nucleic acid extraction and analyzed them using NanoString nCounter from a custom-designed code set including 50 PAM50 genes and 547 CIBERSORT panel genes. Samples were selected to match age and estrogen receptor (ER) status distributions. After removing duplicates and QC-failed samples, 467 samples from Kenya (mean age=50.57, ER+: 71.18%) and 118 samples from Hong Kong (mean age=54.94, ER+: 72.03%) were included in the subsequent analyses. CIBERSORTx was used to estimate relative fractions of 22 immune cell types. PAM50 subtype was defined as Luminal A, Luminal B, HER2-enriched, Basal-like, or Normal-like using the nearest centroid method. Multiple linear regression models were performed to identify the differences in immune cell proportions by ethnicity, adjusting for age, PAM50 subtype, and tumor grade.
Results: After filtering cells with mean fractions <5%, this analysis was focused on T follicular helper, CD8+, resting CD4+, activated NK, resting mast, and macrophage subsets (M0, M1, M2). Compared to Hong Kong, Kenyan samples exhibited higher relative fractions of CD8+ T cells ($p < 0.01$), activated NK cells ($p < 0.001$), and macrophages M2 ($p < 0.05$) but lower fractions of resting CD4+ T cells ($p < 0.05$) and macrophages M0 ($p < 0.01$). Similar results were observed with absolute abundance analyses.
Conclusion: This study reveals distinct immune profiles between Kenyan and Hong Kong BC TMEs. Kenyan samples exhibit a relatively “hot” immune TME with both pro-inflammatory and immunosuppressive components, while Hong Kong samples show a more quiescent or relatively “cold” immune environment. These population-specific differences highlight the potential need for tailored immunotherapy strategies considering unique immune characteristics shaped by genetic and environmental factors. Future analyses will explore associations with clinical outcomes and risk factors to elucidate mechanisms driving these racial disparities.

#5359 Multi-omics characterization of copy number variation in high-grade serous ovarian cancer.

Yuxin Jin[1], Jing Qian[2], Michelle G. Webb[1], David W. Craig[1], Lynda Roman[2], John D. Carpten[1], Rania Bassiouni[1]

[1]Beckman Research Institute of The City of Hope, Duarte, CA,[2]University of Southern California, Los Angeles, CA

Ovarian cancer is one of the most lethal gynecologic malignancies, primarily due to its late-stage diagnosis and intrinsic tumor heterogeneity. Histologically, epithelial ovarian cancer (EOC) accounts for approximately 70% of cases and exhibits distinct molecular and clinical characteristics. High-grade serous ovarian cancer (HGSOC), the most aggressive form of EOC, stands out for its widespread genomic instability and poor prognosis. Copy number variations (CNVs) play a critical role in tumor heterogeneity, treatment resistance, and disease progression. While CNVs have been extensively studied using bulk sequencing approaches, their spatial heterogeneity within tumors remains underexplored. To investigate this, we integrated spatial transcriptomics (10x Genomics Visium Spatial) with whole-exome sequencing (WES) in 24 fresh-frozen HGSOC samples. This dual approach enabled us to validate CNV patterns inferred from spatial data while leveraging WES for a genome-wide view of CNVs. First, spatial transcriptomics data were processed using the Space Ranger pipeline (10x Genomics) to generate spatially resolved gene expression matrices. Tumor purity scores were estimated using the R package ESTIMATE, and were then used to inform CNV inference with the tool InferCNV. This analysis revealed varied CNVs across spatial domains, indicating intratumoral heterogeneity and the existence of subclones with distinct CNV profiles and spatial niches. We further resolved loss of heterozygosity (LOH) in these samples using the R package tLOH. Clonal LOH of chr 17p, encompassing the TP53 locus, was detected in several samples. Pseudotime and spatial trajectory analysis confirmed that chr17 LOH is an early event in the trajectory of CNV development. To validate the CNV and LOH patterns inferred from spatial transcriptomics, we performed WES on paired tumor and normal tissues from the same cohort. WES data were first aligned to the human reference genome GRCh38 using BWA, followed by CNV profiling with GATK CNV. Recurrent CNV patterns, including gains on chromosomes 1q, 3q, 6p, 8q, and 20q, and losses on chromosomes 4, 5q, 6q, 9p, 13q, 14q, and 17, were observed, consistent with previous reports. A comparison between spatially inferred CNV clusters and GATK CNV results showed strong concordance, further validating the robustness of spatial CNV inference. Interestingly, germline BRCA-mutated samples exhibited a higher frequency of CNVs than BRCA wild-type samples, suggesting a link between BRCA status and genomic instability. By placing CNVs in tissue context, our findings highlight the genetic and spatial heterogeneity of HGSOC, and enhance our understanding of the driving role of CNVs in tumor evolution and growth. This abstract was developed with the assistance of generative AI to revise and clarify the text.

#5360 Comprehensive analysis of breast cancer genomic alterations across diverse populations using a large real-world database.

Vasily N. Aushev[1], Samuel Riviero-Hinojosa[1], Carly B. Scalise[1], Rich Green[1], J. Bryce Ortiz[1], Alyssa Antonopoulos[1], Adam C. ElNaggar[1], Angel Rodriguez[1], Minetta C. Liu[1], Alexey Aleshin[1], Laura Chambers[2], Marla D. Lipsyc-Sharf[3]

[1]Natera, Inc., Austin, TX,[2]Wexner Medical Center and James Cancer Hospital, The Ohio State University, Columbus, OH,[3]Division of Hematology/Oncology, UCLA David Geffen School of Medicine, Los Angeles, CA

Background: Breast cancer is genetically and phenotypically heterogeneous, with tumor-specific alterations influencing clinical outcomes. This variability underscores the importance of accounting for factors such as genetic ancestry in genomic studies to better understand tumor biology and improve patient care. However, most genomic studies have focused on individuals of European ancestry, leaving gaps in knowledge about other ancestries. Here, we analyzed genomic data from over 55,000 breast cancer patients in Natera's proprietary Real-World Database to assess the mutational landscape stratified by genetic ancestry.
Methods: Whole-exome sequencing (WES) data of tumor samples from patients with breast cancer were analyzed. WES was performed on tumor tissue for designing Signatera™ molecular residual disease testing, ordered from June 2019 - November 2024. After exclusions, 33,058 patients were included in the analysis. Mutect2 and VarScan2 callers were used for variant calling. Only non-synonymous mutations were included in prevalence analysis. Genetic ancestry was determined using the EthSeq algorithm with four groups: Africa (AFR), East Asia (EAS), Europe (EUR), and South Asia (SAS).
Results: The distribution of stage at diagnosis was: 26.6%, 26.7%, 19.4%, and 14.8% for Stage I, II, III, and IV, respectively; 12.5% of patients had unknown stage. Ancestry distribution was 81.7% EUR, 11.7% AFR, 4.9% EAS, and 1.8% SAS. *TP53* and *PIK3CA* were the most frequently mutated genes, with prevalence rates of 34.8% and 27.2%, respectively. *PIK3CA* variants were the most prevalent, with frequencies of *H1047R* (10.4%), *E545K* (5.2%), and *E542K* (3.0%). Estrogen receptor-negative (ER-) cases exhibited a lower prevalence of *PIK3CA H1047R* mutations and a higher prevalence of *TP53* mutations compared to ER+ cases. For genetic ancestry, individuals of AFR ancestry showed higher mutation rates in *GATA3* (11.2% vs. 9.9%, $p < 0.001$) and *TP53* (48.2% vs. 34.3%, $p < 0.001$) compared to individuals of EUR ancestry. In contrast, EUR individuals exhibited higher mutation rates in *PIK3CA* (29.4% vs. 23.4%, $p < 0.001$), *MAP3K1* (7.4% vs. 5.6%, $p < 0.001$), and *AKT1* (3.2% vs. 2.5%, $p < 0.001$) compared to the AFR group. *PIK3CA* variants *E542K* (3.3% vs. 2.2%, $p = 0.04$) and *E545K* (5.9% vs. 3.2%, $p < 0.001$) were higher in EUR compared to AFR, whereas the *TP53 R213* variant was enriched in AFR individuals when compared to EUR (2.1% vs. 0.9%, $p < 0.001$).
Conclusions: Our analysis reveals differences in breast cancer genomic landscapes across ancestral groups. The variation in somatic alterations between AFR and EUR ancestries suggests potential differences in tumor biology and therapeutic opportunities that may be targeted to improve racial/ethnic disparities in clinical outcomes. Equitable representation in genomic profiling is essential to understand breast cancer and ensure precision oncology benefits all populations.

#5361 Clinical implications of The Cancer Genome Atlas molecular classification system in esophagogastric cancer.

Henry Walch, Raktim Borpatragohain, Justin Jee, Walid Chatila, Christopher Fong, Steven B. Maron, Geoffrey Ku, David H. Ilson, Yelena Y. Janjigian, Abraham J. Wu, Pari Shah, Daniel G. Coit, Manjit S. Bains, Valerie W. Rusch, Bernard Park, Matthew Bott, Katherine Gray, David R. Jones, Michael F. Berger, Nikolaus Schultz, Vivian E. Strong, Daniela Molena, Smita Sihag

Memorial Sloan Kettering Cancer Center, New York, NY

Purpose: The Cancer Genome Atlas (TCGA) project defined four distinct molecular subtypes of esophagogastric adenocarcinoma (EGAC): microsatellite instable (MSI), Epstein-Barr virus associated (EBV), genomically stable (GS), and chromosomally instable (CIN). However, an association between molecular subtype and clinical outcome has not been clearly demonstrated. Given few actionable biomarkers, we investigated the clinical relevance of the TCGA classification system.
Experimental Design: We identified all patients with EGAC whose tumors underwent prospective next-generation sequencing using the MSK-IMPACT assay from 2014 to 2023. We classified all tumors in accordance with TCGA methodology and correlated molecular subtype with high-quality clinicopathologic data.
Results: Among 1,438 included patients, 941 had CIN, 344 had GS, 103 had MSI, and 50 had EBV tumors. CIN was associated with White race, male sex, more-advanced stage, a high frequency of TP53 mutations, cell-cycle and RTK-RAS copy number alterations, and high metastatic potential. GS was associated with a higher proportion of Asians and females, Lauren's diffuse and signet ring histologies, heterogeneous driver alterations, and poor responses to systemic and radiation therapies. MSI and EBV tumors, by contrast, had lower metastatic potential and significantly better survival than CIN and GS tumors, although MSI tumors were associated with the worst rate of response to chemotherapy. Accounting for clinical stage and tumor grade, molecular classification was independently associated with overall cancer-specific survival ($p<0.001$) on Cox multivariable analysis. Furthermore, genomic signatures, patient demographics, pathologic responses to neoadjuvant therapy, patterns of recurrence, and metastatic organotropism differed significantly by molecular subtype. Although most distal esophageal and gastroesophageal junction tumors were CIN, up to 25% of these included GS, MSI, or EBV subtypes in contrast to the TCGA. Random-forest machine learning demonstrated that molecular subtype is more influential in predicting response to treatment than tumor location.
Conclusions: Molecular classification is independently prognostic and may warrant inclusion in future staging and treatment guidelines. Routine molecular profiling is clinically feasible and may play a role in the management of patients to help guide appropriate treatment selection and clinical trial enrollment in place of tumor location.

#5362 Molecular profiling of SW-682, a pan-TEAD inhibitor, as monotherapy and in combination with other treatments across tumor types.

Somdutta Saha, Veronica Jove, Amelia Schirmer, Lorne Celentano, Alma Chanelo, Connor Smith, Sarah Ahn, Jim Smothers, Wenlin Shao, Tai-An Lin, Paula Miliani de Marval, Lei Chen, Robert Dubose

SpringWorks Therapeutics, Inc., Stamford, CT

Hyperactivation of YAP/TAZ signaling (Hippo pathway) can drive oncogenesis across multiple cancer types by binding to the transcription factor TEAD and driving expression of target genes involved in cell proliferation and tumor progression. SW-682, an investigational pan-TEAD small molecule inhibitor, blocks TEAD-dependent transcription by binding to the palmitoylation pocket of all TEAD paralogs. We have previously demonstrated monotherapy efficacy of SW-682 in in vivo models of mesothelioma and head and neck squamous cell carcinoma (HNSCC) harboring Hippo pathway genomic alterations. We have also reported significant benefit of SW-682 in combination with KRAS G12C inhibitor, sotorasib, in KRAS G12C non-small cell lung cancer (NSCLC) models. Here, we sought to characterize the molecular signature of HNSCC, mesothelioma, and NSCLC tumors after TEAD inhibition either with SW-682 alone or in combination with KRAS G12C inhibitors.

To identify downstream targets of TEAD inhibition and compare them with other known Hippo pathway signatures, we performed RNA-seq analysis of tumors following SW-682 monotherapy in mesothelioma and HNSCC CDX (cell-line-derived xenograft) models. We identified TEAD-dependent transcriptional changes for each model that included significant overlap with published YAP/TAZ gene signatures. Additionally, we compared our data with publicly available preclinical RNA-seq data generated with other TEAD inhibitors. To account for experimental variability across datasets, the analysis was divided longitudinally into early (<6 hour) and late (>24 hour) timepoints for each disease model. This meta-analysis confirmed genes identified from RNA-seq and revealed complexities in identifying a universal TEAD-dependent gene signature.

Since hyperactivated YAP/TAZ signaling is also known to be a common resistance mechanism to targeted therapies, we assessed the relationship between dysregulated Hippo signaling and responses to SW-682 and sotorasib combination in a panel of KRAS G12C NSCLC xenograft models. We observed combination benefits more frequently in xenograft models with elevated tumor intrinsic YAP/TAZ activity. However, Hippo pathway gene alterations alone were not sufficient to predict sensitivity to combination of SW-682 and sotorasib. Therefore, we sought to characterize molecular signatures that correlated with combination benefits by analyzing baseline DNA mutation status and RNA expression levels in each model.

In conclusion, these results help elucidate SW-682 molecular signatures across different tumor types and can support combination strategies for SW-682 in clinical investigation. Supported by SpringWorks Therapeutics, Inc.

#5364 Multi-omic analyses reveal epigenetic and transcriptomic reprogramming behind ibrutinib-venetoclax resistance development in mantle cell lymphoma.

Kevin Qiu[1], Jing Gao[1], Qing Yin[1], Allen Hu[1], Zhenjia Wang[1], Loryn Blackburn[1], Bijal Shah[2], Jeffrey W. Craig[1], Craig Portell[1], Michael E. Williams[1], Chongzhi Zang[1], Jianguo Tao[1]

[1]University of Virginia Comprehensive Cancer Center, Charlottesville, VA,[2]Moffitt Cancer Center, Tampa, VA

Mantle cell lymphoma (MCL) is a rare and aggressive form of B-cell lymphoma that is characterized by aberrations in B-cell receptor signaling and BCL-2-mediated apoptotic pathways. MCLs treated with inhibitors of either have shown remarkable response rates but ultimately develop drug resistance, with patients suffering from rapid disease progression and experiencing poor clinical outcomes. Preclinical and clinical studies into the Bruton's tyrosine kinase inhibitor ibrutinib and the BCL-2 inhibitor ABT-199 (venetoclax) revealed that they were synergistic when combined against MCL and showed significant clinical efficacy, respectively. However, through an unclear mechanism, many cases of MCL similarly developed resistance to combination therapy. To elucidate the mechanisms by which MCL develops drug resistance, we induced resistance to BTKi-venetoclax in 2 patient-derived MCL cell lines, Mino and Rec1, and characterized transcriptional and epigenetic changes using bulk and single-cell RNA and ATAC-seq datasets. We conducted differential expression and accessibility analysis between BTKi-venetoclax-sensitive and resistant samples and identified significantly enriched pathways to find molecular processes that differentiate the two. We show that resistant MCL displays changes at the chromatin and RNA level associated with BCL-2 inhibition, including downregulation of E2F and E2F targets such as cyclin D1. However, we observe that both MCL lines that developed resistance to BTKi-venetoclax upregulate BCL-XL to evade BCL-2 inhibition-mediated apoptosis, likely through activation of the alternative JAK/STAT, interferon, NFKB, and Myc pathways, which are also significantly upregulated. These drastic transcriptomic changes provide an explanation of how resistant MCLs bypass both BCL-2 and BCR-signaling inhibition, leading to survival and neoplastic expansion. Additionally, we identify upregulated genes on both a transcriptional and epigenetic level that encode surface proteins that may inform future targeted therapies. These findings provide mechanistic insights into the transcriptomic reprogramming undertaken by mantle cell lymphoma when developing resistance to BTKi-venetoclax and identify potential vulnerabilities conferred by dual-therapy resistance. They suggest that future therapies optimized against MCL require a combination of immunotherapy and targeted therapies designed to counteract transcriptome reprogramming seen during drug resistance.

#5365 Comparison of whole genome sequencing and target capture sequencing in lung cancer.

Koki Fujii[1], Masahiro Sekiguchi[2], Hiroaki Ikushima[1], Kousuke Watanabe[3], Aya Shinozaki-Ushiku[4], Shuichi Tsutsumi[5], Kenji Tatsuno[5], Kuniko Sunami[6], Masaaki Sato[7], Tetsuo Ushiku[8], Katsutoshi Oda[4], Hiroyuki Aburatani[5], Motohiro Kato[2], Hidenori Kage[1]

[1]Department of Respiratory Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan,[2]Department of Pediatrics, The University of Tokyo Hospital, Tokyo, Japan,[3]Next-Generation Precision Medicine Development Laboratory, Graduate School of Medicine, the University of Tokyo, Tokyo, Japan,[4]Department of Clinical Genomics, The University of Tokyo Hospital, Tokyo, Japan,[5]Genome Science Division, Research Center for Advanced Science and Technology, the University of Tokyo, Tokyo, Japan,[6]Division of Genome Biology, National Cancer Center Research Institute, Tokyo, Japan,[7]Department of Thoracic Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan,[8]Department of Pathology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Introduction: Comprehensive genomic profiling (CGP) tests using target capture sequencing are commonly employed clinically to detect gene alterations and to recommend treatment. Whole-genome sequencing (WGS) is capable of detecting all mutations and structural variants of the genome and may offer advantages over CGP. On the other hand, WGS covers the entire genome with a low depth of coverage, which may limit its sensitivity. This study evaluates the concordance of variants detected by WGS and Todai OncoPanel (TOP), a CGP test targeting 737 DNA and 455 RNA cancer-related genes.

Methods: We analyzed 32 consecutive lung cancer specimens collected through surgical resection, including two samples from the same patient, which underwent both WGS and TOP. Fresh frozen samples were used for WGS, while formalin-fixed paraffin-embedded samples were used for TOP. Variants annotated as "likely pathogenic/likely oncogenic" or higher in significance in databases such as ClinVar and COSMIC were compared between the two methods. The t-test and the Wilcoxon rank-sum test were used for comparison.

Results: The clinical characteristics of the cohort were as follows: the median age of 74 years (range: 37-85); 21 males (66%); 10 never-smokers (31%); 20 adenocarcinomas (63%); 10 squamous cell carcinomas (31%). The most frequent variants identified were *TP53* (22 cases, 69%), and *EGFR* (12 cases, 34%). Out of 134 single nucleotide variants (SNVs), short deletions, and insertions detected in at least one test, 65 variants (49%) were detected by both methods, 64 (48%) were exclusive to TOP, and 5 (4%) were exclusive to WGS. The median number of variants detected per sample between these two tests was significantly different (TOP: 3.5 (IQR: 2-6) vs WGS: 2 (IQR:1-3), $p < 0.001$). Twenty-three samples had variants that were only detected by TOP, and 9 samples did not have any. Comparing the variant allele frequency (VAF) of variants detected by TOP, those detected only by TOP had a significantly lower VAF compared with variants detected by both methods (median, 14% (IQR: 10-22%) vs. 22% (IQR: 17-40%)) ($p < 0.001$). There were no significant differences between the two groups in terms of DNA yield (mean 7.0 µg vs. 6.1 µg, $p = 0.37$), DNA Integrity Number (DIN, median 9.2 vs. 9.3, $p = 0.35$), TOP sequencing depth (mean 1095× vs. 1058×, $p = 0.44$), or WGS depth (median 120× vs. 120×, $p = 0.93$).

Conclusion: Significant differences were observed in the variants detected by target capture sequencing and WGS. However, the specimens analyzed using these two methods were obtained from different tumor sections. Therefore, the discrepancies in test results between the two assays may be due to intratumoral heterogeneity, different sample preparation methods, or low VAF. We will further investigate the causes underlying these findings and make a presentation.

#5366 DRAGEN pipeline validation for somatic variant detection in diagnostics using TSO500 panel.

Mehrdad Rakaee[1], Per Niklas Waaler[2], Falah Jabar[3], Krinio Giannikou[4], Elio Adib[4], Sigve Andersen[2], Erna-Elise Paulsen[2], Thomas K.Kilvaer[2], Elisabeth Jarhelle[3], Kristin Aberg[3], Espen Mikal Robertsen[3], Ane Yde Schmidt[5], Christina Westmose Yde[5], Christian Baudet[5], Aslaug Helland[1], Mette Pohl[5], Lill-Tove R. Busund[3], David J. Kwiatkowski[4], Tom Donnem[2]

[1]Oslo University Hospital, OSLO, Norway,[2]UiT The Arctic University of Norway, Tromso, Norway,[3]University Hospital of North Norway, Tromso, Norway,[4]Brigham and Women's Hospital, Boston, MA,[5]Copenhagen University Hospital, Copenhagen, Denmark

Background: The DRAGEN pipeline has recently been released for the analysis of whole genome and exome sequencing data (PMID:39455800). However, its performance in detecting clinically relevant alterations for routine diagnostic practice remains unknown.
Methods: This study includes 108 lung cancer patients consecutively collected from the TNM-I trial (NCT03299478). Tumor-only sequencing of FFPE DNA was performed using the TruSight Oncology 500 (TSO500) panel. Alignment and variant calling were conducted with DRAGEN (TSO500 v2.5.2) and Mutect2-GATK (v2.2), with subsequent annotation and classification using PCGR (v2.1.2) and OncoKB (v3.4.1).
Results: In the DRAGEN pipeline output, 4328 exonic and nonsynonymous variants with a variant allele fraction (VAF) >0.01 and a minimum supporting read count of 5 (normalized by depth) were analyzed. Among these, 214 variants met actionability tiers 1-3 (AMP/ASCO/CAP) and oncogenicity score >3 based on ClinGen/CGC/VICC Oncogenicity Guidelines, with the highest calls observed for *TP53* (44%, n=48) and *KRAS* (34%, n=37). DRAGEN and Mutect2 showed 100% concordance in detecting level 1 variants (OncoKB classifications), distributed as follows: *KRAS* (16 variants), *EGFR* (2 variants), and *BRAF* (1 variant). The median VAF difference between the two pipelines was 0.017, indicating strong alignment in allele frequency for high-confidence variants. Additionally, 174 variants (84%) showed a VAF difference within ±3.0%, suggesting minor VAF calculation variability in a subset of cases. Across all matched variants, DRAGEN reported consistently lower read depth values than Mutect2, with a median depth of 275 for DRAGEN versus 375 for Mutect2. DRAGEN also demonstrated a lower standard deviation in read depth (188 vs. 320 for Mutect2), indicating tighter depth distribution. This depth discrepancy was particularly pronounced in variants with a >3% VAF difference, suggesting that depth differences may contribute to VAF variability between the pipelines. No level-2 OncoKB classifications were detected by either pipeline.
Conclusion: These findings demonstrate DRAGEN concordance with GATK for detecting clinically significant Tier 1 variants, supporting its reliability in routine diagnostics despite read depth inconsistencies. Future studies should further explore DRAGEN performance in detecting copy number variants and fusion calls.

#5367 Distinct molecular characteristics and treatment implications of *TP53* gain-of-function mutations in non-small cell lung cancer.

Hanlin Chen, **Haimeng Tang**, Xue Wu, Qiuxiang Ou

Nanjing Geneseeq Technology Inc., Nanjing, China

Background: TP53 gain-of-function (GOF) effects lead to cellular responses beyond the capabilities of wild-type TP53 and are known to promote cancer progression and tumor growth. Clinical studies linked *TP53* GOF mutations to poorer outcomes in high-methylated metastatic colorectal cancer and advanced pancreatic ductal adenocarcinoma, while their impact on lung cancer remains unclear.
Methods: A total of 486 patients diagnosed with non-small cell lung cancer (NSCLC) with baseline DNA sequencing data were enrolled. Clinical and sequencing data from external two NSCLC cohorts treated with immunotherapy, one treated with ROS1 tyrosine kinase inhibitors, and one with histological data were analyzed to assess relationships between *TP53* GOF mutations and immunotherapy efficacy, TKI efficacy, and histological transformation. The *TP53* GOF mutations are defined by the “The TP53 Database” (https://tp53.cancer.gov/).
Results: Among all patients, 98 are with *TP53* GOF mutations (20.2%), 189 with *TP53* other mutations (38.9%), and 199 with *TP53* wild-type (40.9%). Compared to *TP53* non-GOF mutations, patients with TP53 GOF mutations show higher mutation rates in *PIK3CA* (P = 0.010), *STK11* (P = 0.026), and *CTNNB1* (P = 0.020) but lower in *NTRK1* (P = 0.018); increased *VEGFA* (P = 0.010) but decreased *DLL3* (P = 0.036) and *HRAS* (P = 0.036) amplification rates; and more frequent PI3K (P = 0.026) and WNT (P = 0.040) pathway alterations. Patients with *TP53* GOF mutations have significantly higher tumor mutation burdens (TMB) than those with other *TP53* statuses (P < 0.001). Moreover, *TP53*-mutated patients, both with GOF and other mutations, show significantly higher expression of immune checkpoints, including *CD274*, *PDCD1*, *PDCD1LG2*, *CTLA4*, and *LAG3*, compared to *TP53* wild-type patients. GOF-mutated patients also have higher M1 macrophage and CD8+ T cell infiltration, alongside elevated B cell receptor signaling, whereas patients with other *TP53* mutations display greater expression of inflammatory pathways. Notably, the ratio of *TP53* GOF mutations in *TP53*-mutated patients decreases with higher histological grades (lepidic vs. acinar/papillary vs. solid/micropapillary: 40.0% vs. 31.7% vs. 30.8%). For treatment efficacy, *TP53* GOF mutations are associated to improved outcomes with immunotherapy (mPFS: 5.37 months in GOF mutations vs. 3.03 in wild-type vs. 4.27 in non-GOF mutations) but poorer prognosis with ROS1-TKIs (mPFS: 10.65 months in GOF mutations vs. 19.60 in wild-type vs. 4.95 in non-GOF mutations).
Conclusions: This study demonstrates that *TP53* GOF mutations are associated with poorer TKI efficacy but improved immunotherapy outcomes in NSCLC. Patients with *TP53* GOF mutations also exhibit elevated immune activities and distinct molecular profiles, marked by higher TMB, *VEGFA* amplification rates, and mutation rates in *PIK3CA* and the PI3K pathway.

#5368 The differences in molecular profiles and survival outcomes between early and late-onset glioblastoma.

Hanlin Chen, Jiaohui Pang, **Haimeng Tang**, Xue Wu, Qiuxiang Ou

Nanjing Geneseeq Technology Inc., Nanjing, China

Background: Age influences glioblastoma's molecular characteristics, with the 2021 WHO classification distinguishing pediatric and adult gliomas due to differing molecular profiles and prognoses, as pediatric glioblastomas show unique genetic alterations compared to adults and have a lower rate of malignant transformation. Although the molecular differences and prognosis between pediatric-type and adult-type glioblastoma are well-studied, the impact of age on adult glioblastoma patients remains underexplored.
Methods: Targeted DNA sequencing data from baseline tissue samples of 139 glioblastoma patients were analyzed. Additionally, clinical and sequencing data from 258 glioblastoma patients in the TCGA cohort were utilized for RNA expression and prognosis analyses. Kaplan-Meier survival curves were generated for the TCGA cohort, and the log-rank test was employed to compare survival times between subtypes.
Results: Among all patients in the study cohort, 11 patients were classified as early-onset glioblastoma (age ≤ 40), and 128 patients were with late-onset glioblastoma (age > 40). The results show that *TERT* mutations (P = 0.006), *MGMT* methylation (P = 0.043), and chromosome 7 gain and 10 loss (P = 0.012) are significantly enriched in late-onset glioblastoma patients, whereas *KIT* amplifications (P = 0.043) are more prevalent in early-onset patients. Beyond these genetic distinctions, patients with late-onset glioblastoma (P = 0.030) and positive *MGMT* methylation status (P = 0.050) exhibit higher tumor mutation burdens. The expression levels of chemokine (P = 0.002), IL-17 (P = 0.002), JAK-STAT (P = 0.011), NF-kappa B (P = 0.002), PI3K-Akt (P = 0.027), and TNF signaling pathways (P = 0.002) are elevated in late-onset patients, whereas early-onset patients show higher $CD4^+$ (P = 0.029) and $CD8^+$ (P = 0.077) T cell infiltrations. Notably, both early- and late-onset glioblastoma patients exhibited comparable actionable mutation profiles, except for a higher incidence of *BRAF* mutations in the early-onset group (P = 0.031). Survival analysis further revealed that late-onset (mPFS: 13.0 months in late-onset vs. 30.0 months in early-onset; P < 0.001) and elevated TMB (mPFS: 13.6 months in TMB-high vs. 15.3 months in TMB-low, stratified by the median value; P = 0.043) were associated with a poorer prognosis in patients with glioblastoma.
Conclusions: In summary, late-onset patients have a poorer prognosis, characterized by adverse biomarkers such as TERT promoter mutations and chromosome 7 gain and 10 loss, but are more likely to benefit from temozolomide chemotherapy, while early-onset patients, with a higher frequency of BRAF mutations, may have a higher chance respond to BRAF inhibitors.

#5369 Characterizing the molecular and clinical implications of *NRG1* fusions in NSCLC through integrated RNA and DNA sequencing analyses.

Minyi Zhu, Yang Xu, **Haimeng Tang**, Xue Wu, Qiuxiang Ou

Geneseeq Research Institute, Nanjing Geneseeq Technology Inc., Nanjing, China

Background: *NRG1* fusions are oncogenic drivers in NSCLC, with therapeutic implications underscored by FDA's designation of Zenocutuzumab for *NRG1* fusion-positive cases. Despite this, the molecular characteristics and clinical impacts of distinct *NRG1* fusion types remain poorly understood.

Methods: We retrospectively enrolled 435 NSCLC patients, comprising 78 with *NRG1* fusions and 357 wild-type (WT), from June 2016 to December 2023. We assessed mutational landscapes, tumor mutation burden (TMB), chromosomal instability scores (CIS), and gene expression using broad-panel targeted DNA and RNA sequencing analyses. Survival analyses were conducted in patients from our cohort (N=47) and online TCGA database (N=526).

Results: Among the *NRG1* fusion-positive patients, 65.8% harbored recurrent fusion partners like *CD74-NRG1* and *SLC3A2-NRG1*, with *CD74-NRG1* appearing most frequently (48.1%). These recurrent fusions were associated with significantly fewer *EGFR* and *KRAS* mutations and lower TMB and CIS compared to WT ($P < 0.01$). Additionally, 26 patients had unique singleton fusion partners, with 23 novel fusions identified, including *CEBPD-NRG1* and *BMP1-NRG1,* although validation through tools such as RNA sequencing is warranted in the future. Interestingly, this group showed significant enrichment of mutations in genes like *KRAS*, *MSH2*, *MSH6*, *FANCI*, and *MDM2*, impacting key oncogenic and DNA-repair pathways including Fanconi anemia, mismatch repair, PI3K-AKT, and MAPK pathways ($P < 0.01$). Additionally, the RNA profiling showed upregulation of *DNAJB1* ($P < 0.01$) and *LMNA* ($P = 0.02$) in the singleton group versus WT, linked to poorer overall survival (OS) in the TCGA cohort (HR: 1.52 for both). No significant differences in progression-free survival (PFS) were observed between uncommon and WT groups following the first-line anti-EGFR tyrosine kinase inhibitor (TKI) therapy.

Conclusion: This study revealed the diversity of *NRG1* fusions in NSCLC, identifying novel fusion partners and distinct molecular and clinical features across novel *NRG1+* groups, especially the enriched DNA repair/oncogenic signaling pathways and uniquely upregulated transcription profile. Our study highlights the need of subtyping different *NRG+* tumors to better understand their tumorigenesis and facilitates more personalized and targeted treatment of *NRG1+* NSCLC patients.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Metabolic Reprogramming
Poster Session

#5373 ALDOA reprograms stemness and mitochondrial function in lung cancer by facilitating lactylation of m^6A regulators.

Han-Hsi Kuo, You-Yu Lin, Zhao-Jing He, **Yu-Chan Chang**

National Yang Ming Chiao Tung University, Taipei, Taiwan

Lactic acid-derived lactylation has recently emerged as a novel post-translational modification (PTM). This modification has been shown to regulate the N6-methyladenosine (m^6A) modification mechanism, thereby influencing RNA function. In lung cancer progression, dysfunctional aldolase (ALDOA) forms a positive feedback loop with HIF-1α, promoting lactate accumulation in the tumor and its microenvironment. We observe increased lactate production, hypoxia, acidification of the tumor microenvironment and increased lactylation levels of m^6A regulators. In particular, METTL16, a key m^6A transcription factor, was found to be modified by lactate. We also developed a methylated m^6A RNA immunoprecipitation (MeRIP) platform and identified ATPIF1, an inhibitor of mitochondrial F_0F_1-ATP synthase, as a direct target of lactylation. In our overexpression model, ATPIF1 was degraded following m^6A modification, which activated ATP biosynthesis. In a METTL16 knockout model, ATPIF1 expression was restored, leading to impaired ATP production and mitochondrial membrane potential. In addition, cancer spheroid formation and associated markers were reduced. Our *in vivo* model mirrored these findings, showing lactate-induced acidification and up-regulation of molecules involved in cancer stemness and mitochondrial function within the tumor region. Using multiplex staining, we detected reduced ATPIF1 expression alongside ALDOA overexpression, increased mitochondrial membrane potential and increased expression of OXPHOS-related molecules. In conclusion, we propose that ALDOA accelerates glycolysis in lung cancer progression and increases the global lactylation status of m^6A regulators. These findings highlight lactylation as a critical aspect of metabolic reprogramming in cancer.

#5374 Metabolic reprogramming regulates tyrosine receptor kinase c-Met in prostate cancer.

Songyeon Ahn, Tagari Samanta, Junhyoung Park, Benny A. Kaipparettu

Baylor College of Medicine, Houston, TX

Mitochondrial metabolic reprogramming is known to regulate oncopathways. To understand mitochondria-regulated oncopathways in prostate cancer (PC), we used a transmitochondrial cybrid system. Under a benign prostate cell line PNT1a, cybrids were generated after cytoplasmic fusion from PNT1a (PNT1/PNT1) and PC cell line PC3 (PC3/PNT1). PC3/PNT1 cybrids showed increased tumor stemness, metastasis, and mitochondrial activity with enhanced OXPHOS and fatty acid oxidation (FAO) compared to PNT1/PNT1 cybrids. RNASeq analysis of cybrids identified increased mRNA expression of Mesenchymal Epithelial Transition receptor (c-Met) in cybrids with cancer mitochondria. c-Met is a receptor tyrosine kinase (RTK) and an essential receptor that regulates multiple cellular signaling and homeostasis in mammalian cells. c-Met is often over-expressed in several types of cancers and is thus considered a potential therapeutic target. c-Met activation is known to increase glucose metabolism by upregulating the expression of glycolysis-related genes such as GLUT-1, HK-2, PFK-1, LDH-1, and SIRT1 in tumor cells. However, how tumor metabolism affects c-Met expression is unclear. Etomoxir (FAO inhibitor) and Atovaquone (OXPHOS inhibitor) suppressed c-Met protein expression through the mTOR-P70S6K pathway in PC cells. Rapamycin (mTOR inhibitor) reduced c-Met expression in PC3 and DU145 PC cells. Preliminary analysis suggests that the mitochondrial inhibitors and capmatinib, an FDA-approved c-Met inhibitor, have a combinatorial effect. Collectively, this study suggests that mitochondrial metabolic reprogramming to FAO in PC upregulates c-Met activity.

#5375 Sex-specific differences in response to exogenous asparagine supplementation during colorectal tumor growth is dependent on aromatase activity.

Oladimeji Aladelokun[1], Katherine Benitez[1], Yuying Wang[2], Abhishek Jain[1], Domenica Berardi[1], Georgio Maroun[2], Xinyi Shen[1], Jatin Roper[3], Joanna Gibson[2], Kaelyn Sumigray[2], Sajid A. Khan[2], Caroline H. Johnson[1]

[1]Yale University, New Haven, CT,[2]Yale School of Medicine, New Haven, CT,[3]Duke University School of Medicine, Durham, NC

Studies have shown that sex-related differences in asparagine metabolism are associated with gastrointestinal diseases prognosis. However, the effects of exogenous asparagine on the incidence and phenotype of colorectal cancer (CRC) in both men and women remain unclear. This study describes the relationship between exogenous asparagine supplementation and estradiol secretion which is critical for understanding mechanisms of sex-dependent signaling events. We show that asparagine supplementation causes pronounced sex-dependent alteration in cancer metabolomics using in vivo and in vitro approaches. We intraperitoneally administered asparagine to tumor-bearing immunodeficient male and female Rag2/Il2RG (R2G2) mice, at doses of 1 mg/kg and 10 mg/kg body weight. Asparagine supplementation causes a significant increase in cellular asparagine levels in the tumor-bearing male and female R2G2 mice but increases serum estradiol levels and suppresses tumor growth in females R2G2 mice only. Additionally, we combined transcriptome, metabolome and immunochemical analyses and found that asparagine treatment induces intra-tumoral metabolic changes in colorectal cancer cells based on biological sex. We established that in females only, exogenous asparagine exerts a negative feed-back effect on de novo asparagine synthesis and activates a sub-population of macrophages that secretes estradiol via a Cytochrome P450 Family 19 (CYP19)-dependent mechanism, resulting in improved tumor-specific survival. Conversely, in the male mice, asparagine treatment augments tumor growth by decreasing the number of tumor-associated macrophages and reduces CYP19-mediated estradiol secretion leading to worsened tumor-specific survival. Overall, our results revealed a novel role for exogenous asparagine supply during cancer progression and underscores the importance of understanding mechanisms that control asparagine biosynthesis, especially for patients with CRC.

#5376 Unraveling the metabolic Achilles' heel of pancreatic ductal adenocarcinoma: targeting ref-1 and beyond.

Silpa Gampala[1], Jacqueline Peil[2], Sanya Hayati[1], Sonia Kiran[1], Caleb Cheng[3], Elizabeth A. Potchanant[1], Costas Lyssiotis[4], Chi Zhang[5], Sha Cao[5], Mark R. Kelley[1], Melissa L. Fishel[1]

[1]Department of Pediatrics, Indiana University Simon Comprehensive Cancer Center, Indiana University School of Medicine, Indianapolis, IN,[2]Department of Pediatrics and Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN,[3]Michigan Center for Translational Pathology, Cellular and Molecular Biology Program, University of Michigan, Ann Arbor, MI,[4]Rogel Cancer Center, Departments of Molecular and Integrative Physiology, Internal Medicine, University of Michigan, Ann Arbor, MI,[5]Biomedical Engineering, Oregon Health & Science University, Portland, OR

Background & Objective: Treating pancreatic ductal adenocarcinoma (PDAC) remains exceptionally difficult, with scant advancements in clinical management over the last thirty years. Aptly dubbed the "graveyard of chemotherapy," PDAC poses formidable clinical challenges. Even the most effective first-line chemotherapy regimens merely extend median survival for patients with metastatic disease beyond 12 months. Due to its poorly vascularized and fibrotic environment, tumor cells encounter hypoxia and nutrient scarcity and hence adapt metabolically by engaging processes like autophagy and macropinocytosis. Key genes such as HIF1A, KRAS, and MYC help PDAC reshape tumor metabolism favoring glycolysis thereby, promoting metastasis and therapy resistance. Targeting these cancer-specific metabolic genes offers a promising approach to overcoming PDAC's inherent and acquired chemoresistance. Redox effector factor-1 (Ref-1) activates various transcription factors through its redox activity, including HIF-1α, contributing significantly to metabolic reprogramming and altered gene expression in PDAC. We recently published Ref-1's redox function as crucial for mitochondrial metabolic regulation, and that inhibition of Ref-1 suppresses the TCA cycle, Oxidative phosphorylation (OXPHOS), and tumor growth and metastasis in PDAC models. As further proof of Ref-1's role in regulating OXPHOS, PDAC cells are more sensitive to Ref-1 inhibitors in galactose-containing media than glucose. *Therefore, we hypothesize that Ref-1 inhibitor efficacy can be increased by identifying additional tumor-specific metabolic targets that induce synthetic lethality when combined with Ref-1 inhibition.*

Methods: Two approaches were used to identify Ref-1 synergistic metabolic targets: global metabolomics profiling (Pa03C) and a metabolism-centered CRISPR/Cas9 KO library screening (MiaPaCa2) after treatment with APX2014, a potent Ref-1 redox inhibitor.

Results & Conclusion: The CRISPR screen identified several interesting synthetic lethal genes for combination with Ref-1 redox inhibition, including PRDX1, G6PD, and SEPHS2. Further, integration of the global metabolomics profile with our published scRNA seq data shows significant changes in both substrates and enzyme gene expression of multiple metabolic pathways, with a predicted decrease in TCA cycle and nucleotide biosynthesis, and increased glutaminolysis following Ref-1 inhibition. Ongoing studies include the prediction of combinatorial lethality enabled by co-suppression of Ref-1 and other metabolic targets and validation using our *in vitro* and *in vivo* model of PDAC PDX tumor lines co-cultured with matched cancer-associated fibroblasts.

#5377 FGF1 regulates breast cancer growth and metabolic reprogramming through ETV4.

B. Sankofi, S. Johnson Murguia, W. L. Berry, E. A. Wellberg;
University of Oklahoma Health Sciences Center, Oklahoma City, OK

Breast cancer is the most frequently diagnosed cancer in women worldwide. Obesity increases resistance to breast cancer therapies and patient mortality, particularly for estrogen receptor-positive (ER+) tumors that represent 70% of all cases. Adult weight gain in women with obesity, characterized by adipose tissue expansion, is an independent prognostic factor for breast cancer. In a preclinical model, we found that weight gain promoted ER+ tumor growth after endocrine therapy through adipose-derived fibroblast growth factor 1 (FGF1). To determine the underlying mechanisms, we used cultured ER+ breast cancer cells (MCF7, tamoxifen-resistant MCF7 cells, and UCD12 cells) treated with FGF1, combined with gene expression profiling and metabolic analysis. ETS variant 4 (ETV4), which regulates ER activity and cancer cell glycolysis, was the top gene induced by FGF1 in multiple ER+ lines. ETV4 was shown by others to regulate breast cancer metabolism and stemness, contributing to disease progression. We hypothesized that ETV4 mediates the FGF1-dependent effects on breast cancer glycolytic reprogramming in obesity-associated breast tumors. ETV4 was upregulated in human PDX tumors grown in obese versus lean mice. In invasive human breast cancer specimens, high versus low ETV4 expression predicted a shorter recurrence-free survival for patients with ER+ tumors. ETV4 knockdown in cultured endocrine-resistant breast cancer cells prevented the proliferation and induction of glycolytic genes with FGF1 treatment. Conversely, overexpression of ETV4 was sufficient to increase glycolytic gene expression and enzyme activity, as well as cell proliferation. Taken together, our data suggest a mechanism by which FGF1 supports breast cancer endocrine therapy resistance in the context of obesity through ETV4 induction and glycolytic metabolic reprogramming. Understanding this process may aid in designing effective treatments, especially for patients resistant to current ER-targeted therapies. Furthermore, ETV4 may be a biomarker to identify breast tumors with excess FGF signaling and patients at high risk for progression.

#5378 GLUD1 protects NSCLC from glucose starvation through a novel enzyme-independent function.

Ryan Clay, Lingtao Jin

Molecular Medicine, The University of Texas Health Science Center At San Antonio, San Antonio, TX

Lung cancer is the leading cause of cancer-related deaths among both men and women in the United States with non-small cell lung cancer (NSCLC) accounting for 85% of all lung cancers. However, regardless of genetic background, nearly all NSCLC are dependent on upregulation of glutamine metabolism for survival, and consequently, the nonessential amino acid glutamine becomes conditionally essential to many tumors. Tumor cells generate the bulk of their ATP by upregulating glycolysis and discarding of the majority of glycolytic carbon as lactate, known as the Warburg effect. This lost carbon is replaced by glutamine-dependent anaplerosis of the mitochondrial tricarboxylic acid (TCA) cycle, which maintains a pool of biosynthetic intermediates that contribute to cell growth. Glutaminolysis is also crucial for the regulation of cell proliferation, survival, and chemoresistance by supporting NADPH and GSH synthesis, regulating of redox metabolism, preventing apoptosis, activating of autophagy, as well as influencing oncogenic signalings such as mTOR, Ras, NRF2 and Hippo pathway. Glutamate dehydrogenase 1 (GLUD1) is an enzyme in the glutaminolysis pathway that catalyzes the reversible conversion of glutamate to alpha-ketoglutarate (aKG). GLUD1 acts as an energy sensor and undergoes a high degree of allosteric and post-translational regulation. GLUD1 also seems to play a direct role in the regulation of multiple cell signaling pathways including mTORC1 activation, signals through glutathione peroxidase 1 (GPX1) to regulate redox homeostasis, directly activates p38 MAPK signaling, and activates CamKK2-AMPKa1 signaling to prevent anoikis and promote metastasis. Our data shows knockout of GLUD1 sensitizes cancer cells to nutrient starvation, but expression of enzymatically dead GLUD1 mutants R443S or R463A still protect cancer cells from glucose starvation-induced cell death. We observe a rapid drop in ATP coupled with the loss of AMPK activation in GLUD1 KO cells under glucose starvation. Cell death can be rescued with the direct AMPK activator A769662 or exacerbated by the AMPK inhibitor Dorsomorphin. Inhibition of cysteine import with xCT-inhibiting compounds Erastin and Sulfasalazine, knockdown of xCT, or cysteine/glucose double starvation also rescues cell death. Novel post-translational modifications to GLUD1 were identified by mass spec including acetylation, methylation, and a previously unknown phosphorylation site. In addition, BioID protein proximity labeling identified several new potential interactors for GLUD1 including kinases and glycolysis proteins. We propose the GLUD1 protein possesses a previously undiscovered function that activates AMPK signaling, thereby inhibiting cysteine import and protecting against cell death under glucose starvation.

#5380 Chemotherapy induces a pro-metastatic secretome through cellular senescence.

Aidan Robert Cole, Katherine M. Aird

Department of Pharmacology and Chemical Biology, University of Pittsburgh Medical Center, Hillman Cancer Center, Pittsburgh, PA

High grade serous ovarian cancer (HGSOC) is the most lethal gynecological malignancy, with 70-80% of patients having metastatic disease recurrence within 12 months of treatment with first line therapies. A potential factor in HGSOC metastasis and recurrence is chemotherapy induction of cellular senescence, a stable cell cycle arrest which includes a unique secretome (termed the senescence-associated secretory phenotype - SASP) comprised of cytokines, chemokines, small molecules, and differential usage of nutrients. Prior work found that senescent cells can promote tumor progression and metastasis; however, the direct contribution of the SASP to metastatic recurrence has not been deeply explored. Interestingly, we found that the SASP is sufficient to drive increased HGSOC metastatic tumor nodules *in vivo*. Mechanistically, we found that naïve cancer cells cultured in conditioned media from senescent cells expressed lower levels of multiple extracellular matrix and adhesion related proteins, including fibronectin and integrins. Indeed, the decrease in adhesion factors corresponds to lower cell adhesion and increased cell detachment in 3D conditions. Unexpectedly, experiments depleting SASP proteins suggest a metabolic component of the SASP contributes to the increased metastasis through loss of adhesion factors. We also found that the SASP induced an increase in basal and maximal oxygen consumption, further exhibiting altered metabolism. Using a metabolic CRISPR knockout screen to identify genes required for decreased adhesion after exposure to the SASP, we identified several genes in the electron transport chain, including *NDUFA5*, a subunit of complex I. Consistently, HGSOC patients with low *NDUFA5* expression have upregulated adhesion gene signatures, and *NDUFA5* knockdown or pharmacological inhibition of complex I both increase cell adhesion. *NDUFA5* is also required for SASP induced increase in basal and maximal respiration. Interestingly, use of scavengers to inhibit ROS did not rescue the adhesion phenotype in *NDUFA5* knockout cells, but supplementation with an NAD+ precursor did, implicating *NDUFA5*'s role as an NADH dehydrogenase in its effect on cell adhesion independent of redox status. Together, our data demonstrate that the chemotherapy-induced SASP has a paracrine effect on mitochondrial metabolism in tumor cells, which in turn decreases cell adhesion. We hypothesize this decrease in adhesion will lead to an increase in tumor metastasis, which contributes to recurrence. Future studies will identify the metabolic composition of SASP that leads to paracrine metabolic reprogramming, the mechanism behind *NDUFA5* and respiration's regulation of adhesion through NAD+ regeneration, and its role in SASP-induced dissemination *in vivo*.

#5381 Tumor nutrient stress regulates cancer-associated fibroblast heterogeneity.

Marta Storl-Desmond[1], Kevin Munoz Forti[1], Michał Nizio[1], Alexander Muir[2], Simon Schworer[1]

[1]Section of Hematology and Oncology, Department of Medicine, University of Chicago, Chicago, IL,[2]Ben May Department of Cancer Research, University of Chicago, Chicago, IL

Pancreatic ductal adenocarcinoma (PDAC) is a lethal cancer type with a 5-year survival rate of only 13%. PDAC is unique in that up to 90% of the tumor bulk is comprised of a dense desmoplastic stroma. Enriched within PDAC's extensive stroma are cancer-associated fibroblasts (CAFs). CAFs orchestrate tumor progression through the secretion of signaling molecules that impact cancer and immune cell function, and extracellular matrix proteins that remodel the surrounding stroma. CAFs are functionally and transcriptionally heterogeneous, with three distinct subtype classifications: myofibroblastic (myCAF), inflammatory (iCAF), and antigen-presenting (apCAF). In PDAC, an increased iCAF to myCAF ratio has been associated with worse clinical outcomes; however, efforts to selectively target CAF populations have been met with the challenge of CAF plasticity. A better understanding of the mechanisms underlying CAF state decisions is required before pro-tumor CAFs can be targeted. A key feature of PDAC is impaired perfusion, which leads to regions of hypoxia and altered nutrient availability; however, the significance of this unique metabolic environment to CAF state remains unknown. It has been reported that nutrient stress within the tumor microenvironment directly impacts cancer cell state leading to targetable metabolic adaptions, but how these same stresses affect the CAFs that share this microenvironment have been overlooked. To investigate how the altered nutrient availability of PDAC impacts CAFs, we acclimated pancreatic stellate cells (PSCs), a precursor CAF, to culture in tumor nutrient-relevant media. Characterization of these cells by high-dimensional flow cytometry and RNA-sequencing has revealed that the nutrient environment is sufficient to alter CAF state. CAFs in conditions of tumor nutrient stress are enriched for inflammatory signatures, along with higher expression and secretion of several potent cytokines and chemokines. Changes in CAF state induced by tumor nutrient stress are reversible, highlighting nutrient availability as a dynamic regulator of CAF plasticity. This work demonstrates the importance of the tumor nutrient environment to CAF state decisions. It further suggests that incorporating metabolic features of the PDAC TME increases the fidelity of existing model systems, which may allow uncovering novel regulators of CAF heterogeneity.

#5382 Comprehensive characterization of renal cell carcinomas identifies metabolic reprogramming of the tumor microenvironment associated with disease progression.

Kirsteen H. Maclean[1], Lakshmi Chandramohan[2], Qingyan Au[1], Harry Nunns[1], Jiong Fei[1], Erinn Parnell[1], Anna Juncker-Jensen[1]

[1]NeoGenomics, Aliso Viejo, CA,[2]NeoGenomics, Houston, TX

Background: Renal cell carcinoma (RCC) is one of the most frequent urological cancers with the most common histological subtype being clear cell renal cell carcinoma (ccRCC), an immunologically and histologically diverse tumor which is associated with a poor clinical outcome as up to 40% of ccRCC patients develop metastases. While significant progress has been made in the development of immunotherapy for ccRCC, there are still many unanswered questions about the mechanisms of immune evasion and resistance, and the development of predictive biomarkers for optimal treatment strategies for individual patients. Recently, it has become clear that cellular metabolism may play a significant role in the immune response to ccRCC tumors, affecting both the ability of T-cells to function and the overall composition of the tumor microenvironment (TME). This remodeled TME leads to changes in nutrients and metabolites that can promote an immunosuppressive environment and prevent lymphocyte activation and recruitment, eventually promoting T-cell exhaustion.
Methods: We performed transcriptomic and spatial profiling of 16 ccRCC patient samples across all clinical TNM stages (Stages I-IV) to define the changes in metabolic processes and infiltrating immune cells with advancing disease. Specifically, we included the spatial MultiOmyx™ IF multiplexing assay (NeoGenomics Laboratories, Inc.) using the new MO2.0 workflow with enhanced whole slide capability coupled to the NanoString nCounter® Metabolic Pathways Panel to investigate metabolic reprogramming, cellular stress, and the relationship to the composition of the TME in advancing disease progression in ccRCC.
Results and Conclusions: We identified significant changes associated with the TME, especially immunosuppressive immune cells in advanced disease, coupled to the identification of several metabolism-related genes that correlated with immune infiltrates in ccRCC. Identifying and understanding these metabolic alterations within the different staging of RCC will help in designing new targeted therapies and improving diagnostic tools to better care for patients with this disease.

#5383 Metabolic profiling of compressed stromal cells in the breast peritumor.

Julian Najera, Hao Chen, Scott Howard, Meenal Datta

University of Notre Dame, Notre Dame, IN

Breast cancer represents a significant public health concern for women across the globe. Although the breast peritumor is increasingly recognized for its active role in modulating breast tumor growth and progression, its biophysical relationship with the tumor is poorly understood. Of particular interest is the effect of compressive forces imparted onto the peritumor by the growing mammary tumor. In this study, we found that compression metabolically reprograms stromal cells—namely, fibroblasts and adipocytes—found in the breast peritumor *in vitro*. Using fluorescence lifetime imaging microscopy, we discovered that fibroblasts and differentiated adipocytes under compression adopt a more glycolytic state whereas compressed pre-adipocytes appear to favor oxidative phosphorylation. We were able to confirm this metabolic shift in fibroblasts using bulk RNA sequencing, with pathway analysis showing that compressed fibroblasts significantly downregulate oxidative phosphorylation and strongly enrich glycolysis. Moreover, by measuring glucose uptake, we found that compression moderately increases glucose uptake in fibroblasts. Furthermore, through single cell transcriptomic analysis of breast cancer patients, we discovered that, in relation to those found in normal (compression (-)) tissue, fibroblasts and mature adipocytes in the peritumor (compression (+)) tend to favor glycolysis. It remains to be determined, however, to what degree this metabolic reprogramming is a direct consequence of external tumor compression.

#5384 Targeting PIKfyve-driven lipid homeostasis as a metabolic vulnerability in pancreatic cancer.

Caleb Cheng[1], Jasmine Wisniewski[1], Sydney Peters[1], Ruya Pakkan[1], Jennifer P. Morton[2], Pietro Morlacchi[3], Yuanyuan Qiao[1], Costas A. Lyssiotis[1], Arul M. Chinnaiyan[1]

[1]University of Michigan, Ann Arbor, MI,[2]CRUK Scotland Institute, Glasgow, United Kingdom,[3]Agilent Technologies, Inc., Lexington, MA

Pancreatic ductal adenocarcinoma (PDAC) subsists in a nutrient-deregulated microenvironment, making it particularly susceptible to treatments that interfere with cancer metabolism. For example, PDAC utilizes and is dependent on high levels of autophagy and other lysosomal processes. Although targeting these pathways has shown potential in preclinical studies, progress has been hampered by the challenge of identifying and characterizing favorable targets for drug development. Here, we characterize PIKfyve, a lipid kinase integral to lysosomal functioning, as a novel and targetable vulnerability in PDAC. Employing a genetically engineered mouse model, we established the essential role of PIKfyve in PDAC progression. Further, through comprehensive metabolic analyses, we found that PIKfyve inhibition obligated PDAC to upregulate *de novo* lipid synthesis. PIKfyve inhibition triggered a distinct lipogenic gene expression and metabolic program, creating a dependency on *de novo* lipid metabolism pathways, by upregulating genes such as *FASN* and *ACACA*. In PDAC, the KRAS-MAPK signaling pathway is a primary driver of *de novo* lipid synthesis, specifically enhancing *FASN* and *ACACA* levels. Accordingly, the simultaneous targeting of PIKfyve and KRAS-MAPK resulted in the elimination of tumor burden in numerous preclinical human and mouse models, including endogenous genetically engineered models. Taken together, these studies suggest that disrupting lipid metabolism through PIKfyve inhibition induces synthetic lethality in conjunction with KRAS-MAPK-directed therapies for PDAC.

#5385 Concurrent inhibition of the serine/threonine protein kinase AURKB and the fatty acid oxidation enzyme CPT1A demonstrates synthetic lethality in preclinical glioblastoma models.

Qiuqiang Gao[1], Chang Shu[1], Jeongyeon Mun[1], Zhe Zhu[1], Hasan O. Akman[2], Mike-Andrew Westhoff[3], Georg Karpel-Massler[4], Markus D. Siegelin[1]

[1]Department of Pathology and Cell Biology, Columbia University Medical Center, New York, NY,[2]Department of Neurological Surgery, Columbia University Medical Center, New York, NY,[3]Department of Pediatrics and Adolescent Medicine, Ulm University Medical Center, Ulm, Germany,[4]Department of Neurosurgery, Ulm University Medical Center, Ulm, Germany

Glioblastoma IDH-wild type, an aggressive and treatment-resistant brain cancer, urgently requires novel and more effective therapeutic approaches to enhance patient outcomes. Analysis of public genomic databases has revealed that the serine/threonine protein kinase Aurora kinase B may represent a promising therapeutic target for GBM. This study provided compelling evidence that AURKB is significantly upregulated in GBM tumors, and elevated AURKB levels are associated with poorer survival in GBM patients. Remarkably, the selective AURKB inhibitor barasertib was found to potently suppress the growth of established GBM cell lines, patient-derived xenograft models, and neurosphere cultures at low, nanomolar concentrations. Similarly, genetic depletion of AURKB substantially impaired the growth of patient-derived xenograft lines. Notably, in an orthotopic PDX GBM xenograft model, the loss of AURKB function was found to extend animal survival. Mechanistically, the study revealed that AURKB depletion or inhibition significantly impacts mitochondrial energy metabolism in GBM cells. Paradoxically, the loss of AURKB led to increased cellular respiration and oxidative phosphorylation, facilitated by enhanced fatty acid oxidation. Consistently, the absence of AURKB sensitized GBM cells to the cytotoxic effects of CPT1A inhibition, i.e., fatty acid oxidation inhibition. Furthermore, the BH3-mimetic ABT263 was found to reduce the growth of GBM lines in the context of AURKB loss of function, likely due to the heightened dependence on mitochondrial metabolism. These findings uncover a previously unappreciated role for AURKB in regulating GBM metabolism and provide a strong rationale for further developing AURKB-targeted therapies, potentially in combination with metabolic inhibitors and BH3-mimetics, to improve clinical outcomes for patients with GBM.

#5386 Metabolic regulation of PARP inhibitor response in ovarian cancer cells.

Subin Myong[1], Hardik Shah[2], Benedikt Nagele[1], Anh Q. Nguyen[3], **Sridevi Challa**[3]

[1]The University of Chicago Medicine Comprehensive Cancer Center, Chicago, IL,[2]Metabolomics Platform, The University of Chicago Medicine Comprehensive Cancer Center, Chicago, IL,[3]Obstetrics and Gynecology, The University of Chicago, Chicago, IL

Ovarian cancer (OvCa) is the most lethal gynecologic cancer due to high mortality from therapeutic resistance. Recently, PARP inhibitors (PARPi) have shown efficacy in treating OvCa, and patients with DNA repair pathway defects are now on PARPi maintenance. However, heterogeneous responses and resistance to therapy limit the clinical efficacy of PARPi treatment.

Catalytic activation of the PARP1 enzyme consumes NAD^+, a metabolite coenzyme for redox reactions and a key regulator of energy metabolism. PARP inhibitors can change the metabolic state of cells by inhibiting PARP1 activation and altering cellular NAD^+ levels, but the role of metabolic dysregulation in PARPi resistance remains unclear. Here, we show that PARPi-resistant ovarian cancer cells have elevated NAD^+ biosynthesis through the NAD^+ salvage pathway.

Gene expression analysis using RNA sequencing and metabolomic analysis using metabolic profiling have identified key pathways downstream of NAD^+ biosynthesis that contribute to PARPi resistance. We also demonstrated that the metabolic changes caused by high NAD^+ biosynthesis in PARPi-resistant cells cause cell cycle arrest at the G0/G1 phase and increase cellular quiescence and cell motility. Inhibition of the NAD^+ salvage pathway and reduction of NAD^+ levels within PARPi-resistant cells reverse cellular quiescence and cell migration, ultimately increasing the PARPi response in these cells.

In summary, our results demonstrate that elevated NAD^+ biosynthesis in ovarian cancer cells causes PARP inhibitor resistance by altering the metabolic state of these cells. Furthermore, inhibition of the NAD^+ salvage pathway enhances the efficacy of PARP inhibitor-mediated control of ovarian cancer growth.

Supported by grants from the NCI SPORE and CRF to S.C.

#5387 Modeling physiological lipid availability of tumors to improve in vitro studies of cancer cell lipid metabolism.

Mete E. Ozgurses[1], Vipin S. Rawat[1], Kelly O. Conger[1], Huiping Zhao[1], Patrick B. Jonker[2], Alexander Muir[2], Jonathan L. Coloff[1]

[1]University of Illinois College of Med. at Chicago, Chicago, IL,[2]Ben May Department for Cancer Research, University of Chicago, Chicago, IL

Lipids are highly crucial in cellular physiology and pathology. They are the main structural components of cellular membranes and play critical roles in metabolism, signaling, and other core processes. Many studies of lipid metabolism, both in cancer and other fields, rely on the results of *in vitro* tissue culture experiments. Generally, *in vitro* culture systems use media containing non-physiological levels of nutrients, including high levels of critical nutrients like glucose and glutamine. Recently, several groups have generated culture media with physiological levels of polar metabolites, which can correct some tissue culture-related artifacts. However, these new-generation media still use traditional serum and still represent severe lipid starvation conditions. In lipid-deprived environments, cells respond by activating the sterol regulatory element binding protein (SREBP) pathway, a transcriptional regulator of global lipid metabolic gene expression. Our SREBP transcription factor signature scores comparing human tumor samples to established cancer cell lines growing in TM showed dramatic activation of SREBP in cell lines relative to tumors, supporting traditional tissue culture being a highly lipid-starved environment. We hypothesized that these lipid-starved conditions impact cancer cell behavior in vitro and that developing a more physiological lipid medium will maintain more accurate cell metabolic characteristics. To study this, we have generated two new physiological lipid media - one that mimics lipid levels found in human plasma (HSLM) and one that mimics the lipid levels found in the tumor microenvironment (TILM). The lipidomic analysis confirmed that HSLM closely resembles the lipidome of human serum, while TILM more accurately aligns with the lipid profile of tumor interstitial fluid (TIF). We found that cell lines were unable to survive when cultured in HSLM. However, cancer cell lines adapted to TILM, reduced de novo lipogenesis and proliferation rate and had a similar fatty acid profile to in vivo mouse tumors compared to TM. In the future, we would like to comprehensively study how cells change their metabolism, signaling, and lipid profile to see whether cells cultured in TILM better represent primary tumor metabolism.

#5388 Metastasis suppressor RKIP modulates mitochondrial metabolism in triple-negative breast cancer.

Joana Pinheiro[1], Dongbo Yang[1], Madeline Henn[1], Long Chi Nguyen[1], Olga Martinho[2], Brandon Faubert[1], Marsha Rich Rosner[1]

[1]University of Chicago, Chicago, IL,[2]ICVS/3B's-PT Government Associate Laboratory, University of Minho, Braga, Portugal

Triple-negative breast cancer (TNBC) remains the most aggressive subtype of breast cancer, with a high propensity for metastasis. Tumor cell heterogeneity has been closely linked to metastatic progression, therapy resistance, and disease recurrence in solid tumors, including TNBC. Within tumors, metabolic heterogeneity emerges as cancer cells adapt to diverse environmental pressures, further driving tumor aggressiveness. However, the role of metastasis suppressors, such as Raf Kinase Inhibitory Protein (RKIP), in regulating metabolic heterogeneity is not yet fully understood. Analysis of RNA sequencing data from breast cancer patients revealed a strong correlation between RKIP expression and oxidative phosphorylation (OXPHOS) genes. Together with this, by performing gene set enrichment analysis of bulk RNA sequencing of mouse xenografts we observed an enrichment of hallmark of OXPHOS in RKIP-overexpressing tumors compared to controls. Consistent with these findings, RKIP was shown to modulate oxygen consumption rates in vitro. Using stable isotope tracing with [U-^{13}C] glucose in vivo, we observed higher glucose contribution to citrate labeling in RKIP-overexpressing tumors compared to controls. Through single-cell RNA sequencing, we demonstrate that RKIP reduces overall transcriptional heterogeneity, particularly by decreasing the variability of genes involved in mitochondrial respiration. Importantly, treatment with the OXPHOS inhibitor metformin selectively inhibited the growth of RKIP-overexpressing tumors, while control tumors were unaffected. This study suggests that RKIP or drugs that mimic its action have the potential to promote OXPHOS in tumor cells, presenting an opportunity to suppress tumor growth by subsequent therapeutic targeting of mitochondrial respiration in TNBC.

#5389 Adaptive OXPHOS and antioxidant defense in glucose-deprivation-resistant colon cancer cells.

Elimelech Nesher, Subha Ranjan Das, Bar Levi, Igor Koman

Ariel University, Ari'el, Israel

Cancer cells are generally characterized by exhibiting the Warburg effect, favoring glycolysis over oxidative phosphorylation (OXPHOS) to produce ATP. This leads to an increase in glucose uptake and lactate production, fueling rapid proliferation by providing energy and intermediates for biosynthesis. In parallel, slow angiogenesis resulting in a blood supply insufficient for the tumor's rapid growth leads to glucose starvation in solid tumors. To mimic glucose starvation *in vitro*, we used a model of glucose deprivation in HCT116 cells. A Seahorse study demonstrated that HCT116 cells endured an adaptive state of dormancy during glucose deprivation, shifting their metabolic pathway from glycolysis to OXPHOS. Interestingly, a small percentage of survived cells after dormancy completely resumed their proliferative abilities without glucose supply. We showed that these glucose-deprivation-resistant (GDR) cells have significant activation in the tricarboxylic acid (TCA) cycle, as well as increased levels of antioxidants and their precursors, such as GSSG, GSH, glutamine, glutamate, proline, betaine, citrulline, and arginine. These findings suggest that GDR cells could scavenge reactive oxygen species (ROS) generated during accelerated OXPHOS. Furthermore, the GDR cells demonstrated additional resistance to oxidative stress, induced by hydrogen peroxide. Increased OXPHOS in dormant cells, associated with elevated ROS production, led to significant DNA damage. In turn, enhancing DNA repair mechanisms and boosting antioxidant activity in GDR cells were accompanied with one-carbon metabolism, supporting rapid proliferation by supplying nucleotides for DNA repair and synthesis. We believe that cancer cells which, under glucose starvation, can completely switch their metabolism from glycolysis to OXPHOS are able to survive and regain their proliferative capacity by boosting their antioxidant defenses and improving their DNA repair mechanisms. This phenomenon supports the thesis that targeting cancer cells' metabolic flexibility and antioxidant capacity is an effective anticancer therapeutic strategy.

#5390 Metabolic reprogramming and glutamine addiction in squamous cell carcinoma driven by mutant cyclin D1.

Shuyuan Zhang, Polly Phillips-Mason, J. Alan Diehl

Biochemistry, Case Western Reserve University, Cleveland, OH

Cyclin D1 overexpression contributes to abnormal cell proliferation and tumor development in multiple cancer types. Cyclin D1 overexpression occurs at the highest frequency in head and neck squamous cell carcinoma (HNSCC). Biological events that trigger cyclin D1 overexpression include CCND1 amplification, mutations that impair cyclin D1 degradation, and inactivation of the SCF E3 ubiquitin ligase, Fbxo4 which controls cyclin D1 degradation. We focused on a mutant, cyclin $D1^{T286A}$, which blocks the nuclear export of cyclin D1 and its subsequent degradation by the 26S proteasome. Cyclin D^{T286A} expression can drive a variety of cancer types murine models including endometrial and mammary carcinoma as well as B-cell lymphoma. However, the molecular mechanisms whereby cyclin D^{T286A} drives cancer progression remain to be fully identified. Here, we demonstrate that cyclin $D1^{T286A}$ drives metabolic reprogramming in squamous cancer cells resulting in glutamine addiction via activation of CDK4/6. Notably, cyclin $D1^{T286A}$ triggers downregulation of IDH3 contributing to cell glutamine dependency to enforce oxidative carboxylation benefiting both generation of biosynthetic intermediates and ATP. A decrease of NAD^+/NADH ratio induced by decreased IDH3 results in cellular ATP imbalance and compromised mitochondria which promote cell death under glutamine restriction. Finally, we demonstrate that cyclin $D1^{T286A}$ cooperates with mutant p53 to promote head and neck squamous tumor development in a genetically engineered mouse model. In conclusion, we have uncovered the novel mechanism whereby that cyclin $D1^{T286A}$ drives metabolic reprogramming and HNSCC and our results highlight the potential for targeting glutamine metabolism in cyclin D1-positive HNSCCs.

#5391 The hexosamine biosynthetic pathway promotes metastatic potential upon glutamine deprivation in STK11 null KRAS-driven lung adenocarcinoma.

Shannon Prior, Logan Sands, Sean Lenahan, Cole Royer, Melissa Scheiber, David Seward, Paula Deming

University of Vermont, Burlington, VT

Lung cancer is the leading cause of cancer-related deaths worldwide, with ~15,000 annual US cases presenting with an aggressive lung adenocarcinoma (LUAD) driven by co-mutations in KRAS and STK11 (KS). Loss of STK11 function in KRAS-driven LUAD dysregulates many cellular processes, including metabolism. Specifically, KS cells become “glutamine addicted” and enhance glutaminolysis to drive proliferation. Recent work from our group revealed pro-oncogenic signaling in KS upon glutamine deprivation. We hypothesize that glutamine scarcity promotes metastatic potential in KS via metabolic rewiring. To test this, we employed a cell culture model of KRAS-driven LUAD with and without STK11 (ΔSTK11). At baseline, ΔSTK11 cells demonstrate a hypermetabolic phenotype associated with enhanced glutamine utilization as assessed by Seahorse mitochondrial stress tests. Unexpectedly, deprivation of glutamine led to the detachment of live ΔSTK11 cells, measured by trypan blue exclusion and annexinV/PI staining, that maintained the ability to re-adhere and proliferate in nutrient replete media. Furthermore, ΔSTK11 cells upregulated anti-apoptotic and EMT marker expression in the absence of glutamine. Invasive capacity was assessed using a spheroid model, and while parental spheroids were unaffected by glutamine deprivation, ΔSTK11 spheroids displayed enhanced ameboid-like single cell invasion. To determine the mechanism(s) underlying this metastatic phenotype, we employed heavy nitrogen labeling, which revealed an upregulation of the hexosamine biosynthetic pathway (HBP) upon glutamine deprivation in ΔSTK11 cells. The HBP is an offshoot of glycolysis that serves as a central hub to regulate many cancer fitness pathways via the production of UDP-GlcNAc - a sugar moiety used for generation of glycosylation intermediates or O-GlcNAcylation. Far western blot analysis validated that ΔSTK11 cells have increased O-GlcNAcylation upon decreased glutamine availability. Treatment with FR054, an inhibitor of the intermediate HBP enzyme, PGM3, decreased metastatic potential of ΔSTK11 cells as measured by anchorage independent survival and 3D-spheroid invasion. These results suggest ΔSTK11 cells utilize the HBP as a protective shunt in response to glutamine deprivation, undermining the potential benefit of glutaminase inhibitors (i.e. “glutamine starvation”) as a therapeutic intervention in KS LUAD patients. Future work aims to further elucidate the role of the HBP in promoting metastasis and to determine the mechanism(s) by which ΔSTK11 cells regulate the HBP. Overall, our data reveal novel insight into the molecular mechanisms altered downstream of STK11 loss linking glutamine metabolism with metastatic potential in KRAS-driven LUAD.

#5392 Breast cancer cells that preferentially metastasize to lung are more adaptable to varying glucose levels compared to those that metastasize to the liver.

Marjorie Anne A. Layosa[1], Mike Wendt[2], Stephen Hursting[3], Dorothy Teegarden[1]

[1]Department of Nutrition Science, College of Health and Human Sciences, and the Institute for Cancer, Purdue University, West Lafayette, IN,[2]Department of Internal Medicine, and Holden Comprehensive Cancer Center, University of Iowa, Iowa, IA,[3]Department of Nutrition, Nutrition Research Institute, and Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC

Metastasis is the primary cause of breast cancer-related deaths. We investigated the metabolic adaptations of glucose, a major energy substrate, in breast cancer cells that metastasize to lung versus liver to determine the metabolic adaptations that may support site-specific metastasis. Utilizing a breast cancer model that preferentially metastasizes to the lung (metM-Wnt^{Lung} cells; MLg) or liver (metM-Wnt^{Liver} cells; MLr), we measured ^{14}C-glucose uptake, $^{13}C_6$-glucose flux into metabolic pathways, and viability under varying glucose concentrations. Results show that in 5 mM glucose (normal), MLg is more glycolytic with greater flux of $^{13}C_6$-glucose-derived carbons into the glycolytic intermediates pyruvate and lactate in comparison to MLr. In contrast, MLr showed higher levels of $^{13}C_6$-glucose-derived carbons in the tricarboxylic acid cycle metabolites including oxaloacetate and malate indicative of higher oxidative phosphorylation and higher pyruvate carboxylase (PC) activity. Interestingly, high glucose (25 mM) exposure reduced viability of MLr by 39%, suggesting MLg's adaptability to varying glucose levels. Although glucose uptake similarly increased in all cell lines under high glucose, flux results showed greater oxidative phosphorylation and increased PC activity in MLg, while MLr shifted to a more glycolytic phenotype. These results were consistent with improved oxidative stress protection in high glucose in MLg, but not MLr. Further, MLg deprived of glucose were more viable (9%) compared to MLr, suggesting a potential alternate use of other substrates to overcome glucose deprivation. Inhibiting the rate-limiting enzyme for gluconeogenesis, phosphoenolpyruvate carboxykinase, reduced viability of MLg by 9% compared to MLr. Further, inhibiting the catabolism of glutamine, a gluconeogenic substrate, reduced viability of MLg by 13% compared to MLr, suggesting that MLg utilizes the contribution of glutamine and gluconeogenesis in conditions of low glucose. Overall, breast cancer cells with preferential metastasis to lung are more adaptable to varying glucose concentrations than breast cancer cells with preferential metastasis to liver by shifting metabolism from glycolysis to oxidative phosphorylation under high glucose conditions and utilizing glutamine and gluconeogenesis under low glucose conditions.

#5393 Cancer cells surviving chemotherapy reprogram glucose and glutamine metabolism to combat oxidative stress.

Melvin Li, Luke V. Loftus, Sarah R. Amend, Kenneth J. Pienta

Johns Hopkins University School of Medicine, Baltimore, MD

Therapy resistance is the leading cause of cancer-related deaths across all tumor types. Our group has discovered a novel resistance mechanism where cancer cells enter a reversible non-proliferative state in response to chemotherapy. These cells exhibit a 40-fold increase in cell size and an increase in DNA content when compared to their proliferative counterparts. Cells surviving in this state eventually produce progeny that can repopulate the tumor, modeling cancer recurrence. This resistant phenotype has been reported in cell culture, pre-clinical mouse models, and in human patients. The surviving cells exhibited increased levels of reactive oxygen species (ROS) when compared to the untreated parental controls. It is well known that ROS is produced during oxidative metabolism, and cancer cells utilize a variety of mechanisms to neutralize oxidative radicals. Metabolic reprogramming is one of the main responses to oxidative stress, where cancer cells can better obtain resources from the extracellular environment and repurpose them to replenish their antioxidant defense systems. We hypothesize that cancer cells surviving in this non-proliferative state in response to cisplatin treatment alter their metabolism of major carbon sources to fuel antioxidant-generating pathways. Cells surviving chemotherapy exhibited an increase in glucose and glutamine consumption, but a decrease in lactate excretion and an increase in glutamate excretion when compared to the untreated parental population. We performed parallel labeling experiments with a variety of ^{13}C glucose and glutamine tracers to investigate how these carbon sources are broken down. We observed that surviving cells utilize glucose to fuel the pentose phosphate pathway (PPP), which is one of the major pathways to generate NADPH for ROS detoxification, rather than breaking it down through glycolysis. In addition, surviving cells exhibited decreased glutamine metabolism into the TCA cycle when compared to the parental cells. Thus, we hypothesize that cells surviving chemotherapy are consuming glutamine to generate glutamate for excretion. Single cell RNA sequencing data demonstrated that surviving cells exhibit an enrichment in NRF2 target genes such as SLC7A11, which encodes for a cystine-glutamate antiporter (system Xc) that exchanges glutamate for cystine, a major component in glutathione biosynthesis to combat oxidative stress. We are further characterizing these changes with mathematical modeling-based ^{13}C-metabolic flux analysis. Additionally, we are currently testing if cells surviving chemotherapy are susceptible to PPP and system Xc inhibitors. Our goal is to identify mechanisms of how cells in this resistant state reprogram their metabolism to survive therapeutic stress. Leveraging these key findings will provide insight for better treatment of therapy-resistant cancers and improve patient outcomes.

#5394 Transcriptional reprogramming, metabolic rewiring and tumor immune microenvironment (TIME) reconfiguration: investigating the molecular underpinnings of GRAMD2^{+}AT1 cell-derived murine LUAD.

Angel P. Hunt[1], Aaron Neely[2], Andrea Lanz[3], MauriAnna Richardson[1], Benecia Jackson[1], Zea Borok[4], Crystal N. Marconett[2]

[1]Integrative Translational Sciences, Beckman Research Institute of The City of Hope, Duarte, CA,[2]Beckman Research Institute of The City of Hope, Duarte, CA,[3]Translational Genomics, University of Southern California, Los Angeles, CA,[4]Medicine, University of California, San Diego, La Jolla, CA

Arising from the distal alveolar epithelium, lung adenocarcinoma (LUAD) is the most common histological subtype of non-small cell lung cancer (NSCLC), accounting for approximately 40% of lung cancer cases. The distal lung houses a variety of cell populations in addition to alveolar epithelial type 1 (AT1) and type 2 (AT2) cells. The large, squamous-appearing AT1 cells facilitate gas exchange across the air blood barrier, while their smaller, cuboidal AT2 cell counterparts synthesize and secrete pulmonary surfactants to prevent alveolar collapse. It was previously believed that AT1 cells were terminally differentiated and devoid of tumorigenic properties, while AT2 cells that are capable of both self-renewal and differentiation to AT1 cells were designated as the primary cell of origin of LUAD. Our laboratory previously demonstrated that GRAMD2^{+} AT1 cells could serve as a cell of origin for KRAS-mediated murine LUAD (Yang M. *et al.*, 2023). In the current study, we investigated the functional roles of AT1 cells in EGFR-mediated LUAD tumorigenesis. We performed a longitudinal analysis spanning approximately 15 weeks where H&E-stained mouse lung tissues underwent a double blinded pathology review. We then employed spatial transcriptomic analysis to examine tumor cell composition, and the molecular underpinnings involved in cell of origin specific LUAD tumorigenesis at 5-, 10-, and 15-weeks post-activation of oncogenic transformation. Our findings illustrate that AT1 cell-derived LUAD undergoes significant transcriptional reprogramming, metabolic rewiring, and tumor immune microenvironment (TIME) reconfiguration over time. Our analysis revealed that Gramd2+ AT1 cell-derived tumors at 5 weeks exhibited a bioenergetic reliance upon mitochondrial OXPHOS, while AT1 cell derived LUAD tumors at 15 weeks exhibited metabolic flexibility as they underwent a glycolytic switch. As this metabolic rewiring and transcriptional reprogramming occurred, TIME reconfiguration also took place. At 5 weeks, tumors exhibited a robust anti-tumor immune response and infiltration, which declined by 15 weeks post tamoxifen administration. Furthermore, at 5 weeks there was significant extracellular matrix (ECM) remodeling and stromal promotion of angiogenesis (e.g. Vegfa, and Tgfb). This transcriptional reprogramming and metabolic rewiring may facilitate the observed progressive murine LUAD malignancy that occurs overtime. These findings provide key insights into the interchange between AT1 cell derived LUAD tumors and their TIME revealing potential metabolic vulnerabilities and targetable transcriptional events.

#5395 CDK12 regulates cellular metabolism to promote therapy resistance in glioblastoma.

Jeong-Yeon Mun[1], Chang Shu[1], Qiuqiang Gao[1], Hasan O. Akman[2], Mike-Andrew Westhoff[3], Georg Karpel-Massler[4], Markus D. Siegelin[1]

[1]Department of Pathology and Cell Biology, Columbia University Irving Medical Center, New York, NY,[2]Department of Neurology, Columbia University Irving Medical Center, New York, NY,[3]Department of Pediatrics and Adolescent Medicine, Ulm University Medical Center, Ulm, Germany,[4]Department of Neurosurgery, Ulm University Medical Center, Ulm, Germany

Glioblastoma IDH-wildtype, the most common primary brain cancer in adults, has an exceedingly poor prognosis of only one to two years despite standard treatment involving surgery, temozolomide, and radiation. We evaluated patient-derived xenograft GBM cultures to determine their sensitivity to disrupting the function of CDK12, which was achieved through genetic manipulation, shRNA, or the novel inhibitor SR-4835. GBM metabolism was assessed using extracellular flux analysis and LC/MS-based carbon tracing. The in vivo efficacy was evaluated in orthotopic GBM mouse models. Comprehensive screening approaches identified CDK12 as essential for GBM growth. Consistently, inhibiting CDK12 function led to reduced growth in GBM PDX lines in vitro, but not in human astrocytes. Notably, SR-4835 inhibited GBM growth at low nanomolar concentrations and synergistically enhanced the potency of temozolomide. In vivo, SR-4835 activated cell death machinery related to intrinsic apoptosis. Metabolic analyses demonstrated that the loss of CDK12 function substantially impaired cellular respiration, coupled with the suppression of key metabolic transcriptional regulators, PGC1A and PPARD. This resulted in energy depletion and apoptotic cell death, partially mediated by the pro-apoptotic protein Noxa. The inactivation of CDK12 significantly extended the overall survival time without any harmful side effects in orthotopic mouse models of GBM. In some subjects, it also led to the complete elimination of brain tumors. When used together with temozolomide, blocking CDK12 eradicated brain tumors in all animals studied, indicating that CDK12 is a highly promising treatment target for GBM and providing a solid foundation for future clinical trials involving patients with this devastating disease.

#5396 NDUFA4L2 inhibits oxidative phosphorylation to drive primary tumor escape.

Emily Nicole Arner[1], Erin Q. Jennings[1], Daniel Crooks[2], Christopher J. Ricketts[2], Matthew A. Cottam[1], Kimryn Rathmell[2], Jeffrey C. Rathmell[1]

[1]Vanderbilt University Medical Center, Nashville, TN,[2]National Cancer Institute, Bethesda, MD

Metastasis causes most cancer deaths and cancer cells require metabolic adaptation to spread to new tissues. However, the metabolic requirements of epithelial to mesenchymal transition (EMT) for primary tumor escape are uncertain. Analyses of Renal Cell Carcinoma (RCC) tumors found mesenchymal cancer cells had elevated expression of the mitochondrial electron transport inhibitor NDUFA4L2 and were glycolytic. NDUFA4L2 suppressed oxidative phosphorylation and was essential for EMT and spontaneous metastasis in RCC models. Mechanistically, NDUFA4L2 caused a build-up of TCA cycle intermediates and modified chromatin accessibility of EMT-related loci. This appeared due to altered mitochondrial oxidative phosphorylation, as increased electron transport complex 1 inhibited EMT and Fumarate Hydratase deficiency drove metastasis. Impaired oxidative phosphorylation thus drives EMT, which is essential for primary tumor escape and metastasis.

#5397 Role of pyruvate carboxylase in cancer-associated fibroblasts under tumor nutrient stress.

Michał Nizio[1], Marta Storl-Desmond[1], Kevin Munoz Forti[1], Julien Guillard[1], Shan Cao[1], Kristof Turan[1], Alexander Muir[2], Simon Schworer[1]

[1]Section of Hematology and Oncology, Department of Medicine, The University of Chicago, Chicago, IL,[2]Ben May Department of Cancer Research, The University of Chicago, Chicago, IL

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive and hard-to-treat cancer types. PDAC is characterized by desmoplastic stroma enriched with extracellular matrix (ECM), immune cells and cancer-associated fibroblasts (CAFs). CAFs modulate PDAC progression by producing ECM and signaling molecules that influence cancer and immune cell function. Previous efforts to target CAFs' role in PDAC were unsuccessful but have instead revealed substantial heterogeneity within the CAF population. Two main subtypes of CAFs are tumor-restraining myofibroblastic CAFs (myCAFs) and tumor-promoting inflammatory CAFs (iCAFs). To effectively target CAFs in PDAC, there is a need to find CAF-state-specific vulnerabilities and mechanisms regulating CAF state. We discovered that myCAFs are excluded from hypoxic and nutrient-stressed regions of PDAC, and iCAFs are selectively enriched in those regions. Pyruvate carboxylase (PC) is an enzyme that allows cells to utilize pyruvate as an anaplerotic substrate. Our previous work showed that PC is important for CAFs under nutrient-stressed conditions. Here, we hypothesize that PC is required to promote the iCAF state and support their growth in the nutrient-stressed environments they occupy. To address this hypothesis, we acclimated pancreatic stellate cells (PSCs), CAF precursors in PDAC, to physiological media resembling the concentration of nutrients in human plasma (HPLM - human plasma-like medium) or PDAC (TIFM - tumor interstitial fluid medium). We used CRISPR/Cas9 to delete PC, allowing us to investigate the role of PC in PSCs under physiological conditions. We observed that PC expression is higher in cells cultured in TIFM than in HPLM or conventional RPMI. We confirmed higher PC activity in TIFM compared to HPLM or RPMI cultured cells using stable isotope tracing. These findings indicate that PC expression and activity are regulated by the nutrient conditions present in PDAC and suggest that PC might be selectively important in CAFs under tumor nutrient stress. A better understanding of PC's role in CAFs residing in nutrient-stressed niches in PDAC may lead to the discovery of a novel way of targeting tumor-promoting iCAFs.

#5398 Inhibition of PHGDH suppresses mTOR signaling and metabolic reprogramming in TSC2-deficient lymphangioleiomyomatosis cells.

E. K. Abor, E. Y. Zhang, J. J. Yu;
University of Cincinnati, Cincinnati, OH

Lymphangioleiomyomatosis (LAM) is a rare, progressive neoplastic disease primarily affecting women, characterized by TSC1/2 mutations' driven mTORC1 hyperactivation and the proliferation of abnormal smooth muscle-like cells within the lungs and lymphatics. Despite rapalogs ability to stabilize lung function and limit LAM tumorigenesis, they are cytostatic and tumors regrow post treatment cessation. As such, with mTORC1 being nutrient sensitive, it is of note that understanding and targeting metabolic vulnerabilities in the disease could induce LAM tumor cell death and limit associated lesions. Emerging evidence suggests that serine and the serine biosynthesis enzyme, PHGDH (phosphoglycerate dehydrogenase), may support mTOR-driven anabolic processes in LAM. This study investigated the effects of PHGDH inhibition on mTOR signaling, cell survival, mitochondrial function, and metabolic reprogramming in TSC2-deficient LAM cells. PHGDH expression was evaluated in human LAM lung tissue and nodules using immunohistochemical staining. In HPLM media, TSC2-deficient LAM (621-101) and their control TSC2-add back (621-103) cells were treated with a selective PHGDH inhibitor. mTOR, S6, and their phosphorylated levels were measured via Western blot, and autophagic flux was monitored by immunofluorescence staining targeting LC3-II expression. Mitochondrial membrane potential and biogenesis were assessed using TMRE staining and Western blot targeting PGC-1a and TFAM. Additionally, the effect of PHGDH on LAM cell growth and in metabolic reprogramming in LAM cells was analyzed respectively using CCK-8 and by examining the expression of key Warburg effect and anabolic synthesis markers such as G6PD and PKM2. PHGDH was highly expressed in LAM lung tissues and nodules, implicating its role in LAM progression. PHGDH inhibition in TSC2-deficient LAM cells significantly reduced mTOR, ATF4 and S6 ribosomal protein phosphorylation, suppressed mitochondrial membrane potential, and decreased mitochondrial biogenesis by inhibiting PGC-1a and TFAM protein levels. Furthermore, PHGDH inhibition induced RB1 phosphorylation with a reduction in E2F1 alongside total RB1 protein expression inhibition while, with rapamycin, upregulated autophagy, as indicated by increased LC3-II expression in autophagic flux quantification. This study, thus, discovers a significant LAM cell growth and Warburg effect inhibition by a downregulated G6PD and PKM2 protein expression post PHGDH inhibition. These results suggest that PHGDH supports mTOR-driven metabolic reprogramming in LAM. Targeting PHGDH significantly reduces mTOR signaling, mitochondrial function, and impairs glycolysis and PKM2 mediated Warburg effect in LAM cells. PHGDH inhibition may offer a novel therapeutic strategy to enhance mTORC1 inhibitors' efficacy and overcome resistance in LAM management.

#5399 Placental gene PEG10 promotes onco-fetal metabolic reprogramming in hepatocellular carcinoma.

Dong Yin, Xinyi Yao, Jingyuan Zhang, Yin Zhang, Jianyou Liao, Kaishun Hu, Jiehua He, Daning Lu

Sun Yat-sen University, Guangzhou, China

Tumorigenesis is a complex biological process, accompanied by cellular dedifferentiation and metabolic reprogramming, which similarities to the metabolic characteristics of embryonic development stages. In our research, we focused particularly on the RNA-binding protein PEG10, which is highly expressed in the placenta and found to be similarly overexpressed in liver cancer cells, playing a key role in the process of aerobic glycolysis in tumor cells. This discovery suggests that there may be a phenomenon of fetal-like metabolic reprogramming in hepatocellular carcinoma (HCC), which is of significant importance for understanding the pathogenesis of HCC and for seeking new therapeutic strategies. In hepatocellular carcinoma (HCC) cells, we found that PEG10 enhances the stability of mRNA for key glycolytic genes hexokinase2 (HK2) and Glucose transporter 1 (GLUT1) by inhibiting the STAU1-mediated RNA degradation pathway, thereby promoting aerobic glycolysis in tumor cells. This process not only promotes the proliferation of tumor cells but also reduces the sensitivity of tumor cells to sorafenib. Furthermore, we observed in clinical samples that high expression of PEG10 is closely associated with poor prognosis in HCC patients, further confirming the important role of PEG10 in the development of HCC. Our research also found that retinoic acid can effectively inhibit aerobic glycolysis in tumor cells by suppressing the transcriptional expression of PEG10, thereby inhibiting the growth of tumor cells. In animal models, mice with liver-specific PEG10 gene knockout showed significant resistance to oncogene-induced liver cancer occurrence and development, providing strong evidence for PEG10 as a therapeutic target. These findings not only reveal the biological link between PEG10 and onco-fetal metabolic reprogramming at the molecular level but also indicate from a clinical perspective that PEG10 may be a potential biomarker and therapeutic target for HCC. The high expression of PEG10 is associated with poor prognosis in HCC patients, and the inhibitory effect of retinoic acid on PEG10 expression provides a new strategy for the treatment of HCC. These results provide new ideas for the future diagnosis and treatment of HCC, especially in the development of targeted therapeutic drugs against PEG10, which has important application prospects. Our research has laid a solid foundation for a deeper understanding of the metabolic reprogramming mechanisms of HCC and the development of new therapeutic methods.

#5400 SEPHS2 loss reprograms glucose metabolism to activate the pentose phosphate pathway by stabilizing PCK1 through NAD(+).

Y. Zhang, Q. Zhang, W. Zhang, W. Ding, B. Zhang, S. Xia;
Southern University of Science and Technology (SUSTech Shenzhen), Shenzhen, China

Selenium is an essential trace mineral that plays a crucial role in metabolism, primarily through its incorporation into selenoproteins. However, whether and how selenium modulates glucose metabolism in cancer cells remains unclear. This study focuses on SEPHS2, a key enzyme in synthesizing selenoproteins, which converts hydrogen selenide into selenophosphate. We found that *SEPHS2* knockout inhibits both glycolysis and oxidative phosphorylation (OXPHOS), as indicated by an seahorse assay. Interestingly, *SEPHS2* loss redirects more glucose to the pentose phosphate pathway. Mechanistically, *SEPHS2* knockout elevates NAD+ levels, which are essential for SIRT2/3 enzymatic activity. The increase in the activity of SIRT2/3 leads to deacetylation of PCK1, a posttranslational modification required to prevent PCK1's ubiquitination and subsequent degradation. Thus, *SEPHS2* knockout stabilizes PCK1 by reducing its ubiquitination. Since PCK1 is a key regulator of gluconeogenesis, *SEPHS2* knockout promotes gluconeogenesis while reducing cellular glucose uptake, suppressing glycolysis, down-regulating oxidative phosphorylation and enhancing the pentose phosphate pathway. Collectively, our findings define the selenium-metabolizing enzyme SEPHS2 as a key regulator of glucose metabolism, guiding cancer cells in choosing between glycolysis and the pentose phosphate pathway. This study offers valuable insight into the essential role of selenium metabolism in maintaining glucose homeostasis.

#5401 Revealing the influence of reprogrammed PI3K and glucose metabolism on the progression of hormone-refractory and metastatic prostate cancer.

Prathiksha Prabhakaraalva, Nabila Zaman, Sarah Ann King, Goutam Chakraborty

Icahn School of Medicine at Mount Sinai, New York, NY

Background: Prostate cancer, despite advancements in early detection and therapies, remains an incurable disease when it transform to metastatic and castration-resistant. A significant knowledge gap exists in understanding the mechanistic insights that drives hormone-refractory progression and metastasis. The PI3-Kinase pathway, altered in over >50 % of metastatic prostate cancer cases, plays a pivotal role in oncogenesis and therapy resistant. Although PTEN deletions are well-characterized, the specific contributions of other PI3K pathway components, particularly PIK3R1 mutations, remain underexplored. Our previous study reported that PIK3R1 mutations are highly enriched in metastatic castration-resistant prostate cancer (mCRPC) and are associated with increased glucose uptake detected by FDG-PET scans. Therefore, these mutations are crucial for further investigation.
Method: We generated 22 patients derived naturally occurring prostate cancer specific PIK3R1 mutations by site directed mutagenesis and verified their expression by western blotting. Using CRISPR and shRNA we have also established double PIK3R1-PTEN null prostate cancer cells. Prostasphere experiments was performed to analyzed the impact of these double loss on prostate cell growth in vitro Isogenic pairs of these double-knockout prostate cancer cells were treated with increasing concentrations of insulin in the under serum starvation to study the impact of PIK3R1-PTEN loss on reprogramming of insulin-glucose metabolism and oncogenic signaling pathways associated with this reprogramming
Results: Our results reveal that PIK3R1 mutations exhibit distinct, mutation-specific effects on PI3K-AKT signaling, where some variants induce AKT phosphorylation and destabilize PTEN, while others inhibit AKT and stabilize PTEN, underscoring diverse roles of specific PIK3r1 mutation within the PI3K pathway. Interestingly, PIK3R1-KO cells displayed heightened androgen receptor (AR) pathway activity and enhanced tumor sphere formation, suggesting increased tumorigenic potential. Furthermore elevated levels phosphorylated mTOR in PIK3R1 knockout clones indicate potential mechanisms of oncogenesis, which may further drive metastatic progression. Our primary data also demonstrated that insulin treatment in PIK3R1-PTEN null cells alters androgen receptor (AR) function, indicating a direct association between PIK3R1-PTEN-glucose metabolisms and AR-signaling that is crucial for prostate tumor growth.
Conclusion: Our findings emphasize the critical role of PIK3R1 in both AR and PI3K-AKT-mTOR signaling cascades, offering insights into the complex interplay of PIK3R1 mutations in therapy-resistant prostate cancer. This research underscores the need to explore mutation-specific PIK3R1 effects as we move toward more personalized therapies for lethal mCRPC.

#5402 Metabolic shift towards the *de novo* serine pathway in non-transformed breast cells drives epigenetic plasticity, oxidative DNA damage, and pro-tumorigenic cChanges associated with aging.

Mariana Bustamante Eduardo[1], Gannon Cottone[1], Curtis McCloskey[2], Flavio Palma[1], Shiyu Liu[3], Maria Paula Zappia[4], Abul B.M.M.K. Islam[4], Elizaveta Benevolenskaya[4], Maxim Frolov[4], Jason Locasale[3], Marcelo Bonini[1], Rama Khokha[2], Navdeep Chandel[1], Seema A. Khan[1], Susan E. Clare[1]

[1]Northwestern Univ. Feinberg School of Medicine, Chicago, IL,[2]Princess Margaret Cancer Centre, Toronto, ON, Canada,[3]Duke University, Durham, NC,[4]University of Illinois at Chicago, Chicago, IL

Introduction: A lipid metabolism gene signature is enriched in breast tissue at risk for estrogen receptor negative (ERneg) breast cancer (BC). Fatty acid (FA) exposure alters histone methylation, gene expression and increases metabolic flux through serine, one-carbon, glycine (SOG) and methionine pathways. We hypothesize that FA exposure induces a metabolic shift towards the SOG, increasing S-adenosylmethionine (SAM), altering histone methylation, gene expression, and promoting ERneg BC.
Methods: Proteomics, metabolomics, Reactive Oxygen Species (ROS) measurement, comet assay and H3K4me3 CUT&RUN were performed in MCF-10A cells exposed to octanoic acid (OA). Single-cell RNA-seq (scRNAseq) was performed in breast tissue derived microstructures exposed to OA. Intracellular communication was analyzed using CellChat, and metabolic flux with Compass.
Results: OA increased SAM, glutathione (GSH) and 2-hydroxyglutarate (2-HG); blocking the serine pathway (SSP) prevented these increases. ScRNAseq revealed that OA increased expression of the SSP transcription factor ATF3 and genes PHGDH and PSAT1 in epithelial and stromal compartments. Metabolic flux analysis revealed a significant increase in flux through SSP in Basal BSL1, Luminal Progenitor LP3, and Hormone Sensing HS1 cells after OA exposure. Differential proteomics reveals PHGDH overexpression and downregulation of proteins involved in extracellular matrix (ECM)-receptor interaction and focal adhesion post-OA exposure, along with significant increase in mitochondrial and nuclear ROS ($p < 0.01$). OA exposure also induced DNA damage, likely due to elevated nuclear ROS. OA increased GSH metabolism and ROS detoxification in BSL1. CUT&RUN identified 661 peaks significantly enriched upon OA (FDR < 0.01) in regulatory regions of OA-induced genes involved in neural pathways and BC, including MDK, NGF, and NGFR. CellChat predicted a decrease in ECM-cell interactions, a reduction in cell-cell adhesions, and an increase of secreted signaling upon OA exposure. The strongest secreted signals in OA were AREG (linked to proliferation, growth, and invasiveness), GDF15 (involved in EMT, invasion, and aging), and MDK (linked to neurogenesis, and aging).
Conclusions: We demonstrate an FA-induced shift towards the SOG and methionine pathways that promotes epigenetic plasticity, regulates ROS, and supports the survival of cells with 'inappropriate' phenotypes. These accumulate DNA damage, leading to age-related changes in the mammary gland (elevated ROS, disrupted junctions, altered ECM interactions, and increased MDK/GDF15 expression), all supporting carcinogenesis. Our findings also provide a metabolic explanation for the elevation of PHGDH in 70% of ERneg BCs, despite gene amplification in only 6%, and point to preventive strategies targeting the SSP.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: S/G2/M phase
Poster Session

#5406 Evaluation of antitumor effects of plogosertib, PLK1 inhibitor in biliary tract cancer with BUBR1 as a potential biomarker.

Yoojin Jeong[1], Ah-Rong Nam[1], Kyoung-Seok Oh[1], Jae-Min Kim[1], Ju-Hee Bang[1], Sea Young Choo[1], Hyo Jung Kim[1], Jeesun Yoon[2], Tae-Yong Kim[2], Do-Youn Oh[2]

[1]Seoul National University Cancer Research Institute, Seoul, Korea, Republic of,[2]Seoul National University Hospital, Seoul, Korea, Republic of

Background: Polo-like kinase 1 (PLK1) has been emerging as a promising therapeutic target in solid tumors due to its central role in cell cycle regulation and cancer cell proliferation. In biliary tract cancer (BTC), cell cycle-related factors such as PLK1, CDKs, and Aurora kinase are frequently overexpressed or aberrantly activated, driving tumor progression. However, studies targeting these proteins in BTC remain limited. BUBR1, a critical mitotic checkpoint protein, is essential for maintaining chromosomal stability by ensuring proper chromosome alignment and segregation during cell division. This study evaluated the antitumor effects of a PLK1 inhibitor plogosertib in BTC and identified BUBR1 as a potential biomarker to predict treatment response.
Methods: Total 9 BTC cell lines (SNU-245, SNU-308, SNU-478, SNU-869, SNU-1196, HuCCT-1, TFK-1, SNU-2670, SNU-2773) were used. We used plogosertib (PLK1 inhibitor), ceralasertib (ATR inhibitor) and panobinostat (HDAC2/3 inhibitor). The anticancer effects of plogosertib, both alone and in combination with ceralasertib and panobinostat, were evaluated through MTT assay, Colony formation assay, Annexin-V assay, and Cell cycle analysis. The effect of plogosertib on mitotic arrest was assessed using phospho-histone H3 assay and Immunofluorescence.
Results: Plogosertib monotherapy showed higher anticancer effects in BUBR1-high BTC cells (SNU2773, SNU478) compared to BUBR1-low cells (SNU869, SNU245). Plogosertib promoted mitotic checkpoint complex (MCC) formation in prometaphase, arresting mitosis and ultimately leading BTC cells to undergo apoptosis. However, plogosertib also induced ATR activation, which may limit its efficacy by initiating DNA repair pathways. ATR inhibition by ceralasertib further enhanced the anticancer effects of plogosertib in BUBR1-high cells. In BUBR1-low cells, plogosertib reduced BUBR1 protein expression, possibly through ubiquitination-proteasome-mediated degradation, limiting MCC formation and its therapeutic effects. Among several candidates regulating ubiquitination processes, HDAC2/3 inhibition by panobinostat enhanced the responsiveness of BUBR1-low cells to plogosertib.
Conclusion: BTC cells with high BUBR1 expression are sensitive to the PLK1 inhibitor plogosertib, which shows synergistic effects when combined with an ATR inhibitor. In contrast, BTC cells with low BUBR1 expression which are relatively insensitive to plogosertib, HDAC2/3 inhibition increases the sensitivity to plogosertib. These findings suggest that targeting PLK1 could be an effective strategy for BTC treatment, especially with BUBR1 expression as a potential biomarker to inform optimal combination therapies.

#5407 Identifying synthetic lethality targets in DDX41-mutated MDS/AML.

Sohaumn Mondal, Shizuo Yang, Vincent Maranda, Frederick Vizeacoumar, Franco Vizeacoumar, Yuliang Wu

Department of Biochemistry, Microbiology and Immunology, University of Saskatchewan, Saskatoon, SK, Canada

DDX41 (DEAD-box helicase 41) is a member of the DEAD-box helicase family, critical for RNA metabolism and genome stability. Mutations in *DDX41* are strongly associated with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), particularly in patients carrying inherited mutations or somatic alterations. Among these, the R525H missense mutation in *DDX41* is found in over 70% of affected individuals, impairing helicase activity and contributing to disease pathology. Directly targeting mutant DDX41 (e.g., R525H) with inhibitors remains challenging due to the structural similarity of mutant and wild-type (WT) proteins, as well as the conserved helicase domains shared by the 37 DEAD-box helicases in humans. To overcome these challenges, we are leveraging synthetic lethality (SL) as an innovative therapeutic strategy. Bioinformatic analyses identified potential SL partners of *DDX41*, including *WRN* and *DDX23*. Functional validation experiments revealed that inhibiting WRN helicase selectively impairs the growth of *DDX41*-deficient cells. In *DDX41*-knockout HeLa cells, treatment with WRN inhibitors NSC 617145 and HRO761 significantly reduced cell growth compared to WT controls. Similarly, in *DDX41*-knockdown U2OS cells, WRN inhibitors markedly inhibited cell proliferation compared to WT cells. Future studies aim to elucidate the molecular mechanisms underlying SL interactions between *DDX41* and its partners, providing insights into the therapeutic potential of targeting these vulnerabilities. This work offers a promising avenue for developing novel treatments for MDS/AML patients harboring *DDX41* mutations, ultimately improving clinical outcomes.

#5408 Unraveling c-MYC role in DNA replication origin licensing through non-canonical pathways.

D. Fleifel[1], E. Elkoumy[1], A. Adavikolanu[1], S. Qayyum[2], J. Cook[1];
[1]UNC Chapel Hill, Chapel Hill, NC, [2]Duke University, Durham, NC

Origin licensing is essential for DNA replication, confined to the G1 phase of the cell cycle, where human cells must license thousands of origins to ensure complete genome duplication. Insufficient licensing, or "underlicensing", leads to genome instability and increased sensitivity to chemotherapeutic agents. Altered G1 duration and dysregulated origin licensing are characteristic features of many cancers, potentially contributing to their aberrant cell proliferation. However, how origin licensing rates are controlled in cells with varying G1 lengths is poorly understood. Our research reveals that while cyclin E1 overproduction shortens G1 and causes severe underlicensing, c-MYC overproduction also shortens G1 but uniquely accelerates licensing, preventing underlicensing. *We identified a separation of function c-MYC mutant that fails to induce rapid licensing despite upregulating mRNA levels of licensing factors akin to wild-type c-MYC, indicating the involvement of non-canonical pathways beyond traditional transcriptional mechanisms*. c-MYC influence on global chromatin structure through hyperacetylation is pivotal. Our data show that c-MYC's role in licensing acceleration depends on the recruitment of TRRAP—a transcription cofactor involved in chromatin modification—and the histone acetyltransferase GCN5. Notably, euchromatin is preferred for origin licensing in early G1. We hypothesize that heterochromatin may be rapidly licensed in c-MYC overproducing cells to compensate for potential underlicensing. We are currently profiling early versus late G1 cells overproducing c-MYC to determine the genomic locations of the licensed origins. Unraveling c-MYC downstream pathways regulating origin licensing could revolutionize therapeutic strategies by precisely controlling proliferation in MYC-driven cancers, increasing their sensitivity to DNA-damaging treatments, and paving the way for targeting non-canonical pathways in cancer therapy.

#5409 Asymmetric interdependency among the CDK8 module subunits.

Tzu-Hao Liu[1], Xiao Li[2], Fu-Ning Hsu[3], Mengmeng Liu[1], Rajitha-Udakara-Sampath Hemba-Waduge[1], Jun-Yuan Ji[1]

[1]Biochemistry and Molecular Biology, Tulane University School of Medicine, New Orleans, LA,[2]Lewis-Sigler Institute, Princeton University, Princeton, NJ,[3]Bionova Scientific Inc, Fremont, CA

The CDK8 kinase module (CKM) of the transcriptional cofactor Mediator complex plays a pivotal role in regulating RNA polymerase II-dependent transcription. Dysregulation of the CKM has been implicated in various diseases, as CDK8 overexpression promotes tumorigenesis in colorectal cancer and melanoma, CKM subunits are mutated or amplified in other types of cancers and cardiovascular diseases. Understanding the *in vivo* functions and regulatory mechanisms of the CKM and Mediator complexes is essential for uncovering their contributions to tumorigenesis. Previous studies showed that the loss of CKM subunits in *Drosophila* resulted in distinct phenotypes, particularly in the eyes and wings. In this study, we observed that loss of Med13 and Med12 caused more significant reductions in multiple biological functions compared to the effects observed with CDK8 and CycC. Depleting individual CKM subunits revealed that Med13 has the strongest influence on cell growth and cell proliferation, likely due to differences in the rate of nascent protein synthesis. This hierarchy is followed by Med12 and then the CDK8-CycC complex, suggesting a differential impact of these CKM subunits at the cellular level. Immunoblot analyses further revealed that the stability of CDK8 and Med12 depends on the presence of Med13 in *Drosophila*. These findings support the hypothesis that CKM subunits are asymmetrically interdependent. Furthermore, knockdown of CKM subunits via lentiviral transduction in human LnCaP cells confirmed this asymmetric interdependency in mammalian systems. Taken together, our results suggest the stability and asymmetrical dependence of CKM subunits play a critical role in regulating nascent protein synthesis, cellular growth, and proliferation, with Med13 serving a critical role within the CKM module.

#5410 CDK9 pharmacological inhibition has antitumor activity in marginal zone lymphoma models and can improve the effects of BTK, PI3K, and BCL2 inhibitors.

Alberto J. Arribas[1], Eleonora Cannas[1], Davide Rossi[1], Anastasios Stathis[2], Diane Heiser[1], Francesco Bertoni[1]

[1]IOR - Institute of Oncology Research, Bellinzona, Switzerland,[2]Oncology Institute of Southern Switzerland, Ente Ospedaliero Cantonale, Bellinzona, Switzerland

Background: CDK9 inhibition rapidly down-regulates oncogenes, such as MYC, MCL1, PIM and PI3Ks. Indeed, CDK9 inhibitors, either as a single agent or in combination, can decrease proliferation and induce apoptosis in preclinical models of B cell lymphomas and show promising early signs of activity in B cell lymphoma patients (Diamond et al, CCR 2022; Frigault et al, Can Res Com 2023; Jiang et al, Biomark Res 2024). PRT2527 is a highly selective, ATP-competitive, CDK9 inhibitor with preclinical activity in diffuse large B cell lymphoma, mantle cell lymphoma, and chronic lymphocytic leukemia (Fultang et al, AACR 2024). PRT2527 has shown favorable tolerability, with manageable toxicity in a phase 1 study for solid tumor patients (Patel et al, ENA 2023), and it is now in phase 1 as a single agent and in combination with the BTK inhibitor zanubrutinib for hematological cancers patients (NCT05665530). Here, we studied PRT2527 in models derived from marginal zone lymphoma (MZL), including derivatives with acquired resistance to PI3K, BTK, and BCL2 inhibitors.
Methods: MZL cell lines (VL51, Karpas1718, SSK41, ESKOL, HC1, HAIRM) and their derivatives with secondary resistance to targeted agents obtained by long drug exposure (n.=3 from VL51, n.=1 from Karpas1718, n.=1 from SSK41; Arribas et al, Haematologica 2022, Mol Cancer Ther 2024, ENA 2019, ENA 2020, AACR 2024) were exposed to increasing concentrations of PRT2527 or DMSO (control) for 4 h and then washed out. Anti-proliferative activity was then assessed by MTT assay after 48 h.
Results: Despite the limited exposure time, CDK9 inhibitor PRT2527 exhibited antiproliferative activity in the nM range (average IC50 724nM; 95%CI, 467-981) across the MZL models. The anti-tumor activity was maintained in MZL cell lines with acquired resistance to PI3K, BTK, and BCL2 inhibitors. The combination of PRT2527 with the PI3K inhibitor idelalisib, with the BTK inhibitor ibrutinib, or triple combination with the PI3K inhibitor copanlisib and the BCL2 inhibitor venetoclax was beneficial in all parental cells, and the benefit was maintained, although reduced, in derivatives with acquired resistance to PI3K and BTK inhibitors. Only the VL51 model with resistance to copanlisib/venetoclax benefited from the addition of PRT2527 in the VL51 model, while no advantage was observed with the triple combination in the SSK41 derivative.
Conclusions: CDK9 inhibition with PRT2527 has in vitro antitumor activity in MZL models, which is maintained in models with acquired resistance to PI3K, BTK, or BCL2 inhibitors. CDK9 inhibition with PRT2527 can improve the sensitivity of MZL cells to BTK, PI3K, and BCL2 inhibitors.

#5411 The IL-23 receptor localizes intracellularly in AML and stem cells where it regulates mitotic spindle formation to maintain cell viability.

Nathan Duong[1], Dilshad H. Khan[1], Geethu E. Thomas[1], Yue Feng[1], Rose Hurren[1], Jong Bok Lee[2], Jonathan St-Germain[1], Lily Drimmer[1], Yongran Yan[1], Lan Xin Zhang[1], Dakai Ling[1], Mary L. Ma[1], Neil MacLean[1], Marcela Gronda[1], Vincent Rondeau[1], Brandon D. Brown[3], Laura Matellan[4], Courtney L. Jones[1], Hong Chang[2], Andrea Arruda[1], Stephanie Xie[1], Laurence Pelletier[4], Mark D. Minden[1], Li Zhang[2], Steven M. Kornblau[3], Brian Raught[1], Kevin Jacobs[5], Max G. Jacobs[5], Daniel Goede[5], Vito Spadavecchio[5], Aaron D. Schimmer[1]

[1]UHN Princess Margaret Cancer Centre, Toronto, ON, Canada,[2]Toronto General Hospital Research Institute, Toronto, ON, Canada,[3]MD Anderson Cancer Centre, Houston, TX,[4]Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada,[5]Interlinked Therapeutics, Portland, OR

IL-23 receptor (IL-23R) is canonically a T cell surface cytokine receptor known for its role in inflammatory diseases. In this study, we demonstrated that IL-23R localizes intracellularly in AML where it regulates mitotic spindle formation. Differential gene expressions were analyzed in AML samples compared to normal controls. Of the upregulated processes, the mitotic spindle ontology was most enriched. Analyzing all protein coding genes for their correlation to the mitotic spindle ontology surprisingly returned IL-23R as a top hit. We confirmed IL-23R expression in 7 of 7 tested AML cell lines and 15 of 20 primary AML patient samples. Protein expression was >2 fold higher in AML compared to normal hematopoietic (n=5) and CD34+ sorted cells (n=3).Canonical IL-23R cell surface expression was confirmed in T cells, but minimal amounts were detected on AML cells. Rather, in AML cells, primary AML samples, and AML stem cells, IL-23R was detected intracellularly as determined by 4 different modalities using 4 different epitope targeting antibodies. IL-23R's heterodimeric subunit, IL-12Rβ1, was also detected intracellularly in AML cells. We performed BioID mass spectrometry to identify novel interactors with IL-23R. Pathway analysis of interactors returned mitotic spindle formation as a top pathway. We confirmed interactions between IL-23R and mitotic spindle proteins (NuMA, TMEM201, TACC1, BAG6) via proximity ligation assays in bulk and leukemic stem cell (LSC) sorted AML cells. Furthermore, in bulk AML and LSC sorted fractions, we demonstrated that IL-23R colocalized with the mitotic spindle and centrosomes via confocal microscopy. IL-23R interacted with the mitotic spindle via its (S/T)x(I/L)P motif (amino acids 588-591). IL-12Rβ1 was also found at the mitotic spindle with exogenous IL-23 in culture promoting intracellular translocation of IL-23R. Depletion of IL-23R led to dysregulation of the mitotic spindle with spindle defects such as multipolarity and lagging chromosomes. Additionally, in IL-23R depleted mitotic AML cells, NuMA has decreased localization to mitotic spindle poles thus attenuating its ability to regulate mitotic spindle assembly. Knockdown of IL-23R reduced proliferation and clonogenic growth, reduced marrow engraftment, and increased differentiation of AML cells, thus demonstrating an effect on the leukemia initiating cells. In normal hematopoietic cells, however, knockdown of IL-23R did not impair engraftment. Consistent with this, constitutive homozygote IL-23R knockout mice had no difference in complete blood counts and stem cell number/function compared to wild type mice. In summary, intracellular IL-23R regulates mitotic spindle formation to maintain AML and stem cell growth and viability. We demonstrated a novel function and intracellular localization for IL-23R and its potential as a therapeutic target in AML.

#5412 Developing combination therapies for telomerase-overexpressing cancers.

V. Maranda, F. S. Vizeacoumar, Y. Zhang, L. Kyrylenko, A. Freywald, F. J. Vizeacoumar;
University of Saskatchewan, Saskatoon, SK, Canada

Cancer heterogeneity poses a significant medical challenge, necessitating therapies tailored not only to individual patients but also to the unique cancer clones that exist within diverse microenvironmental contexts. Despite this diversity, nearly all cancer cells share a reliance on telomeres to maintain genomic stability. The telomerase (hTERT) gene is hyperactive in many cancers and has been identified as a potential target for treatment. While this has led to the development of multiple telomerase-targeting approaches, disappointingly, none have been approved in clinical applications yet. To circumvent this concern, our team has applied a genetic approach called synthetic dosage lethality (SDL), to exploit hTERT overexpression to identify potential targets to treat cancer. SDL is a genetic concept, where a normally non-lethal gene inactivation kills cells only in the context of overexpression of another gene like hTERT. Our laboratories have used lentiviral-based pooled CRISPR/Cas9 and pooled shRNA-screening platforms to systematically query the genome and recently identified several SDL partners of hTERT. Through extensive validation using pooled *in vivo* CRISPR/Cas9 screens across multiple cell lines, tumor xenografts, and patient-derived organoids, we identified RMT1, an RNA 2'-O-methyltransferase, as a prominent SDL partner of hTERT. In collaboration with the AtomWise and Thoth Biosimulations companies, we have developed novel inhibitors for RMT1 that preferentially suppress hTERT-overexpressing cell lines growth and viability. We have also performed a large-scale screen of FDA-approved small molecule inhibitors with the goal of repurposing these drugs to target hTERT-overexpressing cancers. Subsequently, we plan to apply these therapies in combination to amplify the efficiency of treatment against cancers, yielding preclinical evidence to support the development of novel cancer therapies. Since hTERT activity is essential in all cancer cells, our findings introduce a transformative approach to overcoming cancer cell heterogeneity by targeting the SDL interactions of hTERT. This strategy also capitalizes on the ubiquitous overexpression of hTERT in all cancer types, enabling the selective eradication of hTERT-dependent malignancies.

#5413 Temporal regulation of c-Myc in endocycling cancer cells facilitates mitotic bypass in response to chemotherapy.

Michael Loycano, Kenneth J. Pienta, Sarah R. Amend

Johns Hopkins University School of Medicine, Baltimore, MD

Chemotherapy resistance remains a major challenge in clinical oncology. The mechanisms that regulate dynamic movement through adaptive cell states to survive through chemotherapeutic stress need to be understood to advance treatment options for patients and move towards curative therapies. In response to various stressors, cancer cells can reprogram their cell cycle, uncoupling DNA replication from mitotic division to acquire a large polyploid phenotype. This alternative cell cycle of repeated G and S phases is known as an endocycle, observed across the tree of life during both development and stress response. Cells exhibiting this phenotype exist in the transient endocycling cancer cell state. Endocycling cancer cells (ECCs) may serve as an actuator of chemotherapy resistance and disease recurrence. Understanding the mechanisms regulating the mitotic-to-endocycle switch and back are crucial to enhance treatments for patients with chemotherapy resistant cancer. A major driver of endocycling described in the literature is the transcription factor, c-Myc, due to its role in governing the G1/S transition. C-Myc is dysregulated in >70% of cancers, functioning as a critical cell cycle driver through transcriptional regulation of cyclins and CDKs, as well as essential metabolic programs necessary for cycle progression. Despite the published roles of Myc in both cancer and endocycling, we have shown through single cell RNA sequencing and western blotting that ECCs exhibit a low-Myc phenotype across multiple contexts. Myc protein levels can be rescued using the proteasome inhibitor, MG132, demonstrating that the low Myc status is driven by proteasomal degradation. To test if Myc degradation serves a mechanistic function, the small molecule inhibitor, MYCi975, was used to inhibit Myc activity at various points in the mitotic cell cycle. Our data shows that loss of Myc activity in cells beyond the restriction point induces mitotic bypass, generating a 4N G1 population. Notably, cells actively replicating their DNA in S phase show high sensitivity to Myc inhibition after skipping to G1, displaying cytotoxic effects not seen in other phases of the cell cycle. Similarly, when ECCs are challenged with the Myc inhibitor, the treatment promotes mitotic bypass and enriches for G0 and G1 populations, primed to enter the next S phase upon release from Myc inhibition. Together, these data suggest a temporal aspect of Myc regulation that has not been described in the literature. Myc activity beyond the restriction point appears to commit cells to mitotic entry, playing a functional role in coupling DNA replication to mitosis. This data supports our hypothesis that the low-Myc status of endocycling cells plays a mechanistic role in permitting mitotic bypass through premature loss of activity in G2. Preliminary data suggests that rescuing Myc activity will force mitotic entry producing mitotic progeny cells.

#5414 The RNA-binding protein HNRNPH1 regulates the expression of cell cycle-related genes to promote glioblastoma progression.

Genaro Villa[1], Paolo Alimonti[1], Joseph Toker[2], Raziye Piranlioglu[1], Andrew Chiocca[1], Alexa Lauinger[3], E. Antonio Chiocca[1], **Marco Mineo**[1]

[1]Brigham and Women's Hospital, Boston, MA,[2]Harvard Medical School, Boston, MA,[3]Carle Illinois College of Medicine, Urbana, IL

Heterogeneous nuclear ribonucleoprotein H1 (HNRNPH1) is a critical regulator of several aspects of RNA metabolism, including RNA stability and splicing. Alteration of the expression of RNA-binding proteins, such as HNRNPH1, has been shown to affect the RNA life cycle. This, in turn, results in the production of abnormal proteins that may promote the development of cancer. While aberrant expression of HNRNPH1 has been linked to several neuronal-related disorders, its role in the progression of glioblastoma (GBM), the most frequent and aggressive primary brain tumor, is still poorly understood. Given our previous findings that HNRNPH1 binds the RNA of several genes involved in cell-cycle regulation and cell proliferation, we hypothesized that HNRNPH1 regulates cell division and glioblastoma growth. Analyses of GBM patient data from The Cancer Genome Atlas database, combined with tissue microarray immunostaining and qPCR analyses of patient-derived tumors and patient-derived glioblastoma cells (PDGCs), revealed that *HNRNPH1* was upregulated in GBM compared to normal brain. Single-cell RNAseq data showed that *HNRNPH1* was more expressed in malignant cells than in other cells within the tumor microenvironment, and its expression was associated with specific cellular states. Knocking out *HNRNPH1* in both U87 cells and PDGCs using the CRISPR/Cas9 system caused the downregulation of several genes involved in cell division, such as *AURKB*, *ESPL1*, *PRC1*, and *UBE2S*. This was associated with an increased number of cells stuck in G2M, reduced cell proliferation, abnormal cell morphology, and an increase in polynucleated cells. Silencing *HNRNPH1 in vivo* suppressed the tumor growth of PDGC-originated intracranial xenografts and had significant survival benefits. Together, our results showed the critical importance of HNRNPH1 in cell cycle progression and tumor growth, with a potential impact on developing novel strategies to overcome the current limitations of GBM therapies.

#5416 Cyclin K deficiency suppresses tumor growth, activates systemic immunity, and confers vulnerability to chemotherapy in pancreatic cancer.

Y. Xiao, Y. Chen, J. Dong;
University of Nebraska Medical Center, Omaha, NE

Pancreatic cancer is a highly lethal disease worldwide, therefore, it is imperative to identify critical targets to inform alternative therapeutics in this disease. Cyclins and cyclin-dependent kinases (CDKs) are key drivers in the hallmarks of cancer, and important targets in clinical trials and cancer treatment. Proven as a druggable target in recent years, however, the function of Cyclin K in pancreatic cancer is still unknown. This study characterizes the role of Cyclin K in tumorigenesis, systemic immunity, and GemTaxol (gemcitabine and paclitaxel) sensitivity in pancreatic cancer, aiming to provide a pre-clinical basis for Cyclin K-targeted therapies in this disease. Data from TCGA and GTEx indicated that Cyclin K expression was high in pancreatic tumors, and elevated expression of Cyclin K was associated with shorter survival. Consistent with this finding, transgenic mouse models suggested Cyclin K expression was induced in KPC ($Kras^{G12D}$; $Trp53^{flox/flox}$; Pdx1-Cre) tumors, whereas homozygous deletion of Cyclin K in KPC mice ($Kras^{G12D}$; $Trp53^{flox/flox}$; Pdx1-Cre; $Ccnk^{flox/flox}$) offered survival benefit. Next, Cyclin K expression was depleted in pancreatic ductal adenocarcinoma (PDAC) cells to investigate the gene function. Inducible knockdown of Cyclin K restricted proliferation and G1-S transition of PDAC cells, while enhanced expression of Cyclin K promoted cell proliferation and cell cycle progression. Cell division cycle 20 (CDC20) was identified by a cell cycle array to mediate Cyclin K-induced cell proliferation. The loss of Cyclin K also hampered PDAC tumor growth in athymic nude mice. Interestingly, in immunocompetent mice, Cyclin K depletion not only attenuated tumor growth, but also led to some levels of complete tumor regression. Cyclin K deficiency-triggered systemic immunity was further examined by rechallenging the tumor-cleared mice with control murine cancer cells. As a result, the tumor clearance rate is 100% in these immunized mice. In addition, RNA-Seq identified immune response and inflammatory response to be the top 10 significantly altered gene ontology terms in Cyclin K-knockdown cells. Importantly, genetic depletion of Cyclin K synergized with GemTaxol to restrain PDAC tumor growth. Furthermore, the combination of GemTaxol and Cyclin K depletion, either by genetic or pharmaceutical methods, markedly induced apoptosis in cells. Finally, two PROTAC degraders (7f and YJ9069) were shown to exert significant anti-tumor growth effect in immunocompetent mice. Together, this work highlights that Cyclin K is a master regulator of tumor growth, immune response, and chemoresistance in PDAC, and a promising target for the treatment of pancreatic cancer.

#5418 Targeting PKMYT1 to preferentially kill tumor cells that have CCNE1 amplifications.

Samantha S. Hodge, Natalie Hill, Kayla Walzer, Ritu Kushwaha, Yihong Guan, Jeremy Fournier, Ester Fernandez-Salas

Arcus Biosciences, Inc., Hayward, CA

BACKGROUND: Cyclin E1 (CCNE1) amplification occurs in endometrial (40%), ovarian (20%), stomach (11%) and breast (3%) cancers and is associated with poor clinical outcomes. CCNE1 amplification deregulates the G1/S checkpoint and causes premature entry into S phase. Consequently, CCNE1 amplified cells depend on the G2/M checkpoint to ensure genomic stability before mitosis. PKMYT1, a member of the WEE1 family of enzymes, phosphorylates and inhibits CDK1 to regulate the G2/M checkpoint and prevent entry into mitosis. Here, we show that PKMYT1 inhibition in CCNE1-high cancer cells results in premature entry into mitosis, catastrophic DNA damage, and cell death. PKMYT1 inhibition also synergizes with chemotherapy to increase CCNE1-high cancer cell killing. Importantly, non-tumorigenic cells are not dependent upon PKMYT1 and do not exhibit DNA damage in response to PKMYT1 inhibition.

METHODS: An isogenic pair of CCNE1-low and CCNE1-high expressing cell lines was generated using lentiviral transduction. PKMYT1 was inhibited using Compound A, a tool small molecule PKMYT1 inhibitor. Inhibition of PKMYT1 was measured by CDK1 pT14 via western blot and HTRF. Cell cycle was monitored by flow cytometry using EdU and DNA content stains. DNA damage was measured by western blot and flow cytometry for yH2AX. Viability was measured with AlamarBlue and Caspase3/7. Bliss Synergy scores were calculated and used to assess synergy after combination treatments.

RESULTS: CCNE1 overexpression increases entry into S phase and dependence on the G2/M checkpoint (CDK1 pT14 and pY15 levels) without changing basal levels of DNA damage. While PKMYT1 inhibition decreases CDK1 pT14 in both CCNE1-low and CCNE1-high cells, only CCNE1-high cells exhibit decreased S phase and increased SubG1 (apoptotic) and G2/M phases. CCNE1-amplified breast and ovarian cancer cells display similar inhibition of CDK1 pT14, reduction of S phase, and increased DNA damage and cell death as a consequence of PKMYT1 inhibition. Combination treatment of PKMYT1 inhibition and chemotherapy synergistically reduced cell viability in CCNE1-amplified cancer cells. Non-tumorigenic cells are not dependent upon PKMYT1 and do not exhibit changes in the cell cycle or DNA damage after PKMYT1 inhibition. In contrast, WEE1 inhibition is detrimental to non-tumorigenic cells and induces cell cycle dysregulation and DNA damage.

CONCLUSIONS: The data presented here provide a rationale for targeting PKMYT1 in cancer cells that are dependent on the G2/M checkpoint to maintain genomic stability, such as CCNE1-amplified tumors. PKMYT1 inhibition selectively kills CCNE1-amplified cancer cells while sparing non-tumorigenic cells that have intact G1/S checkpoints. Our results highlight the importance of developing selective PKMYT1 inhibitors to avoid toxicities related to WEE1 inhibition.

#5419 Cyclin E1 overexpression as a predictive biomarker for PLK1 and WEE1 inhibitor efficacy in ovarian cancer.

Q. Xi[1], A. Kunita[1], M. Ogawa[1], M. Ka[1], S. Nagai[1], A. Matsunaga[2], K. Watanabe[1], T. Fukuda[1], K. Sone[1], K. Kawana[2], K. Katayama[3], H. Kage[1], K. Oda[1];

[1]The University of Tokyo, Tokyo, Japan, [2]Nihon University School of Medicine, Tokyo, Japan, [3]Nihon University, Chiba, Japan

Ovarian cancer remains a lethal malignancy with limited treatment options, particularly for platinum-resistant cases. Key molecular alterations in ovarian cancer include Cyclin E1 overexpression and *TP53* mutations, both of which disrupt cell cycle regulation and contribute to poor prognosis. Cyclin E1 overexpression drives unchecked G1/S progression, promoting treatment resistance. *TP53* mutations, common in high-grade serous carcinoma, impair checkpoints and increase genomic instability. These features underscore the urgency for cell cycle-targeted therapies. PLK1 and WEE1 inhibitors have shown promise by disrupting cell cycle control, but predictive biomarkers remain unclear.

Twelve ovarian cancer cell lines (5 high-grade serous [HGSOC], 5 clear cell [CCOC], and 2 endometrioid [EOC] ovarian carcinomas) were treated with the PLK1 inhibitor volasertib and the WEE1 inhibitor adavosertib to evaluate drug sensitivity, cell cycle effects, and apoptosis *in vitro*, as well as tumor growth *in vivo*. Cyclin E1 expression was elevated in 5 cell lines (2 HGSOC, 2 CCOC, and 1 EOC) and *TP53* was mutated in 6 cell lines (5 HGSOC and 1 EOC) out of 12 cell lines by qPCR and western blotting. Drug sensitivity assays revealed a strong correlation between Cyclin E1 expression levels and sensitivity to both volasertib and adavosertib. Cell lines with high Cyclin E1 expression showed significantly lower IC50 values compared to those with low/normal expression (volasertib: 0.022-0.089 μM vs. 0.311-28.1 μM, $p<0.05$; adavosertib: 0.071-0.346 μM vs. 0.368-90.4 μM, $p<0.01$). Cyclin E1 knockdown reduced sensitivity to both inhibitors ($p<0.001$), supporting its role as a predictive biomarker, whereas *TP53* status did not affect drug response. Flow cytometry showed distinct cell cycle effects of the inhibitors. Volasertib induced M-phase arrest and subsequent cell death, as indicated by a significant increase in the sub-G1 population in high Cyclin E1-expressing cells ($p<0.001$). Similarly, adavosertib dose-dependently increased sub-G1 cells and depleted G1-phase cells in high Cyclin E1-expressing cells ($p<0.001$). These effects were absent in low Cyclin E1-expressing cells. Both inhibitors elevated apoptosis markers and the DNA damage marker, γH2AX, in high Cyclin E1-expressing cells but not in low-expression cells. *In vivo*, volasertib and adavosertib significantly suppressed tumor growth in xenograft models derived from high Cyclin E1-expressing cells ($p<0.001$), with no significant effects observed in low-expression tumors. Both inhibitors induced cleaved PARP and γH2AX in high Cyclin E1-expressing tumors but not in low-expression models. Cyclin E1 overexpression, regardless of *TP53* status, may serve as a predictive biomarker for the efficacy of these inhibitors, thereby offering potential personalized treatment strategies for ovarian cancer.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Therapeutic Targets and Mitochondrial Function
Poster Session

#5423 A focused shRNA library screening identifies MRPL38 as an oncogenic regulator of lung cancer via regulation of mitochondrial homeostasis.

Jieun Seo, So Young Lee, Sangwon Byun, Kyung Chan Park

Personal Genomic Medicine Research Center, Korea Research Institute of Bioscience & Biotechnology (KRIBB), Daejeon, Korea, Republic of

To identify a novel gene that contributes to lung cancer progression, we performed RNA interference genetic screening combined with multi-omics analysis of never-smoker lung cancer cohort (GSE256091). As a result of the screening and MAGeCK analysis, mitochondrial ribosomal protein L38 (MRPL38) was identified as a positive regulator of lung cancer progression. Mitochondrial dysfunction and metabolic reprogramming are key features of lung cancer. Despite their significance, the precise underlying mechanisms of these processes and the role of MRPL38 in lung cancer progression have not been fully elucidated. A significant upregulation of MRPL38 was observed in lung cancer patient tissues, which was associated with advanced tumor stages and poor prognosis. Knockdown of MRPL38 significantly reduced cell viability and colony formation and increased apoptosis in human lung cancer cells. Moreover, MRPL38 has been found to be crucial for maintaining mitochondrial protein translation and homeostasis. Loss-of-function experiments targeting MRPL38 in various lung cancer cell lines resulted in alterations in oxidative phosphorylation (OXPHOS) complex proteins, oxygen consumption rate (OCR), and mitochondrial biogenesis. In MRPL38-deficient cells, the expression levels of proteins encoding subunits of the respiratory chain complexes were significantly reduced, and both basal and maximal mitochondrial respiration were decreased. Furthermore, the inhibition of MRPL38 was found to significantly reduce the mass and number of mitochondria associated with mitochondrial biogenesis. These results suggest that MRPL38, a novel regulator in lung cancer, may promote lung cancer progression by regulating mitochondrial homeostasis.

#5424 Exploring the potential role of PGC-1β in ovarian cancer malignancy.

Laura Formenti, Francesca Abramo, Giulia Dellavedova, Raffaella Giavazzi, **Carmen Ghilardi**

Oncology, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milano, Italy

Mitochondrial metabolism plays a role in tumor development and metastasis. PGC-1β is recognized as a key regulator of mitochondrial biogenesis and metabolic activity. We have recently demonstrated that PGC-1β is a biomarker of addiction to mitochondrial metabolism in ovarian cancer and determines sensitivity to OXPHOS inhibitors. Its role in cancer is largely unexplored, but some reports suggest that PGC-1β exerts pro-tumorigenic actions. We aim at unraveling the influence of PGC-1β on ovarian cancer malignancy and how it regulates ovarian cancer cell functions, besides its metabolic role. To this purpose, murine ovarian cancer cells were silenced for PGC-1β and their behavior characterized in vitro and in vivo. First, we confirmed that PGC-1β silencing caused an alteration of oxidative metabolism by Seahorse analysis, showing the reduction of oxygen consumption. Then, we found that PGC-1β depletion did not affect ovarian cancer cell proliferation in standard culture conditions, but decreased proliferation compared to control cells in starvation conditions. Moreover, PGC-1β silenced cells showed reduced invasive potential, evidenced by decreased migration and invasion, associated to diminished collagenase activity. When injected in immunocompetent mice, PGC-1β silenced cells exhibited delayed tumor growth compared to control cells. In PGC-1β silenced tumor bearing mice ascites formation, a key indicator of disease progression, was reduced compared to controls, along with decreased metastatic dissemination in the peritoneal cavity. Our data suggest that the PGC-1β depletion reduces not only cancer cell ability to survive and proliferate under nutrient limited conditions, but also their invasive potential, leading to a delay in ovarian cancer progression in vivo. Further studies are needed to elucidate the underlying molecular pathways regulated by PGC-1β. (Supported by “HEAL ITALIA” project PE_00000019 to F.A., CUP: B43D22000710006)

#5425 Human mitochondrial ClpXP protease degrades serine phosphorylated substrates.

Y. Feng[1], M. M. Goncalves[2], Y. Jitkova[1], A. F. A. Keszei[1], Y. Yan[1], C. Sarathy[1], J. St-Germain[1], T. M. G. Kenney[1], M. Tcheng[1], V. Trudel[3], R. S. Mancini[4], R. Upadhyay[4], R. Hurren[1], M. Gronda[1], M. Schultz[1], K. Soriano[1], K. Lees[1], N. C. Pomroy[1], S. W. Currie[2], G. G. Prive[1], M. A. Reed[4], A. K. Yudin[3], L. Z. Penn[1], C. H. Arrowsmith[1], B. Raught[1], M. Mazhab-Jafari[1], S. Vahidi[2], A. D. Schimmer[1];
[1]UHN Princess Margaret Cancer Centre, Toronto, ON, Canada, [2]University of Guelph, Guelph, ON, Canada, [3]University of Toronto, Toronto, ON, Canada, [4]UHN Krembil Brain Institute, Toronto, ON, Canada

ClpXP is an AAA+ protease located in mitochondria matrix. In human, ClpXP degrades damaged respiratory chain proteins and is essential for acute myeloid leukemia (AML) cell survival. This complex consists of the tetradecameric ClpP protease and regulatory particles ClpX. In bacteria, ClpXP targets substrates with a co-translationally added SsrA sequence. However, human lacks this tag and the degron for ClpXP remain unidentified. Notably, bacterial ClpXP homologues degrade substrates with phosphorylated arginine (pArg). We hypothesized that phosphorylated amino acids also facilitate substrate degradation by ClpXP in human mitochondria.

Here, we demonstrate that ClpXP selectively degraded phosphorylated α-casein and tau proteins over dephosphorylated forms. Replacing ClpX with ONC201 or *E. coli* ClpA abolished phosphorylation selectivity, indicating ClpX imparts substrate specificity for phosphorylation. Next, we screened a panel of phosphorylated amino acids and peptides for their effect on ClpXP-mediated degradation of α-casein. Phosphorylated serine (pSer) amino acids and peptides inhibited ClpXP protease activity, while phosphorylated tyrosine (pTyr) or pArg did not. Free serine and phosphate also had no effect. Differential scanning fluorimetry showed that pSer stabilized ClpX, consistent with pSer binding to ClpX. We then used hydrogen/deuterium exchange mass spectrometry (HDX-MS) to reveal the ClpX conformational changes upon pSer containing peptides (Ala-pSer-Ala) binding. HDX analysis indicated the ClpX RKL loop as a putative binding site. Mutating this loop in ClpX ($ClpX_{AAL}$) disrupted its interaction with pSer, resulting in loss of specificity for phosphorylated substrates. This suggests ClpXP selectively recruits phosphorylated protein for degradation through its RKL loop.

In AML cells, ClpP knockdown impaired the respiratory chain and increased reactive oxygen species. Through mass spectrometry analysis of ClpXP interactome and their phosphorylation status upon ClpP inhibition, we identified potential ClpXP endogenous substrates SDHA and NDUFA4, components of the OXPHOS complex. Knockdown of ClpX or ClpP increased levels of pSer-SDHA and pSer-NDUFA4 without changing total protein levels. Recombinant ClpXP degraded pSer-SDHA in a dose-dependent manner, with degradation blocked by pSer peptides. ClpXP did not degrade total SDHA, consistent with its preference for phosphorylated substrates. Further analysis showed elevated pSer-SDHA levels only in the insoluble mitochondrial protein fraction after ClpX knockdown, suggesting these might be damaged proteins. In conclusion, ClpXP degrades damaged mitochondrial proteins with serine phosphorylation, maintaining proper mitochondrial function.

#5426 Vesicle-mediated mitochondrial clearance presents an actionable metabolic vulnerability in triple-negative breast cancer.
M. Zuo;
UT MD Anderson Cancer Center, Sugar Land, TX

Selective autophagy of mitochondria is known to promote survival and progression of cancer cells in various malignancies including triple-negative breast cancer (TNBC). Here, we aimed to identify the essential metabolic adaptations that support mitochondrial quality control with the goal to uncover actionable metabolic vulnerabilities with therapeutic potential. Using an integrated approach of proteomics and untargeted and stable-isotope resolved metabolomics, coupled with functional experimental analyses, we define an alternative mechanism to mitophagy enabled by an onco-metabolic program of heightened extracellular sphingomyelin salvaging in TNBC that facilitates extracellular vesicle (EV)-mediated intracellular clearance of mitochondrial damage. Targeting of the cancer cell sphingolipid onco-metabolic pathway via repurposing of eliglustat, a selective small molecule inhibitor of glucosylceramide synthase (UGCG), resulted in ceramide-mediated compensatory mitophagy and subsequent cancer cell death *in vitro* and attenuated tumor growth and prolonged overall survival at clinically achievable doses in an orthotopic syngeneic mouse models of TNBC as well as in human xenograft models. Our study defines a mechanism of aberrant sphingolipid metabolism that underlies an actionable metabolic vulnerability for anti-cancer treatment.

#5427 Targeting mitochondrial protein translation in chemo-refractory triple negative breast cancer.

Mariah J. Berner, Steven W. Wall, Mokryun L. Baek, Audra Lane, Allison S. Greer, Karen Wang, Lacey Dobrolecki, Bing Zhang, Jonathan T. Lei, Michael T. Lewis, Gloria V. Echeverria

Baylor College of Medicine, Houston, TX

Nearly 45% of patients with triple negative breast cancer (TNBC) treated with neoadjuvant chemotherapy have residual tumors that are correlated with worse overall survival. Residual TNBC patient-derived xenograft (PDX) tumors were found to have heightened oxidative phosphorylation (oxphos) and enhanced dependency when pharmacologically targeting the electron transport chain (ETC; PMID 30996079). We have since established *in vitro* models recapitulating this biology and documented pro-mitochondrial effects of conventional chemotherapies in residual cells (PMID: 36813854). Proteomic profiling of pre-treatment TNBC patient biopsies and PDX tumors matched to their responses to carboplatin (CRB), docetaxel (DTX), or the combination revealed mitochondrial translation as a top up-regulated pathway significantly associated resistance (PMID 36001024, PMID 36619972, *Lei JT et al. manuscript in preparation*). Furthermore, on-treatment biopsies were collected from a subset of the TNBC patient samples, and mitochondrial translation pathways were significantly elevated. Leading-edge proteins included several core mitoribosome components and the accessory protein Oxidase (Cytochrome C) Assembly 1-Like (OXA1L). OXA1L plays two critical roles in the mitochondria: 1) it promotes translation termination for the 13 mtDNA-encoded ETC proteins, and 2) it aids in inner mitochondrial membrane insertion of both mtDNA- and nDNA-encoded ETC proteins. Therefore, we hypothesize that mitochondrial translation supported by OXA1L promotes oxphos dependence in residual TNBC cells surviving conventional chemotherapies. Transient siRNA-mediated knock-down (KD) of OXA1L in TNBC cell lines reduced ETC protein levels, reduced mitochondrial ‘respirasome’ supercomplex levels, and diminished oxphos. Notably, the CRB-induced elevation of oxphos characteristic of ‘residual’ cells was significantly diminished by OXA1L KD. Concomitantly, inducible shRNA OXA1L KD cells exhibited significantly enhanced CRB sensitivity relative to control cells. These results highlight the importance of OXA1L for mitochondrial respiratory chain production and functionality. Aiming to translate these findings, we are leveraging the mitochondrial bacterial ancestry to repurpose the FDA-approved antibiotic tigecycline as a chemo-sensitizing drug based on its mitoribosome inhibitory function (PMID 25625193). We demonstrate inhibition of mitochondrial respiratory chain production, respirasome formation, and oxphos with tigecycline. While we observe minimal toxicity to TNBC cells from tigecycline alone, we observe a profound enhancement of efficacy when combined with CRB or DTX both *in vitro* and in an orthotopic PDX model. In summary, our data provide evidence that disrupting mitochondrial translation may be a promising approach to target mitochondrial vulnerabilities in chemo-refractory TNBC cells.

#5428 Detachment from the extracellular matrix induces loss of the mitochondrial phosphatase PGAM5 in cancer cells.

Matthew Fink, Zachary Schafer

Biological Sciences, University of Notre Dame, Notre Dame, IN

Cancer cells often experience altered mitochondrial metabolism due to loss of integrin-mediated attachment to the extracellular matrix (ECM). Relatedly, work from our lab has revealed that ECM-detached cells undergo mitophagy, which subsequently results in the activation of cell death pathways. Cancer cells, however, can circumvent the induction of mitophagy during ECM-detachment to promote their viability, however, the mechanisms underlying mitophagy regulation during ECM-detachment remain poorly understood. The mitochondrial phosphatase PGAM5 is a part of a proteinaceous complex that initiates mitophagy during ECM-detachment, but its regulation in this context has not been extensively studied. Interestingly, we find that certain cancer cell lines have an innate ability to reduce the levels of PGAM5 protein when cultured under ECM-detached conditions. We find that this phenomenon is primarily due to alterations in PGAM5 transcription, rather than from alterations in protein stability. Given that ECM-detachment has been previously shown to impair proliferation, we analyzed PGAM5 expression in ECM-attached cells treated with CDK4/6 inhibitors. Our data suggest that expression of PGAM5 is indeed linked to proliferation, as arresting cells in G1 results in decreased PGAM5 expression. We also examined the consequences of reduced PGAM5 on mitochondrial dynamics. Previous research has discovered that PGAM5 dephosphorylates Drp1, a key regulator of mitochondrial fission. Indeed, we observe that decreased expression of PGAM5 (both during ECM-detachment and after treatment with CDK4/6 inhibitors) results in elevated phosphorylation of Drp1, suggesting an increase in mitochondrial fusion in detached and G1 arrested cells are likely. These data collectively support the conclusion that ECM-detachment causes changes in mitochondrial dynamics as a consequence of impaired proliferation and subsequent loss of PGAM5.

#5429 NRF2 activation sensitizes the Fe-S cluster synthesis pathway to iron restriction.

Maddison A. T. Olley, Nathan P. Ward

Metabolism & Physiology, Moffitt Cancer Center, Tampa, FL

Enhanced mitochondrial metabolism in human lung tumors relative to normal lung and lung tumorigenesis offers a potential therapeutic vulnerability in non-small cell lung cancer (NSCLC) due to the dependence on iron-sulfur (Fe-S) clusters. Recent data indicates that the incorporation of Fe-S clusters into recipient proteins is influenced by iron availability, with possible hierarchical loading when iron is limited. The labile iron pool which Fe-S clusters derive iron from is partly regulated by the transcription factor NRF2, which coordinates restriction of the labile iron pool through increased storage and efflux of free iron when activated. Given the aberrant activation of NRF2 in a significant proportion of NSCLC cases due to amplification or mutations in its negative regulator, KEAP1, we hypothesized that NRF2 regulation of intracellular iron availability may sensitize the Fe-S cluster synthesis pathway in NSCLC when iron is limiting.

To investigate this potential vulnerability, we stratified 14 NSCLC lines based on the activation status of NRF2 and evaluated the sensitivity of Fe-S cluster biology to iron chelation with deferoxamine (DFO). Seahorse analyses of mitochondrial function showed that the decline in mitochondrial respiratory function under iron starvation is exacerbated in NRF2-high cells compared to NRF2-low counterparts. Furthermore, ^{13}C-glucose tracing under DFO treatment revealed a significantly greater reduction in M+2 isotopologue enrichment of TCA cycle intermediates in NRF2-high cells relative to NRF2-low cells. In association with this enhanced mitochondrial metabolic dysfunction, we observe a more rapid loss of Fe-S protein activities such as aconitase and respiratory super complexes I-III and II-III in NRF2-high cells relative to NRF2-low cells following DFO treatment. Importantly, we find that NRF2 knockout protects against the loss of Fe-S protein function in response to iron restriction. Moreover, we find that the loss of mitochondrial iron in response to DFO treatment is dampened in NRF2 knockout cells, indicating that NRF2 coordinates mitochondrial iron availability in a manner relevant to Fe-S cluster biology. Overall, our data indicates that NRF2 activation augments the sensitivity of the Fe-S cluster synthesis pathway to iron restriction in NSCLC, presenting a vulnerability with potential therapeutic relevance.

#5430 Hematopoietic stem cells conditioned media alters the colorectal cancer cells metabolism by disrupting the mitochondrial bioenergetics in vitro.

Sumit Mallick, Sudheer P. Shenoy, Bipasha Bose

Stem Cells and Regenerative Medicine Centre, Yenepoya University, Mangaluru, India

Colorectal cancer (CRC) is a major public health issue, and mitochondrial dysfunction plays a pivotal role in its development. Mitochondria, the "powerhouses of the cell," are particularly important in cancer cells due to their high energy demands. The dynamic nature of mitochondria, including their ability to fuse and divide, is crucial for their role in signal transduction and metabolism. Enhanced mitochondrial fission, a process that divides mitochondria into smaller fragments, promotes the metabolic reprogramming of CRC cells. These reprogramming fuels cell proliferation, metastasis, and resistance to chemotherapy. However, excessive fission can also trigger apoptosis, leading to cell death. Our work shows that hematopoietic stem cells (HSCs) derived conditioned media (CM) altered metabolic activities by altering the mitochondrial oxidative phosphorylation through an excessive mitochondrial fission by upregulating the Drp-1 and downregulating the fusion protein Opa-1 and Mito-fusion proteins. Moreover, HSCs-CM dysregulated the electron transport chain complexes with high dysregulation of complex-III. Our integrated omics and mitochondria-targeted studies provided the dysregulation of the critical proteins involved in mitochondrial bioenergetics in CM treated CRCs. Our findings reveal the therapeutic nature of HSCs-conditioned media for colorectal cancer therapy.

Keywords: Metabolism, Colorectal Cancer, Mitochondrial Fission, Proteomics, Targeted Therapy, Haematopoietic Stem Cells

#5431 The mitochondrial protease, YME1L regulates interferon signaling and is essential for AML growth and differentiation.

Yihe Zhang, Rose Hurren, Yongran Yan, Marcela Gronda, Dakai Ling, Karen Kai-Lin Fang, Andrea Arruda, Mark D. Minden, Aaron D. Schimmer

Princess Margaret Cancer Centre, Toronto, ON, Canada

Yeast Mitochondrial Escape 1-Like 1 (YME1L) is an ATP-dependent metalloprotease located in the inner mitochondrial membrane. Here, we examined the expression and function of this protease in AML. YME1L protein was expressed in 6/7 tested AML cell lines. In 17 of 23 primary AML samples, YME1L protein was > 2-fold higher than normal hematopoietic cells. High YME1L mRNA expression was associated with lower five-year survival rates in AML patients.To assess the dependency of AML cells on this protease, we knocked down YME1L with shRNA in OCI-AML2, NB4, TEX, and THP-1 AML cell lines. YME1L knockdown reduced AML cell proliferation in these tested lines. In addition, the depletion of YME1L reduced the clonogenic growth of leukemia cells, and decreased the engraftment of AML cells into the marrow of immune-deficient NS-GF mice. Thus, YME1L is essential for AML cells and leukemia-initiating cells. To understand the mechanisms of action of this protease and the downstream consequences of its depletion, we measured changes in gene expression after the knockdown of YME1L in OCI-AML2 cells. By RNA sequencing, knockdown of YME1L upregulated genes associated with defense response to viruses, response to type I interferon, and type I interferon signaling pathways, consistent with viral mimicry and induction of interferon (IFN) signaling. Using real-time PCR we confirmed the importance of YME1L in regulating these pathways. Knockdown of YME1L upregulated the expression of beta and gamma IFN, as well as downstream interferon-stimulated genes *ISG15*, *IFI44*, and *IFIT2*. Upregulation of IFN pathways and induction of viral mimicry, can promote the differentiation of AML cells. Therefore, we assessed the differentiation of leukemic cells after the knockdown of YME1L. Knockdown of YME1L induced the differentiation of OCI-AML2 cells as evidenced by upregulation of CD14 protein expression.In summary, YME1L is overexpressed in a subset of AML cells. This protease is essential for AML cells and controls differentiation by regulating IFN and viral mimicry pathways.

#5432 Restoring mitochondrial quantity and quality to reverse Warburg effect and drive tumor differentiation.

Haowen Jiang[1], Sarah Jane Tiche[1], Clifford JiaJun He[1], Junyan Liu[1], Fuyun Bian[1], Mohamed Jedoui[1], Balint Forgo[1], Md Tauhidul Islam[1], Meng Zhao[1], Pamela O. Emengo[1], Bo He[1], Yang Li[1], Albert M. Li[1], Anh T. Truong[2], Jestine Ho[2], Cathyrin Simmermaker[2], Yanan Yang[2], Meng-Ning Zhou[1], Zhen Hu[3], Katrin J. Svensson[1], Daniel J. Cuthbertson[2], Florette K. Hazard[1], Lei Xing[1], Hiroyuki Shimada[1], Bill Chiu[1], Jiangbin Ye[1]

[1]Stanford University School of Medicine, Palo Alto, CA,[2]Agilent Technologies, Santa Clara, CA,[3]Olivia Consulting Service, Redwood City, CA

Reduced mitochondrial quality and quantity in tumors is associated with dedifferentiation and increased malignancy. However, it remains unclear how to restore mitochondrial quantity and quality in tumors, and whether mitochondrial restoration can drive tumor differentiation. Our study shows that restoring mitochondrial function using retinoic acid (RA) to boost mitochondrial biogenesis and a mitochondrial uncoupler to enhance respiration synergistically drives neuroblastoma differentiation and inhibits proliferation. U-^{13}C-glucose/glutamine isotope tracing revealed a metabolic shift from the pentose phosphate pathway to oxidative phosphorylation, accelerating the TCA cycle and switching substrate preference from glutamine to glucose. These effects were reversed by ETC inhibitors or in ρ0 cells lacking mtDNA, emphasizing the necessity of mitochondrial function for differentiation. Dietary RA and uncoupler treatment promoted tumor differentiation in an orthotopic neuroblastoma xenograft model, evidenced by neuropil production and Schwann cell recruitment. Single-cell RNA sequencing analysis of the orthotopic xenografts revealed that this strategy effectively eliminated the stem cell population, promoted differentiation, and increased mitochondrial gene signatures along the differentiation trajectory, which could potentially significantly improve patient outcomes. Collectively, our findings establish a mitochondria-centric therapeutic strategy for inducing tumor differentiation, suggesting that maintaining/driving differentiation in tumor requires not only ATP production but also continuous ATP consumption and sustained ETC activity.

#5435 OXPHOS as a driver of treatment resistance in NSCLC? From computational analysis to preclinical validation.

Katarina Benejova[1], McKenzie Kreamer[2], Nicholas Denko[2], **Martin Benej**[1]

[1]Department of Molecular Medicine and Therapeutics, The Ohio State University Comprehensive Cancer Center, Columbus, OH,[2]Department of Radiation Oncology, The Ohio State University, Columbus, OH

Lung cancer is the leading cause of cancer deaths in the U.S. and worldwide. Up to 90% of all lung cancer cases are non-small cell lung cancer (NSCLC); a complex and highly aggressive disease typical for poor prognosis and high rates of treatment resistance. Clinical management of NSCLC varies according to the stage. High-dose stereotactic body radiation therapy (SBRT) is the standard of care for localized non-resectable NSCLC. In the last decade, first line NSCLC treatment has been substantially reinforced with the introduction of immunotherapy targeting immune checkpoints such as programmed cell death 1 (PD-1). However, despite initial good response, up to 75% of NSCLC patients may experience disease progression and the reasons for this heterogeneity are not fully understood. Several lines of evidence suggest that the highly hypoxic tumor microenvironment in NSCLC contributes to the high rates of treatment resistance. The basis of tumor hypoxia has traditionally been attributed to the oxygen supply deficit as malformed tumor vasculature fails to meet the high demand of the rapidly proliferating tumor mass. However, our preliminary analysis of NSCLC patient datasets in the Cancer Genome Atlas (TCGA) PanCancer dataset revealed a significant correlation between high-level expression of nuclear genes encoding mitochondrial subunits essential for oxidative phosphorylation (OXPHOS), and high-level expression of hypoxia-regulated genes (Buffa hypoxia score). Furthermore, we have observed a direct correlation between copy number amplification (CNA) of several OXPHOS genes and hypoxia levels in patient samples. Because mitochondrial function consumes up to 90% of available cellular oxygen, its activity may be indirectly regulating oxygen availability in the tumor microenvironment by rapidly consuming oxygen upon its delivery to the tumor. Moreover, we have identified that almost 70% of NSCLC patients harbor CNA of these essential OXPHOS genes. To provide mechanistic insight into these clinical observations, we have generated mouse syngeneic NSCLC cell lines with modified mRNA expression levels of OXPHOS genes (representing deletion, diploid and amplified number of copies) identified in our screen and observed a direct effect on the rates of mitochondrial oxygen consumption in vitro and hypoxia levels in vivo, leading to increased resistance to anti-cancer therapy in preclinical mouse model of NSCLC. Our study investigates the previously unknown role of OXPHOS gene copy number in driving mitochondrial oxygen demand and thus contributing to tumor hypoxia and treatment resistance in NSCLC.

#5436 MED8-orchestrated C24 sphingolipid metabolism promotes tumor progression through enhanced tumor cell membrane fluidity and mitochondrial oxidation.

Zhixian Chen, Xiaoxin Zhang, Judy Wai Ping Yam

Department of Pathology, The University of Hong Kong, Hong Kong, China

Mediator complex is a large protein complex containing up to 30 proteins that coactivates the RNA polymerase II (Pol II) enzyme to transcribe all protein-coding and most non-coding RNA genes. Recently, it has been reported that various subunits of the complex may possess distinct functions in cancer progression. After comparing the expression of all the subunits between normal and tumor tissue and analyzing their prognostic potential, we found that mediator complex 8 (MED8) was the most oncogenic gene that consistently predicted poor survival in liver hepatocellular carcinoma (LIHC), kidney renal clear cell carcinoma (KIRC), sarcoma (SARC), and uterine corpus endometrial carcinoma (UCEC) according to TCGA database. Next, we aimed to explore the common functions and mechanisms of MED8 involved in the development of the aforementioned tumor types. Pathway analysis revealed a strong association between MED8 and sphingolipid metabolism. To further study the role of MED8 in LIHC, KIRC, SARC, and UCEC, we established shMED8 stable clones using cell lines of each tumor type followed by metabolomics profiling. Notably, a significant correlation was observed between MED8 levels and a cluster of C24 lipids. ELOVL1, which specifically produced C24 acyl-coAs and linked to C24 sphingolipid synthesis, was confirmed to be positively regulated by MED8. Also, as one of the main groups of C24 sphingolipid, C24 ceramide was found to be reduced by the knockdown of MED8 indicated by the mitigatory phosphorylation of LYN, further underscoring the regulatory role of MED8 in sphingolipid metabolism. Although ceramide is known to initiate apoptosis and suppress tumor development, the metabolism of ceramide into other forms of sphingolipid including sphingomyelin (SM), sphingosine (Sph), and sphingosine-1-phosphate (S1P) has been reported to promote tumor progression. Specifically, C24-SM has been found to increase cell membrane fluidity, contributing to the distant metastasis of tumor cells. Furthermore, S1P has been reported to promote cytochrome c oxidase (COX IV) and mitochondrial respiration. In our studies, MED8 was validated to enhance tumor cell membrane fluidity, COX IV expression, and migratory ability, likely mediated by altered C24-SM and C24-S1P metabolism. Besides being converted into other metabolites, ceramide is involved in small extracellular vesicles (sEV) biogenesis via the complex TSG101-CERT (ceramide transport). Decreased formation of such complex and sEV secretion were observed in shMED8 tumor cells. In addition, MED8-enriched sEV endowed recipient cells with enhanced invasive ability by increasing their membrane fluidity and respiration.

#5437 N-myristoylation inhibitor zelenirstat reduces mitochondrial complex I activity, OXPHOS, glycolysis, and cancer stem cell growth in triple-negative breast cancer (TNBC) cells.

Rony Pain[1], Erwan Beauchamp[2], Morris A. Kostiuk[1], Claudia Y. Acevedo-Morantes[1], Luc G. Berthiaume[1]

[1]Cell Biology, University of Alberta, Edmonton, AB, Canada,[2]Pacylex Pharmaceuticals, Edmonton, AB, Canada

Two mammalian N-myristoyltransferases (NMT1 and NMT2) catalyze the addition of the fatty acid myristate to >200 different proteins and regulate cellular processes such as signal transduction, organelle trafficking, and metabolism. The pan-NMT inhibitor zelenirstat preferentially killed hematologic cancer cells while sparing normal cells, was safely administered, and showed early signs of efficacy in cancer patients during phase I clinical trials.

Normally-myristoylated proteins exposing their un-myristoylated N-terminal glycine resulting from zelenirstat treatment are susceptible for degradation by a glycine-specific N-degron. Using differential mass spectrometry proteomics of *NMT1*/*NMT2* CRISPR/Cas9 knockout (KO) HAP1 cells or HAP1 cells treated with zelenirstat compared to untreated WT HAP1 cells, we identified multiple NMT-specific substrates. Surprisingly, *NMT1* KO statistically reduced the protein levels of only a few signaling proteins, while preferentially reducing the levels of 36 mitochondrial respiratory proteins including the myristoylated complex I assembly factor NDUFAF4.

MISS-54, a gene signature that predicts the sensitivity of cancer or normal cells to zelenirstat, is significantly higher in TNBC than in other breast cancer subtypes or normal breast cells. Zelenirstat abrogated mitochondrial complex I assembly and activity as well as oxidative phosphorylation (OXPHOS) in MDA-MB-231 TNBC cells. The KO of *NDUFAF4* in these cells recapitulated all these results but also increased glucose utilization measured through extracellular acidification rates (ECAR). Zelenirstat significantly decreased respiration through complex I, overall oxygen consumption, ECAR, and the use of glucose as an alternate energy source. Zelenirstat treatment also resulted in pyruvate dehydrogenase inhibition, decreased AMPKβ levels, and decreased the NAD/NADH ratio. Thus, zelenirstat caused significant metabolic disruption that drastically impaired energy production in TNBC cells. We hypothesize that the inability of TNBC cells to switch to glucose utilization upon zelenirstat treatment is due to the loss of myristoylated AMPKβ.

Cancer stem cells (CSCs) have metabolic plasticity and can use either OXPHOS or glycolysis. Since zelenirstat reduced both pathways, we hypothesized that zelenirstat could also reduce the growth of CSCs in vitro. Both zelenirstat and *NDUFAF4* KO individually reduced mammosphere formation and produced dose-dependant additive effects on CSC growth when combined.

Overall, our results indicate that N-myristoylation is required for optimal energy production, cancer cell respiration, and CSC growth. In addition to inhibiting cell growth signaling, our data indicate that zelenirstat reduces OXPHOS and glycolysis, which may reduce CSC survival and prevent metastasis.

#5438 Exploring glioblastoma pharmacotherapy with an anti-epileptic drug: Multi-omic insights and tumor xenograft studies.

Anjali Yadav, Shraddha P. Bhutkar, Vikas V. Dukhande

St. John's University, Queens, NY

Glioblastoma multiforme (GBM) remains one of the most aggressive brain tumors, with a 5-year survival rate of only 6%. GBM resistance to standard-of-care therapies underscores the need for novel treatment approaches. Metabolic reprogramming is a promising hallmark of cancer, indicating a therapeutic avenue. Lactate dehydrogenase (LDH) is upregulated in GBM and adversely affects the survival of GBM patients. In this study, we investigated the repurposing potential of a putative LDH inhibitor Stiripentol (STP), an FDA-approved anti-epileptic drug, to inhibit GBM progression. Using various *in vitro* biological assays and 3D spheroid assays, STP treatment demonstrated a reduction in GBM cell viability and growth. However, the precise mechanism of STP's anticancer activity remains unclear. We aimed to elucidate STP's molecular mechanism and metabolic alterations underlying its anticancer activity in GBM. Additionally, we studied the efficacy of STP through *in vivo* tumor xenograft model. We adopted a multi-omics approach consisting of transcriptomics and metabolomics coupled with bioinformatic analyses. Our results from transcriptomics showed significant alterations in genes such as ALDH1A3, SERPINB2, AOX1, FGF1, TRIM22 and others involved in several biological processes such as oxidoreductase activity, extracellular membrane binding and response to DNA damage. Our metabolomic analysis demonstrated highly dysregulated metabolite enrichment in the Warburg effect, pentose phosphate pathway, glycolytic pathway, and homocysteine degradation upon STP treatment. Our metabolic and transcriptomic analysis bridges the mechanistic gap into the oxidoreductive stress and DNA damage response upon STP treatment. Our tumor xenograft studies showed significant efficacy of STP with decreased tumor volume and tumor weight. Our future studies will further elucidate the mechanistic basis of STP effects in GBM. Taken together, our findings suggest that STP has potential as a novel therapeutic lead for GBM that merits further validation and optimization to enhance its efficacy.

This research was supported by an award from the National Institute of General Medical Sciences of the National Institutes of Health under Award Number R16GM145557 and by funds from the College of Pharmacy and Health Sciences, St. John’s University, NY

#5439 Sphingolipid modulating compounds BXQ-350 and desipramine display synergy in reducing viability across multiple cancer cell types in vitro.

Tariq Arshad, Tim Stephens, Nikhil Wilkins, Robin Furnish, Mike Gazda

Bexion Pharmaceuticals, Inc, Covington, KY

Background: A cancer cell's ability to dictate sphingolipid metabolism is necessary for its survival. Two sphingolipids play a key role in cancer cell survival: Ceramide and Sphingosine-1-Phosphate (S1P), and the relationship between these two is known as the Ceramide-S1P rheostat. High levels of S1P, which favors cancer cell survival, proliferation, chemotherapeutic resistance and immune suppression, have been associated with poorer clinical outcomes; while higher levels of Ceramide, which is associated with cell death signaling and immune activation, lead to more favorable outcomes. Pushing this ratio in favor of Ceramide can weaken cancer cells making them more susceptible to a variety of therapeutic treatments. BXQ-350 is a novel anti-cancer and anti-neuropathy drug consisting of the lysosomal activator protein Saposin C (SapC) and the anionic phospholipid Dioleoyl Phosphatidylserine (DOPS). Together they are an allosteric activator of glucocerebrosidase (GCase) which is responsible for converting glucosylceramides into Ceramide (and glucose). Desipramine is a tricyclic antidepressant that blocks the reuptake of norepinephrine and serotonin. It binds to the inner leaflet of the lysosomal membrane changing the polarity and preventing certain enzymatic activities. Of note, it prevents acid-Ceramidase (ACMase) from converting Ceramide into Sphingosine, the precursor to S1P.
Method: Cells were treated, in vitro, with BXQ-350 and Desipramine for 72hrs. Viability was measuring using the MTT kit from Roche (#11465007001). Synergy was calculated utilizing the online website Synergy Finder (https://synergyfinder.org/). The aim of synergy calculations is to quantitate the change between an assumed no interaction reference model and the observed response when drugs are used in combination. The Zero Interaction Potency (ZIP) model (Yadav, et al., 2015) assumes the single drugs have no effect on the other's potency curve.
Results: Although BXQ-350 and Desipramine concentrations varied between specific cell lines, synergy was observed in colorectal, glioblastoma multiforme and pancreatic cancer cell lines.
Conclusion: Disrupting a cancer cell's sphingolipid metabolism, specifically the Ceramide-S1P rheostat, is a viable strategy for clinical therapeutics. The synergy BXQ-350 and Desipramine combinations show across cancer cell types, with varying oncogenic profiles, suggests that targeting sphingolipid metabolism is an agnostic approach to treating cancer, not related to any specific mutation or cell type. The possibility exists for sphingolipid modulating drugs to be combined with specific chemotherapeutics to increase their efficacy.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Antibody-Based Cancer Therapeutics 3
Poster Session

#5443 IPH4502, a differentiated Nectin-4 exatecan antibody-drug conjugate.

Romain Remark, Cecile Bonnafous, Laura Chiossone, Caroline Soulas, Cyril Perrier, Sivan Bokobza, Rachel Courtois, Julie Lopez, Gregory Fenaux, Olivier Benac, Barbara Carrette, Robin Letay-Drouet, Raja Bonifay, Marion Gaudin, Severine Augier, Lea Simon, Agnes Represa, Ariane Morel, Eric Vivier, Yannis Morel, Nicola Beltraminelli, Carine Paturel

Innate Pharma, Marseille, France

Background: Nectin-4 is a cell-adhesion molecule expressed in many solid tumors. Enfortumab vedotin (EV, PADCEV®), an antibody-drug conjugate (ADC) targeting Nectin-4 with a monomethyl auristatin E (MMAE) payload, is approved for the treatment of urothelial cancer (UC), which exhibits the highest Nectin-4 expression among all solid tumor types. To address the unmet medical need of UC patients who discontinue EV due to toxicity, lack of efficacy, or ineligibility for this approved therapy, and to expand Nectin-4 targeting in tumor indications with lower Nectin-4 expression beyond UC, we have developed IPH4502, a differentiated anti-Nectin-4 ADC conjugated to exatecan with a cleavable hydrophilic linker, and a drug-to-antibody ratio of 8.
Methods: Nectin-4 immunohistochemistry (IHC) was performed on formalin-fixed, paraffin-embedded tissue-microarrays. Internalization was evaluated *in vitro*, and bystander effect was evaluated *in vivo*. *In vivo* studies were also conducted to evaluate anti-tumor activity using syngeneic mouse models, cell line-derived xenografts, and patient-derived xenografts (PDXs) from various indications.
Results: IHC revealed that Nectin-4 is overexpressed in tumor tissues from several cancer types including but not limited to UC, esophageal cancer, triple-negative breast cancer (TNBC), non-small cell lung cancer (NSCLC), and prostate cancer. IPH4502 demonstrates strong bystander killing effect *in vivo,* and high internalization efficiency *in vitro* in comparison to other Nectin-4 ADCs. These attributes contribute to enhanced anti-tumor activity in comparison to EV, across a spectrum of Nectin-4 expression levels, ranging from low to high expression, in PDX models. Moreover, IPH4502 shows efficacy in an *in vivo* model with primary resistance to MMAE due to MDR1 transporter expression and demonstrates anti-tumor activity in a PDX model of UC that acquired resistance to EV. Finally, IPH4502 shows activity in PDX models from indications beyond UC.
Conclusion: The strong internalization and bystander effect of IPH4502 enable an efficient anti-tumor activity in Nectin-4 positive tumor models independent of Nectin-4 expression levels. In addition, IPH4502 shows efficacy in models resistant to EV, indicating that IPH4502 has potential for UC patients relapsing or refractory to EV treatment, as well as for cancer patients treated with MMAE-based ADCs. Finally, IPH4502 shows anti-tumor activity in various preclinical cancer models, including PDX models from different indications, supporting its development beyond UC.

#5444 ImmuMab® mice enable efficient development of antibody therapeutics by unparalleled diversity across multiple formats.

Yuan Li, Wenbao Wu, Yu Wei, Yingjun Zhao, Yu Zhang, Jiwei Zhang

Shanghai Immunocan Biotech Co. Ltd, Shanghai, China

This study highlights the latest advancements of ImmuMab® gene-edited mouse platform, engineered with human immunoglobulin germlines to facilitate efficient antibody therapeutic development across diverse formats, including canonical, heavy-chain-only (HCO), common-light-chain (CLC), or combinations thereof. Utilizing massive-fragment across species *in situ* replacement technology, these mice underwent Mb-scale humanization of immunoglobulin gene fragments in single-step operations, circumventing the cumulative cellular damage associated with traditional transgenic approaches.

These new mice all deliver distinguished immune dynamic profiles featuring rapid initial immune responses and compelling titer plateaus. Since their introductions, the ImmuMab® mouse suite (HK, HKL, HL, HCO, CLC) has consistently delivered highly diversified repertoires across 35+ antibody discovery programs, with no failures due to suboptimal immune responses.

Designed for canonical antibody generation, the ImmuMab HK, HKL and HL mice (the latter two newly introduced), feature up to over 3.2 Mb human Immunoglobulin variable region genes. The ImmuMab HK mice in different project had deliver stable median titers ranging from 72.9k to 656.1k by the third dose, with some maximum titers approaching 1968.3k. Their immune responses exhibit robust resilience against challenging targets, including seven-transmembrane receptors and antigens with over 99% human-mouse homology. Notably, in one hybridoma screening against hROR1, the primary clone positivity rate (OD_{450} > 0.5) exceeded 33%. Selected monoclonal antibodies demonstrating affinities ranging from 0.001 to 1 nM (by BLI) and internalization rates of 20%-90% (Temperature-dependent). Some antibodies further demonstrated superior cytotoxic efficacy (MMAE-conjugated) compared to benchmark antibody-drug conjugates (ADC).

Additionally, one discovery program with ImmuMab Light® (CLC) mice by rapid immunization (1-week intervals) yielded seven candidate antibodies with picomolar EC_{50} values ($EC_{50} < 5 \times 10^{-10}$ M, ELISA) within a single screening cycle. Furthermore, the ImmuMab Heavy® (HCO) mouse enabled rapid heavy-chain-only immune response, reaching titers of 218.7k~656.1k within four weeks and identifying nanomolar heavy-chain-only antibodies with low single-digit nanomolar EC_{50} values for both human and cynomolgus monkey antigens.

The ImmuMab® mouse platform enables efficient development of antibody therapeutics across multiple formats. Its robust immune responses and high repertoire diversity make it a powerful tool for generating high-quality candidates, even against challenging targets.

Keywords: *diversity, fully human antibody, common-light-chain antibody, heavy-chain-only antibody, ImmuMab*

#5445 Discovery and optimization of IDP-001, a bispecific ADC targeting EGFR and CDCP1.

Anna de Regt, Clare Malone, Payam Farahani, Ben Setter, Robert W. Gene, Carol F. Farr, Zachary Caldwell, Brian Woodruff, Gyuna Kim, Hengyu Xu, Heath Klock, Rob Oslund, Niyi Fedyi, Michael Hornsby, Pamela Holland, Scott Lesley

InduPro Therapeutics, Seattle, WA

EGFR is a clinically-validated target that is overexpressed and activated in many human cancers and associated with poor prognosis. However, ADCs targeting EGFR exhibit toxicity due to expression on healthy tissue (1), and EGFR can show heterogeneous tumor expression (2). To engineer a next-generation bispecific EGFR ADC, we utilized InduPro's proprietary MInt^{TM} platform to identify proteins inherently proximal to EGFR on tumor cells. Among multiple proteins in proximity to EGFR, CDCP1 was identified as a TAPA (tumor associated proximity antigen) of interest. Like EGFR, CDCP1 is over-expressed in multiple cancers and plays key roles in oncogenic signaling and malignant transformation (3). Moreover, EGFR signaling induces upregulation and cell surface localization of CDCP1 as well as inhibition of its palmitoylation-dependent degradation (4).

Here we report the development of IDP-001, a novel bispecific ADC targeting EGFR and CDCP1. To identify antibodies that display selective activity only on cells that express both EGFR and CDCP1, we generated antibodies to both targets and then combined them to form over 250 hIgG1-based 1+1 bispecific antibodies with diverse epitopes and affinities. This bispecific library was screened on internalization and cytotoxicity assays, yielding a lead candidate that bound domain III of EGFR with a kappa Fab and bound the membrane-distal domain of CDCP1 with an alpaca-derived VHH. We performed further sequence optimization to affinity tune and humanize this antibody, engineer its Fc, and conjugate it with a DAR of 2 via engineered cysteines to a vcMMAE drug linker to yield our final development candidate, IDP-001. IDP-001 incorporates several design principles that distinguish it from other EGFR ADCs, including possible higher tumor exposure and penetration because of its smaller size. Due to its format and conjugation strategy, IDP-001 exhibits excellent biophysical properties and is highly developable. IDP-001 has potent in vitro cytotoxic activity against cancer cell lines with varying target expression levels. IDP-001 is a first-in-class bispecific ADC with the potential for a wider therapeutic window and best-in-class efficacy.

References

1. Dan-yun Ruan et al., *JCO* 42 (2024) 6013-6013.

2. Passaro A et al *ESMO open* vol. 5,5 (2020) e000919.

3. Khan T et al., *Cancer Res* 81 (2021) 2259-2978.

4. Adams MN et al., *Oncogene* 34 (2015) 1375-1383.

#5446 Development of a novel TCR based bispecific platform for cancer therapy.

Danqing Wu, Li Hu, Lini Huang, Beilei Shi, Lijun Wang, Xuan Wu, Shiyong Gong, Chengbin Wu

Shanghai EpimAb Biotherapeutics Co., Ltd., Shanghai, China

TCR-T therapies emerged as a new generation of therapeutics targeting solid tumors with high specificity but are not “off-the-shelf”. TCR based T-cell engagers follow the same concept, however it remains challenging to generate such molecules containing TCRs, which usually requires extensive engineering and optimization to achieve good solubility and functionality. To solve this problem, we developed a “plug-n-play” technology, namely TCR-Fab in tandem (T-FIT) fusion protein, for generating off-the-shelf TCR based therapeutics. The design of T-FIT was optimized by comparing recombinant production yield and purity in various formats with different spacers, binding valencies and TCR-Fab geometries. The selected T-FIT format showed IgG like production properties across different TCRs and Fabs. Based on the T-FIT format and our proprietary CD3 antibody, we generated 3 TCR based T-cell engagers, targeting NY-ESO-1, MAGE-A3 and Survivin respectively, and validated the T-FIT platform in preclinical functional assays. All three T-FIT molecules demonstrated potent redirected T-cell lysis activity against target positive cells in vitro. The MAGE-A3 and Survivin targeting T-FIT molecules also showed complete tumor inhibition in xenografted models.

#5447 Development and preclinical evaluation of JK06, a biparatopic ADC against 5T4.

Jonathan McNally, Binyam Bezabeh, Jijun Dong, Samuel Murphy

Salubris Biotherapeutics, Gaithersburg, MD

Trophoblast glycoprotein, also known as 5T4, is an oncofetal antigen that is upregulated on a variety of solid tumors including breast, lung, and genitourinary cancers. 5T4 expression levels correlate with stage of cancer progression and are associated with poor clinical outcomes. Herein, we describe the pre-clinical design and validation of JK06, a humanized, quadrivalent, biparatopic antibody-drug conjugate (ADC) that targets 5T4, allowing for efficient and selective delivery of a cytotoxic payload, monomethyl auristatin E (MMAE), to tumor cells. JK06 is engineered from 2 monoclonal antibodies (mAbs) that bind to distinct epitopes of 5T4. The first mAb clone was converted to a single-chain fragment variable (ScFv) format and fused to the C-terminal Fc end of a second mAb. This yields a hybrid molecule with four 5T4 binding sites, targeting two distinct epitopes of the 5T4 antigen. This design increases 5T4 avidity, an important feature given the generally low expression of 5T4 on tumor cells and enhances internalization of the drug. JK06 binds to recombinant 5T4 protein with low picomolar affinity measured by surface plasmon resonance, an approximately 10-fold increase in comparison to the two parental mAbs. JK06 induces internalization into 5T4 expressing tumor cells more rapidly and to a greater extent *in vitro,* than the parental mAbs. The tubulin inhibitor MMAE was conjugated to the Fc backbone of JK06 in a site-specific manner with a cleavable linker at a drug:antibody ratio (DAR) of two. *In vivo* CDX studies utilizing the MDA-MB-461 breast cancer and H1975 lung cancer cell lines in nude mice demonstrated that JK06 induces robust control of tumor growth *in vivo.* A repeat-dose GLP toxicology study, with a once every 3-week (Q3W) dosing schedule, demonstrated that JK06 was well tolerated, and without any observed adverse effects at the tested doses. Toxicokinetic evaluation showed a half-life which projects sustained exposure in humans with a Q3W dosing regimen. JK06 is currently being evaluated in an open-label, dose-escalation and expansion Phase I study to assess the safety, pharmacokinetics, and preliminary efficacy in a basket of solid tumors known to express 5T4.

#5448 Single domain antibodies, EGFR and cancer: a small solution to a big problem.

Adam T. Nelczyk[1], Kimberly J. Anderson[1], Nicholas Wu[2]

[1]Helixon US Inc, Champaign, IL,[2]Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL

The primary purpose of our work is to identify high-affinity, efficacious single-domain antibodies (sdAbs, also known as nanobodies) targeting significant molecular drivers of cancer. The epidermal growth factor receptor (EGFR) family play a prolific role in the development and progression of numerous cancers. The clinical importance of these receptors is exemplified by the fact that 14 small molecule tyrosine kinase inhibitors as well as several monoclonal antibodies (mAbs) have received approval for therapeutic intervention. While these medications have undoubtedly had significant, positive impacts on patient outcomes, loss of efficacy due to mutagenic changes is a persistent issue. Unlike mAbs, sdAbs bind to a substantially smaller epitope, reducing the chances of mutation-induced loss of activity. Additionally, the distinct structure of sdAbs imparts them with highly desirable drug characteristics, such as exceptional tissue penetration, solubility, stability, and binding affinity. Considering these favorable traits, it is no surprise that the pharmaceutical industry is now allocating significant resources towards the development of sdAb-based drugs, with over a dozen in clinical trials, and 2 FDA approvals since 2019. While several anti-EGFR sdAbs have been described, at the time of this abstract submission, we were only aware of one sdAb-based drug targeting an EGFR family member under clinical investigation (I-GMIB-Anti-HER2-VHH1, Precirix). To address this currently unmet need, we first generated a novel sdAb library from 24 naïve crias (immature alpacas). Utilizing an iterative process of magnetic bead panning, mammalian cell display in a high EGFR-expressing cell line, and flow cytometric sorting, we were able to identify several candidates of interest via sequence analysis. Individual evaluation in our reporter cells showed that our candidates were capable of suppressing EGFR signaling, as determined by our fluorometric reporter. Following this validation, we assessed the impact of sdAb-mediated signaling regulation on classical cancer hallmarks. Data from *in vitro* migration and proliferation assays demonstrated that sdAb-antagonism caused significant inhibition of both hallmark properties. These findings were further supported by differential gene expression data, which highlighted significant changes in the expression of well-established regulators of these processes. Taken together, these data demonstrate our sdAb development pipeline can accurately identify multiple candidates capable of inhibiting crucial cancer hallmarks via EGFR antagonism. Ultimately, our goal is to utilize our process across a spectrum of cancers in order to provide patients with a new generation of high-efficacy, low-side effect therapeutics that more precisely and effectively target the primary molecular driver of their malignancy.

#5449 Preclinical development of SCR-A003, a novel topoisomerase I inhibitor-based antibody-drug conjugate (ADC) targeting LIV-1 for solid cancer.

Yayuan Fu, Changyan Chen, Xiaohua Yan, Zhiyong Yu, Zhuangzhi Zhao, Jingli Liao, Zhenyun Tian, Renhong Tang

Simcere Zaiming Pharmaceutical Co, Ltd., State Key Laboratory of Neurology and Oncology Drug Development. Simcere Pharmaceutical Group, Shanghai, China

Background: LIV-1, also known as SLC39A6 or ZIP6, a multi- transmembrane protein with putative zinc transporter and metalloproteinase activity. LIV-1 is expressed in a number of cancers with the highest prevalence in breast, prostate, ovary and melanoma, but a limited presence in vital organs. Currently, the clinical-stage ADC drug targeting LIV-1, Ladiratuzumab vedotin (SGN-LIV1A), is undergoing Phase 2 trials and has demonstrated clinical benefits, thus further underscoring LIV-1 as a promising target for advanced solid tumors. Given the broad spectrum of tumor indications addressable by targeting LIV-1, we utilized Simcere Zaiming' proprietary ADC technology to generate a novel ADC targeted to LIV-1 named SCR-A003.
Method: SCR-A003 is comprised of a LIV-1-targeted monoclonal antibody linked to a novel cytotoxic topoisomerase I inhibitor by a protease-cleavable linker. Pharmacokinetic study was conducted in mice in which SCR-A003 administered IV infusion. In vivo efficacy studies were conducted in immunodeficient mice with human tumor cell-lines including NCI-H838 and OVCAR3.The anti-tumor efficacy of SCR-A003 was evaluated in comparison to SGN-LIV1A analogue.
Result: SCR-A003 had highly specific binding affinity with human and could cross-bind to cyno LIV-1. Moreover, SCR-A003 exhibited excellent internalization and cell killing ability on multiple tumor cells in-vitro assay. SCR-A003, demonstrated specific, dose-dependent in vivo antitumor activity toward LIV-1-positive tumor xenografts representing lung, ovary and breast cancers. Compared to SGN-LIV1A analogue, SCR-A003 exhibited more robust tumor inhibition capacity at lower doses in OVCAR3 and NCI-H838 xenograft models. Additionally, SCR-A003 also showed prolonged serum half-life and higher drug exposure than SGN-LIV1A analogue in pharmacokinetic study.
Conclusions: SCR-A003, exhibited a favorable preclinical profile, with potent in vivo activity toward LIV-1-expressing tumor xenografts representing a range of cancer indications. These data support clinical development of SCR-A003 for the treatment of LIV-1-expressing solid cancers.

#5450 JSKN022, a first in class multi-specific ADC targeting PD-L1 and ITGB6/8 with an innovative DNA topoisomerase I inhibitor demonstrates encouraging efficacy in preclinical models.

Ting Xu, Ling Wang, Yongli Dong, Chuankun Shan

Jiangsu Alphamab Biopharmaceuticals Co.,Ltd., Suzhou, China

Background: Monoclonal antibodies targeting PD-1/PD-L1 have significantly transformed cancer therapy. However, the majority of patients still cannot get benefit from this treatment or may develop resistance afterwards. The increased expression of PD-L1 in tumors makes it an attractive target for ADC development leading to the most advanced program in registration trials. We developed a multi-specific ADC targeting another set of antigens, ITGB6/8. Integrins are cell surface receptors that usually mediate cell to cell adhesion. Integrin β6/8(ITGB6/8), which can only form a heterodimer with the αv integrin subunit, is highly expressed in various tumors. Utilizing our proprietary site-specific glycan conjugation, we generated JSKN022, an innovative PD-L1/ITGB6/8 targeting ADC, which structurally composed of a sdAb (single domain antibody) Fc fusion protein targeting PD-L1 and ITGB6/8, a cleavable linker and a novel DNA topoisomerase I inhibitor (T01, Alphatecan). In this study, the pharmacological activity of JSKN022 was evaluated using preclinical in vitro and in vivo models.
Methods: Based on Envafolimab, we designed a novel sdAb-Fc fusion protein which targeting both PD-L1 and ITGB6/8. Then T01 was homogeneously and stably conjugated to glycan located on Fc. The conjugated product (DAR4) was thoroughly characterized by LC-MS. The binding specificity of JSKN022 and its internalization rate were assessed. Pattern of payload released were analyzed using LC-HRMS. Serum stability was tested across various species. In vitro cell growth inhibition and in vivo antitumor efficacy of JSKN022 were examined using a range of cancer cell lines and CDX models with parental antibody ADC as control.
Results: JSKN022 demonstrated specific binding to the αvβ6/8 protein, without cross-reactivity with other members of integrin family. JSKN022 exhibited a consistent DAR4. Bridging ELISA indicated that JSKN022 can simultaneously bind to both PD-L1 and ITGB6/8. Flow cytometry binding assays revealed that JSKN022 possessed a superior binding capacity compared to both parental antibodies. Correspondingly, the naked antibody of JSKN022 showed an increased internalization rate relative to parental antibodies in HCC4006 and BxPC-3 tumor cells. JSKN022 effectively inhibits the proliferation of antigen-positive cancer cells and exhibited enhanced tumor suppression compared to the single-target ADCs. Furthermore, benefiting from glycan conjugation, JSKN022 demonstrated excellent stability in serum from rats, mice, monkeys, and humans, with minimal T01 release.
Conclusion: This preclinical study suggests JSKN022, as a first in class multi-specific PD-L1/ITGB6/8 ADC, might potentially bring in novelty in the therapeutic approach for cancers that are refractory or resistant to PD-1/PD-L1 inhibitors.

#5451 JSKN021, an innovative site-specific dual-payload bispecific antibody drug conjugate targeting EGFR and HER3 exhibits potent preclinical activities.

Ting Xu, Fan Li, Zhilong Lai, Rupeng Qu

Jiangsu Alphamab Biopharmaceuticals Co., Ltd., Suzhou, China

Background: EGFR and HER3, both belong to the EGFR family, are widely overexpressed in human malignancies, rendering them promising targets for cancer treatment. Cancer is a heterogeneous disease. Inter- and intratumor heterogeneity is believed to be a major factor contributing to recurrence, metastasis and resistance to current socs. To address these therapeutic challenges, we generated JSKN021, a dual payload ADC targeting EGFR/HER3. Both payloads, a novel DNA topoisomerase I inhibitor (T01, Alphatecan) and MMAE, are conjugated to glycan on Fc via cleavable linkers. The conjugation processes were carried out via combination of glycan transferase and click reaction. The streamlined process generated site specific and homogeneous conjugation with DAR4(T01)and DAR2 (MMAE).In this study, the pharmacological activity of JSKN021 was evaluated using relevant in vitro cell killing assays and in vivo models.
Methods: A novel Topo 1 inhibitor T01 and MMAE were stably conjugated to an EGFR/HER3 mAb using a glycan based site-specific manner. The conjugated products were characterized by LC-MS. Binding specificity of JSKN021 was confirmed by ELISA. In addition, release of payload were evaluated by LC-HRMS. At last, in vitro cell growth inhibitory and in vivo antitumor activities of JSKN021 were evaluated using different cancer cell lines and CDX models in comparison to single payload ADCs.
Results: After conjugation, LC-MS results showed that JSKN021 had a homogeneous DAR4 for T01 payload and DAR2 for MMAE. JSKN021 was demonstrated to bind EGFR and HER3 simultaneously. In addition, both T01 and MMAE were detected in cell culture supernatant and suspension of HCC827. Yet, no other metabolites were found detectable. JSKN021 demonstrated excellent stability in rats, mice, monkeys, and human serum, with minimal payload release. The stability was derived from the advantage of glycan based conjugation. JSKN021 inhibited the growth of cancer cells with either Her3 or EGFR or both expression, such as HCC827, MDA-MB-468, A431 and NCI-H1975. Furthermore, JSKN021 showed stronger tumor inhibition efficacy than mono payload ADCs in multiple CDX models.
Conclusion: The preclinical studies suggest JSKN021, as a dual-payload EGFR/HER3 bispecific ADC, might be a promising novel antitumor therapeutic agent.

#5452 Novel tryptophan-based antibody-drug conjugate (ADC) targeting EGFR-positive lung cancer demonstrates robust antitumor efficacy by triggering immunogenic cell death.

Hiroyuki Inoue[1], Kounosuke Oisaki[2], Hiroshi Tagawa[3], Akira Nakao[1], Noriyuki Ebi[1], Takeshi Mori[3], Masaki Fujita[1]

[1]Department of Respiratory Medicine, Faculty of Medicine, Fukuoka University, Fukuoka, Japan,[2]Catalysis Chemistry Research Center, National Institute of Advanced Industrial Science and Technology (AIST), Ibaraki, Japan,[3]Department of Applied Chemistry, Faculty of Engineering, Kyushu University, Fukuoka, Japan

Background and Aim: Antibody-drug conjugates (ADCs), which combine a cytotoxic small molecule drug with an antibody to enable targeted delivery at tumor sites, are emerging as next-generation therapeutic options for non-small cell lung cancer with HER2 mutations. However, clinical development of ADCs for solid tumors has been hindered by hematologic toxicity, such as thrombocytopenia, caused by FcγRIIa-mediated uptake into megakaryocytes (antibody-dependent cellular cytotoxicity: ADCC), as well as the prolonged time and high cost required for linker optimization at cysteine (Cys) or lysine (Lys) residues on antibodies. This study aimed to develop a novel ADC targeting the human EGFR surface antigen, which is expressed in a broad range of lung cancers, through collaborative research in pharmaceutical engineering.
Method: We synthesized novel ADCs using our proprietary ABNO linker, which offers high stability through tryptophan (Trp) modification on anti-EGFR IgG4 antibodies. This approach allows for more homogeneous and stable ADC production at a lower development cost compared to conventional linkers. We assessed whether the synthesized ADCs eliminated ADCC activity in co-culture with NK cells and examined their ability to induce immunogenic cell death by evaluating calreticulin exposure on the surface of dying cancer cells.
Results: We successfully synthesized a novel ADC (Anti-hEGFR(IgG4)-ABNO-DM-1: E4-AD) by conjugating the cytotoxic drug DM-1 to an anti-human EGFR-IgG4 antibody using the ABNO linker. In CKK8 assays, E4-AD demonstrated potent, concentration-dependent cytotoxic activity against EGFR-positive human lung cancer cell lines, with IC_{50} values ranging from 2 to 20 nM, while showing no cytotoxicity in EGFR-negative lung cancer cells. Furthermore, E4-AD effectively abolished NK cell-mediated ADCC in vitro and significantly increased calreticulin exposure on cell surface and HMGB-1 release from dying lung cancer cells, indicating the induction of immunogenic cell death.
Conclusion: These findings suggest that the newly developed E4-AD, with its innovative tryptophan-based linker technology, holds promise as a safer and more effective ADC for treating EGFR-positive lung cancers.

#5453 E-M ADC, a promising EGFR-MUC1 bispecific antibody drug conjugate, with superior anti-tumor effect in a wide range of tumors.

Ziheng Liu, Le Sun, Bing Hu, Lingfeng You, Xinyu Gao, Jin Shao, Shimeng Liu, Beibei Fu, Ying Li, Manting Zhang, Dandan Xiong, Yan Liu, Guangbao Qiu, Ziwei Ren, Zhendong Xue, Yuchang Mao, Huaixin Dang, **Ting Zhang**, Xin Ye, Shuyu Cai, Jun Feng, Min Hu, Feng He

Shanghai Hengrui Pharmaceutical CO., LTD., Shanghai, China

Mucin 1 (MUC1) is a transmembrane glycoprotein with abundant glycosylation. In normal tissues, MUC1 is present at the apical surface of epithelial cells, while epidermal growth factor receptor (EGFR) is present at the basal surface of epithelial cells. Tumor cells lose apical-basal polarity, leading to even distribution of EGFR and MUC1 on cell surface, a phenomenon that allows EGFR and MUC1 to be spatially close together. And EGFR and MUC1 are highly co-expressed in a variety of tumors, e.g., non-small cell lung cancer (NSCLC), esophageal squamous cell carcinoma (ESCC) and breast cancer (BC), among others. Accordingly, bispecific ADCs targeting EGFR and MUC1 preferentially target dual-expressed tumor cells, reducing the binding to normal tissues, meanwhile conjugating with TOPi, which can improve the efficacy. And the EGFR-MUC1 bispecific ADCs have a wider range of indications and patient populations. E-M-TOPi, a EGFR and MUC1 bispecific ADC, consists of a EGFR-MUC1 bispecific antibody (E-M), a cleavable linker and a topoisomerase I inhibitor. TOPi exhibited potent cytotoxicity with good membrane permeability. The binding affinities of E-M to EGFR and MUC1 were optimized to enhance tumor cell selectivity, increase endocytosis, and improve pharmacokinetics (PK). Considering the wide expression of EGFR in normal tissue, the binding affinity of E-M to EGFR was reduced to be weaker than that to MUC1, thereby enhancing the selectivity for EGFR-MUC1 co-expressing tumors while minimizing the toxicity to normal tissues. E-M-TOPi demonstrated significantly stronger EGFR/MUC1 dependent growth inhibition in various tumor cell lines. In HCC827 and HPAC (different EGFR-MUC1 co-expression levels) xenograft mouse models, E-M-TOPi inhibited tumor growth in a dose-dependent manner. Tumor regression was observed at 6 mg/kg of E-M-TOPi and sustained till day 30 in HCC827 model and day 28 in HPAC model. In addition, E-M-TOPi showed good human plasma stability and favorable cyno PK profile. Less than 0.5% of payload was released into human plasma after a 21-day incubation. Half-life of E-M-TOPi was more than 4 days in cyno monkey. In summary, by fine-tuning the EGFR-MUC1 bispecific antibody affinity and incorporating a superior bystander effect, E-M-TOPi holds the potential to be a best-in-class therapeutic candidate.

#5454 DB-1325, a BCMA-targeting antibody-drug conjugate with a Duality Unique Payload, exhibits promising efficacy and safety in preclinical multiple myeloma treatment.

Shengchao Lin, Bing Li, Yu Zhang, Jun Yao, Rong Shi, Yang Qiu, **Haiqing Hua**

Duality Biologics, Shanghai, China

Introduction: B cell maturation antigen (BCMA) overexpression has been found on the surface of the malignant plasma cells in the bone marrow of multiple myeloma (MM) patients. In recent years, innovative medicines targeting BCMA such as antibody-drug conjugates (ADC), T-cell engager and CAR-T have made great progress in the treatment of MM patients. Among them, belantamab mafodotin has been approved by FDA. However, a significant fraction of patients does not respond, or respond for a short period of time, to belantamab mafodotin. Thus, there remains unmet medical needs for BCMA-targeting ADC with novel mode of actions. DB-1325 is a novel BCMA-targeting ADC comprised of belantamab and PDUP5, a Duality Unique Payload (DUP) inhibiting mRNA translation, conjugated through a cleavable linker.

Methods: A proprietary inhibitor of mRNA translation PDUP5 was discovery through a campaign of medicinal chemical design and screening. A cleavable linker was designed to be attached to PDUP5, resulting in LPDUP5. Belantamab was conjugated with LPDUP5 to yield DB-1325. The therapeutic profile of DB-1325, including in vitro and in vivo efficacy, and its safety profile was assessed in preclinical models.

Results: PDUP5 exhibited sub-nanomolar to nanomolar potency in *in vitro* cell killing test on various MM cell lines. DB-1325 (belantamab-LPDUP5) showed robust in vitro anti-proliferation activity in multiple BCMA-expressing cell models (e.g. NCI-H929, KMS-11, MM1S, MOLP8) with activity similar to belantamab mafodotin. In addition, we observed robust efficacy of DB-1325 in several in vivo xenograft models following a single dose treatment. In NCI-H929 CDX model, treatment with DB1325 demonstrated anti-tumor activity superior to belantamab mafodotin when given at an equivalent dose. Furthermore, DB-1325 was well-tolerated at a preliminary toxicology assessment using monkeys, with favorable toxicokinetic profile.

Conclusions: DB-1325 is a novel and promising therapy for MM and currently in preclinical development as a first-in-class ADC with mRNA translation inhibitions as its major mode of action.

#5455 TAVO605, a novel CD318 (CDCP1) ADC, for the treatment of solid cancers that are non-responding or resistant to Enhertu treatment.

Guangmao Mu[1], Fulai Zhou[1], Honglei Bi[1], Zhengxia Zha[1], Sheng Huang[1], Ying Jin[1], Mingcan Yu[2], Chao Han[2], Mark Chiu[2], **Di Zhang**[2]

[1]Tavotek Biotherapeutics, Suzhou, China,[2]Tavotek Biotherapeutics, Lower Gwynedd, PA

Background: CD318, also known as Cub Domain Containing Protein 1 (CDCP1), is a single span transmembrane receptor containing three Cub domains with low level expression in healthy epithelial tissues. The CD318 expression is significantly upregulated in response to RAS transformation in several major types of solid tumors, including breast, lung, pancreatic, colorectal, bladder and prostate cancers. CD318 is an important mediator of aberrant cancer-associated cascades critical for survival, growth, metastasis, and treatment resistance of tumor. The elevated expression of CD318 is associated with poor prognosis and overall survival of cancer patients.

Methods and Results: We discovered several anti-CD318 antibodies with high binding affinities and prepared ADC conjugates with MMAE/MMAF. The CD318-ADC showed potent cytotoxicity to multiple cancer cell lines in *in vitro* cytotoxicity assays. The cytotoxicity activities correlated with the cell surface receptor densities and the binding affinities of CD318 antibodies. In multiple CDX and PDX solid tumor xenograft models involving pancreatic, colon, triple negative breast and non-small cell lung cancer, CD318-MMAE (DAR=4) showed potent tumor growth inhibition activities with efficacies comparable to or better than Enhertu (DS-8201). By employing an anti-CD318 antibody with cross-reactivity to murine CD318, we confirmed that the CD318-ADC with high drug dosing level was tolerable in a mouse toxicity study.

Conclusions: By targeting a novel tumor associated antigen CD318 (CDCP1) with high expression in multiple major solid tumors, our lead CD318-ADC molecule (TAVO605) showed potent cytotoxicity to multiple tumor cell lines as well as in CDX and PDX solid tumor xenograft models. CD318-ADC was tolerable and will be further evaluated in preclinical monkey toxicity studies. Our data supported the further development of TAVO605 as a potent therapy for solid tumors non-responding or resistant to current available treatments, e.g., by Enhertu, especially in tumors with low or no HER2 expression such as triple negative breast cancers. This antibody can also be used in other modalities to control solid tumors.

#5456 Development of human antibodies targeting a novel cancer-associated fibroblast protein identified from spatial transcriptomics.

Jin Yeong Choi[1], Shengjun Li[2], MinKyu Kim[1], Yeonjae Jung[1], Jae Eun Lee[1], Daeseung Lee[1], Hongyoon Choi[1], Hyung-Jun Im[1]

[1]Portrai, Inc., Seoul, Republic of Korea, Seoul, Korea, Republic of,[2]Graduate School of Convergence Science and Technology, Seoul National University, Seoul, Korea, Republic of

Background Cancer-associated fibroblasts (CAFs) are key components of the tumor microenvironment and play a critical role in tumor progression and therapy resistance. Recent advances in spatial transcriptomics (ST) enable precise analysis of gene expression within the spatial context of tissues, offering new avenues for target discovery. Using spatial and single-cell transcriptomic data, we identified a novel target protein, POR-CAF-001, predominantly expressed in CAFs within solid tumor tissues and minimally in normal tissues, making it a promising candidate for therapeutic intervention. **Method** Spatial and single-cell transcriptomics data from human tumor samples were analyzed using our proprietary target discovery program to identify CAF-specific targets. Two human IgG antibodies (POR-CAF-001-01 and POR-CAF-001-02) against the POR-CAF-001 were generated through phage display, ELISA screening and recombinant production in mammalian cells. Surface plasmon resonance (SPR) was employed to validate a binding affinity of antibodies against POR-CAF-001 protein. Also, flow cytometry and immunocytochemistry (ICC) were performed to evaluate the binding specificity of the antibodies. Antibody internalization was assessed via an imaging-based internalization assay using pH-sensitive fluorophore-labeled antibodies, and their internalization was monitored over time. POR-CAF-001-positive, -negative and knockout cell lines were used to confirm binding specificity and internalization. **Results** Differential expression and spatial specificity analyses identified POR-CAF-001 with high expression in various types of tumor tissues with minimal expression in normal tissues. SPR analysis demonstrated binding affinities (KD) of 1.15 nM and 4.1 nM for POR-CAF-001-01 and POR-CAF-001-02, respectively. Flow cytometry and ICC analyses confirmed robust binding of both antibodies to POR-CAF-001-positive cells, with negligible binding in POR-CAF-001-negative and the target knockout cells. Both antibodies showed substantial internalization in POR-CAF-001-positive cells during the internalization assay, while minimal internalization was observed in the negative cell line. **Conclusion** Through our target discovery program with ST data, we identified POR-CAF-001 as a novel CAF-specific target protein and developed two human IgG antibodies against it. These antibodies demonstrated high specificity and successful internalization in target-positive cells. These findings provide a solid foundation for further preclinical development, including antibody-drug conjugates (ADCs), radiopharmaceutical therapy (RPT) or other therapeutic modalities targeting POR-CAF-001. Our study underscores the potential of ST in identifying novel targets and advancing cancer therapy.

#5457 Dual-antigen bridging ELISAs to validate bispecific molecules with CD3-directed T cell engager function.

Connie Zhang, Katarzyna Zientara-Rytter, Moji Samoudi, Sean Green, Kimberly Skuster, Charlene Chang, Mohammad Awad, **Veronique Baron**, Michelle Kinbara, Jonathan Mikolosko, Pavel Shashkin

BPS Bioscience, Inc., San Diego, CA

Bispecific antibodies (bsAbs), designed to recognize two distinct antigens or epitopes, represent a promising class of next-generation biological therapeutics. These macromolecules, capable of engaging multiple targets, have gained significant interest over the past decade for their potential to harness the immune system against specific targets. With additive or synergistic mechanisms of action, bsAbs and other engineered dual-targeting molecules often exhibit greater potency than monoclonal antibodies (mAbs) or combination therapies. By simultaneously bridging two targets, bsAbs provide additional clinical benefits, such as enhanced target cell killing. Therefore, assessing their ability to bind both targets concurrently is essential for advancing their therapeutic potential. CD3-bispecific antibodies (CD3-bsAbs) have emerged as a promising approach in cancer immunotherapy, particularly for hematological cancers. These bsAbs activate T cells by binding both a tumor-associated antigen (TAA) on tumor cells and CD3 on T cells, leading to tumor cell destruction. Recent advancements in bsAb biology and technologies have enabled the development of diverse CD3-bsAb formats, ranging from small fragments with dual variable domains to larger structures incorporating additional domains linked to conventional immunoglobulins. Here, we present dual-target bridging ELISAs for bispecific molecules designed to engage T cells via CD3 binding. These assays are simple, cost-effective, and require no elaborate equipment, making them accessible to most laboratories. By leveraging the bridging capability of bispecific molecules, the method uses an immobilized capture recombinant antigen 1 and a CD3-containing detection reagent (antigen 2) to assess both binding events simultaneously, regardless of molecule format, isotype, or species of origin. Our assays feature low background, high signal-to-noise ratios, and compatibility with purified proteins, human serum, or cell culture supernatants, making them ideal for evaluating bsAb bridging abilities in drug discovery and high-throughput screenings (HTS). Additionally, the all-in-one HRP-conjugated CD3 complex simplifies the protocol by reducing steps what makes these assays less time consuming and more cost effective when compared to surface plasmon resonance (SPR) or cell-based bioassays.

#5459 Accelerating the development of biologics: PRISM high throughput screening enables rapid preclinical characterization of antibodies, ADCs and payloads.

Jillian N. Eskra, Alvin Kalathungal, Aydin Golabi, Bailey Porter, Antonella Masciotti, Nashielli Diaz-Gallegos, Anup Jonchhe, Maha Naim, Lia Petronio, Maggie LeMaitre, Jacob Asiedu, Ashish Bino George, Mustafa Kocak, Melissa M. Ronan, Matthew G. Rees, Jennifer A. Roth

Broad Institute of MIT and Harvard, Cambridge, MA

Biologic agents, such as monoclonal antibodies and antibody-drug conjugates (ADCs), have emerged as a highly promising class of the cancer therapeutics due to their ability to target specific tumor antigens with high specificity, be engineered for selective delivery of cytotoxic payloads, and work through diverse mechanisms of action. While these complex characteristics render biologics highly effective in vivo, they pose difficulty for systematically evaluating these agents in vitro. Here, we describe a multiplexed screening approach to overcome these challenges using the PRISM assay, where antibodies and ADCs are screened across 900+ cancer cell lines. We first investigated a panel of HER2-targeting ADCs conjugated to several different linkers and payloads. We assessed target selectivity and observed a clear association between cell line sensitivity and HER2 expression, with the strongest effects on viability observed in a subset of cell lines overexpressing HER2. We further compared profiles across different ADC targets and payloads to investigate predictive biomarkers of payload sensitivity and characterize bystander killing capabilities. Finally, we developed an antibody internalization reporter (AIR) assay to enable screening of unconjugated monoclonal antibodies. We optimized the AIR methodology using a secondary Fab-drug conjugate which can be used to load drug payload onto any IgG antibody to target and kill cells that are recognized by the primary antibody. To validate this approach, we screened trastuzumab in combination with a secondary Fab conjugated to MMAF via a non-cleavable linker. Comparative profiling with trastuzumab directly conjugated to the same payload revealed similar cytotoxic potency and target selectivity. These results demonstrate how the PRISM AIR method can enable screening of antibody candidates for ADC development to evaluate antigen-specific binding, internalization and cell killing potential. Overall, our study highlights the capabilities of the PRISM assay for investigating antibodies and ADCs.

#5460 C6P, a claudin 6 (CLDN6) directed ADC containing pyrolobenzodiazepine (PBD) for the treatment of advanced solid tumors.

Xiaolan Yu, Shuting Wang, Lingfeng You, Zhibin Xu, Zhendong Xue, Yuchang Mao, Shuyu Cai, Jun Feng, **Ting Zhang**, Xin Ye, Min Hu, Feng He

Shanghai Hengrui Pharmaceutical CO., LTD., Shanghai, China

Claudin 6 (CLDN6), a member of the claudin family of tight junction proteins, is highly expressed in multiple solid tumors such as ovarian and endometrial cancers with limited expression in normal tissues. Here we developed an anti-CLDN6 ADC C6P comprising a Fc-silenced anti-CLDN6 monoclonal antibody, a cleavable linker and a modified PBD (compound A). C6P selectively bound to CLDN6 without cross-reactivity to other claudin family members such as CLDN 3/4/5/8/9. Additionally, it also bound to I143V-mutated CLDN6, a major single nucleotide polymorphism (SNP) in human population. The antibody demonstrated strong binding affinity to several ovarian cancer cell lines with different CLDN6 expression levels and showed good endocytosis. Compound A was an optimized mono-imine PBD dimer with less cytotoxicity than conventional bis-imine PBD dimers. High systemic clearance of this payload was also designed to minimize toxicities. To achieve homogeneous ADC, a glycan mediated site-specific conjugation was used to generate C6P. C6P showed CLDN6-dependent growth inhibition against various cell lines, without effect on CLDN6 negative cells. In PA-1 and OVCAR3 xenograft mouse models, C6P showed a dose-dependent anti-tumor effect and led to robust and sustained tumor regression. C6P demonstrated good stability and safety profiles, likely due to site-specific conjugation and the higher systemic clearance of compound A. Less than 1% payload release was observed in human plasma after a 21-days incubation. C6P was well-tolerated at a single dose of 0.5 mg/kg in cynomolgus monkeys and did not show hematology-related toxicity. The intact ADC/total antibody AUC ratio was above 95%, and the payload was undetectable in the plasma of cynomolgus monkeys after injection with C6P. Overall, the highly specific CLDN6-targeting antibody, the optimized PBD payload, site-specific conjugation and well-controlled DAR value collectively contributed to the potent anti-tumor efficacy, and good safety profile of C6P, supporting its future clinical research.

#5461 BG-C477, a novel topoisomerase 1 inhibitor-based ADC, exhibits antitumor activity in carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5)-expressing preclinical models.

Daochuan He, Yiren Xiao, Zhuo Li, Jie Li, Hongyu Chen, Weijie Li, Huang Yan, Kunji Liu, Yang Lyu, Qingsong Hu, Mengjia Wang, Lai Xu, Zhitao Wan, Xiaomin Song, Yu Shen, Lai Wang

BeiGene Ltd., Shanghai, China

CEACAM5 (CEA) is a cell surface glycoprotein highly expressed in many cancers, including colorectal cancer (CRC), gastric cancer (GC), lung and pancreatic cancers. BG-C477 is a novel ADC that is composed of a CEACAM5-specific antibody conjugated to Top1i payloads (drug-to-antibody ratio = 8) via a cleavable linker. It remains stable in human plasma and mice blood circulation. BG-C477 showed specific binding to CEA-expressing MKN45 cells and stronger internalization activity compared to anti-CEA DM4 ADC. BG-C477 exhibited robust and CEA-dependent cytotoxicity in cell lines with different CEA expression levels. A potent bystander effect of BG-C477 was observed in co-culture killing assay with mixed CEA-positive MKN45 and -negative HCT116 cells, indicating the potential to treat tumors with heterogeneous CEA expression. In the animal studies, BG-C477 demonstrated potent and dose-associated single-agent efficacy in multiple CEA-expressing cell line-derived xenograft (CDX) and patient-derived xenograft (PDX) models. Moreover, BG-C477 exhibited superior antitumor efficacy in GC and CRC PDX models that anti-CEA DM4 ADC was ineffective.
In summary, BG-C477 is a promising CEA targeting ADC with great anti-tumor activity across multiple types of tumors. The first in human study to evaluate the safety, tolerability, pharmacokinetics, pharmacodynamics, and preliminary antitumor activity of BG-C477 is ongoing (NCT06596473).

#5462 AMB303: A novel topo I payload ADC showing efficacy in NSCLC and TNBC, with potential for immune checkpoint combination therapy.

Do-Hyun Nam[1], Yuna Park[2], Byeongkwi Min[2], Wonsik Jung[2], Yunsu Park[2], Hye-Mi Kim[2], Min-A Park[2], Nam-Gu Her[2]

[1]Samsung Medical Center, Seoul, Korea, Republic of,[2]Aimed Bio Inc., Seoul, Korea, Republic of

Background: AMB303 is an ADC targeting ROR1 (Receptor Tyrosine Kinase-Like Orphan Receptor 1), a tumor-specific protein highly overexpressed in tumors. ROR1 is crucial for cancer cell differentiation, proliferation, migration, and metastasis, which drive recurrence and therapy resistance. These tumor-specific functions make ROR1 a highly attractive candidate for ADC development, offering the potential for more precise and effective cancer treatments. To optimize the therapeutic potential of AMB303, a novel topoisomerase I (Topo I) inhibitor payload has been incorporated, designed to overcome the limitations of existing Topo I inhibitors in clinical use. This novel payload aims to enhance the efficacy of AMB303, addressing drug resistance and minimizing the risk of tumor recurrence, offering a more effective approach to cancer treatment.
Method: The novel antibody and drug-linker components of AMB303 were optimally characterized and integrated. The binding affinity and cellular internalization of the AMB303 were assessed in cells with high ROR1 expression. In vivo efficacy was evaluated using a non-small cell lung cancer (NSCLC) patient-derived xenograft (PDX) model, while triple negative breast cancer, colorectal, and ovarian cell-derived xenograft (CDX) models were used to investigate the correlation between in vivo efficacy and target expression levels. Additionally, the combination of AMB303 with mouse PD-1/PD-L1 inhibitors was tested to evaluate antitumor activity in syngeneic mouse models.
Results: AMB303 has demonstrated excellent antitumor efficacy in in vitro and in vivo studies. By combining the new Topo I with an antibody showed target-specific binding affinity and rapid internalization, it also showed stronger cytotoxic effect compared to the clinically validated TopoI. Additionally, AMB303 demonstrated robust antitumor effect in NSCLC PDX and CDX models, including breast and colon cancer. In comparative studies with a benchmark ADC in clinical trials, AMB303 exhibited superior in vivo efficacy in a lung PDX model. In syngeneic mouse models, AMB303 demonstrated potential for enhanced efficacy when combined with PD-1/PD-L1 inhibitors.
Conclusion: AMB303, an ADC conjugated with a novel Topo I inhibitor, is a potent therapeutic designed to target both primary tumor cells and recurrence or relapsed cancer tissues. When combined with immune checkpoint inhibitors, AMB303 demonstrated enhanced therapeutic efficacy, presenting a promising strategy to overcome immune resistance and improve treatment outcomes in cancer therapy.

#5463 AMB304: A next generation ADC with topo I inhibitor, demonstrating robust preclinical efficacy against target overexpression tumors and high tolerability in non-human primate (NHP).

Do-Hyun Nam[1], Yeong-Woo Kim[2], Byeongkwi Min[2], Wonsik Jung[2], Eunsuk Choi[2], Jungsu Shin[2], Nam-Gu Her[2]

[1]Samsung Medical Center, Seoul, Korea, Republic of,[2]Aimed Bio Inc., Seoul, Korea, Republic of

Background: AMB304 is an antibody-drug conjugate (ADC) combining a human IgG1 monoclonal antibody and a high efficacious topoisomerase I inhibitor (Topo I) payload with site-specific conjugation. The target of AMB304 is a cell surface expressed glycoprotein, elevated in a range of tumors, and is associated with cancer survival, growth, metastasis, drug resistance and poor prognosis. This target is highly expressed in various epithelial cancers, including triple negative breast cancer and non-small cell lung cancer. Additionally, advanced malignancies with limited treatment options also demonstrate high target expression, suggesting that AMB304 may provide potential clinical benefits. Furthermore, elevated expression of the target is associated with resistance to anti-tumor drugs, such as EGFR TKIs (e.g., Gefitinib) and trastuzumab, via signaling. In this study, we investigated the cancer-specific therapeutic potential of AMB304, focusing on its preclinical characterization, including anti-cancer efficacy and safety profile.
Methods: Various types of tumor cell lines were treated with AMB304 using tumor spheroid model. For in vivo efficacy study, cancer cell lines or patients derived cells were inoculated subcutaneously in mice xenograft models. Target expression intensity in each tumor model was identified through immunohistochemistry (IHC). The toxicity profiles of AMB304 were analyzed, and its pharmacokinetics were evaluated in cynomolgus monkeys.
Results: AMB304 exhibited robust potency against target-expressing cancer cell lines, as evidenced by cell viability assay and compelling efficacy in various xenograft models, with IHC confirming its tumor regression depends on target expression. It exhibited target-specific binding, efficient internalization, and a strong bystander killing effect. In cynomolgus monkeys, AMB304 was well- tolerated with no significant adverse events at tolerable doses. Its stable linker chemistry minimized payload release, and it showed favorable pharmacokinetics, supporting its clinical potential.
Conclusion: This study demonstrates that AMB304 is potent ADC with significant anticancer efficacy and an excellent safety profile. Moreover, its superior stability in plasma enhances its potential for clinical treatment. Therefore, AMB304 could be developed as a targeted therapy for advanced solid tumors with high target expression.

#5464 A novel T-cell-engager targeting BCMA and GPRC5D with balanced killing efficacy to overcome potential resistance mechanisms in multiple myeloma treatment.

Xiaochuan Ma, Hanxiao Ying, Chunhong Yu, Ting Qiu, Shu Wang, Pu Pu, Zhibin Xu, Zhendong Xue, Shuyu Cai, Jun Feng, **Yi Mao**, Min Hu, Feng He

Shanghai Hengrui Pharmaceutical CO., LTD., Shanghai, China

B-cell maturation antigen (BCMA) and G protein-coupled receptor class C group 5 member D (GPRC5D) are critical surface markers expressed on multiple myeloma (MM) cells. BCMA-targeting T cell engagers (TCEs) and CAR-T therapies have achieved significant success in late-line MM treatment. However, antigen loss or downregulation, as well as point mutations, can result in relapse and resistance. Presence of soluble BCMA in circulation due to protein shedding also jeopardized the efficacy of BCMA-targeted therapies. GPRC5D exhibits both overlapping and independent expression profiles with BCMA, making it a compelling complementary target. Dual-targeting strategies leveraging these two antigens thus offer a promising approach to overcome resistance, improve therapeutic durability, and enhance clinical outcomes. Here, we developed a novel tri-specific TCE targeting BCMA, GPRC5D, and CD3 to optimize T cell-mediated cytotoxicity in MM. This molecule exhibited potent and selective tumor cell lysis in T cell-directed killing assays. Notably, it demonstrated balanced killing activity in cells expressing either BCMA or GPRD5D, reducing dependency on a single target antigen. Moreover, the molecule maintained its efficacy and potency in the presence of high levels of soluble BCMA (sBCMA) and overcame resistance associated with BCMA point mutations observed in patients treated with current approved TCEs. The molecule displayed potent anti-tumor efficacy in vivo in RPMI8226 cell-derived-xenograft models with hPBMC reconstitution. In addition, the molecule was well tolerated in cynomolgus monkeys with favorable pharmacokinetics and deep B cell depletion. In summary, this dual-target TCE offers a promising therapeutic option for addressing resistance of current approved TCEs, and further improving clinical outcomes in MM patients.

#5465 A novel CD3 VHH—based multi-specific T cell engager platform and multiple leads for gastric cancer and auto-immune disease.

Toya N. Baral[1], Jia Yu[2], Weihong Wang[1], Huiyuan Tang[1], John Lee[1], Leo Ng[1], Jing Yu[2], Peng Bo[2], Yong Wang[1], Jiaguo Li[2], Xinhao Wang[1], Qijun Qian[2], **Wenfeng Xu**[1]

[1]Chantibody Therapeutics Inc., Menlo Park, CA,[2]Shanghai Cell Therapy Group Co., Ltd, Shanghai, China

T cell engagers (TCEs) have recent approvals and great potential in the treatment of various diseases such as cancer and auto-immune diseases. The challenge of TCE in solid tumor is the therapeutic window due to limited tumor specific targets, and the CD3 arm requires fine tuning for specific targets and indications. The anti-CD3 used in current TCEs are monoclonal based and are limited in diversity of epitope and potency, which narrows the full potential of TCEs. VHHs being single domain, are ideal for bi- and multi-specific molecule design and engineering. We have identified anti-CD3 VHHs with diverse portfolio using in house llama, alpaca, and camel immunization with cells, protein and mRNA. We utilized VHHMAb® platform to capture full repertoire of the binders. The humanized anti-CD3 VHHs are diverse in epitope, affinity, T cells activation and in T cell dependent cytotoxicity. We have identified NHP cross binding anti-CD3 with great value for pre-clinical evaluation of TCE. We utilized in house developed AI-powered lead design for optimal TCE structure for best synapse between T cell and target cells. AI powered method was also used to design the TCEs for better developability attributes such as expression, solubility, aggregation. The TCEs, we have generated are with all single domain VHHs (anti-CD3 and TAA targeting) and are first of such kind. Colorectal cancer (CRC) has huge unmet clinical need as current treatments have limited clinical efficacy. We have identified targets which are differentially and restrictively expressed in the CRC in comparison to healthy tissues. Beside the tumor specific expression, the specific epitope on target have impact on effective T cell mediated killing of TCE. The membrane proximal domains are desired for TCE. With the VHHMAb® platform we have identified sets of VHHs which target different domains of two novel CRC targets. These VHHs are cross species (hu, NHP and mouse) binders that provide seamless path of pre-clinical to clinical development. With the binders of diverse epitopes on tumor antigen and various affinity and potency anti-CD3 VHH, we have generated TCEs that are functionally the best possible. Therapeutic efficacy, durability and safety are unmet medical needs in auto-immune diseases. For single target therapeutics, relapse due to antigen escape is one of the major problems. We have identified VHHs with NHP cross binding for multiple B cell targets of auto-immune diseases. Due to the single domain and modular nature of VHHs, we have generated TCEs with multi-targeting for better efficacy, durability and potentially overcome the relapse. Conclusion: We have generated diverse humanized anti-CD3 VHHs, different epitope and domain binding VHHs for target antigens. Due to modular nature of VHHs, we have generated multiple potentially FIC and or BIC TCE lead molecules which are composed of all VVHs arms, for CRC and auto-immune diseases.

#5466 7MW4911, a novel cadherin 17-targeting ADC, demonstrates potent efficacy in preclinical models of gastrointestinal cancers.

Rui Wang[1], Peng Fang[2], Wei Zhou[1], Cuicui Guo[1], Xiaoding Tan[2], Hai Wu[1], **Xun Gui**[1]

[1]Mabwell (Shanghai) Bioscience Co., Ltd., Shanghai, China,[2]Jiangsu Mabwell Health Pharmaceutical R&D Co., Ltd., Taizhou, China

Background: Cadherin 17 (CDH17), a member of the 7D-cadherin superfamily, is critically involved in intercellular adhesion, tissue integrity, and intestinal peptide transport. It is predominantly expressed on the basolateral membrane of intestinal and pancreatic ductal epithelial cells, with minimal expression in non-gastrointestinal (GI) tissues. Overexpression of CDH17 is strongly associated with tumor progression and metastasis in colorectal, gastric, and pancreatic cancers, making it an attractive target for therapeutic intervention. Here, we report the development of 7MW4911, a novel ADC designed to selectively target CDH17-positive tumors while sparing normal tissues.
Methods: CDH17 expression as a therapeutic target was validated using comprehensive omics analyses. An anti-CDH17 specific mAb Mab0727 was generated using Mabwell's integrated novel antibody discovery system. The ADC 7MW4911 was constructed by conjugating Mab0727 to the proprietary payload MF-6 via a cleavable linker, achieving a drug-to-antibody ratio (DAR) of 4 using site-specific inter-chain disulfide bond conjugation technology. Binding affinity, internalization, and cytotoxicity of 7MW4911 were evaluated in CDH17-positive GI cancer cell lines. Efficacy was further assessed in cell line-derived xenograft (CDX) and patient-derived xenograft (PDX) models. Pharmacokinetics and safety were studied in cynomolgus monkeys.
Results: Immunohistochemistry (IHC) analysis confirmed CDH17 as a highly specific target, with expression observed in 100% (110/110) of colorectal, 77% (110/142) of gastric, and 55% (58/105) of pancreatic cancer samples. 7MW4911 demonstrated strong binding, efficient internalization, and potent cytotoxicity in CDH17-positive cell lines. *In vivo*, 7MW4911 showed substantial tumor growth inhibition (TGI), with TGI ranging from 29-97% in nine colorectal CDX models, 56-95% in three gastric models, and 81% in one pancreatic model. In PDX models, TGI ranged from 71-99% across nine colorectal and one gastric model. Importantly, 7MW4911 outperformed MMAE-based ADCs in multidrug-resistant CDX and PDX models. Pharmacokinetic studies in cynomolgus monkeys demonstrated a favorable safety profile, with a maximum tolerated dose exceeding 20 mg/kg and minimal off-target toxicity.
Conclusions 7MW4911 demonstrated exceptional preclinical efficacy, including robust activity against multidrug-resistant cancers, along with a strong safety profile. These findings highlight its potential as a transformative therapeutic candidate for CDH17-positive GI cancers, addressing significant unmet needs in oncology.

#5467 Therapeutic potential of antibody-drug conjugate with PARP1 selective inhibitor as a payload: A promising therapeutic approach for potent antitumor efficacy with reduced toxicity.

Jina Koo, Jeong Eun Kim, Hyeon Woong Park, Won Sik Lee, Myongjae Lee

Idience, Seoul, Korea, Republic of

Poly (ADP-ribose) polymerase inhibitors (PARPi) have been extensively studied as anticancer agents that selectively induce cancer cell death by disabling complementary DNA repair pathways. Despite PARPi's initial effectiveness, tumors often develop drug resistance, limiting the long-term effectiveness of these drugs. Combining PARPi with chemotherapeutics or other DNA damage response inhibitors have been explored to enhance the efficacy and overcome resistance. However, these combinations had significantly more side effects, highlighting the need for innovative therapeutic approaches that preserve anti-tumor efficacy while minimizing side effects. Incorporating PARPi as a payload within an antibody-drug conjugate (ADC) may offer a promising strategy for targeted delivery to tumor cells, to overcome drug resistance and to reduce systemic toxicity. To establish proof-of-concept, a TROP2 antibody was conjugated with a PARP1 selective inhibitor using a cleavable linker. *In vitro* cytotoxicity assays were conducted in MDA-MB-436, MDA-MB-468, and HCC1937 cell lines to compare the effects with the naked mAb, ADC, and free payload. An *in vivo* efficacy study was conducted using the MDA-MB-436 subcutaneous xenograft model in NOD-SCID mice. The PARPi payload was confirmed to be PARP1 selective via surface plasmon resonance and trapping assays. *In vitro*, ADC demonstrated potent cytotoxicity in MDA-MB-436 cells, which are known to be PARPi-sensitive with low levels of TROP2 expression, with an IC50 value of approximately 1 nM, whereas the naked mAb showed no cytotoxicity. In contrast, MDA-MB-468 and HCC1937 cells (with high levels of TROP2 expression and homologous recombination proficiency) showed cytotoxicity, only with free payload at high concentrations. *In vivo*, ADC and the free payload induced tumor regression and sustained antitumor effects, whereas the naked TROP2 mAb showed no efficacy. Hematological analysis reveals that the ADC-treated groups showed improved side effects compared to the free payload group. This study highlights the potential of a PARP1 selective inhibitor as an ADC payload, utilizing ADC as a drug delivery system, to achieve substantial antitumor activity with reduced side effects *in vitro* and *in vivo*. These findings emphasize the enhanced safety of PARP1i-based ADC compared to standalone PARP1 inhibitor, enabling novel strategies such as combination therapies or dual-payload ADC apporaches to effectively overcome drug resistance.

#5468 A novel kinase degrader antibody conjugate for the treatment of mCRPC.

Joost C. M. Uitdehaag, Jos de Man, Freek van Cauter, Sander P. W. van Gemert, Yvonne G.T. H. van Mil, Milan J. Hoffmann, Daphne Montizaan, Winfried R. Mulder, Michelle Muller, Martine B. W. Prinsen, Jan Gerard Sterrenburg, Diep Vu, Joeri J. P. de Wit, Erik Ensing, Rogier C. Buijsman

Crossfire Oncology B.V., Oss, Netherlands

Antibody Drug Conjugates (ADCs) represent an efficacious therapeutic modality. However, the classical payloads currently employed are limited in diversity, targeting mostly DNA, topoisomerases or tubulin and are sensitive to developing resistance. The high off-tumor toxicity observed with these payloads furthermore leads to a narrow therapeutic window. To overcome these challenges there is an urgent need for novel payloads that have a different mechanism of action and a more benign free-payload toxicity profile.

Kinases play an essential role in the development and growth of many different cancers and thus form a target class of particular interest for novel payloads. To date, over 80 kinase inhibitors have been developed as therapeutic agents. The vast majority, however, lacks the potency required for an ADC payload. One of the main reasons for this is their inability to abrogate the scaffolding function of kinases, which is known to contribute to their oncogenic signaling. For this reason, heterobifunctional kinase degraders have gained interest. These are small molecules that mediate the degradation of a kinase-of-interest by the proteasome. Their catalytic functionality endows them with high potency. An ADC with a degrader-based payload is generally referred to as a Degrader Antibody Conjugate (DAC).

We selected a cell cycle kinase that is known to be a tumor driver in many types of cancer. Knocking down this kinase using CRISPR technology is cytotoxic to many cancer cell lines representing major tumor indications (DepMap database). Using libraries of kinase ligands, spacers and E3 ligase ligands we identified degraders that rapidly and deeply degrade the kinase target and have potent antiproliferative activity in cancer cell lines, including those resistant to classical payloads. Degrader payloads were further selected for high microsomal lability, predicting rapid liver clearance, contributing to a more benign free payload toxicity profile. Mechanistic studies show that our lead degrader has a unique mode of action that results in complete cell cycle disruption, leading to DNA damage and potent immunogenic cell death.

Our lead degrader was coupled to various maleimide-based hydrophilic linkers and conjugated to clinically validated antibodies to generate DACs. The high hydrophilicity of the linker-degrader combination allows for the generation of DACs up to DAR8 while retaining good monomericity and stability.

Active DACs for various indications were generated, showing the wide applicability of our cell cycle kinase degrader-based payload. Our lead DAC for the treatment of metastatic castrate resistant prostate cancer (mCRPC) shows sub-nanomolar cytotoxic activity, potent target degradation, and effective bystander kill. The lead DAC was tested in a therapeutic *in vivo* xenograft model for prostate cancer and is currently progressing towards clinical development for mCRPC.

#5469 Preclinical safety and efficacy of SOT109, an antibody-drug conjugate targeting cadherin 17 (CDH17) for the treatment of colorectal and other gastrointestinal tract tumors.

Nataliia Kalynovska[1], Diana Hudecz[1], Matous Hrdinka[1], Ilona Prochazkova[1], Eliska Kohelova[1], Lenka Palova Jelinkova[1], Filip Jaburek[1], Tomas Zimmermann[1], Lukas Fojtik[1], Irena Adkins[1], Sarka Stehlikova[1], **Amy Jensen-Smith**[2], Radek Spisek[1], Martin Steegmaier[1]

[1]SOTIO Biotech, a.s., Prague, Czech Republic,[2]SOTIO Biotech, Inc., Boston, MA

Background: Colorectal cancer is the third most prevalent type of cancer, with an incidence of nearly 2 million new cases annually, and ranks as the second leading cause of cancer mortality worldwide. CDH17 is a single-pass transmembrane glycoprotein involved in calcium-dependent cell-cell adhesion and epithelial homeostasis regulation. In normal physiology, CDH17 is predominantly localized on the lateral surfaces of intestinal and pancreatic ductal epithelial cells. However, it is frequently overexpressed and aberrantly localized in gastrointestinal tumors including colorectal, gastric, pancreatic and gastroesophageal carcinomas. We have developed SOT109, a proprietary CDH17 targeting exatecan-based antibody-drug conjugate (ADC) and evaluated its preclinical safety and efficacy.
Results: Immunohistochemical staining of patient tumor and healthy tissue microarrays confirmed CDH17 expression in normal tissues including the colon, stomach and small intestine, with pronounced expression in colorectal, gastric, and gastroesophageal junction tumors, consistent with previously published literature. SOT109 candidates were generated from a fully human CDH17 monoclonal antibody conjugated via a hydrophobic linker to exatecan, a cytotoxic topoisomerase I inhibitor payload. Candidates were selected by rigorous screening for optimal binding epitopes and affinity, together with binding, internalization, and cytotoxicity *in vitro*. SOT109 candidates demonstrated specific binding to CDH17 and selective internalization and killing of target-positive cancer cells. Lead candidates underwent evaluation in several preclinical *in vivo* mouse tumor models, including cell-derived and patient-derived xenografts. Treatment with SOT109 resulted in profound and sustained tumor regressions. The doses tested in these studies were well tolerated in mice, with no dose-limiting toxicities observed. Subsequent studies in non-human primates revealed a favorable pharmacokinetic and safety profile.
Summary: SOT109 targets the overexpressed tumor antigen CDH17 and exhibits potent anti-tumor efficacy coupled with a clean safety profile in preclinical models of colorectal carcinoma. These data suggest that SOT109 holds significant potential as a therapeutic candidate for patients with gastrointestinal malignancies and supports the further clinical development of SOT109.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Drug Discovery Assay Technologies
Poster Session

#5473 Development of a novel fluorescent ELISpot assay using the Celigo^{TM}Image Cytometer.

Wen-Chieh Liao[1], Julia Abdalla[1], **Carolina Franco Nitta**[2], Mackenzie Pierce[2], Bo Lin[2], Jessie Ni[1]

[1]BioLegend, San Diego, CA,[2]Revvity, Lawrence, MA

ELISpot and its variant, fluorescent ELISpot (FluoroSpot), are commonly used methodologies that monitor and detect cytokine and/or antibody secretion from peripheral blood mononuclear cells (PBMCs). Conventional ELISpot and FluoroSpot assays utilize PVDF-membrane plates, which require labor-intensive washing steps that may introduce variability in performance and results. Here, we have developed a novel FluoroSpot assay using polystyrene-, clear-bottom microplates with simplified automated washing steps to minimize operator variability and error. In this work, we assessed multiple critical cellular immune-related activities, including IL-6, IFN-g, and TNF-a secretion in PBMCs using this novel FluoroSpot methodology in comparison to the conventional ELISpot assay. To detect cytokine secretion of IL-6, IFN-g, and TNF-a, the Celigo™ Image Cytometer (Revvity Health Sciences, Inc., Lawrence, MA) was incorporated to capture whole-well images of fluorescently labeled detection antibodies. Results for both methodologies were comparable to one another, validating the use of polystyrene plates on Celigo™ as opposed to PVDF-membrane plates. In addition to FluoroSpot detection, the Celigo™ was used as a quality control check to provide a comprehensive analysis of live cell distribution prior to performing the FluoroSpot assay. Live cell imaging results were used to correlate downstream cytokine-releasing cell spot formation results. Combinational use of the novel FluoroSpot polystyrene plate with the Celigo™ reduced operator variability and errors, while streamlining a multiparametric analysis to identify the number of fluorescent spots, spot morphology (size), and whole-well mean fluorescence intensity values. This methodology may prove useful for researchers interested in monitoring and detecting cytokine and/or antibody-releasing PBMCs.

#5474 QUANTROseq, a transcriptomic based drug discovery platform using time-resolved gene expression profiling: identification of inhibitors and degraders of transcription factors and cell signaling regulators.

Arianna Sabo, QUANTRO Therapeutic Team

QUANTRO Therapeutics GmbH, Vienna, Austria

Transcription factors (TFs) and related signaling pathways have long been considered among the most challenging targets in drug discovery. To address these limitations, QUANTRO Therapeutics has developed QUANTROseq, a pioneering transcriptomic discovery platform designed to match transcriptional dynamic profiles or “fingerprints” triggered by small-molecule drug candidates with those derived from controlled, acute degradation of specific targets. This innovative approach enables the identification of novel inhibitors, modulators, and degraders with unprecedented precision and sensitivity, targeting even previously undruggable proteins. Analysis of our data demonstrated that time-resolved transcriptional profiling not only allows identification of drug candidates in HTS screening, it also reveals the mode of action of these drugs with unprecedented precision and sensitivity, provided that these act on targets that have a direct effect on transcriptional regulation, such as transcription factors (TFs) and signaling pathways (e.g. c-MYC, YAP/TEAD or KRAS). This approach allows for the clear discrimination between molecules that directly act on a specific transcription factors or directly associated regulatory co-factors, and drugs that only indirectly affect its function. The transcriptional fingerprinting approach is employed by QUANTRO at various stages of the drug discovery process: 1. Benchmarking the real mode of action of existing drugs; 2. Interrogating the efficiency, selectivity, and potency of drug candidates during Hit-validation and Hit-to-Lead optimization activities, overcoming the limitation of standard biochemical/biophysical assays in their capacity to inform regarding compounds selectivity; 3. Identifying new mode of action drug candidates that specifically interfere with disease-causing transcriptional programs in cancer, through the proprietary QUANTROseq screening platform. During last year, QUANTROseq screening technology has been employed by QUANTRO in multiple large HTS screening campaigns, which yielded high quality hits against several key oncogenic transcription factors. Screening results will be presented at the conference.

#5475 Leveraging LC-MS workflows to characterize a novel WRN covalent inhibitor.

Kurt Baltier[1], Anthony Tubbs[2], Doug McLaughlin[2], Giulia Bottoni[2], Allison Drew[2], Brian Erickson[1], **Craig Braun**[1]

[1]IQ Proteomics LLC, Framingham, MA,[2]MOMA Therapeutics, Cambridge, MA

Werner protein (WRN) is a helicase with functions critical to DNA repair pathways, and as such is an active target for the development of cancer therapeutics. Notably, WRN has been demonstrated as a synthetic lethality target in DNA mismatch repair deficient (dMMR)/microsatellite instable (MSI-H) tumors owing to its role in the DNA damage response. Accordingly, the development of small-molecule WRN inhibitors is an active and attractive area of cancer research and has the potential to be a powerful new therapeutic modality in several cancers including colorectal, gastric, and endometrial. Here, we report the application of targeted and discovery LC-MS workflows towards the characterization of M6021, a small molecule covalent inhibitor of WRN. A custom targeted LC-MS assay has been developed and optimized for detection and quantification of covalent compound engagement to WRN in preclinical models, including several cancer cell lines and mouse xenograft models. This assay enables reproducible, precise and accurate quantitative characterization of near-complete WRN engagement in clinically relevant biological matrices, despite low endogenous WRN expression and treatment-induced reduction in WRN levels. Plate-based sample preparation and analysis enables high-throughput evaluation of multiple experimental parameters (dose, treatment time, matrix, etc) across meaningful cohorts of replicates. Further, we employed a discovery LC-MS based covalent inhibitor selectivity assay to demonstrate that M6021 is highly selective for WRN, proteome-wide. Using this assay, we demonstrate that the novel WRN inhibitor M6021 is a potent and selective covalent inhibitor of WRN helicase, suitable for use as a high-quality chemical probe for understanding WRN biology in dMMR/MSI-H tumors.

#5476 In vitro real-time evaluation of bystander effects of antibody-drug conjugates.

Xiaoyu Zhang[1], Yun-fei Pu[2], Tian Wang[2], Grace Yang[2], Peifang Ye[2], Nancy Li[1]

[1]Agilent Technologies, Inc., La Jolla, CA,[2]Agilent Technologies, Inc., Hangzhou, China

Antibody-drug conjugates (ADCs) represent a pivotal advancement in biotherapy, leveraging targeted internalization to treat tumors effectively. A critical phenomenon associated with ADCs is the bystander effect, where cytotoxic drugs released from lysed cancer cells diffuse into neighboring tumor cells, enhancing therapeutic efficacy—particularly in tumors with heterogeneous antigen expression. Despite its significance, methods for detecting and evaluating the bystander effect of ADCs remain under development. In this study, we present a robust approach to monitor and analyze the bystander effect of ADCs in real-time using live-cell imaging and impedance measurements with the Agilent xCELLigence RTCA eSight system. Two innovative assays—the in vitro co-culture assay and the conditioned medium transfer assay—were established for this purpose. The cytotoxic effects of DS8201, an ADC with bystander activity, and T-DM1, an ADC without bystander activity, were assessed in these assays. In the co-culture assay, HER2-positive SKBR3 cells and HER2-negative MCF7 cells were co-seeded in a biosensor plate (E-Plate VIEW 96) the day before testing. The results revealed that DS8201 treatment led to the death of HER2-negative MCF7 cells in the presence of SKBR3 cells, indicative of a bystander effect. In contrast, T-DM1 did not affect MCF7 viability under similar conditions. Both DS8201 and T-DM1, however, induced cytotoxicity in HER2-positive SKBR3 cells. In the conditioned medium transfer assay, medium from SKBR3 cells treated with DS8201 significantly reduced MCF7 cell viability, demonstrating a bystander effect. Conversely, conditioned medium from T-DM1-treated SKBR3 cells did not impact MCF7 viability. Notably, neither DS8201 nor T-DM1 exhibited direct cytotoxic effects on MCF7 cells in isolation. In conclusion, we describe two robust methodologies for detecting and evaluating the bystander effect of ADCs using the RTCA eSight system. These approaches provide valuable tools for optimizing the efficacy, safety, and pharmacokinetics of therapeutic ADCs during biopharmaceutical development.

#5477 Cell avidity - the next-gen cell binding to understand the mechanism of action through cell-cell and cell-protein interaction.

Zhou Zhong, **Keith Bailey**, Justin Moser, Bella Perez, Brian Kierce, Michael Savage

LUMICKS, Boston, MA

Traditional assays such as affinity measurements, cytokine secretion, and cytotoxicity provide informative insights but are insufficient for comprehensively characterizing and selecting the most effective cell therapy candidates. The complexity of cell-cell interactions that drive functional outcomes remains inadequately understood. Cell avidity, defined as the aggregate strength of interactions between effector and target cells, serves as a novel pivotal metric for uncovering the mechanism of action behind therapeutic candidates. The z-Movi Cell Avidity Analyzer, part of our versatile Cell Avidity platform, leverages the integration of fluorescence microscopy with precise acoustic force application to measure cell binding under physiologically relevant conditions. This approach allows for a detailed analysis of binding profiles, encompassing cell surface receptors and ligands, cell phenotype, cell status, and more to elucidate potency, safety, sensitivity, and binding kinetics essential for robust therapeutic evaluation. Our platform is designed to support the characterization and screening of a wide array of therapeutic modalities, including molecular binders like antibodies and small molecules, as well as cell-based therapies. By providing extensive insights into the mechanism of action and functional performance, Cell Avidity enables the development of next-generation therapeutics with enhanced safety and efficacy profiles.

#5478 From 2D to 3D: Advancing preclinical platforms in cancer models.

Hussein Chehade, Amber Rowse, David Draper, Anita J. Zaitouna, Daniel Saims, Sheri Barnes, Heidi Nielsen, Scott Wise

Labcorp Drug Development Inc., Ann Arbor, MI

Solid tumors account for about 90% of all adult human cancers. These tumors have a heterogeneous cellular composition that includes cancer cells, stromal cells, immune cells, and more. Developing effective treatments relies on experimental models that can recapitulate the original tumor and effectively predict clinical outcomes. Two-dimensional (2D) cell cultures have been extensively used as tools to predict promising outcomes before proceeding to more expensive animal studies. However, results from 2D models often fail to replicate *in vivo* conditions, necessitating the development of 3D multi-cellular spheroids that better mimic the physiology, architecture, and native microenvironment of parental tumors. In this study, we highlight the differences in drug response between 2D and 3D cancer cell line cultures and how 3D cultures better simulate the response observed in vivo.Human (MDA-MB-231) and mouse (EMT6 & 4T1) cancer cell lines were cultured in 2D as monolayers or in 3D utilizing a species- and histotype-specific extracellular matrices (ECM). Cultures were treated with increasing doses of small molecule drugs (Cisplatin, Paclitaxel, or Gemcitabine) and cell viability was quantified using CellTiter-Glo™ luminescent cell viability assay and/or Celltox™ Green viability dye along with serial imaging quantification. Half-maximal inhibitory concentration (IC_{50}) curves were plotted and quantified using GraphPad Prism™ software.Microscopic imaging revealed morphological differences between 2D and 3D cultures. Unlike 2D cultures, 3D cultures formed either compact or loose spheroids, allowing for cell-cell and cell-matrix interactions that mimic tumor morphology. MDA-MB-231 and EMT6 breast cancer cells showed higher cytotoxicity to Cisplatin in 2D cultures compared to 3D cultures (IC_{50} of 3.5 µM for 2D and 13.47 µM for 3D; IC_{50} of 21.28 µM for 2D and 42.36 µM for 3D respectively). This corresponds to *in vivo* MDA-MB-231 and EMT6 tumors, which show slight tumor growth inhibition post Cisplatin treatment. However, 4T1 cancer cells showed similar Cisplatin cytotoxicity in both 2D and 3D cultures (IC_{50} of 4.21 µM and 5.73 µM respectively), which also aligns with *in vivo* response, where Cisplatin resulted in significant reduction in tumor volume. These *in vitro* results were further confirmed by viability analysis using serial imaging.These findings underscore the efficacy and predictability of this 3D platform in drug development. Not only does this platform help in decreasing the use of laboratory animals, but it also allows to reproduce key aspects of the tumor physiology and architecture. Consequently, 3D cultures have the potential to better predict treatment response and help identify drug resistance mechanisms.

#5479 Development of novel luminescent assays for sensitive and specific quantitation of double-stranded RNA in mRNA vaccines.

Rich Moravec, **Jun Wang**, Jim Hartnett, Mei Cong, Jamison Grailer

Promega, Fitchburg, WI

Personalized mRNA vaccines, designed with patient-specific tumor mutations, are now advancing in clinical trials and showing promising results. Additionally, the delivery of therapeutic proteins (e.g., antibodies) encoded by mRNA is under active development. The large-scale production of mRNA is through the process of in vitro transcription (IVT). A significant byproduct and contaminant of IVT is double-stranded RNA (dsRNA), which is highly immunogenic. dsRNA can trigger inflammation, induce cell death, and inhibit protein translation. Therefore, accurate quantification of dsRNA is essential to ensure the safety and efficacy of mRNA therapeutics. However, current technologies, such as dot blot and ELISA, lack the sensitivity and quantitation needed for robust dsRNA detection. In this work, we describe the development of novel luminescent assays for the sensitive and specific quantitation of dsRNA in IVT products. First, we applied NanoBiT® split luciferase technology to detect dsRNA in mRNA drug substance. In this assay, dsRNA-binding domains are fused to the SmBiT and LgBiT components of NanoBiT®. In the presence of dsRNA, these domains dimerize, reconstituting a functional NanoBiT® luciferase enzyme that generates light proportional to the dsRNA concentration. The assay detects dsRNA longer than 30 base pairs and is agnostic to sequence and size. It exhibits high specificity, with no cross-reactivity to single-stranded RNA or DNA, and a limit of quantitation below 10 pg/ml. As a complementary approach, we developed a cell-based bioassay to detect encapsulated dsRNA within LNPs. Upon endocytosis, LNP-encapsulated dsRNA is recognized by Toll-like receptor 3 (TLR3). This activation is captured via a TLR3-specific promoter driving luciferase expression, offering a physiologically relevant readout of dsRNA bioactivity. Both assays are streamlined in an “add-mix-read” format, requiring no wash steps or media transfers, and deliver robust, sensitive, and reproducible performance. Together, these tools enable precise detection of dsRNA contaminants in IVT products, ensuring the quality and safety of mRNA therapeutics.

#5480 Live cell real-time quantification of drug-target engagement for rapid drug discovery.

Ivan Babic[1], Nikolas Bryan[1], Claire Cunningham[2], Avery Sampson[2], Daniel Starczynowski[2], Elmar Nurmemmedov[1]

[1]CellarisBio, San Diego, CA,[2]Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Drug discovery for challenging drug targets necessitates the proteomic complexities of the cellular milieu for proper folding and function. Therefore, the conventional biophysical methods are not sufficient due to the artificial acellular environment they impose on the target. Cell target engagement is a powerful paradigm in drug discovery, as measures transitions in the thermodynamic state of target protein during engagement with drug molecules. The currently available luminescence-based cell target engagement methods are limited to measurement of end-point signal, poor utility across multiple challenging target families and insufficient resolution and integration for kinetic and mechanistic readouts. We have developed a highly sensitive and versatile fluorescence-based cell target engagement strategy designed for challenging drug targets. The technology enables interrogation of drug targets without interfering with folding, localization and function - all within the physiological milieu of the cell. Importantly, this new method allows for quantitation and monitoring of cell target engagement in real time using live cells. The system seamlessly integrates with readily available real-time systems such as QuantStudio, thus making it highly sensitive and scalable. We share data on challenging drug targets such as Myc, MAPK1, UBE2N and KRAS, and we demonstrate utility of this new methodology for future drug discovery.

#5481 A 3D bioprinted vascularized human tumor-on-a-Chip model for simultaneous evaluation of immunotherapeutic efficacy and safety.

Juliana Navarro-Yepes, Queeny Dasgupta, Purboja Purkayastha, Clara Erice, Carly Truong, Kim Phan, YongDuk Han, Michael Doerfert, Cassio Mello, Kevin Janson, Alma Antonio, Tracy Lin, Ameya Narkar, Sammy Datwani, SoonSeng Ng, Taci Pereira

Systemic Bio, Houston, TX

Immuno-oncology has revolutionized cancer treatment by harnessing the immune system to target tumors. However, developing effective therapies is challenging due to the complexity of the tumor microenvironment (TME), which influences therapy response, and toxicity risks like vascular injury and off-tumor effects. Traditional preclinical models fail to replicate human-specific immune responses and TME complexities, such as immune cell trafficking and tumor immune evasion. Additionally, current safety assessments for immunotherapies are inadequate in predicting systemic risks. These limitations highlight the need for more accurate, human-relevant tumor models. To address these challenges, we developed a 3D vascularized tumor-on-a-chip platform that incorporates key TME features for enhanced clinical relevance. Using 3D bioprinting, we integrated vascular structures with our human vascularized integrated organ system (h-VIOS™), enabling dynamic perfusion and simulating immune cell flow. The vasculature, endothelialized with primary human endothelial cells, forms a functional barrier expressing junctional markers and adhesion molecules, supporting immune cell interactions during T cell trafficking and tumor infiltration, while facilitating immunotherapy safety evaluation. Our model also integrates patient-derived tumor organoids that replicate key tumor characteristics, including heterogeneity and genetic diversity, and simulates TME interactions through co-culture with endothelial and immune cells. In a colorectal cancer model, we demonstrated chemokine-driven T cell infiltration from the endothelialized vasculature into the tumor, confirmed by confocal imaging and flow cytometry. We also modeled T cell responses to immune checkpoint inhibition with pembrolizumab, showing increased tumor killing, evidenced by elevated markers such as IFN-γ, Granzyme B, and cleaved-caspase 3. These results validate anti-PD-1 therapy in our model, highlighting its ability to capture T cell reactivation and tumor killing. In a TROP-2-expressing lung cancer model, we recapitulated the efficacy of the TROP-2 antibody-drug conjugate Sacituzumab govitecan, shown by increased cleaved-caspase 3 and reduced tumoroid viability. We also observed drug-induced vascular injury (DIVI) and off-tumor toxicity, evidenced by upregulation of inflammatory markers (ICAM-1, PECAM, P-selectin, IL-6, TNF-α) in endothelial cells. In conclusion, our 3D vascularized tumor-on-a-chip model outperforms traditional models by accurately replicating the TME and enabling the simultaneous assessment of drug-induced toxicities. This customizable platform facilitates mechanism-of-action studies and holds significant potential for personalized medicine, offering a powerful tool for advancing immuno-oncology research and improving therapeutic outcomes.

#5482 In vitro assays for prediction of ADC hematological toxicities: contribution of antibody, linker, and payload.

Yongzhao Huang[1], **Emer Clarke**[1], Graham A. E. Garnett[2], Manuel Lasalle[2], Jodi Wong[2], Ambroise Wu[2], Araba P. Sagoe-Wagner[2], Elena Barbosa[2], Catalina Suarez[2], Stuart D. Barnscher[2], Jamie R. Rich[2], Raffaele Colombo[2]

[1]Discovery Life Sciences, Seattle, WA,[2]ADC Therapeutic Development, Zymeworks Inc., Vancouver, BC, Canada

Antibody-drug conjugates (ADCs) are one of the fastest-growing therapeutic modalities, with 11 FDA-approved ADCs and more than 250 different ADCs in clinical development. Despite their success, significant hurdles remain. Notably, translating preclinical findings to the clinic remains challenging. Hematological toxicities are commonly associated with many ADCs and may arise from the direct killing of hematopoietic cells by the ADC itself or indirectly from payload released elsewhere in the body. Therefore, development of in vitro assays capable of predicting clinical findings could help improve ADC development and guide the selection of optimal linkers and payloads. Different ADCs were generated by conjugating targeted and non-targeted antibodies to a common microtubule inhibitor, while varying the protease-cleavable linkers. Clinically approved ADCs spanning different payload classes were used as benchmarks. ADC cytotoxicity was evaluated in both antigen-positive and antigen-negative cancer cell lines in vitro. Lysosomal cleavage of different linkers was evaluated in-vitro. ADC linker stability was also evaluated via a neutrophil differentiation assay, using expanded and differentiated CD34+ hematopoietic stem cells, which were treated with various ADCs before assessing CD66b neutrophil maturation. Finally, in vitro off-target toxicity was evaluated using a colony forming cell (CFC) assay to assess cytotoxicity of ADCs and their payloads on erythroid, myeloid, and megakaryocyte progenitors differentiated from hematopoietic stem cells.

In vitro cytotoxicity assays revealed variable potency among the ADCs tested. Some linkers (e.g., VK, FK) resulted in potent ADC cytotoxicity irrespective of the targeting antibody, while others (e.g., VA, VCit, GGFG, K) had target-dependent cytotoxicity in vitro. We identified linkers with different payload release rates in lysosomes, where GGFG and K showed the slowest rates. In vitro off-target and linker stability assays highlight how ADCs may induce toxicity in different healthy cell subsets depending on the ADC components (antibody, linker, and payload). In addition, by comparing results for ADCs and payloads, it is also possible to estimate if these toxicities are likely driven by the conjugated drug, the payload, or both. These results underlined how off-target toxicity and neutrophil differentiation assay may be useful during preclinical development to identify potential off-target effects and to improve the translation of ADC from preclinical to clinical settings.

#5483 High-throughput transcriptomics dose response profiling: identification of functional molecular signatures reflecting efficacy and safety of mTOR inhibitors.

Bruce Seligmann[1], Megan Opichka[1], Monica Hernandez[1], Zhoutao Chen[1], Joanne M. Yeakley[1], Salvatore Camiolo[2]

[1]BioSpyder Technologies, Inc., Carlsbad, CA,[2]BioClavis, Ltd., Glasgow, United Kingdom

Background and Purpose: Recent publications demonstrate the utility of the TempO-Seq® high-throughput transcriptomics (HTTr) assay in generating *in vitro* dose response data for 1751 compounds in MCF-1 cells [Harrill et al, Tox Sci 2024, 202(1): 103-122] and 1201 compounds in U-2OS cells [Bundy et al, Tox & Applied Pharmacol 491 (2024) 117073]. These studies identified biological pathway altering concentrations (BPACs) for functional molecular signature patterns by evaluating 11,037 functional gene signatures for each compound and dose. We applied this same approach to identify novel differences in the modulation of molecular/functional signatures by mTOR rapalogs and ATP-competitive inhibitors.

Methods: To cover a broad range of functional molecular signatures we profiled the selected compounds, at 8 concentrations each, in MCF-7, U-2OS, and HepaRG cells using the commercial TempO-Seq whole transcriptome v2.1 assay. We identified differentially expressed genes between treated and untreated cells using DESeq2, calculated BPACs as previously described [Harrill et. al. Tox Sci 2021, 18(1): 68-89], then identified which of the 11,037 functional molecular signatures were modulated (as published by Harrill and Bundy).

Results: Signature patterns with different BPACs were identified for each compound. Each cell line generated a set of compound modulated molecular signatures that overlapped with one or both of the other cell lines, as well as unique molecular signatures to that cell line. From these data, we identified a set of functional molecular signatures associated with the class of mTOR inhibitors. We also identified molecular signatures that differentiated the rapalogs, which bind to FKBP12 and the mTORC1 complex to inhibit its kinase activity, from the ATP-competitive inhibitors, which inhibit the kinase activity of both mTORC1 and mTORC2. Finally, we identified compound-specific molecular signatures. These data will be presented.

Conclusions: The results demonstrate the utility of HTTr dose response compound profiling in differentiating compound activity using a database of 11,037 functionally annotated molecular signatures. The TempO-Seq assay effectively distinguished these activities for each compound and provided data that reflected potential efficacy and safety. Each cell line contributed unique modulated signatures that were absent in others, offering a more complete view of the activity of each compound. These findings demonstrate the value to therapeutic target focused drug discovery programs of the TempO-Seq HTTr assay in generating functional mechanistic dose response data, and underscore the potential of HTTr TempO-Seq assays to differentiate chemical series not only based on compound structure, but also on functional dose response activity that may impact both efficacy and safety.

#5484 Expanding the role of western blot imaging for oncology research: novel applications for in-cell western assays and single cell analysis using the ChemiDoc Go imaging system.

Michael Kirmiz[1], Deanna Woo[2], Nikolas Chmiel[1], Kenneth Oh[2], **Angelica P. Olcott**[2]

[1]R&D, Bio-Rad Laboratories, Hercules, CA,[2]Bio-Rad Laboratories, Hercules, CA

Western blotting is widely used to detect cellular proteins and nucleic acids in cell lysates; however, its application for analyzing intact cells remains largely unexplored. Here, we evaluate compatibility of Bio-Rad's ChemiDocTM Go Imaging System with chemiluminescence and multi-channel fluorescence detection for in-cell western immunofluorescence assays performed on 96-well plates and microscopy slides. Emphasis was placed on use of the StarBright Blue 700-conjugated secondary antibody due to its strong, stable fluorescence, wide dynamic range, and compatibility with multiplexed assays. In 96-well assays, HEK293 cells were stained with anti-mortalin primary antibodies, followed by StarBright Blue 700-conjugated secondary antibodies. These exhibited a three-fold signal increase compared to control samples lacking the primary antibody. This scalable and high-throughput platform offers a valuable tool for cancer research, particularly for evaluating protein expression in cancer cell lines, facilitating drug discovery, and assessing therapeutic efficacy across diverse cancer models. On microscope slides, HEK293 cells were stained with anti-mortalin primary and StarBright Blue 700 secondary antibodies further demonstrating the imager's strong compatibility with immunofluorescence on fixed, intact single cells. This capability enables simultaneous detection of multiple biomarkers in cancer-related pathways, supporting target validation and pathway analysis in cancer biology. Additionally, the imager was used to validate T cell staining with CD3-labeled fluorescent antibodies prior to multiplexed flow cytometry. This step simplifies the confirmation of T cell staining before performing more complex flow cytometry assays, with direct applications in immuno-oncology. These include monitoring immune cell activation and characterizing tumor-infiltrating lymphocytes (TILs), both critical for developing immune-based cancer therapies. Potential oncology research applications of these novel techniques may include:

- Quantification of protein expression changes following drug treatments or genetic modifications
- Evaluation of protein-protein interactions relevant to cancer signaling pathways
- High-throughput screening of compound libraries
- Provide insights into the analysis of protein localization within entire cells, aiding in biomarker identification and therapeutic targeting efforts.

Together, these findings highlight novel applications of western blot imaging technology as a fast, scalable, and sensitive platform for multiplexed protein analysis, supporting cancer research and therapeutic development.

#5485 A new 30-color spectral flow cytometry panel for T cell characterization.

David Draper, Brogan Yarzabek, Stacey Roys, Anita J. Zaitouna, Philip Lapinski, Amber Rowse, Scott Wise

Labcorp, Ann Arbor, MI

Spectral flow cytometers have facilitated the development of antibody panels with 30 colors or more to provide deep insights into the immune response. With a focus on T cell therapies, Labcorp has developed the 30-color immunophenotyping panel using the Cytek Aurora™ Spectral Flow Cytometer. The panel backbone includes 1 viability dye and 10 lineage markers to profile T cell, NK, and B cell subsets. 19 additional functional markers enable the characterization of activation, exhaustion, cytotoxicity, and proliferation in each subset, plus memory T cell subset delineation. Antibody-fluorophore combinations in a novel 30-color spectral flow cytometry panel were carefully considered and the resulting panel was developed utilizing primary PBMCs cultured with IL-2 and anti-CD3/CD28 to activate T cell subsets. Modulation of the expression levels for each functional marker was validated via comparisons made to unstimulated PBMCs. The most notable changes observed were in the expression of CCR7, CD25, ICOS, and Granzyme B which were expressed at levels between 60-95% of CD8+ T cells. Checkpoints including PD-1, LAG-3, TIM-3, and TIGIT were also upregulated, albeit to a lesser extent (<50% of CD8+ T cells). For memory phenotypes, analysis of CD45RA, CD45RO, CCR7, CD95, CD62L, and CD103 were combined to delineate Tn, Teff, Tte, Tcm, Tem, and Trm subsets. To provide an additional unbiased and wholistic analysis, t-SNE was conducted to deliver a comprehensive interpretation T cell functionality in the context of various exhausted phenotypic profiles. Finally, Nanostring® analysis using the nCounter® CAR-T Characterization panel was performed to further validate the modulation of gene sets underlying the TCR-driven PBMC activation. As expected, the transcriptomic analysis mirrored the flow cytometry immunophenotyping data with regard to activation and immunosuppressive gene modulation downstream of the TCR signaling. Taken together, a lymphoid panel was developed that measured multiple phenotypic and activity markers in T cells. Importantly, our data demonstrate how spectral flow cytometry can be applied to assess T cell therapies in preclinical tumor-bearing mouse models or clinical trials.

#5486 Bioluminescent assays for antibody-drug conjugate development: internalization and Fc-mediated functions.

Julia K. Gilden[1], Christopher Eggers[1], Yitong Li[1], Rod Flemming[1], Nicholas Hess[2], **Gopal B. Krishnan**[2], Frank Fan[1], Brock Binkowski[1], Marjeta Uhr[1], Mei Cong[1]

[1]R&D, Promega, Madison, WI,[2]Promega, Madison, WI

Antibody-drug conjugates (ADCs) combine targeted delivery with potent cytotoxic payloads, utilizing multiple mechanisms of action (MoA). These include direct cytotoxicity following internalization of the ADC by antigen-expressing cells, killing of bystander cells through cleaved payloads, antigen function blockade, and immune-mediated cytotoxicity (e.g. Antibody-Dependent Cellular Cytotoxicity and Antibody-Dependent Cellular Phagocytosis) driven by the antibody Fc region. Comprehensive characterization and potency testing of ADCs require assays that capture each MoA. To address this need, we developed bioluminescent tools to streamline ADC evaluation, focusing on antibody internalization, Fc-mediated effector functions, and bystander killing. Studying bystander killing is particularly important in immunotherapy strategies for cancer, as it allows ADCs to target heterogenous tumors where not all cells express the antigen. The internalization assay employs luciferase-labeled Fab fragments with a quenching mechanism to measure intracellular antibody localization. The assay was validated using HER2-expressing SKOV3 cells with anti-HER2 monoclonal antibodies as model systems. It shows strong time- and antigen-dependent luminescence, achieving over 100-fold signal increases compared to controls, enabling quantitative ADC screening. Additional assays include Fc receptor (FcR) binding assays and cell-based reporter bioassays for ADCC and ADCP activities. The HiBiT technology is a luminescent tagging system that enables precise and sensitive detection of protein interactions and cellular events, making it a powerful tool for evaluating Target Cell Killing (TCK) by effectors and cytotoxic payloads carried by antibodies. To assess payload efficacy, a HiBiT-enabled bystander killing assay was developed, distinguishing ADCs with varying payload properties. Taken together, the internalization assay, bystander killing assay, and Fc effector bioassays, all based on bioluminescent platforms, offer a comprehensive and high-throughput solution for evaluating ADC functionality. With exceptional sensitivity, broad dynamic range, and efficient workflows, these tools enable precise optimization of therapeutic efficacy, delivering significant value for preclinical ADC development.

#5487 IndEx-2: An *in vitro* model to evaluate the impact of target antigen density on antibody and immune therapeutics.

G. Meehan, A. Patakas, H. Findlay, V. Senthivel, T. London;
RoukenBio, Motherwell, United Kingdom

The density of cell-surface receptors targeted by therapeutic agents profoundlyinfluences their safety, efficacy, and pharmacokinetics. Low receptor expression on healthy cells can result in on-target, off-tumor toxicities, while tumor heterogeneity in receptor density may allow low-expressing clones to evade treatment. Traditional *invitro* evaluation relies on panels of cancer cell lines with varying receptor levels, anapproach that is both logistically challenging and experimentally inconsistent. This limitation is particularly pronounced for therapeutics targeting two receptors.To address this, we developed IndEx-2, an *in vitro* cell line platform that employs chemical proximity promoters to achieve a high dynamic range of tuneable and independent expression of up to two target antigens within a single, uniform cell line background. We have demonstrated precise control of antigen density for several receptors ranging from a few hundred up to a million receptors, including a cell line thatallows independent control of two target antigens (CD19 and CD22). We report herethree examples of the utility of the system. In the first, we have compared CLN978, a novel CD19- TCE developed for B-cell malignancies and autoimmune diseases, with Blinatumomab and demonstrated that the former retained its activity in terms of cytotoxicity and cytokine production at much lower levels of CD19 expression and estimate the threshold for each molecule in a cohort of 10 healthy donors. In the second, we assessed the ability of two HER2-targeting CAR-T cells, employing high and low antigen binders, and estimate the number of receptors that resulted in half maximal response (R50) for cytotoxicity and IFNγ production, demonstrating the utility of the system as an “on-target, off-tumor” hazard assessment screening tool. Lastly, using our CD19/CD22 dual-antigen cell line, we compared tandem (“OR”) CD19/CD22-CD3zCD28 and dual (“AND”) CD19-CD3z and CD22-CD28 CAR constructs targetingboth antigens. The tandem CAR exhibited enhanced cytotoxicity when either or both antigens were expressed, whereas the dual CAR required co-expression of both antigens for optimal T-cell activation. These results underscore IndEx-2's robustcapability to evaluate the influence of antigen density on therapeutic safety andefficacy, providing a powerful tool for the development of antibody-based and immune-targeting cancer therapies.

#5488 Identifying novel Ewing sarcoma therapeutics using a high-throughput chromatin accessibility assay.

Darian Williams[1], Andrew McFadden[2], Austin Hepperla[2], Min Shen[1], Tino Sanchez[1], Gordon Stott[3], Mark Henderson[1], Ian Davis[4], Samantha Pattenden[2]

[1]Early Translation Branch, National Center for Advancing Translational Sciences, Rockville, MD,[2]School of Medicine, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC,[3]Frederick National Laboratory for Cancer Research, Leidos Biomedical Research, Inc., Frederick, MD,[4]Department of Pediatrics, School of Medicine, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC

Chromatin dysregulation has been implicated in the development of a wide range of diseases, including cancer, as well as therapeutic resistance. Genetic alterations in chromatin regulatory factors and aberrant activity of transcriptional regulators alter chromatin states and drive tumor growth in cancer. One example of a cancer that is driven by alterations in chromatin landscape is Ewing sarcoma, a highly aggressive pediatric cancer of bones and soft tissues. Ewing sarcoma is driven by an oncoprotein generated by the translocation of the EWSR1 and FLI1 genes, resulting in the fusion oncoprotein (EWSR1::FLI1), which activates noncanonical enhancers and alters the expression of critical genes associated with the disease. EWSR1::FL1 is challenging, if not impossible, to target directly with small molecules, and viable therapeutic targets for Ewing sarcoma have been slow to emerge. To broaden the search for druggable targets we developed a target agnostic screening approach that exploits oncoprotein-specific chromatin accessibility states as a relevant and direct functional readout for identifying novel therapeutics. We developed and optimized a 384-well, high throughput (HT) version of the Assay for Transpose-Accessible Chromatin assay (HT-ATAC-seq) that was applied to 500 EWSR1::FLI1-regulated chromatin regions. Using Ewing sarcoma-derived A673 cells, we conducted a pilot screen of 12,000 small-molecule compounds comprising approved drugs, investigational agents, and bioactive compounds. The screen identified 285 compounds that partially reversed Ewing sarcoma chromatin abnormalities. The set of active compounds included Panobinostat, which is currently in preclinical development for Ewing sarcoma. This pilot screen supports the HT-ATAC-seq assay as a robust approach to identify novel compounds that reverse the chromatin alterations associated with Ewing sarcoma. This approach and assay can be adapted to investigate other cancers or pathologies associated with chromatin state alterations.

#5489 Quantitative intracellular immunoassay for monitoring poly (ADP-ribose) in cancer cells treated with PARP inhibitors.

Hans Layman[1], Dusit Natrakul[2], Maya Kim[2], Lars Duerig[2], Sripriya Ranganathan[2], Belinda Jackson[2], Lalitha Srinivasan[2], Enrique Sanchez-Lemus[2], Chin-Chi Chen[2], Caitlin Hansen[2], Jacob N. Wohlstadter[2]

[1]Meso Scale Diagnostics, LLC, Pacifica, CA,[2]Meso Scale Diagnostics, LLC, Rockville, MD

Poly (ADP-ribose) polymerase (PARP) enzymes play a crucial role in cellular DNA repair mechanisms by catalyzing the addition of poly (ADP-ribose) (PAR) chains to target proteins in response to DNA damage. The overactivity of PARP has been implicated in various pathological conditions, including cancer, where PARP inhibitors (PARPi) have emerged as an important therapeutic strategy. Monitoring PAR levels within different cellular environments is essential for understanding PARP activity and optimizing PARP inhibitor efficacy. This study outlines the characterization and development of a sensitive electrochemiluminescence immunoassay designed to quantitatively measure PAR in multiple cancer cell lines treated with PARP inhibitors.

The PAR assay was developed on the MSD MULTI-ARRAY platform. Multiple anti-PAR antibodies were tested in all possible capture/detection combinations using a wide range of PAR polymer concentrations. An optimal pair was selected based on response linearity and high sensitivity. Cancer cell lines were subjected to treatment with hydrogen peroxide (MCF-7) or cultured under hypoxia (Cos7, HeLa) over 24 hours, followed by whole cell lysate collection for sample detection using the PAR immunoassay to demonstrate assay utility (e.g., linearity, sample detectability, matrix tolerance). To demonstrate the functional application of the PAR immunoassay, three human cancer cell lines were cultured under standard conditions in 96-well plates and treated with varying concentrations (0.1-100 µM) of three PARP inhibitors over time periods ranging from 1 to 24 hours. Cell lysates were harvested, protein was quantified, and lysates were added to the PAR immunoassay to determine PAR concentration. IC_{50} curves were generated to evaluate each PARPi's potency.

Following optimization, the PAR immunoassay demonstrated good sensitivity with an LLOD of 3.34 pM in an assay diluent containing protease and phosphatase inhibitors. Using cell lysates from MCF-7, Cos7, or HeLa cells, assay linearity was demonstrated between 0.156-10 µg protein/well, resulting in a linear concentration range of 7 to 2,630 pM. Upon treatment with PARPi, a time and dose-dependent modulation of PAR levels was observed across all cell lines and inhibitors. Specifically, significant changes in IC_{50} values were found across cell lines and PARP inhibitors tested, indicating the assay's sensitivity for differentiating inhibitory effects of PARP compounds.

This study demonstrates the utility of a novel immunoassay for accurately quantifying PAR in various cancer cell lines subjected to PARP inhibition. This assay offers a robust tool for evaluating PARP inhibitor efficacy and can serve as a valuable asset for researchers in drug development and cellular biology to explore PARP-related cellular responses in different disease contexts.

#5490 Biochemical tools to characterize specific Werner syndrome helicase inhibitors and accelerate drug discovery.

Thibault Annaval[1], Jeremy Lhomedet[1], Amelie Moreau[1], Chantal Rein[1], Alastair King[2], Sophie Raynal[1], **Vanessa Jahnke**[1]

[1]Eurofins Discovery France, Celle-Levescault, France,[2]Eurofins Discovery USA, St-Charles, MO

WRN has various roles in the maintenance of genome stability and is commonly found upregulated in cancers. Therefore, new WRN inhibitors have emerged for selective cancer therapy. Eurofins Discovery researchers have developed assays to accelerate helicase drug discovery focusing on WRN. Relaying on three different biochemical assays: Binding, ATPase and unwinding, these tools can be used to screen, validate, and characterize inhibitors mode of action (MOA). Using clinical-stage WRN helicase inhibitor HRO761 and VVD214, we present a complete MOA study demonstrating that the assays generated by Eurofins Discovery teams are well suited for WRN drug discovery project. The full length protein was used in the three biochemical assays. First protein stability was tested to qualify the protein used in all assays. ATPase activity was performed using DNA double strand substrate with ADP Hunter Plus assay kit (Eurofins DiscoverX). Unwinding activity was performed using the same substrate labelled in 5' end by the fluorophore TAMRA and at the 3' end by the quencher BHQ2. Compounds IC_{50} were validated in the two assays. MOAs were characterized by testing the reversibility of the interaction, substrate competition and ATP cooperativity. Binding analysis was assessed using UHPLC/MS/MS. Methods were first determined and then K_d, B_{max}, k_{on}, k_{off} were analyzed. Stability assay demonstrated that our enzyme is stable with cycle of freezing/thawing. IC_{50}s for ATPase activity correlate well with available publication on the HRO761 and VVD214 with 220 nM and 3.5µM respectively. The panel of selectivity testing, developed by the Eurofins Discovery team, showed high selectivity of the two compounds for WRN. ATPase activity assay pharmacologies of the two compounds were validated in unwinding activity assay . IC_{50}s were 29nM and 6.4µM respectively. Reversibility testing showed that HRO761, is a reversible inhibitor and that VVD214 is an irreversible inhibitor. WRN mass spectrometry binding assay was successfully developed using HRO761, as reference compound. The binding assay using ATP as nonspecific ligand demonstrates a decrease in HRO761 binding to its site suggesting a competition or a negative cooperativity. Enzymatic assay showed that HR0761 is a mixed ATP allosteric inhibitor. Further experiment demonstrates that ATP prevent HR0761 binding when it binds first to the helicase. In this project, we have performed a complete characterization of two different compounds MOA. We were capable of validating HRO761 as an allosteric inhibitor with ATP negative cooperativity and VVD2014 as covalent inhibitor. This demonstration provides strong evidence that the developed assays are well suited for helicase drug candidate discovery, development and characterization. These will help providing innovative solution to fight against cancer an especially the one involving WRN over expression.

#5491 High throughput bioluminescent homogenous assay to monitor N6-methyladenoisne (m6A).

Said A. Goueli[1], Dareen Mikheil[2], Kevin Hsiao[1], WeiWei An[1], Matt Larsen[1]

[1]Promega, Madison, WI,[2]Research and Development, Promega, Madison, WI

N6-methyladenosine (m6A) is a modified nucleoside derived from adenosine, distinguished by the presence of a methyl group at the N6 position. This structural modification enables m6A to play unique roles in cellular signaling, gene expression regulation, and metabolic processes, positioning it as a crucial player in cellular function and disease pathology. Its involvement ushered the beginning of the field of in epitranscriptomics, particularly N6-methyladenosine (m6A)-related pathways, which modulate mRNA stability, translation, and splicing have lent tremendous significance to this form of RNA modification. There are about 7000 mRNAs with m6A modifications and they are highly conserved. Non-coding RNA is mainly regulated by m6A methyltransferase and demethylase, which plays an important biological role in the occurrence and development of tumors. Recent studies suggest that m6A and its derivatives could serve as potential biomarkers for disease diagnosis and as therapeutic targets. Recognizing how important the early detection of m6A is, we have developed an assay that monitors the concentration of m6A so it can be used for screening of m6A-RNA from patients with cancer such as liver cancer. The assay relies on new concept of novel nanoluciferase (nanoluc) complementation where a small peptide (SmBiT) linked to m6A through a linker (m6A tracer) can be recognized by an anti m6A antibody that is linked to a nanoluc complementary fragment (LgBiT) resulting in a bioluminescence upon the addition of nanoluc substrate. The presence of m6A-RNA that is generated in the reaction with RNA substrate, S-adenosylmethionine (SAM) and METTL 3/14 complex can be detected with this assay. Using MTTL3/14 complex we show that SAM is an excellent substrate for this enzyme with a Km of 240nM. The assay does not recognize Adenosine, AMP, ADP, or ATP in the reaction. We will show data in support of this technology to monitor the presence of m6A in methylated RNA. The assay is homogenous, HTS formatted and can be completed in less than one hour. Thus, this assay provides valuable contributions for investigating modulators of the MTTL3/14 complex or FTO demethylating enzymes and facilitates the search for novel therapeutics.

#5492 Application of Optim.AI™ platform for predictive evaluation of immunotherapy combinations in breast cancer.

Sharon Pei Yi Chan, Jhin Jieh Lim, Masturah Mohamed Abdul Rashid, **Edward Kai-Hua Chow**

KYAN Technologies Pte Ltd, Singapore, Singapore

PURPOSE: Successful pre-clinical evaluation of immunotherapy within ex vivo settings require complex systems to accurately represent tumor heterogeneity and the surrounding tumor microenvironment (TME). With expanding immunotherapy combinations being investigated in clinical trials, particularly for breast cancer (BC), there is a growing need for reliable methodologies for functional immune-oncology drug testing in a patient- and subtype-specific manner. We have developed Optim.AI™, a hybrid experimental-analytics platform designed to predict and rank actionable treatment options, with successful application in clinical settings to guide cytotoxic and targeted therapies. However, its potential for evaluating immunotherapy responses remains underexplored. In this study, we present an integrated platform that combines short-term 3D tumor-immune cell co-culture with high content imaging and Optim.AI™ to evaluate immunotherapy-focused drug sets in HER2-positive and triple-negative BC subtypes.

METHODS: BC cells were pre-labeled with cell tracker and seeded in a 384-well format for 3-4 days to allow spheroid formation, and co-cultured with PBMCs. Optim.AI™ combinatorial drug testing was then applied to the co-culture system with up to 12 FDA-approved chemotherapies, targeted agents, monoclonal antibodies, and antibody-drug conjugates. Utilizing real-time fluorescent apoptosis signal as the phenotypic readout, high-content analysis of tumor-specific cell death was evaluated at specific timepoints and used for Optim.AI™ analytics and predictive ranking of combinatorial immunotherapy sensitivities.

RESULTS: Quantitative, real-time, image-based analysis allowed for phenotypic measurement of complex tumor-immune cell interactions within the 3D co-culture system, including immune cell migration, infiltration into spheroids, and subsequent tumor-specific killing. Co-culturing BC spheroids with PBMCs at an optimized effector-to-target ratio induced significant antibody-dependent cellular cytotoxicity, with measurable responses observed within 72 hours post-treatment. Through Optim.AI™, we observed distinct sensitivities towards the different antibody-based therapies, in HER2-positive and triple-negative BC spheroids. These findings underscore the potential of Optim.AI™ to effectively evaluate and rank immunotherapy-based treatment combinations in a physiologically relevant, 3D TME model.

CONCLUSIONS: We present an integrated platform combining high-content screening of a 3D tumor-immune cell co-culture system with Optim.AI™ and demonstrated both visualization and quantification of tumor-specific responses in the context of the TME. Future applications to ex vivo patient samples hold promise for personalized treatment regimens and identifying more effective immunotherapy combinations for BC and other solid malignancies.

#5493 3D bone marrow niche: Scalable and physiologically relevant *ex vivo* drug screening platform for acute myeloid leukemia and multiple myeloma.

Talita Stessuk, Dorrith Verstegen, Sophia Ajala, Hanna Vermeer, Jolie Flach, Marrit Putker, Nataliia Beztsinna, Ludovic Bourre

Crown Bioscience Netherlands B.V., Leiden, Netherlands

Introduction: Preclinical screening of drugs targeting hematological cancers, like Acute Myeloid Leukemia (AML) or Multiple Myeloma (MM), is challenging due to limited *ex vivo* growth and short viability of cells from bone marrow and blood cancers. Consequently, most of the preclinical studies rely on short-term suspension cultures of cell lines and/or primary cells, which undergo spontaneous apoptosis and do not predict the *in vivo* response. Physiologically relevant *in vitro* screening of cells from hematological malignancies requires the recapitulation of the human bone marrow niche (BMN) to provide cell-cell interactions and paracrine factors that are critical for the *ex vivo* proliferation of malignant hematopoietic cells. In our 3D BMN platform, homogeneous and reproducible 3D endothelial-mesenchymal networks that resemble pre-vascularization are used to integrate primary cells isolated from malignant bone marrow disorders and assess Standard of Care (SoC) drug responses
Methods: Bone marrow and whole blood from newly diagnosed and relapsed-refractory (RR) patients with AML and MM were processed to isolate, respectively, CD34+ myeloid blasts and CD138+ myeloma cells. Heterologous mesenchymal stromal cells (MSC) and endothelial cells were embedded in a protein-rich hydrogel in a 384-well format until network formation was microscopically observed. Cryopreserved primary cancer cells were integrated into the 3D BMN and exposed to SoC drug panels for 6-7 days. Quantitative assessment of the SoC drug effects was performed using our proprietary automated high content imaging (HCI) analysis platform. Immunofluorescence (IF) staining and flow cytometry were used for in-depth characterization of BMN-malignant cell co-cultures.
Results: 3D BMN networks were homogeneously distributed throughout the hydrogel and supported the integration of AML and MM cells. Colony-like clusters of cancer cells were observed within the BMN networks, suggesting outgrowth of the cells, which was validated by Ki67 staining. IF staining was also used to characterize and observe the maturation of the BMN over time (CD31, fibronectin). FACS analysis confirmed MSCs immunophenotype and revealed the proportion of myeloid blasts and myeloma cells in the bone marrow of each donor. Dose-dependent responses to Cytarabine and Daunorubicin were observed in primary AML and MM cells integrated in 3D BMN.
Conclusion: The complex bone marrow microenvironment is critical for the maintenance, proliferation, and drug responses of malignant cells from bone marrow disorders. Here, we present a robust and reproducible 3D BMN platform that supports medium-throughput HCI drug screening in primary patient cells from different hematologic malignancies. In addition, our HCI analysis offers the possibility to study off-target effects to the MSCs and endothelial cells within the niche.

#5494 Pursuit of novel first-in-class medicines with a suite of AI-enabled platform technologies.

Jenny Rudnick, Chase Neumann, Kelly Biette, Aurora Blucher, Matias Casas-Selves, Yoon-Chi Han, Gabriela Andrejeva, Rebecca Sarto Basso, Sarah Jo DeVore, Mark Parnell, Christina Egbert, Michaela Hatch, Guillaume Barbe, Carl Brooks, Weston Judd, Vamshi Manda, Zhijuan Cao, Ashraf Saeed, Robin McDougall, Doo-Hyun Kwon, Elizabeth Bruckheimer, Hayley Donnella, Paul Rearden, Joseph Carpenter, Najat Khan, Aimee Iberg, Brandon Probst

Recursion Pharmaceuticals, Salt Lake City, UT

At Recursion, we are on a mission to radically improve patient lives by revolutionizing drug discovery through technological innovation and multi-modal insight generation. Here we describe three preclinical pipeline platform methods that can be assembled in a modular fashion to identify and achieve key stage gate milestones in hit-to-lead and lead-optimization while simultaneously reducing timelines, cutting costs and increasing scale. We show examples from our preclinical pipeline demonstrating the utility of these methods to drive target agnostic discovery of novel first-in-class small molecule oncology therapeutics.(1) Early MoA prediction and derisking: Utilizing our powerful phenomics platform, we can identify starting novel chemical matter predicted to impact disease-relevant biology. However, within most disease-relevant areas of biology, there are strategic or toxicity-based rationales to avoid certain MoAs. By using a combination of phenomics and structural insights, we have built a powerful MoA prediction tool that provides an early view into compound mechanistic insights at the initial hit stage. Hit compounds can then be tested in biochemical assays informed by the predictive model to validate desirable MoAs and/or de-risk undesirable MoAs and therefore advance only those with a desirable path forward. (2) Translational model selection: The pursuit of first-in-class approaches combined with target agnostic discovery presents a challenge to early discovery teams seeking to select best-fit translational models, as the information and predictive value provided by literature is limited. Here we show an example of how we can leverage a combination of public large-data insights, Tempus patient data, DepMap dependency scores, and phenomic relationships to select preclinical translational models that are well suited for testing our hypotheses. Such selection strategies can be automated, enabling rapid execution and decision making in the hit-to-lead stage of discovery. (3) Multimodal SAR: We leverage both phenomics and transcriptomics to drive structure-activity relationship (SAR) optimization in a target agnostic fashion. We integrate these two data layers to select novel chemical entities (NCEs) that drive our biology of interest in both orthogonal assay modalities, enabling us to select compounds with the best in vivo potential. We show an example of how to drive lead optimization SAR by comparing the phenomic and transcriptomic signature of NCEs to those of an efficacious hit compound that potentiates immunotherapy response in vivo, resulting in a polypharmacologic profile that eradicates tumors in 100% of treated animals. Because our approach does not require target-based bespoke cellular or biochemical based potency assays, SAR is highly scalable and is currently being utilized in other programs with a diversity of MoAs.

#5495 Screens to identify TCR-specific epitopes using engineered antigen-presenting cells.

Debbie Deng, Tianbing Liu, Kitt Paraiso, Paul Diehl, **Alex Chenchik**

Cellecta, Inc., Mountain View, CA

Despite the key role of T-cell receptors (TCRs) in adaptive immune responses, experimental validation of interactions between activated TCRs and cognate antigens remains a holy grail in developing novel molecular and cell-based therapeutics. To facilitate efficient screening of TCR-antigen interactions, we engineered both reporter Jurkat-NFAT-GFP cells to express candidate-activated TCRs, and antigen-presenting K562-HLA cells (APC) expressing a library of predicted peptide epitopes. To detect antigen-activation of Jurkat cells interacting with cognate epitopes, the K562 cells were engineered to express a single-chain variable fragment (scFv) which could bind IL2 released by the activated Jurkat-TCR cells. Jurkat-TCR reporter cells co-cultured with K562-APC cells induce expression of the HLA cognate epitope, which enables them to be labeled with PE anti-IL2 antibody and purified by FACS. The cognate epitope is characterized using NGS. To validate this TCR-epitope library screening system, we constructed and expressed a library of ~100 cancer-associated epitopes in K562-HLA_A02:01 cells. The K562-HLA-epitope library was then co-cultured with the Jurkat reporter cells expressing MART1 TCR, NY-ESO-1 TCR or, as a control, CMV TCR. After staining with the IL2 antibody and FACS selection, stained APC cells detected specific epitopes with high specificity for cancer-associated and control TCRs.

#5496 Target density matters: Binding kinetics of antibody-based therapeutics are highly influenced by target expression levels.

Eric Cruickshank[1], Nichole Cerutti[1], Hannah Findlay[1], Timothy London[1], Vivien Hafner[2], Andreas Kratzert[2]

[1]RoukenBio, Motherwell, United Kingdom,[2]Bruker Biosensors (Dynamic Biosensors GmbH), Munich, Germany

Understanding antibody-based therapeutic behaviour under varying target expression levels iscrucial for effective tissue and cell-specific targeting. Traditional *in vitro* evaluations often relyon cancer cell line panels with differing receptor levels, which can be logistically challengingand yield inconsistent results. Standard techniques like SPR are poorly suited for analyzingmolecule binding to eukaryotic cells, as detection depth (~200 nm) covers only ~2% of a typical10-20 µm cell and unstable immobilization limits SPR to cell attachment studies. Similarlimitations apply to BLI and QCM. Other cell-based binding technologies have not gainedwidespread adoption due to challenges in automation, sensitivity, and throughput. Measuringhow binding is influenced by differential target expression is critical for tumor-targetingtherapeutics, as antigen density impacts safety through on-target, off-tumor toxicities andaffects efficacy by enabling tumor escape at low target expression levels.The purpose of this study was to investigate how target antigen density influences the bindingkinetics of therapeutic molecules under physiologically relevant conditions, addressinglimitations inherent to traditional kinetic assays. To achieve this, we utilized the novel IndEx-2system, which employs CHO-K1 cells engineered for tuneable expression of one or two targetantigens via chemically induced proximity-mediated induction. This was paired with single-cellInteraction Cytometry (scIC), a unique biosensor-based technology that measures real-timebinding directly on captured cells. By combining these two systems, we examined the bindingkinetics of trastuzumab to HER2 expressed at varying levels, serving as a model for tumourheterogeneity. HER2 expression was quantified using flow cytometry, while scIC was used toperform kinetic measurements on the same cells. The analysis revealed that higher HER2expression levels prolonged trastuzumab residence time due to avidity effects. Notably,differences in affinity (Kd) across HER2 densities were driven by variations in the dissociationrate (k*off*), rather than the association rate (k*on*), underscoring the critical influence of targetdensity on binding kinetics.This study demonstrates how target density can affect therapeutic binding kinetics, offeringcritical insights into drug efficacy and duration of action. By integrating scIC with the IndEx-2platform, we provide a robust framework for assessing kinetic behaviour of targetedtherapeutics in a cellular context, including bispecific binders and receptor internalisationstudies. These results pave the way for safer, more effective therapeutics, selected in morecomplex, and more relevant, biological systems.

#5497 Sacituzumab govitecan screening in a large organoid panel reveals the importance of functional assays for predicting ADC tumor response.

Marten Hornsveld[1], Jinxi Wang[2], Jun Zhou[2], Xiaolong Tu[2], Peter van Schaik[1], Mariusz Madej[1], Hester Bange[1], Jessie J.J. Wang[2], Ludovic Bourre[3], Marrit Putker[1]

[1]Crown Bioscience Netherlands B.V., Leiden, Netherlands,[2]Crown Bioscience Taicang, Taicang, China,[3]Crown Bioscience Inc, San Diego, CA

Introduction: Antibody-drug conjugates (ADCs) represent an innovative approach in targeted cancer therapy, combining antibodies' specificity with therapeutic modalities such as cytotoxic or targeted chemical compounds. This study presents an ADC testing platform, leveraging patient-derived organoids (PDO) and advanced 3D high-content imaging.
Methods: The efficacy of FDA-approved ADC Sacituzumab Govitecan/SN38 (SG), was evaluated in the CellTiter-Glo (CTG)-based OrganoidXplore panel, a 3D in vitro screening panel of 50 organoid models across 7 indications. The panel was characterized by NGS (WES, RNAseq) but selected without bias for TROP2/TACSTD2 expression. The OrganoidXplore screen was followed up with validating SG and payload responses using CTG and high-content imaging (HCI). ADC target engagement was assessed via immunofluorescence (IF) and ADC responses were validated in a selection of in vivo PDX models.
Results: SG demonstrated a broad differential response (<0.01nM<IC50<50nM) in the OrganoidXplore panel, with 18 models showing strong responses (IC50<0.01nM). Response rates did not correlate with mRNA expression TROP2/TACSTD2. IF staining of the original PDX tumor tissues and organoids confirmed the correspondence between TROP2/TACSTD2 mRNA and protein expression levels, reaffirming that SG sensitivity did not directly correlate with gene and protein expression. Additional studies were performed with SG and SN38 in selected resistant and sensitive organoid models by HCI analysis. Similarly to the CTG assay, TROP2/TACSTD2 protein expression did not correlate with increased SG efficacy, showing that PDO sensitivity was not primarily driven by absolute target levels but rather through the mode of action of ADC uptake and payload sensitivity. Preliminary in vivo ADC efficacy results supported the in vitro findings.
Conclusion: The OrganoidXplore screening platform provides insights into ADC efficacy, and responses and target engagement were validated with HCI. Combined, this demonstrates a lack of correlation between target expression (mRNA and protein) and drug efficacy, and the importance of functional testing for efficacy across a diverse patient population. The unique application of the 3D HCI platform for additional characterization, including ADC binding and uptake assays, will be an essential step to gain insight into the mode of action requirements for ADCs to be effective. These findings highlight the application of functional screening in organoid to better understand the complexity of ADC efficacy and identify responder/non-responder patient profiles to guide on resistance mechanism, in vivo modeling, and biomarker identification for patient stratification.

#5498 Microenvironmental control eliminates edge effect from 96-well plate-based in vitro assays.

Shannon Darou, Graham Jackson, Yan He, Alicia Henn

BioSpherix, LLC, Parish, NY

The most widespread format for *in vitro* drug discovery is the 96-well-plate, but edge effect causes inconsistent results in edge wells due to variations in plate environment. Researchers often avoid using the outermost edge wells, reducing plate capacity by 37 percent, requiring more plates, and increasing assay costs and time. Using the entire plate could save an estimated USD 119M in research costs annually and reduce waste. Finding methods to counteract edge effect is crucial for efficiency, sustainability, and reproducibility of in vitro drug discovery. Previous studies showed that constant thermal control of plating conditions reduces variability in cell distribution in edge wells. Here, we ask the question: can evaporation be eliminated as a difference between edge wells and the other wells of a plate? We plated water in traditional 96-well plates or plates with a water-filled moat around the edges. The plates were covered with either traditional plastic lids that allow gas exchange at the plate edges or an adhesive film that allowed gas exchange across the whole plate. Triplicate plates were incubated for 3 days at 95% relative humidity (RH) in the Xvivo System, a closed system which controls temperature, humidity, and gas levels for cell incubation and handling spaces. Crystal violet dye was added to each well and 0.1ml transferred from each well to a fresh plate. Absorption was read on a plate reader to assess dilution of the dye by the volume of water remaining in each well after incubation (triplicate plates each experiment, n=3 separate experiments). The wells were grouped as four concentric rings, with edge wells as Ring 1, and the center 8 wells as Ring 4. One-way ANOVA with Brown-Forsythe test was used for comparisons. Even at 95 percent RH, traditional plates/lids had edge wells volumes slightly lower than the central rings of wells. There was more overall evaporation from traditional plates covered with film than from the other plate/cover combinations, but there was no difference between the variance in edge wells and other wells. With a moat plate and regular lid, there was no difference between concentric rings on the plate, and there was less overall loss of water across the whole plate. Studies with A549 human lung carcinoma cells also confirmed that the edge effect can be eliminated when temperature and humidity are controlled at all times in a closed cell handling system. We concluded that the proper control of the plate microenvironment in a fully controlled cell handling and incubation environment can allow usage of edge wells for *in vitro* drug discovery, improving efficiency, sustainability, and reproducibility.

#5499 A comparison of spheroid formation using AcroCyte's 3-dimensional R^{3}CE spheroid culture platform *versus* traditional 3D culture methods.

Zichun Wang[1], Di Zhan[1], Qikuan Chen[1], Chao-Jie Yang[2], Jia-Yang Chen[2], Ying C Chang[2], Yinfei Yin[1]

[1]Shanghai ChemPartner Co., Ltd., Shanghai, China,[2]AcroCyte Therapeutics Inc., New Taipei City, Taiwan

Three-dimensional (3D) spheroid culture systems have gained attention due to their evident advantages in mimicking the *in vivo* tumor microenvironment, making them highly valuable for drug testing and cancer research, as the behavior of 3D-cultured cells is more reflective of *in vivo* cellular responses. However, traditional spheroid methods, including ultra-low attachment plates, Matrigel, and soft agar, have limitations, especially for certain cell lines that fail to form stable spheroids. Here, we introduce a novel 3D spheroid culture method using the AcroCyte R^{3}CE plate, and compare it to the generation of spheroids using traditional 3D culture methods. R^{3}CE is a revolutionary scaffold-free single-cell proliferation 3D cell culture platform that can quickly and reliably culture spheroids from a variety of samples including cell lines, circulating tumor cells and biopsies. This technology is highly efficient and can help researchers gain insight into cancer research, clinical treatments, and drug discovery. 20 cell lines with historically poor spheroid formation in standard methods were subjected to the novel AcroCyte R^{3}CE plate culture system which enabled robust and consistent spheroid formation. RNA sequencing (RNA-seq) was performed to compare gene expression profiles between 3D spheroids cultured using the R^{3}CE plate and traditional 2D monolayer cultures. Our analysis identified distinct biomarker expression profiles associated with spheroid cultures, including cancer stem cell-related proteins and key signaling pathways relevant to cell differentiation and tumor biology. The unique biomarker expression profiles of the cancer spheroid cells were validated at the protein level by way of immunofluorescence or western blot. This innovative approach utilizing the R^{3}CE 3D cell culture platform, not only improves spheroid formation across difficult-to-culture cell lines, but also preserves critical biomarker expression profiles and pathways linked to tumorigenesis. These findings highlight the potential of the R^{3}CE 3D culture system to establish a reliable and versatile spheroid panel for high-throughput drug screening, providing a more physiologically relevant model to evaluate drug efficacy and mechanistic insights.

#5500 A customizable, end-to-end platform for the screening and characterization of ADCs.

Zichun Wang, Qiong Wang, Shuyue Wang, Guilan Wang, Qikuan Chen, Wei Xue, Yinfei Yin

Shanghai ChemPartner Co., Ltd., Shanghai, China

Chemotherapy and radiotherapy have been considered the first line of antitumor therapies for patients with advanced or metastatic cancer, however these therapies demonstrate numerous drawbacks and acquired resistance is still inevitable in most cases. In order to address these challenges, scientists have discovered a novel class of cancer-targeted drugs referred to as antibody-drug conjugates (ADCs). ADCs are an emerging class of therapies that conjugate monoclonal antibodies with cytotoxic payloads through a chemical linker, allowing precise delivery of drugs to cancer cells. Trastuzumab deruxtecan (T-DXd) is an ADC composed of a humanized IgG antibody that specifically targets HER2, a tetrapeptide-based cleavable linker and a topoisomerase I inhibitor payload. T-DXd is the second HER2-targeting ADC approved by the FDA after trastuzumab emtansine (T-DM1); approved for HER2-positive unresectable or metastatic breast cancer in the second line and as an adjuvant therapy for residual disease in breast cancer patients with early stage disease. Both ADCs have demonstrated promising clinical efficacy in the treatment of HER2-positive solid cancers, which has sparked great interest in the development of the next generation of ADCs. Emerging evidence indicates that the efficacy of a given ADC depends on a number of key factors including the fine tuning between the combination of the antibody, linker and payload components, how the ADC is processed and it's interaction with the cancer cells. Here we present robust and reproducible data from our comprehensive modular *in vitro* and *in vivo* screening platform, that has been developed to facilitate efficient and streamlined approaches for ADC screening and characterization, and have been validated using T-DXd and T-DM1 as proof-of-concept agents. Our platform offers assays that can evaluate ADC binding, internalization, anti-proliferative activity, ADC effects on cell cycle, apoptosis, and bystander effects. Immunological assays, such as antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent cellular phagocytosis (ADCP) can be employed to assess the induction of Fc effector functions. Additionally, we have validated a number of *in vivo* models including co-inoculation and intracranial xenograft models. Our data demonstrates that both ADCs exhibit strong binding and internalization, with T-DXd showing a pronounced bystander effect due to its payload release via a cleavable linker. Both agents effectively induced ADCC and ADCP, with minimal complement-dependent cytotoxicity observed. The T-DXd bystander effect was also confirmed used an *in vivo* co-inoculation xenograft model and efficacy of both T-DXd and T-DM1 against a human intracranial xenograft model was confirmed. This platform provides a comprehensive and customizable tool for ADC preclinical assessment, supporting the optimization of ADC therapeutic efficacy and safety.

#5501 An integrated end-to-end platform facilitating the evaluation of next generation KRAS inhibitors.

Di Zhan, Lingling Huang, Lei Zhang, Ting Ma, Yuzhou Xu, Yinfei Yin, Zichun Wang

Shanghai ChemPartner Co., Ltd., Shanghai, China

Kirsten rat sarcoma viral oncogene homologue (KRAS) is the most frequently mutated oncogene, and has attracted substantial attention in the preclinical arena. Over the last 40 years, KRAS has been deemed a challenging therapeutic target and has even been described as “undruggable” due to the intrinsic characteristics of KRAS proteins. This has resulted in an urgent and unmet clinical need to target KRAS mutations in KRAS-driven cancer. Recent advances have uncovered a new allosteric site of KRAS (G12C) that facilitates the development of novel covalent allosteric inhibitors targeting this shallow pocket on the surface of KRAS, thereby raising the hope of effectively drugging KRAS. Encouraging clinical trial results have been reported for two KRAS G12C inhibitors, AMG510 (sotorasib) and MRTX849 (adagrasib), with AMG510 being approved by the FDA for clinical use in 2021. Despite the clinical benefits of KRAS G12C inhibitors such as AMG510 and MRTX849, there are still problems that need to be overcome including optimization of clinical efficacy and overcoming drug resistance. Recent clinical research has identified diverse genomic and histological features underlying the resistance of KRAS G12C mutant cancer patients. These resistance mechanisms include co-occurring mutations such as KEAP1, SMARCA4 and CDKN2A, and the acquisition of genetic mutations such as H95D, H96D and R68S in the switch-II pockets. Other non-genetic mechanisms such as reactivation of RAF-MEK-ERK pathway and cell state transition have also been identified. To enable discovery of the new generation of KRAS inhibitors, we have developed a comprehensive KRAS assay platform, which includes biochemical and cell based *in vitro* assays, and *in vivo* efficacy evaluation in KRAS mutant xenograft models. In addition, to allow researchers to have a better understanding of the biological basis of drug resistance, we have established and validated a panel of lung and pancreatic cell models that are resistant to AMG510 and MRTX849 through CRISPR-Cas9 editing and drug-induced approaches. Our continuously evolving assay platform facilitates the discovery and development of new generation KRAS inhibitors and combinational strategies.

#5502 An integrated oligonucleotide synthesis and cellular *in vitro* assay platform to support oligonucleotide-based drug discovery and development.

Di Zhan, Shuyue Wang, Qiuya Huang, Zichun Wang, Wei Xue, Yinfei Yin, Xingquan Ma

Shanghai ChemPartner Co., Ltd., Shanghai, China

Oligonucleotide-based therapeutics, such as small interfering RNAs (siRNAs), represent a transformative approach for gene-specific modulation in various diseases due to their capacity to regulate gene expression and protein function, with reduced toxicity compared to conventional treatments. Our oligonucleotide synthesis platform offers a comprehensive range of specialized monomers, diversified oligonucleotides, and customized solutions for oligonucleotide conjugation. To support research into oligonucleotide drugs, we have established a comprehensive *in vitro* assay platform that includes a series of critical evaluations such as oligonucleotide screening by transfection and RT-qPCR or western blot, oligonucleotide binding and uptake, sub-cellular localization, off-target assessment, and functional efficacy testing. For proof of concept, we employed inclisiran, one of the clinically approved siRNA therapeutics targeting PCSK9, to demonstrate our platform's capabilities. RT-qPCR-based oligo screening assays confirmed the highly effective silencing of PCSK9, with inclisiran achieving >90% reduction in target mRNA, with the IC50 concentration in the single picomolar level. High levels of cellular binding, uptake and subsequent successful co-localization with endosome and lysosome within target cells were observed by means of flow cytometry or high-content imaging. The cellular binding and uptake assay verified that inclisiran maintains high affinity and selective binding of GalNAc, mediating delivery to cellular targets, while the subcellular co-localization studies demonstrated inclisiran effectively trafficking to intracellular compartments, which is essential for its gene-silencing function. Off-target assessments were conducted using a transcriptome-wide approach, which showed minimal off-target effects and confirmed specificity. Functional assessments, which included cellular LDL uptake, further validated the efficacy of inclisiran in modulating PCSK9 expression. In conclusion, we provide an integrated one-stop platform for oligonucleotide-based drug discovery and development, aiming to expedite the early-stage discovery process. Our *in vitro* platform allows for a thorough evaluation of oligonucleotide drugs, covering critical functional and efficacy aspects that are essential for preclinical development. Our data with inclisiran as a model therapeutic confirm the platform's efficiency and robustness in supporting the discovery and optimization of oligonucleotide therapeutics, providing a valuable resource for advancing gene-silencing therapies.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Drug Resistance in Molecular Targeted Therapies 3
Poster Session

#5506 Inactivation of mTOR is a strategy for tumor resistance to ribosome biogenesis inhibition.

Wenjun Fan[1], Lijing Yang[2], Hester Liu[1], Steffie Pitts[1], Niladri Sinha[3], Rajeshkumar N.V.[1], Hariharan Easwaran[2], Rachel Green[3], Marikki Laiho[1]

[1]Department of Radiation Oncology and Molecular Radiation Sciences, Johns Hopkins University School of Medicine, Baltimore, MD,[2]Department of Oncology and The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD,[3]Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD

Increased ribosome biogenesis supports the need for high translation capacity and is a hallmark of cancer. Targeting this process presents a promising strategy for cancer therapy. We have developed first-in-class inhibitors of RNA polymerase I (Pol I) transcription that block the first and rate-limiting step of ribosome biogenesis, rRNA synthesis. We find that Pol I inhibition by BMH-21 leads to a significant decrease in active ribosomes. We found a broad, but heterogenous efficacy in 18 cancer cell lineages. To identify genes that drive potential resistance to Pol I inhibition, we performed genome-wide CRISPR-Cas9 screens in human colorectal carcinoma cells. These positive selection screens identified high-confidence hits leading to drug resistance and included all key positive regulators of the mTORC1 complex. These findings are striking and counterintuitive given that mTOR is a major driver of ribosome biogenesis and cellular translational programs. The findings were validated using chemical inhibition of mTOR by Torin-1, a catalytic mTOR inhibitor and genetic knockout of an activator of mTOR signaling. Consistently, compromised mTOR activity led to resistance to BMH-21. However, loss of mTOR activity did not abrogate Pol I transcription inhibition by BMH-21. To assess the impact on protein translation, we used polysome profiling and found that BMH-21 treatment caused a severe defect in ribosome biogenesis. Surprisingly, mTOR inactivation partially rescued the translation repressed by Pol I inhibition, suggesting that this rescue could be pivotal for cell survival. To profile translational events, we performed Ribo-seq and RNA-seq in the BMH-21 sensitive and resistant cells. These multi-omics results revealed that mTOR inactivation decreased polysome-based translation on 5'TOP mRNAs encoding ribosomal proteins, suggesting that mTOR inactivation reduces ribosome occupation on ribosomal mRNAs. We propose that mTOR inactivation switches off polysome translation to rearrange active ribosomes for translation of survival-essential mRNAs. We further quantified newly synthesized proteins by AHA-labeling combined with TMT-mass spectrometry. These data show that distinct proteins continue to be translated when both Pol I transcription and mTOR activity are blocked. Next, we will examine preferentially translated proteins in resistant cells, and explore combination therapies to circumvent the drug resistance. In summary, we uncover a new non-genetic model of cancer drug resistance termed as translational fitness that enables cells to survive severe translational repression. These findings reveal an unexpected complication by mTOR inhibitory strategies and have implications for exploring effective drug combinations in cancer therapy.

#5507 Mechanisms of resistance to RAS-GTP inhibition in pancreatic cancer.

Joshua H. Choe[1], Julien Dilly[1], Eejung Kim[1], Lick P. Lai[2], Nicole Sindoni[3], Fan Yi Kong[3], Dennis Gong[4], Jaclyn Varga-Wiles[1], Brendan Parent[1], Junning Wang[1], Kyle Seamon[2], Jennifer A. Chan[1], Clint A. Stalnecker[5], Channing J. Der[5], William L. Hwang[6], Brian M. Wolpin[1], David Wildes[2], Joseph D. Mancias[3], Lewis C. Cantley[7], Andrew J. Aguirre[1]

[1]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA,[2]Revolution Medicines, Redwood City, CA,[3]Department of Radiation Oncology, Dana-Farber Cancer Institute, Boston, MA,[4]Massachusetts Institute of Technology, Cambridge, MA,[5]UNC Lineberger Comprehensive Cancer Center, Chapel Hill, NC,[6]Massachusetts General Hospital, Boston, MA,[7]Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA

Targeting oncogenic RAS signaling has the potential to be effective for many RAS-addicted cancers, including pancreatic ductal adenocarcinoma (PDAC). RMC-7977 is a RAS(ON) multi-selective tri-complex inhibitor exhibiting broad-spectrum preclinical activity against both wild-type and oncogenic RAS variants and has shown encouraging anti-tumor activity in preclinical models of PDAC. A related compound, RMC-6236, is under clinical investigation for both RAS-mutant solid tumors and metastatic PDAC. Tumor progression due to acquired resistance is frequently observed for targeted agents, but potential mechanisms of resistance specific to RAS(ON) multi-selective inhibitors have not been characterized. Here, we investigate both on-target and off-target mechanisms of resistance to RMC-7977 in pre-clinical models of PDAC. Using deep mutational scanning and structure-function analysis, we define the landscape of secondary KRAS mutations that confer resistance to RMC-7977. Leveraging insights from ctDNA analysis of patients, we validated putative genetic mechanisms of resistance in PDAC cell line and xenograft models, finding that PI3K pathway activation can drive proliferation under RAS blockade. Furthermore, we performed a multi-omic characterization of human PDAC cell lines and organoids, as well as murine PDAC tumors, to identify factors contributing to RAS independence. Epithelial-to-mesenchymal transition emerged as highly associated with both innate and acquired resistance. To further understand the adaptive signaling that promotes survival in mesenchymal, resistant PDAC cells, we applied phosphoproteomics followed by kinase motif enrichment analysis in resistant cells after RAS inhibition. We determined multiple kinases involved in ECM and cytoskeletal signaling that are activated upon MAPK suppression uniquely in mesenchymal PDAC cells. Targeting these kinases in combination with RMC-7977 reverted resistance in models of innate and acquired resistance, suggesting a potential approach to investigate preventing or overcoming resistance in patients. Collectively, our pre-clinical data suggest that resistance to RAS inhibition with RMC-7977 may arise via multiple mechanisms, among them secondary RAS mutations, PI3K pathway-activating mutations, or cell-state changes that drive adaptive signaling through ECM-signaling pathways.

#5508 Direct inhibition of RAS reveals the features of oncogenic signaling in RAS-mutant cancers.

Santiago Garcia Borrego[1], Michelangelo Marasco[2], Dinesh Kumar[2], Tessa Seale[1], Riccardo Mezzadra[3], Giulia Maddalena[4], Kylie Belanger[1], Soren Cole[1], Bryan Perez[1], Wei Luan[3], Radha Mukherjee[2], Ilinca Aricescu[2], Vladimir Markov[5], Yuxin Zhu[5], Sabrina Arena[6], Alberto Bardelli[6], Elisa de Stanchina[5], Scott Lowe[3], Richard Burkhart[1], Jackie Zimmerman[1], Rona Yaeger[7], Scott Kopetz[4], Neal Rosen[2], Sandra Misale[1]

[1]Cancer Genetics and Epigenetics Program, Johns Hopkins Medicine, Baltimore, MD,[2]Molecular Pharmacology Program, Memorial Sloan Kettering Cancer Center, New York, NY,[3]Department of Cancer Biology and Genetics, Memorial Sloan Kettering Cancer Center, New York, NY,[4]Department of Gastrointestinal Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX,[5]Antitumour Assessment Core Facility, Memorial Sloan Kettering Cancer Center, New York, NY,[6]Department of Oncology, Molecular Biotechnology Center, Torino, Italy,[7]Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY

RAS genes are frequently mutated in cancer, often at hotspots altering codons 12, 13, and 61. The role of mutant RAS in driving overactivation of ERK signaling in cancer has long been elucidated, but its involvement in the oncogenic activation of PI3K is still controversial, and different studies have reported conflicting findings regarding the requirement of RAS for physiological and oncogenic PI3K signaling. One of the reasons for this lack of understanding has been the inability to directly target RAS, which has historically been circumvented through genomic perturbation and murine models. However, these approaches are limited because it is not possible to distinguish between the direct effects of RAS on PI3K and compensatory events that occur as an adaptation to the oncogene. With the discovery of RAS inhibitors, we can now directly interrogate the biological effects of RAS isoforms and hotspot mutations in cancer cells with improved time resolution. Here, we use RMC-7977, a first-in-class RAS·GTP inhibitor (RAS^{MULTI}(ON)), to characterize the features and therapeutic vulnerabilities of mutant RAS signaling in various human cancers. Through a detailed biochemical analysis of the signaling response to RAS inhibition, we reveal that RAS-mediated oncogenic signaling is largely independent of the mutant RAS isoform, rather depending on the mutant codon. Specifically, RAS^{Q61X} is exquisitely sensitive to MAPK pathway inhibition, confers near-complete independence of upstream regulation, and is impaired in its signaling to PI3K/Akt/mTOR. Conversely, RAS^{G12X} enables oncogenic signaling to both MAPK and PI3K/Akt/mTOR by amplifying upstream input from receptor tyrosine kinases (RTKs). Simultaneous RAS and RTK inhibition selectively impairs the growth of RAS^{G12X}-mutant tumors, irrespective of RAS isoform and tumor type, and we investigate the molecular mechanisms behind this synergistic effect. Additionally, we have generated a genetically modified mouse model (GEMM) harboring liver tumors induced by RAS^{G12D} or RAS^{Q61R}. In cell lines derived from these respective models, RAS^{G12D} but not RAS^{Q61R} activates PI3K upon growth-factor stimulation. These findings provide mechanistic insights into how mutant RAS interacts with upstream and downstream partners to maximize oncogenic signaling. Furthermore, our results shed light on the selective pressure driving the emergence of RAS^{Q61X} mutations as a mechanism of resistance against RTK inhibition in colorectal cancer: we show that colorectal cancer cells that acquire RAS^{Q61X} mutations to overcome EGFR inhibition become more independent of upstream signaling than those that acquire RAS^{G12X} and this may explain the predominance of this mutation in colorectal cancer patients who acquire resistance to EGFR inhibitors.

#5509 Mechanisms regulating resistance to KRAS inhibitor driven by DNp63/YAP regulation of tissue stiffness and metabolic alterations in lung adenocarcinoma.

Santanu Adhikary[1], Marco Napoli[1], Suehelay Acevedo[1], Christina L. Carr[1], Jaden R. Baldwin[1], Nicole Hackel[1], Hitendra S. Solanki[2], Yaakov E. Stern[2], Laxmi Swetha Karanam[3], Duy T. Nguyen[3], Eric B. Haura[2], Elsa R. Flores[1]

[1]Molecular Oncology, H. Lee Moffitt Cancer Center, Tampa, FL,[2]Thoracic Oncology, H. Lee Moffitt Cancer Center, Tampa, FL,[3]Bioengineering, H. Lee Moffitt Cancer Center, Tampa, FL

Targeted therapies to the KRAS oncogene have shown significant success in treatment of lung adenocarcinoma (LuAD). Challenges emerge when tumors acquire resistance to these targeted KRAS inhibitors (KRASi). Recent studies have reported several mechanistic pathways associated with the development of resistance which includes nuclear localization of YAP. TP53 mutations are the predominant genetic changes responsible for inducing resistance to targeted therapies and chemotherapy drugs. Mutations in *TP53* lead to the inhibition of the tumor and metastasis suppressor TAp63 and the activation of the oncogenic isoform ΔNp63. We have previously shown that loss of ΔNp63 in KRAS driven LuAD results in fewer tumors indicating an oncogenic role for ΔNp63 in LuAD. Here, we demonstrate that cells and tumors resistant to KRAS G12C inhibitors ($KRASi^R$) exhibit increased expression of ΔNp63 while the sensitive cells express lower levels of ΔNp63. The $KRASi^R$ cells showed increased total and nuclear localization of YAP/TAZ along with increased expression of ΔNp63 in the nucleus. $KRASi^R$ resistant cells also had increased proliferation, mitochondrial membrane potential and mitochondrial fusion, promoting oxidative phosphorylation in $KRASi^R$ LUAD cells. We further found that ΔNp63 is crucial for driving resistance to KRAS G12C inhibitor *in vivo* using genetically engineered mouse models and patient derived xenografts. Therefore, ΔNp63 is an ideal target to overcome acquired resistance to KRAS inhibitors. Additionally, using atomic force microscopy (AFM), we found that $KRASi^R$ cells are stiffer, and that tissue stiffness regulated by YAP/ΔNp63 can also result in KRASi resistance. ChIP-seq analysis for ΔNp63 and YAP bound targets are revealing the molecular basis of the mechanisms driving resistance to KRAS G12C inhibitors.

#5510 Targeting BET, Hippo-YAP and RAS-MAPK signaling interplay in lung cancer and therapy resistance.

Nilanjana Chatterjee[1], Victor Olivas[1], Wei Wu[1], Tracy Tang[2], Ben Powell[3], Trever Bivona[1]

[1]UCSF - University of California San Francisco, San Francisco, CA,[2]Vivace Therapeutics, San Mateo, CA,[3]Kinnate Biopharma, San Francisco, CA

Identifying alternate survival pathways or compensatory mechanisms that limit the response to targeted therapy is critical to discover effective combinatorial strategies for lung cancer treatment and prevention of drug resistance.Using a combinatorial drug screen in a panel of non-small cell lung cancer (NSCLC) cell lines, we identified a functional interplay between RAS-MAPK pathway oncoproteins and BET chromatin regulators in non-small cell lung cancer (NSCLC). We found that combination of BET and RAS-MAPK inhibitors caused pronounced cell death and potent tumor regression/ stasis in several KRAS or BRAF mutant models of NSCLC with potent apoptosis induction, pro-apoptotic BIM upregulation, and pro-survival MYC and BIRC5 (Survivin) downregulation. However, NSCLC cells and tumors with co-occurring alterations in the tumor suppressor STK11 were found to be partially sensitive/ resistant to the BET and RAS-MAPK inhibitors combination. By modeling STK11 deficiency in NSCLC cells and tumors, we further found that STK11 loss creates an unprecedented dependence on YAP/TEAD TFs in lung cancer. STK11 silencing in NSCLC cells resulted in the nuclear translocation of the Hippo pathway effector, YAP and activation of YAP targets, conferred resistance to RAS-MAPK inhibitors and their combination with BET inhibitors and restored cell viability. Furthermore, phenocopying STK11 loss, constitutively active 5SAYAP mitigated the deleterious effects of BET and RAS-MAPK co-inhibition in STK11-proficient NSCLC cells with concomitant upregulation of YAP targets, such as, MYC and BIRC5. Conversely, YAP/TEAD suppression re-sensitized STK11-deficient NSCLC cells and tumors to BET and RAS-MAPK inhibitors combination and induced apoptosis, pronounced loss in cell viability, and tumor regression.This study uncovered a functional interplay between BET chromatin regulators, YAP/TEAD TFs and RAS-MAPK oncoproteins that drives therapy resistance in lung cancer, specifically upon loss of key tumor suppressors, such as, STK11 and suggests rational combinatorial strategies with BET, TEAD and RAS-MAPK inhibitors.

#5511 KRAS inhibition targets and depends on pro-oncogenic HuR in PDAC.

Hen Halamish[1], Yiefe Guo[1], Jennifer M. Finan[1], Vlad Grigoriev[2], Oleh Taratula[2], Alexander Smith[3], Rosalie C. Sears[3], Jonathan Brody[1]

[1]Surgery, Oregon Health and Science university, Portland, OR,[2]OSU/OHSU College of Pharmacy, Portland, OR,[3]Oregon Health and Science university, Portland, OR

The Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation is found in more than 90% of pancreatic ductal adenocarcinomas (PDACs) making it an attractive therapeutic target. However, even with the recent promise of new KRAS inhibitor drugs, the overall enthusiasm is dampened due to reports of drug resistance. Our recent work has shed light on the critical role of Human Antigen R (HuR), an RNA-binding protein, in its role in KRAS signaling and inhibition. Using *KRAS* mutant patient derived models and cell lines derived from genetically engineered mouse models, we surprisingly found that transient siRNA silencing or CRISPR gene deletion of HuR, *ELAVL1* (HuR-KO) led to resistance of 2.5- fold to more than 1000-fold to KRAS inhibitors compared to control cells. Moreover, other relevant therapies for PDAC such as gemcitabine and Oxaliplatin showed no significant difference between our models. Validating this using an *in vivo* orthotopic model, MRTX1133 induced profound tumor regression solely in the wild-type group compared to the HuR-KO cohort. The wild-type treated tumors exhibited a 3.8-fold reduction in weight, contrasting with a mere 1.1-fold change observed in the HuR-KO treated group, as compared to the vehicle-treated group ($p < 0.0001$). Using a lipid nanoparticle loaded strategy to deliver HuR mRNA to HuR-KO cells *in vitro* and *in vivo* rescued the Kras inhibitor resistance phenotype and made the cells sensitive to a Kras inhibitor. A significant increase in tumor-infiltrating T cells was observed in both wild type and HuR ko treated groups ($p < 0.001$), however only treated wild-type group had a significant difference in activated (Tbet) $CD4^+$ and $CD8^+$ cells. Moreover, a significant decrease was observed in DCs and an increase in macrophages between vehicle wild-type group to all the other groups, which suggest that the resistance mechanism cannot be completely attributed to the immune microenvironment. Mechanistically, we applied phospho-proteomic, proteomic and RNAseq analysis on our PDAC models treated and untreated with Kras inhibitors which revealed a possible compensation mechanism that occurs with overexpression of IGF family members genes. Furthermore, a potential connection between HuR and MYC has emerged from our data, as MYC is an established HuR target and driver of Kras inhibitor resistance. More mechanistic studies determining the molecular interaction between HuR and KRAS and KRAS related mRNAs are underway. combined with ongoing studies, we highlight a significant breakthrough in the understanding of PDAC cells addiction to Kras signaling in the context of the pro-oncogenic HuR molecule, revealing that the absence of HuR confers resistance to KRAS inhibitor drugs. Understanding the interplay between KRAS and HuR could offer a novel strategy to disrupt the oncogenic KRAS signaling cascade and overcome the potential inherent and acquired resistance mechanisms of KRAS-mutant PDACs to promising, KRAS-targeted therapies.

#5512 Combining RAS(ON) G12C-selective and RAS(ON) multi-selective inhibitors overcomes sotorasib resistance driven by *KRAS^{G12C}* amplification or *NRAS^{G13R}* mutation.

H. S. Solanki[1], D. Imbody[1], B. Desai[1], D. Miroshnychenko[1], B. Sarcar[1], A. Majumder[1], Y. Zhang[1], U. Rix[1], A. Marusyk[1], M. Singh[2], I. Aronchik[2], E. B. Haura[1];

[1]Moffitt Cancer Center, Tampa, FL, [2]Revolution Medicines, Redwood City, CA

Amplification of the *KRAS^{G12C}* mutant allele and acquisition of NRAS mutations have been reported as resistance mechanisms in tumors progressing on sotorasib and adagrasib. We also identified these alterations in resistance models generated in vitro and in vivo utilizing H358 cells. We evaluated the activity of the RAS(ON) multi-selective noncovalent tri-complex inhibitor RMC-7977, both alone and in combination with the RAS(ON) G12C-selective covalent inhibitor RMC-4998. We generated five sotorasib-resistant cell lines from relapsed tumors of sotorasib-treated H358 xenografts (MR1-MR5) and two vehicle-treated tumors (MV1 and MV2). We created an additional isogenic cell line model, H358-MX1, by treating H358 cells with sotorasib and a SHP2 inhibitor, RMC-4550. Whole exome sequencing (WES) and digital polymerase chain reaction (dPCR) were performed to study mutant allelic fractions. Taqman copy number analysis and fluorescence in situ hybridization (FISH) were performed to assess copy number alterations. The anti-proliferative activity of RMC-7977 alone and in combination with RMC-4998 was evaluated. We identified increased allelic frequency of *KRAS^{G12C}* in MR2 and acquisition of a secondary *NRAS^{G13R}* mutation in H358-MX1 models. Copy number analysis revealed nearly 20 *KRAS* copies in MR2 compared to parental controls. *KRAS* amplification in MR2 was confirmed by dPCR and FISH. RAF-RBD pull down assays confirmed elevated KRAS-GTP and pan-RAS-GTP levels in MR2 cells. A small molecule screen using a customized library revealed that sotorasib had synergistic anti-proliferative activity in vitro with agents targeting the MAPK pathway. We hypothesized that simultaneous suppression of SHP2 and KRAS^{G12C} could elicit more pronounced inhibition of MAPK signaling and tumor growth. Dual inhibition of KRAS^{G12C} and SHP2 provided effective tumor control in vivo for 2 weeks before tumors started to regrow. Treatment with either RMC-4998, RMC-7977, or the combination of RMC-4998 + RMC-4550 did not sustain long-term tumor growth inhibition in this model. However, the RMC-4998 + RMC-7977 combination drove sustained, significant regressions compared to single-agent treatments or other combination regimens. In H358-MX1 model, siRNA-based knockdown of NRAS in combination with sotorasib reduced ERK phosphorylation and inhibited cell proliferation more effectively than NRAS knockdown or KRAS^{G12C} inhibition alone. In H358-MX1 mouse xenografts, RMC-7977 + RMC-4998 significantly inhibited tumor growth compared to single-agent treatments or other combination regimens. These preclinical data suggest that combination of RAS(ON) mutant-selective and RAS(ON) multi-selective inhibitors overcome resistance to KRAS^{G12C} (OFF) inhibitors driven by *KRAS^{G12C}* amplification and secondary *NRAS^{G13R}* mutation in *KRAS^{G12C}* mutated lung cancer models.

#5513 Discovery of SC2073, a potent and mutant selective EGFR degrader that overcomes 3rd generation EGFR inhibitors resistance in NSCLC.

Jihoon Choi[1], **Jun Gyu Kim**[1], Ok Young Lee[1], Young Jun Park[1], Young Min Jeong[1], Chung Sil Lee[1], Sun Ho Choi[1], Hye Yeon Lee[1], Yu Jin Lee[1], Punna Reddy Ullapu[1], Dae Young Lee[1], Hye Sun Lee[1], Jong Ryoul Choi[2], Daseul Yoon[2], Yanghun Tae[2], Ji-Young An[2], Hyo Ju Lee[2], Bong Tae Kim[2], Mi-Kyung Kim[1]

[1]Research Headquarter, Dong-A ST, Yongin-si, Korea, Republic of,[2]New Drug R&D institute, HK inno.N Corp., Seoul, Korea, Republic of

Epidermal growth factor receptor (EGFR) mutations, including the L858R activating mutation in exon 21, are major drivers of non-small cell lung cancer (NSCLC) and are targeted by EGFR tyrosine kinase inhibitors (TKIs). However, despite significant initial clinical benefits, resistance to EGFR TKIs frequently develops due to secondary mutations such as T790M and C797S, which reduce the therapeutic efficacy of second- and third-generation inhibitors. Consequently, extensive efforts have been made to develop new small molecule inhibitors to overcome this resistance, including novel approaches such as proteolysis targeting chimera (PROTAC) technology. We identified a promising heterobifunctional degrader, SC2073, designed to target a unique allosteric binding site on mutant EGFR while sparing wild-type EGFR. SC2073 effectively induced the degradation of EGFR proteins harboring single, dual, or triple mutations through the ubiquitin-proteasome system. It exhibited potent anti-proliferative activity against Ba/F3 cells with common EGFR resistance mutations, including L858R, L858R/T790M, L858R/C797S, and L858R/T790M/C797S as well as NCI-H1975 (L858R/T790M) cells without affecting the expression levels of GSPT1 and SALL4, which are substrates of the E3 ligase protein (CRBN). Notably, SC2073 showed no effect on the proliferation of Ba/F3 and A549 cells expressing wild-type EGFR, thereby minimizing potential toxic side effects associated with wild-type EGFR inhibition. SC2073 demonstrated a favorable pharmacokinetic profile, rapidly degrading mutant EGFR in tumors within 6 hours of oral administration and maintaining efficacy for 24 hours. Further investigations confirmed that oral administration of SC2073 resulted in a dose-dependent, robust anti-tumor activity in allograft and xenograft models resistant to EGFR TKIs by promoting the degradation of mutant EGFR. In this study, we present SC2073, an orally bioavailable, highly potent, and mutant-selective degrader with the potential to provide more durable, safer, and improved therapeutic responses. SC2073 effectively inhibited the proliferation of Ba/F3 cell expressing EGFR mutants while sparing WT EGFR cells and it demonstrated robust anti-tumor activity in EGFR mutation-driven *in vivo* tumor models. Our data suggests that SC2073 could be a promising treatment option for NSCLC patients harboring various EGFR mutations, including L858R, T790M, and C797S, which are associated with acquired resistance to EGFR inhibitors.

#5515 ELVN-001, a highly selective ATP-competitive ABL1 tyrosine kinase inhibitor for the treatment of chronic myeloid leukemia alone or in combination with asciminib.

Stefan David Gross[1], Christopher A. Eide[2], Michael R. Burkard[1], Anna Reister Schultz[2], Yiheng Zhang[3], Joseph E. Aslan[4], Owen J. McCarty[3], Monette A. Aujay[1], Samuel Kintz[1], Brian J. Druker[2], Joseph P. Lyssikatos[1]

[1]EnLiven Therapeutics, Boulder, CO,[2]Knight Cancer Institute, Oregon Health & Science University, Portland, OR,[3]Department of Biomedical Engineering, Oregon Health & Science University, Portland, OR,[4]Knight Cardiovascular Institute, Oregon Health & Science University, Portland, OR

ELVN-001 is a potent, highly selective ABL1 Tyrosine Kinase Inhibitor (TKI) currently being evaluated in a Phase 1a/b clinical trial in patients with BCR-ABL1-driven chronic myelogenous leukemia (CML). Due to its unique binding mode, ELVN-001 retains activity against many of the TKI-associated ABL1 resistance mutations including T315I as well as most of the emergent resistance mutations associated with the allosteric inhibitors like asciminib. While the active-site TKI ponatinib is active against T315I, its poor kinome selectivity limits its clinical utility. In contrast, ELVN-001 is highly kinome-selective thereby avoiding off-target kinases, including KDR, c-KIT, FLT3, PDGFR and SRC, which underlie many of the adverse events associated with the approved active-site TKIs; notably myelosuppression, edema, pleural effusions, hypertension, and certain cardiovascular events. Accordingly, ELVN-001 has demonstrated early signs of efficacy with good tolerability in heavily pre-treated patients with CML. Asciminib represents an important alternative for patients who have become intolerant of active site TKIs or whose disease is relapsed or refractory to treatment. As asciminib inhibits ABL1 by engaging its myristoyl pocket, it is highly kinome-selective and thus demonstrates an improved clinical safety profile. Because asciminib binds outside the kinase domain, it is not sensitive to most mutations conferring resistance to active site TKIs including T315I although a 5-fold higher dose is required to effectively treat this mutation. ABL1 mutations conferring clinical resistance to asciminib (and likely other STAMP inhibitors) have been reported which largely reside within the non-catalytic myristoyl pocket. Importantly, ELVN-001 retains potency against a panel of clinically relevant asciminib resistance mutants in a Ba/F3 cell background. As ELVN-001 and asciminib target distinct non-overlapping regions in ABL1, we evaluated the potential advantage of combining these agents. The mechanistic and functional effects of these drugs both alone and in combination were assessed in cell models of CML including a model of T315I resistance. Results showed that while each compound exerted differentiated effects on the known BCR-ABL1 effector pathways, both exhibited potent antiproliferative and pro-apoptotic activity, especially in combination. Therefore, the combination of ELVN-001 and asciminib was evaluated in a mouse tumor xenograft model of CML. Results demonstrated that combining ELVN-001 and asciminib was not only well tolerated but elicited profound anti-tumor activity. Finally, ENU mutagenesis screens were conducted in a Ba/F3 BCR-ABL1 line with ELVN-001 and asciminib, alone and in combination. Results of these studies and their impact on the clinical utility of this combination will be discussed.

#5516 Deciphering mechanism of resistance to anti EGFR treatment regimen in a NSCLC PDX model.

Philipp Meyer[1], Ruzica Bago[2], Balasz Pograny[2], Kanstantsin Lashuk[1], Diarmuid Kenny[2], Blake Rushing[3], Eva Oswald[1], **Julia B. Schueler**[1]

[1]Charles River Laboratories, Inc., Freiburg, Germany,[2]Charles River Laboratories, Inc., Chesterford Research Park, United Kingdom,[3]Nutrition Research Institute, University of North Carolina at Chapel Hill, Kannapolis, NC

Acquired resistance prevents patients from long term benefits of targeted therapies in many different cancer types. Overactivation of EGFR in malignant cells is a prominent example for acquired resistance since anti-EGFR treatment in NSCLC patients often starts showing loss of activity after a certain period. We have developed three NSCLC PDX lines carrying an acquired resistance towards Gefitinib, a highly selective EGFR inhibitor. All three sublines depict a specific mutational and gene expression pattern. These PDX models were now further characterized by assessment of in vivo sensitivity versus Cetuximab, a chimeric anti-EGFR antibody. The characterization included assessment of tumor growth over time, proteomic and metabolomic analysis at different time points: 24h after treatment, in remission and at regrowth. The parental PDX line and clones 1-3 displayed distinct sensitivity patterns against Cetuximab. The parental PDX was highly sensitive showing no regrowth for >100 days post treatment. Clone2 went into full remission, with only six out of 30 tumors demonstrating regrowth by day 70 post treatment. Clone1 was completely resistant, whereas clone3 showed partial remission and regrowth of 28/30 tumors beginning 21 days after treatment. These sensitivity patterns do correlate with the molecular profile of the different lines, as Clone1 and Clone3 exhibit a downregulation of pTEN in combination with an upregulation of HER2 thereby bypassing survival signals via other RTKs than EGFR. The complete resistance of Clone1 is most likely driven by the NRAS mutation. The proteomics analysis (LC-MS) retrieved a range of 7700 - 8200 proteins per sample. The replicates (n= 4-5 per setting) displayed a Pearson's correlation coefficient >0.95 indicating high data quality across the dataset. A PCA supported that finding with replicates grouping together. In the PCA the original PDX separated from the acquired resistant clones regardless of the treatment and time point. Within those groups samples taken when tumors were in remission separated from the other time points. A deeper analysis comparing individual groups with each other (t-test: limma package, RStudio) revealed an upregulation of proteins that are involved in EMT (TLE1), bad prognosis (UBE2D2 and MAST4), or resistance (SORL1) in the Gefitinib-resistant clone1 under treatment with Cetuximab. The upregulation of TPK1 which was observed as well might give a hint towards new treatment options as the protein is related to sensitivity towards PARP inhibitors. The full analysis of the LC-MS data will elucidate the mechanism behind those resistance mechanism and will help to identify new targets as well as favorable combination regimen for pre-treated NSCLC patients. The currently ongoing metabolomics studies will help to build a more complete picture of the cellular regulations leading to the different outcomes observed in the current study.

#5517 Overcoming sotorasib acquired resistance in KRAS^{G12C} mutant NSCLC by TUSC2 gene therapy.

Ismail M. Meraz[1], Mourad Majidi[1], Renduo Song[1], Shuhong Wu[1], Lihui Gao[1], Feng Meng[1], Chenghui Ren[1], Yi Xu[1], Qi Wang[2], Yuanxin Xi[2], Jing Wang[3], Sung Yun Jung[4], Elizabeth Shpall[5], Jack A. Roth[1]

[1]Thoracic and Cardiovascular Surgery, UT MD Anderson Cancer Center, Houston, TX,[2]Bioinformatics and Computational Biology, UT MD Anderson Cancer Center, Houston, TX,[3]Bioinformatics and Computational biology, UT MD Anderson Cancer Center, Houston, TX,[4]Biochemistry, Baylor College of Medicine, Houston, TX,[5]Stem Cell Transplantation, UT MD Anderson Cancer Center, Houston, TX

Acquired resistance (AR) to sotorasib, an FDA-approved KRAS inhibitor, poses a significant challenge in treating KRAS^{G12C} mutant NSCLC. Despite initial responses, patients invariably develop resistance, necessitating alternative therapeutic strategies. The mechanisms of AR include the emergence of additional mutations in KRAS gene, reactivation of KRAS pathway, or activation of alternative signaling pathways. TUSC2, a potent tumor suppressor gene with immunogenic properties, exhibits multifunctional activity by a) inhibiting signaling pathways, including MAPK and mTOR, b) arresting the growth and proliferation, c) inducing tumor cell death, and d) activating immune responses. This study shows that TUSC2 gene therapy effectively overcomes the AR in KRAS^{G12C} mutant NSCLC. AR patient-derived xenografts (PDX), cell line-derived xenografts (CDX), PDX-derived organoids (PDXO), and cell lines were generated. Both AR cell lines and PDXOs showed >100-fold resistance over sensitive counterparts. The resistant cell lines also showed resistance to adagrasib, another KRAS^{G12C} inhibitor. Whole Exome Sequencing (WES) was performed for AR PDXs, CDX, and cell lines and all retained the KRAS^{G12C} mutation with no additional KRAS mutations. Mass spectrometry (MS) on TC303AR and TC314AR PDXs showed significant sets of differentially regulated proteins in AR vs parental. A significant upregulation of MTORC1 signaling in AR PDXs was found. Proteins involved in the PI3K-AKT-mTOR pathway were significantly upregulated in AR PDX and cells. TUSC2 transfection reduced considerably colony formation and increased the annexin V-positive apoptotic cells in both H23AR and H358AR cells. Re-expression of TUSC2 into AR PDXOs significantly decreased the viability of organoids compared with the empty vector. The H23AR tumors, which exhibited no significant sensitivity to sotorasib, showed robust antitumor effects of quaratusugene ozeplasmid, a lipoplex gene therapy containing the TUSC2 gene in both H23AR and TC314AR PDXs. However, a synergistic antitumor effect was observed when TC314AR PDXs were treated with the combination of TUSC2 and sotorasib. To evaluate the antitumor immune responses, immune-competent humanized-NSG mice are generated by transplanting fresh human cord blood-derived CD34+ stem cells into sub-lethally irradiated NSG mice to reconstitute a human immune system. The robust antitumor effect of TUSC2 was observed in both PDX and xenograft humanized mice. When sotorasib was combined with TUSC2, synergistic antitumor activity was found. This antitumor effect was correlated with significant infiltration of human CD8 T, NK, DC, and M1 MQ, and downregulation of MDSCs and exhausted T cells. TUSC2 therapy, alone or in combination with sotorasib, induced apoptosis, inhibited colony formation, and showed significant antitumor efficacy in KRAS^{G12C} AR tumors

#5518 Targeting FAK by Ifebemtinib synergizes with Pan RAS inhibitors in treating RAS mutant cancers.

B. Zhang, R. Pang, J. Gao, J. Cheng, P. Zhang, N. Qin, L. Liu, Z. Wang;
Inxmed, Shanghai, China

Pan-RAS inhibitors have been developed for the treatment of RAS-mutant cancers. Despite the challenges in achieving clinical benefits, targeting Pan-RAS represents a novel strategy for treating these previously considered untargetable malignancies. Previous studies have demonstrated that targeting FAK enhances the therapeutic efficacy of KRAS G12C inhibition in clinical settings, as hyperactivated FAK signaling is a major mechanism of acquired resistance following KRAS G12C inhibition. In the present study, we observed that Pan-RAS inhibitors can induce acquired hyperactivity of FAK, potentially rendering cancer cells resistant to Pan-RAS inhibition. Co-administration of a clinical-stage FAK inhibitor, Ifebemtinib (also known as Ifebe, IN10018), with RMC6236, a Pan-RAS inhibitor, exhibited synergistic cytotoxic effects in multiple RAS-mutant cancer cell lines. Furthermore, we evaluated the dual regimen in KRAS-mutant and NRAS-mutant animal models, where the combination therapy demonstrated superior tumor growth inhibition compared to monotherapy. Our previous findings indicated that FAK inhibition in conjunction with KRAS G12C/G12D inhibition enhances the anti-cancer effects of PD-1 blockade. Here, we confirmed that the combination of Ifebe and RMC6236 also augments the anti-tumor efficacy of PD-1 antibody therapy, suggesting that this treatment enhancement is independent of the specific RAS mutation type. In summary, our study reveals that the combination of FAK inhibitor Ifebe with Pan-RAS inhibitor exhibits synergistic anti-cancer effects and further improves the outcomes of PD-1 blockade therapy. These preclinical results support the need for further clinical investigation.

#5520 NXP900, a phase 1, first-in-class YES1/SRC inhibitor demonstrates potent single agent activity and synergy with ALK inhibitors in ALK resistant NSCLC models.

Neil O. Carragher[1], Enrique Poradosu[2], Asier Unciti-Broceta[1]

[1]Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, United Kingdom,[2]Nuvectis Pharma Inc., Fort Lee, NJ

Background: NXP900 (eCF506) is a novel potent and selective SRC family kinase (SFK) inhibitor, (IC_{50} of 0.47 nM against YES1) which possesses a unique/first-in-class binding mode-of-action. NXP900 locks its target into its native “closed” conformation, thereby inhibiting both kinase activity and scaffolding function with protein signaling partners (Temps et al. Cancer Res. 2021, 81, 5438-5450). In contrast, multi-kinase inhibitors, including dasatinib and bosutinib, lock SRC in the active “open” conformation (type 1 inhibitors) promoting the association of SFK and signaling partners via allosteric facilitation (Higuchi et al. Cell Rep 2021, 34, 108876). Anaplastic lymphoma kinase (ALK) inhibitors are clinically effective for the treatment of non-small cell lung cancer (NSCLC) patients whose tumors harbor ALK fusion genes. However, the development of resistance to ALK inhibitors in patients is nearly universal. Activation of SFK signaling has previously been shown to mediate acquired resistance to ALK inhibition in NSCLC lines (Yoshida et al. Int J Oncol. 2017, 51,1533-1540, Sato et al., JCO Precis. Oncol., 2022) indicating a potential for NXP900 to overcome an important bypass resistance mechanism and offer therapeutic potential in cancers with acquired resistance to ALK inhibitors.
Materials and Methods: Cell lines: Resistant cell lines to ALK inhibitors were generated from NCI-H2228 (EML4-ALKv3) and NCI-H3122 (EML4-ALKv1). Cell proliferation assays: Cells were diluted in the corresponding ATCC recommended medium and dispensed in a 384-well plate, depending on the cell line used, at a density of 100 - 6400 cells per well. For each cell line the optimal cell density is used and cells were treated with ALK inhibitors lorlatinib and/or NXP900 for 120 h and underwent ATPlite viability assay. Drug combination effects in vitro were studied using synergy scoring methods applied across multiple fixed dose-ratio mixtures.
Results: NXP900 single agent potently inhibited cell proliferation of ALK resistant cell lines, with a median GI50=30nM. At the same concentration range, NXP900 potently inhibited the activating SRC autophosphorylation, and demonstrated synergy in combination with lorlatinib in lorlatinib-resistant cell lines derived from NCI-H2228 (EML4-ALKv3) and NCI-H3122 (EML4-ALKv1) cells.
Conclusions: Despite high response rates to lorlatinib and long duration of response, acquired resistance almost universally arises. Here we demonstrate that NXP900 can potently inhibit cell proliferation of ALK resistant cell lines as a single agent and is synergistic with lorlatinib in NSCLC models harboring different variants of the ALK gene fusion. Altogether, these data suggest that NXP900 may have therapeutic potential in cancers with acquired resistance to ALK inhibitors. A FIH, Phase1 dose escalation study for NXP900 is ongoing.

#5521 Integrative analysis of acquired drug resistance to KRAS(G12D) inhibitors.

Ines Pulido Endrino[1], Laura Gunder[1], Malek Massad[1], Julian Carretero[2], Takeshi Shimamura[1]

[1]University of Illinois at Chicago, Chicago, IL,[2]Universitat de Valencia, Valencia, Spain

KRAS is the most frequently mutated oncogene in cancer and, until recently, targeted therapies against mutant KRAS haven't been successful. However, new breakthroughs in pharmacological design have led to the FDA-approval of two KRAS G12C inhibitors and one inhibitor specific to target the KRAS glycine to aspartic acid in codon 12 (G12D) mutation has shown strong responses *in vitro* and has entered phase I in clinical trials, MRTX1133. KRAS(G12D) is the most common KRAS mutation, being found in 37% of pancreatic ductal adenocarcinomas (PDACs), as well as in 12.5% of colorectal cancer and 4.9% of lung adenocarcinoma (LUAD) patients. MRTX1133, a selective non-covalent inhibitor of the KRAS(G12D) mutation, has demonstrated promising preclinical efficacy and has entered phase 1 clinical trials; however, the acquired drug resistance is expected to emerge. We have generated an acquired resistant model to KRASG12D inhibitors by drug escalation of MRTX1133. Our results show that although the IC50 for parental AsPC-1 cells is 0.2nM, drug resistant AsPC-1M-R cells can grow normally at 10 µM. Expression of KRAS, NRAS but more significantly HRAS are increased in the drug-resistant phenotype, likely to bypass inhibition of KRAS(G12D). Oncogenic KRAS(G12D) signaling isn't essential in AsPC-1M-R cells as determined by a persistent RAS-GTP activity after MRTX1133 treatment due to the emergence of a secondary mutation in KRAS. In fact, tumor proliferation and survival in the resistant phenotype is sustained by increased receptor tyrosine kinase (RTK) phosphorylation. Evaluation of RTK activity demonstrated that resistant tumor cells activated several RTK such as the ErbB and Eph families and MET, predominantly. Dual inhibition with SHP2, SOS or ErbB in combination with MRTX113 didn't exhibit a synergistic effect. Novel therapeutic approaches such as RAS(ON) inhibitors like RMC-6236 or specific KRAS(G12D) PROTAC degraders can significantly inhibit cell proliferation *in vitro* not only in MRTX1133 acquired resistance but also in adagrasib resistance models. To determine the underlying mechanisms of acquired drug resistance to MRTX1133, RNAseq analysis was performed. Complex transcriptomic reorganization shows the upregulation of CD24 -a potential cancer stem cell related gene- and the epithelial to mesenchymal signature highlight the difficulty to target drug resistant cells. These findings emphasize the necessity to design combinatorial treatments to prevent and delay the acquisition of drug resistance in KRAS-driven cancers.

#5522 Cell state modulates the KRAS^{G12C} proximal proteome.

Anurima Majumder, Liznair Bridenstine, Denis Imbody, Kiah Bowers, Dalia Ercan, Bin Fang, Eric Haura

Moffitt Cancer Center, Tampa, FL

Background: KRAS mutations occur in ~25% of lung adenocarcinomas (LUAD), with KRAS^{G12C} being the most common. There has been limited clinical success with KRAS inhibitors due intrinsic/acquired resistance. EMT is a known mechanism by which cancer cells become resistant to targeted therapy. Here, we aim to map protein interactors of KRAS^{G12C} in lung cancer cells using proximity-dependent biotin labeling (BioID) to gain insights into key regulators/effectors of oncogenic signaling and therapy resistance.

Methods: Dox-inducible fusion of KRAS^{G12C} with the BirA biotin ligase was expressed in H358 LUAD cells. To determine alterations in KRAS^{G12C} interactome induced by EMT, BioID/mass spectrometry was performed on BirA-KRAS^{G12C} expressing cells, with or without chronic TGF-β treatment (to induce EMT). Transduced H358 cells treated either with Biotin or Dox alone were used as controls for the BioID experiment.

Results: BioID analysis identified 144 proteins as potential proximal interactors for KRAS^{G12C}, which include known effectors of RAS signaling, such as NF1, RAF1, RASA1, MTOR, as well as novel ones, such as KEAP1, ASAP1, CRIP2, CRK, GULP1, AFDN. Pathway analysis suggests that these proximal proteins are involved in regulation of actin cytoskeleton, RAP1, PI3K-Akt, focal adhesion, and Ras signaling pathways. Our data also shows that TGF-β induced EMT reduces KRAS^{G12C}'s interaction with several of these known effectors, such as MTOR, RICTOR, and RAF1, while gaining new interactors, such as SPRED2, NOTCH2, DEPC1B and F11R. We also observe a reduction in RAS activity, RAS-RAF proximity and pERK/pRSK in TGF-β induced cells compared to parental cells.

Conclusions: We show that BioID system can identify KRAS^{G12C} interactome proteins in a cell line model of LUAD. Our data suggests that upon undergoing TGF-β induced EMT, the KRAS^{G12C} interactome in these cells alter and now couple less with RAS/MTOR signaling effectors. We also observe similar change in sotorasib-resistant H358 cells showing EMT features, where KRASi no longer suppresses ERK signaling. These studies can understand variations in KRAS^{G12C} interactome under epithelial vs mesenchymal state and potentially identify new therapeutic vulnerabilities for cells that have undergone EMT.

#5523 Inhibition of FASN postpones development of resistance to PLX8394 in colorectal cancer.

Mariah E. Geisen, Josiane Weber Tessmann, Courtney O. Kelson, Ellen Beswick, Daheng He, Chi Wang, Abu Saleh Mosa Faisal, Jill M. Kolesar, Yekaterina Zaytseva

University of Kentucky, Lexington, KY

Background: Mutations in proto-oncogene B-Raf (BRAF) occur in about 10% of colorectal cancer (CRC) patients and $BRAF^{V600E}$ is the most common type. This subset of CRC is associated with a reduced response to chemotherapy and poor prognosis compared to BRAF wild-type CRC. While the FDA-approved encorafenib plus cetuximab therapy for BRAF-mutant CRC provides a significant benefit, only 22% of patients respond to this therapeutic approach and resistance ultimately develops in the majority of patients. Therefore, development of new efficacious strategies based on further characterization of resistance mechanisms is needed to improve outcomes for $BRAF^{V600E}$ CRC. We found that development of resistance to PLX8394, a second-generation BRAF inhibitor, is associated with an increase in cellular proliferation, invasion, and lipid metabolism. We identified fatty acid synthase (FASN), a key enzyme in lipid synthesis, as a major lipogenic enzyme overexpressed in PLX8394-resistant cells. Therefore, the goal of this study is to test if inhibition of FASN will enhance the efficacy of PLX8394 in BRAF-mutant CRC.
Methods: HT29, PT130, and PT24249pt, parental and PLX8394-resistant, CRC cells were utilized. The effect of PLX8394 alone and in combination with FASN inhibitors (TVB3664 and C75) was tested using cell viability and colony formation assays. The synergy score was determine using a BLISS synergy model (SynergyFinder 3.0). The development of resistance to PLX8394 in the presence or absence of TVB3664 was evaluated using IC50 analysis, flow cytometry, and western blot.
Results: We found that PLX8394-resistant cells exhibit an increase in FASN expression, oxidative phosphorylation, and triglyceride storage. Combination of PLX8394 and FASN inhibitors led to a significant decrease in cell viability and colony formation as compared to each drug alone. Consistently, we found a significant synergy between PLX8394 and C75 using the BLISS synergy model. FASN-knockdown cells exhibited higher sensitivity to PLX8394 as compared to control cells supporting the role of FASN in resistance to PLX8394. Importantly, an addition of TVB3664 to PLX8394 treatment postpones the development of resistance to PLX8394. Analysis of cell cycle by flow cytometry confirmed a suppression of cell cycle progression via a decrease in a number of cells entering the S phase when cells are treated with combination of PLX8394 and TVB3664. Consistently, we observed a decrease in expression of proteins associated with the cell cycle such as cyclin D, pRB, and E2F expression in this group.
Conclusion: Collectively, these data show that combination of PLX8394 with FASN-targeted therapy at treatment initiation reduces $BRAF^{V600E}$ CRC cell proliferation via inhibition of their cell cycle progression. These findings suggest that targeting FASN could enhance the efficacy and delay the development of resistance to PLX8394 in BRAF-mutant CRC.

#5524 Developing KRAS^{G12C} inhibitor-resistant tumor models for efficacy evaluation of next-generation anticancer therapies.

Jian Feng, Dan Zhang, Aaron Hua, Chenpan Nie, Jessie(Jingjing) Wang, Ludovic Bourre, **Jun Zhou**, Peng Wang

Crown Bioscience, Inc., San Diego, CA

Background: For decades, KRAS was considered undruggable due to the lack of suitable binding sites. However, advancements in bioengineering and chemistry have enabled the approval of targeted therapies. Success was first seen with allele-specific targeting of KRAS^{G12C} in non-small cell lung cancer (NSCLC), leading to the approval of sotorasib (AMG510, Lumakras^{TM}). Despite its clinical benefits, resistance emerged in some patients due to secondary KRAS mutations, which necessitates next-generation or combination therapy development. In this study, we outline the development of KRAS^{G12C} inhibitor-resistant models overcome this hurdle.
Methods: Secondary KRAS mutations (Y96D/C/S, H95D/Q/R, R68S, Q61H, A59T/S, and Q99L) were introduced by CRISPR/Cas9 in MIA PaCa-2 with a homozygous KRAS^{G12C} mutation. Knock-in of point mutation was validated by Sanger sequencing. Cell viability was assessed by CellTiter-Glo (CTG) with AMG510 and MRTX849 (Adagrasib, Krazati^{TM}). RAS-MAPK pathway activity was evaluated by western blot. Xenograft models of MIA PaCa-2 cells with Y96D/C, H95D/Q/R, R68S, Q61H and A59T were established. Additionally, *in vitro* chronic dosing of AMG510 generated AMG510-resistant MIA PaCa-2 and NCI-H358 cell lines were validated by CellTiter-Glo and western blot. RNA-seq identified potential resistance mechanisms. Xenograft models were also established.
Results: A successful homozygous point mutation knock-in was confirmed by Sanger sequencing. Cells expressing double-mutant alleles KRAS G12C Y96D/C/S, A59T/S and R68S showed resistance to both AMG510 and MRTX849, while KRAS G12C H95D/Q/R was more resistant to MRTX849, and KRAS G12C Q61H, Q99L didn't show significant resistance. Persistent phosphorylated ERK (pERK) and pRSK levels indicated sustained RAS-MAPK activity in cells expressing KRAS G12C Y96D, H95D, A59T/S, and R68S, even at high KRAS inhibitor concentrations. Furthermore, a KRAS G12C Y96D/C, A59T, Q61H, R68S and H95D/Q/R double mutant cell-derived xenograft was established in vivo. Additionally, MIA PaCa-2 AMG510-resistant and NCI-H358 AMG510-resistant cells showed resistance to AMG510 and MRTX849 in cell viability assays. RNA-seq data identified c-MET amplification in AMG510-resistant MIA PaCa-2 cell, while FGFR1/3/4 amplification was found in AMG510-resistant NCI-H358 cells.
Conclusion: CRISPR/Cas9-engineered KRAS secondary mutations cell lines displayed differentially resistant profile to KRAS^{G12C} inhibitors, and drug-induced resistant cell models developed *in vitro* displayed KRAS-independent mechanisms of resistance. These novel cell models offer a valuable preclinical platform to evaluate therapeutic strategies to overcome resistance to KRAS-targeted therapies.

#5525 YTHDF3 and RAS-ERK1/2 pathway generate a negative feedback loop to maintain phosphorylation cascade and contribute to EGFR-TKI resistance.

Jaeik Oh[1], Sung-Yup Cho[2]

[1]Department of Translational Medicine, Seoul National University, College of Medicine, Seoul, Korea, Republic of,[2]Department of Biochemistry and Molecular Biology, Seoul National University, College of Medicine, Seoul, Korea, Republic of

About half of East Asian patients with lung adenocarcinoma harbor EGFR mutations, which lead to dramatic initial response to EGFR tyrosine kinase inhibitors (EGFR TKI); however, the resistance to these therapies is ultimately inevitable. Several previous studies on EGFR TKI that typically utilized genomic, epigenomic and transcriptomic data of resistant tumors from patients attributed their resistance to bypass signal activation or downstream target mutations, however, mostly failed to overcome resistance by targeting such signals. The gap between RNA and protein expression, which obscures changes in actual phosphorylation cascade, is one plausible explanation for the previous failures of multi-layer omics studies and genome-wide screening. Given that posttranslational modification contributes to this gap, N6-Methyladenosine, which is dynamically regulated and present in the mRNAs of various genes in the RAS pathway, has emerged as an intriguing target for studying its role in EGFR TKI resistance. Here, we demonstrated that the phosphorylation cascade of EGFR-RAS-ERK1/2 and YTHDF3 regulate each other and are composed of a negative feedback loop between two independent systems. We identified blocking the EGFR signal decreased the protein stability of YTHDF3 while negative regulation of YTHDF3 intensified EGFR-RAS-ERK1/2 signaling. Our findings reveal that YTHDF3 plays a critical role in modulating EGFR TKI resistance, suggesting its potential as a therapeutic target to overcome resistance in lung cancer treatment.

#5526 Inhibition of MEK and AKT synergistically induces melanoma stem cell apoptosis and blocks NRAS-mutant xenograft growth.

Nusrat Islam[1], Ryyan Alobaidi[1], Toni M. Olkey[1], Yogameenakshi Haribabu[1], Mathew Shamo[1], Peter Sykora[2], Cynthia M. Simbulan-Rosenthal[1], Dean S. Rosenthal[1]

[1]Biochemistry and Molecular & Cellular Biology, Georgetown Univ. School of Medicine, Washington, DC,[2]Amelia Technologies, LLC, Washington, DC

Melanoma-initiating cells (MIC) are involved in tumorigenesis, drug resistance and metastasis, and are characterized by upregulated stem cell markers, including CD133. Our previous studies showed that CD133 siRNA increases apoptosis in response to the MEK inhibitor trametinib. The current study employs CRISPR-Cas9 CD133-KO or Dox-induced expression of CD133 to investigate the anti-apoptotic mechanism of CD133 in patient-derived BAKP melanoma cells, harboring an $NRAS^{Q61K}$ driver mutation. CD133-KO cells downregulated pro-survival markers p-AKT and p-BAD, and upregulated pro-apoptotic BAX. On the flip side, Dox-induced CD133 increased expression of p-AKT and p-BAD, and decreased activation of BAX and caspase-3. Hence, an alternate survival pathway may be activated in MICs, wherein CD133 induces AKT phosphorylation and activation, which phosphorylates and inactivates BAD, decreasing BAX activation, and reducing caspases-3 activity and caspase-mediated PARP cleavage, ultimately resulting in apoptosis inhibition and chemoresistance in melanoma. However, trametinib in combination with the pan-AKT inhibitor, capivasertib, can synergistically reduce cell survival even in trametinib-resistant CD133-expressing MICs *in vitro*, as determined by XTT, annexin-FITC, and colony forming assays. Similarly, *in vivo* mouse xenograft studies using Dox-inducible BAKP cells revealed increased rates of tumor growth after induction of CD133 expression in trametinib-treated +Dox mice, an effect that was most effectively suppressed by the combination treatment. Therefore, targeting nodes of the AKT and MAPK survival pathways with trametinib and capivasertib underscores the potential for combination therapies for difficult-to-treat NRAS-mutant melanoma stem cells, paving the path towards improved treatment strategies for patients with high-risk melanoma.

#5527 Optimization of CRISPR-Cas9 library screens in pancreatic cancer organoid models targeting KRAS^{G12D}.

Md Shahadat Hossan[1], Lauryn Flannagan[1], Molly A. Nellen[1], Austin Stram[1], Ethan S. Lin[1], Michela Cadarso[1], C. Dustin Rubinstein[2], Sean M. Ronnekleiv-Kelly[3], Jeremy D. Kratz[1]

[1]Department of Medicine, University of Wisconsin-Madison, Madison, WI,[2]University of Wisconsin-Madison, Madison, WI,[3]Department of Surgery, University of Wisconsin-Madison, Madison, WI

Background: Pancreatic ductal adenocarcinoma (PDAC) has a critical need in systemic therapeutic development, with hallmark activation of the mitogen-activated protein kinase pathway. Novel mutant-specific KRAS inhibitors have been developed, however no method to date can predict patient-specific resistance mechanisms. Here, we optimize a CRISPR-Cas9 lentivirus screen in 3D patient-derived cancer organoids (PCOs) to study resistance mechanisms and guide combination therapy using the KRAS^{G12D} inhibitor MRTX1133.

Methods: PDAC PCOs were derived from surgical specimens. Puromycin (puro) dose-response curves were established using cell viability assays. PCOs were transfected with a lentiviral-based 'druggable genome' CRISPR-Cas9 library of 2,292 unique targets (Millipore-Sigma) overnight (18h) in a liquid phase with the addition of polybrene (5ug/mL). Puro selection was compared for efficiency at 6 days and 12 days. Western blotting was used to confirm Cas9 expression. Multiplicity of infection (MOI) optimization was performed by digital droplet PCR (ddPCR) on selected cells to measure lentiviral genome copy number efficiency. MOI was compared across traditional cell viability assays using CellTiterGlo (CTG, 50% v/v) and cell counting methods.

Results: Molecular profiling of the PDAC PCO line confirmed KRAS^{G12D} mutant by DNA sequencing (SureSelect Cancer, Agilent). Puro dose was selected at 2 μM to exceed the IC_{90} of 1.95 ± 0.1 μM. Western blots confirmed Cas9 presence in selected cells. The KRAS^{G12D} inhibitor MRTX1133 revealed an IC_{50} of 11.16 ± 0.36 nM. The 1:5 cell-to-lentivirus ratio after 6 days of puro selection was selected based on copy number analysis by ddPCR (0.93 ± 0.01). The control, 1:2, and 1:10 ratios after 6 days of selection exhibited copy numbers of 0 ± 0, 0.53 ± 0.13, and 1.52 ± 0.38, respectively. Selection for 12 days yielded higher copy numbers (1.13 ± 0.38 at 1:2, 1.19 ± 0.11 at 1:5, and 1.72 ± 0.43 at 1:5) compared to the 6 day puro selection. Increased selection time also revealed reduced viability compared to the 6 day selection. The 1:5 ratio showing 16.8% ($p<0.0001$) and 22.65% ($p<0.0001$) viability, respectively. Post-selection, the viability of puro-selected, non-transfected cells was 0%, confirming selection efficiency.

Conclusions: Here, we demonstrate the optimization of CRISPR-Cas9 screening with 3D PDAC PCOs using ddPCR to validate the efficiency of transfection. Importantly, the degree of puro selection and lentivirus transfection did confer multiplicity in viral transfections. The ongoing work is aimed at understanding the time course dynamics of gene editing between early (14 day) and late (28 day) resistance to KRAS^{G12D} inhibition using the Model-based Analysis of the Genome-wide CRISPR/Cas9 Knockout (MAGeCK) method. This work extends the efficiency of novel gene editing technologies for screening applications in organoid models of pancreatic cancer.

#5529 Development and characterization of BRAF/MEK inhibitor resistant anaplastic thyroid carcinoma patient-derived xenograft and cell line models.

Ahmad Abubaker[1], Meredith Miller[2], Ying Henderson[1], Brooke Meyers[2], Ningping Feng[2], Stephen Wang[2], Jessica Molkentine[2], Andy Zuniga[2], Virginia Giuliani[2], Stephen Y. Lai[3], Christopher Terranova[2], Anastasios Maniakas[1]

[1]Department of Head and Neck Surgery, The University of Texas MD Anderson Cancer Center, Houston, TX,[2]Translational Research to AdvanCe Therapeutics and Innovation in ONcology (TRACTION), The University of Texas MD Anderson Cancer Center, Houston, TX,[3]Department of Head and Neck Surgery, Radiation Oncoloy, Molecular and Celluler Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX

Introduction: Anaplastic Thyroid Carcinoma (ATC) is one of the deadliest malignancies with 5-month median overall survival (OS). Treatment of BRAFV600E-mutated (BRAFm) ATC with BRAF/MEK inhibitors (BRAF/MEKi) revolutionized disease management and improved median OS to 16 months for this subgroup. However, like other BRAFm cancers, tumors develop resistance within a few months, and patients are often inoperable, making tumor specimen retrieval difficult for studying resistance development. The utilization of patient-derived xenograft (PDX) models and cell lines (CDX) is therefore an integral part of disease mechanism understanding and therapeutics development. To date, there is no BRAF/MEKi resistant ATC model available in the scientific community.
Aim: Report the development and characterization of a BRAF/MEKi resistant ATC cell-line and PDX models.
Methods: Our group previously established and characterized BRAFm ATC PDX models. MDA-ATC1 is BRAFV600E, TP53, TERT, PI3K-mutated. These were implanted in 5-8-week-old nu/nu nude mice and treated with dabrafenib+trametinib (D/T) 15mg/kg+0.5mg/kg, at half or full dose, followed by dose doubling of D once resistance was observed. For CDX modelling, MDA-T220 (BRAFV600E, TP53, TERT, and PI3K mutated, from patient previously treated with D/T) was implanted into 6-12-week-old NSG mice and treated with two D+T concentrations (low: 15mg/kg + 0.25mg/kg and high: 30mg/kg + 0.5mg/kg) once tumors reached ~200mm3. Successful establishment of resistant PDX & CDX models was determined when tumors continued to grow beyond 1000mm3 despite maximum dose escalation, and if growing following cryopreservation for the PDX models.
Results/Discussion: At first passage, resistance to BRAF/MEKi was detected in MDA-ATC1 at week 12 for half and 18 for full dose. On second & third passage, tumors continued to show resistance, even following dose escalation. Resistance in the CDX was observed during the initial treatment period and tumor growth continued under consistent low and high D/T dosing concentrations. The first passage tumors grew rapidly under both dosing concentrations and were subsequently used in the second passage for dose escalation and resistance maintenance studies until tumors reached >1000mm3. At each passage and at study completion, tumors from PDX and CDX models were harvested and evaluated with short tandem repeats, immunohistochemistry for BRAF-status, and standard H&E staining.
Conclusion: To our knowledge, these are the first BRAF/MEKi resistant ATC models described and characterized. We expect these models to play an integral role in drug development and evaluation for this difficult-to-treat population with no adequate therapeutic option to date.

#5530 Combination of BRAF and ACLY inhibition synergistically reduces melanoma cell viability.

Anastasiia Vaska, Michael Ferretti, Daniel A. Ho, Rachel E. Harmon, Robert F. Green, Aaron B. Steiner, **Nancy A. Krucher**

Biology, Pace University, Pleasantville, NY

Fifty percent of Melanoma cases harbor the activating BRAF V600E mutation which prompted the development of BRAF inhibitors Vemurafenib, Dabrafenib and Encorafenib, used clinically in combination with MEK inhibitors Trametinib, Cobimetinib and Binimetinib. Together, these agents serve to inhibit the MAPK pathway, but the PI3K/AKT pathway is often upregulated in response to MAPK pathway inhibition which can lead to clinically important treatment resistance. AKT functions in numerous cellular processes including survival, growth, proliferation, invasion and metabolism. With respect to metabolism, AKT activates lipogenesis by direct phosphorylation and activation of the enzyme ATP-Citrate Lyase (ACLY). ACLY expression and activity are elevated in melanoma and are associated with a poor prognosis. A new inhibitor of ACLY, NDI-091143, was recently identified based on the structure of ACLY and was used in this study. A panel of BRAF mutant melanoma cell lines was used to investigate whether combining the ACLY inhibitor NDI-091143 with the BRAF inhibitor Vemurafenib would enhance the latter's pro-apoptotic effects. Using concentrations of each drug that inhibited cell viability individually by 25%, we found that the combination exhibited synergistic reduction of cell viability in all melanoma lines tested, and similar results were obtained using melanoma cells grown in 3D culture. Normal human keratinocytes treated with the same concentrations of Vemurafenib and NDI-091143 did not exhibit reduced viability. In melanoma cell lines, the combination of BRAF and ACLY inhibition activated the ER stress pathway including phosphorylation of eIF, increased CHOP and ATF4 expression, and induced caspase-mediated apoptosis. In addition, the combination of these agents elicited an increase in reactive oxygen species (ROS) as well as activation of the Jun N-terminal kinase (JNK) pathway, both of which stimulate apoptosis. *In vivo* assessment of this combination treatment in a zebrafish model of melanoma is underway.

#5531 A 3D CRISPR screening identified RET-TKI resistance through MIG6 loss in RET rearranged NSCLC.

X. Wei, K. Uchibori, M. Nishio, R. Katayama;
Japanese Foundation for Cancer Research, Koto City, Japan

RET-fusion positive non-small cell lung cancer (NSCLC) accounts for 1-2% of total population of NSCLC. RET selective tyrosine kinase inhibitors (TKIs) have demonstrated remarkable efficacy, and 2 RET-TKIs have been approved for the treatment of RET-rearranged NSCLC. However, the emergence of acquired resistance which remains unclear largely. To better simulate in vivo tumor conditions, we employed a 3D culture system using LC2/ad (CCDC6-RET) and LCC190 (CCDC6-RET, originally established from RET-TKI naïve patient's pleural effusion) cell lines. RET-TKIs showed significant increased efficacy in this 3D system compared to standard 2D cultures with increased apoptosis pathway and reduced ERBB-mediated MAPK and PI3K/Akt/mTOR signaling pathways.Further, we conducted a genome-wide CRISPR/Cas9 screening in the 3D condition in LCC190, which identified multiple candidate genes including *ERRFI1* (MIG6) contributor to drug-tolerant persister (DTP) cell survival during RET-TKI treatment. Knockout of MIG6, a negative regulator of EGFR signaling, induced significant EGFR activation in the presence of extreme low ligand concentrations (1 ng/mL), leading to resistance through the activation of MAPK and PI3K/Akt/mTOR pathways. These findings highlight the critical role of regulation of MIG6 on EGFR in bypassing RET inhibition. Notably, combining RET-TKIs with EGFR inhibitors (afatinib or cetuximab) markedly reduced the survival of DTP cells *in vivo* and *in vitro* suggesting a potential combination strategy to overcome such resistance. Conversely, overexpression of MIG6 restored sensitivity to RET-TKIs under conditions of low EGFR ligands with reduced level of phosphorylation of EGFR. In conclusion, this study identifies MIG6 as a critical mediator of RET-TKI resistance in NSCLC and highlights the utility of 3D culture systems in uncovering key resistance mechanisms in RET-fusion positive NSCLC. Targeting EGFR in combination with RET-TKIs presents a promising therapeutic strategy to overcome drug-tolerant persistence and improve treatment outcomes in RET-rearranged NSCLC. Besides MIG6, we have also found several candidate genes related to DTP cell survival or RET-TKI resistance from the genome-wide Crispr screening. Further studies are needed to clarify the mechanisms of resistance induced by loss of these candidate genes to comprehensively understand the mechanisms how DTP cells survive against TKI treatment and how such resistance could be overcome by novel treatment methods.

#5532 Combinational targeted therapy effects on EphA2 and KRAS G12C inhibitor against KRAS G12C mutated non-small cell lung cancer cells.

H. Kawachi, T. Yamada, Y. Katayama, T. Koichi;
Kyoto Prefectural University of Medicine, Kyoto, Japan

Background: KRAS is the most frequent oncogene in non-small cell lung cancer (NSCLC), and some KRAS G12C inhibitors have been clinically developed for treatment of KRAS G12C-mutated NSCLC patients. However, these KRAS G12C inhibitors have shown limited efficacy, and developing novel treatment strategy is warranted to improve clinical prognosis in this clinical population. EphA2, a receptor tyrosine kinase overexpressed in various cancers including NSCLC, plays a critical role in tumorigenesis and cancer progression, with high expression levels associated with poor prognosis. Recent studies indicate that targeting EphA2 can effectively inhibit tumor growth and induce apoptosis in KRAS-mutated NSCLC, underscoring its potential as a promising therapeutic target.
Methods: In this preclinical study, we investigated the combined effect and underlying mechanisms of KRAS G12C inhibitors and EphA2 inhibition using multiple human KRAS G12C-mutated lung cancer cell lines.
Results: The combined inhibition of KRAS G12C and EphA2 resulted in enhanced suppression of cell proliferation via increasing cell apoptosis in a subset of these KRAS G12C-mutant lung cancer cells. The KRAS G12C and EphA2 inhibitor combination also suppressed cell migration, compared with the effects in each monotherapy. Cell line-derived xenograft model indicated that the combination of KRAS G12C inhibitor plus EphA2 inhibitor enhanced anti-tumor effects compared with KRAS G12C inhibitor monotherapy. Further examination of apoptosis-related proteins revealed that the combination treatment reduced Mcl-1 expression. Additionally, combining KRAS G12C with an Mcl-1 inhibition led to further increases in cell death and enhanced suppression of cell proliferation.
Conclusions: The combination of KRAS G12C inhibitors and EPhA2 inhibition is expected as the promising strategy for those tumors.

#5533 Drug tolerance mechanisms in patient-derived xenograft models with *epidermal growth factor receptor* mutated tumors treated with tyrosine kinase inhibitors.

Arundhathi Arivajiagane, Quan Li, Nhu-An Pham, Ming Li, Katrina Hueniken, Takamasa Koga, Nikolina Radulovich, Ming-Sound Tsao

UHN Princess Margaret Cancer Centre, Toronto, ON, Canada

Background and Purpose: Epidermal growth factor receptor (EGFR) mutations on exon 18-21 are oncogenic drivers in 10-60% of patients with lung adenocarcinoma. First and second-generation EGFR tyrosine kinase inhibitors (TKIs) have high efficacy but resistance quickly develops, with 60-70% caused by the emergence of T790M secondary mutation. Osimertinib (osi) is effective against common *EGFR* mutations as well as T790M, yet most patients eventually also develop resistance. Understanding post-osi resistance mechanisms is a priority research goal. Our goal is to establish large cohort of patient-derived xenograft (PDX) and/or organoid models with *EGFR* mutations from patients who have progressed on osimertinib and use them to characterize the mechanisms of drug tolerance and resistance.
Methods: We have established six PDX models from tumor biopsies and one organoid-derived xenograft (ODX) from a pleural effusion. To assess models response to osi, donor xenograft tumor fragments were implanted into replicate NOD SCID mice and once tumors reached an average tumor volume of 400 mm^3, they were randomized into vehicle and osi treatment groups. Osi was dosed at 25mg/kg daily oral gavage for 30 days. Excised tumors were profiled by bulk RNAseq in two models PHLC4547 and 4672.
Result: Models were established from tumors of three patients who have progressed on geftinib and osi treatment (PHLC4683^{Ex19delE746_A750del}, PHLC4697$^{L858R/L833V}$, PHLC4547^{L858R}). Additionally, one progressed on gefitinib PHLC4223^{L858R} and one on afatinib and gefitinib PHLC4192 ^{Ex19delE746_A750del}. One progressive on osi PHLC4672$^{L858R/G719}$ and another patient who progressed on afatinib and osi treatment is PHLC4754$^{L858R/T790M/C797S}$. PHLC4672 PDX demonstrated drug tolerance and stable tumor size to osi treatment, all other models showed resistance to osi. However, PHLC4547, 4223, 4683 and 4754 showed initial transient tolerance to osi and resistance quickly followed. PHLC4192 and PHLC4697 were completely osi-resistant. Transcriptomic profiling of osi-resistant PHLC4547 revealed no changes in gene expression profile during all three serial passages of osi-treated tumors. However, PHLC4672 with osi-tolerance revealed downregulation of Hallmark gene set enrichment in pathways of G2M checkpoint, E2F targets, mitotic spindle, glycolysis and estrogen response. The EGFR-TKI responses in patients and PDX models showed high concordance among the resistant models.
Conclusion: The altered pathways in tolerance model appears to be unique to an osi-stable response compared to an osi-resistance response. PDX models from post-TKI patients may be useful to study the drug resistant mechanism.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Drug Resistance in Molecular Targeted Therapies 4
Poster Session

#5539 Single-cell and spatial approaches of identifying targets in endocrine therapy-resistant breast cancer.

Svetlana E. Semina[1], Rosemary Huggins J. Huggins[2], Debra Tonetti[3], Kent Hoskins[4], Geoffrey L. Greene[5], Jonna Frasor[1], Jonathan Coloff[1]

[1]College of Medicine, University of Illinois, Chicago, IL,[2]UChicago Comprehensive Cancer Center, University of Chicago, Chicago, IL,[3]College of Pharmacy, University of Illinois, Chicago, IL,[4]Division of Hematology and Oncology, University of Illinois, Chicago, IL,[5]Ben May Department for Cancer Research, University of Chicago, Chicago, IL

Even though most women with estrogen receptor (ER) positive breast tumors can benefit from endocrine therapy (ET), up to 40% of these patients will experience relapse. The percentage of tumor cells expressing ER varies from 1 to 100%, and some studies suggest that higher expression of ER positively correlates with ET efficiency and reduced rate of tumor relapse. We have previously identified a cell population with active NFκB and integrated stress response pathways that is able to survive selective pressure of ET and contribute to the tumor relapse. However, we and others routinely use models that are highly enriched for ER-expressing cells to study the effect of ET. Much less is known about ET efficiency for breast cancers with a low to moderate number of ER-expressing cells, which tend to be more aggressive. Here, we decided to use patient-derived xenograft organoids (PDxOs) and a single-cell-based approach in order to study how breast cancer models with low to moderate levels of ER expression respond to ET. In addition to the already established PDxOs (HCI003 and HCI017), we developed another two models (UIC013 and UIC020), in which 22%, 30%, 12%, and 7% of cells express ERα, respectively. Using single-cell RNA sequencing (scRNAseq), we established that each PDxO has a model-specific transcriptional profile that is reflected in differentially activated pathways and in the expression of clinically relevant markers associated with more aggressive phenotypes. More specifically, we found that HCI003 and UIC020 are enriched for gene signatures associated with ET resistance and breast cancer stem cells, whereas UIC013 is enriched for invasive/EMT signatures. To understand whether PDxOs are sensitive to ET, we treated each model with fulvestrant and 4-hydroxytamoxifen. We found that some of the cell populations are lost with ET, suggesting that they are responsive to ET, while others showed no change or even enrichment with ET, suggesting that they are resistant to ET. To identify alternative therapeutic options for the ET-resistant cell population, we used DREEP analysis (DRug Estimation from single-cell Expression Profile), a bioinformatic tool that predicts drug response to 450 compounds for scRNAseq dataset. Multiple compounds were predicted to be effective in combination with ET, including vandetanib, an inhibitor of VEGFR2, which is now in a clinical study as an additional treatment to fulvestrant for metastatic breast cancer. We also found several novel therapeutic compounds that can be used to eradicate ET-resistant cell populations. Taken together, these findings indicate that stratifying breast cancer cell populations based on response to ET, rather than ER expression or activity, can lead to the identification of new cell populations, as well as therapeutic strategies to target these populations, thereby improving outcomes for women with breast tumors.

#5540 Deconstructing the lethality of recurrence in ovarian cancer through multi-modal spatiotemporal analysis.

James M. Whipman[1], Francis Jacob[2], Viola Heinzelmann[2], Bernd Bodenmiller[1]

[1]ETH Zurich, Zurich, Switzerland,[2]University Hospital Basel, Basel, Switzerland

Epithelial ovarian cancer (EOC) represents the world's most lethal gynecological malignancy and is commonly associated with chemo-resistance and recurrence. Strikingly, up to 70% of EOC patients will relapse where subsequent care becomes palliative. Owing to a lack of model systems and a scarcity of matched treatment-naïve and recurrent clinical samples, few studies have addressed the complex relationship between first-onset EOC and its respective relapse. Identification of *in situ* tumor phenotypes that resisted initial chemotherapy treatment and initiate recurrent tumors, will allow us to understand which cellular features lead to relapse. This will allow us to better predict tumor recurrence and to ultimately suggest novel therapies. Here we analyze primary and recurrent tumor pairs using highly multiplexed imaging technologies. Through integration of spatial transcriptomics and imaging mass cytometry (IMC), we identify and classify resistance phenotypes in over 320 EOC patients, identifying phenotypes that persist in recurrent tumors. We leverage these technologies to assign novel proteo-transcriptomic resistance modules that allow us to independently observe these phenotypes across multiple single-cell modalities. To uncover phenotype-specific drug sensitivities, we utilized scRNA-seq data to predict *in silico* drug-response and validated these findings through large-scale image-based *ex vivo* drug screening. These screens revealed significant multi-drug resistance (MDR) properties of persistent phenotypes, but unveiled sensitivity to alternate FDA-approved drug classes. Together, our work represents the first-of-its-kind high-dimensional map of resistant and recurrent EOC, offering tangible therapeutic solutions for the treatment and prevention of recurrence.

#5541 Dissecting chemotherapy resistance development in triple negative breast cancer through single cell multiome and lineage tracing.

Chufan Zhang, Chu Pan, Meghan Mcguire, Jennifer Cruickshank, Dave Cescon, Mathieu Lupien

UHN Princess Margaret Cancer Centre, Toronto, ON, Canada

Triple negative breast cancer (TNBC) is a breast cancer subtype that manifests fast disease progression and very poor overall survival. Despite initial response to standard of care, development of therapy resistance remains the major challenge in TNBC clinical management. Delineating the mechanisms underlying resistance development has been impeded by the still unknown cell population of origin and lack of longitudinal molecular profiling. To investigate this question, we applied a CRISPR based lineage tracing system into TNBC cell lines. Longitudinal samples from an Antibody Drug Conjugate (ADC), Sacituzumab Govitican sensitive to resistance were collected in vitro via stepwise drug dose escalation and in vivo via xnenograft serial transplantation. We leveraged cellular barcoding with single cell multiome from 10X genomics to simultaneously capture clonal identity, accessible chromatin and gene expression and hence, directly linked lineage with functional cell states. Preliminary results suggest that TNBC cells adopted distinct cell states shortly after drug exposure with characteristics including upregulation of epithelial-to-mesenchymal transition (EMT) genes, stemness markers and downregulation of immune related pathways. Clonal level analysis revealed only a subset of clones survived initial treatment and are enriched in specific “primed” cell states. Next, we will construct single cell lineage trees and quantify clonal dynamics and cell state transition rates. We will identify and validate regulators of cellular plasticity and explore if potential drug combinations will prevent emergence of resistance cell state. Overall, this study will build a comprehensive map of the phenotypic trajectory towards chemotherapy resistance and pinpoints biomarkers of therapy response in triple negative breast cancer.

#5542 A novel approach for establishing clinically relevant models of chemoresistance.

Jared R. Fischbach, Geoffrey A. Wood, Courtney R. Schott

Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

This study provides a novel methodology for generating *in vitro* chemoresistance models that maximize clinical relevance, aimed at improving the translatability of downstream experimental insights to clinical contexts. Our approach prioritizes an accurate translation of clinical chemotherapy treatment regimens to *in vitro* applications. We present our methodology in this study using canine osteosarcoma (OS), but our approach is designed to broadly apply to other cancer types.

Chemoresistance poses a significant challenge to improving cancer treatment outcomes, as the underlying mechanisms driving it are not well understood. One approach to studying these mechanisms is generating *in vitro* chemoresistance models. However, there has yet to be a consensus on the optimal method for developing clinically relevant chemoresistance models. Many existing guidelines either produce levels of chemoresistance that are not clinically achievable or offer incomplete strategies for producing clinically relevant models.

To establish a clinically relevant model of chemoresistance, we treated canine OS cell lines with carboplatin under conditions designed to precisely mimic the standard-of-care (SOC) treatment regimen used in clinical practice. To translate this clinical regimen to our *in vitro* application, we utilized numerous pharmacokinetic parameters derived from serum concentration curves for carboplatin in dogs treated in accordance with the SOC regimen, including area under the curve (AUC), peak serum concentration (Cmax), time to peak serum concentration (Tmax), and time from treatment initiation to when the serum concentration falls below detectable levels. Utilizing these pharmacokinetic parameters, a biphasic treatment protocol was developed to provide a SOC-equivalent treatment regimen for *in vitro* application. Before commencing model generation, the baseline chemosensitivity of each parental canine OS cell line was determined in triplicate via its respective half-maximal inhibitory concentration (IC50) of carboplatin. These parental cell lines were then split into two identical plates each, with one plate from each cell line treated periodically with our SOC-equivalent regimen, administered every twenty-one days for six cycles, in accordance with the SOC regimen. Untreated cell lines were passaged alongside their treated counterparts as model controls.

Twenty-one days after the final treatment, the IC50s of all treated cell lines and their respective controls will be re-assessed in triplicate to quantify the fold change in chemoresistance induced by our SOC-equivalent regimen. We anticipate that treated cell lines will exhibit appreciable increases in chemoresistance, highlighting the potential of our methodology to establish clinically relevant *in vitro* models of chemoresistance that support future investigations into the underlying mechanisms of chemoresistance.

#5543 High resolution lineage tracing for the study of cancer drug persistence.

Binyanim Zhitomirsky, Jideofor Ezike, Brian P. Danysh, Gad Getz

Broad Institute of MIT and Harvard, Cambridge, MA

Drug-tolerant persister cells (DTPs) are a subpopulation of cancer cells that survive initial anticancer drug treatment despite lacking genetic resistance-driving alterations. DTPs have been shown to serve as an intermediate stage in the evolution of acquired drug resistance, facilitating the adaptation and survival of cancer cells under sustained therapeutic pressure. Recent studies have shed light on the biological characteristics of DTPs during and following drug treatment, describing the various adaptations that cells undergo while transforming into a DTP state. However, little is known about the biology of persistence potential — the cellular and molecular characteristics that prime certain drug-naïve cells to survive initial drug exposure. The main challenge in studying the persistence potential of drug-naïve cells lies in the need to treat cells to determine whether they will persist. To address this issue, we demonstrated the hereditary nature of persistence potential and adopted a high-resolution lineage tracing technology, to develop an assay for the study of persistence potential. This method, utilizing CRISPR-targeted evolving barcodes to reconstruct a lineage tree from a single-cell-derived clone, allows for the placement of persister cells along drug-naïve cells on the lineage tree. Utilizing this technology to study persistence to EGFR inhibitors in EGFR-positive non-small-cell lung cancer cells, we identified events on the lineage tree that alter the persistence potential of specific clades and derived the persistence potential of drug-naïve cells. Combining single-cell RNAseq data with cell persistence potential, we developed a computational pipeline to study the molecular mechanisms that govern the probability of drug-naïve cells to persist through drug treatment. We demonstrated that upregulation of genes related to oxidative phosphorylation correlates with a significant increase in persistence potential. These results agree with previous studies that demonstrate that cells in the DTP state are less dependent on glycolysis and rely more on oxidative phosphorylation for energy production and suggests that drug-naïve cells with elevated persistence potential are primed towards a high oxidative phosphorylation state. Intriguingly, we also described a module of translation-related genes that are negatively correlated with persistence potential, in agreement with recent results that show that persister cells undergo reversible remodeling of mRNA translation. This work introduces a novel approach and computational framework to study persistence potential and allows for the identification of potential drivers of persistence. A better understanding of the unique cellular and molecular traits that drive persistence potential is crucial for the study of drug persistence and development of targeted therapies preventing persistence and enhancing the likelihood of curative treatment.

#5544 Identification, isolation and molecular characterization of drug-resistant sub-populations of pancreatic cancer cells.

Subhendu Roy Choudhury[1], Shixiong Wang[1], Yevhen Akimov[2], Katarina Willoch[1], Biswajyoti Sahu[3], Alfonso Urbanucci[4], Thomas Fleischer[1], Tero Aittokallio[1]

[1]Department of Cancer Genetics, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway,[2]Institute for Molecular Medicine Finland (FIMM), HiLIFE, University of Helsinki, Helsinki, Finland,[3]Centre for Molecular Medicine Norway (NCMM), Faculty of Medicine, University of Oslo, Oslo, Norway,[4]Department of Tumor Biology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway

Pancreatic ductal adenocarcinoma (PDAC) has a poor prognosis, since most PDAC tumors develop resistance to the standard of care (SoC) treatments like chemotherapy, radiation, and targeted therapies. All tumors consist of multiple, genetically related subpopulations of cancer cells that evolve in parallel and display heterogeneity at genomic, epigenetic, or phenotypic levels. As cancer develops, some subpopulations of cancer cells may show faster growth, increased metastatic potential, and resistance toward SoC treatment. A key challenge in PDAC management is understanding chemoresistance driven by cancer subpopulation dynamics. This would broaden our understanding of tumor adaptation to treatments and guide targeting resistant subpopulations to improve therapeutic outcomes. In this study, we identified PDAC cell subpopulations resistant to chemotherapy using our DNA barcoding system B-GLI (barcode-guide lineage isolation). The B-GLI system leverages a highly complex DNA barcode library and CRISPR activation (CRISPRa) to trace and isolate sub-lineages within heterogeneous cell populations by their DNA barcodes. PDAC cell lines PANC-1 and Mia-PaCa-2 were barcoded with the B-GLI barcode library, which consist of two optimized guide RNA binding sites in each barcode. After DNA barcoding, we performed treatments with two SoC chemotherapeutics, gemcitabine + paclitaxel and FOLFIRINOX, to induce a selection pressure in the PDAC cell lines, and then identified the resistant subpopulations through differentially represented barcodes and sequencing. Furthermore, we designed barcode-specific single guide RNAs (sgRNAs) targeting the resistant subpopulations and activated the expression of the puromycin resistance gene by CRISPRa. This allowed us to enrich and isolate chemoresistant subpopulations by puromycin treatment. After having isolated the resistant PDAC subpopulations, we performed ATAC-seq and RNA-seq for molecular characterization of the resistant and parental cell populations, along with phenotypic drug screening with 384 compounds to identify novel treatment vulnerabilities. The compound testing confirmed that the resistant cells were less sensitive to the two SoC treatments, as well as identified selective sensitivity to specific compounds. ATAC-seq and RNA-seq data will allow us to decode whether and how chromatin structure and transcriptomic changes are associated with the treatment resistance, and the confirmation screen of the drug sensitivities will identify potential compounds for future pre-clinical and clinical testing in PDAC models and patients with resistant disease. In summary, this study will identify targeted treatment alternatives, accompanied by molecular biomarkers for chemotherapy resistant PDAC.

#5545 Dynamic Precision™: a scalable tech-bio platform to identify immune-mediated resistance through dynamic monitoring.

Ronan O'Hagan, **Lynda Chin**

Apricity Health, Houston, TX

Background: Understanding immune-mediated resistance requires recognizing the immune system as a complex adaptive system and studying its dynamic responses to drugs in diverse patient populations. Current approaches are hindered by (1) reliance on static, pre-treatment tumor analyses or preclinical models, and (2) sampling bias, as 80% of cancer patients are treated in community settings without enrollment in translational research. We hypothesized that combining dynamic analysis of immune response on treatment with broader patient representation would reveal previously unrecognized resistance mechanisms.

Methods: We developed a tech-bio platform to overcome these limitations. First, applying complex system insights, we pioneered Dynamic Precision™, a AI/ML-based approach to identify emerging resistance targets and blood-based biomarkers from longitudinal patient data. Second, we developed ApricityCare, a HIPAA-compliant virtual care service, to engage diverse populations outside academic institutions in research under the SMART (Serial Molecular and Clinical Annotation of Responses to Therapies) protocol. SMART is IRB-approved for serial liquid biopsy collection, incorporating ApricityCare to enable research-grade data collection. ApricityCare offers 24/7 symptom monitoring and virtual management by nurses augmented with a clinical intelligence co-pilot, ensuring standardized side effect management, documentation, and automated data capture. Coupled with mobile phlebotomy, SMART reduces participation burdens for providers and patients.

Results: We analyzed longitudinal clinical and serial plasma proteomic data from melanoma patients on standard-of-care ICI therapy. Dynamic Precision™ identified early on-treatment signatures predictive of treatment failure and uncovered dynamically regulated immune proteins as potential therapeutic targets. Triangulation with clinical insights revealed druggable immune regulators with validated resistance mechanisms to T-cell checkpoint inhibitors. In parallel, ApricityCare demonstrated its ability to provide proactive symptom monitoring and side effect management while capturing high-quality clinical data without increasing workload for in-clinic care team. Leveraging mobile phlebotomy, SMART has been piloted successfully in multiple states.

Conclusions: We demonstrate here a dynamic approach to identifying immune-mediated resistance mechanisms, with a blood-based biomarker strategy for longitudinal monitoring to detect the molecular signals of resistance months before clinical progression of disease. With ApricityCare-enabled SMART protocol, we have also a scalable platform to bridge the representation gaps, generating more inclusive datasets to elucidate a more diverse range of drug resistance mechanisms. Proof-of-concept data will be presented.

#5546 Sorting to enrich for chemotherapeutic-resistant glioma cells based on unique membrane properties.

V. P. Dang, A. Y. L. Jiang, J. N. Hanamoto, N. S. Lav, C. R. Douglas, D. A. Bota, L. A. Flanagan;
University of California, Irvine, Irvine, CA

The purpose of this study was to leverage dielectrophoresis (DEP) as a novel method for sorting glioma cells based on their innate cell electrophysiological properties. Glioblastoma, astrocytoma, and oligodendroglioma comprise a class of aggressive and deadly brain tumors, termed as diffuse gliomas, with 20,000 cases diagnosed annually in the US. The current standard chemotherapy treatment, temozolomide (TMZ), is insufficient as the 5-year survival rate of glioblastoma patients has remained at roughly 8%. One major contributing factor to treatment inefficacy is the presence of TMZ-resistant cells within tumors that lead to chemoresistance and tumor recurrence. Thus, there is an unmet need to further understand the molecular properties of TMZ-resistant cells which could enable more effective means of targeting them and improve patient outcomes. Here we utilized microfluidic sorting devices and applied DEP, a phenomenon by which non-uniform electric fields induce differential movement of cells, as a novel label-free technique to sort glioma cells based on their membrane electrophysiological properties. With this technique, we successfully sorted cells from glioblastoma and oligodendroglioma established cell lines, which yielded fractions that differed in membrane capacitance, the membrane's ability to store charge. Interestingly, the DEP-sorted cell fractions also exhibited significant differences in TMZ resistance, indicating that DEP can be used robustly to enrich for TMZ-resistant cells across different gliomas. Further, differences in TMZ resistance were coupled with membrane capacitance differences. Since we previously identified links between membrane capacitance and cell-surface glycosylation, we utilized lectin analysis to assess the glycan expression of DEP-sorted oligodendroglioma cells and found inverse correlation between TMZ resistance and mannose glycan expression. Collectively, our study concluded the novel finding that (a) TMZ-resistant glioma cells can be enriched by DEP, and (b) TMZ resistance is correlated with membrane capacitance and cell surface glycosylation. These results could enable downstream molecular characterization of TMZ-resistant cells to uncover less explored mechanisms of TMZ resistance and the development of more effective treatment strategies for gliomas that specifically target membrane properties such as glycosylation.

#5547 High resolution target identification by unbiased forward genetics.

Moritz Horn[1], Georg Michlits[1], Andreia Rocha[1], Christoph Stelzer[1], Josef M. Penninger[2]

[1]JLP Health GmbH, Wien, Austria,[2]Helmholtz Centre for Infection Research, Braunschweig, Germany

Forward genetic screens have revolutionized the field of target discovery, target deconvolution, and target validation, particularly in the oncology field. However, most approaches are limited to the interrogation of non-essential factors and identify gene networks rather than direct target structures. Therefore, the identification of druggable target structures and understanding an active molecules target space remains a challenge in drug discovery and development. To address this, we established a chemical mutagenesis approach in relevant cell types that allows entirely unbiased identification of small molecule targets at amino acid resolution, literally mapping compound-target interaction surfaces. Chemical mutagenesis randomly introduces single nucleotide variants into the entire genome resulting in cell populations which carry substitutions in statistically more than 90% of all amino acids. Challenging such mutagenized cell populations with a drug candidate of interest, followed by next generation sequencing and analysis, reveals direct drug target interactions, drug resistance mechanism, and potential off-targets or concealed efficacies. The alteration of single amino acids thereby allows interrogation of non-essential as well as most essential factors and increases screening resolution to the amino acid level. Several case studies will be shown.

#5548 Single-cell transcriptome analysis reveals the role of myofibroblastic cancer-associated fibroblasts in chemotherapy resistance in pancreatic cancer.

Jiahao Guo[1], Samuele Cancellieri[1], Liangru Fei[1], Tero Aittokallio[2], **Biswajyoti Sahu**[3]

[1]NCMM, University of Oslo, Oslo, Norway,[2]FIMM and Oslo University Hospital, Oslo, Norway,[3]NCMM, University of Oslo and University of Helsinki, Helsinki, Finland

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancer types often diagnosed late due to lack of specific biomarkers and its high capacity to metastasize early. In PDAC, tumor microenvironment (TME) is particularly complex, representing up to 80% of the tumor mass. Thus, better understanding of the cellular states and their controlling transcriptional networks within both malignant and stromal cells is pertinent to improve the survival of PDAC patients. The goal of this project is to elucidate the dynamic non-genetic regulatory mechanisms that contribute to PDAC progression and development of chemoresistance. We have performed in-silico analysis of single-cell RNA-sequencing data from >200,000 cells derived from pancreatic cancer patients with and without neoadjuvant treatment. Based on their gene expression profiles, we identified 12 distinct cell types including three major fibroblast subtypes: regular cancer-associated fibroblasts (CAF), inflammatory CAFs (iCAF), and myofibroblastic CAFs (myCAF). We found that tumors from patients with poor treatment response as well as from untreated patients exhibited a higher proportion of myCAFs compared to treatment-responsive patients. Using specific markers derived from each fibroblast subtype, we applied these signatures to the PAAD cohort from TCGA and found a significant association between the myCAF signature and worse prognosis as well as advanced TNM staging. Functional analyses further demonstrated elevated levels of Wnt/β-Catenin and Notch signaling pathways in the untreated and poor-response groups. Second, we utilized state-of-the-art machine learning methods to model robust relations between transcription factors (TF) and gene regulatory networks from treatment-naïve and neoadjuvant-treated PDAC patients. Our analysis revealed both well-known and new TFs related to pancreatic cell identity and PDAC progression. In-silico perturbation of these TFs was performed to study their effect on gene expression and cell state dynamics. The predictions reported a strong activity from both well-known and newly predicted TFs regarding the cell state transitions and their regulatory role. Currently, we are further characterizing the identified cell states and associated transcriptional programs, and the findings will be presented in the meeting.

#5549 Identification of genes associated with drug persistence/resistance using CRISPR screening and scRNA-seq of ALK rearranged lung cancer.

Ryohei Katayama[1], Takahiro Utsumi[1], Nobuyuki Kondo[1], Ken Uchibori[2], Makoto Nishio[1]

[1]Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo, Japan,[2]Cancer Institute Hospital, Japanese Foundation for Cancer Research, Tokyo, Japan

ALK-rearrangement is found in 3-5% of non-small cell lung cancer (NSCLC), and currently five ALK tyrosine kinase inhibitors (TKIs) have been approved, and several TKIs are in development. These ALK TKIs have contributed to a significant improvement in patient prognosis, such that the median survival of patients with advanced ALK-positive lung cancer has exceeded 5 years. However, it has been a problem that cancers eventually relapse due to drug resistance. To date, many investigators have reported the mechanism of ALK-TKI resistance; secondary mutation(s) in the ALK kinase domain that disrupt ALK-TKI binding, or the activation of bypass signaling pathways that promote cell proliferation even under ALK fusion protein-mediated signaling that is suppressed by ALK-TKIs. However, in a significant number of cases, the mechanism of resistance is still unknown. Therefore, in this study, we first performed a genome-wide CRISPR screen using two ALK-positive NSCLC cell lines derived from patients with ALK-rearranged cancer prior to ALK-TKI treatment. Cas9 and genome-wide sgRNA-introduced cells were cultured with alectinib or lorlatinib for 9 days, and sgRNAs in surviving drug-tolerant cells were analyzed by NGS. Several candidate genes were identified, such as MED12, PTEN, NF2 or MIG6, and confirmed whether knockout of these genes induced drug tolerance/resistance in the ALK-TKI-naive cells. For example, loss of MIG6, an EGFR-binding negative regulator protein, activates EGFR at very low concentrations of EGF (less than 1 ng/mL, which is similar to the healthy human plasma concentration) and induces marked resistance to ALK inhibitors. In contrast, NF2 knockout itself induces drug resistance rather than drug persistence, and we found that brigatinib may be able to overcome the resistance induced by NF2 loss, because brigatinib inhibited ALK as well as several other kinases at single digit nM. As recently published in the NF2 loss Schwannomatosis, NF2 loss induce multiple kinase activation not only mTOR pathway and MAPK pathways, and brigatinib was shown to be an effective treatment option for NF2 loss Schwannomatosis. In addition, from the analysis of alectinib-resistant pleural effusion-derived cells, we found AXL overexpression but not Gas6, a ligand of Axl, in the resistant tumor. Therefore, we performed an in vivo experiment by inoculating Axl-overexpressing ALK-positive cancer cells and analyzed the tumor tissue with or without alectinib treatment. Single-cell RNA sequencing revealed that MMP11-positive cancer-associated fibroblasts (CAFs) and a subset of tumor-associated macrophages (TAMs) were the major sources of Gas6 in the tumor microenvironment. This study uncovered some of the unidentified resistance mechanisms and potential therapeutic strategies to overcome the resistance.

#5550 Understanding therapy resistance in T-cell acute lymphoblastic leukemia.

A. Nasimian, K. Shah, **J. U. Kazi**;
Lunds Universitet, Lund, Sweden

The deregulation of BCL2 family proteins is a key contributor to the development of T-cell acute lymphoblastic leukemia (T-ALL). Pharmacological inhibition of these proteins, particularly through the BCL2-specific inhibitor venetoclax, has emerged as a promising therapeutic approach. However, the clinical efficacy of venetoclax is often compromised due to primary and acquired resistance mechanisms that remain incompletely understood. We employed a deep tabular learning algorithm to develop a drug sensitivity prediction model using T-ALL patient samples. Our findings revealed distinct expression profiles between drug-sensitive and drug-resistant samples. Specifically, in the context of venetoclax sensitivity and resistance phenotypes, we observed associations with several key proteins regulating the cell cycle, cytokine signaling, and mitochondrial apoptosis. Our data suggest a key link between lipid metabolism and beta-catenin signaling, which may cooperate with transcription factors regulating the transcriptional activation of key apoptosis regulatory genes. Together, our results underscore the complexity of therapy resistance and highlight the therapeutic potential of targeting multiple pathways for growth inhibition. Future research should focus on clinical trials exploring this combinatorial approach to overcome resistance and improve patient outcomes in T-ALL.

#5551 From persistence to resistance: insights into the mechanisms of drug tolerance in cancer therapy using patient-derived organoids.

Yasmine Abouleila, Timo Voskuilen, Mayke Doorn, Roel Verkerk, Joris Maas, Carla Verissimo, **Sylvia F. Boj**

HUB Organoids B.V., Utrecht, Netherlands

Resistance to cancer treatment remains a significant obstacle in curing cancer patients. Despite recent advancements in novel cancer drugs, patients often experience treatment resistance even after initially responding to therapy, leading to tumor relapse. To understand how resistance develops against standard cancer therapies, it is essential to study the gradual changes leading to full resistance. Recent research shows that cancer cells can evade the effects of chemotherapy and targeted therapies by entering a reversible, slow-growing phase called the drug-tolerant persister (DTP) state. In this DTP state, cancer cells survive drug treatment long enough to begin accumulating mutations and eventually develop additional mechanisms of irreversible resistance. Patient-derived organoid (PDO) models, also known as HUB Organoids®, are directly derived from patient tissues, making them more physiologically relevant than traditional cell lines. This study highlights the value of PDO models in studying drug persistence and resistance, as they are more likely to reflect in-vivo conditions. Here, we demonstrate the successful development of colorectal cancer (CRC) PDO models that can persist and resist standard therapies. These models were characterized using whole exome sequencing and bulk RNA sequencing to assess their persistent or resistant state and to identify potential pathways and new drug targets involved in resistance. In conclusion, developing persistent and resistant PDO models is essential for understanding the mechanisms behind drug resistance. This research contributes to developing patient-relevant preclinical models to develop more effective and personalized cancer treatments and expands our overall understanding of how resistance develops in cancer patients.

#5553 Acquisition of adaptive and reversible drug-tolerance state to cabozantinib in renal cell carcinoma cell lines.

Angela Zaccagnino, Hiresh Ayoubian, Kerstin Junker

Saarland Univ. Faculty of Medicine, Homburg, Germany

Drug-tolerant (DT) state has been recognized as a transient, adaptive process whereby tumour cells endure therapy with tyrosine kinase inhibitors (TKIs) through non-genetic/epigenetic mechanisms, which might be responsible for driving tumour relapse and therapy resistance. Considering that resistance to the targeted systemic therapies remains challenging in metastatic renal cell carcinoma (mRCC), here we elucidated the DT-state as response to the TKI cabozantinib (CBZ) targeting c-MET/Axl/VEGFR in RCC cell lines.After chronic exposure to CBZ (IC50) for 4 months, the 786-O/DTC, Caki-2/DTC and KTCTL-26/DTC cells were profiled for cell survival (WST-1), proliferation (BrdU), migration/invasion, apoptosis (caspase 3/7) and compared to untreated wild-type (wt) cells. Molecular analyses were carried out via immunoblotting and miRNA expression assays (hs-miRNA microarray and RT-qPCR).The DTC-cell lines were successfully induced. The DT-cell lines maintained viability after long-term therapy with a slow growing state. To investigate the mechanisms that drive DT-state, we compared the DTC and WT cells under CBZ. The DTC-cell lines showed higher proliferation ($p<0.001$), along with lower caspase 3/7 activity in KTCTL-26/DTC and Caki-2/DTC ($p<0.001$). CBZ impaired migration and invasion, except in KTCTL26/DTC, which displayed higher invasion ($p<0.001$). Phospho-TK c-MET was inhibited in DT-cells but not Axl. Yet, c-MET was partially restored in KTCTL-26/DTC. The downstream ERK was unchanged in Caki-2/DTC and KTCTL-26/DTC or boosted in 786-O/DTC. BCL-2 was enhanced in Caki-2/DTC or unaffected in the other two DTC- cell lines hinting at an ability to bypass cell death. The DTC-state was reversible, as the cells recovered cell growth after CBZ-withdrawal implying a dynamic regulation. Among 4 miRNAs, namely hs-miR-551b-3p, hs-miR-34a-5p, hs-miR-424-5p and hs-miR-9-3p that were commonly dysregulated in DTC-cell lines, the hs-miR-424-5p and hs-miR-9-3p (regulation of cell cycle, MAPK signalling and apoptosis) were consensually upregulated in DTC-cells by 2.5-fold change ($p=0.001$), but their expression was reversed after treatment discontinuation. Remarkably, their regulation matched the changes in We established CBZ-DT cell lines and characterised the related spectrum of molecular changes, while considering tumour heterogeneity crucial to drug-adaptation. We identified previously unrecognised miRNAs associated with DT to emphasise the dynamic epigenetic changes that are critical in the initiation of DT-state.

#5554 Intrinsic resistance mechanism of squamous cell lung carcinomas to the multiangiokinase receptor inhibitor Nintedanib through CXCL-1 upregulation in tumor associated fibroblasts.

Natalia Isabel Diaz Valdivia[1], Paula Duch[1], Alejandro Bernardo[1], Marselina Arshakyan[1], Noemi Reguart[2], Derek Radisky[3], Oriol Casanovas[4], Jordi Alcaraz[1]

[1]Unit of Biophysics and Bioengineering, Department of Biomedicine, Universitat de Barcelona, Barcelona, Spain,[2]Thoracic Oncology Unit, Hospital Clinic, Barcelona, Spain,[3]Department of Cancer Biology, Mayo Clinic, Jacksonville, FL,[4]Tumor Angiogenesis Group, ProCURE, Catalan Institute of Oncology, Oncobell, IDIBELL, Barcelona, Spain

Background: Lung adenocarcinoma (ADC) and squamous cell carcinoma (SCC) are the most common lung cancer subtypes. SCC is traditionally resistant to anti-angiogenic therapies, though the mechanisms remain unclear. Nintedanib, a multi-angiokinase receptor inhibitor, is approved for treating ADC in combination with docetaxel, based on selective efficacy in the LUME-1 trial. It is also FDA-approved for idiopathic pulmonary fibrosis due to its antifibrotic properties. Both ADC and SCC feature fibrotic stroma rich in activated tumor-associated fibroblasts (TAFs). Our prior work showed Nintedanib has stronger antifibrotic effects on ADC-TAFs than SCC-TAFs. However, its impact on TAF-driven angiogenesis and the role of TAFs in SCC resistance to multi-angiokinase inhibitors remain unexplored.
Methods: The pro-angiogenic activity of TAFs secretome from ADC and SCC patients was assessed. TAFs were stimulated with TGF-β1 to mimic their activated phenotype, then treated with Nintedanib. Conditioned media from treated TAFs was used to assess endothelial cell migration and vascular network formation. Pro-angiogenic factor secretion was analyzed using antibody arrays, and functional roles of selected proteins were validated in vitro and in vivo using genetic approaches.
Results: Nintedanib inhibited the pro-angiogenic effects of ADC-TAF secretome but paradoxically enhanced angiogenesis in SCC-TAFs, increasing endothelial cell migration in vitro and in vivo. Notably, Nintedanib selectively upregulated CXCL-1 secretion in SCC-TAFs. Silencing CXCL-1 using siRNA in SCC-TAFs abrogated the Nintedanib-induced angiogenesis. Similar findings with other multi-angiokinase inhibitors suggest aberrant CXCL-1 production in SCC-TAFs may drive universal resistance to these therapies.
Conclusion: SCC-TAFs are resistant to the anti-angiogenic effects of Nintedanib, which paradoxically promotes angiogenesis via CXCL-1 upregulation. This study provides the first mechanistic insight into SCC resistance to anti-angiogenic therapies and underscores the critical role of TAFs in modulating therapeutic responses. Targeting CXCL-1 or TAF-mediated pathways may inform alternative anti-angiogenic strategies in SCC, potentially in combination with immune checkpoint inhibitors.

#5555 Potential pathways underlying sorafenib resistance in hepatocellular carcinoma.

Yamile Abuchard Anaya[1], Ana Ayala Pazzi[2], Dennis Kwabiah[1], Veerababu Nagati[1], Ricardo Pequeno Bracho[1], Manish K. Tripathi[1]

[1]Medicine and Oncology ISU, University of Texas Rio Grande Valley, McAllen, TX,[2]Biomedical engineering, UT Southwestern Medical Center, Dallas, TX

Background: Hepatocellular carcinoma (HCC), the most prevalent form of liver cancer, is heavily influenced by socioeconomic and health disparities. In the Rio Grande Valley (RGV), risk factors such as Hepatitis B and C, diabetes, and non-alcoholic fatty liver disease (NAFLD) contribute significantly to HCC prevalence. McAllen, TX, ranks as the most obese city in the United States, compounding the HCC burden in this underserved area. Limited access to screening and early detection often results in late-stage diagnoses and poor outcomes. A deeper understanding of the molecular mechanisms driving HCC, particularly drug resistance, is crucial for improving early detection and treatment strategies. Our lab investigates the role of Y-Box Binding Protein 1 (YBX1) in HCC progression and resistance to sorafenib, the first-line tyrosine kinase inhibitor (TKI) for advanced HCC. YBX1, part of the Cold Shock Domain (CSD) protein superfamily, is a 324-amino acid protein involved in mRNA translation regulation via interactions with microRNAs and non-coding RNAs. While sorafenib shows initial efficacy, resistance frequently develops, and its mechanisms remain poorly understood.
Methods: HCC cell lines were cultured following ATCC guidelines. Lentiviral overexpression plasmids for YBX1, incorporating GFP and a puromycin selection marker, were obtained from Abmgood. YBX1 expression was validated using Western blot and RT-PCR. Functional assays, including invasion, migration, proliferation, colony formation, and Xcelligence impedance analysis, were conducted to assess phenotypic changes. A phosphoproteome array identified kinase pathways influenced by YBX1, while specific kinase activators and inhibitors clarified YBX1-regulated signaling pathways linked to sorafenib resistance.
Results: Analysis of The Cancer Genome Atlas (TCGA) confirmed that YBX1 expression is significantly elevated in HCC tumors compared to normal tissues and correlates with disease progression, metastasis, and poor survival. In vitro studies demonstrated that YBX1 overexpression (OE) enhanced invasion, migration, proliferation, and colony formation, while knockdown (KD) reduced these behaviors. Xcelligence assays further revealed accelerated proliferation in YBX1-OE cells and diminished growth in KD cells. Phosphoproteome analysis identified several kinases modulated by YBX1, and pathway analysis is ongoing to determine their roles in sorafenib resistance.
Conclusion and Future Directions: We established GFP-expressing SK-Hep1 cell lines with stable YBX1 overexpression and knockdown, confirming YBX1's role in driving oncogenic phenotypes in HCC. Ongoing proteomic studies aim to identify YBX1-associated proteins and pathways, with a focus on kinase modulation. These insights will enhance our understanding of sorafenib resistance mechanisms and inform the development of targeted therapies to overcome resistance in HCC.

#5556 Development of osimertinib resistant mouse models for preclinical evaluation of third-generation EFGR-TKI therapies.

Hongyan Sun[1], Yinuo Wang[1], Shiying Guo[1], Jianming Xu[1], Xiang Gao[1], Jun Wang[2]

[1]GemPharmatech Co., Ltd, Nanjing, China,[2]GemPharmatech Co., Ltd, San Diego, CA

Osimertinib was developed as a third-generation EGFR tyrosine kinase inhibitor. Numerous clinical trials have demonstrated that EGFR-TKI targeted drug therapy for non-small cell lung cancer can prolong the survival of patients. However, its efficacy is impacted by acquired EGFR C797 mutations (including EGFR triple mutations Del19/T790M/C797S and L858R/T790M/C797S). To better understand the drug resistance mechanism towards Osimertinib, we have developed both *in vitro* and *in vivo* models associated with Osimertinib resistance for the evaluation of novel therapeutics that overcome Osimertinib resistance.

The human lung adenocarcinoma H1975 is a cell line with double mutations in the EGFR gene (L858R/T790M). To create a model which mimics patients in clinic who received Osimertinib treatment and developed drug resistance thereafter, the C797S mutation was introduced using advanced gene editing technology for site-directed mutagenesis, resulting in the EGFR triple-mutant cell line (H1975 with EGFR L858R/T790M/C797S).

To explore other Osimertinib resistance mechanisms than the C797 mutation, we induced cell lines *in vitro* with multiple rounds of increasing concentrations of Osimertinib. Following the induction of Osimertinib, the IC50 of different cell lines treated by Osimertinib was assessed, confirming increased resistance to Osimertinib. *In vivo* tumorigenicity and efficacy studies with Osimertinib were performed to screen and validate our Osimertinib-resistant models. Currently, we have obtained the EGFR triple mutant H1975 and PC-14 cell lines that exhibit resistance to Osimertinib for *in vitro* and *in vivo* study, which serve as vital tools for studying drug resistance mechanisms and developing innovative treatment strategies.

#5557 Deciphering the interplay of β-catenin/YAP signaling and alveolar lineage plasticity during targeted therapy in non-small cell lung cancer.

Yu-Ting Chou, Wei Wu, D. Lucas Kerr, Victor Olivas, Macey Slota, Trever G. Bivona

UCSF - University of California San Francisco, San Francisco, CA

Molecularly targeted therapies have significantly improved clinical outcomes by directly addressing oncogenic driver mutations in genes such as EGFR. However, despite their initial success, long-term patient survival remains limited due to the emergence of drug tolerance and resistance. This study investigates the molecular mechanisms underlying drug tolerance in response to targeted therapies in non-small cell lung cancer (NSCLC). Using single-cell RNA sequencing of oncogene-driven human NSCLCs at the residual disease (RD) state during active targeted therapy, we observed an enrichment of an alveolar type I/II (AT1/2) mixed lineage program in surviving cancer cells, resembling an alveolar cell-like state. This transition is characterized by increased expression of WNT3A/4 ligands, FZD2 receptors, β-catenin signaling, and AT1/2 signatures in RD cells. Consistent with these clinical observations, preclinical models of drug-tolerant persister (DTP) cells exhibited elevated AT1/2-like transcriptional signatures and target gene expression associated with β-catenin and YAP signaling. Further analysis revealed the formation of a stable nuclear protein complex between β-catenin and YAP in DTP cells, which appears to play a critical role in sustaining drug tolerance. Disrupting the expression and stability of the β-catenin/YAP complex, either through knockdown of these genes or by using Tankyrase inhibitors (e.g. XAV-939 or IWR1) to regulate the β-catenin destruction complex, re-sensitized DTP cells to the EGFR inhibitor Osimertinib. In xenograft mouse models, the combination of Osimertinib with a Tankyrase inhibitor significantly improved tumor growth inhibition compared to Osimertinib alone. This therapeutic strategy also led to a notable reduction in AT1/2 gene signatures, a decrease in nuclear β-catenin and YAP protein expression, reduced stability of these proteins, and suppression of their interaction, further supporting its potential in overcoming drug tolerance. Additionally, we identified specific domains within both β-catenin and YAP proteins that are essential for their interaction. This interdependence underscores the critical roles of β-catenin and YAP in maintaining DTP cell survival and driving alveolar cell-like lineage plasticity. Our findings shed light on the interplay between β-catenin/YAP signaling and alveolar cell-like lineage signatures in residual cancer states. By targeting this axis, we propose a novel therapeutic approach to overcome drug tolerance and improve clinical outcomes for patients with NSCLC undergoing targeted therapy.

#5558 Stress-induced sympathetic stimulation inhibits sorafenib-induced ferroptosis in renal cell carcinoma.

Masaki Ushijima[1], Sei Naito[2], Hiromi Ito[2], Osamu Ichiyanagi[3], Takafumi Narisawa[2], Norihiko Tsuchiya[2]

[1]Urology, Yamagata Prefectural Shinjo Hospital, Shinjo, Japan,[2]Urology, Yamagata University Faculty of Medicine, Yamagata, Japan,[3]Urology, Yamagata Prefectural Kahoku Hospital, Kahoku, Japan

Background and Objective: Cancer patients are often in a chronic state of stress. There have been several reports on the relationship between stress and cancer progression, as well as the development of drug resistance. Our purpose is to investigate the effect of stress-induced sympathetic stimulation on drug resistance, especially on sorafenib, which has been reported to induce ferroptosis, in renal cell carcinoma (RCC).
Methods: First, we investigated the expression of β1- (ADRB1) and β2-adrenergic receptor (ADRB2) in surgical specimens by immunohistochemistry and in RCC cell lines by RT-qPCR. Isoproterenol (ISO), a non-specific β-adrenergic receptor agonist, was added into RCC cell lines ACHN and A498 before the addition of clinically-used-drugs (sorafenib, cabozantinib, lenvatinib, and rapamycin), and cell viability was analyzed by MTS assay. BALB/c nude mice were subcutaneously implanted with ACHN on the right side and ADRB2 knockdown ACHN on the left side of the mice. The mice were divided into the following groups: saline, sorafenib, stress+saline, stress+sorafenib. To explore the mechanisms of sorafenib-induced suppression of cell viability, we examined the effects of sorafenib by adding the ferroptosis inhibitors and inducers.
Result: All examined surgical RCC specimens and RCC cell lines were expressed ADRB1 and ADRB2. Drug resistance due to ISO was observed in sorafenib treatment in ACHN but not in A498. siADRB1 became resistant to sorafenib due to ISO, whereas siADRB2 was not affected. In mouse models, sorafenib inhibited tumor growth in the mock ACHN of control-reared mice. Within the vehicle-treated group, no significant differences in tumor volume due to stress exposure were observed. However, in the sorafenib-treated group, the stress-exposed group showed a greater increase in tumor volume compared to the control-reared group. In the shADRB2 ACHN, no differences in tumor volume changes were observed between the control-reared and stress-exposed groups under sorafenib treatment; both groups showed a suppression of tumor volume increase by sorafenib. In brief, ADRB2-mediated stress stimulation induced sorafenib resistance. Ferrostatin-1, a ferroptosis inhibitor, was able to significantly inhibit the effect of sorafenib in ACHN, but not in A498. The ferroptosis inducers Erastin and RSL3 decreased cell viability in both ACHN and A498 cells, and this induction of ferroptosis was suppressed in the presence of ISO. ISO significantly reduced reactive oxygen species increased by Erastin in ACHN and A-498.
Conclusion: Stress-induced sympathetic stimulation inhibits sorafenib-induced ferroptosis in RCC.

#5559 Receptor tyrosine kinase-like orphan receptor 1 (ROR1) in BTKi-treated mantle cell lymphoma.

A. Sircar[1], S. Parsa[2], L. B. Prickett[1], V. Munugalavadla[2], C. Rhee[1], R. Tourdot[1], A. Nelson[1], M. Ophir[1], W. Zhong[3], M. Sung[1], D. Auclair[1], C. D. Palmer[1];

[1]AstraZeneca US, Waltham, MA, [2]AstraZeneca US, South San Francisco, CA, [3]AstraZeneca US, New York City, NY

Mantle cell lymphoma (MCL) is a rare, aggressive B-cell non-Hodgkin lymphoma with a poor patient overall survival rate. While Bruton tyrosine kinase inhibitors (BTKis) have shown benefit in relapsed/refractory MCL (Wang M, *et al*., 2018; Song Y, *et al*.,2020; Wang ML *et al*., 2013) a portion of patients eventually progress. Here, we explored which biomarkers might be associated with resistance to acalabrutinib, a second generation BTKi, by analyzing samples from MCL patients who were on treatment or progressed on acalabrutinib from the ACE-LY-004 study (NCT02213926), as well as using a pre-clinical MCL cell line model. Receptor tyrosine kinase-like orphan receptor 1 (ROR1) is an important oncofetal protein and a target of clinical relevance in MCL. It was previously attributed to a bypass resistance mechanism to first generation BTKi ibrutinib (Zhang *et al*., 2019). We hence analyzed ROR1 expression levels using available exploratory biomarker flow cytometry data from ACE-LY-004, which revealed that MCL patients who progressed or pre-progressed on acalabrutinib showed a higher percentage of circulating CD5+ROR1+ tumor cells (78.3%; n=7 and 63.6%; n=4 respectively) than patients who continued responding to treatment (23.8%; n=6). Interestingly, although progression was associated with higher frequency of CD5+ROR1+ tumor cells, the per-cell surface ROR1 expression on these cells from patients who pre-progressed/ progressed on acalabrutinib treatment (longitudinal analysis; n=6) was lower. While acute 5-day BTKi treatment in the Jeko-1 MCL cell line led to transiently higher surface expression of ROR1, chronic 4-month exposure of sensitive cells led to ROR1 surface downregulation in acalabrutinib or reversible BTKi acquired resistant (R) Jeko-1 MCL cells. Total ROR1 expression (western blot), was increased in Jeko-1R versus sensitive, suggesting that BTKi chronically treated MCL cells may upregulate intracellular ROR1 and/or partially internalize surface ROR1. To assess potential therapeutic implications of decreased surface expression of ROR1 in MCL post BTKi treatment, we assessed activity of a ROR1-targeted ADC (Zilovertamab vedotin; Wang *et al*., 2021) in BTKi-R cells. In line with downregulated surface ROR1 levels, the response to ZV was lower in BTKi-R compared to sensitive cells. However, Wnt5a (ROR1 ligand) expression was increased in these Jeko-1R cells, indicating a potential increase in ligand-dependent ROR1 activity. These preliminary results highlight the possible association between ROR1 expression on MCL tumor cells and BTKi response. To further dissect the interplay between ROR1 and second generation/reversible BTKi treatment in MCL, studies utilizing a multi-omics integration approach, BTKi-R MCL cell lines, and *ex vivo* propagated MCL patient cell models are ongoing.

#5560 Exploring the role of Wnt, NFκb, and PI3 kinase pathways in oxaliplatin-resistant triple-negative breast carcinoma cell line.

Asif Ahmad[1], Sheerien Rajput[2], Areeb Ahmed[2], Kulsoom Ghias[1], **Azhar Hussain Rajabali**[3]

[1]Biological and Biomedical Sciences, Aga Khan University Hospital, Karachi, Pakistan,[2]Centre for Regenerative Medicine and Stem Cell Research, Aga Khan University Hospital, Karachi, Pakistan,[3]Aga Khan University Hospital, Karachi, Pakistan

Breast cancer (BCa) is one of the major causes of mortality and morbidity in women globally. Triple negative breast cancer (TNBC) is known for its aggressive nature and is associated with high recurrence and metastasis with poor overall survival; 50% of the patients relapse in the first three years. Development of metastasis and drug resistance impacts survival in TNBC patients. The reasons for drug resistance and metastasis are complex and involve dysregulation of different cell signaling pathways, such as Wnt/β-catenin, NFκB and PI3K/AKT. Clinical studies have shown a favorable response to combination therapy using oxaliplatin in the treatment of metastatic breast cancer. This study explored the role of Wnt/β-catenin, NFκB and PI3K/AKT survival pathways in oxaliplatin-resistant TNBC cell line *in vitro*. Oxaliplatin-resistant clones were developed in the TNBC cell line MDA-MB-231 through sequential treatment with increasing doses of oxaliplatin. Differential gene expressions between chemoresistant clones and parental cells revealed the dysregulation of resistance-related genes, such as at 4μM treatment CD44 ($p< 0.001$), COX-2 ($p < 0.001$), ABCG2, BCL-2 ($p < 0.05$) and BAX ($p < 0.01$), alongside a shift towards a mesenchymal phenotype in resistant clones. Signaling pathways were altered, such as the upregulation of β-catenin in the Wnt/β-catenin pathway, Rel-A and NFκB1 in the NFκB pathway, and differential expression of AKT1, PIK3CA and mTOR1 in the PI3K/Akt pathway. Additionally, resistant clones displayed G2/M cell cycle arrest. The findings of the study enhance the understanding of drug resistance mechanisms in triple-negative breast cancer (TNBC) and identify possible targets for therapy.

#5561 Ankyrin repeat domain 1 confers resistance to tepotinib in MET-altered cancers.

Ryunosuke Fujii[1], Tomoaki Higashihara[1], Ken Suzawa[2], Kousei Ishimura[1], Kazuya Hisamatsu[1], Ryota Fujiwara[1], Atsushi Matsuoka[1], Ryo Yoshichika[1], Naohiro Hayashi[1], Yuma Fukumoto[1], Kazuhiro Okada[1], Fumiaki Mukohara[1], Mao Yoshikawa[1], Kazuhiko Shien[2], Shinichi Toyooka[1]

[1]Thoracic, Breast and Endocrinological Surgery, Okayama University Graduate School of Medicine, Dentistry and Phamaceutical Sciences, Okayama, Japan,[2]Thoracic, Breast and Endocrinological Surgery, Okayama University Hospital, Okayama, Japan

Purpose: The mesenchymal epithelial transition factor receptor (MET) is a promising therapeutic target in various cancers, including non-small cell lung cancer (NSCLC). In NSCLC, activation of the MET pathway occurs through diverse mechanisms, influencing cancer cell survival, growth, and invasiveness. These tumors exhibit oncogene addiction to MET and show remarkable responses to MET-Tyrosine Kinase Inhibitors (TKIs). However, the long-term effectiveness of MET-TKIs is usually limited due to acquired drug resistance. The mechanisms underlying primary and acquired resistance to MET-TKIs in NSCLC remain poorly understood. In this study, we investigated the mechanisms to tepotinib in *MET*-amplified and *MET* exon 14 skipping cancer cell lines.

Experimental Design: Two *MET*-amplified lung cancer cell lines, EBC-1 and H1993, and one *MET* exon 14 skipping gastric cancer cell line, Hs746T, were used to establish models of acquired resistance to MET-TKI tepotinib. Resistance mechanisms were explored through functional analyses, comprehensive gene expression analysis using RNA sequencing, and the alterations hypothesized to confer resistance were validated.

Results: Multiple mechanisms contributing to tepotinib resistance were identified. In EBC1 and Hs746T derived resistant cells, *Ankyrin Repeat Domain 1* (*ANKRD1*) was highly expressed, and suppression of *ANKRD1* using shRNA impaired resistance to tepotinib. Additionally, activation of the MAPK pathway was observed in EBC1 derived resistant cells, and inhibition of this pathway with MEK inhibitors had a synergistic effect with tepotinib. All 3 resistant cell lines used in this study showed epithelial-mesenchymal transition (EMT), which may further contribute to the resistance.

Conclusions: In this study, we revealed the different mechanisms of acquired resistance to MET-TKI tepotinib with potential therapeutic application.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Identification of Molecular Targets 2
Poster Session

#5565 Dysfunction in ATP-binding cassette 1 (ABCA1): a driver of intracellular cholesterol accumulation and potential therapeutic vulnerability of bowel cancers.

Daniel J. Gironda, Calvin J. Wagner, Ashok K. Pullikuth, Lance D. Miller

Cancer Biology, Wake Forest University School of Medicine, Winston-Salem, NC

Background: Cholesterol is an integral sterol lipid that influences the homeostatic maintenance of cellular structure, hormone synthesis, and metabolic regulation within the cell. Accumulation of intracellular cholesterol has shown to drive the progression of multiple human cancers. Here we report that mutation or transcriptional repression of the cholesterol exporter, ABCA1, is a common phenomenon conserved in bowel cancers with novel mechanistic and therapeutic implications.
Methods: Whole-exome sequence analysis was conducted on 24 appendiceal cancers (AC) from consented patients. ABCA1 coding mutations and expression levels were analyzed in tumors from The Cancer Genome Atlas (TCGA) and Broad DepMap databases. One AC cell line (Naxorini) was created in house and four commercial CRC cell lines were used in ABCA1 functional studies. Combination treatment of 22-(R)-hydroxycholesterol (OHC) and 9-cis-retinoic acid (CRA) was used to induce ABCA1. ABCA1 knockdown (KD), measures of cellular viability, cell death, apoptosis, and free cholesterol were quantified.
Results: Protein coding mutations in the ABCA1 gene were identified in 7/24 (29%) AC specimens. Mutational analysis of other gastrointestinal cancers shows that ABCA1 is preferentially mutated in mucinous CRCs and mucinous stomach adenocarcinomas compared to their non-mucinous counterparts (X^2 p-value <0.001 and 0.047). ABCA1 mRNA expression levels were significantly lower in CRC and gastric tumors as compared to 28 different tumor types (ANOVA $p<0.001$), and significantly lower in bowel cancer cell lines as compared to 34 different cancer lineages (ANOVA $p<0.001$). 10uM OHC+CRA treatment robustly induced ABCA1 protein expression in ABCA1 wildtype (WT) cell lines (Naxorini, MDST8, COLO201), but not in ABCA1 mutant lines (HCT15, SNU81), significantly induced cell death in ABCA1 WT cell lines (all, $p<0.001$), and decreased free cholesterol accumulation (MDST8, $p=0.010$; Naxorini, $p=0.007$). Importantly, KD of ABCA1 rescued the viability of OHC+CRA treated cells (MDST8, $p<0.001$) demonstrating that OHC+CRA cytotoxicity is dependent on the induction of ABCA1. Furthermore, the KD of ABCA1 in untreated MDST8 cells significantly enhanced cell viability ($p=0.004$) consistent with a growth advantage owing to reduced activity of ABCA1. Analysis of ABCA1-dependent, OHC+CRA induced cell death found alterations in protein levels of Bad, Bcl-2 and HtrA2/Omi (all, $p<0.05$), associating ABCA1 induction with mitochondrial apoptosis.
Conclusions: Dysregulation of cellular cholesterol due to dysfunctional ABCA1, whether by somatic mutation or transcriptional downregulation, may represent an oncogenic addiction conserved in bowel cancers. These findings warrant further investigation into the therapeutic potential of ABCA1 and cholesterol targeting strategies for bowel cancers.

#5566 Comprehensive protein and genomic analysis demonstrate IGF2BP2 promotes cell proliferation via NF-kB signaling pathway in endometrial cancer..

Hiroki Kurahashi, Yuko Watanabe, Kosuke Hiramatsu, Tatsuo Masuda, Mamoru Kakuda, Satoshi Nakagawa, Tadashi Iwamiya, Shinya Matsuzaki, Yutaka Ueda

Obstetrics and Gynecology, Osaka University, Suita, Japan

Introduction: Endometrial cancer (EC) is the most common gynecological cancer. While multidisciplinary therapy including surgery and chemotherapy improves the prognosis, that of refractory cases remains poor. In this study, we aimed to investigate the new treatment target and predictive marker for treatment of refractory EC using comprehensive protein analysis and TCGA genomic data.
Materials and Methods: We performed iTRAQ-based comprehensive protein analysis using EC tissue samples before treatment, and compared protein expression data of refractory and responsive cases. To evaluate the function of Insulin-like growth factor 2 mRNA-binding protein 2 (IGF2BP2) which is highly expressed in refractory EC, we generated IGF2BP2 knockdown (KD) EC cells using siRNA and shRNA and evaluated cell proliferation after IGF2BP2 KD in vitro and in vivo. Moreover, we investigated signaling pathway regulated by IGF2BP2 using protein dataset. Finally, we measured IGF2BP2 cfDNA in the case of EC before and after treatment.
Results: iTRAQ-based comprehensive protein analysis identified 2,299 proteins, and IGF2BP2 had the highest expression in refractory EC. High expression of IGF2BP2 in refractory EC was validated by immunohistochemistry. TCGA data analysis revealed the significant correlation between elevated IGF2BP2 mRNA levels and poor prognosis of EC ($p < 0.01$). Furthermore, we demonstrated that IGF2BP2 KD in EC cell lines significantly suppressed cell proliferation in vitro and tumor growth in vivo ($p < 0.01$, respectively). Moreover, analysis of protein datasets (Cell. 2020) of EC showed that high expression of IGF2BP2 was associated with activation of NF-kB pathway, and IGF2BP2 KD inactivated NF-kB in vitro. Finally, we demonstrated that IGF2BP2 cfDNA was decreased after complete surgery for EC.
Conclusion: IGF2BP2 is highly expressed in refractory EC and contributes to cell proliferation via NF-kB pathway and poor prognosis.

#5567 Targeted immunotherapies in multiple myeloma: focus on flow cytometry markers and therapeutic implications .

Haoting Hsu, Elena Bruni, Claudia Carenza, Karolina Pilipow, Paolo Schiavini, Christine Baer, Gilad Silberberg, BanuPriya Sridharan, Mara Gilardi, Stefano Cairo, Brandon Walling

Champions Oncology, Inc., Hackensack, NJ

Multiple myeloma (MM) is a hematologic malignancy characterized by the clonal proliferation of malignant plasma cells. Despite advancements, MM remains one of the most prevalent hematological malignancies with over 30,000 new cases annually. However, the emergence of immunotherapy through the targeting of specific cell surface proteins has transformed the treatment landscape for MM and highlights the need for the identification and evaluation of unique immunotherapy targets, particularly in relapsed or refractory cases. Drugs to CD38 (Daratumumab & Isatuximab) and SLAMF7 (Elotuzumab) have demonstrated the potency of these therapies, particularly when utilized in combinations. Flow cytometry plays a vital role in diagnosing MM and in evaluating minimal residual disease (MRD), however, it also functions as an excellent method through which to interrogate the expression of unique markers and subpopulations. Here we have utilized Champion Oncology's cryopreserved primary MM patient samples derived from bone marrow aspirates to generate a platform for the interrogation of novel biomarkers utilizing flow cytometry, sequencing, and ex vivo analysis. We have performed cellular deconvolution on these models to sort and interrogate populations based on numerous factors including tumor type, treatment history, and diagnosis. This provides rapid mechanisms to identify populations of interest for unique signatures. These identified targets may then be utilized via flow cytometry to characterize models with an adaptable "drop-in" panel which facilitates the addition of multiple novel targets for rapid screening. These data can be further stratified utilizing the canonical MM markers to evaluate the expression within subpopulations and how these subpopulations evolve throughout treatment. Finally, those models that have been identified can be utilized in ex vivo assays. Using six unique MM models stratified into refractory, naive, and relapsed, we evaluated the ex vivo response to current standard of care (SOC) treatments allowing for drug sensitivity screening and the evaluation of novel target responses. Collectively, these data demonstrate a robust method for rapid identification and evaluation of unique MM targets.

#5568 The estrogen sulfotransferase drives pancreatic ductal adenocarcinoma.

Syamprasad NP, Shijia S. Feng, Donovan Whitfield, Wen Xie

Centre for Pharmacogenetics, University of Pittsburgh School of Pharmacy, Pittsburgh, PA

Background: pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancers, with only a 12% five-year survival rate. The complex nature of PDAC limits treatment options, highlighting the need to identify novel therapeutic targets. Males are more likely to develop PDAC than females, so we performed an initial screening of estrogen-metabolizing enzymes associated with PDAC development. Interestingly, Estrogen sulfotransferase (SULT1E1 or EST) was found to be the most over-expressed in pancreatic ductal adenocarcinoma (PDAC) compared to normal tissues, and it is associated with a poor prognosis for patients. A primary function of EST is sulfonating and deactivating estrogens. Hence, we aimed to investigate the role of EST in PDAC development and progression.
Methods: Human PDAC transcriptome and proteome data were accessed from TCGA and CPTAC databases respectively. The expression pattern of EST in human PDAC tissues and genetically engineered PDAC mice model, KC (K-rasLSL.G12D/+; Pdx-1-Cre) and KPC (K-rasLSL.G12D/+; Trp53R172H/+; Pdx-1-Cre) tissues was determined using IHC and immunofluorescence. MIA PaCa-2 ($KRAS^{MT}$ $TP53^{MT}$ $CDKN2A^{HD}$ $SMAD4^{WT}$) and CFPAC-1($KRAS^{MT}$ $TP53^{MT}$ $CDKN2A^{PM}$ $SMAD4^{HD}$) cells were used to investigate the role of EST in both *in vitro* and *in vivo* models.
Results: In the TCGA pan-cancer atlas, we identified that EST is over-expressed only in PDAC compared to other cancer types. Analysis of single-cell RNA sequencing data, Immunohistochemistry, and immunofluorescence studies revealed that EST is overexpressed in PDAC-specific ductal cells in human tissue, as well as in tissues from KC and KPC mice, compared to normal tissues. Overexpression of EST in MIA PaCa-2 and CFPAC-1 cells increased proliferation, migration, wound healing, and sphere formation, along with significantly less stem cell frequency. Moreover, we observed increased Ki-67 positive nuclei, elevated nuclear Cyclin D1 levels, and the upregulation of cyclin D1 target gene signatures in EST overexpressed cells. Additionally, the overexpression of EST was linked to increased tumor growth *in vivo* in both male and female xenograft models, highlighting EST's significant role in the progression of PDAC. Enzyme activity dead mutant EST overexpression revealed that the pro-PDAC effect of EST depends on its enzymatic activity but is independent of estrogen.
Conclusion: We have uncovered a novel function of EST in the development of PDAC, and it may represent a potential target for treating PDAC.

#5569 FBXW7 functional loss enhances IGF2BP1 levels, implicating oncogenic m^{6}A RNA modification dynamics in colon cancer.

Soo-Jin Lee, Sung-Yup Cho

Department of Biomedical Sciences, Seoul National University, College of Medicine, Seoul, Korea, Republic of

FBXW7 is an E3 ubiquitin ligase and known as a tumor suppressor gene. Considering the high mutation frequency of FBXW7 observed in colon cancer mutation profiling, it is important to identify the interactive partners of FBXW7 for developing effective colon cancer treatments. Many reports have demonstrated diverse changes in cancer development and progression under FBXW7 mutant conditions at transcriptomic and proteomics levels. However, it is unclear how post-transcriptional modifications, especially RNA modifications, are affected under FBXW7 mutant conditions. To find the candidate targets of FBXW7 in protein level, we performed LC-MS analysis under conditions of FBXW7 functional loss. We analyzed gene ontology biological pathway (GOBP) enrichment analysis with up-regulated genes and found gene terms associated with RNA metabolism (such as mRNA metabolic process, positive regulation of 3'-UTR-mediated mRNA stabilization and mRNA transport). As the RNA metabolism is influenced by various types of RNA modifications, we focused on N^6-methyladenosine (m^{6}A) RNA methylation, a prevalent and significant post-transcriptional modification that plays a crucial role in regulating various RNA functions in eukaryotic messenger RNAs. To validate the LC-MS analysis, we profiled m^{6}A regulators (writer, eraser and reader groups) in protein and mRNA level in FBXW7 knock-out and transient knock-down cells. The protein level of IGF2BP1, which belongs to m^{6}A reader proteins, was up-regulated in both FBXW7 knock-out and knock-down conditions, but not in mRNA level. Additionally, the expression of IGF2BP1 increased compared to normal tissue, and expression of IGF2BP1 was associated with decreased survival rates, suggesting that IGF2BP1 plays an oncogenic role, as indicated by the TCGA colon cancer database. Our study suggests that IGF2BP1 is a crucial target in FBXW7 mutant colon cancer.

#5570 Characterizing a CRISPR/Cas9-mediated deletion in the extracellular domain of HER4 in a model of cutaneous melanoma.

Marta Valenti[1], Shubhangi Kandwal[2], Yonglun Luo[3], Georg F. Bischof[4], Darren Fayne[5], John Crown[6], **Denis M. Collins**[1]

[1]Cancer Biotherapeutics Research Group, School of Biotechnology, Dublin City University, Dublin, Ireland,[2]Molecular Design Group, School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland,[3]Department of Biomedicine, Aarhus University, Aarhus, Denmark,[4]Puma Biotechnology, Inc., Los Angeles, CA,[5]Molecular Design Group, School of Chemical Sciences, Dublin City University, Dublin, Ireland,[6]Dept of Medical Oncology, St. Vincent's University Hospital, Dublin, Ireland

Introduction: HER4, a tyrosine kinase receptor belonging to the Human Epidermal Growth Factor/HER/ErbB-family, has a complex biology (four isoforms (JMa-CYT1/2 and JMb-CYT1/2) and tissue specific oncogenic or tumor suppressor roles). Cutaneous melanoma (CM) showed the highest HER4 genomic alteration frequency (17.34%) across 33 cancer types (cBioPortal). To investigate the potential role of HER4 in CM, the SKMEL24 CM cell line was CRISPR/Cas9-edited to contain HER4 with a 30bp deletion (DEL) in the extracellular domain. The HER4 30bp DEL was modelled *in silico* and the SKMEL24 HER4 30bp DEL cell line assessed for HER-family protein expression levels, HER4 isoform RNA levels, and HER4 receptor activation upon neuregulin-1 (NRG-1) stimulation. Moreover, cellular morphology, cellular proliferation and response to the Tyrosine Kinase Inhibitor (TKI) neratinib was assessed.

Material and Methods: All studies compared the wild-type (WT) and the CRISPR/Cas9-edited HER4 30bp DEL SKMEL24 cell line. For *in silico* HER4 3D crystal structure modelling, input files for GROMACS 2020.7 molecular dynamics simulations were generated using CHARMM-GUI. The CHARMM36m force field was used for parameterization of protein chains. The GROMACS in-built tools were used to calculate heavy atom Root Mean Square Deviation (RMSD). HER-family protein levels (EGFR, HER2, HER3, HER4) and their phosphorylated forms (pEGFR Y1173, pHER2 Y1221, pHER3 Y1197, pHER4 Y984/Y1284) were investigated via Western blot. RNA was extracted from cells 24h after seeding using TRIzol, with qRT-PCR used to examine HER4 isoform expression. NRG-1 stimulation was performed in complete growth medium with HER4 receptor activation determined by Western blot. Cellular morphology and proliferation were monitored using the IncuCyte Live Cell Analysis Instrument (Sartorius). Acid phosphatase proliferation assays were used to assess the anti-proliferative effects of neratinib.

Results: *In silico* modelling of the HER4 30bp DEL showed reduced HER4 receptor stability compared to WT (RMSD = 6.465 Å and 6.631 Å, respectively). No changes in expression or activation of EGFR, HER2, or HER3 were detected in the SKMEL24 HER4 30bp DEL cell line. Full-length (180kDa) HER4 expression was decreased and cleaved HER4 expression (80kDa) and activation (pHER4 Y984) were increased in the HER4 30bp DEL cell line. NRG-1 treatment did not activate HER4 but activated EGFR, HER2 and HER3 in the HER4 30bp DEL cell line. The HER4 30bp DEL cell line also displayed a more stellate phenotype, reduced proliferation over 3 days in culture, and an increased sensitivity to neratinib (IC_{50} = 0.17 ± 0.04 µM) compared to the WT (IC_{50} = 0.26 ± 0.04 µM).

Conclusion: These findings suggest HER4 may play a role in cellular morphology, proliferation and response to treatment. Further studies are needed to clarify the specific role of HER4 in CM.

#5571 Identifying and targeting novel kinase regulators for cancer metastasis.

Wenliang Li

University of Texas Health Science Center At Houston, Houston, TX

Background: Metastasis is responsible for >90% of cancer death. However, metastasis is still poorly understood and the current approaches to prevent or treat human metastatic cancers are largely unsuccessful.
Methods and Results: Through RNAi and cDNA functional screening, genomics analysis, and functional validations, we have identified several critical but previously unknown/understudied kinase regulators for metastasis. As an example, shRNA screening revealed that GPCR-kinase 3 (GRK3) is essential preferentially for highly metastatic cancer cells as compared to lowly metastatic cancer cells. GRK3 is significantly overexpressed in metastatic prostate tumors from patients, especially in the aggressive variant of prostate cancers, so called treatment-related neuroendocrine prostate cancer (NEPC). We further found that GRK3 is a key missing link between two prominent phenotypes of NEPC, i.e. angiogenesis and neuroendocrine feature. Mechanistically, GRK3 phosphorylates histone deacetylase 2 (HDAC2) at S394 and enhances HDAC2's epigenetic activity. Through compound library screening, we have identified several compounds that block the kinase activity of GRK3 much more potently than that of GRK2, the closest-related kinase to GRK3. Of note, our GRK3 inhibitors could significantly inhibit NEPC cell growth in culture and in mouse xenografts. Another group of potential metastasis regulators we identified are new EMT-regulating kinases from our kinome cDNA screening for epithelial-mesenchymal transition (EMT). For example, mixed lineage kinase ZAK positively regulates EMT phenotypes and migration of cancer cells in culture, as well as metastasis in mice. ZAK overexpression correlates with poor prognosis in cancer patients. We are carrying out in silicon docking analysis and chemical genomics to identify potent and specific inhibitors for ZAK as research tools and drug candidates for metastatic cancers.
Conclusions: Our robust and unbiased functional screening has identified several novel kinase regulators and potential drug targets for cancer metastasis. For example, GRK3 has emerged as a critical kinase for metastatic prostate cancer cells and a key regulator of aggressive neuroendocrine prostate cancer phenotypes, while ZAK has been identified as a potent new regulator of epithelial-to-mesenchymal transition (EMT).

#5572 The transcription factor hepatic leukemia factor (HLF): a potential therapeutic target in triple negative breast cancer.

Ibrahim Bouakka, Marine Sirerol, Aymeric Silvin, Alicia Tran-Dien, Anna Rocca, Fabrice Andre

INSERM U981 (Gustave Roussy), Paris, France

Pivotal role of immune response in the evolution of triple negative breast cancer has been largely reported in the last decade. Our previous study highlighted the role of HLF gene as part of a four gene signature and was found to be negatively correlated with tumoral immune cells infiltration. We investigated more precisely its implication in the immune response and TNBC tumor development. Bioinformatics analysis of publicly available TNBC patient datasets revealed that in mesenchymal tumor subtypes expressing low levels of HLF upregulation of genes associated with neutrophil attraction and activation pathways was observed in comparison with those expressing high HLF levels. The implication of neutrophils in cancer is contentious as they can function either as active anti tumoral partners or as pro tumoral agents promoting immunosuppression and tumor growth. This ambiguity stems from their high plasticity which enables them to switch roles during tumor progression influenced by signals from tumoral microenvironment in particular those from surrounding tumor cells. In this study we employed CRISPR Cas9 technology to inactivate the HLF gene in TNBC cell lines enabling us to elucidate the downstream effects on the chemokine and protease inhibitor landscape that governs neutrophil infiltration and function within the microenvironment of TNBCs especially in mesenchymal subtypes. In vivo experiments using immunodeficient mouse models revealed that HLF downregulation in TNBC mesenchymal subtypes correlates with increased neutrophil infiltration into the tumor leading to inhibit tumor growth. Altogether our results suggest that a reduction of HLF expression can lead in the early steps of tumor development to the awakening of the anti tumoral function of neutrophils to enforce the necessary immunological tone to tumor elimination. Thus HLF emerges as a potential therapeutic target in order to favor infiltration and function of anti tumoral neutrophils to restore an efficient immune response in the aggressive mesenchymal TNBC subtype.

#5573 Validation of Aspyre Clinical Test for Lung at low input levels: Simplified genomic profiling for NSCLC with minimal tissue requirements.

Christina Xyrafaki[1], Katherine Knudsen[1], Magdalena Stolarek-Januszkiewicz[2], Rebecca Palmer[2], Sam Abujudeh[2], Ryan Evans[1], Eleanor Gray[2], Amanda Green[1], Robert Osborne[2], Barnaby Balmforth[2], Wendy Levin[1], Shari Brown[1]

[1]Biofidelity Inc, Morrisville, NC,[2]Biofidelity Ltd, Cambridge, United Kingdom

Although many assays exist for detecting actionable variants in NSCLC, few of them are suitable for use in low quantity or quality specimens, which significantly contribute to biomarker testing failures. Aspyre Clinical Test for Lung (Tissue) is a robust simplified genomic profiling panel that tests for 114 biomarkers in NSCLC across 11 genes (*ALK*, *BRAF*, *EGFR*, *ERBB2*, *KRAS*, *MET*, *RET*, *ROS1*, *NTRK1/2/3*) and was able to rescue 96% of samples that failed NGS-based testing. Aspyre Clinical Test for Lung (Tissue) was initially validated for 20ng DNA and 6ng RNA input from FFPE lung tissue samples, though it was predicted to have robust performance at even lower inputs. In this study we tested the impact of using low input levels down to 5 ng DNA and 1.5 ng RNA on the sensitivity (LoD95), specificity, analytical accuracy and analytical precision of Aspyre Clinical Test for Lung (Tissue). The study included FFPE lung tissue samples from patients with NSCLC (> 10% tumor content), variant-negative FFPE tissue from tonsil tissue, and contrived samples with controlled variant allele fractions of mutant oligonucleotides in an FFPE-based background.The LoD95 for eleven different single nucleotide variants or insertions/deletions from the Aspyre Clinical Test for Lung panel were established at ≤ 3% VAF, and one SNV at 7.5% VAF. The sensitivity for seven gene fusions, which are detected by Aspyre through RNA, was ≤ 100 copies, and for *MET* exon 14 skipping at ≤ 200 copies. The specificity was 100% with no false positive results for any healthy tonsil tissue samples (30 samples in 60 replicates). The analytical accuracy study found no discordant variant calls between Aspyre and 1) 19 contrived samples at 2x LoD95 or 2) 29 NSCLC patient samples compared to both an orthogonal target-enrichment NGS and Aspyre at higher inputs. Results were replicable and precise across operators, reagent lots, runs, and real-time PCR instruments.This low-input study demonstrates the opportunity to deploy Aspyre Clinical Test for Lung (Tissue) for slide samples with few cells, as well as small biopsy specimens, ensuring reliable genomic profiling even in limited or challenging samples. Combined with a high success rate and a proven 2-day turnaround time, this expands access to precision medicine for NSCLC patients.

#5574 Exploring the therapeutic potential of USP14 in cervical cancer: mechanisms of oncogenesis and metabolic regulation.

Ravi Chauhan[1], Lakshay Malhotra[2], Ashna Gupta[1], Gunjan Dagar[1], Harshul Kapoor[1], Sandeep R. Mathur[3], Ethayathulla Abdul Samath[4], Mayank Singh[1]

[1]Department of Medical Oncology, All India Institute of Medical Sciences (AIIMS), New Delhi, India,[2]Department of Biochemistry, Sri Venkateswara College, University of Delhi, New Delhi, India,[3]Department of Pathology, All India Institute of Medical Sciences (AIIMS), New Delhi, India,[4]Department of Biophysics, All India Institute of Medical Sciences (AIIMS), New Delhi, India

Cervical cancer is the most common cancer among women worldwide, predominantly caused by human papillomavirus (HPV) subtypes, particularly HPV 16 and 18. Although it is largely preventable through vaccination and early detection, cervical cancer remains a significant health issue, particularly in low- and middle-income countries, mainly due to acquired chemoresistance. This study explores the therapeutic potential of deubiquitinating enzymes (DUBs), with a focus on ubiquitin-specific proteases (USPs), in combating oncogenesis in cervical cancer. Using the TCGA database, we assessed the prognostic relevance of USP14, revealing that elevated levels of this enzyme correlate with poorer overall and disease-free survival among cervical cancer patients. In-silico analysis of publicly available gene expression datasets also indicates a significant upregulation of USP14 in cervical cancer tumor tissues compared to their paired normal tissues. USP14 expression was analyzed at both the RNA and protein levels across various cervical cancer cell lines, followed by assays for proliferation, metastasis, and invasion to assess the impact of USP14 depletion. Elevated USP14 expression was observed in the cervical cancer cell lines, and inhibiting USP14 resulted in significant reductions in cell proliferation, metastasis, and invasion. A novel USP14 inhibitor, IU-1, produced similar results. Proteomic analysis indicated that the lactate transporter MCT-4, a crucial metabolic regulator, was dysregulated following USP14 alteration. Immunoprecipitation studies confirmed stable interactions between USP14 and MCT-4. Cycloheximide and Deubiquitination assays further demonstrated that USP14 modulates MCT-4 stability via deubiquitination at specific sites. Additionally, docking studies and molecular dynamics simulations illustrated the interaction between USP14 and MCT-4. Furthermore, the lactate shuttle was evaluated following USP14 inhibition, demonstrating the effect of MCT-4 inhibition on lactate production in cervical cancer cells. Immunohistochemical analysis of retrospective tissue samples assessed the expression of USP14 and MCT-4 and their correlation with patient prognosis. Collectively, our findings highlight the vital role of USP14 in the oncogenic and metabolic processes of cervical cancer, suggesting their potential as therapeutic targets. This research enhances the understanding of the molecular mechanisms driving cervical cancer progression and underscores the need for targeted therapeutic strategies.

#5575 Identification of Novel *RBBP6* transcripts: modulation by *Drimia calcarata* plant extracts.

Zukile Mbita, Kagiso Laka

University of Limpopo, Polokwane, South Africa

Cancer, an assortment of maladies that are epitomized by deregulated cell proliferation, remains a thorn throughout the world. The soaring cancer cases can be attributed to deregulation of cancer-related genes and lack of effective therapeutics. Thus, this work was aimed at understanding the anticancer mechanisms associated with *Drimia calcarata* (*D. calcarata*) plant extracts, targeting cancer-related genes; particularly, *Retinoblastoma binding protein 6* (*RBBP6*) gene. *D. calcarata* phytochemicals were extracted using both water and methanol, and characterized using Liquid Chromatography Mass Spectrometry (LC-MS). The resultant extracts were used to treat various lung cancer cell lines; namely, H1437, H1573 and A549 cells. The inhibitory effects and half maximal inhibitory concentration (IC_{50}) values of *D. calcarata* extracts were established using the (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) and Muse® Count and Viability assays. Using the IC_{50} values, induction of cellular apoptosis was evaluated using both fluorescence microscopy, and the Muse® Cell Analyzer, while gene expression analysis was done using Polymerase Chain Reaction (PCR). The *D. calcarata* extracts, water and methanol, were found to contain a number of compounds with anticancer activities, which include Psoralene ($C_{11}H_6O_3$), Limonin-17-beta-D-glucoside ($C_{32}H_{42}O_{14}$), Oleuropein ($C_{25}H_{31}O_{13}$), Isorhamnetin derivative ($C_{16}H_{12}O_7$), and various uncharacterized compounds. The anticancer cancer activities were demonstrated to involve apoptosis induction, which was measured by both fluorescence microscopy and flow cytometry. Additionally, and most importantly, PCR showed that the *D. calcarata* extracts modulated the expression of novel *RBBP6* splice variants. Intriguingly, the *D. calcarata* plant extracts differentially regulated the expression of novel *RBBP6* transcripts in different lung cancer cells. *D. calcarata* extracts down-regulated *RBBP6* transcripts 1 and 2; however, subjecting the A549 cells to stress, *D. calcarata* extracts and curcumin, resulted in the expression of a novel transcript that has not been identified previous. In H1437 and H1573 cells, this novel transcript is down-regulated by the *D. calcarata* plant extracts. In H1437, the *RBBP6* transcripts 1 and 2 were up-regulated by the *D. calcarata* plant extracts. This suggests that *RBBP6* gene can be targeted for development of novel biomarkers, and *D. calcarata* extracts should be further explored for novel therapeutic targets.

#5576 Identification of inter-organ targets to mitigate cachexia while enhancing tumor suppression by anti-PD-L1.

Se-Young Park[1], Jiwoo Kim[1], Kyuwon Son[2], Haeseung Lee[2], Na-Young Song[1]

[1]Yonsei University College of Dentistry, Seoul, Korea, Republic of,[2]Pusan National University, Busan, Korea, Republic of

Immune checkpoint inhibitors (ICIs), such as anti-PD-L1, have revolutionized cancer therapy but are frequently associated with immune-related adverse events (irAEs). Here, we show a significant correlation between ICI treatment and weight loss in cancer patients. Similarly, anti-PD-L1 therapy exacerbates muscle wasting in a cancer cachexia (CAC) mouse model, despite its anti-tumor effects, suggesting that muscle atrophy may represent a potential irAE. Single-cell RNA sequencing reveals that cytotoxic T-like cells accumulate in the skeletal muscle of CAC mice, directly contributing to muscle wasting. While their depletion prevents cachexia, it compromises the anti-tumor efficacy of anti-PD-L1. To address this discrepancy, we identify cathepsin L (CTSL) as an inter-organ target capable of inhibiting both cancer and cachexia, particularly muscle wasting driven by cytotoxic T cells, through unbiased integrative transcriptome analysis. Notably, CTSL inhibition not only reduces muscle wasting but also enhances the anti-tumor efficacy of anti-PD-L1. These findings highlight CTSL as a promising therapeutic target for cancer cachexia, offering a novel strategy to prevent muscle-specific irAEs while enhancing the benefits of ICIs.

#5578 Role of differentiation driver gene HOXD4 in anaplastic thyroid cancer.

Kaci Kopec[1], Nicole R. DeSouza[1], David Garber[2], Steven A. Hemmerdinger[2], Augustine Moscatello[1], Humayun K. Islam[1], Jan Geliebter[1], Raj K. Tiwari[1]

[1]New York Medical College, Valhalla, NY,[2]Westchester Medical Center, Valhalla, NY

Anaplastic thyroid cancer (ATC) is a rare and extremely aggressive subset of thyroid cancer, noted for its refractory nature and poor prognosis. Conventional cancer treatments are ineffective in treating ATC while targeted therapies are of limited use due to a lack of targetable genetic lesions in this cancer type thus rendering a five-year survival rate of 4%. The low mutational burden of ATC prompted analysis of differential gene expression patterns using NCBI Gene Expression Omnibus publicly available transcriptomic datasets (GSE33630, GSE85457). Bioinformatic analysis of ATC compared to normal thyroid cancer tissue was carried out using GEO2R software. Strikingly, differentiation driver gene Homeobox D4 (HOXD4) was significantly upregulated in ATC patient tissue samples by 3-fold and 12-fold respectively. Kaplan-Meier analysis indicates a significant decrease in survival in patients with high HOXD4 levels (HR: 3.08, logrank: 0.019). Unlike most cancers, ATC lacks differentiation therefore losing its thyroid-like morphology and function. Thus, the marked upregulation of differentiation gene HOXD4 warranted further exploration in vitro. HOXD4 expression was upregulated in ATC cell line T238 by 23-fold compared to immortalized “normal” thyroid epithelial cell line Nthy-ori-3-1. CRISPRi knockdown of HOXD4 in T238 and SW1736, another ATC cell line, was needed for functional analysis rendering HOXD4 knockdown cell lines T238-HOX and SW1736-HOX as well as non-specific oligonucleotides control cell line. Cell proliferation was reduced in T238-HOX by 36%, 29%, 28%, and 38% and SW1736-HOX by 51%, 56%, 59%, and 52% at 24, 48, 72, and 96 hours respectively. Migratory capacity measured via scratch wound assay was significantly reduced in T238-HOX by 31% and 13% and SW1736-HOX at 45% and 22% at 16 and 22 hours respectively. Migration through Boyden chambers decreased by 72% in T238-HOX and 62% in SW1736-HOX and invasion was reduced by 40% and 51% respectively. We are currently evaluating the impact of HOXD4 knockdown on viability, clonogenicity, and senescence. The data reveal the novelty of HOXD4 as a putative carcinogenic driver of ATC and its function as both a diagnostic and prognostic biomarker, with the potential to be used as a therapeutic target. We are currently investigating various differentiating agents and their efficacy in a possible clinical application with or without other therapeutic agents.

#5579 Identification of MTA1 as a new molecular marker in urothelial carcinoma.

G. Campanelli, N. Waxner, N. Parkhomovsky, R. Vanderstichel, C. Yang, A. S. Levenson;
Long Island University, Brookville, NY

Metastasis-associated protein 1 (MTA1), a master transcriptional co-regulator and chromatin modifier, is strongly associated with many types of clinically aggressive cancers. Loss of MTA1 reduces aggressiveness and metastatic potential in cancer cells. A publicly available human dataset predicts that MTA1 might work as a prognostic marker, however, the exact role of MTA1 in the pathobiology of bladder cancer is unknown. We sought to evaluate the expression of MTA1 and associated epithelial-to-mesenchymal (EMT) events using canine urothelial carcinoma (UC) clinical samples as well as cell lines. We found that MTA1 and cyclooxygenase 2 (COX2) are overexpressed in canine UC samples compared to normal bladder samples, whereas E-cadherin (E-cad) levels are higher in normal bladder. The results of samples demonstrated that MTA1 expression correlated with aggressive clinicopathological features such as high tumor-grade, muscular/vascular invasion, and metastasis. The MTA1 expression differed in tumors depending on their localization, with the highest being in the urethra adjoining the prostate. Unexpectedly, higher E-cad levels were detected in metastatic tumor cells compared to primary tumor cells. Further, we analyzed MTA1 and EMT-associated markers in four invasive canine UC cell lines. Using two cell lines, AxA and SH, and their corresponding MTA1 knockdown (shMTA1) clones, we validated the direct link between MTA1 and these markers on mRNA and protein levels. Overall, these findings suggest that MTA1 may represent a key upstream effector tightly associated with COX2 and EMT-mediated events in canine UC. Accordingly, MTA1 may be considered a feasible interceptive and therapeutic target for canine UC treatment.

#5580 Determining the effects of serine-arginine protein kinases inhibition on splicing factors and long non-coding ribonucleic acids regulation in cervical cancer cells.

Lydia Mphahlele, Zodwa Dlamini, **Rahaba Makgotso Marima**

University of Pretoria, Pretoria, South Africa

Cervical cancer (CCa) remains a significant global health challenge, with high morbidity and mortality rates, particularly in developing countries. The pathogenesis of CCa is largely driven by persistent infection with high-risk strains of *Human Papillomavirus* (HPV). Serine-arginine protein kinases are a family of cell cycle-regulated kinases that phosphorylate the serine/arginine (SR) domain-containing proteins in the nuclear patches to intervene with pre-mRNA splicing, signals splicing controls in response to extracellular stimuli and contributes to tumorigenesis. It has been reported in recent studies that the dysregulation of splicing factors and lncRNAs affects the progression of CCa. The involvement of SRPKs with lncRNAs regulation remains unelucidated in CCa. This study aimed to investigate the association of lncRNAs and SRPKs inhibition by SRPIN340 in CCa cells SiHa and C33A. Alamar blue cell viability assay, 84 lncRNA gene panel, bioinformatics analysis and RT-qPCR were used to achieve the aim of the study. SRPIN340 showed no cytotoxicity to CCa. Differential lncRNA gene expression, favoring CCa progressing was observed and these results were verified by increased expressions of MALAT1 and TUG1. The association between splicing factors and lncRNAs is complex, as influence of these 2 parities may be bi-directional. Understating this relationship may unlock diagnostic, prognostic and therapeutic targets in CCa.

#5581 Melatonin and 17β-estradiol treatment modulating TIMP's family gene expression profile in IDCC.

Kelly P. Ferreira, Bruna C. Almeida, **Katia C. Carvalho**

University of Sao Paulo, Faculty of Medicine, Sao Paulo, Brazil

Introduction: *Tissue Inhibitor of Metalloproteinases (TIMPs)* are genes that encodes four (TIMP1-4) proteins that act as endogenous protease inhibitors. They regulate directly the matrix metalloproteinases (MMPs) known to be associated with growth and invasiveness of sundry cancer types, including breast cancer cells. Several evidences have shown that melatonin can present both anti-proliferative and invasiveness role in cancer, while estrogen seems to have the opposite function. Thus, our goal was to evaluate the expression of TIMPs' family compounds in invasive ductal carcinoma cells (IDCC) of breast cancer after treatment with 17β-estradiol (malignancy inducer), melatonin (malignancy inhibitor), isolated and associated with each other.
Methods: IDCC [T47D (ER+/PR+), MCF7 (ER+), and MDA-MB231 (triple-negative)] were cultured as recommended by ATCC. All three cell lines were plated ($5X10^5$ in 6 wells plate) and treated with 17β-estradiol (E2 - 100 nM), Metatonin (Mel - 3000 μM) or both (Mel + E2). After treatment the RNA was extracted using *mirVana miRNA Isolation Kit* (Ambion, CA, USA) and then, purity and concentration of total RNA were accessed. The Reverse transcription synthesis was performed using High-Capacity cDNA Reverse Transcription Kit and RT-PCR was accessed for *TIMP 1 - 4* using TaqMan assay system. The *beta2-microglobulin (B2M)* was established as a housekeeping gene and the relative quantification was determined by 2-DDCT method.
Results: All members of the TIMPs' family were expressed in IDCC tested with significant differences in their expression when compared to control cells. Mel treatment led to *TIMP1, -3* and *-4* mRNA upregulation, while E2 upregulated *TIMP1* but downregulated *TIMP2* in T47D cells. Otherwise, *TIMP1* and *-3* were downregulated and *-2* and *-4* were upregulated in MCF7 cells treated with Mel, while an increase of *TIMP1, -2* and *-4* were observed for E2. In comparison with control group, E2 treatment of MDA-MB231 cells led to upregulation of *TIMPs -1, -3* and *-4*, and Mel upregulated all, except *TIMP4* that was downregulated.
Conclusion: Melatonin and 17β-estradiol treatments seems to modulate negative or positively the gene expression profile of all the assessed members of TIMPs' family in IDCC cells.

#5582 Interrogating the K-ras/p53 transcriptional readout for synthetic lethality applications.

Samuel L. Schach, Farah Mazahreh, A. Mazin Safar

University of Arkansas for Medical Sciences, Little Rock, AR

Some Oncogenes (Onc) are physiologically indispensable or have proven difficult to inhibit (Myc and Ras, respectively). Onc addiction, reflecting the need for specific stress adaptations (SA) to accommodate constant onc signaling, provide a novel approach to disrupt the SA producing synthetic lethality (SL). Broad's dep map CRISPR screens (CS) suggest SA is NOT monogenic. Combinatorial consideration is unavoidable and can only be fruitful if aided by biologic insights. Genetically engineered mouse models confirmed that p53 is sufficient to facilitate full oncogenic abilities of Ras. p53 transcriptional (Tr) network is a mere 110 genes and includes many repressed targets (T) but some that retain Tr activity despite the p53 DNA binding domain mutations (mu). Retained genes (g) are being considered here as putative therapeutic Ts. Identifying indispensable g in Ras onc SA is needed for CRISPR multiplexed screen. We focused on pancreatic adenocarcinoma (PA) due to high prevalence of Ras/p53 and the poor available treatment options. We hypothesize that specific set of p53 retained Tr Ts provide the SA and this may be phenocopied in few wild type (WT) p53 PA. This would guide CS for therapeutic applications. Methods: We probed TCGA and the Broad public databases for expression of the canonical p53 Tr T in Ras & p53 double mutant PA. We reasoned that MDM2 represents the most rational internal control for reduced Tr in basal, unstimulated conditions; all g with higher Tr are scored as retained; the remaining as lost Tr. WT PA were analyzed to identify phenocopy providing additional strength to the hit. The table summarizes the findings at the different levels of scrutiny. Conclusion: Our work represents a rational biologic approach to select a rational set of SL g in the Ras/p53 interdependence circuitry. Such Ts need to be examined by CS to validate their cellular importance. Pathway analysis and considering the possible double negative inhibition of repressed Ts are additional approaches to this analysis.

Tier	Stringency Level	Gene
1	All cases in TCGA and Broad	ANXA4
2	All Cases with >90% concordance; TCGA and Broad	EPS8L2; EPHA2; CDKN1A PTP4A1
	All cases; TCGA 0nly	ENC1; LAPTM5MDM2
	All BROAD only	TRIAP1PLK2BAX
3	>90% concordance; TCGA	TNFRSF10B SUSD6
	All Broad. All -4 cases TCGA	TM7SF3

#5583 Small molecule mediated restoration of PAX3 inhibits malignant features in neuroblastoma cells.

Abinayaselvi Murugan[1], Sowndharyalatha Balasubramanian[1], Narenkumar Muralidharan[1], Ann Mary Alappat Sanjive[1], Prabhuraj Andiperumal Raj[2], **Mathivanan Jothi**[1]

[1]Human Genetics, National Institute of Mental Health and Neurosciences (NIMHANS), Bengaluru, India,[2]Neurosurgery, National Institute of Mental Health and Neurosciences (NIMHANS), Bengaluru, India

Neuroblastoma is the most common extracranial childhood tumor diagnosed in the first year of life. A complete molecular understanding of neuroblastoma is necessary to identify novel therapeutic targets for their management, particularly for high-risk groups. Many transcription factors play an essential role during development, and their expression is restricted to specific cells/tissues after development is completed. *PAX3* belongs to the paired box family of transcription factors involved in various cellular processes like cellular proliferation, migration, lineage specificity, and differentiation. Reexpression of *PAX3* is reported in various childhood tumors, including neuroblastoma; however, its role in tumor development is contradicting. Here, we investigated the contribution of *PAX3* in neuroblastoma in a panel of neuroblastoma cell lines and identified a PAX3-targeted small molecule activator that can alter tumorigenic phenotypes. Specifically, we have analyzed the *PAX3* mRNA, protein, subcellular localization, and its isoform expression in various neuroblastoma cell lines and correlated the results with their tumorigenic potential. Our results showed that most of the PAX3 expressed in these cells are PAX3a/b isoforms that lack PAX3's transactivation domain. Further, we have observed the depletion of *PAX3a/b* isoforms could not alter the malignant properties of neuroblastoma cells. Interestingly, the ectopic *PAX3* expression in the absence of *PAX3a/b* isoform in these cells inhibited malignant phenotypes including growth, colony formation and migration potentials. Furthermore, we have identified potential small molecule *PAX3* activators from our previous small molecule screening. *PAX3* activators effectively inhibited in vitro malignant properties of neuroblastoma cell lines. Our results revealed that tumor inhibitory properties of transcriptionally active *PAX3* and its reactivation using small molecules have great potential in inhibiting neuroblastoma development.

#5584 The critical role of CLIC1 in mediating metformin's antitumoral effects on glioblastoma.

Guido Rey[1], Francesca Cianci[1], Ivan Verduci[1], Sara Torabi[1], Alessandro Fantin[1], Federica Barbieri[2], Tullio Florio[2], Michele Mazzanti[1]

[1]University of Milan, Milano, Italy,[2]University of Genova, Genova, Italy

Glioblastoma (GBM) is the most common and lethal brain tumor and there is a general agreement that GBM stem cells (GSCs) are primarily responsible for its high invasiveness and recurrence. It has been demonstrated that several membrane proteins are upregulated in GSCs and support tumor relapse. However, most of these membrane resident proteins are also involved in essential physiological processes, limiting their use as selective anticancer targets. The ideal target will be a membrane protein only present on the tumor surface. CLIC1 is a metamorphic protein that can transition between cytoplasmic and transmembrane forms (tmCLIC1), with the latter associated with chloride conductance. tmCLIC1 is enriched in the plasma membrane of GSCs, while it is largely absent in healthy cells. Previous experiments demonstrated that impairment of tmCLIC1 downregulated tumor growth and invasion. Thus, this protein could represent a promising antitumoral target. It has been demonstrated that tmCLIC1 is sensitive to metformin, a widely used antidiabetic drug. Previous data showed contradictory results about the use of metformin against solid tumor growth. We have recently demonstrated that in human primary GSCs, tmCLIC1 is the principal target of metformin. There are two main problems to adopt metformin as a chemotherapic molecules on glioblastoma patients: the high drug concentration needed to be effective and the time at which tumor cells are exposed to the drug. The final aim of the present study is to improve metformin-CLIC1 interaction, trying to lower metformin effective concentration. The first step is to uncover the molecular mechanism of metformin binding to tmCLIC1. Our results show how mutating a specific single arginine of tmCLIC1 (R29) leads to the loss of metformin's antitumoral properties. Nonetheless, since arginine and metformin carry the same charge, we tried to investigate more deeply this interaction through a molecular docking simulation. We found that this residue coordinates different negatively charged amino acids, that might create the correct environment in which metformin could bind the protein. The electrophysiological test confirmed that mutating only one of these negatively charged residues partially impairs the effect of metformin on the tmCLIC1 current. Finally, we demonstrated that CLIC1 is essential in metformin antitumoral mechanism against GBM also in zebrafish embryos and in murine model. The project's long-term goal is to develop a personalized medicine against GBM based on the use of metformin.

#5585 DNA methylation and transcription factors regulate overexpression of PVT1 in prostate cancer.

Chinedum C. Udekwu, Colin Finnegan, Murtaza Barkarar, Oluwabusola Ajagbe, Rachel E. Bonacci, Olorunseun O. Ogunwobi

Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI

Population-level genomic analysis of the PVT1 gene, in the 1000 Genomes Project, revealed genetic differentiation between African and non-African populations at PVT1 exons 4A and 4B. Also, PVT1 exons 4A and 4B showed increased expression in prostate cancer (PCa) tissues relative to normal prostate tissues and benign prostatic hyperplasia tissues in men of African ancestry (moAA). We demonstrated that overexpression of PVT1 exons 4A, 4B and 9 caused increased migration and proliferation of prostate epithelial cells. Further, overexpression of PVT1 exon 9 transformed non-tumorigenic prostate epithelial cells into tumorigenic cells that form tumors with neuroendocrine phenotype when implanted in mice. Gene expression is modulated by DNA methylation (DMT), so we investigated the role of DMT as a mechanism of regulating PVT1 expression. Using qPCR analysis, we tested this hypothesis by treating five prostate epithelial cell lines (RWPE-1, WPE1-NA22, DU145, PC-3 and MDA-PCa-2b) with 5-azacytidine (a global DMT inhibitor) and found significantly (p-value <0.05) increased expression of PVT1 exons 1A, 4A, 4B and 9 in the PCa cell lines when compared to RWPE-1 and no treatment. We further identified 13 distinct promoter regions on the PVT1 gene annotated on Ensembl database and compared the strength of the PVT1 exon 9 - associated promoter with the canonical promoter using a luciferase reporter assay. We found that the canonical promoter was stronger in driving the expression of luciferase when compared to the PVT1 exon 9 - associated promoter and the ubiquitin C promoter, a well-established weak promoter. Further, EM-seq of human prostate tissues from men of African ancestry confirmed differential methylation peaks in tumor versus adjacent normal tissues within the PVT1 canonical and PVT1 exon 9 - associated promoter. Additionally, we investigated the role of MYC-PVT1 co-amplification regulation and PVT1 associated extra chromosomal DNAs (ecDNAs) using fluorescence in-situ hybridization. We found that MYC had no regulatory function on the expression of PVT1 exon 9 and there were no PVT1 associated ecDNAs in all the prostate epithelial cell lines used in this study. Lastly, we used bioinformatics to probe the PVT1 canonical and exon 9 - associated promoters for transcription factors. We found NKX2.5 and CDX1 to have the highest binding scores and significantly overexpressed in aggressive PCa. We will further characterize their function in the transcriptional regulation of PVT1. In conclusion, DMT regulates PVT1 exon 9 overexpression; the canonical PVT1 promoter is stronger than the PVT1 exon 9 - associated promoter, PCa in moAA is characterized by hypomethylation at PVT1, and NKX2.5 and CDX1 are putative transcriptional regulators of PVT1 expression in PCa. Understanding of these epigenetic regulatory mechanisms of PVT1 overexpression in PCa in moAA may lead to uncovering novel therapeutic targets in aggressive PCa.

#5586 *In vitro* investigation of the proliferative and metastatic role of CD13 in cancer.

April J. Baral, Gabriel C. Nwokolo, Sarah Mcgowan, Steven D. Shnyder, Robert A. Falconer

Institute of Cancer Therapeutics, University of Bradford, Bradford, United Kingdom

CD13 (Aminopeptidase N,APN) is a glycosylated ectopeptidase with diverse physiological functions including proliferation, differentiation, migration and angiogenesis[1]. CD13 is a marker for neoangiogenesis that is detected in tumor neovasculature, but not in the normal vasculature, contributing with tumor growth, invasion, and metastasis. *In vitro* studies have revealed association between CD13 various cancer types including human glioblastoma[2] and prostate[3] cancers. Thus, CD13 is an appealing target for the delivery of drugs in CD13-expressing tumors which can reduce tumor growth and progression. An example is the aminopeptidase inhibitor, Ubenimex (Bestatin), which has been approved in Japan as an adjuvant to chemotherapy for acute non-lymphocytic leukemia. However, the overlapping substrate specificity of the aminopeptidase family of proteins leads to the challenge of developing a specific CD13 inhibitor. Prostate cancer (PC) and neuroblastoma (NB) are both highly malignant tumors that can spread into the bone marrow, liver, lymph nodes and bones. The effect of CD13 inhibition by Ubenimex in PC cells has previously been investigated with results showing decrease of cell growth *in vitro and in vivo*, however, this was only shown in two PC cell lines, LNCaP and PC-3[3]. Hence, we are expanding this study into additional PC cell lines. Meanwhile, 70% of NB patients demonstrate a metastatic profile and there is currently no evidence yet of a link between NB to CD13. Given that CD13 is a regulator of tumor invasion, investigating the significance of CD13 in PC and NB can deepen our understating in its role in cell proliferation, migration, and metastasis. CD13 protein expression in PC and NB tumor xenografts and cell lines were investigated using western blot analysis, with differential expression identified between the cell lines. The importance of CD13 protein expression status will be reported; cell lines were treated with CD13 inhibitors including bestatin and actinonin, with evaluation of cell proliferation and migration using functional assays (MTT and scratch assays respectively). These data provide evidence towards the importance of CD13 in PC and NB, which will facilitate the development of a novel targeted therapy for CD13-expressing tumors that reduces the toxicity of traditional chemotherapy.

1.Lendeckel U, Karimi F, Al Abdulla R, Wolke C. The Role of the Ectopeptidase APN/CD13 in Cancer. Biomedicines. 2023;11(3).2.Zhang W, Blank A, Kremenetskaia I, Nitzsche A, Acker G, Vajkoczy P, et al. CD13 expression affects glioma patient survival and influences key functions of human glioblastoma cell lines in vitro. BMC Cancer. 2024;24(1):369.3.Wang X, Niu Z, Jia Y, Cui M, Han L, Zhang Y, et al. Ubenimex inhibits cell proliferation, migration and invasion by inhibiting the expression of APN and inducing autophagic cell death in prostate cancer cells. Oncol Rep. 2016;35(4):2121-30.

#5588 Efficacy of TGR-5 inhibition in cholangiocarcinoma using the novel small molecule inhibitor SBI-364.

Enis Ozmert, **Jennifer L. Tomlinson**, Danielle M. Carlson, Rory L. Smoot

Mayo Clinic Hospital-Rochester, Rochester, MN

Background: Cholangiocarcinoma (CCA) is a malignancy of the biliary epithelium with increasing incidence and poor survival. Despite the advances in targeted therapies, treatment success has been hindered by primary or acquired resistance. New therapeutic strategies are necessary to improve outcomes in this patient population. This study investigates the anti-tumor efficacy of TGR-5 inhibition in CCA using a novel small molecular inhibitor, SBI-364.
Methods: Cell viability was assessed using a CellTiter-Glo assay in four unique CCA cell lines treated with SBI-364 at concentrations ranging from 1 nM to 100 µM for 48 hours. Viability was expressed as a percentage relative to untreated controls. Cell migration was assessed using a scratch wound healing assay at 0, 12, and 24 hours following SBI-364 treatment. Migration was reported as the percentage of wound closure relative to the initial scratch width. Apoptosis was assessed using the Caspase-Glo® 3/7 Assay (Promega) after 12 hours of treatment with SBI-364. Caspase activity was normalized to untreated controls and expressed as relative luminescence units.
Results: SBI-364 reduced cell viability in a dose-dependent manner across all CCA cell lines with IC50 values ranging from 4.1-15.8 µM. Higher concentrations of SBI-364 induced increased cytotoxicity. In the scratch assay, untreated CCA cells achieved 100 % wound closure within 24 hours. Treatment with SBI-364 at 10 µM significantly inhibited cellular migration, with reduced wound closure compared to controls ($p < 0.05$). Apoptosis analysis revealed a significant increase in caspase-3/7 activity in three CCA cell lines following treatment with 10 µM SBI-364 for 12 hours ($p < 0.01$).
Conclusion: TGR-5 inhibition using novel small molecule inhibitor SBI-364 demonstrates potent anti-tumor effects in CCA by reducing viability, inhibiting migration, and promoting apoptosis. These findings support further investigation of SBI-364 as a promising therapeutic strategy in CCA.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Kinase and Phosphatase Inhibitors 3
Poster Session

#5591 Development of a BCR-ABL degrader overcoming resistance by BCR-ABL1 kinase domain mutations in chronic myeloid leukemia.

Ye Seul Lim, Na-rae Jeong, Sun-Mi Yoo, Han Wool Kim, So Hyuk Kim, Hyun-Hwi Kim, Yongdae Kwon, Heejung Moon, Hohyun Lee, Jungmin Ahn, **Jihye Lee**, Je Ho Ryu, Song Hee Lee

Ubix Therapeutics, Seoul, Korea, Republic of

Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell disorder characterized by the presence of the BCR::ABL1 fusion gene. Tyrosine kinase inhibitors (TKIs) have revolutionized the treatment of CML, yielding remarkable responses in patients. However, resistance mutations within the BCR-ABL1 kinase domain can arise, leading to treatment failure and disease progression. In this study, we report the identification of potent, orally efficacious BCR-ABL1 PROTACs. UBX-362 exhibits strong degradation activity against BCR-ABL1 proteins in CML cell lines. Consistently, it effectively suppresses cell viability in these cell lines, with low nanomolar IC50 values. Additionally, we evaluated the effects of single and combination therapies with TKIs (Asciminib or Ponatinib) using cell proliferation assays in K562 and Ba/F3 BCR-ABL1 mutant cell lines. Co-treatment of UBX-362 with Asciminib or Ponatinib resulted in a synergistic anti-proliferative effect in K562 cells. Notably, the combination of UBX-362 and Asciminib showed synergistic growth-inhibitory effects in Ba/F3 cell lines harboring BCR-ABL1 mutations. Oral administration of UBX-362 significantly reduced BCR-ABL1 protein levels in tumor tissue and robustly inhibited tumor growth in a CML CDX mouse model. These preclinical findings suggest the potential for the clinical development of a BCR-ABL1 degrader. Furthermore, UBX-362 demonstrates the ability to overcome resistance in CML patients with BCR-ABL1 mutations and shows promise as a candidate for combination therapy with other BCR-ABL1 inhibitors, providing new therapeutic possibilities for CML patients.

#5592 Modeling response prediction to a novel GCN2 kinase activator (NXP800) in patient-derived xenograft cholangiocarcinoma models using a multi-omics approach.

Danielle Marie Carlson[1], Hendrien Kuipers[1], Amro Abdelrahman[1], Erik Jessen[1], Joshua Roldan Kalill[2], Jennifer Tomlinson[1], Jack Sample[1], Akhilesh Pandey[1], Mark Truty[1], Rory Smoot[1]

[1]Mayo Clinic Hospital-Rochester, Rochester, MN,[2]Puerto Rico School of Medicine, San Juan, Puerto Rico

Introduction: Cholangiocarcinoma (CCA) is an aggressive biliary malignancy with poor survival outcomes and multiple mechanisms of resistance to targeted therapies. We previously validated NXP800, a novel GCN2 small-molecule activator, as an effective anti-cancer drug in six CCA cell lines. *In vitro* testing identified upregulation of phosphorylated eIF2a, as well as upregulation of ATF4, in CCA cells treated with of NXP800 compared to vehicle, suggesting that treated cells led to stimulation of stress-induced genes and subsequently, apoptosis. Furthermore, patient derived xenografts (PDX) models treated with NXP800 were found to have decreased tumor size, particularly in models containing *ARID1A, FGFR1*, and *ATM* mutations. Here, we aimed to predict response to NXP800 using sensitivity and resistance signatures from our profiled PDX models.

Methods: GCN2 activation in multiple previously profiled PDX models was evaluated. Whole Exome Sequencing (WES) was done on multiple PDX models to characterize the mutational signature and activity in CCA. Two additional CCA xenografts were expanded into the flank of NOD/SCID mice. Tumor bearing mice were treated with NXP800 (35 mg/kg) or vehicle via oral gavage for 28 days once tumors averaged 120 mm^3. Tumor volume and animal weight were collected twice weekly. Median Efficacy Index (MEI) scores were generated as drug response rates based on tumor growth. Predicted sensitivity and resistance kinase signatures to predict drug response were produced using multi-omics.

Results: Treatment with NXP800 was associated with a statistically significant decrease in tumor size in two additional PDX models, which included *ARID1A* and *BRAF* mutants. Preliminary multi-omics on the PDX models revealed that sensitivity mechanisms were upregulated most in EGFR signaling, while resistance signatures were focused around CSNK2A1 signaling.

Conclusion: Multi-omics analysis revealed a sensitivity mechanism structured around EGFR signaling, providing insights into which patients should be selected for treatment with NXP800. A Phase 1b clinical trial investigating the effects of NXP800 use in patients with advanced CCA is open and currently accepting patients.

#5593 The multi-kinase inhibitor tinengotinib as a novel therapy for advanced prostate cancer.

Brandon Hernandez[1], Jason Somarelli[1], Andrew Armstrong[1], Anaya Dutta[1], Bilal Ashraf[1], Xiaoyan Qiang[2], Yichang Zeng[2], Peng Peng[2], Jean Fan[2]

[1]Medicine, Duke University, Durham, NC,[2]TransThera Sciences, Nanjing, China

Purpose: Metastatic castration-resistant prostate cancer (mCRPC) is responsible for nearly 30,000 deaths each year in the US. While androgen receptor pathway inhibitors (ARPIs) significantly prolong the lives of men with mCRPC, the evolution of resistance to these agents is nearly universal. Among the mechanisms of resistance to ARPIs, studies have pinpointed activation of JAK/STAT and fibroblast growth factor receptor (FGFR) signaling as key mediators of ARPI resistance by mediating lineage plasticity and loss of AR dependence. To target these multiple resistance mechanisms, we interrogated the potential of tinengotinib, a spectrum-selective multi-kinase inhibitor for the treatment of ARPI-resistant mCRPC that targets Aurora Kinase A/B, JAK1/2, FGFRs and VEGFRs. Our study aims to characterize efficacy and resistance mechanisms of tinengotnib in prostate cancer cell lines and to develop novel combinatorial treatment strategies for mCRPC.

Methods: Baseline expression of Aurora A/B, STAT3, JAK1/2, VEGFR1/2/3, FGFR1/2/3, and CS1FR in a panel of prostate cancer cell lines (LNCaP, LNCaP95, LNCaP XER, 22RV1, PC3, and LASCPC-01) was assessed by qPCR and western blots. Single-agent and combination dose-response curves were performed with tinengotinib and standard-of-care agents using IncuCyte imaging and CellTiter Glo. Small molecule inhibition was used to assess the role of tinengotinib targets in driving cancer cell survival and growth.

Results: Tinengotinib potently inhibited growth of all cell lines, including enzalutamide-sensitive and -resistant AR signaling-dependent models (LNCaP, LNCaP95, LNCaP-XER), double negative models (PC-3) and neuroendocrine-like (LASPC-01) lineages, with IC50s ranging from low nanomolar to ~1 μM. Tinengotinib demonstrated additive effects with enzalutamide. Consistent expression of Aurora A and B, JAK2, and FGFR3 was observed across all cell lines while VEGFRs and FGFR1, 2, and 4 showed more variation in expression across lines. Interrogation of potential drivers will be presented.

Conclusion: Preclinical evaluation of tinengotinib shows efficacy across a range of prostate cancer phenotypes and combined effectively with standard agents. Gene expression across this panel of cell lines pinpoints Aurora A and B, JAK2, and FGFR3 as potential mechanisms of action for tinengotinib in prostate cancer. Future studies are aimed at understanding its mechanisms of action and determining its potential as a combination therapy for mCRPC.

#5594 Targeting ribosomal S6 kinase for the treatment of pediatric acute leukemia.

Kailen Mark, Jenny Youm, Ana San Jose Gonzalez, Kathleen M. Sakamoto

Stanford University School of Medicine, Stanford, CA

Pediatric acute leukemia accounts for about one third of all childhood cancers. Although the overall survival has improved to 60-70% for pediatric acute myeloid leukemia (AML) and up to 90% for pediatric acute lymphoblastic leukemia (ALL), standard treatments involve intensive chemotherapy and are associated with significant morbidity and mortality. There are few therapies available for relapsed/refractory pediatric acute leukemia. More targeted, less toxic therapies are needed to improve the outcomes for these patients. Ribosomal S6 kinase (RSK) is a serine-threonine kinase activated downstream of the Ras/MAPK signaling pathway and regulates cell proliferation, protein synthesis, and cell survival. RSK activates the transcription factor CREB, which has been found to mediate the proliferation and survival of myeloid cells. RSK is overexpressed and hyperactivated in pediatric AML and associated with a worse prognosis. Here, we evaluated two novel RSK inhibitors for treatment of pediatric AML and ALL. PMD-026 is a specific RSK inhibitor currently in a Phase II clinical trial for adults with metastatic breast cancer. We treated AML cells with a range of doses of PMD-026 and measured the viable cell number after 72 hours. The IC50 of PMD-026 was 298-560nM in Molm13, MV4;11, and Kasumi-1 cells and 1.02-2.24uM in two pediatric AML patient samples. Daunomycin, a standard chemotherapy agent used to treat AML, had synergistic effects when combined with PMD-026 in MV4;11 cells. To evaluate the effects of PMD-026 on normal hematopoietic stem and progenitor cells, we cultured healthy bone marrow mononuclear cells (10^4) with PMD-026 in methylcellulose containing SCF, GM-CSF, IL-3, IL-6, and EPO. Colony formation was not affected up to 10uM for a 17- to 30-fold therapeutic window. The multikinase inhibitor TAS0612 targets RSK, AKT, and S6K and is in Phase I clinical trials for adult solid tumors. In AML cell lines, the IC50 of TAS0612 was between 181-677nM. TAS0612 did not affect normal hematopoietic colony formation up to 1uM. Furthermore, when testing TAS0612 on ALL cell lines, we found that both B-ALL (REH, Nalm-6) and T-ALL cell lines (Loucy, Jurkat) were highly sensitive to TAS0612 with IC50 values of 56-190nM. Healthy T cell proliferation was not affected by TAS0612 up to 5.41uM for a 28- to 97-fold therapeutic window. Further studies are warranted to investigate the roles of RSK and other targets of TAS0612 in ALL. RSK inhibition is a promising approach for the treatment of pediatric acute leukemia. PMD-026 suppresses the proliferation of AML cells and synergizes with chemotherapy. TAS0612 reduces the growth of AML cells and is also effective at targeting B- and T-ALL cells. Both compounds have minimal toxicity to healthy hematopoietic cells. Therefore, PMD-026 and TAS0612 are strong candidates for translation into future clinical trials for AML and ALL.

#5595 Multiomic profiling identified biomarkers of response to selective Src family kinase inhibition in preclinical models of cholangiocarcinoma.

Hendrien Kuipers[1], Danielle M. Carlson[1], Jack W. Sample[1], Erik Jessen[1], Dong-Gi Mun[1], Aushinie M. Abeynayake[1], Jennifer L. Tomlinson[1], Amro M. Abdelrahman[1], Nathan W. Werneburg[1], Binbin Li[1], Mitesh J. Borad[2], Mark J. Truty[1], Sumera I. Ilyas[1], Gregory J. Gores[1], Rory L. Smoot[1]

[1]Mayo Clinic, Rochester, MN,[2]Mayo Clinic, Phoenix, AZ

Introduction: Src Family Kinases (SFKs) have a crucial role in tumor proliferation, survival, and metastasis. NXP900 is a highly selective SFK inhibitor a novel mechanism of action that locks SFKs in a closed, inhibited conformation, preventing interaction with substrate proteins, unlike other SFK inhibitors like dasatinib. A phase 1 study of NXP900 in patients with advanced solid tumors was recently initiated. Our preclinical studies showed therapeutic efficacy of NXP900 in cholangiocarcinoma (CCA) through decreased YAP, RAF-MAP and PI3K-AKT signaling. Here, we examined mechanisms of sensitivity and resistance to SFK inhibition.

Methods: Secondary resistance was explored by generating a NXP900-resistant human cell line (HuCCT1-R) via prolonged exposure to NXP900, followed by multiomic profiling. Primary resistance was studied in vivo using nine heterogeneous patient-derived xenograft (PDX) tumors implanted in the flanks of NOD/SCID mice. Tumor-bearing mice were treated with vehicle or 40 mg/kg NXP900 daily for up to 4 weeks. To identify factors associated with response, a multiomic approach was used, integrating RNA-Seq, global proteomics, and phosphoproteomics. A hierarchical-all-against-all algorithm (HallA) assessed associations between multiomic features and drug response in tumor growth. Kinase enrichment analysis (KEA3) identified features linked to sensitivity or resistance.

Results: HuCCT1-R cells showed increased pTyr abundancy of TNK2, a protein known to increase PIP3 levels leading to AKT activation, and of DYRK3 and LAMTOR1, both of which can sustain AKT phosphorylation via mTORC, indicating acquired resistance through bypassing inhibited PI3K-AKT signaling to rescue AKT. NXP900 reduced tumor growth rates in 5 of 9 PDX models. Kinases associated with sensitivity in the PDX models included SFKs (SRC, LYN, and FYN), and AKT, PTK2, PRKCZ, EGFR, SYK, and GSK3B, suggesting involvement of the PI3K-AKT pathway in responsiveness to NXP900. Based on tumor inhibition responses in the tested models, we estimated NXP900 response for our additional PDX models, predicting 39% (11/28) of patient tumors may respond.

Conclusion: This study identified molecular markers linked to response to SFK inhibition, which could be utilized for patient selection for NXP900 treatment. Further studies are validating these findings and exploring combination therapies to address resistance mechanisms.

#5596 Novel multikinase CLK inhibitor BH-30236 targets hematological malignancies through alternative splicing regulation.

Nancy Ling[1], Gregory Sims[1], Ping Jiang[1], Wei Deng[1], Dayong Zhai[1], Danan Li[1], Zhenping Wang[1], Yue Hu[1], Joshua Choi[1], Eric Wang[2], Armin Graber[1], Jean Cui[1]

[1]BlossomHill Therapeutics, Inc., San Diego, CA,[2]The Jackson Laboratory for Genomic Medicine, Farmington, CT

RNA splicing dysregulation plays a critical role in tumorigenesis, cancer progression, and therapeutic resistance. Components of the spliceosome machinery are frequently mutated in myeloid malignancies, affecting 40-85% of patients with Myelodysplastic Syndrome (MDS), and 10–25% of Acute Myeloid Leukemia (AML) cases. Recurrent aberrant alternative splicing (AS) patterns have also been detected in AML patients even in the absence of somatic spliceosome gene mutations. Furthermore, genetic screens have shown that the loss of splicing factors sensitizes AML to BCL2 inhibitor venetoclax. As such, splicing modulators are now acknowledged as a promising therapeutic target for hematologic malignancies. CDC2-like kinases (CLKs) play a pivotal role in regulating mRNA splicing *via* the phosphorylation of serine/arginine-rich splicing factor (SRSF) proteins which facilitate pre-mRNA splice site recognition. Consequently, inhibiting CLKs may offer a viable strategy for targeting cancers dependent on aberrant splicing. BH-30236 is a novel, orally bioavailable, ATP-competitive, macrocyclic inhibitor of CLKs, proviral integration for the Moloney murine leukemia virus 3 kinase (PIM3), FMS-like tyrosine kinase 3 (FLT3), and dual-specificity tyrosine-regulated kinase (DYRK) 1/2. BH-30236 potently suppressed SRSF phosphorylation and demonstrated *in vitro* efficacy at nanomolar range across immortalized and patient-derived AML cells, including cells insensitive to venetoclax. To evaluate how BH-30236 impacts mRNA expression and alternative splicing, RNA sequencing was performed on 6 patient-derived AML cells before and after BH-30236 treatment. BH-30236 modulated AS in genes associated with DNA damage response, mRNA splicing, and chromosome remodeling. Gene ontology enrichment analysis of genes downregulated by BH-30236 included terms related to mitochondrial translation, respiratory chain complex assembly, and regulation of cytochrome c release. Specifically, BH-30236 decreased the expression of anti-apoptotic BCL2 family genes MCL1 and BCL2A1, whose expression correlates with venetoclax resistance. Consistent with this, BH-30236 enhanced the potency of venetoclax in AML cell lines, including those with TP53 mutations. The combination of BH-30236 and venetoclax also synergistically suppressed tumor growth or induced deep tumor regression in venetoclax-resistant AML cell-derived xenograft models (Kasumi-1, MOLM-13). These observations support further evaluation of BH-30236 in combination with venetoclax for hematologic malignancies. A Phase 1 study to evaluate the safety, tolerability, pharmacokinetics, pharmacodynamics, and preliminary anti-leukemic activity of BH-30236 in adults with relapsed/refractory AML and higher-risk MDS is currently ongoing (NCT06501196).

#5597 Efficacy of novel urokinase plasminogen activator receptor-associated protein targeted antibody-drug conjugates in patient-derived xenografts of soft tissue sarcoma.

Chao-Chi Wang[1], Agnieszka Wozniak[1], Luna De Sutter[1], Lore De Cock[1], Karo Wyns[1], Ulla Vanleeuw[1], Sander Van Putten[2], Carmel Lynch[2], Patrick Schoffski[3]

[1]KU Leuven, Leuven, Belgium,[2]Adcendo ApS, Frederiksberg, Denmark,[3]University Hospitals Leuven, Leuven, Belgium

Objective: Soft tissue sarcoma is a heterogeneous group of rare malignant tumors with a high unmet medical need. We evaluated the efficacy of two novel antibody-drug conjugates (ADC), ADCE-017 and ADCE-202, each consisting of three components: (1) an antibody targeting urokinase plasminogen activator receptor-associated protein (uPARAP), a collagen receptor highly expressed on mesenchymal tumor cells, (2) a linker, (3) and deruxtecan, a topoisomerase I inhibitor payload. The efficacy of ADCE-017 and ADCE-202 was assessed in 4 in vivo experiments using three different patient-derived xenograft (PDX) models with high uPARAP expression.

Methods: A total of 164 NMRI nu/nu mice were transplanted with PDX models of dedifferentiated liposarcoma (models UZLX-STS5, -STS200) or myxofibrosarcoma (-STS45). Mice were randomized for the following treatments: (1) vehicle control, (2) doxorubicin, (3) targeted antibody ("naked" antibody without payload), (4) ADC-isotype control (deruxtecan linked to a non-specific antibody), (5) uPARAP-targeted ADC. Treatments were given as tail vein injection on days 1, 8 and 15. Tumor volume, bodyweight and general well-being of animals were monitored until day 100 or until reaching the humane endpoint. Statistical analysis was performed with Wilcoxon test and Mann-Whitney U-test, with $p < 0.05$ defined as statistically significant.

Results: Treatment with ADCE-017 and ADCE-202 resulted in significant tumor growth delay in two experiments with STS200, compared to vehicle control and doxorubicin, and tumor volume shrinkage compared to baseline (day 1). Similar results were obtained with STS45, however, in this experiment the ADC-isotype control showed superior tumor volume shrinkage compared to ADCE-017. In STS5, tumor growth delay was observed with ADCE-017 as compared to the vehicle control and ADC-isotype control, with effects being similar to doxorubicin. Based on bodyweight assessment and necropsy of the mice, no severe toxicity was observed.

Conclusion: In general, the novel uPARAP-targeted ADCs ADCE-017 and ADCE-202 showed superior anti-tumor effect in all three soft tissue sarcoma models as compared to vehicle control without any treatment-related side effects. These results warrant further preclinical studies in other types of sarcoma and a clinical evaluation of the ADCs as a potential new treatment option for soft tissue sarcoma.

#5598 First-in-class casein kinase 1 alpha inhibitors for overcoming treatment related resistance in AML and pancreatic cancer.

Pavlina Janovska[1], Tomasz Radaszkiewicz[1], Martin Čulen[2], Ingrid Pristicova[1], Hana Plesingerova[1], Petra Prochazkova[1], Michal Smida[3], Daniel Busa[2], Adriana Ladungova[3], Lukas Maier[4], Stěpan Havel[4], Kamil Paruch[4], Jiři Mayer[2], Alexander Scheer[5], Vitězslav Bryja[1]

[1]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic,[2]3 Department of Internal Medicine, Hematology and Oncology, Faculty of Medicine, Masaryk University, Brno, Czech Republic,[3]Central European Institute of Technology, Masaryk University, Brno, Czech Republic,[4]Department of Chemistry, Faculty of Science, Masaryk University, Brno, Czech Republic,[5]CasInvent Pharma, Brno, Czech Republic

Background: Inhibition of Casein kinase 1 alpha (CK1α) represents a new attractive concept for the therapy of acute myeloid leukemia (AML). Despite this, currently available small-molecule CK1α inhibitors lack sufficient activity and selectivity to fully exploit the therapeutic potential of CK1α inhibition. We have developed a series of potent and highly selective CK1 kinase inhibitors, including the most effective CK1α inhibitors to date. They inhibit all CK1α/δ/ε isoforms, blocking both CK1δ, ε- and CK1α-dependent processes, which leads to higher efficacy and good tolerability. Targeting of CK1 kinases in resistant cancers is a novel concept - several preclinical studies showed the potential of CK1δ/ε targeting to overcome/avoid resistance - e.g. to potentiate Gemcitabine efficacy in pancreatic cancer, or Enzalutamide in prostate cancer. The precise role of CK1α inhibition is still under evaluation; nevertheless, knockout experiments have revealed that CK1α is essential for survival of most cancer cells and could bring benefit when targeted alone or together with CK1δ/ε.

Material and Methods: Cellular target engagement assays, *in vitro* kinase assays, ADME profiling, testing of *in vitro* sensitivity to CK1 inhibition and standard-of-care treatments in cell cultures (cell lines, primary AML samples), *in vivo* efficacy and tolerability studies in mice.

Results: First-in-class CK1 inhibitors were developed and evaluated for their potential in preclinical and clinical settings. These inhibitors exhibited sub-nanomolar activity against CK1α, translating into superior on-target activity in cells compared to the previously reported BTX-A51 compound. High efficacy and safety were observed in both in vitro and in vivo AML models, including Venetoclax-resistant AML, underscoring the significant potential of selective CK1α inhibition. A set of biomarkers predicting sensitivity to CK1α inhibitors, including resistance to Venetoclax (a standard-of-care BCL-2 inhibitor), was identified. Additionally, a Gemcitabine-resistant pancreatic cancer model confirmed the efficacy of combining CK1 inhibition with Gemcitabine, resulting in improved outcomes in resistant in vivo models. Proof-of-concept studies are also underway for melanoma and prostate cancer.

Conclusions: Our findings highlight the therapeutic potential of selective CK1α inhibition for treating Venetoclax-resistant AML. Furthermore, the data suggest that dual targeting of CK1α/δ/ε may help overcome resistance in specific cancers, such as Gemcitabine-resistant pancreatic cancer and melanoma.

This project is co-financed with state support from the Technology Agency of the Czech Republic within the TN Program for the Support of Applied Research, Experimental Development, and Innovation of the National Competence Center (2018 - 2028); project TN02000109

#5599 NXP900, a novel YES1/SRC kinase inhibitor currently in clinical development, blocks YAP1 signaling in NSCLC cell lines.

Boback Kaghazchi[1], Inigo Lanzagorta Calvillo[1], Ben King[1], Enrique Poradosu[2], Neil O Carragher[1], Asier Unciti-Broceta[1]

[1]University of Edinburgh, Edinburgh, United Kingdom,[2]Nuvectis Pharma Inc., New Jersey, United Kingdom

Background: Most kinase inhibitors exert their anticancer effects by targeting the ATP binding site, either binding and locking an active or inactive kinase conformation, with targeting inactive conformation typically leading to a superior selectivity profile[1]. NXP900 (eCF506), a highly-potent SRC family kinase (SFK) inhibitor has previously demonstrated high SFK selectivity and the capability to lock the native “closed” conformation (type 1.5), resulting in inhibition of both enzymatic and scaffolding activities[2] (IC_{50} of 0.47 nM against YES1). Conversely, multi-kinase inhibitors dasatinib and bosutinib target the “open” conformation (type 1), thus promoting SFK association with signalling partners[3]. The aim of this study was to examine the signaling pathways targeted by NXP900, including the Hippo pathway, and unravel potential synergy mechanism of NXP900 with existing targeted therapies against NSCLC. **Materials and methods:** Cell confluence and viability were determined by automated image analysis of confluence and Presto Blue assay, respectively. NSCLC cells were treated with NXP900 for 24 hours prior to fixation and subsequent immunostaining with anti-YAP, anti-YES1, phalloidin and Hoechst. Images were analysed using ImageJ software. mRNA abundance of SRC, YES1, YAP1, NF2/MERLIN, Cyclin D were quantified utilising TaqMan RT-PCR. **Results:** NXP900 inhibited cancer cell proliferation of NSCLC cell lines. NXP900 showed dose-dependent decreases in YAP1 and YES1 nuclear localization across all cell lines and prevented SRC phosphorylation. **Conclusions:** NXP900 prevents YAP1 nuclear localization and cell cycle progression in a panel of NSCLC cell lines, decreasing expression of total YAP1 and YES1. Our data suggests that NXP900, currently in a Phase1 dose escalation study, is an attractive and translatable combination partner that could synergize with existing targeted therapies against NSCLC when the Hippo pathway is dysregulated. **1** C. C. Ayala-Aguilera, T. Valero, A. Lorente-Macias, D. J. Baillache, S. Croke, A. Unciti-Broceta, *J Med Chem*, 2022, 65, 1047-1131. **2** C. Temps, D. Lietha, E. R. Webb, X. F. Li, J. C. Dawson, M. Muir, K. G. Macleod, T. Valero, A. F. Munro, R. Contreras-Montoya, J. R. Luque-Ortega, C. Fraser, H. Beetham, C. Schoenherr, M. Lopalco, M. J. Arends, M. C. Frame, B. Z. Qian, V. G. Brunton, N. O. Carragher and A. Unciti-Broceta, *Cancer Res*, 2021, 81, 5438. **3** M. Higuchi, K. Ishiyama, M. Maruoka, R. Kanamori, A. Takaori-Kondo and N. Watanabe, *Cell Rep*, 2021, 34, 08876.

#5600 BTK T474I with enhanced kinase activity confers growth advantage over BTK-L528W with kinase deficiency in B malignant cells.

Wenjing Zhang[1], Xinxin Zhu[1], Qiming Zhou[1], Jiayun Song[1], Yang Li[2], Bo Li[2], Ming Jiang[2], Xiaoliang Cheng[2], Teiko Sumiyoshi[3], Yangbo Yue[1], Wei Jin[2], Zhirong Shen[2]

[1]BeiGene (Shanghai) Biotechnology Co., Ltd., Shanghai, China,[2]BeiGene (Beijing) Co., Ltd., Beijing, China,[3]BeiGene Co., Ltd., San Mateo,CA, CA

Background: Covalent BTKi have transformed the therapeutic landscape for chronic lymphocytic leukemia (CLL). However, acquired mutations in BTK, often occurring at C481, lead to drug resistance and disease progression. While non-covalent BTKi has demonstrated promising efficacy in CLL patients with BTK C481X mediated resistance, non-C481 mutations within BTK kinase domain confer ineffectiveness to cBTKi and ncBTKi as well. Those BTK mutations most commonly involve BTK T474I and L528W. To compare their role in promoting B malignant cell growth, we developed clonal competition models by co-culturing isogenic B malignant cells harboring BTK T474I and L528W and monitor the clonal dynamics in long term culture.
Methods: The T474I and L528W were introduced to BTK allele of B malignant cell line TMD8. BTK downstream signaling in BTK wildtype or mutant cells with or without anti-IgM stimulation was assessed by western blot. In vitro and in vivo pooled clone models containing both TMD8 wildtype and mutant cells were employed to investigate the clonal competition. The dynamics of individual clones were monitored by measuring the changes of BTK variant allele frequency (VAF) via amplicon sequencing.
Results: Hyperactivation of phosphorylated BTK Y223 in TMD8 BTK T474I cell and very limited BTK Y223 phosphorylation in TMD8 BTK L528W upon IgM stimulation was observed. Enhanced B-cell receptor (BCR) downstream signaling indicative of pPLCγ2, pERK and pAKT were noted in BTK T474I cells compared to those expressing BTK L528W. Those findings suggest that BTK T474I encodes a kinase exhibiting enhanced activity while BTK L528W results in a catalytically inactive form. Clone competition was evaluated using co-culture experiments containing TMD8 BTK T474I, BTK L528W and WT cells in medium with various FBS concentration. T474I clones were significantly expanded while L528W clones were declined in 4 weeks culture period. The outgrowth advantage of BTK T474I was particularly pronounced when cultured in medium with low FBS concentration, which simulated a relatively harsh condition for cell growth. Similar results were observed when those clonal pools were grown as xenografts. An increase in T474I VAF and a decrease in L528W VAF were noted during tumor growth in subcutaneous models incorporating TMD8 wildtype and mutant cells.
Conclusion: Our in vitro and in vivo clonal competition assays provided novel insights into the impact of BTK T474I and L528W on growth fitness. These findings suggested that BTK T474I, characterized by enhanced kinase activity, confers a growth advantage over the kinase deficient form BTK L528W.

#5601 ID11916, a novel androgen receptor antagonist with cGMP-dependent protein kinase activation for next-generation therapeutics in AR positive tumors.

Jeong Eun Kim[1], Ji-Yun Lee[2], Jaebeom Cho[2], Won Sik Lee[1], Myongjae Lee[1]

[1]Idience, Seoul, Korea, Republic of,[2]College of Pharmacy, Chung-Ang University, Seoul, Korea, Republic of

Androgen receptor (AR) signaling plays a crucial role in hormone-dependent cancers. In prostate cancer, AR signaling-targeted therapies, including second-generation AR antagonists such as enzalutamide, are initially effective for castration-resistant prostate cancer, but often develop resistance. AR is expressed in 60-80% of breast cancers and known to contribute to tumor aggressiveness. However, the clinical efficacy of AR-targeted therapies in breast cancer remains limited. Therefore, numerous AR targeted therapies are under development to improve efficacy and overcome resistance. However, given that AR resistance results from the complex interplay of both AR-dependent and AR-independent mechanisms, a strategy targeting both pathways is required. We developed ID11916, a novel orally available AR antagonist that modulates AR-dependent and AR-independent pathways by activating cGMP-dependent protein kinase (PKG). ID11916 effectively blocks androgen binding to AR, nuclear translocation, and androgen-dependent transcriptional activity of AR, while increasing intracellular cGMP levels and activating PKG via phosphodiesterase 5 inhibition. ID11916 demonstrated potent anti-cancer effect in prostate cancer cell lines VCaP and 22Rv1, where enzalutamide showed limited efficacy. In VCaP, where bicalutamide exhibited an agonistic effect, ID11916 showed approximately 60-fold greater anti-proliferative activity (IC_{50}: 2.77 μM) than enzalutamide (IC_{50}: 163 μM). ID11916 demonstrated greater potency in colony formation assays than enzalutamide in AR-positive breast cancer cell lines SK-BR-3 (ID11916 IC_{50}: 0.653 μM vs Enzalutamide IC_{50}: > 30.0 μM) and MDA-MB-231 (ID11916 IC_{50}: 1.96 μM vs Enzalutamide IC_{50}: 18.1 μM). ID11916 also showed superior tumor growth inhibition in xenograft models. In the MDA-MB-453 xenograft model, daily oral administration of ID11916 at 30 mg/kg inhibited tumor growth by 74.7%, compared to 53.0% inhibition with clinically relevant dose of enzalutamide. In a repeated-dose pharmacokinetic study in the MDA-MB-453 xenograft model, oral administration of 30 mg/kg ID11916 showed a 7-fold lower C_{max} and an 8-fold lower AUC compared to enzalutamide. These findings indicate that the enhanced potency of ID11916 compared to enzalutamide is consistently demonstrated *in vivo*. In conclusion, our preclinical data introduce ID11916, a novel compound with a potential of overcoming resistance through both AR-dependent and AR-independent mechanisms by combining AR and PKG modulation. These findings support its potential as a next-generation AR-targeted therapy, offering an alternative to currently available AR targeting approaches.

#5602 Galactokinase 1 is a novel metabolic target against bladder cancer.

Surya P. Singh, Chinthalapally V. Rao, Venkateshwar Madka

Department of Medicine, Center for Cancer Prevention and Drug Development, Stephenson Cancer Center, Hem-Onc, University of Oklahoma HSC, Oklahoma City, OK

Urinary bladder cancer (BCa) is the second most prevalent cancer of the genitourinary tract. Although BCa affects both genders, it is more predominant among men. Most of the tumors are non-invasive at diagnosis, however, high rates of recurrence, heterogeneity, and treatment resistance lead to significant mortality. Therefore, it is necessary to identify and understand the role of novel metabolic targets and explore their therapeutic potential against BCa. Rapidly proliferating cancer cells have high nutritional demand which necessitates metabolic reprogramming and reliance on the alternative nutrients in the tumor microenvironment. Based on the human BCa transcriptome data analysis (TCGA data), we identified that the Galactokinase-1 (GALK1) gene is significantly overexpressed ($p<0.0001$) in human bladder tumors compared to normal tissue, and its expression also correlates with disease stage and poor prognosis. GALK1 is a member of the Leloir pathway that facilitates the initial step of galactose breakdown. Interestingly, we observed abundant expression of GALK1 in various human and rodent BCa cell lines as well as rodent bladder tumor tissues. Hence, we hypothesized that GALK1 may contribute to BCa survival by contributing to the cancer cell proliferation and could serve as a novel target for the management of this malignancy. To understand this, we abrogated GALK1 gene expression in MB49 cells using siRNA and found a significant decrease in cell proliferation with increased apoptosis. Further treatment of various human bladder cancer cells, in vitro with a small-molecule GALK1 inhibitor (Cpd36), resulted in reduced proliferation (IC_{50} = 29.09 - 43.67μM), migration and invasion potential. A similar growth inhibitory effect was also observed in BCa spheroids and tumoroids. Cell cycle analysis indicated that Cpd36 induced significant G1 arrest ($p<0.0001$) in BCa cells and pronounced G2/M arrest ($p<0.0001$). GALK1 inhibition increased mitochondrial ROS production while lowering the mitochondrial membrane potential and triggered apoptosis in BCa cells. GALK1 inhibition induced cell growth arrest was associated with decrease in protein levels of GALK1, PCNA, and Cyclins. Additionally, the induction of apoptosis was associated with increase in cleaved caspase-3 and cleaved poly (ADP-ribose) polymerase (PARP). Further, we investigated the effect of GALK1 abrogation on chemotherapeutic drugs sensitivity. Invitro drug combination data suggested that targeting GALK1 sensitized the BCa cells to cisplatin or gemcitabine as evident from significant reduction in the IC50 of these chemotherapeutic drugs. Based on these findings, GALK1 plays a tumor promoting role in BCa and its inhibition may be a promising therapeutic target for its prevention and treatment.
(Funding supported in part by NCI-P30CA225520 and ACS-134128-IRG-19-142-01 grants).

#5603 A precision polypharmacology approach to co-target EGFR and PI3 kinase: the power of structure-based drug design to generate therapeutic agents with versatility in multiple combination settings.

Elizabeth K. Ziemke[1], Christy L. Frankowski-McGregor[1], Madeline Walker[1], Christopher E. Whitehead[2], **Judith S. Sebolt-Leopold**[3]

[1]Radiology, University of Michigan, Ann Arbor, MI,[2]MEKanistic Therapeutics, Inc., Ann Arbor, MI,[3]University of Michigan, Ann Arbor, MI

Adaptive resistance mechanisms significantly curtail the long term effectiveness of molecular targeted agents directed against a single protein. MTX-531 was computationally designed to selectively target two critical oncogenic drivers, EGFR and PI3 kinase. We recently reported unique structural and pharmacological features of this lead molecule, including strong monotherapy activity against squamous head and neck cancers (HNSCC), which are prone to adaptive resistance when either oncogenic protein is targeted alone (Whitehead et al, Nature Cancer 2024). Based on the central role of the PI3K pathway in driving resistance to a broad spectrum of targeted agents and modalities, we report here pilot studies carried out with MTX-531 to elucidate its potential in combination with other targeted agents, radiation, and immunotherapy. In vivo studies were carried out in KRAS G12D mutant tumor-bearing mice showing that treatment with MTX-531 led to striking enhancement of KRAS inhibitor (MRTX-1133) activity against a broad spectrum of gastrointestinal cancers (pancreatic, colorectal, gastric) as evidenced by a high incidence of durable tumor regressions. In a separate line of investigation, nanomolar potency of MTX-531 against DNA-PK, a PI3K-like family member involved in DNA damage repair, led us to explore the radiosensitizing potential of this molecule in models of HNSCC and triple negative breast cancer. Combination of MTX-531 and daily low dose (2Gy) radiation resulted in prolongation of survival with a high incidence of durable complete regressions in HNSCC. Furthermore, based on the pan-PI3K inhibitory profile of MTX-531, we hypothesized that this molecule would exert immunomodulatory effects to improve therapeutic outcome in the immunotherapy setting. Studies carried out in syngeneic mouse models of pancreatic cancer (KPC) and HNSCC (MOC1) showed that the combination of MTX-531 with anti-PD-1 antibody led to improved survival and increased incidence of objective responses compared to the single agent arms. Collectively, these studies demonstrate the versatility of MTX-531 to serve as the backbone of combination regimens across diverse modalities encompassing treatment with targeted therapies, radiation, or immune checkpoint inhibitors. Based on its promising preclinical profile, MTX-531 represents a first-in-class approach to precision polypharmacology that demonstrates the power of rational structure-based drug design to target multiple resistance drivers in a single molecule.

#5604 Antitumor activity of YH42946, a potent tyrosine kinase inhibitor, in NSCLC harboring EGFR exon 20 insertion mutations.

M. Yu[1], J. Lee[1], K. Lee[2], M. Hong[1], S. Lim[1], B. Cho[1];

[1]Yonsei University College of Medicine, Seoul, Korea, Republic of, [2]Korea Research Institute of Chemical Technology, Daejeon, Korea, Republic of

Introduction: Epidermal growth factor receptor (EGFR) mutations are the most common in non-small cell lung cancer (NSCLC) patients, with around 30% carrying these mutations, of which approximately 4-10% are EGFR exon 20 insertion (ex20ins) mutations. The most common EGFR ex20ins mutations in NSCLC are ASV and SVD variants in the kinase domain. Currently, treatment options for this subset of patients are limited. YH42946 is an orally available tyrosine kinase inhibitor (TKI) targeting EGFR ex20ins mutations, and the potential to be a best-in-class drug candidate to address this unmet clinical need.

Method: Competitive binding affinity was measured using the KINOMEscan screening platform. The inhibitory activity of YH42946 was evaluated through a cell viability assay in Ba/F3 cell lines expressing EGFR ASV, SVD, GY, and HH mutations. A549 cells were used to assess the cellular activity of EGFR wild-type (WT). To further confirm the mechanism of action, western blot analysis was performed on patient-derived cells (PDCs), including YU-1163 (S768_D770dup, SVD) and DFCI-127 (P772_H773insPNP).

Results: Kinome profiling against a panel of various kinases revealed that 100 nM of YH42946 bound strongly to EGFR mutants. In a cell viability assay, YH42946 strongly inhibited cellular activity in Ba/F3 cell lines engineered to express the EGFR mutants ASV, SVD, GY (IC_{50} < 10 nM), and HH (IC_{50} < 30 nM). It demonstrated superior activity compared to Mobocertinib (IC_{50} < 30 nM for ASV and IC_{50} < 30 nM for SVD), Furmonertinib (IC_{50} < 100 nM for ASV and IC_{50} < 100 nM for SVD), and Zipalertinib (IC_{50} < 100 nM for ASV and IC_{50} < 100 nM for SVD), and was comparable to Poziotinib (IC_{50} < 10 nM for ASV and IC_{50} < 10 nM for SVD). In normal cell lines, YH42946 did not inhibit the activity of normal cell lines, largely sparing EGFR WT activity (IC_{50} > 1000 nM). In protein expression analysis, YH42946 effectively inhibited the signaling pathways of p-EGFR, p-AKT, p-ERK1/2, and p-S6 in YU-1163 and DFCI-127 PDC cell lines at a low dose level.

Conclusion: YH42946 is a potent small-molecule inhibitor that selectively targets EGFR ex20ins while sparing EGFR WT in preclinical models, offering potential therapeutic benefits for NSCLC patients with EGFR ex20ins mutations. Additionally, YH42946 has demonstrated effective inhibition of the EGFR pathway. Given these robust activities against EGFR ex20ins, YH42946 is anticipated to provide a significant therapeutic option for NSCLC patients harboring EGFR ex20ins mutations.

#5605 Development of a novel protein phosphatase 2A (PP2A) activator, PPA27, as a potential therapeutic for melanoma.

Hannah Johnson[1], Amandeep Singh[1], Asif Raza[1], Satya Narayan[2], **Arun K. Sharma**[1]

[1]Penn State College of Medicine, Hershey, PA,[2]3Department of Anatomy and Cell Biology, University of Florida, Gainesville, FL

Protein phosphatase 2A (PP2A) is a vital serine/threonine phosphatase acting as a tumor suppressor, often inactivated in melanoma and other cancers. This study builds upon our initial findings on the PP2A activators PPA24 and PPA27, focusing on extended *in vitro* and *in vivo* evaluations to assess PPA27's therapeutic potential against melanoma. In our previous work, both compounds demonstrated selective cytotoxicity in melanoma cell lines. Here, we further characterize PPA27's efficacy across five melanoma cell lines (SK-MEL-2, SK-MEL-28, A375, Mel1241, and 451Lu), showing IC_{50} values ranging from 3.45-8.94 μM, with consistent effectiveness regardless of BRAFV600E mutation status. Mechanistic analyses revealed that PPA27 induced apoptosis in a dose-dependent manner as confirmed by increased caspase-3/7 activation and PARP cleavage. Notably, PPA27 treatment significantly downregulated phosphorylated ERK1/2, particularly in BRAFV600E-positive A375 cells, suggesting differential modulation of MAPK pathways in mutant and wild-type contexts. To validate these findings *in vivo*, the efficacy of PPA27 was assessed in xenograft melanoma mouse models. The compound significantly reduced tumor growth compared to control without any apparent systemic toxicity. Additionally, pharmacokinetic and ADMET analyses indicate that PPA27 possesses excellent bioavailability, stability, and metabolic properties, supporting its potential for clinical translation. Furthermore, combinatorial studies suggest that PPA27 enhances the efficacy of existing BRAF inhibitors, potentially addressing drug resistance issues in melanoma therapy. These findings highlight PPA27's robust therapeutic potential and provide new insights into its mechanism of action. In conclusion, our expanded investigation establishes PPA27 as a promising candidate for melanoma treatment, warranting further preclinical studies before consideration for clinical trials targeting both BRAF-mutant and wild-type melanoma subtypes.

#5606 The novel brain-penetrant paradox breaker BRAF inhibitor RG6344 manifests exceptional activity in BRAF mutant colorectal cancers.

F. Renner[1], J. Eckmann[1], C. Handl[1], N. Kratochwil[1], J. Wichmann[1], G. Schnetzler[1], N. Keshelava[2], H. G. Palmer[3], J. Martinez-Quintanilla[3], I. Chicote[3], J. Verges[3], P. Pettazzoni[1];
[1]F. Hoffmann-La Roche Ltd., Basel, Switzerland, [2]F. Hoffmann-La Roche Ltd., Zurich, Switzerland, [3]Val d'Hebron Institute of Oncology (VHIO), Barcelona, Spain

Despite the approval of the combination Encorafenib/Cetuximab in metastatic colorectal cancers (CRC) harboring BRAF V600E mutations, the therapeutic benefits offered by this regimen remains limited and short lived.
We developed a next generation brain permeable, paradox breaker BRAFi (RG6344, previously identified as Compound Ia in Wichmann et al., Clin Cancer Res 2022) currently undergoing clinical testing (ISRCTN13713551). Here we present its preclinical activity for BRAF mutated CRC.
RG6344 proves paradox breaking properties by not triggering MAPK over-activation in BRAF WT contexts at all levels. For in vivo testing regimens covering IC_{80} (60 mg/kg) and IC_{95} (140 mg/kg) p-ERK inhibition were used.
RG6344 was highly tolerated in all studies as a single agent and in all combination regimens tested clearly highlighting that the lack of paradoxical MAPK activation allows superior target inhibition in vivo. In BRAFi naïve xenograft models (LS411N, HT29) RG6344 as monotherapy outperformed Encorafenib/Cetuximab at all doses tested indicating higher activity of this agent even at doses targeting only IC80 p-ERK inhibition, the same BRAF inhibition levels reached by Encorafenib in CRC approved clinical regimens. RG6344 activity was further strengthened in combination with cetuximab and demonstrated long-lasting responses in PDX models derived from patients that progressed to Encorafenib/Cetuximab therapy supporting the use of RG6344 even in BRAFi experienced patients.
Additional combination studies of RG6344 with FOLFOX in BRAFi naive models resulted in tumor regression and dramatic superiority compared to the same combination with Encorafenib.
These data collectively provide strong preclinical translational evidence for the potentially transformative activity of RG6344 in BRAF mutated CRC.

#5607 Discovery and preclinical evaluation of a potent, orally bioavailable, highly selective, small molecule PTPN2/1 inhibitor.

A. Pushechnikov[1], V. Parchinsky[2], A. Riakhovskii[2], S. Mochalov[2], R. Karapetian[1], A. Burd[3], N. Savchuk[1], I. Dukes[3], R. Abagyan[4];
[1]Expert Systems, Inc., San Diego, CA, [2]ChemDiv, Inc., San Diego, CA, [3]Eilean Therapeutics LLC, Philadelphia, PA, [4]MolSoft LLC, San Diego, CA

Protein tyrosine phosphatase non-receptor type 2 (PTPN2) is ubiquitously expressed, primarily in hematopoietic and placental cells. Critical negative regulator of the JAK-STAT pathway, PTPN2 functions to directly regulate signaling through cytokine receptors, including IFNγ. Thus, enhancing IFNγ sensing and signaling through the inhibition of PTPN2 is a potential therapeutic strategy to improve the efficacy of cancer immunotherapy regimens. Historically, phosphatases are difficult drug targets; due to an intrinsic physicochemical profile of phosphatase inhibitors, finding bioavailable molecules is a great challenge. ZE00-0388 demonstrates sub-nanomolar PTPN2/N1 inhibitory activity and outstanding over 1000-fold selectivity against all other phosphatases. ZE00-0388 demonstrated complete tumor remission in combination with the anti-mPD-1 recombinant mAb therapy in the murine colon adenocarcinoma MC38 in vivo model. ZE00-0388 demonstrates good safety and tolerability; its ADME properties confirm that ZE00-0388 is suitable for further development for combination therapies.

#5608 Design and discovery of BH-30643: A novel, reversible, mutant-selective macrocyclic EGFR inhibitor invulnerable to common resistance mutations.

J. Jean Cui, Eugene Rui, Evan Rogers, Dayong Zhai, Ping Jiang, Zhenping Wang, Yue Hu, Nancy Ling, Danan Li, Joshua Choi, Anindya Sarkar, Levan Darjania, Zhengwei Peng, Wei Deng

BlossomHill Therapeutics, Inc., San Diego, CA

Outcomes on tyrosine kinase inhibitor (TKI) treatment in *EGFR*-mutant NSCLC fall short of the durable benefit observed with next-generation targeted therapies in *ALK* and *ROS1*-driven NSCLC. We reviewed the chemical structures of 30+ existing EGFR TKIs in pursuit of a novel chemical scaffold that could offer a more durable treatment response. First-generation (1G) (e.g. gefitinib) and second-generation (2G) (e.g. afatinib) EGFR TKIs share an anilinoquinazoline scaffold which is vulnerable to T790M gatekeeper resistance. Third-generation (3G) (e.g. osimertinib) EGFR TKIs employ a less potent pyrimidine scaffold and obtain good potency including T790M through introduction of an irreversible binding at C797. However, the dependence of 3G EGFR TKIs on irreversible binding renders these agents vulnerable to C797S resistance. The established EGFR TKI scaffolds additionally have more limited potency against a growing family of non-classic EGFR mutations (atypical and ex20ins). To increase the breadth and durability of EGFR TKIs in *EGFR* mutant NSCLC, we have designed a novel reversible macrocyclic scaffold that fully leverages the characteristics of EGFR active conformation and introduces novel and precise interactions with mutant EGFRs *via* a three-dimensional macrocyclic structure. Our efforts led to the discovery of BH-30643, a potent, orally bioavailable, reversible, ATP-competitive macrocyclic inhibitor selective for mutant EGFRs. BH-30643 demonstrates potent anti-cell proliferation activity against classical mutations (IC_{50}: ex19del 0.73 nM and L858R 0.18 nM), with >250-fold selectivity over wildtype (WT) EGFR (IC_{50}: 191.5 nM). It additionally exhibits ~50-fold selectivity compared to WT HER2 (IC_{50}: 42.2 nM), in contrast to the 2G and 3G EGFR TKIs with limited selectivity over WT HER2, which may contribute to diarrhea as a side effect. The potency of BH-30643 is maintained in the presence of T790M (IC_{50}: ex19del/T790M 0.43 nM and L858R/T790M 0.69 nM), C797S (IC_{50}: ex19del/C797S 0.05 nM and L858R/C797S 0.10 nM) or both (IC_{50}: ex19del/T790M/C797S 0.43 nM and L858R/T790M/C797S 0.54 nM). Potency is additionally evident across a breadth of EGFR mutations including atypical mutations (IC_{50}: 0.05-8.84 nM), and ex20ins mutations (IC_{50}: 1.74-64.68 nM). BH-30643 also showed potent activity against mutant HER2s (IC_{50}: 3.25-18.8 nM), especially in the presence of T780M or C805S resistance mutation. Moreover, BH-30643 showed great selectivity in screens against 372 WT human kinases and 87 safety-related targets. The unique profile of BH-30643 offers a promising strategy to target a range of classical and non-classical *EGFR* activating mutations without vulnerability to common on-target resistance mutations. Such an "OMNI-EGFR^{TM}" inhibitor may represent a new generation of EGFR TKIs with potential to overcome some of the limitations of earlier agents.

#5609 A novel PTPN1/2 inhibitor with high oral bioavailability enhances the antitumoral response.

Angelique Pabois, Alessandra Lopes, Clement Bonnel, Joao Marchante, Theodore J. Sanders, Michael Deligny, Alexis Goffaux, Wilfried Cathou, Aurore Smets, Ines Amendoeira Cabral, Marie-Claire Letellier, Berenice Lossignol, Geraldine Genard, Aura Condila, Celine Dotreppe, Margreet Brouwer, Annelise Hermant, Catherine Hoofd, Reece Marillier, Yvonne McGrath, Steven Van der Plas, **Silvia Pandolfi**

iTeos Therapeutics, Charleroi, Belgium

PTPN1/2 phosphatases are major negative regulators of the antitumor immune response. Inhibiting or depleting PTPN1/2 sensitizes cancer and immune cells to interferon gamma (IFNγ), reshapes the tumor microenvironment and triggers a coordinated antitumor immune response involving myeloid cells, NK, T cells, and cancer cells. Although protein phosphatases were long considered undruggable due to their highly polar catalytic pocket, several small molecule inhibitors of PTPN1/2 have recently been discovered. Most of these inhibitors mimic the phosphate of the natural substrate by the 1,2,5-thiadiazolidin-3-one 1,1-dioxide (TDZ) moiety. Although incorporating a TDZ ensures potency while overcoming the instability of the phosphate group, the resulting inhibitors are very polar. As a consequence, most of the TDZ-containing inhibitors suffer from low oral bioavailability. Nevertheless, 2 small molecules have entered clinical trials. We have discovered a small molecule inhibitor of PTPN1/2 with high specificity and human potency comparable to known PTPN1/2 inhibitors. This novel small molecule displays high exposure in preclinical species, due to its high oral bioavailability. To assess the human and mouse cellular potency of our compound, we measured pSTAT1 in cancer cells upon IFNγ treatment and we observed large discrepancies between human and mouse potency for most PTPN1/2 inhibitors. Whereas previously described PTPN1/2 inhibitors show much higher potency in mouse compared to human cells, our novel compound displays higher potency in human compared to mouse cells. Upon in vivo dosing, our PTPN1/2 inhibitor showed efficacy in a syngeneic model, resulting in tumor growth inhibition, target engagement and pharmacodynamic readout. To further determine efficacy in human cells, we tested our compound on human cancer and immune cells, as well as on Patient Derived Tumor Fragments (PDTFs), an innovative ex vivo technique proven to be highly predictive of patient clinical responsiveness to immunotherapy. In this system, drugs are directly applied to slices of fresh human tumors and treatment-induced changes (e.g. cytokines levels) are measured. In both *in vitro* assays and PDTFs, our PTPN1/2 inhibitor showed signs consistent with a robust anti-tumor response in line with the expected mechanism of action, creating confidence that the molecules will engage the immune system in human patients. Overall, this is the first report of a specific PTPN1/2 inhibitor with competitive human potency and high bioavailability, making it an ideal candidate for clinical development.

#5610 BAY 2927088: a novel treatment option for patients with NSCLC with *HER2* mutations.

Franziska Siegel[1], Kristyna Kotynkova[2], Gizem Karsli-Uzunbas[2], Thibaud Jourdan[1], Melanie Berthold[1], Stephan Siegel[1], Daniel Korr[3], Markus Berger[1], Haruna Tomono[2], Sawyer Andersen[2], Daniel Denney[2], Jeremie Mortier[1], Andrew Cherniack[2], Matthew Meyerson[4], Heidi Greulich[2]

[1]Bayer AG, Berlin, Germany,[2]Broad Institute of MIT and Harvard, Cambridge, MA,[3]Nuvisan ICB GmbH, Berlin, Germany,[4]Dana-Farber Cancer Institute, Boston, MA

Lung cancer is the most prevalent tumor type worldwide, and despite advancements in providing patients with improved therapies, the 5-year overall survival rate is still only 5.8%. About 2-4% of patients with non-small cell lung cancer (NSCLC) carry either a *HER2* exon20 insertion or a *HER2* point mutation, which are potent oncogenic drivers.

The antibody-drug conjugate fam-trastuzumab deruxtecan-nxki was the first targeted treatment option to be approved for patients with *HER2*-mutant NSCLC. However, there is still a significant unmet need for more effective and better tolerated therapies with the potential to improve response rates and response durability.

Here we present preclinical data for BAY 2927088, a highly potent and selective tyrosine kinase inhibitor targeting *HER2*. BAY 2927088 shows potent *in vitro* antiproliferative activity in tumor cell lines carrying *HER2* mutations, and strongly blocks *HER2* phosphorylation, thereby inhibiting *HER2*-mediated downstream MAPK signaling. We also confirmed the strong anti-tumor activity of BAY 2927088 in *in vivo* models with *HER2* exon20 insertions.

To further explore the therapeutic potential of BAY 2927088, we evaluated combination treatment with current standard-of-care therapies in NSCLC *in vitro* and *in vivo*. In addition, we profiled BAY 2927088 vs. other *HER2* tyrosine kinase inhibitors by pooled screening of 866 barcoded human cancer cell lines to identify additional oncogenic dependencies and patient populations that might benefit from treatment with BAY 2927088. We found that BAY 2927088 was also active in a subset of *HER2*-amplified breast and gastroesophageal cancer cell lines.

Single-agent BAY 2927088 is currently being evaluated in clinical trials in patients with NSCLC with *HER2* mutations. Preliminary results demonstrate efficacy and safety in pretreated patients (NCT05099172, NCT06452277).

#5611 Preclinical evaluation of SNH-110: A potent, selective, next-generation RET inhibitor overcoming adaptive drug resistances.

Shaomei Zeng[1], Jing Wang[1], Zanyang Yu[1], Xuezhen Wei[1], Xueyu Li[1], Can Yong[1], Zhiqiang Jia[1], Shuchun Zhao[1], Min Li[1], Liyang Wang[1], Nan Li[1], Zhiqiang Li[1], Yuanqing Tang[2], **Jun Tang**[2]

[1]ScinnoHub Pharmaceutical Co., Ltd., Chengdu, China,[2]Chengdu Brilliant Pharmaceutical Co., Ltd., Chengdu, China

Background: Inhibiting RET kinase is a fundamental therapeutic approach for malignancies related to RET alterations. Despite the remarkable efficacy of pralsetinib and selpercatinib in treating various cancers, resistances due to newly surfacing on-target mutations pose a challenge. The emergence of adaptive mutations at the solvent-front, such as G810C/S/R, coupled with the occurrence of concurrent mutations at both the gatekeeper and solvent front regions, render these medications ineffective. Furthermore, off-target VEGFR2 inhibition can lead to the onset or exacerbation of hypertension, frequently necessitating dose adjustments or termination, consequently hindering cancer treatment outcomes. In response to this urgent challenge, we identified SNH-110, a potent next-generation RET inhibitor.

Methods: The selectivity and activity of SNH-110 were assessed via *in vitro* kinase assays, and its potency was evaluated using modified Ba/F3 cell lines. The *in-vivo* efficacy of SNH-110 was further validated in a variety of RET-driven tumor models.

Results: In a comprehensive analysis of 378 kinases, SNH-110 demonstrated a level of selectivity on par with selpercatinib, but without the concerning interactions with JAKs and VEGFR2. In cellular proliferation tests, SNH-110 exhibited robust inhibition of a range of Ba/F3 cell lines expressing various RET mutations. The IC_{50} values indicated a significant enhancement over selpercatinib, particularly in activities related to G810 mutations. Notably, SNH-110 displayed almost 100-fold selectivity against VEGFR2 in a cellular assay, a non-RET target associated with hypertension. At the anticipated effective concentration, SNH-110 showed no inhibitory effects on JAKs, suggesting a reduced risk of lung infection side effects compared to pralsetinib. SNH-110 demonstrated impressive anti-tumor efficacy across various RET-based models. Administered in doses from 0.1 to 3 mg/kg, SNH-110 significantly reduced tumor growth in various murine models, with inhibition rates between 85-146%. In the Ba/F3 KIF5B-RET G810R model, a 3 mg/kg dose of SNH-110 resulted in an exceptional 99% tumor growth inhibition. It's worth highlighting that SNH-110's effectiveness in controlling tumor proliferation increased with the dose across these models.

Conclusions: SNH-110 stands out as a powerful contender in the field of next-generation RET inhibitors. It showcases impressive efficiency in both *in vitro* and *in vivo* studies, working against a broad array of RET mutations. Importantly, it effectively combats resistance prompted by solvent front mutations, while retaining selectivity over JAKs and VEGFR2. Its remarkable potency allows for a daily dosage as low as single-digit milligrams to be effective in humans, as indicated by animal efficacy models. A submission of an IND application to the FDA and NMPA is planned for 2025.

#5612 Taletrectinib, a next generation selective ROS1 inhibitor, inhibits growth of ROS1 wild-type and ROS1-G2032R xenografts.

H. K. Patel, L. Heller, K. Bowman, Z. Hu, Y. Zhang, I. M. Grossi, M. Pan, G. Hattersley;
Nuvation Bio Inc., New York, NY

The ROS1 gene is altered in approximately 2% of non-small cell lung cancer (NSCLC) patients. ROS1 fusions demonstrate ligand independent activity driving cell growth and survival. While several inhibitors for ROS1 have been FDA approved, including crizotinib, entrectinib and, more recently, repotrectinib, each present unique challenges. Resistance mechanisms such as secondary kinase-domain mutations including ROS1-G2032R can limit the efficacy of crizotinib and entrectinib, while repotrectinib can cause central nervous system (CNS) adverse reactions including dizziness, ataxia and cognitive impairment that have been attributed to inhibition of the tropomyosin receptor kinase B, TRKB. Therefore, a need remains for more tolerable and effective treatment options for ROS1 positive cancers.Taletrectinib is an oral, potent, selective and CNS-active next-generation ROS1 inhibitor specifically designed for the treatment of patients with ROS1-positive NSCLC**.** Herein, we describe taletrectinib and its activity in multiple in vitro and in vivo ROS1 fusion tumor models. Additionally, the antitumor activity of taletrectinib in comparison to other ROS1 inhibitors is examined. Taletrectinib exhibits selectivity for ROS1 over other kinases, including TRKB. Taletrectinib exhibits in vivo activity in models harboring ROS1 wild-type (ROS1-CD74, ROS1-SLC34A2) and ROS1-G2032R resistance mutation fusions (ROS1 G2032R-CD74, ROS1 G2032R-SLC34A2). Moreover, taletrectinib prolonged survival (>70 days) of mice implanted intracranially with a ROS1 fusion xenograft model. Our nonclinical data illustrates that taletrectinib has a profile which exhibits efficacy in ROS1 wild-type-, and mutant-driven cancers while maintaining selectivity against TRKB, resulting in an overall favorable profile. Taletrectinib is being evaluated in patients with locally advanced or metastatic ROS1-positive NSCLC in two Phase 2 single-arm pivotal studies: TRUST-I (NCT04395677) in China, and TRUST-II (NCT04919811), a global study. Based on the integrated analysis of the TRUST-I and TRUST-II clinical studies, an NDA has been submitted for taletrectinib to the U.S. FDA for the treatment of patients with locally advanced or metastatic ROS1-positive NSCLC.

#5613 TQB3002: A potent fourth-generation small molecule EGFR-TKI.

Xun Chen, Lihua He, Xin Tian, Fei Liu, Hongjiang Xu, Ling Yang, Wei Zhao, Xiquan Zhang

Chia Tai Tianqing Pharmaceutical Group Co., Ltd., Nanjing, China

Purpose: EGFR-TKIs have become important targeted therapies for patients with EGFR mutation-positive advanced NSCLC. However, EGFR-TKI resistance, particularly the C797S mutation resulted from the use of third-generation EGFR-TKI therapy, presents clear clinical and scientific concern. Therefore, novel EGFR-TKIs that can effectively inhibit triple mutations are urgently needed. Our clinical candidate, TQB3002, can effectively inhibit EGFR single/double/triple mutations.

Method: The HTRF method was used to test the kinase inhibition activities of TQB3002 against $EGFR^{WT}$, $EGFR^{d746\text{-}750}$, $EGFR^{L858R}$, $EGFR^{d746\text{-}750/T790M}$, $EGFR^{L858R/T790M}$, $EGFR^{d746\text{-}750/T790M/C797S}$ and $EGFR^{L858R/T790M/C797S}$. The anti-proliferative activities of TQB3002 were measured in cell lines including PC-9 ($EGFR^{d746\text{-}750}$), NCI-H3255 ($EGFR^{L858R}$), NCI-H1975($EGFR^{L858R/T790M}$), 32D ($EGFR^{d746\text{-}750/T790M/C797S}$, $EGFR^{L858R/T790M/C797S}$), ect. The anti-tumor activities of TQB3002 were studied in animal models, including five CDX models of PC9($EGFR^{d746\text{-}750}$), 32D($EGFR^{d746\text{-}750/T790M}$), NCI-H1975($EGFR^{L858R/T790M}$) and triple mutations PC9($EGFR^{d746\text{-}750/T790M/C797S}$) and 32D ($EGFR^{L858R/T790M/C797S}$), as well as in two Osimertinib-resistant PDX models.

Results: TQB3002 potently inhibited the kinase activities of EGFR single and double mutations, with IC_{50} of 0.01 - 0.12 nM, which was 4 - 12 times better than that of Osimertinib. It had extremely potent inhibitory activities against the C797S mutant kinases which were resistant to Osimertinib, with IC_{50} of 0.01 - 0.093 nM. In EGFR triple mutant cell lines, TQB3002 had strong inhibitory activities with IC_{50} of 0.05 - 0.42 nM. In EGFR single/double mutant cell lines, it also showed superior or equivalent inhibitory activities compared with Osimertinib. In animal models of EGFR single/double/triple mutations, TQB3002 dose-dependently inhibited tumor growth with good animal tolerance. In the EGFR triple-mutation PDX model, TQB3002 could achieve tumor regression at a lower dose (15 mg/kg) compared with other fourth-generation EGFR-TKIs under development.

Conclusion: With a clearly differentiated chemical structure, TQB3002 has shown strong activities both in-vitro and in-vivo against EGFR single/double/triple mutants, and is much more effective against the C797S mutation resistant to third-generation EGFR-TKIs. In addition, TQB3002 has good druggability and supports clinical oral QD dosing. It has good selectivity in EGFR single/double/triple mutant cells (36.5-fold selectivity over WT), and low risk of DDI and in-vitro cardiotoxicity are also observed in preclinical studies. In the GLP toxicological studies, the toxicity of TQB3002 is comparable to marketed third-generation EGFR-TKIs, with no significant toxicity concerns. IND of TQB3002 has been approved in China and the US, and phase I clinical trial has started in China (NCT06662760).

#5614 Advancing treatment for brain tumors and metastases with brain permeable SHP2 inhibitor I-1000233.

Francesca Puca, Alina Ciammaichella, Danilo Fabbrini, Martina Nibbio, Antonino Cucinotta, Giulio Auciello, Francesco Scalabri, Christian Montalbetti, Alessia Petrocchi, Alessandro Carugo, Carlo Toniatti

IRBM S.p.A., Pomezia, Italy

Background: SHP2, a non-receptor protein tyrosine phosphatase encoded by *PTPN11*, plays a pivotal role as a downstream effector of receptor tyrosine kinases (RTKs) in the MAPK signaling cascade. We report the discovery of I-1000233, a potent and selective SHP2 allosteric inhibitor that is orally bioavailable and penetrates the blood-brain barrier (BBB). This compound holds great potential for treating primary brain tumors and brain metastases associated with dysregulated RTK/RAS/ERK signaling pathways.
Experimental Procedures: Through iterative optimization of the physicochemical and pharmacological properties of a lead compound series, we identified I-1000233 as a SHP2 inhibitor with excellent drug-like characteristics. Preclinical studies included *in vitro* assays to assess potency, *in vivo* pharmacokinetics to confirm BBB penetration, and proof-of-concept studies in orthotopic xenograft models of glioblastoma and brain metastases. Combination studies were conducted using other brain-penetrant agents targeting the MAPK pathway. Additionally, patient-derived glioblastoma stem cell (GSC) models were collected to refine a responder identification (ID) strategy for potential application in personalized medicine.
Results: I-1000233 demonstrated potent *in vitro* inhibition of SHP2 activity (IC50 = 2 nM) with prolonged target residence time. *In vivo*, the compound exhibited strong anti-tumor activity in orthotopic glioblastoma and brain metastasis models, both as a monotherapy and in combination with other brain-permeable inhibitors, underscoring its versatility and potential for combination treatment regimens. Moreover, I-1000233 showed efficacy not only in targeting tumor cells bearing EGFR/RAS/MAPK mutations but also in modulating the tumor microenvironment (TME), enhancing the overall anti-tumor response. Further studies using patient-derived GSC models were conducted to identify biomarkers predictive of response, facilitating the development of precision medicine approaches for SHP2-targeted therapies.
Conclusions: I-1000233 is a potent, selective, and orally available SHP2 inhibitor with demonstrated efficacy in preclinical models of primary brain tumors and metastases. Its ability to penetrate the BBB, modulate the tumor microenvironment, and provide combinatory potential with other agents highlights its promise as a therapeutic candidate for treating SHP2-responsive brain cancers.

#5615 ALRN-0192519 is a potent and selective EGFR L858R mutation inhibitor that demonstrates anti-cancer activity in preclinical cancer models as monotherapy or in combination with osimertinib.

Jiaqi Liang[1], Yezhen Zhang[1], Lisha Xie[1], Jihong Chen[1], Xipan Liu[1], Wenheng Liang[1], Tingru Zhou[1], Xiaobin Zhang[2], Tao Ji[2], David Pirovich[2], Xiang Zhai[2], Yipin Lu[2], Ming Yu[2], John Campbell[2], Fang Li[2], Yaoyu Chen[2], **Lina Gu**[2]

[1]Allorion Therapeutics, Guangzhou, China,[2]Allorion Therapeutics, Natick, MA

Epidermal growth factor receptor (EGFR) therapy with small-molecule tyrosine kinase inhibitors (TKIs) is initially effective in patients with EGFR-mutant lung cancer, but resistance to the treatment typically emerges over time. EGFR allosteric inhibitors bind to a distinct site on the EGFR protein, separate from the ATP-binding site targeted by existing EGFR TKIs, offering a potential strategy to overcome resistance associated with EGFR mutations. Here we report the development and preclinical characterization of ALRN-0192519, a novel, orally bioavailable small molecule mutant-selective allosteric EGFR inhibitor. In an enzymatic assay, ALRN-0192519 potently inhibits the activity of EGFR carrying L858R mutation with 50% inhibitory concentration (IC50) in the sub-nanomolar range. A very low hit rate is observed when ALRN-0192519 is assessed in kinome profiling, further revealing the selectivity of this compound. ALRN-0192519 exhibits single-digit nanomolar potency against the proliferation of BaF3 cells carrying different EGFR mutations and is more than 400× fold selective against BaF3 EGFR WT cells. In addition, X-ray crystal structure analysis revealed ALRN-0192519 as an EGFR allosteric binder. ALRN-0192519 led to tumor regression in multiple EGFR dependent CDX and PDX tumor models with daily oral dosing, while showing no effects on tumor growth in the EGFR WT xenograft tumor model at comparable dosing levels. When combined with osimertinib, ALRN-0192519 delayed the tumor regrowth much longer than osimertinib alone, demonstrating the potential advantage of combination therapy. Taken together, these results support that ALRN-0192519 is a promising EGFR allosteric inhibitor for treating lung cancer patients with the EGFR L858R mutation as either monotherapy or in combination with osimertinib.

#5616 Crystal structure of drug-resistant ROS1 G2032R in complex with zidesamtinib, a clinical-stage ROS1 inhibitor with best-in-class potential.

Joseph M. Magrino, Anupong Tangpeerachaikul, Henry Pelish, Scot Mente

Nuvalent, Cambridge, MA

Introduction. *ROS1* fusions are recurrent oncogenic drivers in non-small cell lung cancer (NSCLC). Tyrosine kinase inhibitors (TKIs) crizotinib, entrectinib, and repotrectinib are FDA-approved for treatment of *ROS1*-positive NSCLC. Approximately 40% of patients progressing on crizotinib harbor the ROS1 G2032R resistance mutation, which is thought to sterically clash with and reduce the activity of many ROS1 TKIs. To address this and other medical needs, zidesamtinib was designed to 1) maintain activity for ROS1 and ROS1 resistant mutants, including G2032R; 2) be brain-penetrant, given the high incidence of brain metastases in ROS1-positive NSCLC; and 3) avoid TRK-family kinases, including TRKB, whose inhibition is associated with neurological toxicity. Here, we disclose the first known crystal structure of ROS1 G2032R, solved in complex with zidesamtinib. Analysis of this structure, combined with computational simulations, provides a molecular explanation for zidesamtinib's high affinity and selectivity for this drug-resistant ROS1 mutant.

Methods. ROS1 G2032R was expressed in Sf9/Sf21 cells, purified via standard nickel chromatography, and crystallized in the presence of zidesamtinib via sitting-drop diffusion methods. Using our and other crystal structures, free-energy perturbation (FEP) calculations were conducted with the Schrodinger-Maestro software to predict affinity changes caused by the ROS1 G2032R mutation for various ROS1 TKIs.

Results. We solved a 2.2 Å crystal structure of ROS1 G2032R in complex with zidesamtinib. The aminopyridine moiety of zidesamtinib forms key hinge interactions with the E2027 - M2029 backbone. There is no apparent steric clash between zidesamtinib and R2032—a feature thought to underlie affinity loss for many prior ROS1 TKIs. FEP calculations predicted affinity changes caused by the G-R mutation for various ROS1 TKIs that correlate well with biochemical inhibition data, with zidesamtinib showing the smallest experimental potency shift between ROS1 and ROS1 G2032R. Our modeling also suggests a steric clash between zidesamtinib and TRKA Y591, providing a rationale for selectivity for ROS1 over TRK-family kinases.

Conclusion. Analysis of our ROS1 G2032R crystal structure and FEP modeling provide a structural explanation for how ROS1 G2032R impairs the binding of many ROS1 TKIs while maintaining high sensitivity to zidesamtinib. The data presented here, together with previously disclosed preclinical and clinical data, support our design of zidesamtinib as a differentiated ROS1 TKI with best-in-class potential for the treatment of ROS1-positive NSCLC.

#5617 Development of an initial triple combination therapy targeting EGFR, AXL, and FGFR for EGFR-mutated non-small cell lung cancer.

Ryota Nakamura[1], Tadaaki Yamada[2], Tomoko Yasuhiro[3], Ryohei Kozaki[3], Koichi Takayama[2]

[1]Japanese Red Cross Kyoto Daini Hospital, Kyoto, Japan,[2]Kyoto Prefectural University of Medicine, Kyoto, Japan,[3]Ono Pharmaceutical Co., Ltd., Osaka, Japan

Background: Based on our prior preclinical research, a clinical trial is currently underway to assess the safety and efficacy of combining the third-generation epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) osimertinib with the AXL inhibitor ONO-7475 in treatment-naive patients with advanced EGFR-mutated non-small cell lung cancer (NSCLC) in Japan (jRCT2051210045). However, even with this combined treatment, the development of acquired resistance is expected in clinical settings. Therefore, we aimed to establish a multidrug combination therapy from the first-line setting to eliminate such resistance.
Methods: We investigated adaptive resistance mechanisms to the combination of osimertinib and ONO-7475 and developed a novel therapeutic strategy to overcome them using in vitro and in vivo experiments.
Results: In an siRNA screening assay, knockdown of fibroblast growth factor receptor 1 (FGFR1) significantly inhibited cell viability in the presence of osimertinib plus ONO-7475. The combination of osimertinib and ONO-7475 activated FGFR1 through the upregulation of FGF2 via the c-Myc pathway. In cell-based assays, triple combination therapy with the pan-FGFR inhibitor BGJ398, along with osimertinib and ONO-7475, significantly enhanced cell apoptosis by increasing the proapoptotic factor Bim and inhibited cell viability compared to the combination of osimertinib and ONO-7475 alone. In cell line-derived xenograft (CDX) models, the combination of osimertinib, ONO-7475, and BGJ398 substantially suppressed tumor regrowth. No apparent adverse effects were observed during this treatment.
Conclusions: Our findings demonstrate that the FGF2-FGFR1 signaling pathway contributes to adaptive resistance to the combination of osimertinib and ONO-7475 in EGFR-mutated NSCLC with high AXL expression. The triple combination therapy of osimertinib, ONO-7475, and BGJ398 showed safety and prevented tumor regrowth in CDX models, suggesting that this regimen has the potential to improve outcomes for patients with EGFR-mutated NSCLC.

#5619 An oral BTK degrader TGRX-3911 overcomes resistance conferred by kinase-proficient and kinase-impaired mutations.

Yanxia Shi, Linxin Li, Fengli He, Li Hu, Xiangyu Hong, Tingting Yan, Shuzhen Jiang, Yingying Zuo, Haoran Tang, Yuanpeng Shen, Huanyin Li, Yixin Ai, **Yihan Wang**

Shenzhen TargetRx, Inc., Shenzhen, China

As a key component of the B-cell receptor (BCR) signaling complex, Bruton's tyrosine kinase (BTK) plays an essential role in B-cell malignancies via both kinase and non-kinase functions. This was revealed by early genetic studies and the emerging kinase-impaired mutations, which activate BCR signaling via intricate protein-protein interactions, conferring resistance to a range of covalent or non-covalent BTK inhibitors (BTKi). Therefore, a new targeting strategy is urgently needed for patients who acquired kinase-impaired mutations of BTK. Targeted protein degradation is a potential and powerful means to modulate the non-catalytic functions. TGRX-3911 is a heterobifunctional molecule that promotes ubiquitination and proteasomal degradation of BTK. In a diffuse large B cell lymphoma (DLBCL) cell line TMD8 featuring chronic active BCR signaling, TGRX-3911 induced rapid (4h) and potent degradation of wildtype (WT) and knock-in mutant BTK proteins including the kinase-proficient C481S and T474I, the kinase-impaired L528W and V416L, and even the compound mutants C481S/T474I, C481S/L528W and T474I/L528W, with DC_{50} < 1 nM and near 100% D_{max}. Functionally, in Ramos cells expressing WT or knock-in C481S, T474I, V416L or L528W mutant BTK, TGRX-3911 diminished anti-IgM induced phosphorylation of BTK (excluding V416L and L528W) and PLCγ2, and strongly blocked Ca^{2+} flux. TGRX-3911 also reduced surface CD86 expression in TMD8 cells, as well as in C481S, T474I, V416L and T474I/C481S mutant cells. These results confirm that degradation of BTK induced by TGRX-3911 abolishes the BCR signaling pathway. TGRX-3911 potently inhibited proliferation of TMD8 parental cells and those harboring knock-in BTK mutations that are resistant to currently approved ibrutinib, zanubrutinib and/or pirtobrutinib, including C481S, T474I, L528W, V416L, A428D, T474I/C481S, T474I/L528W and C481S/L528W (IC50 from 0.4 to 9.9 nM). Remarkably, TGRX-3911 potently inhibited the A428D mutant which is still resistant to current BTK degraders, NX-5948, BGB-16673 and ABBV-101. It also demonstrated higher potency against the T474I/L528W compound mutant. *In vivo*, oral dosing of TGRX-3911 led to tumor shrinkage in a mantle cell lymphoma (MCL) REC-1 xenograft model expressing BTK-C481S, and the effect was superior to pirtobrutinib. No sign of toxicity was observed. In summary, TGRX-3911 has potent and robust degradation activity and effectively suppresses the BCR signaling pathway. It has potent anti-proliferation activity against kinase-proficient, -impaired and even compound mutants of BTK, and has *in vivo* anti-tumor activity. By eliminating both the catalytic and scaffold functions of BTK, TGRX-3911 has the potential to overcome clinical resistance which can hardly be achieved by conventional BTKi. The potential clinical application of TGRX-3911 for patients with B-cell malignancies is worth exploring.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Novel Antitumor Agents 3
Poster Session

#5623 Identification of CGT4255 an EGFR sparing, pan-mutant HER2 clinical development candidate with potential best-in-class brain penetration.

Mark J. Chicarelli, Tanna Bettendorf, Abiezer Blandon, Karyn Bouhana, Richard K. Brizendine, LouAnn Cable, Michelle Crow, Brad Fell, John Fischer, Jennifer Fulton, Anna Guarnieri, Leyla Haygood, Maddie Hillman, Ravi Jalluri, Vijay Kumar, Cori A. Malinky, Rob Rieger, John Robinson, Lee Stunkard, Francis Sullivan, John I. Trujillo, Logan E. Vine, Shannon Winski, Yeyun Zhou

Cogent Biosciences, Boulder, CO

HER2 alterations such as amplification, overexpression, insertions, and point mutations, are established oncogenic drivers in many solid tumors. Activating HER2 mutations are found in 2-4% of advanced lung cancers and have emerged as mechanisms of acquired resistance to targeted therapies. Patients with HER2 mutant lung tumors develop more brain metastasis during treatment than patients with any other oncogenic drivers in lung cancer. Addressing brain metastasis in this group of patients remains a clinical challenge with limited therapeutic options. Approved HER2 tyrosine kinase inhibitors have inferior potency against key mutations and lack sufficient brain penetration to be an impactful treatment option for patients with brain metastasis. Herein, we describe advanced preclinical profiling of an EGFR-sparing, HER2 inhibitor clinical development candidate CGT4255 with potential best-in-class brain penetrance and potent activity across prevalent point mutations and exon 20 YVMA insertions. CGT4255 demonstrates robust efficacy in intracranial tumor growth inhibition studies and shows an additive effect on intracranial tumor shrinkage when combined with a HER2 ADC. Pre-clinical studies with the CGT4255 and T-DXd combination demonstrate enhancement of ADC internalization, thus highlighting a biological rationale for combination therapy.

#5624 The antiviral, Daclatasvir, disrupts ASCL1-EZH2 signaling and impairs survival of neuroendocrine prostate cancer cells.

Rohith Guddeti, Jitender Monga, Craig Rogers, Dhananjay Chitale, Shirish Gadgeel, Jagadananda Ghosh

Henry Ford Health System, Detroit, MI

Management of neuroendocrine prostate cancer (NEPC) is extremely difficult not only because available therapeutic regimens cannot effectively kill NEPC cells but also due to the aggressive, highly metastatic nature of NEPC cells to invade distant organs. Development of effective therapeutic agents for NEPC is urgently needed but is suffering from lack of proper understanding about critical and selective molecular targets. Recent molecular analyses revealed that the pseudokinase, Tribbles 2 (TRIB2), is consistently overexpressed both in the treatment emergent as well as *de novo* NEPC cells and tumors and plays a critical role in the survival and maintenance of neuroendocrine characteristics in NEPC cells. Daclatasvir (DCV), an FDA-approved antiviral agent, was found to directly bind and destabilize TRIB2 protein and prime it for degradation via activation of proteasomes. However, the extent to which DCV impacts the viability and lineage characteristics in NEPC cells remain unclear. We report here that DCV inhibits the growth and survival of NEPC cells by inducing caspase-mediated apoptosis. Mechanistically, DCV disrupts the TRIB2 downstream signal transduction cascade which involves the proneuronal transcription factor, Achaete-scute homolog 1 (ASCL1) and the epigenetic regulator, Enhancer of zeste homolog 2 (EZH2). Interestingly, inhibition of the ASCL1-EZH2 signaling axis by DCV showed an increase in the protein level of the androgen receptor (AR). Moreover, we found that DCV at lower, sub-lethal doses synergize with AR inhibitors, such as enzalutamide, to enhance NEPC cell death, suggesting improved efficacy of these two classes of anticancer agents in combination. Since DCV is well-tolerated in humans, and is approved for treatment of viral hepatitis, our findings position DCV as a promising therapeutic agent for NEPC, a highly aggressive, drug-resistant subtype of prostate cancer with extremely limited therapeutic options.

#5625 EphB4 agonist B4-007 was effectively against high-grade serous ovarian cancer (HGSC) as a single agent in cell-based and patient-derived xenograft models.

Qiu-Xu Teng, Chiyi Xiong, Ping Pan, Thanh Chung Vu, Yunfei Wen, **Chun Li**

UT MD Anderson Cancer Center, Houston, TX

Introduction: This study aimed to assess the therapeutic potential and molecular mechanisms of B4-007, an agonist peptide with high binding affinity (Kd = 7.0 nM) to EphB4, a receptor tyrosine kinase implicated in cancer progression and angiogenesis, in preclinical models of high-grade serous ovarian cancer (HGSC).
Methods: EphB4-agonistic activity of B4-007 or its analogues was confirmed by determining tyrosine-phosphorylation levels of EphB4 (p-EphB4) treated with a gradient of B4-007 (500 nM-10 μM) and incubation times (up to 60 minutes). Two orthotopic HGSC models, including a platinum-resistant patient-derived xenograft (PDX2428) model carried by female NOG mice and a human tumor xenograft OVCAR8-ip2-luc carried by female nude mice were used. On 2 weeks after tumor inoculation, B4-007 was injected intraperitoneally (IP) at a dose of 13 mg/kg, 3 times a week for 6 weeks. After 18 doses, all mice were euthanized, and tumor weight and number of tumor nodules in the peritoneal cavity were recorded. For the OVCAR-ip2-luc mouse model, B4-007 was injected via IP at doses of 13 or 52 mg/kg/injection, three times a week for a total of ten doses. Tumor growth was monitored weekly using bioluminescence imaging.
Results: *In vitro*, B4-007 or its analogues induced EphB4 phosphorylation, with maximal phosphorylation observed at 60 minutes at 1.0 μM. Notably, higher concentrations (10 μM) did not yield further increase in p-EphB4 level. *In vivo*, B4-007 demonstrated significant antitumor efficacy in both HGSC models as a single agent. In the PDX2428-NOG model, tumor burden in the B4-007-treated mice was significantly decreased than those in the control mice ($p<0.05$). In the OVCAR8-ip2-luc model, bioluminescence imaging showed that B4-007 at both doses (13 mg/kg and 52 mg/kg) was effective in suppress tumor growth. However, treatment at the higher dose did not provide additional benefit. B4-007 was well tolerated as reflected by stable mouse body weight across each group. These findings underscore the importance of optimizing dosing schedules and suggest that future studies should prioritize lower concentrations (e.g., <13 mg/kg/injection) to determine the minimal effective dose.
Conclusion: B4-007 demonstrated significant anti-tumor efficacy as a single agent without apparent toxicity in orthotopic HGSC models. Our finding has implications for further developing a novel EphB4-targeted therapy to maximize antitumor efficacy and minimize toxicity.

#5626 Exploring a new therapeutic strategy through hyperactivation of the unfolded protein response pathway in bone cancer treatment.

Yoongyeong Lee[1], Michael Mulligan[1], Brooke Bouwens[1], Jenny Drnevich[1], Keith Bailey[2], Michael Spinella[1], David Shapiro[1], Paul Hergenrother[1], Timothy Fan[1]

[1]University of Illinois at Urbana-Champaign, Champaign, IL,[2]Alnylam Pharmaceuticals, Cambridge, MA

Bone cancer is the third leading cause of cancer-related deaths among children and teenagers. Malignant subtypes such as osteosarcoma (OS), Ewing sarcoma (ES), and chondrosarcoma (CS) originate from distinct cell types within the bone. These malignant bone sarcomas pose significant treatment challenges, particularly in cases of relapse or metastasis due to chemoresistance. Therefore, there is a clinical need for newer, effective therapies for recurrent or relapsed disease. This study explores the therapeutic potential of ErSO, a small molecule previously shown to selectively kill estrogen receptor alpha (ERα)-positive breast cancer cells by hyperactivating the unfolded protein response (UPR) and TRPM4. We evaluated anti-cancer activity of ErSO across multiple bone cancer subtypes. Our findings categorized human bone cancer cell lines, including patient-derived xenograft (PDX) cell lines, into ErSO-sensitive and ErSO-insensitive groups. Surprisingly, unlike in breast cancer cells, ErSO sensitivity in bone cancer cells did not correlate with ERα expression. However, ErSO treatment consistently activated ATF6 and PERK in the UPR pathway in ErSO-sensitive OS cell lines. Furthermore, ErSO-induced cytotoxicity was specifically linked to ATF6 activation. Importantly, we discovered that TRPM4 expression on the membrane was a critical determinant of ErSO-induced cell death. Additionally, ErSO-resistant clones derived from ErSO-sensitive OS cell lines consistently showed a reduction in ErSO-induced ATF6 activation and, in some cases, decreased TRPM4 expression. In mouse models of experimental lung metastasis using ErSO-sensitive human OS cells, a single ErSO injection dramatically reduced tumor burden in the lungs. In models with tumor burdens ranging from low to high, ErSO treatment significantly extended survival. In conclusion, ErSO demonstrated potent anti-cancer activity against certain bone cancers via ATF6 activation, acting independently of ERα expression. These effects were particularly dependent on the expression of TRPM4 on the membrane. Our findings highlight the potential anti-cancer activity of ErSO as a therapeutic agent for bone cancer, including advanced cases with lung metastases. Ongoing studies aim to further evaluate the effectiveness of ErSO in models of spontaneous lung metastasis and chemoresistant OS to support its potential for treating late-stage patients.

#5627 Characterization of the Rac inhibitor CPV-337 in metastatic breast cancer.

Jessica Colon Gonzalez[1], Nilmary Grafals Ruiz[1], Anamaris Torres Sanchez[1], Stephanie Dorta Estremera[2], Cornelis Vlaar[3], Suranganie Dharmawardhane[4]

[1]Department of Biochemistry, University of Puerto Rico School of Medicine, San Juan, PR,[2]Department of Microbiology, University of Puerto Rico School of Medicine and Comprehensive Cancer Center, San Juan, PR,[3]Department of Pharmaceutical Sciences, University of Puerto Rico School of Pharmacy, San Juan, PR,[4]Department of Biochemistry, University of Puerto Rico School of Medicine and Comprehensive Cancer Center, San Juan, PR

Rac and Cdc42 are homologous Rho GTPases that act as anticancer and antimetastatic targets regulating cancer progression and metastasis-associated cell functions such as cell survival, cell polarity, proliferation, migration, and invasion. Our lead compound MBQ-167, a dual Rac/Cdc42 inhibitor (IC_{50} of ~100nM; GI_{50} of 130nM), is currently in Phase I clinical trials for advanced triple-negative breast cancer (NCT06075810). By screening a panel of MBQ-167 derivatives to identify molecules with a range of inhibitory mechanisms that address distinct cancer types, we identified CPV-337, with an IC_{50} of ~50nM for inhibiting Rac activation, via pulldown assays to capture the GTP bound Rac, and a GI_{50} of ~55nM for inhibiting the viability of metastatic breast cancer cells, as measured by MTT assays. However, CPV-337 does not affect the viability of human mammary epithelial cells (HMECs), demonstrating non-toxic effects in primary epithelial cells. Additionally, wound healing assays show that CPV-337 reduces breast cancer cell migration by ~50% at 50nM. These data show that CPV-337 is ~2.5X more effective than MBQ-167. We then compared the efficacy of CPV-337 and MBQ-167 in nude mice with orthotopic tumors established from green fluorescent protein-tagged metastatic HER2-positive breast cancer cells. Results demonstrate that CPV-337 administered at 5mg/kg Body Weight (BW) parallels MBQ-167 treatments of 10mg/kg Body Weight (BW) at 5X a week for 2 months. Compared to the vehicle, both CPV-337 and MBQ-167 reduced tumor growth by ~90-95%, from quantification of the integrated density of fluorescent tumors for 62 days. Lung metastases were inhibited by ~50% in CPV-337 treated mice, and both compounds inhibited liver and kidney metastases by ~95%. At necropsy, blood was collected, and whole blood was analyzed by flow cytometry for the phosphorylated form of the Rac and Cdc42 downstream effector p21-activated kinase (p-PAK), using a fluorescent anti-p-PAK^{T423}antibody, as a measure of Rac activation inhibition. CPV-337 inhibited PAK activation significantly by 37.4%, compared to vehicle. Interestingly, a significant increase in M1 anti-tumorigenic macrophages was observed in splenocytes of mice treated with CPV-337, implicating CPV-337 in the anticancer immune response. Further evaluation of CPV-337's inhibitory mechanism, toxicity, bioavailability, and Rac/Cdc42 regulated processes in the tumor microenvironment are warranted to translate this promising anticancer compound to the clinic.

#5629 TEQ103, a novel therapeutic for the treatment of estrogen receptor alpha positive (ERα+) breast cancer.

Marie-Christine Bissery[1], Eef Hoeben[2], Elisabeth Bertrand[3], Sylvie Maubant[3], Maeva Albanese[3], Olivier Duchamp[3], Isabelle St-Jean[4], Mads K. Dalsgaard[1]

[1]Oncoteq AG, Cham, Switzerland,[2]eScienceQPS, Tongerlo, Belgium,[3]Oncodesign Services, Dijon, France,[4]Universite de Montreal, Montreal, QC, Canada

Background: Breast cancer is the most prevalent female cancer. Approximately 75% of them are ERα+ and are treated with ERα targeting agents. However, a good portion will ultimately relapse. TEQ103 (Sera2, ErSO-TFPy) is a first-in-class small molecule acting through the anticipatory unfolded protein response (aUPR) following dysregulation of calcium homeostasis in ERα+ tumor cells. The goal of this work is to further evaluate the efficacy, the influence of schedules of administration and the pharmacokinetics (PK) of TEQ103.

Methods: TEQ103 was evaluated IV in advanced stage human breast adenocarcinoma MCF-7 implanted in athymic female mice. Mice with approximately 500mm^3 tumors were randomized and 2 schedules evaluated, daily x 10, (0, 1, 2, 4, 15 & 30mg/kg/injection), and intermittent on day1 & 10, (1, 5, 20, 75 & 150mg/kg/injection), 9 mice/group. Tumors were measured with a caliper and efficacy endpoints included tumor growth delay (TGD), complete regressions (CR), and Tumor Free Survivors (TFS). PK evaluation was performed post one IV administrations at 5, 20 & 75mg/kg (6 mice/ dose) over 8 hours in plasma, at 4 and 8h post injection in tumors, and at a late 10 day sampling in both matrices. Plasma and tumor concentrations were measured using LC-MS/MS SCIEX QTRAP 6500+ and PK parameters in plasma were determined using noncompartmental methods (Kinetica Software v5.1).

Results: TEQ 103 was found highly active in MCF-7 tumor bearing mice with a large therapeutic index. With the daily schedule, the lowest active dose was 2mg/kg/injection (i.e., 20mg/kg total dose (TD)) with 2/9 TFS and 44 days TGD for the 7 relapsing mice. With the intermittent schedule, the lowest active dose was 5mg/kg/injection (10mg/kg TD) with 4/9 TFS and 56 days TGD for the 5 relapsing mice. At total dosages equal and above 40mg/kg TD, both schedules lead to 100% TFS (CRs lasted 121 days post tumor induction). This indicates that TEQ103 is schedule independent. Overall TEQ103 was well tolerated and did not induce body weight loss in the dose ranges studied. PK profiles in plasma showed biphasic kinetics of TEQ103 in MCF-7 bearing mice. The C_{max} and $AUC_{0\text{-}8h}$ increase was approximately dose-proportional over the dose range studied. The 4h concentrations in plasma and tumor at 5, 20 and 75 mg/kg were 7.25, 16.7 and 178 ng/mL and 582, 1796 and 5709 ng/g, respectively. There was a very good tumor retention with levels of TEQ 103 detectable 10 days post treatment with 75mg/kg dose.

Conclusion: These results demonstrate the significant durable antitumor effect of TEQ103 driven by the total dose administered regardless of schedule of administration, the wide therapeutic index and the concentration in the tumor tissue at levels markedly above the nM IC50 reached in vitro. These studies form the basis for PKPD Tumor Growth Inhibition modeling that will be used for projection of an efficacious starting dose in a subsequent breast cancer patients' phase 1 clinical trial.

#5630 Ecubectedin as a promising therapeutic agent against gastric cancer through WNT/β-Catenin modulation.

Alejandro Losada, Marta Martinez Diez, Eugenio Bustos-Moran, Ismael Fernandez-Miranda, Maria Jose Guillen, Pablo M. Aviles, **Marcelo Lima Ribeiro**, Carmen Cuevas

Pharma Mar, S.A., Madrid, Spain

Gastric cancer continues to be a major global health challenge due to its high mortality rate, with over half of patients showing significant dysregulation of the WNT/β-catenin pathway. Ecubectedin, a novel transcriptional inhibitor, has shown potent antiproliferative effects across several solid tumors. The aim of this study was to characterize the molecular effects of ecubectedin in gastric cancer, both in vitro and in vivo, focusing on transcriptomic analysis of key regulatory pathways. Ecubectedin´s antineoplastic activity was evaluated through MTT assays in a representative panel of 15 gastric cancer cell lines. Eight cell lines were chosen to assess the effects of ecubectedin on the transcriptome following a 6-hour exposure to 50 nM. Ecubectedin´s in vivo antitumor effects were evaluated in HGC-27 xenograft mouse models. Ecubectedin demonstrated potent antitumor activity in vitro, with GI_{50} values in the low nanomolar range (from 0.3 to 8 nM). Ecubectedin exerts a potent effect as a transcription inhibitor, leading to significant repression of 2,775 genes. Gene set enrichment analysis revealed that Ecubectedin disrupted key signaling pathways, especially WNT/β-catenin. At the protein level, Ecubectedin (50 nM) modulated the WNT/β-catenin pathway and inhibited TCF/β-catenin-mediated transcription. In vivo, a single i.v. dose of Ecubectedin (0.6 mg/kg) inhibited tumor growth by 61% after 7 days in both RNA-seq and in vivo protein data (from tumors in ecubectedin-treated mice) support that ecubectedin represses WNT/β-catenin signaling. In conclusion, ecubectedin shows strong antitumor activity against gastric cancer, modulating the often-dysregulated WNT/β-catenin pathway, highlighting its potential as a promising therapeutic for further clinical exploration.

#5631 ZSTK3744, a novel AhR agonist, demonstrates antitumor efficacy in TNBC cell lines *in vitro* and *in vivo*.

Kayoko Kawai-Asami[1], Takahiro Ohashi[2], Sayuri Terada[2], Kotomi Akatsuka[2], Yuko Nagata[1], Shinsuke Hiramoto[2], Tsubasa Nagasaki[3], Eika Higashi[3], Ryuichiro Ohshita[3], Keiko Fukushima[2]

[1]Pharmacology, Department of Drug Discovery, R&D Center, Zenyaku Kogyo Co., Ltd., Tokyo, Japan,[2]Biosciences, Department of Drug Discovery, R&D Center, Zenyaku Kogyo Co., Ltd., Tokyo, Japan,[3]Organic Synthesis, Department of Drug Discovery, R&D Center, Zenyaku Kogyo Co., Ltd., Tokyo, Japan

Background: Breast cancer is the most commonly diagnosed cancer among women worldwide. Triple-negative breast cancer (TNBC) is the most aggressive form of breast cancer and has limited treatment options. TNBC lacks key molecular targets, making it challenging to treat with conventional therapies and limiting the availability of effective treatment options. Herein, we report a novel small molecule, ZSTK3744, which exhibits potent inhibitory effects on the proliferation of TNBC-derived cell lines both *in vitro* and *in vivo*.
Method: We found a compound, ZSTK3744, that exhibits potent antitumor effects against the TNBC-derived cell line MDA-MB-468. We employed DNA microarray analysis and CRISPR-Cas9 technology to determine the molecular targets of ZSTK3744. Mechanistic studies were performed to evaluate ROS generation, DNA damage, and intracellular signaling pathways in ZSTK3744-treated cells. *In vivo*, ZSTK3744 was administered at a dose of 2.5 mg/kg every four days (days 0, 4, and 8) via tail vein injection in an MDA-MB-468 xenograft model. Tumor size and body wight were measured until day 28. In addition, safety was evaluated in dogs with weekly dose escalation to assess tolerability.
Results: ZSTK3744 exhibited potent antitumor activity (GI_{50}: 0.69 nM) against MDA-MB-468 cells. Target analysis identified ZSTK3744 as an aryl hydrocarbon receptor (AhR) agonist and demonstrated that AhR is essential for its growth-inhibitory effects. ZSTK3744 induced ROS generation, DNA damage, and apoptosis in MDA-MB-468 cells in a dose-dependent manner. *In vivo*, ZSTK3744 demonstrated significant anti-tumor activity, reducing tumor size from baseline and maintaining this reduction throughout the 3-week post-administration observation period. Importantly, ZSTK3744 treatment did not adversely affect the body weight of treated mice. In contrast to aminoflavone, a previously developed AhR agonist associated with severe lung toxicity, ZSTK3744 showed no evidence of lung toxicity in dog studies.
Conclusion: ZSTK3744 demonstrated potent antitumor effects on TNBC-derived cells *in vitro* and *in vivo*, as well as having a favorable safety profile. These results suggest that ZSTK3744 is suitable for development as a novel therapeutic agent for TNBC. Ongoing studies are needed to identify biomarkers that predict response to ZSTK3744.

#5632 HDM2006, A novel and potent HPK1 PROTAC, enhances immune cell activation and induces robust tumor growth inhibition.

Liubin Guo, Zhimin Zhang, Xi Yang, Rongyao Zhu, Zhiping Zhang, Sirui Liu, Hao Pan, Zhao Dong, Chunhua Jiang, **Dongzhou Liu**

Global Drug R&D Center, Huadong Medicine, Hang Zhou, China

Background: HPK1 (Hematopoietic Progenitor Kinase 1) is a member of the MAP4K family, which is a group of kinases that catalyze the phosphorylation of serine or threonine residues in proteins. HPK1 is mainly expressed in hematopoietic and immune cells. Loss of HPK1 kinase function increases cytokine secretion, enhances T cell signaling, viral clearance, and tumor growth inhibition. Therefore, HPK1 is considered as a promising target for tumor immunotherapy. Currently, there are several small molecule inhibitors targeting HPK1 in clinical trials, which only inhibit the kinase activity of HPK1, and cannot completely block its downstream signal transduction, and have low selectivity and poor safety. Therefore, we developed a proteolysis targeting chimera (PROTAC) drug, HDM2006, that specifically targets HPK1 while inhibiting HPK1 kinase activity and deleting its skeletal function, and has better efficacy and safety than small molecule inhibitors. The robust monotherapy and combined with anti-mPD-1 efficacy of HDM2006 makes it a IO drug and also a new option for patients with advanced tumors.
Methods: TR-FRET assay were utilized to assess the target binding activity and ternary complex formation activity. Western blot was utilized to investigate the degradation activity of HDM2006 in human T lymphoma Jurkat cells. The effect of HDM2006 on the secretion of IL-2 and IFN-γ by human Pan-T cells was detected by ELISA. *In vivo* anti-tumor efficacy of HDM2006 monotherapy and combined with anti-mPD-1 was evaluated in murine colorectal cancer MC38 cell allograft models. Oral-bioavailability and repeat-dose GLP toxicity studies were profiled in rats and cynomolgus monkeys.
Results: HDM2006 bound to human HPK1 and E3 ubiquitin ligase CRBN, and formed a ternary complex. Meanwhile, HDM2006 targeted and degraded HPK1 in Jurkat cells, with a DC_{50} of 20.68 nM and a D_{max} of 96.36% (24 h). HDM2006 did not degrade other CRBN substrates, avoiding IMiDs off-target effect. HDM2006 can stimulate hPBMCs to secrete IL-2 and IFN-γ, the maximum activation fold can be more than 9.3-41.6 folds. Furthermore, immune activation upon HPK1 degradation was observed even in immune-suppressed environments (PGE2, adenosine, and T cell exhaustion). *In vivo*, HDM2006 showed efficacy in mouse colorectal cancer model in mono and in combination with anti-mPD-1, and TGI was similar to the small-molecule inhibitor at very low dose. In addition, the results of GLP toxicity studies in rats and cynomolgus monkeys indicated that HDM2006 has a large safety margin, and the NOAEL dose exposure was approximately 435.9-fold and 21.4-fold of the optimal effective dose exposure.
Conclusions: HDM2006 is a novel, potent, highly selective and orally active PROTAC targeting HPK1. Preclinical studies have confirmed that HDM2006 has excellent profile for druggability, safety, and efficacy. HDM2006 has entered the clinical Phase 1 trial.

#5633 Rewiring cancer drivers to induce apoptosis in chronic lymphocytic leukemia.

Weiguo Han[1], Aisharya Bhattacharya[1], Basel Karim[2], Wenzhi Ji[2], Roman C. Sarott[2], Meredith Nix[2], Sai Gourisankar[2], Andrey Krokhotin[3], Sutapa Sinha[1], Zhiquan Wang[1], Tait D. Shanafelt[4], Sameer A. Parikh[1], Nathanael S. Gray[2], Gerald R. Crabtree[3], Neil E. Kay[1]

[1]Hematology, Mayo Clinic, Rochester, MN,[2]Chemical and Systems Biology, Stanford University, Stanford, CA,[3]Pathology, Stanford University, Stanford, CA,[4]School of Medicine, Stanford University, Stanford, CA

Chronic lymphocytic leukemia (CLL) is one of the most common types of leukemia among adults in the US. Despite significant improvement of current novel agent treatments, CLL remains incurable with many eventually experiencing disease relapse. Therefore, it remains of high interest to expand therapeutic options. Transcriptional chemical inducers of proximity (TCIPs) are a new class of anticancer drugs designed and developed by Stanford teams. These agents recruit cancer drivers or transcription factors to activate proapoptotic genes' expression and kill cancer cells. Current TCIPs are produced by covalently linking small molecules that bind B-cell lymphoma 6 (BCL6) to those that bind to transcriptional activators, such as bromodomain-containing protein 4 (BRD4) (BRD4-BCL6, TCIP1) or cyclin-dependent kinases 9 (CDK9) (CDK9-BCL6, TCIP2). In collaboration with the Stanford teams, we have initiated TCIPs cytotoxicity assessment in primary leukemic cells from untreated CLL patients and found heterogeneous but evident drug responsiveness with a panel of TCIPs. Our current work has shown that TCIP2 demonstrated remarkable killing ability of CLL B cells with IC50 of 9nM in 72-hours (h), while the IC50 of TCIP1 was 560nM. TCIPs showed time and dose-dependent drug-associated killing in primary CLL B cells over a 24-72h interval using a flow cytometry-based apoptosis assay. After 24, 48, and 72h exposure of CLL B cells to TCIPs, the IC50 was 1054, 820 and 555nM for TCIP1, and 9, 8, and 9nM for TCIP2, respectively. TCIP2 is designed and produced by covalently linking BI-3812 (BCL6 inhibitor) as a BCL6 binder and SNS-032 (CDK9 inhibitor) as a CDK9 binder with a rigid linker. Comparing TCIP2 to its negative controls (TCIP compounds with only one active binder, either BCL6 or CDK9), TCIP2 exhibited significant cytotoxicity in primary CLL B cells. TCIP2 also showed a significant increase in potency, compared to SNS-032 and BI-3812 alone at 72h. Normal human peripheral blood mononuclear cells (PBMCs) were 6-fold less sensitive to TCIP2 compared to CLL PBMCs with high percentage of CLL B cells. Immunoblotting data showed induction of BCL6 protein level after 20nM TCIP1 treatment in CLL B cells. In samples from relapsed/refractory (RR) CLL patients, TCIPs maintained good cytotoxicity with IC50 of 1805nM for TCIP1 and 84nM for TCIP2 in 24h using a cell viability assay. Our study indicates that TCIPs targeting BCL6 and BRD4/CDK9 have a strong capacity to induce high levels of cell death in primary CLL B cells. This notable killing effect may be due to its unique gain-of-function drug mechanism where BCL6 is induced. This is the first study of TCIPs ability to target CLL B cells from both untreated and RR patients. Further studies to more fully evaluate the preclinical activity of TCIPs and investigate the molecular mechanism(s) for TCIP mediated killing of leukemic B cells will help to define the potential role of TCIPs as a novel therapeutic approach in CLL.

#5634 Macrocycle drug conjugate NOVO-111 induces ribosome biogenesis stress and is efficacious in colorectal cancer xenografts.

Jonathan Arambula[1], Garrett Kimble[2], Calvin Chau[3], Michael Buszczak[2], Jonathan Sessler[3], Krystle Karoscik[1], Zahid Siddik[4], Adam Sedgwick[5]

[1]InnovoTEX Inc, Austin, TX,[2]University of Texas Southwestern Medical Center, Dallas, TX,[3]University of Texas at Austin, Austin, TX,[4]MD Anderson Cancer Center, Houston, TX,[5]Chemistry, Kings College London, London, United Kingdom

Purpose: NOVO-111 is a novel macrocycle drug conjugate (MDC) comprised of an expanded porphyrin conjugated to a lipophilic Pt(IV) analogue of oxaliplatin. NOVO-111 has been shown to overcome both multifactorial and transport-defective mechanisms of platinum resistance. While platinum agents often induce DNA damage repair pathways, it has recently been demonstrated that certain agents such as oxaliplatin induce ribosomal biogenesis stress (RBS) as its primary anticancer mechanism. To further assess the clinical translational potential of NOVO-111, ribosome functional assays and efficacy studies involving human colorectal cancer (CRC) models were evaluated.
Procedures: HCT116 cells with both copies of RPL28 SNAP-tagged at the endogenous locus were assessed for RBS. HCT116+L28SNAP tagged cells were cultured and SNAP-tagged RPL28 was labeled with benzylguanine. Cells were then incubated with 2.5uM NOVO-111 at variable time points then incubated with benzylguanine Oregon Green, fixed with 4%PFA, labeled with Hoechst and mounted in anti-fade Vectashield media. For a given cell (N=43 cells), the number of Oregon Green spots observed in the nucleus was recorded and analyzed. Athymic Nude-Foxn1nu female mice (8 mice/group), with subcutaneously implanted (flank) patient derived xenografts (PDX) using CTG-0401 and CTG-0066 received maximum tolerated doses of oxaliplatin (6 mg/kg) and NOVO-111 (70 mg/kg) i.v. on days 1, 5, 9, and 13. The body weights and tumor volumes were monitored. Mice that had reached maximum tumor burden were euthanized.
Results: The data indicate NOVO-111 induces RBS in HCT116+L28 SNAP cells. The number of puncta and mean intensity of RiboSNAP signal in the puncta increase with NOVO-111 treatment while puncta size is reduced. NOVO-111 and control oxaliplatin were further evaluated in nude mice bearing CRC PDX tumors. Minimal to no body weight loss was observed for either treatment group. The treatments resulted in tumor growth inhibitions (TGI) of 94% and 62% for NOVO-111 and oxaliplatin for CTG-0066, respectively. Survival >80 days revealed a significant increase in survival for the NOVO-111 treatment group. Treatment of CTG-0401 bearing mice resulted in TGIs of 59% and 18% for NOVO-111 and oxaliplatin, respectively. Survival >80 days revealed that a mere 25% of the oxaliplatin treatment group survived, compared to a survival of 88% for the NOVO-111 treatment group.
Conclusions: Results indicate that NOVO-111 is capable of inducing RBS as a potential anticancer mechanism in human CRC cells. In addition, NOVO-111 was found to induce a statistically significant tumor growth delay and increase survival in CRC PDX models compared to oxaliplatin. These data demonstrate that NOVO-111, as an MDC, has a distinct advantage over the current standard of care for CRC.

#5635 In vivo assessment of a potent TEAD inhibitor in a panel of malignant mesothelioma PDX model.

Loreley Calvet[1], Odette Dos-Santos[1], Emmanuel Spanakis[2], Anne Caron[3], Laurence Noblet[1], Isabelle Sanchez[1], Yvette Ruffin[1], Laurent Dassencourt[1], Philipe Prigent[1], David Machnik[4], Olivier Venier[5], Laurent Debussche[6], Iris Valtingojer[1], Valeria Fantin[7], **Sukhvinder Sidhu**[1]

[1]Oncology, Sanofi, Virtry-sur-seine, France,[2]Precision Oncology Data Science, Sanofi, Virtry-sur-seine, France,[3]Molecular & Digital Histopathology, Sanofi, Virtry-sur-seine, France,[4]Small Molecules Medicinal Chemistry, Sanofi, Virtry-sur-seine, France,[5]Syngene, Bangalore, India,[6]Dlog Consulting, Paris, France,[7]Oncology, Sanofi, Cambridge, MA

There has been a growing interest in understanding the involvement of the HIPPO tumor suppressor pathway and its downstream effector, Yes1-associated transcriptional regulator (YAP1), in the context of cancer. The YAP1-TEAD complex plays a pivotal role in controlling transcription, influencing cell proliferation, survival, adaptability, and cell migration. Abnormal activation of YAP1 has been documented in various cancer types, including Malignant Mesothelioma (MM). To assess the proportion of MM cases in which YAP1/TEAD-dependent transcription contributes to tumor initiation and progression and to evaluate the pharmacodynamic effects of a new TEAD inhibitor (Compound A), we previously established a gene expression signature associated with YAP1 activity. This signature was employed to monitor the level of YAP1 inhibition in three Cell Line Derived Xenografts (CDX) and eight Patient Derived Xenografts (PDX) of malignant mesothelioma, and it was used to further assess the effectiveness of compound A in vivo. Here we present, for the first time, the potent in vivo antitumor properties of the novel TEAD inhibitor, compound A, in all of these models and we confirm the reliability of the YAP1-transcription signature as a biomarker for patient selection and for assessing the pharmacodynamic effects of YAP1-TEAD inhibition.

#5636 SKY-1214, a small molecule splicing modulator of members of the Fanconi anemia pathway, exerts anti-cancer activity both as single agent and in combination in multiple myeloma and non-Hodgkin's lymphoma.

Simone Rauch[1], Stefan Reber[1], Montserrat Perez-Salvia[1], Marco Pregnolato[1], Botao Liu[2], Loren Berry[2], Olesia Buiakova[2], Hasane Ratni[1], Brian Gudenas[2], Carlo Cusulin[1], Lauren Shanahan[1], Davide Isaia[1], Corinna Spohr[1], Anna Groener[1], Joseph Duffy[2], **Steven Taylor**[2], Veronica Costa[1], Sergey Paushkin[2]

[1]Skyhawk Therapeutics, Basel, Switzerland,[2]Skyhawk Therapeutics, Waltham, MA

The growing multiple myeloma (MM) and non-Hodgkin's lymphoma (NHL) patient population which becomes refractory to available treatments requires new, effective and convenient therapies with novel mechanisms of action. SKY-1214 is a small molecule that modulates splicing of FANCL and FANCI pre-mRNA, causing exon skipping and introduction of premature termination codons leading to the downregulation of FANCL and FANCI mRNAs and proteins. FANCL and FANCI are critical components of the Fanconi anemia DNA damage repair pathway, which contributes to maintenance of genomic integrity. SKY-1214 shows anti-cancer activity as single agent in multiple MM and NHL cell lines, including models of high-risk MM and NHL. In vivo, SKY-1214 treatment results in tumor growth inhibition and regression in MM and NHL cell line-derived xenograft (CDX) models. The combination of SKY-1214 with small molecule agents used for the treatment of MM and NHL, including vincristine, dexamethasone and selinexor, results in improved inhibition of cell growth and viability in multiple cell lines. In vivo, the combinations result in improved tumor growth inhibition and regression and increased durability of effect upon treatment cessation in CDX models. Here, we describe the results of in vitro combination studies of SKY-1214 with antibody-drug conjugates (ADC) and monoclonal antibodies. Cell growth and viability were assessed using cell viability assays (CellTiter-Glo2.0 or FACS-based assays) in tumor cells monoculture or in co-culture with T cells or NK cells to evaluate the combination with monoclonal antibodies. Combination of SKY-1214 with the BCMA-targeting ADC belantamab mafodotin shows additive and synergistic effect in specific concentration ranges resulting in enhanced cell killing in a panel of MM cell lines. Combination of SKY-1214 with bispecific antibodies, including αCD3-αBCMA, in co-culture with T cells shows additive effect. Additive effect is observed also for the combination of SKY-1214 with monoclonal antibodies in co-culture with NK cells. SKY-1214 treatment shows single agent anti-cancer activity in vitro and in vivo in MM and NHL models and combines well with small molecule agents, ADCs and monoclonal antibodies used for the treatment of these disorders. These data, together with the favorable oral bioavailability and pharmacologic characteristics of SKY-1214 with a distinctive mechanism of action, warrants future development in the clinic.

#5637 A novel KIF18A inhibitor for targeting chromosomal instability in cancer.

Lana Kulyk, Shawn Wright, Jill Hallin, Michael Maestre, Joseph Dennis, Iriny Botrous, Anders S. Christensen, Jan Pencik, Craig Gutierrez, Afsheen Banisadr, Jeeyoung Park, Abby Adams, Chang Zhao, Kelly Chen, Stephen Munoz, Shane Yost, Marcelo Lacerda, Angus Voice, Mary L. Anderson, Bo Liu, Matt Welborn, Chao Zhang, Jeff Hager, Fred Manby, Tom Miller, Hui Zhang, Laurent Gomez, Peter Olson, Zhongdong Huang, Chunmei Zhao

Iambic Therapeutics, Inc., San Diego, CA

Anti-mitotic drugs are frequently used to treat cancer and many work by targeting microtubules and disrupting the mitotic phase of the cell cycle. However, dose-limiting toxicity associated with inhibition of cell division in normal cells limits the clinical benefit of this drug class. The spindle assembly checkpoint (SAC) has emerged as a vulnerability for cancer cells with chromosomal instability due to their increased dependence on proper chromosome alignment. KIF18A is a kinesin family motor protein that promotes chromosome alignment by dampening chromosome oscillation. Targeting KIF18A provides an opportunity for cancer-specific dysregulation of the SAC and therefore a significantly improved therapeutic window. Here we describe a novel series of potent KIF18A inhibitors (IC50 < 30 nM) in both KIF18A ATPase biochemical and OVCAR-3 cell growth inhibition assays. These KIF18A inhibitors demonstrated >300-fold selectivity against other kinesins that are essential for various cellular functions. The high selectivity of these inhibitors is reflected in the lack of in vitro toxicity in normal dividing cells and bone marrow hematopoietic progenitor cells. Furthermore, the anti-proliferative activity is highly correlated with biochemical activity, with increased levels of the mitotic phase biomarker pHH3, and with increased arrest of cells in the mitotic phase. Utilizing the predictive power of Iambic's AI-driven platform, compounds in this series were optimized to exhibit a favorable ADME profile, low risk of drug-drug interaction liabilities and good bioavailability across multiple rodent and non-rodent preclinical species. The lead molecule IAM-K1 in this series is negative in the mini Ames genotoxicity test and has favorable predicted human pharmacokinetics and metabolism. IAM-K1 displays >10 uM direct IC50 inhibition against the major human cytochrome P450 enzymes, including 2C9, 2C19 and 3A4, reducing the risk to alter the metabolism of co-administered drugs. IAM-K1 has robust anti-proliferation activity in a number of cell lines, representing different cancer indications, and is approximately 2-3 fold more potent than a reference compound AMG650. Furthermore, IAM-K1 achieves similar PD and TGI efficacy as AMG650 in vivo at much lower systemic exposure. Repeat oral dosing of IAM-K1 led to durable tumor regression in the OVCAR-3 xenograft mouse model without impacting body weight. The anti-tumor activity is correlated with an increase in pHH3 in xenograft tumors, consistent with the on-target mechanism of mitotic arrest. Together with a predictive biomarker approach, targeting KIF18A with IAM-K1 and other compounds in this series is expected to provide a novel anti-cancer treatment to patients with a wide therapeutic window.

#5638 Preclinical and clinical studies of PEP07, a novel brain penetrating Chk1 inhibitor, on solid tumor treatments.

Chieh-Fang Cheng[1], Hui-Ling Chen[1], Feng_Yu Lee[1], Cheng-Hao Liu[1], Chien-Feng Li[2], Hong-Ren Wang[1]

[1]Pharmaengine Inc., Taipei, Taiwan,[2]Chi Mei Medical Center, Tainan, Taiwan

PEP07 is a potent and selective brain penetrating oral Chk1 inhibitor. Chk1 is involved in the DNA damage response and cell cycle regulation and helps maintain the integrity of the genome during cell division, especially in response to DNA damage or replication stress.
PEP07 showed strong activity in repressing cancer cell growth in vitro and in vivo in different solid cancer models. Over 100 cell lines showed IC50<0.5μM toward PEP07 in vitro, and PEP07 achieved over 90% tumor growth inhibition (TGI) in multiple in vivo models either by monotherapy or combined with standard of care (SoC) treatments. With the property of blood-brain barrier (BBB) penetration, PEP07 was able to inhibit brain cancer in orthotopic models when combined with SoC treatments. In addition, in vitro cell proliferation and transcriptome data were used to train artificial neuron network (ANN) models to identify cancer cells that are sensitive to PEP07. Patient-derived tumor cells (PDCs) were used to validate the prediction of sensitive cells by ANN models.
PEP07 is currently being evaluated in clinical studies for both hematologic and solid cancers. PEP07-102 (NCT05983523), a Phase 1, open-label, multi-center PK/PD and dose-escalation study employs an accelerated titration design combined with a traditional 3+3 approach assessing PEP07 as monotherapy in patients with advanced or metastatic solid tumors.

#5639 A potent aminosteroid (RM-581) that inhibits orally in mice the tumor growth in an orthotopic xenograft pancreatic cancer model.

Donald Poirier[1], Qi Chen[2], Rene Maltais[1], Jenny Roy[1], Forkan Ahamed[2], Shannon Arnold[2]

[1]CHU de Quebec Research Center-Universite Laval, Quebec, QC, Canada,[2]University of Kansas Medical Center, Kansas City, KS

Pancreatic cancer has a very poor prognosis, with a 5-year survival rate of only 10%, making it imperative to develop effective small molecules as new drugs. The aim of this study was to validate the in vitro and in vivo efficacy of a promising endoplasmic reticulum-stress aggravator, the synthetic aminosteroid RM-581, that induces apoptosis, as a potential treatment for pancreatic cancer. When tested on a panel of human (PANC-1, MIACaPa2, L3.6, CAPAN-1, BxPC3) and murine (Pan02) pancreatic cancer cells, RM-581 strongly reduced their proliferation (IC50 = 0.83, 0.45, 2.10, 0.78, 5.75 and 1.77 µM, respectively), which justified its evaluation in a PANC-1-Luc cell-derived orthotopic xenograft model in nu/nu mice. After 5 weeks of oral treatment with RM-581 by gavage (po) daily, in vivo fluorescence imaging (IVIS) imaging showed inhibited tumor growth of 11, 54 and 76% at 10, 40 and 80 mg/kg, respectively, compared to the untreated control group. Compared with a cholesterol biosynthesis inhibitor pravastatin (4 mg/kg/subcutaneous (sc)), the effect of RM-581 was similar at 40 mg/kg/po and greater at 80 mg/kg/po. Inhibitions obtained with RM-581 and pravastatin were also confirmed by tumor weight measurement at necropsy. A comparison with antineoplastic drug docetaxel (4 mg/kg/intraperitoneal/2 x weekly) was however not possible, since several toxicities were found after 2 weeks of treatment, forcing euthanasia of the mice. From histological evaluation (hematoxylin and eosin staining), no differences were found in the liver, kidney and spleen in all groups, which is consistent with the clinical observation of no signs of toxicities and no differences in body weight for RM-581 treatments. In summary, RM-581 is therefore a promising candidate for a possible oral treatment of pancreatic cancer.

#5640 The KRAS^{G12D}inhibitor MRTX1133 demonstrates significant antitumor activity and enhances nanoparticle-paclitaxel chemotherapy outcomes in pancreatic cancer.

Mitchel Ramos, Nicola Grimaldi, Sazzad Hassan, Urs von Holzen, Niranjan Awasthi

Indiana University School of Medicine, South Bend, IN

Objective: Pancreatic ductal adenocarcinoma (PDAC) remains a highly lethal malignancy with an extremely grim prognosis. The combination of nab-paclitaxel and gemcitabine (NPT-GEM) represents the standard treatment for advanced and metastatic PDAC, yielding a median survival of approximately 8.5 months. Genetic studies have identified *KRAS, TP53, p16/CDKN2A*, and *SMAD4* as the most commonly mutated genes in PDAC, with *KRAS* mutations occurring in over 90% of cases, and playing a central role in tumorigenesis. The *KRAS*-mutation subtypes most common in PDAC are G12D (~49%), G12V (~30%), and G12R (~12%). Recent advances in direct KRAS inhibitors provide new opportunities for improving PDAC therapy. MRTX1133, a novel KRAS inhibitor, specifically targets the *KRAS^{G12D}* mutation in both active and inactive states, selectively inhibiting *KRAS^{G12D}*-mutant tumor cells while sparing wild-type KRAS, highlighting its significant therapeutic potential for PDAC.

Methods: *In vitro* proliferation assays were performed on PDAC epithelial cells harboring *KRAS^{G12D}*-mutations, *KRAS^{G12C}*-mutations, or KRAS^{wt} using the colorimetric WST-1 method. Protein expression levels were assessed via Immunoblot analysis. Tumor growth studies were conducted using 4-6-week-old female NOD/SCID mice with AsPC-1 subcutaneous xenografts.

Results:*In vitro* assays demonstrated dose-dependent inhibition of proliferation in *KRAS^{G12D}*-mutant PDAC cell lines (AsPC-1, HPAF-II, SW-1990) treated with MRTX1133 and NPT-GEM. A synergistic effect was observed with the combination therapy. MRTX1133 alone had minimal effects on *KRAS^{G12C}*-mutant Mia PaCa-2 cells and *KRAS^{wt}* BxPC-3 cells. Immunoblot analysis of KRAS^{G12D}-mutant cells (AsPC-1 and HPAF-II) revealed that MRTX1133 treatment reduced phosphorylation of ERK, AKT, S6, MEK, RAF, while increasing apoptosis marker proteins cleaved-PARP-1 and cleaved-caspase-3. In vivo tumor growth inhibition studies exhibited significant delays in tumor growth with MRTX1133 and NPT-GEM, with their combination showing synergistic effects. Compared to the control (average net tumor growth: 327 mm^3), NPT-GEM reduced growth to 129 mm^3, MRTX1133 reduced it to 56 mm^3 and the combination resulted in tumor regression (-4 mm^3). Tumor weight findings corroborated these results. Importantly, no body weight changes were observed in treated mice, indicating no discernable toxicity associated with MRTX1133, NPT-GEM, or their combination.

Conclusion: These findings highlight the significant therapeutic potential of MRTX1133, particularly in combination with standard chemotherapy, for the clinical management of *KRAS^{G12D}*-mutant PDAC patients.

#5641 Role of ceramide synthase 2 in drug-induced ER-stress, Ca2+ release and apoptosis in triple-negative breast cancer cells.

Hissah Alatawi[1], Haritha Nair[1], Garrette Timothy[2], Vulpe Christopher[3], Sharma Arun[4], Narayan Satya[1]

[1]Anatomy and Cell Biology, University of Florida, Gainesville, FL,[2]2Department of Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL,[3]Department of Physiological Sciences, University of Florida, Gainesville, FL,[4]4Department of Pharmacology, Penn State Cancer Institute, Hersey, PA

Breast cancer can be classified in five subgroups: Luminal A (ER+/PR+/HER2-), Luminal B (ER+/PR-/HER2- or ER+/PR+/HER2+), ER-/PR-/HER2+, and ER-/PR-/HER2- (triple-negative). Significant progress has been made in the treatment of hormone-responsive and HER2-overexpressing breast cancer; however, the treatment for TNBC is underdeveloped due to lack of appropriate targeting. Further, all subtypes of breast cancer quickly become resistant to the current available therapy. Hence, there is an urgent need to develop a therapeutic strategy that targets a common molecular pathway, irrespective of their biological markers. Recent studies recognized lipid metabolism as one of the important cancer hallmarks. Among many lipids, ceramides play a crucial role in cell signaling, apoptosis, and the regulation of cell growth and differentiation. Very long-chain fatty acid (VLCFA)-ceramides (C20-C26), which are synthesized by ceramide synthase 2 (CerS2), suppress cancer cell invasion and metastasis. The decreased expression of CerS2 is known as a poor prognostic factor for BC patients. The reduction in CerS-2 expression may be due to genetic mutations, epigenetic modifications, or changes in the cellular microenvironment. Thus, the increased level or the activity of CerS2 can be a better prognostic factor for BC patients. In this effort, we have synthesized a novel biisoquinoline-derivative, DH20931 (US Patent ID: US9914733B2) that causes death of breast cancer cells, including TNBC cells. The spatial molecular modeling and enzymatic assays show that DH20931 interacts with and stimulates ceramide synthase 2 (CerS2) activity, reduces the growth of hormone-responsive, HER2-overexpressing, and TNBC cells. DH20931 treatment increases the level of VLCFA-ceramides, induces lipotoxic endoplasmic reticulum (ER) stress, and ATF4/CHOP/PUMA pathway of apoptosis. Here, we show an additional apoptotic pathway that is linked with DH20931-mediated ER-stress. DH20931 induced ER-stress causes the release of Ca2+ from ER to mitochondria, leading to the release of cytochrome c, that in turn leads to significant increase of apoptosis in TNBC cells. We provide evidence that CerS2 plays an important role in the release of Ca2+ from ER to mitochondria where it forms an active IP3R1 channel complex and reduces the distance between ER and mitochondria. Based on Swiss-ADME analysis and laboratory experiments, DH20931 possess all the properties of a druggable molecule and obeys the Lipinski-rule-of-five. Thus, DH20931 can be developed as a novel therapeutic agent that can induce apoptosis by ATF4/CHOP/PUMA axis and Ca2+-dependent pathways, which are independent to ER, PR and HER2 status. The use of this therapy can potentially reduce the morbidity and mortality of women of all races and ethnic groups with BC of hormone-responsive, HER2-expressing, and TNBC.

#5642 Datopotomab deruxtecan (Dato-DXd), a TROP2 directed antibody-drug conjugate, demonstrates remarkable antitumor activity in uterine serous carcinoma.

Michelle Greenman, Cem Demirkiran, Stefania Bellone, Tobias Hartwich, Blair McNamara, Victoria Ettorre, Niccolo G. Santin, Namrata Sethi, Luca Palmieri, Yang Yang-Hartwich, Katyayani Papatla, Elena Ratner, **Alessandro D. Santin**

Yale University, New Haven, CT

Objective: Uterine serous carcinoma is a rare biologically aggressive type of endometrial cancer associated with a high rate of mortality. Datopotamab deruxtecan (Dato-DXd) is a novel ADC composed of the humanized anti-TROP2 IgG1 monoclonal antibody, a cleavable tetrapeptide linker, and a topoisomerase I inhibitor payload. TROP2 expression is detected in up to 95% of uterine serous carcinoma (USC) cases, making it an excellent target for therapy. We evaluated the preclinical activity of Dato-DXd *in vitro* against primary USC cell lines with various TROP2 expression and *in vivo* in TROP2 overexpressing cell-line derived xenografts in mice.

Methods: Primary USC cell lines were established from chemotherapy-naïve patients at time of surgery and analyzed by flow cytometry for TROP2 expression. USC cell lines with varying TROP2 expression levels were treated with scalar concentrations of Dato-DXd or a non-targeting ADC isotype control (CTL ADC). Flow cytometry-based assays were used to evaluate viability. RepresentativeTROP2 expressing USC were co-cultured with a TROP2 non-expressing USC cell line to evaluate bystander effect. Four-hour chromium release assays were used to evaluate Dato-DXd ability to induce antibody dependent cell cytotoxicity (ADCC) in vitro. Finally, mice xenograft models were established from a selected TROP2 3+ cell line (ie, USC-ARK2) to evaluate the *in vivo* activity of Dato-DXd vs treatment with vehicle, datopotomab (unconjugated antibody), or control (CTL) ADC with an endpoint of overall survival.

Results: *In vitro*, TROP2 overexpressing cell lines demonstrated remarkable sensitivity to Dato-DXd compared to CTL ADC. When high TROP2 USC cells were admixed with TROP2 low-expression cells, significant bystander cytotoxicity was observed ($p=0.03$). Mechanistically, double stranded DNA breakage was demonstrated by increased phosphorylation of the histone H2AX in TROP2 positive cell lines after DATO-DXd exposure vs CTL ADC. In the TROP2 selected cell lines, both Dato-DXd and datopotomab were effective in inducing significant ADCC when challenged with heterologous peripheral blood lymphocytes ($p= \leq 0.05$). *In vivo,* Dato-DXd demonstrated a significant survival advantage in mice xenografted with ARK2 compared to the other treatment groups ($p<0.0001$). By day 10, ARK-2 xenografted mice treated with Dato-DXd had significantly decreased growth when compared to the other three treatments which experienced continued linear growth. The median survival for Dato-DXd was not reached as all mice were still alive at the conclusion of the study on day 60 ($p<0.0001$).

Conclusions: Dato-DXd demonstrates remarkable preclinical activity both *in vitro* as well as *in vivo* against biologically aggressive USC primary cell lines and xenografts. Clinical trials are warranted.

#5643 Aryl hydrocarbon receptor agonist ZSTK3744 overcomes chemotherapy resistance in TNBC.

Takahiro Ohashi[1], Kayoko Kawai-Asami[2], Sayuri Terada[1], Kotomi Akatsuka[1], Yuko Nagata[2], Shinsuke Hiramoto[1], Tsubasa Nagasaki[3], Eika Higashi[3], Ryuichiro Ohshita[3], Keiko Fukushima[1]

[1]Biosciences, Department of Drug Discovery, R&D Center, Zenyaku Kogyo Co., Ltd., Tokyo, Japan,[2]Pharmacology, Department of Drug Discovery, R&D Center, Zenyaku Kogyo Co., Ltd., Tokyo, Japan,[3]Organic Synthesis, Department of Drug Discovery, R&D Center, Zenyaku Kogyo Co., Ltd., Tokyo, Japan

Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer with a poor prognosis compared with other subtypes. Due to the lack of therapeutic targets, such as hormone receptors and human epidermal growth factor receptor 2, systemic chemotherapy remains the primary treatment option for TNBC. However, chemotherapy has limited benefits and some patients acquire resistance, further complicating treatment. New therapeutic approaches are urgently needed to overcome chemotherapy resistance in TNBC. ZSTK3744 is an aryl hydrocarbon receptor agonist developed as a novel therapeutic candidate for TNBC. ZSTK3744 has shown inhibitory effects on the proliferation of various cancer cell lines, including TNBC cell lines, *in vitro*. Moreover, it suppressed tumor growth in TNBC xenograft models. In this study, we evaluated the effects of ZSTK3744 on chemotherapy-resistant TNBC cells. Chemotherapy-resistant MDA-MB-468 cells, derived from TNBC, were developed through long-term exposure to adriamycin or paclitaxel. Compared to wild-type cells, the anti-tumor effects of adriamycin, paclitaxel, and eribulin were weakened in these resistant cells; however, ZSTK3744 maintained its efficacy. Transcriptome analysis was conducted to elucidate the mechanism of chemotherapy resistance, revealing alterations in several resistance-associated genes. In particular, upregulation of the ATP-binding cassette transporter 1 gene (ABCB1) was commonly observed in these cells. The anti-tumor effects of adriamycin, paclitaxel, and eribulin were reduced in cells with high ABCB1 expression, whereas those of ZSTK3744 were unaffected. Moreover, ZSTK3744 significantly inhibited tumor growth in xenograft models implanted with adriamycin-resistant MDA-MB-468 cells, showing a higher potency than eribulin. ZSTK3744 is a promising agent for overcoming chemotherapy-resistance in TNBC.

#5644 Identification of alexidine as a novel small molecule inhibitor of TAZ-TEAD interaction in breast cancer cell migration.

A. Ge[1], Y. Hao[1], K. Klupt[1], Z. Jia[1], Y. Du[2], H. Fu[2], X. Yang[1];
[1]Queen's University, Kingston, ON, Canada, [2]Emory University, Atlanta, GA

Breast cancer (BC) is the most commonly diagnosed malignancy in women, with a risk of distant metastases after a primary tumor has developed. Despite concerns about the aggressive pathology of metastatic BC (MBC), current medical interventions inadequately address the clinical needs of MBC patients, resulting in worse outcomes compared to other BC cases. This highlights the critical requirement for more effective therapies for MBC patients. The Hippo pathway is often dysregulated during tumorigenesis, and one of the Hippo signaling transducers, Transcriptional co-activator with PDZ-binding motif (TAZ), is involved in multiple stages of BC development. Since TAZ mostly interacts with TEAD to facilitates its function, targeting TAZ-TEAD interaction may be a treatment approach for MBC patients. To identify small molecules that inhibit TAZ-TEAD interaction, we established a highly sensitive NanoLuc and TR-FRET applicable biosensor for quantifying protein-protein interaction of TAZ and TEAD. Using this biosensor, we performed an ultra-high throughput screen (uHTS) and identified alexidine as a novel TAZ-TEAD binding inhibitor capable of suppressing TAZ-induced migration and invasion in BC cells. In conclusion, we describe a robust method for screening inhibitors of TAZ-TEAD interaction, contributing to the development of effective cancer treatments.

#5645 Targeting transcription-replication conflict for selective chemotherapy in glioblastoma.

L. Gu, R. J. Hickey, L. H. Malkas;
City of Hope National Medical Center, Duarte, CA

The current standard of care for glioblastoma (GBM) is surgical intervention followed by a combination of temozolomide (TMZ) and radiation therapy. Only 5% GBM patients survive more than five years despite aggressive therapeutic treatment. A significant hurdle to develop target-based therapies for GBM is the complexity and diversity of the disease. Genomic and epigenetic profiling has found a broad range mutational and transcriptional events across this histologically diverse disease. A hallmark of GBM is genomic instability, which is a key factor in its progression and complexity. The fast growing and aggressive GBM cells demand rapid DNA replication and hypertranscription, leading to enhanced transcription-replication conflicts (TRCs). A major source of genomic instability, TCR causes lethal double stranded DNA breaks (DSBs) if not resolved properly. We hypothesize that enhanced TRC is a weak link and a promising target for treating GBM.

Proliferating cell nuclear antigen (PCNA) is a key component of DNA replication machinery and plays an essential role in the DNA replication and repair. Through a rational drug design, we identified a small molecule PCNA inhibitor, AOH1996, which selectively kills cancer cells. Mechanistically, AOH1996 enhances the interaction between PCNA and the largest subunit of RNA polymerase II, RPB1, and dissociates PCNA from actively transcribed chromatin regions. By interfering with TRC resolution as evidenced by induction of R-loop levels in cancer cells, AOH1996 induces DSBs in a transcription-dependent manner. Orally administrable and metabolically stable, AOH1996 can freely pass the blood brain barrier and enter brain tumors. Here, we report that it selectively kills GBM cells as a monotherapy or in combination with TMZ but causes no discernable side effects. Importantly, AOH1996 targets a cancer specific region of PCNA that is a highly conserved through evolution and influences the activity of broad cellular processes that are essential to the growth and survival of cancer cells. GBM cancer cells are, therefore, less likely to develop resistance to AOH1996 through mutagenesis in PCNA. If combined with the existing chemotherapeutic regime and inhibitors of DNA repair pathways, AOH1996 may provide a new and unique therapeutic avenue for exploiting this cancer-selective vulnerability to deliver a durable response in GBM.

#5646 A novel *in vivo* self-assembled peptide-drug conjugate targeting HER2 with potent anti-tumor efficacy overcoming resistance of HER2 ADCs.

Gantian Lyu, Min Han, Yuzhen Qian, Yingbin Zhang, Lin Fu, Hao Wang

Isap (Beijing) Biotechnology Co., Ltd., Beijing, China

Background: Several HER2-targeting antibody-drug conjugates (ADCs) have demonstrated significant clinical effectiveness on HER2-expressing cancer. However, these ADCs still face challenge of disease progression in most patients due to diverse resistance mechanisms. Herein, we report preclinical studies of iSAP-0913, a novel *in vivo* self-assembled peptide-drug conjugate (PDC) targeting HER2. iSAP-0913 demonstrates significant antitumor activity in both *in vitro* and *in vivo* models, overcoming the resistance of HER2 ADCs with Topo I inhibitor payload.

Method: The strategy of *in vivo* self-assembly was leveraged to construct iSAP-0913 with a HER2 targeting peptide possessing binding epitopes located in domain II of HER2, a tubulin inhibitor MMAE, and a self-assembling peptide linker. A series of *in vitro* assays were conducted to evaluate biological functions and anti-tumor activities. The *in vivo* anti-tumor efficacy was assessed in a DS8201-induced NCI-N87 resistance model. Preliminary safety of iSAP-0913 was evaluated by repeated dose toxicity (DRF) study in cynomolgus monkeys.

Results: iSAP-0913 binds HER2 receptor to form nano-assemblies on tumor cells, contributed by the design of self-assembling peptide sequence in linker. As a result, *in vivo* self-assembled PDC monomers accumulate in tumor lesions through assembly induced retention (AIR) effect. In NCI-N87 cells, the payload delivery efficiency of iSAP-0913 was 2-3 times higher than that of HER2 ADCs. Desorption Electrospray Ionization imaging results demonstrated that iSAP-0913 exhibits significantly enhanced tumor tissue penetration compared to HER2 ADCs (DS8201, RC-48) in NCI-N87 xenograft tumor models. iSAP-0913 induced tumor-growth suppression, even regression in *in vivo* models with 1-3 mg/kg dosage. iSAP-0913 also showed more effective tumor suppression with TGI% of 73.4% in xenograft models established with DS8201 induced resistant cells, whereas DS8201 exerted negligible anti-tumor activity (TGI%<19.6%). Tissue distribution studies in NCI-N87 tumor-bearing mice revealed that both iSAP-0913 and MMAE are rapidly cleared from plasma. However, MMAE exhibited an elimination half-life of up to 57 hours in the tumor lesion, which may be attributed to the AIR effect. Preliminary repeat-dose toxicological assessment of iSAP-0913 in pharmacologically relevant species cynomolgus monkey demonstrated that iSAP-0913 is well tolerated with no signs of lung toxicity. TK data showed that free MMAE in plasma is 1/100th of iSAP-0913, without MMAE accumulation during dosage.

Conclusion: Comprehensive preclinical data have supported iSAP-0913 as a promising PDC candidate for HER2-low expression and/or patients insensitive to other HER2-targeting ADCs, particularly as a sequential treatment following resistance to HER2 ADCs. iSAP-0913 is now undergoing preclinical development.

#5647 Targeting HAUS7/gamma-tubulin interaction with HTIP1 suppresses tumorigenesis in esophageal squamous cell carcinoma.

Z. CAI, Y. I. WONG, Y. S. LAW;
The University of Hong Kong, Hong Kong, Hong Kong

Background: Unlimited cell division resulting from cell cycle deregulation is a cancer hallmark. We hypothesized targeting deregulated cell cycle molecule with prognostic significance could be viable to counteract tumorigenesis. Our on-going effort showed HAUS7 was such a molecule highly relevant to esophageal squamous cell carcinoma (ESCC). This research targeted HAUS7/gamma-tubulin interaction using a novel peptide HTIP1, which was strategically designed based on our new understanding on the therapeutic mechanism associated with HAUS7 suppression (attached figure).
Methods: Clinical relevance of HAUS7 upregulation was studied in local and public ESCC cohorts. Physiological microtubule-regulating and tumorigenic functions of HAUS7 relative to other HAUS members were examined in vitro and/or in vivo. A multidisciplinary approach, which included using RNA-seq, mass spectrometry, electron microscopy, in silico predictions, bioinformatics analysis, biochemical methods, cellular assays, immunoassays and animal models, was employed to decode the mechanism underlying HAUS7 suppression. HTIP1 was designed by structure modelling and docking simulation. Its properties, cancer selectivity, anti-tumor efficacy, off-target effect and mechanism of action were comprehensively evaluated. Patient-derived tumor xenograft models were used to provide preclinical evidence.
Results: Among HAUSs, HAUS7 was most essential for regulating microtubule dynamics in ESCC. Its overexpression was correlated with aggressive tumor phenotypes and poor prognosis. Mechanistically, HAUS7 suppression released its binding partner g-tubulin. The free g-tubulin upon phosphorylation seized LMAN1 and together both of them relocalized to the nucleus. Given the protein-transporting function of LMAN1 at endoplasmic reticulum (ER), its removal from the innate location triggered ER stress and apoptosis. Capable to perturb HAUS7/gamma-tubulin interaction, HTIP1 treatment manifested similar anti-tumor effects like HAUS7 suppression, while demonstrating high tumor specificity, negligible off-target effects and more potent than relevant compounds.
Conclusions: We first discovered a unique physiological function and pathological relevance of HAUS7 among other HAUSs in ESCC. A new therapeutic mechanism was dissected under HAUS7 suppression. To target cancer with HAUS7 dependency, a novel peptide HTIP1 was designed to interrupt HAUS7/gamma-tubulin interaction. This invention paved a new strategy to counter tumorigenesis. By translating the scientific evidence to support further R&D of HTIP1, our ultimate goal is to produce a brand new drug.

#5648 Effects of olverembatinib (HQP1351) in combination with BCL-2 inhibitor lisaftoclax (APG-2575) in T-cell acute lymphoblastic leukemia (T-ALL).

Bo Peng[1], Yan Xiong[1], Zhiyan Liang[2], Ping Min[1], Huidan Yu[1], Bingxing Wu[1], Guoqing Zhai[1], Zhou Yu[1], Dajun Yang[1], Yifan Zhai[1]

[1]Ascentage Pharma (Suzhou) Co., Ltd., Suzhou, China,[2]Ascentage Pharma Group Inc., Rockville, MD

Background: T-ALL is a high-risk hematologic cancer arising from malignant transformation of T-cell progenitors, affecting approximately 15% of newly diagnosed pediatric and 25% of adult ALL cases. Survival in children, young adults, and patients with relapsed or refractory disease is poor with limited treatment options. Some T-ALL subtypes depend on pre-TCR/Src signaling and antiapoptotic BCL-2 family proteins for growth and survival. Olverembatinib, a novel multikinase inhibitor, targets oncogenic Src-family kinases (Lck, Fyn, and YES1) essential for T-cell differentiation, survival, and activation. Lisaftoclax is a novel BCL-2 inhibitor under investigation in late-stage clinical trials for multiple hematologic malignancies. Here, we examined the antitumor effects of olverembatinib combined with lisaftoclax in human T-ALL cell lines and explored potential mechanisms of action.

Methods: Cellular proliferation was measured using CellTiter-Glo® assays and apoptosis by flow cytometry. Western blotting was used to elucidate potential mechanisms of action. *In vivo* activity was evaluated using a subcutaneous MOLT4 cell-derived xenograft mouse model.

Results: Olverembatinib in combination with lisaftoclax exhibited strong synergistic antiproliferative effects in T-ALL cell lines MOLT4 and Jurkat, with combination indices of 0.2 and 0.1, respectively; apoptosis analyses revealed that the combination significantly increased the percentage of apoptotic cells compared to either agent alone ($p < 0.0001$). Olverembatinib 10 mg/kg or lisaftoclax 100 mg/kg alone resulted in no or limited tumor inhibition in MOLT4 xenograft model, with treatment-to-control (T/C) values of 113% and 89%, respectively. However, when co-administered, the combination enhanced tumor growth inhibition (T/C, 60%), achieving a synergy index of 1.67. Mechanistically, olverembatinib inhibited Lck activity, and when combined with lisaftoclax, synergistically reduced activity of downstream transcription factor NF-kB p65, leading to downregulation of the key antiapoptotic protein BCL-xL, which is typically upregulated in BCL-2 inhibitor-resistant T-ALL. In addition, the combination downregulated phosphorylation of AKT and GSK3b kinases, resulting in significant degradation of antiapoptotic protein MCL-1 and c-myc, an essential pro-survival oncogene in T-ALL. Increased cleavage of PARP and caspase-3 (markers of apoptosis) was also observed.

Conclusions: Olverembatinib in combination with lisaftoclax demonstrated synergistic antitumor effects in T-ALL, providing a scientific rationale for further clinical evaluation of this novel combination therapy in patients with T-ALL.

#5649 Potentiation of AKR1C3- and P53-dependent AST-3424 activity via PUMA-mediated degradation of Rad51.

J. Duan[1], T. Qi[1], Y. Wang[1], X. Liu[1], Z. Lu[1], J. Yu[1], X. Cai[1], A. Li[1], D. Jung[1], C. Xun[2], Y. Xie[1], F. Meng[1], S. Qin[3];
[1]Ascentawits Pharmaceuticals, LTD, Shenzhen, China, [2]The First Affiliated Hospital of China Pharmaceutical University, Nanjing Tianyinshan Hospital, Hepatobiliary and Pancreatic Tumor Department., Nanjing, China, [3]GI Cancer Center, Nanjing Tianyinshan Hospital affiliated to China Pharmaceutical University, Nanjing, China

AST-3424 is a novel, highly tumor-selective prodrug activated by tumor-specific AKR1C3 to release AST 2660, a toxic bis-alkylating moiety that induces DNA alkylation and cross-linking. Currently undergoing evaluation in various clinical trials for solid and hematologic malignancies, AST-3424 has demonstrated promising efficacy in a Phase II study as a later-line monotherapy for advanced hepatocarcinoma, particularly in patients who have progressed after immunotherapy. Subgroup analysis suggests enhanced clinical benefit in patients with wild-type P53. This study elucidates the mechanism underlying AST-3424's enhanced therapeutic effect in cancer cells expressing high levels of AKR1C3 and wild-type P53. Co-treatment with AST-3424 and the P53 activator nutlin-3 significantly potentiated AST-3424's pharmacological activities, including *in vitro* cytotoxicity, reduced colony formation, apoptosis induction, DNA damage (γH2AX), and G2/M phase cell cycle arrest. Targeted deletion of P53 in P53 wild type cells markedly attenuated AST-3424 activities without affecting its AKR1C3 dependence, highlighting P53's pivotal role in cancer cell sensitivity to AST-3424. Given RAD51's crucial role in homologous recombination repair and genomic stability maintenance, we investigated its involvement in P53-dependent AST-3424 activity. Our findings reveal that AST-3424 activates the P53 signaling pathway while concurrently reducing RAD51 expression exclusively in P53 wild type cells. This P53-dependent reduction of RAD51 is mediated by upregulation of PUMA, which promotes RAD51 ubiquitination and diminishes its half-life. Importantly, the effects of AST-3424 on P53 signaling, RAD51, and PUMA were entirely dependent on AKR1C3, as evidenced by complete reversal of these effects upon treatment with the AKR1C3-specific inhibitor AST-3021. The AKR1C3 dependence confers tumor specificity to AST-3424's regulation of P53, RAD51, and PUMA, as AKR1C3 is highly expressed only in tumor cells. In conclusion, this study demonstrates that AST-3424's pharmacological activity is enhanced in cancer cells with high AKR1C3 expression and wild-type P53. We elucidate the mechanism of tumor-specific enhancement associated with P53-dependent RAD51 degradation, leading to decreased homology-directed DNA repair and synthetic lethality. These insights have significant implications for optimizing AST-3424's antitumor efficacy and potentially improving survival rates in patients with functional P53.

#5650 Small molecule CB001 downregulates cFLIP, potentiates the effects of androgen receptor inhibitors in prostate cancer models, and enhances NK cell activity.

Claire Lin[1], Connor Purcell[2], Shengliang Zhang[2], Vida Tajiknia[2], Wafik S. El-Deiry[2], Benedito A. Carneiro[3]

[1]The Warren Alpert Medical School of Brown University, Providence, RI,[2]Department of Pathology and Laboratory Medicine, Brown University, Providence, RI,[3]Hematology-Oncology Division, Brown University Health, Providence, RI

Background: Androgen deprivation therapy (ADT) and androgen receptor inhibitors (ARi) represent a cornerstone treatment for prostate cancer (PCa). However, resistance often leads to castration-resistant prostate cancer (CRPC). Dysregulation of the extrinsic apoptosis pathway contributes to therapeutic resistance in PCa. Overexpression of FADD-like IL-1β-converting enzyme-inhibitory protein (cFLIP), an endogenous caspase-8 inhibitor, promotes PCa progression and ADT resistance. Targeting cFLIP may represent a novel therapeutic strategy for PCa. This study investigated the potential of CB001, a cFLIP inhibitor, to enhance the effect of ARi and overcome resistance in PCa models.

Methods: We investigated the prevalence of cFLIP molecular alterations in PCa and its impact on patient survival using The Cancer Genome Atlas (TCGA) dataset. We investigated cytotoxic effects of CB001 [0-10μM] in ARi-sensitive (LNCaP) and -resistant (22rv1, DU145, PC3) PCa cell lines. Combination indexing for dual treatment with CB001 [0-5μM] ± ARi (enzalutamide [0-150μM] or darolutamide [0-150μM]) was performed on SynergyFinder. cFLIP, cleaved caspase 8, and cPARP expression were investigated after treatment with CB001 (10μM) ± ARi (10-100μM) at 24 hours by western blot (WB). PCa cells were co-cultured 1:1 with natural killer cells (NKc) in the presence of CB001 (10μM), and tumor cell killing was quantified using the MetaXpress cytoplasmic/nuclear staining software.

Results: Amplifications and deletions of the gene encoding cFLIP were identified in up to 2.7% and 1.34% of PCa tumors in TCGA, respectively. Solid tumors with cFLIP alterations had inferior overall survival compared to wild-type tumors. Single-agent CB001 cytotoxicity displayed a spectrum of sensitivity across different PCa cell lines, with DU145 (3.1μM) and neuroendocrine-phenotype PC3 (11.1μM) requiring higher IC50s than LNCaP (1.1μM) and 22rv1 (0.7μM). CB001 treatment decreased cFLIP expression and increased PARP and caspase 8 cleavage by WB consistent with extrinsic apoptosis pathway activation. CB001 plus ARi was synergistic; combination of CB001 with ARi induced apoptosis in ARi-resistant cell lines 22rv1, DU145, and PC3, reversing apoptosis resistance to single-agent ARi. CB001 also increased immune-mediated NKc killing of 22rv1 ($p=7.3e^{-4}$), DU145 ($p=2.41e^{-4}$), and PC3 ($p=9.83e^{-4}$) cells in co-culture experiments.

Conclusion: CB001 demonstrates potent cytotoxicity and synergizes with ARi in PCa cell lines to overcome resistance to apoptosis with single-agent ARi. In addition to direct tumor cytotoxicity, CB001 enhances NK cell-mediated killing of PCa cell lines. Targeting cFLIP may represent a novel therapeutic strategy for PCa.

#5651 Discovery of AS03157 as a highly potent and orally active antagonist of inhibitor of apoptosis proteins (IAPs).

Yan Xiong[1], Zhou Yu[1], Jiantao Hu[1], Jianyong Chen[1], Xiao Han[1], Zhiyan Liang[2], Yifan Zhai[1], Dajun Yang[1]

[1]Ascentage Pharma (Suzhou) Co., Ltd., Suzhou, China,[2]Ascentage Pharma Group Inc., Rockville, MD

Background: Overexpression of antiapoptotic proteins, such as cellular inhibitor of apoptosis proteins 1 (cIAP1) and 2 (cIAP2) and X-linked IAP (XIAP), occurs in various hematologic and solid cancers and is associated with drug resistance and poor prognosis. Several small molecules are being investigated clinically as monotherapy or in combination therapy in solid tumors. AS03157, a novel and structurally distinct IAP antagonist, was identified with enhanced specificity towards cIAP1 and XIAP, and its pharmacological profiles were characterized *in vitro* and *in vivo.*

Methods: Homogeneous time-resolved fluorescence assays were used to measure the binding of the compound to its target. Detroit 562 cell line was used to test the target degradation. MDA-MB-231 and EVSA-T breast cancer cell lines were used for antiproliferation assay. In vivo efficacy was evaluated in MDA-MB-231-derived xenograft model.

Results: AS03157 strongly bound to cIAP1-BIR3 and XIAP-BIR3 at half-maximal inhibitory concentration (IC_{50}) values of 4.3 nM and 13.7 nM, respectively. AS03157 inhibited cIAP1 with a half-maximal degradation concentration (DC_{50}) of 1.38 nM after treatment for 2 hours in a Detroit 562 pharyngeal carcinoma cell line *in vitro*. AS03157 demonstrated potent growth inhibitory activities in MDA-MB-231 and EVSA-T breast cancer cell lines, with IC_{50} values of 20 nM and 2 nM, respectively. In addition, AS03157 exhibited high aqueous solubility, metabolic stability, as well as high oral bioavailability in both rodent and non-rodent species. Of interest, AS03157 10 and 20 mg/kg administered orally daily demonstrated dose-dependent antitumor activity in an MDA-MB-231-X1 human breast cancer xenograft model, with T/C values of 69.81% and 26.67% on Day 22, respectively.

Conclusions: AS03157 showed promising *in vitro* and *in vivo* activity in preclinical cancer models, and these data provide a rationale for further clinical studies.

#5652 Olverembatinib (HQP1351) in combination with lisaftoclax (APG-2575) overcomes venetoclax resistance in preclinical models of acute myeloid leukemia (AML).

Yan Xiong[1], Zhiyan Liang[2], Ping Min[1], Bingxing Wu[1], Huidan Yu[1], Guoqin Zhai[1], Zhou Yu[1], Dajun Yang[1], Yifan Zhai[1]

[1]Ascentage Pharma (Suzhou) Co., Ltd., Suzhou, China,[2]Ascentage Pharma Group Inc., Rockville, MD

Background: Combining BCL-2 inhibitor venetoclax with hypomethylating agents is the standard of care for older patients with AML or those ineligible for intensive chemotherapy. However, resistance to venetoclax is a major clinical challenge, necessitating alternative therapeutic options. Olverembatinib targets kinases associated with leukemogenesis and venetoclax resistance, including FLT3, KIT, PDGFR, Src family kinases, PI3K, and FGFR. Here, we evaluated the effects of olverembatinib combined with investigational BCL-2 inhibitor lisaftoclax in venetoclax-resistant AML models.

Methods: Primary venetoclax-resistant AML cell line OCI-AML-3 and venetoclax-induced resistant (VEN-RES) *FLT3-ITD* mutant AML cell lines MOLM-13 and MV-4-11 were used for *in vitro* experiments. Cellular proliferation was assessed using CellTiter-Glo® assays, and apoptosis was measured by flow cytometry. Western blotting was used to explore mechanisms of action. Both subcutaneous and systemic models using MOLM-13-VEN-RES models were used to evaluate antitumor effects *in vivo*.

Results: Olverembatinib combined with lisaftoclax synergistically inhibited proliferation in all three VEN-RES AML cell lines vs. either drug alone. Flow cytometry confirmed this, revealing limited apoptotic effects with single agents but a significant increase in apoptosis when combined ($p < 0.0001$). *In vivo*, in a subcutaneous MOLM-13-VEN-RES model, lisaftoclax (100 mg/kg) administered daily for 14 days exerted no antitumor activity, with a treatment-to-control (T/C) value of 96.54%; olverembatinib (10 mg/kg) alone yielded a T/C value of 12.31%. When coadministered, the combination enhanced antitumor activity (T/C value, 5.05%), with a synergistic index of 2.36. This synergy was recapitulated in a systemic MOLM13-VEN-RES model. Mechanistically, western blot analysis showed that the combination synergistically downregulated FLT3 and its phosphorylation, along with downstream proteins such as AKT, STAT, and ERK and antiapoptotic proteins MCL-1 and BCL-xL; notably, significant upregulated phosphorylated FLT3, AKT, MCL-1, and others have been reported to be associated with mechanisms of venetoclax resistance. The combination also promoted upregulation of proapoptotic proteins BAX, BAK, BID, PUMA, and Noxa, resulting in increased cleavage of caspase-3 and PARP-1.

Conclusions: In summary, olverembatinib in combination with lisaftoclax successfully overcame venetoclax resistance in preclinical AML models by downregulating key signaling pathways shown to mediate such resistance. Overall, these findings suggest a potential new therapeutic strategy for venetoclax-resistant AML.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Novel Methods in Small Molecule and Biologics Drug Discovery
Poster Session

#5656 Next-level efficiency: StraightFrom Spleen Isolation Kits - standardizing isolation of untouched B cells with minimal hands-on time.

Sebastian Flade, Amelie Borczewski, Stefano Vergani, Gregor Winkels, Anna Baranska, **Anne Richter**

Miltenyi Biotec B.V. & Co. KG, Bergisch Gladbach, Germany

The study of mouse B cells is essential for advancing our understanding of the immune system, developing new vaccines, and driving progress in immunotherapy through the discovery of new targets. Mouse B cells are abundant in the spleen, one of the major lymphoid organs, making splenic isolation a critical step in B cell research. General workflow to isolate these cells relies on manual mechanical disruption of the mouse spleen and subsequent magnetic purification. However, this combination of methods is time-consuming and operator dependent, with important implication for reproducibility and major impact on downstream application. To address this gap, we at Miltenyi have developed the StraightFromSpleen kit, an all-in-one solution for isolation of highly pure murine B cells from mouse spleen. Briefly, we used the GentleMACS dissociator to disrupt the spleen while labeling unwanted cells with an antibody cocktail. We then performed automated magnetic labeling of cells and isolation of untouched B cells and Pan B cells using the autoMACS NEO Separator. After separation, we measured purity, viability and yield and compared these results with those obtained using a standard isolation kit. Finally, we tested the functionality of the isolated B cells and Pan B cells by culturing then with LPS or in presence of anti-CD40 antibody and IL-4, to promote proliferation, IgG class-switch and differentiation to antibody secreting cells. We demonstrated that the newly developed StraigthFrom Spleen kits allow for isolation of highly viable and pure B cells. Isolated B cells efficiently proliferated in vitro, differentiate to CD138+ antibody secreting cells and were able to class-switch. In conclusion, we have developed a simple, fast, and reliable cell isolation method that significantly accelerates the isolation of functional B cells. This represents an attractive alternative to the laborious multi-step protocols commonly used in academic and industrial laboratories and has the potential to set a new standard for the B cell field.

#5657 Carbon monoxide-releasing molecules as top-notch candidates for prostate cancer therapy.

Gaston Pascual[1], Pablo Sanchis[2], Agustina Sabater[2], Rocio Seniuk[1], Javier Cotignola[1], Elba Vazquez[1], Ayelen Toro[1], Roberta Foresti[3], Roberto Motterlini[3], Geraldine Gueron[1]

[1]IQUIBICEN (UBA-CONICET), Buenos Aires, Argentina,[2]Department of Genitourinary Medical Oncology, IQUIBICEN (UBA-CONICET), Universidad Argentina de la Empresa (UADE), Instituto de Tecnologia (INTEC), The University of Texas MD Anderson Cancer Center, Houston, TX,[3]University Paris-Est Creteil, INSERM, IMRB, Creteil, Paris, France

Carbon monoxide (CO) is an endogenous gasotransmitter with proven anti-inflammatory, anti-apoptotic, and antiproliferative effects. CO-releasing molecules (CO-RMs) have been developed to deliver controlled doses of CO, differing in their CO-release kinetics and chemical structure, showing promise as therapeutic agents in various diseases, including cancer. The main goal of our study is to identify the effectiveness of CO-RMs as a therapeutic strategy to counteract prostatic carcinogenesis. This study aimed to assess the effects of three water-soluble CO-RMs—CORM-3, CORM-401, and CORM-A1—on key hallmarks of prostate cancer (PCa). Using the PCa cell line PC3 we evaluated cell viability across a range of CO-RM concentrations (25-150 µM; 6 h) to identify doses that do not compromise cell viability for further analyses. Confocal microscopy with the DCFH-DA probe revealed that CO-RM treatments significantly reduced reactive oxygen species (ROS) levels ($p<0.001$). Furthermore, CO-RMs enhanced cell adhesion ($p<0.001$) and inhibited cell migration ($p<0.01$). Moreover, mitochondrial integrity, mitochondrial mass and mitochondrial DNA copy numbers were significantly altered ($p<0.0001$), as shown by MitoGreen, TMRE staining and RT-qPCR. Metabolically, PC3 cells treated with CO-RMs exhibited significant reductions in ATP content ($p<0.05$), LDH activity ($p<0.01$) and lactate amounts ($p<0.01$). At the molecular level, CO-RMs repressed markers of oxidative stress (*NFkB*, *SOD-2*), metabolism (*PKM2*, *PDHB*, *ACO2*), proliferation (*Ki67*) ($p<0.01$) and angiogenesis (*VEGF*) ($p<0.05$), as assessed by RT-qPCR. Additionally, analyzing stem-like characteristics, we observed that CO-RMs reduced spheroid size in a hanging drop assay ($p<0.0001$), as well as colony formation in a clonogenic assay ($p<0.01$). As PCa predominantly metastasizes to bone, we further analyzed the potential of CO-RMs to modulate the communication between tumor and bone cells using indirect co-cultures of PC3 cells with osteoblasts precursor cells (MC3T3). CO-RMs decreased osteoblastic marker levels in bone cells (*COL1A1*, *SPP1*, *RUNX2*) ($p<0.01$) and increased *OPG* expression in PCa cells ($p<0.01$) demonstrating that this molecule impaired the crosstalk between both cell types, probably affecting the osteotropism of PC3 cells. This study demonstrates that CO-RMs has a strong effect on modulating key biological processes in PCa cells, highlighting its potential as a therapeutic agent for PCa.

#5658 Overcoming resistance in mCRPC: drug discovery using PDX models and high-throughput drug screening.

Irene Marin-Goni[1], Huanyao Gao[1], Joachim L. Petit[2], Shreya Indulkar[1], Adam M. Kase[3], Cassandra N. Moore[4], Krishna R. Kalari[5], Michael T. Barrett[2], Richard Weinshilboum[1], Winston Tan[3], John A. Copland[6], Liewei Wang[1]

[1]Molecular Pharmacology and Experimental Therapeutics Department, Mayo Clinic, Rochester, MN,[2]Division of Hematology-Oncology, Mayo Clinic, Scottsdale, AZ,[3]Division of Hematology-Oncology, Mayo Clinic, Jacksonville, FL,[4]Division of Hematology-Oncology, Mayo Clinic, Phoenix, AZ,[5]Department of Quantitative Health Sciences, Mayo Clinic, Rochester, MN,[6]Cancer Biology Department, Mayo Clinic, Jacksonville, FL

Background: Second generation androgen receptor signaling inhibitors (ARSIs), such as abiraterone, have demonstrated improved survival in metastatic castration-resistant prostate cancer (mCRPC) patients, however, acquired resistance to these agents often arises. Novel and alternative therapies are needed to combat the ARSI resistant tumor. In the last two decades patient-derived xenograft (PDX) and PDX organoids have become pivotal models in anticancer drug development and biomarker discovery due to their ability to faithfully recapitulate the complex and heterogeneous tumor biology.
Methods: We analyzed RNAseq data from mCRPC patients and corresponding PDX models (PROMOTE study, NCT #01953640) and employed L1000 Chem Perturbation down/up signatures database to find candidate drugs to overcome abiraterone resistance by reverse signature search. We then performed proliferation assay using 49 compounds in three PROMOTE-PDX organoid models (2 abiraterone-resistant and 1 abiraterone-sensitive) using our semi high-throughput drug screening platform.
Results: Overall, we identified a total of 32 compounds with IC50<10μM in at least one of the models, among which, 5 agents are active in all three models and 5 in at least two of the models. (Table 1). Within active compounds we find selective kinase inhibitors (BRAF, JAK2, AKT, FLT3) and histone deacetylase inhibitors (belinostat and pracinostat). Particularly interesting are two drugs that are only active in the abiraterone resistant setting but not the sensitive PDX.
Conclusions: In summary, we leveraged RNAseq data from mCRPC patients and PDX models and performed a high-throughput drug screen in PDX-derived organoid models to identify potential drugs that could overcome abiraterone-resistance in lethal advanced prostate cancer. These data highlight potential drug combinations or subsequent therapeutic strategies for these patients.

Summary of drug screening in PDX-derived organoids

	Abiraterone Sensitive	Abiraterone Resistant-1	Abiraterone Resistant-2
Active (IC50<10μM)	21	16	13
Intermediate (IC50:10-25μM)	3	2	1
Inactive (IC50>25μM)	25	14	35
Number of total drugs tested	49	32	49

#5659 High-throughput screening reveals novel synergistic drug combinations for metastatic castration-resistant prostate cancer.

Jessica D. Kindrick[1], Benjamin C. Brim[1], Kinjal Bhadresha[1], Xiaohu Zhang[2], Keith T. Schmidt[1], Michele Ceribelli[2], Douglas K. Price[1], Cindy Chau[1], Craig J. Thomas[2], William D. Figg[1]

[1]Genitourinary Malignancies Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD,[2]Division of Preclinical Innovation, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD

Metastatic castration-resistant prostate cancer (mCRPC) remains a significant clinical challenge, with patients often developing resistance to androgen receptor (AR)-axis-targeted therapies such as Abiraterone and Enzalutamide. A key resistance mechanism involves the AR splice variant AR-V7, which drives tumor progression and poor prognosis. To address the need for novel, effective treatment strategies, we conducted unbiased high-throughput screening (HTS) to discover highly synergistic drug combinations that improve therapeutic efficacy, overcome resistance mechanisms, and minimize toxicity. Leveraging a robotic automation platform, we quantitatively screened 2,480 mechanistically annotated compounds from an oncology-focused library, targeting over 800 biological pathways, across 8 prostate cancer cell lines representing various stages of disease progression. Single-agent cytotoxicity was evaluated using an area under the 11-point dose-response curve (AUC) metric, identifying therapeutically vulnerable targets in mCRPC, including tubulin, MCL-1, CDK9, BRD4, and PI3K/AKT. This led to the selection of 42 compounds for pairwise study of all possible 861 dual-drug combinations in two AR-V7-expressing mCRPC cell lines, LNCaP-95 and VCaP-CR. Utilizing the Excess Highest Single Agent (HSA) model to quantify synergy/antagonism, this large-scale analysis revealed highly synergistic combinations, particularly between MCL-1 and BcL-xL inhibitors. These results guided the development of a novel triple combination therapy to simultaneously target CDK9-regulated productive RNA polymerase II phosphorylation (Enitociclib), BRD4-mediated epigenetic remodeling (Pelabresib), and pro-survival BcL-xL (Navitoclax). Notably, the triple combination demonstrated robust synergy at low doses, inducing apoptosis as early as 4 hours post-treatment through rapid Caspase 3/7 cleavage. Further mechanistic investigation through RNA-sequencing revealed significant downregulation of key oncogenes, including MYC and AR-V7, alongside disruption of downstream transcriptional pathways. Importantly, the triple combination was validated in patient-derived mCRPC organoid models LuCaP167 and LuCaP136, confirming its translational potential. These findings provide a strong rationale for clinical development of multi-drug targeted combination regimens to overcome AR-V7-driven resistance and deliver durable responses with reduced toxicity for mCRPC patients with limited treatment options. Our approach also highlights the power of HTS to unbiasedly innovate synergistic therapies while uncovering critical biological mechanisms underlying cancer drug resistance.

#5660 Optimizing and integrating high-throughput cell-based assays to enable SAR studies of metarrestin to target cancer metastasis.

Asia S. Almarales[1], Yi-Han Lin[1], Harrison Daly[1], Samarjit Patnaik[1], Abhijeet Kapoor[1], Jing Ran[1], Soumita De[2], Udo Rudloff[2], Juan Marugan[1], Mark Henderson[1]

[1]Early Translation Branch, Division of Preclinical Innovation, National Center for Advancing Translational Sciences, Rockville, MD,[2]Rare Tumor Initiative, Pediatric Oncology Branch, National Cancer Institute, Bethesda, MD

The perinucleolar compartment (PNC), a marker of genome organization / a membrane-less organelle (condensate) adjacent but structurally distinct from the nucleolus, associates with metastatic behavior in cancer cells and has been used as biomarker in high-throughput screening assays to identify small molecules that target metastasis. We discovered metarrestin, an investigational agent that disassembles the PNC in multiple cancer cell lines as a novel treatment for cancer metastasis. Metarrestin is currently in phase I clinical trials. Metarrestin treatment effects phase-separation, rapidly disrupts nucleolar function and inhibits ribosomal RNA synthesis. Pre-clinical structure activity relationship (SAR) studies, to further improve metarrestin's pharmacological properties, are ongoing. These require robust cell-based assays to evaluate PNC disassembly, rRNA synthesis and growth phenotypes. Our prior studies relied heavily on the Incucyte live-cell imaging system to characterize the cytostatic effect of metarrestin derivatives by tracking the growth of treated cells over days. However, this experimental setup requires long machine times and is limited by the capacity of a single Incucyte to accommodate six 384-well format plates. To address these throughput limitations, we optimized an orthogonal viability assay using Cell Titer Glo which measures luminescence and detects ATP in metarrestin treated cells. Using 1536-well plates, we examined the assay performance using cell numbers ranging from 125-1,000/well, and incubation time between 48-96 hr, in two cell lines PANC1, a pancreatic cancer cell line and PC3M, a prostate cancer cell line. Unlike the measurement of cytotoxic effect, which is typically performed at higher cell densities and shorter incubation times, the cytostatic effect was best captured after 96 hr incubation with cells seeded at 250/well in a 1536-well plate. For detection of rRNA inhibition and PNC disassembly in cells, we utilized a rRNA selective fluorescent probe (Probe 1) and a GFP-based reporter of the PNC, respectively, followed by confocal microscopy using an Opera Phenix. Using this suite of cell-based assays, we evaluated the compound of ~160 metarrestin derivatives and performed comparative analyses of compound activities in measurements of PNC disassembly, rRNA synthesis inhibition, and cytostatic growth effect in PANC1 and PC3M cells.

#5661 A 45-color panel for comprehensive immunophenotyping of human leukocyte.

Xiaoyu Zhang[1], Ming Lei[2], Peifang Ye[2], Xiaohuan Wang[2], Yan Lu[2], Garret Guenther[1], Nancy Li[1]

[1]Agilent Technologies, Inc., La Jolla, CA,[2]Agilent Technologies, Inc., Hangzhou, China

Multicolor flow cytometry has advanced significantly with instruments capturing more lasers and detectors. Spectral flow cytometry, which records full emission spectra rather than peak emissions, allows fluorochromes with distinct spectral fingerprints to expand panels beyond the 40+ color limit of traditional methods. Agilent's NovoCyte Opteon spectral flow cytometer, with its five-laser configuration (349nm, 405nm, 488nm, 561nm, 637nm) and 70 fluorescence detection channels, exemplifies this advancement. Using the OMIP-069 panel as a foundation, we expanded it to detect more biomarkers by adding dyes that either fill spectral gaps or possess unique spectral fingerprints despite similar emission maxima. The original OMIP-069 panel allowed characterization of PBMC subsets (e.g., CD4+, CD8+ T cells, B cells, NK cells, monocytes) and included activation/differentiation markers. Our modified 45-color panel introduced five biomarkers: CD31, CD45RO, CD69, CD33, and LAG-3. CD31 and CD45RO identify recent thymic emigrants (RTEs) among CD4+ T cells, CD69 tracks early activated T cells, LAG-3 assesses exhausted T cells alongside PD-1, and CD33 excludes residual monocytes. The results demonstrate that despite incorporating highly overlapping fluorochromes, immune subsets were accurately resolved, preserving dimensionality reduction and clustering outcomes. This highlights the NovoCyte Opteon's excellent performance, enabling high-dimensional panel design and robust biomarker resolution.

#5662 Deep-mining the antibody repertoires of transgenic mouse models with high-throughput single cell BCR and whole transcriptome sequencing.

Ajay Sapre, Alec Salvino, Guillermo Gallareta-Olivares, Efi Papalexi, Jose Jacob, Ashwath Kumar, Charles Roco, Alexander Rosenberg

Parse Biosciences, Inc., Seattle, WA

Transgenic mouse models are excellent tools to model human antibody response against antigens or disease of choice, given their ability to undergo diversification, in vivo affinity maturation, clonal selection and clonal expansion in their natural immune environment. Current antibody discovery methods lack the scale required to fully screen the antibody repertoire of transgenic mouse models to uncover maximum desirable antibody hits per discovery campaign. Here, we demonstrate our novel combinatorial barcoding approach to enable high-throughput single cell BCR and whole transcriptome profiling of transgenic mouse samples with unprecedented sensitivity, scalability, and flexibility.
As a proof of concept, we profiled over 1 million cells from multiple transgenic mice that were either naive or immunized with antigens of interest to obtain hundreds of thousands of paired BCR clonotypes. Using the whole transcriptome data, we detected all B cell subpopulations including plasma cells at single cell resolution. We then used the paired heavy and light chain BCR data to detect productive chains in the majority of B cells revealing both unique and expanded clonotypes. In the expanded clonotype pool we found specific patterns of VDJ utilization and amino acid motifs compared to the naive pool. This method paves the way for high throughput screening of antibody repertoires from transgenic mice to allow for deep mining of repertoires for antibody discovery and therapeutic outcomes.

#5663 High-content screening platform for comprehensive profiling in drug discovery.

Maciej B. Olszewski, Natalia Gostynska, Klaudia Lesniak, Alicja Martyniak, **Magdalena Kieltyka**

Selvita S.A., Cracow, Poland

A robust, multi-modal high-content screening (HCS) platform enables fast and quantitative assessment of cellular phenotypes over a variety of research applications. Our integrated system combines innovative imaging technologies, automated analysis, and cell profiling capabilities to drive drug discovery and biological research programs. Selvita HCS platform features multiparametric cellular phenotyping: simultaneous measurement of morphological, functional, and molecular cellular properties. The data is acquired by automated fluorescence microscopy: high-resolution imaging with multiplexed fluorescent markers. The images are then analyzed, as needed, by standard or machine learning-assisted image analysis. Advanced algorithms are used to accurately extract cellular features. This enables building various kinetic and endpoint assays: cell viability, apoptosis, proliferation, and subcellular localization studies, to name a few. The platform allows multiple application domains, including therapeutic target validation, mechanism of action studies, pharmacological screening and toxicity assessment. Validated across a variety of models under different experimental conditions, our HCS platform generates high-throughput data with high inter-assay consistency. The customizable protocols facilitate adaptable research support across programs in oncology, neuroscience, immunology, and metabolic disease. Examples of successful cases include: tissue analysis with automated identification of areas of interest and subsequent high-resolution rescanning, combined FISH / immunofluorescence studies in target validation, 100+ K compound screening campaign based on reporter cell line and protein-protein interaction study in DNA repair complex assembly, using proximity ligation assay. Future development will focus on expanding the machine learning capabilities, multiplexing potential, and integration of advanced computational biology tools to aid in the improvement of predictive cellular modeling.

#5664 Identifying novel vulnerabilities in clear cell sarcoma through functional and genomic screening.

Laura Leuenberger[1], Larissa Isenegger[1], Yanjiang Chen[2], Miles Burri[1], Sarah Kollar[2], Lara Planas-Paz[2], Peter Karl Bode[3], Agnieszka Wozniak[4], Sebastian Bauer[5], Patrick Schoffski[6], Chantal Pauli[2], Christian Britschgi[7]

[1]Department of Medical Oncology and Hematology, University Hospital Zurich, Zurich, Switzerland,[2]Department of Pathology and Molecular Pathology, University Hospital Zurich, Zurich, Switzerland,[3]Department of Pathology, Cantonal Hospital Winterthur, Winterthur, Switzerland,[4]Laboratory of Experimental Oncology, Department of Oncology, KU Leuven, Leuven, Belgium,[5]Department of Medical Oncology and Sarcoma Center, West German Cancer Center, University Hospital Essen, Essen, Germany,[6]Department of General Medical Oncology, University Hospitals Leuven, Leuven, Belgium,[7]Cantonal Hospital Winterthur, Winterthur, Switzerland

Introduction: Clear Cell Sarcoma (CCSA) is a rare soft tissue sarcoma characterized by specific gene fusions *EWSR1::ATF1* or *EWSR1::CREB1*, leading to the expression of an oncogenic fusion protein. Metastasized CCSA is resistant to conventional chemotherapy, targeted therapy or immunotherapy. This study aims to uncover new therapeutic strategies for CCSA by utilizing a whole-genome CRISPR/Cas9 knock-out screen combined with functional drug screening.
Methods: We performed a genome-wide knock-out screen in the CCSA cell line model KAS using the pooled Brunello sgRNA library (Addgene # 73178, ~4 guides/gene, 19'114 genes). In parallel, three CCSA patient samples and multiple CCSA cell lines (SU-CCS-1, DTC-1, KAS) were treated with a library of 84 solid tumor pan-cancer drugs, and a custom drug library tailored to the hits of the CRISPR screen (70 compounds). Genes identified as critical vulnerabilities in CCSA were validated through single-gene knockouts, and promising therapeutic targets were further mechanistically interrogated and evaluated in a CCSA xenograft model.
Results: Results revealed 1'552 significantly depleted genes (log fold change <-1 and FDR < 0.01) after 21 days, with enriched pathways in proteasome activity, mismatch repair, DNA replication, and, mTORC1 signaling, revealing critical vulnerabilities. Notable highly ranked depleted genes include the transcription factor and CCSA marker *SOX10*, as well as *MDM2, MCL1, CHEK1, HSPA5, PSMA5* and mTOR. Through integration of genetic knock-out and pharmacological inhibition data identified in our dual screening, we were able to identify several overlapping vulnerabilities. Initial validation of the therapeutic potential of PI3K/mTOR inhibition in CCSA cell models *in vitro* revealed an induction of apoptosis upon treatment. We then corroborated those findings in a CCSA xenograft mouse model *in vivo*. Treatment with the two combined PI3K/mTOR inhibitors omipalisib and copanlisib both resulted in significantly improved survival ($P = 0.027$ and $P = 0.036$, respectively) of tumor bearing mice, and reduced tumor growth, each compared to vehicle treatment.
Conclusion: These findings offer new insights into actionable vulnerabilities in CCSA, with PI3K/mTOR inhibitors showing potential for therapeutic development. Future studies will investigate synergistic combinations to further optimize treatment efficacy and validate further vulnerabilities identified by our dual screening approach.

#5665 Targeted cell killing: a versatile platform for cell therapy and cytotoxicity assays in diverse cell lines.

Nicholas J. Hess, Brock Binkowski, Julia Gilden, Gopal Krishnan, Mei Cong, Marjeta Urh

Promega Corporation, Fitchburg, WI, USA, WI

The HiBiT Target Cell Killing Assay (HiBiT TCK Assay) is a streamlined, sensitive method for selectively quantifying target cell lysis in coculture experiments with effector cells. HiBiT is an 11AA peptide that binds with high affinity to LgBiT, forming a bright, luminescent enzyme. For HiBiT TCK, target cells are engineered to express a HiBiT-tagged protein, and release of this protein by dead/dying cells leads to complementation with the cell impermeable LgBiT. This gain-of-signal approach enables precise measurements of target cell killing induced by various effectors, including CAR-T cells and cell killing mediated by bispecific antibodies. Here we demonstrate the use of HiBiT TCK with ADCC, ADCP, TDCC and CAR-T applications. Furthermore, we present data of mRNA transfection to transiently express HiBiT in a variety of target cells, allowing for the rapid and uniform labeling of both dividing and non-dividing cells. This approach extends the HiBiT TCK products beyond use of stable cell lines alone and allows users to screen a variety of cell lines.

#5666 Akos: A novel CAF-targeted therapy to reprogramming the tumor microenvironment to reverse metastatic traits in all solid tumors.

Javier Cerda-Infante[1], Benjamin Prieto[2], Valentina I. Cerda[2], Muriel Nunez[2]

[1]Pontificia Universidad Catolica de Chile, Santiago, Chile,[2]Environ, Santiago, Chile

Introduction: Metastasis is responsible for approximately 90% of cancer-related deaths, yet current therapies fail to effectively address this critical challenge. The tumor microenvironment (TME), particularly carcinoma-associated fibroblasts (CAFs), plays a pivotal role in metastatic progression by driving extracellular matrix (ECM) remodeling, epithelial-to-mesenchymal transition (EMT), and immune evasion. Within metastatic CAFs (mCAFs), we identified a key protein that regulates these processes and developed Akos, a small molecule that selectively inhibits this protein, reprogramming the TME to disrupt the metastatic cascade. This study evaluates the preclinical efficacy, specificity, and safety of Akos across multiple solid tumor models.
Materials and Methods: Primary stromal cell cultures were obtained from needle biopsies of primary tumors from patients with advanced/metastatic cancer (mCAFs), non-metastatic cancer (CAFs), and non-cancerous patients (BAFs). Protein expression was validated through gene profiling, immunohistochemistry, and western blot. In vitro assays were conducted to assess Akos' ability to inhibit mCAF-mediated tumor cell invasion, proliferation, and EMT marker expression. In vivo xenograft models of prostate, breast, colorectal, and pancreatic cancers were utilized to evaluate Akos' effects on tumor growth, weight, and metastatic spread. A spontaneous metastasis assay, replicating the full metastatic cascade, was employed to evaluate its anti-metastatic potential. Toxicology and pharmacokinetic studies were conducted to assess systemic safety and drug stability.
Results: Akos demonstrated significant in vitro efficacy, inhibiting mCAF-driven tumor cell invasion and proliferation while reducing EMT marker expression. In in vivo xenograft models, Akos reduced tumor volume by 50-fold and tumor weight by 30-fold compared to untreated controls. In the spontaneous metastasis assay, Akos completely blocked 100% of metastatic foci across all tested models. Toxicology studies confirmed Akos' safety profile, with no significant off-target effects observed, while pharmacokinetics showed stable systemic exposure.
Discussion: These findings position Akos as a promising therapeutic candidate for targeting the TME to address the unmet need in metastatic cancer treatment. By inhibiting a key protein in mCAFs, Akos effectively disrupts the processes driving metastatic progression, offering a novel approach to halting the spread of cancer. The observed efficacy across multiple tumor types, combined with its favorable safety profile, supports further clinical investigation of Akos as a first-in-class therapeutic for metastatic solid tumors. These results underscore the potential of targeting the TME to improve patient outcomes and advance the standard of care in oncology.

#5667 Epidemiology of tissue factor expression in soft tissue sarcoma.

Francesca La Greca[1], Agnieszka Wozniak[1], Chao-Chi Wang[1], Daniel Gorgels[1], Flavia Paternostro[1], Luna De Sutter[1], Lore De Cock[1], Kimberly Verbeeck[1], Ulla Vanleeuw[1], Raf Sciot[2], Patrick Schoffski[3]

[1]Laboratory of experimental oncology, KU Leuven, Leuven, Belgium,[2]Department of Pathology, Leuven Cancer Institute, University Hospitals Leuven, Leuven, Belgium,[3]Department of General Medical Oncology, Leuven Cancer Institute, University Hospitals Leuven, Leuven, Belgium

Objective: Soft tissue sarcoma (STS) is a heterogeneous group of rare, malignant mesenchymal tumors, comprising over 70 subtypes. Doxorubicin-based chemotherapy, the standard treatment for the majority of advanced or metastatic STS, has limited efficacy, with low response rates and poor disease control. Identifying new molecular targets is crucial for developing more effective treatments and overcoming resistance, considering the high unmet medical need in this indication. Tissue factor (TF) is a transmembrane protein essential for hemostasis and thrombosis. High levels of TF have been observed in cancers, particularly adenocarcinomas, where it promotes tumor growth, invasion, metastasis and angiogenesis. TF expression in tumors and in tumor-associated endothelial cells is currently considered a putative therapeutic target. Consequently, a TF-targeted antibody-drug conjugate (tisotumab vedotin) has been approved by the Food and Drug Administration for the treatment of cervical cancer.As its expression in STS remains unexplored, we aimed to assess the expression of TF in tissue microarrays (TMA) constructed from archival tumor material from patients and patient-derived xenograft (PDX) models.
Methods: Immunohistochemical staining was performed on TMA. Over 550 patient samples with 2-3 cores per tumor were analyzed, covering the 11 most common STS subtypes, such as leiomyosarcoma (LMS), liposarcoma (LPS), angiosarcoma (AS) and myxofibrosarcoma (MFS).In addition, TMA from 31 PDX models from our XenoSarc platform, covering 14 STS subtypes, were included with at least 2 cores per model, with multiple passages of the xenografts to account for potential intra-tumor variability of TF expression during passaging.TMA were evaluated using the scoring: 0 (no expression), 1 (<25% positive cells), 2 (25-50%), and 3 (>50%); scores of 2 or 3 were considered as high expression.
Results: Analysis of a multi-sarcoma clinical TMA revealed a limited number of cases with high expression of the target in only a few samples from AS, LPS, LMS and MFS, subtypes that were selected for further analysis using subtype-specific TMAs from these entities. High expression was found in 22.5% of MFS (27 out of 120 donors), 8.0% of AS (6 out of 79 cases), 4.7% of LMS (6 out of 128 patients), 3.2% of LPS (2 out of 62 patients). Detailed correlations with relevant clinical data are currently ongoing and results will be disclosed at the meeting.In the PDX, high TF expression was observed in 9 out of 31 models in at least one passage. Three out of 4 MFS models showed TF high expression in at least one passage and two high-grade sarcoma models maintained high expression across multiple passages (≥3).
Conclusion: TF expression in STS is generally limited, with MFS showing the highest number of positivity in clinical samples and xenograft models. Nevertheless, the observed frequency appears insufficient to support TF as suggestive therapeutic target across other subtypes of sarcomas.

#5668 Preclinical efficacy of tumor treating fields (TTFields) for small cell lung carcinoma (SCLC).

Rotem Engelman, Lina Somri-Gannam, Talya Borkum, Roni Blatt, Daria Gerasimova, Shay Cahal, Catherine Tempel Brami, Mai Shai, Eyal Dor-On, Itai Tzchori, Adi Haber, Moshe Giladi, Yoram Palti

Novocure Ltd, Haifa, Israel

Purpose: Small cell lung cancer (SCLC) is a highly aggressive malignant neoplasm, accounting for 10-15% of lung cancer cases, characterized by rapid growth, and early metastasis. The standard of care is combination chemotherapy with cisplatin or carboplatin plus etoposide, administered together with immunotherapy and concurrent chest radiotherapy. The current preclinical study aimed to investigate the efficacy of TTFields for treatment of SCLC and examine the effect of TTFields together with the standard-of-care chemotherapies.
Methods: DMS-53 and H196 SCLC cells were treated with TTFields (1 V/cm RMS, 150 kHz, 72 h for DMS-53 cells; 1.62 V/cm RMS, 150 kHz, 120 h for H196 cells), alone or with the addition of different concentrations of cisplatin or etoposide. Efficacy was measured by analyzing cell count, colony formation, and apoptosis induction. The overall effect was calculated by multiplying cell count with colony formation. TTFields efficacy was further evaluated in vivo, in mice orthotopically inoculated with murine KP3 SCLC cells. 7 days after inoculation, TTFields or sham-heat were continuous applied for 9 days. Tumor volume was examined by magnetic resonance imaging (MRI) at treatment end.
Results: TTFields reduced cell count, induced apoptosis, and provided an increased overall effect relative to control in both cell lines. Application of cisplatin or etoposide alongside TTFields showed a synergistic interaction in H196 cells, which was higher for the latter chemotherapy. In DMS-53 cells, the concomitant treatment of TTFields with either chemotherapy showed an additive interaction in cytotoxicity and a synergistic overall effect, which matched the relatively high levels of apoptosis in this cell line. Furthermore, TTFields significantly reduced tumor growth relative to control in the mouse model.
Conclusions: Our results demonstrate efficacy for TTFields for the treatment of SCLC in preclinical models, and a potential advantage for TTFields concomitant with the standard-of-care chemotherapies for this malignancy. Future studies will test the effect of TTFields concomitant with both chemotherapies, in vitro and in vivo; and together with immunotherapy and chemotherapy.

#5669 Performance characterization of the Cellometer Ascend automated cell counter following ISO cell counting standards.

Carolina Franco Nitta, **Yongyang Huang**, Daniel Browe, Allen Lin, Sopaul Hem, Leo L. Y. Chan, Bo Lin, Dmitry Kuksin

Revvity Health Sciences, Inc., Lawrence, MA

In modern therapeutics, cell counting and viability are extremely common, yet vital measurements in research, process development, and manufacturing for novel discoveries and development. Many challenges are associated with these frequent readouts, specifically due to the absence of reference materials, cell sample heterogeneity, stability of living cells, complexity of cell sample preparation, and differences in chemical staining methods. The International Organization for Standardization (ISO) has published two documents to help support cell counting procedures: ISO 20391 - 1:2018 Biotechnology - Cell Counting - Part I: General Guidance on Cell Counting Methods; and ISO 20391 - 2:2019 Biotechnology - Cell Counting - Part II: Experimental Design and Statistical Analysis to Quantify Counting Method Performance. The Cellometer Ascend, Revvity's next generation automated cell counter, has been designed to provide reliable and consistent cell counting and viability measurements as an all-in-one standalone instrument with a user-friendly streamlined workflow allowing for cell counting at a larger concentration range. In this work, we characterized the cell counting performance of the Cellometer Ascend, following ISO cell counting standards. Linearity tests using Jurkat cells showed that Cellometer Ascend reliably covered a concentration range from 2.6×10^4 cells/mL to 4×10^7 cells/mL, with a precision below 10%. A truncated version of the ISO cell counting Part II protocol was developed internally to evaluate the performance and compare quality metrics for four distinct cell counting methods. These methods were compared simultaneously to minimize the variation from cell sample stability and results revealed comparable mean cell counts, precision, coefficient of determination (R^2), and proportionality indices (PI). We further conducted multiple cell counting comparisons using the Bland-Altman comparative analysis, and showed that the Cellometer Ascend was in line with the other three instruments with a less than 6% difference observed in all cases. In addition, we investigated instrument-to-instrument consistency using two different concentrations of fluorescent beads on five Cellometer Ascend instruments. Results from the 5×10^6 beads/mL and 2.5×10^5 beads/mL concentrations showed coefficient of variation (CV) of 0.8% and 2.2%, respectively. In conclusion, the Cellometer Ascend can be used to provide high-quality cell counts and viability readouts according to ISO Cell Counting standards, is comparable to other cell counting methodologies, and could therefore be the benchtop solution for multiple cell counting needs.

#5670 Using molecular docking to develop a novel peptide targeting tumor-associated macrophages based on RXRB recognition.

Nianwu Wang, Hong-Bo Pang

Department of Pharmaceutics, College of Pharmacy, University of Minnesota, Minneapolis, MN

Tumor-associated macrophages (TAMs) play a critical role in tumor progression, and thus have attracted great attention for therapeutic development. However, macrophage-modulating strategies suffer from drawbacks that they may interfere with normal immunity. Therefore, it is desirable to target and modulate macrophages in tumor-specific manner. We previously identified one such peptide, termed CRV, which selectively homes to a variety of solid tumors and bind TAMs within upon systemic administration. This TAM-targeting ability was then attributed to CRV receptor, retinoid X receptor beta (RXRB). While CRV demonstrated desirable properties in tumor homing and TAM recognition *in vivo*, the actual affinity to RXRB was very weak. We hypothesize that peptide variants with a higher affinity to RXRB may exhibit a more specific targeting of TAMs. To test this hypothesis, we employed molecular docking to optimize the CRV sequence. Seven rounds of optimization were carried out to replace one residue of CRV peptide in each round with nineteen other amino acids, and the binding energy was calculated as the selection criterion. This study identified a novel peptide, termed CRH, with an improved predicted binding affinity compared to CRV (-12.2 kcal/mol vs. -8.9 kcal/mol). In vitro assays verified that CRH possesses a higher affinity to RXRB than CRV. Ongoing studies include surface plasmon resonance (SPR) binding analysis and *in vivo* homing evaluations. Overall, we showcase here the identification of a novel peptide to target TAMs, which may enable a more precise drug delivery and TAM modulation.

#5671 Development of HTS enzymatic assays for VPS4A and related ATPases.

Anibal Ramos Martinez, Camdon Yemm, **Ha Pham**, Robert Lowery

BellBrook Labs, Madison, WI

VSP4A and VSP4B (Vacuolar Sorting Protein) are members of the meiotic clade ATPases, which function as oligomers and use ATP hydrolysis to drive protein translocation through their central pore. VPS4B is frequently deleted in several cancers, and VPS4A has been shown to be synthetic lethal with VPS4B in cells and animals, causing cell death accompanied by induction of inflammatory cytokines and anti-tumor responses. To accelerate development of selective VPS4A inhibitors while avoiding off target effects with related ATPases, we are assembling a panel of high throughput biochemical assays using the Transcreener ADP^2 assay for homogenous detection of ATPase activity with a far-red fluorescence polarization (FP) readout. Here we describe development of assays for VPS4A, VPS4B, as well as Spastin, Katanin and Fidgetin, related meiotic clade ATPases that play important roles in microtubule metabolism. Using purified recombinant enzymes, we established reaction conditions, determined kinetic parameters, and optimized the assays for initial velocity detection of ATPase activity with a Z' value greater than 0.7, ensuring a sufficient signal window for inhibitor screening and dose response assays. We validated the VPS4A assay for HTS by screening a collection of bioactives (Tocris 2.0) and confirming two hits in dose response mode: BVT948, a PTP inhibitor, and polygodial, an antifungal that has been shown to inhibit a mitochondrial ATPase, both inhibited VPS4A and VPS4B with low micromolar potency. We then profiled BVT948 and polygodial for inhibition of Spastin, Katanin and Fidgetin: both molecules showed modest selectivity (2-5-fold) vs. Katanin and Fidgetin but had similar or greater potency for inhibition of Spastin. These assays will aid efforts to exploit the VPS4A/4B synthetic lethality therapeutically, including screening and SAR-driven hit-to-lead to minimize off-target effects with related ATPases.

#5672 Detecting tissue susceptibility to celecoxib treatment using a whole-body imaging approach.

Steven Johnson[1], Justin Jeffery[2], Jungwha Lee[1], Ming Zhao[1]

[1]Northwestern University, Chicago, IL,[2]University of Wisconsin Madison, Madison, WI

Purpose: The goal of this study was to evaluate an in vivo imaging technique, which detects phospholipid membrane reorganization, as a molecular marker for tissue response induced by celecoxib as a canonical black box drug which is also being repurposed for anticancer treatment.
Methods: 99mTc-duramycin was used as a phosphatidylethanolamine (PE)-binding imaging agent and was injected intravenously at 1 hour prior to imaging at designated time points. Sprague Dawley rats (male, 8-10 weeks) were treated with a single dose of 200 mg/kg or 1000 mg/kg celecoxib via oral gavage. A pilot study was conducted in duplicates with planar imaging on days 1, 3, 5 and 7 to determine the approximate window of signal changes, then whole-body single photon emission computed tomography (SPECT) was acquired for nontreated control, 200 mg/kg and 1000 mg/kg celecoxib-treated groups (n=12 per group). Blood and tissues from 4 animals in each group were collected for complete blood and serum panels and histopathology. The radioactivity uptake in tissues and organs were determined and statistically compared to the control group using unpaired t test.
Results: The imaging data detected no significant changes with low dose (200 mg/kg) celecoxib treatment, but in a number of tissues/organs in high dose (1000 mg/kg) treatment, including the adipose tissue, blood, brain, upper right quadrant of the abdomen, heart, kidney, skeletal muscle, stomach, and testes. Histopathology analysis is ongoing to validate the imaging findings. Data from complete serum panel detected significantly elevated total protein, albumin and sodium.
Conclusion: The study demonstrated the assessment of tissue susceptibility as a result of celecoxib treatment, which were consistent with known adverse effects of the drug. The current approach will be useful for detecting potential off-target side effects of anticancer treatments and drug candidates.

#5673 Identification of potential therapeutic targets by in vivo CRISPR screening on xenograft models.

Ce Zhong, Lu Bai, Tongtong Wang, Yiying Shao, Lingyun Zhang, Lu Zhang, **Jingqi Huang**

Pharmaron, Inc., Beijing, China

Introduction: CRISPR screens have been successfully used to identify targetable genes for anti-cancer research and drug development. Typically, CRISPR screens are conducted *in vitro* on cell lines, which do not fully capture the influence of the in vivo physiological environment. It is well known that any genetic editing might trigger the adjustment of gene balance to compensate for the modifications in the target cells and tissues. To address the complexity of cellular interactions and the immunological response, in vivo CRISPR screening on a xenograft model is a compelling approach to unravel biological processes where cells are targeted within their natural physiological conditions.
Methods: We have conducted a CRISPR screening approach using the human non-small cell lung cancer cell line A549 by knocking out tumor suppressor genes using a library of 3,188 single guide RNAs (sgRNA). Subsequently, in vivo testing was carried out with the genetically edited A549 cells with or without treatment with the kinase inhibitor Trametinib. At the study endpoint, tumor samples were isolated and analyzed. Amplicon sequencing was used to capture residual sgRNA. MAGeCK was used to calculate the gene enrichment score.
Results: After CRISPR editing, 37 sgRNA corresponding to 20 genes were captured in the edited A549 cell line. In the vehicle-treated A549 xenograft tumors, 45 sgRNA corresponding to 27 genes were captured, of which 17 genes were overlapping with engineered A549 cell culture, 8 upregulated and 9 downregulated. In comparison, in the A549 xenograft tumors of the Trametinib treated group, 38 sgRNA corresponding to 23 genes were discovered, with 15 overlapping genes, 9 upregulated and 6 downregulated. The change in expression levels was abundant. Of the 3,188 sgRNA, the majority of sgRNAs were filtered out in the A549 cell line, under in vivo evolution and the influence of Trametinib treatment. We believe that the pathways related to this small set of identified genes may reveal potential new targets for anti-cancer research and drug development.
Conclusion: The in vivo CRISPR approach offers a valuable and translational tool for discovering novel targets in cancer research, providing insights for new anti-cancer therapies.

#5674 StarBright^{TM} Dyes for flow cytometry, western blotting, microscopy and Genesis System with CelSelect Slides.

Michael Blundell[1], Leon Huang[2], Sharon Sanderson[1]

[1]Bio-Rad Laboratories, Kidlington, United Kingdom,[2]Bio-Rad Laboratories, Hercules, CA

There are many antibody-based applications in the researcher's toolbox that rely on both high-quality antibodies and bright stable fluorescent dyes. Antibodies are usually validated for specific applications and may only work on fresh samples and not work if a sample is fixed. The same application specificity can also be observed with fluorescent dyes. Some dyes are easily bleached, may not be stable after fixation and have spectral profiles that make it impossible to multiplex in some applications. To be able to use multiple applications, researchers may need to purchase three or four versions of an antibody or use sub-optimal choices to keep costs manageable. Here we present data on StarBright Dyes, a range of 33 fluorescent dyes, in a multitude of antibody-based applications. They are bright, have low cross laser excitation, can be fixed in PFA and alcohol-based buffers and work in all protocols without specific buffers, especially when multiplexing. StarBright Dyes have been developed to give researchers high quality data whilst providing flexibility and choice regardless of the application. They work in flow cytometry, allowing very high plex panels to be developed with manageable spillover and spreading, require no special buffer and even allow storage of premixed panels. In western blot their brightness, short exposure time and low background makes them ideal for detection of a single target, or in multiplexing. Their brightness allows easy identification and enumeration in cell enrichment systems such as the Genesis System^{TM}. Finally in microscopy, both wide field and confocal, their photostability, range of emission, and brightness, not only gives more choice, but as they can be used as primaries without the need for secondary amplification can speed up staining protocols and allow panel expansion without species cross reactivity. Here we demonstrate how you can reduce the number of fluorophores and antibodies required for each application and simplify your protocols by including StarBright Dye conjugated antibodies in your experiments.

#5675 ThiOBI® platform, a novel hydrophilic linker for irreversible cysteine-selective biomolecular conjugation, demonstrated potent and durable antitumor activities in various animal models.

Yu-Chao Huang, Ting-Wei Liu, Chi-Huan Lu, Ren-Yu Hsu, Nan-Hsuan Wang, Ya-Chi Chen, **Ming-Tain Lai**, Teng-Yi Huang

OBI Pharma, Inc, Taipei, Taiwan

The ThiOBI® platform represents a cutting-edge approach for the development of next-generation cysteine (Cys)-based drug conjugates including a wide range of cysteine-containing biomolecules, such as nanobodies, antibody fragments, and peptides. This platform leverages a novel hydrophilic stable linker that efficiently delivers the drug to the site of action to enhance therapeutic potential. In addition, an irreversible thiol conjugation enables Cys-selective biomolecular modification providing meticulous control of the drug conjugation process.
Maleimide is a chemical group commonly used in the design of linkers for bio-conjugation, particularly in antibody-drug conjugates (ADCs), where it plays a key role in attaching a cytotoxic payload to the antibody through a linker. Seven FDA approved ADCs used Cys-maleimide chemistry. However, a major limitation of the traditional cysteine-maleimide conjugated ADCs is their instability due to retro-Michael deconjugation, which can lead to premature payload release in circulation. The ThiOBI® platform addresses this challenge by employing a stable hydrophilic linker to prevent such reaction ensuring drug delivery in a more controllable manner. This innovation profoundly improves stability and physicochemical properties of the conjugated biomolecules, minimizes off-target effects, and enhances the overall therapeutic index.
In preclinical studies, ThiOBI®-based ADCs demonstrated potent and durable antitumor activities across a variety of animal models. Notably, the platform possesses substantial flexibility that is capable of incorporating a range of payloads and biomolecular formats, including antibody fragments, nanobodies, and peptides, all of which show enhanced therapeutic activity. This versatility allows for targeting a broader spectrum of cancers and diseases that are not easily addressed by conventional antibody-based ADCs.
The promising preclinical results highlight the versatility and therapeutic promise of the ThiOBI® platform, positioning it as a powerful tool for the development of next-generation drug conjugates. The successful demonstration of potent and durable antitumor activities in animals provides a strong foundation for future clinical development of the biomolecules derived from ThiOBI®.

#5676 TFC Quick ‘n’ Clean, a next-gen platform for high-throughput production of true four-chain bispecific antibodies.

Julia Su[1], Gaozhan Zhang[2], Xiayan Li[2], Dawei Zhang[2], Mengjie Lu[2], Zhumei Feng[2], Jiansheng Wu[2], **Doris Wei**[1]

[1]WuXi Biologics, Cranbury, NJ,[2]WuXi Biologics, Shanghai, China

Introduction: Bispecific antibodies (BsAbs) are a promising class of biologics with substantial therapeutic potential. However, the production of bsAbs, particularly four-chain bsAbs containing two distinct LCs, presents significant challenges. Issues such as LC mispairing, fragmentation, and aggregation complicate purification processes and impact overall yield, purity and throughput. To address these challenges, WuXi Biologics has developed the True Four Chain (TFC) Quick ‘n’ Clean platform. By leveraging the WuXiBody™ backbone, this platform eliminates LC mispairing and enables high-throughput, high-purity production of four-chain bsAbs. This innovative approach accelerates the identification of optimal four-chain bsAb pairings during early-stage drug discovery.

Methods: All experiments were conducted at WuXi Biologics’ Protein Sciences Department. Briefly, the codons of DNA sequence were optimized, and the plasmids were constructed into WuXi Biologics’ proprietary plasmid vector. Transient transfection was performed in CHO cells, with the culture medium harvested after 7 days. The harvested culture medium was clarified and subsequently processed using an AKTA Pure system with a tandem purification strategy using Nickel, anti-DYKDDDDK, and SEC columns. Final products were analyzed using SDS-PAGE, SEC-HPLC, Intact Mass, and LAL assays.

Results: Using the TFC Quick ‘n’ Clean platform, we successfully produced 15 four-chain bsAbs in 20 mL CHO cell cultures within 3-4 weeks. The platform achieved an average monomer purity of 98.23%, an average yield of 3.34 mg, and an average endotoxin level of 0.06 EU/mg. Intact Mass analysis confirmed the absence of impurities such as homodimers and LC mispairing, with heterodimer purity exceeding 99%.

Conclusion: The TFC Quick ‘n’ Clean platform overcomes key challenges associated with four-chain bsAb production by harnessing the WuXiBody™ backbone, high-titer CHO expression systems and advanced tandem purification strategies. This platform provides high-throughput, high-purity production of four-chain bsAbs, enabling the rapid, cost-effective identification of optimal parings to accelerate early-stage drug discovery and development.

#5677 Targeting G protein-coupled receptors in cancer pharmacotherapy: a comprehensive screening platform establishment and application in drug discovery.

Xiaoyan Wang, Dandan Hu, Ziwei Zhang, **Tj (Tiejun) Bing**

ICE Bioscience, Inc., Beijing, China

G protein-coupled receptors (GPCRs) constitute the largest family of membrane protein family in the human genome with over 800 members. They play an important role in various physiological and physiological processes and, such as proliferation, differentiation, neurotransmission, development and apoptosis. Several studies demonstrated that GPCRs play significant roles in the development of cancer, with their involvement in the initiation, growth, and spread of tumors being well-established. Several drugs targeting different GPCRs have been approved for the treatment of cancers, including CXCR4 (C-X-C chemokine receptor type 4) antagonism has been demonstrated to interfere with the communication between tumors and their surrounding stroma, enhance the susceptibility of cancer cells to chemotherapy, and reduce tumor growth and metastatic burden, GnRH (gonadotropin-releasing hormone) antagonists, which are used to treat hormone-dependent tumors like prostate cancer and breast cancer. Despite the increasing recognition of GPCRs' critical functions in cancer pathogenesis, it is noteworthy that only a small number of GPCR targets have been successfully leveraged for the development of anti-cancer treatments. Consequently, comprehensive compound screening platform is particularly important for highlighting the potential of pharmacologically modulating these GPCRs for the development of novel anti-cancer therapeutics.
This research established stable cell lines expressing GPCRs and constructed multiple assay modes for GPCR drug discovery and screening, including in vitro HTRF cAMP assay, calcium flux assay and β-arretin2 NanoBiT assay, which can be used for agonist, antagonist and inverse agonist validation. Meanwhile, several CDX modes are constructed for promisingly conducting in-vivo experiments and biomarker detection. Thus, our GPCR platform can provide comprehensive screening cascade for compound evaluation, support multiple approaches to facilitate anti-cancer drug discovery.

#5678 LC-HRMS based kinetic characterization platform for irreversible covalent inhibitor screening.

Cai Fu, Tong Wu, Li Li, **Tj (Tiejun) Bing**

ICE Bioscience, Inc., Beijing, China

Covalent inhibitors are recognized as indispensable parts in drug discovery and therapeutic researches. The irreversibility of covalent conjugation makes Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) a suitable tool for covalent inhibitor screening. LC-HRMS allows label-free, direct detection of the native protein and the covalent adduct in the sample, providing relative quantification of target engagement. Several methods have been developed for covalent screening using intact protein MS or peptide mapping, including binding site mapping, covalent binding rate determination and Kinact/Ki value measurement.
In this study, the final protein concentration was 1.5 μM and the serial concentrations of positive control AMG510 were from 0.49 μM to 4.00 μM, and the total incubation time was 100 seconds. The intensity of the native protein and covalent adducts were collected on a QE Plus coupled with a C4 column.
A relatively high binding rate was observed by intact mass analysis. And a sequence coverage of GDP-KRASG12C was above 75% using peptide mapping, the specific binding site was confirmed by MS2 data. Kinact/Ki value were fitted using more than six concentrations and five timepoints. This method was used for covalent inhibitors screening, binding site characterization and Kinact/Ki measurement.

#5679 A novel approach for antibody affinity maturation using AI-driven mutation prediction.

Yiran Li, Hao Peng, Hui Zhao, Xiaoyi Han, Panpan Zhang, Jinying Ning, **Feng Hao**

Kyinno Biotechnology Co., Ltd, Beijing, China

Background: Therapeutic antibodies, characterized by their exceptional specificity and efficacy, have been a cornerstone of biotherapeutics development since their discovery. However, achieving high-affinity functional antibodies remains a significant challenge. Traditional affinity maturation methods, such as saturation mutagenesis combined with phage display screening, require extensive library construction and screening, which are time-consuming, costly, and present a high barrier to implementation. Here, we introduce a novel affinity screening approach leveraging AI-based deep learning networks to predict potential mutation sites that enhance affinity. Coupled with high-throughput expression systems, this approach effectively reduces screening scale and rapidly generates higher-affinity antibodies.
Methods: We developed and trained a novel AI model based on deep learning networks to facilitate antibody affinity optimization. The model simulates antigen-antibody docking and predicts affinity improvements by mutating the parent antibody's CDRs, identifying hotspot residues. We constructed the hotspot mutations and high-throughput expression in 96-well deep plates. The expressed antibody in supernatants were captured by protein A, and screened using sandwich ELISA. Mutations showing at least a 1.2-fold increase in OD450 are selected in preliminarily screening for AI to design combination mutations.
Results: For IgG antibodies, computational simulations reduced the initial single-point mutation library size by an order of magnitude, from 10^3 to 10^2 mutations, significantly decreasing library construction and screening costs. This approach enabled the completion of the initial screening within three weeks, with 10% of positive mutations exhibiting an OD450 increase of at least 1.2-fold. For VHH antibodies, optimization resulted in blocking activity increase up to a twofold reduction in IC50 and an 18.89-fold increase in affinity as measured by SPR. Similarly, IgG antibodies exhibited a 6.01-fold improvement in affinity (SPR).
Conclusions: We developed an AI model to simulate antigen-antibody binding and identify mutations for affinity enhancement. These predicted mutations were subsequently validated through experimental screening, confirming improved affinity. This approach enables the rapid and cost-effective generation of high-affinity antibodies.

#5680 MPSA-AB5000: a high-throughput membrane proteins screening array for therapeutic biologics specificity profiling.

Liping Wang, Yuanyuan Sun, Guoqian Wang, Jinying Ning, **Feng Hao**

Kyinno Biotechnology Co., Ltd, Beijing, China

Antibody drug development is lengthy and complex, often hindered by various challenges in clinical advancement. Reports indicate that off-target binding of antibodies may be a significant issue, with approximately 25% of preclinical candidates potentially experiencing these off-target problems. With the rise of new modalities such as CD3 bispecific antibodies, ADCs, and CAR-T therapies, preliminary off-target screening of antibodies has become increasingly important. Despite the use of techniques like immunohistochemistry, ELISA, and flow cytometry (FACS) for off-target testing of antibodies, these methods still exhibit limitations in sensitivity, specificity, and throughput. To address the need for antibody target deconvolution and receptor identification, we developed a novel membrane protein screening array (MPSA) AB5000. MPSA-AB5000 is a high-throughput, cell-based platform for identifying antibody targets and off-targets, enhancing antibody specificity screening and reducing development risks. The MPSA-AB5000 covers diverse human membrane proteins, expressed in live cells to preserve native conformations. C-terminal epitope-tagged clones allow for expression confirmation and cellular localization. To provide the highest level of sensitivity, MPSA-AB5000 uses luciferase reporter cell line detection. The wash-free strategy reduces false negatives, and signal amplification makes remarkable discrimination. Compared to similar technologies, we possess significant advantages in the following aspects: a. High sensitivity and sigh throughput: our technology platform offers not only high sensitivity, capable of detecting subtle interactions but also supports high-throughput screening, allowing for the evaluation of a large number of samples in a short timeframe. b. Avoidance of false negative signals: by eliminating washing steps, our method reduces the risk of false negative signals, ensuring reliable detection of non-specific binding. c. Comprehensive validation approaches: we employ various validation methods such as FACS and ELISA to eliminate false positive signals, enhancing the accuracy and credibility of our results. All the advantages ensure that MPSA-AB5000 is more rapid, simple, and highly sensitive than traditional assays.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Oncogenes, Tumor Suppressor Genes, and Gene Products as Targets for Therapy 2
Poster Session

#5684 PARP inhibitors boost antitumor effects of ADAR1 inhibition regardless of BRCA1 status.

Sophie O'Keefe, Dhillon Parikshak, Marcus Meade, Qiwei Wang

University of Virginia, Charlottesville, VA

Triple-negative breast cancer (TNBC), characterized by the absence of estrogen and progesterone receptors and HER2, is the most aggressive form of breast cancer. Despite recent advances in treatment, patients with advanced TNBC continue to face the worst prognostic outcomes among all breast cancer subtypes. In this project, we aim to develop new and more effective therapies for advanced TNBC. Poly (ADP-ribose) polymerase (PARP) inhibitors (PARPis), a class of drugs that target the DNA damage repair response, are the first and only targeted therapy approved for patients with advanced or metastatic TNBC. PARPis are best known for inducing synthetic lethality in tumors deficient in homologous recombination (HR) repair proteins like BRCA1/2. Thus, their approval is limited to BRCA1/2-deficient breast cancer. We and others recently demonstrated that PARPis can also trigger anti-tumor immunity by activating the STING-type I interferon signaling axis (PMID: 30540933, 35641483). However, it remains unclear whether the immunomodulatory role of PARPis can be exploited to expand their use in TNBC. In this study, we found that PARP inhibition increased interferon-stimulated gene (ISG) expression in both BRCA1-deficient and BRCA1-proficient breast cancer cells. This finding is significant because previous studies suggest that cancers with elevated ISG expression are highly reliant on the RNA editing enzyme adenosine deaminase 1 (ADAR1) for survival, making them susceptible to ADAR1 inhibition (ADAR1i). To investigate this further, we utilized tumor cell lines derived from genetically engineered mouse models (GEMMs) of TNBC and employed siRNA, shRNA, and small molecule inhibitors against ADAR1. Notably, we demonstrated that combining PARPi with ADAR1i synergistically killed TNBC cells, regardless of their BRCA status. Taken together, our results highlight the potential of combing PARPis with ADAR1i for treating TNBC, including BRCA-proficient tumors.

#5685 Targeting the NR2F6-NACC1-NOTCH3 axis: a promising therapeutic strategy to combat tumor progression and resistance in endometrial cancer.

Xiaoqi Sun[1], Xiaofang Guo[1], Tram Le[1], Vaagn Andikyan[2], Thomas J. Rutherford[2], Eduardo Sotomayor[2], Matthew L. Anderson[2], **Adrian Kohut**[2]

[1]University of South Florida, Tampa, FL,[2]University of South Florida/Tampa General Cancer Institute, Tampa, FL

Background: Uterine corpus endometrial carcinoma (UCEC) is the most common gynecologic malignancy, with poor prognoses in advanced, high-grade, or treatment-resistant cases. The transcription factor NR2F6 (nuclear receptor subfamily 2 group F member 6) has emerged as a key regulator of immune evasion, tumor progression, and therapy resistance, suppressing T-cell activation by regulating PD-L1 (programmed death-ligand 1) expression. Nucleus accumbens-associated protein 1 (NAC1), encoded by the NACC1 gene, is a transcriptional repressor critical for tumor growth, metastasis, and drug resistance. NOTCH3 (Notch homolog 3), a downstream regulator, contributes to therapy resistance and tumor aggressiveness. This study investigates the significant therapeutic potential of targeting this axis.

Methods: Gene expression data from 529 UCEC patients were obtained from The Cancer Genome Atlas (TCGA), and survival analyses were performed using R Studio. Microsatellite instability (MSI) cell line HEC1A and microsatellite stable (MSS) cell line KLE were obtained from ATCC. Knockdowns of NR2F6 and NACC1 were performed using small interfering RNA (siRNA). Semi-quantitative real-time PCR (RT-qPCR) and Western blot were used to measure NR2F6, NACC1, NOTCH3, and PD-L1 expression. Cell proliferation was evaluated using Promega assays. NIC3, a small-molecule inhibitor that prevents NAC1 homodimerization and induces proteasomal degradation, was applied to validate findings from siRNA experiments. Statistical significance was determined using paired Student's t-tests.

Results: High expression of NR2F6 and NACC1 was associated with poor survival in UCEC patients (NR2F6: $p = 0.0017$; NACC1: $p = 0.019$). Correlation analyses showed a strong relationship between NR2F6 and NACC1 (Pearson $r = 0.54$, $p < 0.0001$) and a moderate relationship between NACC1 and NOTCH3 ($r = 0.45$, $p < 0.0001$). RT-qPCR and Western blot analyses revealed that NR2F6 knockdown significantly reduced NACC1, NOTCH3, and PD-L1 expression. NACC1 knockdown similarly decreased NOTCH3 and PD-L1 expression, confirming its regulatory role in the axis. Notably, siRNA-mediated knockdowns and NIC3 treatment significantly reduced cellular proliferation across all treated cell lines (HEC1A, KLE, and Colo 684). NIC3 similarly demonstrated the ability to degrade NAC1 protein and reduce downstream NOTCH3 and PD-L1 expression.

Conclusions: This study highlights the therapeutic potential of targeting the NR2F6-NACC1-NOTCH3 axis in UCEC. Validation at both mRNA and protein levels, combined with the observed reduction in cellular proliferation, underscores the promise of this axis as a critical target in high-grade or treatment-resistant tumors. The small-molecule inhibitor NIC3 offers a novel strategy to disrupt NAC1-mediated signaling, and potentially target UCEC.

#5686 Revealing the unique malignant mechanism of gastric-type adenocarcinoma of the cervix.

Hiroaki Yamada[1], Akira Yokoi[2], Kazuhiro Suzuki[2], Kosuke Yoshida[2], Eri Inami[2], Masami Kitagawa[2], Naotake Tsuda[1], Hiroaki Kajiyama[2]

[1]Obstetrics and Gynecology, Kurume University Hospital, Kurume, Japan,[2]Obstetrics and Gynecology, Nagoya University Graduate School of Medicine, Nagoya, Japan

Objective: Gastric-type adenocarcinoma of the cervix (GAS) is an HPV-independent malignancy that accounts for 20-25 % of cervical adenocarcinomas. Compared to usual-type endocervical adenocarcinoma (UEA), GAS is resistant to chemotherapy and radiotherapy, and has a poor prognosis. However, its molecular biological mechanisms remain unclear. This study aimed to identify specific RNA profiles associated with GAS pathophysiology to discover diagnostic and therapeutic targets.
Methods: We examined 15 tumor samples from patients who underwent primary hysterectomy (7 GAS, 8 UEA). From FFPE specimens, only the tumor region was obtained, and miRNA-seq and mRNA-seq were performed. RNA was also extracted from the GAS-derived KGAS cell line, UEA-derived HCA-1 cell line, and normal cervical epithelial HCK1T cell line for miRNA-seq. Extracellular vesicles (EVs) from each cell line were collected, and miRNA-seq was performed by using EV-RNA. Detail functional analyses were performed using cell lines.
Results: miRNA sequencing of FFPE samples, cell lines, and EVs derived from GAS and UEA identified 48 miRNAs highly expressed in GAS tissue, 37 in GAS cell lines, and 41 in GAS-EVs. Further target exploration revealed one miRNA specifically highly expressed in GAS. Additionally, mRNA sequencing analysis of tumor tissue samples with 10 cases (5 GAS, 5 UEA) identified a total of 14,541 genes, and clustering analysis showed that GAS and UEA had clearly distinct expression profiles. Differential expression analysis identified 449 upregulated genes in GAS and 577 downregulated genes in UEA, meeting the criteria of FC ≥ 1 and p-value < 0.05. Among these, the top 30 genes with the highest FC values and basemean ≥ 50 were selected. IPA and GSEA analyses significantly detected cancer-related pathways, including RTK-RAS-PI3K, PDGF signaling, and cell cycle-related pathways. The top 10 significant genes were validated by qRT-PCR, and protein expression was confirmed by immunohistochemistry. Functional validation in cell lines showed that GAS exhibited significantly higher cisplatin resistance ($p < 0.05$) than other cell lines, and suppression of newly identified specific target gene in GAS cells resulted in increasing sensitivity of cisplatin.
Conclusion: This study revealed detailed miRNA and mRNA profiles of GAS and UEA, identifying GAS-specific miRNA and gene expression. Identified genes were confirmed as not only useful as disease markers but also functional targets. These findings contribute to understanding the unique malignant mechanisms of GAS and highlight promising targets for future therapeutic strategies.

#5687 Immune-associated genes as potential therapeutic targets in small cell lung cancer.

Akihiko Miyanaga, Yang Yunchu, Kuniko Matsuda, Takehiro Tozuka, Aya Fukuizumi, Kakeru Hisakane, Naomi Onda, Susumu Takeuchi, Koichiro Kamio, Kazuo Kasahara, Masahiro Seike

Department of Pulmonary Medicine and Oncology, Graduate School of Medicine, Nippon Medical School, Tokyo, Japan

Background: Recently, immune checkpoint inhibitors (ICIs) have been increasingly applied in the treatment of small cell lung cancer (SCLC). While their development has enhanced treatment options for SCLC, the effectiveness of immunotherapy remains limited due to the low expression of Programmed Cell Death Ligand 1 (PD-L1) and the emergence of immunotherapy resistance. As a result, there is a pressing need for new biomarkers to guide SCLC treatment.

Methods: We obtained four microarray datasets from GEO (Gene Expression Omnibus) database and screened differentially expressed genes (DEGs). After the establishment of protein-protein interaction network through Cytoscape, key hub genes were selected via a volcano plot. To validate these results, we used an independent dataset as a test cohort. We also analyzed differences in immune cell infiltration between normal and SCLC samples. Lastly, we identified a marker gene associated with immune cell infiltration and validated its role in SCLC through in vitro experiments.

Results: We screened GEO gene expression database for DEGs, which are immune-related genes, in SCLC. A PPI network analysis revealed seven overexpressed hub genes (*AURKB, BIRC5, TOP2A, TYMS, PCNA, UBE2C, AURKA*) in the SCLC cohort, which were significantly more expressed than in normal cells. Correlation analysis between immune cells and the seven hub genes showed that BIRC5 expression was inversely correlated with monocytes. In SCLC cell lines such as SBC5 and SBC3, downregulation of BIRC5 using siRNA induced apoptosis and inhibited cell activity, tumor invasiveness and proliferative potential. Furthermore, treatment with a BIRC5 inhibitor (YM155) reduced SCLC cell viability and increased apoptosis. Co-culture experiments with SBC3 and monocytes (e.g., THP1) led to decreased BIRC5 expression in SBC3 cells.

Conclusion: We identified *AURKB, BIRC5, TOP2A, TYMS, PCNA, UBE2C*, and *AURKA* genes as potential immune-related therapeutic targets for SCLC. In particular, high BIRC5 expression promotes SCLC progression and may influence the immune microenvironment of the tumor by affecting monocytes.

#5688 DSTYK dependent regulation of autophagy protects against TNF alpha induced apoptosis in lung cancer.

Andrea Pasquier[1], Cristina Viu-Idocin[1], Andrea Arricibita[1], Mirari Echepare[1], Benat Picabea[1], Ane Alava[1], Joaquin Fernandez-Irigoyen[2], Enrique Santamaria[2], Eva Molina[1], Antonio Pineda-Lucena[1], Luis Montuenga[1], **Karmele Valencia**[1]

[1]Center for Applied Medical Research (CIMA), Pamplona, Spain,[2]Public University of Navarra, Pamplona, Spain

The emergence of immunotherapy has significantly advanced the treatment of non-small cell lung cancer (NSCLC), yet acquired resistance remains a critical challenge. Understanding the molecular mechanisms behind tumor cell sensitivity to T-cell killing is essential to overcome resistance and improve treatment outcomes. Previous studies have indicated the roles of T cell-derived tumor necrosis factor-α (TNFα) and nuclear factor κB (NF-κB) signaling in tumor cell killing, with autophagy inhibiting TNF-α-induced apoptosis.Previous research in our lab has identified DSTYK, a dual serine/threonine and tyrosine kinase amplified in NSCLC, as a key regulator of mTOR. DSTYK plays a critical role in preventing TNF-α-mediated $CD8^+$ T cell killing and impairs the response to immunotherapy in non-small cell lung cancer by inhibiting autophagy. However, the specific mechanism by which autophagy constrains TNF-α-dependent apoptosis remains unclear.In this study, we demonstrate that inhibition of DSTYK disrupts ULK1 phosphorylation, leading to RIPK1 autophosphorylation and promoting proapoptotic signaling through TNFR1 complex II. Furthermore, we demonstrated that ULK1 inhibition replicates the effects of DSTYK inhibition, reducing tumorigenic properties and sensitizing cells to apoptosis and necroptosis. *In vivo* experiments in syngeneic and xenograft mouse models corroborated these findings, showing significant tumor size reduction upon ULK1 inhibition. In conclusion, our study identifies DSTYK as a key regulator of the TNFR1 pathway via ULK1 phosphorylation, suggesting that targeting ULK1 could be a viable therapeutic strategy for sensitizing DSTYK-amplified NSCLC tumors to cell death.

#5689 Proliferating cell nuclear antigen as a new therapeutic target in multiple myeloma.

Jeffrey Zonder[1], Bin Bao[1], Amro Aboukameel[1], Sahar Bannoura[1], Husain Y. Khan[1], Hafiz Uddin[1], Mohamad B. Hamami[1], Ali Rehman[1], Ramzi Mohammad[1], Long Gu[2], Linda Malkas[2], Asfar Azmi[1]

[1]Oncology, Wayne State University School of Medicine, Detroit, MI,[2]City of Hope National Medical Center, City of Hope, CA

Background: Multiple myeloma (MM), a plasma cell cancer, is the second most common hematologic malignancy. Despite advances in treatment (proteasome inhibitors, cereblon-targeting immunomodulatory drugs, and anti-CD38 and anti-BCMA immunotherapies) MM remains incurable. Proliferating cell nuclear antigen (PCNA) is an essential protein which is involved in chromatin remodeling, cell cycle progression, and DNA repair and replication, plays a key role in regulation of cell growth and proliferation. A post-translationally modified isoform of PCNA plays a critical role in the pathogenesis of a wide variety of malignant diseases including MM. Increased expression of PCNA has been shown to be associated with an aggressive MM phenotype, making it an attractive therapeutic target.
Methods: We tested the anti-tumor activity of the novel small molecule PCNA inhibitor AOH-1996 (alone and in combination with either bortezomib or venetoclax) against MM cell lines (MM1.S, SKMM2, RPMI-8226). We assessed the cytotoxic anti-MM effects of varying doses AOH-1996 using standard viability assays and also 3D spheroid degradation assays. Changes in gene expression relevant to cell growth, cell-cycle progression, apoptosis, methylation status, and cytokine production following AOH-1996 exposure were analyzed using RT-PCR and Western blotting, as appropriate. We also evaluated the impact of AOH-1996 exposure on miRNA levels. The anti-MM effect of AOH-1996 monotherapy and combinations versus the same cell lines was then further evaluated in xenograft animal model.
Results: AOH-1996 demonstrated dose-dependent reduction of cell growth (2D) and CSC-like sphere formation (3D) of MM1.S, SKMM2, and RPMI-8226 MM cell lines. Either short (2 days) or long (7 days) incubation with AOH-1996 significantly down-regulated the gene expression of selected anti-apoptotic markers (PCNA, CDK4/6, BCL2), cancer stem cell markers (EZH2, vimentin), and inflammatory and pro-survival cytokines (IL-6, VEGF, MIP-1alpha). AOH-1996 treatment down-regulated miR-21, miR-142, and miR-222 levels along with the reduced cytokine production of VEGF. There was a synergic effect on cell growth and CSC-like sphere formation when either bortezomib or venetoclax was added to AOH-1996. AOH-1996 significantly inhibited MM.1S cell line-derived xenograft tumor growth. Additional xenograft studies using other MM cell lines, as well as AOH-1996/bortezomib and AOH-1996/venetoclax combinations are ongoing.
Conclusions: These findings clearly suggest that PCNA is a potential therapeutic target in MM. We have demonstrated for the first time that the PCNA inhibitor AOH-1996 shows potent anti-myeloma activity through its effects on several key tumor-associated genes and miRNAs. AOH-1996 is currently being evaluated in a Phase I trial.

#5690 Targeting MET activation in CDH1 deficient diffuse gastric cancer with peritoneal metastases.

Hannah Sofield[1], Hyun-Kyung Ko[2], Dipti Athavale[3], Yan-ting Yann Zhan[3], Xiaodan Yao[3], Luke Chen[1], Young K. Hong[1], Francis Spitz[1], Generosa Grana[2], Madeline Torres[1], Shumei Song[2]

[1]Department of Surgery, Cooper University Hospital, Camden, NJ,[2]MD Anderson at Cooper University Healthcare, Camden, NJ,[3]Coriell Institute for Medical Research, Camden, NJ

Background: Peritoneal metastasis (PM) is common in advanced gastric cancer (GC). PM has a poor prognosis with a short average survival of 2 to 9 months. Currently, the only treatment option for these patients is palliative chemotherapy, which has poor efficacy. Improving patient survival requires a better treatment approach. Germline mutations in CDH1 (E-cadherin) tumor suppressor gene are associated with hereditary diffuse gastric cancer (HDGC) syndrome, a very aggressive form of GC and lobular breast cancer. Our prior studies on GC PM patient samples have demonstrated increased incidence of CDH1 deficiency (mutations/deletions; 26%) compared to primary tumor samples (9%). Further molecular integrated analysis has shown that CDH1 mutant tumors are correlated with increased expression of MET oncogene, suggesting that targeting MET could be beneficial in the treatment of the subset of PM patients with CDH1 deficiency (somatic or germline). This study seeks to target MET activation in CDH1 deficient diffuse GC cells.
Methods: Three diffuse GC cell lines derived from human PM samples were used: SNU-16 (CDH1 wild-type), GA0518 (CDH1 deletion), and MKN45 (CDH1 mutant). We examined MET and phosphorylated MET (p-MET) expression in all cell lines using qPCR, immuno-blotting and immuno-fluorescence. We then evaluated the *in vitro* effects of two commercially available MET inhibitors, Cabozantinib and Crizotinib. Drug effects on cell viability, colony formation, migration, and downstream MET signaling pathways were assessed.
Results: MET and p-MET expression, and downstream MET signaling were significantly enhanced in CDH1 mutant diffuse GC cell lines compared with the wild-type. Cell viability results demonstrated that CDH1 mutant cell lines were highly sensitive to MET inhibitors. IC50 values showed higher susceptibility for both drugs in CDH1 mutant cell lines, MKN45 (0.01nM and 0.2nM for Crizontinib and Cabozantinib, respectively) and GA0518 (648nM and 824nM) compared to wild-type, SNU16 (1.9uM and 3.2uM). Downstream pathways, PI3K and MAPK, were suppressed at concentrations between 10-50nM. Cell migration and colony formation were inhibited between 10nM-500nM.
Conclusions: CDH1 deficient (mutant/deletion) GC cell lines have increased MET expression and activation (p-MET) compared to wild-type cells. We showed that CDH1 mutant cell lines with high MET activation were susceptible to MET inhibitors. Furthermore, at low concentrations, targeting activated MET led to complete loss of p-MET expression, suppression of its downstream signaling, and impaired cell growth, migration, and colony formation. Thus, blockade of MET signaling could be clinically useful for the treatment of CDH1 deficient diffuse GC PM patients, while CDH1 mutations could serve as a biomarker. Future *in vivo* PDX and syngeneic mouse models are under investigation to validate these results.

#5691 Preclinical evaluation of RMC-7977, a multi-selective RAS(ON) inhibitor, as a therapeutic strategy for KRAS-mutant cholangiocarcinoma.

Rodrigo Entrialgo-Cadierno[1], Iker Feliu[1], Ines Lopez[1], Yaiza Senent[1], Shruthi Narayanan[1], Nicole Nasholm[2], Ruben Pio[1], **Jingjing Jiang**[2], Silvestre Vicent[1]

[1]Center for Applied Medical Research (CIMA), Pamplona, Spain,[2]Revolution Medicines, Redwood City, CA

Background: Cholangiocarcinoma (CCA), comprises approx. 3% of all gastrointestinal tumors, and is characterized by highly dismal prognosis. Therapies predominantly rely on chemotherapy, and treatment advancements have been stagnant over the past decades, underscoring the critical need for innovative therapies. Mutant KRAS is among the most prevalent oncogenes in CCA, highlighting a potential role for KRAS inhibitors as therapeutic avenues. Here, we present preclinical data on RMC-7977, a RAS (ON) inhibitor as a potential therapeutic strategy for CCA with KRAS mutations.

Materials and Methods: Human and murine KRAS mutated cell lines were utilized to determine the effect of RMC-7977 both in vitro and in vivo. H69 immortalized cholangiocytes were virally infected with KRAS mutant isoforms (4B, G12, G13, Q61). RNA-seq and Western blot was conducted to analyze the molecular changes and potential resistance mechanisms. The tumor immune microenvironment was assessed via multiplex flow cytometry. Combinatorial pharmacological treatments were conducted in vitro and in vivo to study resistance mechanisms overcome. Both human and murine cell lines resistant to the RAS(ON) inhibitor were generated. Subsequently RNA seq and WES were performed to identify possible transcriptomic changes and acquired mutations in resistant cells.

Results: RMC-7977 elicited antiproliferative effects in mouse and human CCA cell lines with different KRAS mutations (G12D/G12V/G13C) and H69 expressing various KRAS mutations in vitro. In vivo experiments with human xenografts or mouse allografts revealed heterogeneous antitumor responses ranging from tumor regressions to growth delay. In vitro mouse cell line/CD8+ co-culture experiments, showed RMC-7977 capacity to increase CD8 cytotoxicity. Tumor immune phenotyping of immunocompetent mice showed a notable increase in NK, CD8, and CD4 infiltration, and a reduction of PMN-MDSCs, indicative of a less immunosuppressive microenvironment. RNA-seq and Western blot analysis revealed increased activity of the SHP2, FGFR, and JAK/STAT pathways. Further in vitro testing confirmed the synergistic effects when we combined RMC-7977 plus SHP2 (in parental and resistant models) but also combinatorial benefit in xenograft models in vivo. Addition of the standard of care (Gem/Cis/PD1) to the RAS(ON) inhibitor resulted in significant tumor regression and enhanced anti-tumor activity compared to single treatments. Identification of potential resistant mechanisms related to transcriptomic changes, acquired mutations and CNV in resistant models is currently ongoing.

Conclusion: In summary, our preclinical findings support the clinical testing of a RAS(ON) multi-selective inhibitor, either alone or in combination with other in-pathway or out-of-pathway partners, as a potential therapeutic strategy for CCA treatment.

#5692 Distinct effectors of FSTL1 promote melanoma growth and angiogenesis and FSTL1 blockade mitigates resistance to BRAF and MEK inhibitors.

Umar Patel, **Jiaying Ng**, Chiu Wang Chau, Alex To, Ryohichi Sugimura, Martin Cheung

University of Hong Kong, The - Li Ka Shing Faculty of Medicine, Hong Kong, Hong Kong

Melanoma is a highly lethal form of skin cancer due to its propensity for metastasis through angiogenesis, which confers melanoma initiating cells with resistance to BRAF and MEK inhibitors. However, current drugs for targeting angiogenesis have shown limited efficacy, highlighting the need to develop new anti-angiogenic therapies to effectively combat this devasting malignancy. Here, we found that Follistatin-like 1 (FSTL1) expression is significantly increased in metastatic melanoma, and correlates with higher microvessel density, and poor prognosis. FSTL1 is both required and sufficient for the growth, metastasis, and angiogenesis of melanoma. FSTL1 functions downstream of SOX10-DLC1-FOXK1 to mediate its oncogenic activity, which can be inhibited by FSTL1 neutralizing antibody (FSTL1 nAb). Mechanistically, intracellular FSTL1 promotes G1/S transition in proliferative melanoma cells by activating an AKT/Lamin B2 axis. Secreted FSTL1 activates endothelial expression of TMSB4X to induce nuclear NF-kB-dependent regulation of angiogenesis. Furthermore, FSTL1 expression is selectively upregulated in melanoma-initiating cells that are resistant to BRAF and MEK inhibitors, driving stemness through interactions with the tumor microvasculature. Treatment with FSTL1 nAb depletes melanoma-initiating cells and slows down drug resistance development, suggesting that FSTL1 nAb may be a promising adjunct therapy to BRAF and MEK inhibitors for melanoma treatment.

#5693 Diagnostic and predictive biomarkers for canine prostatic carcinoma: translational therapeutic insights.

Alexandre Matheus Baesso Cavalca[1], Marxa Leao Figueiredo[1], Maricy Apparicio Ferreira[2], Carlos Eduardo Fonseca Alves[3]

[1]Basic Medical Sciences, Purdue University, West Lafayette, IN,[2]Departamento de Reproducao Animal, Universidade Estadual Paulista, Botucatu, Brazil,[3]Universidade Paulista, Bauru, Brazil

The prostate is an organ prone to diseases typical of human aging, with benign and malignant prostatic disorders being among the most common diseases that affect men. There are a wide variety of treatment options for diagnosing prostate cancer in humans, depending on the risk category the disease falls into. The dog is the only mammal, besides man, that spontaneously develops canine prostatic hyperplasia (PH), prostatic atrophy and prostatic carcinoma (PCa), which are associated with age and androgenic hormones. However, unlike PCa in humans, PCa in dogs is not androgen dependent, therefore androgen deprivation therapy is not effective, and animals develop castration-resistant prostatic carcinoma (CRPC). Many treatment modalities usually for human PCa cannot be applied in CRPC. And regarding dogs, unfortunately, there is still no effective therapy for the treatment of these tumors. Available pharmacological treatments are scarce, based primarily on the prescription of anti-inflammatory drugs, with a low degree of survival. Therefore, we intend to carry out new translational studies on human and canine PCa samples, aiming to identify predictive markers and potential common therapeutic targets, to subsequently elucidate the antitumor effect of existing drugs, aiming to develop targeted therapies. The study included 20 animals, with 4 in the control group and 16 in the neoplasia group, under CEUA approval. Samples underwent proteomic analysis, RT-qPCR, RNA sequencing (RNAseq), and Next-Generation Sequencing (NGS) to evaluate gene expression patterns, tumor microenvironment interactions, and immune cell infiltrate estimates. Cross-validation with independent data from canine and human prostate carcinoma databases was performed. Biological triplicates were analyzed, and differential protein and gene expression were assessed using Student's t-test ($p < 0.05$, expression change > 1.5x). A total of 44 proteins, including significantly elevated levels of Vimentin and Peptidase S1, were identified in the neoplasia group. Additionally, 1,412 differentially expressed genes were enriched in prostate cancer-related pathways, with 536,200 SNPs identified across 291 genes in the canine exome. Thirteen genes co-expressed in RNAseq and NGS analyses emerged as potential direct therapy targets. Increased secretion of Vimentin and Peptidase S1 in the neoplasia group highlighted their potential as tumor biomarkers. These findings reveal a significant overlap in tumor progression and biomarker expression between canine and human PCa, offering promising avenues for translational studies to develop effective diagnostic tools and targeted therapies for both species.

#5694 Targeting NAD(P)H: Quinone oxidoreductase 1 (NQO1) for colorectal cancer diagnosis and therapy.

Chaithanya Ganji[1], Santosh Behera[2], Lakkakula V. K.S. Bhaskar[3], Mehmet Akce[4], Bassel F. El-Rayes[4]

[1]Biomedicine, University of Alabama at Birmingham, Birmingham, AL,[2]Biotechnology, National Institute of Pharmaceutical Education and Research-Ahmedabad (NIPER-A), Gandhinagar, India,[3]Zoology, Guru Ghasidas Vishwavidyalaya, Bilaspur, India,[4]Hematology and Oncology, University of Alabama at Birmingham, Birmingham, AL

Background/Objective: Colorectal cancer (CRC) is the third most common cancer in the United States and is associated with poor outcomes. NAD(P)H: Quinone Oxidoreductase 1 (NQO1), an enzyme, shown to contribute to chemoprotection for CRC. This meta-analysis aims to examine the association between NQO1 C609T polymorphisms and CRC risk. Furthermore, the study explores the binding capacity and efficacy of BBI 608 against NQO1 in CRC through molecular docking. The inhibitor will then be tested *in vitro*.

Methods: PubMed, Web of Science, and Google Scholar were used in bibliographic searches. For the NQO1 C609T analysis, 18 studies were used. Data was gathered and subsequently calculated using the pooled odds ratio (OR) (95% confidence interval, CI). Molecular docking, Western Blot, and QRT-PCR were used to determine the molecular function of BBI 608 and NQO1.

Results: The relation between NQO1 polymorphism and CRC risk (TT + CT vs. CC: OR=1.19, 95% CI =1.06-1.34, p<0.001) was significant. Furthermore, stratified investigation based on ethnicity indicated a significant association between NQO1 polymorphism and CRC risk (TT + CT vs. CC: OR=1.17, 95% CI =1.08-1.27, p<0.001). This study indicates that the C609T polymorphism of NQO1 is linked with CRC risk in Asians and Caucasians. Computational approaches were applied to observe the molecular properties of BBI 608 (NQO1 inhibitor) and analyze the exact mechanism of NQO1 on CRC. This investigation provides a detailed understanding of the interaction between NQO1 and BBI 608 and its implication in CRC therapy. *In vitro* studies were performed to support this computational analysis. BBI 608 creates higher cytotoxicity in a dose-dependent manner in CRC cell lines. BBI 608 treatment significantly (p<0.001) reduced the NQO1 expression at protein and RNA levels in both CRC (HCT 116 and RKO) cell lines.

Conclusion: Based on meta-analysis and a computational approach, NQO1 is a viable biomarker and a possible molecular target in CRC. *In vitro*, results showed that inhibiting NQO1 by BBI 608 decreased cell proliferation in both CRC cell lines. Knockout, overexpression, or site-directed mutagenesis is essential for a better understanding of BBI 608-targeted NQO1 and its amino acids.

#5695 Examining the tumor suppressor function of FBXW7 in endometrial cancer.

Sophia E. Agrusa, Swornalata Pukhrambam, Areebah Qazi, Madelyn Maurer, Sneha Aradhya, Jessica Back, Mike R. Wilson

Wayne State University School of Medicine, Detroit, MI

Endometrial cancer (EC) is the sixth most commonly occurring cancer in women, with incidence rates steadily increasing in recent years. The American Cancer Society estimates 67,880 new cases and 13,250 deaths in 2024. A racial disparity is observed in EC as Black patients often have higher grade disease, worse outcome and the more serve subtype of EC, uterine serous carcinoma (USC). The National Cancer Institute Surveillance, Epidemiology, and End Results (SEER) program estimates that per 100,000 cases of EC, Non-Hispanic Black (NHB) patients have a death rate of 9.5 while Non-Hispanic White (NHW) patients have a death rate of 4.7. Disparity is most evident in metastatic USC, since NHW patients have a 5-year survival of 20% while NHB patients only have 5-year survival of 10%. In metastatic disease, FBXW7 mutation rates are higher in NHB patients compared to NHW patients. Using the TCGA database, we found FBXW7 is a tumor suppressor that is frequently mutated in endometrial cancer and has a higher mutation rate in USC compared to endometroid carcinoma. FBXW7 functions as a tumor suppressor through its ubiquitinase activity, in which it targets many oncoproteins for degradation including c-Myc, Notch1, Cyclin E, and mTOR. Previously our lab worked to purify FBXW7 via immunoprecipitation and found that FBXW7 may interact with MLH1, a DNA mismatch repair enzyme. To characterize potential-protein interactions between FBXW7 and MLH1, we transfected endometroid carcinoma cells with FBXW7 or MLH1 plasmids to elevate expression of these proteins and found that overexpression of FBXW7 results in a decrease in MLH1 expression. This may indicate MLH1 is a substrate for FBXW7 and is regulated via FBXW7 mediated ubiquitination. We then generated a FBXW7 knockout model in 12Z cells using a CRISPRCas9 system. With our successful FBXW7 knockout clone, we examined alterations of protein expression via Western Blot analysis. We see an increased expression of mTOR following knockdown of FBXW7, as this is a canonical substrate of FBXW7 ubiquitination. To investigate potential effects on invasion, we examined EMT markers Vimentin and E-Cadherin, where we see a decreased expression of Vimentin but unaltered expression of E-Cadherin following FBXW7 knockout. Due to the mutational frequency of FBXW7 in NHB metastatic UCS, we also examined changes in HER2 expression. Herceptin, which targets HER2, was previously clinically used as a therapy for UCS and had variable success in patients. Here we see a decrease of HER2 following FBXW7 knockout. Using the CRISPR-mediated knockout cells, ongoing studies will investigate cell growth rates and invasion FBXW7 mutant 12Z.

#5696 Hanmi's p53 mRNA and abraxane synergistically promote tumor control in PTX-prefered cancer through p53 stabilization.

Jooyun Byun, Jongmin Hong, Seungheon Baek, Gunwoo Lee, Hocheol Shin, Miyoung Lee, Seung-Hyun Shin, Yong Ho Heo, Daejin Kim, In Young Choi

Hanmi Pharmaceutical Co., Ltd., Hwaseong-si, Korea, Republic of

TP53 is the most frequently mutated tumor suppressor gene in human cancers and plays a critical role as a master regulator of apoptosis and cell cycle. Approximately 50% of human cancers harbor TP53 mutations, which are associated with accelerated tumor progression and resistance to anti-cancer therapies leading to unfavorable prognosis. As the way to restore the loss of function caused by p53 mutations, mRNA-based therapy is expected to yield novel therapeutic outcomes that remain unattainable with current approaches. This study endeavored to characterize the types of p53 mutations that exhibit heightened sensitivity to lipid nanoparticle (LNP)-encapsulated mRNA expressing wild-type p53. The anti-cancer potential of Hanmi p53 mRNA was demonstrated in a large number of cancers with p53 mutations, sparing to normal cells. Notably, p53 null/nonsense mutations were more sensitive to the Hanmi p53 mRNA compared to missense mutations or p53 WT cancer demonstrating a positive biomarker. High-throughput screening was performed to investigate the combination of Hanmi p53 mRNA with standard-of-cares, assessing the synergistic growth inhibition in cancer cells including lung, pancreatic, and breast, which exhibit high frequencies of p53 mutations. Taxanes such as paclitaxel, abraxane or docetaxel exhibited the best combinational synergy with Hanmi p53 mRNA in various p53-mutated cancer cell lines. Specifically, combination of Hanmi p53 mRNA and abraxane led to a dramatic increase in acetylated as well as phosphorylated p53 levels compared to Hanmi p53 mRNA alone. These results suggest that abraxane stabilizes wild-type p53 through acetylation, sustaining its expression and enhancing transcriptional activity in p53-mutated cancer. Restoration of p53 function highly elevated p21 expression, inducing G1 phase cell cycle arrest. Importantly, the combination of Hanmi p53 mRNA and abraxane synergistically amplified apoptotic activity, as evidenced by a marked increase in PARP cleavage. In vivo efficacy assessments of IV administration for LNP-encapsulated Hanmi p53 mRNA in combination with abraxane were conducted in PTX-preferred indication such as lung and ovarian cancer xenograft models. Tumor regression in mice treated with Hanmi p53 mRNA and abraxane was remarkably enhanced, with complete responses observed in 4 out of 5 mice, demonstrating the synergistic efficacy of the combination therapy. NGS-based gene set enrichment analysis conducted on NCI-H1373 xenograft tumors treated with p53-mRNA plus abraxane exhibited up-regulation of apoptotic gene sets compared to vehicle, consistent with our in vitro results. These results highlight Hanmi p53 mRNA exhibits strong synergy with taxanes, providing robust anti-cancer effects in indications requiring such therapeutic approaches.

#5697 Targeting CBL and CBLB ubiquitin ligases to exhaust cancer stem cells in metastatic breast cancer.

Bhopal C. Mohapatra[1], Aaqib M. Bhat[1], Mohsin Raza[1], Haitao Luan[2], Santosh Shrestha[2], Sowmya Kolluru[3], Matthew D. Storck[2], Lusheng Li[1], Fu-Shan Kuo[4], Silvana Leit[4], Fang Qiu[5], Subodh M. Lele[6], David Ciccone[4], Christine Loh[4], Shibiao Wan[1], Vimla Band[1], Hamid Band[2]

[1]Department of Genetics, Cell Biology and Anatomy, University of Nebraska Medical Center, Omaha, NE,[2]Eppley Institute and Fred & Pamela Buffett Cancer Center, University of Nebraska Medical Center, Omaha, NE,[3]Creighton University, Omaha, NE,[4]Nimbus Therapeutics, Boston, MA,[5]Department of Biostatistics, University of Nebraska Medical Center, Omaha, NE,[6]Department of Pathology, Microbiology and Immunology, University of Nebraska Medical Center, Omaha, NE

Introduction: Recurrence, therapy resistance and metastasis remain major challenges in cancer therapy, accounting for nearly all deaths from cancer. The inability of current therapies to eliminate cancer stem cells (CSCs) is widely believed to underlie these challenges. Targeting molecular pathways that help maintain the CSCs could open new avenues to devise durable cancer therapies. The CBL-family E3 ubiquitin ligases (CBL, CBL-B and CBL-C) attenuate tyrosine kinase signaling through ubiquitin-dependent degradation of tyrosine kinase-coupled receptors and signaling components. Gene deletion studies have shown that CBL-B, the predominant family member expressed in mature immune cells, mediates tumor-induced “immune editing” to generate a pro-tumor immune microenvironment. This mechanism has provided one line of evidence to develop chemical CBL-B inhibitors as cancer immune modulators, and candidate inhibitors exhibit anti-tumor activity in mouse implant models, supporting the general idea that CBL-family E3s are potential therapeutic targets. Using mouse genetic models, we have established that CBL and CBL-B are redundantly required for the maintenance of adult stem cell compartments, and that this role is dependent on the core function of CBL/CBL-B as negative regulators of the AKT-mTOR axis downstream of tyrosine kinase signaling. As normal organ stem cell programs are frequently co-opted by CSCs; we used a 3-pronged approach to ask if CBL/CBL-B are required to maintain CSC function.

Methods/Results: 1. Based on higher CBL/CBL-B expression in TNBC cell lines, and TCGA data that CBL/CBL-B mRNA expression positively correlates with shorter patient survival, we engineered Dox-inducible shRNAs in TNBC cell lines and showed that CBL/CBL-B KD impaired cell migration and invasion, reduced the population with CSC markers and impaired orthotopic xenograft tumorigenesis and metastatic seeding. 2. We generated a unique mouse model in which the C3(1)-TAg transgene drives mammary tumors known to resemble human triple-negative breast cancer (TNBC) and floxed-*Cbl and Cblb* genes can be inducibly deleted using tamoxifen (via *CreERT*^{2}). Tumors of these mice established as tumor-derived organoids (TDOs) exhibited significant impairment of growth and CSC signature gene expression upon *Cbl/Cblb* KO. 3. Since the developed “CBL-B” inhibitors target a mechanism shared between CBL/CBL-B, we used two active compounds (NTX-801, NTX-944) and one inactive compound (NTX-616) developed by Nimbus Therapeutics. The active inhibitors mimicked the genetic *Cbl/Cblb* KO to inhibit the mouse mammary TDOs growth, while the inactive analog was without effect.

Conclusions: Our results establish a tumor-intrinsic requirement of CBL/CBL-B to maintain breast cancer CSCs and add a critical second rationale for the use of chemical inhibitors of CBL/CBL-B as targeted therapeutics to counter BC recurrence and metastasis.

#5698 Opposing roles of HOXC6 isoforms in promoting head and neck squamous cell carcinoma.

Ishita Gupta[1], Subhendu Roy Choudhury[2], Anirudh Saxena[1], Madeleine Ndahayo[1], Vera Mukhina[1], Andrey Loginov[1], Nadya Vorobyeva[3], Daria A. Gaykalova[1]

[1]Department of Otorhinolaryngology, Institute of Genome Sciences, University of Maryland Baltimore, Baltimore, MD,[2]Department of Cancer Genetics, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway,[3]Russian Academy of Sciences, Institute of Gene Biology, Moscow, Russian Federation

HOXC6, a member of the homeobox (HOX) family is a homeoprotein transcription factor regulating embryonic development, differentiation, and pathways like TGF-β/smad, PI3K/Akt, and Wnt. Although aberration in HOXC6 promotes onset and progression of several cancers, our previous study indicated an oncogenic role of HOXC6 in head and neck squamous cell carcinoma (HNSCC). Despite its role in HNSCC progression, the specific role of HOXC6 in HNSCC lies nascent. Emerging evidence suggests that alternative splicing events of HOXC6 generate distinct isoforms with diverse biological functions. While the HOXC6 transcript variant 1 encodes the primary isoform, variant 2 differs in the 5' UTR coding region and produces a shorter N-terminal isoform. In this study, we performed in-silico analysis alongside in vitro functional studies to delineate the roles of the primary and short isoforms in HNSCC progression. In-silico analysis revealed isoform-specific expression patterns across HNSCC and correlated with clinical outcomes. Our in vitro data demonstrated that the primary isoform enhances cell proliferation and migration by activating oncogenic pathways, while the shorter isoform counteracts these effects by inhibiting cell proliferation and suppressing metastatic potential. These findings highlight the impact of alternative splicing in modulating HNSCC function and reveal the opposing roles of its isoforms in HNSCC progression. This study underscores the importance of targeting isoform-specific mechanisms for developing biomarkers and therapeutic strategies in HNSCC.

#5699 Development of Elephant *TP53* loaded lipidoid nanoparticles for the treatment of non-small cell lung cancer and colon cancer.

Bhoomi Dholariya[1], Akanksha Patel[1], Joshua Schiffman[2], Aaron Rogers[2], Lisa Abegglen[2], Ketan Patel[1]

[1]St. John's University, Queens, NY,[2]Huntsman Cancer Institute, University of Utah, Salt Lake City, UT

Worldwide, lung cancer and colon cancer are the most frequently diagnosed cancers with high mortality. Targeted therapy, including small molecule inhibitors and monoclonal antibodies of various oncogenic proteins, has improved survival, but the development of resistance remains a significant issue. TP53 is a critical tumor suppressor gene and loss of function occurs in over 50% of all human cancers. In lung and colon cancer, the evolutionary conserved codons of the tumor suppressor gene TP53 are frequently mutated. Among the range of mammalian species, elephants develop less cancer than predicted based on their size and lifespan. The search for potential mechanism of cancer defense in elephants revealed that elephants possess 19 retrogene copies of TP53 in addition to ancestral TP53. Elephant cells exposed to DNA damage undergo significantly more apoptosis than human cells, and increased apoptosis is associated with increased TP53 activity. Specifically, elephant TP53 (EP53) induced mitochondrial mediated apoptosis in human cancer cells. The objective of the current research work was to 1) To investigate the anticancer activity of ancestral elephant TP53 in various non-small cell lung cancer and colon cancer cell lines and 2) develop and optimize elephant TP53 loaded lipidoid nanoparticles. Ancestral elephant TP53 (EP53) showed significant anticancer activity at doses 500 ng-125 ng in A549, H460, HT29 and HCT116 ($p<0.05$). The combination of EP53 with p53 activators and DNA damaging agents is under investigation. Amongst various transfecting reagents screened, Fugene® showed maximal transfecting efficiency for EP53 with minimal reagent toxicity. Apoptotic assay confirmed the anticancer activity of EP53. Building on the promising activity of EP53 in human lung and colon cancer cells; we further investigated formulation strategies to deliver EP53 into tumor effectively. Being a large, anionic, hydrophilic structure, a plasmid/gene poses tremendous challenges in *in vivo* delivery. Non-viral gene delivery by cationic ionizable lipidoid was developed for in vivo delivery of EP53. Lipidoid nanoparticles prepared using DSPC, cholesterol, DOPE, DMG-PEG-2000, DOTAP and C12-200 showed particle size of 73.98 nm with +49 mV zeta potential. High transfection (EP53 with EGFP) with complete binding of the gene (agarose gel electrophoresis) to the lipoid was observed at a 1:10 ratio of gene:lipidoid. Further, *in vitro* assays and tumor marker analysis are currently ongoing.

#5700 Simultaneous expression of MUC13 and N-Myc contributes to diethylnitrosamine-induced hepatocellular carcinoma in rats.

Mohammed Sikander[1], **Shabnam Malik**[1], Swatantra K. Jain[2], Deepshikha P. Katare[3], Murali M. Yallapu[1], Subhash C. Chauhan[1]

[1]University of Texas Rio Grande Valley, McAllen, TX,[2]Hamdard University, New Delhi, India,[3]Amity University, Noida, India

Background: Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality worldwide, with its development intricately linked to environmental factors such as chemical carcinogens and viral infections. Recent studies have highlighted a connection between obesity and non-alcoholic fatty liver disease with modern diets high in fats and processed meats. Despite this, the specific mechanisms by which dietary factors contribute to hepatocarcinogenesis remain inadequately understood. Our study demonstrates that diethylnitrosamine (DEN), a N-nitroso compound found in food additives and tobacco smoke upregulates N-Myc. N-Myc is a member of the Myc family of proto-oncogenes, essential for regulating gene expression, growth, apoptosis, energy metabolism, and differentiation. Additionally, we investigated the relationship between MUC13 and N-Myc, potential oncogenes, in human HCC tumors, proposing a potential link further facilitated by the suppression of miR-145, which contributes to hepatocarcinogenesis.
Methodology: We utilized a chemically induced rat model with DEN; 200 mg/kg bw and 2-acetylaminofluorene (2-AAF; 150 mg/kg bw) to investigate N-Myc and MUC13 expression. Serum levels of AST, ALT, and ALP were measured using Span Diagnostics kits. We assessed N-Myc expression in tumor tissue through immunohistochemical staining and measured miR-145 levels via in situ hybridization. Additionally, we analyzed patient-derived tissue samples to evaluate N-Myc and MUC13 protein expression, supplemented by bioinformatics analyses from various public databases.
Results: Liver sections from the DEN+2-AAF-treated group displayed hepatocyte vacuolization in the centrilobular area, accompanied by variations in nuclear size and significant nuclear atypia in adenomas. Serum levels of AST, ALT, and ALP correlated with liver cancer progression, and DEN treatment notably upregulated MUC13 and N-Myc in liver tissues. In normal tissues, MUC13 and N-Myc showed minimal staining, whereas levels were significantly higher in DEN+2-AAF-induced HCC, suggesting that MUC13 and N-Myc expression is crucial for hepatocyte malignant transformation. A strong correlation between MUC13 and N-Myc expression was observed in human HCC tumors. Additionally, miR-145, a repressor of MUC13, was significantly downregulated in tumor tissues compared to normal controls. Molecular modeling revealed that DEN forms a stable complex with N-Myc through multiple interactions.
Conclusion: This study highlights the pivotal role of MUC13 and N-Myc in the progression of diethylnitrosamine induced HCC.

#5701 MUC13 expression renders radio-resistance in human colorectal cancer cells through NRF2 signaling pathway.

Shabnam Malik, Mohammed Sikander, Daniel Zubieta, Kyle Doxtater, Manish K. Tripathi, Sheema Khan, Murali M. Yallapu, Everardo Cobos, Subhash Chauhan

University of Texas Rio Grande Valley, McAllen, TX

Background: Colorectal cancer (CRC) ranks as the third most commonly diagnosed cancer worldwide and poses a significant public health challenge due to its high incidence and mortality rates. A critical challenge in the management of CRC is the development of radio resistance, which compromises the efficacy of radiotherapy. Identifying novel molecular mechanisms that contribute to radio-resistance in CRC are imperative. Recently, we and others have reported that MUC13 as a pivotal factor in CRC progression and metastasis; however, its role in chemoresistance has not yet thoroughly elucidated. In this study, we have investigated the influence of MUC13 expression on radio-resistance in CRC cells.
Methodology: To evaluate the influence of MUC13 on the radio-resistance of CRC cells, we generated genetically modified cell lines exhibiting either MUC13 overexpression (MUC13-OE) or knockdown (MUC13-KO). These cell lines were subjected to X-ray irradiation (0-6 Gray) to assess the effects of MUC13 modulation on critical cellular functions and tumorigenic features (proliferation, migration, and invasion). Furthermore, we quantified the expression levels of NRF2 and its nuclear localization post-irradiation to delineate the interplay between MUC13 and NRF2 signaling pathways. IHC analysis of patient-derived tissue samples was conducted to ascertain the expression profiles of MUC13 and NRF2 proteins, complemented by bioinformatics analysis utilizing various public databases to investigate the correlation between these two molecules.
Results: Our findings reveal that the ectopic expression of MUC13 (MUC13-OE) in CRC cells caused a higher level resistance to radiotherapy (X-ray)-induced apoptosis as compared to MUC13 knockdown (MUC13-KO) cells. Additionally, MUC13-OE cells exhibited significantly enhanced migratory and invasive capabilities as compared to MUC13 KO cells after radiation treatments. This effect was correlated with the upregulation of NRF2 expression and its enhanced nuclear localization. Interestingly, a robust correlation between the expression levels of MUC13 and NRF2 was observed in human CRC tumors. This observation was supported by bioinformatics analyses. Moreover, we noted modulation of additional oxidative stress regulators, including HO-1, Keap1, and NQO1, which are critical mediators of cellular responses to oxidative stress. Collectively, these results implicate MUC13 as an important modulator of NRF2 signaling pathway.
Conclusion: In this study, we for the first time delineate the influence of MUC13 on CRC radio-resistance. We have also established a relationship between MUC13 and NRF2 expression/subcellular localization, that might contribute to MUC13 mediated radio-resistance in CRC patients. Thus, strategic repression of MUC13 by small molecule inhibitors/siRNAs might improve the therapeutic outcome of radiotherapy in CRC patients.

#5702 Knockdown of COP1 disrupts lipid metabolism and reduces migration ability in hepatocellular carcinoma cells.

Hun-Mo Ryoo[1], Eun-Hye Jeon[2], Keon Uk Park[3], Ilseon Hwang[4], Yun-Han Lee[5]

[1]Internal Medicine, Daegu Catholic University, Daegu, Korea, Republic of,[2]Keimyung University Dongsan Hospital, Daegu, Korea, Republic of,[3]Internal Medicine, Keimyung University Dongsan Hospital, Daegu, Korea, Republic of,[4]Pathology, Keimyung University Dongsan Hospital, Daegu, Korea, Republic of,[5]Molecular Medicine, Keimyung University Dongsan Hospital, Daegu, Korea, Republic of

Hepatocellular carcinoma (HCC) is one of the cancers with the highest mortality rates worldwide, being the sixth most commonly diagnosed cancer globally and the third leading cause of cancer-related deaths. COP1 gene, an E3-ubiquitin ligase, is generally overexpressed in human HCC and that plays an important role in the degradation of p53 tumor suppressor via ubiquitination. We have previously defined the COP1 gene as a therapeutic target in HCC. A recent study demonstrated that COP1 induces nonalcoholic fatty liver disease (NAFLD) in normal hepatocytes and that reducing COP1 expression in the liver significantly improves high-fat diet-induced hepatic steatosis. Thus in this study, we investigated if the function of COP1 was associated with HCC metabolism as well. siRNA-mediated inhibition of COP1 expression suppressed long-term colony formation in both Huh7 and HepG2 HCC cell lines. RNA sequencing of COP1-silenced HCC cells revealed the same directional regulation of 24 (10 down- and 14 up-regulated) genes. Notably, AKR1D1 gene, which negatively regulates bile acid biosynthesis and lipid metabolism, was up-regulated and that TMEM65 gene, which promotes migration and invasion of cancer cells, was down-regulated. Correlation analysis using publicly available databases further supported an inverse relationship between COP1 and AKR1D1 expression in HCC. Subsequent studies showed that COP1 knockdown inhibits synthesis of cellular lipids through the downregulation of GLUT4, DGAT2, FASN, and ChREBPα genes that are functionally involved in glucose transport or fatty acid synthesis. Furthermore, silencing of COP1 significantly reduced the abilities of migration and sphere formation in HCC parental cell population. Collectively, our findings indicate that the function of COP1 is associated with HCC lipid metabolism and migration.

#5703 NDRG1 enhances cisplatin-induced apoptosis by upregulating BMF in ovarian cancer cells.

Yajing Yang[1], Ananya Banerjee[1], Linzhou Wang[1], Na Li[1], Patrick Stevens[2], Elsa Wani[1], Jessica Miao[1], Aidan Li[1], Xiaoli Zhang[1], Qi-En Wang[1]

[1]Radiation Oncology, The Ohio State University, Columbus, OH,[2]The Ohio State University, Columbus, OH

Epithelial ovarian cancer (EOC) is the most lethal malignancy of the female reproductive system, with a five-year survival rate of just 32% for patients diagnosed at distant stages, which accounts for over 48% of cases. Although most EOC patients initially achieve a complete response with surgery and chemotherapy, the majority experience relapse, and recurrent tumors often develop resistance to the original drug regimen. Tumor relapse and acquired chemotherapy resistance are primary contributors to the high mortality associated with EOC. Ovarian cancer is a highly heterogeneous disease, exhibiting extensive variability at the molecular, cellular, and clinical levels. This heterogeneity significantly impacts therapeutic responses, particularly to platinum-based chemotherapies such as cisplatin, leading to variable treatment outcomes among patients. To gain deeper insights into the heterogeneity of ovarian cancer cells in response to cisplatin treatment, single-cell RNA sequencing (scRNA-seq) was conducted on spheroid-cultured ovarian cancer cells exposed to cisplatin. Following a two-week treatment period and a subsequent two-week recovery phase, several distinct clusters of cisplatin-surviving ovarian cancer cells were identified, exhibiting enhanced growth advantages. Analysis of the gene expression profiles of the cell clusters revealed that N-myc downstream regulated 1 (NDRG1) is highly expressed in clusters eliminated by cisplatin, whereas its expression is significantly lower in clusters that survive cisplatin treatment. Further investigation demonstrated that NDRG1 plays a critical role in promoting cisplatin-induced apoptosis. RNA sequencing of NDRG1-knockdown cells identified Bcl2 Modifying Factor (BMF) as a downstream target positively regulated by NDRG1. Given that BMF can bind to Bcl2 protein and functions as an apoptotic activator, these findings suggest that ovarian cancer cells with high NDRG1 expression are more susceptible to cisplatin-induced apoptosis due to NDRG1-mediated upregulation of BMF.

#5705 CHI3L1 overexpression predicts poor prognosis and drives aggressiveness in papillary thyroid cancer.

Pratheesh Kumar Poyil, Abdul K. Siraj, Sandeep Kumar Parvathareddy, Khadija Alobaisi, Rafia Begum, Fouad Al-Dayel, Khawla S. Al-Kuraya

King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia, Riyadh, Saudi Arabia

Chitinase-3-like protein 1 (CHI3L1) is a secreted glycoprotein that is pivotal in regulating inflammation, macrophage polarization, apoptosis, and the process of carcinogenesis. While its expression is markedly increased in various cancer types, its specific role in papillary thyroid cancer (PTC) has not been fully elucidated. In our study, we assessed the expression of CHI3L1 protein in a large cohort of PTC patient samples and investigated its functional significance and underlying mechanisms in PTC cell lines. CHI3L1 over-expression was noted in 38.9% (638/1613) of PTC cases and was significantly associated with aggressive clinicopathological characteristics such as extrathyroidal extension ($p < 0.0001$), advanced T stage ($p < 0.0001$), lymph node metastasis ($p < 0.0001$), distant metastasis ($p = 0.0003$), stage IV tumors ($p = 0.0007$) and tumor recurrence ($p < 0.0001$). Interestingly, a significant association was also noted between CHI3L1 over-expression and phosphor-AKT ($P = 0.0007$) as well as PD-L1 ($P < 0.0001$). In addition, CHI3L1 was associated with shorter recurrence-free survival ($p = 0.0004$). Our *in vitro* studies revealed that inhibition of CHI3L1 reduced cell proliferation and triggered apoptosis in PTC cell lines. Notably, the downregulation of PLK1 impaired the self-renewal capacity of spheroids derived from PTC cells, indicating its role in maintaining cancer stem-like properties. Furthermore, CHI3L1 knockdown resulted in decreased expression of phosphorylated AKT and PD-L1, while its overexpression led to an upregulation of both. These findings underscore the role of CHI3L1 in PTC prognosis and promoting its progression. This study also identifies CHI3L1 as a promising therapeutic target, offering a novel strategy for managing aggressive and treatment-resistant forms of PTC.

#5706 A patient-derived organoid screening platform as a powerful tool to study efficacy of KRAS inhibitors.

Merel Derksen, Mariana Silva, Gerben ten Hag, Nilofar Ehsani, Yasmine Abouleila, Vineeta Adegbenro, Annemarie Buijs, Mayke Doorn, Nikki van Dorp, Javier Frias Aldeguer, Foteini Gkogkou, Yara Hendriksen, Ricardo Korporaal, Ka Wai La, Joris Maas, Francisco Morales-Rodrigues, Daniele Mori, Sumeyra Mucuk, Cesar Oyarce, Nima Salehi, Anna Schepers, Marvin Statia, Roel Verkerk, Timo Voskuilen, Rene Overmeer, Farzin Pourfarzad, **Sylvia F. Boj**

HUB Organoids B.V., Utrecht, Netherlands

Kirsten rat sarcoma (*KRAS*) gene is a proto-oncogene frequently mutated in various cancer types. Upon activation by growth factors such as Epidermal Growth Factor (EGF), KRAS initiates signaling pathways promoting cell proliferation and survival. Activation KRAS mutations are most common in colorectal cancer (CRC), Pancreatic Ductal Adeno Carcinoma (PDAC) and Non-Small Cell Lung Cancer (NSCLC) and are associated with worse disease outcomes. Though KRAS is an interesting target for cancer treatment, the development of specific KRAS inhibitors has, for many years, been challenging due to its small and smooth binding pocket, making KRAS a challenging “druggable” protein. Recently, advances have been made in this area, with the development and approval for clinical use of the KRASG12C inhibitors (Sotorasib and Adagrasib). These developments can pave the way for the design of novel compounds targeting KRAS G12C and other KRAS mutations, which can be accelerated when developed in patient-relevant preclinical models. Patient-derived organoids (PDOs) or HUB Organoids® are generated from the adult stem cells of normal and malignant tissues of epithelial organs, including the colon, pancreas, and lung. HUB Organoids recapitulate tissue physiology, molecular heterogeneity, and morphological and functional characteristics, including mimicking patient response. Organoids are suitable for high-throughput screening and serve as a platform for testing drug efficacy and safety. In this study, we showcase the suitability of our PDO-based platform for screening the efficacy of several KRAS inhibitors in 4 weeks. Eight KRAS inhibitors in different clinical research stages were tested in a panel of over 20 PDOs (including CRC, PDAC and NSCLC) harboring different KRAS mutations (G12C, G12D, Q61R, G13C, G12V and G12S) and using an ATP-based cell viability readout. As expected, various responses were identified among the 20 PDOs. In addition, the results show specific responses of G12C and G12D mutants to KRASG12C and KRASG12D-selective inhibitors, respectively. KRASG12CPDO resistance to KRASG12C inhibitors was alleviated by combinatorial treatment with an EGFR inhibitor, which aligns with the KRASi resistance mechanism driven by EGF signaling. In conclusion, our PDO platform effectively captures the efficacy and specificity of KRAS inhibitors in models with different KRAS mutations. The HUB PDO platform is valuable for studying combinatorial treatments to overcome KRAS inhibitor resistance, highlighting the potential of PDOs in developing novel KRAS inhibitors.

#5707 Augmentation of statin-induced ATF4 stress response enhances the cancer cell-killing effect of statin.

T. Nguyen, G.-B. Tran, J. Ding, H.-f. Ding;
O'Neal Comprehensive Cancer Center at UAB, Birmingham, AL

Statins are a class of drugs widely used to reduce cholesterol levels and have been rediscovered as promising anti-cancer drugs. Statins inhibit HMG-CoA reductase (HMGCR), a rate-limiting enzyme in cholesterol synthesis. We found that statin treatment of neuroblastoma cells triggered stress responses characterized by upregulation of the master stress regulator ATF4. The primary function of ATF4 is to initiate a gene expression program to mitigate stress and promote cell survival. However, ATF4 can also induce cell death under severe or prolonged stress conditions. Our study revealed that combinations of simvastatin with compounds that amplify ATF4 stress signaling synergistically inhibited the growth and survival of neuroblastoma cells. These compounds include the endoplasmic reticulum (ER) stress sensor ATF6 activator, AA147, and the two ATF4 activators, Sal003 and Nelfinavir. The combination of simvastatin with AA147, Sal003 or Nelfinavir significantly increased ER stress marker, XBP1s, and strongly stimulated ATF4-ATF3-CHOP signaling, leading to apoptosis as evidenced by cleavage of caspase 3 and PARP. Moreover, knocking down ATF4 was able to inhibit apoptosis and partially protect neuroblastoma cells from the effects of these drug combinations. These results suggest a strategy to enhance the anti-tumor effect of statins by amplifying ATF4 stress signaling to trigger apoptosis.

#5708 Investigating the role of SMYD3 in medulloblastoma progression and treatment response: Implications for targeted therapy.

Swapna Asuthkar[1], Erick Benjamin Saldes[2], katherine Shishido[2], Ilango Balakrishnan[3], Sujatha Venkataraman[3]

[1]University of Illinois College of Medicine (Peoria), Peoria, IL,[2]Departments of Cancer Biology and Pharmacology,, University of Illinois College of Medicine (Peoria), Peoria, IL,[3]Department of Pediatrics, University of Colorado School of Medicine, Aurora, CO

Medulloblastoma (MB), the most prevalent malignant brain tumor in children, presents significant treatment challenges, particularly in the aggressive Group 3 subtype, which is associated with poor prognosis. Current therapeutic strategies, including surgery, chemotherapy, and radiotherapy, often result in severe side effects and inadequate long-term outcomes. This study investigates the role of SMYD3, a histone methyltransferase known for its overexpression in various cancers, in mediating radioresistance in MB. We analyzed SMYD3 expression levels in MB cell lines and patient samples, revealing a notable overexpression in Group 3 MB, which correlated with diminished patient survival rates. Notably, ionizing radiation exposure resulted in a dose-dependent increase in SMYD3 expression, suggesting a potential adaptive response to treatment. Inhibition of SMYD3 using the small molecule BCI-121 significantly reduced cell viability and enhanced radiosensitivity in vitro. Preliminary in vivo studies utilizing a BALB/c nude mouse orthotopic MB model demonstrated promising outcomes, indicating that targeting SMYD3 may improve therapeutic efficacy. Our findings underscore the critical role of SMYD3 in promoting radioresistance in MB and suggest that SMYD3 inhibition could be a viable strategy to enhance the effectiveness of radiotherapy, particularly in aggressive MB subtypes. Further investigations are warranted to elucidate the underlying mechanisms and to explore the potential for combination therapies aimed at improving treatment outcomes in MB.

#5709 Furin drives colorectal cancer progression and chemoresistance through the TGF-β/ERK signaling pathway.

Abdul K. Siraj, Pratheesh Kumar Poyil, Sandeep Kumar Parvathareddy, Saravanan Thangavel, Fouad Al-Dayel, Khawla S. Al-Kuraya

King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia

Furin, a key member of the pro-protein convertase (PC) family, is unfavorably involved in the proteolytic activation of various precursor proteins. While aberrant expression of furin has been extensively documented in multiple malignancies, its functional significance and mechanistic role in colorectal cancer remain inadequately characterized. We analyzed furin expression in a comprehensive cohort of Middle Eastern colorectal cancer (CRC) patient samples and investigated its functional role and underlying mechanisms using in vitro studies with CRC cell lines. Furin over-expression was noted in 46.9% (530/1131) of CRC cases. A significant association was noted between Furin over-expression and older age at diagnosis of CRC (>50 years; $p = 0.0156$), but no significant association was noted with other clinico-pathological characteristics. However, Furin over-expression was found to be associated with phosphor-ERK1/2 ($p < 0.0001$) and TGFβ ($p < 0.0001$). For *in vitro* analysis, we selected three chemosensitive CRC cell lines—HCT116, DLD1, and LOVO—and generated chemoresistant cells by treating them to prolonged low-dose 5-fluorouracil (5-FU). Our results revealed that 5-FU-resistant CRC cell lines exhibited elevated furin expression, accompanied by increased levels of TGF-β protein and phosphorylated ERK1/2 (pERK1/2). Notably, furin knockdown restored chemosensitivity, highlighting its critical role in drug resistance. Furthermore, we demonstrated that TGF-β treatment upregulated furin and pERK1/2 expression, while forced furin overexpression similarly induced TGF-β and pERK1/2 expression. Collectively, these findings suggest that furin plays a pivotal role in CRC progression and chemoresistance by activating the TGF-β/ERK signaling pathway.

#5710 Anticancer effects of 2,3-*bis*(bromomethyl)-quinoxaline 1,4-dioxide on uterine cancer.

Kaitlyn Mayo, Kennedy Szabo, **Calli A. Davison-Versagli**

Saint Mary's College, Notre Dame, IN

Uterine cancer will claim an estimated 17,610 lives in the United States in the year 2024. Lack of funding and focus have resulted in little advancement in treatment options for uterine cancer patients. Consequently, uterine cancer incidence and mortality have consistently increased since the 1990's in the United States. Thus, further investigation and development of uterine cancer therapies is needed. The Peroxiredoxins (I-VI) family of enzymes is responsible for eliminating reactive oxygen species throughout the cell and has been studied in multiple organisms and diseases, including cancer. The compound 2,3-*bis*(bromomethyl)-quinoxaline 1,4-dioxide (Conoidin A) is a Peroxiredoxin II inhibitor that has been studied in the parasite, *Toxoplasma gondii*. It inhibits the enzymatic activity of Peroxiredoxin II when studied *in vitro* by covalently binding to cysteine. Recent reports identify Peroxiredoxin II as a potential therapeutic target for cancer therapies and indicate that Peroxiredoxin II down-regulation promotes cancer cell death. Thus, the purpose of these studies was to evaluate Conoidin A as a potential therapeutic for uterine cancer. Here, we report that concentrations of 100 µM, 75 µM, and 50 µM of Conoidin A decreased growth of the uterine cancer cell line, HEC-1-A, after 24 hours as measured using crystal violet assays. Conoidin A concentrations of 100 µM, 75 µM, and 50 µM compromised anchorage-independent growth of HEC-1-A cells in soft agar assays. Furthermore, p53 up-regulation (p53+) correlates with poor prognosis in uterine cancer patients. To determine if Conoidin A is capable of decreasing growth of p53+ tumors, increasing Conoidin A concentrations were tested on the p53+ cell line, C-33a, using crystal violet assays. Preliminary studies found Conoidin A abolishes C-33a growth at concentrations of 100 µM, 75 µM, and 50 µM after 24 hours of treatment. These data identify Conoidin A as a potential therapeutic for uterine cancer, including p53+ tumors. Furthermore, these studies establish Peroxiredoxin II as a novel therapeutic target for this disease.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: PARP Inhibitors
Poster Session

#5714 EP102: pharmacological inhibition of METTL3 in tumor growth inhibition and comparative efficacy in combination with anti-PD1 or PARP inhibitors.

Graeme L. Fraser, Catherine Sorlet, Nicolas Parmentier, Killian Oukoloff, Guillaume Dutheuil

EPICS Therapeutics, Gosselies, Belgium

METTL3 is the RNA methyltransferase predominantly responsible for the addition of N-6-methyladenosine (m6A), the most abundant epigenetic modification to mRNA. The prevalence of m6A and the activity and expression of METTL3 have been linked to the appearance and progression of numerous hematological and solid tumor cancers. EPICS has previously presented tumor growth inhibition with its small molecule inhibitors of METTL3 ("M3i") in various models including in SKOV3 (ovarian) in NSG mice. The aim of the current study is to evaluate whether the efficacy of M3i combines favorably with anti-PD1 inhibitors in comparison to PARP inhibitors. M3i compound potency was evaluated *in vitro* by measuring the anti-proliferative effects in cell viability assays following a 72h treatment of A20 (B-cell lymphoma, IC_{50} = 310 nM), SKOV3 (ovarian, IC_{50} = 9 nM) and MDA-MB-231 (TNBC, IC_{50} = 24 nM) cells. Compound M3i-A (30 mg/kg, IP, QD) effectively elicited tumor growth inhibition ("TGI") in SKOV3 (orthotopic implant, NSG mice) but not in A20 (syngeneic mice) either as a standalone therapy nor in combination with anti-PD1. In CD34+ humanized mice, compound M3i-B (20 mg/kg, PO, QD) elicited TGI >60% in the SKOV3 model (SC implant) and >40% TGI in the MDA-MB-231 model (orthotopic implant), but in the latter model with no additional benefit in combination with anti-PD1. SKOV3 is an HR-proficient tumor known to be less sensitive to PARP inhibitors; in a final study, M3i-B effectively prolonged survival in the SKOV3 model in NSG mice (orthotopic implant) and further demonstrated heightened efficacy when tested in combination with Olaparib (50 mg/kg, PO, QD) in data complemented by the resolution of various downstream biomarkers. The current results extend the application of METTL3 inhibitors as a single-agent in the treatment of selected tumors and further suggest that METTL3 inhibitors may be ideally combined with cancer therapies targeting DNA damage response mechanisms, such as PARP inhibitors, as a refined strategy for treatment of solid tumors.

#5715 Enhancing PARP inhibitor efficacy with CDK12/7/9 degradation in prostate cancer.

Fu Gui[1], Baishan Jiang[2], Jie Jiang[2], Marian Kalocsay[3], Anne Fassl[4], Steven P. Balk[5], Adam S. Kibel[1], Li Jia[1]

[1]Department of Urology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA,[2]Department of Cancer Biology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA,[3]Department of Experimental Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX,[4]Department of Urology, University Hospital Frankfurt, Goethe University Frankfurt, Frankfurt am Main, Germany,[5]Department of Medicine and Cancer Center, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA

Current treatments for advanced prostate cancer (PCa) mainly target the androgen receptor (AR) signaling pathway. However, the development of castration-resistant prostate cancer (CRPC) and resistance to AR signaling inhibitors remains a significant clinical challenge.In recent years, the FDA has approved four PARP inhibitors (PARPis) - olaparib, rucaparib, talazoparib, and niraparib - either as monotherapy or in combination with AR signaling inhibitors for CRPC patients carrying deleterious germline or somatic BRCA1/2 mutations or other homologous recombination repair (HRR) gene mutations. CDK12, a kinase associated with the phosphorylation of the carboxy-terminal domain of RNA polymerase II, is crucial for the transcriptional processivity of long mRNA transcripts, including key HRR genes such as BRCA1 and BRCA2. CDK12 alterations, primarily biallelic truncating mutations, have been identified in 5-7% of PCa cases. In this study, we demonstrate that acute CDK12 loss results in BRCA1/2 downregulation and sensitizes CRPC cells to PARP inhibition. However, this sensitization is absent in CRPC adapted to chronic CDK12 loss, where BRCA1/2 expression is restored. Furthermore, degradation of CDK12/7/9 enhances the synthetic lethality of PARP inhibition through acute induction of a "BRCAness" state and AR signaling suppression in PCa. We developed BSJ-5-63, a CDK12/7/9 proteolysis-targeting chimera (PROTAC) degrader, which effectively degrades CDK12 to induce an acute "BRCAness" state, sensitizing cancer cells to PARP inhibitors regardless of their HRR status. In addition, BSJ-5-63 degrades CDK7/9, attenuating AR signaling and further promoting the synthetic lethality of PARP inhibition.Acute CDK12/7/9 degradation by BSJ-5-63 exhibits potent anti-tumor effects in both in vitro and in vivo CRPC models. This study introduces BSJ-5-63 as a promising therapeutic agent that targets both DNA repair and AR signaling pathways, offering potential benefits for a broad patient population.

#5716 XPO1 inhibition synergizes with PARP inhibitor in metastatic castration resistant prostate cancer via the regulation of DNA damage response.

Md. Hafiz Uddin[1], Aaban A. Azmi[1], Laiba M. Monir[1], Amro Aboukameel[1], Husain Y. Khan[1], Sahar F. Bannoura[1], Frank Cackowski[1], Yusra Shao[1], Vinod Shidham[2], Sunil Jaiman[2], Rafic Beydoun[2], Gregory Dyson[1], Seongho Kim[1], Yang Shi[1], Vy Ong[1], Julie Boerner[1], Ramzi M. Mohammad[1], Elisabeth I. Heath[1]

[1]Department of Oncology, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI,[2]Department of Pathology, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI

Background: Dysregulated nuclear protein transport, commonly observed in cancer, results in the mislocalization-mediated inactivation of many proteins. We showed that overexpression of exportin 1 (XPO1) is linked to higher grade and Gleason score in metastatic castration-resistant prostate cancer (mCRPC). Using network topology computational approaches, XPO1 and PARP1 were identified as synthetic lethal partners and we have demonstrated synergy between XPO1 and PARP inhibitors in mCRPC cell lines. This study evaluates the combination's efficacy in cell line-derived (CDX) and patient-derived xenograft (PDX) models, investigating the mechanisms of synergy.
Methods: For the CDx and PDx models, 22rv1 cells and CTG-3581 tissue (Champions Oncology) were grown subcutaneously in ICR-SCID and CEIA/NOG male mice, respectively. XPO1 inhibitor was dosed orally at 10-15 mg/kg twice a week and PARP inhibitor was dosed orally at 50 mg/kg daily. For *in vitro* mechanistic study, 22rv1 cells were subjected to RNAseq, proteomic analysis, and Digital Spatial Profiling (DSP; 10x Genomics Visium platform) after treatment. Data analysis was performed using iPathwayGuide (advaitabio.com) and cLoupe browser.
Results: The XPO1-PARP inhibitors combination showed enhanced anti-cancer efficacy in both CDx and PDx models, with no significant weight loss or organ toxicity such as liver, spleen, and kidney. Survival benefits were evident in the combination group. Immunohistochemistry revealed apoptotic cell death, shown by cleaved caspase 3 and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining. Mechanistic studies with 22rv1 cells showed downregulation of DNA replication genes (e.g., MCM6 and CDC6) and reduced enrichment scores for DNA replication pathways in gene set enrichment analysis. The proteomic analysis highlighted decreased levels of DNA replication modulators HMGB2 and DNAJC9. Besides the suppression of various DNA damage response genes (e.g. BRCA2 and RAD51), XPO1 inhibition also reduced the PARylation of mCRPC cells. DSP analysis of 22rv1 CDx tissue revealed a reduction of unbiased cellular clusters and increased differentially expressed genes in the combination treatment. The DNA replication pathway was among the top 5 pathways modulated by XPO1i treatment in DSP analysis.
Conclusions: Taken together, this study revealed the therapeutic potential of XPO1-PARP inhibitors combination via targeting of the DNA damage response pathway in mCRPC.

#5717 Nesuparib, a dual inhibitor of PARP and tankyrase, is efficacious in preclinical gastric cancer models by regulation of HIPPO and Wnt pathway.

Banyoon Cheon, Jung Young Shin, Dae-In PARK, Jun Kim, John Kim, Hyunju Cha

Onconic Therapeutics, Seoul, Korea, Republic of

Gastric cancer is the fifth most common cancer and it still needs to identify novel actionable targets to bring clinical benefits. Here we explored the efficacy of nesuparib, a dual inhibitor for poly-ADP-ribosyl transferase 1/2 (PARP 1/2) and tankyrase 1/2 (TNKS 1/2) in vitro and in vivo models using BRCA wild type gastric cell lines. In addition to PARP 1/2 inhibition, nesuparib selectively inhibits TNKS 1 and 2 enzymatic activities; TNKS is a member of PARP family, and poly-ribosylates target proteins that are involved in Wnt/β-catenin and Hippo signaling pathways. The poly-ribosylated proteins are subsequently ubiquitinated by E3 ligases and undergo proteasomal degradation. The inhibition of TNKS is known to attenuate cancer promoting signaling like Wnt and Hippo signaling. To evaluate the impact of nesuparib on cell growth, half maximal inhibitory concentration (IC_{50}) of nesuparib, Olaparib, and XAV939 in clonogenicity was compared in BRCA wild type gastric cancer cells. Nesuparib is at least 28-fold more potent than Olaparib and 13-fold more potent than XAV939 among cell lines. To examine how nesuparib modulates Wnt/β-catenin and Hippo signaling through TNKS inhibition, we assessed changes in canonical Wnt/β-catenin and Hippo signaling-related molecules at protein levels. Nesuparib stabilized AXIN, reducing nonphospho β-catenin, c-myc, and cyclin D1. In addition, nesuparib stabilized AMOT proteins and increased phospho-YAP reflecting inhibition of the oncogenic YAP pathway. Given the findings that nesuparib treatment regulates Wnt and Hippo signaling in KATO III and NCI-N87 cells (BRCA wild type), we tested its efficacy as a single or with a combination of irinotecan in these cell line xenograft mouse models. Nesuparib showed superior potency than Olaparib alone in both xenograft models. The combination of nesuparib with irinotecan enhanced the anti-tumor activity compared with nesuparib single treatment. In addition, the combination of nesuparib with irinotecan showed stronger tumor growth suppression than the combination of Olaparib with irinotecan.In summary, these results suggest that nesuparib may exert anti-tumor efficacy in specific types of cancers with pro-cancerous signaling of the Wnt or Hippo pathway by the regulation of TNKS. This warrants a proof-of-concept of anti-tumor effect of nesuparib in gastric cancers and its potentials for clinical benefits.

#5718 Molecular determinants of platinum resistance in ovarian cancer: Linking PARP1, DNMT1, γ-GCS expression, and LINE1 hypermethylation.

L. Devi, A. Kumar, L. Kumar;
All India Institute of Medical Sciences (AIIMS) New Delhi, New Delhi, India

Background: Ovarian cancer is a leading cause of gynecological cancer-related mortality worldwide. Alarmingly, 80% of patients are diagnosed at an advanced stage (FIGO stage III-IV), where the prognosis is poor. The current standard of care for advanced epithelial ovarian cancer (EOC) includes cytoreductive surgery followed by taxane and platinum-based chemotherapy. Despite initial responses, resistance to platinum remains a significant cause of treatment failure and relapse in EOC. This study aims to investigate the molecular underpinnings of platinum resistance by examining the expression and enzymatic activity of PARP1 and γ-GCS and their correlation with DNA methylation patterns in ovarian cancer. Based on existing evidence, we hypothesize that inhibition of PARP1 or γ-GCS may enhance cisplatin (CDDP) sensitivity, offering new therapeutic opportunities. Understanding these mechanisms could pave the way for innovative strategies to overcome chemotherapy resistance in ovarian cancer.
Material and Methods: Peripheral Blood (PB) and Ascitic Fluid (AS) samples were collected from 20 EOC patients. The cohort was divided into two groups: Group A (n=10) included treatment-naive baseline patients, and Group B (n=10) comprised relapsed patients. Additionally, healthy control samples (n=10) were collected for comparison. The study involved:

1. Gene Expression Analysis: Differential expression levels of PARP1, DNMT1, and γ-GCS were evaluated using qPCR.
2. Enzymatic Activity Measurement: Glutathione (GSH) activity was quantified using ELISA.
3. Global DNA Methylation Assessment: LINE1 methylation levels were measured via pyrosequencing to examine epigenetic changes.

The resulting data were correlated with clinico-pathological parameters, including age, stage, CA markers, and hematological indices. Statistical analysis was conducted using GraphPad Prism 6, and a p-value < 0.05 was considered statistically significant.
Results: High γ-GCS expression increases glutathione levels in newly diagnosed ovarian cancer patients, potentially driving platinum resistance. Consistent LINE1 hypermethylation across patients, influenced by various factors, highlights its potential as both a biomarker and a therapeutic target, paving the way for strategies to overcome chemotherapy resistance.
Keywords: Ovarian Cancer, cisplatin, PARP-1, γ -GCS and DNMT1, Glutathione, LINE1.
Abbreviations: Epithelial Ovarian Cancer (EOC), cisplatin (CDDP), Glutathione (GSH), Gamma Glutamyl Cysteine Synthetase (γ-GCS), Poly (ADP ribosyl) polymerase-1 (PARP1), Enzyme-linked immunosorbent assay (ELISA), Quantitative Polymerase Chain Reaction (qPCR), DNA methylation LINE1, Peripheral Blood (PB), Ascitic Fluid (AS).

#5719 Identification of EIK1004 (IMP1707), a CNS-penetrant, potent, and selective PARP1 inhibitor poised for testing in patients with HRD mutant tumors.

Melissa Dumble[1], Ye Edward Tian[2], Ali Tabatabaei[1], Brenda Smith[1], Viola J. Chen[1], Ning Ma[2], Xiaozhu Wang[2], Yuxiao Zhao[2], Ruiyu Zhou[2], Yangzhen Jiang[2], Mu Chen[2], Sui Xiong Cai[2]

[1]Eikon Therapeutics, Inc., New York, NY,[2]IMPACT Therapeutics, Inc., Shanghai, China

INTRODUCTION: Nonselective poly(ADP-ribose) polymerase (PARP) inhibitors have proven to be effective in patients whose tumors harbor mutations in DNA repair proteins, although their efficacy is hampered by dose reductions and suspensions due to on-target toxicity resulting from PARP2-related hematologic suppression. Currently available PARP inhibitors have limited brain penetrance, limiting their utility for central nervous system (CNS) tumors or brain metastases. PARP1 inhibition alone can result in an antitumor effect similar to pan-PARP inhibition; therefore, development of PARP1-selective inhibitors with broad selectivity over PARP2 and brain penetrance is desirable. EIK1004 (previously IMP1707) is a potent, CNS-penetrant, PARP1-selective molecule with robust pharmaceutical properties.

METHODS: Inhibitory activity of EIK1004 was measured using recombinant PARP1 and PARP2 proteins in an in vitro PARylation assay and separately in an in vitro PARP trapping assay. Antiproliferative potential was measured in cell-based 7-day growth assays utilizing MDA-MB-436 (triple negative breast cancer, *BRCA1* mutant), DLD1 (colorectal, *BRCA2* knockout [KO]), and parental cell lines. In vivo antitumor effects were assessed in MDA-MB-436 and DLD1 KO subcutaneous xenograft models grown in NOD/SCID and nude mice, respectively. Brain penetrance and CNS antitumor activity were evaluated using an MDA-MB-436-luc intracranial xenograft model, and target engagement was measured in tumors using Western blot analysis to assess total PAR levels.

RESULTS: EIK1004 potently and selectively inhibits PARP1 enzyme (IC_{50} < 1 nM) with > 800-fold selectivity over PARP2, and potently traps PARP1 on DNA (IC_{50} < 1 nM). As a result of PARP1 inhibition and trapping, EIK1004 inhibits the growth of homologous recombination deficient (HRD) cell lines with potencies of < 5 nM, while having no effect on the growth of cells with intact DNA repair mechanisms. The antitumor activity of EIK1004 was tested in mouse xenograft models. In MDA-MB-436 and DLD1 *BRCA2* KO xenograft models, oral EIK1004 administered once daily at doses ≤ 1 mg/kg led to significant, dose-dependent tumor regression in these 2 models, respectively. In the MDA-MB-436-luc intracranial xenograft model, EIK1004 was shown to result in robust tumor regression at doses ≤ 1 mg/kg daily. In this model, total PAR levels in the tumor were shown to be significantly reduced (~90%) at all dose levels tested.

CONCLUSION: EIK1004 potently and selectively inhibits and traps PARP1, with downstream activity in cells and mouse models. EIK1004 exhibits robust pharmacokinetic properties including brain penetration, high bioavailability across preclinical species, and a safety profile that supports further development. EIK1004 is currently being evaluated in a Phase 1 clinical trial in solid tumors with or without brain metastasis.

#5720 Novel selective FEN1 nuclease inhibitor shows synergy with PARP-targeting drugs.

Jason Munguia[1], Sanjay Agarwalla[1], Dave Martin[1], Junhua Fan[1], Dave Lonergan[1], Celeste Giansanti[2], David Cortez[2], David Puerta[1], Zachary Zimmerman[1], **Konstantin Taganov**[1]

[1]Blacksmith Medicines, Inc., San Diego, CA,[2]Vanderbilt-Ingram Cancer Center, Vanderbilt University, Nashville, TN

Tumors with homologous recombination defects (HRD) due to mutations in BRCA1/2 genes or other genes associated with HR repair are typically sensitive to poly(ADP-ribose) Polymerase 1/2 inhibitors (PARPi), platinum-based drugs or other agents that target DNA repair pathways. Despite initial responsiveness to PARPi, many patients eventually experience disease progression. To that end, novel drug combination strategies involving PARPi plus other DNA replication and repair inhibitors have the potential to achieve more durable responses.

Flap endonuclease 1 (FEN1) is a structure-specific metallonuclease that has been shown to be overexpressed in a variety of tumor types and has been reported to have many synthetic lethality partners, including PARP and BRCA2, making it an attractive target for the development of novel anticancer therapeutics. Utilizing an innovative library of metal-binding pharmacophores (MBPs) and a fragment-based drug discovery approach we identified a novel FEN1-selective chemical scaffold represented by BSM-1516 (IC_{50} of 7 nM and 460 nM in biochemical assays for FEN1 and EXO1, respectively; FEN1 cellular thermal shift target engagement assay EC_{50} of 24 nM).

Examination of proteins on replication forks by iPOND-SILAC-MS in the presence of BSM-1516 revealed rapid enrichment of Okazaki fragment maturation (OFM) proteins FEN1/PCNA/LIG1, PARP1/2 enzymes, poly(ADP-ribose) binders (e.g. CHD1L) and alternative OFM pathway repair proteins XRCC1 and LIG3.

Combination of BSM-1516 with inhibitors of PARP1/2 was strongly synergistic in vitro, enhanced their antiproliferative effect up to 100-fold (an effect not observed in normal fibroblasts) and led to robust activation of ssDNA break repair markers: phospho-Chk1(Ser345), phospho-RPA2(Ser33) and chromatin-bound RPA2.

In vivo PK studies showed that BSM-1516 had oral bioavailability of 40% and $T_{1/2}$ of 2.9 hours in mice. Safety of BSM-1516 was assessed in vitro in lineage-specific differentiation of human hematopoietic CD34+ progenitor cells and in vivo in mice at a daily dose of 120 mg/kg PO and 90 mg/kg IP for 7 days and revealed no signs of hematological toxicity. These collective data support further in vivo testing in PD and efficacy studies either as a single agent or in combination with PARPi.

#5723 XL309 is a potent, selective, and orally bioavailable USP1 inhibitor active as monotherapy and in combination with PARP inhibitors or irinotecan.

Alex Charruyer-Reinwald[1], Aditya Kumar[1], Jimmy Lai[1], Scott Simonovich[1], Christopher Kochansky[1], Amy DeMicco[1], Tina Huynh[1], Avinash Kumar[2], Amit A. Dhudashiya[2], Kishore Narayanan[2], Kiran Aithal[2], Shailesh V. Jadhav[2], Anthony Flores[1], Richard Mansfield[1], Randy Wu[1], Jae H. Chang[1], Andrew Simmons[1], Paige Mahaney[1], Lisa D. Belmont[1]

[1]Exelixis, Alameda, CA,[2]Aurigene Oncology, Karnataka, India

Background: Ubiquitin-specific protease 1 (USP1) catalyzes selective removal of ubiquitin from its substrate proteins. During translesion synthesis (TLS), USP1 cleaves ubiquitin from proliferating cell nuclear antigen (PCNA), facilitating exchange of TLS polymerases for polymerase delta. Inhibition of USP1 prevents the repair of DNA lesions by TLS, resulting in single-stranded DNA gaps which are synthetically lethal with *BRCA1/2* mutations. Poly (ADP-ribose) polymerase (PARP) inhibitors have shown clinical benefit in *BRCA*-mutated cancers, but some patients do not respond, and many responders develop resistance to treatment. USP1 inhibition could potentially benefit patients by synergizing with PARP inhibition or bypassing resistance mechanisms. We report preclinical data for XL309, a potent and selective orally bioavailable small molecule inhibitor of USP1.
Methods: XL309 selectivity was assessed using a DUBprofiler-Cell™ assay. Viability was measured using CellTiter-Glo® in MDA-MB-436 cells. Ubiquitinated PCNA (Ub-PCNA) and PCNA levels after XL309 treatment of cell lines were quantified using global ubiquitination levels and total proteomic analysis (PTMScan and TMT). The antitumor activity of XL309 alone or in combination with saruparib, olaparib, or irinotecan was evaluated in the *BRCA1* mutant MDA-MB-436 cell-derived xenograft (CDX) model or in *BRCA1/2* mutant patient-derived xenograft (PDX) models. Drug-like properties were assessed, and pharmacokinetic (PK) parameters were determined following administration of XL309 in rat, mouse, and dog.
Results: XL309 is a potent inhibitor of USP1 in biochemical and viability assays. XL309 showed selectivity for USP1 versus other ubiquitin-specific proteases, increased Ub-PCNA levels while decreasing PCNA in the global ubiquitination profiling, and exhibited dose-dependent antitumor activity and induction of Ub-PCNA in a CDX model. XL309 exhibited single-agent activity in *BRCA1/2* mutant PDX models. XL309 also elicited durable tumor regression in combination with saruparib and irinotecan in a CDX model, and with olaparib in a PDX model. XL309 showed favorable drug-like properties including high permeability in Caco-2 cells. Low clearance and high bioavailability were observed for XL309 in rat, mouse and dog. Preliminary predictions of human PK parameters estimate a low clearance and long half-life for XL309 in humans.
Conclusions: XL309 is potentially a best-in-class USP1 inhibitor. PK assessments in conjunction with preclinical antitumor activity suggest that once daily oral administration of XL309 may achieve clinical activity alone or in combination with PARP inhibitors. XL309 is currently under investigation in a phase 1 first-in-human trial as monotherapy and in combination with olaparib in patients with advanced solid tumors (NCT05932862).

#5724 Dual inhibition of PARP and STAT3 as a novel therapeutic approach for triple-negative breast cancer cells.

Changyou Shi, Li Pan, Satomi Amano, Jiayuh Lin

University of Maryland, Baltimore, Baltimore, MD

Triple-negative breast cancer (TNBC) is a highly aggressive and metastatic subtype of breast cancer with limited therapeutic options, contributing significantly to patient mortality. This highlights an urgent need for novel treatment strategies. Olaparib, an FDA-approved poly ADP-ribose polymerase (PARP) inhibitor, is effective in human epidermal growth factor receptor (HER2) negative breast cancer, especially in BRCA-mutated subtypes. However, BRCA-mutant TNBC could eventually develop resistance to PARP inhibitors. We recently found that Olaparib treatment induces elevated IL-6 levels in TNBC cells, which may generate resistance to PARP inhibitors and reduce their effectiveness. Furthermore, knockdown of STAT3, a main downstream effector of IL-6 signaling, enhanced TNBC sensitivity to Olaparib. This study explored the combination of Olaparib and a novel orally bioavailable small-molecule STAT3 inhibitor, LLL12B, in human and murine TNBC cell lines. Both Olaparib and LLL12B independently reduced cell viability, migration, and invasion, but their combination demonstrated significantly greater efficacy than either treatment alone. These results support that dual inhibition of PARP and STAT3 is a novel targeted therapeutic strategy for TNBC.

#5725 The potent and selective PARP1 inhibitor and trapper SNV1521 exhibits combination activity with targeted and chemotherapeutics.

Mingming Gao, Qipeng Fan, Zhentian Li, Jun Pan, Renxu Chang, Liangxing Wu, Wenqing Yao, Phillip CC Liu

Synnovation Therapeutics, Inc., Wilmington, DE

SNV1521 is clinical stage PARP1 inhibitor and trapper with excellent potency and selectivity against other MARP and PARP proteins. Although several first generation non-PARP1 selective inhibitors have been approved primarily as monotherapy for BRCA-associated tumors, they have not fully realized the potential of PARP inhibition as a mechanism for potentiation of chemo-, DNA damage response, and radiation therapy because of overlapping toxicities including hematologic suppression. SNV1521 shows exquisite selectivity for PARP1 over PARP2 and other related proteins in biochemical assays compared with both first-generation PARPi and saruparib. In colony formation assays using normal donor HSPCs, SNV1521 exhibited significantly less suppression of the proliferation of both myeloid and erythroid lineages compared with these inhibitors. When comparing with the target efficacious concentration using a BRCA2-deficient cell line versus the NOEL for suppression of heme progenitors, SNV1521 showed an expanded margin versus either olaparib or saruparib. These data support the potential of SNV1521 to combine more effectively with approved and experimental oncology drugs. In cell-based combination experiments, SNV1521 strongly synergized with topoisomerase-1 inhibitors as well ADCs utilizing topo-1 based warheads. Importantly, the combination benefit extended to cell lines that were not intrinsically sensitive to PARP inhibitors suggesting activity in cell models beyond those with functional defects in homologous recombination. In a PDX model of breast cancer, the combination of topotecan with SNV1521 showed deeper tumor regression than either agent alone. Moreover, this activity showed durable anti-tumor activity after cessation of dosing. In summary, these data support the continued clinical development of SNV1521 as monotherapy and also in combination with drugs that are mechanistically synergistic but cannot be effectively combined with less selective PARP1 inhibitors because of overlapping toxicities.

#5726 QLS1103, A highly potent and selective PARP7 inhibitor treating advanced solid tumors.

Yuxing Zhang[1], Xiaoxia Yan[1], Yiming Xu[1], Difei Dong[1], Huijie Guo[1], Junguo Hao[1], Xinrui Shao[1], Ling Li[1], Guanxin Huang[1], Dong Yang[1], Guqin Shi[1], Yanling Xu[2], Daqing Sun[1], Weimei Sun[1], Weikang Tao[1]

[1]Shanghai Qilu Pharmaceutical Research and Development Center Ltd., Shanghai, China,[2]Qilu Pharmaceutical Co., Ltd., Jinan, China

PARP7, a member of the ADP-ribose polymerase family, is an enzyme that modifies proteins by attaching mono ADP-ribose to substrates. This post-translational modification is crucial for a variety of cellular functions, including the regulation of innate immune responses, the control of autophagy, and the facilitation of apoptosis. In some tumor cells, PARP7 may facilitate immune surveillance evasion by inhibiting type I interferon signaling pathways. Additionally, PARP7 has been reported to promote tumor growth in a cell-autonomous manner. Due to its multifaceted functions, PARP7 is an important target for cancer research and therapeutic intervention. QLS1103 is a proprietary, highly potent and selective PARP7 inhibitor. Study findings showed that treatment with QLS1103 may restore Type I IFN responses in tumor cells and trigger anti-tumor immunity in CT26 and EO771 syngeneic models. Analysis of tumor-infiltrating lymphocytes (TILs) revealed that QLS1103 not only increases the percentage of TILs but also enhances the activity of cytotoxic T cells. Furthermore, the combination of QLS1103 with an anti-PD-1 antibody showed a significant survival benefit over monotherapy in the CT26 model, with a complete regression (CR) rate rising to 75% in the combination group, compared to 25% in the monotherapy group. QLS1103 was also evaluated in a lung cancer xenograft model (NCI-H1373) to confirm the cell-autonomous effects of PARP7 inhibition. It was observed that QLS1103 could dose-dependently inhibit tumor growth and even induce tumor regression. QLS1103 has exhibited a favorable safety profile with excellent safety margins in preclinical studies and is currently under investigation in a phase I clinical trial. In conclusion, QLS1103 emerges as a promising candidate for the treatment of advanced solid tumors, either as a monotherapy or in combination with immune checkpoint inhibitors, warranting further investigation in clinical settings to fully realize its therapeutic potential.

CHEMISTRY: Drug Design, Synthesis, and Disposition
Poster Session

#5730 Discovery of a novel antibody-drug conjugate linker-payload with a distinct killing mechanism via prolonged unfolded protein response activation.

Rui Liu, Yushi Chi, Yongqiang Shan, Chenqiang Jia, Yingying Zhang, Xiayan Zhang, Wentao Zhang, Xuemei Tian, Yifan Zhang, Fan Yang, Dongmei Han, Simon Hsu, Jiamin Su, Jijun Yuan, Wan-Jen Yang, Xiaoling Yuan, Zhiyu Ma, Chen Hu

Shanghai Henlius Biotech, Inc., Shanghai, China

Introduction: A specific class of compounds featuring a 3-(4-hydroxyphenyl) indoline-2 scaffold demonstrated potent antitumor activity against cancer cells with elevated TRPM4 expression or estrogen receptor positivity through the sustained activation of unfolded protein response. This mechanism is distinct from existing toxins. Generally, these compounds exhibit low toxicity to most healthy tissues, with gastrointestinal toxicity being the primary adverse effect. Notable examples of this class include ErSO and laxative oxyphenisatin acetate. This study aimed to optimize this scaffold for use as antibody-drug conjugate (ADC) payloads. By selecting antigens with low gastrointestinal expression, this strategy could potentially overcome ADC resistance or synergize with existing ADCs, leading to durable patient responses and improved survival outcomes.
Methods: The cytotoxicity of lead toxin, HLX91-048, and its related ADCs were evaluated in immortalized cell lines and treatment-resistant patient-derived organoids (PDOs) using the CellTiter-Glo® Assay. To create the HER2-targeting ADC, HLX91-048 was linked to a HER2 antibody via a cleavable GGFG linker, resulting in drug-to-antibody ratio (DAR) of 4 or 8 as needed. Flow cytometry was employed to analyze the binding and internalization of the ADCs. Bystander killing assays were performed on the $HER2^-$ MCF-7 cells using conditioned media from $HER2^+$ BT474 cell cultures. *In vivo* efficacy was tested in the BT474 breast cancer model and the DS-8201-resistant NCI-N87R gastric cancer model. The tolerability of HLX91-048 and the ADC was compared in parallel to DXd and DS-8201 in rats.
Results: HLX91-048 demonstrates >10-fold increase in cytotoxicity compared to ErSO, exhibiting sub-nanomolar to nanomolar IC_{50} values across various tumor cell types. When compared to DS-8201 analog the HLX91-048-based ADC showed enhanced cytotoxicity in multiple cell lines, stronger bystander killing, and greater stability in human and monkey plasma. As expected, the HLX91-048-based ADC significantly activates the UPR. The DAR4 HER2 ADC derived from HLX91-048 exhibited dose-dependent antitumor efficacy in both BT474 and NCI-N87 models, leading to substantial tumor regression at higher doses (10-12 mpk). Notably, 5 out of 8 PDOs from patients resistant to various standard treatments remained sensitive to the HLX91-048-based ADC, while showing reduced sensitivity to DS-8201. Lastly, in a pre-toxicology evaluation in rats, both the DS-8201 analog and the HLX91-048-based ADC were well-tolerated at a dose of 60 mpk once weekly for three weeks.
Conclusion: We reported a first-in-class ADC linker-payload featuring a highly differentiated killing mechanism that demonstrated exceptional efficacy and safety in preclinical evaluations. Preliminary toxicology studies in non-human primates have been scheduled.

#5731 BGB-C354, a novel B7H3 ADC with high DAR stability and strong bystander effect, demonstrates robust antitumor activity in preclinical models.

Xiao Ding[1], Charng-Sheng Tsai[2], Mei-Hsuan Tsai[2], Maggie Tang[1], Liu Xue[1], Chi Guan[1], Meiling Tan[2], Yiren Xiao[2], Yuanyuan Xie[1], Kunpeng Lyu[2], Xiaotong Chen[1], **Wenhao Li**[1], Qi Liu[1], Yun Chen[2], Jie Pan[2], Wenjin Yao[1], Yang Lyu[2], Kaiying Jia[1], Chang Song[1], Weiwei Song[1], Ce Wang[1], Fan Wang[1], Zhitao Wan[2], Mingming Guo[1], Beibei Jiang[1], Xiaomin Song[1], Wei Jin[1], Qiansheng Ren[1], Yu Shen[2], Zhirong Shen[1]

[1]BeiGene (Beijing) Co., Ltd., Beijing, China,[2]BeiGene (Shanghai) Research & Development Co., Ltd., Shanghai, China

B7H3 is a type I transmembrane protein belonging to the B7 immunoregulatory family. B7H3 is overexpressed on a wide range of solid tumors such as lung cancers, gastrointestinal cancers and gynecological cancers, but its expression is absent or low in normal tissues, making it an attractive target for anticancer therapies. BGB-C354 is a B7-H3-targeting antibody-drug conjugate (ADC) composed of a humanized anti-B7-H3 monoclonal antibody conjugated via a cleavable linker to a novel TOP1i payload, and the drug to antibody ratio (DAR) is approximately 8. Utilizing a stable ring-open conjugator, BGB-C354 demonstrated improved ADC stability and sustained high DAR in vivo, which may enable more efficient payload delivery to B7H3-expressing tumors. In nonclinical pharmacological studies, BGB-C354 exhibited strong target binding activity, high target-dependent internalization, and potent cytotoxicity toward B7H3-expressing tumor cells. In addition, BGB-C354 demonstrated strong bystander killing effect in the presence of B7H3-expressing cell lines to potentially overcome the tumor heterogeneity. Notably, robust anti-tumor activity was observed in a panel of cell-derived xenograft (CDX) models with varying levels of B7-H3 expression, as well as in patient-derived xenograft (PDX) models. Consistent with its mechanism of action, DNA damage and apoptosis biomarker changes were induced by BGB-C354 in a dose-dependent manner after single dosing in preclinical models. Recently, a phase I study of BGB-C354 has been initiated to investigate its potential safety and preliminary efficacy in patients with B7H3-expressing advanced solid tumors (NCT06422520).

#5732 DXC020, a novel anti-CD54 antibody conjugate with a functional linker, exhibits potent anti-tumor efficacy.

Xingyan Jiang, Tianyu Zhou, Junxiang Jia, Huihui Guo, Yuanyuan Huang, Hangbo Ye, Lingli Zhang, Jun Zheng, Lu Bai, Wenjun Li, Binbin Chen, Qingliang Yang, Robert Zhao

Hangzhou DAC Biotechnology Inc. Ltd, Hangzhou City, China

Intercellular adhesion molecule-1 (ICAM-1, CD54) is a transmembrane glycoprotein of the immunoglobulin superfamily and involved in intercellular adhesion during inflammatory injury, viral infection, and tumorigenesis. The overexpression of CD54 is associated with the aggressiveness of liver cancer, non-small cell lung cancer, triple-negative breast cancer, melanoma, oral squamous cell carcinoma, and pancreatic cancer. Moreover, CD54's role in immune cell adhesion implies that targeting this molecule could potentially enhance the immune response against cancer cells. The identification of CD54 as a candidate gene in prostate tumors exposed to chronic cisplatin administration highlights its potential as a therapeutic target in cancer, including liver cancer. These properties make CD54 a promising target for the development of antibody-drug conjugates (ADCs) and other targeted therapies in hepatocellular carcinoma (HCC) and other malignancies. Through preliminary screening, several monoclonal mouse-derived antibodies with a strong affinity for CD54 were identified. To prevent the human body from developing a human anti-mouse antibody response (HAMA), these antibodies were subjected to a process of mutation and back-mutation to humanization. This meticulous engineering resulted in humanized antibodies with affinities comparable to their murine counterparts, with a dissociation constant (KD) in the pM range. Endocytosis experiments demonstrated that these humanized antibodies effectively internalized within tumor cells, a crucial property for the efficacy of antibody-based therapies. These antibodies were then conjugated with both tubulysin based and CPT-payloads to create series of ADCs. In vitro cytotoxicity assays revealed that many of the ADCs exhibit potent cytotoxicity against a range of cancer cell lines, including non-small cell lung cancer, prostate cancer, breast cancer, liver cancer, and bile duct cancer. In vivo studies using cell-derived xenograft (CDX) models demonstrated that a single, low-dose administration of a CD54-ADC candidate, DXC020, can significantly inhibit tumor growth and even induce tumor regression in cases such as non-small cell lung cancer. These promising results indicated that CD54-ADCs could become a viable candidate for targeted therapy against tumors expressing CD54, offering a new frontier in the treatment of various cancers, in particular for hepatocellular or hepatobiliary carcinoma, which still remains a global health challenge. The successful humanization of the antibodies, their effective internalization in tumor cells, and the robust cytotoxicity of the resulting DXC020 both in vitro and in vivo models, position the CD54-ADC as a promising candidate in further clinical study.

#5733 Design and synthesis of the novel camptothecin analog MF-6 for application into site-specific antibody-drug conjugate.

Xiaowei Cen, Hui Xu, Huikai Zhu, Hua Deng, Zhenzhen Wang, Junwei Xu, Xueying Li, Zhiheng Wu, Yiyan Zhang, Yuhang Chen, Xiaolin Chen, Li Chen, Wei Zhou, Hai Wu

Mabwell (Shanghai) Bioscience Co., Ltd. , Shanghai, China

Introduction: Antibody-drug conjugates (ADCs) have emerged as a revolutionary breakthrough in cancer treatment by precisely targeting tumor cells with high-potency payloads attached to specific antibodies. However, the efficacy and safety of ADCs largely depend on the design and optimization of their payloads. This study leverages the advanced Mtoxin^{Tm} platform to develop a novel ADC payload, MF6, aiming to enhance the antitumor activity of ADCs through precise drug design.

Methods: Supported by the Mtoxin^{Tm} platform, we first modified camptothecin-based compounds by preparing a series of camptothecin analogs with different substituents at the core position. Through in vitro efficacy testing, we screened out a series of compounds with significant antitumor activity and further optimized their attached dipeptide linkers. Subsequently, we conjugated the optimized drug-linker conjugates with anti-Trop2 and anti-B7H3 monoclonal antibodies to form novel ADCs. To comprehensively evaluate the potential of these ADCs, we conducted multiple experiments in vitro and in vivo, including pharmacodynamics, pharmacokinetics, safety, and antitumor activity studies.

Results: The experimental results showed that MF6, as a free molecule, exhibited good drug-killing activity and maintained significant efficacy in multidrug-resistant models resistant to DXd. ADCs constructed using the clinically validated site-specific conjugation technology IDDC™ and our novel payload MF6 demonstrated good uniformity and stability, and exhibited significant antitumor efficacy in multiple CDX models. Additionally, these ADCs showed good bystander killing activity, meaning they could kill nearby tumor cells, thereby further enhancing their antitumor effect. We also found that ADCs synthesized with MF6 had excellent serum and plasma stability and pharmacokinetic properties, providing strong support for their future clinical application.

Conclusions: This study successfully developed a novel ADC payload MF6 based on the Mtoxin^{Tm} platform and initially validated its antitumor activity and safety in multiple *in vitro* and *in vivo* models. These results provide a solid experimental foundation for the further development of MF6-based ADCs for the treatment of various cancers. In the future, we will continue to explore the potential of MF6 and anticipate that MF6-based ADCs will undergo further clinical validation and application, bringing more precise, effective, and safe treatment options to cancer patients.

#5734 Advancing ADC discovery with a highly versatile linker-camptothecin library.

Meijun Xiong, Qingsong Wu, Yanchun Li, Chong Liu, Yajun Sun, Zengyan Mu, Xinju Gao, Yanwen Feng, Haibo He, Paul H. Song, Gang Qin

GeneQuantum Healthcare (Suzhou) Co., Ltd., Suzhou, China

Camptothecin derivatives have gained prominence as payloads in investigational antibody-drug conjugates (ADCs), exemplified by the clinical success of Trodelvy and Enhertu. The precise design of these linker-camptothecins has been instrumental in achieving optimal safety and efficacy for specific therapeutic indications. Growing evidence suggests that a single linker-payload design cannot accommodate the diverse requirements of various therapeutic targets. For instance, while the linker-payload of Enhertu (GGFG-DXd) has shown exceptional efficacy in HER2-targeted therapies, its performance is less promising in TROP2- and HER3-targeted indications. This highlights the critical need for linker-camptothecin systems with greater diversity in drug release mechanisms, kinetics, and payload potency to address varying therapeutic needs. To address the limitations of existing linker-camptothecin designs, including issues with stability, release efficiency, and specificity, we have developed a comprehensive library of linker-camptothecins with enhanced molecular diversity. Our design strategy incorporates a range of stimuli-responsive release mechanisms, such as cathepsin-, legumain-, and glucuronidase-mediated cleavage, as well as pH-sensitive linkers. Multiple camptothecin derivatives, including SN38, Exatecan, and TopoIx, were selected based on their distinct pharmacological properties—such as antitumor efficacy, lipophilicity, and half-life—and were conjugated to various linkers, creating a versatile library with broad applicability. This molecular diversity facilitates optimized release profiles and payload activities, tailored to specific therapeutic scenarios. Leveraging this library, we constructed a series of highly refined ADCs targeting different antigens and evaluated them systematically in both in vitro and in vivo preclinical models. These studies revealed that the ADCs incorporating the novel linker-camptothecin designs demonstrated significantly enhanced therapeutic efficacy and improved safety profiles compared to conventional linker-payload systems. In summary, we have established a novel linker-camptothecin library with diverse release behaviors optimized for specific therapeutic contexts. This approach facilitates the discovery and development of potent ADC candidates with superior efficacy and safety profiles, providing a robust platform for precision drug development targeting diverse indications.

#5735 Design of a peptide-targeted radiotherapy for treatment of bone cancers.

Losha D. Jung[1], Philip S. Low[2]

[1]MCMP, Purdue University, West Lafayette, IN,[2]Purdue University, West Lafayette, IN

According to the American Cancer Society, ~400,000 new cases of malignant bone metastases are diagnosed each year in the United States, with prostate and breast cancers accounting for the largest fractions. Unfortunately, progression of these metastases can lead to bone fractures, spinal cord compressions, hypercalcemia, and severe pain, resulting in a decrement in quality and duration of life. Given the limited effectiveness of existing clinical interventions in alleviating these symptoms, there is a critical need for therapies capable of selectively eradicating bone metastases without harming healthy tissues. To address this problem, we have developed a novel bone cancer-targeted radiotherapy comprised of an acidic oligopeptide that homes specifically to exposed hydroxyapatites (i.e. the mineral component of bone that becomes exposed in bone metastases) linked to a universal chelating agent (e.g. DOTA) that can bind to various cytotoxic radionuclides such as ^{177}Lu, ^{225}Ac, or ^{90}Y. Tail vein administration of the ^{177}Lu-labeled form of the above conjugate into nu/nu mice bearing intratibial C4-2 human prostate cancer revealed pronounced accumulation and retention of the conjugates in the tumor-induced bone lesions, with associated radioactivity decreasing modestly from 38% to 23% injected dose per gram (ID/g) from 4 hours to 240 hours. As the uptake in healthy tissues, aside from the kidneys, was minimal, and renal accumulation declined significantly from 2.8% to 0.13% ID/g over the same 240-hour period, it was concluded that bone metastases could be effectively irradiated without inducing measurable toxicity to healthy tissues. Subsequent therapeutic efficacy studies validated this prediction, demonstrating that serum PSA levels, a biomarker of C4-2 tumor growth, were significantly reduced in mice treated with the targeted radiotherapy, whereas untreated mice experienced disease progression necessitating euthanasia. Comparison of microCT scans between treated and untreated cohorts revealed a substantial reduction in bone lesions in the treated groups. Additionally, pathological examinations showed no detectable harm to kidney, blood, and bone marrow. These findings conclusively suggest that our bone cancer-targeted radiotherapy has the potential to improve survival and quality of life in this challenging patient population while minimizing toxicity.

#5736 Design of CYP1-activated 2,4-diaryl-substituted pyridine analogues with significant anti-proliferative effects in breast cancer cells *in vitro*.

Federico Brucoli, Ketan Ruparelia

De Montfort University, Leicester, United Kingdom

Cytochromes P450-1A1 and 1B1 (CYP1A1 and CYP1B1) are over-expressed in several human malignancies and represent an excellent target for enzyme prodrug therapy of cancer. The extrahepatic CYP1A1 isoform is constitutively expressed in a number of human breast cancers. CYP1B1 has been consistently detected in both pre-malignant tissue and metastatic cancer and its expression is associated with hormone-dependent tumours (e.g., breast and ovary). We have developed lead-prodrugs that selectively targeted CYP1 family enzymes. Here, we report the extensive chemistry work, which included the synthesis of more than 150 analogues, cytotoxicity screening results and CYP1 metabolisation data that led to the identification of a series of 2,4-diaryl-substituted pyridine lead-compounds. In our *in vitro* breast cancer model, the CYP1-activated pro-drugs were highly active against triple negative breast cancer MDA-MB-468 cells (IC_{50}=0.08μM), which constitutively express CYP1B1. The pro-drugs also had significant antiproliferative activity against MCF7 cells, which were induced to express CYP1A1 upon treatment with aryl hydrocarbon agonist 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The hit-compounds exhibited no toxicity in "normal" human breast MCF10A cells (IC_{50}>100μM), which have no basal CYP1 expression. Cytotoxicity assays conducted in the presence of known CYP1 inhibitor α-naphthoflavone resulted in a 300-fold increase in the pro-drugs IC_{50} values from 0.01 to 3μM in MDA-MB-468 cells. Dose-response studies using the NCI 60 human tumour cells panel showed that selected prodrugs were 4-fold more selective towards breast cancer cells (GI_{50}=41nM) compared to the other cell lines tested. *In vitro* metabolism of selected lead-compounds was assessed by separate incubation with human CYP1A1, CYP1A2, CYP1B1 and CYP3A4 enzymes using HPLC methods. Both CYP1A1 and A2 were the most efficient catalysts of the pro-drugs metabolism, followed by CYP1B1. The prodrugs were not metabolised by CYP3A4. Tandem mass spectrometry (MS/MS) fragmentation and HPLC co-elution analysis allowed for the identification of the structure of the main metabolite of a benzodioxole-containing lead-compound that was produced by CYP1A1, CYP1A2 and CYP1B1 isozymes. The CYP1-generated metabolite was synthesised and cytotoxicity screening revealed that the compound exhibited antiproliferative activity with an IC_{50} <0.07μM against all the cell lines used in this study (e.g., MDA-MB-468, MCF10A and MCF7) irrespective of their levels of CYP1 expression. Further investigations on the metabolite mode of action are underway. In summary, we have identified cancer pro-drug chemotherapeutic agents that exhibit significant anti-proliferative activity in a CYP1-enzyme expressing breast cancer *in vitro* model and are suitable candidates for pre-clinical development.

#5737 Preclinical study of a novel long-acting receptor-biased rhIL-2 drug with sustained activation of cytotoxic T cells.

Peipei Cao, Biaxue Zhao, Jun Wang, Chenyang Jiang, Yu Zhuang, Bruce Y. Ma

Zonhon Biopharma Institute, Inc., Changzhou, China

Purpose: Human Interlukin-2 (hIL-2) targets at malignant tumors, such as renal cell carcinoma and melanoma, and has been on the market for many years. Due to its adverse effects, hIL-2 is limited in clinical use. However, with in-depth studies on the mechanism of action of IL-2 related drugs, IL-2 has been found to have a high potential for use in immunotherapy of oncology, setting off the upsurge of developing improved innovative IL-2 related drugs at home and abroad. The joint application of these drugs and PD-1 in clinical trials for malignant tumors displays a better efficacy and huge market potential. A lot of drug companies have developed IL-2 related drugs and now they are conducting phase I and II clinical trials for metastatic solid tumors and other indications. All of these IL-2 related drugs are designed and developed by half-life extension methods such as molecular mutagenesis and PEGylation, while problems are also existed, for example, a weak-effect and severe side effects. Consequently, according to QbD drug design concept, we have combined molecular mutagenesis with PEGylation to design new molecules and screen out feasible drugs through in vitro and in vivo evaluation experiments, and devoted to developing a novel long-acting IL-2 drug of sustained activation of T cells, offering an alternative choice for current tumor immunotherapy.
Method: Facing the defects of these products under development, we carried out molecule design and completed molecular screening of drug candidates through in vitro and in vivo evaluation experiments and pharmacological studies. After that, non-clinical studies were carried out and the comparison with analogous drugs in development was implemented in aspects of efficacy, safety and pharmacy.
Result: ZHB114 was screened out as a drug candidate with a better efficacy and fewer side effects. According to the pharmacological studies, ZHB114 was capable of releasing drug activity in a controlled and sustained manner within a safe therapeutic window, thereby enhancing its safety profile. In a number of animal models bearing tumors, ZHB114 has been demonstrated to possess a significantly higher potency than analogous drugs under development. In some models, ZHB114 can elicit complete tumor growth inhibition. The repeated dosing toxicity studies conducted on rats and cynomolgus monkeys have shown that ZHB114 has a wide security window and minimal adverse effects at efficacious doses. It is noteworthy that ZHB114 has been approved by CDE in China, and now it is in the process of preparing for the approval of FDA.
Conclusion: Compared with other analogous drugs in development, ZHB114 is more potent and is expected to become a novel long-acting IL-2 agent for tumor immunotherapy, with fewer side effects.

#5738 Napabucasin analogues with increased bioavailability and efficacy in fibrolamellar hepatocellular carcinoma.

Shalini Rawat, Jared Alderman, Priscilla Baeza, Lianna Hartshorn, Celeste Villasenor, Jakim Brazil, Barbara Lyons

Chemistry and Biochemistry, New Mexico State Univ. - Las Cruces, Las Cruces, NM

Fibrolamellar Hepatocellular carcinoma (FLC) is a very rare liver cancer which occurs in young adults and children under the age of 40. This cancer is lethal and was first recognized in 1980. The term “fibrolamellar” refers to the fibrous bands that are visible when examining tumor tissue under a microscope. In FLC, a deletion of 400,000 base pairs occurs in a chromosome 19 copy, leading to the formation of a fusion protein comprising heat shock protein DNAJB1 and the catalytic subunit of Protein Kinase A (DNAJB1-PRKACA). Patients show no symptoms in the early stage of the disease. The reported survival rate over the past five years is below 50%. Surgery remains the sole partially effective treatment for FLC, with no currently approved systemic therapies available. The primary model compound of this project is Napabucasin. It is also known as BBI608 is a naphthoquinone natural product isolated from the plants *Newbouldia laevis*, *Ekmanianthe longiflora*, and *Handroanthus impetiginosus*. It has shown wide spectrum anti-cancer activities and is now in trials for cancer treatment. The Napabucasin was found to be a top hit in drug screening showing potency against all FLC patient derived xenografts (PDX) models. Napabucasin is known to work in multiple signaling pathways and have been shown cytotoxic against both PDX and direct-from-patient FLC tumor cells. We anticipate that the organic synthesis and development of optimized Napabucasin analogues, along with the evaluation of their cytotoxicity and proliferation effectiveness compared to the parent compound, Napabucasin, will result in compounds with improved bioavailability and efficacy, particularly in FLC model cell lines (FLX1 and Huh7-Chimera).

#5739 Small molecule modulation of autoinhibition to inhibit DNA binding of ERG and FLI1 - therapeutic for Ewing sarcoma, prostate cancer, and ALL.

John H. Bushweller[1], Kiran Srimath Tirumala[1], Ashish Kabra[1], Adam Boulton[1], Karla Rubio[2], Michael Bond[3], Anglea Su[3], Miguel Rivera[2], Kim Stegmaier[3]

[1]University of Virginia, Charlottesville, VA,[2]Massachusetts General Hospital, Boston, MA,[3]Dana Farber Cancer Institute, Boston, MA

Targeting transcription factor-DNA interactions presents substantial challenges due to the convex and highly positively charged surface on the DNA binding domains that mediate DNA binding. Such a surface is far from ideal for development of drug-like molecules, making this a challenge. Furthermore, as many transcription factors are members of families with highly conserved DNA binding domains, there are also concerns with respect to what level of specificity could be achieved with an inhibitor targeting the DNA binding domain in a family of transcription factors. The 28 member Ets family of transcription factors has numerous members implicated in various cancers. EWS::FLI1 and EWS::ERG fusions are drivers in Ewing sarcoma, ERG overexpression via the TMPRSS2::ERG fusion is a driver in half of prostate cancer, and ERG has been identified as a marker for poor prognosis in AML patients. Based on this, small molecule inhibitors of DNA binding of ERG and FLI1 could have utility for these cancers.To overcome the challenges mentioned above, we chose to target the autoinhibition of ERG and FLI1 to generate inhibitors of their binding to DNA. Numerous transcription factors modulate their activity via autoinhibition and this has been characterized for many of the members of the Ets family including our own work on ERG. By so doing, we hypothesized we could achieve selectivity within the Ets family due to the differing sequences and mechanisms mediating autoinhibition among the different family members.We screened using fragment libraries for compounds inhibiting DNA binding of an autoinhibited ERG construct but were not active against the uninhibited DNA binding domain. Optimization of these gave a fragment with excellent selectivity but limited potency. We subsequently screened for a molecule that could covalently add to ERG and linked a derivative of this to the autoinhibition fragment. Biochemical assays of effects on DNA binding show low μM IC_{50} values and a shift in the K_d of DNA binding of ~2.5-fold for both ERG and FLI1. Importantly, characterization of effects on 7 other Ets family members representing 7 other subfamilies shows the inhibitors are not active against other Ets family members, demonstrating we have achieved a high level of selectivity within the family.We have shown that the ERG/FLI1 inhibitors inhibit the proliferation of Ewing sarcoma cell lines at low μM concentrations and preferentially inhibit Ewing sarcoma cell lines versus other sarcoma cell lines. As EWS::FLI1 is known to bind at enhancers, we evaluated effects on H3K27ac levels at enhancer elements. We observe no global reduction in H3K27ac levels at enhancers but H3K27ac levels at EWS::FLI1 bound enhancers are reduced. In addition, we observe a reduction in the occupancy of RNA Pol II at EWS::FLI regulated genes with inhibitor treatment. Both these results are consistent with an on-target mechanism of action.

#5740 Disruption of the acetylcholine signaling pathway inhibits angiogenesis and tumor growth in human lung adenocarcinoma.

Piyali Dasgupta[1], Eric W. Bow[2], Krista L. Denning[3], Rama S. Gadepalli[2], John M. Rimoldi[2], Yi Charlie Chen[4], Sarah L. Miles[1]

[1]Biomedical Sciences, Marshall University School of Medicine, Huntington, WV,[2]Department of Biomolecular Sciences, University of Mississippi, University, MS,[3]Department of Pathology, Marshall University School of Medicine, Huntington, WV,[4]Department of Biology and Environmental Science, Bluefield State University, Bluefield, WV

Purpose of the study: Lung adenocarcinoma (LUAD) accounts for about 60% of all lung cancers and shows a strong etiological correlation with smoking. Nicotine, the addictive component of tobacco smoke is known to accelerate the growth of human lung tumors by stimulating tumor angiogenesis. The pro-angiogenic effects of nicotine are mediated by nicotinic acetylcholine receptors (nAChRs). The endogenous ligand for nicotine is the neurotransmitter acetylcholine (ACh). The primary aim of this study was to determine if disruption of ACh production (via inhibition of the enzyme choline acetyltransferase; ChAT) could inhibit angiogenesis and suppress the growth of human LUADs. We used a small molecule inhibitor of ChAT namely BW813U for our studies.
Experimental procedures. Primary human microvascular endothelial cells from the lung (HMEC-L) were used for all the experiments. These cells were used between passage 3-7. The anti-angiogenic activity of BW813U was measured in cell culture models by using the “MATRIGEL ASSAY”. The results obtained from the “MATRIGEL ASSAY” were confirmed using chicken chorioallantoic membrane (CAM) angiogenesis experiments. Finally, the anti-tumor activity of BW813U was measured in vivo by using xenograft model systems in SCID mice. Our studies also investigated the signaling pathways underlying the anti-angiogenic activity of BW813U, using chemical inhibitors and siRNA methodology.
Results: BW813U robustly suppressed angiogenesis in Matrigel assays and CAM model systems. The administration of BW813U potently decreased the growth rate of H838 tumors xenotransplanted in SCID mice. Immunohistochemical staining experiments revealed that the anti-tumor activity of BW813U was correlated with decrease of CD31 angiogenic biomarker in H838 tumor sections. The anti-angiogenic activity of BW813U was mediated by the alpha7-nAChR pathway and involved the nitric oxide pathway. Taken together, our studies show that ChAT may be a valuable drug target for the therapy of LUAD.
Conclusions: ChAT antagonists like BW813U decrease the growth of LUAD by suppressing tumor angiogenesis.
Support or Funding Information: Funding for our study was supported by a NIH R15-AREA Grant (2R15CA161491-03) and a Center for Natural Products pilot grant from the WV-INBRE Grant Program (P20GM103434, PI:Gary Rankin). Furthermore, this study was supported in part by an Institutional Development Award (IDeA) Grant number P20GM104932 from the National Institute of General Medical Sciences (NIGMS) and the Research Core B of COBRE, a component of the National Institutes of Health (NIH).

#5741 6-((1,4-naphthoquinone-2-yl)methyl)thio-glucose conjugates, a novel targeted approach for advanced prostate cancer.

Sergey Dyshlovoy[1], Tobias Busenbender[1], Dmitry Pelageev[2], Jessica Hauschild[1], Moritz Kaune[1], Lukas Bockelmann[1], Christoph Krisp[1], Mohamed Elsesy[1], Alexandra Zielinski[1], Thomas Mair[1], Simone Venz[3], Malte Kriegs[1], Konstantin Hoffer[1], Yuri Sabutski[2], Ksenia Borisova[2], Nadja Strewinsky[1], Svetlana Kovach[2], Ekaterina Khmelevskaya[2], Klaus Pantel[1], Hartmut Schluter[1], Victor Anufriev[2], Derya Tilki[1], Markus Graefen[1], Wael Mansour[1], Carsten Bokemeyer[1], Gunhild von Amsberg[1]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany,[2]G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Vladivostok, Russian Federation,[3]University of Greifswald, Greifswald, Germany

The Warburg effect is a shift from oxidative phosphorylation to anaerobic glycolysis, accompanied by an enormous increase in glucose uptake into cancer cells. We have utilized this effect to design a new group of targeted 1,4-naphthoquinone-glucose derivatives conjugated with a novel thiomethylene linker, which were cytotoxic to prostate cancer cells. Compound PeS-9 revealed the highest efficacy and selectivity, which was conditioned by a GLUT-1-mediated uptake. PeS-9 induced androgen receptor degradation followed by downregulation of its signaling. In addition, it increased reactive oxygen species production and induced DNA double-strand breaks. Combinational therapy with PARP-inhibitor olaparib resulted in synergistic effects in homologous recombination-deficient cells. The underlying mode of PeS-9 cytotoxic action involved mitochondria targeting, leading to a loss of mitochondrial membrane potential, release of cytochrome C and AIF, activation of caspases-3 and -9, PARP cleavage, and apoptotic cell death. This process was stipulated by down-regulation of several antiapoptotic factors and induction of endoplasmic reticulum stress. Moreover, drug-induced activation of signaling pathway mediated by kinases p38, JNK1/2, and ERK1/2 was identified as an important factor of the cytotoxic activity. The anticancer activity of PeS-9 could be confirmed *ex vivo* using patients-derived tumoroids as well as *in vivo* resulting in decreased tumor growth and dissemination of prostate cancer cells to the lungs. No serious side effects were observed in animal models. This unique combination of anticancer properties makes PeS-9 an attractive candidate for targeted monotherapy against GLUT-1-overexpressing tumors and as a potential combination partner, especially with PARP inhibitors.

#5742 Synthesis of substituted benzimidazole tetrahydropyridines as anti-breast cancer agents.

Kinfe Ken Redda[1], Madhavi Gangapuram[1], Absera W. Haile[1], Suresh Eyunni[2]

[1]College of Pharmacy and Pharm Sciences, Florida A&M University, Tallahassee, FL,[2]Chemistry, College of Science and Technology, Florida A&M University, Tallahassee, FL

Breast cancer is the most common type of cancer worldwide. In 2023, the World Health Organization (WHO) reported that 2.3 million women were diagnosed with breast cancer, leading to 670,000 deaths. Furthermore, the incidence rates of breast cancer have been rising at 3% each year. Overexpression of COX-2 in breast cancer tissues is associated with poor prognosis, including higher tumor grade, increased metastatic potential, and resistance to therapy. Selective COX-2 inhibitors have demonstrated potential in reducing tumor growth in preclinical and some clinical studies. These inhibitors may also enhance the effectiveness of chemotherapy and radiotherapy. The benzimidazole nucleus is a valuable subunit for the development of pharmaceuticals and biologically relevant molecules. Tetrahydropyridine (THP)analogs are compounds with diverse biological activities, including analgesics, anti-inflammatory agents, and chemotherapeutic agents. Our previous research findings indicated that the THP derivatives exhibited COX-2 inhibition and demonstrated anti-inflammatory effects in rat paw edema assays. The pharmacological activities of THP derivatives depend on the nature of the substituents in the THP ring system. The incorporation of a benzimidazole moiety may enhance the biological activity of these THP derivatives. Thus, the design and synthesis of novel series of benzimidazole-THP derivatives with potential anticancer and anti-inflammatory activities were initiated. In the current investigation, we synthesized numerous analogs maintaining the *N*-(5-(1*H*-benzo[*d*]imidazol-2-yl)-3,6-dihydropyridin-1(2*H*)-yl)benzamide/benzene sulfonamide having modifications on the phenyl ring. We expect that these structural modifications would affect the compounds electron density, lipophilicity and the compounds steric configurations. The starting material 2-(pyridin-3-yl)-1H-benzo[d]imidazole was obtained by the reaction of nicotinic acid, 1,2-phenyldiamine and polyphosphoric acid by stirring in an oil bath at 180° C for 2 h. N-Amination of substituted 2-(pyridin-3-yl)-1H-benzo[d]imidazole by an aminating agent O-(mesitylsulfonyl)hydroxylamine (MSH), followed by ylide formation and further reduction yielded the desired substituted N-(5-(1H-benzo[d]imidazol-2-yl)-3,6-dihydropyridin-1(2H)-yl)benzamide derivatives. Final compounds were analyzed via NMR spectroscopy, IR and elemental analysis. The analogs were evaluated for through docking studies. Two products showed significant anti-inflammatory and anti-breast cancer activities.

#5745 Selective anticancer activity and mechanistic insights of novel compounds identified through NCI-60 cancer cell line screening.

Rakaia S. Abdullah, Md Emran Hossain, Eneye David Ajayi, Mostafa M. A. Aref, Hamed I. Ali

Pharmaceutical Sciences, Texas A&M University, College Station, TX

Targeted cancer therapies aim to maximize efficacy while minimizing side effects. The NCI 60 Human Tumor Cell Line Screen provides a comprehensive platform for identifying compounds with selective anticancer potential across various subtypes. To identify and validate potent anticancer compounds from a 150 compound library, focusing on molecular selectivity, mechanistic insights, and preclinical efficacy. High-throughput screening was performed using the NCI60 platform, followed by cytotoxicity assays (IC_{50}), kinase selectivity profiling, and molecular modeling. Further evaluation utilized 3D spheroid and organoid models, in vivo testing in xenograft models, and mechanistic studies via Western blot to assess apoptosis and pathway modulation. Seventeen compounds (HA45, HA46, HA7, HA12, HA14, HA15, HA16, HA19, HA21, HA23, HA25, HA26, HA64, HA88, HA94, HA96) exhibited significant selectivity, with GI_{50} values in the nanomolar and micro range. Lead compounds HA45 and HA46 against CNS SNB 74 cell line demonstrated potent kinase inhibition profiles and induced apoptosis through disruption of critical signaling pathways. Molecular docking and dynamics simulations revealed stable interactions with target kinases, corroborating structure-activity relationships. Testing in 3D models confirmed their physiological relevance, and in vivo studies showed tumor growth inhibition with minimal toxicity. This study highlights the utility of integrated methodologies for advancing selective anticancer agents toward clinical application. The findings underscore the importance of combining computational, in vitro, and in vivo approaches in the development of novel cancer therapeutics.

#5746 Development of Pt-HDACi complexes to enhance potency and selectivity for advanced cancer therapy.

Rabeya Bosri, Jayden Stahl, Nghia Tran, Chao Feng, Liang Xue, Qinliang Zhao

Department of Chemistry, University of the Pacific, Stockton, CA

Combining standard chemotherapy drugs with low doses of histone deacetylase inhibitors (HDACis) has emerged as a promising approach to enhance cancer treatment efficacy. Incorporation of HDACi onto the platinum (Pt) core as a non-leaving group may improve the potency and selectivity of the Pt anticancer drugs and maintain the synergistic effect. In the newly synthesized inhibitors, the linker domain was modified by including heterocyclic rings or amine groups. One or two amine groups were installed onto the aromatic ring in the protein recognition domain to build monodentate or bidental coordination sites to incorporate Pt. Four HDACi derivatives (L19, L20, L21, L22) were successfully synthesized and fully characterized. Pt-HDACi complexes were obtained by reacting K_2PtCl_4 with bidentate L19 and L20, and $K[PtCl_3(2\text{-picoline})]$ with monodentate L21 and L22. Intermediates and final compounds were analyzed using mass spectrometry and NMR spectroscopy. The purity was confirmed by melting point measurement, analytical HPLC, and/or elemental analysis. Different bioassays, such as enzyme inhibition, cell viability studies with cell lines K562, HCT-116, and A549, and DNA binding assay, have been applied to evaluate their efficacy. All four derivatives and Pt-HDACi conjugates have shown promising enzyme inhibition and potent bioactivities. Particularly, L19 and L21 with a heterocyclic ring in the linker showed the highest potency, with IC50 values significantly lower than the FDA-approved Vorinostat and Panobinostat. Molecular docking studies further supported these findings by revealing stronger binding affinity towards the HDAC enzymes, demonstrating similar trend in inhibition and potency toward cancer treatment.

#5747 Assessment of neuro-pharmacokinetic behavior of BDTX-1535 in brain tumor patients by a validated LC-MS/MS method.

Vahuni Karapetyan, Tigran Margaryan, Nader Sanai, Artak Tovmasyan

Ivy Brain Tumor Center, Barrow Neurological Institute, Phoenix, AZ

BDTX-1535 is an orally available, highly potent, irreversible inhibitor of epidermal growth factor receptor (EGFR), targeting a range of EGFR mutations, including extracellular variants and amplifications commonly expressed in glioblastoma. To assess CNS penetration of BDTX-1535 in glioblastoma patients, a novel bioanalytical method was developed to measure total and unbound BDTX-1535 levels in human brain tumor tissue, plasma, whole blood and cerebrospinal fluid (CSF). The method was validated using plasma as a surrogate matrix over the concentration range of 0.2-500 nM on an LC-MS/MS system. The MS/MS detection was conducted in positive electrospray ionization mode, while chromatography was performed using a Kinetex™ F5 column (50 × 2.1 mm, 1.7 µm) with a total 3.8-minute gradient elution time. The maximum coefficient of variation for both intra- and inter-day precision was 16.3%, with accuracy ranging from 91.5% – 107.2% across all matrices. BDTX-1535 was stable in human whole blood, plasma and brain homogenate for at least 4 hours at room temperature (RT) and 105 days at -20°C, as well as in stock and working solutions for at least 9 hours (RT) and 52 days (4°C). Equilibrium dialysis experiments revealed the unbound fractions of 0.01 and 0.08 for BDTX-1535 in human brain and plasma, respectively. The validated method is currently employed to assess BDTX-1535 levels in human brain tumor tissue, CSF, blood and plasma in an ongoing trial on recurrent high-grade glioma patients (NCT06072586). Initial results of measured total and unbound drug levels in the gadolinium-enhancing and non-enhancing brain tumor regions indicate significant brain penetration ability of BDTX-1535 in high-grade glioma patients.

#5748 Metabolites of the orally potent anticancer agent and endoplasmic reticulum stress inducer RM-581.

Donald Poirier[1], Martin Jutras[2], Rene Maltais[1], Jenny Roy[1], Maude Fleury[1], Camille Gagne[1]

[1]CHU de Quebec Research Center-Universite Laval, Quebec, QC, Canada,[2]Universite de Montreal, Montreal, QC, Canada

The development of new and more effective chemotherapy agents is a biomedical research priority, especially for cancer, still one of the deadliest diseases. To progress towards clinical phase studies with RM-581, an aminosteroid derivative producing promising in vitro and in vivo results against several types of cancers, including some with a poor prognosis such as pancreatic, prostate and breast cancers, it was necessary to address its metabolic stability. Analysis by ultra high-pressure liquid chromatography (UPLC) quadrupole time-of-flight (QTof)-mass spectrometry (MS) showed the gradual disappearance of RM-581, making it possible to determine a half-life of 14 and 15 min in concentrated preparations of mouse and human liver microsomes, respectively, and the presence of 6 metabolites in varying proportions (M1 to M6). The MS/MS spectra of metabolites were investigated to assign sub-structures to fragment ions. These analyses made it possible to conclude that the amino acid proline in the side chain at position C2 of the steroid mestranol was the main site of metabolism and the following mass shift relative to the parent compound were observed for each metabolite relative to RM-581: M1 (+34), M2 (-2), M3 (+16), M4 (+18), M5 (+32) and M6 (+16). Suspecting the formation of RM-581 analogs whose pyrrolidine ring of proline has been opened, we synthesized an aminosteroid where the proline was replaced by a 2-amino-5-hydroxypentanoic acid. Starting from steroid estrone, 4 steps were needed to introduce a piperazine at position C2, an ethynyl at C17α and to methoxylate the OH at C3. The key Fmoc and TBS-diprotected amino acid was then added by a coupling reaction, the Fmoc protecting group removed before adding the quinaldic acid. A cleavage of the TBS protecting group then allowed to obtain the targeted RM-581 analog. After a full chemical characterization of this compound, these chromatographic and MS properties made it possible to formally identify the major metabolite M4 ($M+H^+$ = 665.3684; Rt = 9.11 min) as a primary alcohol derivative. This result also indirectly supports the proposed structures of the other metabolites M6 (aldehyde), M5 (carboxylic acid) and M1 (primary alcohol or oxygen on the quinoline ring). When determining the concentration inhibiting 50% of cell proliferation (IC50) on 8 human leukemia and pancreas cancer cell lines, M4 (IC50 = 17-454 µM) was found to be much less potent than RM-581 (IC50 = 0.78-7.55 µM). The loss of efficiency ranged from 16 to 111-fold, according to cell lines. Furthermore, only M4 was observed when RM-581 was administered by gavage to mice at a 30 mg/kg dose with the plasma analyzed 2 h post-administration. The glucuronidated form of M4 was also detected at 2 h, suggesting the RM-581 elimination pathway and the fact that it does not accumulate in the blood.

#5749 Determination of the mTOR inhibitor temsirolimus and its active metabolite sirolimus in human blood, plasma, cerebrospinal fluid, and brain tumor tissue using a validated LC-MS/MS assay.

Gerold S. Kaplan, Tigran Margaryan, Nader Sanai, **Artak Tovmasyan**

Ivy Brain Tumor Center, Barrow Neurological Institute, Phoenix, AZ

Temsirolimus is an FDA approved mTOR (mammalian target of rapamycin) inhibitor that is presently under investigation in multiple clinical trials for various cancers, including for recurrent high-grade glioma. Here, we report A robust, sensitive, and extremely rapid liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the quantification of total concentrations of temsirolimus and its active metabolite sirolimus in human blood, plasma, brain tumor and cerebrospinal fluid (CSF) samples. The method was validated for the simultaneous determination of both analytes over a concentration range of 1-1000 nM within a total run time of 2.6 min. Simple one-step protein precipitation with methanol was employed to extract analytes from biomatrices. Chromatographic separation of analytes was achieved on Kinetex™ EVO C18 (50 × 2.1 mm, 1.7 µm) column using gradient elution with mobile phases of 5 mM ammonium formate and methanol at a flow rate of 0.5 mL/min and an injection volume of 5 µL. Detection was performed with a Sciex QTRAP 6500+ mass spectrometer, utilizing multiple reaction monitoring mode to register the precursor-to-product ion transitions under positive electrospray ionization. The maximum coefficient of variation for both intra- and inter-day precision was 12.3%, with accuracy ranging from 85.6% - 114.2% in all matrices. Combined temsirolimus and sirolimus samples were determined to be stable at room temperature (RT) in whole blood and brain homogenate for at least 4 hours and in plasma for at least 1 hour. At -80±15°C in a freezer, samples in plasma, brain homogenate, and whole blood were stable for at least 132 days. Stock and working solution stability was also evaluated to be stable for at least 4 hours (RT) and 113 days (-20°C nominal for stock solutions and 4°C for working solutions). The validated method is currently employed in a clinical trial evaluating the efficacy of super-selective intra-arterial infusion for the CNS delivery of temsirolimus in recurrent high-grade glioma patients (NCT05773326) to determine temsirolimus and sirolimus pharmacokinetics in blood, brain tumor, plasma, and CSF.

#5750 Discovery of GSK4418959 (IDE275): A novel, non-covalent, reversible Werner Helicase inhibitor and a new potential therapeutic for the treatment of MSI-H cancers.

Joshua P. Taygerly[1], **Michael P. DeMartino**[2], Brian T. Jones[1], Edward J. Brnardic[2], Joshua E. Cottom[2], Yang Lee[3], Diana M. Munoz[1], Claire Neilan[1], Sunjay Sethi[1], Robert A. Reid[2], Apirat Chaikuad[4], Lisa M. Shewchuk[2], Ethan McSpadden[1], Daniel L. Severance[1], Kira Campbell[2], Philip Landis[2], Jay P. Jain[1], Richard Zhang[1], Leng Nickels[2], Dennis K. Murphy[2], Hans C. Eberl[5], Muzaffar Alam[1], Melissa Fleury[1], Lara K. Leister[2], Tessa Lynch-Colameta[2], James P. Phelan[2], Michael D. VanHeyst[2], Amberly B. Sanford[2], Ann M. Rowley[2], Hongyi Yu[2], Anna Rutkowska-Klute[5], Thilo Werner[5], Stephan Gade[5], Damian Hruszkewycz[2], Tyler Higgins[2], Xin Linghu[1], Ian S. Young[1], An D. Nguyen[1], Sabrina Bedard[2], Eldridge N. Nartey[2], Nanhua Deng[3], Yang Peng[2], Jessica L. Schneck[2], Geeta Sharma[3], Yanhua Rao[2], Paul A. Barsanti[1], Michael A. White[1], Benjamin Schwartz[3]

[1]Ideaya Biosciences, San Francisco, PA,[2]GSK, Upper Providence, PA,[3]GSK, Cambridge, MA,[4]GSK, Stevenage, United Kingdom,[5]GSK Cellzome GmbH, Heidelberg, Germany

Recent large-scale genome wide CRISPR screens identified Werner (WRN) Helicase as uniquely essential to cell survival specifically in the microsatellite instability-high (MSI-H) cancer setting. This synthetic lethal relationship elevated WRN as an attractive precision therapeutic target for the treatment of MSI-H cancers, irrespective of tumor type. High-throughput screening identified a hit characterized by low potency inhibition of ATPase function. This poster describes the systematic optimization of this hit resulting in the discovery of clinical candidate GSK4418959, a novel, potent and selective small molecule that binds to WRN in an allosteric pocket and inhibits ATPase and DNA unwinding functions in an ATP-competitive manner. GSK4418959 phenocopies the genetic silencing studies in cells and organoids and accordingly, elicits complete tumor regression in murine xenograft MSI-H models (CDX and PDX) following oral dosing. Importantly, no response is observed in analogous microsatellite stable (MSS) models, exemplifying the precision effect of the WRN inhibition mechanism. GSK4418959 will be investigated as a potential treatment for patients with MSI-H cancer, including those who have been treated with and failed at least one line of checkpoint immunotherapy for their advanced disease.

CHEMISTRY: Multi-omics for the Cancer Proteome
Poster Session

#5753 Multi-omic analysis links neighborhood disadvantage to elevated stress hormones and tumorigenic markers in ER+ breast cancer patient plasma and tumor samples.

Hannah Heath[1], Oana C. Danciu[2], Garth Rauscher[2], Natalie Pulliam[3], Elona Liko-Hazizi[3], Sarah Friedewald[3], Seema Khan[3], Julie Kim[3], William Gradishar[3], Jonna Frasor[2], Kent F. Hoskins[2], Zeynep Madak-Erdogan[1]

[1]University of Illinois at Urbana-Champaign, Champaign, IL,[2]University of Illinois at Chicago, Chicago, IL,[3]Northwestern University, Chicago, IL

Intro: Estrogen receptor positive (ER+) breast cancer (BC) patients living in disadvantaged neighborhoods experience worse survival compared with patients in advantaged neighborhoods. Chronic stress, indicated by elevated cortisol levels, may mediate this disparity by dysregulating inflammatory responses driving BC tumorigenesis. However, the role of cortisol in ER+ BC remains underexplored.
Objective: To explore associations between pre-treatment plasma cortisol, inflammatory proteins, tumoral gene expression changes, and neighborhood disadvantage in women with primary ER+ BC compared to cancer-free controls.
Methods: Pretreatment plasma samples were collected from women with primary ER+ BC (n=98) and cancer-free controls (n=144). Tumor samples were obtained from a subset of cases (n=77). Samples were from three Chicago hospitals serving disadvantaged or advantaged neighborhoods, as determined by Community Needs Assessment reports. Plasma samples were analyzed via a steroid hormone metabolomics assay and Olink Proteomic's inflammatory proteins panel. Gene expression in tumor samples was analyzed via the Breast Cancer 360 Nanostring panel. Mixed models, adjusted for age and BMI, compared: plasma samples from disadvantaged cases (n=48) vs. controls (n=114), advantaged cases (n=50) vs. controls (n=27), advantaged (n=27) vs. disadvantaged (n=117) controls, and advantaged (n=50) vs. disadvantaged (n=48) cases, and tumor samples from advantaged (n=44) vs. disadvantaged (n=33) cases.
Results: Thirteen steroid hormones, 85 plasma proteins, and 759 tumor tissue genes were detected. Among cases vs. controls, cortisol-related metabolites (cortisol/cortisone ratio, 11-deoxycortisol, 17a-OH-progesterone, androstenedione) were elevated in cases ($p<0.05$). The cortisol/cortisone ratio remained significantly elevated in disadvantaged cases vs. controls ($p<0.01$) but not advantaged cases vs. controls.
In disadvantaged individuals, 62 inflammatory proteins were elevated ($p<0.05$). Forty overlapped between controls-only and cases-only analyses, while 21 were unique to cases, including five weakly correlated with the cortisol/cortisone ratio ($p<0.05$). Seventy-six genes were upregulated in tumors from disadvantaged vs. advantaged cases, including those involved in cell cycle regulation, proliferation, and HIF-1 signaling. Motif analyses showed NFKB as a top motif ($p<0.01$), suggesting dysregulated cortisol signaling. Of the 45 downregulated genes, many were involved in anti-inflammatory pathways ($p<0.05$).
Conclusion: BC diagnosis in disadvantaged individuals is associated with elevated cortisol, potentially contributing to elevated circulating inflammatory proteins and intratumoral gene expression changes that promote tumor progression.

#5754 An LC-MS toolbox for identifying and guiding the development of cysteine-targeted small molecule covalent inhibitors.

Kurt Baltier, Alison Erickson, Craig R. Braun, Ryan Kunz, **Brian Erickson**

IQ Proteomics LLC, Framingham, MA

Covalent inhibitors are a powerful class of therapeutic agents, and have gained particular traction with oncogenic targets. FDA-approved covalent inhibitors have been introduced for treatment of a range of cancers, including mantle cell lymphoma (ibrutinib), non-small cell lung cancer (osimertinib, sotorasib, afatinib), and breast cancer (neratinib). Notably, the recent surge in preclinical covalent inhibitor pipelines across biotech and pharma highlights the methodological and technological advancements in covalent inhibitor research and development. Owing to the mechanism of action of this compound class, mass spectrometry is uniquely positioned as an ideal analysis tool for identification, optimization, and mechanistic characterization of covalent inhibitors across the entire developmental cycle of these therapeutics from library screening to clinical evaluation. IQ Proteomics leverages mass spectrometry to empower biotechnology and pharmaceutical companies as a contract research organization, and has developed a comprehensive, turn-key LC-MS based pipeline for supporting the preclinical development of covalent inhibitors. We present a case study exemplifying this LC-MS pipeline centered on the clinically approved KRAS inhibitor sotorasib. We first describe a high-throughput, proteome-wide cysteine ligandability assay ideally suited for initial identification of library hits and SAR evaluation of preliminary lead compounds, and demonstrate the complimentary utility of multiomics characterization (proteome expression profiling, PTM profiling, etc) to global cysteine profiling data. We further present an optimized targeted LC-MS (PRM) workflow for evaluating target-engagement both indirectly (disappearance of unmodified cysteine peptide) and directly (appearance of adduct peptide) for characterization of preclinical lead compounds over large numbers of in vitro and in vivo samples. We finally evaluate an isobaric multiplexed assay for rapid target engagement characterization of covalent inhibitors in complex matrices. Together, this suite of template assays represents a set of powerful tools for covalent inhibitor discovery, optimization, and therapeutic evaluation that empowers the oncology community as exciting covalent modalities continue to emerge with the potential to treat an increasing number of diseases.

#5755 Expansion of the known functional diversity of human T cells achieved due to the unprecedented resolution of intracellular proteins by mass cytometry (CyTOF).

Laura Polanco[1], Michael J. Cohen[2], Erika L. Smith-Mahoney[1], Ling Wang[2], David King[2], Madison Bailey[1], Christina Loh[2], Anna C. Belkina[1], Amedeo J. Cappione[2], Jennifer E. Snyder-Cappione[1]

[1]Boston University Chobanian & Avedisian School of Medicine, Boston, MA,[2]Standard BioTools, Markham, ON, Canada

Measuring the functional signatures of immune cells comprehensively, spanning the inflammatory (Th1/Th17) and immunosuppressive (Th2/Treg) lineages, provides key insights into several facets of cancer research and therapy. Single-cell detection of cytokines from diverse functional lineages is required to determine mechanisms underlying success/failure of checkpoint blockade, define immunosuppressive activity of the tumor-resident cell subsets and identify immunological biomarkers that predict clinical outcomes. Powerful cytometric research tools, such as fluorescence cytometry, enable important intracellular measurements of functional potential, such as cytokines, phosphorylation events and transcription factors in single cells. However, expanding the number of targets detected per cell by these methods has limitations in commercial availability of compatible conjugates and the technology's resolving capacity for rare subsets, specifically due to spectral signal overlap and autofluorescence.
Many readouts, including cytokines of the Th2/Treg lineages (IL-5, IL-10 and IL-13), have also been notoriously difficult to reproducibly detect in human cells using fluorescence-based cytometry. We predicted that mass cytometry may overcome such limitations and enable better signal resolution for such applications. We evaluated three small (11-12-plex) panels using a full-spectrum flow cytometer and the CyTOF™ XT mass cytometer to address this. Each panel was comprised of surface and intracellular analytes (cytokines, phospho-epitopes or transcription factors) and designed to minimize potential impact of spectral overlap on the resolution of spectral flow data. PBMC were stimulated, split and stained with either fluorochrome- (Cytek Aurora) or metal-conjugated antibodies (CyTOF). Datasets were analyzed by PhenoGraph clustering and visualized with opt-SNE to determine cellular functional diversity.
Overall, data collected on the CyTOF XT system demonstrated superior resolution for many intracellular readouts, including cytokines IL-10 and IL-13, with stimulation-specific events only detected using CyTOF technology. Additionally, improved signal:noise (S/N) provided better resolution of phospho-activation events and transcription factor expression, particularly TOX and Tbet. Further, better S/N with CyTOF technology enabled more accurate population clustering using PhenoGraph, and more distinct functional signatures resulted from mass cytometry datasets compared with fluorescent counterparts. CyTOF XT mass cytometry platform clarifies understanding a sample's immune signatures in unsupervised analysis. Our findings indicate that the CyTOF XT platform could serve as a catalyst for seminal discoveries in immune profiling to drive therapeutic design and advanced disease monitoring in cancer.

#5756 Quantification of SHetA2 in patient-derived ovarian cancer spheroids to study drug uptake and resistance in a physiologically relevant model.

Annelise Huynh[1], Amit Singh[1], Zongkai Peng[1], Doris M. Benbrook[2], Anthony W. G. Burgett[2], Zhibo Yang[1]

[1]Department of Chemistry and Biochemistry, University of Oklahoma, Norman, OK,[2]Department of Obstetrics & Gynecology, University of Oklahoma Health Sciences Center, Oklahoma City, OK

Ovarian cancer presents therapeutic challenges, particularly in advanced stages, where malignant ascites—a buildup of fluid in the abdominal cavity—complicates treatment. Within this ascitic environment, clusters of cancer cells, known as spheroids, form, proliferate, and implant upon various organs, contributing to the metastatic nature of ovarian cancer. As they limit drug penetration and have a hypoxic environment that fosters angiogenesis, spheroids exhibit significant chemoresistance, amplified by the inflammatory and tumor-promoting microenvironment (Peng). Therefore, to reveal the potential mechanisms of chemoresistance in ovarian cancer, directly targeting these spheroids is crucial to develop effective therapies that could help reduce the high mortality rate associated with ovarian cancer. ShetA2 (Sulfur Heteroarotinoid A2) is a novel, promising anticancer drug that exhibits unique potential by inducing apoptosis in cancer cells and disrupting mitochondrial pathways without harming healthy cells (Benbrook). Currently in phase 1 clinical trials, it is vital to understand its efficacy and optimize its therapeutic potential. However, conventional quantification methods for SHetA2 primarily rely on plasma analysis in animal models or human samples, providing only indirect measures and failing to replicate microenvironmental factors in the tumor's 3D structure that inhibit drug access to spheroids (Sharma et al.). Consequently, a direct approach to measure drug concentration in single spheroids is necessary to understand the pharmacodynamics and pharmacokinetics of anticancer drugs (Peng). To address this gap, we developed a highly sensitive nano-flow liquid chromatography-mass spectrometry (nanoLC-MS) method for quantification of SHetA2 in patient-derived spheroids treated ex vivo with cell free-ascites. First, spheroids are washed and lysed. Second, an isotopically labeled compound d4-SHetA2 with known concentration is added to the lysate as the internal standard. Third, a C18 ZipTip is used to clean and desalt the sample to remove competing ions that could reduce detection sensitivity. Lastly, the prepared samples are analyzed using flow ultra-high-performance liquid chromatography (UHPLC) coupled with a high-resolution Thermo Orbitrap mass spectrometer. This integrated approach will provide highly sensitive and accurate quantification of SHetA2 at low concentrations, offering insights into its distribution, penetration, and activity within single patient-derived spheroids. Furthermore, by enabling a more precise and clinically relevant analysis of SHetA2's efficacy in targeting spheroids, this approach will help establish robust criteria for dose selection and scheduling intervals in clinical applications.

#5757 Identification of breast cancer-associated changes to the Rab11 interactome.

Jessica Contreras, Joseph Kainov, Yaneli Betancourt, Oly Khowash, Charlie Yang, Erin N. Howe

Proteomics and Molecular Therapeutics, Rosalind Franklin Univ. of Med. & Science, North Chicago, IL

Breast cancer is the second leading cause of death for American women. Recent advances have improved patient outcomes, but metastatic disease continues to be devastating, and its mechanisms are not fully understood. We made the exciting discovery that Rab11b, a small GTPase that controls endosomal recycling, is required for breast cancer brain metastatic outgrowth, through control of the cell surface proteome. Rab11b interacts with numerous proteins, including GTPase activating proteins (GAPs), guanine exchange factors (GEFs) and motor proteins to facilitate cargo recycling and control the cell surface proteome. We found that Rab11-mediated recycling is dependent on the cellular context, and hypothesize that these changes are driven by dynamic, microenvironment-mediated changes to the Rab11 interactome. To elucidate these changes, we used a proximity labeling approach to identify the Rab11 interacting proteins (the interactome). We used the promiscuous biotin ligase, TurboID, for proteomic analysis of protein-protein interactions. TurboID confers the advantage of detecting highly dynamic changes characteristic of GTPases. To identify the full range of changes to the Rab11 interactome, we are using HEK-293 cells to identify the canonical interactome, immortalized normal breast epithelial cells and triple negative breast cancer cells to identify the breast-specific Rab11 interactome. We have successfully expressed wild-type, dominant negative and constitutively active Rab11a and Rab11b constructs, and shown that they co-localize with endogenous proteins and that biotinylation is indeed spatially restricted. Proteomics studies identified known Rab11 interacting proteins, confirming the reliability of our approach. Importantly, we also identified differences in the canonical and breast cancer-associated Rab11 interactomes, supporting our hypothesis that recycling is context dependent. Current work is focused on validating two such proteins found to be differentially interacting with Rab11 in the different cellular contexts, AnnexinA1 and AnnexinA2. The annexins are phospholipid binding proteins that have a range of functions dependent on cellular location including roles in inflammation and cell trafficking. They've been historically implicated in tumorigenesis, though their functions as tumor suppressors or promoters vary greatly depending on tumor type, subtype, and even stage. Future work will focus on further elucidating the interaction between AnnexinA1/A2 and Rab11 in distinct metastatic microenvironments.

#5758 Identification of MUC13 mediated molecular interactions in pancreatic cancer cells using immunoprecipitation combined with liquid chromatography mass spectrometry analysis.

Anupam Dhasmana, Swati Dhasmana, Rajasekhar Baru, Abigail Gomez, Sheema Khan, Murali M. Yallapu, Subhash Chauhan

Medicine and Oncology ISU, University of Texas Rio Grande Valley, McAllen, TX

Background: MUC13, a recently identified oncoprotein, exhibits aberrant overexpression in pancreatic ductal adenocarcinoma (PDAC) and plays a crucial role in cancer progression. However, the precise molecular interactions mediated by MUC13 that contribute to PDAC progression and metastasis remain incompletely understood. To address this gap, we employed a comprehensive molecular interaction study using an integrated approach combining immunoprecipitation and Liquid Chromatography-Mass Spectrometry (IP-LCMS). This unique study revealed a novel cohort of MUC13-interacting proteins, potentially driving its oncogenic functions in PDAC.
Methodology & Results: Five PDAC cell lines (CaPan2, HPAF-II, HPNE, Panc1, and MiaPaCa) were used for this analysis. Initial LCMS profiling showed high MUC13 expression in HPAFII, moderate levels in CaPan2, and minimal to undetectable expression in Panc1, MiaPaca, and HPNE (normal pancreatic cells). To validate these findings, ELISA, confocal microscopy, and immunoblotting complementary analyses were performed. immunoprecipitation (IP) was performed with MUC13-positive (MUC13+, HPAF-II) and MUC13-negative (MUC13~~-~~, Panc1) cells with our in-house generated unique MUC13 monoclonal antibodies and appropriate IgG control antibodies. The immunoprecipitated proteins were processed for LCMS analysis. This IP-LCMS analysis demonstrated a robust channel signal for MUC13 in positive controls, whereas negative controls yielded minimal or non-significant signals. After normalization across experimental conditions, a total of 54 unique MUC13-interacting proteins were identified. Functional enrichment analysis highlighted the involvement of MUC13 associated interactome with various oncogenesis mechanisms, including central carbon metabolism, PD1/PD-L1 expression and immune checkpoint pathways, adherent junctions, senescence, cell cycle, apoptosis, viral carcinogenesis, diabetic cardiomyopathy etc.
Conclusion: This study established a robust methodology (IP-LCMS) to identify MUC13 interactome, unveiling a cohort of molecular partners potentially mediating MUC13's oncogenic functions in PDAC cells. These findings provide critical insights into MUC13-mediate oncogenic pathways, underscoring its role in cancer progression and therapeutic resistance. This work paves the new way for novel therapeutic strategies targeting precise MUC13-mediated molecular interactions. Key Word: MUC13, Pancreatic Cancer, IP-LCMS, Interactome, Functional Enrichment

#5759 Unraveling the roles of mTORC2 signaling in promoting aggressive glioblastoma characteristics through multi-omics analysis.

Nuttiya Kalpongnukul[1], Rungnapa Bootsri[2], Gea A. Ecoy[2], Tankun Bunlue[2], Napatsakon Youngsanbhu[2], Chatchapon Chuenjit[2], Nuanwan Songcharoen[2], Trairak Pisitkun[1], **Naphat Chantaravisoot**[2]

[1]Research Affairs, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand,[2]Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

The mechanistic target of rapamycin complex 2 (mTORC2) is a pivotal component of the mTOR signaling pathway, regulating critical cellular processes such as growth, proliferation, differentiation, survival, motility, and metabolism. Notably, mTORC2 facilitates actin cytoskeleton reorganization, cell motility, survival, and DNA damage repair. Aberrations in mTORC2 signaling are strongly associated with aggressive features of cancers, including glioblastoma (GBM), driving metastasis, drug resistance, recurrence, and tumor progression. However, unlike mTORC1, the molecular mechanisms governing mTORC2 functions remain less understood. To address this gap, we employed a comprehensive multi-omics approach—integrating transcriptomics, proteomics, phosphoproteomics, and interactomics—to investigate mTORC2-driven mechanisms underlying GBM aggressiveness. By leveraging computational big data analyses, we aimed to uncover therapeutic targets, identify biomarkers, and explore chemotherapy resistance mechanisms. Our findings reveal that mTORC2 signaling is intricately linked to key GBM hallmarks, including enhanced invasion, metastasis, genome instability, deregulated metabolism, and phenotypic plasticity. Disrupting mTORC2 profoundly altered intracellular regulatory mechanisms controlling these traits. Importantly, our analyses identified key molecular players driving these processes. Furthermore, we demonstrated that ATP-competitive inhibitors of mTOR kinase significantly enhanced the efficacy of temozolomide in GBM spheroid models, offering a promising strategy to overcome chemoresistance. These findings highlight the potential of targeting mTORC2-mediated pathways to develop more effective therapeutic strategies for aggressive cancers.

#5760 Mass spectrometry-based proteomic investigation of patient-derived tumoroids employing fast, high-resolution accurate mass technologycatalyzes precision oncology and drug discovery.

Dominique B. Figueroa[1], Kevin Yen-Yu Yang[1], Colin Paul[2], Pradip Shahi Thakuri[2], Anthony Chatman[2], Brett Larsen[1], Julian Saba[1], Tonya Pekar Hart[1], Amirmansoor Hakimi[1]

[1]Thermo Fisher Scientific, San Jose, CA,[2]Thermo Fisher Scientific, Frederick, MD

The inherent heterogeneity of cancer has spurred significant technological advancements to enhance the detailed study required to characterize this complex disease, predominantly defined by uncontrolled changes. Efforts to address and treat this multi-faceted variability are demonstrated by use of patient-derived tumoroids, which model the conditions and complexities observed in patient tumors using an in vitro cell culture system. Despite the strong correlation between patient response and that observed in matched tumoroid models, tumoroid technology has suffered from a lack of standardization in both culture methods and characterization, hindering widespread clinical translation. This work seeks to present robust and standardized methods for the generation and characterization of tumoroid models. Specifically, our methods aim to reduce variability and labor across workflows and spur the adoption of next-generation tumoroid culture and mass spectrometry methods in discovery and development research. Colorectal, lung, breast, and endometrial tumoroids were derived from donor cancer samples in OncoPro™ Tumoroid Culture Medium, a serum-free, conditioned medium-free system with broad applicability across solid cancer indications. After tumoroid cell pellets were collected, samples were digested using the AccelerOme automated sample preparation platform . Peptides were then loaded onto an IonOpticks Aurora Frontier TS 60×75 column, and separated using Vanquish Neo UHPLC system. Data was acquired on anOrbitrap Astral mass spectrometer in narrow window data-independent acquisition (DIA) mode, and the resulting raw fileswere analyzed with Proteome Discoverer using the CHIMERYS™ intelligent search algorithm or Spectronaut with 1% FDR control at the peptide-spectrum match (PSM) and protein level. Proteins identified in the breast cancer model in our experiments were linked to MAPK, ALK, and ERBB2 signaling pathways, as well as protein and RNA metabolism. The next steps in our experiments involve validating the identified proteins in the breast cancer tumoroid model, and understanding the underlying mechanisms to assess the model's efficacy. Future directions will leverage the unique capabilities of mass spectrometry-based proteomics to investigate post-translational modifications, including phosphorylation and glycosylation, which are crucial in signaling and cancer. By integrating cancer tumoroid models, serum-free media, and the Orbitrap Astral mass spectrometer, we aim to streamline precision oncology and drug discovery by minimizing variability in these dynamic models.

#5761 Unbiased phosphoproteomic and metabolic profiling in WT and KRAS mutant colorectal cancer cells reveals molecular interplay between protein regulation and metabolic rewiring.

Ethan Stancliffe[1], Tom Cohen[1], Ashima Mehta[1], Cassandra Kempf[2], Adam Richardson[1], Monil Gandhi[1], Douglas V. Guzior[1], Kevin Cho[2], Gary J. Patti[2]

[1]Panome Bio, St. Louis, MO,[2]Washington University in St. Louis, St. Louis, MO

Introduction: KRAS mutations cause significant metabolic and protein dysregulation in cancer, leading to alterations in cellular signaling pathways, promoting uncontrolled cell growth and proliferation. The metabolic reprogramming of altered KRAS function includes increased glucose, amino acid, and lipid metabolism. The changes in the cellular proteome stem from modifications in protein expression and phosphorylation. Understanding the interplay between these metabolic and protein-level alterations is crucial for developing targeted therapeutic strategies against KRAS-driven cancers. Accordingly, we performed an integrated metabolomic and phosphoproteomic analysis of HCT116 cells with and without a KRAS mutation.
Experimental: HCT-116 cells containing wildtype (WT, n=5) and mutant KRAS (MUT, n=5) were profiled to assess metabolite, protein, and phosphoprotein levels between WT and MUT cells. Metabolomic profiling was completed with LC/MS to capture polar and lipid metabolites. Data was processed through an in-house untargeted metabolomic analysis pipeline. For phosphoteomic analysis, enriched phosphopeptides and flow-through peptides were analyzed with DIA LC/MS/MS. The resulting data was processed with DIA-NN and combined into aggregated protein and phosphosite profiles.
Results: The multi-omic analysis profiled 2,025 metabolites, 6,654 proteins, and 10,034 phosphosites. When considering each data type individually, all three analyte types seperated the WT and MUT cells through PCA analysis, underscoring the magnitude of dysregulation from KRAS mutation. When considering the metabolites, proteins, and phosphosites that reached statistical significance, 85 metabolites and 145 proteins were differentially abundant. Strikingly, 1,036 phosphosites were unique to either KRAS and WT samples, with ~800 of these being unique to KRAS samples. The results of a joint pathway analysis identified >70 pathways that reached significant enrichment ($p<0.05$) and had support at both the protein, metabolite, and phosphosite level. The most enriched pathway was RAS signaling. Other enriched pathways include selenocysteine synthesis, sialic acid metabolism, ROS detoxification, and respiration.
Conclusions: This study showed extensive differential phosphorylation in KRAS mutant cells, suggesting a widespread rewiring of signaling networks thatimpacts diverse cellular functions. The multi-omic approach taken enabled the identification of previously known and novel pathways affected by KRAS signaling, offering potential new targets for therapeutic intervention in KRAS-driven cancers.

#5762 RPPA kinomic profiling of a tumor eradication phenotype induced by SIAH inhibition, a major tumor vulnerability in the EGFR/HER2/RAS signaling pathway, in late-stage and metastatic human cancer cell lines.

Jonathan M. Baker[1], Andrew Howell[2], Daniel J. McWilliams[1], Ashleigh E. Hannah[1], Julia Wulfkuhle[3], Rosa Gallagher[3], Emanuel Petricoin III[3], Amy H. Tang[1]

[1]Department of Biomedical and Translational Sciences, Old Dominion University, Norfolk, VA,[2]Old Dominion University, Norfolk, VA,[3]Center for Applied Proteomics and Molecular Medicine, George Mason University, Manassas, VA

Introduction: Persistent activation of the EGFR/K-RAS pathway is a major driver of tumor malignancy. Therapies attempting to contravene intertwined RAS effector pathways have yet to achieve durable antitumor efficacy. Seven in absentia homologues ($SIAH_1$ and $SIAH_2$) are RING-domain E3 ubiquitin ligases that function as the most downstream signaling gatekeeper of the EGFR/K-RAS pathway. We showed that SIAH inhibition led to tumor eradication against late-stage human cancer cell lines in xenograft mouse models. We propose that SIAH is a major tumor vulnerability whose complete inhibition impedes EGFR/K-RAS pathway activation driving cancer progression. We aim to elucidate the molecular mechanisms underpinning the curative efficacy of our anti-SIAH strategy.

Methods: Reverse phase protein arrays (RPPA) and principal component analysis (PCA) were used to quantify the changes of 300 proteins/phospho-proteins that were significantly up- or down-regulated in response to SIAH blockade. Independent RPPA assays were performed in triplicate on doxycycline (DOX)-inducible MiaPaCa, MDA-MB-231, MDA-MB-468, HeLa, and A549 cell lines in which our SIAH inhibitor, $SIAH_2^{PD}$, expression was controlled by a Tet-ON/OFF system. Four experimental conditions were used: Tet-ON control cells without DOX (group A) and with DOX induction (group B), Tet-ON-$SIAH_2^{PD}$ cancer cells without DOX (no $SIAH_2^{PD}$ expression in group C) and with DOX induction ($SIAH_2^{PD}$ expression in group D). The ratios of D/C/B/A, D/C, D/B, C/A, and B/A were calculated in a pairwise comparison after normalization to GAPDH. Western immunoblotting (WB), immunofluorescence staining (IF), and flow cytometry of the Tet-ON/OFF cancer cells and protein extracts were analyzed at 3-, 5-, and 7-days (+/-) DOX induction in triplicate to validate the results. $SIAH_2^{PD}$-induced protein expression alterations were normalized to β-Actin or α-Tubulin, and the standardized data were quantified. Statistical analyses were performed by one-way ANOVA using GraphPad Prism software.

Results: We identified the altered expression of NFκB, Caspase-3, Caspase-7, and PARP in response to $SIAH_2$ blockade, with independent confirmation by RPPA, WB, and flow cytometry, supporting their roles as a mechanistic readout of SIAH inhibition in human cancer cells.

Conclusion: RPPA cancer pathway mapping provides molecular insights into cancer network rewiring in response to SIAH blockade. This study supports our innovative strategy to design anti-SIAH-based, anti-EGFR/K-RAS targeted therapies to control and eradicate late-stage and incurable human cancers in the future.

#5763 How cancer drugs work, and fail: proteome-wide cellular thermal shift assay (CETSA) to define cellular mechanism of action and resistance of clinical cancer drugs.

Par Nordlund[1], Sara Loof[1], Anderson Ramos[1], smaranda Bacanu[1], Marc Antoine Gerault[1], Ying Yu Liang[2], Wai Leong TAM[2], Nayana Prabhu[2]

[1]Karolinska Institutet, Stockholm, Sweden,[2]Agency for Science, Technology and Research, Singapore, Singapore

Resistance to cytotoxic cancer drugs is considered a key cause of cancer deaths worldwide. Although extensive understanding of cancer drug mechanisms of action (MoA) and resistance has been derived through focused research, our understanding of these process still remains fragmented.
The Cellular Thermal Shift Assay (CETSA) was originally developed in our lab for accessing drug target engagement in intact cells (Martinez Molina, 2013), and is now widely used for this application in academia and industry. However, CETSA can also detect modulations of cellular biochemistry (Dai, 2018), e.g. effects on downstream-of-target pathways, although this application of CETSA remains relatively unexplored. Using a comprehensive and highly sensitive mass-spectrometry implementation of CETSA, we have studied the cellular biochemistry induced by more than 50 clinical cancer drugs. By applying a systematic approach, including time dependent responses to dissect sequential pathway activation, we can confirm components of previously known MoAs for these drugs but also provide comprehensive new insights into their MoA (Ramos 2024). Moreover, parallel studies in sensitive and resistant cancer cell lines provide differential pathway activation in sensitive and resistant cells (Liang, 2021), giving new insights into how cancer cells acquire drug resistance at the biochemical level. Together our data illustrate that CETSA can yield valuable information on modulation of downstream-of-target biochemistry during cancer drug action, information that is not easily accessed with other omics methods. Proteins discovered in some specific pathways also constitute new candidate drug targets for attenuate resistance responses, as well as potential clinical biomarkers.
References: - Martinez Molina D, Jafari R, Ignatushchenko M, Seki T, Larsson EA, Dan C, Sreekumar L, Cao Y and Nordlund Monitoring drug target engagement in cells and tissues using the cellular thermal shift assay. *Science*, 2013 341:84.- Dai L, Zhao T, Bisteau X, Sun W, Prabhu N, Lim YT, Sobota RM, Kaldis P, Nordlund P. Modulation of Protein-Interaction States through the Cell Cycle. *Cell,* 2018 173(6):1481-1494. - Liang YY, Bacanu S, Sreekumar L, Ramos AR, Dai L, Michaelis M, Cinatl J, Seki T, Cao Y, Coffill CR, Lane DP, Prabhu N, Nordlund P, CETSA interaction proteomics define specific RNA modification pathways as key components of fluorouracil-based cancer drug toxicity. *Cell Chemical Biology,* 2021, S2451-9456,00306.-Ramos AD, Liang YY, Surova O, Bacanu S, Gerault M, Mandal T, Ceder S, Langebäck A, Österroos A, Ward GA, Bergh J, Wiman KG, Lehmann S, Prabhu N, Lööf S, Nordlund P. Proteome-wide CETSA reveals diverse apoptosis-inducing mechanisms converging on an initial apoptosis effector stage focused at the peripheral region of the nucleus. Cell Reports, 2024 Oct 22;43:114784.

#5764 The emerging protective role of silymarin in stress associated hepatocellular carcinoma.

Mohammad S. Hussain[1], Yamile Abuchard Anaya[1], Ricardo Pequeno Bracho[1], Amayrani Sanchez[1], Sophia Leslie[1], Kyle Doxtater[1], Subhash C. Chauhan[1], Tamer Oraby[2], Elias George[3], Manish K. Tripathi[1]

[1]Medicine and Oncology ISU, University of Texas Rio Grande Valley, McAllen, TX,[2]School of Mathematical and Statistical Sciences, University of Texas Rio Grande Valley, Edinburg, TX,[3]Clinical Support Services, University of Texas Rio Grande Valley, Edinburg, TX

Background: Texas is projected to have the second-highest liver cancer-related deaths in the nation, with Hispanic populations experiencing the highest mortality rates. In the South Texas Rio Grande Valley (RGV), where ~90% of the population is Latino/Hispanic, socioeconomic disparities, poverty, obesity, diabetes, and low-income levels contribute to heightened stress prevalence. Stress accelerates cancer progression by impairing immune responses, inducing inflammation, and activating the hypothalamic-pituitary-adrenal (HPA) axis, releasing cortisol and leptin, key drivers of oncogenesis. Silymarin, a polyphenolic antioxidant from milk thistle seeds, is a known hepatoprotective agent with benefits in stress-related hypertension, atherosclerosis, and cardiotoxicity. However, its role in stress-induced HCC remains unexplored. This study investigates silymarin's effects on stress-induced proteins in HCC.
Methods: HCC cell lines were treated with cortisol and leptin to simulate acute and chronic stress conditions. Gene expression was analyzed via reverse transcription-polymerase chain reaction (RT-PCR), and overexpression models were established using lentiviral vectors. Proteins and pathways associated with stress were identified through liquid chromatography-mass spectrometry (LC-MS). Identified genes were validated via RT-PCR and western blot analysis in stress-treated and silymarin-treated cells.
Results: LC-MS analysis identified over 3000 proteins, with 6.3% categorized as stress-response proteins. Eleven key stress-associated proteins, including IPO7, SCFD1, TIPRL, SCL25, SERPINB6, TRMT2, and HSP90B1, were validated in leptin- and cortisol-treated SK-Hep1 cells and Malat1 overexpression cell lines. The Cancer Genome Atlas (TCGA) database analysis confirmed these findings in HCC patient cohorts. Notably, silymarin significantly reduced stress-response gene expression in treated SK-Hep1 cells while sparing untreated parental cells. Further pathway analyses and validation in knockdown models are ongoing to elucidate mechanisms underlying these effects.
Conclusion: Silymarin effectively downregulated cortisol- and leptin-induced stress-response proteins in SK-Hep1 cells, highlighting its therapeutic potential in stress-associated HCC. These findings underscore silymarin's role in targeting novel molecular pathways, offering promising strategies to address HCC disparities in underserved populations in the RGV. This research aims to improve outcomes and reduce mortality among high-risk groups, addressing a critical healthcare challenge in Texas.

#5765 Illuminating the kinase activity starmap in NSCLC via tyrosine phosphoproteomics.

N. Zheng[1], Y. Liu[1], J. Yuan[2], Y. Hu[3], Y. Wang[2], T. Zhang[4], A. Yu[1], H. Zhou[1], C. Zhou[1], J. Hou[1], Y. Xu[1], X. Xu[5], H. Ding[1], Y. Zhang[1], R. Zhong[2], M. Yang[2], C. Chang,[2], H. Yan[2], Y. Shang[2], W. Ren[2], S. Li[2], H. Zhong[1], Y. Xiao[1], Z. Zhang[5], M. Han[4], Y. Pang[2];

[1]DeepKinase, Chaoyang, Beijing, China, [2]Department of Bacteriology and Immunology, Beijing Chest Hospital, Capital Medical University/Beijing Tuberculosis and Thoracic Tumor Research Institute, Beijing, China, [3]Department of Medical Oncology, Beijing Chest Hospital, Capital Medical University/Beijing Tuberculosis and Thoracic Tumor Research Institute, Beijing, China, [4]Department of Thoracic Surgery, Beijing Chest Hospital, Capital Medical University/Beijing Tuberculosis and Thoracic Tumor Research Institute, Beijing, China, [5]Jinan University, Guangzhou, China

In lung cancer treatment, understanding kinase activity, particularly tyrosine phosphorylation, is crucial but incomplete. We studied tyrosine phosphoproteomics in 74 patients with non-small-cell lung cancer (NSCLC) using SH2-Superbinder and data-independent acquisition mass spectrometry. We identified 1,048 tyrosine phosphosites, the highest number reported in lung cancer research. We constructed a tyrosine phosphorylation starmap, enabling a novel and intuitive analysis of tumors versus normal adjacent tissues, revealing kinases driving tumor progression and metastasis. Subsequently, patients were stratified by kinase activity for multiple precision medicine perspectives. “Cold” and “hot” tumors were divided based on immune kinase activity, while cancer driver kinase profiling revealed vulnerability to BRD4 inhibitors in wild-type lung cancer cells. EGFR-L858R mutations benefited from combining MEK and EGFR inhibitors. Two distinct kinase activity panels predicted patient survival across different mutation backgrounds. Our findings underscore the diverse kinase activities in NSCLC and highlight the need for personalized therapy based on these profiles.

#5767 Multiplex in situ proximity ligation assay for functional studies of proteins and their interactions in patient samples.

Hampus Elofsson, Desiree Eden, **Olof Hahne**, Linda Arngarden, Frida Tallqvist, Carl-Magnus Clausson, Axel Klaesson, Annica Onell, Sara Bodbin, Caroline Gallant, Agata Zieba Wicher

Navinci Diagnostics AB, Uppsala, Sweden

Detecting protein-protein interactions (PPIs) and their localization is essential for the understanding of protein function and enables deep-diving into processes such as signal transduction, synaptic signaling, regulation of gene expression in cancer, identification of diagnostic markers, patient stratification and more. *In situ* proximity ligation assay (isPLA) is a well-established tool for the study of PPIs in cell and tissue samples. It detects proteins located within 40 nm of each other via antibodies conjugated to oligonucleotides that generate amplified fluorescent or chromogenic signal. The method can be automated and/or combined with multiplexed immunofluorescence (mIF) staining of individual proteins to visualize tissue context e.g., the tumor microenvironment or the immune landscape. However, current isPLA technology has been limited to the detection of a single PPI at a time. We hereby present a new multiplexed mix-and-match approach to isPLA, which overcomes this limitation. We developed a 9-plex assay based on the Naveni® technological solution, suitable for the simultaneous detection of up to nine PPIs or single proteins (here collectively referred to as markers) of the user's choice. Every marker is detected by two directly conjugated primary antibodies (Navenibodies) that participate in the isPLA reaction provided the proximity criterion is fulfilled, leading to specific and strong signal. Additional cycles incorporating other technologies such as mIF or HCR-FISH are also compatible with the method. For isPLA markers, users can choose from a set list of possibilities, including various signal transduction networks such as the RAS/MAPK pathway, the PD1/PD-L1 and PD1/PD-L2 receptor-ligand interactions, various B- and T-cell markers, cell adhesion proteins and more. Thanks to this flexible approach, it was possible to create a tailored 9-plex study, investigating interactions involving and downstream of PD1, as well as activation of nodes in the RAS/MAPK pathway. ERK activation downstream of KRAS mutations has been shown to increase PD-L1 expression in several cancers (e.g., lung and colorectal cancer), which in turn helps tumor cells evade immune detection by binding to PD1 on T-cells, inhibiting T-cell activity and promoting immune tolerance to the tumor. Our data could help indicate which patients would be the best candidates for immune checkpoint or MAPK inhibition therapy. In summary, our multiplex isPLA approach is a sensitive and specific method that visualizes low and high abundant targets in tissues and adds value by uncovering protein interplay in complex signaling networks with implications in translational research.

#5768 Identifying signaling changes that give rise to drug resistance in acute myeloid leukemia through multiomic modeling across diverse model systems.

Juan Camilo Posso[1], Setareh Sharzehi[2], Marina A. Gritsenko[1], McKenna Finley[2], Chelsea M. Hutchinson-Bunch[1], Keith Beadle[2], Karl K. Weitz[1], Melissa Stewart[2], Samantha A. Obermiller[1], Belinda B. Garana[1], Anupriya Agarwal[2], Cristina Tognon[2], Jeffrey Tyner[2], Paul D. Piehowski[1], Elie Traer[2], **Sara J. C. Gosline**[1]

[1]Pacific Northwest National Laboratory, Seattle, WA,[2]Oregon Health and Sciences University, Portland, OR

Acute myeloid leukemia (AML) is a blood cancer that affects ~20,000 people annually, with an overall 5-year survival rate of ~30%. Leukemia cells originate in the bone marrow where normal cell differentiation goes awry, leading to the expansion of immature myeloid cells that retain partial differentiation, or cell state (monocytic, granulocytic, primitive, etc. [Van Galen et al, *Cell* 2019]). However, the mechanism by which cell state shifts cooperate with protein signaling changes to bring about secondary mutations is still unknown. In this work we generated AML cell lines resistant to venetoclax (BCL2 inhibitor), decitabine (hypomethylating agent) and gilteritinib (FLT3 inhibitor) as single agents and in combination and found changes in cell state, protein signaling, and mutational burden. To interrogate the mechanisms behind these changes, we treated four primary AML cells with these drugs (alone and in combination) and captured transcriptomic and proteomic measurements of persistent AML patient cells after 1 month of treatment to compare drug-induced shifts in cell state.

We collected single-cell transcriptomic and global proteomic measurements across two models of venetoclax, gilteritinib, and decitabine resistance: (1) MOLM14 cells treated for up to 6 months with each drug/drug combination, and (2) 4 *ex vivo* patient primary AML cells treated with monotherapy and drug combination for 1 month. In each model system, we measured shifts in cell populations using single cell-derived signatures of cell state based on the transcriptomic data, then calculated differential expression and pathway enrichment statistics of drug resistance models compared to their untreated control using the proteomic data.

In our model of short-term drug resistance, transcriptomic data indicated that leukemia cells acquired a more granulocytic phenotype upon treatment with gilteritinib, became more monocytic with venetoclax treatment, and remained unchanged with decitabine treatment. These shifts occurred with little change in protein abundance (<40 proteins differentially expressed) according to our proteomics measurements of the same samples. Venetoclax combined with decitabine also caused cells to become more monocytic, while combining venetoclax with gilteritinib caused a decrease in monocytic cells as well a noticeable shift (100-500 proteins) in protein expression. Long term drug exposure caused further changes in MOLM14 cells, with many drug-resistant cells becoming more monocytic and exhibiting dramatic changes to protein signaling with up to ~2000 proteins differentially expressed. We are now using these omics measurements to identify putative drug targets that prevent the development of drug resistance. In conclusion, we found that drug-induced protein changes can alter leukemia cell phenotypes that influence mutations and resistance in AML.

#5769 Silibinin profiles the immune microenvironment via mast cell regulation: Potential immunomodulation in cancer prevention and therapy.

N. Mishra, S. Paudel, C. Agarwal, R. Agarwal;
University of Colorado Anschutz Medical Campus, Aurora, CO

Immune cells are critical check points, not only for foreign pathogens, but also for proper growth and proliferation of bodies' own cells. They play crucial and complex roles in cancer, beginning at initiation of malignancy, proliferation of tumors, establishment of the cancer microenvironment, to metastasis and spread of malignant cells throughout the body. In our ongoing studies with silibinin (SB; flavonolignan obtained from milk thistle seeds), we showed that it is an effective preventive natural agent for several cancers including, basal cell carcinoma, prostrate cancer, colon cancer, and hepatocellular carcinoma. SB was also shown to regulate immune responses in several of these studies. Notably, SB treatment was found to significantly regulate mast cell (MC) infiltration and functioning in our completed and ongoing studies. However, since MCs are associated with the functions of other immune cells, it is also important to delve deeply into the downstream effects of SB treatment on other immune components regulated directly and/or indirectly by MCs. Thus, in the present study, in-depth mechanistic aspects of SB treatment on other cells of the tumor immunological microenvironment were determined, leveraging SB regulation of MCs. MCs were derived from bone marrow (BMMCs) of C57BL/6 mice in presence of IL-3 and SCF and confirmed using flow cytometry (dual staining: cKit/Fc€RI). Thereafter, BMMCs were exposed to 25-100 µM SB and two concentrations (25 µM; SB 25 and 100 µM; SB 100) were selected for further experiments. BMMCs treated with SB 25, SB 100, and vehicle control were subjected to proteomics (LCMS on Fusion Lumos mass spectrometer); 3575 proteins were identified using the annotated mouse proteome. Statistical analysis was performed using ANOVA followed by Fisher's posthoc analysis ($p<0.05$, FDR<0.01) and 166 proteins were found to have statistically significant differential expression amongst the three groups (n=4/group). These 166 proteins were selected for further pathway analysis using Ingenuity Pathway Analysis (IPA; Qiagen). Stringent IPA filter settings were used to select pathways specifically *"demonstrated experimentally"* in *"immune cells/cell lines"* for further analysis. Top canonical pathways associated with SB treatment of immune cell regulation via MCs were found to be eukaryotic RNA processing (processing of capped intro-containing pre-mRNA), ISG15 mediated immune responses (ISG15 antiviral mechanism), eukaryotic-translation elongation, and RHO GTPase pathway. Important nodes determined were TNF, CD-28, IL-2, IL-4, IL-15, NR4A1, IFNB, and RAC2, suggesting SB plays an important immunomodulatory role via regulation of MCs. Further studies are warranted to delineate these mechanisms in cancers as well as other injury mechanisms and SB' therapeutic potential (supported by R01 CA140368).

#5770 Single-cell Raman-based omics of the melanoma tumor-immune microenvironment for precision therapy.

Kai Chang[1], Mamatha Serasanambati[2], Baba Ogunlade[1], Ariel Stiber[1], Fareeha Safir[3], Hamish Carr Delgado[1], Amanda Kirane[2], Jennifer Dionne[1]

[1]Stanford University, Stanford, CA,[2]Stanford University School of Medicine, Stanford, CA,[3]Pumpkinseed Technologies, Inc., Palo Alto, CA

Immunotherapy and targeted therapy have altered the standard of care for cancer patients, improving survival rates and cancer control, even in intransigent metastasis. Yet, only 20-40% of patients respond to immunotherapy, and up to 50% experience severe adverse events, limiting therapy application. Notably, cellular heterogeneity and diversity in the makeup of the tumor immune microenvironment (TiME) present extraordinary challenges in finding predictive biomarkers correlating to therapeutic response. Existing profiling methods, even with highly resolved, cutting-edge transcriptomic and proteomic mapping methods remain costly, time-intensive, and do not faithfully capture structural or post-translational modifications actively impacting tumor behavior. Here, we present a high-fidelity, single-cell omics approach based on Raman spectroscopy and machine learning to provide rapid, cellular profiling that enables non-destructive therapeutic response characterization.

Using Raman, we first demonstrate single-cell differentiation and functional state phenotyping of various melanoma and immune cells found in the TiME. Our ML models yield a classification accuracy of >94% across all cell types and parent and derivative cancer lines. We then extend our characterization to track temporal changes across multicellular systems; by investigating cell growth in normal and apoptotic-inducing environments, we show how melanoma-immune cell interactions preserve certain spectral features while introducing new features via cell fusioning under selective pressures. By stimulating cells with small molecule therapeutics serving as growth and inhibiting factors, we demonstrate the potential to monitor melanoma cell plasticity and macrophage polarization changes. Finally, we illustrate the potency of Raman phenotyping by examining the collective drug response in clinically relevant patient melanoma samples and extract pertinent biomarkers associated with small molecule and immunotherapy treatment outcomes. In combination with neural networks, our approach correctly predicts therapy response with >75% accuracy for unseen melanoma patient samples, demonstrating Raman as a powerful rapid, label-free omics approach with groundbreaking potential to advance clinical biomarker efforts and impact the precision treatment decisions of cancer patients.

CLINICAL RESEARCH: Functional and Molecular Imaging and Radiomics
Poster Session

#5774 Enhancing urothelial carcinoma treatment with nectin-4-PET: A predictor of enfortumab vedotin efficacy.

Akhilesh Mishra, Ajay K. Sharma, Kuldeep Gupta, Burles A. Johnson, Jeannie Hoffman-Censits, David J. McConkey, Sridhar Nimmagadda

Johns Hopkins University, Baltimore, MD

Introduction: Enfortumab vedotin (EV), an antibody-drug conjugate targeting nectin-4, has shown significant promise in treating urothelial carcinoma (UC). However, optimizing its efficacy while minimizing treatment-related toxicities necessitates a deeper understanding of EV's target engagement at tumors and its correlation with therapeutic outcomes. To address this, we developed a peptide-based nectin-4 specific positron emission tomography (PET) radiotracer, [^{68}Ga]AJ647, to visualize and quantify EV-induced pharmacodynamics (target engagement) across different EV doses.

Methods: Six UC cell lines representing luminal (HT-1376, UC-14, RT-112) and basal (UC-9, SCaBER, T24) morphologies were used to evaluate in vitro and in vivo nectin-4 specificity of [^{68}Ga]AJ647. Effect of EV on [^{68}Ga]AJ647 uptake was evaluated using competitive binding studies. Target engagement was defined as the percentage reduction in [^{68}Ga]AJ647 tumor uptake, pre-therapy normalized, following EV therapy. In vivo EV target engagement was assessed via PET in HT-1376 xenografts at FDA-equivalent doses (6, 9, 15 mg/kg) on days 1, 3, and 6 post-treatment. Long-term responses to 6 and 15 mg/kg with Nectin-4-PET evaluated target engagement metrics' predictive value.

Results: In vitro assays revealed a strong correlation between [^{68}Ga]AJ647 uptake and nectin-4 expression (R^2=0.85). PET and biodistribution studies confirmed nectin-4-specific uptake across xenografts at 60 minutes post-injection. EV treatment reduced [^{68}Ga]AJ647 uptake in a dose-dependent manner, validating the tracer's ability to measure unoccupied nectin-4. At 24 hours post-treatment, unoccupied nectin-4 levels were similar between 15, 9, and 6 mg/kg groups at approximately 23%. By day 6, these levels diverged significantly, indicating reduced target engagement over time, particularly at lower doses. Further analyses revealed that tumor volume reductions exhibited dose-dependent trends but showed high variability. While pre- and post-treatment [^{68}Ga]AJ647 imaging measures (standard uptake values) were not strongly predictive of therapeutic outcomes, the normalized ΔSUV, reflecting EV-induced target engagement, demonstrated a stronger correlation (r=-0.7877). Mice with less than 25% target engagement showed significantly poorer therapeutic responses, regardless of injected dose. ROC analysis confirmed the predictive power of normalized ΔSUV, with an AUC of 0.87 and a 25% engagement threshold as a robust predictor of positive therapeutic outcomes.

Conclusion: [^{68}Ga]AJ647-PET provides a non-invasive quantitative approach to assessing nectin-4 expression and EV-induced pharmacodynamics. Normalized ΔSUV values, which integrate pre- and post-treatment data, serve as reliable predictors of therapeutic efficacy. These findings highlight the potential of nectin-4 PET to optimize personalized EV dosing.

#5775 Direct prediction of tumor cellular phenotypic composition from multi-parametric MRI in neuroblastoma murine models.

Konstantinos Zormpas-Petridis[1], Matthew Blackledge[2], Louis Chesler[2], Yinyin Yuan[3], Simon P. Robinson[2], **Yann Jamin**[2]

[1]Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy,[2]Institute of Cancer Research, London, United Kingdom,[3]MD Anderson Cancer Center, Houston, TX

Introduction. The clinical prognosis for children with high-risk neuroblastoma, a cancer of the sympathetic nervous system, remains poor. Non-invasive imaging biomarkers of treatment response are essential for precision medicine. Here, we evaluated a supervised machine learning model trained on co-registered MRI-histology maps to predict density maps of key neuroblastoma cell populations (undifferentiated, differentiating, apoptotic) directly from multi-parametric mpMRI in murine models of neuroblastoma.

Methods. Tumour MRI parametric maps for spin-lattice relaxation time (T_1, sensitive to undifferentiated cells), transverse relaxation rate (R_2*, sensitive to red blood cells), and apparent diffusion coefficient (ADC, sensitive to cell density) were co-registered with digitized H&E slides (20x, 0.46µm resolution) from 11 Th-MYCN mice. Automated cell segmentation/classification into 6 categories allowed for the generation of density maps at MRI resolution.

Classical ML models were trained to simultaneously predict 5 histological labels using a supervised regression approach on 9095 MRI voxels (n=4 mice), each represented by 5 normalized input features: R_2*, T_1, and ADC, along with median values of the pixels' square neighbourhood (2px wide). Model performance was evaluated using 10-fold cross-validation and the negative mean-squared-error metric before application to remaining tumours.

Results/Discussion. The random forest classifier achieved the lowest mean-squared-error and smallest variation during the 10-fold cross-validation and was selected as the best model. Extra trees, XGBoost and light-gradient-boosting machines also performed well.

The similarity of the entire MRI-generated maps (for all 11 mice in the dataset) was compared to the histology maps using the mean squared error (MSE) and structural similarity index (SSIM) metrics. The model achieved an average MSE of 0.0037, 0.0089, 0.0016, 0.0014, and 0.0008 for cellular density, undifferentiated neuroblasts, apoptotic neuroblasts, differentiating neuroblasts and red blood cells density respectively. The SSIM indexes were 0.90, 0.88, 0.90, 0.88 and 0.92 for cellular density, undifferentiated neuroblasts', apoptotic neuroblasts', differentiating neuroblasts' and red blood cells density respectively.

Conclusion. This proof-of-concept study demonstrates that mpMRI can characterize neuroblastoma phenotypes and generate quantitative data previously accessible only through digital histology analysis, including cellular density maps. Validation in more diverse datasets could enable "non-invasive" or "virtual" biopsies, capturing entire tumour volume to enhance diagnostic precision, guide surgical planning, and refine response assessment.

#5776 Integrative analysis of regional MRI phenotypes in high-grade glioma: Dimensional reduction of multi-omics imaging and molecular profiles.

Matthew W. Kenaston[1], Matthew Flick[1], Kasthuri Kannan[2], Michele Ceccarelli[3], Nhan Tran[4], Leland S. Hu[5]

[1]Mayo Clinic Alix School of Medicine, Phoenix, AZ,[2]Departments of Translational Molecular Pathology and Neurosurgery, The University of Texas MD Anderson Cancer Center, Houston, TX,[3]Department of Public Health Sciences Research, Miller School of Medicine, University of Miami, Miami, FL,[4]Cancer Biology, Department of Research, Mayo Clinic, Phoenix, AZ,[5]Comprehensive Cancer Center, Radiology, Mayo Clinic, Phoenix, AZ

Introduction: High-grade gliomas (HGG) are aggressive brain tumors with heterogeneous cell populations, including invasive regions driving disease progression and recurrence. Our previous work integrated molecular profiling with spatially matched advanced multiparametric MRI in a cohort of HGG patients with multi-regional sampling, uncovering distinct biological signatures linked to individual advanced MRI phenotypes. Yet, the relationships between MRI phenotypes—and how this potentially informs underlying biology—remains unexplored. Here, we leverage an unsupervised approach to dimensional reduction for a broad array of MRI features and compare regional transcriptomic signatures in a cohort of image-localized HGG samples.
Methods: From our existing data, we selected 20 HGG patients with 73 multi-regional tumor biopsies, each including 18 advanced MRI parameter calculations. With an iterative approach, data was projected using uniform manifold approximation (UMAP) at multiple resolutions and clustered, producing high-confidence stable clusters. We used random forest modeling to assess cluster associations with previously identified signatures. Cluster-specific transcriptomic profiles were detected by DESeq2 and subsequent gene-set enrichment analysis. We further evaluated Pearson correlations and statistical fit with MRI image parameters by performing pathway analysis on genes with significant correlations by image feature, across clusters.
Results: 8 clusters emerged from our analyses. Most advanced MRI features exhibited statistically significant differences across clusters, forming unique patterns of feature behavior. Notably, while these image-based clusters did not correlate well with metabolic profiles, contrast enhancement, or genomics, they did demonstrate distinct and HGG-relevant transcriptomic profiles, with shared and divergent signatures. Across clusters, we also identified several correlations between specific MRI parameters and gene pathway trends. These highlighted combined patterns of multiple MRI features, offering a spectrum of regional phenotypes imperceptible with single feature analysis. We generalize these results to an image-based decision matrix to detect biologic signatures for validation.
Conclusion: Our integrative analysis of MRI mapping stratifies imaged-based patterns to non-invasively assess molecular signatures within HGG. This approach will help elucidate MRI biomarkers that may predict glioma behavior and support future individualized treatment approaches.

#5777 A guidance platform for nanoparticle mediated photothermal therapy for solid tumors.

Ivan Kosik[1], Robert Weersink[2], Margarete Akens[3], Fumi Yokote[4], Sangeet Ghai[1], Gang Zheng[5], Brian Wilson[3]

[1]Princess Margaret Cancer Center, University Health Network, Toronto, ON, Canada,[2]Institute of Biomaterials and Biomedical Engineering, Radiation Oncology, University of Toronto, Toronto, ON, Canada,[3]Medical Biophysics, University of Toronto, Toronto, ON, Canada,[4]Division of Thoracic Surgery, Toronto General Hospital, Toronto, ON, Canada,[5]Princess Margaret Cancer Center, University of Toronto, Toronto, ON, Canada

Introduction Nanoparticle-mediated Photothermal therapy (PTT) is an option for treating solid tumors with low cost and toxicity. Except for a limited set of cancers, PTT in general has not seen widespread clinical adoption, due in part to lack of effective treatment guidance technology. Recent clinical trials here and elsewhere investigating MRI-guided PTT have highlighted 3 critical requirements, including visualization of (1) tumor nanoparticle uptake, (2) temperature distribution during PTT and (3) treatment effect. These trials also highlighted shortcomings of MRI guidance, including high cost and limited access, complexity, speed and indirect functionality, so there is a need for novel direct, real-time PTT-guidance technology. Methods and Results We have designed and constructed a PTT-guidance platform based on hand-held multispectral photoacoustic (PA) tomography enabled by an in-house built frequency-optimized acoustic sensor array designed specifically to capture volumetric PA signals at depths of up to several cm. This is able to quantitatively image bulk-tissue endogenous and exogeneous contrast, including nanoparticle distribution in tumor (Requirement #1). In addition, this platform provides highly sensitive deep-tissue temperature mapping through the temperature dependence of the tissue Gruneisen parameter, matching or exceeding MRI-based thermometry but with direct, real-time precision in an ergonomic hand-held configuration (Requirement #2). Integration of a custom-built diffuse optical tomography (DOT) module into the PAI platform provides measures of the time-dependent tissue optical properties during PTT, particularly scattering that is known to increase 2 to 3-fold with coagulation above ~55°C. Thereby, it provides immediate feedback on the size and location of the photocoagulated lesion (Requirement #3). Results of in vivo and pre-clinical validation studies are presented, showing nanoparticle injection and distribution in tissues, PA thermal mapping and DOT-based proof-of-principle monitoring of PTT treatments in patients with localized prostate cancer. Conclusions These studies indicate that we can provide the three needed functionalities for PTT guidance and that, for the first time, this can be done on a spatial scale relevant to clinical translation. This work is supported by the Terry Fox Research Institute and the Canadian Cancer Society.

#5778 Intra-operative visualization of metastatic sentinel lymph nodes in early breast cancer: An approach to omission of axillary surgery.

Yuanyuan Zhu[1], Wei-Ling Chen[1], Zao Wu[1], Jun-hong Chen[1], Ting-wen Guo[1], **Guo-Jun Zhang**[2]

[1]Xiamen University, Fujian, China,[2]The Breast Center, Yunnan Cancer Hospital, The Third Affiliated Hospital of Kunming Medical Universi, Beijing University Cancer Hospital, Fujian, China

Purpose: Sentinel lymph node biopsy (SLNB) has become the established approach, and is the standard of care for staging clinically node-negative breast cancer. The pathological examination is the golden standard during this procedure. However, its sensitivity is less than 80%, and requires significant time, manpower, and material resources. Noteworthy, the fluorescent imaging in the second near-infrared region (NIR-II) window offers greater tissue penetration and contrast compared to conventional imaging, which is more suitable for detecting the metastatic status of SLN in real-time and dynamically *in vivo.* In this study, we developed a new technology that can quickly identify the metastatic status of SLN both *in vivo* and the resected SLN tissue during the operation.

Methods: 1. Characterization and biosafety of the probe ICG-POL6326 were performed. 2. The targeting efficacy of probe was confirmed through in vitro fluorescence co-localization experiments and *in vivo* fluorescence imaging in tumor-bearing and SLN metastatic mouse model. 3. SLN from mice with lymphatic metastasis were excised and incubated with ICG-POL6326 for subsequent imaging by a NIR-II system to evaluate the efficacy of ex vivo incubation and imaging methods. 4. Ten surgically removed SLN tissues from ten breast cancer patients undergoing resection were incubated with the probe immediately and imaged to identify the metastatic status of SLN. The accuracy of fluorescence imaging was confirmed by pathological diagnosis.

Results: 1. ICG-POL6326 was successfully synthesized with good stability, biocompatibility with high intensity of NIR-II fluorescent imaging. 2. ICG-POL6326 could specifically target 4T1-Luc and MDA-MB-231-Luc cell which with high CXCR4 expression compared with ICG-NHS. 3.ICG-POL6326 group exhibited a 3.15-fold higher maximum signal-to-background ratio (SBR) compared to the ICG-NHS group ($p < 0.0001$) in 4T1-Luc subcutaneous tumor models. 4. ICG-POL6326 was able to detected the metastatic status of SLN in 4T1-Luc SLN metastasis models. the SBR of metastasis SLN was 4.1-fold higher than normal SLN ($p < 0.001$). In addition, via co-incubation of ICG-POL6326, the metastatic SLN exhibited a higher MFI relative to normal SLN with a TNR more than 5 (tumor to normal ratio) ($p < 0.0001$). 5. This new technology yielded more than 90.31% specificity and 100% sensitivity in identifying the metastatic SLN.

Summary: ICG-POL6326 can dynamically evaluate the status of SLN in real-time, with a positive correlation between fluorescence signal and lymph node metastasis. By using ICG-POL6326 to incubate freshly isolated SLN during surgery, the probes can quickly accumulate in SLN invaded by breast cancer cells and identify the metastatic status of SLN. This technology is expected to be used for rapid intraoperative pathological detection and identification of metastatic SLN.

#5779 A comparison of CT/RNASeq and MRI/RNASeq-based radiogenomics for staging muscle-invasive bladder cancer: a pilot study.

Joshua Levy[1], Toru Sakatani[2], Kaoru Murakami[2], Takashi Kobayashi[2], Charles J. Rosser[1], **Hideki Furuya**[1]

[1]Cedars-Sinai Medical Center, Los Angeles, CA,[2]Urology, Kyoto University, Kyoto, Japan

Introduction: Accurate staging of bladder cancer (BCa) assists in identifying optimal treatment (e.g., transurethral resection vs. radical cystectomy vs. bladder preservation). Current clinical tumor staging methods for BCa leverages axial computed tomography (CT) scans due to its speed and cost-effectiveness. Prior research has demonstrated staging uncertainty with CT, leading to under- or over-staging of patients. Improving staging accuracy is crucial for treatment decisions. Recent work has suggested that MRI can better emphasize contrast between abnormal structures and thereby improve stratification, while genomics signatures can identify molecular changes underpinning tumor spread (e.g., collagen remodeling). Thus, we hypothesize that magnetic resonance imaging (MRI)/RNASeq-based radiogenomics can more accurately stage BCa.

Method: A total of 31 matched CT scans, MRI and formalin-fixed paraffin-embedded (FFPE) tissues were collected and made available for analysis. CT and MR images were reviewed by at least two radiologists to generate a consensus BCa T-stage. Next, RNASeq gene expression was profiled from TURBT FFPE tissue using the nf-rnaseq workflow to generate count matrices from the reads. PyRadiomics was used to extract MR/CT features. A radiogenomic analysis was conducted to identify imaging signatures of advanced T-stage through integration of MR/CT imaging and RNASeq data, using Bayesian LASSO for feature selection followed by unpenalized regression modeling, adjusted for age and sex. The performance of image-only and radiogenomic signatures were compared between MR/CT through likelihood ratio testing (LRT).

Results: CT and MRI resulted in the same accuracy of staging pT2 or less. Interestingly, the accuracy of staging >pT2 is better in MR than CT scan, suggesting that MR resolution is superior to CT allowing more accurate staging. CT radiogenomic signatures (p=5.9e-4) stratified patients more effectively than the MR radiogenomics (p=2.9e-3) though changes appeared to be driven by genomic features more than imaging features. In reviewing hierarchical clustering of the radiogenomic features (Fig. 1), we found that MR imaging influenced clustering of the signature more than in CT data, speculating that the MR data delivers more useful information other than tumor stage (e.g. predicting survival and treatment response in addition to stage).

Conclusion: These early findings from the pilot study suggest promising results. The limited sample size restricts our ability to draw definitive conclusions or apply more sophisticated machine learning methods, such as the investigation of how genes may further stratify radiomic features by disease progression and vice versa.

#5780 Prognostic implications of prostatic gonadotropin releasing hormone receptor and androgen receptor expression in non-metastatic prostate adenocarcinoma.

Donghyun Kim

University of Iowa, Iowa City, IA

Background: Suppression of pituitary gonadotropin releasing hormone receptor (GnRHR) is the mainstay systemic therapy for prostate cancer. GnRHR is also expressed in the prostate and its correlation with direct anti-tumorigenic effects have been demonstrated. The inhibition of prostatic androgen receptor (AR) signaling is another potent therapeutic option. Despite their crucial role in prostate cancer proliferation, prognostic implications of prostatic *GNRHR* and *AR* expressions remain uncertain. To investigate the prognostic utility of *GNRHR* and *AR* expressions, a public prostate cancer transcriptomic profile and progression-free survival (PFS) data were reviewed.

Methods: The Cancer Genome Atlas (TCGA) Pan-Cancer project batch-corrected treatment-naïve primary tumor mRNA expression profiles (RNA Seq V2 RSEM) of 324 non-metastatic (M0) prostate adenocarcinoma cases and their associated clinical data were retrieved from the *cBioPortal*. Log-rank Kaplan-Meier was used for survival analysis. The “low” vs. “high” expression cutoffs for *GNRHR* and *AR* were defined as the cohort’s lower 30th and 25th percentiles, respectively.

Results: Prostatic *GNRHR* and *AR* mRNA expressions were heterogeneous, but apart from low *AR* expression in Gleason grade group (GGG) 1, levels were similar across age, anatomical T N stage or all other GGGs. For T2b-4 N0 disease, neither *GNRHR* nor *AR* expression alone was significantly associated with PFS. However, in a subgroup of patients with T2b-3a N0 disease with high *GNRHR*, those with concomitant low *AR* (n=21) were associated with superior 4-year PFS compared to high *AR* (n=119) with hazard ratio (HR) 0.28 (95% CI 0.080-0.976, p=0.046). The composition of GGG was similar between these groups. *GNRHR* or *AR* expression-dependent differential PFS was not observed in patients with T3b-4 N0 disease. For N1 disease, high *GNRHR* (n=38) was associated with superior 4-year PFS compared to low *GNRHR* (n=25) with HR 0.32 (95% CI 0.12-0.85, p=0.007), independent from *AR* expression levels.

Conclusions: High *GNRHR* and low *AR* expression were associated with superior PFS in an anatomical stage-dependent manner. The superior PFS of low *AR* was more pronounced for localized T2b-3a N0 disease, while high *GNRHR* was most pronounced in regional node-positive N1 disease. Prostatic *GNRHR* and *AR* mRNA expression levels may allow tailored strategies in utilizing GnRH agonist/antagonist and AR signaling inhibitors in treating non-metastatic castrate-sensitive disease. Further investigation is warranted.

#5781 Comparing prognostic association of manual vs. artificial intelligence derived quantifications from baseline computed tomography scans in MYSTIC, a global phase 3 trial for treatment of metastatic non-small cell lung cancer.

Harish RaviPrakash[1], Qin Li[2], Kedar Patwardhan[1], John Riskas[3], Shahid Haider[3], Oleksandra Samodorova[3], Jay Hennessy[3], Vignesh Sivan[3], Felix Baldauf-Lenschen[3], Omar Khan[4]

[1]Oncology Data Science, AstraZeneca US, Waltham, MA,[2]Cancer Biomarker Discovery, AstraZeneca US, Waltham, MA,[3]Altis Labs, Inc., Toronto, ON, Canada,[4]Department of Oncology, University of Calgary, Calgary, AB, Canada

Background: Sum of longest diameters (SLD) of target lesions per response evaluation criteria in solid tumors (RECIST) 1.1 is the standard measure of baseline (BL) disease burden, which plays an important prognostic role. Fully automated, artificial intelligence (AI) derived measures from BL computed tomography (CT) scans like imaging-based prognostication (IPRO) and lung volumetric tumor burden (VTB), generated by AI models trained on real-world imaging data, have shown promising association with overall survival (OS).
Methods: BL CT scans acquired in MYSTIC (NCT02453282) were combined across trial arms and retrospectively analyzed. Patients with consent were included in this study. We compared the prognostic association of manually derived SLD with AI derived IPRO and lung VTB using concordance index (c-index), Kaplan-Meier methods, time-dependent area under the curve (TD-AUC), and standardized hazard ratios (HRs) from Cox proportional hazards models.
Results: BL CT scans of 672 patients had available quantifications for comparative analysis. Median OS (mOS) was 12.2 months, 194 patients (29%) were female, 106 (16%) were non-smokers,193 (29%) had squamous cell carcinoma and 201 (30%) had PD-L1 > 50%. SLD, IPRO, and lung VTB, yielded c-indexes of 0.56 (95% CI: 0.54-0.59), 0.61 (0.59-0.64), and 0.57 (0.54-0.60), respectively. Results are shown in Table 1.
Conclusions: BL IPRO has greater association with OS than SLD, and VTB shows similar prognostic association as SLD. AI derived quantifications offer enhanced stratification and may provide an efficient way to analyze treatment effects.

Overall survival strength of association and stratification by variable

Variable	c-index (95%CI)	TD-AUC (12 months)	HR (95% CI)	mOS (months)	
				Above Median	Below Median
SLD	0.56 (0.54-0.59)	0.64	0.76 [0.64, 0.90]	14.6 [6.9, 27.8]	10.3 [4.1, 22.8]
IPRO	0.61 (0.59-0.64)	0.70	0.57 [0.48, 0.68]	16.1 [9.0, 28.4]	8.2 [3.5, 20.3]
Lung VTB	0.57 (0.54-0.60)	0.62	0.68 [0.57, 0.81]	15.5 [7.2, 28.1]	9.8 [4.0, 20.3]

#5782 Effect of tremelimumab in combination with durvalumab and chemotherapy in heterogeneous metastatic lesions: A volumetric analysis.

Q. Li[1], H. RaviPrakash[1], K. Patwardhan[1], J. MacKay[2], A. Shakur[2], Z. Wei[1], J. Parkin[2], M. Parker[2], S. V. Patel[2], R. Stewart[2];
[1]AstraZeneca, Waltham, MA, [2]AstraZeneca, Cambridge, United Kingdom

Background: Patients with metastatic non-small cell lung cancer (mNSCLC) in 1L therapy treated with a limited course of five cycles of the anti-CTLA-4 antibody *tremelimumab (T)* added to *durvalumab (D)* plus four cycles of platinum-based chemotherapy (CTx) experienced a reduction in the risk of death versus CTx alone, especially in non-squamous (NSq) patients. One potential mechanism of anti-CTLA-4 is the diversification of the T-cell response, which may help address tumor heterogeneity more effectively. RECIST based measurements may fail to capture this impact by mainly quantifying target lesions in 2D. Here we assess the ability of volumetric radiographic data to more comprehensively assess the impact of anti-CTLA-4 treatment on tumor growth.
Methods: We analyzed the Computed Tomography (CT) images from 369 NSq patients in the T+D+CTx and D+CTx arms of the Poseidon Phase III trial. Five experienced radiologists annotated and segmented up to 10 lesions in the baseline scans (start of the induction phase), including the RECIST-based target lesions as well as additional measurable non-target lesions (a maximum of 4 lesions per organ). These segmented lesions were tracked, and new lesions were annotated in the follow-up scans of 316 patients at week 12 (end of induction phase) and 276 patients at week 20 (maintenance phase). 3D volume of lesions as well as the RECIST 1.1 lesion diameters were noted and the percentage change in these metrics were observed and compared across the selected trial arms.
Results In comparing the T+D+CTx arm to the D+CTx arm, we observed a significantly larger median volumetric reduction at the end of the induction phase, at both lesion level (69.5% vs 53.4%, $p<0.001$) as well as patient level (65.0% vs 52.3%, $p < 0.001$). Analysis of the volumetric change in metastatic lesions in the liver and adrenal glands showed a similar trend of deeper response in patients given T+D+CTx as compared to D+CTx which was not observed with the 2D diametric change. This deeper response was noted in both the Programmed death-ligand1 (PD-L1, used as a randomization covariate) < 1% and >/=1-49% groups comparing T+D+CTx to D+CTx cohort. Analysis of change in tumor burden from week-12 to week-20 (after the end of CTx) showed that a larger proportion of T+D+CTx treated patients (60.7%) continued with tumor volumetric reduction compared to patients in D+CTx arm (44.6%).
Conclusions This volumetric analysis offers more accurate quantification of tumor size change than the 2D diametric approach. This analysis of the NSq patients suggests that tremelimumab in combination with durvalumab may result in a deeper and longer response in adult patients with Stage IV (metastatic) NSCLC compared to durvalumab-only.

#5783 CT radiomics-based machine learning models for diagnosing para-aortic lymph node metastases in colorectal cancer.

Yingqian Zhou[1], Ximin Pan[2], Qiongwei Tang[3], Zhaoliang Yu[1], Zerong Cai[1], Peiyi Xie[2], Yufeng Chen[1]

[1]Department of General Surgery (Colorectal Surgery) & Biomedical Innovation Center, The Sixth Affiliated Hospital, Sun Yat-sen University, Guangzhou, China,[2]Department of Radiology, The Sixth Affiliated Hospital, Sun Yat-sen University, Guangzhou, China,[3]Guangdong Provincial Key Laboratory of Colorectal and Pelvic Floor Diseases, The Sixth Affiliated Hospital, Sun Yat-sen University, Guangzhou, China

Background: Para-aortic lymph node (PALN) metastasis is a rare but clinically challenging manifestation in colorectal cancer (CRC). Its management is complicated by the high risks associated with radical PALN dissection and the limited diagnostic accuracy of preoperative imaging. This study aims to develop CT radiomics-based machine learning models to predict PALN metastasis in CRC patients.

Methods: A retrospective analysis was conducted on 116 CRC patients who were enrolled between December 2012 and December 2019 and underwent PALN dissection and preoperative enhanced CT imaging within two weeks before surgery. CT images were semi-automatically segmented using 3D Slicer to delineate PALNs as regions of interest, from which a series of shape and texture radiomics features were extracted. Key features were selected using least absolute shrinkage and selection operator (LASSO) regression with cross-validation. Machine learning models, including logistic regression, support vector machine (SVM), extreme gradient boosting (XGBoost), and random forest, were constructed and cross-validated to assess the predictive value of these radiomics features for PALN metastasis.

Results: A total of 1,037 CT radiomics features were extracted from the regions of interest, with six features selected based on their recurrence in over 200 instances. All four machine learning models performed well, with the random forest model exhibiting the most balanced performance: an average accuracy of 0.836, average precision of 0.842, average recall of 0.978, average F1 score of 0.905, and average AUC of 0.809. Compared to traditional diagnostic criteria based on lymph node short-axis diameter, the random forest model significantly improved diagnostic performance, with a net reclassification improvement (NRI) of 0.789 (95% CI, 0.378-1.199, $P < 0.001$) and an integrated discrimination improvement (IDI) of 0.116 (95% CI, 0.024-0.208, $P = 0.014$).

Conclusion: CT radiomics-based machine learning models provide a robust, non-invasive tool for predicting PALN metastasis in CRC, offering superior diagnostic accuracy over traditional imaging metrics. Keywords: CT radiomics, Machine learning model, Para-aortic lymph node, Lymph node metastasis, Colorectal cancer, Prediction.

Funding: Supported by National Key R&D Program of China (No. 2023YFC2507400), grants from the Guangdong Natural Science Foundation (No. 2023A1515011292), the Open Project of Guangdong Provincial Key Laboratory of Artificial Intelligence in Medical Image Analysis and Application (No. 2022B1212010011), National Key Clinical Discipline, The program of Guangdong Provincial Clinical Research Center for Digestive Diseases (No. 2020B1111170004). Yingqian Zhou, Ximin Pan and Qiongwei Tang contributed equally to this study. Corresponding authors: Yufeng Chen, chenyf78@mail.sysu.edu.cn; Peiyi Xie, xiepy6@mail.sysu.edu.cn.

#5784 An image based assay platform for drug screening with a 3D bioprinted vascularized lung cancer model.

Fahimeh Shahabipour, Yuchi Yuchi Chen, Yen-Ting Tung, Marc Ferrer, Min Jae Song

NIH-NCATS (National Center for Advancing Translational Sciences), Maryland, MD

3D tissue models that mimic the physiology of human tissues and organs are being developed in multi-well plate format as predictive assays for drug screening. 3D bioprinting enables the fabrication of tissues with precision and reproducibility in a 96-well plate for medium throughput drug screening. We have established robust protocols for the 3D bioprinting vascularized lung tissue models using human primary pulmonary endothelial cells constitutively expressing green fluorescent protein (GFP), pericytes, and fibroblasts mixed with a fibrin-based hydrogel. We create a vascularized lung tissue model in a ring-shaped structure that facilitates image-based quantification of vasculogenesis and angiogenesis. This bioprinted vascularized lung tissue model was used as a tumor microenvironment to grow lung cancer cells. We incorporated three immortalized lung cancer cell lines, A549, H1975, and H460, constitutively expressing red fluorescent protein (RFP) into the 3D bioprinted vascularized lung tissues. Cancer cells were added as single cells embedded in the hydrogel (1K cancer cells/well) covering the bioprinted vasculature. Fluorescence microscopy was used to quantify the growth and morphology of the cancer cells by measuring total red fluorescence intensity surface roundness and compactness of red fluorescence objects, as well as angiogenesis from the green fluorescence intensity. After 21 days of culture, the three cancer cell lines showed distinct growth morphology and migration patterns. We characterized tumor localization according to the three regions of interest: angiogenesis outwards or inwards and tumor growth inside or outside the bioprinted vasculature area. A549 and H1975 showed a strong tendency to migrate towards the bioprinted vascularized structure, growing as low compact structures. The H460 cell line formed small compact spheroid structures and did not prefer the vasculature area. We also compared cancer cell growth and morphology with and without vascular components to assess any effects of the vasculature on tumor growth. We observed that this study helps demonstrate the usefulness of the bioprinted vascularized lung tissue cancer model as an assay platform for screening for anti-cancer drugs in an assay that includes the tumor microenvironment. ***Keywords:*** 3D bioprinted, 3D cancer-vascularized lung tissue model, drug Screening,

#5785 A novel technique for rapid intra-operative assessment of margins and sentinel lymph node metastasis status in human breast-conserving surgery.

Wei-ling Chen[1], Yuan-yuan Zhu[1], Zhao Wu[1], Jun-hong Chen[1], Ting-wen Guo[1], Li-xin Zhang[2], Guo-jun Zhang[3]

[1]Xiamen University, Xiamen, China,[2]Kunming Medical University, Kunming, China,[3]The Third Affiliated Hospital, Kunming Medical University, Kunming, China

Purpose: The swift and precise evaluation of margin and sentinel lymph node (SLN) metastasis status during breast-conserving surgery is imperative in minimizing the likelihood of requiring additional surgical procedures. Near-infrared optical molecular imaging technology has been extensively utilized in the realm of surgical navigation, representing a significant advancement in the field of surgical oncology. However, due to safety and other concerns, very few effective probes can be applied to clinical trials, hindering the clinical implementation of fluorescence imaging. In this study, a novel technology was developed to rapidly identify tumor areas *in vivo* and *ex vivo* during surgical procedures, and delineate tumor boundaries and SLN metastases to assist surgeons in intraoperative decision-making. **Methods:** For animal studies, an orthotopic breast cancer mouse model, a spontaneous breast cancer transgenic mouse model, and a SLN metastasis mouse model of breast cancer were established. The tumor tissues and SLNs of mouse models above were surgically removed and incubated with a probe of different concentrations for different incubation time, then imaged by a second near-infrared window fluorescence imaging system. For clinical samples, surgically removed breast tissues and SLNs from 13 breast cancer patients were incubated with the targeting probe immediately after intraoperative resection and imaged to identify the tumor area and distinguish the tumor boundary and metastatic SLNs. The accuracy of fluorescence imaging was validated through pathological examination. **Results:** The *ex vivo* animal imaging experiments showed a fluorescence enhancement of tumor tissue and metastatic SLNs. For clinical samples, our results showed that this new technology yielded more than 90 % sensitivity and specificity in identifying the tumor area in the resected breast tissue. The average fluorescence tumor-to-normal ratios were more than three in different incubation conditions. Furthermore, we also used this technique to image metastatic SLNs intra-operatively and showed that metastatic SLNs exhibited a nearly 3-fold increase in fluorescence signal compared to benign SLNs. Calculations on the results of the fluorescence signal in relation to the metastatic status of SLNs yielded values of more than 90 % for sensitivity and specificity. **Conclusions:** The utilization of fluorescently labeled ADC that specifically targets tumor surface proteins combined with intraoperative fluorescence imaging has effectively delineated boundaries in breast cancer specimens and facilitated the detection of metastatic SLNs. This approach has the potential to inform decisions regarding optimal resection strategies in oncology, particularly within the constraints of a limited surgical window.

#5786 Rapidly assess sentinel lymph node metastasis status in breast cancer by a near-infrared fluorescent probe targeting CK.

Junhong Chen[1], Wei-Ling Chen[1], Yuan-Yuan Zhu Zhu[1], Zhao Wu[1], Ting-wen Guo[1], **Guo-Jun Zhang**[2]

[1]XiaMen University, FuJian Province, China,[2]The Breast Center, Yunnan Cancer Hospital, Third Affiliated Hospital of Kunming Medical University, Beijing University Cancer Hospital, YunnanProvince, China

Purpose: At present, surgery is still a main treatment method for breast malignant tumors, but the problems of edema and tumor recurrence faced by patients after surgeries are still relatively common. The main reason for this is closely related to the resection of metastatic sentinel lymph node (SLN) during surgery. The method of identifying SLN by methylene blue (MB) and technetium 99m tracing is not clearly targeted and can't judge the metastatic status of SLNs. Therefore, there is an urgent need to develop tracers that specifically identify the metastatic status of SLN. Our team successfully synthesized ICG-CK, a fluorescent molecular probe targeting pan-CK. Through animal experiments, we found that the probe can identify the metastatic status of SLN *in vivo* and *ex vivo*.

Methods: 1. The penetration and characteristics of the ICG-CK were evaluated. 2. The ability of ICG-CK to recognize different breast cancer cell lines was verified by cell uptake experiments. 3. The ability of ICG-CK to identify the metastatic status of SLN was verified by *in vivo* imaging experiments. 4. The ability of ICG-CK to identify the metastatic status of *ex vivo* SLN was verified by co-incubation with *ex vivo* tissue.

Results: 1. The ICG-CK probe was successfully synthesized, with good stability, biocompatibility and strong NIR-II fluorescence emission ability. 2. ICG-CK could specifically target 4T1 cell which with high CK expression compared with ICG-IgG, and there was a statistically significant difference between the two, $p<0.0001$. 3. *In vivo* imaging experiments, the fluorescence imaging of tumor-bearing mice showed that ICG-CK was easily drained to SLN and targeted to identify metastatic 4T1 tumor cells, producing a strong fluorescent signal, and the SBR of metastatic SLN and normal SLN was 3.34, $p<0.05$. 4. By imaging isolated SLN from tumor-bearing mice, we found that after co-incubation with ICG-CK of isolated SLN, isolated SLN produced a strong fluorescent signal that was significantly different from normal SLN, $p<0.01$.

Summary: Our results show that ICG-CK has a strong ability to identify metastatic SLN compared with normal SLN in breast cancer, both *in vivo* and *ex vivo* imaging experiments, and it can produce a strong fluorescence signal in the near-infrared second region. Therefore, our study provides a basis for further exploration of the use of ICG-CK to assess the metastatic status of SLN in real-time intraoperatively.

#5787 Mutation prevalence in metastatic castration-sensitive prostate cancer based on metastasis timing and risk stratification.

Maria Ovejero-Sanchez[1], Ana Jambrina[1], Ignacio Gonzalez-Ginel[2], Daniel Tello[3], Manuel Balongo[1], Ana Gutierrez-Pecharroman[4], Pedro P Lopez-Casas[3], Alfredo Rodriguez-Antolin[2], Elena Castro[1], David Olmos[3]

[1]Translational Research in Cancer Genetics group, Instituto de Investigacion Hospital 12 de Octubre, Hospital Universitario 12 de Octubre, Madrid, Spain,[2]Urology Department, Hospital Universitario 12 de Octubre, Madrid, Spain,[3]Genomics and Therapeutics in Prostate Cancer, Instituto de Investigacion Hospital 12 de Octubre, Hospital Universitario 12 de Octubre, Madrid, Spain,[4]Department of Pathology, Hospital de Getafe, Madrid, Spain

Introduction and Objectives: Metastatic castration-sensitive prostate cancer (mCSPC) is a clinically heterogeneous disease in which genetic mutations and tumor burden influence patient outcomes. Mutations in some DNA repair genes (e.g. *BRCA1/2* and *ATM*) have been related to prostate cancer development and progression and may serve as therapeutic targets. The GENPRO12 study aims to evaluate the mutational profiles in non-mCSPC and mCSPC patients and to explore its clinical associations.
Material and Methods: This preliminary report includes 109 mCSPC patients, stratified by synchronous/metachronous disease. Each group was further classified into risk/volume (high/low) categories based on CHAARTED and LATITUDE criteria. Tumor samples were collected and analyzed using next-generation sequencing (NGS) ACHILLES test (Olmos et al. Ann Oncol 2024) to identify mutations in 40 genes, including *BRCA1/2, ATM*, and other prostate cancer-related genes. Clinicopathological data were extracted from medical records.
Results: Median age at diagnosis of mHSPC was 72 (range 47-92) with a median PSA 46.9 (0.3-3956). 90 patients had synchronous metastatic disease, 69 were CHAARTED high-volume, 76 were LATITUDE high-risk, and 25 had visceral metastases (17 in the lung only). All but 11 patients had at least 1 alteration in the genes analyzed. The most relevant altered genes are listed in the table. *BRCA2*, *PTEN*, *RB1* Loss of Function (LoF) and *MYC* gain were more frequent in de novo and/or high-volume cases.
Conclusions: Genetic alterations with prognostic and/or predictive value can be detected using prostate-specific NGS assays on archived diagnostic FFPE samples. Their prevalence may vary based on mCSPC clinical presentation, reflecting distinct disease biologies. Updated results, including outcomes and treatment responses, will be presented at the meeting.

Most relevant altered genes

Gene alteration	All patients (n=109)	De novo high-volume (n=61)	De novo low-volume (n=29)	Metachronic high-volume (n=8)	Metachronic low-volume (n=11)
ATM LoF	9 (8%)	3 (5%)	4 (14%)	0 (0%)	2 (25%)
BRCA1 LoF	3 (3%)	0 (0%)	2 (7%)	0 (0%)	1 (9%)
BRCA2 LoF	22 (20%)	13 (21%)	7 (24%)	2 (25%)	0 (0%)
CHEK2 LoF	6 (6%)	5 (8%)	1 (3%)	0 (0%)	0 (0%)
FANCA LoF	12 (11%)	9 (15%)	2 (7%)	0 (0%)	1 (9%)
MYC gain	7 (6%)	6 (10%)	0 (0%)	1 (13%)	0 (0%)
PALB2 LoF	2 (2%)	0 (0%)	1 (3%)	0 (0%)	1 (9%)
PTEN LoF	34 (31%)	21 (34%)	10 (34%)	1 (13%)	2 (18%)
RB1 LoF	29 (27%)	19 (31%)	5 (17%)	3 (38%)	2 (18%)
SPOP mut	13 (12%)	8 (13%)	4 (14%)	0 (0%)	1 (9%)
TP53 LoF	40 (37%)	26 (43%)	11 (38%)	0 (0%)	3 (27%)

#5788 Molecular profiling of NSCLC patients associates CT-based radiomic features with a tumor microenvironment favorable for immunotherapy.

Lauren K. Brady[1], Amogh Hiremath[2], Mohammed Qutaish[1], Kai Zhang[2], Kaustav Bera[3], Anant Madabhushi[2], Sri Sridhar[1], Nora Pencheva[4], Patricia Garrido-Castro[4], Maria Jure-Kunkel[1], Omar Jabado[1], Matthew Loya[1], Erin Henitz[1], Regina Aquino[1], Teng Jin Ong[1], Nathaniel Braman[2], Brandon W. Higgs[1]

[1]Genmab, Plainsboro, NJ,[2]Picture Health Inc, Cleveland, OH,[3]Case Western Reserve University, Cleveland, OH,[4]Genmab, Utrecht, Netherlands

Radiomics provides a powerful, noninvasive way to capture tumor heterogeneity and predict therapy response. However, its clinical utility is limited by feature interpretation. Here, we integrate radiomics with tumor transcriptomic profiles to enhance biological interpretation of CT-based features linked to an immune-responsive tumor microenvironment (TME), in non-small cell lung cancer (NSCLC) treated with acasunlimab, a DuoBody® PD-L1×4-1BB bispecific antibody, +/- pembrolizumab.

This study used contrast-enhanced CT images (n=62) and gene expression (HTG EdgeSeq) from tumor biopsies (n=25) of advanced NSCLC subjects in GCT1046-01 (NCT03917381) and GCT1046-04 (NCT05117242). Gene signatures (n=104) linked to cancer and immune pathways were analyzed. Radiomic features were extracted using Picture Health Px Platform, creating a 4-feature composite radiomics score optimized to assess clinical benefit (one quantitative vessel tortuosity (QVT) and three textural features (Laws, Gabor, and GLCM). Performance and treatment specificity were cross-validated and evaluated in 85 NSCLC patients treated with chemotherapy.

The radiomics score was not associated with baseline BMI, ECOG score, gender, age, or tumor size. Clustering radiomics features with mRNA signatures identified distinct biological pathways, such as immune regulation, angiogenesis and apoptosis. Specifically, QVT was negatively associated with immune regulation and antigen presentation, while positively correlated with hypoxia and tumor immune evasion. In contrast, GLCM correlated with B cell signaling, immune checkpoint activation and lymphocyte infiltration, indicating a TME suitable for immunotherapy. Laws showed positive correlation with antiviral defense and immune activation, and negative association with tumor immune evasion. Gabor was negatively correlated with cancer associated fibrosis and extracellular matrix remodeling.

These results show that integrating radiomics with tumoral mRNA data identified key characteristics of a TME conducive for immunotherapy, advancing the interpretability of AI-derived imaging features. The composite radiomics score was independent of traditional prognostic factors and reflected immunotherapy-specific mechanisms, highlighting its potential as a noninvasive tool for characterizing tumor biology and determining immunotherapy relevance in NSCLC patients.

*equal author contribution, LKB, AH

CLINICAL RESEARCH: Immune Response to Therapies
Poster Session

#5792 Characterization of the tumor microenvironment in a relapsed refractory multiple myeloma patient undergoing daratumumab therapy.

Siobhan B. Cashman[1], Aruna Ayer[2], Teresa Swift[1]

[1]Advanced Technology Group, BD Biosciences, Limerick, Ireland,[2]Advanced Technology Group, BD Biosciences, Miloitas, CA

Multiple Myeloma (MM), a hematological malignancy, is characterized by the clonal proliferation of plasma cells in the bone marrow (BM), which serves as the tumor microenvironment. Despite recent advancements in MM treatment, many patients experience disease progression after multiple therapies. Daratumumab, a human IgG1 monoclonal antibody targeting CD38, has emerged as a breakthrough in the treatment of MM, including in cases of relapsed and refractory MM. However, MM remains incurable and therefore understanding the impact of daratumumab on immune microenvironment may enable the development of better-targeted therapies. This study represents a detailed case of a 45-year-old female with relapsed refractory MM undergoing daratumumab therapy. BM aspirates were collected from the patient before treatment initiation & 7-days post final infusion. To delineate the early cellular and molecular changes induced by daratumumab within the TME, we employed flow cytometry and single-cell whole transcriptome sequencing with CITE-seq. Flow cytometry was used to assess the changes in cell populations within the TME, particularly focusing on $CD8^+$ T cells and NK cells. Single-cell RNA sequencing enabled the high-resolution analysis of both transcriptomic and surface protein changes within individual cells. Post-infusion, we observed an increased percentage in $CD8^+$ T effector memory (T_{EM}) cell subsets accompanied by a corresponding decrease in effector memory expressing CD45-RA (T_{EMRA}). Single-cell sequencing identified heightened activity in the interferon and TNF pathways. Additionally, there was an increased percentage of granzyme B expressing $CD8^+$ T cells. Although we observed no significant change in the percentage of NK cells post-infusion, we did note an increased percentage of $CD56^{Bright}CD16^-$ NK and $CD3^+CD8^+CD56^+$ NK-T-like subsets as well as decreased $CD57^+$ terminally differentiated NK cells. We observed increased transcript expression of *CD160* (encoding the activating receptor CD160/NK1) in the post-daratumumab NK cell cluster. Further, we noted increased activity in the NFκB pathway in post-treatment NK cells. This case study underscores daratumumab's rapid impact on the immune cell landscape within the BM TME. By combining flow cytometry with single-cell whole transcriptome sequencing and CITE-seq this study provided a comprehensive view of the effect of therapy on the MM TME. Through CITE-seq, we identified the dynamic changes in the BM immune cell landscape and captured protein expression patterns specific to targeted cell populations. This approach allowed us to link surface marker expression with gene expression profiles, enabling a comprehensive analysis of the BM TME. For Research-Use Only. Not for use in diagnostic or therapeutic procedures. BD and the BD Logo are trademarks of Becton, Dickinson and Company. © 2024 BD. All rights reserved. NPM-5206

#5793 Measurement of basophil activation using a flow cytometry method for applications in clinical research and clinical trials.

J. J. Stewart, L. Brown;
Translational Drug Development (TD2), Bothell, WA

Background: Basophils are a type of white blood cell that play a role in the immune system, particularly in defending the body against allergens, pathogens, and parasites. Basophils also can contribute to the immune response directed against cancer cells by releasing histamine and other mediators that can enhance inflammation and recruit other immune cells to the tumor site. They express high-affinity immunoglobulin E (IgE) receptors, which are involved in recognizing and responding to antigens presented by cancer cells. Basophils may also play a role in modulating the tumor microenvironment, potentially influencing the effectiveness of immunotherapies and understanding the exact mechanisms make the study of basophil activation an exciting area for developing novel therapeutics.
Methods: Basophil cells can become activated when the antigen loaded IgE antibody recognizes and binds to the Fc receptor (FcεRI) on the basophil surface. Basophil activation also causes certain markers to be upregulated on the surface of the cell that can be measured by flow cytometry to determine basophil activity. The basophil activation test (BAT) was conducted with human whole blood samples using a commercially available basophil activation kit. Whole blood specimens from human donors were collected in EDTA blood collection tubes. Blood was stimulated with FCεR1 and fMLP and staining reagent for 25 - 30 minutes at 37°C. Cells were lysed, washed and acquired on the flow cytometer.
Results: CCR3 was used to identify the basophil population in human whole blood. CD63 and CD203c were then used to identify the unstimulated and stimulated state of the cells. Upon stimulation there was an increase in CD63 and CD203c expression on the surface of the basophils.
Conclusions: These results showed that the basophil activation can be measured using flow cytometry with the increased expression of CD63 and CD203c on stimulated basophils. This method could be used to help scientists in clinical research and clinical trials to monitor allergic diseases, predict responses to immunotherapy, and assess the effectiveness of treatments, including immunoncology drugs.

#5794 Tumor cell targeted granzyme B fusion constructs mediate immunogenic cell death (ICD) in addition to their direct cytotoxic effects.

Khalid A. Mohamedali[1], Brian Aguirre[2], Lawrence H. Cheung[1], SuYin Kok[2], David Casero[3], Madhuri Wadehra[2], Michael G. Rosenblum[1]

[1]Department of Translational Medical Sciences, Texas A&M Univ. Health Science Center, Houston, TX,[2]Department of Pathology and Laboratory Medicine, University of California Los Angeles, Los Angeles, CA,[3]Department of Medicine, Cedars Sinai Medical Center, Los Angeles, CA

Therapeutic agents targeting tumor cells have a cytotoxic effect that primarily results in an apoptotic insult through numerous mechanisms. Recently, immune activation mechanisms directed against tumor cells have been identified as a secondary effect of targeted therapy. In this mechanism, targeted tumor cells release ATP and various intracellular proteins into the intratumoral space including HSP70, HMGB1 and calreticulin. These damage-associated molecular patterns (DAMPs) then decorate nearby undamaged tumor cells where infiltrating host immune cells generate a secondary immune response to these decorated bystander cells. Epithelial membrane protein-2 (EMP2) is highly expressed in invasive breast cancer (BC), including triple negative breast cancer, and represents an attractive therapeutic target. We generated a fusion construct containing human granzyme B fused to an scFv antibody recognizing the EMP2 antigen, tethered through a fragment of an IgG heavy chain (Fc) domain and containing the dimerization domain. The 160 kDa dimeric protein was expressed by HEK-293E cells into conditioned media. The construct was assessed for GrB enzymatic activity, EMP2 binding affinity and cytotoxicity against various breast cancer cell lines. The purified GrB-Fc-KS49 demonstrated exceptional binding to an EMP2 peptide with a K_D of 1 x 10^{-12} M and GrB enzymatic activity was preserved compared to native GrB. The IC_{50} ranged from 40-83 nM against a panel of murine and human EMP2+ BC cell lines. Treatment of mice bearing well-established syngeneic EMT6 or 4T1 breast tumors on a twice weekly schedule (IP, 40 mg/kg total dose) showed a significant (50%) reduction in cell growth and in tumor cell turnover (Ki-67). Importantly, in the 4T1 model tumors, there was a 20-fold increase in CD45+ immune cells and a 60-fold increase in tumor-inhibitory M1 macrophages. We found similar results in the EMT6 tumor model with a significant decrease in tumor supportive M2 macrophages. Studies are ongoing to identify potential directly cytotoxic molecules released from cells intoxicated by GrB-based fusion constructs and to identify DAMP-related proteins which may mediate the secondary immunogenic bystander effect against tumor cells. These data support a proposed “dual mechanism” for GrB-based therapeutics which include a direct, antigen-mediated cytotoxic effect and a secondary, immune-mediated effect which can target both antigen-positive and antigen-negative tumors. Research conducted, in part, by the Clayton Foundation for Research and generously supported by the NCI R21 CA163971.

#5795 Dynamic proteomic biomarkers of survival outcomes with anti-PD-1 immunotherapy in head and neck squamous cell carcinoma.

Md Saiful Islam Sai[1], Kartik Sehgal[2], Ze Zhang[2], Rondi Butler[3], John Wiencke[4], Annette Molinaro[5], Juliana Miller[2], Hannah Stolrow[5], Keisuke Shirai[5], Lucas Salas[5], Devin Kosetler[1], Robert Haddad[2], **Brock C. Christensen**[5], Karl Kelsey[5]

[1]University of Kansas Medical Center, Kansas City, KS,[2]Dana Farber Cancer Institute, Boston, MA,[3]Brown University School of Public Health, Providence, RI,[4]University of California San Francisco, San Francisco, CA,[5]Dartmouth Geisel School of Medicine, Lebanon, NH

Background: Despite advances in anti-programmed death (PD)-1 based immunotherapy in recurrent/metastatic (R/M) head and neck squamous cell carcinoma (HNSCC), challenges remain in identifying who will benefit from these therapies. Current tissue-based biomarkers have many limitations. Due to its accessibility and ease of longitudinal monitoring, there is growing interest in blood-based dynamic biomarkers for immunotherapy outcomes in R/M HNSCC.
Methods: In a prospective multicenter study of patients with R/M HNSCC (n=38) receiving anti-PD-1 based therapy, peripheral blood was collected pre-treatment and post-treatment (cycle 2 day 1). A total of 83 serum-derived cytokine biomarkers were evaluated with the Olink Target 96 Immuno-Oncology panel. Associations of pre-treatment, post-treatment, and the change in cytokine levels (post minus pre) were assessed with overall survival (OS) and progression-free survival (PFS). Unsupervised clustering analysis identified subgroups based on cytokine profiles. Further analyses were conducted using univariable and multivariable Cox proportional hazards and Cox Elastic Net machine learning models.
Results: Among the 83 cytokines, 49 (59%) exhibited a statistically significant within-patient increase from pre- to post-treatment. Further, several cytokines were significantly associated with OS and PFS and 10 associated with OS were shared in both pre- and post-treatment analyses. Generally, increased cytokine levels were associated with poorer outcomes. In analyses of cytokine level change (post minus pre), seven cytokines were significantly associated (FDR0.05) with OS (i.e., VEGFA, HGF, PGF, CCL19, CCL23, MCP3, TNFRSF12A) and three with PFS (i.e., NOS3, MMP12, and CCL23) after adjustment for potential confounders. An unsupervised clustering analysis of patients based on the change in the levels of all 83 cytokines uncovered two subgroups that differed significantly with respect to OS and PFS even after adjustment of potential confounders. A prognostic cytokine score was derived using machine learning analysis using elastic net regularization. Not only were cytokine scores significantly associated with platelet count and, in some cases, neutrophil count, they also provided additional prognostic value beyond established prognostic factors.
Conclusion: Dynamics of serum cytokines related to blood platelets during anti-PD-1-based therapy may serve as prognostic biomarkers in patients with R/M HNSCC.

#5797 Impact of baseline tumor size on response to anti-CD47 immunotherapy in an osteosarcoma mouse model.

R. Roudi, I. Vasylkiv, L. Pisani, T. Liang, R. Saab, H. Daldrup-Link;
Stanford University School of Medicine, Palo Alto, CA

Background and Purpose. Activation of tumor-associated macrophages (TAMs) by CD47 mAb therapy can be monitored with ferumoxytol-enhanced magnetic resonance imaging (MRI). The purpose of our study was to investigate if tumor size at baseline affects TAM response to CD47 mAb, as measured with ferumoxytol-MRI.
Methods. Thirty female NOD scid gamma (NSG) mice with small-sized (tumor volume = 30 mm3), medium-sized (tumor volume = 500 mm3), and large (tumor volume = 1200 mm3) 143B osteosarcoma xenografts were treated with human CD47 mAb (10 mg/kg, day 1, 3, and 5 for 1 week) or PBS (control group). All mice underwent MRI scans at baseline and at one week after CD47 mAb or sham treatment. Post-treatment scans were obtained before and after intravenous infusion of ferumoxytol nanoparticles (30 Fe mg/kg). We measured tumor T2* relaxation times as a quantitive measure of nanoparticle retention in TAM. The tumor ferumoxytol enhancement, calculated as: ΔT2* = 100 * (T2*precontrast - T2*postcontrast) / T2*precontrast, was compared between experimental groups using exact two-sided Wilcoxon rank-sum tests. Histology served as standard of reference.
Results. At baseline, tumors of CD47 mAb and PBS-treated mice demonstrated no significant difference in T2* relaxation time, regardless of tumor size (all $p>0.05$). After CD47 mAb therapy, tumors treated with CD47 mAb demonstrated significantly shorter T2* relaxation times compared to PBS-treated mice (all $p<0.001$). The tumor T2* enhancement was significantly higher in small tumors (ΔT2* = 41.3% +/- 2.182%) compared to large tumors (ΔT2* = 2.93% +/- 2.474%; $p=0.001$), indicating stronger nanoparticle phagocytosis in small sized tumors, apparently due to increased TAM activation. There was not statistically significance difference between T2* relaxation time of small and medium-sized tumors in all time points (all $p> 0.05$). Histopathological correlations confirmed a significantly higher number of CD80-positive TAMs in small sized tumors after CD47 mAb therapy (61.28% +/- 1.77%) compared to large sized tumors (3.38% +/- 0.81%, $p<0.001$).
Conclusion. Tumor size at baseline affects TAM response to CD47 mAb. Smaller osteosarcomas exhibit a more pronounced TAM response to CD47 mAb therapy compared to larger tumors.

#5798 Characterization of neoantigen peptides reveals key factors for sequence design to achieve immunogenicity.

Piriya Wongkongkathep, Worawan Bunrasmee, Jirattha Siriluksana, Korn Chotirosniramit, Pimpayao Sodsai, Nattiya Hirankarn, Trairak Pisitkun

Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

Immunotherapy has become a powerful approach by utilizing immune systems to combat cancer. Therapeutic cancer neoantigen vaccines targeting tumor-specific antigen (TSA) have demonstrated promising results in several clinical trials, particularly in adjuvant settings. Neoantigens are tumor-specific antigens that are derived primarily from somatic mutations or non-canonical sequences, which can be presented on major histocompatibility complex (MHC) complexes to stimulate T cells. However, selecting mutations and designing neoantigen sequences to achieve high immunogenicity remains challenging. Compared with RNA-based vaccines, the peptides are more difficult to design due to solubility issues. We have analyzed immune response data from an enzyme-linked immunospot (ELISpot) assay collected from patients enrolled in Phase I clinical trial of neoantigen peptide vaccine at Faculty of Medicine, Chulalongkorn University, Thailand. A total of 12 patients with metastatic melanoma and renal cell carcinoma were treated with neoantigen synthetic long peptides, derived from 15-20 patient-specific somatic variants. The patients received neoantigen vaccine alone, intramuscularly administered in 5-7 injections, without combination with immune checkpoint inhibitors. A total of 239 peptides have been tested by ELISpot. We have characterized several contributing factors to immune response. Notably, we found no significant difference in immunogenicity based on peptide solubility, gene expression levels, variant allele frequency, or sites of injection. However, neoantigen peptides derived from indel mutations exhibited slightly stronger immune responses, than those from single nucleotide variants (SNVs) or frameshift variants. In conclusion, we identified some key factors that can assist in the design of neoantigen peptide sequence, and help prioritize neoantigen candidates for cancer treatments.

#5799 The immune and metabolic profile in the response to neoadjuvant treatment with immune checkpoint inhibitors in triple-negative breast cancer patients.

Lucia Serrano Garcia[1], Guillermo Quintas[2], Maria Portero Hernandez[1], David Collado Martinez[1], Maria Dolores Torregosa[3], Sara Marin[1], **Mario Mancino**[4], Jose Enrique O'Connor Blasco[5], Antonio Llombart-Cussac[1], Maria Leonor Fernandez-Murga[1]

[1]Hospital Arnau de Vilanova, Valencia (Spain), Valencia, Spain,[2]Health and Biomedicine Department, Leitat Technological Center, Barcelona, Spain,[3]Medical Oncology department Hospital Doctor Peset, Valencia, Spain,[4]Medica Scientia Innovation Research (MEDSIR), Jersey, NJ,[5]Universitat de Valencia, Valencia, Spain

Background: Immune checkpoint inhibitors (ICIs) are a promising option for early triple negative breast cancer (TNBC) treatment, although not all the patients get good responses. The objective of the present work is to research immune and metabolic profiles through non-invasive methods that allow us to differentiate responders (R) and non-responders (NR) patients prior to receive neoadjuvant chemotherapy (NAC) with ICIs.
Methods: 34 TNBC patients treated with NAC and ICIs were enrolled and blood samples were collected before treatment (baseline), after 3 weeks (3W) and at the end of treatment (Endpoint). Patients were classified as responders and non-responders based on whether they achieved histologically complete pathological response respectively after treatment. Cellular immune populations were characterized by flow cytometry. Metabolomic profile was determined by mass spectrometry.
Results: We observed a significant increase in the percentage of total lymphocytes ($p<0.001$), Natural Killer T (NKT) cells ($p=0.01$) and CD8 T cells ($p<0.001$) throughout treatment for the total set of patients. When classified into response (R) and No-response (NR), only the R group obtained a significant increase in NKT ($p<0.001$) and CD8 T cells ($p<0.001$) after therapy. NR group showed higher percentages of Myeloid Derived Suppressor Cells (MDSCs) at all time points. These changes were statistically significant for total MDSCs (3W; $p=0.03$ and endpoint; $p=0.01$) and granulocytic-MDSCs at baseline ($p=0.02$) and endpoint ($p=0.006$). NR patients also had a significant increase of platelet to lymphocyte ratio (PLR) after treatment ($p=0.017$). Metabolomic analysis revealed differences in Tryptophan, cytochrome p450, arginine and proline, nicotine and nicotinamide and Lysine degradation pathways at baseline point between responses ($p<0.05$). At 3W the changes appeared in Tryptophan, steroid hormone biosynthesis, amino sugar and nucleotide sugar, bile acid, purine and cytochrome p450 pathways ($p<0.05$).
Conclusion: Immunotherapy induced an activation of the immune system in patients with TNBC. This activation was more evident in patients in the R group, while in the NR groups had activated immunosuppression mechanisms such as MDSCs and PLR, which are associated with worse prognosis. Both groups of responses showed altered metabolites of different pathways that vary depending on the sample point. The changes observed between R and NR groups could allow establishing an immunosuppression mechanism in TNBC, as well as personalized patterns that predict the effectiveness of NAC with ICIs and help in clinical decision making. This investigation is still in the recruitment phase and metabolomic analysis of endpoint samples, hoping this tendency to continue.

#5802 Somatic variant predictors of cutaneous immune-related adverse events in cancer patients treated with immunotherapy.

Ninghui Hao[1], Jenny Lai[1], Crystal Chang[1], Ahmad Rajeh[1], Chuck Lin[1], Cameron Moseley[1], Arjun Mahajan[1], Matthew Tran[1], Coleen Valery[1], Christopher J. Thang[1], Guihong Wan[1], Alexander Gusev[2], Yevgeniy R. Semenov[1]

[1]Department of Dermatology, Massachusetts General Hospital, Boston, MA,[2]Department of Medicine, Dana Farber Cancer Institute, Boston, MA

Cutaneous immune-related adverse events (cirAEs) affect nearly one-third of patients treated with immune checkpoint inhibitors (ICIs). Identifying patients at high risk for cirAE can help guide monitoring and therapeutic management. Whether tumor somatic mutations are associated with the risk of cirAE development remains unknown. We analyzed tumor somatic single nucleotide variants (SNVs) from a discovery cohort of 733 patients with stage III/IV melanoma receiving ICIs at Dana Farber Cancer Institute and Massachusetts General Hospital. CirAE development was determined by chart review. SNVs were detected by OncoPanel v1-3. Nonsynonymous SNVs were used to calculate tumor mutational burden (TMB) and determine gene mutation status. Logistic regression models were used to test the association between gene mutation status and the binary outcome cirAE, while Cox regression models were used to examine the association between TMB or gene mutation status with time to cirAE development. Models were adjusted for demographic covariates (age at ICI, sex, self-reported race/ethnicity) and clinical covariates (melanoma subtype and ICI type). To validate significant associations identified in the discovery cohort, we used an independent cohort of 792 non-melanoma cancer patients treated with ICIs. Consistent with prior studies, TMB-high (>10 mutations/Mb) was significantly associated with improved overall survival (hazard ratio (HR) [95% CI] = 0.72 [0.53, 0.98]) in melanoma. However, TMB-high was not associated with cirAE development (HR = 0.92 [0.69, 1.22]). Binary logistic regression revealed that somatic mutations in PRKCI (OR = 2.76 [1.27, 6.33]), STAG2 (OR = 2.25 [1.09, 4.80]), and CXCR4 (OR = 2.56 [1.02, 6.80]) were associated with increased odds of cirAE. Furthermore, time-to-event Cox regression identified that somatic mutations in PRKCI, STAG2, and CXCR4 as well as five additional genes (MEN1, AKT3, FANCG, DAXX, H3F3B) were significantly associated with increased risk of cirAE development (nominal p-values<.05). Mutations in CXCR4 replicated in the validation cohort as significantly associated with time to cirAE (HR = 4.02 [1.22, 13.21]) adjusting for cancer type, ICI type, and demographics. Of the CXCR4 mutations identified in patients with cirAE, 5/15 (33%) localized to the C-terminus, were predicted deleterious by SIFT and PolyPhen2, and altered key residues known to be involved in CXCR4 degradation, suggesting a mechanistic link between CXCR4 gain-of-function mutations and increased risk of cirAE. We identified somatic mutations in several genes as significant predictors of cirAE development in melanoma. The association between mutations in CXCR4 and cirAE was replicated in a pan-cancer validation cohort, providing mechanistic insight into cirAE development across cancers.

#5803 Computational modeling of immune cell phenotypes enhances prediction of clinical anti-cancer drug response.

Gema Sanz, Reka Borocz, Bence Czako, Gerold Csendes, Csaba Gor, Gabor Kovacs, Laszlo Merő, Kristof Szalay, **Daniel Veres**, Bence Szalai

Turbine Simulated Cell Technologies Ltd., Budapest, Hungary

Preclinical cancer model systems, such as large-scale drug screens in cancer cell lines, are pivotal for identifying drug-sensitive indications and biomarkers of drug efficacy. However, these systems often lack the tumor microenvironment's complexity, including interactions with immune cells, which play a critical role in modulating drug response. Investigating drug-induced effects on immune cells is experimentally challenging due to the diversity of immune cell types and the complexity of co-culture systems. Computational models of immune cells can help to overcome these limitations and to guide further experiments. Leveraging public post-perturbation single-cell RNA sequencing datasets from various immune cells (including CD8 and CD4 T cells), we trained Turbine's Simulated Cell engine to predict transcription factor (TF) activity changes in 17 immune cell types post-drug treatment. We compared the predicted, drug induced TF activity changes to immune killing effects from public co-culture experiments. Predicted activity changes of CD8 T-cell activator TFs, such as FOSL and MYC, positively correlated with tumor-killing effects (Pearson r = 0.35 and 0.42, respectively). Conversely, inhibitor TFs such as NR4A1 and IRF4 exhibited inverse correlations (Pearson r = -0.27 and -0.47, respectively).To assess clinical relevance, we analyzed oncology clinical trial data from ACTT. We calculated mean overall response rates (ORRs) for indication - drug pairs and compared it to in vitro indication specific relative IC50 values of drugs. Using a linear model to predict ORRs from in vitro cancer cell line drug response data alone, we predicted ORRs with a Pearson correlation of 0.28. Integrating simulated drug induced TF activities of CD8 T-cells improved this correlation to 0.48, showing that combining tumor-intrinsic mechanisms with immune-mediated cell killing enhances the predictive performance of the model. Our findings suggest that augmenting traditional in vitro drug screening with simulated immune cell activity significantly enhances the clinical translatability of preclinical drug response models, paving the way for improved therapeutic strategies.

#5804 Pathologic responses to neoadjuvant immune checkpoint inhibitor (ICI) combinations in head and neck squamous cell carcinoma (HNSCC) associate with specific CD4$^+$ helper and regulatory T cell activation states and clonal plasticity.

M. Brand[1], A. Kulkarni[1], H. Li[2], P. Upadhyay[1], S. I. Chiosea[2], P. M. Santos[1], B. R. Isett[1], M. Joy[1], G. L. Sica[2], U. Duvvuri[3], S. Kim[1], M. Kubik[1], F. Tu[1], T. C. Bruno[2], D. A. A. Vignali[2], A. R. Cillo[2], R. Bao[1], D. P. Zandberg[1], J. Wang[1], L. Vujanovic[1], R. L. Ferris[4];

[1]University of Pittsburgh Medical Center, Hillman Cancer Center, Pittsburgh, PA, [2]University of Pittsburgh, Pittsburgh, PA, [3]NYU Grossman School of Medicine, New York, NY, [4]UNC Lineberger Comprehensive Cancer Center, Chapel Hill, NC

Background: The clinical benefits observed with anti-PD-1 therapy in the recurrent and metastatic setting have sparked greater interest in exploring immunotherapy at an earlier stage of the HNSCC treatment regimen. However, response rates in HNSCC patients are limited, and mechanisms of treatment failure remain poorly understood. In an ongoing neoadjuvant trial, we investigate the transcriptional and spatial dynamics of tumor-infiltrating CD4$^+$ T cells in HNSCC patients treated with neoadjuvant nivolumab (anti-PD-1, Nivo) ± ipilimumab (anti-CTLA-4, Ipi) or relatlimab (anti-LAG-3, Rela). Our goal is to elucidate mechanisms of tumor-infiltrating CD4$^+$ T cells associated with positive clinical response to ICI therapy.

Methods: Pre- and post-treatment tumor samples from HNSCC patients were processed for single-cell RNA-sequencing, T cell receptor (TCR) sequencing and spatial imaging, with sequencing successfully completed for 39 patients. Specific T cell transcripts defined cellular subpopulations, revealing distinct transcriptional and clonal dynamics. Multispectral imaging of tissue sections was performed to evaluate changes in T cell composition within the tumor microenvironment. Pathologic response was linked to observed cellular dynamics to treatment outcomes.

Results: Pathologic response rates were highest in the Nivo+Rela arm (11/15, 73%) versus Nivo+Ipi (7/11, 64%) or Nivo (5/12, 42%). While both combination ICI therapies increased total CD3$^+$ T cell frequencies within tumor and stromal compartments, only Nivo+Ipi led to a significant increase of conventional CD4$^+$ T helper (Tconv) and regulatory T cells (Treg) in both regions. Notably higher CD8$^+$/Treg and Tconv/Treg ratios positively correlated with tumor rejection in the Nivo+Rela arm. In contrast to Nivo+Ipi, Nivo+Rela therapy expanded clonal diversity exclusively within Tconv populations in major responders, with a majority of emerging clones showing effector characteristics. Moreover, Nivo+Rela altered the phenotype of activated Treg to a more naïve or memory-like phenotype in major responders, suggesting a distinct regulatory landscape with implications for long-term tumor control.

Conclusion: Neoadjuvant combination ICI therapies directly influence spatial CD4$^+$ T cell composition and TCR clonal dynamics and modulate the frequency and phenotype of Tconv and Treg cells in the tumor in treatment-specific patterns. These immunological shifts correlate with tumor rejection and improve clinical outcomes. Our findings suggest that a dual ICI approach may enhance response rates beyond those achieved with monotherapies. They also indicate a possible biologic basis in CD4$^+$ T cell activity for precision ICI treatment, supporting more personalized ICI strategies in the neoadjuvant setting.

#5805 Immunomodulatory effects of TG2 in the ovarian cancer tumor microenvironment.

Hao Huang, Ping Xie, Ujin Kim, Longzhen Song, Jianjun Wei, Daniela Matei, Bin Zhang

Northwestern Univ. Feinberg School of Medicine, Chicago, IL

Background: Transglutaminase 2 (TG2), an enzyme highly expressed in ovarian cancer (OC) and secreted in malignant ascites, is a key driver of peritoneal metastasis. Previous research has focused on TG2 activity within cancer cells, while the role of TG2 in the tumor microenvironment remained unexplored.
Methods: To investigate the impact of host TG2 deficiency on antitumor immunity and tumor progression, we injected ID8 or BPPNM cells intraperitoneally (i.p.) into TG2–/– (TG2KO) and wild-type (WT) (TG2+/+) mice. Synergy with PD-1/PD-L1 immune checkpoint blockade was also investigated. Tumor immune infiltrates within the ascites microenvironment or tumors were determined by high-dimensional spectral flow cytometry (CyTEK) and multiplex IHC (mIHC). To validate the spatially correlated expression of TG2 in tumor and stromal regions with immune cell signaling programs, we conducted spatially indexed transcript profiling (GeoMx digital spatial profiling) of FFPE OC sections.
Results: Significantly reduced ascites volume, tumor size, and weight ($p<0.05$) were observed after ip inoculation of ID8 or BPPNM murine cells in TG2 KO vs. WT mice. Survival of TG2 KO mice bearing BPPNM isografts was longer ($p<0.0001$) compared to WT mice (64 vs. 46 days). Treatment with anti-PD-L1 antibody inhibited tumor growth in WT and TG2 KO mice, with reduction in ascites and tumor weight in TG2 KO vs control mice ($p=0.223$ and $p=0.053$, respectively). There was an overall tendency toward increased CD45+ immune cell density within the TG2-/- ascites microenvironment upon anti-PD-L1 treatment. Frequencies of NK and NKT among CD45+ tumor infiltrates in tumor-bearing TG2-/- mice were increased following anti-PD-L1 compared to the control mice. By contrast, the frequency of immunosuppressive TAMs and PMN-MDSCs was decreased. Although there was no difference in the frequency of B cells, dendritic cells (DC), CD4+ Treg, and CD8+ exhausted-like T cells (TEX) between control and TG2-/- mice, stem-like progenitor exhausted CD8+ T cells were more abundant in TG2-/- mice following anti-PD-L1 treatment. There was a trend to increased density of CD8+ T cells in TG2-/- mice after anti-PD-L1. Tumor-promoting PD-L1+CD11b+ myeloid cells were decreased in tumors from TG2-/- mice following anti-PD-L1 compared with control mice.Co-staining of TG2 and CD8 was used for spatial segmentation analysis in human tumors. TG2 expression in tumor cells and $CD8^+$ T cell infiltration were inversely correlated in stromal areas. An in-depth gene pathway enrichment analysis is underway.
Conclusion:The data support the potential of TG2 inhibition to mitigate OC immune evasion and sensitize tumors to immune checkpoint inhibitors.

#5806 Analyzing tumor treating fields (TTFields) therapy combined with checkpoint inhibitors in a GBM mouse model.

S. Lee[1], C. C. Perry[2], K. Cho[1], M. Lim[1], G. Li[1], R. T. Nitta[1];
[1]Stanford University School of Medicine, Palo Alto, CA, [2]Stanford University, Palo Alto, CA

Glioblastoma (GBM) is the most common malignant form of adult brain tumor, with a median survival of around 15 months. Despite multimodal treatments (tumor resection, radiotherapy, and chemotherapy) achieving an effective cure remains a significant challenge due to its highly aggressive nature. Immune checkpoint inhibitors have emerged as a promising strategy to combat GBM; however, limitations have hindered clinical success, largely due to the “cold” and immunosuppressive tumor microenvironment (TME). Previous studies have demonstrated that the TME can transform from “cold” to “hot” by upregulating tumor-specific antigens, thereby enhancing immune cell recognition. Tumor Treating Fields (TTFields) are a non-invasive treatment for solid tumors that employs specific frequency ranges (100-500 kHz) delivered through transducer arrays placed on the body. TTFields are currently being investigated for various tumor types and have received FDA approval and shown efficacy in GBM, pleural mesothelioma and metastatic NSCLC following platinum-based therapy. In our present study, we studied the effects of TTFields treatments on the immune response in GBM by using a syngeneic murine glioma model. Our initial in vitro studies demonstrated that TTFields enhanced the immune response in GBM by inducing immunogenic cell death, which led to the recruitment of immune cells through the release of damage-associated molecular patterns (DAMPs). Additionally, co-culture experiments with splenocytes and GBM cells treated with TTFields showed an increase in activated dendritic cells and macrophages, as well as early activated T cells (CD69/CD4+, CD69/CD8+). We verified these findings by demonstrating TTFields increased T-cell activity (IFNγ and perforin), and enhanced the motility and phagocytic activity in antigen presenting cells (Raw 264.7) and dendritic cells (JawsII). Since our in vitro findings indicated that TTFields enhanced the immune response, we studied the therapeutic potential of directly applying TTFields (200kHz) to a subcutaneous syngeneic GBM mouse model (GL261-luciferase) using the inovivo system. We observed a 2- to 4-fold decrease in tumor volume with mice that received TTFields treatment with the subcutaneous array compared to the control group (n = 8). In our subsequent studies, we anticipate that combining TTFields and the checkpoint inhibitor anti-PD-1 will result in greater tumor regression than TTFields monotherapy. Our findings suggest that concomitant treatment of TTFields with systemic anti PD-1 therapy has the potential to help transform GBM into a “hot” tumor, making it a more attractive target for immunotherapy.

#5807 Differential T cell response to anti-PD1 in breast cancer Sub-types is driven by activity of intra-tumoral immune cells.

Mouniss MM[1], Biswajit Das[1], Kowshik Jaganathan[1], Syamkumar V[1], Moumita Nath[1], Chandan Bhowal[1], Dharanidharan M[1], Saikrishna S[1], Abdul Haseeb[1], Pallavi R[1], Kubera Chandran[1], Rajashekar M[1], Oliyarasi M[1], Mehul Kapur[1], Jayaprakash C[2], Venkatesh T[2], Ganesh MS[3], Amritha Prabha[3], Prakash BV[4], Ravi Krishnappa[5], Upendra K[1], Ritu Malhotra[1], Govindaraj K[1], Pavithira .[1], Mohit Malhotra[1], Nandini Pal Basak[1], **Satish Sankaran**[1]

[1]Farcast Biosciences India Pvt. Ltd., Bangalore, India,[2]DBR & SK Super Speciality Hospital, Tirupati, India,[3]Vydehi Institute of Medical Sciences and Research Centre, Bangalore, India,[4]Sri Lakshmi Multi Speciality Hospital, Bangalore, India,[5]JSS Hospital, Mysuru, India

Immune checkpoint inhibition (ICI) holds great promise for triple-negative breast cancer (TNBC), while it shows limited response hormone receptor positive (ER/PR+) breast cancer (CaBr). We employed the Farcast CaBr TruTumor histoculture platform, that preserves the native tumor microenvironment (TME), to study the role of tumor resident immune cell types in determining T cell activation levels on ICI treatment, in the two CaBr sub-types. Freshly resected tumor tissue samples along with matched blood were collected from consented patients. Tumor explants were generated and distributed into arms and cultured for 72 h. Media was replenished every 24 h and the supernatant was stored. The response to stimulation with anti-CD3 (100 ng/mL) + Interleukin-2 (IL-2, 100 IU/mL) and treatment with Nivolumab (132 µg/mL) was evaluated using cytokine release and flow cytometry based immune profiling. CaBr (n=115) samples had lower immune component than head and neck squamous cell carcinoma (n=113) ($p<0.0001$) but similar to ovarian (n=21) and renal cell (n=52) cancer prior to treatment with ICI. Amongst the two sub-types, immune component in TNBC (n=43) was higher than ER/PR+ subtypes (n=46) ($p<0.05$). TNBC (n=10) showed a greater proportion of lymphocyte (58.46%) compared to myeloid (39.21%) compartment. This bias was not observed in ER/PR+ samples (n=12). Anti-CD3+IL2 stimulation showed similar response between the two CaBr sub-types. Upon treatment with Nivolumab, 2 out of 4 TNBC samples exhibited a response phenotype, with >1.6-fold increase in CD8+GzmB+ cells and interferon-gamma (IFN-γ) release. In contrast, only 1 out of 4 ER/PR+ CaBr samples showed a modest increase of 1.3 fold for CD8+GzmB+ cells with no detectable IFN-γ release. To understand the basis for the differential response in the two sub-types, we studied in detail, one ER/PR+ (H1) and two TNBC (T1 and T2) samples, displaying varying levels of response. T2 and H1 did not show a Nivolumab response phenotype, whereas T1 demonstrated a strong T-cell reinvigoration and tumor cytotoxicity. Interestingly, all three samples showed anti-CD3+IL2 stimulation driven T cell response. tSNE analysis of CTLs in the control arm showed two distinct sub-populations of exhausted CTLs (CD8+PD1+). Population1 (Pop1) was Granzyme B-positive, while population2 (Pop2) was not. Upon anti-CD3 stimulation, Pop1 showed further increase of activation whereas Pop2 did not, indicative of Pop2 being an irreversibly exhausted T cell population. T2 notably had lower Pop1 and Pop2 CTLs, along with highest proportion of monocytes across all three samples pointing towards an immunosuppressive TME. H1 mainly contained over-exhausted Pop2 and negligible Pop1 CTLs. Interplay between different TME immune sub-types, thus influence response to Nivolumab in CaBr. This is effectively captured by the TruTumor platform.

#5808 Differential clinical outcomes of immune vs non-immune based therapeutics in gastrointestinal malignancies: A retrospective, single center experience of early phase studies.

Tarik Demir[1], Nicole Altomare[2], Katrina Dobinda[3], Ruohui Chen[3], Carolyn Moloney[1], Pedro Hermida de Viveiros[1], Aparna Kalyan[1], Young Kwang Chae[1], Devalingam Mahalingam[1]

[1]Division of Hematology and Oncology Developmental Therapeutics Institute, Northwestern University, Chicago, IL,[2]Northwestern University, Chicago, IL,[3]Preventive Medicine; Feinberg School of Medicine, Northwestern University, Chicago, IL

Introduction: Evidence suggest that the immunosuppressive environment of liver parenchyma, leads to a specific resistance to immunotherapy (IO) in patients with liver metastasis (LM). This study seeks to evaluate the outcomes of patients (pts) with advanced gastrointestinal (GI) cancer who have undergone treatment with novel therapeutics (IO vs non-IO agents) within early-phase clinical trials. Further, we aim to identify oncogenic molecular signaling pathways and phenotypes that may indicate a poor response to IO in patients with LM.
Methods: This retrospective study focused on patients treated at Northwestern University from August 2014 to August 2023, who were enrolled in early-phase clinical trials and had CLIA certified next-generation sequencing available. Standard radiologic evaluations were per study, and efficacy based on progression-free survival (PFS) were defined as responders (PFS ≥ 3 months) vs non-responders (PFS < 3 months). Survival curves were calculated using the Kaplan-Meier method. For responder vs non-responder analyses, logistic regression assessed the impact of clinical variables on response, with significance defined as $p \leq 0.05$.
Results: 265 pts participated in this study with 11 pts participating in two studies during the course of their visits to the unit. The median age was 65 years (range 55-72), with 52% being male. Three tumor types were represented: colorectal (49%), pancreatic (43%), and gastroesophageal (8%) cancers. 218 (82%) pts had documented LM. 102(39%) patients were treated with IO based vs 157 (61%) in non-IO based therapeutics. When evaluating efficacy (n=276), the median PFS was 2.28 months, and 43% of patients had a PFS rate of ≥ 3 months. We found that pts with treated non-IO-based therapy were associated with higher PFS, median 3.48m (1.68-6.12m) vs 1.8m (1.4-3.48) for IO-based therapy ($p<0.0001$). In contrast to other studies, LM did not have prognostic significance for patients treated with IO ($p=0.49$). Among IO-based LM patients, KRAS (OR: 6.35, $p=0.028$) and TP53 (OR:4.23, $p=0.044$) pathway mutations were significantly associated with higher odds of non-response. No significant differences were found in the pathways involving APC, CDKN2A, ARID1A, PIK3CA, PTEN, SMAD4, NOTCH1, RB1, FBXW7, and FGF4.
Conclusion: In contrast to findings from earlier studies, our data did not establish a significant association between LM and survival outcomes in patients receiving IO. This discrepancy may result from the variety of IO regimens used in early-phase trials and the limited number of patients. Nonetheless, our findings contribute valuable insights into this complex area, offering important perspectives for future research and potential therapeutic strategies, particularly as IO treatments evolve and more extensive patient data becomes available.

#5809 Spatially resolved immunologic hallmarks of response to neoadjuvant immune checkpoint blockade in metastatic melanoma.

Zichao Liu[1], Xiaofei Song[2], Jodi Balasi[2], Wei-Shen Chen[3], Jiang He[4], Justin He[4], Jonathan Nguyen[2], Carlos Moran-Segura[2], Joseph Johnson[2], Chaomei Zhang[2], Jane Messina[2], Zena Sayegh[2], Nan Sun[2], Douglas Marchion[2], Sean Yoder[2], Vernon K. Sondak[2], Jeffrey H. Chuang[1], Pei-Ling Chen[2]

[1]The Jackson Laboratory for Genomic Medicine, Farmington, CT,[2]H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL,[3]USF Medical Group, Tampa, FL,[4]Vizgen Inc., Cambridge, MA

Background: The groundbreaking SWOG S1801 and NADINA trials herald the use of neoadjuvant immune checkpoint blockade (NICB) as the standard of care for stage III-IV melanomas. The shift from radiographic to pathologic response scoring offers an unprecedented window of opportunity to interrogate 'on-treatment' biospecimens for the immune mechanisms of response and resistance at curative-intent surgeries. To date, single-cell/bulk RNA studies have demonstrated that TCF7+ stem-like CD8+ T cells and tertiary lymphoid structures (TLS) are positive predictors of ICB response. However, how these immune cells organize and communicate within the spatial context of the neoadjuvant tumor microenvironment remains poorly understood.
Methods: We assembled a retrospective cohort of 68 patients with stage 3 melanoma: 35 NICB (Ipilimumab-nivolumab, nivolumab-relatlimab, nivolumab) (14 complete response, 5 partial response, 14 non-response) and 33 treatment-naive (TxN). We dissected the spatial molecular architecture of all tumor beds (n= 68) and 10 uninvolved LNs (5 post-PD-1/5 TxN) using integrative multiplexed error-robust fluorescence in situ hybridization (MERFISH, 305 genes), Vectra multiplex IF and RNAscope multiomics in 38 whole slides and 3 TMAs (77 cores). We also performed single-cell-FFPE-seq using matched blocks (n=12) as an orthogonal benchmark for the MERFISH data.
Results: To analyze these high-dimensional datasets, we built a computational framework that includes 1) cell-typing with scFFPEseq validation, 2) novel spatial quantification methods to compute receptor-ligand (R-L) interactions that account for cell-cell distance and chemical signaling, 3) a graph-based clustering that quantifies germinal centers (GCs)/TLS, B-cell follicles and other spatial topography, and 4) MERFISH/ scRNA imputation via generative AI. Here, we showed that the quantity and size of spatially resolved GC/TLS and B-cell follicles surrounded by TCF7+ stem-like CD4+ and CD8 + T-cells, plasma cells, Tregs and myeloids are associated with positive response to neoadjuvant ICB. Our R-L analysis further revealed the critical importance of CXCR4-CXCL12 between TCF7+ T-cells and M2-myeloids and CCR7-CCL19/21 between TCF7+ T-cells in organizing the immune hubs in NICB response. Lastly, we incorporated scFFPEseq and MERFISH data into a unified latent embedding by environmental variational inference (ENVI) allowing us to impute melanoma phylogenetics, geographically accentuated hypoxic foci, and immune hubs within the spatial MERFISH images.
Conclusions: We leveraged cutting-edge spatial -omics technologies and novel computational methods to resolve the immunologic hallmarks of NICB response in metastatic melanoma. We believe our approach will usher in a new paradigm for the investigation of other cancer biospecimens in the new era of standard of care neoadjuvant immunotherapy.

#5810 Distinct mechanisms of CD28 and 4-1BB costimulation in glofitamab combination therapies for relapsed/refractory non-Hodgkin lymphoma (R/R NHL).

K. Korfi[1], Z. Jiang[2], S. Wilson[3], A. Christiansen[4], W. Leung[5], K. Lechner[6], N. Dimier[3], P. Knuckles[2], K. Reyskens[4], I. Lelios[2], A. Blank[1], A. Epp[6], S. Herter[1], J. Sam[1], P. Caimi[7], L. Falchi[8], R. Houot[9], F. Offner[10], H. Walter[11], H. Ghesquieres[12], G. Cartron[13], W. Townsend[14], G. Gritti[15], F. Bosch[16], M. Dickinson[17], C. Carlo-Stella[18], M. Hutchings[19], F. Morschhauser[20];
[1]Roche, Schlieren, Switzerland, [2]Roche, Basel, Switzerland, [3]Roche, Welwyn, United Kingdom, [4]A4P Consulting Ltd, Sandwich, United Kingdom, [5]Genentech, San Francisco, CA, [6]Roche, Penzberg, Germany, [7]Cleveland Clinic Taussig Cancer Institute, Cleveland, OH, [8]Memorial Sloan Kettering Cancer Center, New York, NY, [9]Centre Hospitalier Universitaire Rennes, Rennes, France, [10]Universitair Ziekenhuis Gent, Gent, Belgium, [11]University of Leicester and University Hospitals Leicester, Leicester, United Kingdom, [12]Hospices Civils de Lyon - Centre Hospitalier Lyon Sud, Lyon, France, [13]Centre Hospitalier Universitaire Montpellier, Montpellier, France, [14]University College London Hospitals NHS Foundation Trust, London, United Kingdom, [15]ASST Papa Giovanni XXIII, Bergamo, Italy, [16]University Hospital Vall d'Hebron, Barcelona, Spain, [17]Peter MacCallum Cancer Center and Royal Melbourne Hospital, Melbourne, Australia, [18]Humanitas University and IRCCS Humanitas Research Hospital, Milan, Italy, [19]Rigshospitalet and University of Copenhagen, Copenhangen, Denmark, [20]Univ. Lille, GRITA - Groupe de Recherche sur les formes Injectables et les Technologies Associees, Lille, France

Background: Two recent Phase I studies (NCT04077723, NCT05219513) investigated CD19-targeted 4-1BB or CD28 costimulatory bispecifics combined with glofitamab in patients with R/R NHL, showing added efficacy benefit but with distinct response dynamics (Hutchings et al; Dickinson et al, ASH 2024). While both costimulators are designed to enhance the anti-tumor effects of T-cell engagers, we present, for the first time, key differences in the mechanisms and response profiles of CD28 and 4-1BB costimulation in these combinations.
Methods: Exploratory biomarker analyses included 28 indolent and aggressive NHL patients treated with RO7443904 (CD19-CD28) and 99 with englumafusp alfa (CD19-4-1BBL) on Cycle 2 (C2) Day 8, after completion of glofitamab step-up dosing, followed by administration on the same day as glofitamab from C3 onwards (Q3W) for a fixed duration of 12 cycles. Peripheral immune profiling was done by flow cytometry, and gene expression and mutational profile of the tumors were characterized by mRNA- and targeted DNA-sequencing. On-treatment pharmacodynamic changes were compared to historical glofitamab data from NCT03075696 (n=140). The cross-trial comparisons were adjusted for differences in baseline characteristics and sample size.
Results: Compared to glofitamab monotherapy, both costimulators boosted and sustained peripheral T-cell activation and proliferation, with 4-1BB primarily expanding HLA-DR+ CD8 cells (1.5-fold), indicating prolonged activation, and CD28 boosting CD4/CD8 proliferation (2-fold). Additionally, 4-1BB further increased both effector and central memory CD8 cells (1.5-fold), while CD28 selectively expanded effector memory CD8 cells (1.5-fold) relative to glofitamab alone. Both costimulators limited PD1+ T-cell expansion in a dose-dependent manner, with 4-1BB mainly affecting Temra cells and CD28 targeting effector memory cells. Complete responses occurred independently of recurrent mutations (e.g. *TP53, CREBBP, B2M*) or cell-of-origin. However, tumor-infiltrating conventional dendritic cells (cDCs) were linked to response to 4-1BB and immunosuppressive macrophages were linked to lack of response to CD28, as determined by gene expression profiling of pre-treatment biopsies.
Conclusion: Our findings reveal distinct mechanisms of CD28 and 4-1BB costimulation with glofitamab, which may explain their different response dynamics. Specifically, 4-1BB promotes a sustained and prolonged immune response, while CD28 triggers rapid T-cell activation and proliferation. Moreover, 4-1BB appears to bypass macrophage suppression, and benefits from cDC-mediated antigen presentation and CD8 T-cell priming. These effects mirror those observed in second-generation CAR T-cell therapies and provide insight into how these off-the-shelf costimulators may be utilized based on tumor biology.

#5811 ABL103, a bispecific antibody targeting B7-H4 and 4-1BB, shows synergistic immune responses with combination of pembrolizumab in the immunosuppressive tumor microenvironment.

Chorong Yang, Eunhee Lee, Yangsoon Lee, Daehae Song, Byungje Sung, Jiseon Yoo, Kyungjin Park, Jonghwa Won

ABL Bio, Inc., Gyeonggi-do, Korea, Republic of

B7-H4 (B7x, VTCN1) is overexpressed in many types of cancers, including breast, ovarian, and endometrial cancers. B7-H4 expression is also observed in tumor associated macrophages and implicated as an immune checkpoint regulating T cell responses. 4-1BB (CD137/TNFRSF9) is an inducible co-stimulatory receptor and expressed in a wide variety of immune cell types, including activated T cells, natural killer (NK) cells, dendritic cells (DCs). Previously, we demonstrated that ABL103, B7-H4x4-1BB bispecific antibody, induced *in vitro* T cell activation only in the presence of B7-H4-expressing tumor cells through B7-H4-dependent 4-1BB clustering, resulting in strong *in vivo* antitumor effects. While ABL103 augmented T cell infiltration and the ratio of intra-tumoral $CD8^+$ T/Treg in tumor microenvironment (TME), no systemic immune response was observed. Mutually exclusive expression pattern of B7-H4 and PD-L1 raised the question on combination effect of ABL103 and PD(L)-1 inhibitors. While most ovarian and triple negative breast cancer patient tissues showed B7-H4 expression, only a few tissues showed PD-L1 expression. To understand how ABL103 modifies TME alone or in combination with immune checkpoint inhibitors, ABL103 was treated alone or in combination with pembrolizumab in two mouse models including MC38/hB7-H4 (immune hot tumors) and B16F10/hB7-H4 (immune cold tumors). In the advanced murine tumor model of MC38/hB7-H4, ABL103 strongly suppressed tumor progression alone or in combination with pembrolizumab. Marked increase of T cell infiltration was observed in the combination group compared to ABL103 alone or pembrolizumab alone. In the rapidly growing B16F10/hB7-H4 tumor model, pembrolizumab alone failed to suppress tumor progression and did not induce T cell infiltration. However, combination of pembrolizumab and ABL103 showed a marked increase of intra-tumoral $CD8^+$ T cells. Interestingly, pembrolizumab induced 4-1BB expression on CD8 T cells, whereas ABL103 induced PD-L1 expression in $CD11b^+$ cells when combined with pembrolizumab. The combination group also showed an increased proportion of MHC I/II double positive macrophages. These results support the interactive immune activation of ABL103 and pembrolizumab, which leads to synergistic activation of T cells and transition of cold TME to inflammatory TME. Overall, our study suggests that ABL103 could be a promising therapeutic antibody as monotherapy as well as combination strategy with pembrolizumab by reinvigorating immune responses in the immunosuppressive TME.

#5812 Single-cell dissection of checkpoint inhibitor hepatitis reveals insights into immune tolerance in the human liver.

Molly Fisher Thomas[1], Thomas Chan[2], Neal Smith[2], Marc Sherman[3], Swetha Ramesh[2], Alice Tirard[2], John McGuire[2], Mazen Nasrallah[4], Kasidet Manakongtreecheep[2], Jessica Tantivit[2], Leyre Zubiri[5], Dejan Juric[5], Ryan Sullivan[5], Nir Hacohen[6], Genevieve Boland[7], Georg Lauer[3], Kerry Reynolds[5], Alexandra-Chloe Villani[2]

[1]Division of Gastroenterology, Department of Medicine, Oregon Health & Science University, Portland, OR,[2]Center for Immunology and Inflammatory Diseases, Department of Medicine, Massachusetts General Hospital, Boston, MA,[3]Division of Gastroenterology, Department of Medicine, Massachusetts General Hospital, Boston, MA,[4]Division of Rheumatology, Department of Medicine, North Shore Physicians Group, Lynn, MA,[5]Division of Hematology-Oncology, Department of Medicine, Massachusetts General Hospital, Boston, MA,[6]Broad Institute of Harvard and MIT, Cambridge, MA,[7]Department of Surgery, Massachusetts General Hospital, Boston, MA

Background: Immune checkpoint inhibitors (ICIs) to treat cancer have revolutionized immuno-oncology. However, therapy is limited by frequent immune-related adverse events, including immune-mediated liver injury from checkpoint inhibitors (ILICI), which occurs in ~1-17% of patients on anti-PD-1 and/or CTLA-4 therapy. While ILICI is often mild, it can cause severe hepatic dysfunction, delay cancer therapy, and require immunosuppressive treatments that may compromise anti-tumor immune responses. ILICI shares clinical and histologic features with autoimmune hepatitis (AIH). Therefore, the study of ILICI and AIH is important for understanding how immune tolerance is lost across a spectrum of inflammatory liver diseases.
Methods: To characterize the cellular and molecular underpinnings of ILICI, we used single-cell and single-nuclei RNA sequencing with paired antigen receptor sequencing to characterize ~300,000 cells from paired liver and blood specimens from 23 patients (9 ILICI, 4 AIH, 3 controls on ICB, 7 controls not on ICB) and ~150,000 peripheral blood cells from an additional 28 control patients on ICB. ILICI was defined by a hepatocellular or cholestatic rise in liver function tests and centrilobular or panlobular inflammatory liver injury. Controls had drug-induced liver injury, hepatic steatosis, or venous outflow obstruction.
Results: Both AIH and ILICI patients showed marked expansion of clonally-related liver *CXCR6*^{Hi} *GZMK*^{Hi} tissue-resident memory CD8 T cells expressing *IFNG* and *GZMB* and multiple CD4 T cell populations including cytotoxic *GZMA*^{Hi} effectors, *CXCL13*^{Hi} effectors, and T regulatory cells. Compared to patients with AIH, those with ILICI showed a marked expansion of intravascular *CX3CR1*^{Hi} CD8 T cells and a relative depletion of tissue dendritic cells while only patients with AIH had expanded plasma B cells. Analysis of matched blood samples from the same patient cohort revealed how cellular and transcriptional signatures in the liver were mirrored in circulating immune cells. Further analysis revealed that patients with ILICI and AIH had a marked interferon stimulated gene signature in a subset of hepatocytes, increased cholangiocyte progenitors, and increased periportal liver endothelial cells, which suggests significant liver parenchymal remodeling. Lastly, we identified a *CXCL10*-expressing cholangiocyte population that was expanded in both AIH and ILICI and was most pronounced in an individual with checkpoint inhibitor-associated cholangitis.
Conclusions: In defining the cellular and transcriptional programs that are altered in ILICI and AIH, we have identified novel immune perturbations implicating PD-1 and CTLA-4 in liver immune tolerance. These discoveries offer promising avenues for the development of targeted diagnostics and therapeutics in the management of liver-related toxicities associated with immunotherapy.

#5813 Evolution of the tumor immune microenvironment (TIME) in hepatocellular carcinoma (HCC) with liver metastases treated with atezolizumab-bevacizumab (INTEGRATE).

Harry Myles Harvey[1], Stephanie Wong[2], Selina Melillo[2], Jehan Vakharia[2], Simone Stone[2], Azin Sayad[1], Ben Wang[2], Pamela Ohashi[2], Rachel Garonce-Hediger[3], Raymond Jang[1], Robert C. Grant[1], Eric X. Chen[1], Jennifer Knox[1], Adrian Sacher[2], Leo M. L. Chan[2]

[1]Department of medical oncology, UHN Princess Margaret Cancer Centre, Toronto, ON, Canada,[2]Department of Immunology, University of Toronto, Toronto, ON, Canada,[3]F. Hoffmann LaRoche, Basel, Switzerland

Background: HCC is the most common form of primary liver cancer and the 3rd leading cause of cancer death globally. Combination of atezolizumab-bevacizumab (AB) demonstrated an impressive 19-month median survival in 1st line HCC (IMbrave-150) and a HR (0.66) that has not been surpassed by newer combinations and remains an important standard of care with an ORR of 32%. Despite advancements in the treatment of advanced HCC, ~30% of patients demonstrate no response to therapy. It is theorized that there is a dynamic interplay between the liver TIME and HCC. We performed an exploratory evaluation of the evolution of the TIME in HCC treated prospectively with AB

Methods: The study was carried out at Princess Margaret Cancer Centre between July 2021 and June 2024. Patients had a histologically confirmed diagnosis of inoperable HCC, Child's Pugh Class A and were receiving AB. Biopsies of tumor and normal liver were performed at pre-treatment and at the time of progression. Blood and stool samples were collected at pre-treatment, 3-4 weeks post initiation of therapy and the time of progression. Blood samples were analyzed for cfDNA, alpha-fetoprotein (AFP) and immune cell phenotyping using 30+ marker spectral flow cytometry panels and stool samples were analyzed for microbiome.

Results: The analysis included 8 treatment response evaluable patients with paired tumor samples, median age of 63 (46-77) years, 5 patients had hepatitis B/C as a risk factor for HCC, 2 non-alcoholic and 1 alcoholic liver disease. 3 patients had progressive disease at 1st interval CT, with 2 patients had ongoing response after >30 months. Patients with a baseline frequency of circulating cytotoxic CD8+ T_{EFF} cells (effector T cells) above the median value (3.78%) exhibited numerically improved PFS (P=0.0265) compared to those with lower frequencies (median PFS: 12.67 months vs. 2.4 months; HR 0.12, 95% CI: 0.01-1.08). T_{EFF} cells are terminally differentiated (TCF1-CD57+), but not exhausted ($PD1^{low}TIGIT^{low}$).

Conclusion: A greater knowledge of the TIME and how it changes with therapy is key to understand which patients are likely to respond to immune checkpoint inhibition. Our findings suggest that enrichment of preexisting, circulating cytotoxic CD8+ T_{EFF} cells may be important for mediating treatment responses to immune checkpoint inhibition in HCC patients. Deep immune profiling is being conducted on serial tumor biopsies using combined single-cell TCR sequencing, and transcriptomic and protein expression analysis (CITE-Seq) to characterize tissue-specific immune responses and identify potential virus-specific T cell clones. This analysis will be updated and has the potential to identify mechanistic biomarkers predictive of response or resistance to treatment.

#5814 Immune-related adverse effects in patients with autoimmune disorders treated with immune checkpoint inhibitors.

Aseel Saadeh[1], Pragosh Saini[2], Favour Nelson[1], Sumera Khan[3], Christian Adonizio[3], Ahmad Hanif[3]

[1]Internal Medicine, Geisinger Medical Center, Danville, PA,[2]Geisinger Commonwealth School of Medicine, Scranton, PA,[3]Hematology and Oncology, Geisinger Wyoming Valley Medical Center, Wilkes Barre, PA

Background: Immune checkpoint inhibitors (ICI) have tremendously improved outcomes for various cancers but can be associated with immune-related adverse effects (IrAE), which can affect multiple organ systems. For patients with pre-existing autoimmune diseases (AD), there is an added risk of exacerbating autoimmunity, either through a flare of prior IrAEs or the development of de novo IrAEs during ICI therapy. Clinical trials leading up to FDA approval of ICIs excluded patients with pre-existing AD, resulting in a lack of data on the specific risks faced by this patient population. Our study aims to assess whether patients with pre-existing AD are more likely to develop IrAEs when treated with ICIs, and if so, whether these IrAEs are more severe.

Methodology: We conducted a retrospective chart review of all patients diagnosed with AD and received ICIs for treatment of a solid tumor malignancy within the Geisinger Health System from January 1, 2017, to December 31, 2023. We analyzed the occurrence, subtype, and severity (grade) of IrAEs based on the Common Terminology Criteria for Adverse Events (CTCAE). Additionally, we assessed steroid use, treatment discontinuation, and hospitalization rates.

Results: A total of 250 patient charts were identified using the cancer registry, 105 of them were excluded due to the absence of a pre-existing AD. Of the 145 patients with a pre-existing AD, 59 (40.7%) developed an IrAE, and 21 (14.5%) developed a severe (grade 3/4) IrAE. Additionally, 44 (30.3%) patients required steroids for the treatment of IrAE, and 12 (8.3%) were hospitalized due to an IrAE. The most prevalent IrAEs were colitis, rash, hypothyroidism, transaminitis, and pneumonitis, which occurred in 52 (35%) cases; of these, 13 (8.9%) were severe (grade ¾).

Discussion: When comparing our results to those of previous studies, we found that the rates and severity of IrAEs were higher in patients with pre-existing AD compared to historical data on patients without AD. A meta-analysis by de Velasco et al. of 21 studies reported an IrAE incidence of 28% for all grades and 5% for grades 3 and 4. Similarly, Silverstein et al. analyzed 3,137 patients treated with ICIs and found that 3.8% were hospitalized due to IrAEs. These significant differences underscore the need for multidisciplinary collaboration and heightened monitoring for IrAEs in patients with AD.

#5815 Both CD4 and CD8 T cells mediate antitumor immunity to prevent development of neoantigen-expressing autochthonous sarcomas.

Sabelo Mndeni Lukhele[1], David G. Kirsch[1], Jonathon E. Himes[2]

[1]Princess Margaret Cancer Centre (UHN), Toronto, ON, Canada,[2]Department of Pharmacology and Cancer Biology, Duke University, Durham, NC

Sarcoma is a rare, aggressive and highly metastatic disease. The efficacy of current treatment approaches including surgery, chemotherapy and radiation is limited hence the recent focus in novel immunotherapy. However, immunotherapy research for sarcoma is impeded by our limited understanding of the antitumor immune responses, largely due to the lack of faithful mouse models expressing known sarcoma neoantigens. Here, we combined CRISPR/Cas9 and sleeping beauty transposase technology to develop an autochthonous orthotopic murine sarcoma model that allows the tumor to coevolve with the immune system and expresses MHCI (mLama4) and/or MHCII (mItgb1) sarcoma neoantigens. We show that expression of mLama4 and/or mItgb1 neoantigens prevented tumor development in a T-cell dependent manner. Indeed, tumors expressing both neoantigens only developed following depletion of both CD4 and CD8 T cells. Similarly, growth of mItgb1-expressing tumors was observed only in CD4 T cell deficient mice. Tumor-specific T cells were detected, using MHC-mLama4/mItgb1 tetramers, as early as ten days post tumor induction and persisted for more than three months. Both CD4 and CD8 tumor-specific T cells were activated, in cycle, and produced effector cytokines IFNγ and TNFα, suggesting that they both mediate antitumor immunity in part by direct killing of tumor cells. Thus, CD4 and CD8 T cells independently contribute to cancer immunosurveillance leading to clearance of neoantigen-expressing sarcomas.

#5816 TCR clonal tracking maps tumor-draining lymph nodes as reservoirs fueling neoadjuvant PD-1 blockade in resectable NSCLC.

Rachel L. Honigsberg[1], Tatiana Cruz[2], Geoffrey Markowitz[2], Liron Yoffe[2], Stephanie Tang[2], Marissa Micheal[2], Ozge Dicle[2], Olivier Elemento[2], Nasser Altorki[2], Jonathan Villena-Vargas[2]

[1]Cornell University, Ithaca, NY,[2]Weill Cornell Medicine, New York, NY

Introduction: PD-1 blockade with platinum chemotherapy (chemo/IO) has transformed treatment for resectable non-small cell lung cancer (NSCLC). However, gaps in understanding the adaptive T cell response impede progress, resulting in a lack of predictive biomarkers and mechanism-informed combination therapies. We hypothesize that novel T cell clones activated in the tumor draining lymph node (tdLN) in response to PD-1 blockade are critical drivers of therapeutic outcomes.
Methods: Tumor, tdLNs (stations 11-12), and peripheral blood (PB) were collected from 16 NSCLC patients (stage I-IIIB). Nine patients received neoadjuvant chemo/IO, while seven were treatment naïve. Samples underwent pathological evaluation and stratified by pathological response based on residual viable tumor. Clonal dynamics were assessed across tissues using single-cell TCR barcoding integrated with transcriptional profiling (scRNASeq) by using machine learning (ML) approaches.
Results: Paired scRNA/TCR sequencing of 670,000 T cells and 110,000 clones from 47 tissue samples revealed deep clonal dynamics across tumor, tdLN, and PB. Tumor matched and expanded ($n \geq 10$) shared TCRs between tumor and tdLN (TM clones) accounted for 5% of clones, 75% of which were found in the PB (3.7% of clonal population). Chemo/IO-treated patients exhibited an increase in expanded TM clonal diversity (69 vs. 47 clones/1000 expanded T cells, $p = 0.07$) and a higher proportion of TM T cells within expanded T cells across all three tissues (83% vs. 68%, $p < 0.05$). Notably, 12 of 111,000 unique TCRs were shared across at least two patients. Clonal fate mapping using TCR sequences and UMAP clustering revealed distinct phenotypic trajectories. TM tdLN-resident clones were enriched in stem-cell quiescence genes (SELL, TCF7, CCR7) and signatures ($p < 0.001$), relative to the cytotoxic and exhausted tumor-infiltrating T cells (TILs) ($p < 0.001$). Chemo/IO patients' tdLN and TILs exhibited T cell activation via AP-1 pathway ($p<0.05$), while untreated patients' TILs were exhaustion. Patients with pathologic response had a less differentiated TM TILs compared to non-responders. ML further identified a 280-gene activation signature indicative of tdLN-linked responses to PD-1 blockade ($p < 0.05$) led by CXCL13 and CXCR6, validated in a resectable murine model. Persisting TM clones circulating in PB 6 months post-resection retained effector-memory phenotypes (CXCR3, TCF7, CD44).
Conclusion: Our findings highlight the distinct role of tdLNs in contributing to treatment responses to PD-1/PD-L1 blockade. This alternative mechanism appears driven by tdLN-mediated T cell activation and tumor infiltration, rather than the reactivation of pre-existing TILs. These insights strongly support the development of pre-lymph node resection treatment strategies, and future approaches targeting lymph node-driven immunity in resectable NSCLC.

#5817 Single cell profiling of diffuse pleural mesothelioma reveals tumor cell plasticity and dynamic immune responses when exposed to platinum, pemetrexed and nivolumab.

Michael Offin, **Matthew Zatzman**, Yonit Lavin, Jennifer L. Sauter, Nataly Naser Al Deen, Nicholas Ceglia, William D. Travis, Rania G. Aly, Andreas Rimner, Annemarie Shepherd, Charles B. Simone, Mark G. Kris, Michelle S. Ginsberg, Andrew Chow, Alvaro Quintanal-Villalonga, Sohrab P. Shah, Charles M. Rudin, Valerie W. Rusch, Prasad S. Adusumilli, Marjorie G. Zauderer

Memorial Sloan Kettering Cancer Center, New York, NY

Background: Diffuse pleural mesothelioma (DPM) is an aggressive malignancy where even patients with potentially resectable disease have poor outcomes. With an evolving understanding of the role of pleurectomy/decortication (P/D) in patients with resectable disease, the need to improve upfront therapies is more urgent. In this single institution prospective pilot study of nivolumab with platinum (cisplatin/carboplatin) and pemetrexed prior to P/D (2 cycles) for patients with resectable DPM (NCT04162015), we report clinical outcomes and multimodal profiling of tumor tissue and plasma samples using single cell RNA sequencing (scRNAseq), whole exome sequencing, and gross pathological assessment. In particular, we perform in-depth exploration of DPM transcriptional heterogeneity, including tumor cell intrinsic changes and microenvironment features associated with clinical responses to ICI.
Results: 22 patients received neoadjuvant chemo-immunotherapy and 19 underwent attempted P/D (2 progressed and 1 declined surgery) within 30 days of the planned resection date. In the 19 patients that went for P/D, the median OS from the start of neoadjuvant therapy was 34mos with a PFS of 14mos (median follow-up: 32mos). scRNAseq was performed on 20 resection specimens from 16 patients with available material, including three patients with matched pre/post resection specimens. We also analyzed single cell transcriptomes from five non-ICI treated DPM samples. We discover that malignant DPM cells exist on a phenotypic continuum between mesothelial-like and fibroblast-like states, defined based on transcriptional similarity to their normal counterparts. Notably, these ‘fibroblast-like’ DPM cells were present in multiple patients, including those pathologically defined as epithelioid, and were predicted to arise from the same ancestral clonal expansion based on single-cell derived copy number profiles. These cells were present in patients prior to receiving ICI, and in certain cases corresponded to samples with gross pathological features such as a spindle-like tumor cell morphology. Focusing on features defining ICI-responsive patients, we find significantly increased abundance of cytotoxic CD8 T cells, ISG^+ (interferon stimulated) T cells, $FOXO1^+CD4^+$ and $CXCL13^+CD4^+$ T cells in patients with PFS greater than 12 months.
Conclusions: Neoadjuvant platinum, pemetrexed, and nivolumab was reliably able to be given to patients with DPM without impacting their ability to undergo P/D. To our knowledge this is the largest cohort of DPM resection specimens analyzed by scRNA-seq and has revealed important insights into the transcriptional heterogeneity of DPM. This in depth characterization provides foundational insights needed to characterize DPM response to chemo-IO and support future biomarker development.

#5818 Incidence and management of steroid-refractory immune checkpoint inhibitor pneumonitis: a single-center retrospective analysis.

MacKenzie Adams[1], Charmi Trivedi[1], Sapana Gupta[1], Jacqueline Chu[1], Curtis Petruzzelli[1], Kanika Malani[2], Rebecca Steuer[3], William Park[4], Sathwik Madireddy[1], Sandeep Jain[3], Benedito Carneiro[3], Matthew J. Hadfield[3]

[1]Brown University Health, Providence, RI,[2]Warren Alpert Medical School of Brown University, Providence, RI,[3]Department of Hematology/Oncology, Brown University Health, Providence, RI,[4]Department of Gastroenterology, Brown University Health, Providence, RI

Introduction: Pneumonitis is a serious and potentially lethal complication of treatment with immune checkpoint inhibitors (ICI). In a rare subset of patients, front-line treatment with steroids is ineffective, necessitating a second-line steroid-sparing agent. We conducted a single-center, retrospective analysis of patients treated with ICIs to determine incidence and outcome patterns of patients who develop pneumonitis.

Methods: We conducted manual chart review on a cohort of 2,820 patients treated with ICIs at our institution from 2015 to 2024. Steroid-refractory pneumonitis was defined as no clinical improvement between 48 hours and up to 14 days after initiation of high-dose corticosteroids. Electronic medical records were then reviewed to identify the clinical features at presentation and overall outcomes.

Results: Of 2,820 patients, we identified 69 with ICI pneumonitis and 11.6% (8/69) with steroid-refractory ICI pneumonitis. The mean age was 69.5 years (range: 42-91) and 53.6% were female (37/69). Most patients had lung cancer (53.6%, 37/69) followed by both renal cancer and melanoma (both: 13%, 9/69), with the treatment goal being palliative for 79.7% (55/69). The mean number of ICI treatments before steroid-refractory ICI pneumonitis diagnosis was 5, compared to 12 for all patients with pneumonitis. Diffuse alveolar damage (DAD) was the most common radiologic pattern amongst steroid-refractory cases (5/8, 62.5%). After corticosteroid failure, patients were managed with: infliximab (n=1), mycophenolate (n=1), a combination of IVIG and infliximab (n=1), no second-line agent in lieu of a longer steroid course (n=1), or hospice alone (n=4); 3/8 (37.5%) required ICU-level of care, 7/8 (87.5%) required some form of oxygen supplementation (ranging from nasal cannula to noninvasive positive pressure ventilation). Ultimately, 6/8 (75%) patients died from steroid-refractory ICI pneumonitis. The patient that received infliximab alone recovered from his pneumonitis but ultimately died from cancer progression. The patient that elected a longer steroid course had recurrent pneumonitis after steroid taper but eventually recovered.

Conclusions: Steroid-refractory ICI pneumonitis is uncommon, only affecting 11.6% of ICI pneumonitis cases at our institution, however, it is responsible for significant mortality. As seen in other studies, steroid-refractory ICI pneumonitis occurred early in ICI treatment. After corticosteroid failure, only 3/8 patients were treated with a second immunosuppressant agent, indicating second-line immunosuppressants may be underutilized in this population. Little data has been generated on outcomes of patients treated with high-dose steroids without second-line therapy. There is a significant need for prospective, randomized trials to determine the best treatment strategies for this patient population.

#5819 Anti-cancer immune effects of PT-112 monotherapy across two disease indications.

Arun Rajan[1], **Tyler D. Ames**[2], Carolina Celades-Errando[1], Christina Y. Yim[2], Shannon Swift[1], Meredith J. McAdams[1], Laercio DaSilva[1], Wiem Lassoued[1], Hana Bagheri[1], Maria T. Congenie[2], Howard I. Scher[3], Alan H. Bryce[4], James L. Gulley[1], Jeffrey Schlom[1], Renee N. Donahue[1]

[1]National Cancer Institute, National Institutes of Health, Bethesda, MD,[2]Promontory Therapeutics Inc., New York, NY,[3]Memorial Sloan Kettering Cancer Center, New York, NY,[4]City of Hope Cancer Center, Phoenix, AZ

Background: PT-112 is a small molecule inhibitor of ribosome biogenesis under development for several cancer indications. Preclinical studies have demonstrated PT-112's ability to induce immunogenic cell death (ICD) and synergize with various anti-cancer agents, including immune checkpoint inhibitors (ICIs). PT-112 has shown durable benefit in patients with advanced cancers, with pre-clinical data supporting an immune-mediated mechanism of action. This report aims to further elucidate the immunological effects of PT-112 through comprehensive immune profiling of blood and tissue samples from ongoing Phase II trials in thymic epithelial tumors (TETs, NCT05104736) and metastatic castration-resistant prostate cancer (mCRPC, NCT02266745).

Methods: Patient samples from the TET trial were analyzed by flow cytometry for 158 subsets of peripheral blood mononuclear cells and serum cytokines and soluble factors. Paired tumor biopsies from a subset of patients were analyzed for changes in immune cell composition before and on treatment. Patient blood samples from the mCRPC trial were analyzed via TCR sequencing to identify novel and expanding T cell clones and to monitor changes in the T cell fraction.

Results: In the TET trial (14 patients), peripheral immunome changes reflective of immune activation included an increase in proliferative CD4+ T cells, activated and proliferative CD8+ T cells and NK cells, including NKp46+ NK cells, and PD-L1-expressing refined monocytes, and a decrease in naïve CD4+ T cells. Furthermore, increases in proinflammatory cytokines IFN-γ and TNF-α, along with decreases in immunosuppressive factors VEGF and TGF-β1 were observed (n = 7). Pooled results from tumor biopsies (n=3) showed an increase in immune cell density with increased expression of markers of immune activation (granzyme B and IFN-γ). In the mCRPC trial, 26/74 (35%) patients showed evidence of marked T cell clonal expansions, many of which were novel clones not detected at baseline. Across the population there was a statistically significant (Wilcoxon signed-rank test, $p < 0.0005$) median increase in the T cell fraction of 20%.

Conclusions: In line with prior non-clinical and clinical findings, data from these ongoing Phase II trials show that PT-112 induces robust signals of immune activation and ICD across the adaptive and innate immune systems and are indicative that PT-112's immune effects play a significant role in its anti-cancer mechanism.

#5820 Spatial and cellular immune landscapes of non-small cell lung cancer in association with response to neoadjuvant chemoimmunotherapy.

Zahraa Rahal[1], Tieling Zhou[1], Alejandra G Serrano[1], Jiping Feng[1], Alessandra Vaccaro[1], Ansam Sinjab[1], John T. Le[1], Xin Sun[1], Michael Wang[1], Wei Hu[1], Jianjun Zhang[1], Tullia Bruno[2], Hai T. Tran[1], Stephen G. Swisher[1], Cheuk Hong Leung[1], J. Jack Lee[1], Jing Wang[1], John Victor Heymach[1], Don Lynn Gibbons[1], Ignacio Ivan Wistuba[1], Annikka Weissferdt[1], Jared K. Burks[1], Luisa Maren Solis Soto[1], Humam Kadara[1], Tina Cascone[1]

[1]The University of Texas MD Anderson Cancer Center, Houston, TX,[2]UPMC Hillman Cancer Center, Pittsburgh, PA

Neoadjuvant and perioperative immunotherapy with checkpoint inhibitors (ICIs) combined with chemotherapy (CT) has improved outcomes for patients with resectable non-small cell lung cancer (NSCLC). However, over half of treated patients experience non-response due to poorly understood mechanisms, underscoring the need to elucidate the factors mediating therapeutic efficacy.

Here, we aimed to dissect the tumor-associated immune landscape in lung tissues from patients with varying levels of residual viable tumor (%RVT) following neoadjuvant chemoimmunotherapy. We analyzed 28 lung tissue samples from 24 patients, including non-squamous (n=17) and squamous (n=7) NSCLCs, with pathological complete response (pCR, n=5), major pathological response (MPR, n=1), and no MPR (n=18). Multimodal spatial transcriptomics (ST) (Visium CytAssist, 10x Genomics) and single-cell RNA sequencing analysis of fixed cells (scFFPE-seq) from consecutive tissue sections was performed in association with therapeutic response and clinicopathological variables.

ST analysis revealed lymphoid aggregates (LAs) and tertiary lymphoid structure (TLS) signatures in both responders and non-responders. Notably, pCRs were enriched in squamous cases. B cells and plasma cells (PCs) were significantly more enriched in responders (pCR and MPR), with CXCL13-CXCR5 ligand-receptor interactions spatially overlapping with TLS signature expression, suggesting a functional interplay that may be crucial in mediating response to therapy. In responders, PCs appeared to differentiate from the LAs in space into the tumor bed, possibly alluding to active humoral anti-tumor immunity. A significant abundance of macrophages, including *CTSK+*, *C1Q*$^{+}$ and M2-like *SELENOP*$^{+}$ subsets, was observed in responders, particularly surroundings TLSs. *IL2RB*$^{+}$ natural killer (NK) cells were also more prevalent in cases with pCR and MPR. The abundance of immune populations was inversely correlated with %RVT; we observed incremental increase in immune proportions with decreasing %RVT. Conversely, non-responders exhibited higher levels of migratory dendritic cells (cDC3) and cDC2 subsets, CD4^{+} naïve T, regulatory T (Tregs), Th17 CD4^{+}, and *GZMK*$^{+}$ exhausted T, Th1, and *FCGR3A*+ NK cells, as well as higher levels of *KIT*$^{+}$ *AHR*$^{+}$ *KLRG1*$^{+}$ group 3 innate lymphoid cells (ILC3).

These findings reveal distinct immune cell compositions and states associated with response to neoadjuvant chemoimmunotherapy in NSCLC. Ongoing efforts aim to characterize lineage-restricted subpopulations and immunogenomic features underlying therapeutic response, which thus could inform biomarker discovery and streamline development of personalized therapeutic strategies to further improve patient outcomes.

#5821 Targeting adenosine receptor signaling enhances anti-PD-L1 therapy efficacy in MTAP-deficient urothelial cancer via reprogramming tumor-associated macrophages.

Ying Mei, Jianfeng Chen, Yuxue Zhang, Yu-Yi Chu, Omar Alhalabi, Jianjun Gao

UT MD Anderson Cancer Center, Houston, TX

Methylthioadenosine phosphorylase (MTAP) deficiency in urothelial cancer (UC) correlates with poor clinical outcomes and reduced response rates to immune checkpoint inhibitor (ICI) therapy. Understanding the mechanisms by which MTAP loss contributes to ICI resistance and identifying therapeutic strategies to enhance ICI efficacy in MTAP-deficient tumors is of critical importance. Using CRISPR-Cas9, we deleted MTAP in the MB49 murine UC cell line and established orthotopic bladder tumors with wild-type (WT) and MTAP knock-out MB49 cells. CyTOF analysis revealed that MTAP-deficient tumors showed a marked reduction in M1-like macrophages (~50%) and a substantial increase in M2-like macrophages (~3-fold). Mechanistically, MTAP-deficient tumors accumulated elevated levels of methylthioadenosine (MTA, >100-fold) in the tumor microenvironment (TME), which drove the polarization of tumor-associated macrophages (TAMs) towards a pro-tumorigenic M2-like phenotype via adenosine receptor (A2A/A2B) signaling. Notably, inhibition of A2A/A2B adenosine receptors significantly enhanced anti-PD-L1 therapy efficacy, achieving an overall tumor growth inhibition of 50% compared to anti-PD-L1 monotherapy. These findings suggest that targeting the adenosine pathway may provide a promising strategy to improve therapeutic outcomes in MTAP-deficient urothelial cancer.

Keywords: Urothelial cancer, MTAP-deficient, immune checkpoint therapy, macrophages

CLINICAL RESEARCH: Immunomodulatory Agents and Interventions
Poster Session

#5825 SWA1211, a next generation HPK1 inhibitor exhibits superior anti tumor efficacy in preclinical studies.

Guanglong Sun, Yuyang Ge, Huting Wang, Desheng Kong, **Carrie Xiang**

Beijing Stonewise Technology Co., Ltd., Beijing, China

Immune oncology is an important breakthrough for cancer therapy represented by immune checkpoints inhibitors like PD-1/L1, CTLA4 antibody drugs. Although this type of drug has become the standard treatment for many kinds of cancers, a large proportion of patients fail to respond to them or become resistant eventually. Therefore, new mechanisms or targets remain urgent to further improve the therapeutic outcomes or overcome the resistance of immunotherapy.Hematopoietic progenitor kinase 1 (HPK1), also known as MAP4K1, belongs to the MAP4K family of protein serine/threonine kinases. HPK1 is a negative regulator of T-cell signaling and has been identified as a promising target to combat cancers by eliciting robust anti-tumor immunity. Many HPK1 inhibitors are under active clinical development.SWA1211 is a best-in-class (BIC) HPK1 inhibitor designed using Stonewise's proprietary 3D molecular generation model. To conquer the challenge of selective inhibition on HPK1, a steric group was designed to extend toward the small hydrophobic pocket formed by Leu23 and Val31 in HPK1, which does not exist in GLK due to the different residue, Ile22. This unique molecular structure design significantly improved potency and selectivity of our compounds, and excellent immune cell activation was achieved (biochemical assay IC_{50}=0.9nM @ATP 1mM; human T-cell activation EC_{50}=9nM). Selectivity study showed a unique kinases inhibition profile with no obvious off-kinase activity related risk.More importantly, when compared to other HPK1 inhibitors developed by leading developers, SWA1211 stimulates T cell activation efficiently in an ever-known broadest concentration range without any exhaustive effect (up to a concentration of 1000 folds of EC_{50}). This unique non-bell-shaped immune cell activation curve translates to unmatched efficacy as no other HPK1 inhibitors have shown in preclinical settings. Single agent of SWA1211 induces tumor shrinkage in Hepa1-6 and EMT6 mouse syngeneic tumor models. GLP toxicity studies indicated ideal therapeutic index was obtained. We believe SWA1211 to be a promising next generation immune therapy for cancer patients.We have completed all IND enabling studies for SWA1211 and the results demonstrated a favorable drug-like profile. We expect to receive the green light for a clinical trial from the FDA and NMPA by March 2025; the clinical trial will be initiated immediately afterward.

#5826 Development of multivalent antibody-based novel therapeutic cancer vaccine in glioblastoma multiforme (GBM) treatment.

Sunghyun Yoon[1], Sungmuk Kang[1], Yeji Byeon[1], Dongmin Chun[1], Hong Kyu Lee[2], Kyung-gi Hyun[1], Sunghyun Byun[1], Eunyoung Cho[1], Yongjun Kang[1], Chaerin Yoo[1], Hong Jai Lee[1], **Jiyeon Hong**[1], Chungmin Lee[1], Kyung-Chul Choi[2], Gyongsik Ha[1]

[1]IMBiologics Corp., Suwon, Korea, Republic of,[2]Chungbuk National University, Cheongju, Korea, Republic of

Glioblastoma multiforme (GBM) is the most prevalent high-grade malignant brain tumor and is associated with a very poor prognosis. Currently, there are no targeted therapies available for this cancer, and only general chemical drugs are being used. This is primarily due to the challenges posed by the blood-brain barrier (BBB) in drug delivery and the lack of sufficient research on its pathogenesis. Recently, a strong association between CMV (Cytomegalovirus) and GBM has been reported in various studies, and therapies targeting CMV are now undergoing clinical trials. Especially, dendritic cell (DC) cancer vaccines represent a promising therapeutic strategy for GBM, with CMV pp65 emerging as a particularly relevant target antigen. Recent clinical trials have provided evidence supporting the efficacy of select DC vaccines. Nevertheless, the production of DC vaccines remains a labor-intensive, costly, and time-consuming process, emphasizing the critical need for innovative approaches to overcome these limitations. In this study, we developed IMB-402, containing the Specific T-cell Activating Modulator (STAM) built on our engineered IgM-based multivalent antibody backbone platform, ePENDY (engineered PENtamer boDY). IMB-402 consists of ten CMV pp65-derived peptide-loaded-HLAs linked to ePENDY to stimulate priming signal of CD8+ T cell by TCR cluster. Also, we linked a CD40 agonistic scFv as a costimulatory signal molecule to the J chain of ePENDY, mimicking cognately licensed dendritic cells (DCs) to prevent T cells from entering an anergic state. We observed that IMB-402 can expand CMV pp65-specific CD8+ T cells and functionally activate these cells. Notably, we found that this effect occurs even in conditions where only CD8+ T cells are present, without any DCs. This highlights the potential of IMB-402 as an alternative to DC vaccines, offering a promising treatment option for immunosuppressive cancer patients whose DCs fail to respond appropriately. Moreover, the superior effects on expansion and activation of CMV pp65-specific CD8 T cells, compared to a competing drug currently in clinical trials, suggest that IMB-402 has the potential to become a successful treatment for GBM. Additionally, synergistic effect of IMB-402 with anti-PD1 observed using a humanized mouse model indicates that IMB-402 is expected to have robust anti-tumor activity in combination with immune checkpoint inhibitors. Overall, these findings suggest that IMB-402 could represent a new paradigm in cancer vaccine technology, highlighting its potential for development as a GBM treatment.

#5827 Hydroxychloroquine increases the tumor killing efficiency via elevating the membrane MHC-I protein levels of tumor cells.

Man Zhang, Chen Huang, Xingming Ma, Wenjia Zhuang, Huajun Jin

Shanghai Juncell Therapeutics Co., Ltd, Shanghai, China

Background: Hydroxychloroquine (HCQ) was firstly developed as an antimalarial drug, while recently it has been used to treat various types of cancer due to its molecular mechanism in inhibiting autophagy process, induction of apoptosis, and the inhibition of CXCL12/CXCR4 pathway in cancer cells. Previous studies found that autophagy promotes immune evasion of pancreatic cancer by degrading major histocompatibility complex class I (MHC-I), so we supposed HCQ treatment might restore or even elevate the MHC-I levels of tumor cells through inhibiting autophagy and then benefit tumor infiltrating lymphocytes (TILs) immunotherapy.
Methods: Malignant melanoma cell line A-375, non-small cell lung cancer (NSCLC) cell line NCI-H2122, ovarian cancer OVCAR-8 and glioblastoma U-87 MG were subjected to HCQ treatment for 48 hours and the protein levels of membrane MHC-I and programmed cell-death ligand 1 (PD-L1) were measured by flow cytometry. IFN-γ was set as a positive control for its well-known function in elevating MHC-I levels. Western blots were carried out to verify the autophagy inhibition. Real-time cell analysis (RTCA) was used to evaluate the in vitro cytotoxicity for transgenic hmTCRT cells specifically recognizing NY-ESO-1 epitope and targeting HCQ treated or non-treated NY-ESO-1 positive A-375 cells. Using the same concentrations as treating tumor cells, 15μM, 20μM HCQ treatment was carried out on TILs in rapid expansion phase to determine whether their proliferation would be affected.
Results: HCQ treatment can significantly elevate the protein levels of membrane MHC-I for tumor cell lines A-375, NCI-H2122, OVCAR-8 and U-87 MG (P value of t-test <0.01), and the elevation can sustain for 72 hours after removing HCQ from the culture system. After HCQ treatment, the surface PD-L1 levels of aforementioned four cell lines didn't change significantly compared to DPBS negative control group, contrarily PD-L1 levels of IFN-γ treatment groups increased significantly. Western blot results indicated that levels of LC3II and p62 increased significantly after HCQ treatment, suggesting that the autophagy process was inhibited. HCQ pre-treatment can significantly elevate the killing efficiency of hmTCRT cells targeting NY-ESO-1 positive A-375 cells. 15μM, 20μM HCQ treatment didn't affect TILs' proliferation, which were the same concentrations as treating tumor cells.
Conclusions: Our studies indicated that HCQ can significantly increase the tumor killing efficiency via elevating the membrane MHC-I protein levels of tumor cells while it doesn't affect TILs' propagation. In addition, its effect on surface PD-L1 is not significant, which is more advantageous than IFN-γ. These results can provide preclinical evidences for HCQ pretreatment before the adoptive cell therapy of TILs in our clinical trials (NCT06375187).

#5828 Development of NK humanized mice models for the in vivo evaluation of NK cell-based therapeutics.

Dong Wang[1], zhixiang zhang[1], zhongbao song[2], xiangnan qiang[1], qingyang gu[1]

[1]WuXi AppTec, Shanghai, China,[2]WuXi AppTec, nantong, China

With the recent advancements in anti-tumor immunotherapy, novel treatments have emerged and progressed rapidly. Several strategies are now being employed to enhance the efficacy of natural killer (NK) cells in tumor immunotherapy, including the use of monoclonal and multi-specific antibodies, as well as immune modulator molecules. Given these developments, there is an urgent need for the establishment of appropriate mouse models to effectively evaluate the therapeutic potential of these emerging immunotherapies.

In this study, we developed a humanized mouse model for the specific reconstitution of functional and durable human NK cells within a murine system. In this model, human NK cells were purified from peripheral blood mononuclear cells (PBMCs) using magnetic bead separation, expanded in vitro, and subsequently engrafted into human IL-15 transgenic NOG mice. We collected blood samples from these mice to monitor human NK cell kinetics and profile using flow cytometry. The therapeutic efficacy of an anti-CD20 antibody was then evaluated in NK-humanized model using the SU-DHL-2 subcutaneous xenograft tumor model. Results indicated that anti-CD20 treatment was ineffective in non-NK-humanized mice. In contrast, in NK-humanized mice, human NK cells significantly inhibited tumor growth via antibody-dependent cellular cytotoxicity (ADCC). Additionally, we assessed NK cell intratumoral infiltration and the expression of activation markers at the end of the in vivo efficacy study, providing further insights into the immune response in the tumor microenvironment.

In summary, we have developed a humanized mouse model capable of sustaining functional human NK cells for extended periods, ranging from weeks to months. This NK-humanized model provides a valuable preclinical platform for evaluating NK-modulating therapies and hold promise as a tool to accelerate the development of novel NK-based immunotherapeutics.

#5830 Combining DNA methylation inhibition and STING agonist in the treatment of metastatic triple-negative breast cancer.

Sofiane Berrazouane[1], Xiaoting You[1], Jack Su[1], Margarita Bartish[1], Rhea Dumitrescu[1], Marios Langke[1], Young Im[1], Benjamin Lebeau[2], Sonia del Rincon[1], Josie Ursini-Siegel[1], Michael Witcher[1]

[1]Oncology, McGill University, Montreal, QC, Canada,[2]Nanyang Technological University, Singapore, Singapore

Triple-negative breast cancer (TNBC) is an aggressive breast cancer associated with early metastatic events leading to a poor prognosis. According to the American Cancer Society, the 5-year relative survival rate is 91% in patients with localized TNBC but only 12% for those with metastatic TNBC. Thus, there is an urgent need to understand the mechanisms that drive TNBC metastasis to uncover more effective therapeutic approaches. In this regard, we first profiled the RNA-Seq and DNA methylation enrichment in 8 metastatic TNBC cell lines with bone, liver and lung organotropism and then compared them to 3 parental TNBC cell lines. The RNA-Seq data showed that the downregulation of IFN-type-1 pathways is associated with TNBC metastatic organotropism in the lung, liver and bone. Surprisingly, the DNA methylation profiling revealed that the IFN-type-1-related genes are under DNA methylation regulation. The inhibition of DNA methylation with decitabine increased the expression of IFNβ gene in metastatic TNBC cell lines. This supports that targeting DNA methylation and stimulating the IFN-type-1 pathway could represent a new vulnerability for metastatic-TNBC. In this regard, the use of STING agonist, a known stimulator of IFN-type-1, synergizes with decitabine in reducing the viability of 6 metastatic TNBC cell lines with lung, liver and bone organotropism. More importantly, the decitabine/STING therapy showed a potent effect in targeting TNBC metastatic lesions in vivo and improved survival. Altogether, this work suggests that the DNA methylation inhibition and the stimulation of the IFN-type-1 pathway represent a new approach to target the metastatic-TNBC.

#5831 ABN202 (αTROP2), a sacituzumab-interferon-beta mutein fusion protein: a novel approach beyond antibody-drug conjugate therapeutics in ADC-naïve and ADC-refractory cancers.

Hee Geon Park[1], Hyun Kyung Lee[1], Myeung Ryun Seo[2], Soo Jin Ryu[2], Sungyoul Hong[3], Chan Gyu Lee[4], Hae Min Jeong[4], Sae Hyung Lee[1], Na Young Kim[1], Young Kee Shin[2], Jun Young Choi[1]

[1]ABION Inc., Seoul, Korea, Republic of,[2]Department of Molecular Medicine and Biopharmaceutical Sciences, Seoul National University, Seoul, Korea, Republic of,[3]Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul, Korea, Republic of,[4]Genopharm Inc., Seoul, Korea, Republic of

Trophoblast cell surface antigen 2 (TROP2) is a cell-surface glycoprotein overexpressed in various solid tumors, including triple-negative breast cancer (TNBC), hormone receptor-positive breast cancer (HR^+ BC), and non-small cell lung cancer (NSCLC). TROP2-targeting antibody-drug conjugates (ADCs) such as Sacituzumab-govitecan (SG) and Datopotamab deruxtecan (Dato-DXd) demonstrated promising clinical efficacy in patients with TROP2-positive cancers. However, TROP2-targeting ADCs exhibit a high incidence of adverse events, a short duration of response, and frequent recurrence. Therefore, there is an unmet medical need for treatments beyond ADC therapeutics, particularly for ADC-naïve and ADC-refractory patients.
ABN202 is an antibody-cytokine fusion protein platform that fuses a potent cytotoxic and immune-modulating cytokine, interferon-beta mutein (IFN-β mutein), with an antibody via flexible protein linker. In previous studies, ABN202 utilizing the TROP2-targeting antibody, Sacituzumab (ABN202, αTROP2), demonstrated potent anti-tumor efficacy by inhibiting cancer cell growth and activating anti-tumor immunity within the tumor microenvironment. In this study, we compared the *in vitro* and *in vivo* anti-cancer efficacy of ABN202 (αTROP2) with those of SG and Dato-DXd in ADC-resistant preclinical models, as well as in xenograft and human IFNAR knock-in (hIFNAR KI) mouse models. The preclinical toxicity profiles of SG, Dato-DXd, and ABN202 (αTROP2) were evaluated using human immune cells and hIFNAR KI mouse models.
In conclusion, ABN202 (αTROP2) demonstrated potent anti-cancer efficacy and a tolerable safety profile compared to SG and Dato-DXd. Our findings suggest that ABN202 (αTROP2) represents a novel therapeutic approach beyond ADCs for both ADC-naïve and ADC-refractory cancers.

#5833 Overcoming immune anergy by targeting tumor macrophages: Rearranging the tumor microenvironment.

Frederick O. Cope, Simon A. Blackburn

Physis International, Columbus, OH

There are remarkable advances in immunotherapy which have transformed solid tumor therapy outcomes for many patients. Immunotherapy rationales include checkpoint inhibitor monoclonal antibodies (CIMA) or chimeric antigen receptor T cell therapy that have become primary treatment options and provide remarkably durable treatment responses that are generally not achieved with standard systemic non-targeted chemotherapy. A hallmark of solid tumors is their ability to induce immune anergy where the target tumor is able to evade immune surveillance. Within the solid tumor micro-environment, a critical element of this anergy maintenance is the presence of tumor-associated macrophages (TAMs). Indeed, the localization of TAMs in the tumor micro-environment is effectively co-opted into contributing toward tumor growth. We have developed two new immune-modulating therapeutic drugs that specifically address the role of TAMs in anergic immune responses. These include the synthetic CD-206 ligand PHY-001, a macrophage phenotype-shifting agent designed to act through CD-206-expressing M2-like TAMs. Its application to solid tumor microenvironments is indicated to result in a shift of TAMs to an antitumor M1-like phenotype. Alternative to TAM phenotype shifting is the induction of apoptosis of TAMs. This too, is effectively achieved through the CD-206 receptor with modification of PHY-001, such that loading of PHY-001 with an elemental anion, results in mitochondrial disruption and TAM apoptosis (Modified PHY001 --> PHY-003). Both agents have been target-tested (CD206) in vivo in humans using an approved isotopically labeled version of the molecule core. Either approach to the modification of TAMs releases the immune micro-environment from the existing anergic lock, resulting in significantly improved adaptive and innate tumor immune responses including increased cancer cell phagocytosis by a] reprogrammed existing TAMs or b] neo-localization of invading immunologically functional macrophages. These two new agents provide a novel approach to solid tumor immunotherapy where they may be used singly or in conjunction with other immunomodulating drugs. Such agents are a rational expansion in available approaches to solid tumor therapy.

#5834 Discovery of a novel Trop2-targeting immune-stimulating antibody conjugate with efficacy in pancreatic cancer models.

M. R. Mullapudi, A. Zhang, S. J. McKay, T. A. Brooks, N. L. Tumey;
Binghamton University, Johnson City, NY

Background: Toll-like receptor (TLR) and Stimulator of interferon genes (STING) agonists are emerging as potent agents in cancer immunotherapy. However, systemic administration of these small molecules carries the risk of severe off-target toxicities, such as cytokine storm. A more targeted delivery approach could enhance specificity and reduce these toxic effects. Immune-stimulating antibody-drug conjugates (ISACs), which utilize TLR or STING agonists conjugated to antibodies via specific linkers, have gained attention for their improved targeting. Our team previously designed a potent TLR7 agonist, E104, and developed a successful E104-based HER2 targeting ISAC. This new study summarizes the identification of a novel Trop2-targeting ISAC with efficacy in pancreatic cancer models.
Methods: We synthesized a library of novel linkers and their corresponding E104 ISACs. These ISACs include both glycosylated and deglycosylated versions of sacituzumab, an anti-Trop2 monoclonal antibody, alongside a control Trop2_mcValCitPABC_E104 ISAC. The ISACs were then tested for NFκB/IRF-7 activation and IL-6 induction in co-culture models and for in vivo efficacy using mouse xenograft models.
Results: Both glycosylated and deglycosylated ISACs exhibited drug-to-antibody ratios (DAR) of 6-8. The next-generation ISACs showed significantly reduced aggregation (<1-2%) compared to the control Trop2_mcValCitPABC_E104 ISAC (30%) and were significantly less hydrophobic, thus imparting an improved PK profile. In co-culture studies using BXPC3/ CFPAC-1 and RAW-Dual mouse macrophage reporter cell lines, deglycosylated ISACs triggered more specific NFκB activation than their glycosylated counterparts. Similar results were observed for the coculture of BXPC3/ CFPAC-1 and Ramos Blue, a human B lymphocyte cell line. Further, co-culture of BxPC-3/CFPAC-1 with mouse splenocytes/bone marrow-derived dendritic cells (BMDCs) resulted in strong antigen-dependent IL-6 induction. In vivo testing of these ISACs in immunodeficient mice with CFPAC xenograft models revealed that one specific ISAC, has efficacy against this pancreatic tumor model. Furthermore, a DAR 4 version of this ISAC, showed complete tumor regression in C57BL/6J mice using a hTrop2-MC38 model.
Conclusion: Pancreatic cancer is aggressive and has a poor prognosis. It has limited treatment options and urgently needs more effective therapies. Toward this end, we have designed and evaluated a library of novel Trop2-targeting ISACs. These engineered ISACs exhibited reduced aggregation and improved pharmacokinetics. One of these achieved complete tumor regression in vivo in two different tumor models leading to the identification of an efficient ISAC for pancreatic cancer. These findings highlight the critical role of linker engineering in the development of more effective ISACs.

#5835 Good quality sixty-eight percent 5-year survival in patients with treatment refractory stage IV lung cancer following treatment with a single agent progesterone receptor antagonist.

Jerome H. Check[1], Diane Check[2], Carrie Wilson[2], Maddison Neulander[2]

[1]Cooper Medical School of Rowan University (CMSRU), Melrose Park, PA,[2]Cooper Institute for Reproductive Hormonal Disorders, P.C., Mt. Laurel, NJ

Both the fetus and malignant tumors utilize membrane progesterone receptors (mPRs) to produce immunomodulatory proteins e.g., the progesterone induced blocking factor (PIBF) to escape immune surveillance. This study evaluated the efficacy of treating stage IV NSCLC or SCLC with single agent oral mifepristone 200-300mg/day. To be included, the cancer had to have progressed despite chemo or immunotherapy or the patient considered likely to die within 1 month. Six patients were evaluated, 4 with NSCLC, 2 with SCLC. Patients were not excluded for co-morbidities or if there were brain metastases. The patients completed a quality of life form every month for the first six months, then every third month, fourth, and six. 3 of 4 NSCLC patients had brain metastases and 2 had COPD with one considered end-stage COPD. 2 were positive for EGFR mutation, the other 2 had no markers to target. One of the two patients with SCLC had no prior therapy since her serum Na+ was 118 mmol/L and PO2 72 mmHg, with extremely rapid progressing SCLC. She was considered by her oncologist to be within two weeks of death with hospice recommended. All six patients showed marked palliative benefits even to the day they died. 3 of 4 with NSCLC lived 5+ years with one still alive. None showed any new metastases and many decreased in size. The 78-year-old woman with severe hyponatremia had a serum Na +of 145 mmol/L and serum PO2 of 100 mmhg one month after treatment and eventually went into full remission. She died at the age of 83 with myocardial infraction with no evidence of cancer. The 68-year-old woman with end stage COPD died after 1.5 years from COPD with her cancer stable from COPD. The 68-year-old male with COPD died at age 73 with pneumonia during the COVID pandemic. His cancer was relatively stable with just slight growth of his primary lung lesion. Both women with NSCLC EGFR + lived five years despite not starting mifepristone until 1 year after treatment with Osimertinib when the cancer was now rapidly progressing with the presence of multiple brain metastasis. One died, but her cancer was stable. We could not ascertain the cause of death. The 65-year-old male with SCLC was clinically doing well after 1 ½ years of mifepristone. However, because the lesions were still present (but smaller) his oncologist enrolled him in a clinical trial requiring him to stop the mifepristone. He died 3 months later. Though some studies find with the new therapies available some patients with stage IV NSCLC may have a 15% 5-year survival from first diagnosis. Though there were only 6 patients in this study, this excellent 5-year survival outcome in previously treated or terminal patients with NSCLC or SCLC strongly suggests the efficacy of single agent mifepristone for advanced lung cancer with no more treatment options available.

#5836 Suppression of TNFα and IFNγ signaling are key molecular events in KRAS^{G12C} mutant cancer cells to confer therapeutic resistance to Sotorasib and promote immune evasion.

M. L. Ibrahim, X. Yu, D. Foresman, Z. Hong, E. Haura, A. Beg;
H. Lee Moffitt Cancer Center, Tampa, FL

The groundbreaking development and FDA approval of the mutant and cancer selective KRAS^{G12C}is Sotorasib and Adagrasib to treat NSCLC and CRC patients have revolutionized the field of cancer biology. While these inhibitors demonstrated efficacy in patients with KRAS^{G12C} mutation, their response was limited and short lived due to rapid and frequent emergence of therapeutic resistance. Multiple genetic alterations in KRAS^{G12C} cancer cells (mainly in RTK and MAPK pathways) have been reported to contribute to their acquired resistance to Sotorasib. However, there is unmet need to fully understand how cancer cells manipulate the immune regulatory signals in the TME to acquire therapeutic resistance and evade antitumor immunity. To this end, we utilized single cell profiling technology to delineate the intricate transcriptomic landscape of tumor cells and tumor infiltrated immune cells in the tumor microenvironment of Sotorasib-resistant tumors. Our single cell RNA seq analysis demonstrated that Sotorasib generated hot TME dominated by effector/cytotoxic T cells and inflammatory macrophages in Sotorasib-responsive CT26^{G12C} colon tumors. Importantly, these Sotorasib-responsive tumors are enriched with immune active tumor populations with highly upregulated TNFα and IFNγ signals. Conversely, these immune-sensitive tumor clusters (with high TNFα and IFNγ signals) were strikingly contracted in Sotorasib-resistant tumors. Furthermore, Sotorasib-resistant tumors exhibited non-inflamed TME dominated by immune suppressive neutrophils and monocytes populations. Intriguingly, Sotorasib failed to suppress or delay tumor growth of resistant tumors even when combined with anti-PD1 immune checkpoint blockade, indicating that KRASi-resistant tumors are in nature less vulnerable to antitumor T cell immunity. In vitro, single-cell imaging flow cytometry revealed that Sotorasib-tolerant cancer cells displayed remarkable reduction in nuclear translocation of NFkB and STAT1 as compared to parental cells, highlighting the importance of TNFα and IFNγ signaling to maintain optimum immune modulatory response of KRAS^{G12C} mutant cancer cells to KRAS inhibition. Collectively, our findings uncovered cancer cell-intrinsic and extrinsic molecular mechanisms underlying clinical resistance to Sotorasib and provide a comprehensive profiling of tumor/immune microenvironment ecosystem in refractory tumors to lay the groundwork for developing promising treatment platforms to overcome this inevitable resistance and achieve optimum clinical outcome.

#5837 Lurbinectedin-induced transcriptional reprogramming: A pathway to sensitise SCLC to immunotherapy.

Joan Salvador Russo-Cabrera[1], Santiago Ponce-Aix[1], Patricia Cozar[1], Alicia Luengo[1], Belen Revuelta[1], Laura Garcia-Redondo[1], Alejandro Navarro[2], Reyes Bernabe[3], Maria Eugenia Olmedo[4], Jose Manuel Trigo[5], Jon Zugazagoitia[1], Luis Paz-Ares[1], Itziar Otano[1]

[1]H12O-CNIO Lung Cancer Clinical Research Unit, Health Research Institute Hospital 12 de Octubre (i+12)/Spanish National Cancer Research Center (CNIO), Madrid, Spain,[2]Medical Oncology Department, Hospital Universitario Vall d'Hebron, Barcelona, Spain,[3]Medical Oncology Department, Hospital Universitario Virgen del Rocio, Sevilla, Spain,[4]Medical Oncology Department, Hospital Universitario Ramon y Cajal, Madrid, Spain,[5]Medical Oncology Department, Hospital Universitario Virgen de la Victoria, Malaga, Spain

Background: For decades, platinum-based chemotherapy plus etoposide (ChT+E) has remained unchallenged at the first-line treatment for extensive-stage-SCLC (ES-SCLC), that is, until the introduction of immune-checkpoint blockade (ICB) anti-PD-L1. ChT+E susceptibility of SCLC relies in the high genomic instability observed in SCLC, further increased by ChT+E and surpassing DNA Damage Repair (DDR) capabilities. In the context of DNA damage and cell-intrinsic immunity, the addition of ICB improves the response of SCLC to ChT+E through tumour immunogenicity. Although initially responding to treatment, almost all patients relapse within the first 6 months, leading to limited options at second-line treatment, Lurbinectedin (LUR) among them. While LUR acts by degrading RNA polymerase II and, therefore, inhibiting transcription, it also causes DNA damage synergising with ICB. 2SMALL is a phase 1/2 study assessing the safety, tolerability and efficacy of LUR plus atezolizumab (ATZ) as second-line treatment for ES-SCLC.

Methods: 2SMALL Phase-I was a dose exploration trial combining increasing doses of LUR plus a fixed dose of ATZ every 3 weeks. Study endpoints included safety profile and LUR recommended dose, as well as, Objective Response Rate (ORR), Progression-Free Survival (PFS) and Overall Survival (OS). Pre-clinically, efficacy of LUR plus anti-PD-L1 mAb was evaluated *in vivo* in a syngeneic immunocompetent mice model for SCLC (*Rb1*$^{-/-}$*Trp53*$^{-/-}$), and *in vitro*/*ex vivo* in several SCLC cell lines and PDX-derived-spheroids.

Results: LUR+ATZ was well tolerated, without unexpected toxicities. ORR were observed in 16/24 patients, including: 3/24 Complete Responses; 13/24 Partial Responses; 5/24 Stable Disease; and 3/24 Progressive Disease. Median PFS was 4.7 months (3.37-7.4) and median OS 14.5 months (9.5-23.4). In pre-clinical in vivo experiments, LUR+anti-PD-L1 achieved tumour rejection in 16/28 mice and 13/16 mice did not show tumour growth when exposed to a rechallenge. At the tumour, LUR increases Antigen Processing and Presentation (APP), activates IFN type-I signalling and promotes immune infiltration when combined with anti-PD-L1, particularly CD8+ T lymphocytes, at Tumour Immune Microenvironment (TIME). Finally, anti-tumour effect was mainly mediated by CD8+ T-cells.

Conclusions: LUR+ATZ anti-tumour activity is remarkable both in patients and pre-clinical *in vivo* experiments. Our results show effective tumour-activating IFN type-I, which increases immunity against tumours and provides the rationale to combine LUR with ICBs.

#5838 Quantification of ERAP-1 inhibition-dependent HLA-I peptides using targeted mass spectrometry for TCR-based therapeutics target discovery.

Anamarija Pfeiffer[1], Lucy Yang[1], Maik Mueller[1], **Daniel Redfern**[1], Yuehan Feng[1], Kris Clark[2], James Bishop[2], Juanita Carrey[2], Sam Humphrey[2], Wayne Paes[2]

[1]Biognosys AG, Schlieren, Switzerland,[2]Greywolf Therapeutics, Oxford, United Kingdom

Human leukocyte antigens (HLA) play a central role in orchestrating immune responses by presenting antigenic peptides derived from both self and foreign proteins. The identification of neoantigens, unique to individual tumors due to somatic mutations, has become a focal point in immunopeptidomics studies. In particular, accurate quantification of immunogenic peptides to evaluate their potential as novel targets for TCR-based therapeutics has been of increasing interest in immuno-oncology and autoimmune diseases, where regulating the immune response is of growing therapeutic importance. Additionally, precise (neo)antigen quantification can guide the development of personalized vaccines and cell therapies, further highlighting the need for robust methods to evaluate peptide presentation in diverse disease contexts.

Current methodologies to measure presented antigens are hampered by low sensitivity. Here, we employed a targeted mass spectrometry technique FAIMS-PRM to overcome these challenges. This method allows for development of highly sensitive targeted assays for the quantification of immunopeptide(s) of interest from minimal sample inputs: 25 million cells or 15mg fresh frozen tissue. Coupled to the spike-in of chemically synthesized internal standards (SIS), FAIMS-PRM enabled the estimation of copy number per cell of immunopeptides, thus providing essential information to assess potential targets in both disease and healthy matrices.

To exemplify the analytical benefits of this workflow, we quantified neoantigens presented by HLA-I complexes in cancer cells upon treatment with a small molecule ERAP1 inhibitor. ERAP1 trims peptide precursors to optimal lengths for loading onto HLA-I molecules in the endoplasmic reticulum, thereby regulating the repertoire of peptides available for recognition by $CD8^+$ T cells. Inhibition of ERAP1 results in an entirely novel repertoire of peptides being presented for immune recognition, generating functional anti-tumor T-cell responses and a novel target space for TCR-based therapies. In this study, a panel of peptide assays containing 30 ERAP1 inhibition-dependent HLA Class I peptides was developed with SIS for each target. This includes peptides from industry-validated TCR targets and from novel targets selected from prior discovery studies. In the tested melanoma and ovarian cancer cell lines, this targeted assay could demonstrate a lower limit of quantification (LLOQ) reaching low single-digit range (1-3 copies) per cell.. Treatment of these cells with ERAP1 inhibitors resulted in a 2- to 10-fold increase in epitope copy number depending on cell type.

In conclusion, we established a robust pipeline for HLA class I peptide quantification which can be routinely applied to estimate copy number per cell in biological samples such as cancer cell lines or tissues for target discovery.

#5839 Mimicking the immunosuppressive tumor microenvironment: a complex primary immune cell tumor killing assay to support candidate vaccine or immunomodulator screening.

Oliver J. Brand, Elizabeth Lieverse, Henry Leonard, Elsenoor Klaver, Lauren Schewitz-Bowers, **Louise S. Brackenbury**

Charles River Laboratories, Bristol, United Kingdom

Recent advances in oncology vaccine strategies, from off-the-shelf to personalized approaches offer a promising new weapon in the cancer treatment arsenal. The ability of these vaccines to exploit host immunity by priming and expanding Tumor Associated Antigen (TAA)-specific T cells presents a tractable tool to eradicate cancer whilst limiting systemic toxicity. TAA-specific $CD8^+$ T cells possess a potent capacity for tumor cell killing, and it is widely accepted that patients with higher frequencies infiltrating the tumor exhibit improved clinical outcomes. However, the complex tumor microenvironment (TME), composed of various stromal and regulatory immune cells such as regulatory T cells (Tregs), cytokines and extracellular matrices is often immunosuppressive and can reduce immune surveillance and favor uncontrolled tumor growth. Building preclinical screening platforms that better reflect the complexity of the TME are therefore crucial to enable better interrogation of therapeutic efficacy. To address this, we have developed a primary human TAA-specific tumor killing platform utilizing a tri-culture of antigen specific $CD8^+$ T cells (TAA-CD8), expanded natural T regs (nTregs) and appropriately selected tumor cells. Mart-1 was chosen as the model TAA to generate proof-of-concept data. This platform overcomes the anticipated low precursor cell frequencies by expanding both antigen specific T cells and nTregs. Following expansion, tumor killing assays (TKA) were established with TAA-CD8 and HLA-matched tumor targets with either: (1) endogenous TAA expression, (2) over-expression of target antigen following lentivirus transduction or (3) target peptide loading of MHC class I with exogenous peptide. Tumor killing was assessed in these cultures in the presence or absence of nTreg by time-lapse imaging (IncuCyte S3, Sartorious), with readouts including both tumor growth and apoptosis. Robust killing following antigen-specific expansion of CD8+ T cells was demonstrated and addition of expanded nTreg to these cultures resulted in reduced tumor cytotoxicity. Therapeutics can be tested for their efficacy in overcoming nTreg-mediated immunosuppression. Complex primary immune cell cultures provide an opportunity to test both immunomodulators and vaccines in vitro, and to better predict responses in patients by mimicking the suppressive nature of the TME. Adoption of translationally relevant platforms early in hit-to-lead campaigns for candidate vaccines or immunomodulators can accelerate drugs to the clinic through early screening.

#5840 PF-08046037, a PD-L1 targeted TLR7 agonist ISAC, drives innate immune activation and anti-tumor efficacy in preclinical in vitro and in vivo models.

Giacomo Tampella, Catalina Sakai, Weiping Zeng, Eleanor Hendrickson, Sasha Lucas, Kung-Pern Wang, Aly Smith, **Ryan A. Heiser**

Pfizer, Inc., Bothell, WA

PF-08046037 (formerly known as SGN-PDL1iT) is an immune-stimulating antibody conjugate (ISAC) designed to induce an immune response against cancer cells by delivering a potent Toll-like receptor 7 (TLR7) agonist to tumor associated antigen-presenting cells (APCs) expressing PD-L1. The ISAC consists of an anti-PD-L1 antibody linked to an imidazoquinoline-based TLR7 agonist using a mannosidase-cleavable maleimidopropionyl-mannose linker (mp-mann). Upon binding to PD-L1 on APCs, PF-08046037 internalizes and releases the payload into TLR7-containing endosomes. To minimize off-target immune activation, PF-08046037 incorporates Fc mutations in the antibody to reduce FcγR binding, and the agonist payload is designed to have low membrane permeability. Through targeting PD-L1-expressing immunosuppressive macrophages and dendritic cells within the tumor and associated immune compartments, PF-08046037 aims to repolarize the suppressive tumor microenvironment, enhance antigen presentation, and establish robust anti-tumor immunity. Here, we present preclinical data demonstrating the potency, targeted delivery, and anti-tumor efficacy of PF-08046037 in both in vitro and in vivo models. In vitro, PF-08046037 induces potent and selective activation of TLR7 reporter cells and PD-L1-expressing human macrophages and dendritic cells. In syngeneic hPD-L-1 .CT26 and hPD-L-1 .MC38 tumor models using human PD-L1-expressing mice, PF-08046037 shows single-agent efficacy and provides anamnestic protection against tumor rechallenge. The in vivo pharmacodynamic effects of PF-08046037 align with its intended mechanism of action, including acute tumor inflammation, loss of CD206+ suppressive macrophages, upregulation of activation and costimulatory molecules on APCs in tumors and tumor-draining lymph nodes (tdLN), and increased activity of cytotoxic cells. Collectively, these data highlight the therapeutic potential of targeted TLR7 agonist delivery to PD-L1-expressing APCs in cancer and support the clinical investigation of PF-08046037 in advanced malignancies.

#5841 SARS-CoV-2 mRNA vaccines sensitize tumors to immune checkpoint blockade.

Adam Grippin[1], Christiano Marconi[2], Sage Copling[1], Priti Gupta[1], Elliana Young[1], Padmanee Sharma[1], Don Gibbons[1], Hai Tran[1], Yon Son Betty Kim[1], Jianjun Zhang[1], Jennifer Wargo[1], John V. Heymach[1], Hector Mendez-Gomez[3], Wen Jiang[1], Elias Sayour[2], Steven H. Lin[1]

[1]UT MD Anderson Cancer Center, Houston, TX,[2]University of Florida, Gainesville, FL,[3]University of Florida, Houston, TX

Background: We previously reported that personalized mRNA vaccines enhance responses to immune checkpoint inhibition (ICI) in part by stimulating IFN-α production. We hypothesized that mRNA vaccines encoding non-tumor antigens, including those targeting SARS-COV-2, would similarly augment responses to ICI.
Methods: We utilized institutional databases to extract clinical data for patients with Stage III/IV non-small cell lung cancer (NSCLC) (n=2406) and metastatic melanoma (n=757) with biopsy specimens between August 2019 and August 2023, as well as a separate “tissue agnostic” cohort with pathology reports quantifying “PD-L1” from August 2020 to November 2023 (n=5,524). We then generated a preclinical model of ICI-resistance with subcutaneous murine B16F0, and analyzed plasma from a cohort of healthy volunteers (n=5) at baseline and 6 hours, 24 hours, 1 week, and 2 weeks after SARS-COV-2 mRNA vaccination with NULISA-Seq and flow cytometry. Differences were assessed with Wilcoxon rank-sum tests, Cox proportional hazards regression, and Gehan-Breslow-Wilcoxon tests.
Results: Median overall survival (OS) was doubled for patients with Stage IV NSCLC who received a SARS-COV-2 mRNA vaccine within 100 days of initiation of ICI compared to patients diagnosed before the pandemic (555 days vs 1120 days, HR 0.5, 95% CI 0.4-0.7) and contemporary patients who did not receive a vaccine (646 days vs 1120 days, HR 0.6, 95% CI 0.4-0.9, p=0.007). OS was unchanged for vaccinated patients who did not receive ICI and those who received non-mRNA vaccines. In the melanoma cohort, SARS-COV-2 mRNA vaccination was associated with substantially improved OS (HR 0.42, 95% CI 0.24-0.74, p=0.003) and progression free survival (HR 0.64, 95% CI 0.44-0.92, p=0.022). In our preclinical model, SARS-COV-2 mRNA vaccines stimulated a surge in many inflammatory cytokines including IFN-α, leading to systemic innate immune activation, expansion, activation, and persistence of tumor-reactive T cells, and increased expression of PD-L1 on tumor cells. SARS-COV-2 mRNA vaccines significantly inhibited growth of B16F0 tumors only when combined with ICI. This effect required Type I IFN signaling but was not recapitulated with systemic IFN-α alone. We then recapitulated these results in human subjects, where SARS-COV-2 mRNA vaccinaties were associated with an 8-fold increase in IL-6 and at least 265-fold increase in IFN-α (p<0.001) in five healthy volunteers. Finally, SARS-COV-2 mRNA vaccination within 100 days of biopsy was associated with a 23% increase in mean tumor proportion score (TPS) of PD-L1 in patients with NSCLC (31% vs 26%, p=0.029) and a 55% increase in mean TPS (14% vs 8.9%, p=0.029) in the tissue agnostic cohort.
Conclusions: SARS-COV-2 mRNA vaccines are associated with a cytokine cascade that likely induces PD-L1 expression in tumors and augments responses to ICIs, leading to improved survival in patients with metastatic melanoma and NSCLC.

#5842 A novel formulated neoantigen DNA vaccine with promising anticancer efficacy in B16F10 mouse melanoma model.

Kempaiah Rayavara, Subeena Sood, Majed Matar, Jessica Kim, John Henderson, Jeff Sparks, Meredyth Kinsella, Olivia Signer, Joseph Rogers, Chelsey Bellmon, **Khursheed Anwer**

IMUNON, Huntsville, AL

Cancer vaccine approaches targeting neoantigens are gaining credence due to their specificity for tumors while sparing healthy tissues. Cancer vaccines based on DNA technology are attractive anti-cancer therapeutic tools due to their ability to elicit an effector T-cell-based immune responses. We report the development of a novel DNA-based cancer vaccine that relies on the delivery of a plasmid DNA vector encompassing immune epitopes of multiple neoantigens delivered with a novel synthetic DNA delivery system with the aim to elicit a broader immune response against cancer. The formulated vaccine was also investigated in combination with different adjuvants to maximize the anti-cancer responses. The vaccine vector encoding the immunogenic epitopes of multiple melanoma neoantigens and flanked by signal peptide at N-terminus and an MHC-targeted domain at the C-terminus end was formulated with a functionalized novel synthetic polymer with or without the adjuvants Poly I:C, diABZI, a STING agonist, or a mouse IL-12 plasmid. Mice were implanted subcutaneously with 0.5×10^6 B16F10 tumor cells on Day 0 and vaccinated intramuscularly with the DNA formulations on Day 2, 9, 16 and 23, and monitored for tumor growth and survival . For cellular responses, mice were injected intramuscularly with the DNA formulations on Day 0, 21, 42 and sacrificed 7 days later for cellular immune response by ELISPOT assay. Vaccination with formulated DNA alone yielded a significant inhibition (60%, $p<0.01$) of tumor growth and improved survival. Addition of Poly I:C, diABZI, or IL-12 plasmid further augmented the tumor inhibition to 90%($p<0.0001$), 94% ($p<0.0001$), and 88% ($p<0.0001$), respectively, and improved the survival outcome. All animals in the adjuvant groups also lived longer. The anti-tumor efficacy results are supported by the onset of robust T-cell responses in all treatment groups supporting the involvement of immune activation in the action of this novel DNA vaccine that does not require a virus or device and can be manufactured rapidly with a simple and cost-effective manufacturing process critical for the viability of cancer vaccines.

#5843 Selective small molecule inhibitor of STAT3 suppresses tumor growth and improves immune checkpoint inhibitor efficacy in *KRAS* mutant lung cancer.

Shanshan Deng[1], Kris Eckols[1], Moses Kasembeli[1], Katherine E. Larsen[1], Nastaran Karimi[1], Michael J. Clowers[1], Anthony Zhang[2], Leticia G. Hamana Zorilla[1], Luisa M. Solis Soto[1], Imran Alibhai[3], Sofia de Achaval[4], Ferdinandos Skoulidis[1], David J. Tweardy[1], Seyed Javad Moghaddam[1]

[1]The University of Texas MD Anderson Cancer Center, Houston, TX,[2]Rice University, Houston, TX,[3]Tvardi Therapeutics, Houston, TX,[4]Tvardi Therapeutics, Inc., Houston, TX

KRAS mutant (KM) lung cancer is the most common molecular subtype of lung adenocarcinoma (LUAD) and is strongly associated with cigarette smoking. KM-LUAD has a poor prognosis as it is resistant to most chemotherapy and targeted therapies. Also, despite recent breakthroughs in development of KRAS inhibitors (KRASi) and immune checkpoint inhibitors (ICIs), only a small sub-population of patients benefit from these therapies and resistance eventually develops. Thus, an urgent unmet medical need exists for alternative strategies that can complement KRASi and/or ICI to improve responses. KM-LUAD is closely associated with tumor-promoting inflammation, particularly the activation of the IL-6/STAT3 pathway. We previously showed in KM-LUAD patients that higher levels of STAT3 mRNA was associated with worse disease-free survival, and that targeting IL-6 in the $CCSP^{Cre}$/LSL-$Kras^{G12D}$ (CC-LR) mouse model of KM-LUAD reduced STAT3 activation and lung tumorigenesis. In this study, we targeted STAT3 directly with a safe and selective small molecule STAT3 inhibitor, TTI-101, that currently is in Phase II studies for treatment of hepatocellular carcinoma and idiopathic pulmonary fibrosis. Starting at 14 weeks of age, when tumors are established, four groups of CC-LR mice (n= 9-16 per group) received TTI-101 alone (100 mg/kg by oral gavage daily), anti-PD-1 mAb alone (10 mg/kg intraperitoneally thrice weekly), TTI-101 plus anti-PD-1 mAb, or no treatment for four weeks. TTI-101 alone reduced lung surface tumor number (LSTN) by 39.5% (22.4 ± 12.0 LSTN; mean ± SD) compared to the no-treatment group (37.0 ± 12.8 LSTN; p=0.02; Welch's ANOVA), while anti-PD-1 mAb alone failed to reduce LSTN (43.8 ± 17.0 LSTN; p=ns). Notably, the combination of anti-PD-1 with TTI-101 further decreased LSTN by 46.4% (12.0 ± 5.7 LSTN) compared to TTI-101 alone (22.4 ± 12.0 LSTN; p=0.04) and by 72.6% (12.0 ± 12.0 LSTN) compared to anti-PD-1 mAb alone (43.8± 17.0 LSTN; p=0.0002). Daily monitoring indicated no significant weight changes or toxicity from either TTI-101 or anti-PD-1 mAb treatment. Thus, STAT3 inhibition using TTI-101, either alone or in combination with anti-PD-1, is a promising new strategy for treating KM-LUAD.

Funding support: This project has been funded by an NIH/NCI R01 grant (1R01CA279452-01A1) awarded to SJM, DJT, and FS. AZ was partly supported by the Carl B. and Florence E. King Foundation Summer Program in Biomedical Sciences, MD Anderson Cancer Center.

#5844 Initial findings on the immunomodulatory effects of histotripsy combined with N-dihydrogalactochitosan in a comparative oncology model of canine osteosarcoma.

Ny Luong[1], Alayna Hay[1], Elliana Vickers[2], Samuel S. K. Lam[3], Wei R. Chen[4], Eli Vlaisavljevich[2], Joanne Tuohy[1]

[1]Virginia-Maryland College of Veterinary Medicine, Roanoke, VA,[2]Virginia Tech, Roanoke, VA,[3]Immunophotonics, Inc., St. Louis, MO,[4]University of Oklahoma, Norman, OK

Background: Osteosarcoma (OS) is the most common primary malignant bone tumor in children and adolescents, associated with poor prognosis, especially in cases with metastatic disease at diagnosis. Canine OS shares many biological and clinical similarities with human OS, making dogs valuable comparative models for OS research. Histotripsy is a non-thermal, non-invasive high-intensity focused ultrasound modality that mechanically disrupts tumor cells, triggering the release of neoantigens and damage-associated molecular patterns (DAMPs) to potentially promote an anti-tumor immune response. N-dihydrogalactochitosan (GC) is a novel immunostimulant shown to enhance systemic immune responses when delivered intratumorally after thermal ablation. However, the combination of GC with a non-thermal ablation modality such as histotripsy remains unexplored. This study evaluates the safety, feasibility, and immunomodulatory effects of combining histotripsy with GC in pet dogs with spontaneous OS.

Methods: Pet dogs with spontaneous OS were treated with a 700 kHz histotripsy system at a1000 Hz pulse repetition frequence and 500-750 pulses/point. Patients underwent 1-5 fractionated histotripsy treatments , ablating the majority of the tumor. After the final histotripsy treatment, GC (10 mg/mL) was injected intratumorally under ultrasound guidance, with the dose proportional to tumor size. Peripheral blood was collected at baseline and 2-, 4-, and 8-weeks post-histotrispy+GC treatment and peripheral blood mononuclear cells (PBMCs) were isolated and immune cell signatures were characterized, serum cytokines and chemokines profiles were evaluated using a Milliplex Canine Cytokine Panel. At the follow up timepoints lung metastases were monitored via thoracic radiographs.

Results: The combination of histotripsy and GC was tolerated, with swelling at the treatment site resolving within one week. At 2 weeks post-treatment, circulating monocytes (CD11b+CD14+) increased with upregulation of CD80 and CD62L expression compared to baseline level. Although the percentage of gated CD11b+CD14+CD80+ monocytes remained stable after two weeks, CD80 expression continued to increase, suggesting sustained monocyte activation. CD62L expression on CD4+ and CD8+ T cells decreased at 2 weeks but increased again by 4 weeks post treatment, potentially reflecting changes in T cell trafficking. Two of three patients remained free of lung metastases at 10 weeks post treatment. Cytokine and gene expression analyses are ongoing and will further inform systemic immune responses.

Conclusion: These preliminary findings suggest that histotripsy combined with GC may induce immune activation and holds promise as a therapeutic strategy for targeting primary tumors and metastases in OS.

#5846 A potent STING agonist induces endothelial PD-L1 and enhances antitumor efficacy of a novel PD-L1/PD-L2 antibody.

Ahmad Salameh, Manuela iezzi, Andrew Lewis, Christine Gagliardi, Laura Conti, Elisabetta Bolli, Chiara Cossu, Martina CECI, Paul Blezinger, Michael A. Curran, Federica Cavallo, James Barlow, Federica Pericle

RD, Immunogenesis, Inc., Houston, TX

Background: The activation of stimulator of interferon genes (STING) has emerged as a promising strategy to reprogram the tumor microenvironment (TME) and enhance antitumor immunity. We previously demonstrated that combining a potent STING agonist (8803, IMGS-203) with a novel dual-specific anti-PD-L1/PD-L2 monoclonal antibody (27907) produced curative responses in checkpoint-refractory tumor models. In this study, we investigated the mechanisms underlying these effects by analyzing the TME of tumors treated locally with 8803 and systemically with 27907 using immunohistochemistry and flow cytometry.
Objective: To evaluate the necrobiotic effects of the STING agonist 8803 combined with the cytotoxic anti-PD-L1/PD-L2 antibody 27907 across multiple tumor models by characterizing the TME and tumor vascularization.
Methods: The efficacy of 8803 was assessed in vivo in combination with 27907 in murine melanoma (B16F10 expressing mouse PD-L2) and mammary adenocarcinoma (TS/A) models. Mice with established tumors received intratumor injections of 8803 (10 ug/dose, twice) and systemic administration of 27907 (10 mg/kg, twice weekly for three weeks). Tumors were harvested 32 days post-challenge and analyzed via H&E staining, IHC, and FACS to evaluate necrobiosis, immune cell infiltration, and vascularization.
Results: The combination of 8803 with 27907 demonstrated robust antitumor activity in both melanoma and breast cancer models. IHC and FACS profiling revealed a significant increase in infiltrating myeloid cells alongside a notable reduction of the immunosuppressive M2 macrophage phenotype (CD206+). Intratumor 8803 markedly upregulated PD-L1 expression in endothelial cells, that were effectively disrupted by systemic 27907, as evidenced by increased caspace-3 and CD62p co-expression in endothelial cells within treated tumors. This endothelial disruption was absent in tumors treated with 8803 alone. Further, western blot and FACS analyses of human and murine endothelial cells co-cultured with 8803 confirmed upregulation of PD-L1 and PD-L2. Critically, PD-L1+/PD-L2+ endothelial cells were selectively eliminated by monocytic-enriched bone marrow-derived myeloid cells and PBMCs in the presence of 27907, suggesting a direct effect of the antibody on tumor vasculature.
Conclusion: Tumors treated with the combination of 8803 and 27907 exhibited extensive necrobiosis and endothelial damage, uniquely observed in the combination treatment group and correlating with curative outcomes. This enhanced antitumor response was associated with increased myeloid infiltration, a shift away from M2 macrophage phenotype, and an elevated effector T-cell population. These findings underscore the potent therapeutic synergy of 8803 with 27907 and support their further development for clinical applications.

#5848 Oxygen-releasing cryogel reinforce dendritic cell function and T-cell mediated tumor immunity in hypoxic microenvironments.

Khushbu Bhatt

Department of Pharmaceutical Sciences, Northeastern University, Boston, MA

Cancer immunotherapy faces significant barriers due to tumor heterogeneity and the hypoxic nature of the solid tumor microenvironment (TME). Hypoxia, defined by reduced oxygen (O_2) tension, induces immunosuppression, impairing dendritic cell (DC) and T-cell function, which are critical for robust anti-tumor immunity. Here, we report on the evaluation of a novel hyaluronic acid (HA)-based cryogel scaffold, termed O_2-cryogels, engineered to counteract hypoxia by releasing O_2 through encapsulated calcium peroxide (CaO_2) particles and catalase enzyme. Our results demonstrate that O_2-cryogels effectively alleviate hypoxia-induced suppression of murine and human DC function. Specifically, they rescue critical DC processes such as antigen uptake, migratory activity, and the expression of activation and co-stimulatory molecules essential for T-cell priming. Moreover, O_2-cryogels enhance the secretion of proinflammatory cytokines, mitigate hypoxia-induced phenotypic switching of mouse DCs to tolerogenic plasmacytoid DCs (pDCs), and suppress the upregulation of hypoxia-induced tolerance markers. These effects translate to restored allogenic T-cell proliferation and improved OT-I cell-mediated cytotoxicity against B16-OVA tumor cells under hypoxic conditions. This study highlights the ability of O_2-cryogels to locally modulate oxygen levels, reinvigorate DC function, and enhance T-cell-mediated tumor cell killing, addressing a critical bottleneck in cancer immunotherapy. By targeting hypoxia, O_2-cryogels offer a novel and promising approach to amplify the efficacy of immunotherapeutic strategies, paving the way for innovative treatments in hypoxia-driven solid tumors.

#5849 Re-Phen, a novel OXPHOS enhancer that reprograms T cell mitochondrial metabolism to potentiate anti-PD-1 immunotherapy.

Boping Jing[1], Qiuxu Teng[1], Guodong Zhang[1], Chiyi Xiong[1], Ping Pan[1], Qizhen Cao[1], Ningbo Zhang[2], Si Chen[2], Weiyi Peng[2], **Chun Li**[1]

[1]UT MD Anderson Cancer Center, Houston, TX,[2]University of Houston, Houston, TX

Introduction: Immune checkpoint inhibitors fail to control most PDAC tumors. Identifying novel ways to enhance immunotherapy in PDAC is highly desired. This study aimed to evaluate a new phenformin analogue, termed Re-Phen, that targeted intratumoral dysregulated metabolism pathways.

Methods: Re-Phen was encapsulated into a polymeric micellar to give MRP. ^{99m}Tc-Phen, a structurally matched radiotheranostic counterpart of Re-Phen was also synthesized and encapsulated into polymeric micelles (i.e. ^{99m}Tc-MRP) for SPECT/CT imaging and biodistribution study. The effects of Re-Phen on mitochondrial membrane potential, generation of reactive oxygen species (ROS), and ATP production were studies *in vitro* with both tumor cells and T cells. Antitumor efficacy of MRP in combination with anti-PD-1 was studied in both subcutaneous and orthotopic models murine Kras* PDAC cells. In addition, immune cells in Kras* tumor and spleen of MRP-treated mice were profiled at 5 days after initiation of treatments.

Results: SPECT/CT with ^{99m}Tc-MRP and biodistribution studies revealed that MRP had higher uptake in tumor and reduced uptakes in the liver and kidneys compared to un-encapsulated Re-Phen. *In vitro,* Re-Phen displayed similar activity as Phen in reducing mitochondrial membrane potential. However, unlike Phen, Re-Phen reduced ROS, increased ATP production by OXPHOS instead of by glycolysis without suppress T cell proliferation. In the orthotopic Kras* model, MRP administered at a dose of 12.5 mg/kg in combination with anti-PD-1 demonstrated superior anti-tumor efficacy, achieving a long-term survival rate of 55.6%. In comparison, mice treated with MRP at 25 mg/kg plus anti-PD-1, anti-PD-1 alone, MRP (12. 5 mg/kg) alone, and untreated control achieved long-term survival rate of 33.3%, 12.5%, 0%, and 0%, respectively. In a subcutaneous Kras* PDAC model, MRP plus anti-PD-1 showed strong abscopal effect as evidenced by suppression of tumor growth in the contralateral tumors that were not treated by a local intervention with irreversible electroporation (IRE). Moreover, MRP significantly increased survival rates when combined with IRE and anti-PD1 in the subcutaneous Kras* PDAC model. The triple combination achieved an 87.5% long-term survival rate, compared to 25% with IRE alone and 25% with anti-PD1 alone. In the subcutaneous pancreatic tumor models, the combination of MRP and anti-PD1 therapy elicited a stronger immune response compared to the untreated and anti-PD1-only groups. This included an increase in CD4+ T cells, CD8+ T cells, and dendritic cells (DCs) in both tumors and spleens, along with a reduction in neutrophils.

Conclusions: Re-Phen is a novel OXPHOS enhancer that induced a robust antitumor immune response, resulting in potentiation of antitumor immunotherapy. Further development of this class of drugs is warranted.

#5850 AXA-042, a systemically administered TLR2/6 agonist, demonstrates innate immune engagement and Th1 polarization in patients with advanced solid tumors.

B. Tran[1], S. Frentzas[2], C. Lemech[3], M. Millward[4], D. Day[2], J. Desai[1], A. Latifi[5], P. Wabnitz[5], P. Kearney[5], **A. V. Galkin**[5];
[1]Peter MacCallum Cancer Centre, Melbourne, Australia, [2]Monash Health, Clayton, Australia, [3]Scientia Clinical Research, Randwick, Australia, [4]Linear Clinical Research, Perth, Australia, [5]Axelia Oncology, Melbourne, Australia

Background: AXA-042 is a novel, systemically delivered synthetic toll-like receptor (TLR) 2/6 agonist in development for the treatment of advanced solid tumors. A first-in-human study evaluating the safety, tolerability, pharmacokinetics, and pharmacodynamics of AXA-042 as monotherapy and in combination with a checkpoint inhibitor in subjects with advanced solid tumors is currently ongoing (ACTRN12622000993796). Cytometry by time-of-flight (CyTOF) analysis was performed to characterize AXA-042 immune modulation signatures in patient whole blood samples.
Methods: AXA-042 was administered intravenously q21d. Informed consent was obtained and whole blood samples were collected pre-dose on C1D1, and 24h and 168h post-treatment (C1D2, C1D15) across three dose levels of AXA-042. Stabilized whole blood samples were immunophenotyped using a 43-marker CyTOF panel.
Results: In pre-dose samples, TLR2 was most frequently expressed on Monocytes (>80%), cDCs (47%) and in 20-30% of CD8, CD4 and γδ-T cell subsets. AXA-042 treatment led to the re-distribution of neutrophil, monocyte and cDC subset frequencies on C1D2, consistent with the expected acute inflammation response to a TLR2 agonist. Furthermore, AXA-042 increased the frequency of activated CD69+ cytolytic NK cells and γδ-T cells on C1D2. A general immune reset to baseline levels occurred for most populations on C1D15. Notably, a decrease in CTLA4+ Tregs and an increase in T-bet+ NK, γδ-T cells and TCF1+ CD8, CD4 T-cell subset frequencies, indicating a potential increase in Th1 polarization and preservation of effector function, was observed on C1D15 (Table 1).
Conclusions: The whole blood immunophenotyping analysis identified early pharmacodynamic markers of innate immune response engagement in response to AXA-042 and demonstrated sustained presence of Th1-polarized NK and T cell subsets with potential for enhanced cytotoxicity and effector function on C1D15.
Table 1.

Subset Frequency	**Timepoint**	**Median % Change (* FDR<0.05, ** FDR<0.01)**		
		Dose Level 1 (n=6)	**Dose Level 2 (n=3)**	**Dose Level 3 (n=3)**
↑CD16lo Neutrophils	**C1D2**	8.7**	38.8**	48.3 **
↓CD16hi Neutrophils	**C1D2**	-7.8**	-37.8**	-48.6 **
↑CD44+ Neutrophils	**C1D2**	13.2	23.6**	17.4**
↓CD39+ Monocytes	**C1D2**	-20.1**	-15.9**	-29.5**
↓CD39+ cDCs	**C1D2**	-14.9**	-12.8*	-5.0*
↓HLA-DR+ Monocytes	**C1D2**	-17.5**	-15.6**	-0.9*
↓CD11b+ Monocytes	**C1D2**	-8.6**	-17.3*	-27.3**
↑CD69+ Cytolytic NKs	**C1D2**	10.5	36.5**	16.7**
↑CD69+ γδ-T	**C1D2**	-0.7 *	14.4	18.2 **
↓CTLA4+ Tregs	**C1D15**	-2.3 *	-11.1 **	-15.9 **
↑Tbet+ Cytolytic NK	**C1D15**	5.5*	30.5**	23.9**
↑Tbet+ γδ-T	**C1D15**	4.4	16.2**	13.1**
↑TCF1+ CD8 TCM T cells	**C1D15**	2.6	14.5*	12.9**
↑TCF1+ CD4 TCM T cells	**C1D15**	0.03	14.5*	9.8**
↑TCF1+ CD8 Naïve T cells	**C1D15**	0.42	21.1*	15.5**
↑TCF1+ CD4 Naïve T cells	**C1D15**	5.3	15.6**	14.7**
↑HLA-DR+ ncMonocytes	**C1D15**	-4.1	9.0*	25.9**
↑HLA-DR+ cDCs	**C1D15**	-0.2	7.4*	32.5*

#5851 The role of IL-9 on NK and T cell infiltration and immune response to promote tumorigenesis in HNSCC.

Samantha L. Nusbaum, Maria Lehn, Vinita Takiar, Dalia El-Gamal, Trisha Wise-Draper

University of Cincinnati, Cincinnati, OH

Head and neck squamous cell carcinomas (HNSCC) are the sixth most common cancer type worldwide and result in 400,000 deaths annually. Interleukin-9 (IL-9) is a pleiotropic cytokine largely produced by CD4+ Th9 cells. IL-9 is known to function in allergic inflammatory processes and is involved in both positive and negative regulation of immune responses. The role of IL-9 in cancer, however, differs depending on the cancer type and the tumor microenvironment, harboring both tumorigenic and anti-tumorigenic effects. The role of IL-9 in HNSCC specifically is poorly understood. We begin here to elucidate the possible functions of IL-9 on the immune system in the context of HNSCC.IL-9 plasma levels were determined by ELISA and cell proliferation was measured using a metabolic absorbance assay. Kaplan-Meier Plotter was used to determine if tumor IL-9 mRNA expression affects survival probability in HNSCC patients. Immune cell infiltration was determined by a novel infiltration assay designed by our laboratory. Briefly, we co-cultured Cal27 spheroids, a 3D model made with the HNSCC cell line Cal27, with peripheral blood mononuclear cells (PBMCs) isolated from HNSCC patients. The spheroids were then dissociated and the percentage of live infiltrating PBMCs was analyzed by flow cytometry. To assess the influence of IL-9 on immune cell killing, NK and CD8+ T cell cytotoxicity was measured by co-culturing CFDA-SE-labeled HNSCC cell lines with HNSCC patient-derived NK or T cells treated with recombinant IL-9 or a neutralizing antibody to IL-9 followed by flow cytometry analysis to determine the percentage of dead target cells.IL-9 plasma levels were found to be significantly increased in HNSCC patients as compared to healthy subjects. Additionally, high tumoral mRNA expression of IL-9 was associated with decreased survival in HNSCC cohorts. IL-9 did not affect tumor cell proliferation in vitro. However, higher concentrations of IL-9 significantly decreased immune cell infiltration into Cal27 spheroids, while a neutralizing antibody to IL-9 reversed these effects. Additionally, while studies are ongoing, IL-9 may decrease NK and CD8+ T cell cytotoxicity against HNSCC cell lines.Since IL-9 did not directly enhance or inhibit HNSCC cell proliferation in vitro, we extended our studies to explore IL-9's potential effects on the immune system. Importantly, IL-9 significantly decreased immune cell infiltration into HNSCC spheroids, suggesting that IL-9 inhibits tumor immune cell infiltration. IL-9 may also decrease immune cell cytotoxicity against HNSCC cells. Taken together, this data suggests that IL-9 negatively regulates the immune system in HNSCC, allowing for tumor growth. Future studies will focus on the effects of IL-9 on immune cell cytotoxicity and immune infiltration in vivo and will utilize single-cell transcriptional profiling to delineate the multifaceted role of IL-9 in the HNSCC immune microenvironment.

CLINICAL RESEARCH: Inflammation, Immunity, and Cancer
Poster Session

#5856 Secondary hemophagocytic lymphohistiocytosis: A systematic review of incidence and prognostic outcomes.

Maha Hameed[1], Gustavo Garcia[1], Kevin C. Zhen[1], Brooke A. Hartenstein[1], Laura D. J. Herrera[1], Utkarsh Acharya[2], Kai He[2], Karen Hamad[1], Wilhelmine Wiese-Rometsch[1]

[1]Florida State University, Tallahassee, FL,[2]The Ohio State University, Columbus, OH

Introduction: Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening condition characterized by immune system dysregulation of natural killer cells, cytotoxic CD8+ T-cells, and macrophages, resulting in malignant inflammation and multi-organ failure. Secondary HLH, often triggered by malignancies, infections, and/or autoimmune disease, typically affects adults. This systematic review investigated the characteristics and prognostic factors associated with secondary HLH.
Methods: A systematic review was conducted in adherence to the PRISMA guidelines. A literature search was performed utilizing Medline, Embase, Scopus, Cochrane, and Clinicaltrials.gov databases without restricting to a starting date. Inclusion criteria encompassed retrospective or prospective, randomized, or non-randomized controlled studies published in English, focusing on adult patients over the age of 18 with secondary/acquired HLH. The primary outcomes of interest were overall survival (OS) and HLH-free/relapsed-free survival.
Results: The systematic review yielded 23 eligible studies, including 2833 patients with a median age of 48.3 years and 1482 (52%) males. Most studies originated from China (48%), followed by Korea (13%) and the USA (13%). Malignancies, particularly lymphomas, and infections, notably Epstein-Barr virus (EBV), were identified as the most common underlying causes of secondary HLH. Two studies involved ICU-related secondary HLH diagnoses, whereby sepsis-associated HLH with gram-negative bacteremia, particularly E. coli (30%), was most implicated. OS was a ubiquitously reported outcome, with only two studies (9%) including HLH-free survival/HLH-relapse-free survival. Most frequently reported statistically significant worse prognostic factors in terms of OS included older age in 8 (35%), low platelets <100 in 7 (30%), and malignancy-associated HLH in 7 (30%) studies. Two studies demonstrated worse prognosis in terms of HLH-free survival. Other poor OS prognostic factors included EBV-associated HLH in 6 (26%), low fibrinogen in 5 (22%), elevated AST in 5 (22%), elevated LDH >400 in 4 (17%), high bilirubin in 4 (17%), albumin <2.8 g/dL in 4 (17%), male sex in 3 (13%), ferritin >2000 in 3 (13%), low hemoglobin in 3 (13%), prolonged APTT in 3 (13%), prolonged PT in 3 (13%), elevated creatinine in 2 (9%), elevated sCD25 in 2 (9%) and elevated IL-10 in 2 (9%). Least frequently reported were prolonged INR, fever, low total T3, and elevated beta-2-microglobulin. One study demonstrated that germline HAVCR2 mutation had a better prognosis.
Conclusion: Older age, thrombocytopenia, and malignancy were the most common poor prognostic indicators for secondary HLH when indexed to outcomes of OS. This review also highlights a potential gap in the global understanding and documentation of secondary HLH, given the notable scarcity of studies from countries outside of China.

#5857 Targeting KDELR2 remodels the immune tumor microenvironment for enhanced anti-cancer response.

Shakti Prasad Pattanayak[1], Boaz Tirosh[2]

[1]Case Western Reserve University School of Medicine, Cleveland, OH,[2]Department of Biochemistry, Case Western Reserve University School of Medicine, Cleveland, OH

Inflammation within the tumor microenvironment (TME) boosts anti-cancer immune responses by activating both innate and adaptive immunity. In situ inflammation enhances antigen presentation, immune cell infiltration and supports immune-cancer cycle. KDEL receptors (R1,R2 & R3) are critical for retaining KDEL proteins in the endoplasmic reticulum (ER). Their compromise can cause a concerted release of pro-immunogenic molecule, such as calreticulin. This can provoke conditions similar to immunogenic cell death (ICD) in the absence of chemotherapy. To address the role of KDEL receptors in immune-cancer interactions, we generated a mouse colon cancer model equipped with doxycycline (DOX)-induced silencing of the KDELRs. We found that in these cells KDEL-R2 isoform controls the intracellular retention of a large number of secretory proteins including calreticulin. Surprisingly, *in vivo* when cells with silenced KDEL-R2 were mixed with normal tumor cells in a 1:10 ratio and treated with DOX, tumors significantly regressed in mice. Analysis of the TME (via flow cytometry, IHC, and IF) revealed a massive infiltration of neutrophils and macrophages, most are polarized to M1. In vitro macrophage polarization studies confirmed that from the silencing of KDEL-R2 confers polarization. Using a novel biotin-labeled probe to detect extracellular PDIs, we confirmed the robust ICD intensity of KDEL proteins in the TME. In athymic Nu/Nu mice, KDEL-R2 silencing also induced tumor regression, underscoring the dominant role of innate immunity. Re-challenge experiments demonstrated T-cell priming and the establishment of immune memory, supported by T-cell and macrophage infiltration in the TME. Additionally, using tumor cells that present an antigenic MHC class I peptide, we demonstrate T cell priming in the absence of vaccination. This study reveals a significant advancement in cancer immunotherapy and potential for synergistic effect with other treatments such as CAR-T therapy and checkpoint inhibitors through immune memory and inflammation-driven immunity.

#5858 Prognostic and predictive role of T cell infiltration in stage III colon cancer (CC) treated with celecoxib: CALGB/SWOG 80702.

Yasutoshi Takashima[1], Chao Ma[1], Qian Shi[2], Andressa Dias Costa[1], Tyler Twombly[3], Juha P. Vayrynen[4], Melissa Zhao[3], Ardaman Shergill[5], Pankaj Kumar[6], Felix Couture[7], Philip Kuebler[8], Smitha Krishnamurthi[9], Benjamin Tan[10], Eileen M. O'Reilly[11], Anthony F. Shields[12], Shuji Ogino[3], Charles S. Fuchs[13], Jeffrey A. Meyerhardt[1], Jonathan A. Nowak[3]

[1]Dana-Farber Cancer Institute, Boston, MA,[2]Alliance Statistics and Data Management Center, Mayo Clinic, Rochester, MN,[3]Brigham and Women's Hospital, Boston, MA,[4]University of Oulu and Oulu University Hospital, Oulu, Finland,[5]The University of Chicago Medical Center, Chicago, IL,[6]Heartland Cancer Research NCORP, Illinois CancerCare PC, Peoria, IL,[7]Hotel-Dieu de Quebec, Quebec City, QC, Canada,[8]Columbus NCI Community Oncology Research Program, Columbus, OH,[9]Cleveland Clinic, Cleveland, OH,[10]Siteman Cancer Center, Washington University School of Medicine, St. Louis, MO,[11]Memorial Sloan Kettering Cancer Center, Weill Cornell Medical Center, New York, NY,[12]Karmanos Cancer Institute, Wayne State University, Detroit, MI,[13]Genentech, South San Francisco, CA

Observational studies have demonstrated that aspirin and/or PTSG2 inhibitor (e.g., celecoxib) use, before or after colon cancer (CC) diagnosis, is associated with a lower risk of recurrence. However, a randomized trial of adjuvant celecoxib therapy in stage III CC did not show improved survival compared to placebo treatment. We therefore hypothesized that immune microenvironment features may identify patient subgroups with stronger survival benefits from anti-inflammatory celecoxib treatment. Drawing from an NCI-Intergroup trial (Cancer and Leukemia Group B [now Alliance for Clinical Trials in Oncology]/SWOG 80702) for patients with stage III resected CC that compared 3 versus 6 months of adjuvant fluorouracil, leucovorin, and oxaliplatin ± 3 years of celecoxib, we conducted tissue-based tumor microenvironment profiling on 1,098 resected tumors using a custom 9-plex, T-cell multiplex immunofluorescence assay coupled with digital image analysis and supervised machine learning. The Kaplan-Meier method was used to describe disease-free survival, based on T cell density, and log-rank testing was performed. Cox proportional hazards models were used to examine unadjusted and adjusted associations between T cell density and disease-free survival (DFS). Two-sided P values ≤0.05 were considered statistically significant. We found that higher $CD3^+$ T cell density was associated with longer DFS with an adjusted hazard ratio (HR) of 0.69 (95% confidence interval [CI] 0.52-0.91) for patients with densities in the highest tertile as compared the lowest tertile (P=0.01). T cell subset analysis showed that stromal regulatory T helper cells (HR 0.42 comparing highest to lowest tertile, CI 0.31-0.58, P<0.001) and stromal memory cytotoxic T cells (HR 0.55 comparing highest to lowest tertile, CI 0.42-0.74, P<0.001) were most strongly associated with longer DFS. Compared to the placebo group, adjuvant celecoxib use associated with improved DFS for patients with low but not high stromal $CD3^+$ T cell density (adjusted HRs of 0.45 [CI 0.31-0.65] for lowest tertile and 1.13 [CI 0.73-1.74] for highest tertile; $P_{interaction}$=0.01). This association was also seen when the analysis was restricted to microsatellite stable tumors (HR 0.50 [CI 0.32-0.80] for lowest density tertile and 1.50 [CI 0.87-2.59] for highest density tertile; $P_{interaction}$=0.01). T cell subset analysis showed that DFS benefit for celecoxib use most strongly associated with stromal memory helper T cell density (HR 0.38 [CI 0.26-0.56] for lowest density tertile and 1.26 [CI 0.80-1.97] for highest density tertile; $P_{interaction}$<0.001). Together, these results show that while higher T cell infiltration is associated with improved DFS, lower T cell infiltration is associated with improved DFS for patients treated with celecoxib. This unexpected finding may inform immunomodulatory treatment strategies for CC.

#5859 Circulating myeloid-derived suppressor cells (MDSC) are increased and correlated with immunosuppression, systemic inflammation and malnutrition in patients with anaplastic thyroid cancer.

M. Shibata, S. Suzuki, K. Kono, S. Takenoshita;
Fukushima Medical Univ., Fukushima, Japan

Anaplastic thyroid cancer (ATC) is one of the most aggressive neoplasm in humans. Myeloid-derived suppressor cells (MDSC) contribute to the negative regulation of immune responses in cancer and are reported to be further increased in patients who immune checkpoint inhibitor is not effective. In order to investigate the pathophysiology of thyroid cancer, peripheral blood mononuclear cells (PBMC) were obtained from 49 patients with thyroid cancer, 18 with non-cancerous thyroid diseases and 22 healthy volunteers. MDSC were detected by flow cytometry (CD14-CD11b+CD33+) and Tcell-mediated immune response is measured by stimulation index (SI) of PHA-stimulated proliferation of lymphocytes. CRP (C-reactive protein) levels were used as inflammatory and serum albumin levels were used as nutritional parameters. MDSC levels were significantly higher in thyroid cancer than in healthy volunteers and non-cancerous patients, and those of ATC was highest among patients with thyroid cancer. SI were significantly lower, and CRP levels were higher in ATC than other histological types of thyroid cancer. The patients with thyroid cancer were divided into 2 groups with the average level of MDSC (1.578 %PBMC) in patients with thyroid cancer. CRP levels were significantly higher, serum albumin levels were significantly lower in high MDSC group than in low MDSC group. These data indicate that MDSCs are increased in patients with ATC. Furthermore, these patients exhibited immunosuppression, enhanced chronic inflammation and nutritional impairment.

#5860 Stimulator of interferon genes protein (STING) expression in tumor cells is linked to favorable disease parameters and a high density of tumor infiltrating inflammatory cells in urinary bladder cancer.

Henning Plage[1], Jacob Schmidt[1], Sebastian Hofbauer[1], Kira Furlano[1], Sarah Weinberger[1], Bernhard Ralla[1], Annika Fendler[1], Nadine Biernath[1], Barbara Erber[1], Florian Ro?ner[1], Simon Schallenberg[1], Sefer Elezkurtaj[1], Maximilian Lennartz[2], Elena Bady[2], Lisa Hornsteiner[2], **Claudia Hube-Magg**[2], Margit Fisch[2], Michael Rink[2], Henrik Zecha[3], Thorsten Ecke[4], Stefan Koch[4], Nico Adamini[3], Ronald Simon[2], Guido Sauter[2], Tobias Klatte[5], David Horst[1], Martina Kluth[2], Stefan Steurer[2], Sarah Minner[2], Thorsten Schlomm[1]

[1]Charite - Universitatsmedizin Berlin, Corporate Member of Freie Universitat Berlin, Humboldt-Universitat zu Berlin and Berlin Institute of Health, Berlin, Germany,[2]University Medical Center Hamburg-Eppendorf, Hamburg, Germany,[3]Albertinen Hospital, Hamburg, Germany,[4]Helios Hospital Bad Saarow, Bad Saarow, Germany,[5]Helios Hospital Bad Saarow, Bad Saarow, Germany

STING is a key element in the cGAS/STING cytosolic DNA sensing pathway which can stimulate immune response through type I interferon (IFN) production. Numerous STING agonists are currently evaluated for their suitability as anticancer drugs. Most previous studies focused on STING expression in inflammatory cells, but STING can also be expressed in cancer cells. To evaluate the potential clinical significance of STING expression in bladder cancer cells, STING was analyzed by immunohistochemistry in more than 2,500 urothelial bladder carcinomas in a tissue microarray format. STING staining in tumor cells occurred in 59.1% of urothelial carcinomas including 31.1% with weak, 11.6% with moderate, and 16.3% with strong staining. Detectable STING expression in tumor cells was associated with histo-pathological features of favorable disease course including absence of invasion ($p<0.0001$), low grade in pTa ($p<0.0001$) and in pT2-4 ($p=0.0018$) tumors, pN0 ($p=0.0036$), L0 (0.0084), and V0 (0.0006) status in pT2-4 cancers, but was not significantly associated with overall, recurrence-free and tumor specific survival. A comparison with previously collected immune cell parameters revealed significant associations between high STING expression in tumor cells and high numbers of tumor infiltrating lymphocytes including various T-cells populations (CD8: $p<0.0001$, CD4: $p=0.0462$, FOXP3: $p=0.0001$), macrophages (M1: $p=0.00003$, M2: $p<0.0001$), dendritic cells ($p<0.0001$), and PD-L1 expression on tumor cells ($p<0.0001$). It is concluded that high STING expression of tumor cells is strongly linked to a high density of tumor infiltrating inflammatory cells which is most likely causative for the significant associations with favorable disease parameters. Given the abundant presence of STING in immune cell rich urothelial cancers, STING agonists may exert significant effects in STING positive cancers.

#5861 Characterizing an immune synapse between plasmacytoid dendritic cells and cancer cells that drives cancer cytotoxicity.

Tobias Wang Bjerg[1], Ian Helstrup Nielsen[1], Sabina Sanchez Hernandez[1], Anders Laustsen[1], Isabella Barbutti[2], Shuli Svetitsky[2], James Lee[2], Rasmus Bak[1], Martin Jakobsen[1]

[1]Aarhus University, Aarhus, Denmark,[2]Francis Crick Institute, London, United Kingdom

Background: Plasmacytoid dendritic cells (pDCs), a unique type of innate immune cells, are known to play an important role in fighting viral infections, especially due to their capacity to sense DNA/RNA nucleic acids by TLR7/-9, and production of type-I interferons. The role of pDCs in driving immunological effects in cancer development and mounting antitumoral responses remains to be elucidated, though few studies describe opposing outcomes in patients based on tumor infiltrating pDCs. These observations come with little evidence explaining the immunosuppressive or tumorigenic state of pDCs.

Methods: To study fundamental anti-tumoral functions of pDCs we utilized a robust and validated *in vitro* stem-cell derived human pDC model. Importantly, this model permits genetic manipulation prior to pDC differentiation and enables large-scale co-culture studies with various cancer types. Using this model, we recently employed a CRISPR library screening approach to identify factors that aid the cytotoxic mechanisms of pDCs following co-culture with tumor cell lines.

Results: Initially, using co-culture assays consisting of hematological, lung, or breast cancer cell lines in combination with *in vitro* generated pDCs, we demonstrated a time-dependent cytotoxic effect using Incucyte live imaging. Importantly, cytotoxic effects were elevated when pDCs had been stimulated with TLR9 agonists. We demonstrated that a direct cell-to-cell interaction was essential for pDC cytotoxicity, and that promoting stronger cell-to-cell interaction, by genetically modifying pDCs to express scFv targeting a tumor antigen, increased the killing effects. CRISPR knock-out of Granzyme B in pDCs did not remove the cytotoxic capabilities of pDCs. We are currently conducting a genetic screen to identify universal factors vital for pDC interactions and cytotoxicity effects in cancer settings. This preliminary data will be presented at the AACR meeting.

Conclusion: Using *in vitro*-generated pDCs we explore an important anti-tumoral function of pDCs. If successful, our work can open for potential new therapeutics and prognostic possibilities in cancer treatment, harnessing the cytotoxic effects of pDCs.

#5862 Combining circular RNA and a silicon membrane-based cell engineering approach to create CAR-Ts with membrane bound IL-2 and IL-12.

Zhihui Song, Andrew Larocque, Sophia Hirsch, Eleni Rogers, Darby Kreienberg, Jacquelyn Hanson, Alec Barclay, Armon Sharei

Portal Biotechnologies, Inc., Watertown, MA

Engineered immune cell therapies have great potential to revolutionize the treatment of many pathologies such as cancer, autoimmunity and infectious diseases. However, limitations remain in some areas including long manufacturing times, inability to engineer critical cell functions, potential for cell exhaustion, and limited access to the disease site as in the case for solid tumors. Portal has developed a novel implementation of mechanoporation technology using a silicon membrane with pores which enables deformation of the cell and poration of the cell membrane, allowing for diffusion of any cargo, charged or uncharged, into the cell. Mechanoporation is a gentle intracellular delivery technique that preserves cell viability and function (DiTommaso, et al., PNAS 2018). It can shorten manufacturing time and enable the introduction of key therapy-enhancing factors via transient modifications shortly before administration. Portal has established methodology for cytoplasmic introduction of many diverse cargo including mRNA, circular RNA, siRNA, proteins, peptides, and CRISPR RNPs to primary immune cells, including both unstimulated and activated T cells, B cells, NK cells, monocytes and iPSCs. In this work, we demonstrated simultaneous expression of a functional CD19 CAR, membrane bound IL-2 and membrane bound IL-12 through delivery of three circRNAs into activated T cells. They are highly expressed for multiple days while maintaining high viability and ensuring robust in vitro killing. The expression of mbIL2 and mbIL12 not only promotes T cell proliferation but also upregulates CD62L and enhances the cytotoxic function of CAR T cells. At clinical scale, we have demonstrated delivery of RNA to over 1 billion T cells while achieving over 90% expression. Portal's technology and approach has the potential to further advance immune cell therapy developments, particularly in multiplexed engineering approaches involving simultaneous delivery of multiple cargos. Coupled with Portal's compatibility with diverse immune cell types and next generation cargos. We believe this simple approach to intracellular delivery can dramatically reduce manufacturing time and cost, easily integrate into existing clinical equipment and enable unique enhancements that underpin a new generation of therapeutics.

#5863 Mapping tumor metastasis and EMT-associated immune changes in HNSCC through spatial transcriptomic analysis.

Chih-Hung Chung, Muh-Hwa Yang

National Yang Ming Chiao Tung University, Hsinchu, Taiwan

Head and neck squamous cell carcinoma (HNSCC) is a globally prevalent malignancy. Standard treatments typically include surgical resection, radiation therapy, and chemotherapy. However, for patients with recurrent or distantly metastatic tumors where surgery or radiation is no longer feasible, the primary treatment strategy shifts to combining targeted therapies with chemotherapy or immune checkpoint blockade (ICI) therapy. Despite these approaches, the overall prognosis remains poor. Therefore, identifying key changes in immune cell composition within the tumor microenvironment of malignant or metastatic tumors is crucial for improving the management and outcomes in HNSCC therapy.

The aim of this study is to analyze changes in the immune microenvironment composition across different HNSCC specimens. We utilized tools including the GeoMx Digital Spatial Profiler (DSP) for imaging-based digitized gene expression analysis. We identified region of interesting (ROI) from distinct tumor regions, such as the tumor invasive front, tumor core, and paired lymph node metastases. Through the Cancer Transcriptome Atlas (CTA) analysis, we identified two distinct epithelial-mesenchymal transition (EMT) gene signatures, which were highly expressed in particularly aggressive tumor tissues. Additionally, within the myeloid activation signature, we observed that immune cell composition in primary tumor site was predominantly neutrophil-activated, whereas in lymph node metastases, macrophages were the dominant immune cells. To further validate these observations, we applied other spatial transcriptomics tools, including 10x Visium and 10x Xenium, to analyze specimens from six HNSCC patients. Transcriptomic trajectory analysis and ligand-receptor pair analysis revealed significant changes in the immune microenvironment of malignant HNSCC tissues.

These results provide crucial insights into head and neck cancer treatment strategies tailored to different tumor microenvironment (TME) characteristics.

#5864 Preclinical investigation of anti-tumor efficacy of allogeneic natural killer cells combined with cetuximab for head and neck squamous cell carcinoma.

Chaeyeon Kim[1], Mina Han[1], Gamin Kim[2], Wonrak Son[1], Jeongah Kim[1], Minchan Gil[3], Yong-Hee Rhee[3], Nam Suk Sim[4], Chang Gon Kim[5], Hye Ryun Kim[5]

[1]Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea, Republic of,[2]Department of Oncology, Yonsei University College of Medicine, Seoul, Korea, Republic of,[3]NKMAX Co., Ltd., Seoul, Korea, Republic of,[4]Department of Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea, Republic of,[5]Division of Medical Oncology, Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea, Republic of

Background: Head and neck squamous cell carcinoma (HNSCC) is a challenging malignancy with limited response rates to conventional therapies, including immune checkpoint inhibitors and cetuximab monotherapy. Despite recent advancements in immunotherapy, a significant unmet need remains owing to suboptimal efficacy. This study investigated the potential of a novel combination therapy using allogeneic natural killer (NK) cells and cetuximab, an anti-epidermal growth factor receptor monoclonal antibody, to enhance anti-tumor efficacy in HNSCC.
Method: Allogeneic NK cells from healthy donors were expanded and tested *in vitro,* and NOG (NOD/Shi-scid/IL-2Rγ null) xenograft mouse models were used to evaluate their efficacy and functionality. *In vitro* assays were employed to evaluate cytotoxicity against HNSCC cells (FaDu) in combination with cetuximab, focusing on the effects of antibody-dependent cellular cytotoxicity (ADCC). *In vivo* experiments assessed the efficacy of high-dose NK cell infusion with cetuximab in reducing tumor growth and improving survival in xenograft mouse models. NK cell infiltration and cytotoxicity were analyzed using flow cytometry and immunohistochemistry.
Results: An increased dose of NK cells was selected for subsequent studies owing to its superior ability to improve survival outcomes and inhibit tumor cell proliferation despite no marked differences growth between the NK-low and NK-high groups. During the selection process for allogeneic NK cells and their partner drugs, interleukin (IL)-2 was selected and tested in the xenograft mouse model. However, the lack of an improved anti-tumor response through the IL-2 combination in vivo prompted us to investigate other combination treatment partners. While IL-2 showed limitations in enhancing NK cell activity in vivo, Combination therapy encompassing allogeneic NK cells and cetuximab demonstrated significantly enhanced cytotoxicity against FaDu cells in vitro, which was attributed to ADCC. In vivo, the combination of allogeneic NK cells and cetuximab effectively managed HNSCC in NOG xenograft mouse model, showing significant reductions in tumor growth and improved survival rates. Additionally, Flow cytometry and immunohistochemistry revealed increased infiltration of NK cells within the tumors, confirming enhanced ADCC-mediated anti-tumor activity. These results highlight the potential of this combination therapy to enhance anti-tumor efficacy.
Conclusion: These findings underscore the potential of combining allogeneic NK cells with cetuximab to improve the therapeutic outcomes in HNSCC. The observed synergy between NK cells and cetuximab highlights a promising therapeutic approach for enhancing NK cell-mediated cytotoxicity through ADCC and, lays a foundation for future clinical trials aimed at addressing unmet needs in HNSCC treatment.

#5865 Novel allosteric STING agonists in combination with DNA methyltransferase inhibitors induce an interferon-driven apoptotic response in *TP53*-mutated acute myeloid leukemia.

Kaushlendra Tripathi[1], Lora Stojanovic[1], Monali BanerJee[2], Sandip Middya[2], Ritesh Srivastava[2], Arjun surya[2], Stephen B. Baylin[3], Michael Topper[3], Maria R. Baer[1], Fervez Rassool[1]

[1]University of Maryland, Baltimore, Baltimore, MD,[2]Curadev Pharma, Noida, India,[3]Johns Hopkins School of Medicine, Baltimore, MD

TP53-mutated acute myeloid leukemia (AML) has dismal outcomes; new therapies are needed, and immunotherapeutic approaches are being explored. STimulator of INterferon Genes (STING) is a key innate immune driver that activates interferon (IFN) and nuclear factor Kappa B (NFκB)/tumor necrosis factor-alpha (TNFα) signaling. We previously reported that phospho (activated)-STING expression is higher in *TP53*-mutated AML and that DNA methyltransferase inhibitors (DNMTis) demethylate epigenetically silenced endogenous retroviruses (ERVs), increasing cytosolic dsRNA and IFN signaling in a viral mimicry mechanism. Moreover DNMTis upregulate STING transcription. Thus STING agonists in combination with DNMTis could be an attractive therapy in *TP53*-mutated AML.

The next-generation allosteric STING agonists CPD 1 and 2 potently bind and activate all human STING variants. While CPD 1 has been extensively used for *in vitro* cellular studies, the more advanced analogue CPD 2 (CRD3874-SI) is currently in Phase I clinical trials in patients with solid cancers (MSKCC) and AML (UMGCCC). We perfomed MTS assays in *TP53*-mutated and -WT AML cell lines and primary cells (N=4) treated with CPD 1 and DNMTi decitabine (DAC) combination *in vitro.* Decreased viability was particularly noted in *TP53*-mutated cells, and the drug combination was synergistic, with combination index <1. To test the transcriptome-wide effects of CPD 1 and DAC, we performed ribosomal depleted RNA-seq on WT and *TP53* CRISPR KO MOLM-14 AML cells after treatment for 3 days. Differentially expressed genes (DEGs) were markedly increased in *TP53* CRISPR KO MOLM-14 treated with CPD 1 and CPD 1 + DAC. Moreover, increased repetitive element (RE) expression was largely restricted to *TP53*-KO cells treated with CPD 1 and CPD 1 + DAC. Pathway analyses for both CPD 1- and CPD 1 + DAC-treated *TP53*-KO cells showed conserved positive enrichment of gene sets associated with Type I/II IFN, TNFα, and apoptosis. Drug combination-specific augmented genes resided in critical interferon gene categories such as Th1 type chemokines (*CXCL10, CXCL11*), RNA detection (*DHX58, EIFAK2, IFIH1, OASL, PARP9, RIGI*), and IFN signaling-induced cell death (*IFI27, IFI44L, IFIT2, MX1, OAS1, OAS2, OAS3, and TNFSF10)*, thus potentially combining substrate, sensors, and effectors in a suicidal interferon-driven apoptosis response in TP53 KO cells. qPCR and apoptosis detection by Annexin V staining validated these findings in *TP53*-mutated vs -WT AML cell lines and patient samples. Treatment with the IFN inhibitor ruxolitinib or the pan-caspase inhibitor ZVAD rescued IFN-driven apoptosis. Finally CPD 1 in combination with DAC significantly reduced leukemia burden in humanized AML mouse models. These results support development of a clinical trial combining CRD3874-SI with DAC for patients with *TP53*-mutated AML.

#5866 Role of inflammatory cytokines and matrix metalloproteinases in colorectal cancer in a Hispanic population.

Elba V. Caraballo, Sheila Natalie Lopez Acevedo, **Camille Zenon-Melendez**, Josue Perez Santiago, Marcia R. Cruz-Correa

University of Puerto Rico Comprehensive Cancer Center, San Juan, Puerto Rico

Background: Colorectal cancer (CRC) is a leading cause of cancer-related deaths in Puerto Rico and the United States. Puerto Rican Hispanics (PRH) experience disproportionately higher CRC incidence and mortality rates compared to the general Hispanic population in the U.S. Biological processes, such as cytokine dysregulation and extracellular matrix remodeling, may contribute to these disparities. However, the molecular mechanisms driving CRC disease progression in PRH remain poorly understood. This study investigates the roles of inflammatory cytokines and matrix metalloproteinases (MMPs) in CRC disease progression.
Methods: We conducted a case-case study using tumor (n=15) and adjacent mucosa (n=15) tissue samples collected from early (n=7) and advanced-stage (n=8) CRC archived cases recruited through the Puerto Rico Familial Colorectal Cancer Registry (PURIFICAR). RNA-seq was performed and analyzed for differential gene expression and fold changes (FC) for key immune-related pathways, including MMPs, interleukin IL-17(IL17), associated cytokines, and chemokines. STRING database was used to identify protein-protein interaction networks and functional associations (≥5 gene connections).
Results: Transcriptomic analysis revealed stage-specific expression patterns. IL-13 was differentially expressed in early-stage tumors, while advanced-stage tumors were characterized by CXCL10 expression Both IL-17A and IL-8 were upregulated in early and advanced stages. Elevated expression levels of IL-17A (log2 FC: 2.72 vs 3.11) and IL-8 (log2 FC: 5.73 vs 3.44) were observed in tumor tissues compared to adjacent mucosa (adj. $p<0.05$). Advanced-stage tumors also showed increased expression of MMP13 and MMP3, indicating extracellular matrix remodeling and invasion. IL-17A-related pathways were consistently upregulated, suggesting their involvement in chronic inflammation, tumor growth, and immune escape.
Conclusions: Our results show that differential expression of IL-17A and IL-8 at early-stage CRC may contribute to immune dysregulation and a tumor-promoting microenvironment. In advanced stages, elevated MMP expression could promote extracellular matrix remodeling, metastasis, and immune evasion. These findings suggest that IL-17A, IL-8, and MMPs are potential therapeutic targets for addressing CRC disease progression and reducing health disparities among PRH.

#5867 Uncovering T-cell receptor clones and immunogenic targets in HER2DX-defined HER2-positive breast cancer.

Victor Albarran-Fernandez[1], Carlota Rubio-Perez[2], Patricia Galvan[3], Oleguer Castillo[3], Paula Blasco[3], Esther Sanfeliu[4], Anabel Martinez-Romero[3], Mercedes Marin[5], Patricia Villagrasa[5], Francisco Pardo[5], Laia Pare[5], Isabel Garcia-Fructuoso[1], Raquel Gomez[1], Elia Segui[1], Barbara Adamo[1], Benjamin Walbaum[1], Olga Martinez-Saez[1], Tomas Pascual[1], Maria Vidal[1], Montserrat Munoz[1], Sonia Guedan[6], Fara Braso[3], Laura Angelats[1], Aleix Prat[7]

[1]Medical Oncology Department, Translational Genomics and Targeted Therapeutics in Solid Tumors, Hospital Clinic, Barcelona, Spain,[2]Medical Oncology Department, Hospital Clinic, Barcelona, Spain,[3]Translational Genomics and Targeted Therapeutics in Solid Tumors (IDIBAPS), Hospital Clinic, Barcelona, Spain,[4]Anatomic Pathology, Translational Genomics and Targeted Therapeutics in Solid Tumors, Hospital Clinic, Barcelona, Spain,[5]Reveal Genomics, Barcelona, Spain,[6]Cellular Immunotherapies for Cancer, August Pi I Sunyer Biomedical Research Institute (IDIBAPS), Hospital Clinic, Barcelona, Spain,[7]Medical Oncology, Translational Genomics and Targeted Therapeutics in Solid Tumors, Reveal Genomics, Hospital Clinic, Barcelona, Spain

Purpose: To explore the role of the immune system in early-stage HER2+ breast cancer (HER2+ BC), focusing on how T-cell receptor (TCR) dynamics relate to the prognostic 14 B-cell gene/IgG immune signature (IGG) included in the clinically available HER2DX genomic test. This study aims to clarify how TCR diversity and targeting of tumor-associated antigens (TAA) contribute to patient outcomes and could inform potential therapeutic strategies.
Experimental design: TCR/BCR clones were identified by PCR amplification and deep sequencing (ImmunoSEQ) in 41 early-stage HER2+ BC samples. CDR3 sequences were cross-referenced with the VDJ database, excluding inconclusive matches (VDJdb score 0-1). Protein expression was analyzed by digital spatial profiling (GeoMx) in 23 samples. In 6 samples, TCR identification was performed through single-cell RNA sequencing (scRNAseq; Chromium). IGG expression in each sample was evaluated using the HER2DX assay and correlated with bulk RNA data from TCGA and MTBC datasets. Spearman's correlation and Wilcoxon tests were used for statistical analysis (R software).
Results: Of the 12,575 TCR clones with predicted targets, 759 (5.9%) showed reliable matches (score 2-3). 53 of them (7%) recognized TAA, primarily MART1 (18.9%), followed by gp100, ABCD3, MAGEA6, KRAS, NY-ESO1, p53, TERT, and others. Most non-human epitopes belonged to common viruses such as Influenza A (31.2%), EBV (30.7%), and CMV (20%). IGG expression was correlated with the number of TCR templates (Cor: 0.47, $p<0.01$), TCR entropy (Cor: 0.60, $p<0.001$), and shared TCR clonotypes between samples (Cor: 0.48, $p<0.01$). IGG was higher in samples with TCR clones against TAA (p50 75.6 vs. 61.1, $p=0.044$). A positive correlation was observed between the number of clones targeting human and viral epitopes (Cor: 0.44, $p=0.013$). The scRNAseq data confirmed that IGG-high samples exhibit greater TCR polyclonality and a higher fraction of cytotoxic CD8+ T cells ($p<0.05$), with upregulated perforin and granzyme A/B expression. IGG correlated with CD27 (Cor: 0.47, $p=0.025$) and CD3 (Cor: 0.52, $p=0.011$) protein levels, as well as the IFN-γ signature in TCGA (Cor: 0.56, $p<0.01$) and MTBC (Cor: 0.71, $p<0.01$) data. Fibronectin correlated with PD1 (Cor: 0.61, $p<0.01$) and CTLA4 (Cor: 0.81, $p<0.001$) levels, and inversely with IGG (Cor: -0.50, $p=0.017$), indicating that IGG-low tumors might have a denser stroma and a more exhausted, less active immune infiltrate.
Conclusions: Early-stage HER2+ BC with high IGG expression is characterized by a robust, polyclonal immune response, with TCR clones targeting both tumor and viral antigens. These findings suggest enhanced immune fitness and may explain IGG favorable prognostic value. The discovery of shared TCR clones across tumors may help identify immunogenic targets, providing new opportunities for developing immune-based treatments or engineered T-cell therapies.

#5868 Differential tumor immunity in limited-stage vs extensive-stage small cell lung cancer.

Jiyeon Kim[1], Jun-Gi Jeong[2], Sehhoon Park[3], Hyun Ae Jung[3], Jong-Mu Sun[3], Jin Seok Ahn[3], Myung-Ju Ahn[3], Se-Hoon Lee[3]

[1]Department of Health Sciences and Technology, SAIHST, Sungkyunkwan University, Samsung Medical Center, Seoul, Korea, Republic of,[2]Samsung Medical Center, Seoul, Korea, Republic of,[3]Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of

Introduction: Small cell lung cancer (SCLC) is an aggressive malignancy characterized by rapid progression and early metastasis. Immunotherapy has become the standard treatment for extensive-stage SCLC (ES-SCLC), but its role in limited-stage SCLC (LS-SCLC) remains under investigation in clinical trials. This study aims to explore the differences in tumor immune microenvironment (TIME) of LS-SCLC and ES-SCLC and to suggest how these differences may influence therapeutic strategies and patient outcomes.
Methods: Samples from 365 patients were collected with histologically confirmed SCLC who had available tissue samples. Whole transcriptome sequencing (WTS) was performed on all samples. The patients were categorized based on clinical staging at the time of biopsy. Using WTS data, immune cell composition was analyzed using CIBERSORTx deconvolution algorithm, and the signature was calculated as the mean of the Transcripts Per Million (TPM) values using curated genes. edgeR was used for differential gene expression and performed GSEA using KEGG pathway database. SCLC subtypes were assigned based on lineage-defining transcription factor (TF) markers (as described by S Park, et al., eBioMedicine, 2024). Statistical significance was evaluated when the P value was below 0.05.
Results: Of the 365 patients, 100 were classified as LS-SCLC, and 265 as ES-SCLC. The median age was 65.3 years for LS-SCLC and 67.8 years for ES-SCLC. The LS-SCLC included 6% never smokers, while the ES-SCLC cohort comprised 8.3% never smokers. Biopsy site distribution was as follows: in LS-SCLC, 28% of tissues were obtained from the lung, bronchus, or trachea, 72% from lymph nodes. Similarly in ES-SCLC, 23% of tissues were from the lung, 69% from lymph nodes, and 9% from other distant organs. Immune cell profiling identified stage-specific differences in the immune landscape. LS-SCLC exhibited significantly higher levels of B cells and activated dendritic cells (DCs), whereas ES-SCLC showed increased proportions of resting DCs and neutrophils. The angiogenesis signature was significantly elevated in ES-SCLC, reflecting distinct immune-modulating effects in TIME. Differential gene expression revealed enrichment of KEGG gene sets related to the peroxisome proliferator-activated receptor (PPAR) signaling and ERBB signaling in ES-SCLC. Subtype distribution based on WTS data showed a significant difference between stages, with the ASCL1 subtype (SCLC-A) more frequent in LS-SCLC and the POU2F3 subtype (SCLC-P) more common in ES-SCLC.
Conclusions: Our findings highlight significant differences in the TIME and molecular subtypes between LS-SCLC and ES-SCLC. These differences may inform the development of tailored therapeutic strategies, ultimately improving patient outcomes by addressing the unique immune and molecular characteristics of LS-SCLC and ES-SCLC.

#5869 Exploring chronic stress-induced hippocampal changes in murine ovarian cancer models and their relevance to depressive-like behaviors.

Luinet Lee Melendez-Rodriguez[1], Luis M. Rivera-Perez[1], Yadiel A. Rivera-Lopez[1], Melanie Ortiz-Leon[1], Jaydiel Casiano-Martinez[1], Alanis P. Torres-Rosado[1], Zuliann S. Galarza-Ruiz[2], Nemesis J. Torres-Rivera[3], Orlando I. Torres-Rodriguez[1], Guillermo N. Armaiz-Pena[1]

[1]Ponce Health Sciences University, Ponce, Puerto Rico,[2]Interamerican University of Puerto Rico, Ponce, Puerto Rico,[3]Pontifical Catholic University of Puerto Rico, Ponce, Puerto Rico

Patients with ovarian cancer (OC) often experience depression, which can negatively impact their quality of life and overall prognosis. Our research group has demonstrated that chronic stress (CS) induces systemic inflammation, contributing to disease progression. Other studies have also highlighted its role in the development of depressive symptoms. Microglia are key regulators of brain inflammation and serve as a primary source of brain-derived neurotrophic factor (BDNF), which is crucial for maintaining synaptic plasticity, neuronal survival, and reducing symptoms of depression. However, the influence of CS and OC on the development of depressive symptoms in OC patients remains poorly understood. This study aims to investigate whether CS and OC can induce changes in the hippocampus (HPC) that may lead to depressive-like behavior in mice with OC. We hypothesize that CS will increase microglial infiltration and inflammatory markers, while reducing BDNF levels and neuronal activity in the HPC of mice with OC. To test our hypothesis, we inoculated 8- to 12-week-old C57BL/6 mice with ID8^{luc} or IG10^{luc} OC cells and subjected them to daily restraint stress for four weeks. Upon sacrifice, we collected and processed their brains for immunofluorescence analysis of BDNF, IBA1, and c-Fos. Our data suggest that CS accelerated tumor growth and led to increased microglial infiltration, decreased BDNF levels, and reduced neuronal activity in the HPC. These findings align with the BDNF hypothesis, which posits that a loss of BDNF in the HPC is directly involved in the development of depression. This study lays the groundwork for further research on how OC and CS can modulate brain activity and behavior.

#5870 Blocking cadherin-11 activates pyroptosis-mediated antitumor immunity via the PD-L1/GSDMD/CCL3 axis in bladder cancer.

Thomas I-Sheng Hwang[1], **An-Chen Chang**[2]

[1]Department of Surgery, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan,[2]School of Oral Hygiene, Taipei Medical University, Taipei, Taiwan

Introduction: Pyroptosis, a form of programmed cell death driven by proinflammatory signals, plays a dual role in inflammation and cancer progression. Cadherin-11 (CDH-11), a type II cadherin, is known to mediate cell-cell and cell-extracellular matrix adhesion. Emerging evidence suggests that CDH-11 contributes to tumor progression and metastasis, making it a potential therapeutic target. In this study, we investigate whether targeting CDH-11 can suppress bladder cancer (BC) cell invasiveness and activate pyroptosis-mediated antitumor immunity.
Materials & Methods: Transwell assays were conducted to evaluate cell migration and invasion abilities. Pyroptosis was measured using lactate dehydrogenase (LDH) assays, while calcein AM staining-based cytotoxicity assays were employed to measure natural killer (NK) cell-mediated cytotoxicity. Inflammatory cytokine levels in BC tissues were determined using a human antibody array. To investigate the role of CDH-11 in tumor progression, a subcutaneous tumor model and a popliteal lymph node model were established to evaluate tumor growth and lymphatic metastasis *in vivo*. Immunohistochemistry staining was utilized to assess CDH-11 protein expression in human BC tissues.
Results: Elevated CDH-11 expression was observed in BC patients compared to healthy individuals, with a positive correlation to advanced clinical stages, lymphatic metastasis, and poor overall survival. In vitro analyses demonstrated that blocking CDH-11 significantly reduced BC cell invasiveness. Notably, CDH-11 blockade induced bubble-like protrusions on the cell surface and increased LDH release, indicative of enhanced pyroptosis. Mechanistically, CDH-11 inhibition activated PD-L1-mediated GSDMD expression, with the GSDMD N-terminal domain forming membrane pores to initiate pyroptosis. Further analysis revealed that CDH-11-induced pyroptosis elevated the release of the proinflammatory cytokine CCL3 within the tumor microenvironment, thereby enhancing natural killer (NK) cell-mediated cytotoxicity against BC cells. In vivo, CDH-11 blockade effectively suppressed tumor growth, lymphatic metastasis, and lung metastasis, highlighting its therapeutic potential.
Conclusions: These findings reveal a novel role of CDH-11, demonstrating that its inhibition suppresses cell invasion and enhances pyroptosis-mediated antitumor immunity through CCL3 in BC. Targeting CDH-11 represents a promising therapeutic strategy for the management of BC.

#5871 Comparative analysis of single-cell immune cell transcriptional profiles in humans, mice, and dogs: insights from normal and tumor states.

Aaron L. Sarver[1], Caitlyn M. Callaghan[2], Marissa Macchietto[2], Lauren Burt[2], Meagan Wojtysiak[2], Courtney H. Labe[2], Taylor DePauw[2], Ashley Schulte[2], Kelly M. Makielski[2], Julia E. Medland[2], Jaime F. Modiano[2]

[1]Institute for Health Informatics Masonic Cancer Center, University of Minnesota, Minneapolis, MN,[2]University of Minnesota, Minneapolis, MN

The immune system plays a critical role in defending against pathogens and surveilling tumors. Understanding immune cell behavior in both health and disease is essential for advancing therapeutic strategies, particularly in oncology. However, there remains a gap in knowledge regarding how immune cell profiles differ across species and how these differences might impact cancer immunology. In this study, we conduct a comparative analysis of single-cell transcriptional profiles of immune cells from three mammalian species—humans, mice, and dogs—under both normal and tumor-bearing conditions. Using high-throughput single-cell RNA sequencing (scRNA-seq), we aim to identify both conserved and species-specific transcriptional signatures that define immune cell function in health and cancer, with the ultimate goal of enhancing translational research and therapeutic development. To generate a comprehensive dataset, we analyzed immune cell samples from both normal and tumor tissues across humans, mice, and dogs. These species were selected for their distinct immunological and clinical features: humans as the primary model for clinical oncology, mice as the standard laboratory model for cancer research, and dogs as a naturally occurring cancer model with genetic and environmental similarities to humans. Immune cells from canine samples were isolated and analyzed using scRNA-seq. Publicly available human, mouse and canine datasets were also incorporated into the analysis. Analyses of these datasets enabled high-resolution characterization of the immune landscape in each species. We developed a pipeline to systematically identify both commonalities and species-specific differences in immune cell transcriptional profiles. In tumor free samples, immune cell profiles were broadly similar across species, with T cells, B cells, macrophages, and dendritic cells as the dominant populations. However, we also observed notable species-specific variations in the transcriptional features of immune cells in normal and tumor tissue. This study provides a comprehensive cross-species analysis of immune cell transcriptional profiles in both normal and tumor states. Our findings highlight the importance of considering species-specific differences when translating preclinical results to human cancer research, emphasizing the value of utilizing multiple animal models, including canines, to enhance our understanding of cancer immunology. These insights are critical for the design of immunotherapies and the optimization of preclinical cancer models.

#5872 The gut microbiota from vasoactive intestinal peptide knock-out mice confers resistance to the growth of leukemia in wild-yype mice.

Kayla Robinson, Shayna Jankowski, Jian Ming Li

Emory Winship Cancer Institute, Atlanta, GA

Mice Resistance to Leukemia via Gut Microbiota Transfer Vasoactive Intestinal Peptide (VIP) regulates adaptive immunity through receptors expressed in T lymphocytes, VPAC1andVPAC2. VIP over production in leukemia suppresses immune function, facilitating cancer progression. VIP-knockout (VIP-KO) mice show a survival advantage against leukemia, likely due to gut microbiota-mediated immune response regulation. Cohousing enables gut microbiota transfer from VIP-KO to wild-type (WT) mice via coprophagy, potentially enhancing WT mice's resistance to leukemia. This study investigates whether cohousing WT and VIP-KO mice alters WT gut microbiota and confers resistance to leukemia challenge. Eighty mice, 42VIP-KO 38WT, were cohoused across 10cages for six weeks. Blood and stool samples were collected pre- and post-cohousing for immune and microbiome analyses. Mice were injected with syngeneic CD1498leukemiacells post-cohousing and monitored for survival. Gender differences in survival outcomes were also assessed. WT mice cohoused with VIP-KO mice demonstrated significant colonization by VIP-KO-derived gut microbiota, including Ruminococcus gnavus and Lactobacillus murinus, characterized by lower gut pH. Cohousing improved WT survival rates after leukemia challenge($p<0.009$), producing long-term survivors. *VIP-KO mice showed the highest presence of R. gnavus after cohousing ($p=0.017$)*Cohousing-induced microbiota transfer enhances leukemia resistance in WT mice through gut microbiota-mediated immune modulation. Survival improvements are independent of gender, underscoring the robustness of this therapeutic strategy. These findings highlight microbiota-targeted therapies, such as fecal microbiota transplantation, as promising approaches for leukemia treatment. Future studies will evaluate combined microbiota transfer and VIP antagonism therapies i.e. ANT308tooptimizeoutcomes.

Wild Type cohousing had a significance of $p<0.001$. Knockout cohousing had a significance of $p<0.009$. Wild type mice had the highest median survival time at 27days.

CLINICAL RESEARCH: Liquid Biopsy: Circulating Nucleic Acids 3
Poster Session

#5876 ctDNA as predictor for systemic therapy response in patients with colorectal cancer: A systematic review and meta-analysis.

Anja Holz, Bidisha Paul, Antonia Zapf, Klaus Pantel, **Simon A. Joosse**

University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Background: The response to systemic therapy against metastatic colorectal cancer (mCRC) is currently assessed by radiologic imaging. However, an increasing number of studies have shown that circulating tumor DNA (ctDNA) as liquid biopsy can be used as an alternative method to assess therapy efficacy. We conducted a systematic review with subsequent meta-analysis of primary studies to assess the predictive value of sequential liquid biopsies in patients with colorectal cancer treated with systemic therapy.
Methods: Randomized, non-randomized, as well as prospective observational studies, reporting on the change in ctDNA concentration in total cell-free DNA over the course of systemic therapy of patients treated for metastatic colorectal cancer to predict treatment response according to RECIST criteria were included.
Results: Forty-one studies involving 4546 evaluable patients with metastatic colorectal cancer were included in the meta-analysis. ctDNA increase during systemic therapy as compared to baseline was significantly associated with progression-free survival (HR: 2.47, 95%CI: 1.97-3.10) and overall survival (HR: 2.18, 95%CI: 1.66-2.86), which were reported in 31 and 22 studies, respectively.
Conclusion: Longitudinal plasma-based ctDNA liquid biopsy has prognostic value and may help to identify those patients that benefit of continuing therapy or therapy switch. Although further standardization and randomized controlled clinical trials are required, based on the available data, we recommend longitudinal ctDNA measurements to be included in clinical practice and inclusion in the RECIST criteria.
Protocol registration: The protocol for this review was registered on PROSPERO (CRD42023420012).

#5877 Feasibility of ctDNA detection with a low-volume blood collection device.

Brad Evans[1], Jordan Feeney[2], Derrick Renner[2], Alexey Aleshin[2], Matthew Rabinowitz[2], Steven Townson[1], **Himanshu Sethi**[2]

[1]Merck & Co., Inc., Rahway, NJ,[2]Natera, Austin, TX

Patient-centric technology for personalized healthcare can transform medical research and disease management by addressing logistical hurdles, and enabling flexible, ongoing patient monitoring with reduced blood volumes. The Tasso+ low blood volume collection device allows a shift from traditional in-clinic blood collection to patient-centric, home, blood collection for volumes up to 1 mL of capillary blood. To demonstrate the utility of low blood volume collection and subsequent molecular residual disease surveillance, we evaluated the capability of the Signatera™ RUO tissue-informed assay (Natera, Inc.) to detect single nucleotide variants (SNVs) using whole blood samples collected by a microsampling device that can collect up to 500 µL, the Tasso+, as compared to traditional venipuncture. We first tested the technical feasibility of the Signatera assay to detect SNVs using a commercial circulating tumor DNA (ctDNA) reference standard (Seraseq ctDNA mutation mix v2, 0.625-5ng) spiked into low blood volumes that are representative of those obtainable from routine Tasso+ collection. Next, we tested the clinical utility of low blood volume collection using commercially obtained traditional venipuncture and Tasso+ samples from 7 cancer patients (pt) with advanced relapsed/refractory solid tumors (bladder n=1, colorectal cancer n=3, lung n=1, melanoma n=2). Venipuncture plasma samples (4 mL, 1mL, 2✕ 500µL) and 1 in the Tasso+ device (250-500µL) were extracted per patient and assessed for ctDNA detection using the patient-informed 16-plex Signatera mPCR-NGS-based RUO ctDNA assay. The feasibility study demonstrated the ability to detect ctDNA at the single variant molecule level down to the lowest quantity of reference standard in the 250µL plasma sample. In the clinical setting, ctDNA was consistently detected among 6 of 7 pts (86%) at the 4 mL plasma volume and in 5 of 7 pts (71.4%) at all other volumes, including the 250µL volume of plasma from Tasso+ samples. The levels of ctDNA detected were generally consistent for most patients across all sample volumes, including that from the Tasso+ device. These results demonstrate that ctDNA can reliably be detected down to the single variant molecule level using the Signatera RUO assay on small plasma volumes collected with the novel Tasso+ microsampling technology. Additional evaluation using larger patient numbers would further validate this technique.

#5878 Landscape of tumor heterogeneity index and tumor mutational burden in non-small cell lung cancer.

Jongwoo Kim[1], Jong Yeob Kim[1], Leeseul Kim[2], Junho Song[1], **Liam Il-Young Chung**[1], Young Kwang Chae[1]

[1]Northwestern University Feinberg School of Medicine, Chicago, IL,[2]University of Chicago, Chicago, IL

Background/Aims
Tumor heterogeneity and tumor mutational burden (TMB) are novel biomarkers associated with the response to immunotherapy in non-small cell lung cancer (NSCLC), but studies report conflicting results. We aim to describe the associations between these biomarkers.
Methods
From records of 77 NSCLC patients, tissue next-generation sequencing (NGS) data and circulating tumor DNA (ctDNA) NGS data, along with corresponding tissue TMB (tTMB) and blood TMB (bTMB) values, were obtained. PyClone was used to estimate tumor heterogeneity from tissue sequencing data. Tumors with ≥3 clusters were defined as having high tumor heterogeneity, and those with <3 clusters as having low heterogeneity. Mutant-allele tumor heterogeneity (MATH), another estimate of tumor heterogeneity, was calculated from tissue and blood NGS data.
Results
Out of the 77 NSCLC cases, 21 cases had high tumor heterogeneity, and 56 cases had low tumor heterogeneity according to PyClone. High tumor heterogeneity was statistically significantly associated with higher tTMB (p-value = 0.02) but was not significantly associated with bTMB. Tumor heterogeneity was not significantly associated with age, sex, smoking status, tumor histology, or metastasis. Tumor heterogeneity was not associated with either tissue MATH (p-value = 0.86) or blood MATH (p-value = 0.30), and there was no significant association between tissue MATH and blood MATH (p-value = 0.96). There was a statistically significant positive correlation between tTMB and bTMB ($r = 0.75$, $p < 0.001$). Neither tissue MATH nor blood MATH showed significant associations with tTMB or bTMB.
Conclusions
High tumor heterogeneity as defined by PyClone was associated with high tTMB but not bTMB. tTMB and bTMB were strongly positively correlated. There were no evident correlations between the estimates of tumor heterogeneity.

#5879 An optimized sample-to-result workflow for detection of tumor mutations and immune sequencing in liquid biopsies.

Anagha Kadam[1], Margaret R. Heider[1], Chen Song[1], Jian Sun[1], Ariel Erijman[1], Nicholas M. George[2]

[1]New England Biolabs, Ipswich, MA,[2]Streck LLC, La Vista, NE

Non-invasive liquid biopsies are helping advance various aspects of disease assessment including early detection, disease monitoring, and therapy selection, while also allowing for improved patient follow-up and economic efficiency over traditional tissue biopsies. Plasma cell-free DNA (cfDNA) applications have recently been adopted in prenatal screening, tumor mutation profiling and post-transplant monitoring. Additionally, combining cfDNA profiling with paired RNA analysis of peripheral blood mononuclear cells (PBMCs) can provide useful insights into biomarkers and immune phenotypes, and present a comprehensive multiomic profile from a single blood draw. Several technical and analytical challenges persist in the liquid biopsy field including immediate preservation of draw time levels of cfDNA, minimizing PBMC lysis during storage, low concentrations of cfDNA with even lower tumor fraction, as well as the need for a higher sensitivity in downstream applications to accurately interpret clinical signals. Here, we present an efficient blood-to-result workflow for tumor-specific mutation detection and immune profiling using contrived samples spiked with known mutation controls. Whole blood was collected in Streck Nucleic Acid BCT enabling preservation of draw time concentration of cell-free nucleic acids and prevention of immune cell lysis. Nucleic acid extraction was performed on the isolated PBMCs and plasma fraction using an optimized protocol for the Monarch Mag Viral DNA/RNA Extraction Kit which features a simplified, magnetic bead-based workflow. Isolated cfDNA was used to prepare sequencing libraries using an optimized library prep workflow to maximize conversion efficiency from cfDNA. Isolated PBMC RNA was utilized to prepare RNA-seq libraries and Immune-seq libraries using the NEBNext UltraExpress RNA Library Prep Kit and NEBNext Immune Sequencing Kit, respectively. Combining these sample storage, extraction, and library prep methods resulted in a high yield of cfDNA libraries with minimal contaminating genomic DNA, enabling improved sequencing data quality, high conversion efficiency, and high sensitivity for clinical research, including detecting spike-in mutations. Transcriptome and immune repertoire profiling from the preserved PBMC in the same blood sample provided complementary biomarker information to enable a comprehensive evaluation of the disease status of these samples.

#5880 Leveraging cell-free DNA methylation biomarkers for pancreatic cancer detection and minimal residual disease monitoring.

Ziqi Zhu, Giancarlo Bonora, Chao Dai, Kemin Zhou, Binggang Xiang, Shidong Jia, Pan Du

Predicine Inc., Hayward, CA

Introduction: Detecting pancreatic cancer from plasma cell-free DNA (cfDNA) is particularly challenging due to the low circulating tumor DNA (ctDNA) shedding associated with pancreatic tumors, especially in localized cases. Pancreatic cancer often exhibits differential methylation across hundreds or thousands of genes. We applied fragment-level DNA methylation analysis to tissue and plasma samples from a pancreatic ductal adenocarcinoma (PDAC) cohort to obtain differentially methylated fragment regions (DMFRs) alongside traditional differentially methylated regions (DMRs). Mutation-based tumor fractions using orthogonal assay were calculated for comparison. Additionally, we developed a PDAC DNA methylation subpanel using patient data based on DMFRs obtained from fragment-level analysis.

Methods: Pancreatic cancer samples from localized (n = 36) and metastatic (n = 117) stages were profiled by methylation-based PredicineEPIC™, PredicineALERT™, and mutation-based PredicineBEACON™ assays. Abnormally methylated fragments were identified using a background model based on healthy plasma donors. A PDAC panel was created using both tissue (n = 45) and plasma (n = 117) samples, as well as public datasets such as TCGA.

Results: Fragment-level analysis identified a comparable number of DMFRs in PDAC plasma and tissue samples, with strong overlap between the two. While DMFRs and DMRs were comparable in the tissue samples, significant fewer DMRs were identified in the plasma samples. This underscores the superior sensitivity of fragment-level analysis for detecting abnormal methylation in the liquid biopsy samples. Furthermore, key PDAC-related biomarkers were more distinctively identified using DMFR analysis. Genome-wide DNA methylation losses were also observed in many pancreatic samples, and combining these with location-specific methylation signals enhanced both the robustness and sensitivity of the assay. The incorporation of the PredicineALERT PDAC panel, informed by the methylation fragment-level analysis, improved concordance between methylation and mutation-based tumor fractions in both metastatic and localized PDAC plasma samples. Sensitivity for minimal residual disease (MRD) detection was also markedly improved using PredicineALERT panel compared to genome-wide methylation profiling using PredicineEPIC.

Conclusion: This study demonstrates the value of fragment-level cfDNA methylation analysis for detecting and monitoring PDAC using plasma samples. DMFR analysis demonstrated greater sensitivity than traditional DMR analysis. In addition, a PDAC panel designed through fragment-level analysis enhanced correlations between methylation abnormalities and mutation-based tumor fractions, offering a robust tool for early detection and disease monitoring in PDAC.

#5881 Transcriptomic profiling of extracellular vesicle subpopulations enables liquid biopsy-based brain tumor classification.

Margie Kinnersley, Claire Seibold, Tre Blohm, Riley Kemp, Sean Lodmell, Amanda Mast, Tia Seibold, Rachel Short Miller, Kelley Van Vaerenberghe, Katie Havranek

FYR Diagnostics, Missoula, MT

Liquid biopsies based on circulating tumor-derived cell-free DNA (ctDNA) have become an increasingly common tool for minimally invasive diagnostic testing. However, as ctDNA does not easily cross the blood-brain barrier, cell-free DNA tests for identifying and classifying brain tumors are of limited utility. Extracellular vesicles (EVs), which carry heterogeneous protein, nucleic acid, and metabolite cargos derived from various cell types including cancer cells, are an attractive alternative to ctDNA that can cross the blood-brain barrier. Because only a small fraction of circulating EVs are derived from diseased cells, current EV-based methods for cancer detection often suffer from low signal-to-noise ratios. To circumvent this issue, we have developed an extracellular vesicle subpopulation enrichment technology that specifically targets EVs derived from diseased cells. EV subpopulation transcriptomics interrogated using machine learning and gene network analyses can distinguish healthy patients, patients with benign tumors, and patients with glioma. Known tissue markers as well as novel features provide the basis for patient stratification. Coupled with a state-of-the-art multi-omics workflow, our novel EV enrichment platform allows us to capitalize upon the powerful diagnostic information contained within EV subpopulations to identify and characterize cancers from standard blood plasma samples.

#5882 A novel NGS-based assay in kit format for tumor-independent cancer monitoring, and evaluation of patient treatment response using cfDNA genomic alteration and methylation duo analysis.

Tali Azenkot[1], Grace Zhao[2], Heng Wang[2], Xiaomu Chen[2], Margherita Corioni[3], Michael Ruvolo[2], Teressa Celma[1], Jeff Pawelek[1], Aswati Aravind[2], Douglas Roberts[2], Yun Bao[2], Bram Herman[2], Annie Wu[4], William Mitchell[1], Kathryn Gold[1], Lyudmila Bazhenova[1], Shengrong Lin[2], Sandip Patel[1]

[1]UC San Diego Moores Cancer Center, La Jolla, CA,[2]Agilent Technologies Inc., Santa Clara, CA,[3]Agilent Technologies Inc., La Jolla, CA,[4]Rensselaer Polytechnic Institute, Troy, NY

Introduction: While circulating tumor DNA-based tests have shown promise in evaluating treatment response, predicting prognosis, and detecting measurable residual disease, tumor-informed personalized strategies have limitations. For example, the need for large tumor samples and the complexity and high cost of personalized testing can be barriers to clinical use. Commercially available genomic and methylation tumor-independent assays require large panels and sample splitting, further limiting their practicality. Both these assays require samples to be sent to centralized CLIA-certified labs. To address these challenges, we explored the Agilent Avida target enrichment technology in a small sample of patients with non-small cell lung cancer (NSCLC). This kit-based solution reduces sequencing costs, is compatible with automation systems like the Agilent Bravo NGS Workstation, and enables decentralized, faster molecular analysis.

Methods: We applied the Agilent Avida circulating cell-free DNA (cfDNA) genomic alteration and methylation combined analysis for tumor-independent cancer monitoring in patients with NSCLC. First, we demonstrated the differential hypermethylation cancer-specific signal in 4 different lung cancer tumors, with low background noise in normal lung tissues, non-cancerous pathological lung tissue, control peripheral blood mononuclear cells, and cfDNA. We then showed feasibility of this cfDNA technology in patients with stage I through III NSCLC. In 4 patients with stage III NSCLC, plasma samples were collected before and 1-3 months after initiating immunotherapy treatment to assess whether the assays correlated with known clinical and imaging markers of disease response.

Results: Using 1 mL of plasma from NSCLC patients, we detected cancer in 8 of 10 stage I patients and 10 of 11 stage II patients. Fifteen of 16 control samples were below the cutoff threshold. Among 4 patients with stage III NSCLC, we performed cfDNA Duo analysis, capturing both DNA mutations and methylation information from one input without sample splitting. The combined genomic and methylation analysis provided information on actionable mutations and tumor mutation burden for these patients. The overall methylation signal correlated with CT imaging-based tumor changes for most cases.

Conclusion: Our results demonstrate the feasibility of the Agilent-Avida DNA/methylation DUO kit for tumor-independent cancer monitoring in patients with NSCLC undergoing immunotherapy treatment.

#5883 Estimation of blood volume collected on plasma-separating dried blood spots using gravimetric and imaging analysis.

Elise C. Dietmann, Michelle D. Stephens, Olivia Krebs, Everlyne N. Nkadori, Pallavi Tiwari, Stephanie M. McGregor, Muhammed Murtaza

University of Wisconsin-Madison, Madison, WI

Background: Analysis of plasma-separating dried blood spots (psDBS) holds promise for circulating tumor DNA (ctDNA) measurement, enabling at-home sample collection, room temperature storage, and easy shipment. However, unlike collection in a blood tube, it is not feasible to directly measure blood volume collected from a fingerstick using psDBS. Here, we evaluate whether analysis of psDBS mass, color, and area can infer blood volume and enable downstream measurement of DNA concentration.
Methods: Whole blood from patients with head and neck, breast, gynecologic, genitourinary, and gastrointestinal malignancies was collected on psDBS devices by the University of Wisconsin-Madison Carbone Cancer Center BioBank. In addition, whole blood from healthy individuals was commercially obtained and between five and nine psDBS were prepared with volumes ranging from 50 to 250 µL. All samples underwent analysis for mass, area, and color. psDBS were imaged using a flatbed scanner. Total area of erythrocytes and mean grey value of center erythrocytes were measured using image segmentation. Using mass of the psDBS membrane, erythrocyte area, erythrocyte mean grey value, and sex as predictors, a linear regression model was developed to predict whole blood volume. Mean absolute percent error (MAPE) was used to assess model accuracy.
Results: One hundred ninety psDBS samples from 30 healthy individuals and 100 psDBS from 100 patients with cancer were analyzed. Using 132 samples from 20 healthy individuals (training set), a linear regression model was fit to the data, showing a very strong correlation between actual and predicted blood volume (MAPE = 7.6, Pearson r = 0.97). The model was then tested using 58 samples from 10 different healthy individuals (independent validation set), as well as psDBS from patients with cancer, and predicted blood volume collected on psDBS with high accuracy (MAPE = 6.8 and 9.3, respectively).
Conclusion: Our results suggest estimating blood volume from psDBS is feasible using gravimetric and imaging analysis. By establishing a linear regression model that incorporates weight, area, and color, we can infer blood volume collected on psDBS samples for healthy individuals and patients with cancer. Ongoing work will incorporate this approach to enable measurement of plasma DNA concentration in blood spots.

#5884 Cancer subtype classification by cfDNA fragmentomics analysis.

Lisha Zhu[1], Chao Dai[1], Shuang Gan[2], Shidong Jia[1], Pan Du[1]

[1]Predicine Inc., Hayward, CA,[2]Huidu Shanghai Medical Sciences, Ltd., Shanghai, China

Introduction: Nucleosome occupancy patterns at transcription factor binding sites (TFBS) exhibit differences between circulating tumor DNA (ctDNA) in cancer patients and normal plasma controls, as well as among different tumor subtypes. Therefore, cell-free DNA (cfDNA) fragmentomics can be employed for cancer subtyping analysis. The tumor fraction in cfDNA can affect the performance of nucleosome profiling, and a minimal tumor fraction is required to obtain high sensitivity of cancer subtyping. This study investigated the detection sensitivity - specifically, the minimal tumor fraction required - for different TFBS analyses.

Methods: We utilized the Griffin framework to classify tumor subtypes based on nucleosome profiling of cancer-specific TFBS from low-pass whole genome sequencing data of cfDNA. Androgen receptor (AR) and ASCL1 binding sites were used to distinguish between AR-dependent prostate cancer (ARPC) and neuroendocrine prostate cancer (NEPC). Estrogen receptor (ER+) ATAC-seq peaks were employed for subtyping of ER-positive breast cancer. For each tumor type, we selected three tumor samples with highest nucleosome profiling abnormality scores for simulation studies. Each cancer sample was down-sampled at various percentages and mixed with six down-sampled normal samples, with three replicates per normal sample using different random seeds, to obtain simulated cancer samples with a serious of predetermined tumor fractions.

Results: The specificity of nucleosome profiling at AR, ASCL1 and ER binding sites was high (> 95%) for all 87 normal samples. The limit of detection (LoD) for tumor fraction of AR binding sites ranged between 10% and 14%, with the average of 12% and standard deviation of 0.0098. Similarly, the LoD for ER binding sites was between 10% and 13%. The LoD of ASCL1 binding sites was higher, ranging from 20% to 25%. When genome-wide DNA methylation data were incorporated, both the specificity and sensitivity of nucleosome profiling increased. Interestingly, we observed methylation loss at AR and ER binding sites in prostate and breast cancer patients respectively.

Conclusion: Prostate and Breast Cancer cfDNA samples sequenced by low-pass whole-genome sequencing can be assigned to subtypes according to nucleosome profiling of AR, ASCL1 and ER binding sites. The minimum tumor fraction required for AR and ER is at least 10%, while for ASCL1 it is as high as 20%. Integrating DNA methylation data into nucleosome profiling analysis can enhance sensitivity and lower the limit of detection, which we are actively investigating.

#5885 Streck Urine Preserve provides cell-free DNA stabilization in whole urine for liquid biopsy.

J. Li, S. Mehta;
Streck LLC., La Vista, NE

Urinary cell-free DNA (ucfDNA) has gained recognition as a critical biomarker for liquid biopsy, as it provides comprehensive and invaluable insights for cancer researchers, especially for urological cancers such as bladder, kidney, and prostate cancers. While urine as a sample matrix offers appealing advantages over blood, including large sample volume and truly non-invasive and easy self-collection, ucfDNA is a challenging and unstable analyte, as it is vulnerable to DNase degradation and contamination from bacterial and genomic DNA. Here, we describe the use of Streck Urine Preserve to minimize pre-analytical variables before ucfDNA extraction and downstream analysis. To determine whether Streck Urine Preserve effectively stabilizes ucfDNA and limits DNA contamination, second void urine was collected from donors, treated with Streck Urine Preserve, and stored at 2 °C to 37 °C for up to 7 days. Following storage, ucfDNA was isolated from 4 or 8 mL urine using a modified automated extraction method with MAGicBead cfDNA Isolation Kit (Zymo Research) on a KingFisher Apex System. Target human ucfDNA concentrations were monitored by ddPCR or dPCR using a housekeeping gene (ACTB) as a surrogate marker. The total bacterial burden in the extracted ucfDNA sample was analyzed using qPCR targeting 16S rRNA. While unpreserved urine samples exhibit rapid ucfDNA degradation during ambient temperature storage, samples stored in Streck Urine Preserve (at a preservative-to-urine ratio range between 1:20 to 1:5) maintained the void-time human ucfDNA target concentration for up to 7 days regardless of temperature (2 °C to 37 °C). Further, a marked increase in bacterial cfDNA contamination was observed in the unpreserved urine sample, whereas samples treated with Streck Urine Preserve displayed minimal changes in Cq values of 16S rRNA in the extracted ucfDNA from void time to day 7. Taken together, these data indicate that Streck Urine Preserve protects ucfDNA targets in the sample from DNase degradation and genomic and bacterial DNA contamination during storage**.** Streck Urine Preserve ensures sample integrity and minimizes pre-analytical variables, providing cancer researchers and assay developers flexibility in sample handling and enhanced confidence in the validity of downstream ucfDNA analysis results. Streck Urine Preserve is for Research Use Only. Not for use in diagnostic procedures. Streck Urine Preserve should only be used for research or the development of new assays.

#5886 Cross-test performance verification of tissue-TMB and a cfDNA somatic variant Next Generation Sequencing (NGS) panel for detection of actionable mutations.

Leisa Jackson[1], Ashley King-Roads[2], Gary A. Pestano[2]

[1]Development, Biodesix, Inc., Louisville, CO,[2]Biodesix, Inc., Louisville, CO

Introduction: Comprehensive tumor profiling by NGS has become widespread in oncology. However, a relatively small number of the developed panels have gained regulatory approvals and/or authorization. Given the need for higher levels of compliance for panels used for clinical laboratory testing, we sought to extend the utility of panels intended for use in blood and tissue testing. In this study we verified the performance of a 52-gene liquid biopsy hotspot panel [evaluated for single nucleotide variants (SNVs) and insertions/deletions (Indels) as low as 0.5% allele frequency (AF) and copy number amplifications (CNAs) down to 1.4x] to be used with formalin-fixed paraffin embedded (FFPE) specimens. In the same study, we evaluated the performance of a well characterized tissue TMB/TML (tumor mutation burden) assay for concordance in somatic variant calling against the hotspot panel. The targeted TMB/TML assay covers 1.7 Mb of the genome (1.2 Mb exome) and detects variants at 5% AF. The performance of the latter panel was previously reported in the harmonization study conducted by the FOCR TMB consortia.

Methods: Twelve de-identified clinical FFPE specimens and a commercial (contrived) FFPE reference material were tested for SNVs and Indels using the validated 52-gene hotspot pan-cancer assay and the TMB/TML panel. Co-occurrence of SNVs/INDELs (n = 40) was observed between the two panels. The reference result for the clinical FFPE specimens was generated by the variant caller function in the TMB/TML bioinformatic workflow and included 14 SNVs. The pan-cancer panel was also used to test for 19 SNVs and 7 Indels present in the contrived FFPE reference specimen.

Results: TMB scores ranged from 1.7-104 Muts/Mb. A high level of inter chip concordance was observed between duplicate runs (R^2 = 0.997). Duplicate NGS libraries were generated from the reference contrived FFPE specimen, and both passed QC. Eleven of 12 clinical samples passed the pan-cancer informatics QC thresholds. Thirteen of 14 SNVs (93%) were detected in the clinical samples (AF range: 13-58%) and eighteen of 19 SNVs (95%) and 5 of 7 Indels (71%) were detected in both replicate tests of the FFPE reference material (AF range:1.9-13.9%). Although no reference was available, one ERBB2 CNA was also detected with the pan-cancer panel (at 10.45x). Since the starting material was DNA (and not RNA or cDNA), fusion variants could not be detected although they are covered by the hotspot panel.

Conclusions: These studies demonstrate extended utility for a previously validated liquid biopsy hotspot pan-cancer panel for the detection of SNVs/Indels and CNAs in FFPE. We simultaneously demonstrated the potential utility of a well characterized TMB panel for detection and reporting of actionable SNVs/Indels. Additional studies are on-going to further validate the TMB panel for actionable variant reporting.

#5887 *NALCN* promoter methylation as a biomarker for metastatic risk in a cohort of non-small cell lung cancer patients.

Eleni Thanou[1], Dora Londra[1], Ioanna Balgouranidou[2], Eleni Eftimiadou[1], Alexandra Delipetrou[1], Emilia Tsaroucha[3], Vasilis Georgoulias[4], Athanasios Kotsakis[5], Evi Lianidou[1], **Athina N. Markou**[1]

[1]Chemistry, University of Athens, Athens, Greece,[2]Department of Medical Oncology, University General Hospital of Alexandroupolis, Medical School, Democritus University of Thrace, Aleandroupolis, Greece,[3]8th Department of Pulmonary Diseases, "Sotiria" General Hospital, Athens, Greece,[4]First Department of Medical Oncology, Metropolitan General Hospital, Athens, Greece,[5]Department of Medical Oncology, University General Hospital of Larissa, Larissa, Greece

Liquid biopsy enables real-time monitoring of tumor development and response to therapy through the analysis of CTCs and ctDNA. NALCN is a sodium leak channel that is frequently involved in tumor evolution and immunity and acts as a tumor suppressor. Deletion of NALCN has been shown to increase cancer metastasis and the number of CTCs in peripheral blood. In this study, we investigated for the first time NALCN promoter methylation in (a) Aza-treated cell lines (A549, TE671, BT20, and MDA-MB-468), (b) paired NSCLC tissues (n = 22), and (c) plasma cell-free DNA (ctDNA) from patients with NSCLC (early stage n = 39, metastatic n = 39) and DNA from 10 healthy donors (HD) using a newly developed highly specific and sensitive real-time MSP method. Treatment with 5′-aza-dC induced the expression of NALCN only in the A549 cell line, suggesting that DNA methylation regulates its expression in certain cancers. The mRNA expression levels of NALCN were quantified in non-small cell lung cancer (NSCLC) and adjacent non-cancerous tissues, and it was found to be underexpressed in 54.5% of tumor tissues, with significantly higher expression in recurrence-free patients ($p = 0.009$) than in patients who relapsed. The NALCN methylation level was not statistically significantly correlated with the corresponding expression ($p = 0.439$), while Kaplan-Meier analysis showed an association between NALCN promoter hypermethylation and worse disease-free intervals (DFIs) ($p = 0.017$). Evaluation of NALCN methylation in ctDNA revealed that it was detected in 5.1% of early and 10.2% of advanced cases. Our results strongly suggest that epigenetic inactivation of NALCN may predict metastasis in NSCLC. Our results should be validated in further studies based on a larger patient cohort to investigate whether DNA methylation of the NALCN promoter could serve as a potential prognostic DNA methylation biomarker and predictor of metastasis in NSCLC.

#5888 Urinary ctDNA tumor fraction monitoring enhances non-invasive assessment of molecular response in bladder-preserving treatment of muscle-invasive bladder cancer.

Jiani Deng[1], Feng Wen[1], **Haoran Tang**[2], Cancan Jia[2], Yue Zhang[2], Peng Zhang[3], Yali Shen[1]

[1]Abdominal Oncology Ward, Division of Radiation Oncology, Cancer Center, West China Hospital, Sichuan University, Chengdu, China,[2]Huidu Shanghai Medical Sciences, Ltd., Shanghai, China,[3]Department of Urology, Institute of Urology, West China Hospital, Sichuan University, Chengdu, China

Introduction: While radical cystectomy (RC) following neoadjuvant therapy remains the standard of care for muscle-invasive bladder cancer (MIBC), bladder-preserving treatments offer a viable option for selected patients to improve quality of life. Rapid detection of therapeutic response is critical for clinical decision-making but remains challenging. Here, we introduce a non-invasive liquid biopsy assay based on next-generation sequencing (NGS) to monitor the molecular response in both urine and blood during neoadjuvant therapy in MIBC patients.
Methods: This ongoing trial has enrolled 20 patients treated with HER2 ADC neoadjuvant therapy, followed by radiotherapy and clinical evaluation to decide between RC or bladder preservation. In a completed pilot cohort of seven patients, both urine and blood samples were collected before and after HER2 ADC treatment to evaluate therapy response. The study utilized PredicineCARE, a targeted NGS assay, to analyze circulating tumor DNA (ctDNA) in urine and blood samples, aiming to detect genetic variations and evaluate tumor fractions in patients.
Results: In the pilot cohort of seven patients, the ctDNA assay detected a greater number of somatic mutations and exhibited higher detection positivity in urine samples compared to blood samples. Specifically, in urine samples, the assay identified 138 somatic mutations at baseline and 18 mutations at the end of therapy, achieving positivity in all seven patients at both time points. In contrast, in blood samples, it identified 11 mutations at baseline and 6 mutations at the end of therapy, with positivity in 5 out of 7 and 4 out of 7 patients, respectively. The overall tumor fractions detected in urine samples were significantly higher at baseline than at the end of therapy (median tumor fraction: baseline 10.6% vs. end of therapy 0.8%; p=0.043), a trend not observed in blood samples. Moreover, through paired comparisons of each patient's urine samples at the two time points, we observed a molecular response—defined as a tumor fraction decrease greater than 20%—in six of the seven patients, and molecular progression—defined as a tumor fraction increase greater than 20%—in one patient. These findings were consistent with the clinical evaluations conducted at baseline and at the end of therapy.
Conclusion: Our study demonstrates that urine-based ctDNA analysis using a non-invasive liquid biopsy assay provides a reliable method for monitoring molecular response during neoadjuvant therapy in patients with MIBC undergoing bladder-preserving treatments. These findings suggest that urine ctDNA analysis can serve as a valuable tool for rapid detection of therapeutic response, aiding clinical decision-making. Further validation in larger cohorts is warranted to establish its utility in routine clinical practice.

#5889 Combined cfDNA and cfRNA enhanced gene fusion detection and drug resistance mechanism insights in mCRPC through combined cfDNA and cfRNA assays profiling.

Haoran Tang, Feng Xie, Yue Zhang, Yutao Feng

Huidu Shanghai Medical Sciences, Ltd., Fengxian District, Shanghai, China

Background: Metastatic castration-resistant prostate cancer (mCRPC) is a complex disease characterized by distinct molecular features related to genomic instability and selective treatment pressures. Cell-free DNA and RNA (cfDNA and cfRNA) found in blood offer potential for disease diagnosis, monitoring, and interrogation of resistance mechanisms by detecting genomic alterations from tumors. Here, we introduce comprehensive cfDNA and cfRNA assays to profile the genomic landscape in mCRPC patients for the purposes of disease diagnosis and monitoring.
Methods: In this retrospective study, plasma samples were collected from 52 patients with mCRPC at treatment baseline. For some patients, additional plasma samples were obtained at both cycle 1 day 1 (C1D1) and cycle 3 day 1 (C3D1) during androgen receptor inhibition therapy. We employed PredicineCARE, a 152-gene cfDNA assay, and PredicineRNA, an 88-gene cfRNA assay, to detect genomic alterations at both DNA and RNA levels in these samples.
Results: The TMPRSS2-ERG fusion is a common genomic alteration in prostate cancer and serves as a potential diagnostic and prognostic indicator. In baseline samples from 52 patients, the cfDNA assay detected a TMPRSS2-ERG fusion positive rate of 25.0%, while the cfRNA assay detected a higher rate of 38.5%, although this difference did not reach statistical significance (McNemar's test, $p = 0.065$). Importantly, using a simultaneous testing strategy with both cfDNA and cfRNA increased the detection rate to 42.3%, which is comparable to the prevalence previously reported in tissue samples. Furthermore, by comparing the same androgen receptor (AR) mutations (W742C and T878A) detected by cfDNA and cfRNA assays at C1D1 and C3D1, this comprehensive analysis illustrates the potential biological mechanisms of acquired drug resistance.
Conclusion: This study demonstrates that combining cfDNA and cfRNA assays enhances gene fusion detection in mCRPC patients and provides insights into the mechanisms of acquired drug resistance. These findings support comprehensive liquid biopsy approaches for improved diagnosis, monitoring, and personalized treatment strategies in mCRPC.

#5890 Personalized detection of circulating cell-free tumor DNA in gynecologic cancer.

Andrea B. Moffitt[1], Jude Kendall[2], Joan Alexander[2], Asya Stepansky[2], Arisa Kapedani[3], Zihua Wang[2], Dan Levy[2], Kenny Ye[4], Abba M. Krieger[5], Marina Frimer[3], Gary L. Goldberg[3], Michael Wigler[2]

[1]Hematology and Medical Oncology, Emory University, Atlanta, GA,[2]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY,[3]R.J. Zuckerberg Cancer Center, Northwell Health, Lake Success, NY,[4]Systems & Computational Biology, Albert Einstein College of Medicine, Bronx, NY,[5]Statistics and Data Science, University of Pennsylvania, Philadelphia, PA

Endometrial and ovarian cancers are among the most common gynecologic malignancies in the U.S., with 5-year overall survival rates of 81% and 50% respectively. Ovarian cancer often presents late due to asymptomatic early stages and nonspecific symptoms in advanced stages. Few markers exist for early detection, and they lack the sensitivity and specificity needed for high-risk screening.

Given the absence of informative protein blood biomarkers, cell-free DNA analysis has become a promising tool for early detection, treatment evaluation, and monitoring. However, previous studies of circulating tumor DNA (ctDNA) in gynecological cancers had limited sensitivity. We adapted MASQ (Multiplex Accurate Sensitive Quantitation), originally developed to measure residual cells in leukemia, to the measurement of ctDNA in solid cancers. MASQ detects patient-specific mutations simultaneously in up to 50 loci with exceptional sensitivity, below one part per million.

This study uses MASQ to measure tumor-specific variants in blood (cell-free DNA) from endometrial and ovarian cancer patients, aiming to determine the range of ctDNA signals at presentation and explore correlations with clinicopathological features and patient outcomes.

We analyzed samples from 46 gynecologic cancer patients (12 ovarian, 34 endometrial) treated at Northwell Health. Samples were collected in collaboration with gynecologic oncology research coordinators and the Northwell Health Biorepository. Peripheral blood was collected at diagnosis (n=46) and post-surgery (n=14), along with surgical tumor specimens. Whole genome sequencing of tumor and blood identified thousands of somatic tumor variants per patient.

For each patient, 30 representative variants were selected for MASQ sequencing. MASQ libraries were generated from cell-free DNA isolated from pre- and post-surgery plasma. Ultrasensitive variant counts across 30 loci were aggregated and analyzed for clinical correlations.

ctDNA signal was detected in 35 of 46 cases at presentation, with variant allele frequencies ranging from below 10^-4 to 10^-1. ctDNA signal was higher in ovarian cancer (13/13 detected) than endometrial cancer, and higher in aggressive Type II endometrial cancer (15/16) vs. Type I (5/13). ctDNA levels correlated with tumor size and copy number imbalance, but not with total cell-free DNA. Detection of ctDNA predicted worse overall survival (p=0.023) and was the strongest predictor of survival in multivariate analysis. Post-surgery follow-up in 10 of 14 patients showed significant ctDNA reduction following treatment, highlighting MASQ's sensitivity in tracking treatment response.

This study highlights the potential of sensitive ctDNA detection for early diagnosis and monitoring in gynecologic cancers. However, limited cell-free DNA abundance necessitates even more sensitive methods to enhance clinical utility.

#5892 Optimized enzymatic conversion method for improved methylation detection in low-input cfDNA samples.

Hong Xu[1], Quan Wang[1], Nan Zhang[1], Zhuoran Jiang[2], Lei Song[2], Ming Yang[2], Jing Zhao[2], Yue An[2], Congchong Wei[2], Yuting Liu[2], Feng Xu[2], Min Li[2], Jing Liu[2], Baoliang Zhu[2], Xiaohui Wu[2], Xueguang Sun[2]

[1]The First Hospital of Jilin University, Changchun, China,[2]Shanghai Weihe Medical Laboratory Co., Ltd, Shanghai, China

Background: Cell-free DNA (cfDNA) is widely utilized in liquid biopsy-based early cancer screening, where DNA methylation serves as a predominant detection marker via next-generation sequencing. Enzymatic Methyl-Seq (EM-Seq) preserve cfDNA integrity and significantly improved methylation marker detection. However, with its expanded application, several limitations have been identified, including insufficient protection of 5-methylcytosine (5mC) in samples below 10 ng, leading to diminished methylation signals and inconsistencies across different input amounts. Additionally, uneven CH conversion results in background noise akin to bisulfite methods. These issues are critical as clinical cfDNA samples of early-stage cancer often fall below the 10 ng threshold, indicating great room for improvement in enzymatic conversion.
Methods: To enhance the compatibility of enzymatic conversion methods with low-input cfDNA samples, we have made a series of improvements, including refining oxidation and deamination reaction systems. We evaluated these optimizations by testing on cfDNA from healthy donors and two cancer patients (colorectal cancer, CRC and gastric cancer, STAD).
Results: The optimized enzymatic conversion method significantly improved library quality, increasing average mapped ratios by 4.24% and 7.53% for healthy samples and for cancer samples, with library complexity rising from 38.04% to 75.52% consequently. The average CpG sites protection efficiency at 1 ng input is nearly equivalent to that at 30 ng input (98.11% vs. 99.27%), and shows a notable improvement over the original method (96.49% vs. 99.11%). The CH non-conversion rate decrease from 0.13% to 0.065%, significantly reducing the background noise. Consequently, more CpG sites are captured with equivalent sequencing depths. Samples with inputs ranging from 1 to 30 ng showed greater consistency in methylation beta values, with an average Pearson's correlation of 0.93, compared to 0.87 for the original method. Sensitivity analysis indicated that optimized method detected more methylation haplotypes in artificially pooled samples with varying cancer cell spike-in ratios: 589 vs. 379 at 0.05%, 935 vs. 502 at 0.1%, 3,326 vs. 1,236 at 0.5%, and 6,318 vs. 2,126 at 1%, enabling detection at lower cancer cell abundances. Using healthy samples as a reference, the optimized method consistently identified more differential haplotypes in CRC and STAD samples at 1, 5, and 30 ng cfDNA inputs, with the disparity between methods decreasing as input increased, confirming its advantages in low-input conditions.
Conclusion: Our optimized enzymatic conversion method provides superior library quality, increased complexity, reduced CH background and enhanced 5mC protection in low-input samples. These improvements enhance the sensitivity for detecting aberrant methylation signals in low-input cfDNA.

#5893 Feasibility of DNA analysis in peritoneal fluid from patients with peritoneal carcinomatosis.

Muhammad Talha Nawaz[1], Muhammad Talha Waheed[2], Kirsten Dennison[1], Bradon McDonald[1], Stephanie McGregor[3], Syed Nabeel Zafar[1], Mustafa Raoof[2], Muhammed Murtaza[1]

[1]Department of Surgery, University of Wisconsin-Madison, Madison, WI,[2]Department of Surgery, City of Hope National Medical Center, Duarte, CA,[3]Department of Pathology, University of Wisconsin-Madison, Madison, WI

Introduction: Gastrointestinal (GI) cancers often metastasize to the peritoneum. Current diagnostic tools for peritoneal carcinomatosis (PC) show limited accuracy. Reliable biomarkers to detect and monitor treatment response for PC are urgently needed. While circulating tumor DNA in plasma is a promising cancer biomarker, contribution of tumor DNA from PC into blood is minimal. We hypothesize that peritoneal fluid tumor DNA (ptDNA) may be useful for PC detection and response monitoring. Currently, there are no established laboratory methods for ptDNA analysis. In this study, we evaluate the feasibility of two DNA extraction methods using peritoneal fluid (PF) samples.

Methods: We evaluated the performance of two cell-free DNA extraction kits based on magnetic beads (MagMax) or silica columns (QIAamp) using commercially obtained PF from two individuals, at 1 mL and 4 mL starting volumes. Based on these results, we evaluated the optimal method further using PF samples from 24 patients with PC from GI cancers (biliary tract, appendiceal, and colorectal primary) undergoing surgery or paracentesis at City of Hope National Medical Center. PF samples were collected immediately before the start of the procedure. We extracted DNA using 4 mL inputs, eluted in 30 µL buffer and assessed using electrophoresis (TapeStation) and fluorometry (Qubit).

Results: In commercially obtained ascites samples, adequate DNA yield and quality was obtained using both kits. Compared to QIAamp, mean DNA concentration was slightly lower using MagMax (24.9 ng/mL [range 13.9 - 35.9] vs. 25.7 ng/mL [range: 16.1-35.3; p=0.91). Similarly, mean fragment length was also shorter using MagMax compared to QIAamp (287.5 bp [range 202-350] vs. 294.3 bp [range: 239-348; p=0.80). In contrast to our prior experience with plasma, peritoneal fluid DNA was observed at higher concentrations (3.7-fold higher) and longer fragment lengths. Based on these results and processing considerations, we selected MagMax kit to evaluate patient PF samples. We observed similar fragment lengths (mean: 281.5 bp, range: 136-369), with concentrations ranging from 0.80 ng/mL to 105 ng/mL in PF (mean: 20.6 ng/mL).

Conclusions: Our results demonstrate the feasibility of DNA analysis using PF samples in patients with PC Furthermore, the yield and quality of DNA extracted is adequate for downstream molecular analyses. We were able to detect differences in PF vs. plasma DNA and observe inter-patient variability in concentration and fragmentation. On-going work will evaluate detection and quantification of peritoneal tumor-specific DNA as a biomarker for PC detection and monitoring.

#5894 Detection of a novel GNA11 processed pseudogene from cfDNA and implications for liquid biopsy.

Jan Wignall, Wen Zhou

Oncology Development, BillionToOne, Inc., Menlo Park, CA

Processed pseudogenes are messenger RNAs that have been reverse transcribed and inserted into the genome. While the functional implications of these processed pseudogenes remain unknown, their presence can lead to variants being mistakenly detected and classified as copy number amplifications and deletions in comprehensive genomic profiling (CGP) assays for cancer treatment selection. False positive amplifications run the risk of patients being prescribed ineffective therapies.

Northstar Select is a liquid biopsy CGP assay which covers 84 genes. In this retrospective analysis of 1800 patients, amplification signals due to a pseudogene were observed in 4 patients on *SMAD4* and 7 patients on *GNA11* and confirmed via orthogonal methods. These samples showed abnormal exonic amplifications and intronic deletions in the *SMAD4* and *GNA11* genes. Splice site variants were present at low variant allele fraction (VAF) on either side of each exon. Notably, the copy number detected does not correlate with estimated tumor fractions or other ctDNA mutations detected in these samples.

Whole genome sequencing (WGS) of selected patients harboring the *SMAD4* amplifications revealed the presence of a poly-A tail and a breakpoint in *SCAI* on chromosome 9, consistent with a pseudogene previously described in the literature. WGS of *GNA11* amplification-positive samples revealed the presence of a novel processed pseudogene in chromosome 20 that has not been previously described, which may have further implications on cancer-driving mutations as *GNA11* is an oncogene. Northstar Select's copy number calling algorithm measures a copy number of 3 for *GNA11*, indicating an additional copy from the pseudogene. qPCR of extracted buffy coat gDNA across the suspected breakpoint in chromosome 20 confirms the insertion and its germline nature. The presence of the same breakpoint in multiple patients suggests this is an ancestral integration event. Similar to the previously described *SMAD4* pseudogene, false positive splice site variants are results of mismapping of reads. In conclusion, we have determined a framework to confirm pseudogene presence that is not tumor DNA derived. Bioinformatic analysis of CGP tests should carefully screen suspicious results for the possibility of as-yet undiscovered processed pseudogenes to avoid reporting such false positive amplifications and deletions.

#5895 Development and analytical validation of a highly sensitive tumor fraction estimation method using blood samples without matched normal controls.

Junmei Wang, Wanshu He, Lisha Zhu, Yong Huang, Xiaohong Wang, Shidong Jia, Pan Du

Predicine Inc., Hayward, CA

Background: Tumor fraction (TF) in cell-free DNA (cfDNA) is a quantitative indicator of circulating tumor DNA (ctDNA) and has become an important biomarker for disease monitoring and tracking therapeutic responses. We developed a longitudinal TF estimation method that does not require matched normal control samples and analytically validated its ability to accurately estimate TF when longitudinal blood samples are available.
Methods: Both reference materials and clinical blood samples were used in the analytical validation. We first determined the limit of detection (LoD) of TF for our PredicineCARE cfDNA assay at targeted sequencing depth of 20,000x using serially diluted reference materials with 12 gradient values from 0 to 4% TF. We further used a sequentially diluted clinical sample (with known TF) with healthy donor cfDNA to a gradient of expected TF values. We evaluated the variant calling and TF calculation algorithm by treating each dilution separately or treating the immediately nearby samples as longitudinal samples. We further evaluated our longitudinal-aware analysis method on 20 clinical subjects tested with PredicineCARE cfDNA assay with ≥2 time points to evaluate the improvement in TF estimation compared to individual estimation.
Results: With the titrated reference materials and manufacturer-labelled somatic variants, we achieved an LoD of 0.01% TF for 30ng cfDNA input and a sequencing depth at 20,000x using PredicineCARE cfDNA assay. When approaching the LoD, the number of reads for the somatic variants becomes more stochastic, which resulted in big variances in the calculated TF values. When independently estimating the TF without referring to matched normal or longitudinal samples, the LoD was 0.5% TF. When the longitudinal sample information was included, the LoD went down to 0.01% TF, contingent on the number of trackable variants. Our simulated results showed that when there are 4 or more tracking variants between longitudinal samples, the LoD can go down to 0.01% TF most of the time. We further applied this longitudinal sample aware dynamic TF estimation method to 20 clinical subjects tested with PredicineCARE cfDNA assay and with ≥2 time points, most showed improved TF estimation with ≥50% of the subjects having questionable somatic/germline calls if called separately.
Conclusions: We developed a dynamic TF estimation methodology that leverages longitudinal samples to improve somatic/germline variant calling and exclude CHIP mutations. Our results demonstrated that with longitudinal samples, the accuracy in TF estimation can approach the ideal situation where all somatic/germline status of the variants are known. Notably, longer interval between time points and a higher number of tracking somatic mutations (≥2) in longitudinal samples generally enhance the effectiveness of this dynamic estimation methodology.

#5896 Tissue naive comprehensive methylation profiling enhances the identification of ctDNA in lung cancer.

Yu Sun, Nafisa Bulsara, Qin Tang, Becky Wang, Neil Peterman, Varahenage Namali Perera, Brian Giacopelli, James Yu, Jason Close, Peter George, Andy Eang, Ashley Linares, Alex Robertson, Nicole Lambert, Yexun Wang

Foundation Medicine, Inc., San Diego, CA

Liquid biopsy of circulating tumor DNA (ctDNA) in plasma has shown to have great clinical value by offering real-time insights into disease progression and response to treatment. Mutation focused cell-free DNA (cfDNA) analysis is limited by low ctDNA levels and is restricted by mutation frequency. DNA methylation is a key epigenetic mechanism for regulating gene expression, gene silencing, and genomic imprinting. In the process of tumorigenesis, aberrant methylation patterns, such as CpG island hypermethylation in promoters of tumor suppressor genes play crucial roles. Tracking the methylation patterns in differential DNA methylation regions (DMRs) in cfDNA can be used to monitor biological changes such as tumor progression or treatment response. Here we developed a novel, multi-omics platform that leverages both genetic and epigenetic information from plasma cfDNA. This platform allows simultaneous extraction of genetic information (mutations, sequence variants) and epigenetic modifications (DNA methylation patterns) at the single-DNA-molecule level. To explore the methylation profiling potential of this platform, we conducted a study using a targeted high-depth sequencing approach on procured plasma cfDNA samples from individuals with lung cancer (n=34) and healthy controls (n=78). Along with the data generation, a robust bioinformatics pipeline was designed and implemented for the analysis, including raw sequence alignment, quality control (QC), methylation status assignment, and cancer associated methylation assessments. The platform demonstrated high sensitivity and specificity in detecting cancer-derived cfDNA in this independent sample set. Overall, approximately 29000 of the DMRs (representing >90% of the designed panel), exhibited aberrant methylation in patient samples. For each individual sample, an integrated methylation score was calculated to quantify the cancerous and non-cancerous cfDNA sources based on methylation profiles. This methylation score effectively stratified samples by both cancer presence and disease stage. The overall detection rate (sensitivity) for this sample set was 0.88 at a specificity of 0.91, with sensitivity of 0.66 in stage I (n=5) and 0.92 in stage II/III (n=29). Notably, this assay maintained high sensitivity for (4/6) samples with low ctDNA levels or minimal mutation burden, as evidenced by a circulating tumor fraction (cTF) below 1e-5, identified by variant allele frequency (VAF).In summary, this assay represents a powerful tool for sensitive and specific ctDNA detection.

#5897 Noninvasive tumor genetic profiling and disease surveillance in mantle cell lymphoma via plasma circulating tumor DNA.

Z. Ouyang[1], R. Zeng[1], S. Wang[2], X. Wu[2], Y. Li[1], Y. He[1], C. Wang[1], C. Xia[1], Q. Ou[2], W. Yang[2], **H. Tang**[3], L. Xiao[4], H. Zhou[1];
[1]The Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University, Hunan Cancer Hospital, Changsha, China, [2]Nanjing Geneseeq Technology Inc., Nanjing, China, [3]Geneseeq Research Institute, Nanjing Geneseeq Technology Inc., Jiangsu, China, [4]School of Basic Medical Science, Central South University, Changsha, China

Background: Mantle cell lymphoma (MCL) is an aggressive subtype of B-cell non-Hodgkin's lymphoma. While plasma circulating tumor DNA (ctDNA) has demonstrated diagnostic and prognostic value in diffuse large B-cell lymphoma and Hodgkin lymphoma, its significance in MCL remains unclear.
Methods: This study analyzed paired treatment-naïve plasma and tissue samples from 34 MCL patients with response data after first-line chemotherapy, targeting 475 genes associated with hematopoietic and lymphoid neoplasms. We assessed the ability of plasma ctDNA to detect tumor-specific genetic alterations and explored its potential as a non-invasive biomarker for predicting treatment response and prognosis.
Results: The most frequently mutated genes in baseline tissues were *CCND1* (93.5%), *ATM* (48.4%), *KMT2D* (25.8%), and *TP53* (25.8%). Subgroup analysis showed that genetic alterations in *CDKN2A* (*P*=0.028), along with those in the B cell antigen receptor (BCR) and endosomal Toll-like receptor (TCR) signaling (*P*=0.004), as well as the PI3K pathway (*P*=0.008), were significantly enriched in the blastoid type of MCL. *ATM* mutations (*P*=0.041) were more prevalent in patients with low scores on the Mantle Cell Lymphoma International Prognostic Index (MIPI), while epigenetic chromatin remodeling pathway alterations (*P*=0.028) were more common in MIPI-high patients. Plasma ctDNA detected 66.3% (118/178) of tissue-based somatic variants, yielding an overall detection sensitivity of 73.3%. The highest sensitivity was observed for structural variants (96.6%), followed by mutations/indels (71.3%) and copy number variants (30.0%). At the patient level, 75% (21/28) of patients with paired tissue and plasma samples exhibited moderate to almost perfect agreement in detecting genomic variants in panel-targetable genes, achieving a kappa coefficient ≥ 0.6. Pretreatment ctDNA levels were highly specific for predicting treatment response (100%), although the sensitivity was suboptimal at 68.2%. Higher ctDNA levels were independently associated with shorter progression-free survival (PFS; *P*=0.002) and overall survival (OS; *P*=0.009). Additional genetic features associated with shorter PFS included mutations in *TP53* (*P*=0.002), *SMARCA4* (*P*=0.023), and *TRAF2* (*P*=0.023). Furthermore, mutations in *TP53* (*P*=0.006) and *TERT* (*P*=0.031) in baseline plasma ctDNA were predictive of reduced OS.
Conclusions: Plasma ctDNA serves as a noninvasive approach for detecting tumor-derived genetic aberrations associated with treatment response and survival outcomes in MCL. These findings underscore its clinical utility for diagnosis, recurrence prediction, and surveillance monitoring in MCL.

#5898 Investigation of EGFR mutations in lung cancer patients through liquid biopsy analysis.

Pin-fei Wei[1], Shang-Gin Wu[2]

[1]Department of Laboratory Medicine, National Taiwan University Hospital, Taipei City, Taiwan,[2]Department of Internal Medicine, National Taiwan University Cancer Center, Taipei City, Taiwan

Objective: Lung cancer is the leading cause of cancer-related mortality in Taiwan as of 2023. Early diagnosis and continuous monitoring are critical for improving patient prognosis. Studies indicate that approximately 50% of lung cancer patients in Asia have EGFR gene mutations. Among patients receiving first- and second-generation targeted therapies, over 50% develop resistance due to T790M gene mutations. The gold standard for lung cancer diagnosis involves pathological tissue examination, including cytological analysis, immunohistochemistry, and genetic testing. Recently, blood-based liquid biopsy for EGFR qPCR gene mutation analysis has emerged as a novel method due to its rapid turnaround time and reduced invasiveness. This study aims to compare EGFR gene qPCR mutation detection via liquid biopsy with tissue biopsy results and assess the clinical utility of liquid biopsy for EGFR qPCR testing.
Methods: From March 2018 to April 2020, we collected 416 peripheral blood samples via venipuncture for EGFR liquid biopsy using the Cobas EGFR Mutation Test v2. Simultaneously, corresponding tissue biopsy samples were obtained for EGFR genetic analysis using Sanger or MASS sequencing. We compared results from both testing methods and statistically evaluated sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and concordance rate.
Results: Among the 416 samples, 280 were from females and 136 from males, aged 32 to 92 years (average age 65). The EGFR mutation test results included: negative (221 cases), deletion in exon 19 (Del 19) (65 cases), L858R (65 cases), Del 19+T790M (34 cases), Del 19+L858R (21 cases), T790M (2 cases), and other mutations (8 cases). Of the 416 cases, 230 lacked available pathological tissue reports for comparison. In the 186 cases with pathology reports, we noted various timing discrepancies between liquid and tissue biopsies. Among 94 patients with both liquid biopsy and tissue biopsy results, the overall concordance rate was 77.7%. Using tissue biopsy as the gold standard, sensitivity, specificity, PPV, NPV, and concordance rates for detecting Del 19, L858R, and T790M mutations were as follows: Del 19 (0.83/1/1/0.86/0.92), L858R (0.67/1/1/0.87/0.89), T790M (0.38/0.93/0.64/0.81/0.79).
Conclusion: EGFR liquid biopsy qPCR can be effectively utilized at lung cancer diagnosis and for monitoring drug resistance during targeted therapy. Compared to tissue biopsy, liquid biopsy qPCR using peripheral blood is a less invasive procedure with improved efficacy and an overall concordance rate deemed clinically acceptable.

#5899 Comparative analysis of transcription factor activities derived from low-pass whole genome sequencing of cell-free DNA and from ATAC-sequencing of tumors.

Ryuji Tamaki[1], Koji Sagane[1], Shuyu Dan Li[2], Taisuke Hoshi[1]

[1]Eisai Co., Ltd., Tokyo, Japan,[2]Eisai Inc., Nutley, NJ

Background: cell-free DNA (cfDNA) in plasma primarily originates from hematopoietic cells in healthy individuals, but in cancer patients, it includes circulating tumor DNA from dead tumor cells. cfDNA mutation analysis is already used in clinical practice for biomarker research and treatment decisions. Recent studies have shown that cfDNA fragmentation patterns reflect tumor tissue chromatin status. Inferring transcription factor (TF) activity from cfDNA TF binding site (TFBS) coverage patterns offer a minimally invasive assay to understand cancer biology with limited sequencing depth. However, the accuracy of TF activity inference has only been examined for a few well-known TFs such as AR and ESR1. We conducted a comparative analysis of 377 TF activities between cfDNA whole genome sequence (WGS) and tumor Assay for Transposase-Accessible Chromatin using sequencing (ATAC-sequencing).
Methods: Two liver cancer cell lines, HepG2 with a gain-of-function CTNNB1 mutation and HuH7 with wild-type CTNNB1, were used to generate xenograft models. ATAC-sequencing/RNA-sequencing and WGS were performed on the tumors and pooled plasma-derived cfDNA from each model. TCF/LEF family TF activities, downstream targets of CTNNB1, were compared between models with different CTNNB1 mutation statuses in both tumor ATAC-sequencing and cfDNA WGS. For 377 TFs with at least 10,000 TFBS, the correlation of TF activities between tumor ATAC-sequencing and cfDNA WGS was examined in each model. Furthermore, tumor model-specific TFs were identified based on ATAC-sequencing, followed by comparison of cfDNA TFBS coverage of these TFs between the two models.
Results: In pilot analysis with TCF/LEF family TFs, both tumor ATAC- sequencing and cfDNA WGS from HepG2 xenograft model showed higher TCF7 and TCF7L2 activities compared to HuH7. In an expanded analysis of 377 TFs, we found a significant and strong correlation between tumor and cfDNA TF activities (Spearman's rank correlation coefficients for HepG2 and HuH7: -0.90 and -0.86, respectively). For tumor model-specific TFs, "HepG2-HIGH" and "HuH7-HIGH" which are composed of 29 and 18 TFs with the highest variance between models, cfDNA TFBS coverage of these two groups of TFs also showed significant differences between tumors ($p<0.05$).
Conclusion: Our results indicate that cfDNA can accurately estimate the activity of over 300 TFs in tumor. To assess the potential utility of cfDNA TFBS analysis in clinical samples, further studies are warranted.

#5900 Tumor-independent cfDNA methylation analysis for cancer monitoring during total neoadjuvant treatment in rectal cancer patients.

Kim Nguyen-Ta[1], Grace Zhao[2], Heng Wang[2], Teressa Celma[1], Lisa Kim[1], Jeff Pawelek[1], Bram Herman[3], Annie Wu[4], Yun Bao[2], Gregory Botta[1], Aaron Miller[1], Hitendra Patel[1], Shengrong Lin[2], Peter Vu[1]

[1]UC San Diego Moores Cancer Center, La Jolla, CA,[2]Agilent Technologies Inc., Santa Clara, CA,[3]Agilent Technologies Inc., Harwell, United Kingdom,[4]Rensselaer Polytechnic Institute, Troy, NY

Introduction: Total neoadjuvant therapy (TNT) is the standard curative-intent treatment for locally advanced rectal cancer (LARC), where patients receive all of their chemotherapy and radiation therapy before surgery. However, monitoring treatment response during TNT can be challenging since radiographic changes are often delayed. Methylation changes in colorectal cancer (CRC) and other cancers are relatively consistent and can be monitored in real time using cell-free DNA (cfDNA), making them a promising strategy for cancer detection and treatment monitoring. This study aims to determine if methylation changes in cfDNA can be used to monitor treatment response in LARC undergoing TNT.
Methods: Plasma of CRC patients stage I-IV and healthy controls were obtained to establish the methylation classifier. Patients with stage II or III rectal cancer undergoing TNT were recruited. Blood samples were collected at baseline and about 6 weeks after the start of treatment. Tumor and normal CRC genomic DNA were analyzed using the Agilent Avida Methyl 3400 DMR panel to generate a methylation index score. Cancer treatment response was assessed by the investigator based on computed tomography and/or magnetic resonance imaging.
Results: We confirmed low background methylation signals in cfDNA and peripheral blood mononuclear cells of healthy controls. Subsequently, we analyzed methylation signals from plasma of CRC patients with stage I (n=20), II (n=12), and IV (n=5) disease. The sensitivity of the assay was 60%, 75%, and 100% for stages I, II, and IV, respectively, while the specificity was 87.5%. Among LARC patients undergoing treatment with TNT, we observed a decrease in methylation index scores compared to baseline in all three patients who experienced a treatment response and an increase in all three patients who experienced disease progression. Notably, the methylation index scores did not correlate with carcinoembryonic antigen values.
Conclusions: Our preliminary results show the feasibility of monitoring rectal cancer treatment response during TNT using a tumor-independent cfDNA methylation analysis. Methylation analysis is a promising technology that may aid in quantifying treatment response to systemic therapy and/or radiation.

#5901 Circulating tumor DNA in non-viral head and neck squamous cell carcinoma: A systematic review and meta-analysis.

V. Helou[1], N.-H. Abdul-Rahman[2], S. Loo[2], S.-J. Gao[2], J. P. Zevallos[1], D. Zandberg[3], M. E. Spector[1], H. D. Skinner[1], R. L. Ferris[4], K. J. Contrera[1];
[1]University of Pittsburgh Medical Center, Pittsburgh, PA, [2]University of Pittsburgh, Pittsburgh, PA, [3]UPMC Hillman Cancer Center, University of Pittsburgh Medical Center, Pittsburgh, PA, [4]University of North Carolina, Chapel Hill, NC

Purpose: Circulating tumor DNA (ctDNA) is a promising non-invasive biomarker offering insights into tumor biology, treatment response, and disease monitoring, particularly for less accessible tumors. Although ctDNA in viral-mediated head and neck squamous cell carcinoma (HNSCC) is widely studied, research into the role ctDNA for non-viral HNSCC is limited. This systematic review and meta-analysis consolidates evidence on ctDNA in non-viral HNSCC and examines its implications for diagnosis, prognosis, and management.
Experimental Design: A comprehensive literature search was conducted in September 2024 across Medline, PubMed, Embase, and the Cochrane Library. Following PRISMA guidelines, two reviewers independently screened titles, abstracts, and full texts. Clinical outcomes were extracted in duplicate into a standardized pilot-tested form. Pooled hazard ratios (HR) were calculated using a fixed-effect model.
Results: Out of 1915 screened studies, 47 studies involving 3,574 patients were included. The median publication year was 2022, with 64% of studies being prospective. The most common ctDNA assays were next-generation sequencing (n=28, 60%) and PCR-based techniques (n=21, 45%). Median assay sensitivity was 80% (range 38-100%), and specificity was 99% (range 60-100%), with twelve studies reporting specificity exceeding 91%. Pre-treatment ctDNA detection
rates ranged from 50% to 100%, with a median of 83%, while post-treatment rates ranged from 28% to 100%, with a median of 48%. ctDNA identified recurrence or progression in 80% of patients (range 40-100%) prior to clinical diagnosis, with five studies reporting 100% detection of recurrence or progression. The median lead time between ctDNA detection and clinically or radiologically confirmed disease progression was 4.6 months (range 1-27 months). On survival analyses, ctDNA detection, SEPT9 methylated ctDNA, and mutated ctDNA were associated with worse overall survival, with pooled HRs of 10.26 (95% CI: 3.58-29.40; *P < .0001*), 1.15 (95% CI: 1.08-1.23; *P < .0001*), and 1.96 (95% CI: 1.28-3.00; *P = 0.002*), respectively. ctDNA correlated with worse progression-free survival (HR 7.32, 95% CI: 4.17-12.86; *P* < .00001) and recurrence-free survival (HR 7.33, 95% CI: 2.75-19.58; *P < .0001*).
Conclusions: ctDNA shows promise for enhancing diagnostic accuracy, monitoring progression and recurrence in non-viral HNSCC. While additional research is necessary, our findings could inform the development of evidence-based clinical guidelines for the personalized management of non-viral HNSCC.

#5902 Quantification of the effect of early-stage cancer on cell-free DNA levels in plasma.

K. Mamis, I. Bozic;
University of Washington, Seattle, WA

Elevated cell-free DNA (cfDNA) levels are often observed in cancer patients. However, the exact effect of cancer on cfDNA concentration is not fully understood. In order to obtain insight into cfDNA dynamics in the presence of early-stage cancer, we analyze a rich, previously published dataset of cfDNA in healthy individuals and patients with stage I-III lung, breast, colorectal, pancreatic, ovarian, esophageal, stomach and liver cancer. We find that, despite the high variance of cfDNA levels among both healthy individuals and cancer patients, early-stage cancer exhibits a systemic effect resulting in a multiplicative, cancer-type specific increase in cfDNA concentration originating from healthy tissue. For each cancer type, we also observe similar multiplicative increases in the concentration of mutant DNA fragments detected in plasma that do not originate from tumor, providing additional validation that increased cfDNA levels in early-stage cancer originate from healthy tissue and not from the tumor. The magnitude of the multiplicative increase in cfDNA differs greatly between the cancer types considered, ranging from a ~1.3-fold increase in lung cancer, to a ~12-fold increase in patients with liver cancer. To determine the mechanism responsible for the increase in cfDNA levels, we employ an additional dataset that reports the cfDNA composition regarding its tissue of origin, in healthy individuals and patients with pancreatic and ovarian cancer. We find that elevated cfDNA levels in cancer patients do not originate from a proportional increase in cfDNA shedding, but from impaired clearance of cfDNA. The impaired cfDNA clearance could stem either from a direct decrease in cfDNA clearance rate in the presence of cancer, or can be due to a saturation effect in the cfDNA clearance mechanism, triggered by smaller increases in cfDNA shedding due to cancer. Our results contribute to the development of quantitative understanding of cfDNA dynamics in the presence of cancer.

#5903 Real world assessment of quality control metrics impacting performance of a commercially available liquid biopsy NGS panel.

Emma Longshore, Nylev Vargas, Colin Cochran, Amanda Weaver, Brittany D'Alessio, Leisa Jackson, Gary A. Pestano

Biodesix, Inc., Louisville, CO

Introduction: NGS panels allow rapid detection of rare variants through detection of targeted nucleic acid (NA) sequences. This technology is capable of sensitive and specific detection of many variants simultaneously in a single specimen. However, assay performance may be impacted by the quality and amount of input of the template. Liquid biopsy assays are then vulnerable to compromised NAs dependent on pre-analytic conditions and the tumor biology. This report reviewed real world data to determine the performance impact of nucleic acid quality and quantity on the detection of the four major variant classes in liquid specimens.

Materials and Methods: Varying input amounts of total nucleic acids (TNA) were examined to determine influence of quantity on passing pre-established Quality Control (QC) for a commercially available, targeted amplicon based NGS assay. Retrospective analysis of approximately 12 months of QC measurements was performed. Reaction input quality control data from 185 batches (2k+ specimens) was collected to analyze frequency of qBittorent failure. Input of TNA was measured by fluorescence, quality by qPCR, and pass/fail in the NGS pipeline by multiple QC metrics. A supervised, linear discriminant analysis (LDA) was conducted to identify significant combinations of primary features that best separate the classes of the five primary quality control metrics and their relation to a final PASS/FAIL QC call.

Results: QC metrics for raw sequencing reads were analyzed using a set of five primary metrics, including minimum mean read length (MRL ≥ 65bp = PASS) and Q-Score [Q = -10log 10 (e)]. Higher Q Scores indicate a smaller probability of error. Q-Score returned a strong negative coefficient correlation in the LDA, indicating as the independent variable, Q-Score, increases, prevalence of the dependent variable, “FAIL” call, decreases. Additionally, SNV/Indels, CNA, and fusion/exon skipping, were called Pass/Fail based on variant detection in the bioinformatics pipeline. The coefficients of linear discriminants identified that a final QC call is most positively influenced by quality control measures Mean Read Length (MRL; > 65) and Reaction Input (> 13ng).

Conclusions: We observed least impact of total nucleic acid input on QC calls for SNV, Indels and Fusions. Modest performance improvements for copy number amplifications (CNA) were observed at the higher input. Overall, there was not a significant impact to any primary or secondary QC metric comparatively in the low input quantity groups. Additional data are being reviewed to trend performance over time.

#5905 A non-invasive, methylation-based NGS assay for sensitive detection and monitoring of bladder cancer in urine.

Feng Xie, Hang Dong, Haoran Tang, Yue Zhang

Huidu Shanghai Medical Sciences, Ltd., Fengxian District, Shanghai, China

Background: Bladder cancer, the sixth most common cancer worldwide, has a high recurrence rate, necessitating frequent and reliable disease monitoring. Current methods, such as cystoscopy, are invasive and can hinder patient compliance. However, detecting therapy response and disease progression on a molecular level still remains challenging. Urinary tumor DNA (utDNA) analysis offers a promising, non-invasive, and highly sensitive diagnostic tool for bladder cancer diagnosis and monitoring. By detecting utDNA methylation profiles, this analysis could enable personalized treatment decisions and improve clinical outcomes.
Methods: We developed PredicineALERT, a methylation-based next-generation sequencing (NGS) assay designed for bladder cancer monitoring using urine. Methylation markers were identified through comparative analysis of profiles from bladder cancer patients and healthy donors. These markers were used to train a model capable of distinguishing bladder cancer from non-cancer samples. The assay's sensitivity and specificity were validated using serially diluted methylated DNA controls to determine its detection limit. To assess the performance of the PredicineALERT assay in detecting tumor fractions, serial urine samples were collected from muscle-invasive bladder cancer (MIBC) patients undergoing neoadjuvant therapies and analyzed using both PredicineALERT and a tumor-informed minimal residual disease (MRD) assay.
Results: A panel of methylation markers was identified from urine samples, enabling the model to accurately differentiate bladder cancer patients from healthy individuals. PredicineALERT demonstrated the ability to detect tumor signals at levels as low as 0.01%, underscoring its potential for minimal residual disease detection and early recurrence monitoring. Comparison with the tumor-informed MRD assay revealed excellent concordance in tumor fraction detection, further highlighting the assay's robustness and reliability.
Conclusion: PredicineALERT is a non-invasive and highly sensitive assay for bladder cancer monitoring. Its design facilitates early recurrence detection while enhancing patient compliance, presenting a promising advancement in bladder cancer management.

CLINICAL RESEARCH: Predictive Biomarkers 6 / Diagnostic Biomarkers 3
Poster Session

#5909 MRD as a predictive biomarker in patients with small cell lung cancer.

J. Chang[1], Q. Zhang[1], D. Dai[2], B. Chen[2], M. Zhang[2], Z. Cai[1], L. Guo[1], S. Huang[1], S. Lyu[1];
[1]National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital & Shenzhen Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Shenzhen, China, [2]BGI Genomics, Shenzhen, China

Background: Despite the high response rate to initial treatment, the clinical outcomes of small cell lung cancer (SCLC) remain challenging. Tumor-informed minimal residual disease (MRD) assessments, leveraging circulating tumor DNA (ctDNA) detection, offer a promising approach for early detection and personalized treatment guidance in SCLC. However, their full potential is yet to be demonstrated.
Objective: This study aims to investigate the predictive value of a plasma-based personalized MRD monitoring assay in SCLC patients.
Methods: We conducted a prospective study, collected clinical data and performed high-throughput whole-exome sequencing (WES) on baseline tumor tissue from SCLC patients. Personalized panels were constructed based on WES data to dynamically monitor MRD through ctDNA extracted from plasma. To evaluate the correlation between MRD dynamics and treatment outcomes, peripheral blood samples were collected at baseline, every 6 weeks ±7 days during treatment, and at disease progression.
Results: Seven SCLC patients with consent were recruited for the study, including those with limited stage (n=2) and with extensive stage (n=5). One patient was diagnosed with combined SCLC. Five patients received platinum-based combination regimens, and the other two received monotherapy irinotecan and anlotinib hydrochloride, respectively. The median age was 60 years (range, 68 to 74 years), and all patients had a smoking history. The MRD positive rate at baseline was 100%. *TP53* and *RB1* genes were the most frequently mutated genes, at 100% and 85.7%, respectively. The MRD elevated up to 48 days prior to disease progression. Patients in the MRD increase group (n=5) experienced progressive disease (PD) finally and the other in the MRD decrease group (n=2) maintained stable disease (SD) or partial response (PR), with one achieving durable MRD clearance after three cycles of sequential chemoradiotherapy. At the end of follow-up, the PFS analysis between the MRD increase and decrease cohorts revealed a clear trend of separation, despite the lack of statistical significance (p=0.06, 95% CI: 1.09-42.81). Moreover, MRD dynamics were more pronounced than the traditional cancer markers, neuron-specific enolase (NSE) and progastrin-releasing peptide (ProGRP), highlighting its high sensitivity and clinical applicability. WES analysis revealed a diverse landscape of genetic alterations, with prevalent missense mutations in genes such as TTN, MUC2, and ZNF676, alongside coding-synonymous variations.
Conclusion: Our study indicates that MRD could predict clinical outcomes and surpass traditional biomarkers, presenting its potential as a biomarker for the management of SCLC patients. Personalized MRD monitoring may provide valuable insights into treatment response and disease progression in SCLC. Further validation with a larger sample size is ongoing.

#5910 Characterizing spatial expression patterns and prevalence of folate receptor alpha in relation to other existing and emerging ovarian cancer biomarkers.

Marian Themeles, Kevin Kolahi, Amita Mistry, Erik Huntzicker, Peter Ansell, Emily Deutschman

AbbVie Inc., Waltham, MA

The ovarian cancer treatment landscape has seen an emergence of biomarker-targeted therapies following accelerated approval of the folate receptor alpha (FRα)-targeting antibody-drug conjugate (ADC) mirvetuximab soravtansine-gynx (MIRV, Elahere) in November 2022 (with full US approval granted in March 2024). Many therapies in development are biomarker-directed ADCs with distinct cytotoxic payloads, which is the major effector component of the ADC. Data suggest mechanisms of resistance may be largely payload specific, meaning patients may only be able to receive a limited number of these therapies; thus making it paramount to identify the therapy from which each individual patient will derive maximal clinical benefit. Eligibility for MIRV is currently evaluated by FRα expression as assessed by the immunohistochemical VENTANA FOLR1 (FOLR1-2.1) RxDx Assay (FOLR1 CDx). FRα is expressed in roughly 95% of high-grade serous ovarian cancer, with around 36% prevalence observed at the cutoff for MIRV eligibility (PS2 ≥ 75%, or ≥75% of viable tumor cells with ≥2+ staining). The goal of this study was to characterize the spatial overlap of FRα expression and prevalence with biomarkers that are currently being or could be leveraged for targeted therapeutics in the ovarian space. FRα expression was evaluated in commercially procured, formalin-fixed, paraffin-embedded (FFPE) primary ovarian tumor specimens using the FOLR1 CDx. HER2, Muc16, Mesothelin, TROP2, CLDN6, B7-H4, CDH6, Tissue factor and Napi2B were also evaluated by immunohistochemistry from serial sections taken from the same FFPE blocks. Intensity staining for each biomarker was correlated to FRα intensity staining to identify overlapping eligible patient populations. In addition to intensity overlap, spatial expression patterns of each biomarker relative to FRα were also evaluated to understand sequencing and combinability options. These data shed light on biomarker co-expression in ovarian cancer to best match patients to the ideal targeted therapy and to best tailor optimal combination therapies in ovarian cancer patients.

#5911 The role of CD53+ subsets and the JAK/STAT3 pathway in regulating cancer stemness and immune escape in esophageal squamous cell carcinoma.

B. Liu[1], H. Zhou[2], L. Tan[2], B. Yao[2], S. Zhang[2], J. Huang[2], M. Yang[1], X. Guan[2];

[1]City University of Hong Kong, Hong Kong, China, [2]The University of Hong Kong, Hong Kong, China

T cell therapy has revolutionized treatment for hematologic malignancies but faces significant challenges in solid tumor applications, including esophageal squamous cell carcinoma (ESCC). In this study, we utilized a dynamic subcutaneous tumor growth model in mice to investigate how ESCC tumors modulate T cell activity through four distinct phases: initial enlargement, reduction, stabilization, and explosive growth. Single-cell sequencing revealed that CD53+ subsets were enriched in the reduction stage and showed their function in stemness regulation and immune escape. Functionally, CD53+ mouse ESCC cell lines showed a higher organoid formation ability when compared with CD53- cells. When co-cultured with T cells, CD53+ ESCC cell lines revealed their capability in regulating T cell exhaustion. Mechanically, GO enrichment analysis and western blotting demonstrated that CD53+ cells activated IL-6/JAK/STAT3 signaling pathways and upregulated their downstream genes such as C-myc and PD-L1. Furthermore, in vivo study showed that JAK inhibitor Ruxolitinib and STAT3 inhibitor C188-9 could efficiently reduce tumor growth and enhance T cell infiltration in CD53+ mouse ESCC cell lines, suggesting a role in tumor proliferation and T cell editing. This study aims to elucidate the biological function of CD53+ subset ESCC cells in tumor progression and verify their role in T cell regulation, offering new therapeutic targets for ESCC treatment.

#5913 Longitudinal blood-based immuno-monitoring reveals distinct immune profiles in colorectal cancer patients with divergent responses to first-line systemic therapy.

Jia Meng[1], **Yong Xiang Gwee**[2], Kai Soon Ng[1], Wei Peng Yong[2], Gloria Hui Jia Chan[2], Hon Lyn Tan[2], Joan Choo[2], Robert John Walsh[2], Jingshan Ho[2], Joe Yeong[1], Cheng Ean Chee[2]

[1]Institute of Molecular and Cell Biology (IMCB), A*STAR, Singapore, Singapore,[2]Department of Haematology-Oncology, National University Cancer Institute, Singapore, Singapore, Singapore

Introduction: Peripheral blood mononuclear cells (PBMCs) are key mediators of immune surveillance and response against malignancies. Profiling PBMC subsets provides insights into immune dynamics in cancer progression and therapeutic response, reflecting systemic immune changes that may mirror the tumor microenvironment. This study aimed to identify differences in PBMC profiles between colorectal cancer (CRC) patients deriving clinical benefit from first-line systemic therapy and those with poor outcomes.

Methods: We recruited patients with locally advanced, unresectable or metastatic CRC initiating first-line systemic therapy. PBMCs were isolated from venous blood samples at two time points: baseline prior to treatment initiation (L1) and before cycle two of treatment (L2). Flow cytometry was used to assess dynamic changes in immune cell subsets for each patient at L1 and L2. Patients were grouped into Cohort A (clinical benefit: partial response or stable disease ≥6 months) and Cohort B (no clinical benefit: stable disease lasting less than 6 months or progressive disease). The GLIMMIX model was used to compare the differences in cell subset proportions between the two cohorts.

Results: Between November 2023 and April 2024, seven patients (median age 64 years, with 71.4% Chinese ethnicity and 57.1% male) were recruited, with four in Cohort A and three in Cohort B. Median progression-free survival was significantly longer in Cohort A (7.0 versus 1.0 months, p=0.029).

Immune profiling revealed distinct differences between cohorts. $CD4^+$ T cell subset patterns were suggestive of immune activity potentially associated with better outcomes in Cohort A compared to Cohort B patients; at L1, Cohort A showed increased $CD4^+$ effector memory T cells (p=0.03) while Cohort B displayed a higher proportion of naïve $CD4^+$ T cells (p=0.015). At L2, $CD4^+$ T cell activation, as indicated by Ki67 expression, trended higher in Cohort A (p=0.09), and was accompanied by a lower Treg population (p=0.022).

Additionally, patterns of B cell subsets of Cohort B at L2 were consistent with an exhausted immune environment which correlated with poorer prognosis, with Cohort B exhibiting elevated expression of CD38 (p=0.007) and PD-1 (p=0.05) compared to Cohort A. These immune profiles may reflect potential mechanisms underlying early progression and treatment resistance, and warrant further investigation as biomarkers for treatment response in CRC.

Conclusions: Dynamic PBMC profiles, particularly $CD4^+$ T cell and B cell subset patterns, show promise as predictive biomarkers for treatment response in advanced CRC. These preliminary findings warrant validation in larger cohorts and highlight the potential of immune profiling to identify patients at risk for early progression, guide therapeutic strategies and improve patient outcomes.

#5914 Development of immunohistochemistry detection kits for claudin family: CLDN6, CLDN9, and CLDN18.2.

Q. Wang, X. Wang, J. Tao, T. Xiao, X. Wang, M. Li, X. Lv, W. Zhao, J. Geng, Y. Liu, Z. Yu, **Y. Li**;
RemeGen Co., Ltd., Yantai, China

Background: Claudins are a family of integral membrane proteins that are critical for the formation of tight junctions in epithelial and endothelial cells. Aberrant expression of Claudin family members is implicated in the progression of various cancers, making them valuable biomarkers for tumor diagnosis, prognosis, and therapeutic targeting. such as CLDN-6, CLDN-9, and the splice variant CLDN-18.2. An antibody drug targeting CLDN18.2 was recently approved by the FDA for the treatment of advanced gastric cancer. Despite their clinical potential, routine assessment of these proteins in cancer diagnostics is limited because of the lack of highly specific detection tools. we developed novel immunohistochemistry (IHC) detection kits for CLDN-6, CLDN-9, and CLDN-18.2 to facilitate their use as diagnostic and therapeutic biomarkers.
Methods: We used AI-designed peptides from the CLDN-6, CLDN-9, and CLDN-18.2 proteins conjugated with keyhole limpet hemocyanin (KLH) as immunogens to immunize the BABL/c mice. The initial IHC antibody candidates were identified by ELISA and further optimized using various structurally facilitated AI approaches. The selected antibodies were evaluated by IHC in formalin-fixed paraffin-embedded (FFPE) CHOK1 cells expressing full-length CLDN6, 9, 18.2 or CLDN 3, 4, 18.1. These IHC antibodies were also subjected to ELISA, Western blotting, and tumor tissue microarray IHC staining to determine the features of IVD products. Furthermore, we conducted intra-day, inter-day, and inter-operator studies to assess the consistency of IHC staining under the conditions of our established system.
Results: The final IHC antibody clones obtained for CLDN6, CLDN9, and CLDN18.2 were #7, #10, and #9B3, respectively. ELISA tests showed that they all had good binding affinity. Tumor tissue microarray staining showed that these antibodies can specifically stain the target protein in paraffin samples from testicular, endometrial, gastric cancer, and other tumor tissues. The WB assay and IHC staining results confirmed their binding specificity. We established a preliminary reaction system for the IHC assay with final working concentrations of 0.25 μg/ml, 0.25 μg/ml, and 20 μg/ml for #7, #10, and #9B3, respectively. The three IHC assays showed good intra-day, inter-day, and inter-operator reproducibility under the current reaction system, demonstrating good robustness as IHC diagnostic products.
Conclusions: We successfully developed IHC antibodies against CLDN6, CLDN9, and CLDN18.2. These antibodies were incorporated into IHC diagnostic kits for clinical and research applications. The IHC kits are sensitive and robust for specific detection in FFPE tissues. They hold promise in enhancing cancer diagnosis, identifying patients for targeted therapies, and improving clinical stratification among patients with Claudin family disorders.

#5915 Real world clinical characteristics of claudin 18.2 expression in patients with pancreatic adenocarcinoma.

Allison Kerper[1], Robert H. Getzenberg[2], Rondell P. Graham[1]

[1]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN,[2]Astellas Pharma Inc, Northbrook, IL

Background: Pancreatic cancer is a frequently fatal disease that is often diagnosed at a late stage associated with a poor prognosis and an increasing impact on cancer related mortality. Despite advances, chemotherapy remains the mainstem of therapeutic interventions for this disease and most of the currently identified molecular alterations are not currently targetable. Therefore, novel biomarkers and strategies are necessary to impact patient outcomes. Claudin-18.2 (CLDN18.2) is a splice variant of the Claudin 18 family and is a component of tight junctions, which seal the paracellular spaces of epithelial and endothelial cells. CLDN18.2 is frequently retained in malignant gastric tissue and overexpressed in pancreatic and gastroesophageal adenocarcinomas. CLDN18.2 overexpression at the message and protein levels has been observed in pre-clinical studies involving pancreatic cancer cell lines and in tumors from pancreatic cancer patients. The goal of this study was to obtain a deeper understanding of CLDN18.2 positivity and CLDN18.2 expression and the associated clinical prevalence, prognostic implications, patient clinical characteristics and relationship with other biomarkers in pancreatic ductal adenocarcinoma (PDAC) patient samples.
Design: This was a single institution/reference laboratory based retrospective study including as many as 320 patients, in which CLDN18.2 positivity as defined by ≥ 75% of the tumor cells expressing moderate to strong membranous staining was examined. The goals were to evaluate CLDN18.2 in tumor samples by immunohistochemistry for prevalence in pancreatic adenocarcinoma patients. Claudin 18.2 expression was assessed by the Ventana (43-14A) Class I IVD that was purchased from Ventana/Roche Diagnostics. In addition to prevalence, CLDN18.2 was evaluated with respect to the clinicopathological features of patients, patient prognosis, adjusted for clinical covariables, response to treatments and relationship to patient demographics, tumor grade and disease status. Patient information has been obtained from electronic medical records and research database to establish clinical course (survival status, metastatic sites, etc.) along with patient demographics.
Conclusions: CLDN18.2 is a novel potentially actionable biomarker in patients with advanced and metastatic pancreatic adenocarcinoma. This study provides important clinical information regarding CLDN18.2 expression in these patients that is important to consider when evaluating biomarkers in these patients. Funding for the study was provided by Astellas Pharma Global Development, Inc.

#5916 Investigating the antiproliferative effects of green synthesized silver and gold unfunctionalized nanoparticles on cervical cancer cell lines.

Nrateng Tsotetsi, Lesetja Motadi, Mpho Choene

Biochemistry, University of Johannesburg, Johannesburg, South Africa

Background: Across the globe cervical cancer is the 4th most common cancers affecting women; however, the burden of CC is greater in Sub-Saharan Africa as it ranks 2nd common cancer and number one cause of deaths. Although cervical cancer can be successfully treated if diagnosed early, many women are diagnosed in advanced stages, rendering current treatments ineffective. Metal nanoparticles which are commonly used in optic imaging of tumours have shown potential as anti-tumour agents when either used alone or in combination with other chemotherapy drugs. Therefore, this study investigated the anti-proliferative potential of Cannabis Sativa synthesized Ag and Au NPs using Me-180 and HeLa human cell lines.

Methods: Metallic Ag and Au nanoparticles were synthesized biologically using Cannabis *sativa* plant. The cell viability was assessed following treatment with Au and Ag NP of 0-200µg/ml in some cases in combination with Hexaxim (DTaP-IPV-HB-Hib) vaccine using alamar blue assayall subsequent assays in combination with. Following treatment, ATP levels, pyruvate kinase activity, effector caspase 3/7 and mitochondrial toxicity were evaluated, to further investigate induction of apoptosis, annexin V staining, Hoechst staining, and deoxyribonucleic acid (DNA) fragmentation analysis by agarose gel electrophoresis were performed. Through siRNA, P53, MDM2 and RBBP6 genes were silenced, and the amplification of pro-apoptotic and cell cycle genes were assed using qPCR.

Results: The Me-180 IC_{50} values of 24.17 ug/m 28,35ug/ml and 37.31 ug/ml for AgNP, AuNP and C. Sativa respectively. The IC_{50} of HeLa were 35.43, 39.02 and 41.45 ug/ml for AgNP, AuNP and C. Sativa respectively. The increased mitochondrial toxicity was consistent with the decrease in ATP levels, pyruvate kinase activity and the decrease in effector caspase 3/7 in NPs treatments. With C. sativa caspase 3/7 activity and ATP levels slightly increased with decreased mitochondrial toxicity. Hoechst staining and DNA fragmentation analysis showed apoptotic bodies in Me-180. In all silenced C. sativa treatments, the expression of P53 remained unchanged with increase expressions of Bax and Bak-1 in Me-180 cell lines whereas in HeLa all were decreased.

Conclusion: Me-180 cell lines that expresses wild-type P53 was more sensitive to apoptosis induction by C. sativa. The increased Bax/Bax expression suggest intrinsic pathway of apoptosis supported by decreased ATP and mitochondrial activity.

#5917 A novel ROR1 antibody with high specificity and sensitivity for predictive biomarker detection in various tumor types.

Xin Zhang, Qianqian Lian, Qian Qian, Dan Lu, Yanling Liu, **Ying Tong**, Qingyang Gu

WuXi AppTec, Shanghai, China

ROR1, a receptor tyrosine kinase-like orphan receptor, is a key mediator of non-canonical Wnt signaling and plays a critical role in cancer progression. ROR1 is selectively and highly expressed in various solid tumors and hematological malignancies while absent in normal adult tissues, making it a promising target for cancer therapy.
Despite this potential, the lack of effective tools has hindered sensitive detection of ROR1 in patient tumor samples by IHC. To address this, we have developed a recombinant rabbit monoclonal antibody in-house, demonstrating superior sensitivity and specificity across multiple methodologies compared to existing antibodies. Additionally, we have established standardized interpretation criteria and performed an extensive epidemiological survey across ROR1-associated cancer types, reporting the following detection rates: 45% in pancreatic cancer (n=40), 39% in ovarian cancer (n=150), 48% in lung adenocarcinoma (n=140), 15% in endometrial cancer (n=97), 18% in triple-negative breast cancer (n=110), 23% in mesothelioma (n=47), and 10% in esophageal squamous carcinoma (n=70).
These results validate this antibody as a reliable tool for clinical biomarker detection, potentially advancing ROR1-targeted cancer treatment.

#5918 Vascular endothelial growth factor A (*VEGF-A*) gene expression profiling in Northern Thai gastric cancer patients and potential biomarker for anti-VEGF therapy selection.

Amonnat Sukhamwang[1], Hathairat Thananchai[2], Supachai Yodkeeree[1], Pornngarm Dejkriengkraikul[1], **Sirikan Limpakan (Yamada)**[3]

[1]Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand,[2]Department of Microbiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand,[3]Division of Upper Gastrointestinal Surgery and Endoscopy, Surgery, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

Background: Vascular endothelial growth factor (Anti-VEGF) drugs are used to treat advanced-stage gastric cancer to inhibit angiogenesis, which is the process of new blood vessel formation that tumors need to grow. These drugs have improved progression-free survival and overall survival when combined with traditional chemotherapy as the second-line treatment. However, the effectiveness of these drugs can vary among patients. Some patients may have tumors that do not express high levels of *VEGF-A*, making the drugs less effective. Additionally, the high cost of these treatments can be a barrier for many patients. This study aims to profile *VEGF-A* gene expression in the Thai population and investigate the relationship between *VEGF-A* and *IL-8*, a poor prognostic marker for advanced-stage gastric cancer, before and after chemotherapy.

Methods: From 2006 to 2018, 149 patients with advanced-stage gastric cancer were collected from Maharaj Nakorn Chiang Mai Hospital in Thailand. These gastric mucosal biopsy tissues were investigated for *IL-8* and *VEGF-A* gene expression using the RT-qPCR technique with specific primer probes for particular genes.

Results: In pre-chemotherapy conditions, 71.15% of 52 cancer gastric mucosal biopsy tissues had higher levels of *IL-8* gene expression than the adjacent normal tissue. In contrast, only 31.51% of 73 cancer tissues had higher levels of *VEGF-A* gene expression than adjacent normal tissue, low expression in 10, 13.7% of patients, and 40, 54.79% of patients showed down-regulate or no expression of *VEGF-A* in cancer tissue in tumor and adjacent normal tissue in the pre-chemotherapy group. Furthermore, when investigating both *IL-8* and *VEGF-A* expression in the same tissues under pre-chemotherapy conditions, the results indicated that most samples had an independent correlation between *IL-8* and *VEGF-A* levels. Specifically, 27.66% of 47 cancer tissues exhibited higher *IL-8* but lower *VEGF-A* levels than adjacent normal tissues in the same patient controls. However, in the subgroup analysis, three of six, 50% of patients with post-chemotherapy conditions showed downregulation of the *IL-8* gene. While only two of nine, 22.22% of patients demonstrated downregulation from post-chemotherapy, and one of nine, 11.11% of patients demonstrated up-regulation post-chemotherapy. There were 6 out of 9, 66.67% patients had no up or down-regulation. Therefore, *VEGF-A* could be used as a specific biomarker for targeted therapy for advanced gastric cancer, as its expression level did not correlate with *IL-8* levels or prior chemotherapy treatment.

Conclusion: The authors found that 23 out of 73, 31.51% of patients had *VEGF-A* expression in pre-chemotherapy conditions. The authors recommend that *VEGF-A* gene expression levels should be tested in patients who intend to use anti-VEGF drugs.

#5919 Performance of the Oncuria-detect bladder cancer test for evaluating patients presenting with hematuria: results from a real-world clinical setting.

Ian Pagano[1], Zhen Zhang[2], Sergei Tikhonenkov[1], Florence Le Calvez-Kelm[3], Steve Goodison[4], Toru Sakatani[5], Kaoru Murakami[5], Takashi Kobayashi[5], Howard Kim[6], Riko Lee[6], Sunao Tanaka[6], Charles J. Rosser[6], **Hideki Furuya**[6]

[1]University of Hawaii Cancer Center, Honolulu, HI,[2]Johns Hopkins Medical Institutions, Baltimore, MD,[3]International Agency for Research on Cancer, Lyon, France,[4]Mayo Clinic, Jacksonville, FL,[5]Urology, Kyoto University, Kyoto, Japan,[6]Cedars-Sinai Medical Center, Los Angeles, CA

Background and Objective: Bladder cancer is the 9th most diagnosed cancer worldwide with high incidences reported in Europe and the United States. Here, we evaluated the real-world performance of a commercially available multiplex immunoassay Oncuria-Detect that detects bladder cancer by simultaneously measuring a panel of 10 biomarker proteins in naturally voided urine samples.
Methods: We tested prospectively collected urine samples from a real-world cohort of 1,002 patients presenting to five USA centers, one European center and one Japanese center with hematuria. Assay results were collated with patient clinical data and a cancer diagnosis was defined by biopsy and pathology. The prevalence of bladder cancer in the cohort was 15.9%.
Results: In a training set (n=684), the Oncuria-Detect assay correctly identified bladder cancer in 94/109 cases, representing a sensitivity of 91.8%, specificity of 71.6% (AUC 0.885; 95% CI 0.843 - 0.927) and with improved performance for high-grade bladder cancer and muscle-invasive bladder cancer (sensitivity 93.6% and 92.1%, respectively). In a test cohort of n =339 the AUC for detecting bladder cancer was 0.850 (95% CI 0.779-0.920).
Conclusions and Implications: The multiplex Oncuria-Detect assay identified bladder cancer with high sensitivity and NPV, for low-grade and high-grade tumors alike. Oncuria-Detect's high NPV could effectively rule out 66% of patients from requiring subsequent cystoscopy.

#5920 Nanomechanical signatures: a breakthrough in personalized diagnosis and treatment optimization for lung cancer.

Jordi Alcaraz[1], Elba Marin[1], Hector Sanz-Fraile[1], Marina Querol[2], Paula Gausa[3], Kate Neal[1], Marselina Arshakyan[1], Marc Rico-Pasto[1], Marc Boada[2], David Sanchez[4], Daniel Martinez[5], Reinier Oropesa Nunez[6], Gitika Srivastava[6], Ahmed Jizawi[6], Carolina Ortiz-Velez[6], Tobias Appenzeller[6], Sara Nizzero[7], Marko Loparic[6], Marija Plodinec[6], Noemi Reguart[2]

[1]Universitat de Barcelona, Barcelona, Spain,[2]Hospital Clinic Barcelona, Barcelona, Spain,[3]Thoracic Oncology Unit, Hospital Clinic Barcelona, Barcelona, Spain,[4]Thoracic Surgery, Hospital Clinic Barcelona, Barcelona, Spain,[5]Anatomophatology, Hospital Clinic Barcelona, Barcelona, Spain,[6]ARTIDIS AG, Basel, Switzerland,[7]ARTIDIS Inc, Houston, TX

Background: Non-small cell lung cancer (NSCLC) is the most common lung cancer subtype and the leading cause of cancer-related deaths worldwide. Advances in treatment have focused on personalized approaches that use targeted therapies and immunotherapies based on protein, DNA, or RNA biomarkers. Assessing molecular biomarkers typically takes 1-2 weeks with challenges such as tissue exhaustion and poor response prediction. Atomic Force Microscopy (AFM) allows for point-of care evaluation of nanomechanical signatures (NS) in hours, offering a fast and accurate method for diagnosis and treatment personalization.
Methods: We used the AFM-based Automated and Reliable Tissue Diagnostics (ARTIDIS) platform to evaluate the diagnostic and prognostic capabilities of NS. Fresh tumor and paired uninvolved pulmonary tissue samples were collected from 70 early-stage NSCLC patients who underwent curative surgical procedures at the Hospital Clinic of Barcelona. Measurements were blinded, and samples were preserved in formalin for further histopathological and molecular analyses.
Results: In this proof-of-concept study, ARTIDIS NS successfully distinguished malignant tumors from uninvolved pulmonary tissue with 88% sensitivity, 86% specificity, and 87% accuracy (n=52). The NS also accurately identified histological subtypes, including adenocarcinoma (ADC, 100% sensitivity) and squamous cell carcinoma (SCC, 83% specificity). ARTIDIS NS identified aggressive features predictive of early recurrence in non-involved samples (82% sensitivity, 72% specificity). Out of 70 patients, 17 had recurrences (median time: 7.7 months), leading to 12 deaths, 10 due to cancer. Remarkably, ARTIDIS NS accurately predicted tumor progression within 10 months post-surgery, achieving 100% sensitivity and 93% specificity in identifying patients at risk of recurrence after curative treatment.
Conclusions: The findings suggest that ARTIDIS NS is a rapid and reliable tool for predicting recurrence and assessing outcomes in NSCLC while preserving tissues for downstream assessment. The ARTIDIS platform not only demonstrated significant accuracy in differentiating malignant tissues, but also showed exceptional precision in predicting early recurrence and progression, effectively identifying high-risk patients. Furthermore, NS identified early recurrence indicators through nanomechanical changes in uninvolved samples, offering insights beyond traditional pathology. A 200-patient extension specifically in neoadjuvant treatment is planned to enhance NSCLC management and improve patient outcomes.

#5921 Correlation between MET amplification/overexpression status and response to MCLA-129 in advanced non-small-cell lung cancer (NSCLC): biomarker results from a phase 1 trial.

Jie Wang[1], Jia Zhong[1], Yu Yao[2], Di Wu[3], Xiujuan Qu[4], Guojun Zhang[5], Haibo Zhang[6], Yu Cao[7], Zhuang Yu[7], Kai Chen[8], Maoqiong Jiang[9], Zhihua Li[10], Jianlin Long[11], Xiubao Ren[12], Huijuan Wang[13], Zhehai Wang[14], Lin Wu[15], Bolin Chen[15], Aimin Zang[16], **Haie Chen**[17], Lin Ma[17], Kan Liu[17], Peiqi Li[17], Lieming Ding[17]

[1]National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China,[2]The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China,[3]Shenzhen People's Hospital, Shenzhen, China,[4]The First Hospital of China Medical University, Shenyang, China,[5]The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China,[6]Guangdong Provincial Hospital of Chinese Medicine, Guangzhou, China,[7]The Affiliated Hospital of Qingdao University, Qingdao, China,[8]The First Affiliated Hospital of Soochow University, Suzhou, China,[9]The Second People's Hospital of Yibin, Yibin, China,[10]Sun Yat-sen Memorial Hospital, Guangzhou, China,[11]Chongqing University Cancer Hospital, Chongqing, China,[12]Tianjin Medical University Cancer Institute & Hospital, Tianjin, China,[13]Henan Cancer Hospital, Zhengzhou, China,[14]Shandong Cancer Hospital, Jinan, China,[15]Hunan Cancer Hospital, Changsha, China,[16]Affiliated Hospital of Hebei University, Baoding, China,[17]Betta Pharmaceuticals Co Ltd, Hangzhou, China

Background: In this ongoing phase 1b study (NCT04930432) of the MET/EGFR bispecific antibody MCLA-129 in patients (pts) with advanced NSCLC, we aimed to gain a deeper insight into the relationship between MCLA-129 and efficacy in the MET amplification (METamp) or protein overexpression (OE) population.
Methods: Eligible pts (≥18 years; ECOG PS 0-1; confirmed metastatic or unresectable NSCLC; and positive EGFR or MET alterations) received MCLA-129 (1500 mg biweekly) intravenously. MET OE was defined as IHC 2+/3+ and METamp was defined based on the gene copy number (GCN). Using a wide range of GCN cut-offs, we aimed to explore its relationship with efficacy outcomes.Results: At data cut-off (March 31, 2024), in 24 NSCLC pts, 6 with MET IHC 2+/3+ and 18 with METamp were evaluable for efficacy. Four out 6 pts with MET IHC 2+/3+ (median No. of previous lines: 1 [0-1]) achieved confirmed PRs (ORR: 66.7% [95% CI 22.3-95.7]), and all these 4 pts harbored concurrent mutations (3 METex14 skipping and 1 EGFR exon20ins). The median DOR and PFS were not reached. Eight out of 18 pts with METamp achieved confirmed PRs (median No. of previous lines: 2 [1-7]) (ORR: 44.4% [95% CI 21.5-69.2]; DOR: NR [2.8-NR]; PFS: 4.8 months [2.7-9.6]). Of 7 pts with METamp (METamp was detected prior to systemic antitumor therapy), 4 pts achieved confirmed PRs, with an ORR of 57.1% (95% CI 18.4-90.1), and all these 4 pts harbored concurrent mutations (2 EGFR exon20ins, 1 METex14 skipping and 1 L861Q). Among 9 pts that progressed on 3rd-generation EGFR-TKIs, 3 pts achieved confirmed PRs. Pts with different cut-offs of MET showed a differentiated ORR (Table).
Conclusions: MCLA-129 showed clinically meaningful antitumor activity in advanced NSCLC with MET OE or METamp, particularly in pts with concurrent mutations. High GCN seemed to be associated with better efficacy in MET-amplified advanced NSCLC, suggesting its predictive properties.

GCN cut-off	N	ORR, % (95% CI)
≥3	15	46.7 (21.3-73.4)
≥4	10	60.0 (26.2-87.8)
≥5	7	71.4 (29.0-96.3)
≥6	6	66.7 (22.3-95.7)
≥7	2	100 (15.8-100)

#5922 B cell subtype is a predictive and pharmacodynamic biomarkers for combination therapy in stage III-IV melanoma: a simon phase II trial.

Teruyuki Mizutani, Amrutesh Puranik, Tomoaki Muramatsu, Joanna Handzlik, Ankita Mitra, Diana Kimono, Stanzin Idga, Eddy Yang, Ramin Herati, Michelle Krogsgaard, Mehnert Janice

Perlmutter Cancer Center, NYU Langone Health, NYC, NY

Background: Assessing biomarkers to predict immune-related adverse events (irAEs) is crucial for optimizing melanoma treatment with immune checkpoint inhibitors (ICIs). T and B lymphocytes are key mediators of autoimmunity and are implicated in the development of irAEs. Here we focus on analysis of specific B cell subsets, as predictive and pharmacodynamic biomarkers for irAEs in a phase II trial combining nivolumab (3 mg/kg), ipilimumab(1 mg/ml), and the IL-6 receptor inhibitor tocilizumab (4 mg/kg) in unresectable/advanced stage III/IV melanoma patients.
Methods: We analyzed cryopreserved PBMC from melanoma patients collected at baseline and week 7, and categorized by irAE severity. Patients were categorized as either experiencing severe irAEs (grade 3-4) (TOX) or no toxicity or grade 1-2 irAEs (NT). We used multi-parameter flow cytometry panel to analyze B cells. PBMCs analyzed by a 35-color antibody B cell panel by spectral flow cytometry. The data underwent quality control, and normalization and initial clustering. CD19+ B cells were concatenated and clustered by FlowJo software. Fifteen B cell clusters was obtained and phenotyped using Cluster Explorer. Statistical analyses were performed using multiple logistic regression and Mann-Whitney U test, with a significance level set at 0.05.
Results: Among the 70 patients administered treatment, we observed a best overall response rate of 57%. Grade 3-5 irAEs occurred in 22% of patients by week 24. We analyzed 58 quality-controlled baseline samples and 54 7-week samples. Fifteen distinct clusters were identified after clustering B cell populations using UMAP and re-clustering using PHATE. Two of these clusters showed significant differences in both multivariate logistic regression and Mann-Whitney U tests. TOX patients had significantly lower baseline naive B cell counts than NT patients ($p=0.016$), suggesting naïve B cells as a predictive marker for toxicity. A significant increase in late memory B cells at week 7 was a key pharmacodynamic marker ($p=0.003$).
Conclusion: Our findings suggest that naive B cells could serve as a toxicity biomarker in combination therapy with ipilimumab, nivolumab, and tocilizumab. Late memory B cells emerged as a promising pharmacodynamic biomarker. Immunological monitoring may help elucidate the mechanisms of irAEs and predict irAE occurrence.

#5923 Human dose-escalation study of CD8$^+$T-cell infiltration in solid malignancies with^{68}Ga-NODAGA-SNA006 by PET imaging.

Y. Wang[1], M. Zheng[1], J. Zhao[1], C. Wang[2], C. Wei[2], S. Zhao[1], Y. Bian[1], N. Dai[1], Y. Zheng[1], S. sang[1], L. Guo[1], C. Huang[1], H. Zhang[1], J. Jiang[1], C. Xu[1], Q. Zhao[1], J. Han[1], T. Xu[2], S. Qin[1], L. Miao[1];

[1]First Affiliated Hospital of Soochow University, Suzhou, China, [2]SmartNuclide Biotech, Suzhou, China

Purpose: A noninvasive method for evaluating the infiltration of CD8$^+$ T cells in tumors is urgently needed for monitoring the response to immunotherapy. This study (NCT05126927) investigated the performance of a ^{68}Ga-NODAGA-SNA006 by positron emission tomography (PET) imaging of CD8$^+$ T cells in patients with solid malignancies.

Methods: This human dose-escalation PET imaging study included nine patients (lung cancer, 7; gastric carcinoma, 1; esophageal carcinoma, 1). Approximately 150 MBq of ^{68}Ga-NODAGA-SNA006 with varying nanobody masses (100 μg, 300 μg, 500 μg, 800 μg) was administered, and PET/computed tomography (CT) scans were performed at 15-30 min, 60-90 min, and 120 min postinjection (*p.i.*). Data regarding biodistribution, pharmacokinetics and radiation dosimetry were evaluated. CD8$^+$ T-cell infiltration in biopsy samples was also measured by IHC staining for correlation analysis with tumor uptake of ^{68}Ga-NODAGA-SNA006 PET.

Results: ^{68}Ga-NODAGA-SNA006 was well tolerated by all nine subjects. The highest radioactive uptake was observed in the spleen, with SUVmax ranging from 60.77 ± 25.05, 66.78±5.64, 70.36±20.94, 56.17 ± 4.69 at 60 min for 100 μg, 300 μg, 500 μg, and 800 μg, respectively, followed by the kidneys and bladder. The SUVmax of kidneys are from 9.20 ± 1.85 to 18.54 ±2.36 for 100 μg to 800 μg. However, the uptake of ^{68}Ga-NODAGA-SNA006 in bladder varied dramatically, depending on the excretion status of patients. Liver uptake decreased with increasing dose of nanobody, maximal (SUVmax: 14.07 ± 2.88) with low nanobody masses (100 μg) and significantly reduced (SUVmax: 6.64 ± 1.30) with high nanobody masses (800 μg). Rapid clearance ($t_{1/2}$<30 min) of ^{68}Ga-NODAGA-SNA006 from whole blood and serum was observed. Furthermore, the uptake of ^{68}Ga-NODAGA-SNA006 in tumors (SUVmean) exhibited a linear relationship with the CD8$^+$ T-cell infiltration in biopsy samples (R^2=0.757, p=0.011), suggesting that the tumor uptake of ^{68}Ga-NODAGA-SNA006 may represent the degree of CD8$^+$ T-cell infiltration in the tumor.

Conclusion: The use of ^{68}Ga-NODAGA-SNA006 is safe, feasible, and well tolerated. ^{68}Ga-NONAGA-SNA006 PET imaging can accurately detect CD8$^+$ T cells with favorable pharmacokinetics, thus providing a feasible method for the noninvasive quantitative assessment of CD8$^+$ T-cell infiltration and monitoring of response to immunotherapy.

#5924 A Tumor-specific, methylation-based algorithm to identify *MTAP* gene deletions via tissue-free circulating tumor DNA.

Sean Gordon, Evan Warner, Geethika Yalamanchili, Jill Tsai, Scott Shell, Shile Zhang, Martina Lefterova, Justin Odegaard, Darya Chudova

Guardant Health, Palo Alto, CA

Background: Methylthioadenosine phosphorylase (MTAP) is a tumor suppressor involved in the methionine salvage cycle. The *MTAP* gene is adjacent to the tumor suppressor *CDKN2A* on the chromosomal locus, 9p21.3. In ~15% of all cancers, the locus 9p21.3 is deleted, and *MTAP* is co-deleted with homozygous loss of *CDKN2A* in >80% of cases. Clinical trials investigating PRMT5 and MAT2A inhibitors have demonstrated potential for inducing synthetic lethality in *MTAP*-deleted tumors. Thus, the characterization of *MTAP* deletion in solid tumors is necessary to identify eligible patients. However, robust detection of biallelic gene deletion in liquid biopsies requires high levels of tumor shedding. Here we show performance of a methylation-informed *MTAP* deletion classifier able to resolve copy loss down to 1% tumor fraction.

Methods: We performed genomic and epigenomic profiling across samples from three distinct pan-cancer cohorts with the Guardant Infinity platform: 1. Discovery cohort of 14,895 minimal-residual disease (MRD) plasma samples from patients after curative-intent treatment for early-stage tumors to identify tumor-specific methylation at the 9p21 locus 2. Discovery cohort of 23,143 plasma samples from late-stage tumors to identify which candidate biomarkers were informative as to *MTAP* status, and 3. Independent validation cohort of ~14,000 plasma samples from late-stage tumors to confirm the association of candidate biomarkers to *MTAP* status. Quantification of tumor-specific DNA was done using an analytically validated methylation-based methodology, and we obtained synchronous mutation, copy-number, and genome-wide methylation data from all samples.

Results: We identified 6 genes within 1MB of the *MTAP* locus (*CDKN2A, CDKN2A-DT, CDKN2B-AS1, DMRTA1*, *MIR31HG*, and *HACD4*) that demonstrated cancer-specific promoter hypermethylation in our MRD discovery cohort. In our late-stage cancer cohort, we observed that hypermethylation of all 6 candidate biomarkers was mutually exclusive with *MTAP* homozygous deletion relative to distal sites. A logistic regression model utilizing methylation of the *MTAP* proximal sites along with distal site hypermethylation was trained on a subcohort of 297 samples balanced for *MTAP* deletion status and tumor fraction. On the independent multicancer validation cohort, the AUC of the *MTAP* predictive model was 0.91. Incorporation of methylation signals increased *MTAP* homdel detection in liquid by 1.3-fold to several fold depending on sample tumor fraction.

Conclusion: We have developed a novel method for improving the sensitivity of *MTAP* biallelic deletion detection in liquid biopsies using tumor-specific methylation. These findings offer biological insight into the relationship between localized deletion and methylation patterns and provide a framework to improve deletion calling in other genes with critical roles in tumor biology.

#5925 Circulating cell-free methylated tumor DNA measurements correlate with plasma VAF-based tumor fraction estimates.

Lee Davis McDaniel, Joseph Schull, David Tsao

BillionToOne, Menlo Park, CA

Introduction: Cell-free DNA (cfDNA) consists of circulating tumor DNA (ctDNA) and DNA shed by non-tumor cells such as immune and endothelial cells. Tumor fraction (TF) is the proportion of ctDNA present in cfDNA. TF is often estimated by summary statistics on somatic variants such as the mean or maximum variant allele fraction (VAF). The Northstar Response (NSR) assay quantifies ctDNA by accurately and precisely counting methylated cfDNA molecules across over 500 loci uniquely methylated in tumor tissue but not in any non-cancerous cells. We hypothesize that the NSR quantification of plasma methylated DNA, via the Tumor Methylation Score (TMS), correlates with tumor fraction as estimated by VAF.

Methods: A pan-cancer cohort of 271 longitudinal blood samples across 74 patients was gathered and run on the Northstar Select (NSS), an 84-gene liquid biopsy panel for somatic variant detection, and NSR assays. Samples were required to have positive detection in both assays to ensure precise TF measurements (max(VAF)>0.01%, TMS>25 methylated molecules). Tumor fractions were estimated by removing germline variants and evaluating the max(VAF) and sum(VAF), with the latter measurement paralleling the summation of methylated molecules used by the TMS. Due to the high variability of tumor mutational burden (TMB) and tumor methylation levels between tumors, correlations between VAF and TMS were calculated on a per-patient basis for patients with at least 3 longitudinal samples.

Results: TMS ranged from 25 to 253k molecules with a mean of 16k; sum(VAF) ranged from 0.06 to 782 with a mean of 67; max(VAF) ranged from 0.06% to 100% with a mean of 34%. TMS correlated most strongly with sum(VAF) with mean(R^2)=0.77, median(R^2)=0.96, whereas for max(VAF), mean(R^2)=0.67, median(R^2)=0.91. A linear regression model between max(VAF) and TMS demonstrated a mean increase of 1.3k methylated molecules per unit percent increase in max(VAF).

Conclusions: Previous studies have demonstrated that VAF decreases are prognostic to targeted therapy response including immune checkpoint blockade and tyrosine kinase inhibitors. However, VAF-based tumor burden measurements are limited by the paucity of somatic variants. In contrast, aberrant DNA methylation is a well-known hallmark of cancer that is an abundant source of ctDNA. The strong, linear correlation between TMS and VAF shown here suggests that TMS is an effective measure of ctDNA burden and can similarly be used to monitor response to therapy. Furthermore, the large amount of methylated DNA in patient samples (mean=16k molecules) suggests that epigenetic approaches can be highly sensitive even at very low tumor fraction (<1%).

#5926 Determining classical-basal transcriptional subtypes of pancreatic ductal adenocarcinoma using circulating tumor DNA epigenomic profiling.

Karl Semaan[1], **Marc Eid**[1], Damien Vasseur[1], Gunsagar S. Gulati[1], Ji-Heui Seo[1], Alexander Jordan[1], Andressa Dias Costa[1], John Canniff[1], Leigh Culnane[1], Elizabeth Andrews[1], Emma C. Coleman[1], Noa Phillips[1], Brad Fortunato[1], Hunter Savignano[1], Rashad Nawfal[1], Razane El Hajj Chehade[1], Talal El Zarif[2], Ze Zhang[1], Paulo Cordeiro[1], Antoine Hollebecque[3], Antoine Italiano[3], Brandon M. Huffman[1], James M. Cleary[1], Jonathan Nowak[4], Jacob E. Berchuck[5], Toni K. Choueiri[1], Kimberly Perez[1], Andrew J. Aguirre[1], Sylvan C. Baca[1], Brian M. Wolpin[1], Matthew L. Freedman[1], Harshabad Singh[1]

[1]Dana-Farber Cancer Institute, Boston, MA,[2]Yale School of Medicine, New Haven, CT,[3]Gustave Roussy, Villejuif, France,[4]Brigham and Women's Hospital, Boston, MA,[5]Emory University, Atlanta, GA

Introduction: Classical (C) and basal (B) transcriptional subtypes are key prognostic biomarkers of pancreatic ductal adenocarcinoma (PDAC). The B subtype is associated with higher rates of metastases, chemoresistance and poor outcomes. Early data suggest that the B subtype may predict response to KRAS inhibition. Current subtyping methods rely on tissue biopsies, which may not capture subtype heterogeneity, and are difficult to implement clinically. Here, we report a novel non-invasive approach for PDAC subtyping based on profiling circulating tumor chromatin.

Methods: RNA-seq, chromatin immunoprecipitation and sequencing (ChIP-seq) for H3K27ac and H3K4me3, assay for transposase-accessible chromatin sequencing (ATAC-seq), and methylated DNA immunoprecipitation sequencing (MeDIP-seq) were performed on PDAC patient derived xenografts (PDX, n = 17). C and B subtypes were called using PURIST algorithm on RNA-seq. Differential features between C and B PDXs for each epigenetic mark were identified using DESeq2. H3K27ac and H3K4me3 ChIP-seq, MeDIP-seq and low pass whole genome sequencing (LPWGS) were then performed on plasma of patients with metastatic PDAC from two clinical cohorts where C and B subtypes were previously determined. Circulating tumor DNA (ctDNA) fraction was estimated from LPWGS data using ichorCNA. A C/B score was derived for each epigenetic feature in individual patients by calculating the ratio of aggregate signal at classical:basal regions. Finally, a plasma integrated epigenomic score (PIES) was developed by combining various epigenetic features and its ability to distinguish subtypes was assessed using the area under the receiver operating characteristic (AUROC) curve.

Results: Overall, 17 PDAC PDXs (C = 13, B = 4) were profiled with ChIP-seq (H3K27Ac, H3K4me3), ATAC-seq and MeDIP-seq. We identified 7,855 differential H3K27ac sites that were also differentially accessible by ATAC-seq, 1,603 differential H3K4me3 sites and 308 differentially methylated regions between the 2 subtypes. We then profiled 53 plasma samples from patients with metastatic PDAC who had previously undergone tissue-based RNA analysis (C = 33, B = 20). The median ctDNA fraction was comparable between the two subtypes (3.1 vs. 3.3%; p=0.4). The H3K27ac and H3K4me3 C/B scores were significantly higher in plasma from patients with classical PDAC ($p<0.001$) compared to basal PDAC. The methylation signature did not distinguish the two subtypes (p=0.2). PIES showed high discriminatory power between classical and basal samples, with an area under the curve (AUC) of 0.84 for all samples regardless of ctDNA fraction and an AUC of 0.88 for samples with >3% ctDNA.

Conclusion: We present a non-invasive approach for distinguishing classical versus basal PDAC based on circulating tumor epigenomic profiles, which could facilitate subtyping to guide clinical decision making.

#5927 Assessing the impact of improved pre-analytic tissue handling on immunohistochemistry and breast cancer subtypes in Ghana.

Patrick Akakpo[1], Sylvester Antwi[2], Kwabena Agbedinu[3], Emmanuel G. Imbeah[1], Foster Amponsah[4], Veneranda Nyarko[5], Harriet Larrious-Lartey[2], Samuel Mensah[6], Josephine Nsaful[5], Valerie Ofori Aboah[2], Moses Dokurugu[7], Michael Nortey[1], Mohammed Sheriff[7], Nelson Affram[8], Jessica Bensenhaver[2], Haythem Ali[2], Ghassan Allo[2], Eleanor Walker[2], Evelyn M. Jiagge[2]

[1]Cape Coast Teaching Hospital, Cape Coast, Ghana,[2]Henry Ford Health System, Detroit, MI,[3]Komfo Anokye Teaching Hospital, Kumasi, Ghana,[4]Eastern Regional Hospital, Koforidua, Ghana,[5]Korle Bu Teaching Hospital, Accra, Ghana,[6]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana,[7]Tamale Teaching Hospital, Tamale, Ghana,[8]Ho Teaching Hospital, Ho, Ghana

Background: The prevalence of triple negative breast cancer (TNBC) in sub-Saharan Africa has been inconsistently reported, with some studies in Ghana citing rates as high as 80%. The discrepancies in TNBC prevalence have been partly attributed to poor tissue handling during the pre- analytic phase, and the lack of 10% neutral buffered formalin for proper fixation. With a higher proportion of TNBCs being reported, many women are not candidates for targeted therapy. This study evaluates the impact of improved pre-analytic tissue handling on the diagnostic outcome of 4000 breast biopsies in Ghana, focusing on molecular subtypes, including TNBC

Methods: A multi-center approach was employed by Precision Medicine for Aggressive Breast Cancer (PMABC) across Ghana to refine pre-analytic tissue handling. Interventions included: (1) training providers on core needle biopsy techniques and supplying them with biopsy needles, (2) providing and training centers on the preparation and use of 10% neutral buffered formalin and (3) conducting workshops and hands-on training on proper tissue handling. Histopathology and immunohistochemistry were performed on 4000 cases at a partner pathology center (Pathologists without Borders) using Ventana BenchMark GX and CAP reporting protocols

Results: Among the 4000 cases analyzed, the mean age was 52.77 +/- 13.45 years (range: 14-101 years), with male breast cancer accounting for 1.6% of cases. Molecular subtype analysis revealed the following prevalence: Luminal A (12.1%) Luminal B (40.7%), HER2-enriched (12.6%), TNBC (29.7%). About 4.9% of cases which were classified as Other, comprised of HER2 equivocal. A comparative analysis of age group and molecular subtype revealed Luminal A is more predominant in the age group 50-69 years, Luminal B, HER2-enriched and TNBC are most prevalent in age group 40-59 year. TNBC was most prevalent in patients younger than 40 years, particularly those under 30. A statistically significant association was observed between age and molecular subtype ($p<0.001$).

Conclusion: The prevalence of TNBC in Ghana was revised to 29.7% (excluding HER equivocal cases) following improved tissue handling practices, contrasting with earlier inflated estimates. TNBC remains the most common subtype in younger patients, underscoring the need for tailored diagnostic and treatment strategies. Notably, molecular subtype varied significantly with age, showing a strong association between age group and subtype ($p<0.001$). This study highlights the importance of ensuring quality pre-analytic practices to ensure correct molecular sub-typing for accurate treatment stratification of breast cancer patients in sub-Saharan Africa to improve outcome.

#5928 Baseline serum protein analysis of patients with interstitial lung disease/pneumonitis (ILD) in 3 trastuzumab deruxtecan (T-DXd) trials: DESTINY-Breast01, DESTINY-Breast04, and DESTINY-Lung01.

Z. Tsuchihashi[1], K. Contrepois[2], K. Kubota[1], J. Zhao[1], X. Wang[1], V. Kumar[1], N. F. Blasco[3], B. Jakubison[4], S. Terrillon[2], Y. Cheng[1], T. Kaji[5], M. Nakamura[5], C. A. Powell[6], E. F. Smit[7], S. Modi[8], P. Newham[9];

[1]Daiichi Sankyo, Inc, Basking Ridge, NJ, [2]AstraZeneca, South San Francisco, CA, [3]AstraZeneca, Barcelona, Spain, [4]AstraZeneca, Gaithersburg, MD, [5]Daiichi Sankyo, Tokyo, Japan, [6]Mount Sinai National Jewish Health Respiratory Institute, New York, NY, [7]Leiden University Medical Center, Leiden, Netherlands, [8]Memorial Sloan Kettering Cancer Center, New York, NY, [9]AstraZeneca, Cambridge, United Kingdom

Background: The mechanism of T-DXd-related ILD remains unknown. Understanding the pathophysiology of drug-induced ILD and identifying biomarkers for patients (pts) at higher risk of T-DXd-related ILD is crucial. Krebs von den Lungen-6 (KL-6) has been analyzed as a biomarker for ILD, but its relevance to T-DXd-related ILD is undetermined. This exploratory analysis aimed to identify candidate ILD risk biomarkers via proteomic analysis and evaluate KL-6 levels in serum samples from pts in 3 T-DXd studies.
Methods: Baseline serum samples were collected from 189 pts in 3 T-DXd clinical trials: DESTINY-Breast01 (DB-01; n = 56; NCT03248492), DESTINY-Breast04 (DB-04; n = 78; NCT03734029), and DESTINY-Lung01 (DL-01; n = 55; NCT03505710). Pts were divided into 3 groups: 94 non-ILD controls, matched 1:1 based on patient characteristics, and 95 pts who later developed ILD, further categorized by worst adjudicated grade (G): G1/2 ILD (n = 74) and G3-5 ILD (n = 21). Available baseline serum samples were analyzed using the SomaScan™ proteomics platform. Baseline and longitudinal serum KL-6 levels were measured by ELISA.
Results: From ~6000 analytes tested, elevated baseline serum interleukin 1 receptor-like 1 (ST2) levels were observed in pts with incident G3-5 ILD in all 3 studies. In DL-01, median levels of baseline ST2 relative fluorescence units (RFU; IQR [Q1-Q3]) in non-ILD pts were comparable to G1/2 ILD cases (n = 27, 3838 RFU [2873-4910] vs n = 20, 4374 RFU [3417-5122]), in contrast, the median baseline ST2 level in G3-5 ILD cases was elevated (n = 8, 8512 RFU [6824-23494]). Elevation of median baseline ST2 levels in G3-5 ILD cases was also observed in DB-01 (n = 6, 7707 RFU [6503-19035]) and DB-04 (n = 7, 5973 RFU [3972-14682]). In DB-01, the median baseline ST2 levels were 3816 RFU (n = 28, [2757-4712]) in non-ILD patients and 3529 RFU (n = 22, [2742-4695]) in G1/2 ILD cases. In DB-04, the median baseline ST2 levels were 4420 RFU (n = 39, [3051-6002]) in non-ILD patients and 4140 RFU (n = 32, [2714-5758]) in G1/2 ILD cases. Baseline KL-6 levels were elevated in G3-5 ILD cases in DB-01. This elevation was not observed in DB-04 or DL-01. Longitudinal sample analysis showed that KL-6 levels decreased with T-DXd treatment, mirroring the reduction in tumor burden observed after treatment.
Conclusions: Proteomics analysis revealed elevated baseline serum ST2 levels as a potential baseline marker for T-DXd-related G3-5 ILD in lung and breast cancer. Luminex™ analysis is ongoing to quantify ST2 levels. The utility of KL-6 as a biomarker for T-DXd-related ILD appears to be limited. KL-6 levels decreased after T-DXd treatment, likely due to tumor shrinkage and reduction of tumor-derived KL-6. Given the limited number of clinical studies included and few G3-5 ILD events, the suitability of these candidate biomarkers needs further investigation.

#5929 High performance miRNA and mRNA signatures that in combination with serum PSA levels predict prostate cancer patients with lymph-node invasion.

Matias A. Bustos[1], Kelly K. Chong[1], Yoko Koh[1], SooMin Kim[2], Eleanor Ziarnik[2], Romela I. Ramos[1], Gianna Jimenez[3], David L. Krasne[4], Warren A. Allen[4], Timothy G. Wilson[3], Dave S. B. Hoon[1]

[1]Dept. Translational Molecular Medicine, Providence Saint John's Health Center, Santa Monica, CA,[2]Dept. Genome Sequencing Center, Providence Saint John's Health Center, Santa Monica, CA,[3]Dept. Urology and Urologic Oncology, Providence Saint John's Health Center, Santa Monica, CA,[4]Dept. Surgical Pathology, Providence Saint John's Health Center, Santa Monica, CA

Background: Nomograms or comparable techniques can be used to determine patients with primary prostate cancer (PCa) which are more likely to have lymph node metastasis (LNM) and will benefit from extended pelvic lymph node dissection (ePLND). However, ~80% of PCa patients undergo unnecessary ePLND, and PCa patients suffer from post-surgical morbid complications. This study aimed to identify both whole transcriptomic mRNA and microRNA (miR) signatures in primary PCa tumors that are associated with the development of regional draining LNM.
Methods: Primary PCa obtained from 88 patients [pN0 (n = 44) and pN1 (n = 44)] were profiled using the same tissue section by two different probe-based captured direct Next-Generation Sequencing assays targeting 19,398 human mRNA transcripts and 2,083 human miRs, respectively. Bioinformatic analyses and publicly available TCGA-PRAD [pN0 (n = 382) and pN1 (n = 70)] and GSE220095 [pN0 (n = 138) and pN1 (n = 17)] databases were used for verification and validation.
Results: A four-mRNA signature (*CHRNA2*, *NPR3*, *VGLL3*, and *PAH*) was commonly found in primary tumor tissue samples from PCa patients who had LNM. The results were validated using the TCGA-PRAD and GSE220095 datasets. The four-mRNA signature identified PCa patients with pN1 status [HTP dataset (AUC = 0.83, p = 1.54e-08), the TCGA-PRAD (AUC = 0.708, p = 2.4e-07), and the GSE220095 datasets (AUC = 0.83, p = 8.7e-06)]. Adding serum PSA values, to the four-gene signatures increased the performance to identify pN1 [mRNA dataset (AUC=0.8487 p=2.18e-09), the TCGA-PRAD (AUC=0.7150, p=8.66e-08), and the GSE220095 datasets (AUC=0.8772, p=4.09e-07)]. Paired miR analyses showed that eight miRs were significantly upregulated in primary PCa that were pN1 ($p < 0.01$). The eight-miR signature significantly identified pN1 patients [miR dataset (AUC=0.8568, p=9.86e-10)], and the performance increased with serum PSA [miR dataset (AUC=0.8626, p=4.66e-10)]. When combining the miR/mRNA signatures (miR-663b, *CHRNA2*, and *PAH*) with serum PSA levels showed the best performance to distinguish pN1 from pN0 patients.
Conclusions: This study found miR/mRNA signatures in primary PCa tumors that in combination with serum PSA levels may complement nomograms for better detection of PCa patients that develop LNM and triage patients into better surgical decision-making.

#5930 The Pancreas Tissue Bank (PCRFTB): Global facilitation of pancreatic research.

Hemant M. Kocher[1], Brian Davidson[2], John Isherwood[3], Stuart Robinson[4], Zahir Soonawalla[5], Lulu Tanno[6], Satyajit Bhattacharya[7], Bilal al-Sarireh[8], Somaiah Aroori[9], Rhiannon Roberts[1], Christine S. Hughes[1], Ahmet Imrali[1], Amina Saad[1], Trupti S. Kolvekar[1], Maryam Abollahyan[1], Dayem A. Z. Ullah[1], Claude Chelala[1]

[1]Cancer Research UK Barts Centre, London, United Kingdom,[2]University College London, London, United Kingdom,[3]University Hospitals of Leicester NHS Trust, Leicester, United Kingdom,[4]Newcastle Hospitals NHS Foundation Trust, Newcastle, United Kingdom,[5]Oxford University Hospitals, Oxford, United Kingdom,[6]University Hospital Southampton, Southampton, United Kingdom,[7]The London Clinic, London, United Kingdom,[8]Swansea Bay University Health Board, Swansea, United Kingdom,[9]University Hospitals Plymouth NHS Trust, Plymouth, United Kingdom

Pancreatic cancer is one of the most devastating diseases. Worldwide, the disease claims >300,000 lives per year with the worst 5-year survival of all common cancers (<7%), with little improvement over the last 50 years. Even though pre-clinical work has led to greater understanding of the patho-physiology of the disease, research into human disease is hampered by lack of availability of well-annotated human samples. With this in mind, Pancreatic Cancer Research Fund Tissue Bank (PCRFTB) was set up as world's first national pancreas tissue bank. PCRFTB facilitated pancreatic research globally by providing access to clinically well-annotated samples collected according to standardised protocols and reliably stored. Informed consent is obtained from patients with suspected benign, pre-malignant and malignant pancreatico-biliary diseases, together with healthy controls to provide contemporaneous controls for case-control studies using blood and its components, urine, saliva in addition to matched fresh frozen and FFPE tissue diseased and healthy tissues. Comprehensive clinical data with 300 data-points per visit include medical history, medications, clinical test results (radiological, blood, tissue, mutational), histological findings, treatment and outcomes which are clinically verified. Since 2015, across nine tertiary referral Centres across England and Wales, we have obtained consent from over 3,700 donors and collected over 110,000 tissue, fluid and cell samples. Patient derived organoids and cancer-associated fibroblasts (CAFs) derived from various primary pancreas tumours using standardised protocols and with certification. The process of consenting, collection, storage and distribution of data and samples are performed, quality controlled and quality assured using over 100 standardised operating procedures in a custom-built Quality Management System (QMS) with additional audits, dashboards and governance to achieve highest quality, robust and reliable research from these valuable human samples. We are in the process of achieving ISO 20387 certification in early 2025.A multidisciplinary PCRFTB Tissue Access Committee (PCRFTB TAC) undertakes in-confidence, rapid-access peer review of applications for access of samples and / or data based on quality of the science, priority, power and supporting preliminary data. Thus far 108 expressions of interest has led to provision of samples and/or data to 50 research projects from commercial and academic institutions globally. Research impact emanating from PCRFTB samples data is enhanced by mandating experimental data return from researchers through the custom-built pancreatic cancer bio-informatics portal Pancreatic Expression Database (PED). PED also allows researchers to access, mine and investigate datasets, preventing duplication of investigation efforts and conserving sample resources.

#5931 Unraveling the genetic complexity of Ph-like ALL: development of a cost-effective diagnostic qPCR panel.

Anita Chopra, Jay Singh, Mercilena Benjamin, Avanish Pandey, Jayanth Kumar Palanichamy, Sameer Bakhshi, Deepam Pushpam, Akash Kumar Jha, Pranay Tanwar

All India Institute of Medical Sciences (AIIMS) New Delhi, New Delhi, India

Background: Advancements in high-throughput sequencing have significantly expanded our understanding of B-cell Acute Lymphoblastic Leukemia (B-ALL). In particular, whole-transcriptome sequencing (WTS) has facilitated the discovery of the Ph-like ALL subtype. However, clinical integration of WTS remains challenging due to high costs and lack of standardized workflows. Ph-like ALL is marked by genetic heterogeneity, including diverse fusions and mutations, which complicates its diagnosis and limits targeted therapeutic options. This genetic complexity correlates with poor prognosis, emphasizing the need for accessible and effective diagnostic approaches.
Aim: To develop a cost-effective, qPCR-based diagnostic panel for its detection and classification in clinical settings.
Methods: A cohort of 394 newly diagnosed B-ALL cases were analyzed using RT-PCR and FISH for initial genetic screening. WTS was employed for comprehensive fusion detection, mutation analysis, and subtype classification. Kaplan-Meier survival analysis was used to evaluate patient outcomes, and a qPCR-based likelihood scoring model was developed based on differential gene expression (DEGs) to identify Ph-like ALL.
Results: Whole transcriptome sequencing revealed that 13.1% of the B-ALL cases have Ph-like phenotype, found in different age groups: 9.7% in pediatric patients (n=15), 20.5% in adolescents and young adults (AYA, n=8), and 50% in adults (n=3). Ph-like ALL cases predominantly exhibited fusions involving JAK-STAT pathway genes (n=10), ABL-class genes (n=9), or NTRK1 (n=1), in addition to a novel RUNX1::FGF13 fusion (n=2). Differentially expressed genes were tested for diagnostic potential, with 9 genes (*FZH2, PON2, CA6, OLFML2A, BAALC, PRKCZ, ZFPM2, TCFL5* and *ENPP2*) demonstrating significant ROC ($p<0.0001$ and sensitivity and specificity >80%) curves in a discovery cohort (n=79). This likelihood model also effectively distinguished Ph-like ALL in a validation cohort (n=150). Additionally, survival analysis revealed significantly worse event-free survival (EFS) and overall survival (OS) in Ph-like ALL patients compared to non-Ph-like patients (EFS: 0% vs. 43.3%, HR 3.40, p=0.006; OS: 0% vs. 45.4%, HR 3.57, p=0.005).
Conclusion: This study underscores the genetic diversity of Ph-like ALL. The poor prognosis of Ph-like ALL, marked by significantly reduced EFS and OS, highlights the need for targeted therapeutic approaches. The cost-effective qPCR-based diagnostic panel provides a promising tool for the early detection of Ph-like ALL, offering potential for improved patient outcomes through timely intervention.

#5932 Molecular determinants of 10-second diagnosis of brain cancer types with laser mass spectrometry.

Alexa Fiorante[1], Michael Woolman[2], David Munoz[3], Lan Anna Ye[4], Taira Kiyota[4], Sunit Das[3], Ahmed Aman[5], Howard Ginsberg[3], Gelareh Zadeh[4], **Arash Zarrine-Afsar**[4]

[1]Medical Biophysics, Univerity of Toronto, Toronto, ON, Canada,[2]University of Toronto, Toronto, ON, Canada,[3]Unity Health Toronto, Toronto, ON, Canada,[4]University Health Network, Toronto, ON, Canada,[5]Ontario Institute for Cancer Research, Toronto, ON, Canada

Surveys of neurosurgical outcome data have suggested suitable extents (aggressiveness) of resection to improve survival for many brain cancer types. Here, personalization of the extent of resection relies on highly subjective intraoperative diagnoses wherein the depth of the diagnostic information depends on the level of experience of the pathologist.
Picosecond InfraRed Laser Mass Spectrometry (PIRL-MS) uses picosecond bursts of mid- infrared laser radiation to extract, in a non-thermal manner, tissue lipids (1) present in cellular membranes, giving cells and nuclei their unique shapes (utilized in morphometric pathology with staining & microscopy) and (2) altered in tumorigenesis due to cross-talk with cellular signalling in metabolism (potentially also revealing cancer molecular subtypes in 10 seconds as shown) for real-time profiling of molecular content unique to each tumour type.
In our laboratory, close to 1,300 frozen brain cancer specimens over 30 different classes of adult and pediatric cancers are being subjected PIRL-MS to build a comprehensive molecular signature library using various supervised and unsupervised dimensionality reduction and data analysis methods. The sensitivity and specificity of the integrated morphometric and molecular diagnosis for pediatric brain cancers with 10-second PIRL-MS was > 96%. This classification could use as little as only 18 tissue lipids whose identities were determined using chromatography and tandem mass spectrometry. Greater than 90% sensitivity and specificity have been obtained for adult brain cancer classifications with 10-second PIRL-MS analysis utilizing 40 lipids spanning fatty acids, phospholipids and ceramides.
PIRL-MS can provide non-subjective differentiation between various brain cancer types with as little as 10 seconds of sampling and analysis time.

#5933 Improved identification of lung neuroendocrine neoplasms using INSM1 (SP493) Rabbit Monoclonal Primary Antibody.

K. Dvorak, R. Sainz, A. Gomez;
Roche, Tucson, AZ

Introduction: About 20% of lung cancers are of neuroendocrine origin. In addition to evaluation of morphological features, these neoplasms can be identified by immunohistochemistry (IHC) using neuroendocrine markers such as synaptophysin, chromogranin A and neural cell adhesion molecule, CD56. Recent studies showed that INSM1 is increasingly used as a diagnostic marker for cancers of neuroendocrine origin, since it is highly sensitive and specific. The primary focus of this study was to assess the sensitivity and specificity of a newly developed assay that is using a new INSM1 (SP493) antibody in detecting neuroendocrine lung cancers and to compare this IHC assay with currently used neuroendocrine markers.
Method: Tissue microarrays containing 292 lung tumors were stained with INSM1 (SP493) antibody, INSM1 (MRQ-70) antibody, CONFIRM anti-Synaptophysin (SP11) antibody, anti-Chromogranin A (LK2H10) antibody, and CD56 (MRQ-42) antibody on a BenchMark ULTRA instrument using OptiView DAB IHC Detection Kit or ultraView Universal DAB Detection Kit and sensitivity and specificity was calculated for each biomarker.
Results: The sensitivity and specificity of this new assay with INSM1 (SP493) antibody were 92.5% and 95.8%, respectively. Similar results were obtained for the lung cancer cases stained with INSM1 (MRQ-70) antibody; the specificity was 90.6% and sensitivity was 95.9%. In contrast, anti-Synaptophysin (SP11) antibody and anti-Chromogranin A (LK2H10) antibody demonstrated considerably lower sensitivity when used to stain these lung cancer cases. The sensitivity was 79.7% and 75.8% for anti-Synaptophysin (SP11) antibody and anti-Chromogranin A (LK2H10) antibody, respectively. The specificity of anti-Chromogranin A (LK2H10) antibody was comparable with the specificity of the INSM1 (SP493) antibody (97.5%); while the specificity of anti-Synaptophysin (SP11) antibody was lower (90.8%). The performance of CD56 (MRQ-42) antibody was suboptimal with the sensitivity of 86.2% and the specificity of 84.9%.
Conclusions: Overall, our data showed that the new IHC assay with INSM1 (SP493) antibody is highly specific and sensitive in detecting neuroendocrine lung tumors.

#5934 Functional biomarkers for bladder cancer.

Tianyu Yang, Karina Aguilar, Joshua van der Eerden, Vinata Lokeshwar

Augusta University, Augusta, GA

Introduction and Objective: Urine tests could offer a less invasive method for identifying bladder cancer, including in patients with hematuria and history of bladder cancer. We previously discovered a first-in-class human Chondroitinase (Chase) that degrades chondroitin sulfate. This Chase is a splice variant of HYAL4 (V1) and promotes a malignant phenotype and chemoresistance in BC cells. The Chase test quantifies enzyme activity by measuring the degradation of chondroitin sulfate using an ELISA-like assay. We evaluated the efficacy of the Chase urine test in identifying bladder cancer.
Methods: Test and validation cohorts came from three centers. The validation cohorts were prospective cohorts and assays were conducted in a blinded fashion. The cohorts included patients with microscopic and gross hematuria, benign genitourinary (BGU) conditions, history (HX) of BC or other genitourinary cancers (prostate and renal cell carcinoma). BGU cases included patients with BPH, cystitis, urinary tract infection, renal insufficiency, hematuria, urethral stricture, urolithiasis, and UPJ obstruction. V1 levels were measured by q-PCR and Chase activity by the Chase urine test.
Results: Chase levels were significantly elevated in both low- and high-grade tumors, correlating strongly with BC across three cohorts ($P<0.0001$; OR: 4.4; 95% CI: 3.3-5.9). The Chase test demonstrated high sensitivity in detecting BC, regardless of grade. Similarly, HYAL-4 (V1) transcript levels were elevated in urine exfoliated cells ($P<0.0001$; OR: 1.5; CI: 1.3-1.7), with 83% sensitivity and specificity, increasing to 86% sensitivity at a lower threshold (79% specificity). In comparison, FDA-approved BTA and NMP22 tests showed <60% efficacy. In cohort 2, Chase achieved 84% accuracy in cases with atypical cytology, while cytology alone had 67% sensitivity and 96.6% specificity. Urinary Chase and HYAL-4 mRNA levels were 4-5 times higher in patients with BC during surveillance than in those without recurrence ($P<0.0001$).
Conclusion: In test and validation cohorts Chase and V1 tests showed high sensitivity to detect BC and to monitor its recurrence. The Chase urine test is a less invasive alternative that could potentially reduce the need for cystoscopies for hematuria work-up and BC surveillance.
Support: 1R01CA227277-05A1; 1R01CA283699-01

#5935 Analytical validation of a robust, integrated multiomics tissue assay powered by the Guardant Infinity platform.

Tingting Jiang, Gayatri Premasekharan, Jeffrey Werbin, Yelia Huo, Ankit Jambusaria, Che-Yu Lee, Claire Olson, Arancha Sanchez, Yueyuan Zhang, Jing Zhao, Andreas Papoutsis, Lauren Lawrence, Jill Tsai, Soni Shukla, Drew Kennedy, Stefanie Mortimer

Guardant Health, Palo Alto, CA

Introduction: Next-generation sequencing (NGS) of tumor tissue is an integral technique to identify actionable alterations and inform oncology treatment. While established, tissue NGS is limited by variability in quantity and quality of both samples and test platforms, which limits its effectiveness. A robust workflow with high sample success rate, providing comprehensive molecular profiling, and fast turn-around time can improve this technology and support patient care. Here, we report the analytical validation of Guardant360 Tissue, a comprehensive molecular profiling test comprising DNA and RNA analysis of 950 genes and epigenomic methylation profiling powered by Guardant Infinity technology.
Methods: The analytical and clinical performance for DNA and RNA profiling was assessed using clinical FFPE samples and reference material at 50-100 ng inputs for DNA (N=556) and 25-50 ng inputs for RNA (N=282). Samples from over 10 common cancer types were analyzed, with tumor fractions as low as 5%, using as little as 6 slides (30 µm) of FFPE tissue as input.
Results: Guardant360 Tissue demonstrated high sample success rates detecting somatic alterations in >98% of DNA and >91% of RNA samples, including challenging specimen types like fine needle aspirates and core needle biopsies. Panel-wide limits of detection (LoD) were 1.8% MAF for SNVs, 1.5% MAF for indels, 0.4% MAF for DNA fusions, 3.5 copies for CNAs, 37.9% TF for gene deletions, 3.3% TF for microsatellite instability-high (MSI-H), 1.8% for promoter methylation, and 15.1 copies for RNA fusions and splice isoforms. Clinical samples demonstrated 100% sensitivity for SNVs (N=4149), indels (N=646), and MSI-H (N=19), and ≥95% sensitivity for CNAs (N=121), DNA rearrangements (N=34) and RNA fusions/splice isoforms (N=59) against FISH, ddPCR or multiple DNA/RNA/methylation based NGS platforms. High clinical specificity was also established at 100% for all guideline approved biomarkers and ≥99.99996% across the entire panel. Guardant360 Tissue TMB score demonstrated a strong linear correlation (R=0.95, Spearman method) and 94.2% categorical concordance (cutoff of 10 mut/Mb) with an orthogonal NGS panel.
Conclusions: Guardant360 Tissue offers a high success rate with minimal input, delivering competitive performance in detecting a wide range of actionable alterations. With a low limit of detection and excellent specificity, this assay achieves comprehensive molecular profiling using as little as 30 µm of FFPE tissue. Its robust capabilities not only enable precise clinical insights but also support the acceleration of novel biomarker discovery through a multiomics approach.

#5936 Landscape of epigenomic tumor fraction in large pan-cancer cfDNA cohort.

Andrew Gross, Jun Zhao, Katie Quinn, Sai Chen, Kyle Chang, Catalin Barbacioru, Tingting Jiang, Carin Espenschied, Kristin Price, Jack Tung, Darya Chudova

Guardant Health, Palo Alto, CA

Introduction: Cell-free DNA (cfDNA) tumor fraction (TF) quantification is an emerging biomarker with many promising applications, including the potential to complement tumor size measurements from imaging. Genomic approaches for TF estimation are limited by the presence of cancer driver alterations, detection sensitivity, and may be confounded by non-tumor derived variants. Therefore, we have previously developed a method using differentially methylated regions to robustly estimate TF (Chen et al AACR 2023). Here we leverage a rich dataset of >50,000 cfDNA samples from >20 cancer types to better understand the variability of epigenomic signals in cancer and non-cancer individuals and inform an update of this TF estimation algorithm.

Methods: We employed a data-driven approach to update the TF algorithm leveraging a large cohort of cfDNA samples with simultaneous genomic and epigenomic profiling. This extensive dataset allowed for robust modeling of non-tumor variability in epigenetic profiles, and better calibration of tumor signals across diverse primary tissue sources shedding signals into plasma. The method update includes a data-informed region selection targeting high pan-cancer signal to noise, as well as more robust accounting for rare sources of non-tumor methylation signals including technical variation and treatment associated tissue damage.

Results: We evaluated the performance of epigenomic TF estimates using 1203 samples with somatic maximum MAF, establishing 88% concordance within 0.2-5X range. Next, samples were computationally titrated into non-tumor background samples, demonstrating consistent concordance with genomic data at up to 10-fold dilutions and maintenance of precision well below the resolution of genomic profiling, largely driven by algorithmic improvements in this updated release. Case studies of discordant samples show the benefit of TF over genomic quantification, especially in scenarios with an ambiguous genomic driver, or low genomic precision. In analytical validation studies of experimental titrations over replicates, linearity and quantitative precision of TF was demonstrated down to an order of ~0.01%, compared to >0.4% for genomic TF.

Conclusions: Our liquid only TF has been analytically validated for use in clinical, research, and trial settings in patients with solid tumors. In addition to its use for absolute quantification, it is incorporated into downstream applications including therapy response monitoring and algorithms for labeling germline variants, clonal hematopoiesis, and wild-type variant calling. Finally, its application in a clinical cohort of over 50,000 samples has led to insights into the dynamics of cfDNA tumor shedding across pre- and on- treatment individuals improving accuracy of quantification and providing results for more patients.

#5937 Development of a protein and clinical multiparametric biomarker panel for assessing pancreatic adenocarcinoma risk.

Nischal Mahaveer Chand[1], A. James Moser[2], Eric M. Grund[1], Poornima K. Tekumalla[1], Madappa N. Kundranda[3], Gregory M. Miller[1], Niven R. Narain[1], Vijay Modur[1], Michael A. Kiebish[1]

[1]BPGbio, Framingham, MA,[2]Beth Israel Deaconess Medical Center, Boston, MA,[3]Banner MD Anderson Cancer, Gilbert, AZ

Detection of pancreatic adenocarcinoma represents a major burden on the healthcare system due to its latent detection, generally in the metastatic stage. To this end, we developed a prospective biomarker clinical trial to clinically and molecularly characterize at risk and confirmed pancreatic adenocarcinoma patients. This multicenter (n=6) clinical trial (NCT 02781012) of PDAC, referred to as Project Survival, combined high-fidelity longitudinal phenotypic characterization and multi-omic profiling to identify biomarkers with diagnostic and therapeutic utility. The study included collecting plasma, serum, buffy coat, urine, saliva, and tumor tissue samples and followed 140 non-cancer patients and 280 PDAC subjects for up to 7 years. Plasma samples were analyzed to identify circulating biomarkers using comprehensive proteomics profiling by LC MS/MS analysis. We first identify proteins that demonstrated a significant change between non-cancer (e. g., relatives with no signs of cancer, pancreatitis patients) and cancer patients. Then, top analytes were examined for impact from confounders (age, gender, BMI, and diabetes) using multivariate regression analysis. Four proteins were selected that demonstrated a robust signal with minimal impact from confounders. Next, multivariate logistic models were fit to distinguish cancer patients from non-cancer patients along with stratification across cancer staging to determine the predictive performance of the four-protein panel. Finally, an additional panel was developed that incorporated clinical features along with the protein panel to further improve predictive performance. We identified a distinct panel of four proteins that produced an optimal predictive diagnostic performance. The protein panel demonstrated ROC AUC of 0.77 for distinguishing cancer (n=273) from non-cancer (n=140) patients (PPV 0.76, NPV 0.70, OR 7.2), followed by ROC AUC of 0.70, 0.75, and 0.85 for distinguishing early stage (n=65), locally advanced (n=117), or metastatic (n=91) patients from non-cancer patients, respectively. Further, adding the four clinical features (Age, History of Pancreatitis, Family History of Cancer, and Weight Loss at Study Entry) further improved the ROC AUCs to 0.85, 0.79, 0.86, and 0.90 respectively. This prospective biomarker-driven clinical trial identified a distinct four-panel biomarker for Pancreatic Ductal Adenocarcinoma. The four proteins individually demonstrated a robust ability to distinguish cancer patients while controlling for confounding factors. The four-protein diagnostic panel showed superior ability to identify cancer patients and showed high predictive performance based on staging predictions. The panel is currently being explored for commercial development.

CLINICAL RESEARCH: Real-World Data and Real-World Evidence: Clinical Informatics and Data Science 2
Poster Session

#5941 Impact of neoadjuvant treatment on the presence of perineural invasion in rectal cancer: a national analysis.

Mohyeddine El Sayed[1], Sassine Youssef[2], Raymond N. Dubois[1]

[1]Hollings Cancer Center, The Medical University of South Carolina (MUSC), Charleston, SC,[2]Department of Biochemistry, The Medical University of South Carolina (MUSC), Charleston, SC

Background: Perineural invasion (PNI) is an independent prognostic factor associated with lower overall and disease-free survival in rectal cancer patients. Several studies have established nomograms predicting PNI presence using clinical data and radiomics. These nomograms will help guide treatment for patients with high risk for PNI. There is limited data on the impact of neoadjuvant therapy on the presence of PNI in pathological specimens. This study assesses whether neoadjuvant treatment reduces the odds of PNI at the time of surgical resection.
Methods: A retrospective analysis was conducted using the National Cancer Database, including patients aged ≥20 years diagnosed with rectal cancer from 2004 to 2020, with documented tumor stage and PNI status. Data collected encompassed demographics, tumor size and grade, TNM stage, and treatment sequences. Patients were categorized based on treatment sequence: surgical resection only (SRT), neoadjuvant treatment (NAT), adjuvant treatment (AT), and perioperative systemic therapy with neoadjuvant radiotherapy (PST/NART). Multivariable binary logistic regression analyzed the impact of neoadjuvant therapy on PNI presence, adjusting for significant covariates. Overall survival was assessed using multivariable Cox proportional hazards regression, adjusting for significant covariates.
Results: Among 38,537 patients, 25.3% received SRT, 38.7% NAT, 11.4% AT, and 24.6% PST/NART. In stage I, patients who received neoadjuvant treatment (NAT + PST/NART) were 50% less likely to have PNI compared to those who did not (SRT + AT) ($P < 0.001$). Stage II, III, and IV patients were 47%, 47%, and 40% less likely to have PNI with neoadjuvant treatment, respectively (all $P < 0.001$). In stage I, NAT patients had a 39% higher mortality risk compared to SRT patients ($P < 0.001$). In stages II and III, PST/NART patients had 38% and 34% lower mortality risks, respectively, compared to SRT patients ($P = 0.003$ and $P < 0.001$). In stage IV, AT patients had an 88% higher mortality risk compared to SRT patients ($P = 0.049$).
Conclusion: Neoadjuvant treatment significantly reduces the presence of perineural invasion across all stages of rectal cancer, potentially improving survival outcomes, particularly in stages II and III. However, the increased mortality risk observed in stage I patients receiving neoadjuvant therapy indicates that benefits may not extend to early-stage disease, underscoring the need for careful patient selection and further prospective studies.

#5942 Association of microsatellite instability status with pathologic complete response and tumor downstaging in locally advanced rectal cancer.

Mohyeddine El Sayed[1], Sassine Youssef[2], Raymond Dubois[1]

[1]Hollings Cancer Center, The Medical University of South Carolina (MUSC), Charleston, SC,[2]Department of Biochemistry, The Medical University of South Carolina (MUSC), Charleston, SC

Background: Two primary genomic instability pathways contribute to rectal carcinogenesis: microsatellite instability (MSI) and chromosomal instability, known as microsatellite stable (MSS). MSI rectal cancers generally have a better prognosis—attributed to enhanced immunogenicity, distinct molecular pathways, and reduced metastatic potential. This study assesses the impact of MSI status on pathologic complete response (pCR) and tumor downstaging after neoadjuvant treatment. We hypothesized that MSI rectal cancers are associated with higher odds of achieving pCR and tumor downstaging.

Methods: A retrospective analysis was performed using the National Cancer Database, including patients aged ≥20 years diagnosed with stage II-III rectal cancer between 2004 and 2020 with documented MSI status. All patients underwent surgical resection, chemotherapy, and radiotherapy. Collected data encompassed demographics, tumor size and grade, TN stage, lymphovascular invasion, perineural invasion, and immunotherapy. MSI status was categorized as MSS, MSI-low (MSI-L), and MSI-high (MSI-H). The primary outcome is pCR, defined as the absence of tumor in the pathological specimen (T0/x, N0/x). Tumor downstaging was assessed by comparing pathological T or N stage with clinical staging. Multivariable binary logistic regression analyzed the impact of MSI status on pCR and tumor downstaging, adjusting for significant covariates.

Results: Of the 5,124 patients included, 93.5% were MSS, 3.1% MSI-L, and 3.4% MSI-H. Immunotherapy was administered to 1.1% of MSS, 1.9% of MSI-L, and 3.5% of MSI-H patients. MSI tumors were less likely to achieve pCR compared to MSS tumors (OR = 0.62; $P = 0.023$). Specifically, MSI-L tumors had significantly lower odds of pCR (OR = 0.40; $P = 0.011$), whereas MSI-H tumors did not differ significantly from MSS tumors. For T downstaging, MSI tumors were less likely to have a reduced pathological T stage (OR = 0.72; $P = 0.009$). MSI-H tumors were associated with lower odds of T downstaging (OR = 0.69; $P = 0.027$), while MSI-L tumors showed no significant impact. Neither MSI-L nor MSI-H status significantly affected N downstaging.

Conclusion: Despite a better overall prognosis of MSI tumors, they are less likely to achieve pathologic complete response and T downstaging following neoadjuvant treatment. Additionally, MSI-L and MSI-H tumors exhibit distinct clinical outcomes in rectal cancer, indicating they should be considered separate entities in clinical management.

#5943 The impact of radiotherapy on prognosis in microsatellite stable rectal cancer: A national analysis.

Mohyeddine El Sayed[1], Sassine Youssef[2], Raymond N. DuBois[1]

[1]Hollings Cancer Center, The Medical University of South Carolina (MUSC), Charleston, SC,[2]Department of Biochemistry, The Medical University of South Carolina (MUSC), Charleston, SC

Background: Approximately 80-85% of rectal cancer cases are microsatellite stable (MSS), indicating a functional mismatch repair (MMR) system. Rectal cancer patients with microsatellite instability (MSI) exhibit better survival rates, mainly due to enhanced treatment responses linked to high tumor mutational burden (TMB). Radiotherapy (RT) induces DNA damage, increasing TMB and immune recognition. We hypothesize that RT improves prognosis in MSS rectal cancer patients.
Methods: A retrospective study was conducted using the National Cancer Database (NCDB), including patients aged ≥20 years diagnosed with MSS rectal cancer between 2004 and 2020 with documented tumor stages. All patients underwent surgical resection, chemotherapy, and immunotherapy. Collected data encompassed demographics, tumor grade, TNM stage, KRAS mutation status, lymphovascular invasion, and perineural invasion. RT was categorized into short-course RT (SC-RT; 25 Gy) and long-course RT (LC-RT; 45-54 Gy). The impact of RT on overall survival was assessed using multivariable Cox proportional hazards regression, adjusting for variables significant in univariate analysis.
Results: Of the 460 patients included, 70.9% received RT, while 29.1% did not. RT recipients demonstrated a 40% reduction in mortality risk compared to non-recipients ($P = 0.001$). Specifically, SC-RT and LC-RT were associated with 39% ($P = 0.042$) and 40% ($P = 0.002$) lower mortality risks, respectively. In stage II-III patients, LC-RT significantly reduced mortality risk (HR: 0.26, $P < 0.001$), whereas SC-RT showed no significant impact. In stage IV patients, neither SC-RT nor LC-RT significantly affected prognosis.
Conclusion: Radiotherapy is associated with improved prognosis in MSS rectal cancer patients, particularly with long-course RT in stages II-III. These findings advocate for clinical trials to further evaluate the role of RT in MSS rectal cancers.

#5944 Trend and treatment pattern of vulvar cancer in a single academic hospital in South Africa.

Adekunle Emmanuel Sajo[1], Edwin Mnisi[1], Cathy Visser[1], Sheynaz Bassa[2], Greta Dreyer[1]

[1]Obstetrics and Gynaecology, University of Pretoria, City of Tshwane, South Africa,[2]Radiation Oncology, University of Pretoria, City of Tshwane, South Africa

The incidence of vulvar cancer is increasing among younger women mainly to the coinfection of HIV and HPV infections which drive majority of cases in sub-Saharan Africa. We aimed to describe the trend and treatment patterns of vulvar cancer at the Steve Biko Academic Hospital, South Africa. This was a retrospective cross-sectional study between 1st January 2012 and 31st December 2022. Data were extracted from the participants' medical records on the demographics, medical history, histologic characteristic and treatment. We analyzed 317 vulvar cancer cases. The mean age was 45±12 yrs. Of the patients, 40% were younger than 40yrs. More than 77% (248) were living with HIV (WLHIV) with a mean CD4 count of 481.8 cell/µL. Of the WLHIV, 82% were virally suppressed. 64% of cases presented in advanced stage (stages III & IV). During the study period, the average age decreased from 56yrs to 40yrs in a linear fashion, p 0.012 while the number of cases rose exponentially. There was a steady decline of cervical cancer cases during the study period in contrast to the rising vulvar cancer. When the study period was halved, the mean age was significantly lower in the later half, 46yys vs 44yrs, p 0.001. Among the cases, the proportion of women with HIV increased steadily from 20% in 2012 to highest proportion of 98% in 2021, p 0.001. 70% of those who had surgery (152) had radical vulvectomy with nodal dissection. Of the total who had radiotherapy (114), only 47% had curative radiotherapy. The mean radiotherapy (RT)completion time was 48 days. 16% (51) of the total patient did not receive any treatment due to ill-health or failure to present for RT. Table 1 shows the comparison of our study with a previous study from the institution (Mnisi et al 2019). Vulvar cancer has transitioned to be a disease of the young. HIV is a major risk factor for vulvar cancer in our environment. Adequate surveillance of the genital area and screening of at risk group might help to stem the tide.

Comparison with previous study from the University of Pretoria

	Mnisi et al 2019	Current study
Study period	2001-2013	2012-2022
Institution	Data from 2 hospitals	single institutional data
Average age	43yrs	45yrs
Proportion of HIV	43.7%	78%
Disease stage	35.9% were advanced	64% were advanced
Proportion of HIV positive below 40yrs	29.6%	98%
Tumour size >4cm	88.8%	78%
Positive nodes	36.7%	35.8%
Total number of cases	134	317
Treatment	40% had surgery	48% had surgery

#5945 Cancer care in rural America: factors associated with acute care utilization following chemotherapy at a community cancer center.

McKenna Deaton[1], Crystal Hattum[1], Sam Gaster[1], Samantha Olson[2], Sheena Lewis[1], Katherine Rehfeld[1], Christina Early[1], Lacey Roberts[1], Maren Ernst[1], Cynthia Kannenberg[1], Mary Hughes[1], Julia Althoff[2], Jamie Arens[1], Tobias Meissner[1]

[1]Avera Cancer Institute, Sioux Falls, SD,[2]Avera Health, Sioux Falls, SD

Background Acute care use (ACU) events, including emergency department visits and hospitalizations following chemotherapy, are costly and often preventable with outpatient interventions. The Centers for Medicare & Medicaid Services track ACU within 30 days of outpatient chemotherapy through the OP-35 measure. While predictive models exist to identify at-risk patients, few have been validated on diverse populations. We aimed to quantify the differences between those who experience ACU and those who do not. Patients and Methods In a retrospective study of 5,575 patients receiving chemotherapy between October 2021 and May 2024, we assessed ACU within 180 days of any chemotherapy visit using institutional records and claims data. Of these, 3,230 patients (57.9%) experienced an ACU event, with a median of 75 days between chemotherapy and ACU. Among those with an ACU event, 2,491 patients (77.1%) experienced an event within 30 days of chemotherapy, 509 (15.8%) within 90 days, and 230 (7.1%) by 180 days. Results Demographics and comorbidities differed significantly between groups. Patients with ACU events were older (66 vs. 64; $p < .0001$), more likely to be former smokers (38% vs. 33%; $p < .0001$) or alcohol users (14% vs. 9%; $p < .0001$), less likely to live rurally (28% vs. 31%; $p = .003$), more likely to use Medicare (59% vs 53%; $p <.0001$), and more likely to be mobility impaired (38% vs 17%; $p <.0001$). These patients were also more likely to have multiple primary tumors (40% vs. 23%; $p < .0001$) and had higher weighted Charlson Comorbidity Index scores (4.52 vs. 3.19; $p < .0001$). There were notable differences between groups in terms of prior hospital utilization. Patients with ACU had a greater number of previous inpatient admissions (7 vs. 4; $p < .0001$) and spent more days hospitalized per admission (4.7 vs. 3.8 days; $p < .0001$). There was also variation in laboratory markers. Patients with ACU events exhibited lower pre-chemotherapy albumin levels (3.83 vs. 4.03 g/dL; $p < .0001$) and higher neutrophil-to-lymphocyte ratios (3.16 vs. 2.79; $p < .0001$), suggestive of greater inflammation. A LASSO logistic regression model was applied to predict ACU within 30-day and 180-day windows, achieving prediction accuracies of 69% and 72%, respectively (AUC: 0.76 and 0.79). Conclusions These findings show various factors are associated with increased risk of ACU, which is essential for determining which patients may benefit most from proactive outpatient support to reduce ACU rates, drive patient satisfaction, and lower costs. This work will be applied prospectively to triage patients at highest risk. Additionally, future work will focus on distinguishing preventable from non-preventable ACU events.

#5946 Five-year real-world survival outcomes of patients with advanced non-small cell lung cancer receiving first-line immunotherapy-based regimens.

David Waterhouse[1], Amanda Shawky[2], Aanal Desai[2], Keith A. Betts[3], Manasvi Sundar[3], Reginald Villacorta[2], David Stenehjem[4]

[1]OHC (Oncology Hematology Care) / Sarah Cannon Research Institute, Cincinatti, OH,[2]Bristol Myers Squibb, Lawrenceville, NJ,[3]Analysis Group, Los Angeles, CA,[4]Department of Pharmacy Practice and Pharmaceutical Sciences, University of Minnesota, Minneapolis, MN

Background: Five-year real-world outcomes of first-line (1L) immunotherapy (IO)-based regimens for advanced non-small cell lung cancer (aNSCLC) were assessed to describe how real-world outcomes correspond to those in clinical trials.

Methods: Adult patients (pts) with aNSCLC from Flatiron Health database receiving 1L IO monotherapy or single-agent IO + chemotherapy from Jan 1, 2016 to Jun 30, 2024 were identified. Pts were excluded if they had *EGFR/ALK* mutations or unknown histology type. Overall survival (OS) and real-world progression-free survival (rwPFS) of each 1L treatment were described using Kaplan-Meier analyses.

Results: A total of 17,070 pts with aNSCLC were included (6,039 pts receiving IO monotherapy; 11,031 pts receiving IO + chemotherapy). Among pts receiving IO monotherapy, the 1-, 2-, 3-, 4-, and 5-year OS rates were 52.7%, 36.4%, 27.3%, 21.6%, and 17.5% and the rwPFS rates were 29.5%, 18.0%, 13.2%, 10.0%, and 7.8% respectively. Among pts receiving IO + chemotherapy, the 1-, 2-, 3-, 4-, and 5-year OS rates were 51.0%, 32.9%, 24.0%, 19.4%, and 15.8% and the rwPFS rates were 27.6%, 15.6%, 10.6%, 8.3%, and 6.9%, respectively. Table 1 presents the 5-year OS and rwPFS rates stratified by histology type, tumor programmed death ligand 1 (PD-L1) expression level, and Eastern Cooperative Oncology Group (ECOG) performance status. Pts with lower PD-L1 expression level (<1%), worse ECOG performance status (≥2), and squamous cell carcinoma had worse survival outcomes, regardless of the type of 1L treatment.

Conclusions: Five-year real-world survival rates for pts with aNSCLC treated with 1L IO-based regimens were lower than those observed in pivotal trials, indicating an unmet need for the management of newly-diagnosed aNSCLC. New strategies are needed to treat aNSCLC. This gap is especially pronounced in pts with squamous histology or PD-L1 <1% emphasizing the need for new treatment strategies for these high-risk groups.

Table 1: 5-year OS and rwPFS rates

	1L IO monotherapy			1L IO+chemotherapy		
	PD-L1<1% N=426	PD-L1 1-49% N=1,040	PD-L1≥50%N=3,861	PD-L1<1% N=3,399	PD-L1 1-49% N=3,265	PD-L1≥50%N=1,939
OS (5-year rate, %)	14.2	11.2	20.0	11.2	15.0	25.2
OS by histology type (5-year rate, %)						
Squamous	12.4	6.2	10.7	11.0	10.9	19.4
Non-squamous	15.0	14.3	23.1	11.3	16.1	26.2
OS by ECOG performance status (5-year rate, %)						
0-1	15.1	15.4	23.5	13.1	16.9	27.0
≥2	7.5	NA	11.1	3.6	8.8	13.9
rwPFS (5-year rate, %)	6.3	5.0	9.0	3.7	5.3	14.1
rwPFS by histology type (5-year rate, %)						
Squamous	7.6	2.9	5.1	4.2	6.0	10.6
Non-squamous	5.3	6.3	10.2	3.6	5.1	14.7
rwPFS by ECOG performance status (5-year rate, %)						
0-1	7.0	7.0	10.6	4.8	5.6	15.3
≥2	NA	NA	5.0	1.1	3.3	7.7

#5947 Efficacy and safety of lorlatinib in first-line and later-line treatment for Chinese patients: a real-world study with retrospective and prospective bidirectional cohorts.

Jiayu Liu, Xin Zhang, Puyuan Xing, Junling Li

Cancer Institute & Hospital.Chinese Academy of Medical Sciences, Beijing, China

Background: Lung cancer is the leading cause of cancer-related deaths worldwide. Lorlatinib, a third-generation ALK inhibitor, is an important treatment for ALK-positive NSCLC. Hyperlipidemia is a common side effect, but its mechanism is not well understood. This study evaluates the efficacy and safety of lorlatinib in the Chinese population and explores the mechanisms of hyperlipidemia.
Method: This study included ALK-positive advanced NSCLC patients treated with lorlatinib between February 2020 and September 2024 at the Cancer Hospital of the Chinese Academy of Medical Sciences. Patients received lorlatinib either as first-line or later-line therapy. Data collected covered demographics, treatment efficacy (ORR, DCR), and survival outcomes using the Kaplan-Meier method. Safety analyses, focusing on hyperlipidemia, were performed with Fisher's exact test.
Result: This study included 63 ALK-positive advanced NSCLC patients, with 26 on first-line and 37 on later-line lorlatinib treatment. Most patients were female (55.6%), nonsmokers (66.7%), and had an ECOG score of 0-1 (98.4%). The median age was 57, and adenocarcinoma was the predominant type (87.3%). The median follow-up was 9.86 months. For first-line patients, the ORR was 85.7%, DCR was 100%, and the 12-month PFS rate was 94.4%, with median PFS not yet reached. For later-line patients, the ORR was 26.7%, DCR was 86.7%, and the 12-month PFS rate was 60.3%, with median PFS also not reached. Among 28 patients with baseline brain metastases (6 on first-line and 22 on later-line therapy), the intracranial ORR was 41.7%, DCR was 100%, and the median intracranial PFS was 30.59 months. The study found that all patients experienced hypercholesterolemia, with most also developing hypertriglyceridemia. Common side effects included peripheral edema, CNS effects, hypertension, weight gain, neuropathy, and joint pain. One patient discontinued lorlatinib due to grade III proteinuria. Patients without brain metastases had milder hypercholesterolemia, while severe hypercholesterolemia was more common in those with brain metastases (37.04% vs. 12.50%, P=0.03). Lipid metabolism testing in 12 patients (5 with brain metastases) showed no significant differences in enzyme levels between patients with and without brain metastases. However, patients on lipid-lowering therapy (n=9) had significantly improved LPL activity compared to untreated patients (n=3) (t=2.69, P=0.038), confirming the effectiveness of lipid-lowering therapy.
Conclusion: Lorlatinib shows strong efficacy for ALK-positive advanced NSCLC, with median PFS not yet reached in both first-line and later-line settings. Hyperlipidemia was common, especially in patients with brain metastases, and lipid-lowering therapy improved LPL activity. Further research into lipid metabolism mechanisms is underway.

#5948 Sequencing of antibody drug conjugates (ADCs) in metastatic breast cancer: A real-world analysis.

Dhairya Gor[1], Maria Jamil[2], Sunita Ghosh[2], Vrushali Dabak[1]

[1]Henry Ford Cancer Institute, Detroit, MI,[2]Henry Ford Hospital, Detroit, MI

Background- Hormone Receptor-Positive/HER2-low (HR+/HER2-low) and Triple Negative Breast Cancer (TNBC) represent distinct subtypes of breast cancer with varying treatment approaches and clinical outcomes. Trastuzumab Deruxtecan (T-Dxd) and Sacituzumab Govitecan (SG), the two ADCs, have emerged as promising therapeutic options for both subtypes. However, there is a lack of real-world data comparing the effectiveness of these agents in different treatment sequences. This retrospective study aims to compare the sequencing of T-Dxd and SG in HR+/ HER2-low and TNBC patients at Henry Ford Cancer Institute(HFCI), with a primary focus on median progression-free survival (mPFS).

Methods -The electronic medical records of patients at HFCI from January 2020 to August 2024 were searched to identify patients diagnosed with metastatic HR+/HER2-low and TNBC who had received both SG and T-Dxd. Patients with incomplete medical records were excluded. Based on the tumor type and sequencing of treatment, patients were divided into four groups: 1) HR+/HER2-low who received T-Dxd followed by SG, 2) HR+/HER2-low who received SG followed by T-Dxd, 3) TNBC who received SG followed by T-Dxd, and 4) TNBC who received T-Dxd followed by SG. The mPFS, median overall survival (mOS), overall response rate (ORR), and disease control rate (DCR) for each of these four groups were plotted using the Kaplan-Meier Method.

Results- A total of 33 patients were initially identified. Of these, 14 patients were HR+/HER2 low (median OS = 18 months, 95% CI 13.737 - 22.263), 14 were TNBC (median OS was 17 months, 95% CI 12.16 - 21.84), and 5 were excluded. Amongst Group 1 patients (n=12), mPFS after receiving T-Dxd was 7.09 months, mOS was 18.07 months, ORR was 33%, and DCR was 75%. On progression, they received SG with mPFS of 3.08 months, mOS of 5.61 months, and DCR of 40%. Amongst Group 2 patients (n=2), mPFS after receiving SG was 1.15 months, and mPFS after receiving sequential T-Dxd was 2.07 months. Amongst Group 3 patients (n=11), the mPFS after receiving SG was 5.06 months, mOS was 15.08 months, ORR was 22.2%, and DCR was 66.7%. Those who progressed and received T-Dxd had an mPFS of 2.16 months, an mOS of 6.3 months, and a DCR of 22.2%. Amongst Group 4 patients (n=3), mPFS after receiving T-Dxd was 7.95 months, mOS was 17.97 months, ORR was 33.3% and DCR was 100%. Those who progressed and received SG had mPFS of 3.08 months, mOS of 5.61 months, and DCR of 40%.

Conclusion- Our retrospective study presents real-world data on the effect of sequencing the ADCs. The overall benefit observed is lesser when a second ADC is used after the first ADC. Contrary to common practice, using T-Dxd followed by SG sequencing, regardless of receptor status, appears more effective in our patient population than the reverse. Further studies analyzing the sequencing of ADCs prospectively with larger cohort populations and identifying biomarkers to aid patient selection can be conducted.

#5950 Assessment of response and overall survival (OS) for various systemic therapies in *ERBB2*-altered advanced urothelial carcinoma (aUC).

J. B. Leary[1], **T. L. Enright**[1], D. Bakaloudi[1], R. Talukder[2], M. T. Schweizer[1], J. E. Hawley[1], B. Montgomery[1], E. Y. Yu[1], A. C. Hsieh[1], T. A. Yezefski[1], P. Grivas[1], A. Khaki[3], R. Raychaudhuri[1]; [1]University of Washington, Seattle, WA, [2]Baylor College of Medicine, Houston, TX, [3]Stanford University, Stanford, CA

Background: A subset of aUCs harbor *ERBB2* alterations. While anti-HER2 therapies in HER2-over-expressing aUC have been promising, relationships between *ERBB2* alterations and response to other systemic therapies are unclear. Based on available data, we hypothesized that *ERBB2*-altered aUC would respond less favorably to immune checkpoint inhibitor (ICI), enfortumab vedotin (EV), and sacituzumab govitecan (SG) than *ERBB2*-wildtype aUC.

Methods: This single-center retrospective study included patients (pts) diagnosed with aUC between 5/6/2016 and 9/23/2022 who underwent panel-based somatic tumor sequencing (120 tissue-based, 9 ctDNA) and received ICI, EV, or SG in first line (1L) or as salvage (2L+). Pts were grouped as *ERBB2*alt aUC, including tumors harboring *ERBB2* amplification (n=13) and/or *ERBB2* mutation (n=9), and *ERBB2*-wildtype(wt) aUC (n=107). Observed response rate (ORR) and OS measured from each therapy start were evaluated. Kaplan-Meier method was used to calculate OS curves. Log-rank and Fisher's exact tests were used to compare OS and ORR between groups, respectively.

Results: 129 pts were evaluable for *ERBB2* status; 22 (17%) were *ERBB2*alt. Baseline characteristics including age, sex, prior extirpative surgery rate, ECOG performance status, proportion of pts with liver metastases, and presence of *de novo* metastasis were similar between groups. One pt with *ERBB2*alt aUC received 1L EV; none received 1L SG. Results are shown in Table 1.

Conclusions: In pts receiving 2L+ ICI, significantly higher ORR and longer OS were noted in pts with *ERBB2*alt vs *ERBB2*wt aUC, contrary to our hypothesis; *ERBB2*alt was also associated with higher ORR to 2L+ EV. Limitations include retrospective design, limited sample size and power for subsets, lack of randomization, selection/confounding biases, and lack of HER2 protein IHC assessment. Results are hypothesis-generating and need external validation.

Table 1. ORR and OS analyses by group

		N	ORR %	OR (95%CI)	p	mOS, months(95%CI)	HR(95%CI)	p
1L ICI	*ERBB2alt*	10	40.0% (4/10)	1.50 (0.37 - 6.05)	0.714	30.0 (7.6 - NR)	0.64 (0.27 - 1.52)	0.3
	ERBB2wt	52	30.8% (16/52)			14.0 (8.5 - 29)		
2L+ ICI	*ERBB2alt*	7	57.1% (4/7)	7.40 (1.27 - 44.00)	0.034	29.2 (4.1 - NR)	0.34 (0.12 - 0.98)	0.046
	ERBB2wt	33	15.2% (5/33)			7.2 (4.1 - 19.8)		
2L+ EV	*ERBB2alt*	9	56.0% (5/9)	6.75 (1.33 - 34.27)	0.026	20.0 (6.9 - NR)	0.66 (0.26 - 1.66)	0.4
	ERBB2wt	32	15.6% (5/32)			11.0 (8.7 - 27.4)		
2L+ SG	*ERBB2alt*	5	40.0% (2/5)	4.44 (0.52 - 38.61)	0.210	38.0 (10.4 - NR)	0.33 (0.07 - 1.54)	0.2
	ERBB2wt	23	13.0% (3/23)			10.4 (6.7 - 25.1)		

#5951 Comparison of abdominal CT alone versus combined chest and abdominal CT on recurrence detection and mortality following gastrectomy for gastric cancer.

Chi Hoon Maeng[1], Yeonjin Kim[2], Hyun Ae Jung[3], Moon Ki Choi[4], Jung Yong Hong[3], Bo-Hyung Kim[5], Woojoo Lee[2]

[1]Hematology-Medical oncology, Kyung Hee University Hospital, Seoul, Korea, Republic of,[2]Public Health Science, Graduate School of Public Health, Seoul National University, Seoul, Korea, Republic of,[3]Hematology-Oncology, Samsung Medical Center, Seoul, Korea, Republic of,[4]Medical oncology, National Cancer Center, Ilsan, Korea, Republic of,[5]Clinical Pharmacology and Therapeutics, Kyung Hee University Hospital, Seoul, Korea, Republic of

Introduction
Recurrence patterns after gastrectomy for gastric cancer are diverse, with intraperitoneal recurrences being common and extraperitoneal also occurring. The optimal surveillance strategy remains unclear. This study examines the clinical significance of follow-up with abdominal CT (computed tomography) alone versus combined chest and abdominal CT.
Methods
We analyzed data on surgery and follow-up CT scans for patients who underwent curative gastrectomy for gastric cancer between 2014 and 2018. The data were sourced from the Cancer Public Library Database, a nationwide public registry established under the Korean Clinical Data Utilization for Research Excellence Project (K-CURE). This newly launched registry is dedicated to research, providing comprehensive clinical data, including information on cancer diagnosis, surgery, summary staging, medications, diagnostic tests, comorbidities, and mortality. Patients were categorized into two groups based on surveillance CT patterns: those followed with abdominal CT alone (Group AA) and those with combined chest and abdominal CT (Group CA). Recurrence detection and all-cause mortality were compared between the groups. Considering variations in CT usage patterns over time, a time-varying Cox model was applied for group comparisons. The analysis was adjusted for key clinical factors, including age, sex, summary stage, co-morbidities, and hospital location.
Results
Of the initial 69,090 patients, 50,813 were analyzed after excluding those with multiple primary cancers, missing, or unclear data. Among them, 44,488 were in Group AA and 6,325 in Group CA. Crude recurrence detection rate was higher in Group CA (13.17%, n=833) compared to Group AA (7.57%, n=3,368), and crude all-cause mortality was also higher in Group CA (21.47%, n=1,358) than Group AA (11.49%, n=5,110). Time-varying Cox regression analysis showed a significantly higher recurrence detection rate in Group CA (HR 1.21, 95% CI 1.12-1.30). Additionally, among patients with recurrence, Group CA was associated with a significantly lower mortality (HR 0.80, 95% CI 0.73-0.87) compared to Group AA. Subgroup analysis revealed higher recurrence detection rates in Group CA across all stages, with a significantly reduction in mortality among locally advanced-stage patients.
Conclusions
This study demonstrated that combined chest and abdominal CT surveillance, compared to abdominal CT alone, improves recurrence detection regardless of stage and contributes to reduced mortality, particularly among patients with recurrent cancer at locally advanced stages. Prospective studies are warranted to establish an evidence-based surveillance strategy following gastrectomy.

#5952 Modulating treatment outcomes of patients with solid tumors in immunotherapy trials: a drug interaction analysis from a phase I unit.

Camila Braganca Xavier, Clark Andersen, JoAnn T. Lim, Julian H. Slade, Stacie A. Bean, Lei Kang, Hung Le, Apostolia Tsimberidou, Aung Naing, David Hong, Ecaterina Dumbrava, Jordi Rodon, Paula Pohlmann, Sarina Piha-Paul, Stephane Champiat, Timothy A. Yap, Tin-Yun Tang, Funda Meric-Bernstam, Siqing Fu

The University of Texas MD Anderson Cancer Center, Houston, TX

The concurrent use of medications can either compromise or enhance treatment (Tx) outcomes in patients with solid tumors (pts) receiving immunotherapy compounds (IO) including monoclonal antibodies (Abs), fusion proteins (FP), immune modulators (IM), oncolytic viruses (OV) and vaccines (VAC). To assess the impact of concurrent medications on Tx outcomes, we conducted a retrospective analysis of pts enrolled in early IO clinical trials at the Department of Investigational Cancer Therapeutics (ICT) at MD Anderson Cancer Center between June 2014 and June 2024. A computerized data extraction tool was used to collect clinical data and identify the prescription of a predefined set of drugs within 30 days of the experimental IO infusion. Primary endpoints were median overall survival (mOS) and median progression-free survival (mPFS), defined as the time from first Tx cycle to death/last follow up or disease progression/death from any cause, respectively. Tumor responses were assessed according to RECIST; disease control rate (DCR) was defined as the proportion of pts achieving partial response (PR) or stable disease (SD) as the best response. Uni and multivariate analysis were performed to correlate variables and outcomes. A total of 827 pts were included (49.3% female, 50.7% male), with the median age of 58 years (range 20-85). The majority of pts (72.9%) were White/Caucasian. The most common tumor types were colorectal (24.5%), head/neck (10.5%) and pancreatic (9.4%), and 85.7% of pts had >2 metastatic sites. The most commonly used therapies were Abs and FP (64.7%) and IM (20.8%), followed by IM + Abs combination (9.2%), OV and VAC (5.3%). The median time from the first appointment to death was 9.26 mo (range 0.85-112). mOS was 6.14 mo (range 0.16-103) and mPFS 2.07 mo (range 0-37.8). Of 744 pts with evaluable response data, the DCR was 38.7%. For IM + Abs, SD and PR were 24% and 6% without antihistamines (AH) and 52% and 15.8% with AH (X2 8.48; p = 0.014). Univariate analyses identified higher body mass index (BMI) and cancer type (immune responsive *versus* immune insusceptible) associated with improved OS while low albumin level, presence of cancer pain, and use of steroids, proton pump inhibitors (PPI), antibiotics (ATB), anti-depressants, anti-emetics, anticoagulant and narcotics were related to worse OS ($p < 0.05$). Multivariate analysis adjusted for BMI, cancer type, albumin level and response to Tx revealed that AH were associated with improved OS (HR 0.752 [0.603,0.938], p = 0.012) while steroids (HR 1.413 [1.101,1.814], p = 0.007), PPI (HR 1.318 [1.02,1.703], p = 0.035) and ATB (HR 1.542 [1.222,1.947], $p < 0.001$) were related to worse OS. In conclusion, concurrent use of medications must be carefully evaluated as part of clinical trial protocols for experimental IO. Optimizing the management of drug prescriptions could improve patient outcomes and enhance the efficacy of experimental Tx.

#5954 Real-world (RW) second-line (2L) treatment (tx) patterns and clinical outcomes in patients (pts) with HER2-overexpressing biliary tract cancer (BTC).

Richard Kim[1], Xiaozhou Fan[2], Javier Sabater[3], Wayne Su[2], Kathleen Hurwitz[4], Kayla Hendrickson[4], Catherine Wiener[4], Phillip Garfin[5], Joan Zape[2], Mark A. Ozog[5], John Bridgewater[6], Juan W. Valle[7], **Farshid Dayyani**[8]

[1]Moffitt Cancer Center, Tampa, FL,[2]Jazz Pharmaceuticals, Philadelphia, PA,[3]Jazz Pharmaceuticals, Oxford, United Kingdom,[4]Target RWE, Durham, NC,[5]Jazz Pharmaceuticals, Palo Alto, CA,[6]UCL Cancer Institute, London, United Kingdom,[7]Cholangiocarcinoma Foundation, Herriman, UT,[8]University of California Irvine Chao Family Comprehensive Cancer Center, Orange, CA

Background: HER2 has emerged as a target for precision therapies in BTC. We describe the RW tx patterns and overall survival (OS) of pts with advanced HER2-overexpressing BTC treated in the 2L.

Methods: This retrospective observational study used the Flatiron Health Electronic Health Record-derived, deidentified database (01/2011 to 12/2023). Pts with HER2-overexpressing (IHC 3+) BTC who received 2L tx ≥6 months (mo) of potential follow-up time were included in the OS analysis. Follow-up began at initiation of 2L tx (index) to the earliest of last recorded activity, death, or end of data. Concordance between HER2 overexpression and amplification was assessed in all pts, regardless of HER2 status, with available testing results from IHC, (F)ISH, and NGS .

Results: Among 29,817 pts with BTC, 36 pts were included in the OS analysis; median age was 67 (range: 49-83) years. 47% of pts received 2L HER2-targeted therapy including pertuzumab + trastuzumab (HP; n=7), trastuzumab + chemotherapy (n=6), trastuzumab deruxtecan (n=2), HP + chemo (n=1), and HP + anti-PD-(L)1 (n=1). Non-HER2-targeted therapies included chemo only (n =14), chemo + anti-PD-(L)1 (n=3), and non-HER2 tyrosine kinase inhibitors ± bevacizumab (n=2). Diabetes and chronic pulmonary disease were more frequent in pts who received non-HER2-targeted therapy (Table). Median OS was 9.9 (95%CI 5.5-29.4) mo overall (HER2-targeted : 13.2 mo; non-HER2-targeted : 8.5 mo; and chemo only: 5.6 mo). Median OS was 8.7 mo in pts with ECOG 0 or 1 (n=21). Among the 80 pts in the concordance analysis, 79% and 90% of pts with HER2 overexpression had HER2 amplification as detected by NGS and (F)ISH, respectively.

Conclusions: The OS of pts with HER2 overexpression in the RW who received chemo only was consistent with previously reported OS for chemo only in 2L BTC regardless of HER2 status. In pts with HER2-overexpressing BTC, HER2-targeted therapy showed a numerical improvement in OS vs. chemo only.

	OverallN=36	HER2-targetedn=17	Non-HER2-targetedn=19	chemo only n=14	ECOG 0-1n=21
Median age (years)	67	66	68	68	66
Female (%)	67	65	68	71	67
ECOG 0-1 (%)	75	69	80	83	100
Anatomical subtype (%)					
GBC	61	65	58	71	62
iCCA	31	23	37	29	33
eCCA	8	12	5	0	5
Diabetes (%)	50	24	74	64	38
Chronic pulmonary disease (%)	36	29	42	43	33
Stage III-IV at initial dx (%)	78	69	86	80	73
Index year 2017-2023 (%)	92	94	90	93	95

#5955 A decade of discovery: Unveiling the prevalence, patient characteristics and histopathologic features of early-onset prostate cancer at a West African tertiary institution.
P. O. Commey;
Korle Bu Teaching Hospital, Accra, Ghana

BACKGROUND: Prostate cancer is commonly regarded as a disease that affects older men, with the average age of diagnosis being 66 years. A notable rise has been seen among men aged 55 years and below (early onset). This demographic shift, particularly pronounced in Sub-Saharan Africa highlights the urgent need for targeted research. The study aimed to determine the prevalence and describe the patient characteristics and histopathologic features associated with prostate cancer in focusing on a demographic that has been relatively underrepresented in prostate cancer research.
DESIGN: A retrospective descriptive study was conducted to evaluate histologically confirmed cases of prostate cancer in men aged 55 years and below at the National Radiotherapy, Oncology, and Nuclear Medicine Centre at the Korle Bu Teaching Hospital in Accra. We used secondary data from medical records over 10 years (2013 to 2022). Data were analyzed using STATA 14.
RESULTS: There were 81 cases of prostate cancer in men aged 55 years and below, comprising 4.5% of all prostate cancers diagnosed over 10 years. The average age at diagnosis was 52 years, ranging from 38 to 55 years. Of these, 98.8% were adenocarcinomas, and 1.2% represented a spindle cell sarcoma. Regarding the Gleason scores, most patients (53.1%) had a primary Gleason grade of 3, followed by 32.1% with a grade of 4. A smaller proportion had grades 5 or 2, and 3.7% of records did not report the primary Gleason grade. The most common secondary Gleason grade was also 3, found in 53.1% of cases, followed by grade 4 in 33.3%. Fewer patients had secondary grades of 5 or 2, and 2.5% of records lacked this information. Grade group 1 accounted for the most frequent pattern, observed in 41.8% of the cases, followed by grade group 4 in 16.5% of cases. The rest of the grade groups, including 2, 3, and 5, were present less frequently. Out of the 81 patients, the majority (48.15%) had evidence of perineural invasion, while 41.98% did not. A small percentage of records (9.88%) did not state whether perineural invasion was present. Interestingly, there was evidence of metastasis in 23.5% of the patients. The most frequently involved metastatic sites were the thoracolumbar vertebrae (14.9%), femur (12.8%), pelvis (12.8%), and liver (8.5%).
CONCLUSION: Given the distinct clinical features and the relatively high prevalence of metastasis in this age group, further research is needed to better understand the underlying biology of early-onset prostate cancer and to devise tailored diagnostic and therapeutic regimen for this unique cohort.

#5956 Glioblastoma in Appalachia: A retrospective analysis.

Umer Rizwan, Gary Monroe, **Sonikpreet Aulakh**

West Virginia Univ. School of Medicine, Morgantown, WV

Introduction: Glioblastoma (GBM) is a fatal malignancy of the brain. It has been observed that patients in rural areas with GBM have worse overall survival. Higher morbidity and mortality in these rural areas have been attributed to lack of optimal access to clinical care and lack of participation in clinical trials. However, the projected number of cases and mortality risk related to GBM do not reflect the reality of Appalachian region. In this retrospective study, we reviewed the survival data of patients with GBM in Appalachia.

Methods: 169 (of 761) patients with grade 4 *IDH* WT GBM from 1/16/2019 through 7/1/2024 were selected for this retrospective study. Patient data, including demographics, insurance status, comorbidities, tumor location, diagnosis date, treatment regimens, treatment start dates, progression dates, *MGMT* methylation status, genetic mutations, and date of mortality, were extracted from electronic medical records and stored in RedCap in a HIPAA compliant manner. De-identified data was exported, organized and processed using Microsoft Excel Version 2409. Progression-free survival (PFS) and overall survival (OS) were calculated using standard Excel functions. A Cox proportional hazards model was employed to estimate the hazard ratios and corresponding p-values.

Results: The median age of GBM diagnosis was 63.4 years. 91 patients were males and 78 were females. 164 patients were Non-Hispanic White. 96 patients with Medicaid, 53 had no insurance, and 11 had Medicare. The mean PFS was 22 months (mo) for the 1st line therapy (n=166), 12 mo for 2nd therapy (n=83), 10 mo for 3rd line therapy (n=28), and 16 mo for the targeted therapy (n=46). The mean OS was 22 mo for first-line therapy (n=61), 19 mo for first/second-line therapy (n=39), and 22 mo for 1st/2nd/targeted therapy (n=20). A significant association was found between RT-TMZ and increased mortality (95% CI 1.2-5.39, p=0.015) compared to RT-TMZ-surgery. In the 2nd line setting, no significant differences in PFS or OS were observed between bevacizumab+lomustine (n=7), bevacizumab+surgery (n=9), bevacizumab +temozolomide (n=8), and bevacizumab (n=41). In the 3rd line setting, a significant difference in PFS was observed between lomustine (95% CI 1.15-48.9, n=4) and bevacizumab (n=11), but not between bevacizumab and bevacizumab+lomustine (n=3). In the 2nd line targeted therapy setting, no significant differences in PFS or OS were observed between osimertinib (n=2), pembrolizumab (n=2), regorafenib (n=3), and abemaciclib (n=3). In the 3rd line targeted therapy setting, no significant differences in PFS or OS were observed between ipilimumab-nivolumab (n=3) and abemaciclib (n=2).

Conclusion: Our preliminary data suggests that mortality related to GBM in Appalachia follows the national trends. It is prudent to conduct rural-specific studies to understand the phenotypic, genotypic, and demographic characteristics.

#5957 A real-world study for cost-effectiveness analysis of nanoliposomal irinotecan in patients with pancreatic adenocarcinoma.

J. Chun[1], J. Lee[1], D. Jang[2], Y. Lee[3], J.-C. Lee[4], J.-H. Hwang[4], B.-G. Kim[1], S. Kim[1], G. Park[1], T. Kim[1], Y. Kim[1], S. Woo[1];
[1]National Cancer Center, Goyang-si, Korea, Republic of, [2]Seoul National University Boramae Medical Center, Seoul, Korea, Republic of, [3]Inje University Ilsan Paik Hospital, Goyang-si, Korea, Republic of, [4]Seoul National University College of Medicine, Seoul National University Bundang Hospital, Seongnam, Korea, Republic of

Background: Systemic chemotherapy plays a crucial role in pancreatic adenocarcinoma (PAC), not only as palliative chemotherapy for unresectable PAC, but also as (neo-)adjuvant chemotherapy for (borderline) resectable PAC. Recently, nano-liposomal irinotecan plus 5-fluorouracil/leucovorin (nal-IRI/5-FU/LV) showed a survival benefit in the global phase 3 NAPOLI-1 trial. However, there is a paucity of studies that have evaluated the effectiveness and cost analysis of nal-IRI in the entire treatment course of PAC. Therefore, this study aimed to evaluate the effectiveness and cost of treatment according to exposure to nal-IRI in the management of PC using an electronic health record data.
Methods: We analyzed the effectiveness and cost according to the exposure of nal-IRI/5-FU/LV treatment during follow-up. Effectiveness was assessed by overall survival (OS) and direct medical costs including costs of imaging or laboratory tests, administration of chemotherapy, surgery, or radiotherapy, supportive care, management of adverse events during inpatient, outpatient, and emergency department were included in this study. Propensity score matching was used to adjust for potential differences in patient characteristics between nal-IRI group and non-liposomal irinotecan group. We defined the incremental cost-effectiveness ratio as the difference in mean total medical costs (per patient) divided by the difference in median OS between two groups.
Results: A total of 1,734 patients diagnosed with PAC were screened, and 705 patients who received at least one cycle of chemotherapy were included in the analysis. Median OS was significantly longer in the nal-IRI group compared to the non-liposomal irinotecan group (28.0 months vs. 18.3 months, P = 0.011). Patients in the nal-IRI group had more hospital visits and admissions during follow-up, the average total cost per patient was was higher in the nal-IRI group ($61,430 vs $39,129). The incremental cost-effectiveness ratio was $2,787 per month of median survival gained ($33,285 per life-year gained).
Conclusions: In this real-world retrospective study, patients treated with nal-IRI/5-FU/LV showed longer OS and the incremental cost-effectiveness ratio was acceptable given the poor prognosis of PAC.
Funding: This work was supported in part by Servier No. 2152460) and in part by the National Cancer Center, Korea (No. 2310280).

#5959 Outcomes of small HER2+ breast cancers including those with micro-metastatic lymph node involvement (SOLEMN HER2).

Uttamjot Kaur[1], Jacob M. Liberman[2], Ajay Dhakal[1]

[1]Department of Hematology and Oncology, University of Rochester Wilmot Cancer Institute, Rochester, NY,[2]University at Buffalo, Buffalo, NY

Background: HER2-positive breast cancer (HER2+BC) accounts for 15-20% of early-stage breast cancers and is treated based on tumor size and Stage. For locally advanced HER2+BC [Stage (AJCC 8th ed, Anatomic) II, III], patients typically receive 2-3 chemotherapy drugs plus two HER2-targeted monoclonal antibodies (HER2-MABs): trastuzumab (T) and pertuzumab (P) before surgery. Stage I cancers are treated differently. Stage IA tumors(≤2 cm, no lymph node involvement) usually receive one chemotherapy [paclitaxel (Tax)] plus T after surgery. However, it's unclear if chemotherapy is needed for a subset with very small tumors [T1a (≤5 mm), T1b (>5 - ≤10 mm)]. Stage IB cancers (≤2 cm tumor with micrometastases in axillary lymph node) may require more aggressive treatment. This study aims to describe various demographic, clinical, pathological characteristics, perioperative treatments, outcomes, including overall survival (OS) and invasive disease-free survival (IDFS) for patients with Stage IB (Cohort A) and very small (T1a/b) Stage IA (Cohort B) HER2+BC.
Methods: This is a single institution retrospective study conducted at URMC with IRB approval. It uses electronic medical records for data collection. Patients diagnosed with Stage IB or very small (T1a/b) Stage IA HER2+BC between January 2010 - June 2024 were included in the study. Patients who had received systemic therapy before surgery were excluded.
Results: Results from Cohort A are submitted in this abstract. Among seven patients included in the study, 43% had a pathological stage pT1a, 14% were pT1b and 43% were pT1c. Four patients received Tax+ T, one patient received Tax+T+P, one received two chemotherapies+T, and one received two chemotherapies+ T+P. There were no recurrence or death after a median follow-up of four years. Investigation into Stage IA cases (n= 91) is ongoing.
Conclusions: This small retrospective study suggests an excellent clinical outcome for Stage IB HER2+ breast cancer patients. A larger study is needed to validate this finding.

#5960 Outcomes of acute myeloid leukemia treated with a hypomethylating agent and venetoclax.

Markie Zimmer[1], Brigid Jacob[2], Mikayla Spica[1], Sunita Ghosh[3], Vrushali Dabak[1]

[1]Hematology/Oncology, Henry Ford Hospital, Detroit, MI,[2]Henry Ford Hospital, Detroit, MI,[3]Department of Public Health, Henry Ford Hospital, Detroit, MI

Introduction: VIALE-A confirmed the benefit of azacitidine (AZA) and venetoclax (VEN) compared to AZA alone in previously untreated patients with AML who were not eligible for intensive induction chemotherapy. Incorporation of this regimen into standard practice has varied across and within healthcare systems due to adjustments in regimen and supportive care use.
Methods: This was a retrospective, single institution analysis of adult patients greater than 18 years of age with AML, treatment related AML (t-AML) or MDS/AML treated with a hypomethylating agent (HMA) (azacitidine or decitabine) and VEN between January 2017 and January 2024. We sought to investigate overall survival (OS) and to explore the trends of regimen and prophylaxis dosing, the use of allogeneic stem cell transplant and outcomes of relapsed and refractory disease.
Results: Thirty patients received VEN and HMA between January 2017 and January 2024. The median age was 72 years. Ten patients were female and 20 were male. Twenty-four patients were Caucasian, 5 were African American and one patient's race was not recorded. Twenty-three patients were diagnosed with AML, 5 t-AML and 2 MDS/AML. Risk stratifying patients by ELN 2022 criteria, 5 (16.7%) were favorable, 6 (20%) were intermediate and 17 (56.7%) were adverse. Within the adverse risk group, 6 had mutated TP53. Patients completed a median of 3 cycles. The mean duration of VEN decreased throughout each cycle, from 21.60 days in cycle 1, to 15.38 in cycle 2, to 8.62 in cycle 3 and 8.10 in cycle 4. The median number of days between the starting of subsequent cycles generally increased (34.5 days from cycle 1 to cycle 2; 39 days from cycle 2 to cycle 3; 42 days from cycle 3 to cycle 4). Twenty-one patients received the treatment in the front-line setting, 7 as salvage therapy and 2 as salvage after allo-HSCT. Three patients proceeded to allo-HSCT a median of 179 days after initial diagnosis. Of all 30 patients, 9 (30%) were in remission, 20 (66.7%) had active disease and one patient's disease status was unknown at the time of data collection. Mean overall survival was 22.67 months (5.92-39.42, 95% CI, p=0.346). Caucasians had greater than 6 times OS than African Americans, (26.12 months, 10.42-41.82, 95% CI versus 4.44 months, 0-10.85, 95% CI, p=0.015). Frontline use of the regimen resulted in a numerically significant difference in OS compared to salvage use (22.67 versus 4.53 months, p=0.200).
Conclusions: In this retrospective analysis of VEN and HMA in patients with AML treated in an urban setting, we found that younger patients, Caucasians, favorable risk disease by ELN 2022 criteria and frontline use resulted in the highest OS. Overall, patients received decreasing number of days of VEN each cycle and had increasing number of days between cycles, suggesting significant treatment reduction and delays, however without compromise to overall survival.

#5961 MRI-based sternocleidomastoid muscle assessment as a predictive marker for sarcopenia and survival outcomes in nasopharyngeal carcinoma patients undergoing concurrent chemoradiotherapy.

Jeng-You Wu[1], Emily Chia-Yu Su[2]

[1]Graduate Institute of Biomedical Informatics, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan,[2]Institute of Biomedical Informatics, College of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan

Purpose/Objective(s): Sarcopenia is strongly linked to poor survival outcomes and increased treatment-related toxicity in solid tumors. Both pre- and post-treatment muscle loss negatively impact prognosis, emphasizing the importance of muscle mass evaluation. This study investigates the sternocleidomastoid (SCM) muscle, an accessible target in head and neck examinations, as a predictive marker for survival outcomes in nasopharyngeal carcinoma (NPC). Using MRI-based assessment, this study aims to establish SCM's utility in prognosis prediction and improve patient management.

Materials/Methods: This multi-institutional study analyzed 976 NPC patients treated at three hospitals between 2002 and 2019. Among them, 504 newly diagnosed patients underwent concurrent chemoradiotherapy, and complete pre- and post-treatment MRI data were available for 286. SCM muscle analysis was conducted using post-contrast T1-weighted MRI at the third cervical vertebra (C3). Measurements included cross-sectional area (SCMA), long axis (SCML), and short axis (SCMS). Derived parameters included SCMAD (SCML + SCMS), SCMAA (SCML × SCMS), and SCMR (SCMS/SCML). Ratios of post/pre-treatment values were calculated to reflect muscle loss. Optimal cutoff points for sarcopenia-related parameters were determined, and predictors of overall survival (OS) were evaluated using Cox proportional hazards regression and Kaplan-Meier survival analysis to visualize OS differences among groups stratified by sarcopenia status.

Results: Among the 286 patients analyzed, 63% were male, and 51.2% were diagnosed between 40 and 60 years old. Advanced stages were common: 25.8% stage III and 40.7% stage IV. Over a median 4.7-year follow-up, 93 patients (31.8%) died. OS rates were 93.2% at one year and 68.9% at five years. Post/pre-treatment SCM parameters (SCMA, SCMS, SCMR) showed no statistically significant results. However, certain parameters approached significance: SCML (cutoff 0.75, $p = 0.1147$), SCMAD (cutoff 0.47, $p = 0.338$), and SCMAA (cutoff 0.26, $p = 0.328$), suggesting trends warranting further investigation.

Conclusion: BMI alone may inadequately reflect muscle loss, whereas SCM parameters measured via MRI offer a reliable method to assess sarcopenia and predict survival. Our findings indicate that SCM provides effective metrics for prognosis in NPC, but further studies with larger samples are needed to identify the most impactful predictive parameter and optimize its clinical utility.

#5962 A multicenter propensity score matched analysis of short and long term outcomes of octogenarian patients undergoing pancreaticoduodenectomy for extrahepatic cholangiocarcinoma.

Y. Choi, H.-S. Jo, D.-S. Kim, Y.-D. Yu;
Korea University, College of Medicine, Seoul, Korea, Republic of

Background: Elderly patients with cancer are at a crossroads to receive either invasive surgical resection or palliative treatment. Due to its invasive nature, surgeons are often reluctant to perform pancreaticoduodenectomy in patients with advanced age, especially octogenarian patients. However, due to enormous advancements in surgical technique and postoperative management, elderly patients with extrahepatic bile duct cancer should also be expected to receive survival benefits from pancreaticoduodenectomy. The aim of this study was to analyze the perioperative and oncological outcomes of pancreaticoduodenectomy for extrahepatic cholangiocarcinoma in octogenarian patients.

Method: A multicenter database (the Korea Tumor Registry System, KOTUS, 24 tertiary referral centered included) was used and patients over 70-years old who underwent pancreaticoduodenectomy for extrahepatic cholangiocarcinoma between 2000 and 2014 were included. They were divided into 2 groups: patients in their seventies (septuagenarians) and in their eighties (octogenarians). A comparative analysis between two groups was performed after the application of propensity score matching. Unadjusted Kaplan-Meier and marginal Cox regression analysis were performed.

Results: A total of 1327 patients were included in the study. After propensity score matching, 114 patients were comparable in each of the main groups. Also, the AJCC TNM (7th) stage (*P*=0.289) and R0 resection rate (*P*=0.061) revealed no significant difference. Postoperative complication rate (54% vs. 56%, *P*=0.329), postoperative mortality (2.7% vs. 1.0%, *P*=0.624), and 5Y-overall survival (58.5% vs. 58.3%, *P*=0.349) gave comparable results. On multivariate analysis, curative resection (HR=5.60, 95% CI=2.24-14.67, *P*<0.000) and lower N-stage (HR=2.19, 95% CI=1.34-3.59, *P*=0.002) were significantly associated with favorable overall survival, whereas age which was represented by the group variable (septuagenarians vs octogenarians) in this study, was not significantly associated (HR=1.01, 95% CI=0.623-1.638, P=0.968) with survival. Moreover, we found only the N-stage was significantly associated with disease-free survival (HR=2.35, 95% CI=1.463-3.776, P<0.000), but not age (HR=0.78, 95% CI=0.490-1.227, P=0.277).

Conclusion: There was no significant difference in postoperative complication and age was not a risk factor for poor overall or disease-free survival. Thus, pancreaticoduodenectomy may be safely performed in octogenarian patients with extrahepatic cholangiocarcinoma. Therefore, the octogenarians patient should not be deprived of the chance for curative resection for extrahepatic bile duct cancer solely because of age.

#5963 Pink to purple: Beyond traditional risk factors of female lung cancer.

Muhammad Rafiqul Islam, Mohammad Hasan Shahriar, Muhammad Ashique Haider Chowdhury, Siara Tasmin, Andrew Craver, Brisa Aschebrook-Kilfoy, Sreenivas Konda, Habibul Ahsan

Institute of Population and Precision health, University of Chicago, Chicago, IL

Background: The pink ribbon for breast cancer awareness is being overshadowed by purple, as lung cancer has become the leading cause of cancer deaths in women. While overall lung cancer rates are declining, younger women in high-income countries are being diagnosed more often than men, suggesting unique risk factors for women beyond smoking. The aim of this study was to identify the beyond-traditional risk factors for female lung cancer in a diverse population within the United States.
Methods: We conducted a nested age-matched case-control study within the All of Us Research cohort, analyzing 4,761 adult female participants (761 cases, 4,000 controls). Comprehensive phenotypic data, including demographic characteristics, reproductive history, and lifestyle behaviors, were extracted from electronic health records. Statistical analysis employed chi-square tests, multivariable logistic regression, and an Extended Gradient Boosting Classifier (XGBC) for risk prediction.
Results: Significant associations emerged between lung cancer risk and multiple factors. Age strongly predicts increased risk in females, with odds ratios rising from 5.14 (95% CI 3.22-8.19, $p<0.001$) in the 46-60-year group to 12.51 (95% CI 8.05-19.44, $p<0.001$) in the 61-95-year group. Smoking was a significant risk factor (adjusted OR: 2.54; 95% CI, 2.13-3.02; $p<0.001$), whereas Hispanic ethnicity (adjusted OR: 0.49; 95% CI, 0.32-0.73; $p<0.001$) compared to non-Hispanic white and high deprivation index (adjusted OR: 0.58; 95% CI, 0.47-0.71; $p<0.001$) had a protective role. Non-natural menopause consistently increased risk (adjusted OR: 1.36; 95% CI, 1.05-1.77; $p<0.01$). The XGBoost (XGBC) model achieved 84% accuracy in risk classification, identifying smoking, age, menopausal history, and deprivation index as primary predictive features compared to other machine learning models. The model revealed distinct risk patterns based on smoking status, age, and reproductive factors, with notable interactions between menopausal status among smokers.
Conclusions: This comprehensive analysis highlights the complex interplay between reproductive, sociodemographic, and lifestyle factors in female lung cancer risk. Our findings emphasize the need for personalized risk assessment strategies considering reproductive history alongside traditional risk factors. The successful implementation of machine learning approaches demonstrates the potential for developing more accurate risk prediction models for female lung cancer.

#5964 Real world efficacy and safety of anlotinib in solid tumors previously treated with immune checkpoint inhibitors.

Changzhen Shang, Xuan Luo, Jinyi Zhong, Yajin Chen

Department of Hepatobiliary Surgery, Sun Yat-sen Memorial Hospital of Sun Yat-sen University, Guangzhou, China

Backgrouds Immune checkpoint inhibitors (ICIs) resistance of solid tumors is common and subsequent treatment options offer limited clinical benefit. Anlotinib is a novel multitarget tyrosine kinase inhibitor (TKI) for tumor angiogenesis and proliferative signaling. Recent preclinical studies have suggested that anlotinib can reverse resistance to ICIs by modulating the immunosuppressive tumor microenvironment (TME). The current real-world study aimed to investigate the efficacy and safety of anlotinib for solid tumors in Chinese patients who have previously accepted ICIs alone or combined treatments.

Methods Data of patients diagnosed with solid tumors and previously accepted ICIs were collected retrospectively from six medical sites in China between Jan 2018 and May 2024. Patients were divided into two groups according to the subsequent treatments: anlotinib alone or combined therapy; other treatmental methods not including anti-angiogenic TKIs. Propensity score matching (PSM) based on age, gender, TNM staging, and lines of therapy was used. Progression-free survival (PFS) was estimated using the Kaplan-Meier method and compared using the log-rank test. Adverse Events were graded by CTCAE v4.03.

Results A total of 911 patients were enrolled, including 539 (59.2%) non-small cell lung cancer (NSCLC), 210 (23.1%) hepatocellular carcinoma, 65 (7.1%) esophageal cancer, 59 (6.5%) gastric cancer, 30 (3.3%) cervical cancer, and 8 (0.9%) endometrial cancer. The median age was 58.0 years (range 51.5-66.0), 74.6% were male, 63.6% were stage IV, and 63.9% had received only first-line treatment. In the total population, after 2:1 PSM, there were 431 and 216 patients in the anlotinib and other treatments group, respectively. The PFS was significantly longer in the anlotinib group than in other treatments group (median, 7.9 months vs 6.2 months; HR 0.79, 95% CI 0.62-1.00; P=0.039). The objective response rate (ORR) was 9.5% (41/431) with anlotinib and 11.6% (25/216) with other treatments. We further compared the outcomes of anlotinib combination therapy with other treatments, and results showed that after 2:1 PSM, the median PFS with anlotinib combination (n=404) was 7.9 months versus 6.1 months with other treatments (n=203) (HR 0.72, 95% CI 0.57-0.91; P=0.002). The ORR was 10.1% (41/404) with anlotinib combination and 11.8% (24/203) with other treatments. Of the 431 patients treated with anlotinib, the most common adverse events (≥5% of patients) were allergy (9.7%), gastrointestinal events (8.6%), bleeding (7.7%), and hand-foot syndrome (5.6%).

Conclusion Our study demonstrated the favorable anti-tumor activity and manageable toxicity of anlotinib treatment in solid tumors previously treated with ICIs, and anlotinib combination may provide more benefit. To our knowledge, this study represents the largest real-world clinical data of anlotinib therapy for solid tumors.

#5965 Brain tumor diagnostic interval and tumor size at detection: Impact on survival, recurrence, inpatient length of stay and neurological deficit.

J. M. Cameron[1], E. Gray[2], P. Brennan[3], M. Baker[1], P. Hall[3], A. Lishman[1], P. Karunaratne[3], G. Tramonti[3], M. Vallet[3];
[1]Dxcover Ltd., Glasgow, United Kingdom, [2]Independent Health Economics Consultant, Edinburgh, United Kingdom, [3]University of Edinburgh, Edinburgh, United Kingdom

Brain tumors commonly present in an emergency setting. However, the majority of brain tumor patients report one or more primary care visits in the preceding months. This suggests opportunities for earlier diagnosis. Early economic evaluation of a brain cancer liquid biopsy identified an evidence gap in quantifying the impact of earlier/later diagnosis on patient outcomes. The aim of this study is to estimate the association between two indicators of earlier/later diagnosis, tumor size and the length of diagnostic interval, and the patient outcomes of cancer-specific survival, overall survival, new or worsened neurological deficit, cancer recurrence and inpatient length of stay in 12 months following diagnosis.
A retrospective cohort study utilising a database of 1400 patients from Southeast Scotland, UK, diagnosed with brain tumors between 2010 and 2020. Diagnostic interval was defined as time from first presentation to radiological diagnosis. Tumor size was defined as the recorded maximum diameter at time of diagnosis. Hazard ratios for cancer-specific and overall mortality, per mm increase in tumor size or per day increase in diagnostic interval, were calculated. Odds ratios for new or worsened neurological deficit and for tumor recurrence were similarly calculated. Linear regression was used to estimate effects on total inpatient bed days. Regression was used for all outcomes to adjust estimates for possible confounding factors. Alternative diagnostic window definitions were explored in sensitivity analysis. Analysis was repeated stratified analysis by brain tumor subtype was used to explore generalisability across subtypes.
There were 1196 eligible patients that were included in the study. The mean tumor size was 40.6mm (SD: 17.5mm). Each 1mm increase in tumor size increases mortality risk by approximately 1%, increases the expected inpatient days by 0.15 days, and the risk of new or worsened neurological deficit by 2%. Diagnostic interval results (in preparation) will be presented at the meeting.
Based on previous estimates of brain tumor growth rates, a tumor with a 40mm diameter at diagnosis (mean diameter in this patient population) would be expected to be 28mm if diagnosed 1 month earlier. Applying the effect size estimates reported above would translate into clinically important differences in outcomes. This highlights the improvement in patient outcomes that could be expected if early intervention is made possible by new diagnostic tools.

#5967 Real-world outcomes of renal cell carcinoma and intracranial metastatic disease.

Madison Sherman[1], Karim Hafazalla[2], Arjun Sahgal[3], Georg Bjarnason[4], Sunit Das[5]

[1]Institute of Medical Science, University of Toronto, Toronto, ON, Canada,[2]Neurosurgery, Thomas Jefferson University, Philadelphia, PA,[3]Radiation Oncology, Sunnybrook Health Sciences Centre, Toronto, ON, Canada,[4]Medical Oncology, Sunnybrook Health Sciences Centre, Toronto, ON, Canada,[5]Neurosurgery, St. Michael's Hospital, Toronto, ON, Canada

Introduction: Intracranial metastatic disease (IMD) is a serious complication of renal cell carcinoma (RCC). However, large-scale data on its clinical characteristics, survival outcomes, and treatment strategies are limited, especially at the population level.
Objective: To examine the clinical features, survival outcomes, and treatment patterns of RCC patients with IMD in Ontario, Canada, and provide insights to optimize patient management.
Methods: This population-based retrospective cohort study included RCC patients diagnosed between 2010 and 2019 in Ontario. Demographics, treatments, and survival data were obtained from provincial healthcare records. Kaplan-Meier and Cox regression analyses assessed overall survival (OS). Propensity score matching (PSM) was used to compare survival outcomes between treatment groups.
Results: Among 13,353 RCC patients (mean age: 62 years), 4.6% developed IMD (71% metachronous, 29% synchronous), with a median time to IMD of 8.3 months [Interquartile Range: 1.3, 25.6]. Median OS was significantly shorter for patients with IMD (20.9 months) versus those without IMD (125.4 months; $p < 0.001$). Synchronous IMD (HR: 2.58) was linked to worse OS. Those without metastases at diagnosis had the longest OS (127.0 months), compared to patients with extracranial metastases alone (13.6 months; HR: 6.89) or synchronous IMD (4.8 months; HR: 14.71). Patients with stage 4 disease at diagnosis who developed metachronous IMD had better OS than those who never developed IMD (17.7 vs. 12.0 months). In patients with IMD, systemic therapy was associated with improved survival (HR: 0.47). Stereotactic radiosurgery (SRS) and/or neurosurgery was associated with improved survival, compared to whole-brain radiotherapy (WBRT) or no IMD-directed treatment (HR: 0.47). Propensity-matched analyses showed that receipt of SRS/neurosurgery was associated with improved OS, compared to WBRT or no IMD-directed treatment (25.9 vs. 13.1 months; HR: 0.55).
Conclusion: This study provides large-scale, population-based insights into the impact of IMD in RCC. Our findings show that SRS/neurosurgery is protective in all subgroups of RCC patients with IMD, informing clinical decision-making and offering valuable guidance for optimizing patient outcomes and resource allocation. Additionally, these findings highlight the need for routine brain imaging in RCC to enable early detection and improve management strategies.

#5968 Prognostic value of cachexia consensus criteria in pancreatic cancer.

April Bell[1], Noah Forrest[1], Steven Tran[1], Amber Willbanks[2], Vijeeth Guggilla[1], Akhil Chawla[1], Jodi Johnson[1], Ishan Roy[2], Theresa Walunas[1]

[1]Center for Health Information Partnerships, Northwestern Univ. Feinberg School of Medicine, Chicago, IL,[2]Shirley Ryan AbilityLab, Chicago, IL

Background: Pancreatic ductal adenocarcinoma (PDAC) carries a poor prognosis due in part to cachexia diagnosed in up to 87% of patients. Cancer-associated cachexia is a muscle wasting syndrome unresponsive to nutritional support and associated with reduced treatment efficacy and higher patient mortality. Defining the relationship between cachexia and patient prognosis remains an unmet need, particularly in settings outside of clinical trials. Here, we applied weight-based markers of cachexia to real-world electronic health record (EHR) data to assess the prognostic ability of current consensus criteria toward PDAC patient survival.

Methods: We utilized EHR data from the Northwestern Medicine Enterprise Data Warehouse to design a PDAC-associated cachexia phenotype from weight-based markers of cachexia. To form our patient cohort, we identified patients by a PDAC diagnostic code billed by an oncology provider from records backdated to 2012. Of 1469 patients,1099 patients had sufficient weight data for cachexia classification at the time of diagnosis, defined by a pathology note indicating the presence of PDAC. We used the classification method from Fearon et al. 2011 to stratify patients by no cachexia, pre-cachexia and cachexia before constructing Kaplan-Meier curves. Our model, validated by manual adjudication of 300 patient charts, had an F1 score of 0.961.

Results: There was no significant difference in PDAC patient median overall survival (OS) comparing patients with no cachexia, pre-cachexia and cachexia, which was 19.04 months, 19.27 months and 16.96 months, respectively. However, there were differences in survival rate at one year, which were 60%, 67% and 59%, respectively (log-rank p=0.04). The pre-cachexia associated 1-year survival benefit was most pronounced in Stage IV patients. With pre-cachexia as the reference, the HRs for no cachexia and cachexia were 1.18 (0.97-1.44) and 1.23 (1.02-1.47), respectively. As compared to patients with no cachexia, those with pre-cachexia and cachexia experienced greater survival benefit with resection and chemotherapy.

Discussion: Cachexia status as defined by the Fearon criteria demonstrated a limited prognostic ability, as patient OS did not vary significantly with stratification. However, pre-cachexia was associated with a statistically significant improved 1-year survival and enhanced response to treatment. The unexpected pre-cachexia associated protective benefit suggests cachexia may serve as a double-edged sword in cancer, an observation deserving of further investigation. Overall, our results indicate that while cachexia status should be considered during PDAC management, better measures of cachexia are needed. Prognostication may be improved in future models by combining weight-based measures with factors that inform patient care, such as cachexia biomarkers and patient functionality.

#5969 Enhancing interobserver agreement in RENAL nephrometry scoring through the integration of center-based endophycity measurements.

Keenan Moore[1], Nicholas Heller[2], Clara Goebel[3], Jayant Siva[2], Jamee Schoephoerster[4], Griffin Struyk[5], Nitin Venkatesh[6], Beatriz Lopez Morato[7], Christopher Weight[2]

[1]Loyola University Chicago Stritch School of Medicine, Maywood, IL,[2]Cleveland Clinic, Cleveland, OH,[3]University of Vermont Larner College of Medicine, Burlington, VT,[4]Oregon Health & Science University, Portland, OR,[5]HealthPartners Regions Hospital, Saint Paul, MN, Saint Paul, MN,[6]Oakland University William Beaumont School of Medicine, Auburn Hills, MI,[7]Autonomous University of Madrid, Madrid, Spain

INTRODUCTION: Since its introduction, the RENAL nephrometry score has proven to be an effective tool in medical decision-making and surgical planning for renal tumors and has been shown to be predictive of several important oncologic and operative outcomes. However, some studies have reported high interobserver variability, particularly within the endophycity or “E” portion of RENAL score. This variability can undermine the utility of the RENAL score in clinical management, as it may lead to inconsistent risk stratification and treatment recommendations. Here, we propose the use of center-based endophycity (CBE) scoring in place of the “E” score within RENAL as a means of facilitating interobserver agreement while maintaining utility.
METHODS: Medical records for patients who underwent radical or partial nephrectomy for suspected renal malignancy at a single institution over a 9-year span were retrospectively collected. Exclusion criteria included unavailable imaging, multifocal disease, and incomplete pathology data. Preoperative imaging was evaluated with six independent observers manually assigning RENAL scores to each case, and three observers assigning CBE scores. CBE scores were determined by approximating the center of the tumor and assessing whether it was interior or exterior to the corticomedullary boundary of the kidney. Exophytic and endophytic tumors were given a score of 1 and 2 respectively. These scores were then integrated in place of the “E” score of RENAL for analysis. Kappa coefficient was used to analyze interobserver agreement, and logistic regression was used for predicting outcomes.
RESULTS: A total of 288 cases met the inclusion criteria for this study. CBE scoring (kappa coefficient 0.77) had significantly higher interobserver agreement than RENAL “E” (kappa coefficient 0.17, $p < 0.0001$). Both traditional RENAL and RENAL with CBE were predictive of ISUP grade ($p < 0.0001$, $p < 0.0001$), tumor necrosis ($p < 0.0001$, $p < 0.0001$), and perioperative blood loss ($p = 0.001$, $p < 0.0001$). Traditional RENAL was predictive of malignancy ($p = 0.001$), while RENAL with CBE was not ($p = 0.058$). However, CBE independently predicted malignancy ($p = 0.039$), whereas RENAL “E” did not ($p = 0.365$).
CONCLUSIONS: These results suggest that the RENAL “E” score is prone to particularly low interobserver agreement. When integrated in place of RENAL “E”, CBE scoring represents a promising modification to the RENAL nephrometry score, offering a more reproducible method for assessing endophycity in renal tumors while preserving much of the predictive power of the original model. These findings have important implications for improving the accuracy and consistency of preoperative renal tumor assessments. Further studies are needed to elucidate currently insignificant yet promising predictive data points from this study and to confirm our findings in other cohorts.

#5970 Differences in survival outcomes based on timing and location of initial systemic therapy in patients with extensive stage small cell lung cancer.

Andrea Y. Lo[1], Zahra Hamedi[2], Taharah Islam[3], Erin Alesi[2], Jonathan Berkman[2]

[1]Internal Medicine, VCU Health System, Richmond, VA,[2]VCU Massey Comprehensive Cancer Center, Richmond, VA,[3]VCU School of Medicine, Richmond, VA

Background: Lung cancer is the leading cause of cancer deaths in the United States with small cell lung cancer comprising 20% of lung cancers and extensive stage small cell lung cancer (ES-SCLC) making up two thirds of these cases. ES-SCLC has a median progression free survival (PFS) of 5.2 months and overall survival (OS) of 13 months when treated with chemoimmunotherapy. Inpatient hospitalization for the patient's first cycle of therapy is often utilized. We aimed to identify whether location of treatment (inpatient vs outpatient) and timing of treatment have significant survival effects.

Methods: A retrospective chart review was done on ES-SCLC patients diagnosed and treated with standard of care systemic therapy between 2018-2023. Date of diagnosis, location of patient's first cycle (inpatient vs outpatient), treatment type and dates, progression dates, death dates, and hospice enrollment dates were obtained. Survival analysis was completed using Cox-regression and log-rank test. Fischer's exact test was used to compare differences in categorical data.

Results: 48 patients were identified—40 received their first cycle outpatient and 8 inpatient. Those who received their first cycle of therapy inpatient had worse OS compared to those who received it outpatient (median 126 days vs 408 days, p=0.002). None of the patients who were initially treated inpatient received immunotherapy (IO) with their first cycle. Proportion of patients who enrolled in hospice did not differ significantly between the inpatient and outpatient groups (80% vs 65%, p=0.70). Patients treated with IO within 6 weeks of diagnosis were found to have worse PFS compared to those treated after 6 weeks (median 178 days vs 236 days, p=0.034). OS did not differ significantly. Patients enrolled in hospice were more likely to have worse OS (HR: 2.2, p=0.05). Those with PFS <6 months did not show significantly different OS if they enrolled in hospice. Amongst patients who had PFS >6 months, hospice enrollment was associated with worse OS compared to those who did not enroll in hospice, (median 465 days vs 908 days, p=.006).

Conclusions: Patients who received their initial cycle of therapy inpatient had worse OS compared to those who began treatment outpatient. At our institution, IO is not administered inpatient. Further analyses show timing of IO did not significantly impact OS. However, those who received IO earlier did have worse PFS. These findings suggest that factors associated with inpatient treatment, such as more aggressive disease or significant medical comorbidities may contribute to the observed worse OS. Additionally, while hospice enrollment was associated with worse OS, the difference disappeared when analyzing patients with PFS <6 months, indicating that hospice enrollment itself may not directly impact survival in this subgroup.

CLINICAL RESEARCH: Targeted Therapies and Combinations 3
Poster Session

#5974 Novel combinational therapeutic strategy in angiosarcoma through PLK1 and mTOR co-inhibition.

Nimod D. Janson[1], Alejandra R. Alzate[2], Annaleigh Powell-Benton[1], Bozhi Liu[1], Ant Murphy[1], Jason A. Hanna[1]

[1]Department of Biological Sciences, Purdue University Institute for Cancer Research, West Lafayette, IN,[2]Purdue University Institute for Cancer Research, West Lafayette, IN

Angiosarcoma (AS) is a rare and highly aggressive vascular sarcoma characterized by a poor prognosis with a 5-year survival rate of only 20-30%. The underlying oncogenic drivers and potential therapeutic targets remain unidentified in most AS cases. Standard treatments, including chemotherapy, surgery, and radiotherapy, demonstrate limited efficacy and are frequently associated with relapse and metastatic progression. These challenges highlight an urgent need to identify novel therapeutic targets and develop effective strategies to improve outcomes for patients with angiosarcoma. We identified Polo-like kinase 1 (PLK1), an overexpressed mitotic kinase, as a potential therapeutic target in angiosarcoma (AS). PLK1, a key mitotic regulator, is a promising candidate for clinical intervention in AS. Through differential gene expression analysis in mouse and human AS models, combined with druggability assessments utilizing publicly available databases and AI-based tools, we have validated PLK1 as a promising therapeutic target for AS. Furthermore, our studies demonstrate the potential combinational synergy of PLK1 and mTOR inhibition using small molecule inhibitors. We have shown that genetic *Plk1* depletion by doxycycline-inducible shRNAs and pharmacological PLK1 inhibition using small molecule inhibitor BI 6727 in AS cells reduces cell proliferation colony forming ability *in vitro* and demonstrates significantly reduced tumor growth *in vivo*. Moreover, our data reveal that genetic depletion of *Plk1* delays tumor onset and significantly improves survival in genetically engineered angiosarcoma mouse models (GEMMs). Previously, we observed activation of the mTOR signaling pathway in AS GEMMs and demonstrated that AS cells are susceptible to mTOR inhibition. In the current study, we observed a synergistic combination of PLK1 inhibition by Volasertib and mTOR inhibition by Rapamycin in AS *in vitro* and *in vivo*. Our objective is to determine the mechanisms and pathways governing the development and progression of AS to generate novel precision-targeted therapies. Our findings conclude that PLK1 is a viable therapeutic target in AS, and the combined inhibition of PLK1 and mTOR represents a promising novel therapeutic strategy for this aggressive cancer.

#5975 Long-acting G-CSF empegfilgrastim with dose-dense chemotherapy for patients with stage II-III TNBC: Defendor Special trial, primary analysis.

Inna Ganshina[1], Elena Artamonova[1], Elena Lubennikova[1], Olga Gordeeva[2], Mariam Manukyan[1], Daria Filonenko[3], Lyudmila Zhukova[3], Irina Sorokina[3]

[1]FSBI "N.N. Blokhin National Medical Research Center for Oncology", MoH, Moscow, Russian Federation,[2]FSBI Federal Research And Clinical Center Of Physical-Chemical Medicine of FMBA named after Yu. M. Lopukhin, Moscow, Russian Federation,[3]A.S. Loginov Moscow Clinical Scientific Center, Moscow, Russian Federation

Background: Dose-dense neoadjuvant chemotherapy (NAC) provides the best efficacy results, irrespective of aPD-1 combinatory agent use for the most aggressive subtype of breast cancer - triple negative (TNBC). Maintaining relative dose intensity (RDI)≥85% of NAC and RCB 0-1 achievement after NAC are significantly associated with improvement of patient's prolonged long-term efficiency outcomes. Neutropenia is one of the keys limiting adverse event (AE) reducing RDI. Long-acting G-CSF is an essential component for balanced RDI' maintenance. Here we present the results of efficacy and safety of original prolonged G-CSF empegfilgrastim for patients' (pts) cohort with TNBC under dose-dense therapy regimen from Defendor Special investigator-initiated trial.
Methods: High-risk stage II-III BC pts (n=60) received NAC: 4 dose-dense doxorubicin/cyclophosphamide (ddAC) with empegfilgrastim → 12 QW paclitaxel/carboplatin. RDI was primary endpoint and presented here for pts who completed the planned regimen. Analyses was performed for the 4ddAC part, paclitaxel/carboplatin part, and whole regimen. Secondary endpoints: pCR (ypT0/is, ypN0), RCB rate and safety are also presented here. NCT04905329.
Results: 60 pts were included and received at least 1 dose of NAC with empegfilgrastim at the time of data cut-off. Pts with initially negative prognosis are enriched in the population: 38 (63%) pts with IIIB/C stages, 36 (60%) pts with G3. 57 (95%) pts completed the planned course of sequential therapy, 3 (5%) pts had disease progression (local or distant) that precluded definitive surgery (initially resistant population). RDI≥85% of the whole NAC course was achieved in 54 (96%) pts. Median RDI for ddAC part was 100% and 94% - for paclitaxel/carboplatin part. pCR rate of operable stages was 50% and 43% - for locally advanced stages. 64% of pts in the whole population switched to the favorable prognosis group due to achieving the RCB level of 0-1. There were 58% of pts with initially stages IIIB/C and RCB 0-1 level was 60% in this group. Neutropenia as a reason for therapy discontinuation was in 1 pts for the whole NAC regimen. Main reasons for premature interruption were neuropathy or thrombocytopenia associated with paclitaxel/carboplatin use.
Conclusions: Empegfilgrastim allows for maintained RDI≥85% of sequential NAC therapy with dose-dense regimen part effectively and safely. Balanced neutrophil count could be able improve efficacy results and switched pts prognosis to favorable for majority of pts. In case of limited access to aPD-1 therapy, prolonged G-CSF (empegfilgrastim) becomes a full-fledged agent in a dose-dense regimen that can determine the outcome for patients with TNBC. Final analysis with long-term outcomes is awaited.

#5976 Importance of molecular diagnostic testing preliminary to personalized therapy in NSCLC patients.

Wiley Maxwell Turner, Raja Flores, Matthew Untalan, Stephanie Tuminello, Emanuela Taioli

Icahn School of Medicine at Mount Sinai, New York, NY

Background: Personalized medicine, including targeted and immunotherapy, has substantially changed the landscape of non-small cell lung cancer (NSCLC). About 30% of NSCLC tumors have targetable mutations, like EGFR, and about 28% of tumors will have a PD-L1 score or tumor mutation burden high enough to warrant immunotherapy; a molecular test prior to therapy assesses eligibility. However, the congruence between testing and treatment, and the effect of treatment without testing on outcomes have yet to be studied.

Methods: Surveillance, Epidemiology and End Results-Medicare linked data was queried for NSCLC patients diagnosed from 2013 (the first year that standard guidelines for molecular testing in lung cancer were available) to 2015 age > 66 years, stage > II with continuous Part A and B and no Part C coverage 1 year prior to 6 months post-diagnosis, and continuous part D coverage for 6 months after diagnosis. Patients treated with targeted therapy: EGFR, ALK1, KRAS, or BRAF; or immunotherapy: CTLA, PD1; were selected for. Patients with a claim for a molecular diagnostic test within 6 months after cancer diagnosis were defined as tested. Demographic and clinical characteristics of those receiving vs not receiving a molecular test were compared, and a multivariable Cox proportional hazards regression was used to assess the association of overall survival with receipt of molecular diagnostic testing, adjusting for sex, race, age, histology, and stage.

Results: There were 911 NSCLC patients treated with a personalized therapy, of which 513 (56.3%) had received prior molecular testing. Black patients were less likely than white patients to receive molecular testing (36.4% vs 59.9%; $p<0.001$). Molecular testing was significantly more frequent in patients with fewer comorbidities ($p<0.001$), adenocarcinoma ($p=0.004$), tumors in the lower lobe ($p=0.037$) or diagnosed at a later stage ($p=0.026$). Only 39.9% of patients receiving EGFR inhibitors were initially tested for an EGFR mutation. Overall, among NSCLC patients treated with personalized therapy, untested patients were at increased mortality risk compared to molecularly tested patients (median survival time 8.22 vs 12.79 months, $p=0.024$). After adjustment, untested patients had a significantly higher mortality risk compared to tested patients (HR_{adj} [hazard ratio]: 1.22; 95% CI [confidence interval]: 1.05-1.41).

Conclusions: Although based on claims data, significant incongruence between receipt molecular testing and personalized therapies exists, with only about half of treated patients having had an appropriate molecular diagnostic workup. This could explain, in part, the higher mortality among treated patients who did not undergo testing. Before expanding personalized therapies, studies should examine whether adequate testing is being coupled with these treatments, and outcomes in non-tested patients.

#5977 Identification and characterization of an allosteric and mutant-selective PI3Kα inhibitor.

Chengshan Niu*, #[1], Shengli Dong*, #[2], Shaoqing Chen*[2], Kaige Ji[1], Maolin Zheng[1], Mingtao Chen[1], Yuanjie Li[1], Meihua Li[2], Zhengfei Guo[2], Hongqiang Li[2], Yu Yu[2], Xinlong Yang[2], Chao Zhou[2], Apeng Liang[2], Yin Zhou[3], Wei Wu[2], Mingyu Jiang[3], Jun Li[3], Yusheng Wu[3]

[1]TYK Medicines, Inc. (Zhengzhou), Zhengzhou, Henan, China,[2]TYK Medicines, Inc., Changxing, Zhejiang, China,[3]TYK Medicines, Inc. (Shanghai), Xuhui District, Shanghai, China

Introduction: The PI3K-AKT-mTOR signaling pathway is pivotal in tumorigenesis. PIK3CA, a PI3K isoform, is one of the most frequently mutated genes, occurring in approximately 14% of cancers and in up to 40% of breast cancers. PIK3CA mutations are highly enriched at “hotspot” amino acid sites in the helical domain (E542K, E545K) and kinase domain (H1047R/L). PI3Kα mutations are often oncogenic drivers of other refractory cancers, including gastric cancer, colon cancer, uterine cancer, and head and neck squamous cell carcinoma. Orthosteric inhibitors of PI3Kα, such as alpelisib and inavolisib have been approved for the treatment of patients with advanced HR+/HER2− breast cancer. However, alpelisib and inavolisib lack mutant selectivity and inhibit WT PI3Kα, which plays a critical role in cellular glucose homeostasis, resulting in dose-limiting toxicity such as hyperglycemia and hyperinsulinemia. Selective targeting of mutant PI3Kα is anticipated to enhance antitumor efficacy and mitigate the toxicity associated with wild-type PI3Kα inhibition in normal tissues. Multiple allosteric and mutant-selective PI3Kα inhibitors have been successfully developed into clinical stage, including LOXO-783 (NCT05307705), RLY-2608 (NCT05216432) and STX-478 (NCT05768139). TY-2291 is an allosteric and mutant-selective PI3Kα inhibitor developed by TYK Medicines Inc.

Results: TY-2291 is a potent kinase inhibitor of mutant PI3Kα with high selectivity over WT PI3Kα. In *in vitro* antiproliferative test, TY-2291 showed better antiproliferative activity against multiple PI3Kα mutant cells than LOXO-783, STX-478, and RLY-2608. In the mouse HCC1954 CDX model, TY-2291 showed potent tumor-inhibitory activity and excellent tolerance. TY-2291 also demonstrated excellent DMPK properties and desirable drug-like properties. In the insulin tolerance test, TY-2291 had no effect on fasting plasma glucose levels after four days of treatment.

In conclusion, we have identified a potent allosteric and mutant-selective PI3Kα inhibitor. Our findings indicate that TY-2291 may significantly enhance the therapeutic index of PI3Kα-mutant cancers in the absence of PI3Kα WT-related toxicities.

# Chengshan Niu and Shengli Dong contributed equally to this work.

*To Whom Correspondence should be addressed to: Shaoqing Chen, Ph.D.; Chengshan Niu, Ph.D.; Shengli Dong, Ph.D.; TYK Medicines Inc., P. R. China, 313100.

#5978 Pharmacological blockade of AURKA and NOTCH3 oncogenic pathways to inhibit cancer cell plasticity in triple negative breast cancer.

Antonino Bonaventura D'Assoro[1], Matthew Goetz[1], Carol Lange[2], Roberto Leon-Ferre[1], Tufia Haddad[1], James Ingle[1], Annapaola Montagner[1], Andrew Takchi[1], Ellie Babadi[3], Lucio Miele[4], Urban Lendahl[5], Urban Lendahl[5]

[1]Mayo Clinic College of Medicine and Science, Rochester, MN,[2]Masonic Cancer Center, Minneapolis, MN,[3]Emory, Atlanta, GA,[4]Louisiana State University Health Sciences Center New Orleans, New Orleans, LA,[5]Karolinska Institute, Stockholm, Sweden

Background: Despite recent advances in the treatment of triple negative breast cancer (TNBC) many patients are still suffering from early recurrence, therapy resistance, and organ metastasis 1 to 3 years post-chemotherapy. TNBC cells with stem cell-like properties are resistant to standard of care chemotherapy and are responsible for early tumor relapse and poor outcome. Up-regulation of PD-L1 expression in Cancer Stem Cells (CSCs) has been associated with immune evasion capacity and promotion of their stem-like properties. In this study we identify two druggable stemness target, Aurora-A Kinase (AURKA) and NOTCH3, with positive feedback on intra-tumoral PD-L1 expression and cancer cell plasticity.

Methods: MDA-MB 231, MDA-MB 231 LM and SUM149-PT TNBC cells were used both in 2D and 3D environment. Unique TNBC cells were established from metastatic PDX models. TNBC cells were treated with Alisertib (AURKA inhibitor, 50nM) or AV353 (NOTCH3 inhibitor, 200ng). Immunofluorescence and immunoblot were used to compare different biomarker expression. ALDH was measured using Aldefluor kit. Apoptotic cells were quantified in real-time using *IncuCyte S3*. *In vivo* studies; $1x10^6$ MDA MB 231/LM cells were injected into the mammary fat pad of NSG-female mice and treated with Alisertib or AV353. Control groups were treated with pacebo.

Results: Our study shows that AURKA and NOTCH3 pharmacological blockade reduced nuclear/undruggable PD-L1, that is not achieved clinically by using FDA-approved immune checkpoint inhibitors (ICIs). Significantly, following stemness-targeted therapy with alisertib or AV353, reduction of intra-tumoral PD-L1 resulted in inhibition of tumor growth, decreased levels of CD44 expression, increased CD8+ T-cell tumor infiltration, impairment in the enrichment of $ALDH^{high}$ CSCs and enhanced chemosensitivity. *In vivo* pharmacological blockade of AURKA and NOTCH3 signaling pathways induced inhibition of tumor growth, apoptosis, and impairment of organ metastases.

Conclusions: This study demonstrates that selective AURKA and NOTCH3 pharmacological blockade results in the inhibition of cancer cell plasticity that is linked to reduction of *undruggable* nuclear PD-L1 in TNBC cells. Our findings provide the strong preclinical rationale to develop novel clinical trials for patients with advanced TNBC that are refractory to chemotherapy and show limited response to FDA-approved ICIs.

#5979 Identification of DNA methylation based prognostic subtype and signature in epithelial ovarian cancer.

Wei Geng, Wei Liu, Lei Liu, Yuxin Chen, Dong Yang, Linqing Chai, Caiyun Huang, Lian Li

Tianjin Medical Univ. Cancer Inst. & Hospital, Tianjin, China

Background: Epithelial ovarian cancer (EOC) is a heterogeneous disease that exhibits different histological types, clinical characteristics, and molecular features. DNA methylation plays a crucial role in the development and progression of cancer and has been utilized for subtyping various tumors. This study focuses on classifying EOC based on DNA methylation and aims to characterize the disease through an integrated analysis of genomic, transcriptomic, and clinical data.
Methods: Genome-wide DNA methylation profiling was performed on 137 EOC tumor tissues using Infinium MehylationEPIC array. Prognosis-related differentially methylated positions (DMPs) were identified through multivariate Cox proportional hazards regression models, and non-negative matrix factorization (NMF) approach was used to classify different subtypes. A prognosis prediction model was constructed using LASSO regression and nomogram was constructed.
Results: Four methylation-based subtypes (MS1-MS4) were identified using 774 DMPs, showing significant differences in prognosis ($P = 2.413 \times 10^{-9}$).The MS1 group showed the best prognosis and the most favorable response to paclitaxel in combination with platinum-based chemotherapy. MS2 is characterized as an immune-excluded phenotype, while MS3 represents a metabolic subtype with a moderate prognosis as determined through differentially expressed genes and functional enrichment analysis. In contrast, MS4, recognized as a hypermethylated subtype, has the poorest prognosis and is marked by the highest methylation levels among the four subtypes. A four-DMP signature was developed to predict prognosis and methylation subtype, thereby enhancing clinical utility.
Conclusion: Together, this study identified a novel molecular subtype of EOC, elucidating the heterogeneity of EOC from an epigenetic perspective and providinga new strategy for personalized treatment options for EOC patients.
Key words: Epithelial ovarian cancer, Molecular subtypes, Chemotherapy, Immune therapy, DNA methylation, Prognosis

#5980 Leveraging large-scale PDO-based assays to optimize antibody-drug conjugate efficacy in digestive cancer.

Lelia Polit[1], Gizem Altay[1], Mia Cabantous[1], Anna-Rose Gryspeert[1], Jerome Cartry[2], Jacques RR. Mathieu[2], Sabrina Bedja[2], Alice Boileve[2], Michel Ducreux[2], Sophie Postel-Vinay[2], Fanny Jaulin[1], Gustave Ronteix[1]

[1]Orakl Oncology, Le Kremlin Bicetre, France,[2]Institut Gustave Roussy, Villejuif, France

Context:
Clinical trials are one of the major barriers in contemporary drug development. Functional assays based on patient-derived organoids (PDO) are a promising new tool for derisking clinical development of new therapies, but their use has been limited due to small cohort sizes and the absence of systematic validation studies. Using the largest cohort of matched PDOs, longitudinal clinical data, transcriptomic and WES data, we explore the use of large PDOs collections to characterize ADC efficacy and affinity with the targeted cells through systematic mapping of the relationship between target antigen abundance and payload efficacy.
Methods:
We have assembled a matched collection of PDOs and transcriptomic data from 135 colorectal (CRC) and pancreatic (PDAC) cancer patients, generated from primary tumour samples and metastatic biopsies. Patients ranged from 31 to 89 years old and had experienced an average of 2.6 lines of treatment prior to the PDO-generating tissue sampling. We used gene expression levels of well-known target antigens such as CEACAM5, HER2, and TROP2 as proxies for protein expression. This data is combined with PDO responses to microtubule and topoisomerase inhibitors, thereby enabling us to explore the relationship between target antigen abundance and payload efficacy. This allows us to generate data-driven hypothesis that were then validated *in vitro* on a pool of CRC and PDAC PDOs.
Results:
We identify patient subgroups by combining the RNA expression level and payload efficacy for ADCs against HER2 and CEACAM5. We systematically matched the RNA expression levels and histology for the corresponding proteins. Then we confirmed the response of the PDOs extracted from the tumors of these patients *in vitro*. We report that large-scale PDO-based assays can be a useful tool to anticipate clinical readouts. This work suggests that well-characterized PDO collections could also help redefine patient populations, increasing the likelihood of identifying those more likely to respond to a given ADC, and thus improving the success rates of clinical studies for new treatments in CRC and PDAC.

#5981 Over 200! The largest BaF3-EGFR engineering cell lines collection, a useful platform for novel drug discovery.

Yao Tang, Guoqian Wang, Tingduo Lv, Jingxiao Xu, Yao Peng, Jinying Ning, **Feng Hao**

Kyinno Biotechnology Co., Ltd, Beijing, China

The epidermal growth factor receptor (EGFR) is a cell-surface receptor tyrosine kinase that belongs to the ERBB family, which includes HER1 (EGFR), HER2, HER3, and HER4. Each of these receptors has three key layers: an input layer, a signal-processing core, and an output layer. EGFR is activated primarily through ligand binding, with ligands such as EGF, TGF-alpha, HB-EGF, AR, EPR, BTC, and epigen. However, mutations or overexpression of EGFR can lead to ligand-independent activation. Once activated, EGFR triggers various downstream signaling pathways, such as PI3K-AKT, JAK-STAT, RAS-MEK/ERK, and PLC-gamma-PKC, which enhance cell proliferation, survival, and resistance to cell death.The overexpression or increased activity of EGFR is linked to several cancers, including non-small cell lung cancer (NSCLC), colon cancer, head and neck cancer (HNC), pancreatic cancer, breast cancer, and brain cancer. Recognizing EGFR as a driver gene has accelerated the development of targeted anticancer therapies, such as monoclonal anti-EGFR antibodies (cetuximab, panitumumab) and small molecule receptor tyrosine kinase inhibitors (TKIs). The first generation of EGFR TKIs, like gefitinib and erlotinib, specifically target mutations such as L858R and exon 19 deletions. To address resistance to these early inhibitors, second-generation EGFR TKIs (afatinib, dacomitinib) were developed. The EGFR T790M mutation is a major mechanism of resistance to both first and second-generation TKIs. Osimertinib, a third-generation TKI, was approved for use in EGFR-mutated NSCLC following the failure of first- and second-generation TKIs, although the EGFR C797S mutation limits its effectiveness. Fourth-generation EGFR TKIs, including BLU-945 and BBT-176, are under clinical evaluation but are not yet approved.We have created more than 200 Ba/F3-EGFR engineered cell lines, making this the largest in vitro and in vivo platform for drug discovery with the widest range of mutant cells. These cell lines include almost all clinically significant EGFR mutations, such as common mutations like L858R and exon 19 deletions, as well as rare mutations like L861Q, S768I, and combinations of mutations like L858R/T790M, Del19/T790M, and triple mutations. These Ba/F3-EGFR engineered cell lines are valuable tools for developing and assessing next-generation EGFR inhibitors and for studying newly acquired resistance mutations in EGFR.

#5982 Mechanisms underlying the anti-cancer activity of new, non-covalent thioredoxin reductase inhibitors.

Abigail Rullo[1], Brenna Flowers[1], Keacha Chang[1], Pavel Petukhov[2], Irida Kastrati[1]

[1]Loyola University Chicago, Maywood, IL,[2]University of Illinois, Chicago, IL

Triple negative breast cancer (TNBC) makes up only 15-20% of all breast cancer cases, yet it contributes to 50% of all breast cancer related mortalities. This highlights the urgent need to develop novel therapeutics for the treatment of TNBC. The thioredoxin (Trx) system is an important cellular antioxidant pathway that plays crucial roles in redox homeostasis and supports proliferation, apoptosis, and gene transcription; Thioredoxin reductases (TXNRDs) are the central enzymes in this pathway. Of note, TXNRDs are elevated in TNBC to help tumors cope with excessive redox stress resulting from deregulated metabolism and replicative stress, thus presenting a potential vulnerability. A series of breakthrough studies by our collaborators identified a novel regulatory and druggable “doorstop” pocket in this family of enzymes to which they have developed inhibitors for. These novel TXNRD(i)s show anti-cancer activity in a panel of TNBC lines, by inhibiting proliferation, inducing cell death, inhibiting clonogenic survival, mammosphere forming efficiency and invasion. Importantly, these TXNRD(i)s also inhibit tumor growth in a xenograft model of TNBC. Our goal is to understand the mechanisms underlying the pleiotropic anti-cancer activities elicited by these TXNRD inhibitors. We hypothesize our novel TXNRD(i)s work, in part, by increasing and shifting the balance of redox stress to block proliferation and induce cell death in TNBC cells. To understand major pathways implicated, we conducted transcriptomics (RNAsequencing) in TNBC cells treated with two lead TXNRD(i)s, 8VP101 and 9VP19. Bioinformatic analysis (GSEA) indicates multiple proliferation and mRNA related pathways predominantly enriched by 8VP101. This is consistent with induction of redox stress. To corroborate this finding, cells were treated with 8VP and ROS levels were determined by incubating cells with DCFDA, a ROS sensitive probe, and analyzed by flow cytometry. We find a significant induction in ROS levels comparable to auranofin, a non-specific covalent inhibitor of TXNRDs. Another predominant pathway emerging from GSEA was regulation of cell cycle. Cell cycle analysis in cells treated with 8VP also shows an accumulation in G1. Based on our data, we conclude that our novel TXNRD(i)s elicit anti-cancer activity by elevating intracellular ROS leading to cell cycle arrest and induction of apoptosis and necroptosis. Our findings have important implications for advancing new therapies for TNBC.

#5984 Overcoming osimertinib resistance in NSCLC with NXP900, a phase 1, highly selective and potent first-in-class total YES1/SRC inhibitor.

Neil O. Carragher[1], Enrique Poradosu[2], Asier Unciti-Broceta[1]

[1]Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, United Kingdom,[2]Nuvectis Pharma inc., Fort Lee, NJ

Background: Mutant-selective epidermal growth factor receptor (EGFR) inhibitors such as osimertinib, demonstrate potent activity in the treatment of EGFR-mutant lung cancer, unfortunately, the development of resistance to such EGFR inhibitors is nearly universal. Recent phosphoproteomic analysis has identified Src family kinase (SFK) activity as an important mechanism of osimertinib resistance (Tozuka et al. JTO Clin Res Rep. 2024, 5(4):100668). NXP900 (eCF506) is a novel potent and highly selective SRC family kinase (SFK) inhibitor, (IC_{50} of 0.47 nM against YES1) which possesses a unique/first-in-class binding mode-of-action. NXP900 locks its target into its native “closed” conformation (type 1.5 inhibitor), thereby inhibiting both kinase activity and complex formation with protein partners (Temps et al. Cancer Res. 2021, 81, 5438-5450). In contrast, multi-kinase inhibitors, including dasatinib and bosutinib, block SRC in the active “open” conformation (type 1 inhibitors) promoting the association of SFK and signaling partners via allosteric facilitation (Higuchi et al. Cell Rep 2021, 34, 108876). NXP900 may therefore represent an optimal drug combination partner with osimertinib to overcome acquired resistance in EGFR mutant lung cancer.
Materials and Methods: Cell proliferation assay - Cells were diluted in the corresponding ATCC recommended medium and dispensed in a 384-well plate, depending on the cell line used, at a density of 100 - 6400 cells per well. To study drug combination effects, mixtures of osimertinib and NXP900 are used across three different fixed concentration ratios to calculate a combination index (CI) value to determine synergistic activity using the Oncolines SynergyFinder^{TM} platform. In vivo studies: Mice transplanted with osimertinib resistant cell lines were treated by oral gavage with single agents or combination after reaching a 100mm^3 threshold volume.
Results: NXP900 and osimertinib combination treatment demonstrates statistically significant and potent synergy in osimertinib resistant NSCLC cell lines in vitro. In vivo studies demonstrate significantly increased duration of anti-cancer response to the NXP900 and osimertinib combination treatment arm relative to corresponding monotherapy arms in an EGFR mutant lung cancer model.
Conclusions: Despite high response rates to osimertinib in EGFR mutant lung cancer patients acquired resistance almost universally arises. Here we demonstrate that NXP900 in combination with osimertinib demonstrates potent synergy and prolonged inhibition of tumor growth in in vitro and in vivo NSCLC models of acquired osimertinib resistance. Altogether, these data suggest that NXP900 may have therapeutic potential in EGFR mutant cancers with acquired resistance to osimertinib. A FIH, Phase1 dose escalation study for NXP900 is ongoing.

#5985 Low-grade serous ovarian cancer is effectively treated with a combination of trametinib and LIFR inhibitor therapeutics.

William C. Arnold[1], Durga Meenakshi Panneerdoss[1], Suryavathi Viswanadhapalli[1], Behnam Ebrahimi[1], Hareesh B. Nair[2], Ratna K. Vadlamudi[1], **Yasmin A. Lyons**[1]

[1]UT Health Science Center at San Antonio, San Antonio, TX,[2]Evestra.inc., San Antonio, TX

Background: Ovarian cancer (OCa) is the most lethal gynecologic malignancy in the United States, with low-grade serous ovarian carcinoma (LGSOC) constituting 10% of cases. LGSOC is characterized by slow progression, resistance to treatment, and mutations in BRAF (33%) and KRAS (35%). LGSOC is often diagnosed at an advanced stage with a 70% relapse rate. Thus, novel targeted therapies are needed. Trametinib, a kinase inhibitor, is used to treat KRAS-mutated cancers, but our studies show that it increases LIFR expression, forming an autocrine LIF/LIFR oncogenic signaling loop that reduces Trametinib effectiveness in LGSOC treatment. This study tests the hypothesis that targeting the LIF/LIFR axis can enhance trametinib's efficacy in treating LGSOC.
Methods: The combination effects of EC359 and Trametinib on LGSOC cells were evaluated using cell viability, colony formation, invasion, and apoptosis assays. Mechanistic studies were performed using Western blotting, RT-qPCR and RNA-seq. The *in vivo* efficacy of trametinib and LIFR inhibitor EC359 as a targeted combination therapy was tested using LGSOC cell-based xenografts. Immunohistochemistry was conducted on the xenograft tumor sections to assess the proliferative index through Ki67 antibody staining.
Results: Trametinib increased the expression of LIFR in LGSOC cells in a time-dependent manner, as confirmed by Western blotting analyses, but not in HGSOC cells. In multiple LGSOC cell models, the combination therapy of EC359 and trametinib exhibited superior efficacy in reducing cell viability, colony formation, as well as increasing apoptosis, when compared to monotherapies with either EC359 or trametinib. The biochemical analyses of these models demonstrated that trametinib treatment activated the LIFR signaling pathway, which included the activation of LIFR downstream effectors such as STAT3, mTOR, and AKT. However, this activation was significantly reduced when combined with EC359. In addition, the growth of LGSOC xenograft tumors was substantially suppressed by treatment with EC359 at a dose of 5mg/kg in cell-based xenograft models in comparison to the vehicle control. Furthermore, the tumor-suppressive effects of trametinib were further enhanced by EC359, which resulted in a further reduction in tumor growth. The proliferative marker Ki67 was significantly reduced in tumors treated with the combination therapy, as evidenced by immunohistochemical analysis, suggesting a potent anti-proliferative effect.
Conclusions: These results indicate that the EC359 + trametinib combination therapy may provide a promising therapeutic approach for the treatment of LGSOC by enhancing the efficacy of trametinib and targeting critical signaling pathways.

#5986 Lurbinectedin/Irinotecan as a new therapeutic combination for small cell lung cancer.

Miguel Ruiz Torres[1], Santiago Ponce[2], Juan Manuel Coya[2], Reyes Bernabe[3], Jon Zugazagoitia[2], Alvaro Ucero[4], Javier Baena[2], Luis Paz-Ares[2], Irene Ferrer[2]

[1]Spanish National Cancer Research Ctr. (CNIO), Madrid, Spain,[2]Hospital Universitario 12 de Octubre, Madrid, Spain,[3]Hospital Universitario Virgen del Rocio, Sevilla, Spain,[4]Universidad Complutense de Madrid (UCM), Madrid, Spain

Small Cell Lung Cancer (SCLC) is one of the most frequent and deadliest types of Cancer, with an overal survival (OS) of less than 14 months and a 2- year survival rate not exceeding 7%. Potentially targetable molecular alterations in kinase genes are uncommon in SCLC, and consequently, treatment has barely changed in the last fifty years. Irinotecan, a Topoisomerase II inhibitor, and Lurbinectedin, an inhibitor of transcription, are the two only recently approved chemotherapy agents employed as monotherapy in second line of treatment. Our group has led a Clinical Trial to test the efficacy of the combination of these two drugs in SCLC in patients previously treated with the standard of care. The Clinical trial shows a consistent reduction in tumor size and a significant improvement in the Progression-free survival (PFS) of patients. To determine the mechanisms behind this promising effect, we have conducted a series of experiments using our platform of Patient-Derived models (Patient Derived Xenografts (PDX), Circulating tumor Cell-Derived Xenografts (CDX) and PDX/CDX-derived organoids/spheroids). Treating our PDX/CDX models faithfully recapitulates the results observed in patients, with a clear increase in survival in mice treated with the combination. Short term treatment of PDX models, as well as 3D cultures of organoids (PDXDO) and spheroids (CDXDS) and 2D cultures of SCLC cell lines, helped us determine that the treatment causes elevated levels of DNA Damage, a blockade in DNA Replication, and finally apoptosis. The results from this study highlight the relevance of the combination of these two already approved drugs in the treatment of SCLC.

#5988 Microscopic colitis - rare events secondary to immune checkpoint inhibitors therapy.

Barbara Radecka[1], Michał Piątek[1], Jacek Pawełczak[2], Ewa Stobiecka[3], Krystian Kowalczyk[1], Patryk Zając[4]

[1]Department of Oncology, University of Opole; Tadeusz Koszarowski Cancer Center in Opole, Opole, Poland,[2]Department of Gastroentrology, Provincial Hospital in Opole, Opole, Poland,[3]Department of Patomorfology, Tadeusz Koszarowski Cancer Center in Opole, Opole, Poland,[4]Department of Clinical Biochemistry and Laboratory Diagnostics, University of Opole; Tadeusz Koszarowski Cancer Center in Opole, Opole, Poland

Introduction: Immune checkpoint inhibitors (ICIs) have become the standard of care for cancer treatment but are associated with immune-related adverse events (irAEs). The combination of Nivolumab and Relatlimab (N+R) was approved in March 2022 for the treatment of unresectable or metastatic melanoma. While colitis is a well-known toxicity of ICIs, microscopic colitis (MC) presenting as watery diarrhea with normal endoscopic mucosal appearance, has not yet been described in Summary of Product Characteristics of N and R.

Description: We present the case of a 54-year-old man undergoing treatment with N+R for recurrent *BRAF*^{V600E}mt cutaneous malignant melanoma with metastases to soft tissues and lymph nodes. Eight weeks after starting therapy, the patient developed grade 3 (per CTCAE v.5) watery diarrhea. Stool tests were negative for infectious agents, including Clostridium difficile. Abdominal imaging with computed tomography did not reveal any signs of colitis. However, due to elevated fecal calprotectin levels (12 times above normal) and N+R treatment, ir-colitis was diagnosed. Intravenous methylprednisolone at 1 mg/kg/day has been administered and led to a temporary reduction of symptoms to grade 1. However, within a week, watery diarrhea worsened again to grade 3 despite ongoing steroid therapy. Due to steroid refractory, another immunomodulator was administered, including two doses of infliximab, without improvement. The patient did not lose weight. Colonoscopy revealed normal-appearing mucosa in the entire colon and terminal ileum. Random biopsies were done throughout the colon and terminal ileum. Histopathological examination revealed inflammatory infiltrates and focal increases in intraepithelial lymphocytosis, confirmed by CD3+ immunohistochemistry across all samples. The treatment with next-generation steroid budesonide (9 mg orally once daily in the morning) was administered, resulting in symptom resolution within two days. Diarrhea did not recur over the following four months. The N+R was finished. The patient received three doses of N+R and achieved a complete response (CR) on imaging, which is ongoing.

Conclusion: Various types of colitis were found secondary to ICIs such as pseudomembranous, granulomatous, or collagenous with endoscopically visible changes in the mucosa. MC is suspected to result from increased immune activity in the gut mucosa. It can develop following ICIs therapy and presents with severe symptoms, often requiring hospitalization, steroids, and other immunomodulators. This case highlights that a normal mucosal appearance on colonoscopy does not exclude immune-mediated colitis. Next-generation steroids, such as budesonide, can be effective in managing MC during immunotherapy. Raising awareness of the uncommon irAEs is essential, as some rare events can lead to fatal outcomes. Radiological, endoscopic, and microscopic pictures will be presented on the poster.

#5989 Investigating the potential of EC359 in inducing ferroptosis for the treatment of type I and type II endometrial cancers.

Emily J. Aller[1], Xue Yang[1], Megharani R. Mahajan[1], Behnam Ebrahimi[1], Paulina Ramirez[1], Hareesh B. Nair[2], Edward Kost[1], Ratna K. Vadlamudi[1], Suryavathi Viswanadhapalli[1]

[1]UT Health Science Center at San Antonio, San Antonio, TX,[2]Evestra Inc., San Antonio, TX

Background: Over the past decade, the diagnostic and mortality rates of endometrial cancer, the most common malignancy of the female reproductive tract, have risen, highlighting the need for improvements in current therapeutic options. Recent studies suggest that targeting the cellular receptor LIFR with the small molecule inhibitor EC359 could be a potential treatment for endometrial cancers. EC359 has been shown to inhibit the viability and invasion of both Type I and Type II endometrial cancers. However, the underlying mechanisms by which EC359 induces cancer cell death remain unclear. Emerging evidence suggests that LIFR signaling may be linked to ferroptosis. The purpose of this study is to explore the potential of EC359 to induce ferroptosis as a therapeutic strategy for Type I and Type II endometrial cancers.
Methods: The effects of EC359 on multiple established and primary endometrial cancer cells were evaluated using colony formation, MTT based cell viability and apoptosis assays. Mechanistic investigations were performed with Western blot, RT-qPCR, transmission electron microscopy (TEM) evaluation, and Flow Cytometry analysis. Patient-derived xenograft (PDX) models were used for preclinical evaluation of EC359.
Results: EC359 was efficient in reducing cell viability and promoting programmed cell death in both Type I and Type II endometrial cancer cells. We have demonstrated that the ability of EC359 to reduce cell viability is markedly diminished when ferroptosis is inhibited by pharmacological agents like Ferrostatin 1. EC359 primarily induced ferroptosis by suppressing the glutathione antioxidant defense system, as demonstrated by mechanistic investigations using Western blot analysis. Accordingly, the viability of endometrial cancer cells is restored by the addition of extracellular cystine. Further, EC359 treatment resulted in the reduction of critical anti-ferroptosis proteins. Flow cytometry analyses confirmed that EC359 treatment resulted in an increase in lipid peroxidation and TEM investigations revealed the induction of mitochondrial death through ferroptosis. Additionally, EC359 substantially inhibited the progression of tumors in both Type I and II endometrial cancer PDX models.
Conclusion: In conclusion, our data indicate that EC359 is a potent inducer of ferroptosis in endometrial cancer cells and has the potential to be used as an additional tool in current endometrial cancer treatment regimens.

#5990 Pertuzumab enhances the antitumor activity of T-DXd in HER2-positive gastric cancer cells.

M. Kang, **K.-J. Kim**, J. Sung, M. Nam, S.-H. Hwang, E. Jung, K. Suh, J.-W. Kim, S. Kim, J. Kim, Y. Kim, J. Kim, K.-W. Lee;
Seoul National University Bundang Hospital, Seongnam-si, Korea, Republic of

Simultaneously targeting HER2 and HER3 pathways is a potentially effective strategy for treating patients with HER2-positive gastric cancer (GC). A recent significant advancement in targeted therapy for HER2-positive GC is the development of trastuzumab deruxtecan (T-DXd). However, the co-administration of HER3 blockade with T-DXd to HER2-positive GC has not yet been explored, making this a pioneering study. Our *in vitro* analysis showed that the cell growth inhibitory effect of T-DXd on HER2-positive GC cells resembled the antiproliferative effect pattern induced by trastuzumab, rather than that caused by DXd. T-DXd reduced the cell viability in a manner closely linked to HER2 expression on the cell membrane. We observed that T-DXd inhibited the phosphorylation of HER3 and, subsequently that of p-AKT S473. The addition of pertuzumab increased T-DXd-induced HER2 internalization and further enhanced its antitumor effect on HER2-positive GC cells, especially when the HER2-HER3 heterodimers are enriched in the cells. By translating our findings into a xenograft model, we showed that combining T-DXd and pertuzumab outperformed single-agent therapy, resulting in significant antitumor effects and apoptosis induction. Our study suggests additional clinical investigations of this new combination strategy using T-DXd and pertuzumab in patients with HER2 positive GC are warranted.

#5991 Novel triple-drug combination strategy for p53 mutant cancers leveraging their DNA damage response liabilities.

Mohammed M. Alruwaili[1], Aidan Mazierski[1], Erica Norris[1], Kyeong Beom Jo[1], Yanqi Guo[1], Priyanka Rajan[1], Henry Withers[2], Thomas Melendy[3], Christos Fountzilas[4], Andrei Bakin[1]

[1]Cancer Genomics and Computational Oncology, Roswell Park Comprehensive Cancer Center, Buffalo, NY,[2]Biostatistics & Bioinformatics, Roswell Park Comprehensive Cancer Center, Buffalo, NY,[3]Department of Microbiology and Immunology, University at Buffalo, Buffalo, NY,[4]Department of Medicine, Roswell Park Comprehensive Cancer Center, Buffalo, NY

Genetic alterations in the tumor suppressor p53 gene (*TP53*) are found in most solid malignancies, including colorectal (CRC), breast (BC), and pancreatic (PANC) cancers. Mutant p53 contributes to cancer progression and metastasis. Current therapeutic options show low selectivity for p53-mutant cancers and frequently exhibit high toxicity. To address this unmet clinical need, we developed a two-drug therapeutic strategy that selectively targets p53-mutant cancers. This novel strategy combines a thymidine analogue TAS102, acting as an inducer of DNA damage, and PARP inhibitor (PARPi) that works as an amplifier of DNA damage. Our two-drug therapeutic strategy showed greater efficacy in p53 mutant cancer models (including patient-derived xenografts (PDXs)) than either drug alone. The two-drug regimen was well tolerated in animal preclinical PDX and cell-derived xenograft models. This two-drug strategy is now being tested in Phase I clinical trial for patients with advanced CRC (NCT04511039). Clinical data showed no major signs of toxicity and increased progression-free survival compared to TAS102 alone. Mechanistically, the incorporation of thymidine analogues into DNA provokes post-replicative repair, generating single-strand DNA break intermediates. Repair of these intermediates is assisted by PARP, while inhibition of PARP increases more lethal double-strand DNA breaks. Cells with p53 wild-type (p53WT) respond with G1 and G2 -checkpoint arrest and efficiently remove thymidine analogues. In contrast, p53 mutant cells, lacking the G1 checkpoint, accumulate in G2 with lethal DNA breaks. The current work investigated the mechanism of the DNA damage response (DDR) and the G2 checkpoint induction in response to the TAS102-PARPi regimen. Analysis of DDR signaling showed that the G2 checkpoint in response to TAS102-PARPi is mediated by ATR kinase that acts through CHK1 and WEE1 kinases. We next examined a drug combination using the two-drug TAS102-PARPi regimen and the G2 checkpoint kinase inhibitors together. The concurrent triple-drug regimen showed high toxicity to non-tumor cells and cancer cells. In contrast, a sequential drug regimen, the two-drug treatment followed by G2-checkpoint inhibitors demonstrated selective toxicity against p53 mutant cancer cells. This sequential triple-drug regimen was further examined in preclinical CRC model in mice. The triple-drug regimen showed substantially greater anti-tumor activity in p53 mutant CRC xenograft model without adverse side effects (based on mouse body weight and peripheral blood analysis). Based on these preclinical studies, we propose a novel triple-drug therapeutic strategy for p53 mutant cancers that has strong potential to improve cancer treatment and meaningfully impact the health of cancer patients.

#5992 In vitro efficacy of novel USP1 inhibitors as a single agent and in combination with SOC in ovarian, prostate, and breast cancer cells independent of BRCA mutational status.

Marina Curcic[1], Mohamed Eltokhy[1], Sanjay K. Srivastava[1], **Partha Pratim Sarma**[2], Prashant Kashinath Bhavar[3], Uday Kumar Surampudi[3]

[1]Texas Tech University Health Sciences Center, Abilene, TX,[2]VeGen Therapeutics, Hyderabad, India,[3]VRise Therapeutics, Cambridge, MA

Background: Ubiquitin-specific protease 1 (USP1) is a key regulator of DNA repair pathways and is implicated in the response to genotoxic stress. Targeting USP1 has emerged as a potential therapeutic strategy for cancer treatment. This study investigates the in vitro efficacy of a series of novel USP1 inhibitor both as a monotherapy and in combination with other treatment agents, in ovarian, prostate, and breast cancer cell lines.
Methods: In vitro efficacy of three different series of USP1i in ovarian (OVCAR-3), prostate (DU-145), and breast (SUM149) cancer cell lines were accessed using cell viability and colony formation assays. Combination included co-treatment with standard chemotherapy agents and determining synergy using composite score.
Results: Treatment with USP1i alone led to significant reductions in cell viability and colony formation irrespective of BRCA mutation status. Furthermore, in combination with USP1 inhibitors enhanced efficacy was seen, leading to a positive shift in IC50 in cell viability assay and a significant increase in apoptosis compared to either treatment alone.
Conclusion: Our results demonstrate the potent in vitro anti-cancer activity of series of USP1 inhibitors, both as single agent and in combination with chemotherapeutic agents in ovarian, prostate, and breast cancer cell lines, irrespective of BRCA mutational status. These findings suggest that USP1 inhibition could provide a promising therapeutic approach for a broad range of cancers, warranting further investigation in preclinical models and clinical trials.

#5993 Identification of predictive biomarkers of response to ADCs by HTS of highly molecularly characterized panels of patient-derived organoids (PDOs).

Yi-Hung Carol Tan, Brandon L. Mapes, Brian M. Larsen, Daniel Gorski, Jessica Barbeau, Shanzah Chaudhry, Megan Spector, Carrie A. Franzen, Richard A. Klinghoffer

Tempus AI, Inc., Chicago, IL

Background: Antibody-drug conjugates (ADCs) such as the HER2-targeting agent trastuzumab deruxtecan, the TROP-2 targeting agent sacituzumab govitecan, and the NECTIN4 targeting agent enfortumab vedotin-ejfv, have resulted in increased clinical response rates in previously difficult to treat solid tumor indications, but only a minority of patients exhibit such responses. Increased understanding of the molecular underpinnings of sensitivity and resistance to ADCs beyond membranous target expression is needed. To that end, we developed a platform for generating, deeply characterizing, and functionally screening extensive panels of patient-derived organoids. Here, to demonstrate feasibility, we report on a screening panel of 60 PDOs representing 10 solid tumor indications and focus on response to ADCs now widely used in the clinical setting: trastuzumab deruxtecan and sacituzumab govitecan.
Methods: PDOs were cultured in the presence of 8-dose dilutions of either trastuzumab deruxtecan or sacituzumab govitecan as monotherapies. Experiments were performed in 384 well plates with each dose in technical triplicate. Cells were plated and drugs were added after 24 hours. Cells were cultured for an additional 6 days with no media refreshes. The screen included staurosporine as a positive control and vehicle negative controls. On Day 7, CellTiter-Glo (CTG) was added to the plates to terminate the experiments and assess cell viability. To evaluate treatment activity, cell viability measurements were normalized to vehicle controls and dose-response curves were generated to estimate key efficacy metrics including IC50, EC50, and inverse area under the curve (iAUC).
Results: ADC specific responses were observed across the 60 models tested. As anticipated, response to ADC exposure was driven by target expression. This was most obvious in response to enfortumab vedotin-ejfv, and also observable to a lesser extent with sacituzumab govitecan and trastuzumab deruxtecan. To better understand other molecular indicators of response, differential gene expression analysis was performed. This revealed additional potential biomarkers of specific ADC response including *VIL1*, *MYO7B*, *HNF4A*, *EFS*, *EPS8L3*, and several of which interestingly regulate cell-cell junction proteins.
Conclusions: The results shown here demonstrate that screening pan indication panels of PDOs can reveal ADC specific differences in efficacy which are rooted in molecular factors that include, but are not limited to target expression. Further analysis is ongoing, including establishing the prevalence of non-ADC target biomarkers in real-world patient populations via the projection of our screen-derived data onto Tempus RWD.

#5994 Investigating druggability of myeloid and lymphoid lineage gene mutations in advanced phase CML by employing drug discovery tools: Implications in fatal blast crisis CML precision oncology.

Fatimah Alabdullah[1], Nawaf Alanazi[1], Abdulkareem AlGarni[1], Kanza Adeel[1], Yaqob Samir Taleb[1], Amer Mahmood[2], Sabreen Alanazi[1], Aamer Aleem[2], Zafar Iqbal[1]

[1]Ministry of National Guard Health Affairs (MNG-HA), Al Mubarraz, Saudi Arabia,[2]King Saud University, Riyadh, Saudi Arabia

Background: Chronic Myeloid Leukemia (CML) is a myeloproliferative stem cell malignancy. Chronic Phase CML (CP-CML) is treatable with overall survival equivalent to the general population. Nevertheless, a proportion of CP-CML progresses to the accelerated phase (AP-CML) and ultimately blast crisis (BC-CML), with the latter having an overall survival (OS) of 3-23 months, making it almost a fatal manifestation. Although BC-CML is a different clinical entity than acute leukemias, it resembles AML (as myeloid BC-CML) or ALL (lymphoid BC-CML). Therefore, this study was designed to find out druggable AML-/ALL-lineage gene mutations in BC-CML

Methods: The study included 141 CML patients. All response criteria were per European LeukemiaNet (ELN) guidelines 2020. Whole exome sequencing (WES) was carried out to find out druggable gene mutations and the druggability of the mutated genes was determined using the online artificial intelligence (AI) tool (https://www.pandrugs.org/#!/query/). SAS/STAT software version 9.4 was used for data analysis (SAS Institute Inc., Cary, NC, USA). For statistical computing, the R package was employed.

Results: Out of 141, 18 (12.8%) patients progressed to AP-CML while 12 (8.5%) to BC-CML. BC-CML patients had poorer response to TKIs and higher mortality rate (75%). WES showed 64 AML-/ALL-associated genes mutated in advanced phase CML patients. Overall WES coverage was about 110X. AP-CML had 1644 variants, whereas BC-CML had 2531 variants, with a 54% gain in mutations from AP-CML to BC-CML (P< 0.000001). Among AML-/ALL- related mutated genes were NPM1 (%1.98), DNMT3A (%1.86), PML (%1.82), AKT1 (%1.62), CBL (%1.30), JAK2 (%0.71), TET2 (%0.59), IDH1 (%0.32), and BCL2. We also found that some FDA-approved drugs for different hematological malignancies (e.g. VENETOCLAX, DECITABINE, AZACITIDINE, BORTEZOMIB, ZANUBRUTINIB, IVOSIDENIB, TAZEMETOSTAT, RUXOLITINIB, PACRITINIB, ARSENIC TRIOXIDE etc.) can effectively target multiple gene groups mutated in our advanced phase CML patients (Table 1-4, Figures 1-4).

Conclusions: NGS analysis of AP- & BC-CML found mutations in many AML-/ALL-lineage genes, which is much higher than previously reported. This shows a huge genetic similarity between BC-CML and AML/ALL. FDA-approved and various novel experimental drugs under clinical trials are available against some of the genes we reported. We conclude that our approach can help in finding drug-targetable gene mutations related to AML-/ALL-lineage & other hematological malignancies in almost every BC-CML patient and provides practical guidance for drug repurposing as well as a gateway for under-trial novel experimental drugs to individualize BC-CML patient treatment.

#5995 Leveraging GPCR overexpression for personalized nanomedicine: A novel approach in ovarian cancer therapy.

R. Khetan[1], P. Eldi[1], N. A. Lokman[2], C. Ricciardelli[2], M. K. Oehler[3], A. Blencowe[1], K. Pillman[1], S. Garg[1], H. Albrecht[1];
[1]University of South Australia, Adelaide, Australia, [2]University of Adelaide, Adelaide, Australia, [3]Royal Adelaide Hospital, Adelaide, Australia

Background and Aims: Genetic heterogeneity in ovarian cancer drives frequent aberrant expression of cell surface proteins including G-protein coupled receptors (GPCRs), which harbour untapped potential for active targeting with nanomedicines. Our goal was to systematically identify GPCRs with significant overexpression in ovarian cancer biopsies and assessing their suitability to develop personalized GPCR-targeted nanomedicines for precise drug delivery.
Methods: We ran a systematic analysis of public RNA-seq gene expression data, to reveal GPCRs with frequent overexpression in ovarian cancer tissues. To validate the relevance of initially identified receptors as targeting candidates, we confirmed their expression in six ovarian cancer cell lines and a selection of patient-derived samples (ascites derived tumour samples and solid tumours), using techniques such as RT-qPCR, flow cytometry, fluorescence imaging and immunofluorescence. This comprehensive analysis provided valuable insights into the prevalence and distribution of candidate receptors across different ovarian cancer probes. Furthermore, we utilized representative ovarian cancer cell lines as *in vitro* models to assess receptor-mediated cellular uptake and internalization processes, to further evaluate potential targets for nanoparticle delivery systems.
Results: Analysis of public RNA-Seq datasets revealed 13 overexpressed GPCRs in cancer tissues compared to healthy tissues. Among these, we focused specifically on the coagulation factor II thrombin receptor (F2R) to highlight its intriguing potential as a therapeutic target. The variable expression of F2R was confirmed in both ovarian cancer cell lines and patient-derived samples. Furthermore, peptide binding to the receptor was successfully demonstrated using flow cytometry and fluorescent imaging, suggesting a promising approach for targeted drug delivery.
Conclusions and Significance: Our findings underscore the importance of personalized targeting approaches in ovarian cancer therapy and highlight the potential of F2R as a promising therapeutic target. By integrating advanced molecular techniques with nanomedicine strategies, we strive to pave the way for more effective and tailored treatments for ovarian cancer patients.

#5996 New therapeutic approaches in NUT carcinoma: An unmet need.

M. Sanchez-Becerra[1], C. Escudero-Iriarte[2], L. Bigot[1], T. Tian[2], P. Aviles-Marin[3], M. Knetki-Wroblewska[4], S. Oreilly[5], M. Bover[6], J. Benitez-Montanez[7], P. Martin-Martorell[8], S. Sandiego Contreras[9], M. Ghigna[1], C. Markellos[10], M. Burgy[11], C. Travert[12], P. Hulo[13], M. Quentin[14], R. Motta Guerrero[15], J. Chapron[16], N. Cozic[1], L. Friboulet[1], Y. Loriot[1], A. Italiano[1], B. Besse[1]; [1]Gustave Roussy, Villejuif, France, [2]Vall d'Hebron Institute of Oncology, Barcelona, Spain, [3]Pharmamar, Madrid, Spain, [4]The Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Poland, [5]The Christie NHS Foundation Trust, Manchester, United Kingdom, [6]Hospital Universitario 12 de Octubre, Madrid, Spain, [7]University Hospital Virgen de la Victoria, Malaga, Spain, [8]Hospital Clinico Universitario de Valencia, Valencia, Spain, [9]Instituto Valenciano de Oncologia, Valencia, Spain, [10]Iatriko Kentro Athinon), Athens, Greece, [11]Institut cancerologie Strasbourg Europe, Strasbourg, France, [12]Hopital Arnaud de Villeneuve-Centre Hospitalier, Montpellier, France, [13]Centre hospitalier universitaire de Nantes, Nantes, France, [14]CHU Clermont-Ferrand, Clermont-Ferrand, France, [15]Centro Oncologico Aliada, Lima, Peru, [16]Hopital Cochin, Paris, France

Background: NUT carcinoma (NC) is a rare, aggressive tumor with poor prognosis and no standard treatment. NC arises from *NUTM1* gene rearrangements, which drive epigenetic dysregulation and uncontrolled proliferation via enhanced RNA polymerase II (RNA-pol II) activity. An analysis of our registry (70 patient) revealed limited benefits from current systemic therapies. Among patients on first-line (26), progression-free survival was modest: immunotherapy (5.4months-mo-, 95%CI 3.6-7.1), chemo-immunotherapy(CTIO) (4.8mo, 95%CI 0.2-8.8), BET inhibitors (4.3mo, 95%CI 1-17.1), and chemotherapy (3.9mo, 95%CI 1.7-52.1). Given Lurbinectedin's (LUR) RNA-pol II-dependent mechanism and its regulation of BRD4, a critical partner of *NUTM1*, we investigated its potential therapeutic value in NC.

Methods: LUR efficacy was assessed in three settings. First, in vitro studies by 4 *BRD4:NUTM1* cell lines treated with escalating doses of LUR for 72 hours, and cell viability evaluated by MTT assays. Second, *in vivo,* with 2 patient-derived xenograft (PDX) models. Finally, under compassionate use, 3 patients with *BRD4:NUTM1*.

Results: NC cell lines demonstrated high sensitivity to LUR, with IC50: 22-122nM. Ongoing PDX model experiments are evaluating in vivo sensitivity. Clinically, a 54-year-old woman with metastatic thoracic NC pretreated with CTIO, showed rapid clinical and radiological response to second-line LUR, with a 3mo duration of response. A 20-year-old man with head and neck metastatic NC pretreated with VDC/IE, had clinical improvement (ECOG-PS, weight gain, and reduced morphine use) after a first cycle (imaging pending for response confirmation). The third patient, recently initiated second-line LUR.

Conclusion: Preliminary results suggest that LUR shows promising activity in NC, both in vitro and in early clinical cases. LUR offers a biologically informed therapeutic strategy in NC. These findings highlight the importance of leveraging tumor biology to guide treatment development for rare and aggressive cancers

Characteristics

Age	38 (0-69)
MenWomen	44 (63%)26 (37%)
ThoracicHead/NeckOther	4026 4
LocalisedLocally AdvancedMetastatic	9 2437
Molecular*BRD4BRD3*OtherUnknown	193840

#5997 Patient-derived cell line and organoid drug screening identifies synergistic PLK1 inhibitor in combination with senolytic ABT-263 for pancreatic cancer.

Madison Jenner[1], Brian Golitz[1], Yi Xu[2], Xianlu Peng[2], Mohammad Anwar Hossain[3], David Drewry[3], Shawn Gomez[4], Jen Jen Yeh[5]

[1]Department of Pharmacology, University of North Carolina, Chapel Hill, NC,[2]Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC,[3]SGC-UNC, University of North Carolina, Chapel Hill, NC,[4]Joint Department of Biomedical Engineering, University of North Carolina at Chapel Hill and North Carolina State University, Chapel Hill, NC,[5]Department of Surgery, University of North Carolina, Chapel Hill, NC

BACKGROUND: Pancreatic cancer (PC) is the 4th deadliest cancer with a 5-year survival rate of only 13%. Chemotherapy remains the standard-of-care to treat PC, but recent retrospective analyses of the Know Your Tumor program found that matched, targeted therapy increases both progression-free survival and overall survival compared to the unmatched chemotherapy group. Therefore, there is a strong need to advance targeted therapy strategies to improve patient outcome in PC.
METHODS: To identify new targeted therapies in PC, we utilized a kinase inhibitor library, KCGS 1.0+, that is composed of clinical kinase inhibitors hitting known targets as well as tool compounds against understudied kinases. We screened KCGS 1.0+ against patient-derived xenograft cell line and organoid models representing both the basal-like and classical subtypes of PC. To assess compound sensitivity, we utilized high-content imaging to monitor cell morphology and CellTiterGlo to determine cell viability.
RESULTS: We found that overall, organoids were more sensitive than cell lines. 11% of KCGS 1.0+ had an IC_{50} of 1μM or less for organoids versus only 2% for cell lines. Several known PC targets sensitive to inhibition were recapitulated such as MAPK, PI3K, and CDK7/9 which provided validation for our screening strategy. A top ranked target was PLK1. Organoids were more sensitive to PLK1 inhibition compared to cell lines and cells of the basal-like subtype showed greater sensitivity compared to classical cells. PLK1 overexpression in a subset of basal-like lines was confirmed by western blot. Previous work has shown PLK1 inhibition induces cellular senescence which promotes therapeutic resistance. In our PC lines treated with PLK1 inhibitor and stained with DAPI, we observed enlarged and irregularly shaped nuclei which is a hallmark of senescence. We confirmed senescence by staining for β-galactosidase in PLK1 treated cells. Since senescent cells upregulate the BCL-2 family, we rationalized we could exploit this dependency and combine PLK1 inhibition with the senolytic BCL-2 inhibitor ABT-263. As expected, inhibition of PLK1 and BCL-2 in combination versus either inhibitor alone resulted in dramatic induction of apoptosis measured by Caspase 3/7 activation.
CONCLUSIONS: Our high-throughput screen of kinases inhibitors has identified PLK1 as a lethal target in PC. PLK1 inhibition induces cellular senescence, a putative escape mechanism. The combination of PLK1 inhibitor with the senolytic, ABT-263 results in greater cell death compared to either inhibitor alone. This combination strategy advances precision oncology and may have significant implications for patients with the more aggressive basal-like subtype in PC.

#5998 Patient-derived organoids and precision medicine: Insights from the PASS-01 clinical trial in PDAC.

Amber N. Habowski[1], Hardik Patel[1], Caitlin Tsang[1], Luce St. Surin[1], Dennis Plenker[1], Fatim M. Kouassi[1], Raditya Utama[1], James Rouse[1], Deepthi Budagavi[1], Grainne M. O'Kane[2], Stephanie Ramotar[2], Anna Dodd[2], Julie Wilson[2], Kenneth H. Yu[3], Faiyaz Notta[2], Robert C. Grant[2], Steven Gallinger[2], Eileen M. O'Reilly[3], Kimberly Perez[4], Andrew J. Aguirre[4], Brian M. Wolpin[4], Dan A. Laheru[5], Daniel A. King[6], Elizabeth M. Jaffee[5], Jennifer J. Knox[2], David A. Tuveson[1]

[1]Cancer Center, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY,[2]Princess Margaret Hospital, University Health Network, Toronto, ON, Canada,[3]Memorial Sloan Kettering Cancer Center, New York, NY,[4]Dana-Farber Cancer Institute, Boston, MA,[5]Johns Hopkins Medicine, Baltimore, MD,[6]Northwell Health Cancer Institute, New Hyde Park, NY

Pancreatic ductal adenocarcinoma (PDAC) is challenging as most patients are diagnosed at an advanced stage with limited treatment options. Tailoring therapies for individual patients is paramount due to the aggressive nature of the disease. Our work explores patient-derived organoids (PDOs) as *in vitro* tumor models to dissect molecular signatures and conduct pharmacotyping, aiming to enhance precision medicine. In the PASS-01 stage IV PDAC clinical trial, biopsies were collected for molecular correlatives, including establishment of PDOs. Tissue was collected from patients at six institutes across the United States and Canada, highlighting our ability to integrate PDOs into a robust, multi-institutional clinical framework. Most biopsies were collected from liver metastases, followed by primary pancreas tumors, peritoneal, omental, lymph node, lung, and brain metastases. KRAS mutation status determined by ddPCR was used to validate neoplastic cells in the organoid cultures and identify pseudonormal outgrowth. Established PDO lines were subjected to high throughput drug screening for 120+ compounds, comprising both standard-of-care and experimental agents. Subsequently, validated PDO lines were expanded, biobanked and harvested for RNA and DNA sequencing. During the trial, 186 biopsies from 183 enrolled patients were shipped to CSHL for PDO establishment. The overall malignant PDO establishment rate was 50% across biopsy sites and patients. Interestingly, PDOs could be generated more often from patients that rapidly progressed ($p<0.001$). Furthermore, by comparing the characteristics of primary biopsies to established PDO, PDOs were more frequently generated from Moffitt subtype classical (64% establishment) compared to basal (35%), and from those with a $KRAS^{minor}$ imbalance (67% establishment) compared with KRAS wild-type (36%), balanced (58%), and $KRAS^{major}$ imbalance (52%). The average time from tissue receipt to first drug screen data was 65 days, with six PDOs screened in under 30 days. This turnaround time enabled PDO therapeutic data to be presented at monthly molecular tumor boards. Consequently, these data were considered alongside other clinical trial correlates to aid in selection of second-line therapies when patients progressed. PDO pharmacoptyping identified sensitivity that correlated with patient outcomes, notably on gemcitabine/nab-paclitaxel. Patients with responder PDOs to the first-line regimen received had improved overall survival and progression-free survival. Additional transcriptomic and genomic analysis of patients and PDOs are ongoing, including identification of molecular markers of therapy response. Moving forward, we aim to continue incorporation of PDOs and tumor molecular profiling to aid in patient therapy selection. We anticipate this work to be crucial as targeted therapies, including KRAS inhibitors, become mainstream in PDAC care.

#5999 Therapeutic delivery of splice switching oligonucleotides to restore p53 function in liposarcoma.

Rafia Rahat[1], Akila S. Venkataramany[1], Matias Montes[2], Ravi Dhital[3], Kevin Cassady[1], Raphael E. Pollock[4], Dawn S. Chandler[1]

[1]The Ohio State University/ Nationwide Children's Hospital, Columbus, OH,[2]Stanford University, Stanford, CA,[3]Nationwide Children's Hospital, Columbus, OH,[4]The Ohio State University, Columbus, OH

Introduction: Dedifferentiated liposarcoma (DDLPS) is a soft tissue sarcoma characterized by genomic amplification of proto-oncogene MDM2 alongside wildtype P53. Disease progression and poor survival rates in DDLPS are driven by MDM2 overexpression, which negatively regulates the tumor suppressor p53. While small molecules such as Nutlin and its derivatives have been employed to inhibit MDM2-p53 interaction, their significant toxicity presents a major limitation. This clinical challenge highlights the pressing need for alternative approaches to reactivate P53 through less toxic mechanisms.

Alternatively spliced isoform of MDM2, MDM2-ALT1 inhibits full-length MDM2 (MDM2-FL) and stabilizes p53 expression. Additionally, MDM2-ALT1 can prevent the activity of MDMX, another negative regulator of p53, and the resulting p53 activation can sensitize tumor cells to p53-dependent chemotherapies. Thus, targeting MDM2 splicing to enhance MDM2-ALT1 production represents a promising non-toxic intervention strategy for DDLPS. Our laboratory has developed Splice Switching Oligonucleotides (SSOs) that can induce MDM2-ALT1 expression and subsequently reactivate p53 function. We hypothesize that *in vivo* delivery of SSOs to p53 wild-type tumor cells will induce MDM2-ALT1 expression and restore p53 activity to reverse the cancer phenotype.

Results: We have synthesized a 20-mer SSO targeting the SRSF2 binding site in MDM2 exon 11, which functions as a positive regulator of MDM2 splicing. These SSOs prevent SRSF2 binding and promote MDM2-ALT1 production. To enhance both *in vitro* and *in vivo* SSO delivery, we incorporated our SSO sequence in an AAV viral vector to be expressed as an anti-sense RNA and validated its ability to induce MDM2-ALT1 splicing *in vitro* using RT-PCR. The SSO sequence expressed by the AAV vector successfully induced MDM2-ALT1 expression. Next, we will measure the p53 activity and perform proliferation assay to validate that SSO expressed from the AAV viral vectors can reduce cellular proliferation through the activation of p53 tumor suppressive activity. Our goal is to facilitate target specific *in vivo* delivery of this SSO construct using AAV viruses or extracellular vesicles in Xenograft tumor models.

Conclusion: This research presents a novel therapeutic strategy by designing SSOs that reactivate p53 tumor suppressor function and developing methods for target-specific delivery. This innovative strategy could potentially revolutionize treatment for DDLPS and other cancers where p53 function is suppressed by MDM2 overexpression.

#6000 Vabametkib plus lazertinib shows promising antitumor activity in advanced NSCLC with MET amplification resistant to 3rd-gen EGFR-TKIs.

Nirmal Rajasekaran[1], Yeongmun Kim[1], Dawoon Jeong[1], Heesoo Ahn[1], Jeesun Kim[1], Yanghee Kim[1], Sangsuk Lee[1], Sunhye Im[1], Young Kee Shin[2], Jun Young Choi[1], Na Young Kim[1]

[1]ABION Inc., Seoul, Korea, Republic of,[2]Department of Molecular Medicine and Biopharmaceutical Sciences, Seoul National University, Seoul, Korea, Republic of

MET amplification (METamp) can emerge as a bypass mechanism of resistance to EGFR tyrosine kinase inhibitors (TKIs), occurring in up to 26% of patients with EGFR TKI-resistant EGFR-mutant non-small cell lung cancer (NSCLC). ABN401 (vabametkib), a highly specific MET inhibitor, is undergoing phase 2 trial (NCT04052971) and shows positive response in metastatic NSCLC patients harboring MET exon 14 skipping. Lazertinib, a 3rd Gen EGFR TKI, is effective against EGFR mutations, including common resistance mutations like T790M. To explore the potential of combination therapy of vabametkib and lazertinib in NSCLC patients, we evaluated the cytotoxic response of vabametkib in combination with various EGFR TKIs in NSCLC cell lines. To explore the feasibility of combination therapy in NSCLC patients, we assessed the drug-drug interaction (DDI) potential of vabametkib with various TKIs, focusing primarily on 3rd Gen EGFR TKI, according to *'In vitro drug interaction studies -Cytochrome P450 enzyme- and transporter-mediated drug interactions'* (Guidance for Industry, US FDA). Additionally, the anti-tumor efficacy of vabametkib plus 3rd Gen EGFRTKI was further validated in EGFR-mutant patient-derived xenograft (PDX) models. The combination of vabametkib and 3rd Gen EGFR TKIs demonstrated strong anti-tumor efficacy (96.6% TGI) in EGFR mutants and high MET-amplified PDX model. In contrast, vabametkib alone and 3rd Gen EGFR TKI alone did not show statistical significance. In drug interaction studies using the basic static model and physiologically-based pharmacokinetic (PBPK) modeling, vabametkib did not alter the PK of the co-administered TKIs. Especially ,the potential for DDI between vabametkib and lazertinib was found to be negligible. These findings support the safe and effective use of vabametkib in combination with lazertinib and other TKIs across a broad range of therapeutic applications. These findings support potential clinical benefits of combination therapy of vabametkib and 3rd Gen EGFR TKI in patients with MET-amplified/overexpressed, EGFRm advanced NSCLC with disease progression after 1st line 3rd Gen EGFR TKIs. Consequently, a clinical trial is underway to evaluate the efficacy and safety of combining vabametkib with lazertinib. The ABN401-003 trial, a phase-2 study comprising cohort-2, is an open-label, multicenter investigation aiming to assess the efficacy and safety in patients who have experienced progression on 1st line 3rd-Gen EGFR-TKIs. Cohort-2 consists of three parts: Part 1 (safety run-in), Part 2 (a randomized dose optimization), and Part 3 (a randomized clinical trial comparing the optimal dose of vabametkib in combination therapy with standard of care).

#6001 Unlocking therapeutic potential of rigosertib as a selective therapy for ovarian cancer.

S. Nath[1], S. Claridge[1], G. Lara Granados[2], M. Al Assaad[1], E. Park[2], J.-A. Cavallo[2], M. E. Ackon[2], L. De Boni[2], S. Baker[2], E. Reddy[2], S. V. Blank[2], O. Elemento[1], R. Brody[2], B. D. Hopkins[1]; [1]Weill Cornell Medicine, New York, NY, [2]Icahn School of Medicine at Mount Sinai, New York, NY

Ovarian cancer has a devastating impact. In 2024 it is estimated that there will be 19,680 new ovarian cancer diagnoses and 12,740 fatalities in the United States. Despite advancements in treatment options, 80% of ovarian tumors recur or progress through treatments leading to a five year survival rate of just 50%. To address this unmet need, we employed a functional genomics approach to identify ovarian cancer specific drug sensitivities. Comparing the impact of each of the 117 drugs in our library across 32 cancer cell lines from 11 tumor lineages we identified Rigosertib, a small molecule RAS mimetic, as a selective agent for treatment of ovarian cancer. This ovarian cancer specific sensitivity to Rigosertib was validated in a panel of ovarian cancer cell lines and patient-derived organoids. This sensitivity fits with the observation that ovarian cancer exhibits a high frequency of alterations in both the PI3K and MAPK pathways, both of which can be negatively regulated through the disruption of RAS interactions. Concordantly, while the interplay between these pathways creates an inverse regulatory relationship by which inhibition of either pathway can lead to activation of the other, we observe that in all of the ovarian lines tested that treatment with Rigosertib leads to increases of MTOR signaling as indicated by phosphorylation of S6 both in vitro and in vivo. Subsequent combination drug screening revealed a cooperative effect between rigosertib and PI3K inhibitor (taselisib), suggesting that combining rigosertib with taselisib which restricts mTOR activation enhances therapeutic efficacy. This combination showed synergy across ovarian cancer cell lines, organoids, and xenograft models, with xenograft studies showing a significant reduction in tumor burden. These findings highlight the potential of rigosertib, especially when paired with PI3K inhibitors, to target the unique mutational landscape of ovarian cancer, offering a promising direction for more effective treatments.

#6002 Tyrosine kinase inhibitor (TKI) plus PD-1 blockade in TKI-responsive MSS/pMMR metastatic colorectal adenocarcinoma (mCRC): Results of a multicenter, phase II trial (TRAP).

J. Zhang[1], Q. Dong[1], Y. Wang[1], Y. Diao[1], X. Cui[2], L. Man[3], J. Zhang[4], Y. Zhang[5], T. Wu[6], T. Chen[7], Y. Dong[3], J. Zhang[8], Y. Huang[9], S. Wang[10];
[1]Liaoning Cancer Hospital & Institute, Cancer Hospital of Dalian University of Technology, Cancer Hospital of China Medical University, Liaoning Key Laboratory of Gastrointestinal Cancer Translational Research, Shenyang, China, [2]Chaoyang Central Hospital, Chaoyang, China, [3]Anshan Cancer Hospital, Anshan, China, [4]Liaoyang Central Hospital, Liaoyang, China, [5]The Second Department of Oncology, General Hospital of Fushun Mining Bureau of Liaoning Health Industry Group, Fushun, China, [6]The Second Hospital of Dalian Medical University, Dalian, China, [7]Benxi Central Hospital, Benxi, China, [8]Panjin Central Hospital, Panjin, China, [9]Liaoyang county Central Hospital, Liaoyang, China, [10]Shengjing Hospital of China Medical University, Shenyang, China

Background: There is an unmet need in immunotherapy for patients (pts) with MSS/pMMR mCRC. PD-1 blockade combined with antiangiogenic therapy has synergistic effects and has shown clinical benefits in these pts in several studies. TRAP (NCT04483219) aims to explore the efficacy and safety of antiangiogenic TKIs combined with PD-1 blockade in TKI-responsive MSS/pMMR mCRC pts refractory to standard treatment.
Methods: Eligible pts were given 1 cycle of TKIs (fruquintinib 5mg or regorafenib 120mg, d1-21, q4w) and grouped by tumor response according to RECIST v1.1: arm A: reduction of target lesion to CR, PR or shrunken SD, or cavitation in metastatic lung lesions, or decrease in the density of liver metastatic target lesions ≥15%; arm B: enlarged SD; arm C: PD. Then arm B pts were given another cycle of TKIs and grouped again. Pts in arm A then received TKIs plus anti-PD-1 antibody (toripalimab 240mg, or sintilimab 200mg, i.v.gtt, q3w, until PD or up to 2 years), pts in arm B continued with TKIs until PD. The Simon 2-stage design tested the null 9-months (m) PFS rate of 27.1% vs. 55% (power = 0.90; α = 0.05). A total of >10 pts reaching ≥ 9-m PFS in 25 pts receiving TKI plus anti-PD-1 antibody is required to reject null hypothesis.
Results: As of Sep 16, 2024 (data cutoff), 58 eligible pts were enrolled. Median age, 60 (34-75); male, 56.9%; ECOG PS 1, 96.6%; KRAS mutation, 48.3%; median prior regimens, 2 (1-4); prior anti-VEGF, 73.7%; prior anti-EGFR, 28.1%. 25 pts entered arm A and 29 entered arm C. Baseline characteristics of arm A were generally consistent with the overall population. In arm A, ORR was 20.0% (95%CI, 6.8%-40.7%). Median PFS was 11.5m (95% CI, 7.31-18.9), 6-m, 9-m and 12-m PFS rates were 87.1% (95% CI, 74.4%-100%), 58.1% (95% CI, 40.4%-83.4%) and 42.6% (95% CI, 25.7%-70.6%), respectively. At median follow-up of 31.1m (95% CI, 29.0-NA), median OS was 24.7m (95% CI, 17.0-NA), 12-m, 24-m and 36-m OS rates were 82.4% (95% CI, 68.1%-99.7%), 53.3% (95% CI, 35.6%-79.6%) and 35.5% (95% CI, 19.2%-65.5%), respectively. Median PFS and median OS were numerically longer in fruquintinib versus regorafenib treated pts (13.21m [95%CI: 7.31-NA] vs 8.95m [95%CI: 6.82-NA], p=0.38; 29.0m [95%CI: 17.0-NA] vs 19.5m [95%CI: 16.9-NA], p=54) and in KRAS wild-type and KRAS mutant pts (13.21m [95%CI: 8.95-NA] vs 7.31m [95%CI: 4.85-NA], p=0.21; 26.6m [95%CI: 17.9-NA] vs 15.0m [95%CI: 14.2-NA], p=0.24). The most common treatment-related adverse events (TRAEs) (total; Grade ≥3) were hand-foot skin reaction (43.8%; 18.8%), hypothyroidism (37.5%; 6.3%), hypertension (31.2%; 25.0%) and proteinuria (31.2%; 6.3%). No treatment related death was observed.
Conclusions: TKIs combined with PD-1 blockade has shown encouraging efficacy with acceptable toxicities in MSS/pMMR mCRC pts responsive to TKI treatment. The regimen could be a preferred approach in pretreated MSS/pMMR mCRC.

CLINICAL RESEARCH: Therapeutic Antibodies, Including Engineered Antibodies 2
Poster Session

#6006 The discovery and development of a CD3 agonistic single domain antibody to accelerate the development of CD3 T cell engager multi-specific antibodies.

Yu Liang[1], Yingyu Li[2], Yi Zhou[2], Cheng Luo[2], Xin Wang[2], Yang Xin[2], Shishuai Shao[2], Tingting Yang[2], Mingzhu Shao[2], Jin Chen[2], Ziyu Chen[2], Sujuan Hong[2], Hai Huang[2], Cheng Chen[2], Hui Wang[2], Miaomiao Song[2], Yuanxin Zhang[2], Shuai Yang[3], Xiaojie Tu[3], Sujuan Wang[3], Li Chen[2]

[1]Discovery, Probio, Inc, Pennington, NJ,[2]Discovery, Probio,Inc, Nanjing, China,[3]Discovery, Legend Biotech, Nanjing, China

CD3 T cell engager (TCE) bispecific antibodies (BsAbs) is a clinically well validated and dominant therapeutic modality of approved bispecific antibodies, contributing to 10 out of 14 marketed BsAb drugs. To develop a TCE BsAb with desired potency and safety profile, a CD3 agonist Ab with appropriate potency and ideal developability is critical. Currently, all approved TCE BsAbs are based on a CD3 agonist Ab in the format of conventional monoclonal antibodies containing both light chain and heavy chain, which may limit the flexibility in multi-specific antibody construction and development. In contrast, considering these limitations, single domain antibodies (sdAb, or VHH) are highly favored in the construction and development of bi- or multi-specific antibodies, mainly due to their smaller size, good stability and developability. Therefore, to ease the development of TCE multi-specific antibodies, we set forth to discover an agonist sdAb of CD3. We first identified a parental CD3 sdAb from an immunized camel phage display library. Through successful humanization and affinity maturation, we obtained a series of variants with a spectrum of binding affinity to CD3 and potency in activating T cells. These CD3 sdAb variants were then used to construct a series of bi- or multi-specific antibodies with ease and flexibility, no matter the TAA (tumor associated antigen) arm is a conventional monoclonal antibody (CD20, HER2, etc.), or sdAb (GPRC5D, etc.). The optimization process focused on fine-tuning the affinity, valency, and geometry of both the CD3 arm and TAA arm. This resulted in multi-specific antibodies with enhanced yield and developability, potent tumor cell killing capabilities, and with reduced cytokine release *in vitro* comparing to clinical benchmark antibodies. In preclinical in vivo studies, these TCE multi-specific antibodies showed favorable pharmacokinetics (PK) and safety profiles along with robust tumor growth inhibition. In summary, we have successfully discovered and developed a series of CD3 agonist sdAbs, showing a spectrum of affinity to CD3 and potency in T cell activation. These CD3 sdAbs have demonstrated a great potential in supporting and accelerating the development of diverse TCE multi-specific antibodies with enhanced ease and flexibility.

#6007 Neoadjuvant pepinemab enhances DC function associated with mature tertiary lymphoid structures and activity of immune checkpoint blockade in patients with metastatic melanoma.

Elizabeth E. Evans[1], Crystal Mallow[1], Terrence L. Fisher[1], Ayana Ruffin[2], Elaine Gersz[1], Maria Scrivens[1], Elizabeth Tuttle[1], Vuyani Matsenjwa[1], Ellen Giampoli[3], Manoj Chelvanambi[4], Brenda Melendez[4], Florentia Dimitrou[4], Alexander J. Lazar[4], Jennifer Wargo[4], Michael Lowe[2], Chrystal M. Paulos[2], Gregory B. Lesinski[2], Maurice Zauderer[1]

[1]Vaccinex, Inc., Rochester, NY,[2]Winship Cancer Institute at Emory University, Atlanta, GA,[3]University of Rochester, Rochester, NY,[4]University of Texas MD Anderson Cancer Center, Houston, TX

Neoadjuvant treatment with immune checkpoint blockade (ICB) improves clinical benefit relative to adjuvant therapy in multiple types of cancer. Window of opportunity studies employing neoadjuvant treatment permit integrated assessments of pathologic response along with assessments of treatment effects and mechanisms of resistance in the tumor microenvironment. Resistance mechanisms including immune exclusion and myeloid suppression have been shown to be regulated by semaphorin 4D (SEMA4D) in preclinical and clinical studies. A neoadjuvant window of opportunity study was designed to evaluate activity and efficacy of pepinemab, SEMA4D blocking antibody, combined with nivolumab (nivo) and/or ipilimumab (ipi) in patients with surgically resectable metastatic melanoma. Consenting patients with resectable stage III melanoma received neoadjuvant nivo alone, pepinemab/nivo, pepinemab/ipi, or pepinemab/nivo/ipi (NCT03769155). Patients underwent surgical resection following six weeks of immunotherapy. In separate studies conducted at University of Texas MD Anderson Cancer Center, patients received neoadjuvant treatment with nivo or nivo/ipi. Surgical tissue underwent high dimensional immune analysis using multiparametric flow cytometry, multiplex immunohistochemistry and Nanostring RNAseq and GeoMX imaging. Pepinemab in combination with nivo (either alone or with ipi) significantly increased tumor-infiltrating B cells and CD4+ T cells within surgical tissue, versus surgery or nivoalone. Compared to nivo alone, pepinemab plus nivo significantly influenced REACTOME pathways involved in degradation of extracellular matrix and signaling by Rho GTPases. Significant changes in DC activation markers including Flt3 ($p=0.03$) and cDC2 marker CD163 ($p=0.0071$), and the the Flt3/Wnt pathway correlated with pepinemab treatment and pathologic response. Multiplex IHC revealed tertiary lymphoid structures (TLS) within the tumor bed following treatment and were increased in patients who did not suffer tumor recurrence. TLS identified in biopsies of patients treated with pepinemab in combination with ICB were larger and more mature, containing high density of activated DCs and follicular DCs, follicular B cells, and Tfh cells expressing CXCR5, characteristic of germinal centers. Mature TLS were significantly increased in the triple combination compared to doublets or nivo alone. Increased density and maturity of TLS and increased T cell clonality correlated with pepinemab treatment and clinical benefit. All patients receiving triple combination therapy (8/8) were recurrence-free at 24 months with mature TLS in the resected tumors (6/6). Pepinemab represents a novel strategy to boost dendritic cell infiltration, activation, and organization of functional TLS to overcome limitations of ICB.

#6009 BGB-B2033, a novel 4-1BB/GPC3 bispecific antibody, exhibits potent in vitro and vivo antitumor activity in preclinical models.

Jie Li[1], Xi Yuan[2], Ruyue Ji[1], Chang Song[2], Yanwen Ma[2], Yuan Zhao[2], Yue Cao[2], Xinyi Liang[2], Yilu Zhang[2], Jingjuan Sun[1], Xiangli Ma[1], Lingmin Zheng[1], Beibei Jiang[2], Xiaomin Song[2], Yu Shen[1], Lai Wang[2]

[1]BeiGene (Shanghai) Biotechnology Co., Ltd., Shanghai, China,[2]BeiGene (Beijing) Co., Ltd., Beijing, China

4-1BB (CD137) is a key costimulatory immunoreceptor expressed on activated T-cells and natural killer cells, making it a promising therapeutic target in cancer. Glypican-3 (GPC3), a membrane-bound heparan sulfate proteoglycan, is overexpressed in approximately 70% to 80% of hepatocellular carcinomas, and extensively expressed in a variety of other solid tumors. GPC3 is recognized as an attractive drug target in precision cancer therapy as it is highly expressed in multiple tumors while minimally expressed in adult normal tissues. BGB-B2033 is a novel immunoglobulin G (IgG)-based bispecific antibody targeting 4-1BB and GPC3 and is under clinical development for the treatment of advanced or metastatic solid tumors in humans. BGB-B2033 binds to its target proteins with high specificity and affinity. Potent and GPC3-dependent functional activities were demonstrated using peripheral blood mononuclear cell (PBMC)-based immune cell activation and cytotoxicity assays. In humanized 4-1BB knock-in mice bearing human GPC3-expressing tumors, BGB-B2033 exhibited potent, dose-associated single-agent efficacy in Hepa1-6/hGPC3 orthotopic tumor model, as well as synergistic antitumor activity in combination with antiPD-1 antibody.
In conclusion, BGB-B2033 is a potent therapeutic agent with strong anti-tumor activity in both in vitro and in vivo studies. The phase 1 study of BGB-B2033, alone or in combination with Tislelizumab is ongoing (NCT06427941).

#6010 Evaluation of BsABN501: A novel T cell engager targeting CLDN3-positive solid tumors.

Saehyung Lee[1], **Sangin Lee**[1], Sunghyun Hong[1], Jaemun Kim[1], Jae Choi[1], Hobin Yang[2], Sungyoul Hong[3], Na Young Kim[1], Jun Young Choi[1], Young Kee Shin[4]

[1]ABION Inc., Seoul, Korea, Republic of,[2]College of Pharmacy, Kyungsung University, Busan, Korea, Republic of,[3]College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul, Korea, Republic of,[4]Department of Molecular Medicine and Biopharmaceutical Sciences, Graduate School of Convergence Science and Technology, Seoul National University, Seoul, Korea, Republic of

Claudin 3 (CLDN3) plays a critical role in tumor initiation, progression, and metastasis and is overexpressed in multiple solid tumors, including breast, ovarian, and small cell lung cancers. Previously, ABN501 was developed as a fully human monoclonal antibody targeting CLDN3, demonstrating strong anti-tumor activity and a favorable safety profile. In this study, we established the proof of concept for BsABN501, a CD3 T cell engager, utilizing the anti-CLDN3 sequence derived from the novel antibody ABN501. BsABN501 specifically binds to CLDN3 while concurrently targeting CD3 on T cells, thereby initiating T cell-mediated cytotoxicity (TDCC) against tumor cells. *In vitro* assays demonstrated that BsABN501 effectively activates peripheral blood mononuclear cells, resulting in potent TDCC against CLDN3-positive cancer cell lines. Notably, BsABN501 exhibited robust cytotoxic activity against CLDN3-expressing tumor cell lines compared to controls. Cytokine release assays further confirmed tumor-specific activation of T cells. Additionally, *in vivo* studies using xenograft mouse models bearing CLDN3-expressing tumors showed that BsABN501 induced significant tumor regression, highlighting its robust anti-tumor activity. In summary, BsABN501 represents a novel and promising therapeutic strategy for the treatment of CLDN3-positivie solid tumors by effectively recruiting and activating T cells to enhance TDCC. Moving forward, we aim to optimize the CD3 sequence and format of BsABN501 to further improve its anti-cancer efficacy and safety, paving the way for its development as a targeted immunotherapeutic for CLDN3-expressing malignancies.

#6011 Neoantigen peptide-loaded anti-CD40-spycatcher antibody as a novel cancer vaccine platform.

Xiaoying Cai[1], Dahee Jung[1], Seung-Oe Lim[2], Steve Seung-Young Lee[1]

[1]Pharmaceutical Sciences, University of Illinois at Chicago, Chicago, IL,[2]Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN

Precision cancer therapy opened up an attractive strategy by therapeutic vaccines targeting neoantigens. Neoantigens are immunogenic and HLA-bound epitopes capable of activating helper and cytotoxic T lymphocytes and not subject to immunological tolerance. However, personalized peptide-based cancer vaccines still have the limitations to formulate and deliver. For example, most vaccine technology was initially based on the use of adjuvants (e.g., alum), which only provides moderate therapeutic effects. To address these issues, we utilize the engineered anti-CD40 agonistic antibody expressing Spy-Catcher003 (αCD40-SC) as a novel antigen presenting cells (APCs)-targeted peptide vaccine delivery platform for effective cancer immunotherapy. Since targeting and activating the CD40 receptor on APCs via anti-CD40 agonistic antibody has been shown to elicit superior T cells priming and boost antigen-specific CD8+ T. Besides, the use of SpyCatcher003, which is a 113-residue protein with reactive Lys31, could trigger spontaneous and irreversible amidation reaction with SpyTag003. SpyTag003/SpyCatcher003 is a protein coupling approach, which can form an intermolecular isopeptide bond between Lys31 and Asp117. Moreover, the protein pair can react with a rate of 5.5×10^5 M^{-1} s^{-1}, which is helpful for potential therapeutic application.Therefore, we hypothesize the efficient and specific delivery, accomplished via SpyCatcher003-expressed anti-mouse CD40 agonistic antibody and SpyTag003-conjugated neoantigen peptide, will ensure the present of neoantigens to T cells and elicit robust and durable specific CD8+ T cell response. Our goal is to directly deliver neoantigen peptide-loaded αCD40-SC to APCs in draining lymph nodes (dLN) as a therapeutic vaccine.

#6012 PSMA x CD3 T-cell engagers show preclinical efficacy for the treatment of prostate cancer.

Peter Bergqvist, Alaa Amash, Kelly Bullock, Lauren Clifford, Patrick Farber, Jessica Fernandes Scortecci, Ingrid Knarston, Tallie Kuang, Ahn Lee, Amy Lee, Cindy-Lee Crichlow, Franco Li, Matt Mai, Stephanie K. Masterman, Janice Reimer, Eduardo Solano Salgado, Raffi Tonikian, Christopher Williamson, Allison Goodman, Lindsay DeVorkin

AbCellera, Vancouver, BC, Canada

Prostate-specific membrane antigen (PSMA) is a clinically validated target in metastatic castration-resistant prostate cancer (mCRPC) that is being prosecuted by a number of different modalities in the clinic. CD3 T-cell engagers (TCEs) targeting PSMA have shown promise in preclinical and early clinical studies, but generating a molecule with a therapeutic window that enables efficacious dosing in patients has been a barrier to development. Here, we present preclinical *in vitro* and *in vivo* data on novel PSMA x CD3 TCEs developed using our TCE platform.

To address the challenges of TCE development for mCRPC, we screened and identified hundreds of diverse PSMA- and CD3-binding antibodies with different affinities, epitopes, and biophysical properties using our proprietary antibody screening platform. From there, we engineered large panels of Orthomab^{TM} PSMA x CD3 bispecifics, varying TCE parameters that impact function. Detailed *in vitro* functional assessment and biophysical characterization assays were conducted to identify antibodies with promising functional and developability profiles.

IgG-like bispecifics comprised of PSMA- and CD3-binding arms with finely tuned affinity for each target were generated. PSMA binding epitopes were assessed using cryo-electron microscopy, and TCE function was measured using *in vitro* T cell co-culture assays. TCEs binding membrane-proximal epitopes drove optimal immune synapse formation, leading to potent killing of cells expressing high (C4-2) and low (22Rv1) levels of PSMA with EC_{50} values in the picomolar range. Molecules show target-dependent T-cell activation with no killing of a low PSMA-expressing cell line (DU-145) *in vitro*. Further, select molecules show robust $CD4^+$/$CD8^+$ T-cell activation and proliferation in the presence of target cells, as well as sustained killing of target cells over time in a repeat challenge assay. Finally, molecules evaluated *in vivo* in a humanized C4-2 xenograft mouse model demonstrated anti-tumor activity and a favorable IgG-like pharmacokinetic profile.

In summary, we engineered and assessed hundreds of PSMA x CD3 TCEs at high-throughput, conducted detailed *in vitro* functional and biophysical characterization, and identified molecules with promising preclinical *in vivo* efficacy that supports further evaluation and development towards the clinic.

#6014 A new generation of Tumor Microenvironment Activated Therapeutic (T-MATE) targeting solid tumors.

Xiaoya Ma, Yang Zhou, Xianzhi Jiang, Juy-Yi Chen, Rodrigo Rivera, Randall Bettman, Thanthar Aye, Ashley Navarro, Daniel Tong, Ricky Cheng, George Wu, **Aude Segaliny**, Yonglei Shang

Amberstone Biosciences Inc, Irvine, CA

T-cell engagers (TCEs) have emerged as potent immuno-therapies, demonstrating significant success in treating hematologic cancers and showing promising clinical benefits in solid tumors. However, their therapeutic potential is often limited by severe side effects, primarily due to cytokine release syndrome and on-target/off-tumor toxicities. To address these challenges, we leveraged Amberstone's Tumor-Microenvironment Activated ThErapeutic (T-MATE) technology to develop bispecific and multispecific TCE antibodies with strong potency in diseased tissues, specifically in the acidic tumor microenvironment, while exhibiting minimal activity in healthy tissue under normal physiological conditions. We have discovered and engineered a set of proprietary pH-dependent CD3 antibodies with varying affinities and binding kinetics. Due to the pH-induced conformational change, TCE T-MATEs offer a wide therapeutic index when paired with antibodies targeting several well-studied tumor associated antigens. Here, we demonstrate the potential of the T-MATE platform by targeting clinically validated tumor associated antigens that are widely expressed in healthy tissues, such as TROP2, FOLR1 and EGFR. All T-MATE TCEs were tested *in vitro* through various cell-based assays, including pH-dependent binding and T-cell-dependent cytotoxicity, and *in vivo* using xenograft models in humanized NSG mice, across several cancer indications (ovarian, endometrial, colon, gastric, pancreatic, breast, and non-small cell lung cancers). All TROP2, FOLR1 and EGFR-targeting TCEs showed robust pH-selectivity in target binding and exhibited potent T cell-dependent cytotoxicity (EC_{50} ~ 1-100 pM) against antigen-expressing tumor cells under acidic tumor conditions, while with minimal activity under physiological conditions. In preclinical xenografted tumor models, these TCE T-MATEs induced significant tumor regression. In Non-Human Primate pharmacology and toxicology studies, TCE T-MATEs exhibited exposure comparable to clinically validated TCEs, with negligible cytokine release and a favorable toxicity profile. These preclinical results confirm that the T-MATE platform offers a superior therapeutic margin, enabling the targeting of widely expressed tumor antigens that were previously considered intractable. Beyond oncology, the T-MATE platform holds promise for a broad range of indications, offering an optimal balance of safety and potency for patients with unmet medical needs. **Acknowledgements:** Ms. Lingrong Zhang for her vivarium support.

#6015 Unveiling RYK: A breakthrough tumor-associated antigen in lymphoma treatment.

Matilde Micillo[1], Beatrice Pirajno[1], Giulia Omezzolli[1], Amelia Fasci[1], Nahal Nabelsi[1], Francesco E. Vallone[1], Victor Navarro-Tableros[2], Elisabetta Bolli[3], Samir Mouhissine[4], Riccardo Moia[4], Candida Vitale[5], Marta Coscia[5], Gianluca Gaidano[4], Brian J. Lannutti[6], Katti A. Jessen[6], Tiziana Vaisitti[1], Silvia Deaglio[1]

[1]Department of Medical Sciences, University of Turin, Turin, Italy,[2]2i3T, Societa Per La Gestione Dell'incubatore Di Imprese e Per Il Trasferimento Tecnologico, University of Turin, Turin, Italy,[3]Department of Molecular Biotechnology and Health Sciences, University of Turin, Turin, Italy,[4]Division of Hematology, Department of Translational Medicine, University of Eastern Piedmont, Novara, Italy,[5]Department of Molecular Biotechnology and Health Sciences and AOU Citta della Salute e della Scienza, University of Turin, Turin, Italy, [6]Solve Theraputics, Inc, San Diego, CA

This work explores expression and therapeutic potential of RYK, a ROR-family receptor tyrosine kinase (RTK) member, in chronic lymphocytic leukemia (CLL) and Richter syndrome (RS). RYK belongs to a family of conserved proteins acting as alternative receptors/co-receptors of Wnt ligands. While the best-known member of the family, ROR1, has been targeted with various therapeutic modalities, little is known for RYK. RNA-sequencing data shows that RYK is expressed in CLL, RS, diffuse large B-cell lymphoma, and Burkitt's lymphoma primary cells and malignant B cell lines of different developmental stages, as also confirmed both at mRNA and protein levels. Like ROR1, RYK expression was restricted to cancer cells, with undetectable levels in B, T, or NK cells, obtained from healthy donors. Given this pattern of expression, an antibody drug conjugate (ADC) against RYK, SLV-404, was generated, consisting of a chimeric antibody targeting the extracellular portion of the receptor, a cleavable linker, and a cytotoxic agent, Dxd, an inhibitor of the topoisomerase I (TOP1) with a target drug-to-antibody ratio (DAR) of eight. Expression data showed that TOP1 is mainly expressed in cancer cells and tumor cells are uniquely sensitive to its inhibition.We then investigated in vitro apoptotic responses following SLV-404 treatment. After 72/96 hours of exposure, a marked apoptotic induction was observed by conventional flow cytometry assays (Annexin V/PI staining) and at the biochemical level by Western blot analysis of PARP1/Caspase 3 cleavage, XIAP reduction, and histone H2A.X phosphorylation. SLV-404 effects were then evaluated in vivo exploiting four established RS patient-derived xenograft models (RS-PDX) and a cell line-derived xenograft model, U-RT1. RS cells were initially injected subcutaneously and left to engraft. Mice were then randomly assigned to two different groups: vehicle (phosphate-buffered saline) and SLV-404 (10 mg/kg), all treated by intravenous administration (iv), once a week for 3 weeks in total. SLV-404 significantly extended animal lifespan of 61 days (P<.04), 36 days (P<.02), 68 days (P<.02), 97 days (P<.01) and 47 days (P<.01), respectively for RS1316, RS9737, IP867/17, RS1050 and U-RT1.We then moved to a systemic model of RS, with leukemic cells injected iv and left to engraft 14 days before administering treatment once a week for 3 weeks in total. Disease spread was then evaluated by flow cytometry analysis of target organs, using tumor-specific human markers. Preliminary data confirm that treatment with SLV-404 resulted in dramatic decreases in RS cells to almost undetectable levels in all compartments, reaching statistical significance in spleen and bone marrow (P<.0001).Together, these results suggest that RYK is a novel tumor-associated antigen, making it a suitable ADC target. Moreover, we demonstrate the therapeutic potential of a novel RYK-based ADC for the treatment of RS.

#6016 AP402, a bispecific antibody targets p95HER2 and CD137, shows a potent antitumor activity in p95HER2-expressing cancers.

Po-Lin Huang, Hsin-Ta Hsieh, Hung-Tsai Kan, Ching-Hsuan Hsu, Kuan-Ming Huang, Hsin-Yu Chen, Jhong-Jhe You

AP Biosciences, Inc, Taipei, Taiwan

Introduction: p95HER2 is the extracellular domain-truncated form of HER2 receptor while preserves the intracellular tyrosine kinase activity to support tumor growth. p95HER2 was found in 30 to 40% HER2-positive breast cancer patients and associated with a more aggressive phenotype and worse prognosis. Due to the lack of extracellular domain, p95HER2 has been recognized as the potential mechanism of trastuzumab-resistance. The unmet need for trastuzumab-resistant patients and exclusive expression in cancerous tissues make p95HER2 as an ideal therapeutic target. Here, AP402 was designed to coordinate two dominant effector cells, natural killer and T cells, against p95HER2-positive cancers.

Experimental procedures: AP402 bispecific antibody was generated by conjugating CD137 single-chain variable fragment (scFv) to the heavy chain C-terminal of p95HER2 antibody (human IgG1). The affinity and specificity of p95HER2 antibodies were checked by biolayer interferometry (BLI) and flow cytometry, respectively. ADCC and ADCP activities of AP402 were determined by NK cells and monocyte-derived macrophages, respectively. AP402-mediated activation of T cells were studied by coculturing T cells with p95HER2-transgenic cells. Antitumor activity of AP402 was studied using humanized mice inoculated with p95HER2-transgenic cancer cells and p95HER2-positive breast cancer patient-derived xenograft (PDX). Safety of AP402 was determined by the 4-week toxicity study in monkeys.

Results: 1. AP402 showed the superior affinity (K_D 0.34 nM) to p95HER2 with the minimal crossreactivity to HER2 (K_D 38.4 nM). 2. AP402 elicited ADCC robustly in p95HER2-NCI-H292 (EC_{50} 0.006 nM) and p95HER2-MDA-MB-231 cells (EC_{50} 0.043 nM), while in SKBR3 cells to a minimal level, but not in the wildtype and CD137-HEK293 cells at all. 3. ADCP was induced by AP402 in macrophages cocultured with p95HER2-NCI-H292 cells. 4. Compared to the mono or combinational treatment of parental monoclonal antibodies, AP402 boosted the most vigorous T cell activation, which intensity corresponded to the expression levels of p95HER2 on cancer cells. 5. AP402 showed the potent antitumor activity in p95HER2-MDA-MB-231 (TGI 92.3%) and p95HER2-NCI-H292 (TGI 73.6%) tumor-bearing mice dosed with 0.4 mg/kg, BIW. Antitumor activity was also shown in mice inoculated with p95HER2-positve breast cancer PDXs (TGI 59%). 6. In monkey Tox study, the exposure of AP402 was approximately dose proportional in doses 25-100 mg/kg with $t_{1/2}$ ~108 hrs. NOAEL: 100 mg/kg.

Conclusions: Nonclinical studies demonstrated that AP402 is efficacious in triggering the effector functions of NK and T cells in vitro and blocking the growth of p95HER2-positive breast and lung tumors in vivo. AP402 was well tolerated in repeat doses up to 100 mg/kg in monkeys.

#6017 L19-mTNF as a novel synergistic combination partner to enhance the efficacy of CD3-based bispecific antibodies in colorectal cancer.

Gudrun Thorhallsdottir, Ramon Benz, Emanuele Puca, Mattia Matasci, Dario Neri, Abdullah Elsayed

Philochem AG, Zurich, Switzerland

T-cell-engaging bispecific antibodies (TCBs) are an expanding modality in immunotherapy, designed to redirect T-cell cytotoxicity toward tumor cells by cross-linking them with tumor-associated antigens[1]. While TCBs have shown significant efficacy in hematological malignancies, their success in solid tumors remains limited[2]. This is likely due to multiple factors, including the low number of T cells in the tumor microenvironment and the presence of immunosuppressive conditions that hinder T-cell activity[3, 4]. In this study, we evaluated the potential of three clinical-stage antibody-cytokine fusion proteins as combination partners to enhance the efficacy of a CEA-targeting CD3-based TCB in a challenging solid tumor model. Using the murine colorectal cancer cell line MC38-CEA, we combined the TCB with L19-mTNF, L19-mIL12, and L19-mIL2. Among these, L19-mTNF demonstrated remarkable synergistic anti-tumor activity, achieving complete tumor regression in treated mice, a significant improvement compared to the limited activity of either agent alone.Our findings suggest that mTNF enhances TCB activity by transforming "cold" tumors into "hot" ones. L19-mTNF appears to increase vascular permeability within the tumor microenvironment, improving T-cell and TCB infiltration and amplifying the immune response.These results highlight the novel potential of L19-mTNF as a powerful combination partner for CD3-based TCBs, offering a promising strategy to overcome the challenges of solid tumor immunotherapy. This preclinical work provides a strong rationale for future clinical translation, underscoring the importance of integrating cytokine-based therapies to maximize TCB efficacy in solid malignancies.

1. Labrijn, A. F., Janmaat, M. L., Reichert, J. M., & Parren, P. W. H. I. (2019). Bispecific antibodies: A mechanistic review of the pipeline. Nature Reviews Drug Discovery, 18(8), 585-608. https://doi.org/10.1038/s41573-019-0028-1 2. Kassner, J., et al. (2024). Current landscape of CD3 bispecific antibodies in hematologic malignancies. Trends in Cancer, 10(8), 708-732.3. Appleman, L. J., & Boussiotis, V. A. (2003). T cell anergy and costimulation. Immunological Reviews, 192, 161-180.4. Mueller, D. L., Jenkins, M. K., & Schwartz, R. H. (1989). Clonal expansion versus functional clonal inactivation: A costimulatory signalling pathway determines the outcome of T cell antigen receptor occupancy. Annual Review of Immunology, 7(1), 445-480.

#6019 AI-guided development of CAR-T drugs.

C. Cheng[1], Y. Ge[2], E. Xie[1], H. Wang[1], **X. He**[1], H. Zhu[2];
[1]Oricell Therapeutics Co.,Ltd., Shanghai, China, [2]Shanghai Key Laboratory of Maternal Fetal Medicine, Shanghai Institute of Maternal-Fetal Medicine and Gynecologic Oncology, Shanghai First Maternity and Infant Hospital, School of Medicine, Tongji University, Shanghai, China

Background: Chimeric Antigen Receptor T-Cell (CAR-T) therapy is an innovative cancer treatment that involves genetically engineering a patient's T-cells to recognize and attack cancer cells.Artificial Intelligence (AI) is playing an increasingly important role in drug development, accelerating drug discovery, optimizing treatment protocols, and enhancing efficacy.Building on the in-depth research of Nobel laureates Demis Hassabis and John Jumper in structural prediction, AI technology has been combined with antibody library techniques. Under the guidance of AI, antibodies targeting the second epitope of FOLR1 were successfully identified from hundreds of candidates.
Methods: In this study, we performed phage display panning for FOLR1, a tumor target, followed by monoclonal identification and sequencing. This resulted in a large number of antibodies targeting the same epitope. Due to the structural characteristics of FOLR1, most antibodies bind to its folate binding site. To obtain antibodies targeting different epitopes and enhance therapeutic efficacy, we combined AI-guided selection with antibody library screening. We conducted NGS sequencing of the antibody library and utilized AI for extensive modeling analysis.
Results: Through next-generation sequencing (NGS) of the antibody library, we obtained a large number of sequences distinct from those obtained through monoclonal sequencing. We modeled and docked these antibodies to predict their targeted epitopes. After analyzing over 300 antibodies with the highest binding potential, we identified one antibody that targets a different epitope. This antibody demonstrated significant binding efficacy and does not compete with our previously identified candidate antibodies.
Conclusions: By integrating AI technology with our antibody library, we successfully identified a second epitope-targeting antibody against FOLR1. This antibody exhibits properties comparable to those obtained through wet lab experiments. As AI technology continues to advance, its role in antibody development will become increasingly significant, transitioning from AI-assisted drug development to AI-guided drug development. Finally, we extend our heartfelt gratitude to Demis Hassabis and John Jumper for their contributions to AI research. Without their foundational work, it would have been very difficult for us to achieve these results.

#6020 Quantitative measurement of Fc-mediated ADCC & ADCP activity of therapeutic antibodies using a robust, universal cell-based assay platform.

Alpana Prasad, Surekha Bonasu, Gaurav Agrawal, Jane E. Lamerdin

Eurofins DiscoverX, Fremont, CA

The clinical success of a broad range of biologics has driven the development of diverse immunomodulatory agents with unique mechanisms of action (MOA) targeting novel antigens. Regulatory guidelines now require evaluation of antibody effector functions, such as antibody-dependent cell-mediated cytotoxicity (ADCC), particularly for lot-release assays that measure immune cell-mediated killing directly rather than surrogate endpoints. Additionally, antibody-dependent cellular phagocytosis (ADCP) has become a key MOA, especially for IND-enabling studies involving antibodies with modified Fc regions. To address these needs, Eurofins DiscoverX® developed the KILR® cytotoxicity assay platform that accurately measures the killing of antigen-expressing target cells in co-culture with immune effector cells. This dye-free, radioactivity-free platform leverages the validated Enzyme Fragment Complementation (EFC) technology and supports various immune-mediated killing mechanisms, including ADCC, CDC, ADCP, and T-cell redirection. Here, we showcase the KILR platform's versatility across multiple antigens (e.g., HER2, CD20, CD19) and effector cell types. Combined with engineered KILR CD16 Effector Cells, the KILR platform provides robust, reproducible assay performance necessary for testing in the quality-controlled environment for commercial lot release. This platform also quantifies ADCP cost-effectively, simplifying traditional FACS or imaging-based workflows. To ensure assay reproducibility, many KILR target cell models are now available in an assay-ready format, with phase-appropriate qualification data supporting their suitability for potency and screening applications.

#6021 Development of JLF025, an anti-mesothelin T cell engager optimized for efficacy and safety.

Xiaodong Xiao[1], Zhidi Pan[2], Junjun Liu[3], Yali Yue[3], Fengzhi Li[4], Shusheng Wang[1], Haiqiu Huang[1], Kaiyong Yang[1], Zhangyi Song[1], Zhenyu Wang[4], Hua Jiang[1], Yueqing Xie[1], Jianwei Zhu[1]

[1]Jecho Laboratories, Frederick, MD,[2]Jecho Institute, Shanghai, China,[3]SJTU, Shanghai, China,[4]Jecho Biopharmaceuticals, Tianjin, China

Mesothelin (MSLN) is a GPI-anchored glycoprotein that is overexpressed in several solid tumors including mesothelioma, pancreatic cancer, ovarian cancer, non-small cell lung cancer, biliary cancer, and triple negative breast cancer with limited expression in normal tissues. This expression profile makes MSLN an attractive target for cancer immunotherapy. m912 antibody that has demonstrated positive effect in CAR T format in clinics is derived from a fully human Fab library. It has a 100% germline sequence, but its binding affinity is relatively poor (KD around 60 nM). To expand applications of the m912 to include the T-cell engager format, we used phage display to improve m912 affinity, and further, developed a bivalent T-cell engaging bispecific format by fusing the humanized OKT3 scFv to the C-terminus of the light chain. When the final lead with a KD of 1.4 nM was incorporated into this format, the resulting bispecific antibody JLF025 showed high potency in T-cell activation and T-cell dependent cytotoxicity in vitro. Interestingly, the cytotoxicity induced by MSLN-490 was not affected by soluble MSLN at a level found in cancer patient sera. Using a co-grafting mouse model in which a mixture of MSLN-positive NCI-N87 cancer cells and human PBMCs was co-injected with JLF025, we demonstrated that JLF025 exhibited a remarkable tumor suppressing activity. The potent tumor suppressing activity was also observed in a xenograft model in which PBMCs were not co-grafted with tumor cells but delivered from the periphery. In addition, combination of MSLN-490 with Atezolizumab or a chemotherapy drug paclitaxel produced a synergistic effect on tumor inhibition and more T cells were found infiltrated into tumors in the combo groups. In hMSLN and hCD3 double knock-in mouse, JLF025 demonstrated acceptable safety profiles. Thus, combination of JLF025 with immune checkpoint inhibitors and chemotherapy drugs represents a promising cancer treatment strategy and could be considered for future clinical trials.

#6022 YH006: a next-generation CTLA-4×OX40 Treg antagonist, demonstrates superior tumor-infiltrating Treg-depletion efficacy and favorable manufacturability.

Sarah Chan, Baihong Liu, Bahetiyaer Huwatibieke, Jing Zhang, Yang Chen, Minjuan Meng, Wenchao Zheng, Lei Zhang, Yi Yang, **Jeremy Lee**

Biocytogen, Waltham, MA

Immune checkpoint inhibitors have revolutionized the treatment of early and advanced cancer. Especially combination of anti-cytotoxic T lymphocyte associated antigen 4 (CTLA-4) antibody and anti-programmed death-1 (PD-1) antibody, has shown remarkable efficacy against various cancers and approved for 7 indications by the US FDA. However, there are still limitations with regard to response rates and dose-dependent immune related adverse events (irAEs) caused by CTLA-4 antibody for fully achieve the optimal therapeutic effect for the combination strategy. YH006 is a monovalent bsAb with effector enhanced Fc domain, generated from Biocytogen RenLite^{TM} common light chain mice, designed to deplete tumor-infiltrating Treg cells with better specificity, based on tumor-infiltrating Treg cells consistently exhibit higher expression levels of both CTLA-4 and OX40 compared to T cells in the periphery across multiple cancer types. YH006 potent to selectively depletion of tumor-infiltrating Tregs and increase the intra-tumoral CD8/Treg ratio, outperform the first and other next generation of anti-CTLA-4 agents in vivo efficacy at low dose. Notably, the antitumor activity of PD-1 mAb was significantly enhanced when combined with YH006 in syngeneic MC38 models at high tumor burden. Ongoing CMC process development has a titer of over 6 g/L at the 20 L scale, and the overall purification yield of >80%. YH006 also has consistent quality from research cell bank to passage 90, suggesting excellent developability.

#6023 Intrabody-mediated TLR2 and TLR9 blockade as new strategy to inhibit inflammation-driven pancreatic cancer growth.

Martin Gasser[1], Thomas Boeldicke[2], Ana Maria Waaga-Gasser[1]

[1]Brigham and Women's Hospital, Harvard Medical School, Boston, MA,[2]Helmholtz Centre for Infection Research, Braunschweig, Germany

Pancreatic cancer (pancreatic ductal adenocarcinoma, PDAC) remains a deadly cancer worldwide with a need for new therapeutic approaches. A dysregulation in the equilibrium between pro- and anti-inflammatory responses with a predominant immunosuppressive inflammatory reaction in advanced stage tumors seem to contribute to tumor growth and metastasis. The current therapies do not include strategies against pro-tumorigenic inflammation in cancer patients. We have shown that the upregulated cell surface expression of Toll-like Receptor (TLR) 2 and of TLR9 inside PDAC cells maintain chronic inflammatory responses, support chemotherapeutic resistance, and mediate tumor progression in human pancreatic cancer. We further demonstrated intracellular TLR2 and TLR9 targeting using specific intrabodies, which resulted in downregulated inflammatory signaling. In this study, we tested, for the first time, an intrabody-mediated TLR blockade in human TLR2- and TLR9-expressing pancreatic cancer cells for its effects on inflammatory signaling-mediated tumor growth. Newly designed anti-TLR2- and anti-TLR9-specific intrabodies inhibited PDAC growth. Co-expression analysis of the intrabodies and corresponding human TLRs showed efficient retention and accumulation of both intrabodies within the endoplasmic reticulum (ER), while co-immunoprecipitation studies indicated both intrabodies interacting with their cognate TLR antigen within the pancreatic cancer cells. Cancer cells with attenuated proliferation expressing accumulated TLR2 and TRL9 intrabodies demonstrated reduced STAT3 phosphorylation signaling, while apoptotic markers Caspases 3 and 8 were upregulated. To conclude, our results demonstrate the TLR2 and TLR9-specific intrabody-mediated signaling pathway inhibition of autoregulatory inflammation inside cancer cells and their proliferation, resulting in the suppression of pancreatic tumor cell growth. These findings underscore the potential of specific intrabody-mediated TLR inhibition in the ER relevant for tumor growth inhibition and open up a new therapeutic intervention strategy for the treatment of pancreatic cancer.

#6024 A novel chemical conjugated anti-CTLA-4 antibody with tumor microenvironment activation.

Cheng Liu, Yuan Liu, Chengli Ding, Ying He

Affinity Biopharmaceutical Co., Ltd., Shanghai, China

Anti-CTLA-4 antibody is considered the “forefront” of immune checkpoint inhibitors as it plays a critical role in preventing CTLA-4 mediated inhibitory signals that downregulate T-cell immune responses, and functioning early at the initial phase of naive T-cell activation. However, CTLA-4 is not a tumor associated antigen and it’s expressed in a variety of normal tissues such as lymph nodes, tonsil and lung, therefore anti-CTLA-4 antibody can trigger severe on-target, off-tumor toxicities and immune-related adverse events (irAEs), substantially restricting its clinical applications and potential. IMD-303 is a novel Tumor MicroEnvironment Activated Antibody (TMEAbody) engineered through chemical conjugation of a legumain-activated chemical linker together with a 20kDa masking group at the variable region. The activating protease, legumain (AEP), is a tumor-specific, acid endopeptidase with tumor site over-expression and a strict specificity for one particular amino acid (Asn), making it a first-in-class, highly specific and pan-solid tumor target in novel drug design. Through screening of TME-activated linkers, glycosylation and masking structures, IMD-303 TMEAbody is inert in circulation as its binding to T cells is blocked in normal tissues, ensuring minimal immunogenicity and systemic toxicities; when entering the Tumor MicroEnvironment (TME), IMD-303 becomes rapidly activated and unmasked by legumain, effectively stimulating CD8+ T-cell proliferation and depleting Treg cells by antibody-dependent cellular cytotoxicity (ADCC). Preclinical pharmacological studies showed that the unmasked IMD-303 exhibited strong binding to CTLA-4, efficiently blocking its interaction with CD80/CD86 in the TME, and high affinity to CD16a, CD32a, and CD64 Fc receptors, enhancing ADCC/ADCP effector functions of Treg depletion locally within the TME. And IMD-303 with masking has greatly improved the safety profile due to the fact that it reduced binding to CTLA-4, CD16a, CD32b (FcγRIIb), and C1q far more than that of CD32a and CD64 in circulation. In animal models, IMD-303 demonstrated strong anti-tumor activities as single agent in multiple syngeneic murine tumor models, and it displayed significant synergistic effects when combined with IMD101 (a TMEA-Cytokine releasing IL-2) in EMT6 tumor model, in which IMD-303 can eliminate the number of Treg cells in tumors that are slightly up-regulated by IMD101. Preclinical toxicology studies showed that the favorable safety profile of IMD-303 allows for continued treatment with repeated dosing, and it’s well-tolerated with a weekly repeated regimen of 200mg/kg in cynomolgus monkeys. All these findings indicate that IMD-303, with its preferential activation within tumors, exhibits superior efficacy and safety performances and has the potential to successfully transform the cold tumor microenvironment which lacks T-cell infiltration and immune responses.

#6025 ABBV-514: an afucosylated CCR8 specific antibody that targets and eliminates key immunosuppressive tumor regulatory T cells.

Amanda Schmidt Paustian[1], Linlin Guo[2], Weiguo Feng[3], Ryan Duggan[1], Mitra Bhattacharyya[1], Andrew McCluskey[4], Kelsey Gallant[4], Christopher Negron[4], Michael McDevitt[2], Cyril Ramathal[1], Diana Chen[2], Maulik Patel[1], Heath Smith[1], Keith Hamel[1], Julie Wilsbacher[1]

[1]AbbVie Inc., North Chicago, IL,[2]AbbVie Inc., South San Francisco, CA,[3]Daiichi Sankyo Inc., Basking Ridge, NJ,[4]AbbVie Inc., Worcester, MA

Regulatory T cells (Tregs) have long been appreciated as a key suppressive cell type that limits anti-tumor immunity, yet it has been challenging to find an effective approach to target this important cell population. CCR8 has recently emerged as a promising target due to its enhanced expression on tumor Tregs compared to other Treg and T effector cell populations. A greater abundance of CCR8+ Tregs is also correlated with poor clinical outcomes in several solid tumor indications. Here we report the discovery and comprehensive preclinical characterization of ABBV-514, a human CCR8 specific afucosylated hIgG1 antibody and a functionally similar mouse CCR8 specific mIgG2a surrogate antibody. Both ABBV-514 and the mouse surrogate specifically bind to and robustly deplete CCR8 expressing cells via ADCC without inhibiting CCL1-stimulated signaling. The mouse surrogate selectively depletes CCR8+ intratumoral Tregs and has strong monotherapy activity in a variety of in vivo syngeneic tumor models including models that are insensitive to anti-PD-1 treatment. We have used scRNAseq and TCRseq studies to provide novel insights into the mechanisms behind the anti-tumor immune response mediated by the CCR8 mouse surrogate ± anti-PD-1 combination therapy. The transcriptional and phenotypic profiles of intratumoral CD8+ T cells are greatly altered upon CCR8+ tumor Treg depletion compared to anti-PD-1. Tumor Treg depletion upon anti-CCR8 treatment leads to enrichment of a distinct effector memory-like CD8+ T cell population with dramatically enhanced Granzyme K expression and reduced expression of exhaustion associated transcripts including TOX and Lag-3. This population is further augmented with anti-CCR8/anti-PD-1 combination therapy. Similarly, ABBV-514 binds human or cyno CCR8 expressing cells and mediates ADCC of CCR8+ Tregs in vitro. ABBV-514 is currently being evaluated in a Phase I clinical trial, both as a monotherapy and in combination with budigalimab, a PD-1-blocking antibody (NCT05005403). Together, these data highlight ABBV-514 as a therapeutic approach to enhance anti-tumor immunity. WF was an employee of AbbVie at the time of the study. All other authors are current employees of AbbVie. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

#6026 HX016-9, a novel PD1xVEGF BsAb, as a new candidate treatment of solid tumors.

H. Ke[1], T. Yang[1], F. Peng[1], J. Li[1], C. Chen[1], L. Zhang[1], S. Xiong[2], F. Zhang[1], **H. Q. Li**[1];
[1]Hanx Bio, Hangzhou, China, [2]Crown Bioscience, Hangzhou, China

With the clinical confirmation of the superior efficacy of AK112, a first-in-class (FIC) PD1xVEGF bispecific antibody (BsAb), in the treatment of NSLCL cancers, over the PD1 monoclonal antibody (mAb), as shown in a head-to-head phase III trial, we set out to engineer a novel and potentially best-in-class (BIC) PD1xVEGF BsAb using a unique PD1 (HX008, an approved PD1-mAb) and a novel VEGF (HX006, a mAb with more favor activity over Avastin) mAb sequences. HX016-9 has 2+2 IgG1 symmetric structure which was constructed by grafting the PD1 binding domain of HX008 onto the N-terminus of HX006. HX016-9 binds PD1 recombinant protein with EC_{50} of 0.13nM (vs. 0.1nM by HX008) and to VEGF recombinant protein with EC_{50} of 0.097nM (vs. 0.042 by HX006) in ELISA assay. It also binds similarly to the $PD1^+$ cell line as HX008 with slightly reduced affinity. A reporter bioassay for PD1 function demonstrated that HX016-9 has EC_{50} of 3.2nM (vs. 1.0nM for HX008), while a reporter- bioassay for the VEGF function demonstrated that HX016-9 has EC_{50} of 12.6nM (vs. 11.4nM for HX006), confirming HX016-9 designed function *in vitro*. Interestingly, HX016-9 demonstrated enhanced reporter activity (up to 5x) in the presence of VEGF than those without, while in contrast, no enhancement for HX008 in the presence of VEGF, similarly as reported for the FIC molecule previously. This enhancement could possibly be interpreted by the formation of the VEGF-HX016-9 complex, thus it facilitating efficient cis-binding to these PD1+ cells and higher avidity binding. This further implicates the enhanced PD1 blocking by HX-016-9 in these cells when VEGF presents. In the huPD1-C57/B6-based HuGEMM mouse, HX016 was shown to have stronger anti-tumor activity over the HX008 and HX006 in huPD-L1/huVEGF-MC38 CRC syngeneic CRC tumor, which can be attributed to both PD1- and VEGF-targeting. In a xenograft A549 NSCLC tumor in immunocompromised mice, HX016-9 also caused anti-tumor activity that can be attributed to anti-VEGF function. These activities are comparable or superior to those of the FIC molecule, warranting the HX016-9 candidacy to move into IND-enabling evaluation and further clinical development.

#6027 Translational medicine studies of BRY812, an anti-LIV-1 antibody drug conjugate for cancer therapeutics.

Qinqin Zhuang[1], Qianqian Tao[1], Xiaobei Zhao[2], Jie Zhu[2], Lei Nie[1], Gang Chen[2], Haibin Wang[1]

[1]Bioray Pharmaceutical Ltd., Hangzhou, China,[2]Bioray Pharmaceutical Co., San Diego, CA

LIV-1, also known as SLC39A6 or ZIP6, belongs to the zinc transporter family and was initially identified as an estrogen-inducible gene in breast cancer. Immunohistochemical (IHC) analysis have revealed escalated LIV-1 expression in estrogen receptor-positive (ER+), hormone-treated tumors (both primary and metastatic sites) as well as ER-/PR-/Her2- (triple-negative) breast cancers. Notably, healthy human tissues exhibit limited LIV-1 expression, primarily in hormone regulated organs (prostate, uterus, and breast). The escalated expression of LIV-1 in breast and prostate cancer, as well as melanoma and other tumors, positions LIV-1 as an excellent target for ADC therapeutics.

BRY812 is a monomethyl auristatin E (MMAE)-conjugated humanized IgG1 ADC against LIV-1. With Bioray's irreversible conjugation method CysLink™, BRY812 enables superior *in vivo* stability and eliminates off-target payload migration. Recent translational medicine studies have shown that BRY812 exhibits favorable efficacy in *in vitro* models of Breast cancer and Endometrial cancer. Additionally, BRY812 has demonstrated synergistic efficacy when combined with PD-1/L1 antibodies in a series of *in vitro* and *in vivo* models. Finally, BRY812 has shown improved efficacy in DS-8201 resistant cell line compared to the parental cell line. These results showed that BRY812, either as a monotherapy or in combination with other drugs, has a significant potential for application in various solid tumor indications, as well as in patients who are resistant to existing therapies.

IMMUNOLOGY: Adaptive Immunity in Tumors / Oncogenic Pathway-Mediated Regulation of Inflammation and Tumor Immunity
Poster Session

#6031 CD4+CD103+ tissue-resident memory T cells correlate with unfavorable prognosis in patients with head and neck cancer.

H. Takahashi[1], S. Ida[2], H. Tada[2], K. Chikamatsu[2];
[1]Ai Komagata Clinic, Aiyukai Medical Corporation, Maebashi, Japan, [2]Gunma University Graduate School of Medicine, Maebashi, Japan

T cell memory is an important mechanism for long-term protection against diverse pathogens such as cancers. Tissue-resident memory T cell (T_{RM}) is a subset of memory T cells that stably resides in tissues and controls local immune homeostasis. In the present study, we investigated the immunological profile and clinical significance of T_{RM} in patients with head and neck squamous cell carcinoma (HNSCC). We analyzed RNA-sequencing data and clinical data obtained from The Cancer Genome Atlas (TCGA) database. In total, 520 cases, including 97 human papillomavirus (HPV)-positive HNSCCs and 423 HPV-negative HNSCCs, were analyzed. T_{RM}-enriched tumors were identified based on the expression of both *CD4/CD8A* and *ITGAE (CD103).* T_{RM}-enriched tumors tended to correlate with HPV-positive status and early T factor. Gene Set Enrichment Analysis revealed the enrichment of pathways relating to cytotoxic immune response in T_{RM}-enriched tumors. Moreover, the expressions of immunostimulatory and immune checkpoint-related genes were elevated in T_{RM}-enriched tumors. Notably, the enrichment of CD4+T_{RM}s in tumors was an independent prognostic factor for shorter progression-free survivals (PFS). We also analyzed peripheral blood mononuclear cells obtained from 77 patients with HNSCC. The increased CD8+CD103+T_{RM}s correlated with HPV-positive status and primary lesion of oropharynx. The proportion of CD4+CD103+T_{RM}s negatively correlated with that of CD4+ naïve T cells, while that of CD8+CD103+T_{RM}s negatively correlated with that of effector T cells. The proportion of CD4+CD103+T_{RM}s positively correlated with Ki-67 expression in CD4+ T cells. Moreover, the elevated CD4+CD103+T_{RM}s was an independent prognostic factor for shorter PFS. In conclusion, we highlighted the clinical and immunological significance of CD103+T_{RM}s in both tumor tissues and peripheral blood of patients with HNSCC. Further characterization of T_{RM}s could lead to the development of novel biomarkers for patients with HNSCC.

#6032 Intratumoral Tregs restrain the immune response against primary and metastatic cancer.

A. Bockman, M. Moreno Ayala, B. Gittins, C. Zhang, S. Benyon, M. DuPage;
UC Berkeley, Berkeley, CA

Advancements in cancer immunotherapies have significantly improved patient outcomes in hematologic malignancies but are dramatically limited by the suppressive function of Regulatory T cells (T_{regs}) in solid cancers. Antibody-based therapeutics, such as anti-CTLA-4 and anti-CCR8, are being tested to selectively deplete intratumoral T_{regs}, with promising preclinical efficacy. However, these therapies have multiple effects that may do more than just deplete intratumoral T_{regs}, such as activate Fc receptors, bind to other types of immune cells, or bind to cells outside of the tumor microenvironment. While T_{regs} highly infiltrate most tumor types, it is unclear whether they function to suppress cancer immunity only in tumor tissue itself or at peripheral sites. To test whether T_{regs} function directly within the tumor microenvironment to suppress antitumor immune responses, we developed a model in which intratumoral (IT) delivery of low-dose diphtheria toxin (DT) in $Foxp3^{DTR}$ mice is used to only deplete IT T_{regs}. We show that mice that receive IT DT have reduced T_{regs} frequencies only in the tumor and do not succumb to lymphoproliferative disease, as observed with systemic T_{reg} depletion. Critically, tumor T_{reg} ablation via IT DT produced more effective and durable tumor control than systemic T_{reg} ablation. Mechanistically, we show that robust tumor control is achieved through enhanced function of effector $CD4^+$ and $CD8^+$ T cells and requires MHC II^+ $CD11b^+$ Batf3- type 2 conventional dendritic cells. Through the use of disseminated tumor models, we find that disrupting T_{reg} suppression within a primary tumor unleashes antitumor immunity against metastatic lesions that are not directly treated. Interestingly, this multifocal tumor control is dependent upon the cooperation between $CD4^+$ and $CD8^+$ T cells and requires matched primary and metastatic tumors. Through in vivo cellular trafficking experiments and blockade of T cell egress from lymphoid tissues we show a high frequency of T_{regs} circulate between local and metastatic tumors to mediate systemic immune effects. Upon disruption of tumor-T_{reg} circulation between lesions, we demonstrate enhanced effector function and decreased disease burden at both tumor sites. These findings identify a tumor-resident T_{reg} niche that limits $CD4^+$-cDC2 mediated antitumor immune responses. Importantly, our results uncover a novel T_{reg} suppressive mechanism that promotes systemic cancer progression.

#6034 Development of a pan-HLA-A02 restricted AFP targeting TCR for both TCR-T and TCR-bispecific therapy.

Junjun Chu, Jiajia Du, Tingfeng Liang, Shifan Wu, Yaolong Chen, Chunguang Yang, Qingjia Yan, Lulu Zhang, **Yanyan Han**

HRYZ Biotech Co., Ltd., Guangzhou, China

Background: Alpha-fetoprotein (AFP) is a well-established tumor-associated antigen, particularly in hepatocellular carcinoma (HCC). Although the AFP specific TCR-T cell therapy has shown promising clinical efficacy in HCC, the HLA restriction limits the patient population. We previously identified a T-cell receptor (TCR) sequence specific to an HLA-A*02:03-restricted $AFP_{158-166}$ antigen epitope. To broaden its therapeutic potential, we mutated the sequence of CDR regions of this TCR to recognize multiple major HLA-A*02 subtypes.
Method: We developed a novel human T-cell-based functional display platform to optimize the original AFP-specific TCR sequence. This platform facilitated the identification of TCR variants with broadened HLA-A*02 subtype recognition. The optimized TCR was engineered into TCR-T cells which were subsequently tested for their ability to recognize and lyse tumor cells both in vitro and in vivo models. Comprehensive safety evaluations were conducted to assess off-target effects. Additionally, the platform was also able to enhance the affinity of TCRs to picomolar level, leading to the development of a TCR bispecific protein.
Results: The original TCR could only recognize and kill AFP158-166 antigen presented by HLA-A*02:03. In contrast, the optimized TCR transduced T cells demonstrated the ability to recognize the same epitope presented by pan-HLA-A*02 molecules, including A*02:01, A*02:03, A*02:06, and A*02:07, significantly broadening their applicability. The pan-HLA-A*02 restricted TCR transduced T cells exhibited superior anti-tumor activity, as evidenced by stronger cytokines production and tumor cell lysis ability in vitro and the capability to completely eradicate tumors in vivo murine models. Toxicology studies revealed no off-target recognition of non-specific HLA or other antigens, indicating a favorable safety profile. Moreover, the high-affinity TCRs (-11M) were linked with an anti-CD3 scFv fragment to form a TCR bispecific protein, which demonstrated substantial anti-tumor efficacy in preclinical models.
Conclusion: To this end, the sequence-optimized AFP-specific TCR-T cells demonstrated robust therapeutic efficacy and a favorable safety profile, expanding potential treatment options for over 60% of patients with pan-HLA-A*02. Moreover, the successful development of a TCR bispecific antibody highlights its promise as an innovative off-the-shelf therapeutic solution, offering improved clinical accessibility and versatility to address the needs of a broader patient population.

#6035 The role of EBV+ nasopharyngeal carcinoma in shaping double-negative B cells to form a pro-metastatic niche.

M. K. Chung[1], N. Kam[2], Y. Zhang[1], Z. Hou[1], K. Islam[1], Y. Zhang[3], G. Lanqi[1], X.-Y. Guan[1], W. Dai[1];
[1]Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pok Fu Lam, Hong Kong, Hong Kong, [2]Laboratory of Synthetic Chemistry and Chemical Biology Limited, Hong Kong, Hong Kong, [3]State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou, China

Introduction: B cells can play an anti-tumor role in the tumor microenvironment (TME) by secreting antibodies and activating other immune cells. In our previous study, IgD-CD27- double-negative (DN) B cells were associated with worse prognoses in nasopharyngeal carcinoma (NPC), a malignancy often associated with Epstein-Barr virus (EBV) infection, implying a potential role in NPC disease progression.
Methods: To characterize DN B cells and identify features associated with NPC clinical outcomes, we analyzed single-cell RNA-sequencing data (scRNA-seq) from NPC tumors, blood samples, and normal nasopharynx tissues (n = 49, 15, and 7), as well as bulk RNA-sequencing (RNA-seq) data from two cohorts of NPC patients with clinical data (n = 77 and 100) respectively. Multiplex Immunohistochemical (mIHC) staining of tumor slides from 40 NPC patients was analyzed for the spatial identification of tumor-infiltrating B cells. To understand the *in vitro* interactions between NPC cancer cells and B cells, tonsil-derived IgD+CD27- naïve B cells were cocultured with EBV-positive (EBV+) or negative (EBV-) NPC43 cells, followed by Flow Cytometry and scRNA-seq for quantifying DN B cells and investigating their interactions with cancer cells.
Results: Trajectory analysis identified DN B cells and Plasma B cells as potential terminal B cell development states in NPC, with both populations enriched in tumors. DN B cells could be classified into two major subpopulations in NPC: DN Branch 1 and DN Branch 2, with the former potentially being influenced by EBV infection and the latter being under stress. RNA-seq data revealed that B-cell-related biological pathways were downregulated in metastatic NPC, while mIHC staining showed that most DN B cells were in NPC tumor nests and were enriched in metastatic NPC. Flow cytometry on our co-culture experiments detected increased DN B cell content in EBV+ NPC43 co-culture (40.0%) compared to EBV- NPC43 (25.9%), supporting our hypothesis that EBV+ NPC malignant cells may be involved in promoting DN B cell development in NPC. Detailed cell-cell interaction analysis indicated that the EGFR-TGFB1, LTBR-LTB, and MIF-TNFRSF14 are potential key interactions between EBV+ NPC cells and naïve B cells, likely contributing to DN B cell development and tumour growth.
Conclusions: DN B cells in NPC may be in a stable development state, with their correlation with distant metastasis suggesting their involvement in B cell dysregulation involved in poor NPC prognosis. Taken together, our results suggest EBV+ NPC cells may receive pro-tumor signals from naïve B cells and promote the development of DN B cells to form a pro-metastatic niche. Future studies are needed for detailed validations of these findings.
Acknowledgments: This study is funded by Theme-based Research Scheme (TBRS) (T12-703/22-R), Health and Medical Research Fund (HMRF) (08192296) from Hong Kong (SAR), P.R. China.

#6036 Mining tumor B cell repertoires for the identification of novel antibody target pairs.

Matthieu Delince, Carlo Zimarino, Julia Steiger, Pattraporn Satitsuksanoa, Justina Rutkauskaite, Eveline Keller, Leandra Zehnder, Lorenza Ferretti, Simone Schmitt, Christoph Esslinger

Memo Therapeutics AG, Schlieren, Switzerland

Tumor-infiltrating B cells and especially B cells organized in tertiary lymphoid structures (TLS) correlate with better prognosis in lung, ovarian and colon cancer patients. TLS are ectopic lymphoid organs which may present B cell follicles of germinal center. B cells in the TLS were found to express typical markers of late-stage maturation, somatic hypermutation and class switch recombination of immunoglobulin genes, indicating a cancer-driven clonal expansion. In some cases, research showed that secreted immunoglobulins of the repertoire were having a direct tumor killing activity. To decipher and then harness the potential of these tumor-trained antibodies, we analyze recombinant cognate repertoires of tumor infiltrating B cells from lung, ovarian and colon cancer surgical resections. We leverage the capabilities of our cutting-edge single-cell microfluidic platform, to extract and clone the comprehensive B cell antibody arsenal from up to a million tumor-derived B cells. The cancer derived antibodies are then expressed as recombinant full-length human IgG maintaining the heavy and light chain pairing of the original B cells. The antibody repertoire obtained as a recombinantly expressed mixture or as individual monoclonals is then screened in a panel of assays designed to identify antibodies with a function of interest. For instance, identification of antibodies showing specific binding to cancer cells. Once identified, the monoclonal antibody is then used to deconvolute novel tumor associated antigens. An initial assessment of the tumor derived antibody repertoire revealed that a typical sample of approximately one cubic centimeter of tumor tissue yielded thousands of antibody-clonotypes. The sequences pattern bore signs of affinity maturation and clonal expansion which may indicate an antigen-driven evolutionary pressure exercised by the tumor and tumor surroundings. Screening the tumor derived antibody repertoire for binding to cancer cell lines, we identified several monoclonal antibodies specifically binding to cancer cells. In vitro, these antibodies show anti-tumor activity with several mechanisms of actions: direct growth inhibition or via effector functions (antibody-dependent cell-mediated cytotoxicity). Some antibodies are strongly internalized and meditate killing when coupled to a toxic payload. The tumor antigens of the antibodies are currently being evaluated as potential therapeutic targets. These promising results may lead us to discover novel tumor pathways and contribute to our further understanding of B cell biology in cancer.

#6037 Circulating CD8 T-cell responses to neoantigens predict responsiveness to PD-(L)1 axis blockade in non-small cell lung cancer.

Celia Ramade[1], Noemie Thebault[1], Clara-Maria Scarlata[1], Francoise Lauzeral-Vizcaino[1], Caroline Fournier[1], Victor Sarradin[1], Anna Salvioni[1], Marie Michelas[1], Daniel Oreper[2], Suchit Jhunjhunwala[2], William Thrift[2], Adric Quade Broadwell[2], Nicolas Lounsbury[2], Kai Liu[2], Meng Xiao He[2], Martine Darwish[2], Catherine Ross[2], Hang Xu[2], Milena Hornburg[2], Amy Heidersbach[2], Fanny Bouquet[3], Catia Fonseca[3], Laetitia Tudeau[3], Nicolas Congy-Jolivet[4], Carlos Gomez-Roca[1], Thomas Filleron[4], Lelia Delamarre[2], Julien Mazieres[4], Jean-Pierre Delord[4], Maha Ayyoub[1]

[1]INSERM U1037 - CRCT, Toulouse, France,[2]Genentech Inc., South San Francisco, CA,[3]Global Product Development-Medical Affairs, F. Hoffmann-La Roche Ltd, Basel, Switzerland,[4]CHU de Toulouse, Toulouse, France

Tumor-specific T cells play a central role in tumor control, yet the attainment of their full tumor rejection potential is hindered by regulatory mechanisms, including expression of inhibitory immune checkpoints. Although immunotherapy by immune checkpoint blockade (ICB) has transformed cancer care, only a proportion of patients experience clinical benefit. Ancillary studies to clinical trials evaluating ICB have revealed an association between high tumor mutational burden (TMB) and treatment efficacy, suggesting that unleashing T cells targeting neoepitopes encoded by somatic mutations could underlie ICB-mediated tumor control or regression. Nevertheless, TMB or tumor neoantigenic burden (TNB) are not consistently related to clinical response, potentially because most studies have addressed the influence of TMB and TNB by *in silico* prediction without systematic analysis of actual neoantigen-specific T cells. We hypothesized that the presence of actual neoantigen-specific T-cell responses would predict response to ICB, rather than the mere presence of a high TMB or TNB. Using whole exome and transcriptome sequencing in a cohort of 29 non-small cell lung cancer patients receiving anti-PD-1/PD-L1, we assessed expressed non-synonymous exonic mutations in each patient's tumor and predicted the encoded neoepitopes restricted by each patient's HLA-I and -II alleles. In our cohort, HLA-I TNB was better correlated to clinical response and progression free survival (PFS) than TMB and HLA-II TNB. We developed a workflow allowing for the assessment of circulating CD8 T-cell responses to more than 400 prioritized neoepitopes, and to 50 long peptides harboring each several predicted CD4 neoepitopes containing the same mutated amino acid. We showed, in 28 patients from whom PBMCs were available, that circulating CD8 T-cell responses to predicted neoantigens were of higher magnitude and breadth in clinical responder compared to non-responder patients. The presence of a CD8 T-cell response to neoantigens was associated with better PFS. No correlation was observed between expressed TMB or TNB (HLA-I or -II) and the magnitude of CD8 or CD4 T-cell responses. Longitudinal ex vivo enumeration of neoantigen-specific CD8 T-cells using HLA/peptide tetramers showed that they had a memory phenotype and that they experienced significant proliferation under therapy. Our results ascertain the contribution of neoantigen-specific CD8 T-cells in therapeutic tumor rejection. They also provide an opportunity, through the analysis of tumor molecular signatures, to unveil the mechanisms supporting or restraining the priming of spontaneous T-cell responses targetable by ICB. This research was a collaborative effort by sites within the imCORE network, made possible through support from F.Hoffmann-La Roche.

#6038 An optimized off-the-shelf whole tumor cell vaccine activates T cells that recognize a diverse antigen repertoire with potential to provide meaningful clinical benefit to patients with NSCLC.

Bernadette Ferraro[1], Justin J. Arndt[1], Grace Tan[2], Nicholas S. LeFlohic[1], Jian Yan[1], Mark L. Bagarazzi[1], Natalia Savelyeva[2], Christian H. H. Ottensmeier[2]

[1]NEUVOGEN Therapeutics, San Diego, CA,[2]The University of Liverpool, Liverpool, United Kingdom

One critical challenge of effective cancer therapies is addressing intertumoral and intratumoral heterogeneity of immunologically visible antigens. Antigenic diversity is a particular challenge for therapies that rely on the induction of a broad repertoire of cytotoxic T cells (CTLs) to eliminate malignant cells. Therapeutic interventions, such as αPD1, can partially mitigate this challenge by rescuing CTLs constrained by immune inhibitory mechanisms, such as intratumoral upregulation of the PDL1/PDL2 pathway.

Cancer vaccines are rapidly advancing as a mechanism to expand the repertoire of effector T cells thereby broadening immune recognition and elimination of tumor cells. Here we provide preclinical evidence that a carefully designed, off-the-shelf vaccine, derived from six immortalized tumor cell lines (NGEN-143) provides the necessary requirements for translation into clinical benefit. Vaccine immunogenicity was enhanced by limiting expression of proteins that suppress immune responses (B7H3, TGFβ1, TGFβ2) and by expression of key cytokines that facilitate activation of dendritic cells (CD40L, GMCSF, IL12).

In human in vitro assays, NGEN-143 significantly increased the breadth and magnitude of T cells activated against tumor associated antigens (TAAs) and shared neoantigens (TSAs) across a wide range of HLA types. IFNγ producing T cells measured by ELISpot (SFU/1e^6 PBMCs) were increased to 10 TAAs (42,972 ± 4,105) and 22 TSAs (2,493 ± 484) compared to unmodified controls (TAAs, 2,889 ± 993; TSAs, 200 ± 71). IFNγ responses were not preferentially directed against a subset of TAAs, TSAs or epitopes. NGEN-143 significantly increased activated T cells with anti-tumor effector function. NGEN-143 preferentially expanded CD8$^+$ polyfunctional (IFNγ$^{+/-}$TNFα$^{+/-}$GzmB$^+$) T cells with effector (CCR7$^-$CD45RO$^+$) and central memory (CCR7$^+$CD45RO$^+$) phenotypes, skewed CD4$^+$ T cells towards T_h1 (Tbet$^+$IFNγ$^+$) over T_h2 (GATA4$^+$IL4$^+$) cells, and increased CD8$^+$ T cell expression of PD1 demonstrating TCR engagement. Cytolytic function of NGEN-143 activated CD8$^+$ T cells was confirmed by caspase-3 mediated killing of tumor cell targets.

Consistent with in vitro data, murine vaccines with immunogenicity enhancing modifications homologous to NGEN-143 significantly improved survival outcome in immunologically 'cold' (B16F10) and 'warm' (CT26) syngeneic tumor models. In both models, vaccine significantly increased tumor infiltration of CD4$^+$ and CD8$^+$ T cells, inhibited tumor growth, and prolonged survival. In the CT26 model, vaccine monotherapy completely inhibited tumor growth (n=7/10) and mice were protected from rechallenge (n=7). Combination with αPD1 synergistically increased vaccine survival benefit.

Our data demonstrate NGEN-143 has all the features to underpin clinical use in patients with NSCLC.

#6039 Triads of dendritic cells, CD4$^+$ T cells, and CD8$^+$ T cells are found within PDA tumors and are associated with intrinsic anti-tumor immune responses.

Sheela R. Damle[1], Jason A. Carter[2], Jose M. B. Pineda[2], Kristin E. Goodsell[2], Lindsay K. Dickerson[2], Shreeram Akilesh[2], Ian N. Crispe[2], Venu G. Pillarisetty[1]

[1]University of Washington/Fred Hutch Cancer Center, Seattle, WA,[2]University of Washington, Seattle, WA

Cytotoxic T cells (CTLs) are the main effectors of anti-tumor immunity, and their activation is dependent on carefully coordinated interactions between antigen presenting cells (APCs) and CD4$^+$ T cells (Th). In a pivotal model, synchronized interaction between a dendritic cell (DC), Th, and CTL were posited as requisite for effective CTL responses. Recent studies have resurrected this hypothesis in the context of anti-tumor immunity and demonstrated the function of 3 cell type clusters in immune checkpoint blockade (ICB) response in murine tumors and human cancer. We hypothesized intratumoral 3-cell type clusters are present in an “immune excluded tumor”, pancreatic adenocarcinoma (PDA), outside the context of immunotherapy. Analysis of single cell RNAseq and PDA tumors identified DC:Th:CTL clusters, defined as DC, Th, and CTL within 20µm radius, occurring with ~15% of DCs. Analysis of multiplex proteomics data from fibrolamellar carcinoma, a rare stromal dominant liver tumor with poor immune infiltration, revealed the presence of DC:Th:CTL clusters. We sought to confirm our findings in a larger dataset using multiplex immunohistochemistry (mIHC) on a tissue microarray (TMA) of >500 patients with untreated resected PDA. Using automatic image analysis software, we identified cell types based on surface marker expression, specifically tumor cells, CTL, Th, macrophages, and non-macrophage APCs. 3-cell type clusters were found in 90% of tumor samples, occurred with ~10% of APCs, located >50µm from tumor cells, and correlated strongly with APC:Th dyads. Weak correlation was found between 3-cell type clusters and isolated cell types, suggesting cluster formation is unlikely to be related to abundance of infiltrating immune cells. DC:Th:CTL clusters were also readily seen in scST of untreated non-small cell lung cancer (NSCLC) and colorectal cancer (CRC). Analysis of PDA, NSCLC, and CRC scST datasets revealed that CTLs within triads were more likely to have an effector-like phenotype. Th within triads were enriched in CXCL13$^+$-like Th phenotype, which previously has been associated with immune triads. Using associated clinical data in the PDA TMA, we found that patients with the highest tertile of APC:Th:CTL clusters had improved overall survival compared to patients with the lowest tertile (HR=0.70, p=0.005). Similar findings were found for patients with the top tertile of APC:Th dyads (HR=0.63, p=6.x10^{-4}), but not APC:CTL or CTL:Th dyads (HR=0.76 and 0.77 and p=0.16 and 0.07, respectively). Overall, our findings demonstrate 3-cell type clusters are readily found in an “immune excluded” tumor like PDA without immunotherapy and are associated with improved patient outcomes. These data are strong evidence for the role of 3-cell type clusters in endogenous anti-tumor immune responses and point toward future opportunities for immunotherapeutic intervention.

#6040 Exploring the impact of analytical factors on B-cell receptor profiling with high-throughput sequencing technologies.

Xiashiyao Zhang[1], Rittika Mallik[2], Miguel Pacheco[2], Shaveta Goyal[3], Mark Izraelson[4], Li Song[5], Henk-Jan van den Ham[6], **Jun Wan**[7], Wenming Xiao[2], BCR-SEQC consortium

[1]Luddy School of Informatics, Computing, and Engineering, Indiana University at Indianapolis, Indianapolis, IN,[2]Center for Drug Evaluation and Research, U.S Food and Drug Administration, Silver Spring, MD,[3]Takara Bio USA, Inc, San Jose, CA,[4]MiLaboratories Inc, San Francisco, CA,[5]Department of Biomedical Data Science, Dartmouth College, Hanover, NH,[6]ENPICOM B.V., 's-Hertogenbosch, Netherlands,[7]Indiana University School of Medicine, Indianapolis, IN

This abstract reflects the views of the authors and should not be construed to represent FDA's views or policies.
High-throughput sequencing of B-cell receptor (BCR) gene rearrangements, along with downstream analysis, provides researchers with the tools to define the B-cell clonal landscape and identify biomarkers for minimal residual disease (MRD). Despite significant advancements in the field over the past decade, comprehensive comparisons on different analytical methods, sequencing technologies, and bioinformatics tools remain largely unexplored.
To bridge this gap, the FDA-led B-cell Receptor Sequencing Quality Control (BCR-SEQC) consortium developed protocols for generating reference materials derived from nine well-characterized B-cell lines and peripheral blood mononuclear cells (PBMCs) from healthy donors. This study aimed to evaluate the impact of key analytical factors—including input material quality and quantity, sequencing depth, sequencing technologies, and bioinformatics workflows—on BCR sequencing (BCR-seq) performance.
The study employed multiple commercial BCR-seq products to prepare libraries with varying input amounts (25ng, 100ng, and 500ng) of reference materials. These libraries were sequenced using three different platforms: Illumina NextSeq 2000, ElementBio Aviti, and MGI DNBSEQ. Subsequently, four bioinformatics workflows (MiXCR, Immcantation, TRUST4, and IGX-Profile) were applied to assemble BCR sequences and determine clonotypes.
This research provided the first comprehensive analysis of how variables in wet-lab and computational workflows influence sequencing-based BCR profiling, specifically in terms of linearity, accuracy, and sensitivity. Additionally, the study developed quality control (QC) metrics to ensure the robustness of assays and analytical workflows.
Ultimately, this work highlights current capabilities and limitations in BCR-seq technology, identifies critical technical challenges, and establishes actionable best practices for reconstructing BCR repertoires from high-throughput sequencing data.

#6041 Targeting the NuRD component, CHD4, impairs Foxp3+ Treg cell production and function and promotes anti-tumor immunity.

Wayne W. Hancock[1], Yan Xiong[2], Liqing Wang[3], Martina Minisini[4], Fanhua Kong[2], Eros di Giorgio[4], Tatiana Akimova[1], Ivan Babic[5], Elmar Nurmemmedov[5]

[1]University of Pennsylvania, Philadelphia, PA,[2]Zhongnan Hospital of Wuhan University, Wuhan, China,[3]Children's Hospital of Philadelphia, Philadelphia, PA,[4]University of Udine, Udine, Italy,[5]CellarisBio, San Diego, CA

Chromatin remodeling enzymes, which reposition nucleosomes throughout the genome to regulate transcription and other processes, include 4 families: chromodomain helicase DNA-binding (CHD), switch/sucrose non-fermentable (SWI/SNF), imitation switch (ISWI), and inositol requiring 80 (INO80). CHD4, a ubiquitous and abundant chromatin remodeling enzyme essential for normal development across a range of tissues, and that is overexpressed in various cancers, is a core member of the nucleosome remodeling and deacetylase (NuRD) complex. Though originally described as a transcriptional repressor, the NuRD complex is now regarded as able to 'fine-tune' gene expression, acting as both an activator and a repressor of transcription. CHD4 is important to the development of lymphocytes but has not been studied in the context of Foxp3+ T-regulatory (Treg) cells. Our work involved genetic deletion of Foxp3 in Treg cells and use of a pharmacologic inhibitor of CHD4 (CHD4i) in studies with WT mice. In basic studies, Foxp3 co-immunoprecipitated with CHD4 and deletion of CHD4 led to decreased Foxp3+ Treg production in the thymus. In the periphery, CHD4-/- Tregs showed decreased Foxp3 expression and suppressive function *in vitro* and *in vivo*, with resultant severe autoimmunity and death by 3 weeks of life. These events were associated with an absence of CNS2 demethylation in the Foxp3 locus and hierarchical clustering map for differentially expressed genes included cytokines and chemokines, leukocyte antigens, transcription factors and genes associated with apoptosis, as well as enrichment of genes associated with regulation of the cell cycle and DNA damage and repair. Thereafter, a DEL screen involving cell target engagement led to discovery of a compound CH41, binding to the CHD4 chromodomain. CH41 impaired Treg suppressive function in vitro and when used in syngeneic tumor models led to inhibition of tumor growth in lung and liver carcinoma models but was ineffective in immunodeficient mice. Efficacy was associated with increased CD4 (IL-2) and CD8 (IFN-g) infiltration and effector cytokine production. Importantly, neither WT tumor bearing mice nor their immunodeficient partners developed evidence of autoimmunity under CHD4i therapy, consistent with our previous data that tumor-associated Tregs have distinctive properties rendering them especially susceptible to immunotherapy versus their peripheral counterparts. We conclude that CHD4 targeting has interesting immunologic effects that include preferentially inhibiting Treg vs. conventional T cell responses, leading to significantly increased anti-tumor immunity.

#6042 Baseline serum level of IgG targeting annexin A2 is predictive of response to immunotherapy in patients with advanced NSCLC.

Matthieu Roulleaux Dugage[1], Francois-Xavier Danlos[1], Melodie Bonvalet[2], Tyler Hulett[3], Sabine Messayke[4], Anne-Laure Voisin[4], Marie Naigeon[5], Jean Jouniaux[5], Aleksei Tikhonov[2], Chifaou Mohamed-Djalim[2], Severine Mouraud[2], Delphine Bredel[2], Scott Paschke[3], Mihaela Aldea[1], Pernelle Lavaud[6], David Planchard[6], Benjamin Besse[6], Nathalie Chaput[5], Aurelien Marabelle[2]

[1]Drug Development Department, Gustave Roussy, Villejuif, France,[2]Immunotherapy Translational Research Lab, Gustave Roussy, Villejuif, France,[3]CDI Laboratories, Baltimore, MD,[4]Pharmacovigilance Unit, Gustave Roussy, Villejuif, France,[5]Laboratory of Immunomonitoring in Oncology, Gustave Roussy, Villejuif, France,[6]Medical Oncology Department, Gustave Roussy, Villejuif, France

Introduction: Despite the success of immune checkpoint blockers (ICBs), predicting which patients will respond to treatment remains a significant challenge, particularly in those with advanced NSCLC. Some circulating antibodies may serve as indicators of a pre-existing antitumor adaptive immune response. This study investigated the potential of baseline serum antibodies as predictors of response and survival in patients with advanced NSCLC undergoing ICB, chemotherapy, or ICB + chemotherapy.
Materials and Methods: Baseline serum samples were collected from 256 patients with advanced NSCLC in two independent cohorts : the PREMIS cohort (NCT03984318, n=195 patients treated with either ICB alone (n=129) or combined chemo-immunotherapy (n=66)) and the MSN cohort (NCT02105168 - n=61 patients receiving first-line chemotherapy). Fluorescence intensities of IgG and IgM binding to individual human proteins were measured using the HuProt arrays containing over 20,000 human proteins. Survival analyses, including Kaplan-Meier, Log-Rank test and Cox regression models, were conducted to determine the predictive value of identified antibodies. Response was defined by RECIST 1.1.
Results: Few protein specific antibody levels were significantly different between responders and non-responders, including five that were significantly higher: ANXA2-IgG, METTL21B-IgG, SPHKAP-IgG, Lupus La-IgG, EDN1-IgG, and EFHC1-IgM. Four isoforms of the ANXA2 protein consistently exhibited elevated IgG antibody levels in responders, with a strong inter-isoform correlation ($R>0.95$, $p<0.0001$). These antibodies were correlated with baseline global IgG levels and antinuclear antibodies (ANA) status but not with tumor histology (squamous or non-squamous) or other clinical characteristics (including PD-L1 staining). ICB-treated patients with higher anti-ANXA2 IgG levels (n=23) had progression-free survival (PFS, Logrank p=0.02) and overall survival (OS, Logrank p=0.008) as compared to intermediate (n=86) and low (n=20) patients in ICB-treated patients. This effect was also suggested in the chemo-immunotherapy group (although not significant) but was absent in patients treated with chemotherapy without ICB. After adjusting for global IgG levels, anti-ANXA2 IgG levels independently predicted PFS and OS in ICB-treated patients.
Conclusion: Pre-existing humoral immunity against ANXA2 predicts response and survival in advanced NSCLC treated with ICB. Annexin A2 (ANXA2) has been implicated in immunosuppression, epithelial-to-mesenchymal transition (EMT), and poorer survival outcomes in cancer patients. Baseline anti-ANXA2 IgG levels could therefore be used as biomarkers to predict benefits of ICB and allow for patient stratification. Also, ANXA2 could become a novel target for NSCLC immunotherapy.

#6043 Role of Rab11 family interacting proteins in immune recognition of breast cancer.

Oly Khowash, Paras Sahani, Christopher Kywe, Kathleen Conway, Khushi Tekale, Jessica Contreras, Erin Howe

Rosalind Franklin Univ. of Med. & Science, North Chicago, IL

Breast cancer is the second leading cause of death for American women, with the majority of morbidity attributable to metastatic disease. Breast cancer brain metastases (BCBM) account for 30% of breast cancer mortality and treatment options remain limited. While immunotherapy holds great promise, objective response rates for metastatic disease are poor. To improve these outcomes, we must understand the context-specific mechanisms used by cancer cells to suppress and evade the immune system. Successful T cell interaction with cancer cells requires proper surface localization of proteins such as MHC complexes and co-activators, all of which are localized within the cell via endosomal protein trafficking. Immunotherapy also relies on CTLA-4 or PD-1/PD-L1 on the cell surface to remove inhibition of T cells and improve the anti-tumor response. How molecular trafficking controls localization of activating and inhibitory proteins to the surface in order to facilitate cancer-immune cell interaction is a key knowledge gap. We previously showed that Rab11b-mediated endosomal trafficking is required for BCBM. Here we investigate how trafficking-mediated control of the cell surface dictates immune recognition and response. Using a targeted CRISPR screen, we found that Rab11 family interacting proteins Rab11fip2 and Rab11fip3 are required for highly immunogenic ovalbumin (OVA) expressing breast cancer cells to be recognized by OT-I CD8+ T cells. Flow cytometry analysis revealed that the decrease in recognition is not due to surface localization of PD-L1 or the MHC/SIINFEKL complex (SIINFEKL is the OVA peptide presented for OT-I recognition). Knockout of Rab11fip2 or 3 did not change cell proliferation or tumor burden in immunocompromised animals, but led to significantly increased tumor growth in immunocompetent animals. This suggests that non-targeting control cells are partially restrained by the immune system, and knockout of either Rab11fip causes loss of immune recognition to allow faster tumor growth. Immunohistochemical analysis revealed a significant decrease of tumor infiltrating T cells in Rab11fip knockout tumors. Cytotoxic CD8+ T cells exhibited the most extreme decrease in infiltration, with CD4+ helper T cells decreasing in only the Rab11fip3 knockout condition. These results indicate that Rab11 may play a role in immune cell recruitment in addition to recognition and activation. We are currently focused on characterizing Rab11 control of the T cell-cancer cell interaction in human breast cancer cell lines. Dissecting the role of Rab11 in tumor cell antigen presentation, immune cell recruitment, T cell cytotoxicity and activation will help determine if inhibition of Rab11-mediated recycling can be used to increase the efficacy of immunotherapy for patients with BCBM.

#6044 T cell-mediated clearance of BCRABL^{+} leukemia cells in transgenic mice model of chronic myeloid leukemia.

Chang Liu, Fang Hui, Xuexiu Fang, Stephanie Mori, John Kenneth Cowell, Tianxiang Hu

Georgia Cancer Center, Augusta, GA

The *BCR-ABL1* fusion gene, which is present in ~95% of chronic myeloid leukemia (CML) patients, encodes a unique amino acid sequence that is exclusively expressed in leukemic cells. Tyrosine kinase inhibitors (TKIs) therapy targeting BCR-ABL1 are widely used as the first-line treatment for CML and has markedly improved the outcome of patients. However, there are still challenges in CML patient management, including the poor prognosis of patients with advanced-phase CML, the adverse effects associated with lifelong dependence on TKIs, and high relapse rates observed after TKI discontinuation. Restored anti-tumor immunity by immune effector cells, especially the NK cells, has been shown to be associated with deep molecular response and treatment-free remission in CML patients. However, there is a lack of direct evidence whether these cytotoxic T lymphocytes (CTLs) can really eradicate the BCR-ABL1^{+} leukemic cells and provide a cure for this disease. Primary murine CML was induced by the withdrawal of tetracycline (TET) from the drinking water of the *SCL:tTA*/*BCR-ABL1* transgenic mice, which served as the donor of BCR-ABL1^{+} leukemia cells for serial transplantation. We performed several engraftment experiments on mice from different strains and subjected them to different treatments. The CML progression in mice engraftment model was measured by white blood cell counts (WBC) and flow cytometry analysis of peripheral blood (PB). Our findings demonstrated that non-irradiated immunocompetent C57BL/6 mice did not develop CML after transplantation of primary leukemia cells, whereas irradiated recipient mice exhibited a typical phenotype of CML, as indicated by a significant increase in the percentage of neutrophil-like CML cells and WBC. Besides, the engraftment of BCRABL1^{+} leukemia cells to immunodeficient NSG mice led to rapid CML progression, indicating a critical role of the immune system in CML control. To test the direct involvement of CTLs, antibody-mediated CD8^{+} T cells depletion was performed, which restored CML progression in C57BL/6 mice. Furthermore, in vitro tumor killing assay revealed that CD8^{+} T cells from transplanted, CML-free mice exhibited enhanced cytotoxic activity against BCR-ABL1^{+} CML cells compared to neutrophil counterparts derived from naïve mice. Consistently, when T cells from tumor-challenged but CML-free mice were transferred together with CML cells into NSG mice, the recipient NSG mice got protected from CML development. Based on these findings, we concluded that CTLs play a key anti-tumor role in the prevention of CML in the mouse CML model. Further investigating these specific cytotoxic T cells and cloning their TCR sequences could provide an experimental basis for the design of TCR-T therapy targeting BCR-ABL1 neo-antigen for the cure of CML.

#6045 MHC II+ VEGFA+ neutrophils induce expansion of regulatory T cells and confer colorectal cancer peritoneal metastasis.

S. Deng, S. Liu, **J. Zheng**;
Sun Yat-sen University Cancer Center (SYSUCC), Guangzhou, China

Peritoneal metastasis is a fatal mode of cancer metastasis that is accompanied by extensive stromal fibrosis and poor prognosis. Increasing evidence suggests that tumor associated neutrophils (TANs) constitute a major component of tumor-infiltrating cells in many types of cancer and contribute to tumor immunity; however, how TANs are connected to adaptive immunity and clinical relevance in peritoneal metastasis remains unclear. Here, we generated and integrated single-cell RNA and spatial transcriptomes in primary tumor, adjacent normal tissues and metastases from 7 cancer types that tend to develop peritoneal metastasis (235 samples from 124 patients). Neutrophils exhibited distinct states in different tissue types. Specially, a terminally differentiated MHC II $^{+}$ VEGFA^{+} neutrophil population with antigen presenting and oncogenic phenotype termed TAN-2 portended peritoneal metastasis and could further induce mesothelium to mesenchymal transition. These fibrotic mesothelial cells exhibit higher CXCL12 levels and recruited more TAN-2 cells via CXCR4. Notably, the antigen-presenting TAN-2 could invoke expansion of regulatory T cells in an antigen-specific manner and was associated with worse survival. Importantly, in vivo TAN-2 depletion or in combination with immune checkpoint inhibitors effectively attenuates peritoneal metastasis. Taken together, we elucidate how MHC II^{+} VEGFA^{+} TANs contribute to peritoneal metastasis, provide novel insights on innate and adaptive immunity, and demonstrate new strategies to enhance cancer immunotherapy effects.

#6046 Combination of RAS(ON) mutant-selective and multi-selective inhibitors sensitizes immune-refractory, RAS-driven preclinical models to immunotherapy.

M. Moreno Ayala, C. Blaj, M. Menard, A. Salmon, J. Lee, S. Ganesh, A. Pham, V. Morton, M. Sandoval, J. A. Smith, E. Quintana;
Revolution Medicines, Redwood City, CA

Oncogenic RAS promotes carcinogenesis by sustaining cell proliferation and orchestrating immune escape. We and others have shown that RAS inhibition combined with immune checkpoint blockade (ICB) prolongs durability of response in immunogenic, RAS-driven preclinical models. Clinical data have shown that most patients treated with KRAS G12C(OFF) inhibitors develop resistance through reactivation of the RAS pathway. We have previously demonstrated that the combination of a RAS(ON) G12C-selective inhibitor with a RAS(ON) multi-selective inhibitor can overcome mechanisms of intrinsic and acquired resistance in an immune-refractory preclinical NSCLC model. Here we evaluated if the RAS(ON) doublet combination of RMC-6291 or RMC-9805, a RAS(ON) G12C-selective inhibitor and a RAS(ON) G12D-selective inhibitor, respectively, with the RAS(ON) multi-selective inhibitor RMC-6236 can sensitize traditionally hard to treat, immune-refractory preclinical models to anti-PD-1. In a KRAS G12C NSCLC syngeneic model, RMC-6291, RMC-6236, and the RAS(ON) doublet induced rapid, transient tumor regressions, but some tumors progressed on treatment. This model is refractory to anti-PD-1 monotherapy and the combination of RMC-6291 or RMC-6236 with anti-PD-1 did not achieve significant combination benefit. RMC-6291 and RMC-6236 as single agents and the RAS(ON) doublet induced a similar favorable modulation of the TME, increasing tumor infiltrating lymphocytes and decreasing myeloid cells. Only the RAS(ON) doublet upregulated MHC class I expression, increasing the possibility of recognition of the tumor by T cells. Notably, the RAS(ON) doublet sensitized this model to anti-PD-1, with the triple combination achieving 100% CRs. In a GEMM-derived, KRAS G12D pancreatic model, the combination of RMC-9805 with RMC-6236 achieved RAS pathway inhibition as evidenced by deeper p-ERK suppression, and significantly increased the depth and durability of the antitumor response compared to either single agent alone. Tumors treated with RMC-9805 plus RMC-6236 showed a more profound transformation of the TME, characterized by a decrease in immunosuppressive myeloid cells, an increase in T cell infiltration, and a significant upregulation of the antigen presentation machinery. Combination of the RAS(ON) doublet with anti-PD-1 induced 90% CRs in this immune-refractory model. Collectively, these preclinical findings suggest that the RAS(ON) doublet combination of RAS(ON) mutant-selective and multi-selective inhibitors can reverse the immune-evasion mechanisms governed by oncogenic RAS and sensitize immune-refractory tumors to ICB to induce durable CRs. These preclinical data support the clinical evaluation of the RAS(ON) doublets in combination with ICB in patients with RAS driven cancers.

#6047 YTHDF proteins modulate gastric cancer immunity by regulating the IFN-γ response through the m6A-dependent degradation of IRF1.

Dongjun Jang, Sung-Yup Cho

Seoul National University College of Medicine, Seoul, Korea, Republic of

N^6-methyladenosine (m6A) RNA methylation is one of the most abundant post-transcriptional modifications in eukaryotic messenger RNAs and plays crucial roles in carcinogenesis and immune responses. YTHDF proteins including YTHDF1, 2, and 3 are ‘reader’ proteins of m6A methylated RNAs and have individual role. A recent report suggests that YTHDFs act redundantly to promote RNA decay. In this study, we found that YTHDF1 was overexpressed in gastric cancer (GC) tissues and correlating with the suppression of cancer immunity, particularly the responsiveness to interferon-gamma (IFN-γ). Overexpression of YTHDF1 reduced the responsiveness to IFN-γ in GC cells, while knockdown studies showed that YTHDF2 and YTHDF3 redundantly affect IFN-γ responsiveness alongside YTHDF1. Analysis of RNA immunoprecipitation (RIP)-sequencing data revealed that IRF1 mRNA, key regulator of IFN-γ signaling, is a direct target of YTHDFs and is degraded in an m6A-dependent manner. Likewise, downregulation of METTL3, which is a major component of m6A methyltransferase complex, using small interfering RNA or catalytic inhibitor increase IRF1 levels and IFN-γ responsiveness. Furthermore, depletion of Ythdf1 reduced tumor growth and increased immune cell infiltration into the tumor, dependent on IFN-γ signaling in mouse syngeneic tumor models. Collectively, our study suggests that YTHDFs modulate cancer immunity by influencing IFN-γ signaling through IRF1 regulation.

#6048 Genome-wide CRISPR screening identifies tyrosylprotein sulfotransferase-2 as a target for augmenting anti-PD1 efficacy.

Yumi Oh[1], Sung-Yup Cho[2], Sujeong Kim[3], Yunjae Kim[3], Hyun Kim[3], Hansoo Park([3], Yongsoo Jang[4], Soobin Park[4], Yong-Chul Kim[5]

[1]Medical Research Center, Genomic Medicine Institute, Seoul National University College of Medicine, Seoul, Korea, Republic of,[2]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Korea, Republic of,[3]Department of Biomedical Science and Engineering, Gwangju Institute of Science and Technology (GIST), Gwangju, Korea, Republic of,[4]School of Life Sciences, Gwangju Institute of Science & Technology, Gwangju, Korea, Republic of,[5]R&D Center, PeLeMed, Co. Ltd., Gwangju, Korea, Republic of

Immune checkpoint therapy (ICT) offers durable responses in select cancer patients, yet resistance remains a significant challenge, prompting the exploration of underlying molecular mechanisms. We propose tyrosylprotein sulfotransferase-2 (TPST2), known for its role in protein tyrosine O-sulfation, as a suppressor of cancer immunity. Through *in vivo* genome-wide CRISPR screening, TPST2 loss-of-function emerged as a potential enhancer of anti-PD1 treatment efficacy. TPST2 suppressed interferon-γ (IFNγ) signaling by sulfating IFNγ receptor 1 at Y397 residue, while its downregulation boosted IFNγ-mediated signaling and antigen presentation. Depletion of TPST2 in cancer cells augmented anti-PD1 antibody efficacy in syngeneic mouse tumor models by enhancing tumor-infiltrating lymphocytes. RNA sequencing data revealed TPST2's inverse correlation with antigen presentation, and increased TPST2 expression is associated with poor prognosis and altered cancer immunity across cancer types. In an effort to develop novel cancer immunotherapies targeting TPST2, we have discovered LDD-5557, a lead compound with potent enzyme inhibitory activity through library screening and structure-based hit to lead optimization process. The lead compound showed an excellent in-vivo PK profile including high oral bioavailability and immunotherapeutic efficacies of significant upregulations of CD8 positive T-cells and antigen processing related genes in a dose-dependent manner by co-treatment with anti-PD1 in a MC38 syngeneic mouse model. These findings underscore TPST2's novel role in cancer immunity and advocate for its consideration as a therapeutic target in ICT-based treatments.

#6049 Fibroblast growth factor receptor modulates IFN-γ response in breast cancer.

Marvis Monteiro[1], Mitchell G. Ayers[1], Michael K. Wendt[2]

[1]Borch Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN,[2]Department of Internal Medicine, University of Iowa, Iowa City, IA

FGFR is amplified in 20% of metastatic breast cancer cells (MBCs) where it promotes growth, sustains mesenchymal plasticity, and mediates drug resistance. Systemic inhibition of FGFR kinase activity increases the numbers of $CD4^+$ and $CD8^+$ T-cells and decreases abundance of myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment. These data indicate an immunosuppressive role of FGFR signaling pathway in MBCs. However, a mechanism by which blockade of FGFR kinase activity affects T-cell presence in the tumor is not well understood. T-cells decrease tumor progression by releasing cytokines such as interferon gamma (IFN-γ). IFN-γ binds to its cognate receptor to activate the JAK-STAT1 signaling pathway, promoting immune recognition of cancer cells. Herein, we investigate the signaling cross talk between FGFR-IFN-γ and the resultant pathophysiological effects on cancer cells. We observe that FGFR signaling reduces STAT1 phosphorylation in breast cancer cells. Conversely, inhibiting FGFR kinase activity in murine mammary carcinoma cells leads to increased STAT1 phosphorylation, increased expression of downstream interferon response genes including the T-cell chemoattractants CXCL9 and CXCL10, and increased cell surface expression of major histocompatibility complex I (MHC-I). A key mediator of the FGFR signaling cascade is SH2 containing protein tyrosine phosphatase (SHP-2). Pharmacological inhibition of SHP-2 causes a decrease in *in-vivo* metastatic burden and an increase in T-cell presence in the tumor. Similar to targeted inhibition of FGFR, blockade of SHP-2 activity also increases STAT1 phosphorylation and expression of IFN-γ response genes. We propose a mechanism in which FGFR mediated activation of SHP-2 dephosphorylates tyrosine 701 of STAT1 thereby tempering anti-tumorigenic effects of IFN-γ. Our findings suggest that increased immunogenicity caused by FGFR pathway inhibition can be leveraged to make tumor cells more susceptible to immune attack and can be used in conjunction with immunotherapy for treatment of MBCs.

#6051 Opposing function of USP5 in MRE11 endonuclease activity to evade anti-tumor immunity.

Chuan-Chun Lee[1], Wan-Rong Wu[1], Liang-Chih Liu[1], Ting-Yi Liao[2], You-Zhe Lin[1], Fang-Ying Lin[1], Yi-Chun Shen[2], Feng-Chi Chung[1], Yuan-Liang Wang[1], Chih-Hao Lu[3], Wei-Chung Cheng[1], Wei-Chao Chang[2], Yi-Chuan Li[1], Chih-Tung Lin[1], Chung-Yu Chen[1], Sheng-Wen Chen[1], Hsin-An Shih[4], Steven Lin[4], I-Wen Chou[1], Chen-Yuan Lin[2], Chang-Feng Chiu[2], **Shao-Chun Wang**[1]

[1]China Medical University, Taichung City, Taiwan,[2]China Medical University Hospital, Taichung City, Taiwan,[3]National Yang Ming Chiao Tung University, Hsinchu, Taiwan,[4]Academia Sinica, Taipei, Taiwan

Proliferating cancer cells frequently encounter replication stress, which is known to generate abnormal cytosolic DNA and trigger anti-tumor immune responses. Thus, the ability to evade immune surveillance is critical for the development and progression of malignant tumors for which the mechanisms are not fully understood. The MRE11 nuclease, essential for maintaining DNA integrity during cell proliferation, plays important roles in processing stalled replication forks during replication stress. This process produces single-stranded DNA (ssDNA) intermediates which activate the cGAS-STING cascade when released into the cytoplasm to trigger anti-tumor immunity. In this study, we demonstrate that in cancer cells experiencing endogenous replication stress induced by the loss of phosphorylation at tyrosine 211 of the proliferative protein PCNA (pY211-PCNA), MRE11 is modified by site-specific polyubiquitinations. The modifications activate the endonuclease activity of MRE11, favoring cytosolic ssDNA production and subsequent immune response, which sensitizes cells to killing by natural killer (NK) cells. In contrast, in cells expressing pY211-PCNA, the ubiquitin-specific protease USP5 is recruited to replication forks, where it deubiquitinates MRE11, selectively reducing its endonuclease activity, suppressing cytosolic ssDNA generation, and blunting the immune response. Elevated USP5 levels correlate with tumor metastasis and poor prognosis in breast cancer patients. Downregulation of USP5 results in enhanced NK cell infiltration and tumor suppression in immune-competent mouse xenograft models, but not in immune-deficient models. Inhibition of USP5 renders patient-derived tumor organoids susceptible to NK cell-mediated killing. Unbiased screening and biochemical verification identified two FDA-approved drugs as potent USP5 inhibitors. Treatment with these agents phenocopies USP5 downregulation by enhancing cytosolic ssDNA production, increasing sensitivity to NK-mediated killing, and tumor suppression in syngeneic mouse models. Furthermore, these treatments sensitize patient-derived tumor organoids (PDTOs) to cytotoxic killing by NK cells and patient-autologous primary peripheral blood mononuclear cells (PBMCs). This mechanistic insight advances our understanding of tumor progression and the programming of immune responses, potentially leading to novel therapeutic strategies that harness anti-tumor immunity.

#6053 Down-regulation of interferon gamma response and other immune-related pathways in *PIK3CA* mutant colorectal cancer.

Alec B. Cornelio, Alexa E. Schmitz, Xingqi Shen, Shirsa Udgata, Cheri A. Pasch, Dustin A. Deming

University of Wisconsin - Madison, Madison, WI

Background:The *PIK3CA* gene, encoding the p110α protein, is commonly mutated across different cancer types, including colorectal cancer (CRC). As 18% of CRCs have a *PIK3CA* mutation, a more comprehensive understanding of its influence on immune processes is needed. Here we sought to isolate and characterize the RNA expression signature of *PIK3CA* mutant CRC at both the tumor and patient-derived cancer organoid (PDCO) level.

Methods:Patient/Originator Specimen (tumor) and Organoid Culture (PDCO) RNA sequencing data of CRC patients with and without *PIK3CA* mutations were sampled from the National Cancer Institute's Patient-Derived Models Repository (NCI PDMR) Database. After filtering out patients with coexisting *BRAF*, *KRAS*, and *NRAS* mutations, patients were separated into two groups: wild-type (WT) and *PIK3CA* mutant (PK). The PDCO dataset contained 15 patients (nine WT vs six PK), and the tumor dataset contained eight patients (four WT vs four PK). Gene Set Enrichment Analysis (GSEA) was performed on the differential expression results comparing these two groups. A cutoff of |log10 false discovery rate (FDR)| ≥ 1.3 was used to determine significance for pathways. Data analysis was performed in R with the following packages: DESeq2, tximport, tximportData, dplyr, readr, magrittr, and ggplot.

Results:In the PDCO data set, two immune pathways were significantly up-regulated in WT compared to PK: interferon alpha (IFNα, FDR q-value < 0.001) and interferon gamma (IFN-γ, 0.018). In the tumor data set, more immune pathways were significantly down-regulated in PK compared to WT. PK had a down-regulation in pathways involved in T cell activation and function like IL6 JAK STAT3 signaling (q = 0.009) and IL2 STAT5 signaling (q = 0.003). Interestingly, in the tumor data set, PK had multiple inflammatory pathways down-regulated compared to WT: TNFA signaling via NFKB, allograft rejection, inflammatory response, complement (all q < 0.001), IFN-γ response (q = 0.004). IFN-γ response was investigated further due to it being significantly down-regulated in PK in both PDCO and tumor data sets. Significant enrichment was seen in common genes for both groups as follows: *PDE4B* (organoid log2FC = -1.24 and tumor log2FC = -3.97), *IRF5* (-1.11 and -3.05), *BTG1* (-0.753 and -2.72), *TNFAIP2* (-0.5 and -2.69), and *CD40* (-0.405 and -2.09). *IRF5* has been implicated in M1 macrophage polarization, *CD40* in CD8 T cell activation, *PDE4B* and *TNFAIP2* in inflammation, and *BTG1* in B cell regulation. Downregulation of these genes hints at PK possibly inhibiting important immune cell markers and the possible mechanisms through which PK is acting.

Conclusions: These analyses indicate mutated *PIK3CA* may play a role in suppressing the immune response in CRC, which can be seen even in PDCOs. Further investigation is needed to determine the mechanisms by which *PIK3CA* is suppressing the IFN-γ response and other immune-related pathways.

#6056 Aging accelerates breast tumorigenesis though cancer immunosuppression.

Shanshan Yin[1], Kay Yeung[2], Jing Yang[2], Peter D. Adams[1]

[1]Sanford Burnham Prebys Med. Discovery Inst., San Diego, CA,[2]University of California, San Diego, San Diego, CA

Age is a major risk factor for breast cancer. However, the mechanisms by which age affects breast cancer initiation and progression is not fully understood. Unfortunately, there is a paucity of mouse with which to study the role of aging in cancer, especially the impact of aging on the epithelial tumor cells-of-origin. To address this for breast cancer, we established a mouse model in which breast cancer was induced de novo in young and old wildtype mice through intraductal injection of a lentivirus carrying the breast cancer oncogene HER2/neu. Consistent with human data, breast tumors in the old mice grew more rapidly than in young mice. Old mice exhibited more aggressive, invasive and higher grade breast tumor lesions. GeoMx whole-transcriptome spatial transcriptomics revealed that old tumor cells upregulated genes associated with invasion, epithelial-to-mesenchymal transition, cell migration, blood vessel development, and extracellular matrix formation, while downregulating genes related to immune response, interferon gamma response, and antigen presentation. Specifically, several chemokines were downregulated in old tumor cells, with Cxcl9 and Cxcl10 (hereafter Cxcl9/10) being the most prominent, an observation confirmed by RNAscope and immunostaining. Expression of Cxcl9/10 is known to attract Cd8+ and Cd4+ T cells through binding to the T cell Cxcr3 receptor, thereby facilitating anti-tumor T cell infiltration into tumors. We observed fewer tumor infiltrating Cd4+ and Cd8+ T cells in old tumors. Simultaneous depletion of Cd4 and Cd8 T cells significantly accelerated tumor growth in young mice but not in old mice. Blocking Cxcr3 in young mice accelerated tumor growth and reduced T cell infiltration, while enhancing Cxcl9/10 expression slowed down the tumor growth in old mice. These results suggest that age-associated downregulation of Cxcl9/10 in tumor cells is responsible for an impaired anti-tumor immune response in aged tissue. Furthermore, scATACseq revealed that Cxcl9/10 gene regions were less accessible in old tumor tissues. Taken together, these results suggest that age-associated downregulation of Cxcl9/10 in tumor cells is responsible for an impaired anti-tumor immune response in aged tissue, with epigenetic rewiring playing a critical role in this process.

IMMUNOLOGY: Antibodies 3: Multi-Target Checkpoint Inhibitors and Immune Activators
Poster Session

#6060 A symmetrical hexavalent trispecific antibody, HH160, targeting PD-1, CTLA-4 and VEGF-A for enhanced anti-cancer effects.

Chunmei Liu, Jianhe Chen, Fang Yang, Fangfang Ren, Jing Shao, Juan Liu, **Bin Ye**, Jianhua Sui

Huahui Health, Ltd., Beijing, China

Background: Antiangiogenic agents, such as the anti-vascular endothelial growth factor A (VEGF-A) antibody Bevacizumab, are among the first targeted therapies in cancer treatment. Immune checkpoint inhibitors (ICIs), including anti-PD-1/L1 and anti-CTLA-4 antibodies, have revolutionized cancer therapy. Combinations of anti-VEGF agents and ICIs have demonstrated enhanced efficacy in treating various types of cancers, including liver cancer, non-small cell lung cancer, and cervical cancer, indicating synergistic anti-tumor mechanisms. Here, we report a novel symmetrical hexavalent trispecific antibody, HH160, which targets PD-1, CTLA-4, and VEGF-A, aiming to enhance therapeutic effects, increase dosing convenience, achieve tumor-specific drug distribution, and reduce treatment-related side effects.
Methods: HH160 was constructed by fusing anti-PD-1 and anti-CTLA-4 nanobodies to the N- and C-termini of the heavy chain of an anti-VEGF-A human IgG1 antibody with a silent Fc, forming a symmetrical hexavalent trispecific antibody. Binding affinity was assessed using Surface Plasmon Resonance, and the EC_{50} and IC_{50} were determined using ELISA and FACS. Immune-related cellular functions were evaluated using luciferase-based reporter assays and Mixed Lymphocyte Reaction (MLR) assays. CTLA-4 mediated cellular internalization was evaluated in Jurkat-PD-1/CTLA-4 cells, and pharmacokinetics (PK) in mice were also assessed. The anti-tumor efficacy of HH160, including anti-tumor immune functions and anti-angiogenesis were assessed respectively in syngeneic hPD-1/hCTLA-4 humanized C57BL/6 mouse tumor models and immunodeficient mice bearing VEGF-A-expressing cells.
Results: HH160 exhibited strong binding to the three targets—PD-1, CTLA-4 and VEGF-A— and effectively blocked their ligand interactions. HH160 showed strong stimulation of IL-2 and IFN-γ secretion in MLR assays, indicating enhanced anti-tumor immune activity and the potential to overcome resistance to single-targeting agents. HH160 was efficiently internalized in Jurkat-PD-1/CTLA-4 cells via receptor-mediated internalization, suggesting its potential to reduce the VEGF-A levels in the tumor microenvironment. HH160 also exhibited a favorable PK profile in BALB/c mice. Significant immune-mediated and anti-angiogenesis-mediated anti-tumor effects were observed in hPD-1/hCTLA-4 humanized mouse tumor models and immunodeficient mice bearing VEGF-A-expressing cells.
Conclusions: Our study demonstrates that HH160 is a promising trispecific antibody for simultaneously targeting PD-1, CTLA4 and VEGF-A. It has the potential to harness the synergistic anti-tumor mechanisms, overcome resistance to monotherapy, and simplify the dosing regimen, supporting further development and clinical investigation.

#6061 Dual PD-L1 blockade and VEGF-A neutralization with the bispecific antibody BNT327/PM8002 shows potent antitumor activity in preclinical models.

Xiaoniu Miao[1], Yifeng Xu[2], Shaogang Peng[2], Weifeng Huang[2], Junying Chen[1], **Maren Kohne**[3], Andrea Imle[3], Alexander Muik[3], Ozlem Tureci[3], Ugur Sahin[3], Andy Tsun[1], Yi Luo[1]

[1]Biotheus Inc., Zhuhai, China,[2]Biotheus (Suzhou) Co., Ltd., Suzhou, China,[3]BioNTech SE, Mainz, Germany

The combined blockade of the PD-1/PD-L1 checkpoint and VEGF-A driven angiogenesis has been shown to reduce immune suppression in the tumor microenvironment, to enhance antitumor immune responses, and exhibit improved antitumor activity compared to each individual treatment. BNT327 (also known as PM8002) is an investigational bispecific antibody (bsAb) composed of a bivalent Fc-silenced anti-VEGF-A IgG1 antibody fused at its C-terminus to two humanized anti-PD-L1 single-domain VHH antibodies. By simultaneously targeting PD-L1 and VEGF-A, BNT327 is designed to combine the two complementary functions of amplifying pre-existing immune responses and anti-angiogenesis. Here, we report preclinical studies characterizing the functional activity of BNT327 *in vitro* and antitumor activity *in vivo*. BNT327 bound with high affinity (*i.e.*, nanomolar or subnanomolar K_D) to recombinant human PD-L1 and VEGF-A, as shown by biolayer interferometry. In cell-based bioluminescent reporter assays, BNT327 inhibited PD-L1/PD-1 signaling and VEGF-A/VEGFR2 signaling with subnanomolar EC_{50} values. In mixed lymphocyte reaction assays using human PBMCs and allogeneic monocyte-derived dendritic cells, BNT327 and the parental anti-PD-L1 VHH dose-dependently increased IL-2 and IFN-γ secretion. Furthermore, BNT327 dose-dependently enhanced $CD8^+$ T-cell mediated tumor-cell killing in an antigen-specific cytotoxicity assay *in vitro*, with the effects being comparable to those of PD-L1 blockade by atezolizumab. *In vivo*, a BNT327 surrogate bsAb targeting human PD-L1 and mouse VEGF-A exhibited dose-dependent antitumor activity in human PD-L1 knock-in mice bearing syngeneic human PD-L1-expressing CT26 tumors. Tumor growth inhibition was superior to individual treatments with PD-L1 blockade (parental anti-PD-L1 or atezolizumab) or with anti-VEGF-A. Furthermore, BNT327 showed antitumor activity in several xenograft models, including A375 melanoma cells, NCI-H1975 lung carcinoma cells, LoVo colon carcinoma cells, and MDA-MB-231 breast carcinoma cells. Antitumor activity was dose-dependent where tested, and superior to single PD-1 (pembrolizumab) or PD-L1 (atezolizumab) checkpoint inhibitor treatment. In conclusion, BNT327 inhibits PD-1/PD-L1 signaling and VEGF-A/VEGFR2 signaling *in vitro* and shows potent antitumor activity *in vivo* both in syngeneic and xenograft models. The safety and efficacy of BNT327 as a monotherapy or in combination is being investigated in multiple clinical trials in patients with solid tumors, including Phase II and Phase III trials investigating the combination with chemotherapeutic agents in triple-negative breast cancer and lung cancer.

#6062 A novel bi-specific antibody targeting CD39 and PD-L1 for enhanced cancer immunotherapy.

Ze Yang*[1], Xiaopei Li*[2], Zhongliang Li*[3], Yongfa Chen*[4], Huajing Wang[2], Xu He[1], Hongwei Sun[3], Xiaowen Lin[3], Ligong Lu[1], Xiaowen He#[2], Meixiao Zhan#[1], Ke He#[5], Wei Zhao#[1]

[1]Interventional Medical Treatment Department, Guangzhou First Pepople's Hospital, the Second Affiliated Hospital, School of Medicine, South China University of Technology, Guangzhou, China,[2]R&D Department, OriCell Therapeutics Co. Ltd., Shanghai, China,[3]Guangdong Provincial Key Laboratory of Tumor Interventional Diagnosis and Treatment, Zhuhai People's Hospital (Zhuhai Clinical Medical College of Jinan University), Zhuhai, China,[4]The Affiliated Dongguan Songshan Lake Central Hospital, Guangdong Medical University, Dongguan, China,[5]Minimally Invasive Tumor Therapies Center, The Affiliated Guangdong Second Provincial General Hospital of Jinan University, Guangzhou, China

While immune checkpoint inhibitors have revolutionized cancer treatment, there remains a significant unmet need for more potent strategies to bolster anti-tumor immunity. This study introduces OriA631-B, a pioneering bi-specific antibody designed to simultaneously engage CD39 and PD-L1. By disrupting both the metabolic checkpoint CD39 and the immune checkpoint PD-L1, OriA631-B aims to amplify therapeutic efficacy.Leveraging advanced antibody engineering, OriA631-B was meticulously crafted to exhibit optimal binding affinity and specificity for its intended targets. In vitro studies revealed that
OriA631-B, rooted in a clinically validated PD-L1 antibody, effectively suppressed CD39-mediated adenosine generation, thereby invigorating effector T cell function and proliferation. In vivo investigations utilizing syngeneic tumor models demonstrated that OriA631-B treatment significantly curtailed tumor growth compared to monotherapy or combination therapies. Safety assessments indicated a favorable toxicity profile, with no substantial adverse events observed.Collectively, these findings highlight the promising potential of OriA631-B as a novel immunotherapeutic agent in cancer treatment. By concurrently targeting CD39-mediated immune suppression and PD-L1-mediated T cell exhaustion, this bi-specific antibody may circumvent resistance mechanisms and enhance T cell engagement with tumor cells, ultimately improving patient outcomes. Further clinical evaluation is warranted to fully assess the therapeutic potential of this innovative bi-specific antibody in cancer immunotherapy.

#6063 ROSE12, a novel anti-CTLA-4 Switch antibody™ with FcγR affinity-enhanced Fc, evokes tumor-selective anti-tumor immune activation by extracellular ATP without systemic immune activation.

Hiroki Hayashi[1], Kanako Tatsumi[1], Hitoshi Katada[1], Yutaka Matsuda[1], Toshiaki Tsunenari[1], Masaki Honda[2], Takayuki Nemoto[2], Shun Shimizu[1], Momoko Miura-Okuda[1], Yuri Ikuta[1], Ami Ito[1], Chika Ogami[3], Chie Kato[2], Masaki Kamimura[1], Tatsuya Kibayashi[1], Chiyomi Kubo[2], Shunichiro Komatsu[2], Yasunori Komori[2], Junko Shinozuka[2], Hiroaki Susumu[1], Honoka Tanno[2], Yasushi Tomii[1], Kenji Nakagawa[1], Hiroaki Nagano[1], Masahiko Nanami[2], Yukari Nishito[1], Nozomi Fujisawa[2], Tomochika Matsushita[2], Saki Michisaka[1], Moe Yoshimoto[1], Hiroaki Wakatsuki[3], Tetsuya Wakabayashi[1], Naoko Wada[1], Otoya Ueda[1], Hiroko Konishi[1], Kenji Kashima[1], Hiroshi Tanaka[1], Mika Endo[1], Takehisa Kitazawa[1], Shimon Sakaguchi[4], Mika Kamata-Sakurai[1], Tomoyuki Igawa[1]

[1]Research Div., Chugai Pharmaceutical Co., Ltd., Kanagawa, Japan,[2]Translational Research Div, Chugai Pharmaceutical Co., Ltd., Kanagawa, Japan,[3]Translational Research Div, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan,[4]Laboratory of Experimental Immunology, Immunology Frontier Research Center, Osaka University, Osaka, Japan

Background: Tregs are considered potential target in cancer immunotherapy, as tumor-infiltrating Tregs express higher levels of CTLA-4. A promising way to evoke effective anti-tumor immunity is to use FcγR affinity-enhanced anti-CTLA-4 antibody to deplete Tregs via ADCC activity. Although anti-CTLA-4 antibodies with non-fucosylated Fc (NF) are being clinically developed, the number of NKs and macrophages that can be activated is limited, resulting in limited efficacy. Therefore, stronger FcγR affinity-enhanced Fc is needed to show efficacy across all tumors. Moreover, systemic Treg depletion can potentially cause autoimmune side effects. Aiming to enhance anti-tumor activity and avoid systemic Treg depletion, we generated ROSE12, a novel FcγR affinity-enhanced anti-CTLA-4 Switch™ antibody. ROSE12 uses our proprietary antibody engineering technology, Switch-Ig™, allowing it to bind to CTLA-4 only in the presence of extracellular ATP, which is well known to be elevated in tumor tissue but tightly regulated in non-tumor tissue. ROSE12 is also utilized ART-Fc® technology that strongly enhances FcγR affinity. Due to this novel engineering, ROSE12 is expected to selectively deplete Tregs in tumor tissue without affecting non-tumor tissues.

Methods: We evaluated ROSE12's in vitro FcγR-affinity, ATP-dependent binding affinity to human CTLA-4, ADCC activity, and selective CTLA-4$^+$ Treg depletion. We assessed in vivo anti-tumor efficacy in human CTLA-4 knock-in (hCTLA-4KI) mice treated with mROSE12, which has the same variable region as ROSE12 with FcγR affinity-enhanced mouse surrogate Fc. We also evaluated mROSE12 and FcγR affinity-enhanced ipilimumab (Ipi-ART)'s intratumoral Treg depletion and T cell activation in non-tumor tissues using flow cytometry.

Results: ROSE12 showed ATP-dependent binding to CTLA-4 and stronger FcγRIIIa-affinity than ipilimumab-NF. Consistent with the binding profile, ROSE12 showed stronger ATP-dependent selective CTLA-4$^+$ Treg depletion among CTLA-4$^+$ T cells than ipilimumab-NF. mROSE12 demonstrated anti-tumor efficacy in a MC38 and PD-L1 therapy-resistant Hepa1-6 bearing mouse model. Moreover, Ipi-ART, but not mROSE12, induced systemic T-cell activation in normal tissue. Consistent with its anti-tumor efficacy, mROSE12 also depleted intratumoral Tregs. Furthermore, ROSE12 was well tolerated in cynomolgus monkeys in a repeated-dose toxicity study.

Conclusion: ROSE12 is a novel FcγR affinity-enhanced anti-CTLA-4 Switch antibody™ with potent anti-tumor efficacy and a wide therapeutic window due to tumor-selective activity. These results strongly support the clinical testing of ROSE12 in the treatment of solid tumors. Currently, ROSE12 is undergoing a phase 1 clinical study (NCT05907980).

#6064 Multiomics preclinical characterization of SAR445877, an anti-PD-1 antibody-IL-15 mutein fusion protein with robust anti-tumor efficacy as monotherapy.

Julien Tessier[1], Joon Sang Lee[1], Ingeborg M. Langohr[2], Caroline Morel[2], Xiangming Li[3], Seth Garren[1], Virna Cortez-Retamozo[3], Dinesh Bangari[2], Donald Jackson[1], Donald Shaffer[3], Angela Hadjipanayis[1]

[1]Precision Medicine & Computational Biology, Sanofi, Cambridge, MA,[2]Translational In vivo Models, Sanofi, Cambridge, MA,[3]Oncology Research, Sanofi, Cambridge, MA

Mechanism of action studies are imperative for translating oncology therapeutics into the clinic informing testable hypotheses and potential combination partners. One class of oncology therapeutics, immune-stimulatory cytokines can augment the antitumor efficacy of checkpoint blockade, but further translational work is required to understand beneficial treatment effects versus unwanted immunotoxicities. SAR445877 (SAR'877, formerly KD050), is a novel antibody-cytokine fusion protein consisting of an Fc silenced human anti-PD-1 IgG1 fused to a mutated interleukin-15 (IL-15)/IL-15 receptor alpha sushi domain fusion, which can cis-activate effector T cells by biasing IL-15 stimulation to T cells expressing PD-1. Here we used immunohistochemistry (IHC), flow cytometry and spatial transcriptomics to characterize SAR'877 treatment effects on residual tumors in a B16-OVA mouse model. Mice were treated with isotype control, anti-mPD-1 (anti-murine PD-1), mntSAR'877 (mIgG2a-mutmIL15) or mSAR'877 (murine surrogate anti-mPD-1-mutmIL15). The mSAR'877 treated animals showed regression of tumors, decreased necrosis and increased tumor immune cell infiltration compared to isotype control, mntSAR'877, and anti-mPD-1. IHC performed on residual tumors treated with mSAR'877 displayed a further decrease of PD-L1+ cells compared to anti-mPD-1 or mntSAR'877. Spatial transcriptomic profiling using the GeoMx DSP instrument revealed significant infiltration of innate immune cells (NK, monocytes, and neutrophils) and adaptive immune cells (CD8 T and CTL cells) into the tumor leading-edge compartment when compared to anti-mPD-1. CD8 T cell and NK cell increases were orthogonally validated by IHC and flow cytometry. Pathway analyses showed these changes were also associated with an upregulation of cytotoxic pathways when compared to controls. Together, these data elucidated the mechanism of anti-PD-1/IL15 showing contributions of NK and CD8 T cells associated with anti-tumor activity observed in mice. These findings suggest that mSAR'877 shows superior efficacy to single agent PD-1 or untargeted IL-15 in mouse tumor models.
Note: all authors are employed by Sanofi and may hold stock and/or stock options

#6065 Preclinical evaluation of LM-168: A next-generation anti-CTLA4 antibody with promising efficacy and reduced toxicity.

Lei Shi, Yun Zhang, Tianen Yao, Xia Qin, Da Fei, Yuan Li, **Runsheng Li**

LaNova Medicines Limited, Shanghai, China

Background: Anti-CTLA-4 antibodies have demonstrated encouraging clinical outcomes across various tumor types, but systemic immune activation leads to severe immune-related adverse events. Thus, there is a medical need for novel CTLA-4 targeting therapies with improved benefit-risk profile. ANP (ATP/ADP/AMP) levels are highly elevated to micromolar levels in the tumor microenvironment (TME), whereas physiological ANP levels in healthy tissues remain in the nanomolar range. LM-168 is engineered to selectively bind CTLA-4 at high ANP level in TME, with weakly binding in healthy tissues, thereby reducing “on target off tumor” toxicity.
Methods: The target binding activity, specificity, and cross-species reactivity of LM-168 were evaluated by flow cytometry. The blocking efficacy of LM-168 against human CTLA-4 was determined by ELISA. *In vivo* efficacy was evaluated using three syngeneic models: monotherapy in MC38 and CT26 models, and combination therapy with anti-PD-1 in MCA205 model. The toxicity of LM-168 was evaluated in 4-week repeated-dose toxicity studies in SD rats and rhesus monkeys.
Results: LM-168 exhibited potent ANP-dependent binding affinity to huCTLA4-CHOK1 cells, with an EC_{50} of 1.56 nM in presence of high levels of ANP. In ANP-dependent binding assays, LM-168 exhibited a favorable specificity margin, achieving a 9.5-fold binding selectivity window. In addition, LM-168 demonstrated strong affinity for CTLA-4 in non-human primate and rodent models. In MC38 syngeneic model with hCTLA4 knock-in mice, LM-168 at 10 mg/kg significantly inhibited tumor growth with a tumor growth inhibition (TGI) of 102.6%. Tumor infiltrating lymphocytes were analyzed after treatment with either LM-168 or ipilimumab. Intratumoral Treg cells were significantly reduced, while CD8+ T cells were increased. Peripheral Treg cell proliferation was lower in LM-168 treated mice compared to those treated with ipilimumab. In CT26 syngeneic model, LM-168 showed comparable anti-tumor efficacy to ipilimumab, achieving a TGI of 67.2% at a dose of 3 mg/kg. The combination of LM-168 with an anti-mPD-1 antibody provided a synergistic anti-tumor effect, achieving 98.4% TGI in the MC205 syngeneic model. 4-week repeated-dose toxicity studies indicated that LM-168 was well tolerated in both SD rats and rhesus monkeys, and 248 mg/kg was considered the highest non-severely toxic dose.
Conclusion: By targeting CTLA-4 conditionally, LM-168 is designed to address the potency and tolerability challenges associated with current anti-CTLA-4 antibodies, and thus has the potential to be a next generation CTLA-4 target therapy with promising clinical efficacy and reduced toxicity. LM-168 is also expected act synergistically with anti-PD-1/PD-L1 therapy. The preclinical data support clinical development of LM-168. It is currently in the FDA IND stage with a first-in-human trial expected in Q1 2025.

#6066 Preclinical evaluation of toxicity reduction for ROSE12, a novel ATP-dependent anti-CTLA-4 Switch antibody™ with FcγR affinity-enhanced Fc.

Moe Yoshimoto[1], Hiroki Hayashi[1], Kanako Tatsumi[1], Hitoshi Katada[1], Toshiaki Tsunenari[1], Hiroaki Susumu[1], Kenji Nakagawa[1], Hiroaki Nagano[1], Saki Michisaka[1], Chie Kato[2], Junko Shinozuka[2], Hiroshi Tanaka[1], Takehisa Kitazawa[1], Mika Kamata-Sakurai[1], Tomoyuki Igawa[1]

[1]Chugai Pharmaceutical, Co., Ltd., Research Div., Yokohama, Kanagawa, Japan,[2]Chugai Pharmaceutical, Co., Ltd., Translational Research Div., Yokohama, Kanagawa, Japan

Introduction: Cytotoxic T-lymphocyte associated protein 4 (CTLA-4) is highly expressed on tumor-infiltrating regulatory T cells (Tregs), which are known to suppress immune activation. To enhance anti-tumor efficacy via antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) activity, numerous Treg depletion antibodies with FcγR affinity-enhanced Fc are currently being developed. However, FcγR affinity-enhanced anti-CTLA-4 antibody may cause autoimmune toxicity due to systemic Treg depletion. In fact, it was reported that anti-CTLA-4 antibody with non-fucosylated Fc, a common FcγR affinity-enhancing Fc, causes autoimmune adverse events, such as colitis. Aiming to enhance anti-tumor activity without inducing systemic Treg depletion, we generated ROSE12, a novel anti-CTLA-4 Switch antibody™ with FcγR affinity-enhanced Fc. ROSE12 applies our proprietary antibody engineering technology, called Switch-Ig™, allowing it to bind to CTLA-4 only in the presence of high extracellular ATP. ROSE12 also utilizes a technology called ART-Fc® to strongly enhance FcγR affinity. Our results here show that ROSE12 avoids toxicity due to its ATP dependency.
Methods: We evaluated CTLA-4 binding affinity and ADCC activity of ROSE12 at varying ATP concentrations in vitro. To evaluate the efficacy and toxicity of ROSE12 in syngeneic mouse models, we established a mouse ROSE12 surrogate Switch antibody™ (mSW-ART) and a conventional mouse anti-CTLA-4 antibody (mCTLA-4-ART), which binds to CTLA-4 in an ATP-independent manner and has the same Fc as mSW-ART. We assessed the anti-tumor efficacy of mSW-ART by measuring the tumor volume in several tumor-bearing syngeneic mouse models. To investigate colonic toxicity, we evaluated diarrhea, colon weight, and colon length in a FM3A-bearing C3H mouse model. The frequency of Tregs in colon lamina propria was also measured using flow cytometry.
Results: ROSE12 both bound to CTLA-4 and exhibited ADCC activity in an ATP dependent manner. In vivo studies revealed that mSW-ART exhibited anti-tumor efficacy against several types of cancers. In the FM3A-bearing C3H mouse model, it was demonstrated that mSW-ART did not cause symptoms of colitis, while mCTLA4-ART did. Further, colonic Treg depletion was not induced by mSW-ART, in contrast to mCTLA4-ART. These results showed that mSW-ART avoids the colitis induced by anti-CTLA-4 Treg depletion antibodies which lack tumor selectivity.
Conclusions: Our study suggests that ROSE12, which has ATP dependency, may mitigate colonic toxicity by avoiding the depletion of Tregs in colon lamina propria. Thus, ROSE12 may show a wide therapeutic window. A phase I clinical trial for patients with solid tumors is ongoing (NCT05907980).

#6067 Preclinical evaluation of XB628: A novel PD-L1 x NKG2A bispecific antibody.

Bee-Cheng Sim[1], Bonnie Hammer[2], Dileep Pulukkunat[2], Jing Chen[1], Annika Lee[2], Mark Bercher[2], Hanying Li[1], Jeffrey Higaki[1], Anna Ritter[2], Seema Kantak[1]

[1]Exelixis Inc., Alameda, CA,[2]Invenra, Madison, WI

Background: Programmed death ligand-1 (PD-L1) is highly expressed by tumor cells and upon binding to PD-1 on T cells, inhibits T cells from killing tumor cells. NKG2A is expressed by NK cells and a subset of CD8+ T cells; upon binding to HLA-E on tumor cells, NKG2A inhibits NK cell-mediated cytotoxicity against tumor cells. Thus, engagement of the PD-L1 and NKG2A immune checkpoint pathways allows tumor cells to evade both adaptive and innate immune surveillance. A phase 2 clinical trial showed a significant clinical benefit with an anti-PD-L1 and anti-NKG2A antibody combination versus an anti-PD-L1 antibody alone,1 suggesting that dual inhibition of the PD-L1/PD-1 and NKG2A/HLA-E axes are complementary strategies. XB628 is a bispecific antibody with two distinct targeted monovalent binding domains, one each for PD-L1 and NKG2A, and is generated using the B-Body® platform. It is designed to kill tumor cells by acting as a dual checkpoint inhibitor targeting PD-L1 on tumor cells and NKG2A on NK cells, and by recruiting NK cells to tumor cells. Preclinical characterization of XB628 is reported here.
Methods: Binding of XB628 to PD-L1 and NKG2A was assessed using cell lines expressing PD-L1 and NKG2A. PD-L1/PD-1 reporter and HLA-E tetramer competition assays were used to assess XB628-induced release of PD-L1/PD-1- and NKG2A/HLA-E-mediated signaling blockade, respectively. Co-localization of tumor and NK cells with XB628 was assessed by flow cytometry using cell lines expressing PD-L1 and NKG2A. T-cell activation was evaluated by measuring interferon-gamma and granzyme B secretion. The ability of XB628 to promote NK-cell mediated tumor cell killing was assessed at varying ratios of tumor and NK cells. In vivo efficacy of XB628 was assessed using a genetically engineered mouse model.
Results: XB628 bound to PD-L1+ cell lines with high affinity and released PD-L1/PD-1 inhibition in a dose-dependent manner; it also bound to NKG2A+ cell lines and released NKG2A/HLA-E inhibition. XB628 induced a high degree of colocalization of PD-L1+ tumor cells and NK cells; an intrinsic property of XB628 not observed with an anti-PD-L1 and anti-NKG2A monoclonal antibody combination. XB628-induced activation of CD8+ T cells. XB628 promotion of NK cell-mediated tumor cell cytotoxicity was greater than anti-PD-1 or anti-NKG2A antibodies alone or in combination. XB628 promoted tumor cell death even when tumor cells significantly outnumbered NK cells. In a genetically engineered mouse model, XB628 demonstrated a significant dose-dependent reduction in tumor volume.
Conclusions: XB628 is a first-in-class, bispecific antibody that simultaneously inhibits both adaptive and innate immune checkpoints by engaging PD-L1 and NKG2A, while also recruiting NK cells to tumor cells. XB628 is highly efficacious in tumor cell killing in vitro and in vivo, supporting further clinical development.
Reference:1. Aggarwal C et al. J Clin Oncol. 2024;42(Suppl.):8046.

#6068 CT111, a novel trispecific PD-1 x CTLA-4 x VEGF single-domain antibody, synergistically targets exhausted T cells and promotes cooperative antitumor effects.

John Lee[1], Huiyuan Tang[1], Jia Yu[2], Weihong Wang[1], Leo Ng[1], Jing Yu[2], Yong Wang[1], Toya N. Baral[1], Jiaguo Li[2], Xinhao Wang[1], Qijun Qian[2], Wenfeng Xu[1]

[1]Chantibody Therapeutics, Inc., Menlo Park, CA,[2]Shanghai Cell Therapy Group Co., Ltd, Shanghai, China

Background: Dual PD-1 and CTLA-4 targeting immunotherapy shows enhanced therapeutic efficacy and is approved across multiple advanced solid tumor indications. In recent clinical trials, combined treatment of anti-PD-(L)1 with anti-VEGF have also been shown to improve clinical outcomes and overcome treatment resistance to either drugs alone. Bispecific antibodies targeting PD-(L)1/CTLA-4 and PD-(L)1/VEGF synergistically potentiates antitumor activity in preclinical models, and more recently, demonstrated enhanced clinical efficacy and tolerability. We developed a de novo, multi-targeting PD-1 x CTLA-4 x VEGF single domain trispecific antibody with enhanced synergistic anti-tumor activity by combining the effects of dual immune checkpoint and VEGF blockade.

Methods: Novel function-blocking anti-PD-1, anti-CTLA-4 and anti-VEGF single-domain antibodies generated from protein-immunized camelids were screened, optimized, and humanized using the VHHMAb® AI-powered platform. Multiple VHH leads were combinatorially screened in different Fc-backbone formats for optimal engagement of all three targets. Binding affinity and specificity were evaluated by protein-based ELISA, cell-based FACS and Octet BLI, and ligand blocking activities were measured by competitive ELISA, cell-based FACS and reporter assays. Multitarget interactions and avidity were measured by competitive ELISA, Octet BLI, and cell-based FACS. Bioactivity of PD-1 and CTLA-4 blockade on primary immune cells was evaluated in CMV-recall and SEB assays for cytokine release, and anti-VEGF bioactivity was determined in a HUVEC proliferation assay. Anti-tumor activity of CT111 was assessed in multiple hPBMC-engrafted humanized xenograft tumor models.

Results: All three de novo VHH antibodies used as building blocks for CT111 were selected based on strong functional activities that were superior to clinical benchmarks in cell-based assays. CT111 exhibited strong binding and optimal ligand blocking activity to human PD-1, CTLA-4 and VEGF alone and simultaneously in different assay formats. In CMV-recall and SEB stimulation assays, CT111 mediated potent IFN-γ and IL-2 secretion compared to clinical mono-targeting Ab combinations and bispecific benchmarks across multiple donors, and CT111 effectively blocked VEGF-mediated signaling and attenuated HUVEC proliferation. CT111 showed significant antitumor activity compared to clinical benchmarks, including AK112 and AK104, in PBMC humanized tumor models.

Conclusion: CT111 is a novel, potential first-in-class single-domain trispecific antibody that can simultaneously target PD-1 and CTLA-4 immune checkpoints on exhausted T cells, while also targeting VEGF-mediated tumor angiogenesis to overcome immune suppression synergistically within solid tumors.

#6069 A bispecific S100A4/TFR antibody to reprogram the GBM tumor microenvironment and target GBM stem cells.

T. Wong[1], J.-S. Leu[1], X. Fan[2], F. Leonard[1], M. Faisal[1], R. Kang[1], Y. Ali[1], N. Abdelfattah[1], N. Zhang[2], K. Yun[1];
[1]Houston Methodist Research Institute, Houston, TX, [2]University of Texas Health Science Center, Houston, TX

GBM (glioblastoma) is the most common and aggressive primary brain tumor in adults. Despite advances in immunotherapy, current treatment modalities have only provided a modest benefit to GBM patients. We propose that enhancing the efficacy of immunotherapy for GBM requires approaches that increase T cell infiltration and reprogram the myeloid cells to a more pro-inflammatory state. We previously reported that S100A4 is a critical regulator of immune suppressive myeloid and T cell phenotypes and also a major regulator of glioma stem cells in GBM. Our preliminary data show that targeting S100A4 in either glioma cells or stromal cells is sufficient to reprogram the tumor immune landscape and extend survival of glioma bearing mice, indicating that S100A4 is a promising therapeutic target. We recently developed a S100A4 blocking monoclonal antibody; however, this antibody does not cross the blood brain barrier (BBB) efficiently to confer significant therapeutic benefit in glioma bearing mice. To overcome this challenge, we generated a bispecific S100A4-TfR antibody (BsA) that allows robust BBB penetration and accumulation in the brain. We assessed efficacy of BsA treatment in a highly aggressive and therapy-resistant, syngeneic mouse model of GBM (SVP). Using ELISA, confocal immunofluorescent microscopy, flow cytometry, and bulk RNA sequencing, we report that BsA treatment accumulates in the gliomas and reprograms the tumor immune microenvironment to increase T cell infiltration and pro-inflammatory activation. Furthermore, BsA treatment in combination with αPD1 checkpoint blockade significantly extends survival in the *SVP* mouse glioma model. Combination treated tumors exhibit extensive CD8 T cell infiltration and increased tumor reactive CD4 and CD8 T cells compared to controls. In addition, the BsA is taken up intracellularly within GBM cells and depletes S100A4 and nuclear SOX2 protein. In vitro treatment of GBM stem cell cultures with the BsA reduced self-renewal, as measured by the secondary sphere formation assay. These results indicate that the BsA is a promising agent to reprogram the GBM immune landscape and target GBM cell stemness through inhibition of both extracellular and intracellular S100A4 protein. We are currently performing in vivo efficacy studies in additional murine glioma models, and deeper mechanistic studies using single cell RNA sequencing and functional assays.

#6070 A novel epitope specific TIM-3 antibody significantly enhanced anti-tumor activities with bispecific format, not with combination strategy.

Xin Dong[1], Bu Tining[1], Bishnu Nayak[2], Jinyu Dong[1], Meiguang Xiong[1], Binbin Wang[1], Dong Wang[1], Haojie Wang[1], Yu Zhang[1]

[1]NeoLogics Bioscience Co. Ltd., Suzhou, Jiangsu, China,[2]NeoLogics Bioscience Co. Ltd., San Diego, CA

Introduction: T-cell immunoglobulin and mucin domain containing-3 (TIM-3) is expressed on T cells, NK cells and some myeloid cells, like macrophage and dendritic cells. Moreover, TIM-3 plays as a negative regulatory receptor and increased expression upon resistance to checkpoint inhibitor therapy. Particularly, recent studies strongly suggest that TIM-3 on myeloid cells, like dendritic cells, play a critical role for anti-tumor activity. Therefore, the dual blockade of PD-1 and TIM-3 holds hopes for enhanced clinical benefits.
Results: we have designed a bispecific anti-PD-1/TIM-3 antibody to optimize the immune response and anti-tumor activities by utilizing a novel epitope-specific anti-human TIM-3 antibody which displays enhancing NK cell activity and improving antigen processing ability of antigen presenting cells (APC), like DCs. This PD-1/TIM-3 bispecific antibody has demonstrated high affinity binding to human TIM-3 and PD-1 in the low nanomolar range. Moreover, it simultaneously binds to TIM-3 and PD-1 receptors through a trans manner, suggesting its potent capacity to enhance the interaction between T-cells and dendritic cells (DCs). In both MLR (mixed lymphocyte reaction) and T cell stimulation assays, the PD-1/TIM-3 exhibits synergistic activity compared to combination of PD-1 mAb and TIM-3 monoclonal antibodies or mAb alone. In addition, we have demonstrated superior anti- tumor activities in PBMC humanized xenograft mouse model as well as syngeneic human PD-(L)1 and human TIM-3 knock-in mice.
Conclusion: A bispecific humanized PD-1/TIM-3 antibody binds to both human PD-1 and TIM-3 strongly and effectively blocks the interaction with its ligand. This bispecific antibody enhances immune responses and anti-tumor activities *in vitro* and *in vivo* as measured by MLR assay, various animal models, etc., supporting the synergistic effects by targeting both acquired and innate immunity with this bispecific antibody.

#6071 PD-1xTGFβR2 bispecific Biclonics® antibody INCA33890 augments human T-cell effector functions in vitro and ex vivo.

Jun Guan[1], Aidan Gilmartin[1], Kenji Kimura[2], Estela Noguera-Ortega[2], Ashwini Kulkarni[1], Pat Feldman[1], Michelle Kinder[1], Cynthia Timmers[1], Alejandro Amador Arjona[1], Patrick Mayes[1], Steven M. Albelda[2], Evgeniy Erulasnov[3], Liang-Chuan Wang[1]

[1]Incyte Corporation, Wilmington, DE,[2]Department of Medicine, University of Pennsylvania, Philadelphia, PA,[3]Department of Surgery, University of Pennsylvania, Philadelphia, PA

Anti-programmed cell death (PD)-1 and anti PD-ligand1 antibodies have produced durable clinical responses in a wide variety of solid and hematologic malignancies; however, their clinical benefits are often limited to a subset of patients. One of the immune resistance mechanisms that limits the activity of PD-1 axis pathway blockers involves activation of the transforming growth factor beta (TGFβ) pathway within the tumor microenvironment (TME). To overcome this immune suppression, a PD-1 and TGFβ receptor 2 (TGFβR2) dual targeting bispecific Biclonics® antibody, INCA33890, was developed. INCA33890 inhibits TGFβR2 preferentially on PD-1-positive cells (Wang L-C, et al. AACR 2023. Abs 2936). In this study, various in vitro and ex vivo cell-based systems were used to evaluate changes in T-cell effector function induced by INCA33890 compared with the anti-PD-1 antibodies, nivolumab or pembrolizumab. We observed that INCA33890 induced higher interferon gamma (IFNγ) levels than nivolumab with and without exogenous TGFβ in an exhausted T-cell mixed lymphocyte reaction (MLR) assay. Additionally, INCA33890 was superior to nivolumab in enhancing T-cell proliferation in the presence of TGFβ. RNAseq analysis of samples from the exhausted MLR assay revealed that, compared with nivolumab, expression of cytotoxic genes (*GZMB*, *GNLY*), proinflammatory cytokines (IFNG), and T-cell activation markers (CD25, 4-1BB) were elevated following INCA33890 treatment in the presence of TGFβ. We next co-cultured T-cell receptor-engineered T cells with non-small cell lung cancer tumor digests to examine the impact of the TME on antigen-specific T-cell responses. INCA33890 increased the percentage of IFNγ-producing T cells compared with pembrolizumab over 72 hours. In summary, our data suggest that co-inhibition of both PD-1 and TGFβR2 by INCA33890 may provide greater therapeutic benefits than an anti-PD-1 antibody alone.

#6072 Engineering and preclinical development of an anti-TROP2/PD-L1 bispecific antibody drug conjugate (ADC) for enhanced anti-tumor efficacy.

Chuan Chen, Liang Tian, Chenpeng Su, Dandan Liu, Jiyuan Tian, Yujuan Li, Bing Yang, Yongxin Shan, Xiaoqian Chen, Jian Peng, Zhenping Zhu

Helixon US Inc., New York, NY

Human trophoblast cell surface antigen 2 (TROP2) and programmed death ligand 1 (PD-L1) are often overexpressed in a variety of human cancers. Both anti-PD-L1 antibodies and TROP2-based antibody drug conjugates (ADCs) have shown significant clinical benefit, and a number of these agents, including atezolizumab and durvalumab (both anti-PD-L1 antibodies) and sacituzumab govitecan (an anti-TROP2-SN38 ADC), have been approved by the US FDA for certain cancer indications. The efficacy of these agents is, however, often limited by the unwanted on-target/off-tumor toxicities and the emergence of resistance of tumor cells. In this study, we engineered a humanized bispecific antibody (bsAb) simultaneously targeting both TROP2 and PD-L1 and produced several drug conjugates utilizing various linkers and cytotoxic payloads. The bsAb ADC binds efficiently to tumor cells that express either or co-express both TROP2 and PD-L1, trigger rapid ADC internalization, and induce potent tumor killing activity. Further, the bsAb ADC also effectively blocks PD1/PD-L1 interaction, and in parallel, induce PD-L1 internalization and down-regulation on tumor cell surface, resulting in significantly enhanced T cell activation for tumor cell killing. In multiple xenografted tumor models, the bsAb ADC demonstrated greater tumor inhibitory activity than their respective monospecific parent antibody ADCs. Taken together, our findings support further development of this bsAb ADC for the treatment of multiple TROP2- and/or PD-L1-expressing cancers, and of those patients who are refractory and/or resistant to TROP2 or PD-L1 single target-based therapies.

#6073 IDP-002 is a PD-1xCD6 bispecific antibody that enables complete PD-1 receptor occupancy and enhances cytotoxic over regulatory T cell responses.

Marc Gavin, Tsadik Habtetsion, Christie Mortales, Hayley Ma, Aniruddh Saxena, Mikaela Rusnak, Jing Song, Ben Setter, Robert Gene, Heath Klock, Carina Xu, Ertan Eryilmaz, Pamela Holland, Scott Lesley

InduPro, Seattle, WA

High response rates observed with PD-1/L1 antagonists have advanced immunotherapy to the forefront of treatment options for multiple cancer types; however, a substantial proportion of partial and non-responders warrants development of novel and more effective approaches. Poor responses to current PD-1/L1 antagonists may result from incomplete receptor occupancy, partial reversal of T cell exhaustion, and proliferative responses in PD-1$^+$ FOXP3$^+$ immunosuppressive regulatory T cells (Tregs) which will suppress anti-tumor effector T cell responses.

Importantly, the recently demonstrated superiority of the PD-1xVEGF bispecific antibody (bsAb) ivonescimab over pembrolizumab in PD-L1$^+$ advanced NSCLC suggests greater PD-1 receptor occupancy (achieved through high-avidity PD-1 binding of ivonescimab/VEGF multivalent complexes) leads to significantly deeper clinical responses.

Enhanced PD-1 receptor occupancy may also be achieved with *cis*-acting bsAbs that target PD-1 and an abundant T cell surface protein, enabling more efficient PD-1 blockade compared to that achieved with conventional mAbs. Furthermore, candidate co-targets can be evaluated not only by expression level, but also for co-stimulatory capacity and differential cell subset expression. For example, low co-target expression on Tregs or co-target signaling that attenuates Treg fitness would skew PD-1 antagonist activity towards effector T cells and reduce therapy-induced Treg responses.

Here, we present IDP-002, a PD-1xCD6 bsAb that facilitates complete PD-1 receptor occupancy and promotes greater proliferative and cytotoxic responses *in vitro* and in tumor-bearing humanized mice compared to current PD-1 antagonist mAbs. Tregs, which express lower levels of CD6, proliferate less efficiently than effector CD4 and CD8 T cells in response to IDP-002. Enhanced effector T cell responses observed with IDP-002 derive from a combination of complete PD-1 blockade and the engagement of unique signaling pathways downstream of CD6. IDP-002 has the potential to become a leading therapeutic option for the next generation of PD-1 antagonists.

#6074 INCA33890, a bispecific antibody targeting PD-1 and TGFβR2, shows enhanced immune responses in models of ovarian cancer.

V. Pandey[1], K. M. Foos[2], I. Pargiolas[2], M. Poussin[2], A. Kulkarni[1], L.-C. Wang[1], P. Mayes[1], D. J. Powell Jr[2];
[1]Incyte Corporation, Wilmington, DE, [2]Center for Cellular Immunotherapies, University of Pennsylvania, Philadelphia, PA

Although immune checkpoint inhibitors (ICIs) targeting pathways involving programmed cell death 1 (PD-1) and its ligand (PD-L1) are promising for some diseases, they have shown modest responses in ovarian cancer patients. Multiple studies have shown that ICI resistance is associated with the prevalence of transforming growth factor (TGF)-β signaling in the tumor microenvironment (TME). In patients with ovarian cancer, TGF-β expression is associated with immunosuppressive features in the TME and poor prognosis. PD-1 and TGF-β inhibitory pathways play independent yet complementary roles in immunosuppression, providing rationale for simultaneous targeting of both pathways. However, agnostic inhibition of the TGF-β pathway has historically led to detrimental side effects. Therefore, we developed a bispecific Biclonics® antibody that conditionally blocks the TGF-β pathway through TGFβR2, only on immunosuppressed T cells that coexpress PD-1. In this study, we tested the efficacy of the dual PD-1- and TGFβR2-binding Biclonics® antibody, INCA33890, in models of ovarian cancer. We used patient ascites (N=6) as a model of ovarian TME. INCA33890 treatment resulted in significantly higher levels of secreted interferon (IFN)-γ compared with control antibodies targeting only PD-1 (pembrolizumab) or TGFβR2 (TGF-1). A significant increase in T-cell activation markers, such as CD137, CD25, and CD69, was also observed in some samples treated with INCA33890 compared with controls. Next, we tested INCA33890 in an autologous coculture of tumor-infiltrating lymphocytes expanded from an enzymatically digested-patient tumor and enriched tumor cells from the same patient. In these cocultures, IFN-γ levels were significantly higher with INCA33890 treatment compared with pembrolizumab; levels were similar compared with TGF-1. For an in vivo assessment, we used double huPD1/huTGFβR2 knock-in mice implanted with ovalbumin (ova) and luciferase-expressing murine ovarian cancer ID8 cells. Mice treated with INCA33890 showed a significant reduction in tumor burden and improved survival compared with controls. A significant increase in tumor-associated ova-specific CD8+ T cells was observed with INCA33890 treatment compared with controls. Phenotypic analysis of CD8+ T cells revealed a skew toward an effector memory subset ($CD44^{high}$; $CD62L^{low}$ expression) with INCA33890 treatment. Thus, INCA33890 showed favorable results in a humanized ovarian cancer model and demonstrated a tumor antigen-specific T-cell response resulting in reduced tumor burden. Altogether, we show that INCA33890 presents a promising immunotherapy option for patients with advanced ovarian cancer by targeting 2 prominent immunosuppressive pathways, leading to a coordinated immune response. Clinical assessment of INCA33890 for various advanced malignancies, including ovarian cancer, is ongoing (NCT05836324).

#6075 The combination of an EpCAMx4-1BB bispecific antibody with PD-1 blockade exhibits antitumor activity in a murine tumor model unresponsive to each individual antibody.

A. Imle[1], S. Fellermeier-Kopf[1], A. Ioan-Facsinay[2], L. Wiedmann[1], M. Kohne[1], A. Toker[1], A. M. Diks[2], T. Ahmadi[3], A. Muik[1], K. Kemper[2], O. Tureci[1], E. Breij[2], U. Sahin[1];
[1]BioNTech SE, Mainz, Germany, [2]Genmab B.V., Utrecht, Netherlands, [3]Genmab Inc., Plainsboro, NJ

Activation of the costimulatory receptor 4-1BB and blockade of the PD1/PD-L1 axis can augment immune responses through distinct mechanisms. DuoBody®-EpCAMx4-1BB (BNT314/GEN1059) is an investigational Fc-silenced bispecific antibody (bsAb) designed to boost antitumor immune responses via EpCAM-dependent 4-1BB agonist activity. We previously showed that combining DuoBody-EpCAMx4-1BB with PD-(L)1 blockade potentiated T-cell effector functions *in vitro* compared to single treatments[1]. Furthermore, combining an EpCAMx4-1BB bsAb with PD-1 blockade exhibited potent antitumor activity *in vivo* in the immunogenic MC38 tumor model. Here we further investigated this combination in *ex vivo* tumor-infiltrating lymphocyte (TIL) assays using resected human colorectal cancer (CRC) specimens and *in vivo* using the less immunogenic B16F10 tumor model. DuoBody-EpCAMx4-1BB promoted $CD8^+$ T-cell and NK-cell expansion in human TIL assays, whereas pembrolizumab alone had minimal effect. Combined treatment with both antibodies further enhanced $CD8^+$ T-cell expansion in three of six specimens tested. The combination of an Fc-silenced bsAb targeting human EpCAM and mouse 4-1BB (hEpCAMxm4-1BB) with anti-mouse PD-1 showed robust antitumor activity in mice bearing human EpCAM-expressing B16F10 tumors. This was evidenced by significantly delayed tumor outgrowth and prolonged survival compared to both single treatments and a nonbinding control antibody, whereas hEpCAMxm4-1BB or anti-PD-1 as single treatments showed no antitumor activity compared to the control. Furthermore, combination treatment led to complete tumor regression in 2 of 11 mice. Flow cytometry analysis of the peripheral blood of tumor-bearing mice showed that hEpCAMxm4-1BB treatment enhanced $CD8^+$ T-cell and NK-cell activation and proliferation, and frequencies of $CD8^+$ T cells with an effector phenotype. The pharmacodynamic effects on $CD8^+$ T cells were further potentiated by the combination with anti-PD-1, whereas anti-PD-1 alone had no effect. NK-cell activation and proliferation was modestly further enhanced by combination treatment, compared to hEpCAMxm4-1BB alone. In addition, the combination significantly enhanced $CD4^+$ T-cell activation and proliferation, compared to each single treatment. In conclusion, combining Fc-silenced hEpCAMxm4-1BB bsAb with PD-1 blockade exhibited antitumor activity *in vivo* in a model unresponsive to each single treatment, suggesting synergistic effects of the combination. The immunomodulatory activity of EpCAMx4-1BB bsAb in mice and in human CRC TIL cultures was further potentiated in combination with PD-1 blockade. These data provide preclinical rationale for the clinical evaluation of DuoBody-EpCAMx4-1BB in combination with PD-1/PD-L1 checkpoint blockade.

[1] Fellermeier-Kopf et al, *J Immunother Cancer* 2024; 12(S2): A833.

#6076 Non-clinical pharmacology of HX044, a novel FIC CTLA4xCD47 bi-specific antibody.

H. Ke[1], T. Yang[1], F. Peng[1], J. Li[1], C. Chen[1], L. Xiong[2], R. Sun[3], L. Zhang[1], F. Zhang[1], **H. Q. Li**[1];
[1]Hanx Bio, Hangzhou, China, [2]Crown Bioscience, Taicang, China, [3]SMOC, Shanghai, China

A bispecific CTLA4-antibody/SIRPα-fusion protein, HX044, was created based on the hypothesis that it would increase antitumor immunity/activities via efficient cis-binding to co-overexpressed CTLA4 and CD47 on regulatory T cells (T_{reg}) within tumor microenvironment while decrease toxicity via lowered affinity to each individual target in peripheral. HX044, as designed as 1+1 asymmetric IgG1 composed of half anti-CTLA4 mAb plus the extracellular CD47 binding domain of SIRPα, binds to human CTLA4 recombinant protein with a EC_{50} at 1.5 nM or human CD47 recombinant protein with a EC_{50} at 8.12 nM, which are 30 times weaker than the bi-valent CTLA4 mAb or 160 times weaker than the SIRPα-Fc fusion protein, respectively. In addition, HX044 exhibited reduced blocking activities on the binding of CTLA-4 to CD80 or CD47 to SIRPα, respectively, with an IC_{50} at 135.8 nM for CTLA4 or 6316 nM for CD47. While HX044 also binds to the respective single-positive cells with reduced binding as compared to the parental single-targeting molecules, HX044 has significantly enhanced binding to the $CTLA4^{hi}/CD47^{hi}$-double positive cells, suggesting an increased avidity via efficient cis-binding. HX044 showed little binding to the CD47-single-positive RBC and minimized binding to platelets, suggesting its potential minimal hematotoxicity usually associated with other CD47-targeting therapeutics. On the other hand, the minimized blocking function of HX044 showed ina CTLA4-ligand blockade function assay may also potentially contribute to its lower risks of inducing immune-related adverse effect (irAE) usually associated with CTLA4 targeting. HX044 mediates strong ADCC toward double target-expressing cells, which is highly dependent on the CTLA4 expression levels. It has comparable FcRn binding as Ipilimumab (Ipi). In the hCTLA4 x hCD47 x hSIRPα HuGEMM mouse model, HX044 was shown to have strong anti-tumor activity to huCD47-MC38 colorectal tumor (classic “hot” tumor) at significant low dose (<0.2mg/kg) and huCD47-B16F10 melanoma tumor (traditionally “cold” tumor) (6.6mg/kg) accompanied by the reduction of TIL-T_{reg}. Notably, the anti-tumor efficacy of HX044 is significantly superior to Ipilimumab or $SIRP_{\alpha}$-Fc fusion protein at equivalent molar doses, respectively., In the weekly repeated dosing study in cynomolgus monkeys, HX044 at 1 mg/kg or 6 mg/kg showed no significant drug-related or non-recoverable toxicity findings, while one animal died due to infusion reactions and one animal was euthanized moribund due to electrolyte imbalance and malnutrition caused by prolonged loose stool in the 40mg/kg group. The Highest Non-Severe Toxic Dose (HNSTD) was determined to be 6 mg/kg. A single-dose intravenous infusion of HX044 PK study showed dose-dependent exposure with half-life around 220 hours at low dose, going down with dose increase. Overall, preclinical data of HX044 support its further clinical testing in cancer patients.

#6077 PLT012, a humanized CD36-blocking antibody, is effective for unleashing anti-tumor immunity and against liver metastasis.

Yi-Ru Yu[1], Sheue-Fen Tzeng[2], Zheng Meng[3], Jaeoh Park[4], Lana Kandalaft[5], Yun-Han Lin[1], Chin-Hsien Tsai[2], Ping-Chih Ho[4]

[1]Pilatus Biosciences, Epalinges, Switzerland,[2]Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, Taiwan,[3]Society of Toxicology, Reston, VA,[4]Department of Oncology, University of Lausanne, Epalinges, Switzerland,[5]Department of Oncology, University of Lausanne, CHUV, Epalinges, Switzerland

Tumor cells employ various mechanisms to evade immune surveillance, including metabolic modulation of the tumor microenvironment (TME). Lipid metabolic stress in the TME leads to upregulation of CD36 in tumor-infiltrating immune cells, impairing anti-tumor immunity by enhancing the survival and suppressive activity of regulatory T cells (Tregs) and promoting ferroptosis and exhaustion in CD8+ T cells. Here, we developed PLT012, a humanized anti-CD36 IgG4 antibody targeting the lipid-binding domain of CD36, with a remarkable safety profile and favorable pharmacokinetics demonstrated in cynomolgus monkey models. Moreover, PLT012 significantly reduces tumor growth and reprograms the immune landscape, enhancing CD8+ T cell effector functions while reducing Treg-mediated suppression. In both immunotherapy-sensitive and resistant hepatocellular carcinoma (HCC) models, PLT012 alone or in combination with PD-L1 blockade induces robust anti-tumor immunity. Importantly, in a syngeneic liver metastasis model, PLT012 decreases immunosuppressive populations, enhances effector T cell responses, and reduces ICI resistance. Notably, *ex vivo* studies with human HCC samples further confirm PLT012's capacity to remodel the immune microenvironment. Taken together, these findings position PLT012 as a first-in-class immunotherapy targeting CD36, addressing critical challenges in HCC and liver metastasis treatment.

#6078 GFS202A, a novel GDF15×IL-6 bispecific antibody for the treatment of cachexia.

Xiangyu He, Jingyang Zhang, Yumei Li, Hongcan Ren, Siyuan Le, Fusheng Zhou, Jiong Lan, Qiang Lu

Genfleet Therapeutics, Shanghai, China

Cancer cachexia is a complicated syndrome characterized by loss of body mass, anorexia and progressive functional impairment. It is associated with intolerance and poor response to therapy, reduced life quality and unfavourable prognosis. Currently, pharmacologic intervention is limited without any FDA approved drugs. GDF15 and IL-6 derived from tumor and immune cells are major drivers of cachexia. GDF15 acts on receptor GFRAL and co-receptor RET which are expressed on neurons localized in area postrema (AP) and nucleus of the solitary tract (NTS) of the hindbrain, subsequently triggers downstream signal. IL-6 initiates trans- and classic- signals upon binding to soluble and membrane IL-6Rα respectively. These pathways together regulate adipose catabolism, muscle proteolysis, and impact appetite through neuronal circuit. GFS202A is a humanized tetravalent GDF15×IL-6 bispecific antibody, the Fc region is engineered to reduce Fc-mediated immune effect. It had high affinity to human GDF15 (K_D 0.05 nM) and IL-6 (K_D 0.313 nM), cross-reacted with cynomolgus monkey counterparts. GFS202A bound to antigens with high specificity, without binding to TGFβ family members GDF-8, BMP-3 and TGFβ1 or other IL-6 family members. GFS202A blocked interaction between GDF15 and GFRAL (IC_{50} 2.653 nM), neutralized GDF15-induced GFRAL/RET signal activation in 293T-luc reporter cells (IC_{50} 0.242 nM). GFS202A can inhibit both trans- and classic IL-6 signal in IL-6-induced BaF3-gp130 and BaF3-IL-6Rα/gp130 cells (IC_{50} 1.967 nM and 2.734 nM). In vivo efficacy was evaluated in tumor-induced cachexia mice. In human fibrosarcoma HT-1080 induced cachectic model, weekly IV administration of GFS202A effectively reversed body weight loss, 1, 4, 12 mg/kg GFS202A resulted in 5.61%, 11.61% and 24.71% tumor-free body weight gain when compared to isotype control. In ovarian cancer TOV21G cachectic model, weekly treatment of 1, 4, 12 mg/kg GFS202A increased tumor-free body weight by 4.55%, 20.62% and 37.35% respectively compared to isotype control. Adipose and muscle tissue weights in GFS202A groups also increased dose-dependently. Noticeably, GFS202A effectively reversed CRP elevation, indicating the function of bispecific antibody in relieving inflammation. In cynomolgus monkey PK study, single dose of GFS202A exhibited linear PK profile, Cmax and AUC increased in proportion with dose at the range of 1~25 mg/kg. GFS202A was well tolerated in cynomolgus monkey toxicity study, with a weekly dose regimen (qw ×5) the NOAEL was 300 mg/kg. Taking together, these preclinical findings show the potential of GFS202A as an innovative treatment for cachexia, with a projected clinical trial initiation in the first half of 2025.

#6079 Two is better than one: First in class anti-TIGIT IL-15 fusion antibody for enhanced tumor killing.

Tejal Bhatt[1], **Joyce Kim**[1], Wondae Kang[2], Rachel Etherington[1], Seulki Cheon[2], Sabrina Nielsen[1], Christian Willberg[1], Hongseok Jo[2], Yu-Hoi Kang[2]

[1]Research and Development, Medimab Biotherapeutics Ltd, Abingdon, United Kingdom,[2]Research and Development, MEDIMABBIO INC., Seongnam City, Korea, Republic of

Introduction: Immune checkpoint inhibitor therapies have revolutionised cancer treatment however, a portion of patients across malignancies become resistant or refractory to existing treatment. We have a first in class anti-TIGIT-IL-15 fusion antibody (MFA011) for treatment of solid tumors. Modes of action including superior tumor cell killing and increased immune effector function was observed in vitro. Significant inhibition of tumor growth and minimum toxicity was observed in 3 different hTIGIT knock-in mouse models. MFA011 has consistently outperformed competitor drugs when assessing pharmacokinetic activity providing a novel therapeutic option for cancer.

Methods: Mechanism of action for TIGIT was measured using a CD155-TIGIT blocking luciferase bioassay platform (Promega). We assessed specificity of IL-15 binding by ELISA and IL-15R signalling by measuring pSTAT5 phospho-staining. Immunotoxicity was assessed by cytokine release assay and off-target effects of IL-15 was measured by flow cytometry. In vitro efficacy was assessed in human PBMC from healthy donors using cytotoxicity assays (IncuCyte, Sartorius), ELISA, and flow cytometry-based assays, including intracellular cytokine staining and immune cell proliferation. In vivo efficacy of MFA011 was assessed using CT26 and Renca bearing BALB/c-hTIGIT knock-in mouse models with i.v. dosing of MFA011. In addition, anti-tumor effects were also observed in Hepa1-6 bearing C57BL/6 hTIGIT knock-in mouse model.

Results and discussion: MFA011 robustly bound and blocked CD155-TIGIT interactions and showed sustained signalling in comparison to unbound IL-15 cytokine alone. NK and CD8+ T cells showed increased proinflammatory cytokine production (IL-2 and IFNγ) and proliferation which led to improved anti-tumor killing observed in cancer derived cell lines. Conversely, regulatory T cells were depleted by antibody-dependent cellular cytotoxicity. Minimal immunotoxicity was observed and IL-15 bound specifically to cells that had been stimulated with MFA011. Reduction in tumor growth and stable biosafety profiles was observed in all mouse models after administration of MFA011.

Conclusion: Dual targeting of TIGIT and IL-15 signalling through MFA011 effectively enhances anti-tumor immune responses while limiting the off-target effects of IL-15 signalling. Our combined in vitro and in vivo data provides strong evidence to pursue MFA011 to IND filing and to commence phase I clinical trials.

#6080 Preferential tumor uptake and retention of EGL-001, an anti-CTLA4 -IL2 mutein fusion antibody achieving selective tumor Treg depletion.

Fiorella Kotsias[1], Alvaro Lladser[2], Marine Dubois[1], Caroline Janot-Sardet[1], Erica Russo[1], Anupama Karnam[1], Yann Courbebaisse[1], Mercedes Tkach[1], Reno Winter[1], Monica Gostissa[1], Pejvack Motlagh[1], Bernard Vanhove[1]

[1]Egle Therapeutics, Suresnes, France,[2]Centro Cientifico y Tecnologico de Excelencia Ciencia & Vida, Fundacion Ciencia & Vida, Santiago, Chile

Background: Regulatory T cells (Tregs) play a pivotal role in the immunosuppressive tumor microenvironment (TME), contributing significantly to resistance against immune checkpoint inhibitors. Targeting Tregs could reinvigorate anti-tumor immune responses. Tumor-associated Tregs express CTLA-4 and CD25 at levels markedly higher than their peripheral counterparts. EGL-001 consists of an anti-CTLA-4 antibody fused to an IL-2 mutein with antagonistic properties. It blocks CD80/86 interaction with CTLA-4 and inhibits IL-2-induced STAT-5 signaling in Tregs. Importantly, EGL-001 induces CTLA-4-dependent CD25 internalization and degradation in early endosomes. Deprived of IL-2 signaling, CD25-negative Tregs undergo apoptosis. Further studies were performed to investigate EGL-001 biodistribution.
Methods: The ability of EGL-001 to selectively localize in the TME, downregulate CD25 on tumor-associated Tregs in a CTLA-4-dependent manner, deplete Tregs, and restore effector T cell (Teff) activity was investigated through in vitro studies by flow cytometry and radiolabeling.
Results: After administering therapeutic doses in the MC38 colorectal carcinoma model, EGL-001 binds to tumor-associated Tregs but not Tregs in the periphery. It was not binding to other T cell types in the TME. Radiolabeling with 125-Iodine enabled the study of distribution in the tumor and other organs. Following the infusion of subtherapeutic doses of 125-I-EGL-001, EGL-001 was largely cleared from all organs by Day 7, whereas tumor retention remained visible up to Day 10. Additionally, Indium labeling of EGL-001 was conducted to longitudinally and quantitatively investigate tumor uptake. 111-In-EGL-001 localized to MC38 tumor sites within 1 hour and accumulated until day 2 when it represented 5% of the infused material. EGL-001 persisted in the TME for several days while being rapidly cleared from peripheral tissues. An assessment of intratumoral distribution revealed that EGL-001 did not diffuse uniformly throughout the tumor tissue but instead concentrated in defined zones, likely corresponding to mononuclear cell infiltrates.
Conclusions: Preclinical studies have demonstrated that EGL-001 exhibits potent anti-tumor activity by depleting Tregs and reactivating anti-tumor immunity. The current data indicates that EGL-001 preferentially acts within the TME, a phenomenon attributed to the strong overexpression of CTLA-4 and CD25 by tumor-associated Tregs compared to other Treg populations. This unique tumor selectivity sets EGL-001 apart from other Treg-targeting agents, presenting a promising strategy to overcome resistance to immune checkpoint blockade. EGL-001 is currently assessed in a First-In-Human clinical trial (NCT NCT06622486).

#6081 Bispecific antibody drug conjugates (bsADCs) targeting DLL3 and B7-H3 demonstrated potent anti-tumor activity in preclinical models of small cell lung cancer (SCLC).

Frank An, Kaiqing Zhang, Liu Yang, Peiran Li, Yu Qi, Jushi Gai, Luheng Du, Gao An, Chengzhang Shang, Yanan Guo, Yi Yang

Biocytogen, Waltham, MA

Lung cancer is the most common type of cancers and the leading cause of cancer-related mortality worldwide, with 2.5 million new cases and 1.8 million deaths in 2022. Small-cell lung cancer (SCLC) accounts for ~15% of all lung tumors, representing the most aggressive high-grade neuroendocrine carcinoma with a five-year survival rate of less than 4%. The majority of patients are diagnosed with extensive stage SCLC (ES-SCLC) with brain metastases in ~80% of cases, yielding a grim 5-year survival rate of under 2%. Many patients experience relapse following front-line therapies, and current second-line treatment options remain limited. Delta-like ligand 3 (DLL3) is highly expressed in SCLC, neuroendocrine tumors, and glioblastoma with minimally or no expression in normal tissues, making it an attractive target for SCLC. B7-H3, an immunoregulatory protein, is overexpressed in several tumor types, including SCLC. Both DLL3 and B7-H3 are validated targets for SCLC. Tarlatamab, a T cell engager targeting DLL3, was recently approved for SCLC. ADCs targeting DLL3 have shown robust anti-tumor activities in clinical trials. Similarly, anti-B7-H3 ADCs have also shown promising clinical potential.

We constructed a series of bispecific ADC (bsADC) that target both DLL3 and B7-H3 (DLL3xB7H3) simultaneously for SCLC and other tumors expressing these markers. We hypothesized that dual targeting could enhance selectivity and potency, address tumor heterogeneity in tumoral DLL3 and B7-H3 expression levels and allow a broader patient population for treatment. The bispecific antibody (bsAb) moieties of the bsADCs were designed in a1+1 format and were conjugated to a novel TOPO1 inhibitor payload with potent bystander effect (DAR 8) and a highly hydrophilic, enzyme-cleavable linker. In vitro, the bsAbs showed superior internalization in double positive cell lines than the parental monovalent arms and outperformed the naked antibodies of DS-7300 and rovalpituzumab (Rova). In vivo studies using a SCLC CDX model, the bsADCs demonstrated robust anti-tumor efficacy that was superior to DS-7300 ADC when conjugated to BLD-1102. The specificity of DLL3 targeting was validated in DLL3 negative CDX and PDX models in vivo. The bsAbs for our lead bsADCs exhibited excellent stability under stress conditions, suggesting their suitability for CMC development. In summary, our DLL3xB7H3 bsADCs showed promising and superior in vitro and in vivo preclinical activities with first-in-class potential for SCLC treatment.

#6082 Enhanced NK cell reactivity with Fc-optimized B7-H3 antibody for colorectal and non-small cell lung cancer.

Sylwia Anna Stefanczyk, Xenija Kaiser, Samuel J. Holzmayer, Ilona Hagelstein, Latifa Zekri, Susanne Jung, Melanie Marklin

Clinical Collaboration Unit Translational Immunology, German Cancer Consortium (DKTK), University Hospital Tubingen, Tubingen, Germany

Colorectal cancer (CRC) and non-small cell lung cancer (NSCLC) are among the deadliest cancers worldwide, especially in advanced stages with limited treatment options. While immune checkpoint inhibitors (ICIs) targeting PD-1/PD-L1 have shown significant potential, their efficacy is limited to a small subset of CRC patients with microsatellite instability-high (MSI-H) tumors, and their use in NSCLC is often limited by severe side effects, further restricting their applicability. This underscores the urgent need for alternative immunotherapeutic approaches employing novel targets to expand clinical benefit.

Here, we present a novel strategy exploiting natural killer (NK) cell mediated antibody-dependent cellular cytotoxicity (ADCC) using a monoclonal antibody (mAb) targeting B7-H3 (CD276). B7-H3, a B7 family immune checkpoint protein, is overexpressed in several tumor types, including CRC and NSCLC, but minimally expressed in normal tissues, making it an ideal target for cancer immunotherapy. Our engineered B7-H3 mAb, 8H8, was modified by introducing amino acid substitutions (S239D/I332E) in the Fc region to create the Fc-optimized variant 8H8-SDIE. This variant shows increased affinity for CD16 on NK cells, thereby enhancing ADCC potential.

Preclinical characterization demonstrated robust B7-H3 expression and specific binding of 8H8-SDIE to CRC (n=5) and NSCLC (n=3) cell lines, with saturation at approximately 1 µg/mL. Co-culture of allogeneic peripheral blood mononuclear cells (PBMCs) with B7-H3-positive CRC and NSCLC cells confirmed that 8H8-SDIE significantly activated NK cells, as evidenced by increased expression of CD69 and CD25. Upregulation of CD107a further indicated NK cell degranulation.

Additional analyses showed that 8H8-SDIE treatment promoted the secretion of IFNγ, TNF, and effector molecules such as granzyme A, granzyme B, granulysin, and perforin, thereby enhancing NK cell-mediated cytotoxicity. In short-term, mid-term and long-term cytotoxicity assays, 8H8-SDIE induced potent target cell-restricted lysis of CRC and NSCLC cell lines, while the iso-SDIE control showed no effect.

In conclusion, 8H8-SDIE is a potent immunotherapeutic candidate that effectively induces NK cell anti-cancer reactivity and represents a promising treatment option for CRC and NSCLC.

#6083 A fully human agonistic antibody against a unique epitope on TREM1 demonstrates immune stimulatory activities both in vitro and in vivo.

L. Yang, D. Liu, Z. Zhang, Y. Jiang, Q. Liu, **Q. Shi**;
Singlomics (Beijing DanXu) Biopharmaceuticals Co., Ltd., Beijing, China

Triggering receptor expressed on myeloid cells-1 (TREM1) is a proinflammatory receptor that amplifies immune responses. TREM1 is expressed on neutrophils, subsets of monocytes and tissue macrophages, and suppressive myeloid populations in the tumor microenvironment (TME), including tumor-associated neutrophils, monocytes, and tumor-associated macrophages. Stimulation by TREM1-mediated inflammatory signaling may promote an immunostimulatory TME and inhibit tumor growth. We developed DX002, a fully human agonistic anti-TREM1 monoclonal antibody. DX002 binds to a unique epitope and has low internalization property. Deep mutational scanning (DMS) of the human TREM1 antigen was applied to map DX002 binding epitope and result showed that DX002 was a ligand-competitive agonist that mimic the function of ligand PGLYRP1 protein. In vitro, Plate-bound DX002 up-regulates the expression of costimulatory molecules on monocytes and induces the production of proinflammatory cytokines. Moreover, soluble DX002 also induces the production of proinflammatory cytokines in PBMCs. In vivo, DX002 exhibits significant tumor growth inhibition activity in MC38 syngeneic model in human TREM1 transgenic mice. Taken together, the TREM1 agonistic antibody DX002 is shown to be active both in vitro and in vivo.

#6084 YH32364 (ABL104), a bispecific antibody against EGFR and 4-1BB, demonstrates potent anti-tumor efficacy through tumor-directed 4-1BB agonism.

Jae-Won Lee[1], Yangsoon Lee[2], Eunjung Lee[1], Hanna Hong[1], Byeong-Yun Ahn[1], Jangbeen Kyung[1], Young Bong Park[1], Junhwan Kim[1], Kyeongsu Park[2], Wonjun Son[2], Jaehyun Eom[2], Hanbyul Lee[2], Kyeongnang Moon[2], Yelim Park[2], Jonghwa Won[2], Se-Woong Oh[1], Yeul Hong Kim[1]

[1]Yuhan Co, Ltd., Seoul, Korea, Republic of,[2]ABL Bio Inc, Gyeonggi-do, Korea, Republic of

The epidermal growth factor receptor (EGFR) regulates cellular proliferation, differentiation, and survival, making it a critical target in cancer therapy. While EGFR-targeted therapies show efficacy in EGFR-overexpressing cancers, their use is limited by the frequent onset of drug resistance, necessitating novel approaches. Combining EGFR inhibitors with immunotherapy, such as targeting the costimulatory receptor 4-1BB (CD137) on activated T and NK cells, holds potential to improve anti-tumor responses. Recent advances in bispecific antibodies (bsAbs) allow for dual targeting strategies. Specifically, bsAbs containing T cell engagers improve immune responses by guiding T cells directly to tumor cells, thus enhancing immune targeting. Additionally, bsAbs that engage both 4-1BB and tumor-associated antigens (TAAs) amplify T cell activation within the tumor microenvironment, increasing the precision and efficacy of immune responses.A novel therapeutic agent, YH32364, was designed to address EGFR-related drug resistance through a dual mechanism: tumor-directed 4-1BB agonism combined with EGFR signal blockade. This approach aims to enhance tumor-localized 4-1BB activation while reducing the risk of excessive 4-1BB stimulation. Its binding affinity and activity were determined using surface plasmon resonance (SPR), a cell-based 4-1BB bioassay and immune mediated tumor killing assays using human peripheral blood mononuclear cells (hPBMCs). *In vivo* anti-tumor efficacy and antitumor immune response of YH32364 were assessed in mice models. 4-Week repeated dose monkey GLP toxicity study was also conducted. SPR analysis revealed high-affinity binding of YH32364 to both human EGFR and 4-1BB. YH32364 demonstrated strong 4-1BB activation and anti-tumor effects in EGFR-expressing cancer cell lines, as shown by IFN-γ secretion and subsequent tumor cell lysis. *In vivo* studies confirmed dose-dependent tumor regression by YH32364 and the induction of adaptive immunity in h4-1BB KI mouse models. Additionally, YH32364 recruited immune cells into EGFR-positive tumors, leading to modulation of the tumor microenvironment. As anticipated, no signs of hepatotoxicity were observed in a 4-week toxicity study in monkeys with no severe adverse findings on mortality, body weight, hematology, clinical chemistry, and other parameters. etc.Collectively, non-clinical studies demonstrate that YH32364 exhibits EGFR-dependent antitumor activity and induces long-term immunologic memory without hepatotoxicity. These findings suggest that YH32364 offers distinct advantages over conventional EGFR-targeted therapies by leveraging 4-1BB-mediated immunotherapy.

#6085 Engineered anti-CD40 agonist antibody as a novel cancer neoantigen vaccine carrier.

Dahee Jung[1], Xiaoying Cai[1], Wonkyung Oh[2], Seung-Oe Lim[2], Steve Lee[1]

[1]Department of Pharmaceutical Sciences, University of Illinois at Chicago, Chicago, IL,[2]Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN

Somatic mutations in cancer cells lead to the expression of neoantigens that can be targeted by personalized vaccines utilizing patient's own host immune system to fight against cancer. However, current personalized cancer vaccines have shown poor clinical outcomes due to the lack of effective delivery methods. To address the issues, our study aims to develop a novel vaccine platform that targets dendritic cells (DCs) and delivers neoantigen peptides more effectively by an anti-CD40 agonistic antibody expressing monovalent streptavidins (αCD40-mSAs). The agonistic anti-CD40 antibody will target and activate DCs, and the mSA compartment will allow the easy and fast loading of two biotinylated payloads (e.g. peptides and RNAs) on each vaccine carrier. CHO cell-based recombinant protein production method was used to biosynthesize αCD40 and αCD40-mSAs. Cloning vectors were reconstructed using mouse IgG2a, incorporating peptide sequences from the anti-mouse CD40 agonist antibody (clone FGK45) and monovalent streptavidin. For the biophysical properties of produced αCD40-mSAs, Surface Plasmon Resonance (SPR) for mouse CD40 and fluorescence polarization for biotin binding of mSAs have been used. Biotin- ovalbumin (OVA) peptide loaded αCD40-mSAs were subcutaneously injected into the footpad of the mice, and the collected lymph nodes were imaged with confocal microscopy and checked subtype of immune cells using flow cytometry. Antigen-specific T-cell responses were evaluated seven days post-injection by collecting lymph nodes and spleen. We successfully produced αCD40-mSAs, confirmed by increased molecular weight on the light chain expressing monovalent streptavidin. Binding assays demonstrated high-affinity interactions with mouse CD40 and effective biotin binding by the mSA moiety. In vivo targeting studies using fluorescently labeled OVA peptides revealed that αCD40-mSAs significantly enhanced peptide uptake in lymph nodes, particularly by cDC1 cells and macrophages. There was a notable increase in antigen-specific T cells, with an increase of OVA-specific $CD8^+$ T cells and increased production of cytokines IL-2 and IFN-γ upon restimulation when OVA peptides were loaded onto αCD40-mSAs. In conclusion, αCD40-mSAs represent an effective delivery platform for personalized cancer vaccines by targeting DCs and improving neoantigen peptide uptake, offering a promising strategy to boost vaccine efficacy in cancer immunotherapy.

#6086 Preclinical safety evaluation of VP1186, a CD47xEpCAM bispecific antibody.

Xiaocheng Chen[1], Xinhua Wang[1], Oi Kwan Wong[1], Leonard Post[1], Laurie Tatalick[2]

[1]Virtuoso Therapeutics, San Mateo, CA,[2]Laurie Tatalick Consulting, PLLC, Redmond, WA

The CD47/SIRPα axis is a critical checkpoint in the innate immune system, and CD47 is recognized as a prominent target for cancer treatment. While the clinical potential of CD47 blockade is evident, significant safety concerns have emerged. One major challenge is that CD47 is also expressed on healthy cells such as red blood cells (RBCs) and platelets. As a result, the first generation of therapies often caused serious side effects, including anemia and thrombocytopenia, which limited their therapeutic utility. EpCAM is a well-established tumor marker that is highly expressed in many epithelial cancers, such as colorectal, gastric, ovarian and lung cancers. We previously introduced VP1186 (also known as VBI-003), a novel CD47xEpCAM bispecific antibody that cross-reacts with both human and cynomolgus monkey antigens with comparable affinities. VP1186 selectively targets cancer cells with high EpCAM expression while sparing healthy cells. This tumor selectivity allows VP1186 to retain its Fc region with full effector function, acting as a potent "eat me" signal to the immune system. Consequently, VP1186 effectively activates macrophages and natural killer (NK) cells within the tumor microenvironment, enhancing the immune system's ability to eliminate cancer cells (*Journal for ImmunoTherapy of Cancer* 2021;9:doi: 10.1136/jitc-2021-SITC2021.274*),* (*Cancer Res* 2023;83(7_Supplement):1870). To further evaluate the safety profile of VP1186, we conducted a GLP-compliant repeat-dose toxicity study in cynomolgus monkeys. The bispecific antibody was administered intravenously at three dose levels as a 30-minute infusion once weekly for five consecutive weeks (Days 1, 8, 15, 22, and 29). The study also assessed the reversibility, progression, or delayed onset of any observed effects following 1-week and 4-week post-dosing periods. All animals were older than 3 years, with body weights ranging from 2.3 to 3.6 kg at the start of dosing. The results showed no clinically significant changes in clinical pathology endpoints, including hematology, clinical chemistry and coagulation, in VP1186-treated animals at doses up to 60 mg/kg/dose. Notably, RBC and platelet counts remained within the normal range across all dose levels, and hemoglobin concentrations stayed above the transfusion threshold throughout the study. No significant changes were observed in pancreatic enzymes, including lipase and amylase. Minimal effects on T cells were observed based on immunophenotyping (IPT) results. Transient but fully reversible changes in B cell and NK cell counts were noted. Pharmacokinetic (PK) analysis confirmed good drug exposure. These findings, combined with the potent anti-tumor activity of VP1186, support its further clinical evaluation as a potential treatment for CD47- and EpCAM-expressing cancers, particularly small cell lung cancer, gastric cancer, ovarian cancer and colorectal cancer.

#6087 A novel Ly6G6D/4-1BB bispecific antibody targeting DNA mismatch repair proficient colorectal cancers shows potent antitumor activities.

Zhiheng Ren, Xiang Li, Shushan Lin, Kuo Zhang, Zhaofeng Li, Xiao Lin, Kaifeng Li, Ju Peng, Mi Zheng, Wen Yang, LiJia Li, Xiaoping Li, **Stewart Leung**

HEC Pharma Co., Ltd, DongGuan, China

HEC921 is a novel bispecific agonistic antibody targeting Lymphocyte Antigen 6 Family Member G6D (Ly6G6D) and the immune agonistic receptor 4-1BB for the treatment of colorectal cancers. Ly6G6D is a tumor associated antigen highly expressed in DNA mismatch repair proficient (pMMR) colorectal cancers frequently resistant to immune check-point therapies. Activating monoclonal antibodies against 4-1BB have shown clinical efficacy but was limited by systemic toxicity. The design of HEC921 enables Ly6G6D-dependent agonism of 4-1BB in the presence of Ly6G6D positive tumor cells, enabling tumor specific T cell activation while minimizing systemic immune toxicity. A high affinity antibody against Ly6G6D and a nanobody against 4-1BB were identified for the construction of HEC921 containing an ADCC-silenced Fc. HEC921 shows potent co-binding to both Ly6G6D and 4-1BB targets and is cross reactive to the cynomolgus orthologues. In an assay using 4-1BB high expression 293 cells with NFκB-luciferase reporter, HEC921 mediated 4-1BB activation in the presence of Ly6G6D positive cells but not Ly6G6D negative cells. HEC921 promoted the production of cytokines IFN-γ, IL-2, and IL-4 in co-cultures of human T cells and Ly6G6D positive tumor cell lines. HEC921 demonstrated effective tumor growth inhibition in xenograft models of Ly6G6D positive tumor lines. Initial drug feasibility and toxicity studies results of HEC921 were favorable. Overall result demonstrated that HEC921 is a promising candidate for Ly6G6D positive cancer immunotherapy.

#6088 The bispecific antibody KA-5377, targeting CD19 and 4-1BB, specifically enhances T cell activity against lymphoma.

Guojin Wu, Jiabei Liang, Hao Peng, Feng Hao, Jinying Ning

Kyinno Biotechnology Co., Ltd, Beijing, China

KA-5377 is a novel bispecific antibody (BsAb) developed to target CD19 and human 4-1BB, addressing the challenge of dose-limiting toxicity associated with traditional 4-1BB agonist cancer immunotherapy. CD19, which is highly expressed in lymphoma, serves as an ideal target for this therapy. By restricting 4-1BB activation to the tumor microenvironment, KA-5377 aims to mitigate systemic toxicity. The 4-1BB nanobody used in KA-5377 was developed through our nanobody antibody discovery platform. KA-5377 is designed in an IgG1 format with a LALA mutation in the Fc region to eliminate ADCC (antibody-dependent cellular cytotoxicity) and ADCP (antibody-dependent cellular phagocytosis) activity, ensuring that the antibody's activity is focused on stimulating 4-1BB in T cells. The bispecific antibody contains two 4-1BB nanobodies at the C-terminal that engage 4-1BB signaling upon binding, and two Fabs at the N-terminal that bind to CD19 expressed on tumor cells. KA-5377 demonstrates high avidity for lymphoma tumor cells expressing CD19, while binding to 4-1BB expressed on 293T cells with significantly lower avidity. Notably, 4-1BB signaling is strongly activated only when the antibody binds to CD19 on tumor cells, with much weaker activation observed when CD19 binding is absent. The difference in EC50 (effective concentration for 50% activation) between the CD19-bound and non-bound states is approximately 100-fold, compared to only a 2-fold difference observed with the benchmark bispecific antibody Englumafusp Alfa.When combined with a low concentration of CD3 antibody to stimulate T cell activation, KA-5377 significantly enhances T cell activation and proliferation in co-culture assays using primary T cells isolated from human PBMCs. In PBMC-humanized immunodeficient mouse tumor models, KA-5377 improves the cytotoxic activity of bispecific antibodies targeting CD20 and CD3 against WSU-DLCL2 tumors, which highly express CD19. Additionally, KA-5377 exhibits a normal half-life in the blood of Balb/c mice and demonstrates good developability. Toxicity studies are currently ongoing. In conclusion, KA-5377, developed using our nanobody antibody discovery platform, represents a promising preclinical candidate for the treatment of Relapsed/Refractory B-cell lymphoma.

#6089 The bispecific antibody KA-3458, targeting CD38 and CD28, specifically enhances T cell activation within the tumor microenvironment.

Guojin Wu, Jiabei Liang, Zhengcheng Guo, Hao Peng, Feng Hao, Jinying Ning

Kyinno Biotechnology Co., Ltd, Beijing, China

KA-3458 is a bispecific antibody (BsAb) designed to target CD38 and human CD28, aiming to enhance T cell activation within the tumor microenvironment, particularly in blood cancers like multiple myeloma and lymphoma, where CD38 is highly expressed. While bispecific antibodies targeting tumor antigens and activating T cell receptor signaling have shown clinical promise, the addition of CD28 co-stimulatory signals may further boost T cell responses against tumors. KA-3458, engineered in an IgG4 format with an identical light chain, was created using our common light chain bispecific antibody discovery platform and knob-into-hole technology. The antibody's two Fabs show high affinity for CD38 and relatively lower affinity for CD28. In in vitro combination assays, KA-3458 significantly enhances T cell activation and cytotoxic activity of CD3 T cell engagers targeting CD20 or GPRC5D, which are critical for the treatment of lymphoma or multiple myeloma, respectively. Cytokine release assays performed with human PBMCs co-cultured with lymphoma cells revealed that KA-3458 enhances the release of INF-γ but not IL-6, in the presence of a CD20 T cell engager, suggesting a favorable safety profile. In PBMC-humanized immunodeficient mouse models, KA-3458 further improves the cytotoxicity of CD3 T cell engagers against CD20 or GPRC5D, targeting tumors in lymphoma or multiple myeloma models, respectively, which strongly express CD38. Additionally, KA-3458 has a normal half-life in Balb/c mice and demonstrates excellent developability. Toxicity studies are currently underway. In conclusion, KA-3458, developed using our common light chain bispecific antibody discovery platform, shows great potential as a preclinical candidate for the treatment of relapsed/refractory B-cell lymphoma and multiple myeloma.

IMMUNOLOGY: CAR Therapies: Emerging Approaches and Combinations
Poster Session

#6093 A novel dual chemokine receptor-expressing CD19 CAR-iPSC-derived NK cells (TB-219) to enhance cancer homing and therapeutic efficacy.

Jin-Jae Sung, Sung-Jin Park, Ji-Hee Kwon, Seung-Ki Baek, **Youngwook Ki**, Do-Won Hwang, Shin-Il Kim, Sung-Chang Lee

Therabest CO., Ltd, Seoul, Korea, Republic of

Introduction: Induced pluripotent stem cell-derived natural killer (iPSC-NK) cell therapies, characterized by exceptional homogeneity and scalable production with a single gene engineering step, are emerging as promising alternatives for the treatment of hematologic malignancies. While NK cells offer a superior safety profile compared to T cells, their limited ability to migrate to the bone marrow (BM) and lymph nodes (LN) significantly hampers therapeutic efficacy and contributes to cancer relapse. To overcome these challenges, we developed an off-the-shelf novel CD19-CAR-dEiNK (TB-219) cell therapy engineered to express dual chemokine receptors to enhance BM and LN homing, incorporating improved persistence and immune evasion capabilities.
Methods: TB-219 was modified to express a CD19 CAR for targeted cytotoxicity, CXCR4 and CCR7 for improved homing to bone marrow and lymphoid tissues, and IL-15RF for autonomous survival support. β2M was knocked out to evade host immune recognition, to avoid host T cell attack. Genetic modifications were achieved via homology-directed repair, followed by iPSC differentiation and bioreactor-based expansion. *In vitro* assays measured cytotoxicity against CD19-positive tumor cells, homing, and persistence, while *in vivo* studies in mouse models assessed tumor control and survival outcomes.
Results: TB-219 exhibited an 80% increase in cytotoxicity against CD19-positive cancer cells compared to unmodified NK cells at an effector-to-target (E:T) ratio of 2:1. Migration efficiency was enhanced by 200% and 400% through the expression of CXCR4 and CCR7 in transwell cell culture system, respectively. Additionally, β2M knockout effectively minimized recognition and elimination by host T cells, thereby improving persistence. Following a single i.v. administration of TB-219, no bioluminescent signals indicative of luciferase-expressing B cell leukemia cancer were detected through day 31, demonstrating complete control of cancer without evidence of relapse in the murine model. By day 39 post-administration, all subjects (Single and multiple injection) in the TB-219 treatment group demonstrated 100% survival, whereas complete mortality was observed in the tumor control group.
Conclusion: The advanced engineering of TB-219 incorporates significant improvements in persistence, cancer homing, and immune evasion, positioning it as a highly promising therapeutic candidate for CD19-positive hematological malignancies. The observed improvements in cytotoxicity, targeting, and survival in preclinical models suggest TB-219 may provide a durable and efficacious treatment option. These findings warrant further clinical investigation, positioning TB-219 as a potent and accessible NK cell therapy for patients with limited therapeutic options.

#6094 Enhanced GPC3-chimeric antigen receptor T cells with secreted bi-specific CD39-PD-L1 antibody for improved liver cancer therapy.

Zhongliang Li*[1], Bing Liu*[1], Huajing Wang*[2], Ke He*[3], Xiaopei Li[2], Dingjie Liu[4], Ligong Lu[1], Xu He#[1], Xiaowen He#[2], **Ze Yang#**[5], Meixiao Zhan#[1]

[1]Interventional Medical Treatment Department, Guangzhou First Pepople's Hospital，the Second Affiliated Hospital, School of Medicine, South China University of Technology, Guangzhou, China,[2]R&D Department, OriCell Therapeutics Co. Ltd., Shanghai, China,[3]Minimally Invasive Tumor Therapies Center, The Affiliated Guangdong Second Provincial General Hospital of Jinan University, Guangzhou, China,[4]Guangdong Provincial Key Laboratory of Tumor Interventional Diagnosis and Treatment, Zhuhai People's Hospital (Zhuhai Clinical Medical College of Jinan University), Zhuhai, China,[5]The Affiliated Dongguan Songshan Lake Central Hospital, Guangdong Medical University, Dongguan, China

Glypican-3 (GPC3)-targeted chimeric antigen receptor (CAR) T cell therapy shows promise for treating liver cancer. However, the immunosuppressive tumor microenvironment (TME), characterized by elevated levels of adenosine and PD-L1 expression, significantly hinders CAR T cell efficacy. We previously demonstrated that a bi-specific antibody, OriA631-B, which targets CD39—an enzyme crucial for converting extracellular ATP into adenosine—and PD-L1, yields significant antitumor effects. Our findings indicate that GPC3 CAR T cells upregulate CD39 upon activation by liver cancer cells, which also express PD-L1.To enhance antitumor efficacy, we engineered GPC3 CAR T cells to secrete a fragment of OriA631-B that targets both CD39 and PD-L1 (termed GPC3 CAR-T-CD39-VHH-PD-L1-ScFv). In a mouse model of liver cancer, GPC3 CAR-T-CD39-VHH-PD-L1-ScFv exhibited superior antitumor activity compared to GPC3 CAR T cells alone. Specifically, while GPC3 CAR T cells inhibited tumor growth, the GPC3 CAR-T-CD39-VHH-PD-L1-ScFv nearly eliminated tumors by day 20, whereas tumors in the GPC3 CAR T group measured around 200 mm^3.This enhanced efficacy is attributed to the localized delivery of the bi-specific antibody fragment, which effectively inhibits adenosine signaling and PD-L1-mediated immune checkpoint blockade.
Our findings suggest that GPC3 CAR-T-CD39-VHH-PD-L1-ScFv represents a novel therapeutic strategy for liver cancer, offering improved efficacy and potentially reduced side effects associated with systemic antibody administration.

#6095 T-Pure^{TM}: A bispecific antibody cocktail for T cell isolation from human blood, enabling simple and affordable point-of-care CAR-T manufacturing.

Ying Xiong[1], Yanping Xie[1], Ann Wang[1], Victor Bass[1], Matt Addington-hall[1], Oxana Slessareva[1], Yang Liu[1], Tony Luo[1], Ibeawuchi Oparaocha[1], Kathy Schonely[2], Kara Anlauf[1], Megan Forrest[1], Jacqueline Schaeffer[2], Shashi Koduru[2], Andrew Worden[2], Mike Kadan[2], Boro Dropulic[1], Zhongyu Zhu[1]

[1]Caring Cross, Inc., Gaithersburg, MD,[2]Vector BioMed, Gaithersburg, MD

Adoptive immunotherapy with engineered T cells, especially chimeric antigen receptor (CAR) T cells, has revolutionized the treatment of leukemia, lymphoma, and multiple myeloma. However, the high production costs of CAR-T therapy severely limit patient access. To address this challenge, we developed T-pure^{TM}, a proprietary bispecific antibody cocktail for isolating T cells from human blood through negative selection. The Bi-specific antibody cocktail selectively links red blood cells (RBCs) to key marker antigens on non-target cells, such as B cell, NK cell, Monocytes, and granulocytes, allowing easy and cost-effective separation using Ficoll density gradient centrifugation. By isolating T cells without engaging their surface receptors, this approach avoids inadvertent activation of isolated T cells, while also preserving cell types like γδ T cells, which show promise for their anti-tumor potential. Additionally, the depletion process efficiently removes CD19-positive cancer cells and can be tailored to eliminate T cell subpopulations like T regulatory (T-reg) cells that could compromise CAR-T efficacy in clinical application. This versatile antibody cocktail can also be adapted to isolate other cell types, such as NK cells, B cells, and monocytes for various biomedical applications. Designed for cost-effective clinical manufacturing, our GMP grade T-pure reagents are commercially available to academic and industry partners for preclinical and clinical applications. This work highlights the ongoing innovation in CAR-T manufacturing and the potential for optimizing cell selection protocols to enhance efficacy and lower the cost of cell therapies.

#6096 Acquired CAR-T therapy resistance in a pre-clinical model of pediatric rhabdomyosarcoma minimal residual disease.

A. M. Lulu, D. A. Cobb, N. Park, L. Liu, D. W. Lee;
University of Virginia, Charlottesville, VA

Introduction: Current treatments for relapsed/refractory or metastatic rhabdomyosarcoma (RMS) are ineffective. Integrin αvβ3 plays a role in progression, metastasis and neovascularization in several cancers. HER2 is expressed on a majority of RMS and contributes to progression and metastasis in other cancers. Our lab previously demonstrated the efficacy of HER2 and αvβ3 CAR Ts in controlling or eliminating pediatric brain tumors. CAR T therapies for solid tumors face numerous obstacles with limited clinical success. While bispecific CAR Ts can overcome heterogeneous antigen expression and antigen modulation, CAR T suppression and tumor evasion is multifactorial. Success requires understanding the mechanisms driving clinical failure. Here we developed a minimal residual disease (MRD) model of CAR T resistant RMS to evaluate the mechanisms driving acquired resistance after profound initial tumor control.
Methods: Control of intramuscular RMS tumors (RH30) by αvβ3 or HER2 CAR Ts was assessed in NSG mice, with CD19 CAR T controls. After 7 weeks, CAR T infiltration, antigen expression and residual disease characteristics were determined by flow cytometry and immunohistochemistry (IHC). In a pilot experiment, mice were treated with a second CAR T dose or rechallenged with a second tumor in the opposite leg. Tumor burden was evaluated by bioluminescence imaging and caliper measurements; MRD and CAR T infiltration were determined by IHC.
Results: Both αvβ3 and HER2 CAR Ts nearly eliminate RMS tumors. However, despite robust initial control and CAR T persistence in both treatment groups, MRD remains. αvβ3 expression minimally decreases following αvβ3 CAR T treatment, although expression is 10-fold higher than the parental cell line. Both αvβ3 and HER2 CAR Ts control tumors in a delayed treatment model, despite HER2 down-regulation with tumor progression. By the time of MRD, 60% of tumor-infiltrating αvβ3 CAR Ts have upregulated multiple exhaustion markers (TIM3, LAG3, PD-1 and TIGIT), in contrast to only 10% of circulating αvβ3 CAR Ts and 10% of tumor-infiltrating CD19 CAR Ts. After αvβ3 or HER2 CAR T treatment, surviving tumor cells upregulate cognate exhaustion ligands (HLA-DR, Galectin3, Galectin9 and PD-L1), consistent with the development of a suppressive tumor environment. Despite this, αvβ3 and HER2 CAR Ts, likely from circulation, control a secondary tumor without delay. Neither the original CAR Ts nor a second dose of CAR Ts eliminate MRD at the primary tumor site.
Conclusions: We developed a pre-clinical model of CAR T resistant MRD in which to evaluate specific mechanisms of acquired tumor resistance and CAR T dysfunction in RMS. Different mechanisms, distinct from antigen modulation, arise based on the CAR treatment. Continued investigations into these mechanisms will ultimately enable superior CAR T cell design that is resistant to tumor-mediated inhibition.

#6097 FcRγ and TLR4 based CARs in DCs modulate antitumor T cell priming.

Shelby Namen, Alex Kim, Gaurav Pandey, Carl DeSelm

Washington University School of Medicine in St. Louis, St. Louis, MO

Background: Immunotherapies have advanced the field of cancer therapy for hematologic malignancies but have not had similar success in solid tumors. Due to their abundance in solid tumors and ability to phagocytose, we developed a chimeric antigen receptor (CAR) macrophage (M) that reduced tumor burden in syngeneic mice. However, in syngeneic mice lacking conventional type 1 dendritic cells (cDC1) the effect was lost suggesting that a mechanism of CAR-M function is through antigen transfer to cDC1s, which then prime an antitumor T cell response.

Purpose: To directly induce antitumor T cell responses, we tested different antitumor CAR constructs in cDC1s (DCs). Toll-like receptors (TLRs) and Fc receptors (FcRs) are major types of receptors in phagocytes, with different downstream signaling; we focused on understanding the impact of TLR4 and FcRγ signaling CARs on DC function. We compared the impact of each CAR on the DC's ability to phagocytose tumor cells, cross-present or degrade the captured antigen, induce costimulatory molecule expression, and cross-prime tumor-specific T cells.

Methods: Mouse bone marrow derived DCs were electroporated with mRNA encoding for anti-B7H3 (B7) TLR4, FcRγ, or truncated (t) CAR. The CAR DCs were cultured with AF488-ovalbumen (ova) loaded MCA-induced sarcoma tumor that expressed human B7 (B7+) or was B7 negative (B7-) to assess uptake of tumor antigen. To determine the impacts on cross-priming, the CAR DCs were cultured with B7+ and B7- ova loaded MHCI negative tumor cells and naïve CD8 OTI T cells.

Results: The FcRγ CAR improved target-specific tumor antigen uptake by >10x compared to CAR(t), while the TLR4 CAR improved tumor uptake by 1.6x. Although the TLR4 CAR DCs did not take up as much antigen, it cross presented the antigen more effectively, as it had 4x more surface expression of MHCI:SIINFEKL compared to the FcRγ CAR. The TLR4 CAR induced slower degradation of antigen compared to the FcRγ CAR. The TLR4 CAR also induced higher levels of CD70, CCR7, and CD86 expression compared to the FcRγ CAR. Both the TLR4 and the FcRγ CAR DCs induced greater proliferation of OTI T cells relative to CAR(t) DCs. The proliferated OTI T cells from the TLR4 condition had a 2.8x increase in tumor killing of ova-loaded, B7- tumor cells, indicating that the quality of the T cell response may differ if the T cells are cross-primed by a DC experiencing TLR4 versus FcRγ signaling at the time of tumor antigen capture.

Conclusion: This data suggests that engineered DCs with either FcRγ or TLR4 CAR led to increased tumor uptake and cross presentation compared to a truncated CAR; however, the two CARs lead to different downstream signaling pathways that impact the amount of antigen cross-presented, activation marker expression, and the quality of the antitumor T cell response. These findings suggest *de novo* antitumor T cell responses can be enhanced and shaped by DCs engineered with CARs having different signaling domains.

#6099 STEAP1 targeting CAR-T cells equipped with collagen-binding IL-12 for advanced prostate cancer.

Koichi Sasaki[1], Vipul Bhatia[2], John K. Lee[2], Jun Ishihara[1]

[1]Department of Bioengineering, Imperial College London, London, United Kingdom,[2]Department of Medicine, UCLA, Los Angeles, CA

Metastatic castration-resistant prostate cancer (mCRPC) is a largely incurable, immunologically cold solid tumor. Six-transmembrane epithelial antigen of the prostate 1 (STEAP1) is a promising target specifically expressed in >85% of mCRPC patients. Chimeric antigen receptor (CAR)-T cells transformed the clinical landscape of blood cancers. However, CAR-T therapy is still not very effective against solid cancers including prostate cancer, in part due to immunosuppressive tumor microenvironment (TME) and antigen heterogeneity. Interleukin-12 (IL-12) is a promising cytokine to address these issues through activation and recruitment of immune cells to the tumor. IL-12 can induce epitope spreading to fight antigen heterogeneity. Previously, armored CAR-T cells that express wild-type (wt) IL-12 were developed, but there is still room for improvement in tuning potency and safety. Adoptive transfer of autologous IL-12-expressing T cells showed objective responses in melanoma patients; however, the trial was limited by toxicity. The result suggests that IL-12 secreted from CAR-T cells are locally produced but still toxic due to the cytokine's diffusion into systemic circulation. Here, we report STEAP1-targeting CAR-T cells that conditionally express collagen-binding domain fused IL-12 (CBD-IL-12) upon recognizing STEAP1 protein. We show that CBD fusion to IL-12 significantly increased the concentration of IL-12 in mouse prostate tumors and suppressed its systemic spread following secretion from the CAR-T cells. This resulted in equivalently high intra-tumoral levels of interferon-gamma (IFN-γ), C-X-C motif chemokine ligand 9 (CXCL9) and granulocyte-macrophage colony-stimulating factor (GM-CSF) without increase of serum alanine aminotransferase (ALT) and T cell infiltration into non-target organs compared to wt IL-12. Flow cytometric analysis showed increase of T cells, NK cells and cross-presenting dendritic cells and decrease of monocytic myeloid-derived suppressor cells (M-MDSCs) in the tumor following CBD-IL-12 expressing STEAP1 CAR-T cell therapy. CBD-IL-12 expressing CAR-T cells combined with anti-PD-1 and anti-CTLA-4 antibodies eradicated an established mouse prostate cancer model without preconditioning and generated anti-tumor immune memory. CBD fusion to potent yet toxic immunotherapies may make a breakthrough in the development of CAR-T therapy that can safely overcome solid tumors.

#6100 Point-of-care manufacturing of FOLR1-directed CAR T cells for the treatment of ovarian cancer.

Christoph Herbel[1], Manuel Martinez-Osuna[1], Larissa Steiner[1], Maria Bethke[1], Leon Osinski[1], Katrin Krischer[1], Janina Brauner[1], Cathrin Bleilevens[1], Jens Kopatz[1], Katja Petry[2], Dominik Eckardt[1]

[1]Miltenyi Biotec B.V. & Co. KG, Bergisch Gladbach, Germany,[2]Miltenyi Biomedicine GmbH, Bergisch Gladbach, Germany

Treatment options for ovarian cancer patients are limited, highlighting a significant unmet need for targeted, durable, and effective therapies. In recent years, adoptive cell transfer therapies have made tremendous progress in the past years. Genetically engineered T cells expressing a chimeric antigen receptor (CAR) are promising drugs that can be directed towards a defined target and have shown efficient, as well as persisting, anti-tumor responses in different indications. Recently, we have reported the preclinical evaluation of a novel FOLR1-targeting CAR directed against ovarian cancer and potentially other FOLR1-expressing tumors. Despite advancements in T cell therapy, producing a functional and robust cell product in the necessary quantities remains challenging. Currently available processes for the preparation of CAR T are laborious and include numerous manual handling steps, which increase the risk of errors and may impose safety risks. This underscores the need for reliable and automated manufacturing processes to ensure high-quality cellular products for immunotherapy. This study aimed to develop an automated, closed, and good manufacturing practice (GMP)-compliant process for FOLR1-specific CAR T cells. Using the CliniMACS Prodigy® Platform, we established a streamlined process in a closed system that meets stringent regulatory requirements while reducing hands-on time. We optimized culture conditions to reduce the process time to 7 days. Starting from a cryopreserved leukapheresis we achieved a clinically relevant yield of FOLR1-specific CAR-engineered T cells. The cellular product mainly consisted of highly viable CAR-expressing T cells with an early memory phenotype. FOLR1-specific CAR T cells manufactured with the optimized process showed specific killing of ovarian cancer cells *in vitro* and in an ovarian cancer xenograft model *in vivo*. Moreover, the automated CliniMACS Prodigy Platform enables point-of-care (POC) manufacturing. In conclusion, we developed a robust, rapid, and efficient process for manufacturing anti-FOLR1 CAR T cells. This platform is well-suited for automated and decentralized POC manufacturing and can be adapted for the production of other CAR T cell therapies.

#6102 BAFF ligand CAR-T cells for hairy cell leukemia variant treatment.

Claire Elizabeth Fritz[1], Derek P. Wong[1], Akshaya Radhakrishnan[2], Philip Rock[3], Richard Burack[3], Reshmi Parameswaran[2]

[1]Pathology, Case Western Reserve University School of Medicine, Cleveland, OH,[2]Medicine, Case Western Reserve University School of Medicine, Cleveland, OH,[3]Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, Rochester, NY

Hairy cell leukemia variant (HCL-v) is a rare B-cell malignancy that is biologically distinct from the classical form (HCL-c) and presents unique treatment challenges. The characteristic genetic or molecular markers of classical HCL include CD11c, CD25, CD103, CD123, the $BRAF^{V600E}$ point mutation, and tartrate resistant acid phosphatase (TRAP) expression. However, HCLv cells have a low immunologic score and don't carry the $BRAF^{V600E}$ mutation or express TRAP. Treatment with the purine nucleoside analogs (PNAs) is effective in a majority of HCLc patients, who can look forward to a normal lifespan. However, HCLv patients are resistant to this treatment, which likely contributes to the significantly shorter prognosis of 6-9 years, in addition to the relative lack of druggable targets. To address poor clinical outcomes for HCL-v patients, we aimed to test a novel cellular therapy for HCL-v. We recently developed a third generation BAFF-ligand chimeric antigen receptor (CAR-T) cell product. B-cell activating factor (BAFF) is a critical factor that promotes B-cell maturation and survival. BAFF binds to three receptors, including BAFF-Receptor (BAFF-R), transmembrane activator and CAML interactor (TACI), and B-cell maturation antigen (BCMA). Because of the pro-survival signaling that BAFF enhances upon binding to its receptors, B-cell cancers tend to exploit this signaling, and serum BAFF levels are often elevated. In a panel of HCLv cell lines and patient-derived splenocytes, we found that HCLv cells have high expression levels of all three receptors. In co-culture assays with fluorescently labeled HCLv patient samples and cell lines, CAR-T cells express significantly elevated markers of degranulation (CD107a) and activation (CD69) and secrete elevated levels of pro-inflammatory cytokines and lytic enzymes relative to control T-cells. BAFF CAR-T cells also display significantly increased cancer cell killing relative to control T-cells. This enhanced cancer cell killing was also observed in an autologous model, where CAR-T cells were generated from a de-identified HCLv patient and co-cultured with the patients' own cancer cells. Similar findings were seen in multiple novel in vivo HCL xenograft models, in which BAFF CAR-T cell treatment (intratumor or intravenous) resulted in decreased tumor burden without significant toxicities.While we have seen success in our in vivo models, we also aim to generate a novel patient-derived xenograft model of HCLv to more accurately model the disease, both for more CAR-T cell translational studies and for more basic investigations into HCLv mechanisms. An additional future direction is to explore whether our BAFF CAR-T cells, which bind three receptors can mitigate antigen escape, the process by which tumor cells downregulate a target to avoid killing. To do so, we will perform single and double receptor knockouts of BAFF-R, TACI, and BCMA and assess if cell killing is intact with in vitro co-culture assays.

#6103 BCMA CAR engineering arms T cells with superior functions post *ex vivo* stimulation by MUC1 peptide-loaded autologous PBMCs.

Kun Yun[1], R. Leo Sakemura[1], Ismail Can[1], Omar Gutierrez Ruiz[1], Ekene J. Ogbodo[1], Truc N. Huynh[1], Claudia Manriquez Roman[1], Olivia Sirpilla[1], Carli M. Stewart[1], Jennifer M. Feigin[1], Grace E. DeFranco[1], Sophia Y. Goldberg[1], Dominic A. Skeele[1], Mariam Stein[1], Dara Missan[2], Latha Pathangey[2], Long Mai[1], Hong Xia[1], Brooke Kimball[1], Lionel A. Kankeu Fonkoua[1], Mehrdad Hefazi[1], Michael W. Ruff[1], Elizabeth L. Siegler[1], Leif Bergsagel[2], Michael Gustafson[2], Sandra Gendler[2], Saad S. Kenderian[1]

[1]Mayo Clinic, Rochester, MN,[2]Mayo Clinic, Phoenix, AZ

Chimeric antigen receptor T (CART) cell therapy has shown clinical successes in B cell hematological malignancies. Two B cell maturation antigen (BCMA)-directed CART cell therapies treating multiple myeloma (MM) were approved by FDA but most patients relapse within 1-2 years. *Ex vivo*-expanded antigen specific T cells as cancer treatment rely on TCR-mediated killing of tumors. We hypothesized that arming *ex vivo* enriched tumor-specific T cells with CARs targeting a different antigen improves T cell functions. First, we expanded mucin 1 (MUC1)-activated T cells from patients with MM through *ex vivo* stimulation by MUC1 peptides-loaded autologous PBMCs for 19 days. Then, we engineered MUC1-activated T cells with 4-1BB-co-stimulated BCMA CAR to generate MUC1 TCR^+BCMA CAR^+ T cells. Baseline T cells without MUC1 stimulation were engineered with BCMA CAR as control. Baseline T cells, MUC1 T cells, baseline BCMA CART cells, or MUC1 BCMA CART cells were cocultured with luc^+ RPMI-8226, a $MUC1^+BCMA^+$ MM cell line, for 3 days. MUC1 BCMA CART cells showed stronger tumor killing than BCMA CART or MUC1 T cells in all 3 patients ($p<0.0001$). MUC1 BCMA CART showed improved proliferation than BCMA CART or MUC1 T cells ($p=0.0765$, $p<0.0001$, respectively) after a 5-day stimulation by RPMI-8226. To exclude possibility of metabolic alterations by 19-day PBMC incubation leading to better T cell functions in MUC1 BCMA CART, baseline and MUC1-stimulated T cells from 3 healthy donors were processed for the same assays. Day-19 BCMA CART cells showed inferior tumor killing compared to baseline BCMA CART in all 3 donors ($p<0.0001$). Lower T cell proliferation was also observed in day-19 BCMA CART ($p=0.0838$). Our data indicate that improved T cell functions in MUC1 BCMA CART were unlikely due to metabolic changes and required stimulation by tumor antigens prior to CAR engineering likely through MUC1 TCR. Additionally, MUC1 T cells, BCMA CART, or MUC1 BCMA CART cells from tested patients were stimulated by RPMI-8226 for bulk RNA sequencing. Both BCMA CART and MUC1 T cells had distinct transcriptomic features from MUC1 BCMA CART cells. Differential gene expression analysis showed upregulation of T cell function-associated genes (*RYR2*, *ITGA1*, *GNLY*, *GZMA*, etc.) in MUC1 BCMA CART vs BCMA CART. Enrichment of T cell function-associated pathways was also observed in MUC1 BCMA CART. Analysis between MUC1 T cell and MUC1 BCMA CART showed upregulation of T cell function-associated genes (*CSF2*, *CRTAM*, *IFNG*, *GZMB*, etc.) and enrichment of immune response pathways in MUC1 BCMA CART. Our findings suggest that equipping *ex vivo*-enriched tumor-specific T cells with CARs targeting a different antigen improves T cell functions *in vitro*. Importantly, this provides a rationale for a therapeutic regimen where patients receive *ex vivo*-enriched tumor-induced T cells followed by CAR engineering. Persistence of the engineered T cells is being investigated

#6104 Comparative evaluation of CAR-expressing T-, NK-, NKT-cells and macrophages in an immunocompetent mouse glioma model.

Payal B. Watchmaker[1], Ryusuke Hatae[1], Akane Yamamichi[1], Keith Kyewalabye[1], Kaori Okada[1], Su Phyu[1], Yitzhar Goretsky[1], Jeffrey Haegelin[1], Psalm Pineo Cavanaugh[1], Marco Gallus[1], Lan Phung[1], Tiffany Chen[1], Hayou Long[1], Pavlina Chuntova[1], David H. Raulet[2], Masaki Terabe[3], Hideho Okada[1]

[1]Neurological Surgery, UCSF, San Francisco, CA,[2]Department of Molecular and Cell Biology, UC Berkeley, Berkeley, CA,[3]Neuro-Oncology Branch, National Cancer Institute, Bethesda, MD

While chimeric antigen receptor (CAR) T-cells are promising, there is a rapidly growing interest in developing other CAR-expressing immune cells. However, to date, no reported studies evaluated these cells side-by-side in immune-competent glioma models. We developed a novel transgenic mouse strain with all hematopoietic cells expressing EGFRvIII-targeting CAR, allowing for rigorous evaluations of CAR-NK, CAR-NKT, and CAR-macrophages compared to CAR-T-cells in a syngeneic mouse SB28-EGFRVIII glioma model. CAR-NK and CAR-NKT-cells demonstrated anti-tumor effects comparable to CAR-T-cells in vitro. A single intratumoral administration of CAR-T and CAR-NKT-cells in combination mediated superior therapeutic efficacy compared to CAR-T-cells or CAR-NKT-cells alone. A single intravenous infusion of CAR-NK-cells following lymphodepletion failed to mediate significant anti-glioma effects associated with inferior persistence to CAR-T cells. Additionally, intratumoral injection of CAR-NK-cells did not confer therapeutic benefit. Contrary to previous reports using human macrophages, CAR-macrophages did not demonstrate enhanced antigen-presentation activity against glioma cells compared to non-CAR macrophages. Intratumorally administered CAR-macrophages failed to demonstrate local persistence or anti-tumor effects in vivo. These data provide a valuable basis as to which CAR-expressing immune cells can mediate effective anti-glioma response in an immuno-competent glioma environment. Our data also suggest that a combination of CAR-T and CAR-NKT-cells may represent a promising therapeutic strategy.

#6105 iPSC-derived HER2 CAR-iNKT cells enhance the activity of immune cells against cancer cells.

Koichiro Shioya[1], Tomio Matsumura[1], Akane Urakami[1], Tomokuni Shigeura[1], Aki Naito[1], Yu-Ching Lin[1], Mio Shiraishi[1], Yuji Mishima[1], Haruhiko Koseki[2], Shinichiro Motohashi[3], Lilin Zhang[1]

[1]BrightPath Biotherapeutics Co., Ltd., Tokyo, Japan,[2]Center for Integrative Medical Sciences, Riken, Yokohama, Japan,[3]Department of Medical Immunology, Graduate School of Medicine, Chiba University, Chiba, Japan

Invariant natural killer T (iNKT) cells directly kill tumor cells through TCR and NKG2D, and also exert anti-tumor activity indirectly by promoting dendritic cell (DC) maturation, activating CD8+T cells and repolarizing macrophages. iNKT cells do not trigger graft versus host disease, making them an attractive cell type for an “off-the-shelf” cell therapy platform. We are developing iPSC-derived iNKT (ipsNKT) cell therapy to overcome manufacturing challenges due to the low abundance of iNKT cells in blood. It is not clear whether ipsNKT cells, especially when the cells are gene edited, still preserve multifunctional properties of native iNKT cells. In this study, we evaluated these intrinsic direct and indirect anti-tumor functions of iNKT cells using HER2-targeting CAR-ipsNKT cells. HER2-CAR-ipsNKT cells were differentiated from HER2 CAR-edited iPS cell line under a feeder cell-free condition and exerted potent cytotoxicity when cultured with HER2 positive HCC1954-CMVpp65 cells. HER2-CAR-ipsNKT cells released cytokines including IL-4, TNF-α and IFN-γ upon CAR-stimulation by HER2 antigen. After co-culturing monocyte derived immature DCs with HER2-CAR-ipsNKT cells, the expression of DC maturation markers like CD80, CD83, CD86 and HLA-DR were markedly upregulated, suggesting that HER2-CAR-ipsNKT cells promote DC maturation. When monocytes were cultured in conditioned medium from HER2-CAR-ipsNKT cells, polarization of M1 macrophages was observed by detecting expression of the M1 macrophages markers using flowcytometry. Intriguingly, conditioned medium of HER2-CAR-T cells did not show the same result. To evaluate whether HER2-CAR-ipsNKT cells can promote cytotoxic T lymphocyte (CTL) activity *in vivo*, HCC1954-CMVpp65 cells were inoculated subcutaneously into NOG-hIL15Tg mice. After the tumor volumes reached 100~200 mm^3, 1×10^5 PBMCs were injected intratumorally, followed by intravenous doses of HER2-CAR-ipsNKT cells. Initially, all animals showed comparable tumor growth, however, the tumor xenografts in the HER2-CAR-ipsNKT cell treatment group started to regress after 3 to 4 weeks of treatment, suggesting an indirect anti-tumor contribution of HER2-CAR-ipsNKT cell by activating-tumor specific CTLs. This speculation is supported by the observation that the CD8+T cells and CMVpp65 tetramer+ cells were increased in both spleen and tumor of the treatment group. This study demonstrated that HER2-CAR-ipsNKT cells promote DC maturation and M1 macrophage polarization, likely due to cytokine release from HER2-CAR-ipsNKT cells as a result of CAR-stimulation, and also activate tumor antigen-specific CD8+T cells, thereby enhancing anti-tumor effect *in vivo*. These results suggest that administration of iPSC-derived CAR-iNKT cells to a patient can improve tumor microenvironment and activate the host endogenous tumor-specific CTLs, potentially achieving a durable therapeutic response.

#6106 A regulated, cell-intrinsic cytokine engineering strategy to enhance the in vitro and in vivo persistence of iPSC-derived CAR-T cell therapies.

Siddarth Chandrasekaran[1], Justin Yoo[1], Michael H. Cadell[1], Laura Prochazka[2], Elisa Martinez[2], Jen F. Lai[1], Muluken S. Belew[2], Amanda AuYeung[2], Chris Saxby[1], Omar Subedar[2], Zahra Sahaf[2], Dylan Lee[2], Sommer Apelu[1], Libin Abraham[1], Chloe Hurling[2], Vaisakh Rajan[2], Shruti Sharma[2], Charlie Liu[2], Richard Carpenedo[3], Shri Joshi[2], Elizabeth Csaszar[2], Emily Titus[2], Chris Bond[1]

[1]Notch Therapeutics, Inc., Seattle, WA,[2]Notch Therapeutics, Inc., Toronto, ON, Canada,[3]Notch Therapeutics, Inc., Vancouver, BC, Canada

Large-scale clinical production of induced pluripotent stem cell (iPSC)-derived CAR-T cells represents a remarkable opportunity in allogeneic cell therapies. Despite the growing advantages offered by iPSCs such as multiplex gene editing and unlimited starting material, most iPSC derived CAR-T cells demonstrate limited clinical proliferation and persistence. This has been partially attributed to existing iPSC differentiation protocols that yield only cytotoxic CD8 T-cells, whereas conventional autologous and donor-derived CAR-T therapies contain a mixture of CD8 and supportive CD4 T-cells. It has been established that cytokine signaling via the JAK/STAT pathway plays an important role in promoting CAR-T cell function. Herein we describe novel engineered receptors that can induce JAK/STAT signaling in iPSC derived CD8 T-cells. We show that iPSC derived CD8 T-cells have a unique JAK/STAT signaling requirement for long-term cytotoxicity and proliferation in our in vitro assays. We show that our engineered receptors can induce JAK/STAT signaling in the absence of exogenous cytokines in culture media. When fully differentiated CD8 T-cells are transduced with engineered cytokine receptors, they show long-term persistence in the absence of exogenous cytokines in vitro. We observed that premature activation of JAK/STAT signaling pathways can skew cells toward a myeloid or innate lymphoid cell lineage. To circumvent this, we designed iPSC clones with an engineered cytokine receptor integrated into a specific locus under the control of context-specific regulatory sequences. These designs exhibited a stringent off-state during differentiation and CAR-dependent transcriptional responsiveness when exposed to target antigen. This data reveals that a regulated strategy can control cytokine receptor expression during differentiation, to preserve phenotype and improve efficacy of differentiated cells. Further, constitutive activation of JAK/STAT signaling may drive adverse side-effects, and we demonstrate that our regulated designs show complete lack of activity in the absence of antigen stimulation. Finally, we evaluated iPSC derived CD8 T-cells harboring the regulated cytokine engineering strategy in vivo. Engineered iPSC derived CD8 T-cells showed tumor growth inhibition and persistence in the absence of exogenous cytokines in a disease relevant lymphoma Raji model. Engineering of appropriate cytokine support and strategies to regulate expression during differentiation is an important first step to enhance the persistence of iPSC derived CD8 T-cells. A major advantage of using iPSC derived CD8 T-cells is their potential for drug re-administration without toxic lymphodepletion and exogenous cytokine support. We believe that cell-intrinsic cytokine support is critical to develop durable and safe allogeneic T-cell therapies for patients.

#6107 Tailoring mRNA expression in T-cell therapy: advancing CAR-T functional optimization.

Gilad Gibor[1], Shai Kilim[1], Neve Tzvi[1], Ortal Harush[1], Orit Itzhaki[1], Ronnie Shapira[1], Elad Jacoby[2], Gal Cafri[3], Yochai Wolf[1]

[1]Immuno-oncology, Sheba Medical Center, Ella Institute, Ramat-Gan, Israel,[2]Center for Pediatric Cell Therapy, Sheba Medical Center, Ramat-Gan, Israel,[3]Immunotherapy and Genetic Engineering, Sheba Medical Center, Ramat-Gan, Israel

Background: T cell engineering is an emerging field in immune-oncology, and new T cell-specific modalities are required, such as gene insertion via mRNA. Cells display diverse functions and identities, requiring tailored approaches for efficient mRNA delivery. Most mRNA constructs contain standard α-globin UTRs, lacking the specificity needed for optimal T cell mRNA expression.
Methods: In our study, we developed modRNA constructs by replacing the standard hemoglobin 5'-UTR with sequences derived from genes highly expressed in normal or exhausted T cells, aiming to enhance mRNA stability and protein expression. mRNA constructs containing reporter genes were electroporated into PBMC-derived T cells, with expression levels measured by flow cytometry.
Results: Interestingly, we observed significant differences, with notable variability in expression arising from the replacement and directly linked to the specific gene substituted. Certain UTRs, such as Interferon gamma (IFNG), showed superior and sustained GFP expression, while others, such as TNF, exhibited reduced activity. Based on these observations, we expressed diverse CAR constructs in T cells, revealing distinct protein turnover and tonic signaling profiles. Specific constructs exhibited rapid decay, while others displayed more stable expression. These turnover patterns suggested significant differences in CAR expression levels across constructs and their impact on tonic signaling, reinforcing earlier findings on the influence of 5'-UTRs in CAR-T expression. Particularly notable were constructs that exhibited significantly lower activity and reduced expression of key activation and well-established hallmark markers of exhaustion. Cytokine secretion assays further confirmed diminished tonic signaling, especially in non-target co-cultures.
Conclusions: These results underscore the critical role of T cell-specific UTRs in fine-tuning CAR-T cell activity and reducing off-target effects.

#6109 Dual targeting of cancer and stromal cells, by combining a FAP-CAR and a PRAME TCR, enhances T cell infiltration and efficient eradication of a wide range of hard-to-treat solid tumors.

Paul Najm, Kathrin Skibbe, Mikhail Steklov, Panagiota A. Sotiropoulou, **Marleen M. Van Loenen**

T-knife Therapeutics Inc., San Francisco, CA

Background: The immunosuppressive tumor microenvironment (TME) is a significant barrier to improving the clinical efficacy of T cell receptor (TCR) T cell therapies in solid tumors. The TME largely consists of stromal cells such as cancer associated fibroblasts (CAF) that support tumor growth and hamper immune responses by forming a physical barrier preventing T cell infiltration and exerting immunosuppressive activity. A common cell surface antigen of CAFs with limited expression in healthy tissue is fibroblast activating protein (FAP). Dual targeting T cells expressing an anti-FAP CAR together with a PRAME-specific TCR and CD8 co-receptor (CoR) were generated using non-viral gene editing. This construct enables depletion of CAFs and disruption of the tumor's supportive stroma and immune barriers, thereby enhancing TCR-T cell infiltration and tumor eradication.

Methods: Ten scFvs against FAP were engineered into 2nd gen 4-1BB CARs and retrovirally transduced into PMBCs. FAP-CAR T cells were selected based on CAR expression using flow cytometry and cytotoxicity against FAP positive target cells. T cells were non virally gene edited with selected FAP-CARs in combination with a HLA-A*02 restricted PRAME-specific TCR and a CD8 CoR. Functionality was tested under repeated antigen stimulation in 2D and 3D in vitro cancer models expressing physiological levels of FAP and PRAME.

Results: Ten anti-FAP CARs bearing different scFvs were screened based on T cell surface expression and functional activity against FAP overexpressing BT-549 cancer cells. An in vitro multi-cellular model was developed consisting of GFP-labelled cancer cells expressing physiological levels of PRAME and immortalized RFP positive fibroblasts expressing physiological levels of FAP. T cells co-expressing a PRAME TCR, CD8 CoR and 4 selected FAP-CARs were generated and tested for cytotoxicity upon repeated stimulation. Two candidates enabled multiple rounds of killing. Then, a multicomponent 3D spheroid model of non-small cell lung cancer (NSCLC) was developed combining cancer cells, monocytes, CAFs and extracellular matrix, mimicking the dual action of TME, raising a physical barrier against T cell infiltration and mediating immune suppression. Constructs containing an anti-FAP-CAR demonstrated a superior infiltration, cytotoxicity and cytokine secretion profile compared to TCR-T cells without CAR armoring.

Conclusion: Dual targeting T cells incorporating both a FAP-CAR and a PRAME TCR demonstrated successful infiltration and effective eradication of both cancer cells and stromal cells within our proprietary, multicomponent 3D spheroid model mimicking the harsh NSCLC TME. This approach holds promise to tackle a major challenge in solid tumor therapy, increasing T cell infiltration and eliciting potent, durable anti-tumor responses.

#6110 Improving CAR T cell potency against low-density targets in rhabdomyosarcoma.

Caroline Piccand[1], Andrea Timpanaro[2], Dila Handan Kaymaz[3], Jochen Karl Rossler[3], Michele Bernasconi[3]

[1]Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland,[2]Ben Towne Center for Childhood Cancer Research, Seattle Children's Research Institute, Seattle, WA,[3]Department for BioMedical Research (DBMR), University of Bern, Bern, Switzerland

Engineering of L1CAM-directed CAR T cells aims to demonstrate high efficacy and better persistence in rhabdomyosarcoma (RMS) models. RMS is the most common pediatric soft tissue sarcoma, and the low overall survival rates for metastatic RMS emphasize the urgent need for improved therapies. Chimeric Antigen Receptor (CAR) Ts have shown remarkable efficacy against hematological malignancies; however, their application in solid tumors remains challenging, mainly due to the absence of ideal targets and to the immunosuppressive nature of the tumor microenvironment (TME). To optimize antitumor activity against RMS and increase CAR T cell persistence *in vivo*, we investigated novel CAR constructs targeting newly identified RMS surface proteins. Through comprehensive RMS surfaceome profiling and validation in patient-derived tumor microarrays (TMAs), we identified L1CAM as attractive target. To evaluate the efficacy of L1CAM-directed CAR T cells against RMS, we generated a panel of anti-L1CAM.CE7 CARs expressing either a long (IgG4CH2CH3, 229aa) or a short (CD28, 35aa) hinge moieties, each paired with either a CD28- or 4-1BB-derived costimulatory domain (CSD).48h co-incubation experiments revealed that anti-L1CAM CAR T cells with long hinge and CD28 CSD showed superior efficacy, killing up to 60% of RMS cells at an Effector-to-Target (E:T) ratio of 1:1 and releasing double amount of IFN-γ compared to other constructs. To corroborate these findings *in vivo*, the selected anti-L1CAM.IgG4CH2CH3.28.3ζ CAR Ts were tested against the orthotopic RMS model Rh30, expressing low copy numbers of L1CAM (~10'000). Despite limited efficacy following the initial CAR T dose, we observed tumor eradication in 2/5 mice and survival benefit in the remaining cohort after administration of a second dose on day 12. Furthermore, by bioluminescence of CAR T cells, we observed long-term persistence of CAR T cells in murine lymph nodes and the abdominal region for up to 50 days post-injection. To enhance therapeutic outcomes and achieve complete remission in 5/5 mice, we modified the anti-L1CAM.IgG4CH2CH3.28.3ζ construct to co-express an additional CD3ζ-mock CAR targeting CD276 - highly expressed on RMS - designed to boost the activation of anti-L1CAM CAR Ts (termed "IF-BETTER" CAR). In vitro screening identified 4-1BB as the optimal CSD for the CD276-associated CAR, which conferred enhanced activity to the L1CAM.IgG4CH2CH3.28.3ζ construct against RD and Rh30 cells, reaching up to ~80% at the E:T ratio of 1:2. Furthermore, the selected IF-BETTER anti-L1CAM CAR Ts improved potency against the challenging Rh4 cells, which express very low copies of L1CAM (~2'000). The selected IF-BETTER CAR Ts are currently tested in preclinical studies. These findings highlight the potential of the IF-BETTER anti-L1CAM CAR T cells as a promising therapeutic strategy for L1CAM^{dim} tumors like RMS.

#6111 Reprogramming ROR1 CAR-T cells with SNIPR logic gating promote safety, while keeping anti-tumor efficacy in a tumor-bearing animal model.

Linyan Deng[1], Yuhua Wan[2], Jingyu Li[1], Jian Hua[2], Di Zhu[3], **Hua Zhang**[1]

[1]SPH Biotherapeutics (HK) Limited, Hong Kong, Hong Kong,[2]SPH Biotherapeutics (Shanghai) Limited, Shanghai, China,[3]Department of Pharmacology, Fudan University, Shanghai, China

CAR-T cell therapies have shown promise in the treatment of cancers. However, off-target toxicities often occur due to heterogeneous and low-abundance target expression by normal tissues. ROR1 is a good tumor target being expressed by many tumor tissues and cells. But normal tissues also express a lower level of ROR1, which sometimes led to patient fatalities in clinical trials with ROR1 CAR-T. Here we developed and demonstrated a two-antigen recognition platform using SNIPR logic gating to control the cytotoxicity of ROR1 CAR-T cells, limiting them to the tumor site *in vivo,* and thus mitigating the risk of attacking normal vital tissues.

Methods: Using SNIPR (synthetic intramembrane proteolysis receptors) (Zhu, et al. *Cell*, 185(8):1431-1443.e16, 2022), we engineered T cells with an inducible ROR1 VHH CAR expression that was controlled by an external sensor protein PD1. We then applied the PD1/SNIPR-based ROR1 CAR-T cells to a tumor-bearing animal model, which could recognize PDL1, commonly expressed in many tumor cells, and then turned on the CAR-mediated killings to those cells.

Results: PD1-specific reporter induction of the SNIPR-based ROR1 CAR-T cells was confirmed *in vitro* using flow, IL2 production, and Incucyte's real-time killing assays when co-culturing separately with PDL1-overexpressing MDA MB231 cells (ROR1+) as a positive control, and other ROR1+ tumor cell lines expressing variable levels of PDL1 at an E:T ratio of 1:1. In the native MDA MB231 and PD1-overexpressing MDA MB231 xenografted animal models, after we infused the CAR-T cells at $4x10^6$ cells/mouse via the tail vein, we found that non-gated ROR1 CAR-T group showing a quicker tumor shrinkage starting D11 at a mean tumor size of $70mm^3$ when treating the native tumor. However, in the gated group treating the same native tumor we observed a delayed and steady decline of the tumor volume starting D15 (the mean $120mm^3$). Its shrinkage continued down to a mean tumor volume of $48mm^3$ D29, almost close to the size (the mean $49mm^3$) of the non-gated ROR1 CAR-T group D29, whereas the gated group treating the PD1-overexpressing MDA MB231 revealed a quick tumor shrinkage D15 (the mean $100mm^3$), leveled the tumor curve D22 (the mean $50mm^3$), and began to show the signs of GHVD on D25.

Conclusion: We successfully demonstrated the therapeutic potential of our two-antigen recognition platform using SNIPR logic gating in an xenografted animal model. This platform enables us to reconfigure and develop safer and more effective CAR products suitable for clinical translation.

Keywords: CAR T; SNIPR; Immunotherapy; ROR1

#6112 Supercharging anti-tumor immunity in hepatocellular carcinoma with PH102: A CAR T-cell-based platform.

V. T. Pham[1], S. Fu[1], V. Sanchez[1], Y.-G. Liu[2], K. Liu[1], A. Bernal[2], L. Sun[1], G. Huang[1];
[1]MD Anderson Mays Cancer Center at UT Health San Antonio, San Antonio, TX, [2]UT Health San Antonio, San Antonio, TX

Hepatocellular carcinoma (HCC) is the 5th leading cause of cancer death in the US among males (7th among females) and remains a major cause of cancer-related death worldwide with especially high prevalence in South Texas (12 per 100,000). HCC is caused by HBV/HCV infections, metabolic dysfunction, and alcoholic steatohepatitis. The majority of HCC patients present clinically with advanced disease and are treated with *ineffective* systemic therapies including chemotherapy and/or immune checkpoint inhibitors. There's a pressing need for an innovative, curative immunotherapy for advanced, unresectable HCC for whom liver transplantation is not possible. The tumor immune microenvironment (TiME) of HCC is a complex milieu of tumor, stromal, and immune cells that interact to modulate tumor growth. We designed a first-in-class precision immunotherapy approach based on established chimeric antigen receptor (CAR) T-cell technology that packages three different targeting approaches into a comprehensive cell-based platform to rebuild the anti-tumor TiME. PH102 is a CAR T-cell-based therapy targeting GPC3 (tumor antigen highly expressed in HCC but not healthy hepatocytes or normal tissues) while co-expressing factors A and B, immune signaling molecules that supercharge anti-tumor immune cells. We have shown exceptional preclinical safety and efficacy of PH102 in multiple solid tumor animal models including a tumor implantation model and a hydrodynamic tail vein injection (HTVI) murine model of *in situ* HCC. PH102 has been demonstrated to significantly improve probability of survival in mice carrying advanced HCC tumors, eliciting powerful elimination of advanced tumors via immune killing of cancer cells. Immunomodulatory factors A and B synergize to induce increased infiltration, expansion, and activation of cytotoxic T-cells, tissue resident memory T-cells, conventional NK cells, liver resident NK cells, and dendritic cells within the TiME. Altogether, PH102's triple-hit approach induces a potent and durable anti-tumor, immune-triad-mediated killing program alongside creating long-term immune memory that persists long after CAR T-cell exhaustion. PH102 is warranted to be translated into a phase I/II HCC trial following final preclinical safety and efficacy validation.

#6113 Cytotoxic and antitumoral effect of an anti-CD44v6 CAR-T against gastric cancer.

gonzalo gonzalez[1], Moonsoo Jin[1], Yanping Yang[1], Elizabeth Ortiz[2], Yogindra Vedvyas[1]

[1]Houston Methodist Research Institute, Houston, TX,[2]Instituto Nacional de Cancerologia, Mexico City, Mexico

Background: Gastric cancer ranks among the top 5 cancer types in terms of both incidence and mortality, with a 5-year overall survival rate of only 29%. CD44 variant 6 (CD44v6) is an isoform of CD44 and is identified in 70% of gastric cancer cases. CD44v6 is considered a marker of poor prognosis, and its expression varies among different types of gastric cancer. CD44v6 has a role in apoptosis resistance, metastasis, and tumor progression, and is shown to be enriched in recurrent tumors. CD44v6 expression in normal tissue is very low and is restricted to certain cell types. Therefore, CD44v6 is a promising target for targeted therapies against gastric cancer. In this study, we aim to develop a chimeric antigen receptor (CAR) T cell therapy targeting CD44v6 and evaluate its functionality, cytotoxic activity and antitumoral effect. The scFv was derived from the monoclonal antibody (mAb) known as bivatuzumab (BIWA-4), a humanized mAb that has been used in clinical studies as radiotherapy and as antibody drug conjugates.
Methods: Plasmid containing the CAR sequence with the BIWA-4 scFv was synthesized, and Jurkat T cell line and primary human T cells were transduced with lentivirus containing the CAR sequence. Jurkat cell line was used to measure binding to recombinant protein and assess CD69 expression after antigen exposure. Primary T cells transduced with CAR were co-cultured at different E:T ratios with gastric cancer cell lines (AGS, SNU 638, and MKN45) with different levels of expression of CD44v6. Target cell killing was measured by flow cytometry, bioluminescence, and fluorescence microscopy. T cell exhaustion and exhaustion phenotype were evaluated prior killing assays. For *in vivo* experiments, NSG mice will be engrafted with gastric cancer cell lines and tumor progression will be evaluated by IVIS.
Results: While high affinity variants of bivatuzumab have been used to construct CAR (BIWA-8), ours is the first study that used medium affinity variant known as BIWA-4. Jurkat cells transduced with the car construct demonstrated specific binding to the recombinant monomeric CD44v6, which triggered surface expression of early T cell activation marker CD69. With varying concentrations of monomeric CD44v6, the affinity of CAR to CD44v6 was determined to be ~100 nM K_D. Transduction efficiency of primary T cells remained stable, reaching ~60% after 10 days of expansion. Most CAR T cells exhibited effector memory phenotype, with low levels of PD1. CD44v6 CAR T killing in vitro was correlated with the E: T ratio and the level of antigen expression, demonstrating specificity of CAR and avoiding off-target effects with CD44v6 negative cells.
Conclusion: We have successfully developed CAR T cells possessing medium affinity against CD44v6 and confirmed CAR-specific killing of CD44v6 positive gastric cancer cells.

#6114 Engineering novel synNotch CAR T cells to precisely and effectively target medulloblastoma.

Maggie Colton Cove[1], Senthilnath Lakshmanachetty[1], Milos Simic[1], Robert Zhu[1], Chanelle Shepherd[1], Lia Cardarelli[2], Sachdev Sidhu[2], Olga Troyanskaya[3], Wendell Lim[1], Hideho Okada[1]

[1]UCSF - University of California San Francisco, San Francisco, CA,[2]University of Toronto, Toronto, ON, Canada,[3]Princeton University, Princeton, NJ

Background: While clinical trials involving chimeric antigen receptor (CAR) T cells have shown promise for treating pediatric brain tumors, factors such as antigen heterogeneity, on-target, off-tumor toxicity and an immunosuppressive microenvironment limit the efficacy of these treatments. To overcome these challenges, we adopted synthetic notch receptor system and developed innovative synNotch-CAR T cell circuits. In this system, the first antigen which is expressed exclusively on the brain, primes the T cells to locally induce the expression of a CAR to completely kill the tumor cells. Here, we investigated the anti-tumor efficacy of synNotch CAR T cells in patient-derived models of Group 3 medulloblastoma, the deadliest subtype of medulloblastoma.
Methods: We utilized a synNotch receptor specific to brevican, a proteoglycan expressed solely in the central nervous system, for brain-specific priming. Human CD3+ cells were stimulated with CD3/CD28 beads for 24 hours and lentivirally transduced to express the synNotch receptor (α-BCAN) and a α-B7H3/IL13Ra2 CAR payload. α-BCAN synNotch-α-B7H3/IL13Ra2 CAR (B-SYNC) T cells were co-cultured with mCherry+ D425 and D283 tumor cells for 4 days and real-time cell growth monitoring was performed using incucyte live imaging. Brevican-priming was accomplished either through brevican-expressing K562 cells or tissue culture plates coated with recombinant human brevican. Tumor cell growth was measured by mCherry fluorescent signal.
Results: B-SYNC T cells completely killed the medulloblastoma cell line, D283, only when primed by K562 cells expressing BCAN. No killing was observed in the absence of priming cells. These cells also maintained killing of a more proliferative medulloblastoma cell line, D425, over an extended period than the constitutive -α-B7H3/IL13Ra2 CAR T cells. Furthermore, when cultured in brevican-coated plates, B-SYNC T cells killed D425 cells at a 17% faster rate than the constitutive -α-B7H3/IL13Ra2 CAR T cells.
Conclusions: B-SYNC T cells show superior cell-killing to constitutive -α-B7H3/IL13Ra2 CAR T cells in in vitro models of Group 3 medulloblastoma. We are currently validating this finding in vivo using patient-derived xenografts. Future studies are aimed at elucidating the mechanisms by which synNotch CAR T cells outperform constitutive CAR T cells. Furthermore, we will investigate the anti-tumor efficacy of synNotch CAR T cells in the more resistant hypoxic medulloblastoma tumor microenvironment.

#6115 MZB1 TCR-like CAR T cell therapy, a new weapon in the war against multiple myeloma and waldenström macroglobulinemia.

Elena Maroto-Martin[1], Marina He[1], Yingjie Zhao[2], Jessica Encinas[1], Sara M. Kolhatkar[1], Roberto Garcia-Vicente[3], Mubin Tarannum[4], Zachary R. Hunter[5], Rizwan Romee[4], Mehmet K. Samur[6], Mariateresa Fulciniti[1], Jianzhu Chen[7], Nikhil C. Munshi[8]

[1]Jerome Lipper Multiple Myeloma Disease Center, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA,[2]Department of Biology, Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA,[3]Department of Translational Hematology, Instituto de Investigacion Sanitaria Hospital 12 de Octubre (imas12), Hematological Malignancies Clinical Research Unit H12O-CNIO, Hospital 12 de Octubre - Centro Nacional de Investigaciones Oncologicas, CIBERONC, Madrid, Spain,[4]Division of Hematologic Malignancies and Transplantation, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA,[5]Bing Center for Waldenstrom's Macroglobulinemia, Dana-Farber Cancer Institute, Boston, MA,[6]Department of Data Sciences. Department of Biostatistics, Dana-Farber Cancer Institute, Harvard T.H. Chan School of Public Health, Boston, MA,[7]Department of Biology, Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Boston, MA,[8]Jerome Lipper Multiple Myeloma Disease Center, Dana-Farber Cancer Institute, Harvard Medical School, VA Boston Healthcare System, Boston, MA

Multiple myeloma (MM) is a plasma cell neoplasm accounting for 10% of all hematological tumors. CAR-T cell therapies have demonstrated unprecedented response rates, resulting in the FDA approval of two BCMA CAR-T products. Despite good initial responses, relapses are common and caused by tumor antigen loss and/or CAR T cell exhaustion. One way to overcome tumor antigen loss is to target a different cancer antigen. However, a limited number of cancer-specific proteins are expressed on the cell surface, where they are accessible to immunotherapies. Normal cells ubiquitously express MHC Class I molecules, which present fragments of cellular proteins to the immune system. Here, we have developed a new generation of CAR T cells able to recognize intracellular antigens presented by an MHC molecule on the cell surface. To identify antigens for a TCR-like CAR T therapy, we combined transcriptomic and proteomic data from MM cell lines, MM patients, and healthy tissues. We found that the intracellular protein MZB1, which is involved in the maturation and function of plasma cells, is highly expressed in MM cells as well as a small subset of B cells including Waldenström Macroglobulinemia (WM) and B-cell lymphoma cells. By computational prediction, we identified putative MZB1 peptides that can be presented in the context of HLA-A*02:01, the most frequent HLA allele worldwide. We used a human scFv antibody yeast surface display library to select monoclonal antibody clones specific to the MZB1:HLA-A*02 complex. By performing binding affinity analysis by flow cytometry in a large panel of MM and WM cell lines with differential expression of MZB1 and/or HLA-A2, we selected two clones with higher binding affinity to the HLA-A2/MZB1 positive cells that were further utilized for CAR design. CAR constructs containing the MZB1-A2 scFv, a 41BB co-stimulatory domain and CD3z signaling domain were generated. MZB1 CAR T cells showed significant cytotoxicity against MZB1$^+$ HLA-A2$^+$ MM and WM cells compared to untransduced T cells, but not against HLA-A2 and/or MZB1 negative cells. Additionally, MZB1 CAR T cells showed higher expression of activation markers and significant increased release of IFNy and Granzyme A/B when co-cultured with MZB1$^+$ HLA-A2$^+$ cells, suggesting enhanced antitumor immune responses. To validate the translational potential of MZB1 CAR T cells we confirmed their tumor-killing activity *ex vivo* across primary MM cells from ND and relapsed MM patients. Importantly, *in vivo* administration of 1x10^7 CAR T cells reduced myeloma burden in a human MM disseminated murine model. In conclusion, we have generated TCR-like CAR T cells that show potent and specific *in vitro*, *ex vivo* and *in vivo* antitumor efficacy against MZB1/HLA-A*02:01 complex on the surface of MM and WM cells. This study provides the logic and framework for similar therapies to be developed against other intracellular targets in MM and other cancers.

#6116 Generation of multi-specific CAR T cells using a novel single-marker selection platform to target medulloblastoma heterogeneity.

Diren Usta, Yushen Feng, Yiyun Chen, Louai Labanieh, Nikoo Jafari, Peng Xu, Sabine Heitzeneder, Elena Sotillo, Crystal Mackall

Stanford Cancer Institute, Stanford, CA

High-risk Group 3 and Group 4 and recurrent medulloblastoma patients have a dismal prognosis, and emerging targeted therapies have not yielded promising results. While CAR T cell therapy has achieved unprecedented success in treating hematologic malignancies, translating this approach to solid tumors has been challenging due to tumor heterogeneity and the immunosuppressive microenvironment. To overcome these obstacles and address the urgent need for new treatments, we aimed to develop an enhanced multi-specific CAR T cell product targeting the antigens GPC2, GD2, and B7H3, which are described to be highly expressed across cancers, notably in tumors of neuroectodermal origin. We first conducted a comprehensive analysis of bulk and single-cell RNA sequencing data to validate the expression of GPC2, GD2, and B7H3 on medulloblastoma samples. Protein expression of these targets was confirmed on medulloblastoma cell lines and PDX models, and confirmation on patient samples using flow cytometry-based Quantibrite analysis is currently ongoing. Based on these findings, we generated tri-specific CAR T cells using the STASH select platform - a method for enriching cells containing multiple genetic modifications via a single selection marker - and performed phenotypic and functional assays in vitro and in vivo. Our initial results show that the selected tri-specific CAR T cells effectively control pooled populations of tumor cells expressing each of the antigens individually or in combination. However, we observed that the phenotype and function are dominated by the CAR with the highest level of tonic signaling, which decreased the overall efficacy of the tri-specific CAR T cells. By implementing downregulation of MED12, we found that the tri-specific CAR T cells demonstrated increased proliferation upon stimulation and enhanced cytotoxic efficacy in repeated stimulation assays, indicating exhaustion resistance. Further T cell engineering, including overexpression of cJUN and in vivo evaluations of antitumor activity in different mouse models are ongoing. In conclusion, our tri-specific CAR T cell product targeting GPC2, GD2, and B7H3 represents a promising novel therapeutic approach to overcoming T cell-intrinsic (exhaustion) and extrinsic (antigen heterogeneity) limitations in treating high-risk medulloblastoma. Ongoing studies aim to further assess their effectiveness and potential for broader clinical application.

#6117 Development of preclinical models for the evaluation of conventional and *in vivo* CAR-T therapies.

Hongyan Sun[1], Xiaoliu Yang[1], Shiying Guo[1], Jianming Xu[1], Xiang Gao[1], Jun Wang[2]

[1]GemPharmatech Co., Ltd, Nanjing, China,[2]GemPharmatech Co., Ltd, San Diego, CA

Conventional CAR-T manufacturing involves *ex vivo* expansion and engineering of T cells either from patients (autologous) or healthy donors (allogenic), which is time- and cost-consuming. The recent advancement of *in vivo* gene editing using lentiviral vectors allows CAR-T generation *in vivo*, which is time- and cost-effective compared to the conventional approaches. To evaluate their *in vivo* anti-tumor efficacy and toxicity, genetically engineered mouse models which express the human-specific target represent a viable choice for preclinical testing. While immunodeficient mouse models are crucial for preclinical evaluation, their utility is often compromised by the development of graft-versus-host disease (GvHD). To overcome this limitation, GemPharmatech has developed a novel NCG-MHC-dKO mouse model, which combined severe immunodeficiency with MHC class I and II molecules knockout to minimize GvHD occurrence and extend treatment window.

We assessed the efficacy of an *in vivo* CAR-T vector using this newly developed model. NCG-MHC-dKO mice bearing luciferase-tagged Nalm6 tumors received intraperitoneal injection of 2×10^7 PBMCs, followed by administration of a single dose of the lentiviral vector encoding an anti-CD19 CAR transgene. Successful *in vivo* gene editing was confirmed by flow cytometry analysis using antibodies specific for CAR in reconstituted human T cells. Tumor burden was monitored longitudinally using an *In Vivo* bioluminescence Imaging System (IVIS®), quantified as total bioluminescent flux. The i*n vivo* CAR-T vector treatment demonstrated robust growth inhibition of Nalm6-Luc tumor in huPBMC-NCG-MHC-dKO mice, with minimal GvHD occurrence throughout the course of the study, while mock-treated groups did not show any obvious tumor growth inhibition, confirming the specificity of the CAR-T vector.

These findings established the NCG-MHC-dKO mouse model with PBMC engraftment as a suitable platform for preclinical evaluation of both conventional and *in vivo* CAR-T therapeutic strategies.

#6118 Engineering p95HER2 CAR T cells for the treatment of HER2-positive tumors.

Ariadna Grinyo-Escuer[1], Marta Bort-Brusca[2], Pablo Ovejero[2], Julia Fernandez Torrente[2], Judit Gago[2], Marta Escorihuela[1], Macarena Roman[1], Joaquin Arribas[2]

[1]VHIO Vall D'Hebron Institute of Oncology, Barcelona, Spain,[2]Hospital del Mar Research Institute, Barcelona, Spain

Background: Clinical implementation of Chimeric Antigen Receptor (CAR) T cells in solid tumors has been more challenging than for hematological malignancies due in part to "on-target, off-tumor" toxicities. The tyrosine-kinase receptor HER2 is a potent oncogene amplified in almost 3% of all cancers. While most HER2-positive tumors diagnosed at early stages have already effective and available treatment, refractory and metastatic tumors still pose an unmet medical need. p95HER2, a truncated version of the HER2, is expressed in 30-40% of HER2-amplified tumors and, unlike HER2, its expression is restricted to tumor cells. Recent research in the CAR T field has explored novel logic gated CAR T cell designs using alternative signaling domains to CD3z and dual antigen targeting strategies to mitigate toxicity. Here we show the use of LINK CAR T cells to selectively target p95HER2 and HER2-positive tumors while sparing normal tissues expressing basal levels of HER2.

Methods: We generated LINK CARs: a CAR incorporating LAT signaling domain and another with SLP-76 domain, targeting p95HER2 and HER2. Then, transduced both CAR constructs into T cells from healthy donors. In vitro cytotoxicity was assessed through co-cultures with cell lines derived from gastric, breast, ovarian, and lung tumors expressing endogenous p95HER2 and HER2. Safety was evaluated by co-culturing the CAR Ts with cell lines from healthy tissues expressing basal HER2 levels. In vivo efficacy was investigated using immunodeficient mice implanted with cell-derived xenografts.

Results: We show the specific activation of these CAR T cells in the presence of both HER2 and p95HER2-expressing tumor cells. No cytotoxicity was observed in cells with only basal HER2 levels. Safety testing across a panel of healthy cell lines with varying HER2 expression confirmed the CAR T cells' specificity. In vivo studies demonstrated the p95HER2 LINK CAR's ability to eradicate tumors from cell lines expressing HER2 and p95HER2, while preserving MCF7 tumors representing tissues with basal HER2 expression. Patient-derived xenograft(PDX) models are currently being tested to evaluate the translational potential of this novel therapeutic.

Conclusions: Our preliminary results show the feasibility of LINK CAR T cells as a targeted therapeutic approach for HER2-positive tumors, leveraging p95HER2 specificity. Experimentation in PDX models and expansion into different tumor types will further validate this findings.

#6119 Efficient generation of active CAR-T cells from healthy donors and lymphoma patients.

Andrew J. Sawyer, Viktor Lemgart, William Kuhlman, Aaron Griset, Mir Ali, Jennifer Richards, Angela Hadjipanayis, Emiko Desvaux, Seth Garren, Chris Hoefler, Haley Nguyen, Laura Strauss, Jing Jiao, Brandon Quido, Laura Powers, Allison Caron, Eyoung Shin, Virna Cortez-Retamozo, Austin Boesch, Dharini Shah, Tiffany Le, Rasika Kunden, Samantha Stewart, Fazila Nasimi, Sampa Maiti, Olga Lihoradova, Christian Mueller, Christopher Borges, Carla Lawendowski, Ulrik Nielsen, Valeria Fantin, Daryl Drummond, Donald Shaffer

Sanofi, Cambridge, MA

CAR T-cells have set a new standard of clinical activity in patients with hematologic malignancies but there are several barriers to broader patient access. Currently, the genetic modification of patients' T cells to produce CAR-T cell therapies is carried out ex vivo before infusing the cells back into the patient, using methods that are complex and hinder widespread use. Here we share a targeted lipid nanoparticle (LNP) encapsulating mRNA to reprogram circulating human T-cells *in vivo*, designed to overcome the significant limitations of current CAR T therapy. The LNP consists of a proprietary ionizable lipid formulation, developed for efficient transfection of T cells and improved tolerability, containing an mRNA encoding a second-generation CD22 CAR. Transfection of human primary T cells was only observed with the addition of a NANOBODY® VHH targeting CD8 to the surface of the LNP. CAR expression was measured for multiple days in up to 80% of CD8+ T cells, with minimal loss of viability and no nonspecific activation. In a whole-blood assay, CAR expression was limited to CD8+ cells (T cells and NK cells), with no CAR expression observed in CD4+ T cells, B cells, or granulocytes. LNP transfected T cells are capable of antigen specific killing. In a serial transfection and rechallenge experiment, cultured T cells were able to maintain cytotoxicity against repeat challenges of Nalm 6 cells but only with additional LNP treatments. The repeated transfected and cellular challenge did not induce exhaustion or disfunction. The in vivo CAR LNP system also efficiently transfected T cells from lymphoma patient PBMCs. Overall levels of in vitro transfection were generally increased in lymphoma patients and the resulting CAR expressing cells were active. CAR expression was greatest in central memory and effector memory T cell phenotypes and decreased in naïve T cells. High-dimensional Spectral Flow profiling identified increased populations of Tem cells in both DLBCL and follicular lymphoma patients, potentially explaining the increased levels of CAR expression. In both healthy donors and lymphoma patient samples, treatment with CAR LNPs significantly reduced B cells, showing the activity of the transfected cells. The capabilities of the LNP system have also been demonstrated in a PBMC humanized mouse model. LNP dosing resulted in expression of CAR in over 80% of circulating human CD8 T cells 6 hours after a single dose. CAR expression was also observed after 4 serial doses, with no substantial loss of CAR expression. Expression of a CAR eliminated any remaining B cells from the PBMC population without severe systemic toxicities. Taken together, we have demonstrated that this targeted LNP system is capable of specific, re-dosable expression of an active CAR that is tolerable for multiple transfections. These are the necessary capabilities to fundamentally alter patient access to CAR-T therapies.

#6120 Combination of a novel allogeneic anti-cMet CAR-NK cell product with therapeutic antibodies in treating solid tumors.

C. Wang[1], T. Liu[1], Q. Wang[1], Y. Gong[1], F. Gao[1], F. Zhou[2], **Z. Cao**[1];
[1]Shanghai Simnova Biotechnology Co., Ltd., Shanghai, China, [2]State Key Laboratory of Neurology and Oncology Drug Development, Simcere Zaiming Pharmaceutical Co, Ltd., Shanghai, China

Both mesenchymal-epithelial transition factor (C-Met) and epidermal growth factor (EGFR) have been acknowledged as significant pan-tumor therapeutic targets. EGFR- and cMet-targeted autologous chimeric antigen receptor (CAR) T therapies have been tested in clinicals. However, the potential toxicity significantly limited their therapeutic outcomes. Compared to T cells, natural killer (NK) cells have a lower risk of inflammatory toxicity and graft-versus-host disease. CAR-NK therapies also can be produced as an off-the shelf product. To prevent antigen escape, we developed cMet targeted NK cells therapy in combination with EGFR targeted therapeutic antibody in treating solid tumors. Our preclinical results demonstrated cMet CAR-NK single treatment or combined with therapeutic antibodies showed better anti-tumor efficacy compared to EGFR/cMet dual-target CAR-NK cells. We verified EGFR and cMet expression in multiple tumor cell lines (HCT116-colon, NUGC4-gastric, and A549/NCI-1975-NSCLC), which were further engineered to express luciferase. EGFR targeted, cMet targeted, and cMet/EGFR dual-targeted CAR structures were designed and transduced with retroviral vectors into expanded NK cells. Flow cytometry was used to compare the transfection rates and phenotyping among different CAR structures. CAR-NK cell functionality was tested using *in vitro* cytotoxicity assays. *In vivo* study was performed in subcutaneously inoculated xenografts with intravenous delivery of cryopreserved CAR-NK cells. Flow cytometry analysis revealed efficient transduction in single targeted CARs and dual-targeted tandem CAR (~80%), but low transduction level in dual-targeted parallel CAR (~40%). All CAR constructs indicated *in vitro* specific cytotoxicity using luciferase- and RTCA-based assays. However, compared to dual-targeted CARs, EGFR and cMet single CARs demonstrated significant better tumor regression and NK cell persistence in peripheral blood in an NCI-H1975 s.c. xenograft mouse model. We demonstrated that combination of cMet CAR-NK with EGFR- or EGFR/cMet-targeted therapeutic antibodies resulted in both potent ADCC and CAR activity, whereas EGFR CAR-NK combo showed compromised *in vitro* tumor killing capacity. Furthermore, cMet CAR-NK solo or combo treatment showed excellent tumor control and survival prolong in different tumor cell line derived xenograft mouse models. These studies have a pivotal role in the evaluation of cMet CAR-NK cell combination therapies against solid tumors and reveal their therapeutic potential. Allogeneic cMet CAR-NK cell therapy and combo may provide safe and effective treatments for pan-tumor patients who have limited therapeutic options.

#6121 A novel allogeneic anti-DLL3 CAR-NK cell therapy in treating small cell lung cancer.

C. Wang[1], T. Liu[1], Q. Wang[1], Y. Gong[1], L. Chen[1], F. Gao[1], F. Zhou[2], **Z. Cao**[1];
[1]Shanghai Simnova Biotechnology Co., Ltd., Shanghai, China, [2]State Key Laboratory of Neurology and Oncology Drug Development, Simcere Zaiming Pharmaceutical Co, Ltd., Shanghai, China

Small cell lung cancer (SCLC) is an aggressively lethal malignancy with a high unmet medical need. Overexpression of Delta-like ligand 3 (DLL3) has been identified as a poor prognostic factor. Adoptive immunotherapy based on chimeric antigen receptor (CAR) T cells has been developed, however with significant adverse events. To address the critical need for an improved therapeutic intervention, we leveraged the ability of DLL3 targeted peripheral blood derived natural killer (PBNK) allogeneic cells therapy in treating SCLC. We describe preclinical *in vitro* and *in vivo* results demonstrating the antitumor efficacy of an off-the-shelf, cryopreserved DLL3 CAR-NK candidate. In addition, we make comprehensive comparison of PB units from different donors to establish criteria of starting materials selection for clinical and commercial supply of CAR-NK cells. PBNK cells were isolated from healthy donors with FcγRIIIA-158 (V/V, V/F) and propagated using feeder cells and cytokine cocktails. Various *in vitro* assays, including flow cytometry phenotyping, freeze-thaw viability/recovery, short-term and serial killing, cytokine releasing against tumor cells, were used for comprehensive function evaluation of PB units. DLL3 targeted CAR-NK cells were engineered by transducing donor-preselected NK cells with a retroviral vector encoding a DLL3 membrane-proximal epitope targeted scFv, a NK-optimized CAR backbone and a secreted IL15 armor. Activity of CAR-NK cells against target-expression SCLC cells was assessed *in vitro* and *in vivo*. PB Donors were selected with excellent NK expansion capacity and freeze/thaw recovery rate, stronger innate cytotoxicity and higher degranulation and intracellular IFN-γ buildup upon co-culturing with different tumor cells. The anti-DLL3 CAR was successfully transduced across all batches of CAR-NK cells derived from selected donors. NK-featured CAR backbone significantly enhanced expansion of NK cells and induced more activated NK phenotype compared to conventional CAR backbone. DLL3 CAR-NK cells was cytotoxic against DLL3$^+$ tumor cells (SHP-77 and NCI-H2171 cells), and DLL3 CAR-NK cells combined with anti-PD-L1 antibodies could further enhance NK cells proliferation in response to tumor cells and could control multiple rounds of tumor cells re-challenge *in vitro*. Using a cell line derived xenograft mouse model, we showed that a single dose of cryopreserved DLL3 CAR-NK cells delayed tumor growth and prolonged mice survival. Synergistic *in vivo* antitumor efficacy and enhanced NK cell expansion in peripheral blood were observed when using CAR-NK combined with anti-PD-L1. Collectively, these results demonstrated the favorable manufacturability, potency, and safety of a DLL3 CAR-NK cells product and its potential as a novel allogeneic treatment option for patients with SCLC.

#6122 Off-the-shelf BCMA/GPRC5D dual targeted CAR-NK cell therapy combined with Daratumumab in treating multiple myeloma.

C. Wang[1], T. Liu[1], Q. Wang[1], Y. Gong[1], F. Gao[1], F. Zhou[2], **Z. Cao**[1];
[1]Shanghai Simnova Biotechnology Co., Ltd., Shanghai, China, [2]State Key Laboratory of Neurology and Oncology Drug Development, Simcere Zaiming Pharmaceutical Co, Ltd., Shanghai, China

B cell maturation antigen (BCMA), and Orphan G protein-coupled receptor, class C group 5-member D (GPRC5D) targeted chimeric antigen receptor (CAR) T cell therapies have been successful as treatment options for refractory/relapse multiple myeloma (RRMM) patients. BCMA/GPRC5D dual-targeting CAR-T to address antigen loss is also being explored. However, CAR-T cell manufacturing and potential toxicities have prevented broad patient access. Natural killer (NK) cells have demonstrated an allogeneic cell therapy modality showing promising antitumor efficacy while exhibiting a more favorable safety profile in clinical testing. We have previously shown the successful engineering of IL15 armored BCMA/GPRC5D dual targeted CAR-NK cell therapy for treating MM (AACR 2023; clinical candidate SNC112). To target MM in a broader and more comprehensive manner, SNC112 were tested in combination with Daratumumab (DARA). To improve antibody-dependent cellular cytotoxicity (ADCC) response, high-affinity variants of CD16 (158V/V polymorphism) peripheral blood donors were selected for manufacturing. To avoid self-inflicted NK-cell-mediated fratricide, the stimulation and expansion protocols were optimized to limit CD38 expression on NK cells without inducing gene editing into scaled manufacturing process. *In vitro* short-term and serial killing assays were performed against MM cell lines to verify CAR functions and evaluate ADCC responses between different manufacturing protocols. The NK purity and surface markers expression were characterized by flow cytometry. The combinational antitumor efficacy was assessed *in vivo* in multiple xenograft models using NPG mice. SNC112 induced near 100% *in vitro* specific cytotoxicity using luciferase-label MM cell lines while non-transduced NK showed no cytotoxicity. SNC112 displayed dual-targeted repetitive tumor-killing potential with a RTCA based serial killing assay using CHOK1-BCMA and/or CHOK1-GPRC5D cells. SNC112 manufactured with optimized protocols showed significantly decrease of CD 38 expression (10% vs 80%), improved freeze/thaw recovery, prolonged *in vivo* persistence, and enhanced ADCC responses. In a CD38-low expression animal model, a sub-optimal dose of SNC112 combined with DARA displayed synergistic increase in tumor targeting through various tumor associated antigens, whereas SNC112 solo and DARA solo resulted in no significant antitumor activity. In a CD38-high expression animal model, both of SNC112+DARA and Non-transduced NK+DARA combos were able to achieve complete clearance of tumor cells. However, only SNC112+DARA could achieve sustained tumor control against re-challenge tumor cells. Studies presented demonstrate the potential of SNC112+DARA combo as a highly effective multi-antigen targeting and a cost-effective off-the-shelf cell therapy in treating MM.

IMMUNOLOGY: Innate Immunity to Tumors
Poster Session

#6126 Natural killer activation drives immunotherapy efficacy in triple-negative breast cancer patients: Novel insights for therapeutic intervention.

G. Manzotti[1], E. Salviato[1], V. Manicardi[1], A. Redavid[1], E. Gasparini[1], M. Ragazzi[1], F. Torricelli[1], E. Vitale[1], G. Talarico[2], F. Bertolini[2], C. Pinto[1], A. Ciarrocchi[1], **F. Reggiani**[1];
[1]AUSL-IRCCS Reggio Emilia, Reggio Emilia, Italy, [2]European Institute of Oncology (IEO), Milan, Italy

Immunotherapy in combination with neoadjuvant chemotherapy is rapidly changing the therapeutic perspective for Triple-Negative Breast Cancer (TNBC) patients, as demonstrated by the KEYNOTE522 trial. Enhancing a proficient immune response to improve neoadjuvant therapy (NAT) efficacy is an urgent clinical need. We have previously shown by applying a morphology-guided digital spatial transcriptome profiling, that Natural Killer (NK) cell infiltration in diagnostic biopsies of TNBC patients was associated with increased chemotherapy success. In this work, we aim to characterize the specific NK landscape associated with NAT response in TNBC. We prospectively enrolled 35 patients with a new diagnosis of TNBC in our Institute and underwent the KEYNOTE522 neoadjuvant schedule. We collected peripheral blood at diagnosis (T0) and after NAT completion before surgery (T1). On peripheral blood mononuclear cells (PBMCs), we performed single-cell RNA sequencing (scRNA-seq) and flow cytometry analysis to profile circulating NKs and compare patients showing either pathological complete or partial response to the KEYNOTE522 schedule. We did not observe a significant difference in NK number or sub-clustering before therapy (T0) when comparing responders to partial responders. However, after the NAT conclusion (T1), NKs from partial responders displayed a prevalent immune tolerant profile, characterized by the down-regulation of NK cytotoxicity-related genes. Besides, $CD16^{-}CD56^{bright}$ NKs were significantly increased in partial responders, whereas $CD16^{+}CD56^{dim}$ NKs were enhanced in complete responders, confirming a divergent activation profile among circulating NKs that was detectable only after NAT. Altogether, our results pointed out the relevance of NK activation in supporting a proficient NAT response in TNBC patients. Still, the molecular mechanisms governing NK cytotoxicity toward TNBC are largely unknown, restraining their clinical exploitation. To fill this gap, we investigated the specific transcriptional landscape associated with NK effector functions in TNBC patients. We applied a predictive bioinformatic approach to reconstruct the hierarchical organization of the regulatory transcription network associated with NK activation in TNBC, identifying a list of top-scoring Transcription Factors (TFs). Among these, we found SMAD3, VEZF1, MAZ, and RXRA as putative novel master regulators of NK cytotoxicity in anti-TNBC response. We developed a CRISPRi-based system to knock down these TFs and confirm the impact of this transcriptional reprogramming on NK effector functions. Collectively, our data unveil a driving role of NKs in triggering NAT efficacy in TNBC patients and that transcriptional reprogramming can be exploited to potentiate NK-based adoptive cell therapies and further enhance immunotherapy efficacy in TNBC patients.

#6127 Genomic instability regulates STING mediated anti-tumor immunity through distinctive cytosolic DNA structures.

S. Mosley, N. Lapa, C. Mowat, K. Baker;
University of Alberta, Edmonton, AB, Canada

Background. Cancer genomes are characterized by instability, with significant levels of DNA damage and mutation due to DNA repair defects. In colorectal cancer (CRC), defects in mismatch repair define the microsatellite instable (MSI) subtype, with stronger immunogenicity and better prognosis than the more common chromosomally instable (CIN) subtype. The mechanisms behind these differences are unknown, but their understanding could identify strategies to improve CIN CRC prognosis. The STING pathway detects foreign DNA in the cytosol, leading to induction of innate and adaptive immune responses. In cancer cells, STING also recognizes self cytosolic DNA (cyDNA) in the form of micronuclei or diffuse fragments generated by endogenous or treatment induced DNA damage. Our lab previously identified this pathway as a key player in the increased cytotoxic T cell infiltration characteristic of MSI CRCs. As MSI CRCs do not contain more cyDNA than CIN, we hypothesize that specific sequence or structural patterns exist within cyDNA from different CRC subtypes that lead to differential STING activation and thereby induction of overall anti-tumor immunity.

Methodology. We first aimed to examine whether cyDNA produced in MSI vs CIN CRC cells differentially induce STING and thereby anti-tumor immunity. CyDNA isolated from MC38 CRC cells with mutations in *Mlh1* (modeling MSI) or *Kras* (CIN) were used to stimulate dendritic cells (DCs), key mediators of anti-tumor immunity. We found equal concentrations of MSI cyDNA more strongly activates STING, and thereby cytotoxic T cells, than CIN cyDNA. CyDNA from MSI CRC patient organoids showed similar results. Next, we aimed to characterize specific changes in cyDNA composition in MSI vs CIN CRC cells and examine how these alterations affect STING signalling. Sequencing identified patterns prevalent in highly stimulatory cyDNAs, including microsatellites. Stimulation with oligos and cyDNA enriched for microsatellites indicate enhanced STING and cytotoxic T cell activation through improved cGAS binding. Likewise, adoptive transfer of DCs activated by microsatellite rich DNAs induced stronger DC and T cell activation in orthotopic CIN tumors. Preliminary data suggests secondary DNA structures may influence this process. Finally, we aim to evaluate the contribution of micronuclei on STING activation in MSI vs CIN CRCs. DC stimulations with micronuclei isolated from MSI and CIN cells indicate MSI micronuclei also lead to enhanced STING activation over CIN micronuclei. We will evaluate micronuclei composition to identify underlying mechanisms.

Significance. CRC is the second most deadly cancer worldwide, and immune recognition is crucial to treatment efficacy. This project will further our understanding of immune differences between MSI and CIN CRCs that may be used to develop therapeutic strategies, such as STING agonists, to improve CIN patient prognosis.

#6128 Targeting DHX9 helicase with the novel small-molecule inhibitor ATX968 induces replication stress and innate immunity in small cell lung cancer.

Takahiko Murayama[1], Jun Nakayama[2], Xueying Ma[1], Siddharth Balachandran[1], Matthew H. Daniels[3], Jennifer Castro[3], Israel Canadas[1]

[1]Fox Chase Cancer Center, Philadelphia, PA,[2]Osaka International Cancer Institute, Osaka, Japan,[3]Accent Therapeutics, Inc, Lexington, MA

Small cell lung cancer (SCLC) is the most lethal type of lung cancer. The uniqueness of this tumor lies in its initial exquisite response to chemotherapy, due to its high level of genomic instability. However, most patients experience relapse within 6 to 12 months. Although the addition of immune checkpoint blockade (ICB) therapy to standard chemotherapy has been approved by the FDA, only a small fraction of SCLC patients responds to immunotherapy because of an immunologically “cold” tumor microenvironment. Recently, using genetic depletion methods, we have demonstrated that targeting DExD/H-box helicase 9 (DHX9) in SCLC cells triggers tumor-intrinsic innate immunity, enhancing immunotherapy response and suggesting an effective and unexplored strategy to convert immunologically “cold” SCLCs into “hot”. DHX9 is a multi-functional RNA helicase capable of unwinding both RNA and DNA duplexes and more complex nucleic acid structures and has been considered an undruggable nuclear protein. Here, using SCLC models we characterized ATX968, an orally bioavailable DHX9 tool inhibitor developed by Accent Therapeutics. *In vitro* treatment with ATX968 induced an aberrant accumulation of R-loops (DNA/RNA hybrids), resulting in replication stress and an increase in DNA damage-derived cytoplasmic DNA in SCLC cells. In addition, ATX968 treatment led to an accumulation of cytoplasmic double-stranded RNA (dsRNA), which mainly derived from repetitive element sequences. Both cytoplasmic nucleic acids contributed to the induction of an innate immune response in SCLC cells. Notably, oral treatment with ATX968 monotherapy resulted in robust and durable tumor regression in SCLC mouse xenograft models, which was tolerable over a 28-day treatment period. Together, these findings suggest that DHX9 is a crucial repressor of tumor-intrinsic innate immunity and replication stress, and inhibitors of DHX9 represent a promising new therapeutic strategy for SCLC treatment and potentially other “cold” tumors characterized by replication stress and genomic instability.

#6129 Cancer cell attenuate tumor-associated macrophage TLR9 pathway through lipid-TFEB-PLD3 axis to induce immune suppression environment in ESCC.

Licheng Tan, Beilei Liu, Hongyu Zhou, Shuang Zhang, Bowen Yao, Jiayi Huang, Xinyuan Guan

University of Hong Kong, The - Li Ka Shing Faculty of Medicine, Pok Fu Lam, China

Introduction: Esophageal squamous cell carcinoma (ESCC) is one of the most threaten malignancies in China. Immunotherapy targeting PD-1/PD-L1 and CTLA-4 has achieved progress, while there remains about 30% patients that did not response. Tumor-associated macrophages (TAMs) are an important component of tumor microenvironment (TME), which may contribute to immunosuppression, tumor progression, and metastasis. Thus, targeting to TAMs in ESCC is with potential translational value. Phospholipase D3 (PLD3) is an exonuclease to degrade single strand DNA/RNA in lysosome to attenuate toll-like receptor 9 signaling (TLR9) in myeloid cells. Mice with PLD3 homozygous deletion sufferd from severe autoimmune disease. However, the role of PLD3 in TME has not been reported. Considering the innate immune inhibition effect of PLD3, we decide to explore its function in TAMs.
Material: We collected normal tissue, tumor, and metastatic lymph node samples from ESCC patients for single-cell RNA sequencing. After quality control, we got a total of 85263 cells and clustered cells by Seurat package. Then we integrated our cohort with 3 other ESCC single-cell sequencing cohorts and focused on macrophages. clusterProfiler package were used to do pathway enrichment analysis. JASPR were used for transcription factor prediction. THP-1 cells were cultured in complete RPMI1640 medium. Mouse bone marrow-derived macrophages (BMDM) were isolated and cultured as mentioned in references. KYSE-30, 180, 410, and 520 cell lines were cultured in complete RPMI1640 medium. mEC25 and mEC35 cell lines were cultured in complete DMEM medium.
Result: After integration and clustering, we found PLD3 was specifically expressed in macrophages among all cell types. After sub-clustering, only a small part of macrophages showed high expression of PLD3, which was defined as PLD3-high macrophages. PLD3-high macrophages presented an enrichment in tumors and metastatic lymph nodes, and pathway analysis revealed an inhibition of innate immune response and upregulation of cholesterol metabolism and lysosome pathway in these macrophages. Immunofluorescence staining verified these macrophages in ESCC patient samples. In vitro results showed that oxidized low-density lipoprotein and tumor cell conditioned medium could increase PLD3 expression both in THP-1 macrophages and BMDM, and attenuated TLR9 downstream cytokines expression. Cholesterol assay found ESCC tumor cells can secret considerable amount of cholesterol to extracellular environment. Since TFEB is the key transcription factor in macrophage lysosome biogenesis, we found TFEB may bind to the promoter region of *PLD3* to start its transcription.
Conclusion: Tumor cells can secret cholesterol to trigger TFEB/PLD3 signaling and suppress TLR9 activation in TAMs to achieve an innate immune inhibition effect in TME.

#6131 Temozolomide induces innate immune responses via the production of double-stranded RNAs in glioblastoma cells.

Natsuki Koide, Tatsunori Nishimura, Miho Suzuki, Keiko Shinjo, Yutaka Kondo

Nagoya University, Nagoya, Japan

Immunotherapy using immune checkpoint inhibitors (ICIs) has revolutionized cancer treatment by enhancing T cell-mediated anti-tumor immunity. However, its efficacy in glioblastoma (GBM) remains limited, as demonstrated by unsuccessful clinical trials. The primary challenge is the low abundance of tumor-infiltrating lymphocytes (TILs) within the GBM tumor microenvironment (TME), which fosters an "immune-cold" or "immune-suppressive" state, rendering the tumor resistant to immunotherapy. The first-line treatment for GBM is temozolomide (TMZ), and several studies have examined whether TMZ improves patient outcomes when combined with ICIs. However, one such trial, Ipi-Glio, did not demonstrate improved outcomes with the addition of ipilimumab to TMZ. In contrast, the MAYA study in metastatic colon cancer patients showed clinical benefits when patients were treated with TMZ followed by low-dose ICIs. As a result, it remains unclear whether TMZ has synergistic effects with ICIs in GBM treatment. To address this question, we consider the following: Does TMZ increase the abundance of TILs within the GBM TME? The recruitment and activation of TILs depend on pro-inflammatory cytokines, including type I interferons (IFN-I), which bridge innate and acquired immunity. Thus, we further investigate: Does TMZ stimulate IFN-I production to enhance TIL infiltration?When the U251 GBM cell line was treated with TMZ, the cells expressed IFNB1, a type of IFN-I, as well as interferon-stimulated genes (ISGs). We also aimed to clarify the pathway through which IFN-I is induced. Several pathways are known to activate IFN-I, so we knocked down the expression of key intermediates in these pathways and treated the cells with TMZ. As a result, the upregulation of IFNB1 and ISGs induced by TMZ was diminished when RIG-I was knocked down. RIG-I is a pattern recognition receptor that detects double-stranded RNA (dsRNA). This finding suggests that the IFN-I induction observed in TMZ-treated GBM cells is linked to the presence of dsRNA within the tumor cells. In this presentation, we will discuss the origins of the dsRNAs and whether TMZ-induced IFN-I is sufficiently strong to transform the "immune-cold" or "immune-suppressive" GBM TME into an "immune-hot" state.

#6132 The Fes kinase plays an innate immune checkpoint role to suppress CD8+ T cell mediated anti-tumor immunity.

Brian J. Laight, Danielle Harper, Natasha Dmytryk, Connie S. Zhang, Changnian Shi, Sameh Basta, Madhuri Koti, Peter A. Greer

Sinclair Cancer Research Institute, Kingston, ON, Canada

Introduction: Immunotherapy is a powerful pillar of cancer therapy that still faces barriers due to cancer-mediated immunosuppression. Immunogenic cell death recruits and activates antigen-presenting cells (APCs) through engagement of pattern recognition receptors (PRRs), leading to pro-inflammatory Signal 3 cytokine production required for full activation of adaptive immune cells, including cytotoxic T lymphocytes (CTLs). The Fes tyrosine kinase suppresses innate immune responses in APCs by inhibiting PRR signaling and regulating cytokine trafficking. In non-cancer contexts, Fes may guard against consequences of overactive innate immunity, such as endotoxic shock or autoimmunity. However, this inhibitory effect on APCs may serve as a checkpoint to suppress successful anti-cancer immunotherapy by obstructing efficient priming of cancer specific CTLs. We hypothesize that Fes inhibition in APCs will improve Signal 3 cytokine signaling, resulting in greater adaptive immunity and tumor control.

Methodology: Using bone marrow derived macrophages (BMDMs) from wildtype (WT) or Fes knockout (*fes*$^{-/-}$) mice, we assessed LPS-induced PRR signalling by immunoblot analysis, production of key Signal 3 cytokines by multiplex ELISA, and the level of cell-associated cytokines by flow cytometry. The ability of BMDMs to prime CTLs was assessed in antigen cross-presentation co-cultures with OT-1 T cells. Using E0771 and B16F10 syngeneic orthotopic mouse engraftment models, we compared the efficacy of doxorubicin and anti-PD-1 to control tumor growth and prolong survival in WT and *fes*$^{-/-}$ mice. Tumors and spleen immune profiles were analyzed by flow cytometry. WT or *fes*$^{-/-}$ BMDMs were adoptively transferred into E0771 and B16F10 tumours to directly assess their relative ability to control tumour growth.

Results: *fes*$^{-/-}$ BMDMs displayed improved PRR signaling, increased IFNβ, IL-12, and TNFα secretion, and greater levels of internal and cell-surface IL-12 following LPS stimulation. In antigen cross-presentation assays, LPS stimulated *fes*$^{-/-}$ BMDMs had improved CTL priming. Tumor control and survival were greater in *fes*$^{-/-}$ mice in both the E0771 and B16F10 engraftment models. Doxorubicin and the combination of doxorubicin and anti-PD-1 were more effective in *fes*$^{-/-}$. This was especially striking in the B16F10 model, which is known to be resistant to PD-1 therapy. Immune profiling of tumors showed increased CTL and NK cell activation and PD-1 positivity, and a shift from predominately M2- to M1-polarized tumor associated macrophages in *fes*$^{-/-}$ mice. Intratumorally injected M1-polarized *fes*$^{-/-}$ BMDMs were better able to control tumor growth in both E0771 and B16F10 models.

Conclusion: Fes acts as an innate immune checkpoint whose inhibition may enhance anti-cancer immunotherapy. We believe this effect is due to an increase in inflammatory signalling, and improved signal 3 cytokine signalling in *fes*$^{-/-}$ APCs.

#6133 Investigating the role of CXCR2 in NK cells in head and neck cancer.

Lindsey M. Bachmann[1], Donatien Kamdem Toukam[2], Maria Lehn[2], Sam Nusbaum[2], Katelyn Jansen[3], Vinita Takiar[4], Dalia El-Gamal[2], Trisha Wise-Draper[2]

[1]University of Cincinnati College of Medicine, Cincinnati, OH,[2]Hematology and Oncology, University of Cincinnati, Cincinnati, OH,[3]University of Cincinnati, Cincinnati, OH,[4]Radiation Oncology, University of Cincinnati, Cincinnati, OH

Head and neck cancer (HNC) is the sixth most common cancer globally, with 90% of HNC comprised of head and neck squamous cell carcinoma (HNSCC). Despite improvements in anticancer therapies, long-term survival rates for patients with advanced-stage HNSCC have remained stagnant for 30 years. Although the field of immunotherapy has primarily focused on the adaptive immune response, accumulating evidence suggests that natural killer (NK) cells are also important. Activation of the C-X-C chemokine receptor 2 (CXCR2) signaling axis has been implicated as a negative prognostic factor in many cancers, including HNSCC. Inhibition of CXCR2 results in reduction of tumor growth and increased infiltration of NK and T cells at least partially through suppressing myeloid derived suppressor cell (MDSC) recruitment. Our laboratory has recently demonstrated that the CXCL1/CXCR2 axis inhibits antitumor functions, including a direct attenuation of NK cellular cytotoxicity *ex vivo*, thereby promoting immune resistance and tumor growth. Here we investigate the effects of pharmacologic or genetic inhibition of CXCR2 *in vivo* using established murine oral cavity tumor models. Wild type C57BL/6 mice were injected into the buccal region with 1.5×10^6 mouse oral cavity (MOC-1) cells. Once tumors reached 50-150mm^3, mice were treated by IP injection 3 times per week for 3 weeks with either vehicle, 10mg/kg of CXCR2 inhibitor (SB225002), 1µg/kg of CXCL1, or the combination. Mice that were treated with the CXCR2 inhibitor (CXCR2i) had decreased tumor growth, increased immune cell infiltration (NK and T cells), and increased anti-tumor cytokines such as IFN-γ and IL-1β in the serum. Interestingly, the antitumor effects of the CXCR2i were abrogated by the addition of CXCL1. Furthermore, to determine if NK and/or T cells contributed to CXCR2i-mediated tumor control, C57BL/6 mice were injected into the flanks with MOC-1 tumors as detailed above and treated with either vehicle or CXCR2i in the presence or absence of antibodies to deplete NK and/or CD8+ T cells. Again, the observed decrease in tumor growth was abrogated in mice depleted of NK cells, CD8+ T cells, or both NK and CD8+ T cells suggesting that both cytotoxic cell types are important for inhibiting tumor growth upon CXCR2 blockade. Visualization of immune cell infiltration within tumor sites via multiplex immunofluorescence staining is ongoing. Next, we explored genetic inhibition of CXCR2 *in vivo*. Interestingly, MOC-1 cells injected into the flanks of C57BL/6 mice in which *Cxcr2* was globally knocked out had a paradoxical effect of enhanced tumor growth, suggesting that *CXCR2* may be important during development for immune cell function. Studies are ongoing to examine how NK cell-specific *CXCR2* knockout influences HNSCC tumor progression using an NK cell conditional *Cxcr2* knockout murine model. These preliminary results suggest that the CXCL1/CXCR2 axis is in fact important for HNSCC tumor control.

#6134 Identifying the mechanism of acquired resistance against cancer immunotherapy targeting innate immunity.

Hitomi Nishinakamura[1], Sayoko Shinya[2], Takuma Irie[1], Shugo Sakihama[3], Takeo Naito[1], Keisuke Watanabe[1], Daisuke Sugiyama[4], Motohiro Tamiya[5], Tatsuya Yoshida[6], Tetsunari Hase[7], Takao Yoshida[8], Kennosuke Karube[9], Yuka Maeda[1], Shohei Koyama[1], Hiroyoshi Nishikawa[1]

[1]National Cancer Center Research Institute, Tokyo, Japan,[2]Discovery and Research, Ono Pharmaceutical Co., Ltd., Osaka, Japan,[3]Laboratory of Hemato-Immunology, Graduate School of Health Sciences, University of the Ryukyus, Nishihara, Japan,[4]Department of Immunology, Nagoya University Graduate School of Medicine, Nagoya, Japan,[5]Respiratory Medicine, Osaka International Cancer Institute, Osaka, Japan,[6]Department of Thoracic Oncology, National Cancer Center, Tokyo, Japan,[7]Department of Respiratory Medicine, Nagoya University Graduate School of Medicine, Nagoya, Japan,[8]Research Canter of Oncology, Ono Pharmaceutical Co., Ltd., Osaka, Japan,[9]Department of Pathology and Laboratory Medicine, Nagoya University Graduate School of Medicine, Nagoya, Japan

Immune checkpoint blockade (ICB) efficiently reactivates effector T cells and induces antitumor efficacy. However, more than half of patients, particularly patients with the immunologically cold tumor microenvironment (TME), failed to experience clinical benefits, even in combination with other drugs. To augment the antitumor efficacy by ICB therapy, various attempts to modify the immunologically cold TME are under investigation. The activation of innate immune cells, particularly antigen-presenting cells (APCs), through pattern recognition receptors, such as Toll-like receptors (TLRs), is essential for the subsequent activation of acquired immunity, including $CD8^+$ T cells. However, several approaches employing ICB and reagents that can activate innate immunity have not shown antitumor efficacy in clinical trials, suggesting the existence of a resistance mechanism(s) against the combination treatment. Here we examine the potential of the combination treatment of OK-432 (Picibanil: a penicillin-inactivated and lyophilized preparation of a low-virulence strain of *Streptococcus pyogenes*), an agonist for multiple TLRs, including TLR2, TLR4, and/or TLR9, with PD-1 blockade against tumors with immunologically cold TME. Although OK-432 treatment effectively stimulated APCs in vitro, many innate immune suppressive cells, such as polymorphonuclear-myeloid-derived suppressor cells (PMN-MDSCs), were co-activated and accumulated in the TME upon OK-432 treatment in a CXCL1-CXCR2-dependent manner (acquired resistance). Accordingly, CXCR2 neutralizing antibody or anti-Ly6G antibody reduced the recruitment of PMN-MDSCs to the TME, consequently the triple combination with CXCR2 neutralizing antibody/anti-Ly6G antibody, OK-432, and PD-1 blockade exhibited a far stronger anti-tumor effect. On the other hand, anti-Ly6G antibody combined with PD-1 blockade sometimes inhibited tumor growth in certain tumor models inherently harboring the TME with abundant PMN-MDSCs (adaptive resistance). To further address the difference, tumor-infiltrating immune cells were comprehensively examined in 10 cancer cell lines; a PMN-MDSC-to-CD8 ratio and an infiltrating $CD45^+$ cells count became biomarkers for the necessity of the triple combination treatment. Thus, we argue that stimulators of innate immunity, such as TLR agonists including OK-432, are a double-edged sword. A stimulator of innate immunity may become an effective cancer immunotherapy by controlling the acquired resistance mechanism, namely, the activation of innate immune suppressive cells in the TME.

#6135 Characterization of monocytic-myeloid populations in the tumor microenvironment using patient-derived organoid xenograft models and macrophage-depleting therapies in esophageal squamous cell carcinoma.

Bryan Chee-chad Lung[1], Alvin Ka-kiu Leung[1], Ian Yu-hong Wong[2], Dora Lai-wan Kwong[1], Anthony Wing-ip Lo[3], Simon Law[2], Wei Dai[1], Maria Li Lung[1], **Valen Z. Yu**[1]

[1]Department of Clinical Oncology, School of Clinical Medicine, LKS Faculty of Medicine, Hong Kong, Hong Kong,[2]Department of Surgery, School of Clinical Medicine, LKS Faculty of Medicine, Hong Kong, Hong Kong,[3]Division of Anatomical Pathology, Queen Mary Hospital, Hong Kong, Hong Kong

Background: Esophageal squamous cell carcinoma (ESCC) is the predominant and highly lethal form of esophageal cancer in Asia. Monocytic-myeloid cells, including monocytes, myeloid-derived suppressor cells (MDSCs), and macrophages, are key components of the tumor microenvironment (TME) and represent promising targets for cancer immunotherapy. Tumor-associated macrophages (TAMs), which likely derive from both resident tissue macrophages and recruited monocyte-derived macrophages, play critical roles in the TME, although the precise origins of TAM subsets remain unclear. Utilizing our latest ESCC patient-derived organoid xenograft (PDOX) model, coupled with single-cell transcriptomic profiling, we aimed to elucidate the distinct subsets of monocytic-myeloid cells within the TME.
Results: We used two distinct monocyte/macrophage-depleting agents to selectively target resident and recruited macrophages in our ESCC PDOX model. Through single-cell profiling of xenografts from treated mice, we analyzed the characteristics and origins of monocytic-myeloid cell subsets in the TME and identified novel biomarkers associated with newly differentiated resident and recruited macrophage populations that contribute to TAMs. Specifically, we found that C1qa is a key marker of fully differentiated mature macrophages in ESCC. Additionally, we identified a bone marrow-derived monocytic-myeloid subset that was negative for C1qa and Cd68, resembling immature macrophages or partially differentiated monocytes. These cells were similar to the conventionally described S100a9-positive MDSCs but displayed distinct biomarker profiles. Using gene set analyses based on the single-cell enrichment index, we characterized the functional features of individual subsets and found that prolonged residence of macrophages within the TME led to polarization towards a pro-tumor M2-like TAM phenotype. Furthermore, we identified a distinct population of resident tissue macrophages expressing LYVE1, which exhibited pronounced angiogenic capabilities and characteristics typical of the M2-like phenotype. Based on molecular and signaling analyses, we speculated on the developmental trajectories and interactions between individual subsets. These findings were further validated in human ESCC samples.
Conclusions: Our findings provide new insights into the roles and origins of diverse monocytic-myeloid populations in the TME, their unique biomarker profiles, and the refined definition of recruited MDSCs, offering potential therapeutic targets for ESCC immunotherapy.
Acknowledgement: We thank the RGC (TRS T12-701/17-R to MLL and SL) and FHB (HMRF 06171566 to VZY) of HKSAR for funding support. We thank DSMZ for providing the KYSE cell lines and HKUMed-CPOS for imaging facilities.

#6136 Efficacy of LPS, flagellin, IFN-g, and sCD40L treated bone marrow-derived macrophages in murine mammary carcinoma.

Robert A. Kurt, Joshua Finn, Melissa Lass, Ernesto Oviedo-Bermudez

Biology, Lafayette College, Easton, PA

Previously we reported that treating bone marrow-derived macrophages (BMDM) with poly(I:C), IFN-γ, and sCD40L generated BMDM with more consistent anti-tumor activity than BMDM generated with the agonists alone. These BMDM also showed significant anti-tumor activity against the EMT6 and 168 murine mammary carcinoma models. However, the BMDM showed no activity against the 4T1 murine mammary carcinoma model. Here we asked whether BMDM generated with IFN-γ and sCD40L in combination with other TLR agonists would have greater anti-tumor activity than the poly(I:C) generated BMDM. For this purpose we generated BMDM and then activated them with IFN-γ, sCD40L, and either LPS, flagellin or R848. BMDM generated with a combination of four agonists (IFN-γ, sCD40L, LPS and flagellin) were also evaluated. Based on *in vitro* analysis these treatments led to a significant increase in nitrite and IL-12 production, and the levels of nitrite and IL-12 were significantly greater than the levels produced by the poly(I:C) generated BMDM. All treatments also led to a significant increase in expression of *il-1b*, *il-6*, *tnf-a*, *il-18*, *ccl5*, *cxcl9*, *cxcl10* and *cxcl11*. Moreover, the LPS, flagellin and R848 generated BMDM often expressed a significantly higher level of cytokines than the poly(I:C) generated BMDM. All treatments led to a significant increase in MHC Class II or CD80 expression, but only BMDM generated with IFN-γ, sCD40L, LPS, flagellin and BMDM generated with IFN-γ, sCD40L and LPS showed a significant increase in both MHC Class II and CD80 expression. Furthermore, BMDM generated by these same treatments showed significant anti-tumor activity against all three murine mammary carcinoma models; EMT6, 168, and 4T1. Depletion studies revealed that the BMDM exerted anti-tumor activity against the 4T1 and EMT6 tumors by recruiting CD8+ T cells and NK cells suggesting that expression of *ccl5*, *cxcl9*, *cxcl10*, and *cxcl11* may be critical for the *in vivo* anti-tumor activity of these BMDM.

#6137 Inhibition of FXR signaling in tumor-associated myeloid cells protects against melanoma progression.

Shiun Chang, Darwin C. Chang, Rachel V. Jimenez, Rosa A. Sierra-Mondragon, Julio A. Vazquez Martinez, Paulo C. Rodriguez

H. Lee Moffitt Cancer Center, Tampa, FL

Our study delves into the complex interplay between the tumor microenvironment and immune cell regulation, with a particular focus on myeloid-derived suppressor cells (MDSCs). A key discovery of our research is the accumulation of bile acids (BAs) within the tumor microenvironment and tumor-infiltrating myeloid cells. BAs significantly enhance the suppressive function of MDSCs and M2 macrophages via activation of Farnesoid X Receptor (FXR).Using melanoma as our primary model. We demonstrate that modulation of MDSCs' suppressive function can be achieved through the targeting FXR using specific agents like GW4064 and z-Guggulsterone (z-GS). Myeloid-specific elimination of FXR via Lysozyme M Cre led to a significant delay in tumor progression. This was accompanied by the reduction of PMN-MDSC and an increased infiltration of CD45 positive immune cells and a surge in the presence of anti-tumor specific T cells. Administration of FXR inhibitor, z-GS, through oral gavage also reduced tumor size in vivo. Additionally, targeting FXR by z-GS or myeloid specific genetic ablation enhances adoptive T cells transfer therapy efficacy. MDSCs with impaired FXR signaling result in reduced mitochondria activity and downregulation of Arg1. Furthermore, an enhanced antigen presentation function is observed in MDSCs and dendritic cells treated with z-GS. Our research presents a compelling case for targeting FXR signaling in myeloid cells as a novel therapeutic strategy and open new pathways for enhancing anti-tumoral immunity.

#6138 Generation of humanized C1Q mice for antibody-mediated CDC therapy research.

Mari Kuraguchi, James Jin, Ruili Lv, Zan Zhang, Jinyi Ma, Jiangfeng Yuan, Xuan Yu, Zhi Zhang, Jiansu Zhang, Xiaofei Zhou

Biocytogen, Waltham, MA

The complement system is an essential part of innate immunity, consisting of more than 30 proteins. A critical element in the pathway is C1q (Complement 1 q), an important recognition molecule in the classical pathway of the complement system that can initiate the classical pathway and plays a major role in bridging innate and adaptive immunity. C1q is also vital in monoclonal antibody (mAb) therapy, as it attaches to the Fc region of antibodies, initiating complement-dependent cytotoxicity (CDC).
Here, we generated a humanized C1Q model (B-hC1Q mice), by replacing the murine C1qa, C1qb, and C1qc genes that encode the full-length protein with the corresponding human C1QA, C1QB, and C1QC coding regions. We confirmed the exclusive expression of human C1Q in the serum of the humanized model at levels comparable to those of wild-type murine C1Q. We also performed classical pathway complement activity analysis on B-hC1Q mice, and the results showed that humanized C1Q performed the proper complement function in mice.
To evaluate the CDC effector of monoclonal antibodies in eliminating cancer cells, we conducted tumor efficacy experiments using B-hCD38-luc E.G7-OVA cells, engineered to express human CD38, injected via the tail vein into B-hC1Q mice. Treatment with Daratumumab analog effectively abolished tumor growth in humanized C1Q mice. Together, the B-hC1Q mice provide *in vivo* platforms to evaluate complement system-related disease and CDC effector functions in tumor therapy.

#6139 Changes in macrophage phenotype and function with aging affects tumor growth and tumor microenvironment in murine cancer models.

Manasa Suresh[1], Marie Durr[1], Xintang Li[1], Bryan Weselman[1], Francisco Tapia Belmonte[2], Nithya Gajendran[1], David Quiceno-Torres[1], Sonia Sebaoui[1], Adhithi Rajesh[3], Matias Hepp[4], Alexis Salas-Burgos[2], Satish Noonepalle[1], Alejandro Villagra[1]

[1]Department of Oncology, Georgetown Lombardi Comprehensive Cancer Ctr., Washington, DC,[2]Pharmacology Department, Concepcion University, Concepcion, Chile,[3]Georgetown University, Washington, DC,[4]Universidad Catolica de la Santisima Concepcion, Concepcion, Chile

Introduction: Tumor-associated macrophages (TAMs) constitute a significant population of immune cells in the tumor microenvironment (TME), mainly exhibiting an anti-inflammatory M2-like phenotype. They secrete angiogenic growth factors that support tumor growth with an immunosuppressive TME and contribute to developing resistance against immunotherapies. Therefore, there is an increasing effort to switch TAMs from protumoral M2-like to antitumoral and pro-inflammatory M1-like phenotype. Aging is a critical risk factor, with global cancer incidences increasing from 45 to 75 years of age and declining thereafter. The reason behind this decline beyond 75 years of age is unknown but needs further investigation. With the underrepresentation of aged population in preclinical and clinical studies, the role of aging in cancer development and the TME are understudied. Considering the multidimensional role of macrophages in the TME, this study determines the effects of age-associated changes in macrophage function and phenotype on tumor development.
Objective: To study the changes in macrophage biology with aging and understand the implications of aging macrophages on the TME in murine cancer models.
Methods: Wildtype mice of ages 6-8 WKS, 3-6 MO, 10-12 MO, and 20-24 MO were implanted with tumor cells (SM1 melanoma, 4T1 breast cancer) to study tumor growth kinetics. Immune cell profiling of TME, including TAMs, was performed using flow cytometry, single-cell RNAseq, and secretome analysis. Bone marrow-derived macrophages isolated from tumor-bearing and healthy mice of the above four age groups were polarized to M1 and M2 phenotypes to study age- and cancer-associated changes in macrophage function, phenotype, and metabolic signature. The changes in macrophage cytokine and chemokine secretion levels with aging were determined by multiplex ELISA assays.
Results: Tumor growth in older mice was significantly slower than in younger mice. A distinct change in immune cell populations within the TME and reduced secretion profile of TAMs with aging was observed from single-cell analyses. Additionally, the single-cell secretome analysis reflected an inefficient monocyte-to-macrophage transition in tumors of older mice. The defect in macrophage polarization to M1- and M2-like phenotypes with aging complemented with the sub-optimal function (phagocytosis, antigen presentation, and migration) and metabolic signatures of aged macrophages.
Conclusions: The phenotypic, functional, and metabolic changes in macrophages with aging significantly affect tumor growth in murine cancer models. Our study further emphasizes the important role of macrophages in the TME and the need for better representation of aged populations in preclinical and clinical studies.

#6140 Therapeutic benefits of innate immunity stimuli in hepatocellular carcinoma.

Longyue Lily Cao, Jonathan Kagan

Boston Children's Hospital, Boston, MA

Beyond personal hygiene, the most impactful public health innovation in human history has been the development of vaccines to protect against lethal infections. Cancer vaccines have long-been envisioned, with minimal clinical success. Hepatocellular carcinoma (HCC) is the most common liver tumor and one of the leading causes of cancer-related mortality worldwide. Unfortunately, most HCC patients fail to respond to widely used T-cell based immunotherapies, likely due to the relatively immunotolerant nature of the disease. Thus, there is an urgent need to develop more effective therapies against HCC. Dendritic cells (DCs) are an attractive immunotherapeutic target given their unique ability to induce anti-tumor T cell responses. Various key DC activities are needed for this function, including migration to draining lymph nodes, tumor antigen presentation, co-stimulation, and production of inflammatory cytokines like interleukin (IL)-1β. Conventional DC stimuli, such as the Toll-Like Receptor ligand lipopolysaccharide (LPS), activate only a subset of these activities. For instance, production of IL-1ß, a cytokine important for memory T cell function, requires a second signal that activates the NLRP3 inflammasome. Commonly used adjuvants that activate NLRP3 inflammasomes and induce IL-1ß release also trigger cell death, undermining the ability for DCs to engage in the other activities required for T cell stimulation. Our lab discovered that the oxidized phospholipid PGPC (1-palmitoyl-2-glutaryl-sn-glycero-3-phosphocholine) can augment IL-1ß release and migration of DCs while maintaining viability, generating a “hyperactive” DC (hDC) state that engender superior T cell responses compared to conventionally activated DCs. To study the anti-tumor benefits of hDCs, we adopted a genetically engineered mouse model of HCC that highly recapitulates human disease. We show that subcutaneous immunizations with hDCs suppress tumor growth and lethality, and that this protective phenotype is NLRP3-dependent. We also show that hDCs are uniquely able to traffic from the subcutaneous immunization site into the liver. Tumors isolated from hDC-immunized mice have an enrichment of antigen-specific CD8+ T cells with more robust IFNγ-production. Furthermore, hDC treatment resulted in a smaller proportion of antigen-specific CD8+ T cells expressing markers of dysfunction, such as PD-1. Remarkably, at 120 days post-tumor induction, there were more antigen-specific tissue resident memory T cells in the livers of mice previously treated with hDCs, and more of these T cells retained the capacity for antigen-specific IFNγ production. Our project demonstrates how novel concepts in innate immunity can be leveraged to engender more potent and durable anti-tumor T cell responses, and to generate vaccines against HCC that can overcome common limitations of conventional immunotherapies.

#6142 Functional reprogramming of neutrophils in the tumor microenvironment.

Filippo Veglia, Alessio Ugolini, Alessandra De Leo

The Wistar Institute, Philadelphia, PA

Despite functional heterogeneity, high frequency of intratumoral neutrophils predicts poor clinical outcome in cancer, including brain tumors. The tumor microenvironment reprograms neutrophils into immunosuppressive subsets that hinder anti-cancer immunity, thereby contributing to tumor growth and poor response to immunotherapy. However, the mechanisms underlying the regulatory reprogramming of neutrophils remain elusive. Here, we report that neutrophils suppress T cell function in mouse and human brain tumor tissues, contrasting with their non-immunosuppressive phenotype in the periphery. The immunosuppressive ability of brain-tumor infiltrating neutrophils was restricted to a subset of highly glycolytic neutrophils expressing the transferrin receptor CD71, which acquired immunosuppressive properties in response to hypoxia. Mechanistically, hypoxia boosted glucose metabolism in $CD71^+$ neutrophils, which was accompanied by high avidity for glucose and production of lactate. Intracellular lactate caused lactylation of histone lysine residues that directly regulated the expression of arginase 1 (ARG1), required for T cell suppression. Pharmacological inhibition of glycolysis, lactate production or the histone lactylation writer CBP/P300, impaired histone lactylation and the expression of ARG1 and, accordingly, reduced the immunosuppressive activity of $CD71^+$ neutrophils. Targeting histone lactylation with the anti-epileptic drug isosafrole blocked the immunosuppressive ability of $CD71^+$ neutrophils, delayed tumor progression and sensitized brain tumors to immunotherapy. Furthermore, a distinctive gene signature characterizing immunosuppressive and glycolytic $CD71^+$ neutrophils correlated with adverse clinical outcomes across diverse human malignancies. Thus, our study identifies hypoxia-driven histone lactylation as a critical determinant of the functional reprogramming of neutrophils within tumors, providing a potential therapeutic strategy to counteract neutrophil-induced immunosuppression.

#6143 Harnessing antiviral innate immunity regulated by *BRCA1 p*seudogene RNA in preclinical cancer models.

Yoo-Jane Han, Jing Zhang, Sulin Wu, Galina Khramtsova, Daniel Peiffer, Olufunmilayo I. Olopade

University of Chicago, Chicago, IL

Background: Antiviral innate immune responses hold intrinsic anticancer benefits by promoting antitumor immunity and thereby increasing the efficacy of chemotherapy and immunotherapy. They mediate essential antitumor responses through several mechanisms, including stimulation of cytokine production and activation of cytotoxic immune cells. Both cancer cells and virus-infected cells threaten the host by expressing damage-associated molecular patterns and by evading control mechanisms of host immune surveillance. Interestingly, current evidence suggests that not only exogenous non-self-RNA but also host-derived pseudogene RNAs regulate antiviral innate immunity.

Methods: Expression of the *BRCA1* pseudogene (*BRCA1P1*)-long noncoding RNA (lncRNA) was measured in 28 different cell lines using qRT-PCR and inhibited using CRISR-Cas9 genome editing tools or antisense oligonucleotides (ASOs). In patient samples, *BRCA1P1*-lncRNA was detected by BaseScope RNA-*In Situ* Hybridization. For phagocytosis assays, pHrodo™ red-positive cells were determined in cancer cells co-cultured with macrophage cells. Patient-derived organoids (PDOs) were established from primary and metastatic breast tumors and transfected with *BRCA1P1*-specific ASOs using electroporation. *In vivo*, mice were humanized with human PBMC and engrafted with breast cancer cells +/- *BRCA1P1*-knockout. Immune infiltration into tumors was measured by flow cytometry and immunohistochemistry.

Results: We discovered the regulation of viral mimicry and antiviral immunity by transcripts of *BRCA1P1*, which serve as immunoregulatory RNAs in pan-cancer cells and preclinical models of human cancers. *BRCA1P1* is expressed in 13 types of human (solid and blood) cancers, with elevated expression in cancer cells compared to normal cells. Depletion of *BRCA1P1* induced antiviral gene expression, stimulated apoptosis, and increased sensitivity to doxorubicin in various cancer cells, with no apoptotic effect on non-malignant cells. Subsequently, the increase in antiviral responses triggered macrophage-induced phagocytosis of *BRCA1P1*-depleted cancer cells. Mechanistically, *BRCA1P1* regulated NF-κB-driven antiviral gene expression in the nuclei of cancer cells. In PDOs of breast tumors, *BRCA1P1* depletion significantly inhibited growth of primary and metastatic tumor PDOs. Lastly, in a humanized mouse model of breast cancer, loss of *BRCA1P1* stimulated cytokine expression and immune cell infiltration into tumors, and reduced tumor growth.

Conclusion: This study identifies an important role for *BRCA1P1* in regulating innate immune defense mechanisms and antitumor responses in pan-cancers and preclinical models of human tumors. This mechanism of antitumor viral immunity regulated by a host-derived pseudogene RNA may guide the development of novel therapies to boost antitumor immune responses in various human cancers.

#6144 Peritoneal and omental macrophages provide anti-tumor immunity in an ovarian cancer model.

Joseph R. Dominguez[1], Jiseon Kim[1], Seung Hyeon Kim[1], Yeonwoo Choi[1], Soroush Tahmasebi[1], Brahm H. Segal[2], Burkhard Ludewig[3], Ki-Wook Kim[1]

[1]Department of Pharmacology and Regenerative Medicine, University of Illinois College of Med. at Chicago, Chicago, IL,[2]Department of Internal Medicine, Roswell Park Comprehensive Cancer Center, Buffalo, NY,[3]Institute of Immunobiology, St. Gallen, Switzerland

The omentum is a visceral adipose tissue that contains fat-associated lymphoid clusters (FALC) to aid in peritoneal immunity. These clusters consist of large aggregates of B cells, T cells, dendritic cells, macrophages, and innate lymphoid cells that participate in the clearance of pathogens. Tissue-resident macrophages (TRM) are immune cells found in every tissue and their function is influenced by the local tissue microenvironment. TRM can be defined by their origins, consisting of subsets maintained either by embryonic precursors or by bone-marrow progenitors. A recent study found embryonically derived TRM express at least one marker amongst Tim4, LYVE1, FOLR2 while bone marrow derived macrophages maintain CCR2 expression. Previous publications have shown the existence of $LYVE1^+$ macrophages surrounding each cluster, here we study an unexamined subset of FALC-associated macrophages that are imbedded within the lymphoid structures. Using confocal imaging, flow cytometry analysis with reporter/ conditional knock-out mice, we have characterized this TRM subset as CX_3CR1^+ $LYVE1^{lo/-}$ $Tim4^-$ and CCR2 dependent. Through Wt1-driven csf1 deletion, we depleted peritoneal and omental-resident (ORM) and found increased ID8 tumor cells in comparison to littermate controls. Lastly, promotion of CX_3CR1^+ ORM and reduction of $LYVE1^+$ $Tim4^-$ ORM led to reduced tumor growth in the omentum in early-stage tumor challenge.

#6145 Serological responses to phosphorylated adducts in pancreatic ductal adenocarcinoma.

Talita Aguiar[1], Shunya Mashiko[1], Kesava Asam[2], Poulomi Roy[1], Shikun Wang[3], Katharina Frank[1], Max Dietzel[1], Luca G. Z. Schahadat[1], Mattea Ausmeier[1], Andrea Hertel[1], Zhe Ran S Susan Duan[1], Bradley Aouizerat[4], Jeanine M. Genkinger[1], Helen Remotti[1], Emmanuel Zorn[1]

[1]Columbia University Irving Medical Center, New York, NY,[2]Dental Translational Research Center, New York University, New York, NY,[3]Department of Biostatistics, Columbia University Irving Medical Center, New York, NY,[4]New York University, New York, NY

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is characterized by late-stage diagnosis and poor survival rates. Identifying biomarkers that can aid in early detection and disease characterization is critical. This study leverages a novel ELISA platform to explore IgG reactivity to chemical adducts in the plasma of PDAC patients compared to healthy donors (HD).
Materials and Methods: Plasma samples from 39 PDAC patients and 40 age- and sex-matched HD were analyzed for IgG reactivity to 93 adducts, including phosphorylated amino acids, advanced glycation end products, and coenzymes. Reactivity to phosphoryl-Thr, -Ser, and -Tyr were further validated and characterized using IgG1-4-specific secondary antibodies. Paired tumor and non-tumoral tissues from a subset of cases underwent immunofluorescence and phosphoproteomic analyses to identify phosphorylation changes.
Results: Serum IgG reactivity to phosphorylated threonine, serine, and tyrosine was significantly higher in patients with pancreatic ductal adenocarcinoma (PDAC) than in healthy donors (HD). A Boruta-based random forest classifier identified these phosphorylated adducts as the most significant target adducts discriminating between the two groups. High-titer IgG responses to these phosphorylated adducts—predominantly IgG1 and IgG3—were observed for PDAC patients. Moreover, patients with metastatic disease exhibited greater IgG reactivity to phosphorylated adducts than those without metastasis. Immunofluorescence analysis demonstrated the accumulation of phosphorylated adducts in PDAC cells, suggesting that their abundance in the tumor triggered the specific antibody response. Additionally, phosphoproteomics revealed dysregulated phosphorylation pathways in PDAC, with 124 proteins showing significantly higher phosphorylation levels in tumor tissue compared to non-tumoral tissue. Gene ontology analysis linked these proteins to processes involving chromatin organization, transcription regulation, and histone acetylation. Essential molecular functions of these aberrantly expressed proteins included chromatin and RNA binding, evoking altered gene expression.
Conclusion: Serum IgG responses to phosphoryl adducts represent a robust biomarker for PDAC detection and may reflect disease progression. These findings highlight the potential of anti-adduct IgG profiles as tools for early cancer detection and therapeutic monitoring.

IMMUNOLOGY: Interplay between Immune System and Radio-, Chemo- and Targeted Therapies 2
Poster Session

#6150 Intramammary gland activation of invariant natural killer T cells with anti-tumor CD4Th1 cells prevents the development of invasive mammary carcinoma.

Camille C. Baumrucker[1], Ganesan Ramamoorthi[1], William Dominguez-Viqueira[2], Brian J. Czerniecki[1]

[1]Clinical Science & Immunology Program, Moffitt Cancer Center, Tampa, FL,[2]Small Animal Imaging Laboratory Core, Moffitt Cancer Center, Tampa, FL

Breast cancer (BC) remains one of the most prevalent cancers in the world thus prompting efforts in risk reduction and prevention. Current strategies for prevention are limited to hormone therapy or prophylactic surgery, which are not without complication. Patients with preinvasive malignant lesions of the breast, including ductal carcinoma in situ (DCIS), if left untreated or after surgical resection, have a higher risk of developing invasive BC (IBC). In addition, clinical studies have shown that a subset of DCIS patients who did not progress to invasive disease died from recurrence or distant metastasis. These findings highlight the need for an effective prevention strategy to halt IBC progression and recurrence. Recently, we reported the critical role of anti-tumor CD4Th1 cells in preventing early BC progression. Other studies have shown that enhancement of invariant natural killer T (iNKT) cells plays a role in preventing mammary carcinoma. However, loss of CD1d expression on mammary epithelial cells can suppress iNKT cells, leading to mammary carcinoma and progression of invasive disease. We tested the efficacy of combined activation of iNKT and CD4Th1 cells with intratumoral delivery of type 1 polarized dendritic cells targeting tumor antigen (HER2-DC1) and α-galactosylceramide (α-GalCer-DC1) in BALB-HER2/neu transgenic mice bearing spontaneous invasive mammary lesions. The intratumoral activation of iNKT-CD4Th1 cells with systemic anti-HER2 antibody therapy resulted in eradication of invasive mammary lesions with improved survival of BALB-neuT mice. We then investigated the efficacy of iNKT-CD4Th1 cells in a BALB-neuT mice preventive model. The combined activation of iNKT-CD4Th1 cells in one mammary gland (mammary gland 5) with systemic delivery of anti-HER2 antibody in BALB-neuT mice before the onset of a preinvasive malignant mammary lesion induced a strong anti-tumor response. Complete prevention of mammary carcinoma was observed in all ten mammary glands (up to 27 weeks of mice age) with improved survival. We further demonstrated that activation of iNKT-CD4Th1 cells in all ten mammary glands of BALB-neuT mice in a preventive model displayed a superior anti-tumor immunity and prolonged tumor prevention with survival benefits. The immune profiling revealed an increased infiltration of iNKT, CD4T, CD4T effector memory, CD4T central memory, CD4T effector, NK, and B cells in the mammary glands of BALB-neuT mice. Importantly, iNKT-CD4Th1 combined activation strategy also decreased the level of myeloid derived suppressor cells and tumor associated macrophages in the mammary glands of BALB-neuT mice. Collectively, these results suggest that iNKT-CD4Th1 cell mediated anti-tumor immunity is an effective strategy for BC prevention.

#6151 The investigation of a new combinatorial immunotherapy strategy to treat platinum-resistant penile cancer.

Yaoyin Li[1], Jad Chahoud[2], Xin Lu[1]

[1]Department of Biological Sciences, Boler-Parseghian Center for Rare and Neglected Diseases, Harper Cancer Research Institute, South Bend, IN, [2]Department of Genitourinary Oncology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL

Penile cancer is a rare malignancy, with penile squamous cell carcinoma (PSCC) accounting for 95% of cases. Human papillomavirus (HPV) infection plays a key role in the carcinogenesis of HPV-positive PSCC. In addition to surgical treatment, multimodal therapies combining neoadjuvant cisplatin-based chemotherapy regimen is required for advanced and metastatic PSCC. However, disease-specific survival analysis showed disappointing results after systemic treatments. To identify an alternative treatment with more favorable outcomes, we investigated the effectiveness of a new combinatorial immunotherapy strategy for refractory PSCC. Here, we identify a therapeutic role of combining poly (ADP-ribose) polymerase inhibitors (PARPi) with anti-PD1 antibody treatment in the SAP (*PB-Cre4+ Smad4L/L ApcL/L PtenL/L*) model of PSCC, which was the first genetically engineered mouse (GEM) model of penile cancer in our previous report (Nat Commun. 2020; PMID 32358507). Mechanistically, PARPi-induced PARP1 trapping and dsDNA micronuclei activated cGAS-STING signaling pathway in both murine derived SA (*PB-Cre4+Smad4L/LApcL/L*) and SAP (*PB-Cre4+ Smad4L/L ApcL/L PtenL/L*) cell lines. HPV overexpression greatly downregulated the expression of type I interferons (IFNα and IFNβ) and pro-inflammatory chemokines (CCL5, CXCL9, CXL10 and CXCL11), while increasing the proliferation and motility of human PSCC cell lines. Furthermore, Niraparib, an FDA approved PARP inhibitor, reversed the effect of HPV overexpression on tumor growth by upregulating the expression of IFN-α/β and PD-L1 in vivo. Overall, this study revealed that the combination therapy of Niraparib and anti-PD1 inhibits the progression of PSCC via reprogramming tumor microenvironment, offering promising strategy for the treatment of refractory PSCC.

#6152 The inhibition of USP18 enhanced cancer immunogenicity in triple-negative breast cancer.

Chartinun Chutoe, Valerie G. Brunton, Alexander Von Kriegsheim

Institute of Genetics and Cancer, The University of Edinburgh, Edinburgh, United Kingdom

Triple-negative breast cancer (TNBC) is one of the most aggressive cancer, which exhibits highly metastasis and shows poorly response to many treatments. Immune checkpoint blockage (ICB) treatment induces anti-tumor effect by innate immune activation for tumor clearance. Nevertheless, ICB was shown to be effective in “hot” tumor microenvironment representing highly immune infiltration. Turning “cold” tumor into “hot” tumor may enhance ICB treatment in TNBC. Type I interferon (IFN) response regulates inflammation and immune modulation. However, IFN-response is abated by USP18, suggesting that inhibiting USP18 could enhance IFN signaling and tumor immunogenicity. To study USP18 inhibition, we employed CRISPR-Cas9 to engineered mutated-USP18 in TNBC cell lines. Mass spectrometry-based proteomic study of mutated-USP18 revealed the induction of IFN-response, cytokine response, MHC-I antigen presentation, while reduced cell proliferation- and cell motility- related pathways under IFN-modulation in TNBC cells. These proteome alterations further caused enhanced-cytotoxic effect of NK cells by inducing apoptotic-mediated cancer cell death in co-culture system. *In vivo* tumors with mutated USP18 also showed increased immune activation, suggesting that this loss of USP18 functions could enhance the immune response within the tumor microenvironment. This may lead to the enhancement of ICB treatment for TNBC. Our finding demonstrated that the inhibition of USP18 potentially enhanced IFN-response mediating hot tumor microenvironment by promoting cancer immunogenicity in TNBC. This inhibition of USP18 approach may hold promise for boosting the effectiveness of ICB therapies in TNBC.

#6153 Overcoming immunosuppression in pancreatic cancer: Efficacy of polyamine blockade and anti-PD-1 combinational therapy.

Joseph A. Goode[1], Diana Gaete-Alvarez[1], Otto Phanstiel[2], Deborah Altomare[1]

[1]Burnett School of Biomedical Sciences, University of Central Florida, Orlando, FL,[2]Department of Medical Education, University of Central Florida, Orlando, FL

Pancreatic ductal adenocarcinoma (PDAC) is an extremely aggressive cancer, with an only a 13% 5-year survival rate. This low survival rate is due to its dense microenvironment, late-stage diagnosis, and limited treatment options. Polyamines, small molecules essential for cell growth, play a critical role here because their dysregulation is linked to PDAC's tumor growth, invasion, and metastasis. Additionally, the tumor microenvironment becomes increasingly “cold,” or resistant to immune responses. This resistance is driven by the recruitment of immunosuppressive populations and immune evasion mechanisms, such as the programmed death-1 (PD-1) pathway, which conceals PDAC from immune detection. This study aims to explore the interaction between polyamines, the PDAC immune microenvironment, and evaluate the effectiveness of combining Polyamine Blockade Therapy (PBT) with anti-PD-1 inhibitors as a novel immunotherapy strategy. Using an orthotopic mouse model of PDAC with syngeneic GFP-Luc2 cells, we assessed the impact of PBT, consisting of Difluoromethylornithine (DFMO) and the polyamine transport inhibitor (PTI) Trimer 44NMe (Trimer PTI), in conjunction with anti-PD-1 therapy. Tumor growth was monitored by bioluminescence in vivo and shifts in immune populations were assessed using flow cytometry. The combination of simultaneous PBT and anti-PD-1 treatment resulted in a significant reduction in tumor mass, decreased levels of immunosuppressive cells, and an increase in anti-tumor immune populations. The combination of Polyamine Blockade Therapy with anti-PD-1 inhibition shows promise as an effective immunotherapy for PDAC, potentially improving patient outcomes by disrupting the polyamine-mediated immunosuppressive network while enhancing anti-tumor immunity.

#6154 Diffusing alpha-emitters radiation therapy in combination with anti-CTLA-4, as well as combination with both ATR inhibitor, and anti-CTLA-4 achieves long-lasting tumor regression in murine pancreatic cancer.

M. Reis[1], P. Marinello[2], A. Rubinstein[2], M. Wasley[2], W. Brito[2], R. Sega[3], V. Bachar[3], G. Sawakuchi[2];
[1]Rice University & MD Anderson Cancer Center, Houston, TX, [2]MD Anderson Cancer Center, Houston, TX, [3]Alpha Tau Medical Ltd, Jerusalem, Israel

Pancreatic cancers (PC) are characterized by their aggressiveness, radioresistance, and immunologically "cold" nature, making them the third leading cause of cancer deaths in the US. Specifically, conventional radiotherapy approaches are limited by collateral damage to adjacent organs. Given the lack of effective treatments, this study investigates a novel therapy combining α-particle radiation through Diffusing Alpha-emitters Radiation Therapy (Alpha DaRT), an intratumoral source that releases alpha emitting atoms into the tumor microenvironment, with ATR inhibition (ATRi) to sensitize immunologically cold PC to anti-CTLA-4 (aCTLA-4). While conventional low linear energy transfer (LET) radiation (0.2 keV/μm) has lower capacity to induce double-strand breaks (DSBs), higher LET α-particles (70-200 keV/μm) generate more complex DSBs and micronuclei, activating an immune response through the cGAS-STING pathway. By combining Alpha DaRT with ATRi, we aim to impair radiation-induced DSB repair and checkpoint arrest, forcing mitotic progression despite unrepaired DNA damage, overstimulating this pathway. We hypothesize that the triple treatment (Alpha DaRT+ATRi+aCTLA-4) sensitizes immunologically cold tumors to aCTLA-4, delaying tumor growth, inducing tumor regression, and extending survival. Mice were implanted with 5×10^5 KPC cells subcutaneously in the left leg. After 7 days of tumor inoculation (day 0), Alpha DaRT sources were implanted. ATRi (or DMSO) was administered via oral gavage on days 0, 1, 2, 7, 8, and 9, at 75 mg/kg. Anti-CTLA-4 (or IgG) was delivered intraperitoneally on days 3, 6, 9, and 12. Tumor volumes were measured three times/week and mice were randomized into eight experimental groups, ranging from triple control to triple treatment. Tumor development was assessed according to tumor volume fold change, relative to Alpha DaRT insertion day. Both Alpha DaRT +ATRi or Alpha DaRT+aCTLA4 significantly reduced tumor development vs. monotherapies. Remarkably, more than 40% achieved complete tumor regression in the Alpha DaRT+DMSO+aCTLA-4 or Alpha DaRT+ATRi+aCTLA-4. Mice in which tumors regressed were re-challenged on day 130 with 5×10^5 KPC cells. Both the double treatment (Alpha DART + aCTLA4) or the triple treatment exhibited significantly smaller tumor development relative to naïve mice. These novel results suggest that the dual combination Alpha DaRT and aCTLA4 or the triple combination Alpha DaRT, ATRi, and aCTLA-4 may be a strategy to induce a robust and long-lasting immune activation in immunologically cold PC.

#6155 The combination of cisplatin and temozolomide triggers immune surveillance through antigen-dependent and independent mechanisms.

Pietro Paolo Vitiello[1], Rosaria Chila[2], Vito Amodio[2], Paolo Battuello[1], Vittorio Battaglieri[2], Sharon Scardellato[2], Gaia Grasso[1], Federica Pisati[3], Claudio Tripodo[2], Achille Anselmo[4], Francesca Clemente[4], Federica Di Nicolantonio[1], Giovanni Crisafulli[2], Benoit Rousseau[5], Luis A. Diaz[5], Giovanni Germano[6], Alberto Bardelli[1]

[1]University of Turin, Torino, Italy,[2]IFOM ETS - The AIRC Institute of Molecular Oncology, Milano, Italy,[3]Cogentech SRL, Milano, Italy,[4]IRCCS San Raffaele Hospital, Milano, Italy,[5]Memorial Sloan Kettering Cancer Center, New York City, NY,[6]University of Milan "La Statale", Milano, Italy

The combination of chemotherapy and immune checkpoint inhibitors (ICIs) has shown dramatic activity in a variety of malignancies. However, the addition of ICIs to standard of care (SOC) chemotherapy is not clinically effective in mismatch repair proficient (MMRp) colorectal cancer (CRC). We have previously reported that priming treatment with temozolomide (TMZ) induces hypermutability and immunotherapy clinical benefit in the molecular subset of CRCs characterized by inactivation of O6-methylguanine methyltransferase (MGMT). We reasoned that the combination of TMZ with cisplatin (CDDP), another active alkylating agent, might similarly increase mutational burden and induce cancer immunogenicity in the larger group of MMRp / MGMT wild-type tumors, which account for more than 80% of CRC cases. We investigated the impact of cytotoxic agents on the immunogenomic features of CRC, focusing on TMZ, CDDP, and their combination, in comparison to the SOC treatments (5-fluorouracil, irinotecan and oxaliplatin). Whole exome sequencing (WES) and RNA sequencing were performed to identify mutational signatures and for neoantigen prediction. We assessed treatment-induced antigenicity and immunoediting by injecting CRC cells that had been treated *in vitro* with chemotherapy into syngeneic immunodeficient and -competent mice. In parallel, we evaluated the activity of chemotherapy and immunotherapy performed *in vivo* in order to capture the effect of chemotherapy in the tumor immune microenvironment. Immune infiltration was assessed using a dedicated flow cytometry panel. We found that combinatorial treatment with CDDP and TMZ induces clonal and subclonal hypermutability resulting in an increase in predicted neoantigens. This combination specifically alters the immune fitness of the tumors and ultimately leads to immunoediting of chemotherapy-induced neoantigens and CD8+ T cell-mediated tumor rejection. Treatment-induced hypermutation and immune surveillance were also confirmed in an immune-refractory breast cancer model. Complete rejection of CDDP- and TMZ-treated tumors is associated with persistent immune surveillance that extends to the untreated counterpart. The same effect is not observed when cells are primed with the clinically approved combination of 5-flourouracil, oxaliplatin and irinotecan (FOLFOXIRI). *In vivo* chemotherapy potentiates immune effectors, allowing for durable responses and complete rejections when anti-PD1 is combined with CDDP and TMZ. These results suggest that rational combinations of alkylating agents can promote immune surveillance by enhancing immunologically relevant hypermutability and antigenicity and by remodeling the immune microenvironment. This can be exploited to promote cancer immunogenicity in otherwise immune-refractory CRC.

#6156 Engineering a PD-1 dependent 4-1BB agonist for combination immunotherapy.

Alana Kramer[1], Damien Ruiz[1], Jonathan Marable[1], Payal Agarwal[1], Deepa Bedi[2], Maninder Sandey[1]

[1]Pathobiology, Auburn University College of Veterinary Medicine, Auburn, AL,[2]Tuskegee University, Tuskegee, AL

First-generation 4-1BB (CD137) monoclonal antibody (mAb)-based agonists demonstrated significant potential in early preclinical and clinical studies by eliciting robust anti-tumor responses. However, their clinical development was hampered by dose-limiting hepatotoxicity, attributed to the systemic activation of 4-1BB receptors on monocytes, which in turn recruited CD8+ T cells to attack hepatocytes. To address this challenge, next-generation therapies have focused on decoupling the hepatotoxic effects of 4-1BB activation from its anti-tumor activity. These strategies utilize bispecific molecules to precisely target and activate 4-1BB receptors within the tumor microenvironment (TME). By binding to tumor-associated antigens, these molecules localize to TME, facilitating the hyperclustering and trimerization required for 4-1BB activation. This approach enhances tumor-specific immune responses while minimizing systemic activation and off-target effects. In this study, we introduce, to the best of our knowledge, the first PD-1-targeted 4-1BB agonist that leverages the PD-1 receptor on exhausted T cells to facilitate 4-1BB hyperclustering and activation. Three distinct murine-specific bispecific constructs were developed *in silico*, each employing an anti-PD-1 nanobody for crosslinking and trimerization of the 4-1BB receptor. Construct 1 was designed to function as a weak agonist in the absence of PD-1, transforming into a strong agonist in its presence. In contrast, constructs 2 and 3 are entirely dependent on PD-1 for hyperclustering and subsequent 4-1BB activation. The constructs were expressed and purified using Expi293F-based stable cell lines. Flow cytometry confirmed their ability to bind specifically to the cognate 4-1BB and PD-1 receptors. A 4-1BB luciferase bioassay was employed to demonstrate the PD-1 dependency of Constructs 2 and 3. As expected, constructs 2 and 3 exhibited no 4-1BB activation in the absence of PD1-expressing cells but transformed into potent agonists in their presence. Construct 1 activated the 4-1BB receptor regardless of PD-1, with its activity being markedly enhanced in its presence. Additionally, constructs 2 and 3 were shown to effectively block the PD-1/PD-L1 interaction. Preliminary preclinical evaluations confirmed that all three constructs function as designed. Following the completion of *in vitro* studies, we are currently conducting *in vivo* analyses using mouse xenograft models to evaluate the therapeutic efficacy, toxicity, and biodistribution of these constructs.

#6157 PAR2 inhibitors reduce resistance to immunotherapy against cancer.

Thibaut Brugat[1], Maleck Kadiri[2], Samya Aouad[2], David Allard[2], Camille Fuselier[2], Emma Skora[2], Antoine Mousson[1], Aurelie Janvier[1], Luc Baron[1], Christel Franchet[1], Anne-Laure Blayo[1], Stephan Schann[1], John Stagg[2]

[1]Domain Therapeutics, Illkirch, France,[2]Centre de recherche de l'universite de Montreal et Institut du Cancer de Montreal, Montreal, QC, Canada

A pan-cancer meta-analysis showed that Protease-activated receptor 2 (PAR2) is one of the genes most significantly associated with resistance to immune checkpoint blockade (ICB) and T cells dysfunction in cancer patients. PAR2 is also upregulated and associated with poor prognosis in several cancer types which makes it a promising therapeutic target in the fight against cancer. However, the potential of PAR2 inhibitors and their mechanism of action in immuno-oncology has not been determined yet. The efficacy of PAR2 knock out and a biased negative allosteric modulator (NAM) of PAR2, RD35-11, in monotherapy or in combination with an anti-PD1 was assessed in the MC38 model. Strikingly, the combination therapy significantly increased the number of complete responses compared with mice treated with immunotherapy alone. As MC38 cells do not express PAR2, this result indicated that RD35-11 promote tumor regression by changing the tumor microenvironment. To investigate this hypothesis, the effect of PAR2 inhibition alone or in combination with an anti-PD1 on tumor-infiltrating immune cells was assessed in vivo by flow cytometry, cytokine measurement, bulk and single cell RNA sequencing. Results obtained showed that a PAR2 NAM reduced the level of immune-regulatory cells (M2 macrophages) and cytokines (e.g. IL10) while promoting antigen presenting cells and pathways. Ex-vivo experiments further demonstrated that PAR2 inhibition reduces IL10 secretion by macrophages while enhancing antigen cross-presentation by dendritic cells. Taken together, our data clearly demonstrate that PAR2 NAM can alleviate resistance to immune checkpoint blockade by reducing macrophage-induced immunosuppression and promoting antigen presentation. Domain Therapeutics has identified a novel PAR2 biased NAM, DT-9045, orally available and with clear competitive advantages. IND-enabling studies are currently ongoing to bring this new hope for cancer patients to the clinic.

#6158 STING-MAVS interaction as novel immune mechanism of DNA-PK-inhibitor in SCLC.

Caterina De Rosa[1], Luisa Amato[1], Concetta Tuccillo[1], Francesca Iommelli[2], Viviana De Rosa[2], Virginia Tirino[3], Federica Papaccio[4], Kavya Ramkumar[5], Qi Wang[6], Jing Wang[6], Alberto Servetto[7], Floriana Morgillo[8], Lauren Averett Byers[9], Fortunato Ciardiello[1], Carminia Maria Della Corte[1]

[1]Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Napoli, Italy,[2]Institute of Biostructures and Bioimaging, National Research Council, Naples, Italy,[3]Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Napoli, Italy,[4]Department of Medicine, Surgery and Dentistry, "Scuola Medica Salernitana", University of Salerno, Baronissi, Italy,[5]Department of Thoracic Head and Neck Medical Oncology, University of Texas, MD Anderson Cancer Center, Houston, TX,[6]Department of Bioinformatics and Computational Biology, University of Texas MD Anderson Cancer Center, Houston, TX,[7]Department of Clinical Medicine and Surgery, University of Naples Federico II, Naples, Italy,[8]Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy,[9]Department of Thoracic Head and Neck Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, TX

Small cell lung cancer (SCLC) is a highly aggressive malignancy characterized by significant replication stress and DNA damage. Recently an "inflamed" subset of SCLC has been identified, showing high expression of genes associated with Stimulator of Interferon Genes (STING)-induced T-cell attracting chemokines, such as CCL5 and CXCL10. The addition of DNA damage repair inhibitors (DDRis) to immunotherapy has been shown to enhance immune responses through STING-dependent activation pathways.This study focuses on the role of DNA-PK inhibition (DNA-PKi), a key regulator of DDR, in the activation of multiple cytosolic DNA/RNA sensors (STING, cGAS, MAVS, IFI16) to elucidate the molecular mechanisms linking DNA damage to immune responses.Treatment-naïve SCLC patients (n=10) and patients undergoing chemotherapy treatment (n=10) were enrolled. Blood samples were collected at baseline and after chemo-immunotherapy to analyze the expression of cGAS, STING, MAVS, and IFI16. Comprehensive transcriptomic analysis, real-time PCR and protein analysis were performed. STING, MAVS and cGAS knockdown and subcellular localisation analysis were also reported.SCLC tumor tissues (n=81) with higher STING-related chemokines (CCL5/CXCL10) exhibited increased expression of genes involved in MAVS pathway (IFITH1, DDX60), suggesting a simultaneous activation of STING and MAVS. DNA-PKi enhanced STING and MAVS more effectively than other DDRis in SCLC patients-derived PBMCs post-cisplatin compared to naïve patients, indicating its unique role in promoting immune responses. No significant changes in cGAS expression were observed in immune cells from SCLC patients, suggesting that the upregulation of STING mediated by DNA-PK inhibition is independent of cGAS, as knockdown of cGAS did not influence the upregulation of STING and p-IRF3 following DNA-PKi treatment. DNA-PKi treatment resulted in novel mitochondrial co-localization and physical interaction between STING and MAVS in PBMCs, leading to improved functional activation via nuclear translocation of p-IRF3.In conclusion, the study posits that DNA-PK inhibition facilitates a non-canonical interaction between STING and MAVS, thereby enhancing the innate immune response against SCLC.

#6159 Transcriptional regulation of DNA damage response and associated inflammatory signaling by CDK7 in prostate cancer.

Chandan Kanta Das[1], Hesham Mohei[2], Brijesh Kumar Verma[1], Muzaffer Kassab[1], Phillip Wulfridge[3], Sharan Venkatesh[1], Mohammed Alhusayan[1], Reyaz ur Rasool[1], Kavitha Sarma[3], Roger A. Greenberg[1], Eric J. Brown[1], Malay Haldar[2], Irfan A. Asangani[1]

[1]Department of Cancer Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA,[2]Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA,[3]Gene expression and Regulation program, The Wistar Institute, Philadelphia, PA

Metastatic prostate cancer (PCa) is incurable and the second leading cause of cancer death among men in the Western world. Although patients initially respond to androgen deprivation therapy (ADT), most eventually develop castration-resistant prostate cancer (CRPC) and metastasize to bone. Second-generation AR signaling inhibitors, such as abiraterone acetate and enzalutamide, have been shown to provide a median survival benefit of 6 to 24 months for metastatic CRPC patients. Therefore, more effective therapies are needed, including novel and innovative therapeutic combination approaches. PARP inhibitors are used primarily in treating patients with DNA damage response (DDR) pathway (e.g. BRCA1/2) mutated cancers because these mutations cause a deficit in homology-directed DNA repair (HDR) that confers sensitivity to these agents. Though BRCA1/2 mutations are relatively rare, genomic defects in other DNA repair genes account for approximately 20% of advanced CRPC. Therefore, PARP inhibitor was recently FDA-approved for metastatic PCa carrying these mutations. However, the rest of the 80% HR-proficient CRPC patients do not benefit from PARPi. Apart from that, prostate tumors are immunologically cold tumors and only 5% of PCa patients respond to immune checkpoint blockers. Using next generation genomics approaches, we have found that inhibiting transcriptional kinase CDK7 in PCa cells creates a BRCA-deficient state (BRCAness) that sensitizes these HR-proficient tumor cells to PARP inhibitors *in vitro* and *in vivo*. Mechanistically, knockdown or inhibition of CDK7 led to transcriptional downregulation of DDR genes through loss of Mediator activity along with accumulation of R-loops resulting in the induction of DNA damage, micronuclei formation with consequent activation of cGAS-STING mediated inflammatory signaling, and synergy with anti-PD1 therapy in a syngeneic PCa model *in vivo* without toxicity. These findings suggest that CDK7 inhibition synergizes with PARP inhibitors and immune checkpoint blockers, offering a promising therapeutic strategy for both HR-proficient and HR-deficient metastatic PCa. This approach has the potential to overcome current limitations of immune checkpoint inhibitors and provides new avenues for targeted combination therapies in advanced PCa treatment.

#6160 Design and preclinical evaluation of a first-in-class covalent reversible inhibitor of IRAK4 with immunomodulatory properties and selective anti-lymphoma effect.

Gema Gorjon de Pablo[1], Nuria Profitos-Peleja[1], Miek Jacobs[1], Aitor Gonzalez-Herrero[2], Jeronimo Parra[3], Elisabetta Mereu[2], Paula Cifuentes[4], Melissa Orphali[4], Jordi Teixido[4], Manel Esteller[3], Jose I. Borrell[4], Roger Estrada-Tejedor[4], Gael Roue[1]

[1]Lymphoma Translational, Josep Carreras Leukaemia Research Institute, Badalona (Barcelona), Spain,[2]Cellular Systems Genomics, Josep Carreras Leukaemia Research Institute, Badalona (Barcelona), Spain,[3]Cancer Epigenetics, Josep Carreras Leukaemia Research Institute, Badalona (Barcelona), Spain,[4]Pharmaceutical Chemistry Group, IQS School of Engineering, Barcelona, Spain

Oncogenic activating mutations in the myeloid differentiation primary response gene 88 (*MyD88*) and the consequent activation of the Toll-like receptor pathway are hallmarks of the activated B-cell-like (ABC) subtype of diffuse large B-cell lymphoma (DLBCL). This cascade includes phosphorylation of interleukin-1 receptor-associated kinase 4 (IRAK4), triggering downstream NF-κB activation and driving tumor survival, proliferation, and chemotherapeutic resistance. Although several IRAK4 small-molecule inhibitors (IRAK4-i) are undergoing clinical evaluation for various cancers, most are non-covalent inhibitors with limited activity and specificity.To address this, we employed computer-aided drug design to create a library of reversible covalent IRAK4-i based on a pyrido[2,3-*d*]pyrimidine scaffold with lysine-targeting warheads. From this library, the ten most promising candidates were synthesized using organometallic reactions and microwave-assisted techniques. Subsequent multikinase screening identified four potential IRAK4-specific inhibitors, with GGP314 emerging as the most potent. GGP314 exhibited IC_{50} values of 6.6-7.3 μM at 24 hours and 1.8-2.8 μM at 72 hours in MyD88mut ABC-DLBCL cell lines, while showing minimal activity against non-ABC-DLBCL cells and normal peripheral blood mononuclear cells (PBMCs). Mechanistically, GGP314 reduced IRAK4 phosphorylation and impaired NF-κB signaling, as evidenced by the downregulation of several target genes. The compound demonstrated antitumor activity in 3D spheroid cultures of ABC-DLBCL cells, achieving a 50-60% reduction in Ki67 levels. However, the cytostatic effect was partially attenuated in the presence of follicular dendritic or mesenchymal stromal cells, though not with M2-polarized macrophages. Single-cell RNA sequencing (scRNA-seq) analysis of 3D spheroids revealed that GGP314 preferentially targets tumor-associated macrophages, shifting the tumor microenvironment towards a pro-inflammatory state via upregulation of interferon-gamma response pathways. Finally, GGP314 efficacy was validated in vivo in an immunocompetent chicken embryo chorioallantoic membrane (CAM) model of MYD88-mutant ABC-DLBCL. Twice-weekly dosing resulted in up to 55% tumor growth inhibition in a dose-dependent manner without systemic toxicity. In conclusion, we report here the development and preclinical validation of GGP314, a first-in-class reversible covalent IRAK4 inhibitor. GGP314 exhibits significant antitumor activity, high specificity, and the ability to modulate both tumor-intrinsic and microenvironment-dependent signals, supporting its potential as a therapeutic candidate for ABC-DLBCL.

#6161 Exploring BRG399, a novel microtubule-binding agent, as an inducer of immunogenic cell death in cancer therapy.

Kaila M. Bennett, Jacob P. Matson, Sylwia Stopka, Juan J. Aristizabal-Henao, Archna Ravi, Andressa L. Mota, Dinesh Chimmanamada, Vivek K. Vishnudas, Stephane Gesta, Maria-D Nastke

BPGbio, Framingham, MA

Cancer therapy is transforming from traditional chemotherapeutic agents (CTAs) to innovative immunotherapies. Despite this shift, chemotherapy remains clinically stable, as CTAs are thought to convert the tumor microenvironment (TME) from immunosuppressive to immune-active by inducing immunogenic cell death (ICD) in tumor cells which leads to stimulation of immune cells. This ICD induction is thought to be crucial for enhancing the efficacy of immunotherapies. Thus, using CTAs as ICD agents—alone or in combination with immunotherapies—may offer significant anti-cancer benefits and new treatment avenues. This study investigates the potential of BRG399, a novel microtubule-binding agent, as an immunogenic cell death (ICD) inducer utilizing preclinical *in vitro* and *in vivo* models. Treatment of cancer cell lines with BRG399 showed a dose-dependent increase in surface calreticulin and secreted HSP70, measured by flow cytometry and enzyme-linked immunosorbent assay (ELISA), respectively. The enhancement of both ICD markers was comparable to that observed with established ICD agents. To explore the potential of BRG399 to exert influence on immune-regulated anti-tumor effects, *in vivo* studies in immune-competent syngeneic models were conducted with measurable endpoints of tumor growth inhibition and TME composition by flow cytometry and TIMS TOF spatial omics. BRG399 displayed anti-tumor activity while largely preserving tumor-associated immune cells and notably modifying the immune cell landscape within the tumor. Our findings suggest that BRG399 acts as an ICD agent, effectively modulating both tumor and immune cells within the TME and highlighting its potential for future combination therapies with immunotherapeutics. Further *in vivo* studies are currently being conducted to comprehensively elucidate the immune-mediated effects of BRG399 aiming to clarify its role in enhancing anti-tumor responses ultimately contributing to the development of more effective cancer therapies.

#6162 RRM1 inhibition enhances anti-tumor immunity by ubiquitination degradation DNMT1 through PI3K/AKT pathway.

Nan Jiang, Jianya Zhou

Zhejiang University, Hangzhou, China

Patients with driver-gene-negative non-small cell lung cancer still need to rely on chemotherapy combined with immunotherapy. Some studies have suggested that chemotherapy drugs can promote the innate immune system, but the mechanism of that is still unclear. Ribonucleotide reductase large subunit (RRM1) is the main target of the commonly used chemotherapy drug gemcitabine, which exerts anti-tumor effects by inhibiting DNA replication and repair. We constructed human A549, Calu-1 cell lines and mouse KP ($KRAS^{G12D}/P53^{KO}$) cell lines with stable knockdown of RRM1 and found that knockdown of RRM1 can significantly inhibit the growth of tumor cells *in vivo* and *in vitro*. RNA-sequencing was used to detect the changes in the transcriptome after RRM1 knockdown. Through GO analysis and KEGG analysis, we found that the DNA damage caused by RRM1 inhibition can cause the non-classical NF-κB signaling pathway to be upregulated, thereby causing an inflammatory response dominated by IL-6. However, at the same time, we found that the RNA recognition innate immune response pathway, which mainly recognizes dsRNA, was also activated. Therefore, we speculate that RRM1 inhibition affects the epigenetic changes of tumor cells. We found that the expression level of DNMT1 protein was downregulated after RRM1 knockdown, and its mRNA level remained unchanged, indicating that post-transcriptional modification of DNMT1 protein occurred. The reduction in DNMT1 protein expression reduced the overall DNA methylation level of the cell, thereby causing the activation of the type I interferon innate immune response pathway dominated by RIG-I/MAVS/TBK1/IRF3. Further mechanistic studies found that RRM1 inhibition mainly reduces the phosphorylation level of AKT protein by inhibiting the intracellular PI3K-AKT signaling pathway, thereby reducing the phosphorylation level of DNMT1 protein, and ultimately promoting the ubiquitination degradation pathway of DNMT1 protein. Therefore, by combining RRM1 inhibitors with DNMT1 inhibitors, the epigenetic drug decitabine (DAC) can further inhibit the expression of DNMT1, thereby promoting the type I interferon innate immune response pathway caused by chemotherapy drugs rather than the inflammatory response dominated by IL6, and exerting a synergistic anti-tumor effect on tumor growth *in vivo*. In summary, our research shows that inhibiting RRM1 can promote DNMT1 ubiquitination degradation through the PI3K/AKT signaling pathway, thereby activating the RNA recognition-induced type I interferon innate immune response pathway to exert anti-tumor effects. Combining with the epigenetic drug DAC can enhance the effect of this pathway, thereby better combining with immunotherapy to exert better anti-tumor effects. This provides a new idea for the treatment of chemotherapy combined with immunotherapy.

#6163 PARP inhibition induces the expression of interferon-response genes in both BRCAm and BRCAwt high grade serous ovarian cancer cells.

Daniel Falcon[1], Alyson Yeckes[1], Ania Klas[1], Andrea Corbet[1], Chelsea Goff[1], Sandra Orsulic[2], Rita Serda[1], Phyllis Gimotty[3], Sarah Adams[1]

[1]University of New Mexico Health Sciences Ctr., Albuquerque, NM,[2]University of California, Los Angeles, Los Angeles, CA,[3]University of Pennsylvania, Philadelphia, PA

Background: Therapeutic synergy of PARP inhibition (PARPi) and CTLA4 immune checkpoint inhibition (ICI) in BRCA1 mutated (BRCAm) ovarian cancer (OC) models was dependent on increased interferon gamma (IFNg) production in the tumor microenvironment (TME). Expression of interferon-response genes in tumor samples is also linked with CTLA4 ICI efficacy in melanoma. In an assessment of interferon-response gene expression in tumor samples from women with BRCAm ovarian cancer enrolled in a phase I/II clinical trial of PARPi and CTLA4 ICI, *IFIT1B* was significantly associated with both treatment benefit (AUC=0.842), and progression-free survival (PFS) (n=30, p=0.029). In this cohort, *IFIT1B* expression was also strongly correlated with other IFIT gene family members, and positively correlated with *CXCL10* expression among subjects with clinical benefit from treatment. In an analysis of TCGA data, *IFIT1B* expression was also significantly associated with PFS among BRCAm patients (n=16, p=0.0306), but not in the larger ovarian cancer cohort (n=299, p=0.1953). IFIT gene family expression is induced by type I and type II interferons, as well as cytosolic nucleic acid sensing. *CXCL10* expression is associated with anti-tumor immunity and is prognostic in ovarian cancer.
Objective: To examine the association between these candidate biomarkers and treatment outcomes, we exposed murine OC cells to PARPi, IFNg, or the combination in vitro, and compared gene expression by qPCR and production of CXCL10 and IFN beta by ELISA.
Results: Treatment with PARPi significantly increased expression of IFIT family members (*Ifit1, Ifit2, Ifit3*) compared with untreated cells, and additionally increased expression in combination with IFNg compared with IFNg exposure alone. PARPi-induced IFIT gene expression was independent of BRCA status and was observed in both PARPi-sensitive and -resistant BRCA1m models. PARPi also significantly increased expression of *CXCL10* by qPCR, and PARPi in combination with IFNg increased levels of secreted CXCL10 by BRCA1m cells compared with IFNg alone. Although expression of *IFNb* was similar across all treatment conditions, high IFN beta secretion was observed in BRCA1m cells treated with PAPRi in combination with IFNg.
Conclusions: These results indicate that PARPi can modulate tumor cell-intrinsic responses to IFNg independent of BRCAm status, including changes in secreted chemokines and cytokines associated with T cell recruitment and immune activation in the tumor microenvironment. With evidence that both IFN signaling and CXCL10 in the TME can impact treatment efficacy and disease outcomes, these data may inform the design of novel combinatorial regimens for ovarian cancer.

#6164 Novel heparin nanoparticles targeting BDCA2 promote immune response.

Greg Kirkpatrick, Caitlin Lemke-Miltner, Rui He, Sue Blackwell, Aliasger Salem, George J. Weiner

University of Iowa, Iowa City, IA

Immune checkpoint inhibitors have revolutionized treatment of many types of cancer, but are not effective for all patients. One proposed mechanism of resistance is lack of local interferon production that results in an immunologically “cold” tumor. This barrier may be overcome by direct immune stimulation with TLR9 agonists. Early phase clinical studies in a variety of cancer types have demonstrated intratumoral injection of TLR9 agonists, as free drug or formulated into nanoparticles (NP), is safe and capable of inducing systemic tumor regression alone or in combination with immune checkpoint inhibitors.

Plasmacytoid dendritic cells (pDCs) are potent producers of type I interferon such as interferon alpha (IFNα). They are key immune system regulators and play a central role in the response to TLR9 agonists. BDCA2 is a cell surface receptor expressed uniquely on pDCs. Signaling through BDCA2 is thought to decrease the immune response initiated by pDCs, particularly in response to TLR agonists. Heparin is a BDCA2 ligand and has been shown to inhibit the TLR9 agonist-driven IFNα response. We developed a novel heparin-coated, polyamidoamine (PAMAM) NP containing a TLR9 agonist to investigate whether heparin-mediated targeting of these NP to pDCs via BDCA2 modulates the pDC response to TLR9. Control NP included those without surface heparin and those without TLR9 agonists. Fresh normal donor human peripheral blood mononuclear cells (PBMCs) were treated with NP for 20 hours. Supernatants were collected for IFNα ELISA to determine whether a functional PBMC response, mediated in large part by pDCs, was stimulated by the NP. Flow cytometric evaluation was used to assess whether NP induced a phenotypic change in pDCs.

Heparin-coated NP containing a TLR9 agonist was more effective than soluble TLR9 agonist at inducing IFNαproduction from PBMCs. Such NP also increased expression of PD-L1 on pDCs. Increased IFNα production and PD-L1 expression were most pronounced with TLR9-containing particles that had a high heparin coating amount. Of note, a low heparin coating amount resulted in reduced IFNα production and PD-L1 expression on pDCs.

The published literature suggests BDCA2 signaling inhibits pDC activation induced by TLR9 agonists. In contrast, here we show that TLR9-containing NP coated with high concentrations of the BDCA2 ligand heparin actually enhances activation of pDCs. Biologically, this suggests signaling via BDCA2 may vary based on the concentration of ligand or how it is presented. Therapeutically, heparin-coated NP containing TLR9 agonists may represent a promising novel approach for stimulating a local immune response, with potential relevance in both cancer immunotherapy and vaccination. Additional studies are ongoing to confirm NP activity is mediated by BDCA2 and to optimize NP composition. This includes assessment of various NP structural components, concentrations and types of BDCA2 ligands, and various TLR agonists.

#6165 Antiprogestin- mediated endocrine therapy activates immunotherapy- responsive programs in hormone receptor positive breast tumors.

Mariana Salatino, Joaquin Pedro Merlo, Tomas Dalotto-Moreno, Magali Berton, Ramiro Martin Perrotta, Andres Elia, Karina V. Marino, Claudia M. Lanari, Gabriel A. Rabinovich

Instituto de Biologia y Medicina Experimental, Buenos Aires, Argentina

The endocrine status of the patient is a critical factor that may modulate the immune response in breast cancer and, accordingly, should be considered during immunotherapy regimens. Given the immunosuppressive and tolerogenic activities of the sexual hormone progesterone and its roles in promoting breast cancer initiation, we aim to evaluate the effect of the antagonist of the classical progesterone receptor, Mifepristone (MIFE), as an endocrine therapy to alter the tumor microenvironment, and particularly to shape an immunogenic and immunotherapy-responsive profile. We evaluated the breast tumor immune landscape both in a mouse model of luminal tumors (C4HD) treated with a synthetic progestin MPA and with MIFE; and in human tumor samples derived from the MIPRA clinical trial (NCT02651844), where breast cancer patients harbouring HR+ tumors were treated with MFP for 14 days before surgery. Using RNA-seq, bioinformatics tools and flow cytometry, we interrogated the C4HD tumor immune infiltration profile after MPA and MIFE treatment, including several global gene expression signatures associated with T-cell exclusion and Immune Checkpoint Inhibitors (ICI) sensitivity and resistance. We observed that treatment with MFP in HR+ luminal breast tumors restrains the progestin-mediated tolerogenic infiltrate composed of Tregs, exhausted CD8+ T cells and TAM macrophages. Importantly, MIFE downregulates the suppressive pathway of IDO, CXCL5, VEGF and Galectin-9, which in turn contributes to the persistence of a population of CD8+ T cells that highly produce granzymes and express lower TIM-3 and PD-1, exhibiting a reinvigorating phenotype. On the contrary, MIFE treatment upregulated several immune-associated genes such as chemokines, granzymes, ICOS-L, EOMES, CD86, CD8 and PD-L1. More importantly, we showed that in both mouse models and human tumors, treatment with MIFE reverts transcriptional signatures associated with T cell exclusion and ICI resistance and significantly upregulates programs associated with immunogenic cell death and PD-1/PD-L1 treatment response. To summarize, our results demonstrate that endocrine therapy as MIFE can foster in HR+ tumors a complete remodelling of the immune landscape, promoting immune cell infiltration and immunogenicity. As a result of the neoadjuvant MIFE treatment, HR+ luminal tumors that traditionally were considered “cold” tumors are now heavily enriched with an immunogenic PD-L1-expressing infiltrate, thus opening a new window of treatment opportunity that may sensitize luminal breast tumors to ICI.

#6166 Identification of immunogenic cell death inducers from Komaroviquinone (KQN)-derived compounds.

Maiko Matsushita[1], Koki Kurita[2], Taisei Fujinami[1], Himari Yamada[2], Yusuke Yamamoto[3], Yutaka Suto[4]

[1]Keio University, Faculty of Pharmacy, Keio Univ., Tokyo, Japan,[2]Keio University, Faculty of Pharmacy, Tokyo, Japan,[3]Division of Cellular Signaling, National Cancer Center Research Institute, Tokyo, Japan,[4]Takasaki University of Health and Welfare, Gunma, Japan

Background: Immunogenic Cell Death (ICD) is a type of cell death accompanied by expression of calreticulin (CRT) and release of ATP and HMGB1, which is induced by photodynamic therapies (PDT), oncolytic viruses and several chemotherapeutic drugs. ICD could enhance the therapeutic effect of other immunotherapies through maturation of dendritic cells and activation of T cells. Multiple myeloma (MM) remains incurable despite of the development of novel therapies including several immunotherapies, such as CAR-T therapy. Therefore, we tried to detect novel drugs which can induce immunogenic cell death in MM cells using a library of Komaroviquinone(KQN) - derived compounds (GTN compounds). We also investigated the mechanism of the ICD induced in MM cells.

Methods: We screened 38 kinds of GTN compounds. MUM24 cell line was treated with each of GTN compounds and the cell surface expression of calreticulin (CRT) and release of ATP and HMGB1 were evaluated by FACS, ENLITEN ATP Assay System® and Western Blotting respectively. We also examined the effect of candidate drugs on differentiation of dendritic cells (DCs) by coculture of human monocyte-derived DCs with drug-treated MUM24 and detection of the expression of CD80, CD83 and HLA-DR by FACS. Production of ROS was also measured by CM-H_2DCFDA assay. Finally, RNA sequence analysis was conducted using total RNA extracted from 4 MM cell lines treated with GTN057.

Results: Five out of 38 GTN derivatives could induce ICD in MM cells. When DCs were co-cultured with drug-treated MUM24, increased expression of HLA-DR, CD80 and CD83 were observed with GTN025, 040,057. Cell surface expression of CRT was not abrogated by antioxidants, despite that those compounds facilitated ROS production. Furthermore, analysis of RNA sequences revealed that ER-stress-related genes were up-regulated by GTN057.

Conclusions: Our results suggest that some of GTN compounds effectively induce ICD in MM cells. Although further investigation using *in vivo* model is needed, those compounds might have synergistic effects when used in combination with other immunotherapies.

IMMUNOLOGY: Innate Immunity to Tumors
Poster Session

#6167 Defining the role of transcriptional coregulator MED15 in immune adaptation in cancer.

Sara Cristiano, Xuanjin Cheng, Stefan Taubert

Medical Genetics, University of British Columbia, Vancouver, BC, Canada

The immune system is widely recognized as an important regulator of cancer progression and metastasis, where immune cells can actively promote or inhibit tumor growth. Initially, immune cells act to eradicate cells with emerging cancerous properties, but cancer cells reprogram these immune cells to instead help promote growth of the cancer. Modulating the immune system is therefore an important approach in cancer therapy, however, the identification of new molecular targets is still needed. A potential target includes MED15, a subunit of the transcriptional coregulator complex Mediator, which interacts with transcription factors (TFs) bound to gene regulatory elements. MED15 upregulation occurs in several cancers and is associated with aggressive clinical presentation and shorter patient survival. The precise role that *MED15* plays in immune responses in cancer, however, is not known. To investigate this, *MED15*KO A549 lung adenocarcinoma cells were used to analyze the expression and secretion of various pro-inflammatory immune response genes. The *MED15*KO cells displayed downregulated expression of the immune genes *CXCL3*, *CXCL5*, and *PTGS2,* and the immune receptor *TLR4*, indicated by qPCR. Decreased expression of the pro-inflammatory modulator COX2 (the product of *PTGS2*) was also observed using immunoblot analysis and decreased secretion of CXCL chemokines and PGE2 (the proinflammatory mediator synthesized by COX2) was observed using ELISA and multiplex assays. The functional effects resulting from this lower expression/secretion of pro-inflammatory mediators were examined, specifically the migration of immune cells towards the cancer cells. A decrease in the migration of THP-1 differentiated macrophages was observed towards the *MED15*KO cells in comparison to WT A549 cells, using a transwell migration assay. To determine how MED15 regulates the expression/secretion of these pro-inflammatory genes a Cut&Run protocol was optimized. This will reveal if MED15 binds chromatin near TFs known to regulate these immune genes, identifying candidate TFs that may cooperate with MED15. In sum, this data suggests that *MED15* is required for the expression and secretion of these immune genes and their ligands, while highlighting an important role for *MED15* in pro-inflammatory immune responses in cancer.

IMMUNOLOGY: Interplay between Immune System and Radio-, Chemo- and Targeted Therapies 2
Poster Session

#6168 CAF-mediated cancer immunotherapy resistance can be overcome via selective inhibition of NOX 2 and 4.

Jihyun Um, Heesu Kim, Hyeji Jang, Eun Sil Lee, Sung Hwan Moon, Soo Jin Lee

Aptabio Therapeutics Inc., Yongin, Korea, Republic of

Cancer-associated fibroblasts (CAFs) are a functionally heterogeneous population that constitutes tumor stroma. CAFs interact with cancer cells and immune cells by secreting various chemokines/cytokines, participating in extracellular matrix (ECM) remodeling and affecting the immune invasion in the tumor microenvironment (TME). Finally, CAFs promote tumor growth and induce resistance to chemotherapy or immunotherapy, making tumors more aggressive associated with poor prognosis in various types of solid tumors. The primary function of NADPH oxidases (NOXs) is to produce reactive oxygen species (ROS). NOX enzymes, which are clinically upregulated in CAFs, have been reported to be critical effectors of tumor fibrosis and immunosuppression of the TME. It suggests that modulation of CAF-mediated immunosuppression via NOX regulation could be a potential strategy for cancer immunotherapy. In this study, NOX2 and 4 are markedly increased in human pancreatic CAFs (pCAFs) compared to human pancreatic fibroblasts (PFs) and knockdown of NOX2 or 4 effectively decreased fibrosis-related markers and immune cytokines/chemokines in pCAFs, respectively. In order to confirm the importance of NOX in CAF functions, we utilized compound-19, a selective NOX inhibitor. Com-19 treatment significantly reduced the expression of fibrosis-related factors and immune cytokines/chemokines in pCAFs. In addition, com-19 inhibited monocyte infiltration and their differentiation into M2 macrophages in CAF-rich tumor-mimicking 3D spheroids *in vitro*. The *in vivo* efficacy was evaluated using a CAF-rich colorectal cancer mouse model. Com-19 inhibited tumor fibrosis and immune cytokines in CAF-rich tumors via NOX inhibition. Importantly, com-19 increased the intratumoral infiltration of CD8+ T cells, resulting in excellent synergistic tumor growth inhibition when co-administered with immune check inhibitors (ICIs) in the model which poorly responses to ICIs. Taken together, the results support that NOXs play a crucial role in CAF-mediated ICIs resistance in cancers and NOX inhibition can be a promising strategy to overcome the resistance. Com-19 effectively regulated the fibrotic and immune-suppressive properties of CAF-rich tumors via NOX inhibition. Therefore, com-19 can potentiate cancer immunotherapy by overcoming CAF-Mediated ICIs resistance. GLP safety/toxicity studies of com-19 have been completed and phase 1 clinical studies are scheduled in 1Q, 2025.

#6169 Immunogenic cell death mediates the anticancer efficacy of high dose vitamin C.

A. Cavaliere[1], M. Macagno[1], F. Maione[1], V. Amodio[2], M. Escobosa Olmo[3], R. Chila[2], S. Lamba[4], P. Vitiello[2], V. Pessei[5], D. Grases[3], E. Perez[3], A. Bartolini[5], N. Congiusta[1], G. Germano[2], A. Sartore-Bianchi[6], T. Troiani[7], M. Esteller[3], E. Porta[3], A. Bardelli[2], **F. Di Nicolantonio**[1];

[1]University of Turin, Candiolo, Italy, [2]IFOM ETS - The AIRC Institute of Molecular Oncology, Milan, Italy, [3]Josep Carreras Leukaemia Research Institute (IJC), Barcelona, Spain, [4]University of Turin, Turin, Italy, [5]Candiolo Cancer Institute, FPO - IRCCS, Candiolo, Italy, [6]Department of Hematology, Oncology and Molecular Medicine, Grande Ospedale Metropolitano Niguarda, Milan, Italy, [7]Department of Precision Medicine, Universita degli studi della Campania Luigi Vanvitelli, Naples, Italy

Background: We previously reported that high-dose Vitamin C (VitC) can synergize with immune checkpoint blockade in immunocompetent mice bearing colorectal, pancreatic and breast tumors. Here, we aim to unveil the molecular mechanisms underlying the above preclinical observations. We hypothesized that VitC could trigger immunogenic cell death (ICD), thereby stimulating an immune-mediated anticancer response.

Methods: Induction of ICD was assessed by the expression or emission of specific markers, including calreticulin, as well as by ex-vivo phagocytosis assays by co-culturing dendritic cells with breast or colon cancer cells treated with VitC. A calreticulin blocking antibody was administered in vivo in combination with VitC to functionally characterize the role of ICD induction in the anticancer effects of vitC. Ex-*vivo* spatial transcriptomic and immunofluorescence analyses were performed to characterize the impact of the treatment on tumor and immune cell populations.

Results: Pharmacological doses of VitC were able to induce oxidative stress as indicated by spatial transcriptomic data and confirmed by ex vivo 8-hydroxyguanine staining in tumor sections. VitC treatment induced ICD markers and among these calreticulin and HMGB1. Although the induction of these danger markers was seen in tumors grown both in immunocompromised and immunocompetent VitC treated mice, anticancer activity was evident only in the presence of an intact immune system. In vivo, a calreticulin blocking antibody effectively prevented its translocation and inhibited HMGB1 release in murine tumors. Notably, preventing calreticulin exposure abrogated the immune mediated anticancer effect of VitC. VitC enhanced the infiltration of natural killers, activated CD8+ T lymphocytes, while it decreased the number of Tregs. Calreticulin blockade impaired the modulation of the immune tumor microenvironment induced by VitC.

Conclusions: VitC treatment can mobilize calreticulin and HMGB1, which are among the established markers of immunogenic cell death. These results, alongside the observation that VitC exerts its maximal antitumor activity only in immunocompetent mice and its anticancer activity is abolished by anti-calreticulin blocking antibody demonstrated that VitC is an ICD inducer when given at pharmacological doses. These findings lend the preclinical rationale and inform the translational analyses planned within the ALFEO clinical trial (Clinical trial information: ECTR2022-502101-15-00), that is investigating pharmacological doses of vitC in combination with nivolumab and ipilimumab in mismatch repair proficient colon cancer patients. Funded by- Next Generation EU - PNRR M6C2 - PNRR-MAD-2022-12376593.

#6170 SPHK2 modulates the tumor-intrinsic response to STING agonism in triple-negative breast cancer cells.

Colette R. Worcester[1], Shane R. Stecklein[2]

[1]Cancer Biology, The University of Kansas Medical Center, Kansas City, KS,[2]Radiation Oncology, The University of Kansas Medical Center, Kansas City, KS

Triple-negative breast cancer (TNBC) accounts for 10-15% of breast cancers and has a poorer prognosis than other breast cancer subtypes. TNBCs with robust immune infiltrates are known to have improved responses to chemotherapy, immunotherapy, and radiotherapy. Stimulator of interferon genes (STING) promotes both innate and adaptive anti-tumor immune cell infiltration through type I interferon production, and we and others have shown that its activation is associated with therapeutic sensitivity in TNBC. Repression of STING expression or activity are commonly observed in various cancer types, and this has been shown to attenuate responses to treatment. TNBCs overexpress sphingosine kinase 2 (SPHK2), which produces the bioactive lipid spingosine-1-phosphate (S1P). SPHK2-mediated S1P biosynthesis has been shown to suppress inflammatory responses in lung injury models via inhibition of STING. In this study, we sought to determine if SPHK2 attenuated STING activity in TNBC cells. We analyzed publicly available RNA-seq data in The Cancer Genome Atlas and found that TNBC patients with higher *SPHK2* expression had significantly worse disease-free survival and overall survival. For *in vitro* studies, we stably overexpressed *SPHK2* in two commonly studied TNBC cell lines with mid-range SPHK2 and STING protein expression: MDA-MB-231 and BT-549. STING was stimulated with the synthetic agonist diABZI, the endogenous agonist 2'3'-cGAMP, or with ionizing radiation. Phosphorylated interferon regulatory factor 3, a downstream event of STING activation, was decreased in *SPHK2* overexpressing cells following diABZI stimulation. We measured type I interferon signaling by analyzing transcriptional activity of interferon-stimulated response elements (ISRE) using a luciferase reporter assay. *SPHK2* overexpressing MDA-MB-231 and BT-549 cells had decreased transcriptional activity of ISRE compared to controls at baseline and after both diABZI and 2'3'-cGAMP treatment, suggesting that SPHK2 suppresses STING-dependent type I interferon production in TNBC. To test a therapeutically relevant model, cells were also exposed to photon radiation. Compared to control cells, *SPHK2* overexpressing cells had decreased radiation induced ISRE activity. The FDA-approved SPHK2 inhibitor opaganib was able to augment diABZI-stimulated ISRE transcriptional activity in a dose-dependent manner. These data suggest that malignancy-associated SPHK2 overexpression may attenuate STING activation in TNBC, and that SPHK2 inhibition may be a therapeutically relevant and viable approach to augment anti-tumor immunity. The interactions between SPHK2 and the STING-interferon pathway warrants further study to develop potential therapeutic combinations to benefit patients with TNBC.

#6171 Flt3 ligand and CD40 agonist-based therapy in breast cancer promotes myeloid cell CXCL9 secretion and CD8 T cell recruitment but is not dependent on CXCL9 for efficacy.

Preteesh L. Mylabathula, Anas Alkhani, James Zhu, Skanda Hebbale, Riyasat Ali, Vivek Mathesh, Isaac Chan, Tao Wang, **Sangeetha M. Reddy**

UT Southwestern Medical Center, Dallas, TX

Background: Breast cancers have defects in antigen presentation, limiting T cell-mediated immunity. Flt3 ligand (Flt3L) is a growth factor that supports the differentiation of DC1 antigen-presenting dendritic cells (DCs), which are deficient in breast tumors, while CD40 agonist (CD40a) activates dendritic cells and also macrophages. We previously demonstrated that the combination of Flt3L, CD40a, and chemotherapy synergizes to improve outcomes in murine breast cancer models.
Methods: C57BL/6 mice (6-8 weeks old) were orthotopically injected with E0771, AT3, and 4T1 breast cancer cells in the mammary fat pad. Tumors were allowed to grow to ~50 mm^3 before mice were randomized into treatment groups. Mice received triplet therapy consisting of pegylated liposomal doxorubicin (PLD) administered via tail vein, followed by Flt3L intraperitoneally (IP) for 5 consecutive days, and CD40a IP one week later. Control groups included monotherapy and doublet therapy. Tumor growth and survival were monitored. Tumors and tumor draining lymph nodes were harvested from E0771 tumor bearing mice for single-cell RNA sequencing (scRNAseq) and spectral flow cytometry. To study if CXCL9 secretion and CD8 T cells mediate effects of therapy, CXCL9 chemokine and CD8 T-cells were systemically depleted with anti-CXCL9 and anti-CD8 antibodies administered IP every 4 and 2 days respectively during treatment.
Results: Triplet therapy led to improved tumor control compared to no treatment, monotherapy, and doublet controls ($p<0.0001$). Treatment led to significant increase in CD8 T cell recruiting chemokine CXCL9 expression from all intra-tumoral cDC populations including, cDC1, cDC2, and mregDCs ($p<0.01$). Additionally, there was a significant increase in an inflammatory macrophage population characterized by high CXCL9 expression. Increased tumor infiltration with CD8 T cells was observed with triplet therapy via both scRNAseq and flow cytometry ($p<0.01$), with an increase in stem-like and effector memory phenotype ($p<0.01$) and a decrease in exhaustion ($p<0.01$). While depletion of CD8 T cells significantly reduced treatment effect, efficacy was maintained in setting of CXCL9 depletion.
Conclusion: Our findings demonstrate that the combination of Flt3L, CD40 agonist, and chemotherapy significantly enhances tumor control in murine breast cancer models through increased recruitment of CD8 T cells which have more stem-like and effector memory phenotype. While significant increase in CXCL9 was observed, the efficacy of treatment was independent of CXCL9, suggesting importance of factors improving CD8 T cell quality and not just quantity. A clinical trial with this combination in metastatic triple negative breast cancer patients is open and recruiting (NCT05029999).

#6172 PI3K/Akt signaling pathway regulates CD155 expression involved in resistance to cancer immunotherapy.

Katsushige Kawase[1], Shusuke Kawashima[2], Tatsuya Nishi[3], Takashi Inozume[2], Takao Morinaga[1], Masahito Kawazu[1], Toyoyuki Hanazawa[4], Yosuke Togashi[3]

[1]Chiba Cancer Center Research Institute, Chiba-shi, Japan,[2]Department of Dermatology, Graduate School of Medicine, Chiba University, Chiba-shi, Japan,[3]Department of Tumor Microenvironment, Faculty of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama-shi, Japan,[4]Department of Otorhinolaryngology/Head & Neck Surgery, Graduate School of Medicine, Chiba University, Chiba-shi, Japan

Although anti-programmed death 1 (PD-1)/PD-1 ligand 1 monoclonal antibodies are effective against various cancers, resistance remains a significant issue in many patients. The immunosuppressive T cell immunoreceptor with Ig and ITIM domains (TIGIT)/CD155 axis has emerged as one of the crucial mechanisms contributing to this resistance. However, the complexities of CD155 expression remain incompletely understood. In this study, we conducted clustered regularly interspaced palindromic repeats (CRISPR) screening to identify key molecules involved in the regulation of CD155 expression and to investigate their roles in modulating CD155 within the tumor microenvironment (TME). CRISPR screening revealed that dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A (DYRK1A) as one of the key regulators of CD155 expression via the PI3K/Akt signaling pathway. In mouse models, inhibition of *Dyrk1a* using CRISPR/CRISPR-associated protein 9 (Cas9) technology or treatment with harmine, a DYRK1A inhibitor reduced CD155 expression, resulting in overcoming the resistance to PD-1 blockade. Moreover, in human head and neck squamous cell carcinoma (HNSCC) cell lines, inhibition of the PI3K/Akt signaling pathway reduced CD155 expression, similar to mouse models. In addition, cetuximab-mediated epidermal growth factor receptor blockade reduced CD155 expression by targeting downstream PI3K/Akt signaling in certain HNSCC cell lines. Clinically, patients with HNSCC harboring high CD155 expression exhibited elevated levels of Akt phosphorylation, consistently indicating that the PI3K/Akt signaling pathway is involved in CD155 expression. Furthermore, high CD155 expression contributed to PD-1 blockade resistance especially in patients who had high $CD8^+$ T cell infiltration. These results emphasized the role of the PI3K/Akt signaling pathway in regulating CD155 expression, which may influence resistance to PD-1 blockade therapies in various cancers, particularly those characterized by an inflamed TME. This study suggests that the reduction of CD155 expression by inhibiting the PI3K/Akt signaling pathway could overcome resistance to PD-1 blockade therapies.

#6173 Preclinical studies of BB-1712: A B7H3-targeting eribulin-containing ADC with the potential to overcome resistance to DNA topoisomerase I inhibitor-containing ADCs.

Y. Wang, B. Xia, L. Cao, J. Yang, C. Feng, F. Jiang, C. Li, L. Gu, Y. Yang, J. Tian, J. Geng, Q. Zhang, Z. Wei, Y. Zhou;
Bliss Biopharmaceutical (Hangzhou) Co., Ltd., Hangzhou, China

B7-H3, a member of the B7 family of proteins, is overexpressed in various solid tumors, including small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), while exhibiting minimal expression in healthy tissues. This unique expression profile positions B7-H3 as a promising target for antibody-drug conjugates (ADCs). Multiple B7H3-targeting ADCs are currently being tested in different stages of clinical trials and several of them have reported antitumor activities in heavily treated patients. In this study, we introduce BB-1712, a novel B7-H3-targeting ADC that incorporates eribulin as its cytotoxic payload, with the potential to treat patients not sensitive to ADCs containing DNA topoisomerase I inhibitors as payload toxin such as DS-7300a. BB-1712 was designed to leverage eribulin's distinct mechanism of action, which effectively inhibits tumor growth through multiple mode of actions including promotion of apoptosis, tumor microenvironment modulation, immunogenic cell death and bystander killing. We conducted extensive preclinical evaluations of BB-1712, focusing on its *in vitro* cytotoxicity and *in vivo* tumor inhibitory activities against a range of B7-H3-expressing cancer cell lines and xenograft models.Our results demonstrated that BB-1712 exhibited binding activity to B7-H3-positive cell lines similarly to that of Infinatamab, and displayed potent cytotoxic effects across multiple human cancer models, including non-small cell lung cancer, breast cancer, gastric cancer and small cell lung cancer cell lines. In xenograft studies, BB-1712 achieved significant tumor regression at well-tolerated doses without obvious toxicity. Notably, BB-1712 effectively overcame resistance to DS-7300a in both SCLC and NSCLC models previously treated with this agent. Pharmacokinetic and toxicology assessments in non-human primates revealed a favorable safety profile for BB-1712 at clinically relevant dosages and administration schedules.In summary, our findings suggest that BB-1712 has promising preclinical efficacy and safety profiles as well as the potential to circumvent resistance mechanisms associated with current therapies. These results support the advancement of BB-1712 into clinical trials for patients with B7-H3-expressing solid tumors.

#6174 Macrophage-based immunotherapy of platinum-resistant ovarian cancer.

Jan Brancewicz, Maciej Bialasek, Bartłomiej Taciak, Ilona Marszalek, Konrad Gabrusiewicz, Tomasz Rygiel, **Magdalena Krol**

Cellis AG, Zurich, Switzerland

Platinum-resistant ovarian cancer remains one of the greatest challenges in oncology, with limited treatment options and poor survival outcomes. To address this unmet need we develop MDC-735, a novel allogeneic, off-the-shelf macrophage-based therapy. MDC-735 leverages the breakthrough TRAIN (TRAnsfer of Iron-binding proteiN) mechanism, discovered by our team, to enable macrophages to deliver drug-loaded ferritin complexes directly to tumor cells. This approach bypasses drug resistance, penetrates hypoxic tumor regions, and overcomes challenges faced by conventional therapies. In preclinical studies, MDC-735 demonstrated remarkable efficacy in multiple solid tumor models, including platinum-resistant ovarian cancer, showing significant tumor shrinkage, long-term animal survival and immune activation. This unique therapy employs allogeneic macrophages loaded with a tubulin polymerization inhibitor, achieving targeted delivery while minimizing systemic toxicity. The ability of MDC-735 to alter the tumor microenvironment and induce immune resistance to recurrence highlights its potential as a game-changing therapy. This innovative therapy promises to transform the landscape of platinum-resistant ovarian cancer treatment, providing a scalable and effective solution for a disease with limited options.

#6175 Sting pathway activation potentiates the antitumor efficacy of doxorubicin in soft-tissue sarcoma.

Wonyoung Choi, Gi Yeon Lee, Sun-Young Kong

National Cancer Center - Korea, Goyang, Korea, Republic of

Background: Systemic treatment for soft-tissue sarcoma (STS) primarily relies on cytotoxic chemotherapy. Doxorubicin remains the key agent for systemic treatment in STS, as effective targeted treatment is unavailable and immune checkpoint inhibitors have shown limited efficacy. This study investigated whether activation of the STING pathway could enhance the antitumor effect of doxorubicin in STS.
Methods: STS cell lines were treated with doxorubicin to assess STING pathway activation, with Sting1 gene deletion used to validate its essential role. In a syngeneic mouse model of STS, the STING agonist ADU-S100 was administered intratumorally in combination with doxorubicin. Tumor-infiltrating $CD45^+$ immune cells were magnetically sorted for RNA sequencing to identify differentially expressed genes and enriched pathways linked to STING activation. These immune-related genes were analyzed for their association with survival in the TCGA-SARC patient cohort.
Results: Doxorubicin treatment in STS cell lines induced cytosolic DNA leakage, triggering STING pathway activation. Sting1 gene deletion in cells attenuated this doxorubicin-induced activation, but did not impact cell proliferation in vitro. In a syngeneic mouse model, doxorubicin suppressed tumor growth in vivo, an effect further enhanced by intratumoral co-administration of ADU-S100. RNA sequencing of $CD45^+$ tumor-infiltrating immune cells revealed upregulation immune response pathways linked with STING signaling. In the TCGA-SARC cohort, patients with higher expression of genes upregulated in STING-activated tumors exhibited improved survival, while those with lower expression showed poorer overall survival.
Conclusion: STING pathway activation by ADU-S100 enhances the antitumor efficacy of doxorubicin in STS, suggesting that combining doxorubicin with ADU-S100 may be a promising strategy for future clinical trials.

#6176 Selective HDAC6 inhibitor ITF3756 induces IL-2 production and CD4 and NK cells activation in MLR assay.

Gianluca Fossati, Chiara Ripamonti, Valeria Spadotto, Michela Bottani, Christian Steinkuhler

New Drug Incubator, Italfarmaco S.p.A., Cinisello Balsamo, Italy

Over the recent years, it has become increasingly clear that epigenetics plays a key role in regulating the anti-tumor immune response. Modification of the altered epigenetic status generated by tumors is a new and promising approach to induce an efficient antitumor immune response. Epigenetic drugs could represent a new avenue for cancer immunotherapy, particularly in combination with immune checkpoint inhibitors. Acetylation of proteins is one of the epigenetic modifications that has a recognized role in shaping the tumor microenvironment and the antitumor immune response. Among the histone deacetylase family, HDAC6 is a peculiar enzyme involved in tumor growth and in the modulation of the immune functions, that has recently taken hold. We have developed ITF3756, a selective HDAC6 inhibitor, currently in Phase I clinical trial, which exerts in vivo antitumor activity that is strictly dependent on a functional immune system. In vitro, it reduces the immunosuppressive function of myeloid cells and promotes their T cell stimulating activity. T cell development is also influenced by driving CD8 T cells towards a more central memory, less exhausted phenotype with an increased effector function upon tumor cells co-culture. These data derived from studies done with purified cells from human Peripheral Blood Mononuclear Cells (huPBMC). To shed light on the activity of ITF3756 in a more complex environment, Mixed Lymphocyte Reaction (MLR) was chosen as a model. MLR is largely used for investigating the activity of immunotherapy compounds including immune checkpoint inhibitors and can give insights on the possible in vivo mechanism of action of the investigated compounds. HuPBMC from different donors were cocultured in the presence or absence of ITF3756. Supernatants and cells were collected at different time-points and analyzed by ELISA and flow cytometry respectively. ITF3756 enhanced the production of IL-2 in the supernatants at 48 and 72h. Flow cytometry analysis showed that CD4 T cells and B cells, but not CD8 T cells, were the major populations where IL-2 was significantly increased in the presence of ITF3756. The increase of IL-2 production by ITF3756 at the earlier time points is correlated with a significant increase of CD4+CD25+ cells at day 6 and with a significant enhancement of the expression of the activator receptor NKG2D in Natural Killer cells (NK). Further analysis of CD4+CD25+ cells showed that ITF3756 activated CD4+CD25+CD127+ cells, but not the T regulatory cells (CD4+CD25+CD127-). Our data indicate that in a multicellular in vitro immune microenvironment, ITF3756 activates CD4 and NK cells and increases the production of IL-2. Given the importance of IL-2 in the tumor microenvironment, these new results, in addition to those previously collected on isolated myeloid and CD8 T cells, support the notion of the use of a HDAC6i such as ITF3756 for a promising immune checkpoint co-treatment therapy.

#6177 Deciphering the molecular determinants of testosterone paradox in prostate cancer.

Abhishek Shetty[1], Lillian Wilson[2], Deven Topiwala[2], Vaishnavi Anand[2], Zhen Wong[1], Yuhan Yang[2], Chandana Kruthiventi[2], Sai Harshitha Dakoor[2], Virginia Bencosme[2], Taylor Blanco[2], Boan Yang[2], Sayyada Kazim[2], Olutosin Owoyemi[2], Nathaniel Brennen[1], Eugene Shenderov[1], Michael Carducci[1], Samuel Denmeade[1], Sushant Kachhap[1]

[1]Oncology, Johns Hopkins Medicine, Baltimore, MD,[2]Johns Hopkins University, Baltimore, MD

Androgen deprivation therapy (ADT) is the mainstay of PC treatment for lethal, recurrent and metastatic prostate cancer (PCa). Although the initial response rate to ADT is high, the therapy invariably fails as cancer cells become resistant to therapy and progress into a castration-resistant stage called castration-resistant prostate cancer (CRPC). Based on a long-standing paradoxical observation that the growth of PCa could be inhibited by supraphysiologic testosterone (SupraT) treatment, we devised a therapy called the bipolar androgen therapy (BAT) to treat the CRPC. BAT involves a rapid cycling of testosterone between two polar extremes from supraphysiological to near castrate levels with each cycle lasting 28 days. Following the significant clinical efficacy of BAT in a subset of men with metastatic CRPC, we elucidated that SupraT can induce a non-apoptotic cell death called ferroptosis and activate innate immune genes in SupraT-sensitive PCa cells. The current study aims to identify molecular determinants of SupraT-induced cell death and immune activation. This study sheds light on the molecular mechanisms involved in the BAT therapeutic response. Understanding and characterizing the molecular determinants of PC response to BAT is crucial as it will help us stratify patients who would benefit from the therapy and also devise novel combination therapies with BAT including immunotherapeutics, for an enhanced clinical outcome.

#6178 Photochemical immune stimulation eradicates primary tumor and induces long-lasting anti-tumor immunity in a murine cutaneous melanoma model.

Layla Pires[1], Carla Calcada[2], Tracy McGaha[2], Gang Zheng[2], Brian Wilson[2]

[1]Biomedical Engineering, Texas A&M University, College Station, TX,[2]Princess Margaret Cancer Center, University Health Network, Toronto, ON, Canada

Background: Photodynamic therapy with light-activation of photosensitizers to induce cell death has been used in the treatment of various tumor types but has been ineffective to date in melanoma due to tumor pigmentation that limits the depth of light penetration. In previous studies in immunodeficient mice using a combination of tumor-cell and vascular targeting we demonstrated eradication of thin primary tumors. Here, we extend this to immunocompetent mice bearing full-thickness primary tumor, investigating both the primary and metastatic responses. Initial evidence of strong immune stimulation was also seen in multifunctional porphyrin-lipid nanoparticles (Porphysomes) as the photosensitizer.

Methods: S91 tumors were induced in the flank of DBA mice by intradermal injection of 10^6 cells in 40μL PBS. Tumor growth was monitored by ultrasound imaging. The main treatment protocol comprised a combination of the clinical photosensitizers: chlorin e6 (1 mg/kg) plus Visudyne (0.8 mg/kg) at 1 h and 15 min drug-light intervals using light fluences of 50 J/cm² at 670 and 690 nm, respectively. Immune biomarkers were measured in spleen, blood, tumor-draining lymph nodes and tumors at 3, 6, 12, 24, 72 h and 5 and 10 d post-treatment. The mice were monitored for 90 d to determine disease-free and overall survival. The functionality of the immune stimulation was demonstrated by tumor re-challenge, abscopal and adoptive immune transfer experiments. Initial studies were also performed using multifunctional porphyrin-lipid nanoparticles (Porphysomes, PS).

Results: The dual-agent protocol resulted in disease-free and overall survival rates >95% at 90 d post-treatment. A robust immune response was triggered, with recruitment and activation of both innate and adaptive immune systems. Upon re-challenge with 10^6 tumor cells at 45d post primary tumor treatment, mice previously treated with PCIS showed no systemic tumor development at 30 d, confirming induction of long-lasting anti-tumor immunity. Adoptive immune transfer was demonstrated by delayed growth of primary intradermal tumor in naïve mice co-injected with splenocytes from previously-treated mice. Abscopal response was seen in mice bearing bilateral tumors when only one tumor was treated. Survival was reduced (20% at 90 d) in $CD8^+$ T cell-depleted mice. PS-mediated treatment exhibited strong immune stimulation even at a fraction of the light dose (25 J/cm^2), as measured by adoptive immune transfer and immune biomarkers.

Conclusions: This study provides the first evidence that, using non-conventional treatment protocols, photodynamic tumor cell kill can eradicate primary pigmented melanoma far beyond the depth of light penetration and markedly impact systemic metastatic disease. PCIS could be used either stand-alone or an adjunctive treatment with surgery, chemotherapy and/or immunotherapy.

POPULATION SCIENCES: Breaking Barriers in Cancer Care: Addressing Disparities and Improving Outcomes
Poster Session

#6182 Receipt of survivorship care plans is associated with self-reported health status for Black female cancer survivors in Maryland.

Camryn Mae Cohen[1], Kassandra I. Alcaraz[2], Lensa S. Keno[1], Mu Jin[3], Avonne E. Connor[1]

[1]Epidemiology, Johns Hopkins Bloomberg Sch. of Public Health, Baltimore, MD,[2]Oncology, Johns Hopkins School of Medicine, Baltimore, MD,[3]Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, NC

Background: Survivorship care plans (SCPs) support the transition from specialized cancer treatment to primary care and have been promoted for improving cancer survivorship. We previously demonstrated that non-Hispanic Black (NHB) female cancer survivors in Maryland are more likely to receive SCPs than their non-Hispanic White (NHW) counterparts. Here, we assessed whether receipt of SCPs is associated with general health of female cancer survivors.

Methods: Using cross-sectional data from the 2011-2023 Maryland Behavioral Risk Factor Surveillance System (BRFSS) survey, we evaluated receipt of SCPs and general health for NHB and NHW female cancer survivors (N, weighted %). A survey-weighted multivariable logistic regression model was used to estimate odds ratios (ORs) with 95% confidence intervals (CIs) for the association between self-reported receipt of a SCP and general health (fair or poor health vs. excellent or very good health); stratified by race, adjusted for time since cancer diagnosis (1-5, 6-10, 11+ years), age (years), income (≤$50k vs. >$50k), education (less than college vs. at least some college or greater) and body mass index (underweight, normal weight, overweight, obese). Individuals on active treatment and <1 year out from their cancer diagnosis were excluded.

Results: Of the 2,854 female cancer survivors, 312 (14%) were NHB and 2,542 (86%) were NHW. Most women received an SCP (73%), with NHB women being more likely to receive a SCP than NHW women (80% vs. 72%). High income and high education individuals were more likely to receive SCPs (76% vs. 72% and 76% vs. 70%, respectively). While most women reported excellent or very good health (75%), NHB vs. NHW women were more likely to report fair or poor health (38% vs. 22%, respectively). NHB vs. NHW women were significantly [OR: 48.31; 95% CI: 5.35-436.02, p=0.001] more likely to report fair or poor general health. However, NHB women who received a SCP were significantly less likely [OR: 0.031; 95% CI:0.003-0.31; p=0.003] to report fair or poor health compared to NHB women who did not receive a SCP. There was no significant difference in health status for NHW women who did vs. did not receive a SCP [OR: 1.20; 95% CI: 0.62-2.36; p=0.587].

Conclusions: Our results indicate that receipt of SCPs post-treatment is important for current health status among NHB women and highlights a potential intervention for improving the general health of minoritized female cancer survivors.

#6183 Social determinants and survivorship care among Black women breast cancer survivors in Maryland.

L. S. Keno[1], **C. M. Cohen**[1], K. E. Dibble[2], K. Visvanathan[1], A. E. Connor[1];
[1]Johns Hopkins Bloomberg Sch. of Public Health, Baltimore, MD, [2]Dana-Farber Cancer Institute, Boston, MA

Purpose: Limited research has examined the impact of social determinants of health (SDOH) on survivorship care experiences exclusively among Black breast cancer (BC) survivors who face survival disparities due their high prevalence of obesity, comorbidities, and inequities in access and quality of care. Survivorship care experiences among Black BC survivors should be examined to identify potential interventions to improve health outcomes. The goal of this study is to test the feasibility of establishing a state-wide cohort study of Black BC survivors residing in Maryland to address this research gap.
Methods: This study will recruit 350 Black women BC survivors, aged ≥18 years, and diagnosed with stage I-III primary BC who have completed recommended adjuvant treatment including surgery, chemotherapy, and/or radiation. Recruitment approaches include targeted Facebook ads, outreach to previous study participants, and mailed recruitment letters from the Maryland Cancer Registry (MCR). Participants complete a 30-40-minute online survey, covering demographic and lifestyle factors, clinical and SDOH characteristics, and survivorship care questions. The study also pilots medical record abstractions with the MCR for cancer characteristics and the state designated health-information exchange, Chesapeake Regional Information System for Patients (CRISP), for follow-up care.
Results: Recruitment began in July 2024, and by September 2024, 61 self-reported Black participants with a prior diagnosis of BC have completed the survey. The average age at survey is 58.96 (standard deviation (SD)= 11.84)) years, with an average age at diagnosis of 46.42 (SD=13.35) years. 40% are married/living as married, 36.7% are college graduates, all have health insurance, and only 20% reported household incomes <$40,000/year. Almost half of the survivors self-reported being diagnosed with stage 1 BC (45.9%), followed by 31.1% stage 2, 18% stage 3, and 4.9% unknown stage. The most common self-reported comorbidities include hypertension (39.3%), diabetes (16.7%), and obesity (12.3%). Related to care, 83.3% have a family doctor, and 73.8% received a treatment summary. Additionally, 56.1% and 54.4% consented to medical record linkages with MCR and CRISP, respectively. Recruitment is ongoing.
Conclusion: This study will inform future interventions by identifying disparities in survivorship care experiences among Black BC survivors and evaluating the impact of this care on health outcomes.

#6184 A literature-based data scanning and prioritization study to guide future systematic literature reviews for WCRF International CUP Global Programme.

Georgios Markozannes, Ahmad Jayedi, Sofia Cividini, Sayada Zartasha Kazmi, Margarita Cariolou, Rita Vieira, Sonia Kiss, Katia Balducci, Dagfinn Aune, Marc Gunter, Amanda Cross, Doris Chan, **Konstantinos Tsilidis**

Epidemiology and Biostatistics, Imperial College London, London, United Kingdom

Background: In 2018, the World Cancer Research Fund (WCRF)/American Institute for Cancer Research published the Third Expert Report (TER) synthesizing the evidence on diet, adiposity, physical activity and risk of 19 cancers and 10 updated evidence-based cancer prevention recommendations. As part WCRF International's Global Cancer Update Programme, we aimed to develop a prioritization support tool to identify what systematic literature reviews (SLR) of the epidemiological evidence published after the TER would help strengthen the TER's conclusions and deepen our understanding on emerging topics.

Methods: We developed a literature scanning and prioritization strategy in six actions. From January 2019 (after TER publication) to February 2024, we searched PubMed for: 1) meta- and pooled analyses, 2) randomized controlled trials (RCTs) and protocols, 3) Mendelian randomization (MR) studies, and 4) prospective cohort studies (≥100,000 participants) on diet, adiposity, physical activity, sedentary behavior and cancer risk. In action 5, we utilized the fail-safe number and conditional power statistics to estimate the number of additional studies needed to change the existing TER meta-analytic findings. Finally, action 6 integrated data from actions 1-5, to construct an exposure-based prioritization ranking across all cancer sites. A prioritization score (PS) was derived by assessing the number of new studies, the concordance of association measures (direction and statistical significance) against the TER, and the volume of new associations not covered in the TER.

Results: We reviewed 366 meta-analyses, 121 pooled analyses, 19 RCTs, 368 MR studies and 359 cohort studies covering 151 exposures and 28 cancers, comparing them to 1371 TER meta-analyses. TER meta-analyses most likely to change from not statistically significant to significant when adding new studies included folate and colorectal cancer, waist circumference and lung cancer in never smokers, poultry and breast cancer, total fat and ovarian cancer, tea and ovarian cancer, and red meat and kidney cancer, but most associations observed in the TER are unlikely to change with additional similar research. The median PS score across exposures was 6 (range -11 to 163). Top ranked exposures with new literature evidence included anthropometric measurements (PS_{height} 40 to PS_{BMI} 163), physical activity (PS 100), sedentary behavior (PS 64), alcohol (PS 52), tea (PS 36), milk and dairy products (PS 29), micronutrients ($PS_{retinol}$ 27 to PS_{iron} 38), vitamins (PS_{B12} 22 to PS_{vitD} 91), soy (PS 24), isoflavones (PS 23), and soft drinks (PS 22). Across exposures, the top cancers were colorectal, breast, lung, liver, and endometrium.

Conclusions: The outputs from the prioritization support tool can help target SLRs that would most usefully contribute to evidence-based cancer prevention recommendations.

#6185 Integrating internships into the CaRE^2 Health Equity Center: A pathway for developing future health equity leaders.

Nazleen Del Rio[1], Brooke Hensel[1], Ileana Guzman[1], Eduardo Ibarra[2], Carolina Aristizabal[2], Rosa Barahona[2], Lourdes Baezconde-Garbanati[2], Mariana Stern[2], John Luque[3], Sandra Suther[3], Fern J. Webb[4]

[1]University of Florida, Orlando, FL,[2]University of Southern California Norris Comprehensive Cancer Center, Los Angeles, CA,[3]Florida Agricultural and Mechanical University, Tallahassee, FL,[4]University of Florida, Jacksonville, FL

The CaRE^2 Health Equity Center's Community Outreach Core (COC) provides internships to prepare students for meaningful careers in cancer health disparities by bridging classroom learning with community work. These internships equipped students with skills in data analysis, community outreach, cancer health education, and cultural humility. Tailored internships provide a unique opportunity for students to gain hands-on experience in health equity research focused on eliminating cancer health disparities. As a full-time intern, one of my first activities was to create a work plan to include objectives, goals and anticipated deliverables of my internship. My orientation included completing ethics training and joining institutionally-approved research studies. I worked directly with community partners and received mentorship from leading scientists and researchers. I helped plan and implement health promotion programs, create materials and organize events designed to increase knowledge about cancer screening, treatment and prevention. I also contributed to outreach activities and analyzed program data which strengthened my skills and broader network in community health. My internship provided me with a comprehensive understanding of health disparities work, preparing me to make a lasting difference in the field. The internship aimed to yield lasting benefits by equipping aspiring health equity advocates with practical skills and opportunities to work directly with others committed to community impact. Interns are encouraged to share their innovative ideas and perspectives on CaRE^2's projects, further enhancing the effectiveness and relevance of its initiatives to meet community needs. Looking to the future, expanding CaRE^2's internship program to include students from all partner institutions Florida A&M University, the University of Florida, and the University of Southern California would further enhance the Center's capacity to conduct research studies and educational programs. Currently, Florida A&M University hosts two 12-week public health internships annually with COC. Overall, interns contribute skills that support COC's aims: increase the number of people informed and prepared to advocate for cancer research (aim 1), share information that increases awareness and knowledge about cancer prevention, screening and ongoing research (aim 2); and provides additional opportunities to disseminate cancer-related information. Internships like those offered by CaRE^2 empower students to engage in health equity. These experiences prepare interns to carry forward culturally sensitive approaches, strengthening CaRE^2's legacy and contributions to a more inclusive healthcare future. For others considering an internship, seek programs with diverse, hands-on opportunities that are designed to help you define your career path and make a meaningful difference.

#6187 Venous thromboembolism in metastatic and non-metastatic head and neck cancer.

Ted Akhiwu[1], May Gao[2], **Nosayaba Osazuwa-Peters**[2]

[1]Medstar Union Memorial Hospital, Baltimore, MD,[2]Duke University, Durham, NC

Background: Venous thromboembolism (VTE) is a comorbidity in many cancer patients, however, data in patients with head and neck cancer (HNC), as well as the burden in patients with metastatic HNC compared to non-metastatic HNC is limited. This study aimed to examine the prevalence, hospitalization outcomes, and correlations of VTE in patients with HNC.
Methods: We utilized retrospective data from the 2016-2020 National Inpatient Sample (NIS) to evaluate VTE in HNC patients. We included adults ≥ 18 years with a diagnosis of HNC using ICD-10 codes. The primary outcome was the odd of VTE among patients with metastatic (M-) vs non-metastatic (NM-) HNC. VTE was defined as deep vein thrombosis (DVT) or pulmonary embolism (PE) using ICD-10 diagnosis codes. Secondary outcomes were the odds of in-patient mortality and prolonged length of stay [LOS (≥ 21 days)] among HNC patients with VTE. We compared demographic, clinical, and hospital characteristics between metastatic and non-metastatic HNC patients using $\chi 2$ for categorical and t-tests for continuous variables. Multivariable logistic regression models were fit to assess the relationship between clinical, patient, and hospital characteristics and study outcomes.
Results: There were 351850 weighted hospitalizations with a diagnosis of HNC between 2016 and 2020 (23.20% M-HNC and 76.80% NM-HNC). Among HNC patients, 2.74% had VTE (3.26% M-HNC vs 2.59% in NM-HNC). The mean age of HNC patients was 64.11 years (± 11.66) [64.55 years (±11.70) NM-HNC vs 62.63 years (±11.40) M-HNC]. Among HNC patients, higher odds of VTE were associated with Black race (aOR: 1.25, 95% CI 1.08 - 1.43), having higher Charlson comorbidity (CCM) scores [aOR 1.34 (1.20 - 1.50)] and lower odds were associated with Asians/Pacific Islanders (PI) (vs White, aOR0.62 (0.44 - 0.84)] and those undergoing major surgery [(aOR 0.63 (0.54-0.74)]. Among NM-HNC, higher odds of VTE were associated with Black race [(vs white, aOR 1.23 (1.04 - 1.45)] and higher CCM [(aOR 1.19 (1.01 - 1.41)]. Among patients with M-HNC, higher odds of VTE were associated with Black race [(vs White aOR 1.30 (1.02 - 1.68)], and higher CCM [(aOR 1.34 (1.09 - 1.64)] and lower odds were noted in Asian/PI [(vs white aOR 0.44 (0.25 - 0.79)]. VTE was associated with increased odds of in-hospital mortality [aOR 2.26 (1.91-2.67)] and prolonged LOS [AOR 3.60 (3.13-4.15)] overall and in both M-HNC [AOR for mortality 2.06 (1.55-2.73) and LOS 3.36 (2.57 - 4.40] and NM-HNC [AOR for mortality 2.35 (1.91-2.89) and LOS 3.73 (3.16 - 4.41)].
Conclusion: VTE is more prevalent in M-HNC patients and is associated with adverse in-hospital outcomes including increased mortality and a prolonged LOS. Black race and higher comorbidity were positively associated with VTE, while Asian/PI race and head and neck surgery were negatively associated. Findings highlight the need for targeted interventions to prevent VTE in high-risk HNC patients and reduce adverse in-hospital outcomes.

#6188 Evaluating impact and progress in the iCCaRE consortium for Black men's approach to reducing prostate cancer disparities.

Ivey Breland, Ernest Kaninjing, Damian Francis

Georgia College & State University, Milledgeville, GA

The purpose of the Inclusive Cancer Care Research Equity for Black men (iCCaRE) Consortium is to address prostate cancer disparities impacting Black men through collaborative research, education, and advocacy. This study evaluates the progress of iCCaRE in achieving its core objectives across five pilot projects supported by multi-institutional partners. Specifically, iCCaRE aims to expand research infrastructure, develop culturally tailored interventions using artificial intelligence to address social determinants of health, explore modifiable social-biological factors affecting patient outcomes, and provide extensive research training for underrepresented minority scientists and advocates. Our methodology involved the creation and implementation of a comprehensive data collection tool via Qualtrics to assess activity outcomes across the Consortium. This tool enabled standardized reporting on publications, grants awarded, media reach, community impact, and additional metrics tied to scientific productivity and partnership engagement. Unpublished data collected this year indicate substantial progress, including an increase in collaborative research outputs, expanded access to prostate cancer care resources, and enhanced engagement with both clinical and community-based partners. These findings underscore the Consortium's effectiveness in fostering scientific collaboration, developing targeted interventions, and creating infrastructure to support sustainable improvements in prostate cancer outcomes for Black men. These accomplishments provide a strong foundation for iCCaRE's transition into Phase 2, with plans to deepen community engagement, expand research training, and enhance data-driven insights through advanced analytics.

#6189 Educational level as a determinant of breast cancer stage at diagnosis.

Xiaying Kuang

The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China

Background: Presently, there is a gap in literature regarding specific demographic characteristics and the stage of breast cancer at diagnosis. Herein, we examined patients' educational levels to explore effective strategies for early breast cancer diagnosis.
Methods: Convenience sampling method was used to select invasive breast cancer patients treated at a tertiary hospital in Guangzhou between January 2018 and March 2023 to determine patients' breast cancer stage at diagnosis and individual characteristics. The associations were analyzed using Student's t-test and the chi-square test, and binary logistic regression analysis was conducted to assess the odds ratios (ORs) for the occurrence of late-stage breast cancer.
Results: The study encompassed a total of 3225 patients diagnosed with invasive breast cancer. Among them, 707 patients were identified with late-stage cancer (stage III-IV), comprising 21.9% of the total sample. Univariate analysis revealed a significant inverse correlation between patients' educational level and the stage of breast cancer at diagnosis, regardless of age stratification. The results of the chi-square test revealed a significant correlation between patients' educational level and their perception of illness ($P < 0.001$), with patients with higher levels of education being more likely to demonstrate a more comprehensive understanding of the disease. Upon controlling for other potential confounding variables, these findings were confirmed by binary logistic regression analysis, which demonstrated that individuals with lower education levels had a significantly increased likelihood of being diagnosed with late-stage breast cancer (adjusted OR = 1.409 for the middle school group and 1.518 for the illiterate and primary school group).
Conclusions: Patients with lower educational levels could be more likely to present with late-stage breast cancer at diagnosis. Therefore, it is imperative to prioritize popular science and early prevention strategies for breast cancer among less educated populations.

#6190 Descriptive analysis of selenium levels in a healthy Malawian subpopulation: Findings from a control study on esophageal squamous cell carcinoma (ESCC).

Emmanuel Uduh, Yukiko Yano, Yingxi Chen, Christian Abnet, Bongani Kaimila, Shiraz Khan

Division of Cancer Epidemiology and Genetics, NIH-NCI, Rockville, MD

Background: Esophageal squamous cell carcinoma (ESCC) is the third most common cancer in Malawi, with an estimated 1,871 new cases and 1,766 deaths in 2024 in Malawi. Although drinking alcohol and smoking tobacco are major causes of ESCC in some populations, the high rates in Malawi are unlikely to be due to these agents. Several East African countries have high incidence rates of ESCC and low soil selenium concentrations. Observational and intervention studies in East Asia have shown that low selenium concentrations are linked to an increased risk of ESCC.
Methods: We used control subjects from a previously reported hospital-based case-control study of ESCC conducted at two hospitals in Lilongwe, Malawi (2017-2020). The study collected questionnaire data and stored fractionated blood. ESCC cases were excluded due to reverse causation for nutritional biomarkers. Instrumental neutron activation analysis (INAA) was used to measure serum selenium concentrations. Using R, we generated descriptive statistics and tested for associations with demographic variables. We also compared this study population to other previously studied populations with high ESCC incidence rates.
Results: We successfully measured 247 control subjects from Malawi and found a median (IQR) serum selenium concentration of 77.3µg/L (65.9-89.5). 79 (31.9%) subjects were below the 72.2 subjects µg/L threshold of the median level of selenium We found no statistically significant associations between serum selenium concentrations and demographic characteristics including sex, education, marital status, and several risk factors for ESCC previously identified in the underlying case-control study. The serum selenium concentrations in these subjects were low and similar to previously studied regions where ESCC is common including both Bomet, Kenya and Linxian, China.
Conclusion: We found low median serum selenium levels in control subjects from a hospital-based case-control study of ESCC conducted in Malawi, a region known to have high rates of ESCC. The range was similar to other populations with high incidence of ESCC. Direct linkage to disease risk will require different study designs, but our findings suggest that selenium deficiency could be a factor in the high burden of ESCC in East Africa.

#6191 Associations of alcohol consumption with expression of CD44, CD24, and ALDH1A1 stem cell markers in incident benign breast biopsy samples.

Rulla M. Tamimi[1], Maisey Ratcliffe[2], Yu Jing J. Heng[3], Brian Sardella[3], Lusine Yaghjyan[4]

[1]Pathology, Cornell University, New York, NY,[2]University of Florida, Gainesville, FL,[3]Pathology, Beth Israel Deaconess Medical Center, Boston, MA,[4]Epidemiology, University of Florida, Gainesville, FL

Purpose: We investigated the associations of alcohol consumption with expressions of stem cell markers CD44, CD24, and ALDH1A1 in cases of incident benign breast disease.
Methods: This study included 721 cancer-free women with biopsy-confirmed benign breast disease (BBD) within the Nurses' Health Study II cohort. Histologically normal breast tissue cores were used to construct tissue microarrays (TMAs). Immunohistochemistry (IHC) was performed on TMA sections to analyze the expression of stem cell markers. For each core, the IHC expression was assessed using semi-automated software and reported as the % of positively stained cells for each of the markers out of the total cell count. All expression measures were log-transformed for analysis. Alcohol consumption was obtained from semi-quantitative food frequency questionnaires. Other covariate information was collected on the initial questionnaire and updated biennially thereafter. We examined associations for recent (at the time of biopsy) and cumulative average alcohol consumption (from all questionnaires before the biopsy), modeled as a continuous variable (drinks/day). Generalized linear regression was used to examine the associations of alcohol with each marker's expression (within stroma and epithelium), while adjusting for known breast cancer risk factors and BBD subtype.
Results: In this study of 721 cancer-free women, the average age at the time of biopsy was 44 (range 27-63), with the majority of participants being premenopausal at the time of biopsy (81.28%). Recent and cumulative average alcohol consumption were not associated with expression of CD44, CD24, or ALDH1A1 in stroma (recent: β per drink/day=0.08, 95% Confidence Interval [CI] -0.16,0.32; β=-0.03, 95% CI -0.11,0.05; β=0.01, 95% CI -0.26,0.29, respectively; cumulative average: β=-0.09, 95% CI -0.38,0.19; β=-0.01, 95% CI -0.10,0.08; β=-0.04, 95% CI -0.36,0.27, respectively) or epithelium (recent: β=0.08, 95% CI -0.04,0.21; β=0.02, 95% CI -0.01,0.06; β=$0.23x10^{-2}$, 95% CI -0.15,0.15, respectively; cumulative average: β=-0.06, 95% CI -0.08,0.20; β=0.04, 95% CI -0.01,0.08; β=-0.01, 95% CI -0.18,0.17, respectively).
Conclusion: Our findings suggest that alcohol use is not associated with the expression of CD44, CD24, and ALDH1A1 in histologically normal breast tissue from benign biopsies.

#6193 Population-level interventions to prevent alcohol-related cancers: A scoping review.

Priyanka Basu[1], Jovan Julien[2], Sangeeta Goswami[3], Anton L.V. Avancena[4]

[1]Texas A&M University School of Medicine, Round Rock, TX,[2]Georgia Institute of Technology, Atlanta, GA,[3]MD Anderson Cancer Institute, Houston, TX,[4]University of Texas, Dell Medical School, Austin, TX

Alcohol use and misuse are associated with at least eight types of alcohol-related cancers, namely cancers of the oral cavity, pharynx, larynx, esophagus (squamous cell carcinoma only), stomach, colon, liver (hepatocellular carcinoma only), and breast in females. Population-level interventions that reduce alcohol use may mitigate this cancer burden. This scoping review examines and synthesizes evidence on the effectiveness of these interventions on cancer-related outcomes.

We followed the PRISMA-ScR guidelines and searched MEDLINE and Web of Science for English-language articles (1987 onwards) using keywords and terms related to interventions, alcohol use, and cancer in titles and abstracts. Only original quantitative research articles evaluating population-level interventions' impact on cancer outcomes were included. Initial data extraction was conducted using Elicit, an AI-based research tool, which was reviewed by the research team for accuracy.

Out of 1,531 studies, 68 full-texts were assessed for eligibility, and 35 were included in the final qualitative synthesis. Most studies were focused on Europe (N=17), Asia and Oceania (N=11), and North America (N=4). Study designs included randomized controlled trials (N=6), observational studies (N=18), and simulation models (N=11). The most common cancer-related outcome was cancer incidence (N=19), followed by cancer mortality (N=11) and knowledge/awareness of alcohol as a carcinogen (N=10). Primary prevention studies (N=31) focused on reducing alcohol-related cancers through educational or media initiatives (N= 13), adherence to clinical guidelines (N=11), and alcohol pricing policies (N=6) like alcohol taxes and minimum unit pricing. Five studies addressed secondary prevention via educational and clinical interventions like screening, health assessment, and treatment of alcohol misuse. No tertiary prevention studies were identified.

This scoping review identified several gaps in our understanding of population-level interventions for alcohol-related cancers. Given the number of ever and current drinkers globally, additional studies are needed on secondary and tertiary prevention strategies. Few studies evaluated alcohol pricing policies despite the long-standing evidence supporting its effectiveness in reducing alcohol use. Meeting cancer prevention guidelines may reduce cancer incidence and mortality, and future research should focus on the most effective and cost-effective ways of translating recommendations into interventions in real-world contexts.

#6194 Unveiling cancer disparities: a task force report on multilevel drivers impacting equitable cancer care among Latinos in the US.

Amelie G. Ramirez[1], Edgar Munoz[1], Lorna Rodriguez-Rodriguez[2], Leon Bernal-Mizrachi[3], Patricia Chalela[1], Jose Lopez[4], Paulo S. Pinheiro[5], Barbara Segarra Vasquez, DHSc[6], Gregory A. Talavera[7], Luis G. Carvajal - Carmona[8], Adolfo Diaz Duque[1], Cliff Despres[1], Edward J. Trapido[9]

[1]UT Health Science Center at San Antonio, San Antonio, TX,[2]City of Hope, Duarte, CA,[3]Emory University, Atlanta, GA,[4]University of Washington, Seattle, WA,[5]University of Miami, Coral Gables, FL,[6]University of Puerto Rico, San Juan, PR,[7]San Diego State University, San Diego, CA,[8]UC Davis - California, Davis, CA,[9]Louisiana State University, New Orleans, LA

Background: Cancer disparities among Latinos in the US are a critical public health issue, with this group experiencing inequitable outcomes across the cancer continuum, from screening to end-of-life care. Latinos have higher rates of certain cancers and poorer outcomes compared to other populations, driven by a complex interplay of environmental, genetic, cultural, behavioral, and systemic factors. While earlier research has highlighted some of these disparities, comprehensive analyses of the multilevel factors affecting cancer care among Latinos are still limited. This report aims to identify these factors to help guide the work of current and future researchers who can address Latino cancer outcomes.
Methods: The Task Force: Latino Researchers Against Cancer (TFLRAC) held structured meetings in 2023 to share expertise. Participants included specialists in oncology, public health, and behavioral sciences who examined environmental, genetic, cultural, behavioral, and systemic contributors to cancer care disparities among Latinos. The goal was to explore the causes and multilevel drivers of health impacting the Latino cancer experience.
Results: Findings show that cancer disparities among Latinos are influenced by multiple factors, including environmental exposures associated with higher pollution levels and occupational hazards, genetic variations affecting cancer risk and prognosis, and cultural and linguistic barriers affecting healthcare decisions. Behavioral factors, such as unhealthy dietary habits, and systemic issues, such as limited access to healthcare, inadequate insurance coverage, and lack of diversity in the healthcare workforce, further contribute to these disparities. Additionally, socioeconomic barriers and restrictive eligibility criteria contribute to the underrepresentation of Latinos in clinical trials, while cultural stigmas, limited support services, and language barriers pose challenges in cancer survivorship.
Conclusions: Reducing cancer disparities among Latinos requires a multifaceted approach, including targeted research on environmental and genetic factors, culturally sensitive interventions, and policy reforms to improve healthcare access and workforce diversity. Collaboration among academia, healthcare providers, Latino communities, government, and industry is essential to achieve equitable cancer care for Latinos.

#6195 Rural and urban disparities in distance to childhood cancer survivorship care.

Amanda S. Thomas[1], David Van Riper[2], Jesse Berman[3], Helen M. Parsons[4], Lucie Turcotte[5], Logan Spector[6], Jenny Poynter[6], Erin Marcotte[6]

[1]Epidemiology & Community Health, University of Minnesota, School of Public Health, Minneapolis, MN,[2]University of Minnesota, Institute for Social Research and Data Innovation, Minneapolis, MN,[3]Environmental Health Sciences, University of Minnesota, School of Public Health, Minneapolis, MN,[4]Health Policy & Management, University of Minnesota, School of Public Health, Minneapolis, MN,[5]Pediatrics, University of Minnesota Medical School, Minneapolis, MN,[6]Division of Pediatric Epidemiology and Clinical Research, University of Minnesota Medical School, Minneapolis, MN

Presently, 1 in 530 young adults (ages 20-39 years) in the US is a survivor of childhood cancer. These individuals are burdened with high risks for chronic disease, disability, secondary cancers, and a shortened lifespan. Because of these late health consequences, survivors are recommended to receive specialized healthcare according to survivorship care guidelines. Despite this, a significant proportion of survivors fail to receive survivorship-directed care, particularly among the adolescent and young adult (AYA) population, suggesting systematic barriers to care. The goal of this analysis was to assess one possible barrier by characterizing where AYA survivors live in the US and their distance to survivorship care centers.

The Children's Oncology Group (COG) Childhood Cancer Research Network (CCRN), a large registry of childhood cancer patients, includes information on cancer diagnosis, place of residence, and initial treatment institution for >50,000 childhood cancer patients treated in North America. In this analysis, CCRN data were linked to LexisNexis via probabilistic matching to derive residential history for each case and up to two parents or guardians and used to evaluate distance to survivorship care. Those with low confidence scores were excluded and the remaining true addresses were geocoded with a 94% good match rate. Distance to care was calculated from the most recent address to nearest COG survivorship center based on those publicly available in the 'COG Late Effects Directory of Services' online database.

Of individuals of AYA age at diagnosis (15-39 years), 3,396 had successfully geocoded case-specific addresses for survivorship at an average of 14.8 years post-diagnosis (survival time). The most common primary diagnoses were leukemias and lymphomas (54.8%) and mean diagnosis age was 16 years (IQR: 15-18). Nearly 60% were male with 15.8% of Hispanic origin and 74% identifying as White. The most populous states for this cohort (CA, TX, FL, NY) comprised about one-third of all survivors. Average straight-line distance to survivorship care among all AYA survivors was 18.2 miles (IQR: 7.9-45.8), with rural residents having to travel more than four times as far than urban to COG-specific centers. Those that had to travel farther for care were primarily in the low/middle socioeconomic classes (Kruskal-Wallis p-value <0.001) with no considerable differences existing based on sex. Among diagnoses, survivors initially treated for CNS tumors and renal cancer were furthest from survivorship care (average 20.5 and 23.3 miles to nearest COG location, respectively).

Treatment at greater distances can become a burden logistically and financially, potentially limiting access to care. As patients transition into survivorship care, distance to care could impact healthcare choices and long-term health outcomes, especially among those with higher-risk initial diagnoses or those from disadvantaged backgrounds.

#6196 Characteristics of individuals with early-onset multiple myeloma in South Carolina.

Amy F. Ballou[1], Tomotaka Ugai[2], Abbas Tavakoli[1], Karen Wickersham[1]

[1]University of South Carolina, Columbia, SC,[2]Harvard Medical School, Boston, MA

Background: The incidence of early-onset cancers, defined as cancer in adults under 50 years, are on the rise. Early-onset multiple myeloma (MM), which is the second most common hematologic cancer in the U.S., is one of early-onset cancer types with increasing incidence. This age/race disparity is believed to be linked to genetic factors and social determinants of health (SDOH), though the precise causes are still under investigation. Data on risk factors for early-onset MM are limited. The incidence rate of MM in South Carolina is higher than the U.S. average. A geospatial analysis study showed differences in average county-level age-adjusted incidence rates (ASR) across U.S. counties, with high ASR found primarily in the Southeast and two major high-high local clusters of the ASR in Georgia and South Carolina. To address this gap, we first examined the characteristics of patients with early-onset MM in South Carolina.
Purpose: To examine 1) the prevalence of patients with early-onset MM in South Carolina and 2) their characteristics and SDOH.
Methods: A retrospective review of electronic medical records (EMR) from 1/1/2020 to 8/30/2023 was conducted to identify patients with early-onset MM (N=23) at a community oncology clinic in South Carolina. Potential cases were identified by EMR reports based on age and diagnosis. EMR confirmed eligibility, and socio-demographics and cancer-related information was entered into a REDCap database. SAS (v.9.4) was used for descriptive statistics.
Results: The total number of MM cancer cases in all ages observed during the study period was 247. Among them, twenty-three cases were diagnosed before age 50 (prevalence: 10.7%). The average age at diagnosis was 44.5 (SD: ±5.29, range: 27-49). The average BMI was 33.87 (SD±9.81). MM stages based on the Revised International Staging System (R-ISS) were as follows: Stage I, N=6, stage II, N=7, stage III, N=4, and missing, N=6. Over half of the individuals with early-onset MM were male (52%). The ethnicity/race was Black (57%), White (39%), and Asian (4%). Other sociodemographic characteristics included unemployment status (35%), rurality (53%), and smoking history (43%). About 52% of early-onset MM patients had commercial insurance, 30% Medicare/Medicaid, 4% no insurance, 4% Department of Corrections, and 8% had veteran insurance. Notably, 43% of early-onset MM patients had high-risk cytogenetics (gain 1q21, gain 1q, del 1/p32/CDKN2C, IGH rearrangement, del 1p, del 17p, del 13q, del TP53, monosomy 13, Trisomy 1) and 18% received allogeneic stem cell transplant.
Discussion: We demonstrated that patients with early-onset MM in South Carolina often have characteristics such as race, unemployment, and high-risk cytogenetics. Future research should explore how genetic, environmental, lifestyle factors and SDOH influence the risk and prognosis of early-onset MM, particularly in areas with persistent poverty and historically disadvantaged communities.

#6197 Examining the impact of food cost and accessibility on nutrition insecurity and food choice among postmenopausal African American breast and endometrial cancer survivors.

Amirah Burton, Zakirah Abdul-Hameed, Mohamed Mubasher, Brian Rivers, Desiree Rivers

Morehouse School of Medicine, Atlanta, GA

Background/Significance- Scientific literature continues to demonstrate the beneficial impact of lifestyle modifications, such as diet, on overall health and in reducing recurrence of breast and endometrial cancers. However, more research is needed to identify and understand how certain structural and social barriers impede cancer survivors' ability to maintain a healthy diet. The purpose of this study is to examine 1) the prevalence of nutrition insecurity and 2) how food cost and accessibility influence food choices and nutrition security status among low-income postmenopausal African American breast and endometrial cancer survivors. This sub-study is part of a larger study funded by the ACS Research Scholar grant.
Methods- Sixteen postmenopausal (50+ years of age) African American women diagnosed with breast or endometrial cancer before 2020 completed cross-sectional surveys assessing nutrition security status and healthfulness choice (ability to meet dietary needs by having control over food options), food accessibility (Neighborhood Environment Walkability Scale), and food expenditures. Nutrition security and healthfulness choice assessment tools were from the Center for Nutrition and Health Impact. Descriptive statistics and univariate analyses were performed to determine nutrition security status, healthfulness choice, average walking distance to food retailers, and food expenditures.
Results- Forty-two percent of participants were nutrition insecure, while 5.3% scored low for household healthfulness choice. Most participants are in closer walking distance to fast food restaurants compared to a supermarket or fruit/vegetable market and purchase most of their food at a supermarket (75%), supercenter (25.8%), or small grocery store (6.3%). Sixty-two percent of participants rated the price of fresh fruits and vegetables as somewhat expensive, 12.5% not expensive, 18.8% very expensive, and 6.3% very inexpensive.
Conclusion- Although many participants experience nutrition insecurity, few participants feel constrained in their ability to meet their dietary healthfulness needs and control their food options. Despite living in closer proximity to fast food restaurants, participants spend more money on food at supermarkets or supercenters. Additional studies are needed to further explore the multi-level factors impacting nutritious food access, diet quality, and health status of African American breast and endometrial cancer survivors.

#6198 Framework for Pacific Island Cancer and Obesity Across the Lifespan: PICOLI, A multi-dataset analysis approach.

A. B. Yamanaka[1], M. B. Laguana[2], M. Chong[3], R. T. Leon Guerrero[2], R. Novotny[1], G. Badowski[2], L. R. Wilkens[3];
[1]University of Hawaii at Manoa, Honolulu, HI, [2]University of Guam, Mangilao, GU, [3]University of Hawaii Cancer Center, Honolulu, HI

Background: Pacific Islanders experience disproportionate rates of obesity and cancer, contributing to morbidity and mortality. Comprehensive analyses examining obesity and cancer across age groups are limited. Objective: We propose to develop a framework identify and harmonize existing datasets to assess lifestyle factors, obesity, and cancer across various age groups, with a focus on Pacific Islander populations.
Methods: We have identified several key datasets for this analysis, including the the Children's Healthy Living Program (CHL), Pacific Islands Cohort on Cardiometabolic Health (PICCAH), Breast Cancer Risk Project (BRISK), and Multiethnic Cohort Study (MEC). These datasets contain approximately 22,000 participants from 2-80 years old, which capture essential factors, such as body mass index, physical activity levels, dietary habits, sleep behavior, tobacco use, and cancer incidence.
Expected Outcomes: The framework will guide future research to provide insights between lifestyle factors, obesity, and cancer risk that evolve throughout life. Anticipated identification of consistent patterns across age groups and potential modifiable lifestyle factors associated with reduced cancer risk. We will contribute valuable knowledge about the long-term effects of lifestyle factors on obesity and cancer risk in Pacific populations. Inform public health strategies tailored to address significant health disparities in this population.
Conclusion: Developing this analytical framework aims to bridge current gaps in understanding the complex relationships between lifestyle factors, obesity, and cancer risk among Pacific Islanders, ultimately contributing to improved health outcomes. Future researchers can apply this framework to existing datasets, facilitating comprehensive analyses across age groups and potentially guiding targeted interventions.

Table 1. Key Datasets for Pacific Island Cancer and Obesity Across the Lifespan Study

Study Name	Children's Healthy Living Program (CHL)	Pacific Islands Cohort on Cardiometabolic Health (PICCAH)	Breast Cancer Risk Project (BRISK)	Multiethnic Cohort(MEC)
Participants, n	1,263	500 dyads	245	~20,000
Age, years	2 - 8y	4 - 10y & 21 - 50y (biological adult parents)	25 - 80y	45 - 79y
Study Design	Cross-sectional and Randomized Controlled Trial	Cohort	Case Control	Cohort
Location	CNMI, Guam, Hawaii	Guam	CNMI, Guam	Hawaii
Health Outcome	Obesity, Acanthosis nigricans	Obesity, Diabetes, Acanthosis nigricans	Breast Cancer, Diabetes	All Cancers, Diabetes
Measured Anthropometry	Height, weight, waist circumference	Height, weight, waist circumference	Height, weight, waist circumference	Height, weight, waist circumference
Lifestyle Measures	Diet, physical activity, sleep	Diet, physical activity, sleep, tobacco use	Diet, physical activity, tobacco use	Diet, physical activity, sleep, tobacco use

#6199 Interlab study supporting hereditary cancer testing in an NGS-focused landscape.

Dana Ruminski Lowe, Deepika Philkana, Krystyna Nahlik, Melissa Berenger, Andrew Anfora, Yves Konigshofer, Catherine Huang, Russell Garlick, Bharathi Anekella

LGC Clinical Diagnostics, Gaithersburg, MD

Up to 10% of all cancers may be caused by inherited genetic changes. Consequently, the increasing prevalence of cancer is driving the growth of the hereditary cancer testing as it is important to implement surveillance and risk-reduction strategies, detect cancer at an early, more treatable stage, and provide tailored treatment and therapies. Panels are often designed to cover hundreds of genes and performing validation and assessing performance requires samples with common mutations in each of those genes. Biosynethic reference materials can address these challenges. Here we describe the development and multisite evaluation of Seraseq® Inherited Cancer DNA Mix v2, which contains genes recommended for testing by the American College of Medical Genetics, the National Comprehensive Cancer Network, and the American Society of Clinical Oncology. Genomic DNA from the GM24385 cell line was blended with 61 biosynthetic DNA variants in 55 different genes including 21 deletions, 16 duplications, 3 insertions, 5 INDELs, 15 SNVs and 1 Inversion. Variant allele frequency (VAF) was targeted to 50% and verified using the Bio-Rad QX-200 Droplet Digital PCR (ddPCR) System. The mix was analyzed with the Axen Hereditary Cancer Panel v2 (Macrogen), the ViennaLab Hereditary Cancer Assay (ViennaLab) on the MiSeq System (Illumina), the Twist Library Preparation and AVITI 2x150 Sequencing Kit Cloudbreak Low Output on the Element Biosciences AVITI (Halo Precision Diagnostics), the GALEAS Hereditary Plus v2 Panel (Nonacus) on the NextSeq 2000 System (Illumina), and the Celemics Library Preparation Kit and Celemics Target Enrichment Kit and the NextSeq 500/550 Kits v2.5 (Illumina) on the NextSeq 500 System (Illumina).All biosynthetic variants were detected within 40-60% by ddPCR. Most NGS assays were not able to detect all variants as many that were included are difficult to detect. Identification of these complex variants required modifications to alignment, variant calling, and annotation settings. For example, RAD51D c.694_715delins is detected as two separate c.694C>T and c.715C>T variants (e.g. at ViennaLab), unless the variant caller is set to merge. At Nonacus, the Boland inversion and BRCA2 and STK11 large deletions were detected by a combination of short-range and long-range paired-end mapping and split-read analysis. An Alu-specific module using repeat masking allowed successful calling of the BRCA2 AluY insertion; both increased detection accuracy from 85.5% to 95.1%.
The performance of the Seraseq Inherited Cancer DNA Mix v2 across NGS assays, supported by robust digital PCR data, demonstrated that these materials have broad compatibility and will allow for assessment of the ability of variant callers to detect complex variants.

#6200 Context-specific strategies for enhancing HPV vaccination: insights from the US and territories in the post-COVID-19 era.

Erika Escabi-Wojna, Mariola Acosta-Rullan, Natalie Alamo-Rodriguez, Grace Velez-Crespo, Vivian Colon-Lopez

Department of Cancer Control and Population Sciences, University of Puerto Rico Comprehensive Cancer Center, San Juan, Puerto Rico

Introduction: Over 42 million people in the United States (US) are affected by Human Papillomavirus (HPV), which causes over 90% of cervical cancer cases. Despite there being an FDA-approved HPV vaccine for cancer prevention, vaccination completion rates significantly vary by state, ranging from 38.5% to 85.2% among adolescents, leaving many vulnerable to this infection and contributing to cancer health disparities.
Objective: This qualitative study aimed to identify effective HPV vaccination strategies and context-specific factors, including the impact of the COVID-19 pandemic, that influence policy adoption and vaccination uptake across the US and its territories, that will offer insights into effective methods for addressing this public health challenge.
Methods: To date, we have purposely sampled key informants, including immunization program managers, health department personnel, and others, from the following states and territories: (1) Puerto Rico, (2) Virginia, (3) D.C., (4) New York, (5) Rhode Island, and (6) California. Inclusion criteria required participants to have expertise in an area related to health or public policy focused on vaccination, at least three years of experience, and knowledge of HPV vaccination policies or strategies. Eligible key informants (n=9) participated in virtual, audio-recorded semi-structured interviews via Microsoft Teams, lasting 45 to 90 minutes. Preliminary analyses were conducted using Dedoose.
Results: Several effective strategies were identified for promoting HPV vaccination, including provider education, the implementation of school vaccination policies, and the use of cancer prevention messaging. Barriers included the spread of vaccine misinformation by anti-vaccine groups, which hindered organizational efforts and complicated the implementation of HPV vaccination strategies. Collaboration with health departments, schools, and immunization programs, emerged as a key facilitator in overcoming these challenges and promoting HPV vaccination uptake. Participants noted that the introduction of the COVID-19 vaccine negatively influenced parents' perceptions of vaccinations, increasing resistance and opposition to HPV vaccination efforts.
Conclusions: Our preliminary results suggest that educating healthcare providers, implementing school vaccination policies, and shifting HPV messaging to focus on cancer prevention are effective strategies for improving vaccination rates. Collaboration between health departments and educational institutions is also crucial. Our analysis highlights the need to address vaccine misinformation and improve perceptions, especially post-COVID-19 vaccine rollout. Future efforts will include expanding participant recruitment to include other states, conducting interviews, and applying thematic analysis to our data.
Funding: NCI grant R01CA232743.

#6201 The factors influencing quality of life among patients with hepatobiliary and pancreatic cancers.

Sieun Lee[1], Sunhee Hwang[2], Junglyun Kim[1]

[1]Chungnam National University, College of Nursing, Jung-gu, Daejeon, Korea, Republic of,[2]nursing, Chungnam National University Hospital, Jung-gu, Daejeon, Korea, Republic of

Background: Hepatobiliary and pancreatic cancers are aggressive malignancies with poor survival rates, largely due to challenges in early diagnosis and limited curative treatment options. These cancers significantly impact patients' quality of life (QOL), which is strongly associated with treatment outcomes and overall survival. Despite its importance, comprehensive research on QOL in these patients remains limited, highlighting the need to identify factors influencing QOL to improve care and outcomes. The purpose of this study is to identify the factors influencing the QOL among patients with hepatobiliary and pancreatic cancers. To describe comprehensive factors associated with QOL, we applied the biopsychosocial model (Engel, 1977) in the study. The study examined comprehensive clinical data, which included individuals' characteristics, symptom assessment, and medical information.
Methods: The patients with hepatobiliary or pancreatic cancers were recruited from a university hospital between September 2022 and November 2023. Along with collecting medical and treatment information, pain, depression, anxiety, and QOL were assessed using the Numeric Rating Scale, Hospital Anxiety and Depression Scale, and Functional Assessment Cancer Therapy-General, respectively. Multiple regression analysis was performed to identify factors influencing the QOL using SPSS 26.0.
Results: Among the 204 participants, the mean age was 66.7 (±8.52) years old;141(69.1%) were males; 107(52.5%) were diagnosed with liver cancer. Depression, anxiety, pain, and serum albumin significantly influenced the QOL, explaining 68.1% of the variance. Patients who were employed or had no metastasis reported significantly higher QOL. Patients who had higher albumin, lower pain, depression, and anxiety reported higher QOL. These findings emphasize the need for psychosocial support, pain management, and nutritional care to improve the QOL in patients with hepatobiliary and pancreatic cancers.
Conclusions: To improve the QOL of patients with hepatobiliary and pancreatic cancers, we suggest providing appropriate psychosocial support, pain control, and nutritional management during the cancer treatment.

#6202 Liquid biopsy multi-omic TempO-Seq platform for efficacy, safety, diagnosis, and subtyping using fingerstick blood.

Bruce Seligmann, Megan Opichka, Monica Hernandez, Zhoutao Chen, Joanne Yeakley, Joel McComb, Gregory Sahagian, Salvatore Camiolo

BioSpyder Technologies, Inc., Carlsbad, CA

Background and Purpose: While liquid biopsy assays are well described for plasma obtained from venipuncture, we used blood from a fingerstick, spotted onto filter paper, to profile gene expression of the whole transcriptome with the TempO-Seq® DBS assay and established a classifier for Alzheimer's Disease (AD) [Seligmann et. al., J. Alz. Dis. 101(2024) 813-822] achieving 98% accuracy classifying patients. Fingerstick samples can be self-collected, making this method a potentially transformative diagnostic tool. We describe expanding this fingerstick liquid biopsy assay to measure DNA variants and protein biomarkers, increasing its diagnostic potential and ability to monitor therapeutic response.
Methods: Blood was spotted onto 903 Proteinsaver cards and dried. Areas from each spot were then punched out into wells to measure the whole transcriptome (mRNA), alternatively spliced isoforms, the whole miRNome (miRNA), select DNA variants, and, after elution, select protein biomarkers providing a multi-omic profile of each sample. Commercial TempO-Seq assays were used, with additional attenuators added to reduce signal from highly expressed genes in blood. TempO-Seq DBS results were benchmarked against standard assays.
Results: Genotyped DNA was spotted and analyzed to derive calling algorithms for AD pathogenic and polygenic risk DNA variants, including APOE4. Assays for Tau and β-amyloid were established. Then patient samples were tested, and clinical diagnosis of AD and plasma biomarker results were correlated to the liquid biopsy protein, mRNA, miRNA, mRNA isoforms, and DNA variant profiles derived from TempO-Seq DBS data. The results demonstrated that the criteria for a definitive diagnosis of Alzheimer's Disease, suitability for immunotherapy, and prognosis via polygenic risk could all be assessed from the same fingerstick blood sample. To demonstrate its applicability monitoring drug response, samples were profiled from a subject administering weekly injections of the GLP-1 agonist (a therapeutic target for Type 2 diabetes and AD) peptide Zepbound. These results will be presented.
Conclusions: The results demonstrate the utility of the TempO-Seq DBS multi-omic liquid biopsy assay of gene expression, DNA variants, and protein biomarkers from a fingerstick. We demonstrate the potential to diagnose disease using AD as an exemplar case, and to monitor drug response. Since fingerstick samples can be self-collected, or collected in any doctor's office or clinic without a lab visit or phlebotomist, this test has the ability to address health disparities and may have the utility to; a) enable early at home diagnosis; b) provide home monitoring of therapy; c) facilitate recruitment of subjects for trials; d) serve as a companion diagnostic; and e) potentially provide a novel approach for early diagnosis of cancer and monitoring of recurrence through a convenient at-home test.

POPULATION SCIENCES: Health Disparities and Systemic Inequities: Lifestyle, Metabolism, and Disparities in Cancer Risk and Outcomes
Poster Session

#6206 The role of lifestyle factors in the disparities of colorectal cancer incidence: Findings from the Multiethnic Cohort Study.

Joel Sanchez Mendez[1], Jonanne Talebloo[2], Adelynn Paik[1], Sihao Han[1], Iona Cheng[3], Anna H. Wu[1], Ugonna Ihenacho[1], Lynne R. Wilkens[4], Loic Le Marchand[4], David V. Conti[1], Christopher A. Haiman[1], Brian Z. Huang[1], Mariana C. Stern[1]

[1]Department of Population and Public Health Sciences, USC - University of Southern California, Los Angeles, CA,[2]University of California Berkeley, Berkeley, CA,[3]Department of Epidemiology & Biostatistics, Unversity of California San Francisco, San Francisco, CA,[4]University of Hawai'i Cancer Center, University of Hawai'i, Mānoa, HI

Introduction: Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer death in the United States (US). In 2018, the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) published a score that allows the combined evaluation of adherence to their 2018 cancer prevention recommendations on diet, nutrition, and physical activity. We investigated associations between this score, and incidence of invasive colorectal adenocarcinoma across different racial and ethnic populations in the Multiethnic Cohort Study (MEC).
Methods: We leveraged data collected on 204,278 Non-Hispanic Black/African American (NHB), Japanese American, Hispanic/Latine (H/L), Native Hawaiian, and Non-Hispanic White (NHW) from the MEC. The WCRF/AICR score (range 0-7) was computed at both baseline (1993-1996) and the third follow-up questionnaire (2003-2008), with higher values representing healthier lifestyle behaviors. Covariate-adjusted Cox-proportional hazards models were used to estimate the association between the WCFR/AICR score and the colorectal cancer diagnosis. Dummy variables for a 1 SD decrease or increase between the baseline and follow-up WCFR/AICR score were generated to assess trends over time in relation to CRC incidence.
Results: A total of 5,993 events were reported, with 5,726 events 1 year or later after the collection of the baseline questionnaire and 1,276 a year or later after the follow-up questionnaire. Participants (55% female) had a mean age at cohort entry of 59.86 years (SD = 8.84), with a median follow-up time of 24 years (IQR = 15.31-25.74). The mean value of the WCRF/AICR score at baseline was 3.60 (SD = 0.89), with a significant decrease on the follow-up questionnaire (mean 3.53; SD = 0.85; paired t-test p-value <0.001). Unhealthier patterns of body mass index, fruit and vegetable, fiber, red meat and processed meat intake were reported at follow-up. We found improvements in the patterns of physical activity, sugar-sweetened drinks, and alcohol consumption. A one unit increase in the baseline WCRF/AICR score was associated with a ~10% decrease in the risk of developing CRC (HR = 0.89; 95%CI = 0.86-0.92). When stratified by race and ethnicity, Hawaiian participants showed the greatest reduction in CRC risk (HR = 0.82; 95% CI = 0.72-0.94), followed by NHW (HR = 0.84; 95% CI = 0.78-0.90), US-born H/L (HR = 0.88; 95% CI = 0.80-0.97) and Japanese (HR = 0.90; 95% CI = 0.85-0.95) (p-heterogeneity <0.001). We saw no association was observed in NHB or non-US-born H/L. There was no evidence of differential CRC risk associated with a 1 SD change (increase or decrease) in the WCRF/AICR score between the two time points.
Conclusions: Higher WCRF/AICR score values were associated with a reduced CRC risk. Moreover, the risk differs by race and ethnicity. Finally, lifestyle changes at later stages in life may not change the risk of developing CRC.

#6207 Trends in breast cancer incidence-based mortality among US women aged 20-49 by molecular subtypes and race.

Yueshiu Lyu, Suleepon Uttamapinan, **Adetunji T. Toriola**

Surgery, Washington University School of Medicine in St. Louis, Saint Louis, MO

Background: Breast cancer incidence is rising in women aged 20-49 years, yet data on trends in mortality for this age group are limited. Mortality varies by several factors, underscoring the need for comprehensive analyses. We, therefore, investigated trends in breast cancer incidence-based mortality (IBM) by race and molecular subtypes.
Methods: We performed a population-based study of women aged 20-49 years diagnosed with breast cancer between 2010 and 2020 using SEER 17-registry data. We applied IBM method to evaluate mortality trends by molecular subtype and race/ethnicity. We used Joinpoint regression models to identify changes in IBM trends, using annual percent changes (APC). Additionally, we evaluated breast cancer survival rates by race/ethnicity.
Results: We analyzed data on 11,661 breast cancer deaths among women aged 20-49 years. Overall, IBM declined from 9.70/100,000 in 2010 to 1.47/100,000 in 2020. The declines occurred in all subtypes and racial/ethnic groups, with some differences in the points of inflexion. IBM for luminal A decreased consistently from 2010, with more marked decline from 2017 (APC, -32.88; 95%CI, -55.17 to -21.30). TNBC followed a similar pattern, with marked decline in 2018 (APC, -32.82; 95%CI, -41.47 to -17.79). Non-Hispanic Black (NHB) women had the highest IBM in 2010 (16.56/100,000) and 2020 (3.41/100,000) compared with Non-Hispanic White (NHW) women (9.18/100,000 in 2010 and 1.16/100,000 in 2020). Declines in IBM in NHB women become pronounced from 2016 (APC, -24.15; 95% CI -34.36 to -17.92), narrowing the gap with other racial groups. Significant declines in IBM for non-Hispanic Asian/Pacific Islander (API) women started earlier from 2013, compared to 2017 for NHW, non-Hispanic American Indian/Alaska Native, and Hispanic women. Survival analyses showed that NHB women had the worst survival outcomes, while NHW and non-Hispanic API had the best.
Conclusion: IBM among US women aged 20-49 years with breast cancer declined significantly from 2010 to 2020, with variations by molecular subtype and race/ethnicity. The rapid decline in IBM after 2016-2018 likely reflects advancements in breast cancer management, particularly in novel treatments. Although NHB women still have the highest IBM, the differences have narrowed in recent years. Efforts should continue to focus on further enhancing access to early screening for high-risk women and effective treatment to fully mitigate racial disparities in mortality.

#6208 Impact of primary site in hepatobiliary and pancreatic lymphomas on overall survival: analysis of SEER database.

JOSEPH SPEAR[1], Carmen Lessani[1], Olivia Davis[2], Silas Day[1], Noha Soror[1], Taha Mahdi Salih Al-Juhaishi[1]

[1]University of Oklahoma Health Sciences Center, Oklahoma City, OK,[2]University of Michigan, Ann Arbor, MI

Introduction: Primary hepatobiliary and pancreatic lymphomas (PHBPL) are a rare and aggressive subset of lymphomas, comprising <0.5% of pancreatic cancers and 1% of extranodal lymphomas; due to limited data on their clinicopathological features and treatment, we used the SEER database to compare overall survival (OS) of PHBPL with those of primary nodal disease.

Methods: We used the SEER database to extract demographic and disease data on patients diagnosed with lymphoma from 2010-15. Primary site was classified into three categories: primary hepatic lymphoma (including biliary tract, gallbladder, liver), primary pancreatic lymphoma (including ampulla of Vater), and primary nodal lymphoma (lymph nodes and spleen). Ann Arbor stages I-II were grouped as early-stage disease, while III-IV were grouped as late-stage. Patient cases were also divided into three distinct groups based on the histologic subtype: aggressive NHL (DLBCL, Burkitt lymphoma, MCL, T-cell lymphoma), indolent NHL (FL, marginal zone lymphoma), and Hodgkin's lymphoma. Summary statistics were used to analyze demographic and disease data. Our outcome of interest was OS defined as time in months from diagnosis to death from any cause. Cox-proportional hazards models and Kaplan-Meier method were used to perform univariate analysis. Multivariate analysis was also performed to control for age, Ann Arbor stage, race, and income. Primary nodal lymphoma was the comparison group for both. Statistical significance was defined using a 95% confidence interval and a p-value < 0.05.

Results: A total of 55,115 cases were included. The most common primary disease site was primary nodal lymphoma (54,454 cases, 98.8%) followed by primary hepatic lymphoma (435 cases, 0.79%) and primary pancreatic lymphoma (226 cases, 0.41%). The median age at diagnosis was 63 years old with majority of patients identifying as male and Caucasian (56.26% and 70.4% respectively). Late-stage lymphoma represented 62.69% of the cohort while early-stage lymphoma represented 37.31%. Primary hepatic lymphoma was associated with worse OS when compared to nodal lymphoma (HR = 2.077, $p < 0.0001$). This finding remained statistically significant even after controlling for age, race, income, and stage (HR = 1.836, $p < 0.0001$). Primary pancreatic lymphoma was also associated with worse OS (HR = 1.226, $p = 0.0349$). This association persisted on multivariate analysis though statistical significance was not met (HR= 1.1182, P = 0.0841).

Conclusions: Hepatic and pancreatic lymphomas have respectively significantly and slightly worse OS than their nodal counterparts. This association persisted on multivariate analysis, though multivariate findings for primary pancreatic lymphoma did not meet statistical significance. These results warrant validation in future prospective studies, including those using risk-adapted approaches to guide therapeutic decisions.

#6209 Different types of diabetes and pancreatic ductal adenocarcinoma risk: A Mendelian randomization and pathway/gene-set analysis.

T. Zhang[1], X. Hua[1], C. Mohindroo[2], X. Wang[1], P. Consortium[1], B. M. Wolpin[3], H. A. Risch[4], L. T. Amundadottir[1], A. P. Klein[5], K. Yu[1], H. Zhang[1], R. Z. Stolzenberg-Solomon[1];
[1]National Cancer Institute, Rockville, MD, [2]National Cancer Institute, Bethesda, MD, [3]Dana-Farber Cancer Institute, Boston, MA, [4]Yale School of Public Health, New Haven, CT, [5]Johns Hopkins School of Medicine, Baltimore, MD

Objective: The associations between different types of diabetes, characterized by distinct pathophysiology and genetic architecture, and pancreatic ductal adenocarcinoma (PDAC) risk are not fully understood. We investigated associations of genetic susceptibility to type 2 diabetes (T2D), its eight mechanistic clusters and type 1 diabetes (T1D) using Mendelian randomization (MR) and maturity-onset diabetes of the young (MODY) pathway/gene-set analysis with PDAC risk.
Method: Using summary-level genome-wide association statistics for T2D (242,283 cases, 1,569,734 controls), T1D (18,942 cases, 501,638 controls), and PDAC (10,244 cases and 360,535 controls) in individuals of European ancestry, we conducted two-sample MR to examine associations of genetic susceptibility to T2D and T1D with PDAC risk, using single nucleotide polymorphisms (SNP) significantly associated with T2D, eight mechanistic TD2 clusters (i.e., β-cell dysfunction with a positive or negative association with proinsulin, residual glycaemic, body fat, metabolic syndrome, obesity, lipodystrophy, and liver and lipid metabolism), and T1D identified from published genome-wide association studies ($P<5\times10^{-8}$) as instrumental variables. We also assessed associations of genetic susceptibility to PDAC risk with T2D and T1D using reverse MR. We used MR-RAPS method as the primary analysis approach complemented with sensitivity analyses using MR-IVW, weighted-median, MR-Egger, MR-PRESSO and MR-PIVW. We applied the Summary-based Adaptive Rank Truncated Product (sARTP) method to evaluate the associations for PDAC and the MODY gene set, which consists of 26 genes identified from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.
Results: Genetic susceptibility to T2D (MR-RAPS: OR=1.10; 95% CI 1.05-1.14), particularly the obesity (OR=1.28; 95% CI 1.15-1.42) and lipodystrophy (OR=1.25; 95% CI 1.04-1.50) clusters, was associated with PDAC risk. T1D showed no association with PDAC risk (OR=1.01; 95% CI 0.99-1.02). Reverse MR found no evidence that genetic susceptibility to PDAC risk was associated with T2D or T1D (P>0.05). Sensitivity analyses using other MR methods supported these findings, with no evidence of pleiotropic effects (MR-Egger intercept P>0.05). The MODY gene-set was significantly associated with PDAC risk ($P=2.5\times10^{-6}$), with *HNF1A*, *HNF1B, FOXA3, HNF4A*, and *HNF4G* as the top five contributing genes.
Conclusion: Our findings support a role of T2D, particularly the obesity and lipodystrophy clusters, in PDAC risk. Additionally, the genomic susceptibility regions of MODY were significantly associated with PDAC.

#6210 The predictive performance of risk prediction models in determining lung cancer incidence in Western and Asian countries: A systematic review and meta-analysis.

Yah Ru Juang, Lina Ang, **Wei Jie Seow**

Saw Swee Hock School of Public Health, National University of Singapore, Singapore, Singapore

Introduction: Numerous prediction models have been developed to identify high-risk individuals for lung cancer screening, aimed at improving early detection and survival rates. However, there has been no comprehensive review and meta-analysis of model performance across different sociocultural contexts. Therefore, this review aims to systematically examine the performance of lung cancer risk prediction models in Western and Asian populations.

Methods: PubMed and EMBASE were searched from inception to January 2023. Studies published in English and proposed a validated model on human populations with well-defined predictive performances were included. Two reviewers independently screened titles and abstracts and the Prediction Model Risk of Bias Assessment Tool (PROBAST) was used to appraise study quality. A random-effects meta-analysis was performed and a 95% confidence interval (CI) for model performance was provided. Between-study heterogeneity was adjusted for using the Hartung-Knapp-Sidik-Honkman test.

Results: Fifty-four studies were included, with 42 from Western and 12 from Asian countries. Most Western studies were designed for ever-smokers (19/42; 45.2%) and the general population (17/42; 40.5%), and only two Asian studies developed models exclusively for never-smokers. Across both Western and Asian prediction models, the three most consistently included risk factors are age, sex, and family cancer history. In 45.2% (19/42) of Western and 50.0% (6/12) of Asian studies, models incorporated both traditional risk factors and biomarkers; 14.8% (8/54) directly compared their biomarker-based models with those incorporating only traditional risk factors, demonstrating better discrimination. Machine-learning algorithms were applied in eight Western models and two Asian models. External validation of $PLCO_{M2012}$ (AUC=0.748; 95% CI: 0.719-0.777) outperformed prediction models such as Bach (AUC=0.710; 95% CI: 0.674-0.745) and Spitz models (AUC=0.698; 95% CI: 0.640-0.755).

Conclusion: Despite showing promising results, the majority of Asian risk models in our study lack external validation. Our review also reveals a significant gap in prediction models for never-smokers. Future research should externally validate existing Asian models or incorporate relevant Asian risk factors in widely-used Western models ($PLCO_{M2012}$) to account for their unique risk profiles and lung cancer progression patterns.

#6211 Personalized risk models based on lung cancer screening history: post hoc analysis of the National Lung Screening Trial (NLST).

Sofia Haddan, Matthew Schabath, Asim Waqas, Ghulam Rasool

Moffitt Cancer Center, Tampa, FL

Introduction: Our group previously revealed (PMID27509046) that lung cancer (LC) screening history is an independent prognostic factor whereby incidentally-detected lung cancer cancers with one or more preceding negative screens is associated with poorer survival outcomes compared to those preceded by only positive screens. As a follow-up study, we sought to develop personalized risk models that considered screening history for incidence cancers, interval LCs, and prevalence LCs.
Methods: Using data from the CT-arm of the NLST, we conducted our independent case-control analyses to develop parsimonious risk models. Controls (n=26,038) were those never diagnosed with LC through the end of follow-up. The four LC case groups were 270 prevalence LCs (diagnosed at baseline screen), 44 interval LCs (diagnosed following a negative screen at any time point prior to the next screening), 206 screen-detected LCs (SDLCs) that had a baseline positive screen, and 164 SDLCs that had a baseline negative screen. For each case-control analysis, univariable analyses identified statistically significant covariates from 47 variables including demographics, smoking history, alcohol use, medical history, family history of LC, and occupational exposures. Then, for each analysis, statistically significant covariates were included into a stepwise backwards selection approach to identify a model with the most informative covariates.
Results: Age was the only covariate found in all four models; pack-years smoked, BMI, and smoking status were found in three of models. For prevalence LCs, the model (AUC=0.711) included age, pack-years smoked, BMI, smoking status, smoking onset age, personal history of cancer, family history of LC, frequency of alcohol consumption, and milling occupation. For interval LCs, the model (AUC=0.734) included age, smoking status, smoking onset age, cigar smoking, marital status, and asbestos occupation. For baseline positive SDLCs, the model (AUC=0.685) included age, pack-years smoked, BMI, emphysema, chemicals/plastics exposure, and milling occupation. For baseline negative SDLCs, the model (AUC=0.701) included age, pack-years smoked, BMI, smoking status, emphysema, sarcoidosis, and sandblasting occupation.
Conclusions: We deployed a rigorous model building approach to identify unique personalized risk models that considered screening history and timing of LC diagnosis. Besides smoking and age, which are inclusion criteria for screening, these models identified other important risk factors including occupational exposures, medical history, alcohol use, personal history of cancer, and family history of LC. These models could be used to provide personalized risk assessment and screening management in screening. Including additional biomarkers (e.g., radiomics, circulating, etc.) could improve the statistical performance of the models.

#6213 T-cell receptor excision circles (TRECs) at birth and risk of childhood cancers.

Jun Tao[1], Paul S. Albert[1], Nellie Gottlieb[2], Eric A. Engels[1]

[1]National Cancer Institute, Rockville, MD,[2]California Cancer Reporting and Epidemiologic Surveillance, Sacramento, CA

Introduction: Acquired immunosuppression is associated with higher risk for infection-related cancers. However, cancer risk among children born with primary immunodeficiency is unknown. Circulating T-cell receptor excision circles (TRECs) are a marker of thymus production of T-cells, which is used to identify severe combined immunodeficiency (SCID) at very low levels during newborn screening. It is unknown whether TREC levels at birth are associated with cancer risk in children.
Methods: The California (CA) and Texas (TX) cancer registries identified cancers in children aged 0-5 years born during 2013-2018. Controls were matched to cases by birth year and sex. TREC levels, measured at birth by the state SCID screening programs were linked to cases and controls. Conditional logistic regression was used to assess the association between tertiles (T) of TREC levels and cancer risk.
Results: We included 1,450 cancer cases and 7,250 controls from TX and 2,564 cases and 12,820 controls from CA. Only 13 newborns in CA and 15 in TX had extremely low TREC level at birth. T2 levels of TREC was inversely associated with cancer risk compared to the lowest (T1), for several cancers (Table 1). However, only acute myeloid leukemia showed an inverse association for T2 and T3 in CA (odds ratio [OR] 95%CI: T2, 0.38, 0.22-0.66; T3, 0.47, 0.27-0.82), but this was not replicated in TX. Both T2 and T3 were associated with increased risk of acute lymphoblastic leukemia in TX (T2, 1.45, 1.05-1.99; T3, 1.49, 1.06-2.08) but not in CA.
Conclusion: SCID is rare among children with cancer. We did not see consistent associations between TREC levels and cancer risk, either across tertile levels or between CA and TX. This indicates that differences in immune function do not contribute to the etiology of the childhood cancers examined. Alternatively, TREC assays are designed to detect children with the abnormally low TREC levels, and higher TREC levels may not reflect immune function.

Table 1. Association between TREC level and childhood cancer risk

	OR (95%CI)	
	Texas	**California**
T1	Ref	Ref
Acute lymphoblastic leukemia		
T2	**0.60 (0.47, 0.75)**	**1.45 (1.05, 1.99)**
T3	0.85 (0.68, 1.07)	**1.49 (1.06, 2.08)**
Central nervous system		
T2	**0.64 (0.48, 0.86)**	1.17 (0.88, 1.57)
T3	0.97 (0.75, 1.27)	1.22 (0.92, 1.65)
Endocrine		
T2	**0.42 (0.24, 0.72)**	0.80 (0.48, 1.35)
T3	0.91 (0.56, 1.46)	0.78 (0.44, 1.35)
Urinary system		
T2	0.98 (0.58, 1.65)	1.20 (0.64, 2.21)
T3	1.54 (0.93, 2.58)	1.04 (0.55, 1.97)
Eye and orbit		
T2	0.77 (0.43, 1.36)	1.02 (0.59, 1.75)
T3	0.80 (0.43, 1.46)	1.21 (0.71, 2.08)
Soft tissue and heart		
T2	0.83 (0.48, 1.42)	0.80 (0.44, 1.43)
T3	1.32 (0.77, 2.30)	0.88 (0.48, 1.60)
Liver and intrahepatic bile duct		
T2	0.70 (0.41, 1.21)	**0.49 (0.27, 0.88)**
T3	0.78 (0.43, 1.39)	0.63 (0.36, 1.12)
Acute myeloid leukemia		
T2	**0.38 (0.22, 0.66)**	0.96 (0.54, 1.72)
T3	**0.47 (0.27, 0.82)**	0.68 (0.36, 1.31)

#6214 Vitamin metabolites in neonatal dried blood spots and risk of retinoblastoma in California.

C. Deng[1], K. C. Paul[2], D. I. Walker[3], D. P. Jones[4], B. R. Ritz[1], J. E. Heck[5];
[1]UCLA Fielding School of Public Health, Los Angeles, CA, [2]David Geffen School of Medicine, Los Angeles, CA, [3]Rollins School of Public Health, Emory University, Atlanta, GA, [4]Division of Pulmonary, Allergy, and Critical Care Medicine, School of Medicine, Emory University, Atlanta, GA, [5]College of Health and Public Service, University of North Texas, Denton, TX

Background: Vitamin intake may influence carcinogenesis through various pathways. In particular, vitamin B6 metabolites have been implicated in cancer development due to their involvement in processes such as oxidative stress and inflammation. However, conclusions remain inconsistent, and epidemiological studies are limited, particularly for retinoblastoma. In this exploratory study, we examined associations between vitamin metabolites in neonatal dried blood spots and retinoblastoma risk in California.
Methods: This study included 497 retinoblastoma cases born between 1983 to 2011 and 893 controls matched by birth year in California. Vitamin feature intensities were measured in neonatal blood spots from the California newborn Genetic Disease Screening Program. Logistic regression was used to evaluate associations between quartiles of vitamin levels and retinoblastoma risk, adjusting for sex, birth year, maternal age, maternal race and ethnicity, maternal place of birth, and socioeconomic status. We also stratified the analyses by retinoblastoma subtypes (i.e., unilateral, bilateral), sex and maternal birthplace (U.S. vs. Mexico).
Results: For vitamin B6 metabolites, the highest quartile of pyridoxamine was associated with a 32% increase in retinoblastoma risk (95% confidence interval (CI): 0.97-1.80), while the highest quartile of 4-pyridoxic acid was associated with a 32% reduction (95% CI: 0.56-1.07), compared to the lowest quartile. Additionally, the highest quartiles of NAD+ were associated with a reduced risk (adjusted odds ratio (aOR): 0.78; 95% CI: 0.57-1.06 and aOR: 0.76; 95% CI: 0.55-1.05). No overall association with retinoblastoma risk was observed for pantothenic acid (vitamin B5), vitamin A, vitamin D2, or methylcobalamin (vitamin B12). After stratification, we did not observe clear dose-response relationships, although some estimates suggested an increased risk. For example, the 3rd quartile of pantothenic acid was associated with an increased risk among female (aOR: 0.65, 95% CI: 0.40-1.04) and children with Mexican-born mothers (aOR: 0.56, 95% CI: 0.30-1.05). The 2nd quartile of methylcobalamin was associated with a 56% increase in retinoblastoma risk among males (95% CI: 1.01-2.44).
Conclusion: Several vitamin metabolites showed a differential abundance in neonatal blood spots of children who later did or did not develop retinoblastoma in California. These results suggest that further research into the role of maternal vitamin intake during pregnancy may hold promise for pediatric cancer prevention.

#6215 Metabolic risk is an important determinant of adipocyte hypertrophy beyond age, BMI and breast density in patients with ductal carcinoma *in situ*.

Charlotta V. Mulder, Mathilde Almekinders, Renaud Tissier, Lennart Mulder, Petra Kristel, **Esther Lips**, Marjanka Schmidt, Jelle Wesseling

Netherlands Cancer Institute, Amsterdam, Netherlands

Background: Ductal carcinoma in situ (DCIS) is a non-obligate precursor to invasive breast cancer, but distinguishing patients with harmless from potentially hazardous DCIS remains a challenge. Consequently, a cornerstone of DCIS research is finding prognostic biomarkers. One such recent biomarker is adipocyte hypertrophy, which has been shown to be prognostic of ipsilateral invasive breast cancer (iIBC) in post-menopausal women with primary DCIS. However, little is known about the correlation between adipocyte size, clinical factors and mammographic density.
Methods: Archival hematoxylin and eosin- stained breast biopsy and excision specimens were retrieved from 669 women diagnosed with primary DCIS between 2000 and 2020 treated at the Netherlands Cancer Institute. These slides were digitized whereafter a machine-learning algorithm using HALO®, was applied to retrieve adipocyte size. Radiology reports were obtained to extract mammographic BI-RADS density. Age at diagnosis, body mass index (BMI), menopausal status and information on comorbidities were extracted from electronic patient records. Associations between adipocyte size and clinical factors and mammographic density were investigated using univariable and multivariable linear regression models. Using a clinically relevant cutoff point for adipocyte size, multivariable logistic regression was performed.
Results: The median age at primary DCIS diagnosis was 55 years (interquartile range (IQR): 49.0 -63.0) and most DCIS lesions were grade 3 (40.9%). The median BMI was 24.1 (IQR: 21.9 - 27.1). The majority of women were post-menopausal (47.8%) and had dense breasts (53.8%). Significant positive correlations were found between adipocyte size and age, BMI and all metabolic comorbidities with the exception of smoking. Strong negative correlations were found between adipocyte size and mammographic density categories C and D. In univariable linear models with age and BMI, metabolic risk was able to further differentiate between patients with and without adipocyte hypertrophy beyond age and BMI. This was confirmed using multivariable logistic regression comparing models adjusted and unadjusted for metabolic risk.
Conclusion: BMI is the strongest predictor of adipocyte hypertrophy in DCIS patients, however metabolic risk further differentiates between patients with and without adipocyte hypertrophy. Future work should be done on collecting long term follow-up and ascertaining subsequent breast cancer in these patients. If successful, BMI and metabolic risk can potentially be incorporated into risk prediction models for subsequent invasive breast cancer.

#6217 Methylation-derived inflammation phenotypes and anal cancer risk among men with HIV.

Shuai Xu[1], Bryan E. Shepherd[2], Tebeb Gebretsadik[2], Anna Junkins[3], Jirong Long[3], Qiuyin Cai[3], Staci L. Sudenga[3]

[1]Epidemiology, Vanderbilt University, Nashville, TN,[2]Biostatistics, Vanderbilt University, Nashville, TN,[3]Epidemiology, Vanderbilt University Medical Center, Nashville, TN

Introduction: Inflammation is a hallmark of cancer. DNA methylation (DNAm) derived immune cell type proportions and circulating inflammation-related protein markers are precise measures of inflammatory phenotypes. We investigated the associations of DNAm-derived inflammation-related phenotypes with risks of anal cancer and precancer (high-grade squamous intraepithelial lesions, HSIL) among men with HIV.

Method: We matched 22 anal cancer cases to 37 controls with normal anal epithelium and 54 HSIL to 90 controls using propensity score methods. DNAm data were profiled by MethylationEPIC v2.0 array on the pre-diagnostic blood biospecimens and processed by R *minfi* and *ChAMP* packages. Immune cell composition, including 4 myeloid (neutrophils, eosinophils, basophils and monocytes) and 8 lymphoid cell sub-lineages (B lymphocytes naïve (Bnv), B lymphocytes memory [Bmem], T helper lymphocytes naïve [CD4nv], T helper lymphocytes memory [CD4mem], T regulatory cells [Treg], T cytotoxic lymphocytes naïve [CD8nv], T cytotoxic lymphocytes memory [CD8mem], and natural killer lymphocytes) were estimated among normalized DNA methylation β values using a reference-based deconvolution algorithm. Proportions of these 12 major immune cell types were estimated to be 100%. Ratios of CD4nv/CD4mem, CD8nv/CD8mem, Bnv/Bmem, CD8/Treg, and neutrophil/lymphocyte were computed. Additionally, 47 DNAm-predicted circulating inflammation-related protein levels were calculated using well-established prediction models. Conditional logistic regression models were built to estimate odds ratios (OR) and 95% confidence intervals (CI) on per standard deviation increase in inflammation phenotypes adjusting for cell type heterogeneities.

Results: Higher CD4nv/CD4mem (OR=2.70, 95%CI:1.09-6.70) and lower CD8/Treg (OR=0.56, 95%CI:0.31-0.99) was positively associated with anal cancer risk at nominal $P < 0.05$. Higher Bnv/Bmem (OR=1.47, 95%CI:1.02-2.12) was linked to increased HSIL risk at nominal $P < 0.05$. Elevated levels of DNAm-derived circulating protein MMP9 (OR=1.86, 95%CI: 1.20-2.88), C5 (OR=1.78, 95%CI: 1.23-2.57), B2M (OR=1.75, 95%CI:1.16-2.65), CRP (OR=2.39, 95%CI:1.32-4.33), MMP12 (OR=1.66, 95%CI:1.15-2.41), CCL11(OR=1.66, 95%CI:1.15-2.38) , TNFRSF1B (OR=1.85, 95%CI:1.20-2.87), HGF (OR=2.10, 95%CI:1.32-3.35), TGFA (OR=2.38, 95%CI:1.31-4.35), VEGFA (OR=1.82, 95%CI:1.21-2.72), CCL17 (OR=1.93, 95%CI:1.26-2.95); and decreased levels of ADAMTS13 (OR=0.47, 95%CI:0.28-0.78) were associated with increased risk of anal cancer and HSIL (combined analysis) compared to controls at false discovery rate (FDR) <5%. No significant associations were observed when comparing cancers with controls or comparing HSIL with controls, separately at FDR<5%.

Discussion: Our findings suggest that inflammation plays a role in anal carcinogenesis. Future research is warranted to validate our results.

#6218 Impact of body composition on tumor proteomics and survival outcomes in clear cell renal cell carcinoma.

Cuthbert M. Mahenge, Rand T. Akasheh, Xuan Nguyen, Ting-Yuan D. Cheng

Cancer Control and Prevention, The Ohio State University, Columbus, OH

Background: The prognosis of clear cell renal cell carcinoma (ccRCC) is affected by a complex interaction of tumor characteristics and patient factors. This study investigates the association between body composition components with survival and tumor proteomics in patients with ccRCC.
Methods: We identified 178 patients from the TCGA-Kidney Renal Clear Cell Carcinoma project with CT images and tumor proteomic data (233 proteins). CT images corresponding to the 3rd lumbar vertebrae were assessed using Slice-O-Matic software for adipose and skeletal muscle tissue areas. The Contal and O'Quigley's method was used to obtain the cutoffs for categorizing patients into high or low groups of adiposity and muscle based on survival. Patients were further classified into four body composition types: (1) High Muscle/Low Adiposity, (2) High Muscle/High Adiposity, (3) Low Muscle/Low Adiposity, and (4) Low Muscle/High Adiposity. Linear regression was used to assess the associations for protein expression. Cox's hazard models were used for survival analysis. All models were adjusted for age, stage, gender, race, and ethnicity.
Results: There was no significant difference in survival based on muscle quantity alone. However, patients with low adiposity had worse survival than those with high adiposity (hazard ratio [HR] = 2.7; 95% CI= 1.2, 5.8). For body composition types, compared to the high muscle/high adiposity group, which had the best survival, the low muscle/low adiposity group had a significantly worse survival (HR = 3.5; 95% CI = 1.5, 8.2). Different muscle/adiposity combinations exhibited distinct protein expression patterns (all false-discovery-rate p-value ≥0.2). Low muscle/low adiposity exhibited a pattern of increased cell survival and proliferation with decreased tumor suppression, while low muscle/high adiposity exhibited increased cell motility and migration. Body composition also significantly affected survival based on expression levels of PREX1 and JAK2. Low muscle/high adiposity (vs. high muscle/high adiposity) was associated with increased mortality among patients with high PREX1 expression (HR =6.72; 95% CI = 1.88, 24.03) but not among those with low PREX1 expression (HR=1.07; 95% = 0.28, 3.99); p-value for interaction = 0.048). Also, the body composition type was associated with increased mortality among patients with high JAK2 expression (HR= 7.50; 95% CI = 1.37, 41.07) but not among those with low JAK2 expression (HR = 1.09; 95% CI= 0.35-3.39; p-value for interaction = 0.065).
Conclusion: Body composition in patients with ccRCC is associated with overall survival and with several tumor protein expressions. The mortality risk can vary based on tumor protein markers. These findings warrant further validation as they highlight the potential utilization of body composition as a prognostic biomarker and therapeutic target.

#6219 Population-based analysis of rurality and its impact on survival in gastric adenocarcinoma.

N. M. Hwang[1], M. Dixon[2];
[1]Rush Univ. - Rush Medical College, Chicago, IL, [2]Rush University Medical Center, Chicago, IL

Background: Despite growing efforts to understand the impact of rurality on cancer survival outcomes, significant gaps remain on how it influences prognosis within specific cancer types. In this study, we examine how residential location (rural vs urban) affects survival outcomes for patients diagnosed with gastric adenocarcinoma.
Methods: Using the Surveillance, Epidemiology, and End Results (SEER) database, we identified 56,371 patients with gastric adenocarcinoma between 2007 and 2020, who were 18 years or older at diagnosis and had complete data for all analyzed variables. Demographics of patients living in rural vs urban areas were compared using Wilcoxon rank sum or chi-squared testing for categorical or continuous variables, respectively. The impact of area of residence on cancer-specific survival (CSS) was assessed through Kaplan-Meier and Cox proportional hazards analysis. A multivariable Weibull regression survival model was used to control for potential confounding covariates.
Results: Most patients lived in urban areas (88.9% vs 11.1%). There were no significant differences in age and stage at diagnosis between rural and urban populations. Rural patients tended to be male ($p < 0.001$) and non-Hispanic White ($p < 0.001$) with lower median household income ($p < 0.001$). Patients residing in rural areas had significantly worse CSS [hazard ratio (HR) = 1.158; 95% CI: 1.122-1.195; $p < 0.0001$]. When stratified by race, the prognostic impact of rurality was absent in Non-Hispanic Black patients ($p = 0.62$). When adjusting for age, sex, race, stage, and income, rurality remained an independent predictor of survival [HR = 1.064; 95% CI: 1.026-1.103; $p = 0.0007$].
Conclusion: Rurality is associated with worse prognosis in patients with gastric adenocarcinoma. Further research is needed to understand the mechanisms of how area of residence contributes to outcome disparities to identify targeted interventions.

Table 1: Weibull multivariable regression model estimates of hazard ratios

Variables	**Hazard Ratio**	**95% CI**	***P-value***
Age	1.02	1.019-1.021	< 0.0001
Sex			
Female	Reference		
Male	0.942	0.921-0.963	< 0.0001
Race			
Non-Hispanic White	Reference		
Non-Hispanic Black	1.077	1.044-1.112	< 0.0001
Hispanic (All Races)	1.018	0.991-1.045	0.202
Non-Hispanic API	0.819	0.790-0.849	< 0.0001
Other	0.959	0.843-1.092	0.531
SEER Summary Stage			
Local	Reference		
Regional	2.19	2.117-2.266	< 0.0001
Distant	7.227	6.997-7.464	< 0.0001
Area of Residence			
Urban	Reference		
Rural	1.064	1.026-1.103	0.0007
Median Household Income			
< $40,000	Reference		
$40,000-$59,999	0.932	0.873-0.995	0.036
$60,000-$74,999	0.86	0.804-0.920	< 0.0001
$75,000+	0.817	0.763-0.874	< 0.0001

#6220 Disparities in cancer incidence among African American and non-Hispanic white participants in the Agricultural Health Study and North Carolina residents.

Somayina C. Ezennia[1], Christine G. Parks[2], Laura E. Beane Freeman[1], Anne Taylor[3], Gabriella Andreotti[1], Stella Koutros[1], Dale P. Sanders[2], Jonathan N. Hofmann[1]

[1]NIH-NCI, Rockville, MD,[2]NIH-NIEHS, Durham, NC,[3]Information Management Services, Inc, Rockville, MD

Disparities in cancer incidence between African American (AA) and non-Hispanic white (NHW) populations in the U.S. are well documented. However, no studies have examined cancer incidence disparities in farming populations. Given unique occupational hazards (e.g., pesticides, zoonotic diseases, diesel exhaust), we hypothesized that racial disparities in cancer incidence may be more pronounced among AA farmers than in the general population.

We investigated cancer incidence among North Carolina (NC) participants in Agricultural Health Study (AHS), a prospective cohort of pesticide applicators and their spouses enrolled between 1993-1997. This investigation included 28,579 participants (1,511 AA, 27,068 NHW), identifying 6,511 cancer cases (432 AA, 6,079 NHW) through 2021. We calculated standardized incidence ratios (SIRs), standardized incidence rate estimates (SREs) and 95% confidence intervals (CI) for overall and site-specific cancers in the AHS relative to the general population in NC.Overall, the SIR was higher for AA in the AHS (SIR = 1.19, CI 1.08 - 1.31) than for AA in NC (SIR = 1.05, CI 1.04 - 1.05), with notably high SIRs for certain sites like prostate cancer, multiple myeloma, and colorectal cancer (Table 1). For prostate cancer and multiple myeloma, we observed the lowest incidence among NHW in the general population and the highest in AA in the AHS cohort. Our findings of greater disparities in cancer incidence among AA in the AHS compared with AA in the general population may be related to differences in certain agricultural exposures or economic and social barriers faced by AA farmers; we plan to further investigate these factors in future efforts. These findings and ongoing efforts are needed to help promote the health and well-being of AA farmers.

#6221 Gastric atrophy is associated with increased risk of esophageal squamous cell carcinoma in Lilongwe, Malawi.

Kadiatou Traore[1], Yingxi Chen[1], Yukiko Yano[1], Paul S. Albert[1], Sung Duk Kim[1], Sanford M. Dawsey[1], Shiraz Khan[2], Christian C. Abnet[1]

[1]National Cancer Institute, Rockville, MD,[2]UNC Project-Malawi, Lilongwe, Malawi

Background: Esophageal cancer (EC) is the 7th leading cause of cancer worldwide, with nearly 500,000 annual deaths. Esophageal squamous cell carcinoma (ESCC), the most prevalent subtype, has high incidence in East Asia, South and East Africa. Malawi has the highest EC incidence rate, almost exclusively ESCC, with risk factors including tobacco use, hot beverages, fuel source, moldy grain consumption, geophagia, poor oral health, and possibly HIV. *H. pylori's* role in ESCC is unclear, with adverse, null, and protective effects across different populations. Gastric atrophy, a potential result of *H. pylori* infection, may increase ESCC risk by promoting gastrointestinal bacterial overgrowth that exposes esophageal mucosa to carcinogens (e.g. nitrosamines). HIV, common in Malawi, may interfere with *H. pylori* antibody tests. Limited data, especially from African populations, leaves a gap in understanding the roles of *H. pylori* and gastric atrophy in ESCC.

Methods: We enrolled 300 ESCC cases and 300 controls, matched by age, sex and region, at two Lilongwe, Malawi hospitals (2017-2020) and collected demographic, health, and behavioral data. We measured *H. pylori* antibodies and gastric atrophy (pepsinogen I (PGI), pepsinogen II (PGII)) via serology. Atrophy was defined as PGI:II <3 or PGI <30µg/L per kit specifications. We adjusted logistic regression models for design variables, known and potential confounders.

Results: *H. pylori* seropositivity was 68% in cases, 80% in controls and significantly associated with lower ESCC risk (aOR 0.54; 95% CI 0.32-0.92). High HIV positivity in cases (21%) may obscure the association due to assay interference. Having a PGI <30µg/L was significantly associated with higher ESCC risk (aOR 4.92; 95% CI 2.04-11.87) while having a PGI:II <3 was marginally associated (aOR 2.14; 95% CI 0.97-4.70).

Conclusion: Serologic gastric atrophy was associated with strongly increased ESCC risk. *H. pylori's* protective effect may be due to measurement interference.

TABLE: Associations between *H. pylori*, Pepsinogen Ratio, Pepsinogen I and ESCC risk

			Base Model[a]		Adjusted Model[b]	
	Cases, n (%)	**Controls, n (%)**	**OR (95% CI)[c]**	**Pvalue**	**aOR (95% CI)[c]**	**Pvalue**
H. pylori Status						
Negative	96 (32%)	61 (20%)	Reference	-	Reference	-
Positive	204 (68%)	239 (80%)	0.53 (0.35-0.81)	0.003	0.54 (0.32-0.92)	0.024
PGI:II ≤ 3						
Negative	260 (87%)	277 (92%)	Reference	-	Reference	-
Positive	40 (13%)	23 (8%)	1.92 (1.02-3.61)	0.043	2.14 (0.97-4.70)	0.059
PGI <30µg/L						
Negative	253 (84%)	285 (95%)	Reference	-	Reference	-
Positive	47 (16%)	15 (5%)	3.79 (1.79- 8.00)	0.001	4.92 (2.04-11.87)	<0.001

Pepsinogen= PG;[a] Adjusted for age, sex and region of origin.[b] Adjusted for age, sex, region of origin, tobacco use, alcohol use, tea temperature, moldy grain consumption, geophagia, main source of cooking and heating fuel, SES score, HIV status, sibling history of esophageal cancer, oral mucosa status, lip depigmentation status, fluorosis grade, decayed/missing/ filled teeth (DMFT) category, and frequency of cleaning teeth with charcoal.[c] Odds ratio (OR) and 95% confidence interval (95% CI) obtained from logistic regression model

#6222 Racial and socioeconomic influences on survival outcomes in small cell lung cancer: Analysis of concomitant radiation, immunotherapy, and demographic variables.

Helen Gandler[1], Pranay Adavelly[1], Parth Anil Desai[2]

[1]Medicine, Lewis Katz School of Medicine, Philadelphia, PA,[2]Fox Chase Cancer Center, Philadelphia, PA

Background: Small cell lung cancer (SCLC) is the most aggressive form of lung cancer, marked by rapid progression, frequent metastases, and poor survival rates. Real-world data from contemporary cohorts is vital to validate current treatments and develop novel hypotheses. SCLC staging typically depends on whether the disease can be encompassed within a definitive radiation field.
Methods: A retrospective cohort study of 381 SCLC patients treated at a single center from January 1, 2014, to December 30, 2022, was conducted. Data included stage at diagnosis, race/ethnicity, treatment modalities (chemotherapy, radiation, and immunotherapy), and survival outcomes. Socioeconomic factors such as insurance status and lifestyle variables (e.g., alcohol and tobacco use) were analyzed. TNM staging was stratified into Early Limited Stage (E-LS, stages I-IIA), Advanced Limited Stage (A-LS, stages IIB-IIIC), and Extensive Stage (ES, stage IV).
Results: Among the 381 patients, 54% were women, 51% were European American (EA), and 29% were African American (AA), the largest minority subgroup. Most (60%) presented with ES disease, while 20% had E-LS and 20% A-LS. Over 96% of patients had a history of smoking. AA patients were more likely to be insured through government programs (Medicare/Medicaid, 87% vs. 72% for EA, p=0.005) but had similar stage distributions. Notably, AA ancestry did not correlate with poorer survival compared to EA patients, unlike trends observed in other cancers. Radiation therapy combined with chemotherapy significantly improved survival in A-LS patients (median overall survival [OS]: 27.42 months vs. 7.5 months without radiation, p=0.0001). In E-LS patients, radiation showed a non-significant trend toward improved survival (median OS: 33.76 months vs. 23.77 months, p=0.09). In ES disease, where radiation was used palliatively or for oligometastatic control, there was no survival benefit (median OS: 6.93 months with radiation vs. 7.33 months without radiation, p=0.90). Immunotherapy significantly extended survival in ES-SCLC patients compared to those not receiving immunotherapy (p=0.03).
Conclusion: This analysis highlights racial and socioeconomic factors in SCLC outcomes, showing AA patients, despite higher reliance on government insurance, had survival outcomes comparable to EA patients—unlike other cancers where disparities persist. Radiation therapy demonstrated the greatest survival benefit in A-LS patients, while its impact in other stages was limited.

#6224 Lifestyle patterns and mortality in a population-based cohort of Black breast cancer survivors.

Bo Qin[1], Shromona Sarkar[1], Tengteng Wang[1], Nur Zeinomar[1], Marley M. Perlstein[1], Coral O. Omene[2], Karen S. Pawlish[3], Kitaw Demissie[4], Christine B. Ambrosone[5], Chi-Chen Hong[5], Elisa V. Bandera[1]

[1]Section of Cancer Epidemiology and Health Outcomes, Rutgers Cancer Institute, New Brunswick, NJ,[2]Clinical Investigations and Precision Therapeutics Program, Rutgers Cancer Institute, New Brunswick, NJ,[3]New Jersey Department of Health, New Jersey State Cancer Registry, Trenton, NJ,[4]Department of Epidemiology and Biostatistics, SUNY Downstate Health Sciences University School of Public Health, Brooklyn, NY,[5]Department of Cancer Prevention and Control, Roswell Park Comprehensive Cancer Center, Buffalo, NY

Background: African American/Black women have a nearly 40% higher breast cancer (BC) mortality rate compared to White women, reflecting a significant health disparity. Although lifestyle modifications may improve BC survival, robust, population-specific estimates quantifying the association between pre-diagnosis lifestyle patterns and mortality among Black women with BC are lacking. This study leverages data from a population-based cohort of Black BC survivors in New Jersey to examine the relationship between pre-diagnosis lifestyle patterns and mortality outcomes in this high-risk group.

Methods: Lifestyle pattern scores for approximately one year before diagnosis were computed based on adherence to the World Cancer Research Fund/American Institute for Cancer Research recommendations, which align with the American Cancer Society guidelines. These scores were calculated using lifestyle factors measured or self-reported during baseline home interviews, including adiposity (BMI and waist circumference), physical activity, and dietary quality (consumption of fruits and vegetables, fiber, ultra-processed foods, red and processed meat, sugar-sweetened beverages, and alcohol). Scores ranged from 0 to 7, with higher scores indicating greater adherence to guidelines. Associations between lifestyle pattern scores and mortality outcomes (all-cause and BC-specific) were analyzed using Cox proportional hazards models and competing risk models to estimate hazard ratios (HR) and 95% confidence intervals (95% CI).

Results: Among 1,710 women (mean age: 54.1 [SD: 10.7]), 391 deaths occurred over a median follow-up of 8.6 years, including 205 BC-related deaths. The average lifestyle score was 2.9 (SD: 1.1). In multivariate models adjusted for age, education, household income, health insurance, marital status, and smoking status, women with the highest scores (5-7) had a 39% lower risk of all-cause mortality (HR = 0.61, 95% CI: 0.39-0.95) and a 31% lower risk of BC-specific mortality (HR = 0.69, 95% CI: 0.37-1.29) compared to those with the lowest scores (0-2), though the latter CI includes the null. Women with the highest lifestyle pattern scores (5-7) also had significantly lower risks of all-cause mortality by 53% over 5 years and 45% over 10 years, respectively. Findings were consistent across subgroups defined by age at diagnosis, tumor subtype, and stage.

Conclusion: High adherence to cancer-related lifestyle guidelines was associated with lower mortality rates among Black BC survivors compared to those with low adherence. These findings suggest that adherence to lifestyle guidelines encompassing multiple lifestyle factors may improve survival outcomes among Black women following a BC diagnosis.

#6225 Association of UV radiation exposure with risk of malignant melanoma and melanoma in situ in the NIH-AARP Diet and Health Study.

Samer Wahood[1], Yufei Li[2], Matthew Kaczynski[1], John Monroe[3], Fatima N. Mirza[4], Linda M. Liao[5], Rashmi Sinha[5], Martin A. Weinstock[4], Abrar A. Qureshi[4], Eunyoung Cho[4]

[1]The Warren Alpert Medical School of Brown University, Providence, RI,[2]Department of Health Law, Policy and Management, Boston University of Public Health, Boston, MA,[3]Norton College of Medicine, Upstate Medical University, Syracuse, NY,[4]Department of Dermatology, The Warren Alpert Medical School of Brown University, Providence, RI,[5]Division of Cancer Epidemiology and Genetics, Department of Health and Human Services, National Cancer Institute, National Institutes of Health, Bethesda, MD

While ultraviolet radiation (UVR) exposure is a risk factor for skin cancer, there is little evidence from prospective studies exploring the association with both malignant melanoma (MM) and melanoma in situ (MIS).1,2 Within the NIH-AARP Diet and Health Study involving 491,367 participants ages 50-71 when the study began in 1995, we investigated the relationship between July erythemal UVR levels and the incidence of MM and MIS prospectively. July erythemal UVR is the average noon-time ground-level erythemal dose measured in July.3 UVR levels were linked to participants' residences at baseline and categorized into quartiles. Participants were predominantly non-Hispanic white with a median age of 62 years. After adjusting for demographic and lifestyle factors, we utilized multivariable-adjusted Cox proportional hazards regression to estimate hazard ratios (HRs) and 95% confidence intervals (CIs). Over 6,611,941 person-years, with a median follow-up time of 15.5 years, 5,034 MM and 3,284 MIS cases were documented. We found a significant trend between higher erythemal UVR levels and increased risk of both MM and MIS. The highest quartile of UVR exposure (>236 J/m^2), compared with the lowest quartile (≤180 J/m^2), was significantly associated with a greater risk of MM; the HR was 1.32 (95% CI:1.22-1.44) for MM and 1.37 (95% CI:1.25-1.51) for MIS (P-trend <0.001 for both). The study underscores the need for heightened awareness and preventative measures for MM and MIS, especially in regions with higher UVR levels. Further research is needed to explore this association across different body sites.

References

1. Lin SW, Wheeler DC, Park Y, et al. Prospective study of ultraviolet radiation exposure and risk of cancer in the United States. Int J Cancer. 2012;131(6):E1015-E1023. doi:10.1002/ijc.276192. Li Y, Liao LM, Sinha R, et al. Fish intake and risk of melanoma in the NIH-AARP diet and health study. Cancer Causes Control. 2022;33(7):921-928. doi:10.1007/s10552-022-01588-53. Ozone and Air Quality. NASA. Accessed September 5, 2023. https://ozoneaq.gsfc.nasa.gov/data/toms/#

#6226 Chinese visceral adiposity index outperforms other obesity indexes in association with increased overall cancer incidence: Findings from prospective MJ cohort study.

M. Wang[1], C. P. Wen[2], D. T. Chu[3], H. Tu[1], W. Li[1], X. Wu[1];
[1]Zhejiang University, Hangzhou, China, [2]National Health Research Institutes, Taiwan, China, [3]MJ Health Management Center, Taiwan, China

Background: Chinese Visceral Adiposity Index (CVAI) is an integrated index to measure visceral adiposity tissue (VAT). This study is to assess the relationship between VAT indices such as CVAI, Visceral Adiposity Index (VAI), Lipid Accumulation Product (LAP) waist circumference (WC) and waist-hip ratio (WHR) with overall and specific cancer incidence in Chinese.
Methods:332,297 individuals from the MJ cohort were tracked from 1996 to 2007. We utilized multivariable Cox proportional hazards models to examine the connections of five baseline visceral obesity indices and cancer incidence.
Results:A robust dose-response correlation between CVAI and the risk of overall cancer incidence was observed. The HRs were 1.45 (95% CI: 1.2-1.76) and 2.03 (95% CI: 1.52-2.72) for males and females when comparing Quintile 5 to the reference. The HRs for WC were 1.27 (95% CI: 1.08-1.49) among males and 1.19 (95% CI: 1.01-1.40) among females, and WHR showed significance in males (HR:1.28; 95% CI: 1.13-1.45), LAP showed significance in females (HR: 1.25; 95% CI: 1.04-1.5). VAI was not related to overall cancer incidence.
Conclusion: The results support CVAI's practicality as a superior clinical marker for predicting cancer incidence in the Chinese population compared to traditional central obesity indices.

#6227 Contemporary incidence of gastroesophageal junction and gastric cancers in the United States, 2016-2021.

Jessie T. Yan[1], Alexander Wu[1], Hongyu Wu[1], Margarita Kouzova[2], Steven P. Stratton[1]

[1]Roche Diagnostics, Santa Clara, CA,[2]Roche Diagnostics, Tucson, AZ

Background: Limited data are available on the contemporary incidence of gastroesophageal junction cancer (GEJC) and gastric (GC). This study aimed to examine the incidence rates and trends of GEJC and GC overall and by gender and race/ethnicity.
Methods: In this cross-sectional study, we used 2016-2021 data from the U.S. Epidemiology, and End Results (SEER) 22 registries. Per the primary International Classification of Diseases for Oncology, 3rd Edition (ICD-O-3) used by SEER, GEJC cases were identified by “C16.0 Cardia” under GC “C16 Stomach”. Age-adjusted incidence rates were calculated using the SEER*Stat software, and annual percentage change (APC) in incidence was analyzed using the Joinpoint regression program.
Results: During 2016-2021, 17,959 GEJC and 65,899 GC cases were identified. For both cancer types, the age-adjusted incidence rate was higher in men than in women (4.42 vs. 1.16 per 100,000/year for GEJC; 12.83 vs. 7.58 per 100,000/year for GC). By race and ethnicity, for GEJC, non-Hispanic white people had the highest rates of new cancer diagnoses (3.05 per 100,000/year) while non-Hispanic black people had the lowest (1.79 per 100,000/year). For GC, non-Hispanic black people had the highest rates of new cancer diagnoses (13.81 per 100,000/year) while non-Hispanic white people had the lowest (7.61 per 100,000/year). By race/ethnicity and gender, the highest incidence rate of GEJC was found among non-Hispanic white men (5.17 per 100,000 person /year). The highest incidence rate of GC was found among the non-Hispanic black men (18.29 per 100,000/year). From 2016 to 2021, there were no significant changes in both GEJC and GC incidence rates overall and by gender, and race/ethnicity. However, when limiting the analysis to more recent years between 2018 and 2021, there was a falling trend in GEJC but a rising trend in GC incidence overall (APC of -4.08% for GEJC and 3.00% for GC) and by gender (APC of -3.67% for GEJC male and -5.69% for GEJC female; 1.61% for GC male and 4.75 % for GC female). The incidence of GEJC among non-hispanic white men and GC among non-Hispanic black men remained stable.
Conclusion: This population-based study highlights the gender and racial/ethnic differences in GEJC and GC incidence in the United States. Ongoing efforts in increasing awareness, prevention, diagnostic, and treatment strategies for these cancers are needed, particularly for sub-populations with the highest incidence. Future studies should investigate the underlying reasons for the increasing trend in gastric cancer.

#6228 Spatial regression analysis for colorectal cancer mortality rates in Puerto Rico: 2012-2016 and 2017-2021.

Brenda C. Torres-Velasquez[1], **Liliana M. Castro-Jimenez**[1], Carlos R. Torres-Cintron[2], Elba V. Caraballo[3]

[1]Division of Shared Resources & Scientific Operations, University of Puerto Rico Comprehensive Cancer Center, San Juan, Puerto Rico,[2]Puerto Rico Central Cancer Registry, University of Puerto Rico Comprehensive Cancer Center, San Juan, Puerto Rico,[3]Division of Cancer Clinical & Translational Research, University of Puerto Rico Comprehensive Cancer Center, San Juan, Puerto Rico

Objective: Despite efforts to improve screening and early detection, colorectal cancer (CRC) remains a leading cause of cancer-related deaths in Puerto Rico. This study aimed to analyze the spatial distribution of CRC mortality rates (MR) in Puerto Rico during two time periods—2012-2016 (before Hurricane Maria) and 2017-2021 (after Hurricane Maria, earthquakes, and the onset of the COVID-19 pandemic) to identify factors contributing to the observed mortality rates.

Methods: Age-adjusted CRC MR for 2012-2016 and 2017-2021 were obtained from the Puerto Rico Central Cancer Registry (PRCCR). Sociodemographic data were sourced from the American Community Survey using the *tidycensus* R package. The study included 34 treatment centers across Puerto Rico and employed Spatial Cross-Regressive Models (SLX) to examine the influence of factors such as distance to treatment centers, median income, transportation access, and age distribution on CRC MR. Statistical analyses were conducted using R version 4.4.1 (R Foundation for Statistical Computing) and R-Studio (Posit Software, PBC).

Results: In simple models, distance to treatment centers significantly impacted CRC mortality in both periods but with reduced influence in 2017-2021 (2012-2016: coefficient = -0.5, $p < 0.01$; lag coefficient = -37.6, $p < 0.01$ vs. 2017-2021: coefficient = -0.37, $p < 0.05$; lag coefficient = -28.9, $p < 0.05$). In multivariate models, additional sociodemographic factors were significant in 2012-2016, including: Proportion of workers without vehicles (coefficient = 3.22×10^{2}, $p < 0.01$; lag coefficient = 2.55×10^{4}, $p < 0.01$), Median income (coefficient = 7.03×10^{-3}, $p < 0.01$; lag coefficient = 5.24×10^{-1}, $p < 0.01$), and Proportion of inhabitants aged 18 or younger (coefficient = 5.6×10^{2}, $p < 0.01$; lag coefficient = 3.95×10^{4}, $p < 0.01$). However, in 2017-2021, these variables were no longer significant, suggesting the impact of systemic disruptions from disasters and the pandemic.

Conclusions: The findings indicate that CRC mortality in Puerto Rico is driven more by sociodemographic factors such as income and transportation access than by geographic proximity to treatment centers. The reduced significance of these factors in 2017-2021 emphasizes the need for further studies to understand the compounded effects of natural disasters and the COVID-19 pandemic. Addressing disparities in transportation, income, and healthcare accessibility is essential to reducing CRC mortality in Puerto Rico.

#6230 Meta-analysis with a total of 4,978,125 cancer patients reveals depression as a major mortality risk factor.

Janos Tibor Fekete[1], Zoltan Ungvari[2], Monika Fekete[1], **Balazs Gyorffy**[1]

[1]Semmelweis University, Budapest, Hungary,[2]University of Oklahoma, Oklahoma, OK

Background: Depression is a prevalent comorbidity in cancer patients, potentially affecting their quality of life, treatment adherence, and biological processes such as immune function and inflammation. Evidence suggests that depression may contribute to poorer outcomes. The objective of our study was to identify studies examining the association between depression and the risk of cancer-specific mortality as well as all-cause mortality in cancer patients.
Methods: A comprehensive search of online databases was conducted for studies published up to October 1, 2024. Only cohort studies that assessed clinical depression using established diagnostic criteria and reported risk estimates for mortality (such as relative risk, hazard ratio, or odds ratio) were included. A random-effects model was used for statistical analysis, and heterogeneity was assessed using Cochran's Q and the I^2 statistic. The analysis was further refined by conducting cancer-type specific analyses for the most common cancers, including lung, breast, colorectal, and prostate cancers. Statistical analysis was performed using www.metaanalysisonline.com web platform.
Results: After screening of 9,306 studies, we included 65 studies with a total of 4,978,125 patients. In these studies, depression was associated with increased cancer mortality in each of the major cancer types. In colorectal cancer, the hazard ratio (HR) was 1.83, 95% CI: 1.47-2.28, indicating an 83% higher risk, while breast cancer showed a 23% higher risk (95%CI: 1.13-1.34). Lung cancer patients had a 59% higher risk (95%CI: 1.36-1.86), and prostate cancer patients faced a 74% higher risk (95%CI: 1.36-2.23). In mixed cancer types, depression was linked to a 38% higher mortality risk (95%CI: 1.20-1.60). Z-score analysis proved sufficient case number to draw a definitive conclusion in each of the sub-cohorts.
Conclusions: Our results suggest that the presence of depression is significantly associated with 23%-83% increase of cancer-specific mortality across various cancer types, including colorectal, breast, lung, and prostate cancers.

#6231 Associations of serum concentrations of α- and γ-tocopherols with cancer mortality.

Yoo-Min Koh[1], Jae Jeong Yang[1], Young-Rock Hong[2], Shama Karanth[1], Dejana Braithwaite[1], Hyung-Suk Yoon[1]

[1]University of Florida, Gainesville, FL,[2]Emory University, Atlanta, GA

Background: While tocopherols' antioxidant and anti-inflammatory properties suggest their potential anti-cancer effects, little is known about the association between circulating tocopherols and mortality outcomes, particularly among cancer survivors. This study aimed to evaluate whether serum concentrations of α- and γ-tocopherols were associated with the risk of death from all causes, cancer, and cardiovascular disease (CVD).
Methods: We incorporated 16,696 US adults aged ≥20 (1,466 cancer survivors and 15,230 cancer-free individuals) from the National Health and Nutrition Examination Survey, 1999-2006. Serum α- and γ-tocopherols were quantified by high-performance liquid chromatography and categorized into quartiles. Mortality and cause of death were ascertained by linkage to National Death Index records through December 31, 2019 (median follow-up of 16.1 years). Cox proportional hazards regression was used to estimate hazard ratios (HRs) and 95% CIs for all-cause, cancer, and CVD mortality after adjustment for comprehensive covariates, including serum cholesterol levels, sociodemographics, lifestyle (e.g., diet, physical activity), and comorbidities. Restricted cubic splines were used to evaluate the potential non-linearity of the associations.
Results: Cancer survivors in the highest quartile of serum α-tocopherol demonstrated a 50% lower risk of cancer mortality compared to those in the lowest quartile (HR=0.50, 95% CI:0.32-0.80, *p*-trend=0.009). Similar protective associations were observed in cancer-free individuals, with those in the highest quartile of serum α-tocopherol showing a 31% reduction in cancer mortality risk (HR=0.69, 95% CI: 0.46-1.02, *p*-trend=0.05). The association between α-tocopherol and cancer mortality was non-linear ($p<0.001$), exhibiting the strongest protective effects at concentrations ranging from 35 μmol/L to 50 μmol/L. All-cause mortality risk was also lower among those with higher α-tocopherol in cancer survivors (HR=0.75, 95% CI: 0.54-1.03, *p*-trend=0.02) and cancer-free individuals (HR=0.86, 95% CI: 0.74-1.00, *p*-trend=0.03). No significant associations were found for γ-tocopherols.
Conclusions: In this nationally representative sample of US adults, higher circulating concentrations of α-tocopherol were associated with a reduced risk of cancer mortality among both cancer survivors and cancer-free individuals. Our findings indicate the potential health benefits of circulating α-tocopherol in reducing preventable deaths and provide population-based evidence for the role of α-tocopherol in cancer survivorship care.

#6232 Pancreatic cancer epidemiology and impacts in patients admitted with sepsis: Insights from a nationwide inpatient sample.

Abdu Mohammed[1], Adamsegd I. Gebremedhen[2], Mamdouh Souleymane[2], Ibrahim Shanti[2], Jennifer Wiese[2], Stephen Roy[2], Toni O. Pacioles[3]

[1]Internal Medicine, Trinity Health System, Steubenville, OH,[2]Internal Medicine, Marshall University Joan C. Edwards School of Medicine, Huntington, WV,[3]Hematology and Oncology, Marshall University Joan C. Edwards School of Medicine, Huntington, WV

Background: Sepsis is a critical public health issue, contributing to high rates of hospitalization and mortality worldwide. While existing studies primarily focus on sepsis in patients with blood cancers or stem cell transplants, limited research addresses the impact of solid tumors, such as pancreatic cancer, on sepsis outcomes. This study examines the epidemiology and outcomes of sepsis in patients with pancreatic cancer, filling a crucial gap in the literature.
Methods: Data from the National Inpatient Sample (NIS) spanning January 2019 to December 2021 was analyzed. Patients with a primary diagnosis of sepsis or severe sepsis were categorized based on the presence of pancreatic cancer. Key comparisons included demographic data, coexisting conditions, and clinical outcomes, with all-cause mortality as the primary endpoint. Secondary outcomes assessed complications, treatments, hospital length of stay, and costs. Statistical analyses employed multivariate regression models, with significance defined as $p < 0.05$.
Results: The analysis included 7,032,966 adult sepsis admissions from January 1, 2019, to December 31, 2021, with 43,779 (0.62%) also having pancreatic cancer. Patients with pancreatic cancer were older (average age 68.5 vs. 64.5 years, $p < 0.001$), predominantly male (56.9% vs. 43.1%, $p < 0.001$), and more frequently had a Charlson Comorbidity Index of 3 or higher (89.9% vs. 44.5%, $p < 0.001$). These patients exhibited a significantly higher risk of all-cause mortality, with an adjusted odds ratio (aOR) of 1.24, (95% CI 1.16 - 1.31). They also had increased odds of venous thromboembolism (aOR 2.65, 95% CI 2.27 - 3.10), gastrointestinal hemorrhage (aOR of 1.105, 95% CI 1.002 - 1.220) and disseminated intravascular coagulation (aOR of 1.19, 95% CI 1.01 - 1.40). However, renal replacement therapy, mechanical ventilation and vasopressors were used less frequently in pancreatic cancer patients (aOR 0.50, 95% CI 0.43-0.59), (aOR 0.78, 95% CI 0.70 - 0.87) and (aOR 0.51, 95% CI 0.46 - 0.56), respectively. Pancreatic cancer in sepsis patients was also associated with shorter hospital stays (6.66 days vs. 7.65 days) with an adjusted incidence rate ratio (aIRR) of 0.69, (95% CI 0.68 - 0.71) and lower hospitalization costs ($86,649 vs. $98,342, aIRR of 0.68, 95% CI 0.66 - 0.70).
Conclusions: Sepsis patients with concurrent pancreatic cancer experienced significantly higher risks of mortality and other adverse outcomes, yet received less aggressive treatment, had shorter hospital stays, and incurred lower hospitalization costs. These findings may reflect a shift toward avoiding aggressive end-of-life care, as advocated by professional societies and governmental organizations. The results underscore the need for customized management strategies to minimize the impact of pancreatic cancer on outcomes for sepsis patients.

#6233 Human cytomegalovirus, tumor characteristics and patient survival in head and neck squamous cell carcinoma.

Heather H. Nelson[1], DeVon Hunter-Schlichting[1], Rondi Butler[2], Karl Kelsey[2]

[1]Masonic Cancer Center, Minneapolis, MN,[2]Brown Unversity, Providence, RI

Human cytomegalovirus (CMV) is a beta-herpes virus that may play a role in head and neck squamous cell carcinoma (HNSCC). CMV is a highly prevalent latent infection among older US adults, and viral reactivation occurs in response to immune suppression and inflammation. Previously, in a study of 184 HNSCC we reported that elevated CMV IgG titers were associated with worse patient survival. Here, we have expanded our evaluation of CMV IgG and patient survival in 983 cases from the CoHANDS study. CMV positive patients had significantly worse HNSCC survival ($p<0.003$); this association remained after controlling for age, sex and stage (HR=1.30, 95% CI 1.02- 1.74). The association between CMV and outcome was independent of the known association between human papilloma virus infection (HPV) and patient survival. Next, we used nCounter gene expression data to evaluate immune infiltration and inflammation in 34 tumors from HNSCC patients with and without active CMV infection; active infection was defined as having DNAemia, the presence of cell-free CMV DNA in serum. Tumors from patients with active CMV infection were more likely to contain CMV transcript ($p<0.06$) and have increased T-cell infiltration ($p<0.05$), however there were no other differences in the immune infiltrate. The Tumor Inflammation Signature (TIS), a gene expression indicator that correlates with response to immune checkpoint inhibitors, was significantly higher in CMV positive patients ($p=0.007$). CMV positive patients were 12 times more likely to have an elevated TIS score (adjusted OR 12.4, 95% CI 1.2 - 124.2). In summary, HNSCC patients who are CMV IgG positive have poor survival and tumors from patients with active CMV infection have both increased T-cell infiltration and elevated tumor inflammation. These findings may have potential implications for immune therapy in HNSCC.

#6234 Association of cultural racism with breast cancer mortality among adults in the United States, 2018-2020.

A. Gupta[1], O. L. Osazuwa-Peters[2], N. Krieger[3], T. Brown[4], T. F. Akinyemiju[2];
[1]Stanford University School of Medicine, Stanford, CA, [2]Duke University School of Medicine, Durham, NC, [3]Harvard T.H. Chan School of Public Health, Boston, MA, [4]Duke University, Durham, NC

Background: Breast cancer is the most common cause of cancer among women in the United States, with persistent disparities in mortality by race and ethnicity. Given the extent to which systemic racism is inextricably part of the social environment, we evaluated the relationship between cultural racism, a poorly examined but prevalent component of systemic racism, and breast cancer mortality overall and by molecular subtype.
Methods: We utilized a novel state-level multi-dimensional latent measure, the cultural racism factor (CRF), incorporating seven indicators (public symbols of the confederacy, online racial animus, racial resentment, anti-immigrant sentiments, lack of White public support for Medicaid expansion, lack of community-oriented policing prevalence, and aggregate implicit bias of White residents; ≤2017 US national data). We applied the CRF in continuous and dichotomized forms (highest vs. lowest two tertiles). Using cancer data from the Surveillance, Epidemiology, and End Results (SEER) Program (2018-2020), we evaluated the associations of CRF with breast cancer mortality via Cox proportional hazards models with clustering by state and adjusting for sociodemographic and clinical characteristics. We examined whether these associations varied by cancer subtype - Luminal A, Luminal B, HER-2 enriched and triple negative (TNBC).
Results: Among the 225,991 patients included in this analysis, 62% were Non-Hispanic (NH)-White, 12% were NH-Black, and 16% were Hispanic. Kaplan-Meier curves by CRF category showed higher survival probabilities among patients living in states within the lowest CRF tertile compared to the highest two tertiles (log-rank $p<0.0001$). For patients with survival times of 10 month or less, those in the highest CRF tertile had 15% higher risk of death relative to patients in the lowest two tertiles after sequentially adjusting for sociodemographic variables (HR: 1.15; 95% CI: 1.01-1.30), and clinical characteristics (HR: 1.15; 95% CI: 1.03-1.27). Additionally, each unit increase in the CRF score was associated with 7% increased hazard of death after adjusting for covariates (HR: 1.07; 95% CI: 1.02-1.11). However, no significant associations were observed among those with longer survival times. In race and ethnicity-stratified models for survival times of 10 months or less, NH-Black (HR: 1.21; 95% CI: 1.10-1.34) and Hispanic (HR: 1.60; 95% CI: 1.13-2.26) patients living in states in the highest CRF tertile had a higher risk of death than their counterparts living in lower CRF states. No significant associations were observed in subtype-stratified models.
Conclusions: Our findings highlight that state-level CRF increases the risk of breast cancer mortality among patients with shorter survival periods, potentially linked to delayed diagnosis, other aspects of inadequate health care, or more aggressive disease.

#6235 Cancer risk behaviors and adverse childhood experiences among cancer survivors.

Oluwole Adeyemi Babatunde[1], Melanie Jefferson[2], Swann Adams[3], Chanita Hughes Halbert[4], Nosayaba Osazuwa Peters[5], Frank Clark[1], Jessica Obeysekare[1], Anusuiya Nagar[1], Eric Adjei Boakye[6]

[1]Prisma Health, GREER, SC,[2]Medical University of South Carolina, Charleston, SC,[3]University of South Carolina, Columbia, SC,[4]University of Southern California, Los Angeles, SC,[5]Duke University School of Medicine, Durham, NC,[6]Henry Ford Health System, Detroit, MI

Introduction: Adults who have experienced Adverse Childhood Experiences (ACEs) are at a higher risk of engaging in health-risk behaviors. While previous studies have examined the connection between ACEs and health-risk behaviors in the general population, there is a scarcity of research specifically addressing cancer survivors. This study investigates the association between three individual ACEs—mental illness in family members, physical abuse, and sexual abuse—and cancer-related risk behaviors (binge drinking and smoking) among cancer survivors.

Methods: Using cross-sectional data from the 2020 Behavioral Risk Factor Surveillance System, we analyzed a sample of 7,896 cancer survivors aged 18 and older. The exposure variables included three ACEs: having a family member with a mental illness, experiencing physical abuse, and experiencing sexual abuse before the age of 18 dichotomized as yes/no. The outcome measures were cancer risk behaviors: smoking status (categorized as current, former, or never smoker) and binge alcohol use (yes or no). Binge drinking was defined as consuming five or more drinks on one occasion for males and four or more drinks for females in past seven days or/and past 30 days of the survey. Weighted multivariable logistic regression models were used to evaluate the association between ACEs and binge drinking, while multinomial logistic regression models assessed the relationship between ACEs and smoking status, adjusting for sociodemographic factors.

Results: In this sample of cancer survivors, 15.5%, 23.6%, and 6.3% reported experiencing the three ACEs: mental illness in family members, physical abuse, and sexual abuse, respectively. Regarding smoking history, 50.9% were never smokers, 34.3% were former smokers, and 15.0% were current smokers, while 7.8% reported binge alcohol use. Adjusted models revealed that cancer survivors with a history of mental illness in family members had significantly higher odds of being current smokers (aOR: 2.15, 95% CI: 1.41-3.26) or former smokers (aOR: 1.70, 95% CI: 1.27-2.29). Similarly, those who experienced physical abuse had increased odds of being current smokers (aOR: 2.29, 95% CI: 1.64-3.20) or former smokers (aOR: 1.53, 95% CI: 1.22-1.91). Survivors who reported sexual abuse had higher odds of being current smokers (aOR: 3.61, 95% CI: 2.18-5.99) or former smokers (aOR: 1.96, 95% CI: 1.15-3.32). However, the adjusted models showed no statistically significant association between the three ACEs and binge alcohol use among cancer survivors.

Conclusion: Among cancer survivors, ACEs are independent predictors of former and current smoking status in adulthood, a well-established risk factor for cancer. As such, individuals with a history of ACEs may constitute a critical target group for preventive interventions designed to mitigate this risk and promote healthier behaviors.

BIOINFORMATICS / COMPUTATIONAL BIOLOGY / SYSTEMS BIOLOGY / CONVERGENT SCIENCE: Computational and Systems Biology Approaches
Poster Session

#6237 Eco-evolutionary guided pathomics analysis to predict DCIS upstaging.

Mehdi Damaghi, Yujie Xiao

Stony Brook University, Renaissance School of Medicine, Stony Brook, NY

Cancers evolve in a dynamic ecosystem. Thus, characterizing cancer's ecological dynamics is crucial to understanding cancer evolution and can lead to discovering novel biomarkers to predict disease progression. Ductal carcinoma in situ (DCIS) is an early-stage breast cancer characterized by abnormal epithelial cell growth confined within the milk ducts. In this study, we show that ecological habitat analysis of hypoxia and acidosis biomarkers can significantly improve prediction of DCIS upstaging. First, we developed a novel eco-evolutionary designed approach to define habitats in the tumor intra-ductal microenvironment based on oxygen diffusion distance. Then, we identify cancer cells with metabolic phenotypes attributed to their habitat conditions, such as the expression of CA9 indicating hypoxia responding phenotype, and LAMP2b indicating the acid adaptation. Traditionally these markers have shown limited predictive capabilities for DCIS upstaging, if any. However, when analyzed from an ecological perspective, their power to differentiate between pure DCIS and upstaged DCIS increased significantly. Second, using eco-evolutionary guided computational and digital pathology techniques, we discovered distinct niches with spatial patterns of these biomarkers and used the distribution of such niches to predict patient upstaging. With a 5-fold validation on the biopsy cohort, we trained a random forest classifier to achieve the area under curve (AUC) of 0.74. Our results affirm the importance of using eco-evolutionary-designed approaches in biomarkers discovery.

#6238 Interactions between ploidy and resource availability shape clonal interference in glioblastoma.

Zuzanna Nowicka[1], Frederika Rentzeperis[2], Vural Tagal[3], Konstantin Maksin[4], Parag Katira[5], **Noemi Andor**[3]

[1]Department of Biostatistics and Translational Medicine, Medical University of Łodź, Łodź, Poland,[2]Icahn School of Medicine at Mount Sinai, New York City, NY,[3]H. Lee Moffitt Cancer Center, Wesley Chapel, FL,[4]Medical Department (WSHIU), The University of Commerce and Services, Poznan, Poland,[5]Department of Mechanical Engineering and the Computational Sciences Research Center, San Diego State University, San Diego, CA

Whole-genome doubling (WGD) transforms a diploid cell into a tetraploid state, making it prone to genomic instability, aneuploidy, and malignancy. Tumors with evidence of past WGD events range from less than 10% in Non-Hodgkin's Lymphoma to almost 60% in germ cell cancer. WGD confers advantages, including resistance to stressors, making it a powerful macroevolutionary force. *Why, then, isn't WGD a precursor for most cancers?* In Glioblastoma **(**GBM**)**, *~86% of tumors lack evidence of WGD in their evolutionary history*. We integrated a multi-modal dataset of 11 histopathology whole slide images (WSIs), 68 medical images, and whole-genome sequencing data (WGS) from six patient-matched primary and recurrent GBM. Three atlases were derived from FDG PET/MRI (glucose uptake), quantitative BOLD imaging (oxygenation), and magnetic resonance elastography (stiffness), capturing variability across brain regions and patient age. Using HATCHet, which infers clone-specific karyotypes from WGS, we quantified GBM clonal composition and its changes between surgeries. Chromosome 7 amplification and chromosome 10 loss were detected in at least one clone in all tumors. **Genetic intra-tumor heterogeneity (coexistence of ≥2 clones) was found in 4/6 patients**, with moderate to strong clonal changes between surgeries in all four. This data informed parameters governing how cells of variable (poly)aneuploidy respond to oxygen and glucose by dying (α_d, s_r), switching to glycolysis (O_th), or migrating (v_max). Two parameters significantly differed among patients: oxygen-dependent death constant α_d (ANOVA p = 1.7E-5) and oxygen level prompting a metabolic shift to glycolysis O_th (ANOVA p = 1.9E-4). **Higher-ploidy GBMs showed elevated** O_th **values, and higher-ploidy clones within tumors had consistently higher** O_th **than coexisting lower-ploidy clones**. Between surgeries, simulated tumors with distinct karyotype compositions progressed at different rates. Whether higher ploidy predicted fast recurrence was however a function of the tumor anatomic location, in line with the hypothesis that environments with limited resources are likely to exert selective pressure against WGD. Compared to intra-brain differences in resource supply, the differences observed between organs can be larger, providing an opportunity to further test this hypothesis. Indeed, the **average ploidy** of 20 different cancer types was **positively correlated with the oxygen** levels recorded in their respective organ of origin (Spearman r = 0.65, P = 0.002) **and negatively correlated to the glucose** uptake rates in the organ of origin (Spearman r = -0.70, P = 0.026). Overall, our findings suggest that metabolic substrate availability in the tissue micro-environment influences cell fate decisions based on karyotype, shaping GBM initiation and recurrence.

#6239 Modeling and simulation of CRS events in low-risk populations with a rapid ramp-up SUD regimen of epcoritamab.

Matthew Putnins[1], Eric Song[1], Merzu Belete[1], Sri Sridhar[1], Han Si[1], Behnam Noorani[2], Mohamed-Eslam Mohamed[2], Manish Gupta[1], Mariana Sacchi[1], Steven Xu[1], Tommy Li[1]

[1]Genmab, Princeton, NJ,[2]AbbVie, North Chicago, IL

Background: Epcoritamab is the only subcutaneous CD3xCD20 bispecific antibody approved for the treatment of adults with different types of relapsed or refractory (R/R) large B-cell lymphoma (LBCL) and R/R follicular lymphoma after ≥2 prior lines of systemic therapy. Cytokine release syndrome (CRS) is a known adverse event with T-cell engagers. A 2-step step-up dosing (SUD) in cycle 1 with CRS prophylaxis consisting of dexamethasone and fluids, involving a priming dose (0.16 mg on day 1) and an intermediate dose (0.8 mg on day 8) before the target dose (48 mg on day 15), has been identified as the optimal SUD for epcoritamab to reduce CRS risk in patients with diffuse LBCL (DLBCL). However, due to the aggressive disease and fast progression, patients with R/R DLBCL with a low risk of developing CRS may benefit from a shorter SUD regimen, allowing for quicker administration of the target dose without significantly increased CRS event rates following treatment.

Methods: We performed model-based simulations for patients with R/R LBCL at lower risk of developing CRS (eg, patients who received prior CAR T therapy) utilizing a previously validated repeated time-to-event model which captures the time course and frequency of grade (G) ≥2 CRS under various SUD regimens. Specifically, we explored regimens that would shorten the 14-day period between the initial priming dose and the target full dose. For each simulated regimen, the predicted event rate of G≥2 CRS was assessed to determine the impact of the modified SUD.

Results: The model was able to accurately predict CRS event risk: approximately 11% of GCT3013-01 DLBCL optimization cohort patients (9/81) had a G≥2 CRS event, with CRS prophylaxis consisting of dexamethasone and adequate hydration, and our model predicted a CRS event rate of ~11%. Patients with prior CAR T therapy are predicted to have even lower CRS risk: ~4% risk of a G≥2 CRS event under the current 2-step SUD regimen, and ~5% risk of a G≥2 CRS event under a rapid ramp-up 1-week SUD (eg, priming on day 1, intermediate on day 4, and target dose on day 8). Despite the small increased risk of a CRS event with the rapid ramp-up SUD, the risk of an event is still lower than the observed G≥2 CRS event rate in the overall GCT3013-01 optimization cohort (ie, ~11%).

Conclusions: Shortening the SUD to 1 week in patients at low risk for CRS (eg, patients with prior CAR T) resulted in a similar G≥2 CRS rate compared with the current SUD regimen, as predicted by the model. Based on these simulations, a 1-week rapid ramp-up SUD may be beneficial in selected patients to deliver the target full dose of epcoritamab in a shorter time without undue risk. Further confirmation of this finding in clinical studies may be warranted.

#6241 Predicting treatment outcomes from adaptive therapy: a new mathematical biomarker.

Kit Gallagher[1], Maximilian A. Strobl[2], Philip K. Maini[3], Alexander R. Anderson[1]

[1]Integrated Mathematical Oncology, Moffitt Cancer Center, Tampa, FL,[2]Theory Division, Cleveland Clinic, Cleveland, OH,[3]Institute of Mathematics, University of Oxford, Oxford, United Kingdom

Standard-of-care cancer therapy regimens are characterized by continual treatment at the maximum tolerated dose, however, this approach often fails on metastatic cancers due to the emergence of drug resistance. An evolution-based treatment paradigm known as 'Adaptive Therapy' has been proposed to counter this, dynamically adjusting treatment to control, rather than minimize, the tumor burden, thus suppressing the growth of treatment-resistant cell populations and hence delaying patient relapse. Promising clinical results in prostate cancer indicate the potential of adaptive treatment protocols, but demonstrate broad heterogeneity in patient response. This naturally leads to the question: why does this heterogeneity occur, and is a 'one-size-fits-all' protocol best for patients across this spectrum of responses?

Using a Lotka-Volterra representation of drug-sensitive and -resistant tumor populations' dynamics, we obtain a predictive expression for the expected benefit from Adaptive Therapy and show that this is effective at identifying the best responders in a clinical dataset. Incorporating techniques from Optimal Control theory, we further derive an analytic expression for the optimal treatment strategy, based on individual patient tumor dynamics. In contrast to prior approaches, this strategy also accounts for clinical limitations such as fixed time intervals between clinical appointments, to produce clinically realistic treatment strategies. We show that this strategy varies significantly between patients, and present a framework to generate individual treatment schedules from a patient's initial treatment data. Finally, we develop metrics to predict which patients have the greatest sensitivity to unplanned schedule changes, such as delayed appointments, allowing clinicians to identify high-risk patients that need to be monitored more closely and potentially more frequently. Overall, the proposed strategies offer personalized treatment schedules that consistently outperform clinical standard-of-care protocols.

#6242 Adaptive therapy in advanced basal cell carcinoma driven by imaging and mathematical modeling.

Jill Gallaher, Masud M A, Zeynep Eroglu, Alexander R. A. Anderson

Moffitt Cancer Center, Tampa, FL

Basal cell carcinoma (BCC) accounts for 80% of skin cancers and in most cases is very treatable. However, due to either neglect or aggressive growth, roughly 1-10% of BCCs are considered advanced and infiltrated into surrounding tissue such that it is challenging to clear without causing morbidity. These advanced cancers are treated with a systemic therapy inhibiting the Hedgehog signaling pathway, such as vismodegib, which is mutated in 85% of BCCs. Whilst Hedgehog inhibitors have a median progression-free survival (PFS) of approximately 1 year, they come with significant toxicity that often disrupts standard treatment scheduling, and even for those that can tolerate it, there is the possibility of treatment resistance. Here we show interim results from an ongoing clinical trial (NCT05651828) where we design an adaptive therapy strategy for BCC to increase PFS by controlling growth whilst reducing toxicity and the risk of treatment resistance. We use measurements and photographic images of skin lesions over time integrated with a mathematical model to adapt treatment scheduling (either on or off) in a dynamic patient-specific manner. We develop a burden metric based on the color and texture of a lesion to define its current state. We also define a system of ordinary differential equations comprising sensitive and resistant cells along with compartments to capture the drug's unique pharmacodynamics to make predictions for future treatments. We fit the model to each patient's lesion dynamics and predict when to start and stop therapy, iteratively driving treatment decisions in our ongoing clinical trial. We find considerable heterogeneity amongst patients due to the number, size, and morphology of lesions and amongst lesions within a single patient. Drug response and clearance rate of the drug may differ between patients and lesions, so pharmacokinetics and pharmacodynamics parameters need to be carefully calibrated to make good response predictions. Importantly, we define a new strategy for treatment of multiple lesions that are responding differently to treatment within the same patient. We can predict BCC patient growth and response dynamics by fitting a mathematical model to a novel tumor burden metric based on size, color, and texture.

#6243 Mathematical modeling of cell-free DNA fragmentation to map chromatin landscapes in health and cancer.

Brian Sun Liu[1], Mengran Zhang[2], Takeshi Sugio[3], Mohammad Shahrokh Esfahani[2]

[1]Institute for Computational & Mathematical Engineering, Stanford University, Stanford, CA,[2]Department of Radiation Oncology, Stanford School of Medicine, Stanford, CA,[3]Divisions of Oncology and Hematology, Stanford University, Stanford, CA

Background: Cell-free DNA (cfDNA) fragments are shed into bodily fluids as a result of apoptosis and necrosis. Profiling plasma cfDNA has emerged as a powerful tool in cancer diagnostics and research, through circulating tumor DNA (ctDNA) fragments that enable genotyping of many tumors, and epigenetic analysis of cfDNA, including fragmentomics. Fragmentomics encompasses the analysis of fragment lengths, end motifs, and central dyads, which reflect epigenetic patterns, to identify the cell of origin for each fragment.
We previously showed that fragment size diversity is strongly correlated with gene expression (Esfahani et al, NBT 2022). We hypothesize that parameters inferred from a mathematical model for the fragmentation process can enhance gene expression inference from cfDNA.
Methods: We followed the mechanistic model previously proposed (Esfahani et al, NBT 2022), and modified it with additional steps. For each transcription start site (TSS), we input the mean and variance of the linker DNA length, fraction of nucleosomes sites occupied, cut frequency, and DNA degradation factor among other factors. Then, in accordance with the given parameters, we generate a sequence of nucleosome units, a sample of nucleosome sites occupied, and a sequence of fragments, and degrade them.
For chromatin landscaping of plasma cfDNA, we used four cfDNA samples profiled by deep whole genome sequencing (~250X). For each of 500 TSSs, we fitted our model to the fragment size distribution from data by minimizing the Jensen-Shannon (JS) divergence between the generated fragment size distribution and the fragment size distribution from the sample. Finally, we performed regression analysis on the fitted parameters to infer gene expression.
Results: We analyzed cfDNA fragmentation spectra at TSSs genome-wide in relation to transcriptome-wide gene expression levels. The regression shows that gene expression has a negative correlation ($P<0.001$) between fraction of nucleosome sites occupied, a positive correlation ($P=0.002$) with the degradation factor, and a negative correlation ($P=0.009$) with the mean linker length.
Conclusions: We show that cfDNA fragmentation can be modeled as a multi-step stochastic process. Through surveying TSSs, we showed significant correlation between certain model parameters and gene expression. Our findings that gene expression is negatively correlated with model parameters for fraction of nucleosome sites occupied and mean linker length is consistent with previous literature suggesting that higher fraction of nucleosome sites occupied and longer linker length lead to suppressed gene expression (Valouev et al, Nature 2011). We envision this model-based approach will enhance gene expression inference that can ultimately improve cancer detection and tissue of origin classification.

#6244 Comparative diversity analysis of microbial composition across tissue and fecal samples in multi-cohort, multi-study cancer analyses.

Tiffany Zhang[1], Kylie Chan[1], Caitlin Guccione[1], Bertalan Takacs[2], Antonio Gonzalez[1], Gail Ackermann[1], Kathleen Curtius[1], Rob Knight[1]

[1]University of California, San Diego (UCSD), San Diego, CA,[2]University of Szeged, Szeged, Hungary

The microbial ecosystems in tumors, surrounding tissues, and the gut (which influences processes throughout the body) offer new opportunities for oncology diagnostics and treatment, but inconsistencies in metadata quality and parameters across abundant public data hinder generalizable conclusions. To address these issues, we analyzed alpha and beta diversity metrics across multiple studies, focusing on how variables like collection site or cancer type influence microbiome profiles.

We compiled a list of over 180 publicly available studies from the European Nucleotide Archive that sequenced human stool and/or tissue. Studies without publications or sufficient metadata were removed, and remaining studies were narrowed to those using 16S rRNA gene amplicon sequencing, leaving 38 studies with 10,796 samples. Using Qiita, an open-source multi-omics platform, samples were split into fecal and tissue. All fecal samples were analyzed as their own category and came from patients with various cancer types (FEC). Each tissue sample was placed into at least one of the following categories: all cancer tissue samples (CAN), tissue from patients with both healthy adjacent biopsies and cancerous biopsies (ADJ), and tissue from healthy donors and matched cancer patients (HEA). Some samples fell into multiple categories, but each category was analyzed independently. After default QC and rarefying, we used QIIME2, a microbiome bioinformatics platform, to apply diversity metrics.

We explored microbial differences across cancer types using Bray Curtis (BC) and Jaccard diversity metrics for tissue (CAN) and fecal (FEC) categories. In the CAN category, PERMANOVA results for both beta diversity metrics showed a statistically significant difference across the six cancer types ($p < 0.00001$). The same result was replicated in the FEC category. In the CAN category, Kruskal-Wallis results for Shannon diversity (SH) showed a significant difference across cancer tumor types ($p = 1.5e-28$). The same result was observed in the FEC category ($p = 4.6e-48$), indicating that cancer type may be related to microbial diversity differences in both contexts. In the ADJ category, significant differences were observed between adjacent and cancerous tissue for both BC ($p < 0.00001$) and SH ($p = 0.017$). In the HEA category, significant differences were found between matched healthy donors and cancer patients for both BC ($p < 0.00001$) and SH ($p = 0.00004$), highlighting variations driven by tissue context and disease state. Finally, our findings reveal substantial and statistically significant variability using both BC and SH diversity when comparing across the unique study ID in both CAN (BC $p<0.00001$, SH $p=3.9e-38$) and FEC (BC $p<0.00001$, SH $p=4.1e-48$) categories. This underscores the need for standardized guidelines in microbiota sample collection and processing to enhance comparability in future analyses.

#6245 Predicting and optimizing the response of breast cancer patients to neoadjuvant therapy using a multiscale computational model.

Mahesh Nandyala[1], Chengyue Wu[2], **Casey E. Stowers**[1], Reshmi Patel[1], Clinton Yam[2], Jingfei Ma[2], David Fuentes[2], Gaiane M. Rauch[2], Thomas E. Yankeelov[1]

[1]The University of Texas At Austin, Austin, TX,[2]The University of Texas MD Anderson Cancer Center, Houston, TX

The high risk for metastasis, poor prognosis, and low overall survival rates in triple-negative breast cancer (TNBC) underline the crucial need for more accurate prediction and optimization of the therapeutic response. The goal of this work is to combine patient-specific imaging with a multi-scale mathematical model to predict and optimize the treatment response of TNBC on an individual basis. This study employed a cohort of 50 TNBC patients from the ARTEMIS trial (NCT02276443) who received standard-of-care neoadjuvant chemotherapy that consisted of four cycles of Adriamycin/cyclophosphamide (A/C) and 12 cycles of paclitaxel. We established a cell-scale model that describes drug transport via vascular delivery, tissue distribution, and cellular uptake and coupled it to a tissue-scale model of tumor size and cellularity to predict treatment response. Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) data were used to inform patient-specific pharmacokinetic parameters to estimate drug concentration in tissue, and *in-vitro* drug uptake data were used to model cellular drug concentration. The tissue-scale model incorporates cell movement, proliferation, and treatment-induced death and was calibrated using DCE-MRI and diffusion-weighted MRI data from pre- and post-two cycles of A/C treatment. Once calibrated on a patient, the model ran forward in time to predict tumor status at the end of A/C. Over the cohort, the concordance correlation coefficient between the predicted and measured changes in total tumor cellularity and volume were 0.96 and 0.91, respectively.We also evaluated hypothetically alternative treatment protocols for each patient by varying the total dose from 180-300 mg/m^2 and the number of cycles from four to eight within a 56-day A/C treatment period. For the standard-of-care total dose of 240 mg/m^2, eight cycles of A/C treatment resulted in a median (range) of 10% (7.12%-14.99%) reduction in total tumor cell count compared to the standard-of-care four cycles. Further, increasing the total dose improved the patients' response. Within the cohort, a median (range) of 36.83% (29.33%-47.41%) reduction of total tumor cellularity was observed for the 300 mg/m^2 total dose over eight treatment cycles.To the best of our knowledge, this is the first multi-scale model calibrated with patient data to generate a testable prediction of treatment response. The methods in this study accurately captured the spatio-temporal dynamics of drug distribution and the response of TNBC to neoadjuvant A/C. Our ongoing work focuses on using this approach to assess the effects of increasing or decreasing the intensity of the treatment on non-responding and responding patients, respectively. Integrating MRI data with pharmacokinetic and mechanistic models has the potential to accurately predict treatment response in TNBC and bring tangible benefits to the patients.

#6246 Graph-based integration of multi-modal data uncovers cell-type specific vulnerabilities and advances toward a multi-scale digital twin.

Ying Zhu, Li Zhao, David Maxwell, Michael Darkoh, Bissan Al-Lazikani

1Department of Genomic Medicine, 2Institute for Data Science in Oncology, UT MD Anderson Cancer Center, Houston, TX

Background: While recurrent cancer drivers have facilitated drug discovery, strong driver signals are elusive in rare and heterogeneous cancers. Importantly, therapeutically actionable molecular vulnerabilities often extend beyond conventional cancer drivers. We previously developed a graph-based AI approach that identified key molecular vulnerabilities across 19 solid tumor types leveraging bulk sequencing data, outperforming standard methods in target hypothesis generation by three fold (https://doi.org/10.1101/2024.06.04.597409). However, a critical factor in cancer progression and therapeutic resistance lies in the interplay between tumor cells with their microenvironment, surrounding tissue and beyond. There is a real need for a generalizable multiscale communication model that captures molecular and cellular interactions within cells, between cells, and across cellular niches and organs. Such a model could pave the way for constructing a comprehensive multiscale digital twin, offering a transformative framework for understanding cancer dynamics and identifying novel therapeutic cancer targets.
Methods: Graph-based computational models provide a powerful framework for analyzing complex biological systems, making them especially effective for any nodal communication, where a node can represent an individual molecule, a cell, a group of cells or an organ. We introduce a probabilistic graph-based approach that integrates data across multiple modalities and scales—from bulk to single-cell resolution—through network fusion to identify cell-specific vulnerabilities. This method, FN-MVP (Fusion Net-Molecular Vulnerability Picker), is, to our knowledge, the first molecular resolution multiscale model able to model intracellular molecular networks together with cell-cell, cell-niche communication. It unifies data from both single cell and bulk data to provide a comprehensive view of these combined modalities.
Results: By leveraging the cooperativity of weak signals within cancer molecular networks, FN-MVP amplifies true molecular vulnerabilities with greater accuracy (significantly higher than bulk MVP, p = 1.3e-3), as validated by genetic perturbation data. Applying FN-MVP to lung cancer patients revealed cell subtype-specific vulnerabilities and identified critical ligands and receptors that drive TME dynamics in a context-specific manner.
Conclusions: These findings underscore the power of this approach in uncovering actionable molecular interactions. FN-MVP represents a foundational step toward building a generalizable multiscale communication model, capable of integrating molecular, cellular, and tissue-level interactions. This progress lays the groundwork for developing a multiscale digital twin, a transformative tool for understanding cancer and advancing therapeutic strategies.

#6247 Artificial intelligence predicts 2021 WHO glioma subtypes from whole slide images.

Shubham Innani[1], W. Robert Bell[1], MacLean Nasrallah[2], Bhakti Baheti[1], Spyridon Bakas[1]

[1]Indiana University, Indianapolis, IN,[2]University of Pennsylvania, Philadelphia, PA

Diagnosis of diffuse glioma according to the WHO 2021 classification criteria mandate the integration of histologic features with molecular profiling. However, molecular profiling is expensive, time-demanding, and when not available leads to the 'not-otherwise-specified' status. We seek interpretable AI-based classification of glioma, as oligodendroglioma, astrocytoma, or glioblastoma, from H&E-stained slides alone. We identified 2,114 multi-institutional whole slide images (WSIs), from two independent retrospective glioma collections, following reclassification according to the WHO 2021 criteria: a) TCGA-GBM/TCGA-LGG (n_{WSI}=1,320, $n_{patients}$=654) & b) EBRAINS ($n_{WSI}=n_{patients}$=794). TCGA data are used for model development, whereas EBRAINS as hold-out data. Each WSI undergoes comprehensive curation to account for any artifacts, such as tissue foldings, glass reflections, and pen markings. We then conduct a quantitative performance evaluation across: i) eight pathology-specific AI foundation models (FM) and an ImageNet-trained AI model that facilitate robust feature extraction, and ii) nine multiple-instance learning (MIL) approaches that are used to aggregate features into slide-level representations, to differentiate across the three glioma classes. Finally, we take into account magnification level combinations of the WSIs (2.5x, 5x, 10x, 20x), in an attempt to mimic the approach that expert neuropathologists follow to histologically assess tissue slides. Our approach yields AUC_{TCGA}=0.979 over a 10-fold cross-validation schema, and generalizable performance on the independent validation ($AUC_{EBRAINS}$=0.963), for the best performing FM and MIL in the multi-magnification setting. Our key findings indicate: i) domain-specific FMs outperform the ImageNet model, ii) MILs yield larger performance contributions when used with ImageNet models than with FMs, iii) Fusion of multiple magnifications adds value on both development and validation datasets. Interpretability analysis through attention heatmaps highlights distinct identifiable morphology features for each glioma class. Oligodendroglioma show uniform, round nuclei with perinuclear halos, microcystic and gemistocytic cells. Astrocytomas contain cells resembling astrocytes with irregular, elongated shaped nuclei and fibrillary cytoplasmic processes. Glioblastomas display highly heterogeneous cellular composition, pleomorphic astrocyte cells, microvascular proliferation, multinucleated giant cells, and pseudo palisading necrotic regions. Determination of glioma classes directly from routine clinically acquired H&E slides can obviate the need for molecular profiling, expedite conclusive diagnosis and hence clinical decision-making, even in underserved regions. Interpretability analysis towards distilled human-identifiable features can contribute in disease understanding.

#6248 Leveraging knowledge graphs and AI to uncover premalignant disease mechanisms for early detection and prevention of lung cancer.

C. Riquelme-Vano, S. Shikhare, M. O'Donoghue, J. Cohen-Setton, M. Carmona, A. Reynolds, K. Bulusu, K. Nurzynska;
AstraZeneca in Cambridge, Cambridge, United Kingdom

Background: Early detection and treatment of lung cancer markedly improve patient outcomes, with a 5-year survival rate of 68-92% for stage I diagnoses compared to 0-10% for stage IV. Targeting early-stage cancer is pivotal in addressing tumour heterogeneity and therapeutic resistance, critical factors for effective treatment development. This study leverages knowledge graphs and AI to identify novel premalignant disease mechanisms at the early lung cancer stage, serving as potential biomarkers and therapeutic intervention opportunities for prevention of advanced disease.
Methods: In this study, we built the Early Cancer Knowledge Graph (ECKG), an integrative framework that connects multi-omics data from both external and AstraZeneca's internal resources, employing advanced machine learning algorithms to uncover novel disease insights and therapeutic opportunities. Our methodology involves mapping the ontological landscape of early-stage lung cancer through AstraZeneca's internal Biological Insights Knowledge Graph (BIKG) and integrating comprehensive multi-omics datasets. Utilising scalable KG build and clustering algorithms, we cluster ECKG into communities based on disease mechanism similarity and rank genes based on their significance in the disease network.
Results: This approach identified multiple potential novel disease mechanisms, including >20 potential targets highlighting intervention opportunities. These targets underwent pathway enrichment analysis, Gene Set Variation Analysis (GSVA), and correlation to hallmarks of cancer at the single-cell level, enhancing our understanding of their biological significance. Key pathways enriched in early lung cancer cells include MAPK/ERK signaling (KRAS, EGFR) and mTOR signaling (mTOR, AKT1/2). Notably, genes influencing immune cell states within the tumour microenvironment (TME), such as IL-1 signaling, immune checkpoints, and immunosuppressant genes like TGFB1, were highly enriched, among others. These findings underscore the importance of the TME in early lung cancer progression. Most of these targets were supported by evidence in an extensive literature review of early lung cancer patient data.
Conclusion: Future research should focus on experimentally validating these targets, identifying potential intervention strategies, and recommending therapeutic approaches, especially leveraging AstraZeneca's early oncology assets. Furthermore, our ECKG framework could be a generalisable blueprint that is capable of expanding to other critical indications of unmet need, e.g., pancreatic and childhood cancers. Ultimately, the goal is to provide a comprehensive, unbiased assessment of the early disease landscape, facilitating strategic decision-making and shifting the focus from cancer cure to prevention.

#6249 Probabilistic causal network models from real-world prostate cancer data identify potential synergistic combinations with CBP/P300 inhibitors.

Veronica Calvo[1], Bonnie Dougherty[1], Ananya Dutta[2], Duo Xu[1], Qi Pan[1], Andrew Armstrong[3], Jens Renstrup[1], Jason Somarelli[2], Eric Schadt[1]

[1]Pathos AI, Chicago, IL,[2]Department of Medicine, Duke University, Durham, NC,[3]Duke University, Durham, NC

The current standard of care for metastatic castration resistant prostate cancer (mCRPC) includes androgen-receptor signaling inhibitors (ARSIs), chemotherapy, PARP inhibitors (PARPi), or PSMA-radioligand therapies. Although these novel therapies have yielded substantial benefits for patients, many fail to respond or quickly develop resistance. Previous work identified key regulatory programs driving AR-altered, ARSI-resistant, late-stage prostate cancer, and further identified and validated that treatment with the CBP/p300 inhibitor pocenbrodib inhibits cancer cell growth both in vitro and in vivo. Here, we focus on identifying targeted therapies where combination with pocenbrodib could improve clinical response in late-stage prostate cancer. Previous analyses identified that treatment of prostate cancer cell lines with pocenbrodib downregulates a group of genes, termed a subnetwork, capturing G2M, E2F, and DNA repair mechanisms, suggesting that combination with a therapy that induces DNA damage or inhibits DNA repair could be additive or synergistic. Cell cycle profiling following treatment of cell lines with pocenbrodib confirmed induction of G2M cell cycle arrest. Next, we systematically ranked current targeted therapies based on publicly available RNA-seq signatures and signatures derived from RWD (e.g. BRCA2 alterations) to identify therapies that target our subnetwork of genes enriched for G2M, E2F, and DNA repair mechanisms. Given that both PSMA-radioligand therapies and PARP inhibitors induce DNA damage or inhibit DNA repair, we hypothesize that these drugs in combination with pocenbrodib would lead to additive or synergistic effects by enhancing the PARPi and PSMA-radioligand efficacy via their known mechanisms of action.

To validate these hypotheses, we treated a panel of 5 genetically-diverse prostate cancer PDX-derived organoids with pocenbrodib, the PARPi olaparib, and in combination. The combination of pocenbrodib with olaparib led to additive or synergistic effects in 3 out of the 5 organoids, consistent with those showing some level of response to olaparib as a single agent. To evaluate the potential for combination benefit with PSMA-radioligand therapy, an in vivo efficacy study was performed using the PSMA+ 22Rv1 prostate cancer xenograft model. Addition of a low dose of pocenbrodib to a single dose of Lu177-PSMA617 (60 MBq) led to better tumor growth inhibition control than either single agent on its own. Mechanistic analyses from RNAseq are ongoing. Together, our integrated omics and experimental analyses support the hypothesis that combination of pocenbrodib with DNA damage-inducing or -inhibiting targeted therapies may provide further therapeutic benefit to prostate cancer patients than single agent therapies alone.

#6250 Clinical significance of co localization index in rectal cancer: a deep learning approach to cell interaction analysis.

Kimihiro Yamashita[1], Tomoki Abe[2], Toru Nagasaka[3], Masayuki Ando[2], Takao Tsuneki[2], Yukari Adachi[2], Takaaki Tachibana[2], Hiroki Kagiyama[2], Tomoaki Aoki[2], Yasufumi Koterazawa[2], Ryuichiro Sawada[2], Hitoshi Harada[2], Yasunori Otowa[2], Naoki Urakawa[2], Hironobu Goto[2], Hiroshi Hasegawa[2], Shingo Kanaji[2], Takeru Matsuda[2], Ryohei Sasaki[4], Yoshihiro Kakeji[2]

[1]Department of Biophysics, Kobe University Graduate School of Health Sciences, Kobe City, Japan,[2]Department of Surgery, Kobe University Graduate School of Medical Science, Kobe City, Japan,[3]Association of Medical Artificial Intelligence Curation, Nagoya City, Japan,[4]Department of Radiation Oncology, Kobe University Graduate School of Medical Science, Kobe City, Japan

Background: Tumor tissue is not just a cluster of cancer cells, but an organized ecosystem in which various cells, extracellular matrices, and various types of liquid factors interact with each other. There are high technical barriers to processing this complex ecosystem in large quantities and quantitatively. Therefore, we have established a histological analysis method using deep-learning-based imaging cytometry (DL-IC) (Abe T, Yamashita K, et al. Anticancer Res. 43:3755 2023). Identification of the constituent cells of tumor tissue and spatial understanding of the tumor immune microenvironment using DL-IC are important for predicting cancer prognosis and treatment efficacy. In this study, we propose a co-localization index (CLI) as a new indicator for analyzing the tumor immune microenvironment using AI.
Purpose: We investigated the impact of the co-localization of cytotoxic lymphocytes and cancer cells on the prognosis of rectal cancer after surgery.
Subjects and Methods: Forty rectal cancer surgical specimens were analyzed using the DL-IC system Cu-Cyto. A bit pattern kernel filtering algorithm was implemented to prevent duplicate cell counting, and its performance on immunohistochemistry (IHC) specimens was evaluated. The accuracy was compared between analyses with and without the algorithm. Cell-cell interactions were quantified using CLI, and the usefulness of CLI as a prognostic indicator was evaluated, particularly focusing on interactions between cancer cells and CD8+ T cells. Additionally, a comprehensive analysis of CLI was conducted using combinations of multiple cell types.
Results: In the training process, where the data size of the training data was scaled, the F1 scores for adenocarcinoma cells, lymphocytes, and CD8+ T cells were 0.589, 0.889, and 0.911, respectively, at a cell number of 1013. The introduction of a bit pattern kernel filtering algorithm improved the accuracy of the determination of each cell type. The 5-year disease-free survival rate was significantly prolonged in the high CLI group between cancer cells and CD8+ T cells (P=0.041), and multivariate analysis also showed that it was an independent prognostic factor. On the other hand, there was no significant difference in the 5-year overall survival rate (P=0.41). Furthermore, it was found that the CLI of the three-way interaction between cancer cells, macrophages, and CD8+ T cells was also an independent prognostic factor.
Conclusion: Cu-Cyto has achieved highly accurate cell determination by introducing a bit pattern kernel filtering algorithm. CLI has shown correlation with patient prognosis as an objective and reproducible quantitative evaluation method for cell-cell interactions. In the future, it will be necessary to elucidate the oncological and biological significance of the combinations of each cell type.

BIOINFORMATICS / COMPUTATIONAL BIOLOGY / SYSTEMS BIOLOGY / CONVERGENT SCIENCE: Computational Immunology and Tumor Microenvironment Characterization Poster Session

#6254 Detection of anti-CMV TCR CDR3s correlates with increased survival for soft tissue sarcoma and stomach adenocarcinoma.

Utsav Kapoor[1], Michael T. Aboujaoude[1], Konrad Cios[1], Andrea Chobrutskiy[2], Boris I. Chobrutskiy[2], George Blanck[3]

[1]Molecular Medicine, USF Health Morsani College of Medicine, Tampa, FL,[2]Pediatrics, Oregon Health and Science University, Tampa, OR,[3]Molecular Medicine and Immunology, USF Health Morsani College of Medicine and Moffitt Cancer Center, Tampa, FL

Introduction: While the role of anti-cytomegalovirus (CMV) T-cell responses in the cancer setting is becoming increasingly recognized, the association between CMV and survival outcomes has not been subject to study through more recent immunogenomic approaches. This study aimed to investigate the association between anti-CMV T-cell receptor (TCR) sequences and improved survival outcomes in two types of cancers where the role of CMV remains currently underexplored, stomach adenocarcinoma (STAD) and soft tissue sarcoma (SARC). The significance of this study is that, to our knowledge, it is the first to identify a potential prognostic role of CMV in both STAD and SARC and highlights the potential for future development of anti-CMV T-cell therapies.
Methods: We extracted TCR recombination reads from whole exome sequencing (WXS) and RNA sequencing (RNASeq) files of STAD and SARC samples from the Cancer Genome Atlas (TCGA) and matched the corresponding TCR complementarity determining region-3 (CDR3) AA sequences represented by these reads to known anti-CMV CDR3 sequences.
Results: Results indicated that, in STAD cases, the recovery of both TRA and TRB recombination reads from WXS files, from either normal blood or tumor samples, which corresponded to AA sequences matching anti-CMV CDR3s, correlated with improved disease-free survival (DFS) (logrank p=0.025) and improved overall (p=0.026), progression-free (p=0.015), and disease specific (p=0.026) survival on an STP comparison (i.e., a comparison at 45 months). Within the SARC WXS dataset, these were associated with better overall survival (OS) (logrank p=0.021) and disease-specific survival (DSS) (logrank p=0.008). However, TCR CDR3s represented by recombination reads recovered from RNAseq files and matching anti-CMV TCR CDR3 AA sequences correlated with lower DFS probabilities (i.e., STAD STP comparison at 45 months, p=0.035, SARC STP comparison at 65 months, p=0.032).
Conclusion: This study reveals a novel association between anti-CMV TCR sequences and survival probability in STAD and SARC. This suggests that TCR CDR3s may be possible indicator of prognosis for STAD and SARC and supports further research into anti-CMV CDR3s as a tool for the development of immunotherapy for these cancers. Additionally, the contrasting results between WXS and RNASeq-derived sequences warrant further investigation into the timing and nature of anti-CMV immune responses in cancer progression. One explanation for these results include the possibility that exposure to CMV prior to onset or progression of cancer may lead to better survival probability, while a potentially active T-cell response to CMV during cancer onset or progression could represent an ongoing comorbidity.

#6255 Patient response to immune checkpoint inhibitors (ICI) informs neoantigen prioritization: Applications to personalized cancer vaccines (PCVs) and ICI response prediction.

R. Burns, S. L. Bristow, J. B. Ortiz, M. Malhotra, J. Hundal, A. M. Rosenfeld, H. A. Costa, A. Aleshin, M. Rabinowitz;
Natera, Austin, TX

Accurate identification of neoantigens that can stimulate a cytotoxic immune response is essential in designing PCVs. Here, we describe the design and performance of a neoantigen prioritization model for PCV design and ICI response prediction.The machine learning (ML)-based neoantigen prioritization model was implemented using tumor and matched normal whole exome sequencing data and clinical information to identify candidate neoantigens. Neoantigens were grouped across combinations of three characteristics: MHC-I binding IC50, T-cell immunogenicity predictions, and inferred gene expression. For each neoantigen combination, ICI response, as measured by progression-free survival (PFS), was compared using Cox proportional hazards tests. Statistically significant hazard ratios were used as a prioritization metric for each neoantigen combination. Model performance for neoantigen prioritization was evaluated using three publicly available datasets (TESLA N=8, NCI N=97, HiTIDE N=10) with known immunogenic, true-positive (TP) neoantigens. The percent of TP neoantigens prioritized within the top 100, 50, and 20 per patient was computed. Furthermore, a real-world clinical cohort (N=543; training 80%, test 20%) and an additional 66 patients with solid tumors from the Bespoke IO clinical study were utilized to validate our model for ICI response prediction as compared to TMB.In this cohort, hazard ratios of candidate neoantigens correlated with higher inferred gene expression and inversely correlated with IC50 and T-cell activation rank. In a separate analysis, we demonstrated that TCGA-based inferred gene expression, in the absence of patient-matched RNAseq, was sufficient to identify immunogenic neoantigens. Across all three test sets, the known TPneoantigens were consistently prioritized in the top 100 (NCI 89.7%, TESLA 91.4%, HiTIDE 96.4%) and top 50 (NCI 77.2%, TESLA 80.0%, HiTIDE 78.6%). Approximately half of TP neoantigens were prioritized in the top 20 (NCI 54.5%, TESLA 54.3%, HiTIDE 46.4%). These results outperformed other methods that have used TESLA data. The classification of patients into non-responders and responders was significantly better by our model for ICI response prediction [melanoma (HR: 13.69, p=0.0005), NSCLC (HR: 5.79, p=0.036) and MSI-H CRC (HR: 23.1, p=0.0001)] compared to TMB [melanoma (HR: 2.56, p=0.07), NSCLC (HR: 1.04, p=0.96) and MSI-H CRC (HR: 3.31, p=0.09)]. Similar trends were observed in the blinded validation cohort [(model: HR=3.16, p=0.006) vs. TMB (HR=1.47, p=0.33)].Our ML-based neoantigen prioritization model is the first to our knowledge to be informed by in vivo ICI response that effectively identifies and prioritizes immunogenic neoantigens for PCV design and ICI response prediction.

#6256 Dissecting tumor-intrinsic programs and immune dynamics driving response to immunotherapy in metastatic melanoma through non-negative matrix factorization decomposition.

Priyanka Solanky[1], Giuseppe Tarantino[1], Yiwen He[2], Xinyu Cui[2], Kathleen Pfaff[3], Aaron R. Thorner[4], Tyler J. Aprati[4], Boyang Zhang[5], Timothy Blosser[6], Jiajia Chen[4], Junko Tsuji[4], Elliot Boblitt[6], Allison Frangieh[3], Hannah Mae Faulkner[3], Marta M. Holovatska[3], Aleigha R. Lawless[7], Michael Manos[3], Emily Robitschek[5], Karla Helvie[3], Tatyana Sharova[7], Dennie Frederick[6], James Liam Fahey[8], Diego Villamarin[7], Sachi Krishna[4], Chanell Mangum[6], Ajit J. Nirmal[8], Domenic Abbondanza[6], Cai McCann[6], Bruce Johnson[3], Alex Shalek[9], Eliezer Van Allen[3], Xiaowei Zhuang[10], Ryan J. Sullivan[7], Barbara Engelhardt[5], Sami Farhi[6], Scott J. Rodig[3], Stephen Hodi[3], Genevieve Boland[7], David Liu[11]

[1]Harvard Medical School, DFCI, Broad Institute, Boston, MA,[2]Immunology, DFCI/Harvard Medical School, Boston, MA,[3]Dana-Farber Cancer Institute, Boston, MA,[4]DFCI, Broad Institute, Boston, MA,[5]Stanford University, Stanford, CA,[6]Broad Institute of MIT and Harvard, Cambridge, MA,[7]Massachusetts General Hospital, Boston, MA,[8]Brigham and Women's Hospital, Boston, MA,[9]MIT, Cambridge, MA,[10]Chemistry, Harvard University, Cambridge, MA,[11]DFCI/Broad Institute, Boston, MA

Background: Understanding the biological mechanisms underlying differential responses to immunotherapy is critical for advancing treatment strategies in metastatic melanoma. We analyzed single-nucleus RNA sequencing data from 66 melanoma samples, including 18 with matched spatial transcriptomics data via MERFISH, across metastatic sites such as lymph nodes, colon, skin, lung, brain, and liver. Our study aimed to identify tumor-intrinsic transcriptional programs and their association with durable clinical benefit.
Methods: We employed non-negative matrix factorization to delineate distinct tumor programs within tumor cells. To assess the robustness and biological relevance of these programs, we conducted hallmark pathway analyses and utilized spatial transcriptomics to examine their associations with biopsy sites, response, and immune infiltration. Comparisons with published datasets were performed to characterize and ensure the stability and applicability of these programs.
Results: We identified five distinct tumor programs with differential enrichment between responders and non-responders. Tumor programs enriched in responders demonstrated strong associations with interferon alpha-beta signaling and antigen presentation pathways. This program was also enriched across diverse lymphocyte populations. Via MERFISH analysis, we classified patients into three categories based on the spatial distribution of immune cells relative to tumor borders: immune-enriched, immune-excluded, and immune-desert. Notably, the program enriched in responders was prevalent in the immune-excluded samples, suggesting that robust signaling pathways can drive therapeutic responses even in the absence of high immune infiltration. In contrast, a mesenchymal-like tumor program was predominant in non-responders, characterized by elevated epithelial-mesenchymal transition activity and diverse tumor-associated macrophage infiltration. This program was particularly enriched in colon metastases.
Conclusion: Our findings underscore the importance of interferon signaling in driving therapy response and the role of mesenchymal-like programs in resistance. The unique association of immune-excluded but therapy-responsive samples with interferon-related pathways raises intriguing questions about tumor-immune dynamics. By integrating single-cell and spatial transcriptomics, this study provides insights into tumor heterogeneity and the tumor microenvironment, offering avenues for targeted therapeutic interventions. Future research will focus on spatial interactions, the characterization of immune subpopulations within tumor programs, and the dynamics of cell-cell communication between tumor and immune cells, aiming to refine and advance personalized treatment strategies.

#6257 Spatial and multi-omic profiling of metastatic colorectal cancer reveals key cellular ecosystems and drivers of heterogeneity.

Chien-Wei Peng[1], Michael Iglesia[1], Adrienne Visani[2], Xiang Li[1], Xiangwei Fang[1], Reyka G. Jayasinghe[1], Jingxian Liu[1], John Herndon[2], Jacqueline Mudd[1], Andrew Houston[1], Preet Lal[1], Siqi Chen[1], Ambrose Plante[1], Andre Targino da Costa[1], Kapur B. Dhami[1], Jennifer Ponce[3], Robert Fulton[3], Michael Heinz[3], Milan Chheda[4], Feng Chen[5], Ryan C. Fields[2], Li Ding[1]

[1]Department of Medicine, Washington University School of Medicine in St. Louis, St. Louis, MO,[2]Department of Surgery, Washington University School of Medicine in St. Louis, St. Louis, MO,[3]McDonnell Genome Institute, Washington University School of Medicine in St. Louis, St. Louis, MO,[4]Department of Neurology, Washington University School of Medicine in St. Louis, St. Louis, MO,[5]Department of Nephrology, Washington University School of Medicine in St. Louis, St. Louis, MO

Colorectal cancer (CRC) metastasis is highly aggressive and associated with poor outcomes. Recent studies have highlighted greater heterogeneity in gene expression within metastatic tumor cells compared to primary tumor cells. However, the underlying factors driving these differences remain poorly understood. We hypothesize that both genetic alterations and the tumor microenvironment play key roles in shaping this heterogeneity. To investigate these aspects, we established a cohort through the Human Tumor Atlas Network composed of 26 primary CRC samples and 41 metastatic CRC samples from multiple organs. We conducted whole exome sequencing (WES), single-nucleus ATAC/RNA-seq (snATAC/RNA-seq), and spatial transcriptomics (Xenium) assays to comprehensively analyze genetic alterations, gene expression patterns, and their spatial relations. Our findings revealed a higher proportion of TP53 and KRAS mutations in metastatic samples compared to primary tumor samples. Analysis of snRNA-seq data identified distinct tumor cell populations characterized by gene signatures from 12 published gene expression modules. Primary tumor cells were predominantly enriched for tumor intestinal stem cell-like and intestinal modules, while metastatic tumor cells showed higher representation of injury repair, epithelial-mesenchymal transition (EMT), osteoblast-like, and neuroendocrine modules. Notably, a microsatellite instability-high (MSI-high) metastatic sample exhibited relatively higher signatures of absorptive intestinal and squamous cancer modules than other microsatellite-stable samples, suggesting a unique gene expression profile in MSI-high CRC metastasis. To spatially study these cancer modules, we defined spatial tumor microregions as regions of tumor cells separated by stromal tissues. Using the Xenium assay, we mapped the gene modules within these microregions and observed significant enrichment of EMT and injury repair modules at the peripheral tumor microregions in metastatic samples. Conversely, the hypoxia module was more prominently enriched at the core of tumor microregions in metastatic samples compared to primary tumors. These findings suggest a strong association between tumor cell location within the microenvironment and their gene expression profiles, shedding light on the spatial dynamics of tumor heterogeneity in CRC metastasis. In summary, our results suggest that the heterogeneity of gene expression in metastatic CRC is driven by genetic alterations and strongly influenced by the tumor microenvironment.

#6258 Inter- and intra-tumoral heterogeneity of TNBC determined by spatial gene transcriptomics.

Fengyuan Huang[1], Deepa Bedi[1], Clayton Yates[2]

[1]Center for Biomedical research, Tuskegee University, Tuskegee, AL,[2]School of Medicine, Johns Hopkins University, Baltimore, MD

Triple-negative breast cancer (TNBC), one of the most aggressive subtypes of breast cancer, is associated with poor prognosis and limited treatment options, particularly when diagnosed at later stages. The extensive inter- and intra-tumoral heterogeneity observed in TNBC further complicates its clinical management. Understanding the molecular difference within this TNBC is crucial for elucidating its biological characteristics and clinical behaviors, as well as for developing personalized therapeutic approaches. In this study, we employ digital spatial profiling (DSP) technology to quantitatively analyze transcript and protein abundance across spatially distinct regions of TNBC, including primary tumor cores, surrounding stroma regions, and metastatic sites. The analysis reveal marked spatial heterogeneity in gene expression profiles, cellular composition, and the immune microenvironment across these regions. Additionally, we identify distinct TNBC subtypes based on unique gene expression patterns. In primary tumor, Basal-like 1 (BL1) emerged as one of the predominant subtypes and was associated with an increased present of memory B cells. We also identified a subgroup resembling unclassified (UNS) subtypes, characterized by low macrophage levels. Moreover, tumor tissues exhibited multiple TNBC subtypes within a single tumor site. This intricate subtyping within the tumor mass underscores the challenges associated with developing effective treatment for TNBC. Our findings demonstrate the power of DSP technology in elucidating the spatial dynamics of TNBC at the molecular level. The identified molecular signatures and subtypes hold potential for improving diagnostic accuracy, enabling targeted drug development, and guiding personalized treatment approaches. Ultimately, this work underscores the utility of spatial transcriptomics for addressing the challenges posed by TNBC heterogeneity and advancing precision medicine for this aggressive cancer subtype.

#6259 Spatial transcriptomics-driven in silico modeling of therapeutic effects of antibody-drug conjugates and their association with treatment response in lung adenocarcinoma.

Sungwoo Bae, Minki Choi, Hongyoon Choi, Dongjoo Lee, Kwon Joong Na, Daeseung Lee

Portrai, Inc., Seoul, Korea, Republic of

Background: Antibody-drug conjugates (ADCs) are targeted therapies that deliver cytotoxic payloads to cells expressing specific antigens. Despite their increasing use in clinical trials for advanced non-small cell lung cancer (NSCLC), many patients experience suboptimal outcomes. While ADC efficacy depends on factors such as antibodies, linkers and payloads, the impact of the tumor microenvironment (TME) remains unclear. This study uses spatial transcriptomics (ST) to investigate the spatial dynamics influencing ADC efficacy within the TME of NSCLC.

Methods: Visium ST datasets from 36 lung adenocarcinoma (LUAD) tissues were analyzed to identify factors influencing ADC efficacy. Key indices included Target Specificity Score (TSS), which reflects the ratio of target expression in malignant versus normal epithelial cells; Target-Linker Match Score (TLM), which measures overlap between target regions and linker enzyme activity; Therapeutic Index (TI), calculated as TSS × TLM; and Intratumoral Target Expression (ITE), which quantifies average target expression. To evaluate the bystander effect, Moran's I index assessed spatial clustering patterns of target expression, and Minimum Target-High Distance (MTHD) assessed the average of the minimum distance from low to high target expression regions. Fifteen ADCs were selected from NSCLC clinical trial data, including targets, linkers, and overall response rates (ORRs). Correlations between spatial indices and ORRs were analyzed to validate the utility of the indices.

Results: Of the 36 tissues analyzed, 3 were excluded due to insufficient or excessive tumor regions. Among the six indices evaluated, only MTHD showed a strong negative correlation with the reported ORRs (Spearman's R: -0.715, $p = 0.003$). This suggests that shorter distances between low and high target expression regions are associated with improved ADC efficacy, highlighting the critical role of spatial homogeneity in the bystander effect. To further evaluate the potential of the indices in identifying ADCs with poor therapeutic efficacy, we compared the indices between ADCs with ORR values below the 10th percentile (ORR Low) and those above (ORR High); only the TSS showed a significant difference ($p < 0.05$). ROC analysis identified a TSS cut-off value of 4.744, yielding an AUC of 0.962 for discriminating between ORR Low and High groups.

Conclusion: Spatial indices derived from spatial transcriptomics, particularly those reflecting target specificity and bystander effect, were found to significantly influence treatment response and predict ADC failure, respectively. This in silico modeling approach offers a promising screening method to identify ADCs with the highest potential for therapeutic efficacy.

#6260 Characterization of the tumor microenvironment's histologic landscape through histology-based deep learning spatial transcriptomic cell-type deconvolution of colon tumors.

Minh-Khang Le[1], Gokul Srinivasan[1], Fred Kolling[2], Keluo Yao[1], Alos Diallo[2], Zarif Azher[1], Louis J. Vaickus[3], Xiaoying Liu[3], Joshua Levy[1]

[1]Cedars-Sinai Medical Center, West Hollywood, CA,[2]Dartmouth College Geisel School of Medicine, Hanover, NH,[3]Dartmouth Health, Lebanon, NH

The tumor microenvironment (TME) is an essential component of cancer biology. TME-derived measures such as tumor-infiltrating lymphocytes proportions (TILs) and tumor-stroma ratio (TSR) are used to evaluate TME composition. We leveraged spatial transcriptomics (ST) data from colon tumors to deconvolve cell-type populations and developed a deep learning (DL) algorithm to infer local ST-derived TIL and TSR estimates reflecting TME heterogeneity from held-out H&E whole slide images (WSIs). A total of 44 colon tumors surgical sections were profiled using matched H&E WSI and Visium ST, with 10 patients profiled for single-cell RNASeq. A pathologist annotated tumor microarchitecture within each slide. A total of 284,770 512x512 pixel^2 image patches were extracted and paired with corresponding Visium ST spots. Using SCANVI, cell type labels from the Human colon cancer atlas (c295) were lifted over to our in-house scRNASeq data. Cells were mapped spatially to estimate spot-level cell-type proportions using Cell2Location (C2L). Cell-type mapping was validated through human-in-the-loop (HITL) testing, where 512-pixel image crops around 400 randomly selected spots were annotated at each pixel. Spot-level TIL/TSR measures were estimated using cell-type proportions from the C2L-transformed ST data and proportion of pixels within each patch assigned to lymphocytes and tumor. Next, Virchow, a state-of-the-art deep learning pathology foundation model, was used to extract spot-level histological features- a Random Forest model was trained on spot-level features to infer cell-type proportions. Spot-level performance for cell-type mapping (evaluated through HITL) and cell-type proportion inference from histology was measured using the area under the curve (AUC) on a held-out test set of 9 patients. HITL tests demonstrated high agreement between H/ST-TIL (AUC=0.71; 95% CI: [0.60-0.82]) and H/ST-TSR (AUC=0.86; 95% CI: [0.81-0.92]). DL spot-level cell-type deconvolution within tumor-only slide regions achieved a macro-averaged AUC of 0.812 (Tumor: 0.85, Mesenchymal: 0.91, Lymphocytes: 0.79, Monocytes: 0.76, Mast: 0.79, Plasma: 0.78). Previous work inferred spatial expression patterns across broader regions, reporting cell-type AUCs of Tumor: 0.93, Mesenchymal: 0.82, Lymphocytes: 0.76, Monocytes: 0.74, and Mast: 0.91. Our results show that DL can infer ST-labeled cell types from H&E WSI, capturing TME features beyond tissue architecture. HITL testing demonstrated strong concordance between derived spot-level ST-TSR/TIL metrics with their histology-based counterparts (H-TSR/TIL), with utility for pathologist validation of spatially mapped cell-type data. This study highlights future potential of DL to capture spatial distribution of TME measures as prognostic biomarkers through ST inference from histology.

#6262 Changes in the spatial architecture of the tumor microenvironment associated with treatment resistance in muscle invasive bladder cancer.

Nikolay Alabi[1], Nicolas Zheng[2], Joshua Scurll[2], Jussi Nikkola[2], Alberti Contreras-Sanz[2], Morgan Roberts[2], Ali Bashashati[1], Peter Black[2]

[1]University of British Columbia, Vancouver, BC, Canada,[2]Vancouver Prostate Centre, Vancouver, BC, Canada

Approximately one-third of bladder cancer (BC) patients are diagnosed with muscle-invasive bladder cancer (MIBC). Despite recent successes in clinical trials with neo-adjuvant therapies such as checkpoint inhibitors and chemotherapy, clinical implementation is hindered by a lack of biomarkers to guide therapy decisions, as not all patients respond to certain treatments. While the prognostic value of immune biomarkers in BC has been studied in the tumor microenvironment (TME), previous studies only evaluate single therapies, lack comparison to untreated groups, and fail to examine post-treatment residual tissue, limiting cross-therapy comparisons. Most studies have also relied solely on immune cell densities, ignoring spatial immune interactions critical for anti-tumor responses. Measuring spatial relationships (SRs) between immune cells in the TME provides a mathematical description of proximity and adjacency, which is more biologically relevant. Associations between SRs, prognosis, and treatment response have been reported across cancer types. For MIBC, analyzing SRs may improve tumor immunology insights and reveal treatment-response biomarkers. We analyzed four MIBC cohorts using multiplex immunofluorescence (mIF, three panels with six markers): 49 pre-NAC, 90 pre-CPI (pembrolizumab), 41 pre-NAC and CPI (nivolumab), and 70 untreated, plus corresponding residual tissue (N=26, 13, 20, and 35). A univariate logistic regression model identified spatial immune features associated with treatment resistance, examining metrics like first nearest neighbor (1NN) distances (e.g., median, Weibull distribution shape/scale) and frequency of cellular triads (interaction patterns). Model coefficients were exponentiated into interpretable odds ratios (ORs) for resistance associations. Multiple immune features were linked to NAC resistance. For example, pre-treatment, a higher Weibull shape parameter for CD4+ T cells and Tregs was associated with resistance to NAC (OR = 0.22, 95% CI [0.05, 0.67], p = 0.02). Similarly, increased pre-treatment abundance of triads involving B cells, CD4+ T cells, and Tregs was associated with NAC resistance (OR = 0.77, 95% CI [0.55, 0.94], p = 0.04). Importantly, these associations were NAC-specific, as no negative prognostic associations were observed in untreated patients (Weibull shape: OR = 2.22, 95% CI [0.71, 8.2], p = 0.19; triads: OR = 0.99, 95% CI [0.97, 1.01], p = 0.33). These findings highlight the potential of spatial immune metrics to refine biomarker identification and improve personalized treatment strategies in MIBC.

#6263 Multi-transcriptomic analysis reveals tumor microenvironment differences associated with anti-EGFR therapy responsiveness between left- and right-sided colorectal cancer.

Atsuki Taniguchi, Shunsuke Kagawa, Shohei Nogi, Nobuhiko Kanaya, Yoshihiko Kakiuchi, Satoru Kikuchi, Shinji Kuroda, Hiroshi Tazawa, Toshiyoshi Fujiwara

Okayama University Hospital, Okayama, Japan

Background: Regarding the sidedness of colorectal cancer, right-sided colorectal cancer (RCRC) and left-sided colorectal cancer (LCRC) originate from the midgut and hindgut, respectively. It is known that in stages III and IV, RCRC has a poorer prognosis compared to LCRC. Furthermore, the prognosis of colorectal liver metastasis (CRLM) also varies depending on the primary tumor's sidedness. Although differences such as MSI-H status, BRAF mutations, and histological types have been noted between right- and left-sided colorectal cancers, clinical observations have recognized that differences in treatment efficacy exist even when these factors are excluded. This study aimed to compare left- and right-sided colorectal cancers based on their responsiveness to anti-EGFR therapy and to elucidate differences in cancer cells and the tumor microenvironment (TME).
Methods: We collected microsatellite stable (MSS) and BRAF mutation-negative cases from six scRNA-seq cohorts, integrating data from 28 right-sided and 57 left-sided cases. Specifically, on the left side, only KRAS wild-type cases that respond to anti-EGFR inhibitors were included. Cancer cells were extracted from the integrated data, and differences in the therapeutic effects of anti-EGFR drugs were analyzed. Subsequently, focusing on cancer-associated fibroblasts (CAFs) that strongly interact with cancer cells, we analyzed differences in cell-cell communication within the TME.
Results
After extracting cancer cells and performing pseudobulk analysis and PCA, significant differences were observed between cancer cells of left-sided and right-sided colorectal cancers. Furthermore, evaluation of copy number variations (CNVs) using inferCNV revealed that left-sided colorectal cancers exhibit higher chromosomal instability (CIN). In analyses related to responsiveness to anti-EGFR therapy, it was confirmed that the expression of EREG (epiregulin), one of the EGFR ligands, was significantly higher in left-sided colorectal cancers. Additionally, focusing on cell-cell communication within the TME mediated by EREG, we found that only cancer cells of left-sided colorectal cancer interact with fibroblasts—especially inflammatory CAFs (iCAFs)—through the EGF pathway.
Conclusion: This study identifies key differences in tumor cell characteristics and the TME between RCRC and LCRC. The higher responsiveness of LCRC to anti-EGFR therapy may be attributed to intrinsic features such as elevated EREG expression and CIN, as well as enhanced interactions with fibroblasts via the EGF pathway. These findings provide insights into the mechanisms underlying the differential efficacy of anti-EGFR therapy based on tumor sidedness.

#6265 Cross-tissue atlas of human disease identifies tumor-specific components in tumor microenvironment.

S. Kim, S. Cheong, S. Lee, Y. Kim, J.-E. Park;
Korea Advanced Institute of Science and Technology, Daejeon, Korea, Republic of

Human diseases affect various cell types within the local niche, but the multicellular responses of human diseases have not been collectively assessed across organs. Here, we categorize multicellular responses of human diseases based on a remapped human cell atlas, incorporating 12 million single-cell transcriptome profiles acquired from an automated data collection pipeline. Additional curation expands the collection up to 46 million cells, covering 160 disease conditions across 49 distinct organ identities. From the re-aligned atlas of single-cell transcriptome data, we constructed a hierarchical cell classification framework comprising 32 major cell types and 165 cell subtype annotations. Systematic profiling of disease and organ association patterns of cellular subtype reveals archetypes of human diseases repeatedly found across organs, including a multicellular phenotype highly specific to the tumor microenvironment. The universal cell atlas could serve as a reference for unbiased deconvolution of spatial transcriptome datasets, revealing spatial distribution patterns associated with immunotherapy responses. Collectively, our study provides foundational resources for cross-tissue cell type annotation, presents a comprehensive multicellular overview of disease-associated responses across human tissues, and highlights multicellular responses in tumor microenvironments.

#6266 Spatial profiling of prognostic differences in the tumor microenvironment between primary and metastatic ovarian cancer using multiplexed protein imaging.

Raymond M. Moore[1], Brenna C. Novotny[1], Chathu L. Atherton[2], Matthew S. Block[3], Bryan M. McCauley[1], Stacey J. Winham[1], Ellen L. Goode[1], Ray Guo[4], Yajue Huang[5], Svetomir N. Markovic[6], Chen Wang[1]

[1]Department of Quantitative Health Sciences, Mayo Clinic, Rochester, MN,[2]Department of Oncology, Mayo Clinic, Rochester, MN,[3]Departments of Oncology and Immunology, Mayo Clinic, Rochester, MN,[4]Department of Laboratory Medicine and Pathology, Mayo Clinic, Jacksonville, FL,[5]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN,[6]5. Departments of Oncology, Immunology, and Internal Medicine, Mayo Clinic, Rochester, MN

Background: The tumor microenvironment (TME) plays a pivotal role in ovarian cancer (OC) progression and prognosis, while also contributing to biological differences between primary and metastatic tumors. This study examines OC TMEs to uncover associations with clinical prognosis and tumor biology by analyzing paired primary and metastatic tumors from the same patients.
Methods: This study focused on 57 stage-IIIC/IV OC patients treated at the Mayo Clinic, all of whom underwent surgical debulking and adjuvant therapies. All patients provided informed consent, and the study received IRB approval. A tissue microarray (TMA) was specifically designed and constructed, containing paired primary and metastatic tissue cores from the same patients. 147 tissue cores from the constructed TMA were profiled using a 35-antibody panel, with 17 antibodies for cell phenotyping and 18 for functional state characterization. Profiling was performed using Cell DIVE, a multiplexed imaging solution at the single-cell level developed by Leica Microsystems. After cell segmentations and classifications, detailed spatial niche and regional analyses were performed at the single-cell level. Immune cell populations, as well as their TME distributions, were characterized to conduct prognostic associations and contrast primary and metastasis differences.
Results: A total of 1,292,988 total cells (after excluding low-density signals and ambiguous classifications) were analyzed, with a median of 8,033 cells per core. Using spatial neighborhood analysis, 12 distinct spatial niches were identified with specific cell types and/or unique functional states. Among these, a macrophage- and fibroblast-enriched niche at the tumor-stroma interface was associated with improved overall survival (p=0.008). Another rare Treg-enriched niche, along with other immune cells, demonstrated protective effects, particularly in patients with residual macroscopic tumors after debulking surgery (p=0.05), while no significant effects were observed in those without visible residual disease (p=0.8). When comparing primary and metastatic tumors, metastatic lesions exhibited significantly increased tumor mass (p=2e-4) and a notable reduction in helper T cells (p=0.04) in tumor areas, as well as significantly increased fibroblast cells in tumor-stroma interfacing regions (p=0.002), reflecting distinct immunosuppressive and tumor-promoting shifts.
Conclusion: This study reveals spatial and cellular differences in primary and metastatic OC TMEs, distribution differences linked to prognosis and surgical outcomes. These findings emphasize the potential of spatially resolved TME profiling to refine adjuvant and neoadjuvant therapeutic strategies.

#6267 Training a machine learning model to predict T cell cytokine secretion function using a novel single-cell flow cytometry method that measures kinetic phenotypes.

Sheldon J.J. Kwok[1], Yulia Shulga[1], Emane Rose Assita[1], Sarah Forward[1], Trevor Brown[1], Pratip Chattopadhyay[2]

[1]LASE Innovation Inc., Waltham, MA,[2]Talon Biomarkers, Whippany, NJ

Advances in high-dimensional single-cell analysis have transformed our understanding of cellular heterogeneity and its role in complex pathologies. Most datasets are acquired through single-cell RNA sequencing, which provides expression of thousands of genes on each cell at a given timepoint. The typical workflow involves identifying potential new cell types via clustering, generation of hypotheses, and followed by post-hoc experiments to test these hypotheses. Technical bottlenecks include the sparse and noisy nature of sequencing data, and the inability to link observations directly to function due to the destructive nature of sequencing.

Supervised machine learning (ML) is an increasingly useful approach to generate powerful predictive models of complex processes. This approach requires both high quality and high quantity of training data. An ideal method to acquire this data would be able to follow individual cells over time while measuring changes in phenotype and function. Here, we describe a novel single-cell method using non-destructive optical barcodes that enables acquisition of dynamic high-dimensional data at scale. We apply this method to generate unprecedented predictive models of single-cell function using a human T-cell activation model.

Our barcoding approach relies on laser particles (Kwok et al., Nat. Biomed. Eng. 2024) to track individual cells over time. T-cells are barcoded, activated, and measured repeatedly over time using a flow cytometer to capture kinetic changes in expression of protein biomarkers (“kinetic phenotyping”). We link these kinetic phenotypes to cytokine secretion function through a stimulation step and cytokine secretion assay. The single-cell phenotype-to-function datasets are then used to train ML models to predict cytokine secretion prior to stimulation.

We observed increased expression of CD25, PD-1 and CD69 in T cells post-activation from 2h to 48h, as expected. However, distinct kinetic patterns were observed. For example, while CD25 increased largely monotonically, there was significant transient expression of PD-1. These observations were consistent across multiple donors. We employed supervised ML (Random Forrest) to train a classification model to predict cytokine secretion from single-cell kinetic phenotypes. The model was validated using 5-fold cross-validation and achieved ROC-AUC values of 0.74 to 0.89 for predicting TNFa, IFNg and IL2 secretion. For each cytokine, we identified the most predictive biomarkers (and timepoint). Finally, we used our classification model to describe T-cell heterogeneity by probability of cytokine secretion and validated our method using conventional analyses. Our approach is expected to be of great utility for predicting single-cell function in complex systems, from immunotherapy development to cancer pathogenesis.

#6268 JANUS: A unified deep learning framework for predicting peptide binding to MHC I and II alleles.

Alan Perez-Rathke[1], Justin Balko[1], Jens Meiler[2]

[1]Vanderbilt University Medical Center, Nashville, TN,[2]Vanderbilt University, Nashville, TN

The accurate prediction of peptide binding to major histocompatibility complex (MHC) molecules is crucial for advancing cancer immunotherapy, particularly in the design of personalized cancer vaccines and in identifying antigens which may be predictive of immunotherapy-related adverse events. Existing computational models for prediction of peptide binding are generally restricted to a single MHC class. We hypothesize that a unified model capable of predicting peptide binding to both MHC I or MHC II alleles can lead to a synergistic improvement in model performance and thereby facilitate advances in cancer neoantigen discovery. Challenges to this approach include finding a universal encoding for both MHC I and MHC II candidate peptide binders as well as addressing data imbalances arising from training with disparate datasets. Using publicly available datasets including from the Immune Epitope Database (IEDB), we developed a novel deep learning framework JANUS (Joint Allele-specific Neural prediction of MHC I/II-binding Universal Sequences) that leverages biological sequence embeddings from a protein large language model and adapts our loss function to resolve data imbalance issues. The model utilizes a feed-forward multi-layer perceptron with dropout layers for regularization. Ultimately, this model explores whether a unified approach demonstrates competitive performance compared to current single class models, particularly regarding increased predictive accuracy for rare or infrequent alleles, or in scenarios with sparse data.

#6269 Advanced single-cell RNA-seq reveals complex interactions between circulating tumor cells and immune subtypes in prostate cancer.

Han Hong Huynh[1], Tzu-Hao Chang[2], Shauh-Der Yeh[3], Chia-Ling Hsieh[4], Shian-Ying Sung[4]

[1]International Master Program for Translational Science, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan,[2]Graduate Institute of Biomedical Informatics, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan,[3]Taipei Cancer Center, Taipei Medical University Hospital, Taipei, Taiwan,[4]International Master and Ph.D. Program for Translational Science, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan

Background: Prostate cancer is marked by extensive cellular diversity within its tumor microenvironment (TME), significantly impacting disease progression and responses to treatment. The study aims to dissect this heterogeneity at the single-cell level, identify distinct subpopulations within key cell types such as circulating tumor cells (CTCs) and T lymphocytes, and uncover potential therapeutic targets.
Methods: We analyzed single-cell RNA-seq data from 16 prostate cancer samples, which included over 170,000 cells. Initially, we performed data integration and normalization using Reciprocal Principal Component Analysis (R-PCA) to mitigate batch effects and enhance data quality. For dimensionality reduction, both PCA and UMAP (Uniform Manifold Approximation and Projection) were applied, allowing us to visualize and identify patterns in the high-dimensional dataset. Clustering of cells was conducted using the k-means algorithm to categorize cells into distinct clusters based on their gene expression profiles. Finally, cell type classification within these clusters was achieved using Support Vector Machines (SVMs), enabling the precise identification of cellular identities and states based on their molecular signatures.
Results: Our comprehensive analysis unveiled a remarkable diversity among the circulating tumor cells (CTCs) within the Taiwanese prostate cancer tumor microenvironment. Specifically, we identified four distinct CTC clusters, each infused with different cellular components that suggest unique roles in tumor dynamics. CTC1 is comprised purely of epithelial cells, presenting a classical phenotype of circulating tumor cells, which underscores the conventional understanding of CTCs in cancer dissemination. Meanwhile, CTC2 is characterized by an infusion with N1 neutrophils, highlighting its potential role in promoting inflammatory responses that could affect tumor growth and patient prognosis. In contrast, CTC3 includes an infusion with N2 neutrophils, indicating a possible contribution to enhanced tumor progression and metastatic potential, thereby suggesting a target for therapeutic intervention to curb tumor spread. Lastly, CTC4 is infused with plasmacytoid dendritic cells, which are known for their role in immune suppression, thereby aiding the tumor in evading immune surveillance.
Conclusion: This study underscores the utility of single-cell technologies in uncovering the complex cellular interactions within prostate cancer. By detailing the unique profiles of CTC subclusters and their associations with specific immune cells, our research provides novel insights into the tumor microenvironment and identifies new targets for immunotherapy. Future research will focus on validating these markers and integrating broader genomic data to refine treatment approaches and enhance patient outcomes.

#6270 AI-derived tumor-infiltrating lymphocytes predicts risk of invasive breast cancer recurrence in ductal carcinoma in situ (DCIS).

Sara Ranjbar[1], Xiaoxi Pan[1], Karina Pinao[1], Caner Ercan[1], Roberto Salgado[2], Hugo M. Horlings[3], Allison Hall[4], Lorraine M. King[5], Carlo Maley[6], E Shelly Hwang[5], Yinyin Yuan[1], Simon Castillo[1], Pingjun Chen[1]

[1]Department of Translational Molecular Pathology, UT MD Anderson Cancer Center, Houston, TX,[2]Department of Pathology, ZAS Hospitals, Antwerp, Belgium,[3]Department of Pathology,, The Netherlands Cancer Institute, Amsterdam, Netherlands,[4]Department of Pathology and Laboratory Medicine, The University of British Columbia, Vancouver, BC, Canada,[5]Duke University School of Medicine, Durham, NC,[6]Center for Evolution and Medicine, Biodesign Institute and School of Life Sciences, Arizona State University, Tempe, AZ

Background: The abundance of tumor-infiltrating lymphocytes (TILs) has demonstrated prognostic value in breast cancer recurrence. However, manual TIL assessment is both time-consuming and prone to inter-observer variability. This study aimed to evaluate the performance of two AI-based TIL scoring pipelines, emphasizing the added value of incorporating tissue-based context insights for automated TILs scoring.

Methods: We compared two in-house AI-based TILs scoring pipelines using the Translational Breast Cancer Research Consortium DCIS cohort. The first pipeline employs single-cell classification to identify epithelial, lymphocyte, stromal, and other cell types, followed by spatial analysis of immune populations relative to epithelial cells through clustering (AI-TILs-cluster). The second pipeline builds on this foundation by integrating a tumor microenvironment segmentation model as context guidance to compute the proportion of lymphoid cells within the epithelium and stromal compartments (AI-TILs-seg). In a cohort of 45 retrospectively collected hematoxylin and eosin (HE)-stained whole-slide tissue sections from 38 patients with available pathologists manual TILs, we evaluated the correlation of both scores and the median manual TIL score across the pathologists. Additionally, we assessed the prognostic value of the AI-derived TILs in 232 patients (530 HE slides) with up to 228 follow-up months (median: 78 months) by examining the association of per-patient AI-TILs with time to invasive breast cancer recurrence. This analysis was conducted using a multivariate Cox Proportional Hazards model that included patient age, tumor grade, DCIS lesion size, and estrogen receptors (ER) and progesterone receptors (PR) status.

Results: AI-TILs-cluster was not significantly correlated with median pathologist TILs, AI-TILs-seg, however, demonstrated a moderate correlation (Spearman's rho = 0.43, p = 0.003). This finding was expected as the automatic scores were not designed to replicate the manual TILs. AI-TILs-seg was associated with an increased risk of invasive breast cancer (Hazard Ratio = 1.067, 95% Confidence Interval: 1.017-1.119, p= 0.008) independent of other variables. In comparison, the multivariate analysis including AI-TILs-cluster did not show significance in predicting the risk of invasive breast cancer.

Conclusion: These findings suggest that incorporating contextual tumor microenvironment segmentation can enhance AI-derived TIL scoring, leading to better alignment with pathologist assessments and improved clinical relevance. Future work will focus on validating this scoring approach in larger cohorts and improving correlation with pathologist TILs.

#6271 Interactions between CD8⁺ T cells and tumor cells decode tumor heterogeneity and provide treatment recommendations for colorectal cancer.

Hongyu Huang[1], Zize Zhao[1], Fan Huang[1], Luoxinyi Yang[1], Shichu He[1], Boya Mu[1], Xiaoyue Liu[1], Ruixi Li[1], Yaqi Guan[1], Jiahao Li[1], Chen Gu[1], Jing Xu[2], Na Li[1]

[1]Nankai University School of Medicine, Tianjin, China,[2]Department of Colorectal Surgery, Tianjin Union Medical Centre, Tianjin, China

Introduction: Individual heterogeneity in colorectal cancer (CRC) remains a major challenge in therapy optimization, despite diagnostic and treatment advances. CD8⁺ T cells, pivotal players in anti-tumor immunity, display diverse functional subtypes shaped by interactions within the tumor microenvironment (TME). However, the specific role of CD8⁺ T cells in mediating heterogeneity through interacting with CRC cells, as well as their influence on the immune response of CRC, remains poorly understood. Here, we employ multi-transcriptomic analyses to discover the factors underlying patient heterogeneity based on interactions between various CD8⁺ T cell subtypes and CRC cells and develop effective, personalized therapeutic strategies.

Methods & Results: Data analysis of CRC patients was conducted by harmonizing information from reputable public databases. Single-cell analysis was used to investigate the functionality and evolutionary trajectories of various CD8⁺ T cell subgroups. Cellular interactions and spatial omics analyses were employed to examine the receptor-ligand pairs between CD8⁺ T cell subtypes and CRC cells. Based on TCGA-CRC data, patients were classified into three prognostically distinct clusters through consensus clustering of the receptor-ligand pairs. Among them, cluster 2 (C2) was the most crucial one with poorest prognosis. TME patterns were predicted with multiple algorithms, and immunotherapy responses were evaluated using the TIDE and EaSleR analysis. Notably, C2 was significantly associated with exhausted CD8⁺ T cells and Tregs, with the lowest response to immunotherapy. High-activity transcription factors for each cluster were predicted using the univariate linear model, while hub genes were identified via WGCNA. Furthermore, functional insights into each cluster were derived from GO and KEGG enrichment analyses. We found that C2 exhibited up-regulated SMADs and SP1, signifying high immunosuppressive potential. Prognostic risk models were built utilizing multivariate Cox regression and LASSO analysis, identifying a predictive model for C2 including GPT, GPX3, etc., with robustness validated. Finally, using the oncoPredict algorithm, we predicted chemotherapy drug sensitivity and discovered that C2 showed a superior response to dasatinib and AZ960, while exhibiting resistance to ulixertinib.

Conclusions: This study provides critical insights into the complex interactions between CD8⁺ T cells and CRC cells, highlighting their pivotal role in tumor heterogeneity. Leveraging these findings, we developed a novel classification system for CRC patients that captured TME heterogeneity and stratified patients into distinct groups. This system lays a foundation for personalized therapeutic strategies based on tumor-immune dynamics, offering significant potential to improve prognosis for CRC patients.

#6273 AI-based serum proteomics models predict survival outcomes in NSCLC patients undergoing immunotherapy.

Junho Song[1], Leeseul Kim[2], Donghoon Shin[3], Jongwoo Kim[1], Taegyu Um[1], Allen Cho[4], Jaeyoung Bae[1], **Liam IL-YOUNG Chung**[1], Jeeyeon Lee[5], Young Kwang Chae[1]

[1]Northwestern University, Chicago, IL,[2]University of Chicago, Chicago, IL,[3]Metrowest Medical Center, Framingham, MA,[4]Weiss Memorial Hospital, Chicago, IL,[5]Kyungpook National University, Daegu, Korea, Republic of

Background: Clinical responses to immune checkpoint inhibitors (ICIs) are highly variable. Pre-treatment serum proteomic biomarkers offer a promising tool to predict response outcomes by capturing the biological mechanisms underlying response variability.
Methods: This retrospective cohort study analyzed pre-treatment serum samples from 158 consecutive locally advanced or metastatic (palliative intent treatment) NSCLC patients treated with ICI monotherapy or ICI-chemotherapy combination. Deep proteomic profiling via mass spectrometry identified protein interaction patterns, and three machine learning models—XGBoost, logistic regression, and multilayer perceptron (MLP)—were evaluated using 5-fold cross-validation. The primary outcomes were progression-free survival (PFS) and overall survival (OS) at multiple time points (3, 6, 9, 12, 36, and 60 months), with performance assessed using the area under the receiver operating characteristic curve (AUC).
Results: The study group had a median age of 70.8 years, with 51.9% female and 67.7% with adenocarcinoma. Smoking history showed 15.8% never smoked, 69.6% were former smokers, and 13.9% were current smokers. Performance status (PS) was 0 in 19.0%, 1 in 54.4%, 2 in 19.6%, and 3 in 5.7%. The median tumor mutational burden (TMB) was 5.3 mut/Mb, with PD-L1 <1% in 31.6%, >1% in 55.7%. Treatment regimens included mono-ICI (38.0%), dual ICI (13.9), and ICI + chemotherapy (48.1%). Median OS was 19.5 months, PFS was 6.6 months. Proteomic-based models showed varying performance across outcomes and time points. For PFS, XGBoost achieved the highest performance at 90 days (AUC=0.67), maintaining moderate prediction at 270 days (AUC=0.65) and 1 year (AUC=0.65). For OS, performance improved over time, with the best results at 1 year (AUC=0.61). Using 4,000 highly variable proteomic mass spectrometry features, risk stratification at 1 year OS showed significant differences between groups (log-rank p=0.05) in PFS, with median PFS of 187 and 283 days for high- and low-risk groups, respectively (HR=1.38, 95% CI: 0.95-2.02, log-rank p=0.09).
Conclusion: Serum proteomics-based AI model can predict PFS and OS in NSCLC patients treated with ICIs, achieving AUCs up to 0.67 for PFS and 0.61 for OS. Larger studies are needed to validate our findings.

#6274 Establishment of a quantitative systems pharmacology platform for syngeneic tumor mouse models: Application in immuno-oncology drug development.

T. Nakayama, A. Kikuchi, K. Toshimoto, H. Sayama, T. Nakazawa, M. Oishi;
Astellas Pharma Inc., Ibaraki, Japan

[Introduction] Quantitative Systems Pharmacology (QSP) modeling is a promising technique for model-informed drug discovery and development, and various QSP models for immuno-oncology (IO) have been published. Syngeneic tumor mice are often used for in vivo pharmacology study, and many kinds of IO QSP models have been reported to understand in vivo data and make prediction. However, published QSP models have varying structures across tumor types that makes it difficult to analyze data across different syngeneic tumor models. In addition, there are few QSP models calibrated by actual data of tumor infiltrating lymphocyte (TIL) dynamics. In this study, we present platform IO QSP modeling for syngeneic tumor mice (MC38, B16F10, CT26, 4T1 and LLC1) with a unified structure based on observed data of TIL dynamics and antitumor efficacy of anti-programmed cell death-1 (anti-PD-1) treatment.
[Methods] (Mouse study for TIL dynamics) Five mouse tumors were inoculated into C57BL/6 (MC38, B16F10, LLC1) or BALB/c (CT26, 4T1). Tumors were sampled at three time points (mean tumor volume was about 50 mm^3, 300-700 mm^3 and 500-2000 mm^3) and immune cells in the tumors were analyzed by flow cytometry. (IO QSP platform development) The structure of IO QSP platform was based on a published QSP model for CT26-bearing mice [1] and modified with reference to a comprehensive IO QSP model for breast cancer in human [2] to improve physiological interpretability of model components. The TIL dynamics data and published anti-mouse PD-1 (anti-mPD-1) antibody efficacy data for syngeneic tumor mice [3] were used for model calibration. The platform model was validated by confirming predictability of combination therapy of anti-mPD-1 antibody with a multiple kinase inhibitor (lenvatinib) for syngeneic tumor mice.
[Results] The IO QSP platform model contains 12 tumor-specific parameters for each tumor type of syngeneic mice and successfully captured the observed TIL dynamics and antitumor effect of anti-mPD-1 antibody treatment. Mechanism of action of lenvatinib was incorporated into the IO QSP platform and calibrated with published data. The final model was successfully validated by comparing simulation and observation of combination therapy of anti-mPD-1 antibody with lenvatinib.
[Conclusions] The IO QSP platform was established for several types of syngeneic tumor mice, which captured TIL dynamics and antitumor efficacy of anti-mPD-1 antibody. This platform model enables us to test a hypothesis by incorporating candidate compounds, to support study design with a translational biomarker and to investigate combination strategies, thus having the potential to facilitate new drug development. [References] [1] Kosinsky Y, et al. J Immunother Cancer. 2018;6(1):17. [2] Wang H, et al. Front Bioeng Biotechnol. 2020;8:141. [3] Georgiev P, et al. Mol Cancer Ther. 2022;21(3):427-439.

#6275 Leveraging the tumor microenvironment (TME) atlas to identify the best PDX-model for pre-clinical testing of TME targeting agents.

Mara Gilardi, Gilad Silberberg, Kurtis Cruz, Stefano Cairo, Marianna Zipeto, Michael Ritchie

Champions Oncology, Inc., Hackensack, NJ

Tumor-infiltrating immune cells play a critical role in tumor progression, metastasis, and resistance to anti-cancer therapies. This is particularly evident in the context of tumor evasion from immune surveillance, which is driven by tumor microenvironment (TME) modulation, ultimately impairing therapeutic efficacy. Consequently, accurately predicting the composition of immune populations within the TME holds great promise for uncovering the complex interplay between immune cells and tumors, as well as for the selection of the optimal pre-clinical model to test new TME-targeted therapeutics. The relevance of experimental settings in preclinical immunotherapy studies is often undermined by the lack of comprehensive TME characterization in the cancer models selected for pre-clinical testing. To address this gap, Champions Oncology generated the TME Atlas. Champions Oncology's preclinical program is built on Patient-Derived Xenograft (PDX), a highly accurate and clinically relevant platform for preclinical research. The PDX bank encloses 1500 models, characterized with transcriptomic, proteomic, and patient treatment history data to guide the model's selection mimicking the future clinical patient cohorts. On top of this, the TME Atlas will provide an additional layer of characterization to refine the model selection based on TME composition, including immune cells and fibroblasts. The TME Atlas derived from the transcriptomic data from Champions Oncology PDX bank and enabled the prediction of TME composition patterns in each specific model. To profile the TME, we applied different computational methods to Champions Oncology's proprietary datasets. The computational tool xCell was identified as the optimal strategy to perform cell-type enrichment analysis for 64 immune and stromal cell types. The implementation of xCell enabled comprehensive profiling of immune infiltration across the entire PDX bank. This detailed characterization of the TME unlocks the selection of optimal models and experimental settings to test TME-engaging drugs. Histological analyses further validated the computational findings enclosed in the TME Atlas. To validate the computational data, we engrafted selected PDX models in humanized mice and assessed TME composition in tumors through Immunohistochemistry (IHC). IHC analyses validated the profile predicted within Champions Oncology TME Atlas. This immune atlas enables the selection of the most suitable PDX models, and humanized hosts to optimize preclinical studies targeting immune and stromal components of the TME. This approach ensures clinically translatable results, that will advance the development of innovative TME targeting strategies.

#6276 Comparative analysis and integration of image-based and high-resolution sequence-based spatial transcriptomics platforms for tumor microenvironment analysis.

Dongjoo Lee, Yeonjae Jung, Jae Eun Lee, Myunghyun Lim, Daeseung Lee, Kwon Joong Na, Hongyoon Choi

Portrai, Inc., Seoul, Korea, Republic of

Background: Advances in spatial transcriptomics (ST) enable precise gene expression analysis within the tumor microenvironment (TME). VisiumHD and Xenium Prime 5K are cutting-edge platforms offering unique strengths in spatial resolution, sensitivity, and multiplexity. This study evaluates their performance metrics in heterogeneous tumor tissues from the same slide, focusing on technical performance, spatial resolution, and cell type identification. Additionally, post-Xenium VisiumHD datasets were generated and integrated to harness the complementary strengths of both platforms. We aimed to investigate a head-to-head comparison in spatially registered ST to provide guidance for researchers in selecting the appropriate platform for specific cancer research objectives.
Methods: ST data were analyzed from tumor samples processed with Xenium Prime 5K followed by VisiumHD. Evaluations included transcript capture efficiency, gene detection sensitivity, and signal-to-noise ratios. Spatial resolution was assessed using cell- and transcript-level metrics, gene expression correlations, and spatial mapping accuracy. Cell type identification focused on assignment accuracy and rare population detection. Custom workflows integrated post-Xenium VisiumHD datasets, combining platform strengths for detailed TME analysis.
Results: Gene expression correlations were highly consistent across platforms. VisiumHD provided superior transcriptome-wide coverage (~18,085 genes), making it ideal for exploratory studies, while Xenium offered 2-3x greater per-gene sensitivity for detecting low-abundance transcripts. Xenium Prime 5K excelled in spatial resolution, with precise cell-level annotations, whereas VisiumHD mapped broader spatial patterns via deconvolution. Integration of Xenium cell segmentation with VisiumHD enabled single-cell transcript analysis with comprehensive gene expression data. This approach improved rare cell population detection and overcame limitations of individual platforms.
Conclusions: VisiumHD is ideal for transcriptome-wide exploratory studies, while Xenium Prime 5K excels in high-resolution, focused cellular analyses. Cross-platform integration bridges gaps between resolution and coverage, enabling robust analysis of complex TMEs. This study provides critical guidance for platform selection and highlights the potential of integrated approaches to advance cancer research through spatial transcriptomics.

#6277 A time-series mouse model identifies an early post-treatment effective liquid biomarker of ICB response in human head and neck cancer.

B. Wang[1], R. Saddawi-konefka[2], L. M. Clubb[3], S. Tang[4], D. Wu[5], S. Mukherjee[1], S. Sahni[1], S. R. Dhruba[1], K. Wang[5], J. Gutkind[3], E. Ruppin[1];
[1]NIH-NCI, Bethesda, MD, [2]UC San Diego School of Medicine, San Diego, CA, [3]UC San Diego, La Jolla, CA, [4]Stanford University, San Jose, CA, [5]University of Illinois Urbana-Champaign, Urbana, IL

Background & Approach: Immune checkpoint blockade (ICB) therapy has shown promise for head and neck squamous cell carcinoma (HNSCC); however, response rates remain limited, with only a subset of patients exhibiting significant clinical benefits. Identifying non-invasive liquid biomarkers to predict ICB response is crucial for optimizing patient selection and treatment strategies. In this study, we generate and analyze time-series RNA and T-cell receptor (TCR) sequencing data from blood in a murine HNSCC model treated with anti-PD-1 therapy to identify potential liquid biomarkers predictive of ICB response
Results: Our analysis reveals significant differences in the clonal expansion and temporal dynamics of effector T cells (Teff) and B cells between responders and non-responders, indicating distinct immune profiles associated with therapeutic outcomes. Notably, these immunological changes are most prominent at early post-treatment timepoints, suggesting that this period is optimal for assessing ICB efficacy and for the potential
identification of predictive biomarkers. Importantly, the Teff and B cell transcriptomic gene signatures identified at these early post-treatment timepoints predict ICB treatment responses in HNSCC patient cohorts without the need for additional training, underscoring their potential clinical utility. Further time-series ligand-receptor interaction analyses uncover potential immune cell communication pathways that may mediate ICB outcomes, offering insights into mechanisms underlying ICB resistance.
Conclusions: Our findings highlight the importance of early post-treatment timepoints for assessing ICB response and suggest blood-based Teff and B cell signatures as effective biomarkers for predicting ICB treatment outcomes in HNSCC patients. This study provides a potential framework for translating findings from murine models to human clinical practice, facilitating the development of personalized immunotherapeutic strategies.

#6278 Reprogramming of the tumor microenvironment via lipid-associated macrophage subtypes after neoadjuvant osimertinib treatment in *EGFR*-mutantnon-small cell lung cancer.

Su-Jin Choi, Jii Bum Lee, Hyo Sup Shim, Byung Jo Park, Chang Young Lee, Min Hee Hong, Byoung Chul Cho, Sun Min Lim

Yonsei University College of Medicine, Seoul, Korea, Republic of

Introduction: Despite treatment with adjuvant osimertinib, some patients experience relapse. NORA is a trial designed to identify persistent drug resistance (DTP) to neoadjuvant osimertinib in resectable non-small cell lung cancer (NSCLC) with *EGFR* mutations (NCT04816838). We aimed to identify tumor-surrounding cellular characteristics that change the tumor microenvironment (TME) after neoadjuvant osimertinib for surgically resected *EGFR*-mutant NSCLC.
Methods: Neoadjuvant osimertinib was given daily for two 28-day cycles followed by surgical resection and adjuvant osimertinib for 3 years. We performed single-cell RNA sequencing (scRNA-seq) on samples from 25 patients with *EGFR* mutations (Exon 19 deletion, N=10; L858R, N=15) before (N=18) and after (N=24) osimertinib treatment. The analysis was based on the Scanpy package in python, and the interaction was performed with Liana. We divided the pathological response according to major pathological response (MPR, N=6) and non-MPR (N=19).
Results: After 8 major clustering from total cells, 60 subclusters were divided. Among them, myeloid cells were classified into 14 clusters. We found 3 macrophage subclusters (MAC1-SELENOP, MAC3-SPP1, and MAC7-PLXDC2) that were significantly increased after treatment with neoadjuvant osimertinib. Macrophage polarization status using the signature scoring method showed that the M2 phenotype was the strongest in the MAC1-SELENOP subcluster, while the M0 signature was high in MAC3-SPP1, and the M1 signature was high in MAC7-PLXDC2. These three macrophage subclusters showed enrichment in lipid metabolism compared to those of pre-neoadjuvant samples. In addition, the MAC1-SELENOP subcluster was significantly increased in non-MPR patients compared to MPR patients. This finding is paramount since MAC1-SELENOP may play a role in modifying the TME through various interactions with epithelial cells with DTP signature.
Conclusion: Among several subclusters, three macrophage clusters enriched in the post-osimertinib samples were associated with lipid metabolism. In particular, the MAC1-SELENOP cluster with a tumor-associated macrophages (TAMs) phenotype had multiple interactions with tumors. This may lead to reprogramming of cancer cells to survive in TMEs. These finding may be applied to identifying DTPs and discovering potential therapeutic applications.

#6280 Exploring the dynamics of plasma proteins and their effect on outcomes in patients with bladder and lung cancer.

Randi Istrup Juul[1], Asbjorn Kjar[1], Nanna Kristjansdottir[1], Iver Nordentoft[2], Lars Dyrskjot[1], Nicolai J. Birkbak[1]

[1]Department of Clinical Medicine, Aarhus University, Aarhus, Denmark,[2]Department of Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark

The immune system's state and composition affects the outcome for patients with cancer. Here we investigated the dynamics of immune-related proteins in relation to treatment or recurrence in muscle-invasive bladder cancer (MIBC) and non-small cell lung cancer (NSCLC).
Using the Olink Proteomics Explore 1536 panel, we measured the level of 1536 proteins across multiple time points (2-6) for 25 patients with MIBC. Additionally, we obtained public data from the TRACERx cohort, where 151 patients with NSCLC had been profiled using the Olink Explore 3072 panel, measuring 3072 proteins at two time points. We investigated the association of the protein levels with immune-related parameters (T cell receptor diversity and relative T cell fraction) and patient outcome using mixed effects models. Significant proteins were further investigated in pathway enrichment analyses using both the Hallmark and Reactome pathways, and selected proteins were investigated individually. Cox Proportional Hazard Ratio was used to evaluate the proteins' association with survival.
We found 70 proteins that were significantly associated with metastatic disease in MIBC with enrichment of proteins in immune-related pathways. Seven proteins were associated with T cell receptor diversity and 19 were associated with the T cell fraction, which were enriched in proliferation pathways. In NSCLC we found 66 proteins that were associated with the T cell receptor diversity, and these were enriched among immune regulation pathways and pathways related to NOTCH signaling and cell-cell communication. Only six proteins were associated with the T cell fraction in the patients with NSCLC, and these were enriched in immune-related pathways. In the NSCLC cohort, we found 400 proteins that were negatively associated with overall survival and 86 proteins that were associated with improved overall survival. Among the 400 proteins, we observed an enrichment of proteins related to the innate immune system.
In conclusion, we find proteins that are associated with outcome or immune-related measures in both cohorts, and combined these proteins are enriched across pathways associated with the immune system, proliferation, or cell-cell communication, all pathways that have a role in cancer development.

#6282 Age-related shifts in the immune system revealed from single-cell RNA-seq using non-negative matrix factorization.

Ragnhild Laursen[1], David Martin-Pestana[2], Nicolai J. Birkbak[1]

[1]Clinical Medicine, Aarhus University, Arhus, Denmark,[2]Bioinformatics Research Centre, Aarhus University, Arhus, Denmark

Unraveling the molecular mechanisms driving aging and identifying potential therapeutic targets for age-related diseases are essential to increase healthspan. The aging immune system undergoes significant alterations, contributing to immune dysfunction and increased susceptibility to diseases. Identifying gene programs associated with immune aging is key to understanding these changes and developing strategies to counteract their effects.
In this study, we leverage non-negative matrix factorization (NMF), an interpretable and powerful dimensionality reduction technique, to identify gene expression programs in the immune system that shift with age. We analyze single-cell RNA sequencing (scRNA-seq) data from the large OneK1K cohort, which includes 981 participants aged 19 to 97 years and encompasses 1.27 million peripheral blood mononuclear cells.
We reveal two distinct age-related transitions in the immune system composition and function occurring with an average peak around 39.3 and 71.8 years of age. These transitions are characterized by the activation or suppression of particular gene programs, reflecting changes in immune cell types, their interactions, and signaling pathways.
Our findings provide a clearer understanding of the molecular underpinnings of immune aging by uncovering gene programs associated with it. These potential biomarkers of immune aging could enhance therapeutic strategies aimed at rejuvenating immune function in aging populations.

#6283 Performance evaluation of tumor microenvironment deconvolution and gene prediction methods in lung cancer.

F. Tang, D. Lee, L. Ji, J. Wu;
Novartis Biomedical Research, Cambridge, MA

The tumor microenvironment (TME) is complex and plays a key role in cancer development, progression and metastasis. Two layers of information are critical for understanding tumor compositions (1) the proportion of each cell type (2) the level of gene expression in each cell type.
Many single-cell profile-assisted algorithms have been developed to infer cell type proportions from bulk RNA-seq data, including CIBERSORTx, MuSic and Scaden, and there are several benchmark studies published to compare the methods. However, most of the deconvolution methods do not support prediction of gene expression in a heterogenous population of tumor cells, and there's no published study systemically comparing the gene expression prediction performance of the methods.
To overcome the limitations, we simulate large-scale of pseudobulk data using scRNA-seq profiling from lung tumors, representing various levels of tumor purities and cell lineages, to compare the cell fraction and gene expression prediction performance of recently published TME deconvolution methods. Our results indicate BayesPrism is the best at predicting gene expression in each of the cell types, and differential gene expression between two groups across cell types, especially in tumor epithelial cells, with higher balanced accuracy and correlation with ground truth. The inclusion of normal epithelial cells in the reference could increase additional risks for tumor deconvolution.
Altogether we evaluate both cell type proportion and gene expression prediction performance of recently published methods and offer a guideline for future benchmarking work. Currently we're working on the downstream application of inferring cellular composition and gene expression in large cohorts of bulk RNA-seq data, allowing for more insights into tumor-intrinsic expression and tumor-microenvironment interactions.

BIOINFORMATICS / COMPUTATIONAL BIOLOGY / SYSTEMS BIOLOGY / CONVERGENT SCIENCE: New Software Tools and Machine Learning Methods
Poster Session

#6287 Bulk transcriptomic analysis with InMoose, the Integrated Multi-Omic Open-Source Environment in Python.

Maximilien Colange, Guillaume Appe, Lea Meunier, Solene Weill, Akpeli Nordor, Abdelkader Behdenna

Epigene Labs, Paris, France

We introduce InMoose, an open-source Python environment for omic data analysis. Due to its wide adoption, Python has grown as a de facto standard in fields increasingly important for bioinformatic pipelines, such as data science, machine learning, or AI. As a general-purpose language, Python is also recognized for its versatility and scalability. InMoose aims at bringing state-of-the-art tools, historically written in R, to the Python ecosystem. Our intent is to provide a drop-in replacement for R tools, so our approach focuses on the faithfulness to the original tools outcomes.
The first development phase has focused on bulk transcriptomic data, with current capabilities encompassing data simulation, batch effect correction, and differential analysis and meta-analysis. InMoose offers a Python implementation of several state-of-the-art tools originally written in R:• ComBat and ComBat-Seq (batch effect correction)• edgeR, DESeq2, limma (differential gene expression analysis)• splatter (RNA-Seq data simulation)
To our knowledge, InMoose is the sole Python implementation of ComBat-Seq, edgeR and limma. InMoose also offers original features:• a quality control report for cohorts built through the batch effect correction features;• a differential gene expression meta-analysis module.
We present the range of capabilities of InMoose, illustrating them with an example workflow. We also compare InMoose with the R original implementations and alternative Python implementations when available. Our experiments show that the results of InMoose are very similar, if not identical, to those of the original R tools. This positions InMoose as a key tool to bridge R and Python ecosystem and to ensure reproducibility and comparability between R-based and Python-based bioinformatics pipelines.
We put the emphasis on making InMoose easy-to-use and open source to reach as many bioinformaticians as possible. Since the first version of InMoose has been made public, multiple users shared their experience with us, and even contributed code enhancements, which led us to update our software and plan to release more bioinformatics tools on Python in the future.
Python has grown as the language of choice for machine learning and AI, and streamlining ML and AI-powered pipelines for biomolecular data has become an essential step to unlock the full potential of biomolecular analysis. Single-cell omic data is well connected to AI/ML tools through scanpy and the scverse ecosystem, but no similar initiative exists for bulk transcriptomic data. Omicverse aggregates existing Python tools into a single-entrypoint, but has not endeavored to port R capacities to the Python world. InMoose addresses this gap, which hinders the smooth integration of bulk transcriptomic data with ML and AI tools.

#6288 Precise characterization of complex repeats in cancer genomes.

Khi Pin Chua[1], Egor Dolzhenko[1], Zev N. Kronenberg[1], Seiya Imoto[2], Seiichi Mori[3], Michael A. Eberle[1]

[1]PacBio, Menlo Park, CA,[2]Institute of Medical Sciences, University of Tokyo, Tokyo, Japan,[3]Genome Center, Japan Foundation for Cancer Research, Tokyo, Japan

The characterization of somatic variation, especially in complex genomic regions, is crucial for understanding the molecular drivers of cancer progression. Accurate PacBio long-read sequencing (HiFi) enables detection of all variant classes, from simple SNVs and INDELs up to complex structural variation, tandem repeats, and changes in epigenetic signatures. Complex and repetitive regions, while fully sequenced by HiFi reads, remain bioinformatically challenging, requiring tailored solutions. Here, we describe new tools to genotype understudied repetitive regions in cancer genomes, a task that has historically posed significant challenges to short-read sequencing.Our software tool, TRGT, resolves the sequence and methylation patterns of complex repetitive regions at a single-nucleotide resolution. A recent algorithmic improvement (vclust module) enables us to type expanded hyper-polymorphic regions, termed variation clusters. Variation clusters usually result in many incorrect variant calls in computational pipelines. By analyzing a variation cluster as a single region, rather than many small variants, we can accurately measure genetic variation. To demonstrate the importance of variation clusters in cancer, we comprehensively resolved repeat regions in five cell lines and 50 clinical tumor samples (with matched healthy tissue). We identified 5,500 new variation clusters across the tumors and an average of 46,220 (out of 4 million, 1%) loci that are contracted or expanded in a tumor compared to its matched normal tissue. We find 3% of these loci can be located within genes found in the Compendium of Cancer Genes, suggesting potentially oncogenic significance. In addition, using TRGT, we were able to detect microsatellite instability (MSI) in a tumor with a compound heterozygous coding mutation in MSH2. Lastly, we further analyzed haplotypes in selected variation clusters to detect and resolve sequences in somatic mosaicism. By incorporating this new class of variants—complex repeats—into our analysis, we significantly enhanced the capability of HiFi reads to detect a comprehensive spectrum of genomic variations. The inclusion of complex repeat genotyping with PacBio HiFi reads will enable a more detailed understanding of cancer genetics and open new avenues for research and clinical applications.

#6289 SCplot: an interactive platform for single-cell RNA-seq analysis in colorectal cancer.

William Kothalawala, **Balazs Gyorffy**

Semmelweis University, Budapest, Hungary

Background: Single-cell RNA sequencing (scRNA-seq) is a powerful tool for detailed characterization of the processes driving cancer and the interactions between different cell types in neoplastic tissues. However, the large volume of resulting data and the complexity of downstream analytic software and statistical approaches can make it challenging for researchers and clinicians without bioinformatics expertise to extract meaningful insights. To address this need, we developed SCplot, an integrated database and interactive online analysis platform for single-cell colorectal cancer (CRC) RNA-seq data.
Methods: SCplot allows users without prior programming experience to explore an integrated single-cell dataset. We compiled eight publicly available raw scRNA-seq CRC datasets from GEO, performed quality control and normalization, combined the datasets, and annotated the cell types. The complete database includes among others 102,195 cancer cells, 19,942 vascular endothelial cells, 25,027 cancer associated fibroblasts, and 7,438 smooth muscle cells. The platform available at www.scplot.com enables downstream analysis features, including differential gene expression, volcano plots, expression heatmaps, and gene-gene correlation analyses across various cell types.
Results: To demonstrate SCplot's utility, we investigated correlations of VEGFA between cancer cells and vascular endothelial cells, cancer-associated fibroblasts, and smooth muscle cells. Notably, the transcription factor JUND was among the top five positively correlated genes to VEGFA in cancer cells in each of these cell types (Pearson's correlation coefficient: 0.27-0.37, $p<0.001$). Other strongly positively correlated genes included those involved in cell signaling (*TCF4*, *CALCRL*, *GNAS*) and angiogenesis (*MCAM*, *COL4A1*, *SOX18*). Conversely, negatively correlated genes were associated with immunoregulation (*B2G*, *FKBP2*), iron metabolism (*FTL*, *FTH1*), apoptosis (*SOD1*), and cell differentiation (*PPDPF*).
Conclusions: These findings provide new insights into VEGF-driven interactions in CRC, revealing potential therapeutic targets to modulate the tumor microenvironment and disrupt angiogenesis. The SCplot platform can be mined to uncover, visualize, and validate cellular interactions in CRC.

#6290 sc2DAT: workflow for targeting tumor subpopulations of single cells.

Giacomo B. Marino, Nasheath Ahmed, Daniel J. B. Clarke, Avi Ma'ayan

Pharmacological Science, Icahn School of Medicine at Mount Sinai, New York, NY

Single-cell RNA sequencing (scRNA-seq) provides enhanced insights into the cellular composition of human tissues. In cancer, normal proportions of single cells are aberrantly dysregulated, and thus targeting subpopulations of cells within a tumor might be more effective than targeting all cells of a tumor. Here we present Single Cells to Drugs And Targets (sc2DAT), a unique workflow for the analysis of bulk and single cell RNA-seq datasets collected from tumors of the Clinical Proteomic Tumor Analysis Consortium (CPTAC). sc2DAT can take in as input single cell or bulk RNA profiles from one or two conditions. When processing scRNA-seq samples, sc2DAT first clusters the single cell vectors of expression to identify cell type subpopulations. It then bulkifies the vectors of expression in each cluster of single cells. For bulk RNA-seq, sc2DAT applies deconvolution with BayesPrism to infer cell-type-specific expression profiles for each identified subpopulation within a tumor. The sc2DAT platform currently has over 20 precomputed single-cell reference datasets derived from human and mouse studies. The expression vectors in each cluster of single cells are analyzed to discover genes that give rise to cell-surface proteins that are highly expressed in the tumor's subpopulation, but lowly expressed across all human healthy cell types and tissues. These identified cell-surface proteins can become immunotherapy targets for selectively elimination of subpopulation of single cells within a tumor. In addition, sc2DAT integrates the LINCS L1000 perturbational gene expression dataset to predict drugs and preclinical compounds that could induce a healthy phenotype in subpopulations of single cells within a tumor. Once datasets are uploaded to sc2DAT, the results are presented in a comprehensive report with an abstract, introduction, methods, results, conclusions, and references sections. The reports have many figures and tables, and it can be exported and shared in several formats including a PDF file. We applied sc2DAT to several CPTAC3 and CPTAC4 datasets to identify cell-type-specific subpopulation targets and reverser compounds, uncovering many potential personalized therapeutic strategies for immunotherapy and small-molecule interventions. sc2DAT is available from: https://sc2dat.maayanlab.cloud.

#6291 CellPyAbility: an integrated toolkit for high-throughput cell viability assays and automated analysis.

James L. Elia[1], Sam L. Friedman[2], Ranjit S. Bindra[2]

[1]Pathology, Yale University, New Haven, CT,[2]Therapeutic Radiology, Yale University, New Haven, CT

Assessing cell viability and proliferation in response to chemical gradients is fundamental to cancer biology research. We provide a cell viability “toolkit” that includes detailed protocols for high-throughput cell viability assays in response to a single agent or two agents in combination at varying concentrations with several advantages over other methods. Additionally, we developed automated software (CellPyAbility) that seamlessly integrates with the provided protocols, outputting detailed tabular and graphical analyses of experiments from bulk raw images. This combined approach significantly lowers the barriers to implementing cell viability assays by simplifying experimental setup and accelerating data analysis.

Our lab often uses short-term, nuclei-based growth delay assays (GDAs) to assess the selectivity of drugs against specific genetic alterations found in tumors. A GDA is conducted using a single 96-well plate with the innermost 60 wells containing two cell lines, a vehicle control, and a log-based gradient of nine concentrations in technical triplicate. Nuclei counting provides several advantages over other common methods. Compared to the commonly used methylthiazol tetrazolium (MTT; reduction-based) or CellTiter-Glo (ATP-based) assays, GDAs provide single-cell resolution of survival; are insensitive to metabolic variability within a cell or between cell lines; are compatible with redox-altering drugs; require simpler methodology; and could be used on live cells using non-toxic nuclear dyes like Hoechst. A disadvantage of the GDA is the computational and temporal cost of the required image analysis. We present CellPyAbility, which rapidly generates dose-response metrics and publication-ready graphics from a folder of unedited, whole-well GDA images in approximately one minute on commodity hardware.

Similarly, a researcher may want to assess how two drugs act in combination with one another across a wide range of concentrations. Our lab uses modified GDAs with simultaneous vertical and horizontal drug gradients, referred to as synergy assays. Synergy assays use three 96-well plates to assess 59 unique drug concentration combinations and a vehicle control in technical triplicate. CellPyAbility includes a synergy analysis module which generates dose-response metrics for each condition and a 3D surface map of cell viability and Bliss independence, a mathematical model for synergy in cellular systems, in approximately three minutes on commodity hardware. By making these tools open-access, we aim to empower cancer researchers with a streamlined, user-friendly platform for the rapid assessment of single- and dual-agent therapies in cell line models.

#6292 Highly sensitive detection of circulating-tumor DNA in plasma using whole genome sequencing.

Sven Bilke, Alex Buerkle, Christine Eng, Theo Heyns, Yuka Suzuki, Yunjiao Zhu, Yuan Ding, Jacob Gibson, Artem Movsesyan, Heather Talia, Pete Taylor, Jacquelyn Truong, Jeff Tsai, Li Liu, Khai Luong

Illumina, Inc., San Diego, CA

Sensitive detection of circulating-tumor DNA (ctDNA) in plasma is increasingly utilized as a biomarker of minimal residual disease (MRD) in assessing oncology therapy response, relapse detection, and as a surrogate endpoint in clinical trials for solid tumors. Both tumor-naïve and informed MRD strategies have been described in literature, some of which are available commercially. Tumor-naïve MRD assays detect tumor-specific biomarkers in cfDNA without the patient's tumor. Although tumor-naïve approach is beneficial in non-resectable tumor cases, tumor-informed assays are generally more sensitive. In a tumor-informed approach, the tumor-specific variants (SNV, indels, MNV, CNV, SV, methylation, etc.) are determined from a patient's tumor and matched-normal, which are subsequently monitored in the patient's cell-free DNA (cfDNA) using a patient-specific bespoke panel, a fixed panel, or whole genome sequencing (WGS). Amongst the tumor-informed approaches, tracking tumor mutations in cfDNA with patient-specific bespoke probes has become a popular approach due to its potential for high analytical accuracy and low sequencing cost. However, bespoke approaches require high DNA input for high accuracy, longer turnaround times, and operational complexity when running a patient-specific assay. Fixed, small panels have the potential to improve operational efficiency and reduce turnaround time. But creating a panel with high sensitivity across multiple tumor types can be a challenge due to tumor type specific mutational rates and patterns. We have developed a simple tumor-informed multi-cancer WGS method that can detect ctDNA down to 10 ppm with low DNA input requirements. WGS of a patient's tumor and matched-normal yields a patient and tumor-specific set of somatic variants for MRD detection. These variants can then be monitored in the plasma using low-pass WGS. Because the WGS data inherently contains the genomic signatures of the tumor, additional biomarkers beyond SNVs that may be more salient of certain cancer types may be additionally assessed without the need to redesign the assay. We demonstrate that this technique can work across a range of tumor mutational burden (TMB) and various tumor types, achieving detection in the range of 10-100ppm.

#6293 Developing and deploying the UCSC Xena gene set enrichment analysis appyter.

Cally Lin[1], Jingchun Zhu[2], Mary Goldman[2]

[1]Stanford University, Stanford, CA,[2]UC Santa Cruz Genomics Institute, Santa Cruz, CA

UCSC Xena (https://xena.ucsc.edu/) is a popular web-based visual integration and exploration tool for multiomic data and associated clinical and phenotypic annotations. Xena users want to be able to run Gene Set Enrichment Analysis (GSEA) to compare gene expression between their two dynamically created sample subgroups and determine which gene programs are upregulated or downregulated. However, the traditional GSEA implementation was not performant enough to support Xena's interactive web-based data visualizations. To overcome this challenge, blitzGSEA, a more performant implementation of GSEA developed in 2022 by the Ma'ayan Lab at Mount Sinai, was integrated to be available within the Xena browser using the Appyter framework, a tool also developed by the Ma'ayan lab. Appyter allows users to use Jupyter Notebooks to run bioinformatic methods without having to interact with the code directly and also allows developers to dynamically code inputs, such as the subgroups created by a user. The framework handles users running analyses concurrently and will cache results if two users run the same analysis, such as part of a workshop. Before development, blitzGSEA was rigorously compared to traditional GSEA to confirm that it provided similar, biologically consistent results with efficient memory usage and runtime. The blitzGSEA Appyter was implemented alongside the addition of a Plotly Dash page to provide users with interactive plots and tables of the analysis results. The blitzGSEA Appyter was then deployed on the production Xena Browser using Docker and an AWS EC2 instance. The blitzGSEA Appyter is now publicly available to Xena users for any sample subgroups they create and averages 500 analyses per month around the world.

#6294 Motif-independent Identification of functional transcription factors from single-cell multiomics data.

Hongpan Zhang[1], Jingyi Wang[1], Zhenjia Wang[2], Chongzhi Zang[2]

[1]Department of Biochemistry and Molecular Genetics, University of Virginia, Charlottesville, VA,[2]Department of Genome Sciences, University of Virginia, Charlottesville, VA

Transcription factors (TF) are proteins that control when and how genes are turned on or off in a cell by binding to specific DNA sequences. The activation of one TF may induce gene expression cascade of other TFs or genes, suggesting the critical role of TFs in cell differentiation and cancer development. Techniques such as ChIP-seq, CUT&RUN and CUT&Tag are widely used to examine TFs' genomic binding activity, but only one or a few TFs can be examined at once, making them low-efficient considering that hundreds of TFs can be typically expressed in a cell. Recently emerging single-cell genomic technologies provide an alternative approach, using computational tools to predict functional TFs from single-cell transcriptomics or chromatin accessibility assays. However, most existing computational methods share similar limitations, including the use of static TF binding motifs and the inability of considering distal enhancer regulation. We developed BARTsc, a precise and time-efficient TF prediction tool for clustered single-cell genomics data and it overcomes aforementioned pitfalls. BARTsc leverages differential features between cell clusters and does model-based predictions of cis-regulatory profiles based on given feature sets. Association scores were then calculated between the predicted cis-regulatory profiles and a collection of TF binding profiles derived from published ChIP-seq datasets. BARTsc implemented a set of handy downstream analysis modules that reveal the TFs that lead to signature gene expression in each cell type, the relative TF activity across cell types and the key regulators for each cell type. By benchmarking against other methods in predicting key regulators, we showed that BARTsc exhibits superior performances in various contexts. A promising impact of this work is that BARTsc can facilitate more precise identification of key regulators driving cancer development and can lead to discovery of new therapeutic targets.

#6296 Unlocking scalable and efficient multiomic analysis of 5- and 6-base genomes.

Sabri Jamal[1], Michael J. Wilson[2], Jean Teyssandier[1], Gary Frewin[1], Mark S. Hill[1], William Stark[1], Nicola Wong[1], Robert Blanshard[1], Andrada Tomoni[1], Tom Charlesworth[1], Nicholas J. Harding[1], Paidi Creed[1]

[1]biomodal, Cambridge, United Kingdom,[2]Princeton University, Princeton, NJ

We present a computational toolkit to analyze 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) Cytosine modifications at scale and describe its performance on a liquid biopsy dataset.

Methylation data has diverse applications in cancer, including early-stage diagnosis through liquid biopsy, classification to guide treatment pathways, and prognosis. However, analyzing methylation data poses significant challenges. Many existing tools are difficult to use and struggle to scale as sample sizes grow. This lack of scalability makes standard tasks, such as identifying differentially methylated regions (DMRs) or summarizing methylation fractions across genomic regions, time- and memory-intensive, often requiring large-scale compute resources. A recently introduced technology, duet multiomics solution evoC, enables the reading of 6-base information from DNA, further increasing the scale and complexity of data that can be extracted from a single sequencing experiment. With this expanded multiomic dataset, the lack of scalability is compounded, hindering interactive data analysis that provides rapid and detailed insight. To address this, we present a fast and scalable array-based python package (modality) for the analysis of 6-base genomes (A, T, G, C, 5-mC and 5-hmC), using multi-core out-of-memory processing to enable extremely efficient computation, even for datasets that are too large to fit into memory.

Our approach enables local analysis of large datasets, scaling to thousands of samples. Unlike existing tools that exceed typical laptop memory with ~10 samples, the modality package efficiently generates hundreds of thousands of features for classification, processing a colorectal cancer liquid biopsy dataset of over 100 samples in minutes on a standard laptop. Furthermore, it runs a complex DMR model (logistic regression with covariates) genome-wide in under an hour, a task infeasible with current tools.

modality combines efficient computation with tools for exploratory (e.g., plotting, methylation summaries) and downstream analyses (e.g., DMR identification, PCA). Designed for efficiency and ease of use, it enables users to rapidly transition from raw data to actionable insights and publication-ready results. As multiomic data become the standard in cancer research, our data structure supports the integration of additional data types, allowing us to handle combined genomic and epigenomic data from solutions like duet evoC. This will enable streamlined and efficient multiomic analysis to uncover deeper biological insights.

#6297 Mapping cellular interactions from spatially resolved transcriptomics data.

J. Zhu[1], Y. Wang[2], W. Chang[1], A. Malewska[1], F. Napolitano[1], J. C. Gahan[1], N. Unni[1], M. Zhao[1], R. Yuan[1], F. Wu[1], L. Yue[1], L. Guo[1], Z. Zhao[3], D. Chen[3], R. Hannan[1], S. Zhang[1], G. Xiao[1], P. Mu[4], A. B. Hanker[1], D. Strand[1], C. L. Arteaga[1], N. Desai[1], X. Wang[5], Y. Xie[1], T. Wang[1];

[1]UT Southwestern Medical Center, Dallas, TX, [2]Cincinnati Children's Hospital Medical Center, Cincinnati, OH, [3]University of Notre Dame, Notre Dame, IN, [4]Yale School of Medicine, New Haven, CT, [5]University of Texas at Arlington, Arlington, TX

Cell-cell communication (CCC) is essential to how life forms and functions. However, accurate, high-throughput mapping of how expression of all genes in one cell affects expression of all genes in another cell is made possible only recently through the introduction of spatially resolved transcriptomics (SRT) technologies, especially those that achieve single-cell resolution. Nevertheless, substantial challenges remain to analyze such highly complex data properly. Here, we introduce a multiple-instance learning framework, Spacia, to detect CCCs from data generated by SRTs, by uniquely exploiting their spatial modality. We highlight Spacia's power to overcome fundamental limitations of popular analytical tools for inference of CCCs, including losing single-cell resolution, limited to ligand-receptor relationships and prior interaction databases, high false positive rates and, most importantly, the lack of consideration of the multiple-sender-to-one-receiver paradigm. We evaluated the fitness of Spacia for three commercialized single-cell resolution SRT technologies: MERSCOPE/Vizgen, CosMx/NanoString and Xenium/10x. Overall, Spacia represents a notable step in advancing quantitative theories of cellular communications.

#6298 The first fully automated, integrated workflow between a small animal imaging tool and animal study management software improves the accuracy, reproducibility, and efficiency of small animal imaging studies.

Sean R. Gallagher[1], Meng Yang[2], Eric Ibsen[3], Antonio Boccia[3], Madalin Florescu[4], Qi Wang[5], Neil Chang[1]

[1]Analytik Jena US LLC, Claremont, CA,[2]R&D, InvivoxBio, San Diego, CA,[3]R&D, studylog, Pacifica, CA,[4]R&D Programming, Cardinal Cognition SRL, Claremont, CA,[5]R&D, Analytik Jena US LLC, Claremont, CA

Preclinical imaging systems and electronic data capture (EDC) technologies are transforming biomedical research and drug discovery by offering powerful, time-saving tools for oncology study data acquisition, analysis, and management. Advanced imaging modalities, such as bioluminescence, fluorescence, CT, MRI, and PET imaging, allow researchers to non-invasively visualize and quantify biological processes in small animal models. These systems support longitudinal studies of disease progression, therapeutic efficacy, and molecular mechanisms, aiding novel drug development while minimizing animal usage through precise data collection. Companies like Analytik-Jena provide high-resolution small animal imaging solutions that help researchers gain deeper insights into biological phenomena. Complementing these innovations, EDC systems like Studylog Animal Study Software streamline preclinical workflows by automating data collection, validation, and analyses. Together, preclinical imaging and EDC technologies drive innovation in biomedical science by enabling robust and efficient data capture. However, before this writing, no integration between preclinical imaging and EDC technologies existed, forcing researchers to rely on a heavily manual process of imaging data association with study animals to generate statistical and graphical results This manual process is fraught with a variety of error types and is extremely time-consuming. Furthermore, access to results and original animal images in the context of the study is absent altogether. This paper reports on the first automated integration of preclinical imaging and animal study workflow software available. Having tools that enable seamless tracking of the animals used for the collective small animal imaging study processes, such as substrates administered, incubation time points, anesthesia records, image collection, animal identification association, -including region of interest (ROI) determination, and tracking—is crucial to improving the accuracy, reproducibility, and efficiency of these studies. Equally important is the accurate transfer of this data into a database, ensuring the integrity and long-term accessibility of study data and results. The novel integration feature enables seamless communication between Studylog and VisionWorks by exchanging data in a standardized format via local methods or internet-based APIs. This allows scientists to initiate workflows in Studylog, process imaging and analysis in VisionWorks imaging software, and then send results back to Studylog with a single click, supporting both local and remote collaboration.

#6300 Global fit normalization for circulating microRNA measured by quantitative PCR.

K. Song, C. Wang, J. W. Kardos, N. Sarkar, Y. Liu, O. Yoon;
Gilead Sciences, Inc., Foster City, CA

MicroRNAs (miRNAs) are small RNA molecules that regulate gene expression at the post-transcriptional level and play a key role in cancer biology affecting cancer cells' proliferation, metastasis, and drug resistance. Cancer cells can release miRNAs into the bloodstream and can potentially be non-invasive markers for cancer diagnosis and prognosis. However, variations in quantitative PCR (qPCR) preparation have limited their clinical utility, and normalization of circulating miRNA qPCR data remains challenging due to low amount of circulating RNA and the lack of universal housekeeping miRNAs. To address these limitations, we have developed Global Fit (GF) normalization, a novel approach that accounts for technical variations from RNA input by fitting a sigmoidal curve to the reverse cumulative distribution of miRNA cycle threshold (Ct) values and subsequently adjusting sample bias through distribution unification. Additionally, we introduce an extended normalization method that employs linear regression (LR) to further correct residual sample bias. The performance of the GF was compared against the commonly used normalization method, Global Mean (GM) using simulated data based on a murine plasma dataset and three public human cancer plasma datasets. In the murine dataset, we demonstrated that sample input bias is reflected by the horizontal shift of the cumulative distribution of Ct values, and GF normalization yielded more aligned distributions compared to GM. Simulation showed that GF outperformed GM by reducing post-normalization variability across scenarios considering different dropout rates and panel sizes. Across multiple simulations of differentially expressed miRNAs, GF showed higher statistical power compared to GM (median 51.9% vs 20.4%), while maintaining similar type I error rates. LR further improved the detection power beyond GF alone. Across three human cancer miRNA datasets, GF consistently demonstrated better correction of circulating miRNA distribution compared to GM. In ANOVA analyses, GF normalization consistently accounted for higher data variability than GM normalization. GF also outperformed other normalizers in stability score (ranked No. 1) as measured by NormFinder. Consequently, GF allowed better detection of biologically meaningful circulating miRNAs in colon and liver cancers, such as miR-210, miR-532-3p and miR-195. These findings support the use of GF normalization in circulating miRNA data analysis to enable more robust biomarker identification for cancer diagnosis and prognosis in clinical settings.

#6301 ARLVPI: A novel machine-learning-informed platform for assessing somatic variant classification and pathogenicity.

Arlen Larry Gyden[1], Nicole Ambroise[2], Tarsus Lam[2], Zehra Ordulu[2], Petr Starostik[2], Kimberly Newsom[2], Mohammed Gbadamosi[1]

[1]College of Pharmacy, Pharmacotherapy and Translational Research, University of Florida, Gainesville, FL,[2]Pathology, Immunology and Laboratory Medicine, UF Health, Gainesville, FL

Cancer comprises several heterogeneous multifaceted diseases characterized by dysfunctional cellular differentiation and excessive proliferation. With its prevalence expected to rise, particularly among middle-aged adults, the burden on pathologists to identify and characterize pathogenic variants increases. Current clinical practices rely on molecular profiling, pathologist interpretation, and supplemental information from disease databases to classify these variants. In this context, variants of unknown significance (VUS) pose a significant clinical challenge due to their uncertain biological and clinical effects that make them inhibitive towards diagnoses and treatment decisions. Representing 40% of Next-Generation Sequencing (NGS) signals, VUS indicates a significant unmet clinical need demanding urgent solutions. To meet this unmet clinical challenge, we utilized 20,756 variants from 415 cancer patient samples to develop the Advanced Risk Locating Variant Pathogenicity Index (ArlVPI). ArlVPI employs a mechanistic model based on information from a random forest classifier and dimensional reduction analyses to identify and flag VUS variants likely to require further investigation from pathologists. In downstream analysis, ArlVPI exhibited a strong correlation with variant pathogenicity and correctly categorized variants as benign or pathogenic with <1% error. With respect to VUS, ArlVPI flagged 16% as requiring potential follow-up, corroborating metrics observed in literature. Furthermore, ArlVPI demonstrated >93% concordance with clinical pathologists in the identification of VUS which were reclassified as pathogenic in an array of solid tumor, hematologic, and myeloproliferative malignancies. Our study illustrates how ArlVPI can be integrated into existing NGS analysis pipelines to support molecular professionals, offering an efficient resource that enhances variant classification and supports targeted cancer treatment strategies. Future work focuses on developing a streamlined application that interfaces with existing clinical diagnostic systems.

#6302 A CellProfiler pipeline for RNAscope analyzing multiple markers across adjacent tissue sections spatially.

Meng-Shin Shiao[1], Natini Jinawath[1], Katherine Copeland[1], Ariestya Indah Permata Sari[2]

[1]Mahidol University, Bangkok, Thailand,[2]Swadaya Gunung Jati University, Cirebon, Indonesia

Understanding the spatial distribution of cancer cells and how they interact with other cell types in the tumor microenvironment (TME) is crucial to elucidate the mechanisms of cancer development and progression. The identification of cell types in the TME relies on the distinct expression profiles of different cell types. Compared to sequencing-based spatial transcriptomics analysis, multiplex nucleic acid *in-situ* hybridization (ISH) at a single-molecule level is more focused and cost-effective. Based on ISH technology, RNAscope developed novel approaches to detect multiple gene expression profiles on tissue sections at a single-molecule level. It is highly sensitive and specific and can simultaneously quantify gene expression at sub-cellular levels. Although the technology has been applied to study spatial gene expression in various cancer types, few have applied it to elucidate the association between different markers in different cell compartments within the TME.This protocol describes an image analysis pipeline for multiplex fluorescent signals detected by RNAscope technology. It was developed based on a previous CellProfiler pipeline, facilitating spatial expression analyses across different cell compartments through a flexible, user-friendly interface for non-computational biologists. We describe specific steps to analyze the expression and spatial arrangement of four endogenous housekeeping genes as quality controls (QCs): *POLR2A*, *PPIB*, *HPRT1*, and *UBC*. We further apply these pipelines to analyze target genes in breast cancer samples, comparing those with and without skin metastasis. The analyses were demonstrated in one sample each using formalin-fixed paraffin-embedded tissue (FFPET) and fresh-frozen tissue (FFT) sections with the ISH steps followed the manufacturer's protocol.

#6303 DrBioRight: an AI-driven chatbot for cancer data analysis.

Han Liang, Jun Li, Wei Liu

UT MD Anderson Cancer Center, Houston, TX

We present DrBioRight, a cutting-edge chatbot designed to streamline omics data analysis by employing large language models (LLMs). Modern omics technologies generate extensive cancer omics datasets, but conventional software often requires significant technical expertise. DrBioRight addresses this challenge by enabling researchers to interact with the platform in a conversational manner, simplifying complex analytical workflows. DrBioRight's chatbot-driven interface allows users to search datasets, perform data analysis, access insights, and generate visualizations by posing natural language questions or commands. This intuitive approach eliminates the need for programming knowledge, making omics research more accessible. Key features include LLM-powered conversational capabilities, real-time task prediction and guidance, collaborative functionalities for research teams, and integration of new tools and datasets via community contributions. By transforming the interaction model from technical to conversational, DrBioRight serves as an intelligent research assistant that democratizes omics data analysis, enhances collaboration, and accelerates scientific discoveries in cancer research.

#6304 RUO LymphoTrack Enterprise Software v3.0.1 - A comprehensive suite to standardize analyses of IG and TCR sequencing data.

T. Nguyen, J. Witsotsky, J. D'Angelo, J. Velazquez-Muriel, A. Arias, J. Jablanovic, J. Miller;
Invivoscribe, Inc., San Diego, CA

This study introduces the LymphoTrack Enterprise Software v3.0.1 (LTE), a high-throughput Research Use Only (RUO) software suite for analyzing all RUO LymphoTrack Assays offered by Invivoscribe on a single platform. LTE provides a comprehensive solution for the analysis of immunoglobulin and T-cell receptor nucleotide sequences. LTE is specifically designed to meet the needs of higher volume laboratories seeking compatibility with a broader range of sequencers and increased automation flexibility. Unlike the desktop RUO LymphoTrack Software - MiSeq for clonality and MRD analyses, which caters to smaller-scale users, LTE offers an advanced enterprise architecture featuring containerization, a REST API for seamless integration, and scalable infrastructure to handle large datasets efficiently. These enhancements make LTE an ideal choice for labs focused on high-throughput analysis and integration into automated workflows. Notably, LTE significantly reduced MiSeq processing time to 2.2 ± 0.34 minutes (n=3 runs, 24 samples each) vs. 30.3 ± 1.50 minutes in RUO LymphoTrack v2.4.6—an ~92.8% reduction. LTE also includes compatibility with more assay and sequencing platforms, supporting Illumina NextSeq1000 and Complete Genomics DNBSEQ-G99, with planned support for NovaSeq6000 in future releases to further increase compatibility. For example, it recognizes DNBSEQ-G99 FASTQ files, performs adapter trimming, and proceeds to clonality analysis, with total analysis times of 20.1 ± 0.56 minutes for a NextSeq550 run (n = 3 runs, 12-25 samples each run), 8.98 ± 2.23 minutes for a NextSeq1000 run (n = 4 runs, 96 samples), and 70.3 ± 6.35 minutes (n = 3 runs, 24 samples each run) for a DNBSEQ-G99 run (including 65.7 ± 7.06 minutes for adapter trimming step). Key advancements in this release include enhanced CDR3 region identification, a critical feature for immunogenomics, enabling rapid and precise immune repertoire and clonality analyses. Testing over 220,000 unique sequences, the RUO LymphoTrack Enterprise v3.0.1 algorithm achieved a CDR3 identification rate of 98.6%, a substantial improvement from the 58.4% rate observed with the previous version. The software also supports multiple immunogenetic databases, including IgBlast, IMGT/V-QUEST, and IMGT/GENE-DB, allowing for customized antibody and T-cell receptor profiling. By offering faster processing, broader platform compatibility, and enhanced analytical capabilities, RUO LymphoTrack Enterprise v3.0.1 provides a robust, all-in-one solution for researchers seeking to streamline immunogenomic workflows. This upgraded version establishes a new standard in cancer genomics, with potential to significantly advance biomarker discovery, immune response characterization, and cancer subtype differentiation.

#6305 hGRN-ChEAR: human transcription factor regulatory network with a knowledge graph interactive user interface.

Anna I. Byrd, John Erol Evangelista, Alexander Lachmann, Ho-Young Chung, Sherry L. Jenkins, Avi Ma'ayan

Pharmacological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY

Gene expression is controlled by transcription factors that selectively bind to DNA to regulate mRNA expression of all human genes. In cancer, gene expression regulation is misconfigured to accelerate uncontrolled cell proliferation. Transcription factors control the expression of other transcription factors, forming a complex gene regulatory network (GRN) with switches, feedback loops, and other regulatory motifs. Many experimental methods and computational tools have been developed to reconstruct GRNs in-silico. Here we present a novel approach to reconstruct the human GRN. By submitting thousands of gene sets from the RummaGEO resource for transcription factor enrichment analysis with ChEA3, we are able to distill a signed and directed edges that connect all human transcription factors to construct a novel human GRN. The GRN has 130,793 signed and directed edges between 703 source and 1,543 target nodes. The network is made accessible via an interactive web-based application called the Human Gene Regulatory Network based on ChEA3 and RummaGEO (hGRN-ChEAR). hGRN-ChEAR enables users to query the GRN by searching for single or pairs of transcription factors, as well as by submitting their gene sets to perform transcription factor enrichment analysis. To demonstrate the utility of hGRN-ChEAR, we systematically identified transcription factor subnetworks that regulate differentially expressed genes in tumors from ten cancer types, and 69 subtypes, profiled by the Clinical Proteomic Tumor Analysis Consortium (CPTAC). hGRN-ChEAR is freely available from: https://hgrn-chear.maayanlab.cloud/.

#6306 Topological arrangement of samples based on high dimensional feature similarities: the NetFlow framework.

Rena Elkin, Jung H. Oh, Joseph O. Deasy

Memorial Sloan Kettering Cancer Center, New York, NY

At the core of exploratory data analysis is the development of effective informatics tools to represent and visualize how samples are organized in a dataset. Cancer heterogeneity between and within cancer types poses challenges for many clustering and dimensional reduction approaches as biological data often vary continuously rather than form distinct clusters. It is therefore essential to develop a visualization tool that can expose the underlying clusters as well as the broader relational structure of the samples in the data. We introduce NetFlow, an open-source software tool designed to address this need. NetFlow serves as a comprehensive and interactive investigative platform to aid researchers in visualizing and analyzing complex biological data to support the generation and evaluation of hypotheses.

NetFlow arranges samples in an undirected graph based on similarities of the underlying high dimensional feature set. It takes biological feature data and provides a visually informative relational topology where each node represents a sample. To incorporate ordering and clustering behavior, the relational topology is derived from the underlying metric structure and constructed by combining the pseudo-ordering of the samples, generated via lineage tracing techniques, with nearest-neighbor information. In addition to classic metrics, NetFlow uses Optimal Mass Transport which offers a network-level and robust metric to compare high dimensional sample profiles with respect to a feature network. NetFlow supports the computation of kernel transformations and diffusion distances to account for local and multi-scale geometry which influence aspects of the similarity structure that will be reflected in the extracted organizational representation.

NetFlow is designed in a modular fashion to facilitate easy integration with diverse computational pipelines. The modules support data input/output, similarity structure derivation, visualization, and down-stream analyses. A key feature of NetFlow is its interactive visualization dashboard for exploring the resulting relational topology and its associations with biological or clinical features. The dashboard offers multiple capabilities for customizing the visualization and enhancing interpretability. For instance, the user can select features of interest to color-code the nodes (i.e., samples) and edges (relationships), and can select samples of interest to identify statistically significant characteristic features. These functionalities empower users to gain deeper insights into the underlying data structure and its potential biological or clinical implications.

NetFlow results in an abstract sample-space network rather than a 2D spatial arrangement. Its topological nature enables interpretable, uncluttered visualizations of relations within and between clusters for identifying novel inter-sample similarities.

#6307 ELViS : An R package for estimating copy number levels of viral genomic segments at base-resolution.

J.-Y. Lee[1], H. Choi[1], J. R. Holt[1], X. Zhao[1], K. A. Hoadley[2], D. N. Hayes[1];

[1]University of Tennessee Health Science Center, Memphis, TN, [2]Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC

Tumor virus is one of the major causes of human cancers, accounting for about 10-20% of their global incidence. Viral oncogenes in the tumor viruses promote tumorigenesis by deregulating host cancer genes. For instance, E6 and E7 proteins of human papillomavirus 16 (HPV16) induce the degradation of the protein products of tumor suppressor genes (TSGs) including p53 and pRb, to promote head and neck squamous cell carcinoma (HNSCC). Therefore, it is imperative to understand how differences in the dosage of viral genes affect cancer biology. However, how the relative and absolute abundance of each viral gene affects tumorigenesis and tumor biology has remained elusive, due to the difficulties in the analysis. Unlike human genomes that exist in two copies per cell, viral genome copies can take dynamic ranges, making copy number analyses challenging. Thus, we developed an R package ELViS that analyzes viral copy number change events from targeted DNA sequencing of whole viral genomes. In the first step, it takes aligned reads in the BAM file to generate base-resolution read depth and finds baseline regions based on the shape of the data. Next, to overcome the uneven base-resolution depths of targeted sequencing data and find copy number events, our framework performs joint segmentation in two-dimensional data space using both normalized read depths and Z-scores. In the simulation dataset, ELViS could detect deletions as small as 50bp and duplications as small as 500bp with F1 score &gt 0.75 at 50X coverage depths. ELViS provides base-resolution copy number change calls, along with publication-ready read depth line plots and cohort-level heatmap visualization of multiple data tracks with integrative clustering results.

#6308 Dory: a computational method for differential chromatin tracing analysis.

Zhaoxia Ma[1], Miao Liu[2], Mengwei Hu[2], Shengyuan Wang[1], Siyuan Wang[2], Chongzhi Zang[1]

[1]University of Virginia, Charlottesville, VA,[2]Yale University, New Haven, CT

The spatial organization of the genome plays a vital role in maintaining the cell type-specific chromatin structures and regulating gene expression. The three-dimensional (3D) genome structures can be measured by sequencing-based techniques such as Hi-C on the ensemble level or by imaging-based techniques such as chromatin tracing on the single-molecule level. Chromatin tracing is a multiplexed DNA fluorescence in situ hybridization (FISH) technique that can directly trace the 3D positions of genomic loci along individual chromosomes in single cells. By designing the primary probes targeting specific genomic regions and dye-labeled secondary probes to indicate their positions, chromatin tracing can construct the 3D trace of chromatin at high resolution using microscopy. However, few computational tools have been developed for differential analysis of chromatin tracing data. Major challenges include the high variability of chromatin traces and missing values in the 3D coordinates of some loci. Here, we present Dory, a statistical method for identifying differential patterns from two groups of chromatin traces without imputation of missing data. Dory measures the spatial distance between each pair of regions in each chromatin trace and employs multi-level statistical tests to quantify the distance difference between the two groups of chromatin traces. The output of Dory includes the differential score for each region pair comparing chromatin traces between two biological conditions. We applied Dory to chromatin tracing data from six cell types in mouse fetal liver and from a mouse lung cancer progression model. We found that the changes in chromatin structure are associated with A-B compartment alterations and promoter-enhancer interaction alterations that cause differential gene expression. Dory is a user-friendly computational tool that can be applied to multiple types of imaging-based 3D genome data analysis for studying genome functions and gene regulation.

#6309 CliPP-on-Web: a dynamic platform for clonal structure identification through penalizing pairwise differences in cancers.

Aaron Wu, Quang Tran, Matthew D. Montierth, Yujie Jiang, Jessica C. Lai, Shuangxi Ji, Wenyi Wang

Bioinformatics and Computational Biology, UT MD Anderson Cancer Center, Houston, TX

Background: Intra-tumor heterogeneity plays a crucial role in tumor evolution and response to therapy, highlighting the need to understand mutation clonality and subclonal architecture to drive advancements in precision cancer treatments. However, existing tools for subclonal reconstruction are typically too slow for large cohort analyses, and lack an accessible web-based interface for widespread use.
Methods: We developed CliPP-on-Web, an interactive platform built with R Shiny, to streamline subclonal analysis for researchers and clinicians. Its back-end algorithm is based on model-based clustering with regularized maximum likelihood estimation, derived from the core methodology of CliPP. This algorithm models variant allele counts using a binomial distribution, incorporating tumor purity, copy number alterations (CNAs), and cancer cell fractions (CCFs) of somatic mutations. By penalizing pairwise differences in CCFs, it effectively clusters mutations without requiring predefined subclone numbers. Optimization is achieved through the Alternating Direction Method of Multipliers (ADMM), ensuring high computational efficiency.
Results: CliPP-on-Web simplifies subclonal analysis by enabling users to process up to 10 samples simultaneously with user-uploaded. Interactive visualization tools, such as Variant Allele Frequency (VAF) and Cellular Prevalence (CP) plots, offer features like zooming, hover details, and adjustable smoothing for enhanced clarity. Pre-loaded integrated datasets from TCGA and PCAWG enable comparative studies, while the Driver Mutation module highlights key cancer mutations with searchable and downloadable results. Capable of processing 100 samples in just30 minutes — far outpacing the ~3 hours per sample required by tools like PhyloWGS — CliPP-on-Web provides efficient and comprehensive subclonal analysis, advancing cancer genomics research.
Conclusions: CliPP-on-Web offers a powerful, accessible platform for analyzing mutation clonality and subclonal architecture across diverse tumor types, facilitating both large-scale studies and individual sample exploration.

#6310 Bridging spatial proteomics and classical histopathology: advanced spatial analysis through H&E-enhanced multiplexed immunoprofiling.

Michael Downing, Ruben Cardenes, Douglas Wood, Gourab Chatterjee, Lorenz Rognoni

Ultivue, Inc., Cambridge, MA

Background: Multiplex immunofluorescence (mIF) is increasingly adopted in translational research due to its ability to provide rich spatial and molecular information. Nevertheless, H&E staining remains the gold standard for clinical diagnosis, revealing essential histological context and morphological features not visible in mIF images. While H&E is routinely performed on serial sections in analytical mIF workflows, biological variability between sections limits its utility. Co-registration of H&E and mIF images from the same slide enables precise colocalization of cells across channels, combining the histological detail of H&E with the spatial insights of mIF to enhance both biological assessment and image analysis.
Overview: This study explored the integration of same-section H&E staining and imaging into mIF workflows using formalin-fixed paraffin-embedded (FFPE) tissue samples from mixed cancer indications. After mIF staining and imaging with InSituPlex™ technology, same-section H&E images were co-registered with the mIF images before incorporation into a standard image analysis pipeline. Co-registration and analysis were performed through the STARVUE™ image data science pipeline, using UltiStacker.AI™ and UltiAnalyzer.AI™. The integration was evaluated across several key applications. --Pathology Assessment: H&E image data was overlaid onto the mIF channels, allowing for the histological context to be directly referenced when identifying the tumor and excluding non-target tissues from the analysis. --mIF-Enhanced H&E: Marker expression from mIF channels was overlaid onto the H&E images, allowing biomarker profiles to be visualized in the histological context. This colocalization enriched the understanding of immune activity beyond what H&E alone can provide. --Tissue Detection: mIF-based tissue detection often struggles with non-stained elements. Co-registered H&E images addressed this limitation by offering additional features for refined tissue-background separation, improving accuracy in measurements such as cell density. --Tumor Segmentation: Cytokeratin (CK) is commonly used to identify tumor epithelia in both brightfield and mIF assays. Tumors lacking CK expression or using non-CK assays pose challenges for segmentation. By leveraging co-registered H&E images, a deep learning model trained on H&E data successfully segmented tumor compartments, enabling accurate mIF-based cell analysis where CK-based methods failed.
Conclusions: The results of this study highlight the enhanced utility of the gold standard H&E stain through integration of co-registered same-section H&E images into mIF workflows. This approach not only enriches tissue analysis but also lays the groundwork for developing novel methods that further broaden the applications of H&E in spatial biology.

#6311 Enabling scalable, precise, and fast spatial biology studies with Orion and OMERO Plus.

Erin Diel[1], Edward Lo[2]

[1]Glencoe Software, Inc., Seattle, WA,[2]RareCyte, Inc., Seattle, WA

The field of spatial biology requires the simultaneous evaluation of 10s to 100s of biomarkers across entire tissue sections, as these complex measurements yield key insights into the organization and state of cells in tissues, and thus are critical for discovery and clinical applications. However, producing such data at speeds consistent with translational applications and enabling responsive visualization of this multidimensional data remain a challenge. We present the integration of fast data acquisition with performant data management and visualization for employing these spatial biology workflows at scale.

RareCyte's Orion is a next generation imaging instrument powering a new paradigm in spatial biology. Utilizing a novel, single round staining technique, Orion spatial biology allows for up to 20 channels (18 marker + 2 autofluorescence channels), same section H&E, and flexible panel design, all within the context of whole-specimen tissue imaging. The combined power of the Orion instrument and workflow enables ultra-fast imaging at a low run cost and preserves the integrity of the tissue sample, providing scale to translational and clinical studies.

Glencoe Software's OMERO Plus is a powerful enterprise image data management platform which enables remote viewing, sharing and analysis of large-scale biological image datasets, including metadata ranging from simple annotations to complex, quantitative descriptions of cells within images. Furthermore, OMERO Plus' open API allows for flexible customization and integration with other tools and pipelines. Additionally, OMERO Plus ensures data integrity and reproducibility by tracking experimental conditions and data provenance.

The seamless integration of Orion and OMERO Plus enables efficient workflows connecting data acquisition to analysis and visualization. Here, we introduce a new visual data mining interface called Pageant, which supports the exploration and classification of millions of cells based on hundreds of features. Using image data from Orion systems, diverse cell types can be classified based on intensity features from 18 markers of interest, in addition to cell morphometric features. Cell-cell interactions can then be studied using the spatial location and distribution of classified cell types. We performed an iterative classification workflow, first by finding marker-positive cell populations, then by creating boolean combinations of these markers into biologically-meaningful cell types, all within a web browser. We report speed improvements in using OMERO Plus and Pageant for remote interactions with analytical data, in some cases greater than 10x. Pageant transforms the scientist's ability to make conclusions from analytical data without code at scale.

#6312 AI-Driven Identification of Drug-Tolerant Persister Cells in Lung Cancer.

Balaji Selvaraj[1], Abinaya Vairam[2], Pablo Andres Moreno Cortez[3], Ramya Tanikanti[2], Anca M. Farcas[4], Yi Wei[5], Martin L. Miller[3], Viia E. Valge-archer[6], Ultan McDermott[4]

[1]R&D IT, AstraZeneca AB, Molndal, Sweden,[2]R&D IT, AstraZeneca India Pvt Ltd, Chennai, India,[3]Oncology Data Science, AstraZeneca plc, Cambridge, United Kingdom,[4]Oncology Bioscience, AstraZeneca plc, Cambridge, United Kingdom,[5]R&D IT, AstraZeneca plc, Cambridge, United Kingdom,[6]External R&D and Strategic Alliances, AstraZeneca plc, Cambridge, United Kingdom

Drug-tolerant persister cells (DTPs) are cancer cells that evade the effects of therapies through a reversible, non-genetic mechanism, leading to therapeutic resistance and cancer recurrence. Single cell RNA sequencing data is a promising modality to study the mechanism behind DTPs' survival. The first step of such studies is to classify persister vs. non-persister cells. Today, bioinformaticians identify persister cells manually from RNA sequencing data; this is a tedious and time consuming task.

This paper introduces the first end-to-end, fully automatic pipeline for DTPs classification using an adapted Transformer-based scGPT model. Our contributions are as follows:

(1) Persister cells are transient and reversible in nature. In our training data, even if a cell is labeled as a persister cell, it is better to inform the model that this cell has certain probability of being a persister cell and some probability of being a non-persister cell. The default one-hot cross-entropy loss of the scGPT model assumes label certainty and will lead to overfitting. To overcome this, we fine-tuned a pre-trained scGPT model using a label smoothing cross-entropy loss that encourages the model to give probabilities to both classes.

(2) Single cell data is sparse; many genes are not expressed. Non-expressed genes bring noise to the model. We leveraged statistical analysis to understand significant genes across cell lines, resulting in a threshold that our pipeline uses to only keep highly variable genes as inputs to the model.

(3) When assigning data points to different cross validation folds, the original scGPT pipeline stratifies them by only constraining on cell types. This will result in imbalanced distribution of data points from different batches in different folds, preventing the model from generalizing to unseen batches. We stratified data using both cell type and batch, mitigating the batch effect.

We evaluated our pipeline on a proprietary data processed through the Persist-seq baseline scRNA-seq pipeline, with 41071 data points, coming from four non-small cell lung cancer cell lines. We performed 4-fold cross validation with each fold using data from 3 cell lines for training and data from the fourth cell line for testing. Averaging over testing folds, our model achieved 90% accuracy, with a 0.89 F1 score. Our finding suggests that transformer-based deep learning pipelines are effective alternatives to traditional manual approaches for persister cell classification. Future research will focus on developing a multi-step approach, where the initial step is to classify cancer cells vs non-cancer cells, followed by persister cell classification.

#6313 Context-aware pathway enrichment clustering and interpretation using large language models.

Yibing Guo[1], Yanhao Tan[2], Li-Ju Wang[2], Chien-Hung Shih[2], Yu-Chiao Chiu[2]

[1]Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA,[2]UPMC Hillman Cancer Center, Pittsburgh, PA

Pathway enrichment analysis is pivotal for elucidating key oncogenic processes from high-throughput cancer profiling. However, the inherent redundancy and interdependence in biological pathway annotations complicate the meaningful interpretation of enrichment results. This challenge necessitates the effective clustering of enriched pathways. Traditional clustering methods predominantly rely on structural matrices, such as gene overlap within hierarchical pathway graphs. However, these methods do not account for the biological context of the experiment, potentially overlooking critical, context-specific insights tied to cancer types, stages, and other key factors. To address these limitations, we present a context-aware approach for clustering and interpretating pathway enrichment results using emerging large language models (LLMs). Our method involves: 1) generating contextually enriched pathway summaries based on original definitions, using LLMs with prompt engineering techniques; 2) embedding these summaries into high-dimensional quantitative representations, to capture pathway-level contextual semantics; and 3) clustering pathways into biologically coherent themes by computing pairwise similarities between embeddings. We applied this approach to a case study involving 144 up-regulated Reactome pathways from pediatric acute myeloid leukemia (AML) samples compared with normal controls in the TARGET dataset. A widely used overlap-based method, EnrichmentMap, yielded clusters with uneven sizes and fragmented groupings. For instance, the largest cluster contained 79 pathways spanning diverse processes, while immune-related pathways formed several separate clusters. In contrast, our LLM-based approach produced more cohesive and interpretable results, with 11 clusters ranging from 5 to 25 pathways. These clusters successfully delineated distinct biological processes, such as platelet activation and coagulation, and chromatin remodeling. Notably, our approach demonstrated the value of contextualizing pathways. For example, the “pentose phosphate pathway” was grouped with iron metabolism and autophagy pathways, emphasizing its role in providing NADPH for iron metabolism, which is relevant to AML. This insight was missed by EnrichmentMap, as this pathway was left unclustered. Additionally, “nuclear events stimulated by *ALK* signaling in cancer” was grouped with oncogenic signaling, reflecting the role of *ALK* in nuclear processes that drive survival, transformation, and apoptosis escape. Overall, this pilot study underscores the potential of LLM-generated contextual pathway summaries and embeddings to produce biologically coherent pathway clusters that highlight their collective roles. Our approach represents a novel strategy for enhancing the interpretability of pathway analysis results, particularly in complex disease contexts such as cancer.

#6314 Development of g-rate based random forest machine learning model to predict overall survival for patients with metastatic prostate cancer.

Mohamed Azzam[1], Harshraj Leuva[1], Mengxi Zhou[2], Benjamin A. Teply[1], Raymond C. Bergan[1], Susan Elaine Bates[2], Shibiao Wan[1], Antonio Tito Fojo[2], **Jieqiong Wang**[1]

[1]University of Nebraska Medical Center, Omaha, NE,[2]Columbia University Medical Center, New York, NY

Metastatic prostate cancer (mPC) is among the most common causes of cancer-related death affecting men worldwide. Currently, predictive biomarkers and models are available to estimate a patient's prognosis, but we lack reliable treatment assessment measures once a patient starts on a treatment. We have developed the ***g***-rate method to measure treatment efficacy, which estimates tumor growth (***g***) and decay (***d***) rates using prostate-specific antigen (PSA) values while the patient is on treatment and have shown that ***g***-rate has a robust inverse correlation with overall survival(OS) in patients with mPC. We have now developed a machine learning model based on g-rate and other clinical measures to predict the survival probability over time for individuals who are receiving treatment for mPC. We included variables of patient demographics, Charlson Comorbidity Index (CCI), mPC history (Gleason score, hormone sensitivity, line of treatment, PSA at diagnosis), variables available at the first visit of the start of treatment such as first PSA and first CBC at start of treatment, and g-rate derived from serial PSA values while on treatment. A random forest machine learning (ML) algorithm was employed to construct the survival prediction model. Harrel's concordance index (C-index), Uno's C-index, and time-dependent area under the receiver operating characteristic (ROC) curve were used for performance evaluation. Permutation importance was applied to assess variable contributions. Our experiment uses 18,018 treatment records of 11,849 unique US veteran patients to train the model. Besides the training data, 4,501 treatment records of 2,963 unique patients are used for model evaluation. The model achieved a remarkably high Harrel's C-index of 0.76 and Uno's C-index of 0.747, with 23.68% of treatment records censored. The time-dependent ROC curve averaged 0.83, demonstrating robust predictive capability. The permutation importance analysis identified ***g***-rate as the most influential variable, followed by the first PSA and first Hgb at the start of treatment. Our g-rate based ML model highlights that it is possible to estimate treatment efficacy early via reliable survival probability prediction while on a treatment for mPC. Once validated, it can be used for both research and clinical purposes to make timely treatment escalation or de-escalation decisions.

#6315 Rapid automatic segmentation of murine lung volumes from micro-CT images using deep learning to reduce analysis burden.

Adam M. Aji[1], Thomas M. Kierski[1], Juan D. Rojas[1], Ryan C. Gessner[1], Yair Reisner[2], Irit Milman-Krentsis[2], John Hazle[3], Kiersten L. Maldonado[3], Charles Kingsley[3], Richard R. Bouchard[3], Tomasz J. Czernuszewicz[1]

[1]Revvity, Durham, NC,[2]Department of Stem Cell Transplantation and Cellular Therapy, MD Anderson Cancer Center, Houston, TX,[3]Department of Imaging Physics, MD Anderson Cancer Center, Houston, TX

Micro-computed tomography (micro-CT) has emerged as a powerful tool for non-invasive small animal imaging, particularly in lung cancer and fibrosis research. This high-resolution imaging modality allows researchers to visualize and quantify lung structures and pathologies with unprecedented detail, enabling sophisticated longitudinal studies. However, the analysis of these complex 3D datasets presents a significant challenge. Manual segmentation of lung tissues from micro-CT images is time-consuming and prone to inter-observer variability, often becoming a bottleneck in study workflows. This analysis burden can impact study design, potentially limiting sample sizes or the frequency of imaging timepoints. As a result, there is a growing need for efficient, automated lung segmentation solutions that can accelerate analysis, improve reproducibility, and ultimately enhance the throughput and statistical power of preclinical lung studies. We address these challenges by training a new fully automatic deep learning model for whole lung segmentation. The goal of this work is to create a single model that generalizes well to data representing various manufacturers, disease states, and fields-of-view. In this pilot study, we collected 321 scans using a SkyScan 1276 (Bruker, Kontich, Belgium) and a Quantum GX2 (Revvity, Waltham, MA, USA). The lungs were segmented from each image by an expert reader. Data were shuffled and divided into groups for training, validation, and testing using a 70%-20%-10% split. Models were trained with different sets of hyperparameters and evaluated on the validation set. The model with the best validation Dice coefficient was selected for analyzing the test set. A linear regression analysis between the predicted and the ground truth test volumes resulted in a R^2 value of 0.96, indicating strong correlation between the output of the model and the human annotator. The average time for the model to compute a single label is roughly 2 seconds, compared to an average of 15 minutes per volume for the expert reader, representing a significant improvement in processing time. Furthermore, the model is fully automatic, reducing the need for user input which might generate user fatigue. Taken together, these results suggest that this deep learning approach may be an effective aid for increasing throughput and reliability for micro-CT workflows in preclinical studies.

#6316 Predictive performance comparison of foundational and CNN models for single-cell immune profiling.

Colwyn Jia Kang Lai[1], Wei Kit Tan[2], Marcia Zhang[3], Timothy Wang[4], Felicia Wee[5], Ai Lin Wang[6], Solomonraj Wilson[4], Tomotaka Ugai[7], Joe Sheng Yeong Poh[5], Jonathan A. Nowak[8], Shuji Ogino[9], Juha P. Vayrynen[10], **Mai Chan Lau**[4]

[1]School of Biological Sciences, Nanyang Technological University, Singapore, Singapore,[2]Bioinformatics Institute, Agency for Science, Technology And Research, Singapore, Singapore,[3]Faculty of Science, National University of Singapore, Singapore, Singapore,[4]Singapore Immunology Network, Agency for Science, Technology And Research, Singapore, Singapore,[5]Institute of Molecular and Cell Biology, Agency for Science, Technology and Research, Singapore, Singapore,[6]National Taiwan University, Taipei, Taiwan,[7]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA,[8]Department of Medical Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA,[9]Program in MPE Molecular Pathological Epidemiology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA,[10]Translational Medicine Research Unit, Medical Research Center Oulu, Oulu University Hospital, and University of Oulu, Oulu, Finland

Introduction

Characterizing the immune contexture is key to understanding the tumor microenvironment, developing biomarkers, and guiding therapeutic strategies. In our previous work on colorectal cancer (CRC), we used a pathologist-guided approach to annotate/count eosinophils and lymphocytes. Their abundance and spatial relationships with tumor cells revealed prognostic significance. While clinically valuable, identifying this demands significant pathologist effort. Advancements in AI, particularly foundational models trained on millions of histology images, have driven significant progress in digital pathology and hold promise to address this gap. However, their application to single-cell tasks remains underexplored. This study investigates the potential of foundation model and compares it with conventional convolutional neural network (CNN) using previously annotated eosinophils and lymphocytes in CRC histology images.

Methods

We used 578 CRC cases in The Cancer Genome Atlas with H&E-stained whole-slide images (WSIs) containing 8,836,393 cells, including 896,322 lymphocytes and eosinophils. For evaluation, 109 WSIs were held out as a test set, with 5 WSIs per density quartile selected from our previous study. The foundational model UNI (11.2M parameters) was fine-tuned and a ResNet18 (303M parameters) model was trained. Performance was evaluated using F1-score and AUROC, with Spearman's correlation accessing WSI-level cell density correlations.

Results

For eosinophil, ResNet18 achieved an F1-score of 0.841, AUROC of 0.986, and Spearman's correlation of 0.679, compared to UNI's 0.847, 0.990, and 0.697, respectively. For lymphocytes, ResNet18 scored 0.697, 0.932, and 0.686, while UNI achieved 0.719, 0.941, and 0.662. ResNet18 performed comparably to UNI despite simpler architecture, at both cell and patient levels. Predicted cell densities generally aligned with the annotated quartiles from previous work, particularly in the 1st and 4th quartiles. Moderate density correlations (0.66-0.69) were observed, but a pathologist's review of misclassified cells revealed imperfections in the semi-supervised cell annotations.

Conclusion

The H&E-based AI model for predicting immune cells offers a scalable alternative to manual annotation, enabling automated quantification of existing immune biomarkers like tumor-infiltrating lymphocytes and tumor-associated tissue eosinophilia. Adaptable to other tumor types, it also opens opportunities to discover novel spatial immune biomarkers by leveraging large H&E cohorts and integrating protein-stained immune cell data with histopathological knowledge. Future work will focus on enhancing performance through weakly supervised approaches using carefully selected training cells, and multi-cell type modeling to better capture the morphological characteristics of diverse cell types in a single model.

PREVENTION / EARLY DETECTION / INTERCEPTION: Development of Chemopreventive Agents and Preclinical Models
Poster Session

#6320 Benfotiamine and fursultiamine prevent glioblastoma cells growth, invasion and migration.
B. Keane, S. Senthil, **K. V. Ramana**;
Noorda College of Osteopathic Medicine, LLC, Provo, UT

Glioblastoma is the most common malignant and aggressive brain tumor in adults. Recent advances in surgical techniques and adjuvant therapies have modestly improved the overall survival rate. Thus, there is a need for novel therapeutic approaches and preventive strategies to control glioblastoma. Lipophilic derivatives of vitamin B1 have been used for many health conditions due to their substantial antioxidant and anti-inflammatory properties. However, their chemopreventive effects in glioblastoma are not known. This study investigated the chemopreventive potential of benfotiamine and fursultiamine using glioblastoma cells. Human glioblastoma (U-87) cell lines grown in Eagle's Minimum Essential Media (EMEM) were treated with various concentrations of benfotiamine and fursultiamine. Cell viability was determined by MTT assay in a time- and dose-dependent manner. Apoptotic cell death was determined by Annexin-V staining and live-dead cell assay kits. The expression of various pro-apoptotic and anti-apoptotic factors and other inflammatory factors, including Bad, Bax, Bcl-2, cIAP-1, cIAP-2, and procaspase 3, was determined using a human apoptosis antibody array. Our results suggest that benfotiamine and fursultiamine prevent the growth of U-87 cells in a dose- and time-dependent manner. Further, they prevented the invasion and migration of glioblastoma cells. They also elevated the caspase-3 activation and reduced the mitochondrial reactive oxygen species. These compounds also regulate the expression of various pro- and anti-apoptotic factors. These findings suggest vitamin B1 derivatives could prevent glioblastoma tumor growth by upregulating the caspase-3-mediated apoptotic signaling pathways. Next, we plan to inject glioblastoma cells into the athymic nude mice xenograft model and examine the growth of glioblastoma cells in the absence and presence of vitamin B1 derivatives.

#6321 Vialinin-A, an antioxidant isolated from edible mushroom *thelephora vialis*, inhibits colorectal cancer growth.

D. Lahori, D. Thornton, **K. V. Ramana**;
Noorda College of Osteopathic Medicine, LLC, Provo, UT

Colorectal cancer (CRC) is the third leading cause of cancer-related deaths in the United States. While CRC screening typically begins at age 45-50, its incidence is rising in younger populations, highlighting the need for diversified treatment options. Genetic predisposition, oxidative stress, and inflammation are recognized as major contributors to the pathogenesis of this disease. Current treatments, such as chemotherapy and surgical resection, often lead to unwanted side effects and risks, including potential cardiotoxicity from chemotherapeutics like anthracyclines. Vialinin-A, a compound isolated from the edible Chinese mushroom *Thelephora Vialis*, has demonstrated antioxidant and anti-inflammatory properties. However, its anti-carcinogenic mechanisms are not well known. Human colon cancer cells such as SW480 and CaCO-2 were treated with varying concentrations of Vialinin-A for 24 and 48 h. Cell viability was determined by MTT assay. Apoptosis was evaluated through the activation of caspase-3 and the use of a human apoptosis array to analyze the expression of key apoptotic markers. Additionally, a multiplex array was used to identify oncogenic, inflammatory, and apoptotic markers in treated cells. Next, we plan to use nude mice xenografts to examine the chemopreventive role of Vialinin-A in vivo. So far, our results indicate that Vialinin-A inhibits CRC cell growth in a dose-dependent manner and suppresses the generation of reactive oxygen species (ROS). Vialinin-A activates caspase-3 in growth factor-treated cancer cells. Furthermore, Vialinin-A modulates the expression of various apoptotic and inflammatory markers in CRC cells, supporting its potential role in anti-carcinogenic pathways. Ongoing experiments aim to further elucidate the molecular mechanisms through which Vialinin-A inhibits CRC growth.

#6322 Lipid-soluble vitamin B1 derivatives prevent pancreatic cancer growth and migration.

Nathan Kobus, Nancy Kaushal, **Kota V. Ramana**

Noorda College of Osteopathic Medicine,, Provo, UT

Pancreatic adenocarcinoma is a lethal type of cancer often diagnosed in the end stages, commonly leading to metastasis. Surgical resection and chemotherapy are not well curative, leading patients to seek alternative therapies via clinical trials. Lipid-soluble derivatives of thiamine, Benfotiamine (Ben), and Fursultiamine (FS) exhibit antioxidative and anti-inflammatory properties. However, their chemo-preventive efficacy is not well known. Therefore, we examined their chemo-preventive potential in pancreatic cancer cells. Human Pancreatic adenocarcinoma (MIA PaCa-2) and metastatic pancreatic adenocarcinoma (BxPC-3) cell lines were incubated for 24 h with varying concentrations (0-100 uM) of Ben and FS. Cell viability was determined by MTT assay. Our results indicate that both Ben and FS prevented the growth of MIA-PaCa2 and BxPC-3 cells in a time- and dose-dependent manner. Ben and FS treatments also increased the expression of caspase-3 and prevented the invasion and trans-well migration of pancreatic cancer cells. Further, human apoptosis array analysis indicates that Ben regulated the expression of various pro-apoptotic factors such as BAD and Bax and anti-apoptotic factors such as Bcl-xL and Bcl-2 in MIA PaCA-2 cells. In addition, human oncology array analysis indicates that Ben regulated the expression of various carcinogenic proteins such as amphiregulin, cathepsin D, HIF-1alpha, osteopontin, p27, and Kallikrein. Next, we planned to examine the in-vivo chemo-preventive efficacy of Ben using nude mice xenografts. So far, our data suggests that treatment with vitamin B1 derivatives is effective against pancreatic cancer cell growth in culture.

#6323 Cancer chemopreventive effects and mechanisms of *Citrus depressa* leaf extracts.

Z.-Y. Su, H.-Y. Chiang;
Chung Yuan Christian University, Taoyuan City, Taiwan

Many diseases such as cancer are closely linked to excessive intracellular oxidative stress and chronic inflammatory responses. Studies have demonstrated that dietary interventions can mitigate or prevent the progression of certain chronic diseases, which reinforces the significance of the 'prevention is better than cure' concept. This study evaluated the antioxidant capacity and chemopreventive mechanisms of *Citrus depressa* leaf (CDL) extracts in mouse JB6 P+ skin cells in inhibiting carcinogenesis induced by the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA). The preparation of various solvent extracts from CDL revealed a richness in flavonoids, which are key bioactive compounds commonly found in citrus fruits. CDL-WE (water extract) and CDL-95EE (95% ethanolic extract) (5-40 μg/mL) both effectively prevented the anchorage-independent growth of JB6 P+ cells with minimal cytotoxicity, but CDL-95EE was found to have superior effects. In addition, CDL-95EE promoted the expression of UGT1A and HO-1 enzymes at the protein level, enhanced the expression of Nrf2 pathway-related genes in mRNA, and decreased TPA-related ROS levels. The protein expression of DNA methyltransferases (DNMTs) and histone deacetylases (HDACs) was also reduced by CDL-95EE, which indicates that it is involved in epigenetic regulation. These findings demonstrate the chemopreventive capacity of CDL and endorse their use in creating agricultural byproducts from this resource.

#6324 Intercepting bladder cancer progression using androgen receptor inhibitor, apalutamide, in a carcinogen BBN-induced rat bladder tumor model.

Venkateshwar Madka[1], Gopal Pathuri[1], Surya Pratap Singh[1], Anil Singh[1], Anh Bao[1], Nicole Stratton[1], Shizuko Sei[2], John L. Clifford[2], Chinthalapally V. Rao[1]

[1]Center for Cancer Prevention and Drug Development, Department of Medicine, OU Health Stephenson Cancer Center, Oklahoma City, OK,[2]Division of Cancer Prevention, National Cancer Institute, Rockville, MD

Bladder cancer (BC) is the 2nd most commonly diagnosed genitourinary cancer. While the majority of cancers are diagnosed and treated at the non-muscle invasive stage; high recurrence rate, treatment resistance, and tumor progression cause significant mortality. Epidemiologic and preclinical data have long demonstrated clear differences in incidence and progression of BC between genders, suggesting strong association with hormonal pathways with development and progression in these tumors. Based on these studies, androgen receptor (AR) signaling pathway emerged as a critical player and a promising target for BC interception. Thus, in the current study, BC preventive efficacy of the nonsteroidal AR antagonist, apalutamide, was evaluated in a N-butyl-N-(4-hydroxyl)-nitrosamine (BBN)-rat BC model. At 7 weeks of age, fisher rats were randomized by weight into 4 groups (30 rats/group/gender: 24 BBN + 6 Vehicle). Starting at 8 weeks of age, rats were administered BBN by oral gavage (150mg/dose; twice weekly for 8 weeks) to induce BC. A week after BBN treatment, rats in each group received apalutamide by oral gavage (5 days/wk) at increasing doses, 0, 7.5, 15, or 30mg/kg BW, until termination. Male and female bladder tumors were assessed at 40 & 50 weeks of age, respectively. Body and organ weights, blood profile, liver and kidney enzyme function analysis suggested non-significant differences, indicating safety of the tested doses. BBN treatment induced bladder tumors in both genders, resulting in significantly larger bladders (~0.75±0.25g) compared to vehicle group (0.12±0.08g) with the incidence of large tumors (≥200mg) in 87% and 75% of male and female rats, respectively. Importantly, apalutamide treatment led to a significant reduction of bladder weights suggesting tumor growth inhibition in treated rats compared to control. Bladder weights were reduced by 60%-65% in males ($p<0.05$) and by 54%-63% in females ($p<0.05$) when compared to the untreated group. Moreover, the incidence of large bladder tumors was also significantly decreased with apalutamide treatment by 30%-47% in males ($p<0.05$-$p<0.01$) and by 32%-50% in females ($p<0.05$) in a dose dependent manner. Histopathological analysis of the tumor demonstrated a significant decrease in papillomas (40%-60% less vs control; $p<0.01$-$p<0.0001$), NMIBC (48%-76% less vs control; $p<0.001$-$p<0.0001$) and MIBC (>77% less vs control; $p<0.001$-$p<0.0001$) at all three doses tested. Biomarker analysis showed reduction in the proliferation markers Ki67 and cyclin D1 in apalutamide treated rats tumors. In conclusion, our study demonstrated that an AR antagonist, apalutamide, can intercept bladder tumor growth and progression in a preclinical rat model and warrants further investigation in clinical trials.

(Project funded 100% with Federal funds from NCI, NIH, DHHS, under Contract 75N91019D00020_75N91022F00002)

#6325 Zeaxanthin augments CD8⁺ effector T cell function by enhancing TCR stimulation.

Freya Q. Zhang[1], Jiacheng Li[1], Rukang Zhang[1], Jiayi Tu[1], Zhicheng Xie[1], Takemasa Tsuji[2], Hardik Shah[1], Matthew O. Ross[3], Ruitu Lyu[3], Junko Matsuzaki[2], Kelly Xue[1], Fatima Choudhry[1], Chunzhao Yin[1], Hamed R. Youshanlouei[1], Syed Shah[4], Michael W. Drazer[1], Marc Bissonnette[1], Yuancheng Li[5], Hui Mao[5], Jun Huang[6], Lei Dong[7], Rui Su[8], Chuan He[3], Kunle Odunsi[2], Jing Chen[1], Hao Fan[1]

[1]Medicine, University of Chicago, Chicago, IL,[2]Obstetrics & Gynecology, University of Chicago, Chicago, IL,[3]Chemistry, University of Chicago, Chicago, IL,[4]Internal Medicine, Rush University Medical Center, Chicago, IL,[5]Department of Radiology and Imaging Sciences, Emory University School of Medicine, Atlanta, GA,[6]Pritzker School of Molecular Engineering, University of Chicago, Chicago, IL,[7]University of Texas Southwestern Medical Center, Dallas, TX,[8]Beckman Research Institute, City of Hope, Duarte, CA

The evolution of diet has played an essential role in shaping human physiology and pathology, including the development of the immune system and responses to environmental stimuli. However, the specific mechanisms underlying the regulatory significance of dietary components in modulating anti-tumor immunity remain largely unknown. Here, we applied a co-culture screen approach using a blood nutrient compound library to identify zeaxanthin, a dietary carotenoid pigment found in many fruits and vegetables that is important for eye health, as an immunomodulator that enhances cytotoxicity of CD8⁺ T cells against tumor cells. Oral zeaxanthin, but not lutein, a zeaxanthin structural isomer, enhances anti-tumor immunity *in vivo*. Integrated multi-omics mechanistic studies reveal that zeaxanthin directly promotes T-cell receptor (TCR) stimulation on CD8⁺ T cell surface, leading to improved intracellular TCR signaling for effector T cell function. Hence, zeaxanthin treatment augments effectiveness of immune checkpoint inhibitor *in vivo* and human TCR-engineered CD8⁺ T cells to induce cell death against co-cultured tumor cells. Our findings uncover a previously unknown immunoregulatory function of zeaxanthin, which has translational potential as a dietary element in bolstering immunotherapy.

#6326 A comparative study of C3H and C57Bl/6 murine models of radiation-induced fibrosis.

Pierre-Antoine Bissey, Justin Williams, Wei Shi, Madison Hossack, Ken W. Yip, Fei-Fei Liu

Research, Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada

Introduction: Approximately 50% of cancer patients are treated with radiation therapy; ~60% of whom will experience significant radiation-induced fibrosis (RF) caused by excessive collagen accumulation. RF reduces tissue function and flexibility, increasing surgical complications during disease recurrence. Accurate and reproducible *in vivo* models are required to elucidate the mechanisms of this condition and to facilitate the development of effective therapeutic strategies. Although there have been comprehensive studies related to model development for other forms of fibrosis, the characterization and comparison of murine RF models remains limited in the literature. This work aims to assess and contrast C3H and C57Bl/6 mice as models for RF.
Methods: RF was assessed in two mouse strains, C3H and C57BL/6. Mice were irradiated using the Precision SmART+ irradiator. C3H mice received 40 Gy, while C57BL/6 mice received 10 Gy, 15 Gy, or 20 Gy. Following irradiation, the tibiofemoral joint angle was measured using X-ray images acquired weekly. At 4-, 8-, 12-, and 16-weeks post-irradiation, skin samples were collected, and collagen deposition was analyzed. Collagen was measured using: a) picrosirius red staining and polarized light microscopy; b) pro-collagen ELISA; and c) the hydroxyproline assay.
Results: The pattern of RF development was unique to each mouse strain. In C3H mice, three phases were observed: an acute response with increased limb stiffness, a recovery phase with reduced stiffness, and a fibrotic phase correlating with collagen accumulation, evidenced by histology and biochemical analyses. At 16 weeks post-irradiation, collagen deposition was observed to increase by 1.48- ($p<0.05$), 1.62- ($p<0.05$), and 1.32-fold *via* picrosirius, ELISA, and hydroxyproline assays, respectively. In C57BL/6 mice, 20 Gy induced RF without a recovery phase, 10 Gy did not induce fibrosis, while 15 Gy induced RF in 50% of cases, revealing unexpected variability despite genetic uniformity. At 16 weeks post-20 Gy irradiation, collagen deposition was observed to increase by 4.2- ($p<0.01$), 1.55-, and 2.69-fold ($p<0.05$) *via* picrosirius, ELISA, and hydroxyproline assays, respectively. Skin thickness following irradiation increased by 1.66-fold ($p<0.05$) in C3H mice, and by 2.35-fold ($p<0.01$) in C57BL/6 mice, compared to non-irradiated controls.
Conclusions: Measuring the tibiofemoral joint angle using weekly X-ray is non-invasive approach in assessing murine RF. The data reveal mouse strain-specific differences in RF. C3H mice may more accurately recapitulate the development of RF in humans. However, due to many more gain- and loss-of-function genetic models, C57BL/6 mice remain essential for investigating RF.

#6327 Prevention of glioblastoma cell growth by Vialinin A.

Chandu Goli, Roxana Nojoumi, Kota V. Ramana

Noorda College of Osteopathic Medicine, LLC, Provo, UT

Glioblastoma is regarded as the most aggressive and common type of primary malignant brain tumor in adults that is characterized by rapid growth of glial cells. It is mainly resistant to therapy and exhibits a universally poor prognosis. Therefore, there is a vital need to explore other alternatives to complement current standard treatment modalities. Recent studies have revealed that Vialinin A, a small antioxidant molecule isolated from the Chinese edible mushroom, has potential antioxidant and anti-inflammatory properties. However, its chemopreventive role is not clear. We now examined the chemopreventive efficacy of this compound in glioblastoma growth and invasion. The different capabilities of Vialinin A and downstream effects, such as cell viability, invasion, migration, and apoptosis induction, were explored in various experiments using human U-87 Glioblastoma cell lines obtained from ATCC. Our results indicate that Vialinin A prevents the growth of glioblastoma cells in a time- and dose-dependent manner. Vialinin A also prevented the invasion and trans-well migration of glioblastoma cells. Further, it also regulated the expression of various pro-apoptotic and anti-apoptotic factors and other inflammatory factors, including Bad, Bax, Bcl-2, cIAP-1, cIAP-2, and procaspase 3. These results suggest that Vialinin A promotes the cancer cell apoptotic pathways to prevent glioblastoma cell growth. Further, we plan to examine how Vialinin A prevents glioblastoma growth in nude mice xenografts.

#6328 Metformin induces cell cycle arrest and inhibits proliferation in *KRAS*-mutated colorectal cancer.

Devanshi Patel, Michelle Pham, Parthiv Medidi, **Holli Ann Loomans-Kropp**

The Ohio State University, Columbus, OH

Purpose: Our objective was to evaluate the potential of metformin for colorectal cancer (CRC) prevention.

Methods: HT-29 (*KRAS* wild-type) and SW837 (*KRAS* G12D mutated) CRC cells were used for *in vitro* analyses. Cells were treated with 10mM (HT-29) or 2mM (SW837) metformin for up to 96 hours. Proliferation was assessed by raw cell counts, CellTiter 96 Aqueous Proliferation Assay, and 0.5% crystal violet staining. Propidium iodide staining was used to evaluate cell cycle and analyzed on a BD FACS Aria III. Experimental results were analyzed using Student's *t*-test or Mann-Whitney U and expressed as the mean +/- standard deviation. Statistical analysis of *in vitro* experiments was performed in Prism 10.0 (GraphPad) and significance was set at $p<0.05$. Sequencing was completed using an Illumina NovaSeq X Plus (Novogene). Differential expression analysis was performed using DESeq2RPackage and enrichment analysis using clusterProfiler and GSEA for Go, KEGG, Reactome, DO, and DisGeNET. Statistical significance was set at an adjusted $p<0.05$.

Results: We found that metformin treatment of CRC cells *in vitro* significantly reduced proliferation and cellular growth after 48 and 96 hours in SW837, but not HT-29 cells. Cell cycle analysis showed that SW837 metformin treatment increased the percent of cells in G1, suggesting G1 cell cycle arrest. Sequencing analysis of SW837 cells identified 2738 and 2492 genes significantly up- and downregulated with metformin treatment, respectively. The top significantly differentially regulated genes were *ATF3*, *SNAI2*, *UPP1*, *GDF15*, and *SLC20A1*. Gene ontology analysis of metformin-treated SW837 cells showed differentially regulated genes involved in chromosome segregation, DNA replication, and cell cycle checkpoint, supporting our findings. Independent validation of top hits and key genes in pathways of interest, including *CDC42, BRIP1, EME1*, and *POLE2*, is ongoing.

Conclusions: Treatment of *KRAS*-mutated CRC cells with metformin significantly reduced cell proliferation and induced cell cycle arrest. Sequencing analysis showed significant regulation of cell cycle and proliferation-associated pathways, supporting our observed phenotype and providing insight into previously unidentified pathways regulated by metformin. The results from this study may have implications for the future use of metformin for CRC prevention.

#6329 Edible Chinese mushroom-derived antioxidant, vialinin A, prevents breast cancer cell growth.

Malak Rafik, Afnan Khan, Kota V. Ramana

Noorda College of Osteopathic Medicine, LLC, Provo, UT

Breast cancer ranks as the second most prevalent cancer worldwide, impacting millions annually, and stands as the leading cancer affecting women. Neoplasia occurs when free radicals, antioxidants, and reactive oxygen species increase in the cell, which can alter protein function and cause mutations and genomic instability. Current breast cancer treatments typically include chemotherapy and radiation. However, different breast cancer cell types have different antioxidative capacities, which allow them to resist these treatments, thereby reducing the efficacy of these expensive treatments. Several vitamins, antioxidants, and anti-inflammatory compounds have been tested for their effectiveness in preventing breast cancer. However, the role of vialinin A, an antioxidant and ubiquitin-specific peptidase inhibitor derived from edible Chinese mushrooms, is unknown. Therefore, in this study, we examined the chemopreventive effect of vialinin A in preventing breast cancer cell growth and migration. Human breast cancer cells (MCF-7) were treated with vialinin A in a time- and dose-dependent manner. Cell viability assay indicates that vialinin A prevents the growth of breast cancer cells in a dose-dependent manner. Further, vialinin also prevented the EGF-induced invasion of MCF-7 cells in a scratch assay. Vialinin A also prevented the EGF-induced trans-well migration of breast cancer cells. Vialinin A also regulated the growth factor-induced expression of various pro- and anti-apoptotic factors in breast cancer cells. Vialinin A also increased the activation of caspase-3 and cleavage of PARP in growth factor-treated breast cancer cells. Next, we plan to examine the chemopreventive efficacy of vialinin A in a nude mouse xenograft model. In conclusion, our results indicate that vialinin A could prevent breast cancer cell growth and invasion, suggesting its potential development as a chemopreventive drug.

#6330 Unveiling the anticancer potential of *Rheum webbianum* in colorectal cancer defense through modulation of key wnt/β-catenin and TGF-β signaling pathways: An in-vitro and in-vivo investigation.

Umer Majeed Khaja[1], Reena Singh[1], Showkat Ahmad Ganie[2]

[1]Bioengineering and Biosciences, Lovely Professional University, Jalandhar, Punjab, India,[2]Department of Clinical Biochemistry, University of Kashmir, Srinagar, Jammu and Kashmir, India

Purpose of the Study: Colorectal cancer (CRC) remains a significant health concern worldwide. Lifestyle and environmental factors are primary contributors to CRC, emphasizing the need for effective preventive strategies. *Rheum webbianum* (RW), a plant with a rich ethnopharmacological background, has demonstrated promising chemopreventive potential. In our previously published study on the ameliorative potential of *Rheum webbianum* extracts in DMH-induced colorectal cancer (U.M. Khaja et al., 2024), the ethanolic extract of RW demonstrated the highest ameliorating potential in both *in-vitro* and *in-vivo* experiments. Building on these findings, this study aims to further investigate the anticancer effects of RW ethanolic extracts through additional *in-vitro* and *in-vivo* assays, focusing on their impact on critical molecular pathways involved in CRC progression.

Materials and Methods: The study evaluated the anticancer potential of RW ethanolic extracts using the HT-29 human colon cancer cell line and a 1,2-dimethylhydrazine (DMH)-induced rat CRC model. In vitro assays assessed the effects of RW on cancer cell viability and migration, using cytotoxicity and scratch wound migration assays. Western blot analysis was conducted to explore the extract's influence on key signaling pathways, including Wnt/β-catenin and TGF-β, both of which are critical drivers of CRC progression and metastasis.

Results: *In-vitro*: The ethanolic extract of *Rheum webbianum* (RW) significantly inhibited the viability of HT-29 cells in a dose- and time-dependent manner, with up to 80% inhibition observed at a concentration of 200 µg/mL. Scratch wound migration assays demonstrated a substantial reduction in cancer cell migration, with progressively stronger inhibitory effects seen at higher RW concentrations. *In-vivo*: In the DMH-induced rat CRC model, molecular analysis revealed that RW extracts suppressed β-catenin expression, indicating inhibition of the Wnt/β-catenin signaling pathway, a hallmark of CRC progression. Furthermore, the extracts downregulated TGF-β1 expression, implicating their role in reducing pro-metastatic signaling. These findings highlight the extract's ability to target critical pathways involved in tumor growth and metastasis.

Conclusion: This study provides compelling evidence of the anticancer potential of RW ethanolic extracts. The results highlight their ability to inhibit cell viability, suppress migration, reduce metastatic behavior, and modulate key signaling pathways associated with CRC progression. These findings emphasize the potential of RW as a natural, plant-based chemopreventive agent and pave the way for future research to isolate active bioactive compounds and explore their applications in cancer prevention and integrative treatment strategies.

#6331 Prevention of lung cancer growth by an edible mushroom-derived p-terphenyl antioxidant, Vialinin-A.

N. Ratna, A. Namit, K. A. Ramana;
Noorda College of Osteopathic Medicine, LLC, Provo, UT

Lung cancer remains the leading cause of cancer-related deaths in the United States, surpassing the combined fatalities from colon, breast, and prostate cancer. In 2024, approximately 234,580 individuals were diagnosed with lung cancer, with projections indicating an increase in 2025. A history of smoking, both first and secondhand, the use of other combustible tobacco products, and hereditary risk factors have been strongly associated with lung cancer. Environmental exposures to radon, asbestos, arsenic, air pollution, and other occupational exposures exacerbate the likelihood of developing the disease. Novel treatment approaches are required to improve outcomes in lung cancer prognosis. Vialinin-A, a ubiquitin-specific peptidase inhibitor derived from Chinese edible mushrooms, T. terrestris and T. vialis, has potent antioxidant and anti-inflammatory properties. However, its potential anti-carcinogenic effects remain unexplored. Therefore, we hypothesize that Vialinin-A, with its potent anti-oxidative and anti-inflammatory effects, could prevent lung cancer growth. Human Non-Small Cell Lung Cancer (NSCLC) cell lines (A549) were used to investigate the potential chemopreventive role of Vialinin-A on lung cancer. An MTT assay was conducted to evaluate EGF-induced cell viability without or with Vialinin-A. The expression of pro-, anti-inflammatory, and apoptotic proteins was then assessed via multiplex antibody arrays. Mitochondrial reactive oxygen species (ROS) and a scratch array were then used to calculate cell migration. Cell death was observed using a live and death assay. Our results suggest that Vialinin-A could prevent EGF-induced lung cancer growth in a dose- and time-dependent manner. The expression of apoptotic proteins such as Catalase, Cleaved Caspase-3, cIAP-1, Claspin, SMAC/Diablo, and Clusterin was significantly upregulated in A549 cells treated with EGF+Vialinin-A. EGF-induced cell migration and invasion were prevented with Vialinin-A treatment. Vialinin-A also decreased the EGF-induced ROS and elevated caspase-3 levels. Thus, our data suggests that by increasing the caspase-3-mediated apoptotic pathway, Vialinin-A could prevent lung cancer growth and migration. Next, we will examine how Vialinin-A prevents lung cancer growth using nude mice xenograft models.

#6332 Targeting PARP for the prevention of BRCA1-deficient triple-negative breast cancers.

Amanda Lanier[1], John Clifford[2], Jamal Hill[1], Darian Coleman[1], Cassandra Moyer[1], Alejandro Contreras[1], Mark Albertella[3], Anna Staniszewska[3], Andrew Reynolds[3], Shizuko Sei[2], Powel Brown[1], Abhijit Mazumdar[1]

[1]UT MD Anderson Cancer Center, Houston, TX,[2]National Cancer Institute, Rockville, MD,[3]AstraZeneca, Cambridge, United Kingdom

Background: Triple-negative breast cancer (TNBC) tumors frequently harbor *BRCA1* gene mutations especially in women with a family history of breast cancer. It has been shown that *BRCA1*-deficient cells are highly sensitive to Poly ADP ribose polymerase (PARP) inhibitors as PARP inhibition and trapping leads to synthetic lethality due to increased genomic instability. PARP inhibitors are therefore effective treatments for breast cancers with *BRCA1* or *BRCA2* mutations and may have potential utility for cancer prevention and risk reduction strategies, in particular for women with germline *BRCA1* or *BRCA2* pathogenic variants. For this study, we hypothesized that PARP inhibitors will prevent the development of TNBCs in a mouse model of mammary tumorigenesis with *BRCA1* deficiency.

Methods: Two studies were conducted in immune competent models: (1) a dose and schedule finding study using syngeneic *BRCA1*-deficient graft model, and (2) a long-term study to determine whether a PARP inhibitor can prevent *de novo* tumor formation. In the dose-finding study, mammary tumor fragments from MMTV-Cre$^{+/+}$/BRCA1$^{co/co}$/p53$^{+/-}$ mice were transplanted into the mammary fat pads of MMTV-Cre$^{+/+}$/BRCA1$^{co/co}$/p53$^{+/+}$ mice. When these mice developed palpable tumors, they were treated by oral gavage with talazoparib or AZD5305. In the second series of experiments designed to determine the preventative efficacy of the PARP inhibitors, mice were treated with PARP inhibitors from 4 months of age until tumors reached 1,500 mm^3. Mice were observed daily for tumor formation and toxicity. Tumor incidence and time to tumor formation were visualized using Kaplan Meier curves and analyzed using the log-rank test.

Results: All three doses and schedules of talazoparib and AZD5305 significantly suppressed the growth of MMTV-Cre/BRCA1$^{co/co}$/p53$^{+/-}$ tumors in the syngraft model. Our current results from the long-term study show that (a) treatment with AZD5305 (at 0.5mg/kg; 5X/week for one week followed by 3 weeks off) significantly (p=0.0001) delays mammary tumor formation in MMTV-Cre/BRCA1$^{co/co}$/p53$^{+/-}$ mice with median survival increasing from 213 days to 383 days, and (b) daily treatment of AZD5305 (5 days treatment, followed by 2 days off) at a lower dose (0.125mg/kg) also significantly delays mammary tumor formation in MMTV-Cre/BRCA1$^{co/co}$/p53$^{+/-}$ mice with median survival increasing from 266 days to 320 days (p=0.004). Long term treatment with AZD5305 at these doses was not associated with any observable toxicity in this study.

Conclusions: Both talazoparib and AZD5305 greatly suppress the growth of syngrafted *BRCA1*-deficient tumors in mice. AZD5305 also delayed the development of TNBC mammary tumors in MMTV-Cre/BRCA1$^{co/co}$/p53$^{+/-}$ mice. Based on these results, in the future, PARP inhibitors may be effective drugs to reduce the risk of *BRCA1*-deficient breast cancer. (Supported by NCI-PREVENT to PB & AM)

#6333 Interactions between endocrine-targeting therapies and short-chain fatty acids in reducing ER+ breast cancer.

Alana Arnone, Adam S. Wilson, Valerie S. Payne, Katherine Ansley

Wake Forest Baptist Comprehensive Cancer Ctr., Winston-Salem, NC

Emerging evidence highlights the gut microbiome as a key player in estrogen receptor-positive (ER+) breast cancer development and recurrence, with breast cancer patients often exhibiting altered gut microbial profiles. Although oral endocrine-targeting therapies have proven effective in treating and preventing ER+ breast cancer recurrence, the risk of therapeutic resistance and tumor recurrence remains decades post-diagnosis. Whether these endocrine targeting therapies interact to shift the gut microbiome and whether modifying diet-microbe-metabolite interactions could enhance the efficacy of these therapies remains largely unexplored. Preliminary data indicate that supplementing with bacterial-derived metabolites, particularly short-chain fatty acids (SCFAs), may impact ER+ breast cancer by influencing tumor initiation, progression, and response to treatment. In both chemical-carcinogen-induced and spontaneous genetic MMTV-PyMT murine models of mammary tumorigenesis, exogenous SCFA supplementation increased tumor-free survival and reduced tumor multiplicity in the context of a high-fat diet. Additionally, in a 4T1.2ER+ syngeneic murine breast cancer model, exogenous administration of SCFA butyrate alone reduced tumorigenesis by modulating apoptosis (cleaved caspase 3) and immune infiltrate (F4/80 macrophages and infiltrating CD8+ T-cells). Further, exogenous administration of all three SCFA (butyrate, acetate, propionate) in mice on high-fat, low-fiber diet significantly reduced tumor growth and enhanced tamoxifen anti-tumor efficacy. SCFA interventions modulated tumor proliferation (Ki67), tumor apoptosis, and enhanced anti-tumor immunity (infiltrating CD8+ T-cells). Plasma butyrate levels were negatively correlated with breast cancer patient BMI, suggesting obesity interactions regulate gut microbial metabolite generation. To explore direct drug-microbe interactions we used an *ex vivo* colonic bioreactor and human donor fecal samples, we show that the aromatase inhibitor (AI) letrozole increased the proportional abundance of several SCFA-producing microbes. Moreover, metagenomic sequencing of DNA isolated from fecal samples obtained from postmenopausal breast cancer patients before and 3 months after AI treatment (NCT05030038) further revealed shifts in gut microbial populations, including increases in the proportional abundance of SCFA-producing species. These changes were associated with decreased circulating inflammatory cytokines. These findings suggest that SCFAs may suppress breast carcinogenesis, promote endocrine-targeting therapy responsiveness, and AI may directly modify SCFA-producing microbiota in breast cancer patients. SCFA may represent a promising adjunctive strategy to improve the outcomes of endocrine-targeting therapies for ER+ breast cancer patients.

#6335 Structure-activity relationship (SAR) studies identify region B capsaicin analogs with growth-inhibitory activity in human small cell lung carcinoma.

Amanda B. Sugrue[1], Kushal J. Modi[1], Reagan S. Light[1], Rama S. Gadepalli[2], John M. Rimoldi[2], Stephen D. Richbart[1], Justin C. Merritt[1], Piyali Dasgupta[1]

[1]Biomedical Sciences, Marshall University School of Medicine, Huntington, WV,[2]Biomolecular Sciences, University of Mississippi, University, MS

Purpose of the Study: The long-term research objective of our laboratory is to identify natural phytochemicals that could have potential applications for the treatment of human small-cell lung cancers (SCLC). Specifically, published data from our laboratory shows that capsaicin (the hot and spicy component of chili peppers) potently abrogates the growth and progression of human SCLC *in vitro* and *in vivo*. Conventionally, capsaicin is recognized as a powerful pain-relieving agent which is present in many over-the-counter creams and lotions. However, the clinical applications of capsaicin as a viable pain-relieving agent (and an anti-cancer drug) are hindered by its adverse side effects, such as gastric irritation, stomach cramps, and burning sensation. Such drawbacks of capsaicin may be circumvented by the rational design of non-pungent capsaicin analogs with comparable/greater bioactivity than capsaicin. The structure of capsaicin may be divided into three regions: Region A (the aromatic ring), Region B (the amide group), and Region C (the hydrophobic side chain). As a first step, we synthesized a panel of compounds with variations in Region C of capsaicin.
Experimental Procedures: The growth-suppressive activity of these Region C capsaicin analogs was evaluated using an MTT-based screening assay. The results of the MTT assay were confirmed by measuring the pro-apoptotic activity of these compounds. Based on our findings, our hit compounds were “ARVANIL” and “DOHEVANIL”. Using these two compounds as the template, we then sought to design analogs that would have different chemical groups in Region B of the molecule. The growth-suppressive activity of these Region B analogs was measured using an MTT-based screening assay in human SCLC cells. The results obtained from the MTT experiments were verified by soft agar assays and experiments in patient-derived organoids.
Results: SAR studies led to the identification of a panel of synthetic Region B capsaicin analogs with varying growth-inhibitory activity in human SCLC cells. The pro-apoptotic activity of these compounds correlates well with their ability to inhibit the viability of human SCLCs.
Conclusions: The Region B capsaicin analogs may have applications in the therapy of human SCLC. Our future studies will examine the anti-cancer activity of selected Region B capsaicin analogs in vivo.
Support or Funding Information Funding for our study was supported by a NIH R15-AREA Grant (2R15CA161491-03) and a Center for Natural Products pilot grant from the WV-INBRE Grant Program (P20GM103434, PI: Gary Rankin). Furthermore, this study was supported in part by an Institutional Development Award (IDeA) Grant number P20GM104932 from the National Institute of General Medical Sciences (NIGMS) and the Research Core B of COBRE, a component of the National Institutes of Health (NIH).

#6336 Preclinical efficacy of TRAIL inducing ONC201 against carcinogen (NNK)-induced lung adenocarcinoma in A/J mouse model.

Karthikkumar Venkatachalam[1], Venkateshwar Madka[1], Gopal Pathuri[1], Anil Singh[1], Anh Bao[1], Nicole Stratton[1], Shizuko Sei[2], Vignesh Gunasekharan[2], **Chinthalapally V. Rao**[1]

[1]Center for Cancer Prevention and Drug Development, Department of Medicine, OU Health Stephenson Cancer Center, Oklahoma City, OK,[2]Division of Cancer Prevention, National Cancer Institute, Rockville, MD

Lung cancer is the 2nd most commonly diagnosed cancer in both genders in the US. Most lung tumors are diagnosed at late stages and distant metastasis drastically reduces 5-year survival to <10%. Hence, intercepting lung tumor progression would aid in reducing tumor burden and cancer mortality. Preclinical and TCGA data demonstrated that the TNF Related Apoptosis Inducing Ligand (TRAIL/TNFSF10) is incrementally downregulated with lung tumor progression. Hence we evaluated ONC201 (Dordaviprone), a orally active TRAIL-inducing small molecule for its cancer preventive efficacy in the tobacco carcinogen Nicotine-derived Nitrosamine Ketone (NNK) driven A/J mouse lung cancer model. Prior to the efficacy studies, five different oral doses of ONC201 (0-200mg/kg BW) were tested in A/J mice (n=6/gender/group) for optimal dose range selection. For efficacy evaluation, A/J mice (6 weeks age; n=20/sex/group) were randomized into vehicle control and intervention groups; and lung tumors were induced by a single i.p. injection of NNK (2.07mg/mouse). Six-weeks after NNK injection, mice were given 0, 25, 50, or 100mg/kg BW ONC201 (in PBS) by oral gavage twice weekly for 28 weeks. Mice were then euthanized and lungs were evaluated for tumor incidence and multiplicity. All of the NNK-injected control mice developed lung tumors (100% incidence); but significant difference ($p<0.001$) was observed in tumor multiplicity between genders (17±2.18 in females (Mean±SEM) Vs 7.7±0.89 in males). ONC201 treatment led to significant decrease in tumor multiplicity at all 3 tested doses in both genders (25-43% less tumors; $p<0.05$-$p<0.001$). Lung tumors were classified as adenomas (AD), and adenocarcinomas (AdCa) based on histological criteria. Control mice developed 2.25±0.19 AD, and 5.45±0.69 AdCa in male; 3.89±0.57 AD, and 11±1.24 AdCa in female mice. While ONC201 treatment reduced the adenomas moderately, AdCa was significantly inhibited at all 3 doses in both genders in a dose dependent manner. In the female mice, AdCa multiplicity was reduced by 27% ($p<0.05$), 53% ($p<0.001$), and 70% ($p<0.0001$) in 25 mg/kg, 50 mg/kg, and 100 mg/kg BW ONC201 respectively. Similarly, AdCa was inhibited by 55% ($p<0.001$), 67% ($p<0.0001$), and 84% ($p<0.0001$) in male mice at the 3 dose levels as compared to controls. No toxicities were observed in all doses tested in both genders. Biomarker analysis of the lung tumors using IHC and Western blotting analysis suggested an induction of TRAIL signaling pathway markers, increased apoptosis, and a decrease in proliferation markers (PCNA, Cyclin D1, EGFR, Ki67). In conclusion, our data indicate a strong preventive efficacy of ONC201 in a preclinical model of lung cancer and warrant further development towards clinical applications for lung cancer prevention in high-risk cohorts. (*Project funded 100% with Federal funds from NCI, NIH, DHHS, under Contract 75N91019D00020_75N91022F00003*)

#6337 A novel PMS2 mutant Lynch syndrome mouse model for cancer prevention studies.

Kajal Biswas[1], Yurong Song[2], Vaishnavi Peddibhotla[3], Brandon Somerville[2], Mary Albaugh[3], Dillon Dierman[3], Rameesha Mustafa[3], Altaf Mohammed[1], Shyam K. Sharan[3], Robert H. Shoemaker[1]

[1]Chemopreventive Agent Development Research Group, Division of Cancer Prevention, National Cancer Institute, Rockville, MD,[2]Vaccine, Immunity and Cancer Directorate, Frederick National Laboratory for Cancer Research, Frederick, MD,[3]Mouse Cancer Genetics Program, Center for Cancer Research, National Cancer Institute, Frederick, MD

Mono-allelic germline pathogenic variants (GPVs) in MMR genes (*MLH1, MSH2, PMS2*, and *MSH6)* predispose individuals to hereditary non-polyposis colorectal cancer (HNPCC), better known now as Lynch syndrome (LS). Bi-allelic GPVs in MMR genes lead to a severe phenotype, constitutional MMR deficiency (CMMRD), that is characterized by early onset of leukemia/lymphoma, colorectal/gastrointestinal tumors, brain tumors, and sarcomas. MMR deficient mouse models have provided many mechanistic and translational insights. *Pms2*-null (*Pms2*$^{ko/ko}$) mice were found to be viable but showed an increase in microsatellite instability and developed lymphomas and sarcomas. One of the most frequently occurring human mutations in *PMS2* is a founder mutation NM_000535.5:c.2002A>G (p.I668V), observed in the Canadian Inuit population (1 in 16 Inuits are carriers). This mutation acts as a de novo splice site that creates a 5 bp deletion resulting in a truncated protein (p.I668*). A mouse model with an equivalent germline mutation (*Pms2c.1993A>G*) results in a splicing defect like that observed in humans and exhibits a significant increase in microsatellite instability and intestinal adenomas on an *Apc* mutant (*Apc*^{tm1Rak}) background. We are now further characterizing this model for intestinal tumor initiation and progression using time course experiments and comparing tumor development by histopathological examination with the *Pms2*$^{ko/ko}$ model. Moreover, we are also examining this model to identify neoantigens, that may lead to development of a new vaccine for *PMS2* mutation carriers.This mouse model can be a valuable tool to test the efficacy of new strategies for cancer prevention in PMS2 mutation carriers. We have developed a luciferase based splicing reporter assay in HEK293 cells that can be used to screen splicing modulators to suppress aberrant splicing of this splice site mutation. The efficacy of these modulators to suppress aberrant splicing in the small intestine of mice can be further tested using this mouse model.

#6338 CRISPR/Cas9 *in vitro* modelling of a VUS mutation found in Peruvian patients diagnosed with hereditary breast cancer.

S. R. Paredes-Moscosso, C. Villegas-Llerena, B. E. Quispe-Bravo, J. Buleje, M. L. Guevara-Fujita, R. Fujita;
Universidad de San Martin de Porres, Lima, Peru

Breast cancer is the most common neoplasm in women worldwide. In Latin America, rapid economic growth has driven epidemiological and demographic transitions that have increased the breast cancer burden. In Peru, breast cancer is the leading malignancy among women, with approximately 10% of cases linked to hereditary factors, primarily mutations in the *BRCA1* and *BRCA2* tumor suppressor genes. Many genetic variants are classified as Variants of Uncertain Significance (VUS), as their biological and clinical impacts remain unknown. In a previous work, our group identified six novel VUS in Peruvian families with hereditary cancer, including the c.5074+28T>A variant found in seven families. The overriding aim of the present study was to model the c.5074+28T>A VUS in a healthy breast tissue cell line (SVCT) using CRISPR/Cas9 technology. Gene editing was performed using ribonucleoprotein complexes (RNPs), whereby 03 different guide RNAs were tested. RNPs were transfected into SVCT cells by electroporation. Transfection and VUS insertion were verified via flow cytometry, T7EI assay, and Sanger sequencing. Single-cell clones were expanded, cryopreserved, and analyzed by PCR and enzymatic digestion (RFLP). Positive clones were further analyzed by Sanger sequencing, where 01 clone carrying the VUS c.5074+28T>A was identified (SVCT-VUS, clone C59). RT-PCR analyses showed no difference between unmodified SVCT cells (ctrl) and clone C59 at the mRNA sequence level. *BRCA1* gene expression was then assessed by qPCR (Taqman assay), where *BRCA1* downregulation was observed on SVCT-VUS compared to unmodified SVCTs. Although RNA from patients carrying the c.5074+28T>A variant was not available for analysis, SVCT-VUS cells showed a comparable *BRCA1* gene expression to MDA-MB-231 breast cancer cell line, which possess a reportedly reduced *BRCA1* mRNA and protein expression levels. Functional studies are still needed to confirm whether c.5074+28T>A affects *BRCA1* expression at a protein level. These findings demonstrate a proof-of-concept for a CRISPR/Cas9 cell-modeling platform to study VUS in Peruvian patients with breast cancer and other diseases.

#6339 Reprogramming SREBP1-dependent metabolism and inflammation in high-risk breast with licochalcone A for the prevention of breast cancer.

Atieh Hajirahimkhan[1], Elizabeth T. Bartom[2], xingyu Guo[3], Kyli Berkley[3], Ruohui Chen[2], Wonhwa Cho[3], Michael Avram[1], Susan E. Clare[1], Seema A. Khan[1]

[1]Surgery, Northwestern University - Chicago, Chicago, IL,[2]Northwestern University - Chicago, Chicago, IL,[3]University of Illinois Chicago, Chicago, IL

Breast cancer (BC) risk-reducing drugs have minimal impact due to their adverse effects and low acceptance. Novel non-endocrine agents with reduced toxicity and efficacy beyond HR+ BC are needed. We recently showed that in high-risk women's breast treated with licochalcone A (LicA) *ex vivo*, changes in metabolism flux led to reduced proliferation, NF-kB-dependent inflammation, and SREBP1-dependent lipogenesis. Additionally, LicA suppressed the proliferation of pre-malignant and malignant breast cell lines (HR+ and HR-) and their xenografts in mice. Now we present confirmatory evidence that LicA prevents BC by reducing SREBP1-dependent metabolism and inflammation. We also show the promising pharmacokinetic (PK) profiles of our novel oral formulations. We performed NanoString metabolism panel assay in MDA-MB-231 (HR-) and MCF-7 (HR+) cells treated with LicA (10 μM, 24 h) and analyzed data by ROSALIND. We also conducted Proteome Integral Solubility Alteration (PISA) proteomics and Enrichr analysis to confirm transcriptomic data and the main targets of LicA. We validated the findings with western blots. In addition, we evaluated spatiotemporal changes in cholesterol concentrations in the inner leaflet of the plasma membrane. Five proprietary novel formulations of LicA were developed and administered orally and intravenously to female BALB/c mice and Sprague-Dawley rats followed by LC-MS/MS analysis of the plasma and mammary tissue, and PK modeling using SAAM II. We observed significant (adj $P < 0.05$) upregulation of anti-inflammatory signals (up to 9-fold) such as HMOX1, and downregulation (up to 6-fold) of NF-kB-dependent inflammatory pathways such as prostaglandin E2 synthesis at the level of genes and proteins. We also observed significant downregulation of SREBP1-dependent lipogenesis signals such as ACAT2, FASN, SCD, and proliferation markers such as MKI67. Proteomics results further revealed a reduction in neddylation responsible for stabilizing SREBP1, and a metabolic shift from lipogenesis to the degradation of fatty acids and branched amino acids, and an enhanced TCA cycle. Western blots revealed significant suppression of SREBP1 (4-fold), and the reduced phosphorylation of PI3K (5-fold) and AKT (6-fold, at Ser 473). These results were confirmed by cholesterol depletion in the inner leaflet of the plasma membrane in HR- (8-fold) and HR+ (4-fold) cells. These results demonstrate that LicA reduces cell proliferation by suppressing SREBP1-dependent lipogenesis and inflammation. Our novel oral formulation of LicA, L13 showed promising PK ($C_{max} \approx 6$ μM, $T_{1/2} = 26$ h) suitable for BC prevention efficacy. Our data suggests that reprogramming SREBP1-dependent metabolism and inflammation by LicA prevents BC regardless of HR status. We will test L13 in immunocompetent models of BC to further establish its preventive efficacy.

#6340 Pancreatic cancer specific *Bmal1*-floxed mice reduced malignant potentials and prolonged survival.

Jinsuk Kim[1], Yukino Oshima[1], Ichie Kajiwara[1], Tomoaki Itaya[1], Tomoya Kuramochi[1], kaori saito[2], Taira Wada[3], Takahiro J. Nakamura[4], Shigeki Shimba[3], Hideaki Ijichi[5], Makoto Sano[1], Takahiro Suzuki[1]

[1]Department of Anesthesiology, School of Medicine, Nihon University, Tokyo, Japan,[2]Department of psychiatry, School of Medicine, Nihon University, Tokyo, Japan,[3]School of Pharmacy, Nihon University, Chiba, Japan,[4]Laboratory of Animal Physiology, School of Agriculture, Meiji University, Kanagawa, Japan,[5]Department of Gastroenterology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

Introduction: Dysregulation of clock genes has been reported in pancreatic ductal adenocarcinoma (PDAC), but the effects of the dysregulation on circadian rhythm, PDAC carcinogenesis, progression and prognosis remain unclear. To investigate the effects of dysfunctional *Bmal1*, a major clock gene, we compared *KPPC* (*LSL-Kras*$^{G12D/+}$*; Trp53*$^{flox/flox}$*; Pdx-1*$^{cre/+}$) and *KPPBC* (*LSL-Kras*$^{G12D/+}$*; Trp53*$^{flox/flox}$*; Bmal1*$^{flox/flox}$*; Pdx-1*$^{cre/+}$) transgenic mice with PDAC.

Methods: Six-week-old *KPPC* and *KPPBC* mice were housed in wheel cages, and the circadian rhythms were measured until the humane endpoint. Tumor status and plasma cytokine levels were determined using histopathological analysis and cytokine array, respectively.

Results: *KPPBC* mice retained wheel activity at the terminal stage and prolonged the survival rate compared to *KPPC* mice. The pancreatic tumor volume of *KPPBC* mice was smaller than that of *KPPC* mice. The proliferative Ki67 index and invasive frequency of PDAC in *KPPBC* mice were lower than in *KPPC* mice. Local MPO$^+$ tumor-associated neutrophils (TANs) and arginase-1$^+$ M2-like tumor-associated macrophages (TAMs) mice decreased in *KPPBC* mice compared to *KPPC* mice, whereas iNOS$^+$ M1-like TAMs and CD8$^+$lymphocytes increased in *KPPBC* mice compared to *KPPC* mice. Indeed, gastrocnemius muscle atrophy with cachexia was observed in both mice groups at the terminal stage, but average area of the gastrocnemiusmuscle in *KPPBC* mice was larger than in *KPPC* mice. Plasma levels of IGF-I and IGF-II in *KPPBC* mice were higher than in *KPPC* mice. The proliferation of PDAC cell lines with *Bmal1*-deficient was slower than that of PDAC cell lines with wild type of *Bmal1* in vitro.

Conclusions: These results suggest that inhibition of the proliferative potential of *Bmal1*-floxed PDAC could result from the direct effect of *Bmal1* deficiency and alteration of tumor microenvironment. Meanwhile, the delay of gastrocnemius muscle atrophy in *KPPBC* mice may be due to an increase in cytokines such as IGF-I and IGF-II.

#6341 Inhibition of 15-PGDH prevents tumor development in the AOM/DSS model.

Mariana Franco Fragoso[1], Filip Goshevski[1], Stephen P. Fink[1], Won Jin Ho[2], Sanford D. Markowitz[1]

[1]Case Western Reserve University School of Medicine, Cleveland, OH,[2]Johns Hopkins University School of Medicine, Baltimore, MD

Patients with ulcerative colitis (UC) face a cumulative risk of developing colitis-associated cancer (CAC) that is significantly higher than that of the general population. This increased risk is directly associated with the inflammatory process that occurs throughout the disease, creating the appropriate environment for the development of neoplastic lesions and impacting treatment decisions for these patients. Therefore, developing a drug capable of both mitigating the inflammatory response associated with ulcerative colitis and preventing tumor development would be an ideal therapeutic approach for UC patients. Using the murine dextran sulfate sodium (DSS) model of acute colitis, the Markowitz lab has previously demonstrated that inhibition of 15-PGDH, the primary enzyme responsible for PGE2 degradation, can markedly enhance tissue repair capacity in the colon as well as multiple additional organs (Science, 2015. PMID: 26068857). We demonstrated that treatment with (+)-SW033291 (‘291), a small molecule 15-PGDH inhibitor, increased the levels of tissue PGE2, near completely reversed colitis mucosal damage and promoted mucosal regeneration by sustaining colonocyte proliferation. We now aim to evaluate the safety of this 15-PGDH inhibitor in the context of CAC by testing ‘291 efficacy in the azoxymethane (AOM)/DSS model. Colon carcinogenesis was induced by a single dose of AOM (10 mg/kg) on day 1 of the experiment followed by 7 days of DSS treatment in drinking water (from days 8 to 15). Animals were treated with daily injections of ‘291 (10 mg/kg, twice daily, i.p.) or it is vehicle for 15 days (from days 8 to 22) and were monitored for signs of disease. Euthanasia occurred on day 47 of the experiment. At the time of sacrifice, colons were isolated and analyzed for the presence of macroscopic lesions and then fixed for histopathology and IHC staining (analyses that are in progress). Macroscopic evaluation of the colons demonstrated that inhibiting 15-PGDH reduced tumor number and tumor burden in the ‘291-treated mice when compared to vehicle controls by 50 and 60%, respectively. Additionally, treatment with ‘291 prevented body weight loss ($p=0.02$) during the disease course. This data indicates that ‘291 can not only reverse epithelial damage after DSS-induced colitis, but also be an effective strategy in preventing the development of colitis-associated dysplastic lesions in the AOM/DSS model. This data reinforces the rationale for developing a 15-PGDH inhibitor therapeutic to be used for patients with UC with the ultimate goal of mitigating colitis symptoms and preventing neoplastic lesion formation.

#6342 Ensuring diverse *in vitro* lung cancer models: development of alveolar epithelial cell lines from individuals of African ancestry.

Bianca Dal Bo[1], Sofia A. Lugo[1], David M. Lee[1], Kweku Ofosu-Asante[2], Kyle R. Phillips[3], Benjamin J. Ryder[3], Yong Huang[3], Nazarius S. Lamango[2], Ite A. Offringa[1]

[1]University of Southern California Keck School of Medicine, Norris Comprehensive Cancer Center, Los Angeles, CA,[2]Florida Agricultural and Mechanical University, Tallahassee, FL,[3]Department of Mechanical and Aerospace Engineering, University of Florida, Gainesville, FL

Lung cancer disproportionately affects Black/African American (B/AA) individuals, particularly men, who experience 12% higher incidence and 15% higher death rates than White/European (W/E) men. Lung adenocarcinoma (LUAD) originates in the alveolar epithelium and is the most common histological subtype of lung cancer for all racial groups. Immortalized human alveolar epithelial cells (ihAEC) are key tools to study the etiology and development of LUAD, as well as effects from environmental exposures. We recently established three ihAEC lines from remnant lung tissue of deceased unidentified W/E individuals using lentiviral transduction of simian virus 40 large-tumor antigen (SV40 LgT) and human telomerase reverse transcriptase (*TERT*) genes. SV40 LgT is an oncogene that promotes constant proliferation, while *TERT* prevents telomere shortening. Lentiviral vectors randomly integrate into the host genome, causing potential unwanted side effects. In addition, integrated transgenes may become silenced in the long-term. Furthermore, viral vectors that deliver oncogenes are a considerable biosafety concern that warrants avoidance whenever possible. Here, we describe an alternative gene-delivery approach that avoids the use of lentiviral vectors, and we use this new strategy to generate ihAEC lines of diverse ancestry. We have constructed a plasmid carrying SV40 LgT, an internal ribosome entry site, and *TERT*, as well as a puromycin resistance gene. The SV40 LgT sequence has been engineered with flanking loxP sites to allow its excision by Cre recombinase, if desired. To direct genomic integration by CRISPR/Cas9, the transgene is surrounded by homology arms that target integration into the adeno-associated virus integration site 1 (AAVS1), a safe harbor region that prevents inserted genes from being silenced. We will electroporate this plasmid into AECs isolated from remnant non-tumor lung tissue that was collected with full consent from lung cancer patients of diverse ancestry, including B/AA men. Development of new ihAEC lines will provide valuable tools for studying responses to environmental exposures, carcinogen detoxification processes, oncogenic transformation, and for testing novel therapies in models distinct from W/E genotypes. This work is an important step towards minimizing lung cancer health disparities caused by the absence of diverse model systems. Supported by grants U54CA233396, U54CA233444, and U54CA233465 from the National Institutes of Health (NIH)/National Cancer Institute (NCI), and the Norris Comprehensive Cancer Center core grant, award number P30CA014089 from the NIH/NCI.

#6344 Effects of psychological stress early in NNK-induced lung carcinogenesis in young female mice.

Dana H. Bovbjerg[1], Laura P. Stabile[1], Beatriz Kanterewicz[1], Jessica Manculich[1], Jessie R. Nedrow[1], Patrick Tarwater[2], Lora H. Rigatti[1], Frank J. Jenkins[1]

[1]UPMC Hillman Cancer Center, Pittsburgh, PA,[2]Texas A&M University, College Station, TX

Tobacco smoke contains many known carcinogens, chief among which is nicotine-derived nitrosamine ketone (NNK). However, even among individuals with high exposure levels, only a minority develop lung cancer. Prior research highlights additional exogenous (e.g., air pollution) and endogenous (e.g., estrogen exposure) factors that may increase risk. Currently lacking in the literature is consideration of possible effects of psychological stress. To experimentally test stress effects, we utilized a well-established mouse lung adenocarcinoma model with NNK exposure of young female mice, along with a mouse stress protocol. We previously reported that repeated exposure to stress before, during and after 4 weeks of exposure to NNK (IP; 2x/week; 3 mg/injection) increased both overall lesion size and the total number of lung lesions compared to controls when assessed 19 weeks post completion of NNK exposure. The present study used the same protocol to test stress effects earlier in the process of NNK carcinogenesis. FVB/N female mice (4-6 wk) bought from a commercial supplier were acclimated for two weeks before study initiation. The repeated stress protocol was then begun (Week-0) for the Stress group (n=40) and continued throughout the study, while the Control group (n=40) remained in their home cages. The stress protocol included social disruption via changes in cage mates at the time of routine cage cleaning (2x/week) and 90-minute restraint stress prior to a cage mate change (1x/week). NNK treatment began on week-1 (IP; 2x/week; 3 mg/injection) and continued for 4 weeks. At 8 and 15 weeks after the end of NNK exposure, 20 mice in each study group were euthanized. Lungs were formalin inflated. Surface lung lesions were counted under a dissecting microscope and lesion sizes were objectively measured (blind) using Motic Images software. Lungs from mice euthanized at 15 weeks were then imbedded in paraffin and serially sectioned (4 μm) to allow H&E confirmation of lesion numbers and sizes by a veterinary pathologist and to provide slides for ongoing immunohistochemical characterization. Statistical analysis (ANOVA) revealed significant main effects of study group, with lungs from stressed mice having larger overall lesion sizes (p=.007), and total number of lesions (p=.012), compared to controls. As expected, those two outcomes were also significantly increased in the lungs from mice euthanized at 15- vs. 8-weeks (no interaction with group). Providing independent corroboration of the Motic imaging results, data from the histological evaluation of lesion sizes and numbers were significantly correlated (p's <.001). Conclusion: We replicated and extended our initial study demonstrating effects of repeated stress in an NNK model of lung cancer in young female mice to earlier phases of NNK-induced lung carcinogenesis.

#6345 Establishing a preclinical cell model to assess the impact of metabolic status on the efficacy of preventive therapies: insights for the COLO-PREVENT trial.

Eve Jarman, Catherine Andreadi, Karen Brown

University of Leicester, Leicester, United Kingdom

Despite numerous advances in colorectal cancer (CRC) treatment, it continues to impose a significant global burden. The COLO-PREVENT polyp prevention trial platform seeks to address this by investigating interventions in 'elevated-risk' cohorts identified through the NHS Bowel Cancer Screening Program (BCSP) by repurposing the drugs aspirin and metformin, as well as the dietary compound resveratrol. The seAFOod polyp prevention trial, which recruited a similar population through BCSP, reported that 82% of participants were classified as overweight or obese (BMI ≥ 25) (Hull, 2018, Lancet). This suggests that COLO-PREVENT participants may also exhibit metabolic dysregulation, potentially modulating the efficacy of preventive interventions. Obesity and type 2 diabetes, both linked to metabolic dysregulation, are well-established risk factors for CRC, supported by both preclinical and epidemiological studies. This study aims to develop an *in-vitro* model of metabolic dysregulation associated with high-fat diet consumption. A non-tumorigenic cell line derived from a colonic tubular adenoma (Williams, 1990, Cancer Res.) was used to explore interactions with therapies under investigation in COLO-PREVENT. Preventive activity will be assessed using MTT assays for cell viability, measurement of free fatty acid levels, analysis of reactive oxygen species (ROS), and confocal microscopy to visualize morphological changes and fatty acid droplet localization in response to treatment. Initial results indicate that clinically achievable concentrations of COLO-PREVENT therapies increase cytotoxicity when cells were cultured under normal conditions. The effect was observed after 72 h of incubation with both single agents and low-dose combinations. Among single agents, only 1 µM aspirin significantly reduced cell viability ($p \leq 0.05$). Low-dose combinations further enhanced cytotoxicity, with 0.5 µM aspirin and 0.005 µM resveratrol increasing cytotoxicity to 37%, while 0.625 µM metformin with 0.0005 µM resveratrol increased cytotoxicity to 65% compared to vehicle control. When exposed to the lipids palmitic acid (PA) and oleic acid (OA) to mimic high fat intake, cells showed enhanced cytotoxicity with PA, while OA exposure had a weak proliferative effect. High concentrations of PA (1 and 5 mM) significantly reduced cell viability, causing 73% and 89% cytotoxicity, respectively, compared to control ($p < 0.001$). In contrast, OA exhibited weak proliferative activity across most tested concentrations. These preliminary findings suggest that metabolic dysregulation may influence the growth of colorectal adenomas and modulate the efficacy of preventive therapies. Further studies are warranted to investigate mechanisms underlying potential interactions and how this might influence precision prevention within the COLO-PREVENT framework.

#6346 The chromatin remodeler SMARCA5 selectively shapes nuclear receptor signaling in African American prostate cancer.

Shahid Hussain[1], Debasis Nayak[1], Sajad A. Wani[2], Isra Elhussin[3], Michael R. Freeman[1], Christopher C. Coss[4], Solomon O. Rotimi[5], Adam R. Murphy[6], Clayton Yates[3], Moray J. Campbell[1]

[1]Cedars-Sinai Medical Center, West Hollywood, CA,[2]University of Cincinnati, Cincinnati, OH,[3]Johns Hopkins School of Medicine, Baltimore, MD,[4]The Ohio State University, Columbus, OH,[5]Covenant University, Ota, Nigeria,[6]Northwestern University, Chicago, IL

SMARCA5 is found in distinct chromatin remodeling complexes where it regulates ATP hydrolysis to control nucleosome positioning required for gene expression and DNA repair. Previously, we established the chromatin remodeling enzyme SMARCA5 is significantly down-regulated in African American (AA) compared to European Americans (EA) prostate cancer (PCa). We have now explored how SMARCA5 distorts the epigenome and impacts transcription, as well as cell phenotypes. Firstly, we revealed that the H3K27ac cistrome had significantly different distributions between AA (RC43N) and EA (HPr1AR) normal cells, and AA (RC43T) and EA (LNCaP) PCa cells. Motif enrichment in H3K27ac revealed significant differences with Helix-Loop-Helix motifs enriched in RC43N compared to HPr1AR; and STAT motifs enriched in RC43T compared to LNCaP. Similarly, nuclear receptor (NR) motifs were distinct with vitamin D receptor (VDR) and orphan receptors (e.g., RORG) enriched in RC43T compared to LNCaP. Next we up-regulated SMARCA5's using dCas9-VP64-activator in the same cells and tested the impact on gene expression with RNA-Seq following treatment with the VDR ligand ($1,25(OH)_2D_3$, 100nM). SMARCA5 regulated ~1200 genes in both RC43T and RC77T, and only ~200 genes in LNCaP. The SMARCA5-dependent genes in AA PCa cells models were significantly enriched for luminal-differentiation genes. Likewise, in the AA compared to EA PCa models, GSEA analyses revealed enrichment for inflammation responses, and distinct NR signaling, such as progesterone signaling. Likewise LISA analyses revealed enrichment in the SMARCA5-dependent genes in AA PCa for progesterone signaling. Finally, we also identified a subset of miRNA related to differentiation that were uniquely regulated in AA PCa models by $1,25(OH)_2D_3$ and a significant number were also SMARCA5-dependent. Ongoing studies are addressing the effect of SMARCA5 on chromatin accessibility using ATAC-Seq. Overall, these studies support the concept the epigenome is shaped by genomic ancestry. The epigenomic-regulator SMARCA5 is significantly downregulated in AA PCa and impacts NRs, including VDR signaling. These findings suggest that African ancestry shapes SMARCA5 functions, NR signaling, and tumor outcomes.

#6347 Epithelial stem cells in tumorigenesis of the lung: lessons from a murine model of lung adenocarcinoma.

Bridget K. Greuel[1], Thomas Danhorn[2], Caroline Hauer[1], Meredith Tennis[1], Alison Bauer[3], Daniel T. Merrick[4], Debashis Ghosh[5], Robert L. Keith[6], York E. Miller[6], Moumita Ghosh[1]

[1]Pulmonary Sciences and Critical Care Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO,[2]Pharmacology, University of Colorado Cancer Center, University of Colorado Anschutz Medical Campus, Aurora, CO,[3]Environmental and Occupational Health, Colorado School of Public Health, University of Colorado Anschutz Medical Campus, Aurora, CO,[4]Pathology, School of Medicine, University of Colorado, Aurora, CO,[5]Biostatistics and Bioinformatics, Colorado School of Public Health, University of Colorado Anschutz Medical Campus, Aurora, CO,[6]Rocky Mountain Veteran Affairs Medical Center, Aurora, CO

Introduction: Lung cancer is the leading cause of cancer-related deaths, with a poor five-year survival rate. The period of premalignancy is marked by structural and functional alterations in the epithelium that eventually progress to cancer. Epithelial stem/progenitor cells are essential for the maintenance and repair of the airway epithelium. Prior research from our group demonstrated a reduction in the function of normal progenitors in human lung premalignancy. However, studying progressive changes in the epithelium as it advances from normal to premalignant is challenging in humans. This led us to conduct a time-course study of urethane-induced lung adenocarcinoma (ADC) in mice to better understand the role of normal tissue stem cells in tumorigenesis.

Methods: Tumorigenesis was induced in A/J mice (12 mice/group) by a single intraperitoneal injection urethane (1mg/gm), and control mice were injected with saline. Lung tissues were harvested at 2-, 4-, 10-, 16-, and 22-week post-injection; the left lobe was used for histological analysis, and the rest of the lung tissue was digested proteolytically to generate single-cell suspensions. Stem cell function was determined by organoid-forming assay, and molecular analysis of organoids was performed to identify genetic changes in stem cell populations.

Results & Conclusions: Histological analysis revealed that the premalignant changes induced by urethane closely resembled the lesions observed in human lung ADC, including adenomatous hyperplasia, nodular hyperplasia, and adenoma. Immunofluorescence staining (IF) with cell type-specific markers to track the progression of these changes identified hyperplasia of club cells ($CCSP^+$) at 2 weeks, followed by proliferation of type II (SPC^+) cells, the formation of adenomas by 10 weeks, and the development of adenocarcinoma at 22 weeks. Quantification of organoids at all time points showed fewer organoids in urethane-treated mice compared to saline at 2 weeks, but the reverse was true at subsequent time points when greater numbers of organoids were made by the urethane-treated mice (*$p<0.01$ for both directions*). IF staining of organoids identified both airway and alveolar organoids, and there was no difference in their proportions between saline and urethane-treated mice. Interestingly, a small number of bronchoalveolar organoids containing both club and type II cells were detected exclusively in the saline-treated mice that were absent in the urethane group. Significantly, whole exome sequencing of organoids identified several oncogenic mutations in organoids from urethane-treated mice but did not detect mutation in Kras, the known driver for urethane-induced ADC. Thus, our results pointed to an early response of stem cells following urethane exposure, with notable shifts in their behavior during premalignant progression to cancer.

#6348 Cholecystokinin receptor blockade modulates in vitro fibrosis gene expression.

Martha Gay, Hong Cao, Jill P. Smith

Medicine, Georgetown University, Washington, DC

Introduction: Cholecystokinin (CCK) receptors, G-protein coupled receptors, play crucial roles in gastrointestinal physiology, but their overexpression in cancer and fibrosis is implicated in disease progression and therapeutic resistance. Recent evidence suggests that activation of CCK receptors in hepatic stellate cells (HSCs) induces fibrosis-associated gene expression, contributing to liver pathology in conditions like metabolic dysfunction-associated steatohepatitis (MASH) and hepatocellular carcinoma (HCC). We have shown that a diet high in saturated fat induces the expression of hepatic CCK- B receptors in a murine model of nonalcoholic steatohepatitis. The CCK receptor antagonist, proglumide, reversed inflammation, fibrosis, and steatosis prevented the development of HCC in the murine Metabolic dysfunction-associated steatohepatitis (MASH) model.
Hypothesis: The blockade of CCK-B receptor signaling using proglumide will reduce the activation of fibrosis-related genes and prevent the progression of liver injury and carcinogenesis in both murine and human in vitro models.
Methods/ murine cells: Using C57BL/6 mice we will follow the standard protocol for isolation of murine HSC. Stellate cells will be isolated and cultured then stimulated with CCK *in vitro* (1-100nM). Another group of stellate cells will be treated with CCK and proglumide to determine if proglumide can prevent activation in fibrosis genes. Stellate cellular protein will be also extracted and subjected to Western analysis for fibrosis/collagen associated proteins. Macrophages release cytokines/chemokines that activate stellate cells. For our co-culture experiments we will culture murine RAW264.7 macrophages with the mouse HSC to activate fibrosis genes and proteins by cytokine /chemokine activation of stellate cells.
Methods/ human cells: We will use the immortalized LX-2 human HSC line to evaluate the activation of the fibrosis genes with addition of CCK to the growth media and examine if proglumide prevents the activation of the LX-2 cells and perform independently CCK-B or CCK-A receptor CRISPR to determine if CCK-induced fibrosis activation is mediated by the CCK-B or CCK-A receptor. Clonal CRISPR CCK-receptor KO stellate cells will also be activated by co- culture and /or with spent media from our human U937 macrophages.Anticipated outcomes: When stellate cells are grown on plastic *in vitro*, they become activated. We know the cytokines / chemokines released from macrophages will stimulate stellate cells, but the stimulation and collagen synthesis may not be as pronounced if the stellate cells were not already in an activated state. Ongoing data collection.
Conclusion: This investigation aims to clarify the molecular mechanisms by which CCK receptor signaling drives hepatic fibrosis and explore the therapeutic potential of proglumide in reversing fibrosis-related gene expression in liver disease.

#6349 Degradation of Aurora Kinase A, Alters Immune Checkpoint Expressions in Prostate Cancer.

S. Shukla[1], A. Venkatesh[1], J.-P. Kanumuambidi[1], R. D. Rodriguez Rosales[1], M. Al-Toubat[1], S. Sparks[1], A. Yamaguchi[1], S. Pogash[2], S. Fletcher[2], K. Balaji[1];
[1]University of Florida, Jacksonville, FL, [2]University of Maryland, Baltimore, MD

Pre-metastatic niche of tumor microenvironment supports tumor progression and regulates cancer cell survival. Aurora kinase A is a key molecule implicated in establishing this niche by promoting tumor cell migration, invasion, and survival within the secondary organ, thus contributing to metastasis. The Cancer Genome Atlas program data suggested increased expression of Aurora Kinase A (AURKA), Ninein (NIN) and Aurora Kinase A Ninein Interacting Protein (AUNIP) in prostate cancer as compared to normal prostate tissue. We are exploring how these interactions might influence immune cell infiltration, both in immune-responsive and immune-suppressive cells. Regulation of immune checkpoint proteins like PD-L1 and LAG3 is crucial for immune evasion by cancer cells. This study investigates the effects of a novel AURKA degrader compound, SP-02-067, on the downstream signaling cascade that regulates immune checkpoints PD-L1 and LAG3. We analyzed expression data of 702 prostate cancer patients using the Tumor Immune Microenvironment Estimation Resource and involved various immune responsive (CD8+; dendritic cells; natural killer) and immune suppressive (T-reg; myeloid derived suppressor cells; neutrophils) cells. Gene expression analysis showed significant correlation between NIN and PD-L1 ($R = 0.12$, $p = 0.01$), NIN and LAG3 ($R = -0.22$, $p < 0.001$). Combined immune responsive and combined immune suppressive cells were significantly associated with AURKA ($R = 0.03$, $p = 0.24$), NIN ($R = 0.1$, $p = 0.1$) and AUNIP ($R = 0.13$, $p = 0.21$) expression. Next, we treated prostate cancer C4-2 cells with varying concentrations of the novel SP-02-067 compound in a time-dependent manner, which led to degradation of AURKA and altered expressions of PD-L1 and LAG-3. Using an siRNA gene silencing approach for AURKA and AUNIP in C4-2 cells showed increased expression of PD-L1 and decreased LAG3 expression. The data suggests that increased expression of PD-L1 may be associated with an increase in tumor infiltrating cells, which could improve the efficacy of subsequent immunotherapy. Spearman correlation showed a positive correlation with infiltrating immune-responsive CD8+ T cells, dendritic cells, and NK cells. Similarly, immune suppressive regulatory T-cells and neutrophils were positively correlated with LAG3. Based on these findings, we suggest that SP-02-067, a novel degrader of AURKA, could be developed as a promising agent for prostate cancer and inspire innovative approaches to overcome drug resistance in prostate tumor-targeted therapy.

Wednesday, April 30, 2025

CLINICAL RESEARCH: Early Detection Biomarkers 2
Poster Session

#6354 ATTUNE: optimizing the treatment journey for lung cancer patients by predicting EGFR mutation from routinely stained histology images.

Talha Qaiser[1], **Harish RaviPrakash**[2], Jose Domingo Salazar[3], Marco Rosati[4], Alma Andoni[5], Guillem Portella[6], Adriana Hepner[7]

[1]Computational Pharmacology and Safety Sciences, AstraZeneca UK, Cambridge, United Kingdom,[2]Oncology Data Science, AstraZeneca US, Waltham, MA,[3]Oncology Data Science, AstraZeneca US, Cambridge, United Kingdom,[4]Cancer Biomarker Discovery, AstraZeneca, Munich, Germany,[5]Cancer Biomarker Development, AstraZeneca US, Gaithersburg, MD,[6]Precision Medicine and Biosamples, AstraZeneca UK, Cambridge, United Kingdom,[7]Clinical Development, AstraZeneca US, Gaithersburg, MD

Background: Identifying targetable mutations through tissue-based molecular testing is part of the standard of care management of patients with NSCLC. Nevertheless, globally up to 50% of lung cancer patients are currently not being tested. Liquid biopsy can provide valuable material for molecular diagnosis; however, low sensitivity, especially in early-disease stages, may prevent its broader applicability in the clinic. Predicting EGFR status from digitalized hematoxylin and eosin (H&E) images using machine learning (ML) could be key to addressing such unmet needs.
Methods: We propose an automated deep learning algorithm that retrospectively analyzes H&E images for EGFR status in a pivotal clinical trial (ADAURA), in early stage resectable EGFRm NSCLC and in a publicly available data from TCGA; totaling more than 1,000 patients. The proposed algorithm includes automated tumor segmentation (trained using all TCGA data) and prediction of EGFR mutation from tumor regions with weakly supervised learning (trained using 80% of ADAURA). The segmentation was validated on ADAURA and the proposed EGFR mutation prediction task was validated on held-out 20% of ADAURA and all of TCGA data.
Results: We outperformed existing published solutions by a margin of 10% in terms of the AUC metric. The model achieved the AUROC of 0.81 ± 0.04 and 0.75 ± 0.01 on over 100 phase III clinical trial patients and over 500 patients in TCGA, respectively. The model can be calibrated to achieve either > 90% sensitivity or specificity, depending on the clinical use case.
Conclusion: The proposed approach does not require any regions marked by pathologists to predict EGFR status and our results reinforce using H&E digital pathology slides with ML to predict molecular status, thereby optimizing the treatment journey for millions of lung cancer patients.

TUMOR BIOLOGY: Heterogeneity of Pediatric Cancers and their Microenvironment
Poster Session

#6439 ROME, a novel membrane protein in vertebrates, is a key modulator of embryonal development and cancer metastasis.

Anna Molotkova, Emre Deniz, Matthew Swift, Eric Glasgow, Jeffrey Toretsky, Aykut Uren

Georgetown University Medical Center, Washington, DC

Despite many advances in cancer treatment, metastasis remains the major cause of mortality: over 90% of cancer-related deaths are due to metastasis. We chose Ewing sarcoma (ES) as a model to study cancer metastasis since less than 25% of ES patients with metastatic disease live beyond five years and there are no effective therapies available to specifically target metastatic disease. Through an *in vivo* genome-wide CRISPR/Cas9 transcriptional activation screen using human ES cells as xenografts, we identified the human *INAFM2* gene as a strong driver of metastasis and therefore named the vertebrate gene and its protein product, ROME (Regulator of Metastasis). Up until now, *INAFM2* has not been studied in-depth and INAFM2 protein expression profile or function is not known. We characterized aspects of ROME expression and function in vertebrate development, physiology, and pathology. Blocking ROME expression in zebrafish embryos resulted in severe developmental defects and early mortality. Single cell RNA sequencing of zebrafish embryos with rome knockdown and RNA sequencing of cancer cell lines with ROME modulation revealed that ROME negatively regulates calcium and canonical Wnt pathways. We also discovered that ROME directly interacts with CAV1, FLOT1, and Vimentin proteins. ROME overexpression increased metastasis of ES xenografts in zebrafish and immunodeficient mice while ROME knockout in ES xenografts reduced metastasis. Human tumor RNA sequencing data revealed a negative correlation between ROME expression and patient survival. In summary, we discovered a previously unstudied human glycoprotein located on the plasma membrane that can regulate cell motility and invasion, is essential for normal development in vertebrates, and increases the metastatic phenotype of human tumor cells.

#6440 Spatial transcriptomics of Ewing sarcoma: evolution of tumor associated extracellular matrix from localized to metastasis-associated primary Ewing sarcomas.

Christopher Kuo[1], Krinio Giannikou[1], Nuoya Wang[2], Mikako Warren[1], Andrew Goodspeed[3], Masanori Hayashi[4], Micha S. B. Raredon[5], James F. Amatruda[1]

[1]Children's Hospital Los Angeles, Los Angeles, CA,[2]Yale School of Medicine, New Haven, CT,[3]University of Colorado, Anschutz Medical Campus, Denver, CO,[4]Children's Hospital Colorado, Denver, CO,[5]Anesthesiology, Yale School of Medicine, New Haven, CT

Objective: To date, the response rate of immunotherapy in Ewing sarcoma (EwS) has been poor. The tumor immune microenvironment (TME) has a vital role in cancer survival and progression with implications in drug resistance and immune escape. However, there is a lack of understanding of the TME of EwS. Although recent single-cell RNA sequencing of EwS and immune cells have revealed a heterogenous transcriptional landscape with distinct subsets of immune cell populations in EwS, little is known about the spatial organization and spatial microenvironmental niches of the TME of EwS.
Methods: We performed Spatial Transcriptomics (ST) on 16 primary pre-treatment biopsied EwS tumors (10 with localized EwS (L-EwS); 6 with metastatic EwS (M-EwS)).
Results: Integrated analysis revealed inter- and intra-tumoral heterogeneity. We inferred the stromal regions of tumors transcriptionally and noted that tumors from L-EwS had significantly more stromal signature than M-EwS (p-value <2.2e-16). Gene set enrichment analysis using Hallmark gene sets revealed that L-EwS tumors were enriched in epithelial mesenchymal transition (EMT) and inflammatory signatures (adjusted p-value <0.05) and M-EwS tumors were enriched in proliferative signatures (adjusted p-value <0.05). We identified a set of 46 unique genes enriched in L-EwS that overlapped with genes in EMT hallmark pathways and were predominantly extracellular matrix related (ECM-r) genes such as *COL3A1, ITGB1, MMP2, ACTA2, TNC, TGFB1*. These ECM-r genes spatially overlapped with the stromal regions of individual tumors. Furthermore, gene composite analysis demonstrated spatial colocalization of macrophages and cancer-associated fibroblasts (CAFs) within the ECM-r regions of the tumors. Spatially informed cell type deconvolution revealed EwS tumor regions that correlated well with histology. Moreover, majority of macrophages/monocytes in L-EwS tumors colocalized with CAFs. Deconvolution analysis also uncovered previously unreported B cell infiltrates and CD8 T cell infiltrative tumors. Spatial cellular microenvironment analysis revealed immune microenvironmental signaling throughout all tumors and unique ECM microenvironmental signaling predominantly in L-EwS compared to M-EwS (p-value <0.05) (TGF-B, cell adhesion and ECM remodeling signals).
Conclusion: L-EwS tumors are enriched in ECM and ECM-related microenvironmental signals compared to M-EwS. Spatial cellular microenvironmental signals reveal that components of the ECM may serve as anchors for tumors to eventually metastasize, nutrients for EwS to proliferate, and most importantly as a communication network with host immune cells. Further studies will be performed on a novel immunocompetent zebrafish genetic EwS model to identify conserved mechanisms of host-tumor interactions and further dissect the role of ECM in EwS.

#6444 PP2A activation targets PAI-1/CCL2 to suppress therapy-induced senescence in neuroblastoma.

Nazia Nazam[1], Maryam Ghufran[2], Morgan L. Brown[2], Sorina Shirley[1], Jerry E. Stewart[2], Michael Ohlmeyer[3], Taylor Schanel[4], Elizabeth A. Beierle[2]

[1]Pediatric Surgery, University of Alabama at Birmingham, Birmingham, AL,[2]University of Alabama at Birmingham, Birmingham, AL,[3]Atux Iskay LLC, Plainsboro, NJ,[4]Department of Radiation Oncology, University of Alabama at Birmingham, Birmingham, AL

Background: Neuroblastoma (NBL) is the most common extracranial childhood solid tumor, and it is responsible for 15% of pediatric cancer deaths. Refractory and relapsed disease are exceedingly difficult to treat and the survival for these children is less than 50%. One hypothesis to explain this poor therapeutic response and tendency for disease recurrence is therapy-induced senescence (TIS) which is a temporary state of growth arrest following treatment. Protein phosphatase 2A (PP2A), a known tumor suppressor, is downregulated in NBL. Preliminary studies indicate that PP2A reactivation reduces TIS in NBL, therefore, we investigated the effects of a novel PP2A activator as a senotherapeutic in NBL.
Methods: We employed the human NBL cell line, SK-N-AS. Topotecan, a common NBL chemotherapeutic, known and confirmed to induce senescence, and ATUX-1215, a novel small molecule PP2A activator were utilized. NBL cells were treated with topotecan and ATUX-1215 alone, as well as in combination. TIS was detected by senescence associated SA-β-galactosidase (SA-β-gal) assay. Colony forming assay detected cell proliferation while immunoblotting identified senescence associated proteins including γ-H2AX, plasminogen activator inhibitor-1 (PAI-1) and C-C motif chemokine ligand 2 (CCL2). R2 Genomics Analysis and Visualization Platform was used for mRNA expression data from human neuroblastoma samples to evaluate the association of *PAI-1* and *CCL2* gene expression with relapse free survival.
Results: PP2A activator reduced SA-β-gal staining and protein expression of γ-H2AX, two markers of senescence, that were induced by topotecan treatment. Further, activation of PP2A in combination with topotecan significantly reduced colony forming potential in NBL cells. Immunoblotting demonstrated the reduction of two mediators and markers of senescence-associated secretory phenotype, PAI-1 and CCL2 with PP2A activation. Clinically, neuroblastoma patients with low PAI-1 as well as CCL2 expression have significantly higher ($p=0.054$, $p=3.74e-03$, respectively) relapse free survival.
Conclusions: PP2A activator, ATUX-1215, holds potential as novel senotherapeutic adjunct by targeting PAI-1 and CCL2. Further investigations into the mechanisms responsible for these findings will be required with goals to advance this therapy to the clinical arena to prevent disease relapse.

#6445 Atypical teratoid rhabdoid tumor development mirrors developmental programs.

F. Yao[1], D. Popovski[1], A. Bondoc[1], J. Rutka[1], T. Pugh[2], A. Huang[1];
[1]SickKids, Toronto, ON, Canada, [2]Princess Margaret Cancer Centre, Toronto, ON, Canada

Atypical Teratoid Rhabdoid Tumors (ATRTs) are pediatric malignancies that arise primarily in young children under the age of 3. While ATRTs are known to segregate into three epigenetically defined subgroups, termed SHH, TYR, and MYC, little is known about the cellular contexts of how these deadly tumors arise and gain tumorigenic properties. We hypothesized that this early onset of disease reflects tumorigenic events in utero. Indeed, previous research has demonstrated maintenance of embryogenesis programs in other pediatric brain tumors.
To characterize the cellular contexts that give rise to ATRTs, we curated 12 single cell and single nuclei RNAseq datasets of human and mouse embryogenesis and developed an atlas and well as a pipeline to match tumors to their most similar embryogenic landmark and timepoint, based on the published cell type identification tool SingleR. We applied this pipeline to two ATRTs single cell datasets generated on the 10X genomics platform: one from primary human samples (N = 33), and one from a tamoxifen inducible ATRT mouse model (N = 8).
To validate the accuracy of our pipeline, we first examined the signatures from our ATRT mouse model. Our pipeline accurately predicted cell types and timepoint within the induction window of E6.5-8.5, suggesting that indeed tumors transcriptionally resemble their cellular contexts.
Results on both human and mouse ATRTs reveals that ATRTs resemble two distinct developmental trajectories: one resembling a gradient of neuronal differentiation from neuroepithelial (NES, SOX2, OTX2) to radial glial cell (TTYH1, ASCL1, FAPB7) types, and a second trajectory characterized by distinctly mesenchymal signatures (COL1A2, PDGFRB). Single cell GSEA showed enrichment of pathways involved in radial glial and mesenchymal signalling, such as WNT, SHH, and PDGFRB signalling. Findings were validated through marker gene immunohistochemistry and spatial transcriptomics.
Taken together, our results demonstrate that ATRTs transcriptionally resemble the developmental contexts of which they arise from. By identifying cellular origins of tumorigenesis, we can exploit this novel information to discover potential therapeutic avenues.

#6446 *Yap1* and CD276/B7-H3: key players in Sex-biased SHH medulloblastoma immune evasion.

Nourhan Abdelfattah[1], Sivaraman Natarajan[2], Han Nhat Tran[1], Thomas Wong[1], Jose Maldonado[1], Rachael McMinimy[3], Hannah Borland[4], Shu-hsia Chen[1], Fernando Camargo[5], James Olson[6], Joshy George[7], Kyuson Yun[1]

[1]Houston Methodist Research Institute, Houston, TX,[2]The Jackson Laboratory for Mammalian Genomics, Bar Harbor, ME,[3]Molecular and Cell Biology, University of California, Berkeley, Berkeley, ME,[4]Borland, College Station, TX,[5]Department of Stem Cell and regenerative medicine, Harvard, Boston, MA,[6]Fred-Hutch, University of Washington School of Medicine, Seattle, WA,[7]The Jackson Laboratory for Genomic Medicine, Farmington, CT

The molecular mechanisms driving sex disparities in cancer prevalence, progression, and treatment outcomes represent a crucial yet understudied area with significant implications for cancer therapy. Medulloblastoma (MB), exhibits a pronounced sex bias in incidence and survival rates, with males experiencing higher incidence and poorer prognoses compared to females. This study focused on the role of *Yap1* in Sonic Hedgehog medulloblastomas (SHH MBs), which revealed an unanticipated sex-biased role of Yap1 in MB immune evasion. Hippo/Yap pathway is one of the top 10 most dysregulated pathways in human cancer. Our in vivo studies uncovered that *Yap1* deletion in a spontaneous SHH MB model significantly extended survival, providing genetic evidence that *Yap1* is a critical oncogene in MB formation. Notably, *Yap1* deletion/inhibition benefitted males, but not females, revealing its sex-biased function for the first time. Integrated multi-omics analyses showed *Yap1* is essential for maintaining MB stem/progenitor cells by activating stemness genes (*Sox2*) and repressing differentiation genes (*Neurod1*, *Zic1/2*) in both sexes. However, *Yap1* plays a more pivotal role in immune evasion in males, particularly by regulating the immune checkpoint molecule; *Cd276*/B7-H3. In males, either CD276 blockade or *Yap1* deletion reverses T cell suppression. In contrast, in females, CD276 blockade or *Yap1* deletion is not sufficient to overcome T cell suppression, and it requires both interventions to activate T cells. Furthermore, our newly discovered transcriptional target gene signatures of YAP1 predict survival in male but not female patients across multiple human cancers, indicating a highly conserved mechanism across species and cancer types. This study highlights sex-specific differences in tumor:immune interactions downstream of YAP1/CD276. In addition, it reveals a novel sex-biased role of YAP1 transcriptional programs, providing molecular entry points for future research on sex differences in immune response. These results underscore the importance of considering sex as a major variable in mechanistic and therapeutic studies involving *Yap1* and CD276, with broader implications for targeted cancer therapies.

#6447 Preclinical efficacy of GCN2 inhibition by KAS-1155 in targeting the integrated stress response in rhabdomyosarcoma.

Da Yeon Kang[1], Hae Won Lee[1], Jong Woo Yoon[2], Jung-Nyoung Heo[2], Youngki Choi[3], Seung Min Hahn[1], Won Ki Ahn[1], **Jung Woo Han**[1]

[1]Department of Pediatrics, Yonsei University College of Medicine, Seoul, Korea, Republic of,[2]Korea Reserch Institute of Chemical Technology, Daejon, Korea, Republic of,[3]Aston Research Institute, Seoul, Korea, Republic of

The integrated stress response (ISR) is an crucial adaptive mechanism that enables the cells to survive microenvironment stress, such as nutrient deficiency. General control non-derepressible 2 (GCN2) and its relevant pathway is essential and pivotal for ISR activation, in which the cells undergo amino acid deficiency. GCN2 phosphorylates Eukaryotic Translation Initiation Factor α (eIF2α) and enhances Activating Transcription Factor 4 (ATF4) expression to regulate the genes involved in physiological responses including cellular adaptation and survival. The roles of GCN2 in cancer survival have been studied and suggested as a target of cancer treatment. Rhabdomyosarcoma is one of the most common cancer types in childhood and adolescence, however, there was little progress in the treatment development over the past two decades. This study evaluated the efficacy KAS-1155, a novel GCN2 inhibitor in two representative rhabdomyosarcoma cell lines. Two rhabdomyosarcoma cell lines (RD, RH-30) were treated with KAS-1155 which is a GCN2 inhibitor produced and provided by Korea Research Institute of Chemical Technology (KRICT). RD was cultured in DMEM, transferred to glutamine-free media for 8 hours, and treated with KAS-1155. The RH30 was initially grown in RPMI media, and the cell line was sub-cultured twice in the glutamine-free media to establish a resistant cell population. This glutamine-starvation-resistant RH30 cell line was treated with KAS-1155 in the same conditions as RD. Cellular viability was assessed using Cell Counting Kit-8(CCK-8) assays. GCN2 pathway activation, including phosphorylated GCN2 (p-GCN2), eIF2α, and ATF4 expression, was analyzed by western blotting. In the glutamin-free media, p-GCN2 was detected after 8 hours in RD cells and, persisted up to 40 hours. In RH30, the p-GCN2 was detectable at 8 hours, and peaked at 40 hours ,and then maintained elevated until 72 hours. Following the 14 hours and 49 hours of glutamine-free media exposure for RD and RH30 respectively, p-GCN2, p-eIF2α and ATF were suppressed successfully with the treatment of KAS-1155 at the concentration of 0.1μM and 1μM for each cell line. Viability assays demonstrated IC50 values of 1.06 μM for RD cells and 1.87 μM for RH30 cells, indicating moderate growth suppression by KAS-1155. GCN2 pathway activation represented the adaptive response in rhabdomyosarcoma cell lines under glutamine-free, amino acid starvation condition. The KAS-1155 inhibited RD and RH30 proliferation suppression at moderate concentrations. This suggests the GCN2 inhibitor may have a role in the treatment of rhabdomyosarcoma, especially in the resistant cell types. Further preclinical investigation is needed to clarify its therapeutic potential and proper use of the GCN2 inhibitor in the rhabdomyosarcoma cell lines.

#6448 Determinants of chemotherapeutic resistance in fusion-positive rhabdomyosarcoma.

T. E. Shaw, F. Shah, J. Toretsky, A. Uren;
Georgetown Lombardi Comprehensive Cancer Ctr., Washington, DC

Rhabdomyosarcoma (RMS) is the most common pediatric soft tissue sarcoma, and is classified as fusion-positive (FP) or fusion-negative (FN) based on the presence of the PAX3::FOXO1 or PAX7::FOXO1 fusions. These chimeric proteins are formed by reciprocal chromosomal translocations and consist of an N-terminal portion of the PAX3 or PAX7 transcription factor fused to a C-terminal portion of the FOXO1 transcription factor. FP status is associated with worse event free survival for patients with RMS. Regardless of fusion status, most RMS patients receive a chemotherapy regimen consisting of some combination of vincristine, actinomycin D, and cyclophosphamide (VAC). Unfortunately, tumors may acquire resistance to these drugs over time, leading to relapse. Our work aimed to uncover genes that drive VAC resistance in FP-RMS using a two-pronged approach. To assess how FP-RMS cells gradually acquire resistance, we cultured FP-RMS cell lines (RH30, RH41, CW9019, U48484, U66788) in sublethal concentrations of vincristine, actinomycin D, or 4-hydroperoxy cyclophosphamide (4-HC, the active metabolite of cyclophosphamide) for multiple passages. We periodically assessed the IC_{50} of cells growing in drugs and increased drug doses as cells developed resistance. We generated four cell lines with a ~5- to ~20-fold increase in IC_{50}. Using RNAseq, we identified differentially expressed genes between the parental and resistant cell lines that may act as drivers of chemotherapeutic resistance. To assess de novo resistance in FP-RMS, we used CRISPR inactivation and activation library screening. We generated FP-RMS cell lines (RH30, RH41, CW9019) stably expressing Cas9 or dCas9-VPR, an endonuclease-dead version of Cas9 fused to three transcriptional activators. We then transduced these cells with a CRISPR inactivation sgRNA library (18,885 gene targets) or a CRISPR activation sgRNA library (19,113 gene targets). Transduced cells were cultured in vincristine, actinomycin D, or 4-HC, and surviving cells were expanded and sequenced to determine which genes provided drug resistance when activated or deleted. We hope that the findings of these experiments will contribute to novel therapeutic targets for patients with VAC-resistant RMS tumors.

#6449 Unique site-specific phosphorylation of PML co-ordinates disease aggravation of neuroblastoma.

Sreenidhi Mohanvelu[1], Poorvi Subramanian[1], Madhi Oli Ramamurthy[1], Sheeja Aravindan[2], Natarajan Aravindan[1]

[1]Oklahoma State University, Stillwater, OK,[2]OU Health Stephenson Cancer Center, Oklahoma City, OK

Neuroblastoma a fatal infant malignancy accounts for 15% of pediatric cancer deaths. The standard treatment for children with neuroblastoma is intensive and include induction, consolidation, maintenance, and salvage therapy. Despite the largely evolving molecular targets for maintenance therapy, retinoic acid (RA) remains the most promiscuous therapy. Restoration of promyelocytic leukemia protein nuclear body (PML-NB) is a crucial cofactor for RA mediated signal transduction, potentially improving the treatment response and outcomes. Phosphorylation at PAS-NA is one of the (if not the only one) crucial phosphorylation site of PML that direct PML degradation through ubiquitin pathway thereby modulating abundance and stability of PML-NB. Herein we investigated the vital association between phosphorylation of PML at PAS-NA and the clinical outcomes of neuroblastoma patients (n=124). To study the spatio-temporal expression of PML and pPML (PAS-NA) at specific tumor sites determined by pathologists, we custom archived the tumors in triplicate in a tissue microarray and effectuated a chromogenic IHC using our custom synthesized antibodies. The analytical endeavor was accomplished by using the modified Multiplex IHC 3.4.9 module integrated into Indica Lab's HALO platform. We observed a strong and definitive inverse correlation of PML to pPML (PAS-NA) expression in the tumor tissues. Negative correlation of pPML (PAS-NA) with patient survival (overall-, relapse-free, progression-free survival) indicating phosphorylation of PAS-NA as a key regulatory mechanism mediating the stability of PML-NBs and impacting the survival outcomes. While PML exhibits an inverse association to disease progression, we observed a proportional increase in pPML (PAS-NA) to the advanced disease stages in neuroblastoma. Furthermore, increased phosphorylation of PML at PAS-NA in patients presenting with metastatic disease or relapse state also emphasizes the critical role of its site-specific phosphorylation in regulating disease aggravation, dissemination and recurrence in neuroblastoma which is mediated by perpetuating PML-NB stability. Together, these results unveil a prominent molecular mechanism that mediates the evolution of neuroblastoma. Critically, these outcomes would have major implications on developing novel molecular targeted therapy and would pave way for achieving desirable clinical outcomes for children affected with this disease. Funding: This work was partially or in full, funded by Department of Defense CA-210339; OCAST-HR19-04; NIH-P20GM103639 to Dr. Aravindan. The work was also supported by NCI Support Grant [P30CA225520], NIGMS [P30GM154635] and a grant from the Oklahoma Tobacco Settlement Endowment Trust [R23-03] to OU Health Stephenson Cancer Center.

#6450 High-throughput screening identified Brefeldin A as a compound that reduces STAT3-dependent survival in acute leukemia and neuroblastoma cells.

Mitsuteru Hiwatari, Masakazu MImaki

Pediatrics, Teikyo University School of Medicine, Tokyo, Japan

Leukemic stem cells exhibit several dysregulated pathways, with the Janus kinase (JAK) and signal transducer and activator of transcription (STAT) pathway being among the most prominent. Additionally, phosphorylated STAT3 is observed following prolonged activation of the anaplastic lymphoma kinase (ALK) ligand-binding site, although the level of phospho-STAT3 induced by ligand-independent activation of ALK is exponentially higher. In ALK-positive tumors, ALK-STAT3 activation promotes tumor cell outgrowth and directly prevents apoptosis. The role of ALK in various ALK-expressing solid tumors, along with the therapeutic use of ALK inhibitors in treating these cancers, has been reviewed in several recent studies. Amplified or mutated ALK is found in approximately 14% of neuroblastomas (NB), one of the most common and aggressive childhood malignancies.In this study, we conducted a high-throughput chemical screen in twenty NB-derived cell lines to identify compounds that could inhibit the oncogenic activity of ALK in NB, using a curated library of around 450 compounds. Brefeldin A (BFA), an inhibitor of protein transport from the endoplasmic reticulum (ER) to the Golgi apparatus, was identified as a promising candidate. Given the increasing evidence linking abnormal expression and activation of STAT3 with leukemia development, suggesting its potential role in leukemia pathogenesis, we next assessed the cytotoxicity and antitumor effect of BFA against 12 T-ALL and 6 AML cell lines.The antitumor effect of BFA, assessed using an MTT assay, revealed potent cytotoxicity across a broad spectrum of hematopoietic malignancies, with particularly strong efficacy observed in 10 T-ALL and 5 AML cell lines. Specifically, T-ALL and AML cell lines were significantly inhibited by BFA, with IC50 values as follows: ICH-TALL-UK, 168.1 nM; MOLT-4, 23.2 nM; Jurkat, 66.6 nM; MOLT14, 97.3 nM; and ICH-TALL-SM, 54.9 nM.In NB cell lines harboring mutated ALK, BFA exhibited notable cytotoxicity. Furthermore, BFA was found to reduce the phosphorylation of ALK in NIH3T3 cells stably expressing mutated ALK. Among the key molecules phosphorylated downstream of ALK, which are critical for the survival of anaplastic large cell lymphoma (ALCL) cells, BFA inhibited the phosphorylation of STAT3, AKT, and ERK.These results suggest that BFA can effectively target the STAT3 signaling pathway, presenting a potential therapeutic approach for leukemia treatment. Moreover, the effects of BFA on ALK-mutated NB cell lines are accompanied by a reduction in the phosphorylation of both ALK and its downstream effector, STAT3. Investigating the antitumor effects of BFA in vivo will be an important next step in the development of future therapeutic strategies.

#6451 Tocilizumab in fibrolamellar carcinoma.

Paul M. Kent[1], Jennifer Hamm[2], Tom Stockwell[3], Jordan Tasse[4]

[1]Pediatric Oncology, FibroFighters Foundation, River Forest, IL,[2]Pediatric Oncology, East Tennessee Children's Hospital, Knoxville, TN,[3]FibroFighters Foundation, Temecula, CA,[4]Interventional Radiology, Rush University Medical Center, Chicago, IL

Background: Fibrolamellar Carcinoma (FLC) is a rare liver cancer of young people. Interleukin-6 (IL-6), is overexpressed in many cancers, including FLC, drives inflammation, angiogenesis, and activation of the JAK-STAT signaling pathway associated with progression, a worse prognosis, and inhibition of T-cell tumor attack. Check-point inhibitor (CPI) drugs induce T-cell tumor attack, but also induce immune-related adverse effects (irAEs), often with elevated IL-6 and eosinophilia. Tocilizumab (TOC), an IL-6 receptor blocker, approved for inflammatory diseases and steroid-refractory CPI irAEs, has been shown in the lab to both mitigate CPI toxicity and promote tumor immunity, while avoiding the toxicity, immune suppression, and tumor promoting effects of steroids, and allowing patients to restart CPIs. We prospectively collected data over 18 months on all FLC TOC patients seen and report on those with irAEs. This is the first report of TOC use in FLC.

Methods: With IRB approval and consent (NCT03793088), data was collected prospectively on all FLC TOC subjects from Apr. 1, 2023 to Dec. 1, 2024.

Results: Overall, 83% of 24 FLC patients with irAEs responded to TOC (13F, median age 24, 50% recent CPI, 92% AE grade 3-4, 1/3 with concurrent steroids). The most common irAEs were ascites, hepatitis, pneumonitis and colitis, with a median serum IL-6 of 163 [<5 pg/ml], median tumor IL-6 overexpression at the 96Th percentile, and median 9% eosinophilia [<2%]. The median tumor volume and circulating tumor DNA response was -20% and -78%, respectively. Proximal follow-up scans showed CR/PR/SD/PD rates of 5%/40%/40%/15%. Most (74%) resumed CPI after TOC treatment and 41% continued TOC as maintenance [Table 1]. Best tumor responses corresponded with CR for irAE, higher IL-6, CPI continuation, and no steroids. No side effects were attributed to TOC (total 174 doses).

Conclusions: TOC used for inflammatory conditions in FLC showed reliable responses, with many patients restarting CPI, and a majority with tumor burden reduction, suggesting a double benefit.

Fibrolamellar Carcinoma Treated with Tocilizumab

Age / Sex	Check Point Inhibitor within 60 days of Event	Inflammatory Condition	NCCN Adverse Event Grade	Time from last CPI dose (months)	Number of Doses of Tocilizumab	Concurrent steroids? Yes / No	irAE response	IL-6 tumor expression (Percentile)	Maximum serum IL-6 level (pg/ml) [<5.0]	Change in circulating tumor DNA (%)	Peripheral Eosinophils (%) [<2%]	Continued on CPI therapy? Yes / No	Continued Maintenance Tocilizumab? Yes / No	Total Tumor Volume % change at Followup
22 / F	Nivolumab	Pneumonitis	4	0	3	No	CR	Not Done	Not Done	Not Done	Unknown	No	No	-100%
25 / F	No CPI ever	Hepatitis, Ascites	4	Not applicable	13	No	CR	100th percentile	116	-100% (went to 0.0)	Unknown	Not Applicable	Not applicable	-90%
45 / F	Nivolumab	Hepatitis, Ascites	2	0	11	No	PR	Not Done	725	Stayed at 0.00	15%	Yes	Yes	-37%
28 / M	Nivolumab	Myositis	3	0	8	No	CR	88th percentile	54	-63%	8%	Yes	Yes	-35%
33 / F	Nivolumab	Cytokine Storm	4	0	10	No	CR	97th percentile	Not Done	-87%	Unknown	Yes	Yes	-35%
22 / M	Nivolumab	Sinusitis	4	0	10	No	CR	Not Done	200	-44%	Unknown	No	Yes	-31%
25 / F	Nivolumab / Ipilimumab	Ascites	4	0	4	No	PR	84th percentile	Not Done	-100% (went to 0.0)	37%	No	No	-30%
29 / M	Nivolumab	Colitis, Sinusitis	4	0	2	No	CR	89th percentile	441	Not Done	Unknown	Yes	Yes	-28%
11 / M	None	Cytokine Storm	4	21	2	Yes	CR	Not Done	350	Stayed at 0.00	1%	No	No	-25%
16 / M	Nivolumab	Myositis, Arthritis	2	0	17	No	CR	94th percentile	Not Done	-100% (went to 0.0)	8%	Yes	Yes	-21%
22 / M	No CPI ever	Hepatitis, Pneumonitis	4	Not applicable	8	Yes	PR	87th percentile	203	-70%	9%	Not applicable	Not applicable	-19%
38 / F	Nivolumab	Cholangitis, Ascites	4	0	4	Yes	SD	<17th percentile	400	-100% (went to 0.0)	5%	Yes	No	-16%
13 / F	None	Cholangitis, Colitis	4	6	1	Yes	PR	Not Done	112	-100% (went to 0.0)	9%	No	No	-15%
26 / F	Nivolumab	Optic Neuritis	3	0	21	Yes	CR	Not Done	Not Done	Stayed at 0.00	0%	Yes	Yes	-8%
20 / F	Nivolumab	Optic Neuritis	4	0	7	No	PR	<17th percentile	7	Not Done	7%	Not Applicable	No	-6%
29 / F	Nivolumab	Optic Neuritis	4	1	21	Yes	CR	Not Done	Not Done	Not Done	Unknown	No	No	No change
15 / M	Nivolumab	Pneumonitis	4	0	5	Yes	SD	96th percentile	125	Stayed at 0.00	39%	No	No	No change
23 / M	Ipilimumab	Hepatitis	3	0	4	No	PD	96th percentile	Not Done	-64%	23%	No	No	+131%
24 / F	Nivolumab	Hepatitis	3	0	4	No	SD	96th percentile	4	Stayed at 0.00	40%	No	No	+219%
22 / M	None	Pneumonitis	4	7	8	Yes	CR	96th percentile	3	Stayed at 0.00	13%	No	No	+220%
29 / M	None	Myocarditis	4	4	1	No	PR	99th percentile	7	Not Done	13%	No	No	Died of Disease
24 / M	None	Colitis	3	4	2	No	PR	96th percentile	422	Not Done	Unknown	No	No	Died of Disease
22 / F	Nivolumab	Psoriasis	2	0	7	Yes	CR	Not Done	Not Done	Stayed at 0.00	Unknown	Yes	Yes	Stayed in Remission
24 / F	None	Myositis, Arthritis	4	3	1	No	CR	Not Done	567	Stayed at 0.00	Unknown	No	No	Stayed in Remission

#6452 Role of obesity in regulation of acute myeloid leukemia progression.

Jieyun Jiang, Samantha R. Melendez, Vivek M. Rangnekar

Radiation Medicine, Markey Cancer Center, University of Kentucky College of Medicine, Lexington, KY

Acute myeloid leukemia (AML) accounts for 15-20 percent of childhood leukemia. Although improved treatment options have increased the overall survival for AML, approximately 30% of the cases tend to relapse. The management of AML patients is an ongoing challenge due to the relapse rates and treatment related mortality. Obesity is a leading cause of preventable death in the United States. Clinical outcome is linked to the degree of genetic heterogeneity of AML and higher body mass index (BMI) at diagnosis. As these underlying factors are associated with poor survival for AML, we are addressing the role and mechanism of obesity-associated factors in progression of AML in mouse models. Since obesity during pregnancy and childhood obesity may be associated with increased risk of developing AML, we tested the growth of the mouse AML cell line C1498 (ATCC.org) in the progeny of obesity-prone C57BL/6J *db/db* and *ob/ob* mice or normal (wild-type) C57BL/6J mice. Our data indicated that both *ob/ob* mice that lack leptin and *db/db* mice that lack the leptin receptor were prone to increased AML growth and showed significant increase in CD33-positive cells compared to wild-type C57BL/6J mice. Kaplan-Meier analysis of survival curves showed statistically significant differences between obese mice and wild type control C57BL/6J mice. Since obesity may change the immune phenotype and thereby affect AML growth, we performed blood cell immunophenotyping in C57BL/6J, *db/db* and *ob/ob* mice before C1498 engraft. We found that the T-cell numbers were significantly lower in the blood of *db/db* and *ob/ob* mice compared to the wild type control C57BL/6J mice. In contrast, myeloid-linage cell numbers were high in the blood of *db/db* and *ob/ob* mice than wild type C57BL/6J mice. The same pattern of immunophenotyping was found in post-AML blood as in pre-AML blood. Pearson Correlation analysis showed that survival of the mice positively correlated with the T-cell population and negatively correlated with myeloid population in blood. As diet-induced obesity (DIO) is the most common cause of obesity in humans, we used C57BL/6J mice that were fed with normal chow diet or high-fat diet (Research Diets, Inc., Catalog # D12492) and tested them for AML growth. Our data showed that obesity associated with high-fat diet also promoted AML growth with decreased survival when compared to normal chow-fed C57BL/6J mice. Taken together, our studies performed in mice with genetically driven obesity or high-fat diet induced obesity indicate that obesity promotes the growth of AML cells and is associated with decreased survival in mice. Our results suggest that T-cell and myeloid cell populations that are modulated by obesity may play an important role in AML progression.

#6453 Dual-omic characterization of the immune landscape of pediatric solid tumors revealed putative epigenetic mechanisms of immune evasion.

Stephanie Bianco[1], Anas Belaktib[1], Virgile Raufaste Cazavieille[1], Charles Joly-Beauparlant[1], Mona Patoughi[1], Lara Herrmann[1], Emeric Texeraud[1], Sylvie Langlois[2], Thomas Sontag[2], Alex Richard-St-Hilaire[2], Vincent-Philippe Lavallee[2], Thai Hoa Tran[2], Sonia Cellot[2], Daniel Sinnett[2], Arnaud Droit[1], **Raoul Santiago**[1]

[1]CHU Laval Research Ctr., Quebec, QC, Canada,[2]CHU Sainte-Justine Research Ctr., Montreal, QC, Canada

Background: There is a general lack of efficacy of immune checkpoint blockades (ICBs) in pediatric solid tumors. Methylation reprogramming is a major player in tumor immune microenvironment (TiME) modeling and ICB resistance. Its role in pediatric-specific TiME has yet to be explored.
Hypothesis: Dual-omic classification, combining DNA-methylation and RNA-sequencing, can identify tumors with epigenetically altered TiME, potentially responsible for ICB resistance.
Objective: Identify and characterize pediatric solid tumors with epigenetically altered immune phenotypes.
Methods: We studied bulk tumor gene expression and DNA-methylation profiles from 184 pediatric extracranial solid tumors. To individualize immune phenotypes, we used similarity network fusion (SNFtool) for dual-omic clustering of immune genes. Clusters were compared for differential expression and differential methylation using volcano3D. We studied the relationship of enhancer methylation to gene expression (ELMER package) to reconstruct regulatory networks (genes and pathways). Transcription factor and cofactor binding motifs were identified to characterize the regulatory element landscape specific to each phenotype. Adjusted p-values <0.01 were deemed significant.
Results: A total of 184 samples were included. SNF clustering identified 3 phenotypes regrouping 52 (28%), 83 (45%), 49 (27%) samples in clusters (cl) 1, 2 and 3, respectively. Cl1 exhibited low expression of immune genes (“cold” phenotype), cl2 overexpressed immune genes (“hot” phenotype), and cl3 featured global hypermethylation (epigenetically “altered” phenotype). Both hot and altered phenotypes overexpressed immune checkpoints (*CD274*, *PDCD1*), T-cell activator chemokines (*CXCL9*, *CXCL10*) and pro-inflammatory pathways, central to ICB sensitivity. However, only hot tumors overexpressed genes and pathways essential for antigen (Ag)-presenting machinery and immune recognition.
The methylation regulatory network indicated that the derepression of genes and pathways related to Ag-presenting machinery (mainly MHC-II and regulators) was specific to hot tumors. In contrast, gene silencing in the altered phenotype relied on hypermethylation/downregulation of the CIITA cofactor domain, the master control of MHC-II genes, interferon-regulated factors (IRFs), and immune-specific master regulators (*SPI1* and *BATF2*). These factors are crucial for anti-tumor immunity and ICB sensitivity.
Conclusion: We demonstrated that DNA-methylation participates in reshaping the TiME of pediatric solid tumors. A subset of tumors with epigenetically altered immune phenotype is characterized by an inflamed immune environment but altered antigen presentation by methylation reprogramming. Future studies should investigate methylation modulators to reverse these mechanisms and enable ICB sensitivity.

#6454 Extracellular matrix topography drives adrenergic to mesenchymal transition in neuroblastoma.

Antonios Chronopolous, Chandra Vemula, Vic Zamloot, Ivan Chavez, Rebekah Kennedy, Shahab Asgharzadeh, **JinSeok Park**

Children's Hospital Los Angeles, Los Angeles, CA

Neuroblastoma (NB), the most common extracranial solid tumor in children, exhibits intra-tumoral heterogeneity with two interconvertible identities: adrenergic (ADRN) and mesenchymal (MES). MES cells display phenotypes associated with metastasis and resistance to therapies compared to ADRN. Thus, the reprogramming of ADRN to MES may contribute to poor NB outcomes, and the process requires further elucidation to improve NB clinical outcomes. The extracellular matrix (ECM), a critical component of the tumor microenvironment (TME), provides structural support and delivers mechanical signals that modulate oncogenic regulation. We demonstrate that high-risk NB contains more topographically aligned ECM fibers than low-risk NB. By using nano-fabricated biomaterials that mimic ECM alignment, we reveal that ECM topography drives the transition from ADRN to MES. This transition involves transcriptional and epigenetic changes, accompanied by the enhanced phenotypic features of MES cells. We also reveal that the Rho-associated kinase and YAP signaling pathways, stimulated by the ECM topography, mediate ECM-driven reprogramming. In summary, ECM-driven transition of NB cells toward MES offers new insights into TME-targeted therapeutic strategies to suppress MES cells and improve NB outcomes.

#6455 Development of a H3K27M inducible and reversible model of diffuse midline glioma (DMG) to characterize tumor microenvironment and epigenetic memory.

Niloofar Khairkhah[1], Sienna L. Galban[2], Megan Faunce[1], Rodrigo T. Cartaxo[1], Carl Koschmann[3], Yue Zhao[4], Stefanie Galban[5]

[1]Department of Radiology, University of Michigan Medical School, Ann Arbor, MI,[2]Davidson School of Chemical Engineering, Purdue University, West Lafayette, IN,[3]Department of Pediatric Neuro-Oncology, University of Michigan Medical School, Ann Arbor, MI,[4]Gilbert S. Omenn Department of Computational Medicine and Bioinformatics, University of Michigan Medical School, Ann Arbor, MI,[5]Departments of Radiology and Surgery, Center for Molecular Imaging, Rogel Cancer Center, University of Michigan Medical School, Ann Arbor, MI

Diffuse Midline Gliomas (DMGs) with a lysine to methionine mutation in Histone 3 are lethal pediatric brain tumors. Despite ongoing efforts to develop new therapies, it remains unclear whether targeting this oncohistone or downstream epigenetic pathway activation are sufficient to halt and eradicate tumors. Here, we introduce a H3K27M inducible and reversible mouse model for DMG that allows precise control of oncohistone expression. This model enables us to study the effects of H3K27M activation, inactivation and re-activation on tumor growth and recurrence and additionally, characterize changes in the microenvironmental and epigenetic memory. We employed the in utero electroporation-based (IUE) delivery method to express the PiggyBac-based expression vectors mutant PDGFRA and dominant-negative TP53 and the newly designed tetracycline inducible H3K27M, along with the transposon. Upon tetracycline induced H3K27M activation, DMG tumors were detected and dissected to generate a new DMG cell line (PPiK27M), which can also be used for intracranial implantations in immunocompetent mice. In addition, we used two patient-derived DMG cell models wherein H3K27M was CRISPR edited (H3K27M-KO) to reintroduce the inducible and reversible H3K27M construct to provide an orthotopic model closely recapitulating human disease. We validated inducibility and reversibility of H3K27M by intracellular flow cytometry and western blotting in the murine and human cell models. We detected characteristic morphological changes and repression of astrocytic markers upon H3K27M re-expression in KO cells. In conclusion, our results indicate that the new inducible and reversible H3K27M IUE model and cells faithfully recapitulate DMG characteristics. We are currently performing single-cell RNA sequencing (scRNA-seq) of tumors to track cellular and molecular alterations in the surrounding tumor immune microenvironment. Additionally, we are characterizing the epigenome to understand whether transcriptional changes persist after inactivation of the oncohistone. Our study provides new tools to mechanistically decipher the role of the oncohistone, and potentially, identify new therapeutic targets for DMG.

#6456 Investigating neuronal signatures in treated fusion positive rhabdomyosarcoma.

Sophie Q. Burke, Roketa Henry, Angelica Oliveras Alsina, Avanthika Balu, Elisabet Olsen, Anand Patel

Oncology, St. Jude Children's Research Hospital, Memphis, TN

Rhabdomyosarcoma (RMS) is the most common pediatric soft-tissue sarcoma with features of skeletal muscle and can be split into two subtypes: fusion positive and fusion negative. Fusion positive-rhabdomyosarcoma (FP-RMS) have a higher likelihood of resurgence over three years after treatment, and patients with recurrent disease experience dismal outcomes with a survival rate under 20%. While long-term treatment responses for this subtype have remained elusive, recent studies have been able to identify the presence of a neuronal cell state in FP-RMS, which has not been observed in FP-RMS cell lines. Furthermore, it is unknown if FP-RMS cell lines can recapitulate this neuronal state. We predicted chemotherapy exposure to be a driving stimulus for lineage transition of FP-RMS cells and examined the neuronal gene expression of chemotherapy-treated FP-RMS cell lines using quantitative PCR (qPCR) and TaqMan Array Cards. We find that treated FP-RMS cell lines induce a dose-dependent increase of expression in neuronal cell markers, and a dose-dependent decrease of expression in muscle cell markers, which suggests the induction of a new cell state in FP-RMS cell lines. For future direction, we will utilize RNA-seq and single cell multi-omics to examine molecular determinates of this linage plasticity. These findings collectively will support future studies by serving as an in vitro model for the study of lineage plasticity in childhood cancer.

TUMOR BIOLOGY: Multi-omics and the Predictive Power of the Tumor Microenvironment
Poster Session

#6461 The spatial cellular landscape of Gleason pattern 3 and 4 prostate tumors.

C. Ak[1], Z. Sayar[1], G. Thibault[1], M. Kuykendall[1], G. Thomas[1], V. Stavrinides[2], Y. Chang[1], **E. S. Eksi**[1];
[1]OHSU Knight Cancer Institute, Portland, OR, [2]University College London, London, United Kingdom

The cellular heterogeneity of prostate cancer cells has been well-established, yet the spatial diversity of stromal cells in the tumor microenvironment remains largely unexplored. In this study, we utilized cyclic immunofluorescence imaging of whole-tissue sections to map spatial relationships between cancer cells and various immune and non-immune stromal populations in primary Gleason pattern 3 and 4 tumors. Our analysis identified over 20 androgen receptor (AR)-positive stromal cell types, many of which have minimal data on their response to AR inhibitors. Notably, we identified distinct AR+ mast cell populations exhibiting unique spatial associations with M2 macrophages and regulatory T cells. Using spatial statistical tools and recurrent cellular neighborhood analysis, we identified disease-specific microenvironments predominantly driven by AR+ stromal cells and uncovered inflammatory gene networks active within AR+ stromal regions. Future work will expand this analysis to spatiotemporal profiling of intermediate-risk patients, aiming to improve tumor stratification for more precise clinical decision-making.

#6463 Interrogating the tumor microenvironment of nasopharyngeal carcinoma by single-cell spatial transcriptomics.

Chee Yit Lim[1], Yi Ren[1], Wei Keat Teo[1], Chor Hiang Siow[1], Kwok Seng Loh[1], Raymond KY Tsang[1], Bingcheng Wu[2], Joshua K. Tay[1]

[1]Otolaryngology - Head & Neck Surgery, National University of Singapore, Singapore, Singapore,[2]Pathology, National University Hospital, Singapore, Singapore

Introduction: Nasopharyngeal carcinoma (NPC) is an Epstein-Barr virus (EBV) associated malignancy characterized by a dense lymphocyte infiltrate and a complex tumor microenvironment (TME). This heterogenous ecosystem influences tumor progression, immune evasion and the variable treatment outcomes observed in NPC patients. Conventional approaches such as bulk RNA sequencing lack the resolution to capture the complex heterogeneity of the TME. Spatial transcriptomics allows us to uncover the heterogeneity and cellular interactions at a single-cell resolution, providing insights into the tumor biology of NPC.

Methods: Recent formalin-fixed, paraffin-embedded (FFPE) NPC tissue samples were selected for analysis with the NanoString CosMx Spatial Molecular Imaging platform. Quality control, dimension reduction and unsupervised clustering at a resolution of 0.6 using the Louvain/Leiden algorithm were performed using Seurat package (v5.1.0). Cluster level annotation was performed using the singleR package using the human primary cell atlas reference. Cell type identities were validated by comparing cluster specific marker genes with canonical markers of known cell types.

Results: Six NPC tissue biopsies were fully interrogated comprising 254 ROIs. Post-filtering, 162,290 cells were examined. A total of 7 clusters were identified, broadly comprising immune, stromal and epithelial cells. Tumor composition was heterogenous (B cell 4.68-50.4%, T cell 0.74-8.78%, endothelial cells 0.91-3.66%, epithelial cells 28.5-66.8%, fibroblasts 1.16%-23.9%, macrophages 6.43%-29.1%, pro B cell CD34+ 0.01-7.1%) despite similar clinical stages III-IV.

Conclusion: We describe the single-cell spatial landscape of nasopharyngeal carcinoma, providing insights into the heterogeneity of the TME. We will evaluate epithelial - immune cell interactions, differential biological processes at the cellular level and compare them with deep gene expression profiles from our larger laser-capture microdissection mRNA-Seq cohort of patients to glean further insights.

#6464 Spatial mapping of the tumor microenvironment for combination therapy design.

A. Bose[1], S. Keezer[2], E. Q. Alonzo[2], S. Struble[1], J. Fisher[2], M. Norton[2], R. A. Heil-Chapdelaine[1], N. F. Diaz Granados[1], V. Agrawal[1];
[1]Leica Microsystems, Inc., Waltham, MA, [2]Cell Signaling Technology, Danvers, MA

Cancer immunotherapy is a promising treatment for many forms of cancer, however only a small subset of patients benefit, and relapse/recurrence frequently occurs due to various resistance mechanisms. To overcome this challenge combinatorial therapeutic strategies are needed targeting multiple steps of the cancer-immunity cycle to achieve long-term efficacy in a large population of cancer patients. It is now known that DNA damage activates the innate immune response and improves immune checkpoint blockade (ICB) efficacy. Combination immunotherapy can target several immunosuppressive elements in the tumor microenvironment and activate multiple steps of the cancer-immunity cycle. For developing an effective combination treatment strategy, including ICB with various other therapeutic modalities, we need to first understand the diverse expression pattern of various factors within tumor immune microenvironment. Multiplex immunofluorescence (mIF) is an important tool for profiling protein expression patterns in tumor tissues. This can provide a framework to understand the sensitivity to a particular combination of therapies and identify potential predictive biomarkers. Here we show spatial proteomics profiling of a diverse range of biomarkers in human colon adenocarcinoma tissue. Our findings demonstrate that spatial proteomics can be tailored to precision oncology using artificial intelligence to predict a patient's response to combination treatment strategies. The Cell DIVE Multiplex Imaging Solution in combination with AI-based image processing software, AIVIA delivers a comprehensive solution for exploring immune landscape of tumor tissue. The broad portfolio of robust IHC- validated antibodies from Cell Signaling Technology (CST) enables the detection of key proteins in the tumor microenvironment (TME). Here, we demonstrate multiplexed Cell DIVE imaging using a novel CST panel to probe human colon adenocarcinoma. The availability of cell type specific biomarkers, combined with the ability to interrogate using multiplexed tissue imaging, provides unprecedented and novel insights and spatial resolution of diverse cell populations in the TME.

#6465 Triple co-culture spheroid models of lung and ovarian carcinoma for the in vitro testing of anti-tumor agents.

Shrey Shah[1], Yongwoon Kim[2], Rock Pulak[2], Gerard G. M. D'Souza[1]

[1]Department of Pharmaceutical Sciences, MCPHS University, Boston, MA,[2]Union Biometrica, Holliston, MA

Tumor spheroids have emerged as superior *in vitro* models for studying solid tumors compared to monolayer cultures due to their ability to replicate key aspects of the tumor microenvironment (TME), including cell-cell interactions, extracellular matrix deposition, and nutrient and oxygen gradients. However, spheroids composed solely of tumor cells, i.e. monoculture spheroids, still lack the non-tumor cell components that contribute to additional *in vivo* TME complexity. This study focuses on developing triple co-culture spheroid models that integrate tumor cells, tissue-specific fibroblasts, and endothelial cells to better replicate the structural and functional features of the TME. Using an optimized liquid overlay technique, triple co-culture spheroids were successfully generated with drug-resistant lung and ovarian tumor cells. These spheroids demonstrated key tumor-like characteristics, such as extracellular matrix production and distinct spatial localization of cell types. Specifically, fibroblasts concentrated at the spheroid core during growth, while endothelial cells localized to the core only in the presence of fibroblasts. When treated with a liposomal formulation previously identified as selectively toxic to drug-resistant tumor cells in monolayer cultures, the triple co-culture spheroids exhibited reduced sensitivity to the treatment in a manner dependent on their composition. These results highlight the potential of triple co-culture spheroids as advanced in vitro models for investigating tumor biology and optimizing therapeutic strategies prior to *in vivo* evaluation.

#6466 Discovery Proteome and Whole Transcriptome Atlas analysis of skin cancer reveals biomarkers associated with immunotherapy response and resistance.

Arutha Kulasinghe[1], Christine Kang[2], Hiromi Sato[2], Brian Filanoski[2], Lori Hamanishi[2], Ashley Heck[2], Margaret Hoang[2], Alyssa Rosenbloom[2], Michael Rhodes[2], Joseph M. Beechem[2]

[1]University of Queensland, Woolloongabba, Australia,[2]Bruker Spatial Biology, Seattle, WA

Cutaneous squamous cell carcinoma (cSCC) is the second most common skin cancer and represents a major global health burden. Approximately 50% of cSCC patients develop primary resistance and 20% will develop secondary resistance to immune checkpoint inhibitors (ICI). There are limited biomarkers that reflect the tumor dynamics predictive of response to ICI therapy and we need to develop a better understanding of the cSCC tumor microenvironment (TME). Enabling simultaneous analyses of proteins and RNAs at ultra-high plex in spatial context, the GeoMx Digital Spatial Profiler (DSP) offers an advanced same slide multiomics workflow and is uniquely positioned to help unravel cellular dynamics throughout the cSCC TME. In this study, we spatially profiled over 1000 proteins (GeoMx Human Discovery Proteome Atlas (DPA)) and over 18,000 transcripts (GeoMx Whole Transcriptome Atlas) using the DSP on formalin fixed paraffin embedded sections of pre-treatment cutaneous skin cancers prior to immunotherapy. Pathology-informed, tumour and stromal regions were sampled across whole tissues to generate histologically relevant assessments of the skin tissues. Covering areas such as immuno-oncology, oncology, immunology, epigenetics, metabolism, cell death, and signaling pathway regulation, the DPA panel supported the discovery of biomarkers and potential therapeutic targets. Our study identified unique tumour and stromal signatures and biomarkers associated with both resistance and sensitivity to immunotherapy at both protein and RNA resolutions. Moreover, coverage across the protein hallmarks of cancer provided deeper insights into the tumour microenvironment dictating clinical responses to therapy. Our study provides an integrated pipeline for discovery based spatial proteomic and transcriptomic assessments of skin cancers.

#6467 Specificity and sensitivity assessment of Xenium in situ platform in multiple human carcinomas for clinical studies.

Jinghao Tian, Tong Lu, Elim Cheung, Lutong Zhang, **Vidyodhaya Sundaram**, Rikita Gakhar

BioChain Institute, Inc., Newark, CA

Spatial transcriptomics is advancing at a rapid pace since being named the Nature ‘Method of the Year’ in 2020. The 10x Genomics Visium and Xenium platform and assays are now providing sub cellular resolution and mapping transcripts to the tissue morphology to study complex biology from whole transcriptomics to precisely targeted gene panels. These assays are of great significance to clinical research in precision medicine and biomarker discovery. However, third party validations on reproducibility, orthogonal concordance, specificity and sensitivity raise questions that are critical to their use. At BioChain Institute Inc. (BioChain), we are running a series of tests on Xenium V1 as well as Visium Cytassist V2 chemistry, using multiple human carcinoma FFPE tissues to address the specificity and sensitivity questions in regard to these platforms. FFPE tissues from BioChain’s repository were screened for quality assessment by our pathologist and molecular scientists for tumor content above 30% as well as the RNA quality. Four carcinoma tissues from Breast, Colon, Lung, and Kidney with high DV200 scores were selected. An array was constructed with these tissues to fit the Xenium slide’s imageable area. Two serial sections were used for the Xenium V1 run per 10x Genomics’ protocol. The Off-the-shelf Human Multi-Tissue and Cancer Panel was used for targeting 377 genes. After the Xenium run completion, these slides were used for H&E staining and imaging as well as Visium V2 assay. The H&E images were annotated by the pathologist and integrated with Xenium images using Xenium Explorer.The data was sub-sampled for the different regions per pathologist’s annotations of stroma, tumor and immune cell regions. Data from these regions were compared between the serial sections from Xenium run as well as the Visium run for specificity and sensitivity. Sensitivity of Xenium data versus the visium data was also compared for these specific regions.We have previously demonstrated reproducibility of Xenium data between serial sections prepared by different operators as well as concordance of the two assays run on the same section. With this study we are zooming into specificity and sensitivity via pathologist annotations of the morphology and the gene expression data across multiple carcinomas. For example, in the breast cancer sample cluster 10 (Figure 1) in the Xenium data corresponded to the immune region as annotated by the pathologist. This cluster was noted to have high expression of immune cell infiltration marker genes such as CD3E, CD8A, CD2, CCL5, PTPRC, etc. Our findings show the sensitivity and specificity of Xenium and Visium assays to delve further into the heterogeneity of tumor microenvironment and patient stratification for precision medicine and biomarker discovery studies.

#6468 Dissecting the immune landscape in EBV+ classical Hodgkin lymphoma following IL-23 inhibition using single-cell hyperplex immunofluorescence analysis: A case report.

Janusz Franco-Barraza[1], Bhaumik Shah[2], Mariia Dmitrieva[1], Joanna Kowal[3], Erica Geneletti[3], Mariusz Wasik[2], Reza Nejati[2], Edna Cukierman[1]

[1]Cancer Signaling and Microenvironment Program, Fox Chase Cancer Center, Philadelphia, PA,[2]Department of Pathology, Fox Chase Cancer Center, Philadelphia, PA,[3]Lunaphore Technologies, Tolochenaz, Switzerland

Background: The immune tumor microenvironment (TME) in Epstein-Barr Virus (EBV)+ classic Hodgkin lymphoma (cHL) displays a T helper (Th) 1 profile typical of effective antitumor response with increased infiltration of CD8+ T-cells. cHL cases showing evidence of interleukin (IL)-23 /IL-17 axis activation are usually EBV-. The TME of EBV+ cHL developed after IL-23 repressive therapy has not been reported. We report the TME characteristics of EBV+ cHL in a psoriasis patient on IL-23 inhibition therapy.
Case Presentation: A 40-year-old male with chronic plaque psoriasis underwent Guselkumab (IL-23 inhibitor) treatment for four months before developing bulky cervical lymphadenopathy. Excisional biopsy revealed Hodgkin and Reed-Sternberg (HRS) cells, predominantly in the interfollicular area, with weak to moderate expression of B-cell markers (PAX-5, CD20, CD79a) along with MUM1, CD30 and CD200. Importantly, HRS cells were strongly positive for EBER by in situ hybridization, showing a latency pattern IIa (LMP-1+, EBNA-2-) with a viral load of 75 IU/ml. A diagnosis of EBV+ cHL, possibly iatrogenic/related to IL23 inhibitor therapy was proposed.
Hypothesis: IL-23/IL-17 axis promoted the reprogramming of the TME, fostering the emergence of EBV-driven HRS cells.
Methods: Excised lymph node sections were analyzed using an automated sequential immunofluorescence (seqIF™) protocol (Lunaphore COMET™), tailored for the TME of cHL. This enabled the spatial mapping of 9 markers (CD20, T-bet, CD4, CD163, CCR4, CCR6, HLA-DR, Ki67, CTLA4). Utilizing the HORIZON™ image analysis software, which integrates single-cell segmentation and rule-based classification algorithms, we identified 8 distinct cell populations, including HRS cells, B cell subsets, Th1, Th2, Th17, Tregs, and macrophage subsets. Additionally, this unbiased approach allowed for the precise measurement of immune exhaustion and proliferation markers expression.
Findings: The seqIF™ analysis revealed an immunosuppressive TME showing predominance of Th2 cells (CCR4+, CCR6-), numerous CTLA-4+ immunosuppressive CD4+ T cells, a very low Th1/Th2 ratio and a significant reduction of Th17 cells. Our study suggests reprogramming of Th17 to Th2 response following IL-23 inhibitory therapy, promoting the development of EBV+ cHL. This contrasts with the Th1 profile reported for the TME of EBV+ cHL.
Conclusions: This single case study highlights the utility of spatial proteomics mapping immune interactions in the TME of EBV+ cHL. A larger study of EBV+ and EBV- cHL cases is planned to further characterize these TMEs, including fibroblastic populations, and identify potential immuno-oncology targets. These findings could guide personalized treatments and identify EBV+ cases unsuitable for IL-23 inhibitors to mitigate cHL risk.

#6469 Defining architectural tissue features and cell composition of IDH-mutant gliomas.

Elisabet Rodriguez Tomas[1], Lina Leiss[1], Rasul Tabiev[1], Fredrik Nysjo[2], Anna Klemm[2], Carolina Wahlby[2], Thomas Olsson Bontell[3], Anna Denes[4], Asgeir S. Jakola[4], Anja Smits[4], Arne Ostman[1]

[1]Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden,[2]Department of Information Technology; Vi3; Image Analysis, Uppsala University, Uppsala, Sweden,[3]Department of Physiology, Institute of Neuroscience and Physiology, University of Gothenburg, Gothenburg, Sweden,[4]Department of Clinical Neuroscience, University of Gothenburg, Gothenburg, Sweden

This study tests the hypothesis that quantitative profiling of cell type composition and tissue architecture in isocitrate dehydrogenase (IDH)-mutant gliomas can provide valuable insights into tumor aggressiveness, treatment response, and survival. Towards this goal, we have established a novel method to analyze cell composition and tissue architectural features in IDH mutant gliomas based on multiplex immunohistochemistry staining and automated image analysis.Two independent clinically well-annotated TMAs, derived from approximately 200 patients with IDH1-mutant tumors, are being analyzed. A multiplex fluorescence antibody panel was designed to include markers for astrocytes ($GFAP^+$/$IDH1\text{-}R132H^-$), glioma cells ($IDH1\text{-}R132H^+$), oligodendrocytes ($IDH1\text{-}R132H^-/SOX10^+$), leukocytes ($CD45^+$), tumor-associated microglia and macrophages (TAMMs) ($IBA1^+$), endothelial cells ($CD34^+$) and perivascular cells ($PDGFR\beta^+$). QuPath software was employed for image analysis. Statistical analyses were performed using Python.For cell classification we firstly created single-core “ground truth data” from 20 cores by manual/visual training of core-specific cell classifiers. Second, we created a “multiple-core-trained” classifier by training on 10 of the ground truth cores and testing on the remaining 10 cores. Comparison between ground truth data and “multiple-core-trained” data indicated overall cell classification accuracy of 0.8.A pilot-study of 20 tumors demonstrated that the most abundant cell types were glioma cells, while leukocytes and perivascular cells were the least abundant. The abundance of glioma and perivascular cells showed good consistency between cores from the same patient (corr = 0.8 for both). In contrast, the abundance of oligodendrocytes and leukocyte cells was less consistent (corr = 0.4 and 0.3, respectively).Analyses of spatial pairing of cell types demonstrated strongest positive spatial enrichment between endothelial cells and pericytes and between endothelial cells and TAMMs. In contrast, glioma cells and endothelial cells showed negative spatial enrichment. Notably, inside-case concordance for these “pair-metrics” was high (0.8-0.9).Analyses aiming to identify co-regulated “pair-metrics” revealed strong positive correlations between the three pair-metrics TAMMs/perivascular cells, TAMMs/endothelial cells and endothelial cells/perivascular cells indicating inter-case variability of multi-cellular vascular niches."Cell abundance metrics" and "cell pair metrics" are currently being explored for their associations with clinical characteristics and their potential to enhance prognostic value relative to 1p19q status and WHO grades.

#6470 Spatial analysis of the tumor microenvironment in diffuse large B-cell lymphoma reveals clinically relevant recurrent cellular neighborhoods.

M. Autio[1], S.-K. Leivonen[1], L. Meriranta[1], M.-L. Karjalainen-Lindsberg[2], T. Pellinen[3], S. Leppa[1];
[1]University of Helsinki and Helsinki University Hospital Comprehensive Cancer Center, Helsinki, Finland, [2]Helsinki University Hospital, Helsinki, Finland, [3]Institute for Molecular Medicine Finland (FIMM), Helsinki, Finland

Introduction: The tumor microenvironment (TME) of diffuse large B-cell lymphoma (DLBCL) has been recently dissected and different TME-based subtypes of DLBCL proposed (Kotlov et al. Cancer Discov. 2021, Steen et al. Cancer Cell. 2021). Previous studies have, however, not systematically explored the spatial organization of the DLBCL TME and its association with biological and clinical factors.
Materials and Methods: We performed multiplex immunofluorescence using a 12-plex antibody panel on diagnostic tumor samples from 99 patients with DLBCL. We characterized B cells, T cells, tumor-associated macrophages (TAMs), and immune checkpoint molecules. We processed images using Ilastik and CellProfiler softwares, and segmented nuclei with a pretrained deep-learning model. We phenotyped the cells using the Phenograph algorithm combined with manual thresholding. We identified recurrent cellular neighborhoods (RCNs) and performed cell-cell interaction analyses using the scimap package (Python 3.9). We correlated the findings with biological and clinical characteristics and survival.
Results: We analyzed over 1.2 x 10^6 single cells with a median of 12 354 cells per patient. We identified 17 cell phenotypes, including various subgroups of B cells, T cells, and TAMs. Of the cell types, B cells were the most abundant (mean 55%), followed by CD4+ helper and CD8+ cytotoxic T cells (means 14% and 10%), and M2-like TAMs (mean 8%) with great heterogeneity between the samples. We identified 10 different RCNs with distinct cellular compositions across DLBCLs. The most common RCN was an immune-poor RCN (30% of the cells), followed by an RCN with high B cell content and a moderate immune cell infiltrate (22% of the cells). We also identified different T cell and TAM-rich RCNs. The RCNs subdivided DLBCLs into immune-poor areas and areas with diverse immune cell infiltrates. Notably, HLA-ABC positive lymphomas consisted of larger proportions of CD4+ T cell-rich RCNs ($p < 0.05$). Defined by the shortest average distance, immune-poor RCNs were located close to the RCNs that had high B cell content combined with moderate immune cell infiltrates but far from the T cell-rich RCNs. However, when CD8+ T cell-rich and immune-poor RCNs were close to each other, the outcome was favorable (progression-free survival (PFS); $p < 0.05$), whereas the proximity of PD-L1+ B cell-rich and CD8+ T cell-rich RCNs was associated with poor survival (PFS; $p < 0.05$). Finally, we correlated RCN proportions with lymphoma microenvironment (LME) and genetic subtypes. Immune-poor RCNs were common in the depleted LME subtype, whereas PD-L1+ M2-like TAM-rich RCNs were more common in the inflamed LME. In contrast, PD-L1+ M2-like TAM-rich RCNs were rare in EZB-type DLBCLs.
Conclusions: Our data show that DLBCL TME is organized into RCNs that are associated with distinct molecular and subtypic features and patient outcomes.

#6471 Spatial multiomics reveals T-cell activation and exhaustion states in the tumor microenvironment.

Steve Zhou, Yifan Wang, **Debia Wakhloo**, Anushka Dikshit, Li-Chong Wang, Maithreyan Srinivasan

Bio-Techne Corporation, Newark, CA

Spatial Multiomics allows for context-based study of the tumor microenvironment (TME). TME consists of diverse cells that collectively influence tumor progression, therapeutic response, and immune activation. CD8 T-cells, essential for antitumor immunity, often become exhausted in the TME. Recent studies show that PD1+ TCF1+ stem-like CD8 T-cells retain differentiation potential and can be rejuvenated through cytokine signaling and immune checkpoint modulation. This detailed spatial profiling of RNA and protein expression in the TME is crucial to understanding immune cell function, activation, and differentiation. Here we introduce a protease-free workflow for RNAscope™ Multiplex Fluorescent v2 assay combining RNA *in-situ* hybridization (ISH) and protein immunofluorescence to study cell marker proteins (CD8, PD1) with RNA targets (*TNFA, TCF7, IFNG*) in a tumor microarray (TMA). The Manual Multiplex Protease-Free workflow enables simultaneous RNA and protein visualization without enzymatic disruption, preserving antigen integrity and tissue morphology. Using this Multiomics assay we can study CD8 T-cell differentiation and activation. Co-detection of *TNFA*, *TCF7*, and *IFNG* transcripts with CD8 and PD1 proteins provides insights into specific T cell phenotypes and their activation/exhaustion status. Data reveal robust co-expression patterns of these targets, particularly in the tumor-cell neighboring stromal and immune cells in breast, cervical and stomach cancers, reflecting immune activation and exhaustion across distinct TME niches. This methodology lays a foundation for refining immunotherapeutic strategies targeting PD1+ TCF7+ CD8 T-cells, enhancing effector functions, and advancing precision oncology interventions.

#6472 Integrative multi-omic analysis of the multiple myeloma bone marrow reveals alterations of the immune microenvironment in treatment naïve patients.

Siobhan B. Cashman[1], Aruna Ayer[2], Teresa Swift[1]

[1]Advanced Technology Group, BD Biosciences, Limerick, Ireland,[2]Advanced Technology Group, BD Biosciences, Milpitas, CA

Multiple myeloma (MM) is defined by the uncontrolled clonal proliferation of plasma cells in the bone marrow (BM). It is one of the most frequent hematological cancers, representing 10% of hematological malignancies in the US. Although recent advancements in therapies have led to improved outcomes, MM remains incurable. Disease development and progression are impacted by alterations in the local immune microenvironment, and dysfunction in both the innate and adaptive immune responses are characteristic of the MM BM microenvironment. With the increasing use of immunotherapeutic strategies to treat MM, there is a growing need to understand the existing immune landscape in these patients. We sought to gain a greater understanding of the immune cell diversity in MM by sampling BM from healthy and newly diagnosed cohorts. A combination of traditional flow cytometry, spectral flow cytometry, and single-cell RNA sequencing (scRNA-seq) with the addition of CITE-seq, enabled the high-resolution analysis of surface protein and targeted immune response-related transcriptomic changes within individual cells. In this study, we found a high degree of inter-patient heterogeneity within the aberrant plasma cell population. We report an expanded population of $CD57^+$ effector memory expressing CD45-RA (T_{EMRA}) cytotoxic T cells, and an overall reduction in T cell effector responses. Our scRNA-seq approach demonstrated a strong correlation of CITE-seq signal with key cell identifying markers. Investigation of the suppressive cell subsets found that overall, these cell frequencies were maintained across health and disease. However, we found altered T_{REG} phenotypes with an increased frequency of a highly suppressive T_{REG} subset in MM patients. In summary, our study provided insight into the significant differences observed in the frequency and functionality of the principal immune populations of the BM microenvironment. By combining flow cytometry with scRNA-seq and CITE-seq, the changes in the immune landscape in the BM of treatment naïve MM patients can be mapped more comprehensively, allowing correlation of surface marker expression with gene expression profiles. For Research-Use Only. Not for use in diagnostic or therapeutic procedures. BD and the BD Logo are trademarks of Becton, Dickinson and Company. © 2024 BD. All rights reserved. NPM-5205

#6473 Determining the spatial composition of immune infiltrate in HER2+ breast cancerHER2+ breast cancer.

Jessica Linak, David Page, Jason Schlumbohm, Fred Robinson, **Sasha Elizabeth Stanton**

Medical Oncology, Earle A. Chiles Research Institute, Portland, OR

Background: Immunotherapy has improved outcomes in triple negative breast cancer but there is less response in the other subtypes. Tumor infiltrating lymphocytes (TIL) infiltrated tumors respond better to immune therapy in other tumor types. We wanted to be able to identify tumor infiltrated verses sequestered tumors and then identify the immune populations of infiltrated/sequestered tumors using spatial localization of immune and breast cancer receptors. To do this we optimized digital spatial profiling using 2 methods (Nanostring GeoMx and 10X Genomics Xenium), immune protein expression using Lunaphore COMET, and hemoxylin & eosin (H&E) TIL evaluation on a single slide. Our goal was to identify an immune infiltrate biomarker that could predict patients that would respond to neoadjuvant immune therapy in HER2+ breast cancer.
Methods: We evaluated 6 HER2+ breast cancer biopsies. Formalin fixed paraffin embedded (FFPE) 10 µM slides from untreated core biopsies were evaluated by the Xenium breast, GeoMx Cancer Transcriptome Atlas, and Comet Lunaphore 21 immune target panels. 4 of the 6 tumors were ER neg PR neg HER2 pos, one was ER neg PR pos HER2 pos, and one was ER pos PR neg HER2 pos. For GeoMX, each ROI was ≥ 250 nuclei and ≥ 400 µM diameter to ensure a sequencing depth of 30X and allow segmentation.
Results: We sequentially stained and evaluated the entire specimen on a single FFPE slide using Xenium, Lunaphore, and H&E. The immune infiltrate for HER2 breast cancer was predominantly sequestered in stroma. In GeoMX, the highest expression in CK positive cells was ERBB2 (p=9.6X10-12), proliferation markers (CCND1 p=1.2X10^{-8}), and cytokeratins (KRT7 p=-1.3X10^{-10}) while in the stroma the highest expression was immune markers (CD163 p=6.1X10^{-12} and CD4 p=6.6X10^{-12}). This was similarly seen in Xenium evaluation where the immune infiltration was predominantly CD163^{+}CD68^{+} macrophages and FOXP3^{+}CD4^{+} T cells in the stroma and ERBB2 and cytokeratins in the tumor. Lunaphore protein evaluation confirmed the findings on the same slide. Even with fewer targets in Xenium, similar results were seen between GeoMx and Xenium but the whole slide was able to be evaluated with Xenium.
Conclusion: Sequential evaluation of Xenium, Lunaphore, and H&E is feasible and allows spatial identification of the TIL populations in HER2+ breast cancer. Consistent with previous studies, most immune infiltrate is localized in the stroma along the immune front and is predominantly CD4^{+} regulatory T cells and CD163^{+} macrophages.

#6474 Comprehensive immunophenotypic profiling in anti-PD-1-responsive and -resistant mouse models reveals perforin-expressing CD4+ T cells as key effectors of cytolytic activity.

Hiroyuki Inoue[1], Takayuki Hamasaki[2], Kazuhiko Inoue[2], Akira Nakao[1], Noriyuki Ebi[1], Hirofumi Minomo[2], Ichiro Nagata[2], Naoto Horai[2], Masaki Fujita[1]

[1]Department of Respiratory Medicine, Faculty of Medicine, Fukuoka University, Fukuoka, Japan,[2]Drug Safety Research Laboratories,, Shin Nippon Biomedical Laboratories, Ltd., Kagoshima, Japan

Immune checkpoint inhibitors targeting the PD-1/PD-L1 axis have dramatically advanced cancer therapy, but resistance mechanisms remain a significant hurdle. This study explores immune changes in syngeneic mouse models sensitive (MC-38) or resistant (LLC1) to anti-PD-1 monoclonal antibody (mAb) treatment. Multicolor flow cytometry was employed to assess immune cell profiles in the tumor microenvironment (TME) and tumor-draining lymph nodes (TdLNs). In the MC-38 model, anti-PD-1 mAb therapy significantly altered immune landscapes, including enhanced dendritic cell (DC) and macrophage populations and decreased myeloid-derived suppressor cells (MDSCs). Upregulated antigen presentation molecules (MHC I/II) and functional markers (CD80, CD86, CCR7, PD-L1) were observed in tumor-associated DCs and macrophages. Notably, there was an increase in tumor-infiltrating CD4+ T cells, CD8+ T cells, including central memory (TCM) and effector memory (TEM) subsets, regulatory T cells (Tregs), natural killer (NK) cells, and natural killer T (NKT) cells. A key finding was the enhanced cytotoxic potential across various lymphocyte subsets, with perforin identified as a pivotal marker among 3 different cytolytic markers, correlating with therapeutic efficacy. Tumor volume inversely correlated with levels of perforin-expressing CD4+ T cells and NKT cells, highlighting their critical roles in antitumor responses. Conversely, the LLC1 model displayed limited immune alterations following anti-PD-1 mAb treatment, emphasizing distinct resistance mechanisms. This study delineates the dynamic immunophenotypic changes induced by anti-PD-1 therapy and identifies perforin, particularly in CD4+ T cells and NKT cells, as a promising biomarker for treatment response. Furthermore, these findings suggest potential combinatorial strategies targeting DC/MDSC ratios and perforin pathways to overcome resistance in refractory tumors.

#6475 Characterizing peri-tumoral tertiary lymphoid structures in non-muscle invasive bladder cancer using a spatial whole transcriptomics approach.

Kartik Sachdeva[1], Priyanka Yolmo[1], Abdulhameed Abdulhamed[1], Sadaf Rahimi[1], David M. Berman[1], Roger Li[2]

[1]Queen's University, Kingston, ON, Canada,[2]Moffitt Cancer Center and Research Institute, Tampa, FL

Background: Intravesical Bacillus Calmette-Guérin (BCG) immunotherapy is the gold standard for high-risk NMIBC, however over half of patients experience early recurrence or disease progression, highlighting the need for reliable predictive biomarkers. Our previous research identified that B cell dominant peri-tumoral tertiary lymphoid structures (TLSs) evolve during chronic carcinogenesis in a subset of patients who experience early recurrence or progression. TLSs generally correlate with favorable outcomes following treatment with immune checkpoint inhibitors, chemotherapy or oncolytic viruses. In contrast, their abundance in peri-tumoral regions in BCG-naïve NMIBC tumors associates with poor prognosis. This highlights the importance of their in-depth characterization and further exploring their biomarker potential in NMIBC.
Methods: Peri-tumoral TLSs and tumor epithelial regions were profiled using the NanoString GeoMx Digital Spatial Profiler based whole transcriptome analysis. Multiplex Immunofluorescence was performed to infer spatial profiles of B, T and myeloid cell functional states as well as immune checkpoint proteins.
Results: Spatial whole transcriptome analysis displayed increased expression of genes associated with antigen presentation, complement activation, B cell recruiting chemokines, and immunoglobulins in the TLSs regions. Tumor epithelial regions located adjacent to TLSs showed elevated expression of genes reflective of a luminal subtype. Increased PD-1+ cells in the peri-tumoral regions and enrichment of luminal subtype genes in the epithelial compartment indicated cancer cell intrinsic aggressive features associated with immune exclusion.
Conclusion: The novel findings from this study will help establish peri-tumoral TLSs as biomarkers for predicting BCG therapy response, enabling early identification of patients who may benefit from alternative immunomodulatory treatments like oncolytic viruses, gemcitabine-docetaxel chemotherapy, or immune checkpoint inhibitors.

#6476 Multimodal single cell analysis of cholangiocarcinoma subtypes reveals distinct immune microenvironments.

H. E. Stumpf[1], J. Yang[2], H. Dong[2], R. L. Smoot[1], S. I. Ilyas[1];
[1]Mayo Clinic College of Medicine and Science, Rochester, MN, [2]Mayo Clinic, Rochester, MN

Cholangiocarcinoma (CCA), an aggressive cancer of the bile ducts, is classified into intrahepatic (iCCA), perihilar (pCCA), and distal (dCCA) anatomic subtypes. Each subtype has a distinct epidemiology and pathogenesis. The tumor immune microenvironment (TIME) of each subtype is poorly understood. CCAs are poorly immunogenic tumors and systemic therapy has had subpar efficacy in CCA subtypes. Thus, we hypothesized that each anatomic subtype has a distinct TIME. To address this hypothesis, we completed single cell sequencing of tumor, liver, and bile duct from patients with either iCCA or pCCA/dCCA. 182,054 cells from paired liver (n=16), tumor (n=16) (9 iCCA, 7 pCCA/dCCA), and normal bile duct (n=9) samples were obtained from treatment-naïve patients undergoing resection from June 2020 to August 2023. The tissues were homogenized and flow sorted for immune ($CD45^+$) populations immediately following resection. The cells then underwent single cell RNA sequencing (scRNA-seq) and cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) with antibodies for 157 proteins. Multiple immunocompetent, syngeneic/orthotopic murine models of iCCA with distinct genetic drivers were used to assess immunotherapeutics (PMID: 38458319). Immune cells of interest were isolated by flow sorting and functionally validated via suppression assays and multiparameter flow cytometry. Additionally, a novel pCCA murine model was established by injection of an $Pik3ca^{H1047R}/p27^{Kip1}$-derived extrahepatic biliary cancer cell line into the porta/hilar tissue plane. Compared to non-involved liver, the bile duct immune microenvironment composition closely resembled that of the tumor. Multimodal analysis demonstrated both human and murine iCCA tumors have a significant upregulation of regulatory T cells (Treg) expressing T cell immunoreceptor with Ig and ITIM domain (TIGIT) compared to controls. Suppression assay with intratumoral $TIGIT^+$ Treg from mouse compared with $TIGIT^-$ Treg indicate $TIGIT^+$ Treg are more potent in inhibiting proliferation of $CD8^+$ T cells. Moreover, TIGIT blockade using a monoclonal antibody resulted in a significant reduction in tumor burden in multiple iCCA murine models, including SB1, a model resistant to anti-PD-(L)1 monotherapy. Meanwhile, human pCCA tumors had a significant increase in IgA^+ plasma cells compared to iCCA tumors. An innovative genetically and anatomically relevant pCCA murine model was developed to interrogate this unique plasma cell population. In summary, these data suggest that iCCA and pCCA have distinct immune microenvironments which likely impacts the differences in response to ICB between the subtypes observed in phase III clinical trials. Additionally, the uninvolved, tumor-adjacent bile duct immune microenvironment resembles that of the tumor, suggesting a pre-malignant niche that facilitates tumor development.

#6477 Tumor microenvironmental alterations and novel biomarkers in metastatic ovarian cancer identified with spatial transcriptomics.

S. Ouedraogo, J. Yu, N. Li, X. Zhan;
Shandong Key Laboratory of Precision Oncology, Shandong Cancer Hospital and Institute, Shandong First Medical University and Shandong Academy of Medical Sciences, Jinan, China

Background: Ovarian cancer (OC) is one of the most common causes of cancer in women in the developed world and causes many cancer-related deaths every year. Patients are frequently discovered at advanced stages of the disease, and metastasis can be detected in 30% of patients at the time of diagnosis, with an additional 30%-40% developing metastases few years later. Current therapy methods for ovarian cancer metastasis are typically ineffectual, and mortality rates for individuals with ovarian cancer metastases are consequently high. As a result, OC remains one of the most serious clinical disorders and a life-threatening risk to women. Investigations into the tumor microenvironment (TME) and biomarkers associated with ovarian cancer metastasis may give critical benefits in understanding the underlying molecular pathways and identifying new targets for clinical diagnosis and treatment.
Methods: We investigated the TME between metastatic platinum sensitive recurrent ovarian cancer (PSROC) tissues and primary OC tissues using sc-RNAseq, spatial transcriptomics with 10x Visium spatial capture technology. The data were analyzed using R, DAVID and Cytoscape software to identify potential biomarkers.
Results: By analyzing the spatial transcriptomic data of PSROC metastases and primary lesions, we identified significant changes in multiple energy metabolism signaling pathways and significantly different tumor-associated macrophage groups. Notably, the metastatic tissue showed fewer B cells, T cells, and macrophages differentiation and infiltration. On the other hand, the metastatic tissue showed more monocyte and DC cell proportion when compared to primary tissue. The spatial location of each cell type in the different tissues was investigated, showing great disparity in the cell arrangement within each tissue type. The average log2FC of the differentially expressed genes (DEG) between the two tissue types was determined, thus providing us with cell and type-specific gene sets. The cutoff values were set to avg_Log2FC >= |0.58| and p_val_adj (FDR) < 0.05. We obtained 1226 DEG ups and 1011 DEG downs. The DEGs were used for KEGG pathways analysis which gave 40 significantly enriched pathways, with notably oxidative phosphorylation, p53 signaling pathway, cell cycle and HIF-1 signaling pathways. By combining KEGG pathway data with PPI and hub-gene analysis we identified potential genes that may play significant role in ovarian cancer metastasis, energy reprogramming and immune escape; TOP2A, CCNB1, CDK1, CCNA2, AURKB, TP53, CD4, EGFR, JUN, HIF1A, FN1 and CTNNB1. These genes will be further investigated through in vitro and in vivo experiments.
Conclusions: Those results provide a new theoretical basis for the diagnosis and treatment of PSROC, which will undoubtedly help in the prevention, molecular diagnosis and development of more precise therapeutic approaches.

#6478 Relapse prediction of stage III MSS colon cancer by certain cell subtype distribution characteristics in tumor and tumor invasive margin.

Hanqing Hu[1], Hongzhe Sun[2], Xia Liu[2], Shuo Han[2], Lin Zhu[2], Miao Wang[2], Zhifu Zhang[2], Na Li[3], **Guiyu Wang**[1]

[1]Department of Colorectal Surgery, the Second Affiliated Hospital of Harbin Medical University, Haerbin, China,[2]Beijing PhenoVision Bio Co., ltd, Beijing, China,[3]Hangzhou PhenoVision Bio Co., ltd, Hangzhou, China

Colorectal cancer (CRC) is a frequent gastrointestinal malignancy with high rates of morbidity and mortality. Previous studies have shown a significant correlation (p<0.001) between immune score and recurrence time, overall survival, and disease-free survival in subgroups of microsatellite stability (MSS) patients with stage II colon cancer. Current study reviewed 17 stage III MSS CRC patient archived FFPE samples including 11 relapse and 6 no-relapse, to investigate their immune cell features and the correlation to relapse after chemotherapy. IHC control, multiplex immunofluorescence (mIF) panel control and the followed mIF staining were conducted using PN 6-Plex Detection Kit (PhenoVision Bio Co., ltd) targeted CD68, CD3, CD20, panCK, PD-L1 and PD-1. The 17 staining slides were scanned, tumor areas were lined out by pathologist based on H&E staining from the serial section of each sample. Non-tumor region, tumor invasive margin (IM) , which was defined as 360μm within and outside of tumor boundary *i.e.* total 720μm, tumor islands and stromal areas were analyzed using PhenoVision mIF AI analysis system trained from Oncotopix Discovery system (Visiopharm). Statistical analysis exhibited significant correlation of MSS mIF results to relapse rate based on binary logistic regression (p<0.05). Positive cell percentage and positive cell densities were both analyzed in 17 samples. When focused in tumor region, there were significant variated PD-1 negative/CD20 positive cells in tumor stromal region between relapse and non-relapse group (6.64% in non-relapse *vs.* 2.58%in relapse, p=0.026). Significant differences were observed in PD-L1/CD3 co-positive cell subtype (p=0.01) between relapse and non-relapse groups. Further focus on tumor and IM region, we observed significant more PD-1 negative/CD20 positive cell subtype in IM region compared to tumor area in relapse groups (0.68% tumor *vs.* 2.21% IM, p=0.0483). Similar trend of less in tumor and more in IM region with CD3 positive and PD-1 negative/CD3 positive cell subtypes was observed in non-relapse group, with p=0.05. Current study indicated that tumor microenvironment carried significant amount variations of cell subtypes between relapse and non-relapse groups. Although there were certain amount of PD-1 negative/CD20 positive cell subtype in relapse group, most located in IM region. The cell distribution feature may be utilized for prognosis prediction of MSS stage III CRCs after chemotherapy to guide patients' health monitoring plans after treatment. Moreover, therapies improve PD-1 negative/CD20 positive and such immune cell subtypes infiltrated into tumor region may improve patient prognosis which need more studies in the field.

#6479 High expression of the exhaustion markers PD-1 and PD-L1 in non-muscle invasive bladder cancer is associated with poor outcome following Bacillus Calmette-Guérin immunotherapy.

T. G. Andreasen, T. Strandgaard, L. Raaby, J. Jensen, L. Dyrskjot;
Aarhus University Hospital, Aarhus N, Denmark

Introduction: The recommended treatment for high-risk non-muscle invasive bladder cancer (NMIBC) includes intravesical instillations of Bacillus Calmette-Guérin (BCG). Despite completing BCG therapy, up to 40 % of patients experience disease recurrence within five years. T cell exhaustion has been associated with poor outcome following treatment with BCG. In this study, we investigated whether T cell exhaustion, characterized by protein expression of PD-1 and PD-L1 in paired samples obtained before and after BCG treatment could provide further insight into BCG response and help predict outcome in patients with NMIBC.
Methods: We included 105 patients with NMIBC, with transurethral resection of bladder tumor (TURBT) samples obtained prior to BCG (n=122) and after in cases with disease recurrence (n=44). Tissue sections from tissue microarrays (TMAs) were stained using immunohistochemistry to analyze the protein expression of the exhaustion markers PD-1 (clone EP239) and PD-L1 (clone 22C3). Data was analyzed using digital pathology software. Cells were scored as positive when detection of a nucleus was combined with a positive PD-1 or PD-L1 stain in both tumor or stromal area (defined by presence of positive pan-CK stain or not, respectively). In total, 167 samples were considered suitable for further analysis (cell count > 200 in tumor and stromal areas). Stratification of samples into high or low expression was based on a median split of the positive cell fraction.
Results: When investigating the fraction of PD-1 and PD-L1 positive cells in the tumors, we observed that PD-1 expression significantly increased with tumor stage in pre- ($p=0.002$) and post-BCG ($p=0.006$) tumor samples. PD-1 expression was also increased in high-grade tumors compared to low-grade tumors in pre-BCG samples ($p = 0.001$). Furthermore, we observed a significant association between tumors of higher stage and high PD-L1 expression in the pre-BCG samples ($p = 0.007$). Patients with low expression of PD-1 and PD-L1 in the pre-BCG tumor samples had a superior high-grade recurrence-free survival (HG-RFS) compared to patients with high PD-1 ($p=0.002$) and PD-L1 ($p=0.021$) expression. In addition, low expression of PD-L1 in pre-BCG tumors was correlated with a better progression-free survival compared to the patients with high PD-L1 expression ($p = 0.013$). When creating a combined score with PD-1 high and PD-L1 high samples, we saw that these patients demonstrated a worse HG-RFS compared to patients with either mixed or low scores ($p = 0.005$).
Conclusion: Protein expression of the exhaustion markers PD-1 and PD-L1 in pre-BCG tumor samples were correlated to higher stage and grade as well as worse HGFRS, indicating that T cell exhaustion plays an important role in resistance to BCG treatment and may be used to guide treatment.

#6480 Single-nucleus RNA sequencing reveals the immunological microenvironment of HPV+ and HPV- penile squamous cell carcinoma.

Andres E. Hernandez-Gonzalez[1], Maria Sanchez-Vazquez[2], Yakshi Ortiz-Maldonado[2], Carlos Rivera-Lopez[3], Brandon Torres-Rivera[4], Hector L. Franco[2], Josue Perez-Santiago[2], Maria Marcos-Martinez[4], Antonio Puras-Baez[3], Magaly Martinez-Ferrer[2]

[1]Department of Pharmaceutical Sciences, University of Puerto Rico - School of Pharmacy, San Juan, Puerto Rico,[2]Division of Cancer Clinical & Translational Research, University of Puerto Rico Comprehensive Cancer Center, San Juan, Puerto Rico,[3]Department of Surgery, Urology Section, University of Puerto Rico - School of Medicine, San Juan, Puerto Rico,[4]Department of Pathology and Laboratory Medicine, University of Puerto Rico - School of Medicine, San Juan, Puerto Rico

Penile squamous cell carcinoma (PSCC) has high morbidity and mortality rates in developing countries. Puerto Rico has the fifth-highest incidence of PSCC globally. HPV-associated PSCC accounts for 20-63% of cases, depending on the geographical area. The tumor microenvironment (TME) plays a crucial role in modulating disease progression. In this study, we seek to characterize the immunological TME in HPV+ and HPV- PSCC using single-nucleus RNA sequencing. We sequenced (Illumina HiSeq, CA, USA) approximately 3,000 nuclei from thirteen fresh PSCC tumor samples (8 HPV- and 5 HPV+) using the 10X Chromium System (10X Genomics, CA, USA). Quality control and filter of raw data was performed using Seurat and UMAP dimension reduction technique was used to cluster the nuclei. Cell types were predicted based on nuclei gene expression using two reference datasets from the Curated Cancer Cell Atlas: Ji 2020 (Skin Squamous Cell Carcinoma) and Kurten 2021 (Head and Neck Squamous Cell Carcinoma). Enrichment and functional analysis of genes that were significantly different between HPV+ and HPV- within each cell type were performed using Metascape. There were 19 distinct cell clusters identified among all samples. Six immune cell clusters were identified: T cells, macrophages, myeloid cells, B cells, dendritic cells, plasma cells. The relative abundance of each cell type was not significantly different between HPV+ and HPV- PSCC. In this study we focused on the T cell, macrophage, and myeloid cell clusters, which are immunologically relevant in cancer. These cell clusters contain differentially expressed genes that belong to pathways associated with protein synthesis (PS), extracellular matrix modulation (ECMM), blood vessel development (BVD), cytokine signaling (CS), and cell motility (CM). In all three immune cell clusters, PS pathway genes such as RPL7, RPL18A, RPL21 were overexpressed in the HPV + tumors. ECMM pathway genes (MMPs, COL1A1), BVD pathway genes (VEGFA), and CM pathway genes (CXCL1, CXCL8) were found to be overexpressed in HPV + tumors. For T cells and macrophages, CS pathways genes such as IL1A and CXCL8 were overexpressed in HPV + tumors. However, myeloid cells overexpressed different genes from the CS pathways in both HPV + (IL18, CCL3) and HPV - (CDKN1A, IFNAR2) tumors. In our analysis of the cancer cell clusters, the top differentially expressed genes among HPV+ tumors were: IL1A, IL24, ATP13A3, CCN1, AREG. Meanwhile the top differentially expressed genes among cancer cells of HPV - tumors were: ATP10B, FXYD3, CSRP2, CYP2C18, and KCTD1. Understanding how HPV infection affects the TME through changes in the molecular pathway interactions is critical for the identification of novel biomarkers and therapeutic targets that could improve treatment outcomes for penile cancer.

#6481 Multiplexed single-cell pharmacotranscriptomics and spatial tumor biology reveals targetable reciprocal cancer-stromal interactions in prostate cancer.

Astrid Murumagi[1], Frida Rantanen[2], Alice Dini[2], Mariliina Arjama[1], Katja Valimaki[1], Elina Multamaki[2], Tuomas Mirtti[3], Antti Rannikko[4], Teijo Pellinen[1], Daniela Ungureanu[2], Olli Kallioniemi[1]

[1]Institute for Molecular Medicine Finland (FIMM), Helsinki Institute of Life Science (HiLIFE), University of Helsinki, Helsinki, Finland,[2]Disease Networks Unit, Faculty of Biochemistry and Molecular Medicine, University of Oulu, Oulu, Finland,[3]HUS Diagnostic Center, Department of Pathology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland,[4]Department of Urology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

Prostate cancer (PCa) remains a leading cause of cancer-related deaths, with castration-resistant metastatic disease posing significant therapeutic challenges. The tumor microenvironment, particularly cancer-associated fibroblasts (CAFs), plays a crucial role in cancer development and in its progression to an aggressive and lethal stage. Previously, we identified a specific CAF subtype expressing fibroblast activation protein and platelet-derived growth factor receptor beta (FAP$^+$/PDGFRβ$^+$) as a strong predictor of poor patient prognosis, associated with PTEN loss and PI3K/pAKT activation in adjacent tumor cells. This study aimed to functionally characterize and pharmacologically inhibit this lethal cancer-stroma interaction phenotype. We first developed a 3D co-culture system using patient-derived primary CAFs and human PCa cells that recapitulates the lethal tumor microenvironment phenotype ex vivo and in vitro, providing robust models for functional studies. Utilizing 96-plex single-cell RNA sequencing with antibody barcoding of live cells, we analyzed the transcriptomic landscape of primary CAFs and assessed the impact of mono- and co-culture conditions on both cell types. Each patient-derived CAF model possessed a unique transcriptional profile, indicating patient-specific CAF characteristics. In 3D co-culture with PCa cells, CAFs exhibited upregulation of FAP and degradome genes, suggesting cancer-induced reprogramming of CAFs to remodel the extracellular matrix and support malignant progression. Concurrently, co-cultured CAFs induced pAKT upregulation in PCa cells, implicating stromal cells in promoting cancer cell proliferation. Hence, we functionally confirmed reciprocal cancer-stroma interactions in co-cultured models, mirroring changes seen in human tumor tissues of aggressive prostate cancers. To investigate potential therapeutic interventions, we applied a 96-plex single-cell pharmacotranscriptomic platform to evaluate drug responses using a library of 30 cancer drugs and key pathway inhibitors. Our findings indicate that dual inhibition of PI3K-AKT signaling in cancer cells and stromal signaling in CAFs could pave the way for combinatorial therapies targeting the reciprocal interactions between PCa cells and CAFs. This approach highlights the opportunity to develop precision cancer medicine strategies aimed at disrupting critical cell interactions within the tumor microenvironment in a tailored fashion.

#6482 Phenoplex™ spatial analysis of whole-slide colon adenocarcinoma imaged with Orion™ 13-plex one-round staining and imaging.

Regan Baird[1], Fabian Schneider[1], Edward Lo[2], Tad George[2], Joshua Nordberg[2]

[1]Visiopharm, Horsholm, Denmark,[2]RareCyte Inc, Seattle, WA

Background: The ability to stain and image tissue at high-plex over an entire slide in a single round enables insights into tissue architecture and the molecular mechanisms of immune and disease processes at unprecedented throughput. Here we investigate a whole slide tissue section of high-grade colon adenocarcinoma, a hot tumor that contains prominent clusters of PD-L1 expression scattered throughout, using single-step high-plex staining and imaging at single-cell resolution followed by the analysis of single-cell phenotypes, tissue segmentation and spatial proximity and nearest neighbor analysis.

Methods: A standard pathology slide was stained in one round with a 13-plex immune-oncology panel then a 21.7 x 18.5 mm area imaged in one round with the Orion™ spatial biology platform (RareCyte). The panel consisted of Hoechst/nucleus, autofluorescence, Ki-67, CD3e, CD4, CD163, CD8a, PD-L1, PD-1, FOXP3, CD68, CD20, Pan-CK, and Vimentin. Analysis was performed on the resulting OME.TIFF file with Phenoplex™ (Visiopharm) using the following pipeline: tissue segmentation into epithelial, necrotic and stromal regions of interest; cell segmentation using the Visiopharm Blueprint APP for DAPI nuclear detection; and phenotyping and spatial biodistribution analysis using the Guided Highplex Workflow.

Results: Data revealed intricate architecture and cellular transitions as tumor cells express varying levels of PAN-CK, with a subset proliferating, and PD-L1 clusters within the lamina propria. Leukocyte clusters are seen interacting with PD-L1+ macrophages and, at higher zoom level, the proximity can be measured of infiltrating T and B cells near the tumor cells. A variety of spatial distance-metric analyses were performed. As an example, neighbor analysis within the stromal ROI showed that PD-L1+ macrophages had a different neighborhood of immune cells within their vicinity compared to PD-L1- macrophages.

Conclusions: These data highlight the importance of sufficient plex, resolution and whole slide context to derive reliable spatial biomarkers of potential prognostic value. The ability to generate such rich data in just hours across a whole section with multiplexed biomarker quantitation makes this approach suited to multi-patient clinical studies. Deep-learning based image analysis enables deeper insights into the TME of cancer samples and enables researchers to robustly and accurately quantify and classify tissue samples to understand biology of disease.

#6483 Powerful end-to-end spatial analyses using precise spatial proteomics.

Charles E. Jackson[1], Matthew H. Ingalls[1], Kayla E. Cashion[1], J Spencer Schwarz[1], Adam Northcutt[1], Arne Christians[2], Oliver Braubach[1]

[1]Bruker Spatial Biology, Saint Louis, MO,[2]Bruker Spatial Biology, Hannover, Germany

Spatial biology supports our understanding of the progression and treatment of cancer by distilling complex cellular interactions into actionable spatial signatures. The most actionable targets to study with spatial methods are protein biomarkers, but non-destructive, high-plex spatial proteomics has been challenging to achieve due to technical limitations posted by available technologies. With the recent advent of enhanced photobleaching in cyclic immunofluorescence (EpicIF™) technology, multiplex immunofluorescence (mIF) can now be achieved more easily and inexpensively. EpicIF technology quickly and safely removes signal from even the brightest organic fluorophores, enabling the use of standard fluorescent antibodies in high-plex spatial proteomic protocols. We leveraged EpicIF technology to design modular mIF assay panels targeting key tumor immune microenvironment (TiME) biomarkers, tissue structural features, and markers enabling accurate cell segmentation. These assay panels were validated for performance (including sensitivity, specificity, and reproducibility) across a range of carcinoma tissues using the CellScape™ Precise Spatial Proteomics platform. Our validated panels enabled robust segmentation of immune, tumor and stromal cell types, ultimately permitting robust and comprehensive end-to-end spatial analysis of the TiME. We identified complex spatial neighborhoods composed of cellular interactions between immune infiltrates containing B-cells and T-cells, with mosaic expression of developmental and activation status markers in the TiME. Together, the use of these modular mIF panels, coined Next-Generation VistaPlex™ Assays, provided a fast foundation for accurate spatial analyses. The pairing of EpicIF technology with ready-to-use mIF assay panels provides the first simple, achievable access to spatial proteomics, including those without previous expertise in spatial biology.

#6484 Single-cell transcriptomic analysis of hepatocellular carcinoma: elucidating intratumoral heterogeneity and immune microenvironment crosstalk.

Qingyang Zhang, Yu-Man Tsui, Vanilla Xin Zhang, Daniel Wai-Hung Ho, Irene Oi-Lin Ng

The University of Hong Kong, Hong Kong, Hong Kong

Background: Hepatocellular carcinoma (HCC), the third leading cause of cancer deaths worldwide, exhibits significant cellular and molecular heterogeneity. The advent of single-cell transcriptomic methodologies has significantly disclosed its intra-tumoral cellular diversity to an unprecedented level. Interactions between cells within the tumor microenvironment (TME) are crucial for cancer progression and affect how tumors respond to current therapies.
Objective: This study aimed to utilize single-cell data to address two main objectives: (1) to dissect the heterogeneous nature of HCC tumors by identifying tumor subclones and their molecular features, key transcription factors, and implicated pathways; and (2) to explore the intricate communication between tumor cells and immune cells, with a focus on the pathways that significantly influence tumor growth and metastasis.
Methods: The tissue samples of HCC were obtained from nine patients undergoing surgery immediately from the operation theater after resection, with informed consent. Dissociated tumor single cells were subjected to scRNA-seq by 10X Genomics platform.
Results: Our analysis identified seven distinct tumor subclones, validating the presence of high tumor heterogeneity. Among these, one subclone, designated as SC6, exhibited particularly distinctive molecular characteristics. Further investigation revealed that NFE2L3 exhibited the highest activity score during the TFs analysis, indicating its significant regulatory role in SC6 cluster, and was intricately associated with pathways involved in extracellular matrix organization and immune regulation. Through cellular communication analysis, we discovered that tumor-associated macrophages (TAMs) and dendritic cells (DCs) exhibited the highest communication intensity with SC6 cluster when acting as the source of ligands. Further investigation revealed critical ligand-receptor pairs, including some integrin family genes functioning as receptors. This interaction suggests that these integrins may engage with ligands secreted by TAMs or DCs within the TME, potentially promoting tumor cell proliferation and migration.
Conclusions: Through comprehensive analysis of scRNA-seq data, we elucidated the intricate heterogeneity within HCC, identifying distinct characteristics of various tumor subclones. Our investigation revealed key transcription factors that predominate in specific subclones. Additionally, we uncovered robust intercellular communication between tumor cells and myeloid cells.

#6485 Prognostic impact of immune cells in vulvar carcinomas.

Zhihao Huang[1], **Elena Bady**[1], Maximilian Lennartz[1], Lisa Hornsteiner[1], Jan Hendrik Muller[1], Monika Kaszubowski[1], Ronald Simon[1], Guido Sauter[1], Sven Mahner[2], Niclas Blessin[1], Nikolaus de Gregorio[3], Rudiger Klapdor[4], Matthias Kalder[5], Elena I Braicu[5], Sophie Furst[2], Maximilian Klar[6], Hans-Georg Strau?[7], Katharina Prieske[1], Linn Wolber[1]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany,[2]LMU Munich, Munich, Germany,[3]Klinikum am Gesundbrunnen, SLK-Kliniken Heilbronn GmbH, Heilbronn, Germany,[4]Hannover Medical School, Hannover, Germany,[5]Charite-University Hospital, Berlin, Germany,[6]University of Freiburg, Freiburg, Germany,[7]University Hospital Martin-Luther-Universitat Halle-Wittenberg, Halle, Germany

Quantification of tumor infiltrating lymphocytes (TILs) and immune checkpoints in the cancer microenvironment has become of increasing interested in immuno-oncology. Vulvar cancer is emerging as a promising target for immune checkpoint blockade therapy. However, only little is known about the presence of TILs and the expression of immune checkpoint molecules in the tumor microenvironment and its predictive value in vulvar cancer. To better comprehend the role of immune cell expression and composition in vulvar cancer, a tissue microarray containing 479 vulvar cancer samples with clinic-pathological data was analyzed by multiplex fluorescence immunohistochemistry including 21 marker proteins to determine the composition of the immune cell infiltrate. Benjamini-Hochberg correction for multiple testing was applied. A total of 479 (85,4%) of the 561 cancers had interpretable results for all 21 markers. The identified immune cell types included cytotoxic-, T-helper- and regulatory T-cells, M1/M2 macrophages, dendritic cells, B-cells, TIM-3, PD-1 and CTLA-4 on immune cells and PD-L1 on immune and tumor cells. Comparison with clinical data revealed significant associations between prolonged progression-free survival and low expression of PD-1 (p=0.008) and TIM3 (p=0.039) on CD8+ T-cells, as well as low PD-L1 expression on M1 macrophages (p=0.046), dendritic cells (p=0.028), and tumor cells (p=0.013). Comparison with the human papilloma virus (HPV) status of the cancers showed a link between high numbers of tumor infiltrating B-cells and a favorable overall survival in HPV+ vulvar carcinomas (p=0.0464, AUC:0.67), but not in the HPV-negative subgroup. The quantification of immune cells alone did not reveal any significant correlation between the overall extent of inflammation and the histopathological parameters of the aggressive disease or the patient outcome. These data suggest that the impact of the immune reaction on the prognosis of vulvar carcinoma patients may be less dramatic than seen for other tumors. However, major differences of the role of anti-tumor immunogenicity between HPV positive and negative cancers, as well as a particular prognostic role of the quantity of B-lymphocytes and the expression of multiple checkpoint proteins is highlighted.

#6486 Prognostic impact of the immune environment in non-small cell lung cancer.

Lisa Hornsteiner[1], Natalia Gorbokon[1], Jonas B Raedler[2], Claudia Hube-Magg[1], Monika Kaszubowski[1], Nina Schraps[1], Katharina Moller[1], Florian Viehweger[1], Florian Lutz[1], Till S Clauditz[1], Christoph Fraune[1], Eike Burandt[1], Ronald Simon[1], Guido Sauter[1], Martin Reck[3], Klaus Raabe[3], Maximilian Lennartz[1], Stefan Steurer[1], **Elena Bady**[1]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany,[2]Harvard University, Boston, MA,[3]German Center for Lung Research, Grosshansdorf, Germany

Lung cancer is the most frequent tumor related reason for death worldwide. It is hoped that a better understanding of the immune environment can foster the development of new immune checkpoint therapies to improve patient management and survival. To study the composition and prognostic impact of the immune infiltrate in non-small cell lung cancer, a tissue microarray containing 798 lung cancer patients, including 441 lung adenocarcinomas, 219 squamous cell carcinomas, was analyzed by multiplex fluorescence immunohistochemistry. A set of 14 antibodies was used to detect cytotoxic-, T-helper- and regulatory T-cells, B-cells, M2 macrophages, and dendritic cells (CD3, CD4, CD8, FOXP3, CD20, CD11c, CD163) expression of immune checkpoint molecules (TIGIT, PD-1, CTLA-4, TIM-3, PD-L1), and immune cell proliferation (Ki67) as well as panCK as an epithelial cell marker for tumor detection. Comparison with clinical data revealed significant associations between a high fraction of intratumoral $CD3^+$ T-cells (p=0.0182), cytotoxic T-cells (p=0.0192) and dendritic cells (p=0.0080) and favorable prognosis, and between a low fraction of intratumoral $CD3^+$T-cells (p=0.0007) and advanced tumor stage. A high Ki-67 labelling index was linked to poor prognosis (p=0.0002) and nodal positivity in all 798 lung cancer patients (p<0.0001), as well as in the subsets of 441 lung adenocarcinomas (p=0.0002) and 219 lung squamous cell carcinomas (p=0.0015). No prognostic relevance was found for the expression of the immune checkpoint molecules TIGIT, PD-L1, TIM-3, CTLA-4 and PD-1 on the immune cells in our series of cancers. In conclusion, a high T-cell infiltrate is linked to beneficial tumor characteristics in non-small cell lung cancers.

TUMOR BIOLOGY: Other Organisms and in Vivo/in Vitro Models
Poster Session

#6490 Patient-derived organotypic tumor spheroids (PDOTS) optimize donor selection in allogenic cell therapy development.

Chunxiao Cui[1], Raluca Dumitru[2], Danielle Hagee[1], Anthony Attardo[1], Kirti Khardenavis[1], Wassim Basheer[2], Sabrina D. Munnik[3], David C. Lopez[3], Gemma Moiset[3], Ketki Bhise[1], Sinal Patel[1], Alyssa Martin[1], **Michael Perricone**[1], Aaron Goldman[4]

[1]Xsphera Biosciences, Inc., Boston, MA,[2]Cell Solutions, Charles River Laboratories, Inc., Northridge, CA,[3]Discovery, Charles River Laboratories, Inc., Leiden, Netherlands,[4]Engineering in Medicine Division, Mass General Brigham, Boston, MA

Introduction: Donor-to-donor variability significantly impacts the process development of cell therapies for cancer. Characterizing the donor-specific functional variances enables donor selection for successful cell therapy development. Ex vivo human models provide unique opportunities to evaluate the functionality of cell therapies in the patient's native tumor microenvironment (TME). Here, using the Xsphera human tumor system, we assessed the specificity, migration, anti-tumor efficacy, and immune modulation of CAR-T cells from different donors to identify the optimal donor characteristics.
Methods: HER2-targeting CAR-T cells were generated from the peripheral blood mononuclear cells (PBMCs) of two healthy donors (CAR-D1 and CAR-D2). HER2+ (ZR-75-30) and HER2- (MDA-MB-468) cell lines were used to generate tumor spheroids. Fresh tumor samples collected from patients with ovarian cancer were partially dissociated into spheroids (PDOTS) ranging from 40-100 µm. Tumor spheroids and PDOTS were loaded into microfluidic devices containing extracellular matrix (ECM) and exposed to CAR-D1 and/or CAR-D2. CAR-T cell migration, proliferation and anti-cancer efficacy were measured and quantified using image-based analysis. RNA transcription was analyzed via NanoString to study tumor response and immune modulation.
Results: Both CAR-D1 and CAR-D2 showed time-dependent tropism through the ECM towards HER2+ tumor spheroids, but not HER2- tumor spheroids. Increased cytotoxicity was observed in HER2+ tumor spheroids with both CAR-T cells, with CAR-D2 showing greater cytotoxicity. CAR-D1 also induced off-target tumor killing in HER2- spheroids, which was not observed in CAR-D2. Nanostring analysis revealed higher cytotoxic signals in CAR-D1, whereas CAR-D2 showed higher antitumor cytokine signaling in HER2+ tumor spheroids and lower anti-tumor activity in HER2- tumor spheroids. In PDOTS derived from ovarian patient tumors (N=3), CAR-D2's cytotoxic effect appeared to be correlated with tumor cells' HER2 expression levels. In high HER2 PDOTS, CAR-D2 led to downregulation of tumor-related genes (ERBB2/HIF1A/EPCAM/EGFR), upregulation of CAR-T activation associated genes (IFNg/GZMB/PRF1/CXCL9/CXCL10/IL1A), and increased CD8 T cell abundances (CD8A/CD8B). In summary, CAR-D2 showed higher target specificity, slower ECM migration, faster spheroids uptake, greater anti-tumor efficacy, and lower off-target cytotoxicity than CAR-D1. Its efficacy in PDOTS appeared to be HER2 expression dependent.
Conclusion: The use of Xsphera's platform to study cell therapies provides a valuable tool for uncovering donor-to-donor variability by differentiating the variability in performance of CAR-T cells derived from different donors. This unique approach will support optimal donor selection and accelerate cell therapy development.

#6491 Advancing precision oncology: Integrating AVI-PDX and patient-derived organoid technologies for enhanced preclinical modeling in immuno-oncology.

Clelia Costechareyre[1], **Romain Teinturier**[1], Jurgen Loskutov[2], Mayrone Mongellaz[1], Madeleine Nadolny[2], Marjorie Lacourrege[1], Loraine Jarrosson[1], Celine Delloye-Bourgeois[1], Lena Wedeken[2], Valerie Castellani[1]

[1]Oncofactory, Lyon, France,[2]Cellphenomics, Berlin, Germany

The AVI-PDX platform enables the creation of miniature, patient-specific tumor models within living organisms, specifically avian embryos. We have developed next-generation, humanized models that incorporate immune cells, expanding the utility of AVI-PDX for investigating immune cell-based therapies and checkpoint inhibitors, both as monotherapies and in combination with standard treatments. Recently, patient-derived organoids have become valuable models for oncology research, offering diverse molecularly characterized resources. However, traditional organoids often lack key components of the stroma and immune microenvironment, limiting their application for studying immune-modulating therapies. To overcome these challenges, we combined AVI-PDX with patient-derived organoid technologies to create an avian patient-derived organoid model, termed AVI-PDO. Here, we describe the generation of a colorectal cancer (CRC) AVI-PDO model.Colorectal tumor organoids from a patient sample were cultured, dissociated, and micro-implanted into avian embryos at established sites within the AVI-PDX model. We conducted a time-course analysis, harvesting embryos 3 to 6 days post-implantation and visualizing them using light-sheet microscopy. Notably, we observed that initially dispersed tumor cells aggregated and reformed 3D organoid-like structures within the embryo. To further characterize these structures, we performed immunolabeling on tissue cryosections, revealing tumor cells organized into spherical formations with central lumens, expressing polarity markers. Additionally, a significant number of Ki67-positive cells were present, indicating active proliferation within these structures. This study demonstrates that our combined AVI-PDX and organoid approach allows the re-creation of organoid-like structures within avian embryo tissues, providing a novel humanized model. This model paves the way for studying immune-based therapies within a whole organism context, offering a comprehensive system that includes systemic signaling and the tissue microenvironment.

#6492 Development of transparent compound zebrafish tumor microenvironment (TME) model system.

Surendra Rajpurohit[1], **Arishia Mishra**[1], Rohan Pasi[1], Shubhra Rajpurohit[2], Sai Nasanally[1], Sreeja Sappati[1], Keyana Williams[1], Timothy Pham[1], Ethan Clegg[1], Kirk Kimmerling[3], Vishal Arora[2], Balakrishna Lokeshwar[1]

[1]Georgia Cancer Center, Medical College of Georgia, Augusta University, Augusta, GA,[2]Cardiology, Medical College of Georgia, Augusta University, Augusta, GA,[3]FiteBac Technology, Marietta, GA

Transgenic (Tg) zebrafish are paramount for studying the phenotypes resulting from overexpression of selected genes globally, or in a tissue-targeted fashion and growing numbers of tissue-specific promoters are becoming available to allow very precise targeting of transgene expression. In our proposed zebrafish glioblastoma experimental therapeutic model system, the first specific aim to develop the homozygous transparent compound transgenic strains from individual transparent transgenic progenies for glioblastoma tumor microenvironment (TME). Development and generation of transparent compound transgenic line *Casper; Tg(mpeg1:mcherry*; *islet1:GFP*) to study CNS resident macrophage, microglial activity and glial cell Interaction. Development and generation of transparent compound transgenic line Casper; Tg(mpeg1:mcherry; myl7:RFP; secA5:YFP) to study microglia, cardiac function, and cell death marker Annexin-5.Development and generation of transparent compound transgenic line *Casper; Tg(mpeg1:mcherry*; *islet1:GFP;* myl7:RFP; secA5:YFP) to study microglia, glial cell, cardiac function and cell death marker Annexin-5. Development and generation of transparent compound transgenic line *Casper; Tg(mpeg1:mcherry;NF-kB:GFP)* to study microglia, NFkB Inflammatory activity. Development and generation of transparent compound transgenic line Casper;Tg(mpeg1:mcherry; myl7:RFP; secA5:YFP; NF-kB:GFP) to study microglia, cardiac function and cell death marker Annexin-5 and NF-kB Inflammatory activity. Development and generation of transparent compound transgenic line *Casper; Tg(mpeg1:mcherry; vgfr:EGFP) and Casper; Tg(mpeg1:mcherry; kdrl:EGFP)* to study microglia and blood vessels and angiogenesis. Development and generation of compound transgenic line *Casper; Tg(mpeg1:mcherry;fli:GFP*) to study microglia, blood vessels, and vasculature. Development and generation of compound transgenic line *Casper:Tg(mpx:GFP);Tg(fms:gal4); Tg(UAS:nfsB.mCherry)* to study neutrophils, macrophages inflammatory process. Development and generation of compound transgenic line: *Casper:Tg(VEGFR2:AcGFP x CREST3-tag RFP-2A-NTR)* to study Crest trigeminal nerve and VGFR blood vessel interaction. By developing a transparent compound transgenic zebrafish model, this specific aim will lead us to explore the glioblastoma tumor microenvironment related biological process alterations and screening of new drugs to improve the diagnosis and treatment of this deadly invasive brain tumor.

#6493 Comparison of NK-92MI cell line vs. primary NK cells derived from peripheral mononuclear blood cells.

Ina Rohleff, Kanstantsin Lashuk, **Julia B. Schueler**

Charles River Laboratories, Inc., Freiburg, Germany

Natural Killer (NK) cell-based therapies are a rapidly growing area in cancer treatment. Preclinical research uses both cell line derived NK cells, such as NK-92MI, and primary NK cells derived from peripheral blood mononuclear cells (PBMCs). Each of these NK cell sources offers distinct advantages and limitations. The NK-92MI cell line, an immortalized and IL-2-independent derivative of the parental NK-92 line, provides a standardized, robust, and easily expandable cell source, making it highly attractive for large-scale experiments. However, NK-92MI cells lack certain activating receptors present in primary NK cells, which may limit their targeting range and reduce their cytotoxic potential against specific cancer types. Conversely, primary NK cells isolated from PBMCs mirror more closely the heterogeneous and receptor-diverse populations present in the human immune system. Yet, primary NK cells also present challenges, including variability between donors, limited proliferation in culture, and complex handling and isolation processes. This study compared the cytotoxic efficacy of the NK-92MI cell line and primary NK cells against HER2+ cancer cells in combination with trastuzumab (anit-HER2 antibody). Using a live-cell imaging platform, we quantitatively assessed the killing dynamics of these NK cell types over time. HER2+ cancer cells (SK-OV3, SK-BR3, HCC1954, JIMT-1) were co-cultured with either NK-92MI cells or PBMC-derived NK cells, and their effect on tumor cell killing was monitored continuously in a 2D and 3D setup. In addition, the migration of different NK cell types through a trans-well membrane was measured to see their migration potential. Key parameters, including the rate of cancer cell death and NK migration activity, were analyzed to determine differences in killing efficiency and sustained potency. The results indicate differential cytotoxic behavior between PBMC-derived NK cells and NK92-MI. In monotherapy the NK92-MI cell line exhibited strong proliferation and increased cytotoxic activity compared to PBMC-derived NK cells, which demonstrated moderate tumor cell killing. However, the treatment with trastuzumab in the presence of the two different NK cell types displayed equally effective killing. Despite JIMT-1 which was resistant towards trastuzumab but displayed reduced growth over time when co-cultured with NK-92MI alone. The migratory capacity of the NK cells was mainly driven by the different CCL2 levels secreted by the four HER2+ cancer cell lines evaluated in the study. In summary, this study highlights the feasibility to use NK cell lines such as NK-92MI for screening and proof-of concept experiments, where the need for scalability and reproducibility is mandatory. However, the limited heterogeneity and lack of certain activating receptors of the NK cell line must be considered when interpreting these studies.

#6494 Genetically characterized canine tumoral cell lines are relevant models to test the efficacy of new targeted therapies: Example of anti-MDM2 targeted therapy.

Pierre Montagne[1], Ulrich Jarry[1], Caroline Confais[1], Edouard Cadieu[2], Aline Primot[1], Nicolai Hoffman[2], Armel Houel[2], Remy Le Guevel[3], Thomas Derrien[2], Catherine Andre[2], Jerome Abadie[4], Christophe Drieu La Rochelle[1], Benoit Hedan[2]

[1]Biotrial, Rennes, France,[2]Institut de genetique et developpement de Rennes UMR6290, CNRS, Rennes, France,[3]UAR Biosit-Oncotrial, Rennes, France,[4]LabOniris, Ecole Nationale Veterinaire de Nantes, Nantes, France

Genetic characterization of cell lines is essential for understanding and predicting sensitivity to anti-tumoral targeted therapies. This study develops a panel of genetically characterized tumoral cell lines to test novel and repositioned anti-tumor therapies, focusing on canine mucosal melanoma (MM) and histiocytic sarcoma (HS) models. We established a diverse panel of MM and HS canine cell lines, leveraging the strong genetic and clinical similarities between canine and human cancers. Using long-read sequencing (Nanopore) technology, we performed comprehensive genetic characterization, identifying single nucleotide variants, structural variations, and copy number alterations. RNA sequencing complemented our genomic analysis by assessing gene expression profiles and potential fusion transcripts. Our methodology involved several key steps:1. Cell line establishment from tumor samples2. Comprehensive genetic profiling3. In vitro drug sensitivity screening4. In vivo validation through mouse xenograft modelsAs a proof of concept, we evaluated Idasanutlin, an MDM2 inhibitor, across our developed cell lines. Our findings demonstrated a clear correlation between TP53 status and drug sensitivity: cell lines with wild-type TP53 were sensitive to the drug, while those with mutated TP53 showed resistance. This result underscores the potential of genetic profiling to guide treatment decisions. The unique value of our approach lies in its ability to:· Identify biomarkers of drug response· Elucidate resistance mechanisms · Discover treatment-responsive subgroups based on genetic profile in previously untreated cancer· Inform the potential of new or repositioned targeted therapiesCanine cancer models offer distinct advantages, including:· Higher incidence of rare cancers in specific breeds· Genetic predispositions from selective breeding· Closer genetic and clinical similarity to human cancersOur cell line panel provides a systematic approach to validate therapeutic strategies tailored to specific genetic contexts. By integrating genomic, transcriptomic, and drug sensitivity data, we create a powerful research tool for both veterinary and human oncology. The research demonstrates the potential of comparative oncology in advancing cancer treatment. Our genetically characterized cell lines offer a comprehensive platform for drug screening and biomarker discovery, ultimately aiming to improve patient care and survival rates for challenging cancers. This work represents a significant step towards more personalized and precise cancer therapies, bridging the gap between preclinical research and clinical application.

#6495 Development of a drug screening platform of glioblastoma utilizing microgel encapsulation.

Brittany A. Payan[1], Annika Carrillo Diaz de Leon[1], Tejasvi Anand[2], Gunnar B. Thompson[3], Brendan A. C. Harley[3]

[1]Bioengineering, University of Illinois at Urbana-Champaign, Urbana, IL,[2]Molecular and Cellular Biology, University of Illinois at Urbana-Champaign, Urbana, IL,[3]Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign, Urbana, IL

Background. We report a method to encapsulate glioblastoma (GBM) cells in maleimide-functionalized gelatin (GelMAL) microparticles for *in vitro* drug screening. GBM is the most common primary malignant brain tumor. Standard of care is surgical resection followed by treatment with the alkylating agent temozolomide (TMZ). GBM cells that evade surgery eventually become resistant to TMZ and lead to recurrence of tumors in patients. Hydrogels have been implemented as matrices for 3D culture that can recapitulate tumor biology and tumor microenvironment. However, bulk hydrogels require large numbers of cells per construct and possess inherent diffusional limits. With only four drugs currently FDA-approved for GBM treatment, there is a need for a platform capable of accelerating the identification of new therapies in a highly controlled and physiologically relevant environment. Hydrogel microparticles allow encapsulation of GBM cells in a tailorable 3D matrix, rapid diffusion to limit spatial hypoxia gradients, and ease of handling for high-throughput screens (HTS).
Methods. U87MG cells are encapsulated in GelMAL microparticles using a flow-focusing microfluidic device. Viability and proliferation of GBM cells is assessed via LIVE/DEAD cell imaging kit and double stranded DNA (dsDNA) quantification, respectively. We record a response from encapsulated cells after TMZ exposure (0-1000 μM) via cellular activity using alamarBlue.
Results. Cellular viability within microfluidic emulsion is limited by shear forces exhibited in the microfluidic device. Here, we report a method to encapsulate GBM cells in microgels and retain cellular viability. Increasing levels of dsDNA suggest maintenance of cellular health in microgels and indicate a proliferative population of encapsulated cells. Metabolic profile of encapsulated cells shows a decrease in cellular activity in response to increasing concentrations of TMZ, concurrent with 2D and bulk hydrogel culture. Ongoing experiments will assess cellular behavior post-movement by liquid handler of encapsulated cells to establish a drug screening platform amenable for HTS.
Conclusion. We have developed a method to reliably encapsulate GBM cells into GelMAL microgels while retaining viability. The recorded cellular activity of encapsulated cells in response to TMZ demonstrates the utilization of microgels as an *in vitro* drug screening platform. We will leverage this platform to examine drug response *in vitro* amenable for high-throughput screening to identify possible therapeutics for TMZ resistant tumors.

#6496 Humanized tridimensional model of osteosarcoma to test immunotherapeutic strategies.

Bobby Dow[1], Federica Banche-Niclot[1], Lorenzo Apolloni[2], Giada Bassi[2], Jason Yustein[3], Monica Montesi[2], Francesca Taraballi[1]

[1]Center for Musculoskeletal Regeneration, Houston Methodist Research Institute, Houston, TX,[2]Institute of Science, Technology, and Sustainability for Ceramics, National Research Council, Faenza, Italy,[3]Winship Cancer Institute and Aflac Cancer and Blood Disorders Center, Emory University, Atlanta, GA

Osteosarcoma (OS) is the most common primary malignant bone cancer, affecting both young adults (ages 10-30) and older adults (over 60). Despite progress in cancer research, survival rates have remained largely unchanged for the past 40 years, particularly for patients with metastatic disease, where 5-year survival rate is about 20%. This lack of progress is partly due to inadequate preclinical models that replicate the complex tumor microenvironment, hindering the development of effective new treatments.

Traditional 2D cell culture models fail to replicate the intricate interactions between tumor cells, the extracellular matrix (ECM), and stromal cells, which are key factors in cancer behavior and therapy response. In contrast, three-dimensional (3D) models offer a more accurate representation of the tumor's architecture, improving predictions of drug efficacy and tumor progression. Moreover, one of the most critical factors influencing OS treatment outcomes is the presence of a subpopulation of cells known as cancer stem cells (CSCs). CSCs contribute to tumor recurrence and metastasis due to their self-renewal and differentiation ability. Furthermore, CSCs are resistant to conventional therapies, such as chemotherapy and radiotherapy. This, coupled with the fact that OS is considered a 'cold tumor,' makes it particularly challenging to treat with immunotherapy.

Our lab is dedicated to developing humanized 3D OS models that closely mimic the tumor microenvironment, integrating OS cells, immune components, and ECM elements such as collagen. These advanced models address the limitations of animal studies by offering a physiologically relevant platform to investigate CSC behavior, drug resistance mechanisms, and tumor-stroma interactions specific to OS. Utilizing patient-derived xenografts (PDX), we generated 3D tumor sarcospheres that recapitulate key features of OS, including high CSC enrichment and resistance to conventional therapies.

To characterize these 3D sarcospheres, we employed CellTiter Glo assays and Live/Dead staining to evaluate viability, flow cytometry for stemness markers (CD44, CD117, and CD133), and PCR analysis for CSC-associated genes (OCT4, Nanog, and SOX2). Our findings consistently demonstrate enhanced CSC populations and distinct tumorigenic features in 3D cultures compared to traditional 2D models.

Additionally, we incorporated immune components in the 3D model by co-culturing OS PDX cells with immune cells (THP-1), further refining the tumor microenvironment. This integrated approach facilitates a comprehensive understanding of tumor cell-immune cell interactions and provides a robust, scalable platform for testing immunotherapeutic strategies. Ultimately, these humanized 3D models aim to address a significant gap between preclinical studies and clinical applications, thus improving the development of novel and effective therapies for OS.

#6497 Modeling HCC molecular and histological features by CRISPR editing of primary hepatocytes.

Lobna Elkhadragy[1], Olayinka G. David[1], Caitlyn C. Castillo[1], Isadora Andre Rosa Lopes1[1], Hannah Liu[1], Luke R. Jordan[1], Luke N. Redlon[1], Grace Guzman[1], Jonathan P. Samuelson[2], Lawrence B. Schook[1], Amaia Lujambio[3], Kyle M. Schachtschneider[4], Ron C. Gaba[1]

[1]University of Illinois at Chicago, Chicago, IL,[2]University of Illinois at Urbana-Champaign, Champaign, IL,[3]Icahn School of Medicine at Mount Sinai, New York, NY,[4]Sus Clinicals, Chicago, IL

Background: Hepatocellular carcinoma (HCC) is an aggressive disease with poor response to current therapies, highlighting the need for more effective therapeutic strategies. Preclinical HCC models are necessary tools for assessment of novel therapies. Pigs offer a valuable disease model due to their similarities with humans and their large size which allows testing clinically relevant procedures. Here, we aimed to develop HCC cell and animal models by precise gene manipulation of primary porcine hepatocytes.
Materials and Methods: Hepatocytes were isolated from minipigs (n=4) and subjected to CRISPR knockout (KO) of tumor suppressor genes, with or without oncogene overexpression (OE). Gene alteration combinations included KO of *TP53*, *PTEN*, and/or *CDKN2A*, with or without c-myc overexpression. Gene alterations were validated by Sanger sequencing, PCR, and Western blotting. The developed cell lines were injected subcutaneously into severe combined immunodeficient (SCID) mice to confirm their tumorigenicity and assess histological features. Cells with *TP53*^{KO}; *PTEN*^{KO}; *CDKN2A*^{KO}; c-myc^{OE} (TPKM) were then autologously injected into pigs (n=2) by percutaneous ultrasound-guided liver parenchymal injection and tumor development was monitored by ultrasound and CT imaging. Tumors from mice and pigs were analyzed by histological and transcriptional analyses.
Results: The induced gene alterations in porcine hepatocytes resulted in the successful development and propagation of four distinct cell lines, all validated for the intended modifications. Injection of these cells into SCID mice resulted in tumor development confirming *in vivo* tumorigenicity. Histological analysis of mouse xenograft tumors identified epithelial neoplasms with multiphenotypic cells, displaying moderate to poor differentiation, and closely mimicking human liver cancer. In pigs, autologous injection of TPKM cells resulted in intrahepatic mass formation within 3 weeks. Histological analysis of the porcine masses revealed neoplastic cells with positive Arginase staining, negative CK19 staining, and intratumoral immune cell infiltration. Transcriptional analysis of the mouse and pig tumors showed activation of pathways associated with cancer cell proliferation and hepatic fibrosis.
Conclusion: This study successfully establishes genetically defined HCC models with histological and molecular features resembling human HCC by CRISPR editing of primary porcine hepatocytes. These innovative HCC cell models provide valuable tools for drug screening and mechanistic studies, and the porcine model is a promising tool that harbors defined tumor driver mutations and is suitable for locoregional therapy testing. Together, these novel HCC cell and animal models offer promising avenues for preclinical evaluation of innovative therapeutic strategies.

#6498 Development of a rat model to study *ESR1* mutations in estrogen receptor-positive breast cancer.

Lillian He[1], Wen Bu[2], Ruixin Xu[2], Yi Li[2]

[1]Rice University, Houston, TX,[2]Baylor College of Medicine, Houston, TX

Estrogen receptor α (ER)-positive breast cancers constitute approximately 70% of all breast cancers and are commonly treated with surgery and endocrine adjuvant therapies. While these treatments significantly reduce mortality, resistance to endocrine therapies often emerges, leading to metastatic disease. One notable mechanism of resistance is *ESR1* gene mutations, which usually alter the ligand-binding domain of ERα. To advance the understanding of *ESR1* mutation-driven tumorigenesis and metastasis, an immunocompetent animal model is essential. Existing models, such as patient-derived xenografts, lack an intact immune system. However, rat mammary tumors are predominantly ER-positive, offering a unique advantage over mouse models, which typically form ER-negative tumors. Despite this, no rat models harboring *ESR1* mutations currently exist. Here, we report the development of a novel ER-positive breast cancer model in rats carrying the *Esr1* D538G somatic mutation, created using a CRISPR-Cas9-mediated homology-directed repair (HDR) precision genome-editing approach. The *Esr1* D538G mutation is paired with a tumor-initiating Neurofibromin 1 (*Nf1*) loss-of-function mutation which was previously shown to establish ER-positive and estrogen-dependent rat mammary tumors. Detailed characterizations of this tumor model will be presented at the conference. This innovative platform provides an essential tool for investigating mechanisms of endocrine resistance in ER-positive breast cancer and for exploring novel therapeutic strategies to treat endocrine-resistant breast cancer.

#6499 Miniaturized 3D cancer models for measuring efficacy of cancer therapeutics.

Jamie Hodgson[1], Hannah Findlay[1], Timothy London[1], Agapitos Patakas[1], Michele Zagnoni[2], Karla Paterson[3], Ronan Mellin[3]

[1]RoukenBio, Motherwell, United Kingdom,[2]Electronic & Electrical Engineering, University of Strathclyde & ScreenIn3D Ltd, Glasgow, United Kingdom,[3]ScreenIn3D Ltd, Glasgow, United Kingdom

Whilst Chimeric Antigen Receptor T-cell (CAR-T) therapy has been proven beneficial in treatinghaematological malignancies and melanoma, their clinical success against the majority of solidtumour types has been limited thus far. The need for physiologically relevant 3D, complex *in vitro*models of cancer is steadily increasing due to the emergence of drugs targeting the immunesystem and the tumor microenvironment (TME). Current *in vivo* and *in vitro* models are notsufficiently representative of the human (TME) and can fail to accurately predict the clinicaloutcome of immunotherapies. Furthermore, an increasing interest in precision treatment of cancerpatients and the rise of anticancer therapies used in combination has highlighted the need forlarge-throughput 3D efficacy tests capable of maximizing data generation using very smallquantities of 3D tumour models, especially including those from clinical samples. To this aim,microfluidic and organ-on-a-chip technologies can facilitate mechanistic studies and, at the sametime, miniaturize investigation of therapy efficacy on relevant human tumor samples.This work aimed to develop novel, high-throughput and miniaturized efficacy assays todemonstrate CAR-T mediated cytotoxicity using the Her2 receptor, a common target over-expressed on many types of solid tumors. $Her2^+$ and $Her2^-$ expressing cancer cells were culturedin 3D within $UpScale^{3D}$ microfluidic platforms (ScreenIn3D Ltd). Hundreds of spheroids wereformed within 48 hours and, subsequently, CAR-Ts with low and high affinity for the target(RoukenBio) were co-cultured with the spheroids over 72-hour incubation at the desired E:T ratio.Viability staining and cell type specific markers were used as readouts. Results showed that CAR-T quickly targeted, disaggregated and specifically killed high $Her2^+$ expressing cells in 3D models.In contrast, CAR-T cells did not elicit statistically significant cytotoxicity in low/non-$Her2^+$expressing spheroids. Immunotherapy treatment was compared to cytotoxicity elicited bychemotherapy agent carboplatin.This versatile microfluidic platform offers high quality and multiplexed efficacy screening assays onspheroid co-cultures, organoids and primary tumour fragments. The platform is designed for self-generation of multiple drug concentration gradients, offering a unique system to miniaturize drugcombination studies using patient's tissue and, at the same time, creating cost-effective and fastimmune-oncology assays for extensive drug combination studies and precision medicine drugdiscovery.

#6500 Application of human inducible pluripotent stem cells (hiPSCs) with CRISPR/Cas9-mediated deletion of *CUX1* as a model for high-risk myeloid neoplasms.

Joseph Michael Cannova[1], Kaina A. Millan[2], Yuqing Xue[2], Aubrianna S. Ramsland[2], Zara Khosravi[3], Adesewa Ogunsusi[4], Matthew Jotte[5], Megan McNerney[6], Michael Drazer[1]

[1]The University of Chicago Medicine Comprehensive Cancer Center, Hyde Park, IL,[2]Biological Sciences Division, The University of Chicago, Hyde Park, IL,[3]Biological Sciences Division, The University of Chicago Medicine, Hyde Park, IL,[4]The University of Chicago, Hyde Park, IL,[5]Pritzker School of Medicine, The University of Chicago, Hyde Park, IL,[6]Pathology and Pediatric Hematology/Oncology, The University of Chicago, Hyde Park, IL

Acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) with cytogenetic abnormalities of chromosome 7 have dismal survival outcomes. Monosomy 7 or deletion of 7q (-7/del7q) are adverse-risk cytogenetic features found in up to 12% of MDS/AML patients and are further enriched in therapy-related MDS/AML. Despite significant scientific effort, no targeted therapy is available for patients with -7/del7q MDS/AML. Several genes are associated with -7/del7q leukemogenesis. Cut-like homeobox 1 (*CUX1*) is a gene encoding a context-dependent transcription factor required for several molecular pathways, including epigenetic signaling. *CUX1* is deleted in -7/del7q MDS/AML and somatically mutated in 2-4% of MDS/AML. *CUX1* mutations and *CUX1* deletions are associated with a poor prognosis, and are known to co-occur with adverse risk *TP53* mutations and a complex cytogenetic background. Thus, there is a need for novel human models of MDS/AML to isolate the effect of *CUX1* alterations. To address the need for high-fidelity models of MDS/AML with high-risk genetic changes, we transfected human inducible pluripotent stem cells (hiPSCs) with *CUX1* targeting gRNA and CRISPR/Cas9 ribonucleotide-protein (RNP) complexes to generate isogenic hiPSC lines bearing $CUX1^{+/-}$ and $CUX1^{-/-}$ genotypes alongside parent hiPSCs and sham transfection lines with a $CUX1^{+/+}$ background. These derivative cell lines underwent rigorous validation by karyotypic G-banding analysis, sequencing, immunoblotting, trilineage germ layer differentiation, and analysis of hiPSC transcriptional and immunophenotypic markers. We hypothesized that the loss of one or both alleles of *CUX1* would result in transcriptional changes similar to *CUX1* mutated and -7/del7q MDS/AML. This model can be further leveraged to identify potential treatment targets. We obtained bulk RNA sequencing of undifferentiated hiPSCs and hiPSC-derived hematopoietic stem and progenitor cells (i-HSPCs) of each genotype ($CUX1^{+/+}$, $CUX1^{+/-}$, and $CUX1^{-/-}$). We identified several differentially expressed genes and novel long non-coding RNAs in hiPSCs with Cas9-mediated *CUX1* deletions, including downregulation of distinct subsets of genes in $CUX1^{-/-}$ and $CUX1^{+/-}$ hiPSCs. Bulk RNA sequencing of $CUX1^{-/-}$ and $CUX1^{+/-}$ i-HSPCs identified an association between reduced expression of thioredoxin-interacting protein (*TXNIP*), a known regulator of autophagy. This study supports the use of hiPSC-based CRISPR/Cas9 gene editing as a novel platform to identify gene-specific alterations in genotypically and cytogenetically complex myeloid neoplasms.

#6501 The SRG RAT supports in vivo human cell xenotransplantation through enhanced tumor microenvironment interactions.

Caitlin M. O'Connnor, Diane Begemann, Kaitlin P. Zawacki, Kelsey Barrie, Fallon K. Noto, Grace Walton, Gabrielle H. Onishi, Jessica Durant, Taylor Branyan, Mike Schlosser, **Goutham Narla**

University of Michigan, Ann Arbor, MI

The use of immunodeficient mice for human tumor engraftment has proven to be an essential model of human cancer, with uses ranging from basic science to translational research. However, low engraftment rates, slow growth, and smaller tumor volumes can be limitations. Previously, we reported a highly immunodeficient rat strain developed by Hera BioLabs, with the functional deletion of both the Rag2 and Il2rg genes on the Sprague-Dawley background (**SRG RAT®**), which lacks B, T, and NK cells. We have shown that the SRG rat supports the growth of multiple PDX and CDX models, however, the mechanisms underlying the supportive growth are not fully understood. Additionally, direct comparison of tumor growth and the rates of engraftment between the SRG rat and NSG mice has remained limited. Here, we subcutaneously engrafted two CDX and five PDX models into SRG or NSG animals and tracked tumor growth. These models included prostate, lung, ovarian, and uterine cancer models. In all cases, the engraftment and tumor growth rates were better supported in the SRG rat compared to the NSG mouse. Interestingly, the SRG rat is not more immunocompromised than the NSG mouse, suggesting alternative mechanisms leading to the supportive growth in the SRG rat. To understand this, we explored potential differences in the tumor microenvironment (TME) between models grown in the two host animals. We analyzed tumors from the H660 CDX model of castrate-resistant prostate cancer grown in either SRG rats or NSG mice using markers of the TME via immunohistochemistry. This analysis showed differences in the vasculature and macrophages between the two host species. We complemented this analysis using Xenium in situ by 10X genomics to allow for single-cell spatial imaging of engrafted tumors. This analysis showed upregulation of the human CXCL2, LAMP3, and other genes in H660 tumors grown in the SRG rat vs the NSG mouse. Interestingly, high expression of these two genes has been linked to poor prognosis in cancer. Combined, our data demonstrate that the SRG rat supports the growth of multiple human cancer types and displays enhanced tumor microenvironment interactions compared to NSG mice.

#6502 Inhibition of VCP in an *in vivo* model of metastatic melanoma selected based on a predictive biomarker demonstrates tumor reduction.

Aofie Campbell, Dora Kallai, Boldizsar Elek, Csilla Hegedűs, Magdolna Djurec, Ivan Fekete, Peter Szikora, Csaba Szantai-Kis, Amrik Basran, Stephen Connolly, **Daniel Veres**

Turbine Simulated Cell Technologies Ltd., Budapest, Hungary

Valosin containing protein (VCP) is a member of the AAA-ATPase family. It is involved in multiple cellular processes, such as protein degradation, DNA damage response, protein quality control and clearance in the endoplasmic reticulum, all of which are important functions often dysregulated in cancer. VCP is a common essential gene and there have been reports of toxicity associated with its inhibition, therefore a predictive biomarker is needed to stratify patients who are selectively vulnerable. Turbine's Simulated Cell model highlighted VCP from an *in silico* target identification screen where a subgroup of melanoma was found to be most sensitive to VCP inhibition (VCPi). These findings were confirmed in a cell line panel covering 12 indications, consisting of 64 cell lines. Next, a simulated drug modifier screen was run using patient relevant avatars with prevalent alterations, which identified MITF as a predictive biomarker of VCPi sensitivity. An *in vitro* melanoma model of MITF dependency, SKMEL5, was used to test our hypothesis. Hypotheses derived from *in silico* predictions were validated using various forms of VCP loss including pharmacological inhibition using a phase 1 VCPi, CB5083, as a reference compound, siRNA and by conditional knockout. The sensitivity of VCPi in melanoma was confirmed *in vitro* and a correlation (R= -0.86) was found between the protein levels of MITF and IC50 values of VCPi. In the SKMEL5 cells, we observed a dose dependent reduction in levels of MITF following VCPi. Next, we sought to test the efficacy of VCPi in the SKMEL5 *in vivo* CDX model. Four weeks of CB5083 dosing using 50 mg/kg 4on/3off schedule was tolerated, resulted in 46% tumor growth inhibition, and this was accompanied by a downregulation of MITF and one of MITFs targets, DCT, in tumor samples post treatment. These studies support the therapeutic potential of VCP inhibitors in the MITF ^{High}/MITF ^{Amplification} subgroup of patients with metastatic melanoma with a poor prognosis and a decreased 5-year survival. Targeting a common essential gene such as VCP is associated with a narrow therapeutic index, however, we have demonstrated efficacy using a relatively low dose of 50 mg/kg . Taken together, we are proposing a patient stratification strategy based on the identification of a predictive biomarker from an *in silico* screen.

#6503 Preclinical insights into chemotherapy-induced alopecia: a focus on mouse and rat models.

Simonetta I. Gaumond, Isabella Kamholtz, Caroline A. Crowley, Claudia Kersting, **Joaquin J. Jimenez**

University of Miami Miller School of Medicine, Miami, FL

Chemotherapy-induced alopecia (CIA) is a prevalent and distressing side effect of cancer treatment, significantly affecting patients' quality of life and highlighting the critical need for preclinical models to investigate its mechanisms and develop effective interventions. Murine models, including both rat and mouse species, are indispensable in advancing our understanding of CIA. This review explores the use of murine models in CIA research, focusing on the most commonly used strains, chemotherapies, and administration methods. Rat models primarily utilized Fischer, Long-Evans, and Sprague-Dawley strains, treated with chemotherapeutic agents such as cyclophosphamide, cytarabine, doxorubicin, and etoposide. Most of these chemotherapies were injected intraperitoneally (IP), with occasional intravenous (IV) or subcutaneous (SQ) injections. Mouse models primarily involved C57/BL mice, with chemotherapy also administered predominantly via IP injection, along with a few IV and one SQ method. The primary agents responsible for inducing alopecia in these models included cyclophosphamide, doxorubicin, and, less frequently, 5-fluorouracil, cytosine arabinoside, etoposide, and paclitaxel. The assessment of alopecia progression relied on visual evaluation, photography, and histological analysis. While these models provide critical insights into CIA mechanisms, variability in outcomes underscore the need for standardized protocols to enhance reproducibility and translational relevance in preclinical CIA research.

#6504 Generation of stable reporter pools in established patient-derived tumoroid models.

Xiaoyu Yang[1], Jacob Delgadillo[1], Garrett Wong[1], Matthew Woods[1], Jakhan Nguyen[1], Colin Paul[2], Chris Yankaskas[2], Pradip Shahi Thakuri[2], Shyanne Salen[2], Sylvia Beam[2], Matt Dallas[2], David Kuninger[2], Jason Sharp[1]

[1]Thermo Fisher Scientific, Carlsbad, CA,[2]Thermo Fisher Scientific, Frederick, MD

Tumoroids are patient-derived cancer cells grown as 3D, self-organized multicellular structures. Recently, tumoroids have emerged as powerful and reliable platforms for preclinical cancer drug discovery due to their ability to retain key characteristics from the original tumor. However, traditional tumoroid culture relies on complex media formulations and labor-intensive culture workflows. To tackle this issue, we have developed the serum-free, conditioned medium-free Gibco™ OncoPro™ Tumoroid Culture Medium for derivation and expansion of tumoroids. Our previous data demonstrated that OncoPro medium enabled the transition of 18 out of 19 previously established tumoroid models of various cancer indications from the National Cancer Institute Patient-Derived Models Repository (NCI PDMR) and another commercial source and preserved their genetic and transcriptional characteristics. Here, we extend this work demonstrating the compatibility of OncoPro medium with many previously established tumoroid lines and present a stable reporter pool generation custom project for a leading pharmaceutical company using a client-provided colorectal tumoroid line. We first tested the ability for the tumoroids to adapt to OncoPro medium and a suspension culture protocol. Tumoroids displayed higher growth rate when cultured in OncoPro medium compared to client-provided media (CM) in both embedded and suspension culture. To enable scalability, suspension culture was selected for downstream reporter pool generation. Firefly luciferase (fLuc)- and green fluorescent protein (GFP)-expressing tumoroids were generated by lentivirus transduction followed by antibiotic selection in OncoPro medium and CM. 5 days after transduction, >90% of cells were GFP-positive, minimizing the selection bias from editing and preserving population heterogeneity. 6 weeks after selection began, GFP-expressing pools evaluated by flow cytometry showed >97% GFP positive cells, while fLuc-expressing pools showed a cell number-dependent increase of luciferase activity in both media systems. Notably, OncoPro medium displayed a considerable advantage for reporter pool expansion, saving >50% of media and hands-on time compared to CM. To test for genetic and transcriptional stability, stable pools were compared with parental cells grown in CM using targeted NGS and bulk RNA sequencing. High correlation coefficients ($r \geq 0.99$ for targeted NGS and ≥ 0.90 for RNA sequencing) were observed in pre- and post-edited pools, suggesting minimal genetic and gene expression drift over the engineering process. Taken together, these findings represent the ability of OncoPro medium to support cell engineering workflows on established tumoroids while preserving characteristics of the parental tumoroids. These tumoroid reporter pools serve as effective and clinically relevant models for evaluating cancer therapies in downstream applications.

#6505 Evaluation of the potential role of K21 pharmacogenomics and its application in the tumor microenvironment and experimental therapeutics by using zebrafish model.

Surendra Rajpurohit[1], Sai Nasanally[1], Pragya Rajpurohit[1], Arishya Mishra[1], Devan Manhiani[1], Rohan Pasi[1], Sharad Purohit[2], Nikhil Patel[3], Kirk Kimmerling[4], Bhupesh Prusty[5]

[1]Georgia Cancer Center, Medical College of Georgia, Augusta University, Augusta, GA,[2]Center for Biotechnology and Genomic Medicine, Medical College of Georgia, Augusta University, Augusta, GA,[3]Pathology, Medical College of Georgia, Augusta University, Augusta, GA,[4]Fitebac Technology, Marietta, GA,[5]INSTITUTE OF MICROBIOLOGY AND VIROLOGY, Riga Stradins University, Riga, Latvia

The zebrafish is an ideal vertebrate model system for glioblastoma experimental therapeutics. Our laboratory is a pioneer in glioblastoma related to zebrafish genomics. We study to identify signature genes through differential expression and pathway analysis and evaluate their potential role in tumor microenvironment (TME) related biological process alterations and screening of new drugs in glioblastoma therapeutics. K21 is an established antimicrobial drug known for its antimicrobial activity. We have examined the potential role of K21 pharmacogenomics in the study of tumor microenvironment and experimental therapeutics by using zebrafish. In this study, the experiments were designed to study the K21 drug impact on zebrafish genomic common pathway enrichment analysis and the comparison of Gene set enrichment analysis (GSEA) between control and K21 drug-treated larvae to define activated and suppress gene ratio. The novel finding reveals the Significant Activated group: regulated to DNA templated transcription, brain development, regulation of transcription by RNA-polymerase-II, monoatomic ion transport, nervous system development, neuron differentiation, monoatomic ion transmembrane transport, anterior/ posterior pattern specification, neuropeptide signaling pathway, chemical synaptic transmission. Significant Suppressed Group: intracellular signal transduction, lipid metabolic process, protein transport, steroid biosynthetic process, endoplasmic reticulum to Golgi vesicle-mediated transport, intermediate filament cytoskeletal, keratinocyte differentiation, and keratinization. Surprisingly, K21 shows significant over-expressed GSEA-like brain development, neuronal differentiation, neuropeptide pathways, regulation of transcription, nervous system development, and significant suppression of innate immune response, T-cell co-stimulation, apoptotic process, lipid metabolism, Neglog10. These significant activations and suppression will be helpful to establish a potential therapeutic application of the K21 drug candidate for glioblastoma tumor microenvironment (TME) and associated ramifications. The outcomes of this study will open a new window in glioblastoma translational research, which explores novel areas to improve the discovery of therapeutic targets and novel agents to improve patient outcomes in an area where current therapies are mostly suboptimal.

#6507 Using induced pluripotent stem cells to model tyrosine kinase inhibitor response heterogeneity in chronic myeloid leukemia.

John Joson Ng[1], Lin Ming Lee[2], Yi Yang Lim[1], Kian Leong Lee[1], Fengcong John Ouyang[1], Vaidehi Krishnan[1], Sin Tiong Ong[1]

[1]Duke-NUS Medical School, Singapore, Singapore,[2]National Cancer Centre Singapore, Singapore, Singapore

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm caused by the fusion of *BCR* in chromosome 22 and *ABL1* in chromosome 9. The resulting BCR::ABL1 fusion protein transforms the hematopoietic stem cell into a leukemic stem cell (LSC), driving malignant proliferation. CML is managed using targeted cancer therapy with tyrosine kinase inhibitors (TKIs), notably imatinib, which binds to the ATP-binding pocket of BCR::ABL1, inhibiting its kinase activity. While most patients respond well to TKI treatment, about 40% of patients experience suboptimal responses and primary resistance, warranting an in-depth exploration of the underlying molecular causes. Here, we employ BCR::ABL1-expressing induced pluripotent stem cells (iPSCs) as a platform for modelling treatment response heterogeneity in CML, and development of novel therapeutic approaches that convert poor TKI-responders into optimal responders.

Our laboratory has previously shown that specific cellular and molecular features in diagnostic patient bone marrow (BM) predict future TKI sensitivity (Krishnan *et al.*, *Blood*, 2023). Optimal responders show an enrichment of erythroid progenitor populations, an LSC gene expression signature indicating increased BCR::ABL1 activity, as well as aberrant expression of a canonical erythroid transcriptional program (ETP) that is known to induce an erythroid lineage bias. Consistent with an LSC-intrinsic mechanism for TKI sensitivity, hematopoietic stem and progenitor cells (HSPCs) from optimal responders exhibited a profound erythroid progenitor bias in colony-forming assays, coupled with an exquisite TKI sensitivity when compared to those with primary resistance. HSPCs from poor responders exhibited a myeloid bias in colony-forming assays, with myeloid colonies being more TKI resistant as compared to erythroid counterparts. We hypothesize that higher *BCR::ABL1* activity in LSCs from optimal responders results in ETP-driven erythroid lineage skewing *in vivo*, generating a higher proportion of erythroid progenitors which are intrinsically TKI-sensitive. To test our hypothesis, we reprogrammed diagnostic CML bone marrow samples from 3 optimal responders and 3 poor responders into iPSCs and validate their pluripotency and that any mutations and chromosomal aberrations are conserved. Examination of the transcriptome reveals that key CML-related gene programs are conserved from the primary sample to their iPSC-derived counterparts, e.g., MYC gene programs in poor responder primary and iPSC-derived samples. We conclude that iPSCs will provide a useful platform for testing hypotheses accounting for response heterogeneity in CML, as well as uncovering novel therapeutic approaches.

#6508 The SRG RAT, an immunodeficient model for immortalized cell line and primary patient derived orthotopic glioblastoma xenograft tumors.

Diane Begemann[1], Elizabeth Fenton[2], Niveen Fulcher[2], Cynthia Dunn[1], Darrell Robertson[1], Cassandra Scutchfield[1], Tanner Keener[1], Cleusa De Oliveira[3], John Kelly[4], Ying Xia[2], John Ronald[4], R. Grace Walton[1], Shilpa Sampathi[1], Valeriya Steffey[1], Michael J. Schlosser[1], Susanne Schmid[5], Matthew O. Hebb[2], Fallon K. Noto[1]

[1]Hera BioLabs, Lexington, KY,[2]Department of Clinical Neurological Sciences, Western University, London, ON, Canada,[3]Department of Anatomy and Cell Biology, Western University, London, ON, Canada,[4]Robarts Research Institute, Western University, London, ON, Canada,[5]Program in Neuroscience, Western University, London, ON, Canada

Human cancer xenografts are vital for understanding tumor biology, growth kinetics, and therapeutic efficacy. Historically, immunodeficient mice have been the standard rodent species for cancer xenografts. However, an immunodeficient rat that supports a variety of human cancer cell types would provide a larger rodent for easier surgical manipulation, serial blood sampling, and combined efficacy, pharmacokinetics, and toxicology studies. Hera BioLabs created a Sprague Dawley *Rag2 -/-, Il2rg -/-* Rat (*SRG RAT®)* that provides a highly supportive environment for growing tumors of human origin. The SRG rat lacks B, T, and NK cells and is more immunodeficient than the Nude rat, suggesting it may be permissive to a wider variety of human cancer types. Here we demonstrate the utility of the SRG rat in supporting intracranial growth of cell- and patient-derived (CDX and PDX) human cancers that grow in the brain. We inoculated female SRG rats with human primary glioblastoma cell lines U87MG-luc and U118MG-luc to establish tumors in the striatum of the rat brain. Additionally, PDX models from both glioblastoma stem cells and adherent, differentiated glioblastoma cells from different human tumors in the brain were isolated, briefly cultured, and inoculated into the striatum of male and female SRG rats. Preliminary biomarker analyses including Ki67, p53, IDH1, and Map2 indicate clinically relevant gene expression. Through a multifaceted approach including in-life bioluminescent imaging, MRI data, and post-mortem histology, we demonstrate robust tumor growth in the brains of SRG rats when established from human glioblastoma CDX, glioblastoma stem cell PDX tumors and differentiated glioblastoma PDX tumors. These data confirm that the SRG rat is an excellent host for studying subtypes of glioblastoma with several clinically relevant markers. These data demonstrate that the SRG rat has a high utility for studies requiring intracranial orthotopic tumor implantation due to its larger size, permissiveness to human xenografts, and ability to support a higher tumor burden when compared to mice. As the most immunodeficient rat commercially available, the SRG RAT supports preclinical testing of brain cancer therapeutics in a larger rodent strain relative to commercially available mouse models.

#6510 The study of the potential role of K21 in cardiovascular genomics and its application cardio-oncology experimental therapeutics by using zebrafish model.

Surendra Rajpurohit[1], Sai Nasanally[1], Shubhra Rajpurohit[1], **Rohan Pasi**[1], Arishia Mishra[1], Devan Manhiani[1], Kailash Jain[2], Kuntal Jain[3], Vishal Arora[4]

[1]Georgia Cancer Center, Medical College of Georgia, Augusta University, Augusta, GA,[2]Cardiology, RNT Medical College, Udaipur, India,[3]OBG and Gynecology, RNT Medical College, Udaipur, India,[4]Cardiology Division, Medical College of Georgia, Augusta University, Augusta, GA

Cardio-oncology is an emerging field that studies cancer therapy-associated cardiotoxicities. Cardiotoxicity is the second leading cause of death among cancer survivors. Developing reliable and effective preclinical models for cardiotoxicity prediction and prevention is essential to improving the long-term cardiac health of cancer patients. Zebrafish is an established model, to study cancer therapeutics for potential cardiovascular toxicity. In this study, we are developing zebrafish pharmacogenomics and experimental therapeutics to address cardiovascular complications induced by cancer drugs and identification and evaluation of potential drug candidates that can mimic the cancer-induced cardio-vascular complexity and mortality of cardio-oncology patients to identify co-treatments that might alleviate the heart disease risk in cancer survivors. K21 is an established antimicrobial drug known for its antimicrobial activity. In this study, we have studied K21 drug impact on zebrafish genomic common pathway enrichment analysis and comparison of Gene set enrichment analysis (GSEA) between control and K21 drug-treated larvae to define activated and suppressed gene ratio. The novel finding reveals the Significant Activated group: regulated to DNA templated transcription, brain development, regulation of transcription by RNA-polymerase-II, monoatomic ion transport, nervous system development, neuron differentiation, monoatomic ion transmembrane transport, anterior/ posterior pattern specification, neuropeptide signaling pathway, chemical synaptic transmission. Significant Suppressed Group: intracellular signal transduction, lipid metabolic process, protein transport, steroid biosynthetic process, endoplasmic reticulum to Golgi vesicle-mediated transport, intermediate filament cytoskeletal, keratinocyte differentiation, and keratinization. In zebrafish, a significant over-expressed GSEA K21 drug has an increased number of genes associated with the development of heart and blood vessels. Some of the gene sets were also associated with heart development in the initial stages. The presence of genes that engage in cardiovascular events is an important indication of cardiotoxicity and vessel damage as a part of cancer treatment. K21 also shows significant over-expressed GSEA like significant suppression of innate immune response, T-cell co-stimulation, apoptotic process, and lipid metabolism. These significant activations and suppression will be helpful to establish a potential therapeutic application of K21 in glioblastoma TME and associated ramifications to serve as potential cardioprotective agents during cancer treatment.

#6511 Immunocompetent large animal model of mesenchymal glioblastoma achieved through somatic gene editing technology.

Nicholas Koes[1], Mandy Taisto[1], Barbara R. Tschida[1], Daniel F. Carlson[1], Maryam Aftabizadeh[2], H. Nayanga Thirimanne[3], Eric C. Holland[3], Massimo D'Apuzzo[2], Christine E. Brown[2], Adrienne L. Watson[4]

[1]Recombinetics, Inc., Eagan, MN,[2]City of Hope, Duarte, CA,[3]Fred Hutchinson Cancer Center, Seattle, WA,[4]Therillume, Inc, Eagan, MN

Glioblastoma multiforme (GBM) is the most common primary brain tumor. GBM tumors are invasive and highly proliferative, with less than 3% of patients surviving five years from initial diagnosis. Current standard of care treatment consists of surgical resection, radiation, and chemotherapy, and despite this aggressive treatment, nearly all patients succumb to disease recurrence. GBM tumors have heterogeneity, high mutation rates, infiltrating growth, and a complicated tumor-immune microenvironment making treatments difficult and complex. Additionally, therapies must cross the blood brain barrier, a significant problem preventing therapeutic efficacy. Novel drugs, promising therapeutic targets, and new targeting strategies frequently fail in late Phase 2 and Phase 3 clinical trials due to lack of efficacy. The therapies often show effectiveness in a subset of patients, but fail in the majority, leading to clinical trial failure. Because GBM clinical trials are not enrolled based on patient specific biomarkers or genetic subtypes, precision medicine approaches have faced an uphill battle for a patient population that is too small for multiple concurrent clinical trials and a disease with a severe lack of predictive preclinical models. GBM tumors fall into three subtypes, mesenchymal, classical and proneural, each with distinct genetic profiles. Variations in progression and survival and differences in therapeutic response suggest that each subtype should be treated with a regimen unique to that subtype. Roughly 30-50% of GBMs are characterized as mesenchymal, the most common and aggressive subtype. In this study, we demonstrate the development of a large animal (mini pig), immunocompetent model of mesenchymal GBM, using stereotactic delivery of gene editing reagents. 50% of injected animals rapidly develop reproducible tumors that genetically and immunologically resemble human tumors, making this an ideal preclinical model for therapeutic development and biomarker identification. Importantly, tumors can be detected within weeks of injection through a simple, cost-effective blood-based assay. Cell lines developed from these tumors show temozolomide sensitivity and provide an opportunity for high throughput drug screening prior to animal trials. Recombinetics is utilizing these animals to identify, develop, and validate novel drugs, prognostic and diagnostic biomarkers, therapeutic devices, and effective drug delivery strategies. In particular, Recombinetics has focused on identifying targeted therapies that previously failed in clinical trials, despite showing efficacy in a subset of patients, and validating the safety and efficacy of these therapies in animals that have a companion biomarker that predicts therapeutic efficacy, in hopes of developing new strategies for clinical trials that will enable biomarker-driven therapeutic development and success.

TUMOR BIOLOGY: Our Commensal Tumor Microenvironment and Its Role in Cancer
Poster Session

#6515 *Candida*-associated microbiome in pancreatic ductal adenocarcinoma.

Carlos H.F. Chan[1], Casandro J. Chan[1], Ashutosh K. Mangalam[2], Po Hien Ear[1]

[1]Department of Surgery, University of Iowa Health Care, Iowa City, IA,[2]Department of Pathology, University of Iowa Health Care, Iowa City, IA

Candida species are commonly found in bile collected during surgery in pancreatic ductal adenocarcinoma (PDAC) patients. We have previously shown that bile and tumor-associated *Candida albicans* are associated with higher pathological stage, worse pathological response to neoadjuvant chemotherapy, worse overall survival, and epithelial-mesenchymal transition gene signature. Here we investigated the association between *Candida* species and other commonly found bacterial species in the biliary system of PDAC patients with biliary stents. Patients undergoing pancreatectomy with available intraoperative bile cultures (aerobic, anaerobic and fungal) were identified in a prospective single-institution cohort. 16S rRNA and ITS sequencing was performed using available frozen banked bile samples from these patients. Relative abundance (RA) was determined as the ratio of an individual microbial count over the total microbial count in a given sample. Bile acid contents in these bile samples were determined using LC-MS methods. Of 135 patients with preoperative biliary stenting, the most commonly found bacteria were *Enterococcus* (56%), *Streptococcus* (47%), *Klebsiella* (47%), *Enterobacter* (26%), *Escherichia* (25%), *Prevotella* (22%), and *Clostridioides* (15%). *Candida* species were found in 36% of these patients. 76% of patients without preoperative biliary stenting had negative bacterial cultures. Using 16S rRNA and ITS sequencing data (N=27), the presence of *Candida* (RA > 0) was significantly associated with the presence of *Enterococcus* species (RA of 24.8% in *Candida*+ samples vs. 7.8% in *Candida*- samples, P=0.046). *Enterococcus* was also found to be associated with more unconjugated bile acids in the bile samples (unconjugated/conjugated bile acid ratios: cholic acid, 3.07 vs. 0.30, P=0.089; deoxycholic acid, 3.57 vs. 0.39, P=0.029; chenodeoxycholic acid, 2.54 vs. 0.35, P=0.045). Our data show *Candida* and *Enterococcus* species frequently co-exist in the biliary system of PDAC patients with biliary stents and that *Enterococcus* species may be responsible for bile acid deconjugation within the biliary system. Whether *Enterococcus* species and unconjugated bile acids promote the colonization and survival of *Candida* within the biliary system remains to be determined in future investigation.

#6516 Comparative microbiome analysis of tumor and normal colorectal tissue across diverse racial and ethnic groups.

S. D. Labrie[1], C. E. Thomas[1], N. Lorona[2], K. R. Curtis[1], H. Yin[1], T. W. Randolph[1], N. Ma[1], J. R. Huyghe[1], S. S. Thomas[1], L. Hsu[1], A. L. Koehne[1], S. Nayemi[1], O. J. Kahsai[1], J. C. Figueiredo[2], L. Li[3], D. G. Redwood[4], T. Thomas[4], C. I. Li[1], A. I. Phipps[1], U. Peters[1], M. Hullar[1];

[1]Fred Hutchinson Cancer Center, Seattle, WA, [2]Cedars Sinai Medical Center, Los Angeles, CA, [3]Ochsner Clinic Foundation, New Orleans, LA, [4]Alaska Native Tribal Health Consortium, Anchorage, AK

Background: Disparities in colorectal cancer (CRC) incidence and outcomes are notable between racial and ethnic groups. While these differences may be associated with various factors, established risk factors do not fully explain observed disparities. Increasingly, evidence suggests that the microbiota associated with the tumor microenvironment may play a role in CRC initiation and/or progression. However, few studies have investigated the microbiome of CRC tumor tissue compared to the microbiome in paired normal tissue as a reference. This study explores the microbial landscape of tumor and normal colorectal tissues from diverse racial and ethnic patients with CRC to understand the microbiome's potential role in CRC disparities.
Methods: We conducted bacterial 16S rRNA gene sequencing of colorectal tumor and normal tissue from 283 individuals of varied racial and ethnic backgrounds (African American, Alaska Native, Hispanic, and Non-Hispanic White). We extracted genomic DNA from tumor and normal colon tissue. We used amplicon sequencing of the 16S rRNA gene to classify the relative abundance of different bacterial amplicon sequence variants (ASVs), genera, and phyla in colon tissue. 8 phyla and 48 genera were identified across all samples. We used linear mixed models to identify differences in alpha diversity (assessed as Shannon diversity) between tumor and normal tissue in all participants combined, as well as within each racial and ethnic group. Linear mixed models were also used to identify associations of specific genera with the tumor and normal tissues. All models were adjusted for sex, race, age, ethnicity, tumor stage, tumor site, and year of diagnosis.
Results: Our analysis identified significant differences in microbial composition and diversity between normal and tumor tissue. Alpha diversity of the microbiota was significantly lower in tumor tissue compared with normal tissues when all racial and ethnic participants were combined ($p < 0.001$). There were also significant differences in alpha diversity between normal and tumor tissue within different racial and ethnic populations ($p < 0.05$) as well as within tumor tissue between different racial and ethnic populations ($p < 0.05$). After accounting for multiple testing, several genera were found to have a lower relative abundance in tumor tissue compared to normal tissue ($p < 0.05$). These included Bacteroides, Parabacteroides, Ruminococcus, Faecalibacterium, and other genera.
Conclusion: This study enhances our understanding of the CRC-related microbiome. By detailing the diversity and abundance profiles of microbial communities in tumor microenvironments versus normal tissues, we identified differences in the distribution and composition of the microbiome in tumor versus normal tissue, as well as identified specific bacterial genera that were found to have a lower relative abundance in tumor tissues.

#6517 Extracellular vesicles derived from vaginal bacteria initiate the progression of early phase ovarian carcinoma at fallopian tubes.

A. Yokoi, E. Asano-Inami, K. Yoshida, Y. Nagao, M. Kitagawa, H. Kajiyama;
Nagoya Univ. Graduate School of Medicine, Nagoya, Japan

[Objective] Microbiota contributes to cancer hallmarks, but the relationship vaginal bacteria and ovarian cancer are entirely unclear. Extracellular vesicles (EVs), including exosomes, play a crucial role in cancer biology, and bacteria secrete EVs, named bacterial EVs (bEV). Recent understanding revealed that high-grade serous carcinoma (HGSC) has raised from fallopian tube epithelial cells (FT cells) but the detailed mechanism how FT cells acquired malignant potential is largely unknown. This study aimed to elucidate the mechanism of initiation of early-phase ovarian carcinoma at fallopian tubes via vaginal bEV.
[Methods] For selecting promising bacteria, vaginal bacterial 16s sequencing data were referred to, and the data from 181 patients with ovarian tumors were analyzed. bEVs were directly collected from patients' fallopian tubes using cellulose nanofiber-based EV sheets (Yokoi A et al. Nat Commun. 2023), and bEV-RNA was extracted. Selected bacteria were cultured with monitoring OD values, and bEVs were isolated using the optimal serial centrifugation method. bEV-RNA were analyzed by small RNA sequencing and validated by qRT-PCR. Functional analyses were performed in vitro and in vivo, and the effect of bEVs on FT cells was investigated.
[Results] Based on vaginal bacterial 16s rRNA sequencing, four indigenous common vaginal bacteria were selected. Those bacteria were successfully cultured and cryo-electron microscopy confirmed isolated bEV. Cell viability analysis showed that none of the bEVs affected the proliferation of FT cells. On the other hand, specific Lactobacillus species promoted cell motility ($p < 0.05$), especially in p53 gain-of-function (GOF) cells. Furthermore, treatment of bEV resulted the sloughed-off features of p53-GOF-FT cells on basement membrane components ($p < 0.05$). These phenotypes mimic the actual progression of FT cells implant to ovaries. In in vivo experiments, although vaginal treatment of bacterial bodies did not reach FT, bEVs were incorporated by FT cells, which were confirmed by confocal microscopy imaging. We collected bEVs on FT from 15 ovarian cancer patients using cellulose nanofiber-based EV sheets, and small RNA sequencing for bEV-RNA revealed that the profiles between HGSC and benign patients were different, and specific sequences of Lactobacillus species were detected. mRNA sequencing for FT cells treated with bEV from specific Lactobacillus species showed that gene expression was significantly altered, and IPA/GSEA analyses identified several cancer-related pathways. Target functional genes in FT cells were identified and validated by qRT-PCR.
[Conclusions] These results demonstrated the unknown early mechanism of ovarian cancer development by pathogenic bEV exposure, and the new target gene alternation was identified in FT cells as the new therapeutic target.

#6518 Sex-biased intratumoral microbiome impact cancer cell proliferation and treatment outcomes in gastrointestinal cancers.

Yuting Shan, Yingbo Huang, Adam Lee, Radwa Elmorsi, Tuyen Phan, R. Stephanie Huang

Experimental and Clinical Pharmocology, University of Minnesota, College of Pharmacy, Minneapolis, MN

Objectives: Sex differences in cancer treatment responses and disease prognosis are well-documented. Our study investigates the intratumoral microbiome, hypothesizing that its composition varies between sexes which contributes to the observed differences. Specifically, our study aims to identify sex-biased intratumoral microbiome and investigate their influence on host gene expression, survival rates, and responses to treatment.

Method: We analyzed intratumoral microbiome abundance in 5 gastrointestinal cancers to identify differentially abundant (DA) microbes between sexes. After initial identification, these microbes were validated using independent datasets. A total of six large-scale intratumoral microbiome datasets were included for discovery and validation. We then assessed differential gene expression and gene set enrichment that are associated with these DA microbes. Experimental examination were carried out for two DA microbiome, Epstein-Barr virus (EBV) and Capnocytophaga, to assess their impact on cancer cell proliferation and responses to standard-of-care (SOC) treatments.

Results: From more than one thousand microbes analyzed, we identified 22 DA microbiome, comprising 17 bacteria, 3 fungi, and 2 viruses, each validated with an independent dataset. EBV is one of the 22 reproducible DA microbiome found to be significantly male-enriched in gastric cancer (GC). Further analysis unveiled the association between intratumoral EBV infection and better overall survival in GC patients. To investigate whether the survival difference partially attributes to difference in response to treatments, we treated EBV positive and negative GC cell lines with 5 SOC chemotherapies. We observed that EBV infection sensitized 3 of 5 SOC treatments (paclitaxel, docetaxel, and 5-FU), while had no effects on the others (cisplatin, oxaliplatin). Moreover, pathway analysis revealed enrichment of inflammatory response, interferon response, and apoptosis pathways in EBV-infected GC. Capnocytophaga, a reproducible female-enriched microbiome in head and neck cancer (HNSC), was found to be significantly associated with improved survival. Further co-culturing experiments found that Capnocytophaga supernatant significantly inhibited HNSC cell proliferation *in vitro*.

Conclusion: Our research identified intratumoral microbiome with sex-specific abundance patterns in gastrointestinal cancers. The identified DA microbes are closely associated with differences in the host transcriptome, drug responses, and survival outcomes. This underscores the potential of the intratumoral microbiome as a significant factor in personalized cancer treatment and management.

#6519 Tumor sidedness of colorectal cancer shows differential abundance of tumor microbiota and plausible implications.

Kuo-Hsing Chen

National Taiwan University College of Medicine, Taipei, Taiwan

Background: The tumor microbiota in colorectal cancer (CRC) plays a significant role in carcinogenesis, progression and metabolic interaction. Studies have shown the microbiota composition may be different between left-sided and right-sided CRC (LCRC and RCRC).

Materials and methods: We conducted a multi-cohort study to investigate the role of tumor microbiota in CRC in Taiwan. Cohort 1 included 140 CRC pairs from the National Taiwan University Hospital biobank. Cohort 2 was a prospective study to recruit patients with newly diagnosed CRC and collect their surgical specimens and physical activity status. Cohort 3 included 30 colon polyps. The V3-V4 regions of the bacterial 16S ribosomal RNA gene were amplified, and the amplicons were sequenced using the MiSeq system. Left-sided (LCRC, descending colon to rectum) and right-sided (RCRC, cecum to splenic flexture of transverse colon) CRC were defined according to the location of primary tumor. An integrated analysis of clinicopathological characteristics and tumor microbiota was conducted. Co-occurrence networks was used to understand the relationships and interactions between different microbial species within a community.

Results: Among 140 CRC in cohort 1, 93 (66.4%) of them were left-sided. There were significantly more CIMP-high tumors in LCRC than in RCRC ($p = 0.0002$). The diversity of microbiota was not different between LCRC and RCRC. LefSe (Linear discriminant analysis Effect Size) analyses revealed many bacteria had significantly higher abundance in LCRC and RCRC (LDA score ≥ 2, $p < 0.05$). The top 3 microbiota were *Bacillaceae, Sphingomonadaceae, Propionibacteriacea* in LCRC and *Enterococcus, Enterococcaceae, Lachnospiraceae* in RCRC. Clinically, high abundance of *Flavobacterium* was significantly correlated to absence of high body mass index (BMI ≥ 27.5 kg/m^2) ($p = 0.0019$) and low abundance of *Lachnoclostridium* was significantly associated with late-stage (stage 3 and 4) ($p = 0.0363$) in right-sided CRC. Co-occurrence networks and pathway analysis showed *Flavobacterium* was negatively associated with multiple nutrients metabolism.

Conclusion: The microbiota composition between LCRC and RCRC was different. The clinical implications of *Flavobacterium* and *Lachnoclostridium* warrants further validation.

#6520 Intratumoral microbiome profiling using high-depth whole-genome sequencing highlighted *Cutibacterium acnes* in chemoresistance in ovarian cancer.

Tatiana V. Karpinets, Xiaogan Wu, Wayne W. Xy, Amma Asare, Sara Corvigno, Joseph Celestino, Ridge T. Rogers, Raymond N. Montoya, Mark S. Kim, Pamela T. Soliman, Shannon N. Westin, P. Andrew Futreal, Amir A. Jazaeri, Anil K. Sood, Jianhua Zhang, **Sanghoon Lee**

The University of Texas MD Anderson Cancer Center, Houston, TX

Background: High-grade serous ovarian cancer (HGSC) is the most lethal gynecologic malignancy, with high recurrence rates and the development of chemoresistance significantly limiting patient survival. While the tumor microenvironment (TME), including extracellular matrix (ECM) remodeling and epithelial-mesenchymal transition (EMT), is known to contribute to chemoresistance, non-genetic factors remain poorly understood. Emerging evidence implicates the intratumoral microbiome in modulating the TME and influencing chemoresistance in several solid tumors, but how composition and diversity of the bacterial communities contribute to the efficacy of neoadjuvant chemotherapy (NACT) in HGSC is not known.
Methods: We analyzed multi-omics data from HGSC samples previously reported. Tumor-associated microbial communities were profiled using metagenomic analysis of whole-genome sequencing data and ECM- and EMT-associated gene expression profiles were evaluated through RNA sequencing. Samples included tumor tissues from 30 patients with complete gross resection (CGR) at primary surgery, neoadjuvant chemotherapy (NACT) excellent responders (NACT-ER), and poor responders (NACT-PR). Each group had 10 patients with a primary tumor site and two synchronous metastatic sites.
Results: Microbial profiling revealed that *Cutibacterium acnes* (CB) is the most common and abundant organism within HGSC intratumoral microbiome. It was more abundant in tumor and metastatic tissue from patients treated with NACT (P=0.001) versus those who had upfront CGR. Integration of RNA sequencing data demonstrated a strong correlation between CB abundance and EMT signature, which emerged as the most significantly enriched hallmark gene set according to the Gene Set Enrichment Analysis. Additionally, tumors enriched in CB exhibited transcriptional signatures associated with aggressive tumor behavior (e.g., enhanced activity of TNFA signaling, adipogenesis, and oxidative phosphorylation). Tumors with high CB abundance displayed a distinct ECM organization, decreased infiltration of T and B cells and increased abundance of fibroblasts, neutrophils, and endothelial cells, potentially indicative of an angiogenic phonotype.
Conclusions: The tumor-associated microbiome, particularly CB, may play a critical role in HGSC chemoresistance through EMT induction and ECM remodeling. This study highlights the potential of microbiome in shaping tumor phenotypes and identifies possible therapeutic targets.

#6521 Tumor colonization with *Candida albicans* increases tumor growth and decreases treatment response by influencing tumor hypoxia and immune activity.

Dennis Grencewicz[1], Alexander Loncar[1], Ruohan Wu[1], Rebecca Hoyd[1], Jinghai Wu[1], Yogita Mehra[1], Shiva Jahanbahkshi[1], Sylvain Ferrandon[1], Matthew Anderson[2], Martin Benej[1], Jiangjiang Zhu[1], Matthew Kalady[1], David Carbone[1], Nicholas Denko[1], ORIEN Microbiome Research Interest Group, Daniel Spakowicz[1]

[1]The Ohio State University College of Medicine, Columbus, OH,[2]The University of Wisconsin-Madison, Madison, WI

Microbes residing in either the gut or inside tumors have been shown to affect tumor growth and therapeutic response in cancer. Our preliminary data supported an association between the presence of *Candida* fungi within tumors and decreased therapy response and survival of rectal cancer patients. However, whether intratumoral *Candida* drive, support, or simply inhabit tumors with poor prognoses remains unclear. Here, we identify that *C. albicans* plays a causal role in modifying tumor physiology and response to treatment.

Immune competent C57BL/6 mice bearing MC38 colorectal or LLC lung cancer flank tumors were gavaged with *C. albicans* or PBS. MC38 mice were treated with 1 fraction of 7.5 Gy radiation and at size endpoint, tumors were stained for fungi and underwent bulk RNA-sequencing. LLC tumors underwent tumor electron paramagnetic resonance monitoring for intratumoral oxygen content, inorganic phosphate, and pH. MC38 cells were cultured in filtered *C. albicans* conditioned media. Metabolomics was performed on the conditioned media. RNA-sequencing from human tumors from the ORIEN database was analyzed for gene expression related to *C. albicans.*

C. albicans translocated to tumors following oral gavage. The presence of *C. albicans* reduced radiation efficacy in MC38 tumors and increased LLC tumor growth ($p<0.05$). LLC tumors from mice gavaged with *C. albicans* had lower pH and decreased oxygen tension. Transcriptional profiling indicated that colonization of these murine tumors and as well as human samples with *C. albicans* showed decreased CD8+ T cell activity and increased glycolytic metabolism. Mechanistic studies further showed that MC38 cells grown in the presence of *C. albicans* metabolites exhibited an increased oxygen consumption rate, and that this conditioned media supported increased mitochondrial beta-oxidation.

Here, we show through *in vitro* assays, murine models, and human samples that *C. albicans* can directly affect tumor growth and treatment resistance, potentially through generating an immune cold and more hypoxic and acidic environment. These data support experimentation to determine clinical relevance of depletion of *C. albicans* or related metabolites from tumors to improve clinical outcomes.

#6522 Sex differences in gut microbial composition during pelvic radiation therapy in experimental models of muscle-invasive bladder cancer.

Eva Michaud[1], Matthew Stendel[1], Jose Joao Mansure[1], Fabio Cury[2], Wassim Kassouf[3]

[1]McGill University Health Centre Research Institute, Montreal, QC, Canada,[2]Radiation Oncology, McGill University Health Centre, Montreal, QC, Canada,[3]Urology, McGill University Health Centre Research Institute, Montreal, QC, Canada

Background: Radical cystectomy is the standard of care for muscle-invasive bladder cancer (MIBC) but significantly impacts patients' quality of life. As not all patients are surgery-eligible, radiation therapy (RT) has emerged as a viable bladder-sparing approach. Yet, 25-30% of patients do not respond and will require salvage cystectomy. To add, while females are less at risk for bladder cancer, they tend to develop more aggressive disease phenotypes, and poorer response to surgery or RT. Determinants of radiation efficacy may reside outside the stole study of the tumor micro-environment (TME), as the gut microbiome, which is implicated in radiation-induced toxicity, may be influential in polarizing tumor responses to RT. Although RT effects on the microbiome have been previously studied, relationship between TME, local immune responses and gut microbes have yet to be described in MIBC.
Methods: C57Bl/6 male (n=20) and female (n=20) mice were treated with 6x6Gy of RT to the bladder or left untreated (n=10 per group) then monitored for 4 weeks without tumor induction. We then generated a N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) bladder tumor model where 3x8Gy radiation was administered to the bladder tumor at week 16 of BBN exposure. After RT was administered, stool samples were collected from all mice for 4 weeks for 16S rRNA-sequencing. Sequence data was converted to metabolic pathways using PiCRUST2. Formalin-fixed tumor tissues were stained by immunohistochemistry for B cell, T cell, macrophages and neutrophil markers.
Results: RT-treated males associated with high abundance of *Akkermansia*, *Bifidobacterium* and *Faecalibaculum*, all promoters gut health and response to anti-cancer therapies in humans. In contrast, RT-treated females selectively clustered according to *Roseburia*, *Romboutsia* and *Bifidobacterium* abundance. Females appeared more resistant to RT-induced dysbiosis at baseline, a protection that is abrogated upon tumor induction. RT treatment following tumor induction led to increased *Clostridium* abundance in females but decreased abundance in males. To add, KEGG pathways analysis predicted increased proteasome and flavonoid synthesis activity in females receiving RT, suggesting higher rates of metabolic conversion in females compared to males. Lastly, tumor immune infiltrates varied in composition and quantity according to both sex and treatment, underlining the sex-specific impacts of RT on both the microbiome and TME.
Conclusion: Our results indicate pelvic RT does affect microbiome composition with significant dysbiosis in males compared to females. Differential composition in both sexes upon tumor induction suggest RT does not affect males and female microbiomes similarly, which may condition distinct tumor immune responses. This is the first study to link sex, microbiome and RT in experimental models of MIBC to our knowledge.

#6523 A multi-omics analysis correlating intratumoral microbial abundance with methylation patterns in hepatocellular carcinoma.

Tianyi Chen, Annie N. Do, Suravi Bajaj, Erin Jang, Rishi Yalamarty, Pranava Gande, Kevin Zhang, Ellie Sang, Wei Tse Li, Jessica Wang-Rodriguez, Weg M. Ongkeko

UC San Diego, San Diego, CA

Background: Liver Hepatocellular Carcinoma (LIHC) is estimated to be the fourth leading cause of cancer worldwide. LIHC is often aggressive, as most patients are diagnosed at advanced stages. Our study aims to understand the molecular mechanisms driving the development and progression of LIHC. The microbiome has been hypothesized to contribute to many diseases, including cancer. Although microbes reside primarily within the gut, they may translocate to the liver by the gut-liver axis. Such microbes may produce metabolites that can regulate epigenetics. DNA methylation influences tumor progression by silencing tumor suppressors and activating oncogenes. Using the theoretical framework of metabolite interactions, we explore the correlation between microbe abundance and methylation patterns to expand research on the pathogenesis of LIHC.
Methods: We analyzed sequencing data for 376 primary LIHC tumor samples and 85 solid tissue normal samples from The Cancer Genome Atlas (TCGA). Using the PathoScope 2.0 framework, we successfully extracted microbial read counts from 446 samples. Microbial contamination correction was performed by three methods: sequencing date, sequencing plate, and overall microbial abundance. Bacterial species identified as contaminants were excluded from downstream analysis. Differential abundance analysis was conducted to identify bacteria species with altered abundance between normal and tumor samples. We used the Kruskal-Wallis test with a Bonferroni-adjusted p-value threshold of 0.05. Methylation data was downloaded from TCGA and filtered by a panel of genes relevant to LIHC oncogenesis. Methylation score disparities between cancer and normal tissues were assessed by the Kruskal-Wallis test. We employed Spearman's rank correlation to identify associations between microbial counts and methylation scores.
Results: We identified 27 differentially expressed microbes and 533 differentially methylated sites in LIHC tissue (p-value < 0.05). Between these microbes and methylation sites, we found 58 statistically significant correlations between the microbial abundance and methylation scores (p-value < 0.05). Notably, species involved in these correlations include the pathogenic *E. coli* O26:H11 str. 11368 and *E. coli* UMN026 and the probiotic *Ligilactobacillus salivarius.* Additional findings indicate significant microbial and methylation correlations, with further analysis underway to integrate these findings with clinical variables including cancer stage and alcohol consumption history. Additionally, microbial metabolic pathways are profiled using the HUMAnN 3.0 pipeline to provide insights into the potential mechanisms by which the microbiome may influence methylation. Further research is needed to elucidate the specific pathways mediating epigenetic modifications induced by the bacterial microbiome.

#6524 In vivo selection of tumoral microbiota identified pro-metastatic anaerobes: A novel experimental platform allowing mechanistic studies leading to the discovery of bacteria-derived molecular therapeutic targets.

Adriana Zingone, Senthil Muthuswamy, **Norihiro Yamaguchi**

National Cancer Institute, Bethesda, MD

Introduction: Tumoral microbiota is a strong predictor of gastrointestinal cancer outcome. However, our efforts in identifying bacterial molecular drivers of cancer progression are hindered by 1. the lack of efficient anaerobic bacteria culturing system, 2. >90% of human gut microbiota are anaerobes. This predicament limits our capability to conduct mechanistic studies in tumor microbiota research. To fill this gap, we sought to establish a novel experimental system allowing the propagation of metastasis-associated tumoral microbiota by taking advantage of the curated culturable human gut microbiota library.
Methods: We established a gnotobiotic system following to a sequential oral gavage of the curated human gut microbiota library in germ-free C57BL/6 mice. Subsequently, the mice underwent intrapancreatic injections of syngeneic KPC pancreatic cancer cells. Liver metastatic foci, primary pancreatic cancer, and the duodenum with their associated bacteria were subject to 16s sequencing and direct culture-based propagation.
Results: To validate the feasibility of our experimental platform in identifying bacterial species despite the expected low biomass, we conducted proof-of-concept experiments by intraspenically injecting KPC cancer cell lines in wild-type C57BL/6 mice. Liver metastatic foci, primary pancreas tumors, and the duodenum were subject to 16s sequencing and direct culture. Bray-Curtis dissimilarity matrix-based PCoA plot revealed an apparent dissimilarity between samples from tumor-bearing mice and the corresponding samples from non-tumor-bearing mock-surgery mice in a sample-site agnostic manner, suggesting a systemic impact from the tumor-bearing state. In the tumor-bearing duodenum and liver samples, there was systemic depletion of the Bacteroidetes phylum. Conversely, there is the enrichment of the Firmicutes phylum. Within the Firmicutes phylum, Clostridia species were particularly enriched in the tumor-bearing duodenum and liver metastatic samples, suggesting a biological pressure in the hypoxic tumors and hypoxic liver microenvironment favoring obligate anaerobes such as Clostridia species.
Conclusion: Our pilot studies suggest the presence of tumor microenvironment-driven biological selection of bacteria. The observed symbiosis in metastasizing tumors between obligate anaerobes such as Clostridia and cancer cells in the hypoxic tumor microenvironment warrants further functional validation, which is uniquely feasible in our proposed system. In summary, we established a novel experimental platform allowing tumor microbiota mechanistic studies to determine bacteria-derived molecular drivers of cancer progression and metastasis.

#6526 Phylogenetic and metagenomic analysis of the human pancreatic cancer microbiome.

Gabrielle Morgan[1], Colby Hunter[2], Michael Hall[2], Narendra Banerjee[2], Erik Armstrong[2], Kanisha McNeil[2], Karington Perry[2], Santanu Bhattacharya[3], **Hirendra N. Banerjee**[4], Krishna K. Rachamala[5], Shamit Dutta[5], Naga M. Nakka[5]

[1]Computer Science, Elizabeth City State University, Elizabeth City, NC,[2]Natural Science, Elizabeth City State University, Elizabeth City, NC,[3]Biochemistry and Molecular Biology,Mayo Clinic College of Medicine and Sciences, Mayo Clinic, Jacksonville, FL,[4]Natural Science, Elizabeth City State University, University of North Carolina, Elizabeth City, NC,[5]Biochemistry and Molecular Biology, Mayo Clinic College of Medicine and Sciences, Jacksonville, FL

Pancreatic cancer ranks third in cancer related deaths in the USA, however, no reliable theragnostic biomarker exists for the disease. The community of bacteria within the body can become dysbiotic and promote cancer through inflammation and other means. The objectives of this study were to determine the identity and abundance of bacterial species in the gut of mice before and after development of pancreatic cancer. As there can be significant variation in the genomes of different strains of bacterial species, a metagenome analysis was also performed. We hypothesized that there would be significant variation in species and genes of the gastrointestinal tract of mice before and after xenografting with pancreatic cancer. A decrease in ecological diversity was also predicted. Fifteen female SCID mice were divided into three cages. To develop the SubQ pancreatic cancer model, 5 X 10^6 Panc1 cells were injected at the flank. Fecal materials were collected prior to tumor cell implantation and after tumor development. DNA was extracted from the fecal material. For phylogenetic analysis the seven variable regions of the 16S rRNA gene were amplified and sequenced. Metagenomic analysis was performed as well. Average tumor size in mice after 40 days was ~ 32 mm^3. Microbial diversity was decreased in two out of the three groups of mice. Principle component analysis showed major differences between all samples. There were at least 15 OTUs in each set of samples specific to pretumor mice and 15 OTUs specific to tumor bearing mice. One genus of bacteria, *Alistipes*, was specific to each set of tumor bearing mice. Taxa at significantly increased abundances in tumor bearing mice included Prevotellaceae and Porpyromonadaceae. Moreover, there were several gene functions that were enriched in tumor bearing mice. This study produced data that demonstrates that there is a link between health status and the gut microbiome. However, this is not a new idea. This study could serve as foundation to establish a gut microbiota profile of pancreatic cancer. Precision medicine is becoming more common due to the decreasing costs of DNA sequencing. The findings of our investigation strengthens the hypothesis that there is an interaction between gut microbiome and pancreatic cancer development and bacteria or bacterial genes could serve as theragnostic biomarker for pancreatic cancer.

Acknowledgement: This research was supported by NIH Grant# T34-GM100831, NSF-NOYCE Graduate student training award, a US Department of Education Graduate student training award to Elizabeth City State University Campus of The University of North Carolina and a University of North Carolina Collaboratory Grant award to Dr. H. Banerjee and Dr. Colby Hunter.

#6527 Ginseng polysaccharides circumvent acquired resistance to anti-pd1 immunotherapy in patients with non-small cell lung cancer.

J. Huang[1], Z. Wang[1], Y. Cao[2], W. Liang[3], **E. L. Leung**[1];

[1]University of Macau, Macau, Macao, [2]Kiang Wu Hospital, Macau, Macao, [3]The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China

In 2021, we demonstrated that ginseng polysaccharides (GPs) enhance the therapeutic efficacy of anti-programmed cell death 1 (PD-1) immunotherapy by modulating the gut microbiota. GPs may serve as a beneficial dietary supplement for non-small cell lung cancer (NSCLC) patients undergoing immunotherapy. We reported GPs increased the anti-tumor response to αPD-1 mAb by increasing the microbial metabolites valeric acid, and decreasing the ratio of Kyn/Trp, which contributed to the suppression of Treg and induction of T_{eff} cells. Besides, the microbial analysis of patients fecal samples indicated that the abundance of *Parabacteroides distasonis* and *Bacteroides vulgatus* was higher in responders to anti-PD-1 blockade than non-responders. In this study, we aim to further validate and extend these previous findings. Therefore, we designed the first World Health Organization-registered clinical trial of traditional Chinese medicine combined with immunotherapy (ChiCTR2100046431) to investigate whether oral administration of GPs can circumvent acquired resistance to anti-PD-1 immunotherapy in patients with advanced NSCLC. Eligible acquired drug-resistant patients continued the use of PD-1 inhibitors as per their initial immunotherapy regimen and initiated oral administration of GPs twice daily upon enrolling in the trial. Longitudinal stool and blood samples were systematically collected from participants. The gut microbiome was profiled through third-generation amplicon sequencing. Simultaneously, comprehensive analyses of blood samples were conducted, utilizing metabolomics and quantification of cytokines. Clinical trial results showed that five patients regained response to anti-PD-1 immunotherapy, with an objective response rate of 50%. Comprehensive analysis of the gut microbiome and blood samples revealed a significant increase in the relative abundance of *Parabacteroides distasonis* after treatment, along with a significant upregulation of tumor necrosis factor-α (TNF-α) and various metabolites levels in the blood. In this study, we report the first case series of patients with advanced NSCLC with acquired resistance to anti-PD-1 immunotherapy who were treated with oral administration of GPs, which resulted in overcoming acquired resistance and regaining a response to immune checkpoint inhibitors. These preliminary data provide valuable clinical clues for overcoming the acquired resistance to PD-1 immunotherapy.

#6528 The role of health behaviors in microbial dysbiosis and lung cancer inequities.

Murugesh Eswaran[1], Briana A. Brock[1], Hina Mir[1], Gabriela Oprea-Ilies[2], Eric Flenaugh[1], James W. Lillard[1], Brian M. Rivers[1], Rajesh Singh[1], Shailesh Singh[1]

[1]Morehouse School of Medicine, Atlanta, GA,[2]Emory University School of Medicine, Atlanta, GA

Lung cancer remains the leading cause of cancer-related morbidity and mortality worldwide. African Americans (AA) are disproportionally affected by the disease, and mortality is higher in AA compared to European Americans (EA). The mortality rate among AA is higher than EA even when diagnosed at an early stage, implying biological differences associated with health behaviors such as smoking and its impact on microbial dysbiosis. A higher mortality rate in AA diagnosed at an early stage compared to EA highlights the biological differences in AA are primarily due to health behaviors such as smoking and its impact on microbial dysbiosis, making AA predisposed to higher risk and mortality. Microbial dysbiosis in lung cancer was analyzed using GEO and All of Us data repositories. Our analysis provides compelling evidence of the interplay between microbial dysbiosis, health behaviors, and racial disparities in lung cancer outcomes. Our study shows that smoking exacerbates microbial imbalances, particularly with the prevalence of *Streptococcus* and its differential impact on AA and EA. Dysbiosis correlates strongly with lung cancer pathogenesis, particularly the abundance of *Streptococcus*, *Haemophilus*, *Neisseria*, and *Pseudomonas*. AA participants show higher susceptibility to *Streptococcus* invasion, with detectable levels in the blood at just 7 cigars daily compared to 23 cigars in EAs. Urine samples of AAs smoking only 17 cigars per day are positive for *streptococcus,* while for EAs the bacteria is detected only when they smoke 20 cigars daily. These findings underscore a biological predisposition that amplifies cancer risk for AAs at lower smoking intensities. The lower thresholds for *Streptococcus* detection among AA populations suggest a heightened biological vulnerability, emphasizing the need for tailored public health interventions and early screening programs in these communities. These results underscore the importance of integrating microbial profiling in personalized medicine approaches and addressing underlying health inequities to improve lung cancer outcomes across diverse populations.

#6530 A comprehensive genomic analysis elucidates the microbial landscape of cancer.

Anders B. Dohlman[1], Robin Mjelle[1], Henry Wood[2], Iris Lee[1], Kevin Jiang[1], Ethan Shurberg[1], Alaina Shumate[1], Blake Sanders[1], Guanxi Qiao[1], Jodi Hirshman[1], Andrew Cherniack[1], Phil Quirke[2], Matthew Meyerson[1]

[1]Medical Oncology, Dana-Farber Cancer Institute, Boston, MA,[2]Pathology and Data Analytics, University of Leeds, Leeds, United Kingdom

There is growing evidence that microorganisms are an important component of the tumor microenvironment. However, conflicting reports regarding the cancer microbiome have left the extent of microbial presence across cancer types unclear, highlighting the need for more accurate methods of profiling of tumor-associated microorganisms. Leveraging the new human genome T2T-CHM13, we developed a new host-subtraction and decontamination pipeline for accurately profiling tumor-associated microorganisms in tumor sequencing data and benchmarked it on simulated and real-world datasets. We used this pipeline to perform a pan-cancer analysis of new high-depth whole-genome tumor sequencing data from The Cancer Genome Atlas (n = 8,840) and the UK 100,000 Genomes Project (n = 15,223). Our analysis revealed that in both cohorts, most cancer types do not harbor a detectable microbiome. However, we identified polymicrobial communities in association with nearly all oral, esophageal, gastric, and colorectal cancers, each of which harbored cancer-type specific microbiomes. Within cancer types, microbial communities differed by anatomical site and tumor subtype. Notably, we observed increased bacterial colonization in microsatellite-instable (MSI) cancers of the upper and lower gastrointestinal tract, and a correlation between bacterial colonization and tumor mutational burden in a pattern that persisted after controlling for MSI status. This trend was driven by a specific community of pathogens including *Fusobacterium animalis,* and was linked to a tumor-specific immune signature using patient-matched RNA-sequencing. Finally, we demonstrate that complete microbial genomes can be assembled from bulk tumor sequencing data, allowing phylogenetic and functional analysis of tumor-resident microorganisms. Together, our analysis helps clarify the microbial landscape of cancer and highlights its potential clinical importance in gastrointestinal malignancies.

#6531 High resolution analysis of Fusobacterium infection in colorectal cancer.

Heiko Dussmann[1], Barry Maguire[2], Caoimbhe Burke[3], Arman Raman[3], John Burke[4], William M. Gallagher[3], Jochen H. M. Prehn[1]

[1]Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland,[2]Physiology and Medical Physics, Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland,[3]UCD School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, Dublin, Ireland,[4]Department of Colorectal Surgery, Beaumont Hospital, Dublin, Ireland

Previous research provided evidence pointing towards an important role for bacterial infection with *Fusobacterium nucleatum* (*F. nucleatum*) and *F. animalis* (previuosly *F. nucleatum subsp. animalis*) in colorectal cancer (CRC) progression. Both are oral commensal Gram negative anaerobes with recent evidence suggesting *F. animalis* predominates in CRC (Zepeda-Rivera et al., 2024). We recently applied spatial transcriptomics to demonstrate local effects of *Fusobacterium* infection on cancer and immune cell gene expression in CRC (Duggan *et al*, Gut Microbes, 2024). While *Fusobacterium* infection was observed in specific patches in the tumors, the precise cell types infected with bacteria and the precise localization of bacteria in cells still requires further elucidation. Commercially available strains of *F. nucleatum* (ATCC 25586) and *F. animalis* (ATCC 51191) were purchased and separately co-cultured with MDA-MB-468 breast cancer cells. Immuno cytochemistry using a commercial Fusobacteria antibody (ANT0084) and a Fusobacteria antiserum (Prof Slade group), along with pancytokeratin and Hoechst staining was undertaken. High resolution confocal microscopy using an LSM 980 Airyscan 2 microscope (Carl Zeiss, Jena, Germany) was performed to create image stacks of optical sections. CRC tissue sections were subsequently prepared and stained by immunohistochemistry (IHC) for pancytokeratin, CD68, and Fusobacteria (Slade antisera). Imaging of the full tumor face was undertaken at a lower resolution using Cell DIVE (Leica Microsystems, Wetzlar, Germany) to select regions rich in Fusobacteria for further study. Selected regions were then imaged using high resolution confocal microscopy. Fusobacteria antisera stained both *F. nucleatum* and *F. animalis* while the commercially produced antibody stained only *F. nucleatum*. Both bacteria can populate the cell surroundings, cell surface, cytoplasm as well as nucleus. The typical morphology of both *F. nucleatum* and *F. animalis* is seen, allowing antibody validation. In CRC tissue intracellular bacteria are seen in both pancytokeratin positive tumor epithelial cells as well as in CD68 (Cluster of Differentiation 68) positive cells (tumor associated macrophages). Tumor associated macrophages (TAMs) have an important role in the tumor immmune microenvironment. In particular, studies have highlighted the importance of M1/M2 macrophage polarization on immune-evasion and prognosis. Our spatial transcriptomic study highlighted reduced M2 polarization as associated with increased Fusobacterial load. The intracellular location of Fusobacteria may suggest a mechanism for altered macrophage polarization. References: Zepeda-Rivera et al, Nature Vol 628, pages 424-432 (2024)Flanagan et al, Eur J Clin Microbiol Infect Dis. 2014 Aug;33(8):1381-90 Duggan et al, Gut Microbes. 2024 Jan-Dec;16(1):2350149.

#6532 Higher *Lachnoanaerobaculum* abundance in the oral microbiome go along with higher levels of tumor-infiltrating lymphocytes and improved survival in head-and-neck cancer patients undergoing radiotherapy.

Alexander Ruhle[1], Mate Krausz[2], Elsa Beatriz Monroy Ordonez[1], Jannis Heyer[3], Andreas R. Thomsen[1], Henning Schafer[1], Peter Bronsert[3], Rebecca Kesselring[4], Ali Al-Ahmad[5], Andreas Knopf[6], Anca-Ligia Grosu[1], Michele Proietti[2], Michael Henke[1], Christopher Berlin[4], Nils Henrik Nicolay[7]

[1]Department of Radiation Oncology, Medical Center, University of Freiburg, Faculty of Medicine, University of Freiburg, German Cancer Consortium (DKTK), partner site DKTK-Freiburg, Freiburg, Germany,[2]Institute for Immunodeficiency, Center for Chronic Immunodeficiency, Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany,[3]Institute for Surgical Pathology, Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany,[4]Department of General and Visceral Surgery, Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany,[5]Department of Operative Dentistry and Periodontology, Center for Dental Medicine, Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany,[6]Department of Otorhinolaryngology, Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany,[7]Department of Radiation Oncology, University of Leipzig Medical Center, Leipzig, Germany

Recent studies suggest a role for the microbiome in modulating anti-cancer treatment responses, but the impact of the oral microbiome on head-and-neck squamous cell carcinoma (HNSCC) treatment outcomes remains largely unexplored, prompting our analysis of its potential influence following definitive (chemo)radiation. Saliva samples of two independent prospective trials including HNSCC patients undergoing definitive (chemo)radiation were analyzed in terms of the oral microbiome composition before treatment initiation using V3-4 16S rRNA sequencing. Significant findings of the saliva microbiome were validated using information on the intratumoral HNSCC microbiome from The Cancer Genome Atlas (TCGA) database. Immunohistochemistry analyses of tumor-infiltrating lymphocytes (TILs) were carried out to reveal potential differences in the tumor microenvironment depending on microbiome findings. A subset of patients received serial [18F]FMISO-PET imaging as part of a prospective study (n=11) and were analyzed regarding a potential link between their oral microbiome and tumoral hypoxia. A total of 92 HNSCC patients treated with definitive (chemo)radiation were analyzed. Median age was 61 years (IQR, 56-66), most patients were male (n=77, 84%), and oropharyngeal cancer was the most common carcinoma (n=53, 58%). Higher salivary levels of *Lachnoanaerobaculum* spp. was associated with longer locoregional recurrence-free (p=0.0115) and overall survival (p=0.0417), which remained significant in the multivariable regression analysis (p=0.049974 [locoregional recurrence-free survival], p=0.0187 [overall survival]). Validation using intratumoral data from the TCGA cohort demonstrated an association of higher intratumoral *Lachnoanaerobaculum* levels with improved overall survival (p=0.0380). Neither the overall prevalence (p>0.9999) nor the time until occurrence of acute higher-grade mucositis (p=0.8414) was influenced by *Lachnoanaerobaculum* abundance. Patients with higher salivary *Lachnoanaerobaculum* levels had more CD4-positive stromal *(*p=0.0269*)* and CD8-positive intraepithelial TILs (p=0.0123). There was an inverse correlation between higher baseline salivary *Lachnoanaerobaculum* levels and a prognostically favorable early resolution of intratumoral hypoxia during chemoradiation (r = -0.6514, p=0.0229). High *Lachnoanaerobaculum* levels in the oral microbiome are associated with better locoregional control, overall survival, and factors predicting improved chemoradiation response in HNSCC patients, such as higher CD8-positive TILs levels and early hypoxia resolution. While *Lachnoanaerobaculum* may serve as a prognostic marker for improved treatment outcomes, further research is required on the mechanistic role of *Lachnoanaerobaculum*.

#6533 Microbial metabolite ammonia disrupts CEACAM1 TGF-β signaling to drive colon cancer progression.

Krishanu Bhowmick[1], Puja Ghosh[1], Xiyan Xiang[1], Xiaochun Yang[1], Taj Mohammad[2], Bibhuti Mishra[3], Md. Imtaiyaz Hassan[2], Adrian R Krainer[4], Srinivasan Dasarathy[5], Keith A Crandall[6], Lopa Mishra[1]

[1]CTR For Translational Research, The Feinstein Institute for Medical Research, New York, NY,[2]Centre for Interdisciplinary Research in Basic Sciences, Jamia Millia Islamia, Jamia Nagar, New Delhi, India,[3]Department of Neurology, Donald and Barbara Zucker School of Medicine at Hofstra/Northwell Health, New York, NY,[4]Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, NY,[5]5Division of Gastroenterology and Hepatology, Cleveland Clinic, Cleveland, Ohio, OH,[6]Computational Biology Institute and Department of Biostatistics and Bioinformatics, Milken Institute School of Public Health, Washington DC, Washington DC, WA

Background: Colorectal cancer (CRC) is rising alarmingly in younger populations, potentially arising from additional risk factors such as obesity, pro-inflammatory microbiomes and accumulation of toxic metabolites. However, how metabolites such as ammonia impact key signaling pathways to promote CRC remains unclear. Our study investigates a critical link between gut microbiome alterations, ammonia, and their toxic effects on the TGF-β signaling pathway, to drive CRC progression.
Methods: Through human TCGA-CRC data analyses, with animal model studies, molecular docking and signaling studies, we examine how gut microbiome alterations, particularly ammonia production, modulate key oncogenic pathways involving Smad3 adaptor, SPTBN1, CEACAM1, and TGF-β. Our work builds on two of our recent publications (*Cell Rep.*2024.43(9):114676 and *Sci Transl Med.2021.13(624): eabk2267*). Results We observe altered microbial populations in an obesity induced mouse model of cancer, where ammonia promotes caspase-3-mediated cleavage of the SMAD3 adaptor βII-spectrin (SPTBN1). Cleaved SPTBN1 fragments form adducts with ammonia that induce pro-inflammatory cytokine expression and disrupt TGF-β signaling. Extending on AlphaFold docking simulations, we identified that ammonia interacts with six polar residues at SPTBN1 (S553, Y556, S663, Y666, N986, and T1178) to form hydrogen bonds that disrupt downstream SMAD3 signaling, altering TGF-β signaling to a protumor genic phenotype. Blocking SPTBN1, through an SPTBN1-specific siRNA blocks ammonia toxicity and restores normal SMAD3/TGF-β signaling by reducing the abundance of SPTBN1-cleaved fragments in SW480 and HCT116 (CRC) cell lines. In addition, our research establishes crosstalk between TGF-β signaling and a microbial sensor, carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), which is significantly overexpressed in CRC patients. We identified CEACAM1-SPTBN1 interactions at specific residues (E517 and Y520) within the immunoreceptor tyrosine-based inhibitory motif (ITIM) of CEACAM1 cytoplasmic domain, identifying a potential axis that is harnessed by the altered microbiome.
Conclusions: Our study identifies mechanistic insights into how microbial metabolites such as ammonia target TGF-β, a major signaling pathway, to promote CRC. These findings underscore the therapeutic potential of targeting SPTBN1 to restore tumor-suppressive TGF-β signaling and improve outcomes for CRC patients.

#6534 Bacterial infection as a modulator of tumor growth in ovarian cancer.

Shinya Nakano, Ryosuke Sonobe, Miho Suzuki, Keiko Shinjo, Yutaka Kondo

Nagoya University, Nagoya, Japan

Ovarian cancer exhibits the lowest survival rate among gynecologic malignancies, emphasizing the critical need for more effective treatment strategies and a comprehensive understanding of its progression mechanisms. Recent studies have suggested that certain microorganisms may contribute to the development and progression of various cancers. Our previous research identified specific microbiota in the uterus and ovaries, which were considered to be sterile. This finding raises the possibility that microbiota within the female reproductive organs may influence the progression of gynecologic cancers. In this study, we investigated whether bacterial communities contribute to the proliferation of ovarian cancer. To confirm the presence of bacteria in ovarian cancer tissues, we performed DNA fluorescence in situ hybridization (DNA FISH) using 16S rRNA probes specific to bacteria on ovarian cancer specimens. Approximately 60% of the tissue samples were found to harbor specific bacteria, predominantly located in the stroma. To evaluate the impact of these bacteria on tumor growth, we developed an in vivo mouse model by introducing bacteria found in ovarian cancer tissues via intraperitoneal injection, followed by intraperitoneal transplantation of mouse ovarian cancer cells. Tumor growth was significantly enhanced in the bacteria-exposed group compared to the control group, indicating that bacterial infection promotes tumor progression. Bacterial infection in the stromal area may induce chronic inflammation conducive to tumor growth, modulate the immune microenvironment, reprogram fibroblasts, and promote angiogenesis. We aim to further discuss these multifaceted roles and their implications in tumor biology and potential therapeutic strategies.

#6535 Association of *Bacteroides fragilis* and enterotoxigenic *Bacteroides fragilis* with specific T-cell subsets in the colorectal carcinoma microenvironment.

Satoko Ugai[1], Yuxue Zhong[2], Yasutoshi Takashima[3], Kosuke Matsuda[2], Claire E. Thomas[4], Daniel D. Buchanan[5], Conghui Qu[4], Li Hsu[4], Steven Gallinger[6], Robert C. Grant[7], Marios Giannakis[3], Meredith Hullar[4], Jeroen R. Huyghe[4], Sushma S. Thomas[4], Ulrike Peters[4], Amanda I. Phipps[4], Jonathan A. Nowak[3], Tomotaka Ugai[2], Shuji Ogino[2]

[1]Harvard T.H. Chan School of Public Health, Boston, MA,[2]Brigham and Women's Hospital, Boston, MA,[3]Dana-Farber Cancer Institute, Boston, MA,[4]Fred Hutch Cancer Center, Seattle, WA,[5]University of Melbourne, Parkville, Australia,[6]Lunenfeld Tanenbaum Research Institute, Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada,[7]Ontario Institute for Cancer Research, Toronto, ON, Canada

Objectives: Experimental studies indicate that *Bacteroides fragilis* (BF), including enterotoxigenic *Bacteroides fragilis* (ETBF), could contribute to colorectal tumorigenesis through immune-mediated inflammation. However, the interactive effect of BF, ETBF, and T cell infiltrates in colorectal tumors is still unclear. In particular, not all T cells are the same, and the implications of different T cell subsets are uncertain; therefore, it is crucial to identify specific T cell subsets associated with BF and ETBF. We hypothesized that infiltration of certain T cell subsets might differ by tumor BF and ETBF status in colorectal cancer tissue.

Methods: The abundance of BF and ETBF was measured by the quantitative polymerase chain reaction in 827 incident colorectal cancer cases that had occurred in the Health Professionals Follow-up Study and Nurses' Health Study. T cell density in intraepithelial and stromal regions was assessed by a customized 9-plex multispectral immunofluorescence assay (CD3, CD4, CD8, CD45RA, CD45RO, FOXP3, KRT, MKI67, and DAPI) with digital image analyses and pathologist-supervised machine learning algorithms. We analyzed the association of the abundance of BF and ETBF with T cell subset densities using logistic regression models adjusted for age at diagnosis, sex, primary tumor location (proximal, distal, rectal), MSI, CIMP status, and *BRAF* and *KRAS* mutation status. We also applied the multivariable-adjusted Cox proportional hazards model using the same covariates to estimate the hazard ratios (HRs) for colorectal cancer-specific mortality according to BF or ETBF status. Two-sided P values <0.005 were considered statistically significant as recommended by the expert statisticians.

Results: Among 827 cases with available tumor BF, ETBF, and T cell data, BF levels were negative, low, and high in 416 (51%), 205 (25%), and 206 (25%) cases, respectively. ETBF levels were negative, low, and high in 753 (91%), 36 (4%), and 38 (5%) cases, respectively. High abundance of BF was statistically significantly associated with a lower stromal $CD3^+CD4^+FOXP3^+$ regulatory T cell density [multivariable OR, 0.61; 95% confidence interval (CI), 0.45-0.84, for BF-high vs. -negative category; P trend = 0.0034]. ETBF was not significantly associated with the densities of any T cell subsets. Neither BF (HR of high vs. negative, 0.84; 95% CI, 0.59–1.26) nor ETBF (HR of high vs. negative, 0.65; 95% CI, 0.34–1.25) was associated with colorectal cancer-specific mortality.

Conclusions: We observed that a high abundance of BF was associated with lower stromal $CD3^+CD4^+FOXP3^+$ regulatory T cell density in the tumor microenvironment. Our findings support the interactive effect of BF on specific T cells, which could explain why colorectal tumors harbor highly variable BF abundances and T cell densities.

TUMOR BIOLOGY: The Soil Matters: Tumor Microenvironment Complexity and Targeting
Poster Session

#6539 Lung cancer microphysiological systems for modeling sex-specific effects of estradiol on treatment response and proangiogenic capacity.

Wills Kpeli, Conner Sweat, Mark J. Mondrinos

Biomedical Engineering, Tulane University, New Orleans, LA

Lung cancer (LC) is the leading cause of cancer deaths in the US, accounting for 20.4% of all cancer deaths. LC incidence and progression exhibits distinct sex differences. Despite higher rates of smoking-related LC in men, premenopausal women never-smokers experience higher age-matched incidence and worse outcomes, suggesting a role for estrogens in disease initiation and progression. Along these lines, combining anti-estrogenic therapy with platinum-based agents such as cisplatin has been shown to enhance cytotoxicity, thereby underscoring the influence of sex hormones on LC treatment responses. Regarding LC progression, estradiol (E2) is known to potentiate angiogenesis in various contexts. A lack of sex-specific cellular models has limited in vitro investigations of sex hormone modulation of LC treatment responses and progression. This study aims to define E2's sex-specific effects on LC growth characteristics and angiogenesis within cisplatin-treated LC tumor microenvironments.
Methods: A549 spheroids were seeded in collagen I + 5% Matrigel and cultured in hormone-depleted medium as a baseline. Proliferation and cytotoxicity post E2 + cisplatin treatment was assessed via Ki67 and caspase 3/7 staining and quantification. A membrane-free organ chip that patterns a vascularized margin tissue and an engineered tumor microenvironment (TME) was used to model LC angiogenesis. Morphometric analysis of lectin-stained vasculature was used to quantify sprouting induced by VEGF gradients or TME-secreted growth factors. Patient-derived LC organoids (LC-PDO) were maintained and expanded in Matrigel. For angiogenesis assays LC-PDO were combined with sex-matched cancer-associated fibroblasts in a collagen I, fibrin, and Matrigel to engineer LC TMEs.
Results: The baseline Ki67 index of 27 +/- 22% in A549 spheroids was decreased to 8 +/- 5% ($p=0.042$) by 25 μM cisplatin. The baseline caspase 3/7 index of 4 +/- 4% was increased to 49 +/- 9% ($p<0.01$) with cisplatin treatment. The addition of 0.1 nM E2 dampened the effect of 25 μM, as evidenced by a Ki67 index of 14 +/- 12% and a caspase 3/7 index of 16 +/- 5%. E2-rich medium significantly increased sprouting angiogenesis induced by externally applied VEGF gradients in female models. Studies using spheroids comprised of cisplatin-resistant A549 derivatives selected for in expansion culture simultaneously displayed features of EMT, pseudo-dormancy, and an increased proangiogenic capacity.
Conclusions: Our proof-of-concept studies with A549 cells demonstrate that E2 decreases cisplatin toxicity. Ongoing work will reveal sex-specific effects of E2 on cisplatin toxicity and sprouting angiogenesis in LC-PDO models. This research could potentially inform the development of sex-specific LC treatment strategies that account for hormonal status and integrate modulators of sex hormone signaling in combination therapies.

#6540 Lung adenocarcinoma cancer cells that metastasize to the brain bring their own soil.

Alejandro Bernardo Suarez[1], Gabriela Caballero[2], Iban Aldecoa Ansorregui[2], Natalia Isabel Diaz-Valdivia[1], Patricia Fernandez-Nogueira[1], Noemi Reguart[3], Jordi Alcaraz Casademunt[1]

[1]Unit of Biophysics and Bioengineering, Department of Biomedicine, Universitat de Barcelona, Barcelona, Spain,[2]Anatomical Pathology, Hospital Clinic Barcelona, Barcelona, Spain,[3]Thoracic Oncology Unit, Hospital Clinic Barcelona, Barcelona, Spain

Lung adenocarcinoma (LUAD) is the most common histologic subtype of non-small cell lung cancer (NSCLC). Unlike other prevalent NSCLC subtypes, LUAD is characterized by an early dissemination, most frequently to the brain, particularly in patients harboring EGFR mutations. Although the mechanisms underlying LUAD metastasis to the brain are poorly understood, tumor-associated fibroblasts (TAFs) are increasingly recognized as major drivers of tumor progression and have also been hypothesized to contribute to tumor dissemination and metastasis. However, current experimental evidence of TAFs in brain metastasis is very limited. To address this gap of knowledge, we aimed to analyze retrospectively common markers of immunosuppressive TAFs phenotypes in LUAD in patient biopsies from primary tumors and paired brain metastases (n=17). These markers included α-SMA, FAP and MYH11, and were analyzed by immunofluorescence. We confirmed the presence of TAFs that were positive for these markers in primary tumors, although α-SMA$^+$ TAFs were dominant, in line with previous observations. Notably, our preliminary analysis in brain metastases revealed numerous cells exhibiting an elongated nuclear morphology comparable to that found in fibroblasts in LUAD primary tumors; moreover, these cells were also positive for either α-SMA, FAP and MYH11 or a mixture of these markers. In further agreement with the primary tumor, α-SMA$^+$ TAFs were dominant, whereas FAP$^+$ TAFs were patient dependent and MYH11$^+$ TAFs accounted for a smaller TAF population. In contrast, these TAF-like cells were negative for standard markers of activated astrocytes and pericytes. Moreover, brain metastasis showed patterns of T-cell exclusion similar to those reported in primary tumors. Because the normal brain is devoid of fibroblasts, these preliminary observations strongly support the presence of bona fide TAFs in brain metastasis. Moreover, the striking similarities between major immunosuppressive TAFs populations in the primary tumors and brain metastases support that disseminated cancer cells from LUAD patients bring their own soil during brain metastasis. Future analysis should validate these observations to ultimately define major immunosuppressive TAFs populations in brain metastasis, which could be used to design more specific therapeutic approaches against these populations.

#6541 Spatial and longitudinal characterization of tumor microenvironment of peritoneal carcinomatosis: Nab-PIPAC phase IB trial.

Laura Grassi[1], Raphael Genolet[2], Valentine Du Bois[1], Manuela Undurraga[1], Thibaud Koessler[1], Jean-Christophe Tille[3], Antonella Diciola[4], Mariagrazia Di Marco[5], Noemie Lang[6], Nicolas Mach[6], remi Peanne[1], Eileen Zhao[1], Nawel Zouggari[1], Julie Terzic[1], Catherine Raimond[6], Patrick Petignat[6], Christian Toso[6], Stefan Monig[6], Martin Hubner[4], Frederic Ris[6], Alexandre Harari[4], **Intidhar Labidi-Galy**[1]

[1]University of Geneva, Geneve, Switzerland,[2]University of Lausanne, Lausanne, Switzerland,[3]Hopitaux Universitaires de Geneve, Geneve, Switzerland,[4]University Hospital of Lausanne, Lausanne, Switzerland,[5]Hopitaux Universitaires de Geneve, Geneve, Switzerland,[6]Hopitaux Universitaires de Geneve, Geneve, Switzerland

Peritoneal carcinomatosis is a major therapeutic challenge in gynecological and gastrointestinal malignancies, associated with poor quality of life and short survival. Little is known about the tumor microenvironment (TME) of peritoneal carcinomatosis resistant to chemotherapy. Using multiplexed immunohistochemistry (FoxP3, CD163, CD8, CD68, FAP and Cytokeratin) and sensitive T cell receptor (TCR) repertoire analysis, we investigated spatial and longitudinal changes of the TME of 54 biopsies collected from 18 patients with peritoneal carcinomatosis (six ovarian cancer, nine gastric cancer and three pancreatic cancer) treated in the Nab-PIPAC phase IB trial (repeated cisplatin and nab-paclitaxel by pressurized intraperitoneal aerosol chemotherapy [PIPAC]). Overall, tumors were cold with low T cell infiltration. There was high intra- and inter-patient heterogeneity of TCR repertoire and immune cell distribution, with different tumor patterns and TME compositions depending on the primary cancer. Ovarian and gastric cancers were enriched in CD163+ tumor-associated macrophages while pancreatic cancer was enriched in FAP+ cancer-associated fibroblasts and FoxP3+ regulatory T cells. TCR clonality was higher in ovarian cancer, as compared to oesogastric cancer, likely reflecting expansion of tumor reactive T cells. Focusing on ovarian cancer, hyperexpanded T cells were more abundant in the tumor core as compared to matched stroma. Our spatial and longitudinal study brings new insights into the TME of peritoneal carcinomatosis, with implications for the future design of precision medicine in this disease.

#6543 Progesterone signaling alters the metabolic profile and immune cell landscape of mammary gland tumors.

Eilidh I. Chowanec[1], Christy R. Hagan[1], Jason Gertz[2]

[1]University of Kansas Medical Center, Kansas City, KS,[2]University of Utah Huntsman Cancer Institute, Salt Lake City, UT

Estrogen and progesterone signaling together drive hormone-receptor positive breast cancer growth and progression. However, there have been limited studies on the independent role that progesterone and its receptor, the progesterone receptor (PR), play in the development of breast cancer. Using the SSM2 tumor cell line, that expresses both the estrogen receptor (ER) and PR, we treated cells with either progesterone or vehicle (EtOH) and performed RNA-seq. The results of these experiments showed that progesterone treatment changes the metabolism of SSM2 tumor cells *in vitro*, altering the amount of free cholesterol and fatty acids in conditioned media. Previous studies in the Hagan lab have shown that progesterone signaling promotes mammary tumor growth and changes the immune landscape of the mammary gland towards immunosuppression. Therefore, we performed a tumor study using orthotopic injection of SSM2 tumor cells. Mice were treated with progesterone or placebo at the time of SSM2 tumor cell implantation. The results of this study showed that progesterone increases SSM2 tumor growth. Additionally, to investigate immune cell changes, we performed scRNA-seq on the progesterone and placebo-treated tumors. ScRNA-seq showed that progesterone-treated tumors harbored less CD3+ T cells than placebo-treated tumors. Additionally, progesterone-treated tumors had a greater number of TREM2+ macrophages than the placebo-treated tumors. The findings of our scRNA-seq data corroborated our *in vitro* findings that progesterone treatment of SSM2 tumor cells changes their metabolic profile. Our findings demonstrate that progesterone signaling promotes an immune suppressed microenvironment in the mammary gland through an increase in TREM2+ macrophages and a decrease in CD3+ T cells. This evidence provides rationale to investigate the use of anti-progestins as a mechanism to overcome the immune-cold tumor microenvironment seen in progesterone receptor positive breast tumors.

#6544 Effects of lipid metabolism on therapy-resistant prostate cancer cell-derived extracellular vesicle function in the tumor microenvironment.

Carolina Restrepo Soto[1], Katherine Carrero Figueroa[2], Carlos Joel Diaz Osterman[2]

[1]Mathematics, UPR - Rio Piedras Campus, San Juan, Puerto Rico,[2]UPR - Comprehensive Cancer Center, San Juan, Puerto Rico

Background: The chemotherapeutic agent docetaxel is a gold standard treatment for advanced, metastatic prostate cancer. However, the development of docetaxel resistance is a challenge to the effective management of the disease. Fatty acid synthase (FASN) is a central enzyme involved in lipid biosynthesis, a process linked to a therapy resistant phenotypes an immunosuppressive microenvironment. We hypothesize that FASN-driven lipogenesis alters prostate cancer cell-derived extracellular vesicle function within the tumor microenvironment. In particular, we will test whether extracellular vesicles from highly lipogenic docetaxel-resistant prostate cancer cells promote alternative macrophage activation.
Methods: Using cell-based models of docetaxel resistance (including both androgen receptor [AR]-positive and AR-negative lines), we first tested whether exposure of U937-derived macrophages to prostate cancer cells influenced the frequency of M1(CD86) or M2 (CD163) marker expression by immunocytochemistry. Next, we isolated extracellular vesicles from chemosensitive and chemoresistant prostate cancer cells and performed Western blots to detect FASN. Ongoing studies are evaluating the role of extracellular vesicles from prostate cancer cells, compared to vesicle-free cultured medium, on macrophage polarization by immunocytochemistry and/or flow cytometry. To assess the clinical relevance and prognostic utility of FASN/macrophage polarization overlap, we performed multiplex immunohistochemistry using human prostate cancer tissue microarrays. FASN and M1 (CD86) and M2 (CD163) macrophage markers were detected and quantified using a Keyence fluorescent microscope.
Results: Our data indicate that FASN is detectable in extracellular vesicles from prostate cancer cells and that the co-expression of FASN with a high M1-to-M2 ratio of macrophage markers in human prostate tumors correlates with aggressive disease based on clinicopathological variables.
Conclusions: These findings suggest that FASN may play a significant role beyond the confines of prostate cancer cells, extending to the tumor immune system and possibly to other cell types in the tumor microenvironment. Further work is needed to define the mechanism and intracellular impact of tumoral FASN uptake by tumor-associated macrophages.
Acknowledgements: Funding was provided by the Puerto Rico Science, Research and Technology Trust through the Catalyzer Research Grant mechanism to C.J.D.O. during his tenure at the Ponce Research Institute, Ponce, Puerto Rico.

#6545 Targeting NOS2 and the tumor microenvironment in ovarian cancer: Switching the immune system from "cold" to "hot".

Gianmarco Melone[1], Ivonne Uzair[1], Mailin Li[1], Maria Chervo[1], Karina Ortega[1], Jenying Deng[1], Wei Qian[1], Jianying Zhou[1], Liliana Guzman[1], Kristina Zambo[1], Harika Nagandla[1], Hong Zhao[1], Christoforos Thomas[1], Fotis Nikolo[1], Keith Chan[1], Lewis Francis[2], Jenny Chang[1], Aireana Phillips[1], Xen Ping Hoi[1]

[1]Houston Methodist Research Institute, Houston, TX,[2]Swansea University, Swansea, United Kingdom

Purpose of the study: The goal of this study is to understand how targeting inducible Nitric Oxide Synthase 2 (NOS2) may alter the ovarian cancer tumor microenvironment. NOS2 is part of many proinflammatory pathways and plays a key role in immune response regulation and cancer cell proliferation. By combining pan-NOS inhibitor L-NMMA with conventional Cisplatin administration, we want to convert the immune environment from “cold” or non-responsive to “hot” or responsive to chemotherapy. **Experimental procedures:** We established an *in vivo* late-stage syngeneic ovarian cancer model by intraperitoneal (IP) injection of ID8 cells in C57BL/6 mice. We administered Cisplatin (2 mg/kg, IP), L-NMMA (4 mg/Kg at day 1, 200 mg/Kg starting from day 2**,** IP), and Cisplatin + L-NMMA combination at the above-mentioned doses starting from week five (ascites formation phase). At the endpoint (11 weeks), ascites was analyzed through flow cytometry to describe the immune population in different treatment regimens. We also developed a NOS2 knockout (KO) human ovarian cancer cell line (OVCAR8) through CRISPR double nickase system. The NOS2 KO and the reduction of NOS2 downstream proteins like ZEB1 and Vimentin were validated through western blot. Sensitivity to Cisplatin for the OVCAR8 KO cell line was tested through RealTime-Glo™ MT Cell Viability Assay. A p-value of <0.05, as measured using the One-way Anova test, was considered significant. **Results:** In comparison to single agents, the combination treatment into ascites showed a significative increase of CD62L- CD44+ markers in $CD4^+$ T cells (35.67% in Cisplatin treatment vs. 59.72% in Combination treatment, $p < 0.05$), increased infiltration of T cells (30.74% in Cisplatin treatment vs. 43.57% in Combination treatment, $p < 0.05$), NK (0.3408% In Cisplatin treatment vs 3.270% in Combination treatment, $p < 0.05$) and NK/NKT cells (2.677% in Cisplatin treatment vs. 7.090% in Combination treatment, $p < 0.05$). A reduction of the ascites formation in the combination treatment group (6.83 cm vs. 6.619 cm abdomen diameter) was also observed. The development of an *in vitro* OVCAR8 NOS2 KO model showed that the depletion of NOS2 can reduce Vimentin and ZEB1 expression together with a reduced KO cell proliferation after treatment with Cisplatin, compared to OVCAR8 parental cell line (90.73% residual proliferation in parental cell line vs 38.17% residual proliferation in the KO clone after 72 h administration of 4 µM Cisplatin). **Conclusions:** Our results showed the potential of the combination therapy to increase the infiltration of NK and NK/NKT cells and T lymphocytes into ascites together with the increase of effector markers in $CD4^+$ T cells. The development of a second *in vivo* model based on the OVCAR8 NOS2 KO cell line will provide a better understanding of the role of NOS2 in the metastasis formation process and the impact of NOS2 on patients' survival.

#6546 Iron accumulation correlates with tumor progression in mouse models of prostate cancer.

Jennifer Rooks[1], Caleb Taylor[2], Asmaa El-Kenawi[1]

[1]Urology, Indiana University School of Medicine, Indianapolis, IN,[2]Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, IN

Tumor-associated macrophages (TAMs) are well-known as anti-inflammatory immune cells that contribute to various facets of prostate cancer development. An emerging aspect of TAMs is their "iron-rich" phenotype. This accumulation of iron can contribute to cancer cell initiation and tumor progression. In the current study, we aim to explore the key role of TAMs in regulating iron balance within the prostate tumor microenvironment (TME). We induced prostate tumorigenesis in the NP mice using Tamoxifen. The NP mice (*Nkx3.1*$^{CreERT2/+}$ *; Pten*$^{flox/flox}$ *; Rosa26-CAG-LSL-EYFP/+* mice) develop prostate intraepithelial neoplasia and localized prostate adenocarcinoma by 12 months of age. We also utilized male TRAMP which spontaneously develops poorly differentiated prostate tumors reminiscent of the neuroendocrine subtype. We then stained prostate tissues using different immune cell markers and Perls"s method which stains "non-heme" iron to identify cells with high abundance of iron. Our results demonstrate that prostate tissues from two distinct transgenic prostate cancer models (adenocarcinoma and neuroendocrine), spanning different developmental stages (12 and 18 months) exhibited high level of non-heme iron in stromal regions compared with non-tumor bearing controls. The areas with high iron abundance exhibited high expression of F4/80 and the iron exporter Ferroportin-1, also known as solute carrier family 40 member 1 (SLC40A1). In summary, we found that the availability of non-heme iron correlates with the abundance of TAMs in prostate tumor microenvironment. Future studies will focus on understanding how TAMs are regulating iron availability in the dynamic tumor environment.

#6547 Ivospemin and doxorubicin cooperatively modulate polyamine metabolism and the tumor immune microenvironment to improve survival in a murine ovarian cancer model.

Cassandra E. Holbert[1], Ashley C. Nwafor[2], Jennifer K. Simpson[3], Tracy Murray Stewart[2], Robert A. Casero, Jr.[2]

[1]Department of Biology, Loyola University Maryland, Baltimore, MD,[2]Department of Oncology, Johns Hopkins School of Medicine, Baltimore, MD,[3]Panbela Therapeutics, Waconia, MN

Polyamines are polycationic alkylamines that are essential for the continual growth and proliferation of cancer cells. The pharmacological modulation of polyamine metabolism is a promising avenue in cancer therapeutics due to the dependency of numerous cancers on continually elevated polyamine levels. Ivospemin, a spermine analogue, has shown efficacy in slowing pancreatic and ovarian tumor progression *in vitro* and *in vivo* and has demonstrated encouraging results in pancreatic cancer clinical trials. We have previously shown that ivospemin exposure decreases polyamine content both *in vitro* and *in vivo* through downregulation of the polyamine biosynthetic enzyme ornithine decarboxylase (ODC) and induction of the polyamine catabolic enzyme spermidine/spermine N^1-acetyltransferase (SSAT). We have also shown that ivospemin increases the efficacy of gemcitabine and topotecan *in vivo* by delaying tumor onset and reducing overall tumor burden. Here we examine the potential of combining ivospemin with doxorubicin, a common chemotherapeutic used in platinum-resistant ovarian cancer. Ovarian adenocarcinoma lines cotreated with ivospemin and doxorubicin exhibit an additive decrease in survival that is associated with upregulation of SSAT and decreased polyamine levels. Using the syngeneic VDID8+ ovarian model, we evaluated the ability of ivospemin to improve response to doxorubicin. Compared to untreated animals, ivospemin and doxorubicin cotreatment increased median survival by more than 200%, delayed tumor onset, and decreased overall tumor burden. Notably, this benefit was lost in an immunocompromised NSG mouse model. Ascites fluid from immune-competent animals was processed for evaluation of polyamine content by HPLC and immunophenotyping by multiparameter flow cytometry. Combination treatment decreased overall polyamine content by 75% and N^1-acetylated spermidine accumulation was observed, consistent with an upregulation of SSAT activity. Compared to single agents, ascites from co-treated animals exhibited an increased presence of CD45+ cells associated with an increase in lymphocyte recruitment. Treatment did not influence peritoneal macrophage numbers, however M1-like macrophages were increased while M2-like macrophages were decreased, resulting in an overall reversal of the M2/M1 ratio in ascites fluid. Cytokine analysis was performed on supernatant from cultured ascites cells to evaluate immune cell activity. Recognizing the non-representative mutational status of the VDID8+ model as a limitation, we are currently evaluating the combination of ivospemin and doxorubicin in genetically defined murine models that better recapitulate human high-grade serous ovarian carcinomas.

#6548 Tumor microenvironment of previously irradiated recurrent head and neck squamous cell carcinoma in humans.

Tarralyn Y. Clark[1], Jeromy Dooyema[1], Nabil Saba[2], Shlomo Koyfman[3], Haydn Kissick[4], Nicole C. Schmitt[5], Christine Lee-Poturalski[6], Tim Chan[3], David Yu[1], Zachary S. Buchwald[1]

[1]Radiation Oncology, Emory University, Atlanta, GA,[2]Hematology and Medical Oncology, Emory University, Atlanta, GA,[3]Radiation Oncology, Cleveland Clinic, Cleveland, OH,[4]Urology, Emory University, Atlanta, GA,[5]Otolaryngology, Emory University, Atlanta, GA,[6]Center for Immunotherapy and Precision Immuno-Oncology, Cleveland Clinic, Cleveland, OH

With increasing annual incidence, head and neck squamous cell carcinoma (HNSCC) is the most common head and neck cancer histology. Approximately half of HNSCC patients will develop recurrent disease. It is, therefore, important to understand the mechanism(s) and factors that may affect patient outcomes including the tumor microenvironment (TME). Previous studies have shown that patients with more TCF1+ PD-1+ CD8+ cells in the tumor prior to anti-PD-1 have superior outcomes. It is unknown whether these cells are present in recurrent tumors following radiation and what prognostic role they serve when patients receive concurrent radiation and anti-PD-1 (nivolumab). In our prior work, we evaluated PBMCs from recurrent HNSCC patients receiving reirradiation and nivolumab and correlated findings with progression free survival (PFS). Results indicated a shorter PFS for patients with a 1.5-fold increase in peripheral blood PD1+Ki67+CD4+ T cells from baseline to week 2 or 4 of treatment, suggesting the further need to assess potential biomarkers in the tumor. Thus, in this study, we provide a phenotypic analysis of cells present in recurrent previously irradiated tumors at baseline and plan to correlate with patient outcomes. Retrospective analysis of patient TME prior to receiving reirradiation and nivolumab was assessed utilizing multiplex immunofluorescence (mIF). T cells within the TME of previously irradiated disease revealed that 48.64% of CD8+ cells were PD-1+ at the time of resection (N=20, range=8.050%-62.16%). Of the PD-1+ CD8+ T cells, a higher percentage of effector TCF1-PD1+CD8+ cells (median= 58.94%, N=20, range= 28.43%-85.90%) were present compared to stem-like TCF1+PD1+CD8+ cells (median= 41.06%, N=20, range= 14.10%-71.57%). Likewise, cell density per mm^2 for effector TCF1-PD1+CD8+ cells (median=46.01, N=20, range= 1.006-265.9) was higher compared to stem-like TCF1+PD1+CD8+ cells (median= 30.81, N=20, range=0.7669-142.7). In parallel, a prospective analysis of PBMCs of standard of care HNSCC patients with no prior history of treatment was assessed longitudinally during radiation by flow cytometry. We found the percentage of CD8+ T cells remained relatively stable throughout radiation treatment though the percentage of CD8 T_{EMRA} cells increased 1.2-fold from baseline to 3 months post treatment. Naïve CD8 T cells, in contrast, experienced a 2-fold decrease from baseline to 3 months post treatment after an initial increase during treatment. Activated CD8 T cells (CD38+ HLA-DR+) decreased gradually from baseline to week 3 of treatment before rebounding 3 months post treatment. These data suggest that previously irradiated tumors still possess the T cell population necessary to mount a robust anti-PD-1 and anti-tumor immune response. Additionally, H&N radiation has an impact on circulating T cell populations in the absence of immunotherapy.

#6549 Immune cell-derived lymphotoxin associated with survival of patients with endometrial cancer.

T. Lau[1], J. Wu[1], K. Chan[1], J. Kwong[2], H. Lee[1], C. Wang[1];
[1]The Chinese University of Hong Kong, Shatin, NT, Hong Kong, [2]Keele University, Keele, Staffordshire, United Kingdom

Background: Endometrial cancer (EC) is a prevalent gynecological malignancy, often associated with poor prognosis in advanced stages. The tumor microenvironment (TME) plays a critical role in modulating immune responses and disease progression. This study aimed to investigate the prognostic significance of lymphotoxin (LT) in EC patients.
Methods: Transcriptomic data from The Cancer Genome Atlas (TCGA) for EC were analyzed to access LT expression level, identified LT-associated gene signatures and evaluate the clinical outcomes. The cellular source of LT was determined by analyzing single-cell RNA sequencing (scRNA-seq) data from public databases. Additionally, these findings were validated in a cohort of 138 EC tissue samples from our center using quantitative PCR (qPCR) and multiplex immunofluorescence (mIF) staining.
Results: Analysis of TCGA data demonstrated that EC patients with high LT expression exhibited significantly improved overall survival (OS) and disease-free survival (DFS) compared to those with low LT expression. Gene signature analysis indicated that high LT expression correlates with increased immune cell infiltration, suggesting a robust anti-tumor immune response. scRNA-seq analysis suggested that LT is predominantly expressed in T cells and B cells in EC samples when compared to normal samples. Validation in our cohort of 138 EC tissues revealed that patients with high LT expression had better OS. EC patients with high LT expression showed high expression of immune cell-related gene signatures, including Territory lymphoid structure signature, Tfh cell signature, Th1 and B cell signature and HEV signature, by qPCR. mIF staining further confirmed that increased infiltration of T cells and B cells in the TME of patients with high LT expression.
Conclusion: Our findings indicate that immune cell-derived lymphotoxin serves as a potential prognostic biomarker in endometrial cancer, associated with enhanced immune cell infiltration and improved patient outcomes. These results highlight the importance of the immune microenvironment in tumor progression and support further exploration of lymphotoxin regulations in TME for identification of therapeutic target in EC.

#6550 Cancer-associated fibroblast-targeted near-infrared photoimmunotherapy induces tumor specific memory T cells from TDLNs.

Akito Shimizu[1], Kazuhiro Noma[1], Tatsuya Takahashi[1], Tasuku Matsumoto[1], Seitaro Nishimura[1], Yasushige Takeda[1], Hijiri Matsumoto[1], Tomoyoshi Kunitomo[1], Masashi Hashimoto[1], Hajime Kashima[1], Naoaki Maeda[1], Satoru Kikuchi[1], Shunsuke Tanabe[1], Toshiaki Ohara[1], Hiroshi Tazawa[1], Hisataka Kobayashi[2], Toshiyoshi Fujiwara[1]

[1]Department of Gastroenterological Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Science, Okayama, Japan, [2]Molecular Imaging Branch, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD

<Introduction> Our laboratory previously showed that cancer-associated fibroblasts (CAFs) suppress antitumor immunity in the tumor microenvironment (TME) and that CAF-targeted near-infrared photoimmunotherapy (CAF-PIT) improves antitumor immunity in the TME and reduces tumor size by TME remodeling. Dendritic cells have been suggested to migrate from the TME to the tumor drainage lymph nodes (TDLNs) and promote the proliferation of tumor-specific memory T cells and their migration to the tumor. We hypothesized that CAFs inhibit this process.
<Methods> To evaluate the effect of memory T cells in the TME on prognosis, we evaluated memory T cells in the TME of clinical specimens of esophageal squamous cell carcinoma using immunohistochemistry with a CD45RO antibody. We also examined its correlation with area of CAF evaluated by α-SMA antibody in previous studies. We evaluated tumor-specific memory T cells and dendritic cells in the TME and ipsilateral inguinal lymph nodes (TDLNs) and contralateral inguinal lymph nodes(non-TDLNs) in flank subcutaneous tumor models of MC38, a mouse colon cancer tumor strain that is highly immunogenic and can be used to evaluate tumor-specific T cells using H-2Kb p15E tetramers. We created CAF-rich models co-inoculated with MEF and evaluated the effect of CAFs, CAF-PIT, and TDLNs using flow cytometry.
<Results> Among patients with esophageal squamous cell carcinoma, those with a high number of CD45RO-positive cells in the TME had a better prognosis. Furthermore, in cases with T2 or deeper, there was a negative correlation with area of CAF which suggested that CAF might inhibit the migration of memory T cells.Evaluation of tumor-specific T cells in a mouse model showed that TDLNs contain more tumor-specific T cells and dendritic cells than non-TDLNs. In addition, tumor-specific T cells were present mainly as effector and central memory T cells in the TME and TDLNs, respectively.In CAF-rich tumor mouse, tumor-specific effector memory T cells in the TME were significantly lower than in CAF-poor tumor mouse, and tumor-specific central memory T cells in the TDLNs were lower. When CAFs were removed by CAF-PIT, the number of tumor-specific effector memory T cells in the TME increased significantly. In addition, resecting TDLNs before FAP-PIT decreased the number of tumor-specific effector memory T cells in the TME and weakened the therapeutic effect.
<Conclusion> CAF-PIT induces tumor-specific memory T cells from TDLNs via TME remodeling.

#6551 Exploring oncolytic viral therapy to target osteosarcoma.

Sumbul Khan, Terri Higgins, Isabella Shimko Lofano, Payal Agarwal

Auburn Univ. College of Veterinary Med., Auburn, AL

Osteosarcoma (OS) is a mesenchymal neoplasm. OS makes up approximately 3% of all malignancies in children. The mortality rate has not changed in the last few decades; therefore, the development of advanced cancer therapies is essential. We hypothesize that next-generation conditionally replicative oncolytic virus with an armed anti-PD1 HcAb will lyse the OS cells and initiate the immune response against tumor cells in the tumor microenvironment.The purpose of the study is to evaluate the oncolytic activity and production/ secretion of anti-PD1 Hcab by conditionally replicative Canine adenovirus armed with anti-PD1 Hcab (CAV 2 AU M3). We have infected canine OS cell lines viz D17-GLP, CF11-GLL, MCKOS and D22-GLP with CAV2 AU M3 and measured its cytotoxicity by performing cell viability assay (Luciferase assay) and cytotoxicity assay (LDH assay). To assess whether the virus can stimulate an immune response or not, we performed an immunogenicity assay (ATP assay). To evaluate the secretion and expression of anti-PD1 HcAb we have performed western blot. Flow cytometry was done to confirm the functionality of anti-PD1 Hcab i.e binding of anti-PD1 HcAb to PD1 receptors and inhibition of PD1 and PDL1 interaction. We have found that CAV2 AU M3 is lytic in OS cell lines (D17-GLP, CF-11-GLL, and MCKOS) in both 2D (Monolayers) and 3D (Tumor spheroids) cell cultures and it also produced and secreted anti-PD1 HcAb, which binds to PD1 receptor efficiently and inhibited the interaction between PD1 and PDL1. We concluded that CAV2 AU M3 is a potent armed oncolytic virus that can lyse and kill OS cells and produce functional anti-PD1 HcAb.

#6552 Siglec1 expression on the tumor-associated macrophage in breast cancer.

Shinyoung Park[1], Chul Hwa Hong[2], Won-Ji Ryu[2], Jeong Dong Lee[3], Ja Seung Koo[4], Min Hwan Kim[5], Joo Hyuk Sohn[5], Hyung Seok Park[6]

[1]Yonsei University College of Medicine, Seoul, Korea, Republic of,[2]Avison Biomedical Research Center, Yonsei University College of Medicine, Seoul, Korea, Republic of,[3]Biomedical Research Institute, Seoul National University Hospital, Seoul, Korea, Republic of,[4]Department of Pathology, Yonsei University College of Medicine, Seoul, Korea, Republic of,[5]Division of Medical Oncology, Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea, Republic of,[6]Department of Surgery, Yonsei University College of Medicine, Seoul, Korea, Republic of

Breast cancer is a common malignant tumor with four distinct subtypes, based on the presence of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (HER2): luminal A, luminal B, HER2-enriched, and triple-negative. There is currently no effective immune checkpoint blockade (ICB) against breast cancer. The tumor microenvironment comprises not only cancer cells, but also fibroblast and tumor-infiltrating immune cells. Macrophages are the largest subset of tumor-infiltrating immune cells, called tumor-associated macrophages (TAMs). It has been reported that Siglec1 is a selective marker of TAM. Siglec1 is also known as CD169 Sialoadhesin, and is typically found on the surface of monocytes and macrophages. Macrophages can be divided into two groups: M1- and M2-like. Although Siglec1 does not belong to either of these, macrophages that express Siglec1 have been associated with immunosuppressive functions, which can facilitate tumor progression. Given the observed differences in Siglec1 mRNA expression among breast cancer subtypes, we postulated that Siglec1 protein expression would also vary between subtypes, resulting in different prognoses. We also evaluated the impact of ICB on Siglec1 expression in an immune co-culture system utilizing patient-derived organoids (PDOs). Formalin-fixed paraffin-embedded blocks were prepared from fresh frozen surgical tissues. Using them, hematoxylin and eosin staining and immunohistochemistry (IHC) were conducted, and the percentage of Siglec1+ cells was obtained from the slides. The relationship between Siglec1 expression and patient baseline characteristics, including age, hormone receptor status, HER2 status, Ki67 level, subtype, histologic grade, and TNM stage, was examined. To evaluate the impact of ICB on Siglec1 expression, PDOs were co-cultured with their own peripheral blood mononuclear cells (PBMCs) and cytokines in minimum medium. Following a 24-hour and 10- to 14-day incubation period, the immune cells were analyzed by flow cytometry. In clinicopathological analyses, no correlation was identified between Siglec1 expression and subtype, but Siglec1 expression was significantly associated with tumor stage. Furthermore, survival analysis revealed a correlation between Siglec1 expression and recurrence and/or mortality. In the co-culture system, Siglec1 was expressed in both M1- and M2-like subsets, and the ratio of Siglec1+ macrophages was decreased when PBMCs were cultured with PDOs. Administration of anti-PD-1 in the models has been observed to alter the proportion of Siglec1+ macrophages compared to the control. In conclusion, the results demonstrated that Siglec1 may be related to both subsets of macrophages and be associated with large tumors and prognosis. Anti-PD-1 treatment may influence the expression of Siglec1+ macrophages. Further investigation is required to ascertain the impact of ICB on Siglec1+ macrophages.

#6553 Ex vivo investigation of estrogen receptor-related macrophages polarization in breast cancer and its prognostic significance.

Daria Kalinska-Lysiak[1], Katarzyna Zielniok[2], Zak Kinsella[1], Radoslaw Zagozdzon[2], Darran O'Connor[1]

[1]Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland,[2]Laboratory of Cellular and Genetic Therapies, Center for Preclinical Research, Medical University of Warsaw, Warsaw, Poland

Breast cancer (BC) is the second most frequent cause of cancer-related deaths. In clinical practice, ER, PR and HER2 assessment in histopathological samples divides BC into biological subtypes and guides treatment decisions. While these subtypes reveal key insights on prognosis they crucially also exhibit distinct immune environments, with ER-positive disease being considered 'immune-cold'. Recently, though immunotherapy has shown efficacy in treating ER-negative tumors, there is a lack of biomarkers that may select patients as good candidates for immunotherapy treatment in BC. In this study, we characterized the immune milieu across tumors of different ER-positivity, challenging the canonical breast cancer ER+/ER- dichotomy. A publicly available RNA-seq dataset (TCGA BRCA) with 481 breast cancer patients and complete information on ER percentage IHC status was analyzed in R Studio (v 4.4.0). We demonstrated that patients with ER IHC expression below 50% (ER-low: <10% and ER-intermediate: 10-49%) exhibit worse prognosis than those with ER-high (above 50%, p-value = 0.0082), throwing doubt onto the sufficiency of the contemporary therapy in ER+ disease for patients of lower ER expression. We have further investigated immune enrichment using the quantiseqr package, and observed gradual changes in immune fractions across three clusters of ER expression (<10%, 11-49%, >50%). While macrophages constitute the most numerous population in ER-high and ER-intermediate tumors, for ER-low patients' cell proportions are more balanced: the fraction of M2 macrophages, B-cells and CD4+ T-cells is reduced whereas macrophages M1, neutrophils, and T-reg increase. Differences between mean abundances in high vs low clusters remain statistically significant for all measured cells except for NK cells. We also noticed effects for M1- and M2-like macrophages by underlying ER expression: the higher the ER expression the more relative abundance of M2 cells, with moderate positive correlation across the tumors with increasing ER deciles (Spearman correlation r = 0.4, p-value < 0.0001). Conversely, M1-like cells decrease at once. Median relative abundance of macrophages M1 had prognostic value in the entire cohort of breast tumors (log-rank test p-value = 0.041), what mostly originated from tumors of high ER levels (n = 151 vs 193, p-value=0.0089), and did not achieve statistical relevance in any other subgroup. While recent in-vitro research postulated existing difference of macrophages' populations in cancers of distinct ER expression, and in environment of variable estrogen levels, this study demonstrates the fluctuation of ER-related M1- and M2-like macrophage proportions in clinical samples. We moreover provide evidence that M1-like macrophages have prognostic relevance in the ER-high subgroup. Further experiments detailing mechanisms of the observed phenomenon are underway.

#6554 Characterization of the tumor immune infiltrate in a colorectal syngeneic model dosed with anti PD1 in combination with radiotherapy or PARP inhibitor.

Theoni (Tania) Katopodi, Charlotte Bell, Pablo Binder, Lyndsey Hanson, Emily Wright, Amy Cantrell, Stewart Brown, Paul Farrington, **Tobias Bunday**, Nick Moore, Lorraine Mooney, Jane Kendrew

Sygnature Discovery, Alderley Edge, United Kingdom

Introduction: Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of many cancers, significantly improving survival outcomes. However, for some indications, phase I and II studies have not demonstrated substantial antitumor activity when ICIs are used as monotherapy. Combining ICIs with poly(ADP ribose) polymerase (PARP) inhibitors (PARPis) or radiotherapy are currently being explored in numerous clinical trials. Therefore, understanding the impact of these therapies on the tumor immune microenvironment (TIME) and the responses after combining radiotherapy, PARPis and ICIs in preclinical tumor models is crucial for guiding drug discovery research.
Methods and Experimental Procedures: The murine colorectal carcinoma cell line MC38 was subcutaneously implanted into C57BL/6 mice. The response of the MC38 cancer model to anti PD1, fractionated radiotherapy, and the PARPi Niraparib was assessed by measuring tumor growth with calipers three times per week. Mice receiving radiotherapy were administered targeted X rays using an Xstrahl CIX3 cabinet irradiator, with animals shielded by lead panels to expose only the tumor for localized treatment. Changes to the TIME following these treatments were evaluated using multi color flow cytometry, and plasma cytokines were assessed by Luminex xMAP technology. Data Treatment with anti PD1 resulted in a mixed response, with tumor regressions observed in a subset of treated animals. The combination of anti PD1 with fractionated radiotherapy significantly enhanced therapeutic efficacy, whereas combining anti PD1 with Niraparib did not yield additional benefits. Characterization of the tumor immune microenvironment (TIME) following these treatments revealed modulation of the intra-tumoral immune infiltrate. Notably, a significant decrease in tumor-associated macrophages and an increase in CD8 cytotoxic T cells were observed in tumors treated with the combination of anti PD1 and fractionated radiotherapy. These results were consistent with the increased efficacy observed in the combination group. However, combining Niraparib with anti PD1 did not show any advantages over the anti PD1 monotherapy which was also reflected by the changes seen when TIME was characterized.
Conclusion: In this study we have characterized and established effective dosing regimens for anti PD1, fractionated radiotherapy, and Niraparib, both as monotherapies and in combination. Additionally, we evaluated changes to the tumor immune microenvironment (TIME) in a syngeneic mouse cancer model. The differences in efficacy and TIME findings with the combinations evaluated demonstrate the value of these models for immuno-oncology (IO) drug discovery research.

#6555 A method for preparing tumor tissues for ex vivo culture that retains T cells within live tumor fragments while preserving tissue viability and T cell function.

Michael Korrer[1], Christina Scribano[1], Pichet Adstamongkonkul[1], Todd Bakken[1], Torey Browning[1], Christian Baltes[1], **Sean Caenepeel**[1], Nicholas Dana[1], Jackie Derrick[1], Thomas Dietz[1], Evan Flietner[1], Hilary Hernan[1], Christin Johnson[1], Payton McDonnell[1], Jacob Nesemeier[1], Josh Porter[1], Abhijeet Prasad[1], Jordyn Richardson[1], Sidney Schneider[1], Rebecca Tarlazzi[1], Noah Van Der Weide[1], Lindsey Vedder[1], Ming (Veronica) Wang[1], Hinco Gierman[1], Andreas Friedl[2], Laura Hrycyniak[1], T.S. Ramasubramanian[1]

[1]Elephas, Madison, WI,[2]University of Wisconsin, Madison, WI

Characterization of response to immunotherapy ex vivo requires preservation of the live tumor microenvironment (TME), including the sequestration of immune cells. Elephas has developed a methodology that incorporates proprietary cutting instruments designed to optimally fragment live tumor resections and core needle biopsies (CNBs), and a proprietary hydrogel that supports the viability of tumor fragments in ex vivo culture while sequestering T cells in their native TME. In this study, tumor specimens (human tumors and humanized PDX) were fragmented using one of two proprietary cutting instruments designed to cut tumor resections into 300 x 300 x 300µm fragments or CNBs into 300µm thick slices. Both tissue form factors were then encapsulated in a propriety hydrogel. Tissue viability was assessed via the Cell Counting Kit 8 (CCK8) assay and in some cases a lactate dehydrogenase assay. Conditioned media from culture plates harboring encapsulated fragments was collected for multiplex cytokine analysis. At the end of ex vivo experimentation tissue was paraffin embedded and formalin fixed for H&E staining and immunohistochemistry was performed to assess T cell retention. Elephas proprietary cutting instruments produced tumor fragments of consistent size and volume. These fragments retained histological characteristics of the original tumor resections from which they were derived and their encapsulation in a proprietary hydrogel improved T cell retention over 2 days of ex vivo culture. Preservation of the native TME was further demonstrated following αPD-1 treatment of tumor fragments with known CDx biomarker status where an enriched cytokine response profile was observed in biomarker positive samples. In CNBs processed on the Elephas platform, overall viability and T cell function were maintained following simulated overnight shipment at 4°C. To overcome challenges associated with the limited amount of tissue in CNBs (e.g. heterogeneity), we developed a treatment strategy where control and treatments were performed sequentially on the same tissue within a single well. Fold changes in the rate of cytokine secretion following sequential (1) control and (2) αPD-1 + STING agonist treatment exhibit improved CVs (%) relative to an inter-well treatment design (control and treatment wells). Additionally, live tumor biopsies were shown to exhibit comparable IFNγ response following treatment with αPD-1 + STING agonist at the experiment start vs. following 20 hrs of control treatment. The Elephas platform preserves overall cell viability and T cell function in the TME of CNBs ex vivo using proprietary cutting instruments and hydrogel encapsulation techniques. Using this preparation method, the Elephas platform optimally captures the heterogeneity in human tumors (resections and CNBs) while allowing for comparisons between treatment conditions.

#6556 Model characterization and tumor immune profile assessment of syngeneic Renca murine renal cell carcinoma in female BALB/c.

Justin Snider, Lauren Kucharczyk, Anita Zaitouna, Sheri Barnes, David Draper, Scott Wise

Laboratory Corporation of America, Ann Arbor, MI

Renal cell carcinoma (RCC) affects over 80,000 people in the U.S. annually, leading to ~15,000 deaths. Although immuno-oncology therapies can yield responses for some patients, complete remissions are rare. This limitation highlights the importance of new therapeutic approaches, necessitating effective animal models for preclinical testing. Syngeneic models, which allow for functional immune systems capable of influencing tumor growth and metastases, are valuable for studying immune responses and immunosuppressive factors. One such model is Renca, derived from a spontaneous renal cortical adenocarcinoma in a BALB/c mouse. Renca is relevant because it varies in immunogenicity and mirrors human RCC in many aspects. This model is a promising tool in immuno-oncology, enabling the testing of new treatments, alone and in combination with other therapies. To ensure reproducible growth, a well-established tumor was collected and processed. The resulting cells where then implanted subcutaneously at two inocula (1.0E+06 and 5.0E+05 cells), with and without dietgel, to observe known weight loss characteristics of the model. Renca demonstrated aggressive growth, with a median tumor volume doubling time of ~3 days, a median time to 2000 mm^3 of ~30 days, disease related mortality of 12.5-25% with dietgel and 37.5%-62.5% without dietgel and disease associated body weight change ranging from -5.1 to 0% with dietgel and -15.1 to -14.6% without dietgel. The baseline tumor immunophenotype in vehicle control treated animals was measured by flow cytometry and revealed myeloid-derived suppressor cells to be the dominant subset in the tumor. With G-MDSCs and M-MDSCs representing 31.5% and 16% of total CD45+ cells, respectively. In addition, tumors were poorly infiltrated with lymphocyte subsets as CD8+ T cells and NK cells each represented less than 0.25% of the CD45+ cell compartment. Furthermore, checkpoint inhibitors including PD-1, TIM-3, and LAG-3 were highly expressed on the T cells present (90.0%, 80.5%, and 74.7% positive respectively), suggesting an immunosuppressed state. Next, response to standard of care (SoC), checkpoint inhibitors, and radiation were evaluated. All treatments had limited impact on tumor regression, but focal beam radiation showed the most significant effect by extending the time to progression. Checkpoint inhibitors were minimal effective, while SoC treatment produced a moderate response. In summary, the Renca model has proven to be a reliable syngeneic tumor model for RCC research. Its immunosuppressive microenvironment is valuable for studying combination therapies that could potentially convert non-immunogenic tumors into more immunogenic tumors, enhancing treatment responsiveness. Future studies will focus on optimizing orthotopic implantation and sampling tumors at varying sizes, which has been shown to impact TIL populations.

#6557 Human 16p11.2 microdeletion suppresses malignant progression of colon cancer through dysregulation of the tumor microenvironment.

Jacob L. Billingsley, Joshua Haebe, Chris Bergin, Tanguy Fenouil, Baptiste Lacoste, Yannick Benoit

University of Ottawa, Ottawa, ON, Canada

Colorectal cancer (CRC) is the third most diagnosed malignancy in Canada, where nearly half of diagnoses occur at late stages. The tumor microenvironment (TME), including host mesenchymal, immune, and endothelial cells, is an important driver of colorectal tumor progression toward late-stage disease. Tumor cells can instruct the TME to support invasion and metastasis via angiogenesis, immunosuppression, and extracellular matrix (ECM) remodeling. Next-generation cancer therapeutics must account for the complexities of the TME. Thus, the discovery of vulnerabilities therein is of critical importance. Our group contributed to a study showing that the hemi-deletion of the 16p11.2 locus (16p11.2+/-), associated with the autism spectrum disorder, significantly impairs cortical vascularization. Given that several genes within 16p11.2 are critical to CRC development, we found that primary CRC tumors with high 16p11.2 locus activity correlated with shorter overall survival rates than low 16p11.2 activity tumors. Pan-cancer hazard ratio analysis showed this relationship with 16p11.2 gene expression is specific to CRC. To study the role of the 16p11.2 locus in CRC tumor progression, we introduced a monoallelic deletion of the 7F3 locus (homologous to human 16p11.2 and termed h16p11.2 for clarity) in the APCmin/AOM tumorigenesis model. CRC tumors developing in the h16p11.2-deficient animals showed a significantly reduced size. Similarly, syngeneic CRC tumor engraftment assays using MC38 cells (h16p11.2 wt) showed delayed tumor growth in h16p11.2-deficient recipients compared to wt controls. CRC tumors developing in an h16p11.2-deficient niche presented significant disruptions in critical elements of the TME. Specifically, 3D mapping of CD31-positive endothelial structures revealed a 50% reduction in blood vessel density for tumors engrafted into h16p11.2-deficient animals. Transcriptome profiling of tumor and tumor-adjacent tissues identified several aspects of the TME perturbed by the h16p11.2-deficient niche, such as extracellular matrix (ECM) composition and enhanced adaptive immune response in macrophages. Polarized light microscopy and immunohistochemistry confirmed ECM gene regulation by showing a reorganization of collagen fiber and SPARCL1 deposition inside CRC tumor mass. Moreover, macrophage infiltration is enhanced within CRC tumors grown in a h16p11.2-deficient TME, with enhanced M1 anti-tumor and reduced M2 pro-tumor polarization. Overall, our data support that a 16p11.2-deficient TME represents an inhospitable niche for CRC tumor growth and progression. Gaining a better understanding of the biological mechanisms associated with lower 16p11.2 locus activity in CRC will reveal novel treatment modalities targeting both tumor and TME tissues.

TUMOR BIOLOGY: Vesicles in the Tumor Microenvironment
Poster Session

#6561 Extracellular vesicles-derived nucleolin as a novel biomarker in neuroblastoma patients.

Veronica Bensa[1], Danilo Marimpietri[2], Martina Morini[1], Martina Ardito[1], Eleonora Ciampi[1], Martina Fragola[3], Massimo Conte[4], Alberto Garaventa[4], Joao N. Moreira[5], Xhuliana Kajana[6], Alessandro Garbarino[6], Gino Tripodi[7], Roberto Bandettini[8], Giovanni Candiano[6], Mirco Ponzoni[1], Chiara Brignole[1], **Fabio Pastorino**[1]

[1]Laboratory of Experimental Therapies in Oncology IRCCS Istituto Giannina Gaslini, Genoa, Italy,[2]Laboratory of Cell Therapy, IRCCS Istituto Giannina Gaslini, Genova, Genoa, Italy,[3]Scientific Directorate, IRCCS Istituto Giannina Gaslini, Genova, Italy, Genoa, Italy,[4]Clinical Oncology Unit, IRCCS Istituto Giannina Gaslini, Genova, Genoa, Italy,[5]CNC-UC - Center for Neurosciences and Cell Biology, Center for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, Faculty of Medicine (Polo 1), Coimbra and Univ Coimbra - University of Coimbra, CIBB, Faculty of Pharmacy, Coimbra, Portugal,[6]Laboratory of Molecular Nephrology, IRCCS Istituto Giannina Gaslini, Genoa, Genoa, Italy,[7]Immunohematology and Transfusion Medicine Unit, IRCCS Giannina Gaslini Institute, Genova, Genoa, Italy,[8]Central Laboratory of Analysis, IRCCS Istituto Giannina Gaslini, Genoa, Genoa, Italy

Background: Neuroblastoma (NB) represents the most frequent form of extracranial solid tumor of children, responsible for 15% of childhood cancer deaths. Recently, cell surface Nucleolin (NCL) was suggested as a target for NB therapy and its expression at RNA level was validated as an independent (adjusting for age, INSS stage, and *MYCN* status) prognostic marker for NB. Extracellular vesicles (EVs) may express different surface proteins derived from the parental cells. Liquid biopsy-derived EVs from NB patients might reveal a possible diagnostic/prognostic role of NCL at the protein level.

Methods: Here we evaluated the expression of small EVs (sEVs)-derived NCL in the peripheral bloods (PB) of low risk- (LR) and high risk- (HR) NB patients at onset and in relapse, and of aged-matched controls (CTRs). PB were firstly centrifuged at 400 g for 10 min at 4°C, then the supernatant was re-centrifuged at 1600 g for 10 min at 4°C to collect plasma. Isolation of EVs was performed from 500 µl of plasma through ultracentrifugation at 20.000 g to remove large EVs and secondly at 100.000 g to isolate sEVs. sEVs were characterized in term of size distribution by using Nano Traking Analysis (NTA). Twenty µg of sEVs-derived proteins were used in Western Blot analysis to evaluate the expression of the EVs markers as well as NCL.

Results: The isolated CD63- and CD9-expressing sEVs range in size between 81.6 and 138.7 nm. Interestingly, particles concentration results significantly higher in the PB derived from HR-NB, both at onset and at relapse, compared to that from both CTR and LR-NB patients. Furthermore, we have found a higher NCL protein expression on sEVs derived from the PB of all NB patients, compared to that derived from CTR. Noteworthy, NCL expression results significantly higher in the sEVs derived from HR-NB patients at onset, compared to that observed in LR-HR patients, suggesting a potential prognostic value of sEVs-derived NCL in clinic.

Conclusion: The extracellular vesicles-derived protein nucleolin in the peripheral blood of patients may represent a prognostic factor for neuroblastoma.

Funding: AIRC IG n. 24397 to PF

#6562 Bro1 proteins govern a multivesicular bodies fate switch to regulate exosome secretion, metastasis and tumor immune evasion.

N. Yeat[1], L.-H. Liu[1], Y.-H. Chang[1], C.-K. Lai[2], R.-H. Chen[1];

[1]Academia Sinica - Institute of Biological Chemistry (IBC), Taipei, Taiwan, [2]Academia Sinica - Institute of Atomic and Molecular Sciences (IAMS), Taipei, Taiwan

Exosomes play pleiotropic tumor-promoting functions and are secreted by fusion of multivesicular bodies (MVBs) with the plasma membrane. However, MVBs are also directed to lysosomes for degradation and the mechanism controlling different fates of MVBs remains elusive. Here, we show that the pro-tumor protein WDR4 enhances exosome secretion through degrading the ESCRT-associated Bro1-family protein PTPN23. Mechanistically, PTPN23 and its paralog ALIX compete for binding to syntenin, thereby directing MVBs towards degradation and secretion, respectively. ALIX, but not PTPN23, recruits actin capping proteins CAPZA1/CAPZB to prevent branched F-actin accumulation around MVBs, thus enabling MVBs trafficking to cell periphery for secretion. Functionally, WDR4/ALIX dependent exosomes preferentially load a set of pro-tumor proteins to potentiate metastasis and immune evasion. Our study highlights a previously unprecedented coupling between biogenesis mechanism and fate decision of MVBs and its importance in exosome cargo landscape, which have potential broad impacts on boosting exosome production and devising cancer therapeutic strategy.

#6563 Mechanisms of extracellular vesicle uptake in chemotherapy resistant and sensitive ovarian cancer: insights into tropism and resistance transfer.

Joana Sousa Esteves[1], Kadie Edwards[1], Jetzabel Garcia-Parra[1], Claire Donnelly[1], Francesca Taraballi[2], Deyarina Gonzalez[1]

[1]Reproductive Biology and Gynaecological Oncology Group, Swansea University Medical School, Swansea, United Kingdom,[2]Center for Musculoskeletal Regeneration, Houston Methodist Research Institute, Houston, TX

Background: Ovarian cancer is the most lethal gynecological malignancy, with approximately 19,000 new cases and 12,000 deaths projected in the US for 2024. Despite therapeutic advancements, including platinum-based chemotherapies, the 5-year survival rate remains at 50.8%, largely due to chemotherapy resistance. Extracellular vesicles (EVs), nanosized particles involved in intercellular communication, play critical roles in ovarian cancer progression by promoting tumor aggressiveness and chemoresistance. This study investigates the uptake mechanisms and potential tropism of EVs derived from chemotherapy-sensitive (SensC-EVs) and chemotherapy-resistant (ChemoRC-EVs) ovarian cancer (OC) cell lines. All EVs were tested for uptake in both cisplatin-sensitive and cisplatin-resistant cell lines to characterize their interactions comprehensively. EVs were isolated via size exclusion chromatography from serous (SKOV3 wild type [wt] and SKOV3 cisplatin-resistant [cis]) and endometrioid (A2780 wt and A2780 cis) OC cells. EV characterization was performed using nanoparticle tracking analysis, and EVs were fluorescently labeled with Vybrant DiD for uptake studies. Endocytosis inhibitors were employed to identify dominant uptake pathways.
Results: In the serous subtype, ChemoRC-EVs exhibited higher tropism towards SKOV3 cis cells, with significant uptake also observed in SKOV3 wt cells. In SKOV3 wt cells, uptake inhibition by dynasore, nystatin, and NAV2729 revealed the predominant involvement of clathrin-mediated, caveolin-mediated, and receptor-mediated pathways. In SKOV3 cis cells, dynasore and NAV2729 confirmed clathrin- and caveolin-mediated endocytosis as the primary uptake mechanisms, with minimal receptor-mediated involvement. Interestingly, SKOV3 wt cells internalized more ChemoRC-EVs than SensC-EVs, potentially contributing to chemoresistance. In the endometrioid subtype, A2780 wt cells demonstrated higher tropism for SensC-EVs compared to ChemoRC-EVs, while A2780 cis cells exhibited similar uptake levels for both types. Uptake inhibition with dynasore and nystatin highlighted clathrin- and caveolin-mediated mechanisms in A2780 wt cells, whereas nystatin indicated caveolin-mediated uptake as the predominant pathway in A2780 cis cells.
These findings suggest that EV uptake mechanisms, and their tropism, vary by OC subtype, resistance status, and EV source. Generally, sensitive cells internalized more ChemoRC-EVs, implicating a potential role in the transfer of resistance traits. Future work will engineer EVs to deliver anticancer agents into OC cells, leveraging their tropism and uptake pathways to overcome chemoresistance. This approach could transform EVs from mediators of resistance into therapeutic vehicles, offering innovative strategies to combat ovarian cancer and improve outcomes.

#6564 Conversion of fibroblasts into Cancer-associated-fibroblasts (CAF) by small extracellular vesicles from EGFR mutated adenocarcinoma: Implication of SDC1 in the establishment of the lung premetastatic niche.

A. Jouida, C. McCarthy, M. P. Keane;
UCD Conway Institute of Biomolecular & Biomedical Research, Dublin, Ireland

Evidence across several cancer types supports the existence of metastasis-supportive microenvironments, called the premetastatic niche (PMN) which form at sites of future metastasis, allowing the colonization and growth of disseminated tumour cells. Extracellular vesicles (EVs) contribute to the establishment of a favourable microenvironment by the development of those niches. EVs and their contents have emerged as a potential source of information on tumour detection and regulatory drivers of tumour progression and metastasis. Recently, we have demonstrated that EVs are mediators of EMT and tumour invasion. We reported that EVs derived from serum of patients with EGFR mutated non-small cell lung cancer (NSCLC) mediates the activation of the PI3K/AKT/mTOR pathway and induce an invasion in epithelial cells through the up-regulation of MMP-9 by promoting partial EMT. This study aims to investigate the impact of EVs on fibroblasts, another key component of the PMN. Using PCR, we observed increased expression of fibroblast activation protein (FAP), COL1a1, MMP-2, and fibronectin in human lung fibroblasts treated with EVs derived from the EGFR-mutated cell line HCC827. Functional assays revealed that these EV-treated fibroblasts exhibited enhanced invasive and migratory abilities, as shown through a modified Boyden chamber assay and a wound healing assay. We also showed that the knock-out of SDC-1 prevents the activation of fibroblast by sEVs. Additionally, in an indirect co-culture system, epithelial cells exposed to EVs were capable of activating and recruiting fibroblasts. Our results suggest that EVs derived from EGFR-mutated adenocarcinoma serum could be a potential contributor to the establishment of a favourable metastatic microenvironment through the remodelling of the stroma. This may provide insights into the mechanisms of lung cancer progression and metastasis. However, additional studies are required to precisely delineate these mechanisms.

#6565 *In-vitro* assessment of the effect of peri-pancreatic adipose tissue derived extracellular vesicles on the tumorigenicity of pancreatic cancer.

A. Arora, V. Sharma, R. Gupta, S. Bhattacharya, S. Pattanaik, A. Aggarwal;
Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India

Background: Pancreatic cancer (PC) is an aggressive malignancy marked by highinvasiveness, metastasis, and resistance to chemotherapy, presenting a significant global healthchallenge. Visceral obesity has been identified as key risk factors of PC. Significant progresshas been made in understanding the relation between visceral obesity and PC, particularly incontext of pro-inflammatory cytokines and adipokines secreted by surrounding adipose tissue.However, various aspects mediating this relationship remain unclear. Recent studiesilluminated the multifaceted role of extracellular vesicles (EVs) as key communicatorsregulating physiological and pathological processes. Despite their recognised significance, nostudy has yet unveiled the specific contribution of peri-pancreatic adipose tissue (PAT) derivedEVs in driving pancreatic tumorigenesis. The aim of this study is to assess the tumorigenicpotential of PAT-EVs in PC.
Methods: The peri-pancreatic adipose tissue was collected from patients undergoing pancreatictumor surgical resection followed by EVs isolation via differential ultracentrifugation andcharacterization using DLS, TEM, Western blot and flow cytometry following MISEV 2023guidelines. Subsequently, the tumorigenic effect of PAT-EVs were determined by treatingPANC-1 cells (pancreatic cancer cell line) with PAT-EVs. The effect on proliferative potentialwas assessed by Colony formation and BrdU assay, while migration potential by Scratch assay. Further, effect on stemness, ECM modulation, EMT and chemoresistance was assessed viaqRT-PCR and western-blot.
Results: The size (50-1000 nm), morphology (round and bilayer) and specific proteins (CD63,CD9, TSG101, Flotilin-1) observed through DLS, TEM, Western blot and flow cytometryrespectively confirmed the presence of EVs. Through *in-vitro* experiments, we observed asignificant increase in cell viability of PC cells with increasing EVs concentration (0, 10, 25,50, 100, 200 µg/mL). Further, we check the effect of 100 µg/mL and 200 µg/mL dose of EVson tumorigenicity of PANC-1 cells. The colony forming assay revealed that surviving fractionof PANC-1 cells increased by 34.5% and 69.13% at 100 µg/mL and 200 µg/mL doses of EVsrespectively. Additionally, the wound closure percentage increased by 62.3 % in 100 µg/mLEV treated PC cells and 74.9% in 200 µg/mL EV treated PC cells. The qRT-PCR analysisshowed an increase in fold change of markers related to stemness (Oct 4, Sox 2, Nanog); ECMmodulation (MMP 2, MMP 9); EMT (N-Cad, Vimentin); proliferation (p53, Ki67);angiogenesis (VEGF) and chemoresistance (ABCA7).
Conclusion: Our results indicate that peri-pancreatic adipose tissue enhances the tumorigenicpotential of PC cells via EVs. The evaluation of PAT-EVs cargo molecules can further elaboratethe molecular-mechanism behind the interactions between PAT and PC cells.

#6566 FTIR microscope imaging and neural network analysis reveal consistent spectral markers of cancer-associated fibroblasts in cells, plasma sEVs, and tissues.

Abhay Mishra[1], Akhilesh Mishra[2], Saroj Kumar[3]

[1]All India Institute of Medical Sciences (AIIMS) New Delhi, New Delhi, India,[2]Johns Hopkins University, Baltimore, MD,[3]Biophysics, All India Institute of Medical Sciences, New Delhi, India

Background: Cancer-associated fibroblasts (CAFs) are pivotal contributors to tumor progression, influencing tumor biology through interactions within the tumor microenvironment. Distinguishing CAFs from normal fibroblasts is essential for understanding their molecular mechanisms and identifying relevant biomarkers. This study utilized Fourier-transform infrared (FTIR) microscope imaging spectra and neural networks (NNs) to classify CAFs and normal fibroblasts and explored the consistency of identified spectral markers across blood plasma small extracellular vesicles (sEVs) and tissue samples.
Methods: FTIR spectra from CAFs and normal fibroblasts were preprocessed using normalization and baseline correction. A feed-forward NN model was developed, employing regularization techniques to reduce overfitting. Model performance was evaluated using 10-fold cross-validation, with metrics including accuracy, area under the curve (AUC), sensitivity, and specificity. NN-based sensitivity analysis identified key infrared (IR) spectral regions for classification. Comparative FTIR analysis of blood plasma sEVs and tissue samples was conducted to assess the reproducibility of identified spectral markers.
Results: The NN model demonstrated high classification performance, achieving 92% accuracy, an AUC of 0.89, and robust sensitivity and specificity metrics. Sensitivity analysis highlighted significant IR spectral regions, including
Amide I and Amide II (proteins): Indicative of changes in protein structure and extracellular matrix remodeling.
Lipid Regions: Reflecting altered lipid metabolism, critical for membrane dynamics and signaling in CAFs.
Nucleic Acid-Associated Regions: Suggesting transcriptional and epigenetic modifications linked to fibroblast activation.
Notably, similar spectral markers were observed in FTIR analysis of blood plasma sEVs and tissue samples, revealing a consistent pattern associated with CAFs. This suggests systemic changes in the tumor microenvironment that are detectable across different biological matrices.
Conclusion: This study highlights the potential of NN-based FTIR spectral analysis as a robust and versatile tool for identifying biomarkers of CAFs. The reproducibility of key spectral markers in blood plasma sEVs and tissue samples underscores the translational potential of this approach for cancer diagnostics and therapeutic targeting of the tumor microenvironment.

#6567 Metabolic reprogramming of pentose phosphate pathway mediated by small extracellular vesicles facilitates tumor metastasis and drug resistance in HCC.

Xiaoxin Zhang, Zhixian Chen, Jiaqi Lai, Judy Wai Ping Yam

The University of Hong Kong, Hong Kong, Hong Kong

Emerging evidence have proved that the interplay between tumor and its microenvironment facilitate tumor metastasis, including the hepatocellular carcinoma (HCC). Therefore, identifying novel target for HCC metastasis and is essential to improve the survival. In recent years, extracellular vesicles (EV) mediated metabolic regulation has been a growing interest in cancer metabolism. For instance, the ectosomal pyruvate kinase M2 isoform (PKM2) was found to facilitate glycolysis reprogramming in monocyte-to-macrophage differentiation and tumor microenvironment remodeling. Compared to directly receiving metabolite or substance from the EV, we proposed that the tumor cells are more likely to obtain proteomics alteration to exert long-term metabolic reprogramming. EVs were extracted from the metastatic and non-metastatic HCC cells. The DEPs were identified by LC-MS/MS. Meanwhile, the metabolomic screening will also be conducted to clarify the differences of metabolite which the HCC cells produced after the treatment of sEVs which derived from metastatic and non-metastatic HCC cell. The expression of G6PD was knockdown by shRNA. The plate cloning and transwell assays were conducted to evaluate the effect of G6PD on the metastasis. Besides, parallel validation of G6PD inhibitors was performed. The expression of gene was validated by real-time PCR. The MTT assay was conducted to test IC50 of sorafenib resistance HCC cells. Western blot was performed to measure expression of different proteins. Our study found that key enzyme involved in pentose phosphate pathway (PPP) was extremely enriched in metastatic HCC derived small extracellular vesicles (sEVs). Inhibition the enzymic activity of sEVs-derived G6PD significantly reduce the invasion and metastasis of HCC tumor cells. Besides, the sEV-G6PD was associated with activation of cancer-associated fibroblast. Moreover, sEV derived PPP enzymes enhanced sorafenib resistance. In conclusion, PPP enzymes derived from sEV in metastatic HCC cells promotes tumor metastasis and sorafenib resistance.

#6568 Extracellular vesicles containing circular RNAs derived from cancer-associated fibroblasts enhance adhesion and tumor formation of gastric cancer cells in the peritoneal tissues.

Yulim Lee, Hoon Hur, In-Hye Ham

Ajou University, Yeongtong-gu, Suwon-si, Gyeonggi-do, Korea, Republic of

Purpose: The poor prognosis of gastric cancer (GC) patients is strongly associated with peritoneal recurrence. Clinically, a high level of cancer-associated fibroblast (CAF) accumulation correlates with an increased risk of peritoneal recurrence after gastric cancer resection. This observation suggested that CAFs may establish metastatic niches in peritoneal tissues, distant from the primary GC. Extracellular vesicles (EVs) have been identified as key transporter for stable communication between donor and recipient cells, even over long distances. Taken together, CAF-derived EV may play a crucial role in creating a metastatic precondition at the mesothelial cells lining the peritoneal tissue. Understanding the mechanisms underlying this process may provide new insight into therapeutic strategies for preventing peritoneal recurrence in GC patients.
Methods: We collected formalin-fixed paraffin-embedded (FFPE) primary GC tissues from the patients who developed peritoneal recurrence (PR group, n=5), and patients without recurrence during follow-up (Non-PR group, n=5). CAF accumulation was assessed using Mason's trichrome staining, and circular RNA (circRNA) arrays were performed on those tissues. The expression of candidate circRNAs in cell lysates and EVs from various GC cell lines and non-cancerous cells including CAFs using qRT-PCR. The effect of CAF-derived EVs on the adhesion between mesothelial cells and cancer cells was evaluated via adhesion assay. Furthermore, CAF culture media and cancer cells were injected into the mouse peritoneum to assess the number of tumor nodules formed.
Results: We observed that CAFs are more significantly accumulate in GC tissues of the PR group compared to the Non-PR group. Six circRNAs were identified as being more abundant in the PR group based on the circRNA array. Among those, that circTSSC2, circALG1, and circALG1L2 were more abundant in CAF cell lysate and CAF-derived EVs compared to GC cells, mesothelial cells, and other immune cells. The resistance to treatments with actinomycin D and RNAse R and the Sanger's sequencing confirmed the circular structure of those RNAs. CAF-derived EVs were shown to enhance adhesion between mesothelial cells and cancer cells in our experimental models. Additionally, CAF-conditioned media significantly increased the number of tumor nodules formed in the mouse peritoneum.
Conclusions: This study suggests that the EVs including specific circRNAs, secreted by CAFs accumulated in primary GC tissues, may contribute to the formation of a pre-metastatic niche in peritoneal tissues of GC patients. Those CAFs-derived EVs could serve as predictive markers for peritoneal recurrence after GC resection and as therapeutic targets to mitigate the risk of peritoneal metastatic recurrence.

#6569 Extracellular vesicle delivery of functional extrachromosomal DNA in FGFR2-amplified cancer of unknown primary.

I. Salamon[1], G. Gallerani[2], G. Storci[1], B. Fontana[2], S. Serravalle[1], F. Valle[3], M. Brucale[3], M. Pagano Mariano[2], A. Cavazzoni[4], R. Roncarati[3], S. Santi[3], M. Bonafe[2], **M. Ferracin**[2];
[1]IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy, Bologna, Italy, [2]University of Bologna, Bologna, Italy, [3]National Research Council, Bologna, Italy, [4]University of Parma, Parma, Italy

The extracellular vesicle (EV) route is essential for cell-to-cell communication. Cancer cells release EVs in the extracellular space, where they can interact with cancer and non-cancer cells, activating specific signaling pathways, modulating tumor microenvironment remodeling and inducing gene expression alterations. We investigated the functional role of EVs released by cancer of unknown primary (CUP), a rare disease (1-3% of novel cancer diagnoses) that presents with metastasis of unknown or uncertain origin and no apparent primary tumor. We derived three CUP cell lines from patient's tumor cells, CUP#55A and S and CUP#96, both characterized by *FGFR2* gene amplification, either in the form of trisomy (CUP#55A), chromosomal homogeneously staining region (CUP#55S) or double minute chromosomes (CUP#96). It has been recently recognized that tumors use extrachromosomal circular DNA (eccDNA) as a way to increase oncogenic amplification, thus conferring resistance to therapy and contributing to a worse survival. We demonstrated that *FGFR2* amplification in CUP cell lines is associated with ecDNA generation and that this ecDNA is loaded as cargo inside EVs and exert a functional activity in nearby cells. The full-length *FGFR2* DNA was detectable inside both small and large vesicles isolated from cell culture medium and we confirmed the circular nature of a fraction of this *FGFR2* ecDNA using DNAse based strategies and visualizing DNA circles in cells and in EVs with atomic force microscopy. The protein surface profile of small and large EV populations revealed the presence of cancer related molecules that mirrored the cells of origin, including EpCAM and CD44, and variable degrees of FGFR2pos EVs. Cancer (NCI-N87) and non-cancer cell exposure (THP1 and HUVEC) to CUP EVs (by direct administration and co-culture) revealed an increase of *FGFR2* DNA copies at 24 hours and its functional transcription in *FGFR2* mRNA after 48 hours in all exposed cells. In addition to oncogenic delivery, CUP#96 EVs induced a polarization of THP1 cells towards M2 subtype, while CUP#55S EVs exerted a proliferative effect on HUVEC. In conclusion, we identified a model of oncogene amplification and EV-mediated delivery recurrent in cancer of unknown primary, whose DNA content was demonstrated to be functionally active in recipient cells and proportional with extrachromosomal DNA generation. This mechanism could contribute to the high metastatic potential of this cancer type. (The research leading to these results has received funding from AIRC under IG 2021 - ID. 25789 project - P.I. Ferracin Manuela)

#6570 Characterization of diverse extracellular nanoparticles and tumorigenic relevance in hepatocellular carcinoma.

Jiaqi Lai[1], Cherlie Lot Sum Yeung[1], Cui Le[1], Lavisha Korani[1], Tung Him Ng[1], Xiaoxin Zhang[1], Zhixian Chen[1], Ruiqi Ma[1], Chi Ming Che[2], Judy Wai Ping Yam[1]

[1]Department of Pathology, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong, Hong Kong,[2]Department of Chemistry, Faculty of Science, The University of Hong Kong, Hong Kong, Hong Kong

Introduction: Tumor-derived extracellular nanoparticles termed exomeres (EM), distinct to small extracellular vesicles (sEV), act as critical mediators of the tumor microenvironment (TME) formation and are associated with tumor progression and therapy resistance in hepatocellular carcinoma (HCC).
Methods: sEV and EM were isolated from various normal and cancer cell lines using serial ultracentrifugation. The physical and biomedical characterization of EM versus sEV was conducted through transmission electron microscopy (TEM) and mass spectrometry (MS) analysis. Potential molecular EM markers were identified based on the distinct proteomic profiles between sEV and EM. The tumorigenic potentials of HCC-EM were evaluated using *in vitro* functional assays and *in vivo* mice models. Relevant oncogenic mechanisms were studied according to the differential cargo contents of HCC- and normal-EM, as well as differential gene expression of the recipient cells by RNA sequencing. The effects of EM on cell cycle progression and metabolic dysregulation were investigated by flow cytometry and analytical metabolomic/lipidomic approaches.
Results: The TEM and MS analysis demonstrated distinct morphological and protein differences between sEV and EM. GALNS and MAN2B1 were two proteins identified as potential EM markers, showing exclusive or significantly higher expression. Functionally, EM released from HCC cells, instead of normal hepatocytes, stimulated cell growth, motility and tumor development. The EM originated from human HCC cells exhibited increased WNT5A, LRP5, and LRP6 levels, activating the PI3K/AKT/mTOR pathway in recipient cells. In addition, EM derived from murine HCC cells were shown to accelerate cell cycle progression by shifting cells through the checkpoint into the G1 phase, and reconstitute the lipid pattern to be enriched with a very-long chained saturated fatty acid, which triggers the metabolic reprogramming.
Conclusions: Collectively, our findings underscore the unique characteristics of EM as a nanoparticle subpopulation that differs from sEV, and that EM derived from HCC can play a significant role in tumorigenesis and metastasis.

#6571 Natural killer cell-derived exosomes encapsulated with IL-12 as nanocarriers for cancer immunotherapy.

Monu Pandey, Vidit Gaur, Jayanta Bhattacharyya

Centre For Biomedical Engineering, Indian Institute of Technology Delhi (IIT Delhi), New Delhi, India

Cold tumors, owing to poor immune cell infiltration, immunosuppressive tumor microenvironment (TME) and low immunogenicity, are marked as a therapeutic challenge in cancer. Immunomodulatory cytokines showed encouraging outcomes in clinical settings by converting TME more immunoresponsive. Among the cytokines, IL-12 has drawn attention for its role in bridging innate and adaptive immunity, by inducing the proliferation and activation of NK and T cells. IL-12 enhances antigen-specific cell-mediated immunity by promoting Th1 immunity that in turn boosts IFN-γ production and inhibits angiogenesis. It also drives M2 to M1 macrophage polarization, enhances the infiltration of tumor-targeting immune cells, and depletes immunosuppressive cells. This action is critical in converting "cold" into "hot" tumors, making them more susceptible to immunotherapy. Additionally, IL-12 has a short plasma half-life and shows systemic toxicity which limits its use in clinical settings. We aim to deliver IL-12 encapsulating inside NK cell derived nanovesicle (NKnvs) which will bring many attributes- small size of NKnvs will facilitate better tumor accumulation, encapsulation will enhance the plasma half-life of IL-12, slow release of payload will-lower the dose-limiting systemic toxicity, NKnvs will carry cytotoxic proteins, miRNAs, and cytokines with tumor-killing and immunomodulatory properties. NKnvs was isolated by differential ultracentrifugation method, the purity of the isolated NKnvs was determined by Western blot. NTA, DLS and SEM was performed to determine the concentration, size and morphology of NKnvs. Next, IL-12 was encapsulated inside NKnvs by probe sonication method and the loading percentage was quantified by ELISA. To visualise the accumulation of NKnvs in different tissues and to determine the plasma half-life, NKnvs was labelled with DiI dye and purified by size exclusion chromatography. NKnvs showed the hydrodynamic radius and zeta potential of 189±20.61 nm and -10.5±10.1 mV respectively. SEM confirmed the spherical morphology of NKnvs. Loading percentage of IL-12 inside NKnvs was found 20.5±0.5 %. NKnvs induced activation and polarization of immune cells, enhancing tumor cell killing. Pharmacokinetic analysis indicated that NKnvs extended the half-life of the payload. The biodistribution study revealed that DiI labelled NKnvs showed enhanced tumor accumulation which also increased till 48 hrs. In conclusion, the developed nanoformulation offers a promising strategy to target the tumor microenvironment, improving therapeutic efficacy and reducing systemic toxicity. Further, the efficacy of immunovesicle (IL-12 NKnvs) can be explored *in-vivo* to determine the further therapeutic benefits associated with the developed nanoformulation.

#6572 High-frequency irreversible electroporation (H-FIRE) enhances extracellular vesicle release and promotes immune system interaction in cancer treatment.

David Ogburn[1], Irving Allen[2], Nikolaos Dervisis[3]

[1]Virginia Tech Carilion School of Medicine, Roanoke, VA,[2]VA-MD College of Veterinary Medicine, Blacksburg, VA,[3]Veterinary Clinical Science, Purdue University College of Veterinary Medicine, West Lafayette, IN

Background: High-Frequency Irreversible Electroporation (H-FIRE) is a non-thermal tissue ablation technique that induces cell death by destabilizing the cell membrane. This innovative approach has demonstrated significant potential in cancer treatment and has been associated with modulation of the tumor microenvironment in both in vivo and in vitro settings. Recent findings from our lab reveal that extracellular vesicles (EVs) released by cells lethally treated with H-FIRE exhibit a unique proteomic profile. These EVs may play a pivotal role in modulating the tumor microenvironment. Building on our preliminary experiments, we hypothesize that EVs released following H-FIRE-mediated tumor ablation actively engage with and influence the immune system.
Methods: Established carcinoma cell lines of breast (4T1) and exocrine pancreas (PanO2) were used. Cells will be treated with sham, sublethal H-FIRE (1500V), and lethal H-FIRE (3000V). The supernatant of the treated cells was collected and EVs were isolated by ultracentrifugation. EVs were then assessed quantitatively via Nanosight. Mouse monocytes (J774a) were then exposed to EVs and evaluated for extracellular vesicle uptake via confocal imaging and evaluation of mean fluorescence intensity.
Results: In quantitative analysis of the EVs from each treatment group, we found a significantly increased number of EVs present within the supernatant of lethally treated cells, in comparison to both the untreated and sublethal treatment groups (4T1 - sham 1.52e11/mL, sublethal 6.89e10/mL, lethal 3.25e11/mL, $p<.0001$ between all groups) (PanO2 - sham 2.18e11/mL, sublethal 1.1e11/mL, lethal 1.34e12/mL, $p<.0001$ when comparing lethal group to other groups). No significant difference in particle size was observed between the treatment groups. J774a macrophages were exposed to TDEVs from each of the treatment groups, and confocal imaging and evaluation of mean fluorescence intensity showed a qualitative and quantitative increase in macrophage uptake of TDEVs from both the lethal and sublethal groups relative to uptake of TDEVs from the sham group. Using TDEV from the 4T1 cell line, average mean intensity was increased in macrophages exposed to EVs from the lethal and sublethal groups relative to those from the sham group (lethal 2.50, sublethal 2.32, sham 2.04, $p<0.0001$ between sham and both treatment groups).
Conclusion: Our study demonstrates that lethal treatment of carcinoma cells with H-FIRE results in a significant increase in extracellular vesicle (EV) release. Furthermore, we provide evidence that EVs derived from H-FIRE-treated cells exhibit enhanced uptake by macrophages. These findings are in support our working hypothesis that H-FIRE exerts some of its effects in cancer immune system modulation through EV release.

#6573 Real-time precision analysis of exosome treatment in cancer immunodynamics using intravital microscopy.

Hyunseok Kim, Hyungjin Kwon, Jenny Junghyon Ohm, Junyoung Park, Sungwoon Yoon, Kubra Akyildiz, Hsuan-Ju Chen

Technical Application Solution, IVIM Technology, Daejeon, Korea, Republic of

Exosomes play a crucial role in cancer therapy, actively engaging in stimulating the tumor microenvironment and recruiting immune cells, as demonstrated by numerous studies. In exosome-based cancer therapy, precision diagnosis of immune mechanisms utilizes technologies such as X-ray, PET, CT, MRI, and bioluminescence analysis. However, further research is needed to fully understand the impact of exosomes on cancer development and suppression. Particularly, the potential functions of exosomes in cancer immunotherapy and drug delivery have garnered significant attention. Therefore, novel precision analysis methods are essential to further advance these well-established functions in cancer therapy. This is because exosomes play a vital role in critical steps of cancer cell therapy, such as efficient trafficking in cancer metabolism, navigation through the tumor microenvironment, entry into the vasculature, and resumption of inhibitory processes.To address these needs, we conducted a study utilizing exosomes in a brain cancer cell xenograft mouse model and analyzed cancer metabolism treated with exosomes using intravital microscopy (IVM), comparing it with a bioluminescence system. First, exosome fluorescence signals in the xenograft mouse model were screened using the IVIS system. Subsequently, intravital microscopy was performed on the same mouse model to track the trafficking of the exosome fluorescence signals.As a result, IVM demonstrated more detailed and precise expression of exosome fluorescence signals in the xenograft mouse model compared to the IVIS system, which also revealed macrophage activity in the same region. While the IVIS system displayed fluorescence signals as contour-like patterns, IVM provided significantly more detailed and meaningful signals, especially in terms of efficacy, based on the morphology of blood vessels observed in the in vivo mouse model. Furthermore, IVM revealed how interactions between exosomes and cancer cells manifest as phenotypic signals. This indicates that exosome activation is expressed not only at the site of cancer cell accumulation in the brain but also directly at the cancer cell site. Such detailed observations are achievable only with the IVM system, as the IVIS system is insufficient to monitor these intricacies in the xenograft model.These findings suggest that IVM systems are likely to align with the trends in new drug development and regulatory guidelines from institutions such as the FDA, EMA, and TGA. Additionally, the efficiency and functionality of exosomes are best expressed and validated through intravital microscopy, demonstrating its potential as an essential precision analysis method.

#6574 Plant-derived vesicles as potential therapy for neuroendocrine prostate cancer.

Sharanjot Saini, Amritha Sreekumar

Augusta University, Augusta, GA

Background: Neuroendocrine prostate cancer (NEPC) is a highly aggressive lethal variant of prostate cancer (PCa) with extremely poor survival times (<1 year). Clinical management of NEPC is currently challenging as there are limited treatment options for these patients. These patients are treated with platinum-based chemotherapy that is associated with toxicity and relapses. This emphasizes the need for newer, more effective therapeutic interventions against this challenging disease. The overall objective of this study was to develop an effective, non-toxic, cheap yet scalable extracellular vesicle (EV)/nanovesicle-based therapeutic intervention against NEPC.
Methods: We isolated intact nanovesicles from pomegranate juice, referred to as POM-EVs, using a combination of ultracentrifugation and filtration. Vesicles were characterized by Nanosight Tracking Analyses and Electron Microscopy. Further, we examined the therapeutic potential of POM-EVs in a panel of prostate cell lines in vitro. Prostate cancer cell lines were treated with POM-EVs for 7 days followed by assessment of cellular proliferation, apoptosis, RNA analyses and Western blot analyses. To understand the mechanistic basis of POM-EV induced alterations in NCI-H660 cells, we performed next generation RNA sequencing in control vs POM-EV treated NCI-H660 cells on Illumina HiSeq platform. To examine the effect of POM-EVs in NEPC, we established LuCaP 145.1 tumors in FOX-Chase SCID mice. Once established, animals were divided into two groups: Control and Test (n=5/group). Test mice were administered POM-EVs via tail vein twice a week. Controls included xenografts treated with PBS. Tumor volumes were measured regularly. Further, studies were conducted to assess active moieties of POM-EVs with therapeutic activity in NEPC.
Results: POM-EVs led to a significant reduction in viability of neuroendocrine cell lines, NCI-H660 and LASCPC-01 as assessed by MTT assay. We further performed apoptosis assay that showed that POM EV treatment led to a significant increase in apoptotic populations. POM-EV treated mice showed significant regression of LuCaP145.1 tumors. Sequencing studies identified that genes involved in ECM-receptor interaction, focal adhesion, proteoglycans in cancer and small cell lung cancer genes were significantly impacted by treatment with POM-EVs. Remarkably, POM-EVs led to significantly attenuated levels of key proneural/neuronal transcription factors.
Conclusions: Systemic administration of POM-EVs can be employed as a novel treatment modality in neuroendocrine prostate cancer.

#6575 Overexpression of fatty acidy synthase increases exosomes secretion in colorectal cancer.

Josiane Weber Tessmann, Yekaterina Zaytseva

Department of Toxicology and Cancer Biology, University of Kentucky, Lexignton, KY

More than 50% of colorectal cancer (CRC) patients develop metastases, with the liver being the most common site of metastasis. Fatty acid synthase (FASN), a key enzyme of de novo lipid synthesis, is overexpressed and strongly associated with metastasis and poor prognosis in CRC. FASN catalyzes the biosynthesis of palmitate, an essential component of structural lipids. Exosomes are composed of a lipid bilayer membrane and carry a wide range of bioactive molecules. Tumor-derived exosomes play a critical role in establishing a pre-metastatic niche (PMN) to support metastasis. Hepatic stellate cells (HSCs) can be activated by tumor signals and transformed to cancer-associated fibroblast, thus, contributing to PMN formation. However, the mechanisms by which exosomes promote HSCs activation and their subsequent contribution to PMN formation during CRC liver metastasis are not fully understood. Therefore, this study aims to investigate the contribution of FASN in exosomes formation, release, and HSCs activation in CRC. To delineate the role of FASN in exosomes formation, FASN-knockdown (FASN KD), FASN-overexpression (FASN OE), and pharmacological inhibition of FASN in CRC cells were utilized. Cells were cultured in media containing exosome-free FBS for 48h. Exosomes were isolated from cell culture media using ultracentrifugation. The concentration and size of exosomes were measured using nanoparticle tracking analysis. Exosomes markers were assessed using western blot. To investigate the functional impact of CRC-derived exosomes, HSCs LX-2 was used. The purity of isolated exosomes was confirmed by assessing the expression of CD9, Alix, TSG101, and syntenin. Secretion of exosomes by FASN-KD cells ($Exo^{FASN\text{-}KD}$) and by cells treated with FASN inhibitors was significantly reduced as compared to control cells. Moreover, FASN expression was decreased in $Exo^{FASN\text{-}KD}$. In contrast, secretion of exosomes by FASN-OE cells ($Exo^{FASN\text{-}OE}$) was significantly increased as compared to control cells. Consistently, we observed an increase in expression of FASN in $Exo^{FASN\text{-}OE}$. To test if FASN expression in CRC cells affects the ability of secreted exosomes to activate HSCs, LX-2 cells were incubated with exosomes from control and FASN-KD CRC cells. We found that the exposure to exosomes from control CRC cells leads to robust activation of LX-2 cells as determined by FAP and α-SMA expression; however, the level of LX-2 activation was significantly reduced when cells were exposed to $Exo^{FASN\text{-}KD}$. In summary, our data suggests that FASN promotes exosomes secretion in CRC. Importantly, the association between the level of FASN expression in CRC cells and activation of LX-2 cells by secreted exosomes suggests a potential role for FASN in PMN formation. Further studies are needed to elucidate the functional importance of FASN in PMN formation via exosomes secretion in CRC.

#6576 Exploring the role of circulating extracellular vesicles in chemotherapy resistance and cachexia in advanced pancreatic cancer.

Shatovisha Dey, Tammy Lo, Alpana Dholakia, Julianna Cuateco, Sabina Swierczek, Richard C. Frank

Nuvance Health, Danbury, CT

Advanced-stage pancreatic (ductal adenocarcinoma) cancer (APC) is an incurable malignancy with an average survival of less than a year. Although combination chemotherapy (FOLFIRINOX, gemcitabine/nab-paclitaxel, liposomal irinotecan-based) regimens offer improved survival compared with single agent gemcitabine, chemotherapy resistance (CR) and cancer cachexia (CC) are universal phenomena near the end of life. CC is an inflammatory process marked by anorexia and weight loss, muscle wasting (sarcopenia), fat depletion (adipopenia) and profound fatigue. CR leads to the uncontrolled growth of tumor cells that accelerates CC. Both processes are thought to be reflected in and in part mediated by circulating extracellular vesicles (EVs), which contain molecular cargo from their tissues of origin. Given the lack of tumor biopsies performed during the treatment course of APC, we analyzed circulating EVs from a patient-derived, serially collected blood biobank to identify proteins that could serve as potential biomarkers of and therapeutic targets for both CR and CC.

In this study, we isolated EVs from nine patients with double- and triple-chemotherapy refractory APC from our Pancreatic Cancer Biobank. Proteomic and phospho-proteomic analyses via mass spectrometry were conducted and differential protein expressions were compared across 3 timepoints: 1. Pre-treatment; 2. Remission; and 3. Chemotherapy resistance towards the end of life. These were also compared against healthy matched controls (n=5).

Our analysis identified 269 differential proteins, with 61 unique to late relapse and 66 unique to pre-treatment, indicating their associations with CR and CC. At late-relapse, 16 proteins were downregulated and 16 were upregulated ($log2FC>1$, $p<0.05$). Top downregulated proteins included RAPGEF1, PPP1CA, LUZP2, PIGG, and NAMPT, while the most upregulated proteins were WNT9B, GFAP, POLK, ADGRB3, and MYH8. Several of these proteins are associated with the Wnt signaling pathway, known to influence cachexia. Phosphoproteomic analysis identified 15 uniquely phosphorylated proteins at relapse, including BRD7 and UBR2, also implicated in Wnt signaling.

This is the first study to longitudinally track proteomic changes in circulating EVs from stage IV, chemotherapy-refractory PC patients, providing a unique perspective on CR and CC. Ongoing validation of these results in additional patient samples, coupled with in-vitro studies exploring the functional and mechanistic roles of these proteins in CR and CC offer the potential to disrupt pancreatic cancer progression and its devastating consequence of cancer cachexia.

#6577 ST6GAL1^{+} exosomes confer treatment resistance in rectal cancer.

J. M. P. Lever, R. Irwin, V. Somasundaram, M. Stiles, M. C. McLeod, K. Hardiman;
University of Alabama at Birmingham, Birmingham, AL

Rectal cancer (RC) is a highly morbid and fatal disease. Locally advanced RC is treated with neoadjuvant chemoradiation. Patients with a complete pathologic response have improved outcomes compared with those with incomplete response and may be able to avoid surgical resection. Among incomplete responders, we have found that there is increased tumor ST6GAL1 protein, which is a glycosyltransferase that adds alpha 2,6-sialic acid to N-glycans. Through knockdown (KD) studies, we have shown that ST6GAL1 causes chemoradiation treatment resistance in RC. Exosomes are small particles that can be transferred between cells. We hypothesized that these exosomes transfer functional ST6GAL1 thereby spreading treatment resistance and can be found in the plasma of a mouse model of RC. In order to test these hypotheses, we cultured colorectal cancer SW620 cells and isolated exosomes by ultracentrifugation from the media. Exosome yield and size were determined using a NanoSight particle analyzer and further characterized using transmission electron microscopy (TEM). Exosomes from ST6GAL1 CV cells were used to treat ST6GAL1 KD. Western blot, immunofluorescence (IF) microscopy, and flow cytometry for alpha 2,6-sialic acid by SNA lectin were utilized. Colony formation assay and cleaved caspase-3 IF staining were performed. Mice bearing a patient derived xenograft model of rectal cancer were treated with chemoradiation daily for 2 weeks (5 days on, 2 days off) and tumor and mouse plasma exosome samples were tested for ST6GAL1. We found that human colorectal cancer cells in culture produced exosomes containing ST6GAL1. These exosomes were 91.2 ± 6.8 nm in diameter and had an umbilicated appearance on TEM. When ST6GAL1 KD cells were treated with ST6GAL1^{+} exosomes, the protein was transferred to KD cells as measured by western blot. IF staining showed that transferred ST6GAL1 protein co-localized to the Golgi apparatus, where it is natively found. Additionally, exosome-treated cells became more resistant to chemoradiation-induced apoptosis and showed improved colony formation (CV 21.7 ± 4.6 colonies vs KD 12.7 ± 3.5 colonies, $p = 0.03$; KD plus exosomes 17.7 ± 3.0 colonies vs CV, $p = 0.38$). ST6GAL1 protein levels increased in tumors from a patient derived xenograft during treatment and peaked at 2 days. We further found that ST6GAL1 protein in exosomes isolated from the plasma of these mice peaked at the same time as in the tumor. In conclusion, rectal cancer exosomes carrying ST6GAL1 spread treatment resistance to previously sensitive cells and can be detected in the plasma of tumor bearing mice. Interfering in exosome-mediated sharing of ST6GAL1 is a promising and entirely novel potential therapeutic strategy for RC. These exosomes may also serve as a biomarker for identifying patients with treatment resistant rectal cancer.

#6578 Effects of the PAX3-FOXO1 fusion oncogene on secreted microvesicle-mediated priming of the pre-metastatic niche in rhabdomyosarcoma.

Yu-Lieh Lin, Monireh Asoudeh, Faiz Ahmad, Pratibha Mishra, Raya Saab

Department of Pediatrics, Stanford University School of Medicine, Stanford, CA

Fusion-positive rhabdomyosarcoma (FP-RMS) is an aggressive childhood soft tissue sarcoma, driven by the expression of a fusion oncoprotein PAX3-FOXO1. It presents with high metastatic potential, with 80% of tumors already metastatic at diagnosis, and a less than 20% cure rate with current available treatment. Paracrine and distant signaling, including via small secreted extracellular microvesicles including exosomes (sEVs), play a critical role in priming the pre-metastatic niche and enhancing tumor metastasis, but their role in FP-RMS, and specifically relating to fusion oncoprotein signaling, has not been studied. In this study, we aim to investigate the role of sEVs in mediating the effects of PAX3-FOXO1 signaling on the tumor microenvironment at the pre-metastatic niche, hypothesized to promote FP-RMS invasion and metastasis. We use the mouse myoblasts C2C12 model, with expression of the fusion oncogene *PAX3-FOXO1*. sEVs are extracted by serial ultracentrifugation and verified by size analysis and western blotting. We utilize *in vitro* cell culture studies as well as immunocompetent mouse models for tail vein injections to study effect of sEVs on the lung tumor microenvironment. We show that sEVs play a key role in enhancing cell migration and invasion of recipient stromal cells by comparing their effects on fibroblasts using treatment with 1) conditioned medium; 2) sEVs extracted from conditioned medium; and 3) an sEV-depleted fraction of the conditioned medium, all derived from PAX3-FOXO1-expressing cells versus controls. Furthermore, through RNA sequencing, we analyze the transcriptome of recipient fibroblasts treated with sEVs derived from PAX3-FOXO1-expressing cells versus those derived from cells expressing empty-vector control and identify signaling pathways differentially influenced by oncogene-modulated sEVs. *In vivo*, we found that sEVs were predominantly taken up by alveolar macrophages in the lung, in addition to other cells. Immunofluorescence staining further revealed that the populations of macrophages and cancer-associated fibroblasts were increased in the lungs of mice receiving sEVs derived from PAX3-FOXO1-expressing cells, as compared to those receiving sEVs from control cells. Ongoing work is focused on dissecting the proteomic and molecular signaling affected by PAX3-FOXO1-modulated sEVs on both fibroblast and immune cell compartments. In conclusion, this study reports that PAX3-FOXO alters the microenvironment of the distant pre-metastatic niche through sEV-mediated distant signaling. Ongoing investigations will provide deeper insights into the mechanisms underlying tumor metastasis in FP-RMS, potentially identifying novel therapeutic molecular targets to address current treatment gaps.

#6579 Elucidating glioblastoma-derived extracellular vesicle cargo using TurboID: Implications for tumor microenvironment adaptation.

M. Russo, E. Norton-Ramos, M. Ulloa Navas, A. Quinones-Hinojosa, H. Guerrero-Cazares;
Mayo Clinic Florida, Jacksonville, FL

Glioblastoma (GBM), the most aggressive primary brain tumor in adults, presents significant challenges due to its universal recurrence and limited survival rates, exacerbated when proximal to the lateral ventricles (LV). The subpopulation of brain tumor-initiating cells (BTICs) plays a pivotal role in tumor initiation and invasiveness, interacting with the microenvironment, particularly the cellular components in the subventricular zone (SVZ). Some mechanisms of intercellular communication present in the SVZ include paracrine, autocrine, direct cell contact, gap junctions, nanotubes, and more recently extracellular vesicles (EVs). EVs are membrane-bound particles released by cells that are central to intercellular communication. They have the ability to carry bioactive proteins, RNA, and other molecules that influence recipient cell behavior.This study investigates the role of EVs in GBM's communication with non-cancer cells. Our findings indicate that BTIC-derived EVs promote migration and proliferation ($*p < 0.02$, $***p < 0.001$) in neural progenitor cells (NPCs) *in vitro*, highlighting the EVs' role in altering the microenvironment. To identify the protein cargo contained in GBM-derived EVs, we utilized the innovative proximity-labeling system TurboID. We successfully induced TurboID expression in primary-cultured human BTICs from GBM patients, enabling the unbiased biotinylation of proteins in EVs. We isolated biotinylated proteins from BTIC lysates and BTIC-EVs, preserving the integrity of EVs, and conducted proteomic analysis via mass spectrometry. Our method marks the first implementation of TurboID for unbiased global labeling of EV protein cargo in primary GBM cells. This approach facilitates the investigation of EV-mediated communication and potential therapeutic targets, contributing to the understanding of GBM's complex interactions with the brain's microenvironment and the identification of biomarkers for improved diagnosis and treatment response.

#6580 Impact of copy number variations on EV-miRNA profiles in ovarian cancer.

Hajime Araki, Yokoi Akira, Kosuke Yoshida, Kazuhiro Suzuki, Hironori Suzuki, Masami Kitagawa, Eri Asano-Inami, Hiroaki Kajiyama

Nagoya Univ. Graduate School of Medicine, Nagoya, Japan

[Objective] Ovarian cancer has poor prognosis due to its late diagnosis and the development of drug resistance, particularly in advanced stages. High-grade serous ovarian carcinoma (HGSOC), the most common subtype, is characterized by extensive genomic alterations, including *TP53* mutations and copy number variations (CNVs). However, the functional impact of CNVs remains largely unclear. This study aimed to investigate how CNVs influence extracellular vesicle (EV)-associated microRNA (miRNA) profiles to identify potential diagnostic biomarkers and therapeutic targets.

[Methods] Clinical and genomic data from 489 ovarian cancer patients in The Cancer Genome Atlas (TCGA) dataset were initially reviewed. From these, *TP53*-mutant cases (n=247) were selected and classified into CNV-high (n=21) and CNV-low (n=62) groups. Additionally, 23 *TP53*-mutant ovarian cancer cell lines were divided into CNV-high (n=17) and CNV-low (n=6) groups. miRNA expression profiles were compared to identify CNV-associated miRNAs. Selected miRNAs were further analyzed through prognostic assessments, tumor validation, and functional assays. Finally, in vitro assays were performed to confirm miRNA expression levels and cisplatin resistance.

[Results] Compared to the CNV-low group, patients in the CNV-high group exhibited shorter OS within 30 months. In 23 cell lines, a comparative analysis of miRNA expression profiles revealed that 7 miRNAs were significantly upregulated, while 9 were downregulated in the CNV-high group ($p < 0.05$). Among the upregulated miRNAs, miR-203a showed the highest expression levels in CNV-high cell lines and was strongly correlated with poor prognosis. Tumor tissue analysis confirmed that miR-203a expression was significantly higher in ovarian cancer tissues compared to adjacent normal tissues ($p < 0.01$), and elevated miR-203a levels were also detected in the serum of ovarian cancer patients. Furthermore, EVs isolated from *TP53*-mutant CNV-high cell lines (CAOV3, OVCAR3, RMUGS) were found to secrete miR-203a in abundance. Functional assays demonstrated that overexpression of miR-203a in these cell lines led to increased resistance to cisplatin, as evidenced by a higher IC50, particularly in the CNV-high group. Knockdown of miR-203a in CNV-high cell lines resulted in enhanced sensitivity to cisplatin and reduced cell proliferation.

[Conclusion] Elevated miR-203a expression was linked to poor prognosis and chemoresistance in ovarian cancer patients with high CNV levels. Detection of miR-203a in EVs indicates its potential as a non-invasive biomarker for early diagnosis. Targeting CNV-associated miR-203a may provide new therapeutic strategies to overcome platinum resistance in HGSOC, offering insights into molecular mechanisms and potential treatment approaches.

#6581 Evaluating the impact of the tumor microenvironment on EV-mediated intercellular exchange of nanoparticles.

Yiqin Li, Hongbo Pang

University of Minnesota, College of Pharmacy, Minneapolis, MN

The limited delivery efficacy of nanoparticles (NPs) continues to impede their clinical translation for cancer therapy. Recent studies have demonstrated that active trans-endothelial pathways dominate NP delivery. While extensive research has focused on endocytosis and subcellular transport, there remains limited understanding of how NPs undergo exocytosis and subsequently re-enter neighboring cells—a process we termed intercellular exchange. Our previous studies established a collagen-based three-dimensional assay designed to specifically quantify the intercellular exchange of NPs and distinguish the contributions of various potential mechanisms. The findings revealed that a significant portion of NPs are transferred within extracellular vesicles (EVs). By modulating EV biogenesis, we successfully altered tumor accumulation and vascular penetration of various NPs in vivo. Here, we further enhance this assay by incorporating critical tumor microenvironment (TME) factors—such as acidity, hypoxia, and the dense extracellular matrix (ECM). This improved model provides a more physiologically relevant platform to evaluate the impact of TME-specific conditions on EV-mediated NP intercellular exchange. Our findings demonstrate that different TME factors exert varying effects on NP transfer efficiency, with ongoing studies evaluating their implications for in vivo delivery. These investigations not only shed light on the mechanisms by which NPs are transported within tumors but also enhance our understanding of the role of EVs in intercellular communication under highly stressed environments.

#6582 HD-PTP modulates anti-tumor immunity via extracellular vesicles in triple-negative breast cancer.

Chenxu Guo, Sheng Sun, Nayana Thimmiah, Ilze Smidt, Shufeng Zhou, Thomas J. Kania, Taisha Joseph, Mark N. Melkonyan, Siang B. Koh, Leif W. Ellisen

Cancer Center, Massachusetts General Hospital, Boston, MA

The tumor immune microenvironment (TIME) is a critical driver for the progression of triple-negative breast cancer (TNBC), directly impacting patient prognosis and treatment response. One emerging mechanism underlying immune evasion in TNBC involves extracellular vesicles (EVs), which act as carriers of immunosuppressive factors (ISFs) that shape the immune landscape. Here, we identify HD-PTP, an ESCRT-associated protein encoded by the *PTPN23* gene, as a key regulator of EV-mediated immune modulation. Clinically, we reveal that HD-PTP loss is frequently observed in TNBC and correlates with immune cold microenvironment, as shown by reduced tumor infiltration levels of lymphocytes, poor response to immune checkpoint inhibitors, and worse overall survival. Additionally, we observe that knockdown of HD-PTP promotes systemic tumor growth only in a syngeneic, immunocompetent TNBC mouse model, but not in the matched, immunodeficient nude mice. Through proteomic profiling of the EVs purified from HD-PTP proficient and deficient cells, we find that HD-PTP downregulation dramatically changes EV protein composition and identify PD-L1 as a major ISF cargo selectively incorporated into EVs upon loss of HD-PTP. Using a co-culture assay with antigen-presenting B cells and PD-1 overexpressing T cells, we show that EVs derived from HD-PTP knockdown TNBC cells significantly reduce IL-2 secretion and impair antigen-dependent T cell activation via EV-associated PD-L1. Mechanistically, HD-PTP inhibition redirects PD-L1 from the plasma membrane to the endosomes, which increases PD-L1 co-localization with the EV marker CD63 and upregulates its subsequent release into EVs. However, blocking EV biogenesis in HD-PTP deficient cells – either by genetic knockdown of Alix or syntenin, or by pharmacological inhibition with GW4869 – reduces EV release of PD-L1, confirming that HD-PTP plays an essential role in regulating EV-associated PD-L1. Perturbation of either PD-L1 expression or EV release by Rab27A in the HD-PTP knockdown tumors abolishes the accelerated growth observed in the immunocompetent mice. Furthermore, we demonstrate that HD-PTP knockdown tumors had significantly fewer infiltrating T cells and altered macrophage polarization, while such immune exclusion was partially reversed by PD-L1 or Rab27A disruption. Collectively, our results unveil a novel tumor suppressor pathway controlling specific ISF cargos in EVs, while HD-PTP loss leads to altered EV-mediated signaling that facilitates immune suppression, remodels TIME, and fosters tumor growth, which eventually leads to inferior patient outcomes.

#6583 Optimized production, biodistribution, and targeted delivery of T cell-derived extracellular vesicles for liver cancer therapy.

Aminah Jaffery[1], Andrea C. Cortes[1], Kiyoyuki Minamiguchi[1], Simone Anfossi[2], Rony Avritscher[1]

[1]Interventional Radiology, University of Texas MD Anderson Cancer Center, Houston, TX,[2]Translational Molecular Pathology, University of Texas MD Anderson Cancer Center, Houston, TX

Background: Extracellular vesicles (EVs) have emerged as potent mediators of physiological and pathological processes. By transporting bioactive molecules such as proteins and RNAs, EVs play a pivotal role in maintaining tissue and organ homeostasis. This unique functionality positions EVs as highly promising therapeutic agents. Notably, their ability to penetrate solid tumors and deliver targeted treatments underscores their potential in oncology. However, the preclinical use of EVs requires the development of robust and scalable production methods. This study aimed to establish standardized protocols for efficient EV production while evaluating their tissue and organ targeting capabilities, with a focus on achieving precise and efficient delivery to the liver. Furthermore, the potential application of EVs in minimally invasive interventions, such as portal vein embolization, opens new avenues for therapeutic delivery to liver tissues.
Methods: EVs were isolated from T cells derived from Buffalo rat spleens and activated with concanavalin A. After 48 hours of culture, EVs were precipitated from the cell culture supernatant. The size and concentration of the isolated EVs were assessed using NanoSight technology. EVs were labeled with IVISense 750 dye and resuspended in either a saline solution or an oil-based contrast agent. These labeled EVs were administered to tumor-bearing rats via three routes: tail vein, portal vein, and intratumoral injections. Biodistribution of the EVs in liver tissue and other organs was analyzed using in vivo and ex vivo imaging. Results: Portal vein injection of saline-suspended EVs demonstrated superior liver targeting, achieving rapid and efficient accumulation in the liver. Intratumoral injection successfully delivered EVs to localized tumor regions, while tail vein injection, despite a slight delay, also enhanced liver biodistribution. Imaging results revealed a strong tropism of EVs for tumor tissues. Interestingly, systemic administration via the tail vein resulted in reduced kidney signals, suggesting urinary clearance of EVs within 72 hours.
Conclusions: This study established a reproducible, standardized protocol for activating spleen-derived T cells, enabling large-scale production of EVs suitable for in vivo applications. The findings highlight the potential of EVs as effective delivery vehicles for therapeutic cargo, particularly for liver-targeted and tumor-specific treatments. Moreover, EV application in minimally invasive interventions, such as portal vein embolization, could further enhance targeted delivery to liver tissues. By optimizing both production and delivery methods, this research lays a foundation for advancing EV-based strategies in cancer therapy, paving the way for future clinical applications.

#6584 Slamming the breaks on extracellular vesicles that cause breast cancer.

A. R. Khaled[1], C. Dang[1], L. Truong[1], S. Laxton[1], J. Velazquez[1], S. Kankipati[2], P. Gopalan[2], S. Litherland[1];
[1]University of Central Florida, Orlando, FL, [2]Orlando VA Healthcare System, Orlando, FL

Breast cancer (BC) recurrence and metastasis are significant causes of death, mainly due to the emergence of therapy-resistant cells. Secreted factors known as extracellular vesicles (EVs), especially small EVs (SEVs) or exosomes less than 100 nanometers in size, have been implicated in the plasticity associated with drug resistance, BC recurrence, and metastasis. EVs derived from BC cells can transform local or distal non-tumor cells, induce the epithelial-to-mesenchymal transition, and trigger uncontrolled proliferation, angiogenesis, and immune suppression of tumor cells. EVs also mediate resistance to hormone therapy and chemotherapy. Given these oncogenic activities, the production of EVs by BC cells presents an attractive target for inhibition. However, the complexity of EV biogenesis and uptake hampers the effectiveness of most pharmacological inhibitors that target single or specific EV synthetic pathways. Because protein synthesis and sorting are the initial steps in the biogenesis of EVs, we inferred that protein folding is a necessary part of EV synthesis. Previous studies revealed that the protein-folding complex, Chaperonin-Containing TCP-1 (CCT), is upregulated in cancer cells to mitigate the damage from misfolded proteins that accumulate due to increased somatic gene alterations and driver mutations. Using RNA sequencing and optimized methods of EV characterization by flow cytometry and nanoparticle tracking analysis (NTA), we determined that CCT has a role in the production of EVs by BC cells. Inhibition of CCT2, the second subunit of the CCT complex, in BC cells deregulated the gene expression of critical components involved in the early stages of EV biogenesis and decreased the release of CCT2-containing EVs. The exogenous expression of CCT2 in BC cells produced small EVs (SEVs) with increased and selective loading of CCT2 RNA and protein cargo. BC cell culture media, enriched for CCT2-containing SEVs using a 0.1 micromolar filter, increased CCT production and activity in recipient cells. Exogenously expressed CCT2 in BC cells also enriched gene expression associated with extracellular exosomes (GO:0070062). Hence, CCT may be essential for the biogenesis of SEVs with transformative properties and a promising therapeutic target for inhibiting cancer-causing EVs. These studies show that CCT directly regulates components of EV biogenesis in BC cells and advances CCT as an essential driver of BC progression through the production of oncogenic EVs.

#6585 Distinct small-EV proteomic landscape mediated by annexin A2 alters recipient cell signaling in triple negative breast cancer metastasis.

Rucha Trivedi, Jamboor K. Vishwanatha

Microbiology, Immunology and Genetics, University of North Texas Health Science Ctr., Fort Worth, TX

Cancer metastasis is promoted through interactions between tumor-derived extracellular vesicles (EVs) and recipient cells in distant organs. The lungs are frequently affected as secondary organs in metastatic triple-negative breast cancer (TNBC), occurring in approximately 20-30% of cases. A membrane-associated protein, Annexin A2 (AnxA2), is significantly upregulated in TNBC tissues and EVs, leading to poor outcomes in patients. It is among the most identified EV proteins and recruits microRNAs and proteins into the EVs. Previous reports from the lab have shown a direct correlation between AnxA2 levels in small-EVs (sEVs) and metastasis in the lungs and brain *in vivo*. This study aims to examine how AnxA2 contributes to the promotion of TNBC lung metastasis mediated by sEVs. MDA-MB-4175 (LM2) lung metastatic TNBC cells were used to downregulate AnxA2 protein, and the metastatic potential was assessed through invasion, migration, and proliferation assays. Downregulation of AnxA2 significantly reduced invasive, migratory, and proliferative potential, as well as decreased plasmin-generating abilities. An optimized differential ultracentrifugation and filtration method was used to isolate sEVs, which were characterized according to MISEV2023 guidelines. sEVs obtained from conditioned cell culture media ranged in size from 30-300 nm and exhibited a distinct double membrane under cryo-electron microscopy. Biological analysis revealed that the sEVs were positive for ESCRT and heat shock proteins, as well as CD9 and CD81 tetraspanins. Following characterization, the sEVs were subjected to quantitative proteomics to assess their protein cargo. Additionally, the uptake by recipient cells and downstream signaling alterations were analyzed. A unique sEV protein landscape was observed, which differentially affected biological processes such as tumor cell viability and proliferation, and the migration of fibroblasts and tumor cells. Altered uptake by recipient non-metastatic TNBC cells and lung fibroblasts was revealed, leading to changes in downstream functional processes. Decreasing AnxA2 expression in TNBC cells diminishes their ability to metastasize by modifying the sEV proteome, thereby influencing interactions with recipient cells at both local and distant sites. These findings uncover a novel role for AnxA2 in promoting TNBC metastasis through sEVs. [This research is supported by the NCI of NIH under Award Number R01CA220273 (JKV), awarded to Dr. Jamboor K. Vishwanatha and partially supported by Cancer Prevention and Research Institute of Texas grant (Award#: RP210046).]

#6586 Targeting cancer cell dynamin activity *in vitro* impacts exosome production and cell proliferation.

Alexander J. Willis, Indira Kondapally, Narjess Hajilou, Paul Grippo, Tolou Shokuhfar, Herbert H. Engelhard, Yolande Chen

University of Illinois at Chicago, Wilmette, IL

Introduction: These experiments aimed to determine the effects of dynamin (DNM) on the exosome production of cancer cells. Researchers can easily collect exosomes, which are circulating extracellular vesicles, from the blood and analyze biological processes from the tumors of origin. This is particularly advantageous in solid tumors with limited tissue available for study.
Methods: Pancreas cancer and glioblastoma cells were grown until confluent, then seeded at equal density with 75 uM dynasore (DNSR), 30 uM Bis-T-23, or control (DMSO) for 24, 48, 72, and 96 hours (h). At the specified time, media was gathered for exosome precipitation, and cells were counted with trypan blue exclusion for proliferation. Exosomes were precipitated by SBI exosome tc precipitation solution, protein yield was measured by nanodrop, and the # of CD63 exosomes was determined using SBI's CD63 ELISA kit. Additionally, the exosomes were fixed on silicon chips for SEM and imaged using the JEOLJEM-ARM200CF. All statistical analysis was done using a t-test: Paired Two Sample for Means.
Results: DNM inhibition had a more significant effect on cell proliferation and exosome precipitation than DNM oligomerization. AsPC cells treated with DNSR had a significant decrease in proliferation compared to DMSO at 72h (1.0×10^5 cells + 3.536×10^4 vs 7.125×10^5 cells + 7.181×10^4, respectively, p=0.005) and 96h (2.125×10^5 cells + 5.154×10^4 vs 6.625×10^5 cells + 7.181×10^4, respectively, p=0.025). U87 (1.5×10^5 cells + 3.536×10^4 vs 4.875×10^5 cells + 8.985×10^4, respectively, p=0.02) cells had decreased proliferation after 96h compared to DMSO. AsPC cells also had a significant increase in proliferation after 96h in response to Bis-T-23 compared to DMSO (1.125×10^6 cells + 1.561×10^5 vs 6.625×10^5 cells + 7.181×10^4, p=0.024). Exosome concentrations were measured in the supernatant and normalized to cell concentrations, AsPC had a significant difference in exosomes per cell in response to DNSR at 72h (25.8 ng/cell + 7.0 vs 2.6 ng/cell + 0.2, respectively, p=0.013) and 96h (10.3 ng/cell + 2.8 vs 2.6 ng/cell + 0.2, respectively, p=0.039), and to Bis-T-23 at 96h (1.7 ng/cell + 0.2 vs 3.1 ng/cell + 0.4, respectively, p=0.013). U87 (14.6 ng/cell + 5.8 vs 3.4 ng/cell + 0.5, respectively, p=0.002) had a significant change in exosomes precipitated per cell at 96h after dynamin inhibition.
Conclusions: Data show extracellular vesicles, specifically exosomes, have been implicated as data transfer units between cells. To be an exosome, a membrane vesicle needs to be 30-90 nm and of endocytic origin enriched with tetraspanin molecules. Here, we demonstrate the impact of targeting DNM activity on the cells' ability to proliferate and the quantity of exosomes produced over time. Exosomes have implications in both tumor suppression and proliferation. Our data shows that targeting DNM and exosomes could aid in the effectiveness of diagnosis or treatment of cancer.

#6587 Plasma-derived exosomes in the frontier of gene therapy development.

Gizem Kursunluoglu[1], Omer Aydin[2], Mukerrem Betul Yerer[3]

[1]Drug Application and Research Center (ERFARMA), Erciyes University, Kayseri, Turkey,[2]Department of Biomedical Engineering, Erciyes University, Kayseri, Turkey,[3]Drug Application and Research Center (ERFARMA) - Department of Pharmacology, Faculty of Pharmacy, Erciyes University, Kayseri, Turkey

Gene transfer refers to the process of incorporating foreign genetic material, such as DNA or RNA, into target cells or organisms. Gene transfer is a dynamic field with significant potential for advancing medicine, biotechnology, and scientific research. Ongoing research and technological developments aim to enhance the efficiency, safety, and precision of gene transfer techniques for various applications. In the transportation of genetic materials such as DNA, miRNA, mRNA, and siRNA, various challenges are encountered, and these challenges are being addressed through the approach of nanobiotechnology.

Exosomes are promising natural carriers for gene delivery systems. Due to their high biocompatibility, stability, and low immunogenicity, exosomes are preferred as cargo carriers. The lipid bilayer membrane of exosomes also provides protection against cargo degradation, making exosomes a desirable candidate for gene delivery. Despite the various strategies developed to facilitate the loading of the desired cargo, such as incubation, electroporation, sonication, extrusion, freeze-thaw cycles, and transfection, insufficient efficiency persists. Our main goal is to develop a highly efficient gene transfer method for exosomes isolated from healthy human blood plasma. In our study, the gene transfer capacities of exosomes isolated from human blood plasma were examined. First, an exosome isolation method from plasma was developed. The most efficient method for exosome isolation has been developed, and for this purpose, both the ultracentrifugation method without gradients and the sucrose gradient have been studied. Based on exosome characterizations with NTA and Western blot, it was determined that the best method was the 30% sucrose gradient. Then, the gene delivery capacity of exosomes as a cargo molecule was examined using FAM-labeled GAPDH-siRNA. In our study, the sonication and the extrusion methods were compared for the loading of genes into exosomes. When evaluating all parametric comparisons, the highest efficiency of gene loading capacity into exosomes was achieved using the sonication method. The gene loading efficiency of exosomes was also determined using FAM-labeled GAPDH-siRNA through fluorescence microscopy and flow cytometry. The cellular uptake of GAPDH-siRNA-loaded exosomes was evaluated based on time and concentration. Cellular uptake within 6-12-24 hours was evaluated. When all these results are evaluated, our study has developed a gene therapy agent to be used for gene therapy purposes, which will guide us in our future gene therapy studies against cancer. In this way, fundamental cases will be provided for more efficient and effective gene therapy through exosomes, which is one of today's most important and favored treatments. Research has been supported by the Erciyes University Scientific Research Projects (BAP) Grant No: TSAÜ-2024-13269.

#6588 Characterization of TGFb1-induced ApoEVs and their potential role in epithelial cell plasticity.

Daxime F. R. Genier, Sarai A. Gomez Perez, Alicia Viloria-Petit

Biomedical Sciences, University of Guelph, Guelph, ON, Canada

Extracellular vesicles (EVs) are membranous particles that carry cargo such as proteins, lipids, and nucleic acids that can be crucial for intercellular communication. Epithelial-mesenchymal transition (EMT) is implicated in tissue remodeling and has been documented in the mammary gland during involution. Transgenic models and clinical studies suggest that both post-lactational and age-related lobular involution may increase susceptibility for breast cancer development. Transforming growth factor-beta 1 (TGFβ1) induces apoptosis of epithelial cells during involution and is also a potent inducer of EMT. TGFβ-induced apoptosis generates a subpopulation of EVs called apoptotic cell-derived EVs (ApoEVs), a potential cell-cell communication avenue that may be crucial for tissue remodeling. Here, we optimized a protocol to generate, isolate, and analyze TGFβ-induced ApoEVs. This may reveal potential EV-mediated signaling in driving EMT, tissue remodeling and cancer susceptibility in the mammary gland.

The murine mammary gland epithelial cell line NMuMG was used, as they are known to both die and undergo EMT in response to TGFβ1 stimulation. NMuMG cells were cultured in DMEM with 10% FBS, 10 µg/mL insulin, and 1% antibiotics. Once 90% confluency was reached, growth media was replaced with starvation media consisting of DMEM, 5% EV-depleted FBS, and antibiotics. After 2.5 hours, one plate was treated with 5 ng/mL of TGFβ1 while the other received PBS. The conditioned media (CM) produced was centrifuged at 400g for 20 minutes to remove debris and then ultracentrifuged for 1 hour at 4ºC at a speed of 10,000g to generate a pellet of large EVs. This was followed by two PBS washes under the same ultracentrifugation conditions. Next, the CM was again ultracentrifuged at a speed of 100,000g to isolate smaller EVs. Both EV populations were resuspended in 10mM Hepes (pH 7.2) and characterized by western blot, dynamic light scattering (DLS), and transmission electron microscopy (TEM).

Western blots for markers of EVs and potential contaminants suggest successful EV isolation. Blotting for apoptotic markers presented results consistent with ApoEVs, which were enriched in the samples obtained by 10,000g ultracentrifugation. DLS analysis showed that EVs released by TGFβ-treated cells were larger and more abundant compared to those released by untreated cells; TEM analysis confirmed these results. Culturing NMuMG cells with either full CM or EV-depleted CM from either control or TGFβ1-treated cells showed morphological changes consistent with cell plasticity in response to EVs released by TGFβ1-treated cells.

Our findings suggest that EVs produced during TGFβ1-induced apoptosis of NMuMG cells contain cargo capable of modulating epithelial phenotype and may aid directly or indirectly in tissue remodeling. Additional biological studies on target cells are necessary to confirm our results.

#6589 Exosomal miR-155-5p from early myeloid-derived suppressor cells promotes breast cancer invasion and metastasis by regulating SIRT1.

Guidong Chen, Jinpu Yu

Tianjin Medical University Cancer Institute and Hospital, Tianjin, China

Early myeloid-derived suppressor cells (eMDSCs) are a heterogeneous group of myeloid cells and have a critical role in the progression of breast cancer. Therefore, exploring the mechanism of communication between eMDSCs and tumor cells is particularly important. In this study, the results showed that eMDSCs co-incubation improved the migration and invasion ability of breast cancer cells by up-regulating the expression of microRNA(miR)-155-5p. Further, we found that eMDSCS-derived exosomes transfer miR-155-5p to breast cancer cells and down-regulate sirtuin 1(SIRT1) expression to promote breast cancer progression. To overcome this challenge, we developed a nanodelivery platform targeting the tumor microenvironment to co-deliver the oligonucleotide inhibitor of miR-155, which demonstrated significant inhibition of tumor growth, migration and invasion in vivo experiments. Overall, our findings suggest that the eMDSCS-derived exosome miR-155-5p promotes invasion and metastasis of breast cancer by targeting SIRT1 and the construction of a tumor microenvironment responsive nanodelivery platform targeting eMDSCs exosome-derived miR-155-5p provides a promising strategy for overcoming breast cancer progression.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Genomic Profiling of Tumors 2
Poster Session

#6594 Constrained hypermutation and absence of TERT promoter mutations in Lynch syndrome-associated urothelial cancer.

Jussi Nikkola[1], **Lauri Ryyppo**[1], Juuso Vuorinen[1], Lauri Moilanen[2], Maarit Ahtiainen[2], Kirsi Pylvanainen[3], Hanna Selin[1], Tuomo Virtanen[1], Matti Nykter[1], Thea Veitonmaki[4], Jukka-Pekka Mecklin[3], Toni T. Seppala[1], Matti Annala[1]

[1]Tampere University, Tampere, Finland,[2]Hospital Nova of Central Finland, Jyvaskyla, Finland,[3]The Wellbeing Services County of Central Finland, Jyvaskyla, Finland,[4]Tampere University Hospital, Tampere, Finland

Lynch syndrome (LS) is a hereditary cancer syndrome characterized by a defective DNA mismatch repair (MMR) system and a high frequency of different cancers, including urothelial cancers (UC) of the upper urinary tract and bladder. We studied the somatic landscape of LS-associated UCs (LS-UC) by analysing 41 tumor samples and 3 urine DNA samples collected from 34 LS-UC patients. We demonstrate that mutations of the telomerase reverse transcriptase (*TERT*) promoter are present in 83% of sporadic UC and are scarcely present (5%) in LS-UC ($p < 0.00001$). As a substitute, all LS-UC patients possessed a 5-methylcytosine deamination (CG>TG) and microsatellite instability driven mutation landscape with highly frequent *ARID1A* (82%), *FGFR3* (80%), and *KMT2D* (78%) mutations. We suggest that the scarcity of *TERT* promoter mutations in LS-UC is due to an inability to create the required GABP binding motif (5'-GGAA) through CG>TG mutation or microsatellite instability. Our data indicates that LS-UC is a distinct disease entity characterized by unique genomic features.

#6595 Lineage markers and driver oncogenes coordinate through gene expression subtypes of lung adenocarcinoma.

Minjeong Kim[1], Wisut Lamlertthon[2], Heejoon Jo[1], Yan Cui[3], Hyo Young Choi[4], Matthew D. Wilkerson[5], Liza Makowski[1], D. Neil Hayes[6]

[1]Division of Hematology and Oncology, Department of Medicine, University of Tennessee Health Science Center, Memphis, TN,[2]Princess Srisavangavadhana College of Medicine, Chulabhorn Royal Academy, Bangkok, Thailand,[3]Department of Genetics, Genomics, and Informatics, University of Tennessee Health Science Center, Memphis, TN,[4]Department of Preventive Medicine, University of Tennessee Health Science Center, Memphis, TN,[5]Department of Anatomy, Physiology, and Genetics, Uniformed Services University, Bethesda, MD,[6]UTHSC Center for Cancer Research, University of Tennessee Health Science Center, Memphis, TN

Background: EGFR-directed therapy marked the beginning of personalized medicine in lung adenocarcinoma (LUAD), where a single gene could be effectively targeted by a specific class of drugs, leading to remarkable treatment responses. After additional targets have been identified, the search for other druggable mutations has slowed despite the need to improve clinical outcomes, underscoring the need for new approaches.

Hypothesis: We hypothesize that gene expression signatures will predict EGFR mutation status in patients and identify cases with similar signaling pathways but without canonical EGFR mutations. Deciphering the role of the EGFR signature in mutant (mt) and wild-type (WT) cases may provide potential therapeutic and classification strategies for understanding LUAD.

Experimental design: To define an EGFR mutation signature ("mSig") as a predictor of EGFR mutation status, we analyzed gene expression and clinical data from four lung adenocarcinoma cohorts. Microarray data from Memorial Sloan Kettering Cancer Center (MSKCC, n=192) served as the training set. For validation, we used combined microarray data from UNC (n=73) and TSP (n=41) as one dataset, and RNA-seq data from The Cancer Genome Atlas (TCGA, n=486) as another. Multiple model evaluation algorithms tested the performance of the predictor. Other key genetic mutations found in TCGA cohort were included in integrative analysis.

Results: Comparing EGFR-mt to EGFR WT tumors identified 1,020 differentially expressed genes in the training dataset as an EGFR mSig. Semi-supervised clustering visualized molecular profiles in both training and validation data. The EGFR mSig achieved an average negative predictive value (NPV) of 96.3% across datasets and was robust across multiple dataset combinations and six machine learning models (AUROC = 0.83-0.95). The EGFR mSig strongly correlated with LUAD subtypes (Bronchioid, Magnoid, Squamoid), particularly with Bronchioid [odds ratio (OR) = 9.2, $p < 0.001$]. EGFR mSig-positive EGFR-mt ("*EGFR-mt/mSig(+)*") tumors were primarily Bronchioid (OR = 4.5, $p < 0.001$). Almost 50% of mSig(+) Bronchioid tumors in fact lacked EGFR mutations, often showing EGFR amplifications or RAS mutations. Two lung lineage markers and driver mutations associated with LUAD subtypes and EGFR activation patterns. Interestingly, *EGFR-mt/mSig(-)* tumors were generally non-Bronchioid, suggesting alternative pathways (e.g., RAS, TP53). RAS/RAF/RTK-mt tumors (*EGFR WT* or *mSig(-)*) showed high dual specificity phosphatase 4 (*DUSP4*) expression, a known repressor of multiple key activated RAS genes. Therefore, *DUSP4* represents an attractive novel target in selected LUAD samples.

Conclusions: Our results define a novel EGFR mSig that identifies EGFR-mt-like tumors. Identifying patients with driver gene mutations lacking EGFR mutations suggests a new approach of identifying new druggable targets.

#6596 Tumor molecular landscape and therapy implications in young BRCA1/2 carriers with breast cancer.

Mwangala P. Akamandisa[1], Mingyi Xia[1], Wilson Cheah[2], Bradley Wubbenhorst[1], Kurt D'Andrea[1], Mengyao Fan[1], Jake Shilan[1], William J. Tapper[2], Ellen Copson[2], Ramsey I. Cutress[2], Diana M. Eccles[2], Susan M. Domchek[1], Katherine L. Nathanson[1]

[1]Perelman School of Med. Univ. of Pennsylvania, Philadelphia, PA,[2]University of Southampton, Southampton, United Kingdom

Women with *BRCA1/2* pathogenic variants (PVs) have a high likelihood of developing young onset breast cancer (BC) compared to noncarriers. In women enrolled in the POSH prospective cohort study of young women diagnosed with BC under age 40 or under age 50 if known *BRCA1/2* positive, no difference in overall survival was observed in *BRCA1/2* carriers vs noncarriers. Treatment options for BC include PARP inhibitors (PARPi) for *BRCA1/2* driven tumors and CDK4/6 inhibitors (CDK4/6i) plus endocrine therapy for ER-positive, HER2-negative tumors. In BC from *BRCA1/2* carriers unselected for age, up to 10% *BRCA1* and 46% *BRCA2* carriers do not have loss of heterozygosity (LOH) at the germline variant locus, suggesting non-*BRCA1/2* tumorigenesis. It is not known what the tumor molecular landscape, including rates of LOH and homologous recombination deficiency (HRD), is in young *BRCA1/2* PV carriers with BC, and whether the tumor molecular features impact disease outcomes or predict therapy response. To elucidate the molecular landscape of breast tumors in young women with *BRCA1/2* PVs, we evaluated treatment naïve primary breast tumors from 136 (86 [63.2%] *BRCA1*; 50 [36.8%] *BRCA2*) PV carriers in the POSH study. 71 (52.2%) of the tumors were ER-positive. We performed whole exome sequencing and called single nucleotide variants, indels, copy number variants, and *BRCA1/2* allele specific LOH in the tumors, calculated HRD scores and tumor mutational burden (TMB), and evaluated single base substitution (SBS) signatures. We found high rates of LOH by gene: *BRCA1* (93%) and *BRCA2* (96%), and by ER-status: ER-negative (94.4%) and ER-positive (93.8%). Mean HRD scores were significantly higher in tumors with LOH compared to nonLOH tumors in both *BRCA1* (57.4 vs 22.6, p<0.0001) and *BRCA2* (43.7 vs 23.5, p=0.005) carriers as well as ER-negative (57.6 vs 22.8, p<0.0001) and ER-positive tumors (46.4 vs 22.8, p<0.001). LOH tumors had higher proportional contribution of HRD-associated SBS3 than nonLOH tumors (0.36 vs 0.22, p=0.048). *BRCA1* LOH tumors had higher median TMB than *BRCA2* LOH tumors (4.8 vs 2.5, p=0.034). Overall survival in women with nonLOH tumors was 100% throughout the follow-up period but was not significantly different from that of women with LOH tumors. Overall survival did not differ by tumor HRD status: analyses were limited by small numbers in the non-LOH and low HRD groups. We found statistically significant enrichment of *AURKA* (OR:4.2, 95%CI:1.3-16.6, p=0.015) and *MYC* (OR:2.4, 95%CI:1.0-5.7, p=0.043) amplification which are associated with resistance to CDK4/6i in ER-positive, HER2-negative tumors from POSH cohort PV carriers compared to noncarriers from the TCGA. Given the high levels of LOH and presence of CDK4/6i resistance associated alterations, our data suggest that PARPi may be preferable over CDK4/6i in young *BRCA1/2* carriers with ER-positive, HER2-negative BC when both therapies are being considered in the adjuvant setting.

#6597 Optimization of DNA and RNA extraction from tumor FFFPE samples and end-to-end automation and analytical validation of the TSO 500 HT v1 library preparation.

Nripesh Prasad, Supriya Singh, Rebecca Beatty, Rachel Rock, Melanie Robinson, Annamaria Szanto, Sara Hildebrand, Boris Umylny, Thomas Halsey

Discovery Life Sciences, Madison, AL

Formalin-fixed paraffin-embedded (FFPE) techniques are cost-effective, long-term preservation methods for clinical samples, enabling next-generation sequencing (NGS) for expanded discovery and diagnostic applications. DNA and RNA extraction and library preparation must be optimized to ensure efficient downstream NGS analysis. We first focused on optimizing the quality and quantity of DNA and RNA recovery from FFPE samples representing various oncology conditions, using the truXTRAC® FFPE Tissue Extraction Kits (Covaris) automated on the Kingfisher Flex instrument (Thermo). We then analyzed the extracted DNA and RNA from FFPE tumor samples, including colorectal, kidney, breast, lung, gastric, and endometrial cancer, along with control samples from Horizon Oncospan and Seraseq, for analytical validation of the TSO 500HT v1 assay. RNA inputs were tested at 20ng, 40ng, and 80ng, while DNA inputs were tested at 20ng, 40ng, and 100ng. The TSO 500HT assay targets 523 genes to assess all DNA and RNA variant types, including SNV, CNV, indels, fusions, MSI, and TMB. For library preparation, 72-sample batches were processed using manual and fully automated workflows, with the automation protocol executed on the Biomek i7 (Beckman Coulter). Sequencing was performed on the Illumina NovaSeq 6000 and NovaSeq X Plus, using a 2x100bp paired-end configuration to generate approximately 50 million DNA reads and 8-10 million RNA reads per library. This approach demonstrated an overall inter-run and intra-run variant detection accuracy of over 90%, indicating highly repeatable and reproducible results. Variability between runs, lots, and operators was ≥99%. Our analysis showed high assay performance, with ≥96% of samples passing Illumina's cut-off metrics, particularly with 40ng DNA and 80ng RNA inputs. The analytical sensitivity for the TSO 500HT assay was benchmarked with ≥95% sensitivity for SNVs, ≥85% for InDels, ≥90% for CNVs, ≥90% for MSI, and ≥90% for TMB. These results provide a high-throughput, automated solution for dual DNA and RNA extraction from FFPE samples, demonstrating a robust, reproducible, and highly accurate targeted sequencing method for variant detection in oncology patients.

#6598 MET gene polymorphisms and breast cancer: A study of Nigerian patients.

Emeka Eze Joshua Iweala[1], Doris N. Amuji[2]

[1]Covenant University, Ota, Nigeria,[2]Department of Biochemistry, Covenant University, Ota, Nigeria

Breast cancer (BC) remains a major cause of cancer-related mortality among women worldwide. Genetic polymorphisms, such as those in the Mesenchymal Epithelial Transition (MET) proto-oncogene, may influence susceptibility and tumor progression as seen in gastric, liver, and thyroid cancer in Japanese and Chinese populations. This study evaluated the association between three MET gene single nucleotide polymorphisms (SNPs) namely rs40239, rs1621, and rs41736 and breast cancer risk and tumor grade among Nigerian women. A case-control study was conducted with 150 participants, comprising 75 breast cancer patients and 75 healthy controls. DNA was extracted from blood samples and genotyped using the TaqMan SNP assay. Genotype and allele frequencies for the SNPs were compared between the groups, and associations with BC risk were evaluated using the chi-square test and odds ratios (ORs). In addition, ordinal regression analysis was performed to assess the relationship between SNP (rs1621) and tumor grade. Genotyping revealed distinct distributions of genotype in both groups. For rs40239, the AA genotype was more frequent in controls (60%) than in cases (17%), though the association with BC risk was not statistically significant (OR=4.706, 95% CI: 0.959-23.096, p=0.062). No significant associations were observed for rs1621 (OR=1.188, 95% CI: 0.516-2.733, p=0.426) or rs41736 (OR=0.954, 95% CI: 0.904-1.006, p=0.262). The mutant allele rs1621 was associated with reduced odds of being in a higher tumor grade (OR=0.475), suggesting a potential protective effect against aggressive tumors. However, this association was not statistically significant (p=0.090, 95% CI: -1.606-0.116). While rs1621 showed a non-significant trend towards a protective role against higher tumor grades, no statistically significant associations were observed between the examined SNPs (rs40239, rs1621, and rs41736) and breast cancer risk. Future research should focus on expanding sample sizes, including diverse African populations, and exploring the role of genetic variants in breast cancer predisposition and progression.

#6599 A comprehensive benchmark study of multiple library amplification solutions for NGS.

Giulia Corbet[1], Caitlyn Mendik[1], Lee French[2], Leo Karamanof[2], Meghan Oddy[2], Andrew Sutherland[2], Philip Benson[1], Josh Haimes[1], Ross Wadsworth[2], Travis Sanders[1], Craig Marshall[1], Kristina Giorda[1], Thomas Harrison[1], Eduard Casas[1], Brian Kudlow[1]

[1]Watchmaker Genomics, Inc., Boulder, CO,[2]Watchmaker Genomics, Inc., Cape Town, South Africa

The emerging field of precision oncology utilizes next-generation sequencing (NGS) to guide decisions based on individual genetic signatures. As demand for these applications grows, minimizing errors and biases introduced during library preparation and sequencing is critical. Advances in sequencing chemistries have improved base quality scores, shifting the focus to library preparation to improve data quality. PCR amplification is a key potential source of error during NGS library preparation; single-base substitutions, insertions, and deletions (indels) introduced by PCR in addition to PCR-induced biases in DNA length and GC content can distort data leading to decreased assay sensitivity and specificity. Improved library amplification, combined with recent innovations in sequencer technology, will increase the reliability of NGS assays and lead to better assay performance.

Herein, we benchmark several commercial PCR amplification systems, including two solutions from Watchmaker Genomics: Equinox, and a re-engineered library amplification mix in development. We employed a UMI-powered NGS assay to quantify GC-uniformity, chimeric reads, base incorporation fidelity, and indel rates in microsatellite sequences. Libraries were sequenced on multiple platforms to leverage the ultra-high base quality scores of recent sequencing chemistries, resulting in low-noise measurements of polymerase fidelity. We also interrogate UMI family depth uniformity, a powerful proxy for template sequence bias and an informative indicator for error-correction efficacy. Furthermore, we assess amplification size bias by comparing the ability to amplify long templates in the presence of short templates. Compatibility with both SPRI and streptavidin beads was assessed. Finally, we test the ability to generate high terminal library yield, which is important for hybridization capture workflows.

These results indicate that while some products may excel in one or two metrics, they dramatically underperform in others. Equinox, in particular the newly engineered variant, demonstrates robust performance across all relevant metrics, making it a leading solution for precise and unbiased library amplification.

#6600 Dissecting genomic features of BCG unresponsive non-muscle-invasive bladder cancer identifies drivers of sensitivity to intravesical gemcitabine/docetaxel.

Matias Vergara[1], Kendrick Yim[2], Kevin R. Melnick[3], Brendan Reardon[1], Timothy N. Clinton[2], Matthew Mossanen[2], Graeme S. Steele[2], Michelle S. Hirsch[4], Natalie M. Rizzo[4], Kent W. Mouw[5], Matthew F. Wszolek[6], Keyan Salari[6], Adam S. Feldman[6], Adam S. Kibel[2], Eliezer M. Van Allen[1], Mark A. Preston[2], Filipe LF. Carvalho[2]

[1]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA,[2]Department of Urology, Brigham and Women's Hospital, Boston, MA,[3]Department of Urology, Brigham and Women's Hospital, Boston, MA,[4]Department of Pathology, Brigham and Women's Hospital, Boston, MA,[5]Department of Radiation Oncology, Dana-Farber Cancer Institute, Boston, MA,[6]Department of Urology, Massachusetts General Hospital, Boston, MA

Introduction: Intravesical Gemcitabine/Docetaxel (GemDoce) is an effective therapy in patients with Bacillus Calmette-Guérin (BCG) unresponsive non-muscle-invasive bladder cancer (NMIBC) with 50% response at 2 years. However, the genomic landscape of BCG-unresponsive NMIBC and whether distinct mutations are associated with response and resistance to sequential intravesical GemDoce is unknown. Our goal is to define mutational features of BCG-unresponsive NMIBC and nominate genomic drivers of response and resistance to GemDoce.
Methods: We identified patients with BCG unresponsive NMIBC who subsequently received intravesical Gem/Doce. We performed whole-exome sequencing of BCG unresponsive bladder tumors (total tumors n=23; Tumor-normal pairs n=3; Tumor-only n=20) prior to GemDoce administration and classified patients in “responders” (recurrence free survival (RFS) > 18 mo) and “non-responders” (RFS < 18 mo). The Broad Institute’s CGA pipeline was used to account for mutation calls, ABSOLUTE was used to define copy number alterations (CNA) and PhylogicNDT was applied to model phylogenetic and evolutionary tumor trajectories.
Results: Median follow-up was 45 months. Median time to HG-recurrence and cystectomy were longer in the responder group (33 mo, 28 mo) in comparison to the non-responder group (3.5 mo, 8 mo). We identified alterations in known bladder cancer genes including *TP53* (10/20 tumors), *KDM6A* (9/20 tumors) and *FGFR3* (5/20 tumors) in both responders and non-responders. Most alterations in *TP53* and *KDM6A* were predicted loss-of-function mutations, consistent with the known tumor suppressor roles for these genes. Mutations in two genes that control cell division and chromosomal stability, *CREBBP* and *STAG2*, were exclusively present in responders to GemDoce. Both responders and non-responders harbor CNA in *CDKN2A* that characterize NMIBC. However, *FGFR3*, *E2F2* and *MDM2* amplifications were exclusively present in non-responders. Phylogenic analysis showed BRCA1 clonal mutations and subclonal BRCA2 mutations only in responders.
Conclusions: BCG unresponsive NMIBC harbor mutational features that biologically explain response to GemDoce. Mutations in *CREBBP* and *STAG2,* and clonal BRCA1 mutations may sensitize tumors to GemDoce resulting in long-term responses to intravesical chemotherapy. Future efforts should focus on determining the presence of *CREBBP* and *STAG2* using immunohistochemistry prior to GemDoce to improve intravesical treatment response in NMIBC.

#6602 Spatial transcriptomic profiling of the mouse distal colon during early carcinogenesis reveals transitional cell states that precede cancer.

Yujin Lee, James C. Fleet

Nutritional Sciences, The University of Texas At Austin, Austin, TX

Early onset colorectal cancer (EOCRC) is increasing yet the mechanism for the accelerated shift to a younger age is unknown. We hypothesize that sporadic gene mutations that are necessary for colon carcinogenesis but not sufficient to cause cancer, create a preneoplastic cell state for increased risk of transformation. To identify transitional cell phenotypes, we used Spatial Transcriptomics (ST) on distal colon samples from Car1-Cre (CAC) mice with Cre expression limited to ~6% of the distal colon/rectum epithelium, where EOCRC is more prevalent. CAC;ROSA$^{mT/mG}$ (CR) mice (where eGFP marks cells expressing Cre) were crossed to those with floxed oncogene alleles to generate mice with one (Apc$^{+/-}$, ACR) or two (Apc$^{+/-}$;Kras$^{G12D/wt}$, AKCR) non-transforming mutations. ACR mice had normal crypt morphology and low adenoma incidence (<5% of mice), while 4-week-old AKCR mice exhibited crypt elongation, and 8-10 wk-old AKCR developed 3-8 advanced adenomas/mouse. Distal colon Swiss rolls (n=9) and tumors (n=3) with phenotypes ranging from normal to malignant were collected and processed using the 10X Visium Spatial Gene Expression System. Sequencing data was processed, and sample data were pooled for analysis using Seurat. Spatial counts were normalized, dimensionality was reduced with PCA, then clustered and visualized as UMAP projections. Clusters were annotated to known cell types, and differential gene expression (DEG) analysis was followed by functional annotation using GO and Pathway terms. In normal tissue, clusters for the crypt base, middle, and top were identified. These spatially restricted clusters were replaced in early AKCR crypts and again in late AKCR crypts. The clusters from late AKCR crypts were distinct from those in ACR tumors, which were different from clusters in AKCR tumors. This indicates the existence of transition states from normal to “not normal, not cancer” to early adenoma to late adenoma. Normal epithelial clusters were enriched for genes controlling typical colonic functions, e.g. metabolism, and transmembrane transport pathways. AKCR crypt clusters lost these functions and gained others, e.g. immune and antimicrobial genes normally associated with Paneth cells (e.g., Defa22, Defa24, Defa30), gene markers associated with CRC tumors (e.g. Egfr, Hamp, Plet1, Wfdc18), genes for xenobiotic inactivation (Maoa, various Cyp, Gst, Ugt, and Sult genes). Compared to late AKCR crypts, AKCR tumor clusters lost even more colonic functions (e.g. downregulation of differentiation marker Krt20 and xenobiotic metabolism markers) and expressed genes related to mitosis, DNA replication, and DNA repair, highlighting a transition to uncontrolled proliferation and genomic instability. Our findings delineate key early molecular transitions in epithelial cells during colon carcinogenesis that may increase the risk of transformation.

#6603 Analytical validation of a new plasma-only bioinformatics classifier to identify variants from clonal hematopoiesis (CH) in cfDNA liquid biopsy assays.

Patrick C. Fiaux, Mingyang Cai, Pegah Safabakhsh, Jeffrey L. Werbin, Che-Yu Lee, Katie Quinn, Marisa Juntilla, Tingting Jiang, Reagan M. Barnett, Errin Lagow, Arielle Yablonovitch, Shile Zhang, Martina Lefterova, Darya Chudova

Guardant Health, San Francisco, CA

Introduction: Distinguishing between tumor derived variants and those from clonal hematopoiesis (CH, or CHIP) can impact clinical decisions. It is essential to develop tools that can accurately differentiate tumor vs CH in clinical tests with expanded gene panels. One method to confirm CH involves sequencing both the buffy coat and plasma samples. However, this approach is cost-prohibitive and has two key limitations: detection sensitivity of buffy coat sequencing for low allele fractions, and the possibility that variants originate from tissues other than the immune cells present in the buffy coat. To address this, we developed a plasma only CH classifier to detect CH variants with high specificity in the Guardant360 test for late-stage cancer patients.
Methods: First, limitations of the buffy coat sequencing were explored by sequencing over 60 healthy donor plasma and buffy coat samples. Second, data with more than 2000 variants observed in over 200 clinical samples across more than 15 cancer types (median age of 69) with paired buffy coats and plasma were utilized to train a boosted tree classifier. The final model was built using 14 features which included variant-level metrics derived from fragmentomics, methylation patterns, and variant allele frequencies, as well as metadata features aggregated from over 250,000 Guardant Health samples and public databases such as COSMIC and GnomAD. The classifier was trained to predict CH variants with high specificity to ensure that informative tumor-derived variants are accurately reported.
Results: In 62 healthy donors, we observed that 18% of variants detected in plasma at low allele frequency were not detected in buffy coat, highlighting the need for bioinformatic solutions to capture CH variants. Our bioinformatic approach was validated on an independent cohort of 173 Guardant360 late-stage, pan-cancer samples, each with paired buffy coat and plasma samples. In a set of clinically actionable genes, consisting of National Comprehensive Cancer Network (NCCN) recommended genes for biomarker testing and canonical CH-associated genes, the classifier successfully detected CH variants with a specificity of 98% and a positive predictive value (PPV) of 99%.
Conclusion: We present here a plasma only CH classifier developed for late-stage cancer patients run on Guardant360 powered by Infinity. The performance of the classifier to detect non-tumor variants in the Guardant360 test achieves a specificity exceeding 98% in clinically relevant genes, with a PPV of 99% in detecting CH variants.

#6604 Characterizing the tumor microenvironment using spatially barcoded archival FFPE tissue: converting single-nucleus RNA-seq into spatial transcriptomics.

Cedric R. Uytingco, Julie Wilhelmy, Bryant Ngo, Jonathan Sakkos, Wanxin Wang, Christina Chang, Christina Fan

Assay Development, Curio Bioscience, Inc., Palo Alto, CA

Identifying the contribution and the role of each individual cell in the spatial context of the tumor microenvironment (TME) is crucial in determining tumor progression, examining the extent of immune infiltration, and identifying potential therapeutic targets. While single-cell sequencing-based techniques help reveal cellular heterogeneity in a cell population, they do not provide spatial context. In turn, recently emerged spatial transcriptomics techniques are challenged by the difficulty in pinpointing the spatial measurement to single cells, requiring either complex cell segmentation or deconvolution algorithms in the data processing.

To address these limitations, we introduce Curio Trekker single-cell spatial mapping kit, a unique platform that turns a single-cell experiment into a spatial experiment, essentially providing a spatial measurement that has true single-cell resolution. It is based on the Slide-tags methodology (Russel et al, 2023) that spatially tags each nucleus within its native tissue environment. This approach utilizes existing single-nuclei sequencing infrastructure to generate high-resolution spatial transcriptomics data. Using archived FFPE samples, we demonstrate the effectiveness of Curio Trekker by aligning 25-30 µm tissue sections to spatially barcoded tiles. Spatial barcodes are cleaved and hybridized to cells and nuclei in the intact tissue, followed by tissue dissociation and nuclei isolation. The tagged nuclei are processed with established snRNA-seq workflows (e.g., 10x Chromium FLEX), producing gene expression and spatial libraries for the individual nuclei in the tissue section. This spatial tagging allows each nucleus to be bioinformatically mapped back to its original tissue location.

We applied this solution to breast cancer biospecimens from different patients using whole transcriptome probes (10x Genomics). The resulting data allowed for detailed characterization of the TME, confidently identifying epithelial-malignant, stromal, and immune cells from each biospecimen. This enabled marker-guided regional selection and differential gene expression analysis based on both cell type and spatial location. Overall, the Curio Trekker platform provides a streamlined, high-resolution solution for profiling cellular heterogeneity and interactions within tumors, seamlessly integrating with existing workflows to generate rapid insights into disease mechanisms and therapeutic development.

#6605 Spatially-resolved single-cell transcriptome landscape of response and resistance to CDK4/6 inhibitors in patients with hormone receptor-positive metastatic breast cancer.

Junko Tsuji[1], Jorge Gomez Tejeda Zanudo[2], Timothy R. Blosser[3], Ha T. Vu[4], Danielle Firer[5], Nick Maus[1], Kathleen L. Pfaff[6], Billie A. Porter[6], Jason L. Weirather[6], Anne Carlisle[6], Allison M. Frangieh[7], Elliot Boblitt[8], Aaron R. Thorner[7], Karla E. Helvie[7], Isabella E. Kallassy[7], Laura K. DelloStritto[7], Ragnhild Laursen[4], Francois Aguet[9], Mendy Miller[1], Melissa E. Hughes[2], Nancy U. Lin[2], Sara M. Tolaney[2], Eliezer M. Van Allen[2], Samouil L. Farhi[10], Xiaowei Zhuang[3], Barbara Engelhardt[4], Scott J. Rodig[6], Alex K. Shalek[8], Aviv Regev[11], Bruce E. Johnson[7], Nikhil Wagle[11], Gad Getz[1], Daniel L. Abravanel[2]

[1]Cancer Program, Broad Institute, Cambridge, MA,[2]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA,[3]Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA,[4]Gladstone Institutes, San Francisco, CA,[5]Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA,[6]Center for Immuno-Oncology, Dana-Farber Cancer Institute, Boston, MA,[7]Center for Cancer Genomics, Dana-Farber Cancer Institute, Boston, MA,[8]Institute for Medical Engineering and Science, Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA,[9]PreDICTA Biosciences, Cambridge, MA,[10]Spatial Technology Platform, Broad Institute, Cambridge, MA,[11]Genentech, Inc., San Francisco, CA

Cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) have significantly improved the treatment of hormone receptor (HR+) positive breast cancer. Clinical trials have demonstrated that addition of CDK4/6i to endocrine therapy (ET) improves both progression-free and overall survival in patients with HR+ metastatic breast cancer (MBC). Nevertheless, their impact remains limited by the eventual development of resistance. While a number of resistance mechanisms have been identified using both preclinical model systems and translational interrogation of patient specimens, these are not apparent in all cases. As part of the Human Tumor Atlas Network, we integrated genomic (whole-exome sequencing; n=47), single-nucleus transcriptomic (snRNA-seq; n=56), and spatial expression profiling (MERFISH and multiplex immunofluorescence; n=22 and n=17, respectively) from 58 clinically annotated MBC biopsy specimens collected before or after treatment with CDK4/6i plus ET to dissect the roles of tumor-intrinsic programs and the tumor microenvironment (TME) in CDK4/6i resistance.

Transcriptomic analysis of the tumor cell compartment with snRNA-seq not only extended prior findings including an association between expression of cell-cycle programs and CDK4/6i resistance, but importantly identified additional programs associated with response and resistance including ciliogenesis, immune evasion, and inflammation. Additionally, tumor-intrinsic gene programs related to epithelial-to-mesenchymal transition had a decreased activity in *ESR1* mutant biopsies, a mutational feature of CDK4/6i-resistant MBC associated with upregulated estrogen receptor pathway activity. In the TME, CD8+ T cells were depleted in the CDK4/6i-resistant biopsies, both before and after treatment. In contrast, the CDK4/6i-sensitive biopsies displayed pro-inflammatory features including an increased number of CXCL9+/CXCL10+ macrophages and clustering of CD8+ T cells in the vicinity of the malignant cells spatially. Notably, MERFISH also demonstrated an association between spatial architecture and CDK4/6i response, with the malignant cells in the CDK4/6i-resistant biopsies dispersed spatially compared to the sensitive biopsies. In summary, to the best of our knowledge, this study represents the largest spatially-resolved single-cell MBC cohort with curated CDK4/6i response status established to date. Our findings offer insights into potential biomarkers for CDK4/6i response based on tumor-intrinsic and tumor-microenvironment mechanisms, as well as therapeutic opportunities.

#6606 Genomic features of histological and molecular subtypes in Japanese ovarian cancer.

Osamu Gotoh, Norio Tanaka, Tomoko Kaneyasu, Seiichi Mori, Tetsuo Noda

Japanese Foundation for Cancer Research, Tokyo, Japan

Ovarian cancer is an intractable disease and comprises a number of histological subtypes that are associated with clinical characteristics such as therapeutic efficacy and likelihood of relapse. Since ovarian cancer exhibits heterogeneity even within a same histological subtype, it is critical to develop a complementary classification framework, in which clinicopathological and biological features are associated with each subtype. Previously, for endometrial cancer, a molecular subtyping scheme based on genomic features was developed by The Cancer Genome Atlas (TCGA) and the scheme has been currently integrated into the system of 2020 WHO classification of Female Genital Tract tumor. A similar classification scheme has been needed developed for ovarian cancer diagnosis for further clinical stratification. Whereas genomic features can be utilized for this purpose because of the tight relationship with clinical manifestations, previous large-scale genomic studies of ovarian cancer have been conducted mainly with high-grade serous (HGS) histology, a major histological subtype in Caucasian. The remaining histologies exemplified by clear cell carcinoma, which is uniquely prevalent in the East Asian population, have not been covered by these studies, and therefore genomic features that can be utilized for molecular subtyping have not been well characterized for non-HGS ovarian cancer. To link genomic features with histological subtypes and to obtain wealthy information for further potential genomic stratification, we conducted genome sequencing analyses of 1,412 Japanese ovarian cancers across 6 major histotypes (clear cell, high-grade serous, endometrioid, mucinous, carcinosarcoma and sex cord-stromal). Using copy number information derived from sequenced data, we identified a novel copy number subtype, which is uniquely associated with clinicopathological and genomic attributes. The results will be presented focusing on the genomic features including germline mutations, somatic driver events and mutational signatures, in association with histological and molecular subtypes.

#6607 Leveraging somatic mutations to distinguish tumor/normal cells in scRNA-seq datasets across different cancer types.

Cindy Kim[1], Eke Snoeren[1], Francesc Muyas[2], **Natasha Markuzon**[3]

[1]Imperial College, London, United Kingdom,[2]AstraZeneca Oncology, Barcelona, Spain,[3]AstraZeneca Oncology, Waltham, MA

Recent advancements in single-cell RNA sequencing (scRNA-seq) have significantly impacted clinical oncology. However, the accurate interpretation of scRNA-seq data remains challenging due to the limitations of current methodologies in distinguishing between different cell types within a tissue sample. Despite substantial efforts to construct comprehensive cell atlases, differentiating malignant from non-malignant cells is still a time-consuming process. Current approaches primarily rely on marker genes, gene expression signatures, and copy number alterations (CNAs), though these methods are not universally applicable to all cancer types. In our study, we explored the potential of leveraging somatic mutations to differentiate tumor and normal cells within scRNA-seq datasets from ovarian and colorectal cancer samples. We conducted a comparative analysis of the effectiveness of CNAs and single nucleotide variants (SNVs), as well as developed a supervised machine learning (ML) method leveraging their combination for improved prediction. The findings highlighted the importance of understanding the biological mechanisms underlying mutational processes for accurate tumor cell detection. Specifically, tumor cells in ovarian cancer samples exhibited a higher prevalence of CNAs, whereas colorectal cancer samples, particularly those with mismatch repair deficiency (MMRd), showed limited utility of CNAs for predicting tumor/normal cell status. In contrast, SNVs proved to be extremely valuable in identifying highly mutated tumor cells in colorectal cancer, with tumor mutational burden (TMB) serving as one of the highly effective predictors. In conclusion, our study utilizes a previously developed framework for detecting somatic mutations in scRNA-seq data for the robust and effective identification of tumor cells and their clusters in tissue samples. This framework is cancer-type agnostic and could help improve tumor cell detection in cancers with a high prevalence of CNAs.

#6608 Integrating HCMI models and tumors with CCLE and TCGA: Advancing cancer modeling and precision oncology.

Dina ElHarouni*[1], Mushriq Al-Jazrawe*[2], Seongmin Choi*[3], **Merve Dede***[4], Toshinori Hinoue*[5], Sean A. Misek*[6], Heeju Noh*[7], Luca Zanella*[7], Moony Tseng[8], Hayley E. Francies[9], Priya Sridevi[10], Rachana Agarwal[11], Cindy W. Kyi[12], Julyann Perez-Mayoral[12], Megan J. Stine[13], Eva Tonsing-Carter[12], James M. Clinton[14], The HCMI Network, Peter W. Laird[5], Calvin J. Kuo[15], Olivier Elemento[16], David L. Spector[10], Andrew D. Cherniack[17], Kyle Ellrott[18], Martin L. Ferguson[19], Rameen Beroukhim[20], Katherine A. Hoadley[21], Nicolas Robine[22], Andrew McPherson[23], Mathew J. Garnett[9], David A. Tuveson*[10], Andrea Califano*[24], Paul T. Spellman*[25], Keith L. Ligon*[26], Daniela S. Gerhard*[12], Louis M. Staudt*[12], Jesse S. Boehm*[27]

[1]Dana Farber Cancer Institute, Broad Institute of MIT and Harvard, Boston, MA,[2]Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Broad Institute of MIT and Harvard, Cambridge, MA,[3]Memorial Sloan Kettering Cancer Center, New York, NY,[4]UT MD Anderson Cancer Center, Houston, TX,[5]Department of Epigenetics, Van Andel Institute, Grand Rapids, MI,[6]Broad Institute, Dana Farber Cancer Institute, Harvard Medical School, Cambridge, MA,[7]Department of Systems Biology, Vagelos College of Physicians and Surgeons, Columbia University Irving Medical Center, New York, NY,[8]Broad Institute of MIT and Harvard, Cambridge, MA,[9]Wellcome Sanger Institute, Cambridge, United Kingdom,[10]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY,[11]Frederick National Laboratory for Cancer Research, Frederick, MD,[12]Center for Cancer Genomics, NCI, Bethesda, MD,[13]Information Management Services, Inc, Rockville, MD,[14]American Type Culture Collection, Manassas, VA,[15]Department of Medicine, Division of Hematology, Stanford University School of Medicine, Stanford, CA,[16]Institute for Computational Biomedicine, Weill Cornell Medicine, New York, NY,[17]Department of Medical Oncology, Department of Genetics, Broad Institute, Dana Farber Cancer Institute, Harvard Medical School, Cambridge, MA,[18]Department of Biomedical Engineering, Oregon Health & Science University, Portland, OR,[19]Center for Cancer Genomics, National Cancer Institute, Bethesda, MD,[20]Departments of Cancer Biology and Medical Oncology, Department of Medicine, Broad Institute, Dana Farber Cancer Institute, Harvard Medical School, Cambridge, MA,[21]Department of Genetics, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC,[22]New York Genome Center, New York, NY,[23]Computational Oncology, Department of Epidemiology and Biostatistics, The Halvorsen Center for Compu, Memorial Sloan Kettering Cancer Center, New York, NY,[24]Department of Systems Biology, Herbert Irving Comprehensive Cancer Center, Columbia University, Chan Zuckerberg Biohub, New York, NY,[25]Departments of Medicine and Human Genetics, University of California Los Angeles, Los Angeles, CA,[26]Department of Pathology, Dana Farber Cancer Institute, Boston, MA,[27]Koch Institute for Integrative Cancer Research at MIT, Broad Institute of MIT and Harvard, Cambridge, MD

The Human Cancer Models Initiative (HCMI) has developed 665 novel cancer models, including organoids and matched parental tumors, providing a significant addition to existing cancer model resources. To evaluate the transcriptional relatedness of HCMI models and tumors to the Cancer Cell Line Encyclopedia (CCLE) and The Cancer Genome Atlas (TCGA), we used the Celligner algorithm to align transcriptomic profiles across datasets, removing systematic biases while preserving intrinsic biological variability.

HCMI models and tumors both exhibited high transcriptional fidelity to TCGA tumors, clustering closely with their respective tumor types in the Celligner-aligned space. Pairwise Euclidean distances showed complementary strengths between HCMI and CCLE models. For example, HCMI models demonstrated significantly closer alignment to TCGA tumors in glioblastoma, breast cancer, and ovarian cancer, while CCLE models performed comparably or better in colorectal cancer. Notably, aligning HCMI tumors to TCGA confirmed their strong transcriptional relatedness, validating the fidelity of these models to their original tumor states. Combining HCMI and CCLE datasets further enhanced the total transcriptional representation across the diversity of TCGA tumors, underscoring the complementary roles of these resources.

The HCMI collection uniquely includes rare cancer types such as nephroblastoma, desmoid tumors, and ampulla of Vater carcinoma, which are absent in CCLE. Celligner analysis confirmed that these rare HCMI models faithfully retained transcriptional features of their corresponding TCGA tumors. This expands opportunities to study rare and clinically challenging cancers that have been underrepresented in preclinical models.

These findings demonstrate the value of integrating HCMI, CCLE, and TCGA datasets. Together, they form a complementary and robust compendium for studying tumor biology, enabling improved cancer modeling and advancing precision oncology.

#6610 Detection of homologous recombination deficiency in targeted long read sequenced tumors using mutational signatures.

Alvin Wei Tian Ng[1], Sheryl Li Lynn Wong[1], Siao Ting Chong[1], Jieru Ye[2], Joanne Ngeow[1]

[1]Lee Kong Chian School of Medicine, Nanyang Technological University Singapore, Singapore, Singapore,[2]National Cancer Centre, Singapore, Singapore

Background: Homologous recombination deficiency (HRD) affects cancer types including breast, ovarian, pancreas and prostate and patients with HR deficient tumors can respond to poly ADP-ribose polymerase (PARP) inhibitors and platinum-based therapies. HRD is associated to the loss of either germline or somatic, *BRCA1* or *BRCA2* and other associated variants. However, a large proportion of tumors do not have an identifiable variant in *BRCA1* or *BRCA2* and other related genes. Patterns of mutations—Mutational Signatures—of HRD can identify changes in DNA sequences due to defective DNA repair and genomic instability in HRD tumors. Current computational tools to detect HRD are based on whole genome sequencing (WGS) and an assessment of robustness of these approaches for clinical implementation are lacking.

Methods: We developed mSigDetect to identify HRD associated mutational signature (SBS3) in breast cancers, sequenced on Oxford Nanopore Technologies (ONT) targeted adaptive sampling technology. We built our mutational signatures classifier based on mutational profiles of 567 breast cancers from the International Cancer Genome Consortium (ICGC) and tested mSigDetect and the targeted panel on synthetic tumors (n=200) and two independent cohorts of breast cancer patients sequenced on Illumina short reads (n=224) and on ONT long reads (n = 38).Using the simulated synthetic tumours, we determined the sensitivity and specificity of our tool. We tested the performance of mSigDetect under different scenarios of tumour cellularity by down sampling and mixing HR deficient cell lines with paired normal lines in a range of 30-90% tumour cellularities. Last, we developed a computational workflow for ONT sequencing, to carry out real-time variant calling and mutational signature identification to reduce the turnaround time of the diagnostic test.

Results: With a target region of 5% of the human genome sequenced, mSigDetect could identify HR deficient tumors in both synthetic (sensitivity = 0.73, specificity = 1) and breast cancer patient tumors (sensitivity = 0.81, specificity = 0.99) compared to WGS. We designed mSigDetect to detect SBS3 in a tumor-only setting, without the need of a paired normal sample. Signature detection of SBS3 using mSigDetect greatly outperformed sequencing whole exome targeted regions. Based on cell line mixing simulations, we recommend sequencing at 45x for 30% tumor purity or higher, or 30X for 50% purity. ONT adaptive sampling identifies HR deficient tumors robustly in a clinical setting, based on targeted sequencing of a tumor-only sample.

#6611 Genome-wide cfDNA fragmentomics combined with machine learning enhance non-invasive prostate cancer detection.

Shuang Gan, Haoran Tang, Feng Xie, Yue Zhang, **Hang Dong**

Huidu (Shanghai) Medical Sciences, Ltd., Shanghai, China

The liquid biopsy approach focusing on DNA molecules offers a promising non-invasive method for prostate cancer diagnosis. Next-generation sequencing (NGS) of targeted gene panels is widely used for detecting somatic mutations but is limited by confounding clonal hematopoietic variants and narrow genome coverage. We propose a solution that encompasses genome-wide DNA fragmentation features for tumor fragment evaluation. This study collected plasma samples from 209 patients with prostate cancer and 49 healthy individuals as controls. Cell-free DNA (cfDNA) was extracted and tested using PredicineSCORE™, a low-coverage whole-genome sequencing assay designed to identify tumor-specific features. Tumor content assessed by mutation-based detection assays served as the reference indicator. Fragmentomics characteristics—including regional coverage, fragment length, and end-motif features—were profiled as tumor markers. Copy number burden and tumor fragment content were refined using the expectation-maximization algorithm. An ensemble machine learning model was then developed to estimate tumor fractions. We found strong correlations between estimated tumor fraction and fragmentomics features. The absolute correlation coefficients for tumor fraction with the relative depth at LYL1 binding sites, the insert size ratio of short reads, and the CCCA end motif were higher than 0.9, 0.75, and 0.7, respectively. To validate these findings, a total of 500 simulated samples with tumor content ranging from 0.1% to 50% were generated by mimicking tumor and normal reads. The ensemble model outperformed single-method approaches, with the XGBoost algorithm achieving high predictive performance (Pearson correlation $r > 0.9$, $p < 0.01$) for predicting tumor fraction in cfDNA. The model demonstrated a limit of detection (LoD) at 2%, surpassing previously proposed models in sensitivity. This study highlights the potential of plasma cfDNA fragmentomics profiling as a highly precise and non-invasive method for quantifying and detecting prostate cancer. The findings support its utility in advancing liquid biopsy applications with improved sensitivity and accuracy.

#6612 Deep and scalable spatial transcriptomics for oncology research.

Joseph W. Foley[1], Wei K. Teo[2], Joshua K. Tay[2]

[1]Picopoint Genomics, Singapore, Singapore,[2]Otolaryngology - Head & Neck Surgery, National University of Singapore, Singapore, Singapore

The emerging field of spatial transcriptomics is revolutionizing histopathology by simultaneously quantifying numerous RNA transcripts *in situ* across a tissue section. However, current technologies provide a broad survey of the spatial environment at the expense of low-complexity genomic data. We present the Picoception RNA platform, which produces deep genomic data from spatially targeted regions of a tissue section. This platform combines laser-capture microdissection (LCM) with comprehensive mRNA-sequencing, which reads all polyadenylated transcripts including pathogens and model organisms. From a single <10 µm section of formalin-fixed paraffin-embedded (FFPE) tissue, Picoception RNA can profile an unlimited number of target regions smaller than 0.1 mm^2, typically reading 200,000-1,000,000 unique molecules each with a dynamic range of more than 100:1.

By isolating small areas of a tissue section, Picoception RNA allows deep molecular profiling of pure, distinct cellular regions such as tumor epithelial vs. normal or immune microenvironment. Combined with the depth of the gene-expression data, the cost-effectiveness of this technique enables statistically powerful analyses from large clinical cohorts. Furthermore, Picoception RNA's compatibility with archived FFPE samples unlocks genomic signals from patients with known clinical outcomes, allowing the process of biomarker and pathway discovery to begin immediately without prospectively recruiting new subjects.

We have applied this technique successfully in a wide range of applications, including oncology clinical trials for targeted therapy and immunotherapy, rare cancers, and cancers with limited biopsy material (including punch biopsies and core-needle biopsies), as well as rodent models. We have had consistent success profiling FFPE samples more than 20 years old.

Picoception RNA complements previous spatial transcriptomics techniques, filling the need for deep genomic analysis at microscopic resolution using large-scale cohorts of FFPE samples.

#6613 Triple-negative breast cancer evolution and therapy response: Breast TRACERx.

Ieva Usaite[1], Thomas B. K. Watkins[2], Olivia Lucas[1], Carlos Marinez-Ruiz[1], Francois Legrand[3], Constance Lamy[4], Marta Jimenez[3], Nusaibah Ibrahimi[5], Stephan Michiels[6], Simone Zaccaria[1], Thibault de la Motte Rouge[7], Anthony Goncalves[8], Jean-Yves Pierga[4], Monica Arnedos[3], Christophe Le Tourneau[4], Fabrice Andre[9], Mariam Jamal-Hanjani[1], Nnennaya Kanu[1], Charles Swanton[10]

[1]University College London (UCL) Cancer Institute, London, United Kingdom,[2]Cancer Research UK Lung Cancer Cenrtre of Excellence, University College London (UCL) Cancer Institute, London, United Kingdom,[3]Unicancer, Paris, France,[4]Institut Curie, Paris, France,[5]Office of Biostatistics and Epidemiology, Gustave Roussy, Villejuif, France,[6]Office of Biostatistics and Epidemiology, Gustave Roussy, Paris, France,[7]Medical oncology department, Centre Eugene Marquis, Rennes, France,[8]Medical oncology department, Institut Paoli Calmettes, Maeseille, France,[9]Institut de Cancerologie, Gustave Roussy, Villejuif, France,[10]Cancer Evolution and Genome Instability Laboratory, The Francis Crick Institute, London, United Kingdom

Introduction: Breast cancer is the most commonly diagnosed cancer and the main cause of cancer-related mortality in women worldwide. Despite advances in our knowledge of the underlying genetic alterations and improvements in treatment, 25-30% of patients with early breast cancers still relapse with distant metastatic disease. Presenting a clear unmet clinical need to improve our understanding of breast cancer evolution, and the drivers of genomic instability in primary breast cancers.
Methodology: We have analyzed multi-region sequencing data from 280 treatment-naïve primary breast cancer tumor regions sampled from 139 patients with triple-negative breast cancer (TNBC) prospectively enrolled into the Breast TRACERx and SCANDARE studies. Here we aim to decipher breast cancer evolution and address the primary study endpoints: determining the relationship between intratumor heterogeneity and clinical outcomes.
Results: In TNBC, 74% of tumors demonstrated a whole-genome doubling event (WGD), whereby 77% of these events were clonal, suggesting that whole-genome duplication is primarily an early event in TNBC evolution. Additionally, 27% of these events where subclonal, and 8% of cases harboured a clonal followed by a second subclonal WGD event. Defects in homologous recombination repair (HRD) occur early in TNBC evolution, which may be driven by the truncal selection of *BRCA1* mutations. Furthermore, HRD levels are significantly higher in BRCA-deficient TNBC tumours, here 17% of TNBC tumours demonstrated either a germline or tumour driver mutation in BRCA in addition to an LOH event in the wild-type allele. BRCA-wild-type tumours were significantly enriched for clonal WGD events, here suggesting increased chromosomal instability early in evolution within these tumours. BRCA-wild-type tumours demonstrated selection for mutation in tumour suppressor genes such as *RB1*, which were not selected for in BRCA-deficient tumours, suggest alternative routes to generating chromosomal instability (CIN) compared to HRD in BRCA-deficient tumours. Amplification of *CCND1* were observed across both BRCA -wild-type and -deficient tumors, however the timing of these events suggests additional CIN in BRCA-deficient tumors later during tumor evolution, with amplifications occurring subclonally, and clonal amplifications in BRCA-wild-type tumors. Parallel evolutionary events occur in 22% of TNBC tumors, demonstrating loss of genes such as *B2M* and LILR receptor family genes, which have been implicated in cancer immune evasion. TNBC tumors further demonstrated additional patterns of immune evasion through parallel gains of genes such as *CD274* and *PDCD1LG2*, suggesting selection for an immuno-suppressed phenotype.
Conclusions: These data demonstrate the evolutionary landscape of primary TNBC tumors and highlight the magnitude of events driving chromosomal instability in TNBC.

#6614 Small cell lung cancer (SCLC) at recurrence has a "colder" tumor microenvironment than primary SCLC according to gene expression profiling.

Farhad Kosari[1], Mohamed Ali[1], Sarah H. Johnson[1], Alex Adjei[2], Julian R. Molina[1], Aaron S. Mansfield[1], Anastasios Dimou[1], Kaushal Parikh[1], Mohamed Shanshal[1], Katherine E. Smith[1], Ailsa G. Luce[1], Anna J. Schwecke[1], Janet Lensing[1], Ying-Chun Lo[1], John C. Cheville[1], George Vasmatzis[1], **Konstantinos Leventakos**[1]

[1]Mayo Clinic Cancer Center Minnesota, Rochester, MN,[2]Cleveland Clinic, Cleveland, OH

Recent studies have described the tumor microenvironment and the key transcription factors in untreated small cell lung cancer (SCLC), but less is known about the salient features in recurrent SCLC (rSCLC). This information could provide valuable insight about the effectiveness of second and later line therapies. In this study, we compared transcriptome profiles (by RNA-seq) of rSCLC against untreated primaries (pSCLC). rSCLC samples (n=23) were obtained from frozen biopsies in patients enrolled in our MC1923 (NCT04607954) phase 2 clinical trial whose disease progressed after chemoimmunotherapy. The pSCLC group (n=73) included publicly available data from fresh frozen primary tumors after surgical resections. Normalized expression data were used in immune deconvolution and gene set enrichment analysis (GSEA) in molecular signature database. Additional GSEA included gene sets describing ASCL1 (A), NEUROD1 (N), ASCL1 and NEUROD1 (AN), and POU2F3 (P) transcription factor activities which are used for subtyping of SCLC. Immune deconvolutions by multiple software algorithms suggested depletion of immune infiltrates in rSCLC compared with pSCLC including dendritic cells, B cells, $CD8^+$ T cells, and M1 macrophages. Furthermore, we found decreased chemotaxis and increased apoptosis of dendritic cells, reduced TNF signaling, and reduced lymphocyte co-stimulation in rSCLC by GSEA. Additionally, in comparisons with pSCLC, rSCLC tumors had significantly lower enrichment scores for POU2F3 activity, representing the SCLC-P subtype ($p < 0.007$). Importantly, the SCLC-P was recently reported to be more immune inflamed than the other A, N, and AN subtypes. Altogether, our analyses provide strong evidence that the tumor microenvironment of rSCLC is significantly more immunosuppressed and with reduced immune cell infiltrates compared to pSCLC. Developing therapeutic strategies aimed at "warming" the tumor microenvironment and enhancing anti-tumor immune activity in ongoing multimodal therapies of recurrent SCLC will likely provide significant benefits to patients.

#6615 Uncovering the landscape of cellular heterogeneity in low- and high-grade endometrial cancer using single cell profiling.

Sabina I. Swierczek*[1], Elizabeth A. R. Garfinkle*[2], Sophia A. Paxton[3], Katherine E. Miller[4], David Doo[5], Leslie Andriani[5], Shatovisha Dey[1], Steven Sieber[6], Alpana Dholakia[1], Syeda Rashid[1], Linus Chuang[5]

[1]Rudy L. Ruggles Biomedical Research Institute, Nuvance Health, Danbury, CT,[2]The Steve and Cindy Rasmussen Institute for Genomic Medicine, Abigail Wexner Research Institute at Nationwide Children's Hospital, Columbus, OH,[3]Biomedical Sciences Graduate Program, The Steve and Cindy Rasmussen Institute for Genomic Medicine, The Ohio State University College of Medicine, Abigail Wexner Research Institute at Nationwide Children's Hospital, Columbus, OH,[4]The Steve and Cindy Rasmussen Institute for Genomic Medicine, Department of Pediatrics, Abigail Wexner Research Institute at Nationwide Children's Hospital, The Ohio State University College of Medicine, Columbus, OH,[5]Department Obstetrics and Gynecology, Nuvance Health, Danbury, CT,[6]Department of Pathology, Nuvance Health, Danbury, CT

Introduction: Endometrial cancer (EC) is the most common gynecologic malignancy in women, with rising incidence and mortality. EC is classified into low-grade (I/II), less aggressive, and high-grade (III), associated with poor prognosis and aggressive behavior. Single-cell RNA sequencing (scRNA-seq) offers insights into the interplay between tumor cells and the tumor microenvironment (TME), improving diagnosis and treatment strategies. This study aims to identify unique cell subtypes and factors distinguishing low-grade from high-grade EC, uncovering therapeutic targets to address tumor progression.

Methods: Tumors from ten treatment-naïve EC patients (four low-grade endometrioid, six high-grade (three endometrioid, three serous), and two age-matched non-malignant endometrial samples were collected at surgery and frozen. Nuclei were isolated from the frozen samples and single-cell libraries prepared using the Chromium Next GEM Single Cell Kit v3.1 according to the manufacturer's recommendations. Data were analyzed using Seurat v5 in R v4.3.3.

Results: Multiple phenotypic subsets of EC cells, T cells, fibroblasts, macrophages, endothelial, and epithelial cells were identified. Six distinct EC cell subsets were present across tumors, showing significant intratumoral heterogeneity. The *MKI67+* EC subcluster predominated in high-grade endometrioid EC, while *CHRM2/3+* EC subclusters were exclusive to high-grade serous EC. Both low- and high-grade endometrioid EC exhibited a high proportion of *PROM1+* (CD133) cancer stem cells. Epithelial-to-mesenchymal transition (EMT) genes (*COL1A2, BNC2, HNF1A-AS1, ITMC2, ZNF492*) were upregulated in high-grade EC compared to low-grade, correlating with aggressive phenotypes and poorer prognoses. High-grade EC showed increased activated T cells (PTPN14+), suggesting immune evasion, interestingly, low-grade EC had the highest proportion of exhausted T cells (TIM3+, PD1+) typically associated with aggressive tumors. Consistent macrophage subtypes were observed across EC cells, indicating T-cell-mediated alterations in the TME between EC grades.

Conclusion: This study provides the first comprehensive comparison of tumor cell diversity and TME components in low- and high-grade EC (endometrioid and serous). These findings, alongside ongoing *in vitro* studies, aim to uncover viable, subtype-specific therapeutic targets. *Both authors contributed equally

#6616 LMNA-FOSB fusions are a recurrent event in meningiomas.

Mateo Gomez Hjerthen[1], Josiah Wong[2], Juan Wang[2], Harrshavasan Congivaram[2], Priscilla K. Brastianos[3], Akash J. Patel[4], Craig M. Horbinski[1], James P. Chandler[1], E. Zeynep Erson Omay[5], Jennifer Moliterno[5], Murat Gunel[6], Stephen T. Magill[1], David R. Raleigh[7], Feng Yue[2], Mark W. Youngblood[1]

[1]Department of Neurosurgery, Northwestern University Feinberg School of Medicine, Chicago, IL,[2]Department of Biochemistry and Molecular Genetics, Northwestern University Feinberg School of Medicine, Chicago, IL,[3]Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA,[4]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX,[5]Department of Neurosurgery, Yale School of Medicine, New Haven, CT,[6]Department of Neurosurgery, Genetics and Neuroscience, Yale School of Medicine, New Haven, CT,[7]Department of Neurological Surgery, University of California San Francisco, San Francisco, CA

Meningiomas are common tumors of the central nervous system associated with a small number of mutually exclusive genetic events. We identify a cohort of seven meningiomas that harbor fusions between the genes *LMNA* and *FOSB*, with no other known meningioma mutations. Previous reports of *LMNA-FOSB* fusions in epithelioid hemangiomas suggest a broader oncogenic potential for this event across tumor types. Lamin A/C is a structural protein involved in chromatin organization expressed ubiquitously in nearly all differentiated cells, while FOSB is an AP-1 family transcription factor implicated in cell proliferation and differentiation. Targeted, whole-genome, and RNA-sequencing approaches revealed a near identical *LMNA-FOSB* fusion comprising exons 1-2 of *LMNA* (chr1) and exons 2-4 of *FOSB* (chr19). The fusion event therefore includes key phosphorylation sites in Lamin A/C such as Ser22 that interact with active enhancers, while preserving the DNA binding domain of FOSB. Expression of these genes was not significantly altered among fusion samples, suggesting neomorphic protein function as an underlying oncogenic mechanism. Transfection of the *LMNA-FOSB* constructs into CH157 and IOMM-LEE meningioma cell lines resulted in changes to chromatin architecture and gene expression, which were validated using Hi-C and ChIP-seq profiling of frozen tumor tissues harboring this event. Our findings suggest that *LMNA-FOSB* fusions represent a novel and recurrent meningioma genetic event, providing new insights into the molecular pathogenesis of these tumors and role of FOSB across cancer.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Genomic Profiling of Tumors 3
Poster Session

#6620 Low interferon expression coupled with 9p21 loss in non-small cell lung cancer (NSCLC) patients associated with distinct somatic landscape, altered immune population, and poorer response to immune checkpoint inhibitor (ICI) therapies.

Natalie Vokes[1], Jessica L. Symons[2], Brooke Rhead[2], Pooja A. Shah[1], Santiago Trevino[1], Konstantinos Levantakos[3], Stamatina Fragkogianni[2], Calvin Chao[2], Jordi A. Rodon[1]

[1]UT MD Anderson Cancer Center, Houston, TX,[2]Tempus AI, Chicago, IL,[3]Mayo Clinic, Rochester, MN

Deletions in 9p21 are common across multiple cancer types including NSCLC. Notably, 9p21 deletions have been implicated in an immune-excluded tumor immune phenotype and worse outcomes following ICIs. Whether this effect is due to *CDKN2A* loss, collateral loss of *MTAP*, or loss of type I interferon genes (*IFNK, IFNA*) has not been fully adjudicated. The Tempus Database (Tempus AI, Inc., Chicago, IL) was queried for de-identified records of patients with NSCLC and DNA (Tempus xT) and RNA (Tempus xR) molecular profiling. *CDKN2A*/*MTAP* deleted samples were defined as those containing biallelic loss of *CDKN2A/B* and *MTAP*. As *IFNK* is not included in the DNA sequencing bait set, *IFNK* status was defined as low in any sample with expression ≤ the first quartile of *IFNK* expression from samples with intact *CDKN2A*/*B* and *MTAP*. Samples with tumor purity < 40% or mixed loss of *CDKN2A*/*B* and *MTAP* were excluded. Association analysis of *CDKN2A*/*MTAP*/*IFNK* status with other genomic events and clinical outcomes was performed. N=16,947 patients were included in the overall analysis, of whom n=1,991 had *CDKN2A/B*/*MTAP* deletion (11.7%, CM^{del}). Significantly more CM^{del} patients had low *IFNK* expression compared to *CDKN2A/B*/*MTAP* intact (CM^{wt}) patients (n=14,956); of CM^{del} (n=1300), 65.3% had high *IFNK* expression ($IFNK^{hi}$) and 34.7% had low *IFNK* expression ($IFNK^{lo}$). Among CM^{del} patients, $IFNK^{hi}$ samples were enriched in never smokers compared to $IFNK^{lo}$ patients (24% vs 18%, p=0.002). CM^{del} was enriched in samples with *EGFR* and *ALK* driver alterations, and depleted in samples with *KRAS* or *TP53* alterations. In both CM^{del} and CM^{wt} contexts, *KRAS*, *EGFR*, and *ALK* alterations associated with $IFNK^{hi}$ expression, whereas *TP53*, *NFE2L2*, *STK11*, *KEAP1*, *SMARCA4*, and *KMT2D* associated with $IFNK^{lo}$ expression. $IFNK^{hi}$ expression within CM^{del} patients was associated with an increase in the estimated proportion of immune cell subpopulations, including CD8 T cells and macrophages (M1 and M2), and a decrease in neutrophils and NK cells. In metastatic/unresectable stage III patients with recorded ICI-containing 1L therapies (n=1,795), patients with CM^{del} and $IFNK^{lo}$ trended toward higher rates of progressive disease (PD) (37%), whereas $CM^{del}/IFNK^{hi}$ had comparable rates of PD to $CM^{wtl}/IFNK^{hi}$ and $CM^{wtl}/IFNK^{lo}$ (28%, 27%, 30%, respectively). $CM^{del}/IFNK^{lo}$ patients treated with non-ICI/TKI therapies (mostly consisting of chemotherapy) had a non-significant trend toward increased rate of PD (63%). CM^{del} associates with driver alterations in *EGFR* and *ALK*, along with worse ICI and chemotherapy outcomes in the context of $IFNK^{lo}$ but not $IFNK^{hi}$ expression. Further work to disentangle the impact of immune infiltrate and the co-mutation landscape will help clarify the underlying associated biology.

#6623 Ultra-deep multi-omics sequencing to identify drivers of glioblastoma recurrence and evolution.

Alexander Bahcheli[1], Philip Zuzarte[1], Sunit Das[2], Jared Simpson[1], Juri Reimand[1]

[1]Ontario Institute for Cancer Research, Toronto, ON, Canada,[2]Univeristy of Toronto, Toronto, ON, Canada

Glioblastoma (GBM) is the most prevalent and deadliest form of brain tumor with a median patient survival of 15 months after diagnosis. However, the mechanisms of tumor recurrence and the molecular alterations contributing to therapy resistance remain poorly understood. To address this challenge, we performed ultra-deep whole transcriptome, whole genome, and nanopore long-read sequencing of matched primary and recurrent GBMs of 12 adult patients with matching germline controls (fresh-frozen blood) and patient treatment and clinical histories. We generated a multi-omics datasets of single nucleotide variants (SNVs), structural variants (SVs), copy number alterations, genome-wide DNA methylation patterns, and transcriptomics. Our long-read sequencing data provided nucleotide-level methylation data to analyze genome-wide methylation patterns and high-quality structural variants for profiling complex genomic rearrangements and identifying episomes. Major changes between primary and recurrent samples in SVs, such as selection for *EGFR* translocations in recurrent tumors, and nucleotide-level methylation patterns, such as increased methylation of the *FUBP1* and *IDH1* promoters in recurrent tumors, were only detectable using long-read sequencing strategies, emphasizing the value of long-read sequencing in evolutionary analyses. We identified distinct mutational processes responsible for primary and recurrent tumor mutations and driving evolution of a recurrent tumor with therapeutic resistance. We found evidence of multi-omics dysregulation of oncogenes including promoter silencing by methylation and SVs selecting for the EGFR-vIII variant. Extensive inter- and intra-tumor heterogeneity and evidence of clonal selection in recurrent tumors was detected from ultra-deep whole genome sequencing, including selection for *EGFR* SNVs and *PTEN* and *RB1* copy number deletions in recurrent tumors. We found an enrichment of SVs and epigenetic modifications in recurrent tumors, such as increased methylation of *CDK4* and *CDK6* promoters, and a depletion of classical tumor drivers, such as *EGFR* and *CDK4* amplifications. Our integrative pathway analysis of multi-omics data of differentially methylated and expressed genes between primary and recurrent GBMs prioritized several glioma pathways enriched in recurrent tumors associated with glioma processes such as gliogenesis and angiogenesis. Our cohort had comparable mutation rates and processes as the Glioma Longitudinal Analysis (*GLASS*) consortium of paired primary-recurrent GBMs. However, our unique dataset of paired primary and recurrent GBMs provides an unprecedented multi-omics, multi-layered view of the genetic, transcriptomic, and epigenetic processes of GBM evolution.

#6624 Comprehensive, multi-omic detection of somatic variants from the GIAB HG008 matched tumor-normal pair using highly accurate long- and short-read whole-genome sequencing.

Alexandra Sockell[1], Khi Pin Chua[1], Christopher Kingsley[1], Dan Nasko[1], Matthew Boitano[1], Young Kim[1], Melanie Wescott[1], Ian McLaughlin[1], Primo Baybayan[1], Jennifer McDaniel[2], Andrew Shaver[1], Justin M. Zook[2], Aaron Wenger[1]

[1]PacBio, Menlo Park, CA,[2]National Institute of Standards and Technology, Gaithersburg, MD

Disentangling the molecular drivers of cancer progression requires a precise understanding of the somatic alterations that take place at the DNA level during tumor development. These include not only small changes like SNVs and indels, but also structural variants, changes in repetitive elements, differential methylation, as well as the haplotype context in which these changes occur. Existing short-read sequencing methods using sequencing by synthesis (SBS) chemistry lack the read length to characterize large structural variants or to span longer repetitive regions as well as to phase variants into haplotypes, while simultaneously lacking the sequencing accuracy to distinguish very low frequency somatic variants from background sequencing error or to sequence through shorter repetitive elements like microsatellites. Long-read PacBio HiFi data addresses the first set of challenges by accurately sequencing long fragments up to 25 kb, while highly accurate short-read sequencing by binding (SBB) chemistry from PacBio addresses the latter by improving raw sequencing accuracy by orders of magnitude, including in homopolymer regions which make up most microsatellites in the human genome.

Here we apply these highly accurate long- and short-read sequencing technologies from PacBio to perform whole-genome sequencing of the newly described HG008 matched tumor-normal pair from the Genome in a Bottle (GIAB) consortium. This reference sample includes an adherent, epithelial-like pancreatic adenocarcinoma (PDAC) cell line as the tumor material, with the matched normal obtained from adjacent duodenal and pancreatic tissue. We perform whole-genome sequencing with both PacBio HiFi (35X normal, 80X tumor) and SBB (50X normal, 100X tumor), followed by alignment and tumor-normal variant calling for all variant types. These accurate long- and short-read sequencing technologies offer a more robust and comprehensive picture of somatic variation in this reference sample and contribute to developing this novel benchmark.

#6625 LinkPrep™ - a comprehensive assay for cancer genome characterization - enabling structural variant detection and discovery of enhancer hijacking.

Ericca Stamper, Jon Torchia, Daniel Hwang, Chu Yuan Wu, Ek Danwanichakul, Cory Padilla, J. Zachary Sanborn, Lisa Munding

Dovetail Genomics, Cantata Bio, LLC, Scotts Valley, CA

Cancer arises from genetic and/or epigenetic disruptions. Currently, no single technology can capture the full range of variation that drives tumorigenesis with high sensitivity. Furthermore, epigenetic changes resulting from somatic variation remain poorly understood. The LinkPrep™ technology, a multi-purpose NGS assay, generates unbiased linked-read libraries through DNA proximity ligation that enable the discovery of structural variants (SVs) while simultaneously capturing 3D chromosome conformation linked to the tumor's epigenetic state. Importantly, LinkPrep data provides uniform coverage, allowing for the detection of single nucleotide variants (SNVs), insertions/deletions (InDels), and copy number variants (CNVs) with similar performance to whole genome shotgun sequencing (WGS). Libraries are generated from a single-day workflow and are compatible with Illumina sequencers.

In a comparison study, a fresh-frozen, clinical ovarian serous adenocarcinoma sample was profiled with LinkPrep (30X), WGS (30X), and RNA-seq (100M reads). Additionally, the same sample stored in FFPE was profiled with Dovetail-FFPE (30X), a linked-read technology designed to extract long-range genomic interactions from FFPE tissue. LinkPrep & Dovetail-FFPE both identified three translocations (tlocs) at high SV-VAF (>30%) between chromosomes 1, 3, and 12. These tlocs were supported by strong evidence (LinkPrep: 589 - 3,282 read pairs, Dovetail-FFPE: 417 - 5,802 read pairs) and confirmed by copy number changes. The tlocs were further validated through PCR and Sanger sequencing. By comparison, RNA-Seq detected only one fusion consistent with tloc(1;12), and WGS identified two tlocs with low specificity (ranked 140 and 374 of 404 putative tlocs). Furthermore, LinkPrep detected a subclonal event present at SV-VAF of 4.5% at tloc (2;15). LinkPrep and WGS produced concordant variant calls that showed >95% overlap with the set of known variants. Similarly, copy number profiles between LinkPrep and WGS showed >98% similarity.

To profile the promoter-enhancer genomic interactions at high coverage (200X), LinkPrep libraries were paired with the Dovetail® Pan Promoter Enrichment Panel. This revealed SV-driven neo-interactions, with interaction strength independent of SV VAF, suggesting biological mechanisms such as enhancer hijacking are at play.

In summary, LinkPrep outperforms WGS and RNA-seq in SV discovery with superior sensitivity and specificity, while maintaining comparable performance to WGS in the detection of SNVs, InDels, and CNVs. Additionally, LinkPrep captures epigenetic information in the same library, providing insights into epigenetic changes and drivers. Taken together, LinkPrep is a powerful tool for comprehensive profiling of the cancer genome - capturing the whole range of genetic variants and the 3D genome in a single dataset.

#6626 Comprehensive, high sensitivity, high resolution, somatic variant detection in solid tumors with LinkPrep - an NGS assay.

Cory C. Padilla, Ericca Stamper, Jonathan Torchia, Daniel Hwang, Mark Wadolkowski, Ek Danwanichakul, J. Zachary Sanborn, Lisa Munding

Dovetail Genomics, Scotts Valley, CA

The characterization of somatic variants, including structural variants (SVs), copy number variations (CNVs), and single-nucleotide variants (SNVs), is essential for understanding tumor biology. Current methodologies for somatic variant profiling are fragmented across several assays and requires ample sample material. We present LinkPrep™ assay, a novel linked-read NGS assay utilizing DNA proximity ligation, optimized for high sensitivity and high-resolution whole genome somatic variant detection. LinkPrep data enables unified profiling of SVs, CNVs, and SNVs/InDels from the same dataset, providing a streamlined approach to comprehensive genomic analysis. We applied the LinkPrep assay to a benchmark cohort consisting of cell lines (ductal carcinoma, HCC1187; myelogenous leukemia, K562) and clinically relevant tumor samples (ovarian serous adenocarcinoma, metastatic ovarian carcinoma, and nasal osteosarcoma). LinkPrep achieved high concordance with whole-genome sequencing (WGS) for CNVs (correlations >89% across all samples) while also detecting SVs with superior mean read support and robust F1 scores (cell lines). Moreover, LinkPrep demonstrated excellent overlap with WGS for SNVs and InDels, exceeding 92% across all samples, and identified critical oncogenic candidates. Notably, LinkPrep uncovered additional high-confidence SVs (including low SV VAF down to 1%) in clinical samples that were missed by WGS, highlighting its enhanced sensitivity for somatic variant detection in low tumor fraction samples. Furthermore, we demonstrate the ability to use LinkPrep data to refine SV breakpoints down to base pair resolution.
The data underscore the utility of the LinkPrep assay in translational research, where precise and comprehensive variant detection is imperative. This study establishes the LinkPrep assay as a next-generation tool for the detection of somatic variants, offering a robust and scalable solution for cancer genomics.

#6627 Clonal architecture analysis of response to PARP inhibition in prostate cancer.

Matias Vergara[1], Alok K. Tewari[2], Jake R. Conway[3], Sabrina Y. Camp[2], Jihye Park[2], Brendan Reardon[2], Wassim Abida[4], David B. Solit[5], Nikolaus Schultz[6], Keyan Salari[7], Eliezer M. Van Allen[2]

[1]Dana-Farber Cancer Institute, Boston, MA,[2]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA,[3]PathAI, Boston, MA,[4]Genitourinary Oncology Service, Memorial Sloan Kettering Cancer Center, New York, NY,[5]Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY,[6]Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY,[7]Department of Urology, Massachusetts General Hospital, Boston, MA

Background: Prior studies have charted the clonal and subclonal evolutionary dynamics (“clonal architecture”) of prostate cancer (PC), although the relationship between PC clonal architecture and therapeutic response remains unclear. PARP inhibition (PARPi) is an approved genomic strategy for treating PCs deficient in homologous recombination (HRD) as determined by specific gene alterations. As clinical responses to PARPi are heterogeneous and not exclusively associated with specific gene mutations, and HRD can exhibit discrete mutational and copy number signatures, we hypothesized that deriving overall tumor and clone-specific mutational signatures in prostate tumors may reveal novel associations between clonal architecture and clinical response to PARPi.

Methods: We analyzed two independent cohorts of PC whole exome sequencing data that passed quality control metrics (Cohort 1: N=845 patients and 1 sample per patient, Cohort 2: N= 185 patients and 21 patients having multiple samples, enriched for patients with known deleterious BRCA2 alterations). For each tumor/normal paired sample, mutations and copy-number alterations were identified and used as input for phylogenetic reconstruction as well as single-nucleotide and copy-number mutational signatures. We examined the relationship between single gene germline or somatic alterations, tumor and clone-specific mutational signatures of HRD such as COSMIC mutational signature 3 (Sig3), and clinical response to PARP inhibition or immune checkpoint blockade. Here we report features of tumor and clone-specific Sig3 positivity (Sig3+) and association with clinical response in these two cohorts.

Results: In Cohort 1, evidence of HRD at the tumor level by Sig3+ was detected in 2% (11/560) of primary tumors and 23.6% (67/285) of metastatic tumors. 31 Cohort 1 patients had germline or somatic alterations in BRCA2. 51.2% (40/78) of Sig3+ tumors had detectable Sig3+ at the individual and clone-specific level, including 12 tumors with only clone-specific Sig3+. Of the 37 patients in cohort 1 treated with PARPi and Sig3+ information, patients with detectable Sig3+ had a lower hazard for progression with treatment at the tumor level (HR = 0.60, p = 0.31), individual clone level (HR = 0.45, p = 0.095), or either (HR = 0.59, p = 0.20) compared to patient tumors with no detectable Sig3+. In Cohort 2, 68.4% (63/92) of primary and 76.7% (89/116) of metastatic tumors were Sig3+. 30 primary and 59 metastatic samples in Cohort 2, the majority of patients in each category, harbored deleterious BRCA2 alterations. 90.1% (137/152) of Sig3+ tumors had detectable Sig3+ at the individual clone level, including 18 with only clone-specific Sig3+. Analysis of clinical outcomes in Cohort 2 is ongoing.

Conclusions: Clone-specific evidence of HRD using clonal architecture and mutational signature analysis is detectable and may identify additional PC patients who could benefit from PARPi.

#6628 Deep dive into the performance of structural variants reported by PGDx elio plasma focus Dx.

Amy Meltzer[1], Jesse Fox[1], Kenneth C. Valkenburg[1], Tonya Watkins[1], Sonomi Dillon[1], Pengfei Gu[1], Ana Perez-Lebron[1], Robert Summersgill[1], Annabelle Culi[1], Nesha Jackson[1], Stephen Higgins[1], Kristy Waszkiewicz[1], Nobel Girmay[1], James Occean[1], Cal Palumbo[1], David Holcomb[1], Kelly Gerding[1], David Riley[1], Sam Angiuoli[1], Aanavi Karandikar[2], Rami Zahr[2], Jennifer Dickey[3], Eric Severson[4], Shakti Ramkissoon[4], Brian Caveney[5]

[1]Research and Development, PGDx Labcorp, Baltimore, MD,[2]Labcorp Oncology, PGDx Labcorp, Baltimore, MD,[3]Regulatory & Quality, PGDx Labcorp, Baltimore, MD,[4]Labcorp Oncology, Labcorp, Durham, NC,[5]Chief Medical and Scientific Officer, Labcorp, Durham, NC

Introduction: Liquid biopsy NGS assays allow for detection of genes clinically relevant to oncology including structural variants such as amplifications and translocations. Due to their low concentration in plasma, structural variants can be difficult to detect with liquid biopsy. Translocations in particular present challenges due to their low prevalence in ctDNA as opposed to RNA. PGDx elio™ plasma focus Dx is an IVD tumor profiling assay which reports 33 genes including difficult to detect structural variants with high sensitivity, specificity, accuracy, and reproducibility. Here, the performance of structural variants is explored using data from analytical validation studies.
Methods: Samples were processed with PGDx elio plasma focus Dx, a NGS based tumor profiling assay capturing 33 clinically relevant genes using cfDNA obtained from plasma. For amplifications, 5 genes are reported (ERBB2, MET, CCND1, CD274, and FGFR2) and for translocations, 3 genes are reported (ALK, NTRK1, and RET). Libraries were sequenced with 150 bp paired end NGS and processed through the EPF software which performs demultiplexing, alignment, and variant calling utilizing DK for amplifications and PARE for translocations. Analytical validation studies were conducted at the gene level across key studies including accuracy, sensitivity, specificity, and reproducibility. For accuracy, results were compared to 5 orthogonal methods with a total of 931 clinical samples across 35 solid tumor types. Specificity was assessed with 68 healthy plasma samples. To assess sensitivity, all amplifications and translocations were established with a dilution series using cell line blends spiked into plasma and confirmed with clinical plasma blends diluted into normal plasma. Performance between clinical plasma and contrived samples was established with a contrived sample functional characterization study. Reproducibility was assessed with an interlaboratory reproducibility (ILR) study across 3 different sites with over 500 total replicates.
Results: The test demonstrated variant concordance of 93.1% PPA and 99.8% NPA for translocations and 90.1% PPA and 100% NPA for amplifications (excluding borderline calls, 1.2-1.8-fold). Analytical specificity was 100% for both amplifications and translocations. Analytical sensitivity (limit of detection, (LoD) 95%) was assessed for each structural variant on the panel and ranged from 0.41% - 0.49% FRF for translocations and 1.3 - 1.7-fold for amplifications. The ILR study resulted in 96.1% average percent agreement (APA) and 99.6% average negative agreement (ANA) for amplifications and 95.3% APA and 99.5% ANA for translocations.
Conclusions: These analytical validation results demonstrate PGDx elio plasma focus Dx detects structural variants with high accuracy, sensitivity, specificity, and reproducibility, supporting the use for precision oncology.

#6629 Spatial immunogenomic analysis of the transformation process in acquired cystic disease of the kidney.

Jun TAKAHASHI[1], Yosuke Tanaka[1], Yusuke Sato[2], Tetsuo Ushiku[3], Hiroyoshi Nishikawa[4], Haruki Kume[2], Hiroyuki Mano[1]

[1]Division of Cellular Signaling, National Cancer Center Research Institute, Tokyo, Japan,[2]Department of Urology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan,[3]Department of Pathology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan,[4]Division of Cancer Immunology, National Cancer Center Research Institute, Tokyo, Japan

Chronic kidney disease (CKD) is one of the leading causes of death, affecting more than 10 % of the general population worldwide. When CKD progresses to the advanced stage known as end-stage renal disease (ESRD), patients require kidney replacement therapy such as dialysis or kidney transplantation. The prevalence of dialysis patients in Japan is 2,535 per million population, which is much higher than those in Western countries, mainly due to the low number of kidney transplants in Japan. One of the critical complications of dialysis is the high incidence of kidney cancer, which is approximately 15 times higher than in the general population. More than 90 % of patients on dialysis for more than 10 years develop acquired cystic kidney disease (ACKD), which has been reported to be associated with the development of kidney cancer. However, the molecular mechanism underlying ACKD-associated RCC(ACD-RCC) has not been elucidated. We performed multi-omic analyses of ACKD especially from a spatial immunogenomic perspective. We collected the data of 26 single-cell transcriptomes and 26 spatial transcriptomes using 10x Genomics platform as well as 42 exome sequencing and 86 RNA sequencing for renal cysts and tumors from ACKD patients. Normal kidney tissues from non-dialysis patients were also analyzed. The mutational landscape of ACD-RCC and ESRD-clear cell renal cell carcinoma(ccRCC) was totally different from that of the sporadic ccRCC. Specifically, ccRCC in ACKD patients was characterized by a higher incidence of *TCEB1* mutations (35.3%). Analysis on the noncancerous tissues in ACKD revealed the unique transcriptomic features of renal cysts in ACKD with a characteristic of inflammatory signaling activation. Specifically, unique immune cell infiltration patterns of lymphocytes and macrophages in ACKD were identified, which are distinct from those in normal kidney tissue. Our integrative single cell and spatial analyses identified renal cyst cluster which is characterized by activation of proximal tubular genes, and our data support that renal cysts and subsequent dialysis-related tumors both originate from proximal tubular cells. Also, we estimated copy number variation in renal cysts and found shared copy number variation with tumors. Furthermore, we identified specific mutations concentrated in the cyst wall using laser capture microdissection, suggesting that a single cyst may have arisen from a single clone. Also, we analyzed intercellular communication and identified important pathways for nurturing cysts formation including signaling pathway. Collectively, our results will contribute to the suppression of cystic kidney and renal tumors, and pave the way for improving the prognosis of patients with ACKD.

#6630 Ancestry and lung adenocarcinoma: exploring ancestry-associated variations in tumor metabolism, mitochondrial dysfunction, and immune dynamics.

Yuxin Jin[1], Yonatan Amzaleg[1], Angel P. Hunt[1], Benecia Jackson[2], Dan Raz[3], Loretta Erhunmwunsee[3], John Carpten[1], **Aaron M. Neely**[1]

[1]Integrative Translational Sciences, Beckman Research Institute of The City of Hope, Los Angeles, CA,[2]Division of Health Equities, Beckman Research Institute of The City of Hope, Los Angeles, CA,[3]Division of Thoracic Surgery, Beckman Research Institute of The City of Hope, Los Angeles, CA

Lung adenocarcinoma (LUAD) is a highly heterogeneous cancer, characterized by diverse genetic mutations, metabolic reprogramming, and complex immune microenvironments. Patients of African ancestry (AFR) often experience distinct clinical outcomes in LUAD (Manojlovic Z, et al. 2017), potentially driven by unique genetic and metabolic adaptations within tumors (Telonis, Aristeidis G et al. 2024, Kap EJ, Seibold P, et al.,. 2016). Global and local ancestry analyses have shown that genetic ancestry can influence tumor behavior, including immune interactions and cellular energy metabolism (Manojlovic Z, et al., Telonis, Aristeidis G et al. 2024). However, there is limited understanding of how AFR ancestry impacts LUAD, particularly regarding tumor metabolism, mitochondrial dynamics, programmed cell death (PCD) pathways, and immune response. The immune microenvironment of LUAD tumors varies widely, and understanding how ancestry affects immune compartment composition and immune cell infiltration could offer insight into observed disparate phenotypic severity and differential responses to therapy. This study aims to uncover the ancestry-linked mutational landscape across tumor metabolism, mitochondrial dynamics, and immune recruitment in LUAD, hypothesizing that these differences may contribute to demonstrative disparities in differential LUAD malignancy severity and patient outcomes. We hypothesize that LUAD tumors in patients of AFR genetic similarity will exhibit unique mutations and transcriptional profiles in metabolic, mitochondrial, and immune-related genes, resulting in distinct metabolic programs, immune infiltration patterns, and differential activation of PCD pathways. These variations could influence tumor progression, immune evasion, and response to treatment in an ancestry-dependent manner. By combining spatial, single-cell, and genetic profiling techniques, this study aims to uncover the ancestry-associated interplay between tumor metabolism, mitochondrial dynamics, PCD, and immune compartment composition in LUAD. Insights from this work could inform ancestry-tailored therapeutic strategies and ultimately improve clinical outcomes for LUAD patients across diverse populations. Admixture analysis highlighted genetic diversity within our diverse LUAD patient cohort as Black patients illustrated predominant AFR genetic similarity with varying proportions of EUR, AMR and SAS similarity. Local ancestry analysis revealed admixture across different regions of the genome demonstrating variations in ancestral contributions within and between individuals of the same cohort and compared to counterparts in other populations. Additionally, we observed associations between high AFR genetic similarity and mutations in metabolic, immune and mitochondrial genes.

#6631 High-resolution characterization of FFPE-derived linked-read libraries for cancer genomics.

Alexander Fortuna, Jon Torchia, Mital Bhatka, Natalie Fredriksson, Daniel Hwang, Ek Danwanichakul, Cory Padilla, J. Zachary Sandborn, Lisa Munding

Cantata Bio, Scotts Valley, CA

Deciphering genomic architecture is crucial for identifying novel disease drivers and understanding cancer initiation and progression mechanisms. While short-read sequencing remains the primary tool in cancer genomics, it may miss important structural variants (SVs), especially large chromosomal alterations. A further challenge to cancer researchers is working with FFPE (formalin-fixed, paraffin-embedded) samples, the most common source of archived material, as this sample type is often at risk of nucleic acid fragmentation, DNA crosslinks, and deamination, further complicating variant discovery. We present a Dovetail-FFPE assay, an MNase-HiC-based method compatible with FFPE samples that generates linked-reads to enable improved sensitivity for SV detection. Here, we evaluate the performance of Dovetail-FFPE against a shotgun sequencing library in FFPE and FF (fresh frozen) using a clinical serous ovarian adenocarcinoma (HGSOC) sample. We generated WGS and Dovetail-FFPE libraries at 30x target coverage and utilized BWA for alignment, with Hi-C breakfinder (Dovetail-FFPE) and Manta (WGS) for SV calling. Both Dovetail-FFPE and WGS datasets were analyzed using DeepSomatic for SNV and intel detection, while CNVs were identified using Purple. Dovetail-FFPE analysis revealed four translocations (tlocs) with strong supporting evidence (1;12, 3;12, 3;4, 2;15). These tlocs, also identified in a MicroC-FF sample and validated by PCR and Sanger sequencing, corresponded with copy number changes and occurred at CNV boundaries. In contrast, WGS detected only two of these four tlocs with lower precision. Somatic mutations in coding regions showed high concordance between methods. The Dovetail-FFPE library successfully recalled all pathogenic variants from the truth set (defined as pathogenic/likely pathogenic significance by ClinVar in the FF WGS sample). Copy number analysis demonstrated strong concordance between Dovetail-FFPE and FF-WGS (89%), recalling a heterozygous deletion of chromosome 1p and gains of 1q and 12. Additionally, shotgun, Dovetail-FFPE, and MicroC-FF showed agreement in predicted purity, ploidy, and percentage of genome altered. Our findings demonstrate that Dovetail-FFPE libraries can effectively detect SVs identified in FF libraries and outperform shotgun libraries. Combined with its capability to identify SNVs, indels, and CNVs, linked-read sequencing proves to be a valuable tool for comprehensive genetic variation discovery.

#6633 Survival implications of *CDKN2A/2B* deletion, *MET* amplification and *PIK3R1* mutation in adult type diffuse glioma.

Yongjae Kim[1], Soyoung Park[1], Soo Jeong Nam[2], Kang-Seo Park[3], Ji Eun Park[4], Hye Hyeon Moon[4], Chang Ohk Sung[2], Sang Woo Song[5], Young-Hoon Kim[5], Chang-Ki Hong[5], Jeong Hoon Kim[5], Shinkyo Yoon[3], Ho-Su Lee[1]

[1]Department of Biochemistry and Molecular Biology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, Republic of,[2]Department of Pathology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, Republic of,[3]Department of Oncology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, Republic of,[4]Department of Radiology and Research Institute of Radiology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, Republic of,[5]Department of Neurological Surgery, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, Republic of

Diffuse gliomas, the most common primary brain tumors, present significant variations in morphology, location, genetic alterations, and treatment responses. However, the relationship between their molecular profiles and disease outcomes remains poorly understood. This study analyzed 470 primary diffuse glioma samples collected between 2017 and 2021 using the next-generation sequencing Oncopanel AMC, following the 2021 WHO criteria, to classify the tumors and analyze their clinical impact on overall survival. Statistical analyses included multivariable Cox regression, reporting hazard ratios (HR) with 95% confidence intervals (95% CI), and Kaplan-Meier survival analysis. The classification resulted in three subtypes: oligodendroglioma (n=68), diffuse astrocytoma (n=63), and glioblastoma (n=339). The median age of glioblastoma patients (64 years) was higher compared with oligodendroglioma (50 years) and diffuse astrocytoma (41 years). Gender and tumor mutational burden showed no significant association with subtypes. Oligodendroglioma was characterized by *IDH* mutations and 1p/19q co-deletion (100%), with frequent mutations in *NOTCH1* (27.9%), *FUBP1* (20.6%), and *ARID1A* (19.1%). Diffuse astrocytoma was marked by *IDH* mutations (100%) and high prevalence of mutations in *TP53* (92.1%) and *ATRX* (79.4%). Glioblastoma showed *CDKN2A/2B* loss (52.5%), *EGFR* amplification (30.4%), and *TP53* mutation (35.1%). The incidence of *MTAP* loss identified using specific version of the Oncopanel was observed to be 2.4% in oligodendroglioma, 14.3% in diffuse astrocytoma, and 45.4% in glioblastoma, with no significant association observed with survival. The median overall survival from the diagnosis were 30.7 months for oligodendroglioma, 27.8 months for diffuse astrocytoma, and 14.9 months for glioblastoma. In glioblastoma patients, a multivariable Cox regression analysis identified significant survival factors: older age≥60years (HR=1.35 [1.03-1.76], p=0.031), lack of MGMT promoter methylation (HR [95% CI]=1.53 [1.16-2.02], p=0.003), impaired performance status (HR [95% CI]=1.68 [1.30-2.16], $p=7.03\times10^{-5}$), bilateral disease (HR [95% CI]=1.71 [1.13-2.57], p=0.010), *PIK3R1* mutation (HR [95% CI]=1.52 [1.05-2.18], p=0.025), *CDKN2A/2B* deletion (HR [95% CI]=1.41 [1.09-1.83], p=0.010), and *MET* amplification (HR [95% CI]=2.33 [1.19-4.54], p=0.013). This study provides insights for improved understanding and potential targeted therapies based on genetic profiling of diffuse gliomas, particularly highlighting the prognostic significance of *CDKN2A/2B* deletion, *MET* amplification, and *PIK3R1* mutation in glioblastoma. These findings may guide future clinical trials drug repurposing, potentially leading to improved outcomes for patients with diffuse gliomas.

#6634 Enhanced detection of gene fusions in sarcoma using Genomic Proximity Mapping in FFPE tissue.

Yajuan J. Liu[1], He Fang[2], Maika Malig[3], Emily Reister[3], Mary Wood[3], Ivan Liachko[3], Stephen M. Eacker[3]

[1]The Permanente Medical Group Regional Genomics Laboratory, Kaiser Permanente, San Jose, CA,[2]University of Washington, Seattle, WA,[3]Phase Genomics, Inc., Seattle, WA

Sarcomas are soft-tissue tumors that pose significant diagnostic challenges for clinical laboratories. With more than 80 subtypes, accurate classification of sarcomas is crucial for providing valuable prognostic and therapeutic guidance. Gene fusions are a hallmark of many sarcoma subtypes, offering pathognomonic markers for diagnosis. Routine pathological diagnosis typically involves hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC), with diagnostic samples most commonly preserved as formalin-fixed, paraffin-embedded (FFPE) specimens. However, FFPE preservation precludes conventional chromosome analysis and presents challenges for molecular tests such as those require high molecular weight DNA (i.e. long read sequencing and optical genome mapping). In addition, current methods, such as fluorescence in situ hybridization (FISH) and targeted RNA-seq panels, have limitations, including the restricted number of genes interrogated, dependence on known gene rearrangement breakpoints or chimera transcripts, and sensitivity to RNA quality, especially in FFPE samples. To address these limitations, we developed a new protocol for genome-wide detection of chromosomal abnormalities using Genomic Proximity Mapping (GPM) technology. GPM is a next-generation cytogenomic technique that employs proximity ligation and low-pass sequencing to identify structural variants and gene fusions across the entire genome. As a DNA-based, genome-wide assay, GPM overcomes the primary constraints of FISH and targeted gene panel by RNA-seq. To evaluate the performance of GPM, we applied it to 12 sarcoma samples that had previously underperformed or failed Anchored Multiplex PCR (AMP) analysis using the FusionPlex solid tumor gene panel. In all cases, GPM not only recapitulated the previous findings but also identified additional pathognomonic fusions in 33% of cases where targeted RNA-seq methods failed due to RNA quality issues or the limited scope of targeted analysis. Overall, GPM detected novel structural variants genome-wide and identified definitive gene fusions were in 8 sarcoma subtypes, achieving a sensitivity >0.9 and specificity of 1. In addition to detecting translocations, inversions, and insertions, GPM can identify novel gene fusions, deletions, duplications/amplifications, and copy-neutral loss of heterozygosity. GPM represents a novel, genome-wide approach to identifying both known and novel gene fusions, as well as other chromosomal abnormalities, in FFPE tissue. It enhances sarcoma classification where other methods fail, addressing the limitations of current cytogenetic and other NGS technologies.

#6635 Use of contrived multiplexed FFPE specimens to evaluate CGP from extraction to variant calling.

Dana Ruminski Lowe, Richard Howard, Edward S. Davis, Andrew Anfora, Yves Konigshofer, Catherine Huang, Russell Garlick, Bharathi Anekella

LGC Clinical Diagnostics, Gaithersburg, MD

Formalin fixed and paraffin embedded (FFPE) samples are a common, yet challenging type of patient sample in comprehensive genomic profiling (CGP). DNA extracted from FFPE is often highly cross-linked, degraded, and fragmented. Yield from patient samples can also be limited, thus optimization of extraction in regard to amount and quality of DNA is crucial to the success of downstream analysis. Contrived reference materials are a convenient sample source to evaluate all steps in a CGP workflow. First, they can be used to optimize and validate extraction as they are readily available and consistent in yield and composition. In addition, they are highly multiplexed, making validation of the analytical part of CGP more efficient and cost effective. Finally, complex, difficult to call variants can be easily included to critically analyze the bioinformatics as well. Here we describe the development and evaluation of Seraseq® FFPE Solid Tumor Reference Material.

GM24385 cells were engineered to carry 74 biosynthetic DNA variants across 62 genes including single nucleotide variants (SNVs), insertions and deletions (indels), structural variants (SVs), and mono- and dinucleotide biomarkers for microsatellite instability (MSI). Variant allele frequency (VAF) was verified using the Bio-Rad QX-200 Droplet Digital PCR (ddPCR) System. The cells were formalin fixed, embedded in paraffin blocks and sectioned at 10 micron thickness. Extraction was performed with various commercially available kits from QIAGEN, Promega, and AutoGen. Yields were analyzed using Qubit dsDNA HS and ddPCR. TapeStation Genomic DNA ScreenTape assay was used to assess fragment size and DNA integrity. The extracted DNA was analyzed with the Illumina TruSight™ Oncology 500 (TSO 500) and the ArcherDx VariantPlex (IDT) NGS assays as well as the Biocartis Idylla IDH1-2 Mutation Assay.

Yields were variable between extraction kits; interestingly, yields determined using Qubit did not always correlate with those measured by ddPCR indicating some kits result in more amplifiable DNA than others. Quality and average fragment size also varied between kits but did not affect downstream analysis. The Idylla platform showed positive detection for all IDH1 and IDH2 variants in the FFPE sections. The average VAF was 26.7% ± 5.7% and 36.3% ± 7.3% as measured by the TSO 500 and VariantPlex assays, respectively. Variants that were originally missed by variant callers could often be seen in raw data files upon further investigation.

This study indicates that use of a multiplexed FFPE reference material that provides a data rich readout of actionable variants can be valuable in the initial assessment, analytic validation, and performance monitoring of all parts of CGP workflows.

#6636 Multi-site evaluation of expanded solid tumor, pan-cancer reference material.

Dana Ruminski Lowe, Patricia Helsel, Praveena Kamineni, Edward S. Davis, Andrew Anfora, Yves Konigshofer, Catherine Huang, Russell Garlick, Bharathi Anekella

LGC Clinical Diagnostics, Gaithersburg, MD

Next generation sequencing (NGS) of biopsy-extracted material remains the gold standard for tumor profiling. Panels designed for this type of testing often target 500+ genes, creating a challenge for analytic validation in a clinical setting. Use of patient-like multiplexed commercial reference materials can be a more cost effective and reliable option versus relying on remnant samples with mutations of interest, especially with more stringent requirements from the FDA and other regulators in the early stages of implementation. Here we describe the development and multisite evaluation of expanded Seraseq® Tumor Mutation DNA Mix v3 reference materials, which contain an expanded variant list to meet the growing needs of NGS tumor profiling assay developers.

Genomic DNA from the GM24385 cell line was blended with 113 clinically relevant biosynthetic DNA variants including single nucleotide variants (SNVs), insertions and deletions (indels), structural variants (SVs), copy number variations (CNVs), and mono- and dinucleotide biomarkers for microsatellite instability (MSI). Three mixes were made: AF7% in which all variants were targeted at variant allele frequency (VAF) of 7% and CNVs at 6 additional copies, AF10% in which all variants were targeted at VAF of 10% and CNVs at 12 additional copies, and Tri-Level in which certain variants were targeted at 4%, 7%, or 10% and CNVs at 3, 6, or 12 additional copies. All VAFs and CNVs were measured using the Bio-Rad QX-200 Droplet Digital PCR (ddPCR) System. DNA quality was analyzed with the Agilent TapeStation Genomic DNA ScreenTape assay. The mixes were analyzed with the Illumina TruSight™ Oncology 500 (TSO 500) assay and additional assays.

All biosynthetic SNVs, indels, SVs, and CNVs were detected near target values and DIN (DNA integrity number) values were >7. The VAFs and CNVs observed in the TSO 500 data correlated well with those that were obtained by dPCR. Variants reported by all evaluation site assays showed good concordance of VAF values. Even when variant callers did not report an expected variant, raw data files were investigated and reads with evidence of those variants were discovered.

The performance of the Seraseq Mutation DNA Mix v3 materials across NGS assays, supported by robust digital PCR data, demonstrated that these materials will allow for assessment of more complex and tumor profiling tests using a single reference sample.

#6637 Whole exome analysis of oral squamous cell carcinoma in local patients at tertiary care hospitals in Peshawar, Pakistan.

Faisal Khan[1], Hina Zamir[1], Khudeja Salim[1], Madina Shirdel[1], Arsalan Riaz[1], Aqsa Hussain[1], Yusra Ilyas[1], Momal Agha[1], Zainab Jahan[1], Saba Saeed[1], Khadim Shah[2], Yasir Rehman[2], Fahim Uddin[2], Zahid Qayyum[2], Zubair Durrani[3], Muhammad Mushtaq Khattak[2], Johar Ali[4]

[1]Precision Medicine Lab, Peshawar, Pakistan,[2]Hayatabad Medical Complex, Peshawar, Pakistan,[3]Rehman Medical Institute, Peshawar, Pakistan,[4]Usman Institute of Technology, Karachi, Pakistan

The Precision Medicine Lab (PML) was established by the Federal Government in 2018 to pilot precision medicine in Pakistan using genomics in oral cancer. Oral squamous cell carcinoma (OSCC)—often called the "poor man's cancer"—constitutes 90% of all head and neck cancers and is the most common cancer in Pakistani males. Supported by a bio-repository of clinical samples and primary cell lines, PML employs a multi-omic approach to identify actionable biomarkers for diagnostic, prognostic, and therapeutic applications.

With ethical approvals from IRBs and informed consent, treatment-naive biopsy-confirmed OSCC patients were recruited from two tertiary care hospitals in Peshawar, Pakistan. The cohort predominantly included older individuals (37.84% aged 41-60 years), late-stage cases (78.46% in stage IV), and self-reported non-alcohol consumers (96.9%). Smokeless tobacco use was reported by 44.6%, while 30.8% reported both smoked and smokeless tobacco use. Tumors were mostly located in the buccal mucosa (29.2%), alveolar region (27.7%), or vestibular region (24.6%). Blood and tumor samples were collected for whole exome sequencing, transcriptomics, and metabolomics, along with saliva for oral microbiome analysis. Combined with clinical history and histopathology, this multi-omic dataset enables machine learning experiments.

For somatic mutation analysis, raw reads were aligned to the GRCh38.p14 genome, and variants were identified using GATK (v4.5). Mutational analyses with maftools (v2.18.0) in R (v4.3) referenced the COSMIC SBS Signature database (v3.2). Results revealed CASP8 (39%), TP53 (38%), FAT1 (29%), and NOTCH1 (29%) as frequently mutated genes. Pathways such as NOTCH (80.3%), RTK-RAS (64.3%), and Hippo (60.7%) were commonly affected. Deletions were observed in over 60% of samples in chromosomal regions 5q13.2, 6p21.32, 6p21.33, 11p15.5, 19q13.42, and 22q11.23, along with loss of heterozygosity at chromosome 17. The top three mutational signatures included those linked to DNA mismatch repair (SBS6).

This study is the first to report the mutational landscape of an OSCC cohort from Pakistan. Our findings highlight potential targets for further investigation and emphasize the utility of a multi-omic approach. With matched primary cell lines for each patient, experimental validation of identified biomarkers is underway to assess their diagnostic, prognostic, and therapeutic relevance. Our integrated dataset offers a resource for machine learning studies to identify novel mutational signatures and actionable biomarkers for OSCC in local populations.

#6638 Identifying cancer germline variants associated with patient prognosis and response by applying clinico-genomics data.

Si Wu[1], Margaret Tsui[2], Robert T. McLaughlin[1], Hiteshi Dehal[1], Conor M. O'Donoghue[1], Jifeng Qian[1], Kevin Zhao[1], Bridget Riley Gillis[3], Fedik Rahimov[3], Josue Samayoa[1], Xi Zhao[1], **Weilong Zhao**[1]

[1]Abbvie South San Francisco, South San Francisco, CA,[2]UC San Francisco, San Francisco, CA,[3]Genomics Research Center, Human Genetics, Abbvie, North Chicago, IL

Germline variants have been recognized as a key determinant in cancer susceptibilities, patient prognosis and treatment outcome. Previous studies have highlighted significant differences in pathogenic variant frequencies across different populations, underscoring the influence of ancestry-specific genetics on cancer risk, survival and drug response. Leveraging extensive real-world data from ConcertAI® electronic health records (EHR) linked with Caris Life Sciences genomic data, we analyzed germline variants in a diverse multi-cancer cohort of 2,776 tumors, including five indications: non-small cell lung cancer (NSCLC, 1,514), colorectal cancer (860), pancreatic (218), gastroesophageal (96), and breast (88) cancers. Notably, we observed a higher proportion of African Americans in breast cancer and more males with gastroesophageal and pancreatic cancers, mirroring US cancer incidence across racial and gender groups. Following germline variant calling by DNA-seq pipeline and annotation by snpeff and snpsift dbSNP, we filtered out low quality and suspected somatic mutations. Utilizing the ClinVar database and the CharGer tool, we identified pathogenic variants in cancer risk genes for each cancer type. When comparing the frequencies of pathogenic variants in ASCO-recommended high-risk genes between our pan-cancer cohort and TCGA, we found that the frequencies were generally comparable. Subsequently, we tested for the association of these pathogenic variants with patient overall survival. Statistically significant associations were detected between patient poor survival and pathogenic variants in *STK11* ($p = 0.005$, in lung NSCLC), *SMAD4* ($p = 0.013$, in colorectal cancer), and *CDKN2A* ($p = 0.034$, in pancreatic cancer), alongside novel associations with *PTH* ($p = 0.012$ and 0.024 in gastroesophageal and pancreatic cancers) and *HFE* ($p = 0.037$, in pancreatic cancer). Intriguingly, Asian patients exhibited a much higher frequency of *PTH* variants (allele frequency > 0.30 %) than other races, while *HFE* variants were more common in Hispanic/Latino populations, reported to cause hereditary hemochromatosis. Although no significant association with immunotherapy (IO) response was detected in the curated NSCLC IO response cohort, the potential links with additional IO markers (e.g., tumor mutational burden, immunologic constant of rejection score, Miracle score) warrant further exploration. Our study describes an innovative approach for investigating the relationships between germline variants and cancer patient prognosis and treatment responses using the EHR linked genomics data — an area often under-explored. Our approach integrates genetic profiling with comprehensive clinical data to characterize germline variants that may serve as predictive biomarkers, ultimately aiming to advance personalized medicine and improve patient outcomes.

#6639 Optimized selection of tumor-informed MRD panels enhances sensitivity of ctDNA detection.

Ashley Acevedo, Ravi Patel, Kyle Trettin, Nafei Xu, Genevieve Gould, Dale Muzzey

Myriad Genetics, Inc., Salt Lake City, UT

Circulating tumor DNA (ctDNA) is a highly sensitive and specific biomarker for molecular residual disease (MRD) and can be by identified by the presence of tumor-specific somatic variants. We have developed a second-generation hybrid-capture-based tumor-informed MRD assay to detect the presence and quantity of ctDNA in plasma-derived cell-free DNA (cfDNA). Our assay surveys the whole tumor genome to identify the superset of high-confidence somatic variant targets that can be used to distinguish ctDNA from non-tumor cfDNA. Each panel consists of up to 1000 targets that are selected based on target-specific and tumor-specific features that impact the representation of tumor variants or the effective molecular depth of a panel, such as somatic call confidence, zygosity, copy number, background error rate and expected molecular depth following capture and sequencing. cfDNA captured using a patient-matched panel undergoes targeted sequencing and that sequence data is fit to a statistical model that evaluates the presence and fraction of ctDNA in the sample. Here, we evaluated the performance of our target-ranking algorithm using contrived mixtures of matched tumor and normal cell lines, with tumor DNA concentrations ranging from 5 to 10,000 parts per million (ppm) and samples processed across multiple days, reagent lots, operators and instruments. Identical sets of contrived samples were analyzed using subsets of panel targets, including top-ranking and bottom-ranking target sets. We found the Limit of Detection with 95% sensitivity (LoD95) for top-ranking targets was 40% of the LoD95 for low-ranking targets, demonstrating the effectiveness of our ranking algorithm in stratifying high and low sensitivity targets. In conclusion, the constituents of tumor-informed MRD panels have a measurable impact on the sensitivity of ctDNA detection and, with optimization, can enhance the resolution of residual disease detection.

#6640 The characteristics of IgD subtype myeloma with distinctive genomic mutational signature and gene expression patterns.

Sheehyun Kim[1], Hyundong Yoon[2], Youngil Koh[1], Sung-Soo Yoon[1]

[1]Seoul National University Hospital, Seoul, Korea, Republic of,[2]Seoul National University Cancer Research Institute, Seoul, Korea, Republic of

Background: Multiple myeloma (MM) is characterized by the neoplastic proliferation of a single clone of plasma cells that produce monoclonal immunoglobulins. Among its subtypes, immunoglobulin D multiple myeloma (IgD MM) is rare, accounting for less than 2% of all MM cases. IgD MM is often associated with rapid disease progression at diagnosis, an aggressive clinical course, and a poor prognosis compared with other MM subtypes.
Methods: Samples were collected from patients diagnosed with MM through bone marrow examination, and myeloma cells were isolated using the CD138-positive marker. Paired datasets (n=37), including whole genome sequencing (WGS) and bulk RNA sequencing (RNA-seq), were generated and analyzed. We utilized whole exome sequencing (WES) and RNA-seq data from the CD138-positive fraction of bone marrow mononuclear cell (BMMC) samples provided by the Multiple Myeloma Research Foundation (MMRF) CoMMpass study.
Results: The WGS data revealed an average of 8,872 single nucleotide variants (SNVs) and 934 insertions and deletions (Indels) of somatic mutations in 37 MM patients, respectively. Using these somatic mutations, we performed a mutational signature analysis to infer the cause of the malignancy and were able to identify different proportions of mutational signatures in each MM patient. Interestingly, among the single base substitution (SBS) 9 signature varied widely between patients, four patients with non-IgG and non-IgA heavy chain and lambda light chain myeloma had significantly high SBS9 signature. Notably, three IgD MM patients were included exclusively in the high SBS9 signature group and not in the low SBS9 signature group ($p = 0.001$). Analyzing RNA-seq data, we found that the high and low SBS9 signature groups had different gene expression patterns. In particular, differentially expressed genes (DEGs) analysis suggested features of IgD myeloma, including high expression of *IGHD,* in high SBS9 signature group. Pathway gene expression analysis showed higher expression of genes associated with the DNA repair system and somatic hypermutation in IgD MM patients. We performed the identical mutation signature analysis on WES data of 784 MM patients from the MMRF database. Of the 30 patients for whom sufficient somatic mutations could be obtained, there were three IgD MM candidates with a high SBS9 signature, whereas there were no IgD MM candidates in the low SBS9 signature group. Similarly, three high SBS9 patients with suspected IgD MM showed characteristic gene expression patterns and upregulation of the DNA repair system.
Conclusions: We found that IgD MM has distinctive features, including high SBS9 mutation signatures and upregulation of genes related to the DNA repair system and somatic hyper-mutation. Further research into IgD MM may reveal the detailed characteristics and underlying mechanisms of IgD MM and lead to tailored treatments.

#6641 Inclusion of INDEL somatic variants in MRD panels improves confidence in ctDNA residual disease detection.

Ravi Patel, Elise Buser, Nafei Xu, Matthew LaBella, Kyle Trettin, Long Tian, Ashley Acevedo, Christopher Battey, Genevieve Gould, Dale Muzzey

Myriad Genetics, Inc., Salt Lake City, UT

Molecular residual disease (MRD) tests detect residual tumor in a patient by identifying the presence of circulating tumor DNA (ctDNA) in cell free DNA (cfDNA). We have developed a highly sensitive MRD test that utilizes panels of hybridization probes targeting somatic variants specific to each patient's tumor. Our tumor-informed MRD assay utilizes whole genome sequencing (WGS) of tumor and normal tissue followed by a somatic calling pipeline to identify tumor variants and generate a panel targeting these variants. The sensitivity of bespoke MRD tests, like ours, is a function of each patient's panel content. A major challenge of ctDNA detection at low tumor concentrations is differentiation of true tumor variants from technical errors. The variants that are most informative for detection of low tumor fraction residual disease are those that are the least likely to occur due to PCR errors or other artifacts during sample preparation. Here we assessed the technical error rates of single nucleotide variants (SNVs) and insertions and deletions (INDELs). In our experiments, many INDELs were less likely to occur due to PCR errors compared to many SNVs, though the error rates of individual variants depended on their genomic context. Several features impacted the error rate of individual INDEL variants in cfDNA including the number of bases inserted or deleted in the INDEL variant and the proximity of the INDEL variant to homopolymer regions. The lower error rate from many INDEL variants resulted in more signal to noise when detecting the presence of the tumor variant. We identified INDEL somatic variants using an ensemble of somatic callers with 99.8% confirmation rate. INDELs were typically less prevalent than SNVs but we observed that INDELs made up as much as 26.4% of a 1000 variant panel. To assess the impact of INDELs on our ability to detect residual tumor, we ran our MRD assay on patients with residual disease. Our detection pipeline was run using a 1000 probe panels that included both INDELs and SNVs or just SNVs alone. INDELs improved the sensitivity of our assay; we observed 60-90%lower error rates for INDELs with more than a single base inserted or deleted compared to SNVs in cfDNA. In conclusion, the likelihood of detecting low abundance ctDNA is higher for panels with INDELs included than for panels without INDELs. Overall, our assay achieved high sensitivity at low tumor concentrations by prioritizing the inclusion of low error rate variants including INDELs in bespoke patient panels.

#6642 Inter-alpha-trypsin inhibitor heavy chain (ITIH) genes: expressional profile in colorectal cancer and potential targeting by using a ribosome inactivating protein.

Aiman Shahzad[1], **Asim Pervaiz**[1], Afraz Numan[2], Sana Iqbal[1], Martin R. Berger[3]

[1]Human Genetics and Molecular Biology, Univ. of Health Sciences, Lahore, Pakistan,[2]Institute of Allied Health Sciences, Univ. of Health Sciences, Lahore, Pakistan,[3]Immunodiagnostic, Bensheim, Germany

Introduction: Expression deregulation of Inter-α-Trypsin Inhibitor Heavy (ITIH) genes at circulatory and tissue levels has been reported in various cancers. These altered levels are linked with worse pathogenesis and low treatment response. ITIH genes are being considered as novel prognostic/ therapeutic targets. Knowledge about ITIH expression profile in primary/metastatic cancers and regulation control via synthetic/natural anticancer compounds deemed crucial. Present study was designed to uncover these facets in colorectal cancer.
Materials & Methods: Total RNA was extracted from frozen colorectal cancer clinical isolates and normal mucosa specimens followed by cDNA synthesis and real-time PCR for expressional analysis of the genes. Labelled rat colorectal cancer cells (CC531) were transplanted into the rat liver via the hepatic portal vein and were re-isolated by FACS after discrete time intervals (3, 6, 9, 14 and 21 days) followed by RNA extraction and cDNA microarray analysis. A fraction of re-isolated cells was cultured *in vitro* for 14 and 22 days to compare the results with those from tumor cells grown *in vivo*. Primary (SW480 and HCT116) and metastatic (SW620) colorectal cancer cell lines were exposed to riproximin (10 and 20ng/ml, 48 hours) followed by RNA extraction, cDNA synthesis, expressional profiling of the genes (ITIH3 and ITIH4) and fold change identification by $2^{\Delta\Delta}$CT method. The results were compared with those of untreated control cells.
Results: Both members of the ITIH family were continuously down-regulated in the clinical isolates. However, ITIH3 gene was more substantially down-regulated (-26fold average, Stage II) in the tissue samples of colorectal cancer patients as compared to ITIH4 gene (-9fold average, Stage II). *In vivo* model mimicking the colorectal cancer liver metastasis depicted a marked increase in the expression of both genes throughout the experimental period (21 days). Precisely, a maximum induction was observed in ITIH3 gene at day 03 of implantation (295fold), while ITIH4 was maximally induced at day 21 (246fold). Exposure with the plant protein (riproximin) induced the expression of ITIH3 gene in the three colorectal cancer cell lines. In contrast, riproximin exposure inhibited the ITIH4 gene in the three colorectal cancer at all applied concentrations.
Conclusion: ITIH3 and ITIH4 genes were consistently down-regulated in clinical isolates. In contrast, a substantial induction was observed in metastatic colorectal liver cancer animal model. Riproximin induced expressional alterations in gene specific format in the colorectal cancer cell lines. Further investigations are needed to understand substantial high levels of ITIH genes during colorectal cancer liver metastasis and potential targeting via riproximin.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Metabolism and Signaling
Poster Session

#6646 Fibroblast growth factor receptor signaling modulates cholesterol storage in a SOAT1-dependent manner to promote mammary tumor cell invasion.

Jennifer E. Tuokkola, Lyndsay E. Reese, Ying Wang, Christine H. O'Connor, Annisa H. Rumahorbo, Kathryn L. Schwertfeger

Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, MN

Signaling by fibroblast growth factor receptors (FGFRs) is active in up to 85% of breast cancers and results in enhanced proliferation, migration, and invasion of tumor cells. Here, we show that FGFR signaling regulates cholesterol metabolism in breast cancer. Specifically, we demonstrate that FGFR activation promotes cellular cholesterol storage by upregulating expression of the enzyme sterol O-acyltransferase 1 (SOAT1). Moreover, we demonstrate that inhibition of SOAT1 attenuates FGFR-driven colony formation and invasion in tumor cells. Finally, we identify a relationship between SOAT1 inhibition and decreased expression of matrix metalloproteinases by tumor cells. Taken together, these findings suggest a previously undiscovered metabolic role for FGFR signaling in breast cancer and present a therapeutic vulnerability that could be targeted in FGFR-driven cancers.

#6647 Targeting DCLK1-mediated glutamine addiction in chemoresistant ovarian cancer.

Samrita Dogra[1], Cole Hladik[2], Sugantha Priya Elayapillai[1], Bethany Hannafon[1]

[1]OU Health Stephenson Cancer Center, Oklahoma City, OK,[2]The University of Oklahoma Health Sciences Center, Oklahoma City, OK

Ovarian cancer (OC) presents significant therapeutic challenges due to its frequent late-stage diagnosis accompanied by distant metastases and the development of chemoresistance. As existing FDA-approved targeted therapies for OC do not adequately address peritoneal metastases, there is an urgent need for innovative treatment strategies that specifically target the molecular and cellular pathways underlying metastasis and disease recurrence. Doublecortin-like kinase 1 (DCLK1) is a serine/threonine kinase with a demonstrated role in cancer cell stemness, epithelial-mesenchymal transition (EMT), disease progression in gastrointestinal and pancreatic cancers and resistance in ovarian and lung cancer. The study objective is to evaluate the functional role of DCLK1 in the peritoneal metastasis of OC and investigate the associated benefits of antagonizing it as a novel strategy to mitigate OC metastasis. Our prior studies demonstrate DCLK1 inhibition reduces the number of visible metastases in an orthotopic OC model in mice. In this study, we used pharmacological inhibition (DCLK1-IN-1), and genetic manipulation (DCLK1 CRISPR-knockout (KO) and over-expression systems) to assess the role of DCLK1 in promoting OC metastasis. We observed that elevated DCLK1 expression enhanced OC cell adhesion to different extracellular matrices including collagen, fibronectin, and laminin. Furthermore, DCLK1 inhibition effectively reduced mesothelial cell clearance relative to control. Targeted gene expression profiling and pathway analysis revealed DCLK1 inhibition in cisplatin resistant (CPR) OC spheroids upregulated genes involved in glutamine metabolism. Using metabolic flux assays, we demonstrated increased oxygen consumption in DCLK1 KO CPR cells that was abrogated by BPTES (glutamine oxidation inhibitor). Furthermore, we show that combined treatment of OC CPR spheroids with DCLK1 inhibitor (DCLK1-IN-1) and glutamine antagonist (DRP-104) showed a synergistic cytotoxic effect. In addition, the novel combination strategy was significantly effective in reducing OC cell adhesion and mesothelial cell clearance relative to single agent alone. Collectively, our findings establish DCLK1 as a key regulator of metastasis and demonstrate that its inhibition induces glutamine dependency in chemoresistant tumor cells. Thus, the combined use of DCLK1 inhibitors (e.g., DCLK1-IN-1) with glutamine metabolism inhibitors (e.g., DRP-104) represents a rational therapeutic approach to mitigate peritoneal metastases in a chemoresistant setting.

#6648 Novel integrated stress response (ISR) kinase inhibitors have activity in multiple cancer cells.

Aurelien Laguerre, Martin Smrcina, Ivy Nai-Jung Hung, Devin K. Porter, Nathan G. Hedrick, Dmitri Rozanov, Mark R. Hansen, **Vikas K. Goel**

Zafrens Inc., San Diego, CA

The integrated stress response (ISR) is an adaptive cellular program that facilitates survival and proliferation under conditions of cellular stress and can be appropriated to contribute to the tumorigenesis of multiple cancers. ISR is activated in response to various cues in the tumor microenvironment and cell-intrinsic factors but is ultimately regulated by four core serine/threonine kinases: GCN2 (activated by amino acid deprivation), HRI (in response to heme deficiency), PERK (unfolded protein response stress), and PKR (dsRNA stress response of infection and oxidative stress). These four ISR kinases play a central role in cancer cell equilibrium, allowing them to proliferate by adapting to adverse conditions, immune evasion, and metastasis. All ISR kinases phosphorylate a common downstream effector, eIF2a, at Ser51. peIF2a^{S51} shuts down cap-dependent translation and results in the up-regulation of ATF4, a transcription factor that controls the transcription of genes essential for cancer cells to adapt to stress and nutrient deprivation. Due to the observation that adaptive compensation and rewiring in cancer cell pathways can occur in response to inhibition of single signaling nodes, it has been suggested that simultaneous inhibition of multiple ISR kinases may be required to improve anti-cancer response or counteract drug resistance. We developed an initial high-throughput ATF4 immunofluorescence (IF) assay to screen chemical matters in low- to no-glutamine culture conditions. We identified three potential subseries of compounds of the type II kinase inhibitor class. Structure modeling and docking suggested that these subseries differ in inhibitory profiles against four ISR kinases, and we decided to focus on two of these subseries to develop potent ISR inhibitors further. Multiple hits (ZF125, ZF163, ZF284, ZF302) demonstrated low nM potency in the cellular target engagement assay (ATF4 IF) and biochemical kinase assay against three ISR kinases. RNA transcriptomic analysis confirmed that ZF-ISR inhibitors downregulate several ISR kinase target genes, such as DDIT3 and SLC1A5, ATF4, CEBPb, and TRIB3. Our lead molecule has also demonstrated high selectivity in a select kinome screen (40 kinases) at 1uM, except for ISR kinases. Zafren's lead ISR (ZF-ISR) inhibitors have shown a growth inhibitory effect in multiple cancer cells with 10mU/ml of ASNase of ASNase. Interestingly, most of these cancer cell lines harbor RAS/RAF/MPAK pathway mutations. DepMAP analysis revealed other potential gene signatures as combination strategies with ZF-ISR inhibitors. As determined by multiple ADME assays, ZF-ISR inhibitors have also shown favorable drug-like property profiles. In-vivo tumor growth inhibition studies are in progress.

#6649 Pharmacological inhibition of HuR deregulates mitochondrial function and reduces mitochondrial activity in cancer cells.

A. M. Vu[1], M. Mehta[1], S. Jayaraman[1], M. Fuller[2], R. Ramesh[1], A. Munshi[1];
[1]University of Oklahoma Health Sciences, Oklahoma City, OK, [2]Michigan State University, East Lansing, MI

The RNA-binding protein, human antigen R (HuR), is overexpressed in many cancer types, and regulates genes linked to tumor growth, angiogenesis, cell cycle, and chemoresistance. HuR overexpression correlates with increased tumor size, invasiveness, and poor prognosis. Inhibiting HuR through pharmacological or RNA interference (RNAi) methods leads to antitumor activity both *in vitro* and *in vivo* against a broad spectrum of human cancers. Molecular mechanisms of antitumor activity include cell cycle arrest, increased ROS production, and enhanced DNA damage culminating in apoptotic cell death. However, the role of HuR in cancer metabolism and mitochondrial dynamics remains largely unexplored. In the present study we investigated the effects of the HuR inhibitor, CMLD-2, on glycolysis, mitochondrial function, ROS production, and mitophagy in human breast (MDA-MB-231) and lung (H1299) cancer cell lines. Both cell lines were treated with vehicle (DMSO) or CMLD-2 (20 μM or 30 μM) and analyzed at 24 or 48 hours post treatment. CMLD-2 treated cells showed a dose- and time-dependent reduction in markers of glycolysis and oxidative phosphorylation (GLUT1, GLUT4, HK2, LDHA), and a decrease in OXPHOS complex proteins. CMLD-2 treatment also led to a significant reduction in the rate of glycolysis (ECAR) and mitochondrial oxidative phosphorylation (OCR) as measured by Seahorse metabolism assays. Transmission electron microscopy (TEM) analysis showed CMLD-2 treatment increased autophagosome formation, mitochondrial fragmentation, and reduced mitochondrial count. Additionally, CMLD-2 treatment increased ROS production, with a 1.3- and 1.6-fold rise in MDA-MB-231 cells at 20 μM and 30 μM, respectively, and a 1.1- and 1.3-fold increase in H1299 cells at 20 μM and 30 μM, as measured by DCFDA fluorescence. Immunoblotting of AMPK-dependent autophagy signaling showed a time- and dose-dependent increase in LKB1, phospho-AMPK (Thr172), phospho-ULK (Ser555), phospho-FOXO3 (Ser413), p62, and LC3-I cleavage in CMLD-2-treated MDA-MB-231 cells. In H1299 cells, CMLD-2 treatment increased the above-mentioned markers except for phospho-ULK (Ser555), suggesting ULK-independent autophagy activation. Confocal microscopy of mitochondria (using Mitotracker Red CMX Ros) and LC3 (using LC3-GFP expression plasmid) in CMLD-2 treated cells, revealed colocalization of mitochondria with autophagosomes in both cell lines, indicating mitophagy. Our study demonstrates HuR inhibition results in mitochondrial dysfunction and significant metabolic changes in cancer cells, providing insights into the potential of HuR-targeted therapies for cancer treatment.

Funding**:** The study was supported in part by a grant received from the National Institutes of Health (R01 CA 282735-01) and American Cancer Society (ACS-DICR: INTR-23-1253708-01-DICR INTR).

#6650 Targeting the PI3K pathway with alpelisib in patient-derived xenografts: a multi-cancer type approach.

Kapur B. Dhami[1], Chiya-Kuei Mo[1], Zhanfang Guo[1], Richard Kurupi[1], Preet Lal[1], Kazuhito Soto[1], Xiyi Wei[1], Michael C. Wendl[1], Jacqueline Mudd[1], Shunqiang Li[1], Sherri R. Davies[1], Jeff Chuang[2], Bingliang Fang[3], Ryan Fields[1], Hui Zhang[4], Meenhard Herlyn[5], Feng Chen[1], Kian-Huat Lim[1], Kian-Huat Lim[1], Cynthia Ma[1], Li DIng[1]

[1]Washington University in St. Louis, School of Medicine, St. Louis, MO,[2]Jackson Laboratory for Genomic Medicine, Farmington, CT,[3]MD Anderson Cancer Center, Houston, TX,[4]Johns Hopkins University, Baltimore, MD,[5]Wistar Institute, Philadelphia, PA

Phosphoinositide 3-kinase (PI3K) pathway has a crucial role in the regulation of cellular proliferation, survival, and metabolism. Frequent aberrant activation of this pathway is one of the most common oncogenic events across various human cancer types including breast (ER+), colorectal, lung, kidney, and skin cancers. We collected and analyzed baseline tandem mass tag (TMT) proteomics data (protein and phosphorylation levels) for 40 samples from 36 PIK3CA PDX models across five cancer types (RCC, Melanoma, CRC, BRCA, and LUAD). Initial Pearson correlation analysis revealed that most samples clustered by cancer type, indicating that tissue specificity contributes more to molecular similarities than oncogenic mutations. When focusing on components of the PI3K pathway, we observed diverse protein levels and activation statuses across the cohort of PDX models. In breast cancer (BRCA), colorectal (CRC), and kidney (RCC) samples, we noted elevated levels of total and phosphorylated AKT, which were lower in lung squamous cell carcinoma (LUSC) and melanoma samples. Interestingly, we also found heterogeneous activation statuses of components in the PI3K pathway in BRCA samples. A subset of BRCA samples exhibited high levels of total AKT and phosphorylation at T479 and S477, alongside elevated TSC1 and TSC2 activation. Since TSC proteins are known to inhibit PI3K pathway activity, their elevation could indicate feedback inhibition of the pathway even with PIK3CA mutations. To further elucidate the diverse treatment response profile of the PIK3CA mutant PDX lines across cancer types in mice, we assembled a cohort of 3 cancer types, BRCA, ccRCC, and PDAC. Despite the varied mutation profiles, we observed that responses to Alpelisib differed among the RCC PDX lines, with insignificant effects in PDMR-274T, no response in RESL4, and a modest effect in RESL10. In the BRCA study, out of 6 treated ER+ PDX lines, we observed significant responses in WHIM9 and WHIM20. Moderate responses were seen in WHIM84 and WHIM18, while HCI-011 and WHIM16 showed no response. Proteogenomic analyses are ongoing to discern response biomarkers.

#6651 β-catenin-mediated aerobic glycolysis: a marker of poor prognosis in triple-negative breast cancer.

Chandra Prakash Prasad[1], Sheikh Mohammad Umar[1], Shruti Kahol[2], Sandeep R. Mathur[2]

[1]Medical Oncology, All India Institute of Medical Sciences (AIIMS) New Delhi, New Delhi, India,[2]Pathology, All India Institute of Medical Sciences (AIIMS) New Delhi, New Delhi, India

Aerobic glycolysis has emerged as a crucial regulator of cancer cell migration and metastasis in various cancers including TNBCs. Characterization of molecular pathways associated with increased aerobic glycolysis in TNBCs could provide key mechanistic insights into the regulators of TNBC progression. Here, we demonstrated for the first time the theranostic potential of the β-catenin-PFKP-LDHA-MCT1 axis that drives aerobic glycolysis in TNBCs. β-catenin and related glycolytic proteins (PFKP, LDHA, and MCT1) were analyzed in triple-negative breast cancer (TNBC) patients (n=98) using immunohistochemistry (IHC). Prognostic significance was assessed through Kaplan-Meier survival analysis and Cox regression. Additionally, we compared the efficacy of two β-catenin inhibitors, XAV939 and Axitinib, in suppressing β-catenin-driven aerobic glycolysis in both *in vitro* and *in vivo* TNBC models. We showed that β-catenin and its associated glycolytic proteins (PFKP, LDHA, and MCT1) are overexpressed in more than 50% of TNBC cases. Kaplan-Meier survival analysis and Cox regression revealed that elevated β-catenin expression, combined with high levels of other glycolytic proteins (β-catenin+MCT1^{high}; β-catenin+PFKP+MCT1^{high}; β-catenin+LDHA+MCT1^{high}; and β-catenin+PFKP+LDHA+MCT1^{high}), was linked to poor prognosis, highlighting the role of β-catenin-regulated aerobic glycolysis in TNBC aggressiveness. Additionally, we found that Axitinib, compared to XAV939, more effectively reduced β-catenin and glycolytic protein expression, lactate production, cell migration, invasion, and colony formation in TNBC cell lines. In conclusion, this study is the first to identify a novel β-catenin-regulated aerobic glycolysis pathway in triple-negative breast cancer (TNBC). We also demonstrated that Axitinib is a more effective inhibitor of this pathway than XAV939.

#6652 PDCD4/mTORC2 axis regulates tumorigenesis through PFKFB3 in NSCLC.

Elham Zokaei, Qing Wang, Hsin-Sheng Yang

Toxicology and Cancer Biology, University of Kentucky, Lexington, KY

Programmed cell death 4 (PDCD4) is a tumor suppressor whose expression is frequently downregulated in various cancers, including lung cancer. PDCD4 has been shown to suppress tumor cell proliferation, migration, invasion, and metastasis in cultured cells and in mouse models. It has also been suggested to play a role in regulating glucose metabolism; however, the detailed mechanisms remain unclear. Using reverse phase protein array, we found that knockdown of PDCD4 upregulated 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3), a critical enzyme in regulation of glycolysis. PFKFB3 converts fructose-6-phosphate to fructose-2,6-bisphosphate to activate the rate-limiting enzyme, 6-phosphofructo-1-kinase, in glycolysis. PFKFB3 expression is upregulated in lung cancer and correlates with tumor stage progression. Inhibition or down-regulation of PFKFB3 in lung cancer cells induces cell apoptosis and inhibits tumor growth in nude mice. Given that PDCD4 inhibits mTORC2 activity, we next examined how mTORC2 affects PFKFB3 expression. We found that mTORC2 interacted with PFKFB3 in NSCLC cells. Using In vitro kinase assays demonstrated that can phosphorylate PFKFB3. Further analysis pinpointed a Ser residue as the target site, leading to the generation of phosphorylation-deficient (S to A) and phospho-mimetic (S to D) mutants. Stability assays revealed that phosphorylation at this specific Ser is essential for maintaining PFKFB3 protein stability; as the phosphorylation-deficient mutant underwent rapidly degradation, whereas the phospho-mimetic mutant showed increased it's half-life. Functional assays showed that WT PFKFB3 promoted high proliferation rates, whereas the phosphorylation-deficient mutant impaired colony formation and proliferation. Taken together, these findings uncover a key mechanism of metabolic reprogramming in NSCLC, in which mTORC2 drives glycolysis by directly phosphorylating PFKFB3, highlighting the therapeutic potential of targeting the PDCD4/mTORC2/PFKFB3 axis in NSCLC treatment.

#6653 Taurine Transporter *SLC6A6* promotes myeloid leukemia progression by regulating glycolysis.

Sonali Sharma[1], Benjamin J. Rodems[1], Cameron D. Baker[2], Christina M. Kaszuba[3], Edgardo I. Franco[3], Takashi Ito[4], Laura M. Calvi[5], Michael W. Becker[5], Bradley R. Smith[6], Paul S. Brookes[7], Joshua C. Munger[6], Jane L. Liesveld[5], Craig T. Jordan[8], John M. Ashton[2], Jeevisha Bajaj[9]

[1]Wilmot Cancer Institute, University of Rochester Medical Center, Rochester, NY,[2]Genomics Research Center, University of Rochester Medical Center, Rochester, NY,[3]Department of Biomedical Engineering, University of Rochester, Rochester, NY,[4]Department of Bioscience and Technology, Fukui Prefectural University, Fukui, Japan,[5]Department of Medicine, University of Rochester Medical Center, Rochester, NY,[6]Department of Biochemistry and Biophysics, University of Rochester Medical Center, Rochester, NY,[7]Department of Anesthesiology, University of Rochester Medical Center, Rochester, NY,[8]Division of Hematology, University of Colorado Anschutz Medical Campus, Aurora, CO,[9]Wilmot Cancer Institute, Department of Biomedical Genetics, University of Rochester Medical Center, Rochester, NY

Interactions of stem cells with their surrounding microenvironment are known to be essential for both normal development and for sustaining self-renewing adult stem cells, such as the hematopoietic stem cells (HSCs). Since cancers often hijack developmental signals for their progression, it is likely that niche-driven signals that sustain HSCs also influence the growth of leukemias arising from mutations in HSCs and early hematopoietic progenitors, such as acute myeloid leukemia (AML) and blast crisis chronic myeloid leukemia (bcCML). To identify candidate cell surface receptors essential for myeloid leukemia progression, we employed a comprehensive approach integrating our *in vivo* genome-wide CRISPR screen and our RNA-seq analysis of human LSCs from distinct myeloid leukemia subtypes. Of these, the taurine transporter (TauT), encoded by *SLC6A6*, was of particular interest since its expression is associated with poor prognosis in acute myeloid leukemia (AML) patients. Our experiments using TauT knockout mice indicate that TauT loss significantly impairs myeloid leukemia progression *in vivo*. An RNA-Seq analysis of freshly isolated $TauT^{+/+}$ and $TauT^{-/-}$ leukemia cells identified a striking decrease in glycolysis associated genes in the absence of TauT. Consistent with this, Seahorse analysis showed significantly reduced basal glycolysis and glycolytic capacity in $TauT^{-/-}$ LSCs as compared to $TauT^{+/+}$ controls. In addition, inhibiting TauT reduced the abundance of key glycolytic metabolites as measured by LC/MS spectrometry. Our experiments showing significant downregulation of mTOR pathway in the absence of TauT indicate that mTOR signaling may regulate glycolysis in leukemia cells. Consistent with a functional role of mTOR downstream of TauT function, our data shows that activating mTOR pathway in $TauT^{-/-}$ cells can rescue expression of glycolysis related genes and leukemic cell growth. Importantly, our experiments inhibiting TauT using shRNAs or small-molecule inhibitors identify a key role of this signal in the growth of patient-derived myeloid leukemia cells. Collectively, our data identify a critical requirement of the taurine-TauT axis in myeloid leukemia progression and indicate that the TauT inhibition may be of therapeutic value in myeloid leukemia.

#6654 Investigating the role of fructose-1,6-bisphosphatase in soft tissue sarcoma.

B. Nance, B. Keith, C. Simon;
University of Pennsylvania, Philadelphia, PA

Glucose metabolism is commonly dysregulated in cancer and other pathological contexts. Fructose-1,6-bisphosphatase (FBP) is a key rate-limiting gluconeogenic enzyme that converts fructose-1,6-bisphosphate to fructose-6-phosphate, which produces glucose and glycogen from noncarbohydrate substrates. Two FBP isoforms, FBP1 and FBP2, are differentially expressed in human tissues. FBP1 is expressed in the primary gluconeogenic organs (liver and kidney) to maintain blood glucose levels under fasting conditions. In contrast, FBP2 expression is primarily restricted to striated muscle where it regulates glycogen synthesis. Our recent work demonstrates that both FBP isoforms function as metabolic tumor suppressors and are significantly downregulated in multiple cancers. In sarcoma cells, ectopic FBP2 decreases cytosolic glycolytic flux and FBP2 interacts with nuclear c-Myc to repress transcription of the gene encoding mitochondrial transcription factor A (TFAM), a transcription factor essential for mitochondrial biogenesis. We aim to expand on the roles of FBP2 expression in sarcomas by investigating its binding partners and the role of its catalytic activity in vitro and in vivo. To explore this, we have ectopically expressed wildtype FBP2 and catalytically inactive FBP2-E98A at similar levels to human skeletal muscle in sarcoma cell lines and observed their impacts on proliferation, glucose metabolism, colony formation, invasion, gene expression, and tumor growth. Interestingly, both enzymatically wildtype FBP2 and catalytically inactive FBP2 can suppress proliferation and colony formation in vitro suggesting that FBP2 has additional non-catalytic functions contributing to its repressive role in cancer cells. To explore additional roles of FBP2 outside of its catalytic function, we have developed a FBP2-BioID2 fusion protein and performed immunoprecipitation mass spectrometry to identify novel binding partners of FBP2. Surprisingly, our RNAseq and proteomics data suggest that ectopically expressed wildtype FBP2 may enrich for gene sets and putatively bind with components of the cytoskeleton.

Furthermore, to investigate the role of FBP2 loss in sarcomagenesis, we have also utilized autochthonous and injury-induced murine models of undifferentiated pleiomorphic sarcoma with and without Fbp2 loss. We aim to fully characterize these models to elucidate the possible roles of FBP2 in tumor latency, size, and metastasis in sarcoma.

Overall, our work will further define the moonlighting roles of FBP2 by elucidating its interactome and the role of FBP2 in coordinating metabolic transitions in sarcomagenesis.

#6655 Meta-analysis of 70 cancer studies suggests altered pH-sensitive potassium channeling associated with 7 oncogenes and/or DNA modifiers as initiators of pH reversal via cytosolic proton chains.

Marie E. Beckner

Brain Health Research Institute, Kent State University, Willoughby Hills, OH

The potassium channel (KCN) gene family (≥ 90 members) is influenced by upstream modifiers and encodes interactive proteins, many also pH-sensitive. Regulated KCN proteins channel K^+ flux to mediate optimal K^+ levels in cells. Small monovalent cations, preferentially K^+, can displace protons from inner cell membrane anions. Released free protons rapidly become hydronium ions (H_3O^+) that react with a "Grotthuss" type water matrix of H-bonded chains of $H_9O_4^+$ & $H_5O_2^+$ in a "microaqueous proton chain" (MAPC) cytosol. The chains undergo domino-like conformational changes involving H^+ that permit protons entering the matrix to incite quick release of different H^+ elsewhere from matrix edges (Adelroth P, BBA, 1757(2006)867-70). Putatively, elevated release of H^+ from cytosolic MAPC near cell exit sites (transporters of H^+, etc.) allows pH reversal. Methods. Meta-analysis was performed on cancer studies selected for published gene expression landscapes (not Supplemental Materials), that lacked KCN bias yet yielded differentially expressed genes (DEG) of the KCN family to determine the proportion of KCN DEG affecting pH-sensitive K^+ flux and to identify associated non-KCN DEG. Heat maps (46 studies), Venn diagrams, tables, phylogenetic trees, etc. were analyzed for count data & with Fisher's Exact testing. Results & Discussion. Thus far in 70 expression (E) studies, E1-E70 (1301 authors), of 29 malignancy types, of the 53 KCN DEG detected, 33 (62.3%) encode H^+-sensitive proteins (135 literature references). 52 of 70 studies (74.3%) contained ≥1 KCN DEG encoding H^+-sensitive proteins. The most frequent KCN DEG, *KCNN4* and *KCNMA1* in 9 and 6 studies, respectively, encode H^+-sensitive proteins. The most common cancers, lung, breast, & glioblastoma, had KCN DEG encoding H^+-sensitive proteins detected in 72.7%, 44.4%, & 85.7% of their respective studies. Also, 50 of 70 studies were in 8 author-designated categories which had relevant DEG present in ≥1 study. Categories in decreasing frequency are Histone/Chromatin/Epigenetic, *TP53*, Epithelial Mesenchymal(Mes) Transition/*TGFβ*/Mes, *EGFR*, *MYC*, *RAS*, *BCL2*, & *MET*. Studies in the 8 categories compared to the other 20 studies differed significantly for detection of KCN DEG encoding H^+-sensitive proteins, $p = 0.0325$. Conclusion. Collectively, malignant gene expression landscapes involving 7 oncogenes and/or DNA modifier scenarios are associated with KCN DEGs encoding H^+-sensitive proteins, possibly as downstream effectors. Potentially, dysregulated inner membrane K^+ levels result in release of membrane-bound protons that (1) incite rapid domino-like proton shifts in a MAPC cytosol resulting in proton release elsewhere for (2) enhanced H^+ efflux achieving (3) pH reversal causing enhanced malignancy via altered enzyme activities, cytoskeletal rearrangements, etc.

#6656 Cancer-derived polyamines regulate adipocyte lipolysis and fat waste in cancer cachexia.

Matias Fabregat, Julia K. Hansen, Hoang V. Bui, Andrea Galmozzi

UW Madison, Madison, WI

Cancer Cachexia (CC) accounts for more than 20% of all cancer-related deaths, rising to 30% in pancreatic cancer cases. Current treatments, which focus primarily on nutritional supplementation to address weight loss, are largely palliative and show limited efficacy. No pharmacologic therapy currently exists for CC. While skeletal muscle loss is a defining characteristic of cachexia, fat wasting often precedes muscle loss and is a stronger predictor of survival than overall body weight loss. Recognizing this, our study aims to identify cancer-secreted factors that drive adipocyte dysfunction and development of cachexia. To achieve this, we measured lipolytic activity in primary adipocytes exposed to conditioned media (CM) derived from various cancer cell lines. Remarkably, cancer cells from tumor types with a high incidence of cachexia, such as pancreatic ductal adenocarcinoma (PDAC) and head and neck cancers, induced significantly greater lipolysis compared to cells from cancers linked to lower cachexia incidence, such as breast cancer. Characterization of cancer-cell-secreted factors identified elevated polyamine levels in pro-cachectic CM. Consistently, we found that data from the Cancer Cell Line Encyclopedia also show an upregulation of the polyamine biosynthetic pathway in pro-cachectic cells. Using a syngeneic PDAC model, we observed that, in tumor-bearing mice, increased circulating lipids and fat loss correlated with elevated polyamine levels in the serum and the adipose tissue compared to sham controls. Mechanistically, high polyamine levels in cultured adipocytes and adipose tissue depots led to hyperhypusination of the eukaryotic translation initiation factor eIF5A. This unique post-translational modification activates eIF5A, enabling it to regulate protein translation, including key enzymes involved in lipolysis. Importantly, inhibiting either polyamine synthesis in cancer cells or eIF5A hypusination in adipocytes effectively blocked the pro-lipolytic effects of cancer cell CM, indicating that polyamines act as cancer-derived signaling molecules to promote lipid mobilization in adipocytes. This study sheds light on tumor-adipose tissue crosstalk and identifies cancer-derived polyamines as key drivers of cachexia. Specifically, polyamines promote the hyperhypusination of eIF5A, thereby enhancing lipolysis. These findings offer a foundation for developing anti-cachectic therapies targeting the polyamine-dependent hypusination of eIF5A. When combined with anti-cancer treatments, such therapies could significantly improve the quality of life and clinical outcomes for cancer patients suffering from cachexia.

#6657 ACLY-mediated acetyl-CoA levels drive bladder cancer progression.

H. Kim[1], J. Lee[2], S.-i. Jo[1], D. Suh[1], H. Lee[1], S. Oh[1], C. Jeong[1], J. Ku[1], C. Kwak[1], S. Park[2], J. Han[1];
[1]Seoul National University Hospital, Seoul, Korea, Republic of, [2]College of Pharmacy Seoul National University, Seoul, Korea, Republic of

Background: As cancers progress, elucidating the underlying mechanisms of metabolic reprogramming caused by genetic alteration in cancer cells may provide a key clue for exploring druggable targets. Targeting unique metabolic reprogramming which contributes to aggressive progression in bladder cancer (BC) remains a major challenge. This study aimed to identify metabolic pathways and druggable targets that influence BC progression and remodel the tumor microenvironment.
Methods: Liquid chromatography mass spectrometry-based untargeted metabolomic profiling was performed on low grade (LG) and high grade (HG) BC tissue samples of patients (N=8). The prognostic value of related metabolic genes was assessed using transcriptomic datasets (TCGA and UROMOL). To investigate the biological mechanisms underlying ACLY-acetyl-CoA-regulated BC progression, we utilized proliferation, transwell invasion, wound healing, colony formation assays, western blotting, immunoprecipitation, and immunohistochemistry, employing T24, J82, UMUC3, and MB49 cell lines and tumor tissues. BC mice models were produced by injection of MB49 cells into the bladder wall and subcutaneous tissues of C57/BL6 and BALB/C nude mice and administrated by daily oral gavage or peritoneal injection of BMS-303141. Tumor growth was monitored longitudinally using caliper measurement and bioluminescence imaging. Altered tumor microenvironment was assess by histological analysis.
Results: Acetyl-CoA was one of the most significantly enhanced metabolites in HG tumor tissues compared to LG tumor tissues. In the acetyl-CoA pathway, ACLY which converts citrate to acetyl-CoA, was found to play a critical role in maintaining tumoral acetyl-CoA levels, firmly linking it to poor prognosis in BC patients. Perturbing ACLY activity through genetic knockdown (siRNA or shRNA) and pharmacological inhibition (BMC-303141) significantly reduced intracellular acetyl-CoA levels, impaired cell proliferation, migration, invasion, and colony formation, suppressed EMT marker expressions (N-cadherin, vimentin), and affected PD-L1 protein levels by acetylation. ACLY and PD-L1 protein expression were positively correlated in BC tissue microarray-based analysis. In line with this, BMS-303141 suppressed tumor growth in both immunocompetent and immunodeficient mice, with more pronounced effects observed in immunocompetent mice, suggesting an additional anti-tumor effect through ACLY-acetyl-CoA-mediated immune modulation.
Conclusion: Our findings indicate that acetyl-CoA is a critical metabolite in BC progression, particularly through ACLY, the primary enzyme responsible for producing intracellular acetyl-CoA in BC cells, linked to increased BC aggressiveness. Targeting ACLY holds therapeutic potential for aggressive BC.
Co-First Authors: HS Kim, J Lee, S-I Jo
Corresponding Authors: JH Han (urohan@snu.ac.kr), S Park (psh@snu.ac.kr)

#6658 Inhibiting glycolysis for the treatment of HGF/MET/TWIST1 driven NSCLC brain metastases.

Xiaxuan Wu[1], Jason Chen[2], Thomas Hintelmann[1], Andrew Zhao[2], Joseph Dosch[2], Rahul Desai[1], Purva H. Rumde[1], Kasey R. Cargill[1], Riyue Bao[2], Vinod Kumar[3], Laura P. Stabile[4], Timothy F. Burns[5]

[1]School of Medicine, University of Pittsburgh, Pittsburgh, PA,[2]UPMC Hillman Cancer Center, University of Pittsburgh, Pittsburgh, PA,[3]Department of Pharmacology & Chemical Biology, University of Pittsburgh, Pittsburgh, PA,[4]Department of Pharmacology & Chemical Biology, University of Pittsburgh, University of Pittsburgh, Pittsburgh, PA,[5]Department of Medicine, Division of Hematology/Oncology, University of Pittsburgh, Pittsburgh, PA

Non-small cell lung cancer (NSCLC) has the highest incidence of brain metastases (BM), with ~40% of patients developing BM. We identified a significant enrichment of *MET* amplification (amp) in NSCLC BM compared to primary lung and liver metastases. MET, a receptor tyrosine kinase, and its ligand, hepatocyte growth factor (HGF), promote proliferation, epithelial-mesenchymal transition, angiogenesis, metastasis and metabolic reprogramming through glycolysis. We have found that *MET* amp NSCLC BM and *MET* altered cells have increased glycolysis compared to *MET* wildtype (WT) BM and cells. We have also shown that TWIST1 is regulated by the HGF/MET pathway, essential for MET-driven NSCLC and MET TKI resistance and responsible for HGF/MET-driven hexokinase II (HK2) expression in *MET* altered cells. HK2, a critical node for metabolic regulation, catalyzes the first step in glucose metabolism. Thus, we hypothesize that targeting glycolysis in MET-driven NSCLC may be an effective therapeutic strategy. We have previously shown that metastatic H1993 (*MET* amp) cells which were derived from the same patient as primary lung H2073 (*MET* WT) cells exhibit increased glycolysis and increased glycolytic dependency. We have now found that TWIST1 was sufficient to drive glycolytic dependency as TWIST1 overexpression (OE) in H2073 cells enhanced sensitivity to the HK2 inhibitor 2-deoxyglucose (2DG), while TWIST1 knockdown in H2073 cells decreased sensitivity to 2DG. Interestingly, TWIST1 OE in H1993 cells increased HK2 levels, leading to 2DG resistance but increased sensitivity to the downstream glycolytic inhibitor, PFK158 with similar or decreased sensitivity to oxidative phosphorylation (OXPHOS) inhibitors. To examine whether glycolytic inhibitors would be effective at the time of MET TKI resistance, we used our *MET* altered models of acquired MET TKI resistance, H1993 CR and UW-21 CR. H1993 CR (low HK2) cells exhibited reduced sensitivity to 2DG but increased sensitivity to PFK158 and AT-101, while UW-21 CR (high HK2) cells showed reduced sensitivity to all three inhibitors compared to parental cells. We then examined whether glycolytic inhibition would be effective against *MET* amp driven EGFR TKI resistance. HCC827R2 (acquired *MET* amp) cells demonstrated increased HK2 protein expression and heightened sensitivity to 2DG and AT-101 compared to HCC827 cells. In a more relevant *ex vivo* brain slice model, we found that H1993 brain colonization was inhibited by 2DG and AT-101 but not OXPHOS inhibitors, while H2073 colonization showed limited response to glycolytic inhibitors but more sensitivity to OXPHOS inhibitors, consistent with our previous findings in patient BM. Remarkably, H1993 CR cells which were resistant to glycolytic inhibition *in vitro* were sensitive to 2DG *ex vivo*. These findings suggest that MET-driven glycolytic dependency plays a significant role in BM, highlighting glycolysis as a potential therapeutic target.

#6659 Defective lipid homeostasis induced by QKI loss is a mutator on gliomagenesis.

Y. Guan, T. Shingu, K. J. Lu, E. Kilinc, T. G. Hart, J. Zhang, Y. Chen, B. Y. S. Kim, J. Hu;
UT MD Anderson Cancer Center, Houston, TX

Glioblastoma (GBM) is the most malignant form of primary brain tumor. It is also one of the deadliest and most treatment-resistant cancers with more than 14,490 cases diagnosed in the United States in 2023. Tumorigenesis is a multistep process that starts with the occurrence of an oncogenic mutation in a single somatic cell. Despite extensive information about mutators for many cancers, the specific mutator driving GBM development remains unclear. Interrogating human glioma genomic and transcriptomic data revealed that defective lipid metabolism is associated with increased structural variants, particularly extrachromosomal DNA (ecDNA) formation. Quaking (QKI) is frequently mutated or deleted in human GBMs. Mechanistically, QKI acts as an RNA binding protein to regulate pre-mRNA stability of genes that regulates lipid components of endolysosomes, particularly the unsaturated fatty acids. We identified that QKI modulates neural stem cells and glioma stem cells lipid metabolism and further maintains membrane integrity in mitochondria and nuclear envelope. Deleting Qki on the *Nestin-CreER^{T2}; Pten$^{L/L}$; Trp53$^{L/L}$* background (QPP) strongly enhances gliomagenesis in mice. Using this model, we found Qki loss promoted somatic driver alterations including focal DNA alterations and oncogene amplification on ecDNA, especially *Pdgfra*/*Kit*, which as common oncogenes in ecDNA across both human and QPP mouse GBMs. Collectively, this work elucidates novel mechanisms underlying defective lipid homeostasis induced by QKI loss acts as a mutator in promoting gliomagenesis through inducing genomic instability. Our study provides a deeper insight into lipid metabolic dysregulation of GBM and a candidate for diagnosis and therapy. It will also contribute to the development of therapeutic strategies that specifically target QKI-depleted GBM.

#6660 Fasting-mimicking diet and starvation escape inhibition as novel low-toxic treatments for kidney cancer.

Samuel Lazzeri, Anastasiya Shmahala, Olga Blazevits, Valter D. Longo

IFOM-FIRC Institute of Molecular Oncology, Milan, Italy

Fasting, or fasting-mimicking diet (FMD), can delay tumor growth and promote tumor regression by making cancer cells more vulnerable to conventional therapies, while promoting protection and resistance in normal cells. FMD, which imitates the beneficial effects of fasting while being more tolerated by patients, activates peculiar survival/growth pathways in different cancer types, which can be identified and inhibited leading to tumor regression with low-toxicity. In the present study, we exploited the beneficial effects of FMD in the treatment of kidney cancer, which remains a major challenge despite the significant therapeutic progress of the past years. We developed a protocol that recapitulates in vitro the main molecular effects of FMD, in which cancer cells are exposed to reduced levels of glucose and serum factors. We observed that renal cancer cells subjected to in vitro FMD up-regulate specific starvation escape pathways, including the ERK-MAPK and the mTOR-S6K signalling. We developed a treatment based on FMD and selective inhibitors of these pathways, which induced cell death in a wide range of renal tumor lines, but not in normal cells, through the impairment of the purine metabolism ultimately leading to oxidative stress. We confirmed that the treatment is very effective in delaying tumor growth and in promoting tumor regression in different kidney cancer subcutaneous xenograft mouse models, attesting its safety and low toxicity on mice. The treatment also reduced metastasis formation upon intravenous injection of renal cancer cells in a syngeneic mouse model. We believe that this study represents a promising start to develop innovative and feasible treatments for kidney cancer, which could potentially also be extended to other cancer types.

#6661 Effect of berberine on cholesterol regulation in pancreatic ductal adenocarcinoma via heat shock factor 1.

Ahmad Hegazi, Rejina Shrestha, Shruti Ghai, Kuo-Hui Su

Department of Cell and Cancer Biology, University of Toledo, Toledo, OH

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is a malignancy characterized by poor prognosis and elevated metabolic activity, including dysregulated lipid metabolism that supports aggressive growth. Cholesterol homeostasis, involving synthesis, uptake, and efflux, is pivotal for tumor progression. The pro-oncogenic transcription factor heat shock factor 1 (HSF1) has been reported to sustain the increasing metabolic demands of cancer cells by directly inhibiting AMP-activated protein kinase (AMPK), leading to an enhanced de novo cholesterol synthesis process and lipogenic phenotype in human melanoma cells. Berberine, a natural alkaloid with anti-cancer properties, has demonstrated efficacy in modulating cholesterol metabolism. However, its role in cholesterol regulation through HSF1 in PDAC remains poorly understood.
Objectives: To investigate the role of berberine in modulating cholesterol homeostasis in PDAC through the regulation of HSF1.
Methods: Multiple human pancreatic epithelial cells and cancer cell lines were treated with berberine to evaluate its effects on cholesterol regulation. Immunoblotting was used to assess changes in key proteins associated with cholesterol metabolism, focusing on determining berberine's impact on cholesterol efflux pathways.
Results: Berberine treatment significantly reduced total HSF1 levels, phosphorylation at Serine326, and downstream target HSP72 in a dose-dependent manner. Berberine treatment also increased AMPK phosphorylation at Thr172 and decreased cholesterol efflux transporter ATP-binding cassette transporter A1 (ABCA1). However, the expression of another cholesterol efflux transporter, ABCG2, remained unaffected by berberine treatment.
Conclusion: This study reveals a novel mechanism by which berberine exerts an anti-cancer effect in PDAC by modulating cholesterol regulation through HSF1.

#6662 Deciphering molecular network complexity of oncogenic PIK3CA through proximity labeling.

Rong Li, Donghee Lee, Md Abdullah, Eslenur Nipa, **Jong Hyuk Kim**

University of Florida, Gainesville, FL

PIK3CA mutations are major drivers for oncogenesis. Mutant PIK3CA continuously activates the PI3K pathway, transmitting oncogenic signals within transformed cells. Despite decades of efforts to target oncogenic PIK3CA for cancer therapy, the precise molecular and functional machinery remains incomplete due to the molecular complexity of the PI3K pathway. Mapping proximity proteins in the oncogenic PI3K pathway and identifying mutant signals will signify a deep understanding of oncogenesis and a broad range of therapeutic applications. In this study, we show that PIK3CA mutant cells establish a unique molecular program that network with distinct proteins, differing from cells with wild-type (WT) PIK3CA. We have developed a bioengineering approach to decode protein-protein interactions of oncogenic PIK3CA and identify key molecular partners, potentially unveiling therapeutic vulnerabilities in cancer cells. First, we applied the proximity labeling technique, based on the fusion of a protein ligase to a protein of interest (POI), featuring proximity-dependent biotinylation of interacting proteins. The biotin ligase BioID2 was utilized to integrate WT PIK3CA or gain-of-function hotspot mutations in E545K or H1047R residues. To optimize the labeling protocol and biotinylation conditions, the BioID2 plasmids were fused with each POI, incorporating multiple combinations of protein linkers and tags, including myc, and red fluorescence protein. A total of 13 plasmids were constructed in mammalian expression vectors, including pcDNA3.1 vector for transient expression, and pHAGE-puro vector for generating stable cell lines. Next, we used fluorescence microscope to screen RFP signals for transfection efficiency and employed immunofluorescence to verify the situations of protein expression and biotinylation with specific antibodies and fluorescence-conjugated streptavidin. Finally, streptavidin pulldown and LC-MS were utilized to identify the proximity proteins. Our technical approach confirmed a high level of reliability and consistency in LC-MS data between POI replicates. We identified approximately 1,000 proximity proteins, of which 436 were shared between samples labeled with WT and mutant PIK3CA. We found that both H1047R and E545K mutants demonstrated altered interactions with the protein GATAP2A. Furthermore, PIK3CA H1047R uniquely interacted with proteins S100A7, FLG2, LGALS7, and PPIB, while E545R specifically mapped proximal proteins SEPTIN7, SERBP1, SNW1, and RNPS1. To further validate our methods, we engineered two cancer cell lines (DHSA-1426 and COSB) to induce PIK3CA H1047R mutations using CRISPR/Cas9. Our ongoing work involves labeling proximity proteins with mutant PIK3CA in these malignant cells, enabling temporal and spatial molecular sensing of oncogenic PIK3CA and its functional interactome in living cancer cells.

#6664 ATF4 is required for PanIN formation and PDAC via effects on nucleotide metabolism and suppression of p53 activity.

Leah M. Ziolkowski, Kay F. Macleod

The Ben May Department for Cancer Research, University of Chicago, Chicago, IL

Pancreatic ductal adenocarcinoma (PDAC) is increasing in incidence in western society and has a 5-year survival rate of less than 13% which while better than it was several years ago, remains one of the worst for all solid tumors. The development of KRAS inhibitors to block the activity of the dominant activated oncogene in human PDAC brings hope that the mortality rate will improve in the coming years. However, as these new therapeutics enter clinical trials, there is an expectation that resistance to KRAS inhibitors will emerge. Thus, the interrogation of additional pathways and the mechanisms of drug resistance are critical to expand the efficacy of KRAS inhibitors and promote patient survival. Our work focuses on the role of *Activating Transcription Factor 4* (ATF4) in PDAC where an ATF4-like gene signature predicts worse survival outcomes for PDAC patients. We show here for the first time that conditional loss of *Atf4* in acinar cells of the adult mouse pancreas prevents KRas-driven PDAC, such that neither pre-malignant pancreatic intra-epithelial neoplasia (PanIN) lesions nor PDAC develops over the lifetime of the animal. We also show that loss of Atf4 alone in pancreatic acinar cells (absent KRas activation) causes increased pancreatitis and marked pancreas shrinkage following treatment with cerulein. We show that loss of Atf4 in adult pancreatic acinar cells leads to DNA damage, induction of p53 (and both p21 and Bax) and increased cell death, that is exacerbated by cerulein treatment. The increased DNA damage was associated with nucleotide depletion and decreased levels of metabolites involved in 1C metabolism which we attribute to the observed loss of expression of Atf4 targets, such as Mthfd2. Together, our data point to a critical role for Atf4 in maintaining nucleotide pools necessary for tumor cell growth and that its loss promotes p53-dependent cell death preventing PDAC initiation and progression.

#6666 Translocation-dependent regulation of nucleotide metabolism in response to drug-induced nucleotide stress.

Zaffar Iqbal Bhat, Iqra Ishrat, XiaoMi Lu, Justin L. Tan

INSTITUTE OF CANCER RESEARCH, Shenzhen Bay Laboratory, Shenzhen, China

Nucleotide metabolism plays a significant role in cancer development and progression. Cancer cells have an increased demand for nucleotides, which are the building blocks of DNA and RNA, due to their uncontrolled proliferation. Targeting nucleotide metabolism is an important strategy for cancer therapy. However, existing therapies have a small therapeutic window as they induce toxicity in cancer and normal cells alike. Little is known about the mechanisms of nucleotide stress-induced toxicity in cells. In prokaryotes, the PyrR protein senses nucleotide levels by binding UMP. This regulates the ability of PyrR to bind the attenuator region of the *pyr* operon transcript, thus altering transcription of downstream nucleotide metabolism genes. PyrR therefore acts as a nucleotide sensor in prokaryotes. The paralog of this protein in mammals is yet to be discovered. It is unknown if similar nucleotide sensing mechanisms occur in mammalian cells. Our study aims focused to identify new nucleotide binding proteins that regulate nucleotide metabolism in humans. Here, we identified human proteins that had conserved homology to regions of the prokaryotic PyrR protein. We found similar amino acid sequences to the UMP binding site of PyrR in some candidate eukaryotic proteins. We analyzed the effect of nucleotide stress induction, via treatment with two clinical nucleotide biosynthesis inhibitors, on candidate protein P. P is normally localized in the cytoplasm but upon inhibitor treatment, P translocates into the nucleus. Knockdown of P resulted in decreased, cell viability, which could be rescued by nucleotide addition. Computational simulations identified potential nucleotide binding sites on P. We mutated the potential binding site aminos acids of P and expressed P mutant protein in cells. Unlike wildtype P, P mutant did not translocate into the nucleus upon nucleotide stress. Chromatin immunoprecipitation with high-throughput sequencing (ChIP)-seq revealed that P binds to the promoter regions of nucleotide metabolic genes. Through these studies, we have identified a novel mammalian protein sensor that transcriptionally regulates nucleotide metabolism through nuclear localization. These findings suggest an additional pathway by which nucleotide metabolism is directly regulated in response to the availability of end-product nucleotides. Knowledge of this pathway could propose ways to improve the therapeutic window of nucleotide biosynthesis-based chemotherapy.

#6667 Dual inhibition of PIKfyve and KRAS/MAPK targets metabolic vulnerabilities of pancreatic ductal adenocarcinoma.

Jasmine P. Wisniewski[1], Caleb Cheng[1], Sydney Peters[1], Ruya Pakkan[1], Pietro Morlacchi[2], Jennifer P. Morton[3], Yuanyuan Qiao[1], Costas A. Lyssiotis[1], Arul M. Chinnaiyan[1]

[1]University of Michigan, Ann Arbor, MI,[2]Agilent Technologies, Inc., Lexington, MA,[3]CRUK Scotland Institute, Glasgow, United Kingdom

Background: Pancreatic Ductal Adenocarcinoma (PDAC) exists within a harsh, nutrient-depleted microenvironment, and is known to use lysosomal processes, such as autophagy, for maintenance of metabolic function. PIKfyve is a lipid kinase that serves as the single source for PI(3,5)P2, a molecule critical for the process of autophagy. Considering the role of PIKfyve in autophagy, we hypothesized it to be crucial for PDAC pathophysiology.

Methods & Results: To evaluate the significance of PIKfyve in PDAC, we employed RNA-ISH and found that PIKfyve was significantly higher expressed in PDAC tissue compared to normal surrounding cells. Performing viability screens across multiple PDAC cell lines, we observed that they were highly sensitive to PIKfyve inhibitors apilimod and ESK981. This data suggested that PIKfyve is crucial for PDAC development.

We then performed a metabolism-focused CRISPR screen with the selective pressure of PIKfyve inhibition to assess the metabolic role of PIKfyve in PDAC. From this, we identifiedthe function of the de novo fatty acid synthesis pathway as necessary for PDAC survival following PIKfyve perturbation. In addition to this pathway being essential upon PIKfyve inhibition, we also noticed dramatic upregulation of fatty acid synthesis genes FASN and ACACA following the activation of SREBP1, a transcriptional regulator of lipogenesis. To determine if this shift translated to a metabolic phenotype, we performed targeted lipidomics and observed a significant increase in sphingolipids.

KRAS, mutated in the majority of PDAC cases, is known to be a major driver of metabolic homeostasis through MAPK signaling. De novo fatty acid synthesis is one such pathway controlled by KRAS signaling; we noticed decreased expression of FASN and ACACA along with sphingolipids following KRAS/MAPK inhibition. Specifically, KRAS/MAPK inhibition attenuated the increase in FASN and ACACA as well as lipidomic reprogramming seen with PIKfyve inhibition.

We investigated the in vivo potential of this combination using a syngeneic orthotopic model of PDAC. Notably, all but one mouse were cured when treated with both ESK981 and MEK inhibitor trametinib. Then employing a KPC model, we observed that mice treated with both ESK981 and MEK inhibitor selumetinib had an increased median survival time by over 5 times.

Taken together, the concurrent targeting of PIKfyve and KRAS-MAPK disrupts lipid homeostasis and has potent antitumor effects, holding promise as a therapeutic treatment strategy.

#6668 MNK2-dependent activation of *de novo* lipid synthesis drives breast cancer brain metastasis.

Jessie Ling Ai Wong[1], Giselle Sek Suan Nah[1], Fatin Nasha Binte Sulaimi[1], Kian Leong Lee[1], Kassoum Nacro[2], Kaustubh Bhinge[3], Amanda E.D. Van Swearingen[3], Carey K. Anders[3], Sin Tiong Ong[1]

[1]Programme in Cancer Stem Cell Biology, Duke-NUS Medical School, Singapore, Singapore,[2]Experimental Drug Development Centre (EDDC), Agency for Science, Technology and Research (A*STAR), Singapore, Singapore,[3]Division of Medical Oncology, Duke Cancer Institute, Duke University Medical Center, Durham, NC; Division of Medical Oncology, Duke Center for Brain and Spine Metastasis, Duke University Medical Center, Durham, NC

Patients with triple-negative breast cancer (TNBC) have poor prognosis, attributed to a ten-fold increased risk of developing brain metastases (compared to patients with other breast cancer subtypes), as well as the limited availability of effective therapies for breast cancer brain metastasis (BCBM). Recent work has revealed that SREBP1, a master transcription factor regulating *de novo* lipid synthesis, is required for BCBM survival in the lipid-depleted brain microenvironment. Given that previous studies have highlighted a link between MAPK interacting protein kinases (MNK) and lipid biosynthetic pathways, we investigated whether MNKs are essential for lipid biosynthesis in BCBM. We found that *MNK2* and *SREBP1* are upregulated in BCBM compared to paired primary cancers (n= 5 pairs, $p<0.05$). This upregulation is also observed in neurotropic 231-Br relative to its parental MDA-MB-231 TNBC cell line. Using a novel brain-penetrant MNK1/2 inhibitor, ETC-501, we show that MNK activity is required for growth of 231-Br cells in lipid-depleted conditions, as well as *de novo* lipid synthesis. Using a combination of immunofluorescence- and immunoblot-based assays, we find that ETC-501 prevents the nuclear translocation of SREBP1 and causes an accumulation of the precursor form of SREBP1 in the endoplasmic reticulum, preventing its processing into the mature form required for nuclear localization, and lipogenic gene activation. Importantly, we find that both genetic & pharmacological inactivation of MNK prevents the establishment of BCBM *in vivo*. In rescue experiments, we find that expression of mature SREBP1c restores lipid droplet levels in ETC-501-treated cells. Our study reveals a critical role for MNK2 in regulating SREBP1 and lipid synthesis, providing new insights into TNBC BCBM. Inhibitors of MNK kinases, such as ETC-501, may offer potential in managing TNBC patients at high-risk for BCBM.

#6669 Vagal perturbation of the gut-brain axis deters cancer-associated cachexia.

Aliesha D. Garrett[1], Xiling Shen[1], Ayelet Erez[2], Naama Darzi[2]

[1]Terasaki Institute for Biomedical Innovation, Los Angeles, CA,[2]Weizmann Institute of Science, Rehevot, Israel

Cancer-associated cachexia (CAC) is a wasting syndrome present in 80% of advanced cancer patients. CAC decreases the quality of life, causes higher mortality, and causes resistance to treatments with increased toxicity. CAC is currently incurable and directly responsible for up to 30% of all cancer deaths.
We find that cancer-induced systemic inflammation disrupts vagal tone via infiltration of innate immune cells into the brain, involving the vagal dorsal motor nucleus of the hindbrain. This disruption leads to CCL2-driven vagal hyperactivation and elevated acetylcholine output to the liver, resulting in the downregulation of hepatocyte nuclear factor 4 alpha (HNF4α), a key regulator of liver metabolism. We further demonstrate that a right cervical vagotomy in mice with lung or pancreatic cancer disrupts the abnormal CNS-liver vagal crosstalk, restoring liver metabolism and feeding behavior, thereby alleviating cachexia symptoms independent of tumor burden. Importantly, electrical, chemical, or surgical disruption of the right vagus nerve, including via a custom wireless implantable device, restores liver metabolism, alleviates CAC manifestations, and synergizes with chemotherapy, significantly improving the overall well-being and survival rates of mice with lung or pancreatic cancer.
Our findings have substantial translational potential for CAC treatment, mainly as non-invasive vagal interventions are already FDA-approved for other therapeutic applications.

#6670 Unraveling bone marrow adipocyte-leukemia interactions: Identifying key signaling pathways for improved leukemia therapies.

J. Rahbani, M. Song, O. Ukomadu, O. Arnold, E. Postich, A. Dhar, C. Saygin;
The University of Chicago Medicine Comprehensive Cancer Center, Chicago, IL

Bone marrow, the soft tissue within our bones, plays a vital role in blood production and also houses fat cells called bone marrow adipocytes (BMAs), which are a natural component of its structure. However, recent studies suggest that these fat cells might contribute to leukemia growth by fostering an environment that aids cancer cell survival and enhances their resistance to treatments like chemotherapy. This issue could be exacerbated in individuals with obesity, as they have an increased amount of bone marrow fat cells, potentially creating an even more protective environment for leukemia cells. Here, we study two aggressive types of leukemia: acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). We believe leukemia cells send specific signals to these fat cells, causing them to change in ways that promote cancer growth and treatment resistance. Our research will investigate these communication signals and the changes in fat cell behavior, with the aim of finding new ways to treat leukemia more effectively. The study has three main goals: (i) understanding how leukemia alters fat cell metabolism; (ii) examining how different eating patterns like calorie restriction and time-restricted feeding affect fat cell activity and (iii) exploring how time-restricted feeding influences leukemia cell survival and metabolism after chemotherapy. Investigating the role of BMAs in leukemia not only deepens our understanding of the bone marrow microenvironment but also opens new avenues for targeted therapies. Over time, this work could revolutionize how we understand and treat leukemia, laying the groundwork for new therapies that target its underlying biochemical mechanisms, and potentially reshaping the future of cancer treatment.

#6671 Metabolic predictors in hepatocellular cancer.

Brandon Mathew[1], Kazufumi Ohshiro[1], Krishanu Bhowmick[1], Xiyan Xiang[1], Srinivasan Dasarathy[2], Adrian R. Krainer[3], Lopa Mishra[1]

[1]The Feinstein Institute for Medical Research, Manhasset, NY,[2]Division of Gastroenterology and Hepatology, Cleveland Clinic, Cleveland, OH,[3]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Background: Obesity and Metabolic dysfunction-associated steatotic liver disease (MASLD) affect nearly a third of the world's population. 20% of MASLD patients progress to Metabolic dysfunction-associated steatohepatitis (MASH). Hepatocellular Cancer (HCC) arises in 4-11% of MASH patients. Recently, we discovered obesity-driven cancers in our mouse models with disruptions to TGF-β signaling (*Cell Rep. 2024.43(9):114676 and Sci Transl Med. 2021.13(624): eabk2267*). Key metabolic enzymes were evaluated in both mouse models and human cirrhotic patients to elucidate their roles in HCC development. Our Mutant mice exhibited significant sarcopenia, with a notable reduction in muscle mass, mirroring the substantial muscle loss observed in human cirrhotic patients. We identified levels of PKM2, Myostatin and SPTBN1 to be significantly altered in our mouse models of obesity driven HCC.
Methods: To investigate the roles of myostatin, SPTBN1, and PKM2 in human HCC progression, serum samples were obtained from 143 patients with cirrhosis and 57 patients diagnosed with HCC. Myostatin and PKM2 serum concentrations were quantified using Somascan proteomic analysis. ELISA was conducted to confirm the observed differences in myostatin and SPTBN1 levels between the cirrhosis and HCC patient cohorts. Additionally, serum samples from 18 cirrhotic patients were longitudinally analyzed over a 5-year follow-up period to monitor their progression to HCC.
Results: Elevated myostatin levels were observed in obese mice. Serum myostatin concentrations were significantly higher in patients with HCC than those with cirrhosis ($p < 0.05$), with a 17% increase in mean levels noted in the HCC cohort. A trend toward increased PKM2 serum concentrations was also observed in HCC patients relative to cirrhosis patients. Somascan proteomic analysis identified elevated myostatin and PKM2 levels in the serum of 18 cirrhosis patients who progressed to HCC over a five-year follow-up period. Validation using ELISA in a prospective cohort ($n = 200$) confirmed the significant difference in myostatin concentrations between HCC and cirrhosis patients ($p < 0.05$). Additionally, serum SPTBN1 concentrations were found to be elevated in patients with virus-related cirrhosis and HCC, further supporting its involvement in disease progression.
Conclusions: These findings highlight the significant role of Myostatin, PKM2, and SPTBN1 in the progression of cirrhosis to HCC. Elevated myostatin levels in obese mice and HCC patients underscore its potential as a biomarker for disease progression. The observed trend of increased PKM2 concentrations in HCC patients further implicates its role in tumorigenesis, particularly in an immunosuppressive tumor microenvironment. Collectively, these results reinforce the importance of these biomarkers in understanding HCC progression and their potential utility in early detection, risk stratification, and targeted therapeutic strategies.

#6672 Lipid droplet accumulation after metformin and 6 aminonicotinamide combinatory treatment of glioblastoma cells.

Cecilia Gasco[1], Ivan Macharashvilli[1], Daniela Perez Visnuk[1], Marina Perona[2], J. Silvio Gutkind[3], **Marcela Solange Villaverde**[1]

[1]University of Buenos Aires, Buenos Aires, Argentina,[2]The National Atomic Energy Commission, Buenos Aires, Argentina,[3]University of San Diego, Moores Cancer Center, San Diego, CA

Glioblastoma (GBM) has the worst prognosis among primary brain cancers. As in many tumors, GBM proliferation is supported by glycolysis and the pentose phosphate pathway (PPP). The PPP enables cancer cells to adapt to anabolic demands, including lipid synthesis, and to restore antioxidant defenses. Metformin (MET), a worldwide prescribed drug for type-two diabetes, impairs the progression of different malignancies by indirectly inhibiting the mammalian target of rapamycin (mTOR). Our previous results showed that the combination of MET and 6-aminonicotinamide (6AN), a PPP inhibitor, strongly potentiates their individual cytotoxic effects on different tumor cells. Thus, the present work aimed to study the effect of MET alone or in combination with 6AN on the mTOR pathway as well as on the activity of acetyl-CoA carboxylase, a key enzyme in lipid synthesis, in the human GBM cell line U251. Results showed that MET (5 mM) alone or in combination with 6AN (25 μM) decreased the mTOR effector, pS6, and increased the presence of the phosphorylated form of acetyl-CoA carboxylase (24 h of treatment, determined by western blot and immunofluorescence, $p<0.05$). Controversially, we also found an increase in the total cell lipid content (BODIPY and oil red staining, analyzed by flow cytometry (FC), $p<0.01$) of MET/6AN-treated cells, which was accompanied by a mild increase in the content of the lipid transporter CD36 (FC, $p<0.01$). In addition, by fluorescence microscopy, we observed an increase in the number and size of lipid droplets of MET/6AN-treated cells. Finally, we also found that MET/6AN promoted a decrease in mitochondrial potential and an increase in oxidative stress and lipid peroxidation (TMRE, DCF and C11-BODIPY respectively, FC, $p<0.01$). Preliminary results indicated that accumulation of lipids inside lipid droplets may prevent lipid peroxidation. These results deserve to be further investigated to better understand the mechanisms involved in the effectiveness of the combination of MET/6AN as a potential strategy to treat GBM.

#6673 Alternate mechanism of translation initiation in TNBC metabolism.

Emilia F. Carlo[1], Erna Mitaishvili[2], Columba de la Parra[3]

[1]Chemistry, Herbert H. Lehman College, City University of New York, New York, NY,[2]Biochemistry, The Graduate Center, City University of New York, New York, NY,[3]Chemistry, Herbert H. Lehman College & The Graduate Center, City University of New York, New York, NY

Triple-negative breast cancer (TNBC) is a highly aggressive and metastatic subtype of breast cancer. Metastasis is considered one of the primary causes of mortality in cancer patients. TNBC has been shown to exhibit advanced cancer hallmarks, such as metabolic reprogramming and selective mRNA translation, which contribute to metastatic progression. Previous studies show that TNBC utilizes an alternate cap-dependent translation mechanism mediated by Death Associated Protein 5 (DAP5). Our studies indicate this alternate translation plays an essential role in metabolic reprogramming. Analysis from genome-wide translatome studies, qRT-PCR, Western blot analyses, as well as metabolomic assays on the TNBC cell line (MDA-MB-231) with non-silencing controls (shNsi) and DAP5-silenced (shDAP5) samples indicate that this alternate translation plays a significant role in the metabolic reprogramming. DAP5 silencing reduces the translation of several glucose metabolism-related mRNAs, independent of their steady-state abundance, and reduces proteins associated with glycolytic metabolism. Targeted metabolomics shows that DAP5 silencing alters metabolites critical in breast cancer energy metabolism. Our findings also suggest that DAP5-dependent translation may selectively regulate metabolic mRNAs under stress conditions when canonical mTORC1/eIF4E cap-dependent translation is attenuated. This alternative translation initiation mechanism suggests this metabolic switch is necessary for malignant progression and metastasis in TNBC cells. These results provide new insights into the molecular mechanisms underlying metabolic reprogramming in metastatic breast cancer and may offer novel therapeutic targets for TNBC treatment.

#6674 Leucine sensing and mTOR activation drive SNAIL-mediated EMT in pancreatic cancer cells.

Hironori Koga[1], Yasuko Imamura[2], Atsutaka Masuda[1], Hiroyuki Suzuki[1], Toshimitsu Tanaka[1], Takahiko Sakaue[1], Hideki Iwamoto[1], Toru Nakamura[1], Takumi Kawaguchi[1]

[1]Div. of Gastroenterology, Dept. of Medicine, Kurume Univ. School of Medicine, Kurume, Japan,[2]Research Center for Innovative Cancer Therapy, Kurume Univ., Kurume, Japan

Background: Pancreatic cancer, characterized by a highly fibrotic and hypovascular tumor microenvironment, possesses unique metabolic adaptations that enable survival and proliferation in malnourished conditions. Aim: This study aimed to compare the metabolic adaptations of pancreatic cancer cells to glucose deprivation with those of liver cancer cells, which are representative of hypervascular tumors.
Methods: Three pancreatic cancer cell lines (BxPC-3, Panc-1, MIA PaCa-2) and two liver cancer cell lines (HuH-7, Hep3B) were analyzed. We examined transcriptional expression levels of genes involved in amino acid transport, including *LAT1*, *CD98*, *CD147*, *EPCAM*, and *ASCT2*; epithelial-mesenchymal transition (EMT), including *SNAIL*, *SLUG*, and *ZEB1*; and cancer stemness, including *CD24*, *CD44*, and *DCLK1*, under glucose-deprived conditions.
Results: Pancreatic cancer cells displayed significantly lower proliferative activity under glucose deprivation compared to liver cancer cells. While glucose-starved cells did not undergo significant apoptosis, they exhibited cell cycle arrest. Pancreatic cancer cells showed mild arrests in both the G0/G1 and G2/M phases, whereas liver cancer cells exhibited a clear G0/G1 arrest. Among amino acid transporters, *LAT1* was uniquely upregulated in pancreatic cancer cells, correlating with increased *SNAIL* (EMT regulator) and *DCLK1* (cancer stemness marker) expression. *LAT1* knockdown abolished *SNAIL* upregulation in glucose-starved pancreatic cancer cells, indicating a mechanistic link. Stimulation of LAT1 by branched-chain amino acids (BCAAs; valine, leucine, isoleucine) under glucose-free conditions induced dose-dependent *SNAIL* upregulation by leucine, accompanied by increased expression of leucine sensors *SAR1B* and *Sestrin2*. This activation triggered mTOR pathway signaling, as evidenced by phosphorylation of S6K1 and 4E-BP1. Conversely, rapamycin inhibited mTORC1 activation, suppressing leucine-induced SNAIL upregulation.
Conclusion: Pancreatic cancer cells reprogram their metabolism to adapt to energy crises via leucine-mediated upregulation of SNAIL, potentially enhancing metastatic capabilities through EMT. Targeting LAT1 or the mTOR pathway may represent promising therapeutic strategies for pancreatic cancer.

#6675 Are malic enzymes a metabolic drug target in the war against cancer?.

B. Krinkel[1], M. Slayton[2], J. Jeon[2], J. Copping[1], J. Flanagan[1], S. Merajver[2], C. Squire[1], K. Loomes[1];
[1]University of Auckland, Auckland, New Zealand, [2]University of Michigan, Ann Arbor, MI

Background: Cancer continues to be a formidable adversary, claiming countless lives globally and fueling the pursuit of more effective therapies to enhance treatment outcomes and quality of life. A hallmark of cancer is the reprogramming of cellular metabolism, including the aberrant biosynthesis of essential metabolites that support survival and rapid cellular proliferation. This metabolic shift often involves increased reliance on glutamine as a fuel source and elevated expression of malic enzyme 2 (ME2) within the mitochondria. ME2 catalyzes the generation of pyruvate and NAD(P)H and is upregulated in pancreatic, melanoma, and lung cancer, making it a promising yet underexplored therapeutic target. We have solved the structure of the active site small molecule inhibitor NPD-389 in complex with ME2 and used virtual screening to identify novel scaffolds to develop into new drug leads.
Methods: We expressed recombinant human ME1-3 isoforms and detailed their enzymatic kinetics with and without inhibitors. Molecular snapshots of the potent malic enzyme inhibitor were trapped within a complete set of X-ray crystal structures for the three isoforms ME1-3, and this structural data was used to conduct the virtual screening of up to 12 million drug-like and fragment molecules to identify novel, potent malic enzyme inhibitors. To complement the multidisciplinary characterization of malic enzyme inhibition, the efficacy of NPD-389 in treating triple-negative breast cancer was assessed.
Results: Our kinetic analyses reveal unique inhibition patterns, particularly for the least studied isoform, ME3. We detailed its substrate and cofactor binding affinities and inhibitory constants for EA, MDSA, and NPD-389. The revelation of X-ray crystallographic visualization of the potent but isoform non-selective inhibitor NPD-389 in complex is a metal-binding mode and the adoption of an unexpected malic enzyme conformation. Virtual screening based on the unique protein conformation identified several new scaffolds with promising inhibitory activity against ME's. These include molecules confirmed by X-ray crystallography to bind in an allosteric site and that, critically, display distinct isoform selectivity. In triple-negative breast cancer cells, NPD-389 inhibited proliferation in most cell lines, suggesting its potential as a lead compound for further development.
Conclusions: Our studies underscore the potential of ME2 as a therapeutic target in cancer treatment. By detailing the binding mode of NPD-389 and those of newly identified selective inhibitors, we provide a robust platform for the future clinical development of potent inhibitors of metabolic pathways driving cancers. We envisage that isoform selective ME drugs could serve as adjunct therapies alongside existing checkpoint inhibitors or chemotherapy, enhancing treatment efficacy and improving patient outcomes.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: microRNAs and Other Noncoding RNAs 1
Poster Session

#6678 Targeting long non-coding RNA LINC00324 using novel plant-derived exosome-like nanovesicles loaded with anticancer epigenetic modifiers effectively inhibits biomarkers of epithelial-mesenchymal transition in breast cancer.

Alaa Ashraf Al_Aaser, Nourhan El-Sayed El-Feel, Ahmed Samir Sultan

Biochemistry, Alexandria University, Alexandria, Egypt

Despite recent advancements in the diagnostics and therapeutics of breast cancer, it continues to be the second leading cause of death among women worldwide. Therefore, identifying new potential biomarkers and molecular targets is essential for enhancing the diagnosis and treatment of BC patients. A wealth of evidence underscores the significant role of long non-coding RNAs in various biological processes, including tumorigenesis, which might offer new biomarkers and molecular targets for improved cancer detection and treatment. In this context, long intergenic non-coding RNA 00324 (LINC00324) has been associated with advanced cancer stage, larger tumor size, lymph node metastasis, and poorer prognosis across several cancer types. However, its biological function and molecular mechanisms in breast cancer remain largely unexplored. This study adopts an innovative approach aimed at challenging existing knowledge and research methodologies, with the goal of making significant advancements in breast cancer research. By exploring the role of LINC00324 as an oncogene in tumorigenesis and progression, the study may uncover crucial downstream oncogenic signaling pathways related to Epithelial-Mesenchymal Transition (EMT), which are linked to the hallmark features of breast cancer pathogenesis. This research could ultimately lead to the identification of a new biomarker for the diagnosis and prognosis of breast cancer, thereby transforming our understanding and treatment of the disease. In this study, we employed an innovative approach to downregulate the expression of LINC00324 across various breast cancer cell lines, ranging from well-differentiated to poorly differentiated types. This was accomplished using isolated novel plant-derived exosome-like nanovesicles (PELNVs), demonstrating specific biological effects, including anti-inflammatory and anti-tumor properties, while exhibiting minimal toxic side effects. PELNVs were a novel vehicle for delivering anticancer epigenetic modifier drugs and specific siRNA targeting LINC00324. Additionally, we investigated several downstream target biomarkers of epithelial-mesenchymal transition, including oncogenic targets such as *Notch1, Notch4, Nanog, s6 kinase, β-catenin*, and *Stat3*, both *in vitro* and *in vivo*. These findings suggest that LINC00324 could serve as a novel biomarker for diagnosis and prognosis.
Outcome: Identifying the molecular classification of breast cancer subtypes based on the expression of individual biomarkers, such as LINC00324, might lead to the development of novel therapeutic biomarkers/targets for early detection, drug resistance, and personalized cancer treatments. Using PELNVs for drug delivery may inspire future research on plant-derived nanovesicles.

#6679 MicroRNA cargo of extracellular vesicles of pancreatic cancer patients have immunoregulatory potential.

Rupinder Kaur[1], Sandeep Bhoriwal[2], Pramod Kumar Gautam[1]

[1]Biochemistry, All India Institute of Medical Sciences (AIIMS) New Delhi, New Delhi, India,[2]IRCH, All India Institute of Medical Sciences (AIIMS) New Delhi, New Delhi, India

Pancreatic ductal adenocarcinoma (PDAC) is characterized by a dismal five-year survival rate of 5-8%. Extracellular vesicles (EVs), nano-sized membrane-bound particles (30-150 nm) present in the circulatory system, play a crucial role in mediating intercellular communication, regulating immune responses, and driving tumor progression. MicroRNAs (miRNAs), integral components of EVs, function as key mediators in cellular signaling pathways. Although the role of exosomal miRNAs (exo-miRNAs) in cancer pathogenesis is well established, their comprehensive therapeutic, diagnostic, and immunomodulatory potential remains largely unexplored. This study established the differential exoMIR profile of PDAC patients using small RNA sequencing on the Illumina platform, compared with healthy controls, post-surgery and follow-up. Statistical analysis revealed hsa-miR-375-3p, hsa-miR-134-5p and hsa-miR-197-3p miRNAs were downregulated in PDAC patients than controls whereas hsa-miR-126-5p, hsa-miR-7847-3p, hsa-miR-197-3p were upregulated post-surgery. Six-month follow-up sample after surgery had hsa-miR-144-3p, hsa-miR-194-5p, hsa-miR-16-5p, hsa-miR-197-5p upregulated and hsa-miR-125b-3p was downregulated as compared to post-surgery sample. These distinct exoMIRs were identified as key regulators of cytokine translation in immune cells. The altered expression of these cytokines is well-known to significantly influence immune activity within the tumor microenvironment. Bioinformatic analyses revealed that these miRNAs, along with their protein targets, are closely involved in oncogenic pathways, underscoring their potential as valuable tools in cancer therapeutics, diagnostics, and prognosis.

#6680 Androgen-regulated miRNA-mRNA interactions in luminal A breast cancer: Insights into metastasis, anoikis resistance, and metabolic pathways.

Jehad A. Yasin[1], Osama M. Younis[1], Leen A. AlKuttob[1], Ramez M. Odat[2], Sofia Adwan[3], Mohammad Abdulla[1], Afaf Mansour[1], Dana Alsafadi[1], Mamoun Ahram[1]

[1]University of Jordan, Amman, Jordan,[2]Jordan University of Science and Technology, Amman, Jordan,[3]Medical Laboratories, American University of Madaba, Amman, Jordan

Background: Luminal A breast cancer (BC), the most common subtype of BC, often expresses the androgen receptor. This study explores androgen-regulated miRNA-mRNA interactions and their roles in metastasis, anoikis resistance, and metabolism.
Methods: Luminal A cell lines, MCF-7 and T47D, and the luminal androgen receptor (LAR) MDA-MB-453 cells, were treated with dihydrotestosterone for 48 hrs, followed by microarray analysis of mRNA and miRNA expression. Expression data were normalized and differentially expressed (DE) transcripts were identified. Gene Set Enrichment Analysis was utilized, and miRNA-mRNA interaction networks were built using multiMiR. Survival analyses, coexpression, and therapy response comparisons were done on luminal A cohorts in TCGA, 'ROC Plotter', and 'KMplotter'. MAVISTO's algorithm was used for network motif discovery.
Results: Seven DEmiRNAs were found in MCF-7 cells and 20 in T47D, both of which were fewer than the 66 found in LAR. A 17-gene androgen response signature was derived by overlapping DEmiRNA-targeted DEmRNAs in luminal A cells with the 'Hallmark Androgen Response' set, including 16 upregulated mRNAs like FKBP5, a hub mRNA regulated by hsa-miR-429, and TPD52, both of which were in T47D and MCF-7. The mean expression of the signature correlated with better overall survival (OS) in luminal A BC (HR = 0.67, 95% CI: 0.45-0.99, n = 1,504) and was higher in endocrine therapy responders compared with non-responders (p = 0.041). However, only three genes in the signature had significant negative epithelial-mesenchymal transition (EMT) scores, while 11 were EMT-promoting. Bi-fan network motifs in signature-related network, featuring two miRNAs co-regulating two mRNAs, were the most enriched. Negative regulation of anoikis, an enriched term, had two upregulated EMT-promoting genes, SNAI2 and CAV1 in MCF-7, both with significant coexpression with downregulated hsa-miR-429 (correlations ≤ -0.29); DEmRNA signature from this term predicted poor OS (HR = 1.8, p = 0.038). Syndecan-mediated signaling was enriched, with SDC2 upregulated and interacting with the downregulated miRNAs hsa-miR-429, hsa-miR-4490, and hsa-miR-890 upon androgen treatment. Similarly, RND3 GTPase was upregulated and interacted with hsa-miR-429. Syndecan and RND3, linked to metastasis, may share a regulatory pathway. Insulin signaling was also enriched; Insulin Receptor Substrate 1 (IRS1) was downregulated and interacted with hsa-miR-429 and hsa-miR-890. In T47D, estrogen response, linked to DEmRNAs FKBP5 and FASN, and previously related to invasion and anoikis resistance, emerged as a key enriched term from miRNA-regulated mRNAs.
Conclusion: We found significant androgen-regulated miRNA-mRNA interactions influencing anoikis suppression, metastasis, and metabolic shifts in luminal A breast cancer, with hsa-miR-429 as a key regulator.

#6681 Identification of a panel of four exosomal microRNAs driving T cell dysfunction in the tumor-immune microenvironment of high-grade serous ovarian cancer.

Michael Chan[1], Li Yang[1], Nan Xiang[2], Dongbing Gao[1], Jianting Sheng[1], Stephen TC Wong[1]

[1]Systems Medicine and Bioengineering, Houston Methodist Neal Cancer Center, Houston, TX,[2]School of Basic Medical Sciences, Anhui Medical University, Hefei, China

Purpose: High-grade serous ovarian cancer (HGSOC), the most aggressive ovarian cancer subtype, has poor outcomes due to late-stage diagnosis and a complex tumor microenvironment (TME). This study investigates exosomal microRNAs (miRNAs) in HGSOC, focusing on their role in immune evasion mechanisms within the TME and their potential as biomarkers for early detection and therapeutic targets. **Methods:** We conducted comprehensive miRNA sequencing on exosomes derived from nine human ovarian cancer cell lines, including those representing high-grade serous ovarian cancer. Bioinformatics analyses were then applied to identify key miRNAs and their target genes potentially involved in T cell exhaustion, stemness, and modulating immune response pathways. Selected exosomal miRNAs were validated through miRNA transfection and RT-qPCR to evaluate their effects on genes related to immune checkpoints, cytokine expression, and immune modulation. Additionally, ex vivo experiments assessed the functional impact of tumor-derived exosomes on CD8+ T cells, including IFN-γ secretion and T cell stemness. **Results:** A critical panel of four exosomal miRNAs — miR-24, miR-191, miR-181c, and miR-146a — were identified through differential expression and pathway enrichment analyses, highlighting their involvement in immune modulation pathways such as IL-4 and IL-13 signaling pathway. In vitro validation confirmed that these exosomal miRNAs significantly altered the expression of key genes associated with immune checkpoints, T cell exhaustion, and cytokine production, reinforcing their role in facilitating immune evasion. Ex vivo experiments demonstrated that tumor-derived exosomes modulate CD8+ T cell function by reducing IFN-γ secretion and impairing T cell stemness. Notably, the encapsulation of these miRNAs within exosomes allows them to circulate systemically and influence both local and distant components of the tumor microenvironment (TME). These findings emphasize the dual role of exosomal miRNAs in shaping the immune landscape of HGSOC—acting as drivers of immune suppression and as promising candidates for non-invasive biomarkers and therapeutic targets. **Conclusions:** The four miRNAs identified in this study are key modulators of the immune landscape within the TME of HGSOC, engaging critical pathways that promote immune evasion and tumor progression. Notably, their presence within exosomes—a circulating vesicle system capable of long-range transport—position exosomal miRNAs as promising biomarkers for non-invasive early detection and as versatile therapeutic targets, offering new opportunities to improve outcomes in this aggressive cancer subtype.

#6682 Circulating Epstein-Barr virus microRNAs associated with Hodgkin lymphoma prognosis.

Soo Mi Lee[1], Cristina S. Bogsan[1], **Jason T. Howard**[2], Abigail G. Wandoff[3], Meng-Xuan Wu[3], Estelle Verburgh[4], Maxine van der Schyff[4], Frank J. Slack[1], Yujing J. Heng[1], Katherine Antel[3]

[1]Beth Israel Deaconess Medical Center, Boston, MA,[2]Mirxes US, Durham, NC,[3]Dana Farber Cancer Institute, Boston, MA,[4]University of Cape Town, University of Cape Town, South Africa

Background: Epstein-Barr virus (EBV) infection is an established causal factor for Hodgkin lymphoma (HL). MicroRNAs (miRNAs) are master regulators of gene expression and are ideal biomarkers. We profiled the circulating expression of 42 EBV miRNAs in HL patients to determine if EBV miRNA(s) can be a noninvasive biomarker for EBV+ HL or disease severity.
Method: Plasma was obtained from 11 patients with HL in a tuberculosis and HIV-endemic setting in Cape Town, South Africa. EBV infection in HL tumor cells were confirmed using a clinical PCR assay. The expression of 42 miRNAs were measuring using a qPCR-based Mirxes ID3EAL^{TM} EBV miRNA panel. Wilcoxon test or Fisher's test were used to compare data between two groups. Spearman's rho was used for correlation analysis. Fold changes were calculated using the $2^{\wedge-\Delta\Delta CT}$ method.
Results: EBV+ HL patients were significantly older (p=0.04; Table 1). No miRNA was associated with EBV or prognostic factors at FDR<0.05. Of interest, miR-BART20-3p expression was 33% lower in EBV+ patients compared to EBV- patients (p=0.052) and did not correlate with age (rho=0.39, p=0.23). MiR-BART17-3p was inversely correlated with stage (rho=-0.61, p=0.047). MiR-BART2-5p correlated with International Prognostic Score (IPS; rho=0.72, p=0.01). miR-BART14-3p was inversely correlated with Eastern Cooperative Oncology Group (ECOG) score (rho=-0.60, p=0.049). MiR-BART11-5p and miR-BART14-5p were higher in patients that reported B-symptoms (11.2-fold p=0.01 and 2.5-fold p=0.02, respectively). In contrast, MiR-BART14-3p and miR-BART17-3p were 33% and 41% lower in patients with B-symptoms (both p=0.03). No miRNA was associated with HIV.
Conclusion: Circulating EBV miRNAs were associated with HL disease severity but not EBV status. Further investigations into these candidate miRNAs will increase our understanding of HL disease progression as well as explore the potential of these miRNAs as therapeutics or therapeutic targets. Table 1.

		EBV+	**EBV-**	*p*
n		6	5	
Age (median [IQR])	35.5 [29.2, 49.2]	24.0 [23.0, 27.0]	0.04	
Sex (%)	Female	1 (16.7)	1 (20.0)	1.00
	Male	5 (83.3)	4 (80.0)	
Stage (%)	I	0 (0.0)	1 (20.0)	0.45
	II	2 (33.3)	0 (0.0)	
	IV	4 (66.7)	4 (80.0)	
IPS (%)	0	1 (16.7)	0 (0.0)	0.84
	1	1 (16.7)	1 (20.0)	
	2	1 (16.7)	0 (0.0)	
	3	1 (16.7)	3 (60.0)	
	4	1 (16.7)	0 (0.0)	
	5	1 (16.7)	1 (20.0)	
ECOG Score (%)	0	1 (20.0)	4 (66.7)	0.13
	1	4 (80.0)	1 (16.7)	
	2	0 (0.0)	1 (16.7)	
B-Symptoms (%)	Yes	3 (50.0)	3 (60.0)	1.00
	No	3 (50.0)	2 (40.0)	
HIV (%)	Positive	2 (33.3)	1 (20.0)	1.00
	Negative	4 (66.7)	4 (80.0)	

#6683 Melatonin and 17β-estradiol suppress TGFB1 and TGFBR2 in IDCs cells by target miR-93-5p.

Bruna Cristine De Almeida, Kelly Pedrozo Ferreira, Jose Maria Soares-Junior, Juciara Costa Silva, Katia Candido Carvalho, Edmund Chada Baracat

Departamento de Obstetricia e Ginecologia, Faculdade de Medicina da Universidade de Sao Paulo, Sao Paulo, Brazil

miR-93-5p exerts an oncogenic effect in a tissue-specific mode, and its activity is related to cancer progression and development. In breast cancer cells, *miR-93* overexpression was associated with proliferation, invasion, migration, and metastasis. In addition, it targets *TGFB1* and *TGFBR2* genes, which are directly involved in mammary development. Activated TGFB1 is able to directly bind the TGFBR2 promoting the cascade of signaling. *TGFB1* modulates many cellular processes. However, changes in its expression may show a controversial role, both inhibiting the development of early-stage lesions (during carcinoma initiation and progression) and participating in invasion and metastasis (in the late stages of tumor progression). It is known that epigenetics events can silencing *TGFB1* pathway signaling. In this context, melatonin (Mel) has gained notoriety due to its oncostatic activities and mainly to modulate miRNAs. Additionally, estrogen signaling is often associated with the onset of breast cancer and directly interacts with *TGFBR2*. Our goal was to evaluate the effects of Mel and 17β-estradiol (E2) treatments in invasive ductal carcinoma cells (IDC) and to assess the expression of *miR-93* and its target, *TGFB1* and *TGFBR2*. IDC cells (MCF7 (ER+) and MDA-MB231 (triple-negative) were treated with 3000 μM Mel and 100 nM E2 isolated, and the two drugs were combined (M+E2), with daily replenishment. RNA was extracted and reverse transcription synthesis was performed. RT-PCR was carried out for *miR-93*, *TGFB1* and *TGFBR2* detection. Relative quantification was determined with ExpressionSuite Software v1.3. ER-positive IDC cells showed significant suppression of *miR-93* expression with Mel (Fold: 0.494) and M+E2 (Fold: 0.488) treatments, compared to control. In addition, *miR-93* expression was significantly lower with M+E2 treatment compared to E2. Lower expression of *miR-93* leads to an increase of *TFGB1*, after the Mel (Fold: 2.607) and M+E2 (Fold: 3.433) treatments, related to control and E2 treatment. This same effect was observed in *TGFBR2* expression, in which the inhibition of *miR-93* promoted the expression of *TGFBR2* after Mel (Fold: 2.551) and M+E2 (Fold: 2.988) treatments. In triple-negative IDC cells, lower expression of *miR-93* was observed in Mel (Fold: 0.868), M+E2 (Fold: 0.814), and E2 (Fold: 0.780) treatments. Suppression of *miR-93* after Mel and M+E2 treatments, increased *TGFB1* expression by more than seven times (Mel Fold: 7.068, and M+E2 Fold: 7.342). However, even though E2 treatment also decreased the expression of *miR-93*, the expression of *TGFB1* did not show significant changes. In addition, *TGFBR2* also presented great expression with inhibition of *miR-93,* after Mel (Fold: 3.005) and M+E2 (Fold: 2.723) treatments. Overall, our findings provided evidence for the significant role of *miR-93* in regulating the TGFB cascade and also introduced Mel as a new therapeutic agent for targeting oncogenic miRNAs in IDC cells. Supported by: FAPESP 2022/04174-2 and 2018/24224-9.

#6684 Aiolos expression regulates epithelial-mesenchymal transition through repressing miR-424-5p in lung cancer cells.

Ying-Shiun Kao, **Jung-Jyh Hung**

National Yang Ming Chiao Tung University and Taipei Veterans General Hospital, Taipei, Taiwan

Introduction: Epithelial-mesenchymal transition (EMT) is one of the major molecular mechanisms inducing tumor invasion and metastasis. The Ikaros family of DNA binding proteins are zinc finger transcription factors. Aiolos overexpression promotes EMT through upregulating the PI 3-kinase/Akt/Twist/MMP16 pathway in lung cancer. However, the roles of Aiolos regulating EMT and metastasis through miRNAs expression in lung cancer has not been well demonstrated in the literature.
Materials and Methods: qRT-PCR was performed to determine miR-424-5p expression in H1299-Aiolos and A549-Aiolos cells. Knockdown of miR-424-5p was performed in H1299 cells, and transfection of miR-424-5p was performed in A549 cells. Western blotting analysis was performed to demonstrated E-cadherin and vimentin expression. Migration assay and matrigel invasion assay were done to determine the EMT phenotypes after miR-424-5p transfection or knockdown in lung cancer cells.
Results: Down-regulation of miR-424-5p in H1299-Aiolos and A549-Aiolos cells was determined by qRT-PCR. Western blotting analysis showed decreased E-cadherin and increased vimentin expression in H1299 cells after miR-424-5p knockdown. Western blotting analysis also showed increased E-cadherin and decreased vimentin expression in A549 cells after miR-424-5p transfection. Migration assay showed that migration increased in H1299-shmiR-424-5p cells, but decreased in A549-miR-424-5p cells. Matrigel invasion assay showed the invasiveness increased in H1299-shmiR-424-5p cells, but decreased in A549-miR-424-5p cells.
Conclusions: Aiolos expression regulates EMT through repressing miR-424-5p expression in lung cancer cells. The results shed light on further understanding of the mechanisms of metastasis in lung cancer.

#6686 Bioinformatic evidence for the involvement of SP1, MYC, and HIF1A in the regulation of miRNAs and histone H2AZ1.

Kotohiko Kimura, Monika Rajput, John Ho Chun, Ru Chih C. Huang

Biology, Johns Hopkins University, Baltimore, MD

We previously proposed the concept that the inhibitors for SP1, MYC, and HIF1A should induce strong anticancer activity by reducing the expression of stem cell-related proteins (Curr. Issues Mol. Biol. 2023, 45, 9262-9283). Then, it was shown that in LN229 and U87MG glioblastoma cells, either tetra-methyl-O-nordihydroguaiaretic acid (M_4N) or tetra-acetyl-O-nordihydroguaiaretic acid (A_4N) suppressed SP1 and only a few stem cell-related proteins and induced only a small amount of cell death while the combination treatment of M_4N with A_4N greatly suppressed the expression of SP1, MYC, and HIF1A, and all stem cell-related proteins examined, and greatly induced cell death. This data provided the biochemical evidences to support the aforementioned concept. In this study we did bioinformatic analysis to seek for further evidences to support this concept. The ontology analysis showed that the proteins associated with SP1, MYC, and HIF1A were specifically involved in the regulation of transcription and that various miRNAs which had been shown to induce either anti- or pro-cancer activity were associated with SP1, MYC, and HIF1A, which suggested that the inhibition of SP1, MYC, and HIF1A could modulate transcription of both coding and noncoding RNAs and affect cancers. It has been known that nucleosomes, containing histone variants H2AZ1, are important for gene transcription initiation and termination, chromosome segregation and DNA double-strand break repair. The network analysis showed that there were intricate connections between three transcription factors (SP1, MYC, and HIF1A) and H2AZ1. These data overall showed that SP1, MYC, and HIF1A in combination played important roles in transcription regulations via multiple ways (modulations of both coding and non-coding RNAs, miRNAs, and H2AZ1) and that the inhibitions of these transcription factors should have strong negative impacts on cancers which required enhanced production of energy and materials for their rapid proliferation. Thus, these bioinformatic data overall supported our concept as well.

#6687 Micromanaging resistance: microRNA expression and chemoresistance in testicular germ cell tumors.

Ratnakar Singh, Kelly Kries, Aayush Gupta, Amy Li, Zeeshan Fazal, Doha Shokry, Raya Boyd, Brayden Rennels, Sarah J. Freemantle, Michael J. Spinella

Comparative Bioscience, University of Illinois at Urbana-Champaign, Urbana, IL

Testicular germ cell tumors (TGCTs) are the most common type of cancer among young men, and incidence has increased over the past 40 years. Approximately 80% of patients with metastatic TGCTs can be cured with chemotherapy; however, those patients that develop resistance to treatment often have a poor prognosis. Despite efforts to identify clear genetic factors that determine chemotherapy sensitivity and resistance in TGCTs, recent research has shifted focus toward the role of epigenetics in maintaining sensitivity to drugs like cisplatin and decitabine. Epigenetic mechanisms, including DNA methylation, histone modifications, and microRNAs (miRNAs), play a crucial role in regulating gene expression. Prior studies in somatic cancers have linked differential expressions of miRNAs to drug resistance. In this study, we hypothesized that the response of TGCTs to cisplatin and decitabine is partially influenced by miRNA expression. Through miRNA sequencing analysis followed by RT-qPCR validation, we identified distinct patterns of miRNA expression that correlate with resistance to cisplatin and decitabine in TGCTs. Consistent with our previous findings on protein coding gene expression, we observed that 54 miRNAs were reciprocally expressed between cells resistant to decitabine and those resistant to cisplatin. In follow-up analysis, we selected a subset of these reciprocally regulated miRNAs and predicted their target genes using miRDB and TargetScan. Downstream miRNA-RNA interactive analysis, enrichment analysis, and validation assays are ongoing and will be presented. Together, our study aims to elucidate the mechanistic roles of miRNAs in chemosensitivity and chemoresistance in TGCTs. The findings from this study will also support precise diagnosis and personalized treatment strategies for patients with TGCTs.

#6688 Differential serum mirnas in obese and diabetic African American colon adenoma patients.

Hassan Brim, Adeyinka O. Laiyemo, **Hassan Ashktorab**

Howard University, Washington DC, DC

Background: Metabolic syndrome, characterized by obesity, diabetes, and other metabolic disturbances, is associated with an increased risk of colon cancer. There is a critical need for assays that can detect biomarkers to enable risk stratification, early diagnosis, and prevention of colon adenomas and colorectal cancer. Serum miRNAs are emerging as promising biomarkers for this purpose.
Aim: The purpose was to elucidate the role of miRNAs in the pathophysiology of colon adenomas in the context of obesity and diabetes.
Methods: We used serum samples from age- and gender-matched African Americans (n=18 per group) to ensure that the comparison of miRNA expression was not confounded by age or gender differences. The groups included patients without adenomas (Normal), patients with adenomas and obesity, and patients with adenomas and diabetes. RNA extraction and miRNA sequencing was rigorous, utilizing the miRNeasy Serum/Plasma Kit and Lexogen Small RNA-Seq Library Prep Kit, respectively. Illumina NextSeq 500 sequencing provided high coverage (15 million reads per sample). The alignment to the UCSC hg38 human genome and the use of the DESeq2 R package for differential expression analysis ensured robust statistical evaluation of miRNA expression changes. We identified miRNAs with significant fold changes (log2 FC ≥ 2 or ≤ -2) and p-values < 0.05.Results: In the Adenoma-Obese group, miR-455, miR-219b, miR-34a, miR-378a, miR-485, and miR-133b showed significant associations with obesity-related metabolic dysregulation. Notably, miR-34a, involved in cell cycle and apoptosis regulation, was upregulated, highlighting its role in obesity-related cancer pathogenesis. In the Adenoma-Diabetic group, miR-455 was upregulated, suggesting its involvement in insulin resistance and adenoma progression, while miR-219b was downregulated, potentially reflecting a compensatory response to hyperglycemia. Ingenuity Pathway Analysis (IPA) revealed that these miRNAs are linked to pathways involved in cancer, diabetes, and lipid metabolism. Key pathways, including insulin signaling, Wnt/β-catenin, and mTOR, underscore the interplay between metabolic disorders and colorectal adenoma progression.
Conclusion: This study highlights distinct miRNA expression profiles associated with adenoma development in obesity and diabetes. miR-34a, miR-378a, and miR-455 emerged as potential biomarkers and therapeutic targets. Future research should validate these findings in larger cohorts to refine their utility in clinical applications.

#6689 Evaluating the influence of blood collection tubes and extraction kits on miRNA recovery in cancer biomarker research.

Eunju Seong, Nicholas M. George, Jordan LaRue, Jing Li, Lisa Bartron

Research and Development, Streck, LLC, La Vista, NE

Cell-free RNAs, especially microRNAs (miRNAs), are emerging as valuable biomarkers in cancer research and other diseases. These small, non-coding RNA molecules circulate in the bloodstream, offering insights into the physiological and pathological states of various organs and tumors. However, the stability of miRNAs in blood samples can be significantly affected by the type of blood collection tube used and the handling of samples. Here, we determine the optimal workflow for miRNA extraction from blood samples by comparing three types of blood collection tubes - citrate, EDTA, and Nucleic Acid BCT (NAC) - paired with three different extraction kits: A, B, and C. The three selected extraction kits differ in their sample lysis mechanisms, which are crucial for minimizing the effects of highly active innate RNases present in plasma. Kit A uses a proprietary lysis reagent containing phenol, allowing for plasma lysis at room temperature and recovering RNA from the aqueous layer following phase separation. Kit B employs two consecutive heated lysis steps at 60 °C with chaotropic agents while Kit C executes plasma lysis at 37 °C using proteinase K and further purifies RNA on an automated magnet-based platform. We observed that when plasma samples were processed within 24 hrs with Kit A per manufacturer's instructions, both RNA spike-in and endogenous miRNA recoveries were notably compromised regardless of tube type, while the mRNA yield was markedly reduced in samples collected in NAC. We were only able to increase recovery with substantial modifications to Kit A's protocol. In contrast, when we analyzed plasma samples with Kits B and C per manufacturer's instructions, we saw consistent isolation of miRNA and mRNA across all three tube types, albeit with slight variations in reproducibility. These data show that the recovery of miRNA and mRNA from plasma samples is significantly influenced by the combination of RNA extraction kit and blood collection tube. The automation capability of Kit C facilitates higher throughput processing, which is advantageous in clinical settings where sample volume and processing time are critical factors. To demonstrate the compatibility of Kit C with NAC, cancer-associated miRNAs, including miR-21-5p and miR-122-5, were quantified in samples collected into citrate, EDTA, and NAC. We observed that these miRNAs are maintained in samples stored in NAC for up to five days, but not those stored in EDTA or citrate. These data underscore the importance of selecting appropriate blood collection tubes and extraction kits to optimize miRNA recovery from plasma samples. The findings indicate that both components significantly influence miRNA quantification. Careful consideration of these factors is crucial for obtaining reliable results in clinical studies involving circulating miRNAs. Nucleic Acid BCT is For Research Use Only. Not for use in diagnostic procedures.

#6690 A novel B7H3-targeting macrocyclic peptide conjugate efficiently delivers siRNAs to cancer cells and tumor endothelium.

Danyan Li[1], Lincy Edatt[1], Jillian Perry[1], Avery Huber[2], William David Green[1], Jarred Michael Green[1], Justin Milner[1], Albert Bowers[2], Chad Pecot[1]

[1]UNC Lineberger Comprehensive Cancer Center, Chapel Hill, NC,[2]Eshelman School of Pharmacy, UNC Chapel Hill, Chapel Hill, NC

Short-interfering RNAs (siRNAs) can treat a wide range of human diseases from viral outbreaks to cancer, however, targeted siRNA delivery remains a significant challenge. Recently, significant efforts have focused on developing novel delivery technologies for siRNAs, targeting tumors and other extrahepatic tissues. B7-H3 (CD276), a B7 family of immune checkpoint proteins, is highly expressed in cancer cells and tumor endothelial cells (TECs). We employed an mRNA display campaign against the hB7-H3 protein and discovered a macrocyclic binding peptide, JP5. Using surface plasmon resonance, we confirmed specific binding of JP5 to both human and murine B7-H3 with a KD of approximately 770nM and 600nM, respectively. Characterization of protease stability showed that greater than 80% of the peptide remains intact after incubation with 50% serum for 24h. Further, our team conjugated JP5 to chemically stabilized siRNAs labeled with a Cy5 fluorophore to observe biodistribution of siRNAs, including cancer cells and and TECs. Mice were implanted with A431 WT or B7H3 KO cells, and upon xenograft tumor formation, mice were given PBS (control) or JP5-siRNA-Cy5 (n=5 mice/group). Gross biodistribution by IVIS imaging was found in the tumors, skin, bowel, liver and kidney, with minimal accumulation in the heart and lungs. Flow cytometry and immunofluorescence revealed that JP5 conjugation directed siRNAs to ~80% of TECs and to 40-60% of cancer cells, with a significant reduction in B7H3 KO cells. In conclusion, the JP5 macrocycle delivers siRNAs to B7H3-expressing cancer cells and TECs in the tumor microenvironment, paving a path forward for sophisticated cancer therapeutics.

#6691 A divalent and chemically modified microRNA-34a with enhanced circulation kinetics and improved endosomal escape for the treatment of triple-negative breast cancer.

Sharjeel Anjum, Shreyas Iyer, Digambar Waiker, Andrea Kasinski

Purdue University Institute for Cancer Research, West Lafayette, IN

Treatment of triple-negative breast cancer (TNBC) remains highly challenging due to the absence of HER2/hormonal receptors and its resistance to conventional therapies. Although surgery, chemotherapy, and radiotherapy are available treatment options, these broad-spectrum therapies are limited by their non-specific targeting and severe side effects, underscoring the need for more precise therapeutic options. Targeted micro RNA (miRNA) therapeutics have gained significant attention in this regard. In most cancers including TNBC, tumor suppressive miRNAs are often lost, leading to unchecked oncogene expression. One such miRNA, miR-34a which is transcriptionally activated by p53, regulates several oncogenic pathways by down-regulating genes like MET, AXL, and C-MYC. Restoring miR-34a in TNBC is therefore being explored as a potential therapeutic strategy. However, clinical use of miRNAs has been limited by their susceptibility to nucleases, immunogenicity, and lack of targeted delivery methods. To address these limitations, our lab developed a chemically modified, folate-conjugated miRNA-34a (FM-FolamiR-34a) that is highly stable and can be specifically delivered to TNBC cells overexpressing folate receptor alpha (FRα). FM-FolamiR-34a effectively downregulated its targets and demonstrated promising antitumor activity. However, due to its small size (~14 kDa), FM-FolamiR-34a was subject to rapid renal clearance, limiting its therapeutic availability. Additionally, endosomal entrapment restricted its cytoplasmic abundance, further reducing its mRNA-targeting efficiency. To overcome these challenges, we developed a novel, chemically modified, folate-conjugated *divalent* miR-34a with enhanced circulation kinetics and improved endosomal escape. This divalent molecule includes two miR-34a duplex copies linked by cleavable deoxythymidine linkers (dT-linkers), which are susceptible to enzymatic cleavage by DNAse II in late endosomes. The dT-linker facilitates miR-34a release from the receptor promoting endosomal escape and increasing cytosolic availability. The dual nature of our construct also raises its molecular weight above the kidney clearance threshold (~30 kDa), preventing rapid excretion and providing additional time for engagement with FRα-expressing cancer cells. Furthermore, delivering two miR-34a strands per receptor doubles the intracellular miR-34a copy number, enhancing cellular availability. We anticipate that our divalent miR-34a will demonstrate superior antitumor efficacy in TNBC compared to the monovalent version (FM-FolamiR-34a). This innovative approach addresses key critical barriers in miRNA-based therapeutics - specifically endosomal entrapment and rapid renal clearance - presenting an effective strategy to target FRα overexpressing TNBC.

#6692 The combination of 5-methyltetrahydrofolate and nigericin enhances the cytosolic release of chemically modified miRNA-34a, significantly boosting its anti-tumoral activity.

Kenan E. Ozcan[1], Sophia A. Matthias[1], Shreyas G. Iyer[1], Ahmed M. Abdelaal[2], Sudarsan R. Kasireddy[3], Philip S. Low[3], Andrea L. Kasinski[1]

[1]Purdue University Institute for Cancer Research, West Lafayette, IN,[2]Microbiology & Immunology, Indiana University School of Medicine, Indianapolis, IN,[3]Department of Chemistry, Purdue University, West Lafayette, IN

A major driver of cancer progression involves the downregulation of tumor-suppressive miRNAs like miR-34a, which regulates key cancer-promoting genes such as MYC, AXL, and BCL-2. Thus, it is not surprising that miR-34a restoration has been proposed as an anticancer strategy. miRNAs, such as miR-34a can precisely target cancer-related genes with limited toxicity, provided they are properly designed. Effective therapeutic design must address RNA specificity, stability, and endosomal escape. Our lab has made significant progress in addressing these unmet challenges, particularly in enhancing specificity and stability of miRNA therapeutics through ligand-mediated delivery and chemical modifications, respectively. Our ligand-mediated delivery strategy capitalizes on overexpression of Folate Receptor 1 (FR1) in cancer cells, and uses the high-affinity FR1 ligand, folate, to deliver miRNAs to cancer cells with high specificity. To overcome the issues with poor instability, we created a fully modified version of miR-34a (FM-34a), increasing its stability by ~400-fold. Despite significant progress in developing FM- 34a as a therapeutic agent, efficient endosomal escape remains a major hurdle. To address this, a small molecule ionophore, nigericin that disrupts endosomal membranes was conjugated to the folate-FM-34a conjugate (Fol-N-FM-34a). However, nigericin alone achieved only modest improvements in escape efficiency, likely due to the inability of folate to dissociate from the receptor at the elevated endosomal pH levels induced by nigericin. To overcome this limitation, a lower affinity ligand of FR1, 5-methyltetrahydrofolate (5-MTHF), was used in combination with nigericin (5M-N-FM-34a). Unlike folate, 5-MTHF retains a similar binding affinity for FR1 at physiological pH but dissociates more effectively at the higher pH levels attainable in the presence of nigericin, thereby enhancing endosomal escape and cytosolic delivery. We validated that the 5-MTHF ligand binds to FR1-expressing cells in both *in vitro* and *in vivo* models and that 5-MTHF-N-FM-34a lead to a higher cytosolic abundance of miR-34a and superior downregulation of miR-34a target genes compared to Fol-N-FM-34a *in vitro*. To evaluate *in vivo* activity both single dose targeting and multi-dose efficacy studies were conducted. 5M-N-FM-34a achieved greater target downregulation and enhanced anti-tumor activity in a $KRAS^{G12D}$ $p53^{-/-}$ genetically engineered mouse model of non-small cell lung cancer in comparison to other ligands. These findings highlight 5-MTHF-nigericin as a promising ligand for targeting FR1-overexpressing tumors.

#6693 Targeting oncogenic miR-135b in colorectal cancer: Computational modeling and small molecule screening for RNA-based therapy.

Berat Demir[1], Lalehan Oktay[2], **Serdar Durdagi**[3]

[1]Department of Molecular Biology and Genetics, Gebze Technical University, Kocaeli, Turkey,[2]Lab for Innovative Drugs (Lab4IND), Computational Drug Design Center (HITMER), Bahcesehir University (BAU), Istanbul, Turkey,[3]Molecular Therapy Lab, Department of Pharmaceutical Chemistry, School of Pharmacy, Bahcesehir University (BAU), Istanbul, Turkey

Colorectal cancer ranks among the leading causes of cancer-related deaths globally. In this study, we identified miR-135b as a differentially expressed microRNA in colorectal cancer through an analysis of datasets from the Gene Expression Omnibus (GEO) database. Target genes associated with miR-135b were subsequently identified, and pathway and functional enrichment analyses were conducted to provide a deeper understanding of the underlying biological processes. We developed a de novo three-dimensional model of miR-135b's tertiary structure, targeting the Dicer cleavage site for small molecule inhibition. Dicer binds to the terminal loop region of the pre-miRNA and cleaves it to produce a double-stranded miRNA duplex. One strand of this duplex, the guide miRNA, is loaded into the RNA-induced silencing complex (RISC), which mediates miRNA-mRNA interactions, leading to post-transcriptional gene silencing. To identify potential inhibitors, molecular docking simulations were conducted using the ChemDiv miRNA-targeted small molecule library (~20,000 compounds). The top-scoring compounds were further explored by screening their commercial analogues in the ZINC library via SwissSimilarity. These analogues were docked at the Dicer cleavage site, and optimized docking scores were obtained. The highest scoring compounds were then subjected to all-atom molecular dynamics simulations, followed by post-simulation analysis to evaluate the dynamic interactions between miR-135b and the selected hit ligands.

#6694 Multiplex RNAi delivers on-versus-off tumor precision in T cells.

Reka A. Haraszti, Xavier Segarra-Visent, Anastasia Kremer, Tatyana Ryaykenen

Department of Medicine II, Hematology, Oncology, Clinical Immunology and Rheumatology, University Hospital Tubingen, Tubingen, Germany

Selective inhibition of graft-versus-host disease (GvHD) while preserving graft-versus-leukemia (GvL) remains a critical challenge in allogeneic stem cell transplantation. More broadly, engineering on-versus-off tumor selectivity represents a central precision medicine problem in T cell-mediated immunotherapies. Evidence suggests that T cell phenotype distributions, defined by transcriptomes and transcriptional regulation, are key determinants of this selectivity. RNA interference (RNAi) therapies, including small interfering RNAs (siRNAs) and microRNA (miRNA) mimics, represent a novel class of precision medicine tools capable of engineering cellular transcriptomes. We identified an siRNA cocktail targeting RAN, WAPAL, and AURKA that achieved GvHD inhibition comparable to approved therapies (35-95% vs. 55-91% reduction in T cell proliferation) while sparing GvL effects (no increase in leukemia proliferation, compared to a 7-20-fold increase with approved drugs). Notably, substituting any siRNA in the cocktail drastically impaired GvHD inhibition (up to a 10-fold reduction), and individual siRNAs against WAPAL and AURKA showed no significant GvHD inhibitory effects. While RAN siRNA alone was highly effective against GvHD *in vitro* (>90% inhibition of T cell proliferation), it compromised bead-activated T cells. Thus, the RNAi-based transcriptome engineering strategy demonstrated that targeting RAN, WAPAL, and AURKA synergistically inhibits GvHD, while also conferring selectivity to this innovative therapeutic approach. The effect of chemical modifications on miRNA function remains poorly understood. In our study, we screened diverse chemical modification patterns to develop miRNA replacement therapies (miR-146a, miR-181a, miR-374b) within the GvHD/GvL context. Silencing performance of miRNA mimics—whether on fully matched reporter targets or natural targets with mismatches—failed to reliably predict miRNA efficacy in inhibiting GvHD *in vitro*. Notably, certain miRNA mimics achieved GvHD inhibition comparable to approved therapies (60-95% vs. 55-91% reduction in T cell proliferation) while either sparing or even enhancing GvL effects (up to an 80% decrease vs. up to a 22-fold increase in leukemia proliferation). Generally, miRNA mimics with higher phosphorothioate content demonstrated stronger GvHD inhibition, whereas no such pattern was observed for GvL outcomes. RNA-Seq analysis revealed that the same marker genes could predict responses to miR-374b mimics in both GvHD and GvL assays, highlighting shared molecular pathways influencing these outcomes. Such analyses may serve as target discovery tool to uncover pathways potentially differentiating between GvHD and GvL. Multiplex RNAi therapies, such as siRNA cocktails or miRNA mimics have the potential to rewrite the T cell transcriptional program and enhance on-versus-off-tumor precision of T cell mediated cancer therapies.

#6695 Lipid nanoparticles with cell specific CRISPR activity against cancer.

Noah Richardson, Sanam Rezaei Benam, Samaneh Maleknia, Reza Shahbazi

Indiana University School of Medicine, Indianapolis, IN

The most prevalent cancer in men is prostate cancer and is the second leading cause of cancer death in men. Initially, metastatic prostate cancer responds to androgen deprivation therapy (ADT) blocking the androgen receptor (AR) signaling pathway responsible for growth and proliferation. Resistance eventually develops however and although systemic androgens are minimal, the androgen receptor remains functional and drives prostate cancer growth in most patients. This advanced form of prostate cancer termed castration-resistant prostate cancer (CRPC) is incurable and largely driven still by the AR. Development of resistance is through several mechanisms including receptor duplications, intratumoral androgen synthesis, constitutively active point mutations, and AR splice variants that confer resistance to current therapies targeting the AR signaling axis The continued biological significance and reliance of AR signaling in CRPC has made the definitive knockout (KO) of the gene an enticing target.

We have developed antibody coated lipid nanoparticles (LNP) to specifically direct CRISPR nanoformulations to cancer cells expressing targetable surface antigens. Our first target is PSMA-expressing CRPC C4-2B cells. Our PSMA-LNP CRISPR formulation has increased safety and specificity with both extracellular and intracellular prerequisites for CRISPR targeting and activity. Intracellular activation of the CRISPR sgRNA complex is mediated by sensing cell-specific miRNA uniquely identified in malignant cells. We have screened miRNA databases and C4-2B cell lines identifying specific miRNA not expressed significantly in other healthy tissues. Using miRNA-flanked sgRNA CRISPR formulations our system once shuttled to PSMA expressing C4-2B cells allows assembly of Cas9 protein and sgRNA in a cell-specific manner causing AR knock out and cell death. In vitro PSMA-LNP delivery of unmodified guides demonstrate 60-95% gene editing efficiency analyzed by T7EI and TIDE analysis in C4-2B cells and control cells. Next using miRNA-flanked sgRNA CRISPR formulations we demonstrate preferrable editing in targeted cancer cell lines. Our time-lapse Incucyte live cell analysis shows preferential targeting of PSMA-LNP to C4-2B cell lines in comparison to control malignant and primary cell lines. Currently, we are continuing our studies through in vivo analysis of PSMA-LNP delivery and gene editing specificity in a CRPC mouse model utilizing our unique CRISPR LNP formulation in addition to exploring other gene targets controllable by microRNA sensing CRISPR therapeutics. Our findings demonstrate the feasibility of targeted CRISPR gene editing to treat prostate cancer and a platform for cancer-cell gene targeting across multiple cancers.

#6696 Exosome delivered combinatorial therapeutic approaches for advanced brain tumors.

Monique Kanitz Ruschel, Isaiah Davis, Karthik Gourishetti, Christian Jacobsen, Deepak Bhere

Dept. of Pathology, Microbiology, and Immunology, University of South Carolina- School of Medicine, Columbia, SC

Glioblastoma (GBM) is an aggressive form of brain cancer that affects over 12,000 people each year in the United States. Current treatments such as surgery, chemotherapy, and radiation have limitations and can often fail to achieve full tumor remission, as the five-year survival rate for this disease is around 7%. Recent research has revealed miRNA (miR), small non-coding RNAs that regulate gene expression, as contributors to the suppression and progression of various cancers, including GBM. Exosomes, tiny vesicles released by cells, show great promise as a cancer therapeutic. Our research focuses on using exosomes enriched with microRNA (miR) to address miR dysregulation in as a delivery system for glioblastoma treatment, as these vessels can cross the blood-brain barrier to deliver the treatment. Our goal is to examine the effectiveness of stem cell-derived exosomes carrying miR-124 and miR-7 as a new approach for treating glioblastoma, as this cancer is known for having downregulation of such microRNAs. Adult human stem cells (SC) are modified with target miR plasmids and then grown in an exosome-depleted medium. The exosomes that carry the miR are then separated, purified, measured, and examined. These exosomes are used in all subsequent research. The effects of exosomes on established and patient derived primary GBM cells are studied using a combination of cell viability tests and microscopy. To understand the molecular mechanisms that underlie the effect of miR modulation on cell proliferation and cell death pathways, a time course immunoblot analysis is carried out. To assess the effects of miR modulation in vivo, orthotopic animal models of GBM will be used.

Our research has shown that stem cell-derived exosomes can be an effective delivery system, asthey can evade the immune system and target specific cells. We have successfully transferred microRNA cargo to our target tumor cells using exosomes. By modulating miR, we have been able to halt tumor progression *in vitro* by targeting multiple pathways involved in GBM growth and progression. Although we are still awaiting the results of our ongoing animal studies, our preliminary data suggests that using stem cell-derived exosomes loaded with miR-124 and miR-7 is a promising approach for treating GBM.

#6697 Role of the miR-101-3p/CALCRL/NEAT1 axis in immune modulation and tumorigenesis in non-small cell lung cancer.

Gulnaz Tabassum, Mansoor A. Syed, Kapil Dev

Department of Biotechnology, Jamia Millia Islamia University, New Delhi, India

Lung cancer (LC), particularly Non-Small Cell Lung Cancer (NSCLC), remains a global health challenge with a five-year survival rate of 17.8%, largely due to delayed diagnosis and limited understanding of tumor progression. MicroRNAs (miRNAs), key regulators of gene expression, play critical roles in immune modulation, metabolism, and tumorigenesis, but their role in immunometabolic reprogramming in LC is underexplored. This study investigated novel molecular targets in LC using integrated bioinformatics and in vitro approaches, focusing on the regulatory interplay between miRNAs, mRNAs, and long non-coding RNAs (lncRNAs). RNA-Seq datasets of lung adenocarcinoma (LUAD) were analyzed to identify differentially expressed genes (DEGs) and miRNAs (DEMs). Immune-related genes (IRGs) were curated and intersected with DEGs to identify differentially expressed immune-related genes (DEIRGs). A protein-protein interaction (PPI) network was constructed, and enrichment analyses were performed to identify hub DEIRGs. A ceRNA regulatory network involving miRNA (miR-101-3p), mRNA (CALCRL), and lncRNA (NEAT1) was constructed and analyzed using survival (KM Plotter) and tumor immune infiltration (TIMER) analyses. Experimental validation included expression analysis of miR-101-3p, CALCRL, and NEAT1 using Real-Time PCR and functional studies involving overexpression of NEAT1 and miR-101-3p via transfection. Real-time PCR, Western Blotting & Dual-luciferase assays confirmed CALCRL as a direct target of miR-101-3p. Functional assays—including cell proliferation (MTT assay), ROS production, mitochondrial membrane potential, ATP content, and apoptosis analysis (FACS)—were performed to assess the biological impact of this regulatory network. This study revealed a novel ceRNA axis involving miR-101-3p, CALCRL, and NEAT1. miR-101-3p was significantly downregulated, correlating with increased expression of CALCRL and NEAT1. Functional studies revealed that NEAT1 overexpression suppressed miR-101-3p while enhancing CALCRL expression, promoting tumor progression. Restoration of miR-101-3p suppressed tumor cell proliferation, induced ROS accumulation, impaired mitochondrial function, reduced ATP levels, and triggered apoptosis, underscoring its tumor-suppressive potential. In conclusion, this study provides a detailed framework for the miR-101-3p/CALCRL/NEAT1 axis, offering novel insights into immunometabolic reprogramming in LC. These findings highlight miR-101-3p as a promising therapeutic target, paving the way for miRNA-based therapies in NSCLC

#6698 Inducible miRNA therapeutics: A multi-targeted strategy for treating diffuse midline gliomas.

Riley Bigger[1], Niloofar Khairkhah[2], Rhea Sridhara[2], Hernando Lopez-Bertoni[3], Stefanie Galban[4]

[1]Departments of Radiology, Surgery, and Biological Chemistry, University of Michigan Medical School, Ann Arbor, MI,[2]Departments of Radiology and Surgery, University of Michigan Medical School, Ann Arbor, MI,[3]Department of Neurology, Johns Hopkins University, Baltimore, MD,[4]Departments of Radiology and Surgery, Center for Molecular Imaging, Rogel Cancer Center, University of Michigan Medical School, Ann Arbor, MI

Diffuse Midline Gliomas (DMGs) are highly aggressive gliomas that account for ~20% of pediatric central nervous system tumors. The H3K27M-altered subset is particularly lethal, with 100% tumor recurrence and no effective long-term treatments. Thus, a desperate need to develop new therapeutic avenues remains. RNA therapies, a new class of therapeutics that include microRNA (miRNA) therapies, utilize RNA mimetics to target and treat cancer and other diseases. Compared to single-target small molecule inhibitors, miRNAs represent potent future tools capable of simultaneously targeting multiple aberrant signaling pathways in DMG to increase therapeutic efficacy and prevent tumor resistance and recurrence. To identify targetable, aberrant gene expression in H3K27M-mutated DMG, we performed RNA sequencing of nascent, newly synthesized RNA in the human DMG cell line SU-DIPG-XIII and its isogenic pair SU-DIPG-XIII-H3K27M-KO, where oncohistone expression is prevented by CRISPR gene editing. We compared transcriptional profiles in isogenic cells to render a list of potential disease/tumor-driving genes, expressed solely in H3K27M DMGs, that can be targeted. Computational analysis using miRNet identified miRNAs hsa-miR-124-3p, hsa-miR-27a-3p, and hsa-miR-146a-5p as promising candidate RNA mimetics to develop as new therapeutic options for DMG H3K27M-altered. These miRNAs are predicted to each target 300-400 genes upregulated specifically in H3K27M DMGs, highlighting the powerful capability of this new class of RNA therapeutics. Notably, preliminary proliferation assays in SU-DIPG-XIII cells showed that DMG-specific miRNAs significantly reduced tumor cell growth, highlighting their therapeutic potential. To evaluate efficacy and cellular consequences of identified miRNAs in a panel of human and murine DMG H3K27M-altered cells, we have employed an inducible lentiviral system that allows for precise on/off gene regulation with miRNA mimetics. This system enables controlled manipulation of gene expression, providing a powerful tool to study the functional consequences of miRNAs in various cellular contexts. Furthermore, the panel of DMGs with inducible miRNA will be utilized for *in vivo* testing in relevant orthotopic models and adapted to the PiggyBac intrauterine electroporation DMG model. Our research will pioneer the use of microRNA mimetics as the first proof-of-concept study of using DMG-specific miRNAs to control aberrant gene expression. While this strategy demonstrates significant promise, we acknowledge that advancements in RNA delivery methods will be essential for translating this approach into a clinical reality in the future. In summary, our findings will provide proof-of-concept for use of a new class of RNA therapeutics for the development of innovative future therapies for DMG.

#6699 Development of a fully modified miR-34a (FM-miR-34a) cancer therapeutic: a translational research study.

Shreyas Ganesan Iyer, Digambar Waiker, Ana C. Betustak, Sharjeel Anjum, Andrea L. Kasinski

Purdue University, West Lafayette, IN

MicroRNAs (miRNAs) regulate gene expression by binding to sites within the 3′ untranslated region (UTR) of target mRNAs and exert negative control through mRNA cleavage or translational repression. In cancer, downregulation of tumor-suppressive miRNAs is common, prompting exploration of miRNA restoration strategies for inhibiting tumor growth and suppressing key oncogenic pathways. MicroRNA-34a (miR-34a), known for its tumor-suppressive properties, has advanced to clinical trials as an anti-cancer agent. However, clinical implementation faces challenges such as precise tumor-targeted delivery, susceptibility to degradation in circulation, rapid renal clearance and hindered cytosolic entry due to endosomal entrapment. We tackled a few obstacles by generating a first-in-class fully modified miR-34a (FM-miR-34a) supported by a ligand-mediated vehicle-free delivery platform which exhibited enhanced stability and sustained target gene repression *in vitro* and *in vivo*. Prior to the generation of FM-miR-34a, the molecule would have degraded in circulation quickly. However, with FM-miR-34a showing increased stability in serum, we can now aim to increase the circulation half-life of the molecule, which would allow more miR-34a to be biologically available. To achieve this, we incorporated an albumin-binding moiety within the delivery ligand, allowing the molecule to bind to human serum albumin, the most abundant protein in blood. This in turn increases the overall molecular weight of the cancer therapeutic, pushing it over the renal clearance limit and thus increasing its circulation half-life. We also observe a potent combinatorial anti-tumor response when FM-miR-34a is combined with paclitaxel, a standard chemotherapy for breast cancer. Paclitaxel induces mitotic arrest at G2/M and apoptosis at G0 and G1/S phases, making it an ideal partner for synergistic effects with miR-34a. This approach will potentially allow for reduced dosing of paclitaxel and contribute to better therapeutic efficacy, thus improving quality of life of triple negative breast cancer patients. The research, thus, aims to contribute to the development of a robust therapeutic agent utilizing the miR-34a restoration strategy, addressing delivery challenges, and exploring synergies with existing treatments for enhanced efficacy against cancer.

#6700 Ligand-directed delivery of chemically modified miR-34a to prostate cancer: enhanced targeting and extended pharmacokinetics.

Digambar Kumar Waiker[1], Ahmed M. Abdelaal[1], Shreyas G. Iyer[1], Ikjot Singh Sohal[1], Esteban A. Orellana[2], Kasireddy Sudarshan[1], Kenan E. Ozcan[1], Andrea P. dos Santos[3], Philip S. Low[4], Andrea L. Kasinski[1]

[1]Department of Biological Sciences, Purdue University, West Lafayette, IN,[2]Department of Molecular and Systems Biology, Geisel School of Medicine, Dartmouth College, Hanover, NH,[3]College of Veterinary Medicine, Purdue University, West Lafayette, IN,[4]Department of Chemistry, Purdue University, West Lafayette, IN

Prostate cancer is a leading cause of cancer-related mortality in men. Current therapeutic options include surgical intervention, chemotherapy, immunotherapy, and localized or systemic radiation therapy. However, advanced metastatic prostate cancers are challenging to treat, underscoring the critical need for more effective strategies. Prostate-specific membrane antigen (PSMA), a transmembrane glycoprotein, is overexpressed up to 1000-fold in ~90% of prostate cancers. The high expression level of PSMA, coupled with highly specific targeting ligands, makes it a promising target for diagnostic and therapeutic applications. While various modalities can be delivered via PSMA, delivery of microRNAs has many advantages including the ability to overcome resistance often observed with single-agent treatments due to the ability of miRNAs to downregulate multiple genes simultaneously. Among the miRNAs with anti-cancer properties, miR-34a is among the most promising. Our work and that of others has determined that restoring miR-34a leads to tumor regression. Unfortunately, *in vivo* delivery challenges remain a bottleneck in advancing miR-34a to the clinic. These challenges can be subclassified into three key issues: 1. achieving specific delivery, 2. avoiding nuclease-mediated degradation, and 3. overcoming poor circulation half-life of the miRNA conjugates. Our lab is dedicated to overcoming these challenges. To address these issues, we developed a miRNA-34a vehicle-free strategy that delivers miR-34a to prostate cancer that comprises three critical attributes. Firstly, a peptidomimetic ligand (PSMA-617) was selected based on its selective specificity for PSMA binding, reducing delivery to non-tumorigenic cells. Secondly, the miR-34a is fully modified (FM-miR34a), containing an alternating pattern of 2′-O-methyl and 2′-fluoro modified sugars and phosphorothioate linkages at the 3′ and 5′ ends of each strand to reduce immunogenicity and to provide exonucleases resistance *in-vivo*. Thirdly, 4-(p-iodophenyl)butyric acid, a small molecule that exhibits high affinity for serum albumin, was added to the conjugate, enhancing the pharmacokinetic properties. Through binding to serum albumin, systemic circulation of the PSMA-617-miRNA-34a conjugate is prolonged, providing additional time to achieve receptor saturation, leading to increased bioavailability and therapeutic efficacy. Overall, our data indicates that bringing all three of these components together into a holist vehicle can enhance the clinical utility of miR-34a for treating prostate cancer.

#6701 CircRNA-derived neoantigen identification in cancer vaccine development.

Yi-Cian Zheng, Chun-Kan Chen

Cell Biology & Physiology, Washington University School of Medicine in St. Louis, St. Louis, MO

Immunotherapy has been a popular topic in combating cancer for its great performance in specificity, versatility and durability recently. Cancer vaccines targeting neoantigens have shown clinical efficacy against cancer, eliciting long-lasting immune response and offering a more selective therapeutic approach with less side effects. However, rare mutation rates and low expression levels of neoantigen make it challenging to identify suitable neoantigens. Traditionally, researchers focus on the canonical mRNA transcriptome and proteome to find potential targets. Recent studies suggest that exploring the non-canonical transcriptome and proteome, particularly circular RNAs (circRNAs), can expand the search. CircRNAs, endogenously formed by the unique back-splicing events during pre-RNA processing, can serve as templates for protein synthesis. Some of these non-canonical peptides are specifically expressed in cancer cells, making circRNAs a valid source for neoantigen discovery. We have developed high-throughput circRNA reporter screening methods and mass spectrometry-based peptidomic workflow to pinpoint translating circRNAs and their derived peptides. Using these techniques, we have identified circRNA-derived neoantigens in breast cancer (BC) cell lines that can be used as possible targets for cancer vaccines. These circRNA-derived neoantigens are immunogenic and can trigger dendritic cell (DC) maturation and T cell activation in vitro. Next, we will test these findings by transferring to in vivo mouse system. We will assess cancer vaccine efficacy by targeting the neoantigens using syngeneic mouse tumor models. Our long-term goal is to apply the circRNA neoantigen identification technology to BC patient samples to facilitate cancer vaccine development and improve cancer immunotherapy. Our work will demonstrate the potential of circRNA-derived non canonical neoantigens in cancer vaccines, serving as a crucial first step to prove their effectiveness in therapy. By expanding our search beyond the traditional transcriptome and proteome, we will explore new targets and transform the neoantigen-based treatment. This innovative effort shifts the current understanding of neoantigen sources and opens up new possibilities for targeted cancer therapy strategies.

#6702 Characterization of the microRNA landscape during airway epithelial differentiation and the key role of the miR-34c-5p-FOSL1 regulatory axis in the transformation to squamous metaplasia.

Dalton Hill[1], Phillip Clapp[2], Ying Du[3], Scott Randell[2], Neil Hayes[4]

[1]Medicine, University of Tennessee Health Science Center, Memphis, TN,[2]University of North Carolina School of Medicine, Chapel Hill, NC,[3]TwinStrand Biosciences, Seattle, WA,[4]University of Tennessee Health Science Center, Memphis, TN

MicroRNAs (miRNA) regulate many cellular functions, including proliferation, differentiation, immune regulation, and apoptosis. Dysregulation of miRNAs plays a key causal role in carcinogenesis across a wide variety of cancers. The miRNA family miR-34/449 has been shown to be important for airway epithelial multiciliogenesis and is down-regulated in asthma. Yet, much remains to be learned about the role of miRNAs in airway epithelial development and differentiation. Moreover, specific causal roles of these miRNAs in the development and progression of airway disease states, such as COPD and squamous cell cancer, is largely unknown. In this study, we characterized the expression of miRNAs and their target mRNA during airway epithelial differentiation. We utilized primary human bronchial epithelial cells grown on an air-liquid interface coupled with mRNA and miRNA sequencing at multiple timepoints to robustly characterize the miRNA-mRNA expression landscape of the airway epithelium throughout differentiation. We identified dynamic changes in expression of many miRNAs and their mRNA targets, including several members of the miR-34/449 family. Within this family, we observed highly induced expression of the family member miR-34c-5p throughout differentiation, while its target FOSL1 - a transcription factor - demonstrated decreased expression throughout differentiation. Furthermore, we demonstrated that inhibition of miR-34c-5p resulted in not only increased expression of FOSL1, but also induced the transition to a squamous metaplasia phenotype. Finally, we showed that in human COPD airway tissue samples, miR-34c-5p is significantly downregulated and FOSL1 is significantly upregulated compared to healthy airway, demonstrating the key role the miR-34c-5p-FOSL1 regulatory axis plays in the transition to squamous metaplasia. This study builds a strong foundation and offers promising future directions for the role of miRNAs in both normal airway epithelial differentiation as well as their role in disease. The discovery of the miR-34c-5p-FOSL1 regulatory axis in the transformation to squamous metaplasia uncovers a key molecular regulator of this preneoplastic state. Further, given the increasing understanding of the role of FOSL1 in carcinogenesis as well as its well-established high expression across many different types of cancers, the tight regulation of FOSL1 by miR-34c-5p uncovers a promising avenue for both further understanding the molecular drivers of carcinogenesis as well as potential therapeutic targets.

#6703 LncRNA UCA1 modulates LDHA expression and drives glucose metabolism in colorectal cancer cells.

Salique H. Shaham, Ricardo Pequeno Bracho, Manish K. Tripathi

Medicine and Oncology ISU, University of Texas Rio Grande Valley, McAllen, TX

Background: Colorectal cancer (CRC) ranks as the third most commonly diagnosed malignancy globally and is the second leading cause of cancer-related deaths in the United States. In 2024, an estimated 152,810 new cases of CRC are projected, with 53,010 fatalities. The prognosis for metastatic CRC (mCRC) remains grim, with a survival rate of less than 15%. Urothelial cancer-associated 1 (UCA1), a long noncoding RNA (lncRNA), is dysregulated in CRC and has been implicated in tumor progression. Our research highlights significantly elevated UCA1 expression in metastatic CRC cells, where its association with the GLUT1 and the metabolic enzyme lactate dehydrogenase A (LDHA) suggests a critical role in promoting the Warburg effect —a metabolic hallmark of cancer.
Methods: To evaluate the oncogenic phenotype, assays for migration, invasion, colony formation, and proliferation were conducted. Lentiviral vectors with puromycin selection were used to generate UCA1 overexpression and knockdown cell lines, and fluorescence-activated cell sorting (FACS) was performed to enrich GFP-expressing cells. Anoikis resistance was induced using PolyHEMA plates, and glucose metabolism markers were analyzed at specific time points through RT-PCR and western blotting. Immunofluorescence was used to assess the localization and expression of relevant proteins. Metabolic assays were performed using the Seahorse xFp.
Results: Metastatic CRC cells (SW620) exhibited significantly higher UCA1 expression compared to oncogenic CRC cells (SW480), particularly after 36 hours of anoikis induction. Enhanced expression of GLUT1, YAP1, and FDFT1 was also observed in SW620 cells. UCA1 overexpressing cell line (SW480+UCA1) also showed enhanced expression of GLUT1, YAP1, and FDFT1 correlating with glucose metabolism modulation during anoikis. The expression of LDHA, a key enzyme in the Warburg effect that converts pyruvate to lactate despite oxygen availability, was markedly elevated in SW620 cells compared to SW480 cells. Similarly, LDHA expression was significantly higher in UCA1-overexpressing SW480 cells compared to vector controls. This suggests a functional link between UCA1 and LDHA in metastatic CRC, supported by published studies linking LDHA with GLUT1 and YAP1.
Conclusion: These findings underscore the pivotal role of lncRNA UCA1 in regulating LDHA expression and driving glucose metabolism via the Warburg effect during anoikis resistance, a critical step in metastasis. Targeting the UCA1-LDHA axis may offer novel therapeutic opportunities for combating metastatic CRC.

MOLECULAR/CELLULAR BIOLOGY AND GENETICS: Pathological Impact of Oncogenes and Tumor Suppressors
Poster Session

#6707 Direct *in vivo* CRISPR screen identifies *BAP1* and *FAT1* as potent tumor suppressors in sarcomagenesis.

Jianguo Huang[1], Xingliang Liu[1], Warren Floyd[2], William Haugh[1], Andrea R. Daniel[2], Zhaoyu Sun[1], Nerissa T. Williams[2], Melissa J. Kasiewicz[1], Yaping Wu[1], Diana M. Cardona[2], Brian Piening[1], John T. Welle[1], Wesley K. Rosales[1], Venkatesh Rajamanickam[1], So Young Kim[2], Eric Xu[2], Lixia Luo[2], Yan Ma[2], Kristianne M. Oristian[2], Omar Lopez[2], Nicholas E. S. Sibinga[3], Rutulkumar Patel[2], Ziqiang Zhang[4], Alexander J. Lazar[5], Corinne M. Linardic[2], Brady Bernard[1], William L. Redmond[1], Walter J. Urba[1], David G. Kirsch[6]

[1]Providence Cancer Institute, Portland, OR,[2]Duke University Medical Center, Durham, NC,[3]Albert Einstein College of Medicine, Bronx, NY,[4]Tongji University School of Medicine, Shanghai, China,[5]University of Texas MD Anderson Cancer Center, Houston, TX,[6]University of Toronto, Toronto, ON, Canada

Undifferentiated pleomorphic sarcoma (UPS) is among the most common soft tissue sarcomas (STS) in adults. For decades, little therapeutic progress has been made for STSs, including UPSs. Targeted therapies for tumors driven by specific genetic mutations have proven to be more effective than standard chemotherapies. However, the molecular pathogenesis of UPSs remains unknown, hindering the development of targeted therapies for UPSs. Approximately 65% of UPSs harbor *TP53* mutations, but somatic mutation of *Trp53* alone is insufficient to induce sarcomas in vivo. The addition of *Rb1* mutation alongside a *Trp53* mutation induces sarcomas in vivo, though with low frequency of tumor onset and slow growth. The role of other genes in facilitating *Trp53*-driven sarcomas is largely unknown. Through a customized *in vivo* CRISPR/Cas9 screen of 35 genes commonly mutated in UPSs, followed by individual gene validation in vivo, we discovered that *Bap1* knockout cooperates with *Trp53* knockout to induce sarcomas in vivo. Furthermore, we demonstrated that *Fat1* deletion increased the onset frequency and growth of *Trp53* and *Rb1*-driven sarcomas in a genetically engineered mouse model. Through multiplex immunohistochemistry and flow cytometry, we found that mouse sarcomas induced by *Trp53* and *Rb1* mutations are significantly enriched with immune cells compared to other mouse sarcomas we generated in vivo. Finally, we show that PARP inhibition may be a potential targeted therapy for *RB1*-loss STSs and BRD4 inhibition may be a potential targeted therapy for *FAT1*-loss STSs.

#6708 RUNX3 restoration in breast tumors leads to profound growth regression via c-Myc inhibition.

Nawaphat Jangphattananont[1], Junichi Matsuo[1], Wei Peng Yong[2], Linda SH Chuang[1], Yoshiaki Ito[1]

[1]Cancer Science Institute of Singapore, Singapore, Singapore,[2]Department of Haematology-Oncology, National University Cancer Institute, National University Health System, Singapore, Singapore

Genetic alterations in the RUNX family genes and their co-factor CBFB are frequently observed in cancer. In breast cancer, RUNX1 and CBFB are identified as significantly mutated genes, while RUNX3 expression is often silenced through hypermethylation. These observations highlight the importance of RUNX genes in mammary homeostasis. Previous studies on luminal-type breast cancer demonstrated that RUNX3 restoration inhibits tumor progression by destabilizing the estrogen receptor α (ERα) protein. However, despite the therapeutic promise of reactivating RUNX3 in breast cancer, its role in hormone receptor-negative subtypes remains inadequately understood. Furthermore, RUNX3 has been reported to exhibit dualistic roles and depending on the cancer type and stage, may function as either tumor suppressor or oncogene. Yet, the molecular mechanisms underlying this duality remain unclear. To investigate the role of RUNX3 in breast pathogenesis, we developed the MMTV-PyMT; Krt8-CreERT2; Rosa26-lsl-RUNX3 mouse model and analysed the effect of conditional induction of RUNX3 expression in Krt8+ mammary epithelial cells and tumor cells within the MMTV-PyMT-driven breast cancer. Without RUNX3 induction, MMTV-PyMT mice developed an average of eight tumors per mouse, and transcriptomic analyses revealed robust activation of the Myc-signaling pathway. Conversely, the induction of RUNX3 significantly suppressed tumor growth, concomitant with a diminished c-Myc expression. This observation underscores the tight relationship between RUNX3 and c-Myc and identifies RUNX3-mediated suppression of Myc pathway as a major aspect of RUNX3 tumor suppressive role. Remarkably, histological analyses of RUNX3-induced breast tumors showed two distinct clusters of cancer cells: proliferating RUNX3-positive cells and non-proliferating RUNX3-positive cells. Spatial transcriptomic profiling revealed elevated c-Myc expression and suppressed RUNX1 expression in the proliferating RUNX3-positive cells compared to non-proliferating RUNX3-positive cells. This observation raises the intriguing possibility that interplay between RUNX1 and RUNX3 determines cellular proliferative potential and implicates RUNX1 as a regulator of RUNX3's paradoxical traits. Consistently, overexpression of RUNX3 in human breast cancer cells induced significant cell death and upregulated RUNX1 expression. Our findings suggest the convergence of RUNX1 and RUNX3 pathways in driving cell states within the tumor microenvironment and establish a causal link between RUNX3, c-Myc activity, and tumor growth dynamics. This study provides critical insights into dual roles of RUNX3 in breast cancer pathogenesis.

#6709 *NF1* mutations in lung adenocarcinoma preclinical models and potential targeted therapies: The crucial role of the RAS-MAPK pathway.

Jean-Stephane Giraud[1], Doriane Gorret[1], Manuela Ye[1], Dominique Lallemand[1], William C. Reinhold[2], Didier Decaudin[3], Ingrid Laurendeau[1], Eric Pasmant[1], Camille Tlemsani[1]

[1]Institut Cochin, Paris, France,[2]Genomics and Pharmacology Facility, National Cancer Institute, Bethesda, MD,[3]Institut Curie, Paris, France

Targeting tumor suppressor genes, such as *NF1,* is a major challenge in cancer. Somatic *NF1* mutations are found in ~15% of lung adenocarcinoma (LUAD) cases. Still, the molecular vulnerabilities and cellular adaptations associated with these mutations remain unclear. Therefore, we aimed to study the functional consequences of *NF1* loss in LUAD and its impact on the RAS/MAPK pathway, as well as potential therapeutic strategies. We established isogenic *NF1*-mutated cellular models (mono- and bi-allelic *NF1* mutations) using CRISPR-Cas9 technology on the HBE4-E6/E7-C1 human bronchial epithelial cell line. Our studies included transcriptomic, phenotypic, and pharmacological studies, utilizing RAS/MAPK and PI3K-AKT-mTOR pathways inhibitors. Differential expression analysis was performed with the “limma” R-package with a 2 × 2 factorial design. We also computed *in silico* data from public drug sensitivity databases of LUAD cell lines. We tested pharmacological combinations in a patient-derived xenograft mouse (PDX) obtained from a LUAD tumor with a homozygous nonsense mutation of *NF1:* c.943C>T, p.(Gln315*) (Variant Allele Frequency ~94%). We confirmed that *NF1* loss-of-function led to the RAS/MAPK pathway activation, while the PI3K-AKT-mTOR pathway was not activated. We observed distinct transcriptomic clusters based on *NF1* mutation status (wild-type [WT], heterozygous, and homozygous). Gene Set Enrichment Analysis found significant *KRAS* signaling down-regulated gene enrichment in *NF1*-mutated clones (q-value=0.059). Moreover, homozygous *NF1*-mutated lung cells exhibited more aggressive characteristics *in vitro* than heterozygous or WT cells: higher proliferation, higher adhesion, and greater migration capacities. In our *in vitro* pharmacological tests, only homozygous *NF1* mutated cells were sensitive to Trametinib (a MEK inhibitor). No sensitivity was observed in these models when treated with the mTOR inhibitor AZD8055 or the PI3K inhibitor Buparlisib alone. Trametinib appeared to modulate the RAS/MAPK pathway more significantly at the transcriptomic level in cells with homozygous *NF1* mutations than in *NF1* WT. Trametinib and Buparlisib synergistically affected the *NF1*-/- model. Analysis of public drug sensitivity databases indicated that inhibitors of the RAS/MAPK and PI3K-AKT-mTOR pathways are effective against *NF1*-mutated LUAD cell lines. We then performed *in vivo* pharmacological tests on the LUAD PDX: Trametinib alone and in combination with Buparlisib resulted in significant tumor volume reductions of 72% and 84%, respectively. Collectively, these findings establish a promising possible efficacy of MEK inhibitors for LUAD patients with *NF1* homozygous mutation.

#6710 The oncogene MYC drives circadian clock disruption and regulates cell plasticity in early lung carcinogenesis.

Juliana Cazarin[1], Cody Naccarato[1], Jamison Burchett[1], Siti Noor Ain Binti Ahmad Shahidan[1], Min Yee[2], Michael O'Reilly[2], Brian J. Altman[1]

[1]Department of Biomedical Genetics, University of Rochester, Rochester, NY,[2]Department of Pediatrics, University of Rochester, Rochester, NY

Circadian rhythms coordinate the compartmentalization of biological processes, such as cell division and metabolism, to the time of the day. Disruption of these rhythms has been implicated in lung carcinogenesis. Circadian disruption accelerates tumor growth in lung adenocarcinoma (LUAD) mouse models, suggesting an oncogenic role of disrupted rhythms. A disrupted circadian clock has been reported in human LUAD, with alterations in circadian gene expression linked to poor prognosis. However, the origins of clock disruption in LUAD and how it supports tumor growth remain unclear. The oncogene MYC has been linked to circadian disruption in cancer cells. MYC or its related family members are mutated or amplified in more than 30% of LUAD cases, with higher MYC expression associated with worse survival. An analysis of public datasets reveals an inverse correlation between MYC expression and the circadian clock gene BMAL1 in human LUAD. BMAL1, a critical component of the mammalian circadian clock, is indispensable for maintaining circadian rhythms, as its deletion leads to their complete disruption in both mice and cells. However, whether MYC directly drives clock disruption in the lung and the extent to which clock dysfunction contributes to MYC-driven carcinogenesis remain unknown. Using organoids derived from alveolar type II cells (ATII), the cell of origin for LUAD, we showed that MYC suppresses BMAL1 expression. MYC also reduced the expression of ATII cell-lineage markers, suggesting activation of a dedifferentiation program. In a LUAD mouse model, three days of MYC overexpression in ATII cells suppressed molecular clock genes, including BMAL1. This was accompanied by increased *Ki67* expression, a marker of proliferation, and the loss of cell-lineage markers early events in lung carcinogenesis. Similarly, in human LUAD, molecular subtypes with reduced epithelial identity markers and poorer survival showed lower BMAL1 expression. These findings suggest a role for the circadian clock in regulating differentiation and tumor progression. Pharmacological restoration of the circadian clock *in vivo* using the RORα agonist SR1078 reduced MYC-induced *Ki67* expression in the lung, indicating that circadian disruption mediates some of MYC's oncogenic effects. Further analysis using CUT&TAG in a mouse lung epithelial cell line revealed that BMAL1 binds to promoters of ATII identity genes (*Sftpb, Abca3*) and cell cycle regulators (*Cdk4*), suggesting that the circadian clock influences both lung epithelial cell identity and proliferation. Overall, we identify MYC as a driver of circadian clock disruption in lung alveolar cells, which may contribute to MYC-induced proliferation and dedifferentiation in early lung cancer. Restoring circadian clock function could represent a potential therapeutic strategy for targeting tumors with MYC amplification.

#6711 Investigating competitive advantage of mismatch repair loss in colonic organoids.

C. Guild, A. Rath, C. Heinen;
University of Connecticut Health Center, Farmington, CT

Lynch syndrome (LS) is an inherited predisposition to developing colon, endometrial, ovarian, and other cancers, affecting about one in 300 people. It is caused by inheriting a pathogenic variant in one copy of a DNA mismatch repair (MMR) gene. Cancers that arise in LS patients are MMR deficient, meaning the cancer cells have lost the remaining, functional MMR allele and thereby lost MMR function. It is known that losing MMR function leads to a drastically increased mutation rate. Additionally, cells in culture that lose MMR function become unresponsive to normally lethal DNA damage caused by alkylating agents. However, the question remains whether this immediate, aberrant DNA damage response contributes to cancer development in LS patients. We hypothesize that loss of this MMR-dependent damage response function provides a competitive advantage to colonic progenitor cells. Importantly, we predict that the strength of this advantage depends on the amount and type of DNA-damaging agents in the environment, such as oxidative stress, that promote expansion of MMR-deficient cells. If this hypothesis is true, it would provide insight into how this selective pressure could be mitigated using chemoprevention, and thereby decrease the incidence of colon cancer in this population.

To test this, we are using stem cell-derived human colonic organoids and CRISPR gene editing to examine differences in survival and proliferation between otherwise syngeneic MMR-deficient (dMMR) and MMR-proficient (pMMR) colonic organoids. We observe that in atmospheric oxygen, dMMR colonic organoids exhibit enhanced budding compared to pMMR colonic organoids, consistent with our hypothesis of a competitive advantage of dMMR colonic cells. Addition of the antioxidant vitamin C decreases the difference in budding between pMMR and dMMR organoids suggesting this difference is mediated by oxidative stress. Additionally, mixed dMMR and pMMR colonic organoid cultures in ambient oxygen become increasingly dMMR overtime, suggesting dMMR colonic progenitor cells have a competitive advantage over pMMR colonic progenitor cells. To explore the mechanism behind this finding, we are analyzing differences in gene expression between pMMR and dMMR colonic organoids. Preliminarily, we have observed increased expression of the senescent marker gene *CDKN2A* (p16) and decreased expression of the colonic stem cell marker *LGR5* in pMMR organoids compared to dMMR organoids. These results suggest that the MMR pathway may promote senescence and/or terminal differentiation in colonic organoid cultures grown in ambient oxygen. Taken together, these results are consistent with our hypothesis that loss of MMR function imparts a competitive advantage in colonic cells in non-transformed colonic organoids that could contribute to cancer formation in LS patients.

#6712 Epigenetic reactivation of a tumor suppressor program in leukemia.

E. Wang[1], C. Han[1], A. Calderon[1], I. Aifantis[2], A. Tsirigos[2], A. Lawal[1], S. H. Rajendran[1], S. Karma[1], H. A. Abbas[3], M. Y. Yassouf[3], Z. Zhang[4], P. Miura[4];
[1]The Jackson Laboratory for Genomic Medicine, Farmington, CT, [2]NYU School of Medicine, New York, NY, [3]MD Anderson, Houston, TX, [4]UConn Health, Farmington, CT

Tumor suppressor genes (TSGs) impart a cellular fitness in human cancers, including acute myeloid leukaemia (AML). In contrast to genetic alterations, silencing of TSGs without direct mutations lacks genomic scars, thereby complicating the clinical management of AML. Here we conducted genome-scale phenotypic CRISPR/Cas9 screens to uncover genes that suppress leukaemia differentiation. We identified ZBTB7A, a transcriptional repressor that is downregulated in leukaemia stem cells (LSCs) and associated with poor survival outcomes in AML patients. Our results show that the mRNA decay factor ZFP36L2 binds to the 3'-untranslated region (UTR) of *ZBTB7A* to induce its mRNA degradation. Mechanistically, the loss of ZBTB7A promotes a dysregulated inflammatory state that drives leukaemia progression. To explore the translational potential, we coupled *in situ* Flow-FISH readout with CRISPR-based screening which identified KDM4 as a therapeutic vulnerability to restore the tumor suppressive function of ZBTB7A. Pharmacologic inhibition of KDM4 elevated ZBTB7A levels, induced terminal leukaemia differentiation, and broad anti-leukemic efficacy across AML subtypes. Inhibition of KDM4 *in vivo* prolonged survival of leukemic mice while sparing normal haematopoiesis. Together, our findings reveal regulatory circuits underlying the tumor suppressive role of ZBTB7A and highlights an epigenetic therapy to restore its function in myeloid malignancies.

#6714 GPR109A expression provides a molecular link between the colonic microbiome and the associated reduced risk of colon cancer formation.

Muthusamy Thangaraju[1], Nanditi N. Thangaraju[1], Sonia Batan[1], Jabuneesa Khanom[1], Kunal Kumar[1], Snigdha Ganjikunta[1], Subash Sundaram[1], Sathish Sivaprakasam[2], Nikhil Patel[3], Santhakumar Manicassamy[4], Puttur D. Prasad[1], Vadivel Ganapathy[2]

[1]Biochemistry and Molecular Biology, Augusta University, Augusta, GA,[2]Cell Biology and Biochemistry, Texas Tech University Health Sciences Center, Lubbock, TX,[3]Pathology, Augusta University, Augusta, GA,[4]Medicine, Augusta University, Augusta, GA

Obesity is associated with an increased risk of colorectal cancer (CRC) in both men and women. It has been shown that a body mass index (BMI) of more than 30 has almost twice the risk of early-onset CRC compared with a BMI of 18 - 23. Although the obesity-associated increase in both CRC risk and progression is associated with several systemic and localized factors, the microbiome could play a critical role in obesity-associated CRC. Using mouse models of CRC, we found that dietary patterns shift the normal colonic microbiota populations. Interestingly, the colonic probiotic *Lactobacillus* abundance negatively correlated with body fat composition, providing evidence that obesity interacts with diet to regulate bacterial populations in the colon. Body fat composition is negatively associated with colonic bacteria-derived host cometabolites, Hippurate, and Butyrate, two agonists of the G-protein coupled receptor 109A (GPR109A). We have shown that GPR109A expression is significantly reduced in human CRC compared to the normal colonic epithelium, and GPR109A activation limits CRC growth and progression. Low GPR109A expression in human CRC limits overall survival. Our preliminary studies show that GPR109A expression is negatively associated with increasing BMI, which in turn reduces the bacterial metabolites Hippurate and Butyrate, especially in African-American (AA) CRC patients than in Caucasian (CA). Dietary supplementation of Hippurate and Butyrate significantly reduced CRC formation, and this effect is mediated through GPR109A. *Gpr109a* knockout mice are more prone to chemical carcinogens, and genetically engineered mouse models (GEMM) of CRC and dietary supplementation of Hippurate and Butyrate do not show significant beneficial effects. Similarly, the ketogenic metabolite beta-hydroxybutyrate (BHB), an endogenous agonist of GPR109A, levels are significantly lower in obese AA CRC patients than in lean AA CRC and CA CRC patients. Dietary supplementation of the ketogenic diet significantly increases the *Lactobacillus* abundance and Hippurate, Butyrate, and BHB levels in *Gpr109a* wild-type mice, but this effect is blunted in Gpr109a knockout mice. Overall, our study provides a novel role of GPR109A in the regulation of the colonic microbiome and its metabolites and associated CRC prevention. Further, our study provides evidence that GPR109A expression links to obesity-associated CRC formation, and GPR109A agonists could have a significant clinical impact on the prevention and treatment of CRC.

#6715 Cross-species investigation of gene copy number and cancer resistance.

Morla Phan[1], Thompson Worden[1], Sarah Adamowicz[2], Stephane Lair[3], Mauricio Seguel[1], Claire Jardine[1], Geoffrey Wood[1], Dirk Steinke[2]

[1]Pathobiology, University of Guelph, Guelph, ON, Canada,[2]Integrative Biology, University of Guelph, Guelph, ON, Canada,[3]Sciences Cliniques, Universite de Montreal, Montreal, QC, Canada

Low cancer incidence rates are observed in large mammals such as elephants and whales, despite their mass and long lifespan hypothetically contributing to a higher lifetime probability of acquiring oncogenic mutations. Few studies have investigated cancer-related genes in these animals. One reported that bowhead whales possess two functional copies of PCNA. Multiple studies have investigated the presence of multiple retrogene copies of TP53 in elephants. Extra copies of genes involved in DNA damage detection and repair could contribute to cancer resistance. Droplet digital PCR (ddPCR) was used to quantify gene copy number for TP53, PCNA, HER2, DLG1, and DLG2 in genomic DNA extracted from frozen skin samples of beluga, narwhal and bowhead whales (n=20 each). Results showed that all 3 whale species had more than one copy of PCNA. Agarose gel PCR showed the simultaneous presence of wild-type PCNA (possessing introns) and variable numbers of pseudogene sequences (lacking introns) within individuals for belugas, narwhals, and bowheads. ddPCR found that elephants did not have increased PCNA copy number, but agarose gel PCR showed the presence of PCNA pseudogenes. Similar patterns were also observed in rhinos, horses, cows and other ungulate species. To investigate copy number loss in cancer, ddPCR was performed on formalin-fixed, paraffin embedded normal and matched tumor tissue of 7 individual belugas from the St. Lawrence estuary, an area that was historically contaminated with industrial carcinogens. Copy number loss was not observed for any investigated tumor suppressor gene in tumor tissue compared to normal tissue. Our results show that bowhead whales are not unique in having multiple copies of PCNA. The presence of incomplete copies with unknown function may influence copy number quantification. Further study is required to understand the significance of the variable PCNA pseudogene copies. Understanding natural copy number variation in tumor suppressor genes may provide insight into risk factors and prevention methods across species.

#6716 Effect of ZFHX3 in endometrial cancer progression.

Sanjeev Ganesh, Areebah Qazi, Swornalata Pukhrambam, Sophia Agrusa, Jessica Long, Katherine Gurdziel, Michael Wilson

Wayne State University School of Medicine, Detroit, MI

Endometrial cancer (EC) is the most prevalent gynecological cancer and the 4th most common cancer in women globally. The frequency of EC is increasing in contrast to other prevalent cancers driven by lifestyle choices and metabolic conditions. Currently, research is focused on molecular mechanisms of tumor initiation and tumor microenvironment. ZFHX3 (ATBF1), is a transcription factor that has roles in embryogenesis steroid hormone regulation. It has been identified as a tumor suppressor gene in multiple cancer types. ZFHX3 mutations have been observed in normal endometrium and reports suggest it is mutated in about 20% of EC cases. Despite these frequent mutations, very few studies fully comprehend the molecular mechanisms behind ZHFX3 inactivation and its subsequent role in initiating EC. Our study aims to find the role of ZFHX3 in initiating EC. We knocked down ZFHX3 in 12Z cells via siRNA, followed by mRNA-seq, to examine its function in the endometrial epithelium. Our preliminary data suggests that ZFHX3 regulates c-MyB, a proto-oncogene, suggesting its role as a tumor suppressor gene in EC. We performed transcriptomic analysis on endometrial cells following the knockdown of ZFHX3 and identified differential expression of cell cycle and cell adhesion-related genes, suggesting important roles for ZFHX3 in oncogenic processes. We also overexpressed ZFHX3 expression in Ishikawa cells and treated them with estrogen and progesterone. These cells were subjected to RNA-seq analysis to identify the different signaling pathways affected by the presence of progesterone. Comparing the 2 RNA-seq data, we found EGR1 to be a common gene. We also investigated the effects of ZFHX3 knockdown in the endometrial epithelium *in vivo* and found an increase in the Ki67 expression and endometrial gland number, indicating endometrial hyperplasia. Future studies will focus on the relation between ZFHX3 and EGR1, its subsequent signaling pathways, and elucidating its role as a tumor suppressor.

#6718 Analysis of the pathological significance of CD109 in pancreatic cancer using the mouse model.

Y. Murakumo, Y. Sakurai, M. Ichinoe;
Kitasato Univ. School of Medicine, Sagamihara, Japan

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive malignancy with a high fatality rate. Because there are few effective treatments for advanced pancreatic cancer, the development of new molecular targeted therapies is desired. CD109 is a glycosylphosphatidylinositol-anchored glycoprotein, whose expression is upregulated in some types of malignant tumors. We recently reported that positive expression of CD109 in tumor cells was significantly associated with poor prognosis in PDAC. In addition, CD109 expression was also detected in the stroma surrounding the tumor cells, suggesting its expression in cancer-associated fibroblasts. The stromal CD109 expression significantly correlated with tumor progression in PDAC. In the present study, we aimed to further investigate the histopathological and prognostic significance of CD109 in PDAC using the mouse model. In the normal mouse pancreas, CD109 expression was almost undetectable by X-gal staining and in situ hybridization. In the PDAC tissues, CD109 expression was detected not only in the tumor cells but also in the surrounding stromal cells, which was similar to that in human PDAC. CD109 expression was predominantly detected in undifferentiated tumor cells rather than differentiated tumor cells. In addition, we generated a CD109-deficient PDAC mouse model line, KPC-CD109KO, and it was revealed that CD109-deficinet KPC mice tended to develop differentiated adenocarcinomas compared with KPC mice with wild-type CD109. However, CD109 deficiency did not affect the survival of KPC mouse. Taken together, CD109 is expressed not only in the tumor cells but also in the stroma in PDAC, and is associated with tumor differentiation in PDAC.

#6719 GULP1 knockout promotes carcinogen-induced bladder cancer by regulating the NRF2-KEAP1 signaling axis.

Chonji Fukumoto[1], Naveen C. Pandey[2], Sylvia S. Sanchez[2], Pritam Sadhukhan[1], Christopher M. Koenig[2], Susmitha R. Pagadala[2], Fenna C. M. Sille[2], Mohammad O. Hoque[1]

[1]Department of Otolaryngology-Head and Neck Surgery, Johns Hopkins University School of Medicine, Baltimore, MD,[2]Department of Environmental Health and Engineering, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD

Urothelial carcinoma of bladder (UCB) is the ninth most commonly diagnosed cancer worldwide. Some key genetic and epigenetic alterations are hallmarks on the initiation and progression of UCB. The nuclear factor E2-related factor 2 (NRF2)-Kelch-like ECH-associated protein-1 (KEAP1) signaling pathway, essential for detoxifying reactive oxygen species, is normally regulated by the interaction of NRF2 with KEAP1and the actin cytoskeleton in the cytoplasm. However, genetic and epigenetic alterations can lead to a constitutive activation of NRF2 through the loss of KEAP1 function. This dysregulation results in NRF2 translocation to the nucleus, triggering the expression of numerous genes. We recently reported that phosphotyrosine binding (PTB) domain-containing engulfment adaptor protein 1 (GULP1) is frequently silenced in UCB by promoter hypermethylation, and interacts with the KEAP1-actin cytoskeleton complex to regulate NRF2. Furthermore, the loss of GULP1 expression led to uncontrolled NRF2 signaling, driving cancer progression and resistance to chemotherapy. Here, to strengthen our previous findings that GULP1 acts as a tumor suppressor gene (TSG) in UCB, we exposed GULP1 knockout (KO) mice to a most commonly used carcinogen for generating UCB model in mice. To this end, we exposed 4-week-old GULP1 KO and wild type (WT) C57BL/6 mice to N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) for 6 to 20 weeks. We then analyzed the resulting bladder tumors using various methods to investigate the NRF2-KEAP1 axis, tumor subtype, and several tumor immune microenvironment (TIME) factors. Our data demonstrates that GULP1 KO exhibits a higher frequency of carcinogen-induced UCB and muscle invasion compared to WT counterparts via regulating the NRF2-KEAP1 axis. Furthermore, GULP1 KO carcinogen-induced tumors appear at earlier time point compared to WT mice. Interestingly, GULP1 KO mice exhibited a characteristic subtype and TIME dynamics that differed from those of WT. In conclusion, our pilot in vivo data suggested that GULP1 may regulate the NRF2-KEAP1 signaling pathway in UCB. Further experiments are ongoing to understand the comprehensive mechanisms of GULP1 involvement in UCB and its association with chemo and immunotherapy response.

#6720 The role of the Co-chaperone UNC45A in breast and prostate cancer.

Asif Elahi, Taoufik Llbiyi, Vamsi Krishna Kommalapati, Yasmeen Jilani, **Ahmed Chadli**

Augusta University Medical Center, Augusta, GA

UNC45A is a key co-chaperone supporting cancer cell proliferation and tumorigenesis. Interestingly, UNC45A is essential for cancer cells but not for normal cell proliferation. However, the mechanism underlying UNC45A regulatory function is not well studied. We have previously shown that UNC45A functions as a glucocorticoid receptor (GR) co-activator to drive the mitotic kinase NEK7 gene transcription. NEK7 is essential for centrosome separation, mitotic spindle formation, and cytokinesis. GR has been implicated in promoting aggressive prostate tumor growth and resistance to therapy. Indeed, GR upregulation is an established mechanism to bypass the androgen receptor blockade therapy in metastatic-resistant prostate tumors. Here, we tested the hypothesis that UNC45A promotes GR-mediated resistance to hormonal therapy. To this end, we used shRNA-mediated gene silencing to evaluate the impact of UNC45A loss on PC3 prostate tumor progression. Our results show that silencing UNC45A reduces cell proliferation *in vitro* and tumor growth *in vivo*. TUNEL analysis and Ki67 staining show that loss of UNC45A triggers DNA damage, reduced cell proliferation, and apoptosis in PC3 tumors grown in the NOD SCID mice model. Immunofluorescence analysis shows a remarkable disorganization of centrosomes in tumor cells expressing UNC45A shRNA. The importance of UNC45A in promoting enzalutamide resistance is being tested in tridimensional spheroid culture and *in vivo* and will be discussed in this meeting. Overall, our results support the hypothesis that UNC45A is a promising cancer therapeutic target and highlight the need for developing UNC45A small molecule inhibitors to treat aggressive diseases such as prostate and breast cancers.

#6721 NUAK2: The key player in ovarian cancer progression - Unraveling the impact of expression and nuclear localization.

Cong Xu, O.G.W Wong, Annie.N.Y Cheung

The University of Hong Kong, Pokfulam, Hong Kong

Background: NUAK2 is a kinase related to the catalytic α-subunits of the AMP-activated protein kinase (AMPK) complexes. Although NUAK2 is amplified in several human cancers, its role in cancer is poorly understood. By immunohistochemical study, we demonstrated that elevated nuclear expressed NUAK2 is associated with poor ovarian cancer patient survival and more aggressive cancer cell behaviour. Exploring the implications of NUAK2 in ovarian cancer could offer valuable perspectives on disease development and patient prognosis.
Aim: To delineate the impact of NUAK2 nuclear localization on cellular mechanisms crucial for tumor proliferation, and metastasis.
Methods: Lentiviral expression vectors harbouring wild type NUAK2 (NUAK2-WT) and NUAK2 NLS mutant (NUAK2-^{375}AAAA^{378}) were used to transduce KF and OVCA420, two ovarian cancer cell lines expressing low level of endogenous NUAK2. Cytoplasmic localization of NUAK2-^{375}AAAA^{378} was confirmed by immunoblotting and immunofluorescence microscopy. The impact on cell growth was evaluated by CCK8 assay and colony formation assay. The ability of the transduced cells in migration and invasion was compared with wound healing assay and transwell assay.
Results: Mutating the NLS excluded NUAK2 from the nucleus. Cells expressing NUAK2-^{375}AAAA^{378} proliferated significantly slower and migrated significantly slower than cells expressing NUAK2-WT.
Conclusion: The study's outcomes indicated nuclear localization is crucial for NUAK2's oncogenic functions in enhancing the proliferation and migration capabilities of ovarian cancer cells. These results underscore the crucial role of NUAK2 in ovarian cancer progression.

#6722 OFD1 as a novel biomarker in cholangiocarcinoma: insights into ciliary dysfunction and tumor growth.

Fabiola Piscopo[1], Anna Barbato[2], Massimiliano Salati[3], Carla Pollastro[2], Giorgia Gitano[2], Alberto Marino[2], Lorenzo Evangelista[2], Luigi Ferrante[2], Antonella Iuliano[4], Luca Reggiani-Bonetti[5], Maria Salatiello[6], Pasquale Pisapia[6], Umberto Malapelle[6], Giancarlo Troncone[6], Massimo Dominici[3], Brunella Franco[1], Pietro Carotenuto[1]

[1]Department of Translational Medical Science, Telethon Institute of Genetics and Medicine (TIGEM), Medical Genetics, University of Naples "Federico II", Naples, Italy,[2]Telethon Institute of Genetics and Medicine (TIGEM), Naples, Italy,[3]Division of Oncology, Department of Oncology and Hematology, University Hospital of Modena, Modena, Italy,[4]Department of Mathematics, Informatics and Economy (DIMIE), University of Basilicata, Potenza, Italy,[5]Department of Medical and Surgical Sciences for Children and Adults, University Hospital of Modena, Modena, Italy,[6]Department of Public Health, University of Naples "Federico II", Naples, Italy

Cholangiocarcinoma (CCA) is a rare and aggressive solid tumor originating from cholangiocytes lining the biliary ducts. It is the second most common primary hepatic malignancy, with an increasing global incidence and limited therapeutic options. Recent studies suggest that the primary cilium acts as a tumor suppressor in the biliary tract by regulating key cancer-related signaling pathways. Moreover, evidence links the reduction or loss of primary cilia to increased aggressiveness in various human cancers. Here, we aimed to identify and functionally characterize primary cilia-associated genes with potential translational relevance for CCA prognosis and treatment. Our study included 120 CCA patients, comprising 51% (36) with intrahepatic CCA and 49% (34) with extrahepatic CCA. Whole-transcriptome and whole-exome profiling were conducted on laser micro-dissected formalin-fixed paraffin-embedded (FFPE) tissue specimens. Bioinformatic analysis identified OFD1 as the top-ranked cilium-associated gene (fold-change ≥ 2.5, $p < 0.01$) among differentially expressed genes in tumor, stromal, and normal tissues. We investigated the role of OFD1 in cilia formation and cell proliferation through in vitro experiments in healthy human cholangiocytes and CCA cell lines. Immunofluorescence staining with ciliary markers revealed that OFD1 silencing promoted ciliogenesis and inhibited tumor growth in CCA cell lines. Western blot analysis confirmed a significant upregulation of ciliary proteins, including PCM1, ARL13B, and acetylated tubulin, following OFD1 silencing. Furthermore, CRISPR/Cas9-mediated OFD1 depletion in CCA cells led to a marked reduction in cell proliferation and restored ciliogenesis. Conversely, OFD1 overexpression in normal cholangiocytes resulted in increased cell proliferation and migration, along with suppression of ciliogenesis. Clinically, higher OFD1 expression was significantly associated with poorer overall survival in CCA patients (10 vs. 39 months; HR 2.33, 95% CI 1.01-5.31, $p = 0.03$). In conclusion, we demonstrated that OFD1 functions as an oncogene in CCA, influencing primary cilia formation and tumor cell growth. Higher OFD1 expression is a significant predictor of worse survival outcomes, identifying OFD1 as a promising biomarker and potential therapeutic target in CCA.

#6723 Targeting RAS in angiosarcoma.

Donghee Lee[1], Eslenur Nipa[1], Erin B. Dickerson[2], Jong Hyuk Kim[1]

[1]University of Florida, Gainesville, FL,[2]University of Minnesota, Minneapolis, MN

Angiosarcomas are aggressive, rare soft tissue sarcomas that form in the lining of blood vessels. Although recurrent mutations in oncogenic drivers, including VEGFR/TIE2 (*KDR*, *FLT4*, *PTPRB*, and *PLCG1*), RAS-MAPK (*KRAS, HRAS*, *NRAS*, and *NF1*), and PI3K (*PIK3CA* and *PTEN*) have been identified in angiosarcomas, attempts to target these pathways using selective or multi-kinase inhibitors have generally failed to produce meaningful clinical responses. RAS proteins act as critical molecular switches that regulate cell signaling, and oncogenic mutations in RAS or VEGFR/TIE2 proteins are strongly implicated in the development of angiosarcoma. However, the mechanisms that govern these mutant pathways and the challenge of developing effective therapeutic interventions have remained elusive. A promising advancement for targeting RAS mutant tumors has been the development of the tri-complex RAS(ON) multi-selective inhibitor, RMC-7977, which simultaneously targets wild-type and mutant KRAS, NRAS, and HRAS in their active, GTP-bound state, offering a potential treatment for angiosarcoma. In this study, we identified *HRAS* Q61R and *NRAS* Q61L mutations in KU-CAS3 and KU-CAS5 angiosarcoma cell lines using Nanopore sequencing. Additionally, we detected *FLT4* amplification and an *NF1* frameshift deletion in HAMON cells, as well as a *PTPRB* deletion in ISO-HAS-B cells. Our results show that RMC-7977 and MEK1/2 inhibitor mirdametinib exhibit strong potency against angiosarcoma cell lines harboring mutations in RAS and VEGFR/TIE2 pathways. Notably, RMC-7977 showed significantly greater anti-proliferation effects in RAS wild-type and *PTEN* frameshift deletion AS5 angiosarcoma cells compared to midrametinib or VEGFR inhibitors. Furthermore, long-term treatment with RMC-7977 inhibited the colony-forming capacity of angiosarcoma cells. These findings suggest that RMC-7977 effectively inhibits the proliferation and survival of angiosarcoma cells. Our ongoing work involves studies to evaluate the *in vivo* effects of RMC-7977 on the the growth of angiosarcomas xenografts containing RAS mutations. We will also investigate the molecular mechanisms underlying oncogenic signaling to elucidate RAS dependency and strengthen the rationale for using RAS inhibitors to treat angiosarcoma. This approach aims to demonstrate that targeting RAS through RAS(ON) multi-selective inhibitors is effective, highlighting the urgent need for clinical trials to advance treatment options for angiosarcoma patients.

#6724 The role of human leukocyte antigen F adjacent transcript 10 in the tumorigenesis of nasopharyngeal carcinoma.

Yusi Liang, Ngar Woon Kam, Zhuoyou YU, Ho Fun LEE

University of Hong Kong, The - Li Ka Shing Faculty of Medicine, Hong Kong, Hong Kong

Nasopharyngeal carcinoma (NPC) is a highly aggressive malignancy prevalent in Southern China and Southeast Asia related to Epstein-Barr virus (EBV) infection and genetic variations at the Human Leukocyte Antigen (HLA) locus. Human leukocyte antigen (HLA)-F adjacent transcript 10 (FAT10) is a ubiquitin-like protein modifier that can modulate its substrates by (1) mediating degradation via the 26S proteasome or (2) altering function through Fat10ylation. Previously studies have found FAT10 overexpression in various cancers, including gliomas and hepatocellular carcinoma, promoting tumorigenesis by altering cell proliferation, metastasis, and angiogenesis. Nevertheless, the precise role of FAT10 in NPC remains ambiguous. NPC public cohort GSE150430 showed high expression of FAT10 in NPC malignant cells. We also found overexpression of FAT10 in both NPC cell lines and patient-derived xenografts (PDXs) by real-time polymerase chain reaction (RT-PCR) and western blot at mRNA and protein level respectively. FAT10 overexpression in both NPC cell line C666-1 and immortalized normal nasopharyngeal cell line NP460 by lentiviral transduction increased proliferation proved by XTT assay, colony formation assay, and ki67 staining assay. Transwell migration and wound healing assay were performed in FAT10 overexpression cancer cell line and normal cell line to show the enhancement of migration ability. ROS production was also increased in these FAT10 overexpression cell lines by using the CellROX Green reagent. However, the western blot result showed that stemness ability cannot be changed in NP460. To conclude, our findings indicated that FAT10 may play a significant role in the tumorigenesis of NPC. Its underlying mechanism will be further explored.

#6725 Introducing new technology for high performance whole exome sequencing with unparalleled hybridization time flexibility and capability for low to high capture-input.

Daniel Saatman, Jinglie Zhou, Ekaterina Star, Roshani Codipilly, Alexandra Wang

Integrated DNA Technologies, Inc., Redwood City, CA

Whole exome sequencing (WES) via hybridization capture is a targeted sequencing approach to specifically enrich the protein-coding regions of the human genome. The human exome comprises ~1% of the entire genome but contains the majority of relevant variants for researchers. Targeting this region saves on sequencing costs and data storage while also allowing greater sequencing depth of regions of interest. However, current hybridization capture workflows are challenging to execute in the laboratory due to high-temperature sample and reagent handling, pre-heating of multiple buffers, long hybridization times, and narrow library input ranges. Widening the input range is desirable because it can allow for fewer PCR cycles in library preparation or enable high multiplexing during hybridization capture, the latter of which can lower reagent costs.

To evaluate targeted sequencing performance across a wide range of sample inputs and hybridization times, we used low to high total input—1-plex 100ng to 12-plex 6000ng—and hybridized for as short as 1 hour up to overnight. We used the Integrated DNA Technologies (IDT) novel xGen™ Hybridization and Wash v3 kit, which features a user-friendly workflow without hot-liquid handling or temperature-sensitive urgency. This performance is benchmarked side-by-side with a current commercial hybridization-capture kit.

Using these methods, we achieve similar targeting, coverage, uniformity, and GC-balance across all samples with minimal impact from low inputs and short hybs. Further, we calculated the reference/alternate (ref/alt) allelic fraction and found no considerable differences between capture conditions, indicating the potential of this workflow for genotyping applications with low-input samples and/or short hybridizations. This flexibility in hybridization time and library input along with the streamlined workflow allows researchers to meet diverse experimental demands and effectively capture the human exome.

#6726 Investigating the tumor suppressive role of LRIG1 in breast cancer disparities.

Amber Yu, Angel H. Pajimola, Kayla S. Ingram, Brittany Jenkins-Lord

Biochemistry and Molecular Biology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

Background: Within the U.S. population, African American (AA) women have a staggering 40% higher mortality rate from breast cancer than European American (EA) women, despite having a lower incidence rate. This disproportionate mortality burden imposed on AA women is rooted in both environmental and genetic causes. We are interested in elucidating molecular and environmental mechanisms that may be contributing to these breast cancer health disparities. Our recent study identified that higher neighborhood-level socioeconomic deprivation was associated with hypomethylation and decreased gene expression for LRIG1 (Leucine-rich repeats and immunoglobulin-like domains 1) in AA women, but not in EA women. LRIG1 has been shown to act as a tumor suppressor, associating with members of the ErbB family of receptor tyrosine kinases (RTKs), which includes the epidermal growth factor receptor (EGFR), HER2, HER3, and HER4. This association of LRIG1 with ErbBs is predicted to result in the degradation of these proteins and the suppression of pro-growth signaling.
Objective: This project aims to determine differences in the protein expression of LRIG1 and ErbB proteins within breast epithelial cell lines (normal epithelial cells, estrogen receptor-positive (ER+), and estrogen receptor-negative (ER-)) derived from both AA and EA women. We hypothesize that there are differences in the protein expression of LRIG1 and ErbB proteins within breast epithelial cell lines derived from AA women and EA women.
Methods: The levels of protein expression of LRIG1 and ErbB proteins in the cell lines were assessed using Western immunoblotting and densitometric analysis. The colocalization of LRIG1/EGFR and LRIG1/HER2 in non-tumorigenic, ER+, and ER- cell lines was assessed through immunofluorescence (IF) cell staining.
Results: Western immunoblotting revealed substantial variation in target protein levels, with notable differences in LRIG1 protein levels by estrogen receptor status, with greater LRIG1 protein expression in ER+ cell lines. IF staining demonstrated limited colocalization between LRIG1 and EGFR as well as LRIG1 and HER2. Future experiments, including co-immunoprecipitation, will aim to determine how LRIG1 and the ErbB proteins are interacting and what factors may be influencing their binding and downstream signaling.
Conclusions: Our results provide insight into LRIG1 and ErbB co-expression in several breast cell lines from AA and EA women. Our next steps will include identifying distinct differences between these cell lines that might be a result of variations in environmental factors, the tumor microenvironment, and genetic ancestry.

#6727 Ecdysoneless (ECD) as a key estrogen-regulated protein driving progression in ER-positive breast cancer.

Farshid Oruji, Asher Rajkumar Rajan, Mohsin Raza, Bhopal Mohapatra, Hamid Band, Vimla Band

Department of Genetics, Cell Biology and Anatomy, University of Nebraska Medical Center, Omaha, NE

Background: Breast cancer (BC) is a heterogenous disease, with estrogen receptor-positive (ER+) BC accounting for over 70% of cases. ER+ BC depend on estrogen signaling for oncogenesis and metastasis. Even though treatments targeting ER pathway have improved patient's outcome, factors driving ER+ BC progression particularly in metastatic remain unclear. One of these factors is Ecdysoneless (ECD), a highly conserved protein from yeast to human. ECD expression correlates with ER expression, ECD overexpression is observed in ER+ BC and ECD overexpression correlates with poor patient survival. In this study, we assessed the relationship of ECD and ER, and the role of ECD in ER+ BC oncogenic traits.
Methods: Bioinformatic analysis of publicly available datasets were explored for the relationship between ER and ECD and survival analysis was performed to examine the link between ECD, ER, and ER target MYC expression. ECD promoter analysis followed by chromatin immunoprecipitation (ChIP) and promoter-luciferase assays were used to assess estrogen-dependent ER recruitment to the ECD promoter. Estradiol (E2) treatments were performed to examine how estrogen signaling affects ECD levels. The effects of ECD overexpression were performed in ER+ breast cancer cells to evaluate changes in in vitro tumorigenic traits.
Results: ECD expression correlated with ER levels in publicly available datasets. Survival data showed patients with elevated levels of ECD in ER+ BC had worse outcome. The ECD promoter contained estrogen response elements, and ChIP assays confirmed E2 mediated recruitment of ER to the ECD promoter. E2 treatment enhanced ECD promoter-luciferase activity. Overexpression of ECD in ER+ cells enhanced oncogenic traits.
Conclusions: We have identified ECD is a novel estrogen regulated protein that plays a critical role in ER-driven oncogenesis in ER+ BC. Current studies are exploring the role of overexpressed ECD in in vivo preclinical mice models and therapy resistance.

#6728 Investigating the effect of male-specific lysine demethylase 5D alteration on oncogenic behavior in human prostate cancer cells.

Sarah Ann S. King, Nabila Zaman, Prathiksha Prabhakaraalva, Goutam Chakraborty

Urology, Icahn School of Medicine at Mount Sinai, New York, NY

Prostate cancer (PC) is the most common urological malignancy, with approximately 11% of men being diagnosed with it during his lifetime and is the second leading cause of cancer-related fatalities in males. One of the challenges in treating advanced, symptomatic prostate cancer is the limited effectiveness of the standard care, androgen deprivation therapy (ADT), in late-stage disease. While somatic Y chromosome (ChrY) loss in men has been associated with an increased risk of several diseases, including prostate cancer, the role of ChrY genes in prostate cancer progression and chemotherapy resistance remains understudied. Based on significant epidemiological and genomic data generated by multiple teams, including ours, this present study critically examines the contributions of the loss of the chromosome Y gene lysine demethylase 5D (KDM5D) to chromosome Y-null prostate cancer progression and resistance to ADT. In our approach to investigating this phenomenon we have employed a number of advanced techniques which interrogate the epigenomic, transcriptomic, proteomic, and behavioral landscape of defined prostate cancer lines with genetically altered levels of KDM5D RNA and protein. Herein, lentiviral transduction was employed to perform CRISPR-mediated KDM5D knockout with three novel gRNAs in each castration sensitive and insensitive prostate lines (LNCaP and 22RV1, respectively). Single cell-derived clones from each of the six lines infected with this CRISPR knockout virus have been isolated and individually expanded to measure the impacts of KDM5D knockout accurately with respect to target expression and Cas9 copy number; to date, several subclones have shown satisfactory reductions in KDM5D expression and are now being subjected to functional and behavioral assays pursuant to analyzing its malignant characteristics, alterations in gene expression, and changes in androgen deprivation and/or chemotherapeutic agent sensitivity. Moreover, we have employed KDM5D addback to create an overexpression model in ChrY-null PC3M cells. Thus far, the loss of KDM5D has been shown to contribute to increased oncogenic behavior in prostate cancer cells and may be associated with a poor patient prognosis. Our preliminary data provide mechanistic evidence that KDM5D expression influences several DNA damage repair pathways in prostate cancer cells .Ultimately, the goal of our group is to identify alternative therapeutic targets, chemotherapeutic agents, and ultimately characteristics of clinically consequential prognostic value in KDM5D/ChrY-null prostate cancers where currently no treatments are available.

#6729 The role of L-MYC in prostate cancer.

Jeyaluxmy Sivalingam, Xiuping Yu

Biochemistry and Molecular Biology, LSU Health Sciences Center - Shreveport, Shreveport, LA

The MYC family of transcription factors, comprising MYC, N-MYC, and L-MYC, regulates essential cellular processes, including proliferation, metabolism, differentiation, and apoptosis. While MYC and N-MYC have been extensively studied in prostate cancer (PCa), the role of L-MYC remains underexplored. To investigate the expression patterns of these MYC family genes across different stages of PCa, we analyzed publicly available mRNA expression data from normal prostate tissues, adenocarcinoma prostate cancer (AdPCa), neuroendocrine prostate cancer (NEPCa), and PCa cell lines representing AdPCa, castration-resistant prostate cancer (CRPCa), and NEPCa (H660). Our analysis revealed elevated MYC expression in AdPCa patients and AdPCa/CRPCa cell lines, whereas N-MYC showed no significant expression across PCa stages. In contrast, L-MYC was prominently expressed in NEPCa patients and the NEPCa cell line H660. RNA-seq data from patient biopsies (PMID: 26855148) further confirmed increased L-MYC levels in CRPCa-NE compared to CRPCa-Adeno. Additionally, neuroendocrine markers (NCAM1, INSM1, ASCL1, and NEUROD1) were co-expressed with L-MYC in NEPCa, underscoring its association with neuroendocrine differentiation. The expression of L-MYC in H660 cells was further validated by RT-qPCR and Western blot. Despite their shared genomic structure and high sequence homology, MYC family genes exhibit mutually exclusive expression patterns in PCa. Transcription factor binding site (TFBS) analysis revealed unique regulatory elements that may contribute to this differential expression, warranting further investigation. Ongoing RNA-seq analysis of L-MYC knockdown in H660 cells aims to identify pathways regulated by L-MYC and provide insights into its potential as a therapeutic target in NEPCa. These findings suggest that L-MYC plays a pivotal role in NEPCa progression and highlight the importance of understanding the regulatory mechanisms driving its unique expression.

#6730 Oncogenic ASPP2kappa (k) drives leukemic transformation and disease progression.

Alessia Ruiba[1], Anna-Lena Ahrens[1], Vanessa Aellig[1], Reka Haraszti[2], Marcus M. Schittenhelm[1], **Kerstin Maria Kampa-Schittenhelm**[1]

[1]Kantonsspital St. Gallen, St. Gallen, Switzerland,[2]Universitatsklinikum Tuebingen, Tuebingen, Germany

Introduction: Despite tremendous efforts and new therapeutics, acute myeloid leukemia (AML) remains difficult to treat and therapy outcome is far from satisfactory for many patients. We have recently identified a novel, oncogenic isoform of the tumor suppressor ASPP2, named *ASPP2k,* which is expressed in >50% of AMLs, and have demonstrated that deregulation of the ASPP2/ASPP2k - p53 axis associates with drug resistance and a more aggressive tumor biology. We now show that ASPP2k drives leukemic transformation and disease progression *in vivo* and proves to be a potent therapeutic target.
Methods: Isogenic AML ASPP2κ silenced (KD) (Molm14, HL60 and ex vivo patient blasts) were established and xenotransplanted into NSG mice. Ba/F3 interleukin-3 dependent pro-B cells, engineered to overexpress ASPP2k were transplanted into immunocompetent C3H mice to study the influence of ASPP2k on the tumor microenvironment. Disease development and progression were monitored using an IVIS Lumina imaging system. In addition, we designed and tested novel, fully chemically modified ASPP2k-specific siRNAs for in vivo application.
Results: All in vivo models demonstrate earlier leukemia development and engraftment as well as a more aggressive course of disease in dependency of ASPP2k expression (lifespan 30 vs 42 days pi for EV and KD, 22d for KI, p<0,001), underlined by higher WBC count (p<0,001), spleno- and hepatomegaly (p<0,0001) early weight loss (p<0,01) and dissemination into brain and lungs (p<0,001). Importantly, Ba/F3 cells lost their IL-3 dependency upon over-expression of ASPP2k, considered a first step of malignant transformation (proliferation +120 fold-change at 96h for KI vs EV). C3H mice transplanted with ASPP2k overexpressing Ba/F3 cells demonstrated higher organ infiltration (eg liver p<0,001) and a strong immune response, as evidenced by upregulation of secretion of pro-inflammatory cytokines like MIP-2, STNFRI, VCAM-1, PF-4, L-selectin, OPG, CD26, MIG proteins, among others as well as significant T-cell infiltration (p<0,01). Most importantly, employing novel, fully chemically modified ASPP2k-specific siRNAs, we were able to silence ASPP2k *in vivo* and again attenuate leukemia progression.
Summary: In summary, we here show that ASPP2k directly contributes to aggressiveness of the disease in vivo and provide a first rational to further develop ASPP2k specific siRNAs as a novel therapeutic approach to combat leukemia. Future studies evaluating ASPP2k as a potential target for therapy are warranted.

#6731 Oncogenic ASPP2k: a key regulator of classical hallmarks of cancer in triple negative breast cancer (TNBC).

Alessia Ruiba[1], Anna-Lena Ahrens[1], Lara Aldinger[1], Lea Stiehl[1], Reka Haraszti[2], Wolfram Jochum[1], Christoph Driessen[1], Marcus M. Schittenhelm[1], **Kerstin Maria Kampa-Schittenhelm**[1]

[1]Kantonsspital St. Gallen, St. Gallen, Switzerland,[2]Medizinisches Universitatsklinikum Tubingen, Tubingen, Germany

ASPP2 is a tumor suppressor, originally characterized as direct p53-binding protein. More recently, ASPP2 was shown to also directly interact with BCL-2 and NFkB, putting ASPP2 in a central position to orchestrate major cancer related pathways. In this context, we have recently reported the identification of a novel, oncogenic isoform of *ASPP2*, named *ASPP2kappa (k),* which is highly and frequently expressed in TNBC. As ASPP2k is characterized by loss of the p53- as well as partial loss of BCL-2 and NFkB-binding sites, its expression impairs major pathways controlling cellular fate, resulting in accelerated proliferation and resistance to chemotherapy. We now show that expression of ASPP2k modulates all classical hallmarks of cancer - fueling a highly aggressive tumor biology in TNBC. Importantly we demonstrate that silencing of ASPP2k slows down tumor growth and inhibits metastasis *in vivo*. Employing our isogenic TNBC ASPP2κ interferenced (KD) cell models (MDA-MB-231 and HS-578T, n=18 per model) we studied the role of ASPP2κ in NSG xenotransplant models. Mice were xenotransplanted with EV or ASPP2k KD cells and tumor engraftment, progression, angiogenesis and metastasis were monitored using an IVIS Lumina imaging system. Tumor engraftment and growth were found to be significantly attenuated in the KD strains (representative data for MDA model: doubling times EV: 7.3d; KD: 12.8d; p<0,0001). EV mice had to be sacrificed between day 40-60 after injection due to tumor size (median OS 51d) while ASPP2k KD only presented with small, localized tumors at day of termination (d72, OS<0,0001). Importantly, 100% of mice bearing ASPP2k expressing (EV) tumors, presented with distant metastasis in multiple different sites at time of sacrifice, while in ASPP2k KD mice, metastasis could be detected in max. one site and only 30% of mice. Regulation of pro-angiogenic and EMT markers as well as involvement of the NFkB pathway were confirmed to play a role in ASPP2k-mediated metastasis. Most importantly, we were able to silence *ASPP2k in vivo,* employing novel, fully chemically modified ASPP2k-specific siRNAs generated for *in vivo* use and confirm potent inhibition of tumor growth also in these models. In summary, we here provide absolutely novel insights into TNBC tumor biology in dependency of ASPP2k and demonstrate first successful attempts to silence *ASPP2κ* employing highly specific siRNAs to target *ASPP2k in vivo.*

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Antibody-Based Cancer Therapeutics 4
Poster Session

#6735 E-cM-Topi, a potent EGFR-cMET bispecific antibody drug conjugate, with superior anti-tumor effect in non-small-cell lung cancer (NSCLC).

Huihui Chen, Le Sun, Bing Hu, Zhiping Zhao, Ziheng Liu, Guangbao Qiu, Dongmei Bai, Ying Li, Ziwei Ren, **Ting Zhang**, Xin Ye, Lingfeng You, Zhendong Xue, Yuchang Mao, Huaixin Dang, Shuyu Cai, Jun Feng, Min Hu, Feng He

Shanghai Hengrui Pharmaceutical CO., LTD., Shanghai, China

EGFR-tyrosine kinase inhibitors (EGFR-TKIs) are standard clinical treatment for NSCLC patients with activating EGFR mutations. However, both intrinsic and acquired resistance to EGFR-TKIs limited their long-term efficacy, posing a significant unmet medical need. The protein overexpression of c-MET has been observed in 5-22% of EGFR-TKIs resistant NSCLC patients. Therefore, a bispecific ADC targeting EGFR and c-MET will shed a light on the management for EGFR-TKI resistance. E-cM-Topi is an EGFR and cMET bispecific ADC, consisting of a Fc-silenced EGFR-cMET bispecific antibody, a cleavable linker and a topoisomerase I inhibitor payload (Topi) with superior bystander effect. The binding affinities to EGFR and c-MET were optimized to enhance tumor-cell-selectivity, increase endocytosis, and improve pharmacokinetics (PK). Considering the wide expression of EGFR in normal tissue, the binding affinity to EGFR was adjusted to be weaker than that to c-MET, thereby enhancing the selectivity for EGFR-cMET-co-expressing tumors while minimizing the toxicity on normal tissues. E-cM-Topi demonstrated strong EGFR/cMET-dependent growth inhibition in various NSCLC cell lines. In HCC827 and EBC-1 xenograft models, E-cM-Topi inhibited tumor growth in a dose-dependent manner. Tumor regression was observed at a single dose of 4.5 mg/kg and sustained till day 35 in HCC827 model and day 20 in EBC-1 model. In addition, E-cM-Topi showed good human plasma stability and favorable cyno PK profile. Less than 0.5% of payload was released in human plasma after a 14-day incubation. Half-life of E-cM-Topi was more than 3 days in cyno monkey. In summary, by fine-tuning the EGFR-cMET bispecific antibody affinity and incorporating a superior bystander effect of payload, E-cM-Topi represents a best-in-class potential. It holds promise as an effective treatment option for EGFR-TKI resistant NSCLC patients.

#6736 Preclinical development of ADCT-241, a novel exatecan-based antibody-drug conjugate targeting PSMA for the treatment of prostate cancer.

Ben Leatherdale, Beccie Martin, Banusha Rajeenthira, Cecile Oblette, Lara Montolio, Abigail Kimpalu, Pedro L. Alves, Hafsah Omar, Alison Faid, Elizabeth Horsley, Lina Baxter, Valenti Gomez, Charlie Britten, Lolke de Haan, Paul W. Hogg, Chris Pickford, Patrick H. van Berkel

ADC Therapeutics, London, United Kingdom

Prostate specific membrane antigen (PSMA) is a 750 amino acid, homodimeric type II transmembrane glycoprotein expressed mainly in prostate tissues, but also at low levels in renal proximal tubules, the small intestine, and the brain. PSMA is known to have two enzymatic functions, firstly in the removal of glutamate from dietary folates in the small intestine, and secondly in hydrolysis of the peptidic neurotransmitter N-acetylaspartylglutamate. In the oncology setting PSMA is a clinically validated target with its expression being upregulated in prostate cancer as well an association with progression and aggressiveness of the disease. Here, the generation and preclinical characterization of ADCT-241, a novel antibody-drug conjugate (ADC) targeting PSMA is described. ADCT-241 is composed of a fully human IgG1 antibody directed against PSMA (anti-PSMA mAb), to which the PL2202 payload, containing the topoisomerase I inhibitor exatecan, has been stochastically conjugated via a cleavable valine-alanine linker with a drug:Ab ratio of 4 (DAR 4). In vitro, anti-PSMA mAb showed good binding affinity for human PSMA and cross-reactivity to rat and cynomolgus monkey PSMA. In addition, ADCT-241 showed specific and potent killing of prostate cancer cell lines, including LNCaP and C4-2, in *in vitro* cytotoxicity assays when compared to a non-binding control DAR 4 ADC containing the PL2202 payload. ADCT-241 showed bystander activity in *in vitro* assays employing PSMA-positive prostate cancer cell lines and PSMA-negative PC-3 cells. *In vivo*, administration of a single intravenous (IV) dose of ADCT-241 was associated with potent anti-tumor activity in prostate cancer xenograft models. Finally, in toxicology studies in rats and cynomolgus monkeys, ADCT-241 was well tolerated at dose levels up to 150 and 75 mg/kg, respectively, following dosing on Day 1 and 22. Circulating serum total antibody, total ADC, and free exatecan concentrations were determined, and showed good *in vivo* stability for ADCT-241 and a half-life of ~8.5 days in cynomolgus monkeys. In summary, ADCT-241 is a novel, stable PSMA-targeting ADC that mediates potent and specific anti-tumor activity in mouse xenograft models and exhibits an excellent tolerability profile in rats and cynomolgus monkeys. The data presented support future clinical development of ADCT-241 for the treatment of prostate cancer indications.

#6737 RAS inhibitors as payloads of ADCs to treat RAS-mutant tumors.

Boyu Zhong, Tony Zhang, Zuyong Deng, Guangming Chen, Chunlan Dong, Liangbao Lai, Erchang Shang

Tyligand Bioscience (Shanghai), Ltd., Shanghai, China

Background: Ras mutation has been identified as one of the major oncogenes in many cancers. Recent breakthroughs in regulating the activity of RAS proteins have led to the discovery of several clinical therapeutic molecules, such as Sotorasib and Adagrasib. However, for patients with RAS mutations, drugs with broader indications, higher potency, better tolerability, and longer efficacy are urgently needed. On the other hand, ADC, as a new precise and efficient drug delivery method, provides a potential solution to the above unmet needs. Here, we disclose a new class of ADCs whose payloads target RAS variant proteins and exhibit excellent antitumor effects and tolerability both *in vitro* and *in vivo*.

Method: Both selective and pan-RAS inhibitors were conjugated to various antibodies via cleavable peptide linkers and ancillary groups to improve druggability. The primary biological activities of payloads and ADCs were examined in a series of cellular proliferation assays. The *in vivo* antitumor effects of the ADCs were evaluated on selected cell-derived xenograft (CDX) mice models, where body weight changes of the test animals were used to estimate the tolerability of the molecules. Both *in vitro* and *in vivo* PK properties of the ADCs were also studied.

Results: The potent KRAS G12D selective inhibitor P1 or pan-RAS inhibitor P2 (KRAS-G12C, G12D, G12S, G12V, G13D, etc.) was successfully conjugated with antibodies such as anti-Her2, Trop2, Claudin18.2, etc., with a maximum DAR value of 8. By optimizing the linker and ancillary group, ADC-1 maintained the high potency of its payload P1 in the antigen-specific cancer cell line GP2D (IC_{50} for P1: 0.5 nM vs ADC-1: 0.4 nM). ADC-1 exhibited excellent anti-tumor effects in animal models. For example, in the GP2D CDX model, a single dose at 10 mpk intravenous injection, 104% TGI (tumor growth inhibition) was observed on day 21. In addition, there was no significant difference in the weight change of the test animals in the ADC treatment group compared with the vehicle group. In both mouse and human plasma, the release of payload P1 from ADC-1 was less than 2% for up to 72 hours. Considering the drug load of approximately 3%wt on ADC, no payload-related safety issues emerged in the study.

Conclusion: ADC containing RAS inhibitor is a new approach to treat RAS mutant tumors. It has shown good selectivity and potency in both *in vitro* and *in vivo* studies on a variety of tumors. It exhibits good PK and physicochemical properties. It also shows an acceptable safety profile. It is a promising measure for treating patients with RAS mutations in the clinic.

#6738 Towards developing a novel therapy for pain: A monoclonal antibody that specifically inhibits voltage-gated sodium channels $Na_v1.7$.

Debin Li, Na Lu, Rui Song, Meimei Liu, **Gavin Zhang**, Huihui Xu, Yuting Xue, Liyan Su, Huaxing Zhu

Novoprotein Scientific Inc., Su Zhou Shi, China

Background: Pain is caused by the transmission of action potentials through nerve fibers to the dorsal root ganglia, ultimately reaching the higher nervous system. Voltage-gated sodium channels (VGSCs) member $Na_v1.7$, encoded by *SCN9A* and primarily localized to the dorsal root ganglion neurons, has been explored as a prominent target for pain management. The $Na_v1.7$ protein is structurally complex. Composed of about 2000 amino acids,this protein possesses four homologous domains, each containing six transmembrane alpha helices. In addition, the Nav family has nine members that share high sequence homology. This structural complexity makes it particularly challenging to develop antibodies that bind to epitopes that cause inhibitory effects on the target. And it's high sequence homologymakes it particularly challenging to develop antibodies with high specificity. Leveraging on our NovoiSMART® technology, we successfully developed NP067, a highly specific and functional monoclonal antibody against $Na_v1.7$.

Methods: It was previously shown that IL-1β upregulates the expression of *ADAMTS1*, and this upregulation is disrupted by the inhibition of $Na_v1.7$. Therefore we measured the expression of *ADAMTS1* under IL-1β induced upregulation, with or without our new antibody NP067. We also compared several known $Na_v1.7$ antagonists and NP067 for their effects to influence the expression of *ADAMTS1*. The specificity of NP067 against $Na_v1.7$ was confirmed by flow cytometry experiments on two cell lines. MCF-7 cells were used for negative confirmation as this cell line expresses SCN5A/SCN7A/SCN8A antigens but not SCN9A. And A549 cells were used for positive confirmation and this cell line expresses SCN5A/SCN7A/SCN8A as well as SCN9A.

Results: NP067 inhibited IL-1β-induced *ADAMTS1* gene expression, and the effects were stronger than other reference molecules. Flow cytometry data showed that NP067 was highly specific to $Na_v1.7$, and the affinity were higher than other reference molecules.

Conclusions: NP067 is a functional and specific $Na_v1.7$ antibody that have great potential in pain treatment. NP067 also shows great potential both as a monotherapy or coupling with other drugs.

#6739 Antibody-drug conjugates with novel payloads are enabled by an ultra-high DAR platform.

Emmalyn Lecky[1], Adam Voelker[2], Isabella Ranieri[1], Andrew Kassick[2], Cassandra Crihfield[2], Christopher Fried[2], Destiny Carpenter[2], David Wilson[2], Robert Fryberger[2], Jingyi Li[2], Amanda Mincey[2], Matthew Cummings[2], Alex Toker[1], Krzysztof Matyjaszewski[3], Antonina Simakova[2], Laura E. Benjamin[2]

[1]Beth Israel Deaconess Medical Center, Boston, MA,[2]BioHybrid Solutions, Pittsburgh, PA,[3]Carnegie Mellon University, Pittsburgh, PA

Antibody-drug conjugates (ADCs) have emerged as a powerful modality to increase the therapeutic index of potent chemotherapy drugs by selectively increasing drug delivery to tumor cells while minimizing exposure to non-tumor tissue. Recent advances have improved efficacy by increasing the drug-antibody ratio (DAR) and optimizing linkers to reduce payload release in plasma and increase release within tumor tissue. Despite this significant progress and the clinical success of recent ADCs, particularly in breast cancer, challenges persist, including managing toxicity and diversifying payload classes to broaden the clinical applications of ADCs. This study demonstrates the engineering and biological potency of ADCs comprised of 50-200 single or mixed kinase inhibitors, enabling the utilization of previously unusable payloads. Small molecules are linked to a specialized polymer scaffold using cathepsin-cleavable linkers and internalized by tumor cells in an antibody target-dependent manner, where they inhibit signaling pathways essential for survival. These HER2-targeted biomolecules combined with payloads inhibiting PI3K/mTOR (apitolisib) or MEK (cobimetinib) demonstrate the use of ADCs as precision medicine to selectively target cancer cells via antibody and payload. For example, many HER2-positive tumors also carry oncogenic mutations in the PI3K pathway rendering those tumors exquisitely sensitive to these ADCs, especially when HER2 expression is low. As shown in Table 1, PI3K pathway mutations can increase the sensitivity to ADCs with apitolisib as a payload, in some cases surpassing the potency of the exatecan derivative DXd in trastuzumab-DXd (DAR8). We will also present data supporting the *in vivo* safety and efficacy of these ADCs which may provide a novel solution to expand the therapeutic landscape, particularly for HER2-positive cancers.

Table 1:

Cell Line	PI3K pathway mutation	HER2 expression	DS-8201 (T-DXd) (IC50 nM)	BHS T- apitolisib DAR100 (IC50 nM)
SUM159	PIK3CA (H1047L)	1+	159.7	9.365
T47D	PIK3CA (H1047R)	1+	12.44	3.859
MCF7	PIK3CA (E545K)	1+	239	4.944
ZR751	PTEN Missense	2+	11.09	9.451
AU565	WT	3+	0.3521	4.28
SKBR3	PIK3CA (H1047)	3+	0.4009	3.346

#6740 Dual targeting of amyloid precursor protein with α-secretase inhibitor and antibody-drug conjugate synergistically reduces tumor progression in colorectal cancer.

Rongzhang Dou[1], Hanwen Xu[2], Ricardo A. Leon-Letelier[1], Ali H. Abdel Sater[1], Yihui Chen[1], Jody Vykoukal[1], Makoto Kobayashi[1], Clemente Aguilar-Bonavides[3], Johannes F. Fahrmann[1], Hiroyuki Katayama[1], Samir M. Hanash[1]

[1]UT MD Anderson Cancer Center, Houston, TX,[2]MSD R&D (China) Co, Shanghai, China,[3]SK Life Science Labs, Greater Philadelphia, PA

Background: Amyloid precursor protein (APP) is a type I transmembrane glycoprotein that is overexpressed in various cancer types and has been reported to play key roles in promoting tumor progression. In this study, we investigated the subcellular expression, biological function, and potential therapeutic strategies targeting the ADAM10-APP axis in colorectal cancer (CRC).
Methods: Single-cell RNA sequencing and spatial transcriptomic analysis were performed to elucidate the enrichment of APP expression in CRC. Liquid chromatography-mass spectrometry (LC-MS) surfaceome analysis was conducted on 14 CRC cell lines to validate APP expression on the cell surface. A tissue microarray (TMA) was used to validate the surface expression of APP. Western blotting, 3D culture, invasion and migration assays, as well as a nude mouse model, were utilized to investigate the biological function of APP. Additionally, an antibody-drug conjugate (ADC) assay targeting APP was utilized to explore its therapeutic potential in CRC.
Results: Multi-omics data revealed that APP is highly expressed in CRC and is specifically enriched in epithelial cells. Through proteomic profiling of human CRC cell lines and validation in a tissue microarray consisting of 140 CRC tumors, we identified APP as significantly enriched on the cell surface. Functional studies showed that knockdown or knockout (KO) of APP in CRC cells led to marked reductions in cell proliferation, colony formation, and invasiveness, effects that were in part reversible upon the addition of soluble APP (sAPP). In xenograft models of colorectal cancer (CRC), APP-KO HCT116 tumor-bearing mice exhibited significantly reduced tumor growth and tumor initiation frequency compared to control HCT116 tumor-bearing mice. We further demonstrated that treatment of CRC cells with GI254023X, a selective ADAM10 inhibitor, increased cancer cell surface APP expression. A combination therapy of GI254023X with an APP antibody conjugated to monomethyl auristatin F (APP-ADC-MMAF) resulted in superior anti-cancer efficacy compared to either treatment alone.
Conclusion: APP is highly expressed on the surface of CRC cells and promotes tumor progression, making it a potential target for ADC therapies. Inhibition of ADAM10 reduces APP cleavage and further enhances its surface expression, highlighting a promising treatment strategy for CRC.

#6741 AT65474, a highly selective anti-CLDN6 ADC with a proprietary payload.

Chen Zhong, Yin Wen Cheng, You Xue Wu, Xi Yun Zhang, Rui Yang Zhao, Qian Wang, Ping Du, Shao Jun Liu, Bin Zou

Axcynsis Therapeutics, Singapore, Singapore

CLDN6 is a membrane-bound oncofetal protein and a promising target for cancers such as ovarian and testicular cancer. AT65474 is an antibody drug conjugate (ADC) candidate composed of a highly selective humanized anti-CLDN6 antibody conjugated to a proprietary payload, namely TCS132, with a drug-to-antibody (DAR) ratio of 4. The antibody is engineered to incorporate AxcynCYS^{TM} technology for site-specific conjugation which can reproducibly achieve high DAR4 ratios of greater than 97% by HIC analysis. The payload, TCS132, is derived from an FDA approved drug and when compared to the parent drug, demonstrates increased potency with broad sub-nanomolar activities across a wide range of cancer cell lines including those resistant to paclitaxel, DM1, MMAE and DXd. Additionally, TCS132 was chemically optimized for better PK properties and demonstrates a much-improved safety profile despite its increased potency over the parent drug. *In vitro*, AT65474 specifically binds to CLDN6 presenting tumor cells and upon internalization into lysosomes, releases the payload effectively inhibiting tumor cell proliferation. AT65474 displays a bystander killing effect that enables it to kill surrounding CLDN6-negative tumor cells, further enhancing its anti-proliferative effects. *In vivo*, AT65474 shows strong anti-tumor activity in CLDN6-positive CDX and PDX models at low doses and long intervals, which reflects the elevated payload levels in the tumors. Furthermore, safety assessment of AT65474 including GLP-compliant toxicology studies in Cynomolgus monkeys and SD rats reveals a good safety profile of AT65474. Taken together, the results of the nonclinical studies support the intended clinical trial, dose selection, and regimen in humans.

#6742 High-affinity human IgG1 antibody against GUCY2C for ADC development: Identification, characterization, and efficacy.

Yunxia Zheng, Xiafen Wu, Junxiang Jia, Huihui Guo, Xiangfei Kong, Gengxiang Zhao, Yuanyuan Huang, Hangbo Ye, Xiaolei Liu, Yi Luo, Xiaoqiang Xie, Xia Zhou, Lu Bai, Wenjun Li, Binbin Chen, Qingliang Yang, **Robert Yongxin Zhao**

HANGZHOU DAC BIOTECHNOLOGY CO., LTD, Hangzhou, China

Colorectal cancer (CRC) is the third most common cancer and the second most common cause of cancer mortality worldwide. Despite the early disease screening techniques could reduce the number of deaths, the treatments for metastatic CRC still remains limited. GUCY2C, a receptor predominantly found in the intestinal epithelium, is a tumor-associated antigen that is overexpressed in gastrointestinal malignancies, particularly colorectal cancer (CRC). GUCY2C offers a super clean therapeutic target, due to restricted expression in normal tissues and high expression in CRC cells, with significantly reduced the on-target-off-tumor toxicities. A high-affinity, high-specificity human IgG1 monoclonal antibody against GUCY2C was obtained from a 100 billion-capacity human Fab phage display library. This antibody, after recombinant expression in CHO cells, was extensively validated both in vitro and in vivo for its suitability in antibody-drug conjugate (ADC) development. The recombinant antibody demonstrated high affinity for its specific antigen, as confirmed by Bio-Layer Interferometry (BLI) and Enzyme-Linked Immunosorbent Assay (ELISA) methods. Flow cytometry further confirmed that the antibody had picomolar (pM) affinity for gastric and colorectal cancer cell lines expressing high levels of GUCY2C. Cellular internalization experiments indicated that the antibody possesses excellent endocytic capabilities, making it particularly suitable for ADC development. The antibody was then conjugated with a linker-payload complex containing a topoisomerase I inhibitor and an antimetabolite moiety, and subjected to a series of in vitro and in vivo evaluations. The ADC achieved in vitro proliferation inhibition in the picomolar range of IC50. Several in vivo CDX model experiments also demonstrated significant tumor suppression efficacy. Moreover, acute toxicity evaluation of the GUCY2C-ADC in diverse preclinical models indicated favorable safety profiles and tolerability. Overall, these compelling preclinical data supports the promising therapeutic potential of this ADC in targeted treatment of CRC.

Keywords: Human IgG1 Antibody, GUCY2C, ADC, High Affinity, Tumor Suppression, In Vitro and In Vivo Validation

#6743 Patient-derived organoids: an *in vitro* platform for evaluating ADC efficacy, potency, and specificity.

Daniele Mori, Claudia Beaurivage, Eider Valle Encinas, Yara Hendriksen, Anna Schepers, Foteini Gkogkou, Joris Maas, Ka Wai Lai, Mariana Silva, Sumeyra Mucuk, Vineeta Adegbenro, Ricardo Korporaal, Merel Derksen, **Sylvia F. Boj**

HUB Organoids B.V., Utrecht, Netherlands

Antibody-drug conjugates (ADCs) are pharmaceutical drugs that selectively deliver cytotoxic agents to cells expressing a specific target antigen (TA). Structurally, ADC consists of a highly specific monoclonal antibody linked to a cytotoxic payload. HUB patient-derived organoids (PDOs) are a unique *in vitro* 3D adult stem cell-based model. They closely resemble the architecture and physiology of the tissue of origin, maintaining the patient-specific genetic and phenotypic characteristics, including surface marker expression. This unique feature makes them a patient-relevant *in vitro* model for ADC screenings. The work presented here demonstrates the potential of the HUB PDO platform in an *in vitro* basket trial. Organoids derived from 7 different tissue origins were used for ADC screening, showcasing the platform's reliability and ability to assess ADC specificity, potency, and efficacy. ADCs directed to different TAs, such as Tissue Factor (F3) or trophoblast antigen 2 (TROP2), were tested, and organoid viability was assessed with CellTiter Glo® 3D after 7 days of treatment.
Further parameters, such as TA expression and the percentage of the cell population expressing TA in each organoid, were evaluated by flow cytometry. The analysis of ADCs targeting F3 revealed an ADC-dependent organoid-killing correlation with F3 expression for a subset of PDOs across all different tumor tissue types. However, as observed in clinical trials, the impact of ADC on the viability of some PDOs was unrelated to F3 expression. The lack of correlation between ADC efficacy and target antigen expression was more evident for the TROP2 ADC. These findings underscore the importance of the study in understanding the differential activity between ADCs. Altogether, these results highlight the value of the PDO platform in assessing ADC target specificity and as a patient-relevant model to study the mechanism behind differential activity between ADCs such as antibody internalization dynamics or bystander effects.

#6744 Why the analytical development of agonistic antibodies is challenging? A comprehensive strategy to unlock their characterization for immunotherapy and autoimmunity.

Gaurav Agrawal, Venkatesh Chari, Luhan Yang, Jennifer Lin-Jones, Alpana Prasad, Jane Lamerdin

Eurofins DiscoverX, Fremont, CA

Checkpoint blockade antibodies, or checkpoint inhibitors, are established as effective cancer therapeutics. A contrasting class of therapeutics, agonistic checkpoint antibodies, has recently emerged for attenuating inflammation in autoimmune diseases. Despite showing clinical promise, their development remains challenging due to limitations in conventional checkpoint assay designs that often fail to replicate physiologically relevant conditions *in vitro* and are sometimes confined to assessing only inhibitory responses.
Here, we introduce a novel checkpoint assay design in a co-culture format involving Fcγ receptor-expressing cells, directly measuring the stimulatory activity of agonistic antibodies. Through testing various classes of Fcγ receptors and diverse cell backgrounds, we have established a specific and robust assay design for several key checkpoint receptors such as CD40, CD137, PD-1, and BTLA. Notably, we have identified previously unrecognized agonistic activity in a clinically approved blockade antibody, underscoring the importance of exploring similar activity in other therapeutics of this nature. The assay is optimized as a thaw-and-use format, exhibiting high reproducibility with ease of execution, and qualified for potency and stability studies as needed for commercial release testing in a quality-controlled environment.

#6745 Generation and validation of a recombinant monoclonal antibody to PAX8: a multi-assay approach.

Amber Miller[1], **Danielle Fails**[1], Jessica Tracy[2], Hailey Wooten[3], Michael Spencer[1]

[1]Immunohistochemistry and Digital Pathology, Fortis Life Sciences, LLC, Montgomery, TX,[2]QC and Validation, Fortis Life Sciences, LLC, Montgomery, TX,[3]QC and Validation, ELISA, Fortis Life Sciences, LLC, Montgomery, TX

Introduction: Paired box 8 (PAX8) is a nuclear protein involved in thyroid follicular cell development and expression of thyroid-specific genes. While primarily associated with thyroid follicular carcinomas and atypical follicular thyroid adenomas, recent research has indicated that this marker is prognostic in small cell lung cancer and positively correlated with Ki67 expression (Tao et al., 2024) -- perhaps opening PAX8 up as a future therapeutic target. Studies such as these, though, require the use of extremely reliable antibodies to generate reproducible and trustworthy data. In congruence with the International Working Group for Antibody Validation's (IWGAV) pillars of antibody validation, a program has been implemented to enhance our existing validation principles including the recombinant monoclonal PAX8.
Methods: Four unique PAX8 peptides were synthesized, purified, and used for rabbit immunization and B-cell sorting. Clones were screened for baseline activity and assessed microscopically for specificity using variable tissue and cell microarrays. Specificity was further assessed via reciprocal IPs, western blot, and flow cytometry. Finally, multiplex immunofluorescence staining was conducted utilizing two separate approaches. First, staining and imaging was done using the Lunaphore COMET™ followed by traditional TSA sequential staining methods and imaged with the Akoya PhenoImager® HT system.
Results: Over 40 clones were screened against OVCAR3 cell lysate using ProteinSimple Simple Western™ technology and stained in tandem across target-specific microarrays using chromogenic immunohistochemistry. This dual screen approach allowed for the narrowing of the substantial clone set down to 8, which was reduced further after in-depth CTWB/IP studies eliminated an additional 5 clones. The remaining 3 clones were examined through expanded chromogenic testing on large (80+ cores) human, mouse, and rat tissue and cell microarrays. Throughout the various assays, PAX8 specificity was assessed utilizing other external antibodies, PAX8-overexpressing cells, and known target localization via literary sources.
Conclusions: In utilizing a multi-assay approach, we were able to narrow the original 40+ clone set to a single, highly-specific monoclonal antibody. This approach is one that the International Working Group for Antibody Validation has been emphasizing as a superior way to produce antibodies that foster reproducibility across research. In future studies, multiplex immunofluorescence staining will be conducted using PAX8 in combination with PAX5 and Ki67 across small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) to evaluate expression rates.
References: 1. Tao, Fengyun et al. Prognostic value of PAX8 in small cell lung cancer. Heliyon. 2024. 10(7): e28251.

#6746 Multi-modal assessment of an anti-HER2 (trastuzumab) antibody-drug conjugate screening assay for breast cancer models.

Michael Steffey, Stacey Roys, **Anita J. Zaitouna**, Philip Lapinski, Kerry-Ann Bright, Sheri Barnes, David Draper, Scott Wise

Labcorp, Ann Arbor, MI

Antibody-drug conjugates (ADCs) represent groundbreaking advancement in the cancer treatment landscape. ADCs combine the specific binding capacity of a monoclonal antibody with a cytotoxic payload that can kill target and bystander cells. The antibody moiety of ADCs confers payloads with the ability to target cancer cells with minimal damage to healthy tissues.
A key consideration in defining appropriate breast cancer treatment regimen is the expression level of human epidermal growth factor receptor 2 (HER-2), targeted with the antibody Trastuzumab. Breast cancers are classified as high, low, or negative for HER-2, which dictates the therapeutic approach. Trastuzumab alone may not be an effective treatment because tumors can either be initially resistant or develop resistance over time. Around 70% of patients experience resistance within the first year of treatment. To overcome this, ADCs such as Trastuzumab-Emtansine (DM1) or Trastuzumab-Deruxtecan (Dxd) can be used at lower doses, reducing the risk of resistance and potentially enhancing treatment efficacy.
Assessment of Trastuzumab ADCs in multiple murine breast cancer models can be costly, but *in vitro* evaluation of various cancer histotypes facilitates the selective identification of the best murine model. Having a robust screening tool is an important asset. To assess Trastuzumab ADCs, we screened five breast cancer cells (BT-474, Hs 578T, MDA-MB-231, MDA-MB-361, SK-BR-3) with varying levels of HER-2 expression. They were evaluated for binding efficiency, competitive inhibition, and cytotoxicity of ADCs. Additionally, the ability of the ADC to kill bystander cells (HER-2 low or negative cells) was assessed using two bystander assay methods.
The half maximal effective concentration (EC_{50}) values for Trastuzumab and Trastuzumab-Dxd were measured on two cell lines. The HER-2 high cell line had a tenfold higher EC_{50} value.
Competitive inhibition of Trastuzumab-ADC binding was tested with a PE labeled Trastuzumab on two cell lines. The Trastuzumab-ADC was able to outcompete Trastuzumab on HER-2 high cells but not on HER-2 low/negative cells. Proliferation assays on multiple cell lines were used to assess the ability of Trastuzumab-DM1 to inhibit cell growth in comparison to DM1 (payload alone) or Trastuzumab (antibody alone). Overall, Trastuzumab-DM1 was better able to inhibit growth of HER-2 positive cells compared to payload or antibody alone.
Two ADC bystander assays were developed and tested. The first involved flow cytometry and the second a luciferase assay. In both assays, bystander cells were more effectively targeted only in the presence of HER-2 positive cells. The ability of ADCs to target bystander cells suggest that even in heterogenous HER-2 expressing tumors, these therapies may still be effective.
Our cutting-edge approaches provide precise insights into the activity and efficacy of Trastuzumab ADCs.

#6747 A next generation ADC: AR153, pH-dependent anti-B7H3 MMAE.

wonhwa kang

Ybiologics, Daejeon, Korea, Republic of

Antibody-drug conjugates (ADCs) demonstrate significant anti-cancer efficacy, but their therapeutic index (TI) can be restricted due to toxicity in normal tissues. To address this limitation, pH-dependent antibodies have emerged as a promising solution. These antibodies exhibit weak binding to their target under physiologically neutral pH conditions but show strong binding in the acidic environment of the tumor microenvironment (TME), improving tumor selectivity and reducing off-target toxicity, thereby increasing the TI of ADCs. B7-H3, a member of the B7 family, is overexpressed in various solid tumors, making it a promising therapeutic target for ADC development. We screened a recombinant human antibody library (Ymax-ABL) and identified pH-dependent antibodies against B7-H3. We developed a pH-dependent anti-B7-H3 antibody (pH-B7-H3 Ab) that shows strong binding under acidic conditions such as TME, and reduced binding under neutral pH, as seen in healthy tissues. We generated ADCs by conjugating a pH-independent anti-B7-H3 antibody (Non-pH-B7-H3 Ab) and the pH-B7-H3 Ab with GGFG-Dxd and vc-PAB-MMAE, achieving drug-to-antibody ratios (DAR) of 3.8, 3.8, 3.5 and 3.9, respectively. Comparative studies revealed that pH-B7-H3-MMAE exhibited lower cytotoxicity than Non-pH-B7-H3-MMAE under neutral pH conditions typical of normal tissues. However, under acidic conditions (pH 6.0), both ADCs displayed comparable cytotoxicity. Additionally, in a B7-H3-expressing CDX model, ADCs demonstrated similar anti-cancer efficacy. Notably, pH-B7-H3 Ab exhibited superior penetration into tumor tissues compared to Non-pH-B7-H3 Ab, highlighting its potential to improve therapeutic outcomes by selectively targeting tumor cells while minimizing harm to normal tissues.

#6748 Comparison of activities and identification of predictive genomic biomarkers of response to trastuzumab and the trastuzumab deruxtecan ADC using the OncoPanel® platform.

Luciano Galdieri[1], Steven Garner[1], Brogan Epkins[1], Emily Schultz[1], Daria Clucas[1], Kaitlyne Powers[1], Justin Lipner[1], Elsa Liu[2], ChunYao Lee[2], Alastair J. King[1]

[1]Eurofins Discovery, Saint Charles, MO,[2]Eurofins Discovery, Taipei, Taiwan

Antibody-drug conjugates (ADCs) represent a novel class of therapeutic agents that allow the targeted delivery of cytotoxic payloads to tumor cells via the selectivity afforded by monoclonal antibodies (mAbs) directed against tumors expressing particular antigens. When the mAb component of the ADC binds to its cell surface antigen, the ADC-antigen complex is internalized by the cancer cell. Once inside the cell, the drug payload is released from the mAb either through a chemical reaction or enzymatic digestion within lysosomes, ultimately allowing the exertion of its cytotoxic effects. In 2013, the first ADC approved for treating solid tumors was the Her2-targeted ado-trastuzumab emtansine, which was developed for metastatic breast cancer. As of June 2024, the US Food and Drug Administration has approved thirteen ADCs, with many more currently undergoing preclinical development and clinical trials. The anti-Her2-targeted ADC trastuzumab deruxtecan (TDxd) features a cleavable enzymatic linker, unlike trastuzumab emtansine, which utilizes a non-cleavable thioether linker. This cleavable linker enables deruxtecan, a topoisomerase I inhibitor, to diffuse out of cells following cleavage, thereby inducing cytotoxic effects even in tumors with low Her2 expression levels. Solid tumors overexpressing Her2—including breast, colorectal, and gastric cancers, as well as Her2-mutated lung cancers—have shown significant responses to TDxd. We evaluated the activity and selectivity of TDxd, along with the unmodified antibody and free payload when administered separately, against a panel of 300 human tumor cell lines. Potencies were assessed from ten-point dose response curves using a four-parameter log-logistic model with custom curve-fitting software to determine IC_{50} and EC_{50} values. In this study, we compare and contrast the potency and efficacy of each tested agent and explore the genomic biomarkers associated with sensitivity and resistance to each of these treatments. Additionally, we have also evaluated the effect of these agents from a broader tissue biology perspective, including use of the BioMAP® cellular phenotypic platform.

#6750 QLS5132, a highly selective anti-CLDN6 ADC with broader therapeutic window.

Ying Huang[1], Zhongcui Sun[1], Shuyong Zhao[2], Xinmei Wang[3], **Ling Zhang**[1], Yang Yang[1], Hua Ying[1], Weikang Tao[1]

[1]Shanghai Qilu Pharmaceutical Research and Development Center LTD., Shanghai, China,[2]Qilu Pharmaceutical Co., Ltd., jinan, China,[3]Qilu Pharmaceutical Co., Ltd., Jinan, China

Background: Claudin-6 (CLDN6), a member of the claudin family, is localized in the tight junctions of epithelial cells to regulate permeability, barrier function and polarity of epithelial layers. CLDN6 is notably upregulated in multiple solid tumor types, including ovarian cancer, testicular cancer, endometrial cancer and non-small cell lung cancer, while exhibiting minimal or no expression in healthy adult tissues. Therapeutic potential of CLDN6 as a target for ADC was unraveled by TORL-1-23, a clinical stage ADC drug with MMAE as the payload from TORL BioTherapeutics, LLC, while QLS5132, a novel CLDN6-targeting ADC in IND-enabling stage, demonstrates superior efficacy and tolerability to TORL-1-23.

Method: QLS5132 is composed of a humanized anti-CLDN6 hIgG1 antibody with no cross-reactivity to CLDN9 conjugated to Qilu's proprietary topoisomerase-1 inhibitor, QLS6916, via a hydrophilic cleavable linker. The payload QLS6916 showed nanomolar activity across a wide range of cancer cell lines in vitro and rapid systematic clearance in rodent models.

Result: QLS5132 showed sub-nanomolar activity in CLDN6-positive cancer cell lines, with faster internalization efficacy and stronger bystander killing activity than TORL-1-23. In *in viv*o pharmacology studies, QLS5132 demonstrated superior antitumor efficacy to TORL-1-23 across a panel of ovarian cancer cell line-derived xenograft (CDX) models with varying levels of CLDN6 expression. Furthermore, QLS5132 displayed synergistic activity when combined with PARP inhibitors in CLDN6-expressing ovarian CDX models, indicating its potential for possible combination with PAPR inhibitors in ovarian cancers. QLS5132 was well tolerated in cyno monkeys, with HNSTD up to 60 mpk Q3W x 4 in non-GLP toxicity study, which is 5 folds higher to TORL-1-23.

Conclusion: QLS5132 showed an expanded therapeutic window with superior efficacy and tolerability to TORL-1-23 in preclinical models. QLS5132 also demonstrated great potential to combine with PARPi in CLDN6 positive ovarian cancers. QLS5132 is expected to offer a novel and promising treatment option either as a single agent or combo with PARPi or other SoCs for patients with CLDN6 expressing tumors. QLS5132 will enter clinical trials.

#6751 AT2604 is a highly efficacious ADC targeting alkaline phosphatases ALPP and ALPPL2.

Bryan K.S. Yeung, Wei Qi Tan, You Xue Wu, Xi Yun Zhang, Yin Wen Cheng, Shao Jun Liu, Chen Zhong, Bin Zou

Axcynsis Therapeutics, Singapore, Singapore

Placental alkaline phosphatases, ALPP and ALPPL2, are highly homologous, membrane-bound proteins involved in fetal development. Despite having limited expression in normal tissues, they are highly differentiated in multiple tumor cells making them ideal targets for antibody drug conjugate (ADC) development. AT2604 is an ADC incorporating an anti-ALPP/ALPPL2 antibody conjugated to monomethyl auristatin E (MMAE) with high DAR 4 homogeneity; and displays strong tumor growth inhibition at low doses in CDX models of gastric and pancreatic cancer. In patient-derived xenograft models of gastric cancer with moderate- and low-antigen expression, AT2604 achieves near-complete tumor regression at 3 and 6 mg/kg (QWx3), respectively. A preliminary toxicity assessment in non-human primates concluded a well-tolerated dose of 10 mg/kg (Q3Wx3) without exhibiting any non-reversible adverse effects.and good plasma stability; implying that AT2604 possesses an improved safety profile over other vedotin-based ADCs.

#6752 Identification and validation of human antibodies targeting a novel pan-adenocarcinoma target discovered via spatial transcriptomics.

MinKyu Kim, Jinyeoung Choi, Jae Eun Lee, Hongyoon Choi, Hyung-Jun Im

Portrai, Inc., Seoul, Korea, Republic of

Background: Adenocarcinoma (ADCA) is a type of cancer that originates in glandular epithelial cells in various organs such as pancreas, colon and lung. Spatial transcriptomics enables the high-resolution mapping of gene expression within the tumor microenvironment, preserving spatial context. This allows for the identification of tumor-specific targets in the context of complex tumor microenvironment, providing insights critical for precision oncology. Through a data-driven target discovery platform which leverages large scale transcriptomic data including ST, a novel target, POR-ADCA-001, was identified. Here, we developed human antibodies that have superior specificity and binding affinity to POR-ADCA-001.
Methods: Panning phage display, filter lift assay, and Solid Phase Extraction Enzyme-Linked Immunosorbent Assay (SPE ELISA) were utilized to screen hit antibodies with strong binding potential to the target protein. Furthermore, ELISA and Surface Plasmon Resonance (SPR) analysis were used to identify lead candidate antibodies (POR-ADCA-001-01, POR-ADCA-001-02) that can effectively target POR-ADCA-001 at low concentrations. Moreover, a cell line overexpressing POR-ADCA-001 (cell-1) and a non-expressing cell line (cell-2) were validated using western blot and immunofluorescence. At last, we examined the effectiveness of the antibodies using flow cytometry, immunofluorescence, and internalization assay.
Results: Spatial and differential expression analyses revealed POR-ADCA-001 as highly expressed in various ADCA tissues with low expression in normal tissues. On ELISA, 28 hits were found and the half maximal effective concentration (EC50) values of them were one or two digit nM. Among them, POR-ADCA-001-01, 02 were selected for further development and demonstrated the binding affinity of 0.84 and 14.8 nM. We demonstrated using flow cytometry and immunofluorescence that the two antibodies bind strongly to cell-1 but hardly bind to cell-2. Internalization assays confirmed substantial uptake of the antibodies in cell-1, with minimal activity in control cell lines.
Conclusion: Our research identified POR-ADCA-001 as a target specific to pan-adenocarcinoma and discovered two human IgG antibodies that demonstrate high specificity and effective internalization in cells that express the target. These antibodies could be utilized for the development of targeted therapeutics such as antibody-drug conjugates (ADCs) or radiopharmaceutical therapies (RPTs). This study emphasizes the promise of ST in uncovering new therapeutic targets and improving cancer treatment.

#6753 Exploring new approaches to widening of the therapeutic index of ADCs with dual-payload design and combination with a novel HMA.

Younghwa Chun, Areum Go, Byeong Sung Lee, Seunggun Shin, Sohui Kweon, Hyemin Han, JaeHyeon Lee, Suhyun Park, Ha Young Lee, Min Young Lee, Hyun Yong Cho, Doo Young Jung

PINOTBIO Inc., Suwon-si, Korea, Republic of

Antibody-drug conjugates (ADCs) have revolutionized targeted cancer therapy by delivering potent cytotoxic agents directly to tumor cells. Recent approvals of ADCs such as trastuzumab deruxtecan, sacituzumab govitecan have significantly improved patient treatment options, underscoring the clinical potential of this therapeutic class. However, single-payload ADCs often face limitations in efficacy. Tumor heterogeneity poses a significant challenge, as targeting cancer cells with a single cytotoxic mechanism may not sufficiently overcome resistance or achieve optimal therapeutic outcomes. These limitations call for the development of novel strategies that widen the therapeutic index by increasing the Maximum Tolerated Dose (MTD) and lowering the Minimum Effective Dose (MED). We have designed and synthesized a novel dual-payload ADC combining two distinct cytotoxic agents: a potent topoisomerase I inhibitor (Top1i) and a microtubule-disrupting agent. This innovative approach allows for a synergistic therapeutic effect, leveraging the complementary mechanisms of action of both payloads within a single ADC. *In vitro* cell viability was determined using the CellTiter-Glo assay against the NCI-N87 and MDA-MB-468 cell lines. These studies demonstrated robust potency of the dual-payload ADC across multiple cancer cell lines. *In vivo* efficacy studies were conducted in HER2-expressing NCI-N87 gastric cancer xenograft model. The tumor-bearing mice were treated with various doses of dual-payload ADC, single-payload ADC, or saline control. Notably, dual-payload ADC displayed superior efficacy compared to single-payload ADC, achieving a tumor growth inhibition (TGI) of 103% ($p < 0.05$). This demonstrates superior tumor growth inhibition of the dual-payload ADC compared to a Top1i-based single-payload ADC and a more favorable safety profile compared to a microtubule inhibitor-based single-payload ADC. Pharmacokinetic (PK) profiling and non-GLP toxicity studies are currently underway to further characterize its suitability for clinical development. Moreover, we have explored combination strategies to further augment the therapeutic index. One promising approach involves the combination of the ADC with NTX-301, a novel 4′-thio-modified analog of decitabine (DAC). NTX-301 enhances p53 stabilization more effectively than DAC, potentially improving the ADC's efficacy through synthetic lethality with BCL2 inhibition. Preliminary data suggest that the ADC+NTX-301 combination produces greater synergy and a more favorable therapeutic index than ADC combinations with traditional hypomethylating agents (HMAs). In this study, we present novel strategies to enhance the therapeutic index of ADCs through a dual-payload design and combination with NTX-301. These results highlight the potential of both approaches for further development in cancer treatment.

#6754 A payload screening and ADC evaluation platform for STING agonist antibody drug conjugates.

Ying Meng, Li Li, Min Kang, Xiao Li, Haiting Dai, **Tj (Tiejun) Bing**

ICE Bioscience, Inc., Beijing, China

Antibody drug conjugates (ADCs) are a class of rapidly developing targeted biotherapeutic drugs, which consist of three elements: antibody, linker, and payload. ADCs combine the high specificity of antibodies with the potency of payloads, delivering the highly efficient small molecule within the target tumor cells.
As one of the new payload types, stimulator of interferon genes (STING) agonist can initiate the innate immune response, enhancing the attack on tumor cells. STING agonist ADCs, allowing tumor-localized activation of STING, may achieve greater anti-tumor activity and better tolerability.
By combining the STING binding assay, different THP-1 reporter assay, we can support for STING agonist activities screening. RT-qPCR assay and WB assay can be applied for demonstrating the molecular mechanisms of candidates. For specific screening and evaluation of HER2 STING agonist ADCs, HER2 overexpression reporter assay, co-culture assays of tumor cell lines or STING knockout tumor cell lines with THP-1, hPBMC, as well as whole blood can be utilized at different project stages.

#6755 Discovery of the synergistic dual-payload antibody-drug conjugate (CTPH-02) by combination of MMAE and novel payloads.

Chang-Sun Lee, Myeong Joo Kim, Da hyun Kim, Soyeon Lim, Jiwoo Lee, Eunseok Choi, Wonbin Ha, Han ah Kim, Jaebeom Park, Seung Chan Kim

Celltrion Pharm, Inc., Incheon, Korea, Republic of

Many ADC drugs in clinical trials are showing poor performance without providing sufficient dose due to dose-limiting toxicity, which is rooted from on-target toxicity related to payload itself. There are many efforts to increase the therapeutic index (TI) of ADC, but research on discovering novel payloads based on unique anticancer mechanisms of action is relatively limited. We have developed unique ADC platform technology by adopting multi-drug payloads concept, where we aim to achieve three different goals, (1) increasing MTD and lowering MED (2) showing cytotoxic activity in antigen low-expressed cell lines (3) overcoming resistance cell lines with better cytotoxic effects. This study began with the search for a drug combination that could maintain or increase efficacy of MMAE even with lower dose of MMAE. Drugs with various mechanisms of action were tested to select a combination that showed a high synergy effect with MMAE, and a cell cycle regulating drug was found to offer the most synergistic combination efficacy with MMAE. By conjugating a dual-payload with two-different mechanisms of action to HER2 antibody(trastuzumab), the resulting ADC(CTPH-02) was confirmed to show synergistic effect in terms of cytotoxicity. Very excitingly, CTPH-02 shows significantly high synergistic efficacy in the case of cell lines with low HER2 antigen expression. These findings based on the right combination of two-different payloads open a new paradigm for ADC drug design strategies in cancer therapy in order to provide treatment options to wider range of cancer patients such as low TPS % with low IHC score.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Cellular Responses to Anticancer Drugs
Poster Session

#6759 *In vivo* tumor growth control by general control nonderepressible 2 targeting agents results from kinase activation.

F. Tameire[1], P. Wojnarowicz[1], C. Dudgeon[1], S. Fujisawa[1], S. Ramurthy[1], O. Reilly[1], C. G. Thomson[2], B. Sherborne[2], S. Taylor[2], F. He[2], P. Pan[2], B. Li[2], E. May[3], A. C. Rigby[1], M. J. Mulvihill[1], N. Bose[1], D. Surguladze[1], **E. S. Lightcap**[1];
[1]HiberCell, Boston, MA, [2]Pharmaron UK Ltd, Hoddesdon, United Kingdom, [3]Watertown Biosciences LLC, Watertown, MA

General Control Nonderepressible 2 (GCN2; *EIF2AK4*) is a serine-threonine kinase in the integrated stress response (ISR) signaling pathway that initiates adaptive responses during nutrient stress conditions. While pharmacological inhibition of GCN2 under nutrient stress conditions induces apoptosis and inhibits tumor growth, GCN2 inhibition without nutrient stress has been reported to have no effect on tumor growth. By exploring an array of GCN2 inhibitors, we demonstrate that multiple agents in fact activate GCN2 in biochemical and cell-based assays at low concentrations and inhibit GCN2 at higher concentrations. Unexpectedly, it is this activation, and not inhibition, of the GCN2 pathway that is associated with decreased viability *in vitro* and tumor growth inhibition *in vivo* across multiple models. Knockdown and knockout experiments show that activation of the ISR by GCN2-targeting agents is dependent on GCN2. We also uncover that Type 1 GCN2-targeting agents do not activate GCN2 whereas non-Type 1 agents activate GCN2 in the absence of stress. We demonstrate that a Type 1 GCN2-targeting agent does not have the same viability effects as the non-Type 1 GCN2-targeting agents. These results provide a clearer understanding of the challenges and opportunities for the clinical development of compounds targeting GCN2.

#6760 Anticancer mechanisms of quercetin revealed via a systems biology approach.

A. Goraieb[1], A. Aggarwal[1], **K. W. Yip**[2];
[1]University of Toronto, Toronto, ON, Canada, [2]University of Toronto, Anticancer.ca, Toronto, ON, Canada

Introduction: Quercetin is a dietary flavonoid found in apples, onions, and berries. Previous *in vitro* and *in vivo* studies have identified its pro-apoptotic, anti-angiogenic, antioxidant, and anti-inflammatory activities, in addition to its ability to sensitize cancer cells to traditional chemotherapies. However, there has not yet been a comprehensive evaluation of its anticancer properties on a large panel of cancer cell lines. We hypothesized that quercetin exhibits varied anticancer effects depending on the particular cell line and its tissue of origin.
Methods: For cell survival assessments, 77 cancer cell lines from the NCI60 dataset were seeded into 96-well plates and incubated for 24 hours. DMSO control or quercetin was then added at 5 ten-fold concentrations to a maximum of 100 µM. 48 hours later, the cells were fixed and stained, and cell viability measured with CellTiter-Glo. Next, for transcriptomic profiling, the L1000 assay from The Broad Institute was used. For these experiments, 10 µM of quercetin was added to 9 core cell lines seeded in 384-well plates. Then, the Affymetrix GeneChip HG-U133 Plus 2.0 Array was used to generate a gene expression signature for quercetin, which was then compared with signatures from other compound and genetic perturbations. Connectivity scores were used to classify the L1000 signatures according to similarity to the gene expression changes produced by quercetin. The data for cell survival and transcriptomic profiling were extracted from PharmacoDB and Clue.io, respectively.
Results: The most sensitive cell line to quercetin was the glioblastoma cell line U-87/H.Fine (IC_{50}=5.03 µM), and the least sensitive was the renal cell carcinoma cell line TK-10 (IC_{50} = 5780.61 µM). Overall, quercetin was most potent against myeloid and prostate cancers and least potent against ovary and fallopian tubes cancers. As indicated by high median tau scores, quercetin administration induced gene expression changes similar to NFKB2, PTK2 and EPCAM knock-downs, which are associated with reductions in inflammation, cell proliferation, and cancer growth and progression. The cellular effects of quercetin were analogous to the administration of other anticancer compounds such as CP466722, rhamnetin, and piceatannol. CP46672 can sensitize tumors to ionizing radiation, rhamnetin acts as an antioxidant, and piceatannol is a naturally occurring anticancer agent.
Conclusions: This study provides a comprehensive *in vitro* examination of the impact of quercetin on the viability and gene expression profiles of cancer cells of various origins. Further research on the molecular changes induced by quercetin in animal models and human subjects would help inform the applicability of quercetin in human cancers and guide its use in cancer prevention and treatment.

#6762 Baseline multiomics and chemotherapy efficacy in preclinical trials using patient-derived xenograft models of cholangiocarcinoma can reveal distinct signatures of chemotherapy response.

Amro M. Abdelrahman[1], Danielle M. Carlson[2], Ellen L. Larson[1], Erik Jessen[3], Dong-Gi Mun[4], Isaac Lynch[5], Alessandro Fogliati[6], Stella Konadu Adjei Antwi[1], Aushinie Abeynayake[2], Akhilesh Pandey[4], Mark J. Truty[1], Gregory J. Gores[2], Rory L. Smoot[1]

[1]Surgery, Mayo Clinic, Rochester, MN,[2]Internal Medicine, Mayo Clinic, Rochester, MN,[3]Quantitative Health Sciences, Mayo Clinic, Rochester, MN,[4]Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN,[5]College of Medicine, University of Iowa, Iowa City, IA,[6]Surgery, University of Milano-Bicocca, Milan, Italy

Background: Cholangiocarcinoma (CCA) is a diverse type of cancer. For several years, platinum-based chemotherapy (Gemcitabine and Cisplatin) has been the standard treatment and serves as the foundation for current immunotherapy combinations. The typical response rates are generally between 20% and 30%. In this study, we present the results of preclinical efficacy trials conducted on CCA patient-derived xenograft (PDX) models, correlating them with the baseline multiomics profiling of these models to identify molecular characteristics linked to treatment response.
Methods: Tumor samples from twenty-eight CCA PDX were used to isolate RNA and protein to be included in multiomics analysis (Phosphoproteomics, Proteomics, and Transcriptomics). A subset of PDX models were treated with various doses of Gemcitabine and Cisplatin. The Median Efficacy Index (MEI) was calculated as the ratio of the difference in median of delta in tumor volume at the last day of the study between the treatment and the control arms divided by the median of delta in tumor volume of the control at the last day of the treatment (positive= growth, negative=inhibition, and 0=no efficacy). A Hierarchical All-Against-All algorithm was utilized to compare multiomics features to the MEI and pathway analysis completed for identified molecular features. Pathway enrichment was calculated using the KEGG database, and random-forest variable selection chose the pathways most influential in predicting sensitivity or resistance.
Results: After conducting preclinical efficacy trials, the 11 tested CCA PDX models were classified into sensitive, moderately sensitive, and resistant models based on the MEI on each treatment arm. The top up-regulated genes were identified for Gemcitabine response (SRPK2, GLK2, and RIOK2) and Cisplatin response (NTRK1, PRPF4B, and SRPK2). The top up-regulated genes related to Gemcitabine resistance were NTRK1, PIP5K1C, and CSNK2A2, while those related to Cisplatin resistance were SRPK1, PRPF4B, and CSNK2A1. Downregulation of the anti-folate resistance pathway is a strong predictor of gemcitabine sensitivity, and the lysine degradation pathway is a strong predictor of cisplatin sensitivity.
Conclusion: Multiomics signatures of cholangiocarcinoma can forecast responses to standard chemotherapy in preclinical models. New markers of resistance to Gemcitabine and Cisplatin were discovered, linked to NTRK1, SRPK1, and SRPK2. These resistance profiles will be tested using suitable targeted therapies on the resistant CCA patient-derived xenograft (PDX) models.

#6763 A cellular platform for systematic comparison of RAS inhibitors using PRISM multiplexed screening.

Matthew G. Rees, Mustafa Kocak, Matthew J. Emmett, Colleen T. Harrington, Anthony Fazio, Alvin Kalathungal, Ursula Widocki, Claudine Mapa, Dennie T. Frederick, Jillian N. Eskra, Laura Doherty, Blanche C. Ip, Melissa M. Ronan, William R. Sellers, Jennifer A. Roth

Broad Institute, Cambridge, MA

The discovery and development of direct inhibitors of KRAS is transforming therapeutic options across diverse cancer types. Here, we use the PRISM collection of nearly 900 cancer cell lines, including more than 150 KRAS-mutant cell lines, to characterize 19 KRAS-targeting inhibitors or degraders, including G12C-, G12D-, and pan-KRAS inhibitors. Agents were tested at 8 doses (threefold dilution) for 5 days across a median of 883 cell lines. For each agent, we assessed its selectivity and correlation with KRAS mutation status and genetic dependency. For each cell line, we categorized its sensitivity to each individual agent and in aggregate by comparing across RAS modulators. For example, all 10 KRAS G12C inhibitors or degraders tested showed significant selectivity for cell lines harboring the G12C mutation, with RMC-6291, adagrasib, divarasib, and sotorasib showing the greatest enrichment. Among G12C-mutant cell lines, all 10 G12C-targeting agents were preferentially active against NCIH358 cells (rank 1-3, median 1.5) while OV56 cells showed much more variability in response (rank 5-623, median 92). Similar trends were observed for pan-KRAS inhibitors (NCIH358: median rank 7; OV56: median rank 655). Further, we characterized a panel of downstream (MEK, ERK) or upstream (SHP2, SOS1, RTK) RAS/MAP kinase pathway modulators in the PRISM assay to understand similarities and differences between direct and indirect RAS pathway suppression. Finally, we also explored orthogonal assay formats, including ultra-low adherent and extended-duration (10-day) screening, to investigate kinetic and environmental factors associated with inhibitor response. Collectively, our work provides a comprehensive profile of the cellular effects of current RAS-targeting therapeutics, and provides a foundation for the characterization of newly-emerging agents.

#6764 Lipophagy-dependent fatty acid oxidation is a metabolic vulnerability for KRAS signaling inhibition in pancreatic cancer.

Junzhang Zhao[1], Ravi Thakur[1], Dezhen Wang[2], Tuo Hu[2], Chunbo He[1], Surendra K. Shukla[1], Kirsten L. Bryant[3], Kamiya Mehla[1], Channing J. Der[3], Pankaj K. Singh[1]

[1]Oncology Science, University of Oklahoma Health Sciences Center, Oklahoma City, OK,[2]Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE,[3]Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC

Pancreatic ductal adenocarcinoma (PDAC) is frequently driven by activating mutations in KRAS, which promote tumor progression through sustained activation of the MAPK (RAS/RAF/MEK/ERK) signaling pathway. This study investigates the metabolic vulnerabilities associated with PDAC by examining the metabolic reprogramming that occurs upon KRAS signaling inhibition via targeting MEK/ERK/KRASG12D, and utilizing metabolomic, lipidomic, and isotope-tracing approaches. Our findings reveal that MEK/ERK/KRASG12D inhibition results in enhanced lipid turnover and fatty acid oxidation while suppressing glycolysis, glucose oxidation, and glutamine metabolism in PDAC cells. Notably, we observed that the increased lipid turnover and fatty acid oxidation are mediated by lipophagy, rather than cytosolic lipolysis. Furthermore, these processes are driven by enhanced nuclear translocation and transcriptional activity of TFEB, a key regulator of lysosomal biogenesis and autophagy. Pharmacological inhibition of fatty acid oxidation, when combined with MEK/ERK/KRASG12D inhibitors, exhibited synergistic anti-tumor effects, significantly reducing the growth of PDAC cell lines and organoids. This combination therapy also decreased tumor burden and improved overall survival in orthotopic cell line-derived and patient-derived xenograft (PDX) mouse models. These results provide novel mechanistic insights into the metabolic adaptations underlying resistance to KRAS signaling inhibition. These studies highlight fatty acid oxidation as a critical metabolic vulnerability in PDAC, offering a promising therapeutic target to enhance the efficacy of KRAS pathway inhibitors.

#6765 Antitumor activity of Selenopsammaplin A analog by targeting histone methyltransferase DOT1L in prostate cancer cells.

Jaeho Han, Sehun Yang, Woong Sub Byun, Sung Chul Jang, Eun Seo Bae, Chae Won Ock, Hyen Joo Park, Hyeung-geun Park, **Sang Kook Lee**

College of Pharmacy, Seoul National University, Seoul, Korea, Republic of

The overexpression of the disruptor of telomeric silencing 1-like (DOT1L), which is a specific methyltransferase for histone H3 at lysine residue 79 (H3K79), has been related to poor outcomes in patients with Castrate-resistant prostate cancer (CRPC). Therefore, targeting DOT1L is considered a potential therapeutic approach to overcome the significant medical challenges of CRPC. In our previous study, we designed selenopsammaplin A (SPA) analogs as non-nucleoside DOT1L inhibitors to suppress human breast cancer cell proliferation and metastasis. However, the antitumor activity and the precise underlying mechanism of SPA analogs in prostate cancer cells remain unclear. Herein, we administered SPA-10091-HCl, a DOT1L-targeting degrader, to effectively hinder the growth and DOT1L-mediated H3K79 methylation in CRPC (PC3 and DU145) cells. Mechanistically, SPA-10091-HCl selectively degrades DOT1L protein through the nuclear ubiquitin-proteasome pathway, thereby suppressing H3K79 methylation in CRPC cells. SPA-10091-HCl inhibits CRPC cell proliferation, migration, and invasion, with the E-cadherin expression upregulation and N-cadherin and vimentin expression downregulation. Additionally, prolonged SPA-10091-HCl treatment induced apoptosis by regulating apoptosis-associated protein expressions. Moreover, SPA-10091-HCl effectively inhibited tumor growth in the PC3 cells-implanted xenograft mouse model without any overt toxicity. These results indicate SPA-10091-HCl as a potential candidate for further development as a chemotherapeutic agent against CRPC.

#6766 Tetraarsenic hexoxide augments the anticancer effects of Artemisia annua polyphenols on both naive and oxaliplatin-resistant HCT116 colorectal cancer cells by downregulating CD44, EGFR and cyclinD1, and increasing DNA damages and intracellular vesicles.

Won Sup Lee[1], Eun Joo Jung [1], Hye Jung Kim[2], Sung Chul Shin[2], Gon Sup Kim[2], Jin-Myung Jung[1]

[1]Gyeongsang National Univ. Hospital, Jinju, Korea, Republic of,[2]Gyeongsang National Univ., Jinju, Korea, Republic of

We previously demonstrated that polyphenols extracted from Korean Artemisia annua L. (pKAL) exerted stronger anticancer activity on oxaliplatin-resistant HCT116 colorectal cancer cells (Oxp-R-HCT 116 cells) than parental HCT116 cells (p-HCT 116 cells). We also demonstrated tetraarsenic hexoxide exerts anticancer activity of pKAL on several cancer cell lines by suppressing NF-κB activity. In this study, we found that tetraarsenic hexoxide significantly enhanced the anticancer activity of pKAL on both Oxp-R-HCT 116 cells and p-HCT 116 cells. The aim of this study is to elucidate how tetraarsenic hexoxide enhance the anticancer effects of pKAL on Oxp-R-HCT 116 cells. CCK-8 assay, phase-contrast microscopy analysis, and morphological analysis with hematoxylin stain revealed that the anticancer effect of pKAL is more enhanced by tetraarsenic hexoxide on Oxp-R-HCT 116 cells even though anticancer effect of tetraarsenic hexoxide alone was not prominent on Oxp-R-HCT 116 cells. This phenomenon was not associated with suppression of some of oxaliplatin resistance-related upregulated proteins (Oxp-RR-Proteins) (β-catenin, neuclephosmin, and SOD), but was associated downregulation of cell survival- and stemness-related proteins (NF-κB, cyclin D1, EGFR, and CD44) that were not up-regulated in Oxp-R-HCT 116 cells. In addition, tetraarsenic hexoxide and pKAL combination treatment enhanced anticancer effect by induction of DNA damage and intracytoplasmic vesicles suggesting autophagic cell death.The detailed western blot analysis for anti-cancer effects of tetraarsenic hexoxide alone did not strongly suppress the expression of β-catenin(one of oxaliplatin resistance-related upregulated proteins) in p-HCT 116 cells as well as Oxp-R-HCT 116 cells while pKAL alone strongly suppressed it. In addition, tetraarsenic hexoxide and pKAL combination treatment enhanced LC3B and P62 while pKAL alone did not enhance them. N-acetylcysteine (NAC) strongly suppressed anticancer effect of induction of DNA damage and intracytoplasmic vesicles that suggested autophagic cell death on Oxp-R-HCT 116 cells. In conclusion, this study suggests that tetraarsenic hexoxide enhanced the anti-cancer effects pKAL by suppressing CD44, EGFR, and cyclin D1, and increasing DNA damages and intracellular vesicles that suggested autophagic cell death. In addition, the anticancer effects of tetraarsenic hexoxide on OXP-R-Hct 116 cells might be associated with cell survival related and ROS pathway, but not associated with downregulating Oxp-RR-Proteins of Oxp-R-HCT 116 cells.

#6767 Apurinic/apyrimidinic endonuclease 2 (APE2) mediates cisplatin ototoxicity by triggering MYH9-dependent mitochondrial toxicity in outer hair cells.

E. Irons[1], Q. Wang[2], W. Zhang[2], F. Zhao[1], M. Yin[2], B. Willard[2], Q. Zheng[1], B. Hu[3], J. Beach[4], R. Prayson[2], J. Yu[2], J. Lin[2], J. Zhao[2];
[1]Case Western Reserve University, Cleveland, OH, [2]Cleveland Clinic Foundation, Cleveland, OH, [3]University at Buffalo, Buffalo, NY, [4]Loyola University Chicago, Chicago, IL

Platinum compounds remain the backbone of chemotherapy regimens for a variety of solid tumors, despite their well-described adverse effects of nephrotoxicity, ototoxicity, and neurotoxicity. Cisplatin ototoxicity results from damage to the cochlear outer hair cells and remains a key dose-limiting toxicity in pediatric cancers, where its incidence reaches as high as 90% in testicular cancer patients. However, the mechanistic underpinnings of cisplatin ototoxicity remains incompletely understood and no treatments exist to prevent its onset. Here we describe a novel pathway mediating cisplatin-induced cell death in outer hair cells dependent on the base excision repair (BER) enzyme apurinic/apyrimidinic endonuclease 2 (APE2). APE2 was highly expressed in cochlear hair cells of patients with cisplatin ototoxicity compared to healthy controls. This was reproduced in vitro, where cisplatin rapidly upregulated APE2 expression and promoted its movement into the mitochondria within hours. APE2 suppressed the activation of ATR and p53 in response to cisplatin, instead promoting the mitochondrial translocation of p53 and intrinsic apoptosis. Knockdown of APE2 protected hair cells from cisplatin-induced apoptotic and non-apoptotic cell death in vitro. In a tamoxifen-induced Cre-lox transgenic mouse model, selective APE2 overexpression in cochlear outer hair cells resulted in loss of outer hair cells, disruption of cochlear architecture, and high-frequency hearing loss. Remarkably, this hearing loss was completely abrogated in mice that also harbored a knockout of MYH9, a non-muscle myosin that is mutated in rare genetic syndromes with hearing loss. We have observed MYH9 to be a binding partner of APE2 and now report that a specific interaction with the proximal coiled-coil domain of MYH9, adjacent to binding sites of its regulatory light chains, mediates this interaction. Consistent with the role of MYH9 in suppressing mitochondrial fission, we found that APE2 incapacitated oxidative phosphorylation and contributed to smaller, fragmented mitochondria. Our data demonstrate a novel mechanism in which the non-canonical DNA repair enzyme APE2 triggers cell death by inhibiting the DNA damage response and triggering MYH9-mediated mitochondrial damage, leading to the cochlear hair cell damage responsible for cisplatin ototoxicity. This work highlights a new therapeutic approach of targeting APE2 to mitigate the off-target toxicities associated with platinum medications.

#6768 Targeting the Her2 and ERα receptors has a significant impact on therapy-resistant ER-positive breast cancer.

DeZha Robinson[1], Hogyoung Kim[1], Alexandra Caroline Selico Dunn[1], Clark Jules Perkins[2], Sihyoung Kim[3], Sichan kim[3], Emily Lauren Schenk-Smith[1], Simak Ali[4], Matthew Burow[5], Emma Elkins[1], Christopher Williams[1]

[1]Division of Basic Pharmaceutical Science, Xavier University of Louisiana - College of Pharmacy, New Orleans, LA,[2]Biology, Xavier University of Louisiana College of Arts and Sciences, New Orleans, LA,[3]Xavier University of Louisiana College of Arts and Sciences, New Orleans, LA,[4]Department of Surgery and Cancer, Imperial College London, London, United Kingdom,[5]Medicine Hematology Medical Oncology, Tulane University School of Medicine, New Orleans, LA

Background: Resistance to endocrine therapy remains a significant clinical hurdle to the management of breast cancer. Mutations in the ESR1 gene (most frequently Y537S) are associated with metastatic disease recurrence in patients previously treated with endocrine therapy. We have shown that silmitasertib, a clinical phase CK2 inhibitor, can inhibit the proliferation of tamoxifen-sensitive and tamoxifen-resistant breast cancer cells in part by disrupting ERα/ERα variant expression. As such, we investigated the antiproliferative and antimigratory efficacy of silmitasertib in CRISPR-edited MCF-7 cells expressing ERαY5237S. Our findings suggest that pharmacological targeting of CK2 may represent an effective strategy in treating ERα-mutated, endocrine therapy-resistant BCa.
Methods: Using parental MCF-7 and MCF-7 ERαY537S cells, we assessed cell proliferation in the monolayer cultures, as well as 3D spheroid growth and wound healing activity using live cell imaging in the presence of silmitasertib. We measured the impact of CK2 inhibition on established mitogenic pathways by RT-PCR and/or immunoblot analysis (Her2, Cyclin D1, S6/pS6, S27, ERK/pERK). Subsequently, we assessed the efficacy of combined exposure to the HER2/EGFR inhibitor lapatinib with or without silmitasertib in both MCF-7 and MCF-7 Y537S cells.
Results: Our studies show that MCF-7 Y537S cells exhibit enhanced sensitivity to both antiproliferative and anti-migratory effects of silmitasertib compared to parental MCF-7 cells. HER2 was also overexpressed in MCF-7 Y537S, and the combination of lapatinib and silmitasertib, revealed an additive effect on cell proliferation.
Conclusion: Our studies suggest that the development of ERαY537S mutations in BCa results in enhanced sensitivity to growth inhibition elicited by CK2 blockade. Our studies provide a convincing rationale for the therapeutic targeting of CK2 in the context of ERα-mutated, endocrine-resistant breast cancer.

#6769 Zanubrutinib (zanu) overcomes BTK V416L resistance in B cell lymphoma models.

Haitao Wang[1], Xiaoyu Feng[2], Jinhong Ren[1], Lanyue Bai[2], Lin Li[1], Chao Cui[2], Lei Shi[2], Yang Li[1], Yangyang Wang[2], Jessica Li[3], Teiko Sumiyoshi[3], Yangbo Yue[2], Wei Jin[1], Zhirong Shen[2]

[1]BeiGene Ltd., Beijing, China,[2]BeiGene Ltd., Shanghai, China,[3]BeiGene Ltd., San Mateo, CA

Introduction: The second-generation BTK inhibitors (BTKis), e.g., zanu, acalabrutinib (acala), have further transformed the therapy landscape of chronic lymphocytic leukemia (CLL). However, acquired mutations in BTK (mainly at position C481) results in covalent BTKi resistance and disease progression. Non-covalent BTKi e.g., pirtobrutinib (pirto) can overcome C481 mutations, but is susceptible to other BTK mutations like the recently described kinase impaired V416L and L528W mutations, which both maintain downstream signaling. However, the impact of V416L on BTKi therapy remains not well characterized. Here, we investigated how V416L affects acala, zanu, and pirto response in B cancer cells.

Method: TMD8 BTK V416L cell lines were generated by CRISPR-Cas9 mediated gene editing. Kinase activity and phosphorylation of BTK Y223 were evaluated in V416L and wildtype (WT) TMD8 cells by Western blot. Structural modeling of human BTK was performed. Viability of TMD8 cells with V416L or WT was measured with CellTiter-Glo assay. Tumor growth inhibition (TGI) was measured in V416L and WT xenograft models. BTK occupancy in TMD8 cells was measured via ELISA with biotinylated probes.

Results: Western blot analysis demonstrates that V416L encodes a kinase with lower enzymatic activity compared to WT indicated by reduced phosphorylation of BTK Y223. However, BTK downstream signaling remains intact. Structural modeling predicts V416L leads to clashes in ATP binding, supporting impairment of kinase activity. TMD8 cells expressing V416L were more sensitive to zanu (IC50=0.8 nM) than to acala (IC50=144.4 nM) and pirto (IC50=2074.8 nM) in cell viability assays. Administration of a clinically relevant dose of zanu (20 mpk BID) resulted in significant regression of TMD8 V416L xenografts compared to vehicle ($p<0.0001$), while a clinically relevant dose of acala (4 mpk BID) did not achieve significant TGI. Neither a higher dose of acala (12 mpk BID) nor 50 mpk BID of pirto showed significant efficacy. Computational structural modeling predicts that V416L creates steric clashes with acala and pirto at the binding site, whereas zanu binding remains unaffected. Consistently, a BTK occupancy assay demonstrated that zanu, but not acala, can efficiently bind to V416L. Furthermore, western blot analysis shows efficient BTK downstream signal inhibition in V416L expressing TMD8 cells treated with zanu, whereas downstream signals are maintained in cells treated with acala and pirto.

Conclusion: These data demonstrate that zanu retains potent antitumor activity against TMD8 cells expressing BTK V416L, whereas acala and pirto may be attenuated by steric clashes. Thus, we hypothesize that clonal expansion of V416L is less likely to occur in patients with CLL treated with zanu, whereas it may lead to resistance in patients treated with acala or pirto. This hypothesis should be validated in clinical studies.

#6770 TFEB-dependent signals confer resistance against chemotherapeutic drug-induced nucleolar stress.

Marjorie Rolland, Florence McDuff, Laure Bessy, Benoit Marchand, **Marie-Josee Boucher**

University of Sherbrooke, Sherbrooke, QC, Canada

In addition to their main role in inhibiting cell growth, chemotherapeutic agents are also known to trigger protective signals that equip cancer cells with resistance mechanisms. Notably, treatment with chemotherapeutics has been associated with increased lysosome-related functions, such as autophagy, which protect cells from death. Our previous research showed that gemcitabine enhances autophagy and lysosomal function, particularly in HeLa and pancreatic cancer cells. However, the precise mechanisms through which this nucleoside analog elicits a protective autophagic response are still elusive. While gemcitabine is designed to interfere with DNA replication, it has also been noted to cause nucleolar stress. In our current study, we sought to determine if gemcitabine induces nucleolar stress and, if so, to examine whether this stress mimics the effects of gemcitabine by triggering a cytoprotective response. Our findings show that: 1) Gemcitabine induces nucleolar stress, indicated by the nucleoplasmic localization of nucleolin and nucleolar morphological changes. 2) Treatment of cells with the nucleolar stress inducer CX5461 leads to an autophagy and lysosomal response, monitored by increased LC3 and LAMP1 puncta, along with elevated levels of mature cathepsin B. 3) Additionally, CX5461-treated cells show an enriched nuclear localization of TFEB, the master regulator of autophagy and lysosomal function, detected by immunofluorescence and subcellular fractionation. The nuclear enrichment of TFEB correlates with the enrichment of faster migrating forms of TFEB (indicative of post-translational modification changes), as detected by immunoblotting. 4) Like gemcitabine, CX5461 impairs cell growth. 5) Nonetheless, clonogenic assays indicate that cells with reduced TFEB function (shTFEB) are more sensitive to CX5461 than control cells. 6) In parallel, shTFEB cells exhibit higher levels of apoptotic markers in response to CX5461 treatment, suggesting that TFEB confers resistance to nucleolar stress. Overall, our data provide the first evidence that nucleolar stress triggers a TFEB-dependent protective response. Conducted in cells without functional wild-type p53 signaling, our findings suggest that TFEB could act as a sensor of nucleolar stress, relaying a protective response and helping cells engage in timely and coordinated actions to cope with stress. Therefore, our results support the involvement of TFEB in chemoresistance. These TFEB-protective signals may be particularly relevant to cancers with high TFEB expression levels, such as pancreatic cancer.

#6772 Heterogeneity of *ex-vivo* tumor responses in primary ovarian cancer tissues.

Chiara Maestri, Ivan Trus, Rajeshwar Nitiyanandan, Jahanvi Kumar, Ricardo J. Parker, William Cance, **Christian Apfel**

SageMedic Corp., Redwood City, CA

Background: While most stage III and IV ovarian cancer patients respond to neo-adjuvant carboplatin and paclitaxel, not all do. Some experience recurrence within 6 months (platinum-resistant), others after 6 months, and a minority achieve remission beyond 5 years. Apart from BRCA mutations, genomic testing offers little guidance on patient-to-patient heterogeneity in tumor responses. To address this, we developed an internally validated functional profiling platform in a CLIA-certified and CMS accredited lab. Our goal was to measure in-vitro heterogeneity of tumor responses to NCCN-recommended treatments to identify the most suitable therapies for patients.

Methods: Ovarian cancer biopsies were shipped overnight and processed the same day using our proprietary functional profiling platform in a physiological culture system with around 2,000 viable cells per well. Tissues were exposed to up to 24 NCCN recommended therapies on the day after for 4 days, cytotoxicity readout with dose response curves within 7 days. Heterogeneity was assessed by the interquartile ratios of the EC50 drug concentrations. Not requiring organoid-like cell growth offers two advantages: a) actionable results for almost all patients and b) a fast turnaround time with an issued report within 10 days. Heterogeneity was evaluated using interquartile ratios of EC50 drug concentrations.

Results: A total of 17 ovarian cancer tissues were processed and exposed to up to 24 NCCN recommended therapies. Interquartile ratios (IQRs) of carboplatin and paclitaxel were 3.62 and 5.22 for the individual drugs, the IQR for the carboplatin/paclitaxel combination was 91.1, suggestive for synergy between both drugs in some, but not other cancer tissues. Gemcitabine's and cisplatin/gemcitabine IQRs were 39.8 and 17.9, respectively, suggestive that patient benefits to gemcitabine or platinum/gemcitabine combination may vary greatly. IQRs for doxorubicin and olaparib were only 5.8 and 3.2, owing to many tissues not showing any tumor response. All p-value comparisons for EC EC50s heterogeneity of tumor responses were <0.001.

Conclusions: Consistent with the clinically known patient-to-patient heterogeneity of tumor responses, such heterogeneities were also observed in our *in-vitro* tissue culture platform. Thus, these results warrant external validation of this already internally validated laboratory developed test (LDT).

#6776 Biological characterization of small molecule eIF4E inhibitors to assess their therapeutic potential in cancer.

Rachel L. O'Rourke[1], Emilio L. Cardenas[1], Jesse Duque[2], Amanda Falzetta[3], Jim Clauwaert[3], Jason Rech[4], John R. Prensner[3], Amanda L. Garner[1]

[1]Medicinal Chemistry, University of Michigan, Ann Arbor, MI,[2]Michigan Center for Therapeutic Innovation, University of Michigan, Ann Arbor, MI,[3]Pediatrics and Biological Chemistry, University of Michigan, Ann Arbor, MI,[4]Medicinal Chemistry, Vahlteich Medicinal Chemistry Core, University of Michigan, Ann Arbor, MI

The dysregulation of translation is a hallmark of cancer that enables rapid changes in the cell proteome to shape oncogenic phenotypes that promote tumor survival. The predominant signaling pathways leading to dysregulation of translational control in cancer are the PI3K-AKT-mTORC1, RAS-RAF-MAPK, and MYC pathways, which all converge on eukaryotic translation initiation factor 4E (eIF4E), an RNA-binding protein which binds to the m^7GpppX cap structure at the 5' end of mRNAs to initiate cap-dependent translation. eIF4E is the rate-limiting factor of translation initiation, and its overexpression is known to drive oncogenic transformation, progression, and chemoresistance across many cancers, establishing it as an attractive therapeutic target. Traditionally, target transcripts of eIF4E have been characterized as mRNAs with long 5'-UTR motifs, but recent studies point to context-dependent translation of eIF4E-sensitive transcripts that is contingent on the regulation status of eIF4E. Therefore, we hypothesize that mechanistically distinct eIF4E inhibitors will elicit different phenotypic responses in tumors based on the dysregulation of select pools of eIF4E-sensitive transcripts. Research in our laboratory focuses on developing mechanistically distinct eIF4E inhibitors, including m^7GpppX prodrug derivatives which bind directly to the cap-binding pocket of eIF4E. Efforts to characterize the effects of these molecules on cap-dependent translation regulation through cell-based phenotypic assays and ribosome profiling will be discussed.

#6777 Characterization of the dual MCT1 and MCT4 inhibitor MCT-IN-1 in MOC1 cell line demonstrates increased apoptosis and compensatory upregulation of glutathione conjugation gene sets.

E. V. Mastrolonardo, V. Diaz Barros, Z. Lin, **H. Samarah**, J. Jackson, A. J. Luginbuhl, A. Linnenbach, U. Martinez-Outschoorn, J. M. Curry;
Thomas Jefferson University, Sidney Kimmel Cancer Center, Philadelphia, PA

Background: Head and neck squamous cell carcinoma, like many solid tumors, upregulates glycolysis regardless of O2 availability via the Warburg effect, which results in accumulation of intracellular lactate. To avoid apoptosis from the resultant decreased pH, these cells export lactate via monocarboxylate transporters (MCT), primarily MCT1 and MCT4, with overexpression of MCT1 and MCT4 having been linked to poor prognosis.
Methods: Mouse Oral Cancer (MOC1) cells were treated with MCT-IN-1, a dual MCT1 and MCT4 inhibitor. Flow cytometry was utilized to characterize apoptosis and S-phase of the cell cycle. Bulk RNAseq data were obtained from both MCT-IN-1-treated and untreated MOC1 cells and analyzed with unbiased gene set enrichment analysis (GSEA). Leading edges of enrichment were analyzed by using the STRING database of protein-protein interactions.
Results: Treatment of MOC1 cells with MCT-IN-1 resulted in decreased tumor cell count ($p<0.01$), increased apoptosis ($p<0.01$), and reduced S-phase of the cell cycle ($p<0.01$) compared to untreated cells. Unbiased GSEA of bulk RNAseq data, contrasting MCT-IN-1 treated versus untreated cells, revealed upregulation of endoplasmic reticulum (ER) stress (NES 1.79, FDR=0.012), suggesting possible mechanism of cell death. In addition, the glutathione conjugation gene set was found to be upregulated (NES 1.75, FDR=0.016), suggesting a possible compensatory response mechanism. Further, GSEA revealed significant enrichment of the oxidative stress and redox pathway (NES 1.74, FDR=0.016), including glutathione transferase and oxidoreductase activities. STRING analysis of upregulations in gene expression revealed a dense protein-protein interaction network, suggesting coordinated roles in detoxification (FDR = 1.00e-16) and cellular stress response mechanisms (FDR = 2.61e-41) in MOC1 cells treated with MCT-IN-1.
Conclusions: We report the first characterization of the dual MCT1 and MCT4 inhibitor MCT-IN-1. Treatment of MOC1 cells in vitro with MCT-IN-1 induces tumor cell apoptosis possibly via upregulation of ER stress and further demonstrates upregulation of glutathione conjugation gene sets, suggesting a possible compensatory mechanism. Validation with in vivo studies and further evaluation of the exact mechanism of these findings are required.

#6778 Targeting the YAP-TEAD interaction in brain metastatic breast cancer cells.

Caitlyn C. Castillo, Mohamed Haloul, Philana Phan, Zongmin Zhao, Ekrem E. Er

University of Illinois at Chicago, Chicago, IL

Breast cancer metastasis to the brain is a devastating stage of breast cancer that presents with cognitive decline and seizures, and currently there are no treatments available. Brain metastases are resistant to immunotherapy, chemotherapy, and targeted therapies. This is partly because the blood-brain barrier and the blood-tumor barriers prevent effective delivery of these treatment modalities at clinically safe doses and partly because we do not know enough about the molecular mechanisms that govern breast cancer brain metastasis. Recent work in mouse models of breast cancer brain metastases revealed that the interaction between Yes Associated Protein (YAP) and Transcriptional Enhanced Association Domain (TEAD) family of transcription factors is an essential step in outgrowth of disseminated cancer cells. Here, we demonstrate a series of time-lapse imaging assays and luminescence based viability assays that we optimized to investigate the impact of YAP-TEAD inhibitors and chemotherapeutic agents on the viability of brain metastatic breast cancer cells *in vitro*. Our results will guide the prioritization of these treatment modalities in pre-clinical *in vivo* models. We will specifically investigate whether one or more of these agents can be loaded onto nanoparticle-engineered macrophages to better overcome blood-brain and blood-tumor barriers for effective delivery and targeted destruction of brain metastases.

#6779 The plant derived compound Simalikalactone D is a potent anticancer agent in triple negative breast cancer cells.

Joshua Nieves[1], Annelis Sanchez[1], Claudia Ospina[2], Pablo Vivas-Mejia[1]

[1]Biochemistry, University of Puerto Rico - Medical Sciences Campus, San Juan, Puerto Rico,[2]Natural Sciences and Mathematics, Interamerican University of Puerto Rico - Bayamon Campus, Bayamon, Puerto Rico

Despite recent advances in systemic therapy, Triple Negative Breast Cancer (TNBC)—accounting for 10-20% of breast cancer cases—remains the subtype with the worst prognosis, marked by a high risk of distant recurrence and death; thus, novel treatments are urgently needed for this deadly disease. Simalikalactone D (SKD) is a potential anticancer compound from *Simarouba tulae*, an endemic plant of Puerto Rico. We observed that the TNBC cells, MDA-MB 231 and MDA-MB 468 respond differently when treated with SKD. Thus, it is critically important to investigate the mechanism of action of SKD in TNBC cells. This study involved a comparative analysis in MDA-MB 231 and MDA-MB 468 cells, and their differential response to treatment with SKD. We performed Tandem Mass Tag (TMT)- based quantitative proteomics, followed by Ingenuity Pathway Analysis (IPA) protein expression analysis to identify distinctly active networks and signaling pathways along with different cellular behaviors between the TNBC cell lines.In the MDA-MB 468 cell line, SKD appeared to play a role in inducing apoptosis through Caspase 3 activation, with apparent PD-1/PD-L1 signaling in Death Receptor pathways. This correlates with reduced cell migration and adhesion in this cell line, which could suggest an apoptotic-mediated loss of integrity. Alternatively, the MDA-MB 231 cells did not show Caspase 3 activation, instead they exhibited an activation of autophagy pathways, including Macroautophagy and Late Endosomal Microautophagy (LEMA). After treatment of cells with SKD, LEMA appeared to act as a compensatory mechanism of survival when Autophagy was impaired. This emphasizes the role of cellular stress responses for maintaining cell viability under SKD treatment.This study highlights the influence of genetic and molecular differences on TNBC cell responses to SKD, reinforcing the clinical importance of personalized medicine. Therapeutic strategies using the genetic and molecular profiles of a patient's tumor could enhance treatment efficacy and improve outcomes, particularly for aggressive cancers like TNBC.

#6780 Discovering novel inhibitors of the chaperone Hsp90 for cancer treatment: A plant-based approach..

Chidera P. Ogbu, Taoufik Llbiyi, Vamsi Krishna Kommalapati, Ahmed Chadli

Augusta University, Augusta, GA

We investigated the effect of small molecules extracted from the latex of Calotropis procera (CPPE) to act as inhibitors of Heat Shock Protein 90 (HSP90), a key molecular chaperone co-opted by cancer cells for survival and proliferation. Current HSP90 inhibitors often activate the heat shock response, reducing their long-term efficacy. While CPPE has been shown to have toxic effects on cancer cells, its exact mechanism of action, including its potential to inhibit HSP90, has yet to be evaluated. Using flow cytometry, gene expression and protein quantification analysis, and mass spectroscopy techniques, we evaluated the effects of CCPE small molecules on triple-negative breast cancer cells (TNBCs) compared to 17-AAG, a known HSP90 inhibitor. We observed a dose-dependent increase in cell death with graded doses of CPPE compared to 17-AAG. Additionally, our findings from the MTT assay and flow cytometry demonstrated that treatment with CPPE led to a decrease in cell viability and the initiation of cell death pathways such as apoptosis and necrosis. Notably, western blot analysis suggests the absence of heat shock responses in cells treated with CPPE compared to 17-AAG. These findings support our hypothesis, indicating that small molecules present in CPPE show promise as candidates for HSP90 inhibitors. Further investigations to gain deeper insights into CPPE mechanisms as well as characterizing its bioactive compounds are ongoing. This research highlights the importance of natural product-based drug discovery in cancer treatment

#6781 Effect of Qingre Lishi Huoxue prescription on pyroptosis and the expression of related proteins caspase-3/GSDME in esophageal cancer cells.

Fuchun Si[1], Gao Si[2]

[1]Hunan University of Chinese Medicine, Henan, China,[2]Peking University Third Hospital, Beijing, China

Introduction: Esophageal carcinoma (EC) is one of the most common malignant tumors posing a threat to human's health, with a high disease incidence and the highest death rate in China. This study aims to screen out the Chinese herbs with strong inhibitory effects on the proliferation of four kinds of EC cells EC9706, EC-1, TE-1 and Eca109 from 300 Chinese herbs, and study the effect of Qingre Lishi Huoxue Prescription(QRLSHXP) on the proliferation and on pyroptosis-related proteins such as Caspase-3 and GSDME of four EC cells.
Methods: The MTT assay was used to assess the effects of 300 Chinese herbs alcohol extracts on the proliferation of the four esophageal cancer cell lines. Based on efficacy and safety profiles, selected herbs were combined to formulate a prescription. Dose-response relationships were determined. Cell morphology changes were observed under an inverted microscope. DNA damage was assessed using TUNEL staining. Lactate dehydrogenase (LDH) release was measured to evaluate cell membrane integrity. Cell death was analyzed using Annexin V/PI staining and flow cytometry. Western blotting and ELISA were performed to detect the expression levels of pyroptosis-related proteins and cytokines.
Results: Eight herbs significantly inhibited all four esophageal cancer cell lines in the initial screening. Toxic herbs were excluded. Four herbs—Sanye Danshen, Jiuguanxue, Shanchagen, and Huaxuedan—were chosen to create the Qingre Lishi Huoxue Prescription (QRLSHXP). This prescription inhibited the proliferation of the cancer cells in a dose- and time-dependent manner.QRLSHXP treatment induced severe cellular swelling, rupture, and content leakage in EC cells. TUNEL staining revealed increased DNA double-strand breaks, indicating enhanced DNA damage. Elevated LDH levels in supernatants suggested compromised membrane integrity. Flow cytometry showed a significant rise in Annexin V/PI double-positive cells, confirming cell death. Western blot analysis indicated increased Cleaved-Caspase-3 and GSDME-N expression, with unchanged total GSDME levels. ELISA results demonstrated significantly higher IL-1β and IL-18 levels in treated groups.
Conclusion: QRLSHXP effectively inhibited the proliferation of EC cells and induced pyroptosis, which was likely associated with the activation of the Caspase-3/GSDME pathway.

#6782 ACADS as a novel target of selinexor activity in diffuse large B cell lymphoma.

K. L. Trkulja[1], W. Tong[1], D. V. Tran[1], L. Meng[1], E. Teh[2], S. Penny[2], D. M. Pinto[2], A. Keating[1], J. Kuruvilla[1], R. C. Laister[1];
[1]UHN Princess Margaret Cancer Centre, Toronto, ON, Canada, [2]National Research Council, Halifax, NS, Canada

Background: Diffuse large B cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma (NHL), with poor outcomes in the relapsed/refractory (R/R) setting. Recent years have identified nuclear export protein Exportin-1 (XPO1) as a negative prognostic factor in several cancer types such as DLBCL. Selinexor is an orally available inhibitor of XPO1 with activity in R/R DLBCL, but knowledge gaps in understanding how XPO1 drives DLBCL are preventing the drug's effective use in the clinic. Here, we analyzed selinexor's effects on DLBCL metabolism and conducted a mass spectrometry analysis of mitochondrial proteins to determine which proteins and pathways relevant to bioenergetics are modulated by selinexor in DLBCL.
Methods: DLBCL cell lines (HBL-1, OCI-Ly2, OCI-Ly8) were treated with selinexor for 24 hours and an XFe96 bioanalyzer was used to measure changes in mitochondrial and glycolytic ATP production. To identify factors driving changes in cellular metabolism, mitochondria were purified from DLBCL cell lines with and without treatment with selinexor. The mitochondrial preparations were digested with proteases and the resulting peptides were identified by mass spectrometry (MS). The sequences of metabolic proteins of interest were analyzed using the LocNES nuclear export sequence identifier (Xu et al., 2014) to determine which of these proteins might be canonical XPO1 cargo molecules. Additional validation of potential XPO1 cargo was performed via nuclear-cytoplasmic fractionation.
Results: Selinexor significantly reduced both glycolytic and mitochondrial ATP production in all cell lines, as measured by extracellular acidification rate and oxygen consumption rate. MS analysis identified 4224 proteins, of which 92 showed a statistically significant change in mitochondrial abundance with selinexor treatment; 61 of these were downregulated, while 31 were upregulated. Several metabolic proteins were affected by selinexor treatment, most notably short-chain acyl-CoA dehydrogenase (ACADS), which catalyzes lipid metabolism via short-chain fatty acid oxidation. ACADS showed a decreased mitochondrial abundance in response to selinexor treatment, suggesting a mechanism by which the drug exerts its metabolic effects via the inhibition of short-chain fatty acid oxidation. Upon further analysis, the primary sequence of the protein was predicted to contain a nuclear export sequence, and subcellular fractionation analysis showed nuclear accumulation of ACADS following selinexor treatment, identifying it as a potential novel XPO1 cargo molecule in DLBCL.
Conclusion: Selinexor inhibits glycolytic and mitochondrial metabolism in DLBCL, in part by blocking the nuclear export of enzymes involved in metabolism such as ACADS, which catalyzes short-chain fatty acid oxidation. Future work will further validate these targets of interest to reveal how selinexor exerts its cytotoxic effects in DLBCL.

#6783 ATM inhibitor AZD1390 sensitizes nasopharyngeal cancer cells to chemotherapy by reversing chemotherapy-induced autophagy.

Syrus Pak Hei Lai, Ngar Woon Kam, Victor Ho-Fun Lee

Department of Clinical Oncology, The University of Hong Kong, Hong Kong, Hong Kong

Background: Nasopharyngeal carcinoma (NPC) is renowned for its high sensitivity towards chemotherapeutic agents. Nonetheless, chemoresistance remains a major reason for treatment failure in NPC. Ataxia telangiectasia mutated (ATM) has a diverse role in cellular homeostasis, regulating DNA damage response, apoptosis and autophagy. Pharmacological inhibition of ATM is hypothesized to disrupt the DNA damage response towards chemotherapy-induced DNA breaks, which in turn enhances the chemosensitivity of tumor cells. However, given the multifaceted role of autophagy in various cell death mechanisms, the interplay between autophagy and DNA damage response remains elusive. Therefore, we aim to investigate the therapeutical potential of ATM inhibitor AZD1390 in NPC via the regulation of autophagy.
Methods: Antitumor effects of AZD1390 in conjunction with cisplatin were evaluated on two NPC cell lines, NPC43 and C17. Cell lines were pretreated with AZD1390 for 2 hours prior to the administration of cisplatin for 72 hours. We assessed the anti-proliferative effects through annexin V/propidium iodide, CellTiter-Glo, XTT, clonogenic assay and cell cycle analysis. The mechanism of action and autophagic activity upon treatment of AZD1390 were examined by means of a combination of mass spectrometry, western blot, autophagy detection kit and immunofluorescence approaches.
Results: Cisplatin treatment with concurrent AZD1390 pretreatment enhanced the anti-proliferative effect in NPC cell lines compared to cisplatin alone. XTT, cell cycle analysis and colony formation assay revealed a reduced tumor cell clonogenicity and proliferation with an accumulation of cell population in the G0/G1 phase upon the combined treatment. However, no significant changes were observed in terms of apoptosis and necrosis. We observed that cisplatin treatment alone induced autophagy, whereas, in conjunction with AZD1390 pretreatment, the cisplatin-induced autophagy was reversed, indicated by the reduced autophagic activity detected by autophagy detection kit and the increased colocalization of LC-3 and lysosome in immunofluorescent staining. These findings were further supported by our mass spectrometry data that the mTOR signaling pathway, indicative of autophagy inhibition, was significantly enriched in cells treated with AZD1390 in combination with cisplatin as compared to cisplatin alone.
Conclusions: Our findings demonstrated that AZD1390 augments the sensitivity of NPC cell lines to chemotherapy through the reversion of chemotherapy-induced autophagy. Therefore, AZD1390 could potentially serve as a chemosensitizer in NPC treatment.

#6784 Effect of novel gold(I) compounds on cell proliferation and signaling protein expression in MDA-MB-231 breast cancer cells.

Alexis Pawlak, Talitha T. Rajah

DePaul University, Chicago, IL

Gold(I) compounds have been gaining prominence in research as alternative metallo-chemotherapeutic strategies against chemo-resistant tumors. However, the cellular and molecular mechanisms by which gold(I) compounds disrupt cellular processes critical to breast cancer cell proliferation are largely unknown. Our study evaluated the effect of eight novel gold(I) chloride compounds (Table 1) on MDA-MB-231 breast cancer cell proliferation and their mechanistic action on signaling proteins (Bax, Bcl_2, pERK1/2). Cell proliferation was assessed by the MTT assay for 24 to 72 hours, and protein expression was analyzed by western blotting and immunodetection after exposure to gold(I) chloride compounds at their respective IC_{50} concentrations. Our results indicate that except for PEt_3AuCl, all compounds inhibited cell proliferation across all exposure times. PCy_3AuCl, $PiPr_3AuCl$, PPh_3AuCl and PMe_3AuCl exhibited the greatest efficacy at low micromolar doses. PPh_3AuCl (2-fold) and PBu_3AuCl (2.5-fold) displayed high Bax/Bcl_2 ratios suggesting proapoptotic effects. All other compounds showed either decreased or unaltered Bax/Bcl_2 ratios. Each compound inhibited pERK1/2, with $PiPr_3AuCl$ (70%), PMe_3AuCl (64%), and PCy_3AuCl (52%) showing the greatest inhibition. Overall, our results suggest PCy_3AuCl, $PiPr_3AuCl$, and PMe_3AuCl inhibited cell proliferation at low micromolar doses most effectively in MDA-MB-231 cells and the cellular mechanism of action likely varies by compound. These could be through altered status of the cell proliferative pERK1/2 protein and/or the apoptotic proteins Bax or Bcl_2 in this cell line. This study provides new insights into the cellular mechanism by which these gold(I) compounds may exert their effects in this triple negative human breast cancer cell line and contributes to the growing evidence supporting the potential of gold(I) compounds in cancer therapeutics.

Table 1. Effect of Gold(I) Compounds on Cell Proliferation

Gold(I) Compound Name	**Concentration Range Tested (µM)**	**48 Hour IC_{50} (µM)**	**Proliferation Inhibition (48 hours) (%)**
PMe_3AuCl	0.1 - 7.5	0.94 ± 0.19	15.8 - 88.4
PEt_3AuCl	1 - 300	-	0 - 1.9
$PiPr_3AuCl$	0.1 - 7.5	0.56 ± 0.36	7 - 99.2
PBu_3AuCl	0.01 - 2.5	6.3 ± 0.39	0 - 45.7
$PtBu_3AuCl$	0.1 - 7.5	2.3 ± 1.2	0 - 98.6
(PTA)AuCl	1 - 100	69 ± 0.78	0 - 68.3
PCy_3AuCl	0.1 - 7.5	0.37 ± 0.21	16.3 - 98.6
PPh_3AuCl	1 - 15	1.78 ± 0.40	15.4 - 99.5

#6785 MicroRNA-lincrna cross-talk in BRCA1-mutated breast cancer and its therapeutic implications.

Olalekan Fadebi, Zodwa Dlamini, **Rahaba Makgotso Marima**

University of Pretoria, Pretoria, South Africa

Breast cancer remains one of the leading causes of cancer-related mortality, characterized by its complexity and resistance to therapy. Triple-negative breast cancer (TNBC), a particularly aggressive subtype, lacks targeted therapies, making it challenging to treat. Long intergenic non-coding RNAs (lincRNAs) have emerged as critical regulators in cancer biology, with lincRNA-BC7 identified as a potential modulator of BRCA1. However, the exact mechanisms through which lincRNA-BC7 interacts with BRCA1. This study investigated the functional role of lincRNA-BC7 in regulating BRCA1 activity and its contribution to the biological response of TNBC cells to Olaparib. Using in breast cancer *in vitro* models. The cell biological response of TNBC cell line (MDA-MB-231) to Olaparib compared to the control group was evaluated. Gene expression analyses by RT-qPCR was done using LincRNA-BC-7 and BRCA1 specific primers. Furthermore, *in silico* bioinformatics analysis to map the molecular pathways involved was employed. This study revealed that miR-663a (has-miR-663a) could act as a sponge for lincRNA-BC7, thereby influencing its stability and expression levels. This interaction leads to alterations in the expression of target genes involved in key cancer hallmarks such as epithelial-mesenchymal transition (EMT), invasion, and metastasis. The competitive endogenous RNA (ceRNA) networks provide a framework for understanding how these non-coding RNAs interact at the post-transcriptional level, potentially affecting the progression of BRCA1-related breast cancer. Notably, both LincRNA-BC7 and BRCA1 gene expression levels were downregulated in Olaparib treated TNBC cells. Understanding the interplay between miR-663a and lincRNA-BC7and how this ceRNA network affects BRCA1 mutated TNBC may open avenues for novel therapeutic interventions.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Drug Resistance in Molecular Targeted Therapies 5
Poster Session

#6789 Identification of DGKA as a regulatory factor for paclitaxel resistance in lung cancer based on CRISPR screen and transcriptome sequencing.

Jiang Luo, Mini Zhang, Jiawen Tang, Qingwei Wang, Yu Yuan, Min Xia, Yufei Yan, Yushuang Tan, Conghua Xie, **Yan Gong**

Zhongnan Hospital of Wuhan University, WuHan, China

Background: Lung cancer is the second most prevalent cancer with the highest mortality rate in the world. Paclitaxel (PTX) is one of the most effective clinical chemotherapeutic agents for the treatment of lung cancer, but a variety of mechanisms induce its chemoresistance including alterations in microtubule-associated proteins, up-regulation of drug efflux systems. Lipid metabolism was reported to impact chemosensitivity, but the underlying mechanisms are to be clarified. Therefore, this study aims to exploring the molecular mechanisms of PTX resistance regulated by lipid metabolism, and to discover novel PTX resistant targets and therapeutic agents.
Methods: Lipid metabolism related sgRNA library was used for CRISPR screen. PTX-resistant lung cancer cells were constructed for transcriptome sequencing. The key regulators were identified via integrating negative enriched genes in CRISPR screen and the differential ones in transcriptome sequencing. The regulation of PTX sensitivity by the key regulators was confirmed in deficient lung cancer cells. Transcriptome sequencing was applied to find downstream pathways, and immunoblotting and RT-qPCR were used to confirm the molecular mechanisms. Mouse models were used to investigate the safety and effectiveness of its inhibitors combined with PTX for lung cancer treatments in vivo.
Results: DGKA is highly expressed in PTX-resistant lung cancer cells, and its deficiency significantly induced PTX sensitivity in lung cancer cells. DGKA knockdown significantly inhibited lung cancer cell proliferation, invasion and migration. In addition, DGKA depletion promoted PYX-induced cell apoptosis and G2/M-phase arrest in lung cancer cells. Transcriptomics showed that the MAPK signaling pathway was significantly enriched after DGKA knockdown, and further experiments confirmed that DGKA inhibition increased lung cancer cell sensitivity to PTX via inhibiting ERK phosphorylation. DGKA inhibitor (R59022) could induce the PTX sensitivity of lung cancer cells safely and effectively *in vivo*.
Conclusion: DGKA promotes PTX sensitivity of lung cancer cells and alleviates PTX resistance through the MAPK pathway, providing preclinical evidence for targeting DGKA as a novel strategy for lung cancer therapy against PTX resistance.

#6790 Exploring sensitivity and resistance to proteasome inhibitors in *TP53* mutated non-small cell lung carcinoma.

Victoria Neely[1], Kranthi Kumar Chougoni[2], Bin Hu[3], Jennifer E. Koblinski[4], Guangrong Zheng[5], Daohong Zhou[6], Swati Palit Deb[7], Sumitra Deb[7], Jorge J. Nieva[2], Senthil K. Radhakrishnan[4], Steven R. Grossman[2], Hisashi Harada[8]

[1]Philips Institute for Oral Health Research, School of Dentistry, Virginia Commonwealth University, Richmond, VA,[2]Department of Medicine, Keck School of Medicine of USC, USC Norris Comprehensive Cancer Center, University of Southern California, Los Angles, CA,[3]Massey Comprehensive Cancer Center, Virginia Commonwealth University, Richmond, VA,[4]Department of Pathology, Massey Comprehensive Cancer Center, Virginia Commonwealth University, Richmond, VA,[5]Department of Medicinal Chemistry, College of Pharmacy, University of Florida, Gainesville, FL,[6]Department of Biochemistry and Structural Biology and Center for Innovative Drug Discovery, Long School of Medicine, University of Texas Health Science Center at San Antonio, San Antonio, TX,[7]Department of Biochemistry & Molecular Biology, Massey Comprehensive Cancer Center, Virginia Commonwealth University, School of Meicine, Richmond, VA,[8]Philips Institute for Oral Health Research, School of Dentistry, Massey Comprehensive Cancer Center, Virginia Commonwealth University, Richmond, VA

Background: Non-small cell lung cancer (NSCLC) is the most common histologic subtype of lung cancer (~80%), with ~50% of tumors carrying a missense mutation in the *TP53* tumor suppressor gene (Onc-p53). These mutants often lead to the acquisition of oncogenic traits such as tumorigenesis and chemoresistance, as well as vulnerabilities that can be targeted for treatment, one of which being elevated basal levels of proteotoxic stress. We have previously shown that NSCLC cells expressing Onc-p53 exhibit elevated 20S proteasome activity which serves as a vulnerability for proteasome inhibitors (PIs) to further augment the proteotoxic stress levels, leading to the accumulation of reactive oxygen species (ROS). However, despite initial effectiveness, these Onc-p53 NSCLC cells develop resistance to PIs with the underlying mechanisms of this resistance remaining largely unknown.

Methods and Results: We have elucidated that bortezomib (BTZ)-induced ROS initiates a cascade including the activation and nuclear translocation of NRF2, followed by the induction of ATF3 and NOXA, and ultimately apoptosis. Furthermore, the combination of BTZ with the dual BCL-X_L/BCL-2 targeting BH3-mimetic compound (ABT-263) or a novel BH3-mimetic PROTAC (753B) synergizes with carboplatin, a standard of care chemotherapy, to selectively kill Onc-p53 NSCLC cells *in vitro* and *in vivo*. A second-generation PROTAC (WH244) also shows efficacy *in vitro*. We now show that the inductions of NRF2, ATF3, NOXA, and apoptosis are strongly suppressed in BTZ-resistant Onc-p53 NSCLC cells, generated by continuous culture in suboptimal doses of BTZ up to double its IC_{50}. Interestingly, the combination of BH3-mimetics with BTZ showed cytotoxicity even in BTZ-resistant cells, suggesting that higher doses of BTZ can be used to overcome BTZ-resistance. Bulk-RNA sequencing of BTZ-sensitive vs. BTZ-resistant cells further identified the induction of PD-L1. This induction was abrogated by BTZ-resistant cells, suggesting that immune checkpoint activation may be playing a role in the resistance to PIs observed in previous clinical settings.

Conclusion: In summary, our findings show that resistance to PIs such as BTZ in NSCLC tumors expressing Onc-p53 involves adaptations that reduce proteotoxic stress and may leverage immune checkpoint pathways. However, combining PIs with BH3-mimetics effectively restores cytotoxicity, even in BTZ-resistant cells. This suggests that targeting anti-apoptotic pathways alongside traditional therapies (e.g. the combination of BTZ, carboplatin, with either ABT-263 or 753B/WH244) could offer a powerful approach to overcome PI resistance in NSCLC, supporting the potential for PI-based precision combination therapies in NSCLC patients.

#6791 Evaluation of apoptotic and immune mediators in responsecurrent chemotherapeutic agents using murine breast cancer cell lines: The role of HSP70 and S100A9.

F. Ozcan[1], H. Alkan[2], F. Koksalar Alkan[2], A. Caglayan[2], S. Ozdatli Kurtulus[3], C. Jones[2], H. Korkaya[2];
[1]Faculty of Science, Istanbul University, Istanbul, Turkey, [2]Karmanos Cancer Institute, Wayne State University, Detroit, MI, [3]University of Health Science of Turkey, Istanbul, Turkey

Doxorubicin, Cytoxan and Docetaxel are commonly used chemotherapeutic agents in breast cancer treatment, primarily inducing cell death by different mechanisms. Combining these with novel compounds may enhance efficacy and reduce resistance. 4-HCP, active metabolite of Cytoxan has shown potential as an anticancer agent, and HSP70 and S100A9 are linked to cancer progression, immune response, and apoptosis. JG-231 is a small-molecule inhibitor targeting HSP70, disrupting its chaperone activity and inducing apoptosis, particularly in cancer cells. This study explores the effects of Doxorubicin, Docetaxel, and 4-HCP, combined with JG-231, on apoptosis and immune responses in EMT6 and 4T1 metastatic breast cancer cell lines, focusing on HSP70 and S100A9. Effective doses were determined by MTT assays based on dose-response curves. EMT6 and 4T1 cells were treated with Doxorubicin, Docetaxel, 4-HCP and JG-231, combined with JG-231 for 24 hours, and apoptotic/immune responses were analyzed through gene expression (Caspase-3, Caspase-7, Bax, Bcl-2, myc, Stat-1, TNF, HSP70, S100A9) using RT-PCR. Protein levels were validated by Western Blot, apoptotic cell death and changes in cancer stem cell populations by flow cytometry.Treatment with Doxorubicin, Docetaxel, and 4-HCP, combined with JG-231 significantly induced apoptosis in EMT6 and 4T1 cells. Increased Caspase-3 and Caspase-7 expression and a higher Bax/Bcl-2 ratio confirmed activation of apoptosis. Annexin V-positive cells were higher in treated groups, validating apoptosis induction. Furthermore, the cancer stem cell population was reduced, especially in the 4-HCP-treated group. HSP70 and S100A9 expression levels increased after treatment, highlighting their roles in stress response and immune modulation. These treatments elevated Stat-1, Caspase-3, Caspase-7, TNF, HSPA1A, HSPA1B, HSPA2 and S100A9, demonstrating a broader effect on apoptosis and immune response. While increased levels of Caspase indicate programmed cell death, higher Stat-1 and TNF levels may suggest immune mediated cytoprotection. These effect when combined with the elevated levels of stress-induced HSP1A/B, HSPA2 and inflammatory S100A9 correlates with inflammation and immune modulation. Together our findings may provide some evidence for stress-induced pathways and immune mediated cytoprotection in response commonly used chemotherapeutics in breast cancer and thus combining molecularly targeted therapeutics like JG -231 with standard of care may be more effective. This study was supported by the Scientific and Technological Research Council of TURKEY (TUBİTAK) Project no: 1919B022410990; 1059B192301226.

#6792 Understanding and enhancing cancer persister cell ferroptosis sensitivity.

Anna E. Stuhlfire, Masayoshi Higuchi, August F. Williams, Ariel H. Nguyen, David A. G. Gervasio, Claire E. Turkal, Suejean Chon, Matthew J. Hangauer

Department of Dermatology and Moores Cancer Center, UC San Diego, San Diego, CA

Cancer persister cells are a diverse subset of tumor cells that survive cytotoxic targeted therapy and contribute to tumor relapse. Identifying and exploiting persister cell vulnerabilities is a promising strategy to prevent acquired drug resistance. We previously showed inhibition of the antioxidant GPX4 selectively induces persister cell ferroptotic death.[1] However, the mechanism for persister cell sensitivity to ferroptosis is poorly understood. First, to address whether low cell density underlies persister cell ferroptosis sensitivity,[2] we generated high density persister cells using prolonged cytostatic drug treatments and found chronic drug stress induces ferroptosis sensitivity independent of cell density. We also performed scRNAseq analysis of persister cells treated with GPX4 inhibitor RSL3 and observed downregulation of oxidative phosphorylation (OXPHOS) genes and upregulation of antioxidant NRF2 target genes among persister cells which survive RSL3 treatment, indicating that OXPHOS and ROS are critical for persister cell ferroptosis sensitivity. Consistent with this, we found that inhibition of OXPHOS with metformin protects persister cells from ferroptosis. Therefore, persister cells depend on OXPHOS for survival[3] yet OXPHOS sensitizes persister cells to ferroptosis. We also explored how disulfiram, which has a wide variety of reported mechanisms of action, selectively kills persister cells.[4] We found that disulfiram-induced persister cell killing cannot be rescued by anti-ferroptosis antioxidants such as ferrostatin, but can be rescued with other antioxidants, indicating that persister cells are vulnerable to additional forms of oxidative death beyond ferroptosis. We then sought to identify additional drugs which could sensitize persister cells to ferroptosis and found that inhibition of the oxidoreductase FSP1, which serves as a backup anti-ferroptosis mechanism to GPX4, enhances GPX4 inhibitor-induced persister cell death. We also found that transient, nontoxic pretreatment with HDAC inhibitors sensitizes persister cells to subsequent single agent GPX4 inhibitor treatment in part by increasing persister cell ROS levels. These findings provide additional mechanistic understanding of the disabled persister cell antioxidant state and reveal therapeutic strategies which may be used to selectively induce ferroptosis in persister cells and potentially spare normal tissue.

1. Hangauer, M. J. *et al. Nature* **551**, 247–250 (2017).2. Wu, J. *et al. Nature* **572**, 402–406 (2019).3. Shen, S. *et al. Cell Rep.* **33**, 108421 (2020).4. Raha, D. *et al. Cancer Res.* **74**, 3579–3590 (2014).

#6793 Characterizing glucose transport at incremental stages of chemoresistance in melanoma.

Emily T. Wang[1], Abbey Michaelson[1], Amy E. Nielson[2], Rock J. Mancini[1]

[1]Miami University, Oxford, OH,[2]Astante Therapeautics, Cincinnati, OH

Multidrug resistance (MDR) presents major challenges in cancer treatment as cancer cells evolve to evade the effects of chemotherapy. This resistance is driven by various cellular mechanisms, including the overproduction of transport proteins that regulate the intake of essential nutrients and the removal of xenobiotics. While the mechanisms underlying drug efflux in MDR cancers are well studied, the role of carbohydrate transport proteins, like glucose transporters (GLUTs), remains poorly characterized as cells become increasingly resistant to chemotherapy. As interest grows in using glycoconjugation for targeted drug delivery, understanding how sugar transport and metabolism are altered in MDR cancers could be crucial for enhancing the specificity and efficiency of these strategies. In this study, we investigated GLUT expression in a melanoma cell line (B16-F10-Luc2) that was subjected to incremental concentrations of the anthracycline doxorubicin (DOX), ranging from 1 nM to 1 μM. As the cells acquired an MDR phenotype, we observed increased efflux activity and decreased cell proliferation rates. At the proteomic level, our analysis showed that GLUT1 and GLUT3 remain stable over the range of resistance levels, while GLUT2 exhibited minimal expression. Interestingly, GLUT4 was transiently upregulated at the 1 μM resistance level and demonstrated insulin responsiveness. We then evaluated GLUT activity and functionality by comparing glucose uptake under normal and starvation conditions and found that glucose deprivation had a limited impact on uptake in both non-resistant and resistant melanoma. To gain a comprehensive analysis into the genomic regulation of GLUTs and metabolic factors associated with MDR, we performed RNA-seq to reveal differential gene expression across various intermediate levels of DOX resistance. Collectively, our findings provide insights into the modular metabolic adaptations of MDR cancers, which could aid in the development of targeted therapies that address GLUT-related defects with drug resistance.

#6794 Elucidating mechanisms of cancer cell persistence to lorlatinib in ALK positive NSCLC.

Toshio Fujino[1], Darlan C. Minussi[2], Christina L. Anastasia[1], Emily Hensley[1], Satoshi Yoda[1], Mari Mino-Kenudson[3], Wei Ping[2], Katya Marjon[2], Xinmeng J. Mu[2], Marco Maruggi[2], Aaron N. Hata[1]

[1]Cancer Center, Masachussets General Hospital, Boston, MA,[2]Pfizer Inc., San Diego, CA,[3]Department of Pathology, Masachussets General Hospital, Boston, MA

ALK fusion-positive lung cancers exhibit deep and durable responses to ALK tyrosine kinase inhibitors (TKIs), however the emergence of acquired drug resistance is inevitable. Current targeted therapies are insufficient to eliminate all cancer cells within a tumor. So called drug tolerant “persister” cells (DTPCs) evade initial treatment, subsequently evolve mechanisms of acquired resistance, and drive disease relapse. Currently, our understanding of the underlying mechanisms driving DTPC survival and evolution in ALK+ fusion NSCLC, especially during treatment with the most advanced ALK lorlatinib, remains incomplete. To identify mechanisms and therapeutic vulnerabilities of DTPC survival in ALK+ NSCLC, we performed bulk and single-cell RNA-seq and ATAC-seq on ALK+ NSCLC patient-derived cell lines and PDX tumors before, during and after treatment with lorlatinib. Tumors exhibited robust initial responses to lorlatinib (> 50% of tumor reduction) followed by a stable residual disease phase and in some cases tumor regrowth after extended treatment, mimicking clinical treatment trajectories. Transcriptomic analysis revealed progressive upregulation of MAPK pathway signaling in DTPC cells in residual tumors and resistant cells in relapsing tumors. Furthermore, we discovered a sub-population of cells with stem-like features present in untreated and residual tumors, potentially representing a pre-existing cell state poised to evade lorlatinib treatment. Finally, we identified several tumor surface proteins upregulated in residual and relapsing tumors that could serve as potential targets for antibody drug conjugate therapies. These results reveal mechanisms driving survival and evolution of DTPCs and identify potential therapeutic targets to prevent resistance of ALK+ NSCLCs to lorlatinib.

#6795 Cystine deprivation overcomes protective effects of hypoxia on ferroptosis in high-grade serous ovarian cancer.

Zonghao Tang, Anil Sood

MD Anderson Cancer Center, Houston, TX

Iron is known to be crucial for high-grade serous ovarian cancer (HGSC) proliferation. HGSC tumors exhibit elevated iron levels compared to normal tissues, making it more susceptible to ferroptosis. Previous studies have shown that ferroptosis inducers can reduce both tumor growth and metastasis in ovarian cancer mouse models. Hypoxia is a prevalent feature of the tumor microenvironment and may protect HGSC from ferroptosis. Induction of ferroptosis can be triggered by inhibitors of GPX4 (GPX4i) or xCT (xCTi), prompting us to consider whether hypoxia may abrogate the induction of ferroptosis in HGSC cells. We tested multiple ovarian cancer cell lines (e.g., OVCAR3, OVCAR4, and OVCAR8) under normoxia and hypoxia. We used both hypoxia chamber (1% oxygen) and CoCl2 to mimic hypoxic conditions and found that both methods significantly inhibited GPX4i-induced ferroptosis by more than twofold (e.g. 5uM RSL3 decreased OVCAR4 cell viability by 80% under normoxia but only 50% under hypoxia), while only having a minimal effect on xCTi-induced ferroptosis. Given that xCTi blocks cystine import, we also examined whether hypoxia affects cystine deprivation-induced ferroptosis. Hypoxia did not protect HGSC cells from cystine deprivation-induced ferroptosis. In conclusion, our study suggests that hypoxia selectively blocks GPX4i-induced ferroptosis in HGSC, while cystine deprivation-induced ferroptosis remains effective, highlighting the potential of targeting cystine import as a more robust approach for treating HGSC.

#6798 Targeting SMAD4^{L535fs} in LNCaP prostate cancer cells: Implications for therapy resistance and sensitivity.

Chizhe Yi, Cindy Sin Ting Chow, Stephen Kwok Wing Tsui

The Chinese University of Hong Kong, Shatin, NT, Hong Kong

Introduction: Prostate cancer is the most common form of cancer and a leading cause of cancer-related death in men worldwide. This disease progresses from an androgen-dependent, non-metastatic state to a highly aggressive, metastatic, androgen-independent phenotype through various intrinsic genetic alterations. One crucial genetic alteration involves SMAD4, a tumor suppressor in the TGF-β signaling pathway, which plays a significant role in promoting aggressive tumor progression. Recent studies have reported that the knockdown of SMAD4 in prostate cancer cells, such as LNCaP, promotes resistance to therapies, including androgen deprivation therapy (ADT) and chemotherapy. The SMAD4^{L535fs} mutation has been implicated in altering cellular proliferation dynamics in LNCaP prostate cancer cells. This study investigates the impact of the SMAD4^{L535fs} mutation on LNCaP prostate cancer cell proliferation and response to chemotherapeutic agents.

Methods: LNCaP prostate cancer cells expressing SMAD4^{WT} and mutant SMAD4^{L535fs} were used to evaluate cell proliferation and viability. Cells were cultured under androgen deprivation conditions and treated with enzalutamide, gemcitabine, or ferroptosis inducers (erastin, ferrostatin-1). Cell viability and spheroid proliferation were assessed using CCK-8 and CellTiter-Glo assays over 72 hours. Apoptosis induction by enzalutamide was evaluated using Annexin V/PI staining. To assess the effects of DNA damage, LNCaP cells were treated with gemcitabine (10 μM), a DNA-damaging agent. For ferroptosis assessment, LNCaP cells were treated with ferroptosis inducers, including erastin (10 μM) and ferrostatin-1 (10 μM), for 72 hours.

Results: SMAD4^{L535fs} significantly enhanced the proliferation of LNCaP spheroids compared to the wild type under androgen deprivation and enzalutamide treatment.Viability assays demonstrated increased resistance to gemcitabine in SMAD4^{L535fs} cells, suggesting a role for SMAD4^{L535fs} in mediating chemoresistance.LNCaP cells treated with ferroptosis inducers showed differential responses, indicating that SMAD4^{L535fs} may influence susceptibility to ferroptosis.

Conclusion: SMAD4^{L535fs} enhances the proliferation and chemoresistance of LNCaP prostate cancer cells, providing insight into potential therapeutic targets for overcoming drug resistance. Further studies are required to elucidate the molecular mechanisms underlying SMAD4^{L535fs}-mediated ferroptosis resistance.

Acknowledgment: This project is supported by the Research Impact Fund (Ref. No.: R4015-19) from the Research Grants Council in Hong Kong

#6799 The interaction of notch signaling and P53 reactivation in endometrial and ovarian cancer therapy.

Daisy Belmares-Ortega[1], Lane E. Smith[1], Jamie L. Padilla[1], Geneva L. Williams[1], Robert J. Lake[1], Hua-Ying Fan[1], Kimberly K. Leslie[2]

[1]Division of Molecular Medicine, University of New Mexico Health Sciences Ctr., Albuquerque, NM,[2]University of New Mexico Health Sciences Ctr., Albuquerque, NM

Across all cancer sites, uterine and uterine cervix cancers have worsened in survival outcomes within the last few decades. Among cancers of the female reproductive organs, endometrial cancer remains the most commonly diagnosed malignancy and is now the most lethal. Deaths from endometrial cancer surpassed those from ovarian cancer just this year. The most aggressive endometrial cancers, serous adenocarcinomas, arise from mutations in p53, leading to loss of the tumor suppressive functions of wild type p53. Endometrial serous adenocarcinomas and ovarian cancers are both associated with an increased recurrence risk. Another aberrant pathway involved in endometrial cancer cell proliferation is Notch signaling, which is hyperactive in the most aggressive endometrial and ovarian cancers. MicroRNA (miRNA) expression profiles have been studied and reveal a signaling protein, NOTCH2 is a likely target related to recurrence. To study the relationship between p53 mutations and Notch signaling, endometrial adenocarcinoma and recurrent ovarian cancer cell lines including KLE, AN3CA, and PEO4 were employed for these studies due to their overexpression of Notch signaling (AN3CA), mutated p53 status (KLE, AN3CA, and PEO4), and as a cancerous recurrence model (PEO4). The curcumin-analogue AKT-100 was utilized as a mutant p53 reactivator agent in our studies to recreate the wild type p53 pro-apoptotic transcriptome. Additionally, our studies included a key down-regulator of the Notch-signaling pathway, auranofin. Auranofin as a Notch inhibitor can be used to prevent recurrence. While AKT-100 and auranofin were active in both cell lines as single agents, KLE cells were less sensitive to Auranofin due to low Notch signaling at baseline compared to AN3CA cells with high baseline activation of Notch. Interestingly, single agent AKT-100 treatment of KLE cells resulted in enhancement of the Notch signaling pathway as a resistance mechanism. This finding set the stage for the dual treatment of KLE cells with AKT-100 and Auranofin to prevent Notch upregulation. The combination of AKT-100 and Auranofin was highly synergistic (Combinatorial Index = 0.25). To confirm the mechanism of drug synergy, RNA sequencing, target protein expression and chromatin immunoprecipitation studies will be employed to confirm the effect of each agent on p53 and Notch signaling. These studies identify a novel combinatorial drug regimen tailored for the treatment of the most aggressive endometrial and ovarian cancers and identify an important mechanism underlying drug synergy.

#6800 Extracellular ATPase inhibitor-chemotherapy treatment of macrophages increases danger signal extracellular ATP to induce inflammatory pyroptotic cell death.

Jasmine M. Manouchehri, Mathew A. Cherian

The Ohio State University Wexner Medical Ctr., Columbus, OH

Among cancers in women worldwide, breast cancer has the highest incidence and takes the most women's lives. Patients with triple negative breast cancer (TNBC) experience worse outcomes than the patients diagnosed with hormone-dependent and human epidermal growth factor receptor-positive breast cancers because of the aggressive and rapidly progressive nature of breast cancer and the shortfall in the availability of specifically targeted therapies for TNBCs. Therefore, there is a demand for unique and fresh therapeutic approaches especially for TNBCs. Extracellular ATP (eATP) has been demonstrated to cause cell death in an autocrine and paracrine manner and is also a known danger signal that causes immune activation. Our previously published data reveals that at high micromolar concentrations eATP can induce cell death through the purinergic P2RX7 and P2RX4 receptors. We also demonstrated that eATP modulates TNBC cell death in the presence of chemotherapy. Despite this, eATP is rapidly degraded to adenosine by extracellular ATPases (eATPases). It should be noted that eATP release and activation of its receptor, P2XR7, are prerequisites for the activation of a complex of proteins known as the NLRP3 inflammasome and subsequent activation of caspase 1 which can lead to the induction of a highly inflammatory form of programmed cell death known as pyroptosis. It is particularly notable that the cell type most associated with this inflammatory form of cell death is macrophages, so we found it compelling to evaluate the effects of ATP signaling in macrophages. The objective of this study was to examine if the application of eATPase inhibitors will augment eATP release from chemotherapy-treated monocytic THP-1 and differentiated THP-1 cells and induce an inflammatory form of cell death known as pyroptosis. We previously showed that heparan sulfate inhibits multiple families of extracellular ATPases. Hence, we treated differentiated THP-1 cells with increasing concentrations of paclitaxel in the presence and absence of five different heparinoids that inhibit heparinase with cell viability and eATP being assessed. Furthermore, we found that there was an increase in caspase 1 activity, indicating increased pyroptosis, in monocytic THP-1 and differentiated THP-1 cells with the combined treatment of the E-NTPDase inhibitors PSB 069 or POM-1 and paclitaxel compared to paclitaxel treatment alone. These results show that eATP modulates monocytic THP-1 and differentiated THP-1 cells responses to chemotherapy, which could be advantageous to sensitize the anticancer effects of macrophages on TNBC cells. The development of efficacious therapies could stimulate an increase the depth and length of responses to chemotherapy and immunotherapy in TNBCs through pyroptosis induction of tumor associate macrophages through enhanced eATP.

#6802 RLIP depletion promotes apoptosis in ovarian cancer.

S. S. Singhal, M. Krishna, P. Kulkarni, D. Horne, R. Salgia;
City of Hope National Medical Center, Monrovia, CA

Ral interacting protein (RLIP) is a multifunctional protein localized and distributed in the plasma membrane, cytosol, and nucleus of the cell. RLIP is ubiquitously expressed in various tissues, including the ovary. In tumorigenesis, RLIP emerges as a common feature for solid tumor growth. RLIP is an anti-apoptotic non-ABC glutathione (GSH)-conjugate transporter involved in receptor-ligand endocytosis, as well as in multispecific drug transport and resistance. The presence of RLIP appears to be indispensable for cancer cells as indicated by its overexpression in various malignant cell lines. Partial inhibition and or depletion of RLIP using antibodies or antisense in the absence of chemotherapy drug causes apoptosis in multiple ovarian cancer (OC) cell lines; in the presence of carboplatin, marked synergy is observed *in vitro* as well as *in vivo*. These findings indicate that RLIP is a good target for cancer cell killing, and that its down-regulation would promote apoptosis through both drug-dependent and drug-independent effects. To examine the effect of specific RLIP depletion on apoptosis, we tested the effect of RLIP antisense in a number of OC cell lines. Growth inhibition and apoptosis were observed in all cases upon RLIP depletion. Cytotoxic synergy between RLIP antisense, RLIP antibodies, and carboplatin was examined in cell culture and xenografts of OVCAR8 OC cells. RLIP inhibition by antibodies was equally as effective as antisense and enhanced carboplatin efficacy in OC xenografts. Taken together, these results show that RLIP is a rational and promising new target for OC therapy. (This work was supported in part by the Department of Defense grant W81XWH-22-1-0331. Funding from the Beckman Research Institute of City of Hope is also acknowledged).

#6803 RNF5-DNAJA1 axis dictates selinexor sensitivity of multiple myeloma by regulating mitochondrial function.

Mengqi Wang

Tianjin Medical University, Tianjin, China

Selinexor is a First-in-Class XPO1 Inhibitor approved for relapsed or refractory multiple myeloma (RRMM), but the overall response rate remains unsatisfactory. The main mechanism by which Selinexor kill MM cells is to suppress the export of tumor suppressor proteins and oncoprotein mRNAs, however other key underlying mechanisms need to be investigated. Here, using a proteomics of nucleocytoplasmic separation assay, we discover that a ring finger containing E3 ligase, RNF5, is a cargo substrate of XPO1 and its nuclear-cytoplasmic transport is correlated with poor clinical therapeutic response. In the interactome of XPO1, we identify that DNAJA1 is a new partner of RNF5 and catalyzes K29-linked polyubiquitination of DNAJA1, which strengthens the interaction between DNAJA1 and HSP70, consequently and antagonizes the interaction of HSP70 and HSF1, therefore activates UPR^{mt} activation and mitochondrial protection. Thus, administration of RNF5 inhibitor inh-02 and DNAJA1 inhibitor 116-9e disrupts UPR^{mt} balance and sensitizes selinexor *in vitro* culture cell lines and in the xenograft and Vk*Myc mouse models. Collectively, our study elucidates the machinery of XPO1-RNF5-DNAJA1 axis in regulating mitochondrial function, and sheds light on developing new strategies to improve the efficacy of treating RRMM patients.

#6804 Sanguinarine overcomes osimertinib resistance by inducing HMOX1-related ferroptosis in osimertinib resistant cells lines and tumors.

Rongzhong Xu, Liubing Lin

Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai, China

Epidermal Growth Factor Receptor (EGFR) gene, as a crucial oncogenic driver, plays a significant role in the occurrence and progression of non-small cell lung cancer (NSCLC). Osimertinib, as the latest generation of EGFR tyrosine kinase inhibitors (TKIs), has shown remarkable efficacy not only in patients with EGFR-sensitive mutations but also in those with rare EGFR mutations. However, similar to patients treated with first- or second-generation EGFR-TKIs, those treated with osimertinib eventually develop resistance Therefore, it is crucial to investigate the mechanisms of resistance and identify novel, rational strategies and drugs to overcome acquired resistance. Here we demonstrated that Sanguinarine, a natural benzophenanthridine alkaloid derived from rhizomes of the plant species Sanguinaria canadensis, which induces ferroptosis in osimertinib-resistance cells, leading to cell death. In this study, we found that sanguinarine induced more cell death in osimertinib-resistant cell lines, compared to osimertinib-sensitive EGFR-mutant cell lines. RNA-seq showed that sanguinarine increased higher expression level of HMOX1 in osimertinib-resistant cell lines than osimertinib-sensitive EGFR-mutant cell lines. Moreover, compared to osimertinib-sensitive cell lines, sanguinarine induces more ROS production, increases Fe^{2+} concentration, and promotes more ferroptosis in osimertinib-resistant cell lines. Interestingly, we found that HMOX1 levels are elevated in tissues from patients with EGFRm NSCLC who relapsed from EGFR-TKI treatment. Also, Osimertinib and other EGFR-TKIs up-regulate HMOX1 expression in EGFRm NSCLSC cells and tumors. Enforced expression of an ectopic HMOX1 gene in Osimertinib-sensitive EGFRm NSCLC cells increased the sensitivity of cells to sanguinarine by inducing more ferroptosis, whereas knockdown of HMOX1 in Osimertinib-resistant cell lines conferred resistance to sanguinarine. Importantly, sanguinarine inhibited more effective in osimertinib resistant EGFRm NSCLC xenograft in vivo than in osimertinib sensitive models. Together, compared to osimertinib-sensitive cell lines, sanguinarine induces more ROS production and increases Fe^{2+} concentration results in more ferroptosis in osimertinib-resistant cell lines. This difference may be due to the overexpression of HMOX1 in Osimertinib-resistant cells, making them more susceptible to the effects of sanguinarine.

#6805 DKC1 promotes colorectal cancer progression and therapy resistance by dysregulating sphingolipid biosynthesis.

U. K. Khan[1], A. Goel[1], S. Nigam[1], N. Chaudhary[2], A. Bajpai[1], A. Praveen[1], A. Shendre[1], S. Carskadon[3], A. Kapoor[4], A. Tewari[4], A. Chandra[5], S. N. Dalal[2], N. Palanisamy[6], B. Ateeq[1]; [1]Indian Institute of Technology Kanpur (IIT Kanpur), Kanpur, India, [2]Advanced Centre for Treatment, Research and Education in Cancer, Tata Memorial Centre, Navi Mumbai, India, [3]Vattikutti Urology Institute, Henry Ford Health System, Detroit, MI, [4]Regency Hospital, Kanpur, India, [5]King George's Medical University, Lucknow, India, [6]Henry Ford Health System, Detroit, MI

Colorectal cancer (CRC) is the second most frequent lethal malignancy globally. It has been associated with limited treatment effectiveness in most cases, leading to modest clinical outcomes with first-line therapies. Despite the recent advancements in the treatment modalities, most patients fail to respond by switching to alternative bypass mechanisms and acquiring drug resistance. This emphasizes an urgent need to discover the underlying mechanistic circuitry in CRC pathogenesis and explore alternative robust drug targets to overcome the lack of treatment response thereby improving the patient's survival. In addition, molecular heterogeneity associated with tumor profiles also influences treatment outcomes, prompting a fundamental need for molecular characterization of colorectal tumors to design targeted therapies. Here, we used a bottom-up approach to delineate the clinical significance of *Dyskerin pseudouridine synthase 1* (*DKC1*) in CRC patients. We observed elevated DKC1 levels in tumor specimens compared to adjacent normal tissue, associated with aggressive disease and poor recurrence-free survival. Moreover, patients acquiring resistance against standard treatment regimen (FOLFOX/FOFIRI) showed increased expression of DKC1. Importantly, loss-of-function studies suggest high DKC1 imparts oncogenicity by promoting cell cycle progression, suppressing cell death, and conferring stem cell-like phenotype. Notably, gene signature profile from DKC1-high patient tumors exhibit similarity to Consensus Molecular Subtype 2 (CMS2) of CRC. We deciphered a positive feedback loop operating between canonical WNT signaling and DKC1, responsible for orchestrating DKC1 expression and its associated tumorigenicity. Comprehensive lipidomic and transcriptomic analyses reveal perturbed sphingolipid metabolism and an abundance of very-long-chain fatty acid ceramides, particularly Cer(d18:2/23:0) and Cer(d18:1/24:0) in DKC1-positive CRC. Mechanistically, Co-IP and ChIP-PCR results confirmed that DKC1 and SOX2 together drive the expression of *SGPP2,* modulating sphingolipid biosynthesis and targeting *SGPP2* in DKC1-positive CRC alleviates oncogenic properties. Taken together, we demonstrate that elevated DKC1 expression imparts chemoresistance, and pharmacological inhibition of DKC1 and WNT signaling effectively attenuates the growth of therapy-resistant patient-derived organoids as well as CRC cells in a synergistic manner. Conclusively, we propose the DKC1/WNT axis as a promising therapeutic target for chemotherapy non-responding CRC patients. The study also highlights the utility of complex sphingolipids as a plasma-based biomarker providing a new paradigm for the non-invasive diagnosis of advanced-stage CRC patients.

#6806 Deciphering the role of BRD2 in acute adaptive response to BET inhibitors across cancers.

Suyakarn Archasappawat, Eunjung Lee, Chang-il Hwang

University of California, Davis, Davis, CA

Bromodomain and extraterminal motif (BET) inhibitors, such as JQ1, are a promising therapeutic strategy against cancer, targeting key epigenetic regulators, particularly BRD4. However, resistance to BET inhibitors poses a significant challenge, necessitating a deeper understanding of adaptive mechanisms. Here, we identified the upregulation of BRD2, another member of the BET protein family, as a conserved response to BET inhibition across multiple cancer types. We hypothesize that this upregulation facilitates cellular adaptation to treatment, reducing drug efficacy and potentially contributing to resistance over time. To test this, we performed BRD2 knockdown, demonstrating that BRD2 depletion sensitizes pancreatic cancer cells to BET inhibitors in vitro. Furthermore, a combination of BRD2 knockdown with JQ1 treatment significantly impaired tumor growth in vivo. Mechanistically, ChIP-seq analysis of BRD2 and BRD4 occupancy upon JQ1 treatment revealed that BRD2 and BRD4 share common binding sites. JQ1 caused a substantial loss of BRD4 from chromatin, while BRD2 was modestly affected. Although JQ1 has a higher affinity for BRD4 than for BRD2, our data suggest that higher cellular abundance of BRD2 compensates for BRD4 function under BET inhibitor treatment, thereby contributing to adaptive resistance and sustaining essential transcriptional programs. These findings highlight BRD2 as a key player in the adaptive response to BET inhibition and a potential therapeutic target to enhance the efficacy of BET inhibitors in pancreatic cancer and other malignancies.

#6807 Targeting EZH2 to overcome osimertinib resistant non-small cell lung cancer.

Christian M. Gosser[1], Xiulong Song[1], Dave-Preston Esoe[1], Avery R. Childress[1], Daniel R. Plaugher[1], Kassandra J. Naughton[1], Riya J. Patel[1], Ryan A. Goettl[2], Jinpeng Liu[2], Christine F. Brainson[3]

[1]Department of Toxicology and Cancer Biology, University of Kentucky, Lexington, KY,[2]Department of Internal Medicine, Markey Cancer Center, University of Kentucky, Lexington, KY,[3]Department of Toxicology and Cancer Biology, Markey Cancer Center, University of Kentucky, Lexington, KY

Non-Small Cell Lung Cancer (NSCLC) is a continual issue across the United States as the leading cause of cancer-related mortalities. Within NSCLC, Epidermal Growth Factor Receptor (EGFR) mutations have emerged as a main genetic driver. Treatment for EGFR mutated lung cancers has evolved over the past two decades, starting with the EGFR tyrosine kinase inhibitors gefitinib and erlotinib, and more recently moving to osimertinib. These treatments are typically successful initially, but resistance to EGFR inhibitors is inevitable for many patients. Findings ways to overcome the myriads of resistance mechanisms that can occur is the key improving treatment outcomes. Our lab studies the Polycomb Repressive Complex 2 (PRC2) that contains the subunit Enhancer of Zeste Homology 2 (EZH2). PRC2 is an epigenetic methyltransferase that facilitates histone H3 lysine 27 tri-methylation (H3K27me3) to silence gene expression. EZH2 has been implicated in many cancers may potentially drive treatment resistance. Our hypothesis is that inhibition of EZH2 will overcome osimertinib resistance by blocking the oncogenic signaling driven by the transcription factor c-MYC. To test our hypothesis, PC9, PC9GR4, and HCC4006 were all made resistant to osimertinib with IC_{50}s of 1μM and above. Following generation of acquired resistance, we found that treatment with the EZH2 inhibitor tazemetostat (EPZ6438) was able to resensitize cell lines to osimertinib. In PC9GR4OR this re-sensitization was accompanied by increased apoptosis. We next tested two cell lines, PC9GR4OR and HCC4006OR, as *in vivo* xenografts, and showed that combination of osimertinib and EPZ6438 led to significant tumor regression that was not achieved by either drug alone. Next, to understand mechanisms of resistance, we performed RNA sequencing on both parental and osimertinib resistant lines treated with vehicle, EPZ6438, osimertinib, or both. GSEA analyses showed that resistance mechanisms for the three lines were not the same. PC9OR upregulated cell cycle targets and c-MYC targets, HCC4006OR upregulated oxidative phosphorylation and c-MYC targets, and PC9GR4OR had an increase in cholesterol homeostasis. RT-qPCR analyses also showed that combination treatment increased antigen presentation (*HLA-A*) and lowered PD-L1 (*CD274*). This combination of changes may produce tumors that are more susceptible to immune surveillance, or that respond better to immune checkpoint inhibitors. Overall, this work highlights the ability of inhibiting EZH2 to overcome multiple osimertinib resistance mechanisms in human lung cancer cells and suggests that this drug combination may prolong the treatment benefit for many lung cancer patients. This work was funded by: Markey Women Strong, Markey STRONG Scholars Program through the American Cancer Society IRG-22-152-34 (HY), T32 CA165990 (DRP), R01 CA237643 + Cure Supplement (CFB, CMG), R01 HL170193 (CFB), P30 CA177558 (Markey Shared Resources)

#6808 DOCK1 as a therapeutic target for EGFR resistance in cholangiocarcinoma.

Kishor Pant[1], Kory Engelstad[1], Estanislao Peixoto[1], Ainhoa Lapitz[2], Jesus M. Banales[3], Adrian Mansini[4], Sergio A. Gradilone[1]

[1]The Hormel Institute, University of Minnesota, Austin, MN,[2]Biodonostia Health Research Institute - Donostia University Hospital, University of the Basque Country (UPV/EHU), San Sebastian, Spain, San Sebastian, Spain,[3]Biodonostia Health Research Institute - Donostia University Hospital, University of the Basque Country (UPV/EHU), San Sebastian, Spain,[4]Rush University Medical Center, Chicago, IL

Cholangiocarcinoma (CCA) is an aggressive cancer of the bile ducts, characterized by poor prognosis and limited treatment options due to late-stage diagnosis and resistance to targeted therapies. Mutations in critical pathways, including the Epidermal Growth Factor Receptor (EGFR) and Fibroblast Growth Factor Receptor (FGFR), drive CCA progression, while resistance mechanisms and mutation weaken the effectiveness of available therapies. Dedicator of Cytokinesis 1 (DOCK1), a guanine nucleotide exchange factor, has emerged as a key player in cancer progression, particularly in modulating cell signaling. In this study, we investigated the therapeutic potential of targeting DOCK1 in CCA via modulating EGFR signaling. Our assessment across five distinct patient cohorts demonstrates that DOCK1 is significantly overexpressed in CCA tumors compared to normal cholangiocytes, underscoring its pivotal role in promoting tumor growth and aggressiveness. Pharmacological inhibition of DOCK1 using the small-molecule inhibitors CPYPP and TBOPP, as well as siRNA-mediated knockdown, resulted in a marked reduction in HuCCT1 and EGI1 CCA cell lines proliferation and migration. This was accompanied by an increase in tumor suppressor proteins p53 and p21, along with a decrease in proliferation markers PCNA and cyclin D1. Moreover, siRNA-mediated reduction of DOCK1 expression significantly induced apoptosis and cytotoxicity, accompanied by elevated levels of cleaved caspase-3 and PARP1, highlighting its role in driving cell death resistance in CCA. Furthermore, DOCK1 inhibition disrupted EGF-stimulated EGFR phosphorylation and downstream signaling through Erk1/2, a critical pathway supporting tumor growth, survival, and therapeutic resistance. These findings position DOCK1 as a central enhancer of EGFR-mediated oncogenic signaling, playing a key role in the aggressive phenotype and therapeutic resistance of CCA. Targeting DOCK1 not only suppressed tumor cells proliferation but also enhanced cell death, offering a dual therapeutic advantage. Our study provides strong evidence supporting DOCK1 as a promising therapeutic target and suggests that its inhibition, particularly in combination with EGFR-targeted therapies, could overcome EGFR mutant and drug resistance mechanisms and improve clinical outcomes in CCA. By unveiling this novel therapeutic strategy, our research lays the foundation for advancing more effective and stable treatments for this aggressive and often deadly malignancy.

#6809 IFITM3/MET axis drives osimertinib resistance through Akt pathway activation in *EGFR*-mutant lung cancer.

Ritsu Ibusuki[1], Eiji Iwama[1], Atsushi Shimauchi[1], Hiroyuki Inoue[2], Daisuke Shibahara[1], Kohei Otsubo[1], Yasuto Yoneshima[1], Isamu Okamoto[1]

[1]Department of Respiratory Medicine, Kyushu University, Fukuoka, Japan,[2]Department of Respiratory Medicine, Fukuoka University, Fukuoka, Japan

EGFR-mutant non-small-cell lung cancer (NSCLC) patients often experience significant clinical benefits from EGFR tyrosine kinase inhibitors (TKIs). However, resistance to these therapies inevitably emerges over time through various mechanisms, limiting the long-term effectiveness of the treatment. The mechanisms of resistance and the duration required for resistance to emerge are highly variable and difficult to predict, highlighting the need for new therapeutic strategies to suppress resistance. In this study, we elucidated the mechanism by which IFITM3, a membrane protein whose expression is upregulated by cytokine stimulation, contributes to reduced sensitivity to the EGFR-TKI osimertinib and promotes the development of resistance through Akt pathway activation. Additionally, we demonstrated that IFITM3 levels are elevated in pre-treatment clinical specimens from *EGFR*-mutant NSCLC patients who developed early resistance to osimertinib, supporting its role in resistance development. Furthermore, Combined treatment with osimertinib and MET inhibitor results in synergistic growth inhibition in NSCLC xenograft mouse models with high IFITM3 expression. These findings provide a rationale for clinical trials investigating the co-administration of MET and EGFR inhibitors in patients with elevated IFITM3 levels to address the heterogeneity of EGFR-TKI resistance mechanisms.

#6810 The mechanism of PRMT5 in causing smoking-induced lung cancer and development of EGFR Tyrosine kinase inhibitor resistance.

Ayesha Khan, Usama Altayeh, Subaranjana Saravanaguru Vasanthi, Mohammad Fazle Alam, Neelu Puri

University of Illinois College of Medicine (Rockford), Rockford, IL

In 2024, lung cancer accounts for 20% of US cancer deaths and 80-90% of NSCLC patients are smokers. PRMT5 (protein arginine methyl transferase 5) is involved in various normal cellular pathways and activates the PI3k-Akt signaling pathways that increase lung tumorigenicity. In NSCLC, EGFR-TKI treatments with Osimertinib initially reduce tumor growth but often lead to resistance in about 19 months. Currently, PRMT5 inhibitors are in clinical trials for several malignancies; further studying PRMT5's role in EGFR-TKI resistance could reveal if these inhibitors enhance TKI efficacy in NSCLC.

We hypothesize that modulating PRMT5 could reduce tumorigenicity and overcome OR resistance. We studied the expression of PRMT5 in both drug-resistant (OR) and drug-sensitive (parental) H3255 and PC9 NSCLC cell lines after treatment with CSE for 24 and 48 hours, followed by western blotting and qPCR. Also, using qPCR the effects of PRMT5i after CSE treatment were studied on inflammatory cytokines.The efficacy of PRMT5i (GSK591) on PC9OR cells were studied using bioprinted with GelMA with a R-Gen 200 bioprinter.

After 24 and 48 hours of CSE treatment PRMT5 gene expression was increased as observed using qPCR in TKI-resistant H3255P/OR (1.5-11 fold) and PC9/OR (1.5-17 fold) ($p<0.05$) cells respectively. Immunoblotting results also indicated a 1.3-2.1-fold ($p<0.01$) upregulation of PRMT5 in these cell lines. PC9P/OR cells were treated with GSK591, and the MTT assay revealed a significant decrease in cell viability to 49% and 46% ($p<0.01$) respectively, in the GSK591 plus OR group as compared to OR treatment group. The cell viability was significantly lowered by 49% in the siRNA plus OR group compared to the siRNA group alone. The treatment groups in both siRNA and PRMT5i treated cells had additive inhibitory effect ($p<0.01$). Studies on inflammatory cytokines in PC9OR cells first treated with CSE and then with PRMT5i showed that CSE alone caused an upregulation of cytokines: TNFα (4-5 fold), IL-1β (1-2.3 fold), and IL-8 (7-9 fold) ($p<0.05$). However, treatment with PRMT5i resulted in a downregulation of these cytokines: TNFα (0.4-0.5 fold), IL-8 (0.5 fold), and IL-1β (0.4-0.6 fold) ($p<0.05$). These findings indicate that inhibiting or downregulating PRMT5 may be effective in reducing CSE-induced oncogenicity. To replicate patient lung tumor microenvironment and enhance the clinical relevance of our findings, we employed 3D bioprinting by accurately layering cells onto biological scaffolds. Preliminary results with PC9OR along with HLF (Human lung fibroblasts) bioprinted spheroids showed 40% inhibition of spheroid count/microscopic field by a combination of PRMT5i and OR compared to OR alone.

In conclusion, we infer that PRMT5 is overexpressed in TKI-resistant cells, leading to increased oncogenic potential. Modulating PRMT5 levels could enhance the effectiveness of EGFR TKIs in NSCLC patients.

#6811 Overcoming temsirolimus resistance in renal cell carcinoma: The potential of bAP15 inhibitor combination therapy.

YI-JU Kao[1], Kuan-Lin Kuo[1], Shih-Ming Liao[1], Chen-Hsun Hsu[1], Yen-ling Chiu[2], Wei-Chou Lin[3], Kuo-How Huang[1], Po-Ming Chow[1]

[1]Department of Urology, National Taiwan University Hospital, Taipei City, Taiwan,[2]Department of medical research, Far Eastern Memorial Hospital, Taipei, Taiwan,[3]Department of Pathology, National Taiwan University Hospital, Taipei City, Taiwan

Renal cell carcinoma (RCC) is a prevalent form of kidney cancer with a high mortality rate, especially in advanced stages. While treatments like tyrosine kinase inhibitors, mTOR inhibitors, and immune checkpoint inhibitors exist, they often have limited efficacy. Temsirolimus, a clinically used mTOR inhibitor, provides a therapeutic option for advanced RCC by hindering cell growth and proliferation, but resistance to this drug can emerge. This study investigated the role of deubiquitylating enzymes UCHL5/USP14 in RCC progression and the potential of their inhibitor, bAP15, to overcome Temsirolimus resistance. bAP15 demonstrated potent anti-cancer activity in RCC cells by inducing apoptosis and cell cycle arrest. Importantly, bAP15 synergized with Temsirolimus in vitro, likely by inhibiting proliferating cell nuclear antigen (PCNA), and enhanced Temsirolimus efficacy in vivo. This combination therapy suppressed tumor growth more effectively than either agent alone. These preclinical findings highlight the potential of bAP15 to overcome Temsirolimus resistance in RCC by targeting UCHL5/USP14. Further research, including clinical trials, is necessary to evaluate the safety and efficacy of bAP15 in RCC patients.

#6812 The third-generation FLT-3 inhibitor, PLM-102, is a promising therapeutic option for venetoclax-resistant AML.

Yun Sun Lee, Min Ji Park, Bongki Ko, Soo Jin Kim, Miran Moon, Soo Yeon Jang, Yong-Chul Kim

Pelemed, Seoul, Korea, Republic of

Venetoclax, a selective inhibitor of the anti-apoptotic protein Bcl-2, has been used as a first-line treatment for acute myeloid leukemia (AML). However, the long-term administration of the drug generally leads to the development of resistance, thereby limiting its therapeutic efficacy. This study aims to evaluate whether PLM-102, an orally available selective and potent FLT3 inhibitor, can overcome venetoclax resistance (VR) in a drug-resistant AML model. To prepare venetoclax-resistant AML cells, the FLT3-ITD-positive MOLM-14 cell line was exposed to increasing concentrations of venetoclax, starting from 10 nM to 1 μM over a period sufficient to induce appreciable resistance. The venetoclax-resistant (VR) cells showed increased phosphorylation of FLT3, AKT, and STAT3 compared to the parental cells. Treatment of the VR cells with PLM-102 ameliorated all activated signaling pathways, restoring them to basal levels more effectively than either tuspetinib or gilteritinib. Additionally, venetoclax, an inhibitor of the anti-apoptotic protein Bcl-2, is known to upregulate MCL-1 as a bypass mechanism. Treatment with PLM-102 reduced MCL-1 and Bcl-2 expression levels and concurrently led to a significant increase in cleaved caspase-3, indicating that PLM-102 induces apoptosis in VR cell lines. In-vivo anti-cancer activities of venetoclax, various FLT3 inhibitors and PLM-102 were compared in the MOLM-14 VR xenograft mouse model. In contrast to the in-vivo study with the parental MOLM-14 model, venetoclax did not show anti-cancer activity in the MOLM-14 VR model, confirming the resistance of VR AML cells in vivo. Notably, PLM-102 demonstrated the highest tumor growth inhibitory (TGI) efficacy compared with those of other FLT3 inhibitors including tuspetinib, gilteritinib and PHI-101. In conclusion, PLM-102 completely inhibited FLT3 kinase and its downstream signaling including AKT and STAT3, inducing apoptosis in VR AML cells along with significant inhibition of the tumor growth in the VR xenograft mouse model. These findings suggest that PLM-102, a third-generation FLT-3 inhibitor, is a promising candidate for overcoming venetoclax resistance and could serve as a therapeutic option for venetoclax-resistant AML.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Mechanisms of Cancer Therapeutics
Poster Session

#6816 Synergistic targeting of nonsense-mediated mRNA decay and heat shock protein 90α for treating lethal prostate cancer.
J. Liu, X. Wang, H. Maasoumyhaghighi, M. Nouri, X. Liu;
University of Kentucky, Lexington, KY

Background: Androgen receptor signaling inhibitors (ARSI) provide modest survival benefits for metastatic castration-resistant prostate cancer (CRPC), but most patients eventually develop resistance, with some progressing to neuroendocrine prostate cancer (NEPC), a highly aggressive form of the disease. There are no effective treatments for ARSI-resistant patients, underscoring the need for new therapeutic strategies. Synthetic lethality, where the combined loss of two genes causes cell death, has shown promise in cancer therapy, as demonstrated by the success of PARP inhibitors. However, synthetic lethal CRISPR-Cas9 screens in prostate cancer (PCa) remain underexplored.
Methods: A combinatorial CRISPR-Cas9 knockout screen was employed to identify synthetic lethal (SL) pairs in ARSI-resistant prostate cancer. Identified SL pairs were validated using in vitro cell viability assays and in vivo models, including cell line and patient-derived xenografts. A luminescence-based nonsense-mediated mRNA decay (NMD) sensor system was utilized to investigate the role of heat shock protein 90α (HSP90α) in NMD. The molecular mechanisms by which NMD components and HSP90α maintain protein and RNA homeostasis in advanced PCa were elucidated using immunohistochemistry, immunofluorescence, immunoblotting, and immunoprecipitation assays.
Results: (1) The combinatorial CRISPR-Cas9 knockout screening identified NMD components and HSP90α emerged as a top novel synthetic lethal pair in ARSI-resistant PCa. (2) Dual knockout of NMD regulators and HSP90α led to cell death in ARSI-resistant PCa. (3) Inhibitors targeting both NMD and HSP90α synergistically suppressed the growth of advanced PCa cells in both in vitro and in vivo models, with minimal effects on normal cells. (4) HSP90α interacts with the NMD regulator, protecting it from aggregation and facilitating its function.
Conclusions: Our research, utilizing an unbiased combinatorial CRISPR-Cas9 double-knockout screen, is the first to identify synthetic lethal pairs in ARSI-resistant prostate cancer. This study not only provides a new therapeutic approach for overcoming drug resistance in advanced PCa but also deepens our understanding of cellular homeostasis by elucidating the interaction between NMD components and heat shock proteins in the context of advanced prostate cancer.

#6817 Targeting XPO1 eliminates cancer stem-like cells and abrogate hepatic metastasis in uveal melanoma.

B. Jin, H. Zou, J. Pan;
Sun Yat-sen University, Guang Zhou, China

Uveal melanoma originating from the uveal tract are a rare form of the malignancy with a biology and clinical feature distinct from its more common cutaneous counterparts. Yet, UM is the most common primary intraocular tumor in adults. Despite effective treatment in primary UM with eye-sparing radiation-based techniques or enucleation, ~50% of UM patients ultimately develop distant metastasis, most commonly in the liver. UM patients with hepatic metastasis have dismal prognosis owing to the limited efficacy of the available treatments. It is fundamental to find new therapeutic targets and develop novel treatment approaches for metastatic UM. XPO1 (also referred as CRM1), a key member of the importin β superfamily of nuclear transport receptors, mediates nuclear export of tumor suppressors and growth factors. We found that XPO1 was highly overexpressed in UM cells and positively correlated with poor prognosis of UM. Depletion of XPO1 by shRNA or its small molecular inhibitor KPT-330 significantly inhibited growth of UM cells *in vitro* and *in vivo*. Treatment with KPT-330 markedly induced apoptosis in UM cells via reducing the protein level of Survivin. Meanwhile, silencing of XPO1 by lentiviral shRNA or incubated with KPT-330 significantly decreased the percentage of ALDH+ cells and dampened self-renewal capacity in UM cells. Targeting XPO1 by KPT-330 profoundly alleviated liver metastasis burden in liver metastasis mouse model. These results suggest XPO1 may be a potential therapeutic target for patients with metastatic UM.

#6818 GPR34-driven damage-response macrophages underlie therapeutic resistance to surufatinib.

Xiaofan Guo, Yuxiao Liu, Yiping Zou, Wenbo Zhu, Peijun Xu, Yuning Song, Jing Huang, Xiaopeng An, **Song Gao**, Jihui Hao

Tianjin Medical University Cancer Institute and Hospital, Tianjin, China

Background: Pancreatic cancer, an immunologically "cold" tumor, poorly responds to immunotherapy. TAMs, key immune cells in its microenvironment, drive tumor progression. Despite recent progress in macrophage-targeted therapies like CSF1R inhibitors, clinical results in pancreatic cancer are disappointing, with unclear mechanisms. Surufatinib, a CSF1R inhibitor, hinders macrophage differentiation into an immunosuppressive state. Surufatinib alters macrophage differentiation pathways, but resistance is mediated by the emergence of damage-associated macrophages. A subset of these macrophages, characterized by high GPR34 expression, is enriched in refractory pancreatic cancer patients. Through activation of the LysoPS-GPR34 axis, these macrophages enhance efferocytosis and subsequently downregulate MHC-I, leading to $CD8^+$ T cell exhaustion.
Method: Tumor immune microenvironment alterations in pancreatic cancer patients after surufatinib treatment were analyzed via scRNA-seq. Multiplex immunofluorescence staining and flow cytometry were performed on tumor tissue microarrays and fresh surgical specimens. GPR34 conditional knockout mice with orthotopic tumors were analyzed via flow cytometry. The mechanisms of $CD8^+$ T cell exhaustion were investigated using in vitro co-culture systems of macrophages and $CD8^+$ T cells. The efficacy of GPR34 antagonists in combination with immunotherapy and chemotherapy was evaluated in spontaneous tumor models.
Result: Single-cell RNA sequencing (scRNA-seq) was performed on tumor specimens from patients enrolled in clinical trials receiving surufatinib plus chemotherapy. The analysis revealed changes in the tumor immune microenvironment, identifying a subset of damage-response macrophages characterized by high GPR34 expression. Notably, this macrophage population was significantly enriched in treatment-resistant patients. These high expression GPR34 macrophages activated damage-response signaling via the lysophosphatidylserine (LysoPS)-GPR34 axis, promoting $CD8^+$ T cell exhaustion through the release of chronic inflammatory factors and enhanced efferocytosis. Furthermore, efferocytosis further promotes the formation of autophagolysosomes in macrophages, leading to increased degradation of MHC-I, thereby suppressing the antigen-presenting function of macrophages. Targeting $GPR34^+$ macrophages significantly improved the efficacy of both immunotherapy and chemotherapy.
Conclusion: Surufatinib alters macrophage differentiation pathways, but resistance is mediated by the emergence of damage-associated macrophages. A subset of these macrophages, characterized by high GPR34 expression, is enriched in refractory pancreatic cancer patients. Through activation of the LysoPS-GPR34 axis, these macrophages enhance efferocytosis and subsequently downregulate MHC-I, leading to $CD8^+$ T cell exhaustion.

#6819 TOMM40/FADS2 expression ratio predicts the sensitivity to mTOR inhibitors in triple-negative breast cancer.

Salman Mohamed Farah[1], **Yuan-Feng Lin**[2]

[1]International Ph.D. Program in Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan,[2]Graduate Institute of Clinical Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan

Background: Triple-negative breast cancer (TNBC) is a subtype of breast cancer that does not express the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2). Because of the negative to ER, PR and HER2, the current targeted therapy is not fully working for TNBC. Deregulated mTOR signaling has been reported to have implications in several human diseases, including cancer. This study aimed to explore the prognostic significance of the mTORC1 gene set and identify potential biomarkers for predictive the therapeutic effectiveness of mTOR inhibitors on TNBC.
Methods: The transcriptional profile of normal tissues and primary tumors derived from TNBC patients was obtained from The Cancer Genome Atlas (TCGA) database and used to generate the differentially expressed genes (DEGs) of mTORC1 gene set from Molecular Signatures Database. Cox regression analysis for the DEGs against overall survival of TNBC patients was employed to identify independent prognostic factors. The Chi-square test of independence was used to study the association among the identified prognostic genes and the clinicopathological characteristics of TNBC patients. RT-PCR, immunohistochemistry (IHC) staining and Western blot experiments were used to detect endogenous expression levels of the identified prognostic genes in a panel of TNBC cell lines. MTT assay were used to assess the sensitivity/resistance of TNBC cells to mTOR inhibitors.
Results: Two potential prognostic genes FADS2 and TOMM40 were identified from 56 differentially expressed genes (DEGs) between normal tissues and primary tumors of TCGA TNBC patients. Where FADS2 is a protective factor (HR= 0.652), TOMM40 (HR= 2.243) serves as a risk factor under overall survival condition. The signature of TOMM40 downregulation and FADS2 upregulation was associated with a favorable overall survival; conversely the higher TOMM40/FADS2 ratio was referred to a poor outcome in TNBC patients. Under overall survival condition, the TOMM40/FADS2 ratio was shown to significantly ($p < 0.05$) associate with age, tumor size, lymph node metastasis, pathologic stage and overall survival event. Notably, cell-based cytotoxicity assay revealed that TNBC cells harboring a higher TOMM40/FADS2 ratio compared to TNBC cells with a lower TOMM40/FADS2 ratio are more sensitive to mTOR inhibitor treatment.
Conclusion: Our results provide the expression ratio of TOMM40/FADS2 genes as a useful biomarker to predict the therapeutic efficacy of mTOR inhibitors in TNBC.

#6820 Artificial intelligence (AI) assisted analysis of plasma cytokine levels in a phase II trial using metronomic chemotherapy on gastrointestinal (GI) cancer patients.

Ryley Stewart[1], Juan C. Silva Espinoza[2], Briana Elise Pinales[2], Cuipeng Qiu[1], Jiangying Zhang[1], Paola Orlandi[3], Vladimir Georgiev[3], Alessandro Michelangeli[4], Kazumasa Fujiwara[5], Giacomo Allegrini[6], Giulio Francia[1], Guido Bocci[3], Maria L. Manca[3]

[1]Biolosciences, The University of Texas at El Paso, El Paso, TX,[2]The University of Texas at El Paso, El Paso, TX,[3]The University of Pisa, Pisa, Italy,[4]Bonn University, Bonn, Germany,[5]Ryukoku University, Kyoto, Japan,[6]Biolosciences, The University of Pisa, Pisa, Italy

We previously reported a metronomic chemotherapy regimen (UFT, a 5-fluorouracil prodrug; 100 mg/twice per day p.o.; plus cyclophosphamide as an i.v. bolus on day 1 and then 50 mg/day p.o.; plus celecoxib 200 mg/twice a day p.o.); evaluated in the COMET trial (ID NCT02926911; Allegrini et al. Angiogenesis 2012: 15(2):275-86) of GI cancer patients. At AACR 2024 we reported analysis of 75 cytokines in the plasma of these patients. Here, we report levels of 22 not previously evaluated cytokines, analyzed for clinical correlations in this trial, in 34 out of the 38 patients. We used AI to perform part of the statistical analysis. AI assisted in the writing of the R or Python programs implemented. We report the identification of baseline plasma levels of soluble Tie-2 (sTie2) and osteopontin as potential predictive biomarkers for the overall survival (OS) of participants to this trial. sTie 2 values showed a positive correlation with OS ($r = 0.43$; $p = 0.019$), while osteopontin was inversely related ($r = -0.41$; $p = 0.024$). Having defined cut-off levels (389 pg/ml for sTie 2 and 412 pg/ml for osteopontin), we observed a stronger association with OS in subgroups of patients above or below the thresholds, as detected by Kaplan-Meier analysis and log-rank tests ($p < 0.01$, in both tests). The chosen cut-offs also were predictive for patients with better Progression Free Survival (PFS). Patients with osteopontin levels below the cut-off had a PFS of 5.5 months, compared to 2 months in patients above the cut-off ($p=0.002$). For sTie2 patients with above cut-off levels had a PFS of 5.9 months versus 2.2 months in patients below the cut-off ($p=0.002$). We also used cytokine levels to generate predictive patient scores. A score of 1 was assigned for each of sTie2 ≤ 388.8 pg/mL, Osteopontin > 412.5 pg/mL, and sHer3 > 185.4 pg/mL baseline levels. We then arranged patients with relation to Stable and Progressive Disease as follows: A) Low Risk (Score 0): Stable Disease at Recist criteria: 62.5% Progressive Disease: 37.5%. B) Intermediate Risk (Score 1): Stable Disease: 45.8% Progressive Disease: 54.2%. C) High Risk (Score 2-3): Stable Disease: 23.1% Progressive Disease: 76.9%. Our results demonstrate an approach using AI to assist in biomarker analysis of clinical trials, and to stratify patients to identify those likely to benefit from metronomic chemotherapy.

#6821 The anticancer effects of polyisoprenylated phosphonyl ester inhibitors (PPEIs) of PMPMEase in pancreatic cancer cells.

Jahnissi Odoom, Amarender Burra, Kweku Ofosu-Asante, Justin Mensah-Mamfo, Joshua Ablordeppey, Nazarius Lamango

Florida A&M University College of Pharmacy & Pharmaceutical Sciences, Tallahassee, FL

Monomeric G-proteins such KRAS, CDC42, RHOA, and RAC1 are polyisoprenylated and methylated to become fully functional. Polyisoprenylated protein methyl transferase (PPMTase) and polyisoprenylated methylated protein methyl esterase (PMPMEase) appear to regulate G-protein function. Previous studies showed that PMPMEase inhibition causes cancer cell death. In the current study, polyisoprenylated phosphonyl ester inhibitors (PPEIs) were developed as *t,t,*-farnesyl-targeted irreversible PMPMEase inhibitors. Experiments were conducted with two pancreatic cancer cell lines, MIA PaCa-2 and PANC-1. NSL-AB-01, NSL-AB-02, and NSL-AB-03 suppressed pancreatic MIA PaCa-2 cells cancer cell viability with EC_{50} values of 4.5, 4.8, and 4.6 μM, respectively, and 5.0, 6.4, and 5.1 μM for PANC-1 cells, respectively. Proliferation was inhibited by 99% and 95% for MIA PaCa-2 and PANC-1 cells at low micromolar PPEIs concentrations, respectively. PPEIs induced caspase activity by over 150% for both cell lines. Phosphorylation of MAPK proteins ERK1/2, MEK1/2, and CRAF were reduced by 77%, 50%, and 42% for MIA PaCa-2 cells, respectively. In PANC-1 cells, ERK1/2 phosphorylation decreased by 75%, while MEK1/2 increased by 140%. The PI3K/AKT proteins, S6 Kinase, PI3K, and AKT, were activated through phosphorylation by 19, 20, and 94%, in MIA PaCa-2 cells. However, opposing effects were observed in PANC-1 cells whereby PDK1 and PI3K phosphorylation were significantly decreased by 30 and 40%, respectively. PPEIs treatment caused actin filaments to become diffused, retracted, and clustered, causing the cancer cells to lose shape and become rounded. These findings show the potential role of PPEIs in disrupting cancer cell proliferation and, consequently, cancer progression.

#6823 Dynamics of KMT2D modulation of the PI3K-Akt pathway and the impact on metastasis in prostate cancer.

Abbas Jawadwala, Surrendra Gulla, Remi Adelaiye-Ogala

University at Buffalo, State University of New York, Buffalo, NY

Despite advances in the treatment of prostate cancers, such as androgen receptor (AR) inhibitors and androgen depreivation therapies (ADT), relapse often occurs, which leads to drug resistant forms of prostate cancer. Understanding the mechanism behind resistance thus becomes important for developing effective treatments. Epigenetic regulators such as KMT2D, a histone 3 lysine 4 methlytransferase, have been implicated in modulating signature pathways associated with cell progression and survival. The PI3K-AKT-mTOR pathway has been studied by many in the field on its role in cancer progression and treatment resistance. However, not as much is known about it being epigenetically regulated. Our preliminary studies demonstrate an association of KMT2D expression/activity and the PI3K-AKT signaling pathway. Interestingly, enrichment analysis in KMT2D knockout treatment-resistant prostate cancer models and their respective controls, demonstrated enriched signatures associated with tumor metastasis and cell adhesion downregulated. While these studies are still preliminary, our results provides an insight to the role of KMT2D in a context dependent mannar, where in an already acquired treatment-resistant state, KMT2D may be promoting a tumor-suppressor-like program and help improve the existing treatment regimen. Ongoing studies will validate our preliminary findings and provide a better understanding of the correlation between KMT2D and these genes along with the PI3K-Akt-mTOR signaling pathway..

#6824 Efficacy and mechanism of surufatinib combination with PD-1 monoclonal antibody and chemotherapy for the treatment of pancreatic cancer with liver metastasis in animal models.

Mengjiao Fan, **Ru Jia**, Guanghai Dai

The Fifth Medical Center, Chinese People's Liberation Army General Hospital, Beijing, China

Background: The phase 1b/2 study of surufatinib plus camrelizumab, nab-paclitaxel and S-1 (NASCA) as first-line therapy has demonstrated superior clinical efficacy compared with nab-paclitaxel and gemcitabine (AG) for metastatic pancreatic adenocarcinoma (PDAC) (NCT05218889), especially in patients with liver metastasis (LM). To delve deeper into the underlying mechanism behind NASCA's effectiveness in the LM group, we conducted animal experiments and utilized multiplex immunohistochemistry (mIHC) technology.
Methods: Two animal models of pancreatic cancer (subcutaneous and LM) were established using KPC cells in C57BL/6 mice. The animals were then randomly assigned to receive placebo, AG, NASCA, and PD-1 plus nab-paclitaxel and S-1 (AS). Human primary pancreatic lesions (PL) and LM lesions were collected, and organ-specific differences were analyzed by using mIHC.
Results: LM changes the effectiveness of NASCA in mice. NASCA outperformed than AG in subcutaneous model. In subcutaneous and LM model, NASCA and AG showed the comparable tumor regression to placebo in subcutaneous tumor, but better than AG in liver tumor. NASCA boosts anti-tumor efficacy in LM by improving immunosuppressive microenvironment. Flow cytometry of peripheral tissues revealed reduced MDSC and PDL1+ macrophages infiltration ($p < 0.05$) and increased NK and activated T cells infiltration ($p < 0.05$) in liver tumors with NASCA. Treg infiltration decreased in both subcutaneous and liver tumors from early (d5) to late (d14) treatment time points. Higher fractions of Treg cells were infiltrated in LM than PL by mIHC: A total of 59 patients with PDAC were enrolled to evaluated the organ-specific response of NASCA therapy. MIHC detected significantly higher fractions of $FOXP3^+$ cells in stroma of LM compared with PL ($p < 0.05$). Patients with $CD68^+CD163^-$ (percent) and FOXP3+ (percent) in stroma may benefit more from NASCA regimen.
Conclusion: LM could alter the efficacy of NASCA in KPC mice through improving immunosuppressive microenvironment. The heterogeneity of mIHC is limited in LM and PL, but patients with $CD68^+CD163^-$ and $FOXP3^+$ in the stroma may benefit more from NASCA regimen, which may contribute to developing precise immunotherapy strategies for PDAC patients with LM.

#6825 Novel therapeutic strategies targeting MECOM rearranged AMLs discovered by unbiased drug screen.

Christine E. Birdwell[1], Warren C. Fiskus[1], Christopher P. Mill[1], Tapan M. Kadia[1], Naval Daver[1], Courtney D. DiNardo[1], Koji Sasaki[1], John A. Davis[1], Kaberi Das[1], Hanxi Hou[1], Surbhi Sharma[1], Rashmi K. Shamanna[1], Michele Ceribelli[2], Kapil N. Bhalla[1]

[1]UT MD Anderson Cancer Center, Houston, TX,[2]NCATS, Bethesda, MD

The MECOM locus at chromosome 3q26.2 encodes for the transcriptional regulator EVI1. In ~10% of *de novo* AML, aberrant overexpression of EVI1 is observed. In approximately 50% of these it is due to inv(3)(q21;q26.2) or due to the chromosomal rearrangement [3q26.2-r], in which GATA2 enhancer is dislocated and drives EVI1 overexpression. 3q26.2-r AMLs tend to be more stem-like, exhibit therapy resistance and poor patient survival. To determine potential druggable targets, we conducted an unbiased drug screen in 3q26.2-r AML cell lines. We screened a library of over 2400 mechanistically annotated drugs (NCATS Mechanism Interrogation Plates), against seven AML cell lines (four 3q26.2-r and three non-3q26.2-r AML). Exploiting the mechanistic redundancy in the library, we identified target-level sensitivities in 3q26.2-r AML cells and relative activity as compared to other AML subtypes. Corroborating previous studies, in 3q26.2-r AMLs, BET protein inhibitors showed absolute sensitivity. We conducted RNA-seq and mass spectrometry analyses after BET inhibitor (BETi) mivebresib (ABBV-075) treatment. The findings revealed a significant overlap with the expression signature determined by RNA-seq and mass spectrometry performed after CRISPR knockout (KO) of EVI1. The dysregulated proteins included MYC, mTOR and TNF-alpha, indicating them to be potential targets or co-targets with BETi in 3q26.2-r AMLs. Since mTOR, PIK3CA, XIAP and Bcl-xL were also identified as druggable sensitivities in 3q26.2-r AML, we tested the activity of the combined mTOR/PI3K inhibitor dactolisib (BEZ235). Exposure to BEZ235 for 48 hours induced moderate levels of apoptosis in 3q26.2-r AML cell lines and patient-derived (PD) AML cells. This was associated with a decrease in the levels of the EVI1 targets, ERG, Bcl-xL, and c-Myc, as well as of c-Myb and EVI1, with simultaneous increase in the cleaved PARP levels. Combination of dactolisib with mivebresib exerted synergistic in vitro lethality in 3q26.2-r AML cell lines and PD AML blasts. Co-targeting with BETi and other identified sensitivities such as the IAP family inhibitor (LCL161) or Bcl-xL inhibitor (navitoclax or A1155463) also exhibited synergistic lethality in 3q26.2-r AML cell lines and PD AML cells. Importantly, compared to the vehicle control, monotherapy with mivebresib (BETi), dactolisib or LCL161 significantly reduced the AML burden and improved survival of the NSG mice in the xenograft models of 3q26.2-r AML. Co-treatment of mivebresib with dactolisib or LCL161 was superior than each drug alone in reducing AML burden and improving survival. These findings demonstrate promising preclinical activity of novel BETi-based combination with IAP or Bcl-xL inhibitor against AML with EVI1 overexpression.

#6826 PRMT5 inhibition is a promising therapeutic strategy for high-risk neuroblastoma.

Parul Suri, Saurabh Agarwal

St. John's University, Queens, NY

Neuroblastoma (NB), a pediatric solid tumor originating from the extracranial sympathetic nervous system, frequently persists as a metastatic, drug-resistant cancer despite recent improvements in dose-intensive chemotherapy, radiation, and surgical approaches. Identifying novel molecular targets and developing effective therapeutic regimens for NB is crucial. PRMT5, an arginine methyltransferase, has emerged as a potential target, with its inhibition demonstrating therapeutic effects in various cancer models. Multiple potent, small-molecule PRMT5 inhibitors are currently in clinical trials or development. In this study, we investigated the pre-clinical effects of a specific PRMT5 targeting small molecule inhibitor, PM5i, on NB growth. Analysis of multiple NB patient datasets revealed an inverse relationship between PRMT5 expression and NB patients' overall survival, underscoring PRMT5 as a potential therapeutic target. Our cytotoxicity and clonogenic assay data demonstrate that PM5i significantly inhibits NB proliferation and colony formation in a dose-dependent manner with IC50 values ranging from 1 to 4 μM across different NB cell lines ($p<0.01$). PRMT5 inhibition by PM5i significantly induced apoptosis with a 2-fold increase in Annexin V-positive cells compared to controls ($p<0.05$) and inhibited cell cycle progression by arresting cells in the S phase with the percentage of cells in the S phase increasing from 22% in controls to 45-50% in treated cells ($p<0.01$). These effects were consistent across different NB cell lines. To recapitulate *in vivo* tumor growth, we employed 3D spheroid models of NB. PM5i treatment significantly inhibited 3D spheroid growth and volume in a dose-dependent manner with about a 2-fold reduction compared to control treatments ($p<0.01$). Molecular analysis revealed that PM5i treatment downregulated the expression of genes involved in critical cell processes by 2 to 3-fold, including AKT, MAPK, and MYCN pathways in NB cells. These pathways are known to play crucial roles in NB pathogenesis and progression. Collectively, our data provide compelling evidence that PRMT5 targeting represents an effective therapeutic strategy for high-risk NB. The potent effects of PM5i on NB cell growth, survival, and key oncogenic pathways highlight the potential of PRMT5 inhibitors in NB treatment. Further development of PRMT5 inhibition strategies and their combination with current chemotherapies may pave the way for efficient clinical translation, holding significant promise for improving outcomes in high-risk NB patients.

#6827 Developing lung adenocarcinoma cell lines to test new drugs and mitigate lung cancer health disparities.

Kweku Ofosu-Asante[1], Sofia Lugo[2], Bianca Dal Bo[2], Matthew A. Gladstone[2], Chunli Yan[2], Daniel J. Mullen[2], Kyle R. Philips[3], Benjamin J. Ryder[3], Yong Huang[3], Ite A. Offringa[2], Nazarius S. Lamango[1]

[1]Florida A&M University College of Pharmacy & Pharmaceutical Sciences Institute of Public Health, Tallahassee, FL,[2]University of Southern California/Norris Comprehensive Cancer Center, Los Angeles, CA,[3]Department of Mechanical and Aerospace Engineering, University of Florida, Gainesville, FL

Lung cancer continues to be the number one cause of cancer-related deaths worldwide and remains a prominent source of cancer health disparities. Black/African American (B/AA) males exhibit a 12% higher lung cancer incidence rate and a 15% higher lung cancer death rate when compared to white males. Lung adenocarcinoma (LUAD) is the most common histological subtype of lung cancer in all ethnic/racial groups, including B/AA individuals. To further examine this phenomenon, we want to create a collection of lung cancer cell lines that would be useful for molecular studies of lung cancer development, progression, development of new targeted therapies, and pre-clinical drug testing. After reviewing the literature (Leon *et al.* 2023, Frontiers in Oncology, Vol. 13, p. 1187585, 2023), we identified only 30 unique B/AA cell lines out of ~800, the majority being from White and Asian patients. Moreover, only 6 of the 30 cell lines are LUAD. In order to mitigate this disparity, we are creating a collection of multiple independent isogenic cell line variants from the existing NCI-H23 lung cancer cell line. NCI-H23 was derived from a B/AA male LUAD patient and harbors a *KRAS*^{G12C} heterozygous mutation. G12C is the most common *KRAS* mutation in LUAD (~40% of *KRAS* mutations) followed by G12V (~20%), G12D (~15%), G12A (~10%), and G13C (~6%). We used CRISPR/Cas9 genome-based editing to replace the *KRAS*^{G12C} mutation with *KRAS*^{G12V}, *KRAS*^{G12D}, *KRAS*^{G12A}, and *KRAS*^{G13C}. We have derived independent cell lines for most mutations. We have performed whole genome sequencing of these cell lines to assess possible off-target effects that may have arisen from the CRISPR/Cas9 engineering. In addition, we will carry out whole genome RNA sequencing to examine the effect of the engineering and/or *KRAS* mutation switching. As a control, we have sequenced the genomes and transcriptomes of three monoclonal cell lines we derived from NCI-H23 cells, to determine what variability ensues with the derivation of monoclonal lines, since that is required to obtain the CRISPR/Cas9 edited cells. We will use this collection of cell lines to study the efficacy of polyisoprenylated cysteinyl amide inhibitors (PCAIs). PCAIs were developed in the Lamango lab to target and suppress hyperactivities of G-proteins in cancers driven by mutant *KRAS*. We will test PCAIs alone, in comparison and in combination anti-KRAS drugs on the new cell lines bearing diverse *KRAS* mutations. Our efforts will provide new cell lines to study lung adenocarcinoma in B/AA subjects addressing a key shortcoming and disparity in the lung cancer research toolkit, thereby facilitating the testing of potential new therapies such as PCAIs in an African ancestry background.

#6828 Therapeutic potential of novel polyisoprenylated phosphonyl ester inhibitors in triple-negative breast cancer.

Joshua K. Ablordeppey, Amarender Burra, Kweku Ofosu-Asante, Jahnissi Odoom, Nazarius S. Lamango

Pharmaceutical Sciences, Florida A&M University, Tallahassee, FL

Hyperactive monomeric G-proteins such as RAS are dominant “drivers” in cancer. In triple negative breast cancer (TNBC), RAS hyperactivity is due to low levels of the RAS-GTPase activating protein, RASA1, a tumor suppressor. KRAS is polyisoprenylated and methylated to become functional. Polyisoprenylated methylated protein methyl esterase (PMPMEase) is overexpressed in various cancers and its specific inhibition causes cancer cell death. Polyisoprenylated phosphonyl ester inhibitors of PMPMEase (PPEIs) were designed to suppress hyperactive RAS-promoted cancers through PMPMEase inhibition. Here, we evaluated the anti-proliferative properties of the PPEIs against two TNBC cell lines, MDA-MB-468 and MDA-MB-231. The PPEIs, NSL-AB-01, NSL-AB-02 and NSL-AB-03 inhibited TNBC cells with EC_{50} values of 3.3 μM, 4.0 μM and 3.6 μM for MDA-MB-468 and 4.1 μM, 5.6 μM and 5.6 μM for MDA-MB-231, respectively. Following 48 h exposure to 1 μM NSL-AB-01, the proliferation of MDA-MB-231 and MDA-MB-468 cells was inhibited by 48% and 8%, respectively. At 2 to 3 μM concentrations, the proliferation of both cell lines was completely blocked. In addition, NSL-AB-01 inhibited the migration of MDA-MB-231 and MDA-MB-468 cells by 95% at 5 μM and 73% at 3 μM, respectively, thereby suggesting potential antimetastatic effects of the PPEIs. Further investigations are in progress to ascertain the molecular mechanisms underlying these effects. Overall, these findings suggest the therapeutic potential of PPEIs as novel agents for TNBC and other cancers with activated G-protein signaling.

#6829 Allele frequency of DPYD genetic variants for fluoropyrimidines metabolism in a Northeast Mexican population.

Maria L. Garza-Rodriguez, Francisco J. Villarreal-Alvarado, Axa N. Vega-Garcia, Valeria J. Gomez-Ordaz, Irasema Martinez-Ramos, Vanessa N. Ortiz-Murillo, Azalea E. Urdiales-Ordonez, Alan Burguete-Torres, Fernando Alcorta-Nunez, Carlos H. Burciaga-Flores, Moises Gonzalez-Escamilla, Oscar Vidal-Gutierrez, **Diana C. Perez-Ibave**

Oncology, Centro Universitario Contra el Cancer UANL, Monterrey, Mexico

Background: Fluoropyrimidine-based (FP) chemotherapy is used to treat solid cancers, including colorectal and breast cancer. The dihydropyrimidinedehydrogenase (DPD) enzyme is in charge of the metabolism of FP; this enzyme is coded by the dihydropyrimidine dehydrogenase (*DPYD*) gene. Genetic variants in*DPYD* have been related to an increased risk of severe toxicity. FP toxicity affects about 30-40% of patients, which in some cases may be lethal. *DPYD* genotyping aims to identify variants that lead to DPD deficiency. The allele frequency of most of the*DPYD* variants in the Mexican population is currently unknown. Variant identification in the Mexican population can help oncologists in the treatment of patients by reducing the chances of toxicity by adjusting treatment without comprising the antitumoral activity. The aim was to describe the frequency *DPYD* gene variants associated with FP toxicity in northeastern Mexico's oncologic population.

Methods: We analyzed seven *DPYD* variants by real-time PCR and Sanger sequencing. Two related to reduced activity (c.1129-5923C->G and c.2846A->T)associated with increased risk for toxicity, and four with null activity (c.1156G->T,c.1905+1G->A, c.1679T->G, c.1898delC, and c.299_302delTCAT) with a higher and severe risk for toxicity. Genomic DNA was isolated from 361 subjects: 117 cancer patients and 244 non-cancer subjects. Allele frequency was calculated for each variant and correlated with clinical data.

Results: We analyzed a total of 361 subjects, including 117 cancer patients, of which68% had an oncological diagnosis of colorectal cancer, followed by breast cancer with 14%. Metastasic and clinical stage III were present in 42% and 34%, respectively. Grade III (G3) gastrointestinal toxicity was the most common, with a presence of31.9%. The variant c.1129-5923 C>G was found in the heterozygous state in 1.2295%(n=3) of non-cancer patients, the c.2846 A>T variant was found in one non-cancer patient 0.4098% (n=1), and in a 1.7094% (n=2) of cancer patients. The rest of the variants were found in a state of native homozygosity. The two patients carrying the c.2846A>T variant presented a G3 and G4 respectively; one patient required dose adjustment, and the other patient suspended the FP treatment.

Conclusions: The allele frequency for two of the seven analyzed variants (c.1129-5923C>G, and c.2846A>T) was higher than reported for the global population (0.00476and 0.005166). *DPYD* genotyping may help to identify people who are at higher risk of developing severe FP toxicity. Personalized medicine allows oncologists to modify the treatment before it begins.

#6830 JZP815, a potent and selective pan-RAF inhibitor, is effective against a spectrum of mutant KRAS and NRAS solid tumor cancers, as monotherapy and in combination, generating a gene expression profile of MAPK inhibition.

Robert Hauptschein[1], Ankur Karmokar[2], Emanuele Loro[2], Yang Yuan[1], Graham Brock[1], **Robin C. Humphreys**[1], Alexey Sorokin[3], Preeti Kanikarla[3], Scott Kopetz[3]

[1]Jazz Pharmaceuticals, Inc., Palo Alto, CA,[2]Jazz Pharmaceuticals, Inc., Cambridge, United Kingdom,[3]University of Texas MD Anderson Cancer Center, Houston, TX

Activation of the mitogen-activated protein kinase (MAPK) pathway by oncogenic mutations in RAS and RAF are frequent drivers of human cancer. Targeting specific components of the MAPK pathway is a precise and rational route to deliver benefits to cancer patients. RAS and RAF proteins are core pathway components that interact directly to mediate MAPK signaling. Direct and indirect inhibition of this key MAPK pathway intersection is critical in driving responses in patients with RAS and RAF mutations. JZP815 (flezurafenib) is an oral pan-RAF inhibitor under development by Jazz Pharmaceuticals. We examined in vitro and in vivo effects of JZP815 pan-RAF inhibition as a single agent and in combination, in multiple RAS and RAF mutant cell and tumor model systems, and those arising from KRAS G12C inhibitor therapy. Lastly, we established a pharmacodynamic (PD) profile indicating JZP815 MAPK pathway engagement. Specifically, in vitro studies in a broad KRAS and NRAS mutant tumor cell line dataset revealed a complex and extensive potency profile of JZP815. Mutant NRAS lines demonstrated significant anti-proliferative response, whereas KRAS mutant lines showed a more diverse response profile. JZP815 inhibited cell proliferation in KRAS mutant cell lines with acquired resistance mutations known to arise in patients after KRAS inhibitor therapy, including acquired KRAS and BRAF mutations. JZP815, in combination with a MEK inhibitor, generated synergistic antiproliferative activity in mutant NRAS melanoma cells lines. In vivo CDX and PDX models showed JZP815 activity against KRAS, NRAS, and BRAF mutant (class 1, 2, 3) xenografts. In BRAF class 1 melanoma PDX models, JZP815 monotherapy generated a more robust antitumor effect compared to 2nd-generation RAF inhibitors. JZP815 demonstrated activity alone and in combination with SOC agents in CRC PDX models, derived from patients who had progressed on KRAS G12C inhibitors. Using bioinformatic analysis, PD profiles were generated that indicated direct impact of JZP815 in MAPK signaling on xenograft tumors bearing KRAS mutations. Whole transcriptome profiling of a KRAS G13D mutant tumor model showed JZP815 suppressed a clinically relevant transcriptional biomarker of MAPK pathway inhibition (MPAS). In addition, the suppression of MPAS gene signature was associated with JZP815 exposure. These PD data support dose- and time-dependent MAPK pathway and target engagement by JZP815 in preclinical models. In summary, JZP815, a potent selective pan-RAF inhibitor, is active against a variety of KRAS, NRAS, and BRAF mutants, and KRAS inhibitor generated mutants, as a single agent and in combination. JZP815 elicits a clinically verified PD profile that could be used to confirm target engagement in patients. JZP815 is currently in phase 1 testing (NCT05557045).

#6831 Systems biology analysis of vitamin D_3 reveals new anticancer properties.

A. M. Sunderji[1], S. Roy[1], A. Aggarwal[1], **K. W. Yip**[2];
[1]University of Toronto, Toronto, ON, Canada, [2]Anticancer.ca, University of Toronto, Toronto, ON, Canada

Introduction: Vitamin D_3 (also known as cholecalciferol) is an essential nutrient and hormone that is obtained through both sunlight-mediated cutaneous synthesis and direct dietary intake. Vitamin D_3 functions to inhibit tumor cell growth by arresting the cell cycle at G0/G1 phases, increasing apoptosis, and reducing nuclear β-catenin levels to prevent the formation of cancer-promoting T-cell factor complexes. This study identified the cancer cell lines most responsive to *in vitro* treatment with vitamin D_3, as well as the compounds and genetic perturbations that are most similar to treatment with vitamin D_3.

Methods: For cell line response analyses, the NCI60 dataset from the US National Cancer Institute was used. Human cancer cell lines were incubated for 24 hours and treated with cholecalciferol for 48 hours at 5 different ten-fold serial dilution concentrations. Sulforhodamine B was used to measure cell survival. To identify genetic and compound perturbations similar to vitamin D_3, data was obtained from the L1000 gene expression assay and CLUE. In this assay, mRNA transcript abundance was measured in 978 human landmark genes and inferred for over 11,000 others to compare gene expression levels to those induced by vitamin D_3.

Results: The least sensitive cell line to vitamin D_3 was the multiple myeloma cell line RPMI-8226 (IC_{50}=56.3 μM), and the most sensitive was the colorectal cancer cell line HCT-116 (IC_{50} = 18.2 μM). Overall, vitamin D_3 was most potent against lymphoid, renal, and melanoma cancer cell lines, and least potent against myeloma cell lines. As indicated by high median tau scores, administration of vitamin D_3 induced gene expression changes similar to OTX2 and ZNF114 knockdowns; OTX2 is a transcription factor associated with neurological organ development while ZNF114 is a zinc-finger protein important for transcription regulation, and both have a biological network connection to vitamin D_3. Vitamin D_3 treatment has similar genetic effects to known therapeutic compounds like VX-702, nelfinavir, and calcipotriol.

Conclusions: This study provides a comprehensive *in vitro* examination of the cancer cell lines most impacted by treatment with vitamin D_3. Further research will help better inform the use of vitamin D_3 in nutrition plans and guide its use in cancer prevention and treatment.

#6832 SYS6041, a next-generation Folate Receptor α-targeted antibody-drug conjugates for the treatment of FRα-expressing malignancies.

Jieru Liu[1], Huan Li[2], **Mo Dan**[1], Xiwu Hui[2], Bin Yao[2], Yang Zhang[1], Linlin Xiao[1], Bin Kang[1], Can Yuan[2], Bin Bao[2], Miaomiao Wei[1], Chunqing Qu[1], Yanling Li[1], Wei Yang[1]

[1]CSPC Pharmaceutical Group Ltd., Beijing, China,[2]CSPC Megalith Biopharmaceutical Technology(Shijiazhuang) Co., Shijiazhuang, China

Folate Receptor α(FRα)exhibits an increased expression on cell surfaces in multiple solid tumors, including ovarian, lung, endometrial cancer, with limited expression in normal tissues. Recently, an antibody drug conjugate (ADC) with a microtubule inhibitor (MTI) payload, was approved by FDA for FRα-expressing platinum-resistant ovarian cancer. However, it can cause severe ocular toxicities and show therapeutic efficacies only in patients with high expression. In order to address the unmet needs of additional patient populations, we sought to develop a next generation FRα-targeting ADC, SYS6041, actively targeting tumors with a broad range of FRα expression, especially moderate or low expression levels. SYS6041 was developed adopting a novel ADC platform with a camptothecin-based topoisomerase 1 inhibitor payload, Exatecan, tethered to a cleavable linker. The drug-to-antibody ratio standed 8.0. Upon binding to FRα, SYS6041 underwent receptor-mediated internalization. Subsequent proteolytic cleavage released the cytotoxic payload Exatecan, leading to DNA strand breaks and consequently disrupting DNA replication and transcription. SYS6041 demonstrated potent cytotoxicities against FRα expressing cancer cell-lines and effective bystander activities towards tumor cells without FRα expression. It demonstrated superior antitumor effects across ovarian endometrial and lung cancer CDX models compared to the MTI-ADC especially in models with low or moderate FRα expression, with a minimum effect dose(MED) at 0.75 mg/kg. In a FRα moderate ovarian PDX model with Olaparib resistance, SYS6041 significantly inhibited tumor growth at 3 mg/kg, whereas the MTI-ADC showed no inhibitory effect at 6 mg/kg. Furthermore, Exatecan demonstrated a significant enrichment in the tumors of mice bearing tumors, achieving a tumor-to-blood ratio of 182 following a dosage of 6 mg/kg of SYS6041.SYS6041 exhibited a stable PK profile with AUC of Exatecan less than 0.01% of that of total Exatecan in ADC. In a GLP toxicity study in cynomolgus monkey, SYS6041 demonstrated an encouraging tolerability with an HNSTD of 50 mg/kg, and favorable PK and DAR stability, and presented a good tolerability profile with therapeutic window (TW)>66 (HNSTD/MED). In conclusion, the distinct mode of action along with the proprietary drug-linker platform resulted in an excellent in-vivo antitumor potency and safety, which supports the potential of SYS6041 as a novel therapeutic agent that may help address unmet needs in patients with moderate and low FRα expressing malignancies.

#6833 Influence of g*uthation S transferase* T1 and M1 polymorphisms on tamoxifen metabolism in malian women with breast cancer.

Brehima Diakite[1], Yaya Kassogue[1], Mamoudou Maiga[2], Oumar Kassogue[1], Fousseyni Diarra[1], Kadidiatou Cisse[1], Aissata Coulibaly[1], Cheick B. Traore[1], Madani Ly[1], Jane L. Holl[3], Lifang Hou[2]

[1]Univ. of Sciences, Techniques & Technologies of Bamako (USTTB), Bamako, Mali,[2]Northwestern University, Chicago, IL,[3]University of Chicago, Chicago, IL

Background: The metabolism of tamoxifen, used in breast cancer treatment, is influenced by the *GSTT1* and *GSTM1* genes. Women were classified as poor (inactivated genes), intermediate (reduced enzyme activity), or rapid (functional genes) metabolizers. This classification helped to understand how genetic variations affected treatment efficacy and side effect risk, promoting a personalized therapeutic approach. This study aimed to analyze the impact of *GSTT1* and *GSTM1* genetic variations on tamoxifen metabolism in Malian women with breast cancer.
Methods: A cohort study was conducted involving 109 Malian women (ages 18-50) diagnosed with ER/PR (+) breast cancer and eligible for tamoxifen treatment. *GSTM1* and *GSTT1* genotypes were determined using multiplex PCR.
Results: A significant correlation was observed between the *GSTM1* gene and family history of breast cancer ($p = 0.026$) as well as tumor size ($p = 0.031$). The genotypic frequencies were 75.2% for functional *GSTM1* and 24.8% for inactive; for *GSTT1*, 56.9% were functional and 43.1% inactive. The categories of metabolizers were distributed as follows: 42.2% (rapid), 14% (intermediate), and 11% (poor), with a significant difference ($p < 0.0001$).
Conclusion: This study highlights the correlation between the *GSTM1* and *GSTT1* genes and factors related to breast cancer. Genotypic variations affect tamoxifen metabolism, emphasizing the importance of genetic factors in optimizing treatment.

#6834 The Region C non-pungent capsaicin analogs ARVANIL and DOHEVANIL sensitize human ovarian cancer cells towards the growth-inhibitory activity of gemcitabine.

K. B. Conley[1], S. D. Richbart[1], K. J. Modi[1], R. S. Light[1], R. S. Gadepalli[2], J. R. Rimoldi[2], T. E. Long[3], Y. Chen[4], P. Dasgupta[1];
[1]Marshall University School of Medicine, Huntington, WV, [2]University of Mississippi, University, MS, [3]Marshall University School of Pharmacy, Huntington, WV, [4]Bluefield State University, Huntington, WV

Purpose of the Study: Ovarian cancer (OC) is the second most common gynecologic cancer in the United States. Ovarian cancer causes more deaths than any other cancer (of the female reproductive system) in women. Combination chemotherapy involving cisplatin and taxanes is the cornerstone for ovarian cancer treatment. The challenge with ovarian chemotherapy is that patients develop cisplatin-resistance (and/or taxane-resistance) upon prolonged exposure to the drug. Gemcitabine is one of the second-line therapies for relapsed ovarian cancer. Several convergent studies show that nutritional phytochemicals can often improve the therapeutic response of chemotherapeutic drugs. The long-term objective of our laboratory is to identify dietary chemicals that may improve the anti-cancer activity of chemotherapeutic drugs. Our published data show that capsaicin (the spicy component of chili peppers) enhanced the pro-apoptotic activity of the chemotherapy drug camptothecin in human small-cell lung cancers. Apart from capsaicin, we have also identified non-pungent capsaicin analogs namely ARVANIL and DOHEVANIL which display greater growth-inhibitory activity in human Ovarian carcinoma cells relative to capsaicin. The objective of the present research project was to explore the combinatorial activity of gemcitabine and these capsaicin analogs in ovarian cancer.
Experimental Procedures: Caspase-3 Activity Assays and Cell Death ELISA assays were used to measure the pro-apoptotic activity of the combination of gemcitabine and Arvanil in human ovarian cancer cells. We also determined the combinatorial pro-apoptotic activity of gemcitabine and dohevanil in human ovarian cancer cells. The results obtained from the apoptosis assays were confirmed using the soft agar assay and the chicken chorioallantoic membrane assay.
Results: Both arvanil and dohevanil sensitized human ovarian cancer cells towards the growth-inhibitory activity of capsaicin
Conclusions: The combination of platinum drugs with capsaicin and capsaicin-like compounds may improve the pro-apoptotic activity of cisplatin in human ovarian cancer.
Support or Funding Information Funding for our study was supported by the NIH R15-AREA Grant (2R15CA161491-02 and 2R15CA161491-03), the Women's Health T3: 3P20GM103434-23W1 (PI: Dr. G Rankin) to PD and MAV and the NIAID-AI151970 grant to TEL. Furthermore, this study was supported in part by the West Virginia IDeA Network of Biomedical Research Excellence (WV-INBRE) grant (NIH grant P20GM103434; PI: Dr. G. Rankin), the National Institute of General Medical Sciences of the National Institutes of Health under the award number P30GM122733.

#6835 Inhibition of platelet activation and antibody-dependent cellular cytotoxicity by the tyrosine kinase inhibitor dasatinib in an *ex vivo* human whole blood system.

Sakthi Srinivasan[1], Ali Zirakzadeh[1], Sofia Ramstrom[2], Erika A. K. Fletcher[1], **Helena Harlin Laven**[1]

[1]Immuneed AB, Uppsala, Sweden,[2]School of Medical Sciences, Orebro University, Orebro, Sweden

Dasatinib is a second-generation tyrosine kinase inhibitor approved for therapeutic use in patients with Philadelphia-positive (Ph+) chronic myeloid leukemia. Dosing of dasatinib is limited by the potential for side effects, including pleural effusion and bleeding disorders. The propensity for bleeding problems is at least in part due to dasatinib inhibiting normal platelet functions. While dasatinib is effective in treating leukemia, the challenge in dosing is associated with a risk for emergence of the T315I mutation of BCR-ABL and potential tumor escape. Thus, the ability to combine dasatinib with other therapeutic agents is desirable and currently under investigation. We have developed an ex vivo test system using circulating fresh human whole blood, enabling direct study of the effects of various compounds on platelet aggregation and activation in a physiologically relevant environment. To see whether the clinical effects of dasatinib could be recapitulated in the test system, we incubated blood from healthy donors in the system with dasatinib prior to stimulation with Thrombin Receptor (PAR1) Activating Peptide 6 (TRAP-6). As predicted, dasatinib significantly inhibited TRAP-6-mediated platelet aggregation and activation as measured by whole blood free platelet counts, platelet expression of CD62P, and the formation of platelet-white blood cell conjugates. At the Cmax concentration, alemtuzumab (anti-CD52) results in antibody-dependent cellular cytotoxicity (ADCC) and release of large amounts of cytokines in the test system, mimicking clinical effects of the drug. We also see a drop in platelet count and platelet activation, correlating with acute effects of alemtuzumab observed in patients. Based on these observations, alemtuzumab was used in the system to investigate whether dasatinib could inhibit platelet activation by a therapeutic drug with known effects on platelets. Dasatinib significantly inhibited the drop in free platelet count and platelet activation seen in response to alemtuzumab. As dasatinib could potentially inhibit multiple signaling pathways, we also investigated whether it had effects on ADCC of CD52+ cell types (lymphocytes and monocytes) by alemtuzumab in the ex vivo system. The decreased lymphocyte and monocyte counts observed in alemtuzumab-treated samples were significantly increased by the addition of dasatinib, whereas non-CD52 expressing cells (such as neutrophils) were not affected. Taken together, the data suggest that the ex vivo test system can be used to investigate the effect of different therapeutic agents on platelet activation and that dasatinib at concentrations that inhibit platelet activation may also inhibit ADCC. Combination therapies involving dasatinib and antibodies with predicted ADCC-dependent effects may thus require further investigation prior to clinical use.

#6836 Analysis of 81 plasma cytokine levels in a phase II trial of gastrointestinal cancer patients treated with metronomic chemotherapy: Incorporation of artificial intelligence and mathematical modeling.

Maria L. Manca[1], Briana E. Pinales[2], Juan C. Silva Espinoza[2], Ryley D. Stewart[2], Diana L. Prospero[2], Mariana N. Frias[2], Mia E. Montelongo[2], Hector M. Padilla[2], Claire E. I. Perucho[2], Brianna E. Ortega[2], Valeria V. Lopez[2], Paola Orlandi[1], Arianna Bandini[2], Mariana J. Silva[2], Marta Banchi[1], Vladimir Georgiev[1], German Rosas-Acosta[2], Kazumasa Fujiwara[3], Alessandro Michelangeli[4], Guido Bocci[1], Giacomo Allegrini[1], **Giulio Francia**[2]

[1]The University of Pisa, Pisa, Italy,[2]The University of Texas at El Paso, El Paso, TX,[3]Ryukoku University, Kyoto, Japan,[4]Bonn University, Bonn, Germany

Metronomic, or continuous low-dose, chemotherapy can inhibit tumor growth via inhibition angiogenesis and immune system activation. At AACR 2024, we reported multiplex analysis of 75 cytokines in 34 out of 38 patients with gastrointestinal (GI) tumors, who were evaluated in a phase II clinical trial of metronomic UFT (a 5-fluorouracil prodrug; 100 mg/twice per day p.o.) and cyclophosphamide (an i.v. bolus on day 1 of 500mg/mq2 and then 50 mg/day p.o.) plus celecoxib (200 mg/twice a day p.o.); the COMET trial (CLINICAL ID NCT02926911; Allegrini et al. Angiogenesis 2012: 15(2):275-86). We now present analysis, including Artificial Intelligence (AI) input (Claude AI built by Anthropic, and Gemini AI built by Google), and mathematical modeling, of plasma levels of 81 cytokines in 34 of the 38 patients. Our analysis included baseline (day 0), days 28 and 56, and days 84 and 112 of the trial, and correlated specific cytokines levels with Overall Survival (OS) and Progression Free Survival (PFS). We now report previosly not evaluated potential biomarkers for this trial, including soluble Her-3 (sHER3). We used AI as confirmatory analysis of data elaboration, firstly performed by R and Stata packages. Building on our previous report of plasma levels of TRAIL and MCP-4 cytokines showing positive correlations with OS, we now report that sHER3 showed an inverse relationship with OS. Patients with sHER3 values ≤185.4 pg/ml presented better prognosis (median OS: 12.1 months) compared to the group with higher levels (median OS: 5.8 months). With regards to PFS, patients with sHER3 levels below the cut-off of 185pg/ml had a better PFS (5.8 months), compared to those above the cut-off (2.3 months; p=0.005). These results add to our previous reporting that TRAIL and MCP-4 levels had a direct association with PFS. As a proof of concept multifaceted analytical approach, we also built mathematical modeling assisted by AI. This approach developed a mathematical model for PFS prediction based on three interacting components: tumor dynamics (by using RECIST measurements), relative pro-angiogenic activity (using baseline VEGF as proxy), and immune response (using baseline IL2 as proxy). AI helped us to optimize the parameters of Ordinary Differential Equations and to predict PFS. Our approach could allow the stratification of GI patients for future metronomic chemotherapy trials, and the potential identification of patients that should instead be offered alternative therapies. Limitations of the present study are that all methods are calibrated on retrospective single-center data. Additional validation will be needed to confirm the robustness of our model.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: New Nonclinical Models for Targets
Poster Session

#6840 FGFR cell lines: A comprehensive approach to drug discovery and evaluation, from small molecules to biologics, covering binding to activation.

Siyu Li, Yao Peng, Yu Wang, Maimaitiming Nuermanguli, Jinying Ning, **Feng Hao**

Kyinno Biotechnology Co., Ltd, Beijing, China

Fibroblast growth factors (FGFs) bind to their receptors (fibroblast growth factor receptors, FGFRs), activating downstream signaling pathways, which play crucial roles in both mitogenic processes, such as embryogenesis and growth, and non-mitogenic processes, such as neuroregulation and metabolic regulation. FGFRs primarily consist of four subtypes: FGFR1, FGFR2, FGFR3, and FGFR4. Abnormalities like high expression or mutations in these receptors can lead to the aberrant activation of their signaling pathways, resulting in tumorigenesis. Consequently, targeting FGFR is a promising strategy for cancer therapy. Current FGFR-targeting drugs, either in clinical use or under development, include small molecule inhibitors, antibodies, antibody-drug conjugates (ADCs), CAR-T therapies, and soluble FGFR receptors. Different cell models are required for efficacy evaluation of various drug types. Kyinno has developed a comprehensive series of FGFR-related cell lines, which include Ba/F3 kinase cells, FGFR overexpression cells, knockout cells, and reporter cells. Among these, Ba/F3 cells have been meticulously engineered to depend on various FGFR point mutations and fusion mutations for survival, showcasing robust capabilities in evaluating FGFR small molecule inhibitors. The FGFR overexpression and knockout cells are crucial for assessing antibody binding, ADC drug endocytosis, and certain radioimmuno conjugates (RICs). Reporter cells, designed based on FGFR downstream signaling pathways, cover a spectrum of wild-type and point-mutated FGFR subtypes, thus effectively evaluating the impact of drugs on downstream signaling pathways. This strategic array of cell models collectively enhances the development and validation of FGFR-targeted therapies, providing invaluable insights and tools for advancing cancer treatment research. By leveraging these diverse cell models, researchers can better understand the mechanisms of FGFR-targeted interventions, ultimately contributing to more effective therapeutic strategies.

#6841 Improved Ba/F3 kinase engineered cell lines: an innovative in vivo screening platform.

Hao Huang, Yunpeng Zhai, Xuyang Duan, Na Li, Siyu Li, Guoqian Wang, Jinying Ning, **Feng Hao**

Kyinno Biotechnology Co., Ltd, Beijing, China

Background: Ba/F3 engineered cells commonly cause tumor metastasis when implanted into immune-deficient mice, resulting in reduced survival time and narrower dosing windows. To overcome this limitation, we have developed a novel approach that modifies these cells without compromising target pathway activation while significantly reducing their metastatic potential. This modification extends both the survival duration and the therapeutic window available for treatment interventions.
Methods: We utilized two distinct cell lines, KC-2400 Ba/F3-TPR-Met-D1228A and KC-4183-Ba/F3-plus-TPR-MET-D1228A respectively. Both cell types were implanted subcutaneously (SC) into NOD SCID female mice. Key parameters such as time to staging at 100 mm^3, time to reach 1000 mm^3, time to first death, dosing window duration, and average tumor volume (ATV) at the point of first death were meticulously recorded and compared between the two groups.
Results: The modified cell line demonstrated a slower progression rate, taking 28 days to reach 1000 mm^3 compared to 18 days for the unmodified group. Survival time increased by 11 days, from 24 days to 35 days, while the therapeutic dosing window expanded from 14 days to 25 days. Futhermore, the ATV at the time of first death was reduced from approximately 1700 mm^3 to around 1300 mm^3, reflecting better tumor growth control.
Conclusion: Engineering Ba/F3 cells to diminish their metastatic capability effectively prolongs survival and broadens the therapeutic window in immune-deficient mouse models. This strategy offers a promising enhancement for preclinical research and holds potential for improving therapeutic outcomes in related clinical applications.

#6842 LgBiT-HiBiT engineered cell lines for protein degradation detection: Comprehensive PROTACs evaluation platform with endpoint and real-time kinetic analysis.

Siyu Li, Shi Qin, Yao Peng, Yunpeng Zhai, Jinying Ning, **Feng Hao**

Kyinno Biotechnology Co., Ltd, Beijing, China

Small molecule drugs have long been integral to disease treatment, but but their limitations have become increasingly apparent over time. The advent of proteolysis-targeting chimeras (PROTACs) offers a promising solution to some of these challenges. PROTACs, also known as hetero-bifunctional compounds, represent a new therapeutic class that includes a specific ligand to bind the target protein, a suitable linker, and an E3 ubiquitin ligase substrate. Upon binding to target proteins, PROTACs recruit an E3 ligase and induce the degradation of target proteins through the ubiquitin-proteasome system. This mechanism allows PROTACs to address protein targets with small-molecule binding ligands that exhibit limited efficacy, broadening the scope of druggable proteins. Additionally, unlike traditional small molecules that merely inhibit protein activity, PROTACs eliminate both enzymatic and non-enzymatic functions of target proteins, reducing the likelihood of drug resistance. Currently, Western Blot (WB) technology is widely used to validate target protein degradation and evaluate the efficacy of PROTACs. While effective for detecting significant protein level changes, WB is time-consuming, lacks precise quantification, and is impractical for monitoring protein degradation at multiple time points due to its high cost and labor intensity. To efficiently and accurately evaluate PROTACs, Kyinno have developed a series of cell models based on the LgBiT-HiBiT technology. LgBiT and HiBiT are high-affinity protein subunits that together constitute NanoLuc luciferase, catalyzing the luminescence of a luciferin substrate detectable by a microplate reader. We have achieved stable overexpression of LgBiT protein in cells and utilized CRISPR-Cas9 gene editing technology to insert HiBiT tags at the N- or C-terminal of endogenous target genes. These cell models are suitable for both endpoint and pharmacokinetic studies. Using a luciferase substrate appropriate for live-cell detection, the luminescence value reflects the current level of HiBiT protein, which correlates with the level of the target protein. Additionally, employing a long-term luciferase substrate allows real-time monitoring of protein levels at each time point. Currently, these cell lines target proteins such as IRAK3, GSPT1, GSPT2, ELAVL1, and GRK2. They support high-throughput screening of PROTACs, molecular glues, lysosome-targeting chimeras (LYTACs), and antibody-based PROTACs (AbTACs), offering a robust platform for advancing drug discovery and development.

#6843 MegaScreen, a useful platform for ADC drug evaluation.

Guoqian Wang, Yao Tang, Jingxiao Xu, Tingduo Lv, Jinying Ning, **Feng Hao**

Kyinno Biotechnology Co., Ltd, Beijing, China

Since the approval of the first antibody-drug conjugate (ADC) , Mylotarg, in 2000, there are now 15 approved ADCs on the market, with over 210 in clinical trials. The development of ADC drugs has entered a flourishing stage, particularly showing significant efficacy and good safety in targets such as HER2, EGFR, Trop2, CLDN18.2, and Nectin-4. The application prospects of ADC therapy in cancer treatment are very broad. ADCs are drugs composed of three parts: a monoclonal antibody, a linker, and a toxin. Among these, the ADC toxin, also known as the ADC payload, is a crucial component of ADC drugs. The toxin is the key to determining the potency of the ADC drug. The payloads of approved ADC drugs are highly toxic, primarily including MMAE/MMAF, calicheamicin, DM1/DM4, SN38/Dxd, etc. These payloads are 1-2 orders of magnitude more toxic than chemotherapy drugs, with some even reaching pM levels. The mechanisms of action of the payloads currently under development mainly include DNA alkylating agents, DNA topoisomerase inhibitors, microtubule disruptors, and RNApolII inhibitors. We have established a substantial cell bank, containing over 800 human tumor cell lines and 100 animal tumor cell lines, covering all types of cancers. Based on this rich cell bank, we are building an Megascreen platform. Through extensive testing, we obtain efficacy data for different ADC payloads across various cell lines, guiding us in selecting suitable cell lines for in vitro evaluation of ADCs. To date, we have tested more than 200 commonly used cell lines particularly breast cancer cell lines. with several ADC palyloads, such as MMAE, SN38, Dxd, exatecan. Our plan is to complete testing of all cells within a year. This Megascreen platform is a large-scale project that will significantly contribute to the screening and evaluation of ADCs.

#6844 In vitro and in vivo screening platform for discovery of JAK2 inhibitors.

Na Li, Xinyu Zhong, Hao Huang, Guoqian Wang, Xuyang Duan, Yunpeng Zhai, Jinying Ning, **Feng Hao**

Kyinno Biotechnology Co., Ltd, Beijing, China

Background: The JAK-STAT signaling pathway functions as a widely utilized mechanism triggered by cytokines, playing a crucial role in regulating various key biological activities , including cell proliferation, cell differentiation, cell apoptosis, and immune response. JAK (Janus Kinase) kinases belong to a family of intracellular non-receptor tyrosine kinases. This family consists of four core members: JAK1, JAK2, JAK3, and TYK2. Among them, JAK2 is particularly significant in mediating signal transmission for hematopoietic growth factor receptors. It facilitates signaling for various cytokines, such as Thrombopoietin (TPO), Erythropoietin (EPO), and Macrophage colony stimulating factor (GM-CSF), thereby regulating the proliferation of hematopoietic cells. Consequently, abnormalities in JAK2 are closely are strongly linked to various diseases, notablypolycythemia vera (PV), myelofibrosis (MF), and essential thrombocythemia (ET). The objective of this study is to assess the effectiveness of JAK2 inhibitors, investigate their underlying mechanisms of action, and determine the optimal dosing regimen to maximize therapeutic benefits while minimizing adverse effects..

Method: The study utilized several cell lines, including Ba/F3-MPL cell line, Ba/F3-EPER-JAK2-V617F cell line, Ba/F3-EPER-JAK2-V617F-mJAK-KO cell line, Ba/F3-MPL-W515L-JAK2 and Ba/F3-MPL-JAK2-V617F cell lines to investigate the effects of fluconazole. *In vitro*, these cell lines were treated with different concentrations of fluconazole and used p-STAT5 alphaLISA to detect the inhibitory effect of fluconazole on downstream signaling pathways. *In vivo*, the cell lines were inoculated into BABL/c nude mice through tail vein injection to form a mouse model of bone marrow proliferative disease caused by JAK2 abnormal activation. We weighed and photographed the liver and spleen, and detected the blood biochemistry and routine of the model mice. We also detected the pathological changes of the spleen through HE staining and reticular protein fibrosis staining.

Results: *In vitro* finding demonstrated that Ruxolitinib significantly inhibited the phosphorylation level of STAT5 downstream of JAK2 in a dose-dependent manner. The *in vivo* experiments revealed that, compared with the sham group, the disease model mice exhibited significantly increased spleen and liver weights. Additionally, higher cell inoculation levels led to shorter survival times, elevated serum ALT and AST levels, and aggravated spleen fibrosis. Treatment with Ruxolitinib significantly alleviated the burden on the liver and spleen and prolonged the survival of the treated mice.

Conclusion: We successfully established both *in vitro* and *in vivo* screening platform for JAK2 inhibitors, providing a valuable tool for optimizing therapeutic strategies for diseases associated with abnormal JAK2 activation.

#6845 Evaluation of individual drug response to tyrosine kinase inhibitor treatment in precision-cut tumor slices of gastrointestinal stroma tumors.

Dina Monch[1], Julia Thiel[1], Meng Dong[1], Annika Maa?[1], Annette M. Staiger[2], Katrin Kurz[2], German Ott[2], Philipp Renner[3], Tobias Leibold[3], Thomas Murdter[1], Matthias Schwab[1], Marc-Hendrik Dahlke[3], Jana Koch[1]

[1]Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany,[2]Department of Pathology, Robert-Bosch-Hospital, Stuttgart, Germany,[3]Department of General and Visceral Surgery, Robert-Bosch-Hospital, Stuttgart, Germany

Gastrointestinal stromal tumors (GIST) are the most prevalent mesenchymal tumor entity of the gastrointestinal tract and serve as a key example for targeted therapies with tyrosine kinase inhibitors (TKI) such as imatinib, in tumors with the most frequent mutations in PDGFRA or c-Kit. Although TKIs have significantly improved the overall survival rates of GIST patients, there are currently no treatment options for imatinib-resistant tumors and tumors without identifiable driver mutations. So far, current sarcoma research mostly relies on expensive mouse models or 2D cell culture that do not integrate the tumor microenvironment (TME). Therefore, clinically relevant, patient-derived 3D GIST models that include cells of the TME are urgently needed. In this study, we tested the feasibility of precision-cut tumor slices (PCTS) as a preclinical model for personalized GIST research. PCTS are patient-derived 3D tissue slices that enable the study of interactions between tumor cells and cells of the surrounding TME. For this, primary GIST tumors were cut into 250µm thick slices and cultured for up to two weeks with or without standard TKI treatment. Tumor morphology and cell composition was analyzed via immunohistochemical (IHC) and multiplex immunofluorescence (mIF) staining, which enabled the simultaneous detection of up to six different markers on one FFPE tissue section. The downstream analysis included a machine-learning based workflow that allowed the comparison of biomarker expression and spatial immune cell distribution in the PCTS stromal and tumor areas before and after treatment. Comparison between primary tumor and PCTS revealed a preserved tumor morphology and heterogeneous cell composition throughout culture. In addition, serum levels of various cytokines were assessed with LEGENDPlex assays and correlated with the PCTS cell composition. The *in vitro* response of PCTS to TKI-based therapies was analyzed via IHC staining and ATP-based viability assays. PCTS from tumors with PDGFRA mutations were expected to be imatinib-resistant, while PCTS from tumors with c-KIT mutations were considered imatinib-sensitive. Sequencing analyses determined the PDGFRA or c-KIT mutational status showing that all patients harbored either a PDGFRA or a c-KIT mutation. PCTS treatment response was compared to the mutational status and if available to clinical follow-up data. Of all PCTS that responded to imatinib therapy, two cases matched the anticipated clinical outcome. PCTS provide a clinically relevant new model system for GIST to explore the development of therapy response to TKI-based treatment and to investigate novel GIST treatments such as immunotherapies and combination treatments. The work is supported by the Robert Bosch Stiftung, Stuttgart, Germany

#6846 Using CRISPR/Cas9 to edit KRAS in a small cell lung cancer cell line.

Myra Elise Castel[1], Neil T. Conlon[1], Georg F. Bischof[2], Ke Wei Gong[3], Neil A. O'Brien[3], John P. Crown[4], **Denis M. Collins**[1]

[1]School of Biotechnology, Dublin City University, Dublin, Ireland,[2]Puma Biotechnology, Inc., Los Angeles, CA,[3]Division of Hematology/Oncology, University of California, Los Angeles, Los Angeles, CA,[4]Department of Medical Oncology, St Vincent's University Hospital, Dublin, Ireland

Introduction: Small cell lung cancer (SCLC) accounts for 10-15% of lung cancers and is the most aggressive subtype. Approximately 16% of SCLCs contain mutations in the *KRAS* oncogene. The $KRAS^{G12C}$ activating mutation is now clinically targetable. There are limited SCLC models containing the $KRAS^{G12C}$ mutation available to generate pre-clinical rationale for clinical studies. This study aimed to develop a protocol for the insertion of the $KRAS^{G12C}$ mutation in the KRAS wild-type SCLC cell line NCI-H1048 using CRISPR/Cas9 technology.

Methods: A protospacer adjacent motif (PAM) was identified near the $KRAS^{G12C}$ mutation site and a single guide RNA (sgRNA) was designed using SnapGene software. The efficiency and specificity of the sgRNA were determined using CRISPRon and CRISPRoff webservers (https://rth.dk/resources/crispr/). A repair template containing the nucleotide substitution was created. The CRISPR/Cas9 plasmid pSpCas9(BB)-2A-GFP (PX458, Addgene) was assembled with a single-stranded DNA oligo matching the sgRNA sequence. The NCI-H1048 cell line was transfected with the DNA assembly product (pKRASm) or the PX548 empty plasmid (control) using Lipofectamine 3000. Cells were sorted by GFP fluorescence 3 days after transfection using a BD FACS Aria III cell sorter. pKRASm- and PX458-transfected cells were seeded for colony formation in 10 cm tissue culture plates. Several pKRASm and PX458 clones were isolated and Sanger sequenced to determine $KRAS^{G12C}$ mutation status. Codons affected by genetic alterations were determined by DNA local alignment using SnapGene software. Cell line growth rates were assessed over 7 days using the Incucyte® live-imaging system.

Results: The transfection efficiency in NCI-H1048 was 0.6% for pKRASm and 1.2% for PX458. No clones with a $KRAS^{G12C}$ mutation were isolated. However, two clones had alterations in the *KRAS* exon 2 sequence. Clone NCI-H1048-29 contained a 15bp in-frame deletion (impacting codons 9-13), and clone NCI-H1048-32 had heterozygous alleles from position 27 (impacting codons 9-37). The regions affected are the P-loop (codons 10-14, phosphate-binding loop) and switch I (codons 30-40, binding interface for KRAS effectors and regulators). The growth of clones NCI-H1048-29 and NCI-H1048-32 was compared to a control clone (PX458-transfected NCI-H1048-9). The *KRAS* alteration significantly reduced the growth of NCI-H1048-29 ($p=0.023$) and NCI-H1048-32 ($p=0.004$) compared to the NCI-H1048-9 control confirming phenotypic impact of the *KRAS* alterations.

Conclusion: The CRISPR/Cas9 method employed resulted in the creation of novel SCLC cell lines with *KRAS* alterations, but further optimization of the protocol is needed to introduce *KRAS* point mutations. The CRISPR/Cas9-edited cell lines created will be used for further *in vitro* study of KRAS biology in SCLC.

#6847 Development and application of DTR mouse models for immune cell ablation and tumor immunotherapy studies.

Yang Yu, Yi Li, Hechun Ma, Shuang Li, Ruilin Sun

GenoBioTX, Sugar Land, TX

Inducing the selective ablation of specific cell types is a powerful approach for studying complex cell-cell interactions, particularly within the immune system. While systemic depletion of immune cells can be achieved using chemicals or cytotoxic antibodies, the diphtheria toxin receptor (DTR)-diphtheria toxin (DT) system provides a more targeted, cost-effective, and minimally invasive alternative. The system leverages the fact that human DTR has a 10^3-10^5 times higher affinity for DT compared to rodent cells. This allows selective cell ablation in mice by introducing the human DTR gene into specific cell populations and subsequently administering DT in vivo.

Using CRISPR/Cas9 technology, we generated a series of DTR mouse models targeting immune cell types, including CD8, Ncr1, Foxp3, CD11c, and seven additional cell-specific targets. These models were created on C57BL/6 or BALB/c genetic backgrounds, with the human DTR coding sequence driven by murine endogenous promoters. Successful gene insertions were validated using GFP or Tomato fluorescent reporter expression. Upon DT administration, these engineered DTR mice exhibited transient immune cell ablation within 48 hours, with cell levels recovering to baseline within 96 hours in the absence of repeated DT dosing.

For functional validation, we used the Ncr1-DTR mouse model in an MC38 tumor inoculation study. DT treatment effectively depleted natural killer (NK) cells in the blood, highlighting the model's utility for immune cell depletion in specific experimental contexts.

Overall, these results demonstrate that DTR mouse models are robust tools for dissecting immune cell functions, exploring intercellular interactions, and evaluating the mechanisms of immune-based therapies.

#6848 Development of a humanized C3 mouse model for preclinical evaluation of therapeutics in C3 glomerulopathy.

Andrei Golovko, Shuang Li, Xiaolei Qiu, Yi Li, Ruilin Sun

GenoBioTX, Sugar Land, TX

C3 glomerulopathy (C3G) is a rare and progressive kidney disease, encompassing C3 glomerulonephritis and dense deposit disease, both driven by dysregulation of the alternative pathway of complement activation. Approximately 50% of patients progress to end-stage kidney disease (ESKD) within 10 years, with limited treatment options currently available.
To address this unmet need, we developed a novel humanized C3 (hC3) mouse model that exhibits a pronounced C3G-like phenotype, characterized by early progression to ESKD and increased mortality. Compared to wild-type mice, hC3 mice display significantly reduced circulating C3 levels, alongside liver and kidney dysfunction confirmed by biochemical and pathological analyses. These mice also demonstrate reduced body weight, elevated morbidity early in life, and a shortened lifespan of approximately 5-6 months. To evaluate potential therapies, we treated hC3 mice with the small interfering RNA drug ARO-C3 at high and low doses. ARO-C3 treatment led to a reduction in serum hC3 levels as early as one week post-administration, with a single dose maintaining suppression for at least five weeks. Importantly, ARO-C3 treatment significantly prolonged survival and improved liver and kidney function, as demonstrated by molecular and histopathological analyses. This hC3 mouse model provides a robust platform for studying complement-mediated diseases such as C3G and for preclinical evaluation of novel therapeutic strategies targeting the complement system.

#6849 RIT1^{M90I} is a driver of lung adenocarcinoma tumorigenesis and resistance to targeted therapy.

Ashley DiMarco, **Mirunalini Ravichandran**, Jeffery Lau, Anthony Lima, Eva Lin, Julie Weng, Luca Gerosa, Pablo Saenz-Lopez Larracho, Thomas Hunsaker, Yang Xiao, Monika Mis, Charles Havnar, Kai Barck, Klara Totpal, Oded Foreman, Mark Merchant, Nicole Sodir, Danilo maddalo

Genentech, Inc., South San Francisco, CA

RIT1 is a RAS-family GTPase that is mutated in 2.4% and amplified in up to 14% of lung adenocarcinoma patients. Yet, the oncogenic potential of RIT1 in the lungs is not completely understood. Many patients with RIT1 alterations are considered 'oncogene-negative', hence they are not eligible for any targeted therapy in the clinic. The role of RIT1 in cancer has been historically understudied due to the lack of *in vitro* and *in vivo* models harboring RIT1 alterations. In this study, we generated the first murine model of RIT1^{M90I}-mutant lung cancer. We found that RIT1^{M90I} was tumorigenic in the lungs and the tumors displayed histopathological features similar to lung adenocarcinoma. We leveraged this new model to assess both *ex vivo* and *in vivo* sensitivity of RIT1 tumors to the SHP2 inhibitor, migoprotafib, in combination with other targeted therapies. Finally, we showed that RIT1^{M90I} can also drive resistance to the KRAS^{G12C} inhibitor, divarasib, and that the combination with migoprotafib can revert this phenotype. Our data shows that RIT1^{M90I} is a bona fide oncogenic driver of NSCLC and mediator of targeted therapy resistance as a co-occurring mutation. Taken together, our study suggests that RIT1-altered cancer patients may benefit from combination treatment with SHP2 inhibitor in the primary and resistance setting.

#6850 A new syngeneic lung cancer model with KRAS and LKB double mutations for evaluating the efficacy of combined KRAS mutation targeted therapy and immunotherapy.

Lei Ci[1], Derek Reznik[1], Kai Zhou[1], Minghui Sun[2], Yi Li[2], Qin Huang[2], Ruilin Sun[1]

[1]GenoBioTX, Sugar Land, TX,[2]Shanghai Model Organisms Center Inc., Shanghai, China

Background: Lung cancer, particularly non-small cell lung cancer (NSCLC), remains the leading cause of cancer-related deaths globally. Genetically engineered mouse models are vital for replicating the genetic and histopathological characteristics of NSCLC, enabling precise manipulation of tumor suppressors and oncogenes. These models facilitate the development of autochthonous tumors within their natural tissue environment. Mutations in LKB1 and KRAS, which frequently co-occur, are associated with resistance to immune checkpoint inhibitors (ICIs) in NSCLC. However, challenges in detecting and quantifying spontaneous lung tumors in mouse models persist. Therefore, there is a critical need for preclinical models with intact immune systems that allow for effective detection and quantification of spontaneous lung cancers. Such models are essential for evaluating the efficacy of therapies targeting KRAS and LKB1 double-mutant NSCLC.
Methods: We developed a novel mouse model (KLL mouse) on the C57BL/6 background, incorporating the KRAS(G12D) mutation, LKB1 deficiency, and firefly luciferase expression. Spontaneous lung cancer was initiated via intranasal delivery of an adeno-associated virus (AAV)-based Cre recombinase system. Tumor progression was monitored longitudinally using micro-CT imaging. From this model, we derived the 279# tumor cell line from spontaneous lung tumors in one mouse (279#). This cell line was used to establish both subcutaneous and orthotopic lung tumor models for therapeutic efficacy studies.
Conclusion: We successfully established a comprehensive preclinical platform for studying NSCLC with KRAS and LKB1 dual mutations. This platform includes a spontaneous lung cancer mouse model, a derived KLL cell line, and associated subcutaneous and orthotopic tumor models. These resources provide valuable tools for investigating optimal treatment strategies for NSCLC, particularly the combination of targeted therapies and immunotherapies.

#6851 An integrated preclinical platform for antibody-drug conjugate development.

Stefano Cairo, Mara Gilardi, Marianna Zipeto, Michael Ritchie

Champions Oncology, Inc., Hackensack, NJ

Antibody-drug conjugates (ADCs) are an innovative class of targeted cancer therapeutics that combine the specificity of monoclonal antibodies with the potent cytotoxic effects of small-molecule drugs. The development of ADCs requires an integrated approach to identify targets, optimize payload delivery, and validate efficacy across ex vivo and in vivo preclinical models. Selecting the right pipeline supporting ADC development is essential. Cell lines often fail to express the specific target antigens leading to discarding or retaining the wrong targets causing clinical failure. For these reasons, ADC development needs clinically relevant models such as patient-derived xenograft (PDX) reflecting clinical trial populations. Champions Oncology has generated a comprehensive preclinical platform to support ADC development by integrating molecular profiling, multi-omics integration and histology data along with functional assays and patient-derived ex-vivo and in vivo models. Champions' PDX bank includes over 1500 models, extensively characterized at a molecular level, and highly reflective of the current clinical trial populations. The deep multi-omics characterization, which includes a proteomic dataset in addition to transcriptomics data, provides a unique advantage in model selection for ADC development. The platform identifies novel targets by analyzing and correlating gene and protein expression in PDX models and validating these findings in clinical tumor samples and normal tissue. This ensures that potential ADC targets exhibit limited overlap with known ADC targets and are differentially expressed in tumor tissues compared to normal tissues, thereby minimizing off-target toxicity. Following target identification, the platform includes steps enabling the discovery and development phase, leveraging Champions' cell-based assays, proprietary ex vivo TumorGraft3D organoid platform, and in vivo model. These models, derived from primary patient tumors, retain the molecular and histopathological characteristics of human cancers enabling a deeper understanding of target biology, ADC internalization, and downstream cytotoxic effects. These analyses are integrated into the workflow to inform ADC construct design, including optimizing antibody specificity, and payload potency. Here we show an example of how Champions Oncology's integrated preclinical ADC development pipeline offers a comprehensive approach for identifying and validating new targets during ADC optimization for therapeutic success. This platform accelerates ADC discovery while ensuring translational relevance, paving the way for next-generation cancer therapeutics.

#6852 Targeted administration of lactate dehydrogenase inhibitor during transarterial of hepatocellular cardinomas in a rat model.

Ryan Baron[1], Ariful Islam[2], George McClung[2], Ryan El Ghazal[2], Nicholas Perkons[2], Alexey Gurevich[2], Jennifer Crainic[2], Daniel Ackerman[2], Nicolas Skuli[2], Gregory Nadolski[2], Stephen Hunt[2], Terence Gade[2]

[1]Drexel University College of Medicine, Philadelphia, PA,[2]Radiology, University of Pennsylvania, Philadelphia, PA

Introduction: Despite improvements in locoregional therapy, recurrence of hepatocellular carcinoma (HCC) remains common, as tumor cells can respond to therapy by undergoing metabolic reprogramming to utilize anaerobic glycolytic pathways via lactate dehydrogenase. In this work, we assessed the growth rates and recurrence rates of HCC in target tumors undergoing transarterial embolization (TAE) with an LDH inhibitor (LDHi), bland TAE (BLAND), and sham therapy (SHAM).

Methods and Materials: Development of autochthonous HCC was induced in 25 Wistar rats using DEN and tumor progressions were tracked with serial MRI until the target tumor reached a volume of 100-300 mm^3. TAE with LDHi were performed using sequential administration of 10 µg/kg LDHi followed by 40-120 µm Embosphere (Merit Medical Systems) (n=13), while bland TAE were performed using Embosphere alone(n=8). An additional cohort underwent a sham TAE procedure without the administration of Embosphere (n=4). Following surgery, rats were serially imaged using T2 MRI, where tumor growth was tracked and monitored for treatment response and potential recurrence. Rats were classified as progressive disease (PD) if there was no decrease in tumor volume by <20%, PR if tumor volume decreased by >20%, and CR if the tumor was eradicated. Recurrence following PR was classified by a >20% increase in tumor volume from the lowest post-treatment volume.

Results: LDHi target tumors had an average %change in tumor volume of -64% from treatment volume, while the BLAND showed a -38% decrease (p=0.068). Results of a Kaplan-Meier survival analysis with a log-rank test showed a significant difference in survival between the groups (p=0.006). All SHAM rats showed no response to treatment and were classified as PD. Treatment responses in the BLAND cohort were2/8 PD and 6/8 PR, while responses in the LDHi cohort were 1/13 PD, 11/13 PR, and1/13 CR. All rats classified as PR experienced recurrence and the time of progression free survival was significantly longer in the LDHi cohort (24 days) as compared to the BLAND (13 days) (p = 0.036).

Conclusion: Administration of LDHi during TAE yields a larger decrease in targeted tumor volume as compared to BLAND TAE, while also providing a statistically significant improvement in progression free survival.

#6853 A standardized drug selectivity profiling platform with a proteome coverage of more than 5,000 proteins.

Tomas Friman, Tuomas Tolvanen, Tove Bostrom, Ying Sun, Alexey Chernobrovkin, Yasmin Andersson, Christina Velasco, Daniel Martinez Molina

Pelago Bioscience AB, Solna, Sweden

Drug hunters typically rely on a combination of biophysical/biochemical binding and efficacy assays to find and prioritize hit compounds in their drug discovery efforts. These activities are pivotal for success where the right assay combination can correlate productive binding (target engagement) with a biological effect. The earlier such correlations can be established, the better, as that speeds up the prioritization process and builds confidence in the lead series that are progressed to lead optimization. Currently, at that stage, selectivity assays for specific protein classes, typically including safety panels of up to 200 proteins, are applied. However, a limited number of proteins will result in a biased selectivity assessment. Instead, an unbiased and proteome-wide selectivity profiling assay, employed early in the drug discovery process, contributes to the correct prioritization of lead compounds. Pelago Bioscience has established a standardized assay for selectivity profiling, based on the Cellular Thermal Shift Assay (CETSA) with mass spectrometry (MS) detection, which enables discrimination between selective and more promiscuous molecules at the proteome level. As an example, we have profiled over 100 different drugs and probes used in clinical oncology and tumor biology research. Investigated drug classes include antimetabolites, alkylating agents, DNA intercalating agents, epigenetic modifiers, kinase inhibitors, and microtubule inhibitors. In the work presented here, we will show how compounds with similar structures and mode of action also display similar CETSA protein shift profiles with regards to intended targets and off-targets. Such reference data allows comparison of novel chemical hits to compounds with an established mode of action. High throughput and data quality, with an assessment of more than 5,000 proteins, enables large compound batches to be assayed also during early phases of drug discovery. This speeds up the process and increases the confidence in lead series and future lead candidates.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Novel Antitumor Agents 4
Poster Session

#6857 The novel transcriptional inhibitor ecubectedin demonstrates strong antitumor activity in patient-derived xenograft models of soft tissue sarcoma.

Daniel Gorgels[1], Chao-Chi Wang[1], Karo Wyns[1], Lore De Cock[1], Luna De Sutter[1], Kimberly Verbeeck[1], Ulla Vanleeuw[1], Maria Jose Guillen Navarro[2], Pablo Manuel Aviles Marin[2], Agnieszka Wozniak[1], Patrick Schoffski[3]

[1]KU Leuven, Leuven, Belgium,[2]PharmaMar, Madrid, Spain,[3]General Medical Oncology, UZ Leuven, Leuven, Belgium

Objective: Soft tissue sarcoma (STS) is a heterogenous group of rare, malignant, mesenchymal tumors. Doxorubicin (DOX)-based chemotherapy is standard of care for advanced/metastatic disease, despite low response rates and poor disease control. We explored the antitumor activity of ecubectedin (ECU; PharmaMar), a novel transcriptional inhibitor related to the ecteinascidin family, in patient-derived xenograft (PDX) models of STS, representing sarcomas with complex genomes and translocation-related entities.

Methods: ECU was studied *in vivo* in 6 PDX models, including two leiomyo- (LMS; UZLX-STS22_2, STS111), two dedifferentiated lipo- (DDLPS; STS204, STS112), one synovial (SySa; STS336) and one *CIC*-rearranged sarcoma (CRS; STS134). Xenografts were transplanted on both flanks of female NMRI *nu/nu* mice (n=257). Mice were randomized to 5 experimental groups and treated by tail vein injection once weekly: 1) vehicle (VEH) (5% dextrose) 5 mL/kg; 2) DOX 5 mg/kg; 3) trabectedin (TRA) 0.15 mg/kg; 4) lurbinectedin (LUR) 0.18mg/kg; 5) ECU 1.2 mg/kg. Treatment lasted 16 days; antitumor activity was assessed by tumor volume (TV) analysis, histopathology and western blot. Group comparisons used the Mann-Whitney U test, and baseline comparisons used the Wilcoxon test. A $p < 0.05$ was considered statistically significant.

Results: ECU-treated tumors were statistically significantly smaller compared to VEH control in all models except STS22_2 on day 16. In 4 out of 6 models the drug caused TV stabilization and in one (STS134) it led to TV shrinkage to 41% compared to baseline. Histopathological analysis confirmed antitumor effects, showing significant changes in mitotic and apoptotic activity compared to VEH-treated tumors (Table 1).

	Relative tumor volume as compared to baseline						Mitotic activity						Apoptotic activity					
Subtypes	**STS22_2**	**STS111**	**STS204**	**STS112**	**STS134**	**STS336**	**STS22_2**	**STS111**	**STS204**	**STS112**	**STS134**	**STS336**	**STS22_2**	**STS111**	**STS204**	**STS112**	**STS134**	**STS336**
VEH	349%	155%	205%	273%	387%	259%	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DOX	243%	170%	148%	154%	177%	161%	↓	↓	↓		↓*		↑*	↑*	↑		↑*	
TRA	254%	137%	172%	135%	230%	167%	↓	↓	↓		↓*		↑*	↑*	↑*		↑*	
LUR	232%	126%	173%	175%	202%	169%	↓*	↓*	↓		↓*		↑*	↑*	↑*		↑*	
ECU	181%	104%	143%	104%	41%	97%	↓*	↓*	↓		↓*		↑*	↑*	↑*		↑*	

Conclusion: ECU has antitumor effects in PDX models of STS, with the most prominent activity seen in STS134. Next step includes investigating the role of DNA repair mechanisms in response to ECU treatment.

#6858 The next generation transcriptional inhibitor PM54 demonstrates strong antitumor activity in patient-derived xenograft models of soft tissue sarcoma.

Daniel Gorgels[1], Chao-Chi Wang[1], Karo Wyns[1], Lore De Cock[1], Luna De Sutter[1], Kimberly Verbeeck[1], Ulla Vanleeuw[1], Maria Jose Guillen Navarro[2], Pablo Manuel Aviles Marin[2], Agnieszka Wozniak[1], Patrick Schoffski[3]

[1]KU Leuven, Leuven, Belgium,[2]PharmaMar, Madrid, Spain,[3]General Medical Oncology, UZ Leuven, Leuven, Belgium

Objective: Soft tissue sarcoma (STS) is a heterogenous group of rare, malignant mesenchymal tumors. Doxorubicin (DOX)-based chemotherapy is standard of care for advanced, metastatic STS, despite low response rates and poor disease control. We explored the antitumor activity of PM54 (PharmaMar), a next generation transcriptional inhibitor related to the ecteinascidin family of agents, in patient-derived xenograft (PDX) models of STS.

Methods: Female NMRI *nu/nu* mice (n=258) were transplanted on both flanks with 6 different PDX models of leiomyo- (LMS; UZLX-STS22_2, STS111), dedifferentiated lipo- (DDLPS; STS204, STS112), *CIC*-rearranged (CRS; STS134) and synovial sarcoma (SySa; STS336). Mice were randomized to 5 treatment groups and treated by tail vein injection once weekly: 1) vehicle (VEH) (5% dextrose) 5 mL/kg; 2) DOX 5 mg/kg; 3) trabectedin (TRA) 0.15 mg/kg; 4) lurbinectedin (LUR) 0.18 mg/kg; 5) PM54 1.2 mg/kg. Treatment lasted 16 days. Antitumor activity was assessed by tumor volume (TV) analysis, histopathology and western blot. The Mann-Whitney U test was used to compare groups, the Wilcoxon test to compare with baseline. A p value of <0.05 was considered statistically significant.

Results: PM54-treated tumors were statistically significantly smaller when compared to VEH-treated PDX on day 16. TV stabilization on day 16 was seen in 5 out of 6 models, while in STS134 the experimental compound led to TV shrinkage to 76% of the baseline (table 1). Histopathological analysis supported the TV analysis showing that PM54 exerted the strongest antitumor effect in all models analyzed thus far, compared to VEH-treated tumors.

	Relative tumor volume as compared to baseline						Mitotic activity						Apoptotic activity					
Subtypes	**STS22_2**	**STS111**	**STS204**	**STS112**	**STS134**	**STS336**	**STS22_2**	**STS111**	**STS204**	**STS112**	**STS134**	**STS336**	**STS22_2**	**STS111**	**STS204**	**STS112**	**STS134**	**STS336**
Vehicle	349%	155%	205%	273%	387%	259%	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DOX	243%	170%	148%	154%	177%	161%	↓	↓	↓*		↓*		↑*	↑*	↑		↑*	
TRA	254%	137%	172%	135%	230%	167%	↓	↓	↓*		↓*		↑*	↑*	↑*		↑*	
LUR	232%	126%	173%	175%	202%	169%	↓*	↓*	↓*		↓*		↑*	↑*	↑*		↑*	
PM54	162%	113%	145%	125%	76%	100%	↓*	↓*	↓*		↓*		↑*	↑*	↑*		↑*	

Conclusion: PM54 has strong antitumor activity in PDX models of STS, with the most pronounced effects in STS134. Further steps include investigation of the role of DNA repair mechanisms in response to treatment with PM54 and the clinical development of this next generation transcriptional inhibitor in STS.

#6859 Targeting scaffold/matrix associated regions in pancreatic ductal adenocarcinoma.

Jack Prochnau[1], Daisy Medina[1], Xena Huang[1], Panneerdoss Subbarayalu[1], Deepika Singh[1], Stanton McHardy[2], Manjeet Rao[1]

[1]UT Health Science Center at San Antonio, San Antonio, TX,[2]University of Texas at San Antonio, San Antonio, TX

Pancreatic cancer is the tenth most common cancer in the United States and is projected to become the second leading cause of cancer-related death by 2030. Most cases arise in exocrine cells and are driven by genetic mutations, such as *KRAS* and *CDKN2A*, as well as modifiable risk factors, including alcohol use, chronic pancreatitis, and obesity. The aggressive nature of pancreatic ductal adenocarcinoma (PDAC), coupled with its complex tumor microenvironment, leads to late-stage diagnoses and a five-year survival rate of 13%. Despite advances in treatment, which include surgical resection, radiotherapy, and chemotherapeutic regimens like gemcitabine and FOLFIRINOX, therapy-related toxicity and chemotherapy resistance remain significant barriers to improved outcomes. One promising therapeutic approach involves exploiting vulnerabilities in the DNA damage response (DDR) pathways unique to cancer cells. While cancer cells rely on an aberrant DDR to sustain unchecked replication, their increased mutational burden renders them particularly susceptible to DNA-targeting therapies. However, existing DNA-targeting treatments are limited by off-target effects, resulting in toxicity and the emergence of resistance. This underscores the need for novel, selective drugs capable of targeting DNA processes specific to cancer cells. We report on a novel carbazole-derived compound that represents a significant advancement in PDAC therapy. Carbazole, a nitrogen-containing heterocyclic molecule, serves as a pharmacophore in therapeutics with diverse applications, including antitumor, antiviral, and anti-inflammatory agents. Our compound demonstrates potent inhibition of PDAC growth and metastasis in both *in vitro* and *in vivo* models while sparing normal cells. Our results reveal that our compound blocks PDAC growth and progression by selectively targeting Scaffold/Matrix Associated Regions (S/MARs), which play critical roles in several processes including increasing the rate of transcription initiation, providing support to origin of replication as well as chromatin organization. By disrupting S/MARs and their associated binding proteins, our compound inhibits specific genes that support replication and DNA repair processes. This study will set the stage for a new paradigm of treating PDAC using a carbazole-derivative that is highly potent, has unique mechanism of action and shows minimal toxicity. Furthermore, its potential to complement existing treatments positions it as a promising candidate for PDAC therapy.

#6860 MTL-004, a promising new therapeutic for post-resection treatment of glioblastoma.

Mike Hudson[1], Elisabeth Bertrand[2], **Sylvie Maubant**[2], Marie Lux[2], Elodie Marie Dit Chatel[2], Jordan Longin[2], Sophie Chaumeron[2], Maxime Beauchet[2], Anne-Pascale Luzy[2], Pauline Bornert[2], Olivier Duchamp[2]

[1]Gordian Pharma LTD, Pentre Bach, Brecon, United Kingdom,[2]Oncodesign Services, Dijon, France

Objectives:
Glioblastomas (GBM), are exceptionally hard to treat, aggressive and genetically heterogeneous tumors. We report the tolerance, biodistribution and excretion of MTL-004 (5-(aziridine-1- yl)-4 hydroxylamino-2-nitrobenzamide) in healthy animals, along with data its effect when delivered as a single intra-cranial dose in a bioresorbable paste (poly DL-lactic acid-co-glycolic acid and poly ethylene glycol (PGLA/PEG)) following surgical resection of primary GBM, on tumor growth and survival.
Experimental procedures
Tolerance and biodistribution of MTL-004:
Healthy Wistar Han male and female rats were subjected to intravenous (IV) escalating or repeated doses of MTL-004. Body weight and behaviour of animals were monitored to assess toxicity. Healthy Wistar Han female rats were subjected to single intracranial (IC) or IV administration of radiolabelled ^{14}CMTL004 to assess its excretion and biodistribution. Sequential collection of blood and organs were performed and radioactivity was measured.
Efficacy of MTL-004 on tumor bearing rats:
On day 0, tumors were orthotopically induced by implanting 9L fragments into the brain of Fischer 344 female and male rats.On day 5, developing tumors were surgically resected, and rats were subsequently treated with either temozolomide (TMZ), the current standard of care model, orally (PO) for 5 consecutive days, a single application of a bioresorbable paste containing MTL-004, or both treatments combined.
Results:
Tolerance and biodistribution of MTL-004:
Based on IV dosing, 5 mg/kg/day was considered to be the maximum tolerated dose of MTL-004. A dose of 1 mg/kg/day was considered to be the highest dose suitable for repeated IV treatments, and was the safe dose selected for all *in vivo* studies.Following IC and IV administration of ^{14}CMTL004, plasma exposure profile and pharmacokinetic half-life were similar. The retention / half-life of ^{14}CMTL004 in brain was longer than in plasma, suggesting
that its elimination, along with metabolites, took longer in brain than in plasma. MTL-004 was mainly excreted through urine.
Efficacy of MTL-004 on tumor bearing rats:
Male and female rats treated with a single IC dose of MTL-004 following tumor resection demonstrated a significantly higher survival compared to both placebo and TMZ groups and remained in apparent good health for the duration of the study.Combination of both PO TMZ and IC MTL-004 doses was assessed in female rats, where no significant increase of survival was observed compared to IC administered MTL-004 alone.
Conclusions:
MTL-004 was found to be safe and well-tolerated at a dose of 1 mg/kg, and was excreted mainly through urine.Following resection, PO TMZ seemed to have a transient impact on tumor growth, but minimal survival gains compared to placebo. In contrast, a single IC treatment with MTL-004 in paste provided prolonged control of tumor recurrence leading to a significantly increased survival of animals.

#6863 Development of MYCN/MYC inhibitors.

Satyanarayana Gadde[1], Sin Wi Ng[1], Natalie Chung[1], Qian Wang[1], Larissa Doughty[2], Tracey Nero[2], Maria Tsoli[1], David Ziegler[1], Louis Chesler[3], Michael Parker[2], Naresh Kumar[4], Belamy Cheung[1], Glenn Marshall[1]

[1]Children's Cancer Institute Australia, Sydney, Australia,[2]Bio21 Institute, University of Melbourne, Melbourne, Australia,[3]The Institute of Cancer Research, London, United Kingdom,[4]School of Chemistry, University of New South Wales, Sydney, Australia

Background: Deregulated expression of MYC family transcription factors is present in more than 70% of human cancers. MYC is an intrinsically disordered protein without known drug binding pockets and is essential for adult cell replication, making clinical development of MYC inhibitors challenging. MYCN amplification and overexpression generate driver signals in the childhood cancers, neuroblastoma (NB), medulloblastoma (MB) and diffuse intrinsic pontine glioma (DIPG).
Methods and Results: We previously reported a small molecule compound, SE486-11, which reduced MYCN protein levels in combination with suberoylanilide hydroxamic acid. We developed more potent analogues of SE486-11 with improved single agent potency, selectivity towards cancer cells and solubility (UNSW-SC compounds). Our lead compound, UNSW-SC-22 is highly effective *in vitro* against MYCN-driven NB and MB cells with sub-micromolar IC_{50} values and displayed a wide therapeutic index. UNSW-SC-22 displayed MYCN- and c-MYC-dependent cytotoxicity and decreased MYCN and c-MYC protein levels in NB and MB cell lines. MYCN and c-MYC protein half-life was decreased ~2-fold, while K-48-linked ubiquitination of MYCN protein was increased by UNSW-SC-22. We show UNSW-SC-22 bound to full length MYCN and c-MYC proteins directly, with KDs of 62.7 µM and 60.5 µM, respectively, by surface plasmon resonance. In-cell target engagement was further confirmed by drug affinity responsive target stability and cellular thermal shift assays. Total RNA from NB and MB cells treated with UNSW-SC-22 showed down-regulation of MYC target genes on GSEA analyses. On *in-vitro* pharmacology profiling against 47 intracellular signalling targets (78 assays) known to cause adverse drug reactions in humans, UNSW-SC-22 demonstrated no significant target activation at <20 µM. UNSW-SC-22 freely crossed the blood-brain barrier, reaching a concentration of 10.8 µM in brain tissue, with a half-life of 1.22 hours. UNSW-SC-22 significantly reduced the tumour burden and improved overall survival of MYCN-driven NB and MB mice. MYCN protein expression was downregulated in tumours collected from mice treated with UNSW-SC-22.
Conclusion: Collectively, our data suggest that MYCN and c-MYC are the direct molecular targets of UNSW-SC compounds. These compounds have strong potential to serve as specific targeted therapy to treat MYCN/c-MYC driven paediatric and adult cancers.

#6864 Overcoming resistance to tubulin-targeting drugs with PM534: an innovative microtubule-targeting compound.

Maria Jose Guillen[1], Maria Jose Munoz[1], Maria Angela Oliva[2], Daniel Torralba[1], Marcelo Lima Ribeiro[1], J. Fernando Diaz[2], Carmen Cuevas[1], **Pablo M. Aviles**[1]

[1]Pharma Mar, S.A., Madrid, Spain,[2]Centro de Investigaciones Biologicas Margarita Salas, CSIC, Madrid, Spain

Tubulin, a GTP-regulated protein essential for microtubule assembly, is a critical target in cancer therapy. However, current tubulin-targeting drugs face limitations due to toxicity and resistance mechanisms, associated with overexpression of either P-glycoprotein or β-III tubulin isotype. This study aimed to evaluate PM534, a novel compound targeting the colchicine-binding domain (CBD) on tubulin, for its potential to overcome these challenges. PM534´s transport properties were investigated applying MDR1-mediated transport assays in Abcb1KO-MDCKII cells. *In vivo*, PM534's antitumor efficacy was assessed in athymic nude mice bearing xenografts of resistant tumors, including LoVo-Doxo cells with Pgp overexpression and HeLa βIII cells with β-III tubulin overexpression. Each xenograft group received a single intravenous (i.v.) dose of PM534 (5 mg/kg), and tumor growth was monitored over a 7-day period. The Km and Vmax kinetic parameters of human MDR1-mediated PM534 transport were determined to be 12.84 µM and 10.3 pmol/min/pmol transporter protein, respectively. *In vivo* data showed that PM534 significantly reduced tumor volumes in resistant models, achieving median tumor volumes of 131.4 mm^3 in LoVo-Doxo xenografts and 163.4 mm^3 in HeLa βIII xenografts by day 7, compared to placebo-treated controls (410.6 mm^3 and 257.4 mm^3, respectively). PM534's efficacy surpassed paclitaxel in these resistant models, as its activity remained unaffected by Pgp overexpression or β-III tubulin presence—both common resistance mechanisms that limit other tubulin-targeting drugs. In conclusion, PM534 demonstrates potent antitumor activity in drug-resistant cancer models, effectively bypassing Pgp-mediated efflux and β-III tubulin-related resistance. These findings support PM534's potential as a promising therapeutic candidate capable of overcoming the limitations of current tubulin-targeting agents in oncology.

#6865 Discovery and characterization of small molecule inhibitors targeting EXO1 for BRCAness cancer therapy.

Yixing Wang, Jessica Hess, Li Zheng, Binghui Shen

City of Hope National Medical Center, Duarte, CA

Human exonuclease 1 (EXO1), a member of the structure specific nuclease family, plays a critical role in maintaining genome stability by processing DNA double-strand breaks (DSBs), nicks, and replication intermediates during DNA replication and repair. As its exonuclease activity is essential for homology-directed repair and replication fork processing, EXO1 has emerged as a compelling therapeutic target, especially in cancers marked by heightened DNA damage and replication stress. Through high-throughput screening of 45,000 compounds, we identified eight distinct scaffolds of EXO1 inhibitors that demonstrated effective and selective inhibition. Two lead compounds, C200 and F684, underwent structural and mutagenesis studies, which confirmed their binding mechanisms. Biochemical assays further validated their potent and selective inhibition of EXO1 activity. Tumor cell profiling revealed that these inhibitors exploit synthetic lethality in BRCA1-deficient cells, emphasizing their specificity and therapeutic potential for targeting BRCAness cancers. Mechanistically, EXO1 inhibition suppressed DNA end resection, increased the accumulation of DSBs, and triggered S-phase PARylation, effectively disrupting DNA repair pathways essential for cancer cell survival. These findings establish EXO1 inhibitors as promising candidates for the treatment of BRCAness cancers and lay the groundwork for further optimization and development of these compounds as targeted therapeutics.

#6867 Triplet combination of monzosertib, a potent CDC7 inhibitor, with DNMT and BCL2 inhibitors is highly active in human AML xenograft mouse models.

Hatsuo Furuichi[1], Hiroko Endo[1], Akinori Arimura[2], Yu Nishioka[1], Masaaki Sawa[1]

[1]Carna Biosciences, Inc., Kobe, Japan,[2]CarnaBio USA, Inc., South San Francisco, CA

Introduction: Cell division cycle 7 (CDC7) is a highly conserved serine-threonine kinase that plays an important role in the initiation of DNA replication and cell cycle progression. Aberrant expression of CDC7 has been reported in many cancers and is thought to promote abnormal proliferation of cancer cells. Thus, CDC7 has been recognized as a potential drug target for treating cancers.Monzosertib (AS-0141) is a potent, selective, orally bioavailable small molecule inhibitor of CDC7, currently under Phase I clinical study in patients with advanced, metastatic, relapsed or refractory malignancies. In the previous studies, we demonstrated that monzosertib exerted strong synergy effects with DNA methyltransferase inhibitor (DNMTi; azacitidine or decitabine) or BCL2 inhibitor (venetoclax) in human acute myeloid leukemia (AML) cell lines.In this study, we evaluated the antitumor efficacy of monzosertib, DNMTi and venetoclax “triplet” therapy in human AML xenograft models.
Method: Fa-CI plot (the Chou-Talalay method) was used to assess combination effects of monzosertib with DNMTi and venetoclax in human AML cell lines. Effects of drug combination on cellular signaling pathway were examined by western blotting. In vivo antitumor activity of monzosertib alone or in triplet combination with DNMTi and venetoclax were evaluated in human AML tumor-bearing mice.
Results: Triplet combination of monzosertib with DNMTi (azacitidine or decitabine) and venetoclax resulted in a synergistic effect on antiproliferative activity in human AML cell lines. Treatment of MV4-11 cells (human AML cell line) with the triplet combination significantly increased in γH2AX, a biomarker of cellular response to DNA damage (DNA double strand breaks). Combining monzosertib with DNMTi (azacitidine or decitabine) and venetoclax resulted in enhanced tumor growth inhibition in a MV4-11 xenograft mouse model without body weight loss.
Conclusions: Monzosertib demonstrated significant synergistic activity in combination with DNMTi (azacitidine or decitabine) and venetoclax in both in vitro and in vivo human AML models. The triplet combination of monzosertib with DNMTi (azacitidine or decitabine) and venetoclax was well-tolerated in these mouse AML models, suggesting the triplet therapy of monzosertib combined with the standard of care (DNMTi plus venetoclax) for AML patients may provide a new therapeutic opportunity in AML.

#6868 Targeting glycolysis in cancer.

Sucheta Telang, Renita Ranjan, Joseph Burlison

University of Louisville, Louisville, KY

The reprogramming of metabolism to increase reliance on glycolysis is a recognized phenomenon in tumors and is frequently driven by oncogenic mutations. In breast cancer, one of these genetic alterations, the increased activity of the human epidermal growth factor receptor 2 (HER2/*ErbB2*) is observed in ~30% of tumors. HER2 activate a variety of downstream effectors (*e.g.* Ras, HIF-1α) that stimulate glycolysis, in part through the activity of a family of enzymes, the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases (PFKFB1-4). The PFKFBs produce fructose-2,6-bisphosphate (F26BP) which activates a rate-limiting glycolytic enzyme, 6-phosphofructo-1-kinase (PFK-1) and therefore important regulators of glycolysis. Preliminary data from our laboratory have determined that the PFKFB4 enzyme was highly expressed in a series of patient - derived $HER2^+$ breast tumors relative to normal breast and additionally found higher expression in paired metastases. We found that silencing PFKFB4 expression in $HER2^+$ breast cancer cells caused a marked reduction in their glycolytic flux and survival. Through in silico screening and optimization, we have identified a small molecule inhibitor of PFKFB4 that selectively inhibits PFKFB4 activity with a resultant decrease in glycolysis and cancer cell growth. We have found that PFKFB4 inhibition also caused a significant decrease in the invasion and migration of $HER2^+$ breast cancer cells *in vitro,* importantly at concentrations well below levels that affect cell viability. We have now examined the effects of PFKFB4 inhibition in a relevant transgenic model of HER2-driven spontaneous mammary tumor formation and have found that the inhibitor caused marked suppression in the growth of $HER2^+$ mammary tumors without signs of systemic toxicity. We additionally have found in preliminary studies that PFKFB4 inhibition decreased the development of metastases in these mice. Taken together, our data strongly support the further evaluation of these inhibitors as a potential strategy for the successful treatment of $HER2^+$ breast cancer.

#6869 A quantitative, conformation-driven screening approach for identification of RNA-targeting small molecule modulators of MYC expression in cancer.

Michelle Kriner, Chuhern Hwang, Brett Israels, Fusayo Koyanagi, Isha Gupta, Wanling Kratzman, Jason Fontana, David Sparkman-Yager

Wayfinder Biosciences, Seattle, WA

Targeting RNA molecules that encode for undruggable proteins involved in cancer is an emerging strategy for small molecule drug development. While a handful of compounds have entered the clinic, progress has been slow due to longstanding challenges in identifying molecules that are both functional and selective. Wayfinder Biosciences has developed an RNA-based sensor technology that addresses these bottlenecks and deployed it to discover compounds that bind to structured elements in the *MYC* mRNA, which is deregulated in a majority of human cancers. The *MYC* 5' UTR is known to contain RNA structures that regulate translation of the c-Myc protein. Using Wayfinder's proprietary computational pipeline, we selected three targetable RNA motifs and designed Wayfinder sensors for each. Wayfinder sensors couple folding of the target RNA motif to folding of a fluorogenic RNA domain and are designed to only generate fluorescence when the target RNA adopts a specific conformation. By screening compound libraries against our sensors, we can identify small molecules that selectively bind to each target RNA structure. Notably, one of the *MYC* motifs we selected represents an alternative conformation that we predict will inhibit interaction with regulatory RNA-binding proteins, enabling us to discover a novel class of modulators of *MYC* expression.

We screened a panel of sensors encoding the *MYC* elements or off-target RNA structures against a library of 50,000 drug-like small molecules to identify compounds that selectively target each *MYC* motif. Hits were validated via dose-response experiments and surface plasmon resonance, an orthogonal biophysical method. Top binders were tested for their ability to reduce cell viability and c-Myc protein levels in a small cell lung cancer cell line (NCI-H82) and a mesothelioma cell line (MSTO-211H), both of which harbor large *MYC* gene amplifications. To date, we have identified multiple hit series that bind to the *MYC* 5' UTR and modulate c-Myc protein levels, leading to significantly reduced proliferation of Myc-amplified cancer cells.

Together, our results validate the ability of Wayfinder's platform to identify selective RNA-binding small molecules that engage their target in *in vitro* models and offer promise for development of cancer therapeutics. Top compounds from this study are currently undergoing medicinal chemistry optimization for potency and selectivity in preclinical models. Future studies will focus on defining the indication space that can be addressed by our *MYC*-targeting molecules and further characterizing their antiproliferative mechanism of action.

#6871 T-Cell redirection to EGFR positive solid tumor with α-TCR nanobody chemically self-assembled nanorings.

Debasmita Paul, Freddys F. Rodriguez, Declan Dahlberg, Abhishek Kulkarni, Lakmal Rozumalski

Department of Chemistry, University of Minnesota, Twin Cities, MN

Cancer immunotherapy involves utilizing a patient's own immune system to identify and destroy cancer cells. Recent advances in this field involve bispecific monoclonal antibodies, genetically engineered T-cells, and immune cell engagers, which have shown success in clinical settings. In this regard, the Wagner Lab has shown that prosthetic antigen receptor (PAR) T-cells, which use chemically self-assembled nanorings (CSANs) can be successfully used to redirect T-cells to target antigens upregulated on cancer cells. CSANs are composed of glycine linked dihydrofolate reductase molecules (1DD) that self-assemble into nanorings upon addition of a chemical dimerizer. This self-assembly can be reversed by the addition of the FDA approved drug, trimethoprim, thus disengaging the T-cells from tumor cells. Bispecific CSANs consisting of alphaCD3 single-chain variable fragment (scFv) recognizing CD3 on T-cells and scaffolds targeted to tumor associated antigens have shown effective eradication of solid tumors such as breast cancer and medulloblastoma both in vitro and in vivo. However, the scFvs pose challenges such as hydrophobicity, aggregation, low stability, and immunogenicity. The structural complexities make production challenging in popular bacterial expression systems. Camelid heavy-chain variable domains, also called nanobodies, have been established as a popular alternative to the scFvs due to their smaller size, increased tissue penetration, low immunogenicity, and ease of production, while retaining similar antigen binding properties. In this project, CSANs composed of 1DD units fused to an antiTCR nanobody, which binds to the T-cell receptor (TCR) on the surface of T-cells, will be used to redirect T-cells to solid tumors. Ongoing experiments show that the antiTCR nanobody has lower binding affinity as compared to antiCD3 scFv. This is beneficial as the non-specific activation of T-cells, which is a drawback of high affinity binders, is weaker in case of the antiTCR nanobody. Further, due to the stability and smaller size of nanobodies, antiTCR-1DD has been genetically modified to fuse a tumor targeting scaffold, thus generating bispecific proteins which can simultaneously engage T-cells and tumor cells. Experiments have shown that perhaps due to its geometry, the bispecific protein is highly potent even at very low doses. Bispecific proteins are clinically translatable, reduction in production load being one of the reasons. The bispecific constructs also lay the foundation of higher order immunotherapeutic tools.

#6872 Repurposing the DNA labeling agent EdU for glioblastoma treatment.

H. Kaanoglu[1], A. Adefolaju[1], C. Fraley[2], M. Shobande[2], R. Dasari[1], B. Mann[1], N. Bell[1], S. Keir[2], S. Hingtgen[1], A. Sancar[1], A. B. Satterlee[1];
[1]University of North Carolina at Chapel Hill, Chapel Hill, NC, [2]Duke University, Durham, NC

Glioblastoma (GBM) is the most common and aggressive type of malignant central nervous system tumors, with over 300,000 new cases diagnosed globally each year. Despite intensive efforts to improve survival with different treatment regimens, GBM is still deemed largely incurable. We previously found that 5-ethynyl-2'-deoxyuridine (EdU), which is commonly used in laboratories to study DNA replication, is recognized by mammalian cells as DNA damage, removed from the genome by nucleotide excision repair, and causes death of proliferating cells. Here, we evaluate the therapeutic potential of EdU for treatment of GBM, as EdU has been shown to cross the blood-brain barrier and incorporate into proliferating GBM cells but not into post-mitotic neurons. We tested the efficacy of EdU against several different model systems, including not only GBM cell lines in *in vitro* cell culture and *in vivo* orthotopic mouse models of GBM, but also living, uncultured GBM patient tumor tissues grown within our Organotypic Brain Slice Culture (OBSC) *ex vivo* platform. In all three model systems, EdU was effective in killing GBM cells. When compared to the standard of care drug Temozolomide (TMZ) in *in vitro* GBM cell survival assays, EdU displayed ED50 values orders of magnitude lower than TMZ in all five GBM tumor cell lines tested. Against two *in vivo* orthotopic brain tumor models, EdU significantly extended survival relative to controls. Using our OBSC *ex vivo* platform, we were able to quantify the capability of EdU to potently kill rapidly dividing cells within heterogeneous GBM patient tumor tissues without harming the healthy OBSC-associated brain tissue. EdU efficacy against a panel of patient GBMs largely correlated with the clinical Ki-67 status of each tumor, save for one tumor that remained unresponsive to treatment with both EdU and TMZ. Overall, these data suggest that (1) EdU has potential to be repurposed as an anticancer therapeutic and is especially adept at killing rapidly proliferating cells with low off-target toxicity; (2) the OBSC platform can measure nuanced differences in efficacy of experimental therapeutics on heterogeneous patient tumor tissues; and (3) OBSCs can continue to help identify potential responders and non-responders to EdU treatment via functional precision testing of patient tumors *ex vivo*. [HK and AA contributed equally to this work.]

#6873 Discovery and characterization of novel ULK1/2 inhibitors and their ability to degrade ATG13, inhibit autophagy, and treat lung cancer.

Patrick Martin Hagan, Allison Limpert, Douglas Sheffler, Huiyu Ren, Nicholas Cosford

Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA

Lung cancer is the leading cause of cancer-related mortality across all populations. Autophagy (ATG) is a cell survival process by which dysfunctional cellular components are removed and recycled to maintain homeostasis through a lysosomal dependent degradation mechanism. ATG upregulation is implicated as a bad actor in several diseases, particularly K-Ras driven non-small cell lung cancer (NSCLC), where it provides nutrients to lung cancer cells in the nutrient poor and hypoxic tumor microenvironment. ATG also contributes to chemotherapeutic resistance, making it a viable pathway for therapeutic intervention. However, the only clinically available drugs targeting ATG are chloroquine and hydroxychloroquine, which are not ATG-specific and have multiple adverse side effects. ATG is an intricate, multistep process beginning with the Unc-51-like autophagy-activating kinases 1 and 2 (ULK1/2) initiation complex. The supramolecular assembly of this complex relies on the adaptor protein ATG13, which binds ULK1/2. Knockout of ATG13 significantly reduces the viability of lung cancer cell lines, indicating that ATG13 is a viable therapeutic target for lung cancer. Furthermore, our group has characterized other autophagy inhibiting compounds that also robustly degrade ATG13 in cells and tissues, leading to cancer cell death. We have developed and optimized a high throughput screening (HTS) assay whereby ATG13 is HiBit tagged in A549 NSCLC cells (ATG13 HiBit assay) to allow identification of compounds that induce ATG13 protein loss, and therefore inhibit ATG. We have also developed a testing funnel that employs multiple assays to characterize compounds that degrade ATG13 independent of cellular toxicity (cell viability assay), confirm their activity against our protein of interest (In Cell Western blot ATG13 assay), measure their ability to inhibit autophagy (GFP-LC3-RFP-LC3ΔG ATG flux assay) and eliminate compounds that cause assay interference (HiBit counterscreen). Using these assays we have identified a novel lead compound which degrade ATG13 and inhibits autophagy resulting in cell death in NSCLC.

#6874 Bkt300, a novel small molecule targeting the protein regulator of cytokinesis 1 pathway in hepatocellular carcinoma.

Amnon Peled[1], **Lika Gamaev**[1], Hanna Wald[2], Rakefet Rosenfeld[2], Orly Eizenberg[2], Arnon Aharon[2], Michal Abraham[2]

[1]Goldyne Savad Institute of Gene Therapy, Hadassah Hebrew University Hospital, Jerusalem, Israel,[2]AlonBio Ltd, Ness Ziona, Israel

Hepatocellular carcinoma (HCC) remains a major cause of cancer mortality worldwide, largely due to its high recurrence rate and chemoresistance. The microtubule-associated protein regulator of cytokinesis 1 (PRC1) has been implicated in HCC progression, associated with poor prognosis, enhanced cancer proliferation, stemness, metastasis, and chemoresistance. PRC1 functions as an oncogenic driver, particularly in HCC cases with elevated expression, where it promotes Wnt signaling and the expression of genes linked to early recurrence. BKT300, a novel targeted anti-cancer agent, was found to inhibit PRC1, offering a potential treatment option for advanced, difficult-to-treat cancers that express high levels of PRC1, such as HCC. This study investigated the effects of BKT300 on HCC cell migration and survival pathways. BKT300 selectively inhibited HCC cell migration, induced G2/M cell cycle arrest, and triggered mitotic catastrophe and apoptosis through the caspase 3 pathway. BKT300 targeted PRC1 by arresting it in a phosphorylated state at T481, concurrently downregulating CDC25C and upregulating p21. siRNA-mediated suppression of PRC1 in HCC cells prevented the activity of BKT300, indicating that BKT300's efficacy is dependent on PRC1 expression. Direct binding of BKT300 to PRC1 was further confirmed through multiple assays. In vivo studies in two HCC mouse models demonstrated significant tumor growth inhibition and tumor regression, underscoring the therapeutic promise of BKT300 for HCC. Overall, BKT300 presents a novel approach to tackling HCC by directly targeting PRC1, potentially improving outcomes in HCC cases with high PRC1 expression and resistance to conventional therapies.

#6875 Preclinical development of a novel small molecule multitarget inhibitor LCI133 for endometrial adenocarcinoma.

Qi Zhang, Vaidehi Mujumdar, Ritchie Delara, Krishnaiah Maddeboina, Dhananjaya Pal, Bharath Yada, Hailey Dryden, Wendel Naumann, Yovanni Casablanca, Erin Crane, Jubilee Brown, Cody Mchale, Donald Durden

Atrium Health Levine, Charlotte, NC

Objective: Endometrial cancer (EC) is the most common gynecologic malignancy with rising mortality and limited targeted therapies. LCI133, an in silico-designed triple inhibitor of CDK4/6-CDK9-AURK, has demonstrated nano-molar potency against PTEN-mutant EC cells lines and favorable in vivo tolerability. Genetically engineered mouse model (GEMM) is the optimal approach to investigate cancer therapeutics in an immunocompetent setting. The objective of this study is to: 1) describe the physiochemical and pharmacokinetic properties of LCI133, 2) establish and characterize the Pgr-Cre; Pten fl/fl GEMM of EC, 3) assess the preliminary effect of LCI133 on GEMM disease burden.
Methods: 1) LCI133 was profiled for target inhibition by cell-free screen and KinomeSCAN. In vivo PK/ADME properties were assessed as described by Veena Bala.5 In vitro potency was assessed by IC50 assay, cell cycle assay, qRT-PCR and immunoblotting at the indicated timepoints for target biomarkers. 2) Pgr-Cre;Ptenfl/fl mice with conditional PTEN deletion were generated by crossbreeding PTEN floxed mice (Ptenflox)with mice expressing Cre under the regulation of the progesterone receptor promoter (PgRCre). Alterations of PTEN/PI3K/AKT pathway were validated by qRT-PCR and immunoblotting. Histopathologic features of female reproductive tract (FRT) were characterized by H&E and immunohistochemistry staining. FRT relative size was normalized to mouse body mass. 3) Beginning at 4-5 weeks of age, formulated LCI133-HCl was administered to Pgr-Cre;Ptenfl/fl mice intraperitoneally daily for 21 days. Animal weight was recorded during treatment. Relative FRT size, pathology and target inhibition were assessed at endpoint.
Results: 1) LCI133 demonstrates favorable preclinical PK/ADME profile. LCl-HCl salt form is superior to free form. 2) We confirmed loss of PTEN in Pgr-Cre;Ptenfl/fl FRT with concomitant increases in pAKT. Endometrial adenocarcinoma with serosal and lympho-vascular invasion in a background of hyperplasia was identified at 7-8 weeks of age with high penetrance. Conditional loss of PTEN correlates with expression of Pgr, predominantly in the endometrium and myometrium. 3) LCI133 treatment was associated with decreased relative FRT size, reduced angiogenesis and target suppression.
Conclusion: In silico-designed LCI133, as a highly selective inhibitor against CDK4/6-CDK9-AURK with nano-molar potency and favorable PK/ADME properties, demonstrated promising in vivo therapeutic index in immunocompetent mouse models.

#6876 A novel PCNA inhibitor AOH1996 demonstrates pre-clinical efficacy in pancreatic ductal adenocarcinoma models.

Sahar F. Bannoura[1], Husain Y. Khan[1], Md Hafiz Uddin[1], Amro Aboukameel[1], Bin Bao[1], Khalil Choucair[1], Yang Shi[1], Rund Nimri[2], Eliza W. Beal[1], Hugo Jimenez[1], Mohammad Najeeb Al Hallak[1], Muhammad Wasif Saif[1], Philip A. Philip[2], Bassel El-Rayes[3], Ramzi M. Mohammad[1], Boris C. Pasche[1], Long Gu[4], Linda Malkas[4], Asfar S. Azmi[1]

[1]Wayne State University, Karmanos Cancer Institute, Detroit, MI,[2]Henry Ford Cancer Institute, Henry Ford Health, Detroit, MI,[3]Department of Hematology and Oncology, University of Alabama at Birmingham, O'Neill Comprehensive Cancer Center, Birmingham, AL,[4]Beckman Research institute of City of Hope, Duarte, CA

Background: Pancreatic ductal adenocarcinoma (PDAC) is an aggressive disease with a dismal prognosis. It is the 9th most common cancer, and the 3rd leading cause of mortality in cancer patients in the United States. PDAC poses a significant clinical challenge partly due to advanced disease stage at diagnosis, poor response to chemotherapeutics, and common recurrence. Proliferating cell nuclear antigen (PCNA) is a ring-shaped clamp protein that encircles DNA and regulates replication and repair. Cancer cells depend on PCNA-mediated DNA repair, and transcription-replication conflict resolution to tolerate DNA damage, thus promoting proliferation. AOH1996 is a novel selective PCNA inhibitor in phase I clinical trials for solid tumors. AOH1996 disrupts the association between PCNA and its numerous binding proteins, thereby inhibiting PCNA functions such as DNA replication, and damage repair. In this study, we investigated the use of AOH1996 in preclinical models of pancreatic ducal adenocarcinoma (PDAC).

Methods: We determined cell viability and growth inhibition by MTT, colony formation and spheroid assays. Drug combinations of AOH1996 with KRAS inhibitors and chemotherapy were analyzed using synergy finder.

Results: We demonstrated that AOH1996 inhibits the growth of a panel of PDAC tumor cells in a dose-dependent manner, in 2D and 3D culture models and clonogenic assays. Cell cycle analysis revealed that AOH1996 causes S-phase arrest in PDAC cells, indicating that S-phase arrest prevents tumor cell growth by targeting DNA replication and repair processes supporting further investigation into AOH1996 to target PDAC. Transcriptomic analysis using RNA sequencing is ongoing to examine the underlying molecular mechanisms of AOH1996 activity. KRAS is the most common oncogenic driver in PDAC and is one of the main synthetic lethal targets with PCNA. Therefore, we examined the combination of emerging KRAS inhibitors with AOH1996 on PDAC models. We found that KRAS G12D inhibitor (MRTX1133) in combination with AOH1996 enhanced cytotoxicity in several PDAC cell lines at lower MRTX1133 concentrations, potentially reducing toxic side effects. We also observed enhanced efficacy of the chemotherapeutic agent oxaliplatin, which is a platinum compound in the FOLFIRINOX regimen, in combination with AOH1996. This indicates that AOH1996 has great potential at improving responses to current and emerging treatments for PDAC patients.

Conclusion: Collectively, our results show that AOH1996 is a promising agent for PDAC treatment which potentiates chemotherapy, and targeted KRAS G12D inhibitors. *In vivo* xenograft studies combining AOH1996 and KRAS inhibitors are ongoing.

#6877 Oxidative stress induced lipid peroxidation as a mechanism of topoisomerase II alpha poisoning by chemotherapeutic agents.

Amy C. Flor, Don Wolfgeher, Stephen J. Kron

Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL

Topoisomerase II alpha (TOP2A) is an ATPase essential for maintaining optimal genomic DNA topology in cells. By relaxing DNA supercoils and entanglements, TOP2A relieves torsional stress to facilitate transcription, replication, and mitosis. In cancer cells, TOP2A is often overexpressed, enabling abnormally rapid genomic replication, in turn facilitating cellular hyperproliferation and driving tumor progression. Thus, TOP2A is a key target of several chemotherapeutic drugs, including etoposide and doxorubicin. While etoposide has long been proposed to stabilize the TOP2A-DNA cleavage complex (TOP2Acc) via interfacial inhibition, we have elucidated a complementary oxidative stress mediated pathway involving lipid peroxidation (LPO) and subsequent generation of reactive lipid electrophiles (LDEs). The LDE 4-hydroxynonenal (HNE) covalently modifies TOP2A at cysteine residues in its ATPase domain, in a DNA-dependent manner. HNE modification inhibits TOP2A catalytic activity and traps TOP2Acc, leading to accumulation of lethal DNA double strand breaks (DSBs). Induction of reactive oxygen species (ROS) by etoposide and doxorubicin were shown by flow cytometry assays, and LPO and HNE-protein adducts were imaged using fluorescence microscopy. Several chemotherapeutic TOP2A poisons significantly depleted reduced glutathione, a key antioxidant. *In vitro*, HNE inhibited DNA decatenation and relaxation activities of purified human TOP2A in kDNA and plasmid DNA assays. Mass spectrometry revealed sites of covalent HNE modification, including Cys216 within the ATPase domain. Malachite green ATPase assays demonstrated that HNE reduced ATP hydrolysis, consistent with disruption of ATP binding. In cell-based studies, HNE directly induced TOP2Acc and DSBs, as detected by trapped in agarose DNA immunostaining (TARDIS) assays and neutral comet assays, respectively. TOP2Acc and DSBs induced by etoposide or doxorubicin were mitigated by a lipid antioxidant, implicating LPO as a mediator of TOP2A targeted drug poisoning. An etoposide resistant cancer cell line exhibited cross resistance to HNE-induced toxicity, further supporting a shared mechanism involving redox metabolism. Proteasome inhibitors including bortezomib exacerbated the persistence of HNE-induced DSBs, indicating that repair of HNE-induced TOP2Acc primarily depends on the proteasome for degradation.

Conclusion: Taken together, our data suggest an underappreciated role for TOP2A as a redox sensor in tumor cells, connecting oxidative stress to DNA damage signaling and creating a target for redox active drugs. This work suggests novel opportunities for improving chemotherapeutic strategies, by leveraging proteasome inhibitors or designing covalent inhibitors targeting Cys216 to optimize therapeutic outcomes and minimize off target toxicities.

#6878 Ag5: a novel quantum cluster therapy targeting mitochondria and antioxidant systems in glioblastoma.

Jayesh Telang[1], Marleen Derweduwe[1], Vanesa Porto[2], Blanca Dominguez[2], Ross Breckenridge[3], Martin Treder[3], Fernando Dominguez[2], Annelies Claeys[1], Isabel Aguirre Alcolea[1], Frederik De Smet[1]

[1]KU Leuven, Leuven, Belgium,[2]Universidade de Santiago de Compostela, Santiago de Compostela, A Coruna, Spain,[3]Arjuna Therapeutics, Novo Milladoiro, A Corunã, Spain

Despite extensive efforts from drug developers, physicians, and patients, the prognosis for glioblastoma (GBM) remains one of the poorest in adult oncology. Current oncology drug development often revisits established technologies, with few truly novel treatments emerging to tackle challenging diseases like GBM. ARJUNA Therapeutics is breaking new ground with quantum cluster technology, creating a unique family of small molecules. The first of these, Ag5, consists of five silver atoms (a quantum cluster) with demonstrated drug-like qualities, including the ability to cross the blood-brain barrier. Ag5 penetrates cells and specifically catalyzes the irreversible inactivation of both glutathione and thioredoxin antioxidant systems in cancer cells, rapidly depleting mitochondrial glutathione and inducing cell death with minimal off-target effects at therapeutic doses. In collaboration with ARJUNA, the Laboratory for Precision Cancer Medicine at KU Leuven, Belgium, screened 20 patient-derived glioma stem cell lines from adult GBM tumors. Ag5 demonstrated cell-killing effectiveness at submicromolar concentrations in approximately 50% of these models, showing activity in both newly diagnosed and recurrent tumor models, as well as in cells from both the core and infiltrating regions. Importantly, tumor cell lines taken from individual patients at various disease stages retained their Ag5 sensitivity, even as they acquired resistance to radiotherapy and/or TMZ. Biomarkers defining sensitivity and resistance to Ag5 in patient-derived GBM lines have been identified, supported by promising preclinical in vivo data. Ag5 has the potential to benefit a significant proportion of GBM patients. A clinical phase 0 neuro-oncology trial at University Hospitals Leuven, Belgium, is planned to test both the biomarker and therapeutic hypothesis for Ag5 in adult GBM patients.

#6879 Inhibition of triple-negative breast cancer progression via ribosomal protein(s) modulation.

Arpit Dheeraj[1], Fernando Jose Garcia Marques[2], Dhanir Tailor[1], Abel Bermudez[2], Praveen Kumar[1], Benedikt Grau[1], Carrsyn B. Haley[1], Sharon Pitteri[2], Sanjay V. Malhotra[1]

[1]Center for Experimental Therapeutics, Knight Cancer Institute, Oregon Health & Science University, Portland, OR,[2]Department of Radiology, Canary Center at Stanford for Cancer Early Detection, Stanford University, Palo Alto, CA

Background: Triple-negative breast cancer (TNBC) is a highly heterogeneous disease with distinct features at the pathological, molecular and clinical levels that are associated with lower survival rates than other subtypes of breast cancer (BC). TNBC is a highly aggressive subtype of breast cancer and represents for 15-20% of all breast cancer cases. TNBC cancers present myriad clinical challenges due to their heterogeneity, poor prognosis, high recurrence, and high incidence of metastasis. Dysregulation of protein translation is a frequent feature of cancer. Translation of mRNAs to proteins i.e., protein synthesis *via* ribosomes, is a fundamental cellular activity. Increased levels of translation, necessary to support rapid cell proliferation, have been reported across different cancer types including TNBC.
Methods: Cell viability and clonogenic assays were performed on a range of TNBC cells, including MDA-MB-231, MDA-MB-468, SUM159, HCC1395, HCC1806, EMT6, and 4T1. Cell cycle distribution was assessed through flow cytometry. LC-MS/MS analysis was performed on TMT-labeled samples for global proteome profiling. CETSA assay was performed to identify the target of CET019. The efficacy of CET019 (30 mg/kg) was tested using tumor (mouse and human cells) and patient derived xenograft studies.
Results: Our studies show CET019 suppresses the clonogenic potential and growth of various triple-negative breast cancer cells. The CET019 treatment causes cell cycle arrest in TNBC cells at the G2/M phase. Proteomics analysis suggests that CET019 specifically affects the protein translation associated pathways. CETSA assay identified the thermal shift of various translation associated proteins, including RPLs and RPS. *In vivo* studies with CET019 found an inhibition of tumor growth kinetics in the mouse xenograft study with different TNBC models, as compared to the control group. We also observed that CET019 inhibited metastatic lung nodules. The results were further substantiated in four Patient Derived Xenograft (PDX) models.
Conclusion: In summary, our study has strong preclinical evidence of CET019 as a promising lead candidate for TNBC treatment. “The complete chemical structures of the compounds used will be disclosed at the time of presentation at the meeting.”

#6880 Rinatabart sesutecan (PRO1184) in combination with standard-of-care therapy exerted potentiated antitumor activity in preclinical cancer models.

Lei Wang, Wanwan Shen, Wenke Qi, Qilin Wu, Yang Xiao

Genmab, Suzhou, China

Background: Rinatabart sesutecan (Rina-S) is an investigational antibody-drug conjugate composed of a human monoclonal antibody that selectively binds to folate receptor alpha (FRα), a novel protease-cleavable highly hydrophilic linker, and a topoisomerase 1 inhibitor, exatecan. Preclinical studies have demonstrated potent antitumor activity of Rina-S monotherapy in a broad range of tumors. In the phase 1/2 GEN1184-01 study (NCT05579366), treatment with Rina-S monotherapy resulted in antitumor activity in patients with heavily pretreated platinum-resistant ovarian cancer (PROC) and endometrial cancer (EC) across various FRα expression levels, with a manageable safety profile. Here we evaluate the combinability and antitumor activity of Rina-S with standard-of-care (SOC) therapies in preclinical cancer models.

Methods: To evaluate the effect of Rina-S on immunogenic cell death (ICD), FRα-expressing cell lines (KB and 3LL-FRα cells) were treated with either Rina-S or exatecan for 24 to 72 hours and then assessed for markers of ICD, including extracellular ATP, HMGB1, and calreticulin. To assess the antitumor activity of Rina-S alone or in combination with SOC therapies, multiple in vivo studies were conducted in various mouse models. Xenograft mouse models inoculated with OC (OVCAR-8 cells) or EC (HEC-1-A cells) received either phosphate-buffered saline (PBS) alone or Rina-S alone, with or without carboplatin (OC), bevacizumab (OC), or olaparib (EC). Syngeneic mouse models of lung cancer or colorectal cancer received either PBS alone or Rina-S alone, with or without an anti-PD1 agent nofazinilmab (CS1003). Antitumor activity was assessed by measuring tumor volume over 30 days and the evaluation durations for lung cancer and colorectal cancer were 20 and 28 days, respectively.

Results: In vitro incubation of FRα-expressing cells with Rina-S or exatecan resulted in upregulation of ICD overall, as measured by ATP, HMGB1, and calreticulin levels. In models of OC and EC, Rina-S combined with carboplatin, bevacizumab, or olaparib produced potentiated antitumor activity compared with single-agent treatment. Combination of Rina-S with an anti-PD1 agent in syngeneic models of lung and colorectal cancer showed a potentiated effect compared with single-agent treatment.

Conclusions: These preclinical studies provide evidence for combination therapy with Rina-S and various SOC therapies and suggest that Rina-S may be investigated in different treatment settings in the clinic. A phase 1/2 study (NCT05579366) is currently ongoing to study the efficacy and safety of Rina-S in combination with carboplatin, bevacizumab, or pembrolizumab for the treatment of platinum-sensitive OC, PROC, and EC, respectively.

#6881 Discovery of ACE-232: a novel and highly potent non-steroidal CYP11A1 inhibitor.

Kuo-Long Yu[1], Hongbin Yuan[2], Sanjeev Kumar[1], Kai Kang[2], Wenming Li[2], Manhua Li[2], Weitao Pan[1], **Wenqian Li**[1], Junmei Wang[1], Zhiming Wen[1], Genshi Zhao[1], Tinggui Yin[1]

[1]Acerand Therapeutics, US, Carmel, IN,[2]Medicinal Chemistry, Acerand Therapeutics, Shanghai, China

CYP11A1 is the first and rate-limiting enzyme in steroid hormone biosynthesis, catalyzing the conversion of cholesterol to pregnenolone and isocaproic aldehyde. Inhibition of this enzyme blocks the entire steroid hormone biosynthesis pathway, including androgens and their precursors, which activate the androgen receptor (AR) and promote prostate cancer growth. Targeting CYP11A1 has led to therapies like ODM-208 (MK-5684), which shows antitumor activity in heavily pretreated mCRPC patients, especially those with AR ligand binding domain (LBD) mutations. Here we report the discovery of ACE-232, a novel, highly potent, and selective CYP11A1 inhibitor. ACE-232 is significantly more potent than ODM-208 (>15x) in inhibiting CYP11A1 in human adrenal adenocarcinoma H295R cells, with an IC_{50} of 0.17 nM compared to ODM-208's IC_{50} of 2.82 nM. Additionally, ACE-232 demonstrates superior potency (>10x) in suppressing testosterone biosynthesis in H295R cells. Preclinical pharmacokinetic studies show that ACE-232 has significantly lower clearance and a longer half-life across multiple species (mouse, rat, and dog), resulting in a flatter pharmacokinetic (PK) curve compared to ODM-208. Together, these properties may enable sustained target engagement with once-daily dosing while minimizing hormone level fluctuations in patients. In castrated mouse models, ACE-232 showed equal or superior efficacy in inhibiting tumor growth at 0.3 mg/kg QD in both VCaP and enzalutamide-resistant xLNCaP models, compared to ODM-208 at 30 mg/kg BID. Furthermore, in a non-castrated mouse model, ACE-232 showed significant efficacy at 1.5 mg/kg BID, whereas ODM-208 at 30 mg/kg BID did not. ACE-232 also demonstrates favorable ADME properties suitable for drug development. In a safety panel screening across 93 targets at 5 μM, ACE-232 exhibited minimal off-target effects. GLP toxicology studies demonstrated a wide safety margin in both rats and dogs, with primary effects observed in the endocrine and reproductive organs, consistent with CYP11A1 inhibition. Based on these findings, an IND filing for ACE-232 with the FDA is planned for December 2024.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Novel Cancer Therapeutic Targets and Drug Delivery Technologies
Poster Session

#6885 Plectin as a novel therapeutic target for pancreatic cancer.

Cody L. Wolf[1], Roxanne K. Ruiz[2], Julien Dimastromatteo[1], Samantha Perez[1], Matthew J. Reilley[3], Kimberly A. Kelly[1]

[1]Biomedical Engineering, University of Virginia, Charlottesville, VA,[2]Department of Molecular Physiology and Biological Physics, University of Virginia, Charlottesville, VA,[3]Department of Medicine, University of Virginia, Charlottesville, VA

Pancreatic ductal adenocarcinoma (PDAC) is the 3rd deadliest cancer in the United States with only a 5-year survival rate of 13%, demonstrating a desperate need for novel therapeutic targets to significantly enhance options for patients. In 2008, *Kelly et al.* pioneered a phage-display-based functional-proteomic approach and identified a cytolinker protein, plectin, which was mislocalized and ubiquitously expressed on the membrane of PDAC. This unique localization of plectin, called cell surface plectin (CSP), has since been found to be expressed on the cell surface of many cancer subtypes, including PDAC, ovarian carcinoma and cholangiocarcinoma, but remains cytoplasmic in normal tissues. Knockdown of plectin and an anti-CSP functional monoclonal antibody revealed the importance of CSP to proliferation, migration, and invasion in cancers. Recently, a phase 1 clinical trial using an anti-CSP antibody, ZB131 (NCT05074472) in CSP positive cancer subtypes showed ZB131 as a viable and safe drug candidate for treatment. In the trial, treatment with ZB31 led to stable disease in 3 out of 9 PDAC patients. In order to more fully understand the mechanism of action of ZB131 and to obtain comprehensive understanding of molecular signaling of CSP in cancer we have employed bioinformatic strategies as well as knock outs using CRISPR/Cas9 inducible systems. Transcriptomic data from bulk and single cell RNA-seq data from human PDAC revealed that CSP overexpressing tumors were enriched for integrin-extracellular matrix interactions, suggesting CSP plays a major role in matrix remodeling and metastasis. Additionally, treatment with anti-CSP antibody led to a decrease in classic signaling cascades associated with cancer progression such as SRC, PRAS40, STAT3, AKT, and ERK1/2. The cumulative work from this research highlights the role of CSP in PDAC and suggests a desperate need for an anti-CSP therapeutic for patients.

#6886 Pharmacokinetics of paclitaxel and cyclopamine co-administered via polymeric micelle in HCC mice.

Emanuel R. Baltrip[1], Lu Dai[1], Diana S-L Chow[1], Chun Li[2]

[1]Pharmacological and Pharmaceutical Sciences, University of Houston, College of Pharmacy, Houston, TX,[2]Cancer Systems Imaging, University of Texas MD Anderson Cancer Center, Houston, TX

Hepatocellular Carcinoma (HCC) is a highly aggressive form of liver cancer that is typically diagnosed at an advanced stage, leaving limited treatment options. The current non-surgical, first-line treatment, Sorafenib, is non-curative and does not improve patient quality of life. Our study involved pharmacokinetic (PK) analysis of Paclitaxel (PTX) and Cyclopamine (CPA) in an HCC mouse model. PTX is a microtubule-stabilizing drug, while CPA is a Hedgehog signaling pathway inhibitor. Together, the compounds arrest the cell cycle at the M and G phases, preventing tumor cell proliferation. The compounds were co-administered using a polymeric micelle formulation, termed M-CPA/PTX, which contained 2.5 mg each of PTX and CPA, with size of 70.63 ± 3.4 nm, PDI of 0.122 ± 0.038, and encapsulation efficiency of ≥80% for both compounds. The PK was evaluated in HCC transgenic mice expressing the C-MYC oncogene, after IV dosing of M-CPA/PTX at 5 mg/kg/drug every other day for 1, 2, and 3 doses, respectively. The injection volume ranged from 100 - 200 µL based on body weight. The mice were randomized into four groups: vehicle (n = 10), 1 dose (n =8), 2 doses (n=7), and 3 doses (n = 10). After the last dose of each group, PK blood samples were withdrawn at specified time points up to 24 hours when the tumor and organs were harvested. Samples were analyzed using a validated LCMS/MS method with an LLOQ of 0.5 ng/mL, following a liquid-liquid extraction with a volume ratio of 5:1 ACN: H_2O (1:1 v/v) to blood sample or tissue homogenates. PK analysis was performed by both 1 and 2-compartment models using Phoenix WinNonlin™ to derive the PK parameters. ANOVA with the Tukey post hoc test was used to compare data across dosing groups with p-value <0.05 for significance. For PTX, the clearance (CL) was 43.17 ± 11.0 L/h/kg, not changing significantly after multiple dosing. The AUC had a notable, but insignificant, increase from 177.1 ± 44.23 h*ng/mL to 193.05 ± 31.76 h*ng/mL. The volume of distribution (V_d) was 91.3 ± 61 L/kg with no appreciable trend across the three groups. For CPA, the CL increased from 23.02 ± 10.34 L/h/kg to 63.48 ± 35.82 L/h/kg and 52.81 ± 31.45 L/h/kg after two and three doses, respectively. The AUC was 253.53 ± 103.2 h*ng/mL after one dose, which decreased to 230.55 ± 99.67 h*ng/mL and 153.96 ± 67.19 h*ng/mL after two and three doses, respectively. The V_d increased from 77.77 ± 12.91 L/kg to 130.57 ± 41.83 L/kg after three doses; however, this increase was not statistically significant. Tumor drug accumulation was also analyzed. In blood, the drug concentrations at 24 hr were below the LLOQ, so the absolute tumor concentration was evaluated. The concentrations were 9.24 ± 5.91 ng/g for PTX and 8.99 ± 4.1 ng/g for CPA. In conclusion, the PK of PTX and CPA in HCC mouse model from single and multiple dosing of M-CPA/PTX were characterized. Multiple dosing yielded no changes in CL and V_d of PTX, but increased CL and decreased AUC in CPA.

#6887 Targeting JMJD6: A Novel Approach in Cancer Therapy?.

Mathilde Calmels, Dorian Brager, **Christophe Marcireau**

Sanofi, Vitry-Sur-Seine, France

The Jumonji Domain Containing 6 protein (JMJD6) is part of the Jumonji C domain-containing protein family and functions as an arginine demethylase and lysine hydroxylase. JMJD6 facilitates demethylation of arginine and hydroxylation of lysine in both histone and non-histone proteins. Its activity on histones is primarily mediated by the JMJC domain, which is involved in demethylating H3R2me2 and H4R3me2. These demethylation events at histone H3 arginine 2 (H3R2me2) and histone H4 arginine 3 (H4R3me2) are crucial for regulating chromatin accessibility and gene transcription. JMJD6 also targets non-histone proteins such as TRAF6, STAT1, RNA helicase A, ERa
, HSP70, EIF3B, EIF3C, etc. Additionally, more than 150 sites of JMJD6-catalyzed lysine hydroxylation on 48 protein substrates have been identified. JMJD6 plays an important role in transcriptional regulation, RNA splicing, inflammation, and immune responses. It downregulates pathways and activities like p53, TGF-β signaling, and Myc-induced apoptosis, while upregulating processes such as CDKs, WNT, MAPK, and autophagy pathways. Abnormal JMJD6 expression is linked to various diseases, including cancer. Elevated JMJD6 expression has been reported in many tumor types, including breast invasive carcinoma (BRCA), lung adenocarcinoma (LUAD), liver hepatocellular carcinoma (LIHC), colon adenocarcinoma (COAD) and rectum adenocarcinoma (READ). High JMJD6 levels are also associated with poor prognosis, making it an attractive target for cancer therapy. In this study, we confirmed the critical role of JMJD6 in a NSCLC cell line and a liver cancer cell line. Using CRISPR/Cas9, we replaced the endogenous JMJD6 promoter with a doxycycline-regulated promoter, creating conditional JMJD6 cells. We will examine the effects of JMJD6 depletion in these cells and assess the complementation results with various JMJD6 alleles.

#6888 Targeting melanoma cells through G-quadruplex formation: mechanism and enhanced efficacy of T-oligo in combination therapy.

Rose Bahari[1], Stephanie M. Lopez[1], Archit Agnihotri[1], Hyatt Alaraj[2], Lawrence Miller[3], Neelu Puri[1]

[1]Biomedical Sciences, University of Illinois College of Medicine Rockford, Rockford, IL,[2]Biomedical Sciences, University of Illinois Chicago, Chicago, IL,[3]Chemistry, University of Illinois Chicago, Chicago, IL

Melanoma is one of the deadliest skin cancers in the world, estimated to cause 7,990 deaths annually. Telomerase binds to the 3' overhang of the telomere, causing telomere elongation and cell survival. Treatment against melanoma aims at targeting the 3' telomere overhang using T-oligo, which is a guanine-rich oligonucleotide homologous to the telomere overhang, preventing the growth of melanoma cells by inducing apoptosis. As a guanine-rich oligonucleotide, T-oligo can form G-quadruplexes (G4), secondary structures of nucleic acids, which we aim to detect using FRET microscopy in melanoma cells. Currently, vemurafenib and trametinib are agents that are used to treat melanoma. We were also interested in studying the combinational treatment of T-oligo with vemurafenib/trametinib to determine if it improves the efficacy of these drugs as compared to monotherapy in melanoma cells. We hypothesize that T-oligo forms G-quadruplexes in the nucleus of melanoma cells, and combination therapy of vemurafenib/Trametinib with T-oligo would be efficacious compared to monotherapy.

#6889 A gold-based antibody-drug conjugate with theranostic potential for synovial sarcoma.

Jeffrey Okojie, Bridger Kearns, Melanie I'anson-Holton, Andralyn McKell, Josh Hanosek, Jared James Barrott

Brigham Young University, Provo, UT

Synovial sarcoma is a rare form of cancer that occurs in the soft tissue adjacent to bones in adolescents and young adults. Although rare, it is ranked as the second most common malignant soft-tissue tumor in children. This cancer has a high metastatic rate with 50% of all cases exhibiting metastasis at the time of diagnosis. The first line of therapy for this cancer is surgery which is effective for the primary tumor, but in patients with metastatic disease, chemotherapy and radiation are the main therapeutic options. These therapies tend to have negative side effects due to their lack of specificity. Antibody-drug conjugates are a promising class of cancer therapeutics due to their specificity and reduced side effects. Of particular interest are gold-based antibody-drug conjugates due to their laser-induced temporal control and theranostic (diagnostic and therapeutic) capabilities. This work sought to develop a novel gold-based antibody-drug conjugate for synovial sarcoma with limited side effects. To accomplish this goal, a potential target receptor, Oncostatin M Receptor (OSMR), was identified. This receptor has been shown to be involved in cancer proliferation and migration when activated by its ligand, Oncostatin M(OSM). OSMR is highly expressed in various cancers including breast cancer. The mRNA and protein expression of OSMR in synovial sarcoma tumors was compared to normal tissues. An anti-OSMR antibody was conjugated to a fluorophore and the conjugate was injected into synovial sarcoma-bearing mice to assess the specificity and targeting ability of the antibody in vivo. Gold nanorods were then synthesized, characterized, and conjugated site-specifically to the anti-OSMR antibody. The over-expression of OSMR in synovial sarcoma tumors as compared to normal tissues was validated. An anti-OSMR antibody conjugated to a fluorophore targeted OSMR-expressing tumors and metastatic cells and discriminated between tumor and normal tissue in vivo. Gold nanorods were successfully synthesized and conjugated site-specifically to an anti-OSMR antibody. These findings suggest OSMR as a viable candidate for a targeted treatment of synovial sarcoma. They also suggest that an anti-OSMR conjugate can target both primary and metastatic tumors. The successful conjugation of gold nanorods to the anti-OSMR antibody opens the possibility of a theranostic approach to synovial sarcoma.

#6890 Knockdown of CD82 sensitizes adult T cell leukemia cells to anti-cancer agents-induced growth inhibition.

K. Fukuchi, T. Ikezoe, I. Ogahara, S. Zhao;
Fukushima Medical Univ., Fukushima, Japan

CD82 belongs to the tetraspanin family and regulates several intracellular signaling pathways. We previously found that CD82 is aberrantly expressed on acute myeloid leukemia (AML) cells and plays a role in the adhesion of these cells to the bone marrow microenvironment. Adult T-cell leukemia (ATL) is a lethal peripheral T-cell malignancy in which HTLV-1-infected T cells transform and proliferate by various mechanisms. This study found that ATL cells expressed aberrant CD82 compared to T cells isolated from a healthy volunteer, as assessed by immunocytochemistry. Interestingly, MTT assay found that MT-1 cells derived from ATL patient became more susceptible to growth inhibition mediated by doxorubicin and etoposide when CD82 was knocked down using the CRISPR-Cas9 technique, although the doubling time of CD82 knockdown MT-1 cells was compatible with parental MT-1 cells. These observations suggest that CD82 may be a promising therapeutic target in ATL. Further experiments are required to elucidate the role of CD82 in ATL cells.

#6892 Ribonucleotide reductase is a potential therapeutic target in colorectal cancer.

Federica Invrea, Consalvo Petti, Claudio Isella, **Enzo Medico**

Candiolo Cancer Institute, FPO-IRCCS, Candiolo, Italy

Colorectal cancer (CRC) is a heterogeneous disease showing significant variability in clinical aggressiveness and treatment response. Primary and acquired resistance limit the efficacy of available treatments, and identification of new therapeutic targets is needed to further improve patients' outcomes. To identify actionable targets, we performed a "drop-out" loss-of-function screening with an shRNA library covering 200 drug-target genes in nine different CRC cell lines, covering the whole spectrum of molecular subtypes of CRC. Multiple screening hits were found. Among them RRM1, encoding a member of the ribonucleotide reductase complex and targeted by the FDA-approved drug clofarabine, was found to be non-dispensable in all cell lines. In vitro drug efficacy studies confirmed sensitivity to clofarabine of most CRC cell lines. In vivo testing on a CRC cell line xenograft confirmed substantial growth inhibition by clofarabine. These results unveil a possible therapeutic opportunity in colorectal cancer.

#6896 A pan-KRAS degrader for the treatment of KRAS-mutant cancers.

Mu-Sheng Zeng[1], Jie Yang[1], Qiao-Li Wang Wang[1], Guan-Nan Wang[1], Jia-Cong Ye[1], Zi-Qian Li[1], Jing-Yun Wang[1], Zhao-Hui Liang[1], Shu-Xin Li[1], Cong Sun[1], Wen-Ting Liao[1], Yi-Jun Gao[1], Jing Wang[1], Yong Mao[2], Chunjing Yu[2], Guo-Kai Feng[1]

[1]Sun Yat-sen University Cancer Center, Guangzhou, China,[2]Affiliated Hospital of Jiangnan University, Wuxi, China

KRAS mutations are highly prevalent in a wide range of lethal cancers, and these mutant forms of KRAS play a crucial role in driving cancer progression and conferring resistance to treatment. While there have been advancements in the development of small molecules to target specific KRAS mutants, the presence of undruggable mutants and the emergence of secondary mutations continue to pose challenges in the clinical treatment of KRAS-mutant cancers. In this study, we developed a novel molecular tool called tumor-targeting KRAS degrader (TKD) that effectively targets a wide range of KRAS mutants. TKD is composed of a KRAS-binding nanobody, a cell-penetrating peptide selectively targeting cancer cells, and a lysosome-binding motif. Our data reveal that TKD selectively binds to KRAS in cancer cells and effectively induces KRAS degradation via a lysosome-dependent process. Functionally, TKD suppresses tumor growth with no obvious side effects and enhances the antitumor effects of the PD-1 antibody and cetuximab. This study not only provides a strategy for developing drugs targeting “undruggable” proteins but also reveals that TKD is a promising therapeutic for treating KRAS-mutant cancers.

#6897 Development of immunomodulatory nanoparticles targeting HER2 for breast cancer therapy.

Mathilde Dacos, Lea Plantureux, Erwan Diroff, Sarah Giacometti, Joseph Ciccolini, **Raphaelle Fanciullino**

Aix-Marseille University, Marseille, France

Nanoparticles (NPs) are emerging as a promising strategy in oncology, offering optimised drug delivery and the potential to modulate immune responses. In this study, we developed an innovative nanoparticle encapsulating docetaxel and functionalised with trastuzumab for the precise targeting of HER2-positive tumours (called ANC for Antibody Nanoparticle Conjufate). Pharmacokinetic analyses were performed using C57BL/6 mice, which were administered free docetaxel (1.6 mg/kg) and trastuzumab (135 ng/kg) via intraperitoneal injection (n = 29), or ANC (nanoparticles at the same docetaxel and trastuzumab doses as the free drugs, n = 40). Blood samples were collected at 0.5, 1, 2, 3, 6, 8, 12, and 24 hours post-injection, and the frozen plasma was analysed using by HPLC-UV. For biodistribution studies, DiR-labelled nanoparticles were administered to C57BL/6 mice, and imaging was performed on a daily basis using an IVIS Spectrum imager. Immunomodulation study was assessed by flow cytometry. The $AUC_{0-\infty}$ for the ANC (4250.79 ng.mL/h) was four times higher than for free docetaxel (1050.3 ng.mL-1.h-1). In addition, the half-life of the liposomal docetaxel (22.14 hours) was significantly three times longer than that of the free docetaxel (7.15 hours). Biodistribution studies in mouse models showed that the nanoparticle remained stable and did not reach the liver until 30 hours after injection. Immunomodulation studies showed that ANC had a different effect on the distribution of lymphocyte subpopulations compared to free drug, with a notable decrease in regulatory T cells (Tregs) in the ANC-treated group, suggesting a potential immunostimulatory effect. The potential immunomodulatory effects of these nanoparticles on anti-tumour immune responses are currently being investigated. These preliminary results demonstrate the dual benefit of targeted drug delivery and immune system engagement offored by these nanoparticles, which could pave the way for novel breast cancer treatments.

Keywords: Nanoparticles, breast cancer HER2 +, immunomodulation, pharmacokinetics, biodistribution

#6898 Identification of a novel first-in-class inhibitor targeting the ADAR1 p150 isoform demonstrates robust anti-tumor efficacy in a syngeneic mouse model.

Avijit Goswami, Sandeep Goyal, Kawaljit Singh, Princy Khurana, **Aditya Kulkarni**

Avammune Therapeutics, Phoenixville, PA

Background: Adenosine deaminase acting on RNA (ADAR1) catalyzes the conversion of adenosine (A) to inosine (I) in double-stranded RNAs (dsRNAs). It exists in two isoforms: nuclear p110 and p150, which shuttles between the nucleus and cytoplasm and is regulated by interferon (IFN) signaling. The p150 isoform contains a Z-DNA/Z-RNA binding domain and suppresses immunogenic dsRNAs and Z-RNAs arising from endogenous retroviral elements. Loss of ADAR1 leads to Z-RNA accumulation, activation of the sensor ZBP1, and RIPK3-driven necroptosis. ADAR1 promotes tumor cell survival and resistance to immune responses, making p150 inhibition a promising strategy to boost anti-tumor immunity. Herein, we report the discovery of a first-in-class Zα-targeted ADAR1 p150 inhibitor for cancer immunotherapy.
Methods: A class of ADAR1 p150 inhibitors (AVA-ADR) were identified through a high-throughput ADAR1p150 binding assay. The ability of these inhibitors to induce interferons was confirmed in various cell-lines. The on-target mechanisms were confirmed by downstream PKR activation and MDA5 mediated upregulation of ISGs. Pharmacokinetic studies demonstrate the oral availability of the AVA-ADR compounds. The anti-tumor efficacy of our ADAR1 p150 inhibitors was evaluated in B16F10 syngeneic melanoma mice model as monotherapy and in combination with anti-PD-1.**Results:** We have identified a potential first-in-class small molecule inhibitor specifically targeting the ADAR1 enzyme via the Zα domain. Our compounds exhibit potent in vitro IFN response via an MDA5-dependent mechanism. Binding studies revealed a series of AVA-ADR compounds with strong sub-micromolar affinity for the Zα-domain. ADME and pharmacokinetic analyses demonstrate favorable PK profiles and oral bioavailability. Our ADAR1 inhibitors demonstrated promising anti-tumor activity in preclinical melanoma models, with notable tumor growth inhibition observed both as monotherapy and in combination with immune checkpoint inhibitors. The combination therapy led to enhanced efficacy compared to either treatment alone. Additionally, markers associated with immune activation were significantly upregulated in tumor samples from the combination group. Target engagement was supported by the development of a PROTAC derivative, which selectively degraded the target protein.**Conclusions:** To our knowledge, our AVA-ADR series of compounds are the first report of a selective small molecule inhibitor of ADAR1 p150. The 2nd generation AVA-ADR compounds described here are stable/orally bioavailable first-in-class ADAR1 inhibitors that demonstrated significant IFN induction in-vitro and in-vivo, demonstrating significant tumor growth inhibition as monotherapy and synergistically in combination with Anti -PD1.

#6899 Investigating the impact of CXCR6 antagonism on Wnt/β-catenin signaling and sorafenib resistance development.

Anastasia G. Chambers[1], Abhishek Shrestha[1], Morgan Reeves[2], Alivia Cullu[1], Lauren Donohue[1], Sergio Duarte[1], Satyamaheshwar Peddibholta[2], Siobhan Malany[2], Ali Zarrinpar[1]

[1]Department of Surgery, University of Florida College of Medicine, Gainesville, FL,[2]Department of Pharmacodynamics, University of Florida College of Pharmacy, Gainesville, FL

Introduction: Due to its heterogeneity, hepatocellular carcinoma (HCC) has a high incidence of resistance and toxicity to its first-line therapy sorafenib. Combinations of drugs can be used to exploit synergistic effects to improve efficacy and reduce adverse events. Upregulation of C-X-C chemokine receptor type 6 (CXCR6) and its ligand has been observed in multiple cancers and has been used as a predictor of cancer recurrence and poor prognosis in HCC. Increased CXCR6 signaling has been shown to promote Wnt/β-catenin signaling, a pathway known to be dysregulated in 50% of HCC and involved in sorafenib resistance. We have developed SBI-457, an orally bioavailable small molecule CXCR6 inhibitor, which decreased tumor growth in a murine HCC model. In this study, we test the efficacy of combining SBI-457 and sorafenib in mitigating the development of resistance and increasing sensitivity to drug treatment in HCC.

Experimental Procedure: An HCC cell line-derived xenograft model was generated by injecting HCC cell line SKHep1 subcutaneously in the flank of male NOD.Cg-Prkdc^{scid}IL2rg^{tm1Wjl}/SzJ (NSG) mice. Mice were treated with vehicle, sorafenib, SBI-457, or sorafenib and SBI-457 in combination; mouse tumor volumes and weights were tracked for the duration of study. Mice were all euthanized at study endpoint, and tissues collected for analysis, including assessment of tumor proliferation, invasion, and CXCR6 and β-catenin expression levels.

Results: SKHep1 tumors treated with sorafenib and SBI-457 in combination were significantly reduced in tumor volume and weight compared to vehicle. Mice treated with SBI-457 alone or in combination with sorafenib maintained stable weight and had no observed toxicity due to treatment. CXCR6 expression was slightly elevated in the sorafenib only treated group and reduced in the groups treated with SBI-457 alone. Western blot and IHC analysis confirmed that treatment with SBI-457 increases active (non-phosphorylated) GSK-3β and decreases β-catenin expression, while sorafenib treatment resulted in an increased phosphorylated GSK-3β and (non-phosphorylated) active β-catenin expression. This sorafenib-induced increase in β-catenin was attenuated by combination treatment with SBI-457.

Conclusion: This study demonstrated that treatment with CXCR6 antagonist, SBI-457, in combination with sorafenib was able to significantly reduce tumor burden compared to vehicle. Additionally, combination therapy seems to attenuate the sorafenib-induced increase in β-catenin expression, indicating that CXCR6 inhibition could help mitigate sorafenib resistance development.

#6901 Imipridones (ONC201, ONC206 and ONC212) modulate MGMT and ClpX expression in DIPG cell lines.

Lanlan Zhou, Shengliang Zhang, Wafik S. El-Deiry

Brown University, Providence, RI

The first-in-class small-molecule imipridone ONC201/Dordaviprone targets mitochondrial protease ClpP to disrupt oxidative phosphorylation and trigger integrated stress response (ISR), TRAIL/DR5, and tumor cell death. ONC201 and analog ONC206 and ONC212 are blood-brain barrier penetrant. Temozolomide (TMZ) is an oral alkylating agent that is generally well tolerated. Epigenetic transcriptional silencing of O6-methylguanine-DNA methyltransferase (MGMT) mediated by MGMT gene promoter methylation is associated with response to TMZ treatment. TMZ has been used in combination with Radiation Therapy (RT) as standard of care for glioblastoma (GBM) with a methylated MGMT promoter since 2005. Our previous studies demonstrated that ONC201/ONC206 synergized with standard-of-care GBM therapies TMZ and RT in GBM cell lines and orthotopic GBM mouse model. Diffuse Intrinsic Pontine Gliomas (DIPG) is a fast growing and highly aggressive pediatric central neural system tumor that starts in the brain stem's glial cells and has a dismal prognosis. DIPG has been recognized to be molecularly distinct from both adult high-grade glioma (HGG) and other non-midline pediatric high-grade gliomas (pHGG). DIPG has almost universally unmethylated MGMT promoters and TMZ has no role in the treatment of this disease. We reported earlier that ONC201 suppresses oxygen consumption rate observed with TMZ alone in DIPG cells, and TMZ has effects on T-cells killing solid tumor cells with high expression of MGMT. In this study we discover that imipridones (ONC201, ONC206 and ONC212) reduce expression of MGMT in DIPG cell lines following treatment with Imipridones -/+ TMZ. ClpX levels were reduced in all glioma cells treated and served as a biomarker of imipridone target engagement. The change of MGMT has the same trend as that of ClpX. These novel findings suggest a potential mechanism of synergy between imipridones and TMZ through suppression of MGMT protein expression in DIPG cells that typically have unmethylated MGMT promoter and are resistant to effects of TMZ. We are going to test this mechanism of synergy and therapeutic efficacy in vivo by treating orthotopic DIPG mouse models with imipridones -/+ TMZ. Our results support further studies combining imipridones (ONC201, ONC206 and ONC212) with TMZ and RT as a reasonable and safe therapeutic option for DIPG.

#6902 Promising therapeutic effects of pyrimidine synthesis inhibition by a novel dihydroorotate dehydrogenase inhibitor in small cell lung cancer.

Bahareh Nourmohammadi[1], Ola A. Elgamal[2], Sandip Vibhute[3], Christopher C. Coss[4], Thomas E. Goodwin[5], Erin Hertlein[2], Joseph M. Amann[6], Chad E. Bennett[4], John C. Byrd[2], David P. Carbone[6]

[1]Biomedical Sciences Graduate Program, College of Medicine, The Ohio State University, Columbus, OH,[2]Division of Hematology and Oncology, Department of Internal Medicine, College of Medicine, University of Cincinnati, Cincinnati, OH,[3]Drug Discovery Shared Resource, Comprehensive Cancer Center, The Ohio State University, Columbus, OH,[4]Drug Development Institute, Comprehensive Cancer Center, The Ohio State University, Columbus, OH,[5]Department of Chemistry, Hendrix College, Conway, AR,[6]Division of Medical Oncology, Department of Internal Medicine, James Thoracic Center, The Ohio State University, Columbus, OH

Small-cell lung cancer is an aggressive subtype of lung cancer with poor prognosis and poor overall survival and comprises approximately 15% of all lung cancers. SCLC, unlike NSCLC, has no known targetable driver mutations and, therefore, targeted therapeutics have been lacking. The frontline therapy for SCLC is platinum-etoposide chemotherapy, a regimen that has been in use for 30 years. Although patients respond well to this combination initially, they almost always relapse shortly after the start of therapy and second line therapies typically provide only a few months of benefit. Clearly, better therapeutics are necessary for the treatment of SCLC. Cancer cells often have dysregulated metabolic pathways. These changes are often necessary to enable the continued proliferation of the cancer cells. Therefore, key metabolic enzymes can have a crucial role in tumor cells survival and can be considered targets for cancer treatment. According to some recent studies, dihydroorotate dehydrogenase (DHODH), which catalyzes the conversion of dihydroorotate to orotate in the pyrimidine *de novo* synthesis pathway, has a particular role in the survival of different types of cancers including leukemia and SCLC. We evaluated the effect of HOSU-53, a novel DHODH inhibitor, on SCLC tumor growth *in vitro* and *in vivo*. We measured IC50s of 18 SCLC cell lines *in vitro* using CellTiter-Glo® Luminescent Cell Viability Assay and most SCLC cells showed sensitivity to HOSU-53 treatment in the low nanomolar range. Additionally, to determine the role of salvage pathway in HOSU-53 sensitivity, we treated the sensitive and resistant cells in parallel with HOSU-53 alone or in combination with exogenous uridine. In the presence of uridine, cell viability was significantly rescued in resistant cells compared to HOSU-53 treatment as single agent. However, we did not observe rescued cell viability in sensitive SCLC cells. These results suggesting that in SCLC cells which are resistant to *de novo* pyrimidine inhibition, activation of salvage pathway may be one of the compensatory mechanisms for tumor cells to sustain their survival. To evaluate the effect of DHODH inhibition on SCLC tumor growth *in vivo*, we treated xenograft mice models with HOSU-53 alone or in combination with etoposide plus cisplatin as the standard chemotherapy for SCLC. We observed a significant decrease of tumor volume when HOSU-53 was used alone and in combination, without significant animal weight loss. Together, these data demonstrated the promising efficacy of HOSU-53 in different SCLC subtypes and support the strategy of targeting DHODH as a potential therapy to treat SCLC. These findings support the initiation of a phase I clinical trial to evaluate the preliminary efficacy and tolerability of HOSU-53; therefore, we have initiated a first-in-human phase I trial of this agent.

#6903 PERMnano: A quick method to develop nanoliposomes and nanosuspensions for oral/parenteral administration to expedite preclinical testing.

Henis Patel, Himaxi Patel, Akanksha Ugale, **Ketan Patel**

St. John's University, Queens, NY

The vast majority of novel drug candidates are hydrophobic in nature, which often presents significant challenges in formulation due to poor aqueous solubility, limiting their drug delivery, systemic concentration, and availability at the target site. Generally, organic solvents (ethanol, PEG, DMSO etc.), cellulose polymers, vegetable oils and surfactants are used to administer drugs for preclinical testing. However, these methods may result in false negative efficacy and poor translational insight. Liposomes (a bilayer phospholipid vesicle) and nanosuspensions (nanostablized drug particles) have emerged as promising delivery systems to overcome these challenges with great translational potential. However, both technologies require a thorough knowledge of excipients, set-up, processing and skilled manpower. This study explores a simple and quick approach for preparing nanoliposomes and nanosuspension of various hydrophobic drugs for oral and parenteral administration. The first objective was to develop an excipient blend for the preparation of liposomes/nanosuspension based on the physicochemical properties of the drug and the route of administration. The second objective was to develop a rapid media milling process using parenterally acceptable zirconia beads. Thus, the study aimed to develop and optimize a premix excipient blend with rapid nanomilling for the preparation of nanomedicine (PERMnano). For liposomes, a pro-liposome powder was prepared through a modified solvent evaporation method, using three different saturated and unsaturated phospholipids, PEGylated DSPE and cholesterol. For nanosuspensions, stabilizer blends (FDA-approved stabilizers for oral and parenteral use) were prepared using a freeze-drying process. Various hydrophobic drugs, including Amphotericin B, Docetaxel, Ibrutinib, BRD4 PROTAC, Sotorasib, Mebendazole, Ebselen, and Irinotecan were suspended into aqueous phase with 1-10% premixed excipient blends for nanoliposomes and nanosuspension. The results demonstrated that both liposomes and nanosuspensions prepared via rapid wet media milling showed desirable physicochemical properties, with particle sizes consistently below 200 nm and with narrow size distribution (PDI < 0.2), indicating uniformity and stability. Entrapment efficiency (%EE) and drug loading (%DL) were significantly influenced by the choice of excipient blend and physicochemical properties of the drug candidate. The resultant formulations were stable and suitable for administration into animals. In conclusion, PERMnano could potentially provide a rapid, translational, and efficient technique for preclinical drug delivery of hydrophobic drug molecules, including beyond the rule of 5 molecules, high dose candidates and PROTACs.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Novel Targets and Pathways
Poster Session

#6906 c-Myc inhibition through targeting MMK3 and c-Myc interaction overcomes acquired resistance to third generation EGFR inhibitors in the treatment of EGFR mutant lung cancer.

Z. Chen, K. Vallega, D. Wang, H. Fu, S. Ramalingam, A. Ivanov, S.-Y. Sun;
Emory University, Atlanta, GA

There is an urgent need to develop effective strategies for overcoming the inevitable acquired resistance to third generation EGFR tyrosine kinase inhibitors (EGFR-TKIs) such as osimertinib, which are approved for the treatment of advanced non-small cell lung cancer (NSCLC) harboring mutant EGFR. Our previous study has demonstrated c-Myc inhibition as a critical mechanism in mediating the therapeutic efficacy of osimertinib against EGFR mutant (EGFRm) NSCLCs/tumors and targeting c-Myc as an effective strategy for overcoming osimertinib acquired resistance. However, lack of specific c-Myc inhibitors restricts the application of this strategy in battling against acquired resistance to third generation EGFR-TKIs. Recently, we developed a novel strategy to inhibit c-Myc via disrupting the interaction of MKK3 and c-Myc, which stabilizes c-Myc protein. In this study, we found that the specific inhibitor of MKK3/c-Myc interaction, SGI-1027, effectively decreased c-Myc levels via disrupting the interaction and promoting c-Myc degradation in different osimertinib-resistant EGFRm NSCLC cell lines that possessed elevated levels of both c-Myc and MKK3 and increased interaction of MKK3 and c-Myc. SGI-1027, but not a p38 (that activates MKK3) or MKK3 kinase inhibitor, when combined with osimertinib or another third generation EGFR-TKI, synergistically decreased the survival of osimertinib-resistant EGFRm NSCLC cells with enhanced induction of apoptosis. In agreement, genetic knockdown of MKK3 led to c-Myc reduction and re-sensitized osimertinib-resistant EGFRm NSCLC cell lines to undergo apoptotic cell death upon osimertinib treatment. Moreover, the combination of SGI-1027 and osimertinib was also significantly more active than either single agent in suppressing the growth of osimertinib-resistant EGFRm NSCLC tumors in mice, indicating enhanced tumor-inhibitory effect *in vivo*. In sensitive EGFRm NSCLC cell lines, osimertinib effectively inhibited MKK3 and c-Myc interaction accompanied with substantial reduction of c-Myc levels, suggesting a critical mechanism by which osimertinib decreases c-Myc levels through promoting c-Myc degradation via disrupting MKK3 and c-Myc interaction. Hence, our findings reveal molecular mechanisms accounting for c-Myc reduction by osimertinib in sensitive EGFRm NSCLC cells and c-Myc elevation in EGFRm NSCLC with acquired resistance; it also suggests a novel strategy to target c-Myc via disrupting MKK3 and c-Myc interaction for overcoming acquired resistance and rationale for developing cancer therapeutic agents that specifically disrupts MKK3 and c-Myc interaction. (This study was supported by NIH/NCI R01 CA245386, UG1 CA233259, U01 CA168449, U01 CA217875, lung cancer SPORE P50CA217691 and Emory Winship Cancer Institute lung cancer research internal funds)

#6907 Novel TACC3 protein-protein interaction inhibitor, AO-252, shows potent activity in prostate, gastric cancers, and triple negative breast cancer with brain metastasis.

Sridhar Vempati, Chaemin Lim, Robbin Frnka, Edward Painter, Sotirios G. Stergiopoulos

A2A Pharmaceuticals, New York, NY

TACC3 (Transforming Acidic Coiled-Coil-Containing 3) is a key player in microtubule and centrosomal processes and is considered to have an important role in causing chromosomal instability, leading to aggressive cancer. TACC3 overexpression is found in multiple cancers and strongly associated with a poor prognosis in those cancer types. TACC3 is a pleiotropic gene which binds to multiple proteins and plays an important role in DNA damage response mechanisms, hematopoiesis, epigenetics, and immune functions. While TACC3 is essential for cancer cells but it is dispensable for normal cells based on conditional knockout in adult mice, making it an ideal treatment target for highly aggressive cancers. We had previously shown that AO-252, a novel small molecule TACC3 protein-protein interaction (PPI) inhibitor, exhibited strong in vivo efficacy in triple negative breast cancer (TNBC) and ovarian cancer. In the present work, we show in vitro and vivo assays that AO-252 shows exceptional efficacy in prostate, gastric cancers and TNBC with brain metastases. In all these cancers, AO-252 exhibited dose dependence on tumor growth inhibition and complete responses in xenograft models. In addition, these responses demonstrated to be durable even after stopping the treatment with AO-252. We also show that AO-252 exerts its activity by destabilizing the C-terminus of TACC3 and disrupting the protein-protein interactions occurring in the C-terminus (EGFR, NOTCH, BRCA1, HIF1b/ARNT, KIFC1, HDAC2, MBD2, DNAPK etc) without blocking the N-terminus interactions. AO-252 is currently in first-in-human (FIH), Phase I clinical trial in TNBC, ovarian and endometrial cancers (NCT06136884). We are in the process of expanding our trial to include prostate, gastric cancer and TNBC with brain metastases.

#6908 Autophagy disruption via PIKfyve inhibition as a novel strategy to enhance immunotherapy responses in multiple myeloma.

Cecilia Bonolo de Campos[1], Dor D. Abelman[1], Ruijuan He[1], Tessa Pelino[1], Ding Yan Wang[1], David S. Scott[1], Zhihua Li[1], Michael St Paul[1], Trevor J. Pugh[1], Olga Issakova[2], Nikolai Sepetov[2], Tak W. Mak[1], Suzanne Trudel[1], A Keith Stewart[1]

[1]UHN Princess Margaret Cancer Centre, Toronto, ON, Canada,[2]PIKSci, Toronto, ON, Canada

Despite advancements in the treatment of multiple myeloma (MM), most patients ultimately relapse due to innate and acquired drug resistance. Our search for novel therapeutic strategies led to the development of PIK001, a potent and selective PIKfyve inhibitor, as a promising therapeutic approach targeting lysosomal function and autophagy. In addition to its robust single agent anti-MM activity, PIK001 presented synergistic activity with relevant anti-MM therapeutics, including selinexor, venetoclax, and pomalidomide (IMiDs), *in vitro*. Notably, PIK001 retained efficacy in IMiD-resistant isogenic human myeloma cell line (HMCL) models, underscoring its potential in drug-resistant MM. To investigate the determinants of PIKfyve resistance, we generated PIK001-resistant HMCLs by culturing three PIK001-sensitive HMCLs (KMS26, KMS11, and JJN3) in escalating doses of the PIKfyve inhibitor (up to 5uM). These isogenic models of resistance were characterized by whole genome and transcriptome sequencing and mass spectrometry-based proteomics. *Ex vivo* downstream effects of PIK001 treatment with 500nM for 16h were further assessed in patient-derived CD138+ MM samples using single-cell multiomic sequencing. PIK001 resistance was associated with a marked upregulation of genes and proteins involved in lysosomal function, autophagy regulation, and cholesterol homeostasis, along with downregulation of MYC targets. These findings were also found in primary patient samples following PIK001 treatment. KMS26 PIK001-resistant showed a clonal *PIKFYVE* kinase domain mutation, previously described in a resistant diffuse large B-cell lymphoma cell line. Importantly, we observed a two-fold increase in canonical and noncanonical Major Histocompatibility Complex (MHC) class I and a four-fold increase in MHC class II gene and protein expression in the PIK001-resistant compared to PIK001-sensitive KMS11. Increased cell surface expression of MHC Class I and II in KMS11 PIK001-resistant was confirmed by flow cytometry. PIK001-resistant KMS26 and JJN3 also presented an upregulation of cell surface expression of MHC Class I and, to a lesser extent, II. Similarly, PIK001 treatment also resulted in increased in MHC Class I gene expression on primary patient samples. Since downregulation or loss of MHC Class I has been shown as a mechanism of immune evasion in cancer, these findings suggest that PIKfyve inhibition may enhance MM immunotherapy responses by upregulating MHC surface expression. This hypothesis aligns with recent studies demonstrating increased tumor-specific MHC Class I expression and improved cancer immunotherapy efficacy following PIKfyve inhibition in solid tumors. Together, these results highlight the potential of PIKfyve inhibitors to synergize with existing anti-MM therapeutics and sensitize MM cells to MHC-dependent immunotherapies via autophagy disruption.

#6909 Exportin 1 as a novel therapeutic target in gastroenteropancreatic neuroendocrine tumors.

Md. Hafiz Uddin[1], Husain Y. Khan[1], Amro Aboukameel[1], Sahar F. Bannoura[1], Hugo Jimenez[1], Grayson Barker[1], Allan M. Johansen[2], Khalil Choucair[1], Sunil Jaiman[3], Gregory Dyson[1], Yang Shi[1], Mohammed N. Al Hallak[1], Nitin Vaishampayan[1], Steve Kim[1], Eliza W. Beal[1], Miguel Tobon[1], Herbert Chen[4], Muhammad W. Saif[1], Jennifer Beebe-Dimmer[1], Anthony F. Shields[1], Ramzi M. Mohammad[1], Philip A. Philip[5], Bassel El-Rayes[6], Boris C. Pasche[1], Asfar S. Azmi[1]

[1]Department of Oncology, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI,[2]Department of Cancer Biology, Wake Forest University, Winston-Salem, NC,[3]Department of Pathology, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI,[4]Department of Surgery, O'Neill Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL,[5]Henry Ford Cancer Institute, Henry Ford Health System, Detroit, MI,[6]Division of Hematology and Oncology, O'Neill Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL

Background: Gastroenteropancreatic neuroendocrine tumors (GEP-NETs) show minimal response to FDA-approved therapies suggesting an urgent need for the identification of novel and effective therapeutic targets. Aberrant nuclear protein transport to the cytoplasm, often observed in cancer, causes mislocalization-dependent inactivation of critical cellular proteins. The major nuclear exporter exportin-1 (XPO1) has been linked to cancer therapy resistance. Nevertheless, the role of XPO1 in GEP-NETs has not been explored. Major GEP-NETs growth regulators such as MEN1, DAXX and mTOR are recognized cargo proteins of XPO1, which regulates their nucleo-cytoplasmic transport. Here we evaluated the role of XPO1 in pancreatic neuroendocrine tumors (pNET) subsistence and therapy resistance.

Methods: We performed immunohistochemistry (IHC) analysis for XPO1 on 145 low-grade pNET tumors, and 35 normal, and established cell lines. Growth inhibition was determined by MTT, colony formation and apoptosis assays. Gene expression was determined using quantitative PCR, and protein expression using western blotting and immunofluorescence (IF) techniques. The impact of XPO1 inhibitor selinexor and analogs was evaluated in pNET 2D and 3D cultures, xenograft and primary explant cultures.

Results: IHC on TMAs confirmed XPO1 over-expression in pNET tumor tissue. Selective inhibitors of nuclear export (SINE) selinexor and eltanexor suppressed the growth of BON-1 and QGP-1 cells at pharmacologically relevant concentrations. Both inhibitors significantly reduced the number and the area of the colonies, and were able to induce apoptosis. Western blot analysis revealed significant induction of PARP cleavage. The pNET marker Chromogranin A was found to be reduced in the XPO1 inhibitors treated cells in IF assay. Selinexor synergized with everolimus and sunitinib leading to superior pNET cell deaths (combination index<1). IF analysis showed nuclear retention of mTOR and MEN1, and perinuclear accumulation of DAXX in the BON-1 cell line. Selinexor suppressed phosphorylation of mTOR and its downstream targets P70S6K and RICTOR. We observed significant inhibition of MEN1 targets including suppression of FANCD2 and SMAD3 and activation of p53. Selinexor given at low dose (15 mg/kg), twice a week for three weeks suppressed the growth of BON-1 and QGP-1 tumors *in vivo*. An examination of residual tumors showed a reduction in mTOR downstream target pS6 and reduced nuclear expression of pAKT. Six-day treatment with 300 nM selinexor demonstrated a marked reduction in the proliferation marker ki67 and nuclear retention of the well-recognized XPO1 cargo FOXO3a in the pNET explant tissue.

Conclusions: This is the first study to reveal the therapeutic potential of novel XPO1-targeted agents for the treatment of a GEP-NET *in vitro* and *in vivo*. XPO1 could be a novel therapeutic target that warrants further clinical investigations in GEP-NETs.

#6910 Synergistic tumor cell killing with dual inhibition of PARG and USP1.

Ramya Ravindranathan, Alexandre A. da Costa, Benjamin P. Lamarre, Sirisha Mukkavalli, Ozge Somuncu, Kalindi Parmar, Geoffrey I. Shapiro, Alan D. D'Andrea

Harvard Medical School, Dana-Farber Cancer Institute, Boston, MA

Poly (ADP-ribose) glycohydrolase (PARG) is an enzyme that aid in DNA repair primarily through the removal of poly (ADP-ribose) (PAR) chains from PARylated proteins. PARG inhibitors are currently in clinical development for patients with tumors exhibiting homologous recombination deficiency (HRD). Recently, we have demonstrated that similar to PARP inhibitors, PARG inhibitors also promote the formation of single stranded DNA gaps (ssGAPs) in preclinical ovarian cancer models. PARGi treatment increases the levels of toxic S phase PARylation resulting from Okazaki fragment processing (OFP) defects. More importantly the cellular sensitivity to PARGi directly correlates with the formation of ssGAPs. Additionally, our recent findings show that inhibitor of USP1, a deubiquitinating enzyme, also promotes the accumulation of ssGAPs during replication in BRCA1-deficient cells, and this phenotype correlates with the drug sensitivity. Interestingly, a CRISPR screen with USP1 inhibitor (USP1i) KSQ-4279 has identified gRNAs against genes encoding proteins in the OFP and base excision repair (BER) pathways, particularly PARG and PARP1, as key sensitizers. Combining PARP inhibitor (PARPi) with USP1i has shown promise in preclinical setting, extending PARPi benefits to tumors without homologous recombination defects and addressing PARPi resistance. In this study, we provide the evidence that PARGi synergizes with USP1i to maximize vulnerability to drug-induced replication stress in a panel of ovarian cancer and breast cancer cells. The combination treatment exacerbated DNA damage and replication stress as detected by an increase in markers such as pKAP1, pRPA, pCHK1 and γH2AX. Notably, USP1i augmented the PARGi-induced S phase PARylation and specifically PARylation at the replication forks. As predicted, the combination treatment induced ssGAP formation in the sensitive cells. Dual inhibition of PARG and USP1 caused an accumulation of unresolved replication intermediates, resulting in mitotic catastrophe as evidenced by a marked increase in micronuclei formation and chromosomal aberrations. Importantly, PARGi and USP1i combination enhanced tumor cell killing in a subset of PARP-inhibitor-resistant BRCA1-deficient tumors. Overall, these results indicate that the combination treatment with a PARGi and a USP1i causes accumulation of toxic PARylation at the replication fork leading to ssGAPs and replication catastrophe. Both PARG inhibitors and USP1 inhibitors are orally available drugs currently in clinical trials. Our data provides novel mechanistic insights into the therapeutic potential of combining these inhibitors, offering preclinical evidence to support its advancement for treating tumors with HRD and those resistant to PARP inhibitors. This combination strategy holds promise for enhancing treatment efficacy while reducing toxicity, paving the way for more targeted and effective therapeutic options.

#6911 Investigating pharmacological targeting of the SLC1A1 aspartate/glutamate transporter.

Pooneh Koochaki[1], Jesse A. Coker[2], Biao Qiu[3], Nancy Wang[2], Todd Romigh[2], Shaun R. Stauffer[2], Olga Boudker[3], Abhishek A. Chakraborty[1]

[1]Cancer Biology, Cleveland Clinic Lerner Research Institute, Cleveland, OH,[2]Center for Therapeutics Discovery, Cleveland Clinic Lerner Research Institute, Cleveland, OH,[3]Department of Physiology & Biophysics, Weill Cornell Medicine, New York, NY

Background: SLC1A1/EAAT3 functions as a trimeric, Na^+-dependent dicarboxylic amino acids (glutamate and aspartate) transporter. Recent studies have identified SLC1A1/EAAT3 as an oncogenic dependency in several cancers, including clear cell renal cell carcinoma (ccRCC), blood-borne tumors, and lung cancer. Unfortunately, despite this transporter's potential as a therapeutic target, pharmacological inhibition of SLC1A1 has remained elusive due to the absence of potent and selective inhibitors. In earlier studies, we investigated a previously described bicyclic imidazopyridine aniline SLC1A1 blocker (which we synthesized in-house, Compd 3e). Compd 3e bound SLC1A1 directly and promoted its thermal stability. Furthermore, Compd 3e treatment replicated the effects of genetic SLC1A1 blockade in terms of both metabolic changes and cytotoxicity of ccRCCs. Importantly, these fitness defects were rescued by supplementation of cell-permeable Asp and Glu.

Results: Here, we resolved the Cryo-EM structure of Compd 3e-bound SLC1A1. These studies showed that Compd 3e operates through a dual inhibitory mechanism. First, Compd 3e occupies a previously uncharacterized 'hidden' cholesterol-enriched pocket within the transporter, competitively disrupting Na^+ binding that is critical for SLC1A1's transport cycle. Second, Compd 3e wedges itself between the trimeric domains, thereby non-competitively arresting the 'elevator' movements necessary for substrate translocation across the membrane. Compd 3e makes several direct contacts with SLC1A1, including with the F99 residue that is not conserved in the other SLC1A family proteins. Mutating this residue into the SLC1A2/1A3-like (F99M) or the SLC1A5-like (F99L) conferred resistance to Compd 3e. These studies explain the mechanistic basis of Compd 3e's selectivity towards SLC1A1. Compd 3e has poor pharmacological properties (solubility, bioavailability, etc.). In silico molecular docking enabled a structure-activity relationship (SAR) campaign to optimize the Compd 3e scaffold. Our biochemical, structural, and functional studies have identified improved SLC1A1 inhibitors.

Conclusion: Our study provides an understanding of the dual mechanisms by which Compd 3e inhibits SLC1A1. These mechanistic discoveries uncover a novel therapeutic avenue for targeting ccRCC (and other SLC1A1-dependent cancers). More broadly, this work highlights the allosteric targeting of solute carriers outside their substrate-binding pocket, which can be leveraged for several other oncogenic SLCs.

#6912 Resolvin E1 sensitizes tumors to immune checkpoint inhibition by stimulating tumor antigen presentation.

Sui Huang[1], Katherine Quinlinvan[2], Eva Rothenberg[2], Michael Gillespie[2], Nandini Krishnamoorthy[3], Wayne D. Klohs[4], John Parkinson[4], Gary Mathias[4], Dipak Panigrahy[2]

[1]Institute of Systems Biology, Seattle, WA,[2]Beth Israel Deaconess Medical Center, Boston, MA,[3]Brigham and Women's Hospital, Boston, MA,[4]Thetis Pharmaceuticals LLC, Essex, CT

Background: Immune-checkpoint inhibitors (ICI) unleash tumor-specific cytotoxic T cells already primed to recognize tumor-associated antigens (TAA). One reason for poor response to ICI is insufficient priming of T cells, which requires cross-presentation of TAA to T cells by innate immune cells. Phagocytosis of apoptotic tumor cell by antigen-presenting innate immune cells is crucial for priming T cells to TAA, but without proper adjuvant signals, it can lead to immunosuppression. The endogenous lipid mediator Resolvin E1 (RvE1), a partial agonist of the BLT1 receptor, promotes phagocytosis of tumor cell debris and has been shown to control solid tumor growth in animal models. However, its effect on antigen presentation remains unknown.
Methods: We studied the efficacy of TP-317, a clinical stage RvE1 drug, in murine tumor models implanted subcutaneously (Lewis Lung carcinoma (LLC), CT26 colon carcinoma, B16F10 melanoma, KPC pancreas adenocarcinoma). After tumor volumes reached ≥ 100mm3, animals were treated with TP-317 (0.3 mg/kg, po, qd; or 300 ug/kg, s.c., q6d) alone or in combination with ICI or chemotherapy or both (n=8-9/group). Tumors were resected at days 9-12 and analyzed by bulk and/or single cell RNAseq.
Results: TP-317 monotherapy suppressed primary tumor growth on average by 31% in LLC, 66% in B16F10, 46% in CT26 and by 36% in KPC, rivaling the canonical ICI + chemo in these models. While TP-317 exhibited additivity when combined with chemotherapy (cisplatin, 5-FU and gemcitabine + paclitaxel in LLC, CT26 and KPC, respectively) or ICI (anti-PD1 +/- anti-CTLA4), TP317 synergistically enhanced tumor control in combination with ICI + chemo or with dual ICI, reducing tumor growth by an additional 60-80% compared to the various dual combinations alone. The effect of TP-317 monotherapy required the presence of CD8 T cells and was largely abrogated in animals co-treated with a BLT1 antagonist. Whole-tumor transcriptomes of resected tumors of TP-317-treated versus control animals displayed a broad upregulation of IFN-γ response genes, including antigen presentation machinery, regulators of MHC loci, immunoproteasome and MHC-I/II, as well as genes activating T-cell, NK cell and dendritic cell interaction. Single-cell RNAseq of B16F10 and LLC located the source of such transcriptional activation to innate immune cells. Intriguingly, antigen presentation genes (including MHC-II) were also markedly induced in tumor cells.
Conclusion: These data demonstrate the potent antitumor activity of TP-317, notably when combined with ICI and chemotherapy. This synergism may be explained by the dual effect of cross-presentation of TAA to prime T cells and presentation of TAA on tumor cells, sensitizing them to cytotoxic T cells. Thus, TP-317 holds promise as novel adjunct treatment for cancers that are currently treated with immunotherapy targeting T cell exhaustion but show limited response.

#6913 Discovery of GSC000580, a novel oral small molecule of MC4R antagonist for treatment of cancer-related body mass loss.

M. Liu, S. Zhao, **B. Lu**, Y. Xia, F. Yang;
Changchun GeneScience Pharmaceutical Co., Ltd., Shanghai, China

Melanocortin 4 Receptor (MC4R) is heavily involved in appetite regulation, body mass and energy homeostasis. MC4R is a GPCR class A receptor that belongs to the melanocortin receptor family. When leptin binds to the receptor, POMC neurons release POMC, which is cleaved to form α-MSH, followed by activating MC4R to result in loss of appetite and weight loss. AGRP released from AGRP neurons inhibits MC4R signaling, which promotes appetite and increases body weight. Weight gain in MC4R KO mice is gene dose dependent. MC4R GOF mutations in human2 are associated with low BMI and LOF mutations are associated with obesity. Agonists of MC4R for treating POMC, leptin receptor and other genetic defects resulting in hereditary obesity, have been approved by the FDA . Antagonists of MC4R are expected to be developed for weight-related disorders like cancer anorexia and other types of anorexia. 27%-58% of patients with advanced cancer have loss of appetite, 33%-75% have anorexia, which significantly increased the risk of postoperative complications and mortality. Current progestogen drugs couldn't offer adequate efficacy and favorable safety. MC4R antagonists with oral convenience may have the potential to recover or increase the appetite and body weight of patients with cancer-related anorexia, thereby improving their quality of life, further promoting the outcome of tumor treatment, and even improving OS. Currently, two MC4R antagonists have progressed to phase I clinical study. TCMCB07, an MC4R antagonist peptide, has shown efficacy in various cancer anorexia rat models but its use is limited due to the requirement for intravenous administration. PF-07258669 is an oral small molecule MC4R antagonist with efficacy in an aged rat model of anorexia.

Here we report the discovery of GSC000580, a novel, highly potent and selective MC4R antagonist that exhibits strong in-vitro activity and has been shown to induce increased food intake and body mass gain in two animal models of anorexia. In vitro, GSC000580 could bind to MC4R receptor with less than 1nM potency with high (500~1000 fold) selectivity across the melanocortin receptor family and potently blocking cAMP production with less than 1nM potency in cellular functional assay. In vivo, GSC000580 demonstrated dose-dependent significant increase of body weight gain and food intake in cisplatin-induced and aging-related anorexia rat models. GSC000580 has a favorable ADME and PK profile in rodents, dogs and NHPs, along with an acceptable safety profile. Additionally, GSC000580 exhibits the ability of brain penetration, with a brain-to-plasma ratio (b/p) of 0.17. GSC000580 demonstrates promise as a potential clinical treatment for cancer-related body mass loss.

#6914 p27 small molecule inhibitors for CDK4/6i-resistant metastatic breast cancer (mBC).

G. Chen, C. Guido, N. Zisman, K. Allamneni, **S. W. Blain**;
Concarlo Therapeutics, Brooklyn, NY

Cyclin-dependent kinases 4/6 (CDKs) are key cell cycle regulators that form trimeric complexes with cyclin D1 and the master regulator p27Kip1, acting downstream of proliferation-driving pathways. CDK4/6 inhibitors (CDK4/6i) like palbociclib have been pivotal for the treatment of ER+ mBC but resistance to these drugs is common. This resistance is due to CDK2 activation, which bypasses CDK4/6 inhibition, restoring cell proliferation and suggesting that effective targeting requires simultaneous inhibition of CDK4/6, and CDK2. CDK4/6i work by dissolving the trimeric CDK4/6-cyclin D1 complex, freeing p27 to inhibit CDK2, initially blocking the activity of all three G1 CDKs, but patients become resistant by reactivating CDK2 by a variety of mechanisms including overexpression of CDK6 and cyclin E or loss of RB and FAT1.

Concarlo's unique approach directly targets the master CDK regulator, p27, to simultaneously inhibit CDK2/4/6 in concert, leveraging p27's ability to turn G1 CDKs ON and OFF during proliferation and quiescence, respectively. Phosphorylation of p27 at Y88 causes p27's C-terminal tail to leave CDK active site, turning the kinases on; without phosphorylation, p27's C-terminal tail remains in the CDK active site, keeping the kinases off. When p27 is phosphorylated at Y88, it becomes a target for ubiquitin-mediated degradation, reducing p27 levels, and reinforcing the unconstrained activity of CDK2. Our studies demonstrated the feasibility and tolerability of targeting p27 in breast cancer in treatment naïve and palbociclib resistant models. Blocking p27 Y-phos with a therapeutic peptide significantly reduced tumor growth in xenograft models, with evidence of CDK2 inactivation, tumor necrosis and increased survival. Moreover, p27 inhibition was well-tolerated during repeat dosing in mice.

Concarlo has identified first-in-class small molecule p27 inhibitors (SMp27i) that act as molecular stabilizers or glues, binding and inactivating the trimeric CDK complex. They block p27 Y-phos and inhibit CDK2/4/6. SMp27i inhibit cell proliferation in treatment-naïve and palbociclib-resistant BC cells but have little effect on normal mammary cells. Target engagement in treated cells showed that Rb, CDK2, and Cdc6 phosphorylation were all inhibited. Total p27 levels increase demonstrating that when p27 Y88-phos is blocked, p27 degradation is reduced and more p27 is present to inhibit CDK2. When combined with approved CDK4/6i, SMp27i enhance their potency. SMp27i have been favorably profiled in in vitro ADME screens, off-target binding screens, and have encouraging in vivo mouse PK. Direct targeting of p27 presents a new treatment paradigm bypassing the challenges of ATP-competitive CDKis, with potential to expand into tumor types with aberrant p27, CDK2/4/6 or RAS/MAPK activity.

#6916 CK2 inhibitor shows anti-tumor activity in Ewing sarcoma preclinical models.

Muhammad Danial, Rajesh Rajaiah, Marudhu Pandiyan Shanmugam, Sholler Chloe, Jeremey Hengst, Abhinav Beeravally Nagulapally, Yasin Uzun, Giselle Saulnier Sholler, Chandrika Gowda behura

Penn State Hershey Cancer Institute, Hershey, PA

Introduction: Ewing sarcoma (EWS) is a highly aggressive bone cancer. EWS-FLI chromosomal fusion is detected in more than 85% of cases and is indispensable for EWS tumor survival and growth. Casein kinase II (CK2) is a serine/threonine kinase encoded by the CSNK2A1 gene that is essential for normal cellular processes and is overexpressed in EWS. Studies report that EWS protein is phosphorylated by CK2 at Ser325 and forced expression of CK2 in 293T cells increased EWS-FL1 protein. Here, we report that CK2 inhibition negatively affects EWS-FLI protein abundance, suppresses its downstream target GLI1, and shows anti-tumor activity in EWS xenografts. CX-4945 (silmitasertib) is a selective small molecule inhibitor of CK2 currently in clinical trial.

Methods: EWS cell lines (n=6) were treated with serial dilution of CX-4945 for up to 48 hours, and cell viability, apoptosis, migration, and invasion potential were analyzed. We measured the mRNA and protein levels of known CK2 and EWS-FLI1 targets. RNA sequencing and gene expression analysis were done to evaluate gene expression changes following treatment with CX-4945. A673 cells were transduced with a clone of GFP+ lentiviral particles expressing CK2a-specific shRNA, and control shRNA, and phenotype was assessed using the above methods. A673 cell line-derived xenograft and patient-derived xenograft of EWS were treated with CX-4945 at a dose of 75mg/kg oral gavage twice daily for 28 days or vehicle. Tumor size and survival was measured.

Results: CX-4945 at inhibitory concentrations of 2-8μM, increased apoptosis, decreased migration, invasion in all cell lines. We confirmed that CX-4945 treatment decreased EWS-FLI protein level, expression of EWS-FLI1 targets (GLI1, RNF2), known CK2 substrates (pAKT s129). Pharmacological and genetic inhibition of CK2 resulted in decreased cell viability, increased apoptosis, and decreased migration and invasion in vitro. Single-drug treatment with CX-4945 achieved 50-80% tumor inhibition and significantly prolonged survival in treated mice after 4 weeks of therapy. Following in vivo treatment with CX-4945, no significant organ toxicity was noted in tumor-bearing mice when compared to vehicle-treated mice. CX-4945 shows synergistic cytotoxic activity with Temozolomide and Irinotecan.

Conclusions: CSNK2A1 expression is high in EWS and correlates with poor overall survival. CK2 inhibition decreases EWS-FLI protein abundance and suppresses its downstream target GLI1. CX-4945 shows excellent anti-tumor activity and target inhibition in EWS xenografts. CX-4945 works synergistically with cytotoxic agents like Temozolomide and Irinotecan. These results support further pre-clinical characterization and clinical development of CX-4945 for treating EWS in combination with cytotoxic therapy. A Phase I clinical trial is evaluating the safety and tolerability of CX-4945 in pediatric solid tumors, including EWS (NCT06541262).

#6917 Developing a novel therapeutic approach to target EGR1 in aggressive breast cancer.

Suguru Ogata[1], Sophia McAneny-Droz[1], Manuel Van Gijsel-Bonnello[1], Verena Martinez-Rodriguez[1], Mark Miller[2], Bingbing Li[2], Naoto T. Ueno[1], Xiangshu Xiao[2], Xiaoping Wang[1]

[1]Cancer Biology Program, University of Hawai'i Cancer Center, Honolulu, HI,[2]Chemical Physiology and Biochemistry, Knight Cancer Institute, Oregon Health & Science University, Portland, OR

Introduction: Aggressive breast cancer, e.g., Triple-negative breast cancer (TNBC) and inflammatory breast cancer (IBC), needs novel targeted therapies. The epidermal growth factor receptor (EGFR) is a central signaling molecule that promotes tumor growth and metastasis and creates an immunosuppressive tumor microenvironment (TME) in aggressive breast cancers. Our research has demonstrated that early growth response 1 (EGR1), which EGFR signaling pathway regulates its expression, is a central mediator to an immunosuppressive TME by regulating the expression of immunosuppressive chemokines, indicating EGR1 is a potential therapeutic target for patients with aggressive breast cancer. However, no inhibitors or therapeutic antibodies currently target EGR1, emphasizing the critical need for novel strategies to inhibit its activity. Because EGR1 gene expression is regulated by the cAMP response element-binding protein (CREB), we hypothesize that suppressing EGR1 expression by inhibiting CREB can downregulate immunosuppressive chemokines and lead to reduced tumor growth in aggressive breast cancer. In this study, we evaluated the effect of the novel CREB inhibitor on IBC and TNBC cell growth and the expression of immunosuppressive chemokines by modulating EGR1 expression.

Methods: We evaluated the effects of the CREB inhibitor on EGR1 gene and protein expression in human IBC and TNBC cells (SUM149, HS578T, HCC1806, and HCC1937) and murine TNBC cells (E0771 and PyMT-N) *in vitro* using real-time PCR and Western blot analyses. Cell sensitivity to CREB inhibitor was assessed using the CellTiter-Blue assay. The expression of EGR1-regulated chemokines (*CCL2*, *CCL20*, *CXCL5*, and *CXCL8*) after CREB inhibitor treatment was examined using real-time PCR.

Results: CREB inhibitor suppressed *EGR1* gene expression in all tested cell lines and reduced EGR1 protein expression in SUM149, HS578T, E0771, and PyMT-N cells. The half-maximal inhibitory concentration (IC_{50}) of TNBC cells to CREB inhibitor was 1.067 μM (HCC1806), 2.342 μM (HCC1937), 3.291 μM (E0771), and 1.622 μM (PyMT-N). Compared to DMSO control, CREB inhibitor treatment reduced the gene expression of *CCL2*, *CCL20*, and *CXCL8* in human TNBC cells and that of *CCL2*, *CCL20*, and *CXCL5* in murine TNBC cells.

Conclusions: The CREB inhibitor suppressed the expression of the EGR1 gene and protein and decreased the expression of EGR1-regulated immunosuppressive chemokines, suggesting that a CREB inhibitor can effectively modulate EGR1 expression and function. We will further investigate the impact of targeting EGR1 with a CREB inhibitor on IBC and TNBC tumor growth and TME modulation *in vivo*.

#6918 TEAD inhibition as a therapeutic strategy for diffuse-type gastric cancer.

Lawrence W. Wu[1], Sung Joo Jang[1], Jimyung Park[1], Cameron Shapiro[1], Tracy T. Tang[2], Ryan H. Moy[1]

[1]Columbia University Irving Medical Center, New York, NY,[2]Vivace Therapeutics, San Mateo, CA

Introduction: Diffuse-type gastric cancer (DGC) accounts for one third of gastric cancers and is associated with poor differentiation, discohesive growth, chemoresistance, and worse survival compared to intestinal-type tumors. DGC is typically genomically stable, and there are no approved DGC-targeted therapies. Recent studies utilizing multi-omic profiling of patient samples and DGC mouse models have identified a tumor-promoting role for Yes-associated protein 1 and transcriptional coactivator with PDZ-binding motif (YAP/TAZ), which interact with TEAD transcription factors to regulate gene expression. Therefore, we evaluated the activity and mechanism of TEAD inhibitors in DGC preclinical models.

Methods: Gastric cancer patient-derived organoids (PDOs) and cell lines were treated with TEAD inhibitors (VT103, VT107) and/or 5-fluorouracil (5-FU) for *in vitro* assays evaluating cell proliferation or invasion. The *in vivo* activity of VT103 and/or 5-FU was tested with a subcutaneous xenograft model using a genetically engineered mouse model of gastric cancer driven by oncogenic $Kras^{G12D}$ and loss of *Trp53*/*Cdh1*.

Results: TEAD inhibition with VT103 and VT107 reduced proliferation, colony formation, and invasion in DGC cell lines and PDOs. We observed no effect of TEAD inhibitors on the viability of a normal gastric tissue cell line. Combining the TEAD1-selective inhibitor VT103 with 5-FU chemotherapy resulted in further reduction of DGC PDO viability relative to monotherapy. In flank tumors, inhibition of the YAP/TAZ-TEAD pathway using VT-103 in combination with 5-FU significantly reduced tumor growth. We hypothesized that TEAD inhibition blocks an epithelial-to-mesenchymal transition (EMT) program and promotes apoptosis. Consistent with this hypothesis, TEAD1 inhibition with VT-103 reduced expression of the EMT marker Slug and increased expression of the apoptosis marker cleaved caspase 3 in tumor xenografts.

Conclusions: The YAP/TAZ-TEAD pathway is an important driver of gastric cancer growth and metastasis, particularly in DGC where it may promote EMT. TEAD inhibition reduces gastric cancer growth and invasion *in vitro* and *in vivo* in preclinical models and demonstrates synergistic activity with 5-FU chemotherapy. These results suggest a potential role for further investigation of clinical efficacy of TEAD inhibitors in patients with DGC.

#6919 Targeting CLIC3 as a novel strategy to inhibit metastasis in breast cancer.

P. Mellor[1], S. Kendall[1], S. Smith[1], J. Olsen[2], H. Sahile[2], B. Page[2], A. Saxena[1], D. Anderson[3];
[1]University of Saskatchewan, Saskatoon, SK, Canada, [2]University of British Columbia, Vancouver, BC, Canada, [3]Saskatchewan Cancer Agency, Saskatoon, SK, Canada

Women with metastatic breast cancer have a 5-year survival rate of only 28%. This study aims to investigate the role of CLIC3 (chloride intracellular channel 3) in breast cancer metastasis and explore its potential as a therapeutic target. CLIC3, secreted by cancer cells and cancer-associated fibroblasts, promotes tumor invasion and angiogenesis through its oxidoreductase activity that inhibits TGM2 (transglutaminase-2). High CLIC3 levels correlate with poor prognosis across all breast cancer subtypes.
CLIC3 was knocked down in metastatic breast cancer cells, which were evaluated for cell migration and invasion using Boyden chambers, non-adherent growth in soft agar, and tumor formation and metastasis in mice. Small molecule inhibitors of CLIC3 activity were identified using an *in silico* screen of >10 million commercially available small molecules and 94 compounds were tested as possible CLIC3 inhibitors (CLIC3i) using recombinant CLIC3. We synthesized over 70 analogs of the top inhibitor for structure-activity relationship analysis, with the most promising inhibitors also assessed for CLIC3 binding using thermal shift assays and migration in scratch assays.
CLIC3 knockdown significantly reduced cell migration, invasion, and growth in soft agar, effects rescued by shRNA-insensitive CLIC3. In vivo, CLIC3 knockdown decreased tumor growth and completely blocked lung metastasis, underscoring its role in the colonization and growth of macroscopic metastases. Four promising compounds with mid-μM IC50 values for CLIC3 activity inhibition were identified (E5, D1, A5, D5). Among them, HA-22-31 emerged as a lead compound (IC50 = 1.7 μM), demonstrating direct binding to CLIC3 and significant inhibition of migration. In conclusion, CLIC3 enhances metastasis in breast cancer through its enzymatic activity. These findings suggest that further development of small molecule CLIC3 inhibitors may represent a promising new strategy for targeting metastatic breast cancer.

#6920 Targeting the AR co-activator CBP/p300 in metastatic castration-resistant prostate cancer (mCRPC).

Ananya Dutta[1], Beatrice C. Thomas[2], Pelumi Olawuni[1], Erik Soderblom[1], Eric Schadt[3], Bonnie Dougherty[3], Andrew J. Armstrong[1], Jason A. Jason A. Somarelli[1]

[1]Duke University, Durham, NC,[2]Nova Southeastern University, Fort Lauderdale, FL,[3]Pathos, New York, NY

Purpose: Standard treatment for metastatic castration-resistant prostate cancer (mCRPC) includes androgen receptor (AR) pathway inhibitors (ARPIs), such as androgen deprivation therapy or enzalutamide. Despite prolonged survival with ARPI therapies, resistance is nearly universal, highlighting the urgent need to identify new resistance mechanisms and therapeutic targets. Methods: We combined systems-level and experimental approaches to identify and test actionable network nodes linked to ARPI resistance as therapeutic vulnerabilities in prostate cancer. Systems-level analyses were performed using weighted gene co-expression network analysis and Bayesian network inference. Networks were overlaid with experimental data, including ENCODE ChIP-Seq data. The *in vitro* efficacy of pocebrodib was evaluated in a series of enzalutamide-sensitive and -resistant prostate cancer cell lines using CellTiter-Glo, colony forming assays and 3D spheroid assays. The *in vivo* activity of pocebrodib was evaluated in patient-derived xenografts. Proteomics and acetylomics was used to interrogate the potential mechanisms of action for pocenbrodib. Results were validated by RT-qPCR, western blotting and flow cytometry. Knockdown studies were performed using non-targeting and SCP2-targeting siRNAs. Results: Multi-layer network inference pinpointed the CREB binding protein (CBP)/p300 complex as a key regulatory node associated with patient outcomes and ARPI resistance. Consistent with this observation, preclinical testing of the CBP/p300 inhibitor, pocebrodib, demonstrated efficacy in both enzalutamide-sensitive and -resistant lines *in vitro*, with IC50s of ~1 μM. Cell growth inhibition was accompanied by downregulation of AR activity, as indicated by RNA-Seq and RT-qPCR. Pocenbrodib also demonstrated tumor growth inhibition of both enzalutamide-sensitive and -resistant patient-derived xenografts and a durable response in a patient with ARPI-resistant prostate cancer as part of the Phase I COURAGE study. Proteomics and acetylomics identified differential acetylation of both histone and non-histone proteins, including a subset of histone acetylation events altered in enzalutamide-resistant cells that are restored upon treatment with pocenbrodib. These analyses also identified enrichment of fatty acid metabolism proteins, including the sterol carrier protein 2 (SCP2) and fatty acid synthase (FASN), suggesting CBP inhibition may cause cells to sense loss of acetyltransferase activity as a reduction in free acetyl-coA, leading to alteration in fatty acid metabolism as a feedback response. Conclusion: Inhibition of the CBP/p300 axis by pocenbrodib is a promising new approach to treat ARPI-resistant prostate cancer. Future studies are aimed at clinical evaluation of pocenbrodib and exploration of downstream signaling axes to identify key biomarkers of response to pocenbrodib.

#6922 MDM2 inhibitor alrizomadlin induces ferroptosis through p53 dependent and independent pathways in TP53 wildtype NSCLC.

Shan Shi[1], Qiu Yun Luo[2], Wen Tao Pan[3], **Miaozhen Qiu**[4], Da Jun Yang[5]

[1]Department of Experimental Research, State Key Laboratory of Oncology in South China, Guangdong Provincial Clinical Research Center for Cancer, Sun Yat-sen University Cancer Center, Guangzhou, China,[2]Department of Clinical Research Center, The Third Affiliated Hospital, Sun Yat-Sen University, Guangzhou, China,[3]Suzhou Ascentage Pharma Inc., Suzhou, China,[4]Department of Medical Oncology, Sun Yat-Sen University Cancer Center; State Key Laboratory of Oncology in South China; Collaborative Innovation Center for Cancer Medicine, Guangzhou, China,[5]Department of Experimental Research, State Key Laboratory of Oncology in South China, Guangdong Provincial Clinical Research Center for Cancer, Sun Yat-sen University Cancer Center, Guangzhou, P. R. China./Suzhou Ascentage Pharma Inc., Suzhou, Jian, Guangzhou, China

Alrizomadlin (APG-115) is a second-generation MDM2 inhibitor that blocks the MDM2-p53 interaction, thereby stabilizing p53 protein and restoring its transcriptional regulatory functions in cell cycle control and apoptosis. Recent study indicated that APG-115 also exerted an anticancer effect in various cancers, but the specific function and underlying mechanism in TP53-wildtype NSCLC has not been elucidated. My project aims to determine whether APG-115 exerts anti-tumor effects by mediating ferroptosis through P53 dependent and non dependent pathways. We repeatedly validated the induction effect of APG-115 on ferroptosis using multiple recognized iron death detection methods, searched for ubiquitinases in public databases, and constructed a mouse tumor model to further verify the in vitro experimental results. In this study, we found that APG-115 exerted potent antitumor efficacy in TP53-wildtype NSCLC both in vitro and in vivo. Further experiments demonstrated that APG-115 induced ferroptosis, thereby facilitating the tumor cell death. Ferroptosis -related pathways genes, such as PTGS2, were significantly enriched after APG-115 treatment. APG-115 promotes PTGS2-mediated ferroptosis through a well-characterized mechanism. Specifically, APG-115 upregulates the expression of the deubiquitinase USP22, a key regulator in protein homeostasis. USP22, once upregulated, acts on PTGS2 by removing its ubiquitin tags through the ubiquitin-proteasome system. This deubiquitination prevents PTGS2 from being targeted for proteasomal degradation, thereby stabilizing the PTGS2 protein. The sustained expression of PTGS2 enhances ferroptosis, ultimately contributing to the cytotoxic effects of APG-115 in tumor cells. Collectively, our study reveals that APG-115 induces ferroptosis in TP53-wildtype NSCLC by stabilizing the expression of PTGS2 protein, providing a new direction for further clinical investigation of APG-115 as a monotherapy or combination therapy.Keywords:Alrizomadlin; Ferroptosis; PTGS2; USP22; Ubiquitin-proteasome system

#6923 The EHD2-Orai1 pro-oncogenic axis is required for calcium-dependent membrane repair in triple-negative breast cancer.

Haitao Luan[1], Azize Cerci[2], Bhopal Mohapatra[2], Timothy Bielecki[1], Matthew Storck[1], Jun-Yul Lim[3], Vimla Band[2], Subu Ramanathan[2], Hamid Band[1]

[1]Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, NE,[2]Department of Genetics, Cell Biology and Anatomy, University of Nebraska Medical Center, Omaha, NE,[3]Department of Mechanical & Materials Engineering, University of Nebraska- Lincoln, Lincoln, NE

Background: Triple-negative breast cancer (TNBC) is an aggressive and highly metastatic breast cancer subtype characterized by poor therapeutic outcomes. Increased invasive capabilities expose tumor cells to higher plasma membrane stress resulting in frequent breaches that are incompatible with cancer cell survival and invasiveness. Upregulation of membrane repair mechanisms has been shown to counteract this inherent tumor cell vulnerability and targeting such mechanisms represents a novel potential therapeutic strategy. Previous studies have shown that plasma membrane repair involves multiple calcium-dependent protein complexes. We have previously demonstrated that EHD2, a member of the EHD (Eps15 homology domain-containing) protein family, functions in this role by sustaining the plasma membrane expression of Orai1, which is required to sustain the pro-oncogenic Store-Operated Calcium Entry (SOCE) pathway. EHD2 has been found to accumulate at plasma membrane repair sites in skeletal muscle models, suggesting the potential role of EHD2-SOCE axis in regulating plasma membrane integrity in breast cancer cells.

Methods: We used CRISPR-Cas9 knockout of EHD2 or Orai1 as well as Orai1 inhibition in TNBC cell lines to examine the role of EHD2-Orai1 axis in the repair of mechanical or streptolysin O (SLO) toxin induced plasma membrane disruptions. The role of EHD2 as a mechano-transducer of external plasma membrane stress leading to Orai1 activation was investigated using micro-cantilevers to indent TNBC cells expressing genetic encoded calcium sensors. Functional linkage of EHD2 and Orai1 was assessed by measuring the impact of gene Knockout or complementation on SOCE and cell migration.

Results: EHD2 knockout impaired the ability of TNBC cells to efficiently repair the plasma membrane disruptions induced by mechanical injury or SLO. Orai1 knockout recapitulated the EHD2 knockout phenotype. A functional linkage of EHD2 and Orai1 was demonstrated by the ability of ectopic constitutively active Orai1, but not its dominant negative form, to rescue the cell migration defects of EHD2 knockout TNBC cells. Indenting the plasma membrane with micro-cantilevers induced rapid Ca^{2+} flux in TNBC cells and accumulation of EHD2 at the sites of deformation. Membrane deformation-induced Ca^{2+} entry was markedly reduced by EHD2 or Orai1 knockout. Orai1 inhibitors impaired the repair of mechanical or SLO induced plasma membrane disruption.

Conclusion: Our results reveal an EHD2-Orai1 axis that is a critical regulator of mechanosensitive Ca^{2+} entry in TNBC cells and required for their plasma membrane repair. Our findings support a role of EHD2-Orai1 axis in mechano-sensing and plasma membrane integrity in TNBC cells under stress. These findings suggest that targeting the EHD2-SOCE axis could present a novel therapeutic strategy in TNBC.

#6926 Targeting plasma membrane $K_{Ca}3.1$ channels reduces tumor size and mitigates gemcitabine-induced fibrosis in a PDAC mouse model.

Z. Petho[1], I. Neumann[1], W. Wilczak[1], M. Rugi[1], S. Sargin[1], S. Schimmelpfennig[1], E. Nass[1], G. Mesquita[1], D. Ciechanska[1], A. Oeckinghaus[1], S. Pillozzi[2], N. Prevarskaya[3], V. Lehen'kyi[3], A. Schwab[1], B. Soret[1];
[1]University of Munster, Munster, Germany, [2]University of Florence, Florence, Italy, [3]University of Lille, Lille, France

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is an aggressive cancer marked by extensive fibrosis and limited therapeutic options. The calcium-activated potassium channel $K_{Ca}3.1$ has been implicated in cancer progression. with Its elevated expression correlatesing with poor survival in PDAC patients. Here, we explored the *in vivo* therapeutic potential of targeting $K_{Ca}3.1$ channels using selective inhibitors in PDAC.

Experimental procedures: Tumor-bearing KPfC ($Kras^{wt/LSL\text{-}G12D}$ $Tp53^{fl/+}$ $PDX1^{Cre+}$) mice were treated with TRAM-34 or maurotoxin, inhibitors of $K_{Ca}3.1$, either alone or in combination with gemcitabine. $K_{Ca}3.1$ expression, and markers of epithelial-to-mesenchymal transition (EMT) were assessed via immunohistochemistry. Tumor progression was evaluated by histological analysis, measuring tumor node size and Sirius Red staining to quantify fibrosis. Distinct effects of plasma membrane and mitochondrial $K_{Ca}3.1$ inhibition were determined by comparing maurotoxin with the membrane-permeable TRAM-34.

Results: $K_{Ca}3.1$ channels were expressed in $CK18^+$ tumor cells and $\alpha\text{-}SMA^+$ cancer-associated fibroblasts. Maurotoxin reduced the tumor node size as compared to vehicle treatment, especially in combination with gemcitabine. In contrast, TRAM-34 and gemcitabine in monotherapy showed no significant impact on tumor node size. Gemcitabine alone induced a marked increase in fibrosis within tumor nodes, an effect not observed with other treatments. Notably, co-treatment with $K_{Ca}3.1$ inhibitors prevented gemcitabine-induced fibrosis and reversed EMT marker expression.

Conclusions: Selective targeting of plasma membrane $K_{Ca}3.1$ channels with maurotoxin reduces tumor node size and mitigates gemcitabine-induced fibrosis in PDAC. These findings highlight the therapeutic potential of $K_{Ca}3.1$ inhibition as a strategy to enhance the efficacy of gemcitabine and limit desmoplasia.

#6927 Novel ferrocene and naphthalene-based analogs block HRH1-signaling to inhibit colon cancer growth.

A. A. Ansari[1], D. Veeragoni[1], A. Azaz[1], S. Bhattacharya[1], H. Ghosh[1], B. Biersack[2], P. Dandawate[1];
[1]University of Kansas Medical Center, Kansas City, KS, [2]University of Bayreuth, Bayreuth, Germany

Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths worldwide, with over 1.8 million incidences and 900,000 deaths annually. CRC persists as a significant clinical challenge, detailing a 5-year survival rate of 14% for patients with distant metastases. CRC exhibits high genetic heterogeneity, which supplements a rich mutational landscape that gives rise to aggressive phenotypes. Therefore, there is a pressing need to identify novel and effective therapeutics to target CRC alongside identifying niche molecular pathways that contribute to pathogenesis. Here, we report the identification and formulation of novel histamine receptor 1 (HRH1) antagonists that inhibit CRC cell growth. Ferrocene (Fc) analogs are interesting due to their excellent lipophilicity, redox nature and anticancer activity. We synthesized a series of Fc and naphthalene-based compounds and tested them against colorectal cancer (CRC) cells HCT-116 and DLD-1. Compounds C10 and C12 inhibited the growth of CRC cells (IC50 = 0.5-1 mM), colony formation and induced apoptosis by downregulating bcl2 and mcl1 levels. We identified that both C10 and C12 bind to histamine receptor 1 (HRH1) using molecular docking studies. HRH1 is overexpressed in CRC cells and tissues compared to noncancerous colonic tissue. Further, histamine treatment activated ERK and GSK3β phosphorylation time-dependently in CRC cells, suggesting the receptor's functionality. Moreover, preincubation of C10 and C12 with CRC cells inhibited histamine-induced ERK and GSK3β phosphorylation, suggesting that C10 and C12 block HRH1 to inhibit histamine signaling. Further, a cellular thermal shift assay (CETSA) showed protection from thermal denaturation in C10 and C12 treated groups, suggesting a protein stabilization and potential binding of C10 and C12 with HRH1. Finally, C10 and C12 treatment (20 mg/kg, i.p., once daily, 21 days) reduced tumor volume and weight in HCT116-xenograft growth in NSG mice. In conclusion, we discovered novel HRH1 antagonists to inhibit colon cancer growth.

#6928 Combination treatment with cisplatin and PG3 for p53-mutated head & neck cancers.

Xiaobing Tian, Wafik S. El-Deiry

Brown University, Providence, RI

More than 90% of head & neck (H&N) cancers are squamous cell carcinoma (HNSCC) that occur from the mucosal epithelial tissue of the oral cavity, oropharynx, and larynx. About 30~40% of stage I or II HNC patients are curable and show improved survival rates after surgery or radiotherapy alone. However, over 60% of stage III or IV HNC patients require chemotherapy. Cisplatin is one of standard chemotherapy drugs for stage III and IV patients. In addition, the mutation frequency of the p53 gene in HNSCC is 65-85%.
DNA damage following cisplatin treatment can activate apoptosis via p53 in p53 wild-type cancer cells. In p53-mutated/deleted cancer cells, DNA-damage drugs can lead to cell death through other mechanisms, such as integrated stress response (ISR). There multiple cisplatin-resistance mechanisms were reported. Nucleotide excision repair (NER) is known as the primary strategy for repair of cisplatin-induced DNA damage. Elevated ERCC1 and ERCC5 expression enhance NER and are associated with cisplatin resistance in HNSCC patients. It was reported that ATF4 downregulation promoted cisplatin resistance in p53 mutated and deleted gastric cancers.
A small molecule PG3 treatment triggers ISR and leads to cell apoptosis through HRI/eIF2α/ATF4/PUMA pathway in p53-mutated and deleted colorectal cancer cell lines. We hypothesize that (1) combination treatment of PG3 with cisplatin can reduce side effects of cisplatin through reducing the dose of cisplatin, which can be achieved by enhanced induction of integrated stress response, (2) PG3 can sensitize cisplatin-resistant and p53-mutated H&N cancer cells to cisplatin through enhanced induction of integrated stress response. The combination treatment shows synergistic effects in p53-mutated FaDu and Cal27 H&N cancer cells. In combination therapy, use of PG3 allows use of a lower cisplatin dose to achieve a therapeutic benefit. The combined treatment enhances ISR induction, and the ISR contributes to cell apoptosis. We identified that cisplatin or the combination treatment activates the HRI/ATF4/NOXA pathway. NOXA mediates Mcl-1 degradation and is responsible for the combination treatment-induced apoptosis.

#6930 Structure activity relationship (SAR) studies identify novel non-pungent capsaicin analogs which display robust growth-inhibitory activity in human ovarian carcinoma cells.

K. J. Modi[1], R. S. Gadepalli[2], J. R. Rimoldi[2], T. E. Long[3], Y. Chen[4], K. C. Brown[1], S. D. Richbart[1], J. C. Meritt[1], S. L. Miles[1], P. Dasgupta[1];
[1]Marshall University School of Medicine, Huntington, WV, [2]University of Mississippi, University, MS, [3]Marshall University School of Pharmacy, Huntington, WV, [4]Bluefield State University, Bluefield, WV

Purpose of the study: The dietary compound Capsaicin is the hot and pungent ingredient of chili peppers. Preliminary data in our laboratory have found that capsaicin displays robust growth-inhibitory activity in a diverse array of human cancers. However, the clinical applications of capsaicin as a viable anti-cancer drug are hindered by its adverse side effects, such as gastric irritation, nausea, stomach cramps and a burning sensation in the gut. Clinical trials which explored the pain-relieving activity of capsaicin found that patients who orally ingested capsaicin discontinued taking the drug due to its unpleasant side effects. The primary aim of our studies was to rationally design non-pungent capsaicin analogs which would retain the anti-cancer activity of the parent molecule.
Experimental procedures. (SAR) studies have shown that the pharmacophore of capsaicin may be divided into three regions: REGION A, B, AND C. The addition of long-chain unsaturated fatty acyl groups in REGION C of capsaicin generated non-pungent compounds which displayed improved pain-relieving properties, relative to capsaicin. We synthesized a panel of compounds which contained a long chain unsaturated fatty acyl side chain in Region C of the capsaicin molecule. Subsequently, we determined the growth-inhibitory activity of these compounds in human ovarian cancer cells (in cell culture and chicken chorioallantoic membrane (CAM) models.
Results: The non-pungent Region C capsaicin analog DOHEVANIL displayed greater pro-apoptotic activity than capsaicin in human ovarian carcinoma cells. Most interestingly, DOHEVANIL did not impact the growth of normal human liver and kidney cells.
Conclusions: The non-pungent Region C capsaicin analog DOHEVANIL may be a promising non-pungent capsaicin analog which displays robust growth-suppressive activity in human ovarian cancer cells.
Support or Funding Information Funding for our study was supported by the NIH R15-AREA Grant (2R15CA161491-02 and 2R15CA161491-03), the Women's Health T3: 3P20GM103434-23W1 (PI: Dr. G Rankin) to PD and MAV and the NIAID-AI151970 grant to TEL. Furthermore, this study was supported in part by the West Virginia IDeA Network of Biomedical Research Excellence (WV-INBRE) grant (NIH grant P20GM103434; PI: Dr. G. Rankin), the National Institute of General Medical Sciences of the National Institutes of Health under the award number P30GM122733.

#6932 Phloroglucinol suppresses metastasis in CAV1 overexpressing cancer cells.

Layla Simon[1], **Keila Torres**[1], Andrew F. G. Quest[2]

[1]Universidad Finis Terrae, Santiago, Chile,[2]Universidad de Chile, Santiago, Chile

Metastasis is the process by which malignant cells dissociate from a primary tumor and disseminate to distant organs or tissues. This phenomenon presents one of the most significant challenges in oncology, as it is strongly associated with poor clinical outcomes and increased mortality rates. Caveolin-1 (CAV1) plays a crucial role in the metabolic reprogramming of cancer cells, particularly by promoting glycolysis, which enhances tumor cell migration and metastasis. This study aimed to evaluate the anti-metastatic potential of phloroglucinol, a monomer of phlorotannins from brown algae with anti-tumorigenic effects. The effects of phloroglucinol were assessed in metastatic breast (MDA-MB-231) and melanoma (B16-F10) cells expressing or not CAV1. Cell viability, migration, hexokinase activity and L-lactate levels were analyzed. C57BL/6 mice (N=5) were injected with B16-F10 cells pre-treated with phloroglucinol, and after 21 days, lung metastasis was assessed. *In vitro*, phloroglucinol significantly inhibited the migration of CAV1-expressing cancer cells and reduced lactate levels in the supernatants of the treated cells. Additionally, phloroglucinol suppressed CAV1-enhanced glycolysis by inhibiting hexokinase activity. *In vivo*, pre-treatment with phloroglucinol reduced the metastatic potential of CAV1-overexpressing cells. These findings suggest that phloroglucinol holds promise as a novel therapeutic agent for preventing cancer metastasis by targeting cancer cell metabolism.
Funding: ANID FONDECYT Iniciación [11230112] (L.S.), ANID-FONDECYT-Regular [1210644] (A.F.G.Q.), Fondo de Investigación Avanzada en Áreas Prioritarias (FONDAP) Grant 15130011 and 1523A0008 (A.F.G.Q.).

#6933 Ru(II)-thymine complex inhibits NF-κB activation, suppressing acute myeloid leukemia stem cells.

Maiara de S. Oliveira[1], Ingrid R. S. B. Dias[1], Rafaela G. A. Costa[1], Ana Carolina B. da C. Rodrigues[1], Suellen L. R. Silva[1], Milena B. P. Soares[1], Rosane B. Dias[2], Ludmila F. Valverde[3], Clarissa A. G. Rocha[1], Alzir A. Batista[4], Rodrigo S. Correa[5], Valdenizia R. Silva[1], Eugenia T. G. Pina[1], **Daniel P. Bezerra**[1]

[1]Oswaldo Cruz Foundation, Salvador, Brazil,[2]State University of Feira de Santana, Feira de Santana, Brazil,[3]Federal University of Sergipe, Lagarto, Brazil,[4]Federal University of Sao Carlos, Sao Carlos, Brazil,[5]Federal University of Ouro Preto, Ouro Preto, Brazil

Acute myeloid leukemia (AML) is a deadly blood cancer caused by leukemia blasts that fail to develop properly. AML has a significant relapse rate, which is attributed to a subset of cells known as leukemia stem cells (LSCs). Herein, we evaluated the ability of a Ru(II)-thymine complex (RTC) with the formula $[Ru(PPh_3)_2(Thy)(bipy)]PF_6$ (where PPh_3 = triphenylphosphine, Thy = thymine, and bipy = 2,2'-bipyridine) to suppress AML LSCs. RTC exhibited strong cytotoxicity against solid and hematologic malignancies and inhibited primary AML LSCs, as evidenced by a decrease in the CD34+CD38- cell population. RTC decreased the number of CD34+ and CD123+ cells in the AML cell line KG-1a, which has a similar population to LSC. RTC-treated NSG mice bearing KG-1a xenografts had a reduced number of leukemic blasts in the bone marrow. RTC-treated KG-1a cells showed increased DNA fragmentation, active caspase-3 and cleaved PARP (Asp 214) levels, and mitochondrial superoxide. The pancaspase inhibitor Z-VAD-(OMe)-FMK, but not the antioxidant N-acetylcysteine, partially reduced RTC-induced cell death in KG-1a cells, demonstrating that RTC causes caspase-mediated apoptosis via an oxidative stress-independent pathway. In molecular mechanism investigations, transcripts of the NF-κB inhibitor NFKBIA were increased, and the level of NF-κB p65 phosphorylated at Ser529 residue was reduced in RTC-treated KG-1a cells. This suggests that RTC may block NF-κB signaling. Altogether, these data suggest the anti-AML potential of RTC in AML LSCs via suppression of NF-κB signaling.

#6934 Acetate-induced transcriptomic alterations reveal potential therapeutic targets in triple negative breast cancer.

Gabriel Borges-Velez[1], Diego N. Bracero-Sanchez[2], Liah M. Roman-Calderon[1], Oscar A. Loperena-Gonzalez[1], Ariana S. Garcia-Lopez[1], Angel J. Amaral-Maisonet[1], Jeannette L. Salgado-Montilla[1], Esther A. Peterson-Peguero[3], **Josue Perez-Santiago**[1]

[1]University of Puerto Rico Comprehensive Cancer Center, San Juan, Puerto Rico,[2]University of Maryland, College Park, MD,[3]University of Puerto Rico - Rio Piedras Campus, San Juan, Puerto Rico

Background: Triple Negative Breast Cancer (TNBC) is the most aggressive subtype of breast cancer and lacks effective therapies. The MDA-MB-231 cell line, derived from TNBC, is used to evaluate new therapeutic strategies. Acetate, a microbial short-chain fatty acid (SCFA), is a key source of acetyl-CoA during metabolic stress and plays a critical role in regulating cancer cell epigenetics and transcriptomics. Acetate's signaling functions may be a significant factor in cancer growth and progression, however the role of acetate in TNBC remains unclear. Here, we evaluated the effects of acetate on the gene expression of TNBC cells.
Methods: We used MDA-MB-231 as our cell model for TNBC. Cells were then exposed with sodium acetate at a concentration of 5mM or growth media (negative control) for 24 hours in triplicates. After exposure, total RNA was extracted (Qiagen RNAeasy Mini kit) and sequenced using the Illumina NextSeq (2 X 75bp). The higher-quality reads were aligned, annotated, and quantified to the human genome (hg38) using STAR and RSEM softwares. Gene expression analysis was performed in R statistical software (packages tximport and DESeq2) and genes with an adjusted p-value < 0.05 were considered significant. Functional and enrichment analyses were performed using Metascape (FDR p<0.05).
Results: There were 1410 differentially expressed genes associated with acetate treatment. Adenylosuccinate lyase (A0A1W2PRX2), a novel described protein, was the highest upregulated gene (log2 fold change: 8.25), while dorsal inhibitory axon guidance protein (DRAXIN) was the most downregulated gene (log2 fold change: -2.70). In addition, there was a significant increase in the levels of kinesin family member 12 (KIF12) and protein phosphatase 1 regulatory inhibitor subunit 1B (PPP1R1B), which have been associated with chemoresistance. Significantly impacted biological processes and pathways include regulation of cell projection organization, response to hormone, chromatin remodeling, cytokine signaling in immune system, signaling by receptor tyrosine kinases, epithelial cell differentiation, response to oxidative stress, and DNA damage response.
Conclusion: Acetate can promote TNBC cell growth and disease progression via inflammatory mechanisms and lipid metabolism. The observed increase and decrease in A0A1W2PRX2 in DRAXIN expression, respectively, highlight these genes as potential novel therapeutic targets, given their association with breast cancer through oncogenic signaling cascades and apoptosis regulation. Furthermore, upregulation of KIF12 and PPP1R1B suggest that acetate may potentially mediating therapeutic resistance in TNBC. These findings provide insights into microbial metabolic byproducts can impact TNBC progression and suggest that targeting acetate-driven metabolic and signaling pathways may offer innovative strategies for treatment of TNBC.

#6935 Combinatorial treatment with small molecule inhibitors C26-A6 and MYCi975 in hepatocellular carcinoma (HCC).

Eva Davis, Ali G. Ermi, Devanand Sarkar

Virginia Commonwealth University, Richmond, VA

Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer. The median overall survival of advanced HCC patients is only 6.5-10.7 months. Identifying novel therapeutic targets and developing effective combinatorial treatment is highly relevant to HCC. Two oncogenes, Astrocyte elevated gene-1(AEG-1) and MYC (coding for c-Myc) are co-amplified in HCC patients and cooperate to promote aggressive, metastatic HCC. MYC is a driver oncogene for HCC and overexpression of MYC alone can initiate HCC. AEG-1 overexpression itself cannot induce HCC but it can significantly accelerate tumor progression once initiated. The objective is to evaluate the therapeutic efficacy of the combination of C26-A6 and MYCi975, small molecule inhibitors of AEG-1 and MYC, respectively, for HCC. Human HCC cell lines SNU-449, SNU-182, HuH-7, and Hep3B were treated with C26-A6 and MYCi975, alone or in combination. Optimum concentration of the inhibitors, to be used in combination, was determined by titration experiments. Cell viability was measured by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] and colony formation assays. Data was analyzed by a two-tailed T-test. Cell invasion was analyzed by Matrigel invasion assay. Differential gene expression was analyzed by RNA-sequencing (RNA-seq) and Ingenuity Pathway Analysis (IPA). In all four cell lines, MYCi975 was more potent than C26-A6 to inhibit cell viability. For MYCi975, MTT assay over 5 days identified the IC_{50} ranging between 2-4 μM, while for C26-A6 IC_{50} was more than 100 μM. A combination of C26-A6 and MYCi975 showed significant and synergistic inhibition in viability. Similarly, the combination treatment showed significant inhibition in cell invasion compared to single agent treatment. SNU-449 cells were treated with C26-A6, MYCi975, or C26-A6+MYCi975 combination for 24 h and RNA-seq was performed. This time point was chosen to identify differentially expressed genes (DEGs) by inhibitor treatment before cell death starts. IPA of DEGs revealed that MYCi975 inhibited pathways associated with cell proliferation, C26-A6 inhibited pathways regulating cell migration, invasion, and metastasis, and the combination inhibited both. Inhibition of several oncogenes, such as STAT3, E2F2, and FOXM1 which are associated with poor prognosis and aggressive HCC, was identified by the combination treatment. MYCi975 inhibited cell proliferation, while C26-A6 inhibited cell invasion indicating that in an *in vivo* setting C26-A6 and MYCi975 combination might inhibit both primary and metastatic tumors and the combination might exert a synergistic inhibitory effect compared to either agent alone. Studies are ongoing to evaluate *in vivo* therapeutic efficacy of the treatment and elucidate the underlying mechanism mediating the effects of the combination treatment.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Protein Kinases and Phosphatases as Targets for Therapy
Poster Session

#6939 MAPKAP kinase 2 regulates inflammatory responses in glioblastoma.

Eleni Panagioti[1], Yi Wen Kong[2], Xueyang Yu[2], Sean E. Lawler[3], Michael B. Yaffe[2], Charles H. Cook[1]

[1]Surgery, BIDMC/Harvard Medical School, Boston, MA,[2]David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA,[3]Department of Pathology and Laboratory Medicine, Legorreta Cancer Center, Brown University; The Warren Alpert Medical School, Brown University, Providence, RI

IDH-wildtype glioblastoma (GBM) is the most common and aggressive primary malignant brain tumor in adults, with 5-year survival rates of only 6% and a median survival of 14-22 months, depending on the MGMT promoter methylation status. Despite advances in treatment, GBM remains highly resistant due to its complex molecular landscape, characterized by rapid proliferation, angiogenesis, immune invasion, and metabolic dysregulation. In this study, we investigate the role of MAPK-activated protein kinase 2 (MK2), a critical kinase in the MAPK signaling pathway, as a potential therapeutic target in GBM. MK2 regulates key cellular processes including inflammation, stress response, DNA damage repair, and cell migration, all of which contribute to GBM progression. Notably, MK2 is highly enriched in microglia and peripherally derived macrophages. Our results demonstrate that MK2 is significantly upregulated in GBM tissues compared to normal brain tissue, and its expression correlates with poor survival outcomes. *In vitro,* MK2 inhibition significantly suppresses glioma cell proliferation, induces G1 cell cycle arrest, and promotes apoptosis, suggesting its potential to limit tumor growth and metastasis. To assess the *in vivo* antitumor effects of MK2 inhibition, we orthotopically implanted GL261 and CT-2A gliomas in MK2 knockout (KO) and wild-type C57BL/6 mice. MK2 genomic knockout is well tolerated in immunocompetent C57BL/6 mice. GL261 tumor cells with intact MK2 signaling effectively implant in hosts with global MK2 deficiency and shows more aggressive tumor growth than in hosts with wild-type MK2. In contrast, there was no effect on tumor growth in the CT-2A model, suggesting that tumor immunogenicity may influence the role of MK2 signaling in GBM. Mechanistically, host MK2 deficiency leads to increased circulating $Gr1^{+}CD11b^{+}$ myeloid-derived suppressor cells (MDSCs) and elevated PD-L1 expression on these cells, indicating a tumor-promoting inflammatory response and an altered immune profile within the myeloid compartment. These findings suggest an important role of MK2 in immune responses to GBM tumors that restricts growth. Ongoing studies are investigating the immunoregulatory function of MK2 using conditional knockout models targeting MK2 specifically in myeloid cells, as well as selective silencing of MK2 in tumor cells through CRISPR/Cas9 *in vivo.* Together, our results highlight MK2 as a possible therapeutic target in GBM, and underscores the importance of tumor-selective MK2 inhibitors and targeted therapies. These approaches could serve as adjunctive treatments to overcome therapy resistance and improve patient outcomes in this devastating malignancy.

#6941 Preclinical efficacy of the CK1 alpha/CDK7/CDK9 inhibitor BTX-A51 in *CIC*-rearranged sarcoma.

Q. Odom-Lewis[1], S. L. Davis[1], S. Pieper[1], C. Chu[2], E. Mazzola[2], R. Liu[2], N. Solimini[2], P. C. Gokhale[2], G. I. Shapiro[2], G. D. Demetri[2], **I.-M. Schaefer**[1];
[1]Brigham and Women's Hospital, Boston, MA, [2]Dana-Farber Cancer Institute, Boston, MA

CIC-rearranged sarcoma is an aggressive mesenchymal neoplasm characterized by high metastatic potential and poor clinical outcomes with median overall survival of only 12 months. The lack of effective systemic therapies highlights the urgent need to identify biologic vulnerabilities for therapeutic targeting. *CIC*-rearranged sarcoma shows molecular dependence on MYC, which can be targeted indirectly through cyclin-dependent kinase (CDK) 9 inhibition. In leukemia, casein kinase 1 alpha (CK1α) inhibition has been shown to have anti-tumor activity through activation of p53. We here investigated the anti-tumor effect of the CK1α/CDK7/CDK9 inhibitor BTX-A51 in preclinical models of *CIC*-rearranged sarcoma and determined the underlying mechanism of response. To evaluate the anti-tumor effect of BTX-A51 *in vitro*, we treated the CIC01, CIC02, NCC-CDS1-X1-C1 (CDS1), and NCC-CDS2-C1 (CDS2) cell lines with BTX-A51 (dose range 0.49-1000 nM) and performed BrdU cell proliferation, CellTiter-Glo cell viability, and Caspase-Glo 3/7 apoptosis assays. Daudi (MYC-driven Burkitt lymphoma) cells were used as control. IC_{50} values were calculated using an AAT Bioquest IC_{50} Calculator. We also assessed the effect of BTX-A51 on cell cycle progression using flow cytometry analysis and measured cell counts. The expression of key signaling proteins after 3, 6, 12, or 24 hours of treatment with BTX-A51 was determined through immunoblotting studies. *In vivo* studies assessing the anti-tumor effect of BTX-A51 in the CIC01 and CIC02 patient-derived xenografts are currently underway, and results will be reported. The CIC01, CIC02, CDS1, and CDS2 cell lines were highly sensitive to BTX-A51 with proliferation IC_{50} values ranging from 13-50 nM. In all 4 cell lines, BTX-A51 substantially inhibited cell viability and induced caspase 3/7-mediated apoptosis. At the same time, BTX-A51 treatment substantially inhibited expression of MYC, MCL-1, MDM2, phospho-RPB1 CTD Ser2 (a substrate of CDK9), phospho-RPB1 CTD Ser5 (a substrate of CDK7), phospho-RB1 Ser795, and RB1, and induced expression of phospho-histone H2A.X Ser13, p53, cleaved PARP (Asp214), and cleaved caspase 3. BTX-A51 treatment for 12 hours reduced the fraction of cells in S phase in cell cycle phase analysis consistent with G1 arrest and significantly reduced cell counts in all 4 cell lines ($P = 0.0381$ each, Wilcoxon rank-sum test). These findings demonstrate that BTX-A51 has anti-tumor activity in preclinical models of *CIC*-rearranged sarcoma. Treatment with BTX-A51 induces caspase 3-mediated apoptotic cell death through combined stabilization of p53 and inactivation of oncogenic drivers. Together with ongoing *in vivo* studies, our data will provide a compelling preclinical rationale for the evaluation of BTX-A51 in patients with *CIC*-rearranged sarcoma.

#6942 Targeting *CCNE1* amplification in gastric cancer.

Sung Joo Jang[1], Lawrence W. Wu[1], Sho Hangai[1], Cameron Shapiro[1], Jimyung Park[1], C. Gary Marshall[2], Ryan H. Moy[1]

[1]Columbia University Irving Medical Center, New York, NY,[2]Repare Therapeutics, Cambridge, MA

Background: Cyclin E (CCNE1) is amplified in multiple cancers including approximately 10% of esophagogastric cancers and 20% of ovarian cancers. *CCNE1* amplification leads to DNA replication stress and chromosomal instability. Prior studies have found that *CCNE1* amplification in gastric cancer is associated with increased liver metastasis, an “immune-cold” tumor microenvironment, and worsened clinical outcomes. At the same time, CCNE1-amplified tumors are vulnerable to loss of membrane-associated tyrosine- and threonine-specific cdc2-inhibitory kinase (PKMYT1). Lunresertib (RP-6306) is a selective, first-in-class, oral PKMYT1 inhibitor that leads to unscheduled mitotic entry and catastrophic DNA damage selectively in *CCNE1*-amplified cells. The purpose of this study is to evaluate the effect of PKMYT1 inhibition in *CCNE1*-amplified gastric cancer.

Methods: Human gastric cancer cell lines derived from patient-derived organoids (PDOs) or mouse CCNE1-overexpressing gastric cancer organoids were treated with lunresertib *in vitro*. Cell proliferation and viability were measured by WST-1, CellTiter-Glo, and colony formation assays. Quantitative reverse-transcription PCR (qRT-PCR) was used to measure RNA expression. Mice bearing syngeneic subcutaneous CCNE1-overexpressing gastric cancer xenografts were treated with lunresertib, anti-PD1, or lunresertib plus anti-PD1. Treatment efficacy was monitored by tumor volume, and tumor-infiltrating immune cells were assessed by flow cytometry.

Results: Lunresertib induced significant cytotoxicity in *CCNE1*-amplified gastric cancer cell lines and organoids measured through multiple cell viability and proliferation assays. Lunresertib induced DNA damage as monitored by increased γH2AX expression in *CCNE1*-amplified gastric cancer cells. Lunresertib also strongly induced *CXCL10* expression, a downstream cytokine of the cGAS-STING cytosolic DNA sensing pathway. While lunresertib or anti-PD1 monotherapy demonstrated moderate tumor growth inhibition *in vivo* in a syngeneic model of CCNE1-overexpressing gastric cancer, the combination of lunresertib and anti-PD1 resulted in the strongest tumor regressions, suggesting possible synergistic activity. Flow cytometry of tumor-infiltrating immune cells identified increased CD4+ T cells and decreased neutrophils with lunresertib plus anti-PD1.

Conclusion: Lunresertib demonstrated synthetic lethality in *CCNE1*-amplified gastric cancer across multiple *in vitro* and *in vivo* experiments. Lunresertib had synergistic effects with anti-PD1 *in vivo* against *CCNE1*-amplified gastric cancer, potentially due to induced DNA damage and inflammatory cytokine induction.

#6943 RV-1521: A broad-spectrum mRNA vaccine targeting KRAS mutations in cancer.

Renxiang Chen[1], Yongsik Bong[1], Wei Liu[2], Ezra Chung[1], David Brown[1], Evan Lewoczko[1], Zachary Dorsey[1], Steven Long[1], Cun Yu Wang[2], **Dong Shen**[1]

[1]RNAimmune, Inc., Baltimore, MD,[2]School of Dentistry, UCLA, Los Angeles, CA

KRAS mutations, particularly G12 and G13 variants, are common in cancers but remain difficult to target therapeutically. While KRAS G12C inhibitors, such as sotorasib and adagrasib, have made significant progress, their mutation-specificity excludes up to 85% of KRAS-mutant cancers, including those with G12D, G12V, and G13D mutations. To address this gap, we developed RV-1521, a broad-spectrum mRNA vaccine targeting multiple KRAS mutations, including G12D and G13D, and evaluated its efficacy in a CT26 colon cancer model in Balb/c mice.

RV-1521 induced robust immune responses, with serum IgG titers increasing 3.5-fold ($p < 0.01$). B cell epitope mapping identified four immunogenic KRAS regions. T cell profiling revealed activation hotspots in peptides P1, P35, and P36, with significant IFN-γ secretion in response to both wild-type and G12D-mutant KRAS epitopes. Flow cytometry analysis showed a 2.7-fold increase in IFN-γ$^+$ CD4$^+$ T cells in splenocytes from vaccinated mice. These results highlight RV-1521's capacity to activate diverse immune mechanisms targeting KRAS-mutant tumor elimination, particularly through CD4$^+$ T cell-mediated responses.

As a monotherapy, RV-1521 demonstrated dose-dependent antitumor efficacy, reducing tumor volume by 50% ($p < 0.001$) and tumor mass by 45%. This was accompanied by a 3-fold increase in CD8$^+$ T cell infiltration and a 2.8-fold increase in granzyme B expression. These findings were consistent with reduced tumor proliferation and enhanced T cell-mediated cytotoxicity. When combined with anti-PD-1 therapy, RV-1521 further amplified its efficacy, achieving a 70% reduction in tumor volume ($p < 0.001$) and a 2.5-fold increase in tumor IFN-γ levels, while preserving cytotoxic T cell activity and reversing T cell exhaustion. Transcriptomic analysis revealed upregulation of immune pathways, including T cell receptor and Th1/Th2 signaling, alongside downregulation of key oncogenic drivers such as EGR1, FOS, and JUN.

Strain-specific analysis revealed stronger immune responses in Balb/c mice compared to C57BL/6 mice, suggesting that genetic background may influence immune activation. Notably, RV-1521 elicited immune responses against both wild-type and mutant KRAS, addressing tumor heterogeneity and minimizing immune escape. The unexpected cytotoxic role of CD4$^+$ T cells further underscores the therapeutic potential of this vaccine.

By targeting multiple KRAS mutations, RV-1521 overcomes the limitations of mutation-specific therapies. Its ability to synergize with anti-PD-1 therapy and elicit a robust, multi-faceted antitumor immune response positions it as a promising strategy for treating KRAS-driven cancers. Ongoing studies will evaluate its safety and adaptability across diverse microenvironments and patient populations.

#6944 Transient pan-RAF inhibitor treatment for melanoma prevention.

Rachel E. Lew[1], Harsha S. Sanaka[2], Venkat R. Chirasani[3], Sharon L. Campbell[3], Christin E. Burd[1]

[1]Cancer Biology and Genetics, The Ohio State University Wexner Medical Center, Columbus, OH,[2]Molecular Genetics, The Ohio State University, Columbus, OH,[3]Biochemistry and Biophysics, University of North Carolina, Chapel Hill, NC

Therapeutic options are currently limited for the 15-25% of cutaneous melanomas driven by an oncogenic *NRAS* mutation. Our previous findings showed that melanoma-associated NRAS substitutions stabilize conformations optimal for BRAF binding, thereby promoting RAF dimerization, proliferative signaling, and ultimately melanomagenesis. Building on these discoveries, we hypothesized that NRAS-mutant melanoma precursors would exhibit substitution-specific sensitivity to pan-RAF inhibition, and that a temporary blockade of RAF signaling would eliminate NRAS mutant-melanocytes in the skin. BRET and NanoBiT complementation assays revealed that pan-RAF inhibitors promote RAS-RAF and RAF-RAF interactions at lower doses in human melanoma cell lines with a weakly melanomagenic NRAS substitution (Q61H) compared to those with a stronger melanomagenic NRAS substitution (Q61R). Surprisingly, these drug-induced RAF complexes were associated with decreased MAPK>ERK signaling and higher drug sensitivity, suggesting that fully inhibited, drug-bound protomers drive increases in NRAS-RAF interactions. To test the mutation-specific sensitivity of NRAS-mutant melanoma precursors in vivo, mouse models of spontaneous NRAS Q61R- or Q61H-driven melanoma were prophylactically treated with a pan-RAF inhibitor for three days and then followed for tumor formation. Consistent with our in vitro data, short-term pan-RAF inhibitor treatment delayed melanoma onset in NRAS Q61H, but not Q61R mice. These findings highlight the critical role of RAF signaling in NRAS-mutant melanoma formation, suggesting that short-term use of effective, targeted therapies could prevent melanoma formation.

#6945 Targeting Chek2-YBX1&3 axis to enhance response to immune checkpoint blockade therapy in gliomas.

Heba Ali[1], Ningjia Zhou[1], Li Chen[1], Vivekanudeep Karri[1], Mahalakshmi Harish[2], Yalu Zhou[3], Vasilios Kalas[4], Karl Habashy[1], Victor A. Arrieta[1], Kwang-Soo Kim[1], Si Wang[1], Joseph Duffy[1], Ragini Yeeravalli[1], Deanna M. Tiek[5], Xiao Song[5], Snehasis Mishra[1], Catalina Leechang[1], Atique Ahmed[1], Prasanna Venkatraman[2], Gabriel J. Rocklin[4], Adam M. Sonabend[1], Crismita Dmello[1]

[1]Department of Neurological Surgery, Feinberg School of Medicine and Northwestern Medicine Malnati Brain Tumor Institute of the Lurie Comprehensive Cancer Center, Northwestern University, Chicago, IL,[2]Protein Interactome Lab for Structural and Functional Biology, Advanced Centre for Treatment Research and Education in Cancer, Tata Memorial Centre, Navi Mumbai, India,[3]Division of Nephrology and Hypertension, Northwestern University Feinberg School of Medicine, The Feinberg Cardiovascular and Renal Research Institute, Chicago, IL,[4]Department of Pharmacology, Northwestern University Feinberg School of Medicine, Center for Synthetic Biology, Northwestern University, Chicago, IL,[5]The Ken and Ruth Davee Department of Neurology, Lou and Jean Malnati Brain Tumor Institute, and Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine, Chicago, IL

Although the immunosuppressive microenvironment of glioblastoma (GBM) is well-established, the tumor-intrinsic mechanisms underlying resistance to CD8+ T cell-mediated killing remain incompletely understood. Our previous study identified Checkpoint Kinase 2 (Chek2) as the top contributor to CD8+ T cell resistance in GBM through an in vivo CRISPR screen. Therapeutic evaluation demonstrated that Chek2 inhibition, combined with PD-1/PD-L1 immune checkpoint blockade, significantly enhanced CD8+ T cell-mediated tumor killing and improved survival in preclinical glioma models. Here, we aimed to elucidate the immunosuppressive function of Chek2. Immunoprecipitation-mass spectrometry (IP-MS) and phosphoproteomics identified the transcriptional repressors and RNA-stabilizing proteins YBX1 and YBX3 as critical interactors of Chek2. Single-gene knockout and overexpression studies of Chek2, YBX1, and YBX3 in multiple glioma cell lines revealed that these proteins positively regulate each other's expression at the protein level. RNA sequencing coupled with ChIP-seq analysis demonstrated that the Chek2-YBX1-YBX3 interaction represses the expression of inflammatory cytokines. Targeting Chek2, YBX1, and YBX3 with the small-molecule inhibitor SU056 enhanced antigen presentation and promoted CD8+ T cell proliferation. Moreover, combining SU056 with PD-1 blockade significantly improved survival in multiple glioma models. Collectively, these findings reveal an immunosuppressive mechanism mediated by the Chek2-YBX1-YBX3 axis and propose its inhibition as a promising therapeutic strategy to enhance responses to immune checkpoint blockade therapies in gliomas.

#6946 Targeting PDGFRa-SHP2 signaling enhances radiotherapy in IDH1 mutant glioma.

Xiaozhou Yu, Xiao Song, Deanna Tiek, Runxin Wu, Maya Walker, Craig Horbinski, Bo Hu, Shi-Yuan Cheng

Northwestern University, Chicago, IL

Background: Isocitrate dehydrogenase mutant (IDH-mut) gliomas represent a distinct subtype of glioma with a somewhat better prognosis. However, despite this, they remain incurable due to the limited availability of effective treatments. Targeting SHP2, a non-receptor protein tyrosine phosphatase, shows promise as a therapeutic strategy for cancers, including IDH wildtype (WT) glioblastoma (GBM). However, it is unknown whether IDH-mutant gliomas respond to SHP2 inhibition.
Methods: Bioinformatic and biological analyses revealed increased expression and activation of the PDGFRα-SHP2-ERK pathway in IDH-mutant gliomas and patient-derived IDH-mut glioma stem-like cells (GSCs). The effects of SHP2 inhibition, alone or with radiation therapy (RT), were assessed through assays including cell growth, sphere formation, cell differentiation, flow cytometry, immunoblotting, orthotopic brain tumor xenografts, and immunohistochemistry.
Results: PDGFRα expression was elevated in IDH-mut gliomas and GSCs, activating the SHP2-ERK pathway. SHP099, a SHP2 inhibitor, reduced GSC tumorigenicity in vitro and in vivo by disrupting SHP2-ERK signaling and promoting differentiation. SHP099 also enhanced cytotoxicity of RT, the standard treatment for IDH-mut glioma, on IDH-mut GSCs and orthotopic glioma models. Mechanistically, the PDGFRα-SHP2-ERK axis is activated in IDH-mut gliomas and RT further activates this pathway. Targeting SHP2 suppressed ERK signaling thereby enhancing the therapeutic effect of RT.
Conclusion: Combining SHP2 inhibition with RT shows promising therapeutic efficacy for IDH-mut glioma by suppressing the activated SHP2-ERK axis.

#6947 CRISPR/Cas9 kinome screen identifies PNKP as a novel fitness gene in HPV-negative head and neck squamous cell carcinoma.

Gurleen Kaur Tung[1], Kate Chatfield-Reed[2], Masaru Miyagi[3], Wendi Quinn O'Neill[4], Quintin Pan[4]

[1]Otolaryngology, Case Western Reserve University School of Medicine, Cleveland, OH,[2]Biology, Yukon University, Whitehorse, YT, Canada,[3]Pharmacology, Case Western Reserve University School of Medicine, Cleveland, OH,[4]Otolaryngology, University Hospitals Cleveland Medical Center, Cleveland, OH

Background: Head and neck squamous cell carcinoma (HNSCC) is the seventh most common malignancy worldwide. Most cases are linked to alcohol, tobacco, and areca nut, while a smaller proportion is driven by high-risk human papillomavirus (HPV). HPV-negative HNSCC is associated with poor prognosis with a 5-year survival rate of 40-50%. This dismal outcome has remained relatively static over the past several decades, highlighting the critical need for the discovery of novel targets and therapeutics to better manage HPV-negative HNSCC patients.**Methodology:** We performed parallel *in vitro* and *in vivo* CRISPR-Cas9 knockout kinome screens to identify kinases essential for the fitness of HPV-negative HNSCC cells. CAL27 Cas9 EGFP cells were transduced with a pooled sgRNA kinome library containing 6,104 sgRNAs at a MOI of ~0.4. After 7 days of puromycin selection, these transduced cells were cultured *in vitro* for either 7 or 14 days or implanted into the flanks of athymic nude mice, with tumor harvesting at 6 weeks. DNA was isolated from both cells and tumors, followed by next-generation sequencing to identify and quantify individual sgRNAs. Fitness genes identified in both the *in vitro* and *in vivo* screens were further validated using shRNA technology in a panel of HPV-negative HNSCC cell lines. **Results:** We identified 24 fitness genes that were common to both our *in vitro* and *in vivo* screening approaches. These kinases are primarily involved in pathways critical for DNA damage and repair, metabolism, and cellular proliferation. Among them, we prioritize the validation of polynucleotide kinase 3'phosphatase (PNKP) as an essential gene in a panel of HPV-negative HNSCC cell lines. shRNA-mediated knockdown of PNKP significantly reduced the cellular proliferation and clonogenic survival of CAL27, Detroit-562 and FaDu cells. Furthermore, PNKP knockdown severely impaired tumorigenicity *in vivo*, with CAL27 and FaDu xenografts showing markedly reduced tumor growth compared to controls. Phosphoproteomic analysis revealed that PNKP knockdown resulted in dephosphorylation of 762 unique sites (log2FC>1.5; FDR p<0.05), with a particular impact on proteins involved in nuclear and mitochondrial DNA repair pathways.**Conclusion:** Our study identified and confirmed PNKP as an essential gene for the survival of HPV-negative HNSCC cells. This discovery highlights the potential of PNKP as a novel therapeutic target, which may lead to the development of innovative therapeutics that could improve the clinical outcomes of HPV-negative HNSCC patients.

#6949 Downregulating the mitotic kinases TTK and NEK2 as a promising approach for the reduction of metastasis in triple-negative breast cancer cell lines.

Alexandra N. Aquino-Acevedo[1], Angel D. Colon-Burgos[1], Marileana Rodriguez-Ruiz[2], Gabriel Rosario-Ortiz[1], Elliott R. Rodriguez-Lopez[1], Esther M. Irizarry-Quintana[3], Gretchen M. Albarran-Acosta[1], Joel A. Orengo-Orengo[1], Melanie E. Cruz-Robles[1], Harold I. Saavedra[1]

[1]Ponce Health Sciences University, Ponce, Puerto Rico,[2]Moffitt U54 Summer Research Program, Ponce Health Sciences University, Ponce, Puerto Rico,[3]U54 REC InterAmerican Undergraduate Program, Ponce Health Sciences University, Ponce, Puerto Rico

Triple-negative breast cancer (TNBC) lacks estrogen, progesterone, and HER2 receptors, making it the most aggressive form of breast cancer. TNBC with distant metastases is nearly untreatable with anti-hormonal or anti-HER2 therapies, leaving chemotherapy as the primary option. TNBC is characterized by uncontrolled cell growth, increased centrosome amplification, genetic instability, and high invasive potential. Non-Hispanic Black (NHB) and Hispanic/Latino (H/L) women have lower 5-year survival rates compared to non-Hispanic White (NHW) women, partly due to later-stage tumor detection and higher TNBC prevalence. Women with a greater proportion of African ancestry exhibit elevated expression of several mitotic kinases, such as TTK and NEK2. TTK helps maintain genomic stability by regulating the spindle assembly checkpoint, while NEK2 regulates centrosome separation and microtubule organization. However, overexpression of these kinases in breast cancer is linked to increased cell growth, chromosomal instability, aneuploidy, and epithelial-to-mesenchymal transition (EMT). Previous data from a tissue microarray confirmed the link between TTK overexpression and markers of proliferation, EMT, and the TNBC subtype. Thus, we aim to measure expression levels of EMT markers and transcription factors, as well as changes in the invasive capacity of metastatic TNBC cell lines, upon downregulation of TTK and/or NEK2. To address this, TNBC cell lines, MDA-MB-231 (NHW) or MDA-MB-157 (NHB), were cultured, followed by TTK and/or NEK2 siRNA-mediated knockdown. Western blot and qPCR analyses measured protein and mRNA expression levels of EMT markers and transcription factors. Invasion assays were performed using Matrigel matrix invasion chambers. Preliminary results indicate that silencing of TTK, NEK2, or both resulted in a reduction of the mRNA expression levels of EMT markers, Vimentin, N-cadherin, or β-Catenin in MDA-MB-231 and MDA-MB-157 cell lines. Western blot results showed a significant decrease in Vimentin protein levels in all treatment groups of MDA-MB-231 cell lines, while TTK/NEK2 knockdown also reduced N-cadherin, β-Catenin, and SNAIL transcription factor. Comparably, results from MDA-MB-157 cell lines showed a significant reduction of β-Catenin protein levels upon NEK2 knockdown. Furthermore, TTK/NEK2 knockdown leads to a decrease in the invasive capacity of both TNBC cell lines. These preliminary data suggest that dual silencing of mitotic kinases TTK and NEK2 may be a promising approach for reducing key proteins involved in EMT processes in TNBC cell lines. Due to their increased expression in women of African descent (e.g. African American and Caribbean Hispanics), targeting mitotic kinases could represent a promising therapeutic strategy, particularly in Afro-descendants with TNBC by limiting tumor growth and metastasis.

#6950 VRK1 as a novel therapeutic target in small cell neuroendocrine carcinoma of the uterine cervix via mitochondrial dysfunction.

Mariya Kobayashi[1], Satoshi Nakagawa[1], Yusuke Ishii[1], Yuji Kamei[2], Mizuki Kanda[3], Tatsuo Masuda[1], Mamoru Kakuda[1], Kosuke Hiramatsu[1], Tadashi Iwamiya[1], Shinya Matsuzaki[1], Masahiro Inoue[4], Yutaka Ueda[1]

[1]Department of Obstetrics and Gynecology, The University of Osaka, Suita, Japan,[2]Department of Obstetrics and Gynecology, Higashiosaka City Medical Center, Higashiosaka, Japan,[3]Department of Obstetrics and Gynecology, Sakai City Medical Center, Sakai, Japan,[4]Clinical Bio-Resource Research and Development, Kyoto University, Kyoto, Japan

[Objective] Small cell neuroendocrine carcinoma of the uterine cervix (SCNEC) is a rare cancer with a poor prognosis, highlighting the pressing need for effective pharmacotherapies. Our previous proteomic analysis identified vaccinia-related kinase 1 (VRK1) as one of the proteins highly expressed in SCNEC compared to squamous cell carcinoma (SCC) and adenocarcinoma (AC). VRK1 is a member of the VRK family of Ser-Thr kinases, and its overexpression is associated with poor prognosis in various cancers. It is also expected to be a potential synthetic-lethal drug target in VRK2-low/null cancers. This study aims to investigate the potential of VRK1 as a therapeutic target for SCNEC.
[Methods] To examine VRK1 and VRK2 expression levels across different histological types of cervical cancer, immunohistochemistry was performed on clinical samples, including five SCNEC cases, 34 SCC cases, and seven AC cases. The effect of VRK1 on tumor growth was assessed in vitro (2D and 3D cultures) and in vivo (xenograft model) by shRNA-mediated knockdown of VRK1 (shVRK1) and its control (shCont). Gene Set Enrichment Analysis (GSEA) was conducted on RNA-seq data extracted from mouse xenograft tumors to compare shCont and shVRK1. Conditions with exposure to reactive oxygen species (ROS) were generated using hydrogen peroxide (H_2O_2). Mitochondrial function was evaluated through changes in mitochondrial membrane potential and mitochondrial protein expression.
[Results] VRK1 expression exhibited variability in SCC and AC but was consistently high across all SCNEC samples. In 2D cultures, VRK1 knockdown demonstrated limited effects on cell proliferation; however, it significantly inhibited tumor growth in 3D cultures and in vivo xenograft models. GSEA revealed that mitochondrial translation and gene expression pathways were the most suppressed in shVRK1 tumors compared to shCont. Subsequent in vitro and in vivo experiments revealed that VRK1 knockdown had a stronger tumor growth inhibitory effect under conditions with exposure to ROS, driven by the combined influence of the tumor microenvironment and the tumor's high energy metabolism, and this was accompanied by mitochondrial dysfunction. Furthermore, VRK2 expression, a potential biomarker of VRK1's effects, was confirmed to be characteristically low in SCNEC.
[Conclusion] SCNEC is characterized by high VRK1 expression and low VRK2 expression. VRK1 knockdown suppresses tumor growth via mitochondrial dysfunction, particularly in vivo. These findings suggest that VRK1 is a promising therapeutic target for the treatment of SCNEC.

#6952 PIM inhibition increases membrane expression of TNFRSF10B(DR5) and combination with recombinant human TRAIL synergizes to reduce renal cell carcinoma cell viability.

Kimberly S. Meza, Wafik S. El-Deiry, Sheldon L. Holder

Brown University, Providence, RI

Renal cell carcinoma (RCC) cases are on the rise globally. RCC tumors' acquired resistance to current RCC therapies necessitates the identification of novel biomarkers and therapeutic strategies. Oncogenic PIM1 kinase is overexpressed in human RCC tissue and cell lines and is associated with worse clinical outcomes in RCC. PIM1 is an emerging clinically relevant biomarker and target in the treatment of RCC. The overexpression of PIM kinases has been previously reported in a glioblastoma model and knockdown or pharmacological inhibition of PIM kinases promoted the increased membrane expression of TNFRSF10B/DR5. Death Receptor 5 (DR5) is the specific receptor to TRAIL, a cancer-selective apoptosis-inducing ligand. A relationship between PIM kinases and regulation of DR5 expression for TRAIL-induced apoptosis is not yet described for RCC. Here we identify a potential link between PIM kinases' involvement in DR5 regulation and TRAIL apoptosis in RCC. RCC and nonmalignant renal epithelial cells were treated with PIM inhibitors (SGI-1776, TP-3654) or vehicle control for 24 hours and then evaluated for membrane DR5 levels via flow cytometry. Low doses (5μM) of PIM inhibitors were sufficient to induce the increased mean membrane expression of DR5 in 786-O cells by more than eleven fold (3.03% control vs 36.07% treated). To investigate synergism between PIM inhibitors and recombinant human TRAIL (rhTRAIL), RCC cell lines were treated with increasing concentrations of SGI-1776 or TP-3654 and rhTRAIL (TLY012) for 24 hours and assessed for viability via Cell Titer Glo assay. Combinations of PIM inhibitors and rhTRAIL reveal that PIM inhibition sensitized RCC cells to TRAIL induced apoptosis. These results reveal novel therapeutic strategies for the treatment of RCC, highlighting PIM kinases as targets to potentiate TRAIL induced apoptosis. Current studies are ongoing to understand the mechanism of PIM kinase involvement in DR5 regulation and sensitivity to TRAIL in RCC.

#6953 Inhibition of Eukaryotic elongation factor 2 kinase (eEF2K) disrupts key processes in colorectal cancer progression and tumor growth.

Nermin Kahraman[1], Ezgi Biltekin[1], Ogun Ali Gul[1], Goknur Kara[2], Izabela Fokt[3], Yasemin M.Akay[4], Waldemar Priebe[3], Metin Akay[4], Bulent Ozpolat[1]

[1]Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX,[2]Department of Neurosurgery, Houston Methodist Research Institute, Houston, TX,[3]Department of Experimental Therapeutics, University of Texas MD Anderson Cancer Center, Houston, TX,[4]Department of Biomedical Engineering, University of Houston, Houston, TX

Colorectal cancer (CRC) is the third most commonly diagnosed cancer with 1.9 million new cases and the second leading cause of cancer-related deaths, with 935,000 deaths reported in 2023. Despite advances in therapy, the 5-year survival rate for metastatic CRC remains as low as 14%, emphasizing the need for novel treatment strategies. Previously, we have validated that Eukaryotic elongation factor 2 kinase (eEF2K) is an atypical alfa kinase as a potential molecular target that is commonly expressed in highly aggressive solid cancers such as triple-negative breast cancer, pancreatic, ovarian, and lung cancers and demonstrated that it acts as a key player in the survival, proliferation, invasion, and tumor growth of cancer cells and promoting cell. In this study, we investigated the role of eEF2K in colon cancer tumorigenesis and progression and its potential as a therapeutic target using a novel eEF2K small molecule inhibitor and siRNA-based knockdown studies. We found that eEF2K expression is highly expressed in CRC cell lines and eEF2K is associated with shorter patient survival and poor prognostic marker in CRC patients (NCI-TCGA patient database), inhibition of eEF2K in various CRC cell lines (HCT116, RKO, COLO320, and with different genetic alterations leads to suppression on cell proliferation, migration, and invasion. Western blot analysis confirmed that treatment with the siRNA and the eEF2K inhibitor reduced the activity of clinically significant signaling molecules including Src, FAK, and Cyclin D1 which are crucial for tumor growth and progression. In vivo, targeting of eEF2K by eEF2K siRNA encapsulated in iron oxide nanoparticles (IONPS) and our novel water soluble small-molecule eEF2K inhibitor suppressed CRC tumor growth of HTC116 and RKO tumors (flank) CRC tumor models in nude mice, with no observed toxicity. Analysis of tumor tissues by immunohistochemistry showed significant inhibition of the Ki-67 proliferation marker and induction of apoptosis by TUNEL staining. Our study provides the first evidence that eEF2K is a clinically significant potential molecular target and contributes to tumorigenesis and progression of CRC. Its effective inhibition is safe and can suppress tumor growth in preclinical CRC models. These promising results lay the foundation for further developing eEF2K-targeted therapies, offering a potential new approach for treating CRC.

#6954 Discovery and characterization of selective mixed-lineage kinase 3 PROTAC degraders for cancer therapy.

D. Mehlich[1], K. Karpinska[1], V. R. Sabbasani[2], M. Lomiak[1], P. Torres-Ayuso[3], K. Wrobel[1], V. Nguyen-Phuong Truong[1], R. Serwa[1], R. E. Swenson[2], J. Brognard[4], A. A. Marusiak[1]; [1]International Institute of Molecular Mechanisms and Machines - Polish Academy of Sciences, Warsaw, Poland, [2]National Heart Lung and Blood Institute, National Institutes of Health, Bethesda, MD, [3]Lewis Katz School of Medicine, Temple University, Philadelphia, PA, [4]National Cancer Institute, National Institutes of Health, Frederick, MD

Mixed-lineage kinase 3 (MLK3) is a member of the MLK family of serine/threonine kinases. MLK3 is well-known to regulate MAPK signaling by acting upstream of ERK, JNK, and p38 pathways. In addition to its canonical role in activating MAPK cascades, MLK3 regulates the activity of various proteins and signaling pathways, including NF-κB, Wnt/β-catenin, and PAK1. Previous studies demonstrated that MLK3 can promote the proliferation, invasive growth, and metastatic potential of human cancers. Triple-negative breast cancer (TNBC) is a subtype of breast cancer that is characterized by poor prognosis owing to its aggressive clinical course and limited targeted treatment options. MLK3 is often upregulated in TNBC and is involved in driving the tumorigenic potential of TNBC cells. Consequently, several small-molecule inhibitors targeting MLK3 were pursued as new investigational drugs for TNBC in preclinical studies. However, the utility of these compounds is limited by their low specificity and multiple off-target effects. Furthermore, recent studies suggested that the pro-oncogenic activity of MLK3 might result from its kinase-independent and scaffolding functions, which are not effectively targeted by conventional small-molecule inhibitors. To overcome the challenges associated with MLK3 inhibition, we developed and characterized first-in-class MLK3 Proteolysis Targeting Chimera (PROTAC) degraders. We synthesized a series of PROTAC compounds by connecting pan-MLK inhibitor CEP-1347 to molecules binding different E3 ligases (VHL, IAP, Cereblon). We found that CEP-1347-VHL-02, a PROTAC based on CEP-1347 and ligand for VHL E3 ligase, was the most potent MLK3 degrader among all candidate compounds. Next, we demonstrated that CEP-1347-VHL-02 effectively degraded MLK3 via the ubiquitin-proteasome system in breast cancer cell lines. By applying biochemical assays and proteomic approaches, we found that CEP-1347-VHL-02 selectively targeted MLK3 but no other MLK kinases. Furthermore, we showed that MLK3 degradation by CEP-1347-VHL-02 led to diminished oncogenic features of TNBC cells, as evidenced by reduced proliferation, migratory potential, cell cycle arrest, and increased apoptosis. These effects were not observed in other breast cancer subtypes upon MLK3 degradation, which indicates the subtype-specific oncogenic functions of this kinase. Collectively, our research provides new opportunities for the selective targeting of MLK3 in cancer and highlights MLK3 degradation as a potential novel treatment strategy in TNBC. In the ongoing studies, we further investigate the pharmacokinetic properties and therapeutic potential of MLK3-targeting PROTACs across various cancer cell lines with high expression of this kinase.

#6955 TTK inhibition as a potential sensitizer to CDK4/6 inhibition in metastatic triple-negative breast cancer cell lines.

Gretchen M. Albarran-Acosta, Magda N. Alvarez Rodriguez, Alexandra N. Aquino-Acevedo, Harold I. Saavedra Lugo

Basic Sciences, Ponce Health Sciences University, Ponce, Puerto Rico

Breast cancer (BC) is the most common and deadly malignant tumor in women worldwide. Triple-negative breast cancer (TNBC) is the most aggressive BC subtype since it is associated with uncontrolled cell proliferation, genetic instability, and metastasis. Advanced TNBC with distant metastasis is untreatable with anti-hormonal or anti-HER2 therapies due to the lack of hormone receptors (estrogen and progesterone) and Human Epidermal Growth Factor 2 receptor (HER2). Therefore, chemotherapy is the primary treatment for this subtype of BC. The main goal of treatment for advanced TNBC patients is to prolong survival while minimizing the toxicities associated with chemotherapy. TNBCs are more prevalent in Non-Hispanic Black (NHB) and Hispanic/Latinas (H/L) with TNBC, who have lower 5-year survival rates when compared to Non-Hispanic White (NHW) women with TNBC. TNBC patients with a higher percentage of African ancestry exhibit increased expression of certain mitotic kinases (e.g. Threonine tyrosine kinase, TTK), which are crucial for genomic stability, relative to NHW. TTK is a mitotic kinase that helps maintain genomic integrity by regulating the spindle assembly checkpoint. In BC, TTK overexpression mediates centrosome amplification, chromosome instability, aneuploidy and early metastasis. Cyclin-dependent kinase (CDK) 4/6 regulates DNA replication by phosphorylating Rb, which signals progression through the G_1 phase of the cell cycle. The CDK4/6 inhibitor Palbociclib, which induces cellular senescence in tumors, is effective in luminal BC patients but not in TNBC patients. Preliminary data indicates that the inhibition of TTK restored levels of the G1/S regulators p21, p27, and Rb. Therefore, our objective is to evaluate if resistant TNBC cells from NHB women may be responsive/sensitive to CDK4/6 inhibition with Palbociclib treatment after inhibition of mitotic kinase TTK, compared to NHW women. We hypothesize that *inhibition of mitotic kinase TTK will restore levels of G1/S regulators, therefore allowing cells to respond to Palbociclib treatment, and decreasing early metastasis and tumor growth.* Inhibitor drug was added at various concentrations 24 hours after seeding MDA-MB-231 and MDA-MB-157 BC cancer cell lines in 96-well plates. After 96 hours, MTT Assay revealed IC_{50} values of 2.52 nM and 5.93 nM for the TTK inhibitor (BAY1217389) and 9.11 nM and 4.95 nM for Palbociclib (PD0332991) in MDA-MB-231 and MDA-MB-157, respectively. These concentrations will allow optimization for dosing in dual therapies. This will allow reducing each drug's levels and toxicity while improving safety and efficacy for clinical use.

#6956 Overcoming stress induced therapy resistance in triple negative breast cancer with the MNK inhibitor EB1.

Marta Cano-Galietero[1], Elena Muro-Blanc[2], Sara Garcia-Ortega[2], Adriana Martinez Cifuentes[1], Jose Ignacio Borrell Bilbao[3], Stefan Hummer[4], Santiago Ramon y Cajal[2]

[1]Spanish Biomedical Research Network Centre in Oncology (CIBERONC), Barcelona, Spain,[2]Translational Molecular Pathology Group, Vall d'Hebron Research Institute (VHIR), Barcelona, Spain,[3]Pharmaceutical Chemistry Group, IQS School of Engineering, Barcelona, Spain,[4]Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain

INTRODUCTION. Therapy resistance and the evasion from immune based therapies represent main caveats of current cancer treatments and both processes critically rely on the activation of stress response pathways, which activate the kinases MNK1/2. While MNKs are non-essential kinases, phosphorylation of eIF4E at S209 is required for oncogenic transformation and correlates with worse prognosis in several human tumors. Targeting MNKs has therefore evolved as a non-toxic strategy in oncology, to improve the efficacy of current treatments. We have identified with EB1 the first in-class Type-II inhibitor of the kinases MNK1/2 with a superior mode of action (Bou-Petit et al, JMC, 2022).
OBJECTIVES. We have previously postulated that targeting MNKs sensitizes cells to therapy induced cellular stress (Martinez et al. PLOSone, 2015). In this work, we describe the impact of EB1 on cell fate in the context of cellular stress and delineate the underling signaling pathways.
METHODOLOGY. The cell line MDA-MB231 was used as model of triple negative breast cancer (TNBC) and results were confirmed in MDA-MB468 and HS578T cells. Effects on cell growth of EB1 alone and in combination with cellular stress or therapeutic agents were studied through crystal violet assay. Alterations in signaling pathways were studied by western blot. Electron and confocal microscopy were used to analyze changes in cellular organelles and their activity. Functional assays on protein translation and the autophagy-lysosomal pathway complement the study. Main results obtained with EB1 were compared to the siRNA mediated knock-down of MNKs and eFT-508 (eFFECTOR Therapeutics, Inc.) as the most advanced Type I MNK inhibitor.
RESULTS. Cell growth assays demonstrate enhanced activity of EB1 in combination with cellular stress or therapeutic agents. Concomitantly, stress response pathways were hyper-activated in the presence of EB1 and protein translation was diminished. Microscopic analysis reveal alterations in the morphology autophagosome/lysosome structures, particularly under cellular stress. Analysis of signaling pathways revealed furthermore a link to the PI3K/AKT/mTOR signaling pathway in stress-compromised cells upstream of protein translation and autophagy. Remarkably, the observed phenotypes could only be observed with EB1 but not with eFT508, suggesting that the mode of action of MNK inhibitors is relevant in this context.
CONCLUSIONS. We demonstrate here that the MNK inhibitor EB1 affects breast cancer cells particularly under cellular stress conditions. We propose that EB1 might represent a novel therapeutic opportunity, to combat cellular stress induced therapy resistance. This might be of particular importance for the treatment of TNBC, in which good initial responses to systemic therapies are in most of cases followed by therapy resistance and tumor relapse.

#6957 Taking a new approach to treating p53 null endometrial and ovarian cancers by combining chemotherapy with tyrosine kinase inhibitors.

Jamie L. Padilla[1], Lane E. Smith[1], Irina V. Lagutina[2], Mara P. Steinkamp[3], Kimberly K. Leslie[4]

[1]Internal Medicine, University of New Mexico Health Sciences Ctr., Albuquerque, NM,[2]CRTC Shared Facility, University of New Mexico Health Sciences Ctr., Albuquerque, NM,[3]Pathology, University of New Mexico Health Sciences Ctr., Albuquerque, NM,[4]University of New Mexico Health Sciences Ctr., Albuquerque, NM

Endometrial and ovarian cancers are of the most common gynecologic cancers, and endometrial cancer incidence and mortality rates are rising. The majority of the highest risk forms of these cancers harbor mutations in the central tumor suppressor p53, encoded by the gene *TP53*. Approximately 30% of mutations such as frame shift alterations, result in a p53 null state as distinguished from the other broad category of p53 mutant tumors that are hotspot single amino acid missense alterations. With the loss of p53-dependent cell cycle checkpoints in p53 null cells, these tumors depend exquisitely on a signaling pathway downstream of p38 MAPK to enforce the G2/M checkpoint by phosphorylating cdc2, the G2/M checkpoint gatekeeper, allowing tumor cells to stop and repair DNA before entering mitosis. Using preclinical cell, organoid and PDX studies we report that adding a tyrosine kinase inhibitor to abrogate the p38 MAPK enforced G2/M checkpoint is synergistic with chemotherapy and results in mitotic catastrophe specifically in p53 null cells. Endometrial p53 null Hec50 and ovarian cancer PDX9 organoids were treated with therapeutically relevant doses of the TKIs erlotinib (an EGFR/MAPK inhibitor) or lenvatinib (primarily targeting VEGFR), paclitaxel alone or the combination of each TKI +paclitaxel and assessed for cell viability using both the CyQuant DNA platform and the Incucyte cell imaging platform. Cells expressing either wild type or missense mutated p53 were used in parallel experiments as comparisons to the p53 null models. Our striking results indicate that the combination of erlotinib and paclitaxel was highly synergistic specifically in p53 null cells with a significant reduction in the IC50 of the paclitaxel when erlotinib was added to the regimen. This result was specific for p53 null models, not for wild type or missense mutated models. Furthermore, the active regimen was erlotinib +paclitaxel, not lenvatinib +paclitaxel, indicating that the effective TKI to achieve synergy must target the EGFR/MAPK pathways, and targeting VEGFR pathways does not produce synergy under these conditions. Studies are now underway in additional cell, organoid and PDX models to validate our initial findings. The impacted kinome is also being assessed to further determine the specific targets of each TKI in the p53 null versus p53 wild type and missense mutated models. By exploring the potential of combining a TKI with chemotherapy specifically for patients with p53 null tumors, we hope to provide a more effective treatment option for this challenging subgroup of patients.

#6958 Combined targeting of AURKA and AURKB as a potential therapeutic strategy to decrease the expression of epithelial-to-mesenchymal transition drivers in triple-negative breast cancers.

Joel Alexis Orengo[1], William Frank Rodriguez[2], Selimar Alvarez Velazquez[3], Alexandra N. Aquino- Acevedo[1], Gabriel Rosario Ortiz[1], Melanie E. Cruz Robles[1], Esther M. Irrizary Quintana[4], Harold I. Saavedra[1]

[1]Basic Sciences, Ponce Health Sciences University, Ponce, Puerto Rico,[2]University of Puerto Rico, Ponce U-RISE program, Ponce Health Sciences University, Ponce, Puerto Rico,[3]Cancer Prevention and Control Research Training Program-UPR Comprehensive Cancer Center, Ponce Health Sciences University, Ponce, Puerto Rico,[4]MCC/PHSU U54 PACHE Partnership Undergraduate Research Program, Ponce Health Sciences University, Ponce, Puerto Rico

Studies have shown a significant health disparity between Hispanic/Latino women (H/L) and women of African ancestry (non-Hispanic black (NHB), Black H/L, and Caribbean H/L) compared to non-Hispanic White women (NHW) as they are more likely to be diagnosed with and to die from triple-negative breast cancer (TNBC). At mRNA levels, NHB women diagnosed with TNBC overexpress mitotic kinases such as AURKA and AURKB relative to NHW. Although these mitotic kinases have a pivotal role in the cell cycle, when dysregulated, they can activate epithelial-to-mesenchymal transition (EMT) drivers that can lead to metastasis and therapy resistance. TNBC is the most aggressive breast cancer subtype that lacks the progesterone (PR), estrogen (ER), and the ERBB2 (HER2) receptor. Therefore, it cannot be treated with biological therapies that are known to be effective against the other molecular subtypes. Thus, the purpose of this study is to investigate if AURKA and AURKB are potential therapeutic targets against TNBC in women of African ancestry. Hence, we hypothesize that co-inhibiting AURKA and AURKB will decrease the expression of EMT biomarkers and transcription factors and, therefore, lead to early metastasis. Using The Cancer Genome Atlas Database (TCGA), we identified higher expression of AURKA and AURKB in NHB women than in NHW women and increased expression of AURKB in NHB women with TNBC. After 4-siRNA-mediated knockdown, immunoblotting and Real-Time qPCR were performed using the MDA-MB-231 cells (NHW) and MDA-MB-157 (NHB) mesenchymal TNBC cells. Preliminary data indicates that the expression of AURKA and AURKB decreased in all cell lines compared to the control group. N-cadherin protein level decreased in MDA-MB-231 after a single inhibition, while the levels of ZO-1 increased after combined inhibition. Vimentin decreased in MDA-MB-157 after AURKB inhibition, while the levels of Snail decreased after combined inhibition. At mRNA levels, AURKA and AURKB significantly reduced in both cell lines and levels of Twist decreased in MDA-MB-231 after co-inhibiting AURKA and AURKB. Preliminary data from invasion assays suggest a decrease in invasion ability after single and combined inhibition in both cell lines. We will perform a prolonged transfection using shRNA to evaluate the expression of these EMT biomarkers. Additionally, we will study tumor growth and metastasis rates in vivo models. In conclusion, these results suggest the role of AURKA and AURKB in TNBC and their potential as therapeutic targets to decrease early metastasis.

#6959 CK2 inhibition exhibits synergistic effect with KIT inhibitors in acute lymphoblastic leukemia.

Katarina Dovat, Daniel Bogush, Julia Randazzo, Marjon Rafie, Stephanie Buczkowski, Rabab Husain, Joseph Schramm, Yali Ding, Chingakham Singh, Dhimant Desai

Penn State University, Hummelstown, PA

Survival for pre-B cell Acute lymphoblastic leukemia (B-ALL) has significantly improved, but High Risk (HR) subgroups continue to result in increased death for pediatric oncology patients. Novel treatment strategies are required to conquer chemotherapy resistance and improve life expectancy for HR B-ALL. Deletion of IKZF1, resulting in decreased IKAROS function, is a feature of high-risk B-ALL that is associated with resistance to chemotherapy and a poor prognosis. Casein kinase 2 (CK2) is a protein kinase upregulated in B-ALL, which phosphorylates IKAROS and reduces Ikaros DNA binding affinity. This results in a loss of IKAROS-mediated transcriptional regulation in B-ALL patients. Previous studies have shown that pharmacologic inhibition of CK2 with CX-4945 recovers IKAROS function as a transcriptional regulator. Receptor tyrosine kinase c-KIT is transcriptionally regulated by IKAROS and is often upregulated in B-ALL. The purpose of this study was to determine whether restoration of IKAROS function as c-KIT repressor via CX-4945 would act in a synergistic manner with c-KIT inhibitor imatinib in B-ALL. B-ALL cells were treated with imatinib over 72-hours to demonstrate the half-maximal inhibitory concentration (IC50). The IC50 was used to determine the range of the concentration of imatinib to be used in association with CX-4945 in a 72-hour synergy analysis of cytotoxicity. Cell viability was determined using WST-1 cell proliferation assay. The data was analyzed using Calcusyn and synergy was specified as a combination index below 0.85. The study was repeated with several different drug concentrations to establish a more precise range of synergy. The cytotoxicity of single drug c-KIT inhibitor, Imatinib, in Nalm6 B-ALL produced an IC50 of 25uM while CK2 inhibitor CX-4945 has IC50 of 4uM. When a combination was administered with a constant dose of CX-4945 at 4uM and different concentrations of imatinib, synergy was detected at several dose combinations. In conclusion, our results demonstrate that CK2 inhibitor, CX-4945, and c-KIT inhibitor, imatinib, exhibit a synergistic therapeutic effect on B-ALL cells. Presented data provide a rational for the use of combination therapy with CK2 inhibitors and imatinib for treatment of high-risk B-ALL, including Philadelphia positive (Ph+) B-ALL.

#6960 Specific BaF3 tumor platform for c-KIT and KRAS mutations.

Ziwei Li, Hating Dai, Tianyu Pang, Jiali Liu, Wenbo Du, **Wen-Jen Yu**

ICE Bioscience INC, Beijing, China

BaF3 cell line from mouse pro-B cell is rely on interleukin-3 (IL-3) for survival and widely used to evaluate the efficacy of kinase oncogenes, downstream signaling, and the blocking ability of small molecule kinase inhibitors by site specific mutation of target genes such as KRAS, EGFR, AKT, c-KIT, etc. However, few was known about the in vivo efficacy of BaF3 tumor models and the differences of efficacy from spontaneous mutation human cell lines. ICE bioscience constructed over 300 BaF3 point mutation stable cell lines as a good platform for high throughput screening in vitro. In this study, two specific BaF3 c-kit mutation tumor models (BaF3 c-KIT-V559-V560 del, BaF3 c-KIT-W557-K558 del-T670I) and BaF3 KRAS-G12C tumor models were established for efficacy evaluation of Imatinib, Sunitinib, and Sotorasib. The results showed that Imatinib 100 mpk inhibited BaF3 c-KIT-V559-V560 del tumor growth (TGI=111.10%, T/C=2.68%) and Sunitinib 40 mpk inhibited BaF3 c-KIT-W557-K558 del-T670I tumor growth (TGI=106.13%,T/C=2.03%), respectively. The efficacy of Sotorasib-30 mpk to Ba/F3 KRAS G12C tumor is significant and reached 89.37% TGI and 26.71% T/C. Although ASP3082 is a KRAS G12D inhibitor, the efficacy of ASP3082-3 mpk to KRAS-G12D GP2d CDX model is not relevant, only 37.29% TGI and 73.25% T/C values were found in this study. Western blotting showed that the BaF3 mutation tumor models are suitable for the drug discovery of specific point mutation anti-cancer drugs.

#6961 Characterization of EGFR C797S-mutant, osimertinib-resistant non-small cell lung cancer cells.

Janneke J. T. M. Melis, Kirsten J. W. Kevenaar, Jonne van Eck, Janneke M. van den End, Yvonne Grobben, Jeffrey J. Kooijman, Guido J. R. Zaman

Oncolines B.V., Oss, Netherlands

Osimertinib is a third-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor that irreversibly binds to active-site cysteine 797 (C797). Originally, it was developed to target the T790M gatekeeper mutation which arises as acquired resistance mechanism to first-generation EGFR inhibitors such as gefitinib. Due to its efficacy and selectivity, osimertinib is now used as first-line treatment for non-small cell lung cancer (NSCLC) patients harboring the activating EGFR mutation L858R or exon 19 deletion. However, a wide range of on- and off-target resistance mechanisms have been observed in clinical settings. Developing relevant models is crucial for designing effective treatment strategies to overcome osimertinib resistance.
The most frequently occurring on-target resistance mechanism against osimertinib is the C797S mutation, which has urged the development of fourth-generation EGFR inhibitors. To enable the profiling of novel EGFR inhibitors and drug combinations on C797S-mutant EGFR models, the mutation was introduced in NCI-H1975 cells via gene-editing with CRISPR/Cas9. NCI-H1975 is an NSCLC cell line harboring the L858R activating mutation and T790M resistance mutation. The cell line is resistant to first-generation EGFR inhibitors such as gefitinib, but remains sensitive to osimertinib. NCI-H1975 cells were transfected with a homology-directed template containing the C797S mutation and were edited via CRISPR/Cas9.
After confirmation that the mutation was introduced in a significant number of cells by DNA sequencing (mutation frequency: 28%), the cell pool was single-cell sorted followed by expansion and analysis of single cell clones. Three cell clones were characterized in detail using whole exome sequencing, quantitative analysis of EGFR surface expression using Quantibrite beads (BD Biosciences), and cell viability assays using ATP as readout in 2D culture and 3D spheroids.
Whole exome sequencing confirmed the presence of the C797S mutation in the three clones. Quantification of cell surface EGFR expression levels revealed two times higher EGFR expression in the clones compared to the parental cell line. In 2D cell viability assays, the C797S-mutant clones were 316-fold less sensitive to osimertinib compared to the parental cell line. In contrast, the fourth-generation EGFR inhibitors BLU-945 and JBJ-09-063 reduced viability of the parental and C797S-mutant clones with similar potency. While potencies in the clones were similar between 2D culture and 3D spheroids, BLU-945 and JBJ-09-063 reached a higher maximum effect in 3D spheroids compared to 2D culture.
The successful generation and characterization of the NCI-H1975 C797S-mutant clones provides a valuable *in vitro* model in which additional next-generation EGFR inhibitors and drug combinations can be profiled to identify therapeutic strategies to overcome osimertinib resistance.

#6963 The impact of aPKC inhibitors on PKC-ι/ζ; and connexin 43 in glioblastoma multiforme.

Grazielly Caroline Teodoro, Mildred Acevedo-Duncan

University of South Florida, Tampa, FL

Glioblastoma multiforme (GBM) is the most aggressive brain cancer in adults, arising from astrocytes. Its treatment is complicated by the blood-brain barrier, tumor infiltration, and genetic diversity, highlighting the urgent need for novel therapies. Our research group focuses on understanding the impact of ICA-S((-Amino-1-[(1R,2S,3R,4R)-2,3-dihydroxy-4-[(phosphonooxy)methyl]cyclopentyl]-1H-imidazole-4-carboxamide), an atypical inhibitor of PKC-ι, and ζ;-Stat (8-hydroxy-1,3,6-naphthalenetrisulfonic acid) inhibitor of PKC- ζ; on GBM proliferation and invasion, as well as their effects on Connexin 43 (Cx43). PKC-ι /ζ; plays a crucial role in GBM tumorigenesis by regulating cell proliferation and invasion. Its overexpression is directly influenced by phosphoinositide 3-kinase (PI3K). PKC-ι/ζ; and its upstream regulator, Src tyrosine kinase, regulate Cx 43, a gap junction protein that facilitates communication between astrocytic cancer cells and surrounding normal cells, thereby promoting cancer progression. The effects of aPKC inhibitors were assessed through proliferation assays on glioblastoma cell lines T98G and U87MG, revealing the impressive efficacy of both ICA-1S and ζ;-Stat. ICA-1S achieved a statistically significant 50% reduction in T98G cell proliferation (p <0.001), while ζ;-Stat resulted in a decrease of approximately 40% in proliferation within T98G cells (p< 0.001). Furthermore, ICA-1S also achieved around 40% reduction in proliferation in the U87 cell line (p< 0.001). Co-immunoprecipitation experiments provided evidence of the association between PKC-ι and Cx 43, as well as between PKC-ζ and Cx 43, in both cell lines. This suggests a direct interaction between these proteins, demonstrating their potential role in mediating cellular signaling and communication in GBM. Western blot analysis showed that ICA-1S downregulated PKC-ι and Cx43, while ζ;-Stat downregulated PKC-ζ and its phosphorylation of Cx43, impacting the PI3K/AKT pathway. Immunofluorescence Analysis of T98G cells showed decreased Cx43 distribution following treatment, suggesting potential disruption of cell communication. Further analysis will investigate the effects of ICA-1S and ζ-Stat on the U87MG cell line, specifically examining the PI3K/AKT pathway using western blotting and immunofluorescence. Moreover, High-Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) will be essential for identifying phosphorylation sites directly impacted by aPKCs and Src in Cx 43. These innovative therapeutic strategies hold promise for overcoming chemical resistance challenges and may enhance patient survival outcomes.

#6964 Dual inhibition of PKMYT1 and WEE1 kinases as a targeted therapy for ATIP3-deficient breast cancers.

Maria M. Haykal[1], Marie Varin de Lapeyriere[2], Alexis Denis[2], Sylvie Rodrigues-Ferreira[3], Clara Nahmias[1]

[1]INSERM U981 (Gustave Roussy), Villejuif, France,[2]IKTOS, Paris, France,[3]Inovarion, Paris, France

ATIP3, a microtubule-associated protein with tumor suppressor functions, is frequently lost in aggressive breast cancers, particularly in 60-80% of triple-negative breast cancer (TNBC), where its absence correlates with poor prognosis. ATIP3 deficiency is associated with heightened aneuploidy, presenting a vulnerability exploitable by targeted therapies. WEE1 kinase, a critical regulator of the G2/M checkpoint, has demonstrated therapeutic promise in this context. Inhibiting WEE1 with AZD1775 selectively compromises ATIP3-deficient cells by exacerbating DNA damage, mitotic abnormalities and chromosome pulverization, ultimately leading to cell death. However, the clinical application of WEE1 inhibitors is constrained by dose-dependent toxicities, necessitating combination strategies to enhance efficacy while reducing toxicity.

To address these challenges, we explored the complementary targeting of PKMYT1, another G2/M checkpoint kinase with distinct yet overlapping functions to WEE1. Using 3D cultures of breast cancer cell lines, we compared the responses of ATIP3-deficient and ATIP3-expressing cells to three PKMYT1 inhibitors—RP-6306, GSK1520489A, and MYT1-IN-I. ATIP3-deficient cells exhibited heightened sensitivity to all three inhibitors, particularly RP-6306, which achieved significantly lower IC50 values. To maximize therapeutic impact, we combined RP-6306 with AZD1775, resulting in a synergistic reduction in cell viability in ATIP3-deficient cells.

Dual inhibition of WEE1 and PKMYT1 intensified replication stress, DNA damage, and abnormal mitoses, exceeding the effects observed with either inhibitor alone. Importantly, the dual-targeting approach enables the use of lower doses of WEE1 inhibitors, potentially mitigating the toxicities associated with high-dose monotherapies.

Our findings establish PKMYT1 as a complementary target to WEE1 in ATIP3-deficient breast cancers. The synergistic effects of their combined inhibition not only enhance therapeutic efficacy but also address key limitations of current WEE1-based therapies. This dual-targeting strategy holds promise for improving outcomes in aggressive breast cancer subtypes. Furthermore, our work highlights the potential of ATIP3 as a predictive biomarker for patient stratification and the rational development of personalized therapies.

#6965 Atypical protein kinase C-ι inhibitor suppresses the proliferation of malignant pancreatic ductal adenocarcinoma cells.

Shreejana Rimal[1], Mildred Acevedo Duncan[2]

[1]University of South Florida, Tampa, FL,[2]Chemistry, University of South Florida, Tampa, FL

Pancreatic cancer is considered to be one of the most lethal malignant cancers as its diagnosis remains elusive if the disease progresses to an advanced stage. Moreover, pancreatic cancer is the third leading cause of cancer-related deaths in the U.S. Chemoresistance to various drugs for pancreatic cancer is the main reason for its aggressive nature. Atypical protein kinase is the family of protein kinases that contain two isoforms -ι and -ζ. These protein kinases which play a significant role in the signaling pathways have been proven to be involved in cancer cell proliferation and metastasis. (5-amino-1-((1R,2S,3S,4R)-2,3-dihydroxy-4-methyl cyclopentyl)-1H-imidazole-4-carboxamide-(ICA-1S), one of the specific inhibitors of Protein Kinase C-ι has been reported to inhibit the function of PKC-ι resulting to a decreased cell proliferation, decreased metastasis, and increased apoptosis. Our study focuses on using ICA-1S to inhibit PKC-ι in two pancreatic adenocarcinoma cell lines: ASPC1 and PANC1. Firstly, 50,000 cells were seeded into 6 well plates and treated with different concentrations of ICA-1S (0.5μM, 5μM,10μM,20μM, and 50μM) in every 24 hours for three days. The drug-treated cells and the control were trypsinized to count the number of cells using Cellometer cell counting chambers. The highest percentage of inhibition by ICA-1S was observed at 10 μM for both the cell lines ASPC1 and PANC1 where the proliferation was observed to decrease at 52% in the case of ASPC1 and 49.8% in the case of PANC1 respectively. ICA-1S concentration of 10 μM is now used for further experiments. Western Blot analysis of both ASPC1 and PANC1 cell lines demonstrated the downregulation of the PKC-ι and PKC-ζ proteins. Similarly, the downregulation of apoptosis-related marker, Caspase 3 led us to hypothesize that apoptosis is induced in the drug-treated pancreatic cancer cells compared to the control. Mutation in KRAS is most common in pancreatic cancer where very few drugs have been approved by the FDA for the treatment of pancreatic adenocarcinoma. Thus, we will on the KRAS regulated pathway for further analysis. Further investigations would include the study of aPKC inhibitor-affected pathways using techniques like WST, immunoprecipitation assay, immunofluorescence, and Western blotting.

#6966 Apparent potency of small molecule inhibitors is influenced by the choice of substrate in LIMK biochemical in-vitro kinase activity assays.

Robert Torka, Andreas Gericke, Frank Totzke, Carolin Heidemann-Dinger, Constance Rademann, Thomas Weber, Daniel Mueller

Scientific Office, Reaction Biology Europe GmbH, Freiburg, Germany

Protein kinases are essential for the regulation of many biological processes, e.g. proliferation, differentiation, migration, and apoptosis. Deregulated kinase activity is observed in tumor cells and altered activity of specific kinases is essential for development and progression of cancer and other diseases. Consequently, regulation of protein kinase activity became a target for therapeutic intervention. Intensive research resulted in the development of many small molecule kinase inhibitors (SMI) being clinically approved as targeted therapies. Functional biochemical in-vitro assays are essential requirements to assess SMIs effects on target kinase activity after validation of specific kinases being molecular drivers of a pathological condition. Only limited information is gained by monitoring the interaction of kinase and compound in pure binding assays, but more conclusive data can be generated by in-vitro kinase activity assays. However, the relevance of results from both approaches depends on how well the assay setup responds to the mode-of-action of the compounds. Recombinant proteins and generic substrates are well established in development of protein kinase assays due to cost and technical feasibility considerations. Especially some generic substrates are highly artificial e.g. the commonly used Tyrosine-kinase substrate Poly(Glu/Tyr)4:1 and may be considered as phosphate group acceptors only. However, such generic substrates have successfully been used for development of numerous clinically approved SMIs. Other kinases however are not compatible with generic substrates and can only be established using less artificial substrates or even require their respective physiological in-vivo substrate for in-vitro activity. These kinases include several members of the RAF and MAPK family. Functional in-vitro assay using a generic substrate are rarely replaced by physiological substrate due to more complex assay conditions and increased cost for drug screening. We compared biochemical in-vitro kinase activity assays for the LIM kinases, LIMK1 and LIMK2, using either generic substrates or the published physiological substrates, CFL1 and CFL2. We further evaluated these results by comparing them in different assays, using radiometric and luminescent read-out technologies. Several published LIMK inhibitors displayed significant differences in the relative potency when tested with generic or physiological substrates. Generic substrates for biochemical in-vitro kinase activity assays in preclinical drug development have been well established and successfully applied in the past. However, our data indicate, that the choice of a more physiological substrate should be considered carefully, as it might significantly increase the relevance and therefore value of the results obtained from early-stage compound screening.

#6967 Suppression of protein X-mediated survivin expression enhance the antitumor effect of irinotecan in colorectal cancer.

Se Yong Park[1], Ju-Hee Kang[1], Jung Ho Hwang[2], Sun-Ju Lee[2], Jun-Won Yun[1], Seung Hyun Oh[1]

[1]College of Veterinary Medicine, Seoul National University, Seoul, Korea, Republic of,[2]College of Pharmacy, Gachon University, Incheon, Korea, Republic of

Survivin, an inhibitor of apoptosis protein, contributes to cancer drug resistance. This study investigated survivin's role in irinotecan resistance in colorectal cancer. RT-PCR and western blotting confirmed increased survivin expression in irinotecan-treated colorectal cancer cells. Knockdown of survivin by using siRNA decreased cell viability, confirming its anti-apoptotic function. Screening potential upstream regulators revealed ‘protein X’ but not ‘protein Y’, as a key mediator of survivin expression. ‘Protein X’ is overexpressed in many cancers and associated with drug resistance. We hypothesized that inhibiting ‘protein X’ could enhance irinotecan's effectiveness. Both genetic inhibition by siRNA and pharmacological inhibition with an ‘protein X’ inhibitor enhanced apoptotic effects of irinotecan in colorectal cancer cell lines. The synergistic effect was particularly pronounced in HCT116 cells, which showed the highest sensitivity to irinotecan. These findings suggest that targeting ‘protein X’-mediated survivin expression may be a promising strategy to overcome irinotecan resistance in colorectal cancer. This approach provides a novel perspective on enhancing the efficacy of irinotecan-based therapies and potentially improving outcomes for colorectal cancer patients. Further in vivo studies and clinical trials are warranted to validate these findings and explore the therapeutic potential of this combination approach.

#6968 Targeting overexpressed NEK2 kinase in esophageal adenocarcinoma.

Lei Chen, Heng Lu, Farah Ballout, Wael El-Rifai, Oliver Gene McDonald, **Dunfa Peng**

University of Miami, Miami, FL

The incidence of esophageal adenocarcinoma (EAC) has rising rapidly during the past four decades in the US and Western countries, becoming the dominant type of esophageal cancer and a significant clinical challenge. However, the prognosis of EAC patients is still poor with 5-year overall survival rate about 20%. There is an urgent need to identify novel therapeutic targets associated with EAC biology. NEK2 is a serine/threonine protein kinase of NEK (NIMA related kinase) family which has 11 members, NEK1-NEK11. NEK2 is frequently overexpressed in human cancers, associated with tumor progression and drug resistance. We detected the overexpression of NEK2 in both mRNA and protein levels in EAC cell lines and primary EAC tissues using qRT-PCR, western blotting, immunohistochemistry. Functionally, we demonstrated that overexpression of NEK2 in EAC cells promoted tumor cell growth. Targeting NEK2 using specific siRNAs and a recent NEK2 specific inhibitor NBI-961 in EAC cells significantly inhibited EAC tumor growth in vitro and in vivo in a xenograft mouse model. RNA-seq analyses indicate that NEK2 inhibition using NBI-961 impaired important signaling pathways such as EMT, IL6-JAK -STAT3, mTOR1 and DNA repair, and enhanced interferon alpha response pathway in EAC cells. Our data suggests that targeting frequently overexpressed NEK2 in EAC is a promising approach.

CHEMISTRY: Lead Identification and Optimization
Poster Session

#6972 Discovery of XNW29016, a PARG inhibitor for HRD cancer therapy.
Y. Hu, H. Wei, D. Wan, B. Ma, J. Xu, S. Wang, L. Kong, Z. Zhang, Y. Li, Z. Wu, Y. Hou, W. Wang, X. Liu, M. Le, J. Qiang;
Evopoint Biosciences Co., Ltd., Suzhou, China

DNA damage repair enzymes are promising targets as genomic instability and DNA repair defects are important characteristics of cancer cells PARylation catalysed by PARP1/2 is a kind of reversible protein modification, which has central functions in maintaining genome stability. Poly (ADP-Ribose) glycohydrolase (PARG) is the dominant eraser of PARylation in the cell, whose activity prevents excessive accumulation of PARylation, promotes the dissociation of repair proteins, and ensures the smooth completion of DNA repair process. Deficiency of PARG leads to accumulation of PARylation and deregulation of DNA repair, which in turn causes cells to be sensitive to DNA damage and replication stress. So PARG is considered as a promising target for treating tumors characterized by DNA repair defects. PARG and PARP are in the same biochemical pathway, but their inhibitors inhibit tumor cell growth through different mechanisms, and therefore tumor cells show different sensitivities to the two inhibitors. Since the efficacy of PARP inhibitors is limited by intrinsic or acquired drug resistance in clinical practice, PARG inhibitors can also be used as an effective supplement for PARP inhibitors. XNW29016 is a rationally designed PARG inhibitor which binds directly to the PARG catalytic domain with *in vitro* biochemical IC_{50} of 29nM against PARG. Treating cells with XNW29016 strongly induced the accumulation of intracellular PARylation, activated DNA damage response signaling pathway and caused cell cycle arrest. XNW29016 demonstrated potent *in vitro* antitumor effects in a variety of PARPi-resistant cancer cell lines such as RMUG-S, KURAMOCHI and HCC1428. SNU601 and HCC1806 cells with acquired niraparib resistance or carboplatin resistance continued to be sensitive to XNW29016, but insensitive to niraparib. This result indicates that patients could still benefit from XNW29016 after treatment with PARP inhibitors and platinum drugs. In line with *in vitro* activities, XNW29016 performs antitumor activity in cell derived xenograft models of breast cancer, ovarian cancer, and gastric cancer. *In vivo* efficacy is dose-dependent and test dosages are well-tolerated, with minimum effective dose at 25 mg/kg/dose twice daily. We observed a time-dependent accumulation of PARylation. XNW29016 has good oral bioavailability and excellent exposure in preclinical pharmacokinetic studies. In toxicological studies, XNW29016 exhibits satisfactory safety profile. Altogether, these preclinical findings warrant further clinical evaluation of XNW29016 in patients with HRD cancers.

#6974 Identification of a potent KRAS (ON) inhibitor with selectivity for mutant KRAS over HRAS and NRAS.

Keith Koch, Kim Alley, Karyn Bouhana, Richard Brizendine, Paul Carlson, Mark Chicarelli, Michelle Crow, Brad Fell, Jennifer Fulton, Anna Guarnieri, Jackie Harrison, John Fischer, Ravi Jalluri, Amber Johnson, Cori Malinky, Macedonio J. Mejia, Brad Newhouse, Scott Niman, Rob Rieger, John Robinson, Mareli Rodriguez, Leah Salituro, Vinny Scarato, Aaron Smith, Francis Sullivan, Yang Wang, Shannon Winski, Silas Wodd, Yeyun Zhou

Cogent Biosciences, Boulder, CO

Oncogenic KRAS gene mutations are prevalent across human cancers, including: pancreatic, lung, and colorectal. In the US alone, it is estimated that over 165,000 cancer patients are affected by KRAS mutations annually. Early successes in the discovery of mutation specific therapeutics for KRAS led to the development of marketed agents adagrasib and sotorasib, which selectively inhibit $KRAS^{G12C}$. While these precision medicines address a critical unmet medical need, they only treat ~11% of patients with oncogenic KRAS mutations. Recent efforts to address this disparity have resulted in the identification of pan KRAS inhibitors that target all clinically relevant mutations. Herein, a novel preclinical pan KRAS (ON) inhibitor, demonstrating picomolar cellular activity across a panel of prevalent KRAS mutations is highlighted. This lead series exemplar has a differentiated mechanism of action compared to the tri complex multi RAS inhibitors currently in development, with selectivity over HRAS and NRAS. In vivo characterization to be presented includes pharmacokinetics, pharmacodynamics measuring inhibition of pERK, and efficacy in KRAS mutant tumor bearing mouse models.

#6976 Identification of novel KAT6A/B inhibitors with enhanced antitumor activity and reduced hematologic toxicity.

Rui Liu, Yushi Chi, Xiayan Zhang, Xuemei Tian, Chenqiang Jia, Zhiliang Lv, Huixin Yan, Qian Zou, Liping Han, Wan-Jen Yang, Jijun Yuan, Xiaoling Yuan, Zhiyu Ma, Yi Zhang, Yongqiang Shan, Chen Hu

Shanghai Henlius Biotech, Inc., Shanghai, China

Introduction: KAT6A/B are promising targets for breast cancer therapy. A phase 1 study of the KAT6A/B inhibitor PF-07248144 showed encouraging clinical results; however, target-associated hematologic toxicity, particularly neutropenia, may hinder dose escalation. KAT6A/B inhibitors that exhibit suboptimal specificity towards other members of the KAT family may lead to increased hematologic toxicity. This study aims to identify potent and highly selective KAT6A/B inhibitors with "fast-on, fast-off" pharmacokinetic profiles *in vivo*, enabling sustained antitumor efficacy while minimizing hematologic toxicity.

Methods: Cytotoxicity of the candidates was evaluated in ZR-75-1 cells using the CellTiter-Glo (CTG) assay. An *in vitro* acetyltransferase assay, employing radiolabeled acetyl-CoA as a substrate, was conducted to assess the inhibitory effects of the candidates. *In vivo* efficacy using mono-agent was tested in a ZR-75-1 breast cancer model, while synergy studies were performed in the patient-derived xenograft (PDX) model LD1-0009-362016, which was resistant to CDK4/6 inhibitors. Top candidates were screened in the WuXi Mini 44 safety panel at a concentration of 10 μM to identify potential off-target effects. Additionally, the inhibition of H3K23 acetylation in ZR-75-1 cells was analyzed by Western blotting.

Results: HLX97-069/053 emerged as the top two candidates, exhibiting superior enzymatic inhibition and enhanced selectivity against KAT5/7/8, along with more potent cytotoxic effects in ZR-75-1 cells, in head-to-head comparisons with PF-07248144. The cytotoxicity of each compound was correlated with their potency to inhibit H3K23 acetylation. Both compounds displayed favorable ADME characteristics, such as high oral bioavailability and excellent pharmacokinetics. In a ZR-75-1 xenograft model, they demonstrated dose-dependent antitumor efficacy with minimal weight loss over a five-week treatment period. Notably, HLX97-069 resulted in less significant reductions in leukocyte, neutrophil, lymphocyte, and monocyte counts compared to PF-07248144 after five weeks of treatment. We propose that the more robust KAT6A/B inhibition and “relatively faster” clearance of HLX97-069 may contribute to its broader therapeutic window. Both compounds were well tolerated at 100 mg/kg in a single-dose acute toxicity study in rats. Additionally, HLX97-069 showed no off-target inhibition in safety panel screening. Compared to PF-07248144, HLX97-053 exhibited significantly stronger synergy with fulvestrant and palbociclib in the LD1-0009-362016 model.

Conclusion: In summary, these preclinical data provide compelling evidence that we have identified novel KAT6A/B inhibitors with best-in-class potential. Confirmation of the final candidate will depend on the outcomes of the forthcoming pilot toxicity studies, with an IND application anticipated by the end of 2025.

#6977 Development of SY-9453, a novel MAT2A inhibitor, for the treatment of MTAP-deficient cancers.

Shuang Liu, Zhenbang Lou, Ying Xin, Chang Lu, Shikang Cheng, Junfen Shi, Yeliu Wang, Sheng Zhao, Yan Zhu, **Hong Luo**, Yinghui Sun

Shouyao Holdings (Beijing) Co., Ltd., Beijing, China

Co-deletion of the *MTAP* gene with the *CDKN2A* gene, which is found in approximately 15% of all cancers, results in sensitization to MAT2A inhibition and provides an option for therapeutic intervention. The encouraging clinical activity of IDE397 provides further proof-of-concept for MAT2A inhibitors in the treatment of MTAP-deficient cancers. Here, we describe SY-9453 as a novel and potent MAT2A inhibitor. In the biochemical assay, SY-9453 potently inhibited the activity of MAT2A enzyme with an IC_{50} of 0.5 nM. In HCT116 $MTAP^{-/-}$ cells, SY-9453 showed very strong inhibition of the cellular SAM production and protein SDMA modification with IC_{50s} less than 10 nM. Moreover, SY-9453 also demonstrated strong inhibitory activity on the proliferation of multiple MTAP-deficient cancer cell lines. Functionally, SY-9453 reduced cellular mRNA splicing, induced cell cycle arrest, and promoted cell apoptosis in a dose-dependent manner. *In vivo* studies showed that SY-9453 has robust anti-tumor activity in several CDX models of colorectal cancer, pancreatic cancer, and non-small cell lung cancer. In the HCT116 $MTAP^{-/-}$ xenograft model, SY-9453 displayed dose-dependent inhibition of tumor growth, and tumor regression was observed after treatment with SY-9453 at 25 mpk, QD. Furthermore, SY-9453 revealed favorable PK properties and tolerability in preclinical studies. In conclusion, the current results demonstrate that SY-9453 is a novel small molecule MAT2A inhibitor with high activity *in vitro* and *in vivo*, providing a rational option for treating cancers with MTAP deficiency.

#6978 Discovery and characterization of BAY-805, a potent and selective DUB inhibitor of ubiquitin specific peptidase USP21.

Norbert Schmees[1], Fabian Goricke[2], Ulrike Scheib[1], Raphael Boehm[1], Namik Akkilic[1], Gerd Wohlfahrt[1], Kirstin Petersen[2], Victoria Vu[3], Leanna Smith[4], Niels Lindner[5], Joerg Weiske[1], Ulf Boemer[1], Krzysztof Brzezinka[1], Philip Lienau[2], Stefan Gradl[2], Hartmut Beck[5], Peter J. Brown[6], Vijayaratnam Santhakumar[3], Masoud Vedadi[7], Dalia Barsyte-Lovejoy[3], Cheryl Arrowsmith[8]

[1]Nuvisan, Berlin, Germany,[2]Bayer AG, Berlin, Germany,[3]University of Toronto, Toronto, ON, Canada,[4]Sickkids Hospital, Toronto, ON, Canada,[5]Bayer AG, Wuppertal, Germany,[6]Structural Consortium SGC, Chapel Hill, NC,[7]Ontario Institute for Cancer Research, Toronto, ON, Canada,[8]UHN Research, Toronto, ON, Canada

Deubiquitinases (DUBs) have recently become of high interest as some members of this protein class show potential for therapeutic interventions. The family of nearly 100 members is divided into 6 sub families. Their action is the cleavage of ubiquitin modifications from proteins, by which the function of the respective protein can change significantly. Due to this catalytic reaction all DUBs share, it is not clear so far how good specificity with potential small molecule inhibitors (SMOLs) can be achieved. Until today, only a handful of selective inhibitors were identified for a small number of protein deubiquitinases. Recent literature suggests that USP21 plays a prominent role in apoptosis, DNA repair, and signal transduction. It down-regulates TNFα-induced NF-κB activation through deubiquitinating of RIP1[15] and was reported to deubiquitinate RIG-I as well as STING to negatively regulate antiviral responses.[16-18] Recent studies indicate the relevance of USP21 in promoting tumor development and growth, including in non-small cell lung cancer[19], bladder carcinoma[20], gastric cancer[21], hepatocellular carcinoma[22], basal-like breast cancer[23], cervical cancer[24], esophageal cancer[25], colorectal cancer[26] and pancreatic cancer[27]. We selected USP21 as an interesting target for oncological treatment with a focus on its immune-oncological potential. A uHTS hit finding campaign on the Bayer 4 million compound library was initiated to identify potential selective inhibitors for USP21. Selectivity was tested against 3 other USP family members and specificity by further interference assays. Binding was cross validated by thermal shift assay (TSA). Surprisingly only a very limited number of compounds passed all criteria, and one cluster was selected for optimization. The poster will show the identification of structural classes, the focus on one series and its optimization starting from the initial 12 µM HTS hit to a single digit nano-Molar probe. The probe BAY-805 shows high selectivity in biochemical assays against a larger number of USP family members and activity in a cell-based NF kB activation assay. This probe gives an opportunity to validate USP21 pharmacology under relevant conditions in cancer.

#6979 Development of orally bioavailable multi-targeted inhibitors of Wnt signaling for the treatment of colorectal cancer.

S. Ginn[1], S. Rokey[1], A. Fistrovich[1], A. McMahon[1], T. Chavez[1], V. Tandon[2], J. Nogales[2], C. Cabel[1], C. Morales[1], S. Daptardar[1], C. Thorne[1], S. Banerjee[2], C. Hulme[1];
[1]University Of Arizona, Tucson, AZ, [2]University Of Dundee, Dundee, United Kingdom

Colorectal cancer (CRC) is a leading cause of cancer-related deaths in the United States, projected to account for 7.6% of all new U.S. cancer cases in 2024. Hyperactivation of the Wnt/β-catenin signaling pathway is a key driver of CRC, with up to 96% of cases showing oncogenic Wnt activation. Increased Wnt activation contributes to tumor stemness, progression, and pathogenesis. Targeted therapies have been developed, but drug resistance can often occur, limiting effectiveness. In this work, a novel series of multi-targeted kinase inhibitors that target downstream members of the Wnt signaling pathway (DYRKs, CLKs, CDK7/8/19), along with PI3Kα, BRAF, and PDGFR have been developed. Our lead compound, DYR895**,** demonstrates improved Wnt signaling inhibition over phase 1b clinical candidate Cirtuvivint (WNT reporter EC_{50}: 5.1 nM vs. 230 nM), while being well tolerated in mouse models (MTD >50 mg/kg) and orally bioavailable (F%: 81%). In an HCT-116 xenograft model, treatment with DYR895 resulted in reduced tumor growth and improved survival. Further optimization of DYR895 with the goal of reducing CNS exposure while enhancing Wnt inhibition led to the discovery of DYR1055, with a 10x improvement in affinity for some target kinases while maintaining key surrogate ADME attributes. Investigations into the efficacy of DYR1055 in disease models are ongoing.

#6980 Discovery of a subtype-selective TEAD inhibitor for cancer treatment.

Won An, YoungKu Kang, Seul Lee, Ye Gi Han, Jiyoon Seok, Sanghoon Kim, Ahreum Kwon, Eunji Ha, **Bora Yoo**, Ji-Duck Kim, Joon Seok Park

Daewoong Pharmaceutical Company Ltd., Yongin-si, Korea, Republic of

The transcriptional enhanced associated domain (TEAD) in the Hippo pathway signaling can regulate development and tumorigenesis. TEAD transcription factors have four subtypes (TEAD1-4) with highly conserved sequence whereas these subtypes show different expression levels depending on the organ. TEAD inhibitors are the emerging therapeutic agents for patients with NF2 mutant tumors. Most TEAD inhibitors block all subtypes of TEAD by targeting palmitoylation site and the interface 3 of YAP and TEAD. Here, we discovered TEAD selective inhibitors targeting the allosteric position of palmitoylation site. Our bicyclic compounds showed the potent in vitro activities on TEAD and cancer cell growth inhibitory effects. We also identified the compound-protein structure through X-ray crystallography and the effect of imidazole part on the TEAD selectivity. Pharmacokinetic properties of TEAD selective inhibitors were acceptable for oral administration. Furthermore, a TEAD selective inhibitor reduced the tumor growth of NCI-H226 xenograft mouse model. Overall, a TEAD selective inhibitor has the potential for developing targeted therapy for NF2 mutant tumors also each TEAD subtype-driven pathologies.

#6981 Discovery and optimization of purine-based CDK11 inhibitors for the treatment of Ewing's sarcoma.

Nada Elkholy, Pompom Ghosh, Hannah Walker-Mimms, Luxin Sun, Dylan Grassie, Ernst Schonbrunn, Derek Duckett, Andrii Monastyrskyi

Drug Discovery, Moffitt Cancer Center, Tampa, FL

RNA splicing, the process of intron removal from pre-mRNA, is a fundamental step in gene expression regulation mediated by the spliceosome. In Ewing's sarcoma, driven by the EWS-FLI1 fusion transcription factor, splicing is essential for generating in-frame fusion transcripts. Cyclin-dependent kinase 11 (CDK11) has been identified as a bona fide kinase responsible for phosphorylating SF3B1, a core component of the U2 snRNP. Both CDK11 and SF3B1 are crucial for EWS-FLI1 pre-mRNA maturation, with inhibition of either impairing splicing and disrupting downstream oncogenic target expression, revealing key therapeutic vulnerabilities in Ewing's sarcoma. These vulnerabilities offer novel therapeutic strategies to target EWS-FLI1-driven malignancy. We have employed structure-based drug design to develop a novel series of purine-based CDK11 inhibitors. Subsequently, machine learning-based quantitative structure activity relationship (QSAR) modeling was employed to optimize the dataset by identifying key structural features correlated with CDK11 activity, enabling a more focused refinement of the inhibitor series. Differential scanning fluorimetry confirmed preferential binding of one series of stereoisomers, indicating a potential for enhanced selectivity across the kinome. Furthermore, radiometric kinase assays further validated the inhibition of CDK11 functional activity. Biochemical and cell-based assays, including NanoBRET target engagement and CellTiter-Glo viability assays were performed to triage compounds. These efforts aim to identify potent and selective CDK11 inhibitors, ultimately advancing our understanding of CDK11's role in Ewing's sarcoma and its therapeutic potential.

#6982 First-in-class inhibitors of the proton-sensing receptor GPR68 for oncology.

Reid Hans Johnson Olsen[1], Michael Prime[2], Bellamy Duncan[2], Berizzi E. Alice[2], Alex Ceroni[2], Fonfria Elena[2], Major Gooyit[3], Mizuho Horioka[2], Katherine Lansu[2], Samuel R. Lawrence[2], Manisha Naik[2], Kostantinos Papachristos[2], Maninder Panesar[2], Martin Redhead[2], Simon Richards[2], David A. Smith-Parkin[2], Piotr Stepien[2], Jake Swadling[2], Diogo M. R. Tomaz[2], Bojan Vilagos[2], Walker S. Andrew[2]

[1]Recursion Pharmaceuticals, Boston, MA,[2]Recursion Pharmaceuticals, Oxford, United Kingdom,[3]Recursion Pharmaceuticals, Chicago, IL

Background: Aggressive tumors are often characterized by a fibrotic and acidic microenvironment. These features are associated with immune dysfunction, reduced survival, and impaired efficacy of chemo- and immunotherapies. One axis mediating these functions acts via GPR68, a proton-sensing receptor thought to be selectively enriched in cancer associated fibroblasts (CAFs). In mouse models, GPR68 deletion is associated with reduced tumor burden and increased overall survival, potentially by reversing immunosuppressive effects of the tumor environment. To date, a single compound purported to be a GPR68 antagonist (FT011) has entered clinical development for fibrosis. There has been limited evaluation of the efficacy of GPR68 antagonists as anti-tumor agents.

Methods: We assessed the efficacy of compounds developed using generative AI on GPR68 signaling pathways, explored GPR68-mediated effects on CAF biology, and evaluated the effects of GPR68 antagonism via CAFs on T-cell mediated cancer cell killing *in vitro.* We evaluated the *in vivo* efficacy of GPR68 antagonism on tumor growth in syngeneic models alone and in combination with immune checkpoint inhibitors.

Results: Expression of GPR68 in patient CAFs was confirmed by qPCR and signaling assays. *In vitro*, GPR68 stimulation induced changes in CAF-secreted factors, which were robustly reversed by GPR68 antagonism. GPR68 activating stimuli had no measured effect on a wildtype fibroblast cell line. GPR68 activation in CAFs led to significant changes in collagen production, which were blocked by GPR68 antagonism. Conditioned media from GPR68-activated CAFs impaired T-cell cytokine release and inhibited the ability of activated primary T-cells to kill PC-3 cancer cells. This effect was reversed by GPR68 antagonism. *In vivo*, GPR68 antagonism significantly reduced tumor growth in multiple aggressive syngeneic models. Tumor material from the 4T1 tumor-bearing animals treated with GPR68 antagonist revealed significantly increased tumor necrosis, suggesting more pronounced effects than those indicated by tumor volume alone. In support of GPR68 antagonism promoting an adaptive anti-tumor immune response, we observed enhanced efficacy when GPR68 antagonism was combined with different immune checkpoint inhibitors in a colon cancer model.

Conclusions: GPR68 acts via CAF signaling to suppress the adaptive immune response to cancer cells. GPR68 antagonism *in vivo* shows anti-tumor activity across multiple syngeneic models suggesting a broad range of potential indications. These effects are improved in combination with immune-checkpoint inhibitors supporting an immune-targeting axis between GPR68, acidic tumors, and the adaptive immune response. We are currently progressing our first-in-class lead molecules through preclinical development and validating potential *in vivo* biomarkers for future human clinical trials.

#6983 Innovative oral STING agonists for cancer therapy.

Zhiyong Qin, Limin Jia, Chengyu Yang, Chuanyun Yu, Yang Lv, Yujie Wang, Pan Li

Henan Genuine Biotech Limited Inc, Henan, China

STING, functioning as an adaptor protein, plays a pivotal role in the induction of type I interferons (IFN) and pro-inflammatory cytokines subsequent to the detection of cytosolic DNA. The development of a non-nucleotide STING agonist amenable to oral administration holds the promise of expanding the clinical utility of STING agonists among cancer patients. Leveraging computer-aided drug design strategies, a cohort of non-nucleotide STING agonists endowed with oral antitumor activities was meticulously designed and synthesized, with the overarching goal of exploring uncharted druggability domains. Among the array of investigated compounds, ZSSW21040164 manifested remarkable IFN-β (Interferon-β) activities within the tumor microenvironment. Rigorous experimental validation unequivocally demonstrated that ZSSW21040164 activates the downstream signaling cascade of STING via a STING-dependent modality. Moreover, ZSSW21040164 exhibited favorable pharmacokinetic (PK) characteristics, notably high oral bioavailability. In murine tumor models bearing H22, MC38, and CT-26 tumors, oral administration of ZSSW21040164 was well-tolerated and had a significant anti-tumor effect with more tumors achieved complete remission, which accompanied by the establishment of durable antitumor immunity. Notably, when ZSSW21040164 was co-administered with a fully murinized anti-mouse PD-1 antibody, the antitumor efficacy of single-agent therapy was substantially augmented. Collectively, these findings strongly suggest that ZSSW21040164 harbors considerable potential for development as an efficacious antitumor agent, warranting further preclinical and clinical investigations. Within this work, a series of aryl-fused heterocycles STING agonists with significant potency towards STING were investigated. The investigation comprised multiple aspects, namely, the identification methodology, pharmacokinetic (PK) properties determination, evaluation of in vitro and in vivo antitumor activities, and the elucidation of relevant structure-activity relationships (SARs). ZSSW21040164, an orally available human STING agonist, emerged as a crucial finding. It is anticipated that this compound will serve as a valuable reference for the discovery and design of human STING agonists suitable for systemic administration in a clinical setting, thereby contributing to the advancement of cancer therapeutics.

#6984 Discovery of selective SMARCA2 inhibitors for the treatments of SMARCA4 mutated cancers.

Seong Heon Kim, Hyuntae Kim, Sukho Lee, Minou Choi, Jong Woo Park, Hyosun Choi, Goeun Yang, Daehoon Kim, Doo-Young Kim, Sunhee Lee, Min Ah Park

Hyundai Pharm, Suwon, Korea, Republic of

BRM and BRG (SMARCA2/4) are two mutually exclusive catalytic ATPase subunits of the BAF complexes. SMARCA4 is frequently mutated in multiple cancers, including about 10% of non-small cell lung cancer (NSCLC). As a result, SMARCA4 deficient cancer cell becomes more dependent on its paralog, SMARCA2, for their survival. Consequently, a selective inhibitor targeting SMARCA2 is a potentially effective therapeutic option for SMARCA4-mutant cancers. We identified HD-10991 and HD-11030 as selective SMARCA2 inhibitors and evaluated their potency in SMARCA2 and SMARCA4 biochemical assays, as well as in Cell Titer-Glo assays using melanoma and NSCLC cell lines. HD-10991 and HD-11030 effectively inhibited SMARCA2 activity with single-digit nanomolar potency by binding to the ATPase domain. Both compounds demonstrated excellent selectivity, showing over 30-fold greater potency against BRM compared to BRG1 in chromatin remodeling assays. HD-10991 and HD-11030 possess desirable drug-like properties for oral administration, including favorable physicochemical characteristics and pharmacokinetic profiles. Systemic exposure through oral administration resulted in dose-dependent anti-tumor effects, consistently leading to tumor growth arrest and regression in SMARCA4-mutant xenograft models, such as SK-MEL-5 and A549 CDX models. Additional in vitro and in vivo studies in SMARCA4-mutant cell lines are ongoing to support the further development of these compounds. These findings, highlighting the anti-tumor efficacy and promising drug-like properties of HD-10991 and HD-11030, underscore their potential as candidates for preclinical studies aimed at therapeutic intervention for patients with SMARCA4-mutant cancers.

#6985 Discovery of potent and selective USP1 inhibitors for the treatments of BRCA-deficient tumors.

Seong Heon Kim, Hwa Sil Kim, Sukho Lee, Jieun Kim, Jinwoo Lee, Jong Woo Park, Yukyung Kim, Daehoon Kim, Doo-Young Kim, Min Ah Park

Hyundai Pharm, Suwon, Korea, Republic of

Poly (ADP-ribose) polymerase 1 (PARP1) inhibitors are the first successfully approved synthetic lethal cancer treatments targeting BRCA1/2-mutated breast and ovarian cancers. However, limited responsiveness among patients with BRCA1/2 mutations and the emergence of resistance to PARP inhibitors highlight the growing need for alternative synthetic lethal drugs, either as monotherapy or in combination with PARP inhibitors. Ubiquitin-Specific Protease 1 (USP1), a member of the deubiquitinating enzyme (DUB) family, plays a crucial role in DNA repair processes. USP1 inhibitors induce cancer cell death by blocking Translesion Synthesis (TLS) and the Fanconi anemia pathway, both of which have been identified as synthetic lethal partners with BRCA1/2 mutations or other homologous recombination deficiencies (HRD). We identified HD-12093 and HD-12136 as potent and selective USP1 inhibitors. Both compounds exhibited strong potency in the BRCA1-mutant triple-negative breast cancer (TNBC) cell line, MDA-MB-436, and demonstrated excellent selectivity for BRCA1-mutant cells over BRCA wild-type cells. HD-12093 and HD-12136 also induced the accumulation of mono-ubiquitinated PCNA, a known substrate of USP1. In PARP inhibitor-resistant cells, these compounds showed synergistic growth-inhibitory effects when combined with a PARP inhibitor, highlighting their strong potential to effectively overcome PARP inhibitor resistance. HD-12093 and HD-12136 exhibit favorable drug-like properties and pharmacokinetic profiles suitable for once-daily oral administration. These results support further development of HD-12093 and HD-12136 as potential best-in-class USP1 inhibitors for BRCA-deficient cancers resistant to PARP inhibitors either as monotherapy or in combination with PARP inhibitors.

#6986 Discovery of trihydroxyflavone from botanical sources targeting the epigenomic driver NSD3 in lung squamous cell carcinoma.

Betty Yangari, Kelsey Zou, Kala Mignone, Huisheng Xie, **Jianliang Zhang**

Chi University, Reddick, FL

Lung squamous cell carcinoma (LSCC) represents a highly aggressive subtype of non-small cell lung cancer, accounting for approximately 30% of all lung cancer cases. Despite its significant prevalence, effective targeted therapies for LSCC remain elusive, contributing to poor clinical outcomes. Recent molecular research has identified NSD3 as a critical oncogenic driver, with gene amplification observed in 60% of LSCC cases. NSD3 catalyzes the di-methylation of histone H3K36, leading to the epigenetic upregulation of key oncogenes such as MYC, CDK1, and CCNB1. Furthermore, the hyperactive NSD3-T1232A mutation has been implicated in accelerating tumor progression. This study aimed to develop and characterize novel small-molecule inhibitors targeting both wild-type NSD3 and the hyperactive NSD3-T1232A mutant in LSCC. Using AI-assisted molecular docking, kaempferol, an anticancer compound derived from medicinal plants, emerged as a promising lead. Comprehensive *in silico* drug design, cellular cytotoxic screening, surface plasmon resonance (SPR) assays, NSD3 chemiluminescent assays, and immunoblotting were employed to identify potent NSD3 inhibitors among kaempferol derivatives. Trihydroxyflavone (THF), one such derivative, demonstrated top DiffDock Confidence and SMANA Affinity scores, indicating a strong potential for NSD3 binding. SPR analysis confirmed the physical interaction between THF and NSD3, yielding favorable binding kinetics with a dissociation constant (KD) of 6.9 μM. Additionally, THF inhibited NSD3-catalyzed histone H3K36 methylation and disrupted critical oncogenic pathways involved in chromatin remodeling, cell cycle control, and mTOR signaling. Cell viability assays revealed that THF significantly reduced the proliferation of LSCC cell lines with NSD3 amplification or gain-of-function mutations, achieving an IC50 of 2.3 μM. In conclusion, our findings demonstrate the therapeutic potential of THF as a targeted inhibitor for NSD3-driven LSCC, laying the groundwork for future preclinical studies aimed at developing selective NSD3 inhibitors to improve outcomes for LSCC patients.

#6987 MYC condensate-modulating small molecules demonstrate robust antitumor activity in animal models of ovarian cancer and other solid tumors.

Ann Boija, Bede Portz, Yingda Ye, Jian-Guo Ren, Thomas Durand-Reville, Kip West, Isaac Klein

Dewpoint Therapeutics, Inc., Boston, MA

The transcription factor MYC is a critical driver of cancer, implicated in 70% of malignancies and amplified in approximately 30% of ovarian cancers, where it contributes to aggressive tumor progression. Despite its pivotal role in oncogenesis, MYC has eluded traditional structure-based drug discovery efforts due to the absence of well-defined drug binding sites and its dependence on highly dynamic interactions. Here, we are deploying condensate biology and phenotypic screening to identify and optimize small molecule condensate modulators (c-mods) to modify MYC oncogenic function. *In vitro*, MYC c-mods dissolve MYC nuclear condensates, selectively repress MYC-driven gene expression, and induce cell killing in a spectrum of ovarian cancer and esophageal cancer cell lines. Advanced MYC c-mods optimized using Dewpoint's AI/ML-powered end-to-end platform are orally bioavailable and yield tumor stasis and regression in *in vivo* models of ovarian cancer at tolerated daily doses. We demonstrate that the antitumor activity of the MYC c-mods extends to additional *in vivo* models from multiple indications and correlates with repressed MYC activity in tumors.

#6989 Advancing AOH1996: Enhanced anticancer activity of next-generation small molecule PCNA inhibitors.

Pouya Haratipour[1], Maryam Zangi[1], Mahshid Yaghoubi[1], Long Gu[2], Jennifer Jossart[2], John J. P. Perry[2], Linda H. Malkas[2], Robert J. Hickey[1]

[1]Cancer Biology and Molecular Medicine, Beckman Research Institute of The City of Hope, Duarte, CA,[2]Department of Molecular Diagnostics & Experimental Therapeutics, Beckman Research Institute of The City of Hope, Duarte, CA

Proliferating cell nuclear antigen (PCNA) plays a critical role in at least nine distinct cellular pathways, including DNA replication and repair, transcription, cell cycle control, apoptosis induction, transcription-replication conflict resolution, and immune surveillance. Tumor cells express an acidic isoform of this protein (caPCNA), whereas non-cancer cells express only a basic isoform (nmPCNA). The absence of the caPCNA isoform in non-cancer cells makes it an ideal target for selectively killing cancer cells while preserving the viability of non-cancer cells. To this end, we developed a small molecule, AOH1996, that selectively inhibits the caPCNA isoform in cancer cells, inducing cell death in these cells while showing no detectable effects on non-cancer cells. AOH1996 directly binds to caPCNA, stabilizes its trimer structure, reduces chromatin association, and selectively inhibits tumor cell growth. Currently, in several phase I and phase Ib clinical trials, AOH1996 exhibits highly selective anti-cancer properties with essentially no off-target toxicities. This study describes our efforts to improve the clinical characteristics, potency, and stability of AOH analogues through a scaffold-hopping strategy that employs structure-activity relationship (SAR) studies and bioisostere replacement. SAR analysis and modification of four unique scaffolds related to AOH1996 led to the discovery of several additional analogues exhibiting enhanced potency, selectivity, and metabolic stability compared to the clinical lead. Over one hundred AOH analogues were designed, synthesized, and initially evaluated for effects on cell growth across eight tumor cell lines. Our results identify top analogues featuring unsymmetrical 3-methoxy-5-methyl groups on the terminal phenoxy moiety, combined with fluorine, nitrile, or chlorine substitutions at the #6 position of 1-naphthoic acid. Among these, AOH-3M5Me-6F and AOH-3M5Me-6CN emerged as the lead analogues, demonstrating up to nine- and seven-fold improved potency and 38% and 68% higher liver microsome stability, respectively. These findings position these analogues for further development as selective PCNA-targeting cancer therapies.

#6990 Profiling Ras inhibitors for effects on GTP hydrolysis using the Transcreener GDP assay.

Camdon Yemm, **Robert Lowery**

BellBrook Labs, Madison, WI

Ras family GTPases are the most frequently mutated proteins in human cancers, most notably missense mutations at G12 and G13 that prolong the activated, GTP-bound state of the protein. Inhibitors that shift the equilibrium toward GDP-bound Ras, such as sotorasib, are proving effective clinically, and efforts to expand upon this strategy are widespread. Current strategies for biochemical screening and analysis of Ras inhibitors rely on the use of fluorescent GTP and GDP analogs to detect effects on GDP release and GTP binding. Though robust and widely used, these methods do not provide information on GTP hydrolysis, a critical part of the GTPase signal transduction process. We have developed methods for detecting GTP hydrolysis by small GTPases using the Transcreener GDP^2 assay, a homogenous, competitive immunoassay for GDP with far-red fluorescence readout. Because GDP release is generally rate limiting, stimulation by GEFs can also be measured by monitoring GDP formation. We have optimized these assays for initial velocity detection of GTPase activity for wild type (WT) and mutant Ras proteins with robustness suitable for high throughput screening and tested a number of compounds reported to inhibit GTP and/or GDP binding. Consistent with expectations, the Ras G12C-specific covalent inhibitor, Sotorasib, potently inhibited KRas G12C (IC_{50} = 9.6 nM) and did not inhibit the wild-type enzyme. MRTX1133, which binds noncovalently to GDP-bound KRas G12D, inhibited the G12D mutant with an IC_{50} of 8.4 nM, and was less potent with the G12V and WT enzymes; IC_{50} = 48 nM and 69 nM, respectively. BI-2865, a pan KRas inhibitor, showed much weaker inhibition of all KRas variants tested, with IC_{50} values of 427 nM, 228 nM and 236 nM for WT, G12D and G12V KRas. We note that these GTPase assays require nanomolar concentrations of protein, and therefore cannot be used to accurately determine the potency of covalent inhibitors, such as sotorasib, that bind with picomolar affinity. However, for non-covalent inhibitors, such as MRTX1113 and BI-2865, measuring GTPase activity is a robust approach for selectivity profiling and may yield mechanistic insights different from those gleaned from guanine nucleotide binding/release assays or biophysical methods, e.g. SPR.

#6991 Targeting NONO as a therapeutic strategy for metastatic castration-resistant prostate cancer (CRPC).

Brian McEllin, Yang Gao, Bodhi Hueffmeier, Andrea Gutierrez, Julia Robbins, Lillian Tatka, Tonibelle Gatbonton-Schwager, Carolyn Allen, Erin Broderick, Daniele Canzani, William Fondrie, Gaelle Mercenne, Lindsay Pino, Alex Federation

Talus Bioscience, Inc., Seattle, WA

Prostate cancer is among the most prevalent cancers among men, with approximately 300,000 new cases diagnosed annually. While androgen deprivation therapy (ADT) is effective in controlling early-stage prostate cancer, treatment options are considerably limited once the disease advances to metastatic castration-resistant prostate cancer (CRPC). This progression underscores the critical need for innovative therapeutic strategies to address this difficult-to-treat stage. Resistance to ADT arises when androgen receptor (AR) signaling becomes active even in the absence of ligands. This resistance is often driven by mutations in the ligand-binding domain (LBD) of AR or the expression of alternative AR isoforms that lack the LBD, with ARv7 being the most prominent. ARv7, plays a crucial role in the progression and treatment resistance of advanced prostate cancer, and is a high priority target for drug development.

A recent report identified an indirect mechanism for inhibiting the AR pathway through targeting NONO (non-POU domain-containing octamer-binding protein), an RNA binding protein which plays various roles in the nucleus, including transcriptional regulation and RNA splicing. Talus Bio has developed a groundbreaking technology, TF-Scan, the first cell-based global screening platform specifically designed to target transcription factors (TF). TF-Scan integrates functional proteomics, automated cell processing, and machine learning to evaluate a drug's ability to modulate the activity of DNA-bound proteins in live human cells. Using our platform, we identified several compounds that bind NONO at the residue C145, stabilizing the protein on the RNA, and blocking splicing.

Ongoing cross-functional studies aim to validate that NONO inhibition can effectively reduce AR and ARv7 splicing, suppress subsequent protein production, and disrupt the AR- and ARv7-dependent transcriptome. This disruption can potentially impede the growth and survival of prostate cancer cells that rely on these pathways. While these findings are promising, the application of NONO inhibition as a treatment for mCRPC is still under investigation. Further studies are necessary to confirm its efficacy and safety in clinical settings.

#6993 Development of synthetic lethal compounds targeting RB1-SUCLA2 deletion in advanced prostate cancer.

Susumu Kohno, Chiaki Takahashi

Kanazawa University, Kanazawa, Japan

The deletion of the tumor suppressor gene RB1 frequently occurs upon metastatic and/or castration-resistant conversion in prostate cancer. This extensive chromosomal loss associated with RB1 deletion also results in the loss of additional neighboring genes. Inhibition of gene products that compensate for these defective deletions can induce tumor-specific synthetic lethality. Our previous research demonstrated that SUCLA2 is collaterally deleted with RB1 often in advanced prostate cancer and that 5-Isopropyl-2-methyl-1,4-benzoquinone (TQ) selectively kills SUCLA2-deficient tumors. In this study, we explored the target of TQ and the mechanism of action. To determine the binding targets of TQ, we synthesized a biotinylated alkyl TQ enabling target identification via mass spectrometry analysis. Four mitochondrial enzymes were identified (W, X, Y, and Z). Enzymatic assays revealed that only enzyme X was inhibited by TQ in a dose-dependent manner. Enzyme X composes qn enzymatic complex consisting of six alpha and six beta subunits, which TQ dissociated. Notably, TQ irreversibly depleted 2-oxoglutarate and glycine in SUCLA2 knockout (KO) cells, while TQ did not affect these metabolites in SUCLA2-reconstituted cells. Adding cell-permeable 2-oxoglutarate prevented TQ-induced apoptosis in SUCLA2 KO cells, suggesting that TQ induces apoptosis by depleting tricarboxylic acid (TCA) cycle intermediates, likely through the inhibition of enzyme X. We are currently developing new TQ derivatives to improve IC50 and pharmacokinetics. This research opens avenues for novel therapeutic strategies targeting metabolic pathways through this distinctive mechanism.

#6994 Reversal of cancer gene expression for novel compound discovery in diffuse midline glioma.

Dmitry Leshchiner[1], Savneet Kaur[2], Eita Uchida[2], Kentaro Mineji[2], Yasuhide Makino[2], Katerina Petrosky[2], Bilal Aleiwi[3], Edmund Ellsworth[3], Rintaro Hashizume[2], Bin Chen[1]

[1]Michigan State University, Grand Rapids, MI,[2]University of Alabama at Birmingham, Birmingham, AL,[3]Michigan State University, East Lansing, MI

Background: Diffuse midline glioma (DMG) H3K27-altered is an aggressive incurable brainstem tumor that targets very young children. Median survival rate of patients is 9 months with 5-year survival rates of less than 1%. Complete resection is not possible due to the tumor location close to vital areas of the brain, and chemotherapy and radiotherapy are only symptomatic treatments used for palliative care. Through the reversal of cancer gene expression, we previously identified mycophenolate mofetil as a repurposed candidate for DMG; however, the clinical applicability of this candidate is limited due to its poor brain penetration. This study aims to identify new potent agents that can cross blood-brain barriers through the screening of a large compound library and lead optimization.

Method: For the screening we used our custom pipeline Gene expression Prediction from chemical Structure (GPS) that allows us to predict gene expression profiles of millions of compounds from chemical structures. With the predicted profiles, we scored compounds based on their reversal of disease gene expression. By screening the Enamine CNS library, we identified novel compounds with the ability to reverse the DMG gene signature we previously identified. Compounds were validated in DMG cell lines using MTS assays. Top hits were then selected for PK studies and maximum tolerated dose studies. To optimize our lead compounds, we ran MolSearch, a multi-objective optimization AI pipeline. We evaluated the final lead in a panel of patient-derived DMG cell lines as well as conducted PK and toxicity studies.

Results: We selected 30 compounds with the lowest reversal score for validation. Out of the 30 compounds selected, 19 had favorable ADME properties such as rule-of-5 compliance, solubility, potential toxicity, synthetic accessibility and predicted metabolic stability. Out of the 19 compounds, 7 showed reduction in cell viability in DMG cell lines. We chose the compound with the lowest IC50 as our lead. PK studies showed acceptable blood barrier penetration but significant clearance of the drug in plasma. To improve these properties, we ran our lead optimalization pipeline MolSearch to generate potential compounds that will retain reversal ability and ADME properties of the lead compound while improving metabolic stability. We are currently evaluating these new
analogs. Together, we have demonstrated that reversal of cancer gene expression could lead to the discovery of novel therapeutic in DMG.

#6995 Novel 4,6-disubstituted-7*H*-pyrrolo[2,3-*d*]pyrimidines as dual mEGFR/AURK inhibitors for non-small cell lung cancer.

Sonali Kurup[1], QiQi Hu[1], Kexin Zheng[1], Samantha Satawa[1], Felix Amissah[1], Erika Lisabeth[2]

[1]Ferris State University, Big Rapids, MI,[2]Michigan State University, East Lansing, MI

The epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) constitute the recommended therapy for non-small cell lung cancer (NSCLC) expressing mutant epidermal growth factor receptor (mEGFR). Resistance to first-generation EGFR-TKIs was addressed by osimertinib, an irreversible EGFR-TKI. However, newer mutations and redundant signaling pathways continue to drive resistance to osimertinib in mEGFR+ NSCLC. There are currently no viable EGFR-mediated treatments for osimertinib-resistant NSCLC. The aurora kinases (AURKA, AURKB) are mitotic kinases involved in centrosome maturation and separation during mitosis and can contribute to tumorigenesis. The AURK inhibitors have resensitized osimertinib-resistant mEGFR+ NSCLC cells when combined with osimertinib. Thus, dual mEGFR/AURK inhibition provides a unique approach for mEGFR+NSCLC. Kurup et al. previously investigated 4-substituted-7*H*-pyrrolo[2,3-*d*]pyrimidines as dual mEGFR/AURK inhibitors. Compounds **1-8** were developed as 4,6-disubstituted-7*H*-pyrrolo[2,3-*d*]pyrimidines to determine their effects as dual mEGFR/AURK inhibitors. The design, synthesis, and evaluation of compounds **1-8** will be presented and discussed. The anticancer effects of dual mEGFR and AURK inhibition in osimertinib-resistant mEGFR+ NSCLC cells will also be presented.

#6996 Integrating high-throughput screening with ligand-based pharmacophore modeling and virtual screening strategies to optimize exonuclease 1 inhibitor design.

J. Hess, L. Zheng, B. Shen;
Beckman Research Institute of The City of Hope, Duarte, CA

Pharmacophores are the specific steric and electronic features of a small molecule that are necessary to ensure interaction with a specific biological target and to trigger or block its biological response. Three-dimensional quantitative structure-activity relationship (3D-QSAR) pharmacophore modeling is a computational approach that utilizes data collected from *in vitro* experiments and molecular docking to map the shared features of known actives, producing a template that can be used to optimize the physical and chemical properties of lead compounds. Our goal is to generate a 3D-QSAR model for exonuclease 1 (EXO1) inhibition to support the pre-clinical development of a potent, EXO1-specific, small molecule inhibitor and define the molecular interactions that drive inhibitor specificity toward EXO1 instead of related nucleases. Construction of this model will facilitate the development of a potent EXO1 inhibitor that is selective, cancer-specific, and synthetic lethal with BRCAness. Additionally, it will establish a structural foundation for enhancing the selectivity of inhibitors targeting highly similar 5' nuclease family members, such as FEN1. Here, we describe our approach to integrating the high-throughput and virtual screening data we have collected for ~365,000 small molecule compounds to map a preliminary 3D-QSAR hypothesis for EXO1 inhibition.

#6997 Impact of KRAS^{G12C} oxidation state on non-small-cell lung cancer response to targeted therapeutics.

Yismeilin R. Feliz Mosquea[1], Maria Luiza Carneiro Buchele[1], Derek Parsonage[2], Jingyun Lee[1], Surbhi Mundra[3], Sharon L. Campbell[3], Leslie B. Poole[2], Cristina M. Furdui[1]

[1]Internal Medicine, Section on Molecular Medicine, Wake Forest University School of Medicine, Winston-Salem, NC,[2]Department of Biochemistry, Wake Forest University School of Medicine, Winston-Salem, NC,[3]Department of Biochemistry and Biophysics, University of North Carolina School of Medicine, Chapel Hill, NC

RAS proteins are critical signaling nodes that regulate cellular growth, with activating mutations in KRAS found in approximately 25% of human cancers. These mutations lead to chronic KRAS activation and sustained oncogenic signaling. Among >130 cancer-associated point mutations in KRAS, 99% cluster at hotspots (G12, G13, and Q61), with G12C mutations predominant in non-small cell lung cancer (NSCLC, ~80%). The G12C mutation introduces a reactive cysteine, enabling covalent drug discovery strategies. Therapeutics such as AMG510 (Sotorasib) target KRAS^{G12C} by locking it in an inactive state; however, resistance arises in ~60% of treated patients. To address this challenge, we aim to investigate how oxidation of KRAS and at Cys12 in KRAS^{G12C} influences its ability to bind covalent inhibitors, potentially offering insights into resistance mechanisms and novel therapeutic strategies. To explore the effects of oxidative modifications on KRAS^{G12C} function, we have started by engineering a recombinant KRAS^{G12C} protein that lacks other cysteines (denoted KRAS$^{G12C\text{-}CL}$) and introduced a Trp substitution (Y137W) to enable fluorescent kinetic analyses (KRAS$^{G12C\text{-}CLW}$; CL stands for cysteine-light). Recombinant protein samples were exposed to oxidants (H_2O_2) in the presence or absence of GDP, subsequently desalted using Bio-Spin Chromatography columns, and then analyzed by mass spectrometry to quantify the kinetics of oxidation to sulfinic acid state (-SO_2H) under these conditions. Further fluorescent kinetic assays of reduced and oxidized KRAS^{G12C} with AMG510 and other KRAS inhibitors showed complex profiles distinguishing inhibitor binding to the reduced and oxidized KRAS^{G12C}. Additionally, we conducted targeted analyses of endogenous KRAS^{G12C} oxidation in Calu-1 non-small cell lung cancer cells. Calu-1 cells treated with 1 mM H_2O_2 for 5 minutes demonstrated rapid oxidation of KRAS^{G12C}. Data will be presented showing the impact of KRAS^{G12C} oxidation on the therapeutic efficacy of clinical and experimental inhibitors. These approaches aim to better evaluate the impact of KRAS^{G12C} oxidation state on inhibitor engagement and therapeutic outcomes, addressing mechanisms of resistance and optimizing treatment strategies.

CHEMISTRY: Targeted Protein Degradation
Poster Session

#7002 Discovery of DXTX-1464: A first-in-class, highly potent and selective degrader of CCNE1/CCNE2 and CDK2 for cancer therapy.

Tim Dwight, Andrew Allen, Vivian Guo, Justin Radatti, Stephane Corbel, Stephane Billotte, Paul Greenspan, MIke Wood, Humphrey Gardner, **Bryce Kenneth Allen**

Differentiated Therapeutics Inc, Oceanside, CA

Introduction: Cyclin E1 (CCNE1) and E2 (CCNE2) when amplified are a hallmark of several aggressive cancers, including ovarian, gastric, and endometrial. These cyclins, when overexpressed, bind and activate CDK2, driving uncontrolled cell cycle progression and contributing to treatment resistance, particularly to CDK4/6 inhibitors in breast cancer. The persistent activation of CCNE1/CCNE2-CDK2 complexes is also linked to poor clinical outcomes in many solid tumors. In addition, kinase-independent functions of Cyclin E have been demonstrated to regulate cell cycle and affect genome stability. Selectively targeting the degradation of CCNE1, CCNE2, and CDK2 offers a promising strategy to dismantle this oncogenic axis, overcome therapeutic resistance, and improve patient outcomes.
Results: Prospective design informed by Auto/dx, a proprietary platform for predicting degrader pharmacology, elucidated multiple series of dual Cyclin E and CDK2 degraders designed to enable preferential stabilization of a ternary complex conformation enabling lysine-specific targeting of a ubiquitination zone between CDK2 and CCNE1/CCNE2, selectively targeting these proteins for degradation via the ubiquitin-proteasome system. These bivalent degraders incorporate a ligand that binds CDK2 and a cereblon-binding moiety, connected by an optimized linker that induces cooperativity and novel protein-protein interaction surfaces between CDK2 and cereblon. Functional studies demonstrated that these degraders effectively eliminated CCNE1, CCNE2, and CDK2 while sparing other cyclins and CDKs. Selective degradation of CCNE1/CCNE2-CDK2 complexes by development candidate DXTX-1464 resulted in potent inhibition of downstream signaling, including phosphorylation of Rb, a critical mediator of cell cycle progression and resulted in the upregulation of p21 and p27. This led to cell cycle arrest, the induction of apoptosis in some contexts, and significant antiproliferative effects in cancer models with CCNE1/CCNE2 dependencies. In vivo studies further validated these results, achieving robust depletion of CCNE1, CCNE2, and CDK2 in tumor models, leading to measurable tumor regression. 14 day DRF studies have indicated DXTX-1464 is well tolerated in rat and dog at doses exceeding the DC90.
Conclusions: Targeted degradation of CCNE1, CCNE2, and CDK2 represents a novel therapeutic approach for cancers characterized by Cyclin E dependencies or CDK2 hyperactivation. By eliminating CCNE1/CCNE2-CDK2 complexes, we propose this strategy will circumvent resistance to CDK4/6 inhibitors in HR+/HER2– breast cancers and will address oncogenic signaling in Cyclin E-amplified or overexpressed solid tumors. These findings support the further development of CCNE1/CCNE2 degraders and provide a rationale for their clinical application in patients with Cyclin E-driven malignancies.

#7003 NX-5948 is a CNS-penetrant catalytic Bruton's tyrosine kinase (BTK) degrader that breaks established design rules for CNS drugs.

Wylie Palmer, Mark Noviski, Paul Auger, Karthik Arumugam, Danielle Bautista, Nivetha Brathaban, Robert Cass, Ming Liang Chan, Ganesh Cherala, Philip Keyes, Aishwarya Kumar, Saloni Malla, Mitchell Lavarias, Filippo Marchioni, Jenny McKinnell, Jun Ma, Ryan Rountree, May Tan, Alexandra Trotier, Sarah Whelan, Davorka Messmer, Frederick Cohen, Gwenn M. Hansen

Nurix Therapeutics, Inc., San Francisco, CA

NX-5948 is a targeted protein degrader of Bruton's Tyrosine Kinase (BTK) in Phase 1 clinical development for the treatment of B cell malignancies. Heterobifunctional degraders, such as NX-5948, occupy beyond Rule-of-Five chemical space where established guidelines for physicochemical properties associated with drug-likeness cannot easily be applied. In contrast to approved central nervous system (CNS) drugs, NX-5948 exceeds several recognized chemical property metrics, such as molecular weight, polar surface area, and hydrogen-bond donor count. In addition, NX-5948 does not conform with in vitro permeability and transporter efflux ratio guidelines that would predict for CNS penetration. Despite these unfavorable properties, NX-5948 shows CNS exposure in preclinical models. As a bifunctional degrader with an event-driven mode of action, NX-5948 induces potent degradation of BTK in primary human B cells and malignant B cells. By performing a series of *in vitro* experiments measuring cellular partitioning and degradation kinetics, we calculated the catalytic efficiency of NX-5948 and demonstrate that one degrader molecule can promote degradation of thousands of copies of target protein. This enables a very low concentration of free drug to sustain pharmacodynamic activity and efficacy *in vivo*. NX-5948 shows dose-dependent brain exposure in rodents with an unbound brain-to-plasma partition coefficient, or Kp,uu, value consistent with CNS penetration. Additionally, NX-5948 degrades BTK in microglia in naive mice and rats with intact blood-brain barriers. NX-5948 also degrades BTK in intracranially-implanted tumor cells and extends survival in an intracranial TMD8 mouse model of CNS lymphoma. In the clinic, NX-5948 is detectable in cerebrospinal fluid of patients with CNS-involved B-cell malignancies, with concentrations that exceed the minimum free plasma level that correlates with BTK degradation. NX-5948 has also demonstrated clinically meaningful responses in patients with primary CNS lymphoma or chronic lymphocytic leukemia with CNS involvement (Hansen, G., AACR San Diego, 2024; Linton, K., EHA Hybrid Congress, Madrid, Spain, 2024), supporting the therapeutic potential of NX-5948 in B-cell malignancies with CNS involvement.

#7004 CT7439 an oral first-in class selective CDK12 and 13 inhibitor and cyclin K degrader: Profiling activity and combination efficacy in an ovarian cancer model.

A. K. Bahl[1], M. Carr[2], S. Thompson[3], A. Campbell[4], A. Leishman[5], E. Ainscow[5];
[1]Bahl (Individual), Dublin, Ireland, [2]Sygnature, Nottingham, United Kingdom, [3]Sygnature Discovery, Nottingham, United Kingdom, [4]Sygnature Disciovery, Nottingham, United Kingdom, [5]Carrick Therapeutics, Dublin, Ireland

CT7439 is a small molecule kinase inhibitor of cyclin-dependent kinases CDK12/CDK13 that received FDA IND approval in 2023. In addition to inhibiting CDK12/13, CT7439 acts as a monovalent glue degrader of cyclin K (CCNK), the cyclin required for CDK12/13 function. Proteomics quantification by DIA LCS/LCSMS mass spectrometry was conducted after exposure to 30 nM CT7439 at 4 & 24 hr. OV-90 cells were cultured in media and exposed to CT7439 at various concentrations and time points. Combination studies were conducted with the PARP inhibitors and Carboplatin. The following parameters were assessed: proliferation/cell survival, confluency formation, CCNK protein levels and DNA damage and repair (DDR) genes. CT7439 inhibited OV-90 cell growth with an IC50 of 4.4nM and this impact on cell growth was significantly attenuated by co-treatment of the cells with proteasome inhibitor. Proteomics analysis revealed that 99 proteins were found to be differentially abundant between samples from CT7439 (30 nM) treated and vehicle at 4 hrs. At 24 hrs 1,666 proteins were found to be differentially abundant. CCNK was the only cyclin impacted by CT7439. Pathway and ontology analysis showed reduced abundance for proteins involved in DDR. DDR genes (BRCA1, ERCC4, RAD51C and POLQ) are inhibited by treatment with CT7439, at concentrations consistent with inhibition of CDK12/13-Cyclin K in the cells. CT7439 activity in OV-90 in cellular assays:

OV-90	Growth	CCNK**	BRAC1*	ERCC4*	RAD51C*	POLQ*	MCL-1*
IC_{50} nM (SD)	4.4	0.66 (0.1)	1.7 (0.4)	0.82(0.3)	1.2 (0.7)	1.9(0.2)	>20

Data is mean ± SD (**Western blot, *qPCR) In addition to single agent activity of CT7439 a strong combination activity between CT7439 and the PARP inhibitors Olaparib, Niraparib and AZD10530 as well as the carboplatin was observed in an OV-90 cellular confluency assay. CT7439 is an orally bioavailable selective CDK12/13 inhibitor and degrader of CCNK. Pre-clinical studies demonstrates clinical potential for monotherapy use in solid tumors and as a combination therapy with agents known to cause a DNA damage response.

#7005 Discovery and characterization of DAC-1522, a novel Trop2-targeting degrader-antibody conjugate for precision oncology.

Chuanjie Chen[1], Qianqian Shen[1], Xinhui Cai[1], Yaqi Ding[1], Xiaoyu Yang[1], Yanfen Fang[1], **Jiangnan Luo**[2], Jian Ding[1], Yi Chen[1], Xuan Zhang[1]

[1]Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China,[2]DaCure Therapeutics, Shanghai, China

Antibody-drug conjugates (ADCs) have revolutionized cancer therapies by enabling the targeted delivery of cytotoxic payloads to tumor cells. However, their efficacy and safety heavily rely on the toxins as their payloads, and the rare availability of toxin types often limits their efficacy and broader expansion of indications. Combining the precision targeting feature of ADCs and diverse options of protein degraders, degrader-antibody conjugates (DACs) have emerged as a next-generation therapeutic modality and offer a promising strategy to overcome these challenges. The human trophoblast cell surface antigen 2 (Trop-2) is a transmembrane glycoprotein widely expressed in various types of tumors. It has been clinically proven as a well-suited antigen for ADCs with its widespread overexpression in cancer cells and much lower expression in normal cells. Despite the clinical success of Trop2-targeting ADCs like IMMU-132 (sacituzumab govitecan-hziy), their dependence on topoisomerase inhibitors as payloads has exposed significant limitations, particularly in addressing both intrinsic and acquired drug resistance. This has created an urgent need for innovative therapeutic solutions. Here we introduce DAC-1522, a novel Trop2-directed DAC, that can potentially overcome the limitations of ADCs. *In vitro* studies have shown that DAC-1522 efficiently degrades the target protein in a dose-dependent manner and exhibits potent antiproliferative activity against multiple human cancer cell lines. Its efficacy is strongly correlated with the level of Trop2, ensuring selective cytotoxicity in Trop2-positive cells and sparing Trop2-negative cells, resulting in a favorable therapeutic window. Notably, DAC-1522 retains uncompromised antiproliferative activity in an ADC-induced acquired drug resistance model, underscoring its potential to overcome the drug resistance after ADC treatments. *In vivo*, DAC-1522 achieves complete tumor regression with a single intravenous injection in mice (human pancreatic cancer BXPC-3 xenograft model), significantly outperforming IMMU-132 in terms of efficacy. No obvious safety concerns were observed. These findings support further preclinical and clinical development of DAC-1522 as a highly promising therapeutic candidate for the Trop2-expressing cancers.

#7006 TY-2719, a potent and selective broad-spectrum mutation-effective EGFR-PROTAC molecule, attenuates acquired resistance to 3rd-generation EGFR TKIs.

Apeng Liang*,#[1], Shengli Dong*,#[1], Shaoqing Chen*[1], Shunxun Han[1], Hongqiang Li[1], Guangbin Liu[1], Xi Wang[1], Zhengfei Guo[1], Yanyan Liu[1], Ling Fang[1], Yi Long[1], Xiang Zhang[1], Wei Wu[1], Chengshan Niu[2], Mingyu Jiang[3], Jun Li[3], Yusheng Wu[3]

[1]TYK Medicines, Inc., Changxing county, Huzhou City, China,[2]TYK Medicines, Inc. (Zhengzhou), Zhengzhou, Henan, China,[3]TYK Medicines, Inc. (Shanghai), Xuhui District, Shanghai, China

Introduction: The epidermal growth factor receptor (EGFR) is a well-known oncogenic driver of non-small cell lung cancer (NSCLC). Approximately 15~50% of NSCLC patients carrying EGFR mutations is reported. Despite the substantial therapeutic efficacy of EGFR tyrosine kinase inhibitors (TKIs), acquired EGFR-TKI resistance presents a significant challenge in the clinical management of NSCLCs. Proteolysis-targeting chimera (PROTAC) represents a novel technology with the capacity to degrade oncogenic proteins, and exhibits significant potential for overcoming drug resistance. Recently, several EGFR PROTAC antitumor drugs have successfully progressed to clinical trials with the aim of addressing drug-resistant EGFR mutations in NSCLC. These included CFT8919 (NCT06641609), HSK40118 (NCT06050980), and HJ-002-03 (CTR20241691). TY-2719 is an oral, highly selective, broad-spectrum EGFR-PROTAC designed by TYK Medicines Inc., to overcome the resistance of 3rd -generation EGFR TKIs.

Results: TY-2719 did not suppress the growth of normal cells, such as NHEK, and NSCLC cells, such as A549 and A431 cells harboring wild-type EGFR. However, TY-2719 has excellent in vitro antiproliferative activity against a number of EGFR mutants, including common mutations, post osimertinib-resistant mutations, and rare mutations. Our data suggest that TY-2719 has superior selective activity in cells containing EGFR mutants compared to wild-type EGFR-expressing cells. It efficiently degraded EGFR carrying L858R/T790M/C797S and 19del/C797S mutations in BaF3 cells with single-digit nanomolar range activity, as evidenced by EGFR-HTRF and western blot assays. Toxicity is an important factor limiting the efficacy of protac therapy, and many toxic effects are caused by ImiD-mediated degradation of CRBN neosubstrates. For example, GSPT1 plays an essential role in the majority of cells; however, GSPT1 may be accidently degraded by PROTACs recruited CRBN. Using a western blot assay, we found that TY-2719 did not degrade IKZF1/2/4, CK1α, and GSPT1. The IC50 values of TY-2719 were greater than 10 μM in the inhibition assays for all eight important CYP450 isoforms involved in drug metabolism. The DMPK characteristics of TY-2719 were excellent in the rat experiments. TY-2719 showed significant antitumor activity and tolerability in xenograft mouse models.

Taken together, we have identified a potent and highly selective, broad-spectrum mutation-effective new EGFR-PROTAC molecule which may grant new therapeutic opportunities to overcome drug resistance of 3rd -generation EGFR TKIs in NSCLC patients.

#Shengli Dong and Apeng Liang contributed equally to this work.

*To Whom Correspondence should be addressed to: Shaoqing Chen, Ph.D; Shengli Dong, Ph.D.; Apeng Liang, Ph.D.; TYK Medicines Inc.

#7007 Identifying novel non-allosteric PIM1 degraders to overcome treatment resistance in triple negative breast cancer.

Sanjay V. Malhotra[1], Benedikt W. Grau[1], Dhanir Tailor[1], Abel Bermudez[2], Fernando Jose Garcia Marques[2], Juliana Geppert[1], Sharon Pitteri[2]

[1]Cell, Development and Cancer Biology, Center for Experimental Therapeutics, Knight Cancer Institute, Oregon Health & Science University, Portland, OR, [2]Department of Radiology, Canary Center at Stanford for Cancer Early Detection, Stanford University School of Medicine, Palo Alto, CA

Background: Breast cancer (BC) is the most common cancer among women, with triple-negative breast cancers (TNBC) being the most aggressive type. Resistance to current mainline therapies is one of the major reasons for increased mortality. PIM1 oncoprotein has been implicated in causing treatment resistance, and therefore, associated with the development and progression of this disease. Several attempts have been made to develop inhibitors of PIM1. However, these inhibitors are not specific and can lead to toxicity by inhibiting the activity of other kinases. Therefore, there is an unmet need to develop a non-orthosteric inhibitor or non-allosteric degrader, which would be more specific and could help in effective treatment of TNBC.
Methods: We synthesized a novel class of PIM1 degraders and confirmed their non-allostatic mode of action experimentally *in vitro*, *in silico* and *in vivo*.
Results: Our lab has designed indole-based compounds and identified initial hits on their PIM1 inhibition properties in a luciferase reporter assay. Further optimization improved the PIM1 inhibition up to 80-90%. However, the *in silico* analysis suggested that the compounds do not interact with the orthosteric center of the protein. This was confirmed by a kinase assay, which revealed that the new compounds do not interfere with PIM1 kinase activity. On the other hand, Bio-layer interferometry (BLI) studies confirmed the physical interaction of our compounds with the PIM1 protein, concluding non-allosteric interaction. The top three compounds were tested further *in vitro*. Treatment with our top candidate(s) inhibits cell proliferation and arrests the cells in G1 phase. This was followed by apoptotic cell death. These findings suggest that our newly designed compounds can indeed interact with PIM1 and hinder its protein function while leaving its kinase activity unaffected. Furthermore, *in vivo* study in the EMT6 orthotopic mice model of the top three compounds suggest that treatment inhibits tumor progression and lung metastasis.
Conclusion: Preliminary results merit the optimization of 'Hit-to-Lead' and development of a suitable candidate to treat TNBC, where PIM1 is the cause of treatment resistance. Successful completion of the proposed work will enable a new combination therapy to reverse chemoresistance.

#7008 Development of a potent SOS1 degrader exhibiting superior combination effects with anti-cancer drugs in various tumor models.

Jiyoung Kim, Ji Youn Park, Yeajin Woo, Soohyun Lee, Beomseon Suh, Han Wool Kim, So Hyuk Kim, Yongdae Kwon, Heejung Moon, Yeeun Kim, Jungmin Ahn, **Jihye Lee**, Je Ho Ryu, Song Hee Lee

Ubix Therapeutics, Seoul, Korea, Republic of

Background: Son of Sevenless 1 (SOS1) is a crucial guanine exchange factor (GEF) for KRAS, facilitating the activation of RAS family proteins by promoting the exchange of GDP for GTP at its catalytic site. It also binds GTP-bound KRAS at an allosteric site, enhancing its GEF activity through positive feedback. Loss or genetic inactivation of SOS1's GEF function reduces the survival of tumor cells with KRAS mutations. Additionally, SOS1 is subject to MAPK-mediated negative feedback inhibition and drugs targeting upstream and downstream nodes relieves SOS1 from this inhibition resulting in pathway reactivation.
Results: UBX-144 demonstrated superior degradation activity, achieving sub-nanomolar DC50 levels in the NCI-H358 NSCLC (non-small cell lung cancer) cell line and in transiently transfected SOS1 mutant cells, where it effectively degraded SOS1. A time-course degradation assay demonstrated half-maximal degradation of SOS1 within 2 hours of treatment. A mechanism of action (MOA) study revealed that UBX-144 degrades SOS1 via the ubiquitination-proteasome system, mediated by CRBN E3 ligase. Both UBX-144 and UBX-164 inhibited cell proliferation across various cancer types, including gastric, colon, breast, lung, brain cancers, and chronic myeloid leukemia (CML). A combination synergy study showed that UBX-204 synergized with various inhibitors in NCI-H358 and MIA-PaCa-2 cell lines. In vivo pharmacodynamic (PD) data showed that UBX-144 effectively degraded SOS1 protein in tumors in a U87-MG xenograft model. Experiments using the glioblastoma cell line derived xenograft model demonstrated a compelling in vivo profile, with 10 mg/kg administration of UBX-144 resulting in significant tumor growth inhibition (TGI: 101%) and maximal degradation values exceeding 95% in tumors.
Conclusion: We have successfully discovered novel SOS1 degraders exhibiting remarkable anti-tumor activities. In particular, the superior anti-cancer effects of combination treatments with other drugs were confirmed across various cancer cell lines, highlighting its potential as an excellent combination partner. We demonstrate that SOS1 degraders provide a promising therapeutic option for RTK/RAS/MAPK-dependent cancer patients.

#7009 Discovery of UBX-306 as a potent, selective, and orally bioavailable HPK1 degrader.

Ae-Ran Choi[1], Na-rae Jeong[1], Sun-Mi Yoo[1], Han Wool Kim[1], So Hyuk Kim[1], Hyun-Hwi Kim[1], Sun-Kyoung Im[2], Miyoung Jang[1], Hohyun Lee[1], Jungmin Ahn[1], Je Ho Ryu[1], **Song Hee Lee**[1]

[1]Ubix Therapeutics Co., Ltd., Seoul, Korea, Republic of,[2]NeoImmuneTech, Inc., Rockville, MD

Background: Hematopoietic progenitor kinase 1 (HPK1), part of the MAP4K family, is a serine/threonine kinase critical for modulating immune cell signaling and function. HPK1 is predominantly expressed in hematopoietic cells, where it acts as a negative regulator of T-cell receptor (TCR) signaling and other immune pathways. Upon activation, HPK1 dampens immune responses by phosphorylating key adapter proteins, such as SLP-76, and promoting their degradation, ultimately inhibiting T-cell activation, proliferation, and cytokine production. Given the critical role of T-cells in anti-tumor immunity, HPK1 has emerged as an attractive target in immuno-oncology for enhancing immune responses.
Results: UBX-306;NT2020 demonstrated robust HPK1 protein degradation, achieving single-digit nanomolar DC_{50} levels in the Jurkat cell line in a dose-dependent manner. The degradation effect of UBX-306-1 is strongly dependent on the ubiquitin-proteasome system (UPS). Both UBX-306-1 and UBX-306-3 significantly enhanced IL-2 secretion in Jurkat cells, demonstrating superior EC_{50} values compared to the warhead of UBX-306, AZ1, or reference HPK1 degrader. Treatment with UBX-306-1 increased IL-2 production in a concentration-dependent manner, even in the presence of immunosuppressive molecules such as PGE2 and NECA. UBX-306-2 effectively degrades HPK1 protein in Ramos, Raw264.7, THP-1, and KG-1 cell lines. In vivo pharmacodynamic (PD) data demonstrated that UBX-306-1 efficiently degraded the HPK1 protein in the mouse blood. UBX-306-1 induced peak degradation of HPK1 at 6 hours after oral administration, with the degradation activity sustained for up to 24 hours. The antitumor efficacy of UBX-306-1 was evaluated in the CT-26 (Colorectal cancer) syngeneic tumor mouse model. Daily oral administration of UBX-306-1 at doses of 10 mg/kg and 30 mg/kg resulted in robust tumor growth inhibition.
Conclusion: We demonstrate that potent HPK1 degraders, effectively activate immune cells and inhibit tumor growth in mouse syngeneic tumor models as single agents. These degraders enhance IL-2 and IFNγ production while overcoming immunosuppressive factors. Further, in vivo studies will deepen our understanding of HPK1 degradation as a promising immunomodulatory strategy for enhancing anti-tumor immunity.

#7010 HDB-82: A promising PROTAC degrader for KRAS G12D mutant cancers.

Mohammad Hassan Baig[1], Yun Seong Jo[1], Sagar Dattatraya Nale[2], Chang Joong Kim[2], Tae Hwan Park[2], Ju Han Bok[3], Dong Min Kim[3], Hye Mi Kim[3], Ji Min Park[3], Jae June Dong[1], Jae Hee Cho[1], Sung Ill Jang[1], Byoung Gon Moon[3]

[1]Yonsei University College of Medicine, Seoul, Korea, Republic of,[2]BNJ Biopharma Inc., Incheon, Korea, Republic of,[3]Handok Inc., Seoul, Korea, Republic of

KRAS G12D mutations, occurring in approximately 30% of solid tumors, represent a significant therapeutic target. While selective KRAS G12C inhibitors have achieved clinical success, effectively targeting the more prevalent G12D mutation remains an unmet medical need. This study investigates the potential of proteolysis-targeting chimeras (PROTACs) as a promising avenue for KRAS G12D-driven cancers, potentially offering advantages over traditional inhibitors through catalytic protein degradation. Targeted protein degradation of mutant KRAS may yield greater efficacy than simply inhibiting the protein. This study focuses on the design, synthesis, and biological evaluation of novel KRAS G12D-targeting PROTAC degraders. Through rigorous optimization, we developed HDB-82, a highly potent and selective PROTAC compound targeting KRAS G12D. HDB-82 effectively induced degradation of the KRAS G12D protein, leading to significant antiproliferative activity against KRAS G12D-mutant cancer cells *in vitro* and *in vivo*. HDB-82 demonstrated potent anti-cancer activity against various KRAS G12D-mutant cell lines, achieving low nanomolar IC_{50} values. It effectively degraded the KRAS G12D protein in various KRAS G12D-expressing cell lines (DC_{50} value ranges from picomolar to sub-nanomolar range). Mechanistic studies revealed significant suppression of downstream MAPK and PI3K/AKT signaling pathways, accompanied by robust induction of apoptosis, and superior antiproliferative activity compared to existing inhibitors. Furthermore, HDB-82 exhibited favorable pharmacokinetic and pharmacodynamic properties in mice. Intravenous administration of HDB-82 (10 mg/kg, q.w.) resulted in substantial tumor growth inhibition across multiple KRAS G12D-positive tumor models. These findings establish HDB-82 as a promising therapeutic candidate for KRAS G12D-mutant cancers, warranting further preclinical investigation and potential clinical development.

#7011 Humanized CRBN knockin mice enable in vivo assessment the efficacy and toxicity of CRBN-targeted protein degraders.

Yi Li, Xiaolei Qiu, Liping Feng, Dongxiao Feng, Ruilin Sun

GenoBioTX, Sugar Land, TX

Protein degraders have emerged as a transformative therapeutic approach in the past decade, enabling targeted degradation of proteins, including traditionally undruggable targets, by facilitating the recruitment of E3 ubiquitin ligases. Among these, molecular glues such as thalidomide and its derivatives, and PROteolysis TArgeting Chimeras (PROTACs), have gained significant attention. Cereblon (CRBN), a key component of the Cullin 4 RING E3 ubiquitin ligase (CRL4), is the most extensively studied target in protein degrader research. CRBN interacts with DDB1, Cullin 4 (CUL4A/B), and RBX1 to form the CRL4^CRBN complex, which mediates substrate recognition and degradation. Thalidomide derivatives bind to CRBN, modulating substrate specificity and promoting substrate degradation.

However, differences at the V388 and E377 residues between human and mouse CRBN proteins (94% homology) impede the antitumor efficacy and teratogenicity of thalidomide in mice, limiting suitable preclinical models. To address this, we developed a humanized CRBN knockin mouse model by inserting the human CRBN coding region (exon 2-11) and a WPRE/polyA cassette into the mouse Crbn locus using ES cell-based gene targeting. This model expresses full-length human CRBN in targeted cells.

We validated human CRBN (hCRBN) expression across multiple tissues, including spleen, liver, brain, and intestines, via RT-PCR and western blot. Lenalidomide and CC-885 treatments of hCRBN splenocytes demonstrated the degradation of substrate proteins CK1α and GSPT1. Pharmacokinetic analysis revealed no significant differences between hCRBN knockin and wild-type C57BL/6 mice. In vivo CC-885 treatment induced on-target toxicity, as evidenced by rapid lethality, GSPT1 degradation, and increased pathological scores in hCRBN knockin mice.

Our humanized CRBN mouse model serves as a robust preclinical platform for evaluating the efficacy and toxicity of CRBN-targeted protein degraders, offering valuable insights for drug development.

#7013 RGT-61159, best-in-class oral small molecule inhibitor of MYB via selective RNASplicing alteration, synergistic anti-tumor aActivity when combined with standards of care in leukemia disease models harboring AML common genetic lesions and with NOTCH inhibitors in ACC disease models.

Norman Lu, **Patricia Soulard**, Xiubin Gu, Chris Yates, Kai Li, Sam Hasson, Ibrahim Kay, Zhiping Weng, Travis Wager, Simon Xi

Rgenta Therapeutics, Inc., Woburn, MA

The oncogenic transcription factor MYB is a master regulator of self-renewal, differentiation and proliferation of hematopoietic cells. MYB is frequently aberrantly expressed in hematologic malignancies and has been revealed to be a central component of oncogenic complexes maintaining aberrant gene expression programs in AML. RGT-61159 is a potent, selective oral small molecule inhibitor of the oncogene MYB via the inclusions of a cryptic exon into MYB RNA transcripts, resulting in the activation of the nonsense-mediated decay pathway promoting MYB mRNA depletion and sequentially MYB protein degradation. The RGT-61159 Phase 1 study in adults with relapsed/refractory ACC or CRC has been initiated (NCT06462183).
Here, we report genomic analyses of different AML cell lines treated with RGT-61159 that provided further insight into key oncogenes (e.g., BCl2, NOTCH, MYC) directly regulated by MYB. To further evaluate RGT-61159's potential to treat leukemia, RGT-61159 single agent was profiled in a battery of murine models of AML harboring the most common genetic lesions (e.g., Flt3 ITD, MLL-fusion and NPM1 mutation). RGT-61159 showed significant anti-tumor activity across the different AML tumor models tested along with robust MYB RNA depletion in tumor cells at tolerated doses.
In addition, the benefit of combining RGT-61159 with a panel of standard-of-care agents for AML treatment was explored in vitro across a panel of AML cell lines harboring different genetic alterations. The data revealed synergistic cell killing activity when RGT-61159 was combined with Flt3 inhibitors (e.g. gilteritinib and midostaurin) in MOLM-13 and MV4.11 cell lines harboring Flt3 IDT mutation, and with menin inhibitors (e.g. KO-539, revemenib) in AML cell lines with NPM1 mutant) or MLL-fusion. Finally, RGT-61159 combination with a Bcl2 inhibitor (venetoclax) resulted in synergistic cell killing activity in AML cell lines carrying NPM1 mutations or AML-1-ETO fusion protein. These studies provide compelling supportive evidence for combining RGT-61159 with standards-of-care for AML treatment in the relevant population of patients with AML. .

#7014 Characterization and validation of PROTACs that recruit KLHL12.

Zoe A. Petros, John Lee Sensintaffar, Anish Kizhakkekkara Vadukoot, Kwangho Kim, Manikandan Thangaraj, Alex G. Waterson, Stephen W. Fesik

Vanderbilt University, Nashville, TN

Proteolysis targeting chimeras (PROTACs) have emerged as a promising strategy for cancer therapy in the field of drug discovery. PROTACs are heterobifunctional small molecules that bind to both a target protein and an E3 ubiquitin ligase, thus facilitating degradation of the target protein of interest. Benefits of PROTACs as an alternative therapeutic strategy include the ability to target inaccessible proteins, the regulation of target protein signaling pathways, and overcoming drug resistance in certain types of cancer. There are more than 600 known E3 ligases in the human genome, but current PROTAC technology has been limited to the use of CRBN, VHL, XIAP, and MDM2. Therefore, the discovery of novel ligands for different E3 ligases could further improve current PROTAC technology. An application that our research group has focused on is exploring the possibility of tissue selective E3 ligases that are overexpressed in cancer cells compared to normal tissue to design tissue selective PROTACs for a target of interest. We have observed that protein levels of the E3 ligase KLHL12 are low in normal tissue and high in cancer cells. Using structure-activity relationship (SAR) by nuclear magnetic resonance (NMR), we identified hits that bound to the KLHL12 E3 ligase, which were then optimized to bind in the range of 10-100 nM K_d by SPR. We then generated KLHL12 based PROTACS that target BRD4 and Bcl-xL using thalidomide and A-115463 derivatives, respectively. PROTAC activity was optimized by measuring protein target degradation in HT29 cells by an in-cell western experiment where our most potent degrader had an average DC_{50} of 5.05 nM and achieved 89.65% degradation. Mechanism studies were carried out to verify that the PROTACs behaved in both a proteasome and target dependent manner. Future studies will support further characterization of KLHL12 by investigating its expression patterns across various cancer tissues, its functional essentiality in cells, and its involvement in protein-protein interactions.

#7015 Intelligent molecular glue discovery by identifying glueable ligase-target pairs at scale.

Phil Burke, Arpita Sen, David Noble, Abhishek Dogra, Ruchi Bansal, Dasha Krayushkina, Natasha Seelam Murakowska, Kerry McGowan, Charles Lin, Yeweon Lee, Randolph Lopez

A-Alpha Bio, Seattle, WA

Targeted protein degradation can be induced by monovalent small molecules, called molecular glues. These molecules modify the substrate-binding interface of E3 ubiquitin ligases and reroute their binding to novel targets, opening the possibility of therapeutically inhibiting proteins previously considered “undruggable”. However, for a molecular glue to sufficiently stabilize a ligase-target interaction and induce degradation, the two proteins must exhibit proper shape complementarity and contain productive amino acid contacts - two proteins selected at random are unlikely to be glued by any small molecule. Identifying novel “glueable” ligase-target pairs requires the discovery and characterization of weak interactions (likely in the micromolar affinity range) between thousands or more potential pairs of human E3 ligases and therapeutically relevant target proteins. The need to evaluate vast numbers of potential protein interactions while maintaining exquisite sensitivity for weak interactions presents a major technical roadblock. To address this challenge, we apply *AlphaSeq*, a high-throughput and highly sensitive experimental platform for measuring protein-protein interactions, to discover and structurally characterize weak interactions between ligases and target proteins. We validate our approach by characterizing known ligase-target interactions, such as between the E3 ligase MDM2 and the tumor suppressor protein p53. We then leverage *AlphaSeq* for the discovery of molecular glues for novel ligase-target pairs and orthogonally validate these interactions with surface plasmon resonance (SPR). Finally, we use *AlphaSeq* mutational data to structurally characterize the interaction interface and inform the intelligent discovery of molecular glues.

#7016 PROTiNb: A novel platform for developing biodegraders targeting intracellular proteins.

Hongzhuan Gu, Lijun Ye, Zexiu Huang, Dan Xu, Lizhen Zhan, Tian Wang, Liang Xiao, Yue Huang, Baiguang Ren, Chang Zhou, Ke Chen, Xiaodong Wu, Di Lu

Guangdong Fapon Biopharma Inc, Dongguan, China

Since the concept of targeted protein degradation (TPD) was first introduced in the 1990s, innovative technologies such as PROTACs and molecular glues have rapidly emerged and advanced, leveraging pathways including the ubiquitin-proteasome system, lysosome-mediated degradation, and autophagy. A notable limitation of PROTAC technology is its dependence on small molecule ligands to engage the target protein or the E3 ligase. These small molecules are often vulnerable to target mutations, which can interfere with binding and are considered a major cause of drug resistance. Furthermore, most existing TPD technologies rely on a limited set of endogenous E3 ligases, primarily CRBN and VHL.Nanobodies, on the other hand, provide larger binding surfaces and greater structural flexibility, allowing for a broader range of target proteins and potentially mitigating resistance risks. Recognizing these advantages, we established PROTiNb (Proteolysis Targeting intra-Nanobody) platform. This platform integrates engineered E3 ligases with VHH nanobodies in RNA form to be translated in the cells, enabling highly efficient and specific protein degradation.

For instance, in GFP-overexpressing cell lines, LaG16-SPOPm (modified SPOP) and GFP enhancer-RNF4m (modified RNF4) demonstrated higher degradation efficiency compared to their wild-type E3 ligase counterparts. Similarly, when fused with KRAS-specific VHH RT22, RT22-SPOPm showed superior degradation activity over RT22-SPOPwt, leading to enhanced inhibition of tumor cell proliferation. Furthermore, the fusion of RNF4m with the PCNA-specific VHH efficiently degraded PCNA in cancer cells, inducing cell cycle arrest and significantly suppression of tumor cell growth in vitro. We inoculated Nude mice with HCT116 cells overexpressing doxycycline-induced KRAS PROTiNb and induced PROTiNb expression by feeding doxycycline. The induction of KRAS PROTiNb significantly inhibited tumor growth. To translate this platform into a therapeutic application, we used a tumor-targeted lipid nanoparticle (LNP) for in vivo delivery. In vitro studies revealed that tumor-targeted LNPs enhanced the delivery specificity and increased the tumor-suppressive potency. Most notably, these nanobody-based degraders exhibited robust activity in vivo, effectively suppressing tumor growth.

In summary, our study demonstrates the versatility of the PROTiNb platform in rapidly generating degraders against intracellular targets once considered undruggable, with promising potential for clinical therapy for cancers.

#7017 Discovery and development of a highly co-operative and potent pan-KRAS degrader.

Dinesh Chikkanna, Madhu Aeluri, Leena Khare, Ravindra MV, Debajyoti Basak, Uday Bhat, Suraj T. Gore, Narmatha V, Shailesh V. Jadhav, K. Y. Vasantha, Sivapriya Marappan, Megha Goyal, R. N. Raghavendra, Amit A. Dhudashiya, Kiran Aithal, T. Jagadeesh Kumar, D. S. Suhas, K. B. Charamanna, P. Dharani, R. Naveenkumar, Rahul B. Chavan , Randeep Bokalial, D. S. Samiulla, Subhendu Mukherjee, Thomas Antony, Kavitha Nellore, Rajesh Eswarappa, Girish Chandrappa Daginakatte, Sanjeev Giri, Susanta Samajdar, Murali Ramachandra

Aurigene Oncology Limited, Bangalore, India

KRAS is one of the most frequently mutated oncogenes in various cancers. KRAS G12D mutation is prevalent in 39% of pancreatic ductal adenocarcinoma (PDAC), 27% of colorectal cancer, 11% of non-small cell lung adenocarcinoma as well as in a subset of other solid tumors. KRAS G12V mutation is found in 31% of PDAC, 20% of colorectal cancer and 19% of non-small cell lung adenocarcinoma. KRASG12C mutations are found in 41% of non-small cell lung adenocarcinoma and 3-5% of colorectal cancers. Despite recent advances in small molecule KRAS inhibitors, a majority of KRAS alterations are not yet addressed. Further, in cases where inhibitors are available, resistance rapidly emerges. Eliminating all mutant KRAS using a targeted protein degradation approach may lead to superior efficacy relative to inhibiting the protein. We have identified a series of degraders through structure-based drug design and our Directed Neo-substrate Degrader (DN_sD) approach, with very high co-operativity, potent pan-KRAS degradation activity and a desirable pharmacokinetic profile. The high co-operativity of pan-KRAS degraders was confirmed though Surface Plasmon Resonance (SPR) analysis. These degraders demonstrated significant degradation of KRAS in multiple KRAS mutant cell lines and showed mechanistic activity via the modulation of pERK levels leading to significant anti-proliferative activity. The KRAS degraders were found to be selective against HRAS and NRAS which was also confirmed using global proteomic analysis. Further, the development candidate molecule demonstrated significant and dose dependent tumor growth inhibition in multiple KRAS mutant xenograft models which was accompanied by sustained KRAS degradation. Notably, the development candidate compound has demonstrated excellent PK and tolerability in rodent and non-rodent tox species. IND enabling studies are ongoing to support clinical development.

#7018 Antibody-degrader conjugates unlock the therapeutic potential of heterobifunctional degraders through antibody-like pharmacokinetics and excellent in vivo efficacy.

Jan G. Felber, Anil P. Jagtap, Philipp Ochtrop, Saskia Schmitt, Isabelle Mai, Sarah Herterich, Paul Machui, Dietmar Konig, simon vogt, Dominik Schumacher, **Jonas Helma-Smets**, Annette Vogl, Marc-Andre Kasper

Tubulis GmbH, Martinsried, Germany

Heterobifunctional small molecules recruiting the proteasomal machinery enable the targeted protein degradation (TPD) of selected proteins and considerably broaden the druggable space for therapeutic use. Many such molecules have seen fantastic success in preclinical and clinical developments, e.g., for targeted degradation of specific oncoproteins that were previously inaccessible for cancer therapy. Even though early hits are quickly generated by adapting the modular concept of bifunctional degraders, most of such molecules are against the central rules of a “drug-like” molecule: they are usually larger in size (800-1500 Da) and often lack sufficient solubility. These early bifunctional degraders hits require exhaustive structure optimization in each case to optimize pharmacokinetics (PK) profiles and bioavailability.
Here, we report Tubulis’ antibody-degrader conjugates that exploit bifunctional degraders as high-potency bioactive payloads to be released specifically inside cells that are targeted by the antibody. Our linker technology ensures high degrader-to-antibody ratios (DARs) and excellent linker stability in blood plasma. Moreover, we liberate the degrader after receptor-mediated uptake of the ADC and show high efficiencies in target protein degradation. Most importantly, our conjugates exhibit antibody-like PK properties that ensure long-lasting plasma circulation of high DAR constructs over weeks, even when bifunctional degraders are employed that have not been optimized for PK properties. Taken together, these factors allow impressive in vivo efficacy of the conjugates after single dose application in preclinical animal models.

#7019 Identification of first-in-class paralog selective SMARCA4 degraders for treatment of hematological and prostate malignancies.

Chandrasekhar Abbineni, Kiran Aithal, Bilash Kuila, Sandeep Vitthal Dukare, Arya Desai, Megha Goyal, Khaji Abdul Rawoof, Gauri Rahul Petkar, Gopinath CH, T Jagadeesh Kumar, Charamanna KB, Rahul B. Chavan, Nandish C, Suraj Tgore, Leena Khare, Samiulla DS, Subhendu Mukherjee, Thomas Antony, Kavitha Nellore, Sanjeev Giri, Rajesh Eswarappa, Girish Daginakatte, Susanta Samajdar, **Murali Ramachandra**

Aurigene Oncology Limited, Bengaluru, India

The BAF (SWI/SNF) chromatin remodeling complex comprises of two mutually exclusive ATPases, SMARCA2 (BRM) and SMARCA4 (BRG1), that affect the mobilization and positioning of nucleosomes on DNA and thereby regulates important cellular functions including transcription, DNA recombination, DNA repair and chromosome decatenation during mitosis. SMARCA4 is frequently overexpressed in several types of cancers. Overexpression has been linked to increased proliferation and survival, as well as aggressive tumors and poor prognosis. SMARCA4 knockdown in these tumors lead to inhibition of proliferation and increased sensitivity to known chemotherapeutic agents, supporting the validity of targeting SMARCA4. In addition, PTEN and SMARCA4 appear to share synthetic lethality relationship based on reported in vitro and in vivo knockdown studies. Considering this well-established biological rationale, selective degradation of SMARCA4 should offer significant clinical benefit in multiple disease settings. Here, we report first-in-class SMARCA4 selective degraders that demonstrate distinct cellular phenotype. As part of the initial design plan, selective SMARCA2/4 Bromodomain inhibitors and specific ligands of several E3 ligases were chosen to arrive at different degrader designs. Several linkers and different exit vectors were considered to construct a variety of hetero-bifunctional degrader molecules. Our proprietary ternary complex modeling algorithm, ALMOND^4 (ALgorithm for MOdeling Neosubstrate Degraders) helped in prioritizing the designs. Shortlisted compounds were synthesized and profiled in multiple cellular assays to understand their potential to degrade SMARCA4 selectively. These compounds exhibited very potent anti-proliferative activity in cell lines of hematological and prostate cancer origin (e.g. MV-4-11, VCaP, etc.) whereas no significant potency is observed in SMARCA4 mutant cell lines. Identified compounds have shown selective degradation of SMARCA4 over SMARCA2 in multiple cell lines. Few potent SMARCA4 selective degraders were optimized for pharmacokinetic properties and further evaluation of tolerability and efficacy in rodents is in progress.

#7020 Chemical probe discovery for PAX3::FOXO1 via small-molecule microarrays.

Y. Xiang, M. Ramos Sala, B. Wu, D. Rana, K. Motoyama, B. Leifer, M. Toure, S. Doyle, A. N. Koehler;
Massachusetts Institute of Technology (MIT), Cambridge, MA

PAX3::FOXO1 (P3F) oncoprotein is a driver of poor patient outcome, contributing to tumorigenesis by dysregulating protein complexes that result in altered chromatin structure and gene expression. We conducted Small-Molecule Microarray (SMM) screening of approximately 10,000 compounds using engineered HEK293 cell lysates, identified KI-P3F-032, a compound that binds silently to the fusion protein, forming the basis for PROTACs development. With this initial results, there is a need to revalidate this preliminary screening result with biophysical data, we validated binding with SPR and developed biologically relevant high-throughput secondary assays to evaluate PROTACs efficacy. We have now developed a PROTAC with strong selective cytotoxicity against P3F positive RMS cell lines. At the same time, we also explored the possibility of indirect targeting of P3F via inhibiting and degrading other transcription regulators such as CDK9. We also developed new SMM assays to identify novel P3F binders using a library of approximately 65,000 small molecules under native P3F positive cell lysates, identifying 90 potential binders. We then developed functional recombinant P3F protein production and purification in our lab to enable pure protein based assays. We hope our research strategy can further advance the current progress on targeting the conventionally undruggable P3F oncogenic fusion and bring insight to targeting disordered oncogenic fusions in other pediatric cancers.

#7021 Discovery of a highly potent and orally bioavailable ER PROTAC degrader for the treatment of breast cancer.

Guan Zhou, Yuanli Wang, Wei Xue, Yongzheng Ding, Jing Cheng, Kanghua Wang, Weiwei Yu, Wenmin Chen, **Fuming Xu**

Gan & Lee Pharmaceuticals, Beijing, China

Breast cancer is the most prevalent invasive cancer among women globally and approximately 70-80% of newly diagnosed breast cancer cases are ER-positive (ER+). Estrogen receptor α (ERα) is a prime target for the treatment of ER+ breast cancer. Despite the development of several effective therapies targeting ERα signaling, clinical resistance remains a major challenge. A promising therapeutic strategy to overcome endocrine resistance in ER+ breast cancer is the development of ER degraders using the proteolysis targeting chimera (PROTAC) technology. Facilitated by our new cereblon ligand library, we have discovered a potent and orally bioavailable ER PROTAC degrader GLR203801, with its binding affinity to cereblon increased by 20-fold than that of ARV-471. GLR203801 exhibited robust protein degradation in both ER wild-type cell lines (DC_{50} < 1 nM, D_{max}> 90%) and mutant cell lines harboring Y537S or D538G mutation (DC_{50} 2-10 nM, D_{max} > 80%). Profound inhibition of the expression of ER-regulated genes GREB1 and PR was observed with GLR203801 in all the tested cell lines. GLR203801 shows more potent cell growth inhibition activity and maximum inhibition is 20%-30% higher than that of ARV-471 in all the tested ER wild-type and mutant cell lines.

GLR203801 was characterized with favorable PK profile *in vivo*, with longer $T_{1/2}$ and higher plasma exposure than ARV-471. GLR203801 displayed significantly enhanced antitumor efficacy than ARV-471 in five ER+ xenograft models (MCF-7, T-47D, ZR-75-1, MCF-7^{Y537S}, and T-$47D^{D538G}$), with tumor regression observed in wild-type xenograft models. PK/PD studies demonstrated that the tumor exposure of GLR203801 was 10 to 20-fold higher than that of ARV-471, and GLR203801 degraded >95% of ER in MCF-7^{WT} and T-$47D^{D538G}$ tumor tissue. In addition, GLR203801 in combination with CDK4/6 inhibitors showed synergistic anti-tumor efficacy in T-47D xenograft model. GLR203801 also effectively inhibited the viability of breast-cancer patient derived ER+ organoids. The good tolerance of GLR203801 in toxicity study both in SD rats and beagle dogs supports it as a promising preclinical candidate for further clinical development.

#7022 Discovery of GLR203701, a highly potent AR PROTAC with favorable PK profile and optimized tissue distribution characteristic.

Xiaobing Liu, Qianqian Zhao, Yuna Zhang, Yichao Ma, Licong Bu, Kanghua Wang, Weiwei Yu, Fuming Xu, **Wenmin Chen**

Gan & Lee Pharmaceuticals, Beijing, China

Prostate cancer is a hormone-dependent tumor, and the androgen receptor (AR) signaling axis drives all stages of prostate cancer, including the lethal, drug-resistant form of the disease termed metastatic castration-resistant prostate cancer (mCRPC). The development of PROTAC targeting AR has become a promising therapeutic strategy to solve the problem of mCRPC drug resistance of prostate cancer.

PROTAC drugs generally have both low solubility and permeability, which often lead to poor oral bioavailability and saturated drug absorption, with the systemic exposure reaching a plateau at lower dose. Phase 1 dose escalation trial showed systemic exposure of ARV-766 reached maximum at 320 mg/day in humans. 100 mg/day and 300 mg/day, were selected as the RP2D. The maximum or saturation exposure determines the window in which PROTAC drugs can be ramped up in clinical trials, which is of great significance to the clinical efficacy.

Recently, our drug discovery campaign led to the discovery of a potent AR PROTAC, named GLR203701. GLR203701 has good FaSSIF (Fasted State Simulated Intestinal Fluid) solubility, which contributed to a significant improvement of oral bioavailability. Moreover, the maximum exposure of GLR203701 was 5-fold higher than that of ARV-766 in rats and its exposure in beagle dogs reached up to 10-fold higher. In VCaP, LNCaP and 22RV1 cells, GLR203701 could degrade more than 90% of AR, with half-maximal degradation concentration (DC_{50}) < 10 nM. Profound tumor growth inhibition (TGI>90%) was observed in all the three xenograft models, even when the compound was dosed once every other day.

GLR203701 was chosen from a STAR (structure-tissue exposure/selectivity-activity relationship) analysis of over 100 pre-screened molecules because of its ideal tissue distribution. GLR203701 had reduced accumulation in liver and kidneys, while achieved 2.7-fold higher exposure in tumor than in plasma. Drug concentration detected in the epididymis and prostate was similar to the blood. Our refined STAR approach has enabled a harmonious balance of drug concentrations among visceral tissues, tumor and disease related organs, benefiting the efficacy/safety. No significant adverse effects were observed when GLR203701 was administered at 120 mg/kg to rats and dogs. Taking the high exposure level and good bioavailability, GLR203701 is expected to generate a board therapeutic window. These preclinical data support the clinical development of GLR203701 for the treatment of mCRPC.

#7023 A phenotypic screening method identifies a potent anti-cancer PROTAC targeting MAP3K1.

Lelisse T. Umeta[1], Jayapal R. Mallareddy[1], Sarbjit Singh[1], Smitha Kizhake[1], Lidia Boghean[1], Amritha Kizhake[1], Suchita Vishwakarma[2], Katelyn L. O'Neill[1], Lisa Humphrey-Brattain[1], Adrian Black[1], Xu Luo[1], Andrew S. Boghossian[3], Matthew G. Rees[3], Melissa M. Ronan[3], Jennifer A. Roth[3], Amarnath Natarajan[2]

[1]UNMC Eppley Institute, Fred & Pamela Buffett Cancer Center, Omaha, NE,[2]Department of Pharmaceutical Sciences, UNMC Eppley Institute, Fred & Pamela Buffett Cancer Center, Omaha, NE,[3]Broad Institute of MIT and Harvard, Cambridge, MA

The identification of Thalidomide, Lenalidomide, and Pomalidomide as Cereblon (CRBN) binders spurred the development of proteolysis-targeting chimeras (PROTACs). PROTACs with CRBN-binding moieties are heterobifunctional molecules that form a ternary complex with CRBN, which recruits the CUL4-DDB1 ubiquitin ligase protein complex, and a protein of interest (POI) and targets it for degradation. Despite numerous ongoing efforts, the structure-based design of PROTACs remains elusive. The current approach relies on the synthesis of a PROTAC library and screening the library in the chosen assay format to identify viable hits. Here, we propose a viability-based phenotypic screening system using HCT116 wild type (WT) POI knock-out ($POI^{-/-}$) and CRBN knock-out ($CRBN^{-/-}$) cells to identify PROTACs that display activity in both a POI and CRBN-dependent manner. For this study, we selected MAP3K1, an oncogenic signaling activator, as our POI and conjugated an ATP-competitive MAP3K1 inhibitor with thalidomide using various linkers to generate the library. The library was subjected to a 3-day viability screen using the above WT, $MAP3K1^{-/-}$, and $CRBN^{-/-}$ cell lines. The screen identified 50-008 as a hit that selectively inhibited the viability of the WT cells but not $MAP3K1^{-/-}$ or $CRBN^{-/-}$ cells. 50-008 was chemically validated in a colorectal cancer cell line and organoid model by making single changes that affect either CRBN binding, MAP3K1 binding, or the linker. Follow-up studies showed that 50-008 inhibits cancer cell proliferation, induces apoptosis, and an S-phase cell cycle arrest in a CRBN and MAP3K1-dependent manner. Furthermore, A Profiling Relative Inhibition Simultaneously in Mixtures (PRISM) screen of 50-008 across ~900 cancer cell lines identified 267 sensitive cancer cell lines (IC_{50} < 0.1μM), including luminal B breast cancer cell line T47D and 131 resistant cancer cell lines (IC_{50} > 10 μM). Mechanistic studies in T47D cells demonstrated that 50-008 degrades MAP3K1 and modulates the activation of key downstream effectors, such as ERK and IKKβ.

#7024 Discovery of highly selective, potent and orally bio-available SMARCA2 degraders.

Chandrasekhar Abbineni, Kiran Aithal, Bilash Kuila, Sandeep Vitthal Dukare, Prasath Kothandaraman, Arya Desai, Megha Goyal, Khaji Abdul Rawoof, Dhaytadak Bhagwan Mahadeo, Gopinath CH, T Jagadeesh Kumar, Charamanna KB, Rahul B. Chavan, Nandish C, Suraj Tgore, Leena Khare, Samiulla DS, Subhendu Mukherjee, Thomas Antony, Kavitha Nellore, Sanjeev Giri, Rajesh Eswarappa, Girish Daginakatte, Murali Ramachandra, **Susanta Samajdar**

Aurigene Oncology Limited, Bengaluru, India

The BAF (SWI/SNF) chromatin remodeling complex comprises of two mutually exclusive ATPases, SMARCA2 (BRM) and SMARCA4 (BRG1), that affect the mobilization and positioning of nucleosomes on DNA and thereby regulate important cellular functions including transcription, DNA recombination, DNA repair and chromosome decatenation during mitosis. Genetic silencing studies have established that the oncogenic activity of tumors lacking SMARCA4 is primarily driven by SMARCA2-containing residual SWI/SNF complex. Thus, targeting SMARCA2 in a SMARCA4 mutant setting is synthetically lethal. Considering this well-established biological rationale, selective targeting of SMARCA2 could be a precision oncology opportunity to meet an unmet medical need. Here, we report the identification and development of highly selective SMARCA2 degrader with oral bioavailability that have demonstrated robust efficacy in SMARCA4 mutant CDX models. SMARCA2 selective degraders were identified by optimizing an assembly of SMARCA2/4 Bromodomain binders, degron connecting linkers and several ligands of specific E3 ligases. Design prioritizations were done using a proprietary ternary complex modelling algorithm, ALMOND (ALgorithm for MOdeling Neosubstrate Degraders). Prioritized designs were synthesized and profiled in multiple cellular assays to understand the mechanistic and functional readouts. Several compounds that potently and selectively degrade SMARCA2 were identified with a distinct phenotype in a panel of SMARCA4 mutant cell lines. ADME optimization led to development of compounds with encouraging oral bioavailability in rodent as well as non-rodent species. Global proteomics indicated highly selective SMARCA2 downregulation in a SMARCA2/4 proficient cell line. Further, the identified degrader demonstrated robust efficacy supported by SMARCA2 degradation in multiple SMARCA4-mutant CDX models, at well-tolerated doses. Repeat dose tolerability profile of the lead compound in non-rodent species is ongoing towards candidate nomination.

#7025 Novel Insights into anti-tumor mechanisms of small-molecule ID antagonists.

Sergey Yaklichkin[1], Yohannes Ambaw[2], Michelle Saoi[3], Justin Cross[3], Robert Benezra[1]

[1]Cancer Biology, Memorial Sloan Kettering Cancer Center, New York, NY,[2]Cell Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY,[3]Donald B. and Catherine C. Marron Cancer Metabolism Center, Memorial Sloan Kettering Cancer Center, New York, NY

Inhibitors of Differentiation (ID) proteins (ID1-4), members of the helix-loop-helix (HLH) transcription regulator family, play critical roles in cancer progression and metastasis. Previously, we developed AGX51, a small-molecule ID antagonist, and its more potent derivative, AGX-A, which target ID proteins for degradation. Loss of ID proteins induces reactive oxygen species (ROS) accumulation and promotes lipid peroxidation, leading to non-ferroptotic cell death in breast cancer (BC) cells. This cell death can be rescued by the lipid scavenger alpha-tocopherol, implicating lipid ROS in the mechanism. To investigate changes in lipid composition, we performed non-targeted LC-MS lipidomic profiling of AGX-A-treated BC cells. This analysis revealed significant increases in specific lipid classes, including phosphatidylinositols (PI) (+47.06%), lysolipids (+23.53%), and phosphatidylcholine (PC) (+6.5%) correspondently, indicating a distinct lipidomic signature compared to classical ferroptotic inducers. Importantly, co-treatment with alpha-tocopherol restored lipid composition to basal levels, supporting the hypothesis that non-ferroptotic lipid peroxidation is a key mechanism underlying these changes. Furthermore, combining AGX-A with the general lipase inhibitor Orlistat significantly enhanced its cell-killing effects. Lipidomic profiling of this combination revealed notable increases in triacylglycerol species, which are likely susceptible to lipid peroxidation, amplifying AGX-A's cytotoxic activity. These findings highlight the therapeutic potential of ID antagonists in inducing a unique, non-ferroptotic cell death pathway. By altering lipid metabolism and exploiting lipid peroxidation, ID antagonists represent a promising strategy for targeting aggressive and drug-resistant tumors.

#7026 Nanobody-PROTACs: Method development towards using single-domain antibodies to target undruggable proteins for degradation.

Caroline Cope, Cristina Tufarelli, Robert Britton

University of Leicester, Leicester, United Kingdom

Proteolysis targeting chimeras (PROTACs) are bifunctional molecules that can bring an E3 ligase (e.g. VHL) into the vicinity of a protein of interest (POI). The E3 ligase poly-ubiquitinates the POI, marking it for degradation via a proteasome. Uniquely, PROTACs have the ability to bind to any chosen amino acid region, meaning there is tailored targeting of the POI, followed by subsequent degradation. This means that PROTACs have the potential to degrade targets previously considered 'undruggable', such as the oncogene, *KRAS*, which is historically difficult to therapeutically target due to lack of deep binding pockets. Typically, PROTACs utilize small molecules to target the POI, however, our work aims to uncover the potential of nanobodies to replace these small molecules. The gold standard for protein targeting remains monoclonal antibodies, however, nanobodies possess the same antigen binding site specificity, whilst possessing a tertiary structure only a tenth of the size. Our work focuses on identifying whether the nanobody-PROTAC has the potential for cellular entry, and thus degradation of its POI. For proof of concept, we utilized an anti-GFP nanobody tagged with AZdye647, synthesized using the Michael addition reaction, followed by SDS-PAGE analysis to assess successful conjugation. This conjugate was then used to treat fixed and live cells expressing enhanced green fluorescent protein (EGFP), followed by fluorescent microscopy to confirm cellular entry and POI targeting. Initial SDS-PAGE analysis revealed the successful conjugation of the AZDye647 to the nanobody. Specific binding within fixed cells was confirmed using fluorescent microscopy, as co-localization of EGFP and the AZdye647-tagged anti-GFP nanobody was revealed. Our data also confirmed that liposomal encapsulation of the AZdye647-tagged anti-GFP nanobody yielded efficient delivery into live EGFP expressing cells. Together, this data provides confirmation that a nanobody-PROTAC would be capable of passing through the cellular membrane, maintaining its integrity and degrading its POI. Further understanding of whether the replacement of AZDye647 with an E3 ligase ligand could result in the degradation of 'undruggable' oncogenes such as *KRAS*, *in vitro*, will confirm whether this is a viable therapeutic approach to patients with *KRAS*-mutant cancers.

#7027 Mass spectrometry-based proteomics for accelerating PROTAC and molecular glue drug discovery.

Michael J. Ford, Ravi Amunugama, David L. Allen, Richard C. Jones

MS Bioworks, LLC, Ann Arbor, MI

Targeted protein degradation (TPD) platforms, including PROTACs and molecular glues, have revolutionized drug discovery as transformative therapeutic strategies. Recent advancements in mass spectrometry-based proteomics have dramatically improved assay speed and sensitivity, providing actionable insights into degradation profiles and drug efficacy on timescales compatible with discovery chemistry. This study evaluates the practical utility of advanced proteomic assays in supporting TPD research, focusing on their role in streamlining drug discovery pipelines. State-of-the-art proteomic workflows were employed to quantify the proteome-wide effects of PROTACs and molecular glues across diverse cellular models. Assay sensitivity, reproducibility, and throughput were rigorously assessed to determine their real-world applicability. Our findings reveal that these enhanced technologies generate robust, high-resolution datasets, capturing the dynamic interplay between target degradation, protein interactions, and cellular responses. This enabled the rapid identification of optimal degrader candidates and informed structure-activity relationship (SAR) studies. Mass spectrometry-based proteomics has emerged as an essential tool for advancing TPD strategies. By combining heightened sensitivity with operational feasibility, these assays offer critical insights that accelerate the development of PROTACs and molecular glues, bridging the gap between foundational research and clinical application.

CLINICAL RESEARCH: Advances in Translational Pediatric Cancer Research
Poster Session

#7031 The role of atypical protein kinase C (aPKC) in the cellular invasion mechanism of diffuse intrinsic pontine glioma (DIPG) and diffuse midline glioma (DMG) and its regulation by ICA-1S and temozolomide (TMZ) inhibitors.

Radwan Ebna Noor[1], Nuzhat Nowshin Oishee[1], Mahfuza Marzan[1], Javad Nazarian[2], Mildred Acevedo-Duncan[1]

[1]Chemistry, University of South Florida, Tampa, FL,[2]Center for Cancer and Immunology Research (CCIR) at Children's National Health Systems (CNHS), Washington, D.C., Children's National Health System, WA

The DIPG develops in the human brainstem area (pons) and the DMG develops in the region covering the pons, midbrain, and thalamus. Due to their locations, surgical resection is not available and sample collection through biopsy is also limited. Moreover, the lack of knowledge of cell signaling regulations is one of the major challenges in treating these lethal pediatric gliomas. To understand the cellular characteristics like migration and invasion of these gliomas our research focuses on the atypical PKC-ι, which plays a major role in tumorigenesis due to its overexpression directly impacted by phosphoinositide 3-kinase (PI3K), phosphatase and tensin homolog (PTEN) and other pathways. In this ongoing research, we are investigating the role of the aPKC-ι/ζ expression levels in correlation with the malignancy of two incurable pediatric gliomas DIPG (SU-DIPG-IV) and DMG (CNMC-D-1008). The study sheds light on a regulatory connection between the isoenzyme-specific signaling pathway for invasion [PKC-ι /Focal Adhesion Kinase (FAK) / Paxillin] and its inhibition by a potent aPKC inhibitor, ICA-1S (5-Amino-1-[(1R,2S,3R,4R)-2,3-dihydroxy-4-[(phosphonooxy)methyl]cyclopentyl]-1H-imidazole-4-carboxamide), alone or in combination with Temozolomide (TMZ) *in-vitro*. In a previous study, we demonstrated that ICA-1S in combination with TMZ decreased the invasion of glioma cell lines by lowering the FAK and paxillin phosphorylation in the PKC-ι/ FAK/ Paxillin pathway. In our current study, we have observed the presence of an immunoprecipitation (IP) complex formation between PKC-ι and FAK indicating the presence of a direct phosphorylation event in SU-DIPG-IV cells during the invasion mechanism. Unlike SU-DIPG-IV, the CNMC-D-1008 cells have shown no such phosphorylation events in the IP experiments. Moreover, the study of the PKC-ι expression level with cellular overgrowth, cell cycle progression, cell migration, apoptosis, and toxicity study of the drugs will also be reported based on different experiments such as western blot, scratch assay, flow cytometry, WST-1 assay, etc. These studies bring insights into cellular processes like invasion and proliferation after the mono/combination therapy of ICA-1S and TMZ in these cell lines. The results presented here shed light on two prospects: that PKC-ι expression level may be established as a biomarker to perform anti-PKC therapy and as an inhibitor of PKC-ι the prospect of ICA-1S as a form of novel personalized treatment for these pediatric gliomas.

#7032 The role of general control nonderepressible 2 (GCN2) kinase in leukemogenesis and therapeutic response to L-asparaginase.

R. Claude, S. Srivastava, M. Zhong, C. Chen, S. Batra, U. Dave, C. Zhang, Y. Fang, K. Staschke, R. A. Wek, J. Zhang;
Indiana University at Indianapolis, Indianapolis, IN

Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer, affecting the lymphoid cells of both B and T lineages. Most ALL cells are auxotrophic for asparagine, a nonessential amino acid for protein synthesis, due to the low expression of asparagine synthetase (ASNS), a rate-limiting enzyme for *de novo* biosynthesis of asparagine. Standard ALL treatment takes advantage of this vulnerability by giving patients L-asparaginase, a bacterial enzyme that depletes the circulating asparagine. However, previous work from our lab and others has shown that some ALL cells become resistant to L-asparaginase treatment through the induction of ASNS expression. Mechanistically, amino acid starvation activates the general control of nonderepressible 2 (GCN2) kinase, leading to the accumulation of the ATF4 transcriptional factor. ATF4, in turn, is recruited to the promoter of the *ASNS* gene to activate its transcription. However, the role of GCN2 kinase in the process of leukemogenesis under nutrient limiting environment has not been established. Here, we found that, in a mouse T-ALL model driven by a KRas(G12D) mutation, ATF4 and ASNS expression were strongly elevated in leukemic tissues. However, germline deletion of the *Gcn2* did not delay leukemogenesis in primary T-ALL and secondary T-ALL generated by transplanting the pre-leukemic bone marrow cells into *Gcn2* wildtype recipient mice. Although GCN2 inactivation does not affect leukemogenesis, it sensitizes T-ALL cells to L-asparaginase treatment *in vitro* and *in vivo*. In addition, a small molecule inhibitor of GCN2, GCN2iB, synergistically inhibits leukemic growth and survival in the mouse T-ALL model and human T-ALL lines where the basal expression of ASNS is low. Finally, we tested a newly developed inhibitor of ASNS (ASX-173), which selectively killed leukemic cells in the absence of exogenous asparagine even though their basal level expression of ASNS is high. Taken together, our results suggest that targeting GCN2 or ASNS will have therapeutic implications in ALL patients who become resistant to L-asparaginase treatment.

#7033 Unraveling *Kiaa1549-Braf* driven brain tumors: From modeling to therapy.

Ylenia Cendon Florez[1], Ram Kannan[2], Matthias A. Karrajannis[1], Darlene Dela Cruz[3], Udi Segal[3], Danilo Maddalo[3], Scott Foster[4], Robert Benezra[1], Andrea Ventura[1]

[1]Memorial Sloan Kettering Cancer Center, New York, NY,[2]Asset and Pipeline Strategy, Schrodinger, New York, NY,[3]Department of Translational Oncology, Genentech, San Francisco, NY,[4]Department of Research Oncology, Genentech, San Francisco, NY

The *KIAA1549-BRAF* (*KB*) fusion is a prevalent genetic event in two distinct central nervous system tumors: pilocytic astrocytomas (PAs) and diffuse leptomeningeal glioneuronal tumors (DLGNTs). This chromosomal rearrangement, consisting of a 2 Mbp tandem duplication at 7q34, results in the formation of a fusion protein absent in normal cells. The KB fusion protein has been described to dimerize the BRAF kinase in a RAS-independent manner, leading to a constitutive activation of the MAPK pathway and promoting tumorigenesis. Despite its significance, the lack of pre-clinical models limits our understanding of how *KB* promotes DLGNTs and PAs development, so effective therapeutic strategies remain elusive. To address this, we aimed to model the KB fusion at its endogenous chromosomal location.

Overcoming the challenges of modeling chromosomal rearrangements, we used the CRISPR-Cas9 gene-editing technique to model the fusion event *ex vivo* and *in vivo* in the mouse brain, leveraging *p53*- or *Arf*-deleted backgrounds. These models allow us to investigate the molecular mechanisms by which *KB* promotes tumor initiation and progression and test the therapeutic potential of Belvarafenib, a class II BRAF inhibitor that targets BRAF dimers.

Our results demonstrate that CRISPR-mediated engineering of KB in *p53*- or *Arf*-deficient backgrounds, both *ex vivo* and *in vivo*, generates CNS tumors resembling human DLGNTs in their histology and growth patterns. Furthermore, targeting the robust MAPK pathway activation in these KB-driven tumor models with Belvarafenib significantly reduced ERK phosphorylation and tumor growth, and significantly increased the survival of treated mice.

These findings demonstrate that *KB* promotes tumor initiation and progression, and highlight the therapeutic potential of Belvarafenib targeting this oncogenic event. Thus, our *KB*-DLGNTs model emerges as a valuable tool to evaluate the efficacy of novel pre-clinical BRAF inhibitors.

#7034 The impact of EphA2 in the malignancy of CIC-DUX sarcomas.

Lisa Toracchio[1], Alberto Bavelloni[1], Caterina Mancarella[1], Lorena Landuzzi[1], Andrea Morini[1], Elisa Simonetti[1], Pier Luigi Lollini[2], Andrea Morrione[3], **Katia Scotlandi**[1]

[1]IRCCS Rizzoli Orthopedic Institute, Bologna, Italy,[2]University of Bologna and IRCCS Azienda Ospedaliera Universitaria di Bologna, Bologna, Italy,[3]Sbarro Institute for Cancer Reseach, Temple University, Philadelphia, PA

Capicua (CIC)-rearranged sarcomas (CDS) are rare and highly aggressive undifferentiated small round cell sarcomas and patients with CDS have a dismal prognosis. Despite being recognized as a distinct entity by WHO 2020, these tumors are still treated according to Ewing sarcoma regimens and new more specific treatment options are urgently needed. Previous work, including data from our group, has identified the IGF-AKT-mTOR signaling pathway as particularly active in these tumors. Here, we highlight the role of the Ephrin system, particularly the EphA2 receptor, in CDS. EphA2-mediated signaling emerged from a core set of 71 genes that clearly distinguish CDS from Ewing sarcoma (PMID: 34903601). Functional studies on three CDS cell lines obtained from patient-derived xenografts (PDX) confirmed the high expression of the receptor, which was constitutively activated on serine residue 897 (S897). EphA2 is highly expressed in embryos during development, declines in later stages and can be re-expressed in cancer. EphA2 is implicated in self-renewal, chemoresistance, and metastasis and its expression correlate with poor patient outcome in several tumors (PMID:33686241). The targeting of EphA2 is thus under investigation. However, EphA2 signaling is complex, occurring through canonical, ligand-dependent mechanisms and/or non-canonical ones mediated by crosstalk with other receptors, kinases or activated by different ligands. In CDS, AKT is hyper-active due to the constitutive activation of IGF2/IGF1R signaling and the intrinsic inhibition of MAPK. AKT induces strong phosphorylation of EphA2 at S897, which leads to a conformational change of EphA2, promoting cell migration and metastasis. This mechanism renders CDS cells resistant to traditional anti-EphA2 tyrosine kinase (TK) inhibitors used as single agents. Therefore, we have successfully tested the efficacy of a combination between ALW-II-41-27, a widely used inhibitor of EphA2 kinase activity, and IGF-1R/AKT inhibitors in preclinical models. In addition, we are testing EphA2 targeted degradation by using HaloPROTAC3 approaches to confirm the impact of targeting EphA2 on CDS cell migration and metastasis.

The research leading to these results has received funding from AIRC under IG 2019—ID. 22805 project—P.I. K. Scotlandi. For HaloPROTAC studies support was provided by the Italian Ministry of Health through the Executive Programme of Cooperation in the field of Science and Technology between Italy and the USA 2023-2025, MAECI-2023-23683465, to C. Mancarella.

#7035 Targeting Ewing sarcoma with a novel RBM39 degrader: DNA damage repair pathway effects.

F. Liu[1], Y. Zhang[1], B. Wang[2], J. Finn[1], J. Tonra[1], L. Huang[1], D. Liu[1], H. Jin[3], X. Liu[1], **D. Lu**[1];
[1]Seed Therapeutics Inc., King of Prussia, PA, [2]University of Washington, Seattle, WA, [3]Vitsgen Therapeutics, Portland, OR

Patients with metastatic Ewing Sarcoma have a five-year survival rate of 15-30%. For 30 years, there are limited approved therapies, including chemotherapy, radiotherapy and surgery, which are associated with significant morbidities and secondary cancers, supporting the need for new impactful therapies. Analysis of the RNA splicing factor RNA Binding Motif Protein 39 (RBM39) in the Cancer Target Discovery and Development (CTD^2) Network showed that Ewing sarcoma cell lines are especially sensitive to RBM39 loss. Here we demonstrate effects on mediators of DNA damage repair in association with significant anti-cancer effects of an orally administered novel RBM39 degrader, ST-00937.
Methods and Results: A next generation RBM39 degrader ST-00937 was designed through structure-based drug design and prioritized based on in vitro anti-cancer potency, metabolic stability and oral pharmacokinetics in mice. Human Ewing sarcoma A673 cells were utilized to explore the effects of ST-00937 in this cancer type. Tumor cell viability was evaluated by CellTiter-Glo® Luminescent Cell Viability Assay (CTG) and anti-tumor efficacy was evaluated in nude mice bearing tumors established with A673 cells. Protein levels in cell lysates and tumor homogenate were measured by Western Blot. Target gene RNA splicing was evaluated by RT-PCR.ST-00937 degraded RBM39 by 6 hours of treatment and reduced in vitro A673 Ewing sarcoma cell viability with an IC50 of 170 nM. These in vitro effects translated to complete A673 tumor regressions in mice by the 11th day of twice daily ST-00937 oral dosing at 30mg/kg, without an effect on body weight. By the third day of treatment, RBM39 was undetectable in A673 tumors. Mechanically both the EWS-FLI1 driver gene fusion in A673 cells and RBM39 degradation are reported to reduce DNA damage repair pathways. Here we report RBM39 degradation in A673 cells significantly increases phosphorylated γH2Ax, a marker of DNA double strand breaks. This DNA damage is thought to contribute to the induction of cell apoptosis detected as increased cleaved caspase-3. Increased DNA double strand breaks is furthermore associated with negative effects on 3 proteins involved in homologous recombination repair of DNA double strand breaks; mis-splicing of BRCA1 (Breast cancer type 1) and ATM (ATM Serine/Threonine Kinase), and reduced protein levels of RAD51D (recombination repairing regulator Recombinase homolog D).
Conclusion: The RBM39 molecular glue degrader ST-00937 therefore demonstrates total tumor regression in a model of Ewing sarcoma. Mechanistic studies indicate that the robust activity of ST-00937 involves deficient homologous recombination repair. Continued research in our lab will add further insights into the mechanism of action of RBM39 degraders in this cancer of high unmet need.

#7036 Co-targeting AR, mTOR, and PI3K inhibitors in DSRCT as a novel therapeutic approach.

Roberto Cardenas-Zuniga, Asmaa G. Ahmed, Kien Ryan Cao, Abid Raza, **Clement Agyemang**, Danh Truong, Joseph Ludwig

UT MD Anderson Cancer Center, Houston, TX

DSRCT (Desmoplastic Small Round Cell Tumor) is a rare soft tissue sarcoma that appears in the abdominal and pelvic area of mainly young males. There is little information about the molecular mechanisms involved in its pathology. Despite existing treatment options such as surgery, chemotherapy, and radiation, the NIH estimates that the five-year survival rate is only 15%, with frequent recurrence. Thus, new molecular targets and more effective therapeutic strategies are needed. Due to the high prevalence in males during puberty and the fact that AR (Androgen Receptor) is expressed in DSRCT, we hypothesized that AR plays an essential role in DSRCT development. We evaluated sensitivity to DHT (Dihydrotestosterone) and its effect on proliferation in the presence of different FBS (Fetal Bovine Serum) concentrations. For this approach, the LNCaP (prostate cancer) cell line was used as control. Our results indicated that DSRCTs responded differently to DHT at different FBS concentrations. We observed that DHT stimulates AR phosphorylation and increases total AR, pS6, and total S6 protein levels. Next, we explored the sensitivity of DSRCT cell lines to Enzalutamide and Darolutamide inhibitors; Temsirolimus (an mTOR inhibitor); Alpelisib (a PI3K inhibitor); and Gedatolisib (a dual mTOR and pan-PI3K inhibitor). Individual drug sensitivity was evaluated using in vitro exposure via the classic dose-response curve assays, measuring viability with CellTiterGlo2.0 reagent to calculate IC50 values. DSRCTs were more sensitive to Darolutamide than Enzalutamide, two different AR inhibitors. Dual targeting PI3K and mTOR with Gedatolisib was more effective than Alpelisib or Temsirolimus alone. Taking lessons learned from prostate cancer, we studied combination assays of AR antagonists combined with PI3K/mTOR inhibitors. Those assays suggested that Darolutamide and Gedatolisib effects were mainly additive, with a slight synergy. We conclude that DSRCT cell lines are responsive to AR blocking and highly sensitive to PI3K/mTOR inhibitors. A co-targeting strategy that jointly inhibits the AR and PI3K/mTOR pathways is being investigated in murine models and might be considered for future studies if the regimens have non-overlapping toxicity profiles.

#7038 Direct targeting of DNMT1 inhibits high-risk neuroblastoma growth through epigenetic reprogramming.

Danielle C. Rouse, Saurabh Agarwal

Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, St. John's University, Queens, NY

DNA Methyltransferase 1 (DNMT1) mediated hypermethylation represents a crucial epigenetic modification implicated in neuroblastoma (NB), a devastating pediatric cancer with a 60% relapse rate. DNMT1, a prominent member of the DNA methyltransferase family of enzymes, catalyzes the transfer of methyl groups to the 5-carbon position of cytosine residues within DNA sequences. While extensive research has established that hypermethylation of CpG islands leads to transcriptional silencing of tumor suppressor genes across various cancer types, the specific molecular mechanisms and therapeutic implications of DNMT1-mediated hypermethylation in NB remained largely unexplored. In this study, we analyzed the publicly available R2 genomic dataset, which revealed the direct correlation between *DNMT1* overexpression and decreased overall NB patient survival rates. Further, we performed a small molecule screening and identified a highly potent and selective DNMT1 inhibitor. Therapeutic targeting of DNMT1 with this inhibitor resulted in significant dose-dependent reductions in multiple hallmarks of cancer progression, including overall cell proliferation, colony formation capacity, and 3D spheroid development. Furthermore, the DNMT1 inhibitor induced cell cycle arrest in the G2/M phase checkpoint and significantly enhanced apoptotic cell death in NB cells. These promising *in vitro* findings were validated through *in vivo* tumor xenograft studies, where the DNMT1 inhibitor significantly inhibited NB tumor growth without any observable toxicity in preclinical models. Further, we employed MethylFlash technology to quantify 5-methylcytosine (5-mc) levels, which verified a significant reduction ($p < 0.0001$) in global DNA methylation status following exposure to increasing concentrations (5, 10, and 20 μM) of the DNMT1 inhibitor. Further molecular analyses revealed profound effects on vital gene and protein expression patterns alongside an enhancement of NB cell sensitivity to conventional chemotherapeutic agents. These findings collectively establish DNMT1 inhibition as a promising therapeutic strategy for NB patients. Our data demonstrates the role of DNMT1-mediated hypermethylation in NB tumorigenesis while establishing that targeted DNMT1 inhibition effectively reduces hypermethylation, thereby suppressing malignant proliferation, inhibiting colony formation, and preventing both 3D spheroid development and in vivo tumor growth. Our data also highlights that the direct DNMT1 targeting approach is a promising epigenetic-based approach for NB treatment. Further mechanistic research of DNMT1 inhibition in NB progression is essential to facilitate the clinical translation of this promising therapeutic strategy.

#7039 Synergistic antitumor activity of the combined PARP1 inhibition and alkylating DNA damage in malignant rhabdoid tumors.

Elena Mironova[1], Sebastian Molinas[1], Vanessa Del Pozo[1], Abhik Bandyopadhyay[1], Zhao Lai[1], Dias Kurmashev[1], Eric L. Schneider[2], Daniel V. Santi[2], Yidong Chen[1], **Raushan Kurmasheva**[1]

[1]UT Health San Antonio Greehey Children's Cancer Res. Inst., San Antonio, TX,[2]Prolynx, Inc., San Francisco, CA

Background: Malignant rhabdoid tumors (MRTs) are among the most aggressive and treatment-resistant malignancies affecting infants, originating in the kidney, brain, liver, and soft tissues. The 5-year event-free survival rate for these cancers is a mere 20%. In nearly all cases of MRT, the *SMARCB1* gene (occasionally *SMARCA4*) - a pivotal component of the SWI/SNF chromatin remodeling complex - is homozygously deleted, although the precise etiology of these tumors remains unknown. While young patients with localized MRT generally show improved outcomes, especially those who are older and have early-stage disease, the overall prognosis remains poor despite optimal standard treatments. This highlights the urgent need for more effective treatment strategies. PARP1 is a widely targeted molecule in cancer treatment and, beyond its role in DNA repair, it participates in transcriptional regulation by recruiting chromatin remodeling complexes to modulate DNA accessibility for RNA polymerases.

Methods: We evaluated the antitumor activity of the PARP1 inhibitor talazoparib (TLZ) in combination with the DNA alkylating agent temozolomide (TMZ) across six MRT xenograft models in CB-17 scid mice. The dosing schedule for this combination followed a previously reported protocol (iScience 2021; 25(2):103725). Time-to-event was defined as 4-fold increase in tumor xenograft volume from the treatment initiation date. Event-free survival (EFS) between treated and control groups was analyzed using Kaplan-Meier method. Objective response categories including partial (PR), complete (CR), and maintained complete responses (MCR), were determined based on previously established criteria (Ped Blood Cancer 2007;49:928). To widen the therapeutic window of the drug combination, we employed PEGylated TLZ (PEG-TLZ), which has been reported to reduce systemic toxicity through slow drug release.

Results: Remarkably, our findings indicate that five out of six MRT xenografts exhibited objective response to PEG-TLZ+TMZ therapy. Notably, *SMARCB1* loss was found to confer a protective effect, correlating with elevated expression of DNA damage and repair proteins in *SMARCB1*-deficient MRT cells. Additionally, MGMT emerged as a potential biomarker predictive of in vivo MRT response to PEG-TLZ+TMZ treatment. In addition, our analysis revealed alterations in signaling pathways associated with the observed antitumor activity.

Conclusions: This study presents a novel and efficacious therapeutic approach for MRT, along with a promising candidate biomarker for predicting tumor response.

#7040 The B7-H3 targeting antibody-drug conjugate (ADC) vobramitamab duocarmazine (vobra duo) is potently effective against a broad panel of pediatric solid tumor xenograft models: A study from the Pediatric Preclinical In Vivo Testing (PIVOT) Consortium.

Haiying Tang[1], Edward Favours[1], Samson Ghilu[1], Peyton Wong[1], Vanessa Del Pozo[1], Abhik Bandyopadhyay[1], Elena Mironova[1], Eric J. Earley[2], Stephen W. Erickson[3], David Groff[4], Beverly A. Teicher[5], Kateryna Krytska[4], Matthew Tsang[4], Yael P. Mosse[4], E. Anders Kolb[6], Matthew Stephens[7], Steve Neuhauser[8], Tim Stearns[8], Jeff Chuang[9], Emily L. Jocoy[10], Jee Young Kwon[9], Carol Bult[8], Malcolm A. Smith[11], John M. Maris[4], Richard G. Gorlick[12], Peter J. Houghton[1], **Raushan Kurmasheva**[1]

[1]Greehey Children's Cancer Research Institute, UT Health San Antonio, San Antonio, TX,[2]Genomics Ltd, Oxford, United Kingdom,[3]RTI International, Durham, NC,[4]Children's Hospital of Philadelphia, Philadelphia, PA,[5]National Cancer Institute, Rockville, MD,[6]The Leukemia & Lymphoma Society, The Leukemia & Lymphoma Society, PA,[7]Macrogenics, Onc., Rockville, MD,[8]The Jackson Laboratories, Bar Harbor, ME,[9]The Jackson Laboratories, Farmington, CT,[10]The Jackson Laboratories, Davis, CA,[11]National Cancer Institute, Bethesda, DC,[12]UT MD Anderson Cancer Center, Houston, TX

Introduction: Vobra duo, a duocarmycin-based humanized ADC (drug-to-antibody ratio is ~2.7) targeting B7-H3, shows robust *in vivo* activity against a range of adult cancer models, and a favorable pharmacokinetic and safety profile in cynomolgus monkeys. Initial results from the single agent phase 1 clinical trial of vobra duo (NCT03729596) showed manageable side effects and promising objective response rates in metastatic castration-resistant prostate cancer. B7-H3 is highly expressed in pediatric solid tumors, and it is emerging as a key target for pediatric oncology. Here we report the antitumor activity of vobra duo against preclinical xenograft models of pediatric solid tumors.

Methods: Ewing sarcoma (ES), rhabdomyosarcoma (RMS), and neuroblastoma (NB) xenograft models were tested with n=1 or n=2 designs. Osteosarcoma (OS) models, due to their slower growth kinetics and lower rates of tumor regression, were tested with n=10 mice per arm. The study was extended to include malignant rhabdoid tumor (MRT), hepatoblastoma (HB), and Wilms tumor (WT) models using n=5 mice per treatment group. vobra duo and control ADC (SYD988 anti-CD20 ADC with the same linker and payload as vobra duo) were provided by MacroGenics, Inc., and were administered at 6 mg/kg as a single administration IP. Time-to-event was defined as 4-fold increase in tumor xenograft volume from the day of treatment. The Kaplan-Meier method was used to compare event-free survival (EFS) between treated and control groups. Objective response categories were determined as described previously (Ped Blood Cancer 2007;49:928-940), with objective responses including partial, complete, and maintained complete responses (PR, CR, and MCR).

Results: Vobra duo induced objective responses in 4 of 11 NB models (3 CR and 1 MCR) and in 3 of 5 OS models (2 MCR). ES models showed objective responses in 2 of 6 models (1 MCR) and 4 of 6 RMS models showed objective responses, all MCR. Control ADC was less active than vobra duo and showed objective responses in 0 of 11 NB, 1 of 5 OS, 1 of 6 ES, and 2 of 4 RMS models. The study was extended to MRT, HB, and WT models. Vobra duo induced objective responses in 2 of 4 MRT with 1 MCR, 2 of 5 HB with 2 MCR, and 3 of 4 WT with 3 MCR. Control ADC only induced objective responses in 1 of 4 MRT and 2 of 4 WT models (all PR), with no objective responses for the 5 HB models.

Conclusions: Vobra duo is potently efficacious across a broad panel of pediatric solid tumor xenograft models supporting clinical development of this agent and other agents targeting B7-H3 for children with cancer.

#7041 The CLK/DYRK inhibitor SM09419 shows potent efficacy across a broad panel of pediatric preclinical xenograft models - A report from the Pediatric Preclinical In Vivo Testing Consortium (PIVOT).

Richard B. Lock[1], Raushan Kurmasheva[2], Kathryn Evans[1], David Groff[3], Ben Watts[1], Kateryna Krytska[3], Christopher M. Smith[1], Faith N. Key[3], Daniella Cofini[1], Miguel Agoncillo[1], Steven Neuhauser[4], Timothy M. Stearns[4], Emily L. Jocoy[5], Jee Young Kwon[6], Jeffrey H. Chuang[6], Beverly A. Teicher[7], Richard Gorlick[8], Yael P. Mosse[9], John M. Maris[9], Peter J. Houghton[2], Carol J. Bult[4], Malcolm A. Smith[7]

[1]Children's Cancer Institute, Lowy Cancer Research Centre, School of Clinical Medicine, UNSW Medicine & Health, UNSW Centre for Childhood Cancer Research, UNSW Sydney, Sydney, Australia,[2]University of Texas Health at San Antonio, Greehey Children's Cancer Research Institute, San Antonio, TX,[3]The Children's Hospital of Philadelphia, Philadelphia, PA,[4]The Jackson Laboratory for Mammalian Genetics, Bar Harbor, ME,[5]The Jackson Laboratory, Sacramento, CA,[6]The Jackson Laboratory for Genomic Medicine, Farmington, CT,[7]National Cancer Institute, Bethesda, MD,[8]The University of Texas MD Anderson Cancer Center, Houston, TX,[9]The Children's Hospital of Philadelphia and the Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA

Introduction: SM09419 is an experimental small-molecule pan-inhibitor targeting CLK and DYRK kinases, which play a key role in RNA splicing and other oncogenic processes. SM09419 exhibited promising activity in preclinical models of *TP53*- and *FLT3*-mutated acute myeloid leukemia, and its analog cirtuvivint (SM08502) exhibited broad preclinical activity and evidence of therapeutic benefit in solid tumors. Cirtuvivint is in clinical trials in adults with AML (NCT06484062) and sarcomas (SELNET-7-2/EuCT#: 2024-511987-10-00). To evaluate the role of CLK and DYRK kinase inhibition in pediatric cancers, SM09419 was tested against panels of pediatric solid tumor and leukemia patient-derived xenografts (PDXs).
Methods: SM09419 was provided by Biosplice Therapeutics and administered orally at 25 mg/kg daily for 5 days per week x 3 weeks for the acute lymphoblastic leukemia (ALL), neuroblastoma (NB), sarcoma (SARC) and hepatoblastoma (HB) models. Tolerability was assessed in groups of 3 non-tumor bearing immune-deficient mice (NSG, CB17 SCID) at 100%, 75% and 50% of the recommended dose of 25 mg/kg. For efficacy testing, a single mouse trial (SMT) design (one vehicle control, one drug treated mouse per PDX) was used for ALL (n=30 PDXs), NB (n=14), HB (n=7), Ewing sarcoma (ES, n=8), rhabdomyosarcoma (RMS, n=5), Wilms tumor (WT, n=2) and malignant rhabdoid tumors (MRT, n=3). For the osteosarcoma (OS, n=6) models a conventional study design of 10 mice per group (vehicle or drug) was employed. Standard PIVOT methods were used to assess event-free survival (EFS T/C = ratio of median EFS for treated versus control animals). Stringent objective response measures (ORMs) modeled after the clinical setting were also used to evaluate activity (Houghton et al., Pediatr Blood Cancer, 2007).
Results: SM09419 was well tolerated at all dose levels with <20% weight loss observed, irrespective of the mouse strain. SM09419 showed objective responses (ORMs of partial response, PR; complete response, CR; maintained CR, MCR) against 20/28 evaluable ALL, 6/14 NB, 2/7 HB, 2/8 ES, 0/2 WT, 0/5 RMS, 0/3 MRT PDXs tested. EFS T/C values ranged from 0.4-9.3 (ALL), 1.2-7.0 (NB), 0.64-2.7 (HB), 1.0-5.9 (ES), 0.37-0.81 (WT), 0.65-1.8 (RMS) and 1.7-2.0 (MRT). No objective responses were observed in the 6 OS models, with 4 showing growth delay (EFS T/C >2).
Conclusions: SM09419 exhibited broad antitumor activity across several pediatric cancers (e.g., ALL and NB), with activity also observed for ES and HB. Lower activity was seen in the RMS and OS models. The promising results support further evaluation of CLK/DYRK as therapeutic targets. Future research directions for these cancers include identifying biomarkers that predict sensitivity to CLK/DYRK inhibition as well as efforts to identify effective combinations with targeted and/or standard-of-care drugs.

#7043 HMGA1 Epigenetic Regulators Drive Relapse in Pediatric B-Cell Leukemia By Amplifying ETV5 and Stemness Networks.

Linda M. S. Resar[1], Jung-Hyun Kim[1], Andrei Thomas-Tikonenko[2], Sarah Tasian[2]

[1]Johns Hopkins University School of Medicine, Baltimore, MD,[2]Childrens Hospital of Philadelphia, Philadelphia, PA

Despite advances in therapy for pediatric B-cell acute lymphoblastic leukemia (pB-ALL), relapse remains a leading cause of death in children with cancer. The gene encoding the High Mobility Group A1 (HMGA1) chromatin regulator is highly expressed in stem cells and hematologic malignancies where high levels portend poor outcomes. In pB-ALL, *HMGA1* is overexpressed with highest levels at relapse. Transgenic mice overexpressing *Hmga1* in lymphoid cells develop aggressive leukemia. To determine how HMGA1 functions in relapsed pB-ALL, we performed multiomic analyses. Here, we uncover a previously unknown role for HMGA1 as an epigenetic gatekeeper of stemness transcriptional networks at relapse. Strikingly, silencing *HMGA1* expression via *CRISPR* or short hairpin RNA decreases proliferation and clonogenicity in vitro while disrupting engraftment and prolonging survival in vivo. Multiomics analyses revealed transcriptional networks governed by HMGA1 that regulate proliferation (G2M checkpoint, E2F), RAS/ERK signaling, hematopoietic stem cells (HSCs), and ETS variant 5 transcription factor (ETV5) targets. *CRISPR*-mediated *ETV5* gene silencing recapitulates effects of *HMGA1* silencing in pB-ALL cell lines. Further, restoring *ETV5* expression in pB-ALL cell lines with *HMGA1* silencing partially rescues anti-leukemogenic effects of *HMGA1* depletion. Mechanistically, HMGA1 binds to AT-rich regions within the *ETV5* promoter and recruits activating histone marks to induce *ETV5*. Moreover, *HMGA1* and *ETV5* are co-expressed and up-regulated in primary blasts from children with relapsed pB-ALL where high *HMGA1* correlates inversely with time to relapse. Artificial intelligence predicted that the histone deacetylase inhibitor (HDACi), vorinostat, targets HMGA1-ETV5 gene networks. In PDX models with pB-ALL blasts from relapse, we found that vorinostat enhances responses to standard B-ALL therapy (dexamethasone, vincristine), depleting circulating blasts and disrupting leukemic engraftment. Our findings reveal HMGA1 as an epigenetic switch that activates *ETV5* and HSC gene networks in relapsed pB-ALL and rational therapeutic target to treat or prevent relapse.

#7044 *In vitro* and *in vivo* activity of the CD127 monoclonal antibody 4A10 in preclinical models of childhood acute lymphoblastic leukemia.

Gisele O. L. Rodrigues[1], Priscila P. Zenatti[2], Joao M. S. Feitoza[2], Robert K. Chen[3], Uksha Saini[4], Julie A. Hixon[1], Ross A. Hamilton[4], Michael J. Heffernan[4], Jose A. Yunes[2], Phillip P. Breitfeld[4], Atul Varadhachary[4], Sarah K. Tasian[3], Scott K. Durum[1]

[1]National Cancer Institute, Frederick, MD,[2]Boldrini Research Center, Campinas, Brazil,[3]Children's Hospital of Philadelphia, Philadelphia, PA,[4]Allterum Therapeutics, Houston, TX

Background: 4A10 is a monoclonal antibody targeting CD127 (IL-7R alpha) under planned clinical development for treatment of patients with relapsed/refractory (r/r) acute lymphoblastic leukemia (ALL). CD127 is endogenously expressed on normal/malignant T cells as a heterodimer with CD132 to form the IL-7 receptor. CD127 is also aberrantly expressed in *CRLF2*-rearranged B-ALL as part of the heterodimeric thymic stromal lymphopoietin receptor (TSLPR). 4A10 binds the extracellular domain of both wild type and mutant CD127 with high affinity and mediates its anticancer activity through a dual mechanism of action: (i) inhibition of IL-7 signaling, as assessed by phospho-STAT5 production, and (ii) induction of receptor-mediated cytotoxicity through antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP), which can facilitate the killing of CD127+ cells. The role of CD127 in the development, maintenance, and relapse of T-ALL is well described. Extensive preclinical data with 4A10 have demonstrated robust anticancer activity in human T-ALL cell lines and patient-derived xenograft (PDX) models of early T-cell precursor (ETP) and other more mature subtypes. 4A10 has demonstrated single agent activity in chemo-naïve, chemo-resistant, and steroid-resistant T-ALL models, as well as augmented activity in combination with chemotherapy. As CD127 is expressed in other hematological cancers, we tested the hypothesis that 4A10 would also have robust anticancer activity across B-ALL subtypes.

Results: In these preclinical studies, we first detected high expression of CD127 in six B-ALL genetic subtypes and potent 4A10-mediated ADCC *in vitro* against KOPN8, REH, and NALM6 cell lines. We then observed robust inhibition of leukemia proliferation and increased survival with 4A10 monotherapy treatment of *CRLF2*-rearranged Ph-like and Down syndrome-associated, *BCR::ABL1*, *TCF3::PBX1*, *ETV6::RUNX1*, and high-hyperdiploid ALL PDX models compared to vehicle. Single agent 4A10 treatment of steroid-resistant KOPN8 cell line xenograft mice further demonstrated *in vivo* activity with some augmentation on dexamethasone addition.

Conclusions: CD127 is an attractive target with a low risk of 'bystander toxicity' given limited CD127 expression in normal tissues as assessed by tissue cross-reactivity studies. In recent non-human primate studies, 4A10 therapy had a tolerable clinical profile with modest and reversible reduction in normal peripheral blood T-lymphocytes. Given the robust activity of 4A10 against multiple B-ALL subtypes, we will seek to extend our planned Phase 1/2A clinical trial assessing the safety and therapeutic efficacy of single agent 4A10 in adults and children with r/r ALL. Ongoing preclinical studies are also evaluating the therapeutic potential of 4A10 against lymphoma and AML based on promising pilot efficacy data.

#7045 Anti-tumor effects following BET inhibition in preclinical models of pediatric and AYA osteosarcoma.

Ryli E. Justice[1], Niknam Riyahi[1], Barbara J. Bailey[1], Erika A. Dobrota[2], Rada Malko[1], Harlan E. Shannon[2], Keiko E. Kreklau[2], Melissa A. Trowbridge[1], Kathryn L. Coy[1], Felicia M. Kennedy[1], Melinda R. Ervin[1], Anthony L. Sinn[1], Christopher Davis[3], Kyle W. Jackson[3], Brian D. Weiss[3], Steven D. Angus[3], M. R. Saadatzadeh[3], Pankita H. Pandya[3], Karen E. Pollok[1]

[1]Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, IN,[2]Department of Pediatrics, Herman B Wells Center for Pediatric Research, Indianapolis, IN,[3]Department of Pediatrics, Indiana University School of Medicine Pediatric Hematology, Oncology and Stem Cell Transplant, Indianapolis, IN

Osteosarcoma (OS) is the most common primary malignant bone sarcoma in pediatric, adolescent, and young adult patients with a five-year survival rate of <30%. This is due, in part, to genomic complexities arising from moderate levels of oncogenic replication stress (RS). While moderate levels of RS cause genome instability that contributes to OS progression, high levels of RS can lead to cell death. Notably, bromodomain and extra-terminal domain (BET) proteins (BRD2,3,4) are epigenetic readers that regulate not only transcriptional networks but also DNA replication and repair. Targeting RS through utilization of BET inhibitors (BETi) is an underexplored treatment option in OS and little is known about BETi-induced molecular changes and adaptive responses in OS. We hypothesize that BETi leads to decreased OS cell growth via not only remodeling of gene expression but also increased DNA damage and non-tolerated RS levels. Effect of BETi (AZD5153, BMS-986378, ZEN-3694) in clinical trials for pediatric and adult solid tumors were investigated. In vitro BETi screening of OS cell lines indicated that bivalent AZD5153 was the most potent BETi compared to monovalent BMS-986378 and ZEN-3694. However, in a longitudinal cell growth delay assay with repeat exposure of BETi at clinically relevant doses, all BETi decreased OS cell growth at similar rates. To interrogate mechanisms of action, RNA-seq analysis of BET/BRD4 inhibition via AZD5153, PROTAC (ARV-825), and BRD4 siRNA in OS cell lines revealed distinct and overlapping patterns of alterations in gene expression. A major challenge in OS therapy is mitigation of invasiveness and targeting OS metastatic foci. In all treatment groups, RNAseq analysis indicated upregulation of tumor suppressor DHRS2 an inhibitor of tumor invasion but also TMPRSS9, a promoter of tumor invasion. This observed duality in tumor invasion mechanisms is an example of the intra-heterogeneity of the tumor response and indicative that a second therapeutic hit is likely necessary for suppression of tumor invasiveness in remaining OS cells following BETi therapy. In vivo studies demonstrate that bivalent AZD5153 significantly suppressed tumor growth in OS patient-derived xenografts (PDXs) derived from both treatment-naïve (patient status: alive-PDX112, PDX124; deceased- PDX96, PDX115) and pre-treated metastatic (patient status: deceased-TT2/HT77) OS compared to vehicle ($p<0.05$). Therapy was well tolerated with similar drug sensitivities in female and male mice. Analysis of in-vivo PDX96 response following AZD5153 therapy indicated increased γ-H2AX, and expression of pro-apoptotic genes, indicative of increased RS and cell death. At the transcript level, increased DHRS2 was evident with no changes in TMPRSS9. These data sets reveal the promise of BETi for treatment of OS PDX derived from treatment naïve patients some of which go on to relapse, and in OS PDX derived from metastatic sites.

#7046 Payload and linker selection in novel osteosarcoma antibody-drug conjugate (ADC) targeting CADM1.

Caterina Longo, Zhongting Zhang, Yifei Wang, Wendong Zhang, Zhaohui Xu, Yanhua Yi, Jonathan B. Gill, Micheal Roth, Xin Zhou, Richard Gorlick

The University of Texas MD Anderson Cancer Center, Houston, TX

Introduction: Our efforts in developing new therapies for osteosarcoma have focused on CADM1 as a novel target for ADC development. After successful proof of principle in vitro and in vivo tests, we developed three GMP manufactured CADM1-targeting ADCs with different linker and payload combinations: CADM1-PEG-Exatecan, CADM1-GGFG-Exatecan (a topoisomerase I inhibitor), and CADM1-Tesirine (a BPD-dimer).
Materials and Methods: We tested 15 of our own osteosarcoma PDX-derived cell lines for cytotoxicity using AlamarBlue and Incucyte Sartorius assays. CADM1-Tesirine was tested at 500 nM and the other two ADCs at 1000 nM due to different cytotoxicity profiles, all three with a 10x dilution. The cells were incubated with the drugs for 120h. Internalization was assessed using Incucyte and Fabfluor Red Labeling Reagent at 2.5 µg/ml for all ADCs and controls, including an isotype control IgG1, bound to the respective linker-payload. The bystander effect was evaluated by incubating CADM1-negative cells with media derived from the 5-day incubation of both CADM1-positive and CADM1-negative cells.
Results: CADM1-Tesirine showed significant cytotoxicity across all tested cell lines (IC50: 0.014 - 5.4 nM). CADM1-GGFG-Exatecan exhibited potent activity in most cell lines (IC50: 1.28 - 115 nM). CADM1-PEG-Exatecan had higher IC50 values (2.15 - 222 nM) but had better internalization than CADM1-GGFG-Exatecan. The fluorescence intensity increased over time, with a significant rise within the first 24 hours, which was not as consistently observed for CADM1-GGFG-Exatecan. CADM1-Tesirine displayed robust internalization across all cell lines, following a trend comparable to CADM1-PEG-Exatecan but often achieving even higher fluorescence intensity, thus proving to be the most internalized of the three ADCs. The bystander effect analysis showed that only the media derived from treated CADM1-positive cells induced cytotoxicity, as evidenced by the decreased growth rate using the Incucyte assay.
Conclusions: All three ADCs demonstrate significant anti-tumor activity, however, CADM1-Tesirine had superior IC50 values and internalization. Results from the bystander experiments indicate target dependent internalization and linker cleavage. We are currently conducting in vivo tests to further evaluate their performance anticipating the conduct of a phase 1/2 trial in patients with recurrent osteosarcoma.

#7047 Impact of MYCN inhibition on lineage state and GD2 expression in neuroblastoma.

Angelle L. Brown[1], Sridhar Veluvolu[1], Matthew Shapiro[1], Connor Hennessey[2], Adam Wolpaw[3]

[1]Center for Childhood Cancer Research, Children's Hospital of Philadelphia, Philadelphia, PA,[2]Cancer Biology Graduate Group, University of Pennsylvania, Philadelphia, PA,[3]Department of Pediatrics, Division of Oncology, Perelman School of Medicine, Philadelphia, PA

Neuroblastoma (NB) is a malignant cancer that originates in the developing peripheral nervous system and accounts for ~15% of all childhood cancer deaths. Some NBs are easily cured, but those classified as high risk require treatment that includes chemotherapy, surgery, autologous stem cell transplant, radiation, and immunotherapy with antibodies targeting the disialoganglioside GD2. Despite this extensive therapy, only ~50% of children survive. Even in these high-risk cases, most of the NB cells respond to initial therapy, but a subset of cells survives (persister cells) and grows back into resistant relapsed disease. Two important factors in relapse are *MYCN* amplification and intratumoral heterogeneity. MYCN, a transcription factor of the MYC family, is amplified in about 40% of high-risk NB cases and is associated with a particularly poor prognosis. Intratumoral heterogeneity is also thought to contribute to relapse. NB cells can exist in two different lineage states: a chemosensitive adrenergic (ADRN) state and chemoresistant mesenchymal (MES) state. With the development of improved pan-MYC inhibitors and the advancement of MYC-targeting therapies into clinical trials, there is a new need and opportunity to investigate how NB responds to MYCN inhibition, how this impacts lineage state, and how this changes GD2 expression.

We investigated the effects of MYCN inhibition in MYCN-amplified ADRN cell lines using the small molecule pan-MYC inhibitor MYCi975. Cell viability assays, western blot analysis, quantitative PCR, and flow cytometry analysis were conducted to evaluate the impact of MYCN inhibition.

We found that treatment with MYCi975 results in a rapid decrease in the protein levels of MYCN, MYCN target genes, and ADRN state markers, but not in new expression of MES state markers or changes in the expression of the immunotherapeutic target GD2. Doses that effectively decrease MYCN result in a loss of cell viability after 3-5 days. However, rare cells survive prolonged treatment (persister cells) and are able to resume proliferation once MYCi975 is removed. These recovered cells demonstrate relative resistance to retreatment with MYCi975 with a ~2-fold increase in the IC50 compared to naïve cells. Additionally, these resistant cells recover basal levels of MYCN and ADRN markers but have decreased surface GD2.

Our findings support the likelihood that some NB cells will survive therapies that inhibit MYCN and provide a model to investigate the identity and potential vulnerabilities of these cells. Furthermore, we find a role for MYCN in maintaining the ADRN lineage state but not in maintaining GD2 expression. However, GD2 is downregulated in resistant cells with implications for the relative timing of MYCN-targeting and GD2 targeting therapies. Ongoing work will be presented on transcriptomic changes after MYCi975 treatment and in resistant cells.

#7048 Molecular and immune landscape of extracranial malignant rhabdoid tumors (eMRT) and atypical teratoid rhabdoid tumors (ATRT): Insights into disease mechanisms.

Maria D. Hernandez Ramirez[1], **Marlena Mucha**[1], Victoria E. Fincke[1], Martin Hasselblatt[2], Christian Vokuhl[3], Michael C. Fruhwald[1], Pascal D. Johann[1]

[1]Pediatrics and Adolescent Medicine, Swabian Children's Cancer Center, University Hospital Augsburg, Augsburg, Germany,[2]Institute of Neuropathology, University Hospital Munster, Munster, Germany,[3]Section of Pediatric Pathology, Department of Pathology, University Hospital Bonn, Bonn, Germany

Rhabdoid tumors are aggressive pediatric cancers characterized by significant alterations in gene expression and tumor microenvironment composition. Our study aims to investigate the molecular mechanisms driving tumor aggressiveness and relapse in extracranial malignant rhabdoid tumors (eMRT) and atypical teratoid rhabdoid tumors (ATRT). Using high-resolution single-cell RNA sequencing and spatial transcriptomics, we analyzed gene expression patterns and cellular interactions within the tumor microenvironment, with a particular focus on immune cell composition and activity.

Previous research has identified distinct DNA methylation subgroups in eMRT, associated with clinical outcomes such as high- and standard-risk profiles. These subgroups, linked to differences in survival rates, relapse frequencies, and SMARCB1 genetic alterations, provide a foundational framework for understanding tumor heterogeneity. Building on this framework, we explored subgroup-specific gene expression profiles and tumor microenvironmental dynamics, moving beyond methylation-based stratification. Pathway enrichment analysis identified key molecular pathways associated with immune evasion and tumor progression, providing insights into the tumor microenvironment and its role in disease progression. We observed enrichment in pathways such as nuclear-transcribed mRNA catabolic process (nonsense-mediated decay), and cytoplasmic translation, which are crucial for sustaining the high proliferative rates of tumor cells. Unique to the high-risk group in eMRT is the enrichment of pathways such as protein phosphorylation and plasma membrane-bounded cell projection assembly, which are critical for oncogenic signaling and invasive mechanisms. Immune cell characterization revealed a higher macrophage count in the eMRT high-risk group compared to the standard-risk group, aligning with their aggressive phenotype, as macrophages facilitate invasion and immune evasion. The high-risk eMRT group also showed elevated levels of growth factors (FGF9, HGF, PDGFC) and invasion-related proteins (SEMA3D, IL1RAPL1), with reduced ALB levels, which suggest enhanced proliferation and metabolic alterations characteristic of aggressive tumors. These findings offer a deeper understanding of the unique molecular and cellular landscapes of rhabdoid tumors, providing valuable insights to inform potential therapeutic targets.

#7049 Harnessing phosphoproteomic profiling for target discovery in high-risk osteosarcoma: A pathway to novel therapies?.

C. M. Perkins[1], Z. Barger[1], A. Fordham[1], A. Salib[1], T. Lim Kam Sian[2], R. J. Daly[2], P. Ekert[1], E. Fleuren[1];
[1]Children's Cancer Institute, Sydney, Australia, [2]Monash Biomedicine Discovery Institute, Melbourne, Australia

Background and Aims: Osteosarcoma (OS) is the most common primary malignant tumor of the bone in children and adolescents and young adults (AYAs). Currently there are no effective targeted treatments for advanced disease, and tumours rarely possess actionable, genomic alterations. Activated (phosphorylated) protein kinase signaling has been identified as a key driver event in subsets of human osteosarcoma. This study aims to utilize a phosphoproteomic approach to identify aberrantly activated kinases in OS, which novel targeted therapies can be directed against.
Hypothesis: Activated kinase signaling is a key driver event in subsets of human OS. Phosphoproteomic profiling of OS tumours will identify aberrantly activated kinases, which if targeted by matched drugs could indicate novel, effective, patient-specific OS therapies.
Methods: Eighteen unique patient-derived xenograft (PDX) models from high-risk (<30% chance of survival) sarcoma patients enrolled in the ZERO Childhood Cancer Precision Medicine Trial (ZERO), including four OS (one with matched disease samples), were subjected to Mass Spectrometry-based phosphoproteomic profiling. Selected activation signatures in OS were validated by Western Blot (WB) and linked to molecular genomics/transcriptomics. Eight drug targets were selected for functional validation in vitro in short and long-term drug viability assays across standard OS (n=3) and newly established OS PDX cell lines from ZERO patients (n=6). Two top-performing novel agents underwent further in vitro combination testing using ZERO's clinically applicable 150-compound drug library and in vivo exploration. Drug mechanism-of-action was investigated by WB and cell cycle analysis.
Results: In OS, we identified ~4900 phosphosites, corresponding to 1843 unique proteins. Eight novel drug targets with elevated phosphorylation across OS patient models were selected for further validation with ten kinase inhibitors. Two clinically applicable inhibitors demonstrated remarkably low IC50 values of <0.05uM or <0.1uM across standard OS and OS PDX cell lines in short-term in vitro assays and were selected for further validation. On-target drug activity and mechanism-of-action was confirmed by WB, where reduced expression of CDK downstream targets was observed. Clonogenic assays further confirmed the remarkable sensitivity of tested cell lines to these drugs. In vitro combination testing revealed synergy with at least three targeted agents.
Conclusion: Our study demonstrates significant anticancer activity of two novel kinase inhibitors in vitro in OS and newly established high-risk ZERO PDX cell lines. Studies exploring the in vivo efficacies of these drugs as single agents and in combinations are underway. This study demonstrates the potential application of phosphoproteomics for the discovery of actionable targets in high-risk OS.

#7050 Preclinical evaluation of the AKR1C3-activated alkylator OBI-3424 in hepatoblastoma: A report from the Pediatric Preclinical In Vivo Testing Consortium (PIVOT).

Filemon Dela Cruz[1], Andrew Kung[1], Raushan Kurmasheva[2], Peter Houghton[2], Daoqi You[1], Glorife Ibanez Sanchez[1], Steven Neuhauser[3], Tim Stearns[3], Jeff Chuang[4], Jee Young Kwon[4], Emily L. Jocoy[5], Yu-Jung Chen[6], Ming-Chen Yang[6], Beverly Teicher[7], Carol J. Bult[3], Malcolm A. Smith[8]

[1]Department of Pediatrics, Memorial Sloan Kettering, New York, NY,[2]Greehey Children's Cancer Research Institute, UT Health San Antonio, San Antonio, TX,[3]The Jackson Laboratory, Bar Harbor, ME,[4]The Jackson Laboratory, Farmington, CT,[5]The Jackson Laboratory, Sacramento, CA,[6]OBI Pharma, Taipei City, Taiwan,[7]Molecular Pharmacology, National Cancer Institute, Bethesda, MD,[8]CTEP, National Cancer Institute, Bethesda, MD

Introduction: OBI-3424 is a DNA alkylating prodrug activated by aldo-keto reductase family 1 member C3 (AKR1C3). Elevated AKR1C3 expression is observed in a variety of tumors including prostate cancer, T-cell acute lymphoblastic leukemia (T-ALL), hepatocellular carcinoma (HCC) and hepatoblastoma. Interim Phase 2 data of OBI-3424 in advanced HCC patients showed encouraging survival benefit and a good safety profile. Evaluation of AKR1C3 expression across a spectrum of pediatric tumors showed highest expression in T-ALL and hepatoblastoma. Prior preclinical testing of OBI-3424 in T-ALL patient-derived xenograft (PDX) models showed significant activity with prolonged complete remissions. Hence, we evaluated the anti-tumor activity of OBI-3424 across a panel of hepatoblastoma PDXs and examined AKR1C3 expression as a response biomarker.

Methods: Tumors from 8 hepatoblastoma PDXs were evaluated for AKR1C3 expression by immunohistochemistry and H-scores assigned following pathology review. OBI-3424 was provided by OBI Pharma and administered intravenously at 2.5 mg/kg weekly for 3 weeks. Tolerability testing was performed in 3 non-tumor bearing immunodeficient mice at 100%, 75% and 50% of the recommended dose (2.5 mg/kg). In efficacy studies, 3-6 mice were treated per treatment group (vehicle, OBI-3424) across the 8 hepatoblastoma PDXs profiled for AKR1C3 expression. Previously described measures assessing event-free survival (EFS T/C = ratio of median EFS for treated versus control animals) and overall objective response measures (ORMs) modeled after the clinical setting were used to evaluate activity (Houghton et al., Pediatr Blood Cancer, 2007).

Results: H-scores for AKR1C3 expression ranged from 50 - 255 (median: 158) with 5 of 8 models having H-scores ≥100. AKR1C3 transcript expression was well correlated with protein expression. OBI-3424 was well tolerated at all dose levels tested with no significant weight loss in 2 different immunodeficient mouse strains. Among 8 models tested, partial responses (PRs) were observed in 3 models and 1 model showed stable disease (SD). Three models were categorized as progressive disease with tumor growth delay (PD2), and 1 model continues to undergo testing. EFS T/C values ranged from 2.34-5.62. Preliminary assessments of response do not appear to consistently correlate with AKR1C3 expression level (e.g., PR and PD2 observed in models with H-scores of 250 and 255, respectively).

Conclusions: OBI-3424 shows promising preclinical activity in hepatoblastoma PDX models inducing objective responses. The role of AKR1C3 expression as a response biomarker for hepatoblastoma requires further investigation. Results from this study along with encouraging interim Phase 2 clinical data for advanced HCC patients may provide rationale for future clinical development of OBI-3424 in hepatoblastoma.

#7051 CCL21 therapy in conjunction with low-dose metronomic cyclophosphamide generates superior responses against neuroblastoma in vivo.

Benjamin Gephart, Gabrielle Brumfield, Donald W. Coulter, Joyce Solheim

University of Nebraska Medical Center, Omaha, NE

Neuroblastoma is a pediatric malignancy that arises from aberrant neural crest progenitor cell division and proliferation. Relapsed and refractory disease often has a poor prognosis, with the long-term survival rate less than 30%. Neuroblastoma is canonically considered to be an immunologically cold and non-immunogenic tumor type, with dense populations of immune suppressive cell populations that have been shown to impair anti-tumor immunity. Despite the significant clinical response in other tumor types, immune checkpoint inhibitors have only shown limited clinical efficacy in neuroblastoma. Superior treatment modalities are needed to increase overall survival with less severe toxicity in the treatment of neuroblastoma. CCL21 is a chemokine that mediates the migration of immune cell populations such as T cells, NK cells, and dendritic cells, which are key immune cell populations for robust anti-tumor immunity. Prior preclinical studies have highlighted the effectiveness of CCL21 therapy as a potential treatment modality for multiple solid tumor types; however, CCL21's effectiveness against neuroblastoma is poorly understood. We sought to investigate the effectiveness of intratumoral delivery of CCL21 therapy against neuroblastoma in vivo. We desired to also characterize the immunomodulatory properties of the alkylating agent cyclophosphamide against neuroblastoma both in vitro and in vivo. Cyclophosphamide is part of the standard-of-care treatment protocol for neuroblastoma and has been shown to possess unique immunomodulatory properties at low doses. Herein, we demonstrate that CCL21 therapy in conjunction with metronomic low-dose cyclophosphamide chemotherapy generates superior controlling of neuroblastoma tumor growth in an immunocompetent, syngeneic Neuro2a mouse model. In our in vitro studies, we show that low-dose cyclophosphamide up-regulates cell surface calreticulin expression, as well as cell surface PD-L1 expression on neuroblastoma tumor cells, suggesting that it has potential to facilitate sensitization to anti-PD-L1 checkpoint blockade. In short, this research shows the therapeutic potential of CCL21 and metronomic low-dose cyclophosphamide chemotherapy in the treatment of non-immunogenic tumor types such as neuroblastoma. Furthermore, this research supports the technical feasibility of a novel in situ tumor immunotherapy strategy for neuroblastoma in a preclinical setting.

#7052 Istiratumab exhibits antiproliferative activity in Ewing sarcoma and rhabdomyosarcoma.

Jerome Salmon[1], Sarah Cherkaoui[2], Floriane Braye[1], Raphael J. Morscher[2], Luc Friboulet[1], Alexandre Bobard[1], Jean-Paul Alves[3], Jean-Pierre Armand[1], **Birgit Geoerger**[1]

[1]Gustave Roussy, Villejuif, France,[2]Kinderspital, Zuerich, Switzerland,[3]Pegascy, Villejuif, France

Istiratumab (prior MM-141) is a fully human tetravalent bi-specific antibody that binds and co-inhibits IGF-1R and ErbB3 (HER3). Istiratumab inhibits ligand binding to abrogate PI3K/AKT signaling resulting in antiproliferative activity in various cancers alone and in combination with mTOR inhibitor everolimus or chemotherapy, including in a human Ewing sarcoma model. Following Phase I and Phase II trials in advanced solid and pancreatic cancer, the molecule was abandoned by Merrimack Pharmaceuticals and was subsequently acquired by Pegascy. To consolidate our decision to proceed with a pediatric study, we explored our MAPPYACTS sequencing data on recurrent or refractory pediatric cancers for alterations in both pathways. Mutations in IGF-1R and HER3 occur rarely in pediatric cancers, some tumors exhibit IGF-1R amplification. Most tumors expressed moderate levels of IGF-1R with high expression in neuroblastoma samples and amplified osteosarcoma; IGF-2R is expressed in most cancers. IGF1 was lowly expressed in neuroblastoma and sarcoma but highly in choroid plexus carcinoma whereas IGF2 was highly expressed in most tumor types, particularly rhabdomyosarcoma, hepatoblastoma and ependymoma that are known to exhibit LOH. HER3 was found expressed in RMS, hepatoblastoma, a proportion of high grade glioma and carcinomas, its ligand NRG1 was found high only in single samples of sarcoma subtypes. Moreover, we observed IRS2 and IRS1 overexpression in about 80% of Ewing sarcoma samples. Overall, the activity of these pathways encouraged us to explore further the antitumor activity of istiratumab in pediatric cancers. We evaluated antiproliferative activity in Ewing sarcoma (TC-71, TC-106, CHLA-99, TC-205), alveolar (Rh41, Rh30) and embryonal (RD) rhabdomyosarcoma of istiratumab alone and in combination with the IRS1/2 inhibitor NT219 or HDAC inhibitor panobinostat. Istiratumab exhibited antiproliferative activity at 0.1 and 1 µM against most cell lines as determined by WST-1 assay. Strong growth inhibition (60-70%) was noted in TC-71 and TC-106, moderate inhibition (26%) in CHLA-99, whereas TC-205 was not sensitive. 35-45% growth inhibition was achieved in rhabdomyosarcoma RD, Rh30 and Rh41, similar to EBV-positive nasopharynx carcinoma C666, which co-expresses both pathways. Antiproliferative effects of istiratumab were enhanced by addition of NT219 at 0.1 µM or panobinostat in the 3 Ewing sarcoma cell lines tested. Similarly, the addition of IGF1 but not NRG1 in low serum growing conditions reinforced the observed activity of istiratumab in Ewing sarcoma cell lines. Evaluations to determine biomarkers for sensitivity are ongoing. Conclusion: Istiratumab has antiproliferative activity in sarcomas and will be evaluated in molecularly selected pediatric malignancies and Ewing sarcoma within the AcSé-ESMART trial.

#7053 Proteomics and integrative pathway analysis to understand the mechanism of action of mithramycin and etoposide combination induced cell death in Ewing sarcoma cells.

Christoffer B. Lambring[1], Khadiza Zaman[1], Elin Stone[1], Natalie Gierat[1], Ameya Bhargava[2], Laszlo Prokai[1], **Riyaz Basha**[1]

[1]University of North Texas Health Science Ctr., Fort Worth, TX,[2]Purdue University, West Lafayette, IN

Ewing's sarcoma (ES) is a highly aggressive cancer that primarily originates in the bones or soft tissues and is predominantly diagnosed in adolescents and young adults. The EWS-FLI1 fusion protein is considered a hallmark of ES, being expressed in approximately 85% of cases. Prognosis for advanced ES remains poor, with survival rates around 30%, and current chemotherapy regimens are associated with severe long-term side effects. Mithramycin has emerged as a promising inhibitor of EWS-FLI1; however, its clinical application is limited by dose-dependent toxicities. We hypothesize that combining mithramycin with standard chemotherapy could enhance therapeutic outcomes through synergistic effects, potentially allowing for reduced doses of both agents to maximize efficacy while minimizing toxicity. To investigate this, we evaluated the combined impact of mithramycin and etoposide on ES cell lines. Using TC205 cells, we further explored the underlying mechanisms through proteomics and integrative pathway analysis (IPA). In this study, we assessed the dose- and time-dependent effects of mithramycin and etoposide in ES cell lines (CHLA10 and TC205) and non-cancerous cardiomyocytes (H9C2) to optimize treatment conditions. In vivo assays demonstrated a significant reduction in tumor growth with combination therapy compared to single-agent treatment. Using TC205 cells, we assessed the impact of mithramycin and etoposide both as monotherapies and in combination. Cells were plated in triplicate for each group (control, mithramycin, etoposide, and mithramycin + etoposide) and treated with optimized doses. After 48 hours, cells were harvested, and protein samples were subjected to proteomics and IPA to elucidate the mechanisms of action of both mono- and combination therapies. The combination of mithramycin and etoposide significantly inhibited cell viability compared to individual treatments. Proteomics analysis identified 517 altered proteins (t-test with BH correction), with 142 upregulated and 375 downregulated. IPA revealed significant changes in markers associated with disease and physiological function networks, including homologous recombination, cytostasis of sarcoma cell lines, cell movement of sarcoma cell lines, and osteoclastogenesis of limb bones. More than 70 disease and function networks and over 200 canonical pathways were significantly affected. Key pathways included eukaryotic translation initiation, cell cycle checkpoints, neutrophil degranulation, and cell cycle control of chromosomal replication. Overall, these findings reveal that the combination of mithramycin and etoposide alters critical networks and pathways, enhancing therapeutic efficacy in ES cell lines.

#7054 Mithramycin and chemotherapy combination induces apoptosis in Ewing sarcoma cells by upregulating reactive oxygen species and associated pathways.

Christoffer B. Lambring[1], Kayla Fure[1], Z'Niyah WIilson[2], Gisel Gutierrez[1], Umesh Sankpal[1], **Riyaz Basha**[1]

[1]University of North Texas Health Science Ctr., Fort Worth, TX,[2]Old Dominion University, Norfolk, VA

Ewing's Sarcoma (ES) is a rare and aggressive bone cancer that primarily affects children and adolescents. The disease is characterized by the presence of the EWS-FLI1 fusion protein, a transcription factor found in 85% of ES tumors that drives oncogenesis and tumor progression. Advanced ES has a grim prognosis with survival rates below 30%. Standard chemotherapy often results in significant long-term morbidities due to side-effects. Mithramycin has shown promise in inhibiting EWS-FLI1, however, its clinical application has not been established due to dose-dependent toxicities. We hypothesize that combining mithramycin with chemotherapy could offer a potential strategy to achieve synergistic effects, enabling lower dosages of both drugs to enhance efficacy while minimizing toxicity. We tested the combination effect of Mithramycin and Etoposide using ES cell lines and determined the response on inducing higher apoptosis through altering reactive oxygen species (ROS). Cardiomyocytes were used to test systemic toxicity against non-cancerous cells. Ewing Sarcoma cells (CHLA 10 and TC205) and cardiomyocytes (H9C2) were cultured and treated with Mithramycin, Etoposide, and their combination using optimized concentrations determined for each drug. Cell viability, Caspase 3/7 activity and ROS production were measured using respective luminescence-based assays. Flow cytometry with Annexin V/7AAD staining evaluated apoptosis levels, and protein expression of apoptosis-related markers, such as c-PARP, was analyzed using a capillary-based Simple Western system. Statistical analyses were performed using GraphPad Prism to evaluate significance. The combination of Mithramycin and Etoposide significantly inhibited ES cell viability compared to either agent alone. Apoptotic markers were markedly elevated in the combination-treated cells and associated with an upregulation of Caspase 3/7 activity and ROS production. Flow cytometry analysis confirmed an elevated apoptotic response, with a higher percentage of Annexin V-positive cells in the combination group. Additionally, Western blot analysis revealed increased c-PARP expression, consistent with enhanced apoptosis. In H9C2 cardiomyocytes, no treatment exhibited significant toxicity at equivalent concentrations, indicating the potential for no cardiac side effects. These results demonstrate the synergistic effects of Mithramycin and Etoposide in targeting cancer cells while sparing healthy tissue. Protein samples were subjected to proteomics and Integrative Pathway Analyses are currently being conducted to precisely elucidate the involvement of ROS-associated markers in understanding the mechanism of action and in vivo efficacy of this combination. Investigating additional biomarkers of response and resistance may provide further insights for personalized therapy.

#7055 An IL1RAP ADC is efficacious in distinct oncofusion-driven pediatric cancers.

Haifeng Zhang[1], Wei Li[2], Xiaqiu Yang[1], Qingfeng Huang[1], Yue Zhou Huang[1], Alberto Delaidelli[1], Andrew Lytle[1], Melanie Rouleau[1], Christian Steidl[1], Dimiter Dimitrov[2], **Poul Sorensen**[3]

[1]BC Cancer Research Institute, Vancouver, BC, Canada,[2]University of Pittsburgh, Pittsburgh, PA,[3]University of British Columbia, Vancouver, BC, Canada

Our recent studies identified the cell surface protein IL-1 receptor accessory protein (IL1RAP) as a crucial regulator of redox homeostasis and a suppressor of ferroptosis (iron-mediated cell death) in Ewing sarcoma (EwS). IL1RAP directly binds to the CD98/xCT plasma membrane antiporter to enhance cystine uptake, thereby replenishing cysteine and glutathione (GSH) pools in EwS cells. Moreover, when extracellular cystine is limited, IL1RAP induces cystathionine gamma lyase (CTH) to activate the transsulfuration (TSS) pathway for *de novo* cysteine synthesis. IL1RAP depletion diminishes GSH pools and induces oxidative stress to trigger *anoikis* and ferroptosis, thereby inhibiting EwS metastatic capacity. Importantly, IL1RAP is minimally expressed in normal tissues, including blood and bone marrow, with placenta as the only normal tissue to express IL1RAP, nominating this protein as a promising immunotherapy target. Here, we uncover that distinct fusion oncoproteins activate IL1RAP expression in cancers of different lineages, including EWS-ETS fusions in Ewing sarcoma (EwS), the ETV6-NTRK3 fusion in diverse tumor types, and the NPM-ALK fusion in anaplastic large cell lymphoma (ALCL). To target IL1RAP in these cancers via immunotherapy, we identified a highly specific IL1RAP binder from phage-display biopanning, and engineered an IL1RAP-directed human IgG based antibody-drug conjugate (ADC; X12-IL1RAP-PBD) linked to the DNA damaging agent, pyrrolobenzodiazepine (PBD). In proof-of-concept studies, our proprietary X12-IL1RAP-PBD induced potent DNA damage, apoptosis, and bystander cell killing in *in vitro*. In preclinical mouse models, X12-IL1RAP-PBD induces dramatic and durable regression of tumor xenografts expressing of the oncofusions at dosing of only 0.2mg/kg. Moreover, essentially no signs of toxicity were noted, with stable body weights and no histological evidence of toxicity in vital organs such as liver, lung, kidney, and brain. Moreover, X12-IL1RAP-PBD fully blocked tail vein injected EwS cell-induced liver changes *in vivo*, again without any toxicity. Thus, this first-in-class IL1RAP-targeting ADC is highly efficacious in preclinical models of multiple oncofusion-driven cancers, warranting further clinical translation.

#7056 The impact of aberrant ZMIZ1 protein expression in pediatric acute lymphoblastic leukemia.

Caroline S. Greenstone[1], Jessica Krugman[2], Danielle W. Cohen[3], John Easton[4], Heather Mulder[4], Ying Shao[4], Xiaotu Ma[5], Nikki A. Evensen[6], William L. Carroll[7]

[1]Barnard College, New York, NY,[2]Pediatric Hematology-Oncology, The Mount Sinai Hospital, New York, NY,[3]Pediatric Hematology-Oncology, NYU Grossman School of Medicine, New York, NY,[4]St. Jude Children's Research Hospital, Memphis, TN,[5]Computational Biology, St. Jude Children's Research Hospital, Memphis, TN,[6]NYU Langone Health, New York, NY,[7]NYU Grossman School of Medicine, New York, NY

While the outcomes for pediatric acute lymphoblastic leukemia (ALL) have improved dramatically over the past 30 years, up to 20% of patients relapse and their prognosis is poor. Collaborators at St. Jude's Research Hospital discovered that mutations in *ZMIZ1* are highly enriched at relapse in a subset of patients. Subsequent studies showed that the transcript level is correlated to event-free survival. Thus, our objective is to study the function of ZMIZ1 in cancer survival under the selective pressures of chemotherapy. To accomplish this, we generated a panel of B-ALL cell lines overexpressing either wild-type (WT), or two different mutant forms of ZMIZ1 found in patients (A291-A305 deletion (Del), or A297T substitution (Sub)) and compared them to an empty vector (EV) control. We performed reverse transcription quantitative PCR (RT qPCR) to confirm mRNA expression, western blots to determine expression of ZMIZ1, as well as cell viability, apoptosis, and proliferation assays to assess functional impact. While full length transcripts were confirmed in all three lines, full length protein was only detected in the WT overexpressing line. All lines expressing the mutant forms of ZMIZ1 demonstrated a lower molecular weight product, suggesting post-translational modification. Phenotypically, we observed profound resistance to prednisolone, a cornerstone of ALL therapy, upon WT (IC50 >1e-1mM) expression compared to EV (IC50=3e-4mM), while cell lines expressing the Del or Sub showed no difference in drug sensitivity (IC50s=3e-4mM). No differences were seen with other commonly used chemotherapies. Since these experiments were done in cell lines with two WT alleles, we then used CRISPR-cas9 to knock out endogenous *ZMIZ1*. Allele specific sequencing for individual clones was confirmed using IDT's rhAmpSeq^{TM} CRISPR kit. *ZMIZ1* complete knockouts (KO) showed no detectable protein expression and had increased sensitivity to prednisolone (IC50=3e-5mM) compared to the Rosa control (IC50=5.7e-5mM) in both cell viability and apoptosis assays. Subsequently, we reintroduced ZMIZ1 (WT and mutants) into KO cells and again only WT had detectable full length protein expression, but now all three lines showed the lower molecular weight product. Resistance to prednisolone was restored completely upon rescue with WT ZMIZ1 (IC50>1e-1mM) compared to the complete KO cells and Rosa. In contrast, mutant cell lines now showed modest resistance to prednisolone (IC50s 4.9e-5mM Del; 5.6e-5mM Sub) compared to KO. Collectively our results indicate a complex relationship between the products of WT and mutant alleles mediated in part by a post-translationally modified species involved in glucocorticoid resistance. A more thorough understanding of how mutations in *ZMIZ1* affect the regulation and drug resistance is vital in determining the clinical significance and evaluating ZMIZ1 as a potential therapeutic target in patients.

CLINICAL RESEARCH: Early Detection Biomarkers 2
Poster Session

#7060 Semi-quantitative lateral flow immunoassay for HPV serology for detection of HPV+ oropharyngeal cancer.

Akshansh Kaushik, Tej Patel, Joshua Eger, Ching Wen Hou, Jennifer Blain Christen, Karen S. Anderson

Arizona State Univ. Biodesign Institute, Tempe, AZ

Background: HPV (human papilloma virus) is a leading cause of 60-70% of oropharyngeal cancer in the US. Antibodies to HPV early antigens are detectable in serum samples up to two decades prior to diagnosis, so that HPV serology are potential biomarkers for screening. We have developed a rapid colorimetric lateral flow assay (LFA) for the semi-quantitative detection of antibodies for HPV16 CE2 and HPV16 E7 antigens.
Methods: Recombinant his-tagged proteins (HPV16 CE2 184-365aa and HPV16 E7) were printed separately on nitrocellulose membranes, with human IgG as a control line. Plasma samples from newly diagnosed HPV+ or p16+ oropharyngeal cases (n=38) and controls (n=46) were selected for assay development and image analysis based on known serostatus. The plasma was applied to the membranes, washed and blocked with casein, and antibodies were detected using a gold-conjugated protein G secondary antibody. A Python program was developed using the OpenCV library for image processing and the scipy.signal library was used to detect bands on the strip as peaks. The area under the curve for these peaks was calculated, with a threshold >700 unit squares. The seropositivity of the LFA was compared with laboratory-based RAPID ELISA HPV serology.
Results: By RAPID ELISA, the sensitivity of CE2 serology was 84% and E7 was 78.9% at >97% specificity for the plasma samples specifically selected for the assay development (not a random or matched cohort). On the LFA, the sensitivity of CE2 serology was 52.6% and E7 was 76.3% at >97% specificity. By LFA, 89.5% of cases were positive for either CE2 or E7 serology at 95.6% specificity, with strong concordance with RAPID ELISA (κ=0.853).
Conclusion: The LFA test demonstrated high concordance for detecting antibodies in plasma samples compared to RAPID ELISA, providing a promising point-of-care tool for large-scale screening of oropharyngeal cancer.

#7061 Biomarkers in TGF-β and corresponding Immune pathway stratify HCC-risk in cirrhotic patients.

Xiyan Xiang[1], Richard L. Amdur[2], Kirti Shetty[3], Herbert Yu[4], Linda L. Wong[5], Nyasha Chambwe[6], Sanjaya K. Satapathy[7], James M. Crawford[8], Lopa Mishra[9]

[1]The Feinstein Institute for Medical Research, Manhasset, NY,[2]The Institutes for Health Systems Science & Bioelectronic Medicine, The Feinstein Institute for Medical Research, Manhasset, NY,[3]University of Maryland, Baltimore, MD,[4]University of Hawaii Cancer Center, Honolulu, HI,[5]Department of Surgery, University of Hawaii, Honolulu, HI,[6]The Institute of Molecular Medicine, The Feinstein Institute for Medical Research, Manhasset, NY,[7]Division of Hepatology, Northwell Health and Donald and Barbara Zucker School of Medicine at Hofstra/Northwell, Hempstead, NY,[8]Department of Pathology and Laboratory Medicine, Donald and Barbara Zucker School of Medicine at Hofstra/Northwell, Hempstead, NY,[9]The Feinstein Institute for Medical Research, New York, NY

Purpose: Hepatocellular carcinoma (HCC) is a leading cause of cancer deaths worldwide, which is mostly diagnosed at advanced and incurable stages. The suboptimal performance of current screening approaches including serum alpha-fetoprotein testing and ultrasound, highlights the urgent need for novel biomarkers to enable risk stratification and early detection of HCC. Based on our previous mechanistic insights with data from human genomic studies available in The Cancer Genome Atlas (TCGA) and from animal models (*Cell Rep. 2024.43(9):114676 and Sci Transl Med. 2021.13(624): eabk2267*), we have identified functional protein markers associated with HCC such as TGFBR2, MSTN, and PKM2. In this study, we present a model based on the TGF-β and associated immune pathways for the HCC stratification in cirrhotic patients that incorporates biologically relevant proteins to capture ongoing pathophysiological changes that drive hepatocarcinogenesis.
Experimental Procedures: A proteomic analysis of 7,000 proteins (SomaScan) utilizing serum samples (55 uL/each) from 311 individuals with cirrhosis, 18 of whom developed HCC by imaging criteria during the median 5-year follow-up. Using a multivariable logistic regression method, we conducted an unsupervised clustering analysis of TGF-β and the corresponding immune pathway and combined with well-described serum biomarkers to create a risk prediction model. We followed this with ELISA validation of the most promising biomarkers.
Results: In our prospective cirrhosis cohort (n=311), 18 HCC cases (5.8%) developed over a median follow-up of 5 years. Using unsupervised hierarchical clustering, we analyzed TGF-β pathway (n=28) and inflammatory/immune response proteins (n=36), grouping patients into two subgroups based on serum protein expression. Among HCC cases, 77.8% showed higher SMAD3 and 66.7% showed higher TGFBR2 expression. Cirrhotic patients with elevated expression of serum HMGB1/IL34/CCL3L1/IL37/SMAD3/TGFBR2 had a six-fold higher HCC risk (10.64% vs. 1.76%). A TGF-β model combining TGFBR2, GPC3, ANGPTL8, and GZMK outperformed the AFP model, with AUCs of 0.88 vs. 0.60 and better sensitivity (0.61 vs. 0.11) at 0.90 specificity. ELISA validation confirmed that MSTN, TGFBR2, and HMGB2 levels were significantly elevated in HCC ($p<0.05$).
Conclusions and Next Steps: This study successfully demonstrates the high performance of our TGF-β and corresponding immune pathway biologically related biomarkers for HCC risk stratification in cirrhotic patients. The next steps will be large-scale validations of this biomarker panel in cirrhotic patients across all racial and ethnic subgroups in Phase II/III studies.

#7062 Potential biomarker role of LCN2, MMP9 and LCN2/MMP9 complex in OSCC.

Smriti Suri[1], Sushmita Ghoshal[2], Jaimanti Bakshi[3], **Arnab Pal**[4]

[1]Biochemistry, Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India,[2]Radiotherapy and Oncology, Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India,[3]ENT, Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India,[4]Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India

Background: Oral Squamous Cell Carcinoma (OSCC) is considered as a major morbidity and mortality in LMICs, with five-year survival rate of less than 50%. Limited availability of early diagnostic markers is one of the important causes of this abysmal outcome. We identified 135 differentially regulated candidate proteins using LC/MS-based shotgun proteomics in the saliva of OSCC patients, of which LCN2 and MMP9 were also found to be significantly upregulated. Dysregulated levels of LCN2, MMP9, and LCN2/MMP9 complex have also been reported in OSCC. To establish the potential role as biomarkers, we evaluated them in OSCC patients.

Methodology: 356 biopsy-proven treatment-naïve OSCC, 26 Oral Pre-Malignant Diseases (OPMDs) and 118 healthy subjects were recruited for the study. 5 ml of unstimulated saliva and 3 ml of whole blood were collected at baseline and during follow-ups. Salivary and serum levels of LCN2, MMP9 and LCN2/ MMP9 complex were estimated using sandwich ELISA. The levels were analysed in various groups and were also correlated with the therapy response and the disease outcome.

Results: Significantly higher (*p≤0.0001*) levels of LCN2 in saliva and serum (median- 609.15ng/ml & 186.18ng/ml) of patients compared to OPMD (median-358.47ng/ml & 124.05ng/ml) and healthy controls (median-95.70ng/ml & 85.53ng/ml) respectively. Salivary and serum levels of MMP9 (median-194.03ng/ml & 3476.755ng/ml) were also found to be significantly higher (*p≤0.0001*) than their levels in OPMD (median-206.50ng/ml & 404.08ng/ml) and control group (median-188.05ng/ml & 540.18ng/ml) respectively. Also, the level of complex of LCN2/MMP9 in saliva and serum (median-145.65ng/ml, median-94.37 ng/ml) was observed to be significantly higher (*p≤0.0001*) than OPMD (median-90.66ng/ml & 55.20ng/ml) and healthy controls (median-70.88ng/ml & 52.76ng/ml) respectively. The increased level of these proteins also correlated with staging and was also found to have a decreasing trend with successful therapy. On ROC analysis, for identifying the ability as a diagnostic marker, significant AUCs for LCN2 in saliva and serum of patients compared to healthy controls were found (0.7446 & 0.6802) with a sensitivity of (88.64% & 77.25%) and specificity of (60.17% & 60.17%) respectively. For MMP9, the AUC for serum was significant (0.9326), with a sensitivity of 85.01% and specificity of 95.54%. AUC for LCN2/MMP9 complex for saliva and serum were also found to be significant (0.7415 & 0.7266) with a sensitivity of (86.74% & 78.80%) and specificity of (52.54% & 63.56%) respectively. Survival curve analysis revealed that pretreatment salivary LCN2 can be used as a predictive marker for OS of OSCC patients with an HR of 1.915 (CI 1.173 to 3.127) and salivary levels of MMP9 for DFS with HR of 2.823 (CI 0.9813 to 8.122).

Conclusion: Baseline LCN2, MMP9, and LCN2/MMP9 complex can be used as potential biomarkers for the diagnosis and prognostication of patients with OSCC.

#7063 Monoclonal antibody evaluation of oxidative DNA base damage in normal and neoplastic breast tissues: development of antibodies specific for 8-OH-adenine and 4,6-diamino-5-formamidopyrimidine (FAPY-adenine).

Zenzeale A. Hudson[1], Sarah McLaughlin[2], Alfredo A. Paredes[3], Gary K. Ostrander[1], Eric H. Holmes[1]

[1]Biomedical Sciences, Florida State University, Tallahassee, FL,[2]Department of Surgery, Mayo Clinic, Jacksonville, FL,[3]Tallahassee Plastic Surgery Clinic, Tallahassee, FL

Breast cancer affects 1 in 8 women and often results from mutations caused by oxidative DNA damage from reactive oxygen species. Two key lesions, 8-hydroxy-adenine (8-OH-Ade) and 4,6-diamino-5-formamidopyrimidine (FAPY-Ade), exhibit two distinct patterns: FAPY-Ade lesions are predominant in normal tissues, while 8-OH-Ade lesions are abundant in cancerous tissues. These shifts reflect tissue redox status, which may provide a pathway to assess future breast cancer risk. Quantitation of these lesions by chemical detection methods is precise but time-intensive and impractical for high-throughput clinical applications. To address this, monoclonal antibodies have been developed and offer a rapid, scalable, and clinically practical alternative for efficiently detecting and quantifying these biomarkers. This innovation can potentially support personalized breast cancer screening strategies. BALB/c mice were immunized with 8-OH-Ade or FAPY-Ade haptens coupled to Keyhole Limpet Hemocyanin (KLH). After three monthly immunizations, the serums of IgG and IgM titers were evaluated. The mice having the highest IgG titer for each antigen were given an intravenous boost, and the spleens were removed three days later for fusions according to standard methods. Using hapten-conjugated BSA-coated 96-well Pro-Bind plates, the ELISA results demonstrated 30-40 positive wells for each antigen. Up to ten positive wells for each antigen were selected and cloned to yield monoclonal antibody-producing hybridomas. The ELISA of the anti-FAPY-Ade antibody, FA5, showed concentration-dependent reduced binding to immobilized BSA-FAPY-Ade hapten conjugate in increasing FAPY-Ade, confirming its FAPY-Ade binding specificity. Immunohistochemistry using normal and cancerous breast tissue revealed significant staining of normal breast tissue compared to cancerous breast tissue using the FA5 antibody. Conversely, the anti-8-OH-Ade antibody, 8A6, displayed weak staining of normal breast tissue with higher staining of cancerous breast tissues. The results demonstrate that monoclonal antibodies specific for 8-OH-Ade and a novel antibody specific for the ring-opening reactive oxygen product, FAPY-Ade, are practical and rapid alternatives to chemical detection methods for their analysis. Immunohistochemistry mirrored past results using chemical detection methods for these oxidized bases, with high FAPY-Ade compared to 8-OH-Ade in normal tissues and the opposite result in cancer-derived tissues. The potential for high-throughput analysis of 8-OH-Ade and FAPY-Ade levels in the DNA of breast tissues may provide information on future breast cancer risk. Further research is underway to evaluate this hypothesis.

#7064 Minimally invasive detection of advanced adenomas in colorectal cancer screening through circulating epithelial cells and somatic DNA aberrations.

Shai Friedland[1], Drew Watson[2], **Zhen Zhang**[3], Alexander Atkins[2], Huangpin Ben Hsieh[2], Jr-Ming Lai[2], Stephen Su[2], Hung-Jen Shao[2], Jen-chia Wu[2], John Sninsky[2], Rui Mei[2]

[1]Standford University, Stanford, CA,[2]CellMax Life, Sunnyvale, CA,[3]Johns Hopkins University, Baltimore, MD

Aim

Advanced adenomas (AAs), precursors to colorectal cancer (CRC), affect 5% of individuals under 50 and 8% of those 50 and older. Current blood-based tests have limited AA sensitivity (12-13%) compared to FIT (23.8%), highlighting the need for improved detection methods. The FirstSight assay combines circulating tumor DNA (ctDNA) and circulating epithelial cells (CECs) to enhance AA detection. Initial results show that FirstSight achieves higher sensitivity and specificity than existing blood-based tests. Here we analyze the contributions of ctDNA and CEC in the detection of AA.

Methods

FirstSight was validated in a study of 1,038 prospectively collected, diverse subjects from a majority screening cohort. CEC data were analyzed using a multi-dye machine learning algorithm. The FirstSight algorithm incorporated patient age, sex, and CRC incidence from SEER statistics to adjust baseline risk and refine probability distributions for improved predictive performance. We estimated the contribution of ctDNA and CEC in the detection of AA, and as an example, the cumulative effect of ctDNA and a single CEC-derived marker GM1.

Results

CEC ($p < 0.01$) and ctDNA ($p < 0.0001$) biomarkers were significant predictors of AA. A CEC marker, GM1, improved specificity for AA. At a prespecified 90% specificity for the detection of advanced neoplasia (CRC+AA), the best single ctDNA marker and the CEC marker GM1 individually had a sensitivity of 20% and 16%, respectively, for AA. The combined use of all ctDNA markers had a sensitivity of 39% for AA and the further addition of GM1 raised the sensitivity to 49% for AA. Finally, the integration of ctDNA, CEC markers, their interaction, and demographics improved FirstSight sensitivity to 54.5% for AA.The contribution of GM1 was significant ($\chi^2 = 9.7272$, $p = 0.02103$); removing GM1 reduced the AUC for AA from 0.8165 to 0.745. Consistent with the CRC dysplasia-malignancy continuum, FirstSight demonstrated higher sensitivity for stage I CRC compared to first-generation blood-based tests (92.3% vs. 54.5%).

Conclusions

The multimodal FirstSight test, combining ctDNA and CEC biomarkers, achieved 54.5% sensitivity and 90.6% specificity for AA detection in a 1,038-subject study. CEC signals are found to be most contributory to the detection of AA and the interaction of a single CEC marker, GM1, with ctDNA further improves AA sensitivity, making the performance not simply additive. FirstSight demonstrates the potential for superior AA detection and effective colorectal cancer prevention strategies. This minimally invasive test offers a patient-friendly alternative, with ongoing validation to confirm its clinical utility.

#7065 Desmocollin3 and tumor immune infiltrate: A bioinformatics evaluation.

Bakulesh M. Khamar[1], Sanjay V. Malhotra[2]

[1]Research & Development Division, Cadila Pharmaceuticals Limited, Ahmedabad, India,[2]Cell, Development and Cancer Biology, Center for Experimental Therapeutics, Knight Cancer Institute, Oregon Health & Science University, Portland, OR

Introduction: Desmocollin-3 (DSC3), a transmembrane glycoprotein is expressed in various cancers like squamous lung, head & neck, Cervical, Melanoma, bladder cancer etc. DSC3 is also a predictive biomarker for efficacy of Mycobacterium w (Mw) vaccine, a heat-killed suspension of a non-pathogenic mycobacterium. It induces a DSC3-specific cell-mediated (CD8+ T cells) immune response and considered to be a barrier for immune infiltrate. Efficacy of anti-PD1/PDl1 therapy depends on tumor infiltrating immune cells. Present study focuses on the key immune genes and their relationship with DSC3 gene.
Materials and Methods: DSC3 and other genes encoding various immune functions (Table) were evaluated across 17 cancers using cBioPortal TCGA PAN CANCER data set. For correlation between DSC3 and other immune genes, samples with a positive Z score for DSC3 or upper quartile DSC3 (lower of two) were used.
Results: DSC3 expression in Squamous lung cancer, Cancer cervix, head & neck, melanoma, colorectal cancer, mesothelioma and adenocarcinoma stomach were found to have major impact with negative Z score for majority of CD8, M1, Th1, T exhaust, M2 and Th2 genes suggesting lower infiltration of immunosuppressive and immunostimulant immune cells. Following genes had negative Z scores in all seven cancers:1. CD8 group: CD8A, CD8B, GZMA, GZMB, GZMK, GZMM, PRF1, TBX12. M1 group: CCL15, CCL20, CCR7, CXCL10, FCGR1BP, CYBB, Il15, 1I15RA3. Th1 group: CXCR3, IL274. Texhast; BTLA, LAG3, PDCD1, SLAMF6, TIGIT 5. Th2: BATF, CXCR6, SOCS1 6. M2: CD163Similar finding of lower magnitude were also seen in bladder cancer, ovarian cancer, thyroid cancer, pancreatic cancer, adenocarcinoma lung, uterine cancer, testicular germ cell cancer, renal cancer, sarcoma, hepatocellular cancer.
Conclusion: DSC3 expressing tumours are likely to have lower TIL (immunostimulant as well immunosuppressive) and may not be ideal candidate for check point inhibitors.

Cytotoxic CD8 T cells									
CD8A	CD8B	CXCL10	CXCL9	GZMA	GZMB	GZMH	GZMK	GZMM	PRF1
TBX21	TNFSF10								
M1 immune response									
CCL15	CCL20	CCR7	CXCL10	CYBB	FCGR1B	FPR2	IL15	IL15RA	IL1B
MARCO									
M2 immune response									
BMP4	CCL13	CD163	CD209	CD63	CD68	CEBPB	HSD11B1	IL4R	MRC1
PPARG	SIGLEC5	STAT6							
T cell exhaustion									
BTLA	CD160	CD274	CD276	CTLA4	EOMES	LAG3	PDCD1	PDCD1LG2	PTGER4
SLAMF6	TIGIT	VSIR							
Th1 immune response									
AIMP1	CCR1	CCR5	CD4	CD44	CXCR3	IFNGR1	IL12RB2	IL18R1	IL27
IL27RA	STAT4								
Th2 immune response									
BATF	CXCR6	EGR2	GATA1	GATA2	GATA3	GATA4	GATA5	GATA6	IL6
IL7R	SOCS1	SOCS2	SOCS4	SOCS5	SOCS6	SOCS7			

#7066 RING1 inhibits smoking-induced lung cancer tumorigenesis by ubiquitination and degradation of CIP2A.

In-ho Jeong, Peter Chang Whan Lee

University of Ulsan College of Medicine, Seoul, Korea, Republic of

Lung cancer is the second incidence rate of all new cancer diagnoses and the leading cause of cancer-related deaths globally. To develop novel diagnostic and therapeutic targets for lung cancer, there are emerging needs for molecular biological research on the occurrence and progression. Among the targets, CIP2A has been studied for promoting various cancer types, but detailed underlying mechanisms are needed to be further investigated. In this study, it was verified that CIP2A is overexpressed in lung cancer tissues, particularly amongst those with a smoking history, compared to adjacent normal tissues. CIP2A knockdown in lung cancer cells efficiently reduced cell proliferation and migration capacity. Through the mass spectrometry analysis on A549 cells overexpressing CIP2A, RING1 was identified as a candidate for E3 ligase of CIP2A. The interaction of the two proteins and ubiquitin chain regulation by RING1 was verified by immunoprecipitation. RING1 knockdown enhanced proliferation and migration of lung cancer cells, and these effects result from the upregulation of CIP2A and its downstream molecules, c-Myc and cyclin B1. To further study a trigger for RING1 expression changes in lung tumorigenesis, cigarette smoke extract (CSE) was applied for the following experiments. CSE treatment depleted RING1 mRNA, including protein expression. Decreased RING1 level led to upregulation of CIP2A and c-MYC. Collectively, these results reveal novel roles of the RING1 and CIP2A in lung cancer progression caused by smoking and propose a potential biomarker candidate for the therapeutic and diagnostic targets.

* Keywords: Lung cancer, UPS, CSE

#7067 Integrating blood-based biomarkers and RNA-Seq Data for enhanced colorectal cancer screening.

Elba V. Caraballo-Rivera[1], Hilmaris Centeno-Girona[1], Camille Zenon-Melendez[1], Sheila Lopez -Acevedo[1], Brenda Torres-Velasquez[1], Josue Perez-Santiago[1], Madeline M. Martir-Ocasio[1], Maria Gonzalez-Pons[1], Marcia R. Cruz-Correa[2]

[1]University of Puerto Rico Comprehensive Cancer Center, San Juan, Puerto Rico,[2]School of Medicine, University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico

Colorectal cancer (CRC) is the second cause of cancer-related deaths in the United States and the first leading cause for Puerto Rican Hispanics (PRH). Despite its preventability through screening, compliance still needs to improve due to the invasiveness of current tools. There is a growing demand for validated molecular biomarker panels for minimally invasive blood-based CRC screening. This study evaluated the diagnostic accuracy of four promising blood-based biomarkers and incorporated RNA-seq data from an independent cohort to provide biological insights into biomarker performance. A retrospective case-control study was conducted in plasma samples from 124 CRC cases and 124 age- and sex-matched controls recruited through the Puerto Rico Familial Colorectal Cancer Registry (PURIFICAR). Biomarkers tested included methylated DNA encoding the Septin-9 gene (*mSEPT9*) using Epi proColon® 2.0 CE, insulin-like growth factor binding protein 2 (IGFBP2), dickkopf-3 (DKK3), and pyruvate kinase M2 (PKM2) by ELISA. Diagnostic accuracy was measured using the receiver operating characteristic (ROC) area under the curve (AUC) and sensitivity and specificity. For the RNA-seq (Illumina) assay, a case-case study was performed on 15 tumor and 15 adjacent mucosa samples from 7 early and 8 advanced-stage CRC cases. Differentially expressed genes (DEGs) with an adjusted p-value < 0.05 were identified between tumor and adjacent mucosa across stages. Pathway enrichment was performed using Gene Set Enrichment Analysis (GSEA) and KEGG databases. Biomarkers and gene expression patterns were cross-validated to identify shared pathways. Diagnostic accuracy for individual biomarkers *mSEPT9*, IGFBP2, DKK3, and PKM2 was 62.9%, 69.7%, 61.6%, and 50.8%, respectively. Combined biomarkers AUC was of 74.4%, outperforming all individual markers except IGFBP2 in advanced-stage CRC. RNA-seq identified 481 DEGs unique to early-stage and 849 to advanced-stage CRC. Genes DKK3 and PKM2, were differentially expressed in both stages. Additionally, DKK1 and DKK2, were uniquely upregulated in early-stage tumors. Dysregulation of *Wnt* signaling, PI3K-Akt signaling, and cytokine-cytokine receptor interaction pathways were observed across stages. Our results show that integrating plasma biomarker analysis with RNA-seq data enables the identification of critical shared pathways, supporting the utility of a multi-omics approach for CRC screening. Plasma biomarkers demonstrated promising diagnostic accuracy, with IGFBP2 as the most accurate individual marker across CRC stages. The differential expression of DKK1 and DKK2 in early-stage tumors highlights their potential as early CRC biomarkers, warranting validation in plasma samples. These findings support the development of minimally invasive blood-based screening tools for CRC, offering a promising strategy for early detection and improved patient outcomes for PRH.

#7068 Diagnostic and prognostic application of blood and tissue cadherin-17 biomarker in pancreatic cancer.

William C. Cho[1], Wing Shan Yu[2], Tsz Chun Hon[2], Elaine T.Y. Cheung[3], Xiao Qi Wang[4], Qi-Hua Zhang[5], Hui-Yan Luo[5], Dennis Anthony Wong[6], Wah Cheuck[3], John M. Luk[6]

[1]Department of Clinical Oncology, Queen Elizabeth Hospital, Hong Kong SAR, China,[2]Tiberias Technology (HK) Limited, Hong Kong SAR, China,[3]Department of Pathology, Queen Elizabeth Hospital, Hong Kong SAR, China,[4]Caleb BioMedical Company Limited, Guangzhou, China,[5]Sun Yat-sen University Cancer Center, Sun Yat-sen University, Guangzhou, China,[6]Arbele Corp., Seattle, WA

Background: Pancreatic cancer (PC) is one of the most lethal cancers, with a 5-year overall survival (OS) rate of ~10%. CA19.9 remains the gold standard biomarker for detecting PC, but its performance is poor, necessitating the exploration of better alternatives. Cadherin-17 (CA17) is overexpressed in several gastrointestinal cancers but shows limited expression in normal tissues, indicating potential diagnostic and prognostic applications in PC. This study aimed to evaluate the diagnostic and prognostic value of CA17 in PC.
Methods: We have newly developed an immunohistochemistry (IHC) platform to compare CA17 expression in PC tissues (n = 127), tumor-adjacent tissues (n = 86), and normal pancreatic tissues (n = 22) obtained from 50 retrospective PC cases, alongside a commercial tissue microarray of PC and normal samples. Digital imaging analysis was performed to quantify CA17 expression with an IHC scoring algorithm that combines the percentage of positive cells and staining intensity (M score). Additionally, 86 retrospective plasma samples were collected from asymptomatic donors and 49 patients with paired pre- and post-treatment plasma. CA17 and CA19.9 levels in plasma were measured using our newly developed highly sensitive and specific CA17 immunoassay and a commercial CA19.9 ELISA kit, respectively.
Results: Normal pancreatic tissues showed negative CA17 IHC results. A significantly higher CA17 M score was observed in PC compared to both normal and cancer-adjacent tissues (median M score: 5.83, 0.38, 0.85, respectively; $p < 0.001$). We observed a significant elevation in blood CA17 levels from asymptomatic donors to pre-treatment stage I/II PC patients, with a decrease in CA17 expression in stage III/IV patients (median in ng/mL: 1.45, 7.14, 2.58, respectively; $p < 0.0001$). Compared to pre-treatment plasma, CA17 levels decreased in post-treatment samples from stages I/II patients, while remaining stable in stages III/IV patients (median in ng/mL: 4.02, 2.42, respectively). Similarly, CA19.9 expression increased progressively from normal donors to stages I/II and stages III/IV patients (median in U/mL: 43, 56, 1446, respectively, $p < 0.0001$). The ROC curve of the CA17 assay showed an AUC of > 0.92, with both sensitivity and specificity of 86% in detecting stages I/II PC, surpassing CA19.9 (55% and 90%, respectively). Stage III/IV PC patients were stratified based on median-high and low CA17 and CA19.9 levels for a 2-year OS analysis, indicating that combining CA17 and CA19.9 in predicting prognosis outperforms each biomarker individually. Patients with both median-high CA17 and CA19.9 levels had a significantly worse OS than those with median-low CA19.9 (median OS: 7 vs 13 months, $p = 0.04$).
Conclusion: CA17 shows promise as a biomarker for early PC detection and prognosis prediction, potentially improving the screening of high-risk individuals for PC and aiding in disease management.

#7069 HPV-related oropharyngeal cancer: Circulating and salivary DNA-based biomarkers for early diagnosis and recurrence monitoring.

Ondrej Bouska[1], Vladimira Koudelakova[1], Zuzana Horakova[2], Marian Hajduch[1]

[1]Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University Olomouc, Olomouc, Czech Republic,[2]Department of Otorhinolaryngology and Head and Neck Surgery, University Hospital Olomouc, Olomouc, Czech Republic

Background: The incidence of oropharyngeal squamous cell carcinoma (OPSCC) has surged worldwide in recent decades. In the Czech Republic, annual OPSCC cases have more than tripled over 30 years, with nearly 800 new cases diagnosed in 2021, surpassing cervical cancer. OPSCC is now predominantly linked to high-risk HPV infection, with about 25% of patients experiencing recurrence within five years. Liquid biopsies (plasma and saliva) are being investigated for their potential in diagnosis, treatment monitoring, and recurrence detection. These efforts focus on DNA-based biomarkers, such as circulating tumor HPV DNA (ctHPV DNA) and oral HPV DNA.
Methods: HPV tumor status was assessed in newly diagnosed OPSCC patients and those in remission using high-risk HPV DNA detection in primary tumor tissue and p16 immunohistochemistry. Only cases positive for both high-risk HPV and p16 were classified as true HPV-related OPSCC. Oral HPV DNA and ctHPV DNA testing were conducted on liquid biopsies (gargle lavage, oropharyngeal swabs, and plasma) before and after treatment, with regular follow-up sampling.
Results: A total of 169 OPSCC patients were enrolled in the study, with 81.8% classified as HPV-related, predominantly involving the HPV16 genotype (98% of cases). Pre-treatment detection of tumor-derived high-risk HPV genotypes in liquid biopsies demonstrated excellent sensitivity: 92% in gargle lavage, 96.3% in oropharyngeal swabs, and 96.3% for ctHPV DNA in plasma, all achieving 100% specificity across sample types. Concordance in high-risk HPV genotype detection between gargle lavage and oropharyngeal swabs reached 94.2% in pre-treatment samples. Among recurrent HPV-related OPSCC cases, ctHPV DNA was detected in 75% (3/4) of cases, with 50% (2/4) also testing positive for oral HPV DNA.
Conclusion: This study aims to validate the combined use of liquid biopsy collection, oral HPV DNA detection, and ctHPV DNA detection for early diagnosis and recurrence monitoring in HPV-related OPSCC patients. Preliminary findings are encouraging, demonstrating the potential for early detection, including in early-stage OPSCC, and highlighting the clinical applicability of these methods for HPV-related OPSCC screening.
This study was supported by the Internal Grant Agency of Palacky University (IGA LF UP 2024_007 and IGA LF 2024_15), the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union - Next Generation EU, EATRIS-CZ (LM2023053), MH CZ - DRO (FNOL, 00098892), and SALVAGE (811100711/37).

#7070 A deeper evaluation of cytokeratin fragment 21-1 as a lung cancer tumor marker and comparison of different assays.

Dianna J. Rowe[1], Timothy A. Khalil[1], Michael N. Kammer[1], Caroline M. Godfrey[1], Yong Zou[1], Cindy Vnencak-Jones[2], David Xiao[1], Stephen Deppen[3], Eric L. Grogan[3]

[1]Allergy, Pulmonary, and Critical Care Medicine, Vanderbilt University Medical Center, Nashville, TN,[2]Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN,[3]Thoracic Surgery, Vanderbilt University Medical Center, Nashville, TN

Studies show CYFRA 21-1 fragments of cytokeratin 19 (CK19) to be promising biomarkers for non-small cell lung cancer (NSCLC). Although previous literature identifies specific CYFRA 21-1 antibody binding epitopes, the exact molecular weight of the CK19 fragment being detected by current assays is not well-documented. It is believed that during apoptosis, CK19 is cleaved by caspases and resulting fragments are released into the serum. A proposed caspase cleavage site is located between amino acids 235 and 238 of its sequence. Upon cleavage, two fragments are released; the fragment bearing the C-terminus of CK19 is characterized as CYFRA 21-1. Two specific antibodies bind to epitopes localized to the CYFRA 21-1 fragment upstream of the caspase cleavage; KS 19.1 is located at aa 311-335 and BM 19.21 at aa 346-367. Although this antibody binding pattern suggests specific targeting of the CYFRA 21-1 fragment, there is no clear consensus on the molecular weight of the CYFRA 21-1 fragment being detected through currently available assays, suggesting that perhaps different assays detect different fragment lengths. Serum samples from 58 patients (lung cancer (N=36), control (N=22)) were used to measure CYFRA 21-1 across four different quantification assays: enzyme-linked immunosorbent assay (ELISA), chemiluminescent assay (ChLIA), electrochemiluminescence immunoassay (ECLIA), and compensated interferometric reader (CIR). In the cancer group, correlation between ECLIA and ELISA was high ($R_{(Pearson)}$=0.948, $r_{(Spearman)}$=0.868) while correlation between ECLIA vs ChLIA and ECLIA vs CIR was low (R = 0.005, r= -0.0593), (R=0.0275, r= 0.167), respectively. In the control group, correlation between ECLIA and ELISA was high (R=0.948, r=0.868) while correlation between ECLIA vs ChLIA and ECLIA vs CIR was low (R= 0.005, r= -0.0593), (R=0.0275, r= 0.167), respectively. Compared to ECLIA, concordance coefficients (p_c) were poor (p_c <0.90) across all assays except for cancers group in ELISA (p_c = 0.913). ECLIA was the only assay to report ranges above 1 ng/mL CYFRA 21-1 (ECLIA, 1.14-21.59 ng/mL; ELISA, 0.79-24.26 ng/mL; ChLIA, 0.062-0.691 ng/mL; 0.08-7.68 ng/mL). Differing sizes of the protein being measured by each assay may have a role in the discrepancies observed. Based upon structural information, a CK19 protein cleaved at one caspase site would result in 18.4 kDA CYFRA 21-1 and 25.8 kDa N-terminus fragments, while many manufactured methods report molecular weights of approximately 30kDa. Given the different CYFRA 21-1 concentration estimates among assays, further characterization of the fragment and its release during epithelial malignancies, such as NSCLC, is imperative to developing effective biomarker assays.

#7072 Lung cancer risk prediction in Asian populations: A study of immune signatures from the Singapore Longitudinal Ageing Studies.

J. Ye[1], J. Meng[1], G. Lai[2], X. Lim[1], D. Goh[1], W. Xu[1], Y. Wu[1], L. Chong[1], D. S. Tan[3], D. W. Lim[1], R. Ho[4], **J. Yeong**[5];
[1]Agency for Science, Technology And Research, Singapore, Singapore, [2]National Cancer Centre Singapore, Singapore, Singapore, [3]National Cancer Centre Singapore, SingHealth Duke-NUS Medical School, Singapore, Singapore, [4]Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore, [5]Agency for Science, Technology And Research, Singapore General Hospital, Singapore, Singapore

Background: The immune system plays a crucial role in the development and progression of cancer, particularly during aging. Given the distinct genetic and environmental variations in Asian populations compared to Western cohorts, we aimed to evaluate the predictive significance of immune markers for early lung cancer detection in an Asian population.
Methods: Two independent cohorts of elderly Asians from the Singapore Longitudinal Ageing Studies (SLAS I/II, NCT03405675) were investigated for immune markers related to lung cancer. Subjects aged 55 years or older, capable of self-ambulation were recruited in 2003 for SLAS I and 2008 for SLAS II (median follow-up period of 17 and 12 years, respectively). Presence of lung cancer served as the clinical endpoint for analysis. Lung cancer diagnoses (69 in SLAS I and 59 in SLAS II) were confirmed through the Singapore National Cancer Registry. Baseline plasma cytokine levels were measured using a 65-plex Human ProcartaPlex Luminex panel (SLAS I: 43 cases, 70 controls; SLAS II: 11 cases, 11 controls). Feature selection was conducted by bootstrapping the summary of 500 differential expression analyses between cases and controls (P<0.05, t-test). Four machine learning algorithms, Logistic Regression, Random Forest, XGBoost, and Support Vector Machine, were used to train models for the prediction of lung cancer using SLAS I, and validated using SLAS II. Characterization of peripheral blood mononuclear cells were performed using a 24-plex flow cytometry on a subset of SLAS II (8 cases, 8 control).
Results: A total of 28 cytokines showed significant differences between cases and controls, with TSLP emerging as the most important in the Random Forest, XGBoost models and Logistic Regression model. Comparing models based solely on demographic factors (age, sex, smoking, and presence of COPD) to models that incorporate demographic and Luminex factors, the mean AUC increased from 0.65 to 0.71. External validation showing an AUC improvement from 0.68 to 0.85. Immuno-profiling using flow cytometry revealed a significantly reduced proportion of γδ T cells and reduced CD57-expression in γδ T cell subsets in lung cancer cases.
Conclusions: The cytokine TSLP may be a biomarker for assessing the risk of lung cancer. TSLP and its interaction with γδ T cells and CD57-expressing γδ T cells may play key roles in aging-related lung cancer development.
Acknowledgements: The work was supported by the A*STAR Biomedical Engineering Programme (C211318003), Industry Alignment Fund-Industry Collaboration Project (I2201E0014; I2001E0065), A*STAR Joint Council Office (1434M00115), A*STAR GAP Funding (I20AGAG602; I23D1AG048), SIgN Immunomonitoring Platform (BMRC IAF 311006 grant; BMRC transition funds #H16/99/b0/011), and Singapore National Medical Research Council (MOH-000323-00; OFYIRG19may-0007; SYMPHONY; MRC/OFLCG/002-2018; OFLCG18May-0028).

#7073 Development and validation of a high-specificity PCR algorithm for ovarian cancer diagnosis.

Eunyong Ahn[1], Se Ik Kim[2], Sungmin Park[1], Sarah Kim[1], Hyunjung Kim[1], Hyejin lee[1], Heeyeon Kim[3], TaeJin Ahn[1], Yong-Sang Song[4]

[1]ForetellMyHealth, Inc., Pohang-si, Gyeongsangbuk-do, Korea, Republic of,[2]Department of Obstetrics and Gynecology, Seoul National University Hospital, Seoul, Korea, Republic of,[3]Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea, Republic of,[4]Department of Obstetrics and Gynecology, Myongji Hospital, Gyeonggi-do, Korea, Republic of

Ovarian cancer (OC) remains one of the most lethal gynecologic malignancies, largely due to difficulties in achieving early diagnosis. To address the limitations of next-generation sequencing (NGS) platforms, particularly their high cost, we developed a qPCR-based method tailored for the early detection of ovarian cancer. Building on our previous research, we systematically selected and evaluated a refined panel of biomarkers optimized for qPCR detection. This study focuses on the development and validation of a highly specific PCR-based diagnostic algorithm, with particular emphasis on detecting high-grade serous ovarian cancer (HGSOC), the most common and aggressive subtype. A total of 30 samples were utilized to train the diagnostic model, supplemented by additional validation using QC-passed (5 benign and 1 cancer samples) and QC-failed samples (1 benign and 9 cancer samples). Biomarker selection prioritized transcripts with low expression in normal and benign tumor samples but significant outliers in ovarian cancer cases. A panel of 12 diagnostic markers and 1 reference marker was finalized based on statistical parameters such as variance, quantiles, and coverage of ovarian cancer-specific aberrations. PCR data were normalized using ACTB as the reference marker. Marker-specific cutoffs were determined from the training data, and a scoring system was devised to effectively differentiate ovarian cancer from non-OC samples. The algorithm achieved 100% specificity within the training set, missing one cancer sample out of 14, resulting in a sensitivity of 92.9%. Validation using QC-passed samples demonstrated a reasonable separation between OC and non-OC groups. The algorithm's robustness was further evaluated using QC-failed samples with challenges such as gDNA contamination and low RNA integrity (RIN), where performance for key markers was largely preserved. In the validation dataset, the algorithm missed one benign sample from the QC-passed group and one cancer sample from the QC-failed group, yielding an overall specificity of 83.3% and sensitivity of 90.0%. To address variability and refine score thresholds, synthetic datasets were generated, enabling the definition of "grey zone" ranges to manage overlapping distributions between OC and non-OC samples. The developed PCR-based diagnostic algorithm achieved exceptional specificity for ovarian cancer detection. This platform shows promise for clinical application in early ovarian cancer diagnosis, particularly for HGSOC, through precise molecular profiling of platelet RNA. Future work will focus on expanding sample diversity and exploring integration with broader biomarker panels.

#7074 Expression and clinical impact of the prostate specific membrane antigen and programmed death-ligand 1 on disseminated tumor cells from early triple negative breast cancer patients.

Anastasios Tolios[1], Vasileios Vardas[1], Ann-Kathrin Bittner[2], Oliver Hoffmann[2], Rainer Kimmig[2], Galatea Kallergi[1], **Sabine Kasimir-Bauer**[2]

[1]Department of Biology, Laboratory of Biochemistry/Metastatic Signaling, Patras, Greece,[2]Gynecology and Obstetrics, Essen University Hospital, Essen, Germany

Background: Triple-negative (TN) breast cancer (BC) is the most aggressive BC subtype resulting in worse outcome. Currently, neoadjuvant chemotherapy (NACT) in combination with an immune checkpoint inhibitor is the standard of care in cases of high-risk early (e)TNBC. With regard to the unmet need of new biomarker research, we recently demonstrated that the presence of the prostate specific membrane antigen (PSMA) as well as the programmed death-ligand 1 (PD-L1) on circulating tumor cells in the blood of TNBC patients (pts) were poor prognostic factors for disease outcome. The present study aimed to investigate the presence and persistence of these markers on disseminated tumor cells (DTCs), already present in the bone marrow (BM) of eTNBC pts, to elucidate their value for current and future treatment strategies.

Methods: 10-20 ml BM of 55 pts (n=55 before and n=48 after NACT; N=44 paired samples) diagnosed between 2014 and 2017, were aspirated and underwent Ficoll-Hypaque density gradient centrifugation, followed by Immunofluorescence triple staining experiments (CK/PSMA/CD45 and CK/PD-L1/CD45) and subsequent VyCAP analysis to identify PSMA and PD-L1 expression on epithelial, cytokeratin (CK)-positive DTCs. Statistical analysis was conducted using the IBM SPSS Software.

Results: In general, PSMA and PD-L1 were upregulated in TNBC pts' DTCs. Before NACT, the $PSMA^+CK^+CD45^-$ phenotype was identified as the most predominant one in 38/55 pts (69%) but rarely present after NACT [4/47 pts (9%); (69% vs 9%, *p<0.001*)]. In 3/4 pts, the $PSMA^+CK^+CD45$ phenotype persisted over the course of NACT, while only in one patient, PSMA-positive DTCs were present exclusively after therapy. The $PD\text{-}L1^+CK^+CD45^-$ phenotype was detected in 21/50 pts (42%) before and in 13/48 pts (27%) after NACT with a persistence of these cells in 6/21 pts. In 13/21 pts, $PD\text{-}L1^+CK^+CD45^-$ DTCs were only present before NACT and 6/13 pts expressed this phenotype after NACT but not at primary diagnosis. The presence of $PSMA^+CK^+CD45^-$ DTCs before NACT was associated with a shorter progression-free (log-rank *p=0.002*, HR=16.1) and overall survival (log-rank *p=0.016*, HR=2.1), respectively. The presence and/or persistence of PD-L1-positive DTCs did not result in any significant correlations with regard to outcome.

Conclusions: PSMA-positive DTCs were identified as a poor prognostic factor with regard to outcome which confirms our data in circulating tumor cells of TNBC. The presence and persistence of PD-L1-positive DTCs is a promising biomarker to stratify pts for checkpoint inhibition and estimate its success.

#7075 Association between homologous recombination deficiency (HRD)-related biomarkers and signatures in advanced solid tumors.

Xiao Yang Jin, Cai Chen, **Xiao Qiao Liu**, Ping Qiu, Alexander Gozman, Razvan Cristescu, Matthew J. Marton

Merck & Co., Inc., Rahway, NJ

Background: Antitumor activity was observed with olaparib plus pembrolizumab in participants with HRD-positive or homologous recombination repair (HRR)-mutated advanced solid tumors. We assessed HRD-related biomarkers and their association with biomarkers that are predictive of response to pembrolizumab.

Methods: HRD-related biomarkers (BRCA mutations, genomic loss of heterozygosity [gLOH], Signature 3 [Sig3; SigMA score]), tumor mutational burden (TMB), and microsatellite instability-high (MSI-H) were analyzed with FoundationOne®CDx using tumor tissue from participants screened for enrollment in KEYLYNK-007 (NCT04123366). Calculation of SigMA score from FoundationOne CDx data was validated using an internal training set (ovarian cancer, n = 44; breast cancer, n = 96) by comparison with deconstructSigs-based Sig3 from paired whole exome sequencing data as a gold standard. Area under the receiver operating characteristic curve (AUROC) was used to assess the association between HRD-related markers and BRCA biallelic mutation. A Wilcoxon rank sum test was used to compare gLOH and SigMA with BRCA biallelic mutation. Associations between all other biomarkers were evaluated using a Spearman correlation coefficient. The data cutoff date was July 14, 2023.

Results: Tumor samples from 2529 participants screened in KEYLYNK-007 were evaluable by FoundationOne CDx; gLOH was evaluable in 1595 participants. Biallelic BRCA mutations were most prevalent in ovarian (8%), prostate (7%), pancreatic (7%), and breast tumors (5%). Among these 4 tumor types, the SigMA score had higher evaluability than the gLOH (100% vs 72%); compared with HRR wild-type tumors, the gLOH and the SigMA score were higher in tumors with biallelic BRCA mutations versus HRR wild-type tumors (P <0.001) with comparable AUROC (0.76 vs 0.71). gLOH (P = 0.036) but not SigMA score (P = 0.092) was also higher in tumors with monoallelic BRCA mutations versus HRR wild-type tumors. Weak to moderate correlations were observed between gLOH and SigMA score, regardless of BRCA mutation status (ρ <0.5). gLOH and SigMA score were not correlated with TMB (ρ = 0.127 and 0.239, respectively) or MSI-H (ρ = –0.091 and 0.026, respectively) across all tumor types.

Conclusions: SigMA score had a similar association with BRCA biallelic mutations when compared with gLOH with higher evaluability. Thus, SigMA score may feasibly provide orthogonal information to detect HRD and should be evaluated further as a potential tool to identify patients who may benefit from treatment with a PARP inhibitor.

#7077 Immunofluorescence microtissue array (IMA) for detection of prognostic cancer stem cell biomarkers.

Madhumita Das[1], Kymkecia Henry[2], Djarie Armstrong[2], Charle Truman[2], Charlie Kendrick[2], Maya S. Saunders[2], Juan E. Anderson[2], **Malcolm J. Lovett**[2], Rose Stiffin[2], Ayivi Huisso[2], Donrie Purcell[2], Marco Ruiz[1], Paulo Chaves[1], Jayanta Kumar Das[2]

[1]Florida International University, Miami, FL,[2]Florida Memorial University, Miami, FL

Cancer is an intricate disease accountable for the deaths of over 10 million people per year in the United States of America. Several scientific studies showed that the cancer stem cell (CSC) markers have prognostic significance in various cancers and are crucial for designing anticancer drugs to lower cancer death. However, there was a lack of rapid, accurate identification, and analysis, of the prognostic cancer stem cell (CSC) biomarkers in numerous cancer patients. In our laboratory, we identified and analyzed prognostic lung cancer stem cell markers (LCSCs) by using the Immunofluorescence microtissue array (IMA) technique in different lung cancer patient's tissue biopsy samples and observed that the increased expression of LCSCs principally, CD44 and CD80 in stage IIIA lung cancer tissues compared to normal lung biopsy tissues. We also investigated pancreatic cancer stem cell biomarkers (PAN CSCs) namely CD44 and CD80 with the IMA technique in pancreatic biopsy tissues. The CD44 fluorescence proved an increased expression in adenocarcinoma pancreatic cell tissues when compared to CD80. We also studied and analyzed the stage progression with ovarian cancer stem cell biomarkers (OCSCs) chiefly CD54 and CD44 using the IMA technique in ovarian cancer patients and normal biopsy tissues. The increased expression of CD44 and CD54 were observed in Stage III ovarian cancer tissues compared to normal ovarian tissue indicating the potential role of these OCSC's biomarkers for the prognosis of ovarian cancer pathogenesis. Our results of prognostic cancer stem cell biomarkers of lung, pancreatic, and ovarian cancers have been analyzed by one-way ANOVA and bioinformatics software (Reactome, Cytoscape PSICQUIC services, STRING) to find underlying molecular mechanism of target gene regulation of increased expression of prognostic CSCs which may give a clue for the prevention and treatment of these cancers. Further research is warranted for these lung, pancreatic, and ovarian CSCs which could be valuable for clinical trials and drug discovery against these CSC biomarkers at early-stage development.

#7079 DNA methylation profiles classifies dysplasia degree in colorectal adenomas.

J. Higareda-Almaraz[1], F. Mancuso[1], P. Canal-Noguer[1], K. Kruusmaa[2], **A. Bertossi**[2];
[1]Universal Diagnostics SA, Madrid, Spain, [2]Universal Diagnostics d.o.o., Ljubljana, Slovenia

Background: Advanced precancerous lesions (APL), precursors to colorectal cancer (CRC), progress from low-grade dysplasia (LGD) to high-grade dysplasia (HGD) through a stepwise accumulation of genomic alterations. Those alterations include mutations, structural variants, and altered methylation patterns, driving dysplastic cells to carcinogenic transformation. Herein, we present a comprehensive analysis of differential DNA methylation profiles to classify dysplasia severity in colorectal adenomas.
Methods: Whole-genome methylation sequencing libraries from 9 HGD and 6 LGD tissue samples were prepared using the NEBNext Enzymatic Methyl-seq kit (EM-seq). To identify differentially methylated regions (DMRs) we have employed two distinct computational tools (DMRcaller and MethylKit) and divided the genome into 1kb bins. DMRs identified by both methods were mapped to genomic features such as genes, gene promoters, CpG islands, and enhancers. Gene ontology and pathway enrichment analyses were performed to elucidate affected biological processes. The identified methylation signature was subsequently investigated in plasma-derived whole-genome EM-seq data from 30 adenoma patients (26 LGD and 4 HGD) and 30 normal samples. Principal component analysis (PCA) and statistical tests were employed to assess its discriminatory power for group classification.
Results: Differential methylation analysis identified 3,751 significant DMRs, with 626 overlapping between both strategies, achieving significant separation of samples by dysplasia grade (FDR = 1.73e-05). The analysis revealed epigenetic changes in genomic clusters regulating DNA-binding transcription factors, tissue morphogenesis, and the Wnt signaling pathway, linking methylation disruptions to dysplasia severity. Furthermore, our methylation signature, when applied to independent plasma samples from HGD and LGD patients, significantly separated the adenoma samples according to their dysplasia grade (FDR = 4.93e-34).
Conclusions: Our analysis enhances the understanding of the molecular biology underlying high- and low-grade dysplastic adenomas. The identified methylation signature, reflecting epigenetic alterations and disruptions in genomic context, allows for the distinction between HGD and LGD in both tissue and plasma samples. This approach advances risk stratification and personalized management for adenoma patients. Further studies are necessary to validate our findings in larger cohorts and to evaluate the potential of this signature in predicting CRC progression.

#7080 Association between atherogenic index of plasma and cancer risk: A prospective cohort study.

Mengbo Xing, Zhangyan Lyu, Wenxuan Li, Yue Cong, Chao Sheng, Hongru Shen, **Kexin Chen**

Tianjin Medical Univ. Cancer Inst. & Hospital, Tianjin, China

Introduction: Cardiovascular disease (CVD) and cancer are among the leading causes of morbidity and mortality globally, with shared mechanisms and risk factors. Patients with CVD face higher risk of multiple cancer subtypes and cancer-related death. The initiation of atherosclerotic plaque formation frequently underlies CVD. Atherogenic index of plasma (AIP), a marker of plasma atherosclerosis, had proved to be a powerful biomarker of CVD. However, few studies have investigated the association between AIP and the risk of overall cancer and common site-specific cancers incidence and mortality in the general population.
Methods: Participants were from the UK Biobank database, a prospective population-based cohort. The exposure variable was the AIP, which was defined as log10 (triglycerides/high-density lipoprotein cholesterol) and participants were categorized into four groups based on their AIP values. The main outcomes included the incidence and death of 20 site-specific cancers: oral, esophagus, stomach, colorectum, liver, pancreas, lung, melanoma, breast, uterus, ovary, prostate, kidney, bladder, brain, thyroid, lymphoma, myeloma, leukemia and mesothelioma cancers. Follow-up time was measured in person years from the baseline enrollment time to the date of cancer diagnosis, death, or the end of follow-up (whichever comes first). We used COX proportional hazards regression model to evaluate the association between AIP and main outcomes. Restricted cubic spline was used for further evaluating the non-linear association.
Results: 401,499 participants were included in this study after excluding baseline cancer and covariates missing, and a total of 55,886 and 14,457 cases of primary cancer and cancer deaths were documented during the median follow-up period of 12.6 years and 14.3 years, respectively. The correlation between AIP and overall cancer risk was not significant. For site-specific cancers, higher AIP was positively associated with lung cancer (LC), endometrial cancer (EC), leukemia and mesothelioma risk, and negatively associated with oral cancer, malignant melanoma and ovarian cancer risk. Especially, higher level of AIP was significantly correlated with higher LC risk, (for Q4-Q1, incidence: 1.19, 95% CI:1.07-1.32, $P = 0.001$, mortality: 1.21, 95% CI:1.07-1.37, $P = 0.002$). Compared to the Q1 group, the higher AIP level group (Q2-Q4) had a higher incidence risk of EC (HR range of 1.25 to 1.39, $P < 0.001$). Furthermore, a U-shaped association was found between AIP and colorectal cancer both in incidence (P for non-linear = 0.012) and mortality (P for non-linear 0.024).
Conclusions: AIP was significantly associated with various cancers risk, especially with higher risk of LC and EC. The results suggested that daily monitoring and management of AIP levels are necessary to reduce cancer risk.

#7081 Body roundness index and its association with cancer incidence and mortality: prospective analyses in the UK Biobank.

Wenxuan Li, Zhangyan Lyu, Mengbo Xing, Haodong Liu, **Kexin Chen**

Tianjin Medical Univ. Cancer Inst. & Hospital, Tianjin, China

Background: Obesity is a well-established risk factor for cancer, yet traditional metrics like Body Mass Index (BMI) have limitations in capturing fat distribution. The Body Roundness Index (BRI) is a novel measure designed to better reflect body fat distribution and its health impacts. This study aims to explore the associations between BRI, cancer incidence, and cancer mortality, and to compare the predictive performance of BRI and BMI in assessing cancer-related risks.

Methods: We utilized data from the UK Biobank to investigate the associations between the Body Roundness Index (BRI) and both cancer incidence and cancer mortality. BRI was calculated using waist circumference and height according to the following formula: $BRI = 364.2 - 365.5 \times \sqrt{(1 - (\text{waist circumference} / (2\pi))^2 / (\text{height} / 2)^2)}$. Cox proportional hazards models were used to evaluate the relationships between BRI and these outcomes. To evaluate the predictive capabilities of BRI and BMI for cancer outcomes, receiver operating characteristic (ROC) analysis and area under the curve (AUC) metrics were employed. The DeLong test was used to statistically compare the AUCs of BRI and BMI.

Results: This study included 315,876 participants, among whom 42,405 (13.42%) cancer cases and 10,519 (3.33%) cancer-related deaths were identified. The mean BRI was significantly higher in individuals with cancer incidence compared to those without (4.33 ± 1.56 vs. 4.05 ± 1.53, $p < 0.05$), and similarly higher in the cancer mortality group compared to the non-cancer mortality group (4.50 ± 1.62 vs. 4.08 ± 1.54, $p < 0.05$). Adjusting for age, sex, ethnicity, household income, education level, smoking status, alcohol consumption, physical activity, and family history of cancer, BRI showed a positive association with both cancer incidence and mortality ($HR_{incidence}$= 1.04, 95% CI: 1.03 - 1.05, $p < 0.05$; $HR_{mortality}$= 1.07, 95% CI: 1.06 - 1.09, $p < 0.05$). Participants were further categorized into five groups based on BRI quintiles. Compared to the lowest quintile (Q1: -0.15 to 2.81), individuals in Q4 (4.24 to 5.19) and Q5 (5.19 to 23.56) showed a 1.08-fold (95% CI: 1.05 - 1.12) and 1.15-fold (95% CI: 1.11 - 1.19) increased risk of cancer incidence, respectively, as well as a 1.09-fold (95% CI: 1.01 - 1.16) and 1.25-fold (95% CI: 1.17 - 1.34) higher risk of cancer mortality. ROC analysis revealed that BRI ($AUC_{incidence}$= 0.55, $AUC_{mortality}$= 0.58) outperformed BMI ($AUC_{incidence}$ = 0.53, $AUC_{mortality}$= 0.54) in predicting both cancer incidence and mortality ($p < 0.05$).

Conclusions: This study demonstrates that BRI is a significant predictor of both cancer incidence and mortality, outperforming BMI in predictive accuracy. These findings highlight the potential of BRI as a more effective tool for cancer risk assessment and prevention strategies.

#7082 Differentially expressed genes as potential biomarkers in patients with suspected prostate cancer in a population of Colombian men.

Dayanne M. Rodriguez-Hernandez[1], Jovanny Zabaleta[2], Alba L. Combita[1], Rafael S. Parra-Medina[3], Carmen L. Roa[3], Mateo Barros Barraza[1], Alvaro Gutierrez[4]

[1]Grupo de Investigacion en Biologia del Cancer, Instituto Nacional de Cancerologia, Colombia, Bogota, Colombia,[2]Stanley S. Scott Cancer Center - Interdisciplinary Oncology, Louisiana Cancer Research Center, New Orleans, LA,[3]Departamento de patologia del Instituto Nacional de Cancerologia, Instituto Nacional de Cancerologia, Colombia, Bogota, Colombia,[4]Clinica Urologica, Urobosque - Clinica Urologica, Bogota, Colombia

Prostate cancer (PCa) is the second most common type of cancer in men worldwide. In Colombia, PCa ranks first in incidence, with an estimated crude rate of 52.6 cases per 100,000 men. A large proportion of PCa are latent and do not progress or affect the lives of patients. The prostate-specific antigen (PSA) test remains the most widely adopted primary screening biomarker for the presumptive diagnosis of PCa. However, PSA testing demonstrates limited utility as a screening tool due to its low specificity, which increases the risk of unnecessary biopsies and the potential for overdiagnosis. Our overarching goal is to identify specific biomarkers that allow the early detection of patients with suspected PCa. We conducted an exploratory study to identify genetic expression profiles associated with the aggressiveness of the disease in a cohort of Colombian patients attending the Urology clinic for the first biopsy at the National Cancer Institute and Urobosque recruited from 2021 to 2024 in Bogotá, Colombia. We performed transcriptome analysis using Illumina high-throughput RNA sequencing (NextSeq2000 system) on 75 paraffin-embedded tissues (FFPE): 36 Gleason-positive cancer samples (Patients) and 39 PCa-negative samples (Controls). Histopathological results according to Gleason Grade Group (GG) were distributed as follows: (GG1 n=1, GG2 n=2, GG3 n=15, GG4 n=5, and GG5 n=13). The mean age was 65 years, and the PSA ranged between 4.34 ng/ml and 191 ng/ml. We first identified differentially expressed genes (DEGs) by status (patient vs. control), and the DEGs were then correlated with age and PSA. Four hundred and seventy-three (473) genes were differentially expressed between patients and controls, 55 of which were identified as putative biomarkers of PCa using the Key Pathways Advisor software (KPA, Clarivate). Of those, 173 DEGs were correlated with PSA levels and 203 with age. We identified 113 DEGs common to the 3 variables (status, PSA, and age). According to the pathway enrichment analysis performed through KPA, *SPON2*, *SERHL*, *KCNA2*, *PKP3*, and *SLC29A2* genes were the most significant ($p < 0.001$). We found that 6 out of the 10 most significant pathways in which our identified DEGs participate are associated with diseases of the genito-urinary tract, including prostate neoplasms, genital diseases, neoplasms, and male urogenital diseases. This is the first Colombian report that performs transcriptome analysis in a cohort of patients with suspected prostate cancer, showing very specific differences between patients diagnosed with prostate cancer versus patients with negative results. The development of strategies that will lead to a decrease in diagnostic biopsies and overtreatment of indolent disease remains a challenge for early detection of prostate cancer. Additional validations are needed to confirm these data.

#7083 Classical and basal like cells in donor tissues reveal phenotypic heterogeneity of pancreatic intraepithelial neoplasia.

Jude O. Okoye, Rosina Carr, Ahmed M. Elhossiny, Alberto C. Olivei, Imade Williams, Lei Sun, Yaqing Zhang, Eileen S. Carpenter, Timothy L. Frankel, Marina Pasca Di Magliano

Surgery, University of Michigan, Ann Arbor, MI

Pancreatic ductal adenocarcinoma (PDAC) is a deadly disease that affects individuals of all ages, with marginal improvements in patient survival rates over the years despite available treatments. Although Pancreatic Intraepithelial Neoplasia (PanIN), an early lesion, is known to precede PDAC, identifying individuals at risk of developing PDAC remains a challenge for clinicians. Recent studies have demonstrated both inter- and intra-heterogeneity of PDAC, as well as the aggressive nature of its phenotypes, but little is known about the heterogeneity of PanIN and its risk of progression in humans. To address this gap, we investigated transplant-incompetent samples from 89 male and female White and African-American patients, ages 21 to 76 years, collected through the Gift of Life program. Pathologic analyses using basic histological techniques revealed an average burden of three PanINs per 2 cm x 2 cm of donor tissue. Single-cell RNA sequencing and protein validation identified distinct classical and basal-like cells, along with expression of cytokines that correlate to the phenotypes previously reported in PDAC. In some patients, subpopulations within the already-established aggressive basal cells were identified. Regardless of cell phenotype, the senescence state of most of the identified PanINs may explain why these patients were not diagnosed with PDAC during their lifetimes. Given that most PDAC develop from PanIN and PanINs are often found adjacent to tumors, we are currently probing upregulated and downregulated pathways in tumor-associated PanINs and donor PanINs with tumor-like features, as well as crosstalk between classical and basal PanINs and their microenvironments. We are also developing a model to provide further insight into the risk of disease progression using spatial transcriptomic analysis. This knowledge could lead to more effective methods for early detection and treatment of the lethal disease.

#7084 Multi-omics information from tumor-derived extracellular vesicles enables biomarker discovery and early detection in non-small cell lung cancer.

Kelley Van Vaerenberghe, Margie Kinnersley, Claire Seibold, Tre Blohm, Riley Kemp, Sean Lodmell, Amanda Mast, Tia Seibold, Rachel Short Miller, Katie Havranek

FYR Diagnostics, Missoula, MT

Liquid biopsy plays an increasingly influential role in clinical decision making by supporting earlier cancer detection, diagnosis, patient stratification, and treatment monitoring. The most common approaches utilize cell-free circulating tumor DNA or circulating tumor cells in the peripheral blood of cancer patients. However, these methods are impeded by miniscule analyte abundance leading to unfavorable signal to noise ratios. As a promising alternative, extracellular vesicles (EVs) have emerged as a complementary liquid biopsy analyte. EVs are cell-derived lipid membrane encapsulated particles carrying diverse protein, nucleic acid, and metabolite cargos. Blood plasma of cancer patients contains EVs from healthy cells and tissues throughout the body, but also contains a population of EVs derived directly from tumor tissue. These tumor-derived EVs (TDEVs) serve as rich sources of insight into tumor biology and disease pathology. To detect TDEVs amongst background noise, we have developed a clinically applicable, multi-omic EV subpopulation interrogation pipeline that robustly profiles TDEVs in blood plasma. We have demonstrated the ability of the pipeline to enrich cancer markers and detect activation of pathways driving oncogenesis across several different cancers, including non-small cell lung cancer (NSCLC). There is a particularly compelling need for liquid biopsy-based solutions in early detection and screening applications, when patient adherence to standard of care screening regimens could be improved by simplifying the procedure to a routine blood draw. Here, we have applied our EV-omic pipeline in combination with machine learning in a cohort of NSCLC patients to robustly identify Stage I and II disease using blood-based EV proteomic and transcriptomic signatures. Biomarker profiles found to be most important for patient stratification combine markers known to be relevant in tumor tissue along with novel features. This work highlights our EV subpopulation technology and its ability to address the major challenge of early cancer detection in liquid biopsy.

#7085 Analysis of gene expression pattern of glycosphingolipid synthases using tumor tissues from breast cancer patients to identify key targets for early diagnosis and protection of recurrence.

Kanchan Chakma[1], Robiul Hasan Bhuiyan[1], Farhana Yesmin[2], Koichi Furukawa[3]

[1]University of Chittagong, Chittagong, Bangladesh,[2]New York University, New York, NY,[3]Chubu University, Kasugai, Japan

Breast cancer become the most common types of cancer in Bangladesh (female), and accounted 13028 new cases and 6783 deaths in 2020. No efficient therapy has been developed although anti-HER2 antibody therapy has been tried, showing some effects. We need to detect breast cancers at early stage of evolution and also of recurrence, and develop more effective way than the current approaches to eradicate breast cancers. In order to achieve these purposes, it seems essential to find breast cancer-specific antigens as a marker and/or therapeutic targets. Recently, some interesting and promising findings on breast cancers have been reported. For example, ganglioside GD2 was reported to be a marker of breast cancer stem cels, and also play roles in the carcinogenesis. GD3, Gb3 have an association in tumorigenesis as well as cel proliferation, adhesion and migration. More important marker antigens of breast cancers might be present. Aims of the study are to identify novel breast cancer-associated glycolipids including their synthetic enzyme genes, using many surgical specimens and sera from breast cancer patients. Tumor and healthy tissues were collected after surgical resection from hospitals and clinics. Samples are immediately transferred to the laboratory. mRNA was extracted using TRIzol method. cDNA was converted from mRNA using Reverse Transcriptase. qRT-PCR was performed to analyze glycosyltransferase gene expression pattern. Expression of glycolipid GD3-synthase (ST8SIA1), GD2-synthase (B4GALNT1), and Gb3 synthase (A4GALT) are analyzed by RT-PCR using tumor tissues from breast cancer patients. GD2-synthase expression level found significantly higher in tumor tissues compared to healthy tissues. GD3 and Gb3 synthases did not show significant difference between tumor tissues and healthy tissues. GD2 are upregulated in breast cancer patients. This gene may be a novel biomarker for breast cancer and may be useful to design therapeutic drug.

#7086 Serological assays for early detection of HPV-associated oropharyngeal cancer.

Mingyue Wang[1], Jermaine Brown[1], John C. Smith[1], Leonid Dzantiev[1], Anu Mathew[1], Martin Stengelin[1], George Sigal[1], Padhma Yuvaraj[2], Karen S. Anderson[2], Jacob Wohlstadter[1]

[1]Meso Scale Diagnostics, LLC, Rockville, MD,[2]Center for Personalized Diagnostics, Biodesign Institute, Arizona State University, Tempe, AZ

Research Use Only (RUO) sample-sparing MULTI-ARRAY serology immunoassays have been developed for human antibodies to several HPV-16 early antigens. These assays are intended to support research of oropharyngeal cancer (OPC) linked to the human papillomavirus (HPV).The HPV virus can infect the mouth and throat and cause cancers of the oropharynx, i.e. the back of the throat, including the base of the tongue and tonsils. HPV-related cancer incidence is increasing dramatically among men and is unlikely to decrease in the foreseeable future, despite vaccination efforts. As a result, this may become one of the most common cancers in middle-aged men in the United States by 2045. The HPV16 genome consists of six early genes (E1, E2, E4, E5, E6, and E7) and two late genes (L1 and L2) that constitute the viral capsid. Antibodies to early HPV-16 antigens are commonly present in those with OPC but are detected in only approximately 1% of the general population. These antibodies are present many years before cancer develops; thus, they are ideal biomarkers for early detection of OPC.Immunoassays for antibodies against HPV-16 early antigens E1, E2, E6, and E7 were developed in a high-throughput multiplexed format using electrochemiluminescence (ECL) detection, and MULTI-ARRAY 10-spot 96-well plates. Each well has an array presenting recombinant antigens manufactured by Meso Scale Diagnostics, LLC. The assay uses 25 uL of 2,500x diluted serum or plasma. The assay format is simple: diluted sample is incubated in the well with the antigen array followed by a wash and detection of bound anti-HPV antibodies using an anti-human IgG antibody labeled with the SULFO-TAG ECL label. Assay performance was evaluated with approximately 200 commercially sourced samples from apparently healthy individuals and 14 samples from individuals known to be positive for at least one HPV-16 early antigen using an established reference method (Programmable Protein (RAPID) ELISA, Anderson 2015). There was excellent agreement between the two methods.This high-throughput MULTI-ARRAY assay may be useful in research applications requiring screening of a large number of samples for HPV-16 antibodies.For Research Use Only. Not for use in diagnostic procedures.

#7087 Sample-sparing immunoassays for early detection of cancer.

Mingyue Wang[1], Jermaine Brown[1], Daniel Lee[1], Salvia Misaghian[1], Taron Gorham[1], Leonid Dzantiev[1], Anu Mathew[1], Martin Stengelin[1], Ji Qiu[2], Karen Anderson[2], Joshua LaBaer[2], George Sigal[1], Jacob Wohlstadter[1]

[1]Meso Scale Diagnostics, LLC, Rockville, MD,[2]Arizona State University, Tempe, AZ

Research Use Only (RUO) sample-sparing MULTI-ARRAY immunoassays have been developed for several well-established cancer markers. The primary focus of this project is the development of assays to support research of suspicious lung nodules, but many of these selected markers are also relevant to other cancer types. These immunoassays include a serology panel, two biomarker panels, and a single-marker assay. The serology panel includes assays to detect autoantibodies to p53, CTAG-1, and CTAG-2. This panel requires 25 µL of 2,500-fold diluted serum or plasma. A biomarker panel requiring 25 µL of 100-fold diluted serum or plasma detects the following biomarkers: CA15-3, SCFR/Kit, ErbB2, IGFBP-2, MIF, MMP-2, MMP-9 (total), REG4, S100A6, and TNF-RI. A second biomarker panel requiring 25 µLof 10-fold diluted serum or plasma detects the following biomarkers: CA125, Ca19-9, CEACAM-5 (CEA), EGFR, VEGFR-1/Flt-1, FLT3L, HE4, MMP-3 (total), and osteopontin. Finally, an assay for the core lung cancer biomarker cytokeratin-19 was developed. All assays had dynamic ranges that span 3-4 logs and are sufficiently sensitive to measure native levels in commercially sourced samples from apparently healthy individuals. The assays were preliminarily evaluated using more than 100 commercially sourced serum or plasma samples from apparently healthy individuals and more than 100 commercially sourced serum or plasma samples from patients with lung or other cancers. Many of the biomarkers had significantly different concentrations in several cancers compared to samples from apparently healthy individuals. These RUO assays may be useful in identifying biomarkers for a multi-marker panel to study early cancer.

CLINICAL RESEARCH: Revolutionizing Cancer Care Through Inclusive Clinical Research
Poster Session

#7091 Disparities in non small cell lung cancer treatment and outcomes among Native Hawaiian and other Pacific Islander populations.

Chalothorn Wannaphut[1], Manasawee Tanariyakul[1], Jared Acoba[2]

[1]Internal Medicine, John A. Burns School of Medicine, University of Hawaii, Honolulu, HI,[2]University of Hawaii Cancer Center, Honolulu, HI

Background:The American Cancer Society and National Cancer Institute, emphasize the critical need to disaggregate data for Asian, Native Hawaiian, and Other Pacific Islander (NHOPI) populations to better understand racial disparities in cancer. NHOPI populations are highly diverse, with distinct genetic, cultural, and socioeconomic backgrounds that can influence cancer outcomes. This study aims to analyze Native Hawaiian, and other Pacific Islander patients with non-small cell lung cancer (NSCLC) to identify disparities in prognostic factors, treatment, and outcomes.
Methods:Data were abstracted from 4,143 NSCLC patients treated at Queen's Medical Center in Honolulu, Hawaii, between 2000 and 2022. Patients were grouped by race: White, Asian, Native Hawaiian, and other Pacific Islander. Kaplan-Meier survival estimates, and Cox proportional hazards models were applied to adjust for demographic, clinical, and treatment-related factors.
Results:Among the 4,143 patients diagnosed with NSCLC, the racial distribution included 971 White patients, 2,324 Asian patients, 637 Native Hawaiian (NH) patients, and 211 Pacific Islander (PI) patients. PI patients were generally younger, more likely to present with late-stage disease, and more often underinsured compared to other racial groups including Native Hawaiians. Median overall survival (OS) was 20.9 months for whites, 20.1 months for Asians, 15.8 months for NH, and 10.3 months for PI ($p<0.001$). Both NH and PI patients were less likely to undergo surgery despite having stage I disease (NH HR 0.52, 95% CI 0.33-0.84; PI HR 0.29, 95% CI 0.12-0.70). Additionally, PI patients less often received systemic therapy for advanced-stage disease (PI HR 0.56, 95% CI 0.38-0.81) compared to whites. In adjusted models without treatment, NH and PI
patients exhibited worse survival compared to whites (NH HR 1.12, 95% CI 1.01-1.26; PI HR 1.16, 95% CI 0.97-1.39). However, when treatment was included in the model, these differences were no longer significant.
Conclusion:Native Hawaiian (NH) and Pacific Islander (PI) patients with cancer should be studied independently due to their unique sociodemographic characteristics and treatment patterns. Both groups showed lower survival rates compared to whites, largely due to disparities in treatment.

#7092 The change in Asian American/Native American/Pacific Islander demographics in interventional clinical trials in Hawai'i.

Aiesha Pradhan, Jangsoon Lee, Naoto T. Ueno, Toshiaki Iwase

University of Hawai'i Cancer Center, Honolulu, HI

Asian Americans, Native Hawaiians, and Pacific Islanders (AA/NH/PI) contribute significantly to the state's ethnic diversity. Despite their substantial presence, these groups face considerable health disparities, particularly in access to and participation in clinical trials. Furthermore, detailed accrual data based on ethnicity is missing as national datasets often aggregate AA/NH/PI populations into a single racial category. The aim of this study is to investigate change in accrual rates to interventional oncology clinical trials over time and determine the patient demographic factors that caused disparities in the trial participation. Through the Clinical Trial Management System at the University of Hawai'i Cancer Center, we collected patient enrollment data for NCI-funded interventional clinical trials (i.e., National Cancer Trials Network, NCTN and NCI Community Oncology Research Program, NCORP trials) conducted within the Hawai'i Cancer Consortium (HCC) between 2005 and 2020. The HCC is constituted by seven entities, including 2 major healthcare organizations in Hawai'i. The dataset comprised of demographic details such as biological sex, age, and race. Among 2,341 study participants who were identified by screening, 870 were enrolled in non-therapeutic studies, while 1,471 were involved in therapeutic trials. Nearly 50% identified as Asian American, 25% as White, and 12% as Native Hawaiian, and 3% as Pacific Islander. The remaining 10% were categorized as Black, American Indian, Multiracial, Other, or Unknown. The number of Asian American participants showed a declining trend until 2014, after which it began to recover. The number of Native Hawaiian and Pacific Islander participants has been stable over this time period. Female participation consistently exceeded male participation over the study period. The annual enrollment of all participants remained low until 2014 but steadily increased, reaching its peak in 2019. This study revealed that local AA/NH/PI accrual has a different trend from the national accrual over time. While the findings suggest that local, continuous community outreach efforts have been effective, male patient accrual remains inequitable. To address this disparity, our center is currently implementing an educational program aimed at Native Hawaiian men to help eliminate mistrust in clinical trial participation.

#7093 Assessing financial toxicity as a correlate of symptom burden in Latina breast cancer patients.

Melissa Gaviria[1], Bharat Narang[1], Ashley M. Rodriguez[1], Francesca Gany[1], Eida M. Castro-Figueroa[2], Victoria Blinder[1], Rosario Costas-Muniz[1]

[1]Memorial Sloan Kettering Cancer Center, New York, NY,[2]Ponce Health Sciences University, Ponce, Puerto Rico

Background/Objective: Depression, anxiety, insomnia, and fatigue often occur concurrently in cancer patients, with Latinos reporting higher burdens compared to non-Latino Whites. These symptoms adversely affect cancer progression and are associated with elevated cancer mortality. Financial toxicity, the financial distress of patients after a cancer diagnosis, has been linked to poorer cancer outcomes. This study aims to explore the relationship between financial toxicity and depression, anxiety, insomnia, and fatigue in Latina breast cancer patients.
Methods: Data was collected from 30 Spanish-speaking Latina breast cancer patients at Memorial Sloan Kettering Cancer Center who endorsed symptoms of depressed mood, insomnia, and fatigue. Depression and anxiety were assessed through the Hospital Anxiety and Depression Scale (HADS), fatigue was measured through the Fatigue Symptom Inventory (FSI), and insomnia was measured using the Insomnia Severity Index (ISI). Financial toxicity was assessed using the FACIT-Cost scale. An independent samples t-test was employed for the analysis, with a p-value of 0.05.
Results: The study participants included 30 Latina breast cancer patients with an average age of 58.9 years. 46.7% of patients were married, and most reported limited English proficiency (86.7%). Most patients had stage 1 or 2 breast cancer (86.7%), were currently receiving treatment (56.7%), and were within five years of their breast cancer diagnosis (73.3%). Patients experiencing higher financial toxicity had significantly higher anxiety scores ($\bar{x}$ = 8.1, SD = 4.5, p = 0.003), depression scores ($\bar{x}$ = 7.6, SD = 3.2, p = 0.001), insomnia levels ($\bar{x}$ = 12.0, SD = 7.0, p = 0.035) and fatigue ($\bar{x}$ = 66.2, SD = 31.0, p < 0.001) compared to those with lower financial toxicity (respectively, $\bar{x}$ = 4.1, SD = 2.5; $\bar{x}$ = 3.5, SD = 2.6; $\bar{x}$ = 8.1, SD = 3.9; $\bar{x}$ = 26.3, SD = 22.9).
Conclusion: Latina breast cancer patients experiencing financial toxicity reported higher levels of depression, anxiety, insomnia, and fatigue compared to those with less financial toxicity, suggesting that financial toxicity has a relationship with symptom burden. Our findings highlight the need to understand how to mitigate financial toxicity to improve cancer care outcomes, or to improve symptom management to reduce financial hardship. Possible interventions might include financial planning with healthcare professionals; leveraging social networks to disseminate financial resource and symptom management information; engaging community health workers and navigators to guide patients and their families toward available financial resources and better symptom management strategies; and building financial self-efficacy and involving family members in financial planning may help lessen the financial distress and symptom burden experienced by Latina breast cancer patients.

#7094 Financial risks in southeast asian patients with cancer.

Nguyen H. Tran, Jordan Mason, Wendy Hanson, Kimberly Steinbert, Minji Lee, Robert McWilliams

Mayo Clinic, Rochester, MN

Background: Financial toxicity can have devastating long-term effects for cancer patients (pts) and their family members which may impact their access to care, adherence and, ultimately, outcomes. Identifying those at greatest risk is key to mitigating costs and distress to pts. Here we report results from a screening questionnaire on financial risks among Southeast Asian (SE-A) pts.

Methods: This retrospective review included pts on the cancer patient registry at Mayo Clinic (Arizona, Florida, Minnesota, Wisconsin, and Iowa) who had completed a clinical visit in an oncology-related department (hematology, medical oncology, radiation oncology, etc) within the past 270 days prior to 6/4/24. Pts completed an electronic social determinant of health questionnaire of which financial risks and transportation needs are the focus of this study: 1) how hard is it for you to pay for the very basics like food, housing, medical care, and heating? 2) in the past 12 months, has lack of transportation kept you from medical appointments or from getting medications. Our primary endpoints include the association between financial risks, active bad debts (defined as having >$5000 owed for high active bad debt, $0-$5000 for some active bad debt), pre-appointment review (PAR) flag status and transportation needs. Descriptive statistics and Chi-square were used to analyze factors associated with financial risks.

Results: We identified 81,731 pts who completed the questionnaire, among whom 387 were SE-A. Compared to non-SE-A and White, 6.5% have some active bad debt vs 3.3% and 3.6% ($p<0.001$), and 1.0% had high active bad debt vs 0.8% and 0.7% ($p<.001$); 58.4% had low financial risk vs 60% and 66% ($p<0.001$) and 2.3% with high risk vs 1.5% and 1.6% ($p<0.001$), 4.1% declined to answer and 29.7% were unknown. Bad debt was associated with PAR flag ($P <0.001$) and financial risk ($P<0.001$). For transportation among SE-A, 2.3% had unmet transportation needs, 1.8% declined/unable to answer, while 17.8% were unknown. Those with transportation needs were associated with active debt and financial risk ($p<0.001$).

Conclusion: In this cohort, we identified SE-A with highest rates of active debt and higher financial risks compared to Non-SE-A and White. A large percentage of patients declined to answer or are unknown. Future pilot questionnaire is in development to identify pts concerns for medical costs, financial strain and the receptivity to be connected to resources/referrals to address them.

#7095 Effectiveness of a breast cancer screening program among non-adherent women in Puerto Rico: A community-based participatory research effort of the Puerto Rico CEAL Community Outreach and Engagement Group.

Norangelys Solis-Torres[1], Karelys Canales-Birriel[1], Nashaly Saldana-Santiago[1], Alondra Mercado-Andino[1], Christian Rivera-Catala[1], Cecilia Marino-Nieto[1], Hector Contreras-Mora[1], Cynthia Perez[2], Vivian Colon-Lopez[1]

[1]Cancer Control and Population Sciences, UPR - Comprehensive Cancer Center, San Juan, Puerto Rico,[2]Biostatistics and Epidemiology, Graduate School of Public Health, University of Puerto Rico, San Juan, Puerto Rico

Background: Breast cancer is the most common cancer (31%) and the leading cause of cancer death (19%) among women in Puerto Rico. The United States Preventive Service Task Force (USPSTF) recommends biennial breast cancer screening by mammography for women aged 40-74. Evidence has documented that Community Health Workers (CHWs) can help increase breast cancer screening rates and improve health equity through community-based approaches.
Objective: The purpose of the study was to assess the effectiveness of a CHW-delivered intervention to increase mammography screening among non-adherent Hispanic women in Puerto Rico.
Methods: PR-CEAL's Community Outreach and Engagement Group conducted an island-wide community-based randomized controlled trial from December 2023 to June 2024. Eligible non-adherent women for breast cancer screening aged 50-74 years were recruited. CHWs collected baseline information on participants' barriers and intentions to schedule an appointment for overdue breast cancer screening using a survey and were randomly assigned to the intervention or a comparison condition. The intervention consisted of a session led by a CHW, where participants received education on breast cancer screening. Participants were randomly allocated into an intervention group, which received information on cancer screening, or a comparison group, which received general health information. Follow-up calls assessed screening status at weeks four and eight, changes in reported barriers, and intent to screen by self-reported confirmation of scheduled appointments. The main outcome variable was having scheduled an appointment for their overdue mammography. Pearson's test was conducted to evaluate the association of having scheduled an appointment among intervention groups.
Results: A total of 89 participants were recruited with an overall median age of 59 (range 55-63) years. Women who received the intervention were RR=1.08 (95% CI: 0.35-3.36) times more likely to report having a higher intent to screen in comparison to the control group; however, the magnitude of this effect was not statistically significant, p-value = 0.898. A positive trend in intent to screen was observed over three time points: baseline (10%), first follow-up (16%), and second follow-up (20%), p-value=0.115.
Conclusion: The intervention demonstrated a positive influence of CHWs on mammography appointment scheduling, but the effect was not statistically significant. Future studies with larger sample sizes are needed to further increase power size estimations and evaluate the impact of CHW-mediated initiatives for cancer prevention and screening promotion in community-based settings.

#7096 Barriers and intention to screen for colorectal cancer among non-adherent women in Puerto Rico: an assessment from the Puerto Rico Community Engagement Alliance (PR-CEAL)..

Karelys I. Canales-Birriel[1], Cynthia M. Perez[2], Norangelys Solis-Torres[1], Nashaly M. Saldana-Santiago[1], Alondra K. Mercado-Andino[1], Christian Rivera-Catala[1], Cecilia Marino-Nieto[1], Hector Contreras-Mora[1], Vivian Colon-Lopez[1]

[1]Cancer Control and Population Sciences, UPR - Comprehensive Cancer Center, San Juan, PR,[2]Department of Biostatistics and Epidemiology, Graduate School of Public Health, University of Puerto Rico, San Juan, PR

Background: Colorectal cancer (CRC) ranks among the top three most diagnosed cancers in men (11.1%) and women (9.5%) in Puerto Rico (PR). Despite efforts to promote early detection, screening rates remain suboptimal, likely due to cultural beliefs, socioeconomic disparities, and limited healthcare access.
Objectives: This study assessed the barriers and intentions for screening among Hispanic women in PR who are non-adherent to FOBT and colonoscopy guidelines.
Methods: PR-CEAL conducted a community based participatory research across various community settings in PR from December 2023 to June 2024, targeting women aged 45-75 who were non-adherent to CRC screening according to the USPSTF guidelines. Eligible women completed an in-person baseline survey where participants provided information about barriers they faced regarding screening and their intentions to screen. To encourage adherence to CRC screening recommendations, community health workers (CHWs) distributed educational materials aligned with USPSTF guidelines. Log-binomial regression models assessed the associations between test modality, barriers, and readiness for screening; *how ready do you feel to schedule an appointment to get tested?* controlling for age and health insurance.
Results: Enrolled women had a median age of 59, 31.2% held a bachelor's degree or higher, 64.9% earned $15,000 or less, 68.2% had public health insurance, and 43.4% married or cohabitating. Readiness for CRC screening differed by test modality ($p < 0.001$), with the highest readiness observed among women in the FOBT group (95.2%, n=118), followed by the colonoscopy group (71.2%, n=84) and the dual modality group (71.4%, n=110). Log-binomial models showed that women in colonoscopy group and women in dual modality group were less likely to be ready for screening compared to FOBT group (RR=0.76, 95% CI: 0.67-0.85; RR=0.74, 95% CI: 0.67-0.83; respectively). Key barriers to CRC screening included the lack of physician recommendation (50.0%, n=198) and being too busy (18.7%, n=74). Women in the dual modality group were more likely than those in FOBT group to report barriers such as not having symptoms (RR=2.15, 95% CI: 1.14-4.04) and being too busy to undergo screening (RR=1.81, 95% CI: 1.07-3.06).
Conclusions: Findings show varying readiness and barriers for CRC screening by test modality. Women in FOBT group demonstrated higher readiness, highlighting the importance of non-invasive tests in promoting screening. Tailored interventions using CHW should address barriers while highlighting the benefits of screening for asymptomatic individuals. Strategies to improve readiness could include enhancing patient education, increasing awareness of screening, and emphasizing the simplicity and accessibility of FOBT.

#7097 Predictors of physician recommendations on cancer screening in women aged 21 to 75 an analysis of sociodemographic, clinical and psychosocial characteristics.

Nitza E. Malave-Velez[1], Ana C. Sala[1], Cecilia Marino Nieto[1], Christian Rivera Catala[1], Cynthia M. Perez[2], Vivian Colon Lopez[1]

[1]Cancer Control and Population Sciences, UPR- Comprehensive Cancer Center, San Juan, PR,[2]Department of Biostatistics and Epidemiology, Graduate School of Public Health, University of Puerto Rico, San Juan, PR

Background: Lack of physician recommendation for cancer screening tests is a critical determinant of cancer screening uptake and remains a major barrier in screening and prevention efforts. This study aims to analyze the sociodemographic, psychosocial, and clinical factors related to lack of physician recommendation in women living in Puerto Rico, ages 21 through 75.
Methodology: A cross-sectional study was conducted by the Puerto Rico Community Engagement Alliance. A total of 500 women, non-adherent to either breast, cervical or colorectal cancer screening were recruited island wide for a randomized controlled trial (RCT) from December 2023 to June 2024. Follow-up calls assessed willingness to undergo either breast, colorectal and cervical cancer screening at four and eight weeks. The main outcome for this analysis was physician recommendation as barrier for screening, categorized as those whose physician did not recommend cancer screening (53%) and those whose physician recommended it (47%). Sociodemographic, clinical, and psychosocial data was collected at baseline. Regression models were conducted to assess the associations between these characteristics and physician recommendation using binary logistic regression. The analysis included models adjusted for age, chronic diseases and high cholesterol. Group differences were analyzed using Mann Whitney u test, alpha 5%.
Results: Participants who lack physician recommendation for cancer screening were significantly older (median=58 years) when compared with those that have received a recommendation (median = 55 years, $p < 0.001$). Significant differences in provider recommendation were also observed among participants reporting chronic diseases in which those participants who lack a physician recommendation are more likely to have diagnosis of heart disease, (15.47% p-value= 0.027), asthma (27.55%; p-value), and high cholesterol (37.74%; p-value). Participants who reported not receiving physician recommendation at baseline had higher willingness to undergo colonoscopy (baseline = 78.88%; first follow-up = 90.30%) in comparison to those who received recommendation (baseline = 63.96%; first follow-up = 78.08%) at both baseline ($p= 0.015$) and during the first follow-up ($p = 0.014$). No significant differences were observed in willingness to undergo other cancer screening test and physician recommendation ($p>0.05$).
Conclusion: Lack of physician recommendation for cancer screening is associated with differences in sociodemographic and clinical characteristics, such as older age and higher prevalence of chronic diseases. We observed statistical differences between willingness to colonoscopy screening at baseline and first follow-up, which highlight missed opportunities during clinical encounters for the promotion of cancer screening in these participants.

#7099 Development of HPV awareness and prevention education materials for Hispanic/Latinx community health educators.

Daisy Montoya[1], Laura Adame[2], Jesus Bastian[1], Najla Dabbagh[1], Yelitza Campos[1], Julie Dang[2], Laura Fejerman[2]

[1]UC Davis, Davis, CA,[2]Comprehensive Cancer Center, UC Davis, Davis, CA

The human papillomavirus (HPV) is the most common sexually transmitted infection in the United States. Although there is no cure for HPV infections, implementation of highly effective preventable measures (screening tests, HPV vaccination) has reduced the incidence of HPV-related cancers. However, the Hispanic/Latinx (H/L) community continues to experience high incidences of HPV infections and some of its related cancers. H/L underutilization of preventative health services can be partly explained by limited health care access, low health literacy levels, and language barriers. To address some of these determinants, the program 'Mas Vale Prevenir' aims to develop culturally and linguistically appropriate HPV related cancer prevention educational materials to train community health educators (Promotores) in California. To inform material development, we conducted 4 informal feedback sessions (N=24) and 2 focus groups with Promotores (N=12) which were analyzed using qualitative methodology (Thematic Analysis). We identified insights related to pre-determined themes: learning preferences (e.g., use medical terminology) and opinions related to content (e.g., liked narrative format, relatability) as well as two emerging themes: importance of content (e.g., normalizing the material, H/L-specific statistics) and the role of Promotores (e.g., bridge between doctors and community). Results from the focus groups analysis were used to refine materials in an iterative manner. Engaging H/L Promotores in the development of health educational materials is essential for their effectiveness as community educators. The next step will be to implement and evaluate the 'Mas Vale Prevenir' program to assess its effectiveness in reducing HPV related cancer health disparities.

#7100 The relationship between social deprivation and immune suppression in Hispanic/Latina patients with breast cancer.

Elexa P. Rallos[1], Sabrina Carrel[2], Michelle Li[3], Batul Al-Zubeidy[4], Edgar Gonzalez[4], Aaron G. Baugh[4], Matthew Jacobo[4], Cheol Park[4], Robert Hsu[4], Dominic Zavala[4], Steven Do[4], Michael F. Press[5], Ming Li[6], Anastasia Martynova[4], Priya Jayachandran[4], Daphne Stewart[4], Chanita Hughes-Halbert[6], Mariana C. Stern[6], Evanthia T. Roussos Torres[4]

[1]Eastern Virginia Medical School, Norfolk, VA,[2]University of South Carolina School of Medicine, Greenville, SC,[3]New York Medical College, New York, NY,[4]Department of Medicine, Division of Oncology, Keck School of Medicine of USC, University of Southern California, Los Angeles, CA,[5]Department of Pathology, Keck School of Medicine of USC, University of Southern California, Los Angeles, CA,[6]Department of Population and Public Health Sciences, Keck School of Medicine of USC, University of Southern California, Los Angeles, CA

Immunosuppressive cells, such as myeloid-derived suppressor cells (MDSCs), prevent tumor infiltrating-lymphocytes (TILs) from mounting an anti-tumoral immune response in solid tumors, including breast cancer. Even though genetic factors, such as race and ethnicity, are suspected to contribute to immunosuppression, social determinants of health—non-medical factors including economic stability, education access and quality, health care access and quality, neighborhood and built environment, and social and community context—may also contribute to observed differences in tumor growth and therapeutic response across different racial and ethnic groups. We hypothesized that the combined effects of Hispanic/Latina origin and higher social deprivation contribute to an increased proportion of immunosuppressive cells in circulation. In this study, our IRB-approved cohort consisted of 131 patients with varied histologic subtypes and stages of breast cancer including 71 Hispanic/Latina (H/L), 37 Non-Hispanic White (NHW), 15 Asian, and 8 African American/Black (AAB) individuals. To assess the proportion of immunosuppressive cells in circulation, we performed flow cytometry analysis of peripheral blood mononuclear cell (PBMC) samples obtained from our cohort. To study social determinants of health across racial and ethnic groups, we utilized the Social Deprivation Index (SDI) as a proxy using each patient's ZIP Code and stratified SDI values into quartiles (where lower quartile indicates less social deprivation). Preliminary data suggested that H/L groups have a greater percentage of circulating suppressive granulocytic (G)-MDSCs (0.31% vs. 0.14%) compared to NHW groups. A similar trend was noted for regulatory T cells with 1.51% in H/L and 1.22% in NHW. This finding also appeared to continue when further adding social deprivation, where groups with both higher social deprivation and H/L origin demonstrated a trending increase in G-MDSCs and monocytic (M)-MDSCs. Preliminary differences in circulating immune cells in in the H/L group could be driven by genetic differences in one or more of the ancestral components and/or by specific exposures that may be more prevalent among H/L women, such as stress, environmental exposures, or others. We are investigating additional data from patient surveys to further inform the observed correlations between SDOH and immune cell differences. Studies are also underway to examine differences in intratumoral immune suppressive cell types in a subset of 13 tumor specimens from 3 H/L and 9 NHW patients with early-stage hormone receptor positive breast cancer. These findings demonstrate the importance of further investigation of the multi-level determinants of response to treatment, especially in patients receiving immune checkpoint inhibitors across diverse racial and socioeconomic populations.

#7101 Envisioning leadership for Latino cancer health equity in prevention and control.

R. F. Trejos, K. Liller;
University of South Florida, Tampa, FL

Background: Latinos are the second-largest ethnic population in the United States of America (USA), and although their cancer rates, for most cancer types, have significantly decreased over the last decade, Latino cancer inequities and disparities continue to result in late cancer diagnosis, cancer treatment-related burden, cancer-related complications, and poor quality of life. Leadership training and skills are needed to adequately prepare healthcare professionals to respond to the complex and ever-evolving nature of cancer inequities. Therefore, this presentation will evaluate current curricular offerings discussing leadership and/or health equity to provide evidence-based recommendations to inform future clinical and community-based curricular and educational approaches.

Methods: We reviewed multiple trainings and educational opportunities currently offered in the USA that discussed or included leadership and/or health equity components and a focus on cancer. Programs were evaluated to understand current gaps in leadership training and the relevance of competency-based education as an effective curriculum development approach to inform the development of future curricular offerings. The literature on leadership for health equity in cancer among Latinos was summarized using the PubMed, Embase (Elsevier), and Scopus (Elsevier) databases following the PRISMA guidelines.

Results: The existing programs in the evidence included the Cancer 101, Éxito Latino Cancer Research Leadership Training, Redes en Acción, and Minority Training in Cancer Control Research programs. Results suggest the importance of developing, implementing, and evaluating formal and informal training programs on leadership that can equip healthcare professionals to respond to the complex and ever-evolving nature of Latino cancer inequities. Most programs included post-baccalaureate youth interested in doctoral-level training. However, absent from the identified curricular offerings are capacity-building training and leadership training, as well as skills that would enable researchers and cancer care providers with the necessary tools to become advocates and patient-driven supporters of patients plagued by cancer and cancer care-related disenfranchisement.

Discussion and Conclusion: Leadership education efforts to tackle Latino cancer inequities are necessary to ensure responding to the unique and complex needs and realities of Latinos in the USA, which can inform clinical and community-based initiatives recognizing the historical and intergenerational factors that shape Latino health.

#7103 p16 predictiveness for HPV-associated head and neck cancer in African Americans compared to Non-Hispanic Whites.

Maria Christiene Lawas[1], Mei-Chin Hsieh[2], Siddhi Patil[3], Anju Duttargi[4], Aishani Kulshreshtha[1], Lucia Martinez Cruz[3], Bailey Garb[1], Shiting Li[1], Nisha D'Silva[1], Maureen Sartor[1], Laura Rozek[5]

[1]University of Michigan Medical School, Ann Arbor, MI,[2]Louisiana Tumor Registry, New Orleans, LA,[3]Georgetown Lombardi Comprehensive Cancer Center, Washington, DC, DC,[4]Georgetown University Medical Center, Washington, DC, DC,[5]Georgetown University Medical Center, Georgetown Lombardi Comprehensive Cancer Center, Washington, DC, DC

This project addresses the need for precision biomarkers for HPV-associated oropharyngeal squamous cell carcinoma (OPSCC) in diverse populations. The immunohistochemistry (IHC) test for the protein p16 is the only clinically approved biomarker for HPV-status in OPSCC and is incorporated into the AJCC v8 staging for OPSCC. It is also used as inclusion criteria for clinical trials, determining the eligibility of patients to receive de-intensified treatment. Literature demonstrates that p16 IHC is estimated to have a sensitivity of 94% and a specificity of ~85-90%. However, the predictive value is lower in populations with lower HPV prevalence, and for these reasons, it is important to study the predictive value of p16 in subpopulations. We investigated the reliability of p16 IHC as a surrogate for HPV+ in a cohort of OPSCC comprised of African Americans (AA) (n=58) and Non-Hispanic Whites (NHW) (n=88) from the Louisiana Tumor Registry (LTR) and University of Michigan (UM). HPV testing was performed on FFPE DNA using bulk RNA sequencing and mapping to high-risk HPV types. Twenty-nine percent (17/58) of AA and 88% (77/88) of NHW were HPV-positive based on RNA. However, 25/58 (43.1%) AA were p16+ and 77/88 (87.5%) of NHW were p16+. The sensitivity of the test for AA and NHW was estimated to be 94.1% and 94.8%, respectively. The specificity of the test for AA and NHW was estimated to be 78.0% and 63.6%, respectively. The positive predictive value (PPV) was significantly lower in AA patients (0.64) compared to NHW (0.948) (p-value = 0.00024, proportions test). In all, the correspondence of p16 and RNA HPV+ was 16/25 in AA OPSCC. Differential expression analysis confirmed that the expression of CDKN2A, the gene responsible for producing p16, was significantly different in HPV RNA positive status versus HPV RNA negative status of any race (logFC ~2-fold, $p < 0.05$) and was not significantly different between NHW HPV RNA positive and AA HPV RNA positive groups (logFC = -0.49, p-value > 0.05). ROC analysis demonstrated that CDKN2A expression had predictive ability for HPV RNA status across all patients (AUC = 0.92), with subgroup analyses showing even higher predictiveness in AA patients (AUC = 0.96) compared to NHW patients (AUC = 0.90). These results indicate that use of p16 IHC for patient stratification in AA OPSCC cases could lead to p16+ HPV-negative cases inappropriately treated as HPV+ tumors, leading to adverse outcomes. This highlights the need for confirmatory HPV testing in OPSCC patients, especially in subpopulations that have a low attributable fraction of HPV, to avoid undertreatment. Our ongoing work will address the use of other HPV-related biomarkers and characterization of pathways that differ between AA and NHW OPSCCs. Prioritizing diversity in patient populations and outcomes research ensures advancements in cancer research reflects all populations, especially those historically underserved.

#7104 Variation in breast cancer molecular sub-types prevalence among ethnic groups within Ghana and implication for tumor biologic studies among African-Americans.

Moses A. Dokurugu[1], Moses Kamita[2], Sylvester Antwi[2], Rose Dampson[3], Patrick K. Akakpo[4], Harriet Larrious-Lartey[2], Valerie Ofori Aboah[2], Foster Amponsah[5], Josephine Nsaful[3], Michael Nortey[6], Kwabena Agbedinu[7], Samuel Mensah[8], Mohammed Sheriff[1], Nelson Affram[9], Ijeoma Aja[10], Alex Mremi[11], Theresia Mwakyembe[11], Mohammed Bharmal[12], Veneranda Nyarko[3], Ali Haythem[12], Eleanor Walker[12], Jessica Bensenhaver[12], Albert Levin[12], Evelyn M. Jiagge[2]

[1]Tamale Teaching Hospital, Tamale, Ghana,[2]Henry Ford Health System, Detroit, MI,[3]Korle Bu Teaching Hospital, Accra, Ghana,[4]Cape Coast Teaching Hospital, Cape Coast, Ghana,[5]Eastern Regional Hospital, Koforidua, Ghana,[6]Cape Coast Teaching Hopsital, Cape Coast, Ghana,[7]Komfo Anokye Teaching Hospital, Kumasi, Ghana,[8]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana,[9]Ho Teaching Hospital, Ho, Ghana,[10]Eastern Regional Hospital, Ho, Ghana,[11]Kilimanjaro Christian Medical Centre, Moshi, Tanzania, United Republic of,[12]Henry Ford Health, Detroit, MI

Background: Triple negative breast cancer (TNBC) is most prevalent in women with Western sub-Saharan African ancestry, including African Americans (AAs) and continental African women. This similarity in risk has been shown to be due in part to the shared genetic ancestry resulting from the trans-Atlantic slave trade. To understand the role of genetic African ancestry in the risk and tumor biology of TNBC in AAs, we established a research collaboration in Ghana (West African, WA), Precision Medicine for Aggressive Breast Cancers (PMABC), to access WA female patients and their tumors for comparative studies with AAs. A principal goal of the PMABC study is to take a more inclusive approach to broadly capture the epidemiologic and tumor biologic characteristics of breast cancer within Ghana.

Methods: To achieve this objective, PMABC is based on a collaborative network of six Ghanaian institution, representing the entire country, and in this first analysis, we describe how patient characteristics, including ethnicity, are related to molecular subtypes of breast cancer in this prospectively collected cohort. We used multinomial logistic regression to evaluate the associations between the molecular breast cancer sub-types (Luminal A, Luminal B, HER2+, TNBC) and patient characteristics.

Results: Sociodemographic data, clinical/pathologic characteristics, and biological samples were collected from a total of 690 consented cases. Among these women the mean age (SD) was 51.37 (13.5) years and mean BMI was 27.9. Importantly, nine different ethnicities were captured in the sample, and 79% of the patients belonged to one of the four main Ghanaian ethnic groups: Akan (Southern, n=352, 51%), Dagomba (Northern, n=36, 5.2%), Ewe (Southeastern, n=93, 13.5%), and Ga (Southwestern, n=61, 8.8%). 38.4% of all the cases were TNBC. There was a significant difference in the risk of developing TNBC among the different ethnic groups; in particular, the Ga ethnic group had the lowest TNBC proportion at 23.7%. Relative to the Akan TNBC prevalence (42.8%) and the Luminal A subtype, the Ga had a 61% decreased risk of TNBC (odds ratio =0.39; p=0.007). Additionally, being menopausal was associated with a decreased risk of developing Luminal B (p=0.029), HER2+ (p=0.018), and TNBC (p=0.027), relative to luminal A subtype. There was also a significant decrease in the age of diagnosis for those with the Luminal B (p=0.008) and HER2+ (p=0.025) subtypes when compared with those who had Luminal A subtype.

Conclusion: These findings are among the first to indicate significantly different risk of TNBC among the geographically diverse ethnicities within Ghana, and they suggest the need for a more refined sub-continental genetic ancestry analysis to effectively determine the impact of genetic African ancestry on the biology of both WA and AA breast cancer.

#7105 Analyses of real-world clinico-genomic and demographic data highlight the multifactorial causes of poor colorectal cancer outcomes in patients of African ancestry.

Sharafudeen D. Abubakar, Henry Walch, Christina I. Lee, Chin-Tung Chen, Kanika Arora, Farheen Shah, Tejiri Agbamu, Michele Waters, Christopher Fong, Justin Jee, Michael F. Berger, Karuna Ganesh, Debyani Chakravarty, Walid K. Chatila, Nikolaus Schultz, Rona Yaeger, Julio Garcia-Aguilar, Francisco Sanchez-Vega

Memorial Sloan-Kettering Cancer Center, New York, NY

Background: Colorectal cancer (CRC) patients of African ancestry (AFR) have been reported to have shorter overall survival (OS) than patients of non-African ancestry (non-AFR). We analyzed real-world clinico-genomic data from a single tertiary cancer center, including treatment and outcomes, to investigate this disparity.
Methods: We analyzed data from 4,310 CRC patients diagnosed after 2014 and treated at Memorial Sloan Kettering Cancer Center (MSKCC), including 270 AFR and 4,040 non-AFR patients. Patients had their tumors sequenced with MSK-IMPACT, a targeted DNA sequencing assay that identifies genomic alterations in 341-505 genes. Genetic ancestry was inferred from sequencing data, and patients with >80% African ancestry were classified as AFR. OncoKB was used to identify clinically actionable genomic alterations, as well as alterations associated with treatment resistance. Type of medical insurance and Yost index based on billing address were recovered from institutional databases. Covariates used in our analyses included primary tumor location, stage at diagnosis, time from diagnosis (Dx) to arrival at MSK and metastatic burden. OS was measured from the time of Dx.
Results: AFR patients had shorter OS (median 39.1 vs. 60.2 months, p<0.001) and presented with more advanced disease: they were more often diagnosed with stage IV disease (51.5% vs 44.3%, p=0.034), tended to arrive later at MSKCC (21.5% vs. 10% arriving >1 year after Dx, p<0.001), more often received prior treatment (29.5% vs 22.1%, p=0.01), and had higher rates of right-sided colon tumors (37.9% vs 21.1%, p<0.001). AFR patients exhibited higher metastatic burden during their clinical history, including higher frequency of liver (70.3% vs 59.3%, p<0.001), lung (57.8% vs 48.6%, p=0.005) and CNS (14.9% vs 8.5%, p=0.001) metastases. They also had lower rates of MSI tumors (6.6% vs 11.1%, p=0.073), fewer FDA-approved targetable alterations (6.1% vs 11.5%, p=0.006 - driven by BRAF), and more alterations resistant to FDA-approved drugs (60.7% vs 45.5%, p<0.001 - driven by KRAS). Overall, Medicaid usage was independently associated with shorter OS (median 29.3 vs. 59.9 months, p<0.001) and later arrival at MSKCC (17.7% vs. 10.4% arriving >1 year after Dx, p=0.024). AFR patients had a higher rate of Medicaid users (7.9 vs. 2.6%, p<0.001), which can partially explain their shorter OS. However, even when Medicaid and non-Medicaid users were analyzed separately, AFR patients still had worse OS (median 26.1 vs. 34.5 months, p=0.069, and 43.7 vs. 60.4 months, p=0.002, respectively).
Conclusions: AFR ancestry and Medicaid use were additive factors associated with poor prognosis. This disparity could be at least partially explained by a combination of clinico-genomic factors, including more advanced disease at diagnosis and delayed arrival at the treating tertiary care center.

#7106 Comprehensive spatial profiling of the tumor immune microenvironment in triple negative breast cancer from Black women.

Felipe Segato Dezem[1], Matt Ingalls[2], Omotoso Ayodele[3], Destiny Burnett[3], Priscila Coelho[3], Carmen Gomez[3], Sara Bodbin[4], Judith Hurley[3], Agata Zieba Wicher[4], Arne Christians[2], Jannik Boog[2], Daniel Jimenez Sanchez[2], Oliver Braubach[2], Sophia George[3], Jasmine Plummer[1]

[1]Center for Spatial OMICs, St. Jude Children's Research Hospital, Memphis, TN,[2]Bruker Spatial Biology, Seattle, WA,[3]Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL,[4]Navinci Diagnostics, Uppsala, Sweden

Normal tissues from Black individuals, regardless of their country of birth or residence, remain critically understudied. This gap in research is particularly concerning, as Black individuals disproportionately experience aggressive pathological diseases, often exhibit resistance or refractoriness to treatment, and face higher rates of premature death. In women, breast cancer is not only more common among U.S. Black women, but it also tends to develop at a younger age compared to other ancestral groups. Furthermore, U.S. Black women are approximately twice as likely as White women to be diagnosed with triple-negative breast cancer (TNBC), a subtype characterized by higher recurrence rates and poorer survival outcomes. To investigate the biological factors contributing to these disparities, we spatially profiled TNBC tumors from U.S. Black and White patients. Our analysis revealed significant differences in the tumor immune microenvironment (TiME). Tumors from Black women exhibited higher immune infiltration, particularly among macrophage and T cell populations. To explore how differences in key immune cell populations could impact immunotherapeutic outcomes, we employed a multiomic spatial proteomics approach, aimed at (1) identifying immune cell populations, (2) mapping their activation states, and (3) revealing PD-1/PD-L1 checkpoint activity. All of this was accomplished with a novel serial CellScape^{TM} workflow, which allows the integration multiplex immunofluorescence (mIF) with and *in situ* Proximity Ligation Assay (*is*PLA). Our mIF assay consisted of more than 30 Vistaplex^{TM} antibodies and efficiently labeled immune cell lineages, activation states and checkpoints. Cell-to-cell interactions and protein-protein interactions at immune checkpoints were then surveyed throughout the TiME using *is*PLA, which enables the imaging-based identification of checkpoint activation via detection of protein-protein interactions. The assay is based on a 40 nm proximity of the interacting partners and uses rolling circle amplification (RCA) to amplify the signal, ensuring high sensitivity and specificity of the detected interaction, in this case, that of PD1 and PDL1. Collectively these results affirmed heightened immune infiltration in tumors from Black woman and, crucially, allowed us to map checkpoint activation on a cell-by-cell phenotypic basis. By leveraging multiomic spatial phenotyping, we detected distinctions in the TNBC TiME from Black women compared to White women. Linking together the occurrence of certain cell phenotypes, their spatial signatures, and their checkpoint activation states. These findings underscore the importance of studying diverse patient populations to better understand tumor biology and inform more equitable therapeutic strategies.

#7107 Racial, gender and socioeconomic disparities in survival outcomes of gastrointestinal stromal tumors: a retrospective analysis from 2000 to 2021.

Asfand Yar Cheema[1], Oboseh J. Ogedegbe[2], Muhammad Faisal Aslam[3], Serhan Unlu[1], Mishaal Munir[4], Kai He[5], Utkarsh Acharya[5]

[1]Cleveland Clinic Foundation/Fairview Hospital, Cleveland Clinic, OH,[2]Trinity Health Ann Arbor Hospital, Ypsilanti, MI,[3]UAB St. Vincent's East Hospital, Birmingham, AL,[4]Lahore Medical and Dental College, Lahore, Pakistan,[5]The Ohio State University Comprehensive Cancer Center, Columbus, OH

Background: Gastrointestinal stromal tumors (GISTs) are rare mesenchymal neoplasms that originate in the interstitial cells of Cajal or precursor cells within the gastrointestinal (GI) tract, predominantly affecting the stomach and small intestine. GISTs represent the most common type of GI mesenchymal tumors, accounting for approximately 1-3% of all GI tumors. In this study, we explored the effect of gender, race/ethnicity, and socioeconomic factors on survival outcomes among patients with GISTs in the United States.
Methods: We extracted data on patients with a histological diagnosis of GISTs from the Surveillance Epidemiology and End Result (SEER) Registry from 2000-2021. The primary outcome of interest was cause-specific survival. According to the International Classification of Disease for Oncology, Third Edition (ICD-O 3) system, the histological code of GISTs is 8936/3. We excluded patients of missing or unknown race, income, location, and patients outside the years of study. Kaplan-Meier methods were used to estimate 5-year survival rates. Cox proportional hazards regression was used for multi-variable modeling of the survival database. The difference between the observed number of events between each group was compared using a log-rank statistical test. Patients were followed up for 5 years, and survival analysis results were censored at this point, with hazard ratios reflecting 5-year overall survival (OS) between groups.
Results: 11,247 patients met the inclusion criteria. The gender split was 5,719 males (50.9%) and 5,528 females (49.1%). Based on gender, males as compared to females have significantly worse outcomes hazard ratio was 1.18 (95% CI 1.1-1.26, p<0.001). Races were categorized into non-Hispanic Black (n=2092, 18.6%), Non-Hispanic White (n=6027, 53.6%), non-Hispanic Asian or Pacific Islander (n=1616, 14.4%), Hispanic (n=1490, 13.2%), and Alaskan-Native/American Indian (n=22, 0.2%). Compared to Hispanic patients, White and Black patients had significantly worse outcomes (HR 1.13, 95% CI 1.01-1.26, HR 1.38, 95% CI 1.22-1.56, p=0.028, p<0.001, respectively), with no significant difference in outcome among other races. Compared to patients with distant metastasis (n=2403), patients with localized disease had significantly better outcomes (HR 0.26, 95% CI 0.24-0.28). When household income was categorized above and below $60,000 per annum, higher household income was also associated with better outcomes (HR 0.86 95% CI 0.78-0.94, p<0.001).
Conclusions: Gender, race/ethnicity, and income disparities all played a significant role in cause-specific survival in patients with GISTs, with improved survival in females, higher household income (>$60K), and Hispanic patients as compared to Whites and Blacks. More efforts and research are needed to elucidate the root cause and mitigate these survival disparities.

#7108 Leveraging race-informed biological models for disparities research in colorectal cancer.

Xiang Han, Stefanie Gerstberger, Kathleen Luckett, Francesco Cambuli, Jaeyop Lee, Andres Rettig, Ahmed Mahmoud, Jonathan Bermeo, Asha Saxena, T.Peter Kingham, William Jarnagin, Philip Paty, Martin Weiser, Michael D'Angelica, Rona Yaeger, Julio Garcia-Aguilar, Francisco Sanchez-Vega, Karuna Ganesh

Memorial Sloan Kettering Cancer Center, New York, NY

Background: Non-Hispanic Black (NHB) patients have a 20% higher incidence of colorectal cancer (CRC) and a 40% higher CRC mortality than non-Hispanic white (NHW) patients. Disparities may be explained by socioeconomic variables (such as smoking or obesity) and access to health care. However, the role of potential biological differences remains unclear. Patient-derived organoid models (PDOs) provide an ideal model system to study biological differences and avoid confounding clinical variables by focusing on the genomic, phenotypic and functional differences in CRC cells *ex vivo*. There is currently a lack of patient-derived preclinical models from NHB populations. We aim to build a public biobank of NHB patients with detailed clinical annotation, chemosensitivity and biological characterization, examining intrinsic tumor cell states in different genomic backgrounds as potential variables in CRC outcomes.
Methods: PDOs were established from surgically resected primary and metastatic CRC samples from NHB and NHW patients, processed through enzymatic digestion and filtration, and cultured in Matrigel in human intestinal stem cell (HISC) media. Genomic features of the corresponding patients were analyzed using the MSK-IMPACT targeted DNA sequencing assay. We assessed the responses of NHB and NHW PDOs to FOLFOX chemotherapy through drug sensitivity assays with 5-FU:oxaliplatin (concentration of 25:1). Dose-response curves were fitted using the two-parameter logistic regression model to calculate median IC50. After dissociation into single cells, PDOs were cultured in HISC and Base (media without intestine-specific growth factors) conditions for 11-14 days and analyzed by bulk RNA-sequencing to capture phenotypic differences associated with canonical intestinal (less aggressive) and non-canonical (more aggressive) cell states.
Results: We aim to establish a biobank of 10 NHB and 10 comparative NHW patients with similar clinicogenomic profiles. To date, 12 PDOs derived from 5 NHB (3 primary and 2 metastasis samples) and 7 NHW (2 primary and 4 metastasis samples) patients are characterized with further PDO collection ongoing. Four samples with KRAS missense mutations were present across the NHB and NHW patient groups (NHB: 80%, NHW: 57%). IC50 of five NHB and five NHW PDOs revealed no significant differences in sensitivity to FOLFOX (median IC50 NHB: 70.3uM; median IC50 NHW: 59.92uM). ssGSEA scores derived from bulk RNA-seq data of five PDOs, comparing three NHB and two NHW patients, exhibited greater variation across lines than within racial groups, with no significant differences between NHB and NHW patients.
Conclusions: Ongoing work is focused on building a larger cohort for wider comparative characterization. The future completed biobank will become a public resource for scientists to access racially diverse PDOs for drug testing and further biological characterization.

#7109 Understanding race-specific differences in the predictive performance of computational outcome models for breast cancer.

William Asinger, Sarah Eskreis-Winkler, Armaan Kohli, Enrico Moiso, Anika Begum, Mehnaj Ahmed, Andrew Aukerman, Jianjiong Gao, Jian Carrot-Zhang, Arfath Pasha, Hong Zhang, Elizabeth Sutton, Sohrab Shah, Pedram Razavi, **Francisco Sanchez-Vega**

Memorial Sloan Kettering Cancer Center, New York, NY

Background: Neoadjuvant chemotherapy (NACT) is the standard of care for locally advanced breast tumors and pathologic complete response (pCR) is the most prominent determinant of favorable outcomes. We built computational predictors of pCR for breast cancer patients (pts) treated with NACT using different data modalities acquired at baseline (clinical, genomic, pathology and radiology) and investigated race-specific differences in their predictive value when pts were stratified into hormone receptor subtypes.
Methods: We analyzed data from 1,135 breast cancer pts who self-identified as Asian (n=151, 13%), Black (168, 15%), Hispanic (154, 14%) or White (662, 58%). Patients were treated with NACT at Memorial Sloan Kettering (MSK) between 2014 and 2021. The cohort included HR-/HER2+(n=183, 16%), HR+/HER2- (353, 31%), HR+/HER2+(339, 30%) and TNBC (260, 23%) pts. Digital pathology whole-slide images from H&E stained pre-NACT tumor biopsies were analyzed for 863 pts. Pre-NACT MRIs were analyzed for 608 pts. Targeted DNA sequencing from MSK-IMPACT was analyzed for 302 pts. Predictors of pCR were built using penalized regression models for the clinical and genomic modalities, and deep learning (DL) architectures for pathology and radiology. Genetic ancestry was computed for pts with sequencing information using published methods. Model performance was quantified as the area under the receiver operating characteristic curve (AUC).
Results: Genetic ancestry labels and self-reported race were highly concordant (297/302, 98% match). The overall pCR rate was 38.5% and varied strongly by receptor subtype, being lowest for HR+/HER2- (13%) and highest for HR-/HER2+ (75.4%). pCR rate also varied across races, being highest for Asians (45%) and lowest for Black pts (34.5%), although this can be at least partially explained by the different representation of receptor subtypes across races (e.g., Black pts had significantly higher rates of TNBC tumors, 37.5% vs. 20.3%, $p < 0.0001$). After controlling for subtype, Hispanics tended to be diagnosed at a younger age (median 46 vs. 50 years, $p=0.02$). We identified several combinations of subtype and data modalities where AUCs varied by race: for example, AUCs for the clinical predictor were higher for Asian pts in the TNBC subtype (0.64 AUC vs. 0.49 mean AUC across all races), while the predictor based on the same modality overperformed among Hispanics of the HR-/HER2+ subtype (0.84 AUC vs. 0.65 mean AUC across all races). Training subtype-specific models instead of a pan-subtype model only improved overall cross-type AUC for Black pts (mean AUC increase of 0.07).
Conclusions: Racial background can impact the predictive performance of computational outcome models in breast cancer and should be factored for method design. The influence of race-specific differences varies across data modalities and receptor subtypes.

#7110 Exploring cardiovascular comorbidities in cancer-directed therapies for patients with breast cancer in the southwestern United States: A two-center analysis.

T. Escobar-Gil[1], E. Sherry[2], A. C. Millhuff[1], V. Ayodele[3], A. Baig[3], V. G. Hanson[1], O. F. Borja-Montes[1], J. M. Nemunaitis[4], M. Mazo-Canola[3];
[1]University of New Mexico Health Sciences Ctr., Albuquerque, NM, [2]University of Texas Health Science Center at San Antonio, San Antonio, TX, [3]University of Texas Health San Antonio Mays Cancer Center, San Antonio, TX, [4]University of New Mexico Comprehensive Cancer Center, Albuquerque, NM

Background: In 2024, approximately 43,700 women in the U.S. are expected to die from breast cancer. This disease is a leading cause of cancer-related mortality, especially among Hispanic women, who face unique risks, including higher cardiotoxicity rates from treatments like anthracyclines and trastuzumab. Nearly 40% of breast cancer patients experience cardiovascular comorbidities such as hypertension and heart failure, worsened by treatment-related toxicities, and cardiovascular disease has emerged as a leading cause of death among breast cancer survivors. Health disparities contribute to these outcomes, with Hispanic women facing barriers to care and a slower decline in cancer-related fatalities compared to non-Hispanic White women. This study investigates these factors within the Southwestern U.S., where the burden of breast cancer is significant.
Methods: This retrospective cohort study analyzed 394 breast cancer patients from two medical centers located in the Southwestern U.S. (195 from the UNMCCC in Albuquerque, New Mexico, and 199 from UTHSCSA Mays Cancer Center in San Antonio, Texas) treated between January 2022 and December 2023. Inclusion criteria encompassed adult patients diagnosed with stage I-III breast cancer, receiving treatment with curative intent, excluding those with incomplete records. Data was extracted from electronic health records, covering demographics, treatment regimens, radiation use, and cardiovascular conditions while adhering to HIPAA regulations.
Results: The study included 394 breast cancer patients, with a mean age of 59.94 years (SD = 12.11) and a mean BMI of 30.12 kg/m² (SD = 6.58). Women comprised 99% of the cohort (n=389). Patients from UTHSCSA had a mean BMI of 31.00 kg/m² (SD = 6.45), while those from UNMCCC had a mean BMI of 29.25 kg/m² (SD = 6.62). Approximately 84.1% identified as Latino (White), with 61.3% (n=241) pre-menopausal and 83.0% (n=327) having hormone receptor-positive cancer. Anthracyclines were given to 21.1% (n=83), and HER2-directed treatments were administered to 14.7% (n=58) of patients. Radiation therapy was utilized in 58.5% (n=113) of the cohort. Hypertension was the most prevalent cardiovascular condition, affecting 46.5% (n=184), followed by diabetes mellitus (26.1%) and hyperlipidemia (29.7%). Cardioprotective medication usage varied, with around 50% not receiving any such treatments.
Discussion: The findings highlight the health challenges of the Latino population, especially concerning hormone receptor-positive breast cancer. The high rates of obesity and cardiovascular comorbidities underscore the need for targeted interventions. Treatment plans should be individualized for each patient's cardiovascular comorbidities while receiving cancer treatment and delivered through a multidisciplinary approach.

#7111 A cross-sectional survey of genomics infrastructure at six NHGRI-funded Diversity Centers for Genome Research.

X. Wang[1], F. A. Fernandez-Lima[1], Z. Bursac[1], S. Black[1], M. Baum[1], J. W. Pettit[1], D. Bedi[2], H. Wang[2], B. Karanam[2], M. B. Davis[3], M. Rachel[3], E. L. Johnson[3], B. Rivers[3], A. Winkler[4], J. Galan[4], J. Blangero[4], S. Williams-Blangero[4], M. Tiirikainen[5], L. Wu[5], Y. Deng[5], G. Pan[5], O. Korolkova[6], A. Ivanova[6], H. Rajakaruna[6], T. Kanagasabai[6], D. B. Borza[6], R. Singh[6], S. Chirwa[6], L. Stewart[6], S. T. Miller-Hughes[6], A. Ramesh[6], E. Madden[7], A. Shanker[6], **Q. Wang**[6];

[1]Florida International University, Miami, FL, [2]Tuskegee University, Tuskegee, AL, [3]Morehouse School of Medicines, Atlanta, GA, [4]University of Texas Rio Grande Valley School of Medicine, Brownsville, TX, [5]University of Hawai'i at Mānoa, Honolulu, HI, [6]Meharry Medical College, Nashville, TN, [7]National Human Genome Research Institute, Bethesda, MD

Background: With the rapid advancements in human genetics and genomics research, it is crucial to build genomics capacity at minority-serving institutions (MSIs), which play a key role in developing diverse workforce in genomics and precision medicine, driven by their commitment to educating and training underrepresented students and trainees. Moreover, MSIs are instrumental in tackling chronic diseases, including cancer, that disproportionately affect minority and underserved populations.

Methods: A comprehensive cross-sectional survey was conducted from August to October 2024 across six MSIs within the Diversity Centers for Genome Research (DCGR) Consortium, established by the National Human Genome Research Institute (NHGRI), to assess their genomic infrastructure and capacity. The surveyed institutions included the Pacific Center for Genome Research at the University of Hawai'i at Mānoa,the University of Texas Rio Grande Valley, Florida International University, Morehouse School of Medicine, Meharry Medical College, and the Carver Genomic Research Center at Tuskegee University, with the last three being Historically Black Colleges/Universities (HBCUs). These MSIs serve diverse and historically underrepresented minority populations, including Native Hawaiian and Pacific Islander, Asian Americans, Hispanic/Latino communities, and African Americans. The survey consisted of 32 questions covering a wide range of topics, including DCGR budget allocations, available sequencing equipment, proteomics instruments, biorepository sample collection and management, etc.

Results: An important aspect of the survey is MSIs' access to cutting-edge next-generation sequencing technologies, a critical component of genomic research infrastructure. Among the six MSIs surveyed, four currently have Illumina short-read sequencers, and three have long-read sequencers, either Nanopore or Ion Torrent. However, none of the institutions owns a PacBio long-read sequencer, an increasingly popular platform for applications where accuracy, read length, and the ability to resolve complex genomic regions outweigh throughput needs. The six MSIs also vary in their computing infrastructure, proteomics instruments, and mass spectrometers, among other resources. Furthermore, all six MSIs own or plan to establish human tissue biorepositories. The survey reveals two common challenges regarding biorepositories across these institutions: (1) effective biorepository management and (2) securing sustainable funding.

Conclusion: These results provide valuable insights into the genomic infrastructure and capacity of the six MSIs in the DCGR Consortium. The data collected and insights gained lay a strong foundation for supporting MSIs and fostering future collaborations among them to tackle the common challenges they face in building robust genomics programs.

#7112 Pattern of beef consumption and prostate cancer risk among males in the United States.

Opeyemi Oreoluwa Bolajoko[1], Daniel W. Lee[1], Catherine Adebukola Oladoyinbo[2], Ernie Kaninjing[3], Samuel O. Antwi[4], Dee M. Glaser-Boivin[4], Robert J. Turesky[5], Folakemi T. Odedina[6]

[1]Mayo Clinic Comprehensive Cancer Center, Jacksonville, FL,[2]Nutrition and Dietetics, Federal University of Agriculture, Abeokuta, Nigeria,[3]School of Health and Human Performance, Georgia College, Milledgeville, GA,[4]Mayo Clinic, Jacksonville, FL,[5]Masonic Cancer Center, University of Minnesota, Minneapolis, MN,[6]Cancer Health Equity Research Program, Mayo Clinic Comprehensive Cancer Center, Jacksonville, FL

Background: Epidemiology studies have linked frequent consumption of well-done cooked red meat with aggressive prostate cancer (CaP) risk, whereas rare intake or consuming medium-cooked meat is not. Providing public information about the impact of well-done cooking and its potential role in CaP risk may foster the adoption of evidence-based lifestyle modification of meat consumption to lower CaP risk. This pilot study aimed to provide preliminary data on the occurrence of CaP and the pattern of consumption of cooked red beef among ethnically diverse males in the US.

Methods: The proposed project is an exploratory study focused on the assessment of the consumption of various beef dishes among ethnically diverse males in the United States. The study included 408 ethnically diverse males from Florida, Georgia, and Texas. Participants were recruited primarily from partnering restaurants, pharmacies, barbershops and collaborating community businesses using QR codes on the study flyer. Participants completed the self-administered survey that captured demographics, as well as information on beef consumption patterns, including cooking methods, frequency and degree of cooking, and the occurrence of CaP. The data were analyzed using descriptive statistics (frequency and percentages) to summarize demographics and variables on beef dishes. Chi-square was employed to determine the relationship between beef consumption patterns, demographic variables, and CaP occurrence.

Results: The study showed that about half (49.8%) of the participants were between ages 44-63 years and 34.2% were 30-43 years old. The participants were quite diverse, as 51.7%, 16.3%, 13.1%, 11.%, and 2.7% were African American, African immigrants, White, Caribbean immigrants, and Hispanics, respectively. Rural dwellers comprised 34.2%, 26.8% were suburban, and 35.7% lived in urban areas. Educational status showed that 22.7%, 27.8%, and 22.7% were completed high school, college graduates, and associate degrees. The beef consumption pattern shows that 23.6%, 33.3%, 40.1%, and 30.3% regularly had boiled, fried, grilled, and barbequed beef, respectively, but 63,2% reported never eating boiled beef. Doneness preferences differed among participants; 26%, 43.9%, 49.3, and 45.1% preferred well-done, while 1.7%, 1.5%, 2..9%, and 2.7% preferred rare, and 9%, 14.5%, 17.4%, and 15.7% preferred medium boiled, fried, grilled, and barbeques beef, respectively. Only 4.4% of the participants reported being diagnosed with CaP in the past. The pattern of beef consumption, such as the degree of doneness, was found for boiled ($p=0.045$), fried ($p<0.001$), grilled ($p<0.001$), and barbequed ($p<0.001$) beef, as well as frequency of consumption of ($p<0.001$), fried ($p<0.001$), grilled ($p=0.001$), and barbequed ($p<0.001$) to be associated with CaP.

Conclusion: Beef consumption pattern was found to be associated with the risk of CaP among participants.

#7113 Barriers to cancer diagnosis and treatment among women in low- and low-middle-income countries.

Sylvester Antwi[1], Kwabena Agbedinu[2], Livingstone Aduse-Poku[3], Foster Amponsah[4], Harriet Larrious-Lartey[1], Samuel Mensah[5], Josephine Nsaful[6], Patrick Akakpo[7], Veneranda Nyarko[6], Michael Nortey[7], Alex Mremi[8], Theresia Mwakyembe[8], Valerie Ofori Aboah[1], Moses Dokurugu[9], Mohammed Sheriff[9], Nelson Affram[10], Evelyn M. Jiagge[11]

[1]Henry Ford Health, Detroit, MI,[2]Komfo Anokye Teaching Hospital, Kumasi, Ghana,[3]University of Florida, Gainesville, FL,[4]Eastern Regional Hospital, Koforidua, Ghana,[5]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana,[6]Korle Bu Teaching Hospital, Accra, Ghana,[7]Cape Coast Teaching Hospital, Cape Coast, Ghana,[8]Kilimanjaro Christian Medical Centre, Moshi, Tanzania, United Republic of,[9]Tamale Teaching Hospital, Tamale, Ghana,[10]Ho Teaching Hospital, Ho, Ghana,[11]Henry Ford Health System, Detroit, MI

Introduction: Globally, the risk of death from cancer is higher in lower to middle income countries despite a reported lower incidence in these countries. This is attributed not only to biological factors, but to limited access to resources for screening, diagnosis, and treatment. We conducted a scoping review to identify barriers to cancer diagnosis and treatment among women in low- and low-middle-income countries, focusing on breast, colorectal, lung, cervix uteri, thyroid, corpus uteri, and stomach cancers.
Methods: Using the Joanna Briggs Institute approach and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) extension for scoping reviews, we systematically searched PubMed, Embase, Cochrane Library, PsycInfo, CINAHL, Scopus, and Web of Science. Eligible studies, published in English, reported barriers to cancer care in low- and low-middle-income countries. Screening and data extraction were conducted in Covidence by two independent reviewers. A total of 29 studies involving 7031 participants were included, employing quantitative, qualitative, and mixed methods designs.
Results: Most studies (79.3%) were conducted in low-middle-income countries, with Sub-Saharan Africa (82.76%) as the predominant region. Breast cancer was the most studied cancer type (59.38%). On average, diagnosis occurred 7.39 months after symptom onset, and treatment began 4.9 months later. Patients traveled an average of 62 km (172.5 minutes) to health facilities. The most common barriers to cancer care were financial difficulties (71.45%), healthcare system challenges (55.17%), health literacy gaps (51.72%), geographical barriers (68.5%), and sociocultural factors. Financial challenges, reported in 65.52% of studies, included limited resources, high costs, and socioeconomic strain. Healthcare system issues, documented in 55.17% of studies, involved resource shortages, misdiagnosis, and delayed referrals. Geographical barriers, noted in 34.48%, included long travel distances and transportation costs. Health literacy gaps, reported in 51.72% of studies, stemmed from inadequate awareness and education among patients and providers. Sociocultural and stigma-related barriers, highlighted in 41.38% of studies, included cultural beliefs, fear of stigma, and spiritual attributions of illness.
Conclusion: This review underscores the multifaceted barriers to cancer care in low-resource settings, emphasizing the need for interventions addressing financial, health system, educational, and sociocultural challenges. Enhanced healthcare infrastructure, community education, and socioeconomic support are critical to improving cancer outcomes in these regions.

#7114 Carboplatin in combination with etoposide for advanced small cell lung cancer complicated with idiopathic interstitial pneumonia: A single-arm phase II study.

M. Matsumoto[1], Y. Minegishi[2], K. Higa[1], A. Fukuizumi[1], N. Onda[1], S. Takeuchi[1], A. Miyanaga[1], A. Gemma[1], M. Seike[1];
[1]Nippon Medical School, Tokyo, Japan, [2]Mitui Memorial Hospital, Tokyo, Japan

Background: Acute exacerbation (AEx) of interstitial pneumonia is the most common lethal adverse event related to the pharmacological treatment of patients with lung cancer complicated with interstitial pneumonia. Although small cell lung cancer (SCLC) is linked to poor prognosis, it exhibits good response to chemotherapy. Few previous research studies have investigated the safety and efficacy of treatment for advanced SCLC complicated with idiopathic interstitial pneumonia (IIP). We conducted a single-arm phase II study to evaluate the safety and efficacy of carboplatin plus etoposide for the treatment of patients with SCLC complicated with IIP.
Methods: Chemotherapy-naïve patients with advanced SCLC complicated with IIP were enrolled. Patients received carboplatin every 21-28 days at a dose of area under the curve 4-6 on day 1 and etoposide at a dose of 80-100 mg/m^2 on days 1-3.
Results: Thirty-one patients were enrolled between December 2009 and December 2022. A median of four cycles of carboplatin plus etoposide were administered. Acute exacerbation of idiopathic interstitial pneumonia was not observed; the rate of AEx was 0% (95% confidence interval [CI]: 0-9.6%, p=0.038). The objective response rate was 83.9% (95% CI: 82.5-85.2). The median progression-free survival and overall survival were 5.9 (95% CI: 4.7-6.8) months and 14.0 (95% CI: 7.6-27.6) months, respectively. The 1-year survival rate was 61%.
Conclusions: The carboplatin plus etoposide treatment was tolerable and effective in SCLC patients complicated with IIP.

#7115 PredicineDIRECT: a one-stop “from sample to result” workflow for improved sample management in oncology clinical trials.

Yong Huang[1], Ao Zhou[1], Pan Du[1], Shidong Jia[1], Qin Luo[2], Alexander Kuklin[1], Myles Walsh[1]

[1]Predicine Inc., Hayward, CA,[2]Huidu Shanghai Medical Sciences, Ltd., Shanghai, China

Background: Liquid biopsy is now a standard in clinical trials, improving therapy selection, prognosis, and biomarker discovery. However, reliance on CROs for sample collection, processing, and storage often leads to delays and inefficiencies. PredicineDIRECT, a genomics-based biorepository system, addresses these challenges by centralizing sample management, enabling rapid and accurate support for clinical trials.

Methods: Using ISO13485-certified GMP kits, Predicine collects and processes blood, urine, and tissue samples globally and manage samples using BioLIMS. Patient-specific SNP barcodes and bioinformatics analyses detect errors like mislabeling or sample swaps. Tumor Fraction (TF) is measured via low-pass whole-genome sequencing to assess tumor burden efficiently. This workflow enables real-time quality control, longitudinal tumor burden evaluation, and optimized sample selection for downstream analysis. Sponsors access data through a client portal, while securely stored samples remain ready for immediate in-house genomic testing.

Results: In a trial cohort, SNP barcoding identified sample mismatches, including one between plasma and buffy coat samples and another at a longitudinal time point. Predicine promptly resolved these discrepancies, communicating actionable solutions to the sponsor before long-term storage. TF analysis of plasma samples revealed molecular progression, guiding selection of key time points for deep sequencing. A case study also demonstrated SNP inconsistencies between plasma and buffy coat samples, highlighting the robustness of PredicineDIRECT's error-detection system.

Conclusion: PredicineDIRECT provides a streamlined, cost-effective solution for clinical trial sample management by centralizing processes in a single laboratory. With integrated SNP barcoding and TF analysis, the system ensures error-free handling, accelerates downstream sequencing, and delivers actionable insights into tumor burden, supporting efficient and informed decisions in oncology trials.

#7117 Short-term surgical outcomes for colon adenocarcinoma: Racial-ethnic comparisons in the Military Health System.

Yvonne L. Eaglehouse, **Sarah Darmon**, Michele M. Gage, Craig D. Shriver, Kangmin Zhu

Surgery, Uniformed Services University of the Health Sciences, Bethesda, MD

Background: Racial-ethnic disparities in receipt of treatment and survival outcomes of colon cancer have been documented in the general U.S. population, with access to care identified as a contributor to the disparities. Less is known about disparities in quality aspects of colon cancer surgery, such as achievement of negative surgical margins, adequate lymphadenectomy, and complication rates. We aimed to study colon cancer surgery and postoperative outcomes in the Military Health System (MHS), which provides access to care regardless of patient demographic or socioeconomic characteristics, to better understand racial-ethnic disparities in surgical oncology care in an equal access setting.
Material and Methods: We used the MilCanEpi database to identify patients aged 18 or older who were diagnosed with stage I-III colon adenocarcinoma between 2001 and 2014 and received colectomy as primary surgical treatment. The occurrence of positive surgical margins, inadequate lymphadenectomy (&lt12 nodes examined), 30-day complications (general: surgical site infection, hemorrhage, myocardial infarction, stroke, thrombosis; or gastrointestinal: bowel obstruction or intra-abdominal abscess), and 30-day inpatient admissions were captured. Multivariable logistic regression models estimated the adjusted odds ratios (AORs) and 95% confidence intervals (CIs) in association with race-ethnicity for each outcome.
Results: The study included 157 Asian, 257 non-Hispanic Black, 110 Hispanic, and 1115 non-Hispanic White patients. Overall, the frequency of positive margins (7.6%), inadequate lymphadenectomy (28.9%), 30-day complications (33.1%), or 30-day inpatient admissions (14.3%) did not differ significantly between racial-ethnic groups (all AORs and their 95% CIs included 1.00). By complication type (general or gastrointestinal), Hispanic patients had a significantly lower likelihood of bowel obstruction (AOR=0.48, 95% CI=0.25, 0.95) compared to non-Hispanic White patients, with no other statistically significant racial-ethnic differences.
Conclusions: In the equal access MHS, there were no overall significant racial-ethnic differences in margins, lymphadenectomy, or 30-day outcomes of colectomy for non-metastatic colon cancer. Our findings highlight the role of access to care in minimizing racial or ethnic disparities in colon cancer surgery and postoperative outcomes in the U.S.

#7118 Clinical characteristics and survival outcomes of ovarian cancer among South Asian women in United States.

Aditi Karki[1], Sumeet Kumar Yadav[2], Muhammad Rafiqul Islam[3], Raja Pramanik[4], Ramila Shilpakar Dangol[5], Zeba Aziz[6], Sandhya Pruthi[7], Maryam Shahi[8], Jamie N. Bakkum-Gamez[9], Scott H. Kaufmann[10], Fergus J. Couch[11], Siddhartha Yadav[12]

[1]Oncology, Mayo Clinic Comprehensive Cancer Center, Rochester, MN,[2]Internal Medicine, Mayo Clinic Health System, Mankato, MN,[3]Oncology, National Institute of Cancer Research and Hospital, Dhaka, Bangladesh,[4]Oncology, All India Institute of Medical Sciences, New Delhi, India,[5]Oncology, National Academy of Medical Sciences, Kathmandu, Nepal,[6]Oncology, Hameed Latif Hospital, Lahore, Pakistan,[7]General Internal Medicine, Mayo Clinic, Rochester, MN,[8]Anatomic Pathology, Mayo Clinic, Rochester, MN,[9]Gynecologic Oncology, Mayo Clinic, Rochester, MN,[10]Oncology Research, Mayo Clinic, Rochester, MN,[11]Experimental Pathology and Laboratory Medicine, Mayo Clinic, Rochester, MN,[12]Oncology, Mayo Clinic, Rochester, MN

Background: Ovarian cancer is the sixth leading cause of cancer deaths among women in the United States. South Asians in the USA, including Asian Indians and Pakistani Americans (AIPA), are a rapidly growing yet under-researched group. This study aims to analyze the clinical features, survival rates, treatment, and outcomes of ovarian cancer among AIPA women in the United States.

Methods: Adult women diagnosed with ovarian cancer between 2000 and 2023 within the Surveillance, Epidemiology, and End Results (SEER) registry were identified utilizing ICD codes. The analysis focused on comparing clinical characteristics and outcomes between AIPA and Non-Hispanic White (NHW)women and excluded all women of other races/ethnicities or unknown race/ethnicity. Baseline characteristics, including age at diagnosis, disease stage, and treatment, were compared between AIPA and NHW women utilizing a chi-squared test for categorical variables and a t-test for continuous variables. The 5-year overall survival (OS) was estimated for each race/ethnicity by the Kaplan-Meier method, and the OS was further compared between AIPA and NHW in a cox-proportional hazard regression analysis adjusting for age at diagnosis, grade, histology, TNM stage, surgical debulking, completeness of debulking surgery, and receipt of chemotherapy. Similar comparisons of baseline characteristics and OS were also performed in the subset of women with high-grade serous ovarian cancer (HGSOC).

Result: A total of 86,485 women met the inclusion criteria, of which 1,179 were AIPA and 85,306 were NHW. AIPA had a significantly younger median age at diagnosis compared to NHW women in the overall population (57 vs. 65 years, $p<0.001$) and among 448 AIPA and 29,677 NHW women with HGSOC (58 vs. 65 years, $p<0.001$). Approximately two-thirds of women in both groups presented with Stage III or IV ovarian cancer at diagnosis and had a serous subtype of ovarian cancer. Similarly, after the exclusion of unknown grade, high-grade tumors accounted for more than two-thirds (75 vs. 72%) of the ovarian cancer cases in both groups. AIPA were more likely to undergo debulking surgery (85 vs. 80%, $p<0.001$) and receive chemotherapy (77 vs. 72%, $p<0.001$) compared to NHW women. The unadjusted 5-year OS for AIPA and NHW women were 56% vs. 43% in the overall population, and 53% vs. 41% among women with HGSOC, respectively. In multivariate analysis of the entire study population, AIPA women had a significantly better OS than NHW women (HR: 0.86, 95%CI: 0.79 - 0.94, $p<0.001$). Among women with HGSOC, a similar trend of better OS was noted in multivariate analysis among AIPA women compared to NHW (HR: 0.88, 95%CI: 0.77 - 1.01, $p=0.07$), although this did not reach statistical significance.

Conclusion: The younger age at diagnosis, along with a generally better prognosis, among AIPA women compared to NHW women, suggests a possible enrichment of DNA-repair gene alterations among AIPA women.

CLINICAL RESEARCH: Immune Monitoring / Clinical Correlates
Poster Session

#7122 Accelerating immuno-oncology research with flexible workflows using modular flex-fit CyTOF panels.

Shakir Hasan, **Ling Wang**, Stephen Li, Christina Loh

Standard BioTools, Markham, ON, Canada

Deep functional profiling of immuno-oncology markers and cytokines in whole blood (WB) reveals immune signatures that inform disease prognoses, immunotherapy selection, and outcomes in cancer patients. WB is best analyzed within 24 hours of collection to capture clinically relevant immune signatures; however, WB collection and cytometric analysis are often performed at different sites, which can lead to significant delays. WB stabilizers can overcome this challenge, yet not all antibody panels are compatible with these reagents. To enable flexible experimental workflows for deep profiling of immuno-oncology markers and cytokines in WB, we developed modular Flex-Fit CyTOF™ panels compatible with stabilizers. High-parameter flow cytometry mediated by CyTOF technology enables single-tube staining to identify 50-plus markers at the single-cell level. CyTOF technology provides exceptional resolution without spectral unmixing or compensation, while Flex-Fit CyTOF panels allow simple and quick panel design by combining a customizable selection of pre-validated subpanels. We built two high-parameter antibody panels for analysis of fresh and stabilized WB. The first panel has 21 markers and identifies 12 immuno-oncology markers in 10 major cell subsets. The second panel has 31 markers and identifies 10 cytokines in 32 cell subsets. Stimulated WB samples from two healthy donors were either freshly stained or stabilized with PROT1, RBC lysed and stained. Liquid antibodies in the panel were pooled together and frozen (-80 °C) as single-use aliquots for surface and intracellular staining to reduce technical variability from staining. Optimal panel design ensured that all markers in both panels could be used to accurately identify target cell populations in PROT1-stabilized WB. Following stabilization, nonspecific staining was not observed when compared with freshly stained samples. Cell populations maintained the anticipated co-expression of markers in PROT1-stabilized WB. Prominent cytokines such as IL-17A, TNF-α, IL-10, etc., could be detected in stabilized WB. Overall, we show that PROT1-compatible high-parameter antibody panels enable functional profiling of immune cells by identifying immuno-oncology markers and cytokines. Compatibility with WB stabilization reagents provides workflow flexibility for carrying out functional profiling in longitudinal and multi-center studies in clinical research and healthcare settings. Furthermore, freezing antibody cocktails is a unique feature of CyTOF flow cytometry, ensuring staining consistency. Together, flexible CyTOF workflows enable fast and convenient panel design for high-parameter cytometric analysis of WB and can facilitate biomarker discovery, immunotherapy selection and tracking disease prognoses in cancer.

#7123 Unravelling temporal dynamics of GD2-CAR T cell therapy in H3K27M-mutant diffuse midline glioma through comprehensive single-cell and multi-omic profiling.

Yiyun Chen, Moksha H. Desai, Zachary J. Ehlinger, Kun Wei Song, Skyler Rietberg, Hossein Daghagh, Mark P. Hamilton, Snehit Prabhu, Sonia Partap, Jasia Mahdi, Elena Sotillo, Bita Sahaf, Sneha Ramakrishna, Zinaida Good, Michelle Monje, Crystal Mackall

Stanford University, Stanford, CA

H3K27M-mutant diffuse midline gliomas (DMG) are aggressive pediatric brain tumors with dismal prognoses and no effective treatments. GD2, a disialoganglioside overexpressed on DMG cells, is a promising target for chimeric antigen receptor (CAR) T cell therapy. In Arm A of the Phase I clinical trial (NCT04196413), 12 patients (11 on study) received GD2-CAR T cells through an initial intravenous (IV) infusion, and if patients had clinical or radiographic improvement, they subsequently received intracerebroventricular (ICV) administrations. This regimen achieved clinical benefit in ten patients, with >50% tumor volumetric reductions in 5 patients and a durable ongoing complete response in one patient (Majzner, Ramakrishna et al. Nature 2022; Monje et al. Nature 2024). However, disease progression ultimately occurred in 11 patients underscoring challenges in therapeutic optimization. To elucidate mechanisms underlying treatment responses and resistance, we performed a comprehensive multi-omic analysis, integrating single-cell RNA and TCR sequencing, mass cytometry, and clinical and molecular datasets. Sequencing 1,062,398 cells from patient apheresis, CAR T products, and cerebrospinal fluid (CSF) samples collected longitudinally at pre-defined trigger events, we reconstructed the dynamic landscape of immune and tumor cell populations in 12 DMG patients. Our analysis revealed profound CAR T cell infiltration into the CSF after treatment, accompanied by increases in endogenous T cell, mast cell, and monocyte populations and a relative decrease in microglia evident in CSF. This works sought to deeply characterize the lymphocyte profiles in these samples. Inflammatory and proliferation pathways were elevated in CAR T cells at early post-ICV time points, transitioning into an NK-like state and TGF-β receptor signaling at later stages. TCR clonal tracking identified persistent CAR T clones, alongside endogenous T cell activation and expansion. Moreover, non-responders to the therapy showed elevated Treg signatures in the CSF. Using CellChat-based cell-cell communication analysis, we uncovered differential cytokine-receptor interactions in responders versus non-responders, offering novel insights into immune-tumor interactions in the context of CAR T cell therapy. By contextualizing single-cell data within multi-omic frameworks and clinical outcomes, this study advances our understanding of the GD2-CAR T cell therapy, the tumor microenvironment, and biomarkers of response and resistance. These findings provide critical insights for optimizing CAR T cell therapies in CNS malignancies and inform the development of next-generation immunotherapies.

#7124 Imaging mass cytometry (IMC) reveals distinct intratumoral B and T regulatory cell populations in responder versus non-responder soft tissue sarcoma (STS) patients before treatment with immune checkpoint blockade (ICB).

Laurence P. Diggs, Elise Nassif Haddad, Susmita Kumari, Varshini Arunkumar, Sammy Ferri-Borgogno, Samuel Mok, Duncan Mak, Angelique Lin, Madeline McAllister, Akshay Basi, **Manoj Chelvanambi**, Javier Gomez, Jared Burks, Davis Ingram, Khalida Wani, Natalie Duarte, Trevor McKee, Alexander Lazar, Jennifer Wargo, Christina Roland, Emily Keung

The University of Texas MD Anderson Cancer Center, Houston, TX

Background: STS are poorly understood cancers for which systemic therapy options are limited. Recent evidence suggests that some patients with two types of STS (undifferentiated pleomorphic sarcoma, UPS; dedifferentiated liposarcoma, DDLPS) benefit from ICB. Presence of tertiary lymphoid structures (TLS) and B cells within the sarcoma tumor immune microenvironment (TME) is associated with response to ICB and longer survival. It remains unclear how immune populations within this TME interact with each other and how they evolve upon ICB exposure. We characterized the TLS composition and spatial relationships between immune populations in untreated biopsies of UPS/DDLPS of responders (R) versus non-responders (NR).

Methods: We completed a phase 2 trial of patients with resectable primary UPS (n=10) and DDLPS (n=17) who received ICB prior to surgery. Pre-ICB treatment biopsies were collected, and IMC was performed using a 34-plex antibody panel to characterize the TME of R vs NR. TLS composition, densities of immune cell subsets, and their spatial relationships were assessed across baseline biopsy specimens within 3 pre-defined regions-of-interest (ROIs): TLS, non-TLS, and TLS-non-TLS interface regions.

Results: We identified distinct TME profiles in R compared to NR prior to ICB treatment. Intratumoral B cell density was higher in R compared to NR patients across pre-treatment biopsies (502 vs 277 cells/mm2, p=0.45) and within interface (1155 vs 710 cells/mm2, p=0.42) and non-TLS (458 vs 257 cells/mm2, p=0.17) regions but lower in TLS regions (506 vs 729 cells/mm2, p=0.52). NR patients tended to have higher densities of Tregs across pre-treatment samples (81 vs 37 cells/mm2, p=0.44) and in TLS (981 vs 824 cells/mm2, p=0.59), interface (119 vs 53 cells/mm2, p=0.14), and non-TLS (66 vs 15 cells/mm2, p<0.01) regions. When examining spatial distributions of TME immune populations, we noted a trend towards greater distances between B cells and T regs in the tumors of R compared to NR patients (78 vs 45 microns, p=0.13) which was also observed in TLS (116 vs 40 microns, p=0.05), non-TLS (76 vs 44 microns, p=0.14), and interface (179 vs 41 microns, p<0.01) regions.

Conclusion: Although presence of intratumoral TLS is associated with ICB response and longer survival among patients with UPS/DDLPS, not everyone benefits. Response to ICB appears to be associated with the immune profile both within and beyond TLS regions of pre-treatment tumors. Lower Treg, higher B cell populations, and greater distances between Tregs and B cells within pre-treatment biopsies may help predict response to ICB for UPS and DDLPS. Studies are ongoing to assess the evolution of these spatial relationships after ICB treatment.

#7125 Distinct CD8+T cell subsets in advanced melanoma patients with liver metastasis.

Shao Tao, Zang Han, Asha Bunyan, Adil Daud, Ajay Maker, James Lee

UCSF - University of California San Francisco, San Francisco, CA

Background: Liver metastases (LM) in melanoma are linked to poor prognosis, reduced immunotherapy efficacy, and survival, likely due to a suppressive liver immune microenvironment. We hypothesized that LM induces distinct, detectable immune changes in blood and tumor biopsies, supported by our prior preclinical findings. We set to immunophenotype tissues from stage IV melanoma patients to identify systemic and local immune changes, uncover immune tolerance mechanisms, and define biomarkers distinguishing LM-associated suppression. These insights aim to inform strategies to overcome liver-specific immune tolerance, enhance immunotherapy predictability and efficacy, and improve clinical outcomes for LM+ melanoma patients.
Methods: Peripheral blood and tumor samples were longitudinally collected from advanced melanoma patients pre, during, and post treatment clinic visits and/or surgeries. Clinical data, including imaging, treatment, and bloodwork, were obtained. Stage IV patients were stratified into two cohorts: LM+ and LM-. Fresh tumor and blood tissues were immunophenotyped by 12-24 color flow cytometry.
Results: In tumor specimens, ICOS expression on CD8+ subsets were significantly reduced in LM+ patients. Three distinct CD8+ T cell subsets were identified correlating with LM status. In peripheral blood, LM+ patients exhibited significantly lower frequencies of HLA-DR+CD8+ and HLA-DR+CD11c+CD11b-CD8+ subsets compared to LM- patients, while the CD11c+CD11b-CD8+ subset was significantly higher. Similarly, in tumor tissue, LM+ patients showed an increased frequency of CD11c+CD11b-CD8+ subset and a decreased frequency of HLA-DR+CD8+ subset. These observations underscore potential functional differences in immunity between LM+ and LM- patients. These subsets warrant further studies on the immune dynamics of melanoma LM.
Conclusions: Our parallel immunophenotyping of melanoma clinical samples is a foundational step towards developing predictive/prognostic biomarkers to distinguish between LM+ and LM- patients and their outcomes. We identified distinct T-cell subsets with differential representation between cohorts, with reduced expression of ICOS in CD8+ subsets and enrichment of CD11c+CD11b-CD8+ subset in LM+ patients. The functional roles of these subsets require further investigation. Given the immune-regulatory nature of the liver tumor microenvironment and clinical manifestations in LM+ patients, we hypothesize that the CD11c+CD11b-CD8+ subset exhibits a regulatory phenotype, contributing to immune suppression and tumor progression. We aim to characterize this subset at phenotypic, transcriptional, and functional levels, elucidating its role in LM-mediated immune modulation. These findings will inform the development of novel biomarkers and therapeutic strategies for melanoma patients with metastatic disease.

#7126 Divergent molecular features associated with immune checkpoint blockade response.

Travis D. Kerr[1], Patrick G. Pilie[2], Hui Dai[2], Amy B. Heimberger[3], Renata Ferrarotto[2], Jeffrey M. Rosen[4], Shiaw-Yih Lin[2], **Daniel J. McGrail**[1]

[1]Center for Immunotherapy & Precision Immuno-Oncology, Cleveland Clinic, Cleveland, OH,[2]MD Anderson Cancer Center, Houston, TX,[3]Northwestern University, Chicago, IL,[4]Baylor College of Medicine, Houston, TX

The advent of immune checkpoint blockade (ICB) has revolutionized cancer management. However, not all patients will benefit from this powerful therapy, necessitating clinical biomarkers and combination treatment strategies to enhance therapeutic efficacy. Recently, high tumor mutation burden (TMB-H) was FDA-approved as a pan-cancer biomarker for treatment with ICB based on a limited sample size and diversity of tumors. In systematic analysis across cancer types, we observed that immune cell infiltration was only associated with TMB in a subset of cancers. Based on this, we divided cancers into “Category I” cancers where TMB is associated with increased immune infiltration, and “Category II” cancers where TMB is not associated with immune infiltration. Using these classifiers, we analyzed the ability of TMB to predict ICB outcomes across over 2,000 patients. We found that TMB-H predicted ICB outcomes in Category I, but not Category II, cancers. As Category II cancers represent many of the most common cancers in the United States, including those of the breast, prostate, kidney, and brain, we next sought to identify biomarkers relevant to this subset of cancers. We identified that defects in DNA replication stress response predicts ICB response in 12 cohorts of Category II cancers, but not Category I cancers. Furthermore, induction of replication stress response defects sensitized otherwise resistant tumors to ICB. Based on these divergent molecular correlates of ICB response, we hypothesized that different mechanisms may underlie ICB activity in these tumors. Using single-cell RNA sequencing, we identified a non-canonical PD1+ dendritic cell population uniquely in mouse and human Category II cancers, and that this population undergoes extensive transcriptional rewiring following treatment with ICB. Together, this work illustrates the need for context-specific biomarkers for treatment with immune checkpoint blockade.

#7127 The correlation between immune status and the therapeutic efficacy of immune checkpoint inhibitors in patients with non-small cell lung cancer with sarcopenia.

Ryo Yamazaki, Satoshi Watanabe, Takaaki Masuda, Tomoya Wakabayashi, Yumi Ando, Kunihiro Shono, Naohiro Yanagimura, Masashi Arita, Miyuki Sato, Tomohiro Tanaka, Koichiro Nozaki, Yu Saida, Kenjiro Shima, Yosuke Kimura, Nobumasa Aoki, Yasuyoshi Ohshima, Toshiyuki Koya, Toshiaki Kikuchi

Department of Respiratory Medicine and Infectious Diseases, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

Background: Our previous prospective study demonstrated that non-small cell lung cancer (NSCLC) patients (pts) with pre-sarcopenia or sarcopenia exhibited poor responses to immune checkpoint inhibitors (ICIs). In this study, we evaluated the relationship between immune status and the efficacy of ICIs in NSCLC pts with sarcopenia.
Method: Pts with stage IV or recurrent NSCLC receiving ICI as first-line treatment at Niigata University Medical and Dental Hospital were eligible. Sarcopenia and pre-sarcopenia were diagnosed with physical performance and skeletal muscle mass. Before ICI treatments, peripheral mononuclear blood cells (PBMCs) were collected, and immune phenotypic data was obtained by flow cytometry.
Result: PBMCs were obtained from 34 pts from December 2021 through September 2024. Six patients and 7 patients were diagnosed with sarcopenia and pre-sarcopenia, respectively. Pts with a higher percentage of Lineage$^-$CD11b$^+$CD14$^+$CD33$^+$DR^{low} monocytic myeloid-derived suppressor cells (M-MDSCs) or Lineage$^-$CD11b$^+$CD15$^+$CD33$^+$DR^{low} polymorphonuclear (PMN)-MDSCs significantly shorter OS (median, 12 months (95% CI, 2.9-26.5) vs 33.1 months (95% CI, 11.4-NR); HR 2.7; P=0.035) after ICI treatments. Further, pts with PD-L1$^+$ cells in MDSCs also had poor OS (median, 12 months (95% CI, 2.9-26.5) vs 33.1 months (95% CI, 11.4-NR); HR 2.6; P=0.0387). The higher percentage of M-MDSC (P=0.0534) and the presence of PD-L1$^+$ cells in MDSCs (P=0.0473) tended to be associated with pts with sarcopenia or pre-sarcopenia.
Conclusion: MDSCs may play a critical role in immune suppression in NSCLC pts with sarcopenia and could be associated with poor OS following ICI treatment. To improve the survival outcome of pts with sarcopenia, it is essential to establish strategies to overcome MDSC-mediated immune suppression.

#7128 Neoantigen expression and expansion of cytotoxic T cell clonotypes in multiple myeloma patients receiving a personalized cancer vaccine post-transplant: Insights from the BM CTN 1401 multicenter trial.

Giulia Cheloni[1], Dimitra Karagkouni[1], Shivani Nanda[1], Lina Bisharat[1], Isabella Saldarriaga[1], Yuling MA[1], Shuoshuo Wang[1], Xanti-Lida Katopodi[1], Yered Pita-Juarez[1], Jessica Liegel[1], Hazal Toros[1], Prateek Pophali[1], David Chung[2], Nina Shah[3], Natalie Callander[4], Binod Dhakal[5], Thinle Chodon[6], Nikhil Munshi[7], Kenneth Anderson[7], Yvonne Efebera[8], Peiman Hematti[5], Hillard Lazarus[9], Ehsan Malek[9], Philip McCarthy[10], Ajay Nooka[11], Krina Patel[12], Aaron Rapoport[13], Robert Soiffer[7], Edmund Waller[11], Marcelo Pasquini[5], Jacalyn Rosenblatt[1], Ioannis Vlachos[1], David Avigan[1]

[1]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA,[2]Memorial Sloan Kettering Cancer Center, New York, NY,[3]AstraZeneca; University of California San Francisco, San Francisco, CA,[4]University of Wisconsin, Carbone Cancer Center, Madison, WI,[5]Medical College of Wisconsin, Milwaukee, WI,[6]Roswell Park Cancer Institute, Buffalo, NY,[7]Dana Farber Cancer Institute, Harvard Medical School, Boston, MA,[8]OhioHealth, Columbus, OH,[9]Case Western Reserve University, Cleveland, OH,[10]Roswell Park Comprehensive Cancer Center, Buffalo, NY,[11]Emory University, Winship Cancer Institute, Atlanta, GA,[12]MD Anderson Cancer Center, Houston, TX,[13]University of Maryland School of Medicine, Baltimore, MD

Background: BMT CTN 1401 is a phase II trial of 140 multiple myeloma (MM) patients undergoing autologous hematopoietic cell transplant (HCT) followed by lenalidomide maintenance with or without a personalized DC/MM vaccine. Vaccination was associated with increased MM-reactive T cells and clonotypic expansion of activated T cells 1-year post-HCT. Here, we identify private or shared antigenic targets in pre-HCT bone marrow (BM) samples, along with corresponding T cell clonotypic patterns. We also further analyzed the peripheral blood (PB) immune landscape of the study patients, exploring the correlation between disease response and the T cell repertoire, including dominant clonotypes.
Method: Pre-HCT BM samples from 16 patients underwent bulk RNAseq and whole exome sequencing. With an in silico-experimental framework developed by our team, we predicted tumor antigens by considering factors including translation potential, RNA expression, peptide-MHC binding affinity, immunogenicity, clonality, and physicochemical properties. 10 samples were further analyzed by single cell (sc) immunoprofiling to assess the antigens expression in MM cells. Additionally, PB samples from 40 study patients underwent sc immunoprofiling to investigate the T cell repertoire in stringent complete response/complete response (sCR/CR, N=26), very good partial response (VGPR, N=9), partial response (PR; N=4) or progressive disease (PD; N=1).
Results: We observed a high prevalence of tumor-specific (neo)antigens, with 50% shared among more than 2 patients. 19 neoantigens were prioritized based on high immunogenicity and expression in multiple patients, including four expressed in immune-privileged tissues (MAGEC1, TPTE, UCHL1). UCHL1 showed an 80-fold higher median expression in MM samples than in healthy controls. We observed a significant enrichment of shared tumor antigenic peptides (n=33) across up to 10 patients, originating from regions not typically expressed or translated, such as retroelements and downstream/upstream open reading frames. PB T cells immunoprofiling revealed higher T cell clonotypic expansion in sCR/CR and VPGR, with sCR/CR associated with higher proportion of cytotoxic CD8 T cells and fewer Tregs 1-year post-HCT. A high degree of TCR sharing between clonally expanded T cells in the PB and BM was observed.
Conclusions: To identify novel immunotherapy targets for MM, we characterized the MM antigenic landscape, identifying personal and shared tumor antigens. In addition, we demonstrated that sCR/CR is associated with clonal expansion of cytotoxic T cells with clonal dynamics in the PB reflecting those in the BM. We are correlating the dominant T cell clonotypes with the identified antigens and assessing the impact of DC/MM fusion vaccination in this setting.

#7129 PDL1 expression on immune cells from solid tumor patients: Implications for tumor immunity and immunotherapy.

Angelina Bisconte, Rachel Owen, Jackie Benko, Jorge Pardo, Samantha Splitt, Deb Phippard

Precision for Medicine, Frederick, MD

Programmed death-ligand 1 (PD-L1) is a key immune checkpoint molecule that regulates immune responses by binding to PD-1 on T cells, contributing to immune tolerance and suppression. PD-L1 expression in solid tumors has become an important biomarker for evaluating the efficacy of immune checkpoint inhibitors in cancer therapy. Characterization of PD-L1 expression on immune cells, particularly CD8+ T cells, in patients with various solid tumors, including melanoma, non-small cell lung cancer (NSCLC), and breast cancer was performed using cryopreserved peripheral blood mononuclear samples using flow cytometry. In addition to PD-L1, co-expression of markers of T cell activation (e.g., CD69, CD86, Ki67) and exhaustion (e.g., PD-1, LAG3) was analyzed to gain a comprehensive understanding of immune cell status. PD-L1 expression was highly variable across patients within solid tumor indications, with elevated expression found on CD8+ T cells, suggesting an upregulation in response to persistent tumor antigenic stimulation. Furthermore, PD-L1+ CD8+ T cells often co-expressed markers of exhaustion, such as PD-1 and LAG-3, indicating a potential role for PD-L1 in T cell dysfunction and immune evasion. These findings underscore the complexity of PD-L1 expression by immune cells and its role in shaping immune responses in solid tumor patients. Through integrating PD-L1 profiling with other markers of immune activation and exhaustion, this highlights the potential of PD-L1 as a predictive biomarker for immunotherapy response and provides insights into the mechanisms of immune evasion in solid tumor cancers.

#7130 Spatially resolved single-cell analysis reveals cGAS-STING-mediated tumor-immune interactions via IFNα as determinants of pembrolizumab sensitivity in triple-negative breast cancer.

Yi Liu[1], **Saranya Chumsri**[2], Yaohua Ma[1], Jodi M. Carter[3], Aziza Nassar[4], Edith Perez[2], Roberto A. Leon-Ferre[5], David Zahrieh[6], David W. Hillman[6], Judy C. Boughey[5], James Ingle[5], Krishna R. Kalari[7], Fergus J. Couch[7], Matthew P. Goetz[5], Keith L. Knutson[1], E. Aubrey Thompson[1]

[1]Mayo Clinic Florida, Jacksonville, FL,[2]Hematology/Oncology, Mayo Clinic Florida, Jacksonville, FL,[3]Laboratory Medicine & Pathology, University of Alberta, Edmonton, AB, Canada,[4]Pathology and Laboratory Medicine, Mayo Clinic Florida, Jacksonville, FL,[5]Hematology/Oncology, Mayo Clinic, Rochester, MN,[6]Quantitative Health Sciences, Mayo Clinic, Rochester, MN,[7]Mayo Clinic, Rochester, MN

Purpose: While an addition of immune checkpoint inhibitor (ICI), pembrolizumab, has improved outcomes of patients with TNBC, early-stage TNBC patients with residual cancer burden (RCB) II/III after neoadjuvant chemotherapy in combination with pembrolizumab (NAP) still have poor outcomes. Previously, we demonstrated that intratumoral HLA-DRA expression and interferon-alpha (IFNα) responses were associated with immune-inflamed phenotype and better outcomes in TNBC patients receiving chemotherapy. This study utilized the CosMx™ SMI to investigate in early-stage TNBC treated with NAP.

Methods: Of 151 samples from TNBC patients treated with NAP at Mayo Clinic, 8 pre-treatment biopsy samples (5 pCR and 3 RCB II/III were analyzed using the CosMx™ SMI platform. Linear mixed models to assess differential expression (DE), adjusting for hierarchical data structures. DE analyses were expressed as log2-fold changes (logFC), with the Benjamini-Yekutieli method. GSEA and IPA for signaling pathway exploration.

Results: 1.7 million cells were identified across 8 samples after stringent quality control. Immune cell profiling revealed higher B cells, plasma cells, plasmablasts, and T cells (CD4 and CD8) in responders, whereas fibroblasts and myeloid cells were enriched in non-responders. DE analysis showed significantly elevated expression of HLA-DRA (logFC 1.955, $p<0.001$), other MHC class I and II components, and IFNα in responders. Tumor cells in responders exhibited the highest IFNα expression across cell types. GSEA and IPA analysis revealed enrichment in interferon signaling, antigen presentation, and the cGAS-STING pathway, which drives IFNα production. Spatial analysis categorized cells by their proximity to tumor cells: within 50 µm, 50-100 µm, and over 100 µm. T cells, particularly CD8 T cells, showed a distance-dependent distribution, with a higher abundance near tumor cells in responders. CD8 T cell activation scores declined with increasing distance from IFNα-positive tumor cells but remained consistently low near IFNα-negative tumor cells. IFNγ-positive CD8 T cells exhibited higher activation compared to IFNγ-negative cells, with activation levels influenced by proximity to tumor cells. Lastly, MHC expression in tumor cells was examined relative to CD8 T cell proximity. HLA-DRA and CIITA expression decreased as tumor cells were farther from CD8 T cells.

Conclusions: This spatially resolved single-cell analysis highlights the pivotal role of tumor-immune interactions in response to pembrolizumab in TNBC. Our data suggest that tumor cells secrete IFNα through the activation of the cGAS-STING pathway, which activates T cells within close proximity, highlighting the potential role of cGAS-STING agonist as well as IFNα signaling and antigen presentation in augmenting TNBC responses to ICIs.

#7131 Integrative single-cell transcriptomic and epigenomic immune profiling reveals parallel insights into cancer research.

Errile Pusod[1], Patricia Schnepp[1], Srikanth Perike[2], Angelica Olcott[2], Elizabeth Dreskin[2]

[1]Bio-Rad Laboratories, Ann Arbor, MI,[2]Bio-Rad Laboratories, Hercules, CA

Introduction: Single-cell technologies have revolutionized immune profiling by enabling the detailed analysis of individual cells, uncovering cellular heterogeneity and complex regulatory mechanisms. Two common single-cell omics include single-cell RNA-sequencing (scRNA-seq) which profiles gene expression by capturing mRNA transcripts, and single-cell ATAC sequencing (scATAC-seq) which profiles gene regulation using hyperactive Tn5 transposase to assess chromatin accessibility. In this workflow, we demonstrate integrative analysis of both technologies that provides comprehensive view of a cell's transcriptomic and epigenomic landscapes.
Methods: Two independent scRNA-seq and scATAC-seq workflows were performed on peripheral blood mononuclear cells (PBMCs) and various cancer cell lines to provide accessible and convenient model system for various oncology applications. Libraries were prepared using Bio-Rad ddSEQ 3' scRNA-seq and scATAC-seq Kits, which uses droplet-based ddSEQ technology for capturing cells with high sensitivity and recovery. Libraries were sequenced using Illumina NextSeq 2000. Data quality was assessed using the Omnition Analysis Software and Seurat was used for downstream tertiary analyses.
Results: Here, we present an integrated analysis of scRNA-seq and scATAC-seq for comprehensive immune profiling of peripheral blood mononuclear cells (PBMCs) and various cancer cell lines. Our findings show consistent correlation of cell types and subtypes identified from each pair of samples. Furthermore, specific differentially expressed genes (DEGs) and differentially accessible regions (DARs) were found to be shared in corresponding cell types such as chromatin peak and increased gene expression of Epiregulin (EREG) in CD14+ monocytes.
Conclusion: Bio-Rad ddSEQ 3' scRNA-seq and scATAC-seq kits provide affordable and scalable method for profiling immune response from thousands of individual cells with increased sensitivity and accuracy. Combined analyses from these two approaches show strong potential for oncology applications. For example, the ability to identify novel immune cell subsets, discover tumor biomarkers, characterize tumor-infiltrating lymphocytes, and reveal regulatory elements driving immune responses in the tumor microenvironment can all be achieved.

#7132 Estimated post-test probabilities of cancers for individuals receiving multi-cancer early detection (MCED) tests.

Candace Westgate[1], Christina Clarke Dur[2], Eric Klein[2], Alpa V. Patel[3], Earl Hubbell[2]

[1]Adventist Health, Saint Helena, CA,[2]GRAIL, Inc., Menlo Park, CA,[3]American Cancer Society, Atlanta, GA

Background: Individuals and their physicians receiving MCED tests may need to interpret the results they receive in the context of their particular cancer risks, both for positive ("cancer signal detected") and for negative ("no cancer signal detected") test results. Using estimated performance characteristics of an on-market MCED test we modeled post-test probabilities after receiving an MCED test result for a variety of typical populations stratified by five year age group and sex, and adjusted for smoking status (unadjusted, ever, former, never), as well as the duration of reduced cancer risk in those who tested negative.

Methods: Using (1) 2006-2015 data (cancer incidence by age, sex) from the Surveillance, Epidemiology and End Reports (SEER) 17, (2) adjustments to incidence by smoking risk factors (3) test sensitivity from the third sub-study of the Circulating Cancer Genome Atlas, and (4) duration of detectable window from the American Cancer Society Cancer Prevention Study III and an exponential distribution for the detection window, we computed post-test probabilities of detection (for positive tests), and depletion of future cancers over various time intervals (for negative tests).

Results: Individuals receiving a negative MCED test result had 42-56% (min-max) the level of late stage (III+IV) cancer diagnosis in the year following blood draw, rising to 72-82% the second year if they did not return for an annual test. Individuals receiving a positive MCED test result with a cancer-signal origin (CSO) returned, had a cancer at that signal location if cancer is confirmed (97% of individual-CSO combinations), with sufficient positive predictive value at each CSO justifying workup (97% of individual-CSO combinations) with few exceptions, such as a breast CSO in males. An important complicating factor is the utilization of conventional screening or imaging tests which can significantly alter the pre-test probability of having certain cancers, for which we provide the example of colonoscopy and its effects over the ten-years post-negative screen.

Conclusions: For common clinical covariates of age, sex, and smoking status this model provides important clinical context for interpreting test reports, including the potential for a significant increase in late stage, deadly interval cancers when testing at longer intervals than one year. Following the CSO estimate in the majority of individuals with positive test results is justified by comparison to usual clinical decision thresholds.

CLINICAL RESEARCH: Liquid Biopsy: Circulating Nucleic Acids 4 / Predictive Biomarkers 1
Poster Session

#7133 Detection of early stage colorectal cancer using cell-free oncRNA biomarkers and AI.

Amir Momen-Roknabadi[1], Mehran Karimzadeh[1], Nae-Chyun Chen[1], Taylor B. Cavazos[1], Jieyang Wang[1], Jeremy Ku[1], Alex Degtiar[1], Akshaya Krishnan[1], Martha Hernandez[1], Alice Huang[1], Selina Chen[1], Dang Nguyen[1], Ti Lam[1], Rose Hanna[1], Lisa Fish[1], Magdalena Gebala[1], Alexx J. Smith[1], Sukh Sekhon[1], Jennifer Yen[1], Jeff Gregg[2], Hani Goodarzi[3], Helen Li[1], Fereydoun Hormozdiari[1], Babak Behsaz[1], Anna Hartwig[1], Lee Schwartzberg[1], Babak Alipanahi[1]

[1]Exai Bio Inc., Palo Alto, CA,[2]UNR School of Medicine, Reno, NV,[3]UCSF, San Francisco, CA

Background
Colorectal cancer (CRC) is the second leading cause of cancer-related mortality worldwide. Early detection offers the best opportunity for effective treatment. Although CRC screening methods exist, there remains a need for a high performing blood test to both improve adherence and detect early stage disease. We have previously identified and developed a novel category of cancer-associated, small orphan non-coding RNAs (oncRNAs), and combined them with generative AI modeling to develop a liquid biopsy platform. This platform has demonstrated high sensitivity and specificity for detection of early stage disease across several types of cancer. In this study, we developed an oncRNA- and AI-based assay and analyzed it in a separate, independent test set to evaluate its generalizability and its ability to detect CRC across different cancer stages.
Methods
We utilized The Cancer Genome Atlas (TCGA) small RNA profiles to discover a library of pan-cancer oncRNAs that were significantly enriched among tumors compared to adjacent normal tissues spanning multiple tissue sites. The diagnostic performance of these oncRNAs was evaluated in plasma using a training and independent test cohort. Our training cohort included 613 samples (388 CRC and 225 asymptomatic controls) from six different sources (mean age: 63.7 years, 35.9% female). 37.6% of the cases were classified as stage I/II and 60.8% as stage III/IV. Further, we acquired 192 samples (113 CRC and 79 asymptomatic controls) from a separate cohort for testing (average age: 62.6 years ; 51.6% female). In the test set, 52.2% of cases were classified as stage I/II and 47.8% as stage III/IV. We processed 1 ml of plasma for each sample through our universal, automated cell-free RNA workflow and sequenced at an average depth of 58 million 100-bp single-end reads. A generative AI model was trained using 5-fold cross-validation (CV) to predict CRC, and then applied on the separate, independent test set.
Results
Our oncRNA-based generative AI model achieved an overall AUC of 0.93 (95% CI: 0.91-0.95) for prediction of CRC versus cancer-free controls in CV, and an overall AUC of 0.95 (0.93-0.98) in the independent test set. At 90% specificity, overall model sensitivity was 81.7% (77.5%-85.4%) by CV in the training data, and 88.5% (81.1%-93.7%) in the test set. For stage I CRC, the model has a sensitivity of 72.1% (59.9%-82.3%, N=68) by CV in the training data, and 80.0% (51.9%-95.7%, N=15) in the test set, both at 90% specificity.
Conclusions
We demonstrate that blood-based cell- free RNA can be used for accurate and generalizable early stage detection of CRC. This approach, leveraging a distinct RNA biomarker, has the potential to overcome performance plateaus seen with conventional DNA-based methods in early-stage CRC detection. It offers a promising alternative for blood-based CRC screening in patients and could complement existing DNA- and methylation-based platforms.

#7138 Whole genome sequencing-based detection of minimal residual disease using individual-specific features.

Jun Hyeok Lim[1], Jeong-Seon Ryu[1], Ji Won Byun[1], Jongsuk Chung[2], **Hyun-Tae Shin**[1]

[1]Inha University College of Medicine, Incheon, Korea, Republic of,[2]Gencurix Inc, Seoul, Korea, Republic of

The detection of minimal residual disease (MRD) following cancer treatment is critical for preventing recurrence and improving patient prognosis. However, existing MRD detection methods often lack the sensitivity required for precise monitoring. We have developed a highly sensitive method for MRD detection using whole-genome sequencing (WGS) analysis of cell-free DNA (cfDNA) with individual-specific features. The approach begins by identifying consensus single nucleotide variants (SNVs) and fusions that are consistently detected by multiple variant callers in the patient's tumor WGS. Using a novel algorithm, we quantify supporting reads at the consensus SNV positions and assess their statistical significance based on the error distribution in the patient's plasma WGS. Additionally, unique sequences corresponding to the consensus fusion positions in the plasma WGS are detected to diagnose MRD. Validation through cancer cell-line mix experiments demonstrated a sensitivity of less than 1:12,800. Furthermore, the method effectively detected MRD in clinical samples from various cancer types, including lung, melanoma, breast, and liver. The limit of detection for the SNV quantification method was determined by the error rate in the individual BAM file, while the fusion sequence detection method exhibited no detection limit due to a limit of blank of zero. This algorithm has been implemented on a cloud platform, allowing individual users to analyze their data by uploading it. This highly sensitive approach for detecting MRD offers the potential to improve cancer patient prognosis significantly. Moreover, this technology holds great promise for broad applicability across various cancer types where cfDNA-based monitoring can provide clinical benefits.

#7139 An optimized extraction and library preparation workflow for sensitive mutation detection in clinical cfDNA samples.

Lauren P. Saunders[1], James Erickson[1], Margaret R. Heider[2], Jian Sun[2], Adrian Reich[2], Ben Lepene[1]

[1]Ceres Nanosciences, Manassas, VA,[2]New England Biolabs, Ipswich, MA

Monitoring cell-free DNA (cfDNA) - small DNA fragments circulating in the bloodstream - provides valuable insights into various tissues throughout the body and diverse health conditions. cfDNA has been particularly useful in tracking cancer progression, although its low abundance can lead to low mutation copy frequencies, especially in early-stage cancers where limited tumor presence yields minimal cfDNA in circulation. Optimizing molecular biology techniques for maximal cfDNA recovery and purity as well as high NGS library conversion efficiency is essential to improve detection of low-frequency cancer mutations.

In this study, several cfDNA next generation sequencing (NGS) library preparation workflows were evaluated for sensitivity and workflow efficiency. The NEBNext UltraExpress DNA library prep kit is a new, fast library prep that has not yet been optimized for cfDNA analysis. We compared it with the NEBNext Ultra II DNA Library Prep Kit, which performs well with cfDNA. Both kits were evaluated downstream of the NEAT Liquid Biopsy kit, which is optimized for gDNA removal using Nanotrap Particle technology and results in a highly concentrated DNA elution. With the ability to concentrate down to 20 µL, this extraction kit pairs well with the NEBNext UltraExpress DNA kit which has a 15 µL input. This allows more of the sample to be analyzed, leading to higher detection of low frequency alleles. Initial workflow assessments were analyzed with mutation spike-ins of 0-5% allele frequency in healthy plasma, then confirmed with clinical samples containing known mutations. All workflows produced high complexity libraries with high quality data and successfully detected spike-in mutations, with expected variant allele frequencies. Mutations were also successfully detected in clinical plasma samples demonstrating the essential need for efficient extraction and library prep methods.

#7140 Non-synonymous mutations in the IFN-γ pathway are predictive of response to immune checkpoint inhibition in NSCLC.

Alexander Azizi[1], Arvind Ravi[1], Marzia Capelletti[2], Nicholas Haradhvala[1], Natalie Vokes[3], Stephen-John Sammut[4], Steven Ressler[2], Milan Radovich[2], Justin Gainor[5], Gad Getz[1]

[1]The Broad Institute, Cambridge, MA,[2]Caris Life Sciences, Dallas, TX,[3]Department of Thoracic/ Head and Neck Medical Oncology, MD Anderson Cancer Centre, Houston, TX,[4]Breast Cancer Now Toby Robins Research Centre, The Institute of Cancer Research, London, United Kingdom,[5]Center for Thoracic Cancers, Massachusetts General Hospital, Boston, MA

Introduction: Baseline disruptions in interferon gamma (IFN-γ) signalling enhance tumor immune elimination post-checkpoint blockade in murine models. In the Stand Up to Cancer-Mark Foundation (SU2C-MF) NSCLC clinical cohort, non-synonymous mutations in IFN-γ pathway genes (IFNGR1, IFNGR2, JAK1, JAK2, STAT1, IRF1 & IRF9; n=26, 8%) associated with better outcomes following PD-(L)1 inhibitors (ICI), including improved radiological responses, progression-free survival, and overall survival (OS) (AACR 2024). Here, we aimed to validate these results in an independent dataset and examine predictive vs. prognostic significance.

Methods: The Caris Life Sciences (CARIS) dataset includes NSCLC patients with standard-of-care biopsies and multi-omic testing. Outcomes were tracked using claims. IFN-γ pathway disruption was defined as non-synonymous mutations in the genes available from CARIS's curated whole exome sequencing data (WES) and deep panel of 592 cancer-associated whole-genes: IFNGR1, JAK1, JAK2 & IRF1. Cox proportional-hazards models (CoxPH) assessed OS from ICI initiation. Co-variables had to be dichotomized: age (<65/≥65), PDL1 TPS (0%/>0%), smoking history (non-smoker/ever smoker), and continuous, including TMB, by median.

Results: Among 1070 patients with pretreatment WES, ICI treatment, and OS data, 88 (8.2%) had IFN-γ pathway mutations. Disruption was associated with significantly improved OS (HR 0.63, p=0.028*). Median OS for patients with disrupted vs. intact pathways was 60.0 months (95% CI: 27.6-NA) vs. 26.2 months (95% CI: 20.5-29.7).

Clinical multivariable CoxPH models incorporating TMB and PDL1 TPS confirmed pathway disruption as independently significant (HR 0.27, p=0.030*); this persisted when including sex, age and smoking (OS: HR 0.66, p=0.05). Biological models, including TMB, PDL1 TPS, and immune biomarkers (CXCL9, CD8A, GZMA, PRF1, IFNG), supported survival benefits (HR 0.65, p=0.046*). Correlation coefficients between pathway disruption and covariates were low (e.g., IFNG, 0.005; PDL1 TPS, -0.04; TMB, 0.14).

To assess prognostic effects, in the CARIS dataset, we evaluated analogous patients with NSCLC who had pretreatment WES, no prior or concurrent immunotherapy, but were treated with chemotherapy (disrupted: n=15; intact: n=143). Pathway disruption did not associate with OS [univariable: HR 1.64, p=0.42; multivariable with TMB and PDL1: HR 1.86, p=0.22]. TCGA lung data also failed to suggest a prognostic impact on OS (disrupted: n=40; intact: n=902) [univariable: HR 0.96, p=0.88; multivariable - continuous TMB, histology, stage, purity, age and sex: HR 1.86, p=0.70].

Conclusion: In the SU2C-MF and CARIS NSCLC cohorts, non-synonymous IFN-γ pathway mutations predict improved ICI outcomes, independently of TMB and other variables, but are not prognostic. IFN-γ pathway disruption could guide immunotherapy stratification in NSCLC.

#7141 Final analysis of the randomized Phase 2 cohort of CM24 with nivolumab and chemotherapy in pancreatic cancer & potential serum biomarkers.

Teresa Macarulla[1], Michael Cecchini[2], Rocio Garcia-Carbonero[3], Talia Golan[4], Ruth Perets[5], Erkut Borazanci[6], Manuel Pedegral[7], Mariano Ponz-Sarvise[8], Mohammed Najeeb Al Hallak[9], Shubham Pant[10], Valentina Boni[11], Omar Saavedra[12], Maria Jose de Miguel-Luken[13], Alexis D Leal[14], Andres J. Munoz[15], Tamara Sauri-Nadal[16], Hava Ben David[17], Tomer Meirson[17], Micheal Schickler[17], Hadas Reuveni[17]

[1]Hospital Universitario Vall d'Hebron, Barcelona, Spain,[2]Yale University School of Medicine - Yale Cancer Center, New Haven, CT,[3]University Hospital 12 De Octubre, Imas12, UCM, Madrid, Spain,[4]Sheba Tel Hashomer Medical Center, Ramat Gan, Israel,[5]Rambam Medical Center, Haifa, Israel,[6]HonorHealth Research Institute, Scottsdale, AZ,[7]START Madrid-FJD Hospital Universitario Fundacion Jimenez Diaz, Madrid, Spain,[8]Clinica Universidad de Navarra, Pamplona, Spain,[9]Karmanos Cancer Institute, Detroit, MI,[10]MD Anderson Cancer Center, Houston, TX,[11]NEXT Oncology, Universitary Hospital Quiron Salud, Madrid, Spain,[12]Next Oncology, Barcelona, Spain,[13]13START Madrid-Centro Integral Oncologico Clara Campal, Sanchinarro University Hospital, Madrid, Spain,[14]University of Colorado, School of Medicine, Aurora, CO,[15]Hospital General Universitario Gregorio Maranon, Madrid, Spain,[16]Hospital Clinic de Barcelona, Barcelona, Spain,[17]Purple Biotech Ltd., Rehovot, Israel

Background: CM24 (mAb) blocks CEACAM1 interactions between cancer cells, immune cells and neutrophil extracellular traps (NET), involved in tumor immune evasion, metastasis and cancer-associated thrombosis, affecting patient (pts) survival. We present the final data for the randomized Phase 2 study (NCT04731467) and significant efficacy in a biomarker-enriched subgroup.

Methods: Advanced/metastatic PDAC pts (n=31) progressing after first line treatment, were randomized to receive CM24 (20mg/kg q2wk), nivolumab (240mg/kg q2wk) and chemotherapy (liposomal irinotecan/5FU/LV) (EXP) vs chemotherapy only (CON; q2wk). Pre-dose serum samples (n=30) were collected. Serum CEACAM1 and myeloperoxidase (MPO; a NET marker) levels were evaluated by ELISA. Biomarkers were analyzed post-hoc and HRs for various thresholds were correlated with survival outcomes to select the greatest range providing a statistical benefit. The selected ranges exclude pts with extremely low or high levels of CEACAM1 and NETs.

Results: Final analysis of EXP vs CON shows OS 7.9m vs 5.6m (Δ=2.3m) HR 0.81 (P 0.575, CI 0.38-1.71), PFS 3.9m vs 2.0m (Δ=1.9m) HR 0.75 (*P* 0.46, CI 0.35-1.61), ORR 25% vs 6.7%, DCR 62.5% vs 46.7%. Most frequent Grade ≥3 adverse events were diarrhea; 4 vs 1 pts, fatigue; 2 vs 0 pts and neutropenia; 2 vs 0 pts. Subgroup analysis of pts with serum CEACAM1 level of 6K-15K pg/mL (n=11/30) demonstrates statistically significant benefits in terms of OS: HR 0.21 (*P* 0.04, CI 0.04-1.06), ΔOS=5.1m, PFS: HR<0.1 (*P* 0.003, CI 0-inf), ΔPFS=2.9m, and ORR 50% vs 0%. Statistically significant results were also seen for 80% of the pts (24/30) with serum CEACAM1 (5-16K pg/mL) or serum NET marker MPO (200-600 ng/mL) demonstrating OS HR 0.39 (*P* 0.039, CI 0.16-0.98) and PFS HR 0.28 (*P* 0.006, CI 0.11-0.73).

Conclusions: Final data show that adding CM24 and nivolumab to chemotherapy improved all efficacy parameters and had a manageable safety profile. Subgroup analyses demonstrate statistically significant reduction in the risk of OS and PFS, and suggest serum CEACAM1 & NET marker as predictive biomarkers for CM24-based therapy, corresponding with CM24 mechanism of action

#7142 Mutations in PBAF, especially in PBRM1, enhance immune checkpoint inhibitor response in lung adenocarcinoma.

Sung Feel Kim[1], Hong Sook Kim[2], Hongui Cha[3], Hyemin Kim[1], Yeong Hak Bang[1], Cheolyong Joe[1], Jun-Gi Jeong[1], Eunjoo Oh[1], Na-Eun Lee[1], Hyun Ae Jung[1], Jong-Mu Sun[1], Jin Seok Ahn[1], Myung-Ju Ahn[1], Sehhoon Park[1], Se-hoon Lee[1]

[1]Division of Hematology-Oncology, Departments of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of,[2]Department of Biological Sciences, Sungkyunkwan University, Suwon, Korea, Republic of,[3]University College London Cancer Institute, Cancer Research UK Lung Cancer Centre of Excellence, London, United Kingdom

Introduction: The SWI/SNF chromatin remodeling complex plays a pivotal role in regulating gene expression and maintaining genomic integrity, with three distinct complexes identified: BAF, PBAF, and ncBAF (also known as GBAF). Each complex contributes uniquely to cellular processes and cancer development. Within the PBAF complex, PBRM1, a key component, has garnered significant interest for its potential to influence the efficacy of immune checkpoint inhibitors (ICIs) in Non-Small Cell Lung Cancer (NSCLC). Despite the critical implications, there remains considerable controversy regarding whether mutations in PBRM1 and other components of the SWI/SNF complexes enhance or impair responses to ICIs. This study seeks to resolve these conflicting perspectives by elucidating the impact of mutations in these key components on the outcomes of ICI therapy in patients with NSCLC, especially lung adenocarcinoma.
Methods: We conducted a retrospective analysis of 4,166 NSCLC patients treated with ICIs at Samsung Medical Center. Among these, 745 met strict inclusion criteria including pre-treatment biopsy and complete RECIST and clinical data. Whole-exome sequencing was performed on 514 patients, focusing on mutations in all the SWI/SNF complex subunits such as ARID1A, ARID1B, ARID2, BRD7, SMARCA4, and PBRM1. Survival analysis techniques were employed to evaluate the association between these mutations and ICI therapy outcomes, calculating median progression-free survival (mPFS) and overall survival (mOS) using hazard ratios (HRs) and log-rank tests.
Results: In the lung adenocarcinoma subset, PBAF complex mutations were significantly associated with improved ICI outcomes, with an mPFS of 3.8 months (HR: 0.71, 95% CI: 0.52-0.98, p = 0.034) and an mOS of 19.4 months (HR: 0.69, 95% CI: 0.50-0.95, p = 0.022). PBRM1 mutations, in particular, resulted in favorable outcomes, with an mPFS of 4.2 months (HR: 0.58, 95% CI: 0.35-0.94, p = 0.018) and an mOS of 24.7 months (HR: 0.54, 95% CI: 0.33-0.91, p = 0.026). These associations were not significant in lung squamous cell carcinoma and other NSCLC subsets.
Conclusion: This study provides evidence that PBAF complex mutations, especially in PBRM1, are associated with improved ICI outcomes in lung adenocarcinoma patients. These findings suggest the potential utility of these mutations as predictive biomarkers for personalizing immunotherapy in this subset of NSCLC patients. Prospective studies are needed to validate these observations and explore the underlying biological mechanisms.

#7143 Development of a peripheral dazostinag (TAK-676) activation RNASeq signature to evaluate STING pharmacodynamic modulation in human solid tumor clinical trials.

Kai Ding, Xin Tong, Radha Ramesh, Samanthi Perera, Yu-Chung Huang, Alex Parent, Jeffrey Raizer, John Gibbs, Neil Lineberry, Vicky Appleman, Somnath Bandyopadhyay, Richard C. Gregory

Takeda Development Center Americas, Inc. (TDCA), Lexington, MA

Dazostinag, a novel STimulator of INterferon Genes (STING) agonist, is currently being evaluated with pembrolizumab for colorectal and head & neck cancers (iintune-1, NCT04420884). RNA sequencing (RNASeq) is a valuable tool for pharmacodynamic (PD) biomarker development, offering comprehensive transcriptome profiling and a wide dynamic detection range. For the iintune-1 Phase 1/2 study, we developed an RNASeq peripheral PD signature for STING activation and demonstrated its utility as a secondary endpoint to measure biological responses to dazostinag.

To develop the PD signature, peripheral blood mononuclear cells (PBMCs) from 7 healthy donors were treated *ex vivo* with vehicle or dazostinag (0.33-10 μM, 6 hours, 3 replicates) and samples were processed for RNASeq. Protein-coding genes with a maximum FPKM ≥ 1 and low variance between replicates were filtered and analyzed utilizing dose-response mixed model for repeated measures. Genes with significant dose responses (FDR ≤ 0.05 and fold change ≥ 1.5 at 0.33 μM) underwent network analysis to identify biologically connected genes.

A 19-gene network with significant response to dazostinag was identified and termed STING_19. Among these genes, 17 are in the interferon (IFN) pathway, consistent with the known IFN activation effect of STING agonists. An independent RNASeq dataset from human PBMCs treated *ex vivo* with dazostinag (0.12-10 μM) was used to evaluate STING_19 against hallmark IFN-α and IFN-γ signatures. STING_19 demonstrated a robust dose-dependent induction by dazostinag, with a median 70-fold increase at 10 μM, greater than the 4 to 6-fold induction of IFN-α and IFN-γ signatures. Additionally, STING_19 induction was confirmed in a syngeneic mouse model, showing a 55-fold increase at 6 hours post-treatment with 1 mg/kg dazostinag.

In the iintune-1 study, for dazostinag single agent arm, STING_19 exhibited a dose-dependent induction in blood, starting from 0.8 mg/kg with a median 18-fold increase at the highest dose of 14mg/kg, indicating a robust PD response. Pembrolizumab combination arm showed a similar induction of STING_19 in response to dazostinag, with pembrolizumab alone at low dazostinag dose (as a surrogate for monotherapy) showing minimum STING_19 induction. Within 0 to 24-hours post-treatment, maximum induction of STING_19 was typically at 24 hours for doses up to 5 mg, and at 10 hours for doses between 7-14 mg, suggesting dose-dependent STING activation dynamics by dazostinag. This induction returned to baseline prior to subsequent weekly doses.

In summary, we developed a robust RNASeq-based peripheral STING activation PD biomarker using *ex vivo* treated PBMCs to evaluate the biological response to STING agonist treatment. This framework could be applied for the development of RNASeq based peripheral PD biomarkers for other investigational assets.

#7144 Integrative analysis of immunogenetics of the HLA-KIR-MIC system to predict clinical outcomes for checkpoint immunotherapy.

Hyun-Sung Lee[1], Priyanka Ranchod[1], Sonali Mitra[1], Hee-Jin Jang[1], Claire Lee[1], Sung Wook Kang[1], Ji Seon Shim[1], Ito Atsushi[1], Gena Lee[1], Tannaz Armaghany[2], Michael Byrd[2], Aihua Yen[2], Quillan Huang[2], Rafee Talukder[2], Shalini Makawita[2], Benjamin Musher[2], Peter Jindra[1], Jinyoung Byun[3], Younghun Han[3], Daniel Wang[4], Bryan Burt[5], Meera Patel[2]

[1]Surgery, Baylor College of Medicine, Houston, TX,[2]Medicine, Baylor College of Medicine, Houston, TX,[3]University of New Mexico, Albuquerque, NM,[4]Medicine, UT South Western Medical Center, Dallas, TX,[5]Surgery, University of California Los Angeles, Los Angeles, CA

Background: Immune-related adverse events (irAEs) have been associated with improved survival during checkpoint immunotherapy in thoracic cancers. However, the relationship between irAEs and survival across various solid tumors remains unclear. This study aims to validate the association between irAEs and prognosis in patients with solid tumors treated with immune checkpoint inhibitors (ICIs) and to identify predictive determinants of irAEs.

Methods: This prospective observational cohort study included 138 patients with solid tumors receiving ICIs or chemo-ICIs, with a median follow-up of 27 months. The primary outcome was the association between irAEs and overall survival (OS). Clinically meaningful irAEs were defined as those requiring treatment delay or discontinuation of ICIs and/or systemic corticosteroids for management of toxic effects. High-resolution HLA/MIC/KIR genotyping and high-throughput plasma proteomics were performed from pre-ICI blood samples to identify determinants to predict irAEs.

Results: Of the 138 patients, 43 (31%) developed irAEs. irAEs were more frequent among responders (12/20 CR (60%) and 4/7 PR (57%)) compared to those with SD (10/31 (32%)) and PD (17/80 (21%)). irAEs were associated with improved OS (median OS with irAEs: 25.8 mo.; without irAEs: 18.5 mo.; HR = 0.56 [95% CI, 0.33-0.95]) and disease-free survival (DFS) (median DFS with irAEs: 17.3 mo.; without irAEs: 4.8 mo.; HR = 0.49 [95% CI, 0.31-0.77]). Furthermore, patients with both durable clinical benefit (DCB) and irAEs demonstrated superior OS (median: 58 mo.) and DFS (median: 34.6 mo.) compared to DCB without irAEs (median OS: 33.4 mo.) and PD (median OS: 13.5 mo.; $P = 1.5 \times 10^{-6}$). Integrated immunogenetic analyses of HLA-A, -B, -C, -E, -F, -G, DRB1, DP, and DQ alleles, as well as MHC-I/II heterozygosity, Bw4/Bw6 (HLA-B), C1/C2 (HLA-C), and centromeric/telomeric KIR groups (AA vs. Bx), revealed distinct clusters for patient stratification. Patients with DCB and irAEs were enriched in more activating immunogenetic genotypes including Bw4 homozygosity, C2 homozygosity, and telomeric Bx KIR genotypes. Furthermore, proteomic analysis of pre-treatment plasma samples showed significant decreases in immunoregulatory proteins and cytokines in patients with DCB and irAEs. Multivariable regression analysis adjusted for cancer type and treatment modality demonstrated that integrative immunogenetics profiles (P=0.004) and circulating proteomic signatures ($P=3.2x10^{-4}$) independently predicted benefit from ICIs while identifying patients at higher risk for irAE development.

Conclusion: This study highlights the utility of integrating immunogenetics and systemic immunity profiling to stratify patients receiving ICIs. Blood-based biomarkers may predict clinical benefit and irAE development, warranting further prospective validation.

#7145 The pan-cancer landscape of *RIT1* genetic alterations.

Matteo Repetto[1], Pier Selenica[2], Soo-Ryum Yang[1], Britta Weigelt[2], Alexander Drilon[1]

[1]Early Drug Development, Memorial Sloan Kettering Cancer Center, New York, NY,[2]Pathology, Memorial Sloan Kettering Cancer Center, New York, NY

Background: Advancements in drug design have resulted in the development of approved and investigational agents targeting KRAS-driven tumors. RIT1 is a GTPase protein that shares homology and oncogenic activation mechanisms with KRAS and plays a role in cell division and resistance to apoptosis. In this study, we aimed to characterize the landscape of *RIT1* alterations across a pan-cancer cohort to assess its frequency and potential as an oncogenic driver.

Methods: Tumor content, ploidy and allelic specific copy number of 86,039 tumor-normal matched samples sequenced clinically with a targeted sequencing panel (MSK-IMPACT) were analyzed with FACETS. Low purity (<20%) and indetermined copy number samples were excluded. Presence of *RIT1* amplifications (Amp), structural variants, oncogenic mutations (Mut), and concurrent OncoKb oncogenic/likely-oncogenic alterations in other genes were evaluated. Co-occurrence and mutual-exclusivity (Co-Me) analyses were performed using DISCOVER. *RIT1*-altered samples were evaluated for genomic complexity compared to wild-type (WT) samples. False discovery rate correction was applied when appropriate.

Results: After removing low-quality samples, 63,270 cancer samples were included, and 1.32% (n=838) were found to harbor *RIT1* oncogenic alterations. Most *RIT1*-alterations observed were Amps (735/838) followed by somatic mutations (Muts) (83/838), concomitant Mut and Amp (2/838) and structural variants (18/838). The most frequent *RIT1* Mut was p.M90I (n=40), followed by p.F82C (n=17), p.A77T (n=11) and R122Q (n=9).Uterine carcinosarcomas (5.5%), hormone receptor-positive breast cancer (4.9%) and triple-negative breast cancer (4.7%) had the highest prevalence of *RIT1* alterations. Restricting the analysis only to Muts, lung adenocarcinoma (LUAD) (n=15; 0.22%) and bladder cancers (n=13; 0.51%) had the highest number of oncogenic *RIT1* Muts. *RIT1*-mutant samples had higher TMBs than *RIT1*-WT samples ($p<0.01$). This association was conserved when accounting for histology in bladder cancer and LUAD ($p<0.01$). *RIT1* Amps were associated with higher fraction of genome altered ($p<0.01$) and co-occurred with amps of neighboring genes *NTRK1* ($p<0.01$) and *AKT3* ($p<0.01$) across most tumor types. DISCOVER assisted Co-Me analysis identified *RIT1* Muts as mutually exclusive with *EGFR* Muts in LUAD ($p<0.01$). Among *RIT1*-mutant samples, 10/15 (66%) LUADs harbored *RIT1* as the sole identifiable oncogene driver alteration, whereas only 3/13 (23%) bladder cancers were otherwise driver negative.

Conclusion: *RIT1* alterations appear to be rare across most tumor types. Higher TMB and rarity of *RIT1* Muts and the observation of *RIT1* Amps frequently co-amplified with other chromosome 1 arm q genes, suggest that these alterations might be primarily passengers. These results have implications for stakeholders interested in developing potential therapies for RIT1 as the landscape of these alterations has not been previously explored.

#7146 Unmasking host immune complexity: Single-cell transcriptomic analysis of circulating PBMCs in non-small cell lung cancer treated with immunotherapy based first line - I3LUNG study.

Arsela Prelaj*[1], Monica Ganzinelli*[1], Alessandro Guidi*[2], Chiara Cavalli*[1], Cecilia Silvestri[1], Antonino Belfiore[2], Laura Mazzeo[1], Claudia Proto[1], Mario Occhipinti[1], Marta Brambilla[1], Teresa Beninato[1], Leonardo Provenzano[1], Luca Inverizzi[2], Giulia Bertolini[3], Daniele Lorenzini[2], Filippo De Braud[1], Luca Agnelli#[2], Andrea Vingiani#[2], **Sabina Sangaletti#**[3], Giuseppe Lo Russo#[1]

[1]Department of Medical Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy,[2]Department of Diagnostic Innovation, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy,[3]Department of Experimental Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

Background and Purpose: Biomarkers for immunotherapy in NSCLC remain inadequate, with PD-L1 continuing to underperform despite its ongoing use. "Ghost biomarkers", such as circulating immune profiling, hold potential to reveal host immunity dynamics and identify ways to enhance immune fitness for improved therapeutic outcomes. This study uses single-cell technologies to analyse circulating immune cells in stage IV NSCLC patients (pts) receiving first line mono-immunotherapy (IO) or chemo (CT)-IO, aiming to identify subpopulation who might benefit or not from treatment.
Methods: Blood samples from 33 stage IV NSCLC pts were collected at the baseline of first-line IO or CT-IO, as part of the Horizon Europe-funded I3LUNG study (NCT05537922) conducted at IRCCS Istituto Nazionale dei Tumori of Milan. Single-cell RNA sequencing was performed by Chromium Single Cell 5' v2 kit (10X Genomics) on PBMCs, enriched for neutrophils sorted by FACS (CD66b+CD15+). After quality control, 30 samples were included in the analysis.
Results: Among 30 pts, 27 were treated with CT-IO and 3 with mono-IO, 25 were adenocarcinoma, 4 squamous and 1 NOS carcinoma. UMAP analysis revealed distinct "swan neck" clusters of immature neutrophils, predominantly expressing LCN2 gene, in pts with disease progression (p-value <0.05, logFC -3,67). These cells were notably absent in pts with complete response or partial response. Conversely, responders showed the enrichment of a mature subset of IGHM TCL1A, IGHD expressing B cells ("smart" B cells) (all genes with p-value <0.05 and logFC >2.5). Of note, TCL1A gene is involved in the formation of tertiary lymphoid structures, which are positively correlated with IO responses. Although based on a very small cohort in which no progressive disease occurred, we observed T and NK cells enrichment in mono-IO treated pts with high PD-L1 expression.
Conclusions: To our knowledge, this is the largest prospective cohort that analyses circulating single-cell PBMCs in stage IV NSCLC pts treated with a first-line IO based. We identified novel circulating immune subsets, such as "swan neck" neutrophils and "smart" B cells, which emerged as promising host biomarkers. These subsets consistently acted as negative and positive predictors, respectively to first-line IO-based therapy.

#7147 Ultra-high throughput single-nuclei RNAs sequencing of malignant pleural mesothelioma samples in the BEAT-MESO clinical trial.

Daria Buszta[1], Maxim Norkin[1], Jonathan Bac[2], Sanjay Popat[3], Anthony Pope[4], Riyaz Shah[5], Toby Talbot[6], Julia Giner[7], Wold-Dieter Janthur[8], Ernest Nadal[9], Annamaria Catino[10], David Gilligan[11], Amy Roy[12], Zoi Tsourti[13], Patrick Vagenknecht[14], Georgia Dimopoulou[15], Roswitha Kammler[16], Stephen P. Finn[17], Enriqueta Felip[18], Solange Peters[1], Rolf Stahel[16], Raphael Gottardo[2], **Krisztian Homicsko**[1]

[1]Oncology, CHUV Lausanne University Hospital, Lausanne, Switzerland,[2]Data Science Center, CHUV Lausanne University Hospital, Lausanne, Switzerland,[3]Department of Medicine, The Royal Marsden Hospital - NHS Foundation Trust, London, United Kingdom,[4]Oncology, Clatterbridge Cancer Centre NHS, Liverpool, United Kingdom,[5]Department of Medical Oncology, Kent Oncology Centre Maidstone, Kent, United Kingdom,[6]Department of Medical Oncology, Royal Cornwall Hospital, Truro, United Kingdom,[7]Department of Medical Oncology, Parc Tauli Hospital Sabadell, Sabadell, Spain,[8]Department of Medical Oncology, Kantonsspital Aarau, Aarau, Switzerland,[9]Department of Medical Oncology, ICO Hospitalet, Hospitalet, Spain,[10]Department of Medical Oncology, IRCCS Istituto Tumori Giovanni Paolo II, Bari, Italy,[11]Department of Medical Oncology, Addenbrooke's Hospital, Cambridge, United Kingdom,[12]Medical Oncology, University Hospital Plymouth, Plymouth, United Kingdom,[13]TOP Statistical Center, Frontier Science Foundation, Athens, Greece,[14]European Thoracic Oncology Platform, Bern, Switzerland,[15]ETOP Statistical Center, Frontier Science Foundation, Athens, Greece,[16]ETOP IBCSG Partners Foundation, Bern, Switzerland,[17]Cancer Molecular Diagnostic Laboratory, St. James's Hospital and Trinity College, Dublin, Ireland,[18]Department of Medical Oncology, Vall d'Hebron University Hospital, Barcelona, Spain

Background: Malignant Pleural Mesothelioma (MPM) is a rare cancer with limited treatment options. Large-scale deep biological description of intratumor heterogeneity at single-cell resolution is still missing. Recent studies of fresh tumor biopsies analyzed only a few patients, limiting resolution of population heterogeneity.
Objective: To describe at single-cell resolution in FFPE tissues the cancer cell and tumor microenvironment heterogeneity of untreated MPM patients.
Method: Of the 400 enrolled patients in the BEAT-MESO clinical trial (NCT03762018), 220 patients had available tumor blocks for single-nuclei preparation. The study compared the chemotherapy and bevacizumab combination with and without atezolizumab (anti-PD-L) treatment. All patients signed the ICFs and approved the translational study. Single nuclei were prepared using the snPATHO protocol followed by 10x Genomics FLEX pipeline. Samples were sequenced on a Novaseq X platform.
Results: 179 of the available 220 samples had sufficient tumor content to perform the assay. In total, we detected 792'000 cells. We defined cell type identities using singleR with a combined reference map from non-small cell lung cancer (NSCLC) and a small mesothelioma cohort. We could readily identify cell types of the tumor microenvironment. The most abundant cell types beside tumor cells were cancer associated fibroblast/CAFS (median of 22% of all cells) and macrophages (14% of all cells). We could also define T cell heterogeneity, whereas CD4 T_{CM} cells were the most abundant and detected in all patients, in contrast with proliferating CD4 or CD8 T cells which were absent in 68% of patients, suggestive of rich abundance of tumor reactive T cells. Among immune checkpoints, the expression of TIGIT was the highest, followed by LAG3 and PD1 expression. Tregs were rare and most frequently expressed TIGIT similar to $CD8_{EM}$ and $CD4_{CM}$ cells. We could also define an important heterogeneity of cancer-associated fibroblasts as well as of endothelial cells. Endothelial cell heterogeneity reflected on the proximity to B cells and tertiary lymphoid structures. In contrast, to previous single-cell RNA seq analysis of a small cohort of MPM, we could readily detect neutrophil granulocytes in the tumors. We will also provide data on matching H&E evaluation of TLSs. Depending on the maturation of clinical data, we will also present comparison with clinical parameters.
Conclusions: Our results show the outstanding power to perform snRNAseq of fixed tissues from clinical trials. The method is readily compatible with large phase III clinical trials, and provide a much deeper insight into tumor heterogeneity than bulk RNAseq with deconvolution would do.

#7148 Exploratory biomarker analysis of trastuzumab deruxtecan (T-DXd) treatment for HER2-positive (HER2+) metastatic colorectal cancer (mCRC) in DESTINY-CRC02 (DC-02).

S. Siena[1], K. Raghav[2], A. Takashima[3], T. Kato[4], M. Van den Eynde[5], F. Pietrantonio[6], Y. Komatsu[7], H. Kawakami[8], M. Peeters[9], T. Andre[10], S. Lonardi[11], K. Yamaguchi[12], J. Tie[13], C. G. Castro[14], J. H. Strickler[15], Y. Shiose[16], M. Koga[17], A. Boran[16], D. Haribhai[16], H. Goto[17], D. Barrios[16], K. Abe[16], **T. Yoshino**[18];

[1]Niguarda Cancer Center, Grande Ospedale Metropolitano Niguarda, Department of Oncology and Hemato-Oncology, Universita degli Studi di Milano, Milan, Italy, [2]The University of Texas MD Anderson Cancer Center, Houston, TX, [3]National Cancer Center Hospital, Tokyo, Japan, [4]NHO Osaka National Hospital, Osaka, Japan, [5]Cliniques Universitaires Saint-Luc, UCLouvain, Brussels, Belgium, [6]Fondazione IRCCS, Istituto Nazionale dei Tumori, Milan, Italy, [7]Hokkaido University Hospital Cancer Center, Sapporo, Japan, [8]Kindai University Faculty of Medicine, Osakasayama, Japan, [9]Antwerp University Hospital, Edegem, Belgium, [10]Sorbonne University, Saint-Antoine Hospital, Paris, France, [11]Veneto Institute of Oncology, IRCCS, Padua, Italy, [12]The Cancer Institute Hospital of JFCR, Tokyo, Japan, [13]Peter MacCallum Cancer Centre, Melbourne, VIC, Australia, [14]12 de Octubre University Hospital, Madrid, Spain, [15]Duke University Medical Center, Durham, NC, [16]Daiichi Sankyo, Inc., Basking Ridge, NJ, [17]Daiichi Sankyo, Tokyo, Japan, [18]National Cancer Center Hospital East, Kashiwa, Japan

Background: T-DXd showed clinically meaningful antitumor activity in patients (pts) with HER2+ mCRC in the phase 2 DC-02 trial (NCT04744831). Exploratory baseline circulating tumor DNA (ctDNA) analyses of *HER2 (ERBB2), PIK3CA,* and *RAS* mutation (mut) status and blood-based tumor mutational burden were reported previously (Raghav et al. 2024). This study reports the relationship between other CRC-related genes and efficacy of T-DXd in HER2+ mCRC.

Methods For this analysis, data were combined from 122 pts enrolled in the 5.4 and 6.4 mg/kg T-DXd cohorts. Transcriptome analysis by RNA sequencing with ribosome depletion was performed on 102/107 available baseline (BL) archival tissue samples. ctDNA from blood collected at BL (n = 120/120) and after 2 T-DXd treatment cycles (C3D1; n = 97/103 paired BL/C3D1 samples) was analyzed at Guardant Health to determine genomic landscape (OMNI, ~500 genes) and molecular response (MR; G360, ~74 genes).

Results: By ctDNA, alterations (any) in *TP53* (84.2%), *APC* (77.5%), *FLT3* (63.3%), and *PREX2* (52.5%) were most common; 82.5% and 24.2% of pts had *HER2* amplification (amp) or mut, respectively. Pts with *APC* mut or ≥ median *ABCB1* (P-gp) transporter gene expression tended to have lower objective response rate (ORR) and shorter median progression-free survival (PFS; Table). PFS appeared to be shorter in pts with *EGFR* amp (Table), although this group also had higher ctDNA levels, which is negatively prognostic. Complete MR at C3D1 was associated with the highest ORR and longest PFS, while ORR was lowest and PFS shortest when MR was absent (Table).

Conclusions: Several genetic biomarkers with known prognostic effects in CRC, including *EGFR* or *APC* alterations, were potentially associated with T-DXd efficacy. MR analyses suggested magnitude of ctDNA reduction at C3D1 could be predictive of T-DXd clinical response. Further analysis is needed to assess these biomarkers' predictive vs prognostic value.

Table

	ORR			**PFS**		
	ORR (95% CI), %	OR (95% CI)	*P* value[a]	Median (95% CI), months	HR (95% CI)	*P* value[a]
BEP (ctDNA) (n = 120)	35.0 (26.5-44.2)	NA	NA	5.7 (4.6-6.9)	NA	NA
BEP (tissue) (n = 102)	32.4 (23.4-42.3)	NA	NA	5.6 (4.6-6.9)	NA	NA
Baseline *EGFR* amplification status (ctDNA)						
AMP ND (n = 70)	38.6 (27.2-51.0)	0.68 (0.30-1.56)	NS (> 0.05)	6.9 (5.5-8.2)	1.7 (1.1-2.6)	0.02
AMP (n = 50)	30.0 (17.9-44.6)			4.5 (3.9-5.7)		
Baseline *APC* mutation status (ctDNA)						
Mut ND (n = 34)	52.9 (35.1-70.2)	0.35 (0.15-0.83)	0.01	8.5 (5.9-NE)	2.8 (1.6-5.0)	< 0.001
Mut (n = 86)	27.9 (18.8-38.6)			5.5 (4.3-5.7)		
Baseline *ABCB1* gene expression (tissue)						
< Median (n = 51)	47.1 (32.9-61.5)	0.24 (0.09-0.64)	0.003	8.1 (5.7-9.8)	2.7 (1.6-4.5)	< 0.001
≥ Median (n = 51)	17.6 (8.4-30.9)			4.2 (2.9-5.5)		
Molecular response at C3D1 (ctDNA)						
BEP (MR) (n = 97)	40.2 (30.4-50.7)	NA	NA	6.7 (5.5-7.4)	NA	NA
CMR[b] (n = 19)	78.9 (54.4-93.9)	NA	NA	8.3 (6.9-NE)	NA	NA
PMR[c] (n = 53)	35.8 (23.1-50.2)	NA	NA	6.8 (5.5-8.2)	NA	NA
AMR[d] (n = 25)	20.0 (6.8-40.7)	NA	NA	4.2 (2.9-5.5)	NA	NA

AMP, amplification; AMR, absent molecular response; BEP, biomarker evaluable population; C3D1, after 2 T-DXd treatment cycles; CMR, complete molecular response; ctDNA, circulating tumor DNA; HR, hazard ratio; MR, molecular response; mut, mutation; NA, not available; ND, not detected; NE, not estimable; NS, not significant; OR, odds ratio; ORR, objective response rate; PFS, progression-free survival; PMR, partial molecular response. [a]*P* ≥ 0.05 is the significance threshold; subgroups were compared using the Fisher exact test for ORR and log-rank test for PFS. [b]CMR = 100% reduction in ctDNA levels. [c]PMR = 50 to <100% reduction in ctDNA levels. [d]AMR = <50% reduction in ctDNA levels.

#7149 The role of TROP2 in the MoA of Dato-DXd and how it underpins the biologic rationale of the novel AI-guided biomarker TROP2 normalized membrane ratio.

Matthew S. Sung[1], Matthew Wilson[2], Dominik Vonficht[3], Armin Meier[3], Chetan Rane[1], Charlie Dunlop[2], Gopi Patel[1], Anna Przybyla[1], Yann Wallez[2], Xiaochuan Liu[4], Ross Hill[2], James Harper[2], Markus Shick[3], Hadassah Sade[3], Danielle Carroll[2]

[1]AstraZeneca US, Waltham, MA,[2]AstraZeneca UK, Cambridge, United Kingdom,[3]AstraZeneca Germany, Munich, Germany,[4]AstraZeneca US, Gaithersburg, MD

Datopotamab deruxtecan (Dato-DXd) is an antibody drug conjugate (ADC) comprised of a humanized anti-TROP2 IgG1 monoclonal antibody attached to DXd, a topoisomerase I inhibitor, via a plasma stable cleavable linker. Dato-DXd is currently under clinical investigation for the treatment of patients with solid tumors including non-small cell lung cancer (NSCLC), hormone receptor positive and triple negative breast cancer. Recently, TROP2 Normalized Membrane Ratio (NMR) was identified as an exploratory predictive biomarker for Dato-DXd, in patients with non-squamous (NSQ), non-actionable genomically altered (AGA) NSCLC in the phase 3 TROPION-Lung01 study. TROP2 NMR is a quantitative scoring algorithm that measures membrane TROP2 expression relative to the combined sum of cytoplasmic and membrane TROP2 expression for every tumor cell in a TROP2 immunohistochemistry (IHC) stained tissue sample. Herein, we further describe the role of TROP2 in the mechanism of action (MoA) for Dato-DXd and the biological rationale linking TROP2 NMR to its pharmacological mode of action. Utilizing an isogenic cell line series in NCI-H322, a NSQ, non-AGA NSCLC cell line, where TROP2 was either knocked-out (KO) or over-expressed, we demonstrate that Dato-DXd binding and efficacy is dependent on TROP2 expression. TROP2-KO resulted in complete loss of Dato-DXd binding, delivery of intracellular DXd, in vitro cytotoxicity and in vivo efficacy of Dato-DXd implicating the MoA of Dato-DXd requires TROP2. However, TROP2 expression was not sufficient to predict Dato-DXd activity in a broad panel of cell lines across multiple indications including NSCLC. In 34 NSCLC cell lines, TROP2 mRNA expression generally correlated with Dato-DXd activity, however, among the TROP2-high NSCLC cell lines (n=17), there was a mixture of Dato-DXd sensitive and insensitive cell lines. As internalization of the Dato-DXd-bound TROP2 complex is central to its MoA, we measured the internalization capacity of Dato-DXd in NSCLC cell lines (n=15). Dato-DXd internalization required binding to cells, however, there was a differential capacity for Dato-DXd internalization across cell lines despite similar levels of Dato-DXd binding. Importantly, characterization of the TROP2 IHC staining pattern revealed a strong correlation between TROP2 NMR, but not membrane nor cytoplasmic TROP2, and Dato-DXd internalization capacity and efficacy across these NSCLC cell lines. This suggests TROP2 NMR can predict the innate internalization capacity of Dato-DXd from TROP2 IHC stained samples. Collectively, these data highlight the key role TROP2 plays in the MoA of Dato-DXd in preclinical NSCLC models and provides the molecular basis underpinning the biological rationale for TROP2 NMR as an innovative biomarker for Dato-DXd activity in NSCLC.

#7150 Elucidating co-mutation landscapes in ALK fusion subtypes: implications for targeted therapy in non-small cell lung cancer (NSCLC).

Yang Zebo[1], Yuanyuan Zhang[2], Songlin Zhang[1]

[1]Department of Cardiothoracic Surgery, Yichang Central People' s Hospital, Yichang, China,[2]Three Gorges University Renhe Hospital, Yichang, China

Background: EML4-ALK is a prevalent ALK fusion variant with diverse breakpoints and subtypes. Different fusion partners and subtypes exhibit varied responses to ALK tyrosine kinase inhibitors (TKIs) and unique resistance mechanisms. Co-mutated genes influence the efficacy of targeted therapies. This study explores the molecular characteristics and co-mutation profiles of NSCLC with non-EML4-ALK fusions and various EML4-ALK subtypes.

Methods: We analyzed 615 NSCLC samples harboring ALK fusions, confirmed by next-generation sequencing (NGS) using a 733-gene panel. Tumor mutational burden (TMB) was defined as the total count of nonsynonymous somatic mutations in coding regions.

Results: Of 615 patients with ALK fusions, 543 had EML4-ALK fusions, with V1 as the most common subtype (40.88%, 222 cases), followed by V3 (36.46%, 198) and non-V1V3 subtypes (22.65%, 123). In the 72 non-EML4-ALK cases, intergenic-ALK fusion(ALK-IR)was the most prevalent (44.44%), followed by HIP1-ALK, KIF5B-ALK, and STRN-ALK.

Analysis of co-mutations showed similar rates across EML4-ALK subtypes [V1: 85.14% (189/222); V3: 83.3% (165/198); non-V1V3: 87.0% (107/123)], while non-EML4-ALK fusions displayed a lower rate (79.16%, 57/72). In V1 and V3 substitutions were the predominant mutation type, whereas amplifications and substitutions were more common in non-EML4-ALK fusions. TP53 was the most frequently co-mutated gene across all EML4-ALK subtypes [~30%]. Apart from TP53, the distribution of co-mutated genes varied among subtypes, with CDKN2A and ALK-IR showing relatively high mutation frequencies across multiple subtypes.

To explore resistance mechanisms, we analyzed mutations associated with ALK-TKI resistance pathways, including RTKsMET, EGFR, SRC, IGF-1R, HER2, and HER3. The non-V1V3 subtype had the highest rate of resistance-related mutations (14.63%), while V1 and V3 displayed similar rates (10.81% and 10.1%). In V1, MET amplification was the primary resistance mechanism (3.15%), absent in other subtypes. Conversely, EGFR mutations were most common in non-V1V3 (5.69%) and V3 (2.53%) and less frequent in V1 (0.90%). ERBB3 amplification was more prevalent in V3 [V1 (0.45%) vs. V3 (1.01%) vs. non-V1V3 (0.81%)], while KRAS mutations were highest in non-V1V3 [V1 (1.35%) vs. V3 (1.52%) vs. non-V1V3 (3.25%)]. These findings indicate subtype-specific resistance patterns, potentially impacting treatment choices and prognosis.

TMB analysis revealed a higher proportion of TMB-H (TMB ≥10) in V3 patients (9.10%) compared to V1 (5.86%) and non-V1V3 (6.21%), suggesting differential responses to immunotherapy among EML4-ALK subtypes.

Conclusion: Distinct co-mutation and resistance patterns among EML4-ALK subtypes, providing insights that support personalized treatment strategies and prognostic assessments for NSCLC patients with various EML4-ALK fusion subtypes.

#7152 High-dimensional, single-cell analysis and transcriptional profiling reveal novel correlatives of response to PARP inhibition plus PD-1 blockade in triple-negative breast cancer.

Alexander P. Gottlieb[1], Gregory J. Baker[2], Jia-Ren Lin[2], Ricardo Pastorello[3], Yu-An Chen[2], Tuulia Vallius[2], Janae Davis[3], Clarence Yapp[2], Sarah E. Church[4], Eric Miller[4], Anniina Farkkila[2], Shaveta Vinayak[5], Melinda L. Telli[6], Sara M. Tolaney[3], Filipa Lynce[3], Joan S. Brugge[7], Alan D'Andrea[7], Geoffrey Shapiro[7], Sandro Santagata[7], Peter K. Sorger[7], Elizabeth A. Mittendorf[1], Jennifer L. Guerriero[1]

[1]Department of Surgery, Division of Breast Surgery, Brigham and Women's Hospital, Boston, MA,[2]Laboratory of Systems Pharmacology, Harvard Medical School, Boston, MA,[3]Breast Oncology Program, Dana-Farber Cancer Institute, Boston, MA,[4]Nanostring Technologies, Seattle, WA,[5]Fred Hutchinson Cancer Center, University of Washington, Seattle, WA,[6]Stanford University School of Medicine, Palo Alto, CA,[7]Ludwig Center for Cancer Research, Harvard Medical School, Boston, MA

Background: TOPACIO was a phase I/II study evaluating the PARP inhibitor (PARPi) niraparib in combination with the anti-PD-1 antibody pembrolizumab in patients with locally advanced or metastatic triple-negative breast cancer (TNBC, n=55), irrespective of *BRCA* status. In the efficacy-evaluable population (n=47) the objective response rate (ORR) was 21% and disease control rate (DCR) 49%. Although activity was greater in *BRCA*-MUT patients (7/15, ORR=47% and 12/15, DCR=80%), durable clinical benefit was seen in patients with *BRCA*-WT tumors (3/27, ORR=11% and 9/27, DCR=33%). Patients with PD-L1^{+} tumors (28/47, 60%) achieved higher ORR (9/28, 32%) than those with PD-L1^{neg} tumors (1/13, 8%). It remains unstudied whether tumor gene expression or immune composition in baseline biospecimens is predictive of treatment response. We conducted exploratory biomarker analyses to uncover gene expression patterns and immune states associated with treatment response.

Methods: Transcriptional profiling of baseline samples was performed using the BC360 (n=41) and PanCancer IO360 (n=42) panels (Nanostring) and multigene signatures were used to measure tumor and immune activities. Transcriptional analysis was paired with high-dimensional, single-cell cyclic immunofluorescence (CyCIF) of samples with adequate tissue for analysis (n=22) to characterize the immune microenvironment at single-cell resolution.

Results: *BRCA*-MUT tumors showed increased downstream interferon signaling and immune infiltration relative to *BRCA*-WT tumors ($p < 0.05$). Downstream interferon and immunoproteasome scores were elevated in objective responders ($p < 0.05$). Genes involved in WNT signaling (*TANKS1*, *TANKS2*, *PARP4*, and *NETO2*) associated with long-term response, defined by those still on therapy and responding at data cut off. Low *NETO2* gene expression strongly associated with better ORR ($p = 0.01$). CyCIF analysis performed on whole tissue sections accounting for 2.97×10^6 single cells revealed that in *BRCA*-WT patients, baseline infiltration of immune cells - cells expressing CD45, CD3 (T cells), CD20 (B cells) or CD68/CD163 (macrophages) positively correlated with favorable PFS ($R=0.81$, $p=0.0004$, Spearman's correlation). Further, increasing fractions of CD8+ and PD-1+ CD4+ T cells strongly associated with favorable PFS ($R > 0.71$, $p < 0.0041$). In *BRCA*-MUT patients, baseline immune composition did not correlate with clinical outcome.

Conclusions: *NETO2* expression, WNT pathway, and interferon signaling associate with response to niraparib plus pembrolizumab. While *BRCA*-MUT tumors showed higher baseline immune cell abundance compared to *BRCA*-WT, immune and T cell infiltration was predictive of PFS duration only in *BRCA*-WT patients, suggesting divergent mechanisms of response to PARPi and ICB dependent on *BRCA* status.

#7153 Subclonal mutation load predicts survival and response to immunotherapy in cancers with low to moderate TMB.

Matthew Montierth[1], Yujie Jiang[2], Kaixian Yu[3], Shuangxi Ji[4], Quang Tran[4], Xiaoqian Liu[5], Jessica Lal[4], Shuai Guo[4], Aaron Wu[6], Seung Shin[7], Ruonan Li[4], Shaolong Cao[4], Yuxin Tang[6], Tom Lesluyes[8], Scott Kopetz[9], Pavlos Msaouel[10], Anil K. Sood[11], Ginny Devonshire[12], Christopher M. Jones[12], Jaffer Ajani[9], Sumit K. Subudhi[13], Ana Aparicio[13], Padmanee Sharma[13], John P. Shen[14], Marek Kimmel[2], Jennifer R. Wang[15], Maxime Tarabichi[16], Rebecca C. Fitzgerald[12], Peter Van Loo[17], Hongtu Zhu[18], Wenyi Wang[19]

[1]Baylor College of Medicine, Houston, TX,[2]Department of Statistics, Rice University, Houston, TX,[3]Department of Biostatistics, The University of Texas M.D. Anderson Cancer Center, Houston, TX,[4]Department of Bioinformatics and Computational Biology, The University of Texas M.D. Anderson Cancer Center, Houston, TX,[5]Department of Statistics, University of California, Riverside, Riverside, CA,[6]Rice University, Houston, TX,[7]Department of Statistics, Korea University, Seoul, Korea, Republic of,[8]Cancer Genomics Group, The Francis Crick Institute, London, United Kingdom,[9]Department of Gastrointestinal Medical Oncology, Division of Cancer Medicine, The University of Texas M.D. Anderson Cancer Center, Houston, TX,[10]Department of Genitourinary Medical Oncology, Division of Cancer Medicine, The University of Texas M.D. Anderson Cancer Center, Houston, TX,[11]Department of Gynecologic Oncology and Reproductive Medicine, Division of Surgery, The University of Texas M.D. Anderson Cancer Center, Houston, TX,[12]Early Cancer Institute, Department of Oncology, University of Cambridge, Cambridge, United Kingdom,[13]Department of Genitourinary Medical Oncology, Division of Cancer Medicine,, The University of Texas MD Anderson Cancer Center, Houston, TX,[14]Department of Gastrointestinal Medical Oncology, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX,[15]Department of Head and Neck Surgery, Division of Surgery, The University of Texas M.D. Anderson Cancer Center, Houston, TX,[16]Institut de Recherche Interdisciplinaire en Biologie Humaine et moleculaire, Universite Libre de Bruxelles, Bruxelles, Belgium,[17]Department of Genetics, Division of Division of Discovery Science, The University of Texas MD Anderson Cancer Center, Houston, TX,[18]Department of Biostatistics, University of North Carolina at Chapel Hill, Chapel Hill, NC,[19]Department of Bioinformatics and Computational Biology, Division of Quantitative Sciences, The University of Texas MD Anderson Cancer Center, Houston, TX

Immune checkpoint inhibitors (ICI) have revolutionized cancer treatment, yet current biomarkers like tumor mutation burden (TMB) and mismatch repair deficiency identify responders in only a small fraction of patients, particularly in low-to-moderate TMB cancers. This is critical as the majority of cancer patients (ex. approximately 90% of TCGA) have TMB<10, the FDA-approved threshold for ICI therapy. As such, there is need to identify biomarkers to predict immunotherapy response in these currently overlooked patients.We developed CliPP, a novel statistical machine learning approach that performs subclonal reconstruction through regularized regression. CliPP achieves 100 - 1000 fold faster computation than existing methods while maintaining accuracy in both simulated (n=5,515) and real patient datasets (n=1,582 whole-genome sequences). To facilitate broad adoption, we deployed CliPP as a user-friendly web application (CliPP-on-web, companion AACR abstract, Wu et al.) facilitating use by clinicians and researchers without computational expertise. Using CliPP, we analyzed 7,279 tumors across 32 cancer types from TCGA, 42 samples from two ICI clinical trials in metastatic prostate cancer, and 613 esophageal adenocarcinomas (EAC) from the OCCAMS consortium. Subclonal mutation load (sML) was calculated as the fraction of subclonal mutations, with comprehensive survival analyses performed.High sML showed opposing effects on survival based on TMB: beneficial in 14 cancer types with low-to-moderate TMB (HR=0.06-0.69, P<0.05), but detrimental in 4 high-TMB cancers (HR=1.48-2.70, P<0.05). In two separate metastatic prostate cancer trials (NCT02113657 and NCT02703623) where TMB failed to predict response, high sML predicted favorable outcomes to ipilimumab (NCT02113657: HR=0.18, P=0.004, NCT02703623: HR=0.1, P=0.007) and was associated with increased CD8+ T cell density (P=0.008). Validation in 613 WGS EAC samples with standardized clinical follow-up confirmed improved survival with high sML in moderate-TMB cancers (HR=0.6, P<0.0001), with effects persistent across disease stages.These findings identify sML as a key feature of cancer and reveal an important relationship between evolutionary dynamics and the tumor microenvironment. Furthermore, sML may serve as an orthogonal approach to identify likely responders to ICI in low-to moderate TMB tumors complementing existing biomarkers and potentially broadening the therapeutic reach of checkpoint inhibitors.

#7154 Phenotypic screening from patient-derived organoids to predict therapeutic response.

Emilie Gontran[1], Leo Fillioux[2], Jerome Cartry[1], Ryme Bouyakoub[3], Sabrina Bedja[1], Alice Boileve[3], Ali Mouawia[3], Anna-Rose Gryspeert[4], Mia Cabantous[4], Gizem Altay[4], Maria Vakalopoulou[2], Stergios Christodoulidis[2], Jacques RR Mathieu[1], Paul-Henry Cournede[2], Fanny Jaulin[5]

[1]Gustave Roussy - INSERM U1279 - Universite Paris-Saclay, Le Kremlin-Bicetre, France,[2]CentraleSupelec - Universite Paris-Saclay, Gif-sur-Yvette, France,[3]Gustave Roussy - INSERM U1279 - Universite Paris-Saclay, Villejuif, France,[4]Orakl Oncology, Le Kremlin-Bicetre, France,[5]Gustave Roussy - INSERM U1279 - Universite Paris-Saclay - Orakl Oncology, Le Kremlin-Bicetre, France

Functional precision medicine (FPM) aims to personalize cancer treatment through ex vivo drug testing on patient tumor cells. Classical biochemical tests (like ATP assay) provide limited insight into how patient cells respond to treatment. Phenotypic markers based on imaging features called "radiomics" offer a promising approach to understanding tumor response. Combining FPM with phenotypic screening (PS), we evaluate the predictive power of biomarkers derived from brightfield time-lapse imaging of patient-derived organoids (PDO) from digestive cancer patients.

We screened 24 patients, including 5 with colorectal (CRC) and 19 with pancreatic (PDAC) cancers, each with varying treatment histories (1 to 8 treatments prior to PDO biopsy and 12 different treatments). PDOs were exposed to the corresponding clinical treatments, and 96-hour post-treatment videos allowed for individual tracking of PDO responses. A total of 103 image features (18 intensity, 75 texture, 10 morphology) were extracted from each PDO. Predictive analysis was conducted at 24h, 48h, 72h, and 96h post-treatment, using single timepoints and time-averaging methods. PDO responses were assessed at 3 treatment doses, and area dose-response (ADR) was calculated using trapezoid estimation. Univariate and multivariate Cox models were applied to assess the association of image features with progression-free survival (PFS) and develop an image-based signature for PFS prediction.

We compared image features extracted from entire movie sequences (49 frames), 24h-averaging (13 frames), 4h-averaging (3 frames), and single timepoints (24h, 48h, 72h, 96h). We found similar results from univariate Cox analyses and focused on single timepoint method, which identified 24 features significantly associated with PFS as early as 48 hours. These included intensity (7), texture (11), and shape (6) features (median C-index=0.63, 95% CI [0.60-0.67]). When combining each feature with clinical factors (disease and treatment), 7 features remained significantly associated with PFS. A subset of 3 non-correlated interpretable features was selected (one per category). A signature built from a texture feature measuring pixel intensity homogeneity, combined with disease and treatment best predicted PFS (C-index=0.72, HR=27.43 95% CI [1.89-399.28], p-value=0.046).

Integrating image biomarkers with clinical variables enhances the predictive power of PDO-based functional drug screening. Extracting features from individual timepoints provides earlier insights than biochemical assays, which typically require 5 days post-treatment. This study encourages further exploration of how PS-based FPM could be implemented in a clinical setting. Future work will focus on validating the image-based signature in an independent cohort and across diverse experimental setups.

#7155 A PD-L1-independent phenotype uncovers responders to combined therapy with immune checkpoint inhibitors in kidney cancer.

Florian A. Buttner[1], Viktor Grunwald[2], Elfriede Noessner[3], Igor Tsaur[4], Jens Bedke[5], Matthias Schwab[1], Elke Schaeffeler[1]

[1]Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany,[2]Department of Urology and Medical Oncology, University Hospital Essen, Essen, Germany,[3]Tissue Control of Immunocytes, Helmholtz Munich, Munich, Germany,[4]Department of Urology, University Hospital Tuebingen, Tuebingen, Germany,[5]Department of Urology and Transplantation Surgery and Eva Mayr-Stihl Cancer Center, Klinikum Stuttgart, Stuttgart, Germany

Immunotherapy with immune checkpoint inhibitors (ICI) has substantially improved the treatment of advanced renal cell carcinoma (aRCC). Since treatment effects vary among patients, identifying biomarkers to predict response is crucial for optimizing clinical management. Expression of programmed death ligand 1 (PD-L1) is associated with better response to ICI treatment, but is not a valid biomarker. To advance patient stratification for aRCC, we analyzed molecular and clinical data from the JAVELIN Renal 101 (n=726) and IMmotion151 (n=823) trials, both of which compared a combination of PD-L1 inhibitors and anti-angiogenic treatments with sunitinib monotherapy. In JAVELIN Renal 101, we found correlations between genes and progression-free survival (PFS) in PD-L1-positive tumors treated with sunitinib. These associations were independent of variations in the proportion of PD-L1-positive immune cells present in the tumors. Clustering of PD-L1-positive tumors based on PFS-related genes revealed an IMmune CHeckpoint Inhibitor-responsive Phenotype (IMCHIP). In PD-L1-positive tumors with IMCHIP, combined therapy with ICI led to significantly longer PFS relative to sunitinib (13.3 months vs. 4.4 months median PFS), while in PD-L1-positive tumors without IMCHIP, PFS was comparable for both treatments (11.1 months). By de-correlating gene expression measurements from PD-L1 expression, the clustering could be extended to PD-L1-negative tumors resulting in two groups (aRCC1/2) that were independent of PD-L1 status. Stratification based on aRCC1/2 and PD-L1 status allowed treatment effects to be categorized into four groups. A significant difference in PFS between treatments arms was only observed in patients with tumors of type aRCC2/PD-L1-positive, which corresponded to the IMCHIP profile and covered 32.4% of JAVELIN Renal 101. These findings could be validated in the IMmotion151 trial, where IMCHIP represented 20% of the cohort. Compared to other biomarkers currently being investigated, the combination of aRCC1/2 with PD-L1 status exhibited the greatest potential as a predictive biomarker for patient selection, which was confirmed in both cohorts. The prognostic potential of the aRCC1/2 subdivision was evaluated in 476 clear cell RCC from TCGA. Here, aRCC2 was associated with significantly worse overall survival and cancer-specific survival in both univariate and multivariable analyses, accounting for age, sex, and clinicopathological parameters (TNM). In summary, categorization into aRCC1/2 proved to be a new risk stratification of aRCC which, in combination with PD-L1 status, enabled the retrospective identification of responders who benefited from combined therapy with ICI relative to sunitinib monotherapy. The analysis of IMmotion151 trial data utilized data generated by Genentech/Genentech Research and Early Development.

#7156 Spatial single-cell transcriptomics identifies tumor niches predictive of response to TKI therapy in non-small cell lung cancer.

Haochen Li, Joseph Lownik, Simeon Mahov, Matthew Gayhart, Jean Lopategui, Karen Reckamp, Akil Merchant

Cedars-Sinai Medical Center, Los Angeles, CA

Non-small cell lung cancer (NSCLC) is the tumor with the highest mortality in the world. Tyrosine kinase inhibitors (TKI) are commonly used treatment options for NSCLC, albeit with significantly different mechanisms of action. Responses to TKIs vary greatly among patients. Biomarkers for determination of response in patients treated with these therapies are needed to improve outcomes for patients and allow for more personalized therapeutic approaches.

In this study, we retrospectively collected 34 patients with stage III or IV EGFR-mutated NSCLC. These patients were treated with TKIs. Responses to TKI therapies were dichotomized to early progression (<12 month) and late progression (>12 month) based on their progression free survival data. We performed 10X Xenium spatial single-cell-resolution transcriptomics and imaging mass cytometry (IMC) on tissue microarrays (TMAs) generated from these patients, representing a total of 91 cores.

In this cohort, we detected an enrichment in low tumor infiltrating lymphocyte (TIL) numbers with high epithelial proportions in early progressors using niche detection methods and neighborhood analysis algorithms. The proportion of tumor cells in these immune cold regions is significantly higher in early-progression patients than the late-progression patients. Amphiregulin, encoded by *AREG*, was significantly upregulated in immune cold tumor regions associated with TKI resistance. *AREG* has been demonstrated to inhibit TKI-induced apoptosis with multiple mechanisms. Additionally, we found increased myofibroblast-like cancer-associate fibroblasts (Myo-CAFs) surrounding the tumor region in the early-progressor group.

We next unbiasedly extracted >144,000 features, including gene expression data, cell types, spatial correlations, and gene correlations. We then used Stabl, which is a feature selection algorithm for isolating a sparse and reliable set of features in predictive models when the number of features is significantly greater than the number of patient samples. Using this platform, we identified a 6-feature program which had an AUC of 0.973 in predicting early vs. late progression in TKI-treated EGFR-mutant NSCLC patients. One of the identified features was the expression level of *ARL14* in Myo-CAFs. We further found these *ARL14*$^{+}$ Myo-CAFs with higher tendency to form spatial niches with tumor cells than other CAFs. The *AREG*$^{+}$ immune cold tumor regions and their surrounding *ARL14*$^{+}$ Myo-CAFs commonly form the spatial niches determining TKI responses.

We identified novel spatial features leading to the TKI resistance in NSCLC using the highly multiplexed nature of spatial single-cell transcriptomics. These spatial biomarkers have a high predictive power in predicting response to TKI treatment and provide a basis for the precise treatment of lung cancer and provide potential targets to overcome treatment resistance.

#7157 Development of a comprehensive multigene assay to predict the prognosis of localized or locally advanced prostate cancer.

Jiyoung Ahn[1], Hyunho Han[2], Jang Hee Han[3], Jongkeun Lee[1], Danbi Lee[1], Kyung Chul Moon[4], Young Deuk Choi[2], Chang Wook Jeong[3], Nam Hoon Cho[5], **Woosung Chung**[1]

[1]DCGen Co., Ltd., Seoul, Korea, Republic of,[2]Department of Urology, Urological Science Institute, Yonsei University College of Medicine, Seoul, Korea, Republic of,[3]Department of Urology, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Korea, Republic of,[4]Department of Pathology, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Korea, Republic of,[5]Department of Pathology, Yonsei University College of Medicine, Seoul, Korea, Republic of

Background: Prostate cancer is the most prevalent malignancy in men worldwide. However, the absence of reliable biomarkers for metastasis due to intra- and inter-tumor heterogeneity complicates the establishment of personalized treatment guidelines. To address tumor heterogeneity, a next-generation sequencing (NGS)-based multigene assay, including gene expression, tumor-related pathways and fusion variants, is proposed for developing a new assay. This study aims to develop and validate a metastasis risk prediction model and a molecular subtype classification model to enable personalized treatment.
Methods: This study included surgical specimens from patients with localized or locally advanced prostate cancer at Seoul National University Hospital and Severance Hospital. Patients were stratified into three cohorts: NED (no evidence of disease; no metastasis within seven years), BCR (biochemical recurrence; PSA elevation within five years), and METS (metastasis within five years). Propensity score matching (PSM) balanced clinical risk factors, and whole transcriptome sequencing was performed on formalin-fixed paraffin-embedded primary tumor tissues to identify differentially expressed genes (DEGs) associated with metastasis. The cancer-related pathways were selected using DEGs in the following methods: Gene Ontology term enrichment analysis and curated pathways, and their scores were calculated using Gene Set Variation Analysis. A Metastasis prediction model was developed using classification and regression algorithms to predict metastatic risk based on DEGs and pathway scores. For molecular subtyping, a improved model was optimized by incorporating previous model with fusion status. Both models were validated using an independent cohort of 172 patients.
Results: Following PSM matching, 354 patients were analyzed, resulting in the identification of 103 DEGs and 33 pathways. Using these features, the metastasis prediction model achieved the highest performance (AUC = 0.904) with the Random Forest algorithm and 0.82 in the validation cohort. This performance surpassed that of regression models and joint models combining classification and regression features. Kaplan-Meier (KM) analysis confirmed the clinical relevance, showing significant differences in 5-year metastasis-free survival (MFS) between high- and low-risk groups ($p < 0.0001$, hazard ratio [HR]: 4.958). The improved subtyping model classified samples into two subtypes: Luminal (69%) and aggressive variant prostate cancer (AVPC, 31%). The 5-year MFS by molecular subtype for all patients (n=526) showed that the AVPC tended to have a worse prognosis (p=0.084, HR: 1.389)
Conclusion: This study can be used to advance risk stratification models for metastasis risk and to inform treatment decisions by comprehensively evaluating gene expression and gene fusion events in prostate cancer.

#7158 Molecular residual disease detection via CAPP-Seq in PTCL patients undergoing stem cell transplantation.

Chandan Sanghera[1], Josie Ford[2], Ruwan Gunaratne[1], Kusha Chopra[2], Anna B. Rider[3], Aliyah R. Sohani,[3], Sean M. McCabe[2], Ronald A. Nemec[2], Yi-Bin Chen[2], Steven McAfee[2], Ephraim Hochberg[2], Jeffrey A. Barnes[2], Caroline MacVicar[2], Eric D. Jacobsen[4], Mwanasha H. Merrill[4], Michael S. Khodadoust[1], David Kurtz[1], Salvia Jain[2]

[1]Department of Medicine, Stanford University, Stanford, CA,[2]Department of Medicine, Massachusetts General Hospital Cancer Center, Boston, MA,[3]Department of Pathology, Massachusetts General Hospital, Boston, MA,[4]Dana-Farber Cancer Institute, Boston, MA

Introduction: Despite advances in therapy, improved biomarkers are needed to guide stem cell transplantation (SCT) in peripheral T-cell lymphoma (PTCL). We hypothesized that molecular residual disease (MRD) detection in pre-SCT apheresis T-cells via Cancer Personalized Profiling by Deep Sequencing (CAPP-Seq) could predict relapse post-SCT.
Methods: Eligible patients had diagnostic FFPE tissue and underwent either autogenic or allogenic SCT from 2005-2024. Autologous stem cells (ASC) harvested at the end of induction chemotherapy were sorted into T-, myeloid, NK/B-cell populations. Whole exome sequencing (WES) was performed on DNA isolated from FFPE, with NK/B-cells as the germline control to identify patient-specific single nucleotide variants (SNVs) and design hybrid-capture panels. A PTCL focused canonical gene panel was designed to capture emerging pathogenic SNVs. The ASC fractions and cell-free DNA (cfDNA) samples were interrogated with both panels as a composite approach to quantify MRD.
Results: 20 AITL and 2 PTCL-NOS cases were included. Median age at diagnosis was 61.3y (IQR 55.4-67.5). In the frontline setting, 6 (27.3%) patients received CHOP, 3 (13.6%) CHOP-azacitidine, 9 (40.9%) CHOEP, 3 (13.6%) BV-CHP, and 1 (4.5%) azacitidine-romidepsin. A median of 25 (IQR 17-37.5) SNVs were recovered per patient. Across all cases *RHOA* was most frequently mutated (86.5%), followed by *IDH2* (54.5%) and *PLCG1* (27.3%), consistent with the known mutation profile of PTCL. MRD+ was defined as the detection of tumor-specific SNVs in the ASC T-cell fraction (T-ASC) as previously described (Newman et al, *Nat Biotechnol* 2016). When limited to canonical PTCL genes, 6/22 patients were T-ASC MRD+. In contrast, when using all SNVs, 14/22 were T-ASC MRD+, demonstrating the benefit of tracking multiple patient-specific SNVs. Shared mutations between hematopoietic lineages, including variants associated with clonal hematopoiesis (CH), are frequently observed in PTCL. Similarly, we found multiple mutations were shared between cell compartments, which limited MRD specificity. To account for this and the effect of CH, SNVs detected in the myeloid and B-cell populations were removed. This significantly improved MRD detection, with 9/10 (90%) T-ASC MRD+ patients relapsing post-SCT, compared to 3/12 (25%) T-ASC MRD- patients (specificity 88.9%, sensitivity 75%, PPV 90%). T-ASC MRD- was associated with superior PFS (p=0.007, HR 5.1, 95% CI 1.4-19.2). Higher levels of T-ASC MRD tumor burden were also associated with inferior PFS (p=0.006, HR 1.4, 95% CI 1.1-1.7). MRD analysis in longitudinal cfDNA samples was performed in a subset of cases and correlated with clinical disease status.
Conclusion: Our PTCL-CAPP-Seq approach enables highly sensitive MRD detection, with T-ASC MRD+ predicting high relapse risk post-SCT. In addition, MRD monitoring in cfDNA samples can help facilitate early treatment decisions.

#7159 Characterizing the role of a *KDM3C* germline polymorphism in chemoradiotherapy outcomes for rectal and head and neck cancers.

Adria Hasan[1], Pragya Priyadarshini[2], Elena V. Demidova[1], Philip Czyzewicz[1], Takahiko Murayama[3], Shreya Shah[1], Mark Andrake[2], Yan Zhou[1], Roland Dunbrack[2], Israel Canadas[3], Thomas J. Galloway[4], Margret B. Einarson[2], Barbara Burtness[5], Erica A. Golemis[6], Joshua E. Meyer[4], Sanjeevani Arora[1]

[1]Cancer Prevention and Control Program, Fox Chase Cancer Center, Philadelphia, PA,[2]Cancer Signaling and Microenvironment, Fox Chase Cancer Center, Philadelphia, PA,[3]Nuclear Dynamics and Cancer, Fox Chase Cancer Center, Philadelphia, PA,[4]Department of Radiation Oncology, Fox Chase Cancer Center, Philadelphia, PA,[5]Department of Internal Medicine, Yale Cancer Center, Yale School of Medicine, New Haven, CT,[6]Department of Cancer and Cellular Biology,, Lewis Katz School of Medicine, Temple University, Philadelphia, PA

Background: Predictive biomarkers for chemoradiotherapy (CRT) are crucial for personalized cancer treatment. We have identified a germline missense polymorphism in lysine modifier, *KDM3C* that significantly associates with CRT outcomes in locally advanced rectal cancer (LARC) and head and neck squamous cell carcinoma (HNSCC). KDM3C regulates cellular processes including double-strand break (DSB) repair and is the first lysine modifier to directly influence BRCA1-mediated DSB repair. We hypothesized that the identified polymorphism, a serine to threonine substitution, modulates CRT responses by altering protein interactions necessary for BRCA1-recruitment to DSB sites.

Methods: Isogenic rectal (RCM-1) and head and neck (Cal-27, SCC9) cancer lines were generated via CRISPR/Cas9-editing; wild-type (WT) and SNP pairs. Immunofluorescence (IF) staining of DSB repair proteins, bulk RNA-sequencing, and colony survival assays were performed. Structure-function studies were conducted using AlphaFold-Multimer (v2.3) and AlphaFold3.

Results: SNP-bearing cells exhibited significantly reduced survival compared to WT cells following treatment with various DNA-damaging agents, including irradiation, platinum-based and other chemotherapeutics, and PARP inhibitors ($p<0.001$, one-way ANOVA). RNA-sequencing revealed downregulation in DNA repair and upregulation in immune response genes in SNP-bearing cells compared to the WT ($q<0.05$). To elucidate the mechanisms behind these results, we explored the regulation of the RNF8-RAP80-BRCA1 pathway in DSB repair, directly influenced by KDM3C. AlphaFold modeling of the FHA domain of RNF8 in complexes with KDM3C peptides (Ser/Thr at SNP position, with/without phosphorylation) showed significantly more stable complexes with KDM3C phospho-peptides, with an order of stability pT>pS>>T>S; aligning with prior studies on FHA peptide binding showing a preference for pThr over pSer. In fact, multiple proteins in BRCA1-mediated DSB repair involve FHA-phospho-peptide interactions, and *KDM3C* SNP region is implicated to play an important role in modulating this process. Supporting AlphaFold predictions, significantly reduced colocalization of RAP80-BRCA1 was observed in SNP-cells versus WT upon irradiation, indicating deficiency in DSB repair ($p<0.01$, Mann-Whitney test). Further, SNP-bearing cells exhibited increased colocalization of cGAS with micronuclei, a marker of genomic instability ($p<0.05$, one-way ANOVA), alongside an upregulated expression of genes associated with innate immune response ($p<0.05$, unpaired t-test).

Conclusion: Results highlight the role of the *KDM3C* SNP in DSB repair, influencing the response to DNA-damaging therapy and triggering broader genomic and immune responses. These findings position this polymorphism as a potential stratification marker to improve CRT efficacy in LARC and HNSCC.

#7160 The distribution of tertiary lymphoid structures serves as a hallmark of hot tumors and is associated with long-term survival and the efficacy of immune checkpoint inhibitors in patients with small cell lung cancer: A retrospective and prospective analysis.

Chaoqi Zhang[1], Peng Wu[1], Le Wang[2], Dexin Shang[1], Junhan Zhou[1], Jingjing Liu[1], Chunqi Lin[1], Nan Sun[1], Jie He[1]

[1]Department of Thoracic Surgery, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing, China,[2]The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China

Tertiary lymphoid structures (TLSs) are ectopic lymphoid organs capable of predicting the outcome and therapeutic response in various tumors. Nevertheless, the distribution and clinical significance of TLSs in small cell lung cancer (SCLC) have never been explored. Here, we enrolled over 1500 cases from 6 centers in this retrospective and prospective analysis to elucidate the immune landscape and predictive value of TLSs in SCLC. By employing immunohistochemistry (CD3, CD20, CD21, and CD23) and RNA-seq analysis, we discovered that a high abundance of TLSs was associated with a lower recurrence rate and long-term survival in patients with resected SCLCs. Univariate and multivariate analyses indicated that the status of TLSs was significantly correlated with a lower risk of relapse and cancer-specific death ($P<0.01$), independent of clinicopathological and molecular classifications (ASCL1, NEUROD1, POU2F3, and YAP1). Through the analysis of single-cell RNA sequencing data from 6 patients and whole slide digital pathology analysis of immunohistochemistry (CD45RO, CD4, CD8, CD14, CD68, CD163, CD57, CD66b, Foxp3, and PD-L1) from more than 600 patients, we found that tumors with a high abundance of TLSs were characterized by a high proportion of effector T cells and a high infiltration of anti-tumor immune cells. More importantly, we demonstrated that the status of TLSs was associated with an improved objective response rate, progression-free survival, and overall survival independently of CD8+ T-cell density in SCLCs with immune checkpoint inhibitors from 2 independent retrospective cohorts and a prospective cohort. In summary, our findings highlight that TLSs is a hallmark of hot tumors in SCLC and represents a promising biomarker for long-term survival and immunotherapy.

#7161 HMGA2 predicts treatment outcome in pancreatic cancer.

N. Yamamoto[1], S. Dobersch[2], I. Loveless[3], A. Samraj[1], G. Jang[4], M. Haraguchi[2], R. Fields[5], D. DeNardo[5], F. Notta[6], H. Crawford[3], N. Steele[3], S. Kugel[2];
[1]University of Washington, Seattle, WA, [2]Fred Hutchinson Cancer Center, Seattle, WA, [3]Henry Ford Health System, Detroit, MI, [4]PanCuRx Research Initiative, Toronto, ON, Canada, [5]Washington University in St. Louis, St. Louis, MO, [6]University of Toronto, Toronto, ON, Canada

The recognition of distinct transcriptional subtypes in pancreatic ductal adenocarcinoma (PDAC) has defined a group of poorly differentiated tumors with worse prognosis, but these findings have yet to reach clinical application. These tumors, termed “basal,” are unique for their loss of epithelial identity and relative chemoresistance compared to “classical” tumors. To develop a prognostic biomarker for the basal subtype, we have identified that the chromatin architectural protein HMGA2 is highly expressed in this subset of cancers. Using a tumor microarray of 580 primary biopsies from a diverse set of PDAC patients undergoing surgical resection, we performed multiplex immunohistochemistry for HMGA2, previously published markers of basal and classical disease, and immune subsets. We then associated patient outcome and known clinical data from 491 of these samples to staining patterns. We found that expression of HMGA2, but not published basal markers CK5 or CK17, predicted overall survival in our cohort. Combination of HMGA2 status with GATA6 status allowed for identification of two key study groups: an HMGA2+/GATA6- cohort with worse survival, decreased CD8+ T cells, and poorer response to gemcitabine-based chemotherapies (n=94, median survival = 11.2 months post-surgery); and an HMGA2-/GATA6+ cohort with improved survival, increased CD8+ T cell infiltrate, and improved survival with gemcitabine-based chemotherapy (n=198, median survival = 21.7 months post-surgery). Importantly, these findings were also true for Black patients, who have been underrepresented in previous subtyping studies. HMGA2 was also predictive of overall survival in RNA sequencing from metastatic tumors in an independent cohort. As a positive nuclear marker for basal disease, HMGA2 complements GATA6 as a dual-indicator test for disease subtype in PDAC. We aim to introduce this novel biomarker in a prospective multi-center clinical trial to further validate its use in selecting chemotherapy regimens and across other under-represented racial groups.

#7162 Association between circulating B cell subtypes and treatment response in patients with metastatic renal cell carcinoma (mRCC).

Zeynep B. Zengin[1], Marice Alcantara[2], Hedyeh Ebrahimi[2], Luis Meza[1], Nazli Dizman[3], Christian Nenninger[2], Jeremy Hall[2], Regina Barragan-Carrillo[2], Joann Hsu[2], Daniella Castro[2], Benjamin Mercier[2], Miguel Zugman[2], Salvador Jaime-Casas[2], Alex Chehrazi-Raffle[2], Abhishek Tripathi[2], Peter Lee[2], Motomichi Takahashi[4], Tanya B. Dorff[2], Marcin Kortylewski[2], Sumanta K. Pal[2]

[1]Yale School of Medicine, New Haven, CT,[2]City of Hope Cancer Center, Duarte, CA,[3]MD Anderson Cancer Center, Houston, TX,[4]Miyarisan Pharmaceutical, Tokyo, Japan

Background: While immune checkpoint blockade (ICB) has transformed the treatment of mRCC, response rates remain suboptimal. Most studies of ICB in cancer have focused on T cells as mediators of antitumor responses, but recent evidence highlights B cells' role in modulating ICB efficacy. CD5+ B cells, a small subset with potential immunosuppressive properties, may contribute to immune evasion through mechanisms such as IL-6/STAT3 signaling. This study investigates the prognostic significance of circulating CD5+ B cells in mRCC patients (pts) treated with cabozantinib/nivolumab with or without CBM588, a live bacterial product.

Methods: This open-label, randomized trial enrolled pts aged ≥18 years with histologically verified advanced or mRCC who had not received prior systemic therapy for metastatic disease. Pts were randomized in a 1:2 ratio to receive either cabozantinib/nivolumab alone (control arm) or in combination with CBM588 (experimental arm). Peripheral blood samples were prospectively collected at baseline and week 13. Samples were incubated with matched patients' sera, diluted to 50% in culture media at 37°C with 5% CO2 for a minimum of 2 hours prior to immunostaining of surface markers, phospho-STAT3, and spectral cytometric analysis using the Cytek Aurora system. A two-sided Wilcoxon and two-sided Mann-Whitney U tests were used to compare B cells levels at the two prespecified time points and between the two arms, respectively. Progression-free survival (PFS) was estimated using the Kaplan-Meier method.

Result: In total, 30 pts (20 male, 10 female) with mRCC were randomized to the control arm (10 pts) or the experimental arm (20 pts). The objective response rate in the control arm was 20%, compared to 79% in the experimental arm (p=0.004). The median PFS in the control arm was 13.4 months and was not reached in the experimental arm at the data cut-off (June 1, 2024; median follow-up: 25.8 months). Of the 30 pts, 27 had evaluable blood samples for the final analysis (8 pts in the control arm and 19 pts in the experimental arm). Pts with <75% CD5+ B cells at baseline (n=13) had a median PFS that was not reached, compared to 13.4 months in those with ≥75% CD5+ B cells (n=14 pts; p<0.01, HR=6.47, 95% CI: 1.40-29.88). Of note, CD5+ B cell levels did not differ significantly at baseline between treatment arms (p=0.43). Additionally, no significant difference in circulating CD5+ B cell levels was observed between baseline and week 13 in either arm (p>0.99 in the control arm vs. p=0.25 in the experimental arm).

Conclusions: High baseline levels of CD5+ B cells are associated with inferior outcomes in patients with mRCC treated with cabozantinib and nivolumab. These findings suggest that CD5+ B cells may serve as a prognostic biomarker and a potential target to improve ICB outcomes in mRCC.

#7163 Noninvasive magnetic resonance imaging measured quantitative perfusion as predictive and prognostic biomarker in glioblastoma.

Durga Udayakumar[1], Limin Zhou[2], Marco Pinho[2], Yiming Wang[2], Ben Wagner[2], Michael Youssef[3], Joseph Maldjian[2], Ananth Madhuranthakam[1]

[1]Radiology, Mayo Clinic, Rochester, MN,[2]Radiology, UT Southwestern Medical Center, Dallas, TX,[3]Neurology and Hematology and Oncology, UT Southwestern Medical Center, Dallas, TX

Glioblastoma is highly vascularized with increased perfusion and prognosis remains dismal, despite improved diagnosis and aggressive multimodal treatment. The current standard of care is maximal surgical resection when feasible, followed by adjuvant radiation with concurrent chemotherapy, temozolomide. However, treatment resistance and tumor recurrence pose a considerable challenge for stratifying patients for optimal treatment planning. Current radiological assessment of glioblastoma therapy response predominantly relies on morphological measures; that may not accurately capture early microscopic changes or therapeutic efficacy. We have developed and validated an advanced imaging method, Arterial Spin Labeling (ASL) MRI that enable noninvasive spatial assessment of the entire tumor. ASL-MRI uses water within the blood as an endogenous tracer and measures perfusion without the need for exogenous gadolinium-based contrast agent. The objective of this study was to investigate ASL measured quantitative perfusion after surgery to predict survival in glioblastoma patients undergoing the standard of care chemoradiation treatment. In this prospective, IRB-approved study, twenty-three adults with glioblastoma participated for longitudinal MRI. Progression-free survival (PFS) and overall survival (OS) were recorded for each patient. A baseline MRI scan was performed prior to chemoradiation treatment and after surgery utilizing a 3D ASL sequence. All structural images were co-registered to T1 post-contrast images. Tumor regions of interest (ROIs) such as enhancing tumor (ET), tumor core, whole tumor, and necrosis were manually segmented. Cerebral blood flow maps were calculated and mean perfusion values (MPV) within ET ROIs were extracted. Scatter plot of MPV and Kaplan-Meier graphs were generated using GraphPad Prism. Based on median survival of 420 days, patients were categorized into short (≤420 days) or long (>420 days) survival. A threshold ASL measured perfusion value of 30 mL/100g/min distinguished survival groups; hyper-perfusion correlated with shorter OS, while hypo-perfusion linked to longer OS. The mean±SD of PFS and OS for patients with shorter survival were 119±42 days and 230±117 days respectively, while for longer survival were 624±285 and 726±210 days respectively, and the values were statistically different (P<0.001for both). Kaplan-Meier curves showed significant OS and PFS differences (p<0.015, Hazard Ratio=3.056). Our data indicate that ASL measured baseline perfusion, after surgery and before chemoradiation treatment can benefit in predicting survival and treatment stratification in glioblastoma patients. ASL measured perfusion can be performed during radiation treatment and could provide valuable information that can be incorporated into emerging treatment strategies such as adaptive radiation.

#7164 Predictive biomarkers and immune dynamics in MSS metastatic colorectal cancer treated with regorafenib, ipilimumab, and nivolumab.

Jian Ye[1], Chongkai Wang[1], Colt Egelston[2], Weihua Guo[2], Peter Lee[2], Mawan Fakih[1]

[1]Department of Medical Oncology and Therapeutics Research, City of Hope Comprehensive Medical Center, Duarte, CA,[2]Department of Immuno-Oncology, Beckman Research Institute of the City of Hope, Duarte, CA

Background: Microsatellite-stable (MSS) metastatic colorectal cancer (mCRC) is largely resistant to immune checkpoint inhibitors (ICIs), posing a significant therapeutic challenge. Our phase I trial of regorafenib, ipilimumab, and nivolumab (RIN) demonstrated a 27.6% overall response rate (ORR) and a median overall survival (OS) of 20 months, with better outcomes in patients without liver metastases. This study aims to identify predictive biomarkers and elucidate the immunological mechanisms associated with response and resistance to RIN therapy in MSS mCRC patients.
Methods: Tumor biopsies from eight patients and peripheral blood samples from 29 MSS mCRC patients undergoing RIN therapy were collected at baseline and during treatment. Comprehensive molecular and immune profiling was performed using RNA sequencing, multiplex imaging (CODEX), flow cytometry, and cytokine assays. Gene expression analyses, immune cell phenotyping, cytokine profiling, and T cell receptor (TCR) repertoire analyses were conducted to characterize the tumor microenvironment (TME) and systemic immune responses.
Results: At baseline, tumors from responders exhibited enhanced cellular proliferation, active DNA repair pathways, and increased expression of stimulator of interferon genes (STING) pathway, indicative of a more immunogenic TME. In contrast, non-responders showed aberrant vascular remodeling and a pro-inflammatory TME. In the peripheral blood, responders displayed a Th1-skewed cytokine profile, while non-responders showed markers of DNA damage, elevated immune checkpoint expression, and upregulation of pathogen-response genes, suggesting immune aging and chronic inflammation. Patients with liver metastases displayed significant immune senescence and metabolic hyperactivation, correlating with poor responses to RIN therapy. Following RIN therapy, responders demonstrated increased infiltration of immune effector cells into the TME, associated with a transient reduction of peripheral $CD8^+$ T cells and natural killer (NK) cells, suggesting their migration into the tumor. This was accompanied by increased markers of proliferation, activation, and exhaustion in peripheral immune cells. Furthermore, RIN therapy induced clonal expansion and diversification of $CD8^+$ T cells in responders, along with upregulation of immune activation signatures.
Conclusions: The RIN regimen can reshape the immunological landscape of MSS mCRC by modulating the TME and systemic immunity. Distinct immune profiles between responders and non-responders, especially in patients with liver metastases, highlight the importance of predictive biomarkers for personalized therapy. Addressing immune senescence and metabolic dysregulation may be key to overcoming resistance in this challenging patient population.

#7165 AI-driven vascular structure analysis predicts immunotherapy response in non-small cell lung cancer.

JunHyeong Park[1], Jaesung Heo[1], Jun Hyeok Lim[2], Woo Kyung Ryu[2], Jeong-Seon Ryu[2], Jeong-Seok Choi[3], Lucia Kim[4]

[1]Department of Radiation Oncology, Ajou University School of Medicine, Suwon, Korea, Republic of,[2]Division of Pulmonology, Department of Internal Medicine, Inha University College of Medicine, Incheon, Korea, Republic of,[3]Department of Otorhinolaryngology-Head and Neck Surgery, Inha University College of Medicine, Incheon, Korea, Republic of,[4]Department of Pathology, Inha University College of Medicine, Incheon, Korea, Republic of

Immune checkpoint inhibitors (ICI) have become the standard treatment for advanced non-small cell lung cancer (NSCLC). PD-L1 immunohistochemistry (IHC), the only approved biomarker for predicting ICI treatment response, shows limited predictive value, particularly in patients with low expression (1-49%). Abnormal tumor vasculature in NSCLC correlates with poor prognosis and hampers treatment efficacy by obstructing drug delivery and suppressing immune cell activation. We developed an AI-based biomarker that predicts immunotherapy response by comprehensively analyzing tumor vascular patterns on contrast-enhanced CT. We developed and trained our model using contrast-enhanced CT data from 1,051 NSCLC patients, focusing on both tumor-associated vasculature and normal lung vessels. We developed the Vessel Risk Score (VRS) based on the Mahalanobis distance between the feature distributions of tumor vessels and normal vessels in immunotherapy-treated patients. For external validation, we analyzed pre-treatment CT images from 321 patients who received immunotherapy at two independent institutions (Ajou University Hospital: 218 patients, Inha University Hospital: 103 patients). All images were reviewed by radiation oncologists. VRS effectively stratified patient outcomes. The VRS Low group (n=160) showed significantly longer progression-free survival (PFS) compared to the VRS High group (n=161) (mPFS: 4.9 vs 2.8 months, p=0.013). In multivariate analysis, VRS proved to be an independent prognostic factor alongside PD-L1 expression for both PFS (HR=1.63, 95% CI=1.26-2.10, p<0.001) and OS (HR=1.60, 95% CI=1.18-2.17, p=0.003). Notably, in patients with low PD-L1 expression (1-49%), where clinical decision-making is challenging, VRS demonstrated superior predictive performance with AUROC of 0.68 (95% CI: 0.60-0.77) for treatment response (CR, PR, SD) and 0.70 (95% CI: 0.60-0.78) for durable clinical benefit (DCB), while PD-L1 showed predictive power below 0.5. VRS showed no significant correlation with PD-L1 (p=0.354), suggesting its complementary role in assessing vascular abnormality. The combination of VRS and PD-L1 expression enhanced prognostic stratification: PD-L1 High/VRS Low patients showed mPFS of 7.2 months and mOS of 23.9 months, while PD-L1 Low/VRS High patients showed mPFS of 2.3 months and mOS of 9.8 months, demonstrating marked prognostic differences. Our CT-based vascular structure biomarker provides an effective, non-invasive method for predicting immunotherapy response in NSCLC patients. VRS is particularly useful in predicting treatment outcomes for patients with low PD-L1 expression and complements PD-L1 expression data. These findings underscore the significance of tumor vascular patterns in NSCLC immunotherapy, potentially enhancing patient selection and facilitating personalized treatment strategies.

#7166 Spatially resolved protein profiling of tumor and immune microenvironment in EGFR mutant NSCLC with transformation to SCLC.

Hong Chen, Bingnan Zhang, Alejandra Serrano, Sharia Hernandez, Wei Lu, Santiago Trevino, Natalie Vokes, Ximeng Liu, Yanhua Tian, Bo Zhu, Monique B. Nilsson, John V. Heymach, Jianjun Zhang, Luisa Solis Soto, Carl M. Gay, Lauren A. Byers, Xiuning Le

UT MD Anderson Cancer Center, Houston, TX

Background: Approximately 3-10% of EGFR-mutant non-small cell lung cancer (NSCLC) cases transform into small cell lung cancer (SCLC) as a resistance mechanism to third-generation EGFR tyrosine kinase inhibitors (TKIs). The mechanisms driving this transformation are poorly understood. This study investigates proteomic differences between pre-transformed EGFR adenocarcinoma (preADC) and post-transformed SCLC (postSCLC) to identify underlying mechanisms and therapeutic vulnerabilities.
Methods: Nanostring GeoMx® Digital Spatial Profiling (DSP) technology was used to analyze seventy protein biomarkers in FFPE patient samples. Two patient cohorts were analyzed: Cohort one included seven patients, with one patient having a preADC sample and six patients having postSCLC samples. Cohort two included three patients, each providing paired preADC and postSCLC samples (3 preADC and 4 postSCLC samples in total). Up to 12 regions of interest (ROIs) were selected by expert pathologists from tumor areas, segmented into Tumor and TME compartments based on the expression of morphology markers (pan-CK, synaptophysin, and CD45).
Results: After integrating both cohorts and correcting for batch effects, UMAP and heatmap visualizations showed clear separation between preADC and postSCLC tumor segments. Differential protein expression analysis revealed that preADC tumors exhibited increased expression of immune regulatory proteins, including IDO1, VISTA, INPP4B, MET, Tim-3, CD45 and STING, indicating active immune evasion. In contrast, postSCLC tumors showed elevated expression of CD56, PARP, BIM, Ki-67 and pan-AKT, which aligns with the high CD56 expression characteristic of SCLC, dysregulated DNA damage repair pathways, and AKT pathway activation implicated in NSCLC to SCLC transformation. Further comparison of protein expression in the TME segments revealed more abundant expression of CD45, CD11c, and CD4 in preADC TME, while postSCLC TME exhibited higher levels of GZMB, CD56, and PARP. FDR < 0.05 was used as the cut-off. These TME findings indicate an "immune-cold" phenotype in SCLC, characterized by scarce immune cell infiltration and increased resistance to apoptosis. In addition, protein expression panels identified promising immune checkpoint as potential therapeutic targets including B7H3 and LAG3 in both preADC and postSCLC samples.
Conclusions: These results highlight molecular and immune changes during the preADC to postSCLC transformation. The shift in tumor and TME protein expressions in postSCLC reflects an increasingly aggressive tumor phenotype, with changes in immune suppression, DNA damage and apoptosis pathways. These findings suggest potential therapeutic targets for EGFR-mutant NSCLC with SCLC transformation.

CLINICAL RESEARCH: Molecular Genetics and Epigenetics of Tumors
Poster Session

#7170 Differential prognostic impacts of the germline APOBEC3B deletion polymorphism in hepato-pancreato-biliary and genitourinary cancers.
Ahmadreza Niavarani, Peyvand Parhizkar Roudsari, Roya Noorbakhsh, Melineh Markarian, Reza Malekzadeh

Digestive Oncology Research Center, Digestive Disease Research Institute, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of

The mutational signatures related to apolipoprotein B mRNA-editing enzyme catalytic polypeptides (APOBECs) are found in many cancers. We aimed to assess the impact of *APOBEC3B* deletion variant on the risk and prognosis of various cancers in the Golestan Cohort Study, Iran, comprising 1,393 cancer cases and 1,806 controls (median age: 69.2 years). The population predominantly consisted of the Turkmen ethnicity (77.5%), with 49.6% of individuals deceased during the study period. The TaqMan genotyping assay was used as a proxy to identify the rs12628403-A/C (linked to intact/deleted *APOBEC3B*) alleles. The *APOBEC3B* deletion was identified in 29.8% of individuals. Cancer cases exhibited a higher frequency of *APOBEC3B* deletion compared to controls, particularly among Turkmen individuals (32.9% vs. 28.5%, OR = 1.23, *P* = 0.019). Increased frequencies were observed for gastric cancer (OR = 1.61, *P* = 0.001) and collective *gastrointestinal (esophageal, gastric, small intestinal,* and *colorectal) cancers* (OR = 1.31, *P* = 0.012) among *APOBEC3B*-deleted individuals. In head and neck cancer, *APOBEC3B* deletion was more prevalent among cases of all origins (OR = 1.83, *P* = 0.036). Survival analyses revealed contrasting effects of the *APOBEC3B* deletion. *APOBEC3B* deletion was significantly associated with longer survival in pancreatic (4.0 vs. 2.7 months; *P* = 0.025) and biliary (29.3 vs. 3.1 months; *P* = 0.047) cancers, as well as the collective *hepato-pancreato-biliary (hepatocellular, pancreatic, gallbladder,* and *biliary) cancers* (4.2 vs. 2.5 months; *P* = 0.002). By contrast, it was predictive of shorter survival in bladder (2.1 vs. 32.8 months; *P* = 1.1e-4) and cervical (5.7 vs. 53.7 months; *P* = 0.017) cancers, and the collective *genitourinary (bladder, cervical, uterus,* and *prostate) cancers* (5.7 vs. 32.8 months; *P* = 1.9e-5). Multivariate Cox regression analyses confirmed a favorable prognostic impact of the *APOBEC3B* deletion in the collective *hepato-pancreato-biliary cancers* (HR = 0.634; *P* = 0.039), while an adverse prognostic impact in the *genitourinary cancers* (HR = 2.45; *P* = 1.9e-4) and meningioma (HR = 6.750; *P* = 0.025). These findings suggest the *APOBEC3B* deletion as a susceptibility and prognostic biomarker in specific cancers, with differential effects based on tumor type. Further studies are warranted to elucidate underlying mechanisms and potential therapeutic implications.

#7171 Comparison of clinical next generation sequencing and whole genome sequencing for acute myeloid leukemia characterization and classification.

Annette A. Prah[1], Derek Van Delden[1], Joan Alexander[2], Asya Stepansky[2], Erin Boyle[3], Nicholas Chiorazzi[3], Jonathan Kolitz[3], Stephanie Boisclair[3], Steven L. Allen[3], David Chitty[3], Andrea B. Moffitt[1]

[1]Department of Hematology and Medical Oncology, Emory University School of Medicine, Atlanta, GA,[2]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY,[3]R.J. Zuckerberg Cancer Center, Northwell Health, Lake Success, NY

Acute myeloid leukemia (AML) is a hematopoietic neoplasm characterized by uncontrolled proliferation of myeloid blast cells. AML is the most common acute leukemia, with over 20,000 new U.S. cases diagnosed annually. The 5-year survival rate varies greatly by age, from 63% in younger patients to 11% in older patients. AML is a genetically heterogeneous disease, with over 25 genes implicated in classification. Clinical next-generation sequencing (NGS) is crucial for AML management, aiding in relapse risk assessment, therapy guidance, and understanding of the genetic landscape. In AML, this typically involves sequencing a panel of 50-500 genes. In practice, only diagnostic samples are tested, without germline controls. In research settings, whole genome sequencing (WGS) is performed on paired diagnosis and remission samples, with remission serving as a germline control. This study assessed the accuracy and utility of AML clinical NGS reports by comparing with matched WGS data. We explored the benefits of germline controls and how discrepancies impact risk classification. 65 AML patients treated in the Northwell Health System and enrolled in an IRB-approved biospecimen study were analyzed. Clinical NGS testing was performed using FoundationOne Heme (n=51) or GenPath OnkoSight (n=14). We analyzed mutation data from clinical NGS reports (median 12 variants per report; range 1-26), identifying DNMT3A, FLT3, TP53, and NPM1 as the most frequently mutated genes, consistent with prior studies. Matched WGS data from 15 patients classified clinically reported variants as somatic (27%; n=57), germline (65%; n=135), or not observed (8%; n=17), using variant allele frequencies (VAFs). There was a strong but imperfect correlation between somatic/germline labels and known/unknown status, provided in the clinical NGS reports: 89% of germline variants were of unknown significance (n=120), while 83% of somatic variants were of known significance (n=43). Clinical NGS reports lacked data such as population variant frequency, pathogenicity scores, and VAFs. Germline and unknown significance variants had higher population frequencies and lower pathogenicity scores than somatic and known significance variants ($p<0.0001$). European LeukemiaNet (ELN) risk groups assigned using clinical NGS data (Adverse: 51%, n=33; Favorable: 29%, n=19) were affected by the inclusion of germline or unobserved variants; excluding those reclassified two patients from Adverse to Intermediate risk. This study finds general agreement between AML clinical NGS and WGS in the overlapping genes. The clinical NGS reports studied here lack annotation of germline and subclonal variant status. We highlight the limitations of clinical NGS without germline controls and its implications for AML classification, clinical decision making, and broader scientific understanding.

#7172 Comprehensive whole genome sequencing (WGS) assay provides diagnostic insight into clinically relevant genomic alterations across myeloid malignancies.

Robert Huether[1], Derick Hoskinson[1], Pavana Anur[1], Raul Torres[1], Karl R. Beutner[1], Kristiyana Kaneva[1], Yan Yang[1], Kelly A. Potts[1], Andrew Frazier[1], Iris Braunstein[1], Brett M. Mahon[1], Michael A. Thompson[1], Kate Sasser[1], Halla Nimeiri[1], Lewis J. Kraft[1], Francisco M. De La Vega[1], Guillermo Garcia-Manero[2]

[1]Tempus AI, Inc., Chicago, IL,[2]M.D. Anderson Cancer Center, Houston, TX

Identification of NCCN and WHO guideline-indicated genetic variation is crucial for diagnosis, risk assessment, and therapeutic decision-making in patients with myeloid malignancies. Current practice often utilizes small mutation panels, cytogenetics (CG), and/or fluorescence in situ hybridization—independent tests that require a separate specimen and have timeline and performance limitations. Whole-genome sequencing (WGS) is a promising alternative with rapid turnaround times and low sample requirements, but evidence showing concordance with other methods is still limited. Here, we report the performance of a WGS assay on patients with acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), myelodysplastic syndromes (MDS) and other hematological malignancies (HM). We show high concordance with matched findings using Tempus xT-Heme (a 648 gene targeted DNA-seq panel), Tempus xR (a whole transcriptome RNA-seq assay), and available cytogenetic results.

All 230 patients in our cohort had targeted DNA panel results, 215 had RNA-seq results, and 10 had cytogenetic data. For the WGS assay, DNA obtained from blood or bone marrow aspirates was used to construct paired-end libraries via tagmentation and WGS to 80X mean coverage with the Illumina NovaSeq-X platform. Data were analyzed using the DRAGEN Platform with custom post-processing filters. We filtered SNV/Indel alterations to 40 genes with VAF ≥10%, 608 recurrent rearrangements, and CNAs greater than 5MB.

In our cohort (68% AML, 18% MDS, 12% CML and 2% other HM) the WGS assay identified 504 reportable SNV/indels, 119 SVs, and 14 large CNAs, which were highly concordant with other assays. Specifically, 99.4% of SNVs/indels and 95.1% of SVs were concordant between xT-Heme and WGS. A subset of guideline-recommended SVs (e.g. RUNX1-RUNX1T1, ELN adverse risk fusion) detected by WGS were not identified by xT-Heme (10.7% [13/121]) but were confirmed via RNA-seq. Large CNAs detected by WGS were 100% concordant (14/14) with available clinically reported CG. Notably, our cohort included 26 FLT3 internal tandem duplications (ITDs) ranging from 12 to 97 nucleotides in length, and 24 of these were identified by WGS —the missed ITDs had xT-Heme VAFs of less than 4%. WGS was less sensitive at low VAFs (targeted sequencing depths of ~600x) that were likely subclonal.

We demonstrate high concordance (97%) between our WGS assay and conventional methods in identifying guideline recommended genomic alterations, including large CNAs, in myeloid malignancies. The ability to obtain large CNA results, historically reserved for CG testing, has the potential to save costs and fill an unmet clinical need globally where cytogenetic resources are limited. Additionally, WGS can identify unique SVs that may be missed by conventional methods and may enhance personalized treatment strategies.

#7173 Comprehensive genomic profiling reveals novel insights into the genomic landscape of extraskeletal myxoid chondrosarcomas.

Carl Philipp Schmitthenner[1], Franziska Evelt[1], Lucas Sebastian Scholl[1], Jessica Janke[1], Anna Kuntze[1], Santina Kirmse[1], Kim Falkenberg[1], Ilka Isfort[1], Ruth Berthold[1], Kornelius Kerl[2], Inga Grunewald[1], Eva Wardelmann[1], Wolfgang Hartmann[1], **Marcel Trautmann**[1]

[1]Gerhard-Domagk-Institute of Pathology, Munster University Hospital, Munster, Germany,[2]Department of Pediatric Hematology and Oncology, Munster University Hospital, Munster, Germany

Background: Extraskeletal myxoid chondrosarcomas (EMCs) are rare mesenchymal neoplasms comprising less than 3% of all soft tissue tumors. EMCs arise mainly from the deep soft tissues of the extremities, accompanied by high rates of recurrence and metastases. The molecular hallmarks of EMCs are cytogenetic *NR4A3* rearrangements, generating chimeric NR4A3 fusion oncoproteins. The most common reciprocal translocation t(9;22)(q22;q12) results in a fusion of the EWS RNA-binding protein 1 gene (*EWSR1*) to the nuclear receptor subfamily 4 group A member 3 gene (*NR4A3* or *TEC*; approximately 75% of cases). Although the oncogenic *NR4A3* fusion transcripts appear to play a crucial role in EMC tumorigenesis and progression, the specific biological function and the mechanism of action remains to be defined. Comprehensive genomic profiling (CGP) and reliable molecular characterization of solid tumors using Next-Generation-Sequencing (NGS) technology has become a key tool to facilitate biomarker-matched therapy selection. With a broad spectrum of therapies already approved or currently tested in clinical trials, rapid and scalable detection of an evolving number of therapeutically relevant genomic alterations and complex biomarkers is required.
Methods: This study combined NGS-based CGP and fusion gene analysis of 69 paraffin-embedded EMC tissue samples to systematically map the genomic landscape of EMC. We used the all-in-one OncoDEEP® in-house kit, integrating both (i) an DNA NGS panel larger than 1MB powered by Twist Bioscience hybrid capture technology in combination with (ii) OncoDNA's bioinformatic data analysis and clinical interpretation software suite (OncoKDM) resulting in an integrated theranostic report. The CE-IVD approved assay covers single-nucleotide variants (SNVs), copy number variants (CNVs), deletions and insertions (indels) and loss of heterozygosity (LOH) in 638 genes and facilitates the assessment of key complex biomarkers / signatures such as homologous recombination deficiency (HRD), tumor mutational burden (TMB), or microsatellite instability (MSI).
Results: Overall, more than 13,600 variants have been identified, categorized as: pathogenic (0.1%), likely pathogenic (1.9%), variant of unknown significance (VUS; 13.3%), likely benign (6.3%) and benign (78.3%). TMB, HRD and MSI status were successfully assessed in more than 88% of all EMC samples. Furthermore, a novel *NR4A3* fusion rearrangement was identified with the majority of EMC samples expressing the chimeric t(9;22) *EWSR1::NR4A3* fusion transcript.
Conclusions: This study provides a comprehensive genomic profile of EMC and uncovers novel genetic alterations, enhancing our understanding of its underlying pathogenesis. Gained genomic insights are anticipated to inform and refine future diagnostic criteria, potentially leading to more precise targeted therapies.

#7174 Multiomics analysis of lung adenocarcinoma manifesting as radiological part-solid features in female never-smokers.

Junsu Choe[1], Noeul Kang[1], Jinyeong Lim[2], Eun Seop Seo[2], Mina Hwang[1], Se-Hoon Lee[1], Myung-Ju Ahn[1], Yoon-La Choi[1], Hong Kwan Kim[1], Woong-Yang Park[2], Sang-Won Um[1]

[1]Samsung Medical Center, Seoul, Korea, Republic of,[2]SAIHST, Sungkyunkwan University, Seoul, Korea, Republic of

Purpose: Early-stage lung adenocarcinomas (LUADs) manifesting as part-solid radiological features have a slowly progressive course to invasive malignancy, but its genomic alterations and tumor microenvironment are poorly understood.
Methods: We analyzed eleven part-solid type LUADs from female never-smokers. Whole-exome sequencing and whole-transcriptome analysis were conducted on samples from seven patients. Single-cell RNA sequencing was performed on samples from four patients, and spatial transcriptomics was conducted on a sample from one patient.
Results: Both the ground-glass and solid components of LUADs exhibited similar characteristics of malignant cells, sharing mutations and showing no significant differences in tumor mutation burden. Compared to the GG component, the solid component showed up-regulation of epithelial-mesenchymal transition (EMT)-related and hypoxia pathways. We identified a cancer-associated secretory (CAS) cell cluster characterized by the expression of the cancer-related gene CEACAM6. CAS cells showed an increasing trend from normal to tumor regions. Interleukin (IL)-1β+ macrophages, a distinct subtype characterized by a pro-inflammatory M1-dominant gene signature, were abundant in solid components. Spatial transcriptomic analysis revealed that IL-1β+ macrophages and CAS cells were co-localized in specific invasive regions of the solid components, which had elevated EMT and hypoxia signatures.
Conclusion: We identified that CAS cells and IL-1β+ macrophages are crucial during the progression from the pre-invasive (GG) to invasive adenocarcinoma (solid component). Targeting CAS cells and IL-1β+ macrophages could be preventive or therapeutic strategies for early LUADs.

#7175 *STAT3/STAT5* mutation predicts shorter overall survival in patients with plasma cell myeloma.

M. T. Ye, Z. Zuo, S. Calin, Y. Yang, M. You;
The University of Texas MD Anderson Cancer Center, Houston, TX

Plasma cell myeloma is a heterogeneous hematological neoplasm characterized by clonal expansion of malignant plasma cells in the bone marrow. Mutations in the RAS-MAPK pathway, such as *KRAS*, *NRAS*, and *BRAF*, have been identified as high-risk factors associated with poor prognosis. However, the clinical impact of *STAT3/STAT5* mutations in plasma cell myeloma has not been well studied. We assessed the clinicopathologic and molecular genetic features and clinical outcomes of 16 myeloma patients with *STAT3/STAT5* mutations identified by next-generation sequencing, including 9 with *STAT3* mutation, 5 with *STAT5A* mutation, and 2 with *STAT5B* mutation, and compared with 32 matched patients without any mutation. *STAT3/STAT5* mutations were detected at initial diagnosis in all 8 patients with initial mutation status available, suggesting *STAT3/STAT5* mutation is an early event in pathogenesis in plasma cell myeloma. *STAT3/STAT5* mutation was the sole mutation in 5 cases and accompanied by other co-existing mutations in 10 cases, with *KRAS/NRAS* (n=5) and *TP53* (n=3) being the most common; in the latter group, *STAT3/STAT5* represented the dominant clone in 4 cases and co-dominant clone in 6 cases. All were missense mutations, with 10 of 16 (63%) located at the SH2 domain. The median variant allele frequency was 9.1%. *STAT3/STAT5*-mutated patients had higher level of serum lactate dehydrogenase, lower level of serum albumin, higher proportion of IgA type paraprotein, and more advanced R-ISS stage. *STAT3/STAT5* mutations were associated with complex karyotype and gain/amplification of *CKS1B*. The median overall survival was significantly shorter for *STAT3/STAT5*-mutated patients (42 months vs 72 months, p<0.0001). In summary, *STAT3/STAT5* mutations are often early events and represent dominant clones in plasma cell myeloma. These mutations are associated with a complex karyotype, more advanced R-ISS stage, and shorter overall survival. Our findings support the routine testing for *STAT3/STAT5* mutations in patients with plasma cell myeloma, highlighting their role in disease pathogenesis and their potential as therapeutic targets.

#7176 ROR1-dependent mutant TP53 and cyclin A1 increases, via NRF2 activation, potentially potentiate genomic instability in chronic lymphocytic leukemia cells with del(17p).
Emanuela M. Ghia, Can Huang, Michael Y. Choi, George F. Widhopf, Thomas J. Kipps

University of California San Diego, La Jolla, CA

Deletion of 17p (del(17p)) and/or TP53 mutated ($p53_{MU}$) and/or complex karyotype (CK) are adverse prognostic factors with chemotherapy or targeted therapy that define high-risk chronic lymphocytic leukemia (CLL). Del(17p) causes loss of one allele of *TP53*. Somatic mutations occur in the other allele of TP53 in about 80% of del(17p) CLL. ROR1 is an embryonic receptor for Wnt5a, which can induce ROR1-signaling leading to activation of ERK1/2, NF-κB and NRF2 target genes in CLL cells. Anti-ROR1 antibody (zilovertamab) therapy, which blocks ROR1-signaling, inhibited expression of such target genes in CLL cells of patients (pts) treated in a phase I study. Prior work also found that pts with CLL cells with high-level ROR1 ($ROR1^{Hi}$) had an inferior progression-free survival and overall survival compared to pts with CLL cells with low-levels of ROR1 ($ROR1^{Lo}$). Our gene set enrichment analysis revealed that NRF2 target genes and cell-cycle control genes were significantly enriched in $ROR1^{Hi}$ versus $ROR1^{Lo}$ CLL cells or in pre-treatment CLL cells (D0) versus CLL cells of the same pts treated with zilovertamab (D28). Most pronounced was CCNA1, which encodes cyclin A1 and is transcriptionally activated by wild-type p53 or most $p53_{MU}$. NRF2 activation can enhance cyclin A1 activation by inducing NRF2 target genes such as NQO1, which can bind $p53_{MU}$ and mitigate its proteasomal degradation. We assessed 493 untreated CLL for ROR1 expression, FISH, and cytogenetics. We found that cases with $ROR1^{Hi}$ CLL had a significantly higher proportion of del(17p) and/or CK than cases with $ROR1^{Lo}$ CLL. This suggests that high-level ROR1-signaling may be conducive to the survival of CLL cells with del(17p) and genomic instability associated with high-level oxidative stress. We examined for this using a CLL-cell line, MEC1, which lacks ROR1 but makes high levels of Wnt5a and has $p53_{MU}$ (Q317fs*29) with del(17p). We also used $MEC1^{Wnt5a-/-}$ cells, in which we deleted *WNT5A*. MEC1 or $MEC1^{Wnt5a-/-}$ cells were transduced with a lentivirus vector encoding ROR1 to generate MEC1-ROR1 or MEC1-$ROR1^{Wnt5a-/-}$ cells. Relative to MEC1 or MEC1-$ROR1^{Wnt5a-/-}$, MEC1-ROR1 cells had significantly increased expression of NRF2 target genes and cell-cycle control genes, NQO1, $p53_{MU}$, and cyclin A1, and increased resistance to drug induced oxidative stress. Similarly, we found that culture of $ROR1^{+}$ del(17p) CLL cells with Wnt5a increased expression of NQO1, cyclin A1, and $p53_{MU}$, and enhanced their resistance to drug induced oxidative stress; this effect of Wnt5a could be blocked by concomitant treatment with zilovertamab. As such, inhibiting ROR1-signaling may reduce resistance to oxidative stress and prove selectively deleterious to CLL cells with del(17p) and CK, thereby mitigating their adverse prognostic implications for pts with CLL.

#7177 Deep genomic analysis of undifferentiated sarcoma.

Jef Baelen[1], Raphael Sciot[2], Ruben Cools[3], Jonas Demeulemeester[3], Isabelle Vanden Bempt[1]

[1]Center of Human Genetics, KU Leuven, Leuven, Belgium,[2]Imaging and Pathology, KU Leuven-UZ Leuven, Leuven, Belgium,[3]Oncology, VIB-KU Leuven Center for Cancer Biology, Leuven, Belgium

Introduction: Undifferentiated sarcomas (USrc) are aggressive mesenchymal tumors that do not show characteristic features and therefore remain a diagnosis of exclusion. USrc typically have a low tumor mutational burden but are genomically complex, carrying many large structural variants (SVs) and copy number aberrations (CNAs). Improving diagnostic practice by adding novel whole genome-technologies based on high molecular weight DNA could increase the sensitivity for detecting genetic alterations in USrc, allowing identification of recurrent aberrations or patterns with clinical implications.

Methods: Snap-frozen tissue from 41 USrc in 38 patients were analyzed by both conventional karyotyping and Optical Genome Mapping (OGM). Chromothripsis was determined based on OGM data and defined as more than eight copy number segments with oscillating copy number in co-occurrence with more than eight chromosomal rearrangements within 50 Mb, or more than 20 rearrangements within 50 Mb. These rearrangements should be interleaved and approximately half of them should be inversion-type aberrations. In addition, we identify known cancer genes (COSMIC Cancer Gene Census) which are amplified/lost or show evidence of disruptive SVs. DNA methylation profiling using EPIC arrays was done on 15 USrc followed by sarcoma classification with the Deutsches Krebsforschungszentrum (DKFZ) sarcoma classifier (v12.3).

Results: 32 out of 41 USrc karyotypes showed at least subclonal whole genome-doubling with complex patterns of SVs and CNAs, including double minutes, ring chromosomes and giant markers. OGM was performed on all USrc biopsies yielding an average coverage of 352X. 25 out of 38 patient samples presented with chromothripsis with an average of 4.3 chromosomes affected (1-13 chromosomes). 70% of amplifications (CN greater than or equal to 5) and 83% of high, focal amplifications (CN greater than or equal to 10) occurred in regions with chromothripsis. Aberrations in 590 COSMIC cancer genes were detected. The most frequently altered cancer genes were *CDKN2A* (42%), *RB1* (34%), *PTPRD* (29%), *MGMT* (26%), and *ARHGEF12* (24%). For 3 patients, biopsies from two different time points were profiled, showing a high proportion of shared aberrations. DNA methylation patterns classified 8 out of 15 analyzed samples as USrc, and the remaining 7 showed no match to any reference sarcoma group. Copy number profiles derived from OGM and DNA methylation arrays were near identical. MGMT promotor hypermethylation was observed in 5 out of 16 cases.

Conclusion: Implementation of OGM improves the detection of SVs and CNAs over standard-of-care. This is demonstrated by the detection of chromothripsis regions and novel recurrent aberrations in USrc. Further investigation to confirm these findings and to identify single nucleotide variants and allele-specific copy numbers are currently ongoing using long-read HiFi sequencing (PacBio).

#7178 Advancing WGS and WTS diagnostic testing for pediatric leukemia: fast-tracking CNV, ploidy, and fusion oncogene detection in 5 days.

Rebecca K. Voss, Maria Cardenas, Meiling Jin, Yen-Chun Liu, Alexa N. Siskar, Stephen V. Rice, Michael N. Edmonson, Jennifer L. Neary, Caleb Gallops, Jiali Gu, Jamie L. Maciaszek, Michael Rusch, Mark R. Wilkinson, David Rosenfeld, Priya Kumar, Jeffery M. Klco, Charles G. Mullighan, Jinghui Zhang, David A. Wheeler, Lu Wang

St. Jude Children's Research Hospital, Memphis, TN

Leukemia is the most common cancer in children, with ~75% being acute lymphocytic leukemia (ALL) and most of the rest acute myeloid leukemia (AML). More than half of pediatric acute leukemias are characterized by chromosome translocations, 40% of B-ALLs by ploidy changes, and a subset of AMLs harbor adverse cytogenetic aberrations defined by large-scale copy number changes (LS-CNV). Whole genome (WGS) and whole transcriptome sequencing (WTS) enable comprehensive molecular diagnosis, but often take weeks. We introduce a rapid workflow integrating WGS and WTS for tumor profiling, using advanced bioinformatic tools to evaluate LS-CNVs, tumor ploidy, and gene fusions, yielding results within 5 working days. Samples with >30% tumor content were used for WGS and WTS. We developed tumor-only WGS-based copy number profiling (WGS-CNP) using the Illumina DRAGEN CNV pipeline and an internal post processing analysis to generate genome-wide CNV results within 2-3 hours post-sequencing. Further on, we developed and automated an algorithm which compares test samples to a normal diploid reference, factoring in DNA gains/losses identified by WGS-CNV analysis to calculate DNA index (DI) for tumor ploidy estimates within minutes. We also implemented Fuzzion2, a rapid pattern-matching algorithm, to identify fusion transcripts from WTS data within ~1 hour. This integrated rapid approach was tested in a total of 117 AML and ALL cases previously diagnosed using standard WGS/WTS pipelines (CREST/CICERO/CONSERTING) and flow cytometry for DI, including 67 cases with characteristic fusion oncogenes, 40 ALLs defined by ploidy change, and 65 cases with LS-CNVs.

For gene fusion detection, Fuzzion2 identified all expected fusion oncogenes with no false positives, according to the established criteria. For LS-CNV, findings between WGS-CNP and the standard pipeline were concordant in all tested cases. For tumor ploidy, WGS-DI showed values concordant with Flow-DI in 33/40 cases. The 7 remaining cases were identified as masked low-hypodiploid by WGS-based CNV and B-allele frequency profiling, of which Flow-DI values were above 1.1 representing the actual tumor DNA content due to genome doubling. When using post-genome doubling (4n) as the ploidy baseline to generate CNVs, the calculated WGS-DI was below 0.75, reflecting the original clone before genome doubling.

Our pilot studies show that the WGS-CNP reliably reproduced previously observed LS-CNVs, accurately established tumor ploidy changes and distinguished masked low-hypodiploidy from hyperdiploidy. Together with the implementation of Fuzzion2 algorithm for gene fusion detection, this integrated rapid approach may detect 80-90% of disease-defining genetic alterations in pediatric acute leukemia with high sensitivity and specificity within 5 days, dramatically expediting the diagnostic process using WGS and WTS.

#7179 Streamlined Glioma diagnosis from formalin-fixed paraffin-embedded (FFPE) tissue: One assay, a fraction of the cost and turnaround time.

M. L. Mimosa[1], J. T. Simpson[1], S. Patel[2], K. Boissinot[2], M. Tiab[2], R. Fattouh[1], R. M. Saleeb[1];
[1]University of Toronto, Toronto, ON, Canada, [2]St. Michael's Hospital, Toronto, ON, Canada

We developed a single diagnostic assay to detect Glioma biomarkers including whole chromosomal and gene copy number variations (CNVs), and single nucleotide variations (SNVs). CNVs and SNVs are traditionally detected using different large/high-throughput platforms that can only exist in big genomic facilities. This leads to very high molecular costs and long turnaround times. The current complex diagnostic algorithm creates delays in patient management while increasing inequity in healthcare as smaller non-centralized labs are unable to conduct glioma molecular testing. To streamline glioma diagnosis and address these challenges, we aimed to use a third-generation sequencing platform called nanopore sequencing. It can simultaneously detect both CNVs and SNVs using small inexpensive tools, but is not yet compatible with formalin-fixed paraffin-embedded (FFPE) DNA.

Therefore, we developed a PCR based assay to create clean nanopore compatible FFPE DNA copies to detect CNVs and SNVs. We established a single nucleotide polymorphism (SNP) microarray-based approach to detect the CNVs. Approximately 300 amplicons were included in the assay design including unique SNP-rich areas to detect the chromosomal heterozygosity. We included all glioma markers required by World Health Organization's (WHO) guidelines including chromosomal CNVs on chr 1, 7, 10, 19; gene CNVs on *CDKN2A, CDKN2B*, *EGFR*; and SNVs on *IDH1, IDH2*, *TERT* promoter, *TP53, ATRX, H3-3A* and *H3C2*. We optimized the testing conditions, and reduced cost and turn-around including streamlining the data analysis using a custom shell script. Lastly, we conducted a mini validation as well as a cost and turn-around time analysis using 40 Glioma FFPE samples.

All our samples had a concordant molecular classification status with the reference results. The concordance, sensitivity and specificity (total accuracy) of the assay were all at 100%. A loss status of chr 1p, chr 19q and chr 10 were determined via a loss of heterozygosity (LOH), and gain status of chr 7 was observed as an allele gain 2:1 SNP pattern. Our total turnaround time (from DNA extraction to data analysis) is 3 business days for an entire batch of samples, which is at least 1/3rd the turnaround time of conventional methods. Our material cost is $150 CAD/sample (including DNA extraction, library preparation and sequencing) which is ~10% of the existing assay costs.

This is the first single streamlined diagnostic test to detect CNVs and SNVs in Gliomas using FFPE DNA. Our assay will increase healthcare equity by allowing smaller labs to adopt molecular testing due to its low assay/capital cost, shorter testing time, and streamlined workflow, helping to overcome many of the existing cancer diagnostic challenges.

#7180 DNA methylation profiling of peripheral blood mononuclear cells from small-cell lung cancer patients treated with hypomethylating agent plus carboplatin.

Elnaz Abbasi Farid[1], Zhen Fu[2], Collin M. Coon[1], Shadia I. Jalal[3], Kenneth P. Nephew[1]

[1]Indiana University School of Medicine, Bloomington, IN,[2]Van Andel Institute, Grand Rapids, MI,[3]Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, IN

Small-cell lung cancer (SCLC), representing 15% of lung cancers, is one of the deadliest malignancies, with a 5-year survival rate under 7%. Despite initial sensitivity to platinum-based chemotherapy, rapid recurrence and treatment resistance remain hallmarks. Epigenetic modifications, particularly DNA methylation, play a critical role in tumor heterogeneity and resistance. Guadecitabine, a hypomethylating agent (HMA), has shown potential when combined with carboplatin. Building on these findings, we conducted a phase II single-arm trial to evaluate this combination as a second-line treatment for extensive-stage SCLC (ES-SCLC). Peripheral blood mononuclear cells (PBMCs) serve as a minimally invasive source to study systemic epigenetic changes, reflecting tumor-specific modifications. To investigate therapy-associated methylation changes, we analyzed PBMCs from 31 patients on NCT0391345 clinical trial testing guadecitabine and carboplatin: 20 paired pre- and post-treatment samples, 6 pre-treatment samples, and 5 post-treatment samples. DNA was extracted using the DNeasy Blood and Tissue Kit, quantified with NanoDrop™ and Qubit spectrophotometry, bisulfite-converted, and analyzed using the Infinium HumanMethylationEPIC v2.0 platform. Data were processed with SeSAMe to generate β-value matrices. Differentially Methylated Positions (DMPs) were identified using a β-value cutoff of 0.2 and an adjusted $P < 0.05$. Functional analysis of significant DMPs was conducted via Ingenuity Pathway Analysis (IPA).Guadecitabine treatment induced 334 hypomethylated and 9 hypermethylated CpG loci, with pathway analysis revealing significant changes in SCLC and NF-kappa B signaling. Key pathways included Rho GTPase signaling (metastasis), pulmonary fibrosis signaling (lung remodeling), p75 NTR signaling (cell survival), and several other signaling pathways (ID1, MYC mediated apoptosis, immunogenic cell death). Deconvolution analysis showed decreased B cells, NK cells, monocytes, eosinophils, and a trend toward reduced CD4+ and CD8+ T cells post-treatment. Conversely, neutrophils significantly increased, suggesting an inflammatory shift in the tumor microenvironment. These findings demonstrate that guadecitabine treatment induces significant CpG methylation changes in PBMCs, impacting genes and pathways critical to cellular signaling, tissue remodeling, and survival mechanisms. The identification of key pathways, including those involved in metastasis, lung remodeling, and tumor cell survival, highlights the potential role of HMAs in modulating epigenetic and biological processes relevant to SCLC progression and therapy.

#7181 Gene enhancers as novel rationale therapeutic targets for impeding colorectal cancer progression.

Flavia Genua[1], Xinxin Shao[2], Ellen Finnegan[2], Christina Caragine[3], Sarah Maguire[4], Paulien Van Minsel[5], Grainne Kirwan[1], Bernard Thienpont[5], Neville Sanjana[3], Weipeng Wang[6], Sudipto Das[1]

[1]Department of Pharmacy and Biomolecular Sciences, Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland,[2]Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland,[3]Department of Biology, New York University, New York, NY,[4]Patrick G Johnston Centre for Cancer Research, Queen's University, Belfast, United Kingdom,[5]Department of Translational Genetics, VIB, KU Leuven, Belgium,[6]Soochow University, Soochow, China

Half of of colorectal cancer (CRC) patients develop metastatic CRC (mCRC) with a low survival rate. Epigenetic abnormalities in promoters and enhancers play a significant role in CRC advancement and hold promise for diagnosis and therapy and over 5,000 variable enhancer loci (VELs) have been shown to be gained/lost in mCRC. Here, the functional impact of these VELs on driving CRC progression was explored. We designed a CRISPRi library including 1,524 VELs gained or lost specifically in CRC cell lines, the top 500 essential genes for the cell line as positive controls, 1,000 non-targeting guides as negative controls and 6 sgRNAs per site, cloned into a lentiviral plasmid. CRISPR screen was applied to the HCT116-dCas9-KRAB-MeCP2 cells for NGS. Anticancer potentials of silenced VELs were evaluated by in vitro assays (i.e MTT assay, apoptosis, cell cycle assay, 3D bioprinting). Chromosomal interactions and epigenetic state were analyzed by Chromosome conformation capture (3C) coupled with sequencing (Hi-C), chromatin immunoprecipitation (ChIP) with massively parallel DNA sequencing (ChIP-seq) and RNA sequencing. We identified 12 depleted VELs causing a significant decrease in HCT116 proliferation after CRISPR-mediated silencing. VEL_1243 and VEL_1084 caused the highest level of cell growth inhibition as well as increased apoptosis and cell cycle arrest in HCT116 cells. After silencing both enhancers, 3D bioprinting showed a reduction in key markers of metastasis. HiC-Seq and transcription factor binding data showed long-range interaction of both enhancers with genes such as MYO3B, UBR3 and GINS3 and enrichment for disease-associated TF including RFX7, and ELF3. RNA seq results showed significant transcriptome-wide expression alterations regulated by VEL_1243 and VEL_1084, exemplified by their impact on disease-associated signaling pathways including PI3K-Akt and MAPK. IFNγ response pathway -which is a critical aspect of anti-tumor immunity - was downregulated following both VEL_1243 and VEL_1084 silencing. Given that immune/inflammatory cells (e.g., tumor-associated macrophages (TAMs), T-cells, natural killer (NK) and cancer-associated fibroblasts (CAFs) are the important components of the tumor microenvironment (TME), we further examined if VELs mediated regulation of TAMs or immune cells controls the TME in a way that promotes tumour growth. M1 were co-cultured with CRC cells knock-down of VELs. Both VELs repression could induce M1 polarization in HCT116 and SW620 cells. Considering M1 macrophages have the capability to promote the Th1 response and engulf and kill target tumor cells, VEL_1084 and VEL_1243 could be potential therapeutic targets for CRC by affecting the polarization of macrophages. VEL_1243 and VEL_1054 regulate CRC-associated phenotypes and warrant further exploration to determine their regulatory role and potential as a novel therapeutic target in CRC.

#7182 EZH2 inhibition as a therapeutic strategy for castration resistant prostate cancer in combination with AR-antagonist enzalutamide.

Shikhar Sharma[1], Jelena Petrovic[1], Sara Linker[1], Whijae Roh[1], Joan Cao[1], Sean Uryu[1], Zhenxiong Wang[1], Yong Zhang[1], Colin A. Flaveny[1], Conglin Fan[1], Tao Xie[1], Kimberly H. Kim[1], Xiaoli Wang[1], Robert A. Rollins[1], Gail P. Risbridger[2], Renea A. Taylor[2], Mitchell G. Lawrence[2], Thomas A. Paul[1], Pei-Pei Kung[1]

[1]Pfizer, Inc., San Diego, CA,[2]Monash Biomedicine Discovery Institute, Monash University, Melbourne, Australia

The histone methyltransferase EZH2 is an epigenetic transcriptional repressor which mediates gene silencing through methylation of H3K27 residues. EZH2 is overexpressed in castration-resistant prostate cancer (CRPC) and has been associated with prostate cancer progression and resistance to anti-androgen therapies. We have developed a potent, selective, SAM-competitive EZH2 inhibitor, PF-06821497 (Mevrometostat). Using genetic and pharmacological approaches, we demonstrate an epigenetic dependency in CRPC to EZH2 inhibition. EZH2 was identified as a hit in a genome-wide CRISPR screen in AR-positive, but not in AR-negative, CRPC models and was further confirmed in independent CRISPR-competition assays. Consistent with genetic data, long-term PF-1497 treatment showed selective anti-tumor activity in AR-positive CRPC cell lines. Anti-tumor activity of PF-1497 was further validated in vivo with dose-dependent tumor growth inhibition observed in correlation with H3K27me3 biomarker inhibition in CRPC xenograft models. EZH2 inhibition induced cell cycle arrest concomitant with transcriptional upregulation of tumor suppressor genes. Interestingly, we found a subset of genes induced upon enzalutamide treatment are also upregulated upon PF-1497 treatment. Transcriptional and epigenetic profiling revealed that genes repressed by AR show enrichment for H3K27me3 indicating a co-repressor function for EZH2. Combination in vitro treatment of PF-1497 with enzalutamide showed synergistic upregulation of such co-repressed genes associated with robust inhibition of proliferation, survival, cell cycle and stem cell pathways. This transcriptional synergy was associated with robust in vivo anti-tumor combination activity of PF-1497 with enzalutamide inducing durable tumor regressions. Similar combination benefit of PF-1497 and enzalutamide was observed across a panel of CRPC PDXs with anti-tumor activity enriched in AR-positive prostate cancer models, including tumors from patients who were refractory to anti-androgen therapies. Taken together, these data highlight the promise of EZH2 inhibition in treating prostate cancer and provide support for ongoing clinical study of PF-06821497 in combination with enzalutamide in mCRPC.

#7183 METTL1-mediated m7G modification promotes 5-FU resistance in colorectal carcinoma.

Silei Sui[1], Yuan Li[1], Rui Su[1], Man Li[2], Ajay Goel[1]

[1]Beckman Research Institute of The City of Hope, Monrovia, CA,[2]The Second Affiliated Hospital of Dalian Medical University, Dalian, China

Background: Colorectal cancer (CRC) ranks among the most common malignancies worldwide. Despite 5-fluorouracil (5-FU) being a cornerstone of CRC therapy, resistance to this chemotherapeutic agent occurs in approximately 50-60% of patients, significantly contributing to poor prognosis. Emerging studies have highlighted the role of METTL1, a methyltransferase mediating N7-methylguanosine (m^7G) modifications, in promoting tumor progression and drug resistance. However, the specific regulatory mechanisms by which METTL1 contributes to 5-FU resistance in CRC remain poorly understood.

Methods: METTL1 expression was analyzed in paired tumor and adjacent normal (AN) tissue specimens in publicly available datasets. Validation of METTL1 dysregulation in CRC tissues and cell lines was performed using qRT-PCR and western blotting (WB), while m^7G levels were assessed by northwestern blot. Functional assays, including cell viability, colony formation, apoptosis, and invasion assays, were conducted to evaluate the role of METTL1 in CRC. RNA sequencing (RNA-seq) and m7G Methylated RNA Immunoprecipitation sequencing (MeRIP-seq) were used to identify downstream targets of METTL1 associated with chemoresistance.

Results: METTL1 was significantly overexpressed in CRC tissues compared to AN tissues ($p<0.001$), with high METTL1 levels correlating with unfavorable clinical outcomes in both public datasets and our clinical cohort ($p<0.05$). Biologically, genetic depletion of METTL1 via CRISPR-Cas9 markedly led to reduced cell proliferation and enhanced apoptosis after 48 hours of 5-FU treatment in HCT116 and SW480 5-FU resistant cell lines. *In vivo*, METTL1 knockdown significantly enhanced the efficacy of 5-FU in a xenograft mouse model, leading to decreased tumor growth. Mechanistically, METTL1-mediated m^7G methylation influenced the translation of mRNAs, including those involved in the TNF and NOTCH1 signaling pathways, both of which are critical in driving 5-FU resistance.

Conclusion: This study identifies METTL1-mediated m^7G modifications as a pivotal mechanism underlying 5-FU resistance in CRC. By regulating the translation of components in the NOTCH1 signaling pathway, METTL1 drives chemoresistance, presenting a potential therapeutic target to enhance the effectiveness of 5-FU-based treatment regimens in CRC patients.

#7184 Novel role of INCB057643, a bromodomain and extra-terminal (BET) protein inhibitor, in myeloid cell regulation and immunosuppressive tumor environment remodeling in myelofibrosis (MF).

Gaurang Trivedi[1], Pat Feldman[2], Natalia Pardo[3], Erin Crowgey[2], Brittney Wass[1], Hamza Celik[1], Betty Lamothe[2], **Diana Alvarez Arias**[2]

[1]Incyte Research Institute, Wilmington, DE,[2]Incyte Corporation, Wilmington, DE,[3]Discovery and Translational Science (DTS), Clarivate Plc, Barcelona, Spain

Dysregulated epigenetic gene expression is a cancer hallmark. BET proteins can bind to acetylated chromatin to regulate transcription factor activity (eg, NF-kB), leading to overproduction of inflammatory cytokines (Kleppe. *Cancer Cell*. 2018). The BET inhibitor (BETi), INCB057643, has been assessed in MF and other cancers (NCT02711137; NCT04279847 [Study 103]). Although NFkB inhibition is proposed as one of the key mechanisms of BETi-mediated activity in MF, the mechanism of BETi is unresolved. This study assessed cellular and molecular mechanisms of BETi in MF. Total RNAseq was performed on whole blood from commercial MF samples treated ex vivo with INCB057643 and samples from patients (pts) enrolled in Study 103 (dose escalation, monotherapy). Differential gene expression (DGE) and gene set enrichment analysis (GSEA) were performed. Plasma biomarkers were analyzed using HuMAP 2.0 (IQVIA^{TM} panel). The impact of INCB057643 on ex vivo differentiation of pt-derived CD34^{+} cells expressing MPN driver mutations was tested at clinical doses. INCB057643 inhibited pathogenic megakaryopoiesis by reducing megakaryocyte (MK) differentiation and proinflammatory cytokine levels, including neutrophil (etc) chemo-attractants, IL-8 and ENA-78. Although IL-8 was produced at similar levels by wild-type and mutant MK progenitors, ENA-78 was produced only by mutant cells. Both cytokines were inhibited by INCB057643. Contrasting with MK, the proportion of erythroid cells was increased. RNAseq of clinical samples showed INCB057643 dose-dependent effects on gene expression. DGE analysis showed differential expression of epigenetic genes, and genes involved in platelet biogenesis; both are known BET functions. DGE analysis of a myeloid gene set representing monocytes, macrophages, dendritic cells, and neutrophils showed dose-dependent decreases in functional genes (*MGST1*, *IDO2*) and immunosuppressive protumorigenic genes (*MARCO*, *SIGLEC11*), but representative T- or B-cell gene expression was either unchanged or elevated (*PRF1*). Consistent with DGE, INCB057643 inhibited proinflammatory cytokine production (eg, RANTES, IL-18 produced by myeloid cells) as well as ENA-78, suggesting reduced inflammatory cell trafficking in bone marrow. Increased expression of MYC is associated with MPN progression; DGE and GSEA showed that hallmark *MYC* targets signatures were reduced by INCB057643. In summary, INCB057643 decreased malignant MK precursors that drive MF. Although data confirmed INCB057643 regulates epigenetic gene expression globally, selective inhibition of myeloid and MK cells, but not other lineages, was observed. INCB057643 inhibited myeloid cells by downregulating immunosuppressive receptors and proinflammatory cytokines, leading to tumor microenvironment remodeling and reduced inflammation.

#7185 Optimization of an epigenetic based combination platform for patients with peripheral t cell lymphoma.

Kallesh D. Jayappa[1], Andrea Joyner[2], Archana Thakur[1], Owen A. O'Connor[1]

[1]Department of Medicine, Division of Hematology/Oncology; Comprehensive Cancer Center, University of Virginia School of Medicine, Charlottesville, VA,[2]Department of Medicine, Division of Hematology/Oncology, University of Virginia School of Medicine, Charlottesville, VA

Introduction: Our previous work demonstrated that combinations of HDAC and DNMT3 inhibitors were synergistic in preclinical models of T-cell lymphoma, and produced compelling activity in patients with relapsed or refractory disease. This combination was identified based on high-throughput chemical screening seeking to identify synthetically lethal combinations in a disease directed fashion. Here, we present a similar approach to rationally identify a third drug to complement the epigenetic-based combinations established to date.

Results: First, we optimized the schedule of an HDACi [romidepsin, (Romi)] and a DNMT3i [5-azacytidine, (Aza)] across T-cell lymphoma lines. Results revealed that simultaneous exposure to Aza+Romi exhibited superior synergistic cytotoxicity compared to all other schedules tested *in vitro*. Using this schedule, we screened a library of drugs for synergy with the cornerstone combination of Aza + Romi. These drugs included agents targeting HMTs, KDMs, SWI/SNF, BET, CDK9, JAK, PI3K, Bcl-2, Bcl-xL, and Mcl-1. The combination drug screen was performed in three representative PTCL lines [H9 (CTCL), SMZ1 (PTCL-NOS), and FEPD (ALCL-ALK^{NEG})] using cell proliferation assay. The synergy between drugs was determined based on excess over Bliss (EOB) scores established using the *Bliss* model of independence (EOB ≥10, synergy; EOB ≥0-10, additive). The results revealed potent synergy between Aza+Romi and drugs targeting HMTs [PRMT5i (mean EOB, 18.5), EZH1/2i (mean EOB, 9.0)], SWI/SNF [SMARCA2-SMARCA4-PBRM1-degrader (mean EOB,15.1)] and kinases [JAK1i (mean EOB, 17.6), PI3Ki (mean EOB, 15.5)]. In further characterization of schedule dependent activity, we noted that preexposure to PRMT5i, EZH1/2i, SWI/SNFi, or JAK1i followed by Aza+Romi treatment produced superior synergy in PTCL cells *in vitro*. The flow cytometric analysis revealed that these combinations induced apoptotic cell death in PTCL cell lines *in vitro* and these drug combinations, except Aza+Romi with SWI/SNFi and PI3Ki, exhibited a broad therapeutic window inducing preferential cell killing in the PTCL lines (cleaved PARP, 66-80%) compared to healthy PBMCs (cleaved PARP, 27-36%). Ongoing studies will decipher mechanisms of action using RNA-Seq and explore the ideal combinations in *in vivo* model of PTCL.

Summary: The development of combination therapy for PTCL patients has been largely predicated on random ad hoc combinations. We describe herein a rational approach to the optimization of a new backbone for the disease. Simultaneous exposure of Aza and Romi coupled to a preexposure to drugs targeting PTMT5, EZH1/2, and JAK1 inhibitors produced potent synergy with a broad therapeutic window. We believe our ongoing investigation on these combinations will soon lead to the development of a new, chemotherapy free combination tailored to treat PTCL.

#7186 Anti-cancer activity of potent and selective EP300 degradation in hematological malignancies.

Claudia Dominici, Qianhe Zhou, Laura La Bonte, Mark Zimmerman, Mei Yun Lin, Wesley Austin

Foghorn Therapeutics, Cambridge, MA

E1A binding protein (EP300) and CREB binding protein (CBP) are highly homologous acetyltransferase paralogs with demonstrated roles as transcriptional co-activators. Dual inhibition of EP300 and CBP has been reported to have anti-proliferative effects in several cancer types. However, it has also been reported to elicit thrombocytopenia, a toxic reduction in the number of platelets in the blood (Nicosia et al., 2023). Hematological malignancy lineages are particularly susceptible to EP300 loss (DepMap, Broad 2024), therefore, developing chemical matter that selectively targets EP300 has the potential to broaden the therapeutic window and improve tolerability. Historically, selectivity has been difficult to engineer due to the high sequence similarity between EP300 and CBP. Using the targeted degradation approach, we have developed a potent and selective heterobifunctional degrader of EP300 with biological activity in hematological malignancy. We observed that selective degradation of EP300 has anti-proliferative activity in a broad range of hematological malignancies in vitro, including diffuse large B-cell lymphoma, multiple myeloma, and follicular lymphoma. We also achieved in vivo efficacy in a Karpas422 GCB-DLBCL xenograft model. Importantly, an investigational safety study was performed in mice at efficacious doses, and no thrombocytopenia was observed. Therefore, leveraging targeted protein degradation to achieve selective EP300 degradation shows promise to be a well-tolerated and effective treatment strategy for advanced hematological malignancies.

#7187 Epigenetic drugs inhibit the oncogenic activity of methyl CpG binding protein 2 (MECP2).

Manish Neupane[1], **Mukesh Kumar**[1], Malela M. Werner[1], Colleen M. Donnelly[1], Alsu Ibragimova[1], Brian Curcio[2], Inna Chervoneva[2], Irina Vasilevskaya[1], Daniel P. Silver[3]

[1]Department of Medical Oncology, Thomas Jefferson University, Philadelphia, PA,[2]Division of Biostatistics, Department of Pharmacology and Experimental Therapeutics, Thomas Jefferson University, Philadelphia, PA,[3]Sidney Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA

Purpose of the study: MECP2 amplification plays a role similar to activated oncogenic RAS in tumorigenesis, activating the MAPK and PI3K signaling pathways. MECP2 amplification and RAS mutations are mutually exclusive across human cancers; solid tumors such as high-grade serous ovarian cancer (HGSOC), triple-negative breast cancer (TNBC) and lung adenocarcinoma have 38%, 33% and 29% rates of MECP2 amplification respectively. The MECP2 protein has DNA- binding activity which is required for oncogenic transformation and binding is dependent on cytosine methylation. Therefore, MECP2 DNA binding may be targeted by inhibiting cytosine methylation with DNMTi. Additionally, since MECP2 protein requires HDAC activity for its stability, treatment with either HDACi and/orDNMTi could be therapeutic strategies in MECP2- dependent tumors.

Experimental Procedures and Results: The MDAMB468 (TNBC) cell line was modified stably with a doxycycline-inducible shRNA construct targeting the 3'UTR of MECP2 (shMECP2-3'UTR) and growth was dependent on MECP2 in culture. The cells were then implanted in nude mice subcutaneously and tumors allowed to reach approximately 150 mm^3 before shRNA was induced. Tumor growth in doxycycline-treated mice immediately ceased whereas tumors in vehicle-treated mice continued to grow, showing dependence on MECP2 activity *in vivo*. To verify that this was an on-target effect, the modified cell line was further infected with lentiviruses carrying both splicing isoforms of MECP2 without the 3'UTR shRNA target. This reconstitution allowed growth as a xenograft at a similar rate regardless of shRNA induction, confirming inhibition was on-target. We then assessed the therapeutic efficacy of HDACi and DNMTi alone and in combination in xenograft (for TNBC and lung) or PDX (HGSOC) models of MECP2-dependent and independent tumors. To determine if growth inhibition was an on-target effect of these drugs, MDAMB468 was stably transduced with activated KRAS or GFP as a control and was injected in nude mice. Activated KRAS rescued the epigenetic drug sensitivity of xenografts, demonstrating that these drugs inhibit tumor growth by preventing the ability of MECP2 to induce RAS-activated growth factor pathways.

Summary: The antitumor effects of the commonly used epigenetic drugs are thought to result from cell reprogramming through re-expression of tumor suppressors that had been silenced by hypermethylation or by repressive chromatin marks. However, in this context, we show that these epigenetic drugs can act directly by interfering with the oncogenic activities of MECP2, culminating in inhibition of key growth factor pathways for transformation.

Conclusion: Targeting MECP2 function with epigenetic drugs provides an unanticipated therapeutic opportunity for treating the subset of tumors that overexpress MECP2.

#7188 INCB057643, a bromodomain and extra-terminal (BET) protein inhibitor, improved bone marrow function and shifted megakaryopoiesis to erythropoiesis in patients with myeloproliferative neoplasms (MPNs).

Hamza Celik[1], Angelo Albertoni[1], Brittney Wass[1], Sarita Sehra[1], Lea Burke[2], Melody Diamond[1], Matthew Stubbs[1], **Diana Alvarez Arias**[1]

[1]Incyte Research Institute, Wilmington, DE,[2]Incyte Corporation, Wilmington, DE

Patients with MPNs share common characteristics, including overproduction of myeloid cells and dysplastic megakaryocytes (MK) in bone marrow. Aberrant cell differentiation results in chronic inflammation, development of bone marrow fibrosis, and ineffective hematopoiesis, often leading to anemia. Dysregulated gene transcription can drive these disease characteristics (AACR 2023; Abs 6274). BET proteins are key epigenetic factors of oncogene transcription. INCB057643, a novel, orally bioavailable BET inhibitor, is being evaluated in clinical trials as monotherapy and in combination with ruxolitinib in patients with MPNs (NCT02711137; NCT04279847). To understand the INCB057643 mechanism of action including MK differentiation and biomarkers of inflammation and fibrosis, we used a physiologically relevant patient-derived mouse xenograft (PDX) model of myelofibrosis transplanted with patient $CD34^+$ JAK2-$V617F^+$ cells. MK and erythroid progenitors in bone marrow were quantified by flow cytometry for CD61 and CD71 markers, respectively. Reticulin staining was used to assess fibrosis. INCB057643 treatment elicited a robust decrease in JAK2 V617F+ hematopoietic stem progenitor cells and MK in bone marrow. Concordant with depletion of pathogenic cells, a significant reduction in serum proinflammatory cytokines and fibrosis was observed in treated mice. Bone marrow samples from patients enrolled in NCT04279847 (monotherapy) were analyzed for CD61 and CD71 by immunohistochemistry. Patient plasma biomarker analyses were performed using HuMAP 2.0 (IQVIA^{TM} panel); total RNAseq was performed on whole blood samples. Consistent with the preclinical models, CD61 staining was decreased in bone marrow after INCB057643 treatment. Moreover, tight MK clusters changed into looser structures with increased distance between MK cells; dysplastic MK morphology changed to a more normal appearance in some patients. Fibrosis score decreased ≥1 grade in 3/5 patients receiving INCB057643 10 mg qd. Moreover, expression of soluble biomarkers and genes associated with fibrosis, such as *TIMP3* and *Col4A2*, were decreased, while adiponectin expression in plasma was increased, especially at ~week 12, suggesting improved bone marrow function. Notably, erythroid progenitors in patient bone marrow were increased at both doses tested (6 mg qd, n=3; 10 mg qd, n=5). Although all patients who received INCB057643 10 mg qd had anemia at screening, treatment resulted in hemoglobin response in 3/5 patients. In conclusion, the results show that INCB057643 specifically inhibits pathogenic MK differentiation, reduces inflammatory cytokine production, and decreases fibrosis. INCB057643 treatment also improves bone marrow function, which results in increased erythroid differentiation and reduced anemia symptoms.

#7189 EED inhibitor APG-5918 renders vulnerability to immunotherapy by rewiring sphingolipid metabolism via nSMase2-ceramide pathway in pancreatic cancer.

Jing Yang[1], Fan Chen[1], Qiuyun Luo[2], Mingyu Lai[1], Jiachen Lu[1], Dajun Yang[3], Miaozhen Qiu[1]

[1]Department of Medical Oncology, Sun Yat-sen University Cancer Center (SYSUCC), Guangzhou, China,[2]The Third Affiliated Hospital, Sun Yat-Sen University, Guangzhou, China,[3]Department of Experimental Research, Sun Yat-sen University Cancer Center (SYSUCC), Guangzhou, China

Background: Pancreatic cancer has a poor prognosis and is primary resistant to immune checkpoint inhibitors (ICIs) partially due to the inhibitory immune microenvironment. To improve the responsiveness of pancreatic cancer to ICIs, researchers have explored various combination therapies, including the use of epigenetic inhibitors. Embryonic Ectoderm Development (EED), a regulatory component of the PRC2 histone modification complex, has emerged as a target of interest. This study aims to investigate the potential effects of combining EED inhibition with PD-1 blockade in treating pancreatic cancer.

Methods: Allograft models of murine pancreatic cancer cell lines, KPC and Panc02, were used to evaluate the combined anti-tumor effects of PD-1 antibody and a novel EED inhibitor, APG-5918. To determine how EED inhibition and PD-1 blockade influenced immune responses, *in vivo* tumor samples underwent single-cell sequencing and flow cytometry to assess immune cell infiltration and gene expression changes. Additionally, lipidomic metabolic sequencing and ELISA were used to measure changes in ceramide levels *in vitro* following EED or SMPD3 inhibition. Macrophage polarization phenotypes were further characterized using RT-qPCR and flow cytometry in the human monocyte lines THP1 and Raw264.7.

Results: Our study demonstrated that the EED inhibitor APG-5918 synergizes with PD-1 antibody to exert anti-tumor effects in immune-competent murine pancreatic cancer allograft models. Single-cell sequencing of mouse tumor tissues revealed, and subsequent flow cytometry confirmed, a significant increase in M1 macrophages and CD8+ T cells in tumors from the combination treatment group compared with other groups. Transcriptomic analysis showed that EED inhibition significantly upregulated SMPD3 expression, which encodes the ceramide-producing enzyme nSMase2, and enriched the sphingolipid metabolic pathway. Quantitative lipidomic analysis revealed an increase in ceramide C16 levels in KPC and Panc1 cells treated with APG-5918, and ELISA confirmed that ceramide levels could be restored following treatment with the SMPD3 inhibitor GW4869. Furthermore, EED knockdown or supplementation with exogenous ceramide significantly promoted macrophage polarization toward the M1 phenotype in the human monocyte lines THP1 and Raw264.7 *in vitro*.

Conclusions: Taken together, our results demonstrate that the novel EED inhibitor APG-5918 can reshape the tumor immune microenvironment by activating the nSMase2-ceramide pathway, overcoming immunotherapy resistance in pancreatic cancer. This promising approach, combining EED inhibition with PD-1 blockade, will be further explored in translational and clinical studies.

#7191 Targeted genomic and epigenomic dual analysis of the same region for an entire single gene.

Grace Q. Zhao[1], Heng Wang[1], Alexandra Harris[2], Yingmin Wang[1], Yingzhen Wang[1], Ling Su[3], Ashraf Wahba[1], Josh Wang[4], Yun Bao[1], Carole Durand[1], Bram Herman[5], Tiffany Dorsey[2], Shengrong Lin[1], Xiaolin Wu[3], Stefan Ambs[2]

[1]Agilent Technologies, Inc., Santa Clara, CA,[2]Molecular Epidemiology Group, Laboratory of Human Carcinogenesis, National Cancer Institute, Bethesda, MD,[3]Cancer Research Technology Program, Frederick National Laboratory for Cancer Research, Frederick, MD,[4]Agilent Technologies, Inc., Annapolis, MD,[5]Agilent Technologies, Inc., Harwell, United Kingdom

Omics analysis for cancer research is increasingly focusing on integrating both genomic and epigenomic data. While most studies often limit methylation analysis to promoter regions and genomic analysis to exons, research suggests the importance of examining omics information for entire genes, including UTRs and introns. Current whole-genome technologies for simultaneous genomic and epigenomic analysis require costly deep sequencing for low tumor content and may suffer from limited coverage in high CG content regions. To address these challenges, we developed a novel strategy that enables simultaneous genomic alteration and methylation dual analysis of the same region within a single gene, offering an affordable solution with improved uniformity, including high CG regions. ARID1A, a critical component of the SWI/SNF complex, is frequently mutated in breast cancer. Low ARID1A expression is associated with poor outcomes in luminal A and HER2-positive subtypes, making it an ideal candidate for investigating genomic alterations and potential methylation-related gene inactivation. To achieve this, we utilized the novel strategy for simultaneous genomic and epigenomic analysis using the Agilent Avida Duo target enrichment system. This approach allowed us to sequentially capture and analyze both genomic and methylation information across the same promoter, intron, and exon regions of the entire gene from a single DNA input without sample splitting. Moreover, the sequencing depth can be independently optimized for each modality. To optimize sequencing budget and coverage uniformity, we divided the panels (90kb) into high-quality and low-complexity regions, primarily introns, and experimented with different panel mixture ratios. With 10ng of fragmented gDNA, we achieved good coverage for both genomic (>1000x unique molecules) and methylation (>100x unique molecules) analysis, using less than 5M PE reads (sequencing clusters) for each omics in the Avida Duo workflow. For uniformity, the percentage of regions with coverage greater than 0.2x of the mean coverage is >90%. Our pilot studies, using tumor genomic DNA, formalin-fixed paraffin-embedded (FFPE) tissue from various cancer types, and cell-free DNA (cfDNA) control samples, successfully
demonstrated the feasibility of our approach for simultaneous targeted genomic and epigenomic analysis of the same region within the entire single gene, even with limited DNA input.
For Research Use Only. Not for use in diagnostic procedures.

CLINICAL RESEARCH: Targeted Therapies and Combinations 4
Poster Session

#7195 Real-world patient characteristics, treatment patterns, and healthcare resource use in patients with metastatic melanoma (mMEL) treated with BRAF-MEK inhibitor targeted therapies (TTS) in the US.

Gino K. In[1], **Kristina Chen**[2], Gautam Sajeev[3], Mu Cheng[3], Jingyi Chen[3], Joanne Chukwueke[3], Jessie J. Lan[3], James Signorovitch[3]

[1]Keck School of Medicine, University of Southern California, Los Angeles, CA,[2]Pfizer, Inc., New York, NY,[3]Analysis Group, Inc., Boston, MA

Background: Real-world evidence on treatment patterns and healthcare resource use (HRU) in patients receiving the targeted therapies encorafenib+binimetinib (ENCO+BINI) and dabrafenib+trametinib (DAB+TRAM) in mMEL is limited.

Methods: Patients initiating ENCO+BINI or DAB+TRAM between June 2018 and March 2023 as first-line (1L) or second-line (2L) treatment for mMEL in the IQVIA Pharmetrics® Plus commercial claims database were included. Patients judged to have received neoadjuvant or adjuvant therapy or treatment for another cancer prior to TT initiation (index date) were excluded. Treatment discontinuation and melanoma-specific HRU were compared between TTs, in 1L and 2L separately. Analyses were adjusted for age, sex, region, index year, time from mMEL diagnosis to index, sites of metastases, NCI score, baseline all-cause HRU and prior immunotherapy (2L analysis only).

Results: In 1L, at baseline, compared to the DAB+TRAM group (n=137), the ENCO+BINI group (n=117) was younger (mean age: 50.6 vs. 54.9 years) and had more patients with lymph node (63.2% vs 49.6%) or bone/bone marrow metastases (28.2% vs 17.5%). In 2L, the ENCO+BINI group (n=60) had higher melanoma-specific emergency room (ER) and outpatient (OP) visit rates than the DAB+TRAM group (n=56) during the baseline period. Treatment discontinuation for ENCO+BINI relative to DAB+TRAM was significantly lower in 1L (adjusted hazard ratio with 95% confidence interval: 0.70 [0.51, 0.96]), and numerically lower in 2L (0.69 [0.43, 1.12]). More ENCO+BINI patients remained on treatment through 12 months (36% vs 21% in 1L; 39% vs 26% in 2L) and 24 months (20% vs 9% in 1L; 27% vs 15% in 2L).Melanoma-specific inpatient (IP) admissions for ENCO+BINI relative to DAB+TRAM were significantly lower in 1L (21% versus 30% with IP admissions in IL; incidence rate ratio [IRR]: 0.45 [0.29, 0.70]) and numerically lower in 2L (37% vs 50% with IP admissions in 2L; IRR: 0.73 [0.43, 1.22]). Rates of OP visits were significantly lower for ENCO+BINI in 1L (IRR: 0.83 [0.77, 0.89]) but significantly higher in 2L (IRR: 1.33 [1.21, 1.46]). There were no differences between the groups on rates of ER visits in either 1L or 2L.

Conclusion: ENCO+BINI was associated with better treatment persistence, lower IP admissions, and lower OP visits in 1L compared to DAB+TRAM, while OP visits for ENCO+BINI were higher than for DAB+TRAM in 2L. This may suggest potential differences in effectiveness or tolerability between TT regimens across treatment settings in this mMEL population. Further contextualization of reasons for resource use in these patients warrant exploration in future studies to help understand their clinical and economic implications.

#7196 Synergistic effect of sotorasib plus trametinib in patients with KRAS^{G12C}-mutated metastatic colorectal cancer.

Silei Sui[1], Marwan Fakih[2], Yuan Li[1], Caiming Xu[1], Ajay Goel[1]

[1]Beckman Research Institute of The City of Hope, Monrovia, CA,[2]City of Hope Medical Center, Duarte, CA

Background: KRAS is altered in approximately 40% of colorectal cancer (CRC), with the KRAS^{G12C} mutant (MUT) being particularly notable for its high prevalence in certain cancers that directly activates the MAPK pathway. Sotorasib, an irreversible, selective KRAS^{G12C} inhibitor, showed promising antitumor activity in *KRAS*^{G12C}-MUT colorectal cancer. However, despite recent FDA approval, the response rate of Sotorasib monotherapy in CRC remains below 10%. Herein, we aimed to investigate the synergistic efficacy for the combination of a MEK1 inhibitor (Trametinib) and Sotorasib in CRC.

Methods: We conducted an integrative analysis of genomic and transcriptomic data, comparing pretreatment cases and those obtained following the development of resistance to Sotorasib. Dose-response curves were generated to determine the IC_{50} values of Sotorasib and Trametinib in CRC cell lines with distinct mutational profiles: SW837 (KRAS^{G12C}) and SW1463 (KRAS^{G12C} and MEK1 MUT). To further investigate the effects of the combination compared to the monotherapy of each drug, we performed a series of functional assays by focusing on perturbations within the MAPK/ERK/AKT signaling pathway.

Results: Among the 27 patients analyzed, 10 were identified with putative resistance phenotype, of which 9 (37%) had acquired MEK1 mutations or amplifications. The Kaplan-Meier analysis for overall survival in CRC patients harboring wild-type and mutant MEK1. Functional analysis demonstrated a synergic effect of combined Sotorasib and Trametinib treatment in SW837 and SW1463 CRC cell lines. In MTT assays, the combination therapy significantly enhanced cytotoxicity compared to either monotherapy alone. Similarly, colony formation assay revealed a marked reduction in the proliferative capacity of CRC cells when treated with the combination therapy. Mechanistically, combination treatment with Sotorasib and Trametinib led to enhanced inhibition of the MAPK/ERK/AKT signaling pathway compared to monotherapy in SW837 and SW1463 cell lines. Furthermore, the introduction of activating mutations in MEK1 (MEK1^{K57T} and MEK1^{K57N}) demonstrated that combinatorial therapies of KRAS^{G12C} inhibitor and MEK1 inhibitors could effectively overcome the drug resistance acquired by these activating mutations.

Conclusion: The combined treatment of Sotorasib and a MEK1 inhibitor, Trametinib, exhibit synergistic effects in drug-induced cytotoxicity and inhibition of MAPK pathways in colorectal cancer lines.

#7197 Targeting FAK exhibits therapeutic effects on interstitial lung disease induced by trastuzumab deruxtecan.

B. Zhang, R. Pang, J. Cheng, J. Gao, N. Qin, L. Liu, Z. Wang;
Inxmed, Shanghai, China

Interstitial lung disease (ILD) is a common pulmonary complication associated with cancer therapy. Several antibody-drug conjugates (ADCs), including trastuzumab deruxtecan (Enhertu), have been implicated in the induction of ILD, thereby limiting their clinical application. Treatment with ADCs must be permanently discontinued if grade 2 or higher ILD-related adverse effects occur. The standard approach for managing Enhertu-induced ILD involves the use of corticosteroids, which can exacerbate the toxicities faced by cancer patients. Our previous studies demonstrated that targeting focal adhesion kinase (FAK) enhances the efficacy of Enhertu and other ADCs in solid tumor treatment. Notably, we have now identified that FAK inhibition by the small molecule inhibitor Ifebemtinib (aka IN10018, Ifebe) can mitigate Enhertu-induced ILD. In an animal model of Enhertu-induced ILD, Ifebe showed recovery trends comparable to those observed with corticosteroids across multiple physiological parameters of mouse respiration. Both inflammatory and ILD injury biomarkers which were activated by Enhertu were significantly reduced with Ifebe monotherapy. Collectively, the combination of FAK inhibition and Enhertu may offer improved therapeutic efficacy while mitigating ILD-related toxicities. These findings warrant further clinical investigation.

#7198 Preclinical efficacy of the combination of MEK inhibitor plus KRAS G12D inhibitor for non-small cell lung cancer with *KRAS G12D*.

Yu Tanaka, Mirai Tsukuda, Gaku Yamamoto, Eri Sugiyama, Hiroki Izumi, Tetsuya Sakai, Shigeki Umemura, Yoshitaka Zenke, Shingo Matsumoto, Kiyotaka Yoh, Koichi Goto, Hibiki Udagawa

National Cancer Center Hospital East, Kashiwanoha, Kashiwa-shi, Chiba, Japan

KRAS G12D is observed in 3 - 8% of non-small cell lung cancer (NSCLC). Currently, no targeted therapies are approved for solid tumors including NSCLC with *KRAS* G12D. MRTX1133 is a selective KRAS G12D inhibitor in advanced development. A previous preclinical data showed that MRTX1133 was effective in pancreatic carcinoma, but might not in NSCLC. Thus, we evaluated the efficacy of MRTX1133 monotherapy and investigated potential therapeutic strategies for NSCLC with *KRAS* G12D using *in vitro* and *in vivo* models. Cell viability assays demonstrated that MRTX1133 was less effective in *KRAS* G12D-mutated NSCLC cell lines. MRTX1133 suppressed phosphorylation of ERK in KRAS G12D-mutated NSCLC cell lines at 2 - 4 hours, but rebound of phosphorylation of ERK was observed at 24 - 48 hours. To identify potential combination drugs that can enhance the efficacy of MRTX1133, we evaluated phosphorylation of receptor tyrosine kinases in *KRAS* G12D-mutated NSCLC cell lines treated with MRTX1133 monotherapy and performed a drug combination screening for *KRAS* G12D-mutated NSCLC cell lines. Although no phosphorylation of receptor tyrosine kinases that could be combination drug candidates were observed, several MEK inhibitors, such as trametinib, selumetinib and binmetinib, enhanced the efficacy of MRTX1133 in cell viability assays. MEK inhibitors suppressed rebound of phosphorylation of ERK by MRTX1133 monotherapy at 24 - 48 hours. The combination of trametinib with MRTX1133 suppressed tumor growth compared to MRTX1133 monotherapy with tolerable toxicity in KRAS G12D-mutated NSCLC *in vivo* models. In conclusion, our findings indicate that the preclinical efficacy of MRTX1133 monotherapy was limited for NSCLC with *KRAS* G12D, and therapeutic potential of MEK inhibitors in combination with MRTX1133.

#7199 Synergistic anticancer effects of milciclib and mirdametinib: mechanistic insights into cell cycle arrest, DNA damage, and senescence.

Xinyu Pei[1], Juifang Fan[2], Anh Nguyen Nguyen[3], Hannah Neiger[3], Imran A. Khan[2], Vladimir Bezrookove[2], Yu Zhou[1], Maureen Veilleux[4], Lily S. Wang[5], Valerie A. Gerriets[3], John K. Cusick[3], Pierre Y. Desprez[2], Li Zheng[1], Mohammed Kashani-Sabet[2], Binghui Shen[1], **Yihui Shi**[2]

[1]Cancer Genetics and Epigenetics, City of Hope, Duarte, CA,[2]California Pacific Medical Center Research Institute, San Francisco, CA,[3]California Northstate University, Elk Grove, CA,[4]University of Lyon, Lyon, France,[5]University of Berkeley, Berkeley, CA

Milciclib, a cyclin-dependent kinase (CDK) inhibitor, has recently been identified as a modulator of pre-mRNA splicing, broadening its therapeutic potential. However, resistance to CDK inhibitors frequently arises due to activation of compensatory pathways, such as the MEK-ERK signaling cascade, which drives unchecked cell cycle progression and circumvents critical checkpoints. RNA sequencing of Milciclib-treated cells revealed significant upregulation of the MEK pathway, highlighting a potential resistance mechanism and supporting the strategy of targeting MEK in combination therapy. To address this challenge, we investigated the combination of Milciclib with the MEK inhibitor Mirdametinib (PD-0325901) across various cancer types, including colorectal cancer. The combination synergistically inhibited cancer cell proliferation by inducing cell cycle arrest at G0/G1 and G2/M phases. It also impaired homologous recombination repair, as evidenced by reduced RAD51 levels, and enhanced DNA damage (γH2AX). Furthermore, Mirdametinib alone and in combination with Milciclib promoted cellular senescence, characterized by the upregulation of p16 and p21. In vivo, the combination treatment significantly inhibited tumor growth in animal models. These findings highlight the complementary mechanisms of action of Milciclib and Mirdametinib. The synergy observed in this combination therapy provides a strong rationale for further clinical investigation.

#7200 PREMOST: an organoid-based functional precision medicine trial in osteosarcoma.

Rina Kuznets[1], Maite Calucho[2], Huyen T. L. Nguyen[2], Scott D. Nelson[3], Sarah M. Dry[3], Jane Yanagawa[4], Arun S. Singh[5], Joseph G. Crompton[4], Lauren E. Wessel[2], Alexander B. Christ[2], Nicholas M. Bernthal[2], Noah C. Federman[6], Bartosz Chmielowski[7], Alice Soragni[8]

[1]Department of Pediatrics, Division of Hematology/Oncology, UCLA, Los Angeles, CA,[2]Department of Orthopedic Surgery, UCLA, Los Angeles, CA,[3]Department of Pathology, David Geffen School of Medicine, UCLA, Los Angeles, CA,[4]Department of Surgery, Division of Thoracic Surgery, David Geffen School of Medicine, UCLA, Los Angeles, CA,[5]Department of Medicine, Division of Hematology & Medical Oncology, UCLA, Los Angeles, CA,[6]Department of Pediatrics, Division of Hematology Oncology, David Geffen School of Medicine, UCLA, Los Angeles, CA,[7]Division of Hematology-Oncology, David Geffen School of Medicine, UCLA, Los Angeles, CA,[8]Department of Orthopaedic Surgery, David Geffen School of Medicine, UCLA, Los Angeles, CA

Pediatric sarcomas are a diverse group of malignancies accounting for ~15% of all childhood solid tumors. Osteosarcomas constitute the most common bone tumor in the pediatric patient population. There are about 1,000 new cases diagnosed each year in the United States, with greatest incidence in adolescence and in the sixth decade of life. Overall, 5-year survival rates are ~60% and as low as ~20-30% for metastatic disease. In the primary setting, neoadjuvant MAP chemotherapy including methotrexate, adriamycin (doxorubicin), and cisplatin combination therapy is the standard of care. However, ~30% of all patients are resistant to MAP therapy, resulting in low degree of necrosis at time of surgery, and rapid recurrences. For recurrent and metastatic disease, NCCN guidelines include several different possible regimens, which have shown some activity in the clinic. For both the neoadjuvant MAP setting and second line therapies, there is no clinically validated biomarker of response to help stratify patients and improve outcomes by selecting the most effective therapy. Our overarching goal is to leverage short-term, patient-derived sarcoma organoids to provide actionable information for treatment guidance. Patient-derived organoids (PDOs) are increasingly used both to investigate tumor growth and for precision medicine, as they recapitulate patients drug responses in many settings (Zhao et al, Nature Reviews Methods Primers 2022). We have an established pipeline to establish and characterize sarcoma patient-derived organoids using an approach that allows us to perform high throughput drug screenings to find individual susceptibilities (Al Shihabi et al, Science Advances 2022; Al Shihabi et al, Cell Stem Cell 2024). Our preliminary correlative studies highlight how sarcoma PDOs can recapitulate therapy outcomes (Al Shihabi et al, Cell Stem Cell 2024). In a small dataset, responses of osteosarcoma organoids established from diagnostic biopsies to MAP neo-adjuvant therapy were found to correlate with both short-term histologic response (necrosis at time of surgery) and long-term patient outcomes (progression-free survival). As such, we have initiated a clinical trial to evaluate the feasibility of developing organoids from these cases and to assess the prognostic accuracy of our platform in predicting treatment outcomes. Here we will discuss the design and preliminary findings of PREMOST (An Organoid-based Functional Precision Medicine Trial in Osteosarcoma, NCT06064682), currently open to patients either suspected of having osteosarcoma (Arm 1) or who have recurrent/metastatic osteosarcoma (Arm 2). Clinical validation of organoid-based platforms is a critical step to deploy these technologies for therapy guidance in the future.

#7201 Targeting SLC38A1 non-conventional glutamine transporter in high risk MDS/AML.

Akansha Jalota[1], Anna Skwarska[1], Divij Verma[1], Kith Pradhan[1], Sovira Chaudhry[1], Gowri Poigaialwar[1], Vijaya Shukla[2], Yaxia Yuan[3], Elvin Wagenblast[4], Daohong Zhou[3], Stefano Tiziani[5], Amit Verma[1], Marina Konopleva[1]

[1]Department of Medicine (Oncology), Blood Cancer Institute, Albert Einstein College of Medicine-Montefiore Medical Center, Bronx, NY,[2]Department of Nutritional Sciences, College of Natural Sciences, The University of Texas at Austin, Austin, TX,[3]Department of Biochemistry & Structural Biology and Center for Innovative Drug Discovery, University of Texas Health Science Center at San Antonio, San Antonio, TX,[4]Department of Oncological Sciences, Tisch Cancer Institute, Black Family Stem Cell Institute, Mindich Child Health & Development Institute and Department of Pediatrics, Division of Pediatric Hematology-Oncology, Icahn School of Medicine at Mount Sinai, New York, NY,[5]Department of Nutritional Sciences, Dell Pediatric Research Institute, College of Natural Sciences & Dell Medical School, The University of Texas at Austin, Austin, TX

Introduction: Myelodysplastic syndrome (MDS) is a malignant clonal hematopoietic disorder characterized by ineffective hematopoiesis, bone marrow dysplasia, and a tendency to transform into acute myeloid leukemia (AML) in about 30% of cases. Cancer cells are known to rely on glutamine as a source of energy. We have reported that inhibition of glutaminase (GLS), a key enzyme in glutaminolysis, with CB-839 combined with 5-azacytidine has synergistic activity in AML/MDS *in vitro* and *in vivo* models and is associated with durable responses in patients with advanced MDS (DiNardo et al., Nat Cancer 2024). Glutamine uptake is tightly regulated by members of the large solute carrier (SLC) superfamily. We found that SLC38A1, a non-canonical glutamine transporter, is significantly and selectively overexpressed in long-term hematopoietic stem cells (LT-HSCs) in patients with MDS and AML and associated with reduced overall survival. Based on these findings, we hypothesize that SLC38A1 represents a promising therapeutic target and that inhibiting critical tumor-specific metabolic pathways will selectively target MDS stem cells responsible for AML transformation.

Methods: Intracellular metabolites were extracted using a modified Bligh-Dyer procedure and analyzed by ultra-high-performance liquid chromatography tandem ultra high-resolution mass spectrometry (UHPLC-HRMS) technology. SiRNA and CRISPR mediated knockdown of SLC38A1 were done in cell lines and patient samples using Amaxa nucleofection. Colony forming assay was done using human methylcellulose enriched media.

Results: Functional relevance of SLC38A1 was shown by metabolic flux analysis of siRNA-mediated SLC38A1 depletion in HEL cells and knockdown of OCI-AML-3 with CRISPR guide RNAs which demonstrated reduced glutamine transport with corresponding reduction of glutamate and aspartate levels. MeAiB (α-(methylamino)-isobutyric acid), a non-metabolized amino acid analogue and competitive inhibitor of system “A” SNAT family transporters including SLC38A1, reduced AML cell viability in MOLM14 and MV4-11 cell lines. Knock-down of SLC38A1 reduced the number of surviving colonies by 37%, 42% and 43% in MOLM13, THP-1 and MDS-L cells, respectively. SLC38A1 KO in MOLM13 and THP-1 cells attenuated cell proliferation and reduced cell viability compared to control cells. SLC38A1 KO in primary AML and high risk MDS patient samples (n=4) reduced cell viability and colony formation. Additional primary samples are being tested and will be reported.

Conclusion: These findings suggest that inhibition of the import function of glutamine transporter SLC38A1 may be therapeutically beneficial to target the metabolic reprogramming in high risk MDS/AML. Chemical library screening is ongoing and has identified initial tool SLC38A1 inhibitors.

#7202 Real-world data and clinical experience from over 100,000 multi-cancer early detection tests.

Marc Matrana[1], Vershalee Shukla[2], Dallas Kingsbury[3], Martin Poliak[4], Jordan Lipton[5], Louis B. Malinow[6], Oliver Venn[7], John Beausang[7], Geoff Stanley[7], Earl Hubbell[7], Kathryn N. Kurtzman[8], Jeffrey M. Venstrom[8], Rita Shaknovich[8], **Candace Westgate**[9]

[1]Ochsner Cancer Institute, New Orleans, LA,[2]Vincere Cancer Center, Scottsdale, AZ,[3]Fountain Life, Naples, FL,[4]Medical Clinic of Houston, Houston, TX,[5]Signature Healthcare, Charlotte, NC,[6]MDVIP, Baltimore, MD,[7]GRAIL, Inc., Menlo Park, CA,[8]GRAIL, Inc. (Affiliation at time of study completion), Menlo Park, CA,[9]Adventist Health, Saint Helena, CA

Background: A multi-cancer early detection (MCED) test, which has been commercially available since April 19, 2021, detects a cancer signal and predicts cancer signal origin (CSO) by analyzing methylation patterns in cell-free DNA. Here, we report real-world clinical experience with >100,000 MCED tests ordered and processed through October 14, 2023.
Methods: A Quality Assurance program was set up to characterize use of MCED testing in a real-world setting and included voluntarily reported outcome data from healthcare providers to assess cancer signal detection rate (CSDR; cancer signal detected results [true + false positives] divided by all tests) and CSO accuracy. This real-world data analysis included 111,080 tests returned to individuals ≥22 years (56% male, median age 58 y [IQR 50-67]). All data were de-identified following the HIPAA-compliant, independent expert determination method. Only the first test administered to each individual was used in this analysis. CSDR was compared to an independent model of CSDR based on estimated cancer incidence from SEER. When available, the proportion of reported diagnosed cancers is broken down by asymptomatic patients (ie, without clinical suspicion of cancer) and stage. CSO prediction accuracy was calculated for reported diagnosed cancers with a known cancer type.
Results: The overall real-world CSDR was 0.91% (1,011/111,080) and increased with age ($p<1e-10$), consistent with the independent CSDR model prediction and previously published data. Among asymptomatic patients with a positive MCED test and a reported cancer status, 49.4% (128/259) received a cancer diagnosis with a median time to diagnosis of 43 days (IQR: 21-94). Excluding recurrences and cases with multiple diagnoses, cancer stage was available for 69 asymptomatic patients, with 59% [41/69] diagnosed with stages I-III. CSO prediction accuracy across the 19 different CSO categories in this dataset was 89% (201/227) and represented 30 distinct AJCC-defined cancer types ultimately diagnosed. Many cancer diagnoses (60.8% [138/227]) were types without recommended screening guidelines, including 18 cases of head and neck, 15 pancreas and gall bladder, and 9 stomach and esophagus cancers.
Conclusions: Real-world CSDR was consistent with previously reported large-scale clinical studies and an independent CSDR model. Although outcomes data is limited by incomplete reporting, a known issue in clinical surveillance data, these data provide valuable insight into real world use of MCED tests. The MCED test reliably detected a cancer signal across a wide range of cancer types, including unscreened cancers. High CSO prediction accuracy guided diagnostic workups, resulting in typically rapid cancer diagnoses. These real-world data demonstrate the successful detection of both conventionally screened and unscreened cancers using the MCED test.

#7203 Systematic pan-cancer functional patient stratification to advance therapy personalization in rare solid tumors.

Jasmina Paluncic[1], Zunamys I. Carrero[1], Juliana Schulz[1], Attila Jady[1], Claudia Dagostino[1], Sara Hrabovska[1], Lino Mohrmann[1], Irina Kerle[1], Maximilian Werner[2], Dorothea Hanf[1], Maria Gabriela Pereira dos Santos[1], Elinor Young[1], Jessica Pablik[3], Ivona Mateska[4], Heike Peterziel[5], Daniel E. Stange[6], Alexander A. Wurm[7], Ina Oehme[5], Vivek Venkataramani[8], Christoph Heilig[9], Simon Kreutzfeldt[9], Benjamin Schmidt[10], Peter Horak[9], Stephan Richter[11], Jurgen Weitz[12], Klaus-Dieter Schaser[13], Daniela Richter[1], Stefan Froehling[9], Christoph Heining[1], Hanno Glimm[1], Claudia R. Ball[1]

[1]Translational Medical Oncology, National Center for Tumor Diseases (NCT/UCC), a partnership between DKFZ and Faculty of Medicine and University Hospital Carl Gustav Carus, Technical University Dresden; Helmholtz-Zentrum Dresden - Rossendorf (HZDR), Dresden, Germany,[2]Department of Internal Medicine I, University Hospital Carl Gustav Carus, Technical University Dresden, Dresden, Germany,[3]Institute of Pathology, University Hospital Carl Gustav Carus Dresden, Dresden, Germany,[4]National Center for Tumor Diseases (NCT/UCC), a partnership between DKFZ and Faculty of Medicine and University Hospital Carl Gustav Carus, Technical University Dresden; Helmholtz-Zentrum Dresden - Rossendorf (HZDR), Dresden, Germany,[5]Hopp Children's Cancer Center Heidelberg (KiTZ); Clinical Cooperation Unit Pediatric Oncology (B310), German Cancer Research Center (DKFZ) and German Cancer Consortium (DKTK); National Center for Tumor Diseases, Heidelberg, Germany,[6]Department of Visceral, Thoracic and Vascular Surgery, University Hospital Carl Gustav Carus Dresden, Dresden, Germany,[7]Translational Medical Oncology, National Center for Tumor Diseases Dresden (NCT/UCC), Germany, a partnership between DKFZ and Faculty of Medicine and University Hospital Carl Gustav Carus, Technical University Dresden, Dresden, Germany; Helmholtz-Zentrum Dresden - Rossendorf (HZDR), Dresden, Germany,[8]Comprehensive Cancer Center Mainfranken (CCCMF), University Hospital Wurzburg, Wurzburg, Germany,[9]Division of Translational Medical Oncology, German Cancer Research Center (DKFZ) and National Center for Tumor Diseases (NCT) Heidelberg; German Cancer Consortium (DKTK), Heidelberg, Germany,[10]Medical Clinic und Polyklinik, University Hospital Hamburg-Eppendorf, Hamburg, Germany,[11]Clinic and Polyclinic for Internal Medicine I, University Hospital Carl Gustav Carus, Technical University Dresden, Dresden, Germany,[12]Department of Visceral, Thoracic and Vascular Surgery, University Hospital and Faculty of Medicine Carl Gustav Carus, Technical University Dresden, Dresden, Germany,[13]University Center for Orthopaedics, Trauma and Plastic Surgery, University Hospital Carl Gustav Carus, Technical University Dresden, Dresden, Germany

Introduction: To date, comprehensive omics sequencing has greatly advanced cancer therapies and precision oncology. However, rare cancers remain challenging due to unique genetic profiles and low prevalence, limiting molecular mechanism-oriented treatment strategies. Comprehensive precision oncology programs enable molecularly informed treatments, benefiting many patients with advanced rare cancers. Nevertheless, therapeutic target selection remains complex, particularly in cases with no or multiple targetable alterations. To improve patient stratification for rare cancers, we developed a high-throughput drug testing strategy that rapidly identifies actionable therapeutic vulnerabilities to support molecular sequencing-based target identification in precision oncology.

Methods: We developed a workflow to generate 3D patient-derived cancer models (PCM) and conduct drug sensitivity testing (DST) from viable patient tissues. Tumor tissue was processed into 3D short-term (ST-PCM) or expandable 3D long-term PCM (LT-PCM), such as organoids and spheroids. Dissociated PCM were seeded on pre-spotted drug test plates containing 87 FDA-approved or clinical trial drugs in five concentrations. Cell viability and drug sensitivity scores (DSS) were assessed after 72 hours for hit identification, followed by discussion in a molecular tumor board.

Results: DSS correlation in replicates across entities and PCM types showed consistent hit classification with low variance (~2.3-8.3%). High DSS values for mutation-targeting drugs validated assay sensitivity, while lack of response in controls confirmed specificity. Screening of >100 PCMs (76 from precision oncology patients) from 17 distinct rare and common tumor entities, including sarcomas, CUP, and others, revealed high feasibility: all LT-PCMs and 97% of ST-PCMs derived from surgical specimens allowed partial or full DST. Despite limited material from small biopsies, i.e., fine needle biopsies, successful screening of drug subsets was achieved in 78% of cases with direct seeding as ST-PCM. Additionally, 24 patient samples were screened after tumor cell expansion in LT-PCM, further expanding the cohort of patients eligible for functional testing. Among all tested PCMs, 91% responded to at least one of the tested drugs ex vivo (range 1-38, median 8). Molecular analysis linked pathway alterations with a higher likelihood of pathway inhibitor response. Importantly, preliminary analysis suggests concordance between ex vivo predictions for drug sensitivity and patient responses.

Conclusion: We established a robust DST strategy that identifies potential therapeutic vulnerabilities to guide personalized treatment recommendations in precision oncology. This approach, feasible even with limited material, has the potential to enhance treatment decision-making and improve outcomes for adults with rare solid tumors.

#7204 Stimulator of interferon genes (STING) protein expression in resected non-small cell lung cancer with EGFR mutation.

Atsushi Osoegawa, Yohei Takumi, Kento Inoue, Eika Kudo, Kazuki Ota, Yuta Abe, Takahiro Sato, Daiki Noda, Miyuki Abe, Naoki Haratake, Kenji Sugio

Oita University, Yufu, Oita, Japan

Background and Objective: In lung cancer with epidermal growth factor receptor (EGFR) mutations (mEGFR), the expression of programmed death-ligand 1 (PD-L1), a predictive marker for the efficacy of immune checkpoint inhibitors (ICI), is generally low, and the effectiveness of ICIs is considered limited. However, a subset of mEGFR cases exhibits high PD-L1 expression, and in such cases, EGFR-tyrosine kinase inhibitors (EGFR-TKIs) have been reported to show reduced efficacy. PD-L1 expression in lung cancer tissue can result from tumor immune responses against neoantigens but may also be induced by the initial immune response via the stimulator of interferon genes (STING) pathway. Moreover, ICI efficacy is reported to be poor in STING-deficient mice. This study, therefore, aimed to analyze the relationship between STING expression and clinicopathological factors, PD-L1 expression, and prognosis in resected mEGFR cases.
Materials and Methods: This study included 40 cases of mEGFR-positive lung cancer surgically resected at our institution between November 2018 and May 2020. Immunohistochemical staining of tumor tissues was performed using an anti-STING antibody (D2P2F), and expression levels were evaluated using the H-score method. EGFR amplification was assessed by qPCR.
Results: Among the 40 cases, 29 were female, with a median age of 72.5 years (range: 42-84 years), and 25 were never-smokers. Pathological stages included stage I in 36 cases and stage III in 4 cases. The EGFR mutation types comprised exon 19 deletions in 16 cases, L858R in 17 cases, and other mutations in 7 cases. PD-L1 expression was negative in 16 cases, 1-49% in 20 cases, and high (≥50%) in 4 cases. STING was expressed in the cytoplasm of tumor cells, with a median H-score of 180 (range: 50-290). When cases were classified into high and low STING expression groups using a cutoff H-score of 150, no significant differences in clinicopathological factors or PD-L1 expression were observed between the two groups. However, a significant correlation was noted between STING expression and EGFR copy number. Prognostic analysis revealed that the high STING expression group had significantly worse outcomes compared to the low expression group (5-year recurrence-free survival: 93% vs. 56%, $p = 0.038$).
Discussion: High STING expression was identified as a poor prognostic factor in mEGFR cases. STING expression correlated with EGFR copy number, suggesting that EGFR copy number may influence STING expression and the malignancy of mEGFR. Further studies are required to investigate the impact of EGFR copy number on STING expression and the malignant potential of mEGFR.

#7205 Targeting diverse pathways for the treatment of KRAS driven non-small cell lung cancer.

R. Stanley[1], S. Flanagan[2], S. Tan[1], E. Dillion[3], A. P. Armstrong[4], J. Naidoo[1], K.-k. Wong[4], G. Cagney[3], T. Ni Chonghaile[1], C. M. Dowling[1];
[1]Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland, [2]University of Limerick, Limerick, Ireland, [3]University College Dublin, Dublin, Ireland, [4]New York University, New York, NY

Non-small cell lung cancer (NSCLC) accounts for nearly 85% of lung cancer cases. 32% of patients with NSCLC harbour a KRAS mutation, and these patients have poor clinical outcomes. The newly approved KRAS inhibitors (KRASi) have shown clinical promise, however most patients eventually develop resistance. Hence, it is critical to understand the mechanism of resistance to KRAS inhibition to fuel effective combinational therapy strategies. We recently demonstrated enhanced sensitivity to Histone deacetylase 6 (HDAC6) inhibitors (HDAC6i) in KRAS driven NSCLC. Hence, we sought to explore the benefit of combining HDAC6i with KRASi in KRAS driven NSCLC. Using two separate cell viability assays, we demonstrated a synergistic response to the KRASi and HDAC6i in combination in KRAS driven NSCLC cell lines. Moreover, we observed a decrease in the migration rate with this combination in comparison to the single drug treatment. We also demonstrated a significant reduction in colony formation following the combination of KRASi and HDAC6i. Finally, we confirmed enhanced sensitivity to this drug combination in three-dimensional (3D) cell cultures. To further decipher the specific mechanism of action of HDAC6 in KRAS driven NSCLC we performed a co-immunoprecipitation of HDAC6 in NSCLC cell lines and analysed by liquid-chromatograph mass spectrometry (LC/MS). We found several epithelial-to-mesenchymal transition (EMT) associated proteins such as Yes Association Protein (YAP) and Vimentin (VIM) bound to HDAC6 in KRAS driven NSCLC cells. Interestingly, bulk RNA sequencing revealed a down-regulation of the EMT pathway in cells treated with a HDAC6i compared to the control. Specifically, in this data, YAP and VIM were down regulated following treatment with HDAC6i, among other EMT markers such as Snai1, Twist, and Zeb1/2. Interestingly, we observe a significant reduction in response to HDAC6i in NSCLC cells overexpressing YAP. Given this data combined with recent literature demonstrating an upregulation of EMT proteins associated with KRAS resistances, we purpose that HDAC6 inhibition could be a mechanism to overcome KRAS resistance. In conclusion, we have demonstrated HDAC6i and KRASi as an effective combination strategy for KRAS driven NSCLC. We also propose that the mechanism of action may be through HDAC6's regulation of EMT, and this may help overcome resistance to KRASi, however this warrants further investigation.

#7206 STING agonist ADU-S100 in combination with vemurafenib induces STING upregulation, immune-stimulatory cytokine modulation and enhancing NK-mediated cytotoxicity in BRAF V600E-mutant melanoma.

Vida Tajiknia, Matthew Hadfield, Andrea Schmidt, Connor purcell, Claire Lin, wafik El Deiry, Sean Lawler

Legorreta Cancer Center at Brown University, Providence, RI

Background: Targeting the mitogen-activated protein kinase (MAPK) pathway with BRAF inhibitors, has significantly advanced the treatment of melanoma patients whose tumor harbor a BRAF V600E/K mutation. therapeutic resistance occurs in the majority of patients, highlighting the need for combination strategies to improve treatment outcomes. The activation of the Stimulator of Interferon Genes (STING) pathway has emerged as a promising approach to boost anti-tumor immunity. This study explores the potential efficacy of combining the STING agonist ADU-S100 (ADU) with vemurafenib in human melanoma cell lines harboring the BRAF V600E mutation, focusing on its impact on tumor signaling, cytokine profiles, and NK-cell-mediated cytotoxicity.

Methods: Human melanoma cell lines with BRAF V600E mutation—SK-MEL-28, SK-MEL-24-SH4, RPMI7951, and SK-MEL-3—were treated with ADU, vemurafenib, or both in combination. Cell viability was assessed, while changes in total STING were measured by Western blot. Cytokine profiles were analyzed using a multiplex Luminex panel. NK cell-mediated cytotoxicity was assessed through co-culture experiments.

Results: Cell viability assays demonstrated treatment with ADU alone did not significantly reduce cell viability. Western blot analysis revealed minimal baseline STING levels in the SH4 cell line, with a marked increase following ADU, vemurafenib, or their combination. The most pronounced upregulation of STING occurred when ADU & vemurafenib were used together. In SK-MEL-28, the combination treatment was the only condition to result in substantial STING upregulation and a corresponding reduction in pERK. Cytokine analysis showed that the combination treatment significantly increased the levels of beta-2 microglobulin (B2M) across all three cell lines, suggesting modulation of immune checkpoint inhibitor resistance. Additionally, the combination treatment led to a marked reduction in CXCL-8, a key mediator of melanoma progression and metastasis, and decreased VEGF levels across all three cell lines. The CXCL5/CXCL13 axis, known to influence responses to targeted immunotherapies, was most favorably modulated under the combination condition. Co-culture with NK cells revealed an increase in tumor cell death in all three cell lines following combination treatment.

Conclusion: The combination of STING agonist ADU-S100 and vemurafenib effectively enhances immune-stimulatory cytokine profiles in BRAF V600E-mutant melanoma. Notably, this approach may be used to promote NK cell-mediated cytotoxicity and modulation of key cytokines associated with immune resistance. This strategy shows promise for overcoming resistance and improving outcomes, warranting further clinical investigation.

#7207 Targeting TGFBR2 neddylation suppresses metastatic/immunosuppressive abilities and enhances responsiveness to BRAF inhibitor in melanoma with BRAF V600E mutation.

Leon Tsung-Ju Lee, Hui-Wen Chiu, Yuan-Feng Lin

Graduate Institute of Clinical Medicine, Taipei Medical University, Taipei, Taiwan

Background: Approximately 50% of melanomas harbor activating BRAF mutations (over 90% V600E). BRAF V600E mutation promotes melanoma progression, including metastasis and immunosuppression. While targeted therapies for metastatic BRAF V600E melanoma exist, resistance limits their efficacy. Crosstalk between TGF-β and receptor tyrosine kinase (RTK) signaling pathway is considered a possible mechanism for drug resistance to RTK inhibitors in melanoma with BRAF V600E mutation. In addition, the post-translational modification of TGFBR2 through a non-canonical protein neddylation pathway has been shown to prevent its proteolytic degradation by the proteasome system, which reinforces the activation of TGF-β signaling axis. This study explores whether targeting TGFBR2 neddylation suppresses metastasis/immunosuppression and enhances BRAF inhibitor response.
Methods: Gene Set Enrichment Analysis (GSEA) was performed to predict the possible pathways suppressed by the treatment with the protein neddylation inhibitor MLN4924 in melanoma cells. Site-directed mutagenesis was employed to generate a TGFBR2 variant with mutation at its neddylation sites Lys556 and Lys567. MTT assays assessed cell survival post-treatment. Transwell cultivation and co-culture with activated T cells were utilized to estimate the cellular migration and immunosuppressive abilities of melanoma cells, respectively. Xenograft implantation was established to estimate tumor growth and metastatic ability of melanoma cells.
Results: GSEA results demonstrated that the treatment with neural precursor cell-expressed, developmentally downregulated gene 8 (NEDD8)-activating enzyme inhibitor MLN4924 significantly suppresses epithelial-mesenchymal transition (EMT) progression and the activity of TGF-β signaling pathways in melanoma cells with BRAF V600E mutation. Conversely, TGFBR2 knockdown in the highly metastatic/BRAF V600E mutation melanoma cell lines RPMI7951 and A2058 suppressed migration and immunosuppressive abilities, but its overexpression in the poorly metastatic/BRAF V600E mutation melanoma cell lines A375 and C32 promoted cellular migration/immunosuppressive abilities and drug resistance to the BRAF inhibitor PLX4720 in vitro. The restoration of wild-type, not Lys556Arg/Lys567Arg mutant, rescued the cellular migration/immunosuppressive abilities and drug resistance characteristics of the TGFBR2-silenced RPMI7951 and A2058 cells. Animal experiments revealed that the overexpression of wild-type, not Lys556Arg/Lys567Arg mutant, enhances the metastatic ability and promotes drug resistance to PLX4720.
Conclusion: Our data suggest that targeting TGFBR2 neddylation might be a novel strategy to combat the metastatic/immunosuppressive and BRAF inhibitor-resistant melanoma with BRAF V600E mutation.

#7208 PLK1 inhibition as a novel therapeutic strategy in HER2+ breast cancer with germline METN375S mutation.

Arpita Datta[1], Calista Jia Ling Ow[1], Charlie Marvalim[1], Natasha Gandasasmita[1], Joline Si Jing Lim[2], Soo Chin Lee[2]

[1]Cancer Science Institute, Singapore, Singapore,[2]Cancer Science Institute, Department of Haematology-oncology, National University Health System and National University Cancer Institute, Singapore, Singapore

Introduction: Germline MET polymorphism (N375S), a variant common in Asians but rare in Caucasians (7.9% in South Asians vs 1-2% in Caucasians), has been previously reported to enhance the aggressiveness of squamous cell carcinoma. Germline MET^{N375S} polymorphism was more prevalent in metastatic compared to early-stage hormone receptor negative (HR-) HER2+ breast cancer (BC) patients in Singapore (19.2% vs 9.2%; p=0.058; n=201) and is significantly associated with poorer relapse-free (p=0.015) and overall survival (p<0.001). In HER2+ BC cell lines, MET^{N375S} enhances an aggressive malignant phenotype *in vitro* compared to MET^{WT}. MET^{N375S} expressing HER2+ clones were not sensitive to MET inhibitors but displayed higher sensitivity to HER2 inhibitors. In this study, we aim to (1) Identify drug classes that are effective against MET^{N375S} HR-/HER2+ BC through high-throughput anticancer drug screening; and (2) establish the molecular mechanism that maintains the vital pro-survival signals in MET^{N375S} HR-/HER2+ BC.

Methods: We generated isogenic HR-/HER2 +BC cell models (SKBR3, AU565) expressing either wild-type MET^{WT} or mutant N375S MET^{N375S} and carried out high-throughput drug screening comprising 414 compounds. Bliss scores were calculated using Synergy Finder to determine the interaction of drugs in combination studies. Immunoprecipitation and immunoblotting analysis evaluated interaction partner and expression of protein in the cell lines.

Results: High-throughput drug screening identified 6 compounds with selective sensitivity to MET^{N375S} cells. Intriguingly, 4 out of 6 compounds target PLK1 (Polo-Like Kinase1), a key mitotic kinase downstream of HER2. The IC50 of the four PLK1 inhibitors was (range is for MET^{WT} 0.2 to 1.1 μM and for MET^{N375S} 0.02 to 0.1 μM) 10-fold reduction in MET^{N375S} cells. Western blot analysis revealed that the N375S mutation led to increased phosphorylation of both MET and HER2, which in turn activated downstream signaling pathways, including SRC and MAPK kinases. MET^{N375S} cells demonstrated elevated PLK-1 protein expression. Co-immunoprecipitation confirmed the interaction between MET^{N375S} and HER2, resulting in hyperactivation of HER2 signaling and enhanced MAPK pathway activation. Notably, HER2 knockdown disrupted this signaling, reduced PLK1 levels, and decreased the cells' sensitivity to PLK1 inhibitors. The combination of the PLK1 inhibitor volasertib with HER2 inhibitor lapatinib was highly synergistic in MET^{N375S} cells, significantly enhancing apoptosis at nanomolar concentrations.

Conclusions: Our findings suggest that MET^{N375S} variant promotes tumorigenesis by hyperactivating HER2 signaling and enhancing PLK1 pathway. These findings highlight the potential of PLK1 inhibitors, as a novel therapeutic option for MET^{N375S} mutant HER2+ BC and supports further evaluation of PLK-1 in HER2+ BC.

#7209 Status-agnostic therapy of breast cancer using antibodies targeting O-glycosylated proteins.

Aaron C. Groen[1], Nisha Shrestha[1], Boris Klebanov[1], Byeongjun Yu[2], Beom Yong Lee[2], Yun-Hee Park[2], Chul-Woong Chung[2], Constantine Theodoropulos[1], Hans H. Wandall[3]

[1]GO Therapeutics, Natick, MA,[2]LigaChem Biosciences, Daejeon, Korea, Republic of,[3]Department of Cellular and Molecular Medicine, Center for Glycomics, University of Copenhagen, Copenhagen, Denmark

We have developed a novel antibody-drug conjugate (ADC), based on the mAb L100, that selectively targets a O-GalNAc-glycoprotein expressed in approximately 20% of all breast cancer. Unlike many ADC programs targeting Breast Cancer, L100 recognizes cancer-specific O-glycosylated versions of proteins do not present on normal tissues circumventing adverse events from on target off tumor binding to healthy tissues. The crystal structure of L100 provides insights into its binding specificity and affinity. L100 exhibits low nanomolar affinity (0.83nM) to the glycoprotein target and has also been developed as a companion diagnostic assay, further validating its potential as a therapeutic target. L100 is conjugated to the microtubule-disrupting agent MMAE and the topoisomerase inhibitor exatecan via a site-specific β-glucuronidase-cleavable linker. This linker is stable in plasma, but selectively releases the payload in tumor tissues, which exhibits a high expression pattern of beta-glucuronidase. In preclinical studies, the L100 ADC (LCB22A) demonstrated potent and selective cytotoxicity against various cancer cell lines (EC50=~4.4 nM) and effectively eliminated tumors in breast cell-derived xenograft (CDX) and patient-derived xenografts (PDX) with MED = ~2mpk. Importantly, L100-MMAE (LCB22A-MMAE) and L100-exatecan (LCB22A-exatecan) were well-tolerated in cynomolgus monkey toxicity studies, with no significant adverse events observed. Additionally, the ADC selectively killed target cells in a human mixed organotypic cancer model without affecting healthy cells, indicating excellent tolerability and safety. By specifically targeting cancer-specific O-glycans expressed only in cancer tissues and employing a tumor-selective payload release mechanism, L100 ADC (LCB22A) offers a promising and safe therapeutic option for treating breast cancer while avoiding the on-target off-tumor toxicity that plague other ADC therapies.

#7210 Predicting drug response heterogeneity in AML: novel AI-enabled proteomics model for a prospective clinical trial.

James T. Sorrentino[1], Salvador Martinez-Bartolome[1], **Afshin Mahmoudi**[1], Antonious Koller[1], Mahan Abbasian[1], Christina Lamm[1], Mohamed Rdait[1], Mary-Elizabeth Percival[1], Jasmine Naru[2], Derek L. Stirewalt[1], Pilgrim Jackson[1]

[1]Yatiri Bio, Inc., San Diego, CA,[2]Fred-Hutch, Seattle, WA

Acute myeloid leukemia (AML) is an aggressive blood cancer with a significant chance of relapse and only a 17% 5-year survival rate for patients refractory to first-line therapy. Current therapies, even when combined with newer agents, have not significantly enhanced long-term survival for many AML patients. One major barrier in the development of effective therapies for AML and their successful deployment for patients, has been the lack of accurate model systems to identify and predict therapeutic efficacy in patient subsets. Current AML cell lines do not predict drug sensitivity because the cell lines harbor strong driver alterations that occur in only a small fraction of patients and their biology has drifted from that of an AML patient. With state-of-the-art culture conditions, we have created over 50 patient-derived hematopoietic stem/progenitor cell (HSPC) models and have measured cellular response across 40 AML therapeutics. Leveraging our pipeline for LC-MS/MS and innovative data integration, we have created a patient landscape with embedded cell models for prospective therapeutic positioning. HSPCs were evaluated using a panel of cell surface markers, and preliminary results show marginal drift from the original patient samples. Unbiased proteomics were generated with peripheral blood samples from 100 AML patients and all ex-vivo models. Pathway overrepresentation and correlation analysis are used to confirm that patient-derived HSPCs are closer in biological context to patient subpopulations than commercially available cell lines. Additionally, global proteomics data from over 2500 samples from 11 public and 5 private datasets across 8 different cancer indications were collected to perform a molecular functional analysis to guide a transfer learning model for matching patients to ex vivo HSPCs. A custom deep learning architecture was built to (1) define global cancer mechanism enrichments of the AML samples and (2) integrate paired ex vivo treatment response. To drive a prospective clinical trial at FHCC, our model was used to select a minimal set of proteins that can accurately separate patient subsets for which there are differential sensitivity profiles across 4 AML therapeutics. For this trial, 40 patients with relapsed/refractory AML or other high-grade myeloid neoplasms will be enrolled and randomized to one of two arms. Arm A: receive standard of care hypomethylating agent therapy (20 patients). Arm B: undergo an ex vivo MS-based targeted assay on the peripheral blood to predict response to various treatment regimens; if a drug is identified through the assay, patients will receive that treatment (20 patients). The primary objective of the trial is to assess the feasibility of a single MS-based assay for the selection of the ideal AML therapeutic for each patient to be used in combination with azacitidine to achieve response, extend survival, and improve quality of life.

#7211 Proteolysis targeting chimeras (PROTACs) of oncogenic RET protein.

Shriya Pandey[1], Yafeng Wang[2], Xueqing Hu[1], Ujjwol Khatri[1], Tao Shen[1], Vivek Subbiah[3], Blaine H. M. Mooers[4], Ting Chao[2], Shaohui Wang[5], Huaxuan Yu[2], Xingmin Sun[6], Jianfeng Cai[2], Jie Wu[1]

[1]Department of Pathology, Peggy and Charles Stephenson Cancer Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK,[2]Department of Chemistry, University of Southern Florida, Tampa, FL,[3]Early-Phase Drug Development, University of Oklahoma Health Sciences Center, Sarah Cannon Research Institute, TN,[4]Department of Biochemistry and Physiology, Peggy and Charles Stephenson Cancer Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK,[5]Department of Biochemistry and Physiology, University of Southern Florida, University of Southern Florida, FL,[6]Department of Biochemistry and Physiology, University of Southern Florida, Tampa, FL

The REarranged during Transfection (RET) kinase is an oncoprotein and a therapeutic target for RET alteration-positive cancer. Two RET-selective protein tyrosine kinase inhibitors, selpercatinib (LOXO-292) and pralsetinib (BLU-667), have been approved for treating RET-alteration-positive cancer. However, achieving a complete response with a RET kinase inhibitor remains a challenge in most patients. In cell cultures, these inhibitors increase the expression of CCDC6-RET fusion protein, which may affect their effectiveness at low concentrations. To tackle this problem, we synthesized and characterized a panel of RET PROTACs for the dual action of inhibiting RET kinase activity and degrading the oncogenic protein. A lead compound, YW-N-7, was selected based on *in vitro* PROTAC analyses and *in vivo* PK data. YW-N-7 exhibited the dual action of specifically inhibiting and degrading RET protein. Proteomic analysis identified RET as the only significantly down-regulated protein in the entire proteome by YW-N-7 compared with the RET kinase inhibitor. In the BaF3/KIF5B-RET tumor model, YW-N-7 inhibited and degraded KIF5B-RET oncoprotein in xenograft tumors in animals, and significantly inhibited tumor growth. This work illustrates the potential of developing a RET PROTAC for simultaneously inhibiting and degrading oncogenic RET kinase for cancer therapy.

#7212 Preclinical analysis of ONC206 and ONC212 with lurbinectedin in pancreatic cancer.

Tej Tummala, Ashley S. S. Uruchurtu, Abbas E. Abbas, Christopher G. Azzoli, Wafik S. El-Deiry

Legorreta Cancer Center at Brown University, Providence, RI

Pancreatic cancer is a devastating disease with a poor five-year survival rate of approximately 12%. Given the poor prognosis of the disease, it is vital to develop novel improved therapeutic regimens. Lurbinectedin is a chemotherapeutic that stalls and degrades RNA Polymerase II after binding to guanine-rich sequences in the minor groove of DNA, stalling transcription and inducing single- and double-stranded DNA breaks. Our group recently described lurbinectedin's potency as both a single agent and combinatorial agent with irinotecan and 5-fluorouracil in pancreatic cancer cell lines. Our group also recently described a synergistic interaction between lurbinectedin and imipridone ONC201/Dordaviprone, an anticancer compound that induces the TRAIL apoptotic pathway, in small cell lung cancer cell lines. We now demonstrate that a combination of lurbinectedin and ONC212, a chemical derivative of ONC201, results in highly efficient and synergistic killing of pancreatic tumor cells in vitro. Under combinatorial conditions, cells seemingly exhibit an upregulation of DR5 and γH2AX and a downregulation of ClpX in the presence of ONC212. Future studies will analyze whether a combination of lurbinectedin and ONC212 or lurbinectedin and ONC206, another imipridone and chemical analogue of ONC201, proves more efficient at killing pancreatic tumor cells in vitro. Our results are developing insights into novel combinatorial therapeutic regimens while investigating the molecular mechanisms underlying synergism.

#7213 Targeting XPO1 to enhance the durability of response of MRTX1133 in pancreatic ductal adenocarcinoma.

Husain Yar Khan[1], Amro Aboukameel[1], Sahar F. Bannoura[1], Md Hafiz Uddin[1], Fulya Koksalar Alkan[1], Ahmet B. Caglayan[1], Hilmi K. Alkan[1], Bin Bao[1], Hugo Jimenez[1], Mohammed Najeeb Al Hallak[1], Khalil Choucair[1], Steve Kim[1], Rafic Beydoun[2], Gregory Dyson[1], Yang Shi[1], Misako Nagasaka[3], Azeddine Atfi[4], Hasan Korkaya[1], Muhammad Wasif Saif[1], Philip A. Philip[5], Bassel El-Rayes[6], Anthony F. Shields[1], Ramzi M. Mohammad[1], Boris C. Pasche[1], Asfar S. Azmi[1]

[1]Oncology, Wayne State University School of Medicine, Barbara Ann Karmanos Cancer Institute, Detroit, MI,[2]Pathology, Wayne State University School of Medicine, Detroit, MI,[3]University of California Irvine School of Medicine, Orange, CA,[4]Oncology, Massey Cancer Center, Virginia Commonwealth University, Richmond, VA,[5]Henry Ford Cancer Institute - Henry Ford Health System, Detroit, MI,[6]Hematology and Oncology, University of Alabama at Birmingham, Birmingham, AL

Introduction: The KRASG12D inhibitor MRTX1133, which targets KRAS in the OFF-state, is currently under evaluation in pancreatic ductal adenocarcinoma (PDAC) patients. However, as seen with other OFF-state KRAS inhibitors, MRTX1133 may provide only limited improvements in disease-free survival due to the emergence of drug resistance. This highlights the need for strategies to enhance the efficacy of KRAS inhibitors in PDAC. The nuclear transport protein, XPO1, plays a significant role in several pro-tumorigenic pathways and has been identified as a therapeutic vulnerability in KRAS-mutant cancers. It is overexpressed in many cancers, including PDAC, and its inhibition causes robust synthetic lethal interaction with oncogenic KRAS, indicating that targeting both XPO1 and KRAS may be beneficial. In this study, using several preclinical PDAC models, we have demonstrated that the second generation XPO1 inhibitor KPT8602 synergizes with and enhances the efficacy of MRTX1133.

Methods: We generated KRASG12D inhibitor-resistant PDAC cells and assessed the effect of KPT8602 on their survival. We further tested different combinations of MRTX1133 and KPT8602 for their antiproliferative effects in 2D and 3D *in vitro* PDAC models. To test the *in vivo* antitumor efficacy of the combination, we used KRASG12D-mutant human PDAC cell-derived xenograft (CDX) and murine PDAC transgenic KPC cell-derived allograft models.

Results: KPT8602 sensitized MRTX1133-resistant PDAC cells to growth inhibition, establishing that KRASG12D inhibitor-resistant cancer cells can potentially respond to KPT8602. Furthermore, treating multiple PDAC cell lines in 2D and 3D cultures with KPT8602 and MRTX1133 at different dose combinations resulted in synergistic inhibition of their viability. At the molecular level, the combination downregulated p-ERK, mTOR, p-4EBP1, DUSP6 and cyclin D1 expression in PDAC cells. Kinome analysis revealed reduced kinase activity for several kinases related to MAPK signaling in the combination treated PDAC cell lysates. Combining subtherapeutic doses of KPT8602 (15 mg/Kg BIW) and MRTX1133 (30 mg/Kg QD) caused significant tumor growth inhibition in multiple subcutaneous and orthotopic PDAC mouse models ($p<0.001$). Also, the combination treatment resulted in significantly enhanced survival in an immunocompetent orthotopic PDAC allograft model ($p<0.001$). Moreover, we have also demonstrated that maintenance with KPT8602 in combination treated mice prevented tumor relapse and resulted in a durable antitumor response in a PDAC CDX model.

Conclusion: This is the first study showing that KPT8602 treatment can overcome resistance to MRTX1133 and synergistically augment the antitumor effects of MRTX1133 in PDAC preclinical models. This combination regimen can potentially prevent or delay drug resistance by making it feasible to achieve a more durable response at a lower dose of KRASG12D inhibitor.

#7214 Real-world concordance analysis of a combinatorial functional precision medicine platform, Optim.AI™, in both solid and hematological cancers.

Masturah Mohamed Abdul Rashid[1], Jhin Jieh Lim[1], Weng Tong Ho[1], Su Pin Choo[2], Donald Yew Hee Poon[3], Daryl Chen Lung Tan[4], William Ying Khee Hwang[5], Yvonne Loh[2], Jeffry Beta Tenggara[6], Chin Hin Ng[7], Hugo Saavedra[1], **Edward Kai-Hua Chow**[1]

[1]KYAN Technologies Pte Ltd, Singapore, Singapore,[2]Curie Oncology, Singapore, Singapore,[3]Dr D.Y.H Poon & Associates Medical Oncology Clinic, Singapore, Singapore,[4]Clinic for Lymphoma, Myeloma and Blood Disorders, Mount Elizabeth Novena Hospital, Singapore, Singapore,[5]National Cancer Centre Singapore, Singapore, Singapore,[6]Division of Hematology and Medical Oncology, Department of Internal Medicine, MRCCC Siloam Hospitals Semanggi, Jakarta, Indonesia,[7]Centre for Clinical Haematology, Singapore, Singapore

PURPOSE: Functional precision medicine aims to expand the landscape of effective drug treatment options beyond standard of care through additional insights from ex vivo phenotypic drug response data. In our previous work, we have demonstrated the clinical feasibility of Optim.AI™, a combinatorial functional precision medicine platform, in identifying effective and resistant treatment options for non-Hodgkin lymphoma and acute myeloid leukemia (AML). This study further investigates the clinical utility and concordance of Optim.AI™ in predicting treatment sensitivity or resistance in a variety of hematological and solid cancers processed at KYAN's clinical laboratory, with notable case studies highlighted.

METHODS: Optim.AI™ utilizes efficiently designed experiments of less than 200 ex vivo combinations to interrogate over 530,000 possible treatment permutations from a 12-drug panel. Optim.AI™ then ranks all clinically actionable treatments within the tested drug panel based on predicted sensitivity or resistance. In this study, we investigated 66 cases from a total of 20 cancer types (5 hematological and 15 solid cancer) and a range of tissue sample sources, including bone marrow aspirate, peripheral blood, tissue biopsy or resections, and pleural or peritoneal fluid. Retrospective concordance analysis was performed by evaluating Optim.AI™-generated tumor cell viability results against the prior lines of treatment for each sample. Prospective data of patients administered with Optim.AI™-predicted treatment were also collected, where applicable.

RESULTS: With an established viability cut-off of 0.5, Optim.AI™ demonstrated a correlation of 88% with prior therapies, which included a range of 36 targeted treatments and chemotherapy agents. Among the 37 solid cancer cases evaluated, 86.5% (p-value < 0.0001) demonstrated concordance to prior line(s) of treatment, while for hematological cancers, 89.7% of the 29 cases (p-value = 0.0005) were concordant with previously observed resistance. Additionally, platform-predicted treatments were associated with positive clinical responses in several cases of hematological and solid cancers, where prospective data were available.

CONCLUSIONS: Our retrospective concordance results demonstrate the clinical applicability and robustness of Optim.AI™ in predicting sensitivity or resistance to cancer treatments. In line with previous prospective clinical studies, these findings illustrate the platform's potential to positively impact treatment recommendations across a broad range of hematological and solid cancers. Future prospective studies to evaluate Optim.AI™-guided treatments are warranted to further confirm the predictive value of the platform and provide evidence for incorporating such functional assays in clinical oncology.

#7215 Targeting EZH2 in castration-resistance prostate cancer using a novel PROTAC.

Qi Chu[1], Wanqing Xie[1], Galina Gritsina[1], Xiaodong Lu[1], Ka Wing Fong[2], Zhiquan Lei[2], Hongshun Shi[1], Lourdes Brea[2], Jonathan Zhao[3], Gary Schiltz[4], Jindan Yu[1]

[1]Urology, Emory University, Atlanta, GA,[2]Department of Medicine, Northwestern University, Chicago, IL,[3]Human Genetics, Emory University, Atlanta, GA,[4]Chemistry, Northwestern University, Evanston, IL

Background: EZH2, a core subunit of the Polycomb Repressive Complex 2 (PRC2), is significantly upregulated in advanced Prostate Cancer (PCa), especially in Castration-Resistant Prostate Cancer (CRPC). However, enzymatic inhibitors of EZH2, such as EPZ-6438, have shown limited efficacy in PCa, while EZH2 knockdown has previously shown to lead to cellular senescence and major growth suppression, warranting the development of Proteolysis Targeting Chimera (PROTAC) to degrade all EZH2 proteins.
Methods: Medicinal chemistry was utilized to develop a plethora of EZH2 PROTACs. Mass spectrometry and western blotting were used to assess target protein levels. RNA-seq and H3K27me3 ChIP-seq analyses were performed in PCA cells to evaluate transcriptional and histone modification changes. Senescence staining and flow cytometry were used to evaluate cellular senescence and analyze cell cycle progression. Cell proliferation was assessed in vitro using CellTiter-Glo and live-cell proliferation assays. In vivo efficacy was evaluated using 22Rv1 and VCaP xenograft mouse models.
Results: Among several EZH2 PROTAC degraders, 6272 was chosen as the lead candidate due to its high specificity and efficiency in degrading EZH2. We showed that 6272 significantly inhibited 22Rv1 and VCaP cell proliferation compared to EPZ-6438, although this effect was not observed in LNCaP cells. RNA-seq analysis revealed that 6272 readily induced cellular senescence pathways, such as CDKN1A (p21) expression, in 22Rv1 and VCaP, but not LNCaP cells. Further, ChIP-seq showed no changes in H3K27me3 at the CDKN1A gene locus, indicating that 6272 regulates these genes in a catalytic function-independent manner. Lastly, we confirmed that 6272 also significantly reduced 22Rv1 and VCaP xenograft tumor growth.
Conclusion: In this study, we developed a novel EZH2 PROTAC with superior efficacy compared to EPZ-6438 in suppressing some prostate cancer cells via rapidly promoting cellular senescence.

#7217 Synergistic anti-cancer effects of dual IGF-1R and EGFR targeting in uveal melanoma.

Pei-Chi Tsai, Charles G. Eberhart, **Su-Chan Lee**

Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD

Uveal melanoma arises from melanocytes in the eye, with approximately 7,000 patients reported worldwide every year. IGF-1R plays an important role in uveal melanoma metastatic progression, and promotes tumor cell proliferation and survival. However, in previously reported clinical trials, targeting IGF-1R did not significantly inhibit uveal melanoma metastatic spread or patient survival. This could be due to compensatory activation of other receptor signaling pathways. Therefore, the aim of this study is to find other activated receptors in uveal melanoma cells following IGF-1R inhibition which could be co-targeted in a novel combination treatment for metastatic uveal melanoma. Using several uveal melanoma cell lines (Mel202, Omm2.3, Omm2.5, Omm1, 92.1, MP38 and MM28), we found that treatment with linsitinib, an IGF-1R inhibitor, increased EGFR protein expression and activity. Targeting IGF-1R alone with linsitinib, or EGFR alone with gefitinib, did not significantly inhibit growth of uveal melanoma cells. However, combination treatment with linsitinib (10μM) and gefitinib (5μM) significantly inhibited both cell growth and anchorage-dependent/independent colony formation abilities in uveal melanoma cultures. Moreover, Linsitinib and gefitinib co-treatment dramatically increased cleaved PARP protein, an apoptotic maker. Our research suggests that dual targeting of IGF-1R and EGFR could be a novel treatment strategy to inhibit uveal melanoma metastatic dissemination and growth.

#7218 Preventive use of trilaciclib in Chinese patient with extensive-stage small cell lung cancer (ES-SCLC) receiving chemotherapy: A multi-center, single-arm, observational real-world study.

Ying Liu[1], Jianqiang Bi[2], Jianxin Chen[3], Meifang Chen[4], Xuesong Chen[5], Yongxing chen[6], Yinghua Ji[7], Yujie Jiang[8], Lu Li[9], Qin Liao[10], Sheng Lin[11], Jie Liu[12], Yi Liu[13], Yu Liu[14], Zhaoting Meng[15], Zhuqing Mu[16], Yunxiang Qi[17], Yanhong Shang[18], Ling Xu[19], Shufeng Xu[20], Lu Zheng[21], Jin Zhou[17], **Ying Cheng**[1]

[1]Jilin Cancer Hospital, Changchun, China,[2]CangZhou Hospital of Integrated Traditional Chinese and Western Medicine, Cangzhou, China,[3]Quzhou People's Hospital, Quzhou, China,[4]Taizhou Hospital of Zhejiang Province, Taizhou, China,[5]Jiangsu Province Hospital, Nanjing, China,[6]Hainan General Hospital, Haikou, China,[7]The First Affiliated Hospital of Xinxiang Medical University, Xinxiang, China,[8]Affiliated Hospital of Youjiang Medical University for Nationalities, Baise, China,[9]West China Hospital of Sichuan University, Chengdu, China,[10]Shulan (Hangzhou) Hospital, Hangzhou, China,[11]The Affiliated Hospital of Southwest Medical University, Luzhou, China,[12]Henan Cancer Hospital, Zhengzhou, China,[13]Shaanxi Provincial People's Hospital, Xi'an, China,[14]HanDan Central Hospital, Handan, China,[15]Tianjin Medical University Cancer Institute and Hospital, Tianjin, China,[16]Xinxiang Central Hospital, Xinxiang, China,[17]Sichuan Cancer Hospital & Institute, Sichuan Cancer Center, School of Medicine, University of Electronic Science and Technology of China, Chengdu, China,[18]Affiliated Hospital of Hebei University, Baoding, China,[19]Anhui Chest Hospital, Hefei, China,[20]First Hospital of Qinhuangdao, Qinhuangdao, China,[21]Ningbo Medical Center Lihuili Hospital, Ningbo, China

Background: Extensive-stage small cell lung cancer (ES-SCLC) is a highly aggressive disease characterized by rapid progression and development of extensive metastases. Standard chemotherapy-based regimens for ES-SCLC often lead to myelosuppression, compromising medication compliance and prognosis. Trilaciclib is a CDK4/6 inhibitor, approved by NMPA in 2022 to decrease the incidence of chemotherapy-induced myelosuppression (CIM) in adult with ES-SCLC. This study aims to evaluate the efficacy and safety of preventive use of trilaciclib prior chemotherapy in Chinese ES-SCLC population under real-world settings.

Methods: ES-SCLC patients suitable for combined treatment of trilaciclib and chemotherapy were enrolled in this study. Eligible participant received 240 mg/m^2 of trilaciclib IV 4 h prior chemotherapy. The primary endpoint was the incidence rate of severe neutropenia (SN). The secondary endpoints included the occurrence of grade 3/4 hematologic adverse events (HAEs), administration rate of G-CSF and ESA, safety, and anti-tumor efficacy of chemotherapy.

Results: A total of 201 ES-SCLC patients receiving at least one dose of trilaciclib combined with chemotherapy were included from 60 centers in China during March 10 2023 to August 10 2024, with median age of 65 (58, 71). Metastases occurred in 90.0% of the subjects at baseline, in which brain metastases accounted for 16%. 92.5% of the patients adopted platinum plus etoposide (E/P) regimen. The mean number of completed chemo-cycle was 3.0 (1.4). In Cycle 1, 1 case (0.6%) of SN occurred in patient receiving second-line (2L) treatment with E/P therapy. Grade 3/4 HAEs were reported in 5.4% of the patients in Cycle 1, including neutropenia (3.6%), anemia (1.2%) and thrombocytopenia (1.8%). During the whole treating period, a total of 5 cases (2.6%) of SN occurred, 4 of which received 2L treatment with E/P, while the remaining one received first-line cis-platinum therapy. 17.5% participants experienced grade 3/4 HAEs during the study, in which neutropenia, anemia and thrombocytopenia accounted for 8.2%, 9.3% and 3.1%, respectively. G-CSF and ESA were used in 2.6% and 1.0% of the patients. As for safety, grade ≥3 AEs were reported in 20.9% of the participants. No AEs-related treatment discontinuation happened. No serious AEs and death occurred. Among 48 patients included into tumor response analysis, the best response to chemotherapy was CR (8.3%), PR (43.8%), SD (39.6%) and PD (8.3%). The objective response rate (ORR) and disease control rate (DCR) reached 52.1% and 91.7%, respectively.

Conclusions: Prophylactic administration of trilaciclib (240 mg/m^2) prior chemotherapy exhibits superior myeloprotective effect and were well-tolerated in Chinese ES-SCLC population, providing evidence for clinical benefit of trilaciclib as a myelo-protectant.

#7219 Targeting the RAS pathway with MEK and pan-RAF inhibition in Philadelphia-like acute lymphoblastic leukemia.

Hong A. De Sa, Isabel Kenna, Jeffrey W. Tyner, Bill H. Chang

Knight Cancer Institute, Oregon Health & Science University, Portland, OR

Philadelphia-like (Ph-like) acute lymphoblastic leukemia (ALL) afflicts close to 30% of adolescents and young adults with ALL and portends a poor response to standard chemotherapy. Ph-like ALL often harbors CRLF2-rearrangements (CRFL2-R) and JAK2 mutations. CRLF2-R and JAK2 can further activate RAS signaling with or without RAS pathway mutations, suggesting a role to target this pathway in Ph-like ALL. Here, we report our results using a novel combination of the MEK inhibitor trametinib (tram) and the pan-RAF inhibitor tovorafenib (tovo) in a CRLF2-R ALL patient-derived xenograft. We identified a CRLF2-R ALL sample from our institution's biorepository. The sample had an IGH-R by cytogenetics, CRLF2 overexpression by flow cytometry, and JAK2 by NGS. The sample was injected into immunodeficient (NSG) mice. Peripheral blood flow cytometry for human CD19 and human CD45 vs mouse CD45 expression was monitored. Following engraftment >20%, mice were sacrificed and mononuclear cells isolated from the spleen were exposed to drugs to assess *in vitro* response. *In vivo* response was then tested in cohorts of 5 mice injected per condition. Upon engraftment, cohorts were treated with vehicle, 1mg/kg/day tram, 12.5mg/kg/day tovo, or the combination daily until moribund. CRLF2-R ALL cells showed *in vitro* sensitivity to tram as assessed by viability and phosphorylation of ERK at low nM concentrations. These cells showed minimal response to tovo at μM concentrations. Combination with tram and tovo did not show added benefit. Upon injection, mice engrafted within 1 month and rapidly succumbed to disease. *In vivo* treatment with single agent or combinations briefly, but significantly improved survival compared to control (Table 1). Targeting the RAS pathway in CRLF2-R ALL has therapeutic potential. Interestingly, addition of the pan-RAF inhibitor tovorafenib to trametinib did not have further therapeutic benefit. These findings begin to shed insight into novel targets for CRLF2-R ALL.

Survival Outcomes

	Median survival (days)	Log-rank p-value to control	Log-rank p-value to combination
Control	3		
Tovorafenib	8	**0.0035	*0.0116
Trametinib	15	**0.0035	ns 0.4235
Combination	13	**0.0035	

#7220 Zongertinib demonstrates potent efficacy against cancer cells harboring HER2 non-tyrosine kinase domain mutations.

Y. Shibata[1], M. B. Nilsson[1], H. Udagawa[2], J. P. Robichaux[1], J. He[1], X. Yu[1], J. V. Heymach[1];
[1]UT MD Anderson Cancer Center, Houston, TX, [2]National Cancer Center Hospital East, Kashiwa, Japan

Activating mutations in HER2 are found in approximately 2-4% of non-small cell lung cancer (NSCLC). Recurrent activating mutations most commonly occur in the tyrosine kinase domain (TKD) encoded by exons 18-21, with exon 20 insertions being the most frequent, followed by non-TKD mutations in the transmembrane domain (TMD) and juxtamembrane domain (JMD). While tyrosine kinase inhibitors (TKIs) such as the HER2-selective inhibitor zongertinib and the pan-HER inhibitors poziotinib, pyrotinib, and afatinib have been tested primarily in patients harboring TKD mutations, their efficacy against non-TKD mutations has been less well characterized. To systematically evaluate HER2 TKI activity against HER2 non-TKD mutations, we established a panel of Ba/F3 cell lines expressing various HER2 mutations including common TKD mutations (e.g. exon 20 insertions YVMA, GSP, and VC), TMD mutations such as V659E, and JMD mutations such as R678Q and evaluated drug sensitivity by Cell Titer Glo assay. Among HER2 TMD mutations, zongertinib showed potent activity with an average IC50 value of 8.72 nM. For HER2 JMD mutations, zongertinib demonstrated an IC50 value of 0.35 nM, and among HER2 TKD mutations, zongertinib treatment yielded an average IC50 value of 6.14 nM. Moreover, calculation of the relative IC50 value, representing the ratio of IC50 values between HER2 mutations and wild-type EGFR, revealed that zongertinib which had an average log IC50 mutant HER2/wild-type EGFR ratio of -2.31, -3.71, -2.46, for TMD, JMD, and TKD HER2 mutations, respectively, exhibited superior selectivity compared to pyrotinib (TMD -1.00, JMD -4.85, TKD -0.55) and poziotinib (TMD 0.09, JMD -3.13, TKD 0.48). This suggests zongertinib has high efficacy and a potentially lower risk of EGFR inhibition-associated toxicity. In addition, we generated a HER2 TMD mutant NSCLC cell line model by expressing HER2 V659E in HCC4006 cells and knocking out endogenous mutant EGFR. Consistent with our results obtained using Ba/F3 models, HCC4006 HER2 V659E cells were sensitive to HER2 inhibition with zongertinib. Our findings indicate that zongertinib demonstrates comparable or more potent inhibitory activity for HER2 non-TKD mutants (e.g TMD and JMD) as HER2 TKD mutations, providing a potential therapeutic option for patients with these HER2 non-TKD mutant cancers.

#7221 Whole genome and transcriptome sequencing can be introduced into the diagnostic workup of sarcoma and pediatric cancer patients.

Svetlana Nikic[1], Kristine N. Jinnett[2]

[1]Precision Oncology Consulting, Barcelona, Spain,[2]Kristine Jinnett Consulting, Dublin, Ireland

Introduction: Limited molecular diagnostics in pediatric cancers and sarcomas hinder accurate diagnosis and therapy selection, due to an inability to detect some clinically relevant large and rare rearrangements. This meta-analysis evaluates the potential of WGS/WGTS to overcome these limitations and improve personalized oncology care.
Methods: A systematic review and meta-analysis of English-language studies (PubMed; 01/08/2024-5/11/2024) was conducted per PRISMA guidelines to evaluate the clinical impact of WG(T)S in pediatric cancers and sarcomas. Included studies were primary research articles with data on clinical impact, validity, utility, and/or feasibility.
Results: A total of 14 studies (5 sarcoma and 9 pediatric) were deemed eligible for inclusion in the study. Analysis revealed a wide turnaround time (TAT) range of 9-71 days. Sequencing depths ranged from 45-100X for tumor and 30-40X for normal samples, whilst feasibility of WG(T)S ranged from 62-100%. Our analysis found that WG(T)S demonstrated significant clinical utility in sarcoma patients leading to a refined/changed diagnosis in 18.6%, actionable findings in 64%, and therapy-informing findings in 36% of cases, on average. In pediatric cancers, WG(T)S led to a refined/changed diagnosis in 22.9%, actionable findings in 42.6%, and therapy-informing findings in 38.5% of cases, on average. We also found that, on average, 7.3% of both sarcoma and pediatric cancer patients harbored germline findings (Table 1).
Conclusions: WG(T)S enhances diagnostic yield by detecting large rearrangements (e.g., structural variants, gene fusions) critical for accurate diagnosis and risk stratification in pediatric cancers and sarcomas. Our meta-analysis demonstrates the clinical utility of WG(T)S, suggesting its potential for routine use in oncology.

Analysis of clinical utility of WG(T)S in sarcoma and pediatric cancer patients. N/A, not availab

Initial Patient Cohort **(No. of patients/samples that underwent genomic profiling)**	Refined Diagnosis	Actionable Alterations	Therapy-informing Findings	Germline Findings	Cancer Type
1,256 (**597**) (Prendergast, Strobl et al, 2020)	3%	55%	N/A	1.7%	Sarcoma
83 (**83**) (Schipper, Monkhorst et al, 2022)	14%	N/A	36%	8%	Sarcoma
73 (**67**) (Watkins, Trotman et al, 2024)	37%	N/A	33%	4%	Sarcoma
200 (**197**) (Öfverholm, Wallander et al, 2024)	7%	N/A	15%	11%	Sarcoma
25 (**15**) (Andrew, Lewin et al, 2024)	32%	73%	60%	13%	Sarcoma
Average %	**18.6%**	**64%**	**36%**	**7.5%**	Sarcoma
247 (**247**) (Wong, Mayoh et al, 2020)	5.2%	39.9%	67%	16.2%	Pediatric cancers
309 (**253**) (Newman, Nakitandwe et al, 2021)	N/A	N/A	25%	18%	Pediatric cancers
36 (**36**) (Trotman, Armstrong et al, 2022)	17%	N/A	22%	8.3%	Pediatric cancers
143 (**114**) (Shukla, Levine et al, 2022)	21%	N/A	15%	18%	Pediatric cancers
300 (**264**) (Villani, Davidson et al, 2023)	6.5%	56%	54%	15%	Pediatric cancers
149 (**117**) (Wadensten, Wessman et al, 2023)	50%	N/A	26%	8.5%	Pediatric cancers
281 (**281**) (Hodder, Leiter et al, 2024)	38%	N/A	7%	7%	Pediatric cancers
87(**79**) (Deyell, Shen et al, 2024)	N/A	32%	64%	12%	Pediatric cancers
384 (**384**) Lau, Khuong-Quang et al, 2024)	N/A	N/A	67%	N/A	Pediatric cancers
Average %	**22.9%**	**42.6%**	**38.5%**	**7.2%**	Pediatric cancers

IMMUNOLOGY: Novel Animal Models / Tumor Antigenicity/Processing and Presentation
Poster Session

#7225 The secreted cytokine Chitinase 3-like-1 promotes breast cancer metastasis.

Adeline Masse[1], Tarek Taifour[2], Yu Gu[1], Virginie Sanguin-Gendreau[1], Dongmei Zuo[1], Bin Xiao[1], Hailey Proud[1], Nancy U. Lin[3], Melissa E. Hughes[3], Kalie Smith[3], Rinath Jeselsohn[3], William J. Muller[1]

[1]Biochemistry, McGill Univ. Goodman Cancer Ctr., Montreal, QC, Canada,[2]Experimental Medicine, McGill Univ. Goodman Cancer Ctr., Montreal, QC, Canada,[3]Medical Oncology, Dana-Farber Cancer Institute, Boston, MA

Breast cancer is the second most common cancer and a major cause of mortality affecting women worldwide. Immunosuppression is a key hallmark of cancer progression, whereby cancer cells suppress the immune system that is programmed to fight the disease. This suppressed tumor immune microenvironment (TIME) facilitates cancer metastatic spread to distant organs, which is associated with treatment resistance, recurrence, and poor prognosis. The Signal transducer and activator of transcription 3 (Stat3) suppresses the immune system, drives cancer progression and metastasis. Genetic ablation of Stat3 in the murine inducible breast tumor model expressing polyoma virus middle T (PyMT), results in delayed tumor onset and reduced tumor growth and metastasis. We have also previously demonstrated that the secreted protein Chitinase 3-like-1 (Chi3l1) is a direct target of Stat3 which promotes immunosuppression through its actions on neutrophils. Chi3l1 recruits neutrophils and induces the formation of Neutrophil Extracellular Traps (NETs) that block T cells from infiltrating the tumor. Targeting both Chi3l1 or neutrophils resulted in increased T cell infiltration and delayed tumor onset. However, the roles of Chi3l1 during the metastatic cascade remained unclear. To further investigate the role of Chi3l1 in metastasis, we generated a novel inducible mouse model that overexpresses Chi3l1 specifically in the mammary epithelial cells. Overexpression of the Chi3l1 protein in the Stat3 knockout PyMT model showed a rescue of the delay in tumor onset. Consistent with our previous studies, Chi3l1 overexpression caused a dramatic influx of neutrophils and the generation of an immune suppressed TIME. In addition, we observe an increase in circulating tumor cells which correlated with a quicker onset and increased incidence of pulmonary metastasis. These results hint that in breast cancer, Chi3l1 overexpression creates a pro-tumorigenic TIME which favorizes tumor initiation and progression as well as induces intravasation of tumor cells into the blood stream, which leads to metastasis to distant sites. Our future studies will investigate whether this phenotype is driven through neutrophils and whether targeting neutrophils would inhibit Chi3l1-mediated metastasis.

#7226 Development of syngeneic tobacco associated oral cancer models using *Trp53* and *Cdkn2a* mutant mice.

Roberto Rangel[1], Roberto Rangel Jr[1], Alanis E. Rodriguez-Rosario[1], Nicholas Balbin[1], Liping Dong[1], Arnoldo Corona[1], Ahmed Tajmim Noor[1], Guillermina Lozano[2], Andrew G. Sikora[1], Jeffrey N. Myers[1]

[1]Head and Neck Surgery, UT MD Anderson Cancer Center, Houston, TX,[2]Genetics, UT MD Anderson Cancer Center, Houston, TX

Background: Immunotherapy has revolutionized cancer treatment by leveraging therapeutic antibodies to block immune checkpoint inhibitors (ICIs). However, despite numerous clinical trials, success remains limited, likely due to tumor models lacking the appropriate genetic mutations or being studied in non-orthotopic sites factors critical to tumor response. To address these limitations, we developed syngeneic oral cancer models featuring defined mutations in *Trp53* and *Cdkn2a*, the most frequently altered genes in human papillomavirus-negative head and neck squamous cell carcinoma (HNSCC). This mutation combination correlates with aggressive disease and poor survival outcomes in HNSCC patients. Using a carcinogenesis approach in single- and double-mutant *Trp53/Cdkn2a* mice, we established a preclinical platform to study HNSCC, a malignancy with limited therapeutic options.

Methods: *Krt14-Cre* transgenic mice carrying *Trp53*-LOF, *Trp53R172H*, *Trp53R245W*, and *Cdkn2a*-LOF mutations were backcrossed onto a C57BL/6 genetic background. Oral carcinogenesis was induced via 16 weeks of low-dose 4-nitroquinoline 1-oxide in drinking water. Tumors from the tongues of treated mice underwent histopathological analysis and tissue culture immortalization. Orthotopic injections of immortalized tumor cells into C57BL/6J mice enabled validation of tumorigenic cell lines. RNA profiling quantified ICIs, cytokines, chemokines, and immune cell markers via qPCR. Sensitivity of the resulting genetically defined ROC tongue tumors to cisplatin, gemcitabine, and anti-PD-1 immunotherapy was assessed.

Results: Mutations in *Trp53* and *Cdkn2a* significantly accelerated tongue tumor development. Mice harboring double germline mutations exhibited more aggressive tumor progression compared to single-mutation or wild-type controls. From these tumors, we established eight syngeneic ROC cell lines, each harboring distinct *Trp53/Cdkn2a* co-mutations confirmed through sequencing. These cell lines, free of mycoplasma and verified to have unique mouse-origin STR profiles, were tumorigenic in C57BL/6J mice. The cell lines displayed variable expression levels of ICIs, cytokines, chemokines, and immune cell markers, and exhibited differential responses to chemotherapy and anti-PD-1 immunotherapy. Importantly, the models demonstrated immune "hot" and "cold" tumor microenvironments, including immune-excluded phenotypes.

Conclusions: Our study establishes syngeneic oral cancer models with *Trp53* and *Cdkn2a* mutations as robust platforms for investigating oral cancer immune evasion and optimizing immune-based therapies. These models provide a complementary tool for human clinical trials by facilitating hypothesis testing and advancing therapeutic strategies targeting the tumor microenvironment and immune system.

#7227 An inducible, spatially restricted mouse model of lung cancer.

Israel Ifeoluwa Odekunle, Sydney Remington, Allison Racela, David J. Seward

Department of Pathology and Laboratory Medicine, University of Vermont Cancer Center, Burlington, VT

Accurately modeling human lung tumor initiation and progression in genetically engineered mouse models (GEMMs) remains a challenge. In contrast to human lung cancer development, where patients typically present with a solitary tumor nodule, the majority of GEMMs produce numerous independent primary lung lesions. Each of these lesions then evolves as an independent tumor, a situation not relevant to human lung cancer biology. The Cre-loxP recombination system is widely used to generate GEMMs, including the inducible, tamoxifen-dependent systems that allow cell-specific conditional activation via promoter regulation. We have used this system extensively to generate KRAS-driven Lung adenocarcinomas arising from club cells via the club cell secretory protein (CCSP) Cre-ERT2 system. While powerful, this model has limitations. Specifically, limiting tumor induction to a single location within the lung is impossible. To address this limitation, we have combined the highly efficient photoactivable Cre (PA-Cre) recombinase mouse (JAX: 033544) with a novel CCSP-FlpO mouse to spatially limit tumor formation based on targeted blue-light exposure. Exploiting the CCSP promoter enables us to limit FlpO recombinase expression to club cells, thereby confining the two light-activatable Cre halves to club cells. Following focal trans-cutaneous blue light exposure, the PA -Cre halves dimerize and induce the oncogenic alleles, KRAS p.G12D, and Serine Threonine Kinase 11 loss, respectively. We assert that utilizing this approach will overcome major limitations inherent in current GEMMs used to study lung cancer and provide a paradigm-shifting platform to study lung cancer initiation and progression.

#7228 Long-term humanization of NOG mice and next-generation NOG strains to induce lineage-specific differentiation of immune cells for assessment of novel immune cell therapies, check point inhibitors, and immune cell engagers for translational immuno-oncology research.

Maria Stecklum[1], Louise Baskin[2], **Jens Hoffmann**[1]

[1]EPO GmbH, Berlin, Germany,[2]Taconic Biosciences, Inc., New York, NY

Background: The preclinical evaluation of novel immune therapies requires humanized immune system (HIS) mouse models. In previous studies we have demonstrated that either peripheral blood mononuclear cells (PBMC), subsets of PBMCs like T and NK cells or CD34+ hematopoietic stem cells (HSC) can be used to establish a HIS model. With the development of next-generation NOG mice, lineage-specific differentiation of immune cell sub-populations of interest can be supported. Transplantation of cell line-derived (CDX) or patient-derived (PDX) tumor xenografts on HIS mice provides a full model for human tumors for the investigation of checkpoint inhibitors (CPI), as well as novel cell therapies and immune cell engagers.
Methods: HSC-humanized mice were generated by i.v. transplantation of CD34+ stem cells to immunodeficient mice. For humanization, NOG mice and next-generation NOG strains were used: NOG, NOG-EXL, hIL-2 NOG, hIL-6 NOG and FcResolv™ NOG mice were compared to each other for lineage-specific differentiation using single donors or a mixed HSC donor pool. Long-term survival was monitored and engraftment of immune cells was analyzed by FACS of blood every four weeks. After termination, spleen, bone marrow and thymus was also screened for human immune cells. In humanized NOG mice, CDX and PDX from different entities were s.c. transplanted and used to evaluate CPI.
Results: Humanized hIL-2 NOG mice showed significantly decreased survival after HSC transplantation in comparison to the other mouse strains and had to be sacrificed within the first 6-8 weeks after HSC transplantation. In the other mouse strains, transplanted HSCs engrafted and differentiated mainly into B and T cells. NOG-EXL mice displayed the highest engraftment, with up to 80% of human cells in the blood, including a higher portion of myeloid cells after 8 to 12 weeks. Humanized NOG, hIL-6 NOG and FcResolv™ NOG mice showed the longest survival rate with over 400 days for single donor transplantation. In humanized NOG mice, selected CDX and PDX tumors successfully engrafted without significant differences in tumor growth compared to non-humanized mice. CPI treatments with Nivolumab or Ipilimumab induced tumor growth delay in selected models.
Conclusions: Next-generation NOG mouse strains are characterized by a lineage-specific differentiation of immune cells depending on integrated human cytokines. Furthermore, we established a human tumor-immune-cell model for NOG mice using different entities of CDX or PDX in combination with CPI. Our human tumor-immune cell models allow preclinical translational studies on tumor immune biology as well as evaluation of new therapies, drug combinations and biomarker identification and validation.

#7229 Humanized mouse models of γδ T engraftment for the testing of cell based anti-cancer therapeutics.

Kevin Tsai, Nancy Zheng, Beau Parry, Destanie Rose, James G. Keck, Li-Chin Yao

Innovation and Product Development, The Jackson Laboratory, Sacramento, CA

One of the major hurdles of T cell-based immune therapy is identifying a T cell receptor that works for all patients. The γδ T cells, which express T cell receptors that can recognize several evolutionarily conserved cell surface molecules commonly become elevated in cancer cells, may solve this problem. There is currently no mouse model capable of expanding these γδ T cell subsets *in vivo*. Using human peripheral blood mononuclear cells (PBMC) engrafted NSG mouse strains, we compared γδ T cell engraftment in mice with or without irradiation to explore whether irradiation can aid the expansion of human γδ T cells and if so, which of our new NSG derivatives is best suited for human γδ T cells expansion.

Non-irradiated or irradiated NSG-MHC I/II double knock-out (DKO), NSG-FLT3- MHC I/II DKO (FDKO), and NSG-SGM3-IL-15 × MHC I/II DKO (SDKO) mice were engrafted with PBMCs. For non-irradiated studies, 7 different donors were used. For irradiated studies, 3 different donors were used. In the irradiated studies, mice were taken down 9 days post engraftment. In the non-irradiated studies, human leukocyte engraftment was monitored via flow cytometry staining of retro-orbital blood for up to 93 days. At indicated time points following engraftment, tissue samples were collected to enumerate the frequency and number of γδ T cells. Robust and rapid γδ T cell expansion was observed in all irradiated NSG derivatives with the SDKO mice showing the highest concentration of circulating γδ T cells 9 days post engraftment (40-70 γδ T cells/μL of blood) followed by the FDKO mice (20-50 γδ T cells/μL of blood) with the DKO mice showing the lowest (1-10 γδ T cells/μL of blood). The frequency of γδ T cells within the hCD45 population was 1-5% in all three strains, which is physiologically similar to that in humans. In addition, γδ T cells were found in the spleen and mucosal sites (e.g. colon and lungs) of engrafted mice. In non-irradiated mice, both SDKO and FDKO mice showed higher γδ T cell engraftment rates relative to the DKO mice up to 93 days post engraftment. However, the expansion rate was slower than that of the irradiated mice: reaching the highest percentage of engraftment 28-42 days post engraftment. The γδ T cells could be recovered from the blood, spleen, and lungs of SDKO and FDKO mice but almost devoid in the DKO mice. Interestingly, with some donors, more γδ T cells were found in the SDKO mice whereas with other donors, in the FDKO mice.

Irradiated SDKO mice showed maximal expansion and recovery of γδ T cells from the spleen, blood, and mucosal sites. By comparison, the γδ T cell expansion was slower in the blood, spleen, and lungs of SDKO and FDKO non-irradiated mice but remained detectable even up to
93 days post engraftment while up to 80% of the DKO mice had lost their γδ T cell engraftment. Studies to investigate anti-tumor capacity of γδ T cells are currently underway.

#7230 New mouse strains NSG-SGM3xIL15xDKO and NSG- FLT3LxDKO are superior to NSG-MHC I/II DKO mice for humanization of peripheral blood mononuclear cells.

Nancy (Xiaoqing) Zheng, Kevin Tsai, Guoxiang Yang, Beau Parry, **Destanie Rose**, James Keck, Li-Chin Yao

The Jackson Laboratory, Sacramento, CA

Background: Peripheral blood mononuclear cells (PBMC)-humanized mice serve as important platforms to evaluate efficacy and toxicity. NSG-MHC I/II double-knock out (DKO) mice have been widely used due to being a humanized T cell model with delayed graft-versus-host disease. However, achieving at least 5% engraftment within a week requires 10-15e6 PBMCs per mouse with irradiation, which accelerates PBMCs to engraft compared to non-irradiated mice. Additionally, engraftment of certain cell populations, such as natural killer (NK) cells and monocytes from PBMC has been challenging in the DKO strain. Therefore, we aimed to develop new mouse strains that can be better humanized with lower PBMC cell counts while gaining a variety of cell subtypes. Next generation DKO strains including NSG-SGM3xIL15xDKO (SDKO) and NSG-FLT3LxDKO (FDKO) engrafted with multiple PBMC donors were compared.
Methods: Nonirradiated SDKO, FDKO, and DKO mice were intravenously (IV) injected with 10e6 (5 donors) or 15e6 (2 donors) PBMCs and retro-orbital breeds were collected for engraftment comparisons for up to 70 days. Additionally, we compared PBMC engraftment of SDKO, FDKO, and DKO with and without irradiation (2 donors) for 70 days.
Results: When mice were engrafted with 10e6 PBMCs without irradiation, SDKO and FDKO achieved higher human CD45+ cells engraftment level in the periphery blood compared to DKO. Depending on the PBMC donors, SDKO or FDKO can achieve the highest engraftment rate sooner and reach the highest human CD45+ cell count among all the three strains. The peak engraftment levels of hCD45+ cells in SDKO or FDKO were 1.5-2.6 times higher than that in DKO. When mice were engrafted with15e6 PBMCs without irradiation, SDKO was engrafted rapidly and reached the highest engraftment level compared to the other two strains. FDKO was engrafted faster and showed a higher level than DKO mice before 28 days post-humanization. hCD3+T cells and hCD19+ B cells were the major cell populations post PBMC engraftment with significantly higher levels in SDKO and FDKO, compared to DKO. In addition, hCD8+ T cells and hCD56+ NK cells were engrafted at significantly higher numbers in SDKO compared to FDKO and DKO. Irradiation helped B cells to engraft at higher levels in SDKO and DKO for short term studies.
Conclusions: The two new mouse strains, SDKO and FDKO, are engrafted with human PBMCs at a faster rate, and higher levels compared to DKO mice. In addition, SDKO provides better engraftments of CD8+ T cells and NK cells. Irradiated SDKO mice reach a higher hCD45+ peak engraftment level compared to the DKO. Thus, SDKO and FDKO may serve as new and improved platforms to assess preclinical immuno-oncology efficacy and toxicity.

#7231 Immuno-oncology drugs fast in vivo efficacy test IO-FIVE for research and precision medicine.

Bin Xie[1], Yang Liu[1], Yu Di[1], Xiaorong Gu[1], Kaimeng Hu[1], Song Xu[1], Loc Van[2], Josh Caggiula[2], Diandong Jiang[1], Danyi Wen[1]

[1]Shanghai LIDE Biotech Co., Ltd., Shanghai, China,[2]LIDE Biotech Co., Ltd., Doylestown, PA

Immunotherapy is becoming a promising cancer treatment for microsatellite-instability-high (MSI-H) pan-cancers, and combination therapy with IO drugs and anti-angiogenesis or antibody-drug-conjugates is considered as a first-line therapy for advanced or unresectable hepatocellular carcinoma and bladder cancer respectively. Though human CD34+HSC and PBMC humanized models are valuable tools to investigate the *in vivo* function and mechanism of immuno-oncology drugs, the allogeneic reaction between targeted cancer tissue and human immune system is a serious issue. Obviously, this could interfere with the fundamental reaction of the testing drugs particularly with mismatched HLA donors. Thus, there is an urgent and unmet need for a preclinical *in vivo* model that effectively mimics patients through autologous immune systems. LIDE's Mini-PDX (mini-patient-derived xenografts) model has been widely applied to test the *in vivo* chemosensitivity of chemical and targeted compounds for more than 3000 clinical biopsies in the last decade. Leveraging this *in vivo* organoid system, we have developed the immuno-oncology version of MiniPDX, named "Immuno-Oncology drugs Fast *In Vivo* Efficacy" test or IO-FIVE. This assay uses patient cancer cells with its microenvironment immune cells and stroma cells isolated from the same fresh biopsy, well mixed and co-inoculated in mini-capsules before implantation into immune deficient mice. Total tissue viability and phenotyping of each cell subsets could be assessed within 2 weeks by Celltiter Glo and flow cytometry analysis respectively. Furthermore, omics study would shed new light for deeper understanding about the mechanism. Using this model, we found that Daratumumab (CD38-Ab) had a tumor suppression effect in less than half of CD38+ acute myeloid leukemia patients (response rate is around 30% in all AML N>60), and laboratory IO-FIVE data guided clinical Sintilimab (PD1-Ab), bevacizumab and capecitabine combo-therapy leads to a stable disease (SD) type for one advanced lung metastatic colon cancer patient with microsatellite stable genotype. Consistent to clinical trials, PD1 antibody (e.g., Sintilimab) monotherapy seems have limited tumor control effect in around one-fourth of ovarian cancer patients (N=11), IO and PARP inhibitor combo-therapy or novel drugs (bispecific antibody, ADCs) may enhance the immunotherapy efficiency and sensitivity. Our IO-FIVE model is offered as a unique *in vivo* functional assay to help do patient stratification as well as MOA studies.

#7232 KDM1A/UTX Mutations Modulate Innate Immune Responses to Influence Immunotherapy Outcomes in Lung Squamous Cell Carcinoma.

Feifei Na, Xiaofeng Deng, Yuying Wang, Zhenkun Zhang, Yang Yang, Liting You, Zuoyu Liang, Yu Liu, Chong Chen

West China Hospital, Sichuan University, Chengdu, China

Introduction: Immunotherapy has come of age for lung cancers, especially lung squamous cell carcinoma (LUSC), which lacks targeted therapies . *KDM6A/UTX* is frequently mutated in LUSC and is associated with poor prognosis. However, the mechanisms by which *KDM6A/UTX* mutations influence the efficacy of immunotherapy in LUSC remain unclear. **Methods:** We developed genetically engineered murine models of LUSC by culturing lung organoids from adult C57B/L6 mice, using Cas9 and sgRNAs to introduce driver mutations of LUSC. These organoids were then orthotopically transplanted into the lungs of recipient C57B/L6 mice to establish in vivo LUSC models. Tumor growth and progression were monitored through live imaging and micro-CT. Histopathological assays were performed to validate the models' accuracy in representing human LUSC. Multi-omics methods were used to analyze the the biological behavior of Kdm6a-mutant tumor cells, their response to immunotherapy, and identify key regulatory target genes. Potential therapeutic targets identified through these analyses are currently under further validation. **Results:** A LUSC mouse models with sg*Trp53*; sg*Pten*; *SOX2*; *Myc; Cas9* (TPSM) was established. Histopathological analyses showed that these models represented the features of human disease. Tumor organoids were successfully developed from these models and serial transplantation confirmed that these organoids remained the characteristics of the original models. Loss of *Kdm6a/UTX* significantly enhanced the growth of LUSC tumor organoids in vivo and promoted metastasis. Notably, LUSC tumors with *Kdm6a/UTX* mutations exhibited a poor response to immune checkpoint inhibitors (ICIs), and showed significantly shortened survival in treated mice. Multi-omics analyses revealed that Kdm6a/UTX mutations upregulated several pathways associated with tumor progression, including focal adhesion, epithelial-mesenchymal transition (EMT), and epithelial cell migration. In contrast, immune-related pathways such as virus response and interferon-β signaling were downregulated. These mutations also resulted in a decreased recruitment of immune cells, including cytotoxic T cells (CD8+), into the tumor microenvironment, potentially contributing to immune evasion and the poor response to immunotherapy. **Conclusion:** The new LUSC mouse models developed in this study provide a platform for investigating the mechanisms of LUSC progression and response to immunotherapy. Our results suggest that *Kdm6a/UTX* play a critical role in modulating the tumor immune microenvironment, promoting immune evasion, and reducing the efficacy of immunotherapy. Further validation of the identified molecular targets and their therapeutic potential is underway.

#7233 An in vivo co-injection model for studying macrophage polarization and therapeutic repolarization in tumor development.

Philipp Metzger, Valerie Oberhardt, Carla N. Castro, **Holger Weber**

Reaction Biology Europe GmbH, Freiburg, Germany

Macrophages play a critical role in tumor development by shaping the tumor microenvironment. In particular, the M2-polarized macrophage phenotype is associated with pro-tumoral functions such as promoting angiogenesis, immune suppression, and tumor progression. Consequently, numerous therapeutic approaches aim to repolarize M2 macrophages to mitigate their tumor-supportive behavior. While these effects are consistently and effectively demonstrated in vitro in our laboratory, translating these findings into in vivo models remains a significant challenge. Here, we present an in vivo co-injection model that allows the detailed study of macrophage-tumor cell interactions and the evaluation of potential therapeutic agents targeting macrophage polarization. First, we isolated monocytes from heathy donors and differentiated them in vitro into various phenotypes (M0, M1, and M2) according to our established protocols. We selected a tumor cell line that secretes high levels of macrophage survival promoting factors and has good in vivo growth characteristics. In this model we co-injected the tumor cells together with macrophages in NXG animals. Our results demonstrate that the co-injected M2 macrophages remains stable for over a week in the tumor microenvironment, closely mimicking the persistence observed in human tumors. Additionally, M0 macrophages undergo differentiation under the influence of the tumor microenvironment, reflecting the dynamic and plastic nature of macrophage polarization in vivo. Marker expression as well as functional properties, such as phagocytotic activity of pHrodo Zymosan particles ex vivo, was analyzed by flow cytometry.
This model provides a biologically relevant system for studying macrophage-targeting therapies, complementing the robust and reproducible in vitro experiments routinely conducted in our laboratory. The presence of the M0 and M2 macrophages over a significant period of time enhance the model's utility for evaluating substances designed to repolarize macrophages in vivo.
In conclusion, this in vivo co-injection model represents a valuable tool for preclinical research into macrophage polarization and its impact on tumor progression. Its potential ability to replicate key aspects of macrophage-tumor cell interactions enables comprehensive investigations of therapeutic strategies targeting the tumor microenvironment. This approach has the potential to accelerate the development of novel treatments aimed at disrupting the pro-tumoral functions of M2 macrophages and enhancing anti-tumoral immune responses.

#7234 Unveiling the role of B7H3 in driving the tumor immune microenvironment and progression of small cell lung cancer.

Mahek Fatima[1], Parvez Khan[2], Md Arafat Khan[2], Asad Ur Rehman[2], Laiba Anwar[2], Aatiya Ahmad[2], Jaewon Lee[3], Imayavaramban Lakshmanan[2], Anish Thomas[4], Dinesh Pradhan[5], Apar K. Ganti[6], Brad St Croix[3], Surinder K. Batra[2], Mohd Wasim Nasser[2]

[1]University of Nebraska Medical Center, Omaha, NE,[2]Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE,[3]Tumor Angiogenesis Unit, National Cancer Institute, Frederick, MD,[4]Developmental Therapeutics Branch, National Cancer Institute, Bethesda, MD,[5]Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE,[6]Department of Hematology-Oncology, University of Nebraska Medical Center, Omaha, NE

Lung cancer is the leading cause of cancer-related deaths globally, with small cell lung cancer (SCLC) representing a highly aggressive subtype. The prognosis for SCLC remains dire due to early metastasis, chemoresistance, high recurrence rates, and limited therapeutic options. Despite the approval of some immunotherapeutic drugs for SCLC, their efficacy is restricted to a small subset of patients. This limited success is attributed to low expression of PD-L1 and the immune-cold tumor microenvironment in SCLC, underscoring the urgent need for new therapeutic targets. Bioinformatic analysis of SCLC datasets, focusing on immune checkpoint regulators, identified B7H3 (CD276) as a promising candidate upregulated in SCLC. This finding was confirmed in SCLC cell lines and human tissue samples. B7H3 is a cell surface protein overexpressed in multiple tumor types, functioning as both an immune modulator and an oncogenic driver. However, its role in SCLC tumorigenesis remains unclear. To investigate the functional involvement of B7H3 in SCLC, we employed CRISPR-Cas9 to knock out the *B7H3* gene in SCLC cell lines. B7H3 knockout significantly reduced transwell migration and wound-healing abilities in SCLC cells. When co-cultured with T-cells, B7H3-proficient SCLC cells suppressed T cell activation, unlike their B7H3-deficient counterparts. To therapeutically target B7H3, we used the antibody-drug conjugate (ADC) m276-SL-PBD in the spontaneous SCLC mouse model RPM (Adeno-CGRP-Cre; $Rb1^{flox/flox}$; $TP53^{flox/flox}$; $LSL\text{-}Myc^{T58A}$) which significantly reduced tumor burden, metastasis, angiogenesis, and increased T cell infiltration. To further elucidate the role of B7H3 in SCLC progression, we generated a novel spontaneous mouse model, RPMC (Adeno-CGRP-Cre; $Rb1^{flox/flox}$; $TP53^{flox/flox}$; $LSL\text{-}Myc^{T58A}$; $Cd276^{fl/fl}$). These mice exhibited a marked decrease in tumor burden, tumor-associated angiogenesis, and metastasis, with a significantly increased T-cell infiltration compared to controls. Transcriptomic analysis of B7H3-proficient/-deficient cells revealed NFIB among the top 50 downregulated genes following B7H3 deletion. NFIB, a key driver of SCLC metastasis, was significantly reduced in B7H3-deficient human and syngeneic (RPMC) cell lines. Metastatic tissues from RPMC mice also showed lower NFIB expression compared to the RPM model. In conclusion, this study highlights the critical role of B7H3 as well as modulation of NFIB expression in regulating SCLC progression. Our findings establish B7H3 as a promising therapeutic target and demonstrate the potential role of CD276 ADCs in overcoming the limitations of current treatments of SCLC. By advancing our understanding of B7H3's role in promoting tumor growth, this study paves the way for designing more effective therapeutic strategies to combat SCLC.

#7235 New mouse strain NSG-SGM3xIL15xDKO is better for PBMC engraftment in Raji-bearing mice of minor immune subsets compared to NSG-MHC I/II DKO mouse.

Nancy (Xiaoqing) Zheng, Guoxiang Yang, James Keck, **Li-Chin Yao**

The Jackson Laboratory, Sacramento, CA

Introduction: PBMC-humanized MHC I/II double knock-out (#025216, DKO) strain has shown strong T cell engraftment with minimal acute GVHD. We recently have developed two new mouse strains, NSG-SGM3xIL15xDKO (SDKO) and NSG-FLT3LxDKO (FDKO), and their engraftment comparison data will be presented in the same meeting. Here we aimed to investigate the engraftment of various immune cell types in SDKO and FDKO mice in the presence of tumor stimulation.
Methods: We assessed PBMC engraftment levels and immune subtypes in irradiated SDKO, FDKO, and DKO implanted with lymphoma (Raji-luc and Nalm6-luc) and breast cancer (MDA-MB-231-luc) xenografts. In addition, we investigated the differences between irradiated and non-irradiated mice of the above three strains implanted with Raji-Luc cells. Whole blood via retro-orbital bleeds and spleen were collected to analyze engraftment and immune cell populations by flow cytometry. Five different PBMC donors were used.
Results: We found irradiated SDKO and FDKO achieved higher PBMC humanization levels compared to DKO. Interestingly, Raji-luc cells stimulated PBMCs to engraft at a significantly higher level compared to PBS, MDA-MB-231-luc and Nalm6-luc cells. hCD3+ T cells were activated (HLA-DR+) in Raji-luc groups. The stimulatory effect of Raji-luc cells was also validated in the three strains without irradiation. PBS treated irradiated mice without tumor stimulation, both SDKO and FDKO had approximately 9-15 folds engraftment of CD19+ B cells and CD56+ natural killer (NK) cells, and some donors showed 2-4 folds engraftment of CD11c+HLA-DR+ dendritic cells and CD64+ macrophages compared to what was seen in DKO mice in early engraftment. After Raji-luc cells stimulation, the engraftments of above cell types were enhanced 3-4 folds over the DKO. Non-irradiated SDKO and FDKO showed a 2-3 folds increase in B cells and NK cells compared to those in DKO mice in early engraftment in peripheral blood in the PBS group. With Raji-luc cells, the engraftments of B cell and NK cells were enhanced 2-4 folds in SDKO and FDKO over DKO mice. Other tumor data will also be presented.
Conclusion: Both PBMC-humanized SDKO and FDKO show improved NK cells and myeloid cells engraftment compared to DKO after tumor antigen stimulation. The response may vary based on PBMC donors and cancer cell lines. Irradiation also helps certain cell types like myeloid cells to engraft compared to no irradiation in the cancer models.

#7236 First successful engraftment of human pancreatic cancer cells in an immunocompromised porcine model to test tumor ablation by high-frequency irreversible electroporation (H-FIRE).

Manali Powar[1], Tamalika Paul[1], Edward Jacobs[2], Sherrie Clark-Deener[1], Christopher Byron[1], Sheryl Coutermarsh-Ott[1], Katie Orr[1], Maxx Steinmann[1], levente Gal[1], Cassandra Poole[1], Carley Elliott[3], Mackenzie Woolls[1], Madeline Mott[1], Julio Arroyo[2], Zaid Salameh[2], Hannah Ivester[1], Rafael V. Davalos[2], Irving Coy Allen[1]

[1]Virginia Tech, Blacksburg, VA,[2]Georgia Tech, Atlanta, GA,[3]Graduate Program in Translational Biology, Medicine and Health, Virginia Tech, Blacksburg, VA

Pancreatic tumor is difficult to treat due to late diagnosis, critical tumor location, limited surgical options, and high metastatic potential. High-frequency irreversible electroporation (H-FIRE) is a non-thermal ablation technique that uses high-voltage electric pulses to create permanent nanopores in cell membranes, disrupting homeostasis and inducing cell death. This study established the feasibility of using H-FIRE to ablate pancreatic tumors in an immunocompromised porcine model were RAG2/IL2RG double knockout pigs. Panc1 human pancreatic cancer cells were orthotopically injected into the pancreas of these pigs. 3 weeks post-injection, successful tumor engraftment and growth were observed during treatment. We successfully accessed and treated the tumors using H-FIRE, with clear ablation zones and preservation of surrounding tissue confirmed by histopathology. The treatment also showed minimal local muscle contraction. This study demonstrated the first robust large-animal model of H-FIRE-treated human pancreatic cancer with safety and efficacy. Future work will explore the potential of these immunocompromised porcine models for developing advanced therapeutic strategies to enhance tumor ablation by H-FIRE.

#7237 Rapid-onset squamous cell carcinoma and spontaneous regression following papillomavirus infection.

Andrea Bilger[1], Ella T. Ward-Shaw[1], Denis L. Lee[1], Renee E. King[1], Shwu-Yuan Wu[2], Michael A. Newton[3], Cheng-Ming Chiang[4], Darya Buehler[5], Kristina Matkowskyj[6], John P. Sundberg[7], Rong Hu[5], Paul F. Lambert[1]

[1]Oncology, University of Wisconsin-Madison, Madison, WI,[2]Biochemistry, The University of Texas Southwestern Medical Center, Dallas, TX,[3]Biostatistics and Informatics, University of Wisconsin-Madison, Madison, WI,[4]Biochemistry; Pharmacology, The University of Texas Southwestern Medical Center, Dallas, TX,[5]Pathology and Laboratory Medicine, University of Wisconsin-Madison, Madison, WI,[6]Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN,[7]The Jackson Laboratory, Bar Harbor, ME

Squamous cell carcinoma (SCC) development in mammals is thought to require months or years from the time that initial changes occur. We show that SCCs induced by the mouse papillomavirus MmuPV1, which are generally diagnosed 4-6 months post infection, can be found as soon as 2 weeks post infection. SCCs developed within 2 weeks in immune-competent, adult FVB (FVB/NTac) mice at sites prone to human papillomavirus (HPV)-induced tumors and cancers in human patients: the base of the tongue, the anus, and the skin. The anterior tongue, less susceptible than the base of the tongue to HPV-induced cancer in humans, was resistant to rapid-onset cancer. The presence of invasive cancer in histological tissue sections was verified by multiple pathologists. These diagnoses were corroborated by the results of a classic test of malignancy: skin tumors obtained two weeks post infection grew ectopically as subcutaneous grafts in immune-deficient hosts. Preventative treatment with a novel BRD4 inhibitor dramatically reduced the frequency of ear skin SCCs present 2 weeks post infection and significantly reduced overall disease severity. Like many human papillomaviruses, MmuPV1 lacks an E5 gene. Expression of HPV16 E5 driven by the Keratin 14 promoter in FVB mice (FVB/NTac-Tg(KRT14-HPV16E5*)33Plam/Plam) significantly increased the frequency of rapid-onset invasive cancers caused by MmuPV1 at the base of the tongue and in the anus. In an E5-transgenic mouse with a persistent MmuPV1-induced ear skin lesion, we detected a lymph node metastasis at 38 weeks - further demonstrating a cancer phenotype. Cancers and less severe dysplasia induced by infection at the base of the tongue or the anus spontaneously regressed within 4 to 8 weeks of infection; however, ear lesions persisted. When CD4+ and CD8+ T cells were depleted, lesions at the base of the tongue and anus also persisted, indicating that spontaneous regression involves a T cell-mediated immune response. These results indicate that papillomavirus infection alone is sufficient to induce rapid onset of invasive cancer, and that persistence of disease depends on the tissue-specific immune microenvironment.

#7238 Quantitative profiling of HLA class I and II antigens and neoantigens in tissue and PBMC samples with an improved mass spectrometry approach.

A. Viode, A. Pfeiffer, L. Raess, M. Tognettti, T. Temu, O. M. Bernhardt, **D. Redfern**, Y. Feng, R. Bruderer, L. Reiter;
Biognosys AG, Schlieren, Switzerland

Human leukocyte antigen (HLA) molecules are critical mediators of immune surveillance, functioning to present antigenic peptides derived from self and non-self proteins to T cells. By facilitating the recognition of pathogenic or abnormal cells, such as cancer cells, HLA molecules play a pivotal role in guiding immune responses and enabling targeted immune interventions. In oncology clinical trials, the use of peripheral blood mononuclear cells (PBMCs) offers significant advantages for immunopeptidomics (IMPX) and potential neoantigen discovery. Unlike solid tissue biopsies, which may be limited in availability and require invasive procedures, PBMCs can be routinely isolated from blood, allowing for serial sampling. PBMCs comprise a diverse population of immune cells, including lymphocytes (T cells, B cells, and NK cells) and monocytes, which collectively serve as a holistic snapshot of the body's immune status, enabling the tracking of immune modulation, and providing pharmacodynamics readout across various disease contexts.

We developed and optimized an unbiased IMPX profiling workflow leveraging native lysis and magnetic bead-based immunoprecipitation for isolating both class I and class II immunopeptides. By refining the lysis and pulldown conditions, we ensured effective extraction and enrichment of HLA complexes from minimal starting material, achieving robust profiling across varying sample types, including cell lines, tissues, and PBMCs. To accommodate the often limited PBMC availability in real-world clinical settings, we tested this pipeline with inputs as low as 5 million PBMCs, achieving high-quality, reproducible IMPX data. Further downstream, LC-MS setup and AI-based bioinformatics solutions were optimized to support streamlined data generation and analysis. Overall, the developed workflow demonstrated high reproducibility and specificity across sample types, identifying robust immunopeptide profiles: (1) For class I immunopeptides, >60% of peptides identified were 9-mers, with >80% predicted as strong binders, exhibiting expected amino acid enrichment at anchor residues; (2) For class II immunopeptides, >50% of peptides were 14-to-16-mers, with >50% predicted as strong binders. Input material ramping tests showed that >2,800 class I immunopeptides could be consistently identified from 2.5 mg of fresh-frozen tissue, and 2,000-3,000 class I immunopeptides from as few as 5 million PBMCs. Moreover, we profiled the immunopeptidomes of several representative cancer cell lines (e.g. SHP77, OV56) with known HLA subtype(s) that are commonly used in TCR therapeutics target discovery.

In summary, we have developed a high-sensitivity, robust pipeline for class I and II immunopeptidome profiling that is compatible with low-input samples, making it suitable for both target discovery and clinical applications.

#7239 Investigating the role of topoisomerase IIB in the regulation of antigen presentation pathway in high-grade serous ovarian cancer.

Shraddha G. Gupta, Sangeeta Kumari, Adam Karami, Aisha Jamil, Sergey Karakashev

Temple University, Philadelphia, PA

Epithelial ovarian cancer (EOC) is the deadliest gynecologic malignancy and accounts for 90% of ovarian tumors. High grade serous ovarian cancer (HGSOC), an aggressive subtype of EOC, develops rapidly and is correlated with poor prognosis. Importantly, HGSOC accounts for 70-80% of deaths related to ovarian cancer. Immunotherapies that have revolutionized the field of cancer therapy demonstrate limited therapeutic efficacy in HGSOC. HGSOC tumors are characterized by low immune cell infiltration and reduced anti-tumor immune response. Hence, it is crucial to develop new therapeutic strategies that improve anti-tumor immune response in HGSOC. Epigenetic suppression of antigen processing and presentation pathway (APP) is one of the major mechanisms adopted by cancer cells to escape T-cell cytotoxicity. APP entails binding of the cytotoxic CD8+ T cells to neoantigen presented by Major Histocompatibility Complex Class I (MHC class I) on the membrane of cancer cells. Neoantigen recognition by CD8+ T cells lead to activation of CD8+T cells and subsequent killing of the antigen-presenting cells (APC). Previous studies have demonstrated that defects in the expressions and functions of APP components correlate with reduced T-cell infiltration, metastasis, and poor overall survival. Our bioinformatics analysis of publicly available ovarian cancer patient dataset from The Cancer Genome Atlas (TCGA) and ovarian cancer cell line database (Depmap) revealed DNA topoisomerase 2-beta (TOP2B) as a potential negative regulator of APP. TOP2B is a topoisomerase that regulates the transcription of its target genes by altering the topological state of DNA. Recently, a small molecule inhibitor, CX-5461, was discovered to specifically target TOP2B. It has also completed Phase I clinical trials for solid tumor and hematological malignancies demonstrating its safety and efficacy in human patients. Understanding the role of TOP2B in cancer progression may lead to the development of a novel therapeutic strategy to improve overall survival of HGSOC patients.

#7240 The role of myeloid cell derived HMGB1 in the development of hepatocellular carcinoma.

Sai Santosh Babu Komakula, Xiaodong Ge, Wei Chen, Romain Desert, Hui Han, Zhuolun Song, Sukanta Das, Dipti Athavale, Nithyananthan Subramaniyam, Chao Wang, Daniel Lantvit, Abid Anwar, Natalia Nieto

Pathology, University of Illinois at Chicago, Chicago, IL

Background: High mobility group box 1 (HMGB1) is a non-histone chromatin-associated protein involved in the pathogenesis of chronic liver disease. HMGB1 is expressed in myeloid cells, including conventional dendritic cells (cDCs), which play a major role in the tumor microenvironment. However, whether intracellular myeloid cell-derived HMGB1 is involved in hepatocellular carcinoma (HCC) is unknown. We hypothesize that intracellular HMGB1 drives cDC maturation towards LAMP3^{+} DCs, allowing effective cytotoxic CD8^{+} T cell responses to reduce HCC.
Methods: We analyzed publicly available scRNA-seq datasets from human HCC for the expression of HMGB1 in all subsets of DCs in HCC tumor and non-tumor tissues and in hepatic draining lymph nodes (dLNs). We generated mice with conditional ablation or overexpression of *Hmgb1* in myeloid cells (*Hmgb1*$^{\Delta Mye}$ and *Hmgb1*$^{KI\ Mye}$). To induce HCC, 14-day-old male mice were injected i.p. with diethylnitrosamine (DEN) and were sacrificed at 5, 6, 8, and 10 months. Histopathological analysis of livers was performed using H&E staining. Immune cell populations were analyzed in tumor and non-tumor tissues using flow cytometry. cDCs were isolated from tumor and non-tumor tissues by FACS, and RNA-seq analysis was performed.
Results: Analysis of scRNA-seq datasets revealed LAMP3^{+} DCs increased in human HCC tumor tissue and hepatic dLNs. LAMP3^{+} DCs displayed increased activation and migration scores. Moreover, HMGB1 was highly expressed in cDCs (CLEC9A^{+} and CD1C^{+}), which can differentiate to LAMP3^{+} DCs. *Hmgb1*$^{KI\ Mye}$ mice were protected from HCC, whereas control mice developed HCC after 8 months, and *Hmgb1*$^{\Delta Mye}$ mice started developing HCC at 5 months. Macroscopic analysis and H&E staining of the livers from *Hmgb1*$^{\Delta Mye}$ mice showed more tumors and higher tumor volume than control and *Hmgb1*$^{KI\ Mye}$ mice. Immunohistochemistry of HCC tumor sections revealed that *Hmgb1*$^{\Delta Mye}$ mice have fewer LAMP3^{+} DCs in tumor tissues than control and *Hmgb1*$^{KI\ Mye}$ mice. Flow cytometry analysis showed that *Hmgb1*$^{KI\ Mye}$ mice had significantly increased cDCs in tumor tissues and dLNs than control and *Hmgb1*$^{\Delta Mye}$ mice. In addition, there was enhanced CD8^{+} T cell apoptosis in the HCC tumor tissues from *Hmgb1*$^{\Delta Mye}$ mice. RNA-seq data of cDCs unveiled that pathways involved in antigen presentation were downregulated in *Hmgb1*$^{\Delta Mye}$ mice and upregulated in *Hmgb1*$^{KI\ Mye}$ mice compared to control mice.
Conclusions: Ablation of myeloid-derived HMGB1 accelerates HCC development in mice. Enhancing HMGB1 expression in myeloid cells increased the number and the antigen presentation activity of cDCs. Therefore, increasing HMGB1 expression in cDCs could be a therapeutic approach to protect from HCC.

#7241 Tumor-derived APi1 inhibits antigen cross-presentation in dendritic cells and impairs anti-tumor immunity in breast cancer.

Erwei Song, Di Huang, **Ruxin Wang**

Sun Yat-sen University, Guangzhou, China

Tumor mutational burden (TMB), usually representing high immunogenicity, could not always predict treatment response of immune checkpoint blockade (ICB). Here, we showed that defective antigen cross-presentation in type 1 conventional dendritic cells (cDC1) was responsible for lacking tumor-specific cytotoxic T lymphocytes (CTLs) in triple-negative breast cancer (TNBC) patients. Mechanistically, we found a tumor-derived protein, termed antigen presentation inhibitor 1 (APi1) , was shuttled via extracellular vesicles (EVs) into the endosomes of intratumor DCs, inhibited antigen cross-presentation by locking antigen binding to HSP90 and precluding their translocation from endosomes to cytoplasm. APi1 knockdown in tumor cells or inhibiting APi1/HSP90 interaction in DCs efficiently promoted antigen translocation and enhanced their cross-presentation, which improved ICB therapeutic responses. Clinically, high tumor APi1 expression was associated with low infiltration of antigen-specific CTLs and poor ICB efficacy in TNBC patients. Therefore, tumor EV-shuttled APi1 locks antigen/chaperone interaction and impairs antigen cross-presentation in DCs. Moreover, targeting APi1 is promising to enhance anti-tumor immunity and reverse ICB resistance.

#7242 Inhibition of ULK1/2-mediated autophagy augments antigen processing and presentation promoting increased antigen specific CD8+ T-cell cytotoxicity.

Jhansi L. Leslie, Umida Djakbarova, Samantha S. Hodge, Logan Chinn, Natalie Hill, Kayla Walzer, Hyunyoung Moon, Xuelei Yan, Anne Van Abbema, Yihong Guan, Connor Rosen, Jenna L. Jeffrey, Ester Fernandez-Salas, Susan L. Paprcka

Arcus Biosciences Inc., Hayward, CA

BACKGROUND: High levels of ULK1/2-mediated macroautophagy (autophagy) are associated with immunologically cold tumors that respond poorly to therapy. Increased autophagy results in decreased antigen processing and presentation (APP) mediated by autophagosomal degradation of neoantigen-generating immunoproteasomes and MHC-I. Here, we demonstrate that inhibition of ULK1/2-mediated autophagy restores APP and increases antigen-specific CD8+ T-cell cytotoxicity.
METHODS: Levels of APP machinery were assessed at baseline and after ULK1/2 inhibition using qPCR, western blot, and/or flow cytometry in both murine and human cancer cell lines. Autophagic flux was evaluated using mCherry-GFP-LC3B reporter cell lines imaged in Incucyte. Localization of MHC-I and LC3B following inhibition of ULK1/2 were assessed by immunofluorescence via microscopy. Levels of peptide-loaded MHC-I were assessed by flow cytometry using antibodies that detected either OVA loaded MHC I or KRAS-peptide loaded MHC I. Antigen-specific CD8+ T-cell cytotoxicity was evaluated utilizing MC38-OVA-mCherry tumor cells and OT-1 CD8+ T-cells in the absence or presence of ULK1/2 inhibition.
RESULTS: Inhibition of ULK1/2 decreases basal levels of autophagy in multiple cancer cell lines. Subcellular localization of MHC-I is dramatically altered by inhibition of ULK1/2 as evidenced by decreases in co-localization of MHC-I with the autophagosome marker LC3B and increases in both total and surface levels of MHC-I. Moreover, levels of peptide-loaded MHC-I increased in multiple cancer cell lines upon ULK1/2 inhibition demonstrating that inhibition of autophagy restores antigen processing and presentation. To determine the functional impact of these changes to AAP, we utilized the OT-1 OVA co-culture system. Inhibition of ULK1/2 results in significantly enhanced killing of the MC38-OVA tumor cells by OT-1 CD8+ T cells. Importantly, the killing is antigen specific since ULK1/2 inhibition did not result in increased killing of non-OVA expressing tumor cells by OT-1 CD8+ T cells.
CONCLUSIONS: Tumors with elevated levels of autophagy are associated with reduced antigen processing and presentation resulting in low immune infiltration and poor responses to therapy. Decreasing autophagy by inhibiting ULK1/2 results in increased antigen processing and presentation, leading to enhanced antigen specific CD8+ T-cell cytotoxicity. Together, these results demonstrate that targeting ULK1/2 enhances antigen presentation and antigen-specific T cell mediated killing of cancer cells.

#7243 Mapping the isoaspartylation site(s) in the N-terminal region of ELAVL4 by using deletion constructs and testing against SCLC patient sera.

Shiori Fukutome, Diego A. Velarde, Davin Pan, Chunli Yan, Pamela R. Duarte, Provati Devi, Ite A. Offringa

University of Southern California Keck School of Medicine, Norris Comprehensive Cancer Center, Los Angeles, CA

Small cell lung cancer (SCLC) is the most aggressive type of lung cancer. Standard of care consists of a platin-based therapy with etoposide, combined with immune checkpoint inhibition. While 2/3 of patients respond, the cancer usually recurs, and patients have a 5-year survival rate of only 8%. New therapies are urgently needed. About 15% of SCLC patients spontaneously develop a low-titer immune response against Embryonic Lethal, Abnormal Vision Drosophila-like 4 (ELAVL4). This protein is expressed throughout the nervous system but in the lung is unique to pulmonary neuroendocrine cells (the main cells of origin of SCLC) and is present on the surface of SCLC cells. Patients with low titer anti-ELAVL4 antibodies show improved response to chemotherapy and significantly improved survival. Our previous work indicates that the anti-ELAVL4 immune response is triggered by isoaspartylation of the unstructured N-terminal ELAVL4 region; asparagine or aspartic residues spontaneously form a succinimide intermediate, followed by hydrolysis resulting in an isoaspartyl link. This creates an antigenic kink in the protein, an appears to favor dimerization/aggregation. The unstructured N-terminal region of ELAVL4 contains 6 asparagine residues and 2 aspartic acid residues. Here we aim to understand which asparagine/aspartic acid residues in the N-terminal region undergo isoaspartylation and contribute to the anti-ELAVL4 immune response. To this end, we have constructed four deletion clones with different regions of the ELAVL4 N-terminal region carrying one or two possible sites of isoaspartylation (residues 1-13, 8-18, 16-29, and 28-38). Recombinant proteins generated from these constructs will be incubated under isoaspartyl-inducing conditions (neutral pH, 37ºC) and will use Western Blot and ELISA assays todetermine reactivity of SCLC patient sera as well as sera from mice immunized with Elavl4 $_{(1\text{-}117)}$. Understanding the sites of isoaspartylation in ELAVL4 will help identify the optimal target for the development of anti-isoaspartylated ELAVL4 monoclonal antibodies, which may provide a novel, targeted immunotherapy for SCLC patients. Supported by the Robert E. and May R. Wright Foundation Transformative Cancer Research Grant, USC's Undergraduate Research Associates Program, and the Norris Comprehensive Cancer Center core grant, award number P30CA014089 from the NIH/NCI.

#7244 Detection of gene fusion-induced neoepitopes and cognate T cell receptors in liposarcoma.

Peter Horak[1], Jonas P. Becker[2], Sven Blobner[1], Julian Hlawatsch[3], David Weber[4], Jonas Forster[2], Annika Baude[1], Johanna Wagner[1], Isabel Poschke[3], Inka Zornig[5], Michael Volkmar[6], Michael Platten[3], Dirk Jager[7], Priya Chudasama[8], Stefan Frohling[1], Angelika Riemer[2]

[1]Translational Medical Oncology, German Cancer Research Center, Heidelberg, Germany,[2]Immunotherapy & Immunoprevention, German Cancer Research Center, Heidelberg, Germany,[3]Immune Monitoring Unit, German Cancer Research Center, Heidelberg, Germany,[4]Expression & Cell Biology, TRON Mainz, Mainz, Germany,[5]Clinical Cooperation Unit Applied Tumor Immunity, German Cancer Research Center, Heidelberg, Germany,[6]Cellular Therapies Platform, Helmholtz Institute for Translational Oncology, Mainz, Germany,[7]Medical Oncology, Heidelberg Univesrity Hospital, Heidelberg, Germany,[8]Precision Sarcoma Research Group, German Cancer Research Center, Heidelberg, Germany

Introduction: Dedifferentiated liposarcomas (DDLS) are malignant soft tissue tumors characterized by focal amplifications on chromosome 12q. Using whole genome and RNA sequencing, we found that these structural alterations generate open reading frames that may lead to potential gene fusions and immunogenic neoepitopes. These may serve as potential therapeutic targets. We present the results of the detection and validation of fusion-derived neoepitopes in eight liposarcoma patient samples. In addition, we investigate the patients' T cell repertoires against the identified fusion-derived (FD) neoepitopes.

Methods: Two variant calling pipelines were employed to identify gene fusions. The FD peptides were used as input for HLA binding prediction. We used mass spectrometry (MS)-based immunopeptidomics for HLA presentation analysis. We performed untargeted MS analysis using data-independent acquisition MS combined with ion mobility separation by high-field asymmetric waveform ion mobility spectrometry. The mass spectra were compared with the human proteome, FD sequences, and libraries of in silico predicted fragment spectra. Targeted MS analysis was performed using the ultra-sensitive parallel reaction monitoring workflow (optiPRM). All identified FD neoepitopes were subjected to Epitope-SPecific Expansion Cultures (ESPEC) testing and the TCR repertoire of expanded T-cells analyzed by deep sequencing for candidate peptide selection.

Results: We identified 17 to 123 fusion events on chromosome 12, resulting in 122 to 1126 neoepitope candidates per patient by sequence analysis. The MS workflow successfully identified neoepitopes in six of the eight samples tested. All neoepitope candidates identified by the untargeted approach were subjected to targeted analysis, regardless of their predicted HLA binding ability. In addition, we targeted the top 20 FD candidates from each patient based on the in silico binding prediction. The targeted analyses confirmed the results of the untargeted approach and provided additional detections in five of the six patients analyzed. The validated FD peptides were used to stimulate autologous peripheral blood mononuclear cells from four patients in ESPECs to identify reactive TCRs. T cell expansion was observed in ESPEC cultures against one to 15 peptides per patient, which were subjected to TCR repertoire analysis by deep sequencing.

Conclusions: We detected bona fide HLA-presenting FD neoepitopes and identified neoepitope-specific T cells in the peripheral blood of DDLS patients. These data may further aid the process of identifying and selecting candidate immunogenic peptides, thereby facilitating the clinical development of personalized treatments for DDLS patients, including cancer vaccines and TCR-based therapies.

#7245 Reversible downregulation of HLA class I in adenoid cystic carcinoma.

Annie Li[1], Bianca L. Gonda[1], Elizabeth M. Codd[1], Adam von Paternos[1], Dawn R. Mitchell[1], Markus D. Herrmann[1], Prinjali Kalyan[1], Samantha E. Flynn[1], Thuc Q. Dzu[1], Chengzhou Gao[1], Edwin Zhang[1], Julia J. Mendel[2], Julia C. Thierauf[1], Peter M. Sadow[1], Thomas Denize[1], Diane Yang[1], Florian J. Fintelmann[3], Ross D. Merkin[4], Atul K. Bhan[1], William C. Faquin[1], Daniel L. Faden[2], Xin Gao[4], Jong Chul Park[4], Lori J. Wirth[4], Stefan T. Kaluziak[1], Anthony John Iafrate[1]

[1]Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA,[2]Otorhinolaryngology, Head and Neck Surgery, Massachusetts Eye and Ear, Harvard Medical School, Boston, MA,[3]Radiology, Massachusetts General Hospital, Harvard Medical School, Boston, MA,[4]Medicine, Hematology-Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, MA

Background: Adenoid cystic carcinoma (ACC), a rare and lethal cancer, has shown low response rates to systemic therapies, such as cytotoxic chemotherapy, tyrosine kinase inhibitors and immune-checkpoint inhibitors (ICIs). Despite numerous clinical trials, no effective treatments for patients with recurrent or metastatic ACC have emerged, and ACC mortality rates remain stagnant. Therefore, we aimed to characterize the ACC immune landscape to understand the poor response rates to ICIs.
Experimental Design: We leveraged automated multiplex immunofluorescence (mIF), RNA in-situ hybridization, scRNAseq Gene Expression analysis and spatial transcriptomics analysis to identify pathways supporting the cold ACC immune environment and molecularly characterize ACC tumors, ACC metastasis, adjacent normal tissues, and normal tissues from regions where ACCs arise. Based on results from these studies, we treated freshly resected ACCs with interferon-γ or a STING agonist in vitro.
Results: mIF demonstrated that ACC tumors are immunologically 'cold', with few tumor-infiltrating T-lymphocytes (TILs) and low PD-L1 expression. The most striking finding, however, was a very low B2M expression in almost all ACCs, with focal expression found in some metastatic cases. Spatial transcriptomics of these focally B2M-positive ACC metastases further identified significantly upregulated genes, including *IRF1*, *GBP1* and *TAP1*, indicating an interferon-γ program that mediated reintroduction of HLA-I/B2M. Upon short-term treatment of primary ACC tissues in vitro with interferon-γ or a STING agonist, we observed strongly upregulated HLA/B2M class I expression. mIF and RNAseq analyses of normal salivary gland and breast tissues revealed a p63+, NFIB+, basal duct cell population with similarly low B2M/HLA class I expression.
Conclusions: Low B2M/HLA class I expression may explain why ACCs are immunologically cold and the lack of response to ICIs. Our findings suggest that the normal cell of ACC origin exists in a B2M/HLA-class I low state, and that pharmacologic manipulation with immune activators, such as STING agonists, can restore HLA/B2M in ACCs, creating a path to urgently needed, effective immunotherapies.

#7246 A natural antibody response associated with improved survival of SCLC patients can be actively induced in a SCLC mouse model and leads to significantly improved survival.

Diego Alejandro Velarde[1], Joseph Valdes[2], Chunli Yan[1], Sarah Elmalh[1], Hannah Lee[1], Daniel J. Mullen[1], Angie Moreno[1], Matthew A. Gladstone[1], Nicky Nie[1], Ming Li[1], Martin Kast[1], Ite A. Offringa[1]

[1]University of Southern California, Los Angeles, CA,[2]University of California San Diego, Los Angeles, CA

Background: Small cell lung cancer (SCLC) has a 5-year survival of just 8%, killing over 20,000 Americans annually, thus new therapies are urgently needed. ~15% of SCLC patients naturally develop anti-Hu (an antibody response against ELAVL4) and show improved response to therapy with significantly extended survival. Rare SCLC patients develop a severe and sometimes lethal autoimmune response against ELAVL4 and can show complete SCLC regression. We determined that the antibody-initiating antigenic epitope consists of ELAVL4 isoaspartylated (isoAsp-ELAVL4) in its unstructured N-terminal region (aa 1-38). Isoaspartylation is an immunogenic type of protein damage that is normally repaired but appears abnormally persist in ELAVL4 in SCLC. IsoAsp-ELAVL4 constitutes a cancer-specific neo-antigen. ELAVL4 has been shown to be expressed on the outside of SCLC cells. In rare cases the anti-isoAsp-ELAVL4 antibody response can lead to autoimmunity through epitope spreading to unmodified ELAVL4.

Methods: Here we tested whether actively inducing an immune response against recombinant isoaspartylated Elavl4 in a mouse SCLC model improves survival. We used a *Trp53*$^{fl/fl}$; *Rb1*$^{fl/fl}$ inducible SCLC mouse model to examine both: whether immunization with isoAsp-Elavl4 prior to SCLC induction is protective, and whether immunization with isoAsp-ELAVL4 extends survival following completion of cisplatin+etoposide therapy. Mice were immunized with a recombinant N-terminal fragment of Elavl4 (aa 1-117) generated in Clear Coli and incubated under isoaspartyl-inducing conditions (phosphate-buffered saline at 37°C for 7 days), then emulsified in incomplete Freund's adjuvant (IFA). The negative control was phosphate-buffered saline in IFA; recombinant Elavl4 naturally undergoes isoaspartylation even after short periods at physiological conditions, preventing its use as a negative control. The trajectories of mice were closely monitored with a detailed body metric score and serum was collected from blood draws every 2 weeks to monitor the anti-Elavl4 antibody response.

Results: ELISA data showed that all isoAsp-Elavl4-immunized animals became immune responsive against isoAsp-Elavl4, and just as in human SCLC patients, a small fraction of control-immunized animals spontaneously developed anti-Elavl4 reactivity. Immunization alone before SCLC induction in the absence of chemotherapy did not improve survival. In contrast, immunization following cisplatin+etoposide therapy significantly extends overall survival ($p = 0.00088$).

Conclusions: An immune response against isoAsp-Elavl4 can be actively induced, and when given after chemotherapy can significantly improve SCLC overall survival in a mouse model.

#7247 Leveraging advanced mass spectrometry for enhanced immunopeptidomics in human and murine cells.

Richard C. Jones[1], Michael J. Ford[1], Daneil A. Bochar[2], Michael R. Pisano[2]

[1]MS Bioworks, LLC, Ann Arbor, MI,[2]AnchorBio Solutions, Ann Arbor, MI

Immunopeptidomics is the study of peptides presented by Class I and Class II Major Histocompatibility Complexes (MHC). Presented peptides originate from a complex intracellular processing pipeline, culminating in the presentation of proteolytically derived fragments on the cell surface bound to MHC molecules. Advances in our understanding of the immune system have spurred significant interest in identifying these peptides and characterizing the immunopeptidome. Recent advances in mass spectrometry have significantly increased sensitivity, enabling unprecedented depth of immunopeptidome coverage and facilitating assays with reduced starting material requirements. To build on recent advancements, we utilized the Orbitrap Astral mass spectrometer, a cutting-edge technology offering superior sensitivity and resolution, to profile immunopeptidomes from human and murine cell lines. Comparative analyses with traditional immunopeptidomics workflows highlighted the Orbitrap Astral's remarkable ability to enhance peptide identification, particularly for low-abundance peptides that were previously challenging to detect. This advancement enabled a deeper and more comprehensive characterization of the immunopeptidome, unveiling valuable insights into antigen presentation. The integration of the Orbitrap Astral represents a major advancement in the field of immunopeptidomics, facilitating unprecedented sensitivity in the profiling of antigen presentation. These findings have important implications for cancer research, particularly in the development of targeted immunotherapies and the broader understanding of immune responses in oncology.

#7248 Expression profiles of 10 common cancer-specific antigens collected for the development of cancer vaccines and T cell therapies applicable to most solid cancer patients.

Tetsuya Nakatsura, Kazumasa Takenouchi, Jun Kataoka, Yusuke Ito, Sae Kikuchi, Hiroki Kinoshita, Kazunobu Ohnuki,, Toshihiro Suzuki, Nobuo Tsukamoto

National Cancer Ctr. Japan, Kashiwa, Japan

We clinical trialed a peptide vaccine derived from the cancer-specific antigen, glypican-3 (GPC3). There have been cases of multiple patients with advanced cancer who responded to the peptide vaccine after just a few doses, and seven patients with refractory pediatric cancer have survived for 10 years without a recurrence, so the peptide vaccine is also expected to be effective in preventing recurrence. On the other hand, we have also seen several cases of GPC3 expression negative cancer recurrence, revealing the limitations of peptide vaccines with a single antigen. To overcome the heterogeneity of individual cancers and apply the antibody to a wide range of solid cancer patients, we collected 10 common cancer-specific antigens, such as GPC3. In this study, we performed immunohistochemical analysis of various solid cancer specimens using antibodies against ROBO1, TGFBI, AFP, EphB4, CLDN1, and LAT1 in addition to GPC3, HSP105α, FOXM1, and SPARC, which we have previously studied. These antigens were frequently expressed in various solid cancers but shown to be rarely expressed, with some exceptions, in non-cancerous normal organs adjacent to the cancer. Even if a normal cell expresses an antigen protein, if the cell does not express HLA class I on its cell membrane, it can escape attack by cytotoxic T lymphocytes (CTL). Many non-cancerous normal cells, including hepatocytes that express HSP105α and pancreatic acinar cells that express CLDN1, do not express or only weakly express HLA class I, and it has been determined that the risk of adverse events from CTLs induced by vaccines containing these antigens or from T cell receptor (TCR)-engineered T cell therapy is low. In our other study, we have identified many peptides derived from these 10 common cancer-specific antigens with higher CTL induction capacity than the GPC3 peptide used in the clinical trial. We are currently developing peptide vaccines and mRNA vaccines. Identifying many TCRs that react to peptides and developing TCR-T cell therapy is also possible. For CTLs to be effective, HLA class I must be expressed on the surface of cancer cells. It was revealed that most solid cancers, except for lung adenocarcinoma and pediatric cancer, frequently express HLA class I on the cell membrane. In addition, it was revealed that the cell membrane antigens EphB4, CLDN1, and LAT1 are frequently expressed in various solid cancers. CAR-T cell therapy targeting these antigens may be a savior for many solid cancer patients. In this study, we clarified the expression profiles of 10 common cancer-specific antigens collected to develop cancer vaccines and T cell therapies that can be applied to most solid tumor patients. We will continue to develop cancer immunotherapies that target these multiple common cancer-specific antigens.

#7249 TME hypoxia drives immunopeptidomic deficiencies in non-small cell lung cancer by blocking translation of interferon stimulated genes within the MHC I pathway.

Matthew G. Smith, Alexis Ramos, Heena Panchal, Adam Mailloux

Microbiology and Immunology, University of Iowa, Iowa City, IA

Loss or reduction in the expression of interferon stimulated genes (ISGs) which augment the MHC class I presentation pathway (MHC1PP) predict poor responsiveness to immunotherapy as these genes are required for tumor rejection mediated through $CD8^+$ T cells. Understanding how these MHC1PP components are regulated is critical for the design of strategies to enhance immunotherapy responses in solid tumors, particularly those deemed "cold" and poorly immunogenic through low-level expression of the MHC1PP. Here, we present evidence that tumor hypoxia, a hallmark of solid cancers, blocks the translation of ISGs comprising the MHC1PP. We first established that tumor hypoxia *in vitro* (2% O_2) blocks IFN-γ induction of MHC1PP components including the immunoproteasome (IP) proteolytic subunits and ER chaperones and transporter proteins involved in generating a peptide-MHC I complex (p-MHC I) via Western blot analysis of human (A549) and murine (LLC) non-small cell lung cancer (NSCLC) lines cultured at normoxia (20% O_2) versus hypoxia. We then assessed the immunopeptidome of A549 cells through immunoprecipitation of intact p-MHC I and analysis of presented antigen via LC-MS based immunopeptidomic workflows. We showed that under normoxia, IFN-γ induction of the MHC1PP results in significantly enhanced antigen diversity and a three-fold increase in total unique tumor antigens, while hypoxia completely abolishes this augmentation of the immunopeptidomic landscape. Mechanistic studies indicate that while MHC1PP components are blocked at the protein level, mRNA transcript levels are largely unaffected as determined by RT-PCR of A549 cultures. 5-methylcytosine (m5C) ELISA experiments showed that hypoxia significantly increases the abundance of m5C modifications on RNA and reversing this accumulation through RNA but not DNA specific methyltransferase inhibitors corresponds with restored protein expression of MHC1PP components in hypoxic A459 cells, suggesting a potential epitranscriptomic mechanism of translation regulation. *In vitro*, restoring oxygen to A549 cells rapidly reverses the blocked induction of MHC1PP components. To corroborate this *in vivo*, subcutaneous LLC tumors were grown on C57Bl/6 mice subjected to whole body hyperbaric oxygen therapy (HBOT) versus controls. Compared to controls, HBOT treated mice had significantly smaller tumors, showed prolonged survival, and exhibited greater $CD8^+$ tumor infiltrating lymphocyte (TIL) activation status. Overall, these studies indicate that tumor hypoxia regulates the translation of ISGs comprising the MHC1PP, potentially driving or contributing to immunologically “cold” TME in solid cancers such as NSCLC, and restoring tumor oxygen *in vivo* is a potential strategy to augment tumor immunogenicity and improve responses to additional treatment modalities.

#7250 Extracellular acidity in the TME interferes with the IFN-γ induction of the immunoproteasome.

Matthew G. Smith, Alexis Ramos, Heena Panchal, Adam Mailloux

Microbiology and Immunology, University of Iowa, Iowa City, IA

The immunoproteasome (IP) is a component of the MHC class I presentation pathway (MHC1PP) that is induced by IFN-γ, functions to augment the immunogenicity of cancer cells by generating optimal tumor antigens from polypeptide precursors, and is correlated with favorable responses to immunotherapy. Understanding tumor intrinsic factors that regulate the IP in cancer is essential for addressing immunologically cold tumors which respond poorly to immunotherapy. Extracellular acidity (pH 6.5) is a hallmark of solid tumors and despite being shown to impair $CD8^+$ anti-tumor immunity, remains understudied as a driver of immune-evasion in cancer. Here, we present data that extracellular acidity *in vitro* can restrict the induction of the catalytically active subunits of the IP, in particular 20s proteasome subunit beta type 9 (β1i) and 20s proteasome subunit beta type 10 (β2i). Various human and murine cancer cell lines (Human: pancreatic ductal adenocarcinoma PANC1, hepatocellular carcinoma HEPG2, non small cell carcinoma A549; Murine: non small cell carcinoma LLC) were cultured in control media (DMEM pH 7.4) versus acidic media (DMEM pH 6.5) and stimulated with IFN-γ to induce the IP. Cell lysates were assessed by Western blot and demonstrated a sharp reduction in the protein induction of β1i and β2i, which are both required for the formation of the mature IP, under acidic conditions compared to controls. RT-PCR analysis of A549 cells indicates that these proteins are blocked transcriptionally as well as translationally. Mechanistically, we demonstrate via Western blot that activation of signal transducer and activator of transcription 1 (STAT1), the critical transcription factor required for IFN-γ induction of the IP, is delayed in acidic conditions compared to controls. We further show that signal transducer and activator of transcription 3 (STAT3), a pro-tumorigenic transcription factor and antagonist of STAT1 transcriptional activity, is activated under acidic conditions in A549 cells and furthermore IFN-γ enhances this STAT3 activation but only under acidic conditions. Functionally, we show via flow cytometry that downstream of the blockade in IP subunit induction that occurs under acidic conditions, surface MHC I expression is diminished. Overall, these studies indicate extracellular acidity in the TME restricts the induction of IP components potentially through a STAT3 dependent mechanism, suggesting that targeting STAT3 activation in acidic tumors may improve tumor immunogenicity and responses to immunotherapy.

IMMUNOLOGY: Novel In Vivo, In Vitro, and In Silico Models
Poster Session

#7254 Mouse models for alleviating GvHD and assessing on-target off-tumor toxicity of bispecific T-cell engagers.

Hongyan Sun[1], Bei Zhang[1], Yunlong Jiang[1], Jianming Xu[1], Xiang Gao[1], Jun Wang[2]

[1]GemPharmatech Co., Ltd, Nanjing, China,[2]GemPharmatech Co., Ltd, San Diego, CA

Bispecific T cell engagers (TCEs), which simultaneously target CD3 on the surface of T cells and a tumor-associated antigen (TAA) located on cancer cells, have shown great promise in tumor treatment. However, multiple factors including targets, indications, dosage and immunogenicity influence the efficacy and safety of TCEs. Furthermore, the intricate mechanism of action may limit the translatability from preclinical study to clinical application. To improve the translation of early research findings into effective therapeutic strategies, we introduced two optimized mouse models for preclinical evaluation of TCEs.

Due to poor cross-species reactivity of anti-CD3 and anti-TAA antibodies, mice that express wild type murine CD3 molecules are not suitable for the evaluation of TCEs. Severe immunodeficient animals engrafted with both human T cells and cancer cells represent an ideal model. However, graft-versus-host disease (GvHD) is often observed, which can make data interpretation challenging. To this end, the NCG-MHC-dKO mouse model was developed by knocking out both murine MHC class I and II molecules in the NCG background. Compared to conventional NCG mice, NCG-MHC-dKO mice alleviated the GvHD response and prolonged the life span of PBMC-engrafted animals, thereby extending the therapeutic window. We have verified the efficacy of talquetamab, a GPRC5D-directed T cell engager, in huPBMC-NCG-MHC-dKO mice inoculated with NCI-H929 cell line. Talquetamab could significantly inhibit tumor growth in NCG-MHC-dKO mice, with the animals maintaining stable body weight and healthy condition throughout the treatment period. These findings highlighted NCG-MHC-dKO mice as an ideal platform for evaluating the efficacy of TCEs.

Although the huPBMC-NCG-MHC-dKO model is very powerful for the efficacy evaluation of TCEs, it cannot be used to evaluate on-target off-tumor toxicity due to the absence of human TAA in mouse tissues with compromised immune systems. To this end, the BALB/c-hCD3EDG mouse model was developed by inserting the entire gene regions including regulatory sequences of the human CD3E, CD3D, and CD3G genes into the mouse genome, while simultaneously deleting the endogenous mouse CD3e, CD3d, and CD3g genes. Replacing the murine CD3 molecules with human counterparts did not impact the immune system of BALB/c-hCD3EDG mice. In addition, we have developed multiple human TAA knock-in mouse models in the BALB/c-hCD3EDG background. The expression of TAAs in these mice resembled that in humans. Together, these models can be used not only to evaluate the efficacy, but also on-target off-tumor toxicity of TCEs.

In conclusion, our mouse models provide valuable tools to increase the translatability of preclinical models to evaluate TCEs.

#7255 Novel anti-PD(L)1 resistance models for post-anti-PD(L)1 drug development.

Hongyan Sun[1], Yan Wang[1], Zhu Fang[1], Jianming Xu[1], Xiang Gao[1], Jun Wang[2]

[1]GemPharmatech Co., Ltd, Nanjing, China,[2]GemPharmatech Co., Ltd, San Diego, CA

Although cancer immunotherapy utilizing blockade of the PD-1/PD-L1 checkpoint has revolutionized the treatment of a wide variety of malignancies, leading to durable therapeutic responses, it is ineffective in many patients who developed drug resistance after initial response. Therefore, the establishment of robust preclinical anti-PD-1 resistance models is of paramount importance for unraveling drug resistance mechanisms and developing novel treatment strategies.

The mechanisms of anti-PD-1/anti-PD-L1 resistance include insufficient presentation of tumor antigens, downregulation of MHC class I molecules, dysregulated IFN-γ signaling pathways, and the immunosuppressive tumor microenvironment.

GemPharmatech has established 3 models of anti-PD-1 resistance. (1) Drug induced model: BALB/c-hPD1 mice were subcutaneously transplanted with CT26 cells and treated with anti-PD-1 (KEYTRUDA®) twice weekly. Non-responsive tumors were excised, dissociated, cultured, and then re-implanted into naïve mice for anti-PD-1 treatment to maintain screening pressure. The induced model of anti-PD-1 resistance was obtained after repeated screening cycles. (2) Genetically engineered model: Engineered anti-PD-1 resistant models were established by knocking out either B2M or STK11 in CT26, which are known to confer specific genetic alterations to anti-PD-1 resistance. (3) Primary resistance model.

CT26 cells acquired anti-PD-1 resistance after a series of KEYTRUDA treatment and re-implantation demonstrated a stable anti-PD-1 resistant phenotype. Compared with parental cell lines, drug-resistant CT26 cell showed a significantly faster tumor growth kinetics in vivo. RNA sequencing data revealed a set of genes disregulated between the resistant and parental cell lines, providing insight into the resistance mechanism to anti-PD-1 therapy. In the engineered PD-1-resistant CT26 model, knocking out STK11 dampen CT26's response towards anti-PD-1 treatment. After ablation of STK11, CD8+ cytotoxic T lymphocyte infiltration was decreased, and myeloid-derived suppressor cells (MDSCs) significantly accumulated in the tumor microenvironment. Additionally, STK11 deletion was associated with reduced PD-L1 expression in tumor cells, suggesting that immunosuppressive microenvironment contributed to the development of resistance.

In conclusion, preclinical anti-PD-1 resistance models are valuable tools for researchers to dissect the complexity of anti-PD-1 resistance mechanisms and provide important guidance for the biomarker discovery as well as development of innovative therapeutic strategies.

#7256 Development of a human immune system reconstituted mouse model without the function of FcγRs expressed on mouse innate immune cells.

Jay Zhang, Yanhui Nie, Dexuan Huang, Xiao Liu, Qingqing Xu, Qiang Liu, Shuangshi Liu, Xiaofei Zhou

Biocytogen, Waltham, MA

Severe immunodeficient mice have been widely used in xenotransplantation research and for validating various immunotherapy methods. We utilized gene editing techniques to knock out the IL2rg gene in NOD scid mice, resulting in a severe immunodeficient mouse model that lacks mature T, B and NK cells, but retains most of the myeloid cells, which we named B-NDG mice (#110586). Human immune cells can be successfully reconstituted in B-NDG mice (#110586). However, due to the lack of effective support from human cytokines, only human T cells can be reconstituted in B-NDG mice (#110586). IL15 is a key cytokine in the development and maturation of NK cells and also promotes the survival of memory CD8+ T cells. When validating the *in vivo* efficacy of therapeutic IgG antibodies using a mouse model with a reconstituted human immune system, the antibodies may not only bind to the reconstituted human immune cells to exert pharmacodynamic activity but also bind to various Fcγ receptors expressed on mouse innate immune cells, such as macrophages and neutrophils. This interaction may lead to antibody neutralization or antibody-dependent cellular phagocytosis (ADCP), affecting the accuracy of the efficacy validation.Biocytogen developed B-NDG hIL15, FcγR KO mice (#112653) in which the mouse IL15 gene was replaced by human IL15 gene. Then the mouse genes Fcgr1, Fcgr2b, Fcgr3 and Fcgr4 were knocked out. Mouse FcγRI, FcγRIIb, FcγRIII and FcγRIV were not detectable in B-NDG hIL15, FcγR KO mice (#112653). Human IL15 was detectable in homozygous B-NDG hIL15, FcγR KO mice (#112653). The concentration of human IL15 in the serum was 112 pg/mL, similar to that in B-NDG hIL15 mice (#110600). The frequency of mouse granulocytes, monocytes, macrophages and DCs in the blood and spleen of B-NDG hIL15, FcγR KO mice (#112653) are similar to that in B-NDG hIL15 mice (#110600). Binding analysis of rituximab analog (in-house) showed that rituximab-bound cells were not detectable in B-NDG hIL15, FcγR KO mice (#112653). B-luc Daudi cells (#310677) were intravenously injected into B-NDG hIL-15, FcγR KO mice (#112653). After 3 days, mice were grouped when the intensity of the imaging signal reached approximately 1.2×10^6 p/sec, at which time human NK cells expanded *in vitro* were engrafted into the mice and the mice were treated with anti-human CD20 antibody rituximab. The results showed that rituximab was efficacious in controlling B-luc Daudi growth in human NK cells engrafted B-NDG hIL15, FcγR KO mice (#112653).

#7257 Humanized GDF15 model for simulating weight loss induced by GDF15 secretion in tumors.

Jay Zhang, Suman Zhao, Ruili Lv, Yongshun Li, Saina Ma, Yanling Wang

Biocytogen, Waltham, MA

GDF15, also known as growth differentiation factor-15, is expressed by multiple tissues and secreted into the serum. It regulates numerous physiological processes, such as affecting food intake and causing weight loss, by binding to the receptor GFRAL. Under normal physiological conditions, GDF15 levels are low; however, its expression significantly increases under pathological conditions such as diabetes and cancer. In some special types of advanced cancers, GDF15 levels can rise and trigger anorexia-cachexia syndrome. We created humanized B-hGDF15 mice by replacing the mouse GDF15 gene with the human GDF15 gene in situ. Additionally, we established a B-hGDF15 MC38 cell line by inserting an exogenous promoter and the human GDF15 CDS sequence at the mouse GDF15 locus in MC38 cells. RT-PCR was conducted to detect mRNA levels of hGDF15 in B-hGDF15 mice, confirming the absence of mouse *Gdf15* expression and the presence of human *GDF15* expression. We performed ELISA to detect the protein expression levels of hGDF15 in B-hGDF15 mice and B-hGDF15 MC38 humanized cell line, confirming successful expression of the hGDF15 protein. When B-hGDF15 mice were inoculated with B-hGDF15 MC38, tumor model was developed successfully, and a trend of body weight loss was observed compared with the wild-type MC38 group. Subsequently, we performed an efficacy experiment in B-hGDF15 mice inoculated with B-hGDF15 MC38. After 7 days of inoculation, the mice were grouped based on body weight and tumor size, and the treatment group received 10 mg/kg of a ponsegromab analog on the same day. We observed significant weight gain in the treated group compared to the control group, indicating that B-hGDF15 mice and B-hGDF15 MC38 provide an excellent preclinical in vivo model for testing GDF15-targeted drugs.

#7258 Enhancing TIL efficacy in NSCLC through epigenetic reprogramming and computational modeling.

Daniel R. Plaugher[1], Avery R. Childress[1], Christian M. Gosser[1], Dave-Preston Esoe[1], Xuilong Song[1], Jinpeng Liu[2], Christine F. Brainson[3]

[1]Toxicology and Cancer Biology, University of Kentucky, Lexington, KY,[2]Cancer Biostatistics, Markey Cancer Center, University of Kentucky, Lexington, KY,[3]Toxicology and Cancer Biology, Markey Cancer Center, University of Kentucky, Lexington, KY

Novel therapeutic protocols are desperately needed to treat non-small cell lung cancer (NSCLC), the deadliest cancer world-wide. Recently, utilizing a patient's tumor-infiltrating lymphocytes (TIL) is proving to be a viable and highly personalized approach. Despite its promise, many obstacles remain, such as refining expansion protocols for better *in vivo* TIL proliferative potential, improving T cell ability for homing-to and targeting tumor cells upon re-infusion, and avoiding IL-2/lymphodepletion related side effects. We hypothesize that inhibiting epigenetic enzyme EZH2 (EZH2i) will improve both TIL expansion and infusion outcomes in NSCLC patients. Yet, there are likely mechanisms of T cell suppression that EZH2i may not overcome. To address and identify these mechanisms, stochastic mathematical modeling of gene signaling in cancer has risen as a useful tool. Thus, we further hypothesize that modern sequencing data and mathematical biology can discover alternative mechanisms of T cell suppression that will suggest novel targets for TIL-combination treatment.

To test these hypotheses, we have tested the EZH2 inhibitor valemetostat with anti-PD1 in a murine model of non-small cell lung cancer. We observed tumor regression and strong IFN-gamma T cell responses in combination treated animals. We also observed up-regulation of both major histocompatibility complexes, greater pro-T cell cytokine signaling, and myeloid populations became more tumor-eliminating. To translate these findings to TILs, we are currently securing NSCLC samples from our Biospecimen Core, establishing patient-derived tumoroids, and expanding TILs *ex vivo*. TILs will be expanded with/without EZH2 inhibitor valemetostat (Val) under "young" protocols. At *ex vivo* endpoint TILs will be assessed for Val-driven differences in viability, differentiation state, and reactivity via flow cytometry. To complement or studies with an *in silico approach*, we are utilizing libraries of publicly available NSCLC data to perform bioinformatic analysis and implemented equation learning techniques to infer NSCLC tumor signaling networks from clinical data between immunotherapy responders and non-responders. Differentiation patterns will be assessed as a function of pseudo-time-course data to inform predictions of signal transduction pathways. Lastly, mathematical phenotype control theory will be used to uncover potential targets within the tumor to boost TIL response in NSCLC.

Given that advanced stage lung cancers remains refractory to most treatments, determining methods to improve precision medicine options such as TIL therapy is an important goal for the field. Our blending of classic bench science with bio-informatics has the potential to deepen our understanding of TIL therapy and to identify new way to improve therapeutic responses.

Funding: T32 CA165990 (DRP), R01 CA237643 (CFB), R01 HL170193 (CFB), P30 CA177558 (Markey Shared Resources).

#7259 An in vivo humanized mouse model for human dendritic cell reconstitution to evaluate efficacy of tumor immunotherapy by B-NDG Flt3 KO mice.

Fengqian Chen, Shujin Zhang, Fengxing Gu, Shaozu Fu, Yanling Wang

Biocytogen, Waltham, MA

Fms-like tyrosine kinase receptor 3 (Flt3) is a membrane protein that is strongly expressed on hematopoietic stem cells (HSCs). FLT3LG (FLT3 ligand) is a growth factor that can stimulate the proliferation, differentiation, and survival of these cells when it binds to its receptor FLT3. The Flt3/Flt3L signaling axis indeed plays an important role in the production of conventional dendritic cells (cDCs) and plasmacytoid dendritic cells (pDCs). Knockout of mouse Flt3 results in a reduction of mouse dendritic cells (DCs), which increases the availability of mouse Flt3L to the human receptor after human HSCs transplantation, thereby supporting the development of human immune cells, particularly dendritic cells.
Biocytogen developed B-NDG Flt3 KO mice by knocking out the Flt3 gene in the NDG background. mFLT3 was detectable in DCs and CD117+ cells of B-NDG mice, but not in homozygous B-NDG Flt3 KO mice. To determine whether the knockout of Flt3 will affect the expression of FLT3LG. Serum were isolated from B-NDG mice and homozygous B-NDG Flt3 KO mice. Expression level of mouse FLT3L were analyzed by ELISA. Mouse FLT3L was detectable in B-NDG mice and homozygous B-NDG Flt3 KO mice. Levels of mFLT3L were substantially elevated in homozygous B-NDG Flt3 KO mice compared to B-NDG mice. To evaluate whether the knockout of Flt3 impacts the frequency of leukocyte subpopulations, Bone marrow cells were isolated from B-NDG mice and homozygous B-NDG Flt3 KO mice, Frequencies of T cells, B cells, NK cells, granulocytes, monocytes, macrophages in B-NDG Flt3 KO mice were like those in B-NDG mice. Frequencies of DCs in B-NDG Flt3 KO mice were lower than those in B-NDG mice.
In summary, the significant decrease of DCs in B-NDG Flt3 KO mice creates an opportunity for the successful reconstruction of human DCs, which could have a significant positive impact on tumor immunotherapy.

#7260 Predicting the starting dose and step-up regimen for first-in-human trial of T-cell engagers using mechanistic mathematical modeling.

Oleg Demin Jr, Galina Kolesova, Dmitry Shchelokov

InSysBio CY, Paphos, Cyprus

The minimal anticipated biological effect level (MABEL) approach is recommended for selecting a safe clinical starting dose for T-cell engagers (TCEs). This approach estimates the minimal recommended starting dose (MRSD) by setting predicted drug exposure in humans below the concentration that results in a desirable percentage of maximal pharmacological activity (PA) observed in vitro (in the range of 20%-50%, e.g., EC20). However, this method may lead to a low MRSD and multiple dose escalations, resulting in sub-therapeutic doses (Saber et al. ***Regul Toxicol Pharmacol***. 2017 Nov;90:144-152). The aim of our study was to apply quantitative systems pharmacology (QSP) modeling to predict the MRSD and step-up dosing regimen for HPN536, a T-cell engager targeting mesothelin (MSLN).

The translational QSP model consists of two parts: an *in vitro* model and a pharmacokinetic (PK), receptor occupancy (RO), and PA model. The *in vitro* model was developed to describe published data on T-cell dependent cytotoxicity, cytokine secretion, and T-cell activation in the presence of various HPN536 concentrations, and to identify EC50 expressed in terms of the number of trimers—complexes of HPN536 with CD3 and MSLN in the immunological synapse (IS) between T-cells and cancer cells. PK parameters were fitted using data on cynomolgus monkeys and translated to humans using a standard allometric scaling approach. The PK/RO/PA model for cancer patients includes the distribution of TCE into the tumor (ovarian cancer), binding of HPN536 with CD3 and MSLN, trimer formation in IS, and PA based on EC50 values identified in the *in vitro* model. PA depends on the number of trimers in the tumor. Variability was implemented in the model, including MSLN expression, PK, T-cell density in the tumor, and tumor size.

Starting dose estimates were calculated as a dose range corresponding to 20%-50% PA. The MRSD range predicted by the model was higher than the range calculated by the conventional MABEL PK-driven approach. The starting dose predicted by the model is 40 ng/kg based on cytokine assays, whereas the starting dose in the phase 1 trial was 6 ng/kg (established by the MABEL PK-driven approach), and CRS grade 3 was observed at 54 ng/kg without dexamethasone pretreatment. The optimal step-up regimen is the one that minimizes peak trimer levels and results in the highest trough trimer level at the target dose. Considering once-weekly administration of the HPN536 target dose, the optimal regimen with two step-ups was as follows: 0.001 mg/kg given on day 1, 0.003 mg/kg given on day 4, and 0.01 mg/kg QW started on day 8 (target dose).

Applying QSP modeling to predict MRSD in addition to the MABEL PK-driven approach might help reduce the number of dose escalation steps, exposing patients to sub-therapeutic doses in first-in-human trials, and optimize step-up dosing regimens.

#7261 Dynamical mathematical models reveal that cell cycle inhibitors promote immunogenicity and T cell cytotoxicity when combined with IL-15 in estrogen receptor positive breast cancer cell lines.

Jiyeon Park[1], Frederick R. Adler[1], Aritro Nath[2], Jason I. Griffiths[2], Patrick A. Cosgrove[2], Kimya Karimi[2]

[1]Department of Mathematics, University of Utah, Salt Lake City, UT,[2]Department of Medical Oncology and Therapeutics Research, Beckman Research Institute, City of Hope National Medical Center, Monrovia, CA

Advanced estrogen receptor positive (ER+) breast cancers are typically treated with endocrine therapy (ET) plus cell cycle inhibitors (CCI); however, nearly 20% of these cancers recur due to treatment resistance. Our previous study of tumor intrinsic properties during CCI plus ET treatment found that CCI-resistant cancer cells are associated with diminished immune-activating signals to cytotoxic CD8+ T cells due in part to reduced interleukin-15 (IL-15) signaling, a potent immunostimulatory cytokine. This finding motivates our study of combination IL-15/T-cell based immune-activating treatment regimen development. However, the fact that CCI also inhibit immune cells challenges this immunotherapy development, creating complex interactions that require mathematical modeling in collaboration with *in vivo* experiments to analyze. To study these complex interactions, we develop a nonlinear ordinary differential equation mathematical model that describes the tumor ecosystem of interacting cancer cells and cytotoxic T cells linked with pharmacokinetics of ribociclib (CCI) and IL-15. We design experimental model using 3D spheroids of CCI-sensitive and resistant cancer cell lines in monoculture or co-cultured with patient-derived cytotoxic T cells and tracked over 5 days of growth. These spheroids were treated with CCI alone or in combination with IL-15. This dynamical model accurately fits experimental data by quantifying the effects of both treatments on the growth rates and interactions between cancers and T cells. Key parameters of our model quantify how treatments impact the promotion of T cell recruitment and T cell activation by cancer cell antigens. These combine to lead to the subsequent killing of cancer cells. Analyzing these parameters under various dose drug combinations drives to three main findings: (1) proliferation of both cell types are suppressed by CCI, while only T cells are activated by IL-15, (2) CCI, despite slowing T cell proliferation, promote immunogenicity and immune cell toxicity in combination with IL-15, and (3) the enhanced immune capacity under this combination treatment is higher in ribociclib-resistant cancer cell lines than in sensitive cell lines. The mathematical modeling reveals that combining immune-activating treatment (IL-15) with a cell cycle inhibitor (ribociclib) outperforms single therapy in limiting cancer cell growth. Furthermore, cancer cells resistant to cell cycle inhibitors are more effectively targeted by this combination therapy. For future work, we will use the mathematical model to propose optimal therapy regimens that minimize the total dosage of the two drugs and maximize treatment response with long-term goal of overcoming recurrence due to drug resistance by strengthening immune surveillance in ER+ breast cancers.

#7262 Organoid-based prediction of immune checkpoint inhibitor response in gastric cancer: A correlation with clinical outcomes.

H. Kim[1], S. Chang[2], H. Jun[2], U.-J. Yun[3], J. Kim[2], H. Kim[4], T. Kim[5], M. Jung[6];

[1]Department of Medicine, Yonsei University College of Medicine, Seoul, Korea, Republic of, [2]Podo Therapeutics, Seongnam-si, Korea, Republic of, [3]Song-Dang Institute for Cancer Research, Yonsei University College of Medicine, Seoul, Korea, Republic of, [4]Department of Pathology, Yonsei University College of Medicine, Seoul, Korea, Republic of, [5]Division of Gastroenterology, Department of Internal Medicine, Yonsei cancer center, Yonsei University College of Medicine, Seoul, Korea, Republic of, [6]Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea, Republic of

Research and development in immune checkpoint inhibitor (ICI) therapy are rapidly advancing across various cancer types. However, accurately predicting treatment responses remains a significant challenge due to factors such as patient-specific immune system variability, tumor heterogeneity, and genetic differences. Current model systems for evaluating ICI efficacy also face limitations. While organoids replicate patient-specific tumor characteristics, their lack of immune components hinders their ability to assess the complexity of immunotherapy. To address these challenges, we developed a co-culture platform combining tumor organoids with immune cells to evaluate ICI drug efficacy and predict patient-specific therapeutic responses. We collected ascites and pleural effusion samples from 88 gastric cancer patients, obtaining 109 samples and successfully establishing 63 organoids, achieving an establishment rate of 80.7%. Immunohistochemical staining and next-generation sequencing (NGS) confirmed the organoids' molecular and histological similarity to the corresponding patient tumors. Peripheral blood mononuclear cells (PBMCs) were isolated from matched blood samples and activated using cytokines such as IL-2. Patient-derived immune cells were then co-cultured with tumor organoids and treated with ICI drugs, including nivolumab, pembrolizumab, and atezolizumab, as well as chemotherapy drugs, to evaluate their anticancer efficacy. Our results showed immune cells localizing around the organoids and inducing concentration-dependent tumor cell death following ICI treatment. Drug response evaluations were conducted on 25 different organoids, and the findings were compared with the clinical responses of the corresponding patients. Among 17 patients who underwent follow-up after anticancer treatments, including immunotherapy, the co-culture platform demonstrated high predictive performance, with an accuracy of 94.12%, sensitivity of 83.33%, and specificity of 100%. Notably, sequential analysis of samples from the same patients revealed that in vitro predictions of resistance to nivolumab corresponded with clinical progression as assessed by the Response Evaluation Criteria in Solid Tumors (RECIST). These findings highlight the robustness of our co-culture platform in predicting ICI responses and underscore its potential to guide personalized immunotherapy strategies for gastric cancer patients. Related patents and rights belong to Podo Therapeutics.

#7263 Development and validation of a machine learning framework for immune phenotype classification and immunotherapy response prediction in lung cancer.

Hyun Woo Park[1], Ki Wook Lee[1], Ji Won Lee[1], Sehhoon Park[2], Young-Jun Jeon[1]

[1]Sungkyunkwan University, Suwon, Korea, Republic of,[2]Samsung Medical Center, Seoul, Korea, Republic of

Tumor immune microenvironment composition significantly influences immunotherapy outcomes in lung cancer, with immune-inflamed tumors demonstrating higher response rates compared to immune-non-inflamed tumors. This study aimed to develop a machine learning framework to classify immune phenotypes in lung cancer using TCGA data and validate its utility in predicting immunotherapy response with real-world patient samples. Existing approaches, such as the Lunit SCOPE technique, have been employed for immune phenotype characterization but face limitations in handling tumor heterogeneity and adapting to diverse datasets, underscoring the need for more robust tools. A baseline machine learning model was constructed using six tree-based classifiers: Random Forest, Extremely Randomized Trees, AdaBoost, Gradient Boosting, XGBoost, and LightGBM. Feature importance scoring identified critical immune-related gene expression profiles, including markers from interferon signaling, T-cell activation, and cytokine regulation pathways. Model optimization incorporated 15 classifiers, leveraging advanced hyperparameter tuning and ensemble learning strategies to improve accuracy, Matthew's correlation coefficient (MCC), and area under the ROC curve (AUROC). Classification training and validation were performed on 447 TCGA lung cancer samples, divided into 80% training and 20% validation subsets, stratified into immune-inflamed and immune-non-inflamed phenotypes based on bulk RNA-seq gene expression datasets. The final model demonstrated robust adaptability to tumor heterogeneity and was independently tested on 87 patient samples labeled through the Lunit SCOPE framework, stratified into immunotherapy responders and non-responders. Correlation analysis assessed the relationship between immune phenotypes and therapeutic outcomes. The optimized model exceeded the performance of Lunit SCOPE, achieving an AUROC >0.90 in classifying immune-inflamed versus immune-non-inflamed samples in the TCGA dataset. Testing on real-world patient samples further confirmed its ability to accurately predict immunotherapy response, supporting the hypothesis that immune-inflamed tumors derive greater benefit from treatment. This machine learning framework demonstrates superior accuracy and adaptability compared to existing techniques, offering significant potential to improve patient stratification, reduce ineffective treatments, and guide precision immunotherapy in lung cancer. Future directions include integrating multi-omics datasets to further refine predictive capabilities and assess its generalizability across other cancer types.

#7264 AI-guided engineering and preclinical characterization of anti-PD1/IL15 mutein bifunctional fusion proteins.

Yun Zhang, Liang Tian, Chenpeng Su, Xiangyu Sun, Ran Hao, Tianhong Li, Dandan Liu, Jiyuan Tian, Xiaoqian Chen, Jian Peng, Zhenping Zhu

Helixon US Inc., New York, NY

IL15, a cytokine of the IL2 family, is pivotal for activating natural killer cells and effector/memory CD8 T cells. Clinical application with various IL15 formulations for cancer therapy, however, has been greatly hindered by its pharmacokinetics and dose-dependent systemic toxicity. A promising approach involves the fusion of IL15 with an anti-TAA antibody or an immune checkpoint antibody, in an attempt to specifically target IL15 to the tumor and its microenvironment while reducing peripheral exposure and toxicity. In this study, we generated a panel of stable IL15 variants (IL15v), utilizing an internally developed AI algorithm, with attenuated binding affinity to IL2Rβ and biological activity. We then engineered a panel of fusion proteins each comprising of a disulfide-bond stabilized IL15v, the IL15Ra sushi domain, and an anti-PD1 antibody IgG. Several lead molecules were produced with good expression level, stability and drugability. These anti-PD1/IL15v fusions not only fully retained their PD1-blocking activity, but also were significantly more potent in promoting the activation of PD1-expressing T cells, comparing to those of PD1-negative cells. In vivo pharmacokinetics and antitumor activity of these anti-PD1/IL15v fusions are currently being investigated. Anti-PD1 targeted delivery of stabilized and attenuated IL15v may provide an effective approach, with an improved safety profile, to reverse the suppressive state of tumor-infiltrating antigen-specific PD1-expressing CD8 T cells.

#7265 Using PBMC-humanized mice to identify optimal autologous CAR T products for maximized efficacy and minimized toxicity at an individual level: a study of CD19, CD22, and CD19x22-specific constructs in multiple PBMC donors.

Won Lee[1], Destanie Rose[1], Beau H. Parry[1], Joshua Gustafson[2], Rebecca Gardner[3], Heather Gustafson[4], James G. Keck[1], Jiwon Yang[1]

[1]Innovation & Product Development, The Jackson Laboratory, Sacramento, CA,[2]Seattle Children's Therapeutics, Seattle Children's Hospital , Seattle, WA,[3]Oncology, St. Jude Children's Research Hospital, Memphis, TN,[4]UW Department of Pediatrics, Ben Towne Childhood Cancer and Blood Disorders Research; Seattle Children's Research Institute; University of Washington, Seattle, WA

Chimeric antigen receptor T-cell (CAR T) therapy has revolutionized cancer treatment by providing targeted eradication of cancer cells. However, the efficacy and toxicity of CAR T cells can vary widely between individuals, posing challenges for patient-specific optimization. To address these gaps, we evaluated the efficacy, expansion, and cytokine induction profiles of CAR T using PBMC-humanized mice. Three autologous CAR T products with 4-1BB co-stimulation were manufactured from the PBMCs of three healthy human donors: CD19-specific, CD22-specific, and CD19x22-specific (dual) CAR T cells. The dual CAR T cells were transduced with lentiviral vectors encoding either CD19- or CD22-specific CARs, resulting in three distinct CAR T cell compositions: those targeting CD19 alone, CD22 alone, or both CD19 and CD22. NSG-MHC Class I/II double knock-out (DKO) mice were irradiated and engrafted with 10 million PBMCs from the same donors used for the autologous CAR T cells. Six days post-humanization, the mice were treated with one of the three autologous CAR T cells. We found that all CAR T products effectively eliminated B cells in vivo within three weeks post-treatment, while CD19 CAR Ts achieved clearance significantly faster than CD22 CAR Ts. Expansion levels of CAR T cells differed significantly by donors and constructs. Overall, mice treated with CD19 CAR Ts showed peak expansion earlier than those treated with the other two CAR Ts. Mice treated with CD22 CAR Ts reached the highest expansion across all donors during the 28-day study period. Notably, one donor treated with CD22 CAR Ts displayed more than a 10-fold higher expansion compared to the other two donors, which corresponded to decreased survival and significant body weight loss. In dual CAR T-treated mice, CAR T cells targeting CD19 alone showed more robust expansion than the others (54 to 99% of total CAR T cells, depending on timepoints and donors). Cytokine induction was both donor-dependent and CAR construct-dependent, particularly for IL-1β, IL-2, IL-3, IL-5, IL-6, and IL-10. In summary, PBMC-humanized mice are a valuable platform to assess individual variability in CAR T efficacy and toxicity. These insights highlight the need for personalized preclinical evaluations to optimize CAR T construct selection and predict patient-specific outcomes.

#7266 Nanotechnology-enabled photochemical immune stimulation in clinical veterinary models of head and neck cancers.

Michael S. Valic[1], Charly J. McKenna[2], Mark Zheng[1], Jason L. Townson[1], Harley H. L. Chan[1], Layla Pires[3], Bridget Bane[2], Sharon Tzelnick[1], Alessandra Ruaro[1], Axel Sahovaler[4], Brian C. Wilson[1], Michelle L. Oblak[2], Jonathan C. Irish[1], Gang Zheng[1]

[1]UHN Princess Margaret Cancer Centre, Toronto, ON, Canada,[2]Ontario Veterinary College, Guelph, ON, Canada,[3]Biomedical Engineering, Texas A&M University, College Station, TX,[4]University College London Hospitals, London, United Kingdom

Background: Photodynamic therapy (PDT) is a non-surgical treatment using light to activate the cytotoxicity of a photosensitizing drug in cancer cells. PORPHYSOMES (PS) are lipid-porphyrin nanoparticles with high tumor uptake and PDT efficacy as well as low skin sensitivity. We have shown that activating PS with 'sub-therapeutic' PDT light dose in mouse models of head and neck (H&N) cancer stimulates immunogenic cell death and generates robust and transferable anti-tumor immunity. Here, we demonstrate the first evidence of this photochemical immune stimulation (PCIS) using PS in veterinary patients with spontaneous H&N tumors.

Methods: Clinical veterinary trials were conducted at the Ontario Veterinary College (AUP# 4548) in companion dogs with resectable H&N cancers. PS were injected IV at 3 mg/kg. Subjects in Arm 1 of the trial underwent surgery 24-48 h post injection. Subjects in Arm 2 underwent sub-therapeutic PDT treatment (671 nm, 12.5~50 J interstitial fiber or 12.5~25 J/cm^2 surface) at 6 or 24 h post injection, followed by resection at 48 h post injection. Blood samples were collected for hematology, biochemistry, pharmacokinetic analysis and immune profiling. During surgery, the tumor and neck lymph nodes were imaged by fluorescence for PS localization before submission for pathology. Cytokine and chemokine levels were measured in plasma/serum pre and post PDT, and tumor and lymph nodes were analyzed to compare for immune cell changes between Arms 1 (control) and 2.

Results: Eight veterinary patients were recruited: 6 thyroid carcinomas, 1 oral squamous cell carcinoma, 1 oral melanoma. Three subjects were enrolled in Arm 1 and 5 in Arm 2. No significant changes in blood counts or biochemistry were detected in any dog post PS injection. The PS half-life in plasma was ~57 h, and the tumor uptake varied between 6 and 60 µg/g (mean 28 µg/g). PDT was well tolerated in all cases, with no treatment-related adverse effects reported. Increases in multiple proinflammatory cytokines and chemokines were detected within 24 h of PDT, including significant increases in IL-8 and TNF alpha. Histology confirmed enrichment in CD68$^+$ macrophages and CD16$^+$ neutrophils in the PDT-treated tumors and in multiple T cell populations in lymph nodes. The relative magnitude of immune responses post PDT correlated weakly with PDT light dose in this small dataset. Of the subjects treated with PDT (7 mo. longest follow-up to date), only 1 tumor recurrence (in lung) was reported.

Conclusions: PS injection and PDT treatments were well tolerated. Sub-therapeutic PDT simulated measurable immune responses in these spontaneous veterinary tumors, analogous to the early responses seen in mouse models, in which the impacts on tumor progression, metastasis and survival were significant. These data provide key proof of concept evidence for PS PCIS in H&N cancers and motivate the continue translation of PS into human clinical studies.

#7267 Development and validation of a novel humanized B-hTRBC1/hTRBC2 mouse model for T cell activation.

Hannah Horton, Zhixia Li, Shufeng Wang, Zhenlan Niu, Lei Zhao, Yanling Wang, Xiaofei Zhou

Biocytogen, Waltham, MA

The gene encoding the TCR constant β chain (TRBC) has two isoforms: TRBC1 and TRBC2. Each TCR (and therefore each T cell) irreversibly selects a TCR β-chain constant region encoded by either TRBC1 or TRBC2 for expression in a mutually exclusive manner. Normal T cells consist of both TRBC1 and TRBC2; however, malignant T cells contain only one of the two isoforms. In the treatment of TRBC1+ or TRBC2+ T cell malignancies, selectively targeting TRBC1 or TRBC2, respectively, can effectively eliminate the malignant clone without significantly compromising the integrity of the T cell compartment, thereby preventing severe immunosuppression.

This study introduces a novel B-hTRBC1/hTRBC2 mouse model for the investigation of T cell activation and includes an extensive analysis of its phenotypic and immunological characteristics. Gene and protein expression of human TRBC1/TRBC2 in these mice were confirmed using RT-PCR and flow cytometry, respectively. The study also found comparable development, differentiation, and distribution of immune cells in the spleen, blood, and lymph nodes between B-hTRBC1/hTRBC2 mice and wild-type C57BL/6 mice. Notably, normal T cell proliferation and cytokine production were observed in vitro in response to anti-mCD3E and anti-hTRBC1 antibody treatments, demonstrating the functionality of humanized T cells in these mice.

In summary, our data indicate that B-hTRBC1/hTRBC2 mice provide a powerful preclinical model for *in vivo*/*ex vivo* evaluation of T cell activation.

#7268 A novel immunocompetent tumor model reveals the immunomodulatory effects of trastuzumab deruxtecan.

J. Steele, J. Balko;
Vanderbilt University School of Medicine, Nashville, TN

The αHER2 antibody-drug conjugate, trastuzumab deruxtecan (T-DXd), has revolutionized the treatment of breast cancer. However, it is being combined with immunotherapy in clinical trials without rigorous preclinical validation. Few studies have explored T-DXd's immunologic impact, and comprehensive preclinical data supporting immunotherapy combinations is lacking. This gap stems from trastuzumab's inability to bind to rodent HER2. Consequently, many existing models rely on human-derived cell lines or xenografts transplanted into immune-deficient mice, thus overlooking T-DXd's adaptive immunologic effects. Other strategies involve inoculating murine tumors expressing human HER2 into immunocompetent mice; however, this approach increases the immunogenicity of the model and often leads to tumor rejection or delayed growth due to xenoantigens arising from sequence differences between mouse and human HER2. To address this, we have developed a robust, immunocompetent tumor model to study trastuzumab-based therapies by minimally editing the mouse HER2 trastuzumab binding region. This tumor model, which we termed HER2^{X}, binds trastuzumab, is nonimmunogenic, and is sensitive to T-DXd treatment. Here we demonstrate the use of this HER2^{X} tumor model to elucidate the immunologic effects of T-DXd. Cytometry by time of flight (CyTOF) analysis revealed significant shifts to proinflammatory, antitumor immune populations, including T cells, NK cells, and M1-like macrophages, in T-DXd treated versus untreated EMT6 HER2^{X} tumors from immunocompetent mice. Accordingly, the proportion of M2-like macrophages, neutrophils, and monocytes were significantly lower in the T-DXd treated tumors. Notably, 60% of T-DXd complete responder mice were also resistant to rechallenge with EMT6 HER2^{X} tumor cells, indicating immunologic memory and potential involvement of adaptive immunity. Furthermore, T-DXd treatment potentiated EMT6 HER2^{X} antitumor responses in immunocompetent mice compared to athymic nude mice. Finally, co-administration of αPD-L1 and T-DXd treatment further increased survival and response compared to either monotherapy in HER2^{X} tumors. Together, these data suggest T-DXd induces a proinflammatory tumor-immune microenvironment, necessitates adaptive immunity for effective antitumor response, and may provide combinatorial benefit with immunotherapy in clinical settings. Further work is needed to determine the mechanism behind these immunomodulatory effects, and how we can leverage this knowledge to inform and improve treatment strategies in patients.

#7269 The humanized CRBN mice model facilitates PROTACs studies based on CRBN ligands.

Mari Kuraguchi, Ying Zhao, Chonghui Liu, Xiao Liu, Jiansu Zhang

Biocytogen, Waltham, MA

CRBN ligands, which have become increasingly popular as E3 ligase ligands, are important in designing PROTACs (Proteolysis Targeting Chimeras) and molecular glue degraders, aiding in targeted protein degradation. CRBN, part of the CRL4 E3 ubiquitin ligase complex, binds to specific proteins and promotes their degradation, a process essential for cell regulation and apoptosis. In multiple myeloma (MM) treatment, drugs targeting CRBN, like thalidomide and lenalidomide, show effectiveness by altering substrate specificity of CRBN, targeting tumor-associated proteins for degradation. CRBN-based PROTACs are emerging as promising cancer treatments by directing CRBN to target proteins for degradation. Biocytogen has developed the B-hCRBN mouse model, engineered to express human CRBN instead of the mouse version by inserting the full-length human *CRBN* CDS into the mouse *Crbn* exons 2-3. Expression of human CRBN mRNA and proteins were exclusively detected in homozygous humanized mice and these mice also demonstrated increased IL2 production in CD4+ T cells in response to lenalidomide treatment, confirming successful generation of the model. In summary, B-hCRBN mice are effective tool for preclinical *in vivo* efficacy and safety evaluation of CRBN-targeted drugs, including CRBN-based small molecules, molecular glues, and PROTAC drugs.

#7270 Novel humanized cell and murine models expressing tumor-associated antigens for ADC toxicity and IO combination evaluation.

X. Wang, R. Wang, K. Lian, R. Lu, J. Feng, J. Zhou, X. Tu, X. Feng, J. Lin, F. Xu, L. Bourre, J. Wang;
Crown Bioscience, Inc., San Diego, CA

Introduction: Antibody-drug conjugates (ADCs) represent a breakthrough therapeutic class that provides tumor-targeted cytotoxicity while stimulating immune responses, offering promising potential when combined with immunotherapies and particularly with PD-1 inhibitors. Traditional xenograft tumor models developed in immunodeficient mice are employed to evaluate ADCs' anti-tumor efficacy. However, these models lack competent immune systems and human antigen expression in normal tissues, limiting their capacity to assess immune responses and toxicity. To address these limitations, we developed HuCell, mouse tumor cells engineered to express tumor associated antigen (hTAA) such as HER2, and HuGEMM, humanized for a specific drug target within mice with a functional immune system. In this study HuCell/HuGEMM humanized mouse models were developed to evaluate the efficacy of ADCs in combination with anti-PD-1 immunotherapy and toxicity.

Methods: Surface expression of human HER2, Trop2, and Nectin-4 on MC-38 HuCell was verified using flow cytometry before and after inoculation on mice. 5×10^6 HuCells were subcutaneously inoculated into hHER2, hTrop2, hNectin4 and hHER2/hPD-1 models in C57BL/6 background or wild-type (WT) models. Tumor-bearing models were treated with monotherapy of trastuzumab-deruxtecan (T-Dxd), sacituzumab govitecan or enfortumab vedotin, or combination therapy with anti-PD-1 antibodies. For the toxicity study, 180 mg/kg T-Dxd and 100 mg/kg trastuzumab-emtansine (T-DM1) were injected into hHER2 mice weekly for two doses by intravenous injection.

Results: A highly HER2-expressing HuCell line (clone #2) successfully grew in hHER2 models but not in WT models, with tumors displaying nearly 100% of human Her2 positive cells with high surface expression (2×10^5 molecules per cell). In dose response study, T-Dxd significantly inhibited tumor growth at 5 mg/kg and 10 mg/kg in C57BL/6-hHER2. Combination treatment of T-Dxd (4 mg/kg+2 mg/kg, QW) with Keytruda (5 mg/kg, BIW×2) resulted in a complete response rate (8/8) with 87% TGI ($p<0.01$) compared to T-Dxd monotherapy (7/8) with 96% TGI ($p<0.001$) in hHER2/PD-1 double knock in mice. In a toxicity study, T-DM1 showed liver toxicity, whereas T-Dxd did not. Both T-DM1 and T-Dxd exhibited notable hematological toxicity, consistent with clinical observations. In the Trop2 and Nectin-4 HuCell/HuGEMM studies, similar results were observed. HuGEMM models demonstrated superior positive cell rate and single-cell antigen expression levels. Furthermore, a synergistic effect was observed when PD-1 therapy was combined with monotherapy.

Conclusion: HuCell and HuGEMM models provide an advanced platform to evaluate ADC drug monotherapy and combination therapies with immune checkpoint inhibitors. Additionally, they offer a sophisticated solution to assess both on-target and off-target toxicity of ADCs.

#7271 SENTI-202 CD33 OR FLT3 NOT EMCN logic-gated gene circuit components selectively target AML while protecting human HSC/HPCs from off-tumor toxicity in a humanized mouse model.

Muharrem Muftuoglu[1], Li Li[1], Mahesh Basyal[1], Jiangxing Lv[1], Lauren B. Ostermann[1], Han Deng[2], Priscilla Wong[2], Tim Lu[2], Brian Garrison[2], Kanya Rajangam[2], Michael Andreeff[1]

[1]Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX,[2]Senti Biosciences, San Francisco, CA

There are no approved chimeric antigen receptor (CAR) natural killer (NK) or T cell therapies for acute myeloid leukemia (AML), due in-part to the scarcity of tumor-specific cell surface targets, which creates potential safety challenges with on-target off-tumor toxicity. SENTI-202 is an innovative CAR NK cell therapy that expresses a potent CD33 OR FLT3 NOT EMCN logic-gated gene circuit, in conjunction with IL-15. The bivalent CD33 OR FLT3 activating CAR targets 2 AML antigens, CD33 & FLT3, enabling targeting of AML stem cells & blasts (Gonzalez, 2023; Kaveri, 2024) and venetoclax-resistant AML cells (Muftuoglu, 2023). The NOT EMCN inhibitory CAR (iCAR) protects hematopoietic stem and progenitors (HSC/HPCs) from off-tumor toxicity (even if the healthy cells are $FLT3^+/CD33^+$), which is a major technological advancement that could enhance safety and hematopoietic recovery in patients. The iCAR recognizes the healthy cell marker endomucin (EMCN), which is expressed on up to 76% of HSCs (Reckzeh, 2018) but not AML cells. Here, we used CD33 OR FLT3 NOT EMCN CAR NK cells to validate iCAR-driven HSC/HPC protection in a humanized mouse model by comparing CD33 OR FLT3 NOT EMCN NK (88% CAR^+) to (1.) Non-engineered NK and (2.) CD33 OR FLT3 NOT HER2 (iCAR control) NK (89% CAR^+). First, we confirmed expected in vitro anti-AML activity (observed 50-70% killing; E:T=1:1) and iCAR-mediated model healthy cell preservation activity (observed ~50% preservation; E:T=1:1), using $EMCN^-$ and $EMCN^+$ cell lines, consistent with our prior results showing the EMCN iCAR preserves primary human HSCs from off-tumor toxicity in vitro (Garrison, 2022). Here, using a humanized mouse model we demonstrated iCAR-mediated in vivo protection of human HSC/HPCs from off-tumor toxicity for the first time in the field. Following successful human HSC/HPC engraftment, mice were given 2 CAR NK cell injections on d0 & d7, then HSC/HPC frequency was assessed on d14. While treatment with the iCAR control NK cells led to a significant decrease in HSC/HPC frequency compared to non-engineered NK (18.15% versus 28.6% of $hCD45^+$; p value=0.00015), treatment with CD33 OR FLT3 NOT EMCN NK cells resulted in a significantly increased HSC/HPC frequency versus iCAR control NK cells (34.2% versus 18.15% of $hCD45^+$; p value=0.00000355). This demonstrates that the EMCN iCAR protects human HSC/HPCs from off-tumor toxicity in vivo, and suggests that the EMCN iCAR may increase the SENTI-202 therapeutic window. In conclusion, the SENTI-202 CD33 OR FLT3 NOT EMCN logic-gated gene circuit enables exceptional efficacy against AML while protecting HSC/HPCs from off-tumor toxicity, demonstrating the potential of SENTI-202 as a targeted and precise therapy for AML. SENTI-202 is currently in Phase 1 clinical development in patients with relapsed/refractory hematologic malignancies including AML (NCT06325748).

#7272 aPD1 response prediction in a human *ex vivo* tumor model: A case study in multiomic approaches for preclinical drug evaluation.

Adam Yalin, Andras Sziraki, Devin Mediratta, Andrew Shuparski, Andy Tu, Mirko Andreoli, Patrick Petrossian, Konstantinos Vlachos, Tomas Zikmund, Dagmar Schierova, Alena Molnarova, Anna Dostalova, Eleni Mimitou, Adeeb Rahman, Lisa Freinkman, Florian Uhlitz, **Evgeny Kiner**

Immunai, New York, NY

Background: Anti-PD1 (aPD1) and other immunotherapies have demonstrated significant clinical impact in cancer treatment. However, the inability to accurately predict which patients will respond to specific therapies and to determine the correct indications or combinations for many immunotherapy drugs has led to clinical trial failures across many tumor indications. To address this, we developed a preclinical *ex vivo* patient derived-tumor fragment (PDTF) platform that leverages single-cell multiomics data and our expansive patient-derived single-cell database AMICA™ to dissect drug mechanism of action (MOA), and identify baseline determinants of molecular *ex vivo* response, which can be used to predict therapeutic response in clinical samples. Using pembrolizumab as a use case, this approach identifies patient-level baseline predictive features of aPD1 therapeutic response and predicts the relative clinical efficacy of aPD1 across patient subgroups.

Methods:

PDTFs from a cohort of renal cell carcinoma (RCC) patients were treated with 10 ug/ml aPD1 or isotype control for 48 hr. Multiplex ELISA was performed on culture supernatants, while pre-culture and treated PDTFs were dissociated and sorted on live immune cells for CITE-seq analysis. The resulting multiomic dataset includes cell abundances, cell type specific RNAs and proteins, clonality of T cell subsets, and secreted proteins. These features were used to stratify patients into molecular responders and non-responders. The pre-culture samples from the same patients were then analyzed using the ImmunoDynamics Engine™, a proprietary machine learning (ML) framework, to determine the baseline features of molecular response. To validate our approach, these baseline features were contextualized using samples from patients with known clinical response to PD1 blockade.

Results:

•aPD1 elicits cytotoxicity and interferon pathway upregulation in PD1-expressing cell types. The MOA of aPD1 in our *ex vivo* system is consistent with several clinical datasets.
•Individual donors could be classified by molecular response to aPD1 across several multiomic features.
•Immune features correlating with response to aPD1 at baseline were identified and found to be predictable of molecular response with reasonable accuracy.
•Molecular response features were validated on baseline tumor samples from patients treated with PD1 inhibitors in the clinic and were found to be predictive of therapeutic prognosis.

Conclusions:

Our *ex vivo* model system and analytical framework uses several modalities to establish the MOA and clinical response features of an immunotherapy agent. Recapitulating the known biology and response of aPD1 builds conviction that extending this framework to
other immunomodulatory drugs and their combinations could enhance patient selection and clinical trial design, ultimately improving clinical outcomes.

#7273 CCR8/CCL1 humanized model for efficacy assessment of CCR8/CCL1-targeting therapies.

Fabiane Sonego, Angela Pappalardo, Gaelle Martin, Yacine Cherifi, Patricia Isnard Petit, Kader Thiam

genOway, Lyon, France

Modulation of tumor microenvironment has shown to be a promising approach to treat cancer. Among diverse mechanisms responsible for tumor immunosuppression, those mediated by regulatory T cells (Treg) infiltrating in tumor are well identified and known to reduce the efficacy of anti-tumor therapies. CCR8 has been identified as a potential therapeutic target due to its role in the immunosuppression induced by Treg. CCL1 is the major CCR8 ligand and CCL1 / CCR8 interaction induces STAT3-dependent up-regulation of FoxP3, CD39, IL-10 and Granzyme B, leading to the enhancement of the suppressive activity of Treg cells. A major challenge in the development of new therapeutic approaches relies on the choice of the right preclinical model, which could mimic the complexity of interactions among different cell types and closely predict the effects of therapeutics in humans. The current therapeutic approaches directed towards the CCR8/CCL1 axis target the receptor CCR8, requiring its humanization for assessment of therapeutics displaying species-specific activity. The development of a physiologically relevant model depends, among others, on the maintenance of a proper interaction of the receptor and ligand. CCR8 has 2 ligands in mouse: CCL1 and CCL8. Mouse CCL8 is able to induce specific calcium flux in human CCR8-transfected mouse cells (Islam *et al.*, Nat Immunol, 2011), suggesting that humanization of CCL8 is not mandatory. However, in *vitro* studies showed that murine CCL1 was unable to interact with human CCR8, leading to the conclusion that the CCR8 / CCL1 axis needs to be humanized. Prior to the mouse model generation, an *in vitro* functional assessment confirmed the functionality of human CCR8 in murine cells. Herein, we describe a novel CCR8 and CCL1 double humanized model. Briefly, CCR8 and CCL1 were humanized. Both human genes are under the control of their endogenous mouse promoter, enabling physiological and regulated expression (no regulatory elements were altered by the gene editing process), which is key for the interpretation of the data obtained with the model. CCL1 is produced and detected in the serum from CCR8/CCL1 humanized mice. CCR8 was expressed on approximately half of the MC38 tumor infiltrated Tregs, while very low or absent expression was observed on Treg in the periphery (spleen, skin and blood). Expression of human CCR8 enabled activity of CCR8-targeting antibodies, as a collaborator has shown that treatment with a CCR8 antibody induces tumor volume reduction ($400mm^3$ vs. $750mm^3$) and increased survival (80% vs 30%) 20 days post MC38 inoculation. Therefore, this model is a valuable tool for efficacy assessment of CCR8-targeting drugs, enabling the investigation of immunomodulatory mechanisms at the tumor microenvironment. This is possible because the mouse immune system is functional and its interplay with mouse stroma and tumor cells enables the assessment of a finely regulated microenvironment.

#7274 A preclinical *in vitro* platform for evaluating the potency of bispecific T cell engagers in 2D and 3D models.

Xuefei Yan, Shiqi Guo, Saskia De Man, Yifan Chen, Jiawei Meng, Michelle Kop, Valeria Teodosieva, Haijuan Yu, Gera Goverse, **Jun Zhou**, Peng Wang

Crown Bioscience, Inc., San Diego, CA

Introduction: Bispecific T-cell engagers (BiTEs) are innovative cancer therapeutics designed to harness the immune system by linking T cells to cancer cells, inducing tumor-specific cytotoxicity, which enhances specificity and reduces off-target effects on normal tissue. In this study *in vitro* assays were established to evaluate the tumor cell-killing and T cell activation of BiTEs, specifically using a claudin18.2-targeted BiTE AMG910 and the monoclonal antibody zolbetuximab.

Methods: Target expression on tumor cells for claudin18.2 and AMG910/zolbetuximab binding to tumor and T cells was evaluated via flow cytometry. Tumor cell killing was assessed after 48h co-culture of tumor cells with human T cells upon treatment by FACS and LDH release assays. T cell activation was evaluated by IFN-γ and IL-2 supernatant release, along with FACS (CD25, CD69, IFN-γ, and Granzyme B). AMG910 was further tested in 3D pancreatic patient-derived xenograft organoid (PDXO) models employing high content imaging (HCI) to assess immune cell activation, infiltration, and organoid killing.

Results: NUGC4-hClaudin18.2 cells exhibited the highest Claudin18.2 protein levels compared to NUGC-4 and SNU-620 cells, despite similar mRNA levels. RNA-seq and FACS showed that Claudin18.2 expression was absent in AGS cells. AMG910 displayed stronger binding to SNU-620 cells and T cells compared to zolbetuximab, while zolbetuximab exhibited stronger binding to NUGC4-hClaudin18.2 cells, with no significant binding difference observed between the two Abs on NUGC-4 cells. In a co-culture with human T cells and tumor cells, AMG910 demonstrated significant dose-dependent killing of SNU-620, NUGC-4, and NUGC4-hClaudin18.2 cells compared to untreated samples, along with elevated IFN-γ and IL-2 secretion. T cell activation was further confirmed by increased expression of CD25, CD69 and intracellular Granzyme B and IFN-γ. No effects were observed in co-culture with AGS cells. Furthermore, AMG910 did not activate T cells in the absence of tumor cells, indicating AMG910's Claudin18.2-dependent T cell activation. In 3D co-cultures, treatment with the T cell engager enhanced migration of activated immune cells (CD69) into the organoids, inducing organoid killing analyzed via HCI.

Conclusion: Successful *in vitro* assays were developed for preclinical BiTEs testing. 2D co-cultures efficiently assess direct tumor cell killing and T cell activation mediated by antibody treatments. Additionally, 3D organoid co-cultures offered a more physiologically relevant setup, mimicking the tumor microenvironment and representing patient diversity. Moreover, the HCI analysis revealed enhanced infiltration of activated T cells upon treatment with the T cell engagers before killing of the organoids. This combination offers a robust platform for in-depth preclinical evaluation of BiTEs.

#7275 A novel PD-1, IL12Rβ1 and IL12Rβ2 humanized mouse model for evaluating the combined efficacy of anti-tumor therapies.

Jade Hsu, Linlin Wang, Fengxing Gu, Meiqi Zhang, Jing Guo, Xiaofei Zhou

Biocytogen, Waltham, MA

PD-1 is an inhibitory immune checkpoint that reduces T cell activity by binding to its ligand PD-L1, which facilitates immune evasion in the tumor microenvironment. In recent years, targeting PD-1 has become a key component of cancer immunotherapy. Interleukin-12 (IL-12) serves as a crucial regulatory factor in both innate and adaptive immunity; it promotes cell proliferation, activates cytotoxic lymphocytes and natural killer (NK) cells, boosts the production of interferon (IFN)-γ, and supports cell differentiation, thereby enhancing the generation of TH1-related immunoglobulins. Given its role in pro-inflammatory responses and immune modulation, IL-12 has the potential to convert tumors from a "cold" state to a "hot" state. Therefore, the combination of IL-12 and PD-1 inhibitors represents a promising strategy for advancing cancer immunotherapy. Biocytogen has developed B-hPD-1 plus/hIL12RB1/hIL12RB2 mice by breeding humanized PD-1mice with humanized IL12RB1 and IL12RB2 mice. To validate the PD-1 expression, splenocytes were collected from C57BL/6 mice and homozygous B-hPD-1 plus/hIL12RB1/hIL12RB2 mice stimulated with anti-mouse CD3ε antibody *in vivo* for 24 hrs. Mouse PD-1 was only detectable in C57BL/6 mice. Human PD-1 was exclusively detectable in homozygous B-hPD-1 plus/hIL12RB1/hIL12RB2 mice, but not in C57BL/6 mice. To validate the functionality of the humanized IL12Rβ1 and IL12Rβ2 receptor complex, $CD4^+$ T cells sorted from splenocytes of C57BL/6 and homozygous B-hPD-1 plus/hIL12RB1/hIL12RB2 mice were stimulated with human IL-12 *in vitro*, and IFN-γ production was detected by ELISA. Mouse IFN-γ were both increased after responsiveness to mIL-12 in C57BL/6 mice and homozygous B-hPD-1 plus/hIL12RB1/hIL12RB2 mice. Mouse IFN-γ were increased after responsiveness to hIL-12 in homozygous mice but not in wild-type mice.

In summary, the PD-1, IL12RB1, and IL12RB2 genes have been successfully humanized in B-hPD-1 plus/hIL12RB1/hIL12RB2 mice, allowing us to assess the combined efficacy of anti-tumor drugs.

#7276 Accurate prediction of clinical immunotoxicity of oligonucleotide therapeutics using ex vivo circulating fresh human whole blood test system.

Erika A. K. Fletcher, Gunilla Tornqvist, Helena Harlin Laven, **Sakthi Srinivasan**

Immuneed AB, Uppsala, Sweden

Few oligonucleotide (ONDs) therapeutics are currently approved for cancer treatment due to their ability to modulate gene expression and protein function and their reduced toxicity compared to traditional chemotherapeutics. Existing preclinical animal models demonstrate varied sensitivities to ONDs for detecting clinical immunotoxicity. Non-Human Primates (NHPs) show a higher sensitivity to phosphorothioate oligonucleotides and tend to overpredict the risk of complement activation. Additionally, NHPs were unable to predict antisense oligonucleotide (ASO) induced cytokine release prior to first-in-human (FIH) trials. A notable example is ISIS 353512 (a 2' methoxyethyl ASO, 2' MOE ASO), which unexpectedly induced IL-6 release in human volunteers. Here, we describe the use of a physiologically relevant ex vivo model based on circulating fresh human whole blood, to accurately predict the clinical immunotoxicity of two FDA-approved ONDs: imetelstat (OND) and inotersen (a 2' MOE ASO). Both ONDs were tested in six donors at three concentrations each 1, 5 and 25 uM), covering the clinical Cmax for both inotersen (at the lowest concentration) and imetelstat (at the highest concentration). This ex vivo test system enables the study of early human immune responses by preserving all blood immune cells, immunoglobulins, and active coagulation and complement cascade systems. The system has previously been validated for predicting infusion reactions of antibody-based drugs prior to FIH trials.Both inotersen and imetelstat are known to cause clinical side effects such as flu-like symptoms. The model system reflects these symptoms with a significant increase in pro-inflammatory cytokine release (IL-8, and TNF-α) observed at the highest dose of both ONDs. Imetelstat demonstrated significant activation of granulocytes and monocytes, correlating with the observed high release of IL-6 and IL-8 cytokines in this model system and its intended action against myeloid tumors. In contrast, inotersen only triggered B cell activation, correlating with the IL-6 release caused by Toll-like receptor (TLR) stimuli in B cells, a response previously observed in patients treated with ISIS 353512. Previous studies indicate that both the investigated ONDs result in complement activation in NHPs, while imetelstat alone caused significant complement activation in the clinic. In line with clinical observations, the high dose of imetelstat (but not inotersen) in our test system showed significantly increased activation of complement factors (C3a, and C5a). Additionally, imetelstat caused a decreasing trend with platelet counts, which correlates with its clinical manifestations of thrombocytopenia. Taken together, the data show that our ex vivo circulating whole blood test system has the potential to predict human immunotoxicity responses to ONDs before FIH trials.

#7277 Development of a macrophage-based screening pipeline to evaluate the immunomodulatory effects of small molecule inhibitors.

Marie Durr, Manasa Suresh, Bryan Weselman, David Quiceno-Torres, Megan Winakur, Satish Noonepalle, Alejandro Villagra

Georgetown University, Washington, DC

Introduction: Immunotherapies targeting adaptive immunity have shown promising results in solid tumors; however, many patients are either unresponsive or develop resistance. Emerging interest in innate immunity has driven novel strategies to target myeloid cells, including tumor-associated macrophages (TAMs). TAMs primarily have an anti-inflammatory, pro-tumoral M2-like phenotype secreting growth and angiogenic factors to promote an immunosuppressive tumor microenvironment (TME). Modulating the TAMs towards a more proinflammatory M1-like elicits anti-tumor responses with enhanced antigen presentation and phagocytic activity. Therefore, understanding the molecular mechanisms driving macrophage phenotype plasticity is essential to maintaining the phenotypic switch from M2-like to M1-like in an immunosuppressive TME. Current high-throughput pipelines for screening and optimizing anticancer drugs focus on the cytotoxic and antiproliferative effects of these compounds, underestimating or ignoring their immunomodulatory effects. In this study, we developed drug screening pipelines to identify small molecule inhibitors impacting the macrophage functional phenotype to identify leading candidates with immunoregulatory properties.
Methods and Analysis: Mouse bone marrow-derived macrophages were polarized to M1-like or M2-like phenotypes and applied in bulk RNA sequencing to identify specific genes associated with each phenotype. We created stable RAW264.7 macrophages expressing luciferase under the control of phenotype-specific promoters to identify targets modulating the macrophage phenotype. Additionally, THP-1 reporter cell lines driven by response elements of inflammatory signaling pathways were used to further evaluate the mechanistic role of small molecule inhibitors on the phenotypic switch in macrophages[MS1] following stimulation of the pathway with appropriate cytokines.
Results and Conclusions: RNA sequencing data identified exclusive genes upregulated with M1 and M2 polarization of macrophages. Based on the gene expression changes and successful validation of the plasmids in RAW264.7 cells, we selected TNF and CXCL5 as genes for M1 and Arg1 and CCL24 for M2 markers. Using the stable reporter RAW264.7 cell lines and the THP-1 reporters for specific pathways, we screened several epigenetic modifiers, including novel HDAC inhibitors. Using this approach, we found selective HDAC6 inhibitors diminished the anti-inflammatory phenotype of macrophages and, upon stimulation with appropriate cytokines, increased pro-inflammatory pathways, such as NFkB. These efforts could enable high-throughput screenings of novel small molecule inhibitors for their immunomodulatory effect on macrophages.

#7278 Non-clinical toxicity studies of S-CP201.

Michael Ahrweiler, Wolfgang Meyer, Daniel Perrissoud, Axel Zander

RDP Pharma AG, Romanshorn, Switzerland

Edelfosine, an anticancer alkylphospholipid, has been widely investigated. Inter alia, it integrates into cell membranes (including mitochondria and ER) and acts via CD95-driven apoptosis, AKT3/PI3K inhibition, phospholipase C and protein kinase C inhibition, and impacts cholesterol metabolism. It exhibits antiangiogenic and antimetastatic effects, selectively targets malignant cells, and has shown efficacy in preclinical xenograft models, (e.g metastatic breast and prostate cancers), and in phase 2 trials across tumor types. The main adverse event was dose-dependent, reversible gastrointestinal toxicity. Many non-clinical toxicity reports on edelfosine are available. Since edelfosine is racemic, its S-enantiomer (S-CP201) was chosen for development due to PK/ PD advantages over the R-counterpart. Key data of a GLP-/ICH S9 and M3-conform, IND-enabling toxicity package for S-CP201 is stated below. In addition to a 4-week dose range-finding (DRF) study, S-CP201 was given in a 13-week rat toxicity study per os (p.o.) at 3, 9, 27, and 40 mg/kg/day (d) (15 rats per sex and group). The 2 high dose groups showed reversible liver enzyme increases. One female in the 27 mg/kg group exhibited an enlarged pituitary; 9/15 males showed seminal vesicle atrophy, and 10/15 tubular vacuolation in the testes. In the 40 mg/kg group, dilated intestines, cecum, and reddened duodenal mucosa were observed. The NOAEL was set at 9 mg/kg/d. In a maximum tolerated dose (MTD)/DRF study in minipigs, escalating doses reached 96 mg/kg/d for 3 d in the DRF phase and 12 mg/kg/d for ≤6 d in the MTD phase. Doses ≥12 mg/kg/d resulted in soft feces, dark duodenal mucosa, and reduced appetite/body weight (BW) loss. MTD was 6 mg/kg/d. Safety pharmacology tests of S-CP201 (core battery) were also conducted. In Wistar rats, single oral doses ≤40 mg/kg showed no notable respiratory effects (whole-body barometric plethysmography). Similarly, single doses ≤14 mg/kg in telemetered male Beagle dogs showed no adverse effects on blood pressure, heart rate, ECG, or body temperature, confirming prior negative hERG assay results. Soft feces were found at doses ≥9 mg/kg, but no other changes in BW or food consumption were noted. In a modified Irwin test, single oral doses ≤100 mg/kg in Sprague-Dawley rats produced no signs of behavioral, neurological, or autonomic effects. A pilot Ames fluctuation test showed no mutagenic potential across a broad concentration range. In a standard Ames test, low mutagenic effects were seen only at high concentrations in 3 of 4 tested S. typhimurium strains. A new Ames test under similar conditions did not reveal mutagenic effects. An in vitro micronucleus assay showed no effects at therapeutic concentrations, and an in vivo micronucleus test in male rats receiving S-CP201 (≤200 mg/kg/d p.o.) showed no clastogenic or aneugenic effects. An in vitro hemolysis test (up to 20 µM) was negative for S-CP201. Overall, this S-CP201 non-clinical safety package supports proceeding to Phase 1 studies.

#7279 A novel humanized IL2 and IL15 receptor mouse model enables preclinical efficacy studies for IL-2 cancer therapeutics.

Kelvin Yin, Alexander Sercel, Linlin Wang, Suman Zhao, Zan Zhang, Zhenlan Niu, Yanling Wang, Xiaofei Zhou

Biocytogen, Waltham, MA

Recombinant human interleukin 2 (IL-2) is effective in treating metastatic renal carcinoma and melanoma, however, severe dose-limiting toxicity restricts patient treatment. There is conflicting evidence from human and murine studies on whether activation of natural killer (NK) cells leads to efficacy or toxicity and if activation of T or NK cells is associated with therapeutic benefit. The development of a humanized mouse model for this system is imperative to evaluate the safety and efficacy of IL-2 targets.

Here, we generated a homozygous humanized hIL-2RB/hIL-2RG/hIL-15/hIL-15RA mouse model which constitutively expresses human IL-2RB, IL-2RG, IL-15, and IL-15RA proteins. The distribution of basal leukocyte subpopulations of blood, spleen, and lymph nodes in humanized B-hIL-2RB/hIL-2RG/hIL-15/hIL-15RA mice is similar and comparable to the distribution found in wild-type C57BL/6 mice. Functionally, the human IL-2 and IL-15/IL-15RA complex elicits a dose-dependent induction of pSTAT5 in humanized mice. Furthermore, using this model, we have detected modest efficacy of a human IL-2 drug in inhibiting MC38 tumor growth *in vivo*.

In summary, B-hIL-2RB/hIL-2RG/hIL-15/hIL-15RA mice provide a powerful preclinical model to clear roadblocks towards *in vivo* evaluation of IL-2 drugs for cancer treatment.

#7280 A novel IL12Rβ1 and IL12Rβ2 humanized mouse model for preclinical toxicity evaluation of human IL-12 analogs.

Kelvin Yin, Alexander Sercel, Fengxing Gu, Jing Guo, Meiqi Zhang, Saina Ma, Jiansu Zhang, Xiaofei Zhou

Biocytogen, Waltham, MA

Interleukin 12 (IL12), a heterodimer cytokine composed of IL12A (p35) and IL12B (p40), is mainly produced by dendritic cells and macrophages in response to antigenic stimulation and its receptor is a protein complex encoded by IL12Rβ1 and IL12Rβ2. IL12 signaling regulates innate and adaptive immunity, influencing the proliferation and activation of cytotoxic lymphocytes and natural killer (NK) cells, stimulating the production of interferon (IFN)-γ, and promoting the differentiation and production of Th1-associated immunoglobulins. IL12 is a promising anti-tumor drug capable of transforming the tumor microenvironment from “cold” to “hot”. To accelerate discovery in this space, Biocytogen has independently developed IL12RB1 and IL12RB2 single KI mice and mated them to subsequently B-hIL12RB1/hIL12RB2 double KI mice to develop a fully humanized model for testing IL12-analog drugs. First, the mRNA expression levels of human IL12RB1 and human IL12RB2 in B-hIL12RB1/hIL12RB2 mice were quantified using RT-qPCR. Additionally, immunophenotyping by flow cytometry shows that the humanization of IL12RB1 and IL12RB2 did not affect the proportions and distribution of immune cells. To validate the functionality of the humanized IL12Rβ1 and IL12Rβ2 receptor complex, $CD4^+$ T cells sorted from splenocytes of C57BL/6 and homozygous B-hIL12RB1/hIL12RB2 mice were stimulated with human IL12 *in vitro*, and IFN-γ production was detected by ELISA. The results indicated that human IL12 induced a significant increase in IFN-γ levels in the B-hIL12RB1/hIL12RB2 mice, while no such effect was observed in wild-type mice. Furthermore, to evaluate the *in-vivo* toxicity of human IL12 analogs, B-hIL12RB1/hIL12RB2 mice were treated with human IL12 at different doses and for varying durations. Body weight was measured daily during treatment. At the end of the experiment, the weights of the liver and spleen were measured, and pathological changes in the liver and spleen were assessed through HE staining. Meanwhile, serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the mice were detected using ELISA. In the *in vivo* toxicity study, human IL-12 administration resulted in a progressive decline in body weight in treated mice over time. Additionally, human IL12 treatment was associated with an increase in the ratios of liver weight to body weight and spleen weight to body weight. Pathological changes in the liver and spleen were also observed, alongside an upward trend in ALT and AST activity. These results suggest the successful humanization of IL12RB1 and IL12RB2 signaling in B-hIL12RB1/hIL12RB2 mice, enabling evaluation of the preclinical toxicity of human IL12 analogs.

#7281 Predicting clinical checkpoint inhibitor responses with TumorGraft3D PBMC co-culture.

Mara Gilardi, BanuPriya Sridharan, Veena Jagannathan, Brandon Walling, Stefano Cairo, Marianna Zipeto, Michael Ritchie

Champions Oncology, Inc., Hackensack, NJ

Although immunotherapy-based therapeutics such as checkpoint inhibitors (CPI) have revolutionized cancer therapy, a lot needs to be done in the field. In preclinical settings, ex vivo models still face significant challenges in translating the results into clinical responses. Traditional approaches, including cell-lines, are limited by their lack of genetic heterogeneity. Similarly, 2D systems fail to capture spatial complexity, significantly reducing their translational relevance. Clinical data on CPI are not available for commercially available cell lines since they were developed before CPI treatments were approved. These limitations hinder the accurate modeling of CPI efficacy and the prediction of therapeutic responses. To address these issues, Champions Oncology leverages a tumor bank of 1500 patient-derived xenograft (PDX) models across solid tumors and hematological malignancies offering 3D co-culture assays specifically tailored for different mechanisms of action. To provide a clinical translational platform that retains human tumor heterogeneity, we developed Champions TumorGraft3D (CTG3D) low-passage 3D PDX-organoid models. The use of proprietary, indication-specific media enabled CTG3D to preserve the genetic, proteomic, and phenotypic diversity of the original tumors. Additionally, their 3D architecture accurately models the cell-cell interactions that are crucial for mimicking immune cell engagement. The in-depth clinical annotation of our bank, which includes detailed patients' clinical history, enables this system to provide predictive insights into the CPI efficacy. In this project, we assessed the translational value of our CTG3D co-culture system, based on its ability to recapitulate the clinical response in patients. We selected models derived from patients displaying resistance or sensitivity to CPI, by leveraging the data collected in Champions Oncology Tumor Bank. Then, we optimized the CTG3D-PBMCs co-culture settings including media and cell ratios. High throughput flow cytometry was used to interrogate the model. We profiled the co-culture with a custom-designed panel allowing for cancer cell killing and immune phenotyping readout in parallel. Beyond immune activation, we were able to determine the correlation of the data obtained from the co-culture with the clinical records of the original patients. In summary, we developed a cutting-edge ex vivo CTG3D PBMC co-culture platform allowing for dynamic evaluation of immune engagement and checkpoint inhibitor responses, therefore providing valuable translational datasets. By overcoming the limitations of traditional assays, CTG3D co-culture systems represent a transformative step forward in preclinical immunotherapy research and the development of personalized cancer treatments.

#7282 Development of an HLA-matched humanized immune system mouse model with primary AML patient samples for immunotherapy testing.

Mara Gilardi[1], Paolo Schiavini[1], Laura Griffin[2], Brandon Walling[1], Paul Heverly[1], Stefano Cairo[1], Philip Dube[2], Monika Buczek[2], Marianna Zipeto[1], Michael Ritchie[1]

[1]Champions Oncology, Inc., Hackensack, NJ,[2]Taconic Biosciences, La Jolla, CA

Acute Myeloid Leukemia (AML) is a devastating hematological malignancy with a global incidence of approximately 120,000 new cases annually and a five-year survival rate below 30%. Despite advances in therapeutic strategies, treatment outcomes remain poor. The complexity of AML, characterized by genetic heterogeneity and clonal evolution, presents significant challenges for the development and testing of novel therapeutics in clinically relevant settings. AML research relies heavily on cell lines and syngeneic models, or immunodeficient models without an added human immune system component. While these systems provide valuable insights, they fail to fully recapitulate the genetic and phenotypic diversity of primary human AML tumors. Traditional models often lack the tumor microenvironment and immune interactions critical for therapeutic evaluation. Furthermore, existing models generally rely on extensively passaged samples, which lose the heterogeneity and clonal diversity intrinsic to AML, undermining their clinical relevance. To address these limitations, we developed an in vivo AML platform using human CD34+ engrafted NOG-EXL mice. NOG EXL was selected as the preferred in vivo model as it has been successfully engrafted with primary AML PDXs, albeit not also with a humanized immune system component. This novel platform was built on a bank of over 50 patient-derived AML models that have been passaged, preserving their native tumor characteristics. Humanized NOG-EXL mice, transgenic for human IL-3 and GM-CSF, were used to promote primary AML engraftment as well as CD34+ stem cell differentiation into a wider repertoire of human myeloid and lymphoid lineages. The models are fully characterized with RNA sequencing, proteomics, diagnostic data, and detailed clinical histories. By retaining the complexity and clonal architecture of primary AML tumors, these models provide a truly translational system for preclinical drug testing. AML donors were evaluated in humanized NOG-EXL. CD34+ engraftment was confirmed in humanized NOG-EXL mice followed by engraftment with AML primary cells. Human immune cell engraftment and AML cancer progression were assessed at different time points through flow cytometry on peripheral blood and bone marrow. A custom flow cytometry panel was used to identify the human immune cells present as well as the main AML sub-populations including the AML progenitors, AML blast,s and monoblasts. This novel approach bridges a critical gap in AML research by offering a robust, clinically relevant platform that reflects real-world disease biology by demonstrating an in vivo model with primary never-passaged AML PDX in the context of a human immune system. The Champions AML humanized model sets a new standard for preclinical evaluation, promising to accelerate the development of personalized therapies and improve outcomes for AML patients worldwide.

IMMUNOLOGY: Overcoming Checkpoint Inhibition and Tumor Suppression
Poster Session

#7286 A first-in-class αPD-1/αVEGF/IL-2v tri-specific fusion protein demonstrates promising anti-tumor efficacy in immune checkpoint inhibitor-resistant tumor models.

Sunjung Cho, Jun-Eui Park, Yongjun Jung, Hyojoo Bang, Jeongseok Heo, Seokchan Kang, Suna Kim, Minwoo Kim, Young Jin Park

Mustbio, Suwon-si, Korea, Republic of

Immune checkpoint inhibitors (ICIs), including anti-PD(L)1 monoclonal antibodies, have been widely approved and used for various cancer types. However, the response rate to ICIs is limited to certain cancer types, largely attributed to the absence or low presence of immune cells within the tumor microenvironment (TME). αVEGF antibodies not only facilitate immune cell infiltration into tumors but also improve the immunosuppressive conditions of the TME. Notably, the combination of αPD-1 and αVEGF antibodies and αPD-1/αVEGF bispecific antibody therapy have demonstrated improved efficacy compared to αPD-1 monotherapy across various cancer types. Despite such advances, boosting immune cell proliferation within the TME remains crucial for achieving superior efficacy in ICI-resistant or refractory patients. αPD-1/αVEGF/IL-2v is a highly TME-selective tri-specific fusion protein comprising αPD-1, αVEGF, and IL-2v. This compound was engineered to activate T cells continuously via αPD-1, enhance tumor-infiltrating lymphocytes (TILs) via αVEGF, and proliferate effector T cells strongly via novel IL-2v. We evaluated the intrinsic activity of each functional arm of the αPD-1/αVEGF/IL-2v fusion protein individually. The αPD-1/αVEGF/IL-2v exhibited blocking activity against PD-1/PD-L1 binding comparable to pembrolizumab, showed VEGF/VEGFR blockade activity similar to that of αVEGF antibody, and effectively induced $CD8^+$ T cell activation comparable to aldesleukin. In vitro trans-endothelial migration assay demonstrated that αPD-1/αVEGF/IL-2v significantly enhanced T cell migration compared to the combination of αPD-1 and αVEGF or αPD-1/αVEGF bispecific antibody. This tri-specific fusion protein also improved the $CD8^+$ T cell/Treg ratio and reduced exhausted $CD8^+$ T cell population during in vitro immune cell expansion in TME mimic condition. Furthermore, αPD-1/αVEGF/IL-2v showed TME selective immune cell activation and cytokine release while exhibiting minimal effects on immune cells in peripheral blood. The murine surrogate of αPD-1/αVEGF/IL-2v demonstrated dose-dependent anti-tumor efficacy, superior to αPD-1 or αPD-1/αVEGF bispecific antibody in both ICI-resistant MC38 and B16F10 tumor models without body weight loss. In conclusion, αPD-1/αVEGF/IL-2v represents a first-in-class tri-specific fusion protein with therapeutic potential to overcome the limitations of αPD-1 or αPD-1/αVEGF therapies.

#7287 Association between *LAG3* expression and immune checkpoint inhibitor (ICI) efficacy in advanced melanoma.

Inderjit Mehmi[1], Jacob mercer[2], Adam Dugan[2], Binyam Yilma[2], Catherine Hegarty-Traverso[2], Calvin Chao[2], Stamatina Fragkogianni[2], Omid Hamid[1]

[1]The Angeles Clinic and Research Institute, Los Angeles, CA,[2]Tempus, Chicago, IL

Predictive biomarkers for first-line (1L) ICI therapy in advanced melanoma are lacking. aLAG-3/PD1 is approved regardless of *LAG3* expression despite evidence of poorer RR and PFS (Relativity-047). The relationship between *LAG3*expression and clinical benefit to 1L non-aLAG-3 ICIs is unknown. We evaluated *LAG3* expression as a biomarker for benefit towards 1L non-aLAG-3 ICIs in a real-world (rw) advanced melanoma cohort.De-identified NGS data from patients (pts; N=367) with stage III-IV (advanced) cutaneous melanoma was analyzed in the Tempus Database. Pts were treated with 1L ICIs, aCTLA4/PD1 (n=222) or aPD1 (n=145). Pts treated with 1L aLAG-3/PD1 were limited and not included. Tissue was collected prior to 1L and sequenced with xT DNA (648-gene panel) and xR RNA assays. *LAG3* high (*LAG3*-H) and low (*LAG3*-L) expressors were defined by median *LAG3* RNA transcripts per million. PD-L1 was determined by IHC. QuanTIseq was used to estimate immune cell proportions. Rw overall survival (rwOS) was calculated from treatment (tx) start to death from any cause. Rw objective response rate (rwORR) was defined as the proportion of pts with a documented complete or partial response within 90 days of tx start. Hazard ratio (HR) was calculated using a Cox proportional hazards (CoxPH) model and p-values using the Wald test. *LAG3*-H was associated with PD-L1+ status (*LAG3*-H 52% vs *LAG3*-L 5.5%) and higher proportions of adaptive immune cells, including CD8+ (2.7% vs 0.1%), B (4.3% vs 3.1%), and inhibitory Treg cells (4.5% vs 2.9%; p<0.001 for all). In the aPD1 cohort, *LAG3*-H (n=77) had higher rwORR vs *LAG3*-L (n=68; 67% vs 15%, p=0.002). In the aCTLA4/PD1 cohort, *LAG3*-H (n=109) had similar rwORR vs *LAG3*-L (n=113; 46% vs 37%, p=0.42). CoxPH analysis found a significant interaction between *LAG3* and 1L ICIs on rwOS (p=0.02), suggesting the ICI effect on rwOS differs between *LAG3*-L and *LAG3*-H (p=0.02). In the *LAG3*-L group, pts treated with aPD1 had reduced rwOS compared to the aCTLA4/PD1 cohort (HR, 1.88, p=0.03), while in the *LAG3*-H group pts treated with aPD1 had higher rwOS compared to the aCTLA4/PD1 cohort (HR, 0.67, p=0.2). *LAG3* was associated with immune microenvironment changes and modulated 1L aPD1 but not aCTLA4/PD1 efficacy in pts with advanced melanoma. Similar to the Relativity-047 trial that showed *LAG3* alters aLAG-3/PD1 efficacy, pts with *LAG3*-H treated with aPD1 had improved outcomes vs pts with *LAG3*-L. However, pts with *LAG3*-H treated with aCTLA4/PD1 had similar outcomes vs pts with *LAG3*-L. Differences in rwOS observed between aCTLA4/PD1 and aPD1-treated pts within the *LAG3* expression subsets indicates potential predictive value of this biomarker. Future studies will prospectively validate these novel findings in pts with advanced melanoma to confirm the finding that aCTLA4/PD1 is optimal for pts with *LAG3*-L and alternative 1L ICIs are best for pts with *LAG3*-H.

#7288 Targeted degradation of mobile immune checkpoints to advance cancer therapy.

Chao Wang[1], Yingqin Hou[2], Jaroslav Zak[3], Kelli A. McCord[4], Qinheng Zheng[5], Shereen Chung[2], Ruben D. Peraza[6], James C. Paulson[7], John R. Teijaro[3], Xu Zhou[8], K. Barry Sharpless[5], Je!rey V. Ravetch[6], Matthew S. Macauley[9], Peng Wu[2]

[1]Department of Molecular and Cellular Biology/Department of Chemistry, The Scripps Research Institute, La Jolla, CA,[2]Department of Molecular and Cellular Biology, The Scripps Research Institute, La Jolla, CA,[3]Department of Immunology and Microbiology, The Scripps Research Institute, La Jolla, CA,[4]Department of Chemistry, University of Alberta, Edmonton, AB, Canada,[5]Department of Chemistry, The Scripps Research Institute, La Jolla, CA,[6]Laboratory of Molecular Genetics and Immunology, The Rockefeller University, New York, NY,[7]Department of Molecular Medicine, The Scripps Research Institute, La Jolla, CA,[8]Division of Gastroenterology, Hepatology and Nutrition, Boston Children's Hospital, Harvard Medical School, Boston, MA,[9]Department of Chemistry/Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, AB, Canada

Immune checkpoint inhibitors (ICIs) targeting T cell-intrinsic checkpoints, such as CTLA-4 and PD-1, have shown promising outcomes across diverse cancer patient populations. However, resistance to these ICIs remains prevalent, especially in patients with an immunosuppressive tumor microenvironment (TME), suggesting the existence of additional immune checkpoints that act through orthogonal mechanisms. To overcome therapeutic resistance, there is an unmet need to identify novel immune checkpoints involved in cancer progression and to develop innovative therapeutic strategies capable of reprogramming the TME by neutralizing tumor-promoting factors. Here, we identify sialic acid-binding immunoglobulin-like lectin (Siglec)-7 and -9 as mobile immune checkpoints acquired by T cells from myeloid cells via trogocytosis in the TME. These trogocytosed Siglec checkpoints suppress T cell activity by dephosphorylating T cell receptor (TCR)-related signaling pathways through engagement with tumor-associated sialoglycans. To target these mobile checkpoints, we leveraged sulfur(VI) fluoride exchange (SuFEx) click chemistry to develop a high-affinity Siglec-7/-9 ligand and converted it into a degrader for lysosomal degradation of both Siglecs. This degrader efficiently reduced Siglec levels in T cells and myeloid cells, significantly restoring T cell function and enhancing anti-tumor immunity. The degrader, particularly when combined with anti-CTLA-4, remodeled the TME and generated durable T cell memory, resulting in excellent tumor control in multiple murine models. These findings underscore the significance of degrading exogenously acquired Siglec checkpoints on T cells as a novel strategy to overcome resistance to established ICIs in cancer therapy.

#7289 High CD8+ T cell TIM-3 expression predicts for sensitivity to TIM-3 based triplet checkpoint inhibitor combinations.

Manali S. Phadke[1], Jiannong Li[1], Sathya Sriramareddy[1], Paulo Rodriguez[1], Brian Ruffel[1], Vincent Luca[1], Ann Chen[2], Keiran S.M. Smalley[1]

[1]Moffitt Cancer Center, Tampa, FL,[2]Huntsman Cancer Foundation, Salt Lake city, UT

Background: Melanoma response to the immune checkpoint inhibitor (ICI) combinations such as anti-PD-1, anti-PD-1+LAG-3 and anti-PD-1+CTLA-4 is not durable. The triplet combination of anti-PD-1+CTLA-4+LAG-3 is being studied clinically in multiple cancers, including advanced melanoma. This study comprehensively describes the mechanism of action of this ICI triplet, and a second triplet of anti-PD-1+LAG-3+TIM-3, in 3 mouse melanoma models with varying levels of sensitivity to ICI.
Methods: Syngeneic mouse models of SM1, SW1 and B16-10 melanoma flank tumors were used to explore the response to anti-PD-1+LAG-3, anti-PD-1+CTLA-4, anti-PD-1+LAG-3+CTLA-4 triplet and anti-PD-1+TIM-3 doublet and anti-PD-1+LAG-3+TIM-3 triplet. We performed flow cytometry on the responding and resistant tumors, tetramer bindings assays, T-cell depletion and IHC to determine the mechanisms of action of these ICI combinations. Additionally, we analyzed scRNA-Seq data from sensitive and ICI resistant human melanoma to determine the overlap in immune checkpoint expression across T -cell subsets.
Results: The PD-1+CTLA-4+LAG-3 triplet combination demonstrated good efficacy in the SM1 and B16-F10 models and was associated with a more favorable immune response such as increased CD69+ CD8+ T cells, reduced exhausted T cells and increased GP100-tumor specific T cells than either doublet. The ICI-resistant SW1 model, did not respond to the PD-1+CTLA-4+LAG-3 triplet and exhibited accumulation of TIM-3+ exhausted CD8+ T cells. Targeting elevated TIM-3 on CD8+ T cells, using the anti-PD-1+LAG-3+TIM-3 triplet, but not the PD-1+TIM3 doublet, restored T cell activity and led to a remarkable anti-tumor activity in SW1 model, with some mice showing complete tumor regression. The PD-1+LAG-3+TIM-3 triplet was also highly effective in the SM1 model showing increased percentage of CD69+ CD8+ and tumor specific T cells and reduced exhausted T cells. scRNA-Seq analysis showed that TIM-3 levels were significantly higher in the CD8+ T cells of melanoma patients failing PD-1 therapy. We also observed a good correlation between LAG-3 and TIM-3 expression in the CD8+ T cells. Hence, co-targeting these two checkpoints may possibly reverse the T cell exhaustion.
Conclusion: In conclusion, our results demonstrate that ICI resistance associated with high TIM-3 expression in the CD8+ T cells can be overcome through use of a PD-1+LAG-3+TIM-3 combination. The observation that high TIM-3 expression in CD8+ T cells is associated with PD-1 resistance suggests the potential to use anti-TIM3 based ICI combinations as a second-line treatment strategy in patients with advanced melanoma.

#7290 Targeting APP with GENA-119B31: A novel approach to overcoming immune checkpoint inhibitor resistance in cancer immunotherapy.

Mi Young Cha, **Kitae Park**, Hyunuk Kim, Hyunkyung Yu, Bu-Nam Jeon, Jiyeong Lee, Youngeun Ha, Sang-Moo Park, Gyeongyeon Kim, Suah Choi, Han-Sol Kim, Hyonam Kim, Sang-Gyun Kim, Hansoo Park

Genome & Company, Suwon-si, Gyeonggi-do, Korea, Republic of

Immunotherapy has profoundly transformed the treatment of advanced cancers, leading to improved clinical outcomes. Despite these successes, many patients remain unresponsive to immune checkpoint inhibitors (ICI). Consequently, there is an urgent need for novel treatments for patients who fail to respond to ICI or develop resistance. We previously identified amyloid precursor protein (APP), a type I transmembrane protein present in T cells. APP interacts with the novel immune checkpoint protein contactin 4 (CNTN4), expressed in tumor cells, and plays a central role in mediating CNTN4's immunosuppressive signal to T cells. Moreover, profiling APP expression in tumor-infiltrating T cells from NSCLC patients treated with anti-PD-1 and chemotherapy, we observed that $CD8^+$ Teff cells of non-responding (SD) patients predominantly exhibited elevated *APP* levels, while those of responding (PR) patients manifested higher levels of *IFNG* and *PDCD1*. Here, we present the *in vitro* and *in vivo* efficacy of GENA-119B31, a humanized monoclonal antibody targeting APP. GENA-119B31 binds strongly to human APP at both protein and cellular levels and effectively blocks the interaction between CNTN4 and APP. The *in vitro* biological activities of GENA-119B31 were evaluated using co-culture conditions with $CD4^+$ or $CD8^+$ T cells and A549 human cancer cells engineered to overexpress OKT3 and CNTN4. GENA-119B31 treatment demonstrated promising T cell-mediated cytotoxicity. For the *in vivo* study, we established CNTN4-overexpressing murine cancer cell lines (CT26/Cntn4 and Pan02/Cntn4) due to the low CNTN4 expression in mouse cells compared to human cells. This allowed for more clinically relevant conditions in efficacy assessment. GENA-119B31 showed strong anti-tumor activity in the CT26/Cntn4 syngeneic liver metastasis murine model, which has been internally confirmed to be resistant to anti-PD-1 therapy and closely mirrors clinical outcomes. The efficacy was demonstrated by a marked reduction in metastatic nodules and a decrease in hepatic damage markers in serum. In our previous study using human cancer TMA slides, pancreatic cancer, known for its resistance to ICIs, was identified as having high CNTN4 expression. In the Pan02/Cntn4 syngeneic subcutaneous murine model, GENA-119B31 showed significant anti-tumor activity, with reductions in tumor volume and weight, whereas no such effects were observed in the Pan02 WT model, which has low CNTN4 expression. These findings indicate that GENA-119B31's anti-tumor activity is mediated through the APP/CNTN4 axis, with CNTN4 expression potentially serving as a biomarker for therapeutic response. This study confirms the potential of APP as a target for cancer immunotherapy through the use of the anti-APP antibody GENA-119B31. Our findings provide preclinical evidence supporting GENA-119B31 as a promising novel therapeutic strategy.

#7291 Advancing cancer immunotherapy: characterizing and leveraging bispecific antibodies targeting immune checkpoints.

Jun Wang, Denise Garvin, Jonathan Mitchell, Pete Stecha, Michael Beck, Jim Hartnett, **Kai Hillman**, Gopal Krishnan, Frank Fan, Julia Gilden, Mei Cong, Jamison Grailer

Promega, Madison, WI

Bispecific antibodies targeting immune checkpoints are transforming cancer immunotherapy by enabling novel mechanisms of action unattainable by monospecific antibodies. These engineered molecules simultaneously engage two distinct epitopes, such as PD-1 and CTLA-4 or TIGIT, to amplify anti-tumor immune responses while minimizing off-target effects. Here we demonstrate the use of cell-based reporter bioassays to provide a robust platform for assessing the functional potency, stability, and specificity of bispecific antibodies. Combination bioassays, such as PD-1 + TIGIT and PD-1 + LAG-3, evaluate synergistic effects on T-cell activation. Additionally, Fc effector function bioassays, including ADCC and ADCP assays, assess the cytotoxic potential and receptor-mediated activity of bispecific formats. These assays ensure precise characterization of mechanisms of action, guiding the refinement of antibody designs.

#7292 HY05350: A trispecific T-cell engager with immune checkpoint inhibitor targeting mesothelin for solid cancer.

Yan Hu, Sanyou Guo, Yujing Zhang, Mengyun Zhang, Bo An, Dan Sun, Ao Fu, Xiaoqiang Chai, Jiangshan Dai, Qing Xiong, Tao Wei

Sichuan Huiyu Seacross Pharmaceutical Technology Co., Ltd, Chengdu, China

Objective: Limited efficacy is the major road block when developing T cell engager (TCE) for solid cancers, which is partly due to the suppressive tumor microenvironment (TME) created by the expression of inhibitory immune checkpoints, such as PD-L1. To enhance anti-tumor activity of T cells, we engineered a trispecific T cell engager (HY05350), targeting CD3 and tumor-associated antigens Mesothelin (MSLN) and PD-L1 for solid cancers.
Methods: CD3 antibody obtained by optimizing affinity of SP34. Alpaca was immunized with MSLN and PD-L1. VHH antibodies were obtained and humanized. Various trispecific formats evaluated by T cell activation, T cell-dependent cytotoxicity (TDCC), cytokine release and PD-L1 checkpoint blockade assays. Anti-tumor efficacy was studied in CDX mouse models, MoA studied by single cell NGS in the CD3xPD1xPDL1 transgenic mice, PK/TK and GLP toxicity evaluated in cynolmogus.
Results: HY05350 in the format of 2(MSLN)+1(PD-L1)+1(CD3) was selected. It could induce strong target- & dose-dependent cytotoxicity in MSLN+ cancer cells and effectively block binding of PD-L1 to T cell PD1. It demonstrated a strong anti-tumor activity in a variety of the mouse CDX models. Cynomolgus GLP toxicity study demonstrated HY05350 was well-tolerated with a toxicity profile comparable to typical CD3 based bispecific antibodies and had an acceptable PK/TK profile with a T1/2 of 12-35 hours. We explored MoA of HY05350 in vitro and in vivo. Our in vitro experiments showed (1) HY05350 could induce a stronger 2nd signal than the bispecific antibody (CD3xMSLN), consistent with the observation by Liu et al (2019) that the CD3xPD-L1 arm of HY05350 could target the PD-L1+ APC cells to activate T cells and induce more effective antitumor immunity than directly bridging T cells to cancer cells; (2) As expected, activation of T cells induced PD-L1 expression in most tested cancer cells. Furthermore, HY05350 showed more potent TDCC activity against MSLN and PDL1 double-positive than single-positive tumor cells, suggesting PD-L1, together with MSLN, could enhance stronger TDCC activity, presumably via a feedforward mechanism, than the bispecific antibody CD3xMSLN; (3) HY05350 induced strong cytotoxicity against PD-L1+ DMSC, suggesting remodeling TME potential. Single cell sequencing analysis of ~10K immune cells of MC38 tumors revealed that in pre- and post-dosed CDX mice, HY05350 induced a wide range of immune responses. In particularly, it significantly increased proportions of three major anti-tumor groups of lymphocytes: T cells, NK cells and interestingly gamma-delta T cells.
Conclusion: We engineered a trispecific T cell engager HY05350 targeting CD3, MSLN and PD-L1 to treat solid cancers. Our in vitro and in vivo studies demonstrated that HY05350 was well-tolerated, induced strong T cell activation, cytotoxicity and potent antitumor efficacy, supporting for further clinical investigation.

#7293 First in class Anti-CLEC-1A myeloid checkpoint antibodies for the treatment of solid tumors with monotherapy and combination therapy efficacies.

Etienne Foucher[1], Mylene Derame[1], Justine Durand[1], Marion Drouin[1], Camille Ligeron[2], Caroline Mary[1], Vanessa Gauttier[1], Isabelle Girault[1], Emmanuelle Wilhelm[1], Virginie Thepenier[1], Cecile Batty[1], Geraldine Teppaz[1], Ariane Desselle[1], Marine Malloci[1], Irene Baccelli[1], Marion Colonello[1], Stephanie Neyton[1], Elise Chiffoleau[3], Aurore Morello[1], Nicolas Poirier[1]

[1]OSE Immunotherapeutics, Nantes, France,[2]OSE Immunotherapeutics and Nantes Universite, INSERM, Center for Research in Transplantation and Translational Immunology, UMR 1064, Nantes, France, Nantes, France,[3]Nantes Universite, INSERM, Center for Research in Transplantation and Translational Immunology, UMR 1064, Nantes, France

The immune checkpoint CLEC-1 (C-type lectin domain family 1 member A) expressed by dendritic cells, macrophages, and endothelial cells, has been shown as a necrotic receptor playing a critical role in immune response modulation during sterile inflammation. Several studies have highlighted CLEC-1's potential as a target for immunotherapeutic strategies. Absence of CLEC-1 signaling by genetic ablation or antibody blockade has been shown to promote anti-tumor response by increasing the cross-presentation of dead cell-associated antigens by type 1 conventional dendritic cells and by enhancing T-cell activation. Furthermore, this CLEC-1 signaling blockade has also recently been shown to increase an acute inflammatory response in a model of liver tissue damage induced by the acetaminophen (APAP) intoxication by exacerbating the recruitment of neutrophils at the site of inflammation. Using LC/mS, we discovered different CLEC-1 ligands, such as TRIM21, HRG and other intracellular or plasma membrane molecules. Although their biological activities following interaction with CLEC-1 remain to be fully investigated, we observed that a cell stress induced by radiation or by different chemotherapeutic class agents such as cyclophosphamide (CPA), oxaliplatin, vorinostat and others, increase the expression of CLEC-1 ligand(s) on necrotic and live cells. Consequently, we found *in vivo* that CPA treatment combined with the absence of Clec1a gene more strongly induced tumor volume reduction than in the presence of Clec1a in fibrosarcoma (MCA) and colorectal (MC38) mouse models. We developed and selected two anti-CLEC-1 antagonist antibodies harboring different properties (e.g. affinity, epitope). Using humanized CLEC-1 mice, we showed, in a orthotopic mouse model of hepatocarcinoma (Hepa1.6) exhibiting resistance to anti-PD1/PDL1 mAb therapy, that these anti-CLEC-1 antibodies in monotherapy significantly increase overall mice survival compared to corresponding isotype control treatment (3 independent experiments, n=32 for each treatment, $p<0.002$). Moreover, we confirmed also the therapeutic effect of these anti-CLEC-1 antibodies in combination with CPA in the ectopic MC38 model (n=18, 2 independent experiments). Altogether, our results further dissect the mechanism of action of the myeloid checkpoint CLEC-1 in its ability to impair anti-tumor immunity and support its target with antagonist antibody for cancer immunotherapy.

#7294 Neuropilin-1 specific antisense oligonucleotides exhibit anti-tumor activity in vivo and significantly increase the efficacy of checkpoint inhibitors.

Andre Maaske[1], Sven Michel[1], Daniel Kokotek[1], Nicole Kirchhammer[2], Anne Sadewasser[1], Julia Festag[1], Melanie Buchi[2], Monika Schell[1], Janani Sekar[1], Stefanie Raith[1], Alfred Zippelius[3], Frank Jaschinski[1], **Richard Klar**[1]

[1]Secarna Pharmaceuticals GmbH & Co. KG, Planegg, Germany,[2]Cancer Immunology, Department of Biomedicine, University Hospital Basel, Basel, Switzerland,[3]Cancer Immunology, Department of Biomedicine; Medical Oncology, University Hospital Basel, Basel, Germany

Introduction: Immune checkpoint inhibitors (ICI) revolutionized the treatment of cancer. However, most patients do not respond to the currently available therapies, or become refractory, which demonstrates the need for the development of novel therapeutic strategies. The transmembrane protein Neuropilin-1 (NRP1) interacts with various receptors and ligands and contributes to an immunosuppressive tumor microenvironment (TME) through multiple mechanisms. NRP1 facilitates the migration of tumor associated macrophages (TAMs), is important for the stability of regulatory T cells, correlates to exhaustion of effector T cells and plays an important role in neoangiogenesis. Furthermore, in several cancers including pancreatic, breast and gastric cancer, high expression of NRP1 has been associated with a poor prognosis. Here we demonstrate the anti-tumor effects of downregulation of NRP1 by locked nucleic acid (LNA) modified antisense oligonucleotides (ASOs) in murine cancer models as monotherapy and in combination with anti-PD-(L)1 antibodies. Furthermore, we provide insights into the potential underlying mechanisms of the observed therapeutic efficacy.
Methods: Highly active and well tolerated NRP1-specific ASOs were identified with our OligoCreator oligonucleotide discovery platform. These were tested in different syngeneic tumor models, after a palpable tumor was established, as monotherapy or in combination with ICIs. Survival, immune infiltration, NRP1 expression, and transcriptomic changes were analyzed.
Results: A robust knockdown of NRP1 was seen in tumor tissue and in individual infiltrating cell types, including T cells and macrophages, which was accompanied by transcriptomic changes in pathways related to inflammation and extracellular matrix remodeling. Mice treated with NRP1 specific ASO had delayed tumor growth up to complete tumor eradication, which was further increased when combined with ICI, whereas ICI alone showed only a low efficacy. The complete responders were re-challenged with the same cancer cell line, alongside previously untreated mice. While the treatment-naïve mice showed rapid tumor growth, all re-challenged mice eradicated tumors quickly, without the need for an additional treatment.
Conclusion: NRP1 specific ASOs are a promising new approach for the treatment of solid cancers. Our data show that the limited efficacy of ICI in the models tested could be improved significantly when combined with the ASO-treatment. Target knockdown was observable in various infiltrating cell types, accompanied by upregulation of genes involved in inflammation and downregulation of genes involved in extracellular matrix organization, which is currently being further analyzed. Based on these promising results, we started preparation for first in human trials to treat patients with solid cancers.

#7295 Reversing resistance to checkpoint inhibitors by treatment with claudin-1 specific antibodies in an orthotopic in vivo model for cholangiocarcinoma.

Romain Desert[1], Zeina Nehme[1], Bocar Kane[1], Emilie Crouchet[1], Frank Juhling[1], Vikas Ranvir[2], Alberto Toso[3], Mathias Heikenwalder[2], Catherine Schuster[1], **Thomas Baumert**[1]

[1]University of Strasbourg, Inserm, Institute for Translational Medicine and Liver Disease (ITM), UMR_S1110, Strasbourg, France,[2]Division of Chronic Inflammation and Cancer, German Cancer Research Center (DKFZ), Heidelberg, Germany,[3]Alentis Therapeutics, Allschwil, Switzerland

Background: Resistance to immunotherapy is a major challenge in the treatment of solid tumors. Cholangiocarcinoma (CCA) exemplifies this challenge by poor outcome and very limited response to approved checkpoint inhibitors (CPI). Claudin-1 (CLDN1) is a transmembrane protein overexpressed in epithelial cancer cells and shown to be a mediator of carcinogenesis, invasion and metastasis. However, the functional role of CLDN1 for the tumor microenvironment (TME) is largely unknown. Using CCA as a model disease, we aimed to study the impact of CLDN1 for the TME and its potential role as a therapeutic target to address CPI resistance.
Methods: CLDN1 expression and its association with TME was analyzed by spatial transcriptomics in CCA patient tissues. Immunocompetent orthotopic animal models of intrahepatic CCA were generated by hydrodynamic tail vein injection (HDTV) of proto-YAP/AKT or the KRAS/p19 oncogenes together with a CLDN1 expressing plasmid. Following tumor development within the liver, mice were treated with monoclonal antibodies (mAbs) targeting CLDN1 and/or PD-1.
Results: Spatial analyses of patient tissues revealed that CLDN1 was overexpressed in cancer cells and CLDN1 expression was associated with oncogenic signaling and reduced T cell infiltration. In orthotopic mouse models for CCA, CLDN1 gain-of-function studies showed enhanced tumor growth and decreased survival. Treatment with CLDN1 mAbs markedly and significantly reduced tumor burden and intratumoral fibrosis and resulted in increased intratumoral CD3+ and CD8+ T cell infiltration as well as modulation of peritumoral macrophage distribution. Gene expression analyses revealed down-regulation of pathways for fibrogenesis, carcinogenesis and up-regulation of pathways for T cell activation and proliferation. Whereas PD1 mAb single agent therapy did not show significant efficacy, the combination of PD-1 with CLDN1 mAbs markedly and significantly improved overall survival.
Conclusions: CLDN1 plays a functional role in the TME by modulating tumor fibrosis and immune cell infiltration. Treatment with CLDN1 mAbs provides an opportunity to overcome resistance and improve the efficacy of CPIs by enhancing tumoral immune responses.

#7296 PF-08046049 synergizes with checkpoint inhibitors to drive CD8+ T cell effector differentiation and tumor clearance in an IO-resistant model.

L. Shehata, M. Ulrich, L. Gutierrez, J. Hartsuyker, R. Giraddi, R. A. Heiser, B. P. O'Connor;
Pfizer, Inc., Bothell, WA

The checkpoint inhibitors anti-PD-1 and anti-CTLA-4 have been clinically successful as therapeutics for melanoma due to their ability to reinvigorate the immune system. However, about half of patients do not benefit from these treatments, suggesting there are still barriers to CD8+ T cell activation or infiltration into the tumor microenvironment (TME). Additionally, while the combination therapy of anti-CTLA-4 and anti-PD-1 (Ipilimumab-Nivolumab) improves response rate and overall survival compared to monotherapies, it also leads to increased toxicity and immune-related adverse events. Recent studies have demonstrated that CD8+ T cell co-stimulation through the 41BB axis is critical for PD-1/PD-L1 blockade efficacy in melanoma, yet those signals may be limited or unavailable to tumor-associated antigen-specific T cells within the TME. However, nontargeted 4-1BB signaling poses a risk of widespread peripheral immune activation and liver toxicity, as was the case for the 4-1BB agonist Urelumab. PF-08046049 (BB228) is a bispecific molecule comprised of a CD228-specific antibody and 4-1BB-directed Anticalin proteins. CD228 is highly expressed in several cancer indications but is low on normal tissues, thus BB228 targets 4-1BB co-stimulation to T cells within the TME. We tested the efficacy of BB228 as a monotherapy and in combination with the current melanoma standard of care (SOC) treatments, anti-PD-1 and anti-PD-1/anti-CTLA-4. In an IO-resistant LL2 model, neither BB228 nor anti-PD-1 monotherapy led to tumor control. However, in combination, BB228 synergized with anti-PD-1 and anti-CTLA-4 to promote tumor clearance. We found that while anti-PD-1 and anti-CTLA-4 can target and expand stem-like CD8+ T cells in both the tumor-draining lymph node and TME, BB228 is able to drive the differentiation of those stem-like cells into effectors, resulting in tumor killing. These data illustrate that BB228 has a distinct and complementary mechanism of action from the current melanoma SOC treatments.

#7297 Enhanced efficacy of CTX-471, a CD137 agonist antibody, in models of immune checkpoint failure via simultaneous blockade of neo-angiogenesis.

Amy Daniel Ulumben[1], Diana I. Albu[1], Rachael Duffy[1], Tristan Lubinski[2], Jason Kong[3], Jessica Zolotaresvsky[3], Melissa Brundin[3], Mei Su[1], Ruturaj Jadhav[3], Bing Gong[3], Thomas Schuetz[4], Kris Sachsenmeier[2], Alberto Visintin[1]

[1]Preclinical Research, Compass Therapeutics, Boston, MA,[2]Translational Research, Compass Therapeutics, Boston, MA,[3]Antibody Discovery, Compass Therapeutics, Boston, MA,[4]CEO and Co-founder, Compass Therapeutics, Boston, MA

CTX-471 is a monoclonal agonistic anti-CD137 antibody currently under investigation in solid tumors. To increase the breadth and duration of response of CTX-471 we set out to investigate its anti-tumor efficacy in combination with anti-angiogenesis agents in murine models. CTX-009 is a bispecific antibody targeting VEGF-A and DLL4 which is undergoing clinical investigation in multiple cancer types, including biliary tract and colorectal cancers. Encouraged by early findings showing activity of the combination in a B2m negative mouse tumor model, engineered to mimic HLA loss in patients, we broadened the exploration of the combinatorial treatment in an additional B2m knockout model using a different murine genetic background ($CT26^{B2m-/-}$, Balb/c). Furthermore, we developed two novel mouse models of immunotherapy resistance without the enhanced NK cell susceptibility bias conferred by complete MHC-I loss due to B2m deletion. CT26 cells with an engineered deletion of the B2m gene were passaged in CT26 tumor-experienced mice and a line of $CT26^{B2m-/-}$ cells that escaped immune rejection was established (CT26 B2m knockout escapers, $CT26^{B2m-/-E}$). For the second model, the H-2k1 MHC-I locus was knocked out in the MC38 cells, resulting in targeted homozygous loss. Using this approach, the expression of the other MHC-I alleles, and therefore natural resistance to NK cells, are expected to be left intact.

Results: Coupling VEGF-A/Dll4 targeting with CD137 agonism markedly increased the anti-tumor efficacy of the individual treatments alone in the $MC38^{B2m-/-}$, $MC38^{H-2k1-/-}$, $CT26^{B2m-/-}$ models as well as in the highly IO resistant $CT26^{B2m-/-E}$ model. Tumor growth inhibition was accompanied by pharmacodynamic evidence consistent with decrease of vascular bed density and enhancement of cellular cytotoxic immunity. CTX-471 monotherapy was still efficacious, albeit blunted, in these IO resistant models. The latter preclinical results mirrored data from a Ph1 clinical trial of CTX-471, where evidence of NK activation following CTX-471 treatment was seen in both blood and tissue samples of patients with known defects in the antigen presentation machinery. The findings presented here suggest that an agonistic anti-CD137 antibody may provide enhanced clinical benefit when employed alongside an anti-angiogenesis agent, such as CTX-009, in tumors where immune checkpoint inhibitors had previously failed.

#7298 DUSP11 is an intracellular innate immune checkpoint in lung adenocarcinoma.

Brian J. Thomas, Xue Bai, Benjamin J. Cryer, Mark A. Daniels, Margaret J. Lange, Donald H. Burke

Molecular Microbiology and Immunology, University of Missouri School of Medicine, Columbia, MO

The major success of immune checkpoint inhibitors (ICIs) to treat cancer highlights both the efficacy of inhibiting immunosuppressive proteins as a means of re-activating the anti-tumor immune response and the generalizability of targeting such proteins (i.e., therapies are clinically useful across many cancer types). While the major focus in immuno-oncology has been to manipulate the adaptive immune system, recent attention has been given to manipulating the innate immune system to treat cancer and/or enhance adaptive responses. In this work we detail the intracellular protein, Dual Specificity Phosphatase 11 (DUSP11), as an innate immune checkpoint (iIC) in lung adenocarcinoma (LUAD), among other cancers. LUAD remains a leading cause of death worldwide, largely due to mutant oncogene acquired therapeutic resistance and a lack of effective alternative therapeutic options, including ICIs. There is a clear unmet need for new methods to treat refractory LUAD. In this work we targeted DUSP11, a phosphatase with high affinity for 5' triphosphate RNA (3pRNA) that has readily been reported as a host factor that aids viruses escape of immune detection. As many cancers show dysregulated RNA transcript processing, we hypothesized that DUSP11 knockdown would increase the presence and detection of immunostimulatory RNA by pattern recognition receptors (PRRs) and activate anti-cancer immune mechanisms. To do this, we engineered multiple Dicer substrate small interfering RNAs targeting DUSP11 and showed that decreased expression at the mRNA and protein levels had significant impacts on cell viability for multiple cancer cell types, including LUAD. The decrease in LUAD cell viability was due to induction of apoptosis, and innate immune responses were significantly upregulated, evidenced by increased expression of interferon-stimulated genes (ISGs), release of type I IFN, and paracrine stimulation of cells expressing IFNAR1. As retinoic acid inducible gene I (RIG-I) was an ISG that was upregulated upon DUSP11 knockdown, we speculated that, as in viral studies, RIG-I was responsible for the anti-cancer phenotype. Indeed, the knockdown of RIG-I, but not other intracellular PRRs, recovered the cell viability and innate immune response phenotypes. To corroborate this, we showed that knocking down DUSP11 enhances the effect of RIG-I agonizing 3pRNA, and we showed that LUAD cells engineered to be deficient in RIG-I do not respond to DUSP11 knockdown. Ongoing studies in mice appear to show that the anti-cancer effect of modulating DUSP11 activity is conserved *in vivo*. Overall, our preclinical studies suggest that DUSP11 is an excellent target to modulate innate immunity and induce LUAD cell death. Early studies suggest that this target and mechanism is generalizable to other cancer types; however, understanding which cancers respond and why, as well as assessing the safety and toxicity of targeting this protein warrant further investigation.

#7299 CS2009: A first-in-class trispecific antibody targeting PD-1, CTLA-4, and VEGFA with potential to be a next-generation backbone therapy with combined checkpoint inhibition and anti-angiogenesis.

Chuan Wang, Ning Zhang, Yilin Huo, Xinling Zhang, Yongwang Li, Jingyu Sun, Xuelian Liu, Yongli Yang, Xueqin Dai, Yuxin Qian, Fei Ma, Yi Sun, Yujuan La, Jianxin Yang

CSTONE Pharmaceuticals, Suzhou, China

Background: CS2009 is a first-in-class trispecific antibody designed to target PD-1, CTLA-4, and VEGFA, with potential to replace current anti-PD(L)1 therapies. CS2009 consists of three functional arms including anti-PD-1, anti-CTLA-4, and anti-VEGFA. Clinical evaluations of bispecific molecules and combination therapies targeting PD-1/CTLA-4 and PD-1/VEGFA have shown favorable synergistic effects. For instance, cadonlimab (targeting PD-1/CTLA-4) and ivonescimab (targeting VEGFA/PD-1) have been approved in China for the treatment of advanced cervical carcinoma, gastric or gastroesophageal junction adenocarcinoma, and EGFR-mutant non-small cell lung cancer, respectively. Here, we present preclinical data that demonstrate CS2009 preferentially targets and reinvigorate PD-1/CTLA-4 double-positive tumor-infiltrating T lymphocytes (TILs); and its anti-VEGFA arm inhibits tumor angiogenesis and promotes vascular normalization, helping to convert an immune-suppressive tumor microenvironment (TME) to an immune-active TME, hence enhancing the tumor growth inhibition mediated by effector TILs.
Methods & Results: The lead molecule of CS2009, J30, was constructed with a bivalent anti-VEGFA arm, a monovalent anti-PD-1 arm, and a monovalent anti-CTLA-4 arm. Preclinical characteristics of J30 have been published previously. To further validate J30 as the lead molecule, J30 was assessed in primary PBMCs assays, which demonstrated its potent checkpoint inhibitory function. Its cross-species binding to PD-1, CTLA-4, and VEGFA were evaluated by Octet (BLI). Its functionality of interrupting the interaction of PD-1/PD-L1, CD80/CTLA4, and VEGFR2/VEGFA was evaluated in the competitive ELISA assays. Its cross-reactivity supported selection of cynomolgus monkey as the relevant species for nonclinical safety assessment. A single-dose PK study (5, 15, or 50 mg/kg, 30-minute IV infusion) and a GLP-compliant 4-week repeat-dose toxicity and safety pharmacology study (0, 25, 50, or 100 mg/kg, 30-minute IV infusion, QW*5) with a 4-week recovery were conducted in cynomolgus monkeys. J30 demonstrated a monoclonal antibody-like PK profile, with terminal half-lives ($t_{1/2}$) around 4 days, with no observed gender differences. All dose levels were well tolerated with the highest non-severely toxic dose (HNSTD) at 100 mg/kg. Further pharmacodynamic analysis demonstrated J30's biological activity in periphery with dose dependency. Both ICOS/Ki67 expression level on CD8 & CD4 T cells were upregulated.
Conclusion: CS2009 is a promising trispecific antibody for the treatment of various advanced solid tumors, with potential to be a next-generation backbone therapy with combined checkpoint inhibition and anti-angiogenesis. Current preclinical data support further clinical investigation of CS2009.

#7300 Resolvin E1 in solid tumor cancer: towards clinical translation of a novel oral agonist targeting the LTB4-BLT1 pathway to sensitize tumors to immune checkpoint inhibition.

Joyce Steinberg[1], Richard Newman[2], Beverly Paperiello[1], Tracy Jennings[1], Wayne D. Klohs[1], Sui Huang[3], Dipak Panigrahy[4], Gary Mathias[1], John Parkinson[1]

[1]Thetis Pharmaceuticals LLC, Essex, CT,[2]CMAX Clinical Research, Adelaide, Australia,[3]Institute of Systems Biology, Seattle, WA,[4]Beth Israel Deaconess Medical Center, Boston, MA

Background: Resolvin E1 (RvE1) and leukotriene B4 (LTB4) are both agonists of the BLT1 receptor (aka LTB4R1) but elicit distinct biological outcomes due to biased agonism. Activation of BLT1 promotes CD8+ T cell recruitment to the tumor, which enables effective immune checkpoint inhibitor (ICI) immunotherapy. However, LTB4, a pro-inflammatory lipid mediator, also promotes tumor progression through neutrophilic inflammation and is found at elevated levels in human cancers. In contrast, RvE1 selectively activates BLT1 to promote phagocytosis of tumor debris without causing inflammation. TP-317, a stabilized RvE1 molecule, effectively suppresses solid tumors in various murine models (CT26 colon carcinoma, Lewis lung carcinoma, B16F10 melanoma, KPCy pancreatic adenocarcinoma) when administered once daily, both alone and with ICI +/- chemotherapy. The effect of TP-317 as monotherapy required the presence of CD8 T cells and was largely abrogated in animals co-treated with a BLT1 antagonist. Whole-tumor transcriptomes of resected tumors of TP-317-treated versus control animals displayed broad upregulation of IFN-γ response genes and antigen presentation machinery, consistent with the synergism observed when given in combination with ICI. TP-317 is being developed as oral therapy for colorectal cancer and non-small cell lung cancer. The present study describes the pharmacokinetics, pharmacodynamics and safety profile of oral TP-317 given as an enteric-coated tablet to healthy volunteers.

Methods: Twenty-four subjects were randomized 3:1 to single oral doses of TP-317 at 10, 40 and 80 mg (n=8/group). Safety, tolerability and PK/PD were assessed using standard methods. Subjects receiving the 80 mg dose underwent frequent CBC testing to assess short-term fluctuations in circulating neutrophils.

Results: Oral TP-317 was well tolerated, achieving peak plasma concentrations of RvE1 that exceeded the EC_{50} for BLT1 activation (~10 nM) by 1.2, 2.8, and 6.7 times at 10, 40, and 80 mg doses, respectively. All subjects receiving the 80 mg dose exhibited transient neutropenia, with neutrophil counts dropping below 1500/mL near plasma tmax and recovering to normal range within about 2 hours. A similar pattern, also without safety concerns, was previously observed in healthy subjects receiving an oral RvE1 drug (150 mg bid) for 10 days.

Conclusions: Oral TP-317 demonstrates good tolerability and achieves adequate systemic RvE1 levels to engage the BLT1 receptor, suggesting a dose range of 40 to 80 mg once per day for patient studies. Transient neutropenia, a known effect of BLT1 activation by RvE1, indicates target engagement in humans. TP-317's unique ability to promote antigen presentation in myeloid and tumor cells offers a novel approach to sensitize tumors to ICI, warranting clinical investigation in cancer patients.

#7301 FP011, A tri-specific immune checkpoint inhibitor targeting PD-1 × TIGIT × PVRIG for cancer immunotherapy.

Yuanyuan Wang, Liang Xiao, Fan Wu, Baiguang Ren, Chang Zhou, Peipei Hu, Ke Chen, Xiaodong Wu, Di Lu, Yongting Huo

Guangdong Fapon Biopharma Inc., Dongguan, China

Immune checkpoint inhibitors, such as anti-PD-1/PD-L1 antibodies, have rapidly changed the treatment paradigm for multiple tumor types. Unfortunately, many patients still remain unresponsive or acquire resistance after initial response to treatment. The complex network of immunosuppressive molecules and their upregulation after initial treatment may contribute to such resistance, and lead to terminal exhaustion and fixed loss of effector functions of T cells. Here, we developed FP011, a tri-specific antibody which can simultaneously block the inhibitory signals of PD-1, PVRIG and TIGIT on effector T cells, so as to better activate T cells to kill tumor cells.

In vitro, by interacting with T cells *in cis*, FP011 can act on T cells with higher avidity, exerting synergistic blockade of these three immunosuppressive signals. In a tri-target reporter assay, both FP011 and the combination group induced much stronger luminescence signal than those induced by bi-specific antibodies and monoclonal antibodies. In an antigen-specific (CMV) T cell activation assay, in which T cells were induced to exhaustion after long-term stimulation with CMV antigens, FP011 also induced a more significant degree of T cell activation than what bi-specific antibodies, pembrolizumab and the combination of anti-PD-1, anti-PVRIG and anti-TIGIT monoclonal antibodies could do. In a PBMC-engrafted NOD-dKO-A375 mice model, FP011 inhibited tumor growth (93%, $P < 0.0001$) in a way significantly better than anti-PD-1 antibody (39.45%, $P = 0.0627$), the combination of anti-PD1, anti-PVRIG and anti-TIGIT antibodies (90% $P < 0.0001$), and another tri-specific positive control (66.18%, $P = 0.0007$). The inhibition of tumor growth was associated with an increase of tumor-infiltrating T cells following the FP011 treatment.

In conclusion, these data provide insight into the efficacy of FP011, both in vitro and in vivo, which indicates that targeting three immune checkpoints simultaneously can synergistically enhance T cell activation to achieve a better tumor-killing effect.

#7302 A Novel Therapeutic Approach : New Drug Candidate with are potent and safe next-generation multi-target immune checkpoint inhibitor.

B.-R. Lee[1], S.-B. Lee[1], C. Yoon[1], H. Hwang[1], S. Jung[1], J. Heo[1], K.-M. Lee[2], H. Kim[1];
[1]Cellemedy Co., Ltd., Seongnam, Korea, Republic of, [2]Korea University College of Medicine, Seoul, Korea, Republic of

Immune checkpoint inhibitors play a crucial role in current cancer treatments, serving as promising therapeutic options across various cancer types. In particular, PD-L1/PD-1 immune checkpoint inhibitors have been at the forefront of this field. However, recent concerns have been raised regarding the risk-benefit ratio of treatment based on PD-L1 expression levels, prompting the need for the development of immune checkpoint therapies that demonstrate high efficacy regardless of whether PD-L1 expression is high or low. Cellemedy's T-sphaera platform based multi-target immune checkpoint inhibitors that simultaneously act on multiple immune targets, regardless of target expression levels. This platform offers several advantages: 1) it introduces biologically active domains that exhibit therapeutic effects, 2) it demonstrates high avidity based on protein structural characteristics, 3) it inherently possesses a cancer-T cell engaging effect when introducing multi-targets, and 4) being human protein-based results in reduced immune-related side effects, contributing to a favorable safety profile and therapeutic index. Cellemedy's multi-target immune checkpoint inhibitors exhibit target-specific cytotoxicity against melanoma cancer cell line. In a non-human toxicity study, those compounds were well tolerated during repeated dosing, providing evidence of a superior safety profile without noticeable side effects. Furthermore, we verified strong efficacy in in vivo studies through T/NK cell engagement and high target avidity following a single-dose administration. Rapid and durable anti-tumor responses have been observed in vivo models. In summary, Cellemedy's T-sphaera is promising multi-target immune checkpoint inhibitor platform that can be designed for various target, not only immune checkpoint blockade but also other tumor receptors, aiming at multiple cancers.

#7303 A novel anti-PD-L1/VEGF bispecific antibody (HLX37) with immune checkpoint inhibition, anti-angiogenic, and antineoplastic activities.

Ge Song, Yu-Syuan Chen, Xiaoling Yuan, Chunlei Ge, Xiansong Xiong, Seng Zhu, Hongqing Wei, Jijun Yuan, Chen Hu, Qingyu Wang, Jun Zhu

Shanghai Henlius Biotech, Inc, Shanghai, China

Introduction: Dual blockade of PD-L1 and VEGF has demonstrated significant clinical benefits in advanced hepatocellular carcinoma (HCC) and non-small cell lung cancer (NSCLC). However, efficacy remains suboptimal in certain indications, potentially due to insufficient levels of anti-VEGF inhibitors within the tumor, as well as vascular dysfunction caused by the disruption of vascular homeostasis via the anti-VEGF inhibitor. The bispecific antibody targeting PD-L1 and VEGF enhances anti-VEGF delivery to the tumor site by binding to tumor PD-L1, promoting synergistic effects of blood vessel normalization and T cell activation within the tumor microenvironment, thus improving anti-tumor activity.

Methods: Flow cytometry and Biacore were employed to evaluate the binding of the bispecific antibody. PD-L1 or VEGF blockade assays were conducted using PD-1-Jurkat cells/PD-L1-APC-CHOK1 or KDR cells. Efficacy was assessed in CDX models with immune humanization, including MDA-MB231 (human breast cancer cell line), NCI-H292 (human lung mucoepidermoid carcinoma cell line), HCT116 (human colon cancer cell line), and SK-MES-1 (human lung cancer cell line) tumor cell lines. Pilot pharmacokinetic and toxicity studies were performed in cynomolgus monkeys.

Results: *In vitro* assays demonstrated that the anti-PD-L1/VEGF bispecific antibody exhibits high binding affinity for both PD-L1 and VEGF, showing dual blockade activity against PD-1/PD-L1 and VEGF/VEGFR2. The anti-PD-L1/VEGF bispecific antibody was shown to possess superior ability to reverse T cell exhaustion. It conferred robust tumor inhibition activity and dose-dependent tumor suppression in the MDA-MB231, NCI-H292, HCT116, and SK-MES-1 models. Compared to the combination group of anti-PD-1 monoclonal antibody and anti-VEGF monoclonal antibody, the anti-PD-L1/VEGF bispecific antibody showed enhanced tumor enrichment. The distribution of anti-PD-L1/VEGF is mediated by both PD-L1 and VEGF, HLX37 was well tolerated in cynomolgus monkeys after three weekly intravenous doses of 100 mg/kg.

Conclusion: HLX37 is an anti-PD-L1/VEGF bispecific antibody with immune checkpoint inhibitory, anti-angiogenic, and antineoplastic properties. It demonstrated strong preclinical efficacy and a favorable safety profile, with enhanced tumor enrichment. It holds promising potential for further application in various types of tumors.

#7304 Cytotoxic PD-L1/PD-L2 dual-specific antibodies couple tumor stroma remodeling with checkpoint blockade to drive rejection of "cold" cancers.

Ahmad Salameh[1], Coline Couillault[2], Anupallavi Srinivasamani[2], Qinying Liu[2], Elisabetta Bolli[3], Christine Gagliardi[1], Guillaume Trusz[2], Nadthakarn Boland[4], Paul Blezinger[1], Dongxing Zha[2], Manuela Iezzi[5], Bianka Prinz[4], Federica Cavallo[3], Federica Pericle[1], **Michael A. Curran**[2]

[1]ImmunoGenesis, Inc., Houston, TX,[2]Immunology, The University of Texas MD Anderson Cancer Center, Houston, TX,[3]Department of Molecular Biotechnology and Health Sciences, University of Torino, Torino, Italy,[4]Adimab, Inc., Lebanon, NH,[5]D'Annunzio University of Chieti-Pescara, Chieti-Pescara, Italy

Introduction: Antibody blockade of either the Programmed Death 1 (PD-1) immune checkpoint receptor on T cells or its cognate ligand PD-L1 on tumor cells and stroma has revolutionized oncology but fails to regress "cold" tumors lacking T cell infiltration. We leveraged the iterative selection capacity of yeast-displayed fully human antibody selection to identify dual PD-L1/PD-L2 specific antibodies (DIPDL) that block their engagement of both PD-1 and B7-1. Finally, we engineered these DIPDL to efficiently kill PD-L1 and PD-L2 expressing tumors and stroma.
Materials and Methods: Antibody binding to PD-Ligands was measured by Octet. The Promega PD-1 assay system was used to measure EC50s for reversal of PD-1 driven T cell inhibition. Retrogenix microarray technology was used to determine specificity of target binding. Surrogate and direct assays were used to measure ADCC and ADCP efficiency. Murine syngeneic tumor models were tested for PD-1 blockade versus DiPDL antibodies to mediate tumor regression. Flow cytometry was used to characterize changes in the tumor immune microenvironment. Human tumor xenografts were implanted into immunodeficient mice to study the cytotoxic potential of these antibodies.
Results: We leveraged multiple antibody engineering technologies to generate dual PD-L1/PD-L2 specific antibodies capable of blocking ligand interactions with PD-1 and B7-1, and also mediating efficient tumor and stromal depletion. These Fc engineered DIPDL kill human PD-L1$^+$ and/or PD-L2$^+$ cells *in vitro* using both NK and myeloid effectors. *In vivo* studies of murine syngeneic tumor models revealed that these DIPDL antibodies significantly outperform PD-1 blockade across both "hot" and "cold" cancers. Tumor microenvironment analysis showed that myeloid stromal depletion correlates with T cell and dendritic cell expansion and therapeutic response. Lastly, treatment of immunodeficient mice bearing human triple negative breast cancer with these cytotoxic DIPDL significantly inhibited tumor growth and reduced myeloid stromal density.
Conclusions: These DIPDL represent a new generation of immunotherapeutic which couple superior PD-1 pathway blockade with direct depletion of both immunosuppressive and T-cell exclusionary stroma and tumors. Across murine models, these DIPDL deliver transformative efficacy overcoming limitations of simple PD-1 blockade and have moved to an ongoing Phase I clinical trial.

#7305 Increasing TCR/antigen sensitivity using anti-CD3 Fabs enhances anti-tumor effects but not immunotoxicity of immunotherapies blocking T cell checkpoint receptors.

Onyekachi Juliet Okpasuo, Chandrashekara P. Mahadevappa, Hien Huynh, Christa Cheatam, Vijay Radhakrishnan, Adam Schrum, Diana Gil

University of Missouri - Columbia, Columbia, MO

Cancer patients treated with immune checkpoint inhibition (ICI), such as monoclonal antibodies (mAbs) that block the inhibitory functions of the T cell receptors, CTLA-4 and PD-1, are at risk of experiencing immune-related adverse effects (IRAEs). These effects may require interruption, neutralization, or discontinuation of treatments, potentially hindering control of cancer progression. The risk for IRAEs is elevated in individuals with underlying autoimmune diseases (AIDs), resulting in this subpopulation of cancer patients being undertreated with ICI therapies. Establishing novel approaches to enhance anti-tumor control by T cells using ICI without increasing concomitant immunotoxicity could benefit a broad range of cancer patients, including those with autoimmune pathologies. Targeting the CD3ε subunit of the T cell antigen receptor (TCR) with a Fab fragment that strictly controls monovalence (Mono-Fab) improves sensitivity to TCR/antigen stimulation and T cell effector function. We have demonstrated that anti-CD3ε Mono-Fabs are effective immunotherapies against murine melanoma, depending on the presence of functional T cells and the availability of tumor-associated antigens (TAAs) in melanoma cells. Here, the efficacy of the anti-mouse CD3ε Mono-7D6-Fab and its potential to induce IRAEs has been compared to ICI therapies using the mAbs anti-PD-1 and anti-CTLA-4 in mice implanted with subcutaneous melanoma. Results indicate that Mono-7D6-Fab enhances the performance of ICIs based on the level of tumor immunogenicity. However, Mono-7D6-Fab did not increase the immunotoxicity risk of either anti-CTLA-4 or anti-PD-1 monoclonal antibodies when combined with these treatments, nor in experiments involving the three types of immunoglobulins, as seen in mice with experimental autoimmune encephalomyelitis (EAE), which reported a worsening of T cell-mediated immune pathology. Additionally, T, B, and innate immune cell subpopulations found in peripheral lymphoid and CNS tissues, which could mediate EAE, were down-modulated in mice treated with Mono-7D6-Fab alone or combined with ICIs while elevated in mice treated solely with ICIs. Similarly, treatment with ICIs alone failed to control tumor growth in the context of underlying autoimmune disease; however, combining Mono-7D6-Fab with ICIs restores the anti-tumor benefit of ICI without further exacerbating autoimmune toxicities. These promising findings justify further experimentation for the eventual translation of anti-human CD3 Mono-Fabs, potentially enhancing and broadening the application of cancer immunotherapies that target T-cell anti-tumor responses.

#7306 Selective and novel ALDH1 inhibitor enhances immune checkpoint blockade therapy in preclinical models of MSS colorectal cancer.

Akinchan Kumar[1], Andrada Constantinescu[2], Ismail Ceylan[2], Mileidys Perez-Alea[2], **Bassam Janji**[3]

[1]Department of Cancer Research, Luxembourg Institute of Health, Luxembourg City, Luxembourg,[2]Advanced BioDesign, Saint-Priest, France,[3]Luxembourg Institute of Health, Luxembourg City, Luxembourg

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths globally and the third most frequently diagnosed cancer type, according to the Global Cancer Observatory. While the emergence of immune-checkpoint inhibitors (ICI) in the past decade has transformed cancer treatment paradigms, their efficacy in CRC is limited, particularly in the microsatellite-stable (MSS) subtype, which constitutes 85% of cases. MSS CRC is characterized by a low mutation burden and an immunosuppressive tumor microenvironment (TME), resulting in poor responsiveness to ICIs. Recent studies have identified elevated ALDH1 expression and its enzymatic products in MSS tumors, with retinoic acid (RA) produced by ALDH1 playing a pivotal role in fostering an immunosuppressive TME. This RA-mediated immunosuppression arises not only from tumor cells but also from tumor-associated immune cells, such as M2 macrophages and myeloid-derived suppressor cells (MDSCs), both of which exhibit high ALDH1 activity. Using syngeneic mouse models of MSS CRC, including MC38-CEA and CT26 cell lines, we evaluated the therapeutic potential of combining ABD0171, a selective ALDH1 inhibitor (ALDH1i), with anti-PD-1 therapy. Our findings reveal that ABD0171 significantly enhances the efficacy of anti-PD-1 therapy, leading to improved survival outcomes in MSS CRC models. This synergistic effect is associated with substantial alterations in the TME immune landscape, including an increase in pro-inflammatory M1 macrophages and reductions in both M2 macrophages and regulatory T cells (Tregs). Mechanistically, ALDH1 inhibition significantly reduces intratumoral RA levels, disrupting a key pathway responsible for the differentiation of immunosuppressive macrophages from monocytes. Ongoing studies are exploring the molecular mechanisms underlying these effects. Overall, our work underscores the potential of selective ALDH1 targeting as a strategy to overcome immunotherapy resistance in MSS CRC, offering new therapeutic opportunities for patients with this challenging cancer subtype.

#7307 Potential role of alpha-2-macroglobulin in versican proteolysis regulation in colorectal cancer.

Yousef H. Gadalla, Somak Chaudhuri, Sean G. Kraus, Anna M. Field, Katherine A. Johnson, Cheri A. Pasch, Dustin A. Deming

University of Wisconsin - Madison, Madison, WI

Background: Versican is a large extracellular glycoprotein that acts as a barrier for immune cell infiltration in colorectal cancer (CRC). The V1 isoform of versican can be cleaved by ADAMTS 4 and 5 into the bioactive fragment versikine with immune stimulatory properties. Despite overexpression of ADAMTS 4 and 5 in CRC, versican may be poorly proteolyzed. ADAMTS 4 and 5 may be inhibited by the presence of an extracellular endopeptidase inhibitor, alpha 2 macroglobulin (A2M). Here we investigate A2M expression in CRC, its correlation with versican proteolysis status, and immune cell infiltration.

Methods: The Cancer Genome Atlas (TCGA) Colorectal Adenocarcinoma PanCancer Atlas dataset was used to stratify CRC patients (n=594) into two cohorts: $A2M^{hi}$ (n=57) (mRNA expression Z-score >1) and $A2M^{lo}$ (n=341) (Z-score <0). Clinical characteristics and differences in gene expression were analyzed between the two groups. CRC patient-derived tumor samples (n=306) were analyzed for protein-level expression of versican (VCAN), versikine (Vkine), A2M, CD8A and CD4 via immunohistochemistry (IHC). A binning scale of 0 - 3 was used to assess staining intensity with 0/1 being low and 2/3 being high expression. The number of CD8A and CD4-positive lymphocytes in direct contact with tumors (tumor-infiltrating lymphocytes; TILs) were counted per high power field (HPF). Th1 (T helper 1 cell) and Treg (T regulatory cell) subsets of CD4+ TILs were further evaluated per 20X field of view (FOV) via dual immunofluorescence.

Results: $A2M^{hi}$ and $A2M^{lo}$ groups have no differences in disease staging (q=.109), sex (q =0.992) or age distribution (q= 0.368). The $A2M^{hi}$ group has higher mRNA expression of VCAN (log_2fold change = 2.40, q <.01), ADAMTS1 (2.3, q<.01), ADAMTS4 (1.74, q <.01), ADAMTS5 (1.47, q<.01), CD8A (1.48, q<.01), CD4 (1.91, q<.01), CXCL9 (1.88, q<.01), and CXCL10 (1.32, q<.01). Out of 306 patient-derived tumor samples, 82.9% of versican proteolytic prominent (VPP) ($VCAN^{Lo}$ $Vkine^{Hi}$) were $A2M^{Hi}$, 76.8% of versican proteolytic weak (VPW) ($VCAN^{Hi}$ and $Vkine^{Lo}$ or $Vkine^{Hi}$) were $A2M^{Hi}$, and 85.1% of versican versikine low (VVL) were $A2M^{Hi}$. There is a trend towards significant interaction between A2M, versican and Th1 cells (p=0.0531), but none exist between A2M, versican and CD8+ T-cells/ Tregs. VPP and $A2M^{Hi}$ samples have significantly higher median Th1s/FOV than VPW and $A2M^{Hi}$ samples (VPP and $A2M^{Hi}$median: 24.5 Th1s/FOV, VPW and $A2M^{Hi}$ median: 9, p = .009).

Conclusion: The expression of A2M can regulate ADAMTS protease function but does not appear to prevent VCAN proteolysis or impact T cell infiltration in CRCs.

#7309 Investigating IL-1β signaling axis and inhibition strategies with small molecules in canine metastatic osteosarcoma: Insights for translational medicine.

Matheus Moreno Passos Barbosa[1], Vishnu V. Krishnamurthy[2], Timothy M. Fan[3]

[1]Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL,[2]High-Throughput Screening Facility, School of Chemical Sciences, University of Illinois at Urbana-Champaign, Urbana, IL,[3]Veterinary Clinical Medicine, University of Illinois at Urbana-Champaign, Urbana, IL

Introduction: Osteosarcoma (OS) is the most common bone tumor in pet dogs and people. While localized therapies, including surgery and ionizing radiation, effectively control bone-related events, options for slowing metastatic progression remain limited and only marginally active. Advances in drug delivery highlight the need for predictive animal tumor models to accelerate therapeutic development for humans. The clinical features, behavior, histopathology, treatment response, and genetics of OS in dogs closely mirror those in humans, making dogs an ideal comparative model. Dogs with spontaneous OS offer valuable insights into OS biology and are particularly useful for evaluating immunotherapeutic strategies. The pro-inflammatory effects of interleukin-1-beta (IL-1β) in the tumor microenvironment may hinder immunological responses against OS in both species. IL-1β is upregulated in the myeloid compartment of canine OS patients and facilitates metastatic progression in other cancers, including breast cancer. This study investigates the biological sources and role of IL-1β in OS metastatic biology and potential blocking strategies with small molecule inhibitors, offering insights for translational medicine.
Methods: Canine OS cells, primary monocytes, monocyte-derived macrophages, and DH82 cells were used to investigate IL-1β secretion and receptor expression. Immunological and proliferative factors under IL-1β exposure were assessed by ELISA and clonogenic assays. RNA NanoString profiling was employed to characterize gene transcripts in both healthy canine lungs and metastatic OS lesions. A high-throughput screening approach evaluated the ability of 80,000 small molecules to inhibit IL-1β intracellular downstream signaling and to inhibit IL-1β biological release and inflammasome activity.
Results: Canine OS cells express receptors related to IL-1β signaling and secrete pro-metastatic factors upon IL-1β exposure. However, OS cells do not secrete IL-1β. Primary monocytes and DH82 cells secrete IL-1β in response to inflammasome and caspase activator molecules. Monocyte-derived M1 macrophages were found to release significantly higher amounts of IL-1β compared to M0 and M2 macrophages. Metastatic lesions in canine OS exhibited distinct gene transcript profiles. Small molecules and structure-related molecules with potent IL-1β inhibition activities were identified.
Conclusions: Our study showed that IL-1β is secreted by monocytes and macrophages, not by OS cells. IL-1β induces the release of pro-metastatic factors by canine OS cells, suggesting its role in OS metastasis. Targeted inhibition of IL-1β may be a potential therapeutic strategy for controlling metastatic progression in OS and collectively underscore the potential value of comparative oncology approach towards successful translation medicine.

#7310 PTPN1/2 inhibition with AC484 inhibits tumor metastasis.

Yue Liu, Im-Meng Sun, Marc Creixell, Jordan S. Brown, Tuan Andrew Nguyen, Jiaxi Wang, Nianxin Yang, Ka Man Li, John Balibalos, Samir Kharbanda, Kayley Hake, Varahram Shahryari, Aaron W. Stebbins, Patrick M. Godfrey, Jonghyeob Lee, Chirag H. Patel, Po-Han Tai, Evangelia Malahias, Wenjun Kong, Kenneth J. Finn, Marcia N. Paddock, Fiona A. Harding, Jonathan D. Powell

Calico Life Sciences, South San Francisco, CA

ABBV-CLS-484 (AC484) is a novel small molecule based immunotherapeutic drug that inhibits both PTPN2 and PTPN1 and is currently under clinical investigation for treating solid tumors. In preclinical syngeneic tumor models, AC484 has been shown to be efficacious as a monotherapy, in conjunction with checkpoint inhibitors (ICBs) in ICB-resistant tumors, as well as in adoptive cellular therapy (Baumgartner et al, Nature 2023). Given the potent efficacy of this immunomodulatory compound, we sought to investigate the potential role of PTPN1/2 inhibition as a means of preventing metastasis. To this end, we established spontaneous metastatic murine models of 4T1 breast cancer and CMT167 lung cancer. In both models, AC484 treatment was able to inhibit metastasis. Notably, AC484 reduced circulating tumor cells and suppressed tumor cell seeding and outgrowth in the lungs. Genetic deletion of PTPN1/2 in tumor cells failed to recapitulate this effect, indicating that PTPN1/2 inhibition was tumor cell extrinsic. Indeed, AC484 was ineffective in inhibiting metastasis in NSG mice, suggesting that it exerted its effects on immune cells. Antibody depletion experiments further demonstrated that both T cells and NK cells play critical roles in AC484-mediated anti-metastatic activity in the C57BL/6 CMT167 model, with NK cells appearing more dominant in the BALB/c 4T1 model. Careful kinetic analysis of these models demonstrated accumulation of myeloid cells, particularly neutrophils and monocyte-derived macrophages, both systemically and locally in the lungs. Importantly, this increase in myeloid population was observed prior to the formation of micro metastases and continued to escalate as lung metastasis advanced. These findings support a role for myeloid cells in altering the microenvironment to promote seeding, survival, and growth of metastatic tumor cells. Interestingly, while AC484 treatment prevented lung metastasis, it did not decrease myeloid cells. Rather, single-cell RNA sequencing of lung tissue from 4T1-bearing mice showed an increase in activation markers of myeloid cells, supporting a role for AC484 in modifying the metastatic microenvironment. Taken together, our data demonstrate the ability of PTPN1/2 inhibition to inhibit metastasis by enhancing anti-tumor immunity and altering the metastasis niche. These data provide the preclinical rationale for pursuing AC484 treatment in the neoadjuvant/adjuvant setting as a means of promoting the efficacy of cancer therapies with curative intent.

#7311 Targeting STAT3 to prevent non-small cell lung cancer in patients with chronic obstructive pulmonary disease.

Katherine E. Larsen[1], Leticia G. Hamana Zorrilla[2], T. Kris Eckols[3], Moses M. Kasembeli[3], Michael J. Clowers[1], Avantika Krishna[1], Maria T. Grimaldo[1], Nastaran Karimi[1], Shanshan Deng[1], Vignesh Gunasekharan[4], Luisa M. Solis Soto[2], David J. Tweardy[1], Seyed Javad Moghaddam[1]

[1]Pulmonary Medicine, UT MD Anderson Cancer Center, Houston, TX,[2]Translational Molecular Pathology, UT MD Anderson Cancer Center, Houston, TX,[3]Infectious Diseases, UT MD Anderson Cancer Center, Houston, TX,[4]Division of Cancer Prevention, National Cancer Institute, Rockville, MD

Non-small cell lung cancer (NSCLC), primarily caused by cigarette smoking, is the leading cause of cancer-related deaths worldwide. Smokers with chronic obstructive pulmonary disease (COPD) face a 3-10 fold increased risk of developing NSCLC compared to smokers without COPD. Lung inflammation and functional deterioration persist in COPD patients, and the risk of developing NSCLC remains elevated. This notes the importance of targeting inflammatory pathways to prevent cancer progression. In COPD patients, lung adenocarcinoma (LUAD) is the most common subtype of NSCLC, with about 30% of cases featuring activating mutations in the Kirsten rat sarcoma viral (*KRAS*) oncogene. Current treatments for *KRAS*-mutant LUAD (KM-LUAD) are often ineffective, warranting novel preventive and therapeutic methods. Our group previously developed a COPD-like mouse model through weekly exposure to an aerosolized lysate of non-typeable *Haemophilus influenzae* (NTHi), a common airway colonizer in COPD patients. Using this model, we showed that COPD-like inflammation promotes KM-LUAD with an essential role for the pro-inflammatory cytokine, IL-6, and activation of signal transducer and activator of transcription 3 (STAT3). Here, we investigated the effects of STAT3 inhibition on KM-LUAD promotion by COPD. We utilized $CCSP^{Cre}$/LSL-$Kras^{G12D}$ (CC-LR) mice, a genetic model of LUAD that develops early-stage lung lesions starting at six weeks of age. Six-week-old CC-LR mice were exposed to aerosolized NTHi lysate or PBS weekly to induce COPD. At ten weeks of age, they received one of three doses (25, 50, or 100 mg/kg) of TTI-101, a selective STAT3 inhibitor, or vehicle control daily for 14 days by oral gavage. We then collected blood, bronchoalveolar lavage fluid (BALF), and lung tissues for complete blood counts, blood chemistry tests, tumor burden, inflammation analysis, and histopathology. Results indicated no significant weight changes or toxicity from TTI-101 treatment. NTHi exposure induced COPD-type neutrophilic inflammation in vehicle treated cohorts that was reduced following the treatment with TTI-101. Tumor burden, both multiplicity and area, also showed a significant decrease in TTI-101-treated mice. Inflammation and levels of tyrosine phosphorylated STAT3 (pY-STAT3) in lung tissues were significantly reduced post-treatment. These findings suggest that STAT3 contributes to KM-LUAD promotion in COPD and that its inhibition with TTI-101 may effectively prevent KM-LUAD associated with COPD. Further research will determine the effects of TTI-101 in preventing NSCLC promotion and identify candidate biomarkers that correlate with NSCLC development and the preventative efficacy of TTI-101. Funding: This project was funded by Federal funds from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, under contract No. 75N91019D00021/75N91022F00002

IMMUNOLOGY: T Cell Engagers and Novel Antibody-Based Therapies
Poster Session

#7315 Engineering and characterization of novel T cell engagers (TCEs) directed against colorectal cancer-associated antigens (CEACAM5, CDH17).

Jinxiu Hou, Liang Tian, Chenpeng Su, Rui Shen, Dandan Liu, Jiyuan Tian, Tingting Gu, Xiaoou Xu, Xiaoqian Chen, Lisha Dong, Chuan Chen, Yongxin Shang, Maowei Wang, Jian Peng, Zhenping Zhu

Helixon US Inc., New York, NY

Colorectal cancer (CRC) is one of the most prevalent and deadliest oncological diseases worldwide. Carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) and cadherin-17 (CDH17) have been shown to be selectively expressed in colorectal tissues, and their level of expression and/or co-expression are significantly up-regulated in malignant diseases. Various experimental therapeutic modalities addressing these TAA targets are currently under active exploration. In this study, we engineered several T cell engagers (TCEs) directed against these targets, in multiple molecular formats, such as TAA/CD3 (1+1), TAA/TAA/CD3 (bivalent or biparatopic, 2+1), and TAA1/TAA2/CD3 (trispecific, 1+1+1), and characterized these TCEs in a battery of assays, including binding, T cell activation and tumor cell-killing, production and developability, as well as anti-tumor efficacy in vivo in various animal models. '1+1', '2+1' bivalent, and '2+1' biparatopic formats were designed and constructed for both CEACAM5/CD3 and CDH17/CD3 bispecific TCEs. A trispecific TCE, CEACAM5/ CDH17/CD3 (1+1+1) was also produced. The cell-binding, cytotoxicity, T cell activation and cytokine release mediated by these TCEs were investigated in vitro. The anti-tumor activity of these TCEs were examined in vivo in human PBMC-reconstituted immune-deficient mice implanted with human CEACAM5- and/or CDH17-expressed tumor xenografts. In these TCE molecules, each building blocks, i.e., αCEACAM5, αCDH17, and αCD3 antibodies, were designed and selected regarding their antigen binding affinity and specific epitope. These are followed by various designing of the molecular formats of the TCEs to ensure the balance of multiple parameters, including antigen/cell binding, T cell activation vs tumor cell killing, production and developability, etc. Our studies identified a CEACAM5/CDH17/CD3 trispecific TCE that was more potent than the bispecific TCEs in killing tumor cells, and was broadly effective in killing tumor cells that either express only CEACAM5 or CDH17, or both. The trispecific TCE was also more efficacious than the single-target based TCEs in inhibiting xenograft tumor growth in animal models. Taken together, these findings support further development of the CEACAM5/CDH17/CD3 trispecific TCE as a promising modality for the treatment of CRC patients.

#7316 QL615, a tetraspecific T cell engager with highly restricted activity on MET and EGFR-positive tumors while providing co-stimulation via CD2.

Yanan Wang, Meihua Luo, Guangjie Guo, Shenda Gu, Joshua Morrissette, Nathan Ngo, Shihao Chen

QLSF Biotherapeutics, South San Francisco, CA

Effective targeting of solid tumors using T cell engagers remains challenging due to the lack of tumor specific antigens (TSA), and the difficulty in achieving sustained anti-tumor response. Frequently, target antigen expression is upregulated on cancer, yet low expression on normal tissues limits the therapeutic window. Moreover, the lack of co-stimulatory signals paired with CD3 in the tumor microenvironment often leads to T cell exhaustion and non-durable responses. To overcome these shortcomings, we have developed a tetraspecific T cell engaging platform, termed TECAD "T Cell Engager with Co-Stimulation Avidity Driven", to address both tumor selectivity and potency by (1) activating T cells only when bound to tumor cells expressing paired antigens, and (2) providing CD3 co-stimulation via conditional CD2 activation. The latter enhances antitumor immune response and overcomes resistance to immune checkpoint blockade therapies. QL615, our lead TECAD candidate, targets MET and EGFR, two clinically validated targets limited by expression on normal tissues. MET and EGFR are typically expressed on non-overlapping normal tissues but frequently colocalized on tumors cells. We have optimized the affinity of MET and EGFR binding domains such that only dual engagement on the target cell provides high avidity binding AND-gated activation. Cell binding assays showed nanomolar EC_{50} on tumor cells expressing both MET and EGFR, whereas binding was negligible on cells expressing only low levels of either MET or EGFR. In vitro killing assays demonstrated over a 1000-fold selectivity in the lysis of double-positive tumor cells. The TECAD's CD2-mediated co-stimulation enhanced in vitro killing of tumor cells ~30-fold over constructs with CD3 redirection alone and showed superior tumor growth control in vivo. QL615 is currently advancing toward a development decision and clinical evaluation.

#7317 A novel LY6G6D x CD3 bispecific T-cell engager demonstrates better tumor killing and minimal cytokine release than a benchmark molecule.

Lini Huang, Mengting Chen, Yu Zhang, Dong Wu, Shiyong Gong, Xuan Wu, Danqing Wu, Chengbin Wu

EpimAb Biotherapeutics, Shanghai, China

Colorectal cancer (CRC) poses a significant global health challenge with high morbidity and mortality rates. Immune checkpoint inhibitors (ICI) have shown remarkable clinical efficacy in patients with mismatch-repair deficient (dMMR) or microsatellite instability-high (MSI-H) colorectal cancer. However, the vast majority of patients with microsatellite-stable (MSS) or microsatellite instability-low (MSI-L) colorectal cancer cannot benefit from ICI treatment. Recently, lymphocyte Antigen 6 Family Member G6D (LY6G6D) has emerged as a tumor associated antigen selectively expressed in MSS and MSI-low colorectal cancers. By using EpimAb's proprietary anti-CD3 antibody panel and bispecific antibody platform, we developed EM1034, a novel LY6G6D x CD3 bispecific T-cell engager (TCE). Compared to a clinical stage benchmark TCE which has monovalent target binding arms and asymmetrical knob-into-hole geometry, EM1034 demonstrated hundred folds increased tumor lysis potency while it induced much lower cytokines *in vitro*. *In vivo* efficacy studies further demonstrated that EM1034 is more potent than the benchmark molecule in xenografted models with different LY6G6D expression levels. Taken together, this study support EM1034 as a promising molecule for the treatment of mCRC patients with LY6G6D positive MSS/MSI-L tumors.

#7318 ZW209, a DLL3 targeted trispecific T cell engager with integrated CD28 co-stimulation, demonstrates safety and potent preclinical efficacy in models of small cell lung cancer.

Desmond Lau, Peter Repenning, Diana Canals Hernaez, Alec Robinson, Diego Perez Escanda, John Zhang, Hamed Shirvani, Catherine Wu, Kurt Stahl, Aditi Deshmukh, Nichole Escalante, Mariana Rocha, Begonia Silva Moreno, Lisa Newhook, Purva Bhojane, Paul A. Moore, Nina E. Weisser, Thomas Spreter von Kreudenstein

Zymeworks BC Inc., Vancouver, BC, Canada

Small cell lung cancer (SCLC) is a highly aggressive and difficult-to-treat malignancy with limited treatment options. Delta-like ligand 3 (DLL3), a cell surface protein overexpressed in SCLC, has emerged as a promising therapeutic target. Bispecific T cell engagers (TCE) targeting DLL3, including tarlatamab which has received accelerated approval, have demonstrated anti-tumor activity in the clinic with an improved duration of response compared to current standard of care, including chemotherapy and immunotherapy. Despite these advances, we believe there is opportunity to improve the rate and depth of response as the clinical activity of bispecific T cell engagers may be limited by low T cell infiltration and poor T cell function characteristic of SCLC tumors, and by the emergence of treatment-related T cell anergy due to T cell stimulation via signal 1 (CD3) only. The incorporation of signal 2 co-stimulation, via CD28 signaling, has the potential to improve response rates by stimulating increased T cell activation, proliferation and survival. To address treatment challenges and enhance the durability and sustainability of T cell activation, we engineered ZW209, a trispecific co-stimulatory T cell engager (TriTCE Co-stim) that optimally engages CD3 and CD28 and redirects and enhances cytotoxic T cell responses to DLL3-expressing tumor cells while maintaining a desired safety profile.

Our development candidate, ZW209, is designed to optimally engage CD3 and CD28 in an obligate *cis* manner, supported by a lack of T cell cross-linking and fratricide. The addition of CD28 co-stimulation in ZW209 demonstrated enhanced DLL3-dependent cytokine induction and T cell proliferation with improved antitumor activity relative to clinical bispecific TCEs benchmarks. In a serial, repeated challenge *in vitro* cytotoxicity assay, ZW209 displayed superior T cell fitness and anti-tumor activity to a bispecific control and clinical benchmark. *In vivo*, ZW209 exhibited potent anti-tumor activity in multiple humanized SCLC xenograft models. In aqueous and solid phase cytokine release assays, ZW209 exhibited minimal DLL3-independent cytokine production. Importantly, a ZW209 cynomolgus cross-reactive surrogate molecule displayed a favorable safety profile as it was well tolerated in non-human primates with repeat dosing at 10 mg/kg with no abnormal clinical signs.

In summary, these preclinical data support that ZW209 promotes improved anti-tumor activity against DLL3-positive cancer cells relative to competitor bispecific TCEs. By integrating CD28 co-stimulation into our trispecific T cell engager, ZW209 has the potential to improve the rate and duration of response for better clinical outcomes.

#7319 AI-guided engineering and development of anti-DLL3-targeting T cell engagers (TCEs).

Chuan Chen, Yue Wu, Liang Tian, Chenpeng Su, Zhaohui Chen, Dandan Liu, Jiyuan Tian, Xiaoou Xu, Xiaoqian Chen, Yang He, Yongxin Shang, Jian Peng, Zhenping Zhu

Helixon US Inc., New York, NY

The aggressive nature of small cell lung cancer (SCLC) and other neuroendocrine cancers (NECs), coupled with their poor prognosis and limited therapeutic options, represent significant challenges in clinical settings. Current first-line treatments, which often include platinum-based chemotherapy combined with programmed cell death protein 1 (PD-1) axis blockade, are associated with short-lived remission and rapid development of resistance, underscoring the critical need for novel therapeutic strategies. Delta-like ligand 3 (DLL3) has emerged as a promising tumor-associated biomarker, with specific overexpression on NECs, particularly SCLC. An anti-DLL3 x CD3 bispecific T-cell engager (TCE), tarlatamab, has demonstrated significant clinical benefits and was recently approved by the US FDA for the treatment of DLL3-expressing SCLC. In this study, we have generated several novel DLL3/CD3-targeting TCEs through the application of our streamlined AI-guided multi-specific development platform. Our AI-driven approach facilitates the engineering and optimization of multiple parameters, including antibody binding epitope, affinity, expression level, stability, and further developability of the candidates in a high-throughput fashion. Our biparatopic TCEs were designed to simultaneously engage two distinct epitopes on DLL3 on tumor cells, and mono-valency binding to CD3 on T cells with moderate to low affinity, in an attempt to enhance T cell-mediated specific cytotoxicity while minimizing potential side effects due to T cell over activation and cytokine production. The affinities and activities of the TCE building-blocks, i.e., the individual anti-DLL3 and CD3 antibodies, and the overall TCE molecules, were fine-tuned through both sequence and molecular format engineering under the guidance of our in-house AI algorithm. Our leading TCE molecule demonstrated a robust DLL3-specific T cell-dependent cellular cytotoxicity towards several tumor cell lines with various DLL3 expression levels, including both cisplatin-sensitive (e.g., NCI-H209, NCI-H526, DMS153) and cisplatin-resistant (e.g., SHP-77) cell lines. The biparatopic DLL3-target TCE also showed significant tumor inhibitory activity in tumor xenograft models including SHP-77, NCI-H82, and DMS-53. Taken together, our data underscore the significant utility of AI-guided platform in antibody/protein engineering, and support further development of our biparatopic anti-DLL3 x CD3 TCE in the treatment of DLL3-expressing SCLC and other NECs.

#7320 KGX105, a conditionally active prodrug of EGFRxCD3 T cell engager induces anti-tumor potency.

Y. Gao, J. Wang, S. Yang, J. Huang, H. Chen, H. Jiang, H. Wu, Y. Wu, **W. Jiang**;
Shanghai KangaBio Co., Ltd., Shanghai, China

T cell engagers (TCEs), are highly potent antitumor therapeutics to trigger T cell cytotoxicity, which have shown clinical benefit in hematologic malignancies while far been less successful in solid tumors. The development of TCEs against solid tumors has been limited by the significant on-target toxicity in normal tissues and cytokine release syndrome. Epidermal growth factor receptor (EGFR) is expressed in a variety of solid tumors. Several EGFR-targeted therapeutics have been approved for the treatment of cancer with either limited efficacy or induction of resistance. EGFR-targeting TCEs offered an alternative solution to overcome these issues. To address these challenges, we have developed KGX105, an EGFRxCD3 TCE prodrug with masked EGFR and CD3 antibody, which could be conditionally activated by tumor-specific proteases, and an albumin binding domain to extend the half-life. This design prevents on-target toxicity in normal tissues and systemic activation of T cells in circulation. Once masks are cleaved by tumor microenvironment (TME)-specific proteases, KGX105 could effectively trigger T cell cytotoxicity toward EGFR-positive tumor cells. To confirm KGX105's masking effect, we compared KGX105 and unmasked KGX105 for their ability in binding, T cell activation and T-cell-induced tumor cytotoxicity. KGX105 showed significantly reduced binding to CD3 and EGFR by >1000-fold and >200-fold, compared to the unmasked KGX105. KGX105 also showed reduced potency by >3000-fold in the T cell activation and by >1000-fold in the tumor cell cytotoxicity, which could be fully recovered after protease digestion, comparable to the unmasked KGX105. Then we conducted *in vivo* antitumor studies in human colorectal cancer HCT116 and COLO205 xenograft models using hPBMC-reconstituted M-NSG mice. KGX105 showed significant anti-tumor efficacy in both tumor models with the TGI% in HCT116 of 95% at 0.3 mg/kg and the TGI% in COLO205 of 68% at 3 mg/kg. No obvious body weight change was observed during the dosing period. The pilot toxic study to evaluate the safety and toxicokinetics of KGX105 is ongoing in *Cynomolgus* monkeys. The studies presented herein support KGX105 as a promising novel therapeutic to target a wide range of EGFR-expressing cancers with the potential to avoid the life-threatening on-target toxicities and systemic cytokine release.

#7321 A novel gamma-delta T cell engager platform for cancer immunotherapy.

Lei Ding, Sadhak Sengupta, Mariska ter Haak, Kate Rochlin, Yanjie Li, Lawrence S. Lamb

IN8bio, Birmingham, AL

Bispecific T cell engagers (TCE) bring effector T cells in contact with cancer cells by binding a pan-T cell receptor sequence and a tumor-associated antigen (TAA) simultaneously, thereby initiating activation and killing properties of the T cell. One of the foremost challenges for the clinical use of TCE is tumor heterogeneity, specifically, the limited expression of specific tumor antigens on the cancer cell surface. Gamma-delta (γδ) T cells are unique in that they recognize multiple stress-associated antigens common to most malignancies regardless of origin. This function is independent of MHC restriction, enabling them to target a broad range of tumors. Here, we report initial findings from a novel γδ T cell engager platform (γδ-TCE), specifically two γδ-TCEs targeting hematologic malignancies, CD33-TCE (CD-33, AML) and CD19-TCE (CD19, B_ALL), while also enabling γδ T cells to engage tumor-associated stress ligands with innate surface receptors such as NKG2D, PVR, and others.

Preliminary in vitro characterization revealed properties suggestive of pan-δ chain binding, such as competing with isoprenyl pyrophosphate activation of Vδ2+ T cells, yet efficiently driving both activation and expansion of Vδ1+ and Vδ2+ T cell subsets from peripheral blood mononuclear cells (PBMC). Robust expansion of a diverse repertoire of γδ T cells in the tumor environment is critical for continued pressure and a persistent anti-tumor response. Cytotoxicity assays show that both CD33-TCE and CD19-TCE measurably enhanced γδ T cell-mediated lysis of CD33+ AML and CD19+ ALL tumor cells, respectively, in a dose-dependent manner. In co-cultures with tumor cells, γδ-TCE- armored γδ T cells showed markedly increased degranulation and the release of pro-inflammatory cytokines, such as IFN-γ, TNF-α, granzymes, and perforin, further highlighting the activation of γδ T cells into a potent cytotoxic state and amplifying their tumor-eradicating abilities.

This novel γδ T cell engager platform represents a promising new approach to cancer immunotherapy, offering an effective strategy for enhancing γδ T cell-mediated tumor cell lysis. Furthermore, the platform's modular and flexible design suggests applications in treating a broad range of malignancies by combining the innate recognition properties of γδ T cells with the targeting of specific tumor-associated antigens, thus expanding its therapeutic potential.

#7322 LBL-058, A novel T cell engager conjugate (TEC) targeting DLL3 with dual tumor suppressive functions..

Sun Jianming, Huang Xiao, Huang Jing, Zhang Peng, Ding Mi, Qin Yurong, Wu Guojin, Mi Ye, Yang Ye, **Jordan Zhu**, Yue Zhao, Shoupeng Lai, Xiaoqiang Kang, Hong Ling

Leads Biolabs, Nan Jing Shi, China

Background :Small cell lung cancer (SCLC) is a highly malignant tumor and accounts for approximately 15% of lung cancer cases. The prognosis of invasive SCLC is poor, with a 5-year survival is 7%. SCLC has a high response to chemotherapy but tends to relapse and resist. In recent years, immune checkpoints have brought breakthroughs in first-line treatment and prolonged OS, but options after the second line are still lacking. Delta-like ligand 3 (DLL3) is an inhibitory Notch ligand that is highly expressed in SCLC and other neuroendocrine tumors, and its expression is limited in normal cells. Nowadays, many DLL3-targeting therapies are in development among them Tarlatamab, which is a T cell engager (TCE), has been approved by FDA for the treatment of adult patients with extensive-stage small cell lung cancer (ES-SCLC) with disease progression on or after platinum-based chemotherapy, at the same time several DLL3 targeting ADCs were also showing some promising results. Thus makes DLL3-targeting therapy an attractive strategy to treat SCLC.
Methods :Here we report a T cell engager conjugate (TEC), LBL-058, comprised of a DLL3-targeted T cell engager conjugated to a novel topoisomerase I inhibitor (TOP1i) payload. Moreover, LBL-058 was designed with a higher affinity targeting DLL3 and a lower affinity targeting CD3 to avoid potential cytotoxicity to T cells mediated by binding to T cells. The binding affinity of LBL-058 to DLL3 and CD3 was determined with Fortebio, while the T cell based activity was measured using several cell-based assays including reporter gene and TDCC assays. Binding of LBL-058 to DLL3 or CD3 induced Internalization and cytotoxicity were also evaluated by several cell-based assays. The anti-tumor activity of LBL-058 was investigated in xenograft mouse model.
Results :The affinity difference between CD3 and DLL3 has been fine-tuned, LBL-058 binds more easily to DLL3-positive cells. In CD3 reporter gene assay, LBL-058 could activate the NFAT reporter element through DLL3 antigen-dependent CD3 cross-linking. LBL-058 not only induce potent TDCC and T cell activation but also selectively deliver cytotoxicity payload to DLL3-positive tumor cells. In animal models, LBL-058 treatment induced durable tumor regression in xenograft tumor model.
Conclusion:LBL-058 has the potential to be a First-In-Class DLL3-targeted TCE ADC, which combines the anti-tumor properties of TCE and ADC, and shows a potent and durable anti-tumor activity and is expected to provide clinical benefits for the treatment of patients with SCLC.

#7323 LBL-043, a novel LILRB4xCD3 T cell engager, for the treatment of relapsed/refractory multiple myeloma.

Yujia Dang, Xiao Huang, Yurong Qin, Xiaoya Liu, Min Chen, Duqing Jiang, Guojin Wu, Mi Ye, Jianming Sun, **Hong Ling**, Shoupeng Lai, Xiaoqiang Kang, Jordan Zhu

Leads Biolabs, Nanjing, China

Background: Multiple myeloma (MM) is a clonal plasma cell proliferative disorder characterized by abnormal increase of monoclonal immunoglobulins. Despite newly developed novel therapeutic agents have greatly improved the outcome of MM patients in the past decades, MM is still an incurable hematological malignancy. Several CD3 bispecific immunotherapies that harness T cells against MM are at various stages of preclinical and clinical development, but most of them are hampered by the refractory and resistant mechanisms. Leukocyte immunoglobulin-like receptor B4 (LILRB4) is an immune checkpoint inhibitory receptor, which is highly expressed on monocytic blasts of AML and myeloid-derived suppressive cells. Most recently it is discovered also highly expressed in MM, in particular rrMM(relapsed/refractory MM). It does not express on hematopoietic stem cells and progenitor cells, therefore make it a promising target for hematological malignancies. Herein a novel T cell engager targeting LILRB4, LBL-043, is developed to specifically kill LILRB4 expressing MM cells.

Methods: LBL-043 is a bispecific antibody targeting LILRB4 and CD3, designed using proprietary LeadsBody^{TM} platform, which is a 2:1 format with two VHH arms targeting LILRB4 with high affinity and one scFv arm targeting CD3 with fine-tuned affinity. The binding affinity of LBL-043 was determined with Fortebio and flow cytometry, and the function activity was measured using reporter gene and TDCC assays on MM cell lines. The anti-tumor activity was investigated in B-NDG/hu-PBMC reconstructed mice implanted with NCI-H929. In addition, the potential toxicity profile of LBL-043 was examined in a non-GLP dose range finding (DRF) study in cynomolgus monkeys via IV infusion, once a week for a total of 4 doses.

Results: The binding affinity of LBL-043 to LILRB4 and CD3 protein was 0.724 nM and 35.7 nM, respectively. In CD3 reporter gene assays, LBL-043 could activate the NFAT signaling only in the presence of LILRB4+ cells. And LBL-043 was shown a potent TDCC activity in different LILRB4 expression level MM cells. In NCI-H929 CDX mouse model, LBL-043 was shown significant anti-tumor activity. DRF Study in Drug Toxicology Assessments demonstrated that LBL-043 has good safety profile in cynomolgus monkeys. There were no LBL-043 related changes of cardiac parameters, clinical chemistry, hematology, urinalysis, organ weight and gross pathology (except for decreased size of thymus).

Conclusion: LBL-043, a novel bispecific antibody targeting CD3 and LILRB4, redirecs T cells to effectively kill MM cells by simultaneously binding MM cells and T cells. It is also shown a great anti-tumor efficacy in NCI-H929 CDX mouse model. DRF of LBL-043 (0.2~10 mg/kg) in cynomolgus monkeys was well tolerated, and HNSTD was 10 mg/kg. These data for the first time show LBL-043 as a promising therapeutic bispecific antibody for the treatment of MM patients.

#7324 ONO-4685: A novel PD-1/CD3 bispecific T-cell engager (TCE) for the treatment of T-cell malignancies.

N. Tateshita[1], N. Watanabe[2], T. Shimbo[1], A. Morimoto[2], K. Takeda[1], T. Tanihiro[2];
[1]Ono Pharmaceutical Co., Ltd., Mishima-gun, Japan, [2]Ono Pharmaceutical Co., Ltd., Tsukuba-shi, Japan

Mature T and NK-cell neoplasms are of mature T-cell or NK-cell origin and comprise approximately 10% to 15% of non-Hodgkin's lymphomas (NHLs) all over the world. While new therapies with novel mechanisms of action have become available, their therapeutic outcomes are limited and most subtypes of the neoplasms have a poor prognosis. Therefore, identification of new therapeutic approaches is still an unmet medical need.Programmed cell death 1 (PD-1) is an inhibitory receptor expressed and increased on normal activated T and B cells that promotes T-cell apoptosis, anergy and functional exhaustion. PD-1 is also expressed on multiple different cancer types including major T-cell lymphomas/leukemias: Angioimmunoblastic T-cell lymphoma (AITL); Nodal T-cell lymphoma with T follicular helper (Tfh) phenotype; Follicular T-cell lymphoma; Peripheral T-cell lymphoma (PTCL) not otherwise specified (PTCL-NOS); Cutaneous T-cell lymphoma (CTCL) mycosis fungoides (CTCL-MF); CTCL Sézary syndrome (CTCL-SS), and; Adult T-cell leukemia/lymphoma (ATLL). Thus, PD-1 is a novel potential therapeutic target for T-cell lymphomas/leukemias.ONO-4685 is a first-in-class bispecific TCE antibody that binds to human PD-1 and CD3 to engage T-cells with PD-1 expressing tumor cells. TCEs have recently shown clinical anticancer success with some hematologic malignancies and solid tumor types.Here we performed preclinical studies to evaluate the therapeutic value of ONO-4685 to treat PD-1 expressing T-cell malignancies.To evaluate the effect of ONO-4685 on the cytolytic activity of T-cells, peripheral blood mononuclear cells (PBMCs) from healthy donors as well as PD-1 expressing T cell lymphoma/leukemia cell lines (ILT-Mat, HPB-ALL, and SUP-T1) were co-cultured in the presence of ONO-4685. ONO-4685 induced potent cytolytic cell killing of the cell lines. To assess the effect of ONO-4685 on T-cells, the intracellular expression of T-cell granzyme B was evaluated in *in vitro* cytotoxicity assay. ONO-4685 induced granzyme B expression in $CD4^+$ and $CD8^+$ T cells in the presence of PD-1-expressing target cells (ILT-Mat). Moreover, ONO-4685-mediated cytotoxicity on PD-1 expressing target cells was observed in the PBMCs from ATLL patients. *In vivo* anti-tumor activity of ONO-4685 was evaluated in PBMC-humanized mice xenograft models using PD-1 expressing human T cell lymphoma/leukemia cell lines (SUP-T1 and TL-Mor). ONO-4685 treatment showed suppressive effects on tumor growth in a dose-dependent manner.Taken together, these preclinical data support the clinical development of ONO-4685 as a novel TCE targeting PD-1 expressing hematologic malignancies. A Phase I clinical study in the USA is ongoing to evaluate the safety and tolerability of ONO-4685 in relapsed or refractory patients with PTCL and CTCL (NCT05079282).

#7325 Pharmacologic characterization of a highly specific and potent CLDN6xCD3 T cell engager (TCE), ARC101, for the treatment of CLDN6+ cancers.

Sanjaya Singh, Danlin Yang, Joseph Erhardt, Theresa McDevitt, Scott R. Brodeur, Bridget Larkin, Cassandra Lowenstein, **Desmond Lewis**, Tabitha Soto, Brinda Shah

Third Arc Bio, Spring House, PA

Claudin 6 (CLDN6) is an oncofetal protein involved in tight junction formation and is responsible for regulating cell polarity, adhesion and permeability. CLDN6 has recently emerged as an ideal target for novel oncology therapeutics, given the expression in various solid tumors, with limited normal tissue expression. High-levels of CLDN6 cell-surface protein expression are commonly detected in testicular cancer and epithelial ovarian cancer (EOC), with lower frequency expression in additional solid tumors such as gastric-, cervical-, endometrial-, and lung cancer. Using normal human tissue microarrays (TMA) and a highly selective commercially available CLDN6 antibody, minimal staining of CLDN6 was confirmed across a large panel of normal tissues by IHC, where sparse and limited apical staining of CLND6 was detected in pancreas, stomach, salivary gland, kidney and uterine tissues. High CLDN6 expression in ovarian tumors was demonstrated with the same selective antibody, confirming CLDN6 as a unique solid tumor target with limited normal tissue expression. ARC101 is a bispecific antibody that targets CLDN6 on tumor cells and CD3 on T cells, leading to T-cell-mediated tumor cell lysis. Generation of CLDN6-specific therapeutic antibodies has been challenging given the high degree of homology that exists between CLDN6 and CLN9, and to a lesser extent CLDN4. ARC101 was selected as a therapeutic candidate, due to the high degree of specificity obtained with this molecule. ARC101 bound to and induced cytotoxicity of a CLDN6 overexpressing SKOV3 ovarian cell line. No significant binding or cytotoxicity was observed for CLDN9 or CLDN4 overexpressing SKOV3 cell lines, underscoring the high specificity of ARC101 for CLDN6 over other highly homologous claudins. ARC101 bound to CLDN6-positive human ovarian cancer cell lines OVCAR3, PA1, and OV90, while binding to OVCAR3 cells was completely abolished when CLDN6 expression was deleted via knock out. ARC101 induced potent T cell activation and cytotoxicity at low effector to target (E:T) cell ratios across the panel of CLDN6-positive cells (2D and 3D models), with no activity observed on the CLDN6-negative OVCAR3 knock out cell line. In vivo, ARC101 demonstrated potent and dose-dependent anti-tumor activity in an ovarian tumor xenograft model. Overall, ARC101 is a highly specific CLDN6-directed T-cell engager with significant therapeutic potential for CLDN6-positive solid tumors. A Phase 1 first-in-human (FIH) clinical trial of ARC101 (NCT06672185) is planned to commence in 2025.

#7326 ATG-201, a novel T-cell engager (TCE) effectively depletes B cells with reduced risk of CRS for the treatment of B cell malignancies and B cell related autoimmune diseases.

Gang Bian[1], Huiling Liu[2], Tengteng Li[1], Zaoshun Hu[2], Peng Chen[1], Jay Mei[3], **Bing Hou**[3]

[1]Shanghai Antengene Corporation Limited, Shanghai, China,[2]Antengene (Hangzhou) Biologics, Hangzhou, China,[3]Antengene Corporation, Shaoxing, China

Background: CD19-targeted therapies, such as chimeric antigen receptor (CAR)-T or T-cell engagers (TCE), have been approved for the treatment of B cell malignancies. By depleting autoreactive B cells, CD19-targeted CAR-T have shown early yet promising efficacy in treating patients with B cell-driven autoimmune diseases. However, the clinical application of TCE continues to be greatly hindered by the unfavorable pharmacokinetics and toxicity associated with cytokine release syndrome. Here we developed a "2+1" CD19 x CD3 TCE, ATG-201, which effectively depletes B cells with minimal risk of CRS and IgG-like PK profile. It demonstrates potent anti-disease *in vivo* efficacy in mouse models for lymphoma and autoimmune diseases.
Methods: ATG-201 was constructed by introducing a high affinity, novel conformational epitope-targeted anti-CD3 single chain fragment variable (scFv) to the hinge region of one of the heavy chains of a CD19 monoclonal antibody. It was evaluated in a series of *in vitro* studies for binding affinity, T cell dependent cytotoxicity (TDCC) cytokine release, and drug developability. The *in vivo* anti-lymphoma efficacy and pharmacodynamic effect were evaluated in Raji xenograft model. The tissue reside B cell depletion was assessed in CD34+ hematopoietic stem cells humanized NDG-hIL-15 mice. Pharmacokinetic parameters of ATG-201 was evaluated in normal Balb/c mice.
Results: ATG-201 showed limited CD3+ T cell binding before CD19-crosslinking. It activated T cells and only in the presence of CD19+ cells. It induced potent TDCC against CD19 positive cells with pM range IC50. ATG-201 demonstrated stronger naïve B cell depletion activity with much lower cytokine release compared to benchmark TCEs *in vitro*. ATG-201 showed potent anti-lymphoma activity in PBMC-humanized subcutaneous Raji xenograft model with significant augment of infiltrated CD8+ T cells in tumor microenvironment and limited proinflammatory cytokines detected in plasma. Single dose ATG-201 completely and deeply depleted B cells in blood, bone marrow and spleen of CD34+ cells humanized NDG-hIL-15 mice. ATG-201 exhibited a mAb-like pharmacokinetic profile in Balb/c mice with a T1/2 of ~300h. ATG-201 was stable at high concentration (125mg/ml) and under stress conditions. The surrogate CD19xCD3 TCE demonstrated potent efficacy in mouse systemic lupus erythematosus model, inhibiting the lymph node swelling and auto-antibody producing.
Conclusion: ATG-201 demonstrates CD19-dependent CD3 binding and activation, inducing effective B cell depletion *in vitro* and *in vivo* with low cytokine release, which provides potential for the treatment of B cell malignancies and B cell related autoimmune diseases.

#7327 Harnessing FcγR glycoengineering to enhance anti-tumor monoclonal antibody efficacy.

Bowie Yik-Ling Cheng, Raquel M. Centeio, David Kung-Chun Chiu, Taia T. Wang

Stanford University, Stanford, CA

New strategies to enhance the activity of anti-tumor monoclonal antibodies (mAbs) that mediate cytotoxicity are needed. Glycoengineering of certain anti-tumor mAbs has been employed to increase their cytotoxic activity; however, the benefits of this approach are limited to specific patient groups. Beyond mAb engineering, an additional strategy involves modifying Fc gamma receptors (FcγRs), the receptors for IgG antibodies on effector cells, to augment specific antibody effector functions including cytotoxicity. One method for improving FcγR binding to mAbs is through glycoengineering, though the potential to modulate anti-tumor mAb activity via *in vivo* FcR glycoform engineering remains unexplored. We investigated the impact of FcγR glycosylation on therapeutic mAb activity and demonstrated that modifying FcγR glycoforms can enhance antibody-mediated anti-cancer responses. Underprocessed N-linked glycoforms were associated with improved binding affinity of Rituximab and Trastuzumab to FcRs, which enhanced the antibody-dependent cellular cytotoxicity activity of human peripheral blood mononuclear cells against target cells expressing CD20 or HER2, respectively. In vivo, glycoengineering of FcγRs improved the therapeutic efficacy of both a wild-type and an afucosylated anti-tumor mAb, demonstrating that this approach can further potentiate the activity of mAbs that have already been engineered for increased cytotoxicity. These findings underscore the potential of FcγR glycoengineering as a strategy to optimize mAb-based cancer immunotherapy.

#7328 Anti-tumor activity of anti-body drug conjugates in patient-derived xenografts of metastatic breast cancers patients.

Elodie Montaudon[1], Rania El Botty[1], Heloise Derrien[1], Ahmed Dahmani[1], Laura Sourd[1], Gabriel Champenois[2], Andre Nicolas[2], Didier Meseure[2], Guillaume Dutertre[3], Anne Vincent-Salomon[2], Laura Courtois[4], Julien Masliah Planchon[4], Ivan Bieche[4], Florence Coussy[5], Luc Cabel[5], Elisabetta Marangoni[1]

[1]Translational Research Department, Institut Curie, Paris, France,[2]Department of Pathology, Institut Curie, Paris, France,[3]Surgery Department, Institut Curie, Paris, France,[4]Genetics Department, Institut Curie, Paris, France,[5]Medical Oncology Department, Institut Curie, Paris, France

Background: Antibody-drug conjugates (ADCs) have emerged as highly effective new therapeutics for metastatic breast cancer (mBC). Sacituzumab govitecan (SG), an anti-TROP2 ADC, has been approved for the treatment of ER+/HER2- and triple negative mBC and trastuzumab deruxtecan (T-DXd), an anti-HER2 ADC, has been approved for HER2-amplified and HER2-low mBC. Despite the significant improvement in patients' survival, several hurdles remain to overcome, including the development of predictive biomarkers and the optimal sequential strategy. The aim of this study was to evaluate the response to SG and T-DXd ADCs in PDX models established from mBC and to identify potential biomarkers of response and resistance.
Material and Method: Biopsies from metastases or breast tumors from stage IV patients progressing on standard of care treatments were engrafted in nude mice. PDX were characterized at the genomic level and by RNAseq. HER2 expression was scored by IHC analysis by a clinical pathologist. The anti-tumor efficacy of T-DXd and SG was tested *in vivo* on 20 and 8 PDX respectively as compared to standard care treatment (endocrine therapy, CDK4/6 inhibitors, PARP inhibitors and chemotherapy).
Results: Forty-two PDXs were established, 33 (79%) from metastases and 9 (21%) from breast tumors of mBC. At the genetic level, PDX exhibited the typical molecular pattern of mBC with frequent alterations in the *PIK3CA*, *AKT1*, *ESR1* and *FGFR1* genes. Both T-DXd and SG treatment resulted in strong anti-tumor activity in multiple PDX, with 70 % and 75% of the models showing partial or complete response, respectively. Most of the models show similar responses to T-DXd and SG, with few PDX showing a different response pattern. Among these, a *BRCA1* mutated PDX showing cross resistance to talazoparib, CDK4/6 inhibitor, chemotherapy and T-DXd, was found to be a highly responder to SG treatment. T-DXd and SG were also highly efficient in *ESR1* mutated PDX established from patients progressing on aromatase inhibitors, including a model with cross resistance to different SERD. HER2 expression at baseline did not predict response to T-DXd. GSEA analysis identified different gene sets associated with T-DXd resistance, including KRAS signaling, EMT and hypoxia. Pharmacodynamic experiments in PDX responder and resistant to T-DXd and SG are ongoing to identify pharmacodynamic biomarkers.
Conclusion and Perspective: In summary, we established PDX of mBC, characterized for HER2 expression, genomic alterations and response to T-DXd and SG. Both ADCs showed high anti-tumor activity in multiple PDX with resistance to standard treatments including CDK4/6 inhibitors, SERD and PARP inhibitors. Response to T-DXD was not correlated with HER2 expression while T-DXd resistance was associated to an enrichment of EMT and hypoxia gene sets.

#7329 Overcoming systemic toxicity of IL-12-based immunotherapy by tumor-targeting and IL-12 attenuation.

Jihoon Chang, **Byoung Chul Lee**

Kanaph Therapeutics, Seoul, Korea, Republic of

With the recent success of immune checkpoint inhibitors, there is a renewed interest in cytokines as promising agents for cancer immunotherapy. Cytokines are well-established immunomodulators, and several inflammatory cytokines, including IL-12, have demonstrated potent anti-tumor activity. However, systemic administration of IL-12 has been associated with severe immune related adverse events, significantly limiting its therapeutic application. To overcome such narrow therapeutic window, we developed KNP-101, a bispecific Fc-fusion protein consisting of anti-FAP and attenuated IL-12. KNP-101 accumulates in the tumor microenvironment via anti-FAP and thus induces IL-12 activity at the tumor site. In addition, additional mutations were introduced into IL-12 to further mitigate systemic toxicity. Although the IL-12 activity was attenuated, KNP-101 mouse surrogate maintained significant anti-tumor potency in various mouse syngeneic models with a single intravenous injection. In combination therapies with anti-PD-L1, KNP-101 mouse surrogate demonstrated a synergistic anti-tumor effect. Analysis of tumor infiltrated lymphocytes via FACS demonstrated that the effects were mediated by immune cell infiltration. In naïve mouse toxicity test, KNP-101 mouse surrogate was tolerable up to 150 ug/head and no survival issue was observed. Similar toxicity studies were performed in tumor-bearing mice and, KNP-101 mouse surrogate showed safe profiles being tolerable up to 500 ug/head with no survival issues. Noticeably, KNP-101 mouse surrogate induced far less IFNg in serum compared to null/IL-12 suggesting that systemic toxicity was greatly reduced. Furthermore, KNP-101 was tolerable at doses up to 2.4 mg/kg in repeat-dose non-GLP studies conducted in cynomolgus monkey. Notably, KNP-101demonstrated that systemic toxicity of IL-12 therapy can be overcome by tumor-targeting and IL-12 attenuation strategies. KNP-101 exhibited a wide therapeutic window, by showing potent anti-tumor activities and significantly improved safety profiles. These attributes position KNP-101 as a promising therapeutic option for patients facing resistance to current cancer therapies.

#7330 MDNA113: A tumor targeting and conditionally activated anti-PD1-IL2^{SK} to enhance the therapeutic index.

Scott Rowlinson, Minh D. To, Qian Liu, Rosemina Merchant, Fahar Merchant, **Aanchal Sharma**

Medicenna Therapeutics, Toronto, ON, Canada

Background: To improve systemic tolerability of potent cytokines including anti-PD1/IL2 immunocytokines, conditional activation using tumor associated proteases have been gaining attention. MDNA113 (masked anti-PD1-IL2^{SK}), a Bifunctional SuperKine for Immunotherapy (BiSKIT), offers key distinguishing features including (i) next-generation IL2 Superkine (IL2^{SK}) with 'β-enhanced not-α' receptor selectivity, and (ii) a masking domain that also functions as a tumor targeting domain. MDNA113 incorporates an IL-13Rα2 selective IL13 superkine (IL13^{SK}) that facilitates tumor targeting and promotes durable accumulation within IL-13Rα2 expressing tumors thereby amplifying activation and therapeutic activity at the tumor site. In addition, the IL13^{SK} partially masks the IL2^{SK}, sterically hindering its systemic activity to improve tolerability while maximizing localization and activity of anti-PD1-IL2^{SK} within the tumor microenvironment (TME) which promotes synergy between IL2 receptor (IL-2R) agonism and immune checkpoint blockade by cis binding.

Methods: IL-2R signaling and PD1/PD-L1 blockade were evaluated using in vitro cell based reporter assays and human PBMCs. Tolerability, pharmacodynamics, efficacy and mechanistic studies were conducted in mouse syngeneic tumor models.

Results: MDNA113 demonstrated reduced IL-2R agonism while maintaining PD1/PD-L1 blockade compared to non-masked anti-PD1-IL2^{SK} in cell-based assays. This was reflected in reduced p-STAT5 signaling in human CD8+T cells, accompanied by decreased PBMC proliferation. Upon in vitro cleavage by proteases, full IL-2R agonism was restored as intended. In mice, MDNA113 exhibited better tolerability than naked anti-PD1-IL2^{SK}, correlating with reduced peripheral lymphocyte expansion. Systemic administration of MDNA113 showed comparable efficacy to anti-PD1-IL2^{SK} in mouse syngeneic tumor models, consistent with the designed protease mediated activation of MDNA113 through release of the IL13^{SK} mask within TME. Furthermore, MDNA113 achieved complete tumor regression in mice harboring IL-13Rα2 expressing solid tumors. These mice were also resistant to tumor growth when rechallenged with the same tumor antigen in the absence of any additional treatment, indicative of an underlying tumor specific memory response. Tumors from mice treated with a single dose of MDNA113 showed an elevated influx of CD8^{+}GrzB^{+} T cells, consistent with synergy between IL-2R agonism and immune checkpoint blockade. In addition, MDNA113 demonstrated superior efficacy compared to single-agent anti-PD1 when tested in a neo-adjuvant setting in an orthotopic 4T1.2 triple negative breast tumor model.

Conclusion: MDNA113 is conditionally active anti-PD1-IL2^{SK} designed by leveraging IL13^{SK} as a dual tumor targeting and masking domain for enhanced tolerability and robust efficacy in both therapeutic and neoadjuvant settings.

#7331 Antitumor activity of amivantamab in diffuse pleural mesothelioma.

Shinichiro Suzuki, Lin Yang, Jennifer Ayers-Ringler, Ezequiel J. Tolosa, Kuan-Li Wu, Janet L. Schaefer-Klein, Farhad Kosari, Aaron S. Mansfield

Mayo Clinic, Rochester, MN

Background: Diffuse pleural mesothelioma (DPM) is a rare and aggressive cancer arising from mesothelial cells of the pleura. Over 2,500 new cases are diagnosed annually in the United States, mainly attributable to asbestos exposure. The prognosis is poor, and while nivolumab (PD-1 inhibitor) and ipilimumab (CTLA-4 inhibitor) have been approved for first-line treatment of unresectable DPM, the three-year survival rate is only 23%. DPM tumors express high levels of epidermal growth factor receptor (EGFR) and mesenchymal-epithelial transition (MET), but targeted therapies like erlotinib (EGFR tyrosine kinase inhibitors) have shown no clinical benefit. We hypothesized that amivantamab, a bispecific antibody that targets both EGFR and MET, may have activity against DPM through antibody-dependent cellular cytotoxicity (ADCC).
Objective: The purpose of this study was to assess the potential of amivantamab to mediate ADCC in tissue culture experiments with DPM cell lines.
Methods: We assessed DPM for the expression of EGFR and MET using available data from bulk RNA sequencing. We also examined protein expressions of EGFR and MET by Western blot analysis in five epithelial (EM-Meso, H2452, H2461, Mero-41 and Mero-95), three sarcomatoid (H2373, H2596 and ONE58), and one normal mesothelial (MeT-5A) cell lines. Co-culture experiments were performed with the DPM and normal cell lines and NK cells in the presence of amivantamab to evaluate ADCC.
Results: We identified high levels of EGFR and MET expression in both epithelial and sarcomatoid cases of DPM from 183 patients. This finding was corroborated by Western blot analysis of all eight DPM cell lines. In vitro experiments demonstrated that amivantamab induced dose-dependent ADCC in DPM cell lines, with robust NK cell-mediated ADCC observed across all tested DPM lines. Notably, sarcomatoid-type DPM cells were more sensitive to amivantamab-induced ADCC than epithelial-type cells. These findings suggest that amivantamab may offer therapeutic benefits for DPM tumors with high EGFR and MET expression.
Conclusion: This study provides preclinical evidence supporting the potential of amivantamab as a novel therapeutic option for DPM, especially in sarcomatoid tumors. Ongoing in vivo studies including in patient-derived xenograft (PDX) models will further evaluate the efficacy of amivantamab in DPM.

#7332 Investigating genes and pathways determining sensitivity or resistance to anti-L1cam ADCs in lymphomas.

Filippo Spriano[1], Giulio Sartori[1], Chiara Tarantelli[1], Luciano Cascione[1], Sara Napoli[1], Flavio Mehli[2], Gunther Spohn[2], Francesco Bertoni[1]

[1]IOR - Institute of Oncology Research, Bellinzona, Switzerland,[2]Elthera AG, Zurich, Switzerland

Background: L1 cell adhesion molecule (L1CAM) is a glycoprotein initially characterized for its role in neuronal migration, cell adhesion, axonal growth, and synaptogenesis. In recent years, it has been identified in various solid tumors, in which it promotes invasion, metastasis, and resistance to therapies. We previously demonstrated the expression of L1CAM in a subset of lymphomas, mainly mantle cell lymphoma (MCL) and cutaneous T-cell lymphomas, determining *in vitro* and *in vivo* sensitivity to anti-L1CAM antibody-based therapies (Spriano et al., AACR-NCI-EORTC 2023). This project aimed to define genes and pathways contributing to the anti-lymphoma activity of L1CAM targeting antibody-drug conjugates (ADCs) in L1CAM-positive lymphomas. We used the Z138 MCL cell line as a model system exposed to two ADCs, sharing the same antibody backbone but with different payloads, the DNA targeting SG3199 (ADC6) and the tubulin targeting MMAE (ADC4), respectively.
Methods: We generated stable Cas9 or dCas9-VP64 expressing Z138 cells, then infected them with the Brunello (LOF) or the Calabrese (GOF) library. After the appropriate selection, cells were split to obtain the baseline representation of sgRNA distribution (T0) and then left untreated or treated with either of the two anti-L1CAM ADCs (exp arms) for two weeks. MAGeCK-MLE was used to model sgRNA read counts and assess the sgRNA β values and statistical enrichment (FDR<0.1). Combination treatments were performed, exposing cells to increasing concentrations of both drugs for three days, followed by an MTT assay.
Results: L1CAM expression was strongly associated with sensitivity to ADC4 but not to ADC6. As already reported and demonstrated also by us, the SG3199 payload is highly potent, rendering its activity less dependent on L1CAM levels. Among the genes sustaining resistance to ADC4, we identified FBXW7 and SOX11 (known L1CAM regulators), kinesins involved in microtubule assembly, MDR1, BCL2, CASP9, and ARID1A; TP53, BAX, CD20, CD19, RC3H1, and FCGR1B were identified as genes providing sensitivity to ADC4. Among the genes promoting resistance to ADC6, we identified RAD51B, ATR, BCL2, CHD4, and genes involved in the mitochondrial respiratory chain. Among the genes related to ADC6 sensitivity, we identified CDKN2B, RIPK1, MAPK9, RC3H1, NFKB1A, and RIF1. Finally, due to the observed involvement of ATR and BCL2 in the resistance to ADC6, we tested the addition of an ATR inhibitor (elimusertib) or a BCL2 inhibitor (venetoclax) to ADC6 in the Z138, achieving a strong synergism and additivity/synergism, respectively.
Conclusion: LOF and GOF CRISPR-Cas9 screenings identified genes related to resistance or sensitivity to anti-L1CAM ADCs that could be potentially used as therapeutic targets or biomarkers.

#7333 PD-L1-directed ISACs target host immune cells to drive powerful antitumor immune responses in a manner distinct from conventional PD-1/PD-L1 blockade.

Po Y. Ho, Rishali K. Gadkari, Shahid Khan, David T. Omstead, Jennifer E. Melrose, Matthew N. Zhou, Katelynn A. McEachin, Karla A. Henning, William G. Mallet, Steven J. Chapin, Romas A. Kudirka, Shelley E. Ackerman, Michael N. Alonso, **Justin A. Kenkel**

Bolt Biotherapeutics, Redwood City, CA

Background: Monoclonal antibodies targeting PD-1/PD-L1 have demonstrated clinical activity in numerous indications, but a large proportion of patients do not respond to anti-PD-(L)1 therapy, underscoring the need for alternative strategies. PD-L1 can be expressed by tumor cells as well as immune cells in the tumor microenvironment (TME) and tumor-draining lymph nodes. In particular, antigen-presenting cells (APCs) including macrophages and dendritic cells (DCs) can express high levels of PD-L1 and play a key role in regulating T cell responses through the PD-1/PD-L1 pathway. We are developing a novel class of molecules called Immune-Stimulating Antibody Conjugates (ISACs) that consist of tumor- or TME-targeting antibodies coupled to innate immune stimuli such as TLR7/8 agonists. Here, we present preclinical data demonstrating that PD-L1-targeted ISACs can engage both PD-L1-expressing tumor cells and APCs to drive robust immune responses and antitumor activity. Our results indicate a critical role for immune cell-expressed PD-L1 in antitumor activity and suggest a mechanism of action uncoupled from PD-1/PD-L1 blockade.

Methods: ISACs were prepared using proprietary anti-human PD-L1 antibodies and TLR7/8 agonist payloads. Anti-mouse PD-L1 ISACs were made using antibodies from Bioxcell. In vitro activity was assessed in human whole blood, tumor/DC cocultures, and purified PD-L1+ myeloid cell populations. In vivo efficacy studies were performed in syngeneic and xenograft tumor models. PD-L1 knockout tumor cells were generated using CRISPR/Cas9.

Results & Conclusions: PD-L1 ISACs induced robust, target-dependent activation of human myeloid cells cocultured with PD-L1-expressing tumor cells, as measured by secretion of cytokines such as IL-12 and TNFα. PD-L1 ISACs also strongly activated macrophages and DCs polarized to express high levels of PD-L1, demonstrating the ability to directly activate PD-L1-expressing APCs. Systemically administered PD-L1 ISACs were well tolerated in mice and showed improved efficacy over anti-PD-L1 antibodies, with tumor regression including complete responses observed in syngeneic and xenograft models. Mice with complete responses in syngeneic models rejected tumors upon rechallenge, indicating induction of immunological memory. Knockout of tumor PD-L1 expression in a syngeneic model had no impact on efficacy, suggesting that targeting of PD-L1-expressing host immune cells is critical for antitumor activity. PD-L1 ISACs exhibited similar efficacy whether or not the anti-PD-L1 antibody used blocked binding to PD-1, indicating that checkpoint blockade is not a major mechanism of action. These data demonstrate that PD-L1 ISACs represent a compelling new approach to treat cancer, leveraging mechanisms that are distinct from and potentially complementary to conventional PD-1/PD-L1 blockade.

#7334 Targeting Nectin-4 with a first-in-class triple MMAE/dual TOP1i payload ADC showing synergistic and durable activity across all target expression levels and favorable tolerability.

Isabella Attinger-Toller, Philipp Probst, Romain Bertrand, Rachael Fay, Lia Kallenberger, Patrick Maurhofer, Ramona Stark, Emma Renard, Roger Santimaria, Dragan Grabulovski, Bernd Schlereth, Philipp R. Spycher

Araris Biotech AG, Au, Switzerland

The Araris' site-specific and one-step linker conjugation technology aims at generating stable, safe and highly potent ADCs without the need for antibody engineering prior to payload conjugation. We here present a novel Nectin-4 targeting triple-warhead ADC using a combination of MMAE and two different topoisomerase-1 inhibitors (TOP1i, DAR2+2+2) designed to treat a broad range of Nectin-4 expressing solid tumors. With this novel ADC we aim to 1) effectively kill heterogenous and resistant tumors by delivering multiple cytotoxic payloads to the same tumor cell, 2) control and limit bystander activity to avoid excessive toxicities and 3) maximize payload delivery through stable linker-payload coupling resulting antibody-like ADC pharmacokinetic exposure profiles. The triple-warhead ADC was shown to be highly homogenous and pure with a DAR of 6, as desired. The ADC showed high stability under stressed conditions with no signs of aggregation, which we attribute to the hydrophilic nature of the peptide-linker-payload design. On target-positive cancer cell lines, the ADC showed high and target-specific cytotoxic activity. It further showed excellent stability in mouse, cyno and human sera with no linker-payload deconjugation or linker cleavage, and the DAR remaining unchanged. Most importantly, the ADC was extremely stable in circulation showing an exposure profile like the unmodified parent antibody in rodents. Notably, the tolerability in rats showed an HNSTD of more than 20 mg/kg in a dose-range-finder study (vs. Enfortumab vedotin (EV): 5mg/kg). No significant skin or hematological toxicities were observed, implying that the triple warhead did not result in toxicities exceeding the mono-payload toxicity profiles in rats. Strikingly, in a receptor high-expressing breast cancer SUM-190PT xenograft model, the triple warhead ADC administered as a single dose at 0.5 mg/kg on day 0 led to long-lasting and complete tumor regression (more than 100d), indicating synergistic activity of the different payloads. Interestingly, the combination using the respective MMAE (DAR2) and TOPi (DAR2+2) ADCs, each dosed at 0.5 mg/kg, showed no response whatsoever and neither did Enfortumab vedotin (EV) at this dose, the current Nectin-4 targeting standard of care in mUC. Further, in a low Nectin-4-expressing TNBC PDX model, the high anti-tumor activity was confirmed leading to high and complete anti-tumor responses at 2.5 mg/kg compared to EV and Sacituzumab govitecan, the FDA-approved ADC for TNBC. In conclusion, the first-in-class Nectin-4 targeting triple-payload ADC leads to very high anti-tumor efficacy in CDX and PDX models and is well tolerable in rats. We show for the first time, that combination of multiple payloads in one ADC can lead to synergistic effects in mouse models while still being well tolerable.

#7335 Comparison of ADCC-mediated efficacy of zolbetuximab in hCD16A-CB17SCID humanized mice and CB17SCID WT mice bearing the SNU-601 tumor model.

Rong Wang, Xiaoze Wang, Kaixia Lian, Rongfei Lu, Haijuan Yu, Jie Lin, Ludovic Bourre, Jingjing Wang

Crown Bioscience, Inc., San Diego, CA

Introduction: The mechanisms of action of cytotoxic monoclonal antibodies (mAbs) include FcγR-dependent mechanisms to induce antibody-dependent cell cytotoxicity (ADCC). In mice, ADCC relies on mFcγRIII expressed on NK cells, while human NK cells depend on hFcγRIIIA (hCD16A). However, human IgG1 display significant affinity differences between mouse and human FcγRs, which can impact their efficacy in preclinical models. Additionally, testing an affinity-enhanced Fc-engineered antibody in a mouse without the human FcγR counterparts provides limited predictive value. Therefore, preclinical mouse models expressing human FcγRs are essential to evaluate clinical Fc potent antibody. In this study, a hCD16A-CB17SCID humanized (HuGEMM) mouse model, featuring hCD16A knock-in on mouse NK cells, was used to evaluate the ADCC function of the CLDN18.2 mAb zolbetuximab. Using SNU-601 cell line, a high expression hCLDN18.2 model, the efficacy of zolbetuximab in hCD16A-CB17SCID mice was compared to CB17SCID WT mice in SNU-601 xenograft.

Methods: Surface expression of human CD16 on NK cells from peripheral blood (PB) and spleen of hCD16A-CB17SCID HuGEMM mice was verified by flow cytometry. *In vivo* studies involved 1×10^7 SNU-601 cells subcutaneously inoculated into hCD16A-CB17SCID HuGEMM models or CB17SCID WT models. Tumor-bearing models were treated with zolbetuximab (10 mg/kg, i.p., BIW×13 doses) or hIgG1 (10 mg/kg, i.p., BIW×13 doses) as an isotype control. At the study endpoint, NK cell function related markers in PB, tumor tissues and hCLDN18.2$^+$ tumor cell apoptosis from SNU-601 tumors were assessed by flow cytometry.

Results: hCD16A expression was confirmed on NK cells from PB and spleen in hCD16A-CB17SCID HuGEMM mice. SNU-601 tumor showed similar growth profile as was observed in hCD16A-CB17SCID HuGEMM and WT models. Significant tumor growth inhibition (TGI) of SNU-601 tumor was observedwith zolbetuximab compared to the isotype control, with hCD16A-CB17SCID HuGEMM models showing 55% TGI, higher than the 33% TGI observed in CB17SCID WT models. At the study endpoint, similar percentages of mNKp46$^+$ mCD49b$^+$ NK cells, mCD107a$^+$ mNKp46$^+$ mCD49b$^+$ NK cells, and mKi67$^+$ mNKp46$^+$ mCD49b$^+$ NK cells were observed between hCD16A-CB17SCID HuGEMM and CB17SCID WT models. Apoptosis analysis indicated that zolbetuximab induced apoptosis in hCLDN18.2$^+$ tumor cells compared to control groups in both hCD16A-CB17SCID HuGEMM and CB17SCID WT models.

Conclusion: This study indicates that hCD16A-CB17SCID HuGEMM models exhibit enhanced tumor growth inhibition in response to zolbetuximab treatment compared to CB17SCID WT models, demonstrating the potential of this model as a more effective preclinical model for evaluating ADCC-mediated therapies.

#7336 B7-H7 is a novel ADC target for solid tumors and shows potent activity with multiple payload-linker technologies.

Emilien Loeuillard, Bijan A. Etemad-Gilbertson, Susannah L. Hewitt, Jamie L. Strand, Riale T. Gilligan, Silvia Ferrati, Tatiana I. Novobrantseva, Matthew Rausch

NextPoint Therapeutics, Boston, MA

Introduction: B7-H7 is a member of the B7 family, with strong expression in multiple cancer indications in addition to its known role as an immune regulator. B7-H7 upregulation is correlated with poor patient outcomes. Furthermore, its normal tissue expression is limited making B7-H7 very attractive for targeting tumors. NextPoint has therefore explored B7-H7 as a novel antigen to target antibody drug conjugates (ADCs) to solid tumors. Antibodies previously raised against distinct B7-H7 epitopes, with cross-reactivity to the cynomolgus protein, were produced as ADCs and multiple distinct payload-linker technologies were applied. These were tested for *in vitro* and *in vivo* activity, and for their developability characteristics enabling lead selection of the most potent compounds.

Methods: ADC binding efficacy was assessed by flow cytometry on endogenous B7-H7+ and overexpressing B7-H7 cell lines. Internalization was tracked with Fabfluor-pH reporter conjugated to ADCs using Incucyte® imaging. *In vitro* cytotoxicity was evaluated by luminescence-based cell viability assays with CellTiter-Glo® to detect ATP in the media. The pharmacokinetics of each ADC was assessed in rats by measurement of both total and drug-conjugated antibody. The *in vivo* activity was tested against B7-H7-expressing xenograft tumors in immunodeficient mice.

Results: The majority of B7-H7-targeted ADCs had similar on-cell binding capacities as parental antibodies. Internalization analyses showed that after 4h, over 50% of cells had internalized the compounds with an optimal concentration of 0.3 nM ADCs. Multiple B7-H7 targeted ADCs demonstrated cytotoxicity against tumor cell lines, with an *in vitro* EC50 under 0.3 nM on average and under 0.1 nM for the most potent compounds. Rat pharmacokinetics demonstrated half-lives above 7 days for the lead compounds and acceptable payload stability across technologies. *In vivo*, ADC leads were tested in several *in vivo* tumor models that cover a wide range ofB7-H7 protein expression. Selected ADC leads with distinct linker-payload technologies demonstrated tumor activity in multiple *in vivo* tumor models. Body weight changes were minimal following dosing of the most active ADCs in these mouse systems.

Conclusions: Collectively, these preclinical studies establish B7-H7 as an attractive ADC targeting antigen and demonstrate its activity with multiple clinically relevant payload-linker technologies. These data support further development of lead compounds as a promising treatment for patients with B7-H7-expressing tumors.

#7338 Evaluation of a novel target Claudin1 in preclinical models of nasopharyngeal carcinoma.

K. Chin[1], C. Hui[1], C.-H. Wong[1], G. Chung[1], W. Lai[2], A. Toso[3], L. Manenti[3], L. Lili[1], C. Tsang[1], Q. Tao[1], E. Hui[1], A. Chan[1], K. Lo[1], B. Ma[1];
[1]The Chinese University of Hong Kong, Hong Kong, Hong Kong, [2]Prince of Wales Hospital, Hong Kong, Hong Kong, [3]Alentis Therapeutics AG, Allschwil, Switzerland

Introduction: Epstein-Barr virus (EBV)+ve nasopharyngeal carcinoma (NPC) is prevalent in Southern China where patients with recurrent/ metastatic disease can be treated with chemotherapy and/or immune-checkpoint inhibitors. Claudin 1 (CLDN1) is a member of the tight junction (TJ) family of proteins that is overexpressed in some cancers with their non-junctional (NJ) epitopes exposed at the epithelium. This study investigated CLDN1 as a novel therapeutic target in NPC and evaluated the preclinical activity of ALE.C04 - a first-in-class, highly specific monoclonal antibody targeting NJ-CLDN1, in NPC models.
Method: Expression of NJ-CLDN1 was assessed in three EBV+ve NPC cell lines (C666-1, NPC43 and C17C), four patient-derived- xenografts (PDXs) and NPC biopsies (n=44). The anti-proliferative effect of ALE.C04 was evaluated in NJ-CLDN1+ve NPC cell lines by clonogenic and invasion assay, co-cultured with or without human-derived natural killer (NK) cells. Results are validated in PDX models (C15, C17). ALE.C04 was administrated either as a single agent or in combination with chemotherapy or bevacizumab.
Results: NJ-CLDN1 were overexpressed in two NPC cell line (NPC43 and C17C), three PDXs (C15, C17 and X2117) and biopsies (80%). Interestingly, NJ-CLDN1 was not detected in PDX Xeno-666 and its derived cell line, C666-1. A non-significant correlation trend was found between h-score with overall survival. Treatment with ALE.C04 suppressed colony formation of NJ-CLDN1+ve cell lines, with more significant effect observed in C17C. Effect of ALE.C04 as a single agent is modest on the growth of NJ-CLDN1+ve positive cell lines and C17 PDX model. Whilst in combinatorial approach, ALE.C04 showed synergism with gemcitabine as tumor growth was suppressed in C17 PDX. In contrast, ALE.C04 monotherapy significantly suppressed growth in C15 PDX, resulting in substantial changes in gene expression profile (via RNA-Sequencing) compared to the control group. The results of the *in vitro* coculture assay and *in vivo* combination with gemcitabine and bevacizumab will be presented.
Conclusion: The results generated to date provide a proof-of-concept of targeting NJ-CLDN1 signaling in NPC using ALE.C04, especially in combination with chemotherapy or anti-vascular agent. ALE.C04 is now being evaluated in patients with NPC and other head and neck cancers in a phase 1 clinical trial (NCT06054477).

#7339 A highly efficacious next-generation CEACAM5 (CEA)-targeted Boltbody™ ISAC for the treatment of colorectal, pancreatic and lung tumors.

Rishali Gadkari, Andrew Luo, David T. Omstead, Jess L. Nolin, JingJing Li, Chi-Ling Fu, Cecelia I. Pearson, Cindy Kreder, Karla Henning, Matthew N. Zhou, Stefan Chun, Shahid Khan, Romas Kudirka, Han Kim, Robert Veneziale, Lu Xu, William G. Mallet, Shelley E. Ackerman, Michael N. Alonso, **Paul Ponath**

Bolt Biotherapeutics, Redwood City, CA

Background: CEACAM5 is a well-characterized cell-surface antigen highly expressed on multiple solid tumors, particularly colorectal, pancreatic, and non-small cell lung cancers. Despite its favorable properties as a cancer target, no therapies targeting CEA have been approved to date. We are developing a novel class of molecules, Immune-Stimulating Antibody Conjugates (ISACs) with next-generation TLR7/8 agonists to activate tumor-infiltrating myeloid cells and initiate a broad innate and adaptive anti-tumor immune response.[1]
Methods: A panel of anti-CEACAM5 antibodies was tested for binding affinity and specificity; CEACAM5-mediated antibody-dependent cellular phagocytosis (ADCP) by myeloid cells; and myeloid-mediated tumor cell killing. A subset of the antibodies was chemically conjugated to a proprietary next-generation TLR7/8 agonist and assessed in myeloid cell activation assays in vitro and tumor growth inhibition in vivo.
Results: Our lead CEA-targeted ISAC binds with high affinity and selectivity to CEACAM5+ cell lines and tumors. In vitro, ISAC binding to tumor cells drives efficient ADCP by myeloid cells and stimulates production of critical immune-activating cytokines including IL-12p70, IFNγ, and TNFα. The next-generation TLR7/8 agonist payload is active in mice where it inhibits the growth of CEACAM5+ xenograft tumors in immune-compromised mice. In CEACAM5 transgenic mice, treatment with the CEA-targeted ISAC results in complete tumor regression of CEACAM5+ syngeneic tumors including models with low CEACAM5 expression. Failure of CEACAM5+ cell lines and CEACAM5-parental cell lines to form tumors upon rechallenge demonstrates development of immunological memory and epitope spreading during initial treatment. In cynomolgus monkeys the CEA-targeted ISAC was well-tolerated with no significant drug-related adverse events up to 15 mg/kg, the highest dose tested.
Conclusions: This pre-clinical data set demonstrates the antigen-dependent induction of a robust innate and adaptive immune response by a next-generation CEA-targeted ISAC and the potential to treat CEACAM5+ human cancers.
References: [1] Ackerman S, Pearson C, Gregorio J. Immune-stimulating antibody conjugates elicit robust myeloid activation and durable antitumor immunity. Nature Cancer 2021; 2: 18-33. https://doi.org/10.1038/s43018-020-00136-x

POPULATION SCIENCES: Innovations in Prevention and Cancer Care: Addressing Disparities, Inflammation, Survivorship, and Modifiable Risk Factors
Poster Session

#7343 Factors associated with self-reported fatigue among long-term metastatic breast cancer survivors.

Chloe M. Hery, Juan Peng, Ashley Pariser Davenport, Michelle J. Naughton

The Ohio State University Wexner Medical Center, Columbus, OH

Purpose: Fatigue is one of the most often reported symptoms among breast cancer patients and affects patients' quality of life and emotional and physical functioning. This study aimed to examine the association between self-reported fatigue and sociodemographic, clinical, and psychosocial factors among metastatic breast cancer (MBC) survivors.
Methods: 232 patients from the Ohio State University Stefanie Spielman Comprehensive Breast Center, who had been diagnosed with MBC for ≥1 year, were asked to complete a one-time, online survey. The survey assessed the patients' quality of life, self-rated health, symptoms, supportive care needs, and reflections on their cancer diagnosis and treatment. Participants were asked to rate their level of fatigue in the past 7 days on a scale of 0 (no fatigue) to 10 (very severe fatigue). A multivariate generalized linear model (GLM) was used to determine the sociodemographic (age, race, education, income, rural/urban residency), psychosocial (self-rated health, quality of life, social support, sleep quality, anxiety), and clinical factors (time since diagnosis, treatment types, pain, symptoms) associated with patients' fatigue.
Results: Patients were on average 59.6 years old (±12.7) and 5.3 (±4.8) years from their diagnosis with MBC. Most were non-Hispanic and white (91%), approximately half had a college education (51.7%), and over a quarter resided in rural counties (28%). The results indicated that overall, 53% rated their quality of life as very good or excellent. The patients' average level of fatigue was 4.3 points (±2.4) out of 10 points possible. In multivariate models, self-rated quality of life, pain, and sleep quality were significantly associated with fatigue after controlling for age, time since diagnosis, self-rated health, education level, rural/urban residency, and treatment type (oral therapy only vs. IV therapy). Higher quality of life (β=-0.35, p=0.0003) and better sleep quality (β=-0.14, p=0.02) were associated with lower levels of fatigue. In contrast, higher levels of pain were associated with greater fatigue (β=0.37, p<0.0001).
Conclusions: Understanding and treating the long-term symptoms and unique experiences of MBC survivors is important in improving their quality of life and long-term survival. Fatigue does not necessarily resolve after treatments have concluded and MBC patients are often on treatment for long periods of time. Addressing methods to improve patients' sleep quality and pain levels may improve their fatigue and quality of life and alleviate the symptom burden experienced by these patients.

#7344 Family history of hematologic malignancy and prognosis across lymphoma subtypes: Novel association with poor outcomes in follicular lymphoma.

George Cholack[1], Raphael Mwangi[2], Thomas M. Habermann[3], Verena Hinder[2], Rosalie Griffen[2], Dennis Robinson[2], Andrew L. Feldman[4], Thomas E. Witzig[3], Stephen M. Ansell[3], Brian K. Link[5], Yucai Wang[3], Carrie A. Thompson[3], Susan L. Slager[2], Matthew Maurer[2], Anne J. Novak[2], James R. Cerhan[2]

[1]Internal Medicine, Mayo Clinic School of Graduate Medical Education, Rochester, MN,[2]Department of Quantitative Health Sciences, Mayo Clinic, Rochester, MN,[3]Department of Medicine, Division of Hematology, Mayo Clinic, Rochester, MN,[4]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN,[5]Department of Medicine, University of Iowa Hospitals and Clinics, Iowa City, IA

Family history of hematologic malignancy (FHHM) is an established risk factor for developing lymphoma, but studies examining associations between self-reported FHHM and lymphoma prognosis during the recent treatment era are limited. Utilizing a prospective cohort study, we evaluated associations between FHHM and prognosis for common lymphoma subtypes, including diffuse large B-cell (DLBCL), follicular (FL), marginal zone (MZL), mantle cell (MCL), Hodgkin (HL), and T-cell (TCL) lymphomas. We used the Mayo Clinic component of the Molecular Epidemiology Resource, a prospective cohort study of newly diagnosed lymphoma patients enrolled from 2002 to 2015. Self-reported FHHM (HL, NHL, leukemia, or multiple myeloma) in a first-degree relative was obtained via questionnaire. Primary outcomes were overall survival (OS) and event-free survival (EFS; time from diagnosis to disease progression, initiation of second-line therapy, or all-cause death). Secondary outcomes were failure to achieve 24 months of event-free survival (EFS24; associated with aggressive disease and poor outcomes) and lymphoma-specific survival (LSS; time from diagnosis to death from lymphoma or lymphoma treatment-related complications). We used multivariable Cox proportional hazards regression to estimate hazard ratios (HRs) and 95% confidence intervals (CI) for the association of FHHM with OS, EFS, and LSS, and logistic regression to estimate odds ratios (ORs) and 95% CI for the association with failure to achieve EFS24. All models were adjusted for sex and a lymphoma subtype specific prognostic index. The cohort consisted of 650 DLBCL, 679 FL, 291 MZL, 178 MCL, 254 HL, and 196 TCL patients. The prevalence of FHHM ranged from 9.7% in TCL to 14% in FL. FHHM was not associated with OS in any subtype, but MCL patients with FHHM showed an inferior EFS (HR=1.70, 95% CI 1.02-2.85; p=0.04). For EFS24, FHHM was associated with failure to achieve EFS24 only in FL (OR=1.57, 95% CI 0.99-2.48; p=0.05). Similarly, FHHM was associated with inferior LSS in FL patients (HR=1.98, 95% CI 1.11-3.53; p=0.02). In a treatment subgroup analysis of FL patients, the FHHM associations with failure to achieve EFS24 (OR=2.48, 95% CI 1.04-5.74; p=0.04) and inferior LSS (HR=2.68, 95% CI 1.09-6.58; p=0.03) strengthened among those treated with frontline immunochemotherapy (IC). In summary, there were no evidence of associations between FHHM and OS across lymphoma subtypes, but MCL patients with FHHM displayed inferior EFS. FL patients with FHHM were more likely to not achieve EFS24 and to ultimately die of their lymphoma or a lymphoma treatment-related complication. This finding was stronger in more aggressive FL requiring frontline IC. This novel finding would suggest that genetic or environmental factors associated with FHHM may underlie pathways that impact disease aggressiveness and early clinical failure in FL.

#7345 Differences in complete screening adherence across age, income, and education groups.

Kierstin M. Faw, Jamal R. Winn, Robert A. Smith, Ahmedin Jemal, Parichoy Pal Choudhury, Priti Bandi, Jordan A. Baeker Bispo, Alpa V. Patel

American Cancer Society, Atlanta, GA

There is limited knowledge of the sociodemographic characteristics of individuals who are fully adherent to all recommended cancer screening tests. Understanding these cancer screening gaps may provide insights into subpopulations who may benefit from interventions to increase overall early cancer detection. The Cancer Prevention Study- 3 is a prospective cohort study established by the American Cancer Society (enrollment 2006-2013). Between enrollment and 2018, cancer-free participants reported cancer screening tests (mammography, colonoscopy, sigmoidoscopy, stool testing, and low dose lung computed tomography (CT) scans) and year(s) of each screening. Eligibility for each test was determined using 2018 American Cancer Society guidelines. For each person, an adherence score was calculated based on the number of eligible screenings and whether they were adherent. Patterns of adherence were compared across age, education, and income groups using an ANOVA to determine statistical significance. 129,927 individuals were included in this analysis. Men had a higher proportion of no screening adherence (17%), and complete adherence (81%) compared to women, 8% and 67%, respectively. For those 45-55 years old (N=37,393), approximately 46% were not completely adherent, compared to only 24% of those > 55 years old. Likewise, study participants who had an annual income <$50,000 or a high school degree or less, had a higher proportion of individuals with partial or no adherence versus those who had an annual income ≥$100,000 or a college degree or higher.

	No Adherence N (%)	**Partial Adherence N (%)**	**Complete Adherence N (%)**	**p-value**
Age				
45- 55 years old	6,977 (18.66)	10,044 (26.86)	20,372 (54.48)	
>55 years old	5,282 (5.71)	17,231 (18.62)	70,021 (75.67)	<0.0001
Income				
<$50,000	2,354 (11.73)	5,290 (26.35)	12,429 (61.92)	
$50,000-100,000	3,809 (8.74)	9,480 (21.76)	30,271 (69.49)	
≥$100,000	5,783 (9.12)	11,884 (18.74)	45,733 (72.13)	<0.0001
Education				
≤High School Graduate	1,168 (11.38)	2,611 (25.44)	6,485 (63.18)	
Some or technical college	3,522 (9.91)	8,573 (24.11)	23,457 (65.98)	
≥ College Graduation	7,079 (8.97)	15,014 (19.03)	56,804 (72.00)	<0.0001

Given the significant differences in adherence by age, income level, and education, consideration of bundled screening (getting multiple screenings during a single visit), especially for some populations, may provide an opportunity to increase complete screening adherence, potentially reducing the number of cancers diagnosed at an advanced stage and increasing survival.

#7346 Disparities in sarcoma care prior to initiation of treatment among patients with newly diagnosed sarcoma.

Lauren Li, Nam Bui, Kristen Ganjoo, Minggui Pan

Division of Oncology, Stanford University School of Medicine, Saratoga, CA

Purpose: Disparities in the outcomes for patients with sarcoma have been reported, however, few studies have investigated the disparities in sarcoma care and the related etiologies. We investigated the care disparities and associated factors along the segments of the adult sarcoma care journey from the onset of symptoms to the establishment of diagnosis and sarcoma consult prior to the initiation of treatment.

Patients and Methods: This cohort included patients with newly diagnosed soft tissue or bone sarcoma (including benign musculoskeletal neoplasms) who were referred to Stanford Medicine for establishing sarcoma care, from July 2020 to April 2024. We investigated the interval from the onset of symptoms (pain and/or palpable mass) to the first appointment with a primary care provider (PCP wait-time), the interval from first PCP appointment to obtaining the first imaging study for workup such as MRI, PET/CT or a Ct scan (imaging wait-time), the interval from the initial PCP appointment to obtaining the tissue biopsy for establishing the diagnosis (biopsy wait-time), and the interval from the referral date for sarcoma consult to obtaining the sarcoma consult (sarcoma consult wait-time. We also performed a survey among the physicians and non-physician staff within the Stanford sarcoma program to understand the perception of acceptability of wait-time more than 5 weeks.

Results: Among 402 eligible patients, approximately one third had PCP wait-time longer than 5 weeks, young adults and Hispanic patients had the highest rate of such long wait-time. Approximately 20% of patients had imaging wait-time longer than 5 weeks, young adults had the highest rate of such long wait-time (30%). There were approximately 5% of patients with sarcoma consult wait-time longer than 5 weeks. However, nearly 80% of patients had biopsy wait-time longer than 5 weeks, African American and Hispanic patients had the highest rate of such long wait-time. In addition, compared to public insurance, there were more patients with private insurance that were associated with PCP wait-time, imaging wait-time, sarcoma consult wait-time and biopsy wait-time longer than 5 weeks. Our survey among the physicians and non-physician staff within the Stanford sarcoma program showed that wait-time longer than 5 weeks was considered unacceptable by a vast majority of responders.

Conclusion: Substantial disparities in sarcoma care related to age group, ethnicity and insurance type appear to exist in the multiple segments of sarcoma care journey prior to the initiation of treatment. Biopsy wait-time appears to be the segment of sarcoma care with the highest rate of long wait-time and significant ethnicity disparities.

#7347 Disparities in breast cancer outcomes among South Asian women: A comparative analysis with White patients.

Jasmin Hundal, Yanwen Chen, Abhay Singh

The Cleveland Clinic, Taussig Cancer Institute, Cleveland, OH

Background: South Asians (SA) are among the fastest-growing racial and ethnic immigrant populations in the United States. Despite this, limited research exists on their breast cancer (BC) outcomes compared to other populations. This study examines disparities between SA and White breast cancer patients.
Methods: Data from female BC patients aged 18 years or older, diagnosed between 2004 and 2021 were obtained from National Cancer Database. For “RACE”, code “15”, “16”, “17” [“Asian Indian or Pakistani, NOS (formerly code 09)”, “Asian Indian”, “Pakistani”] were considered as the “South Asian”; the code “01” as “White”. Cox proportional hazards models were used to estimate the effect of race/ethnicity on OS unadjusted, or adjusted for age group, insurance status, Charlson-Deyo comorbidity score, socioeconomic indicators (percent without a high school degree, and median income quartiles), facility type, urban or rural residence, treatment types (chemotherapy, radiation, surgery, hormone therapy), and year of diagnosis. All analyses were done via SAS/STAT 15.3, or R 4.2.2.
Results: SA were significantly younger at diagnosis, with 37.5% under the age of 50 compared to 21.5% of White (p-value<0.001). A larger proportion of SA were privately insured (62.9%) compared to White (53.7%), with fewer relying on Medicare (17.3% vs. 36.9%) (p-value<0.001). SA were also more likely to reside in areas with higher median income and education levels. Regarding clinical presentation, SA had higher advanced-stage diagnoses (12.8% Stage III-IV) compared to White (11.6%) (p-value<0.001). Tumor subtype distributions revealed that 45% of South Asians had HR+/HER2- cancers compared to 43.3% of Whites, and the prevalence of Triple-Negative breast cancer was slightly higher in SA (7.9%) than in White (6.0%) (p-value<0.001). Treatment patterns showed nearly identical rates of 63.1% SA and 63.2% of Whites received hormone therapy (p-value=0.741), with slightly different surgery rates (93.0% vs. 93.7%) (p-value<0.001). However, SA was less likely to receive chemotherapy (24.7% vs. 17.4%) (p-value<0.001) but had comparable radiation therapy rates (57.1% vs. 57.8%) (p-value=0.055). Survival analyses indicated better outcomes for SA in HR+/HER2- cancers across all stages (e.g., Stage I: HR 0.560, p<0.001) and in HR-/HER2+ cancers at advanced stages (e.g., Stage IV: HR 0.528, p=0.002). Mortality greater than 90 days after surgery was lower for South Asians (81.9%) compared to White (86.1%) (p-value<0.001).
Conclusion: SA exhibit several distinctive characteristics compared to White BC patients, including a younger age at diagnosis, higher socioeconomic status, and better survival outcomes. These findings underscore potential differences in tumor biology, healthcare access, and treatment patterns. Future research should explore systemic, genetic, and social determinants underlying these distinctions.

#7348 Long-term outcomes of pediatric craniopharyngiomas: multi-center comparative study.

Vitor Nagai Yamaki[1], Jai Sidpra[2], Bruno Santana Peres[3], Catuto Domingos Alexandre Quianga[1], Guilherme Jose Costa Borsatto[1], Joao Paulo Mota Telles[1], Ines Nobrega Silva[4], Asthik Biswas[4], Kshitij Mankad[4], Hani Marcus[5], Noor ul Owase Jeelani[6], Sueli Mieko Oba-Shinjo[1], Hamilton Matushita[1], Suely Kazue Nagahashi Marie[1], Kristian Aquilina[6]

[1]Neurology, University of Sao Paulo, Sao Paulo, Brazil,[2]Development Biology and Cancer section, University College of London, London, United Kingdom,[3]Radiology, University of Sao Paulo, Sao Paulo, Brazil,[4]Neuroradiology, National Hospital for Neurology and Neurosurgery, London, United Kingdom,[5]Neurosurgery, National Hospital for Neurology and Neurosurgery, London, United Kingdom,[6]Neurosurgery, Great Ormond Street Hospital, London, United Kingdom

Background: Craniopharyngioma (CP) account for 80% of childhood hypothalamo-pituitary tumors and, though benign, their proximity to the visual pathways, hypothalamus, and pituitary incurs significant long-term neurologic and endocrine morbidity. Informed surgical decision-making has the potential to reduce morbidity and improve outcomes. There remains a high degree of variability in how these lesions continue to be managed.
Methods: This multicenter retrospective observational study aimed to compare long-term outcomes of CP at two pediatric neurosurgery units (GOSH, UK, and USP, Brazil), delineating management paradigms associated with better prognosis. CP treated over a 20-year period were identified and standardized clinical outcomes recorded for comparative analysis.
Results**:**123 patients were identified (USP=52; GOSH=71). No demographic differences were identified between series (mean age: USP=9.2±4.6; GOSH=8.2±4.1 years). Headaches (USP=75%; GOSH=62%) and visual deficits (USP=46%; GOSH=38%) were the most common presenting symptoms. Growth hormone deficiency at presentation was more common in the USP cohort (USP=62.5%; GOSH=35%; p=0.009). There was significantly more hypothalamic invasion in the GOSH cohort (Paris grade 2: GOSH=67.0%; USP=37.5%; p=0.026), but significantly larger cystic volumes in the USP cohort (USP=27.5±37cm^3; GOSH=10.5±8.9cm^3; p=0.004). These differences led to different surgical approaches, with more subtotal resections at USP (78%) and more cyst aspirations at GOSH (62%). 80% of patients at GOSH received RT compared to 32% at USP. Mean follow-up at GOSH was 7.5±4.8 years compared to 10.1±5.6 years at USP. Cognitive outcome did not vary. GOSH cohort presented higher rates of levothyroxine replacement (USP=67%; GOSH=86%; p=0.017). GOSH exhibited better hypothalamic outcomes (p=0.08) and better visual outcomes (field deficits: GOSH=44%; USP=66%; p=0.01). In multivariate analysis (n=123), subtotal/gross-total resection (OR=0.17; 95%CI:0.05-0.55, p<0.01) and older age at diagnosis (OR=0.91, 95%CI:0.82-0.99, p=0.04) were associated with longer progression-free survival.
Conclusions: Comparative analysis of two large craniopharyngioma series identified different paradigms of surgical management driven by clinical-radiological presentation with similar long-term outcomes. Subtotal/gross-total resection was not related to worse clinical outcome or better progression-free survival.

#7349 Impact of HLA loss on NK cell infiltration and resistance to immunotherapy.

Olamide Adefioye, Youngsoo Han, Lucas Yeung, Aidan Shen, Jesse Wang, Zhaohui Wang, Cheng-chi Chao, **Chongming Jiang**

Terasaki Institute for Biomedical Innovation, Los Angeles, CA

Background: Clinical resistance to immunotherapy remains a significant challenge in cancer treatment, with many patients requiring combination therapies to achieve optimal therapeutic outcomes. Tumor cells often evade immune detection by down-regulating Major Histocompatibility Complex (MHC)-I or Human Leukocyte Antigen (HLA) expression, effectively escaping T-cell-mediated immunity. However, this loss may enhance tumors susceptible to Natural Killer (NK) cells, which are capable of recognizing and targeting cells with diminished HLA expression. Elucidating the interplay between HLA heterogeneity, NK cell infiltration, and the tumor immune microenvironment (TIME) is critical for addressing resistance and improving immunotherapy efficacy. This study investigates the HLA loss and its impact on tumor-infiltrating NK cells in the TIME.

Methods: Utilizing single-cell RNA-seq data from human pan-cancer datasets, we crafted 18 distinct pan-cancer NK signatures reflective of NK presence. Pan-cancer datasets, including lung squamous cell carcinoma (LUSC) and lung adenocarcinoma (LUAD), were analyzed using gene set enrichment analysis (GSEA), correlation heatmaps, and survival analyses to evaluate NK cell activity, HLA expression, and immune microenvironment characteristics. The T and NK cell ratio was explored as a potential biomarker of HLA heterogeneity. Linear Discriminant Analysis (LDA) was also employed to evaluate the separation of high and low NK activity groups, though these results were inconclusive.

Results: HLA loss patients exhibited increased NK cell infiltration, likely driven by diminished inhibitory signaling in the absence of HLA molecules. Significant correlations between the T and NK cell ratio and HLA heterogeneity were observed, suggesting its potential as a biomarker for immune profiling. High NK activity was associated with improved survival outcomes, underscoring the protective role of NK cells in the TIME. Hot tumors showed higher NK cell infiltration and lower HLA heterogeneity compared to cold tumors, highlighting distinct patterns of immune cell recruitment.

Conclusion: Tumor-infiltrating NK cells represent a promising target for overcoming therapy resistance associated with HLA loss. Although the LDA results were inconclusive, the observed associations between NK signatures, HLA heterogeneity, and the T and NK cell ratio lay a solid foundation for further investigation. These findings suggest that NK cells and the T and NK ratio could serve as both biomarkers and therapeutic targets in immunotherapy-resistant cancers. Future studies should validate these findings across independent datasets and explore NK-targeted combination therapies.

#7350 Association between radioactive iodine treatment for hyperthyroidism and incident cancer.

Harry D. Momo[1], Megan R. Haymart[2], Xu Shi[3], Alison M. Mondul[1]

[1]Department of Epidemiology, University of Michigan, Ann Arbor, MI,[2]Division of Metabolism, Endocrinology, and Diabetes, University of Michigan, Ann Arbor, MI,[3]Department of Biostatistics, University of Michigan, Ann Arbor, MI

Background: Radioactive iodine (RAI) has been one of the main treatments for hyperthyroidism since the 1940's. However, its long-term safety, particularly regarding cancer risk, remains unclear and controversial. This study aimed to evaluate the association between RAI treatment for hyperthyroidism and overall cancer risk as well as risk of site-specific cancers.
Methods: We conducted a retrospective cohort study using the University of Michigan Precision Health database, which integrates clinical and administrative data from multiple sources, including records from electronic health records, pharmacy, cancer registries, and death registries. Our analytic sample included Michigan Medicine primary care patients aged 18 and older newly diagnosed with hyperthyroidism between 2006 and 2019 with no previous cancer diagnosis. Information on types of treatment for hyperthyroidism (RAI, anti-thyroid medications or thyroidectomy) was abstracted from electronic health records. Participants were followed until cancer diagnosis, death, or end of study (December 2022). Data for key covariates including age, sex, race, comorbidities, BMI, smoking and alcohol history were extracted. Associations between hyperthyroidism treatment and risk of overall and site-specific cancers with at least 20 cases (Breast, Melanoma, Cervix, Prostate, Brain/CNS, Bladder and Colon) were estimated using Cox proportional hazards models. Treatment type was modeled as a time-dependent variable (RAI vs. other treatment) and the analysis included both age-adjusted (age adj) models and multivariable (mv) models which adjusted for age, sex, BMI, comorbidities and severity of hyperthyroidism. MV models for breast, cervix, and prostate cancer did not include sex as these models were restricted to women or men, as appropriate.
Results: A total of 6435 participants were included in the analysis, contributing 52,782 total person-years of follow-up. A total 487 cases of cancer were diagnosed. Compared to patients treated with either medication or thyroidectomy, RAI treatment was not significantly associated with an increased risk of overall cancer (age adj-HR = 0.93 [95% CI: 0.77-1.13], p=0.46; mv-HR= 1.02 [95% CI: 0.73-1.41], p=0.93). In site-specific cancer analyses, RAI treatment was associated with a significant increase in risk of breast cancer in the age adj-HR = 1.70 [95% CI: 1.16-2.40], p=0.04 and remained significant in the multivariable model HR = 2.24 [1.21-4.17], p=0.03). No significant associations were identified between RAI treatment and other site-specific cancers.
Conclusions: In this cohort study, RAI treatment for hyperthyroidism was associated with a significantly increased risk of breast cancer but showed no consistent association with overall cancer risk.

#7351 Financial toxicity and satisfaction with cancer treatment among patients with multiple myeloma: An analysis of the MMRF's CureCloud initiative.

M. A. Fiala[1], E. Lepisto[2], C. Amadi-Mgbenka[2], J. Schulman[2], G. Mulligan[2], H. Cho[2];
[1]Washington University School of Medicine in St. Louis, St. Louis, MO, [2]Multiple Myeloma Research Foundation, Norwalk, CT

Background: Multiple myeloma (MM) is one of the most expensive cancers to treat and many patients with MM experience financial toxicity. However, there are limited studies of financial toxicity and its sequalae in this population. In this study, we aimed to explore financial toxicity in a national cohort of patients with MM. Moreover, we aimed to determine if financial toxicity was associated with satisfaction with one's cancer treatment.
Patients and Methods: This study analyzed data from the Multiple Myeloma Research Foundation (MMRF) CureCloud initiative (NCT03657251). CureCloud is a longitudinal study which collected biospecimens, clinical data, and patient reported outcomes (PROs), the COmprehensive Score for Financial Toxicity (COST) and the Cancer Therapy Satisfaction Questionnaire (CTSQ).
Results: In total, 214 participants completed the PROs. The mean age was 68, 60% were male, and 94% were white. The median time from MM diagnosis to the baseline survey completion was 6 months. The mean COST score was 29 (SD=10) and 37% had a COST score less than 26 and were considered to be experiencing financial toxicity. Younger patients were more likely to be experiencing financial toxicity than older patients. For each 10 years decrease in age, the odds of financial toxicity increased by 52% (aOR 1.52; 95% CI 1.20-1.87; p<0.01). Patients who resided in less affluent neighborhoods were also more likely to be experiencing financial toxicity. For each $10,000 decrease in median household income of one's home census tract, the odds of financial toxicity increased by 87% (aOR 1.87; 95% CI 1.20-2.49; p=0.01). Participants experiencing financial toxicity had a mean CTSQ score 6.7 points lower than those without (b = -6.7; 95% CI [-2.7]-[-10.6]; p < 0.01). A subset of patients (n = 75) completed a second COST survey approximately one year later. Financial toxicity status was relatively stable; the status of 81% of patients was the same at both time points. In patients experiencing financial toxicity at baseline, 67% (18/27) were still experiencing it at the later time point. An additional 10% (5/48) of patients not experiencing financial toxicity at the initial survey were at the later time point.
Conclusions: Over one-third of patients with MM experienced financial toxicity and financial toxicity was associated with reduced satisfaction with MM treatment. Younger patients and those who live in less affluent neighborhoods were more likely to experience financial toxicity. These segments of the MM population can be targeted for interventions aimed to reduce financial toxicity.

#7352 Gemcitabine-docetaxol-cisplatin and everolimus in advanced soft tissue sarcomas: Clinical outcomes and prognostic biomarkers.

Wen-Chi Wu, Chueh-Chuan Yen

Taipei Veterans General Hospital, Taipei, Taiwan

Background: Soft tissue sarcomas (STSs) are rare mesenchymal tumors with poor prognosis. While anthracycline-based regimens remain first-line treatment, subsequent options are limited. This study evaluated a novel combination regimen of gemcitabine, docetaxel, cisplatin with everolimus (G-GTC/E), which combines conventional cytotoxic agents with targeted therapy.
Methods: A retrospective analysis was conducted between June 2009 and September 2024 on STS patients treated with G-GTC/E at our institution. The regimen was administered every three weeks: Day 1 - gemcitabine 800 mg/m2 and everolimus 10 mg; Day 8 - gemcitabine 60 mg/m2, cisplatin 60 mg/m2, docetaxel 60 mg/m2, and everolimus 10 mg; Day 15 - everolimus 10 mg. We analyzed baseline characteristics, inflammatory markers, and treatment outcomes. Statistical analyses included Kaplan-Meier survival estimates and Cox proportional hazards models for prognostic factors.
Results: Among 74 enrolled patients with advanced STSs, the ORR was 46% (CR 2.7%, PR 43.2%) with a DCR of 68.9%. The mOS was 16.2 months (95% CI: 10.9-21.4) and mPFS was 5.9 months (95% CI: 3.3-8.5). Histological subtypes included LMS (32.4%), UPS (18.9%), angiosarcoma (6.8%), synovial sarcoma (6.8%), LPS (5.4%), MPNST (5.4%), and others (24.3%). Survival varied significantly across subtypes, with synovial sarcoma showing the most favorable mOS at 29.8 months (95% CI: 0-76.3), followed by LMS at 21.7 months (95% CI: 9.6-33.9), while UPS showed the poorest survival of 8.6 months (95% CI: 0-20.8). Primary sites included extremities (40.5%), abdominal/thoracic visceral organs (29.7%), retroperitoneum (13.5%), trunk (10.8%), and head and neck (5.4%). Head/neck sarcomas had the longest mOS at 27.4 months (95% CI: 12.2-42.6), followed by retroperitoneal (24.8 months; 95% CI: 16.5-33.0) and visceral sarcomas (23.6 months; 95% CI: 14.0-33.3). For prognostic factors, elevated CRP, neutrophil-lymphocyte-ratio, platelet-lymphocyte-ratio, and systemic inflammatory index predicted poor OS. Monocyte-lymphocyte-ratio showed similar trend without significance (p=0.119). All inflammatory markers suggested inferior PFS without statistical significance. Low albumin levels predicted poor outcomes in both OS and PFS. Grade 4 hematological toxicities included neutropeniq (40%), thrombocytopenia (35%), and anemia (10%). Grade 3/4 diarrhea and skin rashes occurred in 16.2% and 25% of patients, respectively.
Conclusion: G-GTC/E demonstrated promising efficacy as a second-line treatment for advanced STS. Careful monitoring for thrombocytopenia, diarrhea and skin reactions is essential. Elevated inflammatory markers and low albumin levels predicted poor outcomes, suggesting their potential utility in patient stratification for G-GTC/E therapy in advanced STS.

#7353 Evaluating the LinC CHW program's effect on emergency department visits and health outcomes in cancer patients.

Tishiya Carey, Alexis Roach, Elizabeth Hansen

University of Chicago, Chicago, IL

This study examines the impact of the Liaisons in Care (LinC) Community Health Worker (CHW) program on emergency department (ED) utilization and survival outcomes among cancer patients at the University of Chicago Medicine. Designed to reduce ED visits by enhancing patient engagement, the LinC program provides targeted support through follow-ups, resource provision, and coordinated care. Our cohort included 72 cancer patients enrolled in LinC from January 2022 to December 2023, each with at least one recorded home visit or extensive engagement through other encounters. Key engagement metrics indicated that 83% of all encounters were CHW-initiated, with 77% conducted virtually. ED utilization decreased, with the average number of visits per patient dropping from 1.68 visits before program enrollment to 0.81 visits post-enrollment. Patients experienced an average interval of 102 days between ED visits and a 30-day readmission rate of 35.3%. The median survival time for the cohort was 557 days, with a one-year survival probability of 66.7%. Kaplan-Meier analysis revealed a significant difference in survival outcomes based on diagnosis timing: patients diagnosed within six months of enrollment had a lower median survival time (284 days) compared to those diagnosed before six months of enrollment (835.5 days; $p = 0.005$).These findings highlight the potential of CHW programs to improve healthcare outcomes for high-risk cancer populations by enabling timely interventions and providing access to resources. The results support the expansion of similar CHW-led initiatives to reduce health disparities and enhance the quality of life for cancer patients. Future analyses will incorporate social determinants of health (SDOH) to further refine engagement strategies and optimize CHW allocation across service lines.

#7354 Patient reported outcome validation within the Voyage Study cohort.

E. J. Kirsch, J. A. Grimm, S. M. Stoddard, W. Z. Fan, K. A. Bohn, K. J. Yost, L. J. Finney Rutten, J. E. Olson;
Mayo Clinic, Rochester, MN

Background: Epidemiologic studies commonly obtain important exposure and outcome information from patient self-report although these may be influenced by imperfect understanding and/or faulty recall. The Voyage Study is a large-scale, prospective cohort study of long-term health outcomes among participants with a valid multi-target stool DNA (mt-sDNA) test order (N=150,047). The purpose of this analysis was to evaluate the validity of participant reported mt-sDNA tests, colorectal cancer (CRC), and/or aerodigestive cancers (ADC, including cancers of the head, neck, oral, esophageal, lung, liver, stomach, pancreas, anus) among respondents.

Methods: Participants from across the Unites States enrolled in the study completing questionnaires at enrollment and 1 year. The year 1 questionnaire collected data on completion and outcome of their mt-sDNA test, follow up colonoscopies and other procedures as well as a diagnosis of CRC or ADC. The study team contacts those who reported either CRC or ADC for permission to obtain copies of medical records. Records are then reviewed by a trained nurse abstractor and evaluated for validity. Mt-sDNA test results were available from Exact Sciences.

Results: At the time of this analysis, year 1 questionnaire data were available on 135,035 participants. Abnormal mt-sDNA results (n=12,928) were self-reported by 13,420 participants with 96.3% accuracy. Of the 111,556 self-reported normal results, 93.4% (n=104,150) were reported correctly. The validation process for the Voyage Study is ongoing with 860 self-reported CRC and 318 self-reported aerodigestive cancer diagnoses to date. Complete data are available on 155 individuals with a self-report of a new CRC diagnosis and 63 individuals with a self-report of aerodigestive cancer. Abstraction confirmed 47% (n=73) of those reported as true CRC diagnoses. Participants were better at correctly reporting at least one aerodigestive cancer (73%, n=46).

Discussion and Conclusions: Patient reported medical record validation poses a unique challenge in today's world. The process of requesting participant authorization followed by institutional requests to release the medical records to the research team is slow and painstaking. We found that patients are able to correctly report their mt-sDNA tests but found a relatively low proportion of reported cancer diagnoses reported correctly. This underscores the need to continue to validate key outcomes rather than relying solely on participant responses. As the number of outcomes validated increases, the study team will look at a variety of social and economic factors to better understand what contributes to or hinders an accurate self-report.

#7355 Understanding health belief profiles and their role in medication adherence and quality of life among cancer survivors: insights from a multimethod study.

Meng-Jung Wen[1], Olayinka O. Shiyanbola[2]

[1]Health Services Research in Pharmacy, University of Wisconsin-Madison, Madison, WI,[2]Clinical Pharmacy, University of Michigan, Ann Arbor, MI

Background: More than half of cancer survivors don't engage in taking oral anticancer medications (OAMs) due to negative perceptions, leading to relapse, hospitalizations, and higher medical costs. This multimethod study explored cancer survivors' health belief schemas to understand their decision-making processes around OAM adherence and its impact on quality of life.
Methods: This cross-sectional study surveyed 323 cancer survivors nationally to assess beliefs about cancer, OAM adherence, and health-related quality of life (HRQoL), using latent profile analysis to identify subgroups based on health beliefs. Hierarchical regression examined subgroup associations with adherence and HRQoL, while qualitative content analysis of eight interviews provided deeper insights into participants' cancer experiences.
Results: Three health belief profiles were identified: **(1)** optimistic and adaptive (n = 43, 13%), **(2)** conscious but hesitant (n = 99, 31%), and **(3)** overwhelmed and unaware (n = 181, 56%), with significant differences in belief patterns. Regression analysis showed that health belief profiles explained 49.1% of the variance in medication adherence and 28.6% in HRQoL, adjusted for clinical and demographic factors. Cancer survivors in Profile 3 had the lowest adherence (mean = 20.98, SD = 9.0, P<.001) and HRQoL (mean = 14.45, SD = 7.8, P<.001), with *Risk of Recurrence* (β = -.15, p = .01), *Cancer Consequences* (β = -.17, p = .01), and *Personal Control* (β = -.12, p = .05) as significant strong predictors of nonadherence. Qualitatively, participants reported key consequences of cancer including fatigue and medication side effects, social and structural barriers such as altered dynamics with friends and family, social isolation, and financial strain caused by unaffordable healthcare costs and reduced work capacity. Those who perceived a higher risk of recurrence showed greater tolerance for uncomfortable side effects and adhered more consistently to OAMs. Additionally, compared to the overwhelmed and unaware group, participants who maintained optimism and adaptability in managing their cancer, embraced positive thinking and actively engaged in self-management practices, such as maintaining a healthy diet, exercising, and setting alarms for medication taking.
Conclusion: This study identified distinct health belief profiles among cancer survivors, highlighting their impact on medication adherence and HRQoL. Survivors with overwhelmed and unaware beliefs exhibited the lowest adherence and HRQoL, emphasizing the importance of addressing negative perceptions and enhancing personal control to improve outcomes. Tailored interventions targeting specific belief profiles, particularly those at higher risk of nonadherence, may foster better self-management and adherence, ultimately improving quality of life for cancer survivors.

#7356 Feasibility, acceptability, and preliminary efficacy of acceptance and commitment therapy in women living with metastatic breast cancer.

Ashley Hatch[1], Joanna B. Torzewski[2], Jessica L. Thomas[3], Heidy N. Medina[1], Fiona S. Horner[4], William J. Gradishar[5], David Victorson[3], Frank J. Penedo[6], Michael H. Antoni[6], Patricia I. Moreno[1]

[1]Department of Public Health Sciences, University of Miami Miller School of Medicine, Miami, FL,[2]Department of Psychiatry and Behavioral Sciences, Northwestern University Feinberg School of Medicine, Evanston, IL,[3]Department of Medical Social Sciences, Northwestern University Feinberg School of Medicine, Evanston, IL,[4]Department of Psychology, Carnegie Melon University, Pittsburgh, PA,[5]Department of Medicine, Northwestern University Feinberg School of Medicine, Evanston, IL,[6]Department of Psychology, University of Miami, Miami, FL

Purpose: While metastatic breast cancer (BC) has no cure, treatment advances are helping women live longer. However, these women often experience debilitating treatment symptoms and emotional distress. Acceptance and commitment therapy (ACT) is an evidence-based intervention that improves mood and quality of life while allowing for the feelings of grief and loss that are expected when facing a life-limiting prognosis. There is a paucity of randomized controlled trials of ACT among women with metastatic BC. This study examined acceptability, feasibility, and preliminary efficacy of a tailored ACT intervention in a randomized pilot trial.
Procedures: Women with stage IV BC (N=30) were randomized 1:1:1 to ACT, usual care, or Cognitive and Behavioral Stress Management (CBSM). ACT and CBSM included 8 weekly group sessions (90 minutes each) delivered via videoconference. Participants completed FACT-B and PROMIS measures at baseline, mid-intervention, post-intervention and 1-month follow-up. Cohen's D effect sizes were calculated.
Results: On average, women were 59.7 years old and 5.6 years from metastatic BC diagnosis. Enrollment rate was 88.5% (31/35), randomization rate was 96.8% (30/31), and retention rate was 90% (9/10 for ACT, 9/10 for usual care, 9/10 for CBSM). 100% of women in both ACT and CBSM attended at least half of the 8 sessions and 88.8% attended at least 6 sessions. 94.4% of women in ACT and 88.2% in CBSM would recommend the intervention to another woman living with metastatic BC. The effect sizes at post intervention were greater than .8 for ACT vs. usual care, while only anxiety and meaning were greater than .8 in CBSM vs. usual care (Table 1).
Conclusion: Findings demonstrate high feasibility and acceptability for ACT and CBSM in metastatic BC. A full-scale randomized control trial is needed to determine the stability of effects and whether ACT has robust effects on outcomes of interest and optimizes HRQoL among women with metastatic BC.

#7357 Mediators of inflammation modify the association between clonal hematopoiesis and incident hematologic malignancy in ARIC.

Elizabeth A. Platz[1], Vernon A. Burk[1], Meng Ru[1], Sergiu Pasca[2], Lukasz P. Gondek[2], Katherine Y. King[3], Ajibike D. Lapite[3], Anna Prizment[4], Corinne E. Joshu[1], Pradeep Natarajan[5], Margaret A. Goodell[3], Christie M. Ballantyne[3], Koichi Takahashi[6]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD,[2]Johns Hopkins University School of Medicine, Baltimore, MD,[3]Baylor College of Medicine, Houston, TX,[4]University of Minnesota, Minneapolis, MN,[5]Harvard Medical School, Boston, MA,[6]MD Anderson Cancer Center, Houston, TX

Background: Clonal hematopoiesis (CH) is associated with a higher hematologic malignancy (HM) risk, and risk increases with variant allele frequency (VAF). We determined whether inflammatory factors modify the association between CH and HM.

Methods: We conducted a prospective cohort analysis of 9,978 participants (55.9% female, 29.5% Black) in the Atherosclerosis Risk in Communities (ARIC) study who were 44-80 years old and did not have a cancer history. Median follow up was 20.9 years. CH (VAF≥2%: 9.8%; VAF≥10% [large CH]: 4.7%) was identified from whole exome sequencing data constrained to 74 genes (Bick et al. *Nature* 2020). Incident HM (N=434) was ascertained mainly by cancer registry linkage. Aspirin was assessed at study visits by review of containers of prescribed and over the counter drugs used in the last 2 weeks. Plasma proteins were measured by SomaScan® 5K assay. We prespecified interleukin (IL)-6, interferon-gamma (IFN-gamma), IL-8, IL-1beta, and tumor necrosis factor (TNF)-alpha. We estimated the association between CH and HM stratified by aspirin use and plasma protein levels (median; 8,693 participants) using Cox regression adjusting for age, sex, race, and risk factors for HM.

Results: The association between large CH (vs no CH) and HM was weaker in aspirin users (HR=1.58, 95% CI 0.91-2.72, p-trend [across no, small, large CH]=0.26) than in non-users (HR=2.02, 95% CI 1.29-3.15, p-trend=0.008). The association between large CH and HM was stronger in participants with higher (HR=2.11, 95% CI 1.29-3.44, p-trend=0.10) than lower (HR=1.38, 95% CI 0.77-2.48, p-trend=0.56) plasma IL-6. Median VAF in those with large CH was comparable between higher (18%) and lower (16%) IL-6 strata, as was percentage of common genes mutated (*DNMT3A:* higher 48%, lower 48%; *TET2:* higher 21%, lower 23%). Patterns of association between CH and HM by strata of IFN-gamma (higher: p-trend=0.014; lower: p-trend=0.46) and IL-8 (higher: p-trend=0.01, lower: p-trend=0.46) were similar to IL-6. In contrast, for TNF-alpha strata, the association between large CH and HM was weaker in participants with higher (p-trend=0.20) than with lower (p-trend=0.06) levels, and the percentage with mutated *DNMT3A* appeared to differ by TNF-alpha level (higher 51%, lower 43%). Associations between large CH and HM were comparable in the higher (p-trend=0.05) and lower (p-trend=0.12) IL-1beta strata.

Conclusions: Regular aspirin use may dampen CH-associated hematologic malignancy risk. Higher plasma levels of some pro-inflammatory cytokines, especially IL-6, may exacerbate CH-associated hematologic malignancy risk. While IL-6 is produced by immune cells with *DMNT3A* and/or *TET2* mutations, the similar VAF and percentage of mutated gene by IL-6 level suggests that IL-6 is not simply marking CH extent. Laboratory experiments are needed to establish cause for these inflammation mediators. Support: NHLBI, NCI, NPCR

#7358 Cigarette smoking is associated with worse prognosis in myelodysplastic syndromes and causes inflammation and hematopoietic alterations in murine models.

T. Verma[1], D. Verma[1], S. Sahu[1], K. Pradhan[1], R. Aggarwal[1], S. Jasra[2], S. S. Dehghani[1], H. Zhang[1], M. Spodek[1], I. Mantzaris[1], M. Konopleva[1], M. Goldfinger[1], A. Verma[1], D. Hosgood[1], A. Shastri[1], Y. Zou[1];

[1]Albert Einstein College of Medicine, Bronx, NY, [2]University of Vermont Medical Center, Burlington, VT

Introduction: Cigarette Smoking has been associated strongly with solid tumors but the causative links with myeloid malignancies have not been well elucidated. Also, the effects of smoking on hematopoiesis have not been explored in vivo.

Results: A highly diverse cohort of Myelodysplastic syndrome (MDS) patients (N=128) including 37% Hispanic and 29% African American subjects was studied. Overall survival was found to be significantly reduced in subjects with previous smoking histories (N=78) when compared to never smokers (N= 58), (P value = 0.04; Median Survival 2130 days vs 4620 days). This was not due to significant differences in age, sex, IPSS, blast counts, mutations between the groups. Next, to determine the effects of smoking in vivo, we optimized a cigarette smoke machine that allowed us to expose mice (C57B/6) to 6 cigarettes a day, 3 times a week for one month. Mice exposed to cigarette smoking exhibited signs of reduced activity and showed raised levels of inflammatory mediators, Interleukin-1β (IL1β) and histamine, while the anti-inflammatory protein Interleukin -10 decreased when compared to controls. Mice exposed to cigarette smoking showed reduced white cell counts. Increased hemoglobin was also seen reflecting exposure to smoke. To assess for qualitative stem cell alterations we performed competitive transplantation experiments using CD45.2 donors exposed to smoking using CD45.1 recipients. Smoking exposure led to reduced donor chimerism at 4 months after transplants, when compared to controls. This difference was reduced at 8 and 12 months, demonstrating that smoking exposure led to reduced progenitor functionality, reflected in early chimerism defects. Cigarette smoke condensate (CSC) collected from the smoke machine's artificial lung was chemically analyzed and showed high levels of nicotine and known carcinogens Acetaldehyde, Formaldehyde, Benzo(a)pyrene, Toluene, Benzene, and Nitrosamines (NNN, NAT, NAB, NNK). We administered CSC via intratracheal injections chronically for 4 months (3 times a week) to an independent cohort of mice. Mice again demonstrated an increase in inflammatory IL-1β and histamine and murine bronchial macrophages treated with CSC exhibited to an inflammatory transcriptomic signature and proteomic validation of increased IL-1β. Lastly, treatment of primary human CD34+ stem and progenitors with CSC resulted in a decreased production of hematopoietic cell colonies, validating the in vivo findings of bone marrow suppression.

Conclusion: We demonstrate that history of cigarette smoking is associated worse overall survival in MDS. We also demonstrate that exposure to cigarette smoking leads to inflammation and hematopoietic alterations in vivo, potentially accounting for adverse clinical outcomes in hematologic malignancies.

#7359 Systemic chronic inflammation is associated with quality of life in the Cancer Disparities Research Network.

Holli Ann Loomans-Kropp[1], Yengeniya Gokun[2], Priya Das[2], Aaliyah Beneby[3], Ting-Yuan Cheng[2], Electra Paskett[2], Cancer Disparities Research Network Cohort Workgroup

[1]The Ohio State University Wexner Medical Ctr., Columbus, OH,[2]The Ohio State University, Columbus, OH,[3]Central State University, Wilburforce, OH

Purpose: The objective of this study was to examine the association between chronic inflammatory (CI) biomarkers and self-reported quality of life (QoL) measurements among medically underserved and minority individuals in the Cancer Disparities Research Network (CDRN).
Methods: We utilized 190 plasma samples collected as part of the CDRN. Briefly, the CDRN was a nationwide initiative intended to improve participation of minority and underserved populations in biobanking research. Eligibility criteria for CDRN was age 40-74, living in an Appalachian County or self-identified as African American (AA)/Black, Hispanic, or Asian, and meeting one of the following criteria: living in a medically underserved area, low literacy, a household income below the federal poverty level in 2015 or being uninsured or receiving subsidized health insurance. For inclusion in the current study, participants needed to have a completed baseline survey and provided a blood sample. CI biomarkers were measured using the ProcartaPlex Human Inflammation Panel 20plex. Concentrations were log-transformed prior to analysis. Self-reported QoL in the past week was measured using a Likert scale (0-10) for anxiety, fatigue, and distress, which were categorized as none, mild, moderate, and severe. Spearman correlation coefficients and adjusted generalized linear regressions were used to assess the relationships between QoL measures and biomarkers. Model covariates included socioeconomic status, age, sex, race, aspirin and nonsteroidal anti-inflammatory drug use, frequency of moderate exercise, and history of arthritis, asthma, depression, high cholesterol, and diabetes.
Results: Among the participants included in our study, 32% were White, 34% were AA/Black, 30% Asian, and 4% Other. We found statistically significant positive correlations between 10 (30.3%) CI biomarkers and anxiety, 6 (18.1%) biomarkers and fatigue, and 4 (12.1%) biomarkers and distress. gp130 was strongly correlated with anxiety and APRIL, MMP-1, and IL-19, were correlated with all three measures (anxiety, fatigue, distress). Adjusted results showed that moderate distress was associated with higher concentrations of APRIL ($p<0.01$), while mild and moderate anxiety were associated with higher concentrations of MMP-1 ($p=0.01$ and $p=0.04$, respectively). Mild ($p=0.03$), moderate ($p=0.04$), and severe ($p=0.02$) anxiety was associated with higher gp130 concentration. Race was a significant covariate in our models, suggesting baseline differences in CI markers across self-reported race.
Conclusions: We demonstrated the association between CI biomarkers APRIL, MMP-,1 IL-19, and gp130 with self-reported QoL measures, specifically anxiety and distress. Moreover, we showed that subjective QoL perceptions was associated with systemic CI, suggesting the importance of understanding the mind-body inflammatory connection.

#7360 Characterizing the immune microenvironment of Nigerian breast tumors by subtype.

Jovita Byemerwa[1], Drew Neish[1], April Deveaux[1], Omolola Salako[2], Adetola Daramola[3], Olusegun Alatise[4], Gabriel Egun[5], Tomi Akinyemiju[1]

[1]Population Health Sciences, Duke University School of Medicine, Durham, NC,[2]College of Medicine & Lagos University Teaching Hospital, Lagos, Nigeria,[3]Lagos University Teaching Hospital, University of Lagos, Lagos, Nigeria,[4]Population Health Sciences, Obafemi Awolow University Teaching Hospital, Ile-Ife, Osun State, Nigeria,[5]University College Hospital, University of Ibadan, Ibadan, Oyo State, Nigeria

Background: Breast cancer (BC) bears significant and well-established disparities in aggressiveness and mortality, disproportionally affecting women of African descent. While socioeconomic factors might explain disparities in BC outcomes in women of African descent, intrinsic tumor biology is now recognized as a contributing factor in BC mortality. Clinical and molecular features of breast tumors in African American women strikingly resemble those of West African women, both characterized by early onset and a high prevalence of the aggressive triple-negative breast cancer subtype, suggesting the shared West African genetic ancestry might play a factor in BC aggressiveness. However, there is a paucity of studies that have clearly characterized the breast tumor microenvironment (TME) in West African women, a critical knowledge gap whose addressing might open therapeutic opportunities to address BC disparities in women of African descent. In this study, we characterized the immune repertoire of BC from Nigeria by receptor subtype.
Methods: We used the Nanostring technology Immune Exhaustion (IE) Panel to characterize the immune repertoire of Nigerian breast tumors by subtype. The Nanostring IE panel targets 785 genes across 47 pathways involved in immune activation, suppression, exhaustion and memory. RNA was isolated from breast tumors of 48 newly diagnosed, treatment naïve breast cancer patients with either estrogen receptor-positive (ER+), HER2-enriched, and Triple-negative Breast Cancer (TNBC) subtypes from Nigeria. After hybridization of the RNA with fluorescent barcoded IE panel probes and counting of hybridized probes, quality control and normalization of the raw data were performed using the Nanostring nSolver Software. Analysis of differential gene expression, pathway scoring, cell type profiling, and gene set enrichment by receptor subtype was performed using the nSolver Software Advanced Analysis Package 2.0, NanostringTM).
Results: Six differentially expressed genes (DEGs) were significantly upregulated or downregulated in ER+ breast cancers vs TNBC. The CHRD gene (associated with TGF-beta signaling) was the most significantly upregulated in ER+ BC vs TNBC. In contrast, the SHC4 gene (associated with cytotoxicity) was the most significantly downregulated in ER+BC vs TNBC. Pathway scoring indicated that ER+ BC had decreased fatty acid metabolism, hypoxia response, and glycolysis and glucose import pathway scores compared to TNBC. No differences in B, T, and NK cell immune exhaustion scores were observed between various BC subtypes.
Conclusion: We observed differences in gene expression and metabolic pathways by subtypes, with the ER+ BC subtype having lower fatty acid metabolism, and glycolysis and glucose import pathway scores in comparison to TNBC. Our findings highlight new insights that could open therapeutic opportunities to improve BC outcomes among women of African descent.

#7361 Scientific rationale and successful implementation of biospecimen collection in the NCI Connect Cohort for Cancer Prevention.

Nicolas A. Wentzensen[1], Stephanie Weinstein[1], Amanda Black[1], Erin Schwartz[1], Hannah P. Yang[1], Michelle Brotzman[1], Paul Albert[1], Laura Beane-Freeman[1], Amy Berrington[2], Jonas De Almeida[1], Jonine D. Figueroa[1], Montserrat Garcia-Closas[2], Nicole Gerlanc[1], Gretchen Gierach[1], Rena Jones[1], Peter Kraft[1], Charles Matthews[1], Habib Ahsan[3], Brisa Aschebrook-Kilfoy[3], Chun-Hung Chan[4], Robert Greenlee[5], Stacey Honda[6], Ben Rybicki[7], Blythe Ryerson[8], Katherine Sanchez[9], Mark Schmidt[10], Kevin Sykes[9], Larissa White[11], Jeanette Ziegenfuss[12], Stephen Chanock[1], Christian Abnet[1], Mia Gaudet[1]

[1]NCI Div. of Cancer Epidemiology & Genetics, Bethesda, MD,[2]ICR, London, United Kingdom,[3]University of Chicago, Chicago, IL,[4]Sanford Health, Sioux Falls, SD,[5]Marshfield, Marshfield, WI,[6]KP Hawaii, Honolulu, HI,[7]Henry Ford Health System, Detroit, MI,[8]KP Georgia, Atlanta, GA,[9]Baylor Scott and White, Dallas, TX,[10]KP Northwest, Portland, OR,[11]KP Colorado, Denver, CO,[12]Health Partners, Minneapolis, MN

Introduction: The Connect for Cancer Prevention Study is a new prospective cohort with repeated exposure assessment and long-term follow-up to study the cancer continuum from initiation, multi-step carcinogenesis, diagnosis, to outcomes in a diverse US population. Over 50,000 participants towards the goal of 200,000 have been recruited so far at 10 integrated healthcare sites across the United States. Goals of Connect include studies of cancer etiology, risk prediction, and early detection. Biospecimens are a critical component for exposure assessment and biomarker measurement. We summarize the scientific rationale and successful implementation of biospecimen collections in Connect.

Methods: A biospecimen collection protocol was informed by literature review, expert consultations, and pilot studies evaluating how different blood collection tubes and processing protocols influence DNA yield and quality, as well as nucleic acid and protein-based biomarkers. Baseline biospecimens include a 45ml blood draw, a urine collection, and a mouthwash sample. Biospecimens are collected in dedicated research labs or at clinical sites with home collection of mouthwash samples. A cell-free DNA collection for multi-cancer early detection is implemented at research collection sites. All biospecimens are shipped to an NCI laboratory for processing and long-term storage. Process metrics include sample completeness, sample deviations, temperature logging, and needle-to-freezer time, among others.

Results: As of November 2024, 35,790 participants of 53,005 enrolled (68%, ranging from 57% to 75% across sites) donated blood and urine samples, with baseline collections still ongoing. Among collections, 58% were from clinical sites, and 42% from research labs. Over 80% of biospecimens collected at research labs were received at NCI within one day, while 70% of biospecimens collected at clinical sites were received within 2 days. Over 95% of all biospecimens were received within 4 days. The return of home-collected mouthwash samples was 77%. Among 276,228 biospecimen tubes collected, 87% were complete with no deviations recorded. Over 90% of participants submitted a short survey relevant for sample collection after the biospecimen visit. Blood collections are planned every three years to study different exposure windows and biomarker changes within individuals. More frequent collections are considered among participants at high risk of cancer.

Conclusions: Connect combines EHR data, state-of-the-art surveys, and biospecimen collections to address critical questions of cancer etiology and prevention. We successfully implemented a robust and efficient biospecimen collection approach at 10 recruitment sites across the U.S. The timeline and process for biospecimen access for the research community and initial biospecimen activities will be presented at AACR.

#7362 Connect for Cancer Prevention Study: a modern prospective cohort.

Mia M. Gaudet[1], Amy Berrington de Gonzalez[2], Christian C. Abnet[1], Paul Albert[1], Jonas S. Almeida[1], Stephanie Weinstein[1], Amanda Black[1], Hannah P. Yang[1], Michelle Brotzman[1], Laura Beane-Freeman[1], Laura Beane-Freeman[1], Jonine Figueroa[1], Neal D. Freedman[3], Nicole M. Gerlanc[1], Gretchen L. Gierach[1], Rena R. Jones[1], Peter Kraft[1], Charles Matthews[1], Habibul Ashan[4], Briseis Aschebrook-Kilfoy[4], Chun-Hung Chan[5], Robert T. Greenlee[6], Stacey A. Honda[7], Benjamin Rybicki[8], Katherine Sanchez[9], Kevin Skyes[9], Mark A. Schmidt[10], Larissa L. White[11], Jeanette Y. Ziegenfuss[12], Stephen J. Chanock[1], Montse Garcia-Closas[2], Nicolas A. Wentzensen[1]

[1]NCI Div. of Cancer Epidemiology & Genetics, Bethesda, MD,[2]Institute of Cancer Research, London, United Kingdom,[3]NCI DCCPS, Bethesda, MD,[4]University of Chicago, Chicago, IL,[5]Sanford Health, Sioux Falls, SD,[6]Marshfield Clinic, Marshfield, WI,[7]Kaiser Permentae - Hawaii, Honolulu, HI,[8]Henry Ford Health, Detroit, MI,[9]Baylor, Scott and White Health, Dallas, TX,[10]KP Northwest, Portland, OR,[11]KP Colorado, Denver, CO,[12]Health Partners, Minneapolis, MN

Background: Reaching the national goal of reducing cancer mortality by 50% within 25 years will require improvements in cancer early detection and prevention in addition to treatment. Modern prospective cohorts that capture new and emerging exposures to research cancer etiology are critical to achieve these goals. Profound societal and technological changes in the last decade present opportunities and challenges for the recruitment, engagement, and retention of participants in new cohorts.
Methods: The Connect for Cancer Prevention Study is a modern cohort of adults, 30-70 years old without a prior cancer diagnosis, recruited from 10 U.S. integrated health care systems. Participants provide information and biospecimens at enrollment and at regular intervals during at least 20 years of follow up. Exposure and outcome information will be captured through online surveys, electronic medical records, medical imaging, geospatial linkages of 20-year residential histories, wearable sensors, and linkages with the National Death Index and the state cancer registries. Collected biospecimens include blood, urine, saliva, and fecal samples, as well as precursor and tumor tissue specimens. Data systems follow F.A.I.R. (findability, accessibility, interoperability, and reuse of digital assets) principles to maximize data and tool re-usability.
Results: In 2021, recruitment commenced among an eligible catchment population of more than 3.2 million patients. As of November 2024, over 53,000 of the expected 200,000 participants consented; 87% completed at least some baseline activities to date. Baseline recruitment is expected to be complete in 2027. The current study population has a median age of 53 (IQR: 42-61) years, 67% are female, 63% completed at least a bachelor's degree, and 88% report good to excellent overall health. Among the cohort, 61% of participants self-report as White, 14% self-report as multi-racial, 11% as Black, African American or African, 5% as Asian, and 4% as Hispanic, Latino, or Spanish among other categories. The portion of the latter category is expected to increase as recruitment ramps up at a site with a high Hispanic catchment population, which started enrollment in 2023. The proportion of individuals who have ever smoked cigarettes is 30%, whereas 9% have ever used marijuana, 3% have ever smoked cigars, and 2% have ever vaped nicotine containing electronic cigarettes. The median body mass index of participants is 30 kg/m^2 (IQR: 24-36 kg/m^2). Approximately, 30,000 incident precursor and 8,000 cancer diagnoses are expected to occur during the first 10 years of follow up.
Conclusions: Connect for Cancer Prevention Study combines novel approaches in epidemiology and data science to provide a valuable resource for the scientific community to study cancer etiology, natural history, risk prediction, early detection, and survivorship. Individual-level data is expected to be released to the scientific community in 2026.

#7363 Providers' beliefs, perceived skill, and perception of organizational norms relate to their delivery of cigarette smoking intervention to patients seen in healthcare centers serving rural and medically underserved areas of Texas.

A. D. Siddiqi[1], M. Britton[1], T. A. Chen[2], I. Martinez Leal[1], A. Bergey[2], T. Goulbourne[2], T. Ousley[2], T. Williams[3], K. Casey[3], L. R. Reitzel[1];
[1]UT MD Anderson Cancer Center, Houston, TX, [2]University of Houston, Houston, TX, [3]Integral Care, Austin, TX

Rural and medically underserved populations have elevated smoking rates, yet evidence-based cessation care is not often provided in healthcare settings serving these communities, leading to a greater burden of smoking-related cancers. Social cognitive theory (SCT) factors - cognitive, behavioral, and environmental - influence providers' smoking intervention delivery. Here, we examine the veracity of these relations in a novel setting, healthcare centers serving rural and medically underserved areas of Texas, to inform workplace capacity building to improve care for smokers and prevent cancers. Providers (N=347) from 19 healthcare centers took an e-survey and reported on 1) their beliefs about smoking: whether their patients a) are concerned about smoking and b) want to quit (cognitive); 2) perceived treatment skills: whether they a) have the skills to help their patients quit smoking and b) are knowledgeable about referral options (behavioral); and 3) workplace norms: whether tobacco cessation counseling is an important part of their center's mission (environmental). Smoking intervention practices (the 5A's: Ask, Advise, Assess, Assist, Arrange) for past-month patient contacts were self-reported. Linear mixed models tested for associations between SCT constructs and the 5A's, controlling for center type. Overall, 31.41% of providers believed their patients were concerned about smoking and 29.11% thought they wanted to quit; 48.99% endorsed treatment skills and 53.89% were knowledgeable of referral options; and 48.99% endorsed that tobacco treatment was an important part of their center's mission. Providers Asked 68.31% of patients about their smoking status; of the smokers seen, providers Advised 58.68% to quit, Assessed interest in quitting among 51.90%, Assisted 35.87% to quit, and Arranged follow-ups for 22.79%. Relative to those who did not, providers who believed patients wanted to quit Asked a greater proportion of their patients about their smoking status. Providers who reported having the skills to help patients quit smoking or knowing about referral options delivered Ask to more patients and Advise, Assess, Assist, and Arrange to more smokers. Providers who believed that tobacco cessation was an important part of their center's mission delivered Ask to more patients and Advise and Assess to more smokers. All ps <0.02. Workplace interventions providing education to improve the accuracy of providers' smoking-related beliefs, training to bolster their smoking treatment and referral skills, and efforts to reflect the importance of a shared smoke-free mission may reduce gaps between the current and the recommended best practices in smoking care provision in centers serving patients in rural and medically underserved areas of Texas.

#7364 Culturally sensitive, virtually supervised, home exercise improves cognition in Latina and Hispanic breast cancer survivors: results from the ROSA Trial.

Elizabeth Cox[1], Paula Gonzalo-Encabo[2], Rebekah L. Wilson[1], Mary K. Norris[3], Danny Nguyen[3], Cami N. Christopher[4], Jocelyn Greer[3], Christina M. Dieli-Conwright[4]

[1]Dana-Farber Cancer Institute/Harvard Medical School, Boston, MA,[2]Dana-Farber Cancer Institute/Universidad de Alcala, Madrid, Spain,[3]Dana-Farber Cancer Institute, Boston, MA,[4]Dana-Farber Cancer Institute/Harvard Medical School/Harvard T.H. Chan School of Public Health, Boston, MA

Background: Breast cancer diagnosis and treatment contribute to cognitive impairment, including executive and memory dysfunction, which reduce quality of life. Breast cancer is the most common cancer diagnosis in Hispanic and Latinas, who are often diagnosed at later stages, requiring more aggressive treatment that can increase severity of cognitive impairment. Exercise can safely and effectively improve cognition in cancer survivors, but Latina/Hispanic breast cancer survivors (LHBCS) often do not meet physical activity guidelines and face exercise barriers such as lack of enjoyment and knowledge, self-consciousness, and discouragement. Previous research on exercise and cognition is inconclusive among LHBCS, possibly due to inadequate attention to these barriers. Culturally sensitive, virtually supervised, home exercise interventions may reduce barriers and improve cognition in LHBCS.
Aim: This exploratory analysis aims to determine the efficacy of virtually supervised, home exercise to improve cognition in LHBCS enrolled in the ROSA Trial (NCT04717050).
Methods: LHBCS (n=64) were randomized to a 16-week progressive aerobic and resistance exercise group (EG) or attention control group (CG). The EG completed ~90 min sessions led virtually by an exercise trainer, and the CG completed static stretching 3 days/week. Barriers were addressed with Spanish-language materials and staff, and delivery in the home. At pre- and post-intervention, participants completed NIH Cognition Toolbox tasks: i) List Sort Working Memory to assess working memory (ability to hold and manipulate information in mind, a component of executive function), and ii) Picture Sequence Memory to assess episodic memory (ability to learn and recall experiences in daily life). Residual change scores quantified pre- to post-intervention changes in performance. T-tests assessed changes within and between groups; when model assumptions were violated, Mann-Whitney U tests were applied.
Results: Thirty-nine LHBCS were included in the analysis ($\bar{x}_{age}$=55.39, SD=10.18). The EG (n=21) showed significant improvement on List Sort Working Memory (p=0.05, d=0.40) and Picture Sequence Memory (p=0.01, d=0.50) tasks from pre- to post-intervention, while the CG (n=18) showed no significant changes (p>0.05). No significant differences were detected between groups (p>0.05).
Discussion: The findings suggest culturally sensitive, virtually supervised, home, progressive combined exercise improves components of executive function and episodic memory in LHBCS. Our results align with evidence that exercise enhances these cognitive domains in BCS. Lack of differences between groups may reflect limited power or variability in individual responses. Future analyses will assess additional cognitive domains and effect mediators (e.g., cardiorespiratory and physical fitness).

#7365 Use of wearable technology for physical activity and health monitoring in individuals diagnosed with cancer.

Gillian Gresham[1], Cami N. Christopher[2], Rikuta Hamaya[2], Aditi Hazra[2], JoAnn E. Manson[2], Howard D. Sesso[2]

[1]Cedars-Sinai Medical Center, Los Angeles, CA,[2]Brigham and Women's Hospital, Boston, MA

Background: Wearable devices provide an opportunity to remotely collect objective data on a patient's physical activity (PA) and sleep patterns, blood pressure (BP), blood glucose, and other health-related metrics. To date, the prevalence of use and role of wearable devices in older adults diagnosed with cancer remains unclear. We sought to evaluate patterns and characteristics associated with wearable device use.
Methods: A cross-sectional survey on wearable device use was nested within the COSMOS trial, a completed randomized trial of cocoa extract and multivitamin supplementation among U.S. older adults that began in 2015. A 2023 follow-up survey included questions about the usage and frequency of use of any wearable device (activity tracker, BP monitor, or glucose monitor), and willingness to share data for future research. We included participants who completed the 2023 survey and had a self-reported history of cancer diagnosis at baseline, except for non-melanoma skin cancers and compared survey responses to individuals who did not report a history of cancer. Multivariable logistic regression analyses were conducted to assess characteristics associated with wearable device usage adjusted for age, sex, race, income, body mass index (BMI), PA level, and diet quality.
Results: Of the 18,552 COSMOS participants with 2023 survey data, 3,045 reported a cancer history, 62% were female with a median age 73 years (interquartile range=8.3). There were 780 (26%), 1161 (38%), and 60 (2%) participants with a cancer history reporting usage of an activity tracker, BP monitor, or glucose monitor, respectively. Activity tracker usage was lower in those with vs without a cancer history (n=15,507) (26% vs 29%, p=0.0003), while BP monitor usage was higher (38% vs 36%, p=0.023). Among those with a cancer history and reporting any wearable device usage, 78% used activity trackers, 19% used BP monitors, and 66% used glucose monitors most or all of the time. The percentage of individuals willing to share their data was higher for those with a history of cancer compared to those without, for all devices, with statistically significant differences for activity trackers (84% vs. 81%, p=0.02). In multivariable logistic regression, age (Odds ratio (OR): 1.04 per 1-year decrease), female sex (OR: 1.55), higher income (OR: 2.03), BMI (OR: 1.02), and PA level (OR: 1.07) were each statistically significantly ($p<0.05$) associated with increased odds of activity tracker use. BMI also was significantly associated with use of BP and glucose monitors ($p<0.05$).
Conclusions: Older adults with a history of cancer have a high prevalence of wearable device usage, and are willing to share their data for research purposes. This highlights the potential to leverage existing trials to recruit participants in future studies involving wearables and collect valuable insights on their daily activity and biometric data.

#7366 Associations of inflammatory dietary patterns and predicted pro-inflammatory hs-CRP scores with health-related quality of life among breast cancer survivors.

Ji Yeong Kim[1], Sang-Eun Moon[1], Hyun Jeong Cho[1], Zisun Kim[2], Hyun Jo Youn[3], Jihyoung Cho[4], Jun Won Min[5], Yoo Seok Kim[6], So-Youn Jung[7], Eun Sook Lee[7], Young Bum Yoo[8], Se Kyung Lee[9], Seho Park[10], Jung Eun Lee[1]

[1]Department of Food and Nutrition, Seoul National University, Seoul, Korea, Republic of,[2]Department of Surgery, Soonchunhyang University Bucheon Hospital, Bucheon, Korea, Republic of,[3]Department of Surgery, Jeonbuk National University Medical School, Jeonju, Korea, Republic of,[4]Department of Surgery, Keimyung University School of Medicine, Daegu, Korea, Republic of,[5]Department of Surgery, College of Medicine, Dankook University, Cheonan, Korea, Republic of,[6]Department of Surgery, College of Medicine, Chosun University, Gwangju, Korea, Republic of,[7]Research Institute and Hospital, National Cancer Center, Goyang, Korea, Republic of,[8]Department of Surgery, Konkuk University Medical Center, Seoul, Korea, Republic of,[9]Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of,[10]Division of Breast Surgery, Department of Surgery, Yonsei University College of Medicine, Seoul, Korea, Republic of

JYK and S-EM contributed equally to this study.

Backgrounds: Diet may modify chronic inflammation, which has a potential link with health-related quality of life (HRQoL) and cancer prognosis. This study investigated the associations of inflammatory dietary patterns and predicted pro-inflammatory hs-CRP scores with HRQoL levels among breast cancer survivors.

Methods: This cross-sectional study included a total of 717 breast cancer survivors recruited from nine hospitals in Korea. Dietary intake was assessed using 3-day dietary records (DRs) or a validated food frequency questionnaire (FFQ). The HRQoL of the participants was assessed using the Short Form-36 (SF-36) Health Survey, which comprises eight domains. To examine the inflammatory dietary pattern, the reduced rank regression (RRR) was performed with the intake amount of each food group and the plasma levels of five inflammatory biomarkers (high-sensitivity C-reactive protein (hs-CRP), Interleukin (IL)-6, IL-8, adiponectin, and tumor necrosis factor (TNF)-α). The factor loadings were multiplied by the standardized daily intake (g) of each food group to calculate the inflammatory dietary pattern scores. The least square-means (LS-means) and 95% confidence intervals (95% CIs) of the HRQoL scores were estimated according to the quartiles of the inflammatory dietary pattern score using the general linear model (GLM). Additionally, a predicted pro-inflammatory hs-CRP score was derived using a previously developed model and its association with HRQoL was examined by using the GLM.

Results: The inflammatory dietary pattern was characterized by high intakes of refined rice, noodle, seafood (excluding fish), sugar-sweetened coffee, carbonated or sugar-sweetened beverages, along with low intakes of salted vegetables and processed fish. Higher inflammatory dietary score was associated with lower scores on HRQoL mental health domain (p for trend = 0.034), physical functioning domain (p for trend = 0.017) and bodily pain domain (p for trend = 0.057). Higher predicted pro-inflammatory hs-CRP score was associated with lower scores on HRQoL physical component summary domain (p for trend = 0.001), physical functioning domain (p for trend = 0.003) and bodily pain domain (p for trend = 0.018).

Conclusions: The inflammatory dietary pattern score and the predicted pro-inflammatory hs-CRP score may be inversely associated with the HRQoL levels in breast cancer survivors.

#7368 Serum concentrations of per- and polyfluoroalkyl substances and mosaic loss of chromosome Y among men in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial.

Jongeun Rhee, Vicky C. Chang, Weiyin Zhou, Mitchell J. Machiela, Jonathan N. Hofmann, Mark P. Purdue, Sonja I. Berndt

Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD

Per- and polyfluoroalkyl substances (PFAS) are persistent organic pollutants detectable in the serum of most U.S. adults. Some PFAS are established or possible human carcinogens. Mechanistic studies in human cells and animals suggest PFAS may have genotoxic effects through indirect DNA damage caused by induction of oxidative stress or other mechanisms; however, evidence from human populations is limited. We investigated the relationship between serum PFAS concentrations and mosaic loss of chromosome Y (mLOY) as a biomarker of genotoxicity and genomic instability among men. We analyzed blood-derived DNA from 2,377 men (aged 55-76 at blood collection) in three nested case-control studies within the PLCO Cancer Screening Trial. mLOY was detected using high-density genotyping array intensity data for chromosome Y. Pre-diagnostic serum concentrations of four PFAS [perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), perfluorononanoate (PFNA)] were measured. Using study-specific multivariable logistic regression, we estimated odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between serum PFAS and any detectable mLOY (overall mLOY) or mLOY affecting ≥10% of cells (expanded mLOY) among cases and cancer-free controls, separately. Results stratified by study and case-control status were summarized using a random-effects meta-analysis. Overall mLOY was detected in 22% of all men and 8.3% of all men had expanded mLOY. We found a suggestive positive association between serum PFHxS concentrations and mLOY (doubling in serum concentration, summary $OR_{continuous}$=1.14, 95%CI=0.93-1.40; summary OR highest vs. lowest quartile=1.22, 95% CI=0.55, 2.69, P-trend=0.09). The magnitude of the association was stronger for those with a higher proportion of affected cells (expanded mLOY; doubling in serum concentration, $OR_{continuous}$=1.19, 95% CI=0.89-1.59). No associations were observed with other PFAS. In conclusion, in this large population-based study, we observed suggestive evidence of a positive association between elevated serum PFHxS concentrations and mLOY. As the first study to evaluate PFAS in relation to mLOY, our findings, if confirmed, may provide new insight into PFHxS effects affecting genome maintenance, relevant for cancer development.

#7369 Self-reported barriers to healthcare and cancer screening rates: results from the All of Us Research Program.

Kevin H. Kensler[1], Anjile An[1], Aaron Gurayah[2], Faith Morley[1], Meenakshi Davuluri[2], David M. Nanus[3], Bashir Al Hussein Al Awamlh[2], Rulla M. Tamimi[1]

[1]Population Health Sciences, Weill Cornell Medicine, New York, NY,[2]Urology, Weill Cornell Medicine, New York, NY,[3]Medicine, Weill Cornell Medicine, New York, NY

Introduction: Identification of healthcare access-related factors linked to lower use of cancer screening can inform targeted policies and interventions to mitigate these barriers and ameliorate screening disparities. We evaluated how barriers to healthcare influence cancer screening rates for five cancer types in a diverse population.

Methods: We identified cohorts of participants within the *All of Us* Research Program (2017-2022) meeting U.S. Preventive Services Task Force (USPSTF) guideline eligibility criteria for breast, colorectal, cervical, lung, or prostate cancer screening. Participants self-reported whether nine potential barriers had led them to delay seeking medical care in the past year. Using these items, a barrier burden was calculated by summing the number of affirmative responses (range 0-9), and exploratory factor analysis was performed to identify latent classes of barriers. USPSTF guideline-concordant receipt of cancer screening was assessed in the linked participant electronic health record. Multivariable-adjusted odds ratios (OR) were estimated for the association between each barrier, the barrier burden, and factor scores and receipt of cancer screening, accounting for participant sociodemographic characteristics.

Results: Compliance with screening guidelines varied by cancer site: breast (42%, n=31,827 eligible), cervical (28%, n=37,770), colorectal (42%, n=68,895), lung (11%, n=2,737), and prostate (36%, n=8,807). The most cited barriers were concerns about out-of-pocket costs, nervousness about seeing a provider, and inability to get time off work, though barrier frequency varied across cancer sites. Participants who were younger, female, had lower income or educational attainment, or identified as Hispanic or non-Hispanic Black were more likely to report 3+ barriers to care. Participants reporting 3+ barriers to care had lower screening rates relative to those who reported no barriers for breast (OR 0.66, 95% CI 0.60-0.72), cervical (OR 0.77, 95% CI 0.72-0.83), colorectal (OR 0.77, 95% CI 0.72-0.82), lung (OR 0.65, 95% CI 0.37-1.09), and prostate cancer (OR 0.78, 95% CI 0.64-0.95). Three latent factors were consistently identified across cancer sites reflecting cost concerns, logistical barriers (e.g. transportation), and competing obligations (e.g. time off work). In multivariable analyses, cost concerns were associated with lower screening rates for breast, cervical, and colorectal cancer, logistical barriers for breast, cervical, colorectal, and prostate cancer, and competing obligations for breast and prostate cancer.

Conclusions: The cumulative burden of barriers to access and barriers related to cost concerns, logistics, and competing obligations were associated with lower cancer screening rates. Policies and interventions are needed to target multiple dimensions of access simultaneously to increase cancer screening uptake.

#7370 Evaluations of lifestyle factors with biliary tract cancer risk in cohort studies including 742,906 Asian adults.

Valerie Gunchick, Wei Zheng

Department of Medicine, Division of Epidemiology, Vanderbilt University Medical Center, Nashville, TN

Biliary tract cancer (BTC) is a rare, aggressive malignancy and incidence has globally increased 76% from 1990 to 2017. BTC incidence is higher in Asia than Europe and North America, yet studies evaluating BTC risk in Asian populations are limited. Herein we evaluate tobacco smoking, alcohol consumption, BMI, physical activity, and dietary factors with BTC risk. We evaluated 742,906 adults enrolled in 14 cohort studies as part of the Asia Cohort Consortium. Represented countries include China, Japan, Korea, Bangladesh, India, and Iran. Participants completed baseline surveys which collected demographics, lifestyle characteristics, and medical histories, and were followed to identify cancer diagnoses. Participants were removed due to data only on cancer death, a baseline history of cancer or stroke, among other reasons. Cox regression was used to estimate hazard ratios and 95% confidence intervals describing risk of first primary incident BTC in pooled cohort data. Models were adjusted for age, sex, education, cohort study, diabetes, and BMI, where applicable. Some evaluations were stratified by sex due to differing smoking and drinking behaviors. The analyzed cohort includes 451,053 participants with a median age of 54 years, follow-up time of 15.0 years, and 1,300 incident BTC cases. More men than women reported ever smoking or drinking (76.9% vs 7.0%, 63.3% vs 8.0%, respectively). Alcohol consumption was positively associated with BTC risk in men [HR (95% CI) comparing >=60 to 0 grams/day was 1.48 (0.99-2.20), p-trend=0.02]. In both sexes, duration of smoking was positively associated with BTC risk [comparing >40 to 0 years: 1.40 (0.81-2.43), p-trend=0.008] as was high BMI [comparing ≥27.5 to 18.5-22.9: 1.36 (1.11-1.66), p-trend=0.002]. Physical activity was not associated with BTC risk [comparing moderate to minimal activity: 1.00 (0.77-1.28), p-trend=0.76]. Given that our study suggests that obesity, alcohol, and smoking may play an important role in the etiology of BTC, mitigating these conditions could combat the rising incidence of this fatal malignancy. Future plans include dietary evaluations, study-specific estimates combined with meta-analysis, and sensitivity analysis studying BTC death.

POPULATION SCIENCES: Socioeconomic, Lifestyle, and Biological Drivers of Cancer Risk and Survival: Clinical Outcomes and Treatment Guidelines
Poster Session

#7373 The dual influence of treatment and age on survival in patients with glioblastoma.

James Fahey[1], **Manon Penco**[2], Emma Federico[1], Erica Power[1], Michael Egozi[1], Dayna Sloane[1], Isaac Ng[1], Kayeromi Gomez[3], Kristen L. Lauing[4], Ishan Roy[5], Theresa Walunas[6], Kevin P. Barton[7], Douglas E. Anderson[1], Anand V. Germanwala[1], Vikram C. Prabhu[1], Jigisha Thakkar[1], Derek A. Wainwright[1]

[1]Department of Neurological Surgery, Loyola University Medical Center, Maywood, IL,[2]Department of Cancer Biology, Loyola University Medical Center, Maywood, IL,[3]Biostatistics Collaborative Core, Clinical Research Office, Loyola University Medical Center, Maywood, IL,[4]Department of Cancer Biology, Loyola University Chicago Stritch School of Medicine Loyola Medicine, Maywood, IL,[5]Department of Physical Medicine and Rehabilitation, Shirley Ryan Ability Lab, Northwestern University Feinberg School of Medicine, Chicago, IL,[6]Department of Medicine, Division of General Internal Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL,[7]Division of Hematology/Oncology, Loyola University Medical Center, Maywood, IL

Background: Glioblastoma (GBM; IDHwt) is primarily diagnosed at ≥65 years of age with a peak incidence between 70-79 years old. Increased age is a strong detrimental factor. Older patients have significantly poorer treatment outcomes for both immune-based- and standard of care (SOC)-therapies. In addition to treatment response, maladaptive age-dependent effects have been attributed to decreased physical resilience, immune system alterations, and lack of tolerance to therapies. Yet, there is insufficient research investigating age-dependent prognostic factors in electronic health record (EHR) patient data, particularly in the diverse cohort of patients that Loyola University Medical Center (LUMC) serves.
Methods: Patients were identified through ICD-9/10 definitions for brain cancer and 192 IDHwt GBM patients were chart reviewed and confirmed. Demographic, co-morbid, mutational, and treatment data was compiled. Analysis of overall survival (OS) was performed using the survminer package in R 4.0.2. Data were stratified by patient age and used to construct univariate and multivariate Cox proportional-hazard models with statistical significance assessed using log-rank tests.
Results: GBM patients ≥65 years old (n=103, median OS=221 days) experienced worse OS ($p<0.001$) compared to those<65 years of age (n=89, median OS=464 days). Older patients declined treatment more frequently ($p=0.002$) (OR=2.95) and had higher Charlson Comorbidity Index (CCI) scores ($p<0.001$). After removing patients who declined treatment (n=143), this age-influenced outcome disparity persisted ($p=0.019$). After further restricting to only patients who completed a full course of SOC radio-chemotherapy (n=125) (young: n=71, median OS=543 days) (old: n=54, median OS=495 days), OS differences between age groups lost significance ($p=0.061$). Among patients in this cohort, MGMT methylation ($p=0.027$) and initial tumor resection ($p<0.001$) was associated with increased OS. The development of neurocognitive symptoms within 6 months of diagnosis ($p<0.001$), higher initial CCI ($p=0.019$), and initial KPS under 80 ($p=0.002$) were independently associated with poorer OS. When accounting for initial KPS and resection status, OS differences between age groups were significant ($p=0.01$). Unlike the older cohort, younger patients had a significant association between increased body mass index and improved OS ($p=0.017$) while cachexia/significant weight loss was associated with worse OS ($p=0.015$).
Conclusion: Though the statistical significance is missing in the completed treatment cohort, likely due to sample size, clinical trends show younger patients have better survival outcomes. The older cohort's decreased physical resilience, as evidenced by the increased pre-existing comorbidities and higher CCI scores, could be driving their decreased participation and may suggest additional biological etiologies for the discrepancies in OS.

#7374 Body size and composition in relation to PI3K/AKT/mTOR signaling: insights into cancer risk and outcomes - a systematic review.

Rand Lucine Talal Akasheh[1], Cuthbert Mario Mahenge[1], Faiza Kalam[1], Anita Adib[1], Stephanie Schulte[2], Ting-Yuan David Cheng[1]

[1]Department of Internal Medicine, The Ohio State University Wexner Medical Center, Columbus, OH,[2]Health Sciences Library, The Ohio State University, Columbus, OH

Objective: To synthesize evidence from studies investigating the relationship between body size and composition with PI3K/AKT/MTOR signaling in the context of cancer risk and outcomes.
Methods: A systematic search of the literature was conducted in Web of Science, Embase, and Pubmed as of January 2019. The inclusion criteria entailed studies that reported any anthropometric measure of body size and/or composition; and any measure of gene, RNA, or protein expression in PI3K/AKT/MTOR signaling, or that directly or indirectly interacts with the pathway in tumors, precancerous lesions, adipose, or muscle tissue; and included people with cancer or precancerous lesions, cancer survivors, high-risk populations, or/and populations with average risk for studying cancer risk.
Results: The search strategy resulted in 6258 studies, of which 1198 studies were excluded by deduplication. Titles and abstracts of the remaining 5087 studies were screened and 4471 studies were found irrelevant and therefore excluded, leaving 316 studies for full-text screening. Of these, 173 studies were excluded leaving 143 studies that met the inclusion criteria and were used in full-text extraction. The included studies focused on gastrointestinal cancers (n = 50: 35 colorectal, 2 upper GI, 3 gastric, 4 esophageal with 1 on Barrett cases, 5 pancreatic, and 1 hepatic); gynecological cancers (n = 34: 32 endometrial with 4 on normal endometrium of at-risk women, 1 cervical, 1 ovarian); breast cancer (n = 31: with 2 on normal breast tissue of at-risk women); male reproductive cancers (n = 7: 6 prostate, 1 Leydig cell); lung cancer (n = 6); thyroid cancer (n = 6); renal cancer (n = 2); skin cancer (n = 1); and cancer cachexia across cancer sites (n = 6). Body size and composition modulate many components of PI3K/AKT/MTOR pathway, in addition to upstream and downstream effectors. Numerous studies indicate that obesity modifies the relationship between molecules in the PI3K/AKT/MTOR pathway and cancer survival, while alterations in this pathway may also affect how body size and composition relate to cancer survival. However, inconsistency in findings was observed due to heterogeneity in study design, exposure variables, reported effect estimates, population characteristics, cancer and tumor features, and methods of outcome measurements. This heterogeneity precluded a meta-analysis. Instead, a qualitative synthesis was conducted to integrate key insights and trends.
Conclusions: The review highlights the broad impact of obesity on cancer biomarkers and outcomes and emphasizes the need for standardized study design and consistent reporting in future research to facilitate meta-analyses and robust conclusions. Most studies relied solely on BMI as a measure of body size, revealing a substantial research gap in examining the relationship between body adiposity and muscularity with PI3K/AKT/mTOR signaling.

#7375 The epidemiology and outcomes of protein-energy malnutrition in lung cancer patients admitted with sepsis: A population-based study.

Adamsegd Isac Gebremedhen[1], **Abdu Osman Mohammed**[2], Mamdouh Souleymane[1], Reesha Bodiwala[1], Awasi Marcel[1], Avantika Thapa[3], Muhammad O. Jamil[1]

[1]Marshall University Joan C. Edwards School of Medicine, Huntington, WV,[2]Trinity Health System, Wintersville, OH,[3]Anwar Khan Modern Medical College, Dhaka, Bangladesh

IntroductionLung cancer patients are at high risk for infection-associated complications. Protein-energy malnutrition (PEM) is common among lung cancer patients. PEM is associated with worse outcomes and longer hospital stays in various cancers, but its impact on lung cancer patients with sepsis is not well studied.This study aims to explore how PEM affects in-hospital outcomes in lung cancer patients admitted with sepsis.MethodWe identified adult patients with lung cancer from the National Inpatient Sample who were admitted with sepsis as the principal diagnosis from 2018 to 2021, using ICD-10 codes. The cohort was categorized based on the presence or absence of protein-energy malnutrition (PEM), and complex sampling weights were applied to ensure national representativeness. Our primary outcome was all-cause mortality, and we analyzed several secondary outcomes. Multivariate regression models evaluated outcome disparitiesbetween the groups, with statistical significance set at $p < 0.05$.ResultsWe identified a total of 186,275 adults with lung cancer who were admitted with the principal diagnosis of sepsis from 2018 to 2021. Among these, 51,065 patients (27.4%) had a concurrent diagnosis of PEM.The PEM group had a higher proportion of male (57.9% vs 53.9%, $p<0.001$) and non-white (28.3% vs 22.1%, $p<0.001$) patients. Patients with PEM had higher odds of all-cause mortality with an aOR (adjusted odds ratio) of 1.11 (95% confidence interval [CI] 1.05-1.18). They also had higher odds of respiratory failure (aOR 1.15, 95% CI 1.10-1.21), mechanical ventilation use (aOR 1.11, 95% CI 1.02- 1.21), septic shock (aOR 1.15, 95% CI 1.09-1.21) and vasopressor use (aOR 1.17, 95% CI 1.04-1.31).Furthermore, they experienced longer hospital stays (8.9 vs 6.5 days) with an adjusted incidence rate ratio (aIRR) of 1.32 (95% CI 1.30-1.35) and higher hospitalization charges ($109,925 vs $81,376) with aIRR of 1.27 (95% CI 1.23-1.31).ConclusionOur study revealed that lung cancer patients with PEM admitted with sepsis had significantly higher odds of all-cause mortality and other adverse outcomes compared to those without PEM. They also experienced longer hospital stays and higher hospitalization charges. These results underscore the critical need to address PEM in lung cancer patients and adjust inpatient care to minimize complications in this high-risk population.

#7377 Intrauterine device use and ovarian cancer risk among 11 case-control studies and 7 prospective cohort studies.

Jennifer M. Mongiovi[1], Ana Babic[2], Allison F. Vitonis[3], Naoko Sasamoto[3], Mark K. Townsend[4], Brett M. Reid[5], Mollie E. Barnard[6], Holly R. Harris[7], Lauren Peres[8], Britton Trabet[9], Brooke L. Fridley[10], Katie M. O'Brien[11], Anita Koushik[12], Renee Turzanski Fortner[13], Jennifer A. Doherty[9], Joelle M. Schildkraut[14], Shelley S. Tworoger[4], Kathryn L. Terry[3], Ovarian Cancer Association Consortium, Ovarian Cancer Cohort Consortium

[1]Harvard T.H. Chan School of Public Health, Boston, MA,[2]Department of Medical Oncology, Dana Farber Cancer Institute, Boston, MA,[3]Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Boston, MA,[4]Division of Oncological Sciences and the Knight Cancer Institute, Oregon Health & Science University School of Medicine, Portland, OR,[5]Department of Cancer Epidemiology, Moffitt Cancer Center, Moffitt, FL,[6]Slone Epidemiology Center, Boston University Chobanian and Avedisian School of Medicine, Boston, MA,[7]Program in Epidemiology, Fred Hutchinson Cancer Center, Seattle, WA,[8]Department of Cancer Epidemiology, Moffitt Cancer Center, Tampa, FL,[9]Huntsman Cancer Institute at the University of Utah, Salt Lake City, UT,[10]Biostatistics and Epidemiology Core, Children's Mercy Kansas City, Kansas City, MO,[11]Epidemiology Branch, National Institute of Environmental Health Sciences, National Institutes of Health, Durham, NC,[12]Department of Oncology, Faculty of Medicine and Health Sciences, McGill University, Montreal, QC, Canada,[13]Department of Research, Cancer Registry of Norway, Norwegian Institute of Public Health, Oslo, Norway,[14]Department of Epidemiology, Emory University, Rollins School of Public Health, Atlana, GA

Introduction: Recently, intrauterine device (IUD) use has increased and oral contraceptive (OC) use has decreased. While OC use is known to reduce epithelial ovarian cancer (EOC) risk, previous studies of IUD use and EOC risk have shown inconsistent associations. Here, we examined the association between primarily non-hormonal IUD use and EOC risk by leveraging harmonized individual-level data from 11 case-control studies, 10 in the Ovarian Cancer Association Consortium and the African American Cancer Epidemiology Study, and 7 prospective cohort studies from the Ovarian Cancer Cohort Consortium.

Methods: Contraception use was self-reported through questionnaires. Study-specific odds ratios (OR) and 95% confidence intervals (CI) were estimated using unconditional logistic regression (case-control studies) or time varying polytomous logistic regression models (cohort studies), adjusted for age, duration of OC use, parity, and family history of breast or ovarian cancer. Heterogeneity between studies was assessed for case-control and cohort studies separately using Cochran *Q*. There was no evidence of heterogeneity between studies, thus, data were pooled within each study design. Case-control and cohort pooled results were combined by random effects meta-analysis. Associations were estimated for overall EOC risk and by histotype (high-grade serous, low-grade serous, endometrioid, clear cell, mucinous). Heterogeneity across histotypes was evaluated using a Wald test for case-control and cohort models separately.

Results: Among case-control studies, data were pooled for 11,033 cases and 13,358 controls. A total of 1,933 (17.5%) cases reported ever IUD use compared to 2,656 (19.9%) controls. Among cohort studies, data were pooled for 629,008 participants with 3,549 incident cases. A total of 72,546 (11.5%) participants reported ever IUD use at enrollment. The associations of ever vs. never IUD use and EOC risk were similar in case-control (pooled OR: 0.92; 95% CI: 0.86-0.98) and cohort studies (pooled OR: 1.00; 95% CI: 0.88-1.13). When meta-analyzed, there was a suggestive 5% lower odds of overall EOC among IUD users (OR: 0.94; 95% CI: 0.88-1.01; p-het = 0.26). We observed heterogeneity by histotype in case-control studies (p=0.0001) but not in cohort studies (p=0.77). After meta-analysis, results suggested that IUD use was associated with a lower risk of mucinous (OR: 0.85; 95% CI: 0.73-0.98), clear cell (OR: 0.69; 95% CI: 0.64-0.86), and low grade serous EOC (OR: 0.75; 95% CI: 0.55-1.01).

Conclusion: IUD use was not associated with an increased risk of EOC in this analysis of over 12,000 ovarian cancer cases. Our findings suggest there may be an inverse association between IUD use and risk of mucinous, clear cell, and low grade serous EOC. Analyses are ongoing and will include assessment of effect modification by parity and birth cohort, as well as consideration of timing of IUD use.

#7378 Trends in head and neck cancer: Incidence, mortality and distant metastasis disparities from SEER data.

L. Mazhar, L. Zhang, J. Ireland, V. Walter, L. Wang, J. M. Yingst, S. I. Allen, J. Foulds, J. Muscat;
Penn State College of Medicine, Hershey, PA

Introduction: Head and neck cancer (HNC) is the seventh most common cancer globally. Approximately 10% of HNCs present with distant metastases (DM) at diagnosis, with a median survival of less than one year. Lower socioeconomic status (SES) has been associated with advanced stage HNCs. Socioeconomic disparities have been reported in HNC incidence and mortality. However, the role of SES among individuals with HNC who develop DM remains less explored. We analyzed trends in the HNC incidence and mortality rates in the U.S. and identified sociodemographic predictors of DM using data from the Surveillance, Epidemiology, and End Results (SEER) database.
Methods: This study used publicly available SEER Data from 2000-2022. Descriptive statistics were calculated for the full incident cohort (N=303,196: human papilloma virus (HPV) related HNC: 85,531; non-HPV: 217,665) and the subset of cases with DM (n=33575). Age-adjusted incidence and mortality rates per 100,000 population and annual percentage changes (APC) were calculated using SEER*Stat version 8.4.4. Poisson regression was used to identify sociodemographic predictors of DM such as age, sex, race/ethnicity, marital status, income, and metropolitan/non-metropolitan status using SAS version 9.4. The 95% confidence intervals (CIs) and p-values for incidence rate ratios (IRR) were reported.
Results: Age-adjusted incidence and mortality rates of HNC were 14.9 and 2.6 per 100,000 population, respectively. Slight decreases in incidence (APC: -0.2; 95% CI: (-0.4, -0.1); p<0.0001) and mortality (APC: -0.6; 95% CI: (-0.8, -0.4); p<0.0001) were observed. Oropharyngeal cancers (HPV-related) exhibited increased incidence (APC: 3.9; 95% CI: (3.1, 4.7); p<0.0001) and mortality (APC: 3.0; 95% CI: (2.5, 3.5); p<0.0001). Laryngeal cancers had decreased incidence (APC: -2.6; 95% CI: (-2.7, -2.4); p<0.0001) and mortality (APC: -2.1; 95% CI: (-2.2, -2.0); p<0.0001). About 11% of HNC cases had DM, with higher rates for individuals aged 50-64 years (IRR: 1.09; 95% CI: (1.06, 1.13) ; ref: 20-49 years), males (IRR: 1.21; 95% CI: (1.18, 1.24)), non-Hispanic (NH) Asian or Pacific Islanders (IRR: 1.50; 95% CI: (1.44, 1.56)); ref: NH White) and those with an income of $40K-$74,999 (IRR: 1.61; 95% CI: (1.48-1.75); ref: $75K or more). Advanced age at diagnosis (80+ years: IRR: 0.89; 95% CI: (0.85, 0.93)), being married or partnered (IRR: 0.84; 95% CI: (0.82, 0.86); ref: others) and living in non-metropolitan areas (IRR: 0.89; 95% CI: (0.86, 0.92); ref: metropolitan areas) were associated with lower rates of DM.
Conclusions: The incidence and mortality rates of HNCs have declined since 2000 whereas the rates have increased for HPV associated HNC. Our study highlights the need for understanding the causes of more advanced disease in subgroups of the population, and to refine the risk categories such as specific Asian or Pacific Islander populations and geographic classification.

#7379 Exploring the epidemiology, clinical outcomes, and healthcare burden of multiple myeloma patients with atrial fibrillation hospitalized for sepsis: a population-based study.

Adamsegd I. Gebremedhen[1], Abdu Mohammed[2], Mamdouh Souleymane[1], Yifang Song[2], Yonas Fetle[1], Mahmoud Abdallah[3], Muhammad O. Jamil[3]

[1]Internal Medicine, Marshall University Joan C. Edwards School of Medicine, Huntington, WV,[2]Internal Medicine, Trinity Health System, Steubenville, OH,[3]Hematology and Oncology, Marshall University Joan C. Edwards School of Medicine, Huntington, WV

Introduction: Multiple myeloma (MM) patients face a high risk of infection-related complications, and many also develop atrial fibrillation (AF), partly due to disease-driven inflammation and treatments like chemotherapy and immunotherapy. While AF is known to worsen outcomes in various cancers, its specific impact on MM patients with sepsis remains unclear. This study investigates how AF influences outcomes in MM patients hospitalized with sepsis, shedding light on an important yet underexplored aspect of their care.
Methods: We identified adult patients with MM from the National Inpatient Sample who were admitted with the principal diagnosis of sepsis from 2018 to 2021, using ICD-10 codes. We divided the cohort based on the presence or absence of AF and applied complex sampling weights to ensure national representativeness. Our primary outcome was all-cause mortality, and we analyzed several secondary outcomes. We used multivariate regression models to evaluate outcome disparities between the groups, with statistical significance set at $p < 0.05$.
Results: We identified 56,970 adults with MM admitted with sepsis between 2018 and 2021, of whom 15,565 (27.3%) had AF. The mortality rates were 14.0% for the entire cohort and 17.9% for the AF group. Patients with AF were older (74.7 vs. 69.0 years, p<0.001), had a higher proportion of male patients (63.2% vs.56.2%, p<0.001), and a higher proportion of white patients (71.5% vs. 59.6%, p<0.001). They also had a higher comorbidity burden (Charlson Comorbidity Index > 3: 89.8% vs. 79.2%, p<0.001). Patients with AF had higher odds of all-cause mortality with an adjusted odds ratio (aOR) of 1.60 (95% confidence interval [CI] 1.41-1.81). They had higher odds of respiratory failure (aOR 1.50, 95% CI 1.36-1.64), mechanical ventilation use (aOR 1.66, 95% CI 1.41-1.96), septic shock (aOR 1.60, 95% CI 1.45-1.78) vasopressor use (aOR 1.64, 95% CI 1.33-2.01), and renal replacement therapy use (aOR 1.69, 95% CI 1.43-2.01). Furthermore, they experienced longer hospital stays (8.6 vs. 7.8 days) with an adjusted incidence rate ratio (aIRR) of 1.14 (95% CI 1.09-1.19) and higher hospitalization charges ($110,329 vs. $99,756) with aIRR of 1.20 (95% CI 1.13-1.28).
Conclusion: This study indicates that MM patients with sepsis and AF have worse outcomes, such as higher mortality, increased need for critical treatments (including mechanical ventilation, vasopressors, and renal replacement therapy), longer hospital stays, and higher medical expenses. These results highlight the necessity for careful and individualized management of MM patients with AF, especially when they develop sepsis.

#7380 Association of weight loss promoting medications with male breast cancer incidence, tumor grade, and stage: a SEER-Medicare nested case-control study.

Aniruddha B. Rathod[1], Biai Dominique Elmir Digbeu[2], Jenil R. Patel[3], David S. Lopez[4]

[1]Department of Epidemiology, Fay W. Boozman College of Public Health, University of Arkansas for Medical Sciences, Little Rock, AR,[2]Department of Biostatistics and Data Science, University of Texas Medical Branch, Galveston, TX,[3]Department of Epidemiology, Human Genetics and Environmental Sciences, UTHealth Houston School of Public Health, Dallas, TX,[4]Peter O'Donnell Jr. School of Public Health,, University of Texas Southwestern Medical Center, Dallas, TX

Background: Obesity has been associated with an increased incidence of male breast cancer (MBC), including the high-grade and advance-stage of the disease. With the rising use of weight loss-promoting medications (WLPMs), including the use of metformin, there is a growing interest in their potential to positively influence cancer outcomes. Yet, there is a poor understanding about the impact of WLPMs and metformin on the incidence of MBC, stage and grade, presenting a significant gap in research.
Methods: We conducted a nested case-control study using the Surveillance, Epidemiology, and End Results (SEER)-Medicare database. The cohort included 2,444 MBC cases and 12,068 matched controls (1:5 by birth year and diagnosis date), all of whom had continuous Medicare Part A/B enrollment for at least one year between 2008 and 2019. Pre-diagnostic exposure to WLPMs and metformin was determined through prescription records. Multivariable weighted conditional logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for MBC incidence, advanced-stage cancer (stage 4), and high tumor grade (grade 3).
Results: Among MBC cases, 83% were Non-Hispanic White, 34% were >=80 years, 75% had hypertension, 56% had hyperlipidemia, 36% had diabetes and 16% used metformin over 6 months prior to index date. Metformin use was associated with a reduced risk of MBC incidence (OR 0.64, 95% CI 0.54-0.74), and high tumor grade (OR 0.57, 95% CI 0.41-0.78). However, it was linked to an increased likelihood of advanced-stage cancer (OR 1.79, 95% CI 1.26-2.56). WLPMs did not significantly affect the incidence, tumor grade and stage of MBC.
Conclusion: Our findings suggest that metformin may reduce the risk of MBC incidence but could be associated with advanced-stage disease at diagnosis. Further research is needed to explore the effects of WLPMs on MBC outcomes.

#7381 Esophageal carcinoma association with chronic liver disease and cirrhosis mortality risk: A population based retrospective study.

Olanipekun Lanny Ntukidem[1], Oboseh John Ogedegbe[1], Sakshi Bai[2], Asfand Yar Cheema[3], Ayobami Gbenga Olafimihan[4], Nosakhare Paul Ilerhunmwuwa[5], Utkarsh Acharya[6], Kai He[6]

[1]Trinity Health Ann Arbor, Ypsilanti, MI,[2]Henry Ford Jackson Hospital, Jackson, MI,[3]Taussig Cancer Center, Cleveland, Ohio, Cleveland, OH,[4]John H. Stroger, Jr. Hospital of Cook County, Chicago, IL,[5]University of Pittsburgh, Pittsburgh, PA,[6]The Ohio State University Comprehensive Cancer Center, Columbus, Ohio, Columbus, OH

Introduction: The American Cancer Society estimates about 22,370 new esophageal cancer (EC) cases and about 16, 130 deaths in the United States for 2024. While the rates of esophageal cancer have decreased slightly in recent years, the chronic liver disease and cirrhosis (CLD & C) mortality rates are upwardly trending. We holistically analyzed the CLD & C mortality risk in EC patients given the multiple risk factors that are common to these diseases.

Methods: Using the Surveillance, Epidemiology and End-Result (SEER) Research Data, 17 registries and Multiple Primary Standardized Incidence Ratio (MP-SIR) registries, a retrospective study of patients diagnosed with esophageal cancer between 2000 and 2021 was conducted. We estimated the standardized mortality ratios (SMR) by dividing the observed chronic liver disease and cirrhosis death rates in our sample over expected dates of the general population, matched to age, gender and ethnicity.

Results: Analysis of the risk based on race revealed a much higher risk of CLD & C in American Indian/Alaska Natives compared to other races. American Indian/Alaska Natives had SMR 555.37 (CI 114.53-1623.04, $p < 0.05$), Asian/Pacific Islanders SMR 89.56 (CI 32.87-194.94, $p < 0.05$), Black SMR 44.52 (CI 21.35-81.88, $p < 0.05$), Whites SMR 20.49 (CI 17.27-24.14, $p < 0.05$). Females had a higher risk of CLD & C mortality (SMR 36.46, CI 22.84-55.18, $p < 0.05$) compared to males (SMR 20.98, CI 17.65-24.76, $p < 0.05$). We also analyzed mortality risk based on staging of esophageal cancer. Localized EC SMR was 24.12 (CI 17.78-31.98, $p < 0.05$), Regional EC SMR 16.09 (CI 11.98-21.26, $p < 0.05$), Distant EC SMR 47.03 (CI 30.43-69.42, $p < 0.05$). Based on age, patients aged <60 years SMR 31.09 (CI 24.10-39.49, $p < 0.05$), patients aged 60-69 years SMR 24.71 (CI 18.99-31.61, $p < 0.05$) and patients aged 70+ years SMR 12.45 (CI 8.51-17.57, $p < 0.05$).

Conclusion: These data should inform screening and preventive strategies for esophageal cancer patients. It is well known that esophageal cancer and CLD & C share multiple risk factors. American Indian/Alaska Natives was noted to have a much higher SMR compared to other races. Primary and Secondary preventive modalities must be strategized to tackle these disparities.

#7382 Trends in next generation sequencing (NGS) testing by social determinants of health for metastatic cancer patients, 2016-2022.

Clementine Adeyemi[1], Louise B. Murphy[2], Tim Bancroft[3], Kristin Moore[2], Stephanie Gallagher[3], Amy Nguyen[2], **Gieira S. Jones**[1]

[1]351 N Sumneytown Pike P.O. Box 1000, Merck, North Wales, PA,[2]Optum Life Sciences, Eden Prairie, MN,[3]Optum Life Sciences, North Wales, PA

Introduction: Next Generation Sequencing (NGS) testing is a precision medicine tool that uses genomic information from patient tumors to inform treatment decisions and direct patient access to targeted therapies. Social determinants of health (SDOH) *partially explain* barriers to healthcare access, which can impact clinical outcomes. Real-world studies examining impact of SDOH on receipt of genomic testing are limited. Objective: To examine annual incidence rates (IR) of NGS testing overall and by SDOH indices in endometrial, lung, and colorectal cancer patients.
Methods: This retrospective cohort study included patients diagnosed with advanced and recurrent endometrial cancer (aEC), metastatic non-small cell lung cancer (mNSCLC), or metastatic colorectal cancer (mCRC), from July 2016 to September 2022, in the Optum Research Database. Annual incident rates (per 365,000 person-days) were calculated for NGS testing and stratified by 5 SDOH domains: social isolation, financial stress, food insecurity, housing insecurity, and transportation difficulty. SDOH measures were categorized as low, medium, or high unmet needs. Full-year NGS testing IRs are reported for trend analysis.
Results: The study examined 14,624 aEC, 33,608 mNSCLC, and 20,437 mCRC mostly White patients (69%), with median age (years): 69 aEC, 72 mNSCLC, and 70 mCRC. NGS testing rates varied across tumors during the study follow up-period with the highest overall rates observed in mNSCLC (146.9), followed by CRC (73.8) and aEC (19.3). IRs increased over time for all cancer groups (1.5X higher in 2021 than 2017 for mNSCLC, and 2X higher for aEC and mCRC). Annual NGS testing trends were similar for racial/ethnic groups in aEC and mCRC, but, Black and Hispanic mNSCLC patients had the two lowest testing rates (IR: 139.3 and 134.8 respectively) compared to White (IR: 147.3), and Asian patients (IR: 186.3). In mCRC patients, those with the highest level of unmet need had the lowest IRs for all SDOH domains except food insecurity; a similar pattern was seen in mNSCLC. Before 2019, rates were lower in aEC patients with high financial stress and food insecurity vs. with those with low unmet need; this trend reversed from 2019-2021. Changing testing trends in aEC patients may result from the issuance of a Medicare national coverage determination for NGS testing.
Conclusion: NGS testing rates have increased for mCRC, mNCSLC and aEC patients since 2017, however, rates differed by level of SDOH. Targeted interventions focused on increasing NGS testing among patients with more unmet SDOH needs could be impactful. Further investigation into the social factors that influence patient access and treatment decisions may help remove existing barriers to optimal patient care.

#7383 Plant-based diet index and risk of hepatocellular carcinoma: Findings from a prospective cohort study.

Yen Thi-Hai Pham[1], Renwei Wang[1], Jaideep Behari[2], Jian-Min Yuan[1], Hung N. Luu[1]

[1]UPMC HIllman Cancer Center, University of Pittsburgh Medical Center, Pittsburgh, PA,[2]Division of Gastroenterology, Hepatology, and Nutrition, Department of Medicine, University of Pittsburgh Medical Center, Pittsburgh, PA

Background: Hepatocellular carcinoma (HCC) accounts for ~90% of primary liver cancer cases, one of leading causes for cancer incidence (>900,000 new cases/year) and mortality (>800,000 deaths/year) worldwide. The established risk factors for HCC are chronic infection with hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol abuse and obesity; among which about two-thirds of HCC cases are attributable to viral hepatitis. Recently, plant-based diets, including overall plant-based dietary index (PDI) and healthful plant-based dietary index (hPDI), which were based on overall intake and sub-categories of food items that deem to be “healthfulness” to better account for the quality of plant foods, have received a great attention due to their potential benefits to chronic diseases. Data on the association between PDI and risk of HCC are sparse. We, thus, prospectively evaluated the associations between PDI and hPDI scores and HCC risk.

Methods: We used data from the Singapore Chinese Health Study, a prospective cohort study of 63,275 participants aged 45-74 living in Singapore. Both PDI and hPDI scores were derived from the semi-quantitative food frequency questionnaire at baseline. The HCC incident cases were identified through record linkage analysis with the Singapore Cancer Registry. Cox proportional hazard regression method was used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for HCC in relation with PDI and hPDI scores. A nested case-control study (197 HCC incident cases and 465 matched controls) was also performed among the cohort participants. Conditional logistic regression model was used for the case-control analysis to examine the association between PDI and hPDI scores with risk of HCC with further adjustment for HBsAg seropositivity status.

Results: After 17.6 years of follow-up with 819,573 person-years, we identified 561 incident HCC cases. We found an inverse association between PDI and HCC risk ($HR_{per\text{-}SD\ increment}$=0.87, 95% CI: 0.79-0.95; P_{trend}=0.009). Compared with the lowest quartile, the HRs (95% CIs) of HCC for quartiles 2, 3, and 4 of PDI score were 0.81 (0.64-1.01), 0.75 (0.59-0.95), and 0.73 (0.56-0.93), respectively. Similar pattern was also found between hPDI and HCC risk ($HR_{per\text{-}SD\ increment}$=0.86, 95% CI: 0.79-0.94; P_{trend}=0.002). Furthermore, this inverse association pattern between PDI and HCC risk was found only in HBsAg-negative individuals in the case-control study ($OR_{per\text{-}SD\ increment}$=0.79, 95% CI: 0.64-0.98; *P*trend =0.04).

Conclusions: Our findings showed that higher plant-based dietary index score was associated with lower risk of HCC. Our findings have public health implications for primary prevention against the development of HCC, particularly relevant in populations such as the United States where HCC incidence rate has been increasing in the past 3 decades dur to high prevalence of obesity, but not due to HBV and/or HCV infection.

#7384 Population attributable fraction of cancer incidence obesity and physical inactivity, in 2020 in the Republic of Korea.

SuJung Byoun

National Cancer Center - Korea, Goyang-si, Korea, Republic of

Abstract Purpose: Cancer is the leading cause of death in Korea. This study aimed to analyze the impact of two major risk factors, obesity and physical inactivity, on cancer incidence and to estimate the population attributable fraction (PAF) for cancers attributable to these factors in Korea as of 2020.
Materials and Methods: For obesity-related cancers, 13 types of cancers (e.g., pharyngeal cancer, gastric cancer, pancreatic cancer, liver cancer) were selected based on the World Cancer Research Fund (WCRF) recommendation. Obesity was categorized using BMI as follows: 16.0 ≤ BMI < 18.5 (underweight), 18.5 ≤ BMI < 23.0 (normal weight; reference), 23.0 ≤ BMI < 25.0 (overweight), 25.0 ≤ BMI < 30.0 (obesity), and BMI ≥ 30.0 (severe obesity). Physical inactivity was defined as having activities less than 600 MET·min/week. Exposure prevalence for obesity and physical inactivity in 2005 was derived from the Korea National Health and Nutrition Examination Survey (KNHANES) assuming 15 years of latency. Relative risks (RR) for cancer types were derived from meta-analyses of relative risks (RR) estimated from the four Korean cohort studies, including the Korean Genome and Epidemiology Study (KoGES), KNHANES, the health examination data from National Health Insurance Service (NHIS), and the Korean Cancer Prevention Study-II (KCPS-II) using Cox proportional hazards models. Confounding variables such as age, smoking status, alcohol consumption, and BMI were adjusted The PAF for each cancer type and overall cancer incidence was calculated using Levin's formula.
Results: For obesity-related cancers, the PAF for overall cancer incidence was 2.35% (5,794 cases attributed to obesity in 2020) for the total population, 2.26% (2,935 cases) for men, and 2.45% (2,859 cases) for women. Among specific cancers, the highest PAFs were observed for kidney cancer (21.09%, men), pharyngeal cancer (27.07%, men), and endometrial cancer (29.38%, women). For physical inactivity, the PAF for overall cancer incidence was 0.39% (464 cases) for the total population, 0.47% (78 cases) for men, and 0.97% (387 cases) for women. Among cancer types, colorectal cancer showed the highest PAF (0.50%, 399 cases) for the total population, while breast cancer in women accounted for 65 cases (PAF 0.26%).
Conclusion: Obesity and physical inactivity significantly contribute to cancer incidence in Korea, particularly for cancers such as kidney, endometrial, colorectal, and breast cancer. These findings underscore the need for targeted public health strategies to manage obesity and promote physical activity as part of comprehensive cancer prevention efforts. Further research should focus on refining risk estimates and exploring additional environmental and genetic factors affecting cancer incidence.
Key Words: Obesity, physical inactivity, population attributable fraction, cancer risk, BMI, physical activity

#7385 Shifting landscapes of cancer-related lifestyle risk factors among U.S. young adults under age 50.

Sina Azadnajafabad, Xiaoyu Zong, Nicholas Jeschke, Yichen Sun, Duo Ren, Yin Cao, on behalf of Cancer Grand Challenges Team PROSPECT

Division of Public Health Sciences, Department of Surgery, Washington University School of Medicine, St. Louis, MO

Introduction: The rising incidence of early-onset cancers among younger adults in the U.S. and globally has been alarming over the past decades. Understanding the evolving patterns of cancer-related lifestyle factors in younger populations is crucial for identifying key risk factors for targeted prevention and emerging exposures for etiological research.
Methods: We utilized the National Health and Nutrition Examination Survey (NHANES) (1999-2023) to characterize age-specific patterns and trends of established risk factors for multiple cancers, including body mass index (BMI), current smoking, alcohol consumption, and physical activity, among adults under age 50. We also examined emerging dimensions of these behaviors, such as weight history, e-cigarette use, binge drinking, and sedentary behaviors.
Results: Across all age groups under 50, mean BMI steadily increased (27.7 to 29.5 kg/m^2), with a sharper increase in later survey cycles for age groups 30-39 and 40-49. The proportion of overweight/obese children and adolescents increased (30.0% to 33.8%). Trends for highest self-reported lifetime weight also followed an upward trajectory (185 to 202 lbs), with 30-39 age group reporting the highest figures in the past decade. Current smoking prevalence decreased across all age groups throughout this period (29.0% to 14.2%), but 30-39 age group exhibited a stabilizing trend recently. While the mean age of smoking initiation among current smokers decreased slightly (17.5 to 17.0 yrs), adolescents reported smoking a full cigarette at a much younger age in the past decade (14.8 to 13.1 yrs). Meanwhile, prevalence of recent e-cigarette use rose sharply (2.8% to 9.0%), with the highest rates in 20-29 age group. While alcohol consumption decreased slightly across all age groups (3.3 to 3.0 drinks/day among drinkers), prevalence of binge drinking more than doubled (11.4% to 27.5%). The 20-29 age group consistently reported highest levels of alcohol intake and binge drinking. Younger adults showed higher prevalence of moderate/vigorous physical activity (58.5% to 61.3%); however, the daily time spent on such activity declined (229 to 206 minutes), particularly among 20-29 and 30-39 age groups. Sedentary behavior time increased for all age groups (342 to 371 minutes), with the highest level observed in adolescents and recent rising trends in 20-29 and 30-39 age groups.
Conclusion: Analyses of nationally representative data in the U.S. revealed substantial shifts in the landscape of cancer-related lifestyle risk factors, underscoring the urgent need to understand the biological impact of age of initiation, cumulative and peak exposures, emerging behaviors, and age-specific exposure patterns on cancer initiation and progression in younger populations. These insights are crucial for informing the development of tailored, life-stage-focused prevention strategies to combat early-onset cancers.

#7386 Economic deprivation and cardiovascular disease risk among long-term breast cancer survivors.

Robina Josiah Willock[1], Daniel Parks[2], Samah Nabi[3], Desiree Rivers[1], Chaohua Li[4]

[1]Community Health and Preventive Medicine; Cancer Health Equity Institute, Morehouse School of Medicine, Atlanta, GA,[2]Community Health and Preventive Medicine, Morehouse School of Medicine, Atlanta, GA,[3]Undergraduate Medical Education, Morehouse School of Medicine, Atlanta, GA,[4]National Center for Primary Care, Morehouse School of Medicine, Atlanta, GA

Background and Purpose: Disparities in cardiovascular disease (CVD) mortality among breast cancer (BC) survivors are well documented. There has been notable progress in identifying and mitigating risk factors for poor health outcomes and improved quality of life for long-term BC survivors. However, the unique impact of structural determinants of health, and the intersections of the community-level and individual-level economic deprivation that perpetuate inequities in BC incidence, screening, treatment options, and risk of comorbidities, are still not cogently described in the literature. Persistent, chronic, or transient poverty exacerbates CVD risk and poor health outcomes among BC survivors. This observational study examined the associations between measures of economic deprivation and CVD outcomes in a national sample of female BC survivors.

Methods: This study used National Health Interview Survey 1999-2022 cohort data to examine the prevalence of CVD, (*angina, stroke, myocardial infarction and coronary artery disease*) among female BC survivors. We evaluated correlational and logistic models of association between socio-demographic characteristics, health behaviors, comorbid conditions, and family poverty-income ratio (PIR) as well as interaction terms between PIR and select predictor variables.

Results: The impact of family PIR varied across different age cohorts and race/ethnicity categories. Race was not independently associated with CVD risk in this population, but significantly moderated the relationship between poverty status and CVD risk among Black breast cancer patients compared to White and Asian breast cancer patients OR=0.08 (95% CI 0.01-0.65) Additionally for middle-aged breast cancer survivors (45-64 yrs), higher socioeconomic status offers substantial protection against CVD (OR=0.0405; 95% CI 0.17-0.96) compared to patients <45yrs and older patients (≥65yrs). While at very advanced ages (>85yrs), the protective effect of higher socioeconomic status becomes less relevant in determining CVD risk. Higher PIR (>5 and 1-4) confers strong protective effects against CVD particularly during the initial seven-year period after BC diagnosis and even among women who seek routine follow-up care (OR=0.52; 95% CI 0.30-0.92).

Conclusions and Implications: Economic deprivation as measured by the poverty income ratio is signaled as an important determinant of cardiovascular disease (CVD) risk among certain subpopulations of breast cancer patients, and its effect is magnified when intertwined with other determinants and sociodemographic characteristics.

#7387 Trends in cancer prevalence among people with type 2 diabetes: an analysis using National Health Interview Survey.

Pranali G. Patel[1], Chaitali S. Dagli[1], Nada Al-Antary[2], Mrudula Nair[2], Abdulghafoor Alani[2], Oluwole A. Babatunde[3], Nosayaba Osazuwa-Peters[4], **Eric Adjei Boakye**[2]

[1]University of Alabama at Birmingham, Birmingham, AL,[2]Henry Ford Health System, Detroit, MI,[3]Prisma Health, Greer, SC,[4]Duke University School of Medicine, Durham, NC

Background: Type 2 diabetes (T2D) is associated with an increased risk of cancers including colorectal (CRC), breast, endometrial, liver, pancreatic, kidney, bladder, lung, and esophageal. Research indicates that individuals with T2D experience a higher incidence of CRC, potentially due to shared risk factors or biological pathway. This study aims to examine and compare trends in the prevalence of CRC to other cancers (in this study includes breast, endometrial, liver, pancreatic, kidney, bladder, lung, and esophageal) among respondents diagnosed with T2D.
Methods: We analyzed data from the 2013-2023 National Health Interview Survey (NHIS), a nationally representative, cross-sectional survey of the U.S. non-institutionalized population that employs a multistage probability sampling design. Adults aged >18 years with a T2D diagnosis, currently on medications, and diagnosed with cancer were included. Joinpoint regression was used to estimate trends in CRC prevalence compared to other cancers among individuals with T2D through annual percent change (APC).
Results: The study included a total weighted sample of 11,766,072 respondents, consisting of 1,893,687 individuals with CRC and 9,872,385 individuals with other cancers. Most respondents were aged 65+ (70%), female (66%), non-Hispanic White (71%) and married (56%). Overall, we observed an increase of 9% per year in the proportion of CRC cases between 2013 and 2015, although not statistically significant, followed by a significant decrease of 4% per year between 2015 and 2023. In contrast, the proportion of other cancer cases remained relatively stable. For females with CRC, the proportion increased by 25% per year from 2013 to 2015, although not statistically significant, but significantly decreased by 8% per year from 2015 to 2023. While among females with other cancers, the proportion decreased by 4% per year between 2013 and 2015, although not statistically significant, but significantly increased 1% per year between 2015 and 2023. The proportion of males with CRC increased 1% per year from 2013 to 2016 and later decreased by 4% per year from 2016 to 2023; neither of which were statistically significant. The proportion of other cancers among males significantly increased by 2% per year from 2013 to 2021 and remained stable between 2021 and 2023.
Conclusion: CRC prevalence has declined overall since 2015 among individuals with T2D. However, sex differences persist, with notable decrease among females in the same time frame. Meanwhile, other cancer trends have remained stable. These findings highlight the complex relationship between T2D and cancer, which may be influenced by various factors such as gender, age, and possible common underlying mechanisms. Further research is needed to explore the underlying causes of these trends and disparities among males and females.

#7388 Trends of diagnosis disclosure among individuals with plasma cell disorders.

Akshay Dalvi[1], Maria Malik[2], Andriy Derkach[3], Ana Sahagun[4], Jorge A. H. Martinez[4], Gia Merlo[5], Jennifer Ahlstrom[4], Jay R. Hydren[4], Saad Z. Usmani[6], Susan Chimonas[7], Robin Ortiz[8], Urvi Shah[6]

[1]Department of Chemistry, University of Illinois Chicago, Chicago, IL,[2]Department of Internal Medicine, New York University Langone Health, New York, NY,[3]Department of Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY,[4]HealthTree Foundation, Lehi, UT,[5]Departments of Nursing and Psychiatry, New York University Grossman School of Medicine, New York, NY,[6]Multiple Myeloma Division, Memorial Sloan Kettering Cancer Center, New York, NY,[7]Center for Health Policy and Outcomes, Memorial Sloan Kettering Cancer Center, New York, NY,[8]Departments of Pediatrics and Public Health, New York University Grossman School of Medicine, New York, NY

Multiple myeloma, along with precancerous conditions such as monoclonal gammopathy of undetermined significance (MGUS) and smoldering myeloma, represent the most common plasma cell disorders (PCDs) around the world. Coping with such disorders is often a challenging prospect for patients, and the disclosure or withholding of information pertaining to the patient's diagnosis or prognosis may have a significant impact on the patient's perception of their care. We hypothesize that patient perceptions of diagnosis disclosure will be largely positive due to the various psychosocial benefits conferred by the sharing of information with friends and family. In order to investigate these diagnosis disclosure patterns among individuals with PCDs and determine areas for interventions that utilize social support strategies to help patients navigate their diagnosis, we invited participants with PCDs through the HealthTree Cure Hub to complete a 34-question online survey regarding their disclosure trends (n= 510). Participants generally reported sharing their diagnosis with at least one person (98%), with the most common responses being a spouse, domestic partner or significant other (60%) and friend(s) (10%). Most participants that chose to disclose their diagnosis experienced a positive impact in their coping (79%) and became closer with others (55%), whereas patients who chose not to disclose their diagnosis generally reported feeling more isolated in their relationships (56%). Despite some reports of certain negative experiences associated with sharing a PCD diagnosis, the majority of participants expressed no regrets over sharing their diagnosis (87%). Based on these findings, patients generally perceive sharing their diagnosis among their primary social networks to be beneficial to their coping. Oncologists may also be advised to promote the utilization of social support to navigate a PCD diagnosis.

#7389 Examining ovarian cancer disparities: The influence of demographics, insurance coverage, and travel distance on survival outcomes.

Sejong Bae

Medicine, University of Alabama at Birmingham, Birmingham, AL

Background: Ovarian cancer continues to be a devastating disease despite advancements in medicine. Significant disparities in care and outcomes exist, particularly among marginalized populations, due to limited healthcare access and the considerable impact of travel distance. Patients in remote or underserved areas often face delays in diagnosis and treatment, adversely affecting survival rates. This study underscores the critical need for ongoing research into early detection methods and personalized treatment strategies, aiming to enhance survival rates and quality of life for ovarian cancer patients while addressing barriers to equitable healthcare access.
Methods: For the national-level analysis, data from the National Cancer Database (NCDB) was utilized, focusing on ovarian cancer patients in the East South-Central region treated at Academic/Research programs, diagnosed between 2004 and 2017, with a minimum of five years of follow-up. At the local level, data was sourced from the Tumor Registry at the O'Neal Comprehensive Cancer Center. Travel distance in the O'Neal data was determined using zip codes and categorized into tertiles—short, intermediate, and long distances. Multivariable logistic regression models were employed to identify factors associated with five-year mortality, adjusting for distance, demographics, cancer stage, and insurance status at the national level.
Results: Both national and local analyses revealed that being Black, older age, and having a higher cancer stage were associated with poorer odds of survival. Nationally, Medicaid recipients (vs. those with private insurance) had worse survival odds. Travel distance was not significantly associated with five-year mortality in the South at the national level. However, data from O'Neal showed that among ovarian cancer patients diagnosed from 2006-2015, longer travel distance was associated with lower five-year mortality compared to those with shorter travel distances to the treatment center.
Discussion: Ovarian cancer remains a lethal disease with significant disparities, affecting Black patients, older individuals, and Medicaid recipients the most. While travel distance did not impact survival rates nationally, the O'Neal data revealed an unexpected association between longer travel distances and better survival rates, likely due to access to specialized care. These findings highlight the urgent need for equitable healthcare access and continued research to improve early detection and treatment strategies.

#7390 Differences in cancer and all cause mortality among non Hispanic Black American adults by nativity and duration of U.S. residence: Findings from the 1997-2018 NHIS-NDI record linkage study.

A. K. Abubakar[1], P. T. Nguyen[2], M. Rahman[1];

[1]St. Luke's International University, Tokyo, Japan, [2]Hitotsubashi Institute for Advanced Study (HIAS), Hitotsubashi University, Tokyo, Japan

Background: Non-Hispanic Black (NHB) Americans bear a high burden of cancer and all-cause mortality. Previous studies show that foreign-born individuals generally have lower cancer mortality rates, though declines are more pronounced among U.S.-born NHBs. This study examined differences in cancer and all-cause mortality risk between U.S.-born and foreign-born NHB adults as well as by duration of U.S. residence among the foreign-born.

Method: We used pooled data from the 1997-2018 National Health Interview Survey, linked to the National Death Index with follow-up through December 31, 2019, including 90,487 adult NHB participants. Cox regression models were used to estimate hazard ratios by place of birth and duration of residence in the U.S. after adjusting for sociodemographic and health behavior factors.

Result: The intra-racial disparity in cancer mortality has been continuously observed over the past several decades among NHBs by their place of birth (foreign-born versus US-born). Multi-pronged research strategies are needed to understand this disparity as well as to plan appropriate interventions to address this gap among NHB.

#7391 Longitudinal changes in the gut microbiome among newly diagnosed colorectal cancer patients.

Maria F. Gomez[1], Stephanie R. Hogue[1], Jessica R. Burns[1], Seth I. Felder[2], Esther Jean-Baptiste[1], Adetunji T. Toriola[3], Jane C. Figueiredo[4], Christopher I. Li[5], David Shibata[6], Victoria Damerell[7], Christoph Kahlert[7], Erin M. Siegel[1], Rashmi Sinha[8], Emily Vogtmann[8], Sheetal Hardikar[9], Mary Playdon[9], Leigh Greathouse[10], Biljana Gigic[7], Cornelia M. Ulrich[9], Doratha A. Byrd[1]

[1]Cancer Epidemiology, H. Lee Moffitt Cancer Center, Tampa, FL,[2]Department of Gastrointestinal Oncology, H. Lee Moffitt Cancer Center, Tampa, FL,[3]Division of Public Health Sciences, Department of Surgery, Washington University School of Medicine and Siteman Cancer Center, St. Louis, MO,[4]Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA,[5]Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA,[6]Department of Surgery, University of Tennessee Health Science Center, Memphis, TN,[7]Department of General, Visceral and Transplantation Surgery, Heidelberg University Hospital, Heidelberg, Germany,[8]Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institute of Health, Rockville, MD,[9]Department of Population Health Sciences, University of Utah, Salt Lake City, UT,[10]Department of Human Sciences and Design, , Robbins College of Health and Human Sciences, Baylor University, Waco, TX

Background: The gut microbiome may be linked to colorectal cancer (CRC) prognosis. Few studies characterized the gut microbiome longitudinally across CRC treatment. Herein, we investigated the gut microbiome dynamics before and up to two years after surgery among newly diagnosed CRC patients.
Methods: We extracted DNA from stool collected longitudinally at baseline (pre-surgery) and at 6, 12, and 24-months after in the ColoCare Study. We characterized fecal bacteria using 16S rRNA gene sequencing (16S; N=133; baseline and 6 months only) and whole-genome shotgun sequencing (WGS; N=41; all timepoints). We estimated rarefied alpha diversity (richness and Shannon index) and beta diversity (Bray Curtis). We calculated centered-log ratio transformed relative abundances of *a priori*-selected taxa and functional genes (WGS only). We used permutational multivariate analysis of variance to estimate the percentage explained in variability of the Bray Curtis distance matrix. We estimated average differences in the microbiome metrics across time using linear mixed effects models adjusted for study center and treatment.
Results: In total, 43% of the cohort received adjuvant therapy and 24% received neoadjuvant therapy. On average, from baseline to 6 months, alpha diversity decreased, with statistically significant declines in the Shannon index for 16S (β = -0.32, 95% CI = -0.47, -0.17; *P* <0.001) and WGS (β = -0.38, 95% CI = -0.56, -0.20; *P* <0.001). WGS alpha diversity tended to somewhat recover at 12 and 24 months. Bray Curtis distance variation was primarily explained by subject (e.g., for 16S: 75%, *P*=0.03); whereas timepoint explained about 3% of variation in 16S and WGS Bray Curtis distance (all *P* < 0.05). Among 16S genera (presented per 1-unit increase in log ratio), there was an average 0.33-unit increase in *Fusobacterium* (95% CI = 0.05, 0.62; *P*= 0.02) and a -0.98-unit decrease in *Prevotella* (95% CI = -1.41, -0.56; *P* <0.001) abundance over 6 months. At the species-level (WGS), there were progressively stronger decreases from baseline up to 24 months in multiple potentially pathogenic *a priori* bacteria. For example, the changes β's (95% CIs) in *Porphyromonas asaccharolytica* were -1.33 (-2.11, -0.57; *P*<0.001) at 6 months, -1.53 (-2.33, -0.74; *P*<0.001) at 12 months, and -1.18 (-2.05, -0.31; *P*=0.01) at 24 months. Acetolactate synthase gene abundance, involved in branch chain amino acid synthesis, increased on average from baseline to 6 and 12 months.
Conclusion: We found that microbial diversity tended to decrease over time, recovering somewhat around a year. We found that the abundance of multiple oral-originating species previously associated with CRC presence and prognosis tended to decrease over time (except *Fusobacterium* in the less detailed 16S data). Overall, our intriguing findings highlight that the microbiome may shift after tumor removal. Next steps will interrogate these shifts as they relate to clinical outcomes.

#7392 The effects of socioeconomic deprivation on the tumor microenvironment of bladder cancer.

Tom Marco[1], Kenneth Barker[1], Jesse Ritter[1], Numaan Mahmood[1], Rishab Srivastava[1], Amith Rao[2], Isaac Zarif[1], Ricardo Estrada Mendizabal[3], Sachin Deshmukh[4], Edgar Tapia[4], Jose Guillen-Rodriguez[1], Juan Chipollini[1], Alejandro Recio-Boiles[1]

[1]University of Arizona, Tucson, AZ,[2]Banner University Medical Center, Tucson, AZ,[3]Mayo Clinic, Phoenix, AZ,[4]CARIS Life Sciences, Phoenix, AZ

Background: Bladder cancer patients with lower socioeconomic status (SES) often present with more aggressive disease and face poor outcomes, likely due to complex interactions with risk factors linked to lower SES. However, little is known about the effect of low SES on bladder tumor biology at the molecular level. This study sought to characterize better the impact of low SES on the bladder tumor microenvironment.

Methods: This is a retrospective analysis of 93 patients (63 male and 30 female) ranging from age 33 to 92 presenting to the University of Arizona Cancer Center with bladder cancer from 2016-2023. Each patient's neighborhood level of SES was scored using the Area Deprivation Index on a scale of 1-100, with 1 representing the highest SES and 100 the least. These patients were then split into tertile 1: 1-33 (T1: N=16), tertile 2: 34-67 (T2: N=43), and tertile 3: 68-100 (T3: N=34), and each patient's tumor microenvironment and mutational landscape was characterized and compared utilizing NGS (592, NextSeq; WES, NovaSeq) and WTS Sequencing (NovaSeq) (Caris Life Sciences, Phoenix, AZ). Statistical significance was determined using chi-square and Mann-Whitney U ($p<0.05$).

Results: Our study had no significant difference between age, sex, and race between the three tertiles. Hispanic ethnicity was significantly higher in T3 (26.5%) when compared to T2 (7%) and T1 (0%), p=0.013. The frequency of TMB-H (≥10 mutations/megabase) was significantly lower in T3 (15.6%) when compared to T2 (51.2%, p=0.02) and T1 (50%, p=0.01). There was significantly less T cell CD8+ infiltration in tertile 3 (0.02%) when compared to T2 (0.43%, p=0.04) and T1 (0.37%, p=0.08), and less dendritic cell infiltration in T3 (0%) when compared to T2 (0.1%, p=0.13) and T1 (0.64%, p=0.008). T regulatory cells were lower in T3 (1.37%) compared to T2 (2.13%, p=0.09) and T3 (2.02%, p=0.24). IHC-PD-L1 and gene expression for immune checkpoint genes, MHC-Class I, and MHC-Class II did not vary significantly between tertiles. KMT2C-mt was highest in T2 (21.1%) compared to T1 (3.12%, p=0.03) and T3 (6.67%). There was no other significant differences in mutation prevalence between the three tertiles.

Discussion: In our study, lower SES bladder cancer patients had a lower amount of CD8+ cells, dendritic cell infiltration, and a low frequency of TMB-H, indicating a lower level of adaptive immune response present in the tumor microenvironment. Lower levels of adaptive immune activity have been associated with more aggressive tumor behavior and worse outcomes in bladder cancer patients. These findings provide a partial explanation of the molecular mechanisms underlying the poor prognosis observed in low SES patients with bladder cancer. Larger-scale studies exploring the impact of SES on tumor biology are essential as they hold significant therapeutic and preventive implications.

#7393 Occupational benzene exposure and solid cancers: A systematic review and meta-analysis.

P. Boffetta[1], M. Seyyedsalehi[2];
[1]Stony Brook University, Stony Brook, NY, [2]University of Bologna, Bologna, Italy

Benzene, an aromatic hydrocarbon, is an established cause of leukemia. The association between benzene exposure and solid cancers is currently being investigated. We conducted a systematic review and meta-analysis to assess the association between occupational benzene exposure and the incidence and mortality of breast, female genital, kidney, bladder, prostate, testis, esophageal, gastric, colorectal, liver, gallbladder, pancreas, lung, head and neck, brain, and skin cancers. We included cohort and case-control studies referenced in the 2018 IARC Monograph on benzene and also reviewed studies published up to April 2024 in PubMed, SCOPUS, and EMBASE. We evaluated the quality of the studies using a modified version of the Newcastle-Ottawa Scale. We used forest plots of relative risk (RR) to present and analyze the data and employed a random-effects model to address heterogeneity between studies. Stratified analyses were performed to explore potential effect modification. Our meta-analysis included 80 independent studies, and the findings are summarized in Table 1. The results of our study indicated an association between overall occupational benzene exposure and breast, kidney, colorectal, head and neck, and brain cancer and a dose-effect association between benzene exposure and lung, hepatobiliary, bladder cancer. In addition, non-significant RR>1.25 were derived for ovarian and gallbladder cancer. Additional well-designed studies, incorporating detailed exposure assessment and control for potential confounders, are required to clarify these relationships. It is essential to implement occupational safety measures, such as proper ventilation, personal protective equipment, and workplace monitoring, to minimize exposure and safeguard workers in industries where benzene is present.

Table 1: The combined relative risk (RR) of occupational benzene exposure and various types of solid cancer.

Cancer type	**RR (95%CI), I2, p value**	**Number of risk estimates**	**Publication bias**
Breast	**1.08 (1.03-1.14), I^2=38.6%, p=0.03**	23	0.03
Cervix	1.24(0.89-1.73), I^2<0.001%, p=0.21	6	0.43
Endometrium	1.21(0.87-1.68), I^2=0.001%, p=0.26	4	0.36
Ovary	1.28(0.77-2.14), I^2=57.7%, p=0.35	6	0.50
Kidney	**1.20(1.03-1.39), I^2=91.6%, p<0.001**	31	0.81
Bladder	1.07(0.97-1.18), I^2=44.4%, p=0.003	35	0.75
Prostate	1.00 (0.93-1.08), I^2=12.6%, p=0.27	29	0.12
Testis	1.09(0.53-2.23), I2=1.09%, P=0.80	5	0.32
Esophagus	1.06(0.87-1.30), I^2=68.7%, p<0.001	27	0.004
Stomach	1.07(0.85-1.33), I^2=89.5%, p<0.001	35	0.60
Colorectal	**1.10 (1.06-1.14), I^2=0.0%, p=0.95**	28	0.90
Liver	0.94 (0.80- 1.11), I^2=13.0%, p=0.31	15	0.23
Gallbladder	1.48 (0.86- 2.53), I^2=71.9%, p<0.001	8	0.37
Pancreas	0.97 (0.87- 1.09), I^2=21.6%, p=0.16	26	0.28
Lung	1.00(0.93-1.07), I^2=84.9%, p<0.001	45	0.16
Head and neck	**1.27(1.00-1.64), I^2= 0%, P =0.05**	29	0.10
Brain	**1.14(1.01-1.29), I2=22.1%, p=0.13**	32	0.05
Skin melanoma	0.98(0.79- 1.22), I^2=21.2%, p=0.22	14	0.70
Skin non-melanoma	1.14(0.90- 1.43), I^2=40%, p=0.01	18	0.08

#7394 Investigating the synergistic carcinogenic effect of tobacco and marijuana smoke: A retrospective chart review study.

Ryan P. Kimball[1], Paul J. Wilkinson[1], Eric C. Lis[1], Jacqueline W. Poole[2], Ryan W. Walters[3]

[1]Creighton University School of Medicine, Omaha, NE,[2]Division of Pulmonology, Critical Care, and Sleep Medicine, Creighton University, Omaha, NE,[3]Department of Clinical Research and Public Health, Creighton University, Omaha, NE

Introduction: Tobacco is the leading preventable cause of lung cancer, and both duration and quantity of tobacco smoking are positively correlated with risk of lung cancer.[1-2] Marijuana has long been speculated to increase the risk of developing lung cancer due to many shared carcinogens with tobacco and its ability to cause cancer cells to grow in vitro.[3] While a potential synergistic carcinogenic effect of marijuana and nicotine has been suggested, no clinical studies have yet confirmed this.[4-5]

Methods: The electronic medical record system was utilized to identify patient age at diagnosis and lung cancer stage at diagnosis in patients with biopsy-confirmed lung cancer in the CHI Health System (Omaha, NE) from January 1, 2017 to December 31, 2022. A total of 156 patients with a history of smoking both tobacco and marijuana concurrently met inclusion criteria. Another 135 patients with a history of smoking tobacco (but not marijuana) were matched as controls according to shared demographic data as well as quantity and duration of tobacco use. One way analysis of variance (ANOVA) and chi-square analysis was used to compare data between groups.

Results: The average age at diagnosis of patients who smoked tobacco and marijuana was 61.3 ± 7.5, compared to 64.0 ± 7.8 for patients who smoked only tobacco (p-value = 0.041). Of patients who smoked tobacco and marijuana, 83 (53%) had stage III or IV lung cancer at time of diagnosis, compared to 75 (56%) patients who smoked only tobacco (p-value > 0.05).

Conclusions: These results suggest that the concurrent use of marijuana and tobacco is associated with a lower age at diagnosis of lung cancer compared to the use of tobacco alone. There was no difference in proportion of patients with advanced stage lung cancer at time of diagnosis between groups. These findings warrant further research into a potential additive carcinogenic effect of smoking tobacco and marijuana concurrently.

References: 1. Peto R, Lopez AD, Boreham J, Thun M, Heath C Jr. Mortality from tobacco in developed countries: indirect estimation from national vital statistics. Lancet. 1992;339(8804):1268-1278. 2. Alberg AJ, Brock MV, Ford JG, Samet JM, Spivack SD. Epidemiology of lung cancer: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest. 2013;143(5 Suppl):e1S-e29S. 3. Tashkin DP. Marijuana and Lung Disease. Chest. 2018;154(3):653-663. 4. Mehra R, Moore BA, Crothers K, Tetrault J, Fiellin DA. The association between marijuana smoking and lung cancer: a systematic review. Arch Intern Med. 2006;166(13):1359-1367. 5. Fligiel SE, Roth MD, Kleerup EC, Barsky SH, Simmons MS, Tashkin DP. Tracheobronchial histopathology in habitual smokers of cocaine, marijuana, and/or tobacco. Chest. 1997;112(2):319-326.

PREVENTION / EARLY DETECTION / INTERCEPTION: Genetic and Environmental Determinants of Risk and Screening Methods
Poster Session

#7397 A multi-ancestry polygenic risk score improves lung cancer risk stratification across diverse populations.

N. Adler[1], T. Chen[2], J. Byun[3], C. Amos[3], Q. Lan[4], D. C. Christiani[2], M. Johansson[5], J. McKay[5], M. T. Landi[4], G. Liu[6], L. Le Marchand[7], The International Lung Cancer Consortium, E. J. Parra[8], L. Kachuri[9], H. Zhang[4], R. J. Hung[1];

[1]University of Toronto; Lunenfeld-Tanenbaum Research Institute, Sinai Health, Toronto, ON, Canada, [2]Harvard T.H. Chan School of Public Health, Boston, MA, [3]University of New Mexico, Albuquerque, NM, [4]National Cancer Institute, Bethesda, MD, [5]International Agency for Research on Cancer, World Health Organization, Lyon, France, [6]Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada, [7]University of Hawaii Cancer Center, Honolulu, HI, [8]University of Toronto, Toronto, ON, Canada, [9]Stanford University, Stanford, CA

Background: Currently implemented lung cancer risk prediction models consider a limited set of risk factors and insufficiently stratify patients for screening, leaving a large proportion of lung cancer patients, particularly those from non-European ancestries, ineligible for low-dose CT scans. Polygenic risk scores (PRS) have demonstrated potential for improving lung cancer risk prediction, but are predominantly derived from participants of European-ancestry, limiting their applicability to other populations. This study aims to develop a novel multi-ancestry PRS for lung cancer and evaluate its ability to enhance equitable risk stratification.

Methods: We constructed multi-ancestry PRS using the largest available lung cancer genome-wide associate studies (GWAS), which include 33,023 European cases and 339,471 controls, 11,506 East Asian cases and 179,654 controls, and 2,379 African American cases and 6,908 controls. We trained four genome-wide multi-ancestry PRS models (PRS-CSx, JointPRS, CT-SLEB, and PROSPER) using a reference panel of 1,287,077 SNPs, and compared them to a previously published 128-SNP PRS based on known lung cancer risk loci. PRS models were validated in 1,047 lung cancer cases and 214,403 controls from the All of Us Research Program biobank and their performance was evaluated in a pooled multi-ancestry analysis as well as stratified by genetic ancestry.

Results: PRS-CSx demonstrated the highest performance among all tested methods, with an adjusted-AUC (conditional on sex, age, and the top 16 principal components) of 0.60 (95% CI: 0.58-0.62) and an OR of 1.43 (95% CI: 1.35-1.52) in the pooled analysis. Comparable performance was observed in the European ancestry subgroup (OR: 1.48, 95% CI: 1.37-1.59, 697 cases, 112,145 controls). The PRS-CSx PRS also performed well in the African American population (OR 1.36, 95% CI: 1.17-1.58, 178 cases, 47,701 controls), and in the Admixed American/Latino population (OR: 1.34, 95% CI: 1.03-1.74, 55 cases, 32,936 controls). Individuals in the top decile of the PRS distribution had a 1.96-fold increased risk of lung cancer (95% CI: 1.60-2.40) compared to the average group in the 40-60th percentile. Furthermore, on average, individuals in the top decile reached a 1.5% 5-year absolute risk of lung cancer 8 years earlier than those with average PRS values.

Conclusions: Multi-ancestry PRS outperform a single-ancestry PRS model. Integrating a multi-ancestry PRS into a lung cancer risk prediction model can improve risk stratification, addressing disparities in screening eligibility across diverse populations. This approach has the potential to improve early detection and reduce lung cancer mortality equitably across ancestry groups.

#7398 Defining prostate cancer polygenic risk score categories: Reference populations and methods used impact risk interpretation.

B. Guo[1], A. Sahimi[2], X. Sheng[3], S. Cheng[4], F. Chen[3], C. A. Haiman[3], D. V. Conti[3], B. Darst[1];
[1]Fred Hutchinson Cancer Center, Seattle, WA, [2]New York University Grossman School of Medicine, New York City, NY, [3]University of Southern California/Norris Comprehensive Cancer Center, Los Angeles, CA, [4]University of Washington School of Public Health, Seattle, WA

Background: Polygenic risk scores (PRS) could effectively identify individuals at higher risk of prostate cancer (PCa) and may have notable implications for screening. However, PRS risk classification depends on the comparison population.
Objective: We investigated how different reference populations and methods for defining PRS risk categories affect interpretation.
Methods: We evaluated a multi-ancestry PCa PRS in 6,194 cases and 24,693 controls from self-identified non-Hispanic White (WH), African American (AA), Japanese American (JA), and Latino American (LA) populations in the Multiethnic Cohort (MEC). The PRS was based on 451 variants identified in a prior PCa GWAS across four ancestries. MEC participants were categorized into PRS deciles using 1) PRS distribution of MEC controls within each population or across all populations combined; 2) MEC controls' PRS distribution adjusted for the first 10 principal components (PCs); 3) PRS distribution from external reference populations within each matched continental ancestry or across all ancestries; and 4) individualized PRS deciles calculated as a linear combination of ancestral proportions and external reference populations' decile cutoffs. Reference populations for methods 3 and 4 included 1,890 EUR, AFR, EAS, and AMR individuals from 1KGP and the PAGE Global Reference Panel, chosen for their close genetic resemblance to respective populations based on PCA. Additionally, 41,825 EUR, 18,636 AFR, 1,263 EAS, and 9,888 AMR individuals from All of Us (AoU), defined by genetic ancestry, were also used as reference population for methods 3. Logistic regression models adjusted for age and the first 10 PCs were used to evaluate the association between PRS deciles and PCa risk.Results: Non-population-specific reference group cutoffs approaches consistently had higher PRS predictive ability than population-specific cutoffs for the top 90%-100% PRS decile across all methods and populations. For instance, the OR for AA men in the top 90%-100% PRS decile was 4.37 [3.49-5.48] and 5.29 [3.85-7.27] when using the PRS distribution of MEC controls within each population and across all populations combined, respectively. Similarly, the OR was 4.60 [3.35-6.30] and 5.32 [4.18-6.79] when using 1KGP/PAGE, and 4.73 [3.74-6.00] and 5.41 [4.14-7.07] when using AoU as reference population. There was high variability in cases and controls selected in the top 90%-100% PRS decile across methods. For instance, the top decile included 9,447/1,264, 485/440, and 750/872 cases/controls when using MEC controls, 1KGP/PAGE, and AoU, respectively, to define non-population-specific PRS decile cutoffs for AA men.
Conclusion: PRS interpretation and individual risk categorization are highly dependent on reference populations and methods used to define risk categories, which have critical clinical implications when implementing PRS.

#7399 Polygenic risk score-based smoking and drinking behaviors and head and neck squamous cell cancer risk by HPV status.

T. Hou[1], A. Thakral[2], K. Hueniken[1], T. Dudding[3], M. Gormley[3], S. Virani[4], A. Olshan[5], B. Diergaarde[6], T. Waterboer[7], K. Smith-Byrne[8], P. Brennan[8], D. Neil Hayes[9], E. Sanderson[3], C. Brown[1], S. Huang[1], D. Conway[10], C. Canova[11], S. Bratman[1], A. Spreafico[1], L. Richiardi[12], J. D. Almeida[1], J. Davies[13], L. Bierut[14], M. Dong[15], P. Lagiou[16], A. Lagiou[17], J. Polesel[18], C. Healy[19], M. Nygard[20], A. Agudo[21], L. Alemany[21], W. Ahrens[22], I. Holcatova[23], A. Znaor[8], D. Goldstein[1], O. Espin-Garcia [24], R. Hung[13], W. Xu[1], J. J. Lee[25], G. Liu[1];
[1]UHN Princess Margaret Cancer Centre, Toronto, ON, Canada, [2]University of California, San Francisco, San Francisco, CA, [3]University of Bristol, Bristol, United Kingdom, [4]International Agency for Research on Cancer, Paris, France, [5]University of North Carolina, Chapel Hill, NC, [6]University of Pittsburgh, Pittsburgh, PA, [7]German Cancer Research Center (DKFZ), Heidelberg, Germany, [8]World Health Organization, Lyon, France, [9]University of Tennessee Health Science Center, Memphis, TN, [10]University of Glasgow, Glasgow, United Kingdom, [11]University of Padua, Padova, Italy, [12]University of Turin and CPO-Piemonte, Turin, Italy, [13]Sinai Health, Toronto, ON, Canada, [14]Washington University in St. Louis, St Louis, MO, [15]University of Toronto, Toronto, ON, Canada, [16]University of Athens, Goudi, Greece, [17]University of West Attica, Athens, Greece, [18]Centro di Riferimento Oncologico di Aviano (CRO) IRCCS, Aviano, Italy, [19]Trinity College Dublin, Dublin, Ireland, [20]Norwegian Institute of Public Health, Oslo, Norway, [21]Centro de Investigacion Biomedica en Red de Epidemiologia y Salud Publica, Madrid, Spain, [22]University of Bremen, Bremen, Germany, [23]Charles University, Prague, Czech Republic, [24]Western University, London, ON, Canada, [25]Sinai Health System, Toronto, ON, Canada

Objective: HPV-positive and HPV-negative head and neck squamous cell carcinoma (HNSCC) are distinct entities from an epidemiological and biological standpoint. We aimed to evaluate the predictive performance of polygenic risk scores (PRS), which could be a surrogate when behavioral data are missing or deemed unreliable, for tobacco smoking (Smoking Initiation [SI], Age of Smoking Initiation, Cigarettes Per Day [CPD], Smoking Cessation) and alcohol consumption (Drinks Per Week [DPW]) and their impact on HNSCC risk stratified by HPV status.
Methods: We analyzed data from 8013 participants in the VOYAGER consortium, including 4716 HNSCC cases and 3297 controls. PRS for the 5 phenotypes were generated based on the genome-wide significant variants ($P < 5\times10^{-8}$, $r^2 < 0.001$) from the GSCAN consortium. Logistic regression models were used to evaluate the association between PRS and HNSCC risk, separately in HPV-positive and negative patients. A corresponding phenotype analysis based on actual smoking and alcohol behaviors is included for comparison.
Results: All 5 PRS significantly predicted corresponding behaviors in the expected direction. For HPV-positive patients, higher PRS for SI and DPW were associated with a higher HNSCC risk. For HPV-negative patients, higher PRS for SI, CPD, and DPW had a higher risk of HNSCC. Phenotype analyses confirmed the predictive value of PRS and showed strong correlations between all 5 behaviors and HNSCC risk in both HPV-positive and negative patients.
Conclusion: PRS-based models of smoking and drinking behaviors and are associated with HNSCC risk in both HPV-positive and negative patients. These findings highlight the role of a genetic predisposition to these behaviors as a surrogate marker influencing the risk of HNSCC.

Table. PRS Analyses for Smoking and Drinking Behaviors in Predicting HNSCC Risk by HPV Status.

PRS of smoking and drinking behaviors predicted HNSCC risk				
HPV status	Behaviors	OR	95% CI	*P*
Positive	Smoking Initiation	1.10	1.05-1.16	1.18×10^{-4}
	Age of Smoking Initiation	1.01	0.96-1.06	0.681
	Cigarettes Per Day	0.99	0.94-1.04	0.676
	Smoking Cessation	1.02	0.97-1.07	0.495
	Drinks Per Week	1.24	1.18-1.30	3.50×10^{-16}
Negative	Smoking Initiation	1.07	1.01-1.13	0.041
	Age of Smoking Initiation	0.98	0.93-1.05	0.595
	Cigarettes Per Day	1.06	1.01-1.13	0.047
	Smoking Cessation	1.05	0.99-1.12	0.090
	Drinks Per Week	1.24	1.16-1.32	1.82×10^{-11}

#7400 Prevention outpatient clinic: evidence-based, risk-stratified counseling and prevention research.

Ursula M. C. Will

Prevention Outpatient Clinic, German Cancer Research Center, Heidelberg, Germany

The Prevention Outpatient Clinic (POC), the centerpiece of the National Cancer Prevention Center (NCPC), is being established in Heidelberg through a long-term partnership between the German Cancer Research Center and the German Cancer Aid. This initiative aims to systematically expand evidence-based, personalized cancer prevention across Germany, integrating all essential elements of prevention, from basic research and clinical trials to public counseling and the training of prevention specialists, under one roof. Inspired by the Comprehensive Cancer Centers model, the NCPC seeks to create a comprehensive and sustainable framework for cancer prevention. At the POC, visitors complete a digital questionnaire before engaging in a personalized consultation with medical experts. A specialized prevention counseling guide is used to develop tailored, evidence-based recommendations aimed at improving overall health and addressing specific cancer risk factors. This guidance encourages positive lifestyle changes, such as smoking cessation, healthier dietary choices, weight management, and increased physical activity. To further support these changes, the POC provides programs that focus on behavioral therapy, motivational techniques, psychoeducation, group nutritional counseling, and physical activity initiatives. The clinic collaborates with external partners to offer additional services, such as specialized smoking cessation programs, dietary advice, targeted exercise opportunities, age-appropriate cancer screenings, and genetic counseling, ensuring a comprehensive approach to prevention. In addition to its counseling services, the POC is deeply integrated into cancer prevention research. Participants are invited to contribute biosamples (e.g. blood, urine, stool, saliva) for a long-term registry study, forming a valuable resource for future research. Collaborative studies with DKFZ and partner institutions explore areas such as genetic risk profiling, biomarker discovery, innovative early detection methods, and digital prevention tools. Dedicated research studios enable in-depth exploration of the roles of nutrition and physical activity in cancer prevention. Data collected from these activities feed into a comprehensive database, driving the ongoing refinement of prevention strategies and the development of innovative interventions. Regular follow-up appointments ensure sustained support and allow the POC to monitor the effectiveness of its services, adapting them based on emerging scientific evidence. Once piloted and refined, the POC model will be scaled to other locations across Germany. This expansion will make personalized prevention counseling and participation in cutting-edge research accessible to all, empowering citizens nationwide to actively engage in reducing cancer risks and improving long-term health outcomes.

#7401 Ancestral impact on family history of breast cancer and high-risk mutations among women from Lima, Peru.

Amanda F. Corpuz[1], Xiaosong Huang[1], Elad Ziv[2], Susan L. Neuhausen[3], Laura Fejerman[1], Tatiana Vidaurre[4]

[1]University of California, Davis, Davis, CA,[2]University of California San Francisco, San Francisco, CA,[3]City of Hope Comprehensive Cancer Center, Duarte, CA,[4]Instituto Nacional de Enfermedades Neoplasicas, Lima, Peru

Family history of breast cancer (FHBC) and carrying a high penetrance mutation are strong predictors of breast cancer risk. Breast cancer associated genes have been characterized and pathogenic/likely pathogenic variants (P/LP) identified in different populations. However, characterization of family history and high-risk mutations within the Indigenous American genomic background is still understudied. The Peruvian Genetics and Genomics of Breast Cancer Study (PEGEN-BC) is a case cohort of ~2,000 breast cancer patients from Lima, Peru. Demographic, and clinical information was collected from all participants. Targeted sequencing of 40 susceptibility genes was performed in 1,364 women from the PEGEN-BC study. Variants were called by GATK HaplotypeCaller and annotated by ANNOVAR with the ClinVar database (v20240611). Global ancestry proportions (Indigenous American, European, African, and East Asian) were estimated for another project using the program Admixture (v1.3.0, k = 4). Mean differences of proportions between groups based on FHBC (1st to 3rd degree relatives) were tested using t-test and chi-squared test respectively. The association between FHBC and P/LP mutation status was tested using a logistic regression model stratified by ancestry (dichotomized: above and below the median European genetic ancestry), including age at diagnosis as a covariate. The average age of the study participants was similar between those with FHBC (49.6 years, standard deviation (sd)=11.1) and those without FHBC (49.9 years, sd=11.1). Average Indigenous American ancestry was 77% (sd=16) among participants with FHBC and 70% (sd=19) among those without ($p<0.0001$) and European ancestry was 21% (14) and 17% (11) respectively ($p<0.0001$). There were 62 (4%) carriers of P/LP variants among individuals without FHBC and 44 (10%) among those with FHBC ($p<0.0001$). Among participants with European ancestry below the median (<18%), the association between FHBC and P/LP status was not significant. However, among women with European ancestry above the median, the odds of carrying a P/LP variant if they had FHBC were 4.1 times (95%CI 2.16-7.77) higher than if they did not have FHBC ($p<0.0001$). These results may reflect disparities in patients knowledge of family history by ancestry, which correlates with factors such as socioeconomic status and level of education, as well as difference in the proportion of genetic variants contributing to familial aggregation of risk in different genetic ancestry backgrounds.

#7402 High frequency of germline mutations among breast cancer patients in Jamaica.

Patrick Roberts[1], Rory Thompson[2], Richard Shaw[1], Alison Ayoub[3], Imani Hewitt[3], Derria Cornwall[1], Natalie Guthrie-Dixon[4], Camille Ragin[5], Simone Ann Marie Badal[3]

[1]Department of Surgery, Radiology, Anesthesia & Intensive Care, Univ. of the West Indies - Mona Campus, Kingston, Jamaica,[2]Department of Pathology, Univ. of the West Indies - Mona Campus, Kingston, Jamaica,[3]Basic Medical Sciences, Univ. of the West Indies - Mona Campus, Kingston, Jamaica,[4]Caribbean Institute for Health Research, Univ. of the West Indies - Mona Campus, Kingston, Jamaica,[5]Fox Chase Cancer Center, Philadelphia, PA

Breast cancer (BCa) is the most common cancer among Jamaican women, with 60-70% presenting at advanced stages (III or IV). Incidence rates are slightly lower (43.1 per 100,000) than global rates (47.8 per 100,000) and mortality rates are notably higher (18.1 per 100,000 versus 13.6 per 100,000). This study investigated germline testing in Jamaican women with BCa to examine clinicopathologic associations and to compare the utility of two genetic testing panels within this population. A retrospective, cross-sectional study was conducted on 178 Jamaican women with pathologically confirmed BCa at 8 hospital sites during 2018 - 2024. Cancer data extracted from patients' medical records included BCa type, cancer surgeries performed, metastatic sites, tumor stage, molecular markers (ER, PR, HER2, Ki67) and documentation of family history of cancer. Germline genetic testing was performed using comprehensive multigene panel tests from either Invitae (n=126) or Blueprint Genetics (n=52). The Invitae panel assessed 84 genes associated with hereditary cancer syndromes, while the Blueprint panel examined 160 genes. Both panels included core BCa susceptibility genes (BRCA1, BRCA2, PALB2, CHEK2, ATM). Data underwent quality control measures including removal of duplicates, standardization of formats, appropriate handling of missing values, and validation of outliers prior to statistical analysis using STATA 18 Software. The mean age was 44.5±10.5 years with most patients having at least one variant classification (36.5%). Just over 40% and 34% had Luminal A/B and triple negative molecular subtypes respectively, and 36% had advanced stage. Variants classified as pathogenic or likely pathogenic (P/LP) were identified in 15.7% of cases. P/LP status was significantly associated with multiple variant status ($p<0.001$) with 57.1% of cases with P/LP status having two or more gene variants overall when compared to 42.4% with VUS status. Of the 75 genes examined amongst the cases, 10.7% had mutations in *BRCA2*. Thirty-two of the 255 variants classified were unique P/LP variants. Of these variants, *BRCA2* had the most positive (30.6%); c.3109C>T p.(Gln1037*) being the most common P/LP variant overall (8.3%). Other high frequency variants were c.303T>G p.(Tyr101*) in *BRCA1* and c.258+2T>C in *SBDS*. A greater proportion of patients carried P/LP variants when tested by Blueprint Genetics (19%) in contrast to 14% when tested by Invitae. No genetic variants were identified for 32.6% of participants. Our findings reveal a high frequency of germline mutations in Jamaican BCa patients. This supports the need for implementing germline testing as part of treatment planning and to ensure the utilization of targeted therapies. Further investigations of broader gene panels beyond the traditional BCa susceptibility genes may be warranted for better understanding of the germline mutation landscape in this population.

#7403 Colorectal neoplasia rates in Li Fraumeni syndrome.

Sophia R. Spiegel[1], Caitlin Orr[1], Mikia J. Croom[2], Nicolas U. Edgar[3], Sophie R. Cahill[4], Anh N. Le[1], Madeline Good[1], Jo Anson[3], Soren Feola[3], Casey Mehrhoff[5], Anne Naumer[3], Jennie Vagher[3], Luke D. Maese[5], Judy E. Garber[4], Asaf Maoz[4], Bryson W. Katona[6], Suzanne P. MacFarland[2], Kara N. Maxwell[1]

[1]Division of Hematology-Oncology, Department of Medicine, Perelman School of Med. Univ. of Pennsylvania, Philadelphia, PA,[2]Children's Hospital of Philadelphia, Philadelphia, PA,[3]Huntsman Cancer Institute, University of Utah, Salt Lake City, UT,[4]Division of Cancer Genetics and Prevention, Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA,[5]Department of Pediatrics, University of Utah School of Medicine, Primary Children's Hospital, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT,[6]Division of Gastroenterology, Department of Medicine, Perelman School of Med. Univ. of Pennsylvania, Philadelphia, PA

Background and Aims: Li Fraumeni Syndrome (LFS) is an inherited cancer predisposition syndrome with up to 90% lifetime cancer risk and is caused by *TP53* pathogenic germline variants (PGVs). Current NCCN guidelines for LFS recommend colonoscopy for colorectal cancer (CRC) screening every 2-5 years, beginning at age 25 or 5 years before earliest known familial CRC, based on recent literature suggesting ~5-7% of LFS patients develop CRC. However, no data exists on findings of screening colonoscopy in LFS patients to support use of earlier and more frequent screening. In this study, we determined colon neoplasia detection rates of colonoscopy in LFS patients.

Methods: We analyzed CRC diagnoses from our entire LFS cohort (n=220 adults) and fully documented screening colonoscopies (n=123) from January 2019 - August 2024 in a cohort of adult LFS patients (n=90). *TP53* PGVs were classified based on variant type and functional effect into dominant negative (DN) loss of function (LOF) missense, truncating LOF variants, or hypomorphic variants. Analysis is ongoing of validation cohorts at Huntsman Cancer Institute and Dana Farber Cancer Institute and of pediatric data at these institutions.

Results: Of 220 adult LFS patients, 15 (6.8%) had a diagnosis of CRC at a median (IQR) age of 39(32-46). Compared to LFS patients without CRC, patients with CRC were more likely to be male (73% vs 26%, p=0.0004), but not more likely to have a DNLOF/LOF vs hypomorphic PGV (30% vs 39%, p=0.78). Of 90 adult LFS patients with fully documented colonoscopies, the average age at time of data abstraction was 42.6 ± 12.5 y/o, 72% were female, and 73% of patients had a prior malignancy. 31% of PGVs were DNLOF missense, 24% were LOF truncating variants, 38% were hypomorphic, and 7% were unknown. Overall detection rates for any neoplastic finding, adenoma (ADR), serrated lesion (SDR), advanced precancerous polyp, and CRC were 34%, 24%, 9%, 4%, and 0.8% respectively. Detection rates for any neoplastic finding, adenoma, advanced adenoma, serrated lesion, advanced serrated lesion, and CRC were similar between patients aged 25-44 (34%, 32%, 8%, 13%, 0 and 3% respectively) and 45-75 (36%, 28%, 6%, 6%, 0 and 2%), with no significant difference by gender. No significant difference in precancerous polyp (p=0.8438) and colon neoplasia (p=1) detection rates was found in patients with hypomorphic *TP53* PGVs compared to DNLOF/LOF PGVs.

Conclusion: Our data show that LFS patients have a lower ADR (24%) but similar SDR (9%) to the endoscopic benchmarks in standard risk adults (35% and 6%, respectively); however, LFS patients undergo earlier and more frequent screening, suggesting that LFS patients have comparable polyp burdens at an earlier age. These data support the NCCN guidelines for earlier and more frequent colonoscopy in LFS patients.

#7404 Screening patterns among high-risk individuals for pancreatic cancer at a tertiary academic center.

Alice Ahyoung Lee, Naoko Fushimi, Audrey Sutton, Andrea Leung, Sharon Pneh, Walter Park

Stanford University, Palo Alto, CA

Background: Multiple guidelines have developed in the last decade on recommendations for pancreatic cancer screening among high-risk individuals (HRIs). The International Cancer of the Pancreas Screening (CAPS) Consortium, American Gastroenterology Association (AGA), and American Society for Gastrointestinal Endoscopy (ASGE) are three widely known societies that have each published screening guidelines. At our tertiary care hospital, we perform annual pancreatic cancer screening for individuals meeting high-risk screening criteria by any of the 3 above guidelines. We aimed to describe real-world screening patterns within our cohort.

Methods: Patients followed by Stanford's Benign Pancreas Clinic were identified as HRIs if they met screening criteria per the 2019 CAPS, 2020 AGA, and/or 2022 ASGE guidelines and either underwent at least 1 screening modality or were counseled on pancreatic cancer screening. Screening was deemed completed for a calendar year if the HRI underwent either an abdominal MRI with contrast or an EUS of the pancreas. Patterns of annual pancreatic cancer screening were reviewed retrospectively.

Results: A total of 341 HRIs were identified from March 2009 to July 2024. 97 (28.4%) met high-risk criteria due to family history only, while 244 (71.6%) met high-risk criteria due to a high-risk hereditary mutation. The mean follow-up time from first screening encounter was 4.6 years. 51.4% of patients completed at least 50% of their recommended annual screenings. There were no significant differences in gender, race/ethnicity, age at first encounter, or risk factor among those in the lowest quartile of successfully screened patients compared to the entire cohort.

Conclusions: It is difficult to achieve high compliance rates for annual pancreatic cancer screening of high-risk individuals as recommended by the CAPS, AGA, and ASGE. Analysis of our lowest performing quartile suggest no disparity by gender, race/ethnicity, age at first encounter, or risk factor.

	All HRIs (n=341)	Lowest quartile of screened patients (n=86)	P-value
Gender (%)			0.51
Female	268 (78.6)	71 (82.6)	
Male	73 (21.4)	15 (17.4)	
Race/Ethnicity (%)			0.88
Non-Hispanic White	224 (65.7)	60 (69.8)	
Asian	47 (13.8)	10 (11.6)	
Hispanic	38 (11.1)	8 (9.3)	
Black	6 (1.8)	1 (1.2)	
Other/Unknown	26 (7.6)	7 (8.1)	
Age at first encounter (%)			0.96
<50	59 (17.3)	15 (17.4)	
50-59	131 (38.4)	33 (38.4)	
60-69	99 (29)	23 (26.7)	
70-79	50 (14.7)	14 (16.3)	
≥80	2 (0.6)	1 (1.2)	
Risk factor (%)			0.85
Family history only	97 (28.4)	26 (30.2)	
Hereditary mutation	244 (71.6)	60 (69.8)	
Mean follow-up time	4.2	3.6	
Mean years screened	2.0	0.02	
Mean % successful annual screening	52.4%	2.1%	

#7405 Temporal evolution of early signs and symptoms of early-onset colorectal cancer in diverse populations: Insights from the All of US Research Program.

Ruiyi Tian, Chongliang Luo, Xiaoyu Zong, Cassandra D. L. Fritz, Philip R. O. Payne, Yin Cao

Washington University In St. Louis, St. Louis, MO

Introduction: The incidence of early-onset colorectal cancer (EOCRC, diagnosed before age 50) has risen sharply over the past three decades. Current screening guidelines are inadequate for detecting EOCRC in individuals under the recommended screening age, emphasizing the urgent need for identifying pre-diagnostic signs and symptoms and their temporal evolution patterns to inform early detection.
Methods: A total of 347 incident EOCRC cases within the NIH All of Us Research Program (1991-2022) with ≥1 year of pre-diagnostic electronic health record (EHR) were matched with up to four controls. Signs and symptoms were classified as chronic (vs non-chronic) based on whether required ongoing medical care or impacted daily functioning for ≥12 months. Poisson regression models and dynamic network analyses were used to identify signs and symptoms from 1 year to 3 months before diagnosis and characterize their co-occurrence network within 2 years to diagnosis. Longitudinal trajectory analysis was used to examine the evolution of symptom intensity over 3-month sliding windows from 2 years to diagnosis.
Results: From 1 year to 3 months pre-diagnosis, 16 non-chronic signs and symptoms were significantly associated with increased EOCRC risk, with the highest incidence rate ratios (IRRs) for blood in stool (IRR 12.9, 95% CI 4.4-38.1) and hemorrhage of rectum and anus (11.4, 5.0-26.0). Network analysis identified three symptom clusters centered on abdominal pain: (1) hemorrhage of the rectum and anus, which connects to blood in stool, acute pain, and anal/rectal conditions; (2) benign neoplasm of the colon; and (3) nausea and vomiting, back pain, syncope and collapse, and urinary tract infection, which interconnect. Clusters 1 and 2 co-occurred increasingly from 2 years to 3 months pre-diagnosis, with connections between clusters 1 and 3 emerging in the final 3 months. Longitudinal trajectory analysis demonstrated a steady increase in symptom intensity, accelerating 9 months before diagnosis, for abdominal pain, hemorrhage of rectum and anus, blood in stool, benign neoplasm of colon and anal and rectal conditions.
Conclusion: Mapping symptom networks and their temporal evolution offers a promising path to enhance EOCRC detection. Integrating these insights into precision diagnostics can align with advanced prevention frameworks, improving outcomes for younger at-risk populations.

#7406 Noncontrast screening whole body MRI with diffusion-weighted imaging for multi cancer detection: a retrospective case series study.

Candace Westgate[1], Rebecca Nayeri[2], Madhurima Datta[3], Jeffrey Venstrom[3], Rodrigo S. Pompa[3], Pratik Shingru[3], Saqib Basar[3], Sam Hashemi[3], **Yosef Chodakiewitz**[3]

[1]Adventist Health - St. Helena Women's Center, St. Helena, CA,[2]Department of Medicine, Division of Hematology/Oncology, Greater Los Angeles VA Healthcare Center, Los Angeles, CA,[3]Prenuvo, Redwood City, CA

Introduction: Single-cancer screening (SCS) methods improve survival in some cancers but miss ~70% of cancer deaths from unscreened cancers. Multi-Cancer Detection (MCD) strategies aim to broaden early detection. We evaluate the MCD yield of screening WB-MRI (sWB-MRI) in a real-world clinical setting.
Methods: In this retrospective single-center study, we reviewed sWB-MRI cases from 2022 with 12 months follow-up, excluding active cancer patients. The noncontrast, multiparametric sWB-MRI with Diffusion-Weighted-Imaging (1.5T) covered head to ankles. Radiologists provided structured reports for primary care physician (PCP) review. Follow-up data was gathered through direct-to-patient phone calls, focusing on capturing any interval history of biopsy and histopathologic outcomes.
Results: Of 1,011 subjects (52% male, mean age 56 ± 28 years, median follow-up 14 ± 2 months), indications included proactive health (64%), general concerns (18%), and specific symptoms (18%). 9 radiologists reported the scans, with 5 reporting 90% (12%, 15%, 15%, 16%, & 32%). Results were reviewed with PCPs in 92.3% of cases. sWB-MRI led to targeted Tissue Sampling (TS) in 50 cases (4.9%). To isolate simple diagnostic-motivated TS (dTS) for further focused analysis, we excluded TS from clinically-indicated procedures beyond simple diagnostics: dTS = 41 (4.0%).
Histopathology-confirmed Cancer Detection Percentages (CDP) with 95% CI were: Overall sWB-MRI CDP (O-CDP) = 2.2% (1.37-3.28%); dTS CDP = 51% (35-67%).
O-CDP by age (# subjects, CDP %): <35 years (36, 0), 35-49 years (288, 1.4), 50-64 years (393, 2.6), 65-79 years (265, 3.0), >79 years (29, 0). Two false negatives (0.2%) were breast cancers. Of 22 detected cancers, 64% were retrospectively estimated localized, 36% regional/distant. 86% of cancers detected occurred in patients who did *not* indicate specific symptoms for their sWB-MRI reason. Of sWB-MRI detected cancer, 68% lacked SCS methods, while 32% had SCS options.
Non-cancer clinically significant diagnoses (CSDs) included benign masses, aneurysms, liver disease, and pneumonia, prompting clinical action. Study limitations included reliance on patient-reported outcomes and lack of comprehensive medical records, limiting analysis of intermediate diagnostics and follow-up testing.
Conclusions: sWB-MRI prompted pathologically-proven cancer diagnoses across diverse anatomical regions, including those outside standard SCS coverage. Prospective studies involving standardized sWB-MRI reporting frameworks (such as ONCO-RADS), larger cohorts, and designs that capture comprehensive intermediate diagnostic processes and long-term health outcomes, are essential to refine protocols and assess clinical validity (e.g., sensitivity, specificity, predictive values) and utility (e.g., patient outcomes, healthcare system impact).

#7407 Generalizability of an AI-based mammogram risk score (MRS) for breast cancer among racially and ethnically diverse women.

Shu Jiang[1], Debbie L. Bennett[2], Peter Kraft[3], **Graham A. Colditz**[1]

[1]Surgery, Public Health Sciences, Washington University School of Medicine in St. Louis, St. Louis, MO,[2]Radiology, Washington University School of Medicine in St. Louis, St. Louis, MO,[3]National Cancer Institute, Bethesda, MD

Breast cancer remains the most common cancer diagnosed among women worldwide. While risk prediction models hold great promise for enhancing preventive breast cancer management, a major challenge lies in accurately identifying women at elevated risk across diverse racial and ethnic groups. Mammogram risk scores (MRS), an AI-driven feature summarizing mammographic texture, have shown potential to accurately classify 5-year breast cancer risk in racially diverse populations.[1] However, similar prediction performance across groups does not necessarily ensure the model's generalizability. To address this, we analyzed data from two large North American cohorts—Emory and British Columbia—encompassing over 226,000 racially diverse women, including Non-Hispanic White (NHW), Non-Hispanic Black (NHB), East Asian, South Asian, and Indigenous women. We assessed the distribution of MRS by race and ethnicity and observed little variation in median MRS between the two cohorts overall, among race and ethnicity groups within each cohort, or between cohorts within race and ethnicity (where such comparisons were feasible). We further examined the association between MRS and breast cancer risk both overall and stratified by race/ethnicity. Statistically significant associations were found, with hazard ratios (HR) per standard deviation increase in MRS ranging from 2.03 (95% CI: 2.00-2.06) to 2.15 (95% CI: 1.93-2.40) after adjusting for age. This significant association was consistent across racial/ethnic groups: NHB (2.12, 95% CI: 1.92-2.51), East Asian (2.10, 95% CI: 2.02-2.18), South Asian (2.09, 95% CI: 1.72-2.49), and Indigenous women (2.19, 95% CI: 1.65-2.79). Prediction accuracy, as assessed using the 5-year AUC, also showed no statistically significant differences by race/ethnicity within each cohort. Our findings indicate no clinically meaningful differences in MRS performance across these groups, suggesting broad applicability. The MRS could be effectively used in clinical practice to estimate age-specific breast cancer incidence, including for individuals with mixed ancestry or self-reported identities commonly encountered in real-world settings. 1. Jiang S, Debbie B, Rosner B, Tamimi R, Colditz G. Development and validation of dynamic 5-year breast cancer risk model using repeated mammograms. JCO Clinical Cancer Informatics, In press. 2024.

#7408 Factors influencing false positive low-dose computed tomography-based lung cancer screening in an urban, safety-net system.

Rutu Rathod[1], Urooj Wahid[2], Asha Kandathil[3], Mary Gwin[4], Sheena Bhalla[4], Song Zhang[2], Andrea Semlow[5], Vijaya Natchimuthu[5], Sarah Malone[2], George Oliver[6], David H. Johnson[4], Megan A. Mullins[2], David Gerber[2], Rasmi Nair[2]

[1]Department of Epidemiology, Fay W Boozman College of Public Health, University of Arkansas for Medical Sciences, Little Rock, AR,[2]Peter O'Donnell Jr. School of Public Health, UT Southwestern Medical Center, Dallas, TX,[3]Radiology, UT Southwestern Medical Center, Dallas, TX,[4]Internal Medicine, UT Southwestern Medical Center, Dallas, TX,[5]Parkland Health, Dallas, TX,[6]Parkland Center for Clinical Innovation, Dallas, TX

Background: False-positive results from low-dose computed tomography (LDCT)-based lung cancer screening can result in unnecessary follow-up tests, increased patient anxiety, and higher healthcare costs. We evaluated the prevalence of false-positive LDCT results and assessed factors associated with these LDCT results within an integrated, urban safety-net healthcare system serving a diverse and underserved population. Design: Retrospective cohort study.
Setting: Electronic health records data from Parkland Health, an integrated safety-net provider in Dallas, Texas (January 1, 2017-May 31, 2022), with a 1-year follow-up period through May 31, 2023.
Outcome: False-positive LDCT was defined as positive screen (lung RADS 3, 4A, 4B or 4X) followed by a negative completed work-up or no diagnosis of lung cancer after 12-month follow-up.
Analysis: Univariable and multivariable logistic regression models were used to assess the associations between patient characteristics (patient age, sex, race/ethnicity, preferred language, insurance status, comorbidity score, smoking status and smoking pack-years) and a false-positive LDCT.
Results: A total of 2525 LDCTs were included from 2029 patients. Of these, lung cancer was diagnosed in 12 patients (0.6%), and 394 (19.4%) were classified as false-positive. Median age was 62 years, 59% were men, 49% were non-Hispanic Black, 16% were Hispanic, and the median pack-years were 40. In both univariable and multivariable analyses, age was significantly associated with false positive LDCT results. Patients aged 65-70 years (OR=1.81, 95% CI: 1.06-3.10) and >70 years (OR=2.02, 95% CI: 1.15-3.54) had higher odds of false-positive results compared to those aged 50-54 years.
Conclusion: In our integrated safety-net healthcare system, approximately one in 5 patients had a false-positive LDCT result, and age was a significant contributor. Patients aged 65 years and older were more likely to experience false-positive findings, suggesting that age-related factors may influence the diagnostic accuracy of LDCT. Future studies are needed to replicate these findings and further minimize unnecessary follow-up interventions, particularly in older populations.

#7409 Effectiveness of lung cancer screening by sex and tumor histology: Extended, pooled analysis of the ITALUNG and LUSI trials, with comparison to findings in the NLST.

Rudolf Kaaks[1], Francisco O. Cortes-Ibanez[1], Stefan Delorme[1], Erna Motsch[1], Verena Katzke[1], Claus-Peter Heussel[2], Hans-Ulrich Kauczor[3], Giulia Picozzi[4], Giuseppe Gorini[4], Francesca Maria Carozzi[5], Laura Carrozzi[6], Eugenio Paci[4], Donella Puliti[4], Mario Mascalchi[7]

[1]German Cancer Research Center, Heidelberg, Germany,[2]Thoraxklinik, Heidelberg University Hospital, Heidelberg, Germany,[3]University Hospital Heidelberg, Heidelberg, Germany,[4]Institute for Cancer Research, Prevention and Clinical Network (ISPRO), Florence, Italy,[5]University of Pisa, Pisa, Italy,[6]University Hospital of Pisa, Pisa, Italy,[7]University of Florence, Florence, Italy

Introduction: Low-dose computed tomography (LDCT) screening can reduce lung cancer (LC)-related mortality, but questions remain about the duration of this effect and differences by sex and tumor histology.
Methods: Extended follow-up data from the ITALUNG and LUSI trials were pooled to examine screening-related tumor stage-shifts and to estimate relative hazards for LC-related mortality, by sex and histology. Findings were compared to, and additionally combined with, those from the US National Lung Screening Trial (NLST).
Results: In ITALUNG-LUSI, screening yielded a 30% reduction of overall LC mortality up to 8 years after final screening (dilution-adjusted HR=0.70[0.51-0.96]). This reduction, however, was more pronounced for women (HR=0.49[0.25-0.96]) than men (HR=0.78[0.54-1.11]), which was confirmed in combined data of ITALUNG-LUSI plus NLST (6 years post-screening, women: HR=0.74[0.61-0.90], men: HR=0.93[0.80-1.06]; $p_{heterogeneity}$=0.06). Analyses of stage-shifts and relative mortality hazards suggested that screening reduced mortality by non-small cell and non-squamous tumors in both sexes. In women (18-20%), more than among men (5-9%), screening also resulted in frequent detection of adenocarcinomas with lepidic growth. Finally, in both ITALUNG-LUSI and NLST, LDCT screening reduced the incidence and mortality for small-cell lung cancer (SCLC) among women (HR=0.63[0.43-0.94], all trials combined) but not among men ($p_{heterogeneity}$=0.04).
Conclusion: LC-related mortality reduction by LDCT screening may last until 8 or more years after cessation, and appears to be stronger for women than men, due to reduced incidence and mortality of SCLC in women only. The latter might be caused by removal of (likely EGFR-mutated, and slowly growing) adenomatous precursor lesions that over time would transform into SCLC (lineage plasticity). These findings may have major implications for the optimization of screening programs in terms of eligibility criteria and screening intervals

#7410 Make it better and faster: a retrospective study examining the introduction of an agnostic, reflexive, and expanded pathological protocol for lung cancer patients in Saskatchewan, Canada.

Nicholas Jette[1], John Decoteau[2], Hui Wang[3], Darryl Yu[2], Mary Kinloch[2]

[1]Department of Surgery, University of Saskatchewan, Saskatoon, SK, Canada,[2]Department of Pathology, University of Saskatchewan, Saskatoon, SK, Canada,[3]Department of Chemical and Biological Engineering, University of Saskatchewan, Saskatoon, SK, Canada

Background: Lung cancer evaluation for Saskatchewan patients involves an initial histologic or cytologic diagnosis rendered to the thoracic surgeon, followed by an oncologist consult. The oncologist initiates tissue biomarker testing for advanced stage patients at the first patient visit, and treatment guidance is determined once this is available. This multi-step process ranges 25-39 days from biopsy to treatment-critical information. We sought to reduce this delay and increase actionable mutation rates by implementing stage-agnostic, pathologist-initiated, expanded biomarker reflex testing. We also sought to determine if these changes would lead to improved times to treatment amongst the various populations throughout our unique provincial population considering pathologic testing for lung cancer patients occurs in one of the major population centers, Saskatoon.
Methods: 1064 patients were included in the study and examined 18 months of data pre-and post-algorithm change. The differences between actionable mutation rate were examined using a T-test. Multivariable logistic regression models were used to estimate confidence intervals (CI) for the associations between independent factors and time to treatment from biopsy. The initial algorithm of advanced stage, parallel immunohistochemistry (IHC) for ALK, ROS, 22c3 PDL1 and DNA next-generation sequencing with ThermoFisher Oncomine for KRAS/BRAF/EGFR was updated to stage agnostic DNA triage panel for KRAS/BRAF/EGFR and PDL-1 IHC with all driver-negative patients reflexed to a ThermoFisher Oncomine Fusion panel with 49 actionable mutational targets.
Results: 394 patients (pre algorithmic change) and 670 (post change) were examined. An increased detection rate of actionable mutation of 6.7% was noted with post-algorithmic changes. This included an increase of X ALK and Y ROS patients and X MET14 and NTRK patients. Biopsy to biomarker reporting decreased from 40.4 days to 29.7 days (-26%). Furthermore, prior to the implemented change, biopsy to treatment time was 30 days for patients residing near or in Saskatoon, and 37 days for those residing near or in Regina, and 28 and 30 days post algorithmic change, respectively.
Conclusions: Patient biomarker results at the first oncologist visit are paramount to timely treatment of the patient. Saskatoon was able to maintain the same turnaround time (TAT) despite an increased number of patients being tested. Regina, despite being a major center, still shows an increased TAT due to the time it takes to transfer the patient material across the province. While an updated protocol increases biomarker equity for patients across the province, interventions need to be targeted to ensuring patients do not suffer untimely delay by not being in a biomarker testing center.

#7411 Screening of nasopharyngeal cancer in a multi-ethnic healthy Singaporean cohort.

Ashlynn Chen[1], Arthur Tan[2], Jia Hui Isabelle Jang[2], Song Tar Toh[2], Wei Yee Wan[3], Chwee Ming Lim[4]

[1]Duke-NUS Medical School, Singapore, Singapore,[2]Department of Otolaryngology-Head and Neck Surgery, Singapore General Hospital, Singapore, Singapore,[3]Department of Pathology, Singapore General Hospital, Singapore, Singapore,[4]Singapore General Hospital, Singapore, Singapore

Background: Epstein-Barr Virus (EBV)-positive Nasopharyngeal Cancer (EBV-NPC) is highly prevalent in Asia, with annual incidences of 20-100 per 100,000. In Singapore's multi-ethnic population, EBV IgA serologies targeting viral capsid antigen (VCA) and early antigen (EA) are commonly performed for NPC screening. However, the longitudinal variability of EBV IgA serologies and its relationship with NPC risk remains unclear. This study evaluates the longitudinal patterns of EBV IgA serologies and NPC development in a tertiary medical center.
Methods: Retrospective analysis of asymptomatic individuals referred for NPC screening at Singapore General Hospital between 2008-2021 was performed. Demographics, EBV IgA serologies titers, plasma DNA PCR, nasoendoscopy, biopsy, and MRI post-nasal space (PNS) findings were collected. A positive family history was defined as any first-degree relative with a prior history of NPC. Patients with only one serology test and screening of less than 1 year were excluded.
Results: 4586 patients had EBV IgA serologies performed and 1977 met our inclusion criteria. Patients (45.6% male, 54.4% female; aged 12-90) had a median follow-up of 207.1 months (range: 12-5125 months) and were predominantly Chinese (95%), with 1.16% Indian, 1.66% Malay, and 2.17% from other ethnicities. All were screened using nasal endoscopy examination (n=11,423) and 725 biopsies were performed. Adjunctive tests including MRI PNS (n=876) and EBV DNA-PCR (n=392) were also performed as per physician's discretion. EBV serologies were repeated in 2-5 times (61%), 6-10 (21.3%), or >10 (18.1%) over the screening period. Titre fluctuations showed no clear pattern: 61.96% were positive for only one serology type, 12.13% were persistently positive for both, 23.4% had decreasing titers, and 2.42% had increasing titers. Six patients (0.3%) developed NPC, all ethnic Chinese, asymptomatic, and aged 50 on average. Among these, 3/6, 1/6, and 2/6 had Stage I, III, and IV NPC at diagnosis, respectively. Two had a family history of NPC. The mean detection time was 61 months, and follow-up averaged 12.7 years. Serology titer patterns were persistently positive or increasing. Increased EBV EA-IgA (2/6) or VCA-IgA (2/6) titers were observed 1-2 years before diagnosis. One patient had a normal biopsy but suspicious MRI, leading to repeat biopsy and NPC diagnosis.
Conclusion: In this longitudinal heterogeneous healthy multi-ethnic NPC screening cohort, the NPC detection rate was 0.3%. Fluctuation of EBV IgA serologies was variable, and increasing titers of both Ea and VCA serologies were identified in 33.3% of NPC. Future evaluation of high risk cohorts and cost-effectiveness of NPC screening are warranted.

#7412 The relationship between lifestyle factors and polygenic risk score with prostate cancer risk in the Multiethnic Cohort.

R. W. Hughley IV[1], A. Wang[2], P. Wan[3], X. Xiong[4], W. Xiong[3], D. Bogumil[3], S. Park[5], L. Wilkens[5], L. Le Marchand[5], D. Conti[3], C. Haiman[3], F. Chen[4];
[1]USC - University of Southern California, Waldorf, MD, [2]Harvard T.H. Chan School of Public Health, Boston, MA, [3]USC - University of Southern California, Los Angles, CA, [4]USC - University of Southern California, Los Angeles, CA, [5]University of Hawaii Cancer Center, Honolulu, HI

Introduction: Behavior modifications for men at high genetic risk of prostate cancer (PCa) have the potential to prevent or delay disease onset. In this study, we evaluated whether the association of a previously developed healthy behavior score with PCa risk varies across levels of genetic susceptibility, as measured by a multi-ancestry polygenic risk score (PRS) in the Multiethnic Cohort (MEC).
Methods: We conducted a nested case-control analysis of 6,096 incident PCa cases and 24,684 controls among African American (13%), Latino (23%), Japanese American (35%), Native Hawaiian (7%), and White (22%) men in the MEC. Among the PCa cases, 2,049 were aggressive cases and 776 were lethal cases. We calculated the 2015 Prostate Cancer Behavior Score from the baseline questionnaire, which comprised six modifiable factors (smoking, BMI, physical activity, intake of tomatoes, processed meat, and fatty fish), with each factor scored as 1 for meeting healthy thresholds and 0 otherwise. This score ranged from 0 to 6 with a higher score indicating a healthier behavior pattern. The PRS of 451 risk variants was constructed with weights from the largest multi-ancestry genome-wide association study for PCa. In the overall study and within each racial/ethnic group, we evaluated the association of the behavior score (categorized as 0-2, 3, 4, 5-6) with risk of overall, aggressive, and lethal PCa in logistic regression analyses and assessed interactions between the behavior score and PRS (categorized as 0-50%, 50-75%, and 75-100%) on PCa risk using the likelihood ratio test (LRT). A $P < 0.05$ was considered statistically significant.
Results: Comparing the highest to the lowest category of the behavior score (5-6 vs. 0-2), there was a suggestive 29% (95% CI: 0.47-1.04, P=0.09) lower risk of lethal PCa in the overall study. Although not statistically significant, a similar inverse association was observed in Whites (OR=0.36), Japanese Americans (OR=0.26), and Latinos (OR=0.26) but not in African Americans (OR=1.62). When stratified by PRS category in the overall study, this inverse association appeared to be stronger for men in the 75-100% PRS category (OR=0.55, 95% CI: 0.27-1.04, P=0.07) than those in the lower PRS categories (ORs 0.72-0.97, P >0.9). These differences in the association of behavior score with risk of lethal PCa across PRS categories were not statistically significant ($P_{LRT} = 0.56$). No interaction was observed between the behavior score and PRS on risk of overall and aggressive PCa.
Conclusion: Findings from this multiethnic study provided further support that the 2015 Prostate Cancer Behavior Score was associated with a reduced risk of lethal PCa. However, there was limited evidence that this association was modified by PCa PRS, which could be due to the relatively small number of lethal PCa cases in our study. Further investigations in larger diverse populations are warranted to confirm our findings.

#7413 Germline susceptibility to single and multiple primary cancers utilizing an ancestry adjusted case-control analyses.

Vignesh Ravichandran, Ying Liu, Irina Ostrovnaya, Kaitlyn Tkachuk, Aliya Khurram, Yelena Kemel, Alicia Latham, Diana Mandelker, Chaitanya Bandlamudi, Michael Conry, Saloni Sinhas, Syed Aijazuddin, Nikolaus Schultz, Michael Berger, Mark Robson, Zsofia Stadler, Kenneth Offit, **Vijai Joseph**

Memorial Sloan Kettering Cancer Center, New York, NY

Background: Germline pathogenic variations (PV) increase heritable risk for cancer and are utilized to target tumor-specific prevention and early detection. However, inherited cancer associations may be pleomorphic, penetrance and expressivity may vary and may be modified by location of the PV, ancestry and polygenic or non-genetic factors. This study aims to determine the magnitude and spectrum of risk for specific tumor types and multiple primary cancer (MPC) for genes commonly tested in patients at familial risk for cancer or undergoing tumor-normal analysis for a cancer diagnosis.

Methods: We conducted a retrospective cohort study of patients consenting to MSK-IMPACT, a matched tumor-normal sequencing assay, over a five-year period. We collected data on cancer onset, race, ethnicity, and, where consent was obtained, clinical information through cBioPortal and the MSKCC clinical databases. MPC were classified using IARC guidelines. Non-cancer control data was sourced from gnomAD. Clinically actionable pathogenic variants in cases and controls were identified using the automated variant curator PathoMAN and manual curation in a subset. Ancestry adjusted weighted generalized linear model was used to estimate risk for single primary (SP), MPC, and MPC-pairs. Biallelic losses were analyzed from paired tumors and normal.

Results: 28,000 individuals across 22 cancer types were tested for 100 cancer predisposition genes (CPG). MPC had a higher rate of carrier frequency for germline PV in known or novel CPG compared to single primary (SP) cases (SP =15%, MPC=21%). In the analysis including MPC cases, we discovered or replicated novel associations including *BRCA2* and biliary cancer (OR=4.5, CI=2.2-8), *ATM* (OR=4.8, CI=2.1-9.2) and *BRCA2* and mesothelioma (OR=8, CI=3.1-16.6); *NBN* (OR=4.2, CI=2.3-6.9) and lung cancer, *PALB2* and germ cell cancer (OR=7.8, CI=1.9-20.8). Novel associations were observed with *RECQL4* and breast, bowel, cervix, lung cancers and uveal melanoma, and *RECQL* with breast, lung and bowel cancers. *SLX4* was associated with prostate, soft tissue and ovarian cancers. PV in *RECQL4, MSH2* and *MLH1* were enriched in patients with two independent compared to single bowel tumors. In the MPC group, as expected, mismatch repair genes *MSH2, MSH3* and *MLH1* were associated with increased risks for stomach, bowel, and uterine cancer, and TP53 with multiple breast cancer primary tumors. The MPC cohort demonstrated pleiotropy of *BRCA1/2*, *NF1, ATM, TP53 and RECQL4* with several tumor types, with biallelic losses in tumors supporting several of these associations. We did not replicate reported associations of *PALB2* and ovarian cancer and *CHEK2* and colon cancer.

Conclusions: Ancestry adjusted risk estimates among a real-world case-cohort of primary and multiple primary cancer cases revealed several novel associations and did not confirm some prior associations with cancer phenotypes.

#7415 Association between sleep traits and risk of non-Hodgkin's lymphoma subtypes: a mendelian randomization study in the International Lymphoma Epidemiology (InterLymph) Consortium.

Pankhil Shah[1], Brittany Crawford[1], Anwar Merchant[1], Brenda Birmann[2], Angelica Macauda[3], Michelle A. T. Hildebrandt[4], Aaron Norman[3], Neil E. Caporaso[5], Meredith Yeager[5], Michael Dean[5], Immaculata De Vivo[6], Lynn Goldin[5], Nicola J. Camp[7], Rosalie Griffin[8], Delphine Casabonne[9], Federico Canzian[10], Pelin Unal[10], Elad Ziv[11], Catherine R. Marinac[12], Alexandra Nieters[13], Stephen J. Chanock[5], Mitchell J. Machiela[5], Michael Conry[14], Charlie Zhong[15], Hanla A. Park[16], Simon Cheah[17], Jonathan N. Hofmann[5], Elizabeth E. Brown[18], Celine Vachon[3], Susan Slager[19], Sonja Berndt[5], Sophia S. Wang[20], Vijai Joseph[21], James McKay[16], Henrik Hjalgrim[22], Lara Sucheston-Campbell[23], Karl Smith-Byrne[24], Alyssa Clay-Gilmour[1]

[1]Arnold School of Public Health, University of South Carolina, Columbia, SC,[2]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA,[3]Division of Epidemiology, Department of Quantitative Health Sciences, Mayo Clinic, Rochester, MN,[4]Department of Lymphoma/Myeloma, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX,[5]Division of Cancer Epidemiology & Genetics, National Cancer Institute, National Institute of Health, Bethesda, MD,[6]Brigham and Women's Hospital and Harvard Medical School; Harvard TH Chan School of Public Health; Harvard Radcliffe Institute, Boston, MA,[7]Division of Hematology and Huntsman Cancer Institute, University of Utah, Salt Lake City, UT,[8]Department of Quantitative Health Sciences, Mayo Clinic, Rochester, MN,[9]Catalan Institute of Oncology and Centro de Investigacion Biomedica en Red: Epidemiologia y Salud Publica, Madrid, Spain,[10]Research Group Genomic Epidemiology, German Cancer Research Center, Heidelberg, Germany,[11]Department of Medicine, University of California San Francisco Helen Diller Family Comprehensive Cancer Center, San Francisco, CA,[12]Department of Medicine, Harvard Medical School and Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA,[13]Institute for Immunodeficiency, Faculty of Medicine and Medical Center, University of Freiburg, Freiburg, Germany,[14]Clinical Genetics Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY,[15]Population Science, American Cancer Society, Atlanta, GA,[16]Genomic Epidemiology branch, International Agency for Research on Cancer/World Health Organization, Lyon, France,[17]Cancer Council Victoria and University of Melbourne, Melbourne, Australia,[18]Department of Pathology, School of Medicine, University of Alabama at Birmingham, Birmingham, AL,[19]Division of Computational Biology, Department of Quantitative Health Sciences and Division of Hem, Mayo Clinic, Rochester, MN,[20]Department of Computational and Quantitative Medicine, City of Hope, Duarte, CA,[21]Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY,[22]Statens Serum Institut, University of Copenhagen, Rigshospitalet, and Danish Cancer Society, Copenhagen, Denmark,[23]Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI,[24]Cancer Epidemiology Unit, University of Oxford, Oxford, United Kingdom

Introduction: With over 80,000 new cases of non-Hodgkin's lymphoma (NHL) diagnosed annually, modifiable risk factors remain unclear. Circadian disruption, linked to cancers like breast and prostate, affects immune cells (e.g., natural killer cells and T-helper cells) by altering their trafficking and proliferation. Some studies (PMID: 30566672, 27611440) suggest long sleep may increase overall NHL risk, including a Mendelian randomization (MR) study (PMID: 32895918). However, no research has examined the causal relationship between sleep traits, including long sleep, and NHL risk, by subtypes. We hypothesize that sleep traits are causally associated with NHL subtypes in European populations, testing this using MR with data from the International Lymphoma Epidemiology Consortium (InterLymph).

Methods: We conducted two-sample MR using genome-wide association studies (GWAS) summary data for sleep traits and NHL subtypes. Only independent, genome-wide significant ($p < 5\times10\text{-}8$) Single Nucleotide Polymorphisms (SNPs) were selected as valid instruments, sourced from the UK Biobank. Sleep traits included chronotype (153 SNPs), insomnia (48 SNPs), sleep duration (78 SNPs), excessive daytime sleepiness (37 SNPs), short sleep (27 SNPs), and long sleep (8 SNPs). NHL subtype data were from InterLymph: follicular lymphoma (6,508 cases / 64,183 controls), mantle cell lymphoma (1,169 cases / 61,603 controls), Waldenstrom macroglobulinemia/lymphoplasmacytic lymphoma (WM/LPL: 1,697 cases / 59,333 controls), and chronic lymphocytic leukemia (8,522 cases / 67,653 controls). The primary analysis used inverse-variance weighted (IVW) random-effects, with MR-Egger for pleiotropy adjustment.

Results: Using the IVW method, we did not identify any statistically significant causal association between sleep traits and the risk of NHL subtypes, but did yield several intriguing trends. Long sleep (sleep duration >= 9 h per night) showed a marginally increased NHL risk [Odds ratio (OR) range: 1.51 - 247.9] across each subtype. Short sleep (sleep duration < 7 h per night) showed a similar trend [OR range: 1.25 - 1.81], except for WM/LPL, where we noticed a negative trend. The confidence limit for long sleep was imprecise due to the small number of variants. We did not notice any significant directional horizontal pleiotropy or weak instrument bias.

Conclusions: We found no conclusive evidence of a relationship between sleep traits and four NHL subtypes. However, we see suggestive trends of long and short sleep associated with higher risk of some NHL subtypes. These findings align with the previous studies, which showed long sleep was associated with higher risk of NHL overall. Future directions include expanding our analysis to additional NHL subtypes, generating polygenic risk scores, and conducting stratified analyses.

#7416 Prevalence of IL28B single nucleotide polymorphism Rs12979860 and cytomegalovirus (CMV) among allogeneic stem cell transplant in leukemia children.

Mohamed A. Eltokhy[1], Israa Gamal[2], Omar A. Eltoukhy[3], Youssif Madney[4], Mohamed M. Farag[5], Tarek Mansour[6], Sanjay K. Srivastava[1], Sahar Radwan[7]

[1]Department of Immunotherapeutics and Biotechnology, Texas Tech University Health Sciences Center, Abilene, TX,[2]Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt,[3]Department of Clinical Pharmacy, Misr International University, Cairo, Egypt,[4]Department of Immunotherapeutics and Biotechnology, National Cancer Institute, Cairo University, Cairo, Egypt,[5]The Regional Center for Mycology & Biotechnology, Al-Azhar University, Cairo, Egypt,[6]Department of Virology and Immunology, National Cancer Institute, Cairo University, Cairo, Egypt,[7]Microbiology and Immunology Dept., Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt

Cytomegalovirus (CMV) remains a significant cause of morbidity and mortality among allogeneic stem cell transplant (allo-SCT) recipients especially in pediatric leukemia patients. IFN-λ family: interleukin (IL)-28A, -28B, and -29 and IFN-λ4. All have antiviral properties similar to IFN-α. In vivo, IFN-λs are mostly secreted by dendritic cells (DC) and macrophages. IL-28B signals through a heterodimeric receptor consisting of IL-28R1 chains and IL-10Rβ, hence inducing an antiviral state. It has been shown that screening allo-SCT recipients for the presence of defined polymorphisms in genes involved in the regulation and activation of innate and adaptive immune responses may help to predict their individual risk of viral infection and disease. Our study aims to investigate the prevalence of single nucleotide polymorphism (SNP) (rs12979860), upstream of the IL28B gene among allo-SCT recipients and to investigate whether that SNP has any effect on the incidence rate and the features of active CMV infection in cancer children receiving stem cell transplantation. Thirty-five patients undergoing allo-SCT in 57357 children's cancer hospital were included in the study (age range,2-20 years). A total of thirty-five whole blood samples were collected. DNA Extraction from Blood Samples was performed by using ThermoScientific GeneJet genomic DNA purification kit, IL28B SNP Analysis L28B (rs12979860) genotyping was performed by RT-qPCR (Applied Biosystems TaqMan SNP genotyping assay) using QuantStudio ViiA7 system, and routine screening of CMV was performed once a week by real-time PCR (ViroGene real-time CMV quantification kit). The prevalence of single nucleotide polymorphism (SNP) (rs12979860), upstream of the IL28B gene among allo-SCT in cancer children was as follows: C/C carriers were 34.3%, C/T carriers were 42.8% and T/T carriers were 22.8%, 37.1% of patients had one or more episodes of active CMV infection during the first 100 days following allo-SCT. Patients carrying the homozygous T allele had a shorter duration of initial active CMV infection than those carrying the homozygous or heterozygous C-allele. Also, Patients carrying the homozygous T allele show a lower magnitude of CMV DNA viral load within their initial episodes of CMV infection compared to the C/C and C/T groups. Data from the current study showed a protective effect of the homozygous T allele of IL28B SNP (rs12979860) against CMV infection in cancer patients undergoing allo-SCT, T/T carriers showed a lower incidence of active CMV infection episodes occurring within 100 days after allo-SCT, those episodes were shorter in duration and lower in magnitude compared to patients carrying C/C and C/T allele. These findings emphasize the potential for personalized risk assessment and management strategies in pediatric allo-SCT recipients based on IL28B SNP profiling.

#7417 Biological, clinical and epidemiological risk factors for oropharyngeal cancers in people with HIV in Puerto Rico.

Juliana M. Serrano-Rodriguez[1], Jurelis Torres-Reyes[1], Yakshi N. Ortiz-Maldonado[1], Carlos A. Sola-Morla[2], Gabriel Borges-Velez[1], Jeannette Salgado-Montilla[1], Maria M. Sanchez-Vazquez[1], Magaly Martinez-Ferrer[1], Ramon F. Gonzalez-Garcia[3], Josue Perez-Santiago[1]

[1]Division of Clinical and Translational Cancer Research, University of Puerto Rico Comprehensive Cancer Center, San Juan, Puerto Rico,[2]School of Medicine, Universidad Central del Caribe, San Juan, Puerto Rico,[3]School of Dental Medicine, University of Puerto Rico Medical Science Campus, San Juan, Puerto Rico

Background: People with HIV (PWH) are at a higher risk of developing cancers. Periodontal disease (PD), which is highly prevalent in PWH, is a chronic inflammatory disease associated with increased risk factor for oropharyngeal cancers as well as lung, pancreatic and colorectal cancers. Dysbiosis of the oral microbiome and oral human papillomavirus (HPV) infection can increase cancer risk. Puerto Rico (PR) has disparities in HIV infection and HPV-related malignancies, and socio-economic disadvantages that further increase cancer risk, but none of these risk factors have been evaluated altogether in PWH, particularly in PR. In this study, we evaluated the prevalence of PD in Puerto Rican PWH and analyzed other risk factors associated with oropharyngeal cancers in relation to PD.
Methods: We collected saliva and oral rinse samples as well as PD status from 165 sexually active and virally suppressed PWH. We also collected sociodemographic, clinical and lifestyle variables through a questionnaire. In a subset of participants, we evaluated HPV infection and genotype (DNA ELISA kit HPV SPF10 and RHA kit HPV SPF10-LiPA2, n=135), the oral bacteriome (16s sequencing, n=98) and oral mycobiome (ITS sequencing, n=32), in relation to PD as factors for increased oral cancer risk. Diversity, taxonomic assignments, differential abundance analysis and statistical analysis were performed using QIIME2 and R-statistical software.
Results: High prevalence of PD (86%) was observed, with moderate (60%) and severe (27%) being the most abundant. PWH with PD had significantly lower median CD4 count than PWH without PD (753cells/µl vs. 895cells/µl p=0.029). HPV+ PWH had a 95% prevalence of PD, with the prevalence of severe PD increasing to 35%. Furthermore, PD was also significantly associated with higher bacterial and fungal phylogenetic diversity (p=0.048 and p=0.028, respectively) and higher bacterial richness (p=0.063). In addition, significantly higher abundance of fungal pathogenic species was observed in PD, characterized by higher relative abundance of *Candida*.
Conclusion: Overall, high prevalence of PD significantly associated with lower median of CD4 count was observed in Puerto Rican PWH, which may contribute to a higher risk for oropharyngeal cancers, as well as other distal cancers. Moreover, dysbiosis of the oral microbiome and oral HPV infection associated with PD could contribute to subsequent inflammation and oral tissue damage which can further increase cancer risk. Understanding the mechanistic interplay between these cancer risk factors is essential to establish targets for early prognosis and personalized treatment approaches and better preventive public health strategies to reduce oropharyngeal cancers.

#7418 HPV and EBV coinfection in Puerto Rican people with HIV: insights into oropharyngeal cancer risk.

Jurelis Torres-Reyes[1], Oscar A. Loperena-Gonzalez[1], Jeyra L. Perez-Lozada[2], Juliana M. Serrano-Rodriguez[1], Gabriel Borges-Velez[1], Maria M. Sanchez-Vazquez[1], Magaly Martinez-Ferrer[1], Josue Perez-Santiago[1]

[1]UPR - Comprehensive Cancer Center, San Juan, Puerto Rico,[2]San Juan Bautista School of Medicine, Caguas, Puerto Rico

Background: People with HIV (PWH) are disproportionately affected by Human papillomavirus (HPV), a major risk factor for oropharyngeal cancers. Additionally, PWH are more susceptible to acquiring opportunistic infections, such as Epstein-Barr Virus (EBV). EBV may lead to the development and progression of Burkitt's and Hodgkin's lymphoma and other types of cancers. HPV-related malignancies coupled with EBV infection have been associated with an increased risk of cervical cancer, however the relationship between EBV and HPV in in the oral cavity, particularly in PWH has not been investigated. In this study, we evaluated the prevalence of EBV in PWH and assessed their relationship with oral HPV infection due to their potential impact in immune suppression and oropharyngeal cancer development.
Methods: Saliva samples, sociodemographic, and clinical data from 91 sexually active subjects (68 PWH and 23 people without HIV [PWOH]) from a community clinic in San Juan, Puerto Rico. We analyzed for oral HPV infection (34 HPV+ and 57 HPV-) and genotyped HPV using the DNA ELISA kit HPV SPF10 and RHA kit HPV SPF10-LiPA25 that detects 40 HPV subtypes. We quantified EBV levels in saliva using qPCR. Statistical analyses of levels of EBV were assessed in relation to HPV status using R-statistical software.
Results: PWH had a median CD4 count of 791 cells/µL. The prevalences of EBV in PWH was 88% (60/68) vs in PWOH was 74% (17/23). In addition, the prevalence of EBV was higher in males than females (90% vs 63%). PWH with HPV had a 62% (21/34) of high-risk HPV genotypes, with HPV-18 being the most prevalent subtype 38% (8/21). Furthermore, EBV detectability had a trend with HPV status (p=0.07), especially in males with HPV showed higher detectability of EBV (p=0.002). In a multivariate model, EBV detectability was associated with biological sex after adjusting for HIV status and HPV status (p=0.004).
Conclusions: Higher prevalence of EBV were observed in males than females. The trend observed between EBV detectability and HPV status, suggest a potential association between these viral infections, which could have implications for immune regulation in PWH. The high prevalence of HPV in PWH, and the association with EBV, particularly in males may indicated a need to underline the importance of HPV vaccination and regular screening in this population. We aim to increase the sample size to further validate the association of EBV and HPV in Puerto Rican PWH and include sociodemographic and lifestyle variable to investigate how socio-cultural characteristics may influence this association.

#7419 Polygenic risk scores and risk of second cancers among older adult cancer survivors.

Clara Bodelon, Adriana Lori, Christina C. Newton, Ying Wang, Parichoy Pal Choudhury, Hyuna Sung, Ahmedin Jemal, Lauren R. Teras, Alpa V. Patel

American Cancer Society, Atlanta, GA

Background. The etiology of second primary cancers among survivors of older adult-onset cancers is largely known. There is limited data as to whether genetic predisposition increases the risk of second cancers among older adult-onset cancer survivors.
Methods. This analysis was conducted among men and women enrolled in the Cancer Prevention Study II Nutrition cohort who were diagnosed with a first non-metastatic incident cancer between 1992 and 2015. Survivors were followed-up until 2017 for the diagnosis of a second primary cancer at a different organ. Analyses were restricted to participants with genetic data, younger than 85 years at their initial diagnosis, unrelated and of European ancestry. We used externally validated polygenic risk scores (PRSs) for the prediction of overall (overall-PRS; 179 variants), breast (BC-PRS; 313 variants) and prostate (PC-PRS; 451 variants) cancers in the general population. Adjusted Cox proportional regression models were used to compute hazard ratios (HRs) and 95% confidence intervals (CIs) to estimate the risk of second primary cancers associated with the PRSs, modeled as continuous and quartile variables.
Results. This cohort included 16,356 survivors of a first primary cancer (58.1% male). The median age at this diagnosis was 73 years (interquartile range (IQR): 68, 77), with 72.2% of the cancers being localized. The most common first primary cancers were prostate (35.0%), breast (19.1%) and colorectal (9.5%). Cancer survivors were followed-up for a median of 8.6 years (IRQ: 4.1, 13.9) for the incidence of a second primary cancer. We found that the PRSs were associated with significant increased risks of second cancers. Specifically, 1-standard deviation (sd) increased of the overall-PRS increased the risk of any second cancer by 7% (HR=1.07, 95% CI: 1.03, 1.12); 1-sd increased of the BC-PRS increased the risk of breast cancer among female survivors of a non-breast cancer by 59% (HR=1.59, 95% CI: 1.35, 1.86); and 1-sd increased of the PC-PRS also increased the risk of prostate cancer in male cancer survivors of a non-prostate cancer by 59% (HR=1.59, 95% CI: 1.39, 1.82). Compared to the lowest quartiles of each of the PRSs, those in the highest quartile of the overall-PRS had a 16% increased risk of any second cancer (HR=1.16, 95% CI: 1.04, 1.30); those in the highest quartile of the BC-PRS had a 4-fold increased risk of breast cancer (HR=4.07, 95% CI: 2.43, 6.80); and those in the highest quartile of the PC-PRS had a 3-fold increased risk of prostate cancer (HR=3.05, 95% CI: 2.10, 4.43).
Conclusions. In this cohort of older cancer survivors, we found that externally validated PRSs for the prediction of overall and breast and prostate cancers in the general population were associated with higher risks of developing second primary cancers. These PRSs may provide valuable information to improve clinical risk stratification among survivors and opportunities to enhance surveillance for second cancers.

BIOINFORMATICS / COMPUTATIONAL BIOLOGY / SYSTEMS BIOLOGY / CONVERGENT SCIENCE: Artificial Intelligence and Machine/Deep Learning
Poster Session

#7423 Modelling COPD to lung cancer progression using machine learning frameworks.

Tej Pandya[1], Marcellus Augustine[1], Kevin Litchfield[1], Nuno Rocha Nene[2], Charles Swanton[1]

[1]The Francis Crick Institute, London, United Kingdom,[2]University College London, London, United Kingdom

Background
Chronic obstructive pulmonary disease (COPD) is a lung condition characterised by chronic respiratory symptoms due to abnormalities of the airways that causes persistent, often progressive airflow obstruction and independent risk factor for lung cancer. However, the mechanisms linking COPD to lung cancer remain poorly understood, particularly the role of mutual risk factors such as air pollution and smoking. Emerging evidence challenges that environmental carcinogens can be purely mutagenic, and suggests that chronic inflammation also facilitates cancer progression and can be compounded in genetically susceptible individuals. This study aimed to investigate any gene-environment interactions contributing to lung cancer development in patients with COPD.
Methods
We used machine learning frameworks to solve this problem given the limitations of conventional methods on this high-dimensional and multi-modal data. Using germline SNPs, measurements of air pollution through PM 2.5, and clinical information from the UK Biobank, a cohort of 488,377 participants, we created models to predict whether patients with COPD would get lung cancer. We tested multiple frameworks optimised for tabular data such as eXtreme Gradient Boosting (XGBoost) and TabNet, in addition to Elastic Net, to be able to independently capture different facets of the data and account for potential non-linear interactions not modelled in linear approaches. As a secondary aim, we focussed on COPD patients with at least a ten-year post-diagnosis survival, minimizing bias from mortality effects on cancer progression.
Results
In (n=24,094) individuals with COPD, 1,312 progressed to develop lung cancer by the date of censorship. Patients progressing to lung cancer were significantly older at enrolment, had greater exposure to air pollution, and smoked more heavily (all $p < 0.001$). Using an 80:20 train-test split, XGBoost and Elastic Net models outperformed TabNet ($p< 0.01$ by DeLong's test) in predicting lung cancer risk, with XGBoost achieving the highest ROC-AUC on the held-out test set (0.73, 95% CI: 0.72-0.75). Restricting the inclusion criteria to ten-year COPD survivors notably improved prediction accuracy (ROC-AUC = 0.87, 95% CI: 0.85-0.90) and identified SNPs related to lung function and inflammation (such as RAGE) as important risk factor of lung cancer in patients in COPD patients.
Conclusions
This study demonstrates that integrating machine learning with epidemiological data enables high-accuracy modelling of lung cancer risk in patents with COPD, emphasizing a potential role of inflammation mediating PM 2.5 gene-environment interactions. These findings underscore the need for research on the biological mechanisms driving inflammation-associated cancer risk in COPD for patient stratification, as well as the potential for applying machine learning frameworks methods to other cancers to explore tumorigenic inflammation.

#7424 Simplifying oncology terminology: Leveraging large language models for patient education.

Yuqing Wang[1], Inae Park[1], Simo Du[2], Zhengrui Xiao[1], Yizhou Chen[3]

[1]Montefiore Medical Center, Albert Einstein College of Medicine, The Bronx, NY,[2]Jacobi Medical Center, Albert Einstein College of Medicine, The Bronx, NY,[3]Neuroscience Institute, Carnegie Mellon University, Pittsburgh, PA

Introduction: Large language models (LLMs), such as OpenAI's ChatGPT-4, are designed to process natural language and generate responses to text-based prompts. While these models have shown promise in addressing clinical and patient-related inquiries, they are not specialized in medicine and lack integration with dedicated medical knowledge databases, leading to potential inaccuracies. On the other hand, healthcare professionals and models explicitly trained in medical texts frequently rely on specialized terminology, which can create significant barriers to clear and patient-friendly communication. This study aims to utilize LLMs to translate complex medical terminology into easy-to-understand explanations, focusing on hematology and oncology fields where communicating medical concepts to the public is particularly challenging. Our objective is to develop a solution that ensures explanations are both accurate and accessible, bridging the gap between technical medical knowledge and patient comprehension.

Methods: We curated a dataset of cancer-related terms and their explanations from two sources: the National Cancer Institute (NCI) Dictionary, which provides detailed medical definitions, and simplified explanations based on National Comprehensive Cancer Network (NCCN) guidelines for patients. Using Meta's LLaMA 7 B-based chat model, we implemented retrieval-augmented generation (RAG) to enable the model to access the NCI Dictionary as needed. To fine-tune the model to generate patient-friendly explanations, we applied LoRA-based supervised fine-tuning (SFT). Readability was measured using the Flesch Reading Ease Score (FRES) and Dale-Chall Readability Formula (DCRF). Improvements in accessibility were quantified through a two-sample t-test, comparing the mean readability scores of the model's outputs against the baseline.

Results: The fine-tuned model demonstrated significant improvements in both accessibility and readability, achieving a 5% and 4% increase in the FRES (baseline: 69.25; output: 72.60, $P < 0.01$) and DCRF (baseline: 9.50; output: 9.15, $P < 0.01$), respectively. Preliminary results also indicate the model's capability to translate entire paragraphs of dense medical text into patient-friendly explanations. Expert validation on a larger scale is currently underway, further solidifying the model's potential to revolutionize patient communication in oncology. This innovative approach sets a new standard for leveraging advanced language models in healthcare education.

Conclusion: We successfully trained a large language model specifically designed to simplify complex oncology medical terms into patient-friendly language. By serving as a reliable tool for delivering precise and accessible medical information, it holds the potential to reduce the workload of healthcare providers and enhance patient understanding in clinical settings.

#7425 AttentionAML: An attention-based deep learning model for accurate identification of childhood acute myeloid leukemia subtypes.

L. Li, J. D. Khoury, J. Wang, S. Wan;
University of Nebraska Medical Center, Omaha, NE

Acute myeloid leukemia (AML) is a rapidly progressing hematopoietic malignancy characterized by aberrant clonal expansion of abnormal myeloid progenitor cells, typically leading to bone marrow failure and compromised hematopoiesis. Pediatric AML encompasses over 20 molecular subtypes defined by genetic alterations, such as chromosomal alteration, gene fusions, mutations, or tandem duplications. Each subtype exhibits a specific response to treatment and prognosis, underscoring the critical need of accurately identifying subtypes for effective clinical management and tailored therapeutic approaches. Traditional wet lab approaches to identify AML subtypes primarily rely on morphological analysis, cytogenetic analysis, immunophenotyping, or molecular profiling, which can be labor-intensive, costly, and technically challenging for clinical practice applications. To address these challenges, we propose AttentionAML (Attention-based Multilayer Perceptron Model for Identifying Acute Myeloid Leukemia Subtypes), a novel deep learning approach that leverages the power of attention mechanisms and multilayer perceptron (MLP) to accurately classify AML subtypes based solely on transcriptomic profiling. Compared with standard MLP models, the attention-based MLP model can capture both global and local information within high-dimensional gene expression data by focusing on the most relevant features, enhancing the performance of the model in AML subtyping. Our model is trained on different cohorts of 1,656 AML patients with known subtypes, utilizing RNA-seq data from publicly available databases such as Therapeutically Applicable Research to Generate Effective Treatments (TARGET) initiative and the St. Jude Cloud Genomics Platform. To evaluate the performance and generalizability of AttentionAML, we conducted rigorous 100 times five-fold cross-validation tests. Our results suggested that AttentionAML consistently outperformed both conventional machine learning models (e.g., ensemble SVM) and standard deep learning models (e.g., MLP model), with remarkable performance across different metrics, including the accuracy of 97.15%, precision of 97.13%, recall of 97.15%, F1 score of 97.02%, and the Matthews correlation coefficient (MCC) of 0.9674. In addition, by leveraging the hidden layer embedding information, AttentionAML gave clearer and more insightful visualization of the global landscape of all AML patients compared to tSNE, providing better separation of AML subtypes and preserving both local and global data structures. We believe that AttentionAML represents a significant progression in characterizing pediatric AML, which can potentially improve downstream risk stratification, optimize treatment strategies, and advance precision medicine for AML as well as other pediatric, adolescent, and young adults (AYA).

#7426 Leveraging deep learning to enable precision medicine via the IMPROVE benchmarking framework.

Natasha Koussa[1], Sara Jones[1], Jeremy Jacoboson[2], Yannick Mahlich[2], M Ryan Weil[1], **Sara J. C. Gosline**[2]

[1]Frederick National Laboratory for Cancer Research, Frederick, MD,[2]Pacific Northwest National Laboratory, Seattle, WA

The rise of targeted therapies for cancer treatment has fueled the development of algorithms that take the molecular profile (e.g. mutations, transcripts, etc.) of a tumor and predict the effect a drug has on this tumor. Recent advancements in deep learning have enabled the prediction of a cell's response to untested, potentially novel drugs using molecular measurements [1]. To assess the clinical reliability of these algorithms, we first need to measure their performance across a range of data and model systems (e.g. cell lines, organoids, etc.). Toward this end, we introduce a benchmarking framework that enables the comparison of deep learning models for drug response across both cell line and organoid datasets.

We built a fully provenanced, federated data collection pipeline that standardizes gene names, chemical structure information, and recalculates drug response curves for 10 different datasets from 4 different model system types, each accessible via our coderdata Python package (https://pnnl-compbio.github.io/coderdata/) which properly formats and processes the data for machine learning ingest. We integrated this package into the IMPROVE benchmarking framework (https://github.com/JDACS4C-IMPROVE) to evaluate the performance of eight machine learning algorithms that either leverage gene expression data alone [2-5], or with copy number [6,7], mutation[8], and methylation data[9] added. For each algorithm we calculate the performance when using different data splitting methods as well as within-dataset and cross-dataset performance, including multiple model system types.

To date, we have collected over 4,100,000 drug-cell datapoints across ~55,000 drugs and ~1600 samples and 4 types of model system. Using our framework we evaluate (1) the effects of proper data splitting in cross-dataset analysis within a single dataset, (2) cross-dataset predictions within model systems, and (3) the ability to predict across different model systems. We are currently extending the organoid data to better enable the benchmarking of tools across the model systems and add algorithms to our benchmarking framework. We plan to use this framework to perform robust assessment of clinical applicability of AI/ML in precision medicine.

References: **1.** Baptista, et al. PLOS Comput Biol. 2023 Mar 23;19(3):e1010200. **2.** Jiang, et al. Brief Bioinform. 2022 May 1;23(3):bbac100**. 3.** Nguyen, et al. IEEE/ACM Trans Comput Biol Bioinform. 2022;19(1):146-54. **4.** Jin, et al. HiDRA: Hierarchical Network for Drug Response Prediction with Attention. J Chem Inf Model. 2021 Aug 23;61(8):3858-67. **5.** Ke, et al. In: Advances in Neural Information Processing Systems [Internet]. Curran Associates, Inc.; 2017. **6.** Ma, et al, BMC Bioinformatics. 2022 Apr 15;23(4):129. **7.** Tang, et al. Sci Rep. 2021 Feb 4;11(1):3128. **8.** Li, et al. Brief Bioinform. 2024 May 1;25(3):bbae153. **9.** Liu, et al. Bioinformatics. 2020 Dec 30;36(Supplement_2):i911-8.

#7427 Use of large language models for clinical data abstraction from oncologic medical records.

Jillian Nelson[1], Monica Agrawal[2], Den E Bloodworth[1], Divya Gopinath[2], James Mullenbach[2], Peter J. Briggs[1], Dominique Connolly[2], David Sontag[2], Alpa V. Patel[1]

[1]American Cancer Society, Atlanta, GA,[2]Layer Health, Boston, MA

Introduction: Abstraction of clinical data from unstructured medical records is a labor-intensive process. Large language model (LLM)-based systems can accelerate the availability of data for research use.

Participants and Methods: The American Cancer Society's Cancer Prevention Study-3 is a large, nationwide prospective cohort study of ~300,000 cancer-free participants enrolled between 2006-2013. Participants who self-reported a diagnosis of breast, colorectal, ovarian, or any blood cancer on the 2021 follow-up survey were consented for the retrieval of medical records (available as a single PDF) from diagnostic and treatment facilities. Guidelines, e.g. ontologies and decision trees, were generated to precisely define abstraction tasks, and relevant research data were annotated via human abstraction. This project then used 300 breast cancer-related medical records to develop (n=200) and test (n=100) the performance of Layer Health's LLM-based platform Distill to abstract seven data elements from the medical record: cancer behavior, laterality, neoadjuvant therapy status, and presence of key biopsy or surgery with associated procedure dates. Both an answer and corresponding evidence from the record were provided by their algorithms; this evidence was used to conduct quality control on a small fraction of data points that were automatically flagged by inter-variable consistency checks. An ACS oncology data specialist adjudicated disagreements between human and LLM-based abstraction to update ground truth labels. Accuracy and F1 statistics were then calculated between ground truth and LLM-derived results.

Results: Results on the test set (n=100) were available on the same day; adjudication of 25 discrepancies between human and LLM-based abstraction found 12 LLM errors and 13 human errors. Cancer behavior and laterality were abstracted with 100% accuracy by the LLM system. Neoadjuvant therapy (yes/no) was abstracted with 99% accuracy (F1=0.99). Surgical and biopsy procedures (yes/no) were abstracted with 98% (F1=0.99) and 96% (F1 = 0.98) accuracy, respectively. 94% of identified key biopsy dates were accurate within 1 day, and 96% were accurate within 30 days. 96% of identified key surgery dates were accurate within 1 day, and 97% were accurate within 30 days.

Conclusion: LLM-based systems offer a time-efficient and accurate solution to accelerate abstraction of key data elements for use in oncologic research, including complex multi-step variables.

#7428 Predicting acute care utilization following cancer treatment using machine learning: A patient-centered approach to precision oncology.

Arman Koul[1], Mohana Roy[2], Behzad Naderalvojoud[1], Tina Hernandez-Boussard[1]

[1]Biomedical Informatics, Stanford University School of Medicine, Stanford, CA,[2]Medical Oncology, Stanford University School of Medicine, Stanford, CA

Introduction: Unplanned acute care utilization (ACU) after receipt of cancer treatment is a significant challenge in oncology, often reflecting care gaps and driving higher costs. To address this, the Centers for Medicare & Medicaid Services (CMS) have designated ACU as a critical quality metric (OP-35). Predicting patients at risk and understanding root causes of ACU can enable interventions that improve outcomes for patients. This work applies advanced machine learning models, integrating structured and unstructured data, to predict ACU in diverse patients across varying malignancy categorizations and treatments.

Methods: This study utilized data on 18,143 patients from a comprehensive, multisite cancer center, to develop machine learning models predicting ACU within 30 days of cancer therapy initiation per OP-35 criteria. Predictions for each therapy line were generated separately. Predictions utilized structured data from medical records and when earlier therapy lines were received outside the center, large language models were applied to clinical notes to encode this unstructured data into the model. Demographic variables, including race, ethnicity, and insurance, were included to identify potential biases. The model, trained on 2010-2019 data and validated on 2020-2024 data, used mean imputation for missing values and employed XgBoost and Random Forest algorithms for supervised learning with hyperparameter tuning to ensure the best trade-off between bias and variance in the model.

Results: The 30-day ACU prediction model showed strong predictive performance, with an XGBoost model achieving an Area Under the Receiver Operating Characteristic Curve (AUROC) of 0.84 (95% CI: .82-.85). Performance was further analyzed across OP-35 categories, with the highest accuracy in predicting ACU due to anemia (AUROC: .87, 95% CI: .86-.88) and infection (AUROC: .86, 95% CI: .85-.87). Incorporating later therapy lines enhanced predictive accuracy by capturing the complexities of advanced disease.

Conclusions: This study highlights the potential of ML to advance precision oncology by predicting ACU following cancer therapy—a critical indicator of patient-centered care quality. By incorporating data from multiple therapy lines and leveraging advanced modeling techniques, it addresses complexities such as disease progression and diverse treatment histories. Decomposing predictions by ACU causes offers actionable insights for timely interventions. These findings highlight the importance of integrating advanced analytics into clinical workflows to improve outcomes, reduce costs, and support data-driven decision-making in oncology.

#7429 Illuminating the dark genome in cancer using artificial intelligence.

M. Alvarez-Torres, X. Fu, R. Rabadan;
Columbia University Irving Medical Center, New York, NY

Understanding the role of noncoding regions of the human genome in cancer requires innovative, high-resolution approaches. Artificial intelligence (AI) is transforming cancer research by providing advanced tools to identify, analyze, and interpret cancer genomes. This study aims to 1) discuss how noncoding mutations in key regulatory elements and other regions can contribute to cancer development, and 2) evaluate the leading AI models used for analyzing noncoding variants, focusing on their evolution and application in cancer research. We present examples of mutations in promoters, e.g. TERT [Huang et al., Science 2013] APC [Snow et al., Clin Gen 2015]), enhancers (a SNP in gliomas [Yanchus et al., Science 2022]), and super-enhancer hypermutation in B-cell lymphomas [Bal et al., Nature 2022; Leeman-Neil et al., Nat Gen 2023]. These cases demonstrate the functional impact of noncoding variants in tumorigenesis. However, for many noncoding regions and mutations, our understanding of the underlying mechanisms, binding motifs, and their roles in gene regulation remains limited. Here, AI can play a crucial role by analyzing large genomic datasets to uncover hidden patterns and interactions. This study compares AI models developed over the past decade, including DeepSEA[Zhou et al., Nat Meth 2015], DeepBind [Alipanahi et al., Com Bio 2015], DeltaSVM [Lee et al., Nat Gen 2015], Basset [Kelley et al., Gen Res 2015], CADD [Rentzsch et al., Nuc Ac Res 2018], Expecto [Zhou et al., Nat Gen 2018], DeepC [Schwessinger et al., Nat Meth 2020], Enformer [Avsec et al., Nat Meth 2021], and GET [Fu et al., Nature 2024 (in press)]. We assess and scored key factors such as model complexity, data integration, cell-type specificity, interpretability, and usability within cancer research. Older models (2015-2018) score lower in complexity than recent ones (2018-2024), reflecting simpler architectures due to earlier computational limitations. In contrast, newer models leverage advanced computational power and deep learning, enhancing their ability to capture complex biological patterns and improve data integration and cell-type specificity. However, usability in practical research does not correlate with publication year, as user-friendly interfaces and comprehensive documentation play crucial roles in application, regardless of model complexity. In conclusion, AI has proven instrumental in elucidating the role of noncoding mutations in cancer, revealing intricate regulatory patterns and previously unknown mechanisms. Over 11,000 publications cite the models discussed, underscoring their scientific impact. Such efforts will support predictive models that can generalize across diverse contexts, advancing genomic research and its implications for cancer biology. Our work highlights the potential of these AI models to assist researchers, particularly those less familiar with computational techniques, in advancing cancer genomics.

#7430 Multicenter study on an artificial intelligence model for predicting NSCLC recurrence.

Junho Lee[1], Daehwan Lee[2], Kiwon Lee[2], Ho Yun Lee[3], Tae Jung Kim[3], Yeong Jeong Jeon[3], Junghee Lee[4], Seong Yong Park[4], Jong Ho Cho[4], Hong Kwan Kim[4], Yong Soo Choi[4], Sehhoon Park[5], Jong-Mu Sun[5], Se-Hoon Lee[5], Jin Seok Ahn[5], Myung-Ju Ahn[5], Hyun Ae Jung[5]

[1]Division of Medical Oncology-Hematology, Department of Internal Medicine, Kyung Hee University Hospital, Kyung Hee University College of Medicine, Seoul, Korea, Republic of,[2]Spidercore Inc., Daejeon, Korea, Republic of,[3]Department of Radiology and Center for Imaging Science, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of,[4]Department of Thoracic and Cardiovascular Surgery, Sungkyunkwan University School of Medicine, Samsung Medical Center, Seoul, Korea, Republic of,[5]Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of

Background:Accurate and practical prediction of recurrence in early-stage NSCLC is essential for managing patient outcomes and tailoring personalized treatment strategies. This study updated and validated a previously developed transformer-based model for NSCLC recurrence prediction, which integrates clinical, pathological, laboratory, and radiologic data, while proposing strategies to enhance its generalizability.Methods:The model was developed and internally validated using data from Samsung Medical Center (SMC) and Kyung Hee University Hospital (KH) collected from 2008 to 2024. The primary outcome was predicting recurrence within one year after the monitoring point. This study demonstrates the timely provision of risk scores (RADAR score) and determined thresholds and the corresponding area under the curve (AUC).
Results:A total of 16,828 patients was enrolled (12,208 with stage I; 2,702 with stage II; and 1,918 with stage III) Development set was 11,341 and validation set was 5,487. The median age was 64 years (range: 13-96). Females accounted for 44.3%, while males made up 55.7%. The median follow-up duration was 32.9 months, and 18.7% of patients experienced recurrence. The percentage of never-smokers was 49.7%, and ex-smokers or current smokers comprised 50.3%. Median RFS in total population was 140.9 months (95% CI, 134.0-147.9). The AUC for predicting relapse within one year of the monitoring point was 0.804. However, depends on the institute, AUC was 0.855 for SMC data (2008-2022), however 0.756 for SMC data (2023-2024) and 0.719 for KH data. We developed updated version of RADAR model by using multi-center data and validation. The updated RADAR model showed 0.858 of AUC with sensitivity of 74.5% and specificity of 82.1% (AUC=0.865 in stage I, AUC=0.756 in stage II, and AUC=0.731 in stage III).Conclusions:This study addresses the challenges of generalizability in NSCLC recurrence prediction models across diverse data sources. The integrated model showed improved overall performance, emphasizing the importance of multi-institutional data integration for enhancing prediction reliability. Future work will focus on feature selection to mitigate data heterogeneity and optimize model performance. Further external validation and refinement are necessary to support the implementation of precision medicine tailored to individual patients.

#7432 Transformer-based glioma patient recurrence prediction model infers novel genetic markers.

Ko-Hong Lin, Kai Zhang, Dung-Fang Lee, Xiaoqian Jiang

UT Health Houston, Houston, TX

Approximately 90% of glioma patients experience recurrence after initial treatment, leading to poor prognosis and short survival time. While established genetic markers, such as IDH mutation and 1p/19q codeletion, are linked to glioma prognosis, there is a critical need to identify novel targets associated with recurrence to overcome therapeutic challenges. In this study, we developed a transformer-based deep learning model to predict glioma recurrence by integrating multi-omics data, including mutations and copy number variations, from the Glioma Longitudinal Analysis (GLASS) Consortium. Our model outperformed traditional machine learning approaches, including support vector machines, logistic regression, and decision trees, achieving the best performance when utilizing mutational features alone, while copy number variations were less informative. Feature importance analysis identified key contributors to glioma recurrence, including frequently mutated genes such as TP53, ATRX, and PTEN, as well as novel markers like MUC16, TTN, and CIC. Our approach represents an early application of state-of-the-art deep learning to dissect the genomic drivers of glioma recurrence, offering valuable insights for developing targeted therapies to improve patient outcomes.

#7433 Predicting overall survival (OS) differences using longitudinal AI-driven imaging-based prognostication (IPRO) in patients with advanced non-small cell lung cancer (aNSCLC).

Mohammed A. Alvi[1], John Riskas[1], Shahid A. Haider[1], Vignesh Sivan[1], Oleksandra Samorodova[1], Jay Hennesy[1], Duoaud Shah[1], Felix Baldauf-Lenschen[1], Marina Salluzzi[2], Ronald Bridges[3], **Omar F. Khan**[4]

[1]Altis Labs, Inc., Toronto, ON, Canada,[2]Calgary Image Processing and Analysis Centre, University of Calgary Cumming School of Medicine, Calgary, AB, Canada,[3]Department of Medicine, University of Calgary Cumming School of Medicine, Calgary, AB, Canada,[4]Department of Oncology, University of Calgary Cumming School of Medicine, Calgary, AB, Canada

Background: Early prediction of overall survival benefit associated with cancer treatments remains a key challenge in clinical trials and patient care. Standardized measures such as the Response Evaluation Criteria in Solid Tumours (RECIST) v1.1 are used in clinical trials to define tumour response and progression, but are subject to inter- and intra-observer variability, and are infrequently used in day to day clinical practice. Imaging-based prognostication delta (IPRO-Δ) is an artificial intelligence system trained on longitudinal changes in rich features extracted from serial real-world imaging data, automatically predicting survival from pairs of baseline (BL) and follow up (FU) thoracic computed tomography (CT) scans.

Methods: IPRO-Δ was evaluated in an external real-world dataset of 194 aNSCLC patients treated with first line systemic therapy at 17 cancer centers, with pretreatment BL and FU (8±4 weeks after treatment initiation) scans. IPRO-Δ generated survival predictions at week 8 for each patient. Kaplan-Meier analyses and hazard ratios (HR) evaluated the association of IPRO-Δ quartiles with OS, with the highest quartile representing progressive disease, the lowest treatment response, and the middle stable disease.

Results: The cohort included 19 stage IIIB (9.8%) and 175 stage IV (90.2%) aNSCLC patients, with 94 females (48.5%) and a median age of 64 years. Most tumours were adenocarcinoma (n = 147, 75.8%), followed by squamous cell (n = 18, 9.3%), large cell (n = 5, 2.6%) and other histologies (n = 24, 12.4%). Table 1 shows survival stratification amongst IPRO-Δ quartiles for the overall population and the subgroup of EGFR+ patients.

Conclusion: IPRO-Δ successfully predicted survival differences amongst aNSCLC patients using BL and week-8 CT scans. Future work will compare IPRO-Δ to RECIST v1.1, potentially leading to IPRO-Δ's use as a surrogate endpoint in aNSCLC trials.

Table 1. Overall survival based on IPRO-Δ quartile risk stratification.

	Overall Population (n = 194)			**EGFR Positive Population (n = 79)**	
	Median OS, mths (95% CI)	*HR (95% CI)*		*Median OS, mths (95% CI)*	*HR (95% CI)*
Treatment Response Quartile	16.07 (12.93 - 40.3)	ref	**Treatment Response Quartile**	29.4 (16.07 - 50.9)	ref
Stable Disease Quartiles	14.75 (12.3 - 19.2)	1.31 (0.91 - 1.89)	**Stable Disease Quartiles**	20.2 (16.93 - 24.8)	1.58 (0.89 - 2.8)
Progression Quartile	7.43 (5.03 - 10.9)	2.11 (1.39 - 3.2)	**Progression Quartile**	10.2 (7.43 - 26.6)	2.71 (1.41 - 5.19)

#7434 Cloud-based machine learning for enhanced tumor classification in cancer genomics: an end-to-end solution for whole slide imaging data.

Jovana Babić, Nevena Nikolić, Milan Kovačević, Rowan Beck, **Tariq Khoyratty**, Zelia Worman

Seven Bridges Genomics Inc., Boston, MA

The digitization of histological slides into high-resolution Whole Slide Images (WSIs) has revolutionized pathology workflows, providing unparalleled insights into tissue morphology and playing a vital role in advancing cancer diagnostics and treatment. Tasks that were once time-consuming and subjective can now be automated and enhanced with machine learning (ML) and artificial intelligence (AI) solutions. These include image analysis systems capable of extracting diagnostic insights such as tumor classification, subclassification, and grading, aiming to improve accuracy, reproducibility, and efficiency in histopathology workflows. To lower the technical barrier to researchers in accessing and utilizing advanced ML, we developed a comprehensive solution for classifying WSIs by disease or tumor subtype, based on the morphological characteristics of the tissue. Building on the NCI Cancer Research Data Commons' (CRDC) cloud infrastructure, we take advantage of hosted data from the Imaging Data Commons (IDC) and Human Tumor Atlas Network (HTAN) paired with the computational resources available in the Cancer Genomics Cloud (CGC), powered by Velsera and funded by the NCI, to host a reproducible WSI ML solution. This solution includes data preparation and preprocessing, model training, evaluation, and predictions, within an interactive analysis environment. The analysis harnesses the computational capabilities of the CGC platform to efficiently process large-scale datasets. To facilitate data preparation, we integrated tools previously developed on the CGC to extract regions of interest (ROIs) from WSIs, enabling the creation of expanded datasets by generating tumor tissue patches for model training. To further enhance classification performance, a consensus model approach was employed—combining predictions from multiple models to improve robustness and accuracy. This work demonstrates the power of coupling AI-ready harmonized imaging data, available from within the CRDC, with advanced ML techniques, and showcases an end-to-end ML workflow on the CGC. By hosting the solution on an accessible and interactive system, users will be able to seamlessly apply the analysis to their own datasets, gaining meaningful insights with minimal technical barriers. The combination of best practices in data preparation, model development, and consensus-driven accuracy improvement makes ML for histopathology more accessible to users, enabling further research and deeper insights into cancer etiology and diagnostics.

#7435 CRCFound: A colorectal cancer CT image foundation model based on self-supervised learning.

Jing Yang[1], **Du Cai**[2], Junwei Liu[3], Zhenfeng Zhuang[1], Fengao Wang[3], Chenghang Li[4], Chuling Hu[2], Baowen Gai[2], Yiping Chen[5], Yixue Li[3], Liansheng Wang[1], Feng Gao[2], Xiaojian Wu[2]

[1]Xiamen University, Xiamen, China,[2]The Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou, China,[3]Guangzhou National Laboratory, Guangzhou, China,[4]The Hong Kong University of Science and Technology, Guangzhou, China,[5]Sun Yat-sen University, Guangzhou, China

Background: Colorectal cancer (CRC) is a significant global health threat. Accurate risk stratification is essential for determining the optimal treatment plan, yet existing deep learning models perform poorly in the preoperative diagnosis of CRC and lack generalizability due to insufficient annotated data. To address these issues, we propose CRCFound, a self-supervised learning-based CT image foundational model for CRC.
Methods: A total of 6,332 CRC patients with preoperative CT images were collected from 2008 to 2019 at the Sixth Affiliated Hospital of Sun Yat-sen University. Among them, 5,137 unlabeled CT images were used for pretraining, and 1,195 images were reserved for fine-tuning and validation. Comprehensive benchmark tests were conducted on six diagnostic and two prognosis tasks in comparison with other models. Model interpretation was also explored to gain a more profound understanding of the model's behavior and decision-making process.
Results: CRCFound demonstrated excellent performance and generalization ability. For preoperative diagnosis of TNM stage, the average AUC of CRCFound for T, N, M, and overall TNM stage reached 0.889, 0.847, 0.830, and 0.774. In the MSI and CMS diagnosis tasks, it also showed good predictive performance (AUC 0.952 and 0.810, respectively). For prognosis prediction, CRCFound_CT was an independent risk factor for disease-free survival (hazard ratio (HR)= 4.62, 95%CI = 2.7-7.88, P < 0.001) and overall survival (HR = 7.78, 95%CI = 4.12-14.7, P < 0.001). Feature visualization using the t-SNE algorithm demonstrated that CRCFound could effectively differentiate samples in multiple tasks. Visualization of the attention map indicates that CRCFound can focus more effectively on the critical areas of the tumor than traditional supervised learning models, thus providing more accurate predictions.
Conclusion: CRCFound addresses the challenge of insufficient annotated data and performs well in a wide range of downstream tasks, offering a promising solution for accurate diagnosis and personalized treatment of CRC patients.

#7436 DeepSomatic: A SNV and small-indel somatic caller using deep neural networks.

Daniel E. Cook[1], Jimin Park[2], Pi-Chuan Chang[1], Alexey Kolesnikov[1], Lucas Brambrink[1], Juan C. Mier[1], Joshua Gardner[2], Brandy McNulty[2], Samuel Sacco[2], Ayse Keskus[3], Asher Bryant[3], Tanveer Ahmad[3], Jyoti Shetty[4], Yongmei Zhao[5], Bao Tran[4], Giuseppe Narzisi[6], Adrienne Hellend[6], Byunggil Yoo[7], Irina Pushel[7], Lisa Lansdon[7], Chengpeng Bi[7], Adam Walter[7], Margaret Gibson[7], Tomi Pastinen[7], Midhat S. Farooqi[7], Nicolas Robine[6], Karen H. Miga[2], Andrew Carroll[1], Mikhail Kolmogorov[3], Benedict Paten[2], Kishwar Shafin[1]

[1]Google, Mountain View, CA,[2]UC Santa Cruz Genomics Institute, Santa Cruz, CA,[3]Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD,[4]Sequencing Facility, Cancer Research Technology Program, Frederick National Laboratory for Cancer Research, Frederick, MD,[5]Sequencing Facility Bioinformatics Group, Biomedical Informatics and Data Science Directorate, Frederick National Laboratory for Cancer Research, Frederick, MD,[6]New York Genome Center, New York, NY,[7]Children's Mercy Hospital, University of Missouri-Kansas City School of Medicine, Kansas City, MO

DeepVariant is a highly accurate germline variant caller that applies deep dearning to classify germline variants with high accuracy. Here, we present DeepSomatic, which applies DeepVariant's convolutional neural network (CNN) to accurately call somatic mutations from paired tumor-normal sequencing and tumor-only sequencing. To develop DeepSomatic, we adapted the input pipeline to accommodate tumor-normal pairs. DeepVariant model inputs consist of a set of pileup images, also referred to as "channels", that represent features extracted from sequence data at candidate sites. Features include base, base quality, mapping quality, and haplotype. Channel sets from both tumor and normal samples are combined. In this way, our model can compare tumor and normal reads and learns to distinguish germline variants from somatic mutations.

For training, DeepSomatic uses sequence data from the SEQC2 consortium, which has established a benchmarking dataset for HCC1395 (a triple-negative breast cancer cell line) across a variety of sequencing technologies. Additionally, we have supplemented the SEQC2 data with additional sequencing from cell lines (H2009, H1437, HCC1954, HCC1937, Hs578T) to further improve our training datasets and enable benchmarking across different cancer types. We have also developed in silico samples that replicate varying levels of tumor purity and normal-sample contamination. Using these data, we have trained models on whole-genome data from Illumina, PacBio, and Oxford Nanopore (ONT).

We report that DeepSomatic outperforms existing somatic callers across sequencing technologies. For example, we observe that our DeepSomatic Illumina model has a SNP F1 score of 0.983 on held-out chr1 HCC1395 data, which outperforms ClairS (F1=0.969), and Strelka2 (F1=0.952). Similarly, our PacBio model has a SNP F1=0.95 compared to ClairS (F1=0.935). Finally, our ONT model achieves an F1=0.869, which is an improvement over ClairS (F1=0.863). We similarly observe that DeepSomatic outperforms on F1 scores for indels as well for Illumina, PacBio, and ONT models. We plan to continue improving DeepSomatic through improvements to the software, models, and training data. We welcome community feedback.

#7437 AI-guided discovery of small molecules targeting eIF4F complex to inhibit oncogenic mRNA translation in castration-resistant prostate cancer.

Fengze Jin, Puja Singh, Hanyong Chen, Yibin Deng

Department of Urology, The University of Minnesota Medical School, Minneapolis, MN

Effective treatment of castration-resistant prostate cancer (CRPC) remains a significant clinical challenge. Our structure-guided biochemical studies have revealed that the interaction between eukaryotic initiation factors eIF4A and eIF4G within the eIF4F complex plays an essential role for prostate cancer cell survival and CRPC tumor growth. Genetic disruption of the eIF4A-eIF4G interaction in cancer cells selectively inhibits oncogenic mRNA translation, significantly reduces prostate cancer cell survival, and suppresses the *in vivo* growth of xenografted CRPC tumors. Despite its promise as a therapeutic target, no chemical drug has been developed that effectively disrupts the eIF4A-eIF4G complex to block oncogenic mRNA translation for cancer therapy. To enhance the likelihood of discovering highly biologically active compounds targeting the eIF4A-eIF4G complex, we are developing and utilizing artificial intelligence (AI)-guided “Deep Docking (DD)” algorithms with Schrödinger Molecular Docking platform. This approach enables to develop 'end-to-end' algorithms for structure-based virtual screening (SBVS) of ultra-large chemical libraries containing billions of synthesizable molecules, including novel and unchartered areas of the chemical universe. Building on our resolved structure of the human eIF4A-eIF4G complex, we performed AI-guided SBVS of a chemical library containing 1.5 billion of molecules. By integrating our optimized computational filtering and validation approaches, we identified and selected top-ranked promising candidates for chemical synthesis. Experimental validation of the synthesized compounds demonstrated their abilities to disrupt the eIF4A-eIF4G interaction, block the translation of oncogenic mRNAs such as MYC, MDM2, BCL2, and MCL1, and significantly reduce the survival of CRPC cells. These results demonstrate the effectiveness of our AI-guided platform for structure-based ultra-high chemical library screening, highlighting its potential to accelerate the discovery of drug-like molecules targeting the eIF4F complex. Translationally, the experimentally validated compounds could be advanced into first-in-class therapies for castration-resistant prostate cancer (CRPC) characterized by aberrant activation of the eIF4F complex.

#7438 Mapping T cell reactivity against the human genome using machine learning uncovers target specific TCRs against novel tumour antigens.

Nathaniel J. Davies[1], Amalia Martinez[1], Maria Busz[1], David Cook[1], Xiaoyan Pan[1], Juan Bolivar[1], Simon Wright[1], Eleanor Bagg[1], Graham Ogg[2], Sophie Johnson[1], Sophie Wells[1], Sophie Richard[1], Samantha Drennan[1], Samuel Ward[1], Sarah Leonard[1], Thomas L. Andresen[1]

[1]T-Cypher Bio Limited, Oxford, United Kingdom,[2]MRC Translational Immune Discovery Unit, University of Oxford, Oxford, United Kingdom

Introduction: T cell engagers (TCEs) are a class of targeted therapies with demonstrated clinical potential in solid tumors. A main challenge currently facing TCEs is the availability of clean cancer targets with a wide therapeutic window. Intracellular targets presented to T cells by HLA complexes are strongly linked to disease mechanisms and are therefore very attractive, but drugging HLA-presented targets has been challenged by our ability to map the specificity of T cell repertoires. We here present the T-Cα platform, which represents a fundamental change in TCR discovery methodology, enabling us to discover >10,000 TCRs per week against 100s of targets. This data has enabled us to train machine learning (ML) models and to demonstrate the ability of these models to extrapolate beyond the training data. In effect, this results in the discovery of novel therapeutically relevant TCRs and enhances our understanding of the interface between T cell reactivity and the human genome.

Experimental Procedures: We used the T-Cα platform to discover more than 50 novel targets across all areas of the genome, including regions previously considered non-coding. We screened billions of T cells from 100s of donors against these targets and other targets of interest. We generated single-cell transcriptomic data as part of the screens and predicted the potency of each TCR at the discovery stage. For the purposes of model development, we generated matched negative data with non-binding TCRs during the course of the screens. These datasets, representing 10,000s of TCR-epitope pairs, were collated alongside available public data and a machine learning model was trained to predict TCR specificity. The TCRs were functionally validated downstream for potency and cross-reactivity.

Results: We have generated, to our knowledge, the largest high-quality database of TCR-epitope pairs against 100s of targets. We demonstrate that with this data, alongside matched negatives, we can train models that are able to predict TCR specificity with high concordance with known epitope pairings. We used the model to predict the specificity of unseen TCRs against novel targets of therapeutic interest in oncology. We further demonstrate that the model can extrapolate beyond the training data and identify novel target-specific TCRs suitable for the development of TCEs.

Conclusions: We have developed a uniquely high-throughput, high-content TCR discovery platform with which we are able to discover >10,000 specific TCRs per week against 100s of targets. We have trained a machine learning model which can accurately extrapolate to unseen TCRs, enhancing our understanding of T cell reactivity against the human genome. With the T-Cα platform, we have built a pipeline of novel T cell engagers for the treatment of solid tumors, the first of which we expect to enter IND enabling studies mid 2025.

#7439 Label-free 3D virtual papanicolaou staining of urine cytology using deep learning and holotomography.

Tal Lifshitz[1], Geon Kim[1], Su-Jin Shin[2], Kwang Suk Lee[3], YongKeun Park[1]

[1]Physics, Korea Advanced Institute of Science and Technology, Daejeon, Korea, Republic of,[2]Department of Pathology, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea, Republic of,[3]Department of Urology, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea, Republic of

The Papanicolaou stain is commonly used to assess cells for carcinoma in urine cytopathology. However, due to the nature of cytology, cells are often imaged overlapping one another, which makes them hard to distinguish and complicates the assessment process. The staining procedure not only introduces changes to the cells, but it is also lengthy and expensive. Here, we present a deep learning model that creates virtual Papanicolaou stained 3D images from label-free 3D holotomography images of urine cytology. We acquired 38 3D holotomography images of urine cytology samples using the Tomocube HT-X1, after which the samples were stained and 38 matching whole slide images were acquired. The image pairs were preprocessed to flatten the 3D holotomography images into 2D, and image registration to align the image pairs. This produced 10,693 image pairs of size 256x256 px, 8670 of which were used to train a modified version of the CycleGAN model. After the model was fully trained, the z-stack 3D holotomography images were input into the model one by one, resulting in a 3D virtual stained image. Our results show that our model was able to virtually stain the test sample (SSIM = 0.691 ± 0.097, PSNR = 19.9 ± 5.8, MSE = 0.017 ± 0.011) to produce an accurate 2D image, and also virtually visualize the Papanicolaou stained test samples in 3D. The presented work has potential in aiding practitioners by shortening the standard staining process. Through visualizing virtually stained urine cytology samples in 3D, our work provides the information needed to accurately assess cases of overlapping cells and cell clusters.

#7440 Natural language processing algorithms allow retrospective glioblastoma phenotyping by identifying true wild-type isocitrate dehydrogenase gliomas in electronic health records.

V. Guggilla[1], N. Forrest[1], A. Bell[1], K. Nandoliya[1], E. Houskamp[1], S. Tran[1], R. Lukas[1], J. Johnson[1], I. Roy[1], D. Wainwright[2], T. Walunas[1];
[1]Northwestern Univ. Feinberg School of Medicine, Chicago, IL, [2]Loyola University Stritch School of Medicine, Maywood, IL

Background: Glioblastoma (GBM), the most common primary brain tumor in adults with a devastating median overall survival time of 12 months, was reclassified by the World Health Organization in 2021 to only include gliomas with wild-type isocitrate dehydrogenase (IDHwt). This presents a challenge in studying retrospective cohorts of patients diagnosed with outdated definitions of GBM, which may include tumors with IDH mutations. To address this, we developed and validated natural language processing-based models in electronic health record (EHR) data designed to identify patients with IDHwt gliomas. We also developed models for identifying important prognostic markers in GBM, such as the methylation status of the O6-methylguanine-DNA-methyltransferase (MGMT) promoter, to further demonstrate the utility of these tools and EHR data in studying GBM.
Methods: We manually adjudicated 1,500 pathology documents from Northwestern Medicine (NM) containing the case-insensitive word, “glioblastoma”, for the presence of a diagnosis of IDHwt glioma as well as the methylation status of MGMT. In parallel, we implemented the National Library of Medicine’s MetaMap tool on these documents to identify biomedical concepts contained within the text. These biomedical concepts were used as features in training a series of regularized logistic regression models that identify the IDH mutation and MGMT methylation status. We next constructed Kaplan-Meier survival curves, stratifying patients by MGMT methylation. Finally, we evaluated the performance of these models at Loyola University Medical Center (LUMC) to determine their efficacy at a secondary healthcare institution.
Results: The best performing model in identifying IDHwt gliomas had an F1-measure of 0.88 in the holdout test set of documents from NM. Comparing patients with hyper-methylated and unmethylated MGMT showed significant differences in median overall survival times regardless of the MGMT identification method used ($p < 0.001$). Finally, the best performing IDHwt glioma identification model had an F1-measure of 0.96 when implemented at LUMC. 15% of patients identified at LUMC were Hispanic or Latino, compared to only 5% at NM ($p < 0.05$).
Discussion: Our results suggest that we successfully designed and implemented models to identify patients with true IDHwt gliomas. Importantly, these models displayed excellent performance at a secondary healthcare institution, demonstrating that they can be utilized to identify ethnically diverse and multi-site GBM cohorts. Furthermore, our characterization of the NM GBM cohort recapitulated known trends in survival patterns, serving as a secondary validation metric and demonstrating the utility of EHR data in studying GBM in clinical settings.

#7441 Machine-learning-based inference of clinical metadata from gene expression data.

Valentin Bernu, Guillaume Appe, **Abdelkader Behdenna**, Antoine Gaston, Julien Haziza, Akpeli Nordor

Epigene Labs, Paris, France

Secondary analysis of omic datasets holds great potential in accelerating precision oncology research and drug development. Despite this potential, a major challenge lies in harmonizing clinical information across diverse data sources. Although natural language processing techniques perform well in harmonizing many clinical variables, their performance diminishes when dealing with more intricate ones (e.g., histological type). Here, we introduce a method to infer clinical information directly from molecular data.

We identify the most informative genes from the gene expression data using an analysis of variance for each clinical variable. Then, we train machine learning classifiers (XGBoosts) on the selected features. The Cancer Genome Atlas (TCGA) pancancer database is used for training and Gene Expression Omnibus (GEO) database is used for independent testing. Expert scientists labeled clinical variables from both sources. Due to underrepresentation in TCGA, some classes of clinical variables are excluded from the training and test sets. TCGA encompasses 11,000 transcriptomic profiles spanning 33 cancer types, while the 210 aggregated GEO datasets include 7,077 transcriptomic profiles across 13 cancer types. A total of 15,430 genes are profiled in both TCGA and these GEO datasets.

We assessed our methods on five clinical variables. The encouraging accuracy of current models for biopsy site, sample type, and histological subtype suggests that they are effectively capturing the underlying patterns. For primary site and histological type classifications, the models achieved high accuracies and macro-averaged F1 scores: 93% accuracy with a 0.89 F1 score across 11 classes for primary site, and 96% accuracy with a 0.91 F1 score across 6 classes for histological type.

This method offers a significant opportunity to complement expert data labeling, addressing the challenge of scaling clinical data harmonization for the secondary analysis of omic data across multiple applications in cancer drug discovery and development. Our findings are particularly encouraging for inferring clinical details related to primary sites and histological types, with cross-database training and testing further boosting its generalizability. The strong performance across these two clinical variables underscores the potential of our approach. Nonetheless, the performance has been evaluated on a limited subset of classes of the clinical variables, highlighting the need for further validation with more diverse datasets to further unlock the potential of the method.

#7442 Transforming breast cancer treatment decisions: AI tools improve Ki67 risk assessments with 90 pathologists.

Amanda Dy[1], Ngoc-Nhu Jennifer Nguyen[2], Melanie Dawe[3], Dimitrios Androutsos[1], Susan Done[3], April Khademi[1]

[1]Electrical, Computer, & Biomedical Engineering, Toronto Metropolitan University, Toronto, ON, Canada,[2]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada,[3]Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada

A Ki67 proliferation index (PI) of ≥20% determines high-risk status and guides treatment decisions in HR+/HER2− early breast cancer. The Ki67 PI, defined as the ratio of Ki67+ tumor cells to total malignant cells, is labor-intensive and subjective to score. AI support is necessary for routine and reliable clinical use. This is the first large-scale, international study on AI-aided Ki67 PI risk assessments in breast cancer. Ninety pathologists scored the continuous PI from 0% to 100% for 10 breast cancer tissue microarrays (TMA) with and without AI support. The AI tool provided a continuous PI and a Ki67+/- tumor nuclei overlay. The AI tool was developed and validated independently of the pathologists and data used in this study (PMID: 38218973). Using gold-standard manual counts, two pathologists established the ground-truth (GT) PI scores for each TMA, ranging from 7% to 28%. The pathologists' PI scores were classified into low (<20%) and high risk (≥20%). The risk classification accuracy, sensitivity and specificity relative to the GT were obtained for manual and AI-aided scores, yielding 900 paired classification metrics (90 pathologists × 10 TMAs). Table 1 summarizes the classification metrics showing improvements with AI support for all demographics. The AI tool achieved 100% risk classification accuracy. Manual misclassification and AI-aided correction rates are also summarized in Table 1. The overall manual misclassification rate was 26%, and AI support successfully corrected 95% of these cases. The McNemar's test, a nonparametric statistical test for paired binary data, assessed statistical differences in risk classification between manual and AI-aided scoring, an asterisk denotes significance (p<0.05). AI support can improve the clinical reliability of PI scoring for risk classification and treatment decisions. AI integration into routine practice can ensure consistent Ki67 use, improving drug response predictions and patient outcomes.

Risk classification metrics, misclassification rates and correction rates, (*) denotes p<0.05.

Demographics (n, %)		**Manual Accuracy (mean [95% CI])**	**Manual Accuracy (mean [95% CI])**	**Manual Specificity (mean [95% CI])**	**Manual Misclassification Rate (%)**	**AI-aided Accuracy (mean [95% CI])**	**AI-aided Sensitivity (mean [95% CI])**	**AI-aided Sensitivity (mean [95% CI])**	**AI-aided Correction Rate (%)**
All Pathologists* (90, 100%)		0.74 [0.71, 0.77]	0.44 [0.38, 0.49]	0.94 [0.92, 0.96]	26% (233/900 scores)	0.89 [0.87, 0.91]	0.76 [0.72, 0.81]	0.98 [0.96, 0.99]	95% (221/233 scores)
Career Stage (7 preferred not to answer)	Resident/Fellow* (39, 47%)	0.78 [0.71, 0.79]	0.47 [0.39, 0.55]	0.94 [0.90, 0.97]	25% (98/390 scores)	0.89 [0.86, 0.92]	0.78 [0.72, 0.85]	0.96 [0.94, 0.97]	93% (91/98 scores)
	Practicing* (13, 16%)	0.74 [0.66, 0.81]	0.46 [0.33, 0.60]	0.92 [0.87, 0.98]	26% (34/130 scores)	0.89 [0.84, 0.95]	0.79 [0.68, 0.90]	0.96 [0.92, 1.0]	94% (32/34 scores)
	Retired* (31, 37%)	0.73 [0.68, 0.78]	0.39 [0.31, 0.48]	0.95 [0.92, 0.98]	27% (84/310 scores)	0.89 [0.86, 0.93]	0.74 [0.67, 0.82]	0.96 [0.98, 1.0]	98% (82/84 scores)
AI Implementation Timeline (1 preferred not to answer)	<5 Years* (27, 30%)	0.73 [0.68, 0.78]	0.46 [0.37, 0.56]	0.91 [0.86, 0.95]	27% (73/270 scores)	0.91 [0.87, 0.94]	0.81 [0.73, 0.88]	0.98 [0.95, 1.0]	95% (69/73 scores)
	5-10 Years* (16, 18%)	0.74 [0.67, 0.81]	0.39 [0.27, 0.51]	0.97 [0.93, 1.0]	26% (42/160 scores)	0.87 [0.82, 0.92]	0.72 [0.61, 0.83]	0.97 [0.93, 1.0]	93% (39/42 scores)
	AI is Not Ready* (30, 34%)	0.74 [0.69, 0.79]	0.42 [0.34, 0.51]	0.95 [0.92, 0.98]	26% (78/300 scores)	0.89 [0.86, 0.93]	0.76 [0.68, 0.83]	0.98 [0.96, 1.0]	97% (76/78 scores)
	Do Not Know* (16, 18%)	0.77 [0.70, 0.83]	0.47 [0.35, 0.59]	0.97 [0.93, 1.0]	23% (37/160 scores)	0.89 [0.84, 0.94]	0.77 [0.66, 0.87]	0.97 [0.93, 1.0]	95% (35/37 scores)
Proficiency in Ki67 Scoring (6 preferred not to answer)	Strong* (37, 44%)	0.73 [0.68, 0.77]	0.43 [0.35, 0.51]	0.93 [0.89, 0.96]	27% (101/370 scores)	0.89 [0.86, 0.92]	0.78 [0.72, 0.85]	0.96 [0.94, 0.99]	93% (94/101 scores)
	Neutral* (25, 30%)	0.77 [0.72, 0.82]	0.52 [0.42, 0.62]	0.93 [0.89, 0.97]	23% (58/250 scores)	0.90 [0.86, 0.94]	0.78 [0.70, 0.86]	0.98 [0.96, 1.0]	98% (57/58 scores)
	Weak* (22, 26%)	0.74 [0.68, 0.80]	0.40 [0.30, 0.50]	0.97 [0.94, 1.0]	26% (57/220 scores)	0.88 [0.84, 0.92]	0.73 [0.63, 0.82]	0.98 [0.96, 1.0]	95% (54/57 scores)
Current Workplace (4 preferred not to answer)	Academic Health Sciences Centre* (24, 28%)	0.75 [0.70, 0.81]	0.55 [0.45, 0.65]	0.89 [0.84, 0.94]	25% (59/240 scores)	0.88 [0.84, 0.92]	0.79 [0.71, 0.87]	0.94 [0.91, 0.98]	90% (53/59 scores)
	Community Hospital* (16, 19%)	0.74 [0.67, 0.81]	0.50 [0.38, 0.62]	0.90 [0.83, 0.96]	26% (42/160 scores)	0.88 [0.83, 0.93]	0.77 [0.66, 0.87]	0.96 [0.92, 1.0]	90% (38/42 scores)
	Diagnostic Clinic* (13, 15%)	0.74 [0.66, 0.81]	0.35 [0.22, 0.48]	1.0 [1.0, 1.0]	26% (34/130 scores)	0.90 [0.85, 0.95]	0.75 [0.63, 0.87]	1.0 [1.0, 1.0]	100% (34/34 scores)
	Non-Academic Health Sciences Centre Teaching Hospital* (4, 5%)	0.70 [0.56, 0.84]	0.56 [0.32, 0.81]	0.79 [0.63, 0.95]	30% (12/40 scores)	0.93 [0.84, 1.0]	0.81 [0.62, 1.0]	1.0 [1.0, 1.0]	100% (12/12 scores)
	University (3, 3%)	0.83 [0.70, 0.97]	0.83 [0.62, 1.0]	0.83 [0.66, 1.0]	17% (5/30 scores)	0.83 [0.70, 0.97]	0.92 [0.76, 1.0]	0.78 [0.59, 0.97]	80% (4/5 scores)
	Not Working* (26, 30%)	0.73 [0.68, 0.79]	0.34 [0.25, 0.43]	1.0 [1.0, 1.0]	27% (69/260 scores)	0.90 [0.86, 0.94]	0.75 [0.67, 0.83]	1.0 [1.0, 1.0]	100% (69/69 scores)
Pathology Discipline (12 preferred not to answer)	Clinical* (29, 37%)	0.74 [0.69, 0.79]	0.38 [0.29, 0.47]	0.98 [0.95, 1.0]	26% (76/290 scores)	0.90 [0.87, 0.94]	0.76 [0.68, 0.84]	1.0 [1.0, 1.0]	100% (76/76 scores)
	Anatomical* (19, 24%)	0.76 [0.70, 0.82]	0.64 [0.54, 0.75]	0.84 [0.78, 0.91]	24% (45/190 scores)	0.87 [0.82, 0.92]	0.83 [0.74, 0.91]	0.89 [0.84, 0.95]	80% (36/45 scores)
	Neuro* (9, 12%)	0.71 [0.62, 0.80]	0.31 [0.16, 0.46]	0.98 [0.95, 1.0]	29% (26/90 scores)	0.88 [0.81, 0.95]	0.72 [0.58, 0.87]	0.98 [0.95, 1.0]	92% (24/26 scores)
	Pediatric* (9, 12%)	0.69 [0.59, 0.78]	0.39 [0.23, 0.55]	0.89 [0.81, 0.97]	31% (28/90 scores)	0.90 [0.84, 0.96]	0.75 [0.61, 0.89]	1.0 [1.0, 1.0]	100% (28/28 scores)
	Micro* (6, 8%)	0.75 [0.64, 0.86]	0.38 [0.18, 0.57]	1.0 [1.0, 1.0]	25% (15/60 scores)	0.90 [0.82, 0.98]	0.75 [0.58, 0.92]	1.0 [1.0, 1.0]	100% (15/15 scores)
	Hema (3, 4%)	0.73 [0.58, 0.89]	0.33 [0.07, 0.60]	1.0 [1.0, 1.0]	27% (8/30 scores)	0.90 [0.79, 1.0]	0.75 [0.51, 0.99]	1.0 [1.0, 1.0]	100% (8/8 scores)
	Forensic (3, 4%)	0.77 [0.62, 0.92]	0.42 [0.14, 0.70]	1.0 [1.0, 1.0]	23% (7/30 scores)	0.90 [0.79, 1.0]	0.75 [0.51, 0.99]	1.0 [1.0, 1.0]	100% (7/7 scores)

#7443 Feasibility of automatic spleen volumetry from 3D ultrasound images of mice using deep learning.

Adam M. Aji[1], Thomas M. Kierski[1], Juan D. Rojas[1], Bincy John[2], David Harris[2], Ryan C. Gessner[1], Craig McMannus[1], Jeffrey Peterson[1], Tomasz J. Czernuszewicz[1]

[1]Revvity, Durham, NC,[2]Charles River Laboratories, Morrisville, NC

The spleen plays a critical role in immune function and blood filtration. Interestingly, the spleen's volume changes in response to various diseases. For example, enlargement of the spleen (splenomegaly) can arise because of cancer (solid and liquid tumors), liver diseases (MASH), Lupus, or various infections. Conversely, atrophy or shrinking of the spleen can arise from conditions such as graft-versus-host-disease, inflammatory bowel, or sickle cell disease. Though the size of the spleen is a relatively well-established biomarker that may be useful in several preclinical studies, it is currently difficult and costly to measure for large cohorts of mice over multiple timepoints.

It is possible to measure spleen volume and weight ex vivo at a terminal timepoint, but this approach is not amenable to longitudinal tracking. Alternatively, imaging using ultrasound or magnetic resonance can be used to interrogate the spleen noninvasively and repeatedly, but this method requires data to be segmented in 3D. Segmentation often creates a bottleneck in study design because it is time consuming. Furthermore, measurements derived from human-annotated data are prone to inter-user variability and user error.

Towards addressing these challenges with measuring spleen volume in longitudinal preclinical studies, we trained an AI model to segment spleens from a diverse set of 3D ultrasound images. In total, 180 images were acquired in mice from a Vega ultrasound scanner (Revvity, Waltham, MA, USA). These images were varied in terms of animal strain, positioning, field of view, and disease state with splenic volumes ranging between approximately 20 mm^3 and 150 mm^3. These data included samples from healthy controls and mice with conditions including solid tumors, blood cancer, Lupus, and graft versus host disease which were annotated by expert human readers. Data were shuffled and split into training and validation groups with an 80-20 split and the training set was used to train a modified U-Net model.

Inference on the validation set after training yielded a Dice coefficient of 0.86, and the total processing time for the 36 images was 49.0 seconds (1.36 sec/image) on an NVIDIA RTX 3090 GPU. A linear regression analysis between the ground truth volumes and the volumes predicted by the model resulted in a R^2 value of 0.85. These results suggest that this problem is an excellent candidate for automation using artificial intelligence, particularly with an automated ultrasound scanner such as the Vega where positioning is highly reproducible. The training set size (N=144 images) is quite small by conventional deep learning standards, and we expect the segmentation accuracy to increase further as more images are added to the training pool. Future work will focus on collecting more in vivo images from a variety of murine models.

#7444 Automated quantification of small animal liver tumor volume in longitudinal MR imaging using deep learning.

Thomas M. Kierski[1], **Adam M. Aji**[1], Juan D. Rojas[1], Ryan C. Gessner[1], Laura Beretta[2], Suet Ying Kwan[2], John Hazle[3], Kiersten L. Maldonado[3], Charles Kingsley[3], Richard R. Bouchard[3], Tomasz J. Czernuszewicz[1]

[1]Revvity, Durham, NC,[2]Department of Molecular and Cellular Oncology, MD Anderson Cancer Center, Houston, TX,[3]Department of Imaging Physics, MD Anderson Cancer Center, Houston, TX

In preclinical imaging studies with small animals, tracking tumor growth accurately over time can be challenging due to the time-consuming, labor-intensive, and error-prone nature of manual segmentation of 3D images. Orthotopic and metastatic cancer models can be particularly problematic as additional radiologic expertise is required to detect tumors within the context of deep anatomy, and individual animals may contain many nodules (ten or more) requiring many hours of segmentation effort. Therefore, the goal of this study was to develop and validate a deep learning model for automatic volumetric measurement of murine liver tumors in MR images to increase the practicality and adoptability of longitudinal imaging in preclinical research.

We describe a method for automatically quantifying tumor volume over time using a dataset of 4.7T T2-weighted MR images taken from a study involving a genetic model of spontaneous liver cancer. The dataset included 894 images from 198 unique animals collected using a Biospec 47/40 (Bruker, Kontich, Belgium) over a span of 30 weeks. Each image was manually segmented by an expert annotator and tumor burden was tracked longitudinally to generate growth curves. A modified U-Net model built on the MONAI framework was trained to segment tumors.

On a held-out test subset of 177 images (20% of the overall dataset), the model's predicted overall tumor burden showed a strong correlation with the human ground truth, achieving an R^2 value of 0.88. The model processed each image in 0.34 seconds on a machine with an NVIDIA RTX 3090 GPU, significantly faster than the 5-10 minutes required for manual annotation. This efficiency translates to a substantial reduction in overall annotation time for studies with large datasets.

This study demonstrates the feasibility of using deep learning for liver tumor segmentation in MR images. The proposed method offers a reliable and efficient alternative to manual segmentation, potentially improving the accuracy and speed of volumetric measurements in preclinical cancer research.

#7445 Deep learning prioritizes cancer mutations that alter protein nucleocytoplasmic shuttling to drive tumorigenesis.

Z.-X. Liu, K. Yu, Y. Zheng, J.-F. Lin;
Sun Yat-sen University Cancer Center (SYSUCC), Guangzhou, China

Genetic variants can affect protein function by driving aberrant subcellular localization. However, due to limitations in existing computational tools, comprehensive analysis of how mutations promote tumor progression by influencing nuclear localization is currently lacking. Here, for the first time, we systematically characterized potential shuttling-attacking mutations (SAMs) across cancers. The deep learning model Predicting Shuttling-Attacking Mutations (pSAM) was the leading tool constructed for the *ab initio* decoding of protein sequence determinants essential for subcellular shuttling, including classical nuclear localization signals and unconventional sequences such as fragments embedded in DNA/RNA-binding domains. Leveraging cancer mutations across 11 cancer types, we found that SAMs constitute about 6.7% of the mutations and enrich functional genetic variations and critical genes in cancer; for example, SAMs in transcription factors prevent them from entering the nucleus and subsequently cause abnormal expression of downstream targets. We experimentally validated a dozen SAMs, among which R14M in PTEN, P255L in CHFR, R674C in NFE2L1 and R144C in KAT8 were identified to disrupt the nuclear localization through interfering their interactions with importins. Further studies confirmed that the altered nucleocytoplasmic shuttling triggered by SAMs in PTEN and CHFR rewired the downstream signaling pathways and eliminated their function of suppressing cancer progression. Thus, this study greatly contributes to the understanding of the molecular details of nucleocytoplasmic shuttling and reveals the variants mediating aberrant protein subcellular localization and dysfunction in cancers and diseases.

#7446 Stratification of cell therapies in solid tumor organoids using deep learning-derived imaging metrics.

Luca Lonini[1], Madhavi Kannan[1], Geoffrey Schau[1], Stanislaw Szydlo[1], Vignesh Krishnaraja[1], Sonal Khare[1], Daniel Rabe[1], Michael Streit[1], Mary Flaherty[1], Brandon Mapes[1], Mark Prytyskach[2], Stefan Kiesgen[3], Gurpanna Saggu[2], Megan McAfee[2], Kate Sasser[1], Justin Guinney[1], Richard Klinghoffer[1], Chi-Sing Ho[1]

[1]Tempus AI, Inc., Chicago, IL,[2]Takeda, Cambridge, MA,[3]BioNTech, Cambridge, MA

Background. High-throughput screening of immunotherapies is crucial for identifying promising candidates against various cancer indications and accelerating the pre-clinical development of cell therapies. Two technologies have shown promise in quantifying therapy effectiveness: patient-derived organoid (PDO) models co-cultured with cell therapies, and deep learning-based computer vision, which facilitates large-scale, automated image analysis to extract quantitative metrics of treatment efficacy and cell biology effects. Here, we use deep networks to predict PDO viability from brightfield images and compute explainable features which are used to stratify a large cohort of cell therapies, tested on multiple tumoroid lines and indications.
Methods. Time-lapse confocal microscopy was used to record images of a large cohort of 27 cell therapies co-cultured with 15 different patient-derived tumor organoids (PDO) lines, across 8 cancer indications (breast, colorectal, endometrial, gastric, head and neck, liver, lung, pancreas) over the course of 72 hours, and at 2 different effector-to-target concentrations. PDO viability was measured by terminal TO-PRO-3 staining. A convolutional deep network was trained to perform label-free predictions of PDO viability from brightfield images at each timepoint (N=11,110) and evaluated on a held-out test set (N=2,112). Deep learning segmentation models were further used to co-localize tumoroid and immune cells, and extract a set of 6 explainable features - or spatial phenotypes - which quantified 1) tumoroid area, 2) cell apoptosis intensity and 3) temporal dynamics, as well as 4) immune proliferation, 5) immune infiltration and its 6) temporal dynamics. We fitted a linear model to measure their contribution in predicting terminal viability, and used hierarchical clustering to assess associations with exposure to different therapies.
Results. Our bright-field model of viability was found to be highly concordant with ground truth viability from terminal vital dye stain on a held-out test set, representing 11 TO lines across 8 distinct cancer types (Pearson's r=0.76). A linear model of spatial phenotypes showed high correlation with terminal viability (Pearson's r=0.70), while clustering of spatial phenotypes stratified samples into groups of non-engineered and engineered therapies with and without an additional compound.
Conclusion. Our study highlights that imaging-derived metrics from brightfield imaging can quantify treatment potency and cell phenotypes in ex-vivo screens. The approach pursued here is highly scalable and permits the analysis of large scale screening experiments to assess the impact of cell therapies on PDOs. Using explainable features further allows to stratify therapies into biologically meaningful groups based on the measured phenotypic readouts.

#7448 Developing virtual staining algorithm of colon cancer organoids using holotomography and deep learning.

Juyeon Park, Geon Kim, YongKeun Park

Korea Advanced Institute of Science and Technology, Daejeon, Korea, Republic of

Colon cancer organoids hold great potential for studying the development of colon cancer. To assess their physiological and developmental status, both morphological parameters (e.g., cell shape and volume) and functional parameters (e.g., presence of proliferating cells or pluripotent stem cells) are crucial. However, current fluorescence labeling methods are invasive, potentially damaging or altering the organoids, making them disposable. To overcome this limitation, it is essential to develop a non-invasive method for monitoring organoids for long-term. We propose a solution that integrates holotomography and deep learning. Holotomography is a label-free imaging technique that measures the 3D refractive index distribution of samples without requiring any exogenous labeling. While this technique operates within a label-free framework, the resulting images provide only structural information and lack functional information. This limitation makes it challenging for researchers to distinguish cellular and subcellular structures with specific function. We address this challenge by leveraging deep learning to provide both structural and functional information through virtual staining of label-free images. Specifically, we construct a paired dataset using correlative holotomography, which captures both label-free images and their corresponding fluorescence images. Using supervised learning, we train a neural network to map label-free images to their corresponding fluorescence counterparts. Once trained, the neural network can automatically predict virtual fluorescence images from label-free inputs without exposing the organoids to any exogenous chemicals. To validate the accuracy of the trained neural network, we first acquire label-free images of the organoids and input them into the network. After obtaining the network-generated virtual fluorescence images, we chemically stain the same organoids. Finally, we compare the chemically stained images with the virtual images produced by the neural network to evaluate its performance. By combining the safety of label-free imaging with the precision of artificial intelligence, our technology eliminates critical barriers to organoid research and translation. This breakthrough accelerates the development of scalable and safe organoid therapies, bringing us closer to a future where lab-grown organoids revolutionize regenerative medicine and save lives.

#7449 Evaluating the effectiveness of machine learning models using polygenic risk scores and clinical factors for breast cancer risk stratification.

Lannawill Caruth[1], Roukiatou Sore[2], Sarah Ehsan[2], Anne-Marie McCarthy[2], Shefali Setia-Verma[1]

[1]Department of Pathology and Laboratory Medicine, Perelman School of Med. Univ. of Pennsylvania, Philadelphia, PA,[2]Dept. of Biostatistics Epidemiology & Informatics, Perelman School of Med. Univ. of Pennsylvania, Philadelphia, PA

Breast cancer is the most common cancer among women in the United States, aside from skin cancer. Early detection and treatment significantly reduce mortality rates, highlighting the importance of effective risk prediction methods. This study evaluates the predictive performance of multiple machine learning models that include polygenic risk scores (PRS), clinical and demographic factors. We compared three predictive models using 313 SNPs model from PGS catalog and incorporated age, body mass index (BMI), five genetic principal components (PC1-5), and breast density as predictor variables. Logistic Regression (LR), Multilayer Perceptron (MLP), and Probabilistic Graphical Model (PGM) were implemented to assess breast cancer risk. MLPs are a type of artificial neural network that consists of layers of interconnected "neurons" that work together to process and learn from data. Each layer processes the information and passes it to the next layer, allowing the model to learn complex patterns and detect non-linear relationships in the data, which makes it useful for predictions. The selected PGM implementation uses Bayesian statistics to determine feature importance and correlation. The model then uses this information to predict the probability an instance belongs to a group (e.g. case or control). Among 3,000 African ancestry women from the Penn Medicine BioBank, model performance varied. Logistic Regression demonstrated the highest predictive accuracy at 0.69, followed by Probabilistic Graphical Model at 0.64 and Multilayer Perceptron at 0.63. Our study suggests limited interaction between PRS and clinical factors in breast cancer prediction. These findings aligns with prior research demonstrating the largely independent nature of polygenic risk scores from traditional clinical risk factors. These findings underscore the challenges in developing more sophisticated risk prediction models and highlight the need for alternative approaches to capture the complex genetic architecture of breast cancer risk Subsequent research will incorporate moderate and high penetrance genes to enhance the predictive power of our risk assessment models, potentially improving precision in breast cancer risk stratification.

#7450 Leveraging large language models' own knowledge for obtaining and exploring gene embeddings.

Dailin Gan[1], Siyuan Zhang[2], Xin Lu[1], Jun Li[1]

[1]University of Notre Dame, Notre Dame, IN,[2]The University of Texas Southwestern Medical Center, Dallas, TX

Transfer learning is increasingly prevalent in gene expression analysis. Large transformer-based models generate embeddings from genes, which are used to construct machine-learning models for various downstream tasks. Given the high cost of training these large models, recent work has utilized ChatGPT-4, a pre-trained language model, to produce embeddings from manually curated NCBI gene descriptions. However, this method does not leverage the vast pre-trained knowledge of ChatGPT-4. In this work, we propose directly querying ChatGPT-4 about genes and converting its responses into embeddings rather than using static NCBI descriptions. For each gene, we independently asked ChatGPT-4 four specific questions designed to elicit ChatGPT's knowledge about each gene's general characteristics, regulatory functions, gene structure, and implications in cancer research. We evaluate our approach by comparing the embeddings it produces with those derived from NCBI descriptions. The comparison suggests, notably, that ChatGPT-4's knowledge closely aligns with that of NCBI despite differences in presentation. Furthermore, ChatGPT-4 reveals more detailed and comprehensive information about many genes. We also used the ChatGPT-answer-derived gene embeddings to distinguish long and short-range transcription factors, dosage-sensitive and insensitive genes, and bivalent, Lys4-methylated, and non-methylated genes. Results indicate a preference for ChatGPT-answer-derived gene embeddings in downstream applications by machine learning models. Overall, our findings suggest that direct querying of ChatGPT-4 offers clear advantages for generating gene embeddings.

#7451 Efficacy of chemoradiotherapy in older patients with advanced stage head and neck cancer: A propensity and machine learning analysis.

Rasheed Omobolaji Alabi, Alhadi Almangush, Antti Makitie

Research Program In Systems Oncology, University of Helsinki, Haartman Institute, Helsinki, Finland

Background: The rising incidence of head and neck squamous cell carcinoma (HNSCC) is linked with both human papillomavirus and aging population. As a result, there is an emerging need for developing management strategies for older patients with advanced-stage HNSCC. This study aims to use machine learning (ML) techniques to examine the efficacy of definitive, concurrent chemoradiotherapy (CRT) for older patients (≥65 years) with stage III-IV HNSCC. We used permutation feature importance (PFI) to compare the efficacy of CRT with single-modality radiotherapy (RT) for the overall survival (OS) of this HNSCC subgroup. Finally, we compared the results obtained using ML analysis with propensity-score matched (PSM) analysis to clarify the efficacy of CRT in this patient population.
Methods: A total of 3570 patients with HNSCC were obtained from the Surveillance, Epidemiology, and End Results (SEER) Program to build the ML model. We compared voting ensemble and extreme gradient boosting algorithms for stratifying older (≥65 years) HNSCC patients into OS risk groups. Furthermore, the PFI analysis was based on stacked ML models to evaluate the efficacy of CRT regarding OS of older (≥65 years) HNSCC patients. Then, we performed a 1:1 propensity-score matched (PSM) analysis to reduce treatment selection bias and analyze the prognostic role of CRT.
Results: Our ML model showed a performance accuracy of 85.2% in predicting OS in this subgroup of HNSCC. The aggregate feature importance showed that CRT is the most important feature for OS in this group of HNSCC patients. In addition, patients with stage IV disease showed better survival than those with stage III. The PSM confirmed the efficacy of CRT compared to RT alone.
Conclusions: This study showed that CRT may be more feasible and effective than RT alone for older patients with stage III-IV HNSCC. This further emphasized that advanced-stage treatment decisions should rather be made according to other patient-related factors than chronologic (calendar) age alone. Therefore, validating these models with multi-institutional datasets and testing them in the context of clinical trials is warranted to confirm the efficacy of CRT over RT alone for older patients (≥65 years) with stage III-IV HNSCC.

#7452 MAGNETICA: Machine-augmented generation of novel enhanced tomographic imaging for tumor insight and cancer assessment.

Hosein Barzekar, Mahyar Abbasian, **Morteza Rezanejad**, Mohammed Qutaish, Neal Sherkar, Deepti Nagarkar, Karen Stephansen, Scott Ogden, Lauren K. Brady, Brandon W. Higgs

Genmab, Plainsboro, NJ

Background: Quantitative features extracted from radiology images can reveal tumor characteristics beyond what the human eye can detect. While promising for identifying imaging biomarkers, the limited availability of patient 3D CT images in early-phase cancer trials is a major bottleneck. Recruiting patients with specific tumor types is difficult, leading to small, non-diverse datasets that lack statistical power for decisions on drug dosing, safety, and mechanism. Image augmentation could generate diverse, synthetic patient images to simulate tumor profiles, model disease progression, and increase biological variation while preserving patient privacy. However, current models do not meet these goals. To address this, we introduce MAGNETICA, a novel framework that integrates clinical features with anatomical structures for synthetic 3D CT image generation.
Methods: We used the NSCLC-Radiomics (Lung1) dataset - which includes pretreatment CT scans, manual 3D gross tumor volume delineations by radiation oncologists, and clinical outcome data from 422 NSCLC patients, 36% were squamous histology and 68% were stage III - to train our model. A Variational Autoencoder (VAE) processed 3D CT volumes, preserving anatomical structures while compressing them into latent features, which were input into a Latent Diffusion Model (LDM) incorporating key clinical characteristics into its latent space. Clinical variables—including age, gender, tumor stage (T, N, M), histology, and survival time were transformed into textual descriptions using a sentence transformer model. These embeddings were integrated into the LDM to condition image generation. To ensure anatomical consistency, ControlNet was used to condition the generation on organ and lesion maps, producing realistic, patient-specific CT images with lesion labels. Synthetic images were validated with automated anatomical reconstruction scoring to ensure fidelity.
Results: The model demonstrated strong alignment between synthetic and real 3D CT scans. The Mean Absolute Error between synthetic and real images was 0.12. For the radiomic feature “Total Energy,” the Kolmogorov-Smirnov test had a statistic of 0.07, and the Kullback-Leibler divergence was 3.81×10^{-11}, indicating no significant differences. Moreover, an automated anatomical annotation score validated that 98.9% of our VAE-generated CT synthetic images were high-quality, scoring 0.8 or higher.
Conclusions: Consistent with our hypothesis, the synthetic 3D CT scans closely resembled real images, paving the way for capturing key disease characteristics. MAGNETICA advances synthetic imaging by bridging AI-driven generation and clinical needs. By augmenting early-stage trials with diverse, clinically relevant synthetic CT scans, this approach has potential to empower analyses that inform on dose selection, safety monitoring, and precision medicine.

BIOINFORMATICS / COMPUTATIONAL BIOLOGY / SYSTEMS BIOLOGY / CONVERGENT SCIENCE: Identification of New Cancer Vulnerabilities Through Convergence Science Poster Session

#7456 Overcoming ICI resistance with CXCR1/2 antagonism in advanced HCC.

Jeric Hernandez, Kabeer Munjal, Emma Kartalia, Madelena Brancati, Howard L. Li, James Leatherman, Ervin Griffin, Christopher J. Thoburn, Won Jin Ho, Marina Baretti, Daniel J. Zabransky, Elizabeth M. Jaffee, Mark Yarchoan, Mari Nakazawa

Oncology, Johns Hopkins University School of Medicine, Baltimore, MD

Background: Despite the recent success of immune checkpoint inhibitor (ICI) based regimens in advanced stage HCC, clinical outcomes remain poor with a median overall survival (OS) of under two years. There is an unmet need to improve responses to, and durability of, ICI based therapy in HCC. IL-8 is a chemokine promoting the attraction of myeloid cells via receptor CXCR1/2 and has been shown to have numerous pro-tumorigenic roles in the tumor immune microenviroment (TiME). Recent work by others has shown that high plasma IL-8 concentration is associated with inferior outcomes for patients receiving ICI therapy across multiple solid tumors, but its relevance to outcomes in patients with HCC is not well characterized.
Methods: We prospectively collected whole blood from patients with HCC prior to initiation of standard of care immunotherapy-based regimens at Johns Hopkins (IRB #00267960). Plasma cytokines were profiled via Luminex bead-based immunoassay. To profile the local effects of IL-8 on the TiME, we performed IHC (anti-IL8, anti-CD3, anti-CD8, and anti-CD206) on resected HCC tumor tissue from systemic treatment naive patients (n=13). Finally, to understand the consequences of IL-8 inhibition via CXCR1/2 antagonism, we utilized a syngeneic murine HCC model (RIL-175 in female C57BL/6) treated with medicated SX-682 (anti-CXCR1/2) alone and in combination with intraperitoneal injections of anti-PD1+anti-VEGF.
Results: The final clinical cohort included 41 patients with advanced HCC. Patients were predominantly male (82.9%) with Barcelona Clinic Liver Cancer (BCLC) stage C disease (75.6%) who received treatment with atezolizumab + bevacizumab (51.2%). In a multivariable Cox model, baseline plasma IL-8 above or equal to median was associated with inferior progression free survival (PFS; hazard ratio [HR] 3.78, p=0.01) and OS (HR 2.32, p=0.07) after controlling for BCLC stage, albumin-bilirubin (ALBI) index, baseline alpha fetoprotein (AFP), regimen type, and baseline concentration of other cytokines with relevance in HCC (IL-6, IL-10, and VEGF-A). IHC on resected tumor specimens revealed negative correlation between IL-8 staining and CD8 cell density (R=-0.2, p=0.041) and positive correlation with CD206 staining (R=0.24, p=0.05). In our murine model, combination treatment with anti-CXCR1/2, anti-PD1, and anti-VEGF was associated with reduction of tumor volume at day 36 compared to control (p<0.001), anti-CXCR1/2 (p=0.006) and anti-PD1+anti-VEGF (p=0.06).
Conclusions: Consistent with prior reports in other solid tumors, high plasma IL-8 concentration was associated with inferior survival on ICI based regimens in HCC. We hypothesize that IL-8 signaling promotes M2 macrophage polarization and in turn results in reduced effector T cell infiltration in the TiME. These results provide rationale for targeting the CXCR1/2 signaling axis in advanced HCC.

#7457 Personalized treatment for hepatocellular carcinoma (HCC) patients.

Jinhee Kim[1], Miseol Son[1], Da Som Kim[1], Yunjeong Oh[1], Naeun Jeon[1], Inhee Roh[2], Jayeon Kim[2], Vinay Tergaonkar[3], Do-Hyun Nam[4], Pierce Kah-Hoes Chow[5], Yong Jae Shin[4]

[1]Innovative Institute for Precision Medicine, Samsung Medical Center, Seoul, Korea, Republic of,[2]Samsung Medical Center, Seoul, Korea, Republic of,[3]Department of Pathology, Yong Loo Lin School of Medicine, NUS, Singapore,[4]Department of Neurosurgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of,[5]Duke-NUS Medical School, National Cancer Centre, Singapore, Singapore

Predicting drug response is crucial for personalized medicine, especially in cancer research and drug resistance studies. Accurate prediction of drug response, such as the half-maximal inhibitory concentration (IC50) in cell culture models, is vital for establishing personalized treatment models. In particular, patient-derived cell culture models have garnered increasing attention due to their precise prediction of therapeutic responses in targeted drug therapies. However, the prediction of drug response in hepatocellular carcinoma (HCC) patient-derived cells (PDCs) has not been fully explored. In this study, we aimed to predict personalized drug responses to anticancer drugs in HCC. We characterized hepatic stem-like cancer cells using various human HCC cell lines (Hep3B, SkHep1, PLCPRF5, MHCC-LM3, HepG2, Huh-7). Different types of cell culture media were tested to identify HCC-specific conditions. Anticancer agents such as sorafenib and regorafenib were found to increase drug resistance in 3D cell culture models of HCC. A high-throughput screening of 300 media conditions, each with varying growth factor compositions, was conducted, and cell viability was assessed in 17 HCC patient-derived PDC models. Drug resistance profiles varied among HCC patients, with some demonstrating sensitivity to sorafenib and lapatinib, while others showed resistance to tivantinib. Next-generation sequencing (NGS) revealed unique mutations across different HCC patients. In particular, one individualized HCC patient showed the best drug response to lenvatinib and sunitinib. Furthermore, our prediction model revealed that lapatinib exhibited higher drug resistance, mirroring patterns observed in tumor cells associated with regulatory T cells, tissue-resident memory T cells (TRM), and tumor-associated macrophages (TAMs). These findings suggest that our approach offers a promising framework for predicting drug responses in HCC, supporting its application in personalized medicine.

#7459 The anticancer effects of brusatol are mediated by PPAR-γ/ME1-driven glycolysis in intrahepatic cholangiocarcinoma.

Xiaoqing Wang[1], Caiming Xu[2], Kai Luo[3], Yuhan Fang[1], Goel Ajay[4], Guixin Zhang[3]

[1]Institute (College) of Integrative Medicine, Dalian Medical University, DaLian, China,[2]Department of General Surgery, The First Affiliated Hospital of Dalian Medical University, DaLian, China,[3]Department of General Surgery, The Second Affiliated Hospital of Dalian Medical University, DaLian, China,[4]Department of Molecular Diagnostics and Experimental Therapeutics, Beckman Research Institute of City of Hope, Biomedical Research Center, Monrovia, Monrovia, CA

Background: Intrahepatic cholangiocarcinoma (ICC) is a malignant tumor characterized by its dense fibrotic microenvironment, which is associated with chemotherapy resistance and poor outcome. Hence, developing novel strategies to improve the outcome of ICC is essential. Brusatol, a steroidal molecule isolated from *Brucea javanica*, exhibits various biological activities, including anti-inflammatory and anticancer effects. Although the molecular mechanisms underlying the anti-cancer effects of brusatol in ICC remain unclear, the present study aims to investigate its therapeutic potential in ICC cells.

Methods: We conducted a series of in vitro experiments on human ICC cell lines (HCCC-9810 and HuCCT1) to assess the impact of brusatol on cell proliferation and glycolysis regulation. Transcriptomic sequencing and pathway enrichment analysis was performed to identify specific signaling pathways associated with brusatol treatment in ICC cells. Furthermore, the pathway was further validated through a series of mechanistic studies.

Results: Brusatol significantly inhibited the proliferation, migration, and invasion of ICC cells in a concentration-dependent manner ($p < 0.05$). Glycolytic activity was markedly suppressed by brusatol, as evidenced by a reduction in glucose consumption, ATP production, and lactate secretion in ICC cells. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay confirmed the downregulation of glycolysis-related genes, including GLUT1, HK2, PFK1, and LDHA. RNA sequencing and pathway enrichment analysis suggested that brusatol inhibits the PPAR-γ/ME1 signaling pathway ($p < 0.05$), which likely regulates glycolysis in ICC cells. Subsequently, RT-qPCR and western blotting results corroborated the RNA-seq findings. Interfering with PPAR-γ expression via siRNA or inhibiting the PPAR-γ pathway with Rosiglitazone resulted in a marked reduction in glycolytic activity ($p < 0.01$), further reinforcing the critical role of the PPAR-γ/ME1 signaling axis in the regulation of glycolysis. These findings underscore the potential of brusatol as a therapeutic agent that effectively disrupts glycolytic metabolism in ICC by targeting the PPAR-γ/ME1 pathway.

Conclusion: Our findings provide compelling evidence that the PPAR-γ/ME1 signaling pathway mediates the anti-cancer effects of brusatol, supporting its potential as a safe and effective integrative treatment approach for ICC. This potential offers hope for the development of new and improved treatments for ICC.

#7460 Unveiling NEPC vulnerabilities through single-cell analysis and drug screening.

Ziyu Zeng[1], Yan Liu[1], Aktar Ali[2], Xin Lu[1]

[1]Biological Sciences, University of Notre Dame, Notre Dame, IN,[2]Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN

Neuroendocrine prostate cancer (NEPC) represents an aggressive, treatment-resistant disease that emerges following androgen deprivation therapy. Despite growing evidence supporting transdifferentiation from luminal cells, the cellular origins and developmental trajectories remain poorly understood. Through comprehensive integration of single-cell RNA sequencing data from 318 human prostate cancer samples and 179 mouse models, analyzing over one million cells, we identified distinct neuroendocrine subpopulations as well as both convergence and diversity among non-neoplastic cells (especially fibroblasts and immune cells). These ongoing efforts are expected to provide an unprecedented comprehensive view of prostate cancer heterogeneity and unravel critical cellular and molecular differences between treatment-induced and de novo NEPC. Using our established dual-luciferase reporter system in NEPC cells, we screened 290 compounds and identified 19 AR-inducing compounds and 44 NEPC-selective compounds. Among these, several promising candidates showed significant therapeutic windows, including compounds targeting inflammatory pathways and epigenetic regulators, with one compound demonstrating 100-fold selectivity for NEPC cells compared to AR-positive prostate cancer cells. Additionally, we found that while NEPC cells retain basic AR signaling machinery, AR expression alone was insufficient to restore sensitivity to AR pathway inhibitors, highlighting the complexity of targeting cellular plasticity in NEPC. This integrative approach combining computational analysis with experimental validation provides new insights into NEPC development and presents novel therapeutic opportunities for this aggressive disease. Our ongoing work aims to validate these findings through cross-species comparative analyses and detailed mechanistic studies.

#7461 Identifying molecular vulnerabilities in rare cancers using a novel graph-based therapeutic discovery model.

Li Zhao[1], Ying Zhu[1], Randy Chu[2], Haifeng Zhu[2], Stephanie T. Schmidt[1], Yuntao Yang[1], Chunjie Jiang[1], Mark Knafl[2], Xingzhi Song[2], Xiaogang Wu[2], Jianhua Zhang[2], Sabitha Prabhakaran[2], Scott Woodman[2], Timothy Heffernan[3], Elizabeth Burton[2], Rare Tumor Initiative Team, Andy Futreal[2], Bissan Al-Lazikani[1]

[1]Genomic Medicine, Institute for Data Science in Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX,[2]Genomic Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX,[3]TRACTION, The University of Texas MD Anderson Cancer Center, Houston, TX

Background: Rare cancers, as defined by the WHO criteria with an incidence of fewer than 6 cases per 100,000 people annually, pose significant challenges in diagnosis and treatment due to their low prevalence, heterogeneity, and limited therapeutic options. Patients with rare cancers represent a historically neglected population. Traditional statistical approaches predominantly focus on altered genes, overlooking molecular vulnerabilities that are not explicitly mutated but could still be exploited therapeutically.
Methods: We analyzed a cohort of over 1,000 patients spanning more than 120 distinct rare cancer types (Futreal, A., "Rare cancer patient profiling and engagement", AACR Annual Meeting 2022). By systematically evaluating whole-exome sequencing and RNA sequencing data, we identified genes representing key alterations across the cohort. To uncover both altered and latent molecular vulnerabilities, we applied an integrative, generalizable graph-based AI model explicitly designed for therapeutic discovery. Unlike traditional methods, this model integrates multimodal patient data into a network-based framework, connects genomic alterations with latent dependencies, and identifies hidden signals of molecular vulnerabilities, irrespective of their mutational status. Results were validated using public resources, including the DepMap dataset, without incorporating cell line data as inputs or for model training.
Results: Unsupervised clustering revealed 15 molecular subgroups across the cohort. Some clusters, such as CNS (Brain and Spine), exhibited high molecular homogeneity (e.g., 92% of cluster members), while heterogeneous diagnoses like sarcoma were distributed across four clusters, reflecting distinct molecular subtypes. For each cluster, we constructed networks to predict molecular vulnerabilities. Each subgroup yielded at least five prioritized targets associated with FDA-approved or late-stage investigational drugs. Additionally, we identified novel targets that hold promise for innovative drug discovery, offering new avenues for therapeutic exploration. Validation using DepMap data confirmed significant enrichment of these predicted vulnerabilities. Further integration with PRISM and the Genomics of Drug Sensitivity in Cancer (GDSC) datasets refined and prioritized key therapeutic opportunities.
Conclusions: This study demonstrates the power of a graph-based therapeutic discovery model to identify key molecular vulnerabilities in rare cancers. By uncovering both altered and latent dependencies, we have laid the foundation for safe and effective therapeutic development in these neglected malignancies. This integrative approach provides a scalable framework for advancing precision oncology in rare tumors, addressing a critical unmet need for novel and repurposed therapeutic options.

#7463 A linker platform for antibody drug conjugates (ADCs): expanding the therapeutic window.

David Yi, Wen Zhang, John Liu, Suk Lee, Alice Chen

Baylink Biosciences Inc., Palo Alto, CA

Although ADCs have demonstrated great clinical promise, their therapeutic window remains limited due to the complexity of this drug format. Factors contributing to this narrow safety window include the instability of the linkers, accelerated plasma clearance due to the hydrophobicity of the ADCs, and off-target toxicity caused by nonspecific uptake by non-antigen expression healthy cells, free payload pre-release in the circulation system, low tumor retention due to heterogeneity of tumor antigen level and ADC DAR value By preserving the essential functions of the antibody and the payload, an optimized linker can significantly enhance ADC therapeutic window by increasing hydrophilicity and stability while reducing non-specific endocytosis by non-tumor cells. We have designed a panel of novel linkers focused on improving hydrophilicity and stability of ADC, with a particular emphasis on reducing its off-target endocytosis. A key feature of our linkers is the inclusion of a negative charged side chain and hydrophilic chaperone, which not only minimizes non-specific uptake by non-tumor cells but also greatly enhances its hydrophilicity. The side chain linker with this special architecture allows efficient enzymatic cleavage to ensure rapid payload release intracellularly, and lead to superior bystander cell killing effect than the ADC with GGFG-Dxd linker system. One example exploiting the proprietary linker is Trastuzumab-BL-001-exatecan with a drug-to-antibody ratio (DAR) of 8, demonstrated hydrophilicity comparable to Trastuzumab-GGFG-Dxd (biosimilar) in HIC analysis, with no aggregation detected by analytical SEC. This ADC exhibited excellent stability, maintaining high solubility, and cytotoxicity under stressed conditions. It also resisted deconjugation in the presence of high concentration of N -acetylcysteine and showed exceptional stability in human and mouse plasma. Pharmacokinetic studies in rats indicated favorable pharmacokinetic properties, while efficacy testing in the NCI-N87 tumor model revealed comparable performance to Trastuzumab-GGFG-Dxd. Additionally, BLB-101 ADC, featuring the same linker, was well tolerated in non-human primates following repeated administration. In conclusion, our novel linker designs offer significant advantages, with features that could expand the therapeutic window of current ADCs by improving their stability, reducing off-target effects, increasing bystander activity, and maintaining efficacy. We are also expanding the utility of this class of linkers to enable ubiquitous design of dual-payload-ADC Degrader-Antibody-Conjugates.

#7465 Clustered macrophages cooperate to eliminate tumors via coordinated intrudopodia.

Dennis E. Discher, Lawrence Dooling

University of Pennsylvania, Philadelphia, PA

Macrophages often pervade solid tumors, but observations that macrophage clusters might associate with patient survival have remained largely unexplored. We observe dynamic macrophage clusters in tumors under conditions that maximize cancer cell phagocytosis, and our reductionist approaches to cluster formation reveal pathways and roles for tumor-intrusive pseudopodia. Aggregates form over hours on low-adhesion substrates after ‘M1’ polarization of macrophages with interferons, including Tcell-derived cytokines, and yet clusters prove fluid on timescales of minutes. Clusters also sort from M2 macrophages which are induced by an interleukin and that disperse on the same substrates. M1’s upregulate cell-cell adhesion receptors but suppress actomyosin contractility, and while both pathways contribute to cluster formation, decreased cortical tension was predicted to unleash pseudopodia. Macrophage neighbors in tumor spheroids indeed extend intrusive pseudopodia or ‘intrudopodia’ in between adjacent cancer cell junctions - at least when phagocytosis conditions are maximized, and coordinated intrudopodia help detach and individualize cancer cells for rapid engulfment. Macrophage clusters thereby provide a cooperative advantage for phagocytosis to overcome solid tumor cohesion.

#7466 Advancing tumor characterization and immune phenotyping through multiomic in situ profiling of RNA and proteins.

Thanutra Zhang, Smritee Dadhwal, Joshua Thao, Anatalia Robles, Cheyenne Christopherson, Suyog Pathare, Kristen Pham, Minkyung Park, Hsin Wen Chang, Janine Hensel, Cedric Espenel, Hayato Ikoma, Gabriele Girelli, Paul Ryvkin, Veronica Gonzalez Munoz

10x Genomics, Inc., Pleasanton, CA

Cancer continues to be one of the leading causes of death and financial burden on public health worldwide. Despite advances in detection and treatments, there is still a significant level of uncertainty in predicting how an individual's cancer will respond to treatment due to tumor heterogeneity among different patients and multiple subclones from the same individual. A more comprehensive understanding of cancer cell populations, their molecular interactions, and their microenvironment can offer valuable insights into optimal treatment strategies and patient stratification. This can be achieved through the integrated detection of both RNA and protein targets, enabling a deeper exploration of the molecular mechanisms driving cancer.

Here, we analyzed formalin-fixed, paraffin-embedded (FFPE) human tissue sections from diverse tissue types using spatial multi-omics technologies from 10x Genomics. A new add-on capability enables visualization and analysis of protein expression for key immuno-oncology markers including markers for immune cell typing, cell structure, proliferation, and differentiation, as well as immune checkpoint markers (e.g., PD-1, PD-L1, LAG-3, and VISTA). Simultaneous spatial profiling of 480 genes and 28 proteins, integrated with H&E imaging, provided detailed insights into tissue architecture, tumor origins, immune cell infiltration, and the immune status of the tumor microenvironment.

The combined RNA and protein profiling method is a powerful approach for validating RNA-based findings and providing definitive evidence at the functional biological level. Protein data also offers critical insights into the expression and function of different isoforms that are challenging to discern at the RNA level. For instance, isoforms such as CD45RA and CD45RO, which arise from alternative splicing of the PTPRC gene, are often indistinguishable using RNA profiling alone due to the shared exonic sequences in their transcripts. However, protein-level analysis can differentiate these isoforms and enables a more precise understanding of their distinct roles in the immune response, including the delineation of naïve and memory T-cell subsets.

Furthermore, RNA expression does not always directly correlate with protein levels, underscoring the importance of integrating both data types. For instance, discrepancies between RNA and protein expression were observed for β-catenin, encoded by the CTNNB1 gene, across tissues. β-catenin plays a pivotal role in tumor progression through its dual functions in cell adhesion and signaling. By integrating RNA and protein data, researchers can uncover the complexities of post-transcriptional regulation, subcellular localization, and their combined influence on cancer progression, providing deeper insights into disease mechanisms and potential therapeutic targets.

#7467 Unveiling racial disparities in prostate cancer using an integrative genomic and transcriptomic analysis.

Ahmed Fadiel[1], Abdalla Elbialy[1], Akshay Sood[2], Shang-Jui Wang[2], Peng Wang[2], Anil V. Parwani[2], Gennady Shvets[3], Nagireddy Putluri[4], Jenny Li[2], Xuefeng Liu[2]

[1]University of Chicago, Chicago, IL,[2]Ohio State University, Columbus, OH,[3]Cornell University, Ithaca, NY,[4]Baylor College of Medicine, Houston, TX

Significant racial disparities are found in prostate cancer patients. African American (AA) individuals have a ~64% higher incidence and 2.3 times increased mortality rate compared to Caucasian (CA) counterparts. This study integrates RNA and exome sequencing from prostate cancer tissue in both populations to uncover genetic and transcriptomic differences driving these disparities. We analyzed 52 prostate cancer samples (27 AA, 25 CA) using RNA-seq and exome sequencing. RNA-seq analysis revealed 1003 upregulated and 1589 downregulated genes in AA samples compared to CA samples. Pathway analysis showed enhanced activity in immune response and cellular interactions in AA samples, with notable regulation by histone-associated transcription factors (HIST1H1A, HIST1H1D, and HIST1H1B). X2K analysis identified enrichment of EZH2 and SUZ12 transcription factors, core components of the PRC2 complex, suggesting epigenetic regulation involvement in AA prostate cancer. Exome sequencing identified 5419 mutations unique to AA samples and 1146 unique to CA samples. After filtering race-specific variants, we identified 266 unique mutations affecting 20 genes in AA samples and 415 unique mutations impacting 21 genes in CA samples. Key genes with high-impact mutations in AA samples included TP73 (tumor suppression), XYLB (metabolism), ALDH4A1 (oxidative stress), PTPRB (cellular signaling), and HLA-DRB5 (immune response), potentially contributing to disease progression and therapy response disparities. The distinct molecular profiles identified between AA and CA prostate cancers, particularly in immune response pathways and epigenetic regulation, suggest potential therapeutic targets. These findings highlight the importance of considering genetic and epigenetic variations in developing tailored therapeutic approaches to improve treatment efficacy and reduce mortality rates across diverse populations.

Keywords: Racial disparities, Prostate cancer, Genomics, Transcriptomics, Epigenetic regulation, Differential analysis

#7468 Androgen response and prognostication in luminal androgen receptor-positive TNBC patients: Defining a novel signature.

Osama Mustafa Younis, Jehad Amer Yasin, Afaf Mansour, Mohammad Abdulla, Dana Alsafadi, Mamoun Ahram

University of Jordan, Amman, Jordan

Triple negative breast cancer (TNBC) is an aggressive form of cancer that has been subclassified into six molecular subtypes. One subtype, the luminal androgen receptor (LAR), which is characterized by over-expression of the androgen receptor (AR), confers the worst survival. In this work, we aimed to elucidate the androgen-regulated expression profile and molecular pathways involved in the survival of LAR patients. Microarray and bioinformatic analyses of gene expression and microRNA (miRNA) levels were performed following treatment of MDA-MB-453 cells with 10 nM dihydrotestosterone (DHT) for 48 hours. Differentially expressed genes were overlapped with GSE245554, which treated MDA-MB-453 cells with a selective androgen receptor degrader. Overlapped genes with opposing expression patterns were considered androgen-dependent. Shortlisted genes were run through univariate, multivariate, and Kaplan-Meier-based signature collection using the METABRIC cohort. Validation was conducted across the KMplotter and TCGA cohorts. Finally, gene set enrichment analysis (GSEA) between high and low signature expression was carried out using enrichr, and miRNA-mRNA interactions were identified through multiMiR. An 11 gene signature involving FASN, CYFIP2, HSD11B2, EPS8, DALRD3, NANS, MGAT4A, LRRN1, SPATA13, SSR1, and GPCPD1 (LARsig) was curated. LARsig was prognostically significant in the TNBC and LAR cohorts using overall survival (OS) and recurrence-free survival (RFS) metrics. In the high LARsig cohort, LAR patients had a median RFS of 40 months, in comparison to 120 months of the low LARsig cohort (HR = 2.37, 95% CI: 1.39-4.04, $p < 0.01$). A Cox regression-based risk score was inserted into a multivariate Cox model with age, grade, and tumor stage which revealed a consistent OS prognostic significance (HR = 2.41, 95% CI: 1.30-4.49, $p < 0.01$). GSEA showed an immunosuppressive environment where downregulated genes had terms like TNF-alpha signaling, IFN-gamma signaling, apoptotic processes, and cellular defense response. Upregulated genes showed terms consistent with stress response such as macromolecule catabolic process, autophagy, cell cycle, macro-autophagy, and negative regulation of apoptosis. Lastly, FASN appears to play the biggest biological role in our signature. Six differentially expressed miRNAs had validated interactions with FASN including miR-20b-3p (Fold Change = 3.36, FDR p value = 0.0132). MiR-20b-3p had positive co-expression (R = 0.19, p = 0.01) with FASN in the TCGA TNBC cohort. Overall, our study presents the first androgen response signature in LAR patients, and elucidates the possible mechanisms underlying patient survival and treatment resistance. Employment of our risk score for patient stratification in future androgen blockade-based trials could be of clinical utility.

#7469 Molecular insights and biology of low-PSA high-risk prostate cancer.

Nabila Zaman[1], Adriana M. Pedraza[2], Sarah Ann King[1], Prathiksha Prabhakaraalva[1], Natasha Kyprianou[1], Ashutosh K. Tewari[2], Goutam Chakraborty[1]

[1]Department of Urology, Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY,[2]Department of Urology, Icahn School of Medicine at Mount Sinai, New York, NY

Background: Aggressive prostate cancer (PCa) often progresses to castration-resistant disease (CRPC), which becomes incurable and lethal. Although most PCa tumors are initially responsive to androgen deprivation therapy (ADT), duration of this response varies, and relapse typically occurs as disease transitions to CRPC in most patients. Although there have been advancements in molecular genetics and genome sequencing, early identification of lethal versus non-lethal forms of PCa remains difficult. A substantial number of PCa patients are present with high-risk tumors that produce low levels of prostate-specific antigen (PSA) at diagnosis. Research suggests that therapeutic outcomes for these patients may be unfavorable, as they may not respond as effectively to ADT or androgen receptor pathway inhibitors (ARPI). As a result, this high-risk PCa with low PSA secretion may represent an understudied subtype of the disease.

Methods: Between 2013 and 2023, a total of 3619 PCa patients underwent robotic-assisted radical prostatectomy (RARP). Patients were divided into four groups based on PSA levels: very low (<2.5 ng/ml), low (2.6-5.0 ng/ml), medium (5.1-8.0 ng/ml), and high (>8.0 ng/ml). ANOVA and Pearson's chi-squared tests were performed to compare clinical characteristics among these groups. In addition, gene expression profiles from TCGA PCa cohort were analyzed to assess differential PSA/KLK3 mRNA expression within cancer cells. To further investigate, single-cell clones from LNCaP parental population were selected to study molecular characteristics of tumor cells with low PSA expression.

Results: We observed that patients with high-risk PCa and very low PSA levels (<2.5 ng/ml) experienced higher rates of biochemical recurrence than those with high-grade cancer who had PSA levels above 2.5 ng/ml. Furthermore, we observed a significantly higher occurrence of seminal vesicle invasion (p=1.058e-05) and neurovascular invasion (p=0.0047) in both very low and high PSA groups compared to other two groups. Notably, very low PSA group showed a higher rate of lymph node invasion (p=0.00095) than other three groups. Transcriptomic analysis of TCGA cohort showed genes upregulated in very low PSA group compared to high PSA group, were significantly enriched (p<0.001) in metastatic castration-resistant prostate cancer (mCRPC). Furthermore, our study of LNCaP-derived naturally occurring low-PSA clones demonstrated enhanced migratory potential and higher expression of EMT markers compared to parental LNCaP cells, which express high levels of PSA.

Conclusions: Our study reveals a distinct subtype of localized PCa characterized by low PSA levels while still displaying high-risk features. This subtype is particularly invasive and linked to potentially poor outcomes, highlighting the necessity for further investigation to gain deeper understanding of its aggressive biology and characteristics that remain largely unexplored.

#7470 Extensive genome sequencing on tissue vs large ctDNA panel: what clinical impact?.

Marie Morfouace, Etienne Rouleau, Marc Deloger, Ludovic Lacroix, Voreak Suybeng, Sofiane Taleb, Claudio Nicotra, Maud Ngo Camus, Fabrice Barlesi, Fabrice Andre, Antoine Italiano, Benjamin Besse, Arnaud Bayle

Gustave Roussy, Villejuif, France

Background: Molecular profiling is critical for personalizing cancer treatments, with approaches spanning from tissue DNA sequencing to liquid biopsy analysis of circulating tumor DNA (ctDNA). While both techniques provide valuable insights, the comparative clinical impact of tissue-based genomic profiling versus ctDNA-based profiling in tailoring treatments remains insufficiently explored.
Methods: We are investigating the comparison between wide genomic sequencing of tissue and ctDNA profiling with a large panel to evaluate their ability to guide treatment decisions in cancer patients. At Gustave Roussy, Comprehensive genomic profiling (CGP) on ctDNA is routinely used to guide treatment selection for patients with advanced metastatic cancers, in therapy failure after 1st line. In the same patient population, Extensive Genome Sequencing (EGS: WGS, WES, WTS) can also be proposed, from fresh frozen material. To assess the relevance of these approaches, we identified a cohort of patients from Gustave Roussy with prescription of both EGS on fresh frozen tumor tissue and CGP on ctDNA, (Foundation One Liquid CDx Assay, 324 genes, tumor mutational burden [TMB], microsatellite instability) and were discussed in Gustave Roussy's molecular tumor board. This study will evaluate and compare the capacity of these methods to tailor treatment strategies in this population
Results: Between April 2021 and Oct 2024, 202 patients with both EGS and CGP were identified. Failure rate of EGS on tissue was 18.8% (due to lack of sufficient tissue for 36 patients and non-contributive for 2 patients). Conversely, failure rate of CGP on ctDNA was 8.4% (non-contributive for 17 patients). Out of the 147 evaluable patients, main tumor types were HNSCC (n=32, 22%), endocrine tumors (n=26, 18%), gynecological tumors (n=16, 11%), sarcoma (n=13, 9%) and pancreatic tumors (n=14, 9%). The median turnaround time between reception of the sample and results from the molecular analysis was 10 [5:37] days for CGP versus 95 [25:724] days for EGS. We compared the therapeutic recommendations provided with both analyses, after discussion during a molecular tumor board. Independently of the time between tissue sampling and liquid biopsy (median: 111 days), treatment recommendations were concordant in 83% of cases. For 13 cases (8.8%), alterations were identified only on tissue (deletion (n=4), mutation (n=6), genomic signature (n=3)).
Conclusion: Our analysis on a small cohort of patient shows that EGS and CGP have a high concordance rate in terms of therapeutic recommendations when both are contributive. Given organizational barriers of EGS on tissue (cost, turnaround time, difficulty to obtain tumor material of sufficient quality), a sequential strategy could be interesting.

#7471 Mutational profiles of pancreatic cancer patients from Eastern Asia using somatic exome sequencing.

Jacquelyn Reuther[1], Taro Shibuki[2], Megan Hawley[1], Hideaki Bando[3], Keir Violaceae[1], Makoto Ueno[4], Kenji Ikezawa[5], Takuji Okusaka[6], Teiichi Sugiura[7], Keiko Kamei[8], Yoshito Komatsu[9], Michiaki Unno[10], Hiroki Yukami[11], Takuji Iwashita[12], Emily Westheimer[1], Kumiko Umemoto[13], Robert Daber[1], Takayuki Yoshino[14], **Jianhua Zhao**[1], Masafumi Ikeda[2]

[1]Labcorp Genetics, Iselin, NJ,[2]Department of Hepatobiliary and Pancreatic Oncology, National Cancer Center Hospital East, Kashiwa, Japan,[3]Department for the Promotion of Drug and Diagnostic Dev., Division of Drug and Diagnostic Dev., National Cancer Center Hospital East, Kashiwa, Japan,[4]Department of Gastroenterology, Kanagawa Cancer Center, Yokohama, Japan,[5]Department of Hepatobiliary and Pancreatic Oncology, Osaka International Cancer Institute, Osaka, Japan,[6]Department of Hepatobiliary and Pancreatic Oncology, National Cancer Center Hospital, Tokyo, Japan,[7]Division of Hepato-Biliary-Pancreatic Surgery, Shizuoka Cancer Center Hospital, Shizuoka, Japan,[8]Department of Surgery, Kindai University Faculty of Medicine, Osakasayama, Japan,[9]Division of Cancer Chemotherapy, Hokkaido University Hospital, Sapporo, Japan,[10]Department of Surgery, Tohoku University Graduate School of Medicine, Sendai, Japan,[11]Cancer Chemotherapy Center, Osaka Medical and Pharmaceutical University, Takatsuki, Japan,[12]First Department of Internal Medicine, Gifu University Hospital, Gifu, Japan,[13]Department of Clinical Oncology, St. Marianna University School of Medicine, Kawasaki, Japan,[14]Department of Gastroenterology and Gastrointestinal Oncology, National Cancer Center Hospital East, Kashiwa, Japan

Introduction: Pancreatic adenocarcinoma (PC) is one of the most lethal diseases. The incidence of PC is rising in the Asia-Pacific population, with some of the highest rates in Japan. As such, the utilization of molecular testing has increased, especially for advanced disease, to identify molecular drivers and therapeutic targets.
Methods: 146 patients from Japan (ages 45-81) who were diagnosed with PC underwent exome-based, tumor molecular profiling (TMP) (Labcorp Genetics Inc). 96/146 (65.8%) patients had unresectable tumors. Results were correlated with clinical information. Variants were interpreted according to the AMP/ASCO/CAP guidelines. Signaling pathway analysis was conducted using the Reactome database, with significance determined by binomial test with corrected p-values (FDR; Benjamini-Hochberg procedure). Fisher's exact test was utilized for statistical assessment of clinical parameters.
Results: TMP detected 404 Tier 1/2 somatic variants in 135 (92.5%) patients. Genes known to be frequently mutated in PC were identified in the cohort: KRAS (122/146; 83.6%), TP53 (108/146; 74.0%), SMAD4 (35/146; 24.0%) and CDKN2A (25/146; 17.1%). A potential enrichment of hotspot U2AF1 (5/146; 3.4%) codon 34 mutations was identified, compared to published frequencies in PC in cancer databases (1%) that trended toward statistical significance (p=0.07). Pathway analysis identified a statistically significant enrichment of mutations in several kinase signaling pathways including PI3K/AKT (p-value: 2.1e-11; FDR: 9.8e-09), ERBB2 (p-value: 5.3e-07; FDR: 1.2e-04), and RAS/MAPK (p-value: 4.2e-06; FDR: 5.5e-04). Mutations associated with homologous recombination or mismatch repair pathways were statistically associated with early age of onset (<50 years of age; p= 0.036). Hotspot KRAS mutations were associated with unresectability (p=0.002), late tumor stage (stages 3-4, p=0.026), and metastasis (p=0.010).
Conclusion: Alterations enriched in targetable pathways were identified in the patient cohort in addition to statistically significant findings associated with more aggressive PC. TMP testing can aid in the identification of alterations that are pertinent to the identification of relevant therapies and clinical trials as well as identify PC patients with potentially more aggressive disease.

#7472 Metabolic mechanisms underlying late recurrence in hormone receptor positive breast cancer.

Bryce Ordway, Isabella Vianna, Johannes Kreuzer, Wilhelm Haas, Dennis Sgroi, Leif W. Ellisen

Mass General Cancer Center, Boston, MA

Hormone receptor positive (HR+) breast tumors are anomalous in their tendency for late recurrence. In the clinic, an mRNA ratio of HOXB13 to IL17RB (H/I) from the primary tumor is used to determine a patient's risk for late recurrence and benefit of extended adjuvant hormonal therapy. Little is known about the tie between the H/I ratio and the biology leading to late recurrence. Herein we present data from primary breast tumors, *in silico* analyses and cell-based models that support our hypothesis that dysregulation of one-carbon metabolism and the SAM cycle is linked to the H/I signature and contributes to epithelial-mesenchymal plasticity (EMP), which has been shown to mediate the late recurring phenotype. We conducted proteomics analysis of 86 HR+ breast tumors and identified Choline Dehydrogenase (CHDH) as the most significant differentially expressed protein between HOXB13 high and low groups; with CHDH being associated with HOXB13 low tumors. Notably, RNA expression of CHDH is highly correlated with expression of IL17RB due to a shared promoter region. These links with both HOXB13 and IL17RB led us to hypothesize that CHDH contributes to the phenotype of late recurrence. To determine if signatures of differences in CHDH function could be seen in H/I low and high cell lines we used RNA expression and metabolomics data from the DepMap portal. Betaine, a standard readout metabolite for CHDH function, was significantly elevated in H/I low cell lines, consistent with our previous observations. We also observed that 1-methylnicotinamide, produced by Nicotinamide N-methyltransferase (NNMT), was identified as the metabolite most upregulated in H/I high cell lines. CHDH and NNMT both contribute to one-carbon metabolism in the SAM cycle, with opposing influences. Previous studies have demonstrated that dormancy and late recurrence in the context of HR+ breast cancer is driven by epithelial-mesenchymal plasticity (EMP). To test the association between SAM flux and differing epithelial and mesenchymal phenotypes, we created a CHDH:NNMT score and correlated this against epithelial-mesenchymal phenotypes in patient tumors from the TCGA Firehouse Legacy dataset. A significant association was found in all tumor types, with a conglomerate p-value across tumor types of $<10^{-300}$. We also observed high SAM levels in tumors associated with markers of an epithelial-like state. We then demonstrated that shRNA knockdown of NNMT is sufficient to increase expression of epithelial proteins in cell lines displaying a mesenchymal phenotype. We will next test the ability of CHDH and NNMT to alter EMP in breast cancer cells and identify EMP associated metabolic pathways. In total, we have demonstrated that metabolic enzymes regulating SAM flux are highly associated with late-recurrence in HR+ breast tumors. Ongoing experiments will assess how metabolism controls epithelial-mesenchymal plasticity that may mediate the phenotype.

#7474 Comprehensive multi-omics characterization of gastric cancers with proteogenomic insights.

Lindsey K. Olsen[1], Yuefan Wang[2], Hui Zhang[2], Bing Zhang[1], Clinical Proteomic Tumor Analysis Consortium (CPTAC)

[1]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX,[2]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD

Gastric cancers are one of the leading causes of cancer-related mortality worldwide. Characterized by high tumor heterogeneity, the treatment of gastric cancers remains challenging. Prior attempts to elucidate the tumor heterogeneity of gastric cancers have included subtyping it into differing clinical and molecular subtypes. For example, the main histological type, adenocarcinoma, has intestinal and diffuse subtypes. At the genomic level, gastric cancers are classified as either Epstein-Barr virus (EBV), microsatellite instability (MSI), chromosomal instability (CIN), or genomically stable (GS). Transcriptomic subtyping has further revealed subtype and clinical prognosis relationships: MSI, microsatellite-stable microsatellite stable with epithelial-to-mesenchymal transition phenotype (MSS/EMT) (poor prognosis), and MSS/TP53 active or inactive (intermediate prognosis). However, these subtypes are still poorly understood at the functional level. To address this gap, the Clinical Proteomics Tumor Analysis Consortium (CPTAC) conducted a comprehensive multi-omic characterization of 159 gastric tumors and 31 normal adjacent tissues. Moving beyond conventional genomic and transcriptomic analyses, we employed a novel multi-omics approach that encompasses the proteome, phosphoproteome, glycoproteome, metabolome, and additional “omes” such as ubiquitinome, acetylome, and protein-protein interactome. In addition, to better understand the unique role of the microbiota in gastric cancers, we integrated the entire microbiome genome and transcriptome data into the whole analysis. By combining these 12 layers of data and microbiome analysis together, our study provides unprecedented proteogenomic insights, advancing our understanding of the molecular mechanisms of gastric cancer oncogenesis and aiding in the development of novel precision oncologist therapies. In this study, we classified gastric cancers by molecular and clinical subtypes, revealing both shared and subtype-specific biological features at the multi-omic level. We highlight the associations of post-translational modification (PTM) alterations to immune and metabolic pathways. Proteogenomic analysis uncovered promising new surface targets and potential biomarkers for existing targeted therapies. Further in-depth analyses of PTMs and protein-protein interactions have uncovered insights into intracellular signaling pathways and PTM crosstalk with therapeutic potential. Additionally, the impact of microbiome on immune responses and PTM patterns underscores the interplay between microbiota and cancer phenotypes, providing valuable insights for advancing precision medicine in gastric cancers. Overall, our study provides the most comprehensive multi-omics atlas for gastric cancers, offering deep insights into its cancer biology and molecular heterogeneity.

#7475 A comprehensive single-cell atlas of gastric cancer reveals malignant cell state dynamics and plasticity in tumor progression and metastasis.

Enyu Dai, Yanshuo Chu, Yang Liu, Ruiping Wang, Yibo Dai, Jaffer A. Ajani, Linghua Wang

The University of Texas MD Anderson Cancer Center, Houston, TX

Background: Gastric cancer exhibits diverse malignant cell states that contribute to intratumor heterogeneity (ITH), a fundamental factor that drive tumor progression, metastasis, and treatment resistance. While previous single-cell RNA sequencing (scRNA-seq) studies have provided novel insights into ITH in gastric cancer, they are often constrained by small sample sizes and limited transcriptional coverage.
Methods: In this study, we curated, annotated, and integrated scRNA-seq datasets from 21 studies, comprising 626 samples from 316 individuals, including healthy donors and patients with precancerous lesions and gastric cancer. Within the epithelial compartment, malignant and non-malignant cells were distinguished using three complementary computational methods. To refine malignant cell subclustering, we developed a novel approach that incorporates patient-specific copy number variations, minimizing the impact of inter-patient heterogeneity on clustering analysis.
Results: Following rigorous quality control and data integration, unsupervised clustering analysis classified 2,088,266 high-quality cells into six major cell types or compartments: B cells, T cells, innate lymphoid cells, myeloid cells, stromal cells, and epithelial cells. Within the epithelial cell compartment, we identified 12 distinct malignant cell states characterized by unique expression profiles and phenotypic characteristics, such as cell proliferation capacity and lineage-specific plasticity. These cancer cell states exhibited substantial variation in the activity of hallmark cancer signaling pathways, with their presence and composition closely linked to tumor aggressiveness and clinical outcomes. Proliferative cancer cells and translationally active cancer cells predominated in intermediate tumor stages, dormant cells were prevalent in advanced stages, and epithelial-mesenchymal transition (EMT) states were enriched in metastatic tumors. Additionally, we identified a malignant cell population with high expression of intestinal lineage markers in patients with signet ring cell carcinoma, and these findings were validated using spatial transcriptomic.
Conclusions: This study presents a comprehensive single-cell transcriptomic atlas of gastric cancer epithelial cells, providing valuable insights into transcriptional ITH. Our findings define distinct malignant cell states, elucidate their dynamics, and highlight their clinical significance, establishing a framework for understanding how these states contribute to disease progression and metastasis.

#7476 Characterizing the molecular drivers of terminal duct lobular unit involution and associations with breast cancer in African American women.

Jeri Danielle Hughes[1], Angel Hokulani Pajimola[1], Brittny Davis-Lynn[2], Sharon Fan[2], Mustapha Abubakar[2], Renata Cora[3], Alexandra Harris[2], Gretchen Gierach[2], Brittany Jenkins[1]

[1]Biochemistry & Molecular Biology, Johns Hopkins Bloomberg Sch. of Public Health, Baltimore, MD,[2]National Cancer Institute, Bethesda, MD,[3]Division of Cancer Epidemiology and Genetics, National Instiutes of Health, Bethesda, MD

Background: Terminal duct lobular units (TDLUs) are the functional units of the mammary gland, consisting of a terminal duct and the surrounding lobular tissue. These structures are key in the process of milk production and secretion, particularly in women of reproductive age. TDLUs are important in the context of breast pathology, as they can be sites of various benign and malignant changes, such as fibroadenomas or breast cancer. TDLU involution refers to the process of regression (or shrinking) of TDLUs as women age, particularly after menopause. Incomplete or delayed involution may be associated with an increased risk of breast cancer, as persistent epithelial tissue may remain susceptible to malignant changes. Differences in TDLU involution have been observed in the context of self-reported race, generating a gap in knowledge regarding the differential molecular regulation of TDLU involution by race and genetic ancestry. Our work aims to identify driver genes and pathways that regulate this process, as well as understand how these pathways are epigenetically modified, and possibly differ by race.
Methods: Normal breast tissue from voluntary research biopsies were obtained from 94 pre-menopausal women (47 African American, 47 European American) between 2009-2019 from the Komen Tissue Bank. We performed both bulk RNAseq analyses and the EPIC850K methylation array to determine differences in cancer- and immune-related pathways associated with high and low TDLU counts (counts per mm^2) stratified by self-reported race.

Results: In African American women, 56 genes showed significant changes in gene expression associated with differential expression by TDLU counts per mm^2, indicating differences in TDLU involution. Of these genes, LMOD1 and MFGE8 were associated with TDLU count (high/low) and were more significantly differentially expressed among African American women than European American women. LMOD1 is an actin-regulating protein that regulates cell shape, motility, and cytoskeleton remodeling. MFGE8 is a milk-fat globule epithelial growth factor that also mediates phagocytosis of apoptotic cells. Ingenuity Pathway Analysis revealed LMOD1 and MFGE8 interact via androgen receptor signaling in breast cancer. Further studies will investigate epigenetic modifications in these genes and those related directly to TDLU involution.
Conclusions: Studying TDLU involution is crucial for understanding both the normal aging process of the breast and its implications for breast cancer risk. Understanding the molecular mechanisms behind TDLU involution, such as immune regulation and genetic factors, can help identify individuals at higher risk for breast cancer and inform preventive strategies.

#7477 Conquering overtreatment of ductal carcinoma in situ through integrative team science.

Jelle Wesseling[1], Proteeti Bhattacharjee[2], Esther H. Lips[2], Elinor J. Sawyer[3], E. Shelley Hwang[4], Alastair M. Thompson[5], Grand Challenge PRECISION Consortium[6]

[1]Department of Pathology, Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital, Amsterdam, Netherlands,[2]Division of Molecular Pathology, Netherlands Cancer Institute, Amsterdam, Netherlands,[3]School of Cancer and Pharmaceutical Sciences, Faculty of Life Sciences and Medicine, Guy's Cancer Centre, King's College London, London, United Kingdom,[4]Department of Surgery, Duke University School of Medicine, Durham, NC,[5]Department of Surgery, Dan L Duncan Comprehensive Cancer Center, Baylor College of Medicine, Houston, TX,[6]Netherlands Cancer Institute, Amsterdam, Netherlands

Ductal carcinoma in situ (DCIS), often misclassified as early-stage breast cancer, rarely progresses to invasive disease. Yet, the inability to distinguish between indolent and aggressive DCIS leads to widespread overtreatment, burdening thousands of women globally. Addressing this, the PRECISION team, under the Cancer Grand Challenges initiative, has achieved remarkable advancements, driving a paradigm shift in DCIS management. PRECISION exemplifies next-level team science, merging expertise from 10 institutions across the USA, UK, and the Netherlands. Spanning disciplines such as epidemiology, molecular biology, AI, pathology, clinical expertise, health outcomes, and patient advocacy, the team's interdisciplinary approach enabled transformative breakthroughs.
Key achievements include:

1. Understanding DCIS Biology: Using innovative patient-derived DCIS-MIND models and single-cell genomic techniques, PRECISION revealed that genetic changes alone do not dictate progression. These findings challenge long-held assumptions, emphasizing the need for comprehensive approaches to assess risk.
2. Risk Stratification Methodologies: Novel biomarkers, AI-driven morphometric analyses, and an RNA-seq classifier reliably differentiate high-risk from low-risk DCIS. For example, the RNA-seq classifier identified low-risk DCIS with a 98% negative predictive value, paving the way for treatment de-escalation.
3. Testing Active Surveillance: Clinical trials embedded in PRECISION' (COMET, LORD, LORIS) explored the safety of active surveillance, potentially replacing invasive treatments for low-risk DCIS with cost-effective outcomes. Patient engagement and shared decision-making tools further enhance personalized care strategies.

PRECISION's success stems from dynamic international collaborations and integration of advanced technologies. Its findings not only redefine DCIS management but also establish a blueprint for addressing other low-risk malignancies. By focusing on biology-based, patient-centered solutions, PRECISION ensures that treatment aligns with the true nature of the disease, paving the path to transform DCIS care worldwide. To do so, the team aims to develop dynamic risk forecasting techniques, powered by evolutionary AI, drawing inspiration from geoscience methodologies. The team also plans to establish a Global Virtual DCIS Monitoring Clinic, enabling worldwide participation. This initiative will support personalized care, foster shared decision-making, and minimize clinic visits through standardized data collection and seamless online follow-ups.

#7478 Lung cancer gene regulatory networks reflect smoking history and age-related metabolic pathway alterations.

Camila Lopes-Ramos[1], Enakshi Saha[2], Jeong Yun[3], Craig Hersh[3], Edwin Silverman[3], Dawn DeMeo[3], John Quackenbush[4], Kimberly Glass[1]

[1]Harvard T.H. Chan School of Public Health; Brigham and Women's Hospital; Harvard Medical School, Boston, MA,[2]University of South Carolina, Columbia, SC,[3]Brigham and Women's Hospital; Harvard Medical School, Boston, MA,[4]Harvard T.H. Chan School of Public Health, Boston, MA

Background: The rising incidence of lung cancer among individuals without a history of smoking highlights the need to explore biological mechanisms underlying this phenomenon. Our study aims to identify gene regulatory mechanisms that drive lung cancer risk among never-smokers by analyzing how gene regulatory networks differ between individuals with and without lung cancer, depending on their smoking history.
Methods: We used RNA-Seq data from the Lung Tissue Research Consortium (LTRC) collected via TOPMed, comprising non-cancerous lung tissue samples from 344 individuals with non-small cell carcinoma and 329 lung tissue samples from individuals without cancer. Across all, 18% reported no history of smoking. We ran differential gene expression analysis by voom, adjusting for age, sex, COPD status, and examining statistical interactions between smoking (never/former) and cancer status. We analyzed sample-specific transcription factor-gene regulatory networks generated by PANDA-LIONESS. We used linear regression to evaluate the association between gene targeting score (measured by gene indegree) and the interaction between smoking and cancer status. We ran gene set enrichment analysis with genes ranked by the corresponding smoking by cancer interaction coefficients.
Results: Differential expression analysis of lung tissues from individuals with and without cancer showed that genes overexpressed in cancer are enriched for canonical cancer pathways, such as p53, MAPK, and WNT signaling pathways (FDR<0.05). We found a significant interaction between cancer and smoking status, indicating these cancer-related pathways had higher enrichment in former-smokers compared to never smokers. Gene regulatory network analysis indicated that these differential expression patterns are possibly driven by increased transcriptional targeting of these cancer-related pathways. Among never-smokers, individuals with cancer showed higher targeting of metabolic pathways (e.g., fructose and mannose, glutathione, phenylalanine, histidine, arginine, and proline metabolism) compared to lung tissue from individuals without cancer (FDR<0.05), highlighting a possible role of metabolic pathways in tumor development, uniquely among never-smokers. A negative correlation between metabolic pathway targeting scores and age was observed exclusively in individuals with cancer without a history of smoking (Mean Pearson R=-0.24).
Conclusion: Our findings reveal transcriptional targeting differences in lung cancer by smoking history. Among individuals without a history of smoking, the increased targeting of metabolic pathways in cancer, which is pronounced in younger compared to older individuals, may contribute to the higher risk of early-onset lung cancer among never-smokers, offering new avenues for understanding and addressing the disease in populations without a history of smoking.

#7479 Single-cell OncoTreat: Novel therapeutic candidates by high resolution inference and validation of drug sensitivities in heterogeneous cholangiocarcinoma tumors.

A. Obradovic[1], L. Tomassoni[1], D. You[2], C. Drake[1], F. Dela Cruz[1], A. Kung[2], A. Califano[1];
[1]Columbia University Irving Medical Center, New York, NY, [2]Memorial Sloane Kettering, New York, NY

Cholangiocarcinoma is an aggressive biliary adenocarcinoma with limited treatment options. Stromal infiltration and intratumoral heterogeneity in this tumor type prevents identification of actionable dependencies from bulk-tissue profiles. While cholangiocarcinoma illustrates these challenges, they are common across many other aggressive tumors.To address these challenges, we introduce a single-cell RNA-Sequencing (scRNA-Seq) framework for prioritization of drugs to treat heterogeneous tumor cell subpopulations. Single-cell analyses can bypass limitations of sample size in a precision medicine approach by providing large tumor cell “cohorts” even from individual patients, with the advantage that cell subpopulations can be effectively distinguished. Unfortunately, >80% of genes in each cell profiled by scRNA-Seq are typically undetectable, limiting discoverability of therapeutic targets and drug sensitivities. The VIPER algorithm addresses this by inferring the *transcriptional activity* of a protein from expression of its downstream target genes. By then identifying aberrantly activated proteins, each cell can be matched to an empiric database of cell line drug perturbation RNA-Seq, ranking drugs which empirically inhibit the proteins aberrantly activated in each cell. We refer to this approach as single-cell OncoTreat.Analysis of tumor cells across six patients by this approach has revealed three distinct sub-phenotypes of cholangiocarcinoma cells, with conserved proteomic signatures, and distinct predicted drug sensitivities. A seventh patient recapitulated these phenotypes, with drug predictions validated in a low-passage, patient-derived xenograft (PDX) model. The top-ranked drug by integrated analysis of all tumor cells was plicamycin, which at sub-cluster level only two of three clusters were predicted to respond to. Conversely, dacinostat was inferred to be active against all three tumor-cell sub-phenotypes. Tumor growth studies in PDX demonstrated growth rate control with both plicamycin and dacinostat, but much improved survival time with dacinostat, outperforming gemcitabine + cisplatin standard of care. Post-treatment scRNA-Seq confirmed predicted subpopulation-specific effects, such that dacinostat depleted all three tumor sub-phenotypes, while plicamycin depleted only the two predicted, allowing the third to reconstitute a treatment-resistant tumor. To our knowledge, this is the first report of dacinostat as an HDAC inhibitor effective in cholangiocarcinoma treatment, *in vivo*. Ultimately, this approach suggests promising candidates for follow-up trials in cholangiocarcinoma, and is highly generalizable to elucidate complementary dependencies of other aggressive, heterogeneous tumors.

#7480 Spatial Groups provide a pan-tumor spatial transcriptomic biomarker of immunotherapy response.

Vivek Behera[1], Hannah Giba[2], Ue-Yu Pen[2], Anna Di Lello[1], Benjamin A. Doran[2], Alessandra Esposito[1], Apameh Pezeshk[1], Christine Bestvina[1], Justin P. Kline[1], Marina C. Garassino[1], Arjun S. Raman[2]

[1]Department of Medicine, Section of Hematology/Oncology, University of Chicago, Chicago, IL,[2]Department of Pathology, Duchossois Family Institute, University of Chicago, Chicago, IL

Immune checkpoint blockade (ICB) has transformed cancer therapy and is now approved in every type of cancer, yet it remains difficult to identify the ~ 20% of patients that will benefit from such therapy. Explainable models that predict ICB response can potentially spare ‘responders’ from toxic chemotherapies while identifying alternative options for ‘non-responders’. The only widely approved ICB biomarker of response, PD-L1, treats TME cells as individual components and is only weakly predictive of outcomes. Recent studies have suggested that biomarkers built on cell-to-cell spatial proximity, such as tumor cell to CD8+ T cell distance, can improve upon PD-L1 in predicting ICB efficacy. Yet, there is a lack of generalized approaches for identifying tumor spatial biology relationships relevant to ICB response, especially considering the wealth of information generated by high-resolution spatial biology techniques. Our approach was (1) to integrate spatial transcriptomics datasets from 96 tumors across twelve tumor types and (2) to apply statistical covariation approaches to identify organizing principles of spatial biology that would facilitate the comparison of these datasets. The basis of this approach is our identification of a conserved unit of pan-tumor spatial organization that we termed ‘Spatial Groups’. Spatial Groups (SGs) are multi-cellular units demonstrating coordinated transcriptional programs contained within spatial domains ranging in size from tens of microns (dozens of cells) to mm-scale (large regions of a tumor). SGs are hierarchically organized such that large-scale SGs exert contextual field effects on their nested small-scale SGs. For example, we found that T cell presence in large-scale SGs impacted metabolic pathway shifts occurring within nested medium-scale SGs, which in turn impacted cell adhesion programs within nested small-scale SGs. We found that SGs revealed immune-related spatial biology patterns that we hypothesized would serve as a genome-wide spatial biomarker of ICB response. To test this idea, we performed a retrospective study of 16 patients with metastatic non-small cell lung cancer treated with ICB or chemotherapy-ICB in the frontline setting. Unlike PD-L1 status, our SG-derived biomarker was highly significant in predicting progression-free survival after therapy initiation. Additionally, analysis of SGs in non-responder patients provided insights into patient-specific spatial biology architectures limiting ICB efficacy. In summary, our discovery of Spatial Groups provides a general framework for using spatial biology to identify pan-tumor ICB response.

#7481 Genomic aberration-derived signature in lung adenocarcinoma improves patient prognosis and therapy stratification.

Xiang Wang[1], Zijian Zhang[1], Jian-Rong Li[1], Jianjun Zhang[2], Chao Cheng[1]

[1]ICTR, Baylor College of Medicine, Houston, TX,[2]Department of Thoracic/Head and Neck Medical Oncology, MD Anderson Cancer Center, Houston, TX

Background: Lung cancer is the leading cause of cancer-related mortality worldwide, with a poor prognosis due to late-stage diagnoses. While advances in targeted therapies and immune checkpoint blockade therapies (ICBT) have improved survival outcomes for subsets of non-small cell lung cancer (NSCLC) patients, the clinical heterogeneity and variable response rates to these therapies remain significant challenges in precision medicine. Identifying robust biomarkers for stratifying patients and tailoring personalized treatments remains a critical unmet need.
Methods: We developed the Genomic Aberration-derived Signature for Patient Stratification (GASPS), a novel statistical framework integrating genomic and transcriptomic data. GASPS models the effects of driver genomic aberrations—mutations, amplifications, and deletions—on gene expression to define actionable gene signatures. Using lung adenocarcinoma (LUAD) data from The Cancer Genome Atlas (TCGA), we identified and validated driver gene signatures across independent NSCLC datasets, including TRACERx. The clinical relevance of these signatures was assessed for their ability to predict prognosis and treatment response.
Results: GASPS defined 38 driver genomic aberration signatures, which consistently outperformed genomic aberration status in predicting patient outcomes. Hierarchical clustering of signature scores stratified patients into subgroups with distinct survival patterns. Notably, the EGFR-Mut and EGFR-Amp signatures demonstrated contrasting prognostic and predictive associations: EGFR-Mut was linked to favorable prognosis and chemotherapy sensitivity, whereas EGFR-Amp was associated with poor prognosis and increased tumor proliferation. The STK11-Mut signature emerged as a robust biomarker for resistance to ICBT, correlating with reduced immune infiltration and a "cold" tumor microenvironment. Validation across multiple datasets confirmed the generalizability of these findings.
Conclusion: The GASPS framework provides a powerful tool for integrating genomic and transcriptomic data to derive clinically actionable gene signatures. These signatures offer superior prognostic and predictive capabilities, enabling better patient stratification and advancing personalized treatment strategies for NSCLC. While demonstrated in LUAD, this framework is adaptable to other cancer types, paving the way for broader applications in precision oncology.

#7482 Mapping the temporal landscape of breast cancer using epigenetic entropy.

Daniel L. Monyak[1], Shannon T. Holloway[1], Graham Gumbert[1], Lars J. Grimm[1], Shelley Hwang[1], Jeffrey R. Marks[1], Darryl Shibata[2], **Marc D. Ryser**[1]

[1]Duke University School of Medicine, Durham, NC,[2]University of Southern California, Los Angeles, CA

Background: The age of a newly diagnosed breast cancer encodes valuable etiologic and prognostic information, yet it is considered a known unknown. Here we develop an entropy-based epigenetic clock that measures the age of individual breast cancers and quantifies the evolutionary-ecological forces that modulate growth.
Methods: Using 450K methylation array data from 634 invasive breast cancers and 79 normal breast tissue samples, we identified 500 fluctuating CpG (fCpG) sites whose methylation states exhibit unbiased oscillations. After external validation of the fCpG set in an independent cohort (n=146), we derived an entropy-based epigenetic clock index (ECI) as a measure of tumor mitotic age. We correlated the ECI with tumor features and gene expression (GSEA) and quantified its relationship with the tumor microenvironment (CIBERSORTx). We validated the ECI in 40 patients with paired primary-metastasis samples and used mathematical models to estimate the calendar ages of individual tumors.
Results: Compared to older tumors (higher ECI), younger tumors (lower ECI) had increased proliferation, higher grade, and larger size. Younger tumor age was strongly associated with the fraction of genome altered (R, -0.56; P<0.0001). Luminal A cancers were older compared to luminal B and basal cancers (P<0.001 each, Wilcoxon). Mitotically younger tumors were enriched for gene pathways related to proliferation and cell cycle control, whereas older tumors were enriched for immune pathways and immune-related signaling pathways. The extent of immune infiltration, and T-cell infiltration in particular, increased with mitotic tumor age. These findings suggest that a tumor's effective growth rate is shaped by the competition between intrinsic growth potential and microenvironmental pressures (e.g., immune surveillance). In patients with paired primary-metastasis samples, the 500 fCpGs were strongly correlated within but not between patients (median R, 0.73 vs. 0.08; P<.0001, Wilcoxon). In 36/40 patients, the metastasis was younger than the primary, providing support for the interpretation of the ECI as a measure of tumor age. Anchoring the median age at a consensus estimate of 3 years, the inferred calendar ages among 413 cancers ranged from 0.2 to 35.2 years (IQR: 1.5-5.6 years). There were notable differences in median tumor ages by molecular subtype, ranging from 1.0 years in basal cancers to 6.5 years in luminal A cancers.
Conclusions: This entropy-based epigenetic clock measures tumor growth on a scale of months to years, which contrasts with existing clocks that track longer timescales associated with biologic tissue aging and cancer risk. By delineating old and stable from young and aggressive tumors, the clock has potential applications in risk stratification and early detection. Although developed for breast cancer, the clock provides a scalable technology to characterize the broader temporal landscape of solid tumors.

#7483 A new spatial multi-omics approach to deeply characterize human cancer tissue using a single slide: a new spatial muti-omics approach to deeply characterize human cancer tissue using a single slide.

yasmine adimi[1], clement Levin[2], Emilia Puig Lombardi[2], Marion Classe[2], Cecile Badoual[1]

[1]Inserm U970, Paris, France,[2]Sanofi R&D, Paris, France

Introduction: In digital pathology, extracting comprehensive data from a single tissue sample is critical for enhancing diagnostic precision and therapeutic strategies. Techniques like multiplex imaging and spatial transcriptomics offer unprecedented depth of information. This study introduces a novel and innovative approach combining for the first time Phenocycler® (with a 34-marker panel) and Visium® spatial transcriptomics on the same tissue section of squamous cell carcinoma of the upper aerodigestive tract.
A single tissue section was analyzed using Phenocycler® (PP) for proteomics with a 34-marker panel targeting microenvironment and structural proteins, followed by H&E staining and Visium® spatial transcriptomics (ST) to map RNA expression. This approach allowed for the thorough assessment of immune cell spatial distribution and transcriptomic profiles, while evaluating the impact of proteomic treatment on RNA integrity and gene expression.
Results: Despite initial concerns about RNA degradation due to multiplex staining treatments, RNA integrity in the PP+ST sample was comparable to ST-only samples, as indicated by similar total gene expression distributions. The PP+ST sample exhibited high-density feature points across the tissue, suggesting that proteomic pre-treatment did not impair subsequent transcriptomic analysis. Unsupervised UMAP visualization revealed similar clustering patterns between PP+ST and ST-only samples, indicating preserved cellular heterogeneity and molecular signatures. Consistent spatial distributions and clustering were observed between Phenocycler® pseudospots and Visium® data, with significant correlations in the spatial distribution of key cell types, including malignant clusters and immune cells.
This study is a pioneering demonstration of the feasibility and efficiency of integrating spatial proteomics and transcriptomics on the same tissue section, guaranteeing high fidelity in molecular profiling. This combined method is crucial for deepening the understanding of the tumor microenvironment, with potential implications for improving diagnostics and personalized cancer therapies. The ability to analyze multiple data layers on the same cells enhances the development of precise AI-based diagnostic tools and personalized treatment strategies.

#7487 Genetic and genomic knowledge, attitudes, and beliefs among Hispanic/Latinos with colorectal cancer.

Charite Ricker[1], Daisy Hernandez[1], Itzya Ulloa[2], Elena Taylor[2], Blanca Ovalle[3], Bo Yuan[4], Juan Pablo Lewinger[2], Rosa Barahona[3], Caryn Lerman[2], Julie O. Culver[5], Mariana Stern[3], John Carpten[6], Heinz-Josef Lenz[2], **Lourdes A. Baezconde-Garbanati**[3]

[1]Department of Medicine, Division of Medical Oncology, Keck School of Medicine of USC, Los Angeles, CA,[2]Norris Comprehensive Cancer Center, Keck School of Medicine of USC, Los Angeles, CA,[3]Keck School of Medicine of USC, Los Angeles, CA,[4]Department of Population and Public Health Sciences, Keck School of Medicine of USC, Los Angeles, CA,[5]Department of Medicine, Keck School of Medicine of USC, Los Angeles, CA,[6]City of Hope Comprehensive Cancer Center, Duarte, CA

Background: Knowledge, attitudes, and beliefs (KAB) about genetics impacts engagement in genetics and genomics research, but there is a lack of data about these constructs among Hispanic/Latinos (H/L).
Methods: Adult H/L patients with colorectal cancer were recruited at a public (Los Angeles General) and a private (USC Norris Cancer Hospital) hospital to complete a cross-sectional survey assessing KAB. Recruitment and survey administration was done in Spanish or English.
Results: Preliminary analysis of 56 surveys found that most participants were from the public hospital (93%), born outside the US (84%), Spanish speaking (71%), and male (63%); median age was 55 years and acculturation was low [1.89 (SD 1.25); scale 1-5]. Low to mid-range scores were found for genetic literacy [3.45 (SD 1.49); scale 1-7], genetic knowledge [9.63 (SD 6.03); scale 0-25], cancer knowledge [2.20 (SD 1.24); scale 0-5]; and tumor genomics knowledge [3.62 (SD 0.78); scale 0-6]. Although 51% agreed they would want to be contacted for future research, 33% expressed uncertainty. When asked about genetic research, > 90% agreed it is medical progress. Almost 90% would want to know if their disease was hereditary, >80% would inform relatives about genetic results, and 29% agreed they were concerned about genetic testing impacting their ability to stay in this country.
Conclusion: Participants had limited genetic literacy, genetic, cancer and tumor genomics knowledge. Most expressed interest and positive feelings towards genetic testing and genomic research; however, there were uncertainties and concerns to consider in future research to improve H/L engagement in genetics research.

BIOINFORMATICS / COMPUTATIONAL BIOLOGY / SYSTEMS BIOLOGY / CONVERGENT SCIENCE: Tumor Evolution, Clonal Progression, and Spatiotemporal Dynamics
Poster Session

#7488 Prenatal exposure to chemotherapy increases the mutation burden in human neonatal hematopoietic stem cells.

I. Struys[1], C. Velazquez[1], J. Ubels[2], C. L. LeJeune[1], M. van Roosmalen[2], A. Rosendahl Huber[3], A. van Leeuwen[2], W. Bossuyt[1], B. Thienpont[1], T. Voet[1], K. Van Calsteren[1], L. Lenaerts[1], R. van Boxtel[2], F. Amant[1];

[1]KU Leuven, Leuven, Belgium, [2]Princess Maxima Center, Utrecht, Netherlands, [3]Institute for Research in Biomedicine, Barcelona, Spain

Background. Nowadays, chemotherapy is included in the standard of care for cancer treatment during pregnancy, and is considered clinically safe for the child's cognitive, cardiac and brain development up until early adolescence. However, whether prenatal exposure to maternal chemotherapy treatment has a mutagenic impact on the fetal genome, remains unexplored. Patients and methods. We investigated mutation accumulation in hematopoietic stem and progenitor cells (HSPCs) from neonates born to pregnant cancer patients treated with chemotherapy (n=13), as well as healthy pregnant women (n=9) and untreated pregnant cancer patients (n=4). From every subject, cord blood, as a source for neonatal DNA, was collected at delivery, whereafter single HSPCs were isolated and clonally expanded for subsequent whole-genome sequencing (15ξ). Signature analyses was performed using the MutationalPatterns R package and comparison with the COSMIC database.
Results. The mutational burden identified in HSPCs from neonates born to untreated pregnant cancer patients and to healthy controls was similar (mean of single base substitutions (SBS)=41.2±9.1 and 39.9±14.3 respectively; p=0.97) and reflected the expected mutation burden from healthy individuals at birth. In contrast, HSPCs from neonates prenatally exposed to varying chemotherapy regimens, showed an increased number of somatic mutations (mean SBS=89.7±59.2; p=0.0038). Mutational signature analyses attributed the excess mutations to either clock-like processes, which are active during normal cellular aging, or to direct mutagenesis by platinum-based drugs in the case of neonates prenatally exposed to platinum-containing regimens. Our findings in early neonatal tissue are consistent with mutational signatures previously identified in healthy cells of cancer survivors directly exposed to chemotherapeutic drugs and are validated via direct *in vitro* exposure of cord blood HSPCs from healthy individuals to single chemotherapeutic agents frequently used to treat pregnant women.
Conclusion. This study demonstrates that environmental mutagenic exposure during pregnancy can increase somatic mutation accumulation in the fetus. Given that detrimental early life exposures can adversely affect health outcomes later in life, our study highlights the need for further research into the impact of environmental maternal exposures associated to genomic insults during the perinatal period.

#7489 Archetype analysis links heterogeneity in premalignant lesions to diverging features of lung adenocarcinoma.

Kelley Anderson[1], Linh Tran[2], Kostyantyn Krysan[2], Gregory A. Fishbein[3], William D. Wallace[4], Emily Green[1], Hanqiao Liu[1], Xiaohui Xiao[1], Gang Liu[1], Erin Kane[1], Avrum Spira[1], Steven M. Dubinett[2], Eric Burks[5], Sarah Mazzilli[1], Marc Lenburg[1], Jennifer Beane[1]

[1]Section of Computational Biomedicine, Boston University Chobanian & Avedisian School of Medicine, Boston, MA,[2]Department of Medicine, David Geffen School of Medicine at University of California, Los Angeles, CA,[3]Department of Pathology & Lab Medicine, David Geffen School of Medicine at University of California, Los Angeles, CA,[4]Department of Pathology, Keck School of Medicine of University of Southern California, Los Angeles, CA,[5]Department of Pathology, Boston University Chobanian & Avedisian School of Medicine, Boston, MA

Lung cancer is the leading cause of cancer-related death. Adenocarcinoma (LUAD), the most common form of lung cancer, is a heterogeneous disease with high variability in histopathologic features and clinical outcomes. Atypical adenomatous hyperplasia and adenocarcinoma *in situ* are the premalignant lesions (PML) in LUAD pathogenesis. The molecular features of PML that lead to aggressive LUAD are poorly characterized. We hypothesized that transcriptomic changes in PML are linked to distinct clinicopathologic features that may identify aggressive PML before they become invasive LUAD. We examined lung resections from patients with early-stage LUAD where adjacent tissue contained premalignant histology. Normal, PML, and LUAD areas were laser-capture micro-dissected, and RNA was isolated and then profiled using bulk RNA-sequencing (n=41 patients, n=177 samples). We derived a set of recurrent gene modules (n=9 modules) in LUAD PML using samples from our cohort (n=80 PML samples) and three publicly available data sets (n=57 LUAD PML samples). Using these modules, we identified four PML archetypes, where the vertices of a polytope define each archetype. Based on pathway (Gene Set Enrichment Analysis, FDR <0.01) and signature enrichment, we identified the four archetypes as: normal-like, inflammation, cell adhesion, and proliferation. Analyzing the expression of published gene signatures suggests that the immune environment and epithelial cell plasticity may play different roles in different archetypes. Archetypes also differ with regard to a gene-expression signature for predicted immunotherapy response, with the proliferation archetype predicted to have a poor response, and the inflammation archetype predicted to have a good response to immunotherapy (logistic regression, $p<0.05$). An advantage of the archetyping approach is that archetype space is continuous, and samples from other cohorts can be placed within the PML-derived polytope based on their transcriptomic profile. We found that the PML-derived archetypes were related to clinical features in studies of LUAD. The proliferation archetype was associated with a decrease in disease free survival in multiple datasets (cox-proportional hazard model, $p<0.05$ for each tested cohort), and aggressive LUAD histology (linear model, $p<0.05$ for each tested cohort). While past studies demonstrated transcriptional differences between tumors and PML, our archetyping approach focuses on PML-specific gene expression patterns using multiple cohorts to increase the diversity of PML samples studied. Gene expression differences among archetypes were related to pathway dysregulation and prognosis. Characterizing PML based on transcriptomic features may improve our understanding of divergent mechanisms of carcinogenesis, and enable identification of aggressive PML capable of developing into invasive LUAD.

#7490 Uncovering rare cellular populations in esophageal preneoplasia through genetic perturbation tracking and scRNA-seq.

J. Jang, K.-P. Ko, J. Zhang, J.-I. Park;
UT MD Anderson Cancer Center, Houston, TX

The early detection and characterization of rare cellular populations in preneoplastic lesions are crucial for understanding cancer initiation and progression, as well as for identifying novel therapeutic targets. Rare cell populations, often overlooked due to their low abundance, play pivotal roles in shaping the tumor microenvironment and driving disease development. In this study, we employed a lineage tracing system based on tracing vectors introduced into a 2D cell line model of esophageal preneoplasia. This approach allowed us to track genetic perturbations that accumulate during cell proliferation, enabling precise identification of distinct cellular lineages. By coupling this lineage tracing method with single-cell RNA sequencing (scRNA-seq), we achieved high-resolution transcriptomic profiling, uncovering rare cellular populations with unique molecular and lineage-specific characteristics. The rare populations identified through this integrated approach exhibited a quiescent phenotype, suggesting their involvement in lesion persistence and resistance to therapies targeting proliferative cells. Transcriptomic analysis revealed distinct genetic signatures and key pathways associated with these populations, offering potential avenues for therapeutic intervention and biomarker development. Our findings demonstrate the value of combining genetic perturbation-based lineage tracing with scRNA-seq to uncover rare and functionally significant cellular populations. This study provides new insights into the cellular dynamics underlying esophageal cancer initiation and progression, laying the groundwork for future research aimed at understanding the roles of these populations and developing targeted therapies.

#7492 "Baseline-informed" unsupervised multiplexed CyTOF analysis enhances detection of minimal/measurable residual disease in acute myeloid leukemia.

Mahesh Basyal[1], Muharrem Muftuoglu[1], Li Li[1], Jiangxing Lv[1], Yukun Tan[2], Ken Chen[2], Michael Andreeff[1]

[1]Leukemia, UT MD Anderson Cancer Center, Houston, TX,[2]Department of Bioinformatics and Computational Biology, UT MD Anderson Cancer Center, Houston, TX

Multiparameter flow cytometry (MFC) and molecular techniques are the cornerstone for detecting acute myeloid leukemia (AML) minimal/measurable residual disease (MRD). However, some MRD(-) patients relapse, suggesting current methods miss persistent leukemia due to suboptimal detection limits or distinct leukemia-associated phenotypes(LAP). This highlights the need for improved MRD detection methods. Challenges in MFC-based MRD detection include reliance on limited markers, incomplete LAP detection, clonal evolution, surface-marker restrictions, and the need for specialized expertise. These limitations underscore the need for methodologies capable of capturing diverse and dynamic AML proteomic landscape. Single-cell proteomics via CyTOF uniquely dissects high-dimensional features, enabling comprehensive and unbiased leukemic cell characterization. We hypothesized that CyTOF, combined with unsupervised analysis, enhances MRD detection by identifying diverse proteomic features in a feature-agnostic manner. To explore this, we conducted integrated CyTOF analysis of 60 AML patients and 12 bone marrow (BM) samples. This revealed diverse AML proteomic profiles distinct from HSCs/HSPCs indicating unsupervised methods capture aberrant leukemia profiles in a feature-agnostic manner. Next, AML MRD was modeled by mixing barcoded primary AML cells (n=5) and cell lines (Kasumi, Molm13, OCI-AML3) with healthy BM at dilution ratios from 1:10 to 1:100,000. A 50-parameter CyTOF panel and UMAP analysis assessed MRD detection sensitivity. UMAP analysis showed that primary AML cells and cell lines were well-segregated up to a 1:1,000 dilution, with progressive loss of resolution at higher dilutions. At a 1:100,000 dilution, AML cells became indistinguishable from healthy HSCs/HSPCs, highlighting the limitations of UMAP in detecting rare populations. Baseline-informed unsupervised analysis—leveraging phenotypically similar baseline AML cells—restored clustering sensitivity at a 1:100,000 dilution (23 AML cells in 2.3 million BM cells). Furthermore, we extended these findings by conducting integrated CyTOF analysis on pre- and post-therapy primary AML samples (n=5). This "informed" analysis improved AML MRD detection and demonstrated its potential for clinical trials. This approach enabled detailed characterization of metabolic, signaling, apoptotic, and phenotypic features of AML MRD, providing deeper insights into its complexity and surpassing the limited insights of current MRD detection tools. In conclusion, multiplexed CyTOF analysis combined with an "informed" unsupervised analytic approach significantly enhances AML MRD detection and characterization. In addition to enumerating MRD cells with high sensitivity, CyTOF enables detailed proteomic profiling, offering insights that could guide AML MRD therapy.

#7493 Spatially resolved insights into epithelial-stromal co-evolution in pancreatic ductal adenocarcinoma.

Ahmed M. Elhossiny[1], Padma Kadiyala[2], Alexander Bray[3], Jamie Mills[3], Hannah Watkoske[4], Arvind Rao[1], Jude Okoye[4], Atul Deshpande[5], Jiaqi Shi[6], Elana J. Fertig[7], Filip Bednar[2], Yaqing Zhang[4], Eileen S. Carpenter[8], Timothy Frankel[2], Marina Pasca di Magliano[9]

[1]Department of Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI,[2]Department of Surgery, University of Michigan, Ann Arbor, MI,[3]Department of Internal Medicine, Division of Hematology and Oncology, University of Michigan, Ann Arbor, MI,[4]Rogel Cancer Center, University of Michigan, Ann Arbor, MI,[5]School of Medicine, Johns Hopkins University, Baltimore, MD,[6]Department of Pathology, University of Michigan, Ann Arbor, MI,[7]Institute for Genome Sciences, Greenbaum Comprehensive Cancer Center, Department of Medicine, University of Maryland, Baltimore, MD,[8]Department of Internal Medicine, Division of Gastroenterology and Hepatology, University of Michigan, Ann Arbor, MI,[9]Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, MI

Pancreatic ductal adenocarcinoma (PDAC) remains one of the deadliest cancers, with a five-year survival rate of 13%. The tumor microenvironment (TME) in PDAC includes a heterogeneous population of cells. In genetically engineered mouse models, the TME evolves alongside the formation and progression of epithelial precursor lesions to cancer. However, the evolution of the TME in human samples has not been thoroughly characterized, primarily due to limited access to pre-malignant pancreatic tissue. Leveraging a collaboration with Gift of Life Michigan, an organ donor organization, we previously reported that precursor lesions are abundant in the normal human pancreas (*Carpenter et al., Cancer Discovery, 2023*). To date, we have collected over 100 human pancreata, a dataset that enables us to map the characteristics of these lesions and their surrounding microenvironment across variables such as age, sex, and race. Here, we aim to model the co-evolution of the stroma and neoplastic epithelium during tumor progression. We utilized spatial transcriptomics technology with the 10x Visium platform, analyzing over 20 human pancreata during the discovery phase. We applied a spatially aware clustering approach to delineate distinct tissue domains across normal, tumor-adjacent, and tumor samples. We leveraged a previously established single-cell RNA-seq atlas (n=41) to deconvolute cell-type fractions and cell-type-specific gene expression profiles within each spatial transcriptomics spot. As a result, we identified distinct epithelial structures (e.g., Acinar, Ducts, PanINs, tumor) as well as stromal domains (e.g., tertiary lymphoid structures (TLS), fibrotic regions). We hypothesized that stromal gene expression is influenced by the proximity to and the density of different epithelial structures. Therefore, we employed a kernel density estimation approach to model gene expression in stromal cells, specifically fibroblasts and macrophages, as a function of epithelial-specific density. This analysis uncovered distinct characteristics of the stroma surrounding precursor lesions compared to tumor stroma, emphasizing spatially regulated gene expression programs that likely drive TME remodeling in PDAC. Future directions include performing cell-cell interaction analysis to further elucidate epithelial-stromal crosstalk, complemented by in vitro functional experiments to validate the pro- or anti-tumorigenic roles of the identified markers. This work lays the foundation for spatially resolved insights into PDAC biology, informing therapeutic strategies targeting the TME.

#7494 An integrative systems-level framework reveals SMARCD3 resistance regulon signatures vulnerable to MEK1/2 inhibitors in ER+BC cells resistant to everolimus.

Eric F. Medina, Elena Farmaki, Jason I. Griffiths, Vince K. Grolmusz, Andrea H. Bild, Aritro Nath

Beckman Research Institute of The City of Hope, Duarte, CA

Estrogen receptor-positive breast cancer (ER+BC) represents nearly 75% of all breast tumors, with nearly 20-40% patients eventually developing metastases. Everolimus is an mTOR inhibitor which has been approved for use in combination with exemestane in patients with metastatic ER+BC. However, many patients eventually develop resistance to everolimus leading to poor survival outcomes. Systematic characterization of the molecular phenotypes underlying everolimus resistance could reveal new therapeutic targets for metastatic ER+BC. We analyzed transcriptomic profiles from multiple pairs of everolimus resistant or sensitive ER+BC cell lines, to identify signaling mechanisms that distinguish resistant cells both at baseline and under everolimus treatment. Using linear mixed-effects models, we identified strong activation of the growth-factor signaling meta-phenotype in resistant cells at baseline, which persisted even under everolimus treatment. This meta-phenotype included alternate growth signaling mechanisms including IGF1R/insulin, ESR1, RAS and MAPK signaling. To understand the regulatory mechanism behind alternate growth-factor signaling activation, we adopted a network reconstruction architecture to identify SMARCD3 regulons as key transcriptional regulatory networks in resistant cells. In a neo-adjuvant trial for ER+BC patients, the expression levels of SMARCD3 and its regulon's activity were significantly elevated in tumors refractory to everolimus. To develop a therapeutic strategy against everolimus resistance, we used transcriptomic and drug sensitivity profiles of 926 cancer cell lines treated with 395 compounds. We found that the activation of SMARCD3 regulons was consistently associated with sensitivity to several known MEK1/2 inhibitors, including trametinib. To validate these findings, we evaluated the efficacy of everolimus in combination with trametinib and demonstrate that the combination treatment significantly reduced the growth of resistant cells compared to everolimus. Our results demonstrate that everolimus resistant ER+BC cells utilize alternate growth-factor signaling to escape therapeutic control. We found that this phenotype is strongly linked with activation of the SMARCD3 regulons and can be therapeutically targeted using MEK1/2 inhibitors.

#7495 The evolving ECM architecture shapes unique evolutionary trajectories of breast tumor immune escape and elimination.

Yijia Fan, Jason Tomas George

Biomedical Engineering, Texas A&M University, Houston, TX

Purpose: Cancer progression remains a clinical challenge due to tumor heterogeneity. Effective T cell infiltration is critical for mounting an anti-tumor response. Tumors are classified as “hot” or “cold” based on the extent of T cell infiltration. In many cancers, particularly breast cancer, extracellular matrix (ECM) geometry evolves with tumor progression, characterized by Tumor-Associated Collagen Signatures (TACS). Specifically, empirically observed ECM topologies are random fibers (TACS1), circumferentially aligned fibers (TACS2), and radially arranged fibers (TACS3). Clinically, patients with TACS3+ breast cancer exhibit poorer survival compared to TACS3- cases, but the underlying mechanisms and the role of evolving ECM geometry in tumor evolution and tumor-T cell interaction remain unclear. The lack of biomarkers or methods to track TACS evolution underscores the need for physical models linking ECM geometry to tumor-immune co-evolution.
Methods: To address this, we developed the EVO-ACT (EVOlutionary Agent-based Cancer T cell interaction) model, built upon the Gillespie algorithm.
Results: Our analysis of the three TACS geometries revealed distinct T cell migration patterns and efficiencies: TACS3 demonstrated the highest efficiency, TACS1 intermediate, and TACS2 the lowest. While TACS geometry significantly influences T cell infiltration, it does not act as an absolute barrier; stronger chemokine gradients enhance T cell infiltration across all TACS types. Notably, TACS3 facilitates immune recognition and correlates with higher survival in our simulations, which contrasts with the clinically observed lower survival of TACS3+ breast cancer patients. As TACS3 is typically linked to late-stage breast tumors with phenotypic adaptations, we incorporated mechanisms such as EMT into our model to address this discrepancy. The progression from TACS2 to TACS3, coupled with EMT, more accurately reproduced clinical outcomes. Considering the mesenchymal phenotype’s ability to upregulate immune checkpoint molecules such as PD-1/PD-L1, we further explored TACS-specific tumor evolution and its impact on responses to immune checkpoint inhibitors and patient survival. Our findings reveal lower survival in TACS3/EMT+/PD-L1+ cases compared to TACS2/EMT-/PD-L1+ cases, highlighting that TACS3-associated poor survival arises not solely from ECM geometry but from late-stage cancer adaptations such as EMT and upregulated immune checkpoint expression.
Conclusions: This study underscores the complex interplay between evolving ECM geometry and tumor-T cell interaction dynamics, offering a new explanation for TACS-associated survival differences. It also provides novel insights into TACS-specific responses to PD-1/PD-L1 inhibitors and their implications for patient survival.

#7496 Evolutionary trajectories inferred by selective epistasis and phylogenetic analysis reveal the malignant progression of intraductal papillary mucinous neoplasms to pancreatic ductal adenocarcinoma.

Derek C. Song[1], Nic Fisk[2], Margaret Moore[3], Vincent L. Cannataro[4], Jeffrey D. Mandell[1], Luisa Escobar-Hoyos[5], Leanne Brown[5], Lu Qiao[5], John W. Kunstman[5], Jeffrey P. Townsend[3]

[1]Yale University, New Haven, CT,[2]University of Rhode Island, Kingston, RI,[3]Yale School of Public Health, New Haven, CT,[4]Emmanuel College, Boston, MA,[5]Yale School of Medicine, New Haven, CT

Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of cancer death, with high mortality rates attributable to late detection when metastases have already occurred. Early-stage PDAC is challenging to detect due to its frequently asymptomatic nature, lack of biomarker sensitivity, and inaccessible anatomical location. Pre-malignant intraductal papillary mucinous neoplasms (IPMNs) are detectable through imaging and can progress to malignant PDAC, offering a unique opportunity for early interventions such as prophylactic resection. However, identification of IPMNs that will become malignant is challenging, and pancreatic resection comes with significant morbidities. Thus, characterizing the evolutionary trajectories by which IPMNs progress to PDAC could both guide surgical decision-making and reveal targetable mechanisms to prevent malignant development.

Here, we quantify the evolutionary selection for mutations that lead to IPMNs and compare that result to the selection of known drivers of PDAC. We infer a protective effect of KLF4 mutation in IPMNs on subsequent progression to PDAC that arises due to antagonistic selective epistasis with KRAS and other high-impact contributors to PDAC. We then investigate the molecular etiology of IPMNs and PDACs at different histopathological grades, finding that the mutational processes observed in each are markedly alike, suggesting instead that microenvironmental and epistatic dynamics of the heterogenous proto-tumor drive progression. Finally, to detail prospective mutational trajectories by which IPMNs progress to KRAS-positive PDACs, we leverage selective epistatic interactions to map likely mutational orderings in PDACs and compare those orderings to the sequence of mutations reconstructed via phylogenetic analysis of IPMN lineages.

#7499 Driver mutations propel subclonal evolution and spatial patterns of tumor growth in intrahepatic cholangiocarcinoma.

E. John[1], P. Shukla[1], A. Jain[1], A. Lau[1], T. Lesluyes[2], H. Ogilvie[1], M. Tarabichi[3], J. Roszik[1], Y. Yuan[1], P. Van Loo[1], L. Kwong[1], Y. Chun[1];
[1]UT MD Anderson Cancer Center, Houston, TX, [2]University Cancer Institute Toulouse Oncopole, Toulouse, France, [3]Universite Libre de Bruxelles, IRIBHM, Belgium, Belgium

Intrahepatic cholangiocarcinoma (ICC) is a highly lethal cancer of the bile ducts that exhibits a diversity of driver oncogenic mutations, several of which have been shown to be frequently subclonal. However, existing tumor evolution studies in ICC have been limited by either the number of sub-regions assessed or a paucity of driver mutation heterogeneity. Here, we conducted a multi-omic analysis of 90 tumor regions from 11 discrete tumors in 10 patients, identifying subclonal driver mutations in 5 of the patients, in the genes *PBRM1*, *PIK3CA*, *TP53*, *ARID1A*, *BRAF*, *MLH1*, and *CDKN2A*. This high-number multi-region approach also allowed us to unambiguously identify distinct subclonal evolutionary branches and map them to the spatial location of the samples. Importantly, each subclonal driver mutation was tightly associated with these defined branches, implicating them as a frequent mechanism of driving subclonal evolution. Additionally, subclonal branching showed a spatial correlation on surgical resection depicting the strength of multi-region sequencing in treatment. In total, we conclude that while subclonal branching can be driven by a number of factors, the acquisition of bona fide driver mutations strongly propels subclonal evolution and spatial patterns of tumor growth.

#7500 Joint reconstruction of single-cell evolutionary lineages and fate transition trajectories from long-read single cell transcriptomes.

Yueying He[1], Yuanqing Yan[2], Cheng-Kai Shiau[1], Lina Lu[1], Minhua Wang[1], Taisuke Kaihou[2], Chitaru Kurihara[2], Kazutaka Fukumura[3], Rajul K. Ranka[4], Yudai Miyashita[2], Timothy Pan[1], Arvind Bhimaraj[5], Daniel Brat[6], Jason Huse[5], Ankit Bharat[2], Ruli Gao[1]

[1]Department of Biochemistry and Molecular Genetics, Northwestern Univ. Feinberg School of Medicine, Chicago, IL,[2]Department of Surgery, Northwestern Univ. Feinberg School of Medicine, Chicago, IL,[3]Department of Anatomical Pathology, University of Texas MD Anderson Cancer Center, Houston, TX,[4]Department of Cardiovascular Sciences, Houston Methodist Research Institute, Houston, TX,[5]Department of Cardiology, J.C. Water Jr. Transplant Center, Houston Methodist Research Institute, Houston, TX,[6]Department of Pathology, Northwestern Univ. Feinberg School of Medicine, Chicago, IL

Computational inference of endogenous genetic alterations from single cell transcriptomic data opens an avenue of measuring both evolution and fate markers in individual cells, in particular, the emerging long-read single cell RNA sequencing (LR-scRNAseq) technologies allowed detection of multi-layered mutations across whole transcript bodies of thousands of genes in thousands of cells in parallel. However, robust tools for integrating single cell genetic evolutionary lineage and fate transition trajectory from these powerful data are missing. To address this, we developed EvoFATE, an unsupervised graph learning model, to automatically reconstruct unified roadmaps of single cell evolutionary lineages and fate transition trajectories by discovering representations using endogenous mutations as evolutionary markers and gene expressions as fate remodeling markers. EvoFATE recovered complex evolutionary fate dynamics in simulations and blood samples where diverse cell types had known lineage orders. We applied EvoFATE onto LR-scRNAseq data of patient-matched lung metastatic tumors and identified the evolutionary fate paths of tumor cells during both metastatic seeding and clonal expansion stages. To summarize, EvoFATE robustly integrates genetic evolutionary lineages with cell fate transition trajectories to resolve complex fate remodeling dynamics and evolution-fate synchronies using cell endogenous markers, enabling precise tracing of temporal changes in cells under real pathophysiology conditions without genetic manipulations.

#7502 Radiation-induced transcriptomic reprogramming of mesenchymal glioblastoma stem cells reveals key pathways and potential targets.

Grace Rosner[1], Abigail Goen[1], Godwin Peasah-Darkwah[1], Jonathan Rodgers Gochicoa[1], Alos Diallo[1], Gokul Srinivasan[2], Elisa Bu Sha[3], Joshua Levy[4], **Kelli B. Pointer**[5]

[1]Dartmouth College, Hanover, NH,[2]UCLA/Caltech, Los Angeles, CA,[3]Rutgers University, New Brunswick, NJ,[4]Computational Biomedicine, Cedars-Sinai, Los Angeles, CA,[5]Radiation Oncology, Dartmouth College, Hanover, NH

Background: Glioblastoma (GBM) is a highly aggressive primary brain tumor with a median survival of less than two years. Despite standard therapy of surgery, radiation, and chemotherapy, GBMs recur. These recurrences may partially be due to glioblastoma stem cells (GSCs). Various subpopulations of GSCs exist, likely attributing to different resistant niches. The mesenchymal (MES) subpopulation may drive radiation (RT) resistance. The transcriptomic shifts that define GSC adaptation to RT remain unknown. This study explores the transcriptomic reprogramming of MES GSCs following RT to identify resistance mechanisms.

Methods: Using dataset GSE162931 from the GEO database and MES gene signatures from PMID:31327527, we analyzed two patient-derived GBM cell lines pre- and post-10 Gy RT. Data for the two cell lines were preprocessed and integrated with Seurat, followed by clustering and UMAP visualization with 2D kernel module-score weighted density plots. Cluster proportion changes between conditions were statistically examined using Chi-squared or Fisher's tests with adjusted p-values. Differential expression analysis revealed conserved and unique gene markers distinguishing treated from naïve conditions, visualized in volcano plots and heatmaps. A final score integrating module scores and cluster proportions quantified MES traits at the single-cell level. All analyses were conducted in R studio version 4.2.3.

Results: UMAP, density plots, and cluster proportion changes indicate significant shifts in MES GSC distributions post-RT, suggesting RT selection for specific transcriptomic profiles. Highly MES subclusters exhibit distinctive RT-responsive gene expression patterns. The final scoring revealed enhanced MES traits in post-RT clusters. Distinct clustering patterns in MES GSCs highlight diverse adaptive strategies. Differential expression analysis reveals upregulation of DNA repair genes (SAT1, NDRG1, GADD45A), enhancing genomic stability post-RT, and stemness markers (NES, GDF15), emphasizing self-renewal capacity. Genes supporting oxidative stress management (SQSTM1, DDIT4) were upregulated, while downregulated metabolic genes (PGK1, LDHA) indicate a shift towards oxidative phosphorylation. Gene Set Enrichment Analysis identified enriched pathways related to DNA repair, cell migration, and differentiation regulation.

Conclusion: These findings underline the transcriptomic plasticity in MES GSCs that drive RT resistance. Potential targets like AKAP12 and S100A4, essential for maintaining stem-like traits and MES phenotypes, present new therapeutic targets that could be used to combat resistant GSC populations, aiming to reduce tumor recurrence and enhance GBM patient outcomes.

#7503 Detection of genomic alterations in lenvatinib-resistant liver cancer cell lines.

JungHyun Kim[1], Ji Young Kim[1], Gamin Han[1], BoYoon Kim[2], Hoon Kim[2], Yeup Yoon[1], Wonseok Kang[3]

[1]Samsung Advanced Institute for Health Sciences and Technology (SAIHST), Sungkyunkwan University, Seoul, Korea, Republic of,[2]Department of Biopharmaceutical Convergence, Sungkyunkwan University, Suwon, Korea, Republic of,[3]Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of

Hepatocellular carcinoma (HCC), the most common type of primary liver cancer, is the sixth most prevalent tumor worldwide and the fourth leading cause of cancer-related deaths. Lenvatinib, a multi-targeted tyrosine kinase inhibitor (TKI), is a first-line therapy for HCC patients who are ineligible for immunotherapy. However, like other targeted therapies, its efficacy is often compromised by the development of resistance. Understanding the molecular alterations driving this resistance is critical to improving therapeutic outcomes in HCC. In this study, lenvatinib-resistant human HCC cell lines were established by exposing parental sensitive cells to increasing concentrations of lenvatinib. Resistant Hep3B and Huh7 cell lines were successfully developed, exhibiting higher lenvatinib-specific IC_{50} values compared to their sensitive counterparts. To investigate the mechanisms underlying drug resistance, whole-genome sequencing (WGS, average depth: 30X) was performed, identifying resistance-specific genomic alterations, including copy number variations (CNVs), mutations, and structural variations (SVs). Emerging evidence suggests that chromosomal instability (CIN) plays a critical role in resistance evolution through structural rearrangements and focal amplifications. To reconstruct these amplified regions, we utilized AmpliconArchitect, followed by classification into chromosomal amplification (ChrAmp) and extrachromosomal DNA (ecDNA) using AmpliconClassifier. The number of focal amplifications increased from 4 to 7 in resistant Hep3B cells and from 14 to 18 in resistant Huh7 cells. Notably, newly identified oncogenes were detected within these amplicons, including *ZIC2* in Hep3B and *EGFR* in Huh7. Despite the emergence of new amplicon profiles in resistant cells, most retained their original classifications as ecDNAs or ChrAmps, suggesting that clones carrying these amplicons exhibit an intrinsic resistance phenotype. Ongoing studies involving single-clone isolation and further genomic characterization aim to refine and expand these findings. In conclusion, we successfully established lenvatinib-resistant HCC cell lines and characterized their resistance-specific genomic alterations. Our results highlight the role of CIN in driving resistance under the selective pressure of targeted therapies, offering potential insights for improving therapeutic strategies in HCC.

#7504 Integrated multiomic analysis to infer longitudinal patterns of ecDNA in IDH-wildtype integrated multiomic analysis to infer longitudinal patterns of ecDNA in IDH wild-type glioblastoma.

Dacheng Zhao[1], Kevin Anderson[2], Amit Gujar[1], Megan Costa[1], Djamel Nehar-Belaid[2], Frederick Varn[2], Elise Courtois[2], Paul Gabrie[2], Paul Robson[2], Hyo-Eun Moon[3], Anna Golebiewska[4], Sun Ha Paek[3], Simone Niclou[4]

[1]Yale University School of Medicine, New Haven, CT,[2]The Jackson Laboratory for Genomic Medicine, Farmington, CT,[3]Seoul National University, Seoul, Korea, Republic of,[4]Luxembourg Institute of Health, Luxembourg, Luxembourg

Extrachromosomal DNA (ecDNA), a prevalent mechanism of gene amplification, drives oncogene overexpression and is associated with poor patient survival. Over 60% of primary glioblastoma (GBM) cases harbor ecDNA-mediated oncogene amplifications. To better understand the functional consequences of ecDNA amplification, we performed a comprehensive analysis of ecDNA distribution in tumors using longitudinally sampled multiomic data from 35 GBM patient specimens. This dataset integrates 68 whole-genome sequencing (WGS) datasets, 243,459 transcriptomic and chromatin accessibility profiles derived from the same nuclei. We developed a single-nucleus ATAC-seq-based pipeline to classify ecDNA status at single-cell resolution. To validate the robustness of this pipeline, we utilized patient-derived neurospheres (n = 2), generating multiomic datasets and over 100 fluorescent in situ hybridization (FISH) images per cell line for structural validation. This approach enabled the identification of ecDNA-containing regions in each patient sample through WGS data and the inference of single-cell-level copy number variations. In total, we classified the ecDNA status of over 86,345 malignant cells. Our findings demonstrated that ecDNA copy numbers remain stable during tumor progression, with no significant changes in the proportion of ecDNA-containing cells over time. Notably, we observed that oncogenes amplified on ecDNA are associated with distinct tumor cell states. By applying non-negative matrix factorization and a random forest machine learning algorithm, we extracted gene programs linked to ecDNA that are enriched for cell-cycle regulation and chromatin regulating. These results provide new insights into the regulatory landscape of ecDNA-driven glioblastoma and highlight its potential role in shaping tumor cell identity and progression.

#7505 Comprehensive in-silico molecular analysis of early-onset gastric cancer identifies novel genes implicated in disease characterization and progression.

Fernan Gomez-Valenzuela, Ian Silva, Ignacio Nicolas Retamal, Javiera Garrido, Benjamin Garcia-Bloj, Tomas De Mayo Glasser, Matias Munoz-Medel, Juan Alejandro Godoy, Marcelo Garrido

Centro de Oncologia de Precision, Universidad Mayor, Santiago, Chile

Gastric cancer, a prevalent and fatal form of cancer worldwide, is manifested at different age ranges during the lifespan. Approximately one-third of newly diagnosed gastric cancer cases are early onset gastric cancer (EO-GC), which affects individuals under the age of 50. EO-GC tends to be more aggressive than late-onset gastric cancer (L-GC), with a faster and multifocal disease progression. Moreover, EO-GC is associated with early metastatic disease. Recent research has underscored the need for a deeper understanding of EO-GC that promotes therapeutic approaches specific to EO-GC. These characteristics emphasize the need to understand their differential molecular characteristics deeply. We analyzed The Cancer Genome Atlas Stomach Adenocarcinoma (TCGA-STAD) transcriptomic expression data as a proxy to understand if age may be positioned as a variable associated with a specific gene expression pattern in gastric cancer. Using transcriptomic and clinical data, we performed a comprehensive bioinformatic analysis to explore molecular differences between EO-GC (n = 32) and L-GC (n = 345). Principal component analysis (PCA) revealed distinct transcriptomic clusters separating EO-GC and L-GC cases, particularly when categorized by decades, highlighting their molecular divergence. Next, we performed differential gene expression analysis (DGEA) using the 'DESeq2' and 'edgeR' R packages, identifying several significant genes that distinguish EO-GC from L-GC. Among these, *KLHL4*, *MAGEL2*, *RNLS*, and *CYP8B1* were upregulated in EO-GC, while *CLDN6*, *MIOX*, *PNMA5*, and *ACTL8* were downregulated. Consequently, we performed a functional analysis of differentially expressed EO-GC genes related to cytoskeleton regulation, cell adhesion, and metabolism pathways, which may contribute to its aggressive phenotype. Furthermore, some EO-GC genes were associated with poorer survival outcomes. Last, we observed that the DNA methylation score was higher for EO-GC cases. In conclusion, our findings highlight the critical role of differential genetic and epigenetic landscapes of EO-GC and propose these molecular features for its diagnosis and prognostic evaluation. These insights could inform the development of personalized therapies to improve outcomes for younger GC patients.

#7506 A gene program association study (GPAS) in prostate cancer reveals novel gene modules associated with plasticity and metastasis.

Hanbing Song[1], **Junxiang Xu**[2], Paul Allegakoen[1], Yih-An Chen[1], Hannah N. W. Weinstein[1], Arda Demirci[1], Isaac Salzman[1], Keila Velazquez-Arcelay[1], Margaret Tsui[1], Jon Akutagawa[1], Julia H. Pham[1], Peter R. Carroll[1], Matthew R. Cooperberg[1], Ezequiel Goldschmidt[3], Nancy Greenland[4], Joshua D. Campbell[2], Franklin W. Huang[1]

[1]Department of Medicine, University of California, San Francisco, San Francisco, CA,[2]Department of Medicine, Boston University, Boston, MA,[3]Brain Tumor Center, University of California, San Francisco, San Francisco, CA,[4]San Francisco Veterans Affairs Medical Center, San Francisco, CA

Objective: Prostate cancer (PCa) is a heterogeneous disease both within and across individuals. Genomic alterations, epigenetic changes, and interactions with cells in the tumor microenvironment (TME) contribute to different states in tumor cells. Single-cell RNAseq (scRNAseq) can provide insights into the transcriptional heterogeneity of tumor cells and cell types in the TME. However, most scRNAseq studies are limited by small sample sizes. Here, we performed the first gene program association study in PCa to identify gene modules and immune cell types associated with intra-tumoral heterogeneity and metastasis.
Methods: 14 datasets were merged to create a harmonized scRNAseq atlas. Copy number inference and boosted gradient tree models were trained to identify the tumor cells. Celda was used to identify co-expression modules. Linear mixed effect models were used to associate modules with clinical phenotypes. Finally, a transformer model similar to RoBERTa for annotation of PCa datasets was developed and validated in 2 independent datasets.
Result: We compiled an atlas of 560,492 cells from 128 PCa patients with localized primary PCa, Castration resistant PCa (CRPC), and metastatic PCa from 7 different sites. 166 co-expression modules were identified across 106,998 tumor cells. 2 cell-cycle modules were consistently upregulated across all metastatic sites while prostate lineage and antigen presentation modules were consistently downregulated. Different metastatic sites exhibited unique module expression patterns. For example, a module of FOLH1 and SPON2 was upregulated specifically in brain metastasis while erythroid modules were found in liver metastasis. 4 different modules were significantly higher in neuroendocrine prostate cancers (NEPC). Each NE tumor was not monolithic but expressed a unique combination of these modules. A novel subtype of NEPC tumors was identified which predominantly expresses the HES1 module instead of the canonical NE module. We developed a novel scoring system to identify NEPC tumors in bulk RNA-seq data using a combination of NE-associated modules which performed better than previously established gene set signatures (AUC = 0.938). Within the TME, we detected a T-cell cluster signified by heat shock protein genes enriched in African American PCa patients than European American patients. Furthermore, a transformer neural network was developed for automated annotation of scRNAseq PCa datasets which achieved a 91% accuracy in validation datasets.
Conclusion: We generated a comprehensive atlas of cells from PCa, characterized novel gene programs that defined tumors cells in localized and metastatic samples, identified a novel subtype of NEPC, and developed a robust computational tool to predict these states in new studies. These findings can potentially be used clinically to stratify high-risk patients with localized disease.

#7507 Towards transparent and context-specific annotation of driver and passenger mutations in cancer.

M. Madhumita[1], J. Teague[1], P. Beer[2], Z. Sondka[1];
[1]Wellcome Sanger Institute, Hinxton, United Kingdom, [2]University of Glasgow, Glasgow, United Kingdom

Cancer arises from the accumulation of somatic genetic alterations, with only a subset acting as "drivers" that promote malignant transformation. Distinguishing between driver and passenger mutations is fundamental for both clinical and research applications, yet current *in-silico* variant annotation tools often fall short. Our previous evaluation of multiple algorithms revealed a concerning reliance on pre-computed, black-box models that are rarely updated and typically limited to missense mutations. These tools also lack transparency and biological context, leading to inconsistent predictions that hinder effective cancer driver identification.

Building on these findings, our current study emphasizes the need for a new framework in cancer genomics. This framework should be characterized by explainable, unbiased approaches, long-term support, and the integration of recurrence data as a marker of positive selection. To address the above limitations, we leverage 20 years of manually curated COSMIC data. By systematically analysing mutation peaks or hotspots within cancer-associated genes, we uncover patterns indicative of positive selection, a hallmark of driver mutations. Our approach is enriched by integrating additional biological metrics, including dN/dS ratios, which measure selection pressure on genes, and allele frequency data from diverse human populations to account for background genetic variation. Furthermore, we incorporate gene-specific functional insights to enhance the contextual relevance of our predictions.

Our integrative methodology aims to establish a robust, reproducible set of rules for annotating driver mutations, leveraging multiple layers of evidence to reduce biases and increase predictive accuracy. By prioritizing algorithmic transparency and the inclusion of biologically informed data, we lay the foundation for a more reliable classification system that adapts to emerging discoveries in cancer genomics. Ultimately, our work seeks to transform cancer mutation annotation into a reproducible and clinically actionable process, paving the way for better-targeted therapies and more precise diagnostic tools. Through this, we aspire to bridge the existing gaps in driver mutation identification and to propel cancer genomics towards its full potential in precision medicine.

#7508 Transcriptomic analysis of polyisoprenylated cysteinyl amide inhibitors treatment of pancreatic and breast cancer reveals changes in apoptotic and angiogenic factors.

Kweku Ofosu-Asante, Amarender Burra, Jahnissi Odoom, Joshua Ablordeppey, Nazarius S. Lamango

Florida A&M University College of Pharmacy & Pharmaceutical Sciences, Tallahassee, FL

The aggressive nature of KRAS-driven cancers and the lack of effective targeted therapies makes them extremely challenging to manage. The polyisoprenylated cysteinyl amide inhibitors (PCAIs) were designed to mimic the polybasic C-terminal hypervariable (HVR) region of KRAS and related proteins. The HVR and polyisoprene modifications are essential for their functional localization. Disrupting the polyisoprenylation-dependent interactions abrogates RAS signaling interactions, affording opportunities for controlling hyperactive G-proteins in cancers. Previous studies have demonstrated the effectiveness of PCAIs against cancer-progression phenomena various cancer cell lines. Hence, we determined the effect of the PCAIs on the gene expression in PANC-1, MIA PaCa-2 and MDA-MB-231 cells, to better understand biological responses elicited by cancer cells on exposure to PCAIs at gene level. Following 3 µM NSL-YHJ-2-27 treatment of MIA PaCa-2 cells, total RNA was extracted, sequenced and analyzed using Partek Flow. DESeq2 tool was used to analyze a total of 88 genes that were significantly differentially expressed. Out of the 88 genes, 85 were upregulated and 3 downregulated. Notable genes such as tumor suppressor gene, suppressor of cytokine signaling 1 (*SOCS1*) and autophagy-related protein 9 (*ATG9L*) were significantly upregulated by 2-fold and 3-fold, respectively. Moreover, treatment with 3 µM NSL-AB-51 of both PANC-1 and MDA-MB-231 cells resulted in 9 and 28 genes, respectively, being differentially expressed. The gene endothelin-1 (*EDN1*) which promotes cell proliferation, survival and neovascularization was significantly downregulated by one-fold in PANC-1 cells. Genes such as BCL-2 binding component 3 (*BBC3*) that has been shown to promote apoptosis and angiopoietin-like 4 (*ANGPTL4*) that inhibits tumor angiogenesis were significantly upregulated by 2-fold and 4-fold, respectively. These effects on the transcriptome strongly corroborate the phenotypic characteristics of the PCAIs against cell viability and proliferation, migration, invasion and angiogenesis and offers more opportunities to further understand and develop the PCAIs as potential agents to treat KRAS-driven cancers.

#7509 Path-Omics: Elevated plasma cell infiltration into aneuploid epithelium predicts Barrett's esphagus progression to adenocarcinoma.

Caner Ercan[1], **Xiaoxi Pan**[1], Thomas G. Paulson[2], Matthew D. Stachler[3], Carlo C. Maley[4], Yinyin Yuan[1]

[1]Translational Molecular Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX,[2]Translational Science and Therapeutics Division, Fred Hutchinson Cancer Center, Seattle, WA,[3]Department of Pathology, University of California San Francisco, San Francisco, CA,[4]Arizona Cancer Evolution Center, Arizona State University, Tempe, AZ

Background

Barrett's esophagus (BE) is a precancerous condition characterized by the metaplastic replacement of esophageal epithelium in response to gastroesophageal reflux. Although the progression rate to invasive carcinoma is low, identifying high-risk patients remains a critical unmet need, especially as BE prevalence rises. While molecular and immune microenvironment changes associated with BE progression are documented (Nowicki-Osuch 2021), there is a gap in reliable pathology-level indicators to assess cancer risk. This study aims to uncover cancer-relevant changes in BE using computational pathology approaches.

Methods

We analyzed a unique dataset comprising 742 esophageal biopsy slides from 138 patients, and with DNA content abnormalities, including aneuploid (n=125 slides from 26 patients), determined by flow cytometry. Clinical follow-up data for cancer progression was available for 117 of these patients. Using computational pathology models, we trained a multiple-instance learning framework for aneuploid prediction (Lu 2021, Ercan 2024) on 45 slides, and a single-cell detection model based on the ACformer architecture (Huang 2023), fine-tuned on 13,343 cells. Spatial immune cell distribution within the epithelium was quantified using the Morisita-Horn index (MHI), which measures spatial overlap between immune cells and BE cells.

Results

The MIL-based aneuploid prediction model achieved a balanced accuracy of 74.3% and an AUC of 0.81 on the test set (n= 388), while the single-cell detection & classification model reached accuracies of 73.7% for detection and 98.3% for classification (in epithelium, lymphocyte, plasma cell, eosinophil, neutrophil and stromal cell classes). Immune cell MHI was significantly elevated in aneuploid biopsies ($p < 10e-4$, n=125). Aneuploid was found to strongly correlate with cancer progression ($p < 10e-5$, n=33). In aneuploid samples, immune cell infiltration analysis revealed that increased immune cell abundance and higher plasma cell MHI scores were significantly associated ($p < 10e-4$, $< 10e-4$, respectively) with cancer progression in the vicinity of aneuploid BE epithelium, consistent with the observations in (MK Strasser 2023).

Conclusion

This study demonstrates the potential of Patho-Omic tools to predict aneuploid in BE biopsies and identify cancer progression-associated features directly from routine pathology slides. By combining slide-level aneuploid prediction with single-cell detection, we highlight the role of immune cell spatial dynamics in cancer progression. These findings lay the groundwork for stratifying BE patients by progression risk through biopsy image analysis.